Proteomic profiling of primary cells to investigate effects of non-genotoxic carcinogens and mechanisms of chemoprevention.

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Abstract

There are compounds affecting an organism upon incorporation in such a toxic manner, that they are able to promote and in some cases even initiate tumour in tissue without any direct interaction with the respective cellular DNA. This characteristic is eponymous for a toxicological classified group of compounds called non-genotoxic carcinogens (NGCs). Their cancerogenic capacity arises from molecular mechanisms, which act in an indirect way, such as by affecting the regulating of gene expression via signal transduction or histone modification (acetylation, methylation), wherefore they are also sometimes called epigenetic carcinogens. This transcriptionally deregulation disrupts metabolic pathways and as a consequence may cause endocrine alterations or oxidative stress, are cytotoxic, trigger a pro-inflammatory response or act immune suppressively. Other important modes of action are a dysbalance of apoptotic and proliferative effectors and an inhibition of gap junctional intercellular communications. The lack of a direct interaction with DNA and the various effects caused by these compounds in organisms makes it hard to detect them via assays and thereby causes problems e.g. during drug development, because of their late discovery. So far there is no short-term assay available and the only safe way to determine, if a compound may act in a non-genotoxic manner or not, is to use long-term assays. It may be possible to outline groups of NGCs with similar modes of action, such as peroxisomal proliferators (wyeth-14,643 or nafenopin) or p-450 inducing compounds (phenobarbital), and define group specific biomarkers. Nevertheless, the exact modes of action of NGCs are still unknown, because of their complexity and potential to act tissue-spanning.

We have set us to investigate relatively well-studied NGCs and define modes of action and subsequently biomarkers for them, which should serve as basis for other compound groups and the development for future short-term assays. NGCs are metabolised in the liver and thereby affect the cells there. These cells are comprised of the hepatocytes and the non-parenchymal cells, which are neglected most of the time in investigations of NGCs, missing out the opportunity to study the epithelial-mesenchymal interactions. We used rats as model species, because it is know that rodents are more sensitive towards the effects of NGCs modes of action than humans, raising the issue of human relevance in some cases. Our model compounds were nafenopin, a fibrate, and phenobarbital, a barbiturate, which were used to treat the rats *in vivo* or the isolated primary cells *in vitro*. For the purpose of analysing the hepatocytes and the non-parenchymal cells, we decided to use a powerful screening method, shotgun proteomics, which allows analysing the (sub-cellular) proteome of cells by utilising a combination of 1D SDS-PAGE

and LC-MS/MS. By comparing the proteomes of cells, alterations in the protein expression become apparent, which may give references for further studies. To summarise the results, we found various noteworthy alterations in hepatocytes and non-parenchymal cells, which were already known as well as novel ones suggesting that an autocrine and paracrine cell-cell interaction is important for NGCs modes of action.

We also investigated inflammatory-related effects of clinical relevance. It should be commented that nowadays acute inflammation is not problematic by using drugs such as antiphlogistics. Disease linked to chronic inflammation, such as cancer or auto-immune disease, tell a different story, because by treating the patients' chronic inflammatory response and not the primarily diseased cells, such as endothelial cells, the cure would be short-lived. Originating from the bone marrow, immune cells are replenished all the time and will become activated by the diseased cells of the affected tissue. As a context to the NGC study, the NGCs, we investigated, affected proteins involved in the immune system and with that immune cells, such as Kupffer cells or monocytes and t-lymphocytes deriving from the blood.

The peripheral mononuclear blood cells (PBMCs), which are mainly comprised of monocytes and t-lymphocytes, were the cells of interest for our study. By comparing the proteomes of untreated and inflammatory activated PBMCs, we were able to determine cell specific inflammation-related proteins. This short-list of proteins was used for subsequent investigations, namely a study of coffee and mould fungi extracts. The investigation of the effects of coffee *in vivo* and mould fungi extracts *in vitro* on the proteome of naïve and inflammatory activated PBMCs aimed to find alterations in the protein expression of the candidates in the short-list. As a result, we noted an activating as well as an enhancing effect of coffee on naïve and inflammatory activated PBMCs, respectively, while the mould fungi extracts showed a weak anti-inflammatory capacity.

Kurzfassung

Es gibt Substanzen die sich bei einmaliger oder chronischer Inkorporation auf den Organismus in einer Art und Weise toxisch auswirken können, dass sie Tumor promovieren und in manchen Fällen sogar initiieren können, ohne dabei direkt mit der zellulären DNA zu interagieren bzw. diese zu schädigen. Diese Eigenschaft ist auch namengebend für diese Klasse von Substanzen, den nicht-gentoxischer Kanzerogenen (toxikologische Klassifizierung; NGK). Dass diese Substanzen dennoch Kanzerogen wirken können begründet sich auf molekulare Mechanismen, die über indirektem Weg agieren, z.B. durch die Einflussnahme auf die Regulation der Genexpression über Signaltransduktion oder Histon-Modifikationen (Acetylierung, Methylierung), weshalb sie auch als epigenetische Kanzerogene bezeichnet werden. Diese transkriptionelle Deregulation führt zu einer Störung von metabolischen Stoffwechselwegen, welche in Folge unter anderem: oxidativen Stress ausüben, endokrine Modifikationen hervorrufen, Zytotoxisch und/oder Entzündungsfördernd sind, Immunsuppressiv wirken als auch die Apoptose, die Proliferation oder die interzelluläre Kommunikation beeinflussen können. Das Fehlen von direkter Interaktion mit DNA und die Vielseitigkeit der Eingriffe in den Zellmetabolismus macht es schwierig diese Eigenschaft bei Substanzen festzustellen, was z.B. bei der Entwicklung von Medikamenten ein frühes Erkennen problematisch macht. Es ist jedoch möglich NGK in Gruppen mit ähnlichen Wirkmechanismen zusammen zufassen, z.B. peroxisomale Proliferatoren (Wyeth-14,643 oder Nafenopin) oder die P-450 induzierenden Substanzen (Phenobarbital), um gruppenspezifische Biomarker zu definieren. Die Kenntnis über die exakten Wirkungsweisen dieser Substanzen, die auch gewebsübergreifend wirken können, ist aufgrund ihrer Komplexität bis heute unvollständig.

Unsere Aufgabe war es nun bekannte nicht-gentoxische Kanzerogene zu analysieren und Wirkmechanismen bzw. Biomarker zu finden, die als Grundlage für weitere Substanzgruppen als auch für zukünftige Kurzzeittests dienen können. Da nicht-gentoxische Substanzen in der Leber metabolisiert werden, wirken sie auf die Leberzellen, welche sich aus den Hepatozyten (parenchymale Zellen) und den mesenchymalen Zellen, die bei Untersuchungen zumeist vernachlässigt werden, zusammen setzen. Als Tiermodell verwendeten wir Ratten, da bekannt ist, dass Nagetiere sensitiver auf NGK reagieren, wobei dadurch in manchen Fällen eine humane Relevanz in Frage gestellt werden kann. Als zu untersuchende Substanzen wählten wir Nafenopin, aus der Gruppe der Fibrate, und Phenobarbital, ein Barbiturat, aus, mit denen die Ratten *in vivo* bzw. die isolierten primären Zellen *in vitro* behandelt wurden. Für die Analyse

entschieden wir uns für Shotgun Proteomics als Screening-Methode. Bei dieser Technik wird das Proteom sub-zellulärer Fraktionen von Zellen mittels einer Kombination aus 1D SDS-PAGE und LC-MS/MS analysiert. Durch den Vergleich der Proteome von Hepatozyten und der mesenchymalen Zellen kristallisierten sich Unterschiede in der Proteinexpression heraus, die Anhaltpunkte für weitere Untersuchungen liefern könnten. Zusammenfassend sei erwähnt, dass es uns bei beiden Substanzen gelang zahlreiche nennenswerte, bekannte als auch bis dahin unbekannte Veränderungen in Hepatozyten und mesenchymalen Zellen festzustellen, wobei eine autokrine und parakrine Zell-Zell Interaktion eine wichtige Rolle zu spielen scheint.

Des Weiteren haben wir uns mit einem klinisch relevanten Thema beschäftigt, der Entzündung. Es ist zu bemerken, dass heutzutage akute Entzündungen durch die Medikation von Antiphlogistika gut in den Griff zu bekommen sind. Ein bedeutendes Problem verursachen jedoch die chronischen Entzündungen, die bei einer Großzahl von Erkrankungen eine tragende Rolle spielen, nicht zuletzt bei Autoimmunerkrankungen und Krebs, wobei eine Behandlung ausschließlich der Entzündung und nicht der tatsächlich erkrankten Zellen, z.B. Endothelzellen, nur einen kurzfristiger Erfolg durch das Hemmen von z.B. T-Lymphozyten verspricht, da im Knochenmark permanent Immunzellen nachproduziert werden, welche weiterhin durch erkrankte Zellen im betroffenen Gewebe entzündlich aktiviert werden. Um einen Kontext zu den nicht-gentoxischen Kanzerogenen zu schaffen sei zu erwähnen, dass diese Substanzen das Immunsystem beeinflussen können, wie z.B. Kupffer-Zellen als auch Monozyten und T-Lymphozyten aus dem Blut und daher eine entzündliche Komponente nicht auszuschließen ist.

Nun haben wir uns mit dem Proteom von primären humanen peripheren mononuklearen Blutzellen (PBMCs) beschäftigt, welche sich Großteils aus Monozyten und T-Lymphozyten zusammensetzen. Durch den Vergleich der Proteome von unbehandelten und entzündlich aktivierten PBMCs konnten wir zellspezifisch entzündungsrelevante Proteine definieren. Diese Liste an Proteinen wurde anschließend für zwei weitere Studien herangezogen. Bei den Studien handelte es sich zum einen um eine Kaffeestudie und zum anderen um eine Schleimpilzstudie, wobei die Auswirkungen von Kaffee *in vivo* und der Schleimpilzextrakte *in vitro* auf das Proteom von naive und entzündlich aktivierte PBMCs untersucht wurden. Dabei konnten wir feststellen, dass Kaffee eine aktivierende Wirkung auf naive als auch entzündlich aktivierte PBMCs hat, während die Schleimpilzextrakte eine schwach-hemmenden Wirkung auf entzündlich aktivierte PBMCs erzielten.

1. Introduction

The main topic of my thesis was to investigate effects of non-genotoxic carcinogens (NGCs) on protein level. My supervisor Prof. Reingard Grabherr (BOKU) attached me to the clinical proteomics group of Prof. Christopher Gerners at the cancer research institute of the medical university in Vienna. At the MUW the group of Prof. Bettina Grasl-Kraupp also participated in this EU-IMI funded project. Her group conducted all animal experiments.

1.1 Non-genotoxic carcinogens

Generally it is known that chemical compounds may cause cancer by directly affecting DNA and are thus called genotoxic carcinogens. During drug development they can be easily detected via various assays, such as the Ames bacterial reverse mutation assay, and are thus not a big issue in this process. Another story is a group of chemical compounds that do not directly interact with DNA, yet may still cause cancer via various molecular mechanisms or modes of action, respectively. These compounds are called non-genotoxic carcinogens (NGCs). These NGCs may affect multiple and various tissues and organs of an organism causing a dysbalance of the usual homeostasis, in a microenvironment as well as altering or triggering cell-cell interaction between (distant) tissues. Because of that variety of possible effects, the modes of action of most NGCs are poorly understood or even recognised as such. Nevertheless, it is known that these effects may also affect initiated and mutated cells, exerting a selective proliferation of preneoplastic cells and in that way NGCs may act as tumour promoters. Some NGCs are known to have even tumour initiating capabilities. Possible molecular mechanisms by which NGCs act are via epigenetic changes such as hypo- and hypermethylation of CpG sites, chromatin modifications and miRNA regulated mechanisms.[1,2] Endocrine effects, inhibition of gap junctional intercellular communications, immune modulation, and/or profound disturbances in the epithelial-mesenchymal interactions.[3] It is known that during carcinogenesis the microenvironment may gain a pivotal role supporting preneoplastic and neoplastic cell growth via an altered vasculature, deviated immunological activities and altered interstitial extracellular matrix (ECM).[4,5] Further possible modes of NGC actions in rodents may be cytotoxicity followed by regenerative growth accompanied by a pro-inflammatory status. Involvement of inflammatory mechanisms may be accompanied by enhanced production of reactive oxygen species (ROS) and reactive nitrogen species (RNS).[6,7] These two species can serve as signalling molecules, but may also be of relevance in carcinogenesis, possibly causing endogenous DNA damage, which may be

responsible for a weak genotoxic potential of NGCs. As related to that, when considering the liver, cells of the microenvironment such as Kupffer cells, which are liver resident macrophages, may be key players, which trigger an inflammatory response upon activation by releasing cytokines to the surrounding cell, e.g. paracrine signalling via interleukins and the production of ROS.[8] Furthermore, so far there is no common mechanism known via which all NGCs act. That said NGC action is quite miscellaneous and diverse, so that only NGCs which act via similar modes of action, such as the phenobarbital-like cytochrome P450 inducers can be grouped at best.

1.1.1 Approach

The long term aim of the IMI project was to establish short-term assays, which should enable a secure detection of NGC action, when testing chemical compounds early during drug development. To accomplish that, our part in the project was to pinpoint early events and hypothesise about possible mode of action. Our approach for this and other clinical, physiological as well as toxicological issues was proteomics. Proteomics aims to capture as much as possible of the whole proteome, the entirety of proteins present in cells, tissue or body at a given time point, such as a snap-shot. It is the aim of proteomics to identify the proteins, determine their abundance as well as the optional search for modifications, such as glycosylations or phosphorylations. A common approach to tackle clinical issues is the study of dynamic changes, which is usually performed in proteomics by comparing the abundance of the proteins deriving from control material, which could be primary cells isolated from healthy tissue of donors or cell lines, with test material, e.g. tissues material deriving from diseased patients or somehow treated cells. This allowed us the establishment of reference proteome profiles for various cells as well as cells in different functional states. During data evaluation proteins of interest can be further studied regarding protein interaction by using software for pathway analyses, such as cytoscape or reactome. Challenges of proteomics are, amongst others, the complexity of the samples as well as the dynamic range of the proteins to be analysed. The proteome of a cell contains more than 20,000 different proteins, whereby results of such a complex sample at one go would be rather disappointing with the techniques available today. One restriction would be the speed of the mass analyser, with its limited number of ions it can handle per time or cycle, resulting in a loss of information. Another prominent effect would be the ion suppression, which causes a masking of low abundant proteins caused by other proteins

or matrix components, such as salts resulting in an inefficient formation of droplets or the evaporation of them, and thereby reduces the amount of charged ions in the gas phase.[9] Concerning the dynamic range, the proteins of plasma isolated from the blood of donor's ranges from mg/ml concentrations of high abundant proteins, such as albumin and globulin, down to pg/ml concentrations, which are comprised by chemokines and other low abundant proteins. To improve the results, a reduction of the complexity is required. This can be done during sample processing by in-gel separation techniques or via chromatographic means, such as reverse-phase chromatography, whereby the sample may be directly eluted into an ion source of a tandem-MS/MS. An alternative chromatographic approach would be a fractionation of the samples by using e.g. a salt gradient, which is often done beforehand an in-solution digest. An additional option is the sub-cellular fractionation into cellular sub-compartments, because cell compartments vary in percentage of cell coverage and by that in protein amount. The analyses of sub-cellular fractionations compensate for that issue and even allow a semi-quantitative comparison of reference proteomes of different cells from different tissue.

Two proteomic approaches were majorly used during this endeavour. The first approach was the 2D SDS-PAGE, a 'Top-Down' approach, which means that the analyses start at the protein level and provides first results relative fast. Moreover, this method allows follow-up in-depth analyses, such as protein identification via MS analyses of corresponding peptides.[10] We used this approach for the inflammation project as well as for pre-screening to verify the functionality of our experimental setup at the NGC project. 2D SDS-PAGE combines the isoelectric focusing (IEF, 1st dimension) with a SDS-PAGE as the 2nd dimension. We further improved this method, concerning sensitivity and the resulting biological information, by metabolically labelling the samples with sulphur isotopes ^{35}S linked to cysteines and methionines during cell culture for 24 hours before cell lyses. The resulting autoradiograms give specific semi-quantitatively information about the newly synthesized proteins. Thereby alterations in the proteome can be found quickly and in most cases allow an early rough interpretation of the data. A variation to that is the 2D-DIGE, a quantitative technique which is essentially the same as the 2D SDS-PAGE, but includes an additional step. Hereby, the proteins of the samples are labelled via covalently linked fluorochromes, such as the CyDyes from GE or the G-Dyes from DyAgnostics, previous to the IEF. This approach allows multiplex analyses, a quantitative comparison of multiple samples on one or more gels in one step, and also permits an identification of proteins later on.

The second approach was a combination of 1D SDS-PAGE and LC-MS/MS analyses, which is also called shotgun proteomics. This 'Bottom-Up' approach is used for proteomic profiling, the generation of reference proteome maps, which starts with the MS data of peptide fragments, the fragment ions, and ends with the protein data.[11] The 1D SDS-PAGE separates the proteins by their molecular weight, which also helped us to get rid of disruptive salts and to reduce the sample complexity. During the LC-MS/MS analyses this reduction is an optimization to get the best results out of samples. This reduction, in combination with the gradient elution of the reversed-phase LC, enabled us to analyse the samples by having manageable protein amounts at the MS analyser at any given time point. Following the 1D SDS-PAGE separation the sample fractions were digested with trypsin, a serine protease cleaving proteins mainly at the carboxyl side of the amino acids lysine or arginine, which is incorporated in the algorithm of the fragment ion to peptide assignment step. The final step was the LC-MS/MS analysis. In our case we used two Agilent HPLCs, whereby the first was used as the capillary pump, loading the sample onto the trapping column and the second provided the nano-flow of the mobile phase for the octadecyl carbon chain bonded silica (C18) column, the separation column. Both columns are incorporated into an HPLC-Chip from Agilent with the advantages of an easy setup, standardised columns as well as low dead volumes, which is helpful concerning sample carryover. As stated above an acetonitrile gradient elution was utilized, which further separated our samples fractions according to their hydrophobicity previous to the MS/MS analyses using an ESI-ion trap (electrospray ionization) and collision induced dissociation (CID – using helium). The MS or raw data, which are basically measured m/z values of the ions from the sample, were processed using the SpectrumMill software and the UniProtKB/Swiss-Prot protein database from Pride (EBI). This database is currently the most trustworthy and publicly available database to assign ions to peptides and to determine the amino acid sequences and the protein names. For the evaluation of the MS data, which is a huge amount of data to handle, we used the GPDE[12], a biological protein database that provides tools or meta-analyses of proteomics experiments. Because of our standardized sample processing, the sub-cellular fractionation and by determining the emPAI value (Exponentially Modified Protein Abundance Index; 'an approximate, label-free, relative quantitation of the proteins in a mixture based on protein coverage by the peptide matches in a database search result')[13] for each protein, we were able to compare all of our reference proteome profiles of interest with each other, which allowed us to determine alterations in the proteome.

Now I want to introduce the experimental concept of the NGC study.

1.1.2 Experimental concept of the NGC investigation

The most common target organ of NGCs in rodent models is the liver. About 40% of all NGCs tested so far are hepatocarcinogens. As yet, research on the action of NGCs has been focusing mainly on hepatocytes (HCs), the major parenchymal cells of the liver which may give rise to liver cancer. A role of non-parenchymal liver cells (NPCs) in NGC-driven hepatocarcinogenesis has been mostly neglected,[8,14] probably because these cells do not transform upon drug treatment. However, NPCs, which consists mainly of Kupffer, endothelial, and stellate cells, may also be targeted by NGCs and may contribute considerably to the selective proliferation of preneoplastic and neoplastic HCs via release of paracrine growth factors or other growth-enhancing stimuli. *Figure 1* presents the schematic structure of the liver, including the most important liver resident cells. As an example, it was demonstrated that the involvement of NPCs such as Kupffer cells, essentially liver resident macrophages, and their inflammatory activity may aggravate the genotoxic effects of chemical compounds such as NNM (N –nitrosomorpholine).[15]

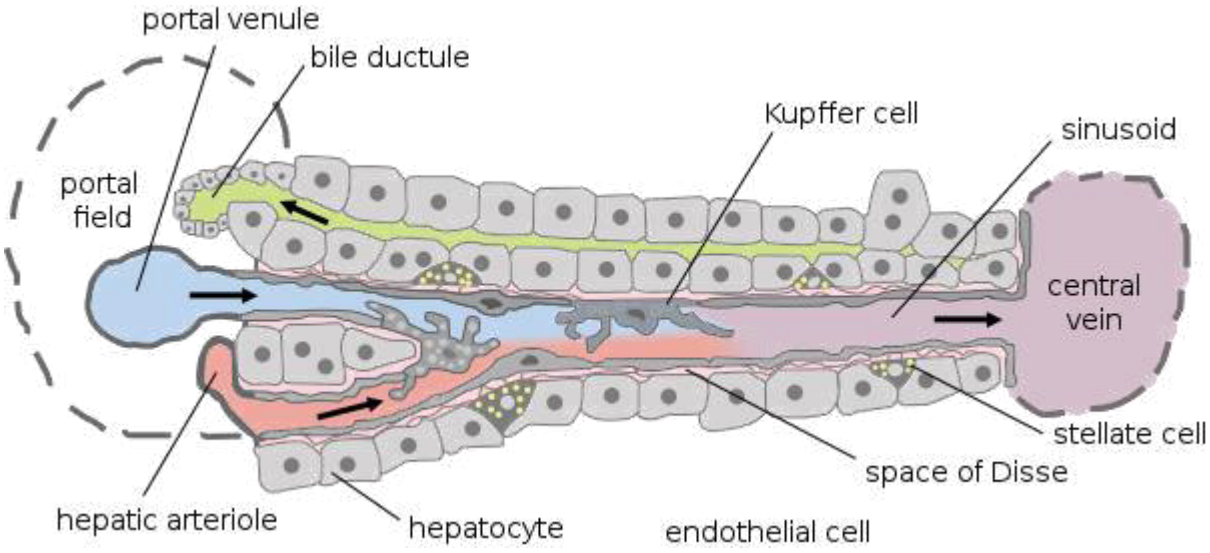

Figure 2. Schematic structure of the liver. All relevant liver cells and their localization as well as the blood vessels are depicted in this figure. Figure was taken from Wikipedia.

The next step was the choice of the model species. Being the most common hosts for animal testing and because of their, compared to humans, more prominent susceptibility or sensitivity to NGCs, rodents were our model species. Our corporation partners have been working with rats

for years and because of the higher yield of protein amount compared to a mouse model, we decided to work with this animal model. For the treatment, we decided to start with the *in vitro* treatment of isolated HCs and NPCs and analyse *in vivo* treated rats subsequently. To summarize, we analysed HCs and NPCs of rat livers separately, either treated the isolated cells *in vitro* or the animals *in vivo*. Two substances, nafenopin and phenobarbital, were chosen for our investigation, whereby we spent the majority of our research efforts on phenobarbital.

1.1.3 Phenobarbital

Phenobarbital (PB; 5-Ethyl -5-phenylbarbituric acid) is a barbiturate, which is used for epilepsy treatment and anaesthesia preparation. In the past PB was used as a soporific, but was withdrawn from the market for this utilization because of the risks of addiction and intoxication, when abused. PB has many known side-effects, including being a teratogenic agent. Beyond that, it was demonstrated that PB affects organisms via yet not fully understood non-genotoxic mechanisms that may lead to liver cancer at a later time point, whereby the tumour promoting effects can be verified by applying long-term rodent carcinogenicity assays.[7,16–20] The deliberate effect of PB is its binding to $GABA_A$ receptors (*gamma*-aminobutyric acid receptor A), whereby it augments the effectiveness of the neurotransmitter GABA, which is the most important repressor of the central nervous system in mammals. In addition PB inhibits the most important stimulating neurotransmitter by blocking the AMPA receptor (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor).

In mammals PB is detoxified via the drug metabolizing enzymatic system in the liver, usually in three sub-sequent phases – modification, conjugation and excretion. Because of PBs lipophilic character, it is not effectively removed from the organism via urine and to get rid of it, the organism has to metabolise PB in the liver beforehand. *Figure 2* is split into two parts, where part a) depicts the chemical structure of PB and glucuronic acid, and part b) shows the 2 steps of metabolisation of PB in the liver. PB is converted into hydrophilic products via hydroxylation and a subsequent conjugation of polar moieties. The first phase is performed by phase I drug metabolizing enzymes, such as the cytochrome P450 oxidases, which attach polar or other reactive groups to compounds such as PB. This reaction is also called functionalization reactions and in case of PB adds an OH-group (hydroxylation reaction) to it. In the second phase, biosynthetic reactions are performed by phase II drug metabolizing enzymes, such as UDP-glucuronyltransferases and glutathione S-transferases, which conjugate or transfer polar groups,

such as glucuronic acid or sulphate, to the previously modified site. This introduction of a hydrophilic endogenous species results in tagged hydrophilic molecules, which can now be excreted from the cells by membrane transporters of the multidrug resistance protein (MRP) family followed by renal excretion or excretion via the bile ductus.[21–23]

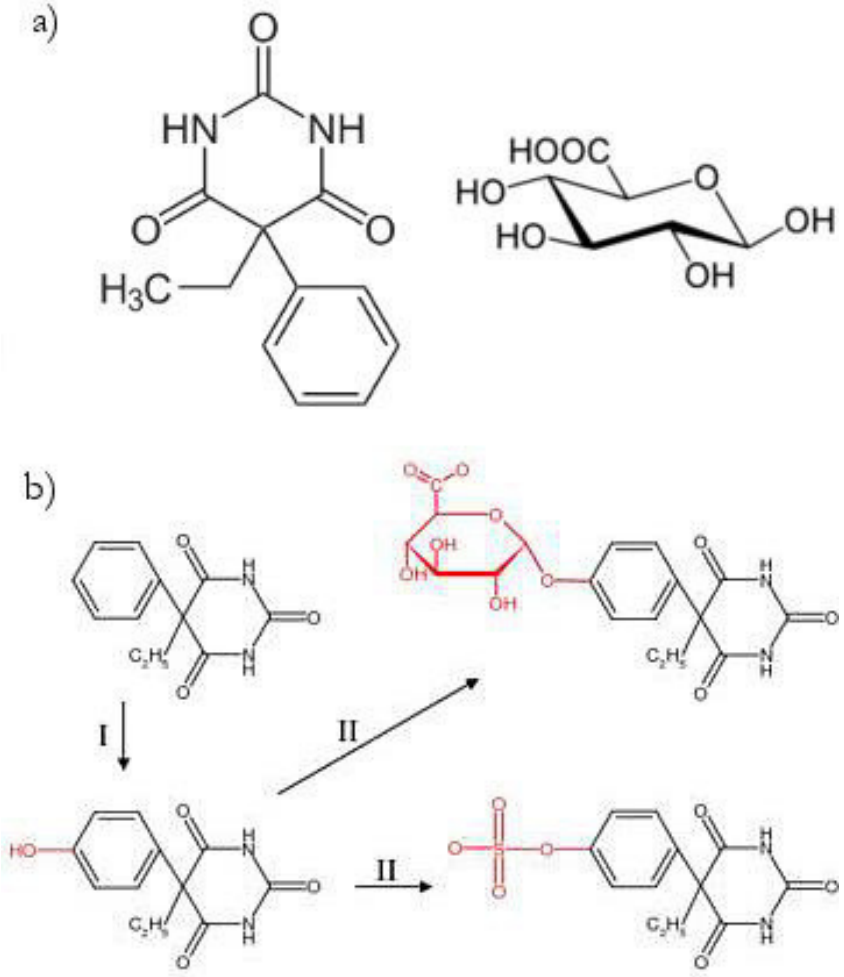

Figure 2. Chemical structure of phenobarbital and its metabolisation. a) chemical structure of PB and glucuronic acid. b) The two steps of metabolisation of PB in the liver. In step I PB is hydroxylated by phase I drug metabolizing enzymes, such as the cytochrome P450 oxidases. In a second step a polar residue is added to this OH-modified location via phase II drug metabolizing enzymes, such as UDP-glucuronyltransferases (glucuronidation) and glutathione S-transferases (sulphate).
Figures from *2a)* were taken from www.wikipedia.com, while *figure 2b* was taken from the Biochemical Pharmacology course notes of Prof. Michael Palmer, University of Waterloo[23]

Beyond that common pathway, PB has been described to interact with the constitutive androstane receptor (CAR) and the pregnane X receptor (PXR), xenobiotic-sensing nuclear receptors, triggering a signal transduction cascade leading to an induction of cytochrome P450 genes, response elements such as members of the CYP2B and CYP3A subfamily.[7,24–28] Furthermore, it was shown that PB acts through other mechanisms such as oxidative stress[29], which may correlate with the P450 induction[30], and by that drive tumour promotion by inducing proliferation[31,32] in HCs, non-receptor mediated endocrine modifications and inhibition of gap junction intercellular communications, regulating growth and differentiation[7]. It was demonstrated that PB promotes tumour formation by stimulating selective growth of different subtypes of liver preneoplastic lesions, known as gamma-glutamyltranspeptidase-positive eosinophilic-clear cell lesions.[15,33] Gamma-glutamyltranspeptidase is a single-pass type II membrane protein that initiates the extracellular glutathione breakdown, transfers glutamyl moieties from glutathione to amino acids as well as supports the maintenance of the intracellular glutathione level. The PB growth promoted gamma-glutamyltranspeptidase-positive cells show elevated occurrence of the glutathione S-transferase subunits Yb1 and Yb2, meaning an elevated level of glutathione S-transferases Mu and P, which are phase II drug metabolizing enzymes. To conclude, we were able to reproduce these already described PB-induced alterations in our analyses. Moreover, we identified novel PB-induced proteins involved in various molecular events, which may be one of the modes of action of PB. These findings may support future investigations in this field.

1.1.4 Nafenopin

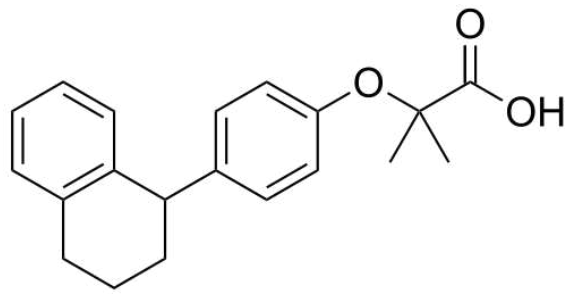

Figure 3. Chemical structure of nafenopin. The figure was taken from www.wikipedia.com.

The second chemical compound we investigated was nafenopin {NAF; 2-Methyl-2-[4-(1,2,3,4-tetrahydronaphthalen-1-yl)phenoxy]propanoic acid}, which is part of the peroxisome proliferator (PP) class of non-genotoxic carcinogens.[34] NAF is a fibrate, a hypolipidemic agent - a lipid lowering drug, affecting the cholesterol distribution of low density lipoproteins (LDL) and high density lipoproteins (HDL) and was applied to lower serum lipids of human patients in the past, until arising evidence of its hepatocarcinogenic potential in rodents and a possibility to be of human relevance. Just as is the case with PB, the exact modes of action by which NAF act are unknown. Nevertheless, three causal modes of action were identified for PP in rodent liver: activation of PPARα, perturbation of cell proliferation and apoptosis and selective clonal expansion, by which NAF acts as tumour promoter.[35] As stated above NAF acts via the peroxisome proliferator-activated receptors (PPARs) of cells, such as HCs and Kupffer cells, of the liver.[36] The PPARs are nuclear receptors that comes in three isoforms, the α, ß/δ and γ isoform, whereby PPARα is the isoform found in the liver. Upon ligand binding, they form heterodimers with retinoid X receptors (RXRs) and act in HCs as transcription factor for genes of the lipid metabolism, peroxisomal genes and growth regulating genes.[37] It was demonstrated that NAF synergizes with the epidermal growth factor (EGF), a liver mitogen, and the transforming growth factor alpha (TGF-α) causing a clonal expansion of initiated rat HCs.[34] NAF may also suppress spontaneous apoptosis of HCs and apoptosis induced by transforming growth factor β1 (TGFβ1), the physiological negative regulator of liver growth.[38] NAF also affects the NPCs by e.g. pro-inflammatory activating the Kupffer cells, resulting in an increase of the NOS and ROS activity and cause a oxidant-dependent NF-ϰß pathway induction with a sub-sequent secretion of tumour necrosis factor alpha (TNF-α).[36,39] As a consequence, the secretion of

cytokines, such as TNF-α, by NPCs may mediate a proliferative response in HCs leading to NAFs tumour promoting effect as well as to hepatomegaly in rodents. Another mechanism, by which NAF acts on HCs, is the protein kinase C mediated inhibition of gap junctional communication via the post translational modification of connexin 32, a tumour suppressor. The conformational change caused by the phosphorylation of serine in connexin 32 causes a loss of function, and by that a mechanism to regulate the growth of neighbouring cells which may be an important factor during tumour promotion.[40] Regarding the selective clonal expression, it was demonstrated that NAF amplifies gamma-glutamyltranspeptidase -negative weakly basophilic foci (WBF).[33]

NAF was the first NGC we investigated by conduction *in vitro* experiments by treating isolated HCs and NPCs, which were obtained from perfused rats, separately for one day. After approximately halve a year of studying NAF the consortium changed the focus of the IMI projects to the investigation of PB. Nevertheless, even during this relative short period we were able to identify NAF related alterations in the proteomes of HCs and NPCs, which will be discussed later.

1.2 Inflammation and nutritional intervention

We have the hypothesis, that (an induced) inflammation may play a role in the modes of action of substances showing non-genotoxic activity. As described above, there are indications in the literature that Kupffer cells may be involved in the modes of action of non-genotoxic carcinogens; they might even be key players. Actually during our investigation of NAF and PB, we found proteins up-regulated that are involved in inflammatory response, which would corroborate with the literature and will be discussed later. Furthermore, activated immune cells and their inflammatory activity, e.g. paracrine signalling via secretion of cytokines, makes them important players in chronic inflammatory diseases and cancer, where inflammation is a hallmark.[41–45] Peripheral blood mononuclear cells (PBMCs) are immune cells, which are involved in many diseases. They are blood cells with a round nucleus, such as lymphocytes (75% t-cells), macrophages and monocytes – representatives of the adaptive and the innate immune system. If not activated by some stimuli, these cells circulate the organism in a quiescent state and keep watch for immune related elicitors. Upon induction by e.g. pathogens, PBMCs respond in a calibrated and inflammatory way to clear the threat from the organism. During the process

PBMCs may also inflict collateral damage to the surrounding cells, which is usually regenerated after clearing of the threat. Inflammatory response involves several biological functions, such as interferon response, leukocyte migration, NF-κB signalling, proliferation of t-cells and regulation of cell death.[10] Lymphocytes and monocytes are the main constituents of the PBMCs, whereby t-cells contribute more than two third to the lymphocytes. Upon activation t-cells may cause tissue damage, arising from their intrinsic immune response, which is also characteristic for chronic inflammation as well as autoimmune disease.[46] Monocytes become activated upon encountering damaged tissue (so called damage-associated molecular pattern molecules; DAMPS) and potential pathogens (so called pathogen-associated molecular patterns; PAMPS), and subsequently differentiate into macrophages and dendritic cells. Macrophages phagocytise, process damaged tissue as well as pathogens, and perform anti-gen presentation, usually in the same tissue. Dendritic cells also process and present antigens, but upon activation they migrate to the lymph nodes to interact with cells of the adaptive immune system, such as b-cells and t-cells. Both cell types are also responsible for the targeted activation and regulation of t-cells. As a consequence, t-cells may activate endothelial cells, fibroblasts, monocytes as well as macrophages and maintain an acute or chronic inflammatory state in the tissue.[47] In short, monocytes are part of the innate immune system and during the immune response fulfil the functions of phagocytosis, antigen presentation and pro-inflammatory cytokine production of lymphokins, interleukins and chemokines such as TNF, Il-1 and 12. T-lymphocytes are part of the adaptive immune system assume various tasks depending on their sub-type, which are helper-, cytotoxic-, memory-, regulatory-, γδ- and NK t-cells. Usually they are activated upon encounter with antigen presenting cells, such as macrophages, dendritic cells or b-cells, recognising molecules/antigens bound to the MHC II molecule (major histocompatibility complex II). T-helper cells secrete cytokines to attract and direct macrophages, neutrophils and t-lymphocytes, while NK and cytotoxic t-cells eliminate the threat, such as virus infected cells. *Figure 4* presents an overview of the hematopoiesis, which is the formation of cellular blood components evolving from stem cells produced in the bone marrow (hematopoietic cells).

Manuscript 3.2 (p. 66ff) is about the proteomes of inflammatory activated PBMCs and describes the basics of the inflammatory project in detail. We compared all the reference proteomes of LPS, PHA, LPS+PHA treated and untreated human PBMCs with each other, and by doing so we were able to distinguish between proteins, which are specifically occurring in single cell types, proteins found only in a specific cell type, e.g. dendritic cells, monocytes and t-cells; proteins that

are specifically shared between two or more cell types and proteins found to be common in many cells. To conclude this study was the basis for sub-sequent investigations of the inflammatory modulating effects of coffee and mould fungi extracts on human PBMCs. The established reference proteome maps and the resulting protein short-list supported us during data interpretation of the following investigations.

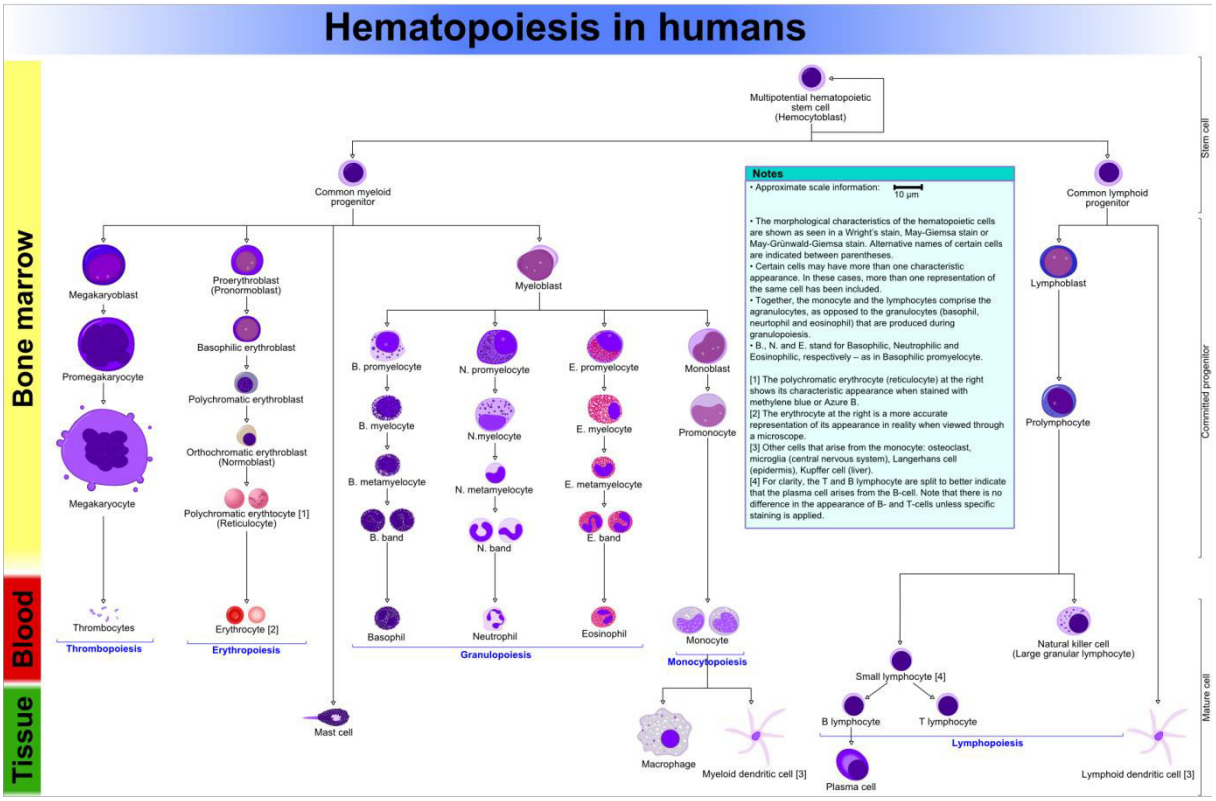

Figure 4. Hematopoiesis in humans. This figure depicts an overview of the evolution of cellular blood components, including monocytes and t-lymphocytes. The left side informs about the localization of the cells in their various evolutionary states. This picture was taken from www.wikipedia.com.

1.2.1 Approach

As to our approach, the whole procedure is described in detail in the *manuscript 3.2 (p. 66ff)*. In short, we used isolated primary PBMCs deriving from the donated blood of voluntary human study participants. We investigated the reaction of the PBMCs to inflammatory activators, such as the *in vitro* stimulation of t-cells with phythaemagglutinin (PHA) and monocytes with lipopolysaccharide (LPS). We conducted shotgun proteomics, as described for the NGC study, in a systematic strategy based fashion by establishing of reference proteome profiles of the investigated cells at different functional states. Compared to the NGC study, we relied more extensively on 2D SDS-PAGE, especially on metabolic labelling via autoradiography.

1.2.2 Coffee

Coffee is one of the most popular beverages of our society. Coffee beans are rich of bioactive constituents, such as caffeine, chlorogenic acids, diterpenes and trigonelline, to name only a few.[48] It was demonstrated, that the consumption of coffee prevents the endogenous formation of oxidized DNA bases in PBMCs.[49] Further, the consumption of coffee has an impact on ROS related diseases, which may be due to the inactivation of ROS via direct scavenging[50,51] and the induction of antioxidant enzymes in rodents[52,53]. Indeed these mechanisms are also interesting for the NGC study, when considering ROS and RNS capability of inducing DNA damage and genomic instability. But it is unclear if the ROS and RNS products of inflammatory activated macrophages, such as the Kupffer cells, and neutrophils will even be stable long enough to reach and interact with the DNA of an adjacent epithelial or parenchymal cell, such as HCs, or stimulates the ROS accumulation within the parenchymal cells via paracrine signalling.[54]

Coffee consumption may also affect liver relevant modes of actions of NGCs via mechanisms, such as the increased expression of phase I drug metabolizing enzymes, e.g. UDP-glucuronosyl transferases and glutathione S-transferases, and the activation of Nrf-2.[52,53] During our cooperation with Prof. Knasmüller (MUW), we investigated the effects of coffee and its modulating effects on naïve and inflammatory activated primary human PBMCs. Our working thesis was that coffee has an activating effect on PBMCs and thereby changes the resulting proteome profiles. To investigate the alimentary effects of coffee upon this part of the immune system of humans, we analysed the PBMCs isolated from the blood of human study participants, prior and posterior to coffee consumption and generated two fractions from each time point by

treating one of the fractions with a combination of LPS and PHA, activators of monocytes and t-lymphocytes. This setup and the use of 2D autoradiography enabled us to find alterations in the proteome of proteins previously identified to be induced during inflammatory activation in PBMCs. The results indicate that primary human PBMCs are notably affected by coffee in both functional states, the untreated and the inflammatory stimulated cell state. Monocyte and t-lymphocyte specific proteins, which are known to be induced upon inflammatory activation, were found to experience an enhancement in their expression, pointing toward an agitating effect of coffee upon the immune system in the acute phase.

1.2.3 Extracts of mould fungi

A German-based company commissioned us with testing mould fungi extracts, which should be incorporated orally and are supposed to have an anti-inflammatory effect in humans. I hereby state, that we had no benefit, financially or otherwise, from either outcome and conducted an unbiased investigation. At that time we performed the coffee experiments as stated above and with little extra effort we subsequently investigated the effect of these substances on human derived primary PBMCs. They were extracts from cultures of Aspergillus niger, Mucor racemosus and Penicillium chrysogenum. The results indicate, that the extracts from these mould fungi exert a moderate anti-inflammatory influence upon the activated PBMCs by down-regulating the protein expression of inflammation-related proteins and thereby dampening the effects of the inflammatory stimuli somewhat.

1.3 Objectives of the thesis

There are a few topics that are considered within this thesis, whereby all investigations are tackled on protein level via proteomic methods. The major issue of my thesis was to investigate early modes of actions of two NGCs, namely NAF and PB, on rodent liver cells. These two compounds are known to affect organisms differently, when considering molecular mechanisms of NGC action. In the liver it is known that NAF acts as a peroxisome proliferator, while PB acts as a cytochrome P450 inducer, so we investigated representatives from two groups of NGCs. The aim within the scope of my thesis was to establish reference proteome maps for these two compounds by using rodent liver cells as our model system. To distinguish parenchymal and non-parenchymal contribution to NGC activity, we analysed the respective HCs and NPCs separately.

These reference proteome maps were then used to find NAF or PB induced alterations in the proteome. Proteins found down- or up-regulated may indicate the contribution of some NGC relevant molecular events, which would be further validated as probable biomarkers by other members of the IMI project.

During my time as PhD student other sub-topic emerged that were of relevance for my thesis. It is described and our results demonstrate that the immune system may contribute to the modes of action of NGCs, such as by affecting Kupffer cells in the liver. That means that inflammation may be part of NGC relevant molecular mechanisms, at least to some extent, and, moreover, inflammation is an important hallmark for cancers. Because of these reasons, our aim was to improve our knowledge of inflammatory activated immune cells in a human relevant setting. Therefore we investigated inflammatory activated primary human PMBCs, which are majorly comprised of lymphocytes, macrophages and monocytes. PBMCs are important cells of the adaptive (lymphocytes) and the innate (monocytes, may differentiate into macrophages or dendritic cells) immune system patrolling the blood stream and monitor our organisms for infections. Adjoining this study we investigated alimentary modulating effects of substances, which may have a chemo-preventive capability. We were intrigued by Prof. Knasmüllers investigations of coffee effects, considering amongst others the ROS scavenging ability of some coffee compounds. Incidentally, ROS may also contribute to modes of action of NGCs, when considering the involvement of activated Kupffer cells. Therefore we analysed the modulating effects of coffee on inflammatory activated PBMCs of probands, which had consumed coffee. The aim was to pin-point coffee modulated alterations of immune cell specific proteins in the proteome. A cooperation partner from Germany was interested in this experimental setup, resulting in a second adjoining study. In this study we investigated the modulating effects of extracts deriving from mould fungi on inflammatory activated PBMCs. The goal was the same as before, to find mould fungi extract induced alterations in the proteome of inflammatory activate PBMCs.

2. Achievements during my time as PhD student

Now follow the thesis relevant achievements, which are conveniently separated into achievements of the NGC and the PBMC study. Related to the stated lists of the established reference proteome maps, we always generated the proteomes of three sub-cellular fractions – namely the cell supernatant or secretome, the cytoplasm and the nuclear extract.

2.1 Proteomic analyses of non-genotoxic carcinogens

After receiving the treated or untreated primary HCs and NPCs, we performed the sub-cellular fractionation and all sub-sequent sample processing steps, sample analyses and data evaluation as described in detail within the manuscripts. We were able to generate proteome reference maps for the following cells in various functional states and in most cases by using biological and technical replicas. The resulting PRIDE files are xml-files, which include the information of the resulting MS data and our assignment to proteins. These files were uploaded to the PRIDE database, which is a public available database. To give numbers, we analysed 144 cell fractions, each with hundreds of assigned proteins, and evaluated them respectively. The evaluation of the data allowed a short-listing of proteins, which may be of relevance for NAFs and PBs modes of action, respectively.

- Rat male primary HCs untreated
- Rat male primary NPCs untreated
- Rat female primary HCs untreated
- Rat female primary NPCs untreated
- Rat male primary HCs treated *in vitro* with PB
- Rat male primary NPCs treated *in vitro* with PB
- Rat male primary HCs treated *in vitro* with interleukin-1 beta
- Rat male primary HCs treated *in vitro* with interleukin-6
- Rat male primary NPCs treated *in vitro* with LPS
- Rat male primary HCs treated *in vivo* with PB
- Rat male primary NPCs treated *in vivo* with PB
- Rat male primary HCs treated *in vitro* with NAF
- Rat male primary NPCs treated *in vitro* with NAF

The manuscript, concerning the investigation of PB, is resubmitted and will be published in a peer-reviewed journal (*see manuscript 3.1; p.28ff*). The effects of NAF on proteomes of HCs and NPCs were evaluated, but not published and will be presented in the results and discussion section.

2.2 Proteomic analyses of PBMCs

We performed all steps from the blood sampling of the human probands till the evaluation of the MS data and the 2D SDS-PAGE, respectively. Concerning MS data, we generated and analysed the following reference proteome maps.

- Human PBMCs untreated
- Human PBMCs treated *in vitro* with LPS
- Human PBMCs treated *in vitro* with PHA
- Human PBMCs treated *in vitro* with LPS+PHA

As stated above, the investigations of inflammatory activated PBMCs rely more extensively on the evaluation of 2D SDS-PAGE of metabolically labelled samples, which are listed below. There were at least three biological replicas for each functional state.

- Human PBMCs untreated
- Human PBMCs treated *in vitro* with LPS+PHA
- Human PBMCs after coffee consumption
- Human PBMCs treated *in vitro* with LPS+PHA after coffee consumption
- Human PBMCs treated *in vitro* with the extract of Aspergillus niger
- Human PBMCs treated *in vitro* with the extract of Penicillium chrysogenum
- Human PBMCs treated *in vitro* with the extract of Mucor racemosus
- Human PBMCs treated *in vitro* with LPS+PHA & Mucor racemosus extract
- Human PBMCs treated *in vitro* with LPS+PHA & Penicillium chrysogenum Human PBMCs treated *in vitro* with LPS+PHA & Aspergillus niger extract

The evaluation of the data concerning inflammatory activated PBMCs was published in a peer-reviewed journal (*see manuscript 3.2; p.66ff*). Summarizing the achievements of the PBMC studies, we were able to generate a short-list of proteins induced by inflammatory activation of PBMCs.

This protein list includes cell type associations, which enabled us to differentiate between cell type specific proteins, proteins shared by more than one cell type and common proteins. Concerning the effects of coffee and mould fungi extract on the proteome of human PBMCs, we evaluated the respective 2D autoradiograms considering the modulating effects on the proteins, which were found induced in the proteome of inflammatory activation of PBMCs. We analysed the effect of coffee and mould fungi on these proteins in untreated as well as in the inflammatory activated cell state.

2.3 Off-topic achievements

As part of a corporation with the rheumatology of the AKH, we conducted proteomic analyses of mice spleen, looking for alterations in the expression of specific proteins, which may be of relevance during rheumatism. Another corporation was with the dermatology of the AKH. I contributed to one of the melanoma projects, which resulted in a publication, see Paulitschke *et al.*[55]

3. Manuscripts

The two following manuscripts, with context to my thesis, were and will be published by peer-reviewed journals, respectively. The first manuscript is about the investigation of PB in our NGCs study, while the second manuscript presents our results from the investigation of inflammatory activated PBMCs.

3.1 Phenobarbital induces alterations in the proteome of hepatocytes and mesenchymal cells of rat livers.

Author list:

Philip Klepeisz, Sandra Sagmeister, Verena Haudek-Prinz, Melanie Pichlbauer, Bettina Grasl-Kraupp, Christopher Gerner

This manuscript (*p.29 – p.65*) was resubmitted to PlosOne, where it is currently reviewed.

Figures and tables of this manuscript are presented starting with *p. 52ff.*

The supplementary tables S1 & S2, two excel tables containing the summarized proteome profiling results obtained with HCs (S1) and NPCs (S2) derived from PB treated rats (*in vivo)*, will be available as downloadable files upon publication.

Phenobarbital induces alterations in the proteome of hepatocytes and mesenchymal cells of rat livers.

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Abstract

Preceding studies on the mode of action of non-genotoxic hepatocarcinogens (NGCs) have concentrated on alterations induced in hepatocytes (HCs). A potential role of non-parenchymal liver cells (NPCs) in NGC-driven hepatocarcinogenesis has been largely neglected so far. The aim of this study is to characterize NGC-induced alterations in the proteome profiles of HCs as well as NPCs.

We chose the prototypic NGC phenobarbital (PB) which was applied to male rats for a period of 14 days. The livers of PB-treated rats were perfused by collagenase and the cell suspensions obtained were subjected to density gradient centrifugation to separate HCs from NPCs. In addition, HCs and NPC isolated from untreated animals were treated with PB *in vitro*. Proteome profiling was done by CHIP-HPLC and ion trap mass spectrometry.

Proteome analyses of the *in vivo* experiments showed many of the PB effects previously described in HCs by other methods, e.g. induction of phase I and phase II drug metabolising enzymes. In NPCs proteins related to inflammation and immune regulation such as PAI-1 and S100-A10, ADP-ribosyl cyclase 1 and to cell migration such as kinesin-1 heavy chain, myosin regulatory light chain RLC-A and dihydropyrimidinase-related protein 1 were found to be induced, indicating major PB effects on these cells. Remarkably, *in vitro* treatment of HCs and NPCs with PB hardly reproduced the proteome alterations observed *in vivo*, indicating differences of NGC induced responses of cells at culture conditions compared to the intact organism.

To conclude, the present study clearly demonstrated that PB induces proteome alterations not only in HCs but also in NPCs. Thus, any profound molecular understanding on the mode of action of NGCs has to consider effects on cells of the hepatic mesenchyme.

Keywords: mass spectrometry, proteome profiling, phenobarbital, non-genotoxic carcinogens, non-parenchymal cells, hepatocytes, inflammation,

Introduction

Screening assays, which enable the early detection of potential carcinogenic activities, are of crucial importance for safe drug development strategies. Chemical compounds may cause cancer by directly affecting DNA and are thus called genotoxic carcinogens. This type of compounds is easily detectable as such by the application of well-established *in vitro* assays, such as Ames bacterial reverse mutation assay, mammalian forward mutation assays and detection of chromosomal aberrations. Furthermore, *in vivo* assays are routinely used which include rodent erythrocyte micronucleus assay, mammalian bone marrow chromosomal aberration assay and assays for somatic cell gene mutation in endogenous genes. Chemical carcinogens which do not affect DNA directly are called non-genotoxic carcinogens (NGCs) [1]. In contrast to genotoxic carcinogens, there are no sufficiently accurate and validated short-term assays that may allow detection of NGCs [2–6]. Currently employed assays necessitate long-term rodent carcinogenicity assays causing high efforts, costs and time requirement as major drawbacks. In order to overcome these problems, deeper insights into NGC-relevant mechanisms are urgently required, which may be obtained by the application of a screening technology such as proteome profiling.

According to current knowledge, a characteristic effect of many NGCs is a deviation of tissue homeostasis resulting in organ growth based on a dysbalance between cell replication and cell death by apoptosis [7,8]. This dysbalance acts also on mutated/initiated cells. By this mechanism NGCs enhance the selective proliferation of preneoplastic cells and exert tumour promoting effects. Possible molecular mechanisms of the tumour promoting effects of NGCs comprise epigenetic changes such as hypo- and hypermethylation of CpG sites, chromatin modifications, and miRNA regulated mechanisms [9], but also endocrine effects, inhibition of gap junctional intercellular communications, immune modulation, and/or profound disturbances in the epithelial-mesenchymal interactions [6]. It is known that during carcinogenesis the microenvironment may gain a pivotal role supporting preneoplastic and neoplastic cell growth via an altered vasculature, deviated immunological activities and altered interstitial extracellular matrix (ECM) [10,11]. Further possible modes of NGC actions in rodents may be cytotoxicity followed by regenerative growth and a pro-inflammatory status. Involvement of inflammatory mechanisms may be accompanied by enhanced production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [7,8]. These two species may have relevance in carcinogenesis via signalling function and possibly cause endogenous DNA damage, which may be responsible for a weak genotoxic potential of NGCs.

The most common target organ of NGCs in rodent models is the liver, i.e., about 40% of all NGC tested so far are hepatocarcinogens. Hitherto, research on the action of NGCs has been focusing mainly on hepatocytes (HCs), the major parenchymal cells of the liver which eventually give rise to liver cancer. A role of non-parenchymal liver cells (NPCs) in NGC-driven hepatocarcinogenesis has been mostly neglected, because these cells do not transform [12,13]. However, NPCs, which consists mainly of Kupffer, endothelial, and stellate cells, may also be targeted by NGCs and may contribute considerably to the selective proliferation of preneoplastic and neoplastic HCs via release of paracrine growth factors or other growth-enhancing stimuli. Here, as a model NGC we chose phenobarbital (PB), a barbiturate known to be a potent tumour promoter in rodent liver [14–18]. PB has been described to interact with the pregnane X receptor (PXR) and the constitutive androstane receptor (CAR) triggering a signal transduction cascade leading to an induction of cytochrome P450 genes such as members of the CYP2B and CYP3A subfamily [9,19–23]. Furthermore, it was shown that PB acts through other mechanisms such as oxidative stress [24], which may correlate with the P450 induction [25], driving tumour promotion by inducing proliferation [26,27] in HCs, non-receptor mediated endocrine modifications and inhibition of gap junction intercellular communications, regulating growth and differentiation [18].

Proteome profiling is a powerful technique to observe molecular consequences of drug action. Cells may respond to drug actions via the synthesis of new proteins. Cells synthesize proteins in order to overcome biological challenges. Therefore, the identification of drug-induced proteins may give important hints to better understand the way of action of drugs. Furthermore, if the drug-induced proteins display restricted expression patterns, they may be used as indicative marker proteins. For comprehensive investigation, we analysed subcellular fractions including the secretome of isolated HCs and NPCs separately applying LC-MS/MS analysis. We hypothesized that NGC-driven hepatocarcinogenesis may involve NPCs and specifically considered a potential contribution of inflammatory activities of this tissue compartment, as known for the pathogenesis of hepatocellular carcinoma in humans [28]. Therefore we treated HCs and NPCs with pro-inflammatory cytokines *in vitro* in order to identify proteome signatures characteristic for such events. Induction of such a signature by PB treatment could thus indicate the involvement of inflammatory processes in drug action. Furthermore, we investigated PB effects induced by *in vivo* treatment of animals in comparison to PB effects observed upon *in vitro* treatment of isolated primary cells, including both HCs and NPCs. This strategy provided the unique opportunity to differentiate direct drug effects on the isolated cell types *in vitro* from indirect drug effects modulated by complex epithelial-mesenchymal interactions in the intact organism. This approach may thus give novel insights into the mode of action of this prototypic NGC. To conclude, the aim of the present study was to investigate proteome alterations in HCs as well as NPCs, which are caused by the non-genotoxic carcinogen phenobarbital (PB) *in vitro* as well as *in vivo*.

Material and Methods

Animals and treatment

Male Wistar rats were obtained from and kept at the "Division for Decentralized Biomedical Facilities of the Medical University of Vienna" under standardized SPF-conditions. Phenobarbital (PB, 5-Ethyl-5-phenyl-barbituric acid-sodium salt; Fluka, 04712) [29,30], admixed to drinking water, was administered to three rats which were 8 to 10 weeks old at a daily dose of 50mg/kg bodyweight. Two times a week the PB concentrations in the drinking water were adjusted to the body weights and the amount of water consumed. Five controls and four animals for the *in vitro* experiments were treated with tap water only. Animals were sacrificed by exsanguination under CO_2-asphyxation during the liver perfusion. The experiments were approved by the "Committee of Animal Protection of the Austrian Ministry of Sciences" (permission number 66009/157 II10b/2009) and performed according to Austrian regulations in accordance with the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" by the National Academy of Sciences.

Separation of liver cells and primary cultures

The following procedure was used for the *in vitro* and the *in vivo* experiments. To isolate and cultivate hepatocytes (HCs) and non-parenchymal cells (NPCs) at a functional state as naïve as possible, livers of rats were perfused with collagenase (Worthington CLS-2) as described before [31,32]. In the resulting cell suspension HCs were isolated from NPCs by low speed centrifugation followed by discontinuous density gradient centrifugation using Percoll [33]. The purity was found to be an average of 95.4% for hepatocyte fractions and 99.8% for NPC fractions. Cell preparations were used for further experimentation when the viability exceeded 90%, as determined by the trypan blue exclusion assay. HCs and NPCs were subsequently seeded on collagen-coated 6-well plates. HCs were seeded at a density of $4x10^5$cells/well in Williams' medium E (Invitrogen) supplemented with glutamax, HEPES, gentamycin, H2 mix, ascorbat and 10% FCS. NPCs were seeded at a density of 3-$4x10^6$cells/well in RPMI 1640 medium (Gibco Ltd.) supplemented with gentamycin and 10% FCS at 37°C for 2 hours. To determine the purity of the isolated cell fractions, cells were counted using microscope pictures and the ImageJ software (National Institutes of Health). After an attachment period of 2 hours cells were switched to serum-free medium (Williams medium E and RPMI 1640 medium, both supplemented as above without the 10% FCS) and kept at 37°C for further 24 hours in order to collect cell supernatants.

In vitro treatment of primary HCs and NPCs

In addition to the procedure described above, the *in vitro* treatment of cultures commenced 2h after plating of cells deriving from four untreated rats. Two rats were used for the cytokine treatment, while the other two were used for the PB treatment. HCs were treated with 10ng/ml interleukin-1ß (R&D Systems) and 5ng/ml interleukin-6 (R&D Systems) [34] for 24 hours, which induce the acute phase plasma protein

synthesis in HCs [35–38]. 10ng/ml lipopolysaccharide (LPS, Sigma-Aldrich) was applied to the medium of NPCs for 24 hours [39–43]. HCs and NPCs were treated with 1mM PB (Fluka) for 24 hours.

Cellular sub-fractionation and protein sample processing (see also [44]*)*

The serum-free supernatants were sterile filtered using a 0.2µm cellulose acetate filter (Whatman). One part of this filtrate was precipitated by adding 5x volume of -20°C tempered p.a. ethanol (Merck) and subsequent storing at -20°C for at least overnight. The other part was directly stored at -80°C for subsequent analyses by ELISA. During all steps samples were kept on ice. For harvesting of cytoplasmic and nucleic protein fraction, cells were gathered in isotonic buffer (10mM HEPES/NaOH pH=7.4, 10mM NaCl, 3.5mM MgCl2, 1mM EGTA, 0.25M Sucrose and 0.5% Triton X-100) and protease inhibitor mix (1mM PMSF; aprotinin, leupeptin and pepstatin, [1µg/ml] each). The cells were disrupted by sheer stress caused by syringing the cell lysates through 23G needles. The cytoplasmic fraction, in the supernatant, was separated from nuclei and membrane proteins as well as debris by centrifugation at 2300xg and 4°C for 5min and was subsequently precipitated by ethanol tempered to -20°C. The nuclei protein fraction was extracted from the remaining residue via a 10min incubation with an extraction buffer (10mM Tris/HCl pH=7.4, 1mM EDTA, 500mM NaCl) which act through osmotic pressure followed by a 1:10 dilution with a NP40 buffer (10mM Tris/HCl pH=7.4, 1mM EDTA, 0.5% NP40), to reduce the final NaCl concentration, for 15min. Nucleic proteins were separated from debris by centrifugation at 2328xg and 4°C for 5min and precipitated by ethanol tempered to -20°C.

After precipitation all fractions were centrifuged at 4700xg and 4°C for 25min. The resulting protein pellets were dissolved in sample buffer (7.5M urea, 1.5M thiourea, 4% CHAPS, 0.05% SDS, 100mM DTT) in a volume according to the protein amount / pellet size.

Shotgun analysis - 1D-SDS PAGE, in-gel tryptic digestion & MS analysis ('bottom up')

For 1D-SDS PAGE we used a 4% stacking and a 12% resolving polyacrylamide gel. Protein samples were loaded onto the gel and the electrophoresis ran until complete separation of a pre-stained molecular marker (Dual Color, Biorad, Hercules, CA) was visible. Gels were fixated with 50% methanol/10% acetic acid and MS compatible silver stained as described by Mortz, E. *et al* [45]. For tryptic digestion samples were cut into lanes to group proteins with a similar molecular weight, which improved the following LC-MS/MS analyses. These lanes were cubed and the proteins were destained, reduced and alkylated (see Supplementary Material) before digestion with trypsin (sequencing grad, Roche) at 37°C over night as described before [46]. After elution the peptide solutions were analysed by LC - MS/MS.

For reversed-phase chromatography we used a nano-flow LC (1100 Series LC system, Agilent) combined with the HPLC chip technology (Agilent). The chips consist of a 40nl Zorbax 300SB-C18 trapping column and a Zorbax 300SB-C18 (75µm x 150mm) separation column. The flow rate was 400nl/min,

using a gradient from 0.2% formic acid and 3% ACN to 0.2% formic acid and 50% ACN over 40min or 60min for supernatants or the other two fractions, respectively.

Peptide identification was performed by MS/MS fragmentation analysis using an ion-trap mass spectrometer (XCT-Ultra, Agilent) combined with already described HPLC chips, which are eventually an orthogonal nanospray ion source (Agilent). For peak list-generation and spectrum identification, of the MS/MS data we used Spectrum Mill MS Proteomics Workbench software (Version A.03.03, Agilent). We searched our MS/MS data against the UniProtKB/Swiss-Prot protein database (Version 10th August 2010; 519,348 entries). The settings were as follows: max. of 2 missed cleavages, minimum scored peak intensity (%SPI) of 70%, precursor mass tolerance of +/-1.5Da, product mass tolerance +/- 0.7. Two types of modification were considered, both arising during sample preparation. Carbamidomethylation of cysteine (deliberately) was set as fixed modification and oxidized methionine (artefact) was set as post-translational modification.

Generating protein maps and their interpretation

The resulting peptide-protein assignment list was reviewed by hand considering the following parameters. Spectrum Mill peptide sequence score values (sequence matching probability) of >13 counted as valid sequence assignments. Peptide sequences were valid, when at least one peptide sequence scored as much and did not occur in other proteins of this cell type. An estimated error rate was calculated by searching the sequences against a non-sense reversed database. Furthermore we included peptides scoring between 9 and 13 only if precursor m/z value, retention time and MS2 pattern were found similarly in at least one of our previous experiments and the peptide was thereby scoring above 13. With respect to protein inference, we chose the smallest number of proteins required to explain all observed peptides as described for ProteinProphet [47]. As our protein identification algorithm includes manual selection, we cannot calculate an exact false discovery rate.

Proteome analysis using emPAI (exponentially modified protein abundance index) values[48], comparisons and PB specific alterations were recorded and interpreted using the GPDE (Griss Proteomics Database Engine), a database specifically engineered for the identification and characterization of marker proteins [49].

ELISA

Arginase-1 protein levels were determined by a kit (Uscn Life Science Inc.; Houston, TX 77036) according to the user manual.

Results

Isolation and proteome profiling of primary rat hepatocytes and non-parenchymal cells

Primary rat cells were obtained from untreated rats and rats treated with PB *in vivo*. Cell isolation was performed by liver perfusion followed by separation into hepatocytes (HCs) and non-parenchymal cells (NPCs) (see *figure 1*). Cells were cultured for 24 hours to allow accumulation of secreted proteins. Subsequently, cells were fractionated into cytoplasm and nuclear extract. The protein fractions were further separated by SDS-PAGE and forwarded to proteome profiling via LC-MS/MS as described.

We restricted our analyses to proteins which were identified by two peptides or more in two or more independent experiments. By this approach, we identified 1148 proteins in HCs and 1213 proteins in NPCs with an overlap of 966 proteins in both cell compartments. The 182 proteins being apparently specific for HCs indeed comprise a large number of known liver-specific proteins including, apolipoproteins and other serum proteins, cytochrome P450 isoenzymes, sulfotransferases, UDP-glucuronosyltransferases and many others (*table 1-5, table S1 & S2*). Furthermore, the 247 proteins identified in NPCs included many proteins known to be expressed in stromal cells. Representative for leukocytes are the surface antigens CD37, CD47, CD96 and CD166 as well as the chemokines CXCL1, CXCL2, and CCL6. Amongst known marker proteins for endothelial cells we identified endothelial cell-specific molecule 1, endothelial nitric oxide synthase and septin-2 (vascular endothelial cell specific protein 11), while MMP-3 and various collagens are characteristic for the stellate cells.

In vitro treatment of isolated primary cells

HCs were treated with IL-6 and NPCs with LPS, respectively. Upon treatment for 24 hours, the induction of several pro-inflammatory proteins was observed which confirms the responsiveness of these cells to stress stimuli under our experimental conditions (*figure 2*).

To investigate direct drug effects, primary untreated cells were treated with 1mM PB for 24 hours and analysed by proteome profiling. Remarkably, this treatment had hardly any measureable effect on the proteome composition of HC and NPCs. A single protein was apparently induced in HC and two proteins only in NPCs, respectively. Proteins indicating stress such as chaperones including the heat shock protein family, stress-induced phosphoprotein, heme oxygenase 1 and DNAJ homolog subfamily members were not or hardly induced.

In vivo treatment of animals with phenobarbital

13 proteins were found to be newly induced in the cytoplasmic fraction of HCs by PB, including phase I drug metabolizing enzymes such as amine oxidase, phase II enzymes such as UDP-glucuronosyltransferases and glutathione S-transferases, the chaperone rotamase D, glutathione synthetases and the proto-oncogen c-Raf. The induction of estradiol 17-beta-dehydrogenase 8 may

indicate alterations in steroid hormone homeostasis. The induction of CYP2B, probably via CAR, is a known positive control for PB action.

Table 1, 2 and 3 depict the most significant proteins found up-regulated in the secretome, cytoplasm or nuclear extract of HCs and NPCs in response to *in vivo* PB exposure. Proteins found up-regulated in the secretome of HCs are involved in the acute-phase response, inflammation response and the action of drugs. In the cytoplasm of HCs we found 82 proteins up-regulated. These proteins preferentially act in the acute-phase response, cell growth, immune response, inflammatory response as well as in response to cell stress, oxidative stress and wounding. In the nuclear extract of HCs we found 182 proteins up-regulated. These proteins are involved in nucleosome assembly, nucleocytoplasmic transport, mRNA processing, translation, protein localization to the nucleus, RNA processing, removal of superoxide radicals, anti-gen processing and presenting, protein methylation and cell redox homeostasis as well as are major nucleolar proteins of growing eukaryotic cells and proteins of the nuclear matrix.

In NPCs, 12 proteins were found newly induced, comprising proteins related to inflammation such as PAI-1 and S100-A10, cell migration such as kinesin-1 heavy chain, myosin regulatory light chain RLC-A and dihydropyrimidinase-related protein 1 as well as altered immune cell functional state such as acetyl-CoA carboxylase 1 and ADP-ribosyl cyclase 1. Proteins found up-regulated in the secretome of NPCs are involved in the acute-phase response, inflammation response, and action of drugs and oxidative species. In the cytoplasm of NPCs we found 108 proteins up-regulated. These proteins exert function in tissue, acute phase, cell redox homeostasis, cell cycle, response to drug, cell adhesion, vesicle-mediated protein transport, stress response, positive regulation of MAPKKK cascade, cellular response to cell-matrix adhesion, response to ROS. In the nuclear extract of NPCs we found 78 proteins up-regulated which act on transcription regulation, mRNA processing, mRNA and protein transport, response to DNA damage stimulus and DNA repair.

Table 4 and 5 depict selected proteins found only in untreated rats, which means a down-regulation of these proteins in HCs and NPCs in response to *in vivo* PB exposure. This rather stringent selection criterion for down-regulated proteins improves the reliability of these results. In HCs, 6 proteins were found only in untreated rats, including the E3 ubiquitin-protein ligase UBR4, a protein involved in in membrane morphogenesis and cytoskeletal organization as well as the glutamine synthetase, which catalyses the production of glutamine and 4-aminobutanoate (gamma-aminobutyric acid, GABA). In NPCs, 28 proteins were found only in untreated rats, comprising proteins involved in cell adhesion, cell proliferation, liver development and protein transport.

Table S1 and S2 present the summarised proteome profiling results obtained with HCs (S1) and NPCs (S2) derived from PB treated rats (*in vivo*).

Pathway analysis of the in vivo data via Reactome

The alterations in the proteome were further analysed by Reactome, a public peer-reviewed pathway database from CSHL, OICR and EBI, which is cross-referenced to bioinformatics databases and allows the assignment of a given protein to one or more molecular pathways. When searching for the positively PB-induced proteome alterations, in both HCs and NPCs, proteins assigned to the categories of "gene expression", "metabolism of proteins" and "3`UTR-mediated translational regulation" were most prominent among the top listed events (see *table 6 & 7*). With respect to molecular pathways, in HCs "Peroxisomal lipid metabolism", "Class I MHC mediated antigen processing & presentation" and "Asparagine N-linked glycosylation" were listed on top (see *table 8*). In contrast, in NPCs "Protein folding", "Dissolution of Fibrin Clot" and "Platelet Adhesion to exposed collagen" were listed on top (see *table 9*).

ELISA verification of arginase-1 variations

To verify selected LC-MS/MS data in a quantitative fashion, we conducted an ELISA for rat arginase-1. Arginase-1 was chosen, because of its ability to diminish anti-tumour immunity by interfering with the activation of T-cells [50]. LC-MS/MS results indicated a PB induced decrease in protein secretion of arginase-1 by HCs in case of *in vivo* treated rats. The ELISA results confirmed our LC-MS/MS results as demonstrated in *figure S1*. In the secretome arginase-1 concentration was found decreased by a factor of 2.3. In the cytoplasmic protein fraction, arginase-1 abundance was found induced by PB more than three-fold, which corresponds very well to the LC-MS/MS results.

Discussion

The aim of this study was to investigate molecular mechanisms induced by treatment of rats with the non-genotoxic carcinogen phenobarbital (PB) by means of proteome profiling. This approach was used to pinpoint crucial events not previously recognised by other technical approaches. Current mechanistic considerations on non-genotoxic carcinogenesis include altered cell-cell interactions, epigenetic changes endocrine effects, inhibition of gap junctional intercellular communications and immune modulation [6], which may be of crucial importance for initiation as well as promotion and progression [10]. However, the significance of an altered epithelial-mesenchymal dialogue and the role of NPCs for NGC-driven hepatocarcinogenesis have not been investigated so far.

Teufehlofer *et al* has described that chemical compounds, including genotoxic hepatocarcinogens, may induce the superoxide radical production by Kupffer cells and may thus contribute to DNA damage and an increased occurrence of mutations [51]. Laskin *et al* and Roberts *et al* have found that NGCs, such as PB and peroxisome proliferators, may also activate Kupffer cells to release the pro-inflammatory cytokine TNFa [12,25]. Furthermore, stromal liver cells may be induced to secrete survival factors, which may act

as tumour promoters [52,53]. Therefore, an improved understanding of non-genotoxic compounds has to consider drug effects on stroma cells, even if these cells are not transformed to cancer cells. In order to assign molecular events caused by NGCs to the different cell types of the liver, we isolated primary cells by liver perfusion and separated them into parenchymal HCs and NPCs as described previously [54,55]. Here, we isolated cells from untreated and PB-treated animals in order to investigate *in vivo* effects.

Cells obtained from untreated livers were also treated with PB *in vitro*. This experimental approach is limited mainly due to the de-differentiation of primary cells during prolonged *in vitro* cultivation. To avoid this issue, we chose a 24 hours treatment period to ensure a meaningful data interpretation. In the *in vitro* part, functional activation by interleukin-6 or LPS resulted in well detectable proteome alterations in HCs or NPCs (data not shown). Any cytotoxic substance will cause a cell stress response, which results in an increased expression of chaperones, especially the heat shock protein family. Under the present experimental conditions PB application *in vitro* hardly induced such a stress response and generally exerted marginal effects. A reason for that may be that the dedifferentiation of HCs in culture during the first 24 hours hampers the reaction of HCs to PB, namely the induction of drug metabolising enzymes. Nevertheless, proteins induced *in vitro* were also found to be induced in the *in vivo* experiments, some of which are presented in *figure 2A*. *Figure 3* highlights the diverse response of HCs and NPCs upon *in vitro* (A) and *in vivo* (B) PB treatment. This observation suggests that *in vivo* PB treatment has effects being more profound and largely different from those obtained by *in vitro* treatment. This may be due to the fact that the milieu in the intact organism is required to enable the full response of liver cells to NGCs and that the disrupted cell-cell interactions *in vitro* and the tendency towards de-differentiation under artificial culture conditions compromise such a response.

The presently observed PB-induced proteome alterations in the stromal cells of the liver indeed suggest profound functional alterations. There was strong induction of pleckstrin, which is a positive regulator of platelets causing an aggregation of these sensitive cells, indicating that PB may trigger a wound healing cascade. ADP-ribosyl cyclase 1 (CD38) is an immunity-related protein induced in NPCs upon PB action. This protein usually regulates B cell function by influencing the intra-cellular Ca^{2+} concentration [56]. It was also demonstrated that it affects the migration capabilities of dendritic cells and as a consequence it also affects T cells [57].

The increased secretion of PAI-1, cathepsin L1, MMP-10 and V-CAM 1 indicates some inflammatory activation of these cells. Remarkably, however, a profound inflammatory activation as observed upon LPS-treatment was not evident (*figure 2*). Cox-1 (Q63921), an important mediator of inflammatory signalling, was observed induced by PB in stroma cells in one animal only, which may be due to low protein concentration at the limit of detection. The expression of the inflammation-related protein MX-1 [58] in the control stroma cells, however, indicates that the presently employed cell manipulation steps

may also have altered the inflammatory activity state in an artificial way, rendering clear conclusions, with respect to inflammatory pathways, difficult. The present observations still indicate some complex regulatory actions of PB which are related to but still distinct from classical inflammatory activation warranting further investigation.

Many effects of PB on HCs, as observed in our *in vivo* experiments, have already been identified applying techniques other than proteomics, e.g. the induction of phase-I and phase-II drug-metabolising enzymes [59], redox-regulating enzymes as well as the proto-oncogen c-RAF were observed by proteome profiling (*table 1-3*) [60–62]. As part of the drug metabolism, we found Cytochrome P450 2B1 and other isoforms, sulfotransferase 1A1 and glutathione S-transferase alpha-2 (GST-A2) up-regulated in HCs. Increased glutathione S-transferase levels may indicate increased oxidative stress [63]. It has been proposed that an accumulation of ROS has many effects on cells such as an increased proliferation, DNA mutation rates [64] and genetic instability [65]. Reproducing these and other well-known PB effects by our currently employed proteome profiling analyses supports the notion that this strategy was valid. Furthermore, we were able to observe a few molecular events which have not yet been described. This includes the induction of estradiol 17-beta-dehydrogenase 8 in HCs (*table 2*) and the down-regulation of estrogen sulfotransferase 1 in NPCs (*table 5*) by PB, which may indicate alterations of the estrogen metabolism. Estrogens are important growth factors and potential tumour promoters [66] and may exert anti-inflammatory activities [67]. Whether PB may act via estrogen activity modulation deserves more detailed investigations, e.g. by including female rats in the PB study. Furthermore, ectonucleotide pyrophosphatase 3 (E-NPP 3) was found to be induced, which has been observed in association with neoplastic bile duct diseases [68].

The nuclear proteins presently observed to be induced by PB actually indicate that cells were finally exposed to DNA stress. This interpretation is supported by the PB-induced expression of DNA topoisomerase I, poly [ADP-ribose] polymerase 1 and sister chromatid cohesion protein PDS5 homolog B. These proteins are known to be involved in DNA damage sensing as well as DNA-repair. These findings were somewhat unexpected, as PB is known to be non-genotoxic. However, it may increase the hepatocellular production of ROS by Cyp450 induction or ROS production by activated NPCs and may thus evoke some marginal DNA repair activity. This demonstrates the potential difficulties to group chemical compounds unequivocally to the categories of genotoxic or non-genotoxic carcinogen.

Interestingly it was demonstrated that inflammation-related ROS formation and signalling may lead to carcinogenesis of epithelial cells and a phenotypic change. This includes dissolution of cell-cell contacts, cytoplasmic redistribution of E-cadherin and up-regulation of integrins, as evidenced in the present experiments by the up-regulation of integrin-linked protein kinase and matrix metalloproteinases such as MMP-10. Furthermore, under these conditions MMP activity may correlate with invasiveness as

determined by Matrigel invasion assays [69]. An PB-induced formation of ROS is evidenced by the observed increase of ROS responding enzymes such as retinal dehydrogenase 1 and mitochondrial superoxide dismutase [Mn] in HCs and plasminogen activator inhibitor 1 (PAI-1) in NPCs. as well as by the induction of DNA repair proteins described above.

The present investigation of primary cells by proteome profiling may be considered as essential for the achievement of biological relevance of the employed model, but on the other hand, also accounts for the experimental limitation of the present study with respect to reliable quantification of potential marker proteins. Shotgun proteomics as presently employed using nano liquid chromatography and ion trap mass spectrometry may give a quite comprehensive overview to cell activities, but results in rather semi-quantitative data hardly accessible to stringent statistical analysis. However, the present observation of PB-induced proteome alterations especially in NPCs is a first but important step to improve our understanding of the complex mode of action of non-genotoxic carcinogens.

It is evident from the current results that the analysis of drug effects on isolated cell model systems will hardly represent the mode of action *in vivo*. Furthermore, the contribution of tumour-promoting molecules associated with ROS to the effect of PB is clearly evidenced by the induction of several marker proteins signifying oxidative stress and associated DNA damage. Only the complete comprehension of direct and indirect consequences will enable a strategy to identify a panel of marker molecules with sufficient specificity for the unequivocal indication of non-genotoxic carcinogen activity.

Supplementary data description

Figure S1. ELISA verification of arginase-1 variations. This figure depicts the arginase-1 variations in 1) secretome and 2) cytoplasm of HCs using the quantitative data from the ELISA and 3) the semi-quantitative data from the LC-MS/MS analyses (description of how to read this sort of presentation see figure 2), which are altered accordingly. Arginase-1 concentration decreases in the secretome and increases upon PB treatment of rats. Part 4) presents the values used to generate these figure, whereby the emPAI values were used for part 3).

Table S1 and S2. Summary of proteome profiling results obtained with HCs (S1) and NPCs (S2) derived from PB treated rats (in vivo). Column names, which are labelled 'analysis' and 'reference', refer to the experiments of the isolated primary cells, HCs and NPCs, deriving from rats treated *in vivo* with PB (analysis) and untreated animals (reference). "found", specificity of protein identification; accession, Uniprot accession numbers; name, protein names; "analysis_fractions", subcellular fractions in which the protein was identified in untreated samples; "analysis_peptides", number of distinct peptides identified per proteins in untreated samples; "analysis_nuclei_expcount", number of positive identifications in nuclear fractions compared to

the total number of experiments; "analysis_nuclei_empai", calculated emPAI values in the nuclear fraction; "analysis_nuclei_empai_stdev", standard deviation thereof. These terms are used in the same way referring to the secretome as well as the cytoplasm. After the values obtained for the treated samples (analysis) all values are listed again referring to untreated samples (reference). The last three columns list difference values for the emPAI values obtained for nuclei, secretomes and cytoplasms, respectively.

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Author contribution towards the manuscript

Conceived and designed the experiments: Christopher Gerner, Bettina Grasl-Kraupp, Verena Haudek-Prinz, Sandra Sagmeister. Performed the isolation of the primary cells, animal & cell treatment: Sandra Sagmeister, Melanie Pichlbauer. Experiments – proteomics: Philip Klepeisz, Christopher Gerner. Analyzed the proteome data: Christopher Gerner, Philip Klepeisz. Contributed reagents/materials/analysis tools: Christopher Gerner, Bettina Grasl-Kraupp. Wrote the manuscript: Christopher Gerner, Bettina Grasl-Kraupp, Philip Klepeisz.

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Figure legend

Figure 1. Microscope images of HCs and NPCs in culture. These pictures depict representative areas of untreated, PB *in vitro* as well as *in vivo* treated HCs and NPCs in culture extracted from microscopic images of equal magnification.

Figure 2. Proteome alterations induced by *in vitro* treatment of primary cells. Part A) shows schematic representations of a cell and her three sub-compartments, namely the supernatant, the

cytoplasm and the nucleus. The intensity of red represents the degree of amount of the selected protein found in the respective compartment in contrast to the other experiments. The higher intensity of red corresponds to a higher occurrence. This allows an easy comparison of the expression levels of a protein in different experimental setups.

NPCs induce the secretion of IL-1beta and TNF-alpha upon inflammatory stimulation with LPS. *In vitro* treatment with PB induced coronin-7 and ADP-ribosyl cyclase 1, which both are also induced by *in vivo* treatment. The expression of Hsp90, a stress response related protein, was increased upon LPS and PB treatment. Prostaglandin, a protein involved in promotion of proliferation in normal and preneoplastic cells, was induced upon LPS and in vivo PB treatment. HCs respond hardly to the *in vitro* treatment with PB. Treatment with IL-6 specifically induced the acute phase protein T-kininogen-2. UDP-glucuronosyltransferase 2B37 and the chaperone peptidyl-prolyl cis-trans isomerase D were induced by both *in vitro* stimulation experiments as well as by the *in vivo* treatment with PB. Carbamoyl-phosphate synthase is part of the urea cycle and has to be found in all four categories.

Proteins in NPC: (1) **O35828** Coronin-7, (2) **P16599** Tumor necrosis factor, (3) **P34058** Heat shock protein HSP 90-beta, (4) **Q63264** Interleukin-1 beta, (5) **Q63921** Prostaglandin G/H synthase 1, (6) **Q64244** ADP-ribosyl cyclase 1

Proteins in HC: (1) **P07756** Carbamoyl-phosphate synthase [ammonia], (2) **P08932** T-kininogen 2, (3) **P19488** UDP-glucuronosyltransferase 2B37, (4) **Q6DGG0** Peptidyl-prolyl cis-trans isomerase D

Part B) demonstrates the distribution of distinct proteins within the three fractions, supernatant, cytoplasm and nuclear protein fractions, underneath the respective treatment of the cells, which gives an overview of the responsiveness of the cells.

Abbr.: SN –proteome of the supernatant, Cyt – proteome of the cytoplasm, NE – proteome of the nuclear extract

Figure 3: Distribution of distinct proteins, when comparing controls with PB-treatment from the *in vitro* and *in vivo* sample pools, respectively. This figure demonstrates the distribution of distinct proteins found in HCs and NPCs during the pooled A) *in vitro* and B) *in vivo* experiments, while including only proteins found with at least 2 peptides. The up- and down-regulation of proteins were neglected in this qualitative comparison.

Figures 1-3

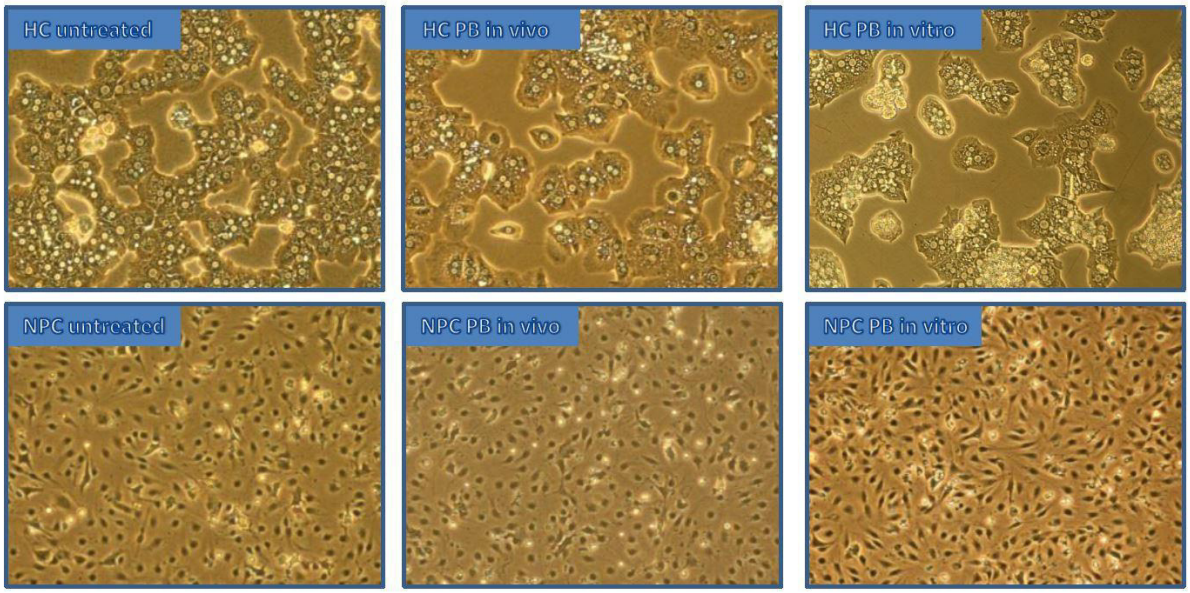

Fig. 1: Microscope images of HCs and NPCs in culture.

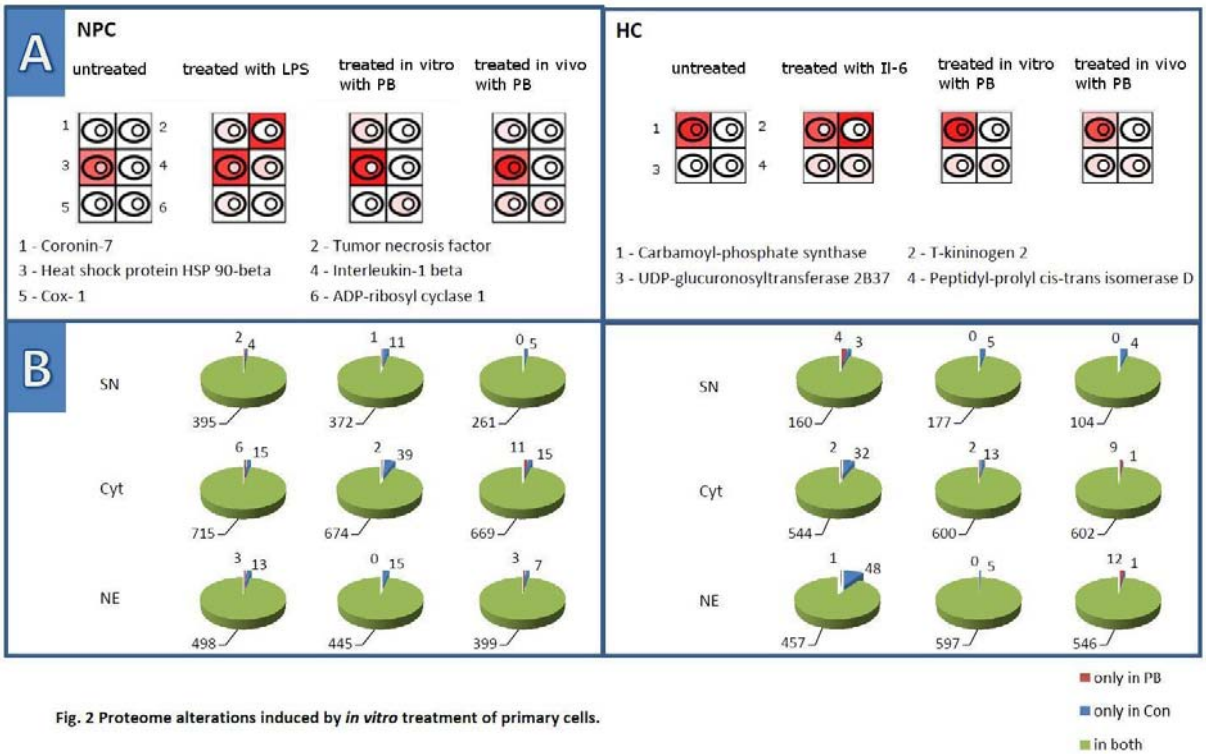

Fig. 2 Proteome alterations induced by *in vitro* treatment of primary cells.

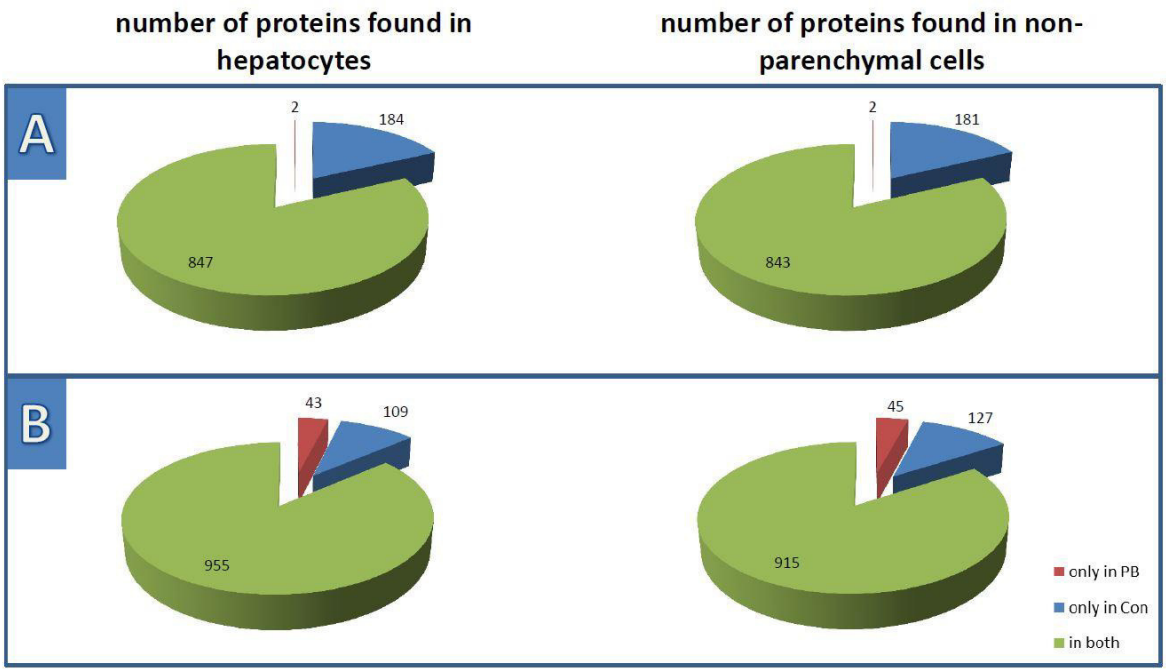

Fig. 3: Distribution of distinct proteins when comparing the untreated and the PB *in vitro* or *in vivo* treated sample pool, respectively.

Table 1. ***Selected proteins found up-regulated in the secretome of HCs and NPCs isolated from rat livers, when treated with PB*** **in vivo.** *These up-regulated proteins of interest were selected out of 666 and 1044 distinct proteins, whereby the selected proteins had a relative difference of emPAI values of at least 40% and 50% for HCs and NPCs, respectively. Furthermore, the proteins had to fulfill the criteria of being represented in at least 50% of the experiments with at least 2 peptides. For convenience, the proteins are sorted according their Go terms.*

Hepatocytes / secreted protein fraction		
Accession number	Protein name	GO - biological process
P24090	Alpha-2-HS-glycoprotein	acute-phase response
P06238 [t]	Alpha-2-macroglobulin	acute-phase response, response to glucocorticoid stimulus
P02650	Apolipoprotein E	cellular response to growth factor stimulus
P02454	Collagen alpha-1(I) chain	cellular response to transforming growth factor beta stimulus, response to corticosteroid stimulus
P06759 [t]	Apolipoprotein C-III	inflammatory & drug response
P02680	Fibrinogen gamma chain	inflammatory response

Non-parenchymal cells / secreted protein fraction		
Accession number	Protein name	GO - biological process
P29534 [t]	Vascular cell adhesion protein 1 (V-CAM 1)	acute & chronic inflammatory response
P12346	Serotransferrin (Transferrin) (Beta-1 metal-binding globulin) (Liver regeneration-related protein LRRG03)	acute-phase response
P07154	Cathepsin L1 (Major excreted protein) (MEP) (Cyclic protein 2) (CP-2)	autophagic cell death, proteolysis, response to organic cyclic compound
P22985	Xanthine dehydrogenase/oxidase	bone resorption, contributes to the generation of reactive oxygen species.
P11232	Thioredoxin (Trx)	cellular response to drug
P02761	Major urinary protein (MUP) (Alpha-2u-globulin)	cellular response to lipid, positive regulation of gene expression
P10760	Adenosylhomocysteinase (AdoHcyase) (S-adenosyl-L-homocysteine hydrolase)	chronic inflammatory response to antigenic stimulus
P07152 [t]	Stromelysin-2 (SL-2) (Matrix metalloproteinase-10) (MMP-10)	Collagen degradation
P18484	AP-2 complex subunit alpha-2 (Alpha2-adaptin)	endocytosis, intracellular protein transport
P15978	Class I histocompatibility antigen, Non-RT1.A alpha-1 chain	immune response

Q63228	Glia maturation factor beta (GMF-beta)	inhibition of proliferation of tumor cells
P31720	Complement C1q subcomponent subunit A	innate immune response
Q711G3 [t]	Isoamyl acetate-hydrolyzing esterase 1 homolog	lipid catabolic process
P14841	Cystatin-C (Cystatin-3)	positive regulation of cell proliferation, response to drug and toxin
P20961 [t]	Plasminogen activator inhibitor 1 (PAI-1) (Endothelial plasminogen activator inhibitor) (Serpin E1)	response to reactive oxygen species, tissue regeneration

[t] – Evidence at transcript level only, [PB] –found only in PB treated rats

Table 2. ***Selected proteins found up-regulated in the cytoplasmic protein fraction of HCs and NPCs isolated from rat livers, when treated with PB*** **in vivo.** *These up-regulated proteins of interest were selected out of 1283 and 1336 distinct proteins, whereby the selected proteins had a relative difference of emPAI values of at least 50% for HCs and NPCs, with the same exclusion criteria as table 1. For convenience, the proteins are sorted according their Go terms.*

Hepatocytes / cytoplasmic protein fraction		
Accession number	Protein name	GO - biological process
P09034 [t]	Argininosuccinate synthase	acute-phase response, cellular response to interferon-gamma, response to drug, liver development
P12346	Serotransferrin	acute-phase response, response to organic cyclic compound
Q6P791	Regulator complex protein LAMTOR1	cell growth, cholesterol homeostasis, positive regulation of MAPK & TOR signaling cascade
Q8K581 [t]	Thioredoxin domain-containing protein 9	cell redox homeostasis
P15709 [t]	Bile salt sulfotransferase	drug metabolic process
P00176	Cytochrome P450 2B1	drug metabolic process
P05178 [t]	Cytochrome P450 2C6	drug metabolic process
P04903 [t]	Glutathione S-transferase alpha-2	drug metabolic process
P17988	Sulfotransferase 1A1	drug metabolic process

P08011	Microsomal glutathione S-transferase 1	drug metabolic process
P09875 [t, PB]	UDP-glucuronosyltransferase 2B1	drug metabolic process
P19488 [t, PB]	UDP-glucuronosyltransferase 2B37	drug metabolic process
P97675	Ectonucleotide pyrophosphatase / phosphodiesterase family member 3 (E-NPP 3) (B10)	immune response
P07151	Beta-2-microglobulin	immune response, antigen processing and presentation of peptide antigen via MHC class I
P80254	D-dopachrome decarboxylase	inflammatory response
P51647	Retinal dehydrogenase 1	response to oxidative stress, response to organic cyclic compound
P55053	Fatty acid-binding protein, epidermal	response to wounding
Q66HA8	Heat shock protein 105 kDa	stress response

Non-parenchymal cells / cytoplasmic protein fraction		
Accession number	Protein name	GO - biological process
P11497 [PB]	Acetyl-CoA carboxylase 1	acetyl-CoA metabolic process, fatty acid biosynthetic process, response to organic cyclic compound
Q4KM33 [t, PB]	Pleckstrin	actin cytoskeleton reorganization, hemopoietic progenitor cell differentiation, positive regulation of platelet activation
P12346	Serotransferrin	acute-phase response, response to organic cyclic compound
Q70VB1 [PB]	G-protein coupled receptor family C group 6 member A	calcium-mediated signaling, response to amino acid stimulus
P85972	Vinculin	cell adhesion
Q91Y81	Septin-2 (Vascular endothelial cell specific protein 11)	cell division
Q9ESH6 [t]	Glutaredoxin-1	cell redox homeostasis
P85845	Fascin	cellular response to cell-matrix adhesion, liver development, cell motility
Q2PQA9 [t, PB]	Kinesin-1 heavy chain	cytoplasm organization, vesicle transport along microtubule
P00176 [PB]	Cytochrome P450 2B1	drug metabolic process, response to organic cyclic compound
P07323 [PB]	Gamma-enolase	glycolysis, gluconeogenesis, response to organic cyclic compound
P97675 [PB]	Ectonucleotide pyrophosphatase/phosphodiesterase family member 3	immune response
P67779	Prohibitin	organ regeneration, response to cytokine stimulus, response to drug, response to stress

Q64244 [t, PB]	ADP-ribosyl cyclase 1	positive regulation of B cell proliferation, cell growth & vasoconstriction and response to drug
Q99J82 [t]	Integrin-linked protein kinase (cell-cell junction)	positive regulation of MAPKKK cascade, positive regulation of cell migration, positive regulation of cell proliferation
Q5U204 [t]	Ragulator complex protein LAMTOR3	positive regulation of TOR signaling cascade, cellular protein localization
P13832 [t, PB]	Myosin regulatory light chain RLC-A	protein targeting to plasma membrane, regulation of cell shape
Q62950 [PB]	Dihydropyrimidinase-related protein 1	pyrimidine base catabolic process
Q5FVC7 [t, PB]	Arf-GAP with coiled-coil, ANK repeat and PH domain-containing protein 2	regulation of ARF GTPase activity
P05943 [PB]	Protein S100-A10	regulation of cell differentiation, regulation of cell growth
P25235 [t]	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2	response to drug
P20961 [t]	Plasminogen activator inhibitor 1 (PAI-1)	response to reactive oxygen species, tissue regeneration, positive regulation of receptor-mediated endocytosis
O88600	Heat shock 70 kDa protein 4	stress response
Q7TP47 [t]	Heterogeneous nuclear ribonucleoprotein Q (hnRNP Q) (Liver regeneration-related protein LRRG077)	tissue regeneration, mRNA processing
Q68FP1	Gelsolin (Actin-depolymerizing factor)	tissue regeneration, regulation of cell adhesion
P50399	Rab GDP dissociation inhibitor beta	vesicle-mediated protein transport
Q5U2R7 [t, PB]	LDLR chaperone MESD	Wnt receptor signaling pathway

[t] – *Evidence at transcript level only,* [PB] *–found only in PB treated rats*

Table 3. ***Selected proteins found up-regulated in the nuclear extract protein fraction of HCs and NPCs isolated from rat livers, when treated with PB*** **in vivo.** *These up-regulated proteins of interest were selected out of 1081 and 957 distinct proteins, whereby the selected proteins had a relative difference of emPAI values of at least 50% for HCs and NPCs, with the same exclusion criteria as table 1. For convenience, the proteins are sorted according their Go terms.*

Hepatocytes / nuclear extract protein fraction		
Accession number	Protein name	GO - biological process
P28064	Proteasome subunit beta type-8 (Proteasome subunit beta-5i)	anti-gen processing and presenting, fat cell differentiation
P13383	Nucleolin (Protein C23)	associated with transcription
Q6TRW4 [t, PB]	Sister chromatid cohesion protein PDS5 homolog B	cell division
Q5I0H9 [t]	Protein disulfide-isomerase A5	cell redox homeostasis, response to stress
Q9WUL0 [t]	DNA topoisomerase 1	cellular response to stress
P27008 [PB]	Poly [ADP-ribose] polymerase 1	DNA damage response, detection of DNA damage
P05183 [PB]	Cytochrome P450 3A2	drug metabolic process, oxidative demethylation
O09171	Betaine--homocysteine S-methyltransferase 1	methionine biosynthetic process, protein methylation
Q4KM65 [t]	Cleavage and polyadenylation specificity factor subunit 5	mRNA polyadenylation
Q62780	Probable ATP-dependent RNA helicase DDX46	mRNA processing
P17136 [t]	Small nuclear ribonucleoprotein-associated protein B (snRNP-B)	mRNA processing
O35821 [t]	Myb-binding protein 1A (PAR-interacting protein) (PIP)	nucleocytoplasmic transport, transcription (DNA-dependent)
Q6LED0	Histone H3.1	nucleosome assembly
Q00715	Histone H2B type 1*	nucleosome assembly
Q6P747 [t, PB]	Heterochromatin protein 1-binding protein 3	nucleosome assembly
P62914	60S ribosomal protein L11	protein localization to nucleus, translation
P07895	Superoxide dismutase [Mn], mitochondrial	removal of superoxide radicals
Q6AYB5 [t, PB]	Signal recognition particle 54 kDa protein	SRP-dependent cotranslational protein targeting to membrane
Q6P7R8 [t, PB]	Estradiol 17-beta-dehydrogenase 12	steroid biosynthetic process
Q63396 [t, PB]	Activated RNA polymerase II transcriptional coactivator p15	transcription, DNA-dependent

Q6PDV7	60S ribosomal protein L10	translation
P05765	40S ribosomal protein S21	translation
P24050 [t]	40S ribosomal protein S5	translation
Q71TY3 [t]	40S ribosomal protein S27	translation
P43244	Matrin-3 (Nuclear scaffold protein p130/MAT3)	chromatin organisation

Non-parenchymal cells / nuclear extract protein fraction		
Accession number	Protein name	GO - biological process
P41516 [t]	DNA topoisomerase 2-alpha	DNA topological change, response to drug
O08629	Transcription intermediary factor 1-beta (TIF1-beta) (epithelial to mesenchymal transition)	epithelial to mesenchymal transition, positive regulation of transcription (DNA-dependent)
Q68FY1	Nucleoporin NUP53	mRNA & protein transport
Q6AY87 [t]	THO complex subunit 6 homolog (WD repeat-containing protein 58)	mRNA processing
Q4V898	RNA-binding motif protein, X chromosome	mRNA splice site selection, positive regulation of transcription (DNA-dependent)
Q00566	Methyl-CpG-binding protein 2 (MeCp-2 protein)	negative regulation of transcription from RNA polymerase II promoter, transcription (DNA-dependent)
Q9Z2Y1	Protein timeless homolog (rTIM)	positive regulation of circadian rhythm, response to DNA damage stimulus
Q9JIL3	Interleukin enhancer-binding factor 3	protein methylation, transcription (DNA-dependent)

** Exact isoform could not be distinguished with our resources*

[t] – Evidence at transcript level only, [PB] –found only in PB treated rats

Table 4. ***Selected proteins found down-regulated in HCs isolated from rat livers, when treated with PB*** **in vivo.** *These down-regulated proteins of interest were selected out of 389 proteins. These proteins were found exclusively in the livers of untreated rats and had to fulfill the criteria of being represented in at least 50% of the experiments with at least 2 peptides.*

Accession number	Protein name	GO - biological process
Secreted protein fraction		
Q2TL32 [t]	E3 ubiquitin-protein ligase UBR4 (N-recognin-4) (Zinc finger UBR1-type protein 1)	Ubl conjugation pathway; together with clathrin, forms meshwork structures involved in membrane morphogenesis and cytoskeletal organization
Cytoplasmic protein fraction		
P08516	Cytochrome P450 4A10 (CYPIVA10) (Cytochrome P450-LA-omega 1) (Cytochrome P452)	arachidonic acid metabolic process
P09606	Glutamine synthetase (GS) (Glutamate--ammonia ligase) (Glutamate decarboxylase)	ammonia assimilation cycle, glutamine biosynthetic process
P04182	Ornithine aminotransferase, mitochondrial (Ornithine--oxo-acid aminotransferase)	L-proline biosynthetic process
Q5PPL3 [t]	Sterol-4-alpha-carboxylate 3-dehydrogenase, decarboxylating	Cholesterol biosynthesis, Steroid biosynthesis
Nuclear extract protein fraction		
Q9ES53	Ubiquitin fusion degradation protein 1 homolog (UB fusion protein 1)	proteasomal ubiquitin-dependent protein catabolic process

[t] – *Evidence at transcript level only,*

Table 5. ***Selected proteins found down-regulated in NPCs isolated from rat livers, when treated with PB*** **in vivo.** *These down-regulated proteins of interest were selected out of 484 proteins. These proteins were found exclusively in the livers of untreated rats and had to fulfill the criteria of being represented in at least 50% of the experiments with at least 2 peptides.*

Accession number	Protein name	GO - biological process
Secreted protein fraction		
Q5XI22	Acetyl-CoA acetyltransferase, cytosolic (Cytosolic acetoacetyl-CoA thiolase)	liver development, cellular response to nutrient
P52844 [t]	Estrogen sulfotransferase, isoform 1 (EST-1)	estrogen metabolic process
P09606	Glutamine synthetase (GS) (Glutamate--ammonia ligase) (Glutamate decarboxylase)	ammonia assimilation cycle, glutamine biosynthetic process
P14095	Growth-regulated alpha protein (C-X-C motif chemokine 1) (Cytokine-induced neutrophil chemoattractant 1) (CINC-1)	acute inflammatory response, immune response
P10868	Guanidinoacetate N-methyltransferase	S-adenosylhomocysteine metabolic process
P04176	Phenylalanine-4-hydroxylase (PAH) (Phe-4-monooxygenase)	L-phenylalanine metabolic process
Cytoplasmic protein fraction		
Q5DWV2 [t]	Cadherin-7	homophilic cell adhesion
O35796	Complement component 1 Q subcomponent-binding protein, mitochondrial (Glycoprotein gC1qBP)	negative regulation of interferon-gamma & interleukin-12 production, positive regulation of apoptotic process
Q64611	Cysteine sulfinic acid decarboxylase (Sulfinoalanine decarboxylase)	carboxylic acid metabolic process
P10818	Cytochrome c oxidase subunit 6A1, mitochondrial (Cytochrome c oxidase polypeptide VIa-liver)	mitochondrial respiratory chain complex IV
P08683	Cytochrome P450 2C11 (CYPIIC11) (Cytochrome P-450(M-1))	xenobiotic metabolic process
P63095	Guanine nucleotide-binding protein G(s) subunit alpha isoforms short	adenylate cyclase-activating dopamine receptor signaling pathway
P14659 [t]	Heat shock-related 70 kDa protein 2 (Heat shock protein 70.2)	multicellular organismal development, response to stress
P27881	Hexokinase-2 (Hexokinase type II) (HK II)	cellular glucose homeostasis, apoptotic mitochondrial changes
Q920F3	KH domain-containing, RNA-binding, signal transduction-associated protein 2 (SLM-1)	regulation of transcription, DNA-dependent
O08816	Neural Wiskott-Aldrich syndrome protein (N-WASP)	actin polymerization or depolymerization

P04182	Ornithine aminotransferase, mitochondrial (Ornithine--oxo-acid aminotransferase)	L-proline biosynthetic process
P22062	Protein-L-isoaspartate(D-aspartate) O-methyltransferase (PIMT)	S-adenosylhomocysteine metabolic process
P12928 [t]	Pyruvate kinase isozymes R/L (L-PK)	ATP biosynthetic process
Q53B90 [t]	Ras-related protein Rab-43	protein transport
Q6BBI8 [t]	Ubiquitin-fold modifier-conjugating enzyme 1 (Ufm1-conjugating enzyme 1)	protein ufmylation
Nuclear extract protein fraction		
P21531	60S ribosomal protein L3 (L4)	translation
P08753	Guanine nucleotide-binding protein G(k) subunit alpha (G(i) alpha-3)	cell cycle, adenylate cyclase-inhibiting G-protein coupled receptor signaling pathway
P63095	Guanine nucleotide-binding protein G(s) subunit alpha isoforms short (G-alpha-8)	adenylate cyclase-activating dopamine receptor signaling pathway, heterotrimeric G-protein complex
P14659 [t]	Heat shock-related 70 kDa protein 2 (Heat shock protein 70.2)	multicellular organismal development, response to stress
P17955	Nuclear pore glycoprotein p62 (62 kDa nucleoporin) (Nucleoporin Nup62)	cell death, negative regulation of cell proliferation
P62961	Nuclease-sensitive element-binding protein 1 (CCAAT-binding transcription factor I subunit A) (DNA-binding protein B) (EFI-A) (YB-1)	CRD-mediated mRNA stabilization
Q498U4	SAP domain-containing ribonucleoprotein (Nuclear protein Hcc-1)	regulation of transcription, DNA-dependent

[t] – *Evidence at transcript level only,*

Table 6. Events represented by up-regulated proteins found in HCs upon PB treatment of rats. *This indicates effects on molecular events via positively PB-induced proteome alterations.*

name of the event	un-adjusted probability of seeing N or more genes in this event by chance	number of genes in the query which map to this event	total number of genes involved in this event
Metabolism	7.40E-17	62	1033
Gene Expression	3.40E-03	25	654
Metabolism of proteins	1.90E-10	26	283
3' -UTR-mediated translational regulation	4.60E-11	19	134
Signal Recognition (Preprolactin)	1.40E-08	15	112
Signal Recognition (Preproinsulin)	1.60E-08	15	113
DNA Replication	2.80E-01	7	241
Cell Cycle	3.20E-01	11	422
Apoptosis	2.00E-02	9	185
Signal Transduction	1.00E+00	14	1710
Cdc20:Phospho-APC/C mediated degradation of Cyclin A	2.50E-03	7	85
Developmental Biology	9.60E-01	5	418
Immune System	9.30E-01	4	319
Membrane Trafficking	1.70E-02	5	69

Table 7. Events represented by up-regulated proteins found in NPCs upon PB treatment of rats. *This indicates effects on molecular events via positively PB-induced proteome alterations.*

name of the event	un-adjusted probability of seeing N or more genes in this event by chance	number of genes in the query which map to this event	total number of genes involved in this event
Metabolism of proteins	4.90E-08	20	283
Gene Expression	7.90E-03	20	654
3' -UTR-mediated translational regulation	2.80E-06	12	134
Metabolism	1.60E-04	33	1033
Signal Recognition (Preprolactin)	1.30E-04	9	112
Signal Recognition (Preproinsulin)	1.40E-04	9	113
Apoptosis	3.30E-04	11	185
DNA Replication	2.50E-01	6	241
Cell Cycle	3.20E-01	9	422
Signal Transduction	1.00E+00	18	1710
Cdc20:Phospho-APC/C mediated degradation of Cyclin A	3.50E-03	6	85
Developmental Biology	1.90E-01	10	418
Muscle contraction	1.10E-01	3	66
Membrane Trafficking	1.20E-01	3	69
Cell-Cell communication	6.80E-01	2	133

Table 8. ***Molecular pathways represented by up-regulated proteins found in HCs upon PB treatment of rats.*** *PB treatment of rats may exert a perturbation or up-regulation of the pathways, listed in this table, in HCs. Total number of proteins states the number of different proteins present in the pathway in this database. The matching column gives the proteins number of the number of different proteins also found in our data. The last column shows the calculated percentage value of the previous two columns.*

Pathway name	Total number of proteins	Matching proteins in data	% in data
Peroxisomal lipid metabolism	21	4	19%
Class I MHC mediated antigen processing & presentation	16	3	18%
Asparagine N-linked glycosylation	24	3	12%
Bile acid and bile salt metabolism	27	3	11%
Eukaryotic Translation Elongation	109	12	11%
Eukaryotic Translation Initiation	145	16	11%
Eukaryotic Translation Termination	104	12	11%
RAF/MAP kinase cascade	10	1	10%
SRP-dependent cotranslational protein targeting to membrane	129	14	10%
Translation	178	18	10%
Platelet Adhesion to exposed collagen	11	1	9%
Phase II conjugation	67	6	8%
Biological oxidations	149	11	7%
Lipid digestion, mobilization, and transport	26	2	7%
Membrane Trafficking	69	5	7%
Metabolism of proteins	283	21	7%
Processing of Capped Intronless Pre-mRNA	14	1	7%
Metabolism of amino acids and derivatives	194	12	6%
Fatty acid, triacylglycerol, and ketone body metabolism	90	5	5%
Formation of Fibrin Clot (Clotting Cascade)	36	2	5%
Metabolism of non-coding RNA	19	1	5%
Phase 1 - Functionalization of compounds	84	5	5%
Regulation of Apoptosis	79	4	5%
Signaling by Wnt	69	4	5%

Table 9. ***Molecular pathways represented by up-regulated proteins found in HCs upon PB treatment of rats.*** *PB treatment of rats may exert a perturbation or up-regulation of the pathways, listed in this table, in NPCs.*
(description see table 6)

Pathway name	Total number of proteins	Matching proteins in data	% in data
Protein folding	35	5	14%
Dissolution of Fibrin Clot	10	1	10%
Platelet Adhesion to exposed collagen	11	1	9%
Signaling by Wnt	69	6	8%
Asparagine N-linked glycosylation	24	2	8%
Lipid digestion, mobilization, and transport	26	2	7%
Regulation of Apoptosis	79	6	7%
Regulation of DNA replication	87	6	6%
Eukaryotic Translation Termination	104	7	6%
Fatty acid, triacylglycerol, and ketone body metabolism	90	6	6%
Eukaryotic Translation Elongation	109	7	6%
Class I MHC mediated antigen processing & presentation	16	1	6%
Rap1 signalling	16	1	6%
Eukaryotic Translation Initiation	145	9	6%
SRP-dependent cotranslational protein targeting to membrane	129	8	6%
APC/C-mediated degradation of cell cycle proteins	100	6	6%
Regulation of mitotic cell cycle	100	6	6%
Metabolism of nucleotides	69	4	5%
Metabolism of amino acids and derivatives	194	11	5%
Metabolism of proteins	283	16	5%
Translation	178	10	5%
Signal amplification	18	1	5%
Semaphorin interactions	79	4	5%
Synthesis of DNA	120	6	5%

3.2 Proteome signatures of inflammatory activated primary human peripheral blood mononuclear cells

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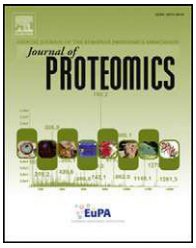

Proteome signatures of inflammatory activated primary human peripheral blood mononuclear cells☆

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ABSTRACT

Proteome profiling is the method of choice to identify marker proteins whose expression may be characteristic for certain diseases. The formation of such marker proteins results from disease-related pathophysiologic processes. In healthy individuals, peripheral blood mononuclear cells (PBMCs) circulate in a quiescent cell state monitoring potential immune-relevant events, but have the competence to respond quickly and efficiently in an inflammatory manner to any invasion of potential pathogens. Activation of these cells is most plausibly accompanied by characteristic proteome alterations. Therefore we investigated untreated and inflammatory activated primary human PBMCs by proteome profiling using a 'top down' 2D-PAGE approach in addition to a 'bottom up' LC–MS/MS-based shotgun approach. Furthermore, we purified primary human T-cells and monocytes and activated them separately. Comparative analysis allowed us to characterize a robust proteome signature including NAMPT and PAI2 which indicates the activation of PBMCs. The T-cell specific inflammation signature included IRF-4, GBP1and the previously uncharacterized translation product of GBP5; the corresponding monocyte signature included PDCD5, IL1RN and IL1B. The involvement of inflammatory activated PBMCs in certain diseases as well as the responsiveness of these cells to anti-inflammatory drugs may be evaluated by quantification of these marker proteins.
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1. Introduction

Peripheral blood mononuclear cells (PBMCs) are very important immune players and therefore involved in a large number of diseases. In healthy individuals, these cells circulate in a quiescent state monitoring potential immune-relevant events. Encountering any kind of disease-related abnormalities may elicit strong inflammatory responses in these cells which are targeted at the elimination of potential pathogens but may also cause severe side effects and malicious symptoms. Inflammatory activation engages several biological functions such as leukocyte migration, proliferation of T-cells, interferon response, NF-κB signaling and regulation of cell death. Proteome profiles characteristic for such functional cell states have not yet been comprehensively investigated in inflammatory activated PBMCs.

Proteomics is a powerful screening technology aiming at the high throughput analysis of complex samples mainly using mass spectrometry [1]. Several studies have been performed in order to analyze proteome profiles of quiescent PBMCs, resulting in the classification of proteins according to functional groups and cellular localization [2] or cell type specificity [3]. PBMCs consist of several different cell types and proteome analyses of these cells result in great complexity of data, especially when including the consideration of different functional states. Additionally, the heterogeneity of biological samples and methodological challenges may obstruct straight interpretation of data [4,5].

Indeed, PBMCs consist of different cell types with lymphocytes and monocytes as main constituents and more than two thirds of the lymphocytes represented by T-cells. Furthermore, each of these cells can occur at different functional states, especially when cells from diseased persons are considered. Activated T-cells may account for intrinsic immune response-related tissue damage characteristic for chronic inflammation, autoimmunity and graft versus host disease [6]. Monocytes act as important regulators of T-cell activities. Upon encounter with a potential pathogen, monocytes become activated resulting in differentiation into macrophages. Macrophages present phagocytized and processed material on their cell surface and thus induce the targeted activation of T-cells. Activated T-cells in turn may further activate endothelial cells, fibroblasts and monocytes/macrophages thus maintaining an acute or chronic inflammatory state [7]. Recently, differential PBMC protein expression was shown to support the diagnosis of ulcerative colitis and Crohn's disease [8] as well as of systemic lupus erythematosus [9] or rheumatoid arthritis [10].

While the role of the immune system for several diseases such as pathogen-related disorders is obvious and well understood, the detailed regulatory mechanisms involved in disorders such as chronic inflammatory diseases, atherosclerosis, Alzheimer and cancer are not yet fully understood and therefore in the focus of current research [11–14]. The proteomic characterization of PBMCs and of functional cell states thereof may help to indicate and assess the contribution of these cells to certain diseases.

While current data interpretation strategies for large-omics data sets rather rely on network analysis [15], we seek a systematic strategy based on the establishment of reference proteome profiles. Especially, we focus on the characterization of proteins specifically expressed in individual cell types at defined functional states. We have already characterized proteins specifically expressed in inflammatory activated as well as tolerogenic primary human dendritic cells [16]. Our research group has also been working with PBMCs focusing on oxidative stress [17] and nutritional intervention [18].

In the present work, in order to generate further reference proteome profiles, we focus on PBMCs which were inflammatory activated *in vitro* with LPS (lipopolysaccharide) and PHA (phytohaemagglutinin) and subsequently analyzed using 2D-PAGE ('top down'), as well as an LC–MS/MS based shotgun approach ('bottom up') [19]. Proteome profiles were recorded and compared to those of control PBMCs using the Griss Proteomics Database Engine (GPDE), a database specifically engineered for the identification and characterization of marker proteins [20]. In order to relate the observed proteome alterations to the corresponding cell type of origin, we isolated and stimulated T-cells (with PHA) and monocytes (with LPS), the two main cellular constituents of PBMCs, separately before subsequent analysis. We thereby successfully identified proteins that are newly expressed or up-regulated both in T-cells and monocytes. Additionally, we identified proteins specifically induced in T-cells and monocytes, respectively, upon activation. Knowledge of these marker proteins may reveal the involvement of inflammatory-activated T-cells or monocytes in biological samples, which may strongly support the interpretation of more complex clinical proteomics data whenever inflammatory activated PBMCs may be involved.

2. Material and methods

2.1. *Blood samples*

PBMCs of six individuals were isolated. From each donation we created four aliquots. Two were used for metabolic labeling (untreated and treated) and subsequently analyzed by 2D-PAGE. The other two aliquots were fractionated and further processed for shotgun analysis. Per group, fractionation resulted in six cytoplasmic fractions. Three of them were analyzed twice, resulting in a total of nine shotgun analyses. Five nuclear extracts were successfully isolated and analyzed, while only two secretomes were successfully analyzed. T-cells and monocytes were isolated from four independent blood donations and the corresponding cytoplasmic aliquots were processed for shotgun analysis. In case of two T-cell preparations, aliquots were treated in two different ways (PHA and ionomycin/PMA, respectively). The corresponding PRIDE-experiments are listed in Supplementary Table 2. We have not generated PRIDE-files for the spots identified in 2D-gels.

2.2. *Isolation and cultivation of PBMCs*

PBMCs were isolated from fresh blood (blood samples from volunteers) of healthy donors with written consent of each donor and the approval of the Austrian Ethics committee (no. 297/2011). For the isolation of PBMCs, 50 ml full blood were diluted with RPMI1640 medium (Gibco Ltd., Paisley, Scotland) and supplemented with 2 mM L-glutamine, 100U/ml penicillin,

100 μg/ml streptomycin (Sigma-Aldrich, St. Louis, MO) and 1000U heparin (EBEWE Pharma, Unterach, Austria). 35 ml of the mixture were then carefully overlaid above Ficoll-Paque (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) and centrifuged at 500 g for 30 min at 14 °C. PBMCs were collected from the interphase and were then either re-seeded in diluted autologous plasma or, if used for subsequent cell purification, washed with RPMI Heparin medium and MACS buffer (PBS 1% Human Serum Albumin (Aventis Behring, Vienna, Austria)/5 mM EDTA (Gibco Ltd., Paisley, Scotland) and counted [21,22].

2.3. Monocyte and T cell separation

T-cells and monocytes were separated by magnetic sorting using the MACS technique (Miltenyi Biotec, Bergisch Gladbach, Germany), including the use of MACS buffer, Streptavidin MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany), CS columns (Miltenyi Biotec) and the VarioMACS separator. T-cells were obtained by negative selection, which was done by depletion of the PBMCs flowtrough from non-T-cells using an antibody mix containing anti-CD14 (MEM 18) for monocytes, anti-CD16 (3G8) for granulocytes and NK-cells, anti-CD19 for B cells (BU 12) and anti-CD33 (4D3) for monocytes, thrombocytes and myeloid progenitors, all at concentrations of 10 μg/ml. For monocytes up to 1×10^9 cells were positively enriched by incubating the PBMCs with 15 μg/ml of biotinylated anti-CD14 (VIM13, MEM 18) to label the monocytes [23].

2.4. FACS analysis of purified T-cells and monocytes

This method was applied to verify the purity of isolated T-cells and monocytes. The cell suspension (5×10^5 cells/assay) was resuspended in 50 μl Beriglobin (CSL Behring) and kept for 10 min on ice. 20 μl of the following PE or FITC-conjugated mouse antibodies were used at a concentration of 20 μg/ml each: VIAP (clone 2D5), CD3 (UCHT1) and CD4 (vit4)/CD8 (Vit8) provided from the Institute of Immunology from Otto Majdic; CD45 (HI30)/CD14 (TüK4), CD3 (S4.1)/SJ25-C1, CD3 (S4.1)/HLA-DR (Tü36) and CD56 (MEM188) from Caltag. Antibodies were prepared in Micronic-tubes and 50 μl of the cell suspension was added, mixed and incubated for 30 min at 4 °C. Dead cells were labeled by addition of ethidiumbromide or propidiumiodide (1:100). The tubes were kept on ice until they were analyzed by flow-cytometry. Purity of monocytes was found to be above 95%, for T-cells above 98% (see Supplementary Fig. 1). In case of monocytes, we did not collect sufficient cell amounts to allow the analysis of nuclear extracts and secreted protein fractions.

2.5. ^{35}S-metabolic labeling for measuring of protein synthesis

After isolation PBMCs were reseeded in diluted plasma of the donor in the presence of ^{35}S-labeled methionine and cysteine (Trans35label, Biomedica, MP Biomedicals) for 6 h at 37 °C. The induction of new protein synthesis which is observed by autoradiography is highest within the first few hours after stimulation [24], therefore here we chose 6 h labeling time.

2.6. Inflammatory stimulation

For activation of PBMCs for 2D-PAGE, cells were resuspended in blood plasma and treated with 1 μg/ml lipopolysaccharide (LPS, Sigma-Aldrich) [25], inducing an immune response in monocytes via the toll-like pathway (Myd88) [26–28], and 5 μg/ml phytohaemagglutinin (PHA, Biochrom) [29], a lectin acting mitogenic in T-cells causing proliferative cell division [30], for 6 h at 37 °C. For shotgun analysis, PBMCs were diluted in RPMI medium to 1×10^6 cells/ml and treated as above with a combination of LPS and PHA (same concentrations) for 24 h in order to provide the cells sufficient time to cumulate newly synthesized proteins [31]. Isolated monocytes or T-cells were treated with 1 μg/ml LPS or 5 μg/ml PHA for 24 h, respectively. Alternatively T-cells were activated by 1 μM ionomycin/100nM PMA (phorbol 12-myristate 13-acetate) for 24 h [32–34].

2.7. Sub-cellular fractionation

The *in vitro* treated cells were grown in culture medium for 16 h and further in serum-free medium for 8 h (together 24 h) to collect the secretome. To minimize unspecific effects on cells due to the *in vitro* culturing conditions we obtained controls either by directly processing isolated PBMCs or after 8 h of incubation time.

2.7.1. Secretome protein isolation

After that period of time the supernatant was sterile-filtered through a 0.2 μm filter and precipitated overnight by addition of ethanol tempered to −20 °C.

2.7.2. Cytoplasmic protein isolation

The isolation of cytoplasmic proteins was performed as described by Gundacker et al. [35]. During all steps samples were kept on ice. Cells were lysed in isotonic lysis buffer (10 mM HEPES/NaOH, pH 7.4, 0.25 M sucrose, 10 mM NaCl, 3 mM $MgCl_2$, 0.5% Triton X-100) supplemented with protease inhibitors (pepstatin, leupeptin and aprotinin, each at 1 μg/ml; 1 mM PMSF) and pressed through a 23 g syringe to induce cell lysis. The cytoplasmic fraction was separated from nuclei by centrifugation at 3500 rpm and 4 °C for 5 min and precipitated overnight by addition of ethanol tempered to −20 °C.

2.7.3. Nuclear fraction protein isolation

The nuclear pellets swelled up in extraction buffer (500 mM NaCl) for 10 min followed by a 1:10 dilution in NP-40 buffer for 15 min, to reduce the NaCl concentration. The nuclear protein fraction was separated from debris by centrifugation at 3500 rpm and 4 °C for 5 min and precipitated overnight by addition of ethanol tempered to −20 °C. Afterwards, all protein samples were dissolved in sample buffer (7.5 M urea, 1.5 M thiourea, 4% CHAPS, 0.05% SDS, 100 mM DDT).

2.8. 2D-PAGE ('top down')

Cytoplasmic proteins were loaded by passive rehydration on IPG strips pH 5–8, 17 cm (BioRad, Hercules, CA) at room temperature. Isoelectric focusing (IEF) was performed in a stepwise fashion (1 h 0–500 V linear; 5 h 500 V; 5 h 500–3500 V

linear; 12 h 3500 V). After IEF, the strips were equilibrated with 100 mM DTT and 2.5% iodacetamide according to the instructions of the manufacturer (BioRad). For SDS-PAGE the Protean II xi electrophoresis system (BioRad) was used. IPG strips were placed on top of 1.5 mm 12% polyacrylamide slab gels and overlaid with 0.5% low-melting agarose with bromophenol blue. After electrophoresis, gels were stained with a 400 nM solution of Ruthenium II tris (bathophenanthroline disulfonate) (RuBPS) as described before [36]. For this purpose the gels were fixated in 50% methanol/7% acetic acid overnight, next day washed two times for 30 min with 20% methanol, then stained for 6 h with 400 nM RuBPS, and destained overnight in 15% methanol/7% acetic acid. Fluorography scanning was again performed with the FluorImager 595 at a resolution of 100 μm [37]. After scanning the fluorescence of the gels, the gels were dried for subsequent autoradiography. Dried gels were inserted into cassettes including a phosphor screen as detector for ß-radiation of the ^{35}S labeled proteins. These phosphor screens were scanned with the PhosphorImager SI MAC (Molecular Dynamics) with 100 microns. Gels were warped to a reference gel with the TT900 S2S software (version 2006.0.2389, Nonlinear dynamics, Carlsbad, CA) and evaluated with the Progenesis software PG200 (version 2006, Nonlinear) using the "same spot" algorithm. Only protein spots which displayed a more than two-fold increase on average of the corresponding normalized integrated spot intensity were considered as differently regulated and were further analyzed by mass spectrometry.

2.9. *1D-PAGE for subsequent shotgun analysis ('bottom up')*

Protein fractions (supernatant, cytoplasm and nuclear extracts) were loaded on 12% polyacrylamid gels, electrophoresis was performed until complete separation of a pre-stained molecular marker (Dual Color, Biorad, Hercules, CA) was visible. After fixation with 50% methanol/10% acetic acid and subsequent silver staining, gel lanes were cut out of the gel and digested with trypsin as described below.

2.10. *MS-compatible silver staining procedure*

SDS-PAGE gels were fixed with 50% methanol, washed and sensitized with 0.02% $Na_2S_2O_3$. The gels were stained with 0.1% $AgNO_3$ ice cold for 20 min, rinsed with bi-distilled water and subsequently developed with 3% Na_2CO_3/0.05% formaldehyde as previously described [38].

2.11. *Protein digestion with trypsin*

Spots were cut out from 2D-gels. SDS-gels were cut into slices. After destaining, reduction with DTT and alkylation with iodacetamide, proteins were digested with trypsin (sequencing grade, Roche) at 37 °C overnight as described before [39]. After elution, the peptides were forwarded to LC–MS/MS analysis.

2.12. *Mass spectrometry analysis*

For the identification of isolated 2D spots, the corresponding peptides were loaded on a Zorbax 300SB-C8 (5 μm, 0.3 mm, 5 mm) column and separated by nanoflow LC (1100 Series LC system, Agilent, Palo Alto, CA) with a Zorbax 300SB-C18 (5 μm, 75 mm) column at a flow rate of 250 nl/min using a gradient from 0.2% formic acid and 3% acetonitrile (ACN) to 0.2% formic acid and 45% ACN over 12 min. In case of shotgun analysis, peptides were separated by nanoflow LC (1100 Series LC system, Agilent, Palo Alto, CA) using the HPLC-Chip technology (Agilent) equipped with a 40 nl Zorbax 300SB-C18 trapping column and a 75 μm × 150 mm Zorbax 300SB-C18 separation column at a flow rate of 400 nl/min, using a gradient from 0.2% formic acid and 3% ACN to 0.2% formic acid and 50% ACN for over 60 min. Peptide identification was accomplished by MS/MS fragmentation analysis with an iontrap mass spectrometer (XCT-Ultra, Agilent) equipped with an orthogonal nanospray ion source. The MS/MS data analysis, including peak list-generation and spectrum identification, was done using the Spectrum Mill MS Proteomics Workbench software (Version A.03.03, Agilent) allowing for two missed cleavages and searched against the SwissProt/UniProtKB protein database for human proteins (Version 12/2010 containing 20,328 entries) allowing for precursor mass deviation of 1.5 Da, a product mass tolerance of 0.7 Da and a minimum matched peak intensity (%SPI) of 70%. Due to previous chemical modification, carbamidomethylation of cysteines was set as fixed modification. Oxidation of methionine was the only post-translational modifications considered here. The apparent positive matches found within the search results for peptides having a SpectrumMill peptide score higher than 13 when using the corresponding reversed database compared to the true database were consistenly less than 1% (documented in the PRIDE XML files). Peptides scoring between 9 and 13 were included only if precursor m/z value, retention time and MS2 pattern were found similarly in at least one of our previous experiments and the peptide was thereby scoring above 13. With respect to protein inference, we chose the smallest number of proteins required to explain all observed peptides as described for ProteinProphet [40]. As our protein identification algorithm includes manual selection, we cannot calculate an exact false discovery rate. All identification details including MS2 spectra are fully documented in the PRIDE-XML files available at www.ebi.ac.uk/pride (experiments 22162–22200, 26890–26904, Table S2).

2.13. *Data evaluation of shotgun analyses*

The PRIDE-XML files were loaded into a local version of the GPDE, the software can be downloaded freely from http://sourceforge.net/projects/gpde/. For uploading the files, the parameters "species", "tissue", "cell type" and "cell state" as well as "sub-cellular fraction" were set accordingly. Data replicates become assembled as described [20], the emPAI (exponentially modified protein abundance index) values were calculated according to Ishihama et al. [41] using the "Data Analysis" tool. The cell symbols were obtained using the "Protein Expression" tool. In Figs. 3 and 4, snapshots of the database output screens are shown.

3. Results

3.1. *2D-PAGE of control and LPS/PHA-treated PBMCs*

Primary cells present a significant challenge to proteome research because of their intrinsic heterogeneity, instability

and sensitivity to any environmental alteration. Therefore, we applied strict standard operating procedures to minimize the differences between the working procedures. PBMCs from six individual donors were isolated under sterile conditions, transferred back into plasma of the corresponding donor and metabolically labeled for 6 h by the addition of ^{35}S-methionine/cysteine. One aliquot was treated with LPS, an activator of monocytes, and PHA, an activator of T-cells (see Section 2). 2D-PAGE separation of cytoplasmic proteins, fluorescence detection and subsequent autoradiography allowed us to record a marked increase in protein synthesis in the stimulated cells (Fig. 1). The comparison of the activated cells with the untreated controls considering both fluorescence and autoradiographic protein detection identified several proteins to be specifically induced as exemplified in more detail for IFIT-2 (Fig. 2). Selected protein spots of corresponding unlabeled cell preparations were excised, digested with trypsin and analyzed by mass spectrometry. 14 proteins displayed a more than two-fold increase on average of the corresponding normalized integrated spot intensity and were identified using MS as indicated in Fig. 1. Although the autoradiographic spot patterns of the individual donors showed some variations, the inflammation-induced alterations were of very high conformity (Fig. S1).

3.2. *Shotgun analysis of control and LPS/PHA-treated PBMCs*

Additionally, unlabeled cell aliquots of similar experiments were analyzed using shotgun proteomics. Eight hours after treatment, when inflammation-induced protein synthesis was apparently up-regulated most significantly (Fig. 2), shotgun analysis did not reveal several of the alterations observed in the 2D gels (data not shown). However, after 24 h of treatment, most of the proteins found induced by means of 2D-PAGE were as well identified by shotgun proteomics. Shotgun proteomics requires some threshold protein amounts which may require the accumulation of several hours of successful protein synthesis. As specifically detecting protein synthesis, autoradiography may therefore provide more contrasting results than determination of protein amounts (Fig. 2). Furthermore, we observed that *in vitro* culturing of PBMCs for several hours without treatment was sufficient to induce some inflammatory activation of cells. The immune cells may easily recognize the environmental change accompanying blood processing and respond to it. Consequently, we processed untreated cells directly after isolation. Then, cytoplasmic, nuclear and secreted protein fractions were separated by SDS-PAGE, digested with trypsin and analyzed by mass spectrometry as described previously [3]. For data analysis only proteins identified with at least two distinct peptides and in at least two independent experiments were considered. Based on these conditions, 1496 proteins were identified in the untreated cells and 1497 proteins in the inflammatory activated cells. 1424 proteins of these were common to both groups (Supplementary Data Table S1). All identification details including MS2 spectra are fully documented in the PRIDE-XML files available at www.ebi.ac.uk/pride (experiments 22162–22200, 26890–26904, Table S2).

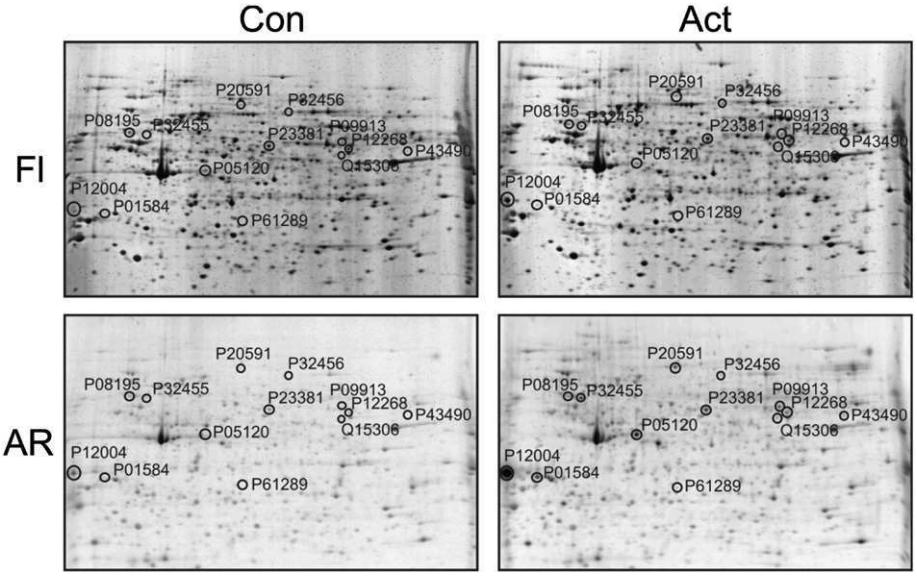

Fig. 1 – Comparison of the cytoplasmic protein fractions from untreated and inflammatory activated PBMCs by fluorescence detection and autoradiography from 2D gels. Cytoplasmic proteins of untreated PBMCs (Con) and, by LPS and PHA, inflammatory activated PBMCs (Act) were separated by 2D-PAGE, stained with the fluorescence dye RuBPS, dried and exposed to phosphor-screens. The first row (Fl) features the fluorescence images, while the second row shows the autoradiography (AR) images. The fluorescence images provide qualitative and quantitative information about the overall protein composition of the PBMCs. The autoradiographs display proteins newly synthesized during the labeling period. UniProtKB/SwissProt accession numbers indicate proteins which were identified as specifically induced or up-regulated upon inflammatory activation.

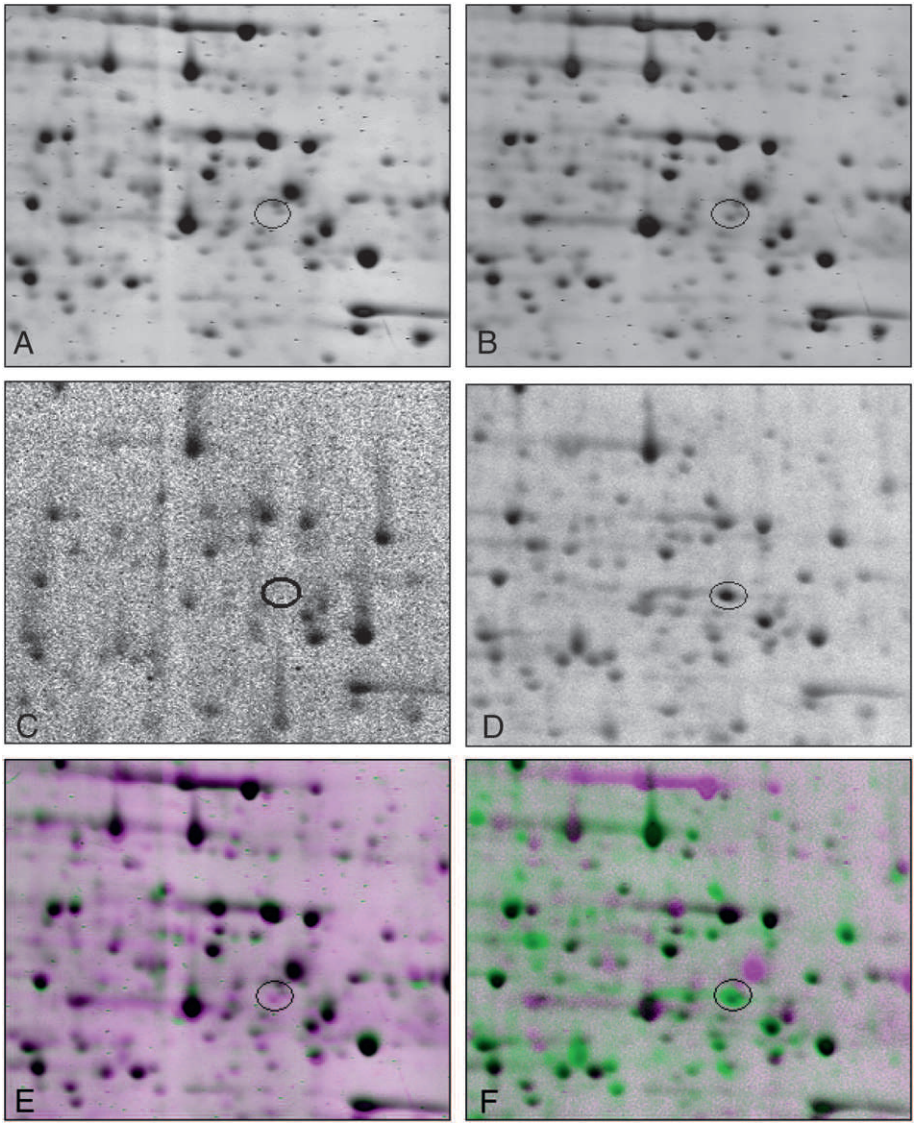

Fig. 2 – Evidence for induced protein expression by 2D-PAGE. The fluorescence pattern of inflammatory activated PBMCs (B) of a representative donor was very similar to the corresponding control (A). The newly detectable spot was identified as IFIT-2 (P09913). The corresponding autoradiograph demonstrated very high ^{35}S incorporation of this protein (D) and absence of detection in the control (C). Image overlays of fluorescence detection of activated (magenta) with the control (green) demonstrate the specificity of this protein expression (E). Image overlay of autoradiography of the activated sample (green) with the corresponding fluorescence detection (magenta) demonstrates the very high labeling of the otherwise hardly detectable spot (F).

3.3. *2D-PAGE in combination with results from shotgun analysis*

18 cytoplasmic and 6 nuclear proteins were considered as specifically expressed upon activation as they were reliably identified by shotgun analysis with three or more distinct peptides in at least three out of the six donors and were not detected in the controls. The 18 cytoplasmic proteins including corresponding gene-ontology terms [42] are listed in Table 1. 13 of these proteins were confirmed to be induced using 2D autoradiographs (Table 1), and only one protein (IFIT2) was found using 2D-PAGE and remained undetectable using shotgun analysis (Fig. 1). One of the newly induced proteins was identified as guanylate-binding protein 5, a UniProtKB entry listed only with "evidenced at transcriptional level". The corresponding mass spectrometry data are summarized in Fig. 3 and clearly evidence the expression of this protein in activated PBMCs. The specifically induced 6 nuclear proteins were MCM7 (P33993), SATB1 (Q01826), OAS2 (P29728), G3BP1 (Q13283), EIF2AK2 (P19525) and MCM5 (P33992).

To obtain a rough estimate of relative protein abundances, we calculated the average emPAI values for all proteins determined in inflammatory activated PBMCs, over all biological replicates (Table S1). 10 cytoplasmic and 4 nuclear proteins consistently displayed a three-fold or higher increase of the average emPAI (Table 2) compared to controls.

Table 1 – Grouping of proteins which were specifically expressed in inflammatory activated PBMCs according to their expression in purified and activated T-cells and monocytes. All proteins listed here were found specifically expressed in activated PBMCs with three or more distinct peptides in at least three out of six donors and were not detected in the controls. T-cells and monocytes, the main constituents of PBMCs were purified, activated and analyzed separately. The induced proteins were compared to those observed in activated PBMCs. Proteins identified in both T-cells and monocytes, in only one cell type or in none were assembled into groups. Proteins independently observed to be up-regulated when using 2D-PAGE are indicated by "x" in the column "2D". A selected "biological process" GO term is listed for each protein.

		2D	GO - biological processes
I) Proteins induced in both activated T-cells and monocytes			
P08195	4F2 cell-surface antigen heavy chain	x	Leukocyte migration, cell growth
P05120	Plasminogen activator inhibitor 2 (PAI-2)	x	Anti-apoptosis
P43490	Nicotinamide phosphoribosyltransferase	x	Positive regulation of cell proliferation
P61289	Proteasome activator complex subunit 3	x	Regulation of apoptotic process
II) Proteins induced in activated T-cells only			
Q00653	Nuclear factor NF-kappa-B p100 subunit		Proliferation
Q15306	Interferon regulatory factor 4 (IRF-4)	x	T cell activation
P32456	Interferon-induced guanylate-binding protein 2	x	Interferon-gamma-mediated signaling pathway
P12004	Proliferating cell nuclear antigen (PCNA)	x	proliferation
P12268	Inosine-5′-monophosphate dehydrogenase 2	x	GMP biosynthetic process
P80217	Interferon-induced 35 kDa protein (IFP 35)	x	Type I interferon-mediated signaling pathway
Q99873	Protein arginine N-methyltransferase 1		Cell surface receptor linked signaling pathway
Q96PP8	Guanylate-binding protein 5	x	GTP binding
P32455	Interferon-induced guanylate-binding protein 1		Interferon-gamma-mediated signaling pathway
III) Proteins induced in activated monocytes only			
O14737	Programmed cell death protein 5		Apoptotic process
P01584	Interleukin-1 beta	x	Positive regulation of T cell proliferation
P18510	Interleukin-1 receptor antagonist protein (IL-1ra)	x	Immune response
IV) Proteins induced in activated PBMCs, but not isolated cells			
P20591	Interferon-induced GTP-binding protein Mx1	x	Type I interferon-mediated signaling pathway
P14902	Indoleamine 2,3-dioxygenase 1 (IDO-1)		Tryptophan catabolic process

3.4. *Shotgun analysis of isolated T-cells and monocytes*

T-cells and monocytes are well known for their distinct functions during inflammation. We therefore tried to relate the activation-induced proteome alterations to the corresponding cell type of origin. T-cells and monocytes were isolated from the PBMC cell mixtures of four individual donors, characterized with respect to cell purity by FACS analysis (Fig. S2), and treated with PHA and LPS for 24 h, respectively. Two T-cell aliquots were treated with Ionomycin/PMA for 24 h as an alternative to activation with PHA, the results were largely similar to those obtained with PHA. 16 of the 18 proteins observed to be induced in activated PBMCs were again identified in these purified and activated cell populations; 4 of them in both, purified and activated T-cells and monocytes, 9 proteins were identified in activated T-cells, 3 proteins in activated monocytes, while 2 proteins, IDO-1 and MX1, remained undetectable in the isolated cells (Table 1). The latter two proteins were thus found induced in the natural cell mixture only, but not in the purified cell populations. IDO1 has been described to be induced in human macrophages and monocyte-derived dendritic cells upon interaction with T-cells [43,44]. MX1 was identified in all 2D-gels and almost all shotgun results from activated PBMCs and also in mature dendritic cells investigated by LC–MS/MS in a previous study [16]. The absence of MX1 expression in the LPS-treated monocytes, which was independently reproduced in the 2D gels thereof (data not shown) may indicate that the isolated cells did not gain the full inflammatory activation state. The full inflammation state was therefore only obtained when the natural cell mixture of PBMCs was present during activation. We interpret this finding as indication for cell cooperation between different PBMCs.

Remarkably, no protein was found induced in the purified cell populations, which has not been identified in the activated PBMCs. These observations lead us to the classification of these 18 proteins into four groups, which we consider as functional signatures (Table 1); group I: signature for activated leukocytes; group II: signature for activated T-cells; group III: signature for activated monocytes; and group IV: signature for the cooperation of activated T-cells and monocytes.

3.5. *Data interpretation*

In this study we used a sub-cellular fractionation approach which was shown to increase the experimental reproducibility of proteome profiles [35]. Additionally, it supports cross-comparisons of protein expression patterns in specific sub-cellular compartments. We have extended our in-house developed data analysis platform, the GPDE [20], to visualize the average protein abundance (calculated by using the average emPAI of biological replicates) between the different cell types. These abundance values are represented as colored cell symbols for a given selection of proteins and cell types. The average emPAI value is translated into a color code with intensities

Overview

Coverage	Fractions	Identified in
24.06	secreted, cytoplasm, nuclei	-

Scores

Agilent Spectrum Mill Score: 461

Sequence

MALEIHMSDPMCLIENFNEQLKVNQEALEILSAITQPVVVVAIVGLYRTGKSYLMNKLAGKNKGFSVASTVQSHTKGIWI
WCVPHPNWPNHTLVLLDTEGLGDVEKADNKNDIQIFALALLLSSTFVYNTVNKIDQGAIDLLHNVTELTDLLKARNSPDL
DRVEDPADSASFFPDLVWTLRDFCLGLEIDGQLVTPDEYLENSLRPKQGSDQRVQNFNLPRLCIQKFFPKKKCFIFDLPA
HQKKLAQLETLPDDELEPEFVQQVTEFCSYIFSHSMTKTLPGGIMVNGSRLKNLVLTYVNAISSGDLPCIENAVLALAQR
ENSAAVQKAIAHYDQQMGQKVQLPMETLQELLDLHRTSEREAIEVFMKNSFKDVDQSFQKELETLLDAKQNDICKRNLEA
SSDYCSALLKDIFGPLEEAVKQGIYSKPGGHNLFIQKTEELKAKYYREPRKGIQAEEVLQKYLKSKESVSHAILQTDQAL
TETEKKKKEAQVKAEAEKAEAQRLAAIQRQNEQMMQERERLHQEQVRQMEIAKQNWLAEQQKMQEQQMQEQAAQLSTTFQ
AQNRSLLSELQHAQRIVNNDDPCVLL

Peptides

Sequence	Unique	Id. count	Start	End	Score	Fraction
IDQGAIDLLHNVTELTDLLK	✔		134	153	20.23	cytoplasm
NLVLTYVNAISSGDLPCIENAVLALAQR	✔		293	320	24	cytoplasm
NSPDLDRVEDPADSASFFPDLVWTLR	✔		156	181	18.6	cytoplasm
NLEASSDYCSALLK	✔		397	410	14.4	cytoplasm
SLLSELQHAQR	✔		565	575	10.47	cytoplasm
VNQEALEILSAITQPVVVVAIVGLYR	✔		23	48	15.11	cytoplasm
VQLPMETLQELLDLHR	✔		341	356	12.68	cytoplasm

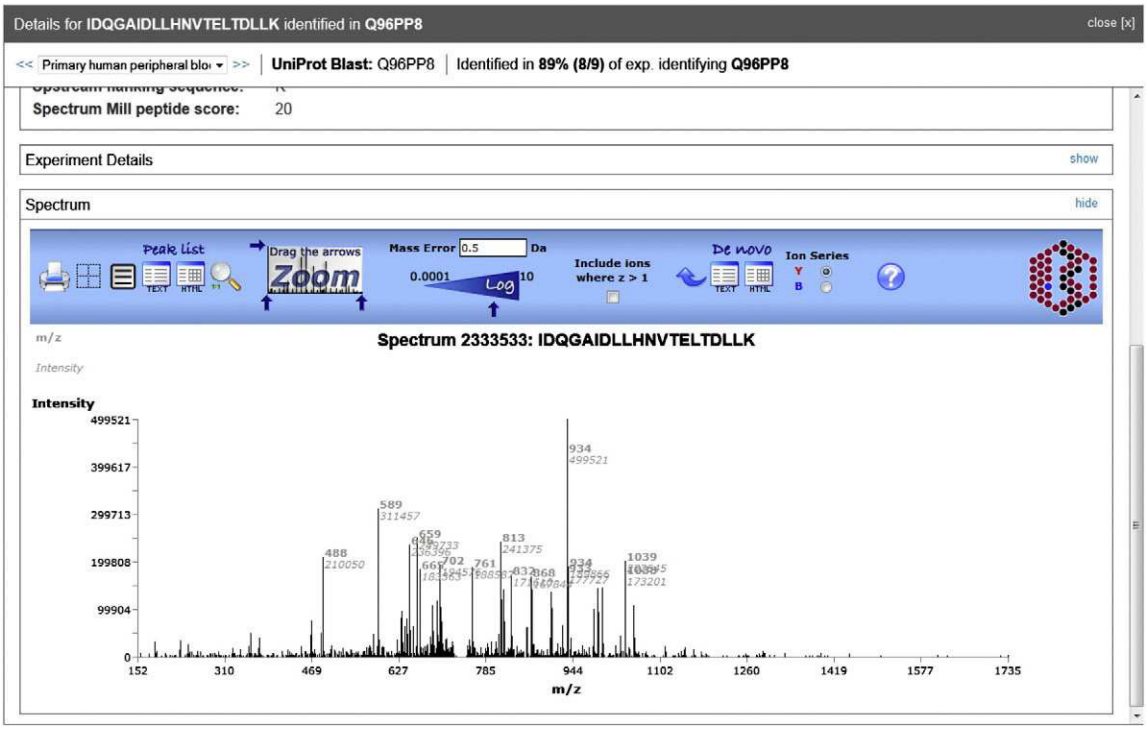

Fig. 3 – Identification of guanylate binding protein 5 (Uniprot Q96PP8). Readouts of the GPDE are shown and suggest safe identification of the indicated protein. Within the amino acid sequence of the protein, the identified peptides are underlined, summing up to sequence coverage of 24.06%. The identified sequences of all present experiments are listed indicating whether the peptide sequence is unique within the human proteome and the number of identifications within the set of data (first peptide: identified in 8 out of nine experiments identifying Q96PP8) as well as amino acid positions within protein sequence, scores and fractions. Below a single MS2 spectrum of the indicated peptide sequence is shown.

corresponding to the found emPAI values. Cells are symbolized by a small inner circle for the nuclear fraction, an outer circle for the cytoplasm and an outer frame for the secretome (Fig. 4).

Using this tool the specificity of functional proteome signatures can easily be visualized. Fig. 4 shows a single representative of each of the above described groups as well

Table 2 – Proteins specifically up-regulated in inflammatory activated PBMCs as demonstrated by shotgun proteomics. The assessment of quantitative alterations was based on the calculation of the emPAI (exponentially modified protein abundance index) value for a protein identification in activated versus control PBMCs. We considered the up-regulation of a protein in activated PBMCs as relevant only if the corresponding average emPAI value exceeded three-fold the average value of the untreated PBMCs and was increased in this way in at least three independent experiments. Pep-con and Pep-act, number of distinct peptides identified in untreated or inflammatory activated PBMCs respectively; exp-con and exp-act, number of experiments with a positive protein identification in control or activated PBMCs compared to the total number of the respective experiments; emPAI-con and emPAI-act, average emPAI value determined for a protein identification in the cytoplasmic or nuclear protein fraction of control and activated PBMCs respectively.

		pep-con	exp-con	emPAI-con	pep-act	exp-act	emPAI-act
Cytoplasmic proteins up-regulated							
P42224	Signal transducer and activator of transcription 1-alpha/beta	2	3/9	0.044	14	6/9	0.208
P49327	Fatty acid synthase	5	3/9	0.038	28	6/9	0.159
P23381	Tryptophanyl-tRNA synthetase, cytoplasmic	4	8/9	0.121	20	8/9	0.458
P61247	40S ribosomal protein S3a	10	4/9	0.146	13	6/9	0.523
Q14152	Eukaryotic translation initiation factor 3 subunit A	15	4/9	0.031	17	5/9	0.11
P46776	60S ribosomal protein L27a	5	5/9	0.179	6	5/9	0.624
P62263	40S ribosomal protein S14	11	8/9	1.347	13	8/9	4.248
P46781	40S ribosomal protein S9	8	4/9	0.181	13	6/9	0.569
P83731	60S ribosomal protein L24 (Ribosomal protein L30)	5	1/9	0.212	5	7/9	0.664
O00571	ATP-dependent RNA helicase DDX3X	9	1/9	0.049	16	5/9	0.149
Nuclear proteins up-regulated							
Q9NR30	Nucleolar RNA helicase 2	6	2/5	0.163	25	5/5	0.777
P10144	Granzyme B	1	2/5	0.145	10	4/5	0.679
Q13765	Nascent polypeptide-associated complex subunit alpha	0	0/5	0	5	5/5	0.648
Q01469	Fatty acid-binding protein, epidermal	0	0/5	0	4	3/5	0.532

as a protein not affected by inflammatory activation. The cytoplasmic part of the cell symbol representing GAPDH (Q96PP8) is of similar intensity in all cell types shown in Fig. 4. GAPDH can therefore be regarded as a baseline similar to a loading control in Western experiments. GAPDH was detected in the secretome of inflammatory activated PBMCs but not in the secretome of untreated controls. This clearly indicates the presence of dead cells which have released cytoplasmic proteins into the supernatant. NAMPT (P43490) is shown as a representative of group I: it is undetectable in the untreated PBMCs, T-cells and monocytes but positively identified in all inflammatory activated cells. PCNA (P12004) is shown as a representative of group II: it is up-regulated in activated T-cells but not identified in monocytes and dendritic cells. Remarkably, PCNA was detected in the nuclear fraction of untreated PBMCs which is compatible with the known sub-cellular location of this cyclin [45]. However, the newly induced synthesis of this protein upon induction of cell proliferation needs to take place in the cytoplasm. Indeed, PCNA was detected in the cytoplasmic protein fraction of activated PBMCs. IL-1beta (P01584), representative of group III, was found to be induced in monocytes but not in T-cells. IL-1beta was previously described to be up-regulated in LPS-activated monocytes [46]. IDO1 (P14902) was identified in activated PBMCs only, thus representing group IV.

4. Discussion

The results of most proteome experiments are long protein lists which may be difficult to interpret. To support biological data interpretation we already classified PBMC-derived proteins according to their cellular origin [3]. We identified proteins specifically occurring in single cell types as well as proteins shared between two or more cell types, and proteins common to many cell types [39]. Analyzing a complex biological sample and identifying therein proteins known to be selectively expressed may thus identify the corresponding cell type in the sample. Proteome signatures specific for cell types in a defined functional state could further support interpretation of complex data. In this study we therefore present reference proteome profiles of inflammatory activated white blood cells, including cell type-specific inflammation signatures.

PBMCs are the immediate players of inflammatory responses and mainly consist of lymphocytes and monocytes [47–49]. B-cells are producers of specific antibodies, while T-cells are important regulators as well as effectors of inflammatory processes. Monocytes are the most important partners of lymphocytes with a complex repertoire of cellular functions comprising phagocytosis, presentation of antigens and paracrine regulation of inflammation. In addition to the analysis of the bulk PBMC mixture we have included the selective analysis of the two most abundant constituents, i.e. purified T-cells and monocytes. B-cells and other leukocyte subtypes which are present in relatively much smaller amounts were not considered here.

For a robust and reliable assessment of function-related proteome alterations we applied two different analysis strategies, a ‘top down’ and ‘bottom up’ approach, in parallel [19]. 2D-PAGE is a well-established ‘top town’ technology providing accurate quantitative protein expression patterns. However, 2D-PAGE is limited with respect to the number of proteins accessible for quantification and a rather lab intensive technique which is hard to automatize [50]. However, by the application of metabolic labeling, a very sensitive measure for the induction of protein synthesis was achieved (Fig. 2). In order

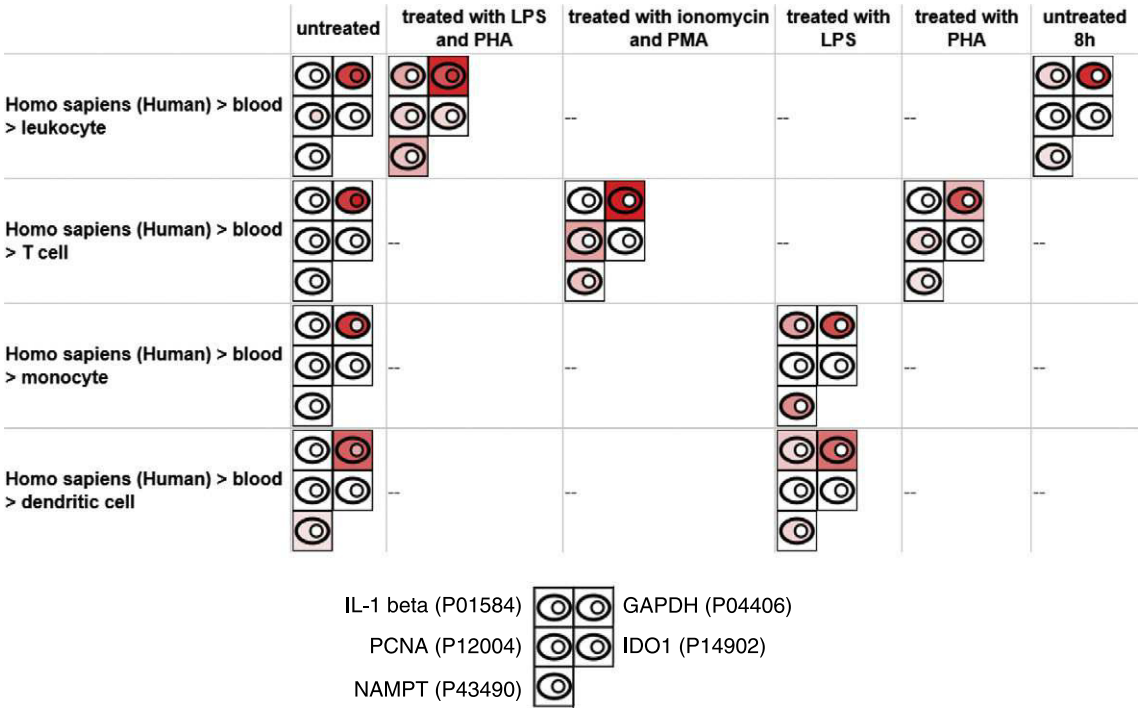

Fig. 4 – Comparative analysis of protein expression across different cell types and functional cell states. Each cell symbol represents the protein expression of a single protein for a single cell type. Average emPAI values were calculated, increased color intensities correspond to increased emPAI values. All positive identifications were reproduced in at least three different donors, white fields indicate negative finding in six donors. The inner circle represents identification in the nuclear extracts, the outer circle in the cytoplasm and the outer frame in the secreted protein fraction. Five proteins were selected: 1, interleukin-1 beta (P01584); 2, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (P04406); 3, proliferating cell nuclear antigen (P12004); 4, indoleamine 2,3-dioxygenase 1 (IDO-1) (P14902); 5, Nicotinamide phosphoribosyltransferase (NAmPRTase) (P43490). GAPDH may serve as a kind of loading control, the emPAI in the cytoplasm was very similar in all cells presented here. NAmPRTase was found upregulated in T-cells as well as monocytes and monocyte-derived dendritic cells, thus representing a member of the first group of the proteome signature. PCNA was strongly induced in T-cells only, thus identifying activated T-cells (second group). IL-1beta, representative for group 3 was already detectable in untreated PBMCs cultured for eight hours and strongly induced in LPS-treated monocytes and dendritic cells but not in T-cells. IDO-1 was strongly induced in PBMCs (leukocytes) treated with LPS and PHA, but not in activated T-cells, monocytes and dendritic cells. The ladder observation may indicate characteristic cooperation upon inflammatory activation between the cells.

to identify more proteins and enable database-supported data interpretation, we complemented this approach with the 'bottom up' shotgun approach, our second analysis strategy.

Table 1 presents the newly synthesized proteins identified upon inflammatory activation of PBMCs using LPS and PHA. Corresponding gene ontology terms are also listed in order to give insights into known biological functions of the proteins. As can be seen, all induced proteins relate to known consequences of inflammatory activation such as leukocyte migration (4F2 cell-surface antigen heavy chain) [51], proliferation of T-cells (PCNA) [52], regulation of cell death (Proteasome activator complex subunit 3, Programmed cell death protein 5) [53,54], NF-κB signaling (NF-kappa-B p100 subunit) [55] and interferon response (interferon regulatory factor 4, interferon-induced guanylate-binding protein 2, interferon-induced 35 kDa protein, interferon-induced GTP-binding protein Mx1) [56–59]. As T-cells and monocytes have different biological tasks it is not surprising that these cells also display different responses to activation. T-cells induced proteins related to the induction of proliferation (PCNA) and several interferon-responsive proteins. Monocytes, which have no capability to proliferate, rather expressed proteins regulating cell death (programmed cell death protein 5) as well as proteins acting in a paracrine fashion (IL-1beta). By assembling these proteins according to their expression specificity we obtained inflammatory signatures of T-cells and monocytes. Knowledge of these specifically expressed proteins may reveal the involvement of inflammatory activated T-cells or monocytes in biological samples, which may strongly support the interpretation of complex clinical proteomics data when inflammatory activated PBMCs are involved.

The application of our standard proteome analysis procedure and data processing system enabled us to include previously published data into the present comparative analysis. Such cross-experimental comparisons may further support data validation and interpretation. To give an example: some subtle inflammatory activation of cells was evidenced in the untreated cells kept in culture for 8 h. *In vitro* cultivation without any treatment apparently induced small amounts of IL-1beta and NAMPT, indicating activation of monocytes (Fig. 4). This is the reason why we used directly processed cells as controls as we

were not focusing on the specific effects of the inflammatory agonists but rather on the finally obtained cell states. This observation also demonstrates the great sensitivity of the PBMCs to cell manipulation *ex vivo*. Here we also present a comparison to previously published proteome profiles of primary monocyte-derived dendritic cells (DCs) [16]. While none of the inflammation signature members were identified in the immature DCs, eight members of the inflammation signatures were also identified as up-regulated in the inflammatory activated DCs. Remarkably, three members of group II, the signature of activated T-cells, were identified in activated DCs. This finding may be somewhat unexpected as dendritic cells are close relatives to monocytes. However, it is in line with the previously described observation that DCs express surface markers not found in monocytes but characteristic for T-cells during maturation in the thymus [60]. Considering activated DCs, which are not members of PBMCs, we found, amongst others, interferon lambda-1 and C-X-C motif chemokine 9 [16]. These proteins were not identified in the activated PBMCs and may thus represent members of an inflammatory signature of DCs.

It is our aim to extend these systematic analyses of functional signatures of different cell types eventually resulting in signatures which are highly specific for each single cell type and cell state. Such knowledge would support a fully automated assessment of biological proteome profiles with respect to the presence of certain cells in defined cell states. Such assessments could greatly support the identification or recognition of pathophysiologic pathways in individual samples. A recent paper presenting proteome profiles of human vulvar cancer samples investigated characteristic features of samples derived from patients suffering from early relapse of disease. Interestingly, the proteins correlating with such unfavorable clinical situation are all contained in our lists of proteins which were found induced or up-regulated in inflammatory activated PBMCs (Tables 1, 2). This finding suggests that the invasion of inflammatory activated leukocytes may have been the characteristic feature of tumor tissue derived from these early relapsing patients. Such recognized pathophysiologic events could then be specifically targeted by pharmacologic means. This is exactly what we intended to achieve with our approach: to provide means for researchers to interpret complex data with respect to functional aspects in order to create clear hypotheses which may then become verified subsequently.

5. Outlook

The assessment of cell type-specific activation states out of a proteome profile of a complex clinical sample should support different issues: to recognize involved pathologic mechanisms and to assess individual variations thereof. It may also help to monitor drug effects. It can be assumed that drug-induced down-regulation of inflammation will be accompanied by the down-regulation of members of the inflammatory signatures. Monitoring the expression rate of such proteins may therefore provide novel means in order to assess drug effects and drug efficiency in an individualized fashion.

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4. Material and Methods

This section will cover the material and methods, which were used during the investigations of coffee and mould fungi effects on inflammatory activated PBMCs and which are not already described.

4.1 Investigation of coffee effects

The material and methods used during these investigations are same as described in detail in *manuscript 3.2* and only the study plan will be presented in this context. In short, we drew 48ml of blood from a peripheral vein from four independent sober human probands and isolated the PBMCs subsequently. After the blood sampling the study participants consumed two cups of Nespresso©, which was chosen because of its standardised content. Half an hour later the probands were sampled a second time and the PBMCs were isolated again. The obtained PBMCs were organised into four groups, which were groupings of the two time points, prior and posterior to the coffee consumption, and the untreated or treated *in vitro* setup, whereby a combination of 1µg/ml lipopolysaccharide (LPS, Sigma-Aldrich) and 5µg/ml phytohaemagglutinin (PHA, Biochrom) was used to inflammatory activate the PBMCs. All samples were metabolically labelled with ^{35}S and kept in their respective blood plasma at 37°C for 6 hours. After incubation the cells were lysed and sub-cellular fractionated. The cytoplasmic protein fractions were analysed via 2D SDS-PAGE with two technical replicas, as described in *manuscript 3.2; section 2.8*.

4.2 Investigation of mould fungi effects

As mentioned above, a detailed presentation of this section can be found in *manuscript 3.2*. The study plan was as follows. We drew 48ml of blood from a peripheral vein of one sober human proband, who has also participated in the coffee study, and isolated the PBMCs subsequently. The obtained PBMCs were organised into the following groups:

- untreated
- treated *in vitro* with LPS + PHA
- treated *in vitro* with the extract of Aspergillus niger
- treated *in vitro* with the extract of Penicillium chrysogenum
- treated *in vitro* with the extract of Mucor racemosus
- treated *in vitro* with LPS+PHA & the extract of Mucor racemosus
- treated *in vitro* with LPS+PHA & the extract of Penicillium chrysogenum
- treated *in vitro* with LPS+PHA & the extract of Aspergillus niger

All samples were metabolically labelled with ^{35}S and treated with 1µg/ml lipopolysaccharide (LPS, Sigma-Aldrich), 5µg/ml phytohaemagglutinin (PHA, Biochrom) and/or 20µl (1:5000 dilution of the stock solution) of the respective mould fungi extract. Cells were kept in the respective blood plasma at 37°C for 6 hours. After incubation the cells were lysed and sub-cellular fractionated, whereby we only considered the cytoplasmic protein fractions for this study. The cytoplasmic protein fractions were analysed via 2D SDS-PAGE as described in *manuscript 3.2; section 2.8*.

5. Results & Discussion

In this chapter only unpublished results will be presented and discussed. This comprises the results of the investigations of NAF as well as the effects of coffee and mould fungi extracts on untreated and inflammatory activated PBMCs. The results of the investigation of PB and inflammatory activated PBMCs are described in detail in *manuscript 3.1 and 3.2*.

5.1 Nafenopin – NGC study

Peroxisome proliferators, such as NAF, are known to be inducers of hepatocarcinogenesis in rodent livers, whereby NAF binds to the PPARα as ligand. PPARα is also known as NR1C1, a nuclear receptor localised in the nuclear membrane of hepatocytes, which usually binds steroid- and thyroid-hormones as ligand and upon activation dimerises with RXR in the nucleus and regulates the expression of peroxisomal genes as well as genes involved in the lipid metabolism and growth regulation. Cells use enzymes, such as catalases and peroxisomal oxidases, to cope with oxidative stress (ROS). Furthermore, it is responsible for degrading long-chain fatty acids and alcohols to acetyl coenzyme A. An activation of PPARα via NAF may perturb cell proliferation and apoptosis resulting in a selective clonal expansion of initiated cells.[37] But it is recognised, that NAF may act via other modes of action, which are still unknown. As we have done in our PB investigation, we also considered the NPCs for this investigation, in expectation of a contribution of the mesenchyme.

To investigate probable modes of action of NAF, we analysed the proteome of *in vitro* treated HCs and NPCs isolated from rats. For that purpose NAF was dissolved in dimethyl sulfoxide (DMSO), an organic (polar aprotic) solvent, which is known to have a weak toxic potential on its own and by that affects liver cells in their protein expression pattern. Therefore, we used the proteomes of DMSO treated HCs and NPCs as controls for evaluating NAF effects. We started with analysing the proteomes of DMSO versus NAF *in vitro* treated HCs and NPCs using 2D SDS-PAGE (not shown). Alterations in the protein expression were detected upon NAF treatment, corroborating our experimental setup and we continued with shotgun proteomics. The resulting tables of the alterations in protein expression/abundance in the secretome, cytoplasmic and the nuclear extract protein fractions (only for NPCs) of HCs and NPCs can be reviewed in the *attachment section (p.108)*. As already mentioned in the introduction, we were forced to abandon the completion of our proteome analyses on NAF pre-maturely. Therefore we ended up with

partly limited data sets on basis of which we had to investigate NAF. The data sets of the supernatants of HCs and NPCs are complete with the data of four and three experiments, respectively. We had two data sets for all the cytoplasms, except for the DMSO treated HCs where we only had one data set at hand. We have analysed the nuclear protein fraction of the NPCs only once. To compensate this dilemma, we stringent during evaluation by included only proteins, which were found with at least two peptides, in at least two experiments with a minimum alteration of ±50%. Detailed information about the proteins was taken from the UniProt database, which is also cross-reverenced with Pubmed.

In HCs several proteins were newly induced upon NAF treatment of the cells, such as peroxisomal proteins e.g. 3-ketoacyl-CoA thiolase B (P07871), a protein involved in the fatty acid metabolic process, hydroxysteroid dehydrogenase-like protein 2 (Q4V8F9) and acyl-coenzyme A thioesterase 1 (O88267), which are known to be induced by peroxisome proliferators via the peroxisome proliferator-activated receptors (PPARs) and catalyse the hydrolysis of acyl-coenzyme As (acyl-CoAs) to the free fatty acid and CoA and a potential regulatory capability of these three molecules. Other noteworthy events are the induction of drug metabolizing enzymes such as the liver carboxylesterase 3 (Q63108) and UDP-glucuronosyl-transferase 1-1 (Q64550), which are responsible for the glucuronidation of toxic xenobiotics, such as NAF. Furthermore, several different ribosomal sub-units are induced, glutathione peroxidase 4 (P36970), a mitochondrial protein which acts in response to oxidative stress and apolipoprotein E (P02650) which are involved in the catabolism of lipoproteins. We also found proteins, which were found only in the proteome of DMSO treated HCs, such as the proteasomal proteins proteasome subunit alpha type-2 (P17220) and proteasome subunit beta type-5) (P28075), which are part of the ubiquitin-dependent protein catabolic process. Other proteins are the pyridoxal kinase (O35331), which is involved in the negative regulation of apoptotic process; glutathione S-transferase theta-2 (P30713), a protein involved in the glutathione metabolic process, where it catalyses the inactivation of reactive sulfate esters of carcinogenic arylmethanols[56]; annexin A1 (p35) (P07150), which is involved in hepatocyte differentiation and regulation of cell proliferation and mitochondrial peptide methionine sulfoxide reductase, a protein which acts as a repair enzyme for inactivated proteins via oxidation. 131 proteins were found to be up-regulated by more than 50%, while 82 of these proteins were up-regulated by more than two times, upon NAF treatment in comparison to DMSO treatment. Thereon, 149 proteins were down-regulated by more than 50%, while 96 of this group were down-regulated by a factor ≥2.

As was the case with HCs, we also found noteworthy alterations in the proteome of NPCs. Amongst the newly induced proteins in NAF *in vitro* treated NPCs were the argininosuccinate synthase (P09034), which may control blood pressure and is known to be induced during the acute-phase and during liver development, several t-complex protein 1 subunits (P28480, Q7TPB1, Q6P502, Q68FQ0) acting as chaperons, transthyretin (P02767), which is involved in the thyroid hormone metabolic process, cytoplasmic aconitate hydratase (Q63270), an enzyme being an ion sensor and thereby helps to maintain the cellular iron ion homeostasis, interferon-inducible double stranded RNA-dependent protein kinase activator A (Q4V8C7), which is part of the production of siRNA that are inhibiting the translation and induction of apoptosis and lon protease homolog 2, peroxisomal (Q3MIB4), a serine protease that is known to be induced in response to organic cyclic compounds and thereby may regulate peroxisomal fatty acid beta-oxidation, which is known to be affected by NAF. There were also proteins, whose expressions were suppressed upon NAF *in vitro* treatment. Ubiquitin thioesterase OTUB1 (B2RYG), usually induced in response to DNA damage and DNA repair, DNA-binding protein A (Cold shock domain-containing protein A; Q62764), a protein that seems to act as a negative regulator of translation, cell surface glycoprotein MUC18 (Q9EPF2) which plays a role in cell adhesion and vascular wound healing, C-type lectin domain family 4 member F (P10716), a Kupffer cell specific protein hypothesised to be involved in endocytosis as well as DNA-(apurinic or apyrimidinic site) lyase (APEX nuclease) (P43138), a protein with diverse functions such as DNA repair and it plays a pivotal role in the cellular response to oxidative stress. When it comes to regulation, we found 256 proteins up-regulated, while 319 proteins were down-regulated in their expression.

For the purpose of investigating the molecular biological effects of NAF on the proteome of HCs and NPCs, we used reactome, a peer-reviewed pathway database developed, amongst others, by EMBL-EBI (UniProt database), which enabled us to cross-reference our lists of newly induced, suppressed as well as up- and down-regulated proteins with molecular events and pathways. These results can be reviewed in *table 1* and *2* for the HCs as well as *table 3* and *4* for the NPCs. *Table 1* and *3* give the top 50 affected pathway of the up- and down-regulated HCs and NPCs, while *table 2* and *4* present the top molecular events. The tables give the hypernyms of sub-pathways or molecular events affected by NAF. Within reactome itself, it is possible to go down to the level of information of specific reactions and even further, if the links to the UniProt database are used. As presented in the tables for HCs, metabolism in general, metabolism of

lipids and lipoproteins (peroxisomal; NAF is a hypolipidemic agent) and metabolism of amino acids and proteins are among the most prominent functions altered in HCs. The second important effect of NAF is the alteration of proteins associated with the adaptive and the innate immune system, indicating a paracrine communication or signalling with immune cells such as the liver resident Kupffer cells[8], which is supplemented by the up-regulation of proteins with an antigen presenting function (altered expression of proteins related to the MHC-I and II) and phagocytosis, t-lymphocytes recruited from the nearby blood vessels, b-cells and probably with Stellate cells, which may act as coordinators[14]. The NAF induced drug metabolism and signal transduction was to be expected and the altered protein expression of proteins acting in response to ROS and NOS may be part of NAFs metabolisation, but may also be predecessor reactions for modes of actions of NAF by affecting e.g. the signalling capacity of NOS or enabling a ROS induced DNA damage, depending on the level of ROS.[39] Furthermore, there are effects on the haemostasis and the activation, signalling, aggregation and degranulation of platelets, which may be associated to the release of growth factors (activated platelets) such as insulin-like growth factor 1, which was found up-regulated in the secretome of HCs, and wound healing. There are also numerous alterations in expression of proteins related to translation, protein folding that may be related to stress (chaperones), the Wnt-pathway (cell fate specification, proliferation and cell migration), glucose metabolism, the citric acid cycle (TCA), respiratory electron transport, mitotic cell cycle with its check points and phases, nerve growth factors (NGF) and, very important, apoptosis. In NPC we see the same events and pathways, with some differences in respect to the magnitude, affected by NAF.

To conclude, even at this early time point of NAFs effects on the cells the results from the secretome suggest that cell-cell interactions, autocrine and paracrine, between HCs and NPCs are taking place, or at least were attempted because of our *in vitro* setting, similar to the PB experiments. Co-culture experiments of HCs and NPCs or *in vivo* experiments will improve our knowledge in that direction. Moreover, the alteration of various peroxisomal proteins supports the notion of ROS and NOS in context to the peroxisome proliferation (via PPARα) to be part of the modes of action of NAF. The NAF induced alterations of proteins related to the immune system, growth factors, the metabolism, translation, the Wnt-pathway and apoptosis in combination with the known occurrence of hepatomegaly in rodents indicate the modes of action for a possible tumour induction via DNA damage caused by an increased ROS formation by the peroxisomes, cytotoxic damage when metabolising NAF and/or via immune cells during an acute

inflammatory respond. Because of an altered apoptotic and proliferative protein setting in the cells, a following regenerative growth, favouring initiated HCs, is conceivable.[34,36,57]

Table 1. Top 50 pathways found to be affected by NAF in HCs. The two sub-tables give the lists of pathways which may be a) enhanced or b) repressed, because of the NAF induced alterations in protein expression. The pathway names below are the hypernyms of the affected sub-pathways to allow a better overview. The columns 2 to 4 state the total number of proteins already incorporated into the database, the number of proteins of our data cross-reverenced with pathways of the database and the coverage of the uploaded data.

a) up-regulated in HCs

Pathway name	Total number of proteins	Matching proteins in data	% in data
Metabolism	1519	66	4%
Immune System	990	29	2%
Metabolism of proteins	484	26	5%
Gene Expression	790	26	3%
Signal Transduction	2022	21	1%
Adaptive Immune System	494	19	3%
Cell Cycle	543	19	3%
Metabolism of lipids and lipoproteins	604	19	3%
Cell Cycle, Mitotic	474	16	3%
Hemostasis	518	15	2%
Innate Immune System	550	14	2%
Biological oxidations	159	12	7%
Platelet activation, signaling and aggregation	223	12	5%
Phase II conjugation	66	11	16%
M Phase	243	11	4%
Translation	74	10	13%
Metabolism of amino acids and derivatives	156	10	6%
Apoptosis	182	10	5%
Mitotic Metaphase and Anaphase	207	10	4%
Protein folding	34	9	26%
Eukaryotic Translation Initiation	72	9	12%
Class I MHC mediated antigen processing & presentation	118	9	7%
Disease	587	9	1%
Regulation of mRNA Stability by Proteins that Bind AU-rich Elements	84	8	9%
Response to elevated platelet cytosolic Ca2+	111	8	7%

b) down-regulated in HCs

Pathway name	Total number of proteins	Matching proteins in data	% in data
Metabolism	1519	51	3%
Metabolism of lipids and lipoproteins	604	12	1%
Immune System	990	12	1%
Metabolism of amino acids and derivatives	156	11	7%
Hemostasis	518	11	2%
Signal Transduction	2022	10	0%
Platelet activation, signaling and aggregation	223	9	4%
Metabolism of carbohydrates	233	9	3%
Innate Immune System	550	9	1%
Response to elevated platelet cytosolic Ca2+	111	8	7%
Metabolism of nucleotides	85	7	8%
The citric acid (TCA) cycle and respiratory electron transport	142	7	4%
Metabolism of proteins	484	7	1%
Fatty acid, triacylglycerol, and ketone body metabolism	167	6	3%
Disease	587	6	1%
Cell Cycle	543	6	1%
Adaptive Immune System	494	6	1%
Apoptosis	182	5	2%
Cell Cycle, Mitotic	474	5	1%
Gene Expression	790	5	0%
Respiratory electron transport, ATP synthesis by chemiosmotic coupling, and heat production by uncoupling proteins.	100	4	4%
Membrane Trafficking	117	4	3%
Mitotic G1-G1/S phases	152	4	2%
Signalling by NGF	374	4	1%
Signaling by the B Cell Receptor (BCR)	248	4	1%

Table 1 continued; a) up-regulated in HCs

Pathway name	Total number of proteins	Matching proteins in data	% in data
S Phase	154	8	5%
Fatty acid, triacylglycerol, and ketone body metabolism	167	8	4%
Signaling by the B Cell Receptor (BCR)	248	8	3%
Developmental Biology	480	8	1%
Metabolism of porphyrins	23	7	30%
Signaling by Wnt	73	7	9%
MHC class II antigen presentation	78	7	8%
Regulation of Apoptosis	82	7	8%
Regulation of DNA replication	90	7	7%
APC/C-mediated degradation of cell cycle proteins	101	7	6%
M/G1 Transition	107	7	6%
Regulation of mitotic cell cycle	101	7	6%
DNA Replication	130	7	5%
Membrane Trafficking	117	7	5%
Synthesis of DNA	123	7	5%
Cell Cycle Checkpoints	152	7	4%
Mitotic G1-G1/S phases	152	7	4%
The citric acid (TCA) cycle and respiratory electron transport	142	7	4%
Transmission across Chemical Synapses	177	7	3%
Axon guidance	315	7	2%
Neuronal System	266	7	2%
Fcgamma receptor (FCGR) dependent phagocytosis	91	6	6%
Metabolism of carbohydrates	233	6	2%
Signalling by NGF	374	6	1%
Semaphorin interactions	82	5	6%

b) down-regulated in HCs

Pathway name	Total number of proteins	Matching proteins in data	% in data
Peroxisomal lipid metabolism	23	3	13%
Metabolism of nitric oxide	25	3	12%
Lipid digestion, mobilization, and transport	25	3	12%
Translocation of GLUT4 to the Plasma Membrane	35	3	8%
Amyloids	55	3	5%
Signaling by Wnt	73	3	4%
Phase II conjugation	66	3	4%
Regulation of mRNA Stability by Proteins that Bind AU-rich Elements	84	3	3%
Regulation of DNA replication	90	3	3%
Regulation of Apoptosis	82	3	3%
Toll-Like Receptors Cascades	137	3	2%
Synthesis of DNA	123	3	2%
Regulation of mitotic cell cycle	101	3	2%
M/G1 Transition	107	3	2%
DNA Replication	130	3	2%
Class I MHC mediated antigen processing & presentation	118	3	2%
APC/C-mediated degradation of cell cycle proteins	101	3	2%
Signaling by Rho GTPases	186	3	1%
S Phase	154	3	1%
Mitotic Metaphase and Anaphase	207	3	1%
M Phase	243	3	1%
Cell Cycle Checkpoints	152	3	1%
Biological oxidations	159	3	1%
alpha-linolenic (omega3) and linoleic (omega6) acid metabolism	11	2	18%
Binding and Uptake of Ligands by Scavenger Receptors	17	2	11%

Table 2. Top 20 molecular events found to be affected by NAF in HCs. The two sub-tables give the lists of molecular events which may be a) enhanced or b) repressed, because of the NAF induced alterations in protein expression. The first column gives the p-value, while the following columns state the number of proteins of our data linked to the specific event, again labeled with a hypernym (column four) and the total number of proteins involved in this event.

a) up-regulated in HCs

Un-adjusted probability of seeing N or more proteins in this Event by chance	Number of proteins in your query which map to this Event	Total number of proteins involved in this Event	Name of Event
1.4e-19	62	1546	Metabolism
3.9e-06	23	604	Metabolism of lipids and lipoproteins
5.5e-17	22	156	Metabolism of amino acids and derivatives
4.0e-07	12	142	The citric acid (TCA) cycle and respiratory electron transport
1.4e-05	11	167	Fatty acid, triacylglycerol, and ketone body metabolism
9.7e-04	10	227	Platelet activation, signaling and aggregation
1.0e-05	9	104	Platelet degranulation
1.8e-05	9	111	Response to elevated platelet cytosolic Ca2+
1.2e-09	7	17	Branched-chain amino acid catabolism
1.4e-08	7	23	Peroxisomal lipid metabolism
2.5e-06	6	30	Bile acid and bile salt metabolism
3.3e-05	6	46	Pyruvate metabolism and Citric Acid (TCA) cycle
2.3e-03	6	100	Respiratory electron transport, ATP synthesis by chemiosmotic coupling, and heat production by uncoupling proteins.

b) down-regulated in HCs

Un-adjusted probability of seeing N or more proteins in this Event by chance	Number of proteins in your query which map to this Event	Total number of proteins involved in this Event	Name of Event
4.9e-15	51	1546	Metabolism
1.8e-02	12	523	Hemostasis
4.8e-02	12	604	Metabolism of lipids and lipoproteins
1.7e-06	11	156	Metabolism of amino acids and derivatives
1.3e-03	9	227	Platelet activation, signaling and aggregation
1.8e-03	9	239	Metabolism of carbohydrates
2.6e-05	8	104	Platelet degranulation
4.3e-05	8	111	Response to elevated platelet cytosolic Ca2+
3.0e-05	7	77	Glucose metabolism
5.6e-05	7	85	Metabolism of nucleotides
1.3e-03	7	142	The citric acid (TCA) cycle and respiratory electron transport
4.4e-03	6	132	Membrane Trafficking
1.3e-02	6	167	Fatty acid, triacylglycerol, and ketone body metabolism

Table 2 continued; a) up-regulated in HCs

Un-adjusted probability of seeing N or more proteins in this Event by chance	Number of proteins in your query which map to this Event	Total number of proteins involved in this Event	Name of Event
1.1e-06	5	15	Mitochondrial Fatty Acid Beta-Oxidation
1.1e-06	5	15	Synthesis of bile acids and bile salts via 7alpha-hydroxycholesterol
5.5e-06	5	20	Synthesis of bile acids and bile salts
3.8e-06	4	9	Beta-oxidation of very long chain fatty acids
9.9e-06	4	11	alpha-linolenic (omega3) and linoleic (omega6) acid metabolism
9.9e-06	4	11	alpha-linolenic acid (ALA) metabolism
2.3e-04	4	23	Citric acid cycle (TCA cycle)

b) down-regulated in HCs

Un-adjusted probability of seeing N or more proteins in this Event by chance	Number of proteins in your query which map to this Event	Total number of proteins involved in this Event	Name of Event
5.6e-06	5	23	Citric acid cycle (TCA cycle)
6.4e-05	5	37	Translocation of GLUT4 to the Plasma Membrane
7.3e-05	5	38	Gluconeoproteinsis
1.9e-04	5	46	Pyruvate metabolism and Citric Acid (TCA) cycle
2.9e-05	4	16	Synthesis and interconversion of nucleotide di- and triphosphates
4.4e-03	4	57	Exocytosis of platelet alpha granule contents
3.0e-02	4	100	Respiratory electron transport, ATP synthesis by chemiosmotic coupling, and heat production by uncoupling proteins.

Table 3. Top 50 pathways found to be affected by NAF in NPCs. The two sub-tables give the lists of pathways which may be a) enhanced or b) repressed in NPCs, because of the NAF induced alterations in protein expression. *See table 1* for the table description.

a) up-regulated in NPCs

Pathway name	Total number of proteins	Matching proteins in data	% in data
Metabolism	1519	66	4%
Immune System	990	29	2%
Metabolism of proteins	484	26	5%
Gene Expression	790	26	3%
Signal Transduction	2022	21	1%
Adaptive Immune System	494	19	3%
Cell Cycle	543	19	3%
Metabolism of lipids and lipoproteins	604	19	3%
Cell Cycle, Mitotic	474	16	3%
Hemostasis	518	15	2%
Innate Immune System	550	14	2%
Biological oxidations	159	13	8%
Phase II conjugation	66	12	18%
Platelet activation, signaling and aggregation	223	12	5%
M Phase	243	11	4%
Translation	74	10	13%
Metabolism of amino acids and derivatives	156	10	6%
Apoptosis	182	10	5%
Mitotic Metaphase and Anaphase	207	10	4%
Protein folding	34	9	26%
Eukaryotic Translation Initiation	72	9	12%
Class I MHC mediated antigen processing & presentation	118	9	7%
Disease	587	9	1%
Regulation of mRNA Stability by Proteins that Bind AU-rich Elements	84	8	9%
Response to elevated platelet cytosolic Ca2+	111	8	7%
S Phase	154	8	5%
Fatty acid, triacylglycerol, and ketone body metabolism	167	8	4%
Signaling by the B Cell Receptor (BCR)	248	8	3%

b) down-regulated in NPCs

Pathway name	Total number of proteins	Matching proteins in data	% in data
Metabolism	1519	66	4%
Immune System	990	24	2%
Signal Transduction	2022	24	1%
Hemostasis	518	21	4%
Gene Expression	790	21	2%
Metabolism of lipids and lipoproteins	604	18	2%
Platelet activation, signaling and aggregation	223	16	7%
Adaptive Immune System	494	16	3%
Axon guidance	315	15	4%
Developmental Biology	480	15	3%
Metabolism of proteins	484	15	3%
Response to elevated platelet cytosolic Ca2+	111	12	10%
Metabolism of carbohydrates	233	12	5%
The citric acid (TCA) cycle and respiratory electron transport	142	11	7%
Cell Cycle	543	11	2%
Cell Cycle, Mitotic	474	11	2%
Innate Immune System	550	11	2%
Disease	587	11	1%
Apoptosis	182	10	5%
Metabolism of nucleotides	85	9	10%
Metabolism of amino acids and derivatives	156	9	5%
Signalling by NGF	374	9	2%
Semaphorin interactions	82	8	9%
Fatty acid, triacylglycerol, and ketone body metabolism	167	8	4%
Processing of Capped Intron-Containing Pre-mRNA	161	8	4%
Transmission across Chemical Synapses	177	8	4%
Neuronal System	266	8	3%
Muscle contraction	67	7	10%

Table 3 continued; a) up-regulated in NPCs

Pathway name	Total number of proteins	Matching proteins in data	% in data
Developmental Biology	480	8	1%
Metabolism of porphyrins	23	7	30%
Signaling by Wnt	73	7	9%
MHC class II antigen presentation	78	7	8%
Regulation of Apoptosis	82	7	8%
Regulation of DNA replication	90	7	7%
APC/C-mediated degradation of cell cycle proteins	101	7	6%
M/G1 Transition	107	7	6%
Regulation of mitotic cell cycle	101	7	6%
DNA Replication	130	7	5%
Membrane Trafficking	117	7	5%
Synthesis of DNA	123	7	5%
Cell Cycle Checkpoints	152	7	4%
Mitotic G1-G1/S phases	152	7	4%
The citric acid (TCA) cycle and respiratory electron transport	142	7	4%
Transmission across Chemical Synapses	177	7	3%
Axon guidance	315	7	2%
Neuronal System	266	7	2%
Fcgamma receptor (FCGR) dependent phagocytosis	91	6	6%
Metabolism of carbohydrates	233	6	2%
Signalling by NGF	374	6	1%
Semaphorin interactions	82	5	6%

b) down-regulated in NPCs

Pathway name	Total number of proteins	Matching proteins in data	% in data
Regulation of mRNA Stability by Proteins that Bind AU-rich Elements	84	7	8%
Respiratory electron transport, ATP synthesis by chemiosmotic coupling, and heat production by uncoupling proteins.	100	7	7%
Membrane Trafficking	117	7	5%
Neurotransmitter Receptor Binding And Downstream Transmission In The Postsynaptic Cell	131	7	5%
Signaling by EGFR in Cancer	214	7	3%
NGF signalling via TRKA from the plasma membrane	251	7	2%
Eukaryotic Translation Initiation	72	6	8%
Translation	74	6	8%
MHC class II antigen presentation	78	6	7%
Fcgamma receptor (FCGR) dependent phagocytosis	91	6	6%
Extracellular matrix organization	233	6	2%
M Phase	243	6	2%
Signaling by EGFR	209	6	2%
Signaling by GPCR	1026	6	0%
Translocation of GLUT4 to the Plasma Membrane	35	5	14%
Class I MHC mediated antigen processing & presentation	118	5	4%
L1CAM interactions	105	5	4%
Mitotic G2-G2/M phases	134	5	3%
Mitotic Metaphase and Anaphase	207	5	2%
Lipid digestion, mobilization, and transport	25	4	16%
Intrinsic Pathway for Apoptosis	41	4	9%
Opioid Signalling	70	4	5%

Table 4. Top 20 molecular events found to be affected by NAF in NPCs. The two sub-tables give the lists of molecular events which may be a) enhanced or b) repressed, because of the NAF induced alterations in protein expression. *See table 2* for the table description.

a) up-regulated in NPCs

Un-adjusted probability of seeing N or more proteins in this Event by chance	Number of proteins in your query which map to this Event	Total number of proteins involved in this Event	Name of Event
7.0e-09	60	1546	Metabolism
2.0e-07	29	526	Metabolism of proteins
3.5e-02	28	1021	Immune System
1.7e-02	24	790	Gene Expression
5.3e-03	19	518	Adaptive Immune System
2.4e-02	19	604	Metabolism of lipids and lipoproteins
2.5e-02	18	564	Cell Cycle
4.7e-02	16	523	Hemostasis
1.5e-03	12	227	Platelet activation, signaling and aggregation
2.1e-04	11	156	Metabolism of amino acids and derivatives
1.3e-07	10	58	Chaperonin-mediated protein folding
3.0e-07	10	63	Protein folding
2.8e-03	10	182	Apoptosis
2.9e-02	10	258	M Phase
1.1e-09	9	27	Actin/tubulin:prefoldin complex associates with CCT/TriC
1.1e-09	9	27	Prefoldin mediated transfer of substrate to CCT/TriC

b) down-regulated in NPCs

Un-adjusted probability of seeing N or more proteins in this Event by chance	Number of proteins in your query which map to this Event	Total number of proteins involved in this Event	Name of Event
5.1e-11	70	1546	Metabolism
2.0e-02	19	523	Hemostasis
3.4e-02	18	518	Adaptive Immune System
3.0e-03	16	339	Axon guidance
4.3e-04	14	227	Platelet activation, signaling and aggregation
7.2e-04	14	239	Metabolism of carbohydrates
1.5e-05	11	104	Platelet degranulation
2.8e-05	11	111	Response to elevated platelet cytosolic Ca2+
2.6e-04	11	142	The citric acid (TCA) cycle and respiratory electron transport
5.9e-06	10	77	Glucose metabolism
6.5e-03	10	182	Apoptosis
9.2e-05	9	85	Metabolism of nucleotides
7.1e-03	9	156	Metabolism of amino acids and derivatives
4.0e-04	8	82	Semaphorin interactions
3.0e-02	8	167	Fatty acid, triacylglycerol, and ketone body metabolism
1.5e-05	7	38	Gluconeoproteinsis

Table 4 continued; a) up-regulated in NPCs

Un-adjusted probability of seeing N or more proteins in this Event by chance	Number of proteins in your query which map to this Event	Total number of proteins involved in this Event	Name of Event
1.6e-09	9	28	Cooperation of Prefoldin and TriC/CCT in actin and tubulin folding
1.2e-05	9	74	Translation
7.4e-04	9	126	Class I MHC mediated antigen processing & presentation
1.0e-03	9	132	Membrane Trafficking

b) down-regulated in NPCs

Un-adjusted probability of seeing N or more proteins in this Event by chance	Number of proteins in your query which map to this Event	Total number of proteins involved in this Event	Name of Event
8.0e-04	7	70	Muscle contraction
6.2e-03	7	100	Respiratory electron transport, ATP synthesis by chemiosmotic coupling, and heat production by uncoupling proteins.
2.6e-02	7	132	Membrane Trafficking
2.6e-02	7	133	Formation of the Spliceosomal B Complex

5.2 Effects of coffee the protein expression of PBMCs

By using 2D SDS-PAGE in combination with ^{35}S metabolic labelling, we were able to detect newly synthesized proteins with a detection limit of 1ng, which is a rather good sensitivity in terms of 2D gels. For comparison, with silver gel staining it is possible to detect a protein spot consisting of 200ng of protein. The most sensitive labelling method, currently available for 2D gels, is the 2D DIGE fluorescence labelling (or similar), where the detection limit is situated between 100 and 200pg of protein. With modern MS instruments fg amounts of analytes can be measured in 10µl (injection). Nevertheless, the detection of only newly synthesised proteins is a major advantage, when analysing molecular mechanisms by highlighting alterations in the protein expression.

With the data of our PBMC study as reference at hand, in the following investigations we constrained our effort on proteins found induced or up-regulated upon inflammatory activation in the 2D gels of cytoplasms of PBMCs (*see figure 1 and table 1 of manuscript 3.2; p. 66ff*). *Table 5* depicts the summarised results of the alterations in expression of these proteins using ImageJ. The proteins in the short-list are separated into groups of monocyte and t-cell specific proteins, proteins found in both cell types, when incubated separately and together including all cell types of PBMCs isolated from the blood, as well as proteins found only during the combined incubation of monocytes and t-cells during the stimulation phase. Because of the semi-quantitative nature of the 2D autoradiography, including the small variations in applied protein amounts, the resulting protein spot intensity values were approximated and summarised in this table, giving percentage values of change in protein expression of the respective proteins. The third column states the intensity of the protein spot, which is equivalent to the protein amount found in the cytoplasm of the respective PBMCs. This information may help to assess the actual impact of a protein on the cell and its functional state, when considering amongst others the enzyme activity. When we look at the results of the proteomes of untreated primary human PBMCs, it becomes apparent that the consumption of coffee alone is enough to enhance or, in some cases, even induce the protein expression of inflammation-related proteins. Furthermore, by comparing the proteomes of activated PBMCs deriving from probands prior against posterior of coffee consumption, the results indicate that the PBMCs of the probands, which had consumed coffee, reacted remarkably stronger to the inflammatory stimuli. This is demonstrated by our data, where a consistent enhancement of the protein expression of inflammation-related proteins is observable. Therefore coffee seems to convey an improved immune activity to

PBMCs in the acute phase. Amongst others, the heightened protein expression of interleukine-1 beta in monocytes and its subsequent secretion indicates an enhanced paracrine signalling for t-lymphocytes to proliferate. This idea is augmented by the fact that the protein expression of proliferating cell nuclear antigen (PCNA), a protein involved in t-cell proliferation, is also increased in t-lymphocytes. Moreover, the protein expression of acute phase proteins involved in the interferon-mediated signalling pathways, and interferon regulatory factor 4 (IRF-4) are noteworthy increased.

There are indications that a chronic consumption of coffee may have a positive effect, leading to changes in concentrations of inflammation-associated mediators in the blood.[58] A significant down-regulation of Il-18 in the serum is evident.[58] The results of Il-6 and CRP, c-reactive protein of the pentraxin family - an acute phase protein, which is synthesised in the liver and is secreted into the blood, are controversial in various publications, with indications of no change in the protein levels[58], an increase of 50% and 30% in the blood respectively[59] and even a reduction of CRP and e-selection (CD62, an inflammation-associated cytokine secreted by endothelial cells) in the blood was observed[60].

To conclude, we hypothesise that the consumption of coffee boosts the immune reaction of PBMCs against PAMPS (e.g.) during the acute phase, which may indeed result in a positive short-term effect upon the immune system.[49,51,61]

5.3 Effects of mould fungi extracts on inflammatory activated PBMCs

The proteome analyses of the effects of the mould fungi extracts deriving from Aspergillus niger Mucor racemosus and Penicillium chrysogenum were conducted according to the study protocol of the other PMBC investigations. As before, we investigated the proteome of isolated primary human PBMCs, in an untreated as well as an inflammatory stimulated functional cell state, via 2D autoradiography. The results are presented in *table 6* using the already established short-list of proteins of *table 1* in *manuscript 3.2 (p. 66ff).* According to our analyses, the extract of Aspergillus niger has some effect on naïve PBMCs. There is some increase in the protein expression of proliferating cell nuclear antigen (PCNA) and 4F2 cell-surface antigen heavy chain, proteins which are involved in the migration and proliferation of t-lymphocytes, indicating a weak immune response. On the other hand proteins involved in the type I interferon-mediated signalling pathway were found to be down-regulated. The extract of Mucor racemosus

demonstrates bivalent aspects, with weak positive and negative alterations in protein expression. Interestingly the extract of Penicillium chrysogenum showed, at least in some cases, a noteworthy down-regulation of this set of inflammation-related proteins even in untreated PBMCs. Our investigations on PBMCs in *manuscript 3.2* showed that cultivation exerts a 'weak' inflammatory stimulus upon naive PBMCs. It is easily conceivable that this effect was modulated by the mould fungi extracts to various degrees.

The results from the inflammatory activated PBMCs treated with the extracts of the mould fungi are similar for all three mould fungi, but again with different 'effectiveness'. The protein expression of the short-listed proteins was tendentially weakly down-regulation by Aspergillus niger extract, which indicates a rather weak anti-inflammatory potential of the extract. The extracts of Mucor racemosus and Penicillium chrysogenum had a stronger anti-inflammatory effect on the protein expression of the inflammatory activated PBMCs, where proteins involved in the migration and proliferation of t-lymphocytes and the immune activity were noticeably down-regulated.

To conclude, the results from the 2D autoradiograms indicate an anti-inflammatory potential of the mould fungi extracts, with the extract of Aspergillus niger having the weakest effect on PBMCs. The down-regulation of inflammation-relevant proteins in PBMCs demonstrates at least a soft damping capability of the mould fungi extracts, if applied directly onto the cells. If the results of the *in vitro* setup hold true for the *in vivo* application, with possible metabolisation taking place and an unknown final concentration of the effectors in the blood, has yet to be determined.

Table 5. The effects of coffee on naive and inflammatory activated primary PBMCs. The column of the level of intensity gives information about the amount of protein, whereby a 'low' intensity is equivalent to a relative low amount of newly synthesised protein. The proband columns are divided into two separate sub-columns, one for the results of the activated and the other for the untreated functional state of the PBMCs. The percentage values give information about the change in intensity, which is equivalent to the change in amount of newly synthesised protein. It should be noted that, because of the semi-quantitative nature of the method, the percentage values are approximated and should not be taken at face value. ('i' – newly induced, 'x' - not found, '-' – no change)

Accession number	Protein name	Level of intensity of the respective spot		Proband 1		Proband 2		Proband 3		Proband 4		GO - biological processes
I) Proteins induced in both activated t-cells and monocytes		Act	Con	Act	Con	Act	Con	Act	Con	Act	Con	
P08195	4F2 cell-surface antigen heavy chain	medium	low	+55%	+30%	+10%	+60%	-5%	x	+80%	+45%	leukocyte migration, cell growth
P05120	Plasminogen activator inhibitor 2 (PAI-2)	high	medium	+85%	+75%	+50%	-20%	+30%	-30%	+25%	+15%	anti-apoptosis
P43490	Nicotinamide phosphoribosyltransferase	low	x	+45%	x	+150%	x	+25%	x	+50%	x	positive regulation of cell proliferation
P61289	Proteasome activator complex subunit 3	low	x	+15%	x	+120%	+40%	+35%	x	+65%	+10%	regulation of apoptotic process
II) Proteins induced in activated t-cells only												
Q15306	Interferon regulatory factor 4 (IRF-4)	low	x	+40%	x	+30%	x	+50%	x	+60%	+15%	T cell activation
P32456	Interferon-induced guanylate-binding protein 2	medium	x	+125%	x	+30%	x	+35%	x	+105%	i	interferon-gamma-mediated signaling pathway
P12004	Proliferating cell nuclear antigen (PCNA)	high	high	+40%	+80%	+45%	+15%	+10%	+25%	+10%	+40%	proliferation
P12268	Inosine-5'-monophosphate dehydrogenase 2	high	low	+80%	+100%	+65%	+35%	+40%	x	+80%	+65%	GMP biosynthetic process
P32455	Interferon-induced guanylate-binding protein 1	high	low	+70%	+70%	+85%	-	+20%	x	+95%	+35%	interferon-gamma-mediated signaling pathway
III) Proteins induced in activated monocytes only												
P01584	Interleukin-1 beta	high	x	+60%	x	+135%	x	+20%	+20%	+50%	-	positive regulation of T cell proliferation
IV) Proteins induced in activated PBMCs, but not isolated cells												
P20591	Interferon-induced GTP-binding protein Mx1	high	x	+130%	x	+10%	x	+55%	x	+135%	+155%	type I interferon-mediated signaling pathway
p09913	Interferon-induced protein with tetratricopeptide repeats 2 (IFIT-2)	high	x	+35%	x	+100%	x	+30%	x	+110%	x	type I interferon-mediated signaling pathway
p23381	Tryptophan--tRNA ligase, cytoplasmic	high	low	+100%	+60%	+110%	+50%	+35%	-35%	+50%	+105%	tryptophanyl-tRNA aminoacylation

Table 6. The effects of mould fungi extracts on naive and inflammatory activated primary PBMCs. The effects of mould fungi extracts on naive and inflammatory activated primary PBMCs. This table is structured such as *table 1*, with the exception that the proband columns are substituted by the mould fungi columns.

Accession number	Protein name	Level of intensity of the respective spot		Aspergillus niger		Mucor racemosus		Penicillium chrysogenum		GO - biological processes
I) Proteins induced in both activated t-cells and monocytes		activated	untreated	activated	untreated	activated	untreated	activated	untreated	
P08195	4F2 cell-surface antigen heavy chain	medium	low	-10%	+20%	-35%	+25%	-20%	-30%	leukocyte migration, cell growth
P05120	Plasminogen activator inhibitor 2 (PAI-2)	high	medium	-10%	-10%	-25%	-10%	-20%	-45%	anti-apoptosis
P43490	Nicotinamide phosphoribosyltransferase	low	x	-20%	x	-35%	x	-40%	x	positive regulation of cell proliferation
P61289	Proteasome activator complex subunit 3	low	x	-10%	x	-25%	x	-15%	x	regulation of apoptotic process
II) Proteins induced in activated t-cells only										
Q15306	Interferon regulatory factor 4 (IRF-4)	low	x	-10%	x	-25%	-15%	-40%	x	T cell activation
P32456	Interferon-induced guanylate-binding protein 2	medium	x	-10%	x	+5%	+25%	-	x	interferon-gamma-mediated signaling pathway
P12004	Proliferating cell nuclear antigen (PCNA)	high	high	-	+20%	-5%	-	-5%	-25%	proliferation
P12268	Inosine-5'-monophosphate dehydrogenase 2	high	low	-15%	-30%	-15%	-	-30%	-40%	GMP biosynthetic process
P32455	Interferon-induced guanylate-binding protein 1	high	low	-30%	-65%	-40%	-50%	-20%	-70%	interferon-gamma-mediated signaling pathway
III) Proteins induced in activated monocytes only										
P01584	Interleukin-1 beta	high	x	-15%	x	-30%	-	-15%	x	positive regulation of T cell proliferation
IV) Proteins induced in activated PBMCs, but not isolated cells										
P20591	Interferon-induced GTP-binding protein Mx1	high	x	-15%	-15%	-30%	+25%	-10%	-20%	type I interferon-mediated signaling pathway
p09913	Interferon-induced protein with tetratricopeptide repeats 2 (IFIT-2)	high	x	-15%	x	-20%	x	-60%	x	type I interferon-mediated signaling pathway
p23381	Tryptophan--tRNA ligase, cytoplasmic	high	low	-15%	-	-20%	-10%	-5%	-20%	tryptophanyl-tRNA aminoacylation

6. Summary

Non-genotoxic carcinogen (NGCs) is a term for chemical compounds, being able to promote and in some cases even initiate cancer in an organism by modes of action, which are devoid of direct interaction with DNA. These mechanisms are based on perturbation of molecular pathways and affecting gene expression by events such as triggering signal transduction or histone modification, to name only a few. Despite all efforts NGCs are poorly understood. A better understanding of NGCs is a necessity, when it comes amongst others to modern drug development. Any advancement that leads to the establishment of validated short-term assays, which may indicate compounds with such a carcinogenic potential, are welcomed and would increase the safety of newly developed drugs considerably, allowing an early exclusion of such substances in the process. That said, up to now there are no such short-term assays available, which are reliable enough to be used in the clinical trial phase. To tackle this issue, we decided to conduct a screening of the proteome of liver resident cells, because the liver is the foremost organ for drug metabolisation, usually making it the main target for so-called non-genotoxic carcinogens (NGCs). In our investigation we used rodent model organisms treated with nafenopin or phenobarbital, two known NGCs, because in general rodents show a marked susceptibility to non-genotoxic compounds compared to Homo sapiens. These two compounds are rather well understood and suited us well for this purpose.

Our approach using LC-MS/MS allowed us to identify a large number of proteins, including low abundant proteins, with a high accuracy and confidence. When comparing the results in a semi-quantitative manner using emPAI values, alterations in the proteomes became evident. The experimental setups were rats treated *in vivo* with PB as well as isolated primary liver cell, HCs and NPCs, treated *in vitro* with NAF and PB. The results of NAFs and PBs proteome profiling data underline the complexity to interpret NGCs modes of action, but support the notion of an involvement of the epithelium and the mesenchyme (NPCs) as well as cell-cell interactions. As it was already mentioned in *manuscript 3.1*, any potential marker protein needs to indicate an essential common step within this rather complex chain of events. Such events could be the response of cells towards ROS formation, a pro-inflammatory or apoptosis/proliferative signature or other stress-related effects. Furthermore, the data conveys the sentiment that for NGCs to deploy their complete modes of action an intact organism is essential, where all cell-cell and systemic interactions are feasible. Other members of the IMI project were also able to delve

deeper into NGCs modes of action using different approaches, such as Affymetrix or PCR, to ensure a wide spectrum of data diversity. E.g. the groups Dr. Moggs and Dr. Rémi Terranova were able to learn that phenobarbital mediates an epigenetic switch at the CAR target gene Cyp2b10.[62] The aim would be to combine all results in one data base, which was actually done, allowing an easy access to all the data enabling a thoroughly data mining, such as the analysis of pathway networks and clusters, using bioinformatics. The next step would be to establish for each sub-group of NGCs a representative panels of biomarkers, which are being validated using state-of-the-art quantitative methods such as multiple reaction monitoring (MRM) for proteomics.

To conclude the studies on the primary PBMCs, monocytes and t-lymphocytes constitute the main cells of the PBMCs circulating in the blood. By monitoring the organism and triggering an immune response upon encountering DAMPS or PAMPS, they are a very important part of the immune system. Again shotgun proteomics using a LC-MS/MS and metabolic labelling in combination with 2D SDS-PAGE were used. The latter method is very useful, when it comes to highlighting newly synthesised proteins. Using these methods, we were able to identify proteins specific for a cell type or shared by more than one cell, which were expressed upon giving an inflammatory stimulus *in vitro*, simulating an acute phase response. With the established short-list of inflammation-related proteins, we performed two follow-up studies investigating effects of coffee *in vivo* and mould fungi extracts *in vitro*. The data clearly indicate that coffee has an activating effect on the protein expression of inflammation-related proteins in naïve PBMCs and had an enhancing effect on these proteins in inflammatory activated PBMCs. For an organism this could mean a positive effect by enhancing the immune reaction of these cells. On the other hand it has to be investigated, how coffee affects the immune response in chronic disease, as in literature the hypotheses are bivalent. The results on mould fungi extract treated PBMCs indicate a weak anti-inflammatory capacity, but *in vivo* studies have to be conducted to determine usefulness in organisms.

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Attachments

This chapter presents the summarised proteome profiling results of the NAF experiments. The results are separated into the following supplementary tables:

Column names, which are labelled 'analysis' and 'reference', refer to the experiments of the isolated primary cells, HCs and NPCs, deriving from rats treated *in vitro* with nafenopin (NAF) or DMSO, respectively. For each specific protein there is information about its UniProt accession number, protein name, number of peptides by which it was found in the experiments and the corresponding calculated emPAI value. In the tables where proteins are listed, which were found in the proteome of both experimental setups, NAF and DMSO, additional information is available. There are columns for 'expcount' (experiment count; number of positive identifications compared to the total number of experiments of a specific sub-cellular fraction) and difference in emPAI values. Furthermore, there are two columns giving information about the alteration in protein expression or, to be more specific, the difference in detected protein abundance. These values are calculated by means of the respective emPAI values, and the columns are designated as 'x-times down- or up-regulation' as well as 'alteration in %', different aspects of the same meaning.

The corresponding discussion can be found in chapter *5.1 Nafenopin – NGC study (p.82)*.

Supplementary table 1. HC - secretome

found only in NAF samples	min. 2 experiments, min. 2 peptides		sorted according to the magnitude of the emPAI value	NAF and DMSO each 4 exp
accession number	protein name	analysis peptides	analysis secreted emPAI	
P0CC09	Histone H2A type 2-A (Histone H2A.2)	4	1,53	
P02706	Asialoglycoprotein receptor 1 (ASGP-R 1) (Hepatic lectin 1) (HL-1)	5	0,301	
Q6LED0	Histone H3.1	2	0,233	
P19999	Mannose-binding protein A (MBP-A) (Mannan-binding protein)	2	0,214	
P55797	Apolipoprotein C-IV (Apo-CIV) (Apolipoprotein C4) (Apolipoprotein E-linked) (ECL)	2	0,212	

only in ref/DMSO	min. 2 experiments, min. 2 peptides		sorted according to the magnitude of the emPAI value
accession number	protein name	reference peptides	reference secreted emPAI
P60868	40S ribosomal protein S20	2	0,556
P19945	60S acidic ribosomal protein P0 (60S ribosomal protein L10E)	4	0,24
P62161	Calmodulin (CaM)	2	0,212
O35331	Pyridoxal kinase (Pyridoxine kinase)	2	0,156
P04797	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (38 kDa BFA-dependent ADP-ribosylation substrate) (BARS-38)	9	0,156
P17220	Proteasome subunit alpha type-2 (Proteasome component C3) (Macropain subunit C3) (Multicatalytic endopeptidase complex subunit C3)	2	0,155
P30713	Glutathione S-transferase theta-2 (GST class-theta-2) (Glutathione S-transferase 12) (GST 12-12) (Glutathione S-transferase Yrs-Yrs)	3	0,155
P38918	Aflatoxin B1 aldehyde reductase member 3 (AFB1-AR) (Aflatoxin B1 aldehyde reductase member 1) (rAFAR1)	2	0,122
P97584	Prostaglandin reductase 1 (PRG-1) (NADP-dependent leukotriene B4 12-hydroxydehydrogenase) (15-oxoprostaglandin 13-reductase) (Dithiolethione-inducible gene 1 protein) (D3T-inducible gene 1 protein)	3	0,105
P16617	Phosphoglycerate kinase 1	4	0,101
Q8VIF7	Selenium-binding protein 1 (Selenium-binding protein 2) (56 kDa selenium-binding protein) (SBP56)	5	0,077
Q62975	Protein Z-dependent protease inhibitor (PZ-dependent protease inhibitor) (Serpin A10) (Regeneration-associated serpin 1) (RASP-1)	2	0,075
P07150	Annexin A1 (Annexin-1) (Annexin I) (Lipocortin I) (Calpactin-2) (Calpactin II) (Chromobindin-9) (p35) (Phospholipase A2 inhibitory protein)	2	0,068
Q63150	Dihydropyrimidinase (DHPase) (Dihydropyrimidine amidohydrolase) (Hydantoinase)	3	0,064

found in both	min. 2 exp, min. 2 peptides, delta of ≥1.45 or 45% upon NAF treatment				sorted according to the magnitude of the regulation				
accession number	protein name	analysis peptides	analysis secreted expcount	analysis secreted emPAI	reference peptides	reference secreted expcount	reference secreted emPAI	emPAI diff secreted	x-times up-regulated	alteration in %
P23680	Serum amyloid P-component (SAP)	7	3 von 4	1.069	9	4 von 4	0,688	0,381	1553,77907	155277,907
P04636	Malate dehydrogenase, mitochondrial	13	1 von 4	1.154	14	3 von 4	0,867	0,287	1331,026528	133002,6528
P11030	Acyl-CoA-binding protein (ACBP) (Diazepam-binding inhibitor) (DBI) (Endozepine) (EP)	4	3 von 4	1.025	3	2 von 4	0,778	0,247	1317,48072	131648,072
P05545	Serine protease inhibitor A3K (Serpin A3K) (Contrapsin-like protease inhibitor 1) (CPI-21) (Kallikrein-binding protein) (KBP) (Growth hormone-regulated proteinase inhibitor) (GHR-P63) (Serine protease inhibitor 2) (SPI-2) (SPI-2.3) (Thyroid hormone-regula	14	4 von 4	1.173	11	4 von 4	0,994	0,179	1180,080483	117908,0483
P02770	Serum albumin	38	4 von 4	2.452	39	4 von 4	2,52	-0,068	973,015873	97201,5873
P09656	Serine protease inhibitor Kazal-type 3 (Calcium transport inhibitor) (Caltrin) (Pancreatic secretory trypsin inhibitor II) (PSTI-II)	3	1 von 4	2.981	4	1 von 4	5,31	-2.329	561,393597	56039,3597
P02680	Fibrinogen gamma chain	5	1 von 4	0,403	2	1 von 4	0,145	0,258	2,779310345	177,9310345
P07151	Beta-2-microglobulin	6	4 von 4	2.811	3	3 von 4	1.181	1,63	2,380186283	138,0186283
P81827	Urinary protein 1 (UP-1) (Liver regeneration-related protein LRRG05)	3	4 von 4	1,74	1	4 von 4	0,778	0,962	2,236503856	123,6503856
P11348	Dihydropteridine reductase (HDHPR) (Quinoid dihydropteridine reductase)	5	1 von 4	0,719	5	2 von 4	0,323	0,396	2,226006192	122,6006192
Q7TP52	Carboxymethylenebutenolidase homolog (Liver regeneration-related protein LRRG072)	4	1 von 4	0,334	4	1 von 4	0,155	0,179	2,15483871	115,483871
Q5XI73	Rho GDP-dissociation inhibitor 1 (Rho GDI 1) (Rho-GDI alpha)	4	1 von 4	0,311	3	1 von 4	0,145	0,166	2,144827586	114,4827586

Supplementary table 1. HC - secretome

accession number	protein name	analysis peptides	analysis secreted expcount	analysis secreted emPAI	reference peptides	reference secreted expcount	reference secreted emPAI	emPAI diff secreted	x-times up-regulated	alteration in %
Q63276	Bile acid-CoA:amino acid N-acyltransferase (BACAT) (Glycine N-choloyltransferase) (Kan-1) (Long-chain fatty-acyl-CoA hydrolase)	7	1 von 4	0,318	7	2 von 4	0,149	0,169	2,134228188	113,4228188
P09034	Argininosuccinate synthase (Citrulline--aspartate ligase)	16	1 von 4	0,241	8	2 von 4	0,115	0,126	2,095652174	109,5652174
P18292	Prothrombin (Coagulation factor II)	4	3 von 4	0,111	1	1 von 4	0,054	0,057	2,055555556	105,5555556
P08661	Mannose-binding protein C (MBP-C) (Mannan-binding protein) (Ra-reactive factor polysaccharide-binding component p28A) (RaRF p28A)	3	2 von 4	0,406	2	3 von 4	0,2	0,206	2,03	103
P52759	Ribonuclease UK114 (14.5 kDa translational inhibitor protein) (Perchloric acid soluble protein)	11	4 von 4	5.423	10	3 von 4	2.835	2.588	1,91287478	91,28747795
P08025	Insulin-like growth factor I (IGF-I) (Somatomedin)	4	2 von 4	0,911	2	2 von 4	0,48	0,431	1,897916667	89,79166667
P26644	Beta-2-glycoprotein 1 (Beta-2-glycoprotein I) (Beta(2)GPI) (Apolipoprotein H) (Apo-H)	5	3 von 4	0,293	3	2 von 4	0,181	0,112	1,61878453	61,87845304
P02761	Major urinary protein (MUP) (Alpha-2u-globulin) (Alpha(2)-euglobulin) (Allergen Rat n I)	22	4 von 4	13.407	19	4 von 4	8.297	5,11	1,61588526	61,58852597
Q9Z0V6	Thioredoxin-dependent peroxide reductase, mitochondrial (Peroxiredoxin-3) (PRX-3) (PRx III)	5	2 von 4	0,228	4	1 von 4	0,145	0,083	1,572413793	57,24137931
P16391	RT1 class I histocompatibility antigen, AA alpha chain	3	3 von 4	0,197	3	3 von 4	0,126	0,071	1,563492063	56,34920635
P15257	Hepatocyte nuclear factor 1-alpha (HNF-1-alpha) (Liver-specific transcription factor LF-B1) (LFB1) (Transcription factor 1) (TCF-1)	2	2 von 4	0,181	1	1 von 4	0,116	0,065	1,560344828	56,03448276
Q01177	Plasminogen	11	4 von 4	0,265	10	3 von 4	0,176	0,089	1,505681818	50,56818182
Q8VHZ8	Down syndrome cell adhesion molecule homolog	2	2 von 4	0,025	1	1 von 4	0,017	0,008	1,470588235	47,05882353

found in both	min. 2 exp, min. 2 peptides, delta of ≤-1.45 or -45% upon NAF treatment					sorted according to the magnitude of the regulation				
accession number	protein name	analysis peptides	analysis secreted expcount	analysis secreted emPAI	reference peptides	reference secreted expcount	reference secreted emPAI	emPAI diff secreted	x-times down-regulated	alteration in %
P62963	Profilin-1 (Profilin I)	5	3 von 4	0,476	6	1 von 4	2.162	-1.686	-4542,016807	-454101,6807
P62898	Cytochrome c, somatic	7	3 von 4	0,789	4	1 von 4	1.783	-0,994	-2259,82256	-225882,256
P06866	Haptoglobin (Liver regeneration-related protein LRRG173)	17	4 von 4	0,945	21	4 von 4	2.079	-1.134	-2200	-219900
P02401	60S acidic ribosomal protein P2	3	2 von 4	0,811	3	2 von 4	1.658	-0,847	-2044,389642	-204338,9642
P04041	Glutathione peroxidase 1 (GSHPx-1) (Cellular glutathione peroxidase)	7	4 von 4	0,656	11	4 von 4	1.266	-0,61	-1929,878049	-192887,8049
Q05982	Nucleoside diphosphate kinase A (NDP kinase A) (Tumor metastatic process-associated protein) (Metastasis inhibition factor NM23)	8	3 von 4	0,735	8	3 von 4	1.206	-0,471	-1640,816327	-163981,6327
Q00715	Histone H2B type 1	6	4 von 4	0,747	6	2 von 4	1.187	-0,44	-1589,022758	-158802,2758
O55004	Ribonuclease 4 (RNase 4) (RL3)	4	4 von 4	0,983	4	3 von 4	1.165	-0,182	-1185,147508	-118414,7508
P07824	Arginase-1 (Type I arginase) (Liver-type arginase)	20	2 von 4	0,995	16	3 von 4	1.139	-0,144	-1144,723618	-114372,3618
Q64240	Protein AMBP	17	4 von 4	1,5	16	4 von 4	1.565	-0,065	-1043,333333	-104233,3333
P02767	Transthyretin (Prealbumin) (TBPA)	10	4 von 4	4,43	9	4 von 4	1.739	2.691	-392,5507901	-39155,07901
Q9Z1P2	Alpha-actinin-1 (Alpha-actinin cytoskeletal isoform) (Non-muscle alpha-actinin-1) (F-actin cross-linking protein)	2	1 von 4	0,034	17	1 von 4	0,764	-0,73	-22,47058824	-2147,058824
Q9QXQ0	Alpha-actinin-4 (Non-muscle alpha-actinin 4) (F-actin cross-linking protein)	5	1 von 4	0,033	9	1 von 4	0,345	-0,312	-10,45454545	-945,4545455
P06238	Alpha-2-macroglobulin (Alpha-2-M)	4	3 von 4	0,04	20	3 von 4	0,238	-0,198	-5,95	-495
O88989	Malate dehydrogenase, cytoplasmic (Cytosolic malate dehydrogenase)	6	1 von 4	0,096	9	4 von 4	0,49	-0,394	-5,104166667	-410,4166667
Q5M8C6	Fibrinogen-like protein 1	2	3 von 4	0,101	6	2 von 4	0,475	-0,374	-4,702970297	-370,2970297
P32755	4-hydroxyphenylpyruvate dioxygenase (4-hydroxyphenylpyruvic acid oxidase) (HPPDase) (F Alloantigen) (F protein)	8	1 von 4	0,077	7	1 von 4	0,346	-0,269	-4,493506494	-349,3506494
P19112	Fructose-1,6-bisphosphatase 1 (FBPase 1) (D-fructose-1,6-bisphosphate 1-phosphohydrolase 1)	15	2 von 4	0,172	11	1 von 4	0,688	-0,516	-4	-300
P55051	Fatty acid-binding protein, brain (Brain-type fatty acid-binding protein) (B-FABP) (Fatty acid-binding protein 7) (Brain lipid-binding protein) (BLBP)	4	1 von 4	0,212	3	1 von 4	0,778	-0,566	-3,669811321	-266,9811321
P25093	Fumarylacetoacetase (FAA) (Fumarylacetoacetate hydrolase) (Beta-diketonase)	7	2 von 4	0,19	9	2 von 4	0,68	-0,49	-3,578947368	-257,8947368
Q7M0E3	Destrin (Actin-depolymerizing factor) (ADF)	7	1 von 4	0,179	6	1 von 4	0,638	-0,459	-3,56424581	-256,424581
Q6QMY6	Tsukushin (Tsukushi) (Leucine-rich repeat-containing protein 54) (Early insulin-induced hepatic gene protein) (EIIH)	1	1 von 4	0,166	3	1 von 4	0,585	-0,419	-3,524096386	-252,4096386
P13221	Aspartate aminotransferase, cytoplasmic (Transaminase A) (Glutamate oxaloacetate transaminase 1)	7	1 von 4	0,072	8	2 von 4	0,245	-0,173	-3,402777778	-240,2777778
Q9Z339	Glutathione S-transferase omega-1 (GSTO-1) (Glutathione-dependent dehydroascorbate reductase)	2	1 von 4	0,129	3	1 von 4	0,438	-0,309	-3,395348837	-239,5348837
P11915	Non-specific lipid-transfer protein (NSL-TP) (Propanoyl-CoA C-acyltransferase) (Sterol carrier protein 2) (SCP-2) (Sterol carrier protein X) (SCP-X) (SCP-chi) (SCPX)	12	2 von 4	0,122	8	1 von 4	0,413	-0,291	-3,385245902	-238,5245902

Supplementary table 1. HC - secretome

accession number	protein name	analysis peptides	analysis secreted expcount	analysis secreted emPAI	reference peptides	reference secreted expcount	reference secreted emPAI	emPAI diff secreted	x-times down-regulated	alteration in %
O35077	Glycerol-3-phosphate dehydrogenase [NAD+], cytoplasmic (GPDH-C)	10	3 von 4	0,126	11	3 von 4	0,419	-0,293	-3,325396825	-232,5396825
P45592	Cofilin-1 (Cofilin, non-muscle isoform)	4	1 von 4	0,179	5	2 von 4	0,555	-0,376	-3,100558659	-210,0558659
P55159	Serum paraoxonase/arylesterase 1 (PON 1) (Serum aryldialkylphosphatase 1) (Aromatic esterase 1) (A-esterase 1)	5	2 von 4	0,172	4	1 von 4	0,52	-0,348	-3,023255814	-202,3255814
P20961	Plasminogen activator inhibitor 1 (PAI-1) (Endothelial plasminogen activator inhibitor) (Serpin E1)	4	4 von 4	0,157	11	4 von 4	0,467	-0,31	-2,974522293	-197,4522293
Q9WVK7	Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial (HCDH) (Short-chain 3-hydroxyacyl-CoA dehydrogenase) (Medium and short-chain L-3-hydroxyacyl-coenzyme A dehydrogenase)	8	3 von 4	0,11	6	4 von 4	0,308	-0,198	-2,8	-180
P10959	Liver carboxylesterase 1 (Carboxyesterase ES-1) (E1) (ES-THET) (Esterase-2) (Retinyl ester hydrolase) (REH) (Neutral retinyl ester hydrolase) (NREH)	3	3 von 4	0,08	4	3 von 4	0,202	-0,122	-2,525	-152,5
P06214	Delta-aminolevulinic acid dehydratase (ALADH) (Porphobilinogen synthase)	2	2 von 4	0,116	5	4 von 4	0,285	-0,169	-2,456896552	-145,6896552
P61459	Pterin-4-alpha-carbinolamine dehydratase (PHS) (4-alpha-hydroxy-tetrahydropterin dehydratase) (Phenylalanine hydroxylase-stimulating protein) (Pterin carbinolamine dehydratase) (PCD) (Dimerization cofactor of hepatocyte nuclear factor 1-alpha) (Dimerizati	5	3 von 4	0,542	5	1 von 4	1,31	-0,768	-2,41697417	-141,697417
Q03336	Regucalcin (RC) (Senescence marker protein 30) (SMP-30)	11	3 von 4	0,224	11	4 von 4	0,531	-0,307	-2,370535714	-137,0535714
P26772	10 kDa heat shock protein, mitochondrial (Hsp10) (10 kDa chaperonin) (Chaperonin 10) (CPN10)	10	2 von 4	0,376	8	1 von 4	0,874	-0,498	-2,324468085	-132,4468085
P62986	Ubiquitin-60S ribosomal protein L40 (Ubiquitin A-52 residue ribosomal protein fusion product 1)	3	1 von 4	0,311	4	1 von 4	0,719	-0,408	-2,311897106	-131,1897106
Q5I0P2	Glycine cleavage system H protein, mitochondrial	1	2 von 4	0,233	2	1 von 4	0,52	-0,287	-2,231759657	-123,1759657
Q3T1J1	Eukaryotic translation initiation factor 5A-1 (eIF-5A-1) (Eukaryotic initiation factor 5A isoform 1) (eIF-5A) (eIF-4D)	8	1 von 4	0,212	7	1 von 4	0,468	-0,256	-2,20754717	-120,754717
P21744	Insulin-like growth factor-binding protein 4 (IGF-binding protein 4)	1	2 von 4	0,145	4	3 von 4	0,319	-0,174	-2,2	-120
P13255	Glycine N-methyltransferase (Folate-binding protein)	9	3 von 4	0,431	10	3 von 4	0,94	-0,509	-2,180974478	-118,0974478
P36972	Adenine phosphoribosyltransferase (APRT)	4	1 von 4	0,179	3	1 von 4	0,389	-0,21	-2,173184358	-117,3184358
P40307	Proteasome subunit beta type-2 (Proteasome component C7-I) (Macropain subunit C7-I) (Multicatalytic endopeptidase complex subunit C7-I)	1	3 von 4	0,179	2	1 von 4	0,389	-0,21	-2,173184358	-117,3184358
P14942	Glutathione S-transferase alpha-4 (Glutathione S-transferase Yk) (GST Yk) (GST 8-8) (GST K) (GST A4-4)	3	2 von 4	0,166	4	3 von 4	0,359	-0,193	-2,162650602	-116,2650602
Q63556	Serine protease inhibitor A3M (Serpin A3M) (Serine protease inhibitor 2.4) (SPI-2.4)	3	4 von 4	0,129	6	4 von 4	0,277	-0,148	-2,147286822	-114,7286822
Q6AYQ8	Fumarylacetoacetate hydrolase domain-containing protein 1	6	2 von 4	0,145	5	1 von 4	0,311	-0,166	-2,144827586	-114,4827586
O70351	3-hydroxyacyl-CoA dehydrogenase type-2 (3-hydroxyacyl-CoA dehydrogenase type II) (Type II HADH) (3-hydroxy-2-methylbutyryl-CoA dehydrogenase) (17-beta-hydroxysteroid dehydrogenase 10) (17-beta-HSD 10) (Mitochondrial ribonuclease P protein 2) (Mitochondria	20	3 von 4	0,455	15	1 von 4	0,968	-0,513	-2,127472527	-112,7472527
Q5M889	Apolipoprotein F (Apo-F) (Liver regeneration-related protein LRRG151)	2	3 von 4	0,129	3	2 von 4	0,274	-0,145	-2,124031008	-112,4031008
P23457	3-alpha-hydroxysteroid dehydrogenase (3-alpha-HSD) (Hydroxyprostaglandin dehydrogenase)	8	2 von 4	0,083	5	3 von 4	0,175	-0,092	-2,108433735	-110,8433735
P04762	Catalase	16	3 von 4	0,143	14	3 von 4	0,301	-0,158	-2,104895105	-110,4895105
P41562	Isocitrate dehydrogenase [NADP] cytoplasmic (IDH) (Cytosolic NADP-isocitrate dehydrogenase) (Oxalosuccinate decarboxylase) (NADP(+)-specific ICDH) (IDP)	6	1 von 4	0,062	6	1 von 4	0,129	-0,067	-2,080645161	-108,0645161
Q6P6R2	Dihydrolipoyl dehydrogenase, mitochondrial (Dihydrolipoamide dehydrogenase)	8	1 von 4	0,064	8	1 von 4	0,133	-0,069	-2,078125	-107,8125
P50137	Transketolase (TK)	8	1 von 4	0,054	9	2 von 4	0,112	-0,058	-2,074074074	-107,4074074
P97852	Peroxisomal multifunctional enzyme type 2 (MFE-2) (D-bifunctional protein) (DBP) (17-beta-hydroxysteroid dehydrogenase 4) (17-beta-HSD 4)	13	1 von 4	0,051	6	1 von 4	0,105	-0,054	-2,058823529	-105,8823529
P02793	Ferritin light chain 1 (Ferritin L subunit 1)	9	2 von 4	0,408	9	3 von 4	0,833	-0,425	-2,041666667	-104,1666667
P19804	Nucleoside diphosphate kinase B (NDP kinase B) (P18)	11	3 von 4	1.084	12	3 von 4	2.187	-1.103	-2,017527675	-101,7527675
Q6DGG1	Abhydrolase domain-containing protein 14B	5	2 von 4	0,34	6	4 von 4	0,653	-0,313	-1,920588235	-92,05882353
O09171	Betaine--homocysteine S-methyltransferase 1	15	3 von 4	0,316	16	4 von 4	0,594	-0,278	-1,879746835	-87,97468354
P00502	Glutathione S-transferase alpha-1 (Glutathione S-transferase Ya-1) (GST Ya1) (Ligandin) (GST 1a-1a) (GST B) (GST 1-1) (GST A1-1)	4	4 von 4	0,365	7	4 von 4	0,673	-0,308	-1,843835616	-84,38356164
Q62930	Complement component C9	2	3 von 4	0,087	6	3 von 4	0,16	-0,073	-1,83908046	-83,90804598
P02764	Alpha-1-acid glycoprotein (Orosomucoid) (OMD)	3	3 von 4	0,283	6	4 von 4	0,52	-0,237	-1,83745583	-83,74558304
P04764	Alpha-enolase (2-phospho-D-glycerate hydro-lyase) (Non-neural enolase) (NNE) (Enolase 1)	14	2 von 4	0,163	7	2 von 4	0,298	-0,135	-1,828220859	-82,82208589
P62804	Histone H4	6	4 von 4	1.857	6	3 von 4	3.379	-1.522	-1,819601508	-81,96015078
Q7TMA5	Apolipoprotein B-100 (Apo B-100)	23	4 von 4	0,062	33	3 von 4	0,112	-0,05	-1,806451613	-80,64516129
Q9WUW3	Complement factor I (C3B/C4B inactivator)	2	1 von 4	0,11	8	3 von 4	0,198	-0,088	-1,8	-80

Supplementary table 1. HC - secretome

accession number	protein name	analysis peptides	analysis secreted expcount	analysis secreted emPAI	reference peptides	reference secreted expcount	reference secreted emPAI	emPAI diff secreted	x-times down-regulated	alteration in %
P07895	Superoxide dismutase [Mn], mitochondrial	5	2 von 4	0,244	4	2 von 4	0,437	-0,193	-1,790983607	-79,09836066
P04903	Glutathione S-transferase alpha-2 (Glutathione S-transferase Ya-2) (GST Ya2) (GST 1b-1b) (GST A2-2)	3	3 von 4	0,319	7	4 von 4	0,563	-0,244	-1,764890282	-76,48902821
P25113	Phosphoglycerate mutase 1 (Phosphoglycerate mutase isozyme B) (PGAM-B) (BPG-dependent PGAM 1)	5	2 von 4	0,202	6	2 von 4	0,356	-0,154	-1,762376238	-76,23762376
P08649	Complement C4	7	4 von 4	0,056	10	3 von 4	0,097	-0,041	-1,732142857	-73,21428571
P05197	Elongation factor 2 (EF-2)	17	2 von 4	0,036	12	3 von 4	0,061	-0,025	-1,694444444	-69,44444444
P18757	Cystathionine gamma-lyase (Gamma-cystathionase) (Probasin-related antigen) (PRB-RA)	8	2 von 4	0,408	12	2 von 4	0,688	-0,28	-1,68627451	-68,62745098
P11232	Thioredoxin (Trx)	3	2 von 4	0,334	2	2 von 4	0,556	-0,222	-1,664670659	-66,46706587
P04904	Glutathione S-transferase alpha-3 (Glutathione S-transferase Yc-1) (GST Yc1) (GST 2-2) (GST AA) (GST A3-3)	5	2 von 4	0,311	7	4 von 4	0,508	-0,197	-1,633440514	-63,34405145
P84245	Histone H3.3	3	3 von 4	0,233	2	2 von 4	0,376	-0,143	-1,613733906	-61,37339056
P31044	Phosphatidylethanolamine-binding protein 1 (PEBP-1) (HCNPpp) (23 kDa morphine-binding protein) (P23K)	5	4 von 4	0,623	8	4 von 4	0,999	-0,376	-1,6035313	-60,35313002
P07872	Peroxisomal acyl-coenzyme A oxidase 1 (AOX) (Palmitoyl-CoA oxidase)	17	2 von 4	0,103	8	3 von 4	0,165	-0,062	-1,601941748	-60,19417476
P83121	Urinary protein 3 (UP-3)	1	3 von 4	0,778	2	3 von 4	1,24	-0,462	-1,593830334	-59,38303342
P62260	14-3-3 protein epsilon (14-3-3E) (Mitochondrial import stimulation factor L subunit) (MSF L)	5	1 von 4	0,212	7	1 von 4	0,334	-0,122	-1,575471698	-57,54716981
P60711	Actin, cytoplasmic 1 (Beta-actin)	5	1 von 4	0,318	7	2 von 4	0,501	-0,183	-1,575471698	-57,54716981
Q9QX79	Fetuin-B (Fetuin-like protein IRL685)	1	3 von 4	0,11	2	4 von 4	0,172	-0,062	-1,563636364	-56,36363636
Q02974	Ketohexokinase (Hepatic fructokinase)	4	3 von 4	0,138	4	3 von 4	0,215	-0,077	-1,557971014	-55,79710145
P14408	Fumarate hydratase, mitochondrial (Fumarase)	6	1 von 4	0,068	4	2 von 4	0,104	-0,036	-1,529411765	-52,94117647
Q63716	Peroxiredoxin-1 (Thioredoxin peroxidase 2) (Thioredoxin-dependent peroxide reductase 2) (Heme-binding 23 kDa protein) (HBP23)	16	4 von 4	1.776	20	4 von 4	2.655	-0,879	-1,494932432	-49,49324324
P35704	Peroxiredoxin-2 (Thioredoxin peroxidase 1) (Thioredoxin-dependent peroxide reductase 1) (Thiol-specific antioxidant protein) (TSA)	2	2 von 4	0,34	4	4 von 4	0,495	-0,155	-1,455882353	-45,58823529
P02650	Apolipoprotein E (Apo-E)	17	4 von 4	1.926	23	4 von 4	2.799	-0,873	-1,453271028	-45,3271028

Supplementary table 2. HC - cytoplasmic protein fraction

found only in NAF samples	min. 2 experiments, min. 2 peptides, DMSO only 1 expermient		sorted according to the magnitude of the emPAI value	NAF 2 exp, DMSO 1 exp
accession number	protein name	analysis peptides	analysis cytoplasm emPAI	
P07871	3-ketoacyl-CoA thiolase B, peroxisomal (Beta-ketothiolase B) (Acetyl-CoA acyltransferase B) (Peroxisomal 3-oxoacyl-CoA thiolase B)	11	1.014	
P23358	60S ribosomal protein L12	6	0,911	
P60868	40S ribosomal protein S20	2	0,778	
P62832	60S ribosomal protein L23	2	0,585	
P13471	40S ribosomal protein S14	3	0,556	
P62850	40S ribosomal protein S24	3	0,553	
O88267	Acyl-coenzyme A thioesterase 1 (Acyl-CoA thioesterase 1) (Inducible cytosolic acyl-coenzyme A thioester hydrolase) (Long chain acyl-CoA thioester hydrolase) (Long chain acyl-CoA hydrolase) (CTE-I) (LACH2) (ACH2)	6	0,532	
P83732	60S ribosomal protein L24 (L30)	2	0,468	
Q63228	Glia maturation factor beta (GMF-beta)	3	0,376	
P63326	40S ribosomal protein S10	4	0,323	
Q9R1Z0	Voltage-dependent anion-selective channel protein 3 (VDAC-3) (Outer mitochondrial membrane protein porin 3)	3	0,317	
Q4V8F9	Hydroxysteroid dehydrogenase-like protein 2	4	0,263	
P62914	60S ribosomal protein L11	2	0,263	
Q64550	UDP-glucuronosyltransferase 1-1 (UDPGT 1-1) (UDP-glucuronosyltransferase 1A1) (B1)	5	0,244	
P13086	Succinyl-CoA ligase [GDP-forming] subunit alpha, mitochondrial (Succinyl-CoA synthetase subunit alpha) (SCS-alpha)	2	0,228	
Q03248	Beta-ureidopropionase (Beta-alanine synthase) (N-carbamoyl-beta-alanine amidohydrolase)	3	0,179	
Q66X93	Staphylococcal nuclease domain-containing protein 1 (p100 co-activator) (100 kDa coactivator) (p105 coactivator) (SND p102)	9	0,174	
Q5XIE6	3-hydroxyisobutyryl-CoA hydrolase, mitochondrial (3-hydroxyisobutyryl-coenzyme A hydrolase) (HIB-CoA hydrolase)	3	0,148	
Q0VGK3	Glycerate kinase	2	0,143	
Q99PF5	Far upstream element-binding protein 2 (FUSE-binding protein 2) (KH type-splicing regulatory protein) (KSRP) (MAP2 RNA trans-acting protein 1) (MARTA1)	5	0,134	
P36970	Phospholipid hydroperoxide glutathione peroxidase, mitochondrial (PHGPx) (Glutathione peroxidase 4) (GSHPx-4)	2	0,129	
Q03346	Mitochondrial-processing peptidase subunit beta (Beta-MPP) (P-52)	2	0,119	
Q08163	Adenylyl cyclase-associated protein 1 (CAP 1)	2	0,115	
Q8K4G6	MACRO domain-containing protein 1 (Protein LRP16)	2	0,11	
P55159	Serum paraoxonase/arylesterase 1 (PON 1) (Serum aryldialkylphosphatase 1) (Aromatic esterase 1) (A-esterase 1)	5	0,11	
P02650	Apolipoprotein E (Apo-E)	17	0,101	
P16638	ATP-citrate synthase (ATP-citrate (pro-S-)-lyase) (Citrate cleavage enzyme)	5	0,1	
Q63108	Liver carboxylesterase 3 (Carboxyesterase ES-3) (pI 5.5 esterase) (ES-HTEL)	3	0,086	
Q07205	Eukaryotic translation initiation factor 5 (eIF-5)	2	0,077	
Q58FK9	Kynurenine--oxoglutarate transaminase 3 (Kynurenine--oxoglutarate transaminase III) (Kynurenine aminotransferase III) (KATIII) (Cysteine-S-conjugate beta-lyase 2) (Kynurenine--glyoxylate transaminase)	2	0,075	
P38659	Protein disulfide-isomerase A4 (Endoplasmic reticulum resident protein 72) (ER protein 72) (Endoplasmic reticulum resident protein 70) (ER protein 70) (Calcium-binding protein 2) (CaBP2)	3	0,075	
P50475	Alanyl-tRNA synthetase, cytoplasmic (Alanine--tRNA ligase) (AlaRS)	3	0,072	
Q9WTT6	Guanine deaminase (Guanase) (Guanine aminohydrolase) (GAH)	2	0,064	
Q5U300	Ubiquitin-like modifier-activating enzyme 1 (Ubiquitin-activating enzyme E1)	2	0,053	
Q63617	Hypoxia up-regulated protein 1 (150 kDa oxygen-regulated protein) (ORP-150)	2	0,047	
Q5XHY5	Threonyl-tRNA synthetase, cytoplasmic (Threonine--tRNA ligase) (ThrRS)	2	0,042	

Supplementary table 2. HC - cytoplasmic protein fraction

only in ref/DMSO	min. 2 experiments, min. 2 peptides		sorted according to the magnitude of the emPAI value	only 1 experiment of DMSO cyt
accession number	protein name	reference peptides	reference cytoplasm emPAI	
Q923M1	Peptide methionine sulfoxide reductase (Protein-methionine-S-oxide reductase) (PMSR) (Peptide-methionine (S)-S-oxide reductase) (Peptide Met(O) reductase)	2	0,259	
P28075	Proteasome subunit beta type-5 (Proteasome epsilon chain) (Macropain epsilon chain) (Multicatalytic endopeptidase complex epsilon chain) (Proteasome subunit X) (Proteasome chain 6)	2	0,136	

found in both	min. 2 exp, min. 2 peptides, delta of ≥2 or 50% upon NAF treatment				sorted according to the magnitude of the regulation				only 1 experiment of DMSO cyt	
accession number	protein name	analysis peptides	analysis cytoplasm expcount	analysis cytoplasm emPAI	reference peptides	reference cytoplasm expcount	reference cytoplasm emPAI	emPAI diff cytoplasm	x-times up-regulated	alteration in %
P84079	ADP-ribosylation factor 1	6	1 von 2	1.512	1	1 von 1	0,166	1.346	9108,433735	910743,3735
Q06647	ATP synthase subunit O, mitochondrial (Oligomycin sensitivity conferral protein) (OSCP)	11	2 von 2	1.767	2	1 von 1	0,334	1.433	5290,419162	528941,9162
P04916	Retinol-binding protein 4 (Plasma retinol-binding protein) (PRBP)	11	1 von 2	1.894	11	1 von 1	0,425	1.469	4456,470588	445547,0588
P62898	Cytochrome c, somatic	7	2 von 2	2.712	4	1 von 1	0,668	2.044	4059,88024	405888,024
Q63362	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 5 (NADH-ubiquinone oxidoreductase 13 kDa-B subunit) (Complex I-13kD-B) (CI-13kD-B) (Complex I subunit B13)	9	2 von 2	2.831	3	1 von 1	0,778	2.053	3638,817481	363781,7481
Q9WVA1	Mitochondrial import inner membrane translocase subunit Tim8 A (Deafness dystonia protein 1 homolog)	3	2 von 2	1.307	1	1 von 1	0,389	0,918	3359,897172	335889,7172
B0K020	CDGSH iron sulfur domain-containing protein 1 (MitoNEET)	4	2 von 2	1.075	1	1 von 1	0,334	0,741	3218,562874	321756,2874
Q9Z2L0	Voltage-dependent anion-selective channel protein 1 (VDAC-1) (Outer mitochondrial membrane protein porin 1)	9	2 von 2	1.207	3	1 von 1	0,438	0,769	2755,707763	275470,7763
P11240	Cytochrome c oxidase subunit 5A, mitochondrial (Cytochrome c oxidase polypeptide Va)	7	2 von 2	1.361	2	1 von 1	0,52	0,841	2617,307692	261630,7692
Q02253	Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial (Malonate-semialdehyde dehydrogenase [acylating]) (Aldehyde dehydrogenase family 6 member A1)	16	2 von 2	1.226	8	1 von 1	0,668	0,558	1835,329341	183432,9341
P62959	Histidine triad nucleotide-binding protein 1 (Adenosine 5'-monophosphoramidase) (Protein kinase C inhibitor 1) (Protein kinase C-interacting protein 1) (PKCI-1) (17 kDa inhibitor of protein kinase C)	4	2 von 2	1.225	2	1 von 1	0,668	0,557	1833,832335	183283,2335
P00406	Cytochrome c oxidase subunit 2 (Cytochrome c oxidase polypeptide II)	3	1 von 2	1.154	2	1 von 1	0,668	0,486	1727,54491	172654,491
P09034	Argininosuccinate synthase (Citrulline--aspartate ligase)	16	2 von 2	1.241	8	1 von 1	0,778	0,463	1595,115681	159411,5681
Q6AYQ8	Fumarylacetoacetate hydrolase domain-containing protein 1	6	2 von 2	1.111	5	1 von 1	0,719	0,392	1545,201669	154420,1669
O09171	Betaine--homocysteine S-methyltransferase 1	15	2 von 2	1.183	16	1 von 1	0,812	0,371	1456,896552	145589,6552
Q7M0E3	Destrin (Actin-depolymerizing factor) (ADF)	7	2 von 2	1.276	6	1 von 1	0,931	0,345	1370,56928	136956,928
P21775	3-ketoacyl-CoA thiolase A, peroxisomal (Beta-ketothiolase A) (Acetyl-CoA acyltransferase A) (Peroxisomal 3-oxoacyl-CoA thiolase A)	12	2 von 2	1.263	7	1 von 1	0,957	0,306	1319,749216	131874,9216
P14604	Enoyl-CoA hydratase, mitochondrial (Short-chain enoyl-CoA hydratase) (SCEH) (Enoyl-CoA hydratase 1)	13	2 von 2	1.621	10	1 von 1	1,31	0,311	1237,40458	123640,458
P63039	60 kDa heat shock protein, mitochondrial (Heat shock protein 60) (HSP-60) (60 kDa chaperonin) (Chaperonin 60) (CPN60) (Mitochondrial matrix protein P1) (HSP-65)	35	2 von 2	2.818	22	1 von 1	2,34	0,478	1204,273504	120327,3504
P00884	Fructose-bisphosphate aldolase B (Liver-type aldolase)	10	2 von 2	1.075	9	1 von 1	0,957	0,118	1123,301985	112230,1985
Q3T1J1	Eukaryotic translation initiation factor 5A-1 (eIF-5A-1) (Eukaryotic initiation factor 5A isoform 1) (eIF-5A) (eIF-4D)	8	2 von 2	1.805	7	1 von 1	1,61	0,195	1121,118012	112011,8012
P22791	Hydroxymethylglutaryl-CoA synthase, mitochondrial (HMG-CoA synthase) (3-hydroxy-3-methylglutaryl coenzyme A synthase)	15	2 von 2	1.394	12	1 von 1	1,31	0,084	1064,122137	106312,2137
P00481	Ornithine carbamoyltransferase, mitochondrial (Ornithine transcarbamylase) (OTCase)	16	2 von 2	1.244	14	1 von 1	1,61	-0,366	772,6708075	77167,08075
P07896	Peroxisomal bifunctional enzyme (PBE)	20	2 von 2	0,716	2	1 von 1	0,094	0,622	7,617021277	661,7021277
O88618	Formimidoyltransferase-cyclodeaminase (Formiminotransferase-cyclodeaminase) (FTCD) (58 kDa microtubule-binding protein)	13	2 von 2	0,812	4	1 von 1	0,141	0,671	5,758865248	475,8865248
P29266	3-hydroxyisobutyrate dehydrogenase, mitochondrial (HIBADH)	6	2 von 2	0,604	1	1 von 1	0,11	0,494	5,490909091	449,0909091
P50170	Retinol dehydrogenase 2 (Retinol dehydrogenase type II) (RODH II) (29 kDa protein)	4	1 von 2	0,551	1	1 von 1	0,116	0,435	4,75	375
P02767	Transthyretin (Prealbumin) (TBPA)	10	2 von 2	0,966	9	1 von 1	0,212	0,754	4,556603774	355,6603774
Q64565	Alanine--glyoxylate aminotransferase 2, mitochondrial (AGT 2) ((R)-3-amino-2-methylpropionate--pyruvate transaminase) (Beta-alanine-pyruvate aminotransferase) (Beta-ALAAT II) (D-AIBAT)	5	2 von 2	0,237	1	1 von 1	0,062	0,175	3,822580645	282,2580645

Supplementary table 2. HC - cytoplasmic protein fraction

accession number	protein name	analysis peptides	analysis cytoplasm expcount	analysis cytoplasm emPAI	reference peptides	reference cytoplasm expcount	reference cytoplasm emPAI	emPAI diff cytoplasm	x-times up-regulated	alteration in %
P12785	Fatty acid synthase	17	1 von 2	0,3	5	1 von 1	0,08	0,22	3,75	275
Q64057	Alpha-aminoadipic semialdehyde dehydrogenase (Alpha-AASA dehydrogenase) (Betaine aldehyde dehydrogenase) (Delta1-piperideine-6-carboxylate dehydrogenase) (P6c dehydrogenase) (Aldehyde dehydrogenase family 7 member A1) (Antiquitin-1)	10	2 von 2	0,553	2	1 von 1	0,15	0,403	3,686666667	268,6666667
P81155	Voltage-dependent anion-selective channel protein 2 (VDAC-2) (Outer mitochondrial membrane protein porin 2) (B36-VDAC)	4	2 von 2	0,468	1	1 von 1	0,136	0,332	3,441176471	244,1176471
P49432	Pyruvate dehydrogenase E1 component subunit beta, mitochondrial (PDHE1-B)	4	2 von 2	0,438	1	1 von 1	0,129	0,309	3,395348837	239,5348837
P07872	Peroxisomal acyl-coenzyme A oxidase 1 (AOX) (Palmitoyl-CoA oxidase)	17	2 von 2	0,729	8	1 von 1	0,216	0,513	3,375	237,5
P70473	Alpha-methylacyl-CoA racemase (2-methylacyl-CoA racemase) (2-arylpropionyl-CoA epimerase)	3	1 von 2	0,334	2	1 von 1	0,101	0,233	3,306930693	230,6930693
Q63060	Glycerol kinase (Glycerokinase) (ATP:glycerol 3-phosphotransferase) (ATP-stimulated glucocorticoid-receptor translocation promoter) (ASTP)	3	2 von 2	0,233	1	1 von 1	0,072	0,161	3,236111111	223,6111111
P28492	Glutaminase liver isoform, mitochondrial (GLS) (L-glutamine amidohydrolase) (L-glutaminase)	4	2 von 2	0,176	1	1 von 1	0,055	0,121	3,2	220
P97562	Peroxisomal acyl-coenzyme A oxidase 2 (3-alpha,7-alpha,12-alpha-trihydroxy-5-beta-cholestanoyl-CoA 24-hydroxylase) (3-alpha,7-alpha,12-alpha-trihydroxy-5-beta-cholestanoyl-CoA oxidase) (Trihydroxycoprostanoyl-CoA oxidase) (THCA-CoA oxidase)	3	1 von 2	0,142	1	1 von 1	0,045	0,097	3,155555556	215,5555556
P11915	Non-specific lipid-transfer protein (NSL-TP) (Propanoyl-CoA C-acyltransferase) (Sterol carrier protein 2) (SCP-2) (Sterol carrier protein X) (SCP-X) (SCP-chi) (SCPX)	12	2 von 2	0,588	8	1 von 1	0,189	0,399	3,111111111	211,1111111
A2VCW9	Alpha-aminoadipic semialdehyde synthase, mitochondrial (LKR/SDH)	4	2 von 2	0,115	1	1 von 1	0,037	0,078	3,108108108	210,8108108
P63029	Translationally-controlled tumor protein (TCTP) (Lens epithelial protein)	3	2 von 2	0,79	2	1 von 1	0,259	0,531	3,05019305	205,019305
P24329	Thiosulfate sulfurtransferase (Rhodanese)	6	2 von 2	0,65	4	1 von 1	0,222	0,428	2,927927928	192,7927928
Q63010	Liver carboxylesterase B-1 (Liver microsomal carboxylesterase)	5	1 von 2	0,377	2	1 von 1	0,136	0,241	2,772058824	177,2058824
Q64591	2,4-dienoyl-CoA reductase, mitochondrial (2,4-dienoyl-CoA reductase [NADPH]) (4-enoyl-CoA reductase [NADPH])	4	2 von 2	0,238	1	1 von 1	0,086	0,152	2,76744186	176,744186
Q8CG45	Aflatoxin B1 aldehyde reductase member 2 (rAFAR2) (Succinic semialdehyde reductase) (SSA reductase)	3	2 von 2	0,286	1	1 von 1	0,105	0,181	2,723809524	172,3809524
P70584	Short/branched chain specific acyl-CoA dehydrogenase, mitochondrial (SBCAD) (2-methyl branched chain acyl-CoA dehydrogenase) (2-MEBCAD) (2-methylbutyryl-coenzyme A dehydrogenase) (2-methylbutyryl-CoA dehydrogenase)	4	2 von 2	0,205	1	1 von 1	0,077	0,128	2,662337662	166,2337662
Q8CHM7	2-hydroxyacyl-CoA lyase 1 (2-hydroxyphytanoyl-CoA lyase) (2-HPCL) (Phytanoyl-CoA 2-hydroxylase 2)	4	2 von 2	0,179	1	1 von 1	0,068	0,111	2,632352941	163,2352941
Q1HCL7	UPF0465 protein C5orf33 homolog	3	2 von 2	0,155	1	1 von 1	0,059	0,096	2,627118644	162,7118644
Q5I0C3	Methylcrotonoyl-CoA carboxylase subunit alpha, mitochondrial (MCCase subunit alpha) (3-methylcrotonyl-CoA carboxylase 1) (3-methylcrotonyl-CoA:carbon dioxide ligase subunit alpha) (3-methylcrotonyl-CoA carboxylase biotin-containing subunit)	6	2 von 2	0,117	2	1 von 1	0,045	0,072	2,6	160
Q62651	Delta(3,5)-Delta(2,4)-dienoyl-CoA isomerase, mitochondrial	7	2 von 2	0,459	2	1 von 1	0,179	0,28	2,56424581	156,424581
P12346	Serotransferrin (Transferrin) (Siderophilin) (Beta-1 metal-binding globulin) (Liver regeneration-related protein LRRG03)	26	2 von 2	0,256	20	1 von 1	0,103	0,153	2,485436893	148,5436893
P16617	Phosphoglycerate kinase 1	6	2 von 2	0,334	4	1 von 1	0,136	0,198	2,455882353	145,5882353
Q9ER34	Aconitate hydratase, mitochondrial (Aconitase) (Citrate hydro-lyase)	12	2 von 2	0,327	3	1 von 1	0,134	0,193	2,440298507	144,0298507
Q68FS4	Cytosol aminopeptidase (Leucine aminopeptidase 3) (LAP-3) (Leucyl aminopeptidase) (Proline aminopeptidase) (Prolyl aminopeptidase)	12	2 von 2	0,499	3	1 von 1	0,205	0,294	2,434146341	143,4146341
P11232	Thioredoxin (Trx)	3	2 von 2	0,778	2	1 von 1	0,334	0,444	2,329341317	132,9341317
Q63270	Cytoplasmic aconitate hydratase (Aconitase) (Citrate hydro-lyase) (Iron-responsive element-binding protein 1) (IRE-BP 1) (Iron regulatory protein 1) (IRP1)	15	2 von 2	0,36	4	1 von 1	0,155	0,205	2,322580645	132,2580645
P09811	Glycogen phosphorylase, liver form	9	2 von 2	0,236	3	1 von 1	0,102	0,134	2,31372549	131,372549
O55171	Acyl-coenzyme A thioesterase 2, mitochondrial (Acyl-CoA thioesterase 2) (Acyl coenzyme A thioester hydrolase) (Very-long-chain acyl-CoA thioesterase) (MTE-I) (ARTISt/p43)	6	2 von 2	0,61	3	1 von 1	0,269	0,341	2,267657993	126,7657993
Q66HF1	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial	15	2 von 2	0,546	5	1 von 1	0,243	0,303	2,24691358	124,691358
P35435	ATP synthase subunit gamma, mitochondrial (F-ATPase gamma subunit)	8	2 von 2	0,912	4	1 von 1	0,413	0,499	2,208232446	120,8232446
Q07066	Peroxisomal membrane protein 2 (22 kDa peroxisomal membrane protein)	2	1 von 2	0,425	1	1 von 1	0,194	0,231	2,190721649	119,0721649
P12001	60S ribosomal protein L18	2	1 von 2	0,425	2	1 von 1	0,194	0,231	2,190721649	119,0721649
Q68FY0	Cytochrome b-c1 complex subunit 1, mitochondrial (Ubiquinol-cytochrome-c reductase complex core protein 1) (Core protein I) (Complex III subunit 1)	4	2 von 2	0,19	1	1 von 1	0,089	0,101	2,134831461	113,4831461

Supplementary table 2. HC - cytoplasmic protein fraction

accession number	protein name	analysis peptides	analysis cytoplasm expcount	analysis cytoplasm emPAI	reference peptides	reference cytoplasm expcount	reference cytoplasm emPAI	emPAI diff cytoplasm	x-times up-regulated	alteration in %
P80067	Dipeptidyl peptidase 1 (Dipeptidyl peptidase I) (DPP-I) (Cathepsin C) (Cathepsin J) (Dipeptidyl transferase)	3	2 von 2	0,183	1	1 von 1	0,086	0,097	2,127906977	112,7906977
P41123	60S ribosomal protein L13	2	1 von 2	0,259	1	1 von 1	0,122	0,137	2,12295082	112,295082
Q64602	Kynurenine/alpha-aminoadipate aminotransferase, mitochondrial (KAT/AadAT) (Kynurenine aminotransferase II) (Kynurenine--oxoglutarate aminotransferase II) (Kynurenine--oxoglutarate transaminase II) (2-aminoadipate transaminase) (2-aminoadipate aminotransfe	3	2 von 2	0,176	1	1 von 1	0,083	0,093	2,120481928	112,0481928
P19945	60S acidic ribosomal protein P0 (60S ribosomal protein L10E)	2	2 von 2	0,233	4	1 von 1	0,11	0,123	2,118181818	111,8181818
Q641Y2	NADH dehydrogenase [ubiquinone] iron-sulfur protein 2, mitochondrial (NADH-ubiquinone oxidoreductase 49 kDa subunit) (Complex I-49kD) (CI-49kD)	3	2 von 2	0,135	1	1 von 1	0,064	0,071	2,109375	110,9375
Q8VHT6	Arsenite methyltransferase (S-adenosyl-L-methionine:arsenic(III) methyltransferase) (Methylarsonite methyltransferase)	3	2 von 2	0,158	1	1 von 1	0,075	0,083	2,106666667	110,6666667
P35738	2-oxoisovalerate dehydrogenase subunit beta, mitochondrial (Branched-chain alpha-keto acid dehydrogenase E1 component beta chain) (BCKDH E1-beta)	2	1 von 2	0,212	1	1 von 1	0,101	0,111	2,099009901	109,9009901
Q02974	Ketohexokinase (Hepatic fructokinase)	4	2 von 2	0,212	4	1 von 1	0,101	0,111	2,099009901	109,9009901
P21533	60S ribosomal protein L6 (Neoplasm-related protein C140)	2	1 von 2	0,179	1	1 von 1	0,086	0,093	2,081395349	108,1395349
Q01205	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex, mitochondrial (Dihydrolipoamide succinyltransferase component of 2-oxoglutarate dehydrogenase complex) (2-oxoglutarate dehydrogenase complex component E2) (	3	2 von 2	0,179	1	1 von 1	0,086	0,093	2,081395349	108,1395349
Q9QUL6	Vesicle-fusing ATPase (N-ethylmaleimide-sensitive fusion protein) (NEM-sensitive fusion protein) (Vesicular-fusion protein NSF)	2	1 von 2	0,083	1	1 von 1	0,04	0,043	2,075	107,5
P13697	NADP-dependent malic enzyme (NADP-ME) (Malic enzyme 1)	2	1 von 2	0,122	1	1 von 1	0,059	0,063	2,06779661	106,779661
P21213	Histidine ammonia-lyase (Histidase)	4	2 von 2	0,105	1	1 von 1	0,051	0,054	2,058823529	105,8823529
Q07071	Glucokinase regulatory protein (Glucokinase regulator)	2	1 von 2	0,113	1	1 von 1	0,055	0,058	2,054545455	105,4545455
Q5XHZ0	Heat shock protein 75 kDa, mitochondrial (HSP 75) (Tumor necrosis factor type 1 receptor-associated protein) (TRAP-1) (TNFR-associated protein 1)	3	2 von 2	0,08	1	1 von 1	0,039	0,041	2,051282051	105,1282051
Q64428	Trifunctional enzyme subunit alpha, mitochondrial (TP-alpha)	14	2 von 2	0,573	6	1 von 1	0,28	0,293	2,046428571	104,6428571
P46462	Transitional endoplasmic reticulum ATPase (TER ATPase) (15S Mg(2+)-ATPase p97 subunit) (Valosin-containing protein) (VCP)	11	2 von 2	0,267	4	1 von 1	0,131	0,136	2,038167939	103,8167939
P97608	5-oxoprolinase (5-oxo-L-prolinase) (5-OPase) (Pyroglutamase)	2	1 von 2	0,063	1	1 von 1	0,031	0,032	2,032258065	103,2258065
O89000	Dihydropyrimidine dehydrogenase [NADP+] (DHPDHase) (Dihydrouracil dehydrogenase) (Dihydrothymine dehydrogenase)	3	2 von 2	0,075	1	1 von 1	0,037	0,038	2,027027027	102,7027027
P85972	Vinculin (Metavinculin)	2	1 von 2	0,05	5	1 von 1	0,025	0,025	2	100
Q5XIF6	Tubulin alpha-4A chain (Tubulin alpha-4 chain) (Alpha-tubulin 4)	5	2 von 2	0,359	2	1 von 1	0,186	0,173	1,930107527	93,01075269
P68370	Tubulin alpha-1A chain (Tubulin alpha-1 chain) (Alpha-tubulin 1)	5	2 von 2	0,359	2	1 von 1	0,186	0,173	1,930107527	93,01075269
P52847	Sulfotransferase family cytosolic 1B member 1 (Sulfotransferase 1B1) (DOPA/tyrosine sulfotransferase)	6	2 von 2	0,623	3	1 von 1	0,334	0,289	1,865269461	86,52694611
P97852	Peroxisomal multifunctional enzyme type 2 (MFE-2) (D-bifunctional protein) (DBP) (17-beta-hydroxysteroid dehydrogenase 4) (17-beta-HSD 4)	13	2 von 2	0,652	6	1 von 1	0,35	0,302	1,862857143	86,28571429
P15650	Long-chain specific acyl-CoA dehydrogenase, mitochondrial (LCAD)	4	2 von 2	0,298	2	1 von 1	0,16	0,138	1,8625	86,25
P46953	3-hydroxyanthranilate 3,4-dioxygenase (3-hydroxyanthranilic acid dioxygenase) (HAD) (3-hydroxyanthranilate oxygenase) (3-HAO)	7	2 von 2	0,65	4	1 von 1	0,35	0,3	1,857142857	85,71428571
Q924S5	Lon protease homolog, mitochondrial (Lon protease-like protein) (LONP) (Mitochondrial ATP-dependent protease Lon) (Serine protease 15)	7	2 von 2	0,123	2	1 von 1	0,068	0,055	1,808823529	80,88235294
P17764	Acetyl-CoA acetyltransferase, mitochondrial (Acetoacetyl-CoA thiolase)	11	2 von 2	0,752	5	1 von 1	0,417	0,335	1,803357314	80,33573141
P04636	Malate dehydrogenase, mitochondrial	13	2 von 2	2.018	14	1 von 1	1.154	0,864	1,748700173	74,87001733
P09118	Uricase (Urate oxidase)	7	2 von 2	0,584	3	1 von 1	0,334	0,25	1,748502994	74,8502994
Q4KLP0	Probable 2-oxoglutarate dehydrogenase E1 component DHKTD1, mitochondrial (Dehydrogenase E1 and transketolase domain-containing protein 1)	12	2 von 2	0,427	5	1 von 1	0,248	0,179	1,721774194	72,17741935
P31210	3-oxo-5-beta-steroid 4-dehydrogenase (Delta(4)-3-ketosteroid 5-beta-reductase) (Delta(4)-3-oxosteroid 5-beta-reductase) (Aldo-keto reductase family 1 member D1)	7	2 von 2	0,479	4	1 von 1	0,28	0,199	1,710714286	71,07142857
Q71UE8	NEDD8 (Ubiquitin-like protein NEDD8) (Neddylin)	3	1 von 2	0,995	2	1 von 1	0,585	0,41	1,700854701	70,08547009
P04764	Alpha-enolase (2-phospho-D-glycerate hydro-lyase) (Non-neural enolase) (NNE) (Enolase 1)	14	2 von 2	0,944	7	1 von 1	0,562	0,382	1,679715302	67,97153025
P24368	Peptidyl-prolyl cis-trans isomerase B (PPIase B) (Rotamase B) (Cyclophilin B) (S-cyclophilin) (SCYLP) (CYP-S1)	8	2 von 2	0,732	5	1 von 1	0,438	0,294	1,671232877	67,12328767
P30713	Glutathione S-transferase theta-2 (GST class-theta-2) (Glutathione S-transferase 12) (GST 12-12) (Glutathione S-transferase Yrs-Yrs)	5	2 von 2	0,556	3	1 von 1	0,334	0,222	1,664670659	66,46706587

Supplementary table 2. HC - cytoplasmic protein fraction

accession number	protein name	analysis peptides	analysis cytoplasm expcount	analysis cytoplasm emPAI	reference peptides	reference cytoplasm expcount	reference cytoplasm emPAI	emPAI diff cytoplasm	x-times up-regulated	alteration in %
P55051	Fatty acid-binding protein, brain (Brain-type fatty acid-binding protein) (B-FABP) (Fatty acid-binding protein 7) (Brain lipid-binding protein) (BLBP)	4	2 von 2	0,778	3	1 von 1	0,468	0,31	1,662393162	66,23931624
P40112	Proteasome subunit beta type-3 (Proteasome theta chain) (Proteasome chain 13) (Proteasome component C10-II)	3	2 von 2	0,48	2	1 von 1	0,292	0,188	1,643835616	64,38356164
Q6AYZ1	Tubulin alpha-1C chain (Tubulin alpha-6 chain) (Alpha-tubulin 6)	6	2 von 2	0,48	3	1 von 1	0,292	0,188	1,643835616	64,38356164
P25113	Phosphoglycerate mutase 1 (Phosphoglycerate mutase isozyme B) (PGAM-B) (BPG-dependent PGAM 1)	5	2 von 2	0,449	6	1 von 1	0,274	0,175	1,638686131	63,86861314
P35434	ATP synthase subunit delta, mitochondrial (F-ATPase delta subunit)	2	2 von 2	0,422	1	1 von 1	0,259	0,163	1,629343629	62,93436293
P12075	Cytochrome c oxidase subunit 5B, mitochondrial (Cytochrome c oxidase polypeptide Vb) (Cytochrome c oxidase subunit VIA*)	2	2 von 2	0,376	1	1 von 1	0,233	0,143	1,613733906	61,37339056
Q8CFN2	Cell division control protein 42 homolog	2	2 von 2	0,376	1	1 von 1	0,233	0,143	1,613733906	61,37339056
P13437	3-ketoacyl-CoA thiolase, mitochondrial (Beta-ketothiolase) (Acetyl-CoA acyltransferase) (Mitochondrial 3-oxoacyl-CoA thiolase)	19	2 von 2	2.165	10	1 von 1	1.346	0,819	1,608469539	60,84695394
P11598	Protein disulfide-isomerase A3 (Disulfide isomerase ER-60) (Endoplasmic reticulum resident protein 60) (ER protein 60) (Endoplasmic reticulum resident protein 57) (ER protein 57) (58 kDa microsomal protein) (p58) (HIP-70) (Q-2) (58 kDa glucose-regulated p	9	2 von 2	0,392	4	1 von 1	0,245	0,147	1,6	60
P55053	Fatty acid-binding protein, epidermal (Epidermal-type fatty acid-binding protein) (E-FABP) (Fatty acid-binding protein 5) (Cutaneous fatty acid-binding protein) (C-FABP) (DA11)	2	2 von 2	0,309	1	1 von 1	0,194	0,115	1,592783505	59,27835052
P61589	Transforming protein RhoA	2	2 von 2	0,263	1	1 von 1	0,166	0,097	1,584337349	58,43373494
Q7M767	Ubiquitin-conjugating enzyme E2 variant 2 (Ubiquitin-conjugating enzyme variant MMS2)	3	2 von 2	0,263	1	1 von 1	0,166	0,097	1,584337349	58,43373494
Q9WVK3	Peroxisomal trans-2-enoyl-CoA reductase (TERP) (RLF98) (Peroxisomal 2,4-dienoyl-CoA reductase) (PX-2,4-DCR1)	2	2 von 2	0,244	1	1 von 1	0,155	0,089	1,574193548	57,41935484
P17220	Proteasome subunit alpha type-2 (Proteasome component C3) (Macropain subunit C3) (Multicatalytic endopeptidase complex subunit C3)	2	2 von 2	0,244	2	1 von 1	0,155	0,089	1,574193548	57,41935484
P16303	Carboxylesterase 3 (Liver carboxylesterase 10) (Carboxyesterase ES-10) (Fatty acid ethyl ester synthase) (FAEE synthase) (pI 6.1 esterase) (ES-HVEL)	5	2 von 2	0,244	2	1 von 1	0,155	0,089	1,574193548	57,41935484
P85834	Elongation factor Tu, mitochondrial	4	2 von 2	0,208	2	1 von 1	0,133	0,075	1,563909774	56,39097744
P19511	ATP synthase subunit b, mitochondrial (ATPase subunit b)	2	2 von 2	0,172	1	1 von 1	0,11	0,062	1,563636364	56,36363636
P09367	L-serine dehydratase/L-threonine deaminase (L-serine deaminase) (SDH) (L-threonine dehydratase) (TDH)	2	2 von 2	0,172	1	1 von 1	0,11	0,062	1,563636364	56,36363636
P63245	Guanine nucleotide-binding protein subunit beta-2-like 1 (Receptor of activated protein kinase C 1) (RACK1) (Receptor for activated C kinase)	2	2 von 2	0,172	2	1 von 1	0,11	0,062	1,563636364	56,36363636
P62919	60S ribosomal protein L8	2	2 von 2	0,19	1	1 von 1	0,122	0,068	1,557377049	55,73770492
Q0D2L3	Agmatinase, mitochondrial (Agmatine ureohydrolase) (AUH)	3	2 von 2	0,19	1	1 von 1	0,122	0,068	1,557377049	55,73770492
P17046	Lysosome-associated membrane glycoprotein 2 (Lysosome-associated membrane protein 2) (Lysosomal membrane glycoprotein type B) (LGP-B) (LGP-96) (LGP-110) (CD107 antigen-like family member B)	2	2 von 2	0,163	1	1 von 1	0,105	0,058	1,552380952	55,23809524
P00786	Cathepsin H (Cathepsin B3) (Cathepsin BA)	3	2 von 2	0,156	1	1 von 1	0,101	0,055	1,544554455	54,45544554
P07153	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1 (Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 67 kDa subunit) (Ribophorin-1) (Ribophorin I) (RPN-I)	2	2 von 2	0,077	1	1 von 1	0,05	0,027	1,54	54
P17475	Alpha-1-antiproteinase (Alpha-1-antitrypsin) (Alpha-1-proteinase inhibitor) (Serpin A1)	23	2 von 2	0,132	21	1 von 1	0,086	0,046	1,534883721	53,48837209
P15083	Polymeric immunoglobulin receptor (Poly-Ig receptor)	6	2 von 2	0,075	7	1 von 1	0,049	0,026	1,530612245	53,06122449
Q561S0	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10, mitochondrial (NADH-ubiquinone oxidoreductase 42 kDa subunit) (Complex I-42kD) (CI-42kD)	2	2 von 2	0,127	1	1 von 1	0,083	0,044	1,530120482	53,01204819
P08461	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial (Dihydrolipoamide acetyltransferase component of pyruvate dehydrogenase complex) (Pyruvate dehydrogenase complex component E2) (PDC-E2) (70 kDa mitoch	2	2 von 2	0,093	1	1 von 1	0,061	0,032	1,524590164	52,45901639
P14882	Propionyl-CoA carboxylase alpha chain, mitochondrial (PCCase subunit alpha) (Propanoyl-CoA:carbon dioxide ligase subunit alpha)	2	2 von 2	0,064	1	1 von 1	0,042	0,022	1,523809524	52,38095238
Q5I0G4	Glycyl-tRNA synthetase (Glycine--tRNA ligase) (GlyRS) (Diadenosine tetraphosphate synthetase) (AP-4-A synthetase)	3	2 von 2	0,073	1	1 von 1	0,048	0,025	1,520833333	52,08333333
Q5BJQ0	Chaperone activity of bc1 complex-like, mitochondrial (Chaperone-ABC1-like) (aarF domain-containing protein kinase 3)	2	2 von 2	0,088	1	1 von 1	0,058	0,03	1,517241379	51,72413793
P25093	Fumarylacetoacetase (FAA) (Fumarylacetoacetate hydrolase) (Beta-diketonase)	7	2 von 2	0,887	9	1 von 1	0,585	0,302	1,516239316	51,62393162
Q9Z1A6	Vigilin (High density lipoprotein-binding protein) (HDL-binding protein)	5	2 von 2	0,065	2	1 von 1	0,043	0,022	1,511627907	51,1627907

Supplementary table 2. HC - cytoplasmic protein fraction

found in both	min. 2 exp, min. 2 peptides, delta of ≤-2 or -50% upon NAF treatment		sorted according to the magnitude of the regulation					only 1 experiment of DMSO cyt		
accession number	protein name	analysis peptides	analysis cytoplasm expcount	analysis cytoplasm emPAI	reference peptides	reference cytoplasm expcount	reference cytoplasm emPAI	emPAI diff cytoplasm	x-times down-regulated	alteration in %
P10868	Guanidinoacetate N-methyltransferase	2	2 von 2	0,179	5	1 von 1	1.276	-1.097	-7128,49162	-712749,162
P31399	ATP synthase subunit d, mitochondrial (ATPase subunit d)	5	2 von 2	0,866	8	1 von 1	3.125	-2.259	-3608,545035	-360754,5035
P04904	Glutathione S-transferase alpha-3 (Glutathione S-transferase Yc-1) (GST Yc1) (GST 2-2) (GST AA) (GST A3-3)	5	1 von 2	0,501	7	1 von 1	1.581	-1,08	-3155,688623	-315468,8623
P04041	Glutathione peroxidase 1 (GSHPx-1) (Cellular glutathione peroxidase)	7	2 von 2	1,18	11	1 von 1	2.981	-1.801	-2526,271186	-252527,1186
P29419	ATP synthase subunit e, mitochondrial (ATPase subunit e)	2	2 von 2	0,931	3	1 von 1	1.683	-0,752	-1807,73362	-180673,362
O35077	Glycerol-3-phosphate dehydrogenase [NAD+], cytoplasmic (GPDH-C)	10	2 von 2	0,612	11	1 von 1	1.031	-0,419	-1684,640523	-168364,0523
P10111	Peptidyl-prolyl cis-trans isomerase A (PPIase A) (Rotamase A) (Cyclophilin A) (Cyclosporin A-binding protein) (p31) (p1B15)	7	2 von 2	1,18	8	1 von 1	1.929	-0,749	-1634,745763	-163374,5763
P67779	Prohibitin	8	2 von 2	0,832	8	1 von 1	1.228	-0,396	-1475,961538	-147496,1538
P04797	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (38 kDa BFA-dependent ADP-ribosylation substrate) (BARS-38)	10	2 von 2	0,972	9	1 von 1	1.371	-0,399	-1410,493827	-140949,3827
P52759	Ribonuclease UK114 (14.5 kDa translational inhibitor protein) (Perchloric acid soluble protein)	11	2 von 2	5,31	10	1 von 1	6.943	-1.633	-1307,532957	-130653,2957
P04762	Catalase	16	2 von 2	0,926	14	1 von 1	1.075	-0,149	-1160,907127	-115990,7127
P62630	Elongation factor 1-alpha 1 (EF-1-alpha-1) (Eukaryotic elongation factor 1 A-1) (eEF1A-1) (Elongation factor Tu) (EF-Tu)	10	2 von 2	0,965	9	1 von 1	1.043	-0,078	-1080,829016	-107982,9016
P52873	Pyruvate carboxylase, mitochondrial (Pyruvic carboxylase) (PCB)	35	2 von 2	1,06	25	1 von 1	1.001	0,059	-944,3396226	-94333,96226
Q9WUC4	Copper transport protein ATOX1 (Metal transport protein ATX1) (ATX1 homolog protein Rah1)	1	1 von 2	0,778	5	1 von 1	9	-8.222	-11,56812339	-1056,812339
P04903	Glutathione S-transferase alpha-2 (Glutathione S-transferase Ya-2) (GST Ya2) (GST 1b-1b) (GST A2-2)	3	2 von 2	0,145	7	1 von 1	0,968	-0,823	-6,675862069	-567,5862069
P09527	Ras-related protein Rab-7a (Ras-related protein p23) (Ras-related protein BRL-RAS)	1	1 von 2	0,136	4	1 von 1	0,668	-0,532	-4,911764706	-391,1764706
P00502	Glutathione S-transferase alpha-1 (Glutathione S-transferase Ya-1) (GST Ya1) (Ligandin) (GST 1a-1a) (GST B) (GST 1-1) (GST A1-1)	4	2 von 2	0,228	7	1 von 1	0,968	-0,74	-4,245614035	-324,5614035
P48037	Annexin A6 (Annexin-6) (Annexin VI) (Calcium-binding protein 65/67) (CBP 65/67)	1	2 von 2	0,037	4	1 von 1	0,157	-0,12	-4,243243243	-324,3243243
P02692	Fatty acid-binding protein, liver (Liver-type fatty acid-binding protein) (L-FABP) (Fatty acid-binding protein 1) (Squalene- and sterol-carrier protein) (SCP) (Z-protein) (p14)	15	2 von 2	3.794	16	1 von 1	15.681	-11.887	-4,133104902	-313,3104902
Q63716	Peroxiredoxin-1 (Thioredoxin peroxidase 2) (Thioredoxin-dependent peroxide reductase 2) (Heme-binding 23 kDa protein) (HBP23)	16	2 von 2	1.608	20	1 von 1	6.499	-4.891	-4,041666667	-304,1666667
P61459	Pterin-4-alpha-carbinolamine dehydratase (PHS) (4-alpha-hydroxy-tetrahydropterin dehydratase) (Phenylalanine hydroxylase-stimulating protein) (Pterin carbinolamine dehydratase) (PCD) (Dimerization cofactor of hepatocyte nuclear factor 1-alpha) (Dimerizati	5	2 von 2	0,376	5	1 von 1	1,31	-0,934	-3,484042553	-248,4042553
P06866	Haptoglobin (Liver regeneration-related protein LRRG173)	17	2 von 2	0,086	21	1 von 1	0,28	-0,194	-3,255813953	-225,5813953
P21913	Succinate dehydrogenase [ubiquinone] iron-sulfur subunit, mitochondrial (Iron-sulfur subunit of complex II) (Ip)	3	2 von 2	0,19	5	1 von 1	0,532	-0,342	-2,8	-180
P14046	Alpha-1-inhibitor 3 (Alpha-1-inhibitor 3 variant II) (Alpha-1-inhibitor III)	51	2 von 2	0,039	54	1 von 1	0,108	-0,069	-2,769230769	-176,9230769
Q9JM53	Apoptosis-inducing factor 1, mitochondrial (Programmed cell death protein 8)	4	2 von 2	0,14	6	1 von 1	0,369	-0,229	-2,635714286	-163,5714286
P22734	Catechol O-methyltransferase	10	2 von 2	1.054	12	1 von 1	2.652	-1.598	-2,516129032	-151,6129032
Q6P7Q4	Lactoylglutathione lyase (Methylglyoxalase) (Aldoketomutase) (Glyoxalase I) (Glx I) (Ketone-aldehyde mutase) (S-D-lactoylglutathione methylglyoxal lyase)	4	2 von 2	0,389	4	1 von 1	0,931	-0,542	-2,393316195	-139,3316195
P08010	Glutathione S-transferase Mu 2 (GSTM2-2) (Glutathione S-transferase Yb-2) (GST Yb2) (GST 4-4)	19	2 von 2	1.438	22	1 von 1	3.329	-1.891	-2,315020862	-131,5020862
P35704	Peroxiredoxin-2 (Thioredoxin peroxidase 1) (Thioredoxin-dependent peroxide reductase 1) (Thiol-specific antioxidant protein) (TSA)	2	2 von 2	0,34	4	1 von 1	0,778	-0,438	-2,288235294	-128,8235294
P45592	Cofilin-1 (Cofilin, non-muscle isoform)	4	2 von 2	0,408	5	1 von 1	0,931	-0,523	-2,281862745	-128,1862745
Q9EQX9	Ubiquitin-conjugating enzyme E2 N (Ubiquitin-protein ligase N) (Ubiquitin carrier protein N) (Bendless-like ubiquitin-conjugating enzyme)	2	2 von 2	0,284	3	1 von 1	0,638	-0,354	-2,246478873	-124,6478873
P97576	GrpE protein homolog 1, mitochondrial (Mt-GrpE#1)	2	2 von 2	0,202	3	1 von 1	0,438	-0,236	-2,168316832	-116,8316832
P97584	Prostaglandin reductase 1 (PRG-1) (NADP-dependent leukotriene B4 12-hydroxydehydrogenase) (15-oxoprostaglandin 13-reductase) (Dithiolethione-inducible gene 1 protein) (D3T-inducible gene 1 protein)	2	2 von 2	0,163	3	1 von 1	0,35	-0,187	-2,147239264	-114,7239264
P63102	14-3-3 protein zeta/delta (Protein kinase C inhibitor protein 1) (KCIP-1) (Mitochondrial import stimulation factor S1 subunit)	4	2 von 2	0,163	6	1 von 1	0,35	-0,187	-2,147239264	-114,7239264

Supplementary table 2. HC - cytoplasmic protein fraction

accession number	protein name	analysis peptides	analysis cytoplasm expcount	analysis cytoplasm emPAI	reference peptides	reference cytoplasm expcount	reference cytoplasm emPAI	emPAI diff cytoplasm	x-times down-regulated	alteration in %
Q562C4	Methyltransferase-like protein 7B (Associated with lipid droplet protein 1) (ALDI)	2	2 von 2	0,136	2	1 von 1	0,292	-0,156	-2,147058824	-114,7058824
Q9JLJ3	4-trimethylaminobutyraldehyde dehydrogenase (TMABADH) (Aldehyde dehydrogenase family 9 member A1)	3	2 von 2	0,136	4	1 von 1	0,292	-0,156	-2,147058824	-114,7058824
Q920D2	Dihydrofolate reductase	1	1 von 2	0,145	2	1 von 1	0,311	-0,166	-2,144827586	-114,4827586
Q9WUS0	Adenylate kinase isoenzyme 4, mitochondrial (Adenylate kinase 3-like) (ATP-AMP transphosphorylase)	1	1 von 2	0,145	2	1 von 1	0,311	-0,166	-2,144827586	-114,4827586
P38918	Aflatoxin B1 aldehyde reductase member 3 (AFB1-AR) (Aflatoxin B1 aldehyde reductase member 1) (rAFAR1)	1	2 von 2	0,122	2	1 von 1	0,259	-0,137	-2,12295082	-112,295082
P07379	Phosphoenolpyruvate carboxykinase, cytosolic [GTP] (PEPCK-C) (Phosphoenolpyruvate carboxylase)	2	1 von 2	0,113	4	1 von 1	0,239	-0,126	-2,115044248	-111,5044248
P50399	Rab GDP dissociation inhibitor beta (Rab GDI beta) (Guanosine diphosphate dissociation inhibitor 2) (GDI-2) (GDI-3)	1	1 von 2	0,072	3	1 von 1	0,15	-0,078	-2,083333333	-108,3333333
O70199	UDP-glucose 6-dehydrogenase (UDP-Glc dehydrogenase)	3	2 von 2	0,096	3	1 von 1	0,199	-0,103	-2,072916667	-107,2916667
Q5SGE0	Leucine-rich PPR motif-containing protein, mitochondrial (130 kDa leucine-rich protein) (LRP 130) (Leucine rich protein 157) (rLRP157)	1	1 von 2	0,019	2	1 von 1	0,039	-0,02	-2,052631579	-105,2631579
P07632	Superoxide dismutase [Cu-Zn]	7	2 von 2	1.693	8	1 von 1	3.329	-1.636	-1,966331955	-96,63319551
P29147	D-beta-hydroxybutyrate dehydrogenase, mitochondrial (BDH) (3-hydroxybutyrate dehydrogenase)	5	2 von 2	0,378	7	1 von 1	0,743	-0,365	-1,965608466	-96,56084656
P04905	Glutathione S-transferase Mu 1 (GSTM1-1) (Glutathione S-transferase Yb-1) (GST Yb1) (GST 3-3)	21	2 von 2	2.232	25	1 von 1	4.337	-2.105	-1,943100358	-94,31003584
P31044	Phosphatidylethanolamine-binding protein 1 (PEBP-1) (HCNPpp) (23 kDa morphine-binding protein) (P23K)	5	2 von 2	1.154	8	1 von 1	2.162	-1.008	-1,873483536	-87,34835355
P18297	Sepiapterin reductase (SPR)	3	2 von 2	0,437	4	1 von 1	0,778	-0,341	-1,780320366	-78,03203661
Q4KM73	UMP-CMP kinase (Cytidylate kinase) (Deoxycytidylate kinase) (Cytidine monophosphate kinase) (Uridine monophosphate kinase) (Uridine monophosphate/cytidine monophosphate kinase) (UMP/CMP kinase)	3	2 von 2	0,406	4	1 von 1	0,719	-0,313	-1,770935961	-77,09359606
P56571	ES1 protein homolog, mitochondrial	3	2 von 2	0,356	4	1 von 1	0,624	-0,268	-1,752808989	-75,28089888
O35244	Peroxiredoxin-6 (Antioxidant protein 2) (1-Cys peroxiredoxin) (1-Cys PRX) (Acidic calcium-independent phospholipase A2) (aiPLA2) (Non-selenium glutathione peroxidase) (NSGPx) (Thiol-specific antioxidant protein)	9	2 von 2	1.025	11	1 von 1	1.783	-0,758	-1,739512195	-73,95121951
P04785	Protein disulfide-isomerase (PDI) (Prolyl 4-hydroxylase subunit beta) (Cellular thyroid hormone-binding protein)	7	2 von 2	0,301	8	1 von 1	0,52	-0,219	-1,727574751	-72,75747508
P63259	Actin, cytoplasmic 2 (Gamma-actin)	5	2 von 2	0,26	4	1 von 1	0,445	-0,185	-1,711538462	-71,15384615
P60711	Actin, cytoplasmic 1 (Beta-actin)	5	2 von 2	0,26	7	1 von 1	0,445	-0,185	-1,711538462	-71,15384615
Q920L2	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial (Flavoprotein subunit of complex II) (Fp)	3	2 von 2	0,12	4	1 von 1	0,198	-0,078	-1,65	-65
Q07523	Hydroxyacid oxidase 2 (HAOX2) ((S)-2-hydroxy-acid oxidase, peroxisomal) (Long chain alpha-hydroxy acid oxidase) (Long-chain L-2-hydroxy acid oxidase)	7	2 von 2	0,6	8	1 von 1	0,978	-0,378	-1,63	-63
P02761	Major urinary protein (MUP) (Alpha-2u-globulin) (Alpha(2)-euglobulin) (Allergen Rat n I)	22	2 von 2	2.246	19	1 von 1	3.642	-1.396	-1,621549421	-62,15494212
Q66HD0	Endoplasmin (Heat shock protein 90 kDa beta member 1) (94 kDa glucose-regulated protein) (GRP-94)	4	2 von 2	0,103	4	1 von 1	0,166	-0,063	-1,611650485	-61,16504854
P15651	Short-chain specific acyl-CoA dehydrogenase, mitochondrial (SCAD) (Butyryl-CoA dehydrogenase)	5	2 von 2	0,35	6	1 von 1	0,562	-0,212	-1,605714286	-60,57142857
Q9Z0V5	Peroxiredoxin-4 (Peroxiredoxin IV) (Prx-IV) (Thioredoxin peroxidase AO372) (Thioredoxin-dependent peroxide reductase A0372) (Antioxidant enzyme AOE372)	2	2 von 2	0,274	3	1 von 1	0,438	-0,164	-1,598540146	-59,8540146
P19234	NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial (NADH-ubiquinone oxidoreductase 24 kDa subunit)	3	2 von 2	0,259	3	1 von 1	0,413	-0,154	-1,594594595	-59,45945946
P38652	Phosphoglucomutase-1 (PGM 1) (Glucose phosphomutase 1)	8	2 von 2	0,233	7	1 von 1	0,369	-0,136	-1,583690987	-58,36909871
Q99NA5	Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial (Isocitric dehydrogenase subunit alpha) (NAD(+)-specific ICDH subunit alpha)	2	1 von 2	0,202	3	1 von 1	0,318	-0,116	-1,574257426	-57,42574257
P80254	D-dopachrome decarboxylase (D-dopachrome tautomerase)	10	2 von 2	2.572	9	1 von 1	4.012	-1,44	-1,559875583	-55,98755832
Q5XI73	Rho GDP-dissociation inhibitor 1 (Rho GDI 1) (Rho-GDI alpha)	4	2 von 2	0,323	3	1 von 1	0,501	-0,178	-1,551083591	-55,10835913
Q63150	Dihydropyrimidinase (DHPase) (Dihydropyrimidine amidohydrolase) (Hydantoinase)	3	2 von 2	0,135	3	1 von 1	0,205	-0,07	-1,518518519	-51,85185185

Supplementary table 3. NPC - secretome

found only in NAF samples	min. 2 experiments, min. 2 peptides		sorted according to the magnitude of the emPAI value	NAF and DMSO each 3 exp
accession number	protein name	analysis peptides	analysis secreted emPAI	
Q66H59	N-acetylneuraminate lyase (NALase) (N-acetylneuraminic acid aldolase) (N-acetylneuraminate pyruvate-lyase) (Sialic acid lyase) (Sialate lyase) (Sialate-pyruvate lyase) (Sialic acid aldolase)	5	0,233	
P21775	3-ketoacyl-CoA thiolase A, peroxisomal (Beta-ketothiolase A) (Acetyl-CoA acyltransferase A) (Peroxisomal 3-oxoacyl-CoA thiolase A)	3	0,156	
P17046	Lysosome-associated membrane glycoprotein 2 (Lysosome-associated membrane protein 2) (Lysosomal membrane glycoprotein type B) (LGP-B) (LGP-96) (LGP-110) (CD107 antigen-like family member B)	2	0,105	
Q6P9V9	Tubulin alpha-1B chain (Alpha-tubulin 2) (Tubulin alpha-2 chain)	12	0,089	

only in ref/DMSO	min. 2 experiments, min. 2 peptides		sorted according to the magnitude of the emPAI value
accession number	protein name	reference peptides	reference secreted emPAI
B2RYG6	Ubiquitin thioesterase OTUB1 (Deubiquitinating enzyme OTUB1) (OTU domain-containing ubiquitin aldehyde-binding protein 1) (Otubain-1) (Ubiquitin-specific-processing protease OTUB1)	1	0,136
Q5RKI0	WD repeat-containing protein 1	7	0,118
Q9EPF2	Cell surface glycoprotein MUC18 (Melanoma-associated antigen MUC18) (Melanoma cell adhesion molecule) (Gicerin)	3	0,093
P10716	C-type lectin domain family 4 member F (C-type lectin superfamily member 13) (C-type lectin 13) (Kupffer cell receptor)	3	0,091

found in both	min. 2 exp, min. 2peptides, delta of ≥2 or 50% upon NAF treatment				sorted according to the magnitude of the regulation				
accession number	protein name	analysis peptides	analysis secreted expcount	analysis secreted emPAI	reference peptides	reference secreted expcount	reference secreted emPAI	emPAI diff secreted	x-times up-regulated	alteration in %
P10111	Peptidyl-prolyl cis-trans isomerase A (PPIase A) (Rotamase A) (Cyclophilin A) (Cyclosporin A-binding protein) (p31) (p1B15)	14	3 von 3	1.084	14	3 von 3	0,862	0,222	1257,540603	125654,0603
P08010	Glutathione S-transferase Mu 2 (GSTM2-2) (Glutathione S-transferase Yb-2) (GST Yb2) (GST 4-4)	17	3 von 3	1.518	19	3 von 3	1,25	0,268	1214,4	121340
Q497B0	Omega-amidase NIT2 (Nitrilase homolog 2)	10	3 von 3	1.048	9	3 von 3	0,864	0,184	1212,962963	121196,2963
P40112	Proteasome subunit beta type-3 (Proteasome theta chain) (Proteasome chain 13) (Proteasome component C10-II)	5	3 von 3	1.184	5	3 von 3	0,992	0,192	1193,548387	119254,8387
P62804	Histone H4	13	3 von 3	16.015	15	3 von 3	14,91	1.105	1074,111335	107311,1335
P14141	Carbonic anhydrase 3 (Carbonic anhydrase III) (CA-III) (Carbonate dehydratase III)	20	3 von 3	4.372	20	3 von 3	4,78	-0,408	914,6443515	91364,43515
P52759	Ribonuclease UK114 (14.5 kDa translational inhibitor protein) (Perchloric acid soluble protein)	11	3 von 3	4.877	13	3 von 3	5,85	-0,973	833,6752137	83267,52137
O35331	Pyridoxal kinase (Pyridoxine kinase)	4	1 von 3	0,468	2	2 von 3	0,101	0,367	4,633663366	363,3663366
P62630	Elongation factor 1-alpha 1 (EF-1-alpha-1) (Eukaryotic elongation factor 1 A-1) (eEF1A-1) (Elongation factor Tu) (EF-Tu)	15	1 von 3	0,374	10	1 von 3	0,083	0,291	4,506024096	350,6024096
P46844	Biliverdin reductase A (BVR A) (Biliverdin-IX alpha-reductase)	9	1 von 3	0,823	8	2 von 3	0,228	0,595	3,609649123	260,9649123
Q8VBU2	Protein NDRG2 (NDRG1-related protein) (Antidepressant-related protein ADRG123)	3	1 von 3	0,468	2	1 von 3	0,136	0,332	3,441176471	244,1176471
P68255	14-3-3 protein theta (14-3-3 protein tau)	7	1 von 3	0,369	9	1 von 3	0,11	0,259	3,354545455	235,4545455
P14740	Dipeptidyl peptidase 4 (Dipeptidyl peptidase IV) (DPP IV) (T-cell activation antigen CD26) (GP110 glycoprotein) (Bile canaliculus domain-specific membrane glycoprotein)	10	1 von 3	0,304	16	3 von 3	0,095	0,209	3,2	220
P25113	Phosphoglycerate mutase 1 (Phosphoglycerate mutase isozyme B) (PGAM-B) (BPG-dependent PGAM 1)	9	2 von 3	0,887	8	2 von 3	0,284	0,603	3,123239437	212,3239437
Q63081	Protein disulfide-isomerase A6 (Protein disulfide isomerase P5) (Calcium-binding protein 1) (CaBP1) (Thioredoxin domain-containing protein 7)	11	3 von 3	0,238	8	1 von 3	0,08	0,158	2,975	197,5
P07335	Creatine kinase B-type (Creatine kinase B chain) (B-CK)	5	3 von 3	0,345	3	3 von 3	0,126	0,219	2,738095238	173,8095238
P10719	ATP synthase subunit beta, mitochondrial	29	2 von 3	0,185	28	1 von 3	0,07	0,115	2,642857143	164,2857143

Supplementary table 3. NPC - secretome

accession number	protein name	analysis peptides	analysis secreted expcount	analysis secreted emPAI	reference peptides	reference secreted expcount	reference secreted emPAI	emPAI diff secreted	x-times up-regulated	alteration in %
Q63610	Tropomyosin alpha-3 chain (Tropomyosin-3) (Gamma-tropomyosin) (Tropomyosin-5)	29	3 von 3	1,11	28	3 von 3	0,475	0,635	2,336842105	133,6842105
P85970	Actin-related protein 2/3 complex subunit 2 (Arp2/3 complex 34 kDa subunit) (p34-ARC)	10	2 von 3	0,325	9	2 von 3	0,143	0,182	2,272727273	127,2727273
P22734	Catechol O-methyltransferase	6	1 von 3	0,54	3	2 von 3	0,244	0,296	2,213114754	121,3114754
P23358	60S ribosomal protein L12	2	1 von 3	0,468	7	1 von 3	0,212	0,256	2,20754717	120,754717
Q5U2Q3	Ester hydrolase C11orf54 homolog	5	2 von 3	0,284	2	2 von 3	0,129	0,155	2,201550388	120,1550388
Q711G3	Isoamyl acetate-hydrolyzing esterase 1 homolog (Hypertrophic agonist-responsive protein B64)	4	3 von 3	0,281	3	2 von 3	0,129	0,152	2,178294574	117,8294574
P21670	Proteasome subunit alpha type-4 (Proteasome component C9) (Macropain subunit C9) (Multicatalytic endopeptidase complex subunit C9) (Proteasome subunit L)	3	1 von 3	0,413	3	2 von 3	0,19	0,223	2,173684211	117,3684211
P62836	Ras-related protein Rap-1A (Ras-related protein Krev-1)	6	1 von 3	0,359	6	1 von 3	0,166	0,193	2,162650602	116,2650602
P07895	Superoxide dismutase [Mn], mitochondrial	4	2 von 3	0,334	4	2 von 3	0,155	0,179	2,15483871	115,483871
Q5XFX0	Transgelin-2	15	3 von 3	0,86	16	3 von 3	0,402	0,458	2,139303483	113,9303483
P18420	Proteasome subunit alpha type-1 (Proteasome component C2) (Macropain subunit C2) (Multicatalytic endopeptidase complex subunit C2) (Proteasome nu chain)	2	1 von 3	0,245	2	2 von 3	0,116	0,129	2,112068966	111,2068966
P31210	3-oxo-5-beta-steroid 4-dehydrogenase (Delta(4)-3-ketosteroid 5-beta-reductase) (Delta(4)-3-oxosteroid 5-beta-reductase) (Aldo-keto reductase family 1 member D1)	2	1 von 3	0,179	2	2 von 3	0,086	0,093	2,081395349	108,1395349
P16617	Phosphoglycerate kinase 1	10	2 von 3	0,136	8	2 von 3	0,066	0,07	2,060606061	106,0606061
P97852	Peroxisomal multifunctional enzyme type 2 (MFE-2) (D-bifunctional protein) (DBP) (17-beta-hydroxysteroid dehydrogenase 4) (17-beta-HSD 4)	3	1 von 3	0,105	2	1 von 3	0,051	0,054	2,058823529	105,8823529
Q6AYC4	Macrophage-capping protein (Actin regulatory protein CAP-G)	3	1 von 3	0,389	5	3 von 3	0,207	0,182	1,879227053	87,92270531
P19945	60S acidic ribosomal protein P0 (60S ribosomal protein L10E)	7	1 von 3	0,688	7	1 von 3	0,369	0,319	1,864498645	86,4498645
P09495	Tropomyosin alpha-4 chain (Tropomyosin-4) (TM-4)	18	3 von 3	0,618	24	3 von 3	0,352	0,266	1,755681818	75,56818182
Q66HD0	Endoplasmin (Heat shock protein 90 kDa beta member 1) (94 kDa glucose-regulated protein) (GRP-94)	17	1 von 3	0,166	10	3 von 3	0,095	0,071	1,747368421	74,73684211
Q63716	Peroxiredoxin-1 (Thioredoxin peroxidase 2) (Thioredoxin-dependent peroxide reductase 2) (Heme-binding 23 kDa protein) (HBP23)	23	3 von 3	3.005	22	3 von 3	1.723	1.282	1,744051074	74,40510737
P62839	Ubiquitin-conjugating enzyme E2 D2 (Ubiquitin-protein ligase D2) (Ubiquitin carrier protein D2) (Ubiquitin-conjugating enzyme E2-17 kDa 2) (Ubiquitin-conjugating enzyme E2(17)KB 2)	2	2 von 3	0,811	1	1 von 3	0,468	0,343	1,732905983	73,29059829
P05197	Elongation factor 2 (EF-2)	19	2 von 3	0,238	10	3 von 3	0,139	0,099	1,712230216	71,22302158
P62775	Myotrophin (Protein V-1) (Granule cell differentiation protein)	4	1 von 3	0,995	2	1 von 3	0,585	0,41	1,700854701	70,08547009
P28077	Proteasome subunit beta type-9 (Proteasome subunit beta-1i) (Proteasome chain 7) (Macropain chain 7) (Multicatalytic endopeptidase complex chain 7) (Really interesting new gene 12 protein) (Low molecular mass protein 2)	4	1 von 3	0,778	4	1 von 3	0,468	0,31	1,662393162	66,23931624
Q9R0J8	Legumain (Asparaginyl endopeptidase) (Protease, cysteine 1)	6	3 von 3	0,227	4	2 von 3	0,137	0,09	1,656934307	65,69343066
P01041	Cystatin-B (Cystatin-beta) (Stefin-B) (Liver thiol proteinase inhibitor)	3	2 von 3	0,48	2	2 von 3	0,292	0,188	1,643835616	64,38356164
P34064	Proteasome subunit alpha type-5 (Proteasome zeta chain) (Macropain zeta chain) (Multicatalytic endopeptidase complex zeta chain)	3	1 von 3	0,585	3	1 von 3	0,359	0,226	1,629526462	62,95264624
P29410	Adenylate kinase 2, mitochondrial (AK 2) (ATP-AMP transphosphorylase 2)	6	3 von 3	0,299	7	3 von 3	0,188	0,111	1,590425532	59,04255319
P10868	Guanidinoacetate N-methyltransferase	2	2 von 3	0,284	1	2 von 3	0,179	0,105	1,586592179	58,65921788
P14942	Glutathione S-transferase alpha-4 (Glutathione S-transferase Yk) (GST Yk) (GST 8-8) (GST K) (GST A4-4)	3	2 von 3	0,263	1	1 von 3	0,166	0,097	1,584337349	58,43373494
P61983	14-3-3 protein gamma	7	2 von 3	0,357	10	3 von 3	0,226	0,131	1,579646018	57,96460177
P62494	Ras-related protein Rab-11A (Rab-11) (24KG)	3	2 von 3	0,214	5	2 von 3	0,136	0,078	1,573529412	57,35294118
Q5XI22	Acetyl-CoA acetyltransferase, cytosolic (Cytosolic acetoacetyl-CoA thiolase)	3	1 von 3	0,304	2	1 von 3	0,194	0,11	1,567010309	56,70103093
P0C169	Histone H2A type 1-C	6	2 von 3	3.454	4	2 von 3	2.205	1.249	1,566439909	56,64399093
P42123	L-lactate dehydrogenase B chain (LDH-B) (LDH heart subunit) (LDH-H)	9	3 von 3	0,423	5	2 von 3	0,273	0,15	1,549450549	54,94505495
P36972	Adenine phosphoribosyltransferase (APRT)	10	2 von 3	2.205	11	3 von 3	1.432	0,773	1,539804469	53,98044693
P04182	Ornithine aminotransferase, mitochondrial (Ornithine--oxo-acid aminotransferase)	5	1 von 3	0,25	4	2 von 3	0,163	0,087	1,533742331	53,37423313
P70615	Lamin-B1	3	2 von 3	0,066	3	2 von 3	0,044	0,022	1,5	50

found in both	min. 2 exp, min. 2 peptides, delta of ≤-2 or -50% upon NAF treatment	sorted according to the magnitude of the regulation

accession number	protein name	analysis peptides	analysis secreted expcount	analysis secreted emPAI	reference peptides	reference secreted expcount	reference secreted emPAI	emPAI diff secreted	x-times down-regulated	alteration in %
P00173	Cytochrome b5	4	2 von 3	0,292	7	1 von 3	1.783	-1.491	-6106,164384	-610516,4384
P01946	Hemoglobin subunit alpha-1/2 (Alpha-1/2-globin) (Hemoglobin alpha-1/2 chain)	8	1 von 3	0,259	5	1 von 3	1.512	-1.253	-5837,837838	-583683,7838

Supplementary table 3. NPC - secretome

accession number	protein name	analysis peptides	analysis secreted expcount	analysis secreted emPAI	reference peptides	reference secreted expcount	reference secreted emPAI	emPAI diff secreted	x-times down-regulated	alteration in %
Q9R063	Peroxiredoxin-5, mitochondrial (Peroxiredoxin V) (Prx-V) (Peroxisomal antioxidant enzyme) (PLP) (Thioredoxin reductase) (Thioredoxin peroxidase PMP20) (Antioxidant enzyme B166) (AOEB166)	13	3 von 3	0,673	17	3 von 3	1.763	-1,09	-2619,61367	-261861,367
Q05982	Nucleoside diphosphate kinase A (NDP kinase A) (Tumor metastatic process-associated protein) (Metastasis inhibition factor NM23)	6	2 von 3	0,514	10	3 von 3	1.296	-0,782	-2521,400778	-252040,0778
P04041	Glutathione peroxidase 1 (GSHPx-1) (Cellular glutathione peroxidase)	9	3 von 3	0,585	9	3 von 3	1.084	-0,499	-1852,991453	-185199,1453
P04636	Malate dehydrogenase, mitochondrial	15	3 von 3	0,9	15	3 von 3	1.272	-0,372	-1413,333333	-141233,3333
P11030	Acyl-CoA-binding protein (ACBP) (Diazepam-binding inhibitor) (DBI) (Endozepine) (EP)	4	1 von 3	0,778	5	2 von 3	1.075	-0,297	-1381,748072	-138074,8072
O09171	Betaine--homocysteine S-methyltransferase 1	15	3 von 3	0,876	17	3 von 3	1.164	-0,288	-1328,767123	-132776,7123
P85973	Purine nucleoside phosphorylase (PNP) (Inosine phosphorylase)	27	3 von 3	4,84	30	3 von 3	3.508	1.332	-724,7933884	-72379,33884
P09811	Glycogen phosphorylase, liver form	22	2 von 3	0,05	17	2 von 3	0,296	-0,246	-5,92	-492
P23097	Collagenase 3 (Matrix metalloproteinase-13) (MMP-13) (UMRCASE)	2	2 von 3	0,086	5	1 von 3	0,509	-0,423	-5,918604651	-491,8604651
Q63342	Dimethylglycine dehydrogenase, mitochondrial (ME2GLYDH)	4	1 von 3	0,041	5	1 von 3	0,224	-0,183	-5,463414634	-446,3414634
P16086	Spectrin alpha chain, brain (Spectrin, non-erythroid alpha chain) (Alpha-II spectrin) (Fodrin alpha chain)	38	1 von 3	0,022	99	3 von 3	0,104	-0,082	-4,727272727	-372,7272727
P23785	Granulins	1	1 von 3	0,075	4	1 von 3	0,334	-0,259	-4,453333333	-345,3333333
Q3SWT0	Platelet endothelial cell adhesion molecule (PECAM-1)	1	1 von 3	0,056	4	1 von 3	0,245	-0,189	-4,375	-337,5
P29534	Vascular cell adhesion protein 1 (V-CAM 1)	2	2 von 3	0,053	4	1 von 3	0,227	-0,174	-4,283018868	-328,3018868
O89000	Dihydropyrimidine dehydrogenase [NADP+] (DHPDHase) (Dihydrouracil dehydrogenase) (Dihydrothymine dehydrogenase)	4	1 von 3	0,037	4	1 von 3	0,155	-0,118	-4,189189189	-318,9189189
Q4KLZ6	Bifunctional ATP-dependent dihydroxyacetone kinase/FAD-AMP lyase (cyclizing)	6	3 von 3	0,09	8	2 von 3	0,377	-0,287	-4,188888889	-318,8888889
Q6P734	Plasma protease C1 inhibitor (C1 Inh) (C1 esterase inhibitor) (C1-inhibiting factor) (Serpin G1)	1	1 von 3	0,077	6	2 von 3	0,319	-0,242	-4,142857143	-314,2857143
Q4KM73	UMP-CMP kinase (Cytidylate kinase) (Deoxycytidylate kinase) (Cytidine monophosphate kinase) (Uridine monophosphate kinase) (Uridine monophosphate/cytidine monophosphate kinase) (UMP/CMP kinase)	5	2 von 3	0,145	7	1 von 3	0,501	-0,356	-3,455172414	-245,5172414
P97697	Inositol monophosphatase 1 (IMPase 1) (Inositol-1(or 4)-monophosphatase 1) (Lithium-sensitive myo-inositol monophosphatase A1)	4	1 von 3	0,136	5	1 von 3	0,468	-0,332	-3,441176471	-244,1176471
Q5M7U6	Actin-related protein 2 (Actin-like protein 2)	3	1 von 3	0,086	5	1 von 3	0,28	-0,194	-3,255813953	-225,5813953
Q63270	Cytoplasmic aconitate hydratase (Aconitase) (Citrate hydro-lyase) (Iron-responsive element-binding protein 1) (IRE-BP 1) (Iron regulatory protein 1) (IRP1)	6	3 von 3	0,049	7	2 von 3	0,158	-0,109	-3,224489796	-222,4489796
P14659	Heat shock-related 70 kDa protein 2 (Heat shock protein 70.2) (Testis-specific heat shock protein-related) (HST)	1	1 von 3	0,051	3	1 von 3	0,162	-0,111	-3,176470588	-217,6470588
Q8CFM6	Stabilin-2 (Fasciclin, EGF-like, laminin-type EGF-like and link domain-containing scavenger receptor 2) (FEEL-2) (Hyaluronan receptor for endocytosis)	6	2 von 3	0,026	15	1 von 3	0,081	-0,055	-3,115384615	-211,5384615
Q6P6R2	Dihydrolipoyl dehydrogenase, mitochondrial (Dihydrolipoamide dehydrogenase)	9	2 von 3	0,208	10	1 von 3	0,645	-0,437	-3,100961538	-210,0961538
P46953	3-hydroxyanthranilate 3,4-dioxygenase (3-hydroxyanthranilic acid dioxygenase) (HAD) (3-hydroxyanthranilate oxygenase) (3-HAO)	4	1 von 3	0,105	6	2 von 3	0,299	-0,194	-2,847619048	-184,7619048
P18484	AP-2 complex subunit alpha-2 (Adapter-related protein complex 2 alpha-2 subunit) (Adaptor protein complex AP-2 subunit alpha-2) (Alpha2-adaptin) (Plasma membrane adaptor HA2/AP2 adaptin alpha C subunit) (Alpha-adaptin C) (Clathrin assembly protein complex	22	1 von 3	0,035	22	2 von 3	0,091	-0,056	-2,6	-160
P02262	Histone H2A type 1	9	2 von 3	3.454	9	2 von 3	8.537	-5.083	-2,471627099	-147,1627099
P18421	Proteasome subunit beta type-1 (Proteasome component C5) (Macropain subunit C5) (Multicatalytic endopeptidase complex subunit C5) (Proteasome gamma chain)	6	3 von 3	0,264	6	2 von 3	0,64	-0,376	-2,424242424	-142,4242424
Q3T1J1	Eukaryotic translation initiation factor 5A-1 (eIF-5A-1) (Eukaryotic initiation factor 5A isoform 1) (eIF-5A) (eIF-4D)	4	1 von 3	0,212	6	2 von 3	0,495	-0,283	-2,33490566	-133,490566
Q9EQS0	Transaldolase	2	2 von 3	0,132	7	3 von 3	0,288	-0,156	-2,181818182	-118,1818182
P63018	Heat shock cognate 71 kDa protein (Heat shock 70 kDa protein 8)	23	3 von 3	0,187	17	2 von 3	0,406	-0,219	-2,171122995	-117,1122995
Q4KM35	Proteasome subunit beta type-10 (Proteasome subunit beta-2i) (Proteasome MECl-1) (Macropain subunit MECl-1) (Multicatalytic endopeptidase complex subunit MECl-1) (Low molecular mass protein 10)	2	1 von 3	0,122	4	1 von 3	0,259	-0,137	-2,12295082	-112,295082
P97584	Prostaglandin reductase 1 (PRG-1) (NADP-dependent leukotriene B4 12-hydroxydehydrogenase) (15-oxoprostaglandin 13-reductase) (Dithiolethione-inducible gene 1 protein) (D3T-inducible gene 1 protein)	1	1 von 3	0,105	2	1 von 3	0,222	-0,117	-2,114285714	-111,4285714
Q64640	Adenosine kinase (AK) (Adenosine 5'-phosphotransferase)	1	1 von 3	0,116	2	1 von 3	0,245	-0,129	-2,112068966	-111,2068966

Supplementary table 3. NPC - secretome

accession number	protein name	analysis peptides	analysis secreted expcount	analysis secreted emPAI	reference peptides	reference secreted expcount	reference secreted emPAI	emPAI diff secreted	x-times down-regulated	alteration in %
Q5I0D7	Xaa-Pro dipeptidase (X-Pro dipeptidase) (Proline dipeptidase) (Prolidase) (Imidodipeptidase) (Peptidase D)	3	1 von 3	0,08	2	1 von 3	0,166	-0,086	-2,075	-107,5
P34058	Heat shock protein HSP 90-beta (Heat shock 84 kDa) (HSP 84)	19	2 von 3	0,113	12	1 von 3	0,233	-0,12	-2,061946903	-106,1946903
P38659	Protein disulfide-isomerase A4 (Endoplasmic reticulum resident protein 72) (ER protein 72) (Endoplasmic reticulum resident protein 70) (ER protein 70) (Calcium-binding protein 2) (CaBP2)	8	1 von 3	0,049	2	1 von 3	0,101	-0,052	-2,06122449	-106,122449
P12346	Serotransferrin (Transferrin) (Siderophilin) (Beta-1 metal-binding globulin) (Liver regeneration-related protein LRRG03)	8	2 von 3	0,077	3	1 von 3	0,158	-0,081	-2,051948052	-105,1948052
Q9Z339	Glutathione S-transferase omega-1 (GSTO-1) (Glutathione-dependent dehydroascorbate reductase)	3	3 von 3	0,177	6	2 von 3	0,356	-0,179	-2,011299435	-101,1299435
P00787	Cathepsin B (Cathepsin B1) (RSG-2)	4	3 von 3	0,177	6	2 von 3	0,356	-0,179	-2,011299435	-101,1299435
P10860	Glutamate dehydrogenase 1, mitochondrial (GDH 1) (Memory-related gene 2 protein) (MRG-2)	28	2 von 3	0,375	26	1 von 3	0,753	-0,378	-2,008	-100,8
O35763	Moesin (Membrane-organizing extension spike protein)	19	3 von 3	0,241	21	3 von 3	0,478	-0,237	-1,98340249	-98,34024896
Q64119	Myosin light polypeptide 6 (Smooth muscle and nonmuscle myosin light chain alkali 6) (Myosin light chain alkali 3) (Myosin light chain A3) (Myosin light chain 3) (MLC-3) (17 kDa myosin light chain) (LC17)	8	2 von 3	0,309	8	2 von 3	0,612	-0,303	-1,980582524	-98,05825243
P84245	Histone H3.3	2	2 von 3	0,376	3	2 von 3	0,697	-0,321	-1,853723404	-85,37234043
P06761	78 kDa glucose-regulated protein (GRP-78) (Heat shock 70 kDa protein 5) (Immunoglobulin heavy chain-binding protein) (BiP) (Steroidogenesis-activator polypeptide)	35	3 von 3	0,154	17	3 von 3	0,284	-0,13	-1,844155844	-84,41558442
P51635	Alcohol dehydrogenase [NADP+] (Aldehyde reductase) (Aldo-keto reductase family 1 member A1) (3-DG-reducing enzyme)	5	3 von 3	0,292	8	2 von 3	0,537	-0,245	-1,839041096	-83,90410959
P46462	Transitional endoplasmic reticulum ATPase (TER ATPase) (15S Mg(2+)-ATPase p97 subunit) (Valosin-containing protein) (VCP)	25	3 von 3	0,429	20	2 von 3	0,784	-0,355	-1,827505828	-82,75058275
P23965	3,2-trans-enoyl-CoA isomerase, mitochondrial (Dodecenoyl-CoA isomerase) (Delta(3),Delta(2)-enoyl-CoA isomerase) (D3,D2-enoyl-CoA isomerase)	5	2 von 3	0,286	6	2 von 3	0,5	-0,214	-1,748251748	-74,82517483
P82995	Heat shock protein HSP 90-alpha (Heat shock 86 kDa) (HSP 86)	13	3 von 3	0,09	10	3 von 3	0,157	-0,067	-1,744444444	-74,44444444
Q68FT5	Betaine--homocysteine S-methyltransferase 2	3	1 von 3	0,105	3	3 von 3	0,183	-0,078	-1,742857143	-74,28571429
P23457	3-alpha-hydroxysteroid dehydrogenase (3-alpha-HSD) (Hydroxyprostaglandin dehydrogenase)	3	2 von 3	0,083	3	3 von 3	0,142	-0,059	-1,710843373	-71,08433735
P13697	NADP-dependent malic enzyme (NADP-ME) (Malic enzyme 1)	2	2 von 3	0,091	3	2 von 3	0,155	-0,064	-1,703296703	-70,32967033
P62959	Histidine triad nucleotide-binding protein 1 (Adenosine 5'-monophosphoramidase) (Protein kinase C inhibitor 1) (Protein kinase C-interacting protein 1) (PKCI-1) (17 kDa inhibitor of protein kinase C)	3	1 von 3	0,292	3	2 von 3	0,48	-0,188	-1,643835616	-64,38356164
Q4V7C7	Actin-related protein 3 (Actin-like protein 3)	9	3 von 3	0,258	14	3 von 3	0,423	-0,165	-1,639534884	-63,95348837
P19132	Ferritin heavy chain (Ferritin H subunit)	5	3 von 3	0,2	4	3 von 3	0,319	-0,119	-1,595	-59,5
P85972	Vinculin (Metavinculin)	17	1 von 3	0,185	31	3 von 3	0,294	-0,109	-1,589189189	-58,91891892
Q6P6V0	Glucose-6-phosphate isomerase (GPI) (Phosphoglucose isomerase) (PGI) (Phosphohexose isomerase) (PHI) (Autocrine motility factor) (AMF) (Neuroleukin) (NLK)	7	3 von 3	0,179	7	2 von 3	0,284	-0,105	-1,586592179	-58,65921788
P04785	Protein disulfide-isomerase (PDI) (Prolyl 4-hydroxylase subunit beta) (Cellular thyroid hormone-binding protein)	15	3 von 3	0,193	12	2 von 3	0,306	-0,113	-1,585492228	-58,5492228
P27139	Carbonic anhydrase 2 (Carbonic anhydrase II) (CA-II) (Carbonate dehydratase II)	1	2 von 3	0,166	2	2 von 3	0,263	-0,097	-1,584337349	-58,43373494
P03957	Stromelysin-1 (SL-1) (Matrix metalloproteinase-3) (MMP-3) (Transin-1) (PTR1 protein)	7	3 von 3	0,256	7	2 von 3	0,405	-0,149	-1,58203125	-58,203125
P00481	Ornithine carbamoyltransferase, mitochondrial (Ornithine transcarbamylase) (OTCase)	11	2 von 3	1.194	14	2 von 3	1.886	-0,692	-1,579564489	-57,95644891
Q6DGG1	Abhydrolase domain-containing protein 14B	4	3 von 3	0,297	3	3 von 3	0,468	-0,171	-1,575757576	-57,57575758
Q66HG4	Aldose 1-epimerase (Galactose mutarotase)	1	2 von 3	0,136	3	2 von 3	0,214	-0,078	-1,573529412	-57,35294118
Q6AYQ8	Fumarylacetoacetate hydrolase domain-containing protein 1	4	1 von 3	0,145	3	2 von 3	0,228	-0,083	-1,572413793	-57,24137931
Q6IRK9	Plasma glutamate carboxypeptidase (Liver annexin-like protein 1) (LAL-1) (Hematopoietic lineage switch 2 related protein) (Hls2-rp)	4	3 von 3	0,13	5	2 von 3	0,204	-0,074	-1,569230769	-56,92307692
P07151	Beta-2-microglobulin	5	3 von 3	2.031	6	3 von 3	3.187	-1.156	-1,569177745	-56,9177745
P25086	Interleukin-1 receptor antagonist protein (IL-1ra) (IL1 inhibitor)	1	1 von 3	0,129	3	2 von 3	0,202	-0,073	-1,565891473	-56,58914729
Q9QXQ0	Alpha-actinin-4 (Non-muscle alpha-actinin 4) (F-actin cross-linking protein)	29	3 von 3	0,154	25	3 von 3	0,241	-0,087	-1,564935065	-56,49350649
P05369	Farnesyl pyrophosphate synthase (FPP synthase) (Farnesyl diphosphate synthase) (Cholesterol-regulated 39 kDa protein) (CR 39)	1	2 von 3	0,101	2	2 von 3	0,156	-0,055	-1,544554455	-54,45544554
O35077	Glycerol-3-phosphate dehydrogenase [NAD+], cytoplasmic (GPDH-C)	7	3 von 3	0,237	7	3 von 3	0,366	-0,129	-1,544303797	-54,43037975
P47942	Dihydropyrimidinase-related protein 2 (DRP-2) (Turned on after division 64 kDa protein) (TOAD-64) (Collapsin response mediator protein 2) (CRMP-2)	2	2 von 3	0,059	3	2 von 3	0,091	-0,032	-1,542372881	-54,23728814
P0CG51	Polyubiquitin-B	6	1 von 3	0,066	4	2 von 3	0,101	-0,035	-1,53030303	-53,03030303

Supplementary table 3. NPC - secretome

accession number	protein name	analysis peptides	analysis secreted expcount	analysis secreted emPAI	reference peptides	reference secreted expcount	reference secreted emPAI	emPAI diff secreted	x-times down-regulated	alteration in %
P31000	Vimentin	2	3 von 3	0,08	2	2 von 3	0,122	-0,042	-1,525	-52,5
P62963	Profilin-1 (Profilin I)	10	3 von 3	3.756	12	3 von 3	5.704	-1.948	-1,518636848	-51,86368477
P02696	Retinol-binding protein 1 (Cellular retinol-binding protein I) (CRBP-I) (Cellular retinol-binding protein) (CRBP)	4	3 von 3	0,363	5	3 von 3	0,55	-0,187	-1,515151515	-51,51515152
Q6P7Q4	Lactoylglutathione lyase (Methylglyoxalase) (Aldoketomutase) (Glyoxalase I) (Glx I) (Ketone-aldehyde mutase) (S-D-lactoylglutathione methylglyoxal lyase)	8	3 von 3	0,555	8	3 von 3	0,84	-0,285	-1,513513514	-51,35135135
P52303	AP-1 complex subunit beta-1 (Adapter-related protein complex 1 subunit beta-1) (Adaptor protein complex AP-1 subunit beta-1) (Beta-adaptin 1) (Beta-1-adaptin) (Golgi adaptor HA1/AP1 adaptin beta subunit) (Clathrin assembly protein complex 1 beta large cha	17	2 von 3	0,078	19	1 von 3	0,118	-0,04	-1,512820513	-51,28205128
P32755	4-hydroxyphenylpyruvate dioxygenase (4-hydroxyphenylpyruvic acid oxidase) (HPPDase) (F Alloantigen) (F protein)	7	3 von 3	0,282	9	3 von 3	0,426	-0,144	-1,510638298	-51,06382979
Q63598	Plastin-3 (T-plastin)	5	2 von 3	0,053	2	2 von 3	0,08	-0,027	-1,509433962	-50,94339623

Supplementary table 4. NPC – cytoplasmic protein fraction

found only in NAF samples	min. 2 experiments, min. 2 peptides		sorted according to the magnitude of the emPAI value	NAF and DMSO each 2 exp
accession number	protein name	analysis peptides	analysis cytoplasm emPAI	
P0C169	Histone H2A type 1-C	6	2.931	
P02401	60S acidic ribosomal protein P2	4	1.154	
P02767	Transthyretin (Prealbumin) (TBPA)	4	0,623	
P62859	40S ribosomal protein S28	2	0,585	
Q5U2Q3	Ester hydrolase C11orf54 homolog	5	0,202	
Q63270	Cytoplasmic aconitate hydratase (Aconitase) (Citrate hydro-lyase) (Iron-responsive element-binding protein 1) (IRE-BP 1) (Iron regulatory protein 1) (IRP1)	6	0,156	
P52844	Estrogen sulfotransferase, isoform 1 (EST-1) (Sulfotransferase, estrogen-preferring) (Estrone sulfotransferase) (Sulfotransferase 1E1) (ST1E1)	2	0,093	
P09034	Argininosuccinate synthase (Citrulline--aspartate ligase)	3	0,075	

only in ref/DMSO	min. 2 experiments, min. 2 peptides		sorted according to the magnitude of the emPAI value
accession number	protein name	analysis peptides	analysis cytoplasm emPAI
P04903	Glutathione S-transferase alpha-2 (Glutathione S-transferase Ya-2) (GST Ya2) (GST 1b-1b) (GST A2-2)	5	0,323

found in both	min. 2 exp, min. 2peptides, delta of ≥2 or 50% upon NAF treatment				sorted according to the magnitude of the regulation					
accession number	protein name	analysis peptides	analysis cytoplasm expcount	analysis cytoplasm emPAI	reference peptides	reference cytoplasm expcount	reference cytoplasm emPAI	emPAI diff cytoplasm	x-times up-regulated	alteration in %
P62898	Cytochrome c, somatic	6	1 von 2	2.594	4	1 von 2	0,292	2.302	8883,561644	888256,1644
P02091	Hemoglobin subunit beta-1 (Hemoglobin beta-1 chain) (Beta-1-globin) (Hemoglobin beta chain, major-form)	11	2 von 2	3.012	2	2 von 2	0,468	2.544	6435,897436	643489,7436
P55053	Fatty acid-binding protein, epidermal (Epidermal-type fatty acid-binding protein) (E-FABP) (Fatty acid-binding protein 5) (Cutaneous fatty acid-binding protein) (C-FABP) (DA11)	10	2 von 2	1.913	4	1 von 2	0,425	1.488	4501,176471	450017,6471
Q6P7S1	Acid ceramidase (AC) (Acylsphingosine deacylase) (N-acylsphingosine amidohydrolase)	9	1 von 2	1.291	3	1 von 2	0,318	0,973	4059,748428	405874,8428
P63259	Actin, cytoplasmic 2 (Gamma-actin)	12	2 von 2	1.111	3	1 von 2	0,318	0,793	3493,710692	349271,0692
P07151	Beta-2-microglobulin	5	2 von 2	1.307	6	1 von 2	0,389	0,918	3359,897172	335889,7172
Q62658	Peptidyl-prolyl cis-trans isomerase FKBP1A (PPIase FKBP1A) (FK506-binding protein 1A) (FKBP-1A) (Rotamase) (Immunophilin FKBP12) (12 kDa FK506-binding protein) (12 kDa FKBP)	5	2 von 2	1.075	2	2 von 2	0,334	0,741	3218,562874	321756,2874
P02262	Histone H2A type 1	9	1 von 2	2.728	9	1 von 2	0,931	1.797	2930,182599	292918,2599
P06761	78 kDa glucose-regulated protein (GRP-78) (Heat shock 70 kDa protein 5) (Immunoglobulin heavy chain-binding protein) (BiP) (Steroidogenesis-activator polypeptide)	35	2 von 2	1.988	17	1 von 2	0,688	1,3	2889,534884	288853,4884
P63039	60 kDa heat shock protein, mitochondrial (Heat shock protein 60) (HSP-60) (60 kDa chaperonin) (Chaperonin 60) (CPN60) (Mitochondrial matrix protein P1) (HSP-65)	26	1 von 2	1.994	18	1 von 2	0,73	1.264	2731,506849	273050,6849
Q64119	Myosin light polypeptide 6 (Smooth muscle and nonmuscle myosin light chain alkali 6) (Myosin light chain alkali 3) (Myosin light chain A3) (Myosin light chain 3) (MLC-3) (17 kDa myosin light chain) (LC17)	8	2 von 2	1.913	8	2 von 2	0,728	1.185	2627,747253	262674,7253
P30904	Macrophage migration inhibitory factor (MIF) (Phenylpyruvate tautomerase) (L-dopachrome tautomerase) (L-dopachrome isomerase) (Glutathione-binding 13 kDa protein)	3	2 von 2	1.154	2	1 von 2	0,468	0,686	2465,811966	246481,1966
P31044	Phosphatidylethanolamine-binding protein 1 (PEBP-1) (HCNPpp) (23 kDa morphine-binding protein) (P23K)	10	2 von 2	1.658	6	2 von 2	0,778	0,88	2131,105398	213010,5398
P11598	Protein disulfide-isomerase A3 (Disulfide isomerase ER-60) (Endoplasmic reticulum resident protein 60) (ER protein 60) (Endoplasmic reticulum resident protein 57) (ER protein 57) (58 kDa microsomal protein) (p58) (HIP-70) (Q-2) (58 kDa glucose-regulated p	22	1 von 2	1.404	20	2 von 2	0,666	0,738	2108,108108	210710,8108
Q00728	Histone H2A type 4 (Histone H2A, testis) (TH2A)	3	1 von 2	1.683	2	1 von 2	0,931	0,752	1807,73362	180673,362
P08699	Galectin-3 (Gal-3) (Galactose-specific lectin 3) (Mac-2 antigen) (IgE-binding protein) (35 kDa lectin) (Carbohydrate-binding protein 35) (CBP 35) (Laminin-binding protein) (Lectin L-29)	4	1 von 2	1.154	3	1 von 2	0,668	0,486	1727,54491	172654,491
P08009	Glutathione S-transferase Yb-3 (Chain 4) (GST Yb3) (GST class-mu 3)	9	1 von 2	1.081	10	1 von 2	0,688	0,393	1571,22093	157022,093
B2RZ78	Vacuolar protein sorting-associated protein 29 (Vesicle protein sorting 29)	4	1 von 2	1.154	3	1 von 2	0,778	0,376	1483,290488	148229,0488
P04764	Alpha-enolase (2-phospho-D-glycerate hydro-lyase) (Non-neural enolase) (NNE) (Enolase 1)	16	2 von 2	1.154	14	2 von 2	0,812	0,342	1421,182266	142018,2266

Supplementary table 4. NPC – cytoplasmic protein fraction

accession number	protein name	analysis peptides	analysis cytoplasm expcount	analysis cytoplasm emPAI	reference peptides	reference cytoplasm expcount	reference cytoplasm emPAI	emPAI diff cytoplasm	x-times up-regulated	alteration in %
P61459	Pterin-4-alpha-carbinolamine dehydratase (PHS) (4-alpha-hydroxy-tetrahydropterin dehydratase) (Phenylalanine hydroxylase-stimulating protein) (Pterin carbinolamine dehydratase) (PCD)	7	1 von 2	1.848	6	1 von 2	1,31	0,538	1410,687023	140968,7023
P62332	ADP-ribosylation factor 6	5	1 von 2	1.276	4	1 von 2	0,931	0,345	1370,56928	136956,928
P84079	ADP-ribosylation factor 1	5	1 von 2	1.154	4	1 von 2	0,848	0,306	1360,849057	135984,9057
Q63610	Tropomyosin alpha-3 chain (Tropomyosin-3) (Gamma-tropomyosin) (Tropomyosin-5)	29	2 von 2	1.211	28	2 von 2	0,892	0,319	1357,623318	135662,3318
Q6RUV5	Ras-related C3 botulinum toxin substrate 1 (p21-Rac1)	6	1 von 2	2.162	5	1 von 2	1,61	0,552	1342,857143	134185,7143
P05065	Fructose-bisphosphate aldolase A (Muscle-type aldolase)	8	1 von 2	1.031	9	1 von 2	0,859	0,172	1200,232829	119923,2829
P48500	Triosephosphate isomerase (TIM) (Triose-phosphate isomerase)	13	2 von 2	1.837	16	2 von 2	1,7	0,137	1080,588235	107958,8235
O35244	Peroxiredoxin-6 (Antioxidant protein 2) (1-Cys peroxiredoxin) (1-Cys PRX) (Acidic calcium-independent phospholipase A2) (aiPLA2) (Non-selenium glutathione peroxidase) (NSGPx) (Thiol-specific antioxidant protein)	14	2 von 2	1.172	14	2 von 2	1,99	-0,818	588,9447236	58794,47236
P01946	Hemoglobin subunit alpha-1/2 (Alpha-1/2-globin) (Hemoglobin alpha-1/2 chain)	8	1 von 2	5,31	5	1 von 2	0,585	4.725	9,076923077	807,6923077
Q02253	Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial (Malonate-semialdehyde dehydrogenase [acylating]) (Aldehyde dehydrogenase family 6 member A1)	7	1 von 2	0,468	5	1 von 2	0,066	0,402	7,090909091	609,0909091
Q64428	Trifunctional enzyme subunit alpha, mitochondrial (TP-alpha)	13	1 von 2	0,572	4	1 von 2	0,086	0,486	6,651162791	565,1162791
O35763	Moesin (Membrane-organizing extension spike protein)	19	2 von 2	0,66	21	2 von 2	0,1	0,56	6,6	560
Q9QXQ0	Alpha-actinin-4 (Non-muscle alpha-actinin 4) (F-actin cross-linking protein)	29	2 von 2	0,543	25	2 von 2	0,104	0,439	5,221153846	422,1153846
Q6Q0N1	Cytosolic non-specific dipeptidase (CNDP dipeptidase 2)	4	1 von 2	0,346	1	1 von 2	0,077	0,269	4,493506494	349,3506494
Q64057	Alpha-aminoadipic semialdehyde dehydrogenase (Alpha-AASA dehydrogenase) (Betaine aldehyde dehydrogenase) (Delta1-piperideine-6-carboxylate dehydrogenase) (P6c dehydrogenase) (Aldehyde dehydrogenase family 7 member A1) (Antiquitin-1)	4	1 von 2	0,322	1	1 von 2	0,072	0,25	4,472222222	347,2222222
P0C2X9	Delta-1-pyrroline-5-carboxylate dehydrogenase, mitochondrial (P5C dehydrogenase) (Aldehyde dehydrogenase family 4 member A1)	5	1 von 2	0,252	2	1 von 2	0,058	0,194	4,344827586	334,4827586
P11980	Pyruvate kinase isozymes M1/M2 (Pyruvate kinase muscle isozyme)	4	1 von 2	0,227	1	1 von 2	0,053	0,174	4,283018868	328,3018868
B0BNA5	Coactosin-like protein	6	2 von 2	0,915	7	2 von 2	0,233	0,682	3,927038627	292,7038627
P55051	Fatty acid-binding protein, brain (Brain-type fatty acid-binding protein) (B-FABP) (Fatty acid-binding protein 7) (Brain lipid-binding protein) (BLBP)	5	1 von 2	0,778	3	1 von 2	0,212	0,566	3,669811321	266,9811321
P56574	Isocitrate dehydrogenase [NADP], mitochondrial (IDH) (Oxalosuccinate decarboxylase) (NADP(+)-specific ICDH) (IDP) (ICD-M)	9	1 von 2	0,638	3	1 von 2	0,179	0,459	3,56424581	256,424581
P18421	Proteasome subunit beta type-1 (Proteasome component C5) (Macropain subunit C5) (Multicatalytic endopeptidase complex subunit C5) (Proteasome gamma chain)	6	2 von 2	0,515	6	2 von 2	0,145	0,37	3,551724138	255,1724138
P62243	40S ribosomal protein S8	5	1 von 2	0,585	5	1 von 2	0,166	0,419	3,524096386	252,4096386
P70623	Fatty acid-binding protein, adipocyte (Adipocyte-type fatty acid-binding protein) (A-FABP) (Fatty acid-binding protein 4) (Adipocyte lipid-binding protein) (ALBP)	10	2 von 2	2,79	8	2 von 2	0,809	1.981	3,448702101	244,8702101
P70580	Membrane-associated progesterone receptor component 1 (Acidic 25 kDa protein) (25-DX) (Ventral midline antigen) (VEMA)	5	1 von 2	1,61	3	1 von 2	0,468	1.142	3,44017094	244,017094
Q5U204	Mitogen-activated protein kinase scaffold protein 1 (Mitogen-activated protein kinase kinase 1-interacting protein 1)	4	2 von 2	0,885	2	1 von 2	0,259	0,626	3,416988417	241,6988417
P47875	Cysteine and glycine-rich protein 1 (Cysteine-rich protein 1) (CRP1)	3	1 von 2	0,438	1	1 von 2	0,129	0,309	3,395348837	239,5348837
Q8R431	Monoglyceride lipase (MGL) (Monoacylglycerol lipase) (MAGL)	3	1 von 2	0,438	1	1 von 2	0,129	0,309	3,395348837	239,5348837
P05197	Elongation factor 2 (EF-2)	19	2 von 2	0,514	10	2 von 2	0,155	0,359	3,316129032	231,6129032
Q6AYE2	Endophilin-B1 (SH3 domain-containing GRB2-like protein B1)	3	1 von 2	0,318	1	1 von 2	0,096	0,222	3,3125	231,25
Q64591	2,4-dienoyl-CoA reductase, mitochondrial (2,4-dienoyl-CoA reductase [NADPH]) (4-enoyl-CoA reductase [NADPH])	4	1 von 2	0,28	2	1 von 2	0,086	0,194	3,255813953	225,5813953
Q64550	UDP-glucuronosyltransferase 1-1 (UDPGT 1-1) (UDP-glucuronosyltransferase 1A1) (B1)	3	1 von 2	0,241	1	1 von 2	0,075	0,166	3,213333333	221,3333333
Q6P7B0	Tryptophanyl-tRNA synthetase, cytoplasmic (Tryptophan--tRNA ligase) (TrpRS)	3	1 von 2	0,212	1	1 von 2	0,066	0,146	3,212121212	221,2121212
Q60587	Trifunctional enzyme subunit beta, mitochondrial (TP-beta)	3	1 von 2	0,218	1	1 von 2	0,068	0,15	3,205882353	220,5882353
Q64573	Liver carboxylesterase 4 (Carboxyesterase ES-4) (Microsomal palmitoyl-CoA hydrolase) (Kidney microsomal carboxylesterase)	8	1 von 2	0,719	3	1 von 2	0,225	0,494	3,195555556	219,5555556
P21213	Histidine ammonia-lyase (Histidase)	4	1 von 2	0,162	4	1 von 2	0,051	0,111	3,176470588	217,6470588
P10688	1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase delta-1 (Phosphoinositide phospholipase C-delta-1) (Phospholipase C-III) (PLC-III) (Phospholipase C-delta-1) (PLC-delta-1)	3	1 von 2	0,142	1	1 von 2	0,045	0,097	3,155555556	215,5555556
Q920L2	Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial (Flavoprotein subunit of complex II) (Fp)	3	1 von 2	0,145	1	1 von 2	0,046	0,099	3,152173913	215,2173913

Supplementary table 4. NPC – cytoplasmic protein fraction

accession number	protein name	analysis peptides	analysis cytoplasm expcount	analysis cytoplasm emPAI	reference peptides	reference cytoplasm expcount	reference cytoplasm emPAI	emPAI diff cytoplasm	x-times up-regulated	alteration in %
O35509	Ras-related protein Rab-11B	5	1 von 2	0,833	2	1 von 2	0,274	0,559	3,040145985	204,0145985
P29266	3-hydroxyisobutyrate dehydrogenase, mitochondrial (HIBADH)	5	1 von 2	0,688	2	1 von 2	0,233	0,455	2,9527897	195,27897
P55260	Annexin A4 (36 kDa zymogen granule membrane-associated protein) (ZAP36) (Annexin IV) (Annexin-4) (Lipocortin IV)	5	1 von 2	0,532	2	1 von 2	0,186	0,346	2,860215054	186,0215054
Q66H12	Alpha-N-acetylgalactosaminidase (Alpha-galactosidase B)	5	1 von 2	0,468	4	1 von 2	0,166	0,302	2,819277108	181,9277108
P16303	Carboxylesterase 3 (Liver carboxylesterase 10) (Carboxyesterase ES-10) (Fatty acid ethyl ester synthase) (FAEE synthase) (pI 6.1 esterase) (ES-HVEL)	5	1 von 2	0,433	2	1 von 2	0,155	0,278	2,793548387	179,3548387
Q63010	Liver carboxylesterase B-1 (Liver microsomal carboxylesterase)	5	1 von 2	0,377	3	1 von 2	0,136	0,241	2,772058824	177,2058824
Q4KLZ6	Bifunctional ATP-dependent dihydroxyacetone kinase/FAD-AMP lyase (cyclizing)	6	1 von 2	0,377	8	1 von 2	0,136	0,241	2,772058824	177,2058824
P09495	Tropomyosin alpha-4 chain (Tropomyosin-4) (TM-4)	18	1 von 2	0,887	24	2 von 2	0,33	0,557	2,687878788	168,7878788
Q9Z1P2	Alpha-actinin-1 (Alpha-actinin cytoskeletal isoform) (Non-muscle alpha-actinin-1) (F-actin cross-linking protein)	18	1 von 2	0,444	18	1 von 2	0,182	0,262	2,43956044	143,956044
P26772	10 kDa heat shock protein, mitochondrial (Hsp10) (10 kDa chaperonin) (Chaperonin 10) (CPN10)	5	2 von 2	1,31	5	2 von 2	0,553	0,757	2,368896926	136,8896926
P61107	Ras-related protein Rab-14	4	1 von 2	0,778	2	1 von 2	0,334	0,444	2,329341317	132,9341317
Q6LED0	Histone H3.1	7	1 von 2	0,874	3	2 von 2	0,376	0,498	2,324468085	132,4468085
P22791	Hydroxymethylglutaryl-CoA synthase, mitochondrial (HMG-CoA synthase) (3-hydroxy-3-methylglutaryl coenzyme A synthase)	9	1 von 2	0,748	5	1 von 2	0,322	0,426	2,322981366	132,2981366
Q6AYQ8	Fumarylacetoacetate hydrolase domain-containing protein 1	4	1 von 2	0,719	3	1 von 2	0,311	0,408	2,311897106	131,1897106
P01041	Cystatin-B (Cystatin-beta) (Stefin-B) (Liver thiol proteinase inhibitor)	3	1 von 2	0,668	2	1 von 2	0,292	0,376	2,287671233	128,7671233
P00406	Cytochrome c oxidase subunit 2 (Cytochrome c oxidase polypeptide II)	2	1 von 2	0,668	4	1 von 2	0,292	0,376	2,287671233	128,7671233
P13437	3-ketoacyl-CoA thiolase, mitochondrial (Beta-ketothiolase) (Acetyl-CoA acyltransferase) (Mitochondrial 3-oxoacyl-CoA thiolase)	7	1 von 2	0,668	7	1 von 2	0,292	0,376	2,287671233	128,7671233
P62630	Elongation factor 1-alpha 1 (EF-1-alpha-1) (Eukaryotic elongation factor 1 A-1) (eEF1A-1) (Elongation factor Tu) (EF-Tu)	15	2 von 2	0,506	10	2 von 2	0,228	0,278	2,219298246	121,9298246
P07895	Superoxide dismutase [Mn], mitochondrial	4	2 von 2	0,54	4	2 von 2	0,244	0,296	2,213114754	121,3114754
P84817	Mitochondrial fission 1 protein (FIS1 homolog) (rFis1) (Tetratricopeptide repeat protein 11) (TPR repeat protein 11)	2	1 von 2	0,468	1	1 von 2	0,212	0,256	2,20754717	120,754717
P04762	Catalase	15	1 von 2	0,753	12	2 von 2	0,343	0,41	2,195335277	119,5335277
P07335	Creatine kinase B-type (Creatine kinase B chain) (B-CK)	5	1 von 2	0,425	3	1 von 2	0,194	0,231	2,190721649	119,0721649
Q9ERR2	COMM domain-containing protein 5 (Hypertension-related calcium-regulated gene protein) (HCaRG)	2	1 von 2	0,389	1	1 von 2	0,179	0,21	2,173184358	117,3184358
P97682	Endothelial cell-specific molecule 1 (ESM-1) (PG25)	2	1 von 2	0,389	2	1 von 2	0,179	0,21	2,173184358	117,3184358
P62278	40S ribosomal protein S13	5	1 von 2	0,389	5	1 von 2	0,179	0,21	2,173184358	117,3184358
B0BN18	Prefoldin subunit 2	2	1 von 2	0,359	1	1 von 2	0,166	0,193	2,162650602	116,2650602
P14942	Glutathione S-transferase alpha-4 (Glutathione S-transferase Yk) (GST Yk) (GST 8-8) (GST K) (GST A4-4)	3	1 von 2	0,359	1	1 von 2	0,166	0,193	2,162650602	116,2650602
P23965	3,2-trans-enoyl-CoA isomerase, mitochondrial (Dodecenoyl-CoA isomerase) (Delta(3),Delta(2)-enoyl-CoA isomerase) (D3,D2-enoyl-CoA isomerase)	5	1 von 2	0,492	6	2 von 2	0,228	0,264	2,157894737	115,7894737
O35264	Platelet-activating factor acetylhydrolase IB subunit beta (PAF acetylhydrolase 30 kDa subunit) (PAF-AH 30 kDa subunit) (PAF-AH subunit beta) (PAFAH subunit beta)	2	1 von 2	0,334	2	1 von 2	0,155	0,179	2,15483871	115,483871
Q6AXY0	Glutathione S-transferase A6 (GST class-alpha member 6)	3	1 von 2	0,334	2	1 von 2	0,155	0,179	2,15483871	115,483871
Q9EST6	Acidic leucine-rich nuclear phosphoprotein 32 family member B (Proliferation-related acidic leucine-rich protein PAL31)	6	1 von 2	0,334	7	1 von 2	0,155	0,179	2,15483871	115,483871
Q66HD0	Endoplasmin (Heat shock protein 90 kDa beta member 1) (94 kDa glucose-regulated protein) (GRP-94)	17	2 von 2	0,53	10	2 von 2	0,246	0,284	2,154471545	115,4471545
B2RYG6	Ubiquitin thioesterase OTUB1 (Deubiquitinating enzyme OTUB1) (OTU domain-containing ubiquitin aldehyde-binding protein 1) (Otubain-1) (Ubiquitin-specific-processing protease OTUB1)	2	1 von 2	0,292	1	1 von 2	0,136	0,156	2,147058824	114,7058824
P08542	UDP-glucuronosyltransferase 2B17 (UDPGT 2B17) (UDP-glucuronosyltransferase 2B5) (UDPGT 2B5) (17-beta-hydroxysteroid-specific UDPGT) (RLUG38) (Testosterone, dihydrotestosterone, and beta-estradiol-specific UDPGT) (UDPGTr-3)	4	1 von 2	0,322	2	1 von 2	0,15	0,172	2,146666667	114,6666667
P63326	40S ribosomal protein S10	5	1 von 2	0,311	3	1 von 2	0,145	0,166	2,144827586	114,4827586
P34058	Heat shock protein HSP 90-beta (Heat shock 84 kDa) (HSP 84)	19	2 von 2	0,917	12	2 von 2	0,43	0,487	2,13255814	113,255814
Q66HR2	Microtubule-associated protein RP/EB family member 1 (APC-binding protein EB1) (End-binding protein 1) (EB1)	2	1 von 2	0,274	1	1 von 2	0,129	0,145	2,124031008	112,4031008
Q02765	Cathepsin S	3	1 von 2	0,274	2	1 von 2	0,129	0,145	2,124031008	112,4031008

Supplementary table 4. NPC – cytoplasmic protein fraction

accession number	protein name	analysis peptides	analysis cytoplasm expcount	analysis cytoplasm emPAI	reference peptides	reference cytoplasm expcount	reference cytoplasm emPAI	emPAI diff cytoplasm	x-times up-regulated	alteration in %
P56571	ES1 protein homolog, mitochondrial	3	1 von 2	0,274	2	1 von 2	0,129	0,145	2,124031008	112,4031008
P38983	40S ribosomal protein SA (Laminin receptor 1) (LamR) (37/67 kDa laminin receptor) (LRP/LR) (Laminin-binding protein precursor p40) (LBP/p40) (37 kDa laminin receptor precursor) (37LRP) (67 kDa laminin receptor) (67LR)	7	1 von 2	0,274	7	1 von 2	0,129	0,145	2,124031008	112,4031008
P28064	Proteasome subunit beta type-8 (Proteasome subunit beta-5i) (Proteasome component C13) (Macropain subunit C13) (Multicatalytic endopeptidase complex subunit C13)	3	1 von 2	0,259	3	1 von 2	0,122	0,137	2,12295082	112,295082
P18422	Proteasome subunit alpha type-3 (Proteasome component C8) (Macropain subunit C8) (Multicatalytic endopeptidase complex subunit C8) (Proteasome subunit K)	4	2 von 2	0,259	3	2 von 2	0,122	0,137	2,12295082	112,295082
P17046	Lysosome-associated membrane glycoprotein 2 (Lysosome-associated membrane protein 2) (Lysosomal membrane glycoprotein type B) (LGP-B) (LGP-96) (LGP-110) (CD107 antigen-like family member B)	2	1 von 2	0,222	1	1 von 2	0,105	0,117	2,114285714	111,4285714
B2RYW9	Fumarylacetoacetate hydrolase domain-containing protein 2	2	1 von 2	0,245	1	1 von 2	0,116	0,129	2,112068966	111,2068966
P09606	Glutamine synthetase (GS) (Glutamate--ammonia ligase) (Glutamate decarboxylase)	2	1 von 2	0,202	3	1 von 2	0,096	0,106	2,104166667	110,4166667
Q794E4	Heterogeneous nuclear ribonucleoprotein F (hnRNP F)	3	1 von 2	0,212	2	1 von 2	0,101	0,111	2,099009901	109,9009901
Q9EQV6	Tripeptidyl-peptidase 1 (TPP-1) (Tripeptidyl aminopeptidase) (Tripeptidyl-peptidase I) (TPP-I)	2	1 von 2	0,186	1	1 von 2	0,089	0,097	2,08988764	108,988764
P50554	4-aminobutyrate aminotransferase, mitochondrial (Gamma-amino-N-butyrate transaminase) (GABA transaminase) (GABA aminotransferase) (GABA-AT) (L-AIBAT) ((S)-3-amino-2-methylpropionate transaminase)	2	1 von 2	0,15	1	1 von 2	0,072	0,078	2,083333333	108,3333333
Q63108	Liver carboxylesterase 3 (Carboxyesterase ES-3) (pI 5.5 esterase) (ES-HTEL)	2	1 von 2	0,179	1	1 von 2	0,086	0,093	2,081395349	108,1395349
Q07205	Eukaryotic translation initiation factor 5 (eIF-5)	2	1 von 2	0,16	1	1 von 2	0,077	0,083	2,077922078	107,7922078
P04182	Ornithine aminotransferase, mitochondrial (Ornithine--oxo-acid aminotransferase)	5	1 von 2	0,16	4	1 von 2	0,077	0,083	2,077922078	107,7922078
P24268	Cathepsin D	3	1 von 2	0,166	1	1 von 2	0,08	0,086	2,075	107,5
P23457	3-alpha-hydroxysteroid dehydrogenase (3-alpha-HSD) (Hydroxyprostaglandin dehydrogenase)	3	2 von 2	0,172	3	2 von 2	0,083	0,089	2,072289157	107,2289157
Q5XIM9	T-complex protein 1 subunit beta (TCP-1-beta) (CCT-beta)	3	1 von 2	0,116	1	1 von 2	0,056	0,06	2,071428571	107,1428571
Q5U2V4	Putative phospholipase B-like 1 (LAMA-like protein 1) (Lamina ancestor homolog 1) (Phospholipase B domain-containing protein 1)	2	1 von 2	0,145	3	1 von 2	0,07	0,075	2,071428571	107,1428571
P31000	Vimentin	2	1 von 2	0,122	2	1 von 2	0,059	0,063	2,06779661	106,779661
P47942	Dihydropyrimidinase-related protein 2 (DRP-2) (Turned on after division 64 kDa protein) (TOAD-64) (Collapsin response mediator protein 2) (CRMP-2)	2	1 von 2	0,122	3	1 von 2	0,059	0,063	2,06779661	106,779661
P08503	Medium-chain specific acyl-CoA dehydrogenase, mitochondrial (MCAD)	2	1 von 2	0,155	1	1 von 2	0,075	0,08	2,066666667	106,6666667
P07687	Epoxide hydrolase 1 (Epoxide hydratase) (Microsomal epoxide hydrolase)	2	1 von 2	0,136	1	1 von 2	0,066	0,07	2,060606061	106,0606061
Q99N27	Sorting nexin-1	2	1 von 2	0,119	1	1 von 2	0,058	0,061	2,051724138	105,1724138
Q5XHZ0	Heat shock protein 75 kDa, mitochondrial (HSP 75) (Tumor necrosis factor type 1 receptor-associated protein) (TRAP-1) (TNFR-associated protein 1)	3	2 von 2	0,08	1	2 von 2	0,039	0,041	2,051282051	105,1282051
Q9JI85	Nucleobindin-2 (DNA-binding protein NEFA)	2	1 von 2	0,125	1	1 von 2	0,061	0,064	2,049180328	104,9180328
Q9Z2Q1	Protein transport protein Sec31A (SEC31-like protein 1) (SEC31-related protein A) (Vesicle-associated protein 1)	2	1 von 2	0,073	1	1 von 2	0,036	0,037	2,027777778	102,7777778
P02761	Major urinary protein (MUP) (Alpha-2u-globulin) (Alpha(2)-euglobulin) (Allergen Rat n I)	10	2 von 2	2.245	7	2 von 2	1.154	1.091	1,945407279	94,5407279
P29457	Serpin H1 (47 kDa heat shock protein) (Collagen-binding protein) (Colligin) (GP46)	9	1 von 2	0,585	5	1 von 2	0,301	0,284	1,943521595	94,35215947
P50137	Transketolase (TK)	16	2 von 2	0,586	12	2 von 2	0,306	0,28	1,91503268	91,50326797
P00502	Glutathione S-transferase alpha-1 (Glutathione S-transferase Ya-1) (GST Ya1) (Ligandin) (GST 1a-1a) (GST B) (GST 1-1) (GST A1-1)	8	2 von 2	1,05	6	2 von 2	0,557	0,493	1,885098743	88,50987433
P35435	ATP synthase subunit gamma, mitochondrial (F-ATPase gamma subunit)	5	1 von 2	0,778	4	1 von 2	0,413	0,365	1,88377724	88,37772397
P19945	60S acidic ribosomal protein P0 (60S ribosomal protein L10E)	7	1 von 2	0,688	7	1 von 2	0,369	0,319	1,864498645	86,4498645
P15650	Long-chain specific acyl-CoA dehydrogenase, mitochondrial (LCAD)	5	1 von 2	0,45	3	1 von 2	0,25	0,2	1,8	80
P30713	Glutathione S-transferase theta-2 (GST class-theta-2) (Glutathione S-transferase 12) (GST 12-12) (Glutathione S-transferase Yrs-Yrs)	4	2 von 2	0,437	2	2 von 2	0,244	0,193	1,790983607	79,09836066
P04904	Glutathione S-transferase alpha-3 (Glutathione S-transferase Yc-1) (GST Yc1) (GST 2-2) (GST AA) (GST A3-3)	6	1 von 2	0,968	7	2 von 2	0,557	0,411	1,737881508	73,78815081
P22734	Catechol O-methyltransferase	6	2 von 2	0,556	3	2 von 2	0,334	0,222	1,664670659	66,46706587
P82995	Heat shock protein HSP 90-alpha (Heat shock 86 kDa) (HSP 86)	13	2 von 2	0,532	10	2 von 2	0,32	0,212	1,6625	66,25
P62161	Calmodulin (CaM)	3	1 von 2	0,778	2	1 von 2	0,468	0,31	1,662393162	66,23931624
Q5XIH7	Prohibitin-2 (B-cell receptor-associated protein BAP37) (BAP-37)	7	1 von 2	0,585	5	1 von 2	0,359	0,226	1,629526462	62,95264624
P62083	40S ribosomal protein S7 (S8)	7	1 von 2	0,54	5	1 von 2	0,334	0,206	1,616766467	61,67664671
Q9WVK3	Peroxisomal trans-2-enoyl-CoA reductase (TERP) (RLF98) (Peroxisomal 2,4-dienoyl-CoA reductase) (PX-2,4-DCR1)	5	1 von 2	0,54	6	1 von 2	0,334	0,206	1,616766467	61,67664671

Supplementary table 4. NPC – cytoplasmic protein fraction

accession number	protein name	analysis peptides	analysis cytoplasm expcount	analysis cytoplasm emPAI	reference peptides	reference cytoplasm expcount	reference cytoplasm emPAI	emPAI diff cytoplasm	x-times up-regulated	alteration in %
P04785	Protein disulfide-isomerase (PDI) (Prolyl 4-hydroxylase subunit beta) (Cellular thyroid hormone-binding protein)	15	2 von 2	0,464	12	2 von 2	0,287	0,177	1,616724739	61,67247387
P97532	3-mercaptopyruvate sulfurtransferase (MST)	5	1 von 2	0,501	2	1 von 2	0,311	0,19	1,610932476	61,09324759
A7VJC2	Heterogeneous nuclear ribonucleoproteins A2/B1 (hnRNP A2/B1)	10	1 von 2	0,35	14	1 von 2	0,222	0,128	1,576576577	57,65765766
P28075	Proteasome subunit beta type-5 (Proteasome epsilon chain) (Macropain epsilon chain) (Multicatalytic endopeptidase complex epsilon chain) (Proteasome subunit X) (Proteasome chain 6)	3	2 von 2	0,214	1	1 von 2	0,136	0,078	1,573529412	57,35294118
Q68FY0	Cytochrome b-c1 complex subunit 1, mitochondrial (Ubiquinol-cytochrome-c reductase complex core protein 1) (Core protein I) (Complex III subunit 1)	3	1 von 2	0,292	3	1 von 2	0,186	0,106	1,569892473	56,98924731
P02793	Ferritin light chain 1 (Ferritin L subunit 1)	13	2 von 2	3.454	14	2 von 2	2.205	1.249	1,566439909	56,64399093
P04897	Guanine nucleotide-binding protein G(i) subunit alpha-2 (Adenylate cyclase-inhibiting G alpha protein)	3	1 von 2	0,28	5	1 von 2	0,179	0,101	1,56424581	56,42458101
Q68FS4	Cytosol aminopeptidase (Leucine aminopeptidase 3) (LAP-3) (Leucyl aminopeptidase) (Proline aminopeptidase) (Prolyl aminopeptidase)	11	2 von 2	0,324	15	2 von 2	0,208	0,116	1,557692308	55,76923077
P15999	ATP synthase subunit alpha, mitochondrial	23	1 von 2	1.918	22	1 von 2	1.233	0,685	1,555555556	55,55555556
P35565	Calnexin	3	1 von 2	0,225	2	1 von 2	0,145	0,08	1,551724138	55,17241379
P21775	3-ketoacyl-CoA thiolase A, peroxisomal (Beta-ketothiolase A) (Acetyl-CoA acyltransferase A) (Peroxisomal 3-oxoacyl-CoA thiolase A)	3	2 von 2	0,156	1	1 von 2	0,101	0,055	1,544554455	54,45544554
P46462	Transitional endoplasmic reticulum ATPase (TER ATPase) (15S Mg(2+)-ATPase p97 subunit) (Valosin-containing protein) (VCP)	25	2 von 2	0,632	20	2 von 2	0,41	0,222	1,541463415	54,14634146
Q9R0J8	Legumain (Asparaginyl endopeptidase) (Protease, cysteine 1)	6	2 von 2	0,137	4	1 von 2	0,089	0,048	1,539325843	53,93258427
P62959	Histidine triad nucleotide-binding protein 1 (Adenosine 5'-monophosphoramidase) (Protein kinase C inhibitor 1) (Protein kinase C-interacting protein 1) (PKCI-1) (17 kDa inhibitor of protein kinase C)	3	2 von 2	0,723	3	2 von 2	0,48	0,243	1,50625	50,625
Q9EQX9	Ubiquitin-conjugating enzyme E2 N (Ubiquitin-protein ligase N) (Ubiquitin carrier protein N) (Bendless-like ubiquitin-conjugating enzyme)	6	2 von 2	0,833	4	2 von 2	0,555	0,278	1,500900901	50,09009009

found in both	min. 2 exp, min. 2 peptides, delta of ≤-2 or -50% upon NAF treatment				sorted according to the magnitude of the regulation					
accession number	protein name	analysis peptides	analysis cytoplasm expcount	analysis cytoplasm emPAI	reference peptides	reference cytoplasm expcount	reference cytoplasm emPAI	emPAI diff cytoplasm	x-times down-regulated	alteration in %
Q4QRB4	Tubulin beta-3 chain (Neuron-specific class III beta-tubulin)	6	1 von 2	0,101	12	1 von 2	2.162	-2.061	-21405,94059	-2140494,059
Q4KM73	UMP-CMP kinase (Cytidylate kinase) (Deoxycytidylate kinase) (Cytidine monophosphate kinase) (Uridine monophosphate kinase) (Uridine monophosphate/cytidine monophosphate kinase) (UMP/CMP kinase)	5	2 von 2	0,311	7	1 von 2	1.254	-0,943	-4032,154341	-403115,4341
Q9Z0W7	Chloride intracellular channel protein 4 (Intracellular chloride ion channel protein p64H1)	6	1 von 2	0,413	8	1 von 2	1.512	-1.099	-3661,016949	-366001,6949
O70351	3-hydroxyacyl-CoA dehydrogenase type-2 (3-hydroxyacyl-CoA dehydrogenase type II) (Type II HADH) (3-hydroxy-2-methylbutyryl-CoA dehydrogenase) (17-beta-hydroxysteroid dehydrogenase 10) (17-beta-HSD 10) (Mitochondrial ribonuclease P protein 2) (Mitochondria	9	2 von 2	0,863	12	1 von 2	2.875	-2.012	-3331,402086	-333040,2086
P62828	GTP-binding nuclear protein Ran (GTPase Ran) (Ras-related nuclear protein) (Ras-like protein TC4)	3	1 von 2	0,585	7	1 von 2	1.929	-1.344	-3297,435897	-329643,5897
P85973	Purine nucleoside phosphorylase (PNP) (Inosine phosphorylase)	27	2 von 2	0,999	30	2 von 2	3.133	-2.134	-3136,136136	-313513,6136
Q497B0	Omega-amidase NIT2 (Nitrilase homolog 2)	10	2 von 2	0,389	9	2 von 2	1.067	-0,678	-2742,930591	-274193,0591
Q05982	Nucleoside diphosphate kinase A (NDP kinase A) (Tumor metastatic process-associated protein) (Metastasis inhibition factor NM23)	6	2 von 2	0,931	10	2 von 2	2.205	-1.274	-2368,421053	-236742,1053
Q00715	Histone H2B type 1	6	2 von 2	0,811	8	2 von 2	1.649	-0,838	-2033,292232	-203229,2232
P18666	Myosin regulatory light chain 12B (Myosin RLC-B) (Myosin regulatory light chain 2-B, smooth muscle isoform) (MLC-2) (Myosin regulatory light chain 20 kDa) (MLC20) (Myosin regulatory light chain MRLC2)	4	1 von 2	0,701	7	1 von 2	1.424	-0,723	-2031,383738	-203038,3738
P25093	Fumarylacetoacetase (FAA) (Fumarylacetoacetate hydrolase) (Beta-diketonase)	9	1 von 2	0,778	11	1 von 2	1.512	-0,734	-1943,44473	-194244,473
P52555	Endoplasmic reticulum resident protein 29 (ERp29) (Endoplasmic reticulum resident protein 31) (ERp31)	4	1 von 2	0,624	6	1 von 2	1.069	-0,445	-1713,141026	-171214,1026
P13255	Glycine N-methyltransferase (Folate-binding protein)	7	1 von 2	0,778	9	1 von 2	1.239	-0,461	-1592,544987	-159154,4987
Q9WTT6	Guanine deaminase (Guanase) (Guanine aminohydrolase) (GAH)	17	2 von 2	0,92	17	1 von 2	1.246	-0,326	-1354,347826	-135334,7826

Supplementary table 4. NPC – cytoplasmic protein fraction

accession number	protein name	analysis peptides	analysis cytoplasm expcount	analysis cytoplasm emPAI	reference peptides	reference cytoplasm expcount	reference cytoplasm emPAI	emPAI diff cytoplasm	x-times down-regulated	alteration in %
P62260	14-3-3 protein epsilon (14-3-3E) (Mitochondrial import stimulation factor L subunit) (MSF L)	15	2 von 2	0,852	16	2 von 2	1.103	-0,251	-1294,600939	-129360,0939
P46844	Biliverdin reductase A (BVR A) (Biliverdin-IX alpha-reductase)	9	1 von 2	0,823	8	1 von 2	1.015	-0,192	-1233,292831	-123229,2831
P63102	14-3-3 protein zeta/delta (Protein kinase C inhibitor protein 1) (KCIP-1) (Mitochondrial import stimulation factor S1 subunit)	12	2 von 2	0,832	12	2 von 2	1.025	-0,193	-1231,971154	-123097,1154
Q5XI73	Rho GDP-dissociation inhibitor 1 (Rho GDI 1) (Rho-GDI alpha)	12	2 von 2	0,968	10	2 von 2	1.133	-0,165	-1170,454545	-116945,4545
Q8R491	EH domain-containing protein 3	19	1 von 2	2,07	18	1 von 2	1.894	0,176	-914,9758454	-91397,58454
Q6P9T8	Tubulin beta-2C chain	21	1 von 2	5,19	18	1 von 2	3.642	1.548	-701,734104	-70073,4104
P02692	Fatty acid-binding protein, liver (Liver-type fatty acid-binding protein) (L-FABP) (Fatty acid-binding protein 1) (Squalene- and sterol-carrier protein) (SCP) (Z-protein) (p14)	12	2 von 2	6.104	17	1 von 2	45.416	-39.312	-7,440366972	-644,0366972
Q8CFM6	Stabilin-2 (Fasciclin, EGF-like, laminin-type EGF-like and link domain-containing scavenger receptor 2) (FEEL-2) (Hyaluronan receptor for endocytosis)	6	1 von 2	0,026	15	1 von 2	0,138	-0,112	-5,307692308	-430,7692308
P97576	GrpE protein homolog 1, mitochondrial (Mt-GrpE#1)	2	2 von 2	0,129	5	1 von 2	0,624	-0,495	-4,837209302	-383,7209302
P16086	Spectrin alpha chain, brain (Spectrin, non-erythroid alpha chain) (Alpha-II spectrin) (Fodrin alpha chain)	38	2 von 2	0,086	99	1 von 2	0,385	-0,299	-4,476744186	-347,6744186
P62271	40S ribosomal protein S18	5	1 von 2	0,179	8	1 von 2	0,638	-0,459	-3,56424581	-256,424581
P85971	6-phosphogluconolactonase (6PGL)	6	1 von 2	0,166	7	2 von 2	0,585	-0,419	-3,524096386	-252,4096386
Q5EB77	Ras-related protein Rab-18	1	1 von 2	0,145	3	1 von 2	0,501	-0,356	-3,455172414	-245,5172414
P36970	Phospholipid hydroperoxide glutathione peroxidase, mitochondrial (PHGPx) (Glutathione peroxidase 4) (GSHPx-4)	1	1 von 2	0,129	3	1 von 2	0,438	-0,309	-3,395348837	-239,5348837
Q63768	Adapter molecule crk (Proto-oncogene c-Crk) (p38)	1	1 von 2	0,129	4	1 von 2	0,438	-0,309	-3,395348837	-239,5348837
P06214	Delta-aminolevulinic acid dehydratase (ALADH) (Porphobilinogen synthase)	8	2 von 2	0,253	9	2 von 2	0,852	-0,599	-3,367588933	-236,7588933
P97519	Hydroxymethylglutaryl-CoA lyase, mitochondrial (HMG-CoA lyase) (3-hydroxy-3-methylglutarate-CoA lyase)	1	1 von 2	0,11	3	1 von 2	0,369	-0,259	-3,354545455	-235,4545455
P62944	AP-2 complex subunit beta (Adapter-related protein complex 2 beta subunit) (Adaptor protein complex AP-2 subunit beta) (Beta-2-adaptin) (Beta-adaptin) (Plasma membrane adaptor HA2/AP2 adaptin beta subunit) (Clathrin assembly protein complex 2 beta large c	10	1 von 2	0,084	10	1 von 2	0,274	-0,19	-3,261904762	-226,1904762
Q32KJ6	N-acetylgalactosamine-6-sulfatase (Chondroitinsulfatase) (Chondroitinase) (Galactose-6-sulfate sulfatase) (N-acetylgalactosamine-6-sulfate sulfatase) (GalNAc6S sulfatase)	1	1 von 2	0,07	3	1 von 2	0,225	-0,155	-3,214285714	-221,4285714
Q63150	Dihydropyrimidinase (DHPase) (Dihydropyrimidine amidohydrolase) (Hydantoinase)	6	1 von 2	0,064	8	1 von 2	0,205	-0,141	-3,203125	-220,3125
P11442	Clathrin heavy chain 1	46	2 von 2	0,116	63	1 von 2	0,37	-0,254	-3,189655172	-218,9655172
P50475	Alanyl-tRNA synthetase, cytoplasmic (Alanine--tRNA ligase) (AlaRS)	1	1 von 2	0,035	3	1 von 2	0,109	-0,074	-3,114285714	-211,4285714
P18484	AP-2 complex subunit alpha-2 (Adapter-related protein complex 2 alpha-2 subunit) (Adaptor protein complex AP-2 subunit alpha-2) (Alpha2-adaptin) (Plasma membrane adaptor HA2/AP2 adaptin alpha C subunit) (Alpha-adaptin C) (Clathrin assembly protein complex	22	1 von 2	0,035	22	1 von 2	0,109	-0,074	-3,114285714	-211,4285714
P62138	Serine/threonine-protein phosphatase PP1-alpha catalytic subunit (PP-1A)	4	1 von 2	0,222	15	1 von 2	0,65	-0,428	-2,927927928	-192,7927928
P85970	Actin-related protein 2/3 complex subunit 2 (Arp2/3 complex 34 kDa subunit) (p34-ARC)	10	1 von 2	0,194	9	1 von 2	0,557	-0,363	-2,871134021	-187,1134021
Q02974	Ketohexokinase (Hepatic fructokinase)	6	2 von 2	0,217	6	1 von 2	0,616	-0,399	-2,838709677	-183,8709677
Q6P686	Osteoclast-stimulating factor 1	2	2 von 2	0,155	3	2 von 2	0,437	-0,282	-2,819354839	-181,9354839
Q63617	Hypoxia up-regulated protein 1 (150 kDa oxygen-regulated protein) (ORP-150)	4	1 von 2	0,129	10	1 von 2	0,354	-0,225	-2,744186047	-174,4186047
P25113	Phosphoglycerate mutase 1 (Phosphoglycerate mutase isozyme B) (PGAM-B) (BPG-dependent PGAM 1)	9	2 von 2	0,202	8	2 von 2	0,554	-0,352	-2,742574257	-174,2574257
Q08163	Adenylyl cyclase-associated protein 1 (CAP 1)	5	2 von 2	0,204	8	1 von 2	0,54	-0,336	-2,647058824	-164,7058824
Q8VIF7	Selenium-binding protein 1 (Selenium-binding protein 2) (56 kDa selenium-binding protein) (SBP56)	10	2 von 2	0,263	10	1 von 2	0,682	-0,419	-2,593155894	-159,3155894
P52303	AP-1 complex subunit beta-1 (Adapter-related protein complex 1 subunit beta-1) (Adaptor protein complex AP-1 subunit beta-1) (Beta-adaptin 1) (Beta-1-adaptin) (Golgi adaptor HA1/AP1 adaptin beta subunit) (Clathrin assembly protein complex 1 beta large cha	17	1 von 2	0,16	19	1 von 2	0,397	-0,237	-2,48125	-148,125
P07943	Aldose reductase (AR) (Aldehyde reductase)	4	2 von 2	0,217	7	2 von 2	0,529	-0,312	-2,437788018	-143,7788018
P22985	Xanthine dehydrogenase/oxidase	21	2 von 2	0,163	20	1 von 2	0,389	-0,226	-2,386503067	-138,6503067
P00173	Cytochrome b5	4	2 von 2	1.154	7	2 von 2	2.712	-1.558	-2,350086655	-135,0086655
P49911	Acidic leucine-rich nuclear phosphoprotein 32 family member A (Leucine-rich acidic nuclear protein) (LANP)	5	1 von 2	0,334	5	1 von 2	0,778	-0,444	-2,329341317	-132,9341317
P19112	Fructose-1,6-bisphosphatase 1 (FBPase 1) (D-fructose-1,6-bisphosphate 1-phosphohydrolase 1)	14	1 von 2	1.081	16	1 von 2	2.511	-1,43	-2,322849214	-132,2849214
P10888	Cytochrome c oxidase subunit 4 isoform 1, mitochondrial (Cytochrome c oxidase subunit IV isoform 1) (COX IV-1) (Cytochrome c oxidase polypeptide IV)	2	1 von 2	0,292	4	1 von 2	0,668	-0,376	-2,287671233	-128,7671233

Supplementary table 4. NPC – cytoplasmic protein fraction

accession number	protein name	analysis peptides	analysis cytoplasm expcount	analysis cytoplasm emPAI	reference peptides	reference cytoplasm expcount	reference cytoplasm emPAI	emPAI diff cytoplasm	x-times down-regulated	alteration in %
Q9EPB1	Dipeptidyl peptidase 2 (Dipeptidyl peptidase II) (DPP II) (Dipeptidyl aminopeptidase II) (Quiescent cell proline dipeptidase) (Dipeptidyl peptidase 7)	3	1 von 2	0,292	6	1 von 2	0,668	-0,376	-2,287671233	-128,7671233
P54313	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-2 (Transducin beta chain 2) (G protein subunit beta-2)	2	1 von 2	0,233	6	1 von 2	0,52	-0,287	-2,231759657	-123,1759657
P54311	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1 (Transducin beta chain 1)	2	1 von 2	0,233	6	1 von 2	0,52	-0,287	-2,231759657	-123,1759657
P07632	Superoxide dismutase [Cu-Zn]	9	2 von 2	2.588	11	2 von 2	5.756	-3.168	-2,224111283	-122,4111283
Q80T18	Glia maturation factor gamma (GMF-gamma)	1	1 von 2	0,212	2	1 von 2	0,468	-0,256	-2,20754717	-120,754717
P62142	Serine/threonine-protein phosphatase PP1-beta catalytic subunit (PP-1B)	2	1 von 2	0,212	11	1 von 2	0,468	-0,256	-2,20754717	-120,754717
P41498	Low molecular weight phosphotyrosine protein phosphatase (LMW-PTPase) (Low molecular weight cytosolic acid phosphatase)	2	2 von 2	0,179	3	2 von 2	0,389	-0,21	-2,173184358	-117,3184358
Q498E0	Thioredoxin domain-containing protein 12	1	1 von 2	0,166	2	1 von 2	0,359	-0,193	-2,162650602	-116,2650602
A1L108	Actin-related protein 2/3 complex subunit 5-like protein (Arp2/3 complex 16 kDa subunit 2) (ARC16-2)	1	1 von 2	0,166	2	1 von 2	0,359	-0,193	-2,162650602	-116,2650602
Q5XII0	Mammalian ependymin-related protein 1 (MERP-1)	2	1 von 2	0,166	2	1 von 2	0,359	-0,193	-2,162650602	-116,2650602
Q920P6	Adenosine deaminase (Adenosine aminohydrolase)	5	2 von 2	0,271	7	1 von 2	0,585	-0,314	-2,158671587	-115,8671587
Q5U316	Ras-related protein Rab-35	1	1 von 2	0,155	2	1 von 2	0,334	-0,179	-2,15483871	-115,483871
P13668	Stathmin (Leukemia-associated phosphoprotein p18) (Metablastin) (Oncoprotein 18) (Op18) (Phosphoprotein p19) (pp19) (Pr22 protein) (Prosolin) (pp17)	1	1 von 2	0,155	2	1 von 2	0,334	-0,179	-2,15483871	-115,483871
P97829	Leukocyte surface antigen CD47 (Integrin-associated protein) (IAP)	1	1 von 2	0,155	2	1 von 2	0,334	-0,179	-2,15483871	-115,483871
P07154	Cathepsin L1 (Major excreted protein) (MEP) (Cyclic protein 2) (CP-2)	9	2 von 2	0,181	7	2 von 2	0,389	-0,208	-2,149171271	-114,9171271
P06757	Alcohol dehydrogenase 1 (Alcohol dehydrogenase A subunit)	3	2 von 2	0,101	4	2 von 2	0,217	-0,116	-2,148514851	-114,8514851
P13086	Succinyl-CoA ligase [GDP-forming] subunit alpha, mitochondrial (Succinyl-CoA synthetase subunit alpha) (SCS-alpha)	1	1 von 2	0,145	2	1 von 2	0,311	-0,166	-2,144827586	-114,4827586
O35077	Glycerol-3-phosphate dehydrogenase [NAD+], cytoplasmic (GPDH-C)	7	2 von 2	0,143	7	1 von 2	0,304	-0,161	-2,125874126	-112,5874126
Q920J4	Thioredoxin-like protein 1 (Thioredoxin-related protein)	1	1 von 2	0,129	2	1 von 2	0,274	-0,145	-2,124031008	-112,4031008
Q711G3	Isoamyl acetate-hydrolyzing esterase 1 homolog (Hypertrophic agonist-responsive protein B64)	4	1 von 2	0,129	3	1 von 2	0,274	-0,145	-2,124031008	-112,4031008
Q6PCT3	Tumor protein D54 (Tumor protein D52-like 2)	1	1 von 2	0,122	2	1 von 2	0,259	-0,137	-2,12295082	-112,295082
P19234	NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial (NADH-ubiquinone oxidoreductase 24 kDa subunit)	2	1 von 2	0,122	2	1 von 2	0,259	-0,137	-2,12295082	-112,295082
Q99MZ8	LIM and SH3 domain protein 1 (LASP-1)	1	1 von 2	0,122	3	1 von 2	0,259	-0,137	-2,12295082	-112,295082
P15978	Class I histocompatibility antigen, Non-RT1.A alpha-1 chain	3	1 von 2	0,105	5	1 von 2	0,222	-0,117	-2,114285714	-111,4285714
P14562	Lysosome-associated membrane glycoprotein 1 (LAMP-1) (120 kDa lysosomal membrane glycoprotein) (LGP-120) (CD107 antigen-like family member A)	1	1 von 2	0,096	2	1 von 2	0,202	-0,106	-2,104166667	-110,4166667
P18614	Integrin alpha-1 (CD49 antigen-like family member A) (Laminin and collagen receptor) (VLA-1)	3	1 von 2	0,101	6	1 von 2	0,212	-0,111	-2,099009901	-109,9009901
Q5RJR2	Twinfilin-1	1	2 von 2	0,101	2	1 von 2	0,212	-0,111	-2,099009901	-109,9009901
Q03248	Beta-ureidopropionase (Beta-alanine synthase) (N-carbamoyl-beta-alanine amidohydrolase)	2	1 von 2	0,086	4	1 von 2	0,179	-0,093	-2,081395349	-108,1395349
Q8VHF5	Citrate synthase, mitochondrial	1	1 von 2	0,077	2	1 von 2	0,16	-0,083	-2,077922078	-107,7922078
P13444	S-adenosylmethionine synthase isoform type-1 (AdoMet synthase 1) (Methionine adenosyltransferase 1) (MAT 1) (Methionine adenosyltransferase I/III) (MAT-I/III)	3	1 von 2	0,08	3	1 von 2	0,166	-0,086	-2,075	-107,5
Q5RKI0	WD repeat-containing protein 1	3	1 von 2	0,056	7	1 von 2	0,116	-0,06	-2,071428571	-107,1428571
Q9Z1X1	Extended synaptotagmin-1 (E-Syt1) (Membrane-bound C2 domain-containing protein) (vp115)	2	1 von 2	0,071	4	1 von 2	0,147	-0,076	-2,070422535	-107,0422535
P15429	Beta-enolase (2-phospho-D-glycerate hydro-lyase) (Muscle-specific enolase) (MSE) (Skeletal muscle enolase) (Enolase 3)	3	2 von 2	0,075	3	1 von 2	0,155	-0,08	-2,066666667	-106,6666667
Q4FZU2	Keratin, type II cytoskeletal 6A (Cytokeratin-6A) (CK-6A) (Keratin-6A) (K6A) (Type-II keratin Kb6)	3	1 von 2	0,055	3	1 von 2	0,113	-0,058	-2,054545455	-105,4545455
P13635	Ceruloplasmin (Ferroxidase)	1	1 von 2	0,038	2	1 von 2	0,078	-0,04	-2,052631579	-105,2631579
P97536	Cullin-associated NEDD8-dissociated protein 1 (Cullin-associated and neddylation-dissociated protein 1) (p120 CAND1) (TBP-interacting protein of 120 kDa A) (TBP-interacting protein 120A)	2	2 von 2	0,043	3	1 von 2	0,088	-0,045	-2,046511628	-104,6511628
Q6IMF3	Keratin, type II cytoskeletal 1 (Cytokeratin-1) (CK-1) (Keratin-1) (K1) (Type-II keratin Kb1)	3	1 von 2	0,053	3	1 von 2	0,108	-0,055	-2,037735849	-103,7735849
Q62812	Myosin-9 (Cellular myosin heavy chain, type A) (Myosin heavy chain 9) (Myosin heavy chain, non-muscle IIa) (Non-muscle myosin heavy chain A) (NMMHC-A) (Non-muscle myosin heavy chain IIa) (NMMHC II-a)	21	1 von 2	0,056	39	1 von 2	0,114	-0,058	-2,035714286	-103,5714286
Q66HA8	Heat shock protein 105 kDa (Heat shock 110 kDa protein)	1	2 von 2	0,038	2	1 von 2	0,077	-0,039	-2,026315789	-102,6315789

Supplementary table 4. NPC – cytoplasmic protein fraction

accession number	protein name	analysis peptides	analysis cytoplasm expcount	analysis cytoplasm emPAI	reference peptides	reference cytoplasm expcount	reference cytoplasm emPAI	emPAI diff cytoplasm	x-times down-regulated	alteration in %
P14740	Dipeptidyl peptidase 4 (Dipeptidyl peptidase IV) (DPP IV) (T-cell activation antigen CD26) (GP110 glycoprotein) (Bile canaliculus domain-specific membrane glycoprotein)	10	1 von 2	0,425	16	1 von 2	0,859	-0,434	-2,021176471	-102,1176471
Q9JLA3	UDP-glucose:glycoprotein glucosyltransferase 1 (UGT1) (UDP-glucose ceramide glucosyltransferase-like 1) (UDP--Glc:glycoprotein glucosyltransferase)	1	1 von 2	0,023	2	1 von 2	0,046	-0,023	-2	-100
Q2PQA9	Kinesin-1 heavy chain (Conventional kinesin heavy chain) (Ubiquitous kinesin heavy chain) (UKHC)	2	1 von 2	0,03	2	1 von 2	0,06	-0,03	-2	-100
P68255	14-3-3 protein theta (14-3-3 protein tau)	7	2 von 2	0,399	9	2 von 2	0,771	-0,372	-1,932330827	-93,23308271
P05712	Ras-related protein Rab-2A	3	1 von 2	0,501	5	1 von 2	0,968	-0,467	-1,932135729	-93,21357285
P35213	14-3-3 protein beta/alpha (Protein kinase C inhibitor protein 1) (KCIP-1) (Prepronerve growth factor RNH-1)	6	2 von 2	0,24	9	2 von 2	0,46	-0,22	-1,916666667	-91,66666667
P62494	Ras-related protein Rab-11A (Rab-11) (24KG)	3	1 von 2	0,468	5	1 von 2	0,896	-0,428	-1,914529915	-91,45299145
P97697	Inositol monophosphatase 1 (IMPase 1) (Inositol-1(or 4)-monophosphatase 1) (Lithium-sensitive myo-inositol monophosphatase A1)	4	1 von 2	0,468	5	1 von 2	0,896	-0,428	-1,914529915	-91,45299145
P68511	14-3-3 protein eta	8	1 von 2	0,688	11	1 von 2	1,31	-0,622	-1,904069767	-90,40697674
P04642	L-lactate dehydrogenase A chain (LDH-A) (LDH muscle subunit) (LDH-M)	21	2 von 2	0,656	19	2 von 2	1,24	-0,584	-1,890243902	-89,02439024
Q66H59	N-acetylneuraminate lyase (NALase) (N-acetylneuraminic acid aldolase) (N-acetylneuraminate pyruvate-lyase) (Sialic acid lyase) (Sialate lyase) (Sialate-pyruvate lyase) (Sialic acid aldolase)	5	1 von 2	0,369	5	1 von 2	0,688	-0,319	-1,864498645	-86,4498645
P29147	D-beta-hydroxybutyrate dehydrogenase, mitochondrial (BDH) (3-hydroxybutyrate dehydrogenase)	6	1 von 2	0,269	7	1 von 2	0,487	-0,218	-1,810408922	-81,04089219
Q03336	Regucalcin (RC) (Senescence marker protein 30) (SMP-30)	11	2 von 2	0,349	12	2 von 2	0,622	-0,273	-1,782234957	-78,2234957
P19804	Nucleoside diphosphate kinase B (NDP kinase B) (P18)	10	2 von 2	2.205	13	2 von 2	3.916	-1.711	-1,775963719	-77,59637188
P14604	Enoyl-CoA hydratase, mitochondrial (Short-chain enoyl-CoA hydratase) (SCEH) (Enoyl-CoA hydratase 1)	8	2 von 2	0,315	9	2 von 2	0,553	-0,238	-1,755555556	-75,55555556
P00884	Fructose-bisphosphate aldolase B (Liver-type aldolase)	11	2 von 2	0,556	12	2 von 2	0,972	-0,416	-1,748201439	-74,82014388
Q9WVK7	Hydroxyacyl-coenzyme A dehydrogenase, mitochondrial (HCDH) (Short-chain 3-hydroxyacyl-CoA dehydrogenase) (Medium and short-chain L-3-hydroxyacyl-coenzyme A dehydrogenase)	6	2 von 2	0,233	7	2 von 2	0,399	-0,166	-1,712446352	-71,24463519
P51635	Alcohol dehydrogenase [NADP+] (Aldehyde reductase) (Aldo-keto reductase family 1 member A1) (3-DG-reducing enzyme)	5	2 von 2	0,186	8	2 von 2	0,31	-0,124	-1,666666667	-66,66666667
P52944	PDZ and LIM domain protein 1 (C-terminal LIM domain protein 1) (Elfin) (LIM domain protein CLP-36)	4	1 von 2	0,468	6	1 von 2	0,778	-0,31	-1,662393162	-66,23931624
P0C5H9	Mesencephalic astrocyte-derived neurotrophic factor (Protein ARMET) (Arginine-rich protein)	2	1 von 2	0,425	3	1 von 2	0,701	-0,276	-1,649411765	-64,94117647
P62282	40S ribosomal protein S11	3	1 von 2	0,389	6	1 von 2	0,638	-0,249	-1,640102828	-64,01028278
P62749	Hippocalcin-like protein 1 (Neural visinin-like protein 3) (NVL-3) (Visinin-like protein 3) (VILIP-3)	2	1 von 2	0,334	3	1 von 2	0,54	-0,206	-1,616766467	-61,67664671
P10536	Ras-related protein Rab-1B	3	1 von 2	0,292	3	1 von 2	0,468	-0,176	-1,602739726	-60,2739726
Q9R1T3	Cathepsin Z (Cathepsin Y)	2	1 von 2	0,274	3	1 von 2	0,438	-0,164	-1,598540146	-59,8540146
P00787	Cathepsin B (Cathepsin B1) (RSG-2)	4	1 von 2	0,274	6	1 von 2	0,438	-0,164	-1,598540146	-59,8540146
Q4KM35	Proteasome subunit beta type-10 (Proteasome subunit beta-2i) (Proteasome MECl-1) (Macropain subunit MECl-1) (Multicatalytic endopeptidase complex subunit MECl-1) (Low molecular mass protein 10)	2	1 von 2	0,259	4	1 von 2	0,413	-0,154	-1,594594595	-59,45945946
O09171	Betaine--homocysteine S-methyltransferase 1	15	2 von 2	0,514	17	1 von 2	0,812	-0,298	-1,579766537	-57,9766537
Q68FR9	Elongation factor 1-delta (EF-1-delta)	3	1 von 2	0,222	4	1 von 2	0,35	-0,128	-1,576576577	-57,65765766
P16391	RT1 class I histocompatibility antigen, AA alpha chain	6	2 von 2	0,198	5	2 von 2	0,309	-0,111	-1,560606061	-56,06060606
P18420	Proteasome subunit alpha type-1 (Proteasome component C2) (Macropain subunit C2) (Multicatalytic endopeptidase complex subunit C2) (Proteasome nu chain)	2	1 von 2	0,116	2	2 von 2	0,181	-0,065	-1,560344828	-56,03448276
Q7TQ94	Nitrilase homolog 1	2	2 von 2	0,122	3	2 von 2	0,19	-0,068	-1,557377049	-55,73770492
Q5U300	Ubiquitin-like modifier-activating enzyme 1 (Ubiquitin-activating enzyme E1)	9	1 von 2	0,362	13	1 von 2	0,563	-0,201	-1,555248619	-55,52486188
P50123	Glutamyl aminopeptidase (EAP) (Aminopeptidase A) (AP-A)	2	1 von 2	0,071	3	1 von 2	0,109	-0,038	-1,535211268	-53,52112676
P07153	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1 (Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 67 kDa subunit) (Ribophorin-1) (Ribophorin I) (RPN-I)	7	1 von 2	0,103	6	1 von 2	0,158	-0,055	-1,533980583	-53,39805825
P48004	Proteasome subunit alpha type-7 (Proteasome subunit RC6-1)	6	2 von 2	0,301	6	2 von 2	0,46	-0,159	-1,528239203	-52,82392027
P52873	Pyruvate carboxylase, mitochondrial (Pyruvic carboxylase) (PCB)	12	1 von 2	0,395	18	1 von 2	0,603	-0,208	-1,526582278	-52,65822785
Q5U211	Sorting nexin-3	8	1 von 2	2.415	10	1 von 2	3.642	-1.227	-1,508074534	-50,80745342
P10719	ATP synthase subunit beta, mitochondrial	29	2 von 2	2.291	28	1 von 2	3.437	-1.146	-1,500218245	-50,02182453

Supplementary table 5. NPC – nuclear protein fraction

found only in NAF samples	min. 2 peptides		sorted according to the magnitude of the emPAI value	NAF and DMSO each 1 exp
accession number	protein name	analysis peptides	analysis nuclei emPAI	
P18445	60S ribosomal protein L27a	2	0,389	
Q99J82	Integrin-linked protein kinase	3	0,212	
Q5RJR8	Leucine-rich repeat-containing protein 59 (Protein p34)	2	0,202	
Q09073	ADP/ATP translocase 2 (ADP,ATP carrier protein 2) (Adenine nucleotide translocator 2) (ANT 2) (Solute carrier family 25 member 5)	3	0,194	
Q6IFV3	Keratin, type I cytoskeletal 15 (Cytokeratin-15) (CK-15) (Keratin-15) (K15) (Type I keratin Ka15)	2	0,136	
P21396	Amine oxidase [flavin-containing] A (Monoamine oxidase type A) (MAO-A)	2	0,125	
Q4V8C7	Interferon-inducible double stranded RNA-dependent protein kinase activator A (Protein activator of the interferon-induced protein kinase) (Protein kinase, interferon-inducible double stranded RNA-dependent activator)	2	0,122	
B5DFC8	Eukaryotic translation initiation factor 3 subunit C (eIF3c) (Eukaryotic translation initiation factor 3 subunit 8) (eIF3 p110)	3	0,12	
Q6P502	T-complex protein 1 subunit gamma (TCP-1-gamma) (CCT-gamma)	4	0,119	
Q6AXR4	Beta-hexosaminidase subunit beta (Beta-N-acetylhexosaminidase subunit beta) (Hexosaminidase subunit B) (N-acetyl-beta-glucosaminidase subunit beta)	2	0,066	
P28480	T-complex protein 1 subunit alpha (TCP-1-alpha) (CCT-alpha)	3	0,059	
Q7TPB1	T-complex protein 1 subunit delta (TCP-1-delta) (CCT-delta)	4	0,058	
Q499N5	Acyl-CoA synthetase family member 2, mitochondrial	6	0,056	
P15178	Aspartyl-tRNA synthetase, cytoplasmic (Aspartate--tRNA ligase) (AspRS)	2	0,055	
Q68FQ0	T-complex protein 1 subunit epsilon (TCP-1-epsilon) (CCT-epsilon)	2	0,054	
Q8K1P7	Transcription activator BRG1 (ATP-dependent helicase SMARCA4) (BRG1-associated factor 190A) (BAF190A) (Protein brahma homolog 1) (SNF2-beta) (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A member 4)	2	0,048	
Q4KLP0	Probable 2-oxoglutarate dehydrogenase E1 component DHKTD1, mitochondrial (Dehydrogenase E1 and transketolase domain-containing protein 1)	2	0,045	
Q66HF1	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial	4	0,044	
Q3MIB4	Lon protease homolog 2, peroxisomal (Lon protease-like protein 2) (Lon protease 2) (Peroxisomal Lon protease)	2	0,041	

only in ref/DMSO	min. 2 peptides		sorted according to the magnitude of the emPAI value
accession number	protein name	reference peptides	reference nuclei emPAI
P62845	40S ribosomal protein S15 (RIG protein)	2	0,585
P43138	DNA-(apurinic or apyrimidinic site) lyase (APEX nuclease) (APEN) (Apurinic-apyrimidinic endonuclease 1) (AP endonuclease 1) (REF-1) (Redox factor-1)	4	0,445
Q62764	DNA-binding protein A (Cold shock domain-containing protein A) (Muscle Y-box protein YB2) (RYB-A) (Y-box-binding protein A)	3	0,334
P62907	60S ribosomal protein L10a	2	0,274
P61621	Protein transport protein Sec61 subunit alpha isoform 1 (Sec61 alpha-1)	2	0,259
Q5M9G3	Caprin-1 (Cytoplasmic activation- and proliferation-associated protein 1) (GPI-anchored protein p137) (GPI-p137)	3	0,259
Q3B8Q2	Eukaryotic initiation factor 4A-III (eIF-4A-III) (Eukaryotic translation initiation factor 4A isoform 3) (ATP-dependent RNA helicase eIF4A-3) (ATP-dependent RNA helicase DDX48) (DEAD box protein 48)	2	0,166
P25235	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2 (Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 63 kDa subunit) (Ribophorin II) (RPN-II) (Ribophorin-2)	4	0,133
P63331	Serine/threonine-protein phosphatase 2A catalytic subunit alpha isoform (PP2A-alpha)	2	0,101
P41777	Nucleolar and coiled-body phosphoprotein 1 (140 kDa nucleolar phosphoprotein) (Nopp140) (Nucleolar 130 kDa protein) (Nucleolar phosphoprotein p130)	2	0,096
Q5U301	A-kinase anchor protein 2 (AKAP-2)	2	0,047

Supplementary table 5. NPC – nuclear protein fraction

found in both	min. 2peptides, delta of ≥2 or 50% upon NAF treatment	sorted according to the magnitude of the regulation								
accession number	protein name	analysis peptides	analysis nuclei expcount	analysis nuclei emPAI	reference peptides	reference nuclei expcount	reference nuclei emPAI	emPAI diff nuclei	x-times up-regulated	alteration in %
P01946	Hemoglobin subunit alpha-1/2 (Alpha-1/2-globin) (Hemoglobin alpha-1/2 chain)	8	1 von 1	1.512	5	1 von 1	0,259	1.253	5837,837838	583683,7838
Q6LED0	Histone H3.1	7	1 von 1	2.511	3	1 von 1	0,874	1.637	2872,997712	287199,7712
Q71TY3	40S ribosomal protein S27	2	1 von 1	1.154	1	1 von 1	0,468	0,686	2465,811966	246481,1966
P02091	Hemoglobin subunit beta-1 (Hemoglobin beta-1 chain) (Beta-1-globin) (Hemoglobin beta chain, major-form)	11	1 von 1	1.154	2	1 von 1	0,468	0,686	2465,811966	246481,1966
P06761	78 kDa glucose-regulated protein (GRP-78) (Heat shock 70 kDa protein 5) (Immunoglobulin heavy chain-binding protein) (BiP) (Steroidogenesis-activator polypeptide)	35	1 von 1	1.703	17	1 von 1	0,778	0,925	2188,946015	218794,6015
P62630	Elongation factor 1-alpha 1 (EF-1-alpha-1) (Eukaryotic elongation factor 1 A-1) (eEF1A-1) (Elongation factor Tu) (EF-Tu)	15	1 von 1	1.807	10	1 von 1	0,887	0,92	2037,204059	203620,4059
P29314	40S ribosomal protein S9	8	1 von 1	1.404	5	1 von 1	0,73	0,674	1923,287671	192228,7671
P68370	Tubulin alpha-1A chain (Tubulin alpha-1 chain) (Alpha-tubulin 1)	16	1 von 1	1.555	14	1 von 1	0,817	0,738	1903,304774	190230,4774
P62856	40S ribosomal protein S26	3	1 von 1	1.154	2	1 von 1	0,668	0,486	1727,54491	172654,491
Q63081	Protein disulfide-isomerase A6 (Protein disulfide isomerase P5) (Calcium-binding protein 1) (CaBP1) (Thioredoxin domain-containing protein 7)	11	1 von 1	1.154	8	1 von 1	0,711	0,443	1623,066104	162206,6104
P11598	Protein disulfide-isomerase A3 (Disulfide isomerase ER-60) (Endoplasmic reticulum resident protein 60) (ER protein 60) (Endoplasmic reticulum resident protein 57) (ER protein 57) (58 kDa microsomal protein) (p58) (HIP-70) (Q-2) (58 kDa glucose-regulated p	22	1 von 1	1.276	20	1 von 1	0,828	0,448	1541,062802	154006,2802
P63039	60 kDa heat shock protein, mitochondrial (Heat shock protein 60) (HSP-60) (60 kDa chaperonin) (Chaperonin 60) (CPN60) (Mitochondrial matrix protein P1) (HSP-65)	26	1 von 1	1.404	18	1 von 1	1,04	0,364	1350	134900
P63018	Heat shock cognate 71 kDa protein (Heat shock 70 kDa protein 8)	23	1 von 1	1.154	17	1 von 1	0,945	0,209	1221,164021	122016,4021
Q6P9T8	Tubulin beta-2C chain	21	1 von 1	1.873	18	1 von 1	1,61	0,263	1163,354037	116235,4037
Q63716	Peroxiredoxin-1 (Thioredoxin peroxidase 2) (Thioredoxin-dependent peroxide reductase 2) (Heme-binding 23 kDa protein) (HBP23)	23	1 von 1	6.499	22	1 von 1	7,66	-1.161	848,4334204	84743,34204
Q6URK4	Heterogeneous nuclear ribonucleoprotein A3 (hnRNP A3)	9	1 von 1	1.291	12	1 von 1	2,02	-0,729	639,1089109	63810,89109
P60711	Actin, cytoplasmic 1 (Beta-actin)	20	1 von 1	2.981	22	1 von 1	5,31	-2.329	561,393597	56039,3597
P48721	Stress-70 protein, mitochondrial (75 kDa glucose-regulated protein) (GRP-75) (Heat shock 70 kDa protein 9) (Peptide-binding protein 74) (PBP74) (mtHSP70) (Mortalin)	9	1 von 1	0,139	1	1 von 1	0,044	0,095	3,159090909	215,9090909
P82995	Heat shock protein HSP 90-alpha (Heat shock 86 kDa) (HSP 86)	13	1 von 1	0,136	10	1 von 1	0,044	0,092	3,090909091	209,0909091
P27008	Poly [ADP-ribose] polymerase 1 (PARP-1) (NAD(+) ADP-ribosyltransferase 1) (ADPRT 1) (Poly[ADP-ribose] synthase 1)	5	1 von 1	0,157	2	1 von 1	0,06	0,097	2,616666667	161,6666667
Q4G061	Eukaryotic translation initiation factor 3 subunit B (eIF3b) (Eukaryotic translation initiation factor 3 subunit 9) (eIF-3-eta)	7	1 von 1	0,297	3	1 von 1	0,118	0,179	2,516949153	151,6949153
P62703	40S ribosomal protein S4, X isoform	6	1 von 1	0,874	3	1 von 1	0,369	0,505	2,368563686	136,8563686
P62718	60S ribosomal protein L18a	5	1 von 1	0,668	2	1 von 1	0,292	0,376	2,287671233	128,7671233
Q9R1Z0	Voltage-dependent anion-selective channel protein 3 (VDAC-3) (Outer mitochondrial membrane protein porin 3)	5	1 von 1	0,551	5	1 von 1	0,245	0,306	2,248979592	124,8979592
Q64591	2,4-dienoyl-CoA reductase, mitochondrial (2,4-dienoyl-CoA reductase [NADPH]) (4-enoyl-CoA reductase [NADPH])	4	1 von 1	0,389	2	1 von 1	0,179	0,21	2,173184358	117,3184358
Q8VID1	Dehydrogenase/reductase SDR family member 4 (NADPH-dependent carbonyl reductase/NADP-retinol dehydrogenase) (PHCR) (Peroxisomal short-chain alcohol dehydrogenase) (PSCD) (NADPH-dependent retinol dehydrogenase/reductase) (NDRD)	2	1 von 1	0,292	2	1 von 1	0,136	0,156	2,147058824	114,7058824
P97532	3-mercaptopyruvate sulfurtransferase (MST)	5	1 von 1	0,311	2	1 von 1	0,145	0,166	2,144827586	114,4827586
P62919	60S ribosomal protein L8	3	1 von 1	0,259	1	1 von 1	0,122	0,137	2,12295082	112,295082
P62755	40S ribosomal protein S6	2	1 von 1	0,233	1	1 von 1	0,11	0,123	2,118181818	111,8181818
P19139	Casein kinase II subunit alpha (CK II alpha)	2	1 von 1	0,233	1	1 von 1	0,11	0,123	2,118181818	111,8181818
Q05962	ADP/ATP translocase 1 (Adenine nucleotide translocator 1) (ANT 1) (ADP,ATP carrier protein 1) (Solute carrier family 25 member 4)	2	1 von 1	0,202	1	1 von 1	0,096	0,106	2,104166667	110,4166667
P50878	60S ribosomal protein L4 (60S ribosomal protein L1)	2	1 von 1	0,15	1	1 von 1	0,072	0,078	2,083333333	108,3333333
P20961	Plasminogen activator inhibitor 1 (PAI-1) (Endothelial plasminogen activator inhibitor) (Serpin E1)	24	1 von 1	0,179	21	1 von 1	0,086	0,093	2,081395349	108,1395349
P84092	AP-2 complex subunit mu (Adapter-related protein complex 2 mu subunit) (Adaptor protein complex AP-2 subunit mu) (Mu2-adaptin) (AP-2 mu chain) (Plasma membrane adaptor AP-2 50 kDa protein) (Clathrin assembly protein complex 2 medium chain) (Clathrin coat	2	1 von 1	0,133	1	1 von 1	0,064	0,069	2,078125	107,8125
Q9Z118	Regulator of differentiation 1 (Rod1)	2	1 von 1	0,16	1	1 von 1	0,077	0,083	2,077922078	107,7922078
Q4QQW4	Histone deacetylase 1 (HD1)	2	1 von 1	0,145	1	1 von 1	0,07	0,075	2,071428571	107,1428571

Supplementary table 5. NPC – nuclear protein fraction

accession number	protein name	analysis peptides	analysis nuclei expcount	analysis nuclei emPAI	reference peptides	reference nuclei expcount	reference nuclei emPAI	emPAI diff nuclei	x-times up-regulated	alteration in %
A4L9P7	Sister chromatid cohesion protein PDS5 homolog A	2	1 von 1	0,043	1	1 von 1	0,021	0,022	2,047619048	104,7619048
Q62780	Probable ATP-dependent RNA helicase DDX46 (DEAD box protein 46) (Helicase of 117.4 kDa)	2	1 von 1	0,061	1	1 von 1	0,03	0,031	2,033333333	103,3333333
P27952	40S ribosomal protein S2	5	1 von 1	0,585	3	1 von 1	0,318	0,267	1,839622642	83,96226415
P29457	Serpin H1 (47 kDa heat shock protein) (Collagen-binding protein) (Colligin) (GP46)	9	1 von 1	0,389	5	1 von 1	0,218	0,171	1,78440367	78,44036697
Q1JU68	Eukaryotic translation initiation factor 3 subunit A (eIF3a) (Eukaryotic translation initiation factor 3 subunit 10) (eIF-3-theta)	5	1 von 1	0,092	3	1 von 1	0,054	0,038	1,703703704	70,37037037
Q63396	Activated RNA polymerase II transcriptional coactivator p15 (Positive cofactor 4) (PC4) (SUB1 homolog) (p14)	3	1 von 1	0,874	2	1 von 1	0,52	0,354	1,680769231	68,07692308
P17077	60S ribosomal protein L9	3	1 von 1	0,701	3	1 von 1	0,425	0,276	1,649411765	64,94117647
Q06647	ATP synthase subunit O, mitochondrial (Oligomycin sensitivity conferral protein) (OSCP)	12	1 von 1	1.738	10	1 von 1	1.054	0,684	1,648956357	64,89563567
P29147	D-beta-hydroxybutyrate dehydrogenase, mitochondrial (BDH) (3-hydroxybutyrate dehydrogenase)	6	1 von 1	0,61	7	1 von 1	0,374	0,236	1,631016043	63,10160428
P24049	60S ribosomal protein L17 (Amino acid starvation-induced protein) (ASI) (L23)	4	1 von 1	0,585	3	1 von 1	0,359	0,226	1,629526462	62,95264624
Q63507	60S ribosomal protein L14	3	1 von 1	0,468	2	1 von 1	0,292	0,176	1,602739726	60,2739726
P0CG51	Polyubiquitin-B	6	1 von 1	0,468	4	1 von 1	0,292	0,176	1,602739726	60,2739726
Q66HD0	Endoplasmin (Heat shock protein 90 kDa beta member 1) (94 kDa glucose-regulated protein) (GRP-94)	17	1 von 1	0,413	10	1 von 1	0,259	0,154	1,594594595	59,45945946
P05426	60S ribosomal protein L7	3	1 von 1	0,369	3	1 von 1	0,233	0,136	1,583690987	58,36909871
Q68FR9	Elongation factor 1-delta (EF-1-delta)	3	1 von 1	0,35	4	1 von 1	0,222	0,128	1,576576577	57,65765766
Q794E4	Heterogeneous nuclear ribonucleoprotein F (hnRNP F)	3	1 von 1	0,334	2	1 von 1	0,212	0,122	1,575471698	57,54716981
Q64428	Trifunctional enzyme subunit alpha, mitochondrial (TP-alpha)	13	1 von 1	0,28	4	1 von 1	0,179	0,101	1,56424581	56,42458101
P60123	RuvB-like 1 (49 kDa TATA box-binding protein-interacting protein) (49 kDa TBP-interacting protein) (DNA helicase p50) (Pontin 52) (TIP49a)	3	1 von 1	0,25	2	1 von 1	0,16	0,09	1,5625	56,25
P39052	Dynamin-2	6	1 von 1	0,104	2	1 von 1	0,068	0,036	1,529411765	52,94117647
Q9JIL3	Interleukin enhancer-binding factor 3	3	1 von 1	0,142	2	1 von 1	0,093	0,049	1,52688172	52,68817204

found in both	min. 2 peptides, delta of ≤-2 or -50% upon NAF treatment	sorted according to the magnitude of the regulation

accession number	protein name	analysis peptides	analysis nuclei expcount	analysis nuclei emPAI	reference peptides	reference nuclei expcount	reference nuclei emPAI	emPAI diff nuclei	x-times down-regulated	alteration in %
P18666	Myosin regulatory light chain 12B (Myosin RLC-B) (Myosin regulatory light chain 2-B, smooth muscle isoform) (MLC-2) (Myosin regulatory light chain 20 kDa) (MLC20) (Myosin regulatory light chain MRLC2)	4	1 von 1	0,194	7	1 von 1	1.424	-1,23	-7340,206186	-733920,6186
P62853	40S ribosomal protein S25	1	1 von 1	0,292	4	1 von 1	1.783	-1.491	-6106,164384	-610516,4384
P00406	Cytochrome c oxidase subunit 2 (Cytochrome c oxidase polypeptide II)	2	1 von 1	0,292	4	1 von 1	1.783	-1.491	-6106,164384	-610516,4384
P00173	Cytochrome b5	4	1 von 1	0,292	7	1 von 1	1.783	-1.491	-6106,164384	-610516,4384
P23358	60S ribosomal protein L12	2	1 von 1	0,468	7	1 von 1	2.831	-2.363	-6049,145299	-604814,5299
P62138	Serine/threonine-protein phosphatase PP1-alpha catalytic subunit (PP-1A)	4	1 von 1	0,492	15	1 von 1	2.675	-2.183	-5436,99187	-543599,187
P62282	40S ribosomal protein S11	3	1 von 1	0,389	6	1 von 1	1.683	-1.294	-4326,478149	-432547,8149
P45592	Cofilin-1 (Cofilin, non-muscle isoform)	11	1 von 1	0,638	12	1 von 1	2.728	-2,09	-4275,862069	-427486,2069
O70351	3-hydroxyacyl-CoA dehydrogenase type-2 (3-hydroxyacyl-CoA dehydrogenase type II) (Type II HADH) (3-hydroxy-2-methylbutyryl-CoA dehydrogenase) (17-beta-hydroxysteroid dehydrogenase 10) (17-beta-HSD 10) (Mitochondrial ribonuclease P protein 2) (Mitochondria	9	1 von 1	0,311	12	1 von 1	1.254	-0,943	-4032,154341	-403115,4341
P63088	Serine/threonine-protein phosphatase PP1-gamma catalytic subunit (PP-1G) (Protein phosphatase 1C catalytic subunit)	4	1 von 1	0,445	11	1 von 1	1.754	-1.309	-3941,573034	-394057,3034
Q4V7C7	Actin-related protein 3 (Actin-like protein 3)	9	1 von 1	0,389	14	1 von 1	1.471	-1.082	-3781,491003	-378049,1003
P16086	Spectrin alpha chain, brain (Spectrin, non-erythroid alpha chain) (Alpha-II spectrin) (Fodrin alpha chain)	38	1 von 1	0,385	99	1 von 1	1.437	-1.052	-3732,467532	-373146,7532
P52925	High mobility group protein B2 (High mobility group protein 2) (HMG-2)	3	1 von 1	0,54	7	1 von 1	1.738	-1.198	-3218,518519	-321751,8519
Q9WVK3	Peroxisomal trans-2-enoyl-CoA reductase (TERP) (RLF98) (Peroxisomal 2,4-dienoyl-CoA reductase) (PX-2,4-DCR1)	5	1 von 1	0,334	6	1 von 1	1.054	-0,72	-3155,688623	-315468,8623
P19945	60S acidic ribosomal protein P0 (60S ribosomal protein L10E)	7	1 von 1	0,369	7	1 von 1	1.081	-0,712	-2929,539295	-292853,9295
P62859	40S ribosomal protein S28	2	1 von 1	0,585	2	1 von 1	1.512	-0,927	-2584,615385	-258361,5385
P09495	Tropomyosin alpha-4 chain (Tropomyosin-4) (TM-4)	18	1 von 1	2,29	24	1 von 1	5.723	-3.433	-2499,126638	-249812,6638
Q9JJ54	Heterogeneous nuclear ribonucleoprotein D0 (hnRNP D0) (AU-rich element RNA-binding protein 1)	6	1 von 1	0,874	11	1 von 1	2.162	-1.288	-2473,684211	-247268,4211

Supplementary table 5. NPC – nuclear protein fraction

accession number	protein name	analysis peptides	analysis nuclei expcount	analysis nuclei emPAI	reference peptides	reference nuclei expcount	reference nuclei emPAI	emPAI diff nuclei	x-times down-regulated	alteration in %
Q02874	Core histone macro-H2A.1 (Histone macroH2A1) (H2A.y) (H2A/y)	4	1 von 1	0,52	7	1 von 1	1.081	-0,561	-2078,846154	-207784,6154
P04797	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (38 kDa BFA-dependent ADP-ribosylation substrate) (BARS-38)	12	1 von 1	0,616	12	1 von 1	1.154	-0,538	-1873,376623	-187237,6623
P62890	60S ribosomal protein L30	3	1 von 1	0,995	4	1 von 1	1.512	-0,517	-1519,59799	-151859,799
P62832	60S ribosomal protein L23	4	1 von 1	0,995	4	1 von 1	1.512	-0,517	-1519,59799	-151859,799
Q3T1J1	Eukaryotic translation initiation factor 5A-1 (eIF-5A-1) (Eukaryotic initiation factor 5A isoform 1) (eIF-5A) (eIF-4D)	4	1 von 1	0,778	6	1 von 1	1.154	-0,376	-1483,290488	-148229,0488
P62260	14-3-3 protein epsilon (14-3-3E) (Mitochondrial import stimulation factor L subunit) (MSF L)	15	1 von 1	0,778	16	1 von 1	1.154	-0,376	-1483,290488	-148229,0488
P02262	Histone H2A type 1	9	1 von 1	9	9	1 von 1	12.895	-3.895	-1432,777778	-143177,7778
P04041	Glutathione peroxidase 1 (GSHPx-1) (Cellular glutathione peroxidase)	9	1 von 1	0,848	9	1 von 1	1.154	-0,306	-1360,849057	-135984,9057
O35763	Moesin (Membrane-organizing extension spike protein)	19	1 von 1	0,842	21	1 von 1	1.024	-0,182	-1216,152019	-121515,2019
Q9QXQ0	Alpha-actinin-4 (Non-muscle alpha-actinin 4) (F-actin cross-linking protein)	29	1 von 1	0,931	25	1 von 1	1.131	-0,2	-1214,822771	-121382,2771
P18484	AP-2 complex subunit alpha-2 (Adapter-related protein complex 2 alpha-2 subunit) (Adaptor protein complex AP-2 subunit alpha-2) (Alpha2-adaptin) (Plasma membrane adaptor HA2/AP2 adaptin alpha C subunit) (Alpha-adaptin C) (Clathrin assembly protein complex	22	1 von 1	1,13	22	1 von 1	1.058	0,072	-936,2831858	-93528,31858
Q68A21	Transcriptional activator protein Pur-beta (Purine-rich element-binding protein B)	1	1 von 1	0,101	5	1 von 1	0,616	-0,515	-6,099009901	-509,9009901
Q09167	Serine/arginine-rich splicing factor 5 (Delayed-early protein HRS) (Insulin-induced growth response protein CL-4) (Pre-mRNA-splicing factor SRP40) (Splicing factor, arginine/serine-rich 5)	1	1 von 1	0,101	5	1 von 1	0,616	-0,515	-6,099009901	-509,9009901
P13471	40S ribosomal protein S14	3	1 von 1	1.371	7	1 von 1	6.499	-5.128	-4,740335522	-374,0335522
B2GUZ5	F-actin-capping protein subunit alpha-1 (CapZ alpha-1)	5	1 von 1	0,105	6	1 von 1	0,492	-0,387	-4,685714286	-368,5714286
P13437	3-ketoacyl-CoA thiolase, mitochondrial (Beta-ketothiolase) (Acetyl-CoA acyltransferase) (Mitochondrial 3-oxoacyl-CoA thiolase)	7	1 von 1	0,186	7	1 von 1	0,817	-0,631	-4,392473118	-339,2473118
Q62847	Gamma-adducin (Adducin-like protein 70) (Protein kinase C-binding protein 35H)	1	1 von 1	0,053	4	1 von 1	0,227	-0,174	-4,283018868	-328,3018868
P85972	Vinculin (Metavinculin)	17	1 von 1	0,05	31	1 von 1	0,214	-0,164	-4,28	-328
P55770	NHP2-like protein 1 (High mobility group-like nuclear protein 2 homolog 1) (OTK27) (U4/U6.U5 tri-snRNP 15.5 kDa protein)	1	1 von 1	0,233	3	1 von 1	0,874	-0,641	-3,751072961	-275,1072961
P24329	Thiosulfate sulfurtransferase (Rhodanese)	9	1 von 1	0,222	9	1 von 1	0,823	-0,601	-3,707207207	-270,7207207
P70580	Membrane-associated progesterone receptor component 1 (Acidic 25 kDa protein) (25-DX) (Ventral midline antigen) (VEMA)	5	1 von 1	0,212	3	1 von 1	0,778	-0,566	-3,669811321	-266,9811321
P35704	Peroxiredoxin-2 (Thioredoxin peroxidase 1) (Thioredoxin-dependent peroxide reductase 1) (Thiol-specific antioxidant protein) (TSA)	7	1 von 1	0,212	6	1 von 1	0,778	-0,566	-3,669811321	-266,9811321
P31399	ATP synthase subunit d, mitochondrial (ATPase subunit d)	5	1 von 1	0,194	5	1 von 1	0,701	-0,507	-3,613402062	-261,3402062
P07895	Superoxide dismutase [Mn], mitochondrial	4	1 von 1	0,155	4	1 von 1	0,54	-0,385	-3,483870968	-248,3870968
Q91XU1	Protein quaking (RqkI)	1	1 von 1	0,105	3	1 von 1	0,35	-0,245	-3,333333333	-233,3333333
B5DEH2	Erlin-2 (Endoplasmic reticulum lipid raft-associated protein 2) (Stomatin-prohibitin-flotillin-HflC/K domain-containing protein 2) (SPFH domain-containing protein 2)	1	1 von 1	0,089	3	1 von 1	0,292	-0,203	-3,280898876	-228,0898876
Q66H12	Alpha-N-acetylgalactosaminidase (Alpha-galactosidase B)	5	1 von 1	0,08	4	1 von 1	0,259	-0,179	-3,2375	-223,75
Q5XIH7	Prohibitin-2 (B-cell receptor-associated protein BAP37) (BAP-37)	7	1 von 1	0,08	5	1 von 1	0,259	-0,179	-3,2375	-223,75
Q5U2V4	Putative phospholipase B-like 1 (LAMA-like protein 1) (Lamina ancestor homolog 1) (Phospholipase B domain-containing protein 1)	2	1 von 1	0,07	3	1 von 1	0,225	-0,155	-3,214285714	-221,4285714
P52873	Pyruvate carboxylase, mitochondrial (Pyruvic carboxylase) (PCB)	12	1 von 1	0,028	18	1 von 1	0,087	-0,059	-3,107142857	-210,7142857
Q68FU3	Electron transfer flavoprotein subunit beta (Beta-ETF)	10	1 von 1	0,274	10	1 von 1	0,833	-0,559	-3,040145985	-204,0145985
Q5M7U6	Actin-related protein 2 (Actin-like protein 2)	3	1 von 1	0,179	5	1 von 1	0,509	-0,33	-2,843575419	-184,3575419
P70582	Nuclear pore complex protein Nup54 (54 kDa nucleoporin) (Nucleoporin Nup54)	2	1 von 1	0,145	5	1 von 1	0,403	-0,258	-2,779310345	-177,9310345
A7VJC2	Heterogeneous nuclear ribonucleoproteins A2/B1 (hnRNP A2/B1)	10	1 von 1	1.228	14	1 von 1	3.062	-1.834	-2,493485342	-149,3485342
Q8CFM6	Stabilin-2 (Fasciclin, EGF-like, laminin-type EGF-like and link domain-containing scavenger receptor 2) (FEEL-2) (Hyaluronan receptor for endocytosis)	6	1 von 1	0,138	15	1 von 1	0,329	-0,191	-2,384057971	-138,4057971
P62914	60S ribosomal protein L11	2	1 von 1	0,359	4	1 von 1	0,848	-0,489	-2,362116992	-136,2116992
Q498U4	SAP domain-containing ribonucleoprotein (Nuclear protein Hcc-1)	2	1 von 1	0,359	4	1 von 1	0,848	-0,489	-2,362116992	-136,2116992
P62744	AP-2 complex subunit sigma (Adapter-related protein complex 2 sigma subunit) (Adaptor protein complex AP-2 subunit sigma) (Sigma2-adaptin) (Sigma-adaptin 3b) (Plasma membrane adaptor AP-2 17 kDa protein) (Clathrin assembly protein 2 small chain) (Clathrin	1	1 von 1	0,334	2	1 von 1	0,778	-0,444	-2,329341317	-132,9341317
P68101	Eukaryotic translation initiation factor 2 subunit 1 (Eukaryotic translation initiation factor 2 subunit alpha) (eIF-2-alpha)	3	1 von 1	0,318	6	1 von 1	0,738	-0,42	-2,320754717	-132,0754717

Supplementary table 5. NPC – nuclear protein fraction

accession number	protein name	analysis peptides	analysis nuclei expcount	analysis nuclei emPAI	reference peptides	reference nuclei expcount	reference nuclei emPAI	emPAI diff nuclei	x-times down-regulated	alteration in %
P86252	Transcriptional activator protein Pur-alpha (Purine-rich single-stranded DNA-binding protein alpha)	1	1 von 1	0,259	2	1 von 1	0,585	-0,326	-2,258687259	-125,8687259
P35434	ATP synthase subunit delta, mitochondrial (F-ATPase delta subunit)	2	1 von 1	0,259	3	1 von 1	0,585	-0,326	-2,258687259	-125,8687259
Q5M883	Chloride intracellular channel protein 2	6	1 von 1	0,259	6	1 von 1	0,585	-0,326	-2,258687259	-125,8687259
P11240	Cytochrome c oxidase subunit 5A, mitochondrial (Cytochrome c oxidase polypeptide Va)	6	1 von 1	0,233	2	1 von 1	0,52	-0,287	-2,231759657	-123,1759657
Q8CFN2	Cell division control protein 42 homolog	4	1 von 1	0,233	3	1 von 1	0,52	-0,287	-2,231759657	-123,1759657
Q3MIE0	Enoyl-CoA hydratase domain-containing protein 3, mitochondrial	3	1 von 1	0,233	4	1 von 1	0,52	-0,287	-2,231759657	-123,1759657
P62161	Calmodulin (CaM)	3	1 von 1	0,212	2	1 von 1	0,468	-0,256	-2,20754717	-120,754717
Q3SWU3	Heterogeneous nuclear ribonucleoprotein D-like (hnRNP D-like)	2	1 von 1	0,212	4	1 von 1	0,468	-0,256	-2,20754717	-120,754717
P13803	Electron transfer flavoprotein subunit alpha, mitochondrial (Alpha-ETF)	7	1 von 1	0,202	6	1 von 1	0,445	-0,243	-2,202970297	-120,2970297
P12749	60S ribosomal protein L26	1	1 von 1	0,179	2	1 von 1	0,389	-0,21	-2,173184358	-117,3184358
P20280	60S ribosomal protein L21	1	1 von 1	0,166	2	1 von 1	0,359	-0,193	-2,162650602	-116,2650602
Q68FY1	Nucleoporin NUP53 (35 kDa nucleoporin) (Nuclear pore complex protein Nup53) (Nucleoporin Nup35)	1	1 von 1	0,166	2	1 von 1	0,359	-0,193	-2,162650602	-116,2650602
Q5XII0	Mammalian ependymin-related protein 1 (MERP-1)	2	1 von 1	0,166	2	1 von 1	0,359	-0,193	-2,162650602	-116,2650602
Q63945	Protein SET (Liver regeneration-related protein LRRGR00002) (Phosphatase 2A inhibitor I2PP2A) (I-2PP2A) (Template-activating factor I) (TAF-I)	1	1 von 1	0,155	2	1 von 1	0,334	-0,179	-2,15483871	-115,483871
P60825	Cold-inducible RNA-binding protein (A18 hnRNP) (Glycine-rich RNA-binding protein CIRP)	1	1 von 1	0,136	2	1 von 1	0,292	-0,156	-2,147058824	-114,7058824
P62271	40S ribosomal protein S18	5	1 von 1	1.276	8	1 von 1	2.728	-1.452	-2,137931034	-113,7931034
P35435	ATP synthase subunit gamma, mitochondrial (F-ATPase gamma subunit)	5	1 von 1	0,122	4	1 von 1	0,259	-0,137	-2,12295082	-112,295082
P48500	Triosephosphate isomerase (TIM) (Triose-phosphate isomerase)	13	1 von 1	0,122	16	1 von 1	0,259	-0,137	-2,12295082	-112,295082
P08010	Glutathione S-transferase Mu 2 (GSTM2-2) (Glutathione S-transferase Yb-2) (GST Yb2) (GST 4-4)	17	1 von 1	0,11	19	1 von 1	0,233	-0,123	-2,118181818	-111,8181818
Q5I0H9	Protein disulfide-isomerase A5	1	1 von 1	0,072	2	1 von 1	0,15	-0,078	-2,083333333	-108,3333333
Q66H20	Polypyrimidine tract-binding protein 2 (Neural polypyrimidine tract-binding protein) (PTB-like protein)	2	1 von 1	0,072	2	1 von 1	0,15	-0,078	-2,083333333	-108,3333333
Q9WTT6	Guanine deaminase (Guanase) (Guanine aminohydrolase) (GAH)	17	1 von 1	0,064	17	1 von 1	0,133	-0,069	-2,078125	-107,8125
O08984	Lamin-B receptor (Integral nuclear envelope inner membrane protein) (NBP60)	1	1 von 1	0,068	2	1 von 1	0,141	-0,073	-2,073529412	-107,3529412
Q561S0	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10, mitochondrial (NADH-ubiquinone oxidoreductase 42 kDa subunit) (Complex I-42kD) (CI-42kD)	3	1 von 1	0,083	2	1 von 1	0,172	-0,089	-2,072289157	-107,2289157
P14668	Annexin A5 (Annexin-5) (Annexin V) (Lipocortin V) (Endonexin II) (Calphobindin I) (CBP-I) (Placental anticoagulant protein I) (PAP-I) (Placental anticoagulant protein 4) (PP4) (Thromboplastin inhibitor) (Vascular anticoagulant-alpha) (VAC-alpha) (Anchorin	12	1 von 1	0,083	14	1 von 1	0,172	-0,089	-2,072289157	-107,2289157
P04256	Heterogeneous nuclear ribonucleoprotein A1 (hnRNP A1) (hnRNP core protein A1) (Helix-destabilizing protein) (HDP) (Single-strand RNA-binding protein)	6	1 von 1	0,778	10	1 von 1	1,61	-0,832	-2,06940874	-106,940874
Q7TT49	Serine/threonine-protein kinase MRCK beta (CDC42-binding protein kinase beta) (DMPK-like beta) (Myotonic dystrophy kinase-related CDC42-binding kinase beta) (MRCK beta)	1	1 von 1	0,018	2	1 von 1	0,037	-0,019	-2,055555556	-105,5555556
P84586	Heterogeneous nuclear ribonucleoprotein G (hnRNP G) (RNA-binding motif protein, X chromosome)	1	1 von 1	0,058	2	1 von 1	0,119	-0,061	-2,051724138	-105,1724138
Q66HC5	Nuclear pore complex protein Nup93 (93 kDa nucleoporin) (Nucleoporin Nup93)	1	1 von 1	0,037	2	1 von 1	0,075	-0,038	-2,027027027	-102,7027027
P09895	60S ribosomal protein L5	6	1 von 1	0,389	7	1 von 1	0,778	-0,389	-2	-100
Q62812	Myosin-9 (Cellular myosin heavy chain, type A) (Myosin heavy chain 9) (Myosin heavy chain, non-muscle IIa) (Non-muscle myosin heavy chain A) (NMMHC-A) (Non-muscle myosin heavy chain IIa) (NMMHC II-a)	21	1 von 1	0,311	39	1 von 1	0,607	-0,296	-1,951768489	-95,17684887
P69897	Tubulin beta-5 chain	20	1 von 1	1.154	20	1 von 1	2.162	-1.008	-1,873483536	-87,34835355
Q64119	Myosin light polypeptide 6 (Smooth muscle and nonmuscle myosin light chain alkali 6) (Myosin light chain alkali 3) (Myosin light chain A3) (Myosin light chain 3) (MLC-3) (17 kDa myosin light chain) (LC17)	8	1 von 1	1.031	8	1 von 1	1.894	-0,863	-1,837051406	-83,70514064
P49242	40S ribosomal protein S3a (V-fos transformation effector protein)	3	1 von 1	0,304	5	1 von 1	0,557	-0,253	-1,832236842	-83,22368421
Q9JLT0	Myosin-10 (Cellular myosin heavy chain, type B) (Myosin heavy chain 10) (Myosin heavy chain, non-muscle IIb) (Non-muscle myosin heavy chain B) (NMMHC-B) (Non-muscle myosin heavy chain IIb) (NMMHC II-b)	3	1 von 1	0,042	5	1 von 1	0,072	-0,03	-1,714285714	-71,42857143
P04644	40S ribosomal protein S17	2	1 von 1	0,585	3	1 von 1	0,995	-0,41	-1,700854701	-70,08547009
P11442	Clathrin heavy chain 1	46	1 von 1	1.377	63	1 von 1	2.322	-0,945	-1,68627451	-68,62745098
P62850	40S ribosomal protein S24	2	1 von 1	0,52	3	1 von 1	0,874	-0,354	-1,680769231	-68,07692308
Q5XIE0	Acidic leucine-rich nuclear phosphoprotein 32 family member E	2	1 von 1	0,52	3	1 von 1	0,874	-0,354	-1,680769231	-68,07692308

Supplementary table 5. NPC – nuclear protein fraction

accession number	protein name	analysis peptides	analysis nuclei expcount	analysis nuclei emPAI	reference peptides	reference nuclei expcount	reference nuclei emPAI	emPAI diff nuclei	x-times down-regulated	alteration in %
Q63413	Spliceosome RNA helicase Bat1 (56 kDa U2AF65-associated protein) (ATP-dependent RNA helicase p47) (DEAD box protein UAP56)	6	1 von 1	0,52	9	1 von 1	0,874	-0,354	-1,680769231	-68,07692308
P61354	60S ribosomal protein L27	2	1 von 1	0,468	3	1 von 1	0,778	-0,31	-1,662393162	-66,23931624
Q6PDU1	Serine/arginine-rich splicing factor 2 (Splicing component, 35 kDa) (Splicing factor SC35) (SC-35) (Splicing factor, arginine/serine-rich 2)	2	1 von 1	0,359	3	1 von 1	0,585	-0,226	-1,629526462	-62,95264624
P10111	Peptidyl-prolyl cis-trans isomerase A (PPIase A) (Rotamase A) (Cyclophilin A) (Cyclosporin A-binding protein) (p31) (p1B15)	14	1 von 1	0,359	14	1 von 1	0,585	-0,226	-1,629526462	-62,95264624
P63159	High mobility group protein B1 (High mobility group protein 1) (HMG-1) (Amphoterin) (Heparin-binding protein p30)	7	1 von 1	1.683	8	1 von 1	2.728	-1.045	-1,620915033	-62,09150327
P19511	ATP synthase subunit b, mitochondrial (ATPase subunit b)	5	1 von 1	0,233	5	1 von 1	0,369	-0,136	-1,583690987	-58,36909871
P68255	14-3-3 protein theta (14-3-3 protein tau)	7	1 von 1	0,233	9	1 von 1	0,369	-0,136	-1,583690987	-58,36909871
P24368	Peptidyl-prolyl cis-trans isomerase B (PPIase B) (Rotamase B) (Cyclophilin B) (S-cyclophilin) (SCYLP) (CYP-S1)	16	1 von 1	3.281	18	1 von 1	5.158	-1.877	-1,572081682	-57,20816824
Q8VHV7	Heterogeneous nuclear ribonucleoprotein H (hnRNP H) (Ratsg1)	2	1 von 1	0,194	3	1 von 1	0,304	-0,11	-1,567010309	-56,70103093
P21533	60S ribosomal protein L6 (Neoplasm-related protein C140)	2	1 von 1	0,179	3	1 von 1	0,28	-0,101	-1,56424581	-56,42458101
O55012	Phosphatidylinositol-binding clathrin assembly protein (Clathrin assembly lymphoid myeloid leukemia protein) (rCALM)	3	1 von 1	0,145	3	1 von 1	0,225	-0,08	-1,551724138	-55,17241379
Q9WVC0	Septin-7 (CDC10 protein homolog)	2	1 von 1	0,145	4	1 von 1	0,225	-0,08	-1,551724138	-55,17241379
Q5RKI0	WD repeat-containing protein 1	3	1 von 1	0,116	7	1 von 1	0,179	-0,063	-1,543103448	-54,31034483
P43244	Matrin-3 (Nuclear scaffold protein p130/MAT3)	2	1 von 1	0,08	3	1 von 1	0,122	-0,042	-1,525	-52,5
Q5M7W5	Microtubule-associated protein 4 (MAP-4)	2	1 von 1	0,083	4	1 von 1	0,126	-0,043	-1,518072289	-51,80722892
Q5FVM4	Non-POU domain-containing octamer-binding protein (NonO protein)	8	1 von 1	0,624	11	1 von 1	0,947	-0,323	-1,517628205	-51,76282051

Nomenclature

2D	two dimensional
DIGE	difference gel electrophoresis
DMSO	dimethyl sulfoxide
emPAI	exponentially modified protein abundance index
HCs	hepatocytes
LC	liquid chromatography
LPS	lipopolysaccharide
MS	mass spectrometry
NAF	nafenopin
NGC	non-genotoxic carcinogen
NPCs	non-parenchymal cells
PAGE	polyacrylamide gel electrophoresis
PB	phenobarbital
PBMCs	peripheral blood mononuclear cells
PHA	phytohaemagglutinin
ROS	reactive oxygen species
SDS	sodium dodecyl sulphate

Curriculum vitae

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Personal

Marital status: engaged, 1 child
Nationality: Austria
Age: 34 (27th May 1979)
Place of birth: Vienna

Professional

Research assistant and Ph.D. position — 06/2009 – 07/2012
At the Department of Cancer Research; Medical University of Vienna — expected end of the
Ph.D. modus of the University of Natural Resources and Life Sciences — PhD – 10/2013 (doctoral viva)
Clinical Proteomics – Prof. Dr. Christopher Gerner (advisor)

Thesis – 'Proteomic profiling of primary cells to investigate effects of non-genotoxic carcinogens and mechanisms of chemoprevention.'
EU/IMI-MARCAR project
Project coordinator: Jonathan Moggs, Ph.D. (Novartis)

Tasks performed:

- Clinical relevant proteomic analyses of, amongst others, primary human cells, deriving from various tissues, e.g. PBMCs, liver, skin
- Data evaluation
- Design and development of methods
- Supervision of research projects of students in our laboratory
- Writing manuscripts for publications and filing of several applications for research projects to the ethics commission of the MUW

Methods conducted: shotgun proteomics (LC-MS/MS), 2D-PAGE (autoradiography (metabolic labelling), DIGE and other staining types), ELISA, Western blot, immunofluorescence staining (Chamber Slides), PCR;

Data evaluation, writing a publication and the PhD thesis as well as the preparation for the doctoral viva — 07/2012 – 10/2013

Education

University of Natural Resources and Life Sciences, Vienna 06/2009 – 10/2013
Doctoral studies of Natural Resources and Life Sciences

University of Natural Resources and Life Sciences, Vienna 10/2000 – 10/2008
Food Chemistry and Biotechnology (degree - MSc) (15 semesters)
Focus on biotechnology

Technical University of Vienna 10/1999 – 06/2000
Electrical engineering

Basic military service – pioneer; Klosterneuburg 10/1998 – 05/1999

TGM Wexstraße; Vienna (polytechnic) 10/1994 – 05/1998
- continued in another department
Biomedicine (school leaving examination)

HTL Ettenreichgasse; Vienna (polytechnic) 09/1993 – 06/1994
Communications engineering

AHS Pichelmayerstraße; Vienna 09/1989 – 06/1993
Elementary level

Primary school Aspernallee; Vienna 09/1985 – 06/1989

Miscellaneous

Full-time as tobacconist since 04/2013

Diploma thesis - Dr. Friedemann Hesse and Prof. Nicole Borth (advisors) 02/2007 – 03/2008
ACBT at the Institute of Applied Microbiology; BOKU Vienna
'Proteomic analysis of hypo-thermically cultivated CHO cells'
Tasks: cell culture procedures and proteomic analysis via 2D-DIGE

Part-time as tobacconist (store) 2001 – 2009

Internships
Vamed; building service - NRZ Rosenhügel 2004
Novartis GmbH Vienna; microbiological laboratory
Tasks: a.o. molecular cloning 2003
Novartis GmbH Vienna; HSE 2002
IKEA (joinery) 1998
OMV (office, bookkeeping, repository) 1995, 1996, 1997

Languages

- German: mother tongue,
- English: fluent (spoken and written)

Personal Interests

- family and friends
- sport - sports climbing, martial arts, snowboarding
- travelling
- outdoor activities

Peer-reviewed publications

Proteome signatures of inflammatory activated primary human peripheral blood mononuclear cells.
Verena Haudek-Prinz†, Philip Klepeisz†, Astrid Slany, Johannes Griss, Anastasia Meshcheryakova, Verena Paulitschke, Goran Mitulovic, Johannes Stöckl, Christopher Gerner
† These authors contributed equally to this work.
Journal of Proteomics 2012 Dec 5;76 Spec No.:150-62. PMID: 22813876

Phenobarbital induces alterations in the proteome of hepatocytes and mesenchymal cells of rat livers.
Philip Klepeisz, Sandra Sagmeister, Verena Haudek-Prinz, Melanie Pichlbauer, Bettina Grasl-Kraupp, Christopher Gerner
This manuscript is resubmitted to PLoS One, where it is currently reviewed.

Proteome Analysis Identified the PPARγ Ligand 15d-PGJ2 as a Novel Drug Inhibiting Melanoma Progression and Interfering with Tumor-Stroma Interaction
Verena Paulitschke, Silke Gruber, Elisabeth Hofstätter, Verena Haudek-Prinz, Philipp Klepeisz, Nikolaus Schicher, Constanze Jonak, Peter Petzelbauer, Hubert Pehamberger, Christopher Gerner, Rainer Kunstfeld
PLoS One. 2012;7(9):e46103. PMID: 23049949

References

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