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Serum proteomics of lung adenocarcinomas induced by targeted overexpression of c-raf in alveolar epithelium identifies candidate biomarkers

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Abbreviations:

c-raf, serine-threonine kinase of the Raf family; **NSCLC,** non-small cell lung carcinomas; **ras,** rat sarcoma; **SCLC,** small cell lung carcinomas; **SP-C,** surfactant protein C

Keywords: Serum / c-raf / Lung adenocarcinoma / Biomarkers / 2-DE

Abstract

We previously reported a proteome map of lung adenocarcinomas in c-raf transgenic mice. We now extend our initial studies to serum proteins at early stage (1 month) and advanced stages of tumorigenesis (12 months). Notably, serum proteins from wildtype and tumor bearing mice were extracted with a lysis buffer containing 5 mol/L urea, 2 mol/L thiourea, 40 mmol/L Tris, 4% CHAPS, 100 mmol/L DTT, 0.5% BioLyte 3-10, separated by 2-DE and studied by image analysis. On average 400 protein spots per gel were excised and analyzed by MALDI-TOF MS. We identified 45 common and 5 uniquely expressed proteins in wildtype and tumor bearing mice. Apart from uniquely identified proteins we observed for n=9 proteins differential expression when wildtype and tumor bearing mice were compared. This included serpins and other protease inhibitors, lipocalins, transthyretins, globins and immunoglobulins. Notably, we demonstrate significant regulation of alpha-1-antitrypsin, alpha-2-macroglobulin, hemoglobin subunit alpha, vitamin D-binding protein, major urinary proteins and transthyretin (up to 8-fold) in serum of lung tumor bearing mice. Disease association of these proteins in human malignancies has been reported. Thus, an identification of regulated serum proteins in this lung cancer disease model provides excellent opportunities for the search of novel biomarkers.

1 Introduction

In 2002, an estimated 1.2 million new cases of death caused by lung cancer were diagnosed worldwide (American Cancer Society, 2002). Smoking is considered as the major risk factor and accounts for > 80% of all diagnosed cases [1]. Other risk factors include inhalation of radioactive compounds, heavy metals, asbestos and petrochemicals [2].

In general, lung tumors are classified by histological phenotypes and are divided into small cell lung carcinomas (SCLC) and non-small cell lung carcinomas (NSCLC). The latter group is further divided into adenocarcinoma, large cell carcinoma and squamous cell carcinoma. Notably, classification of human lung carcinomas by mRNA expression profiling revealed distinct subclasses of adenocarcinomas that might arise from bronchial, Clara and alveolar epithelium [3]. Recent data suggest a significant rise in alveolar malignancies and may account for as many as 30% of all adenocarcinomas [4].

One of the molecular causes leading to NSCLC has been linked to enhanced mitogen activated kinase signalling of the ras-raf cascade resulting in high capacity cell division and lung tumor formation [4]. Note, raf is an essential serine/threonine kinase constituent of the MAPK signalling pathway and a downstream effector of the central signal transduction mediator ras. Both, ras and raf are encoded by proto-oncogenes which become oncogenes when mutated. The MAPK pathway is dysregulated in a remarkable proportion of human malignancies through aberant signalling upstream of the protein and by activating mutations of the protein itself, both of which confer a proliferative advantage. Therapeutics targeting c-raf in NSCLC and SCLC have therefore been evaluated [5, 6].

We are specifically interested in the role of c-raf in lung cancer biology and therefore studied disease onset and progression in a transgenic mouse model [5]. These mice overexpress an activated form of c-raf-1, which mimics the effect of c-ras activation [7]. The transgene was targeted to alveolar epithelial cells through use of the surfactant protein C promotor (SP-C). In transgenic mice, the first

morphological changes occurred in distinct areas of the lung within the first 2 months. After eight to ten months the whole lung was morphologically changed showing typical features of lung adenocarcinomas [5].

At present, the tumor proteome of c-raf-induced lung adenocarcinomas is unknown. Disease proteomics, however, may provide new insights into the molecular events associated with lung carcinogenesis. Previous works from our laboratory aimed at identifying pulmonary proteins expressed in lung tissue of c-raf-induced lung tumors and of differentially expressed proteins for their putative value in diagnostics and therapy [8]. We now extend our initial findings to the serum proteome of c-raf transgenic mice and report *de novo* expression of tumor associated proteins and regulation of serum proteins.

2 Materials and methods

2.1 Biological material and protein extraction

2.1.1 SP-C/c-raf model

SP-C/c-raf transgenic lung tumor bearing mice were obtained from the laboratory of Prof. Ulf Rapp (University of Würzburg, Germany). A detailed description of the SP-C/c-raf transgenic line is given in [7] and [8]. Blood serum of tumor bearing mice (n=6, aged 1 month and n=15, aged 12 months) and blood serum of wildtype mice (n=4, aged 1 month and n=12, aged 12 months), that served as controls, were studied. Lung tumors arose multifocally. Starting as adenomatous hyperplasia (AAH), first morphological changes in distinct areas of the lung appeared within the first 2 months, only visible through histopathology. Macroscopically, tumors were visible after 4 months. For our studies we used 1 month old mice at an early stage of tumor development, and 12 months old mice with advanced tumor growth. At this point (12 months) almost the entire lung is tumorous (see Fig. 5 for histopathology). Hematoxylin and eosin staining

were used for histopathology of tumors. We compared expression of pulmonary proteins of tumor bearing with those of wildtype mice.

2.1.2 Serum sample preparation

Blood serum was collected from the *vena cava* and allowed to clot for 2 h at room temperature. The clotted material was removed by centrifugation at 3000 rpm for 15 min. Hemolysis was not observed. The sera obtained from the blood samples were frozen immediately without any further treatment in liquid nitrogen and stored at -80°C until further analysis. The protein concentration of serum was determined by the Bradford protein assay (Protein Assay Dye Reagent Concentrate, Bio-Rad), using bovine gamma globulin as the standard. Extraction yields ranged from 80 to 90 µg/µL for both wildtype and tumor samples.

2.2 2-DE

2.2.1 IEF

In the first dimension, proteins were separated by IEF with precast IPG strips (pH 3-10, non-linear gradient and pH 4-7, linear gradient; both 170x3x0.5 mm, Bio-Rad). Of the total proteins, 1 mg was diluted in a lysis buffer (5 mol/L urea, 2 mol/L thiourea, 40 mmol/L Tris, 4% CHAPS, 100 mmol/L DTT, 0.5% BioLyte 3-10; Bio-Rad) to obtain a total volume of 350 µL per strip. Focused IPG strips were rehydrated at 50 V for 12 h. IEF was performed at 20°C with a maximum voltage of 10 kV and a maximum current of 50 µA per strip. Each sample was analyzed in triplicate. After IEF, IPG strips were stored at -80°C until SDS-PAGE.

2.2.2 Reduction and alkylation

After IEF, IPG strips were equilibrated in 10 mL reducing buffer (2% DTT in 10 mL equilibration buffer containing 6 mol/L urea, 30% glycerin, 2% SDS, 0.05 mol/L

Tris-HCl, pH 8.8) for 15 min, followed by 15-min equilibration in 10 mL alkylation buffer (400 mg iodoacetamide in 10 mL equilibration buffer).

2.2.3 SDS-PAGE

SDS-PAGE was performed in a Protean-plus Dodeca™ Cell (Bio-Rad) using self-cast polyacrylamide gels (200 x 205 x 1.5 mm; 12% T). Gels were run in parallel in 0.025 mol/L Tris/ 0.192 mol/L glycine/ 0.1% SDS at 10°C with a constant voltage of 70 V. The 2-DE standards (Bio-Rad) used for M_r and p*I* calibration were as follows: hen egg conalbumin type I (76 kDa; p*I* 6.0, 6.3, 6.6); BSA (66.2 kDa; p*I* 5.4, 5.6); bovine muscle actin (43 kDa; p*I* 5.0, 5.1); rabbit muscle glyceraldehyde 3-phosphate dehydrogenase (36 kDa; p*I* 8.3, 8.5); bovine carbonic anhydrase (31 kDa, p*I* 5.9, 6.0); soybean trypsin inhibitor (21.5 kDa; p*I* 4.5); and equine myoglobin (17.5 kDa; p*I* 7.0).

2.3 Protein staining

Gels were fixed overnight in 500 mL 30% ethanol/ 2% phosphoric acid, washed three times for 20 min each in 500 mL 2% phosphoric acid and equilibrated with 500 mL 2% phosphoric acid/ 18% ethanol/ 15% ammonium sulfate. Colloidal CBB staining of proteins was initiated by addition of 6 mL staining solution (2% CBB G250, Roth) to 500 mL of equilibration solution. Gels were stained for 48 h and thereafter washed once with 500 mL water for 20 min.

2.4 Image analysis

Gel images were scanned with the Molecular Imager Pharos FX (Bio-Rad). Spot detection, quantification and comparison of 2-D protein patterns was done with the PDQuest 8.0 software (Bio-Rad). Background and vertical streaks were removed from each gel image and spots were digitized by Gaussian fit. For quantification a "matchset" of all gels was prepared and the absorbance of individual protein spots from 2-D gels was assessed. The raw quantity of each spot

in a member gel was divided by the total intensity value of all the pixels in the image (i.e., total density in gel image); this normalization procedure of the PDQuest program assumed that the total density of an image (background density plus spot density) will be relatively consistent from gel to gel. After generation of the analysis sets, the selected spots were excised and transferred to 96-well microtiter plates (ABgene) by the EXQuest spot cutter (Bio-Rad).

2.5 Protein identification by MALDI MS

2.5.1 In-gel digestion

Each of the CBB-stained gel plugs was dehydrated in 50 µL acetonitrile (ACN), rehydrated/washed with 50 µL ammonium hydrogencarbonate solution (50 mmol/L) and then dehydrated with 50 µL ACN. Digestion with 20 ng/µL trypsin (Sequencing grade, Promega) was performed at 37°C for 4 h. Resulting peptides were extracted with 5 µL 1% TFA in an ultrasonic bath (Sonorex, Super RK 514 BH, Bandelin).

2.5.2 MALDI-TOF analysis

Extracted peptides were spotted directly onto a 600 µm/384 well AnchorChip sample target (Bruker Daltonics) using the affinity preparation protocol recommended by the manufacturer (matrix: CHCA). The MALDI mass spectra were obtained using a Bruker Ultraflex II TOF/TOF mass spectrometer equipped with a 384-sample scout source (Bruker Daltonics). An external peptide calibration standard containing the following fragments was used to calibrate the instrument: angiotensin II ($[M+H]^+$ 1046.54); angiotensin I ($[M+H]^+$ 1296.68); substance P ($[M+H]^+$ 1347.74); bombesin ($[M+H]^+$ 1619.82); ACTH clip 1–17 ($[M+H]^+$ 2093.09); ACTH clip 18–39 ($[M+H]^+$ 2465.20); somatostatin 28 ($[M+H]^+$ 1347.47) (Bruker Daltonics). Peptide masses were searched against the Swiss-Prot database employing the MASCOT program (in-house MASCOT-server) [9] for protein identification. Database searches were performed taking into account

carbamidomethyl modification of cysteines and possible oxidation of methionine, and allowing one missed cleavage. A mass inaccuracy of <100 ppm was required for PMF. For further consideration, only those proteins were assumed to be identified that were annotated from corresponding spots in at least three gels with a MASCOT score >>53 being clearly separated from the next best match. MS/MS analysis was performed when necessary. Identified proteins were sent to the Proteinscape™ database (Protagen) and checked individually for further consideration.

3 Results and discussion

3.1 Serum proteomics of SP-C/c-raf model mice

As described in the materials and methods, we used a thiourea-containing lysis buffer to extract proteins from serum [10, 11]. Proteins were separated within pH ranges of 3-10 and 4-7 and visualized with the colloidal CBB (CCB) stain. With the CCB stain approximately 400 spots/gel were detected. Figure 1 depicts a serum reference map for wildtype mice (pH 3-10).

3.2 Identification of serum proteins in SP-C/c-raf mice by MS

About 400 spots per gel were excised from CCB-stained gels. Protein spots from three parallel gels of each sample were analyzed by PMF using MALDI-TOF MS after tryptic in-gel digest. Identification was carried out by Swiss-Prot database searches with MASCOT. When needed, protein identification was confirmed by additional MS/MS experiments.

In tumor bearing and wildtype mice, 45 common (Tab. 1) and 5 unique serum proteins were identified by MS and MS/MS. Our identification of serum proteins fits best to those of Duan *et al.* [12], who reported 38 unique proteins and of Wait *et al.* [13] with 28 distinct proteins (Fig. 2). Overall, we identified 24 serum

proteins not reported by the afore-mentioned investigators. Furthermore 8 serum proteins identified in this study are also expressed in lung tissue as reported previously [8] and included A1AT4_MOUSE (spot no. 4), A1AT6_MOUSE (spot no. 5), ACTG_MOUSE (spot no. 8), ALBU_MOUSE (spot no. 10), APOA1_MOUSE (spot no. 11), HBA_MOUSE (spot no. 25), TRFE_MOUSE (spot no. 41) and TTHY_MOUSE (spot no. 42).

3.3 Differential expression of serum proteins in SP-C/c-raf transgenic mice

Nine proteins were found to be differentially expressed (matched in >3 different gels) with a minimum of 2-fold regulation, whereas five proteins were found to be exclusively expressed (see 3.4) when extracts of wildtype and lung tumor serum proteomes (aged 12 months) were compared. Table 2a and 2b and Figure 3a and 3b depict examples of differentially expressed proteins from wildtype and tumor bearing mice. Additionally, extracts from wildtype and tumor bearing mice at an age of 1 month were analyzed to compare expression between early and late stages of tumor development. Seven proteins were regulated in both tumor stages (Tab. 2a). Prominent examples are discussed below in terms of their regulation in human malignancies, overexpression of the c-raf kinase and regulation at different stages of cancerogenesis. Fig. 4 depicts a comparison of average regulation of these proteins between mice, aged 1 month and 12 months.

Spot no. 3 and no. 5 were identified as **alpha-1 antitrypsin** isoforms (A1AT, Swiss-Prot accession no.: Q00896 and P81105; A1AT3_MOUSE and A1AT6_MOUSE). A1AT is a secretory glycoprotein produced mainly in the liver and monocytes. It is the most abundant serine protease inhibitor in human plasma. It predominantly inhibits neutrophil elastase and therefore prevents breakdown of lung tissue. The deficiency of A1AT is an inheritable disorder characterized by reduced serum levels of A1AT. Protease inhibitors Z (PiZ) and protease inhibitors S (PiS) are the most common deficient genotypes of A1AT [14]. In previous studies A1AT was shown to be regulated in lung cancer [15, 16, 17]. Notably, Woodworth

et al. reported induction of the c-raf kinase in hepatocellular carcinoma along with overexpression of A1AT [34]. Likewise, we demonstrate overexpression of A1AT in tumor bearing mice at early (1 month) and advanced stages (12 months) of lung tumor development.

Spot no. 7 was identified by PMF as **alpha-2-macroglobulin** (A2MG, Swiss-Prot accession no.: Q61838; A2MG_MOUSE) from the protease inhibitor I39 family. This plasma-specific protein is able to inhibit all four classes of proteinases by a unique "trapping" mechanism [18]. More than twenty years ago A2MG was reported to be a lung tumor marker being significantly increased in human tumor patients [19]. A2MG levels, however, were slightly increased in patients with pulmonary emphysema and pneumonia as well [15]. In the study of Misra and coworkers incubation of prostate cancer cells with A2MG caused induction of c-raf expression [35]. We observed a > 2-fold upregulation of this protein in tumor bearing mice. Note, A2MG regulation at initial stages of cancerogenesis was repressed or unchanged. We propose alpha-2-macroglobulin as a lung cancer dependent candidate biomarker for advanced stages of disease.

Spot no. 25 was identified as **hemoglobin subunit alpha** (HBA, Swiss-Prot accession no.: P01942; HBA_MOUSE). This protein is one of the subunits of hemoglobin that belongs to the globin superfamily and is a heme-containing protein in the red blood cells, involved in binding and/or transporting oxygen from the lung to various peripheral tissues. Experiments have demonstrated that the expression of HBA was upregulated upon specific apoptotic stimuli like cytokine deprivation or cisplatin treatment in a hematopoietic pro-B cell line [20]. These data indicate that HBA is a new and crucial factor in apoptosis, supporting the mitochondrial pathway. Moreover, HBA was regulated in ovarian cancer [36]. Angiogenic studies with 17-DMAG, an orally bioavailable heat shock protein 90 modulator, decreased hemoglobin levels in vivo and reduced c-raf-1 expression in vitro [37]. In serum of tumor bearing mice, HBA was overexpressed.

Spot no. 36 was identified as a **major urinary protein** (MUP). MUPs belong to

the lipocalin superfamily, bind and slowly release male-specific pheromones in deposited scent marks. Likewise, females also express these proteins, consistent with their role in encoding individual signatures in scent marks [21]. Urinary proteins had been used as biomarkers for lung cancer, such as pseudouridine [22]. In a genomics approach MUP was found to be repressed in lung adenocarcinomas [23]. In our study, however, MUP was upregulated in the serum of tumor bearing mice. Both, at early stage by 6-fold (Swiss-Prot accession no.: P11589; MUP2_MOUSE), and at advanced stages of tumorigenesis by > 4-fold (Swiss-Prot accession no.: P04938; MUP8_MOUSE). A c-raf dependent regulation of MUPs was not reported so far.

Spot no. 42 was identified as **transthyretin**, also known as prealbumin (TTR, Swiss-Prot accession no.: P07309; TTHY_MOUSE). TTR is a regular blood protein which belongs to the same-named superfamily. It carries thyroid hormones, such as thyroxine and tri-iodothyronine from bloodstream to tissues. Furthermore transthyretin facilitates retinol transport through interaction with the **retinol binding protein** (RBP, spot no. 38, Swiss-Prot accession no.: P00724; RETBP_MOUSE). Mice lacking transthyretin expression have dramatically lower levels of retinol and RBP as well as cellular RBP and was shown to be associated with malignant transformation of ovarian surface epithelium [24, 25]. Notably, Zhang *et al.* reported a truncated form of transthyretin to be repressed in human patients with ovarian cancer [26] and suggests its use as a biomarker [27]. A recent proteomic study demonstrated, however, upregulation of transthyretin in human lung adenocarcinomas. In particular, the TTR monomer may be a blood marker for cerebrospinal fluid barrier disruption that occurs, for instance, in cerebral metastasis [28], but might also be regulated in carcinomas [29]. A downregulation of transthyretin by more than 6-fold was reported for ovarian [30] and lung cancer patients. We observed an initial 1.3-fold upregulation of this protein at an early stage of lung cancer (1 month), but a more than four-fold downregulation in mouse serum at advanced stages of tumor growth (12 months). The role of c-raf in the regulation of transthyretin is uncertain. Nonetheless, results from the present study and those reported by others point to TTR as an interesting

candidate biomarker.

Based on PMF, spot no. 43 was identified as **vitamin D-binding protein** (DBP, Swiss-Prot accession no.: P21614; VTDB_MOUSE) and regulated in serum of tumor bearing mice. This protein belongs to the ALB/AFB/VDB family and can be found in plasma, ascitic fluid, cerebrospinal fluid, urine and on the surface of many cell types. In plasma, this multifunctional protein acts as a vitamin D sterol carrier and binds the actin monomers, thereby preventing its polymerization. DBP associates with membrane-bound immunoglobulin on the surface of B-lymphocytes, furthermore with membrane-bound IgG Fc receptor of T-lymphocytes [31]. Deglycosylation of serum DBP led to immunesuppression in cancer patients [32]. According to Hlavaty *et al.* DBP may be of utility as a serum biomarker in the early detection of prostate cancer [33]. > 2-fold upregulation of DBP at advanced stages of lung cancerogenesis is a novel finding. Note, 1,25-dihydroxyvitamin D3, a vitamin D3 metabolite, caused activation of c-raf in human keratinocytes [38]. Additionally, in our studies DBP was downregulated by 1.3-fold in serum of lung tumor bearing mice at early stage of tumor growth (1 month). These findings warrant an evaluation of DBP as a candidate biomarker in cancer patients.

3.4 Exclusive expression of serum proteins in SP-C/c-raf transgenic mice

Notably, five proteins were found to be exclusively expressed either in wildtype or tumor bearing mice, aged 12 months, which are discussed below.

A soluble form of the **epidermal growth factor receptor** (EGFR, spot no. 46, see Tab. 2b, Swiss-Prot accession no.: Q01279; EGFR_MOUSE) was exclusively identified in serum of wildtype mice. EGFR is a membrane-bound tyrosine kinase, but soluble forms were also found in body fluids. Binding of EGF to EGFR leads to dimerization and internalization of the EGFR complex. Through phosporylation of tyrosine residues the EGFR connects to the MAPK/ERK pathway, with c-raf as a key member, thereby stimulating DNA synthesis and cell proliferation. As reviewed by Mendelsohn *et al.*, the EGFR is a suitable target in the drug treatment of cancers.

EGFR is overexpressed, dysregulated or mutated in many epithelial malignancies, and activation of EGFR appears important in tumor growth and progression [39]. EGFR was proposed as a serum biomarker in breast and ovarian cancer [40]. Studies on human sera by Baron and coworkers demonstrated repression of sEGFR in ovarian cancer [41, 42, 43].

The role of serum **properdin** (spot no. 50, see Tab. 2b, Swiss-Prot accession no.: P11680; PROP_MOUSE) in tumor bearing mice was investigated by Schwartz *et al.* more than fourty years ago [44, 45]. In our studies, properdin was found exclusively expressed in wildtype mice. Properdin, also known as factor P, is a positive regulator of the alternate pathway of complement. It binds to and stabilizes the C3- and C5-convertase enzyme complexes. It is known that properdin participates in some specific immune responses. It plays a part in tissue inflammation as well as in the engulfing of pathogens by phagocytes. In addition, properdin is known to support neutralization of some viruses. As a component of the alternative pathway for complement activation, properdin complexes with another protein, C3b, to stabilize the alternative C3 convertase (C3bBb).

The **immunglobulin gamma-2B chain C region** (spot no. 47, see Tab. 2b, Swiss-Prot accession no.: P01867; GCBM_MOUSE) was found to be exclusively expressed in wildtype mice, whereas the **immunglobulin J chain** (spot no. 48, see Tab. 2b, Swiss-Prot accession no.: P01592; IGJ_MOUSE) and **immunglobulin kappa chain V-III region MOPC 70** (spot no. 49, see Tab. 2b, Swiss-Prot accession no.: P01656; KV3C_MOUSE) were found exclusively expressed in tumor bearing mice. To the best of our knowledge, their role in lung cancer is unknown.

4 Concluding remarks

Based on the initial works of our laboratory [8] we now extended our investigations to the serum proteome of lung tumor bearing mice. Two stages of tumor development were studied (1 month and 12 months). Protein expression of tumor bearing mice was compared with those of wildtype, e.g. non-transgenic animals. In total, 8 proteins identified in tissue of lung tumor bearing mice were also identified in serum. A total of 50 proteins were identified in serum, some of which were specifically regulated or exclusively expressed either in tumor bearing or wildtype mice. The biological functions of the identified proteins associated with tumorigenesis are not always clear. Notably, we found alpha-1-antitrypsin (A1AT) and alpha-2-macroglobulin (A2MG) to be upregulated in the serum proteome of 12 months old mice and according to literature its regulation is linked to c-raf overexpression [34, 35]. We extend early findings for their regulation in tissue [15, 16]. A1AT was upregulated in 1 month old mice as well, whereas expression of A2MG was downregulated in 1 month old mice. Hemoglobin subunit alpha was upregulated in serum samples of lung tumor bearing mice (12 months) and may play a role in apoptosis [20]. We found major urinary proteins to be upregulated both, in early and late stages of tumor development. MUPs may serve as biomarkers for lung cancer [12, 22]. Upregulation of vitamin D-binding protein (DBP) in advanced stages of lung cancer is a novel finding. A role for c-raf activation by a vitamin D metabolite in human keratinocytes has already been reported [38]. We found expression of transthyretin to be upregulated in 1 month and repressed in 12 month old tumor bearing mice. Therefore, previously reported findings fit well with our results of regulated serum proteins. We thus propose their in-depth validation as biomarker candidates for the detection of lung adenocarcinomas.

Figure captions

Figure 1: 2-D reference map of blood serum from wildtype mice stained with CBB.

Figure 2: Comparison of works investigating the mouse serum proteome. Duan and coworkers reported 38 mouse serum proteins, while Wait *et al.* reported 28 proteins. We identified 50 proteins, 24 of them were novel and not reported so far. 14 proteins were in common.

Figure 3a/b: Examples of differentially expressed serum proteins of tumor bearing (T) and wildtype (C) mice. Protein spots of interest are marked by circles and/or arrows. **a:** differentially expressed serum proteins from mice, aged 1 month. **b:** differentially expressed serum proteins from mice, aged 12 months.

Figure 4: Comparison of protein expression profiles. Comparison of expression profiles of regulated serum proteins between lung tumor bearing and wildtype mice (1 month and 12 months). T/C: average ratio between n tumor bearing (T) and n wildtype (C) mice. T/C = 1.0: no regulation, T/C < 1.0: proteins are downregulated in tumor bearing mice, T/C > 1.0: proteins are upregulated in tumor bearing mice.

Figure 5: Histopathology of tumorigenesis in the lung of c-raf transgenic mice. Lung tumors arose multifocally. Starting as adenomatous hyperplasia (AAH), first morphological changes in distinct areas of the lung appeared within the first 2 months. This figure shows advanced stages of tumorigenesis (12 months), when almost the entire lung is tumorous.

Table captions

Table 1: **Protein identification in 2-DE maps of serum proteins from wildtype and SP-C/c-raf transgenic lung tumor bearing mice, identified by MALDI-MS.** See supplementary table 1 for detailed information.

Table 2a/b: **Quantification of regulated proteins from 2-D gels. a:** Protein expression profiles from tumor bearing (T) and wildtype (C) mice, aged 1 month. **b:** Protein expression profiles from tumor bearing (T) and wildtype (C) mice, aged 12 months. Significant differential expression of protein spots that were quantified from 2-D gels. Quantification of protein abundance was done using the PDQuest 2-D software (Bio-Rad) by measuring the normalized OD (arbitrary units, AU) of each protein spot. The change in abundance of the proteins is expressed by the calculated ratio between average tumor (T) and wildtype (C) protein expression values. Exclusively expressed proteins are marked by T or C.

Supplementary Table 1: **An overview of mouse serum proteins.** The Mascot score, the number of identified peptides, their sequence and the protein coverage of the best hits are shown for each identified protein. O@M: Abbreviation for oxidation at the amino acid methionine.

The authors have declared no conflict of interest.

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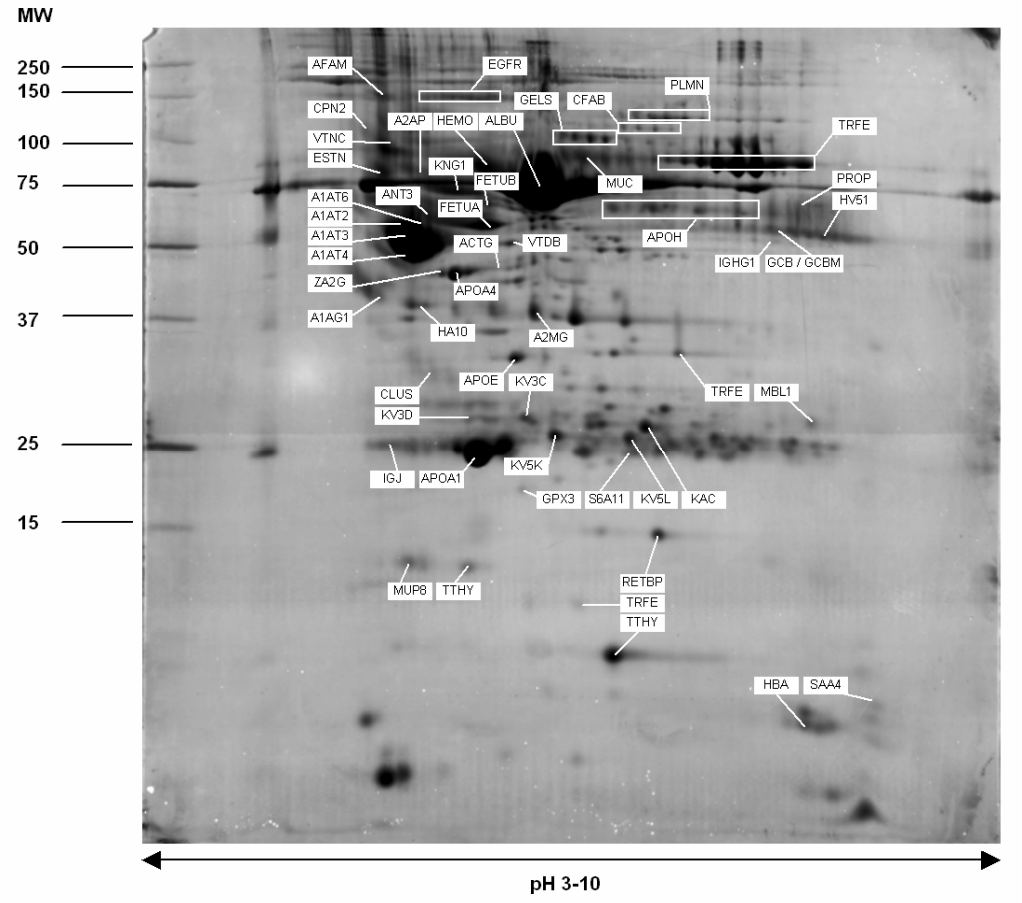
Figure 1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VTNC
ESTN
KNG1
FETUB
MUC
PROP
HV51
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ANT3
FETUA
A1AT2
A1AT3
ACTG
VTDB
APOH
A1AT4
IGHG1
GCB / GCBM
ZA2G
APOA4
A1AG1
HA10
A2MG
APOE
KV3C
TRFE
MBL1
CLUS
KV3D
KV5K
IGJ
APOA1
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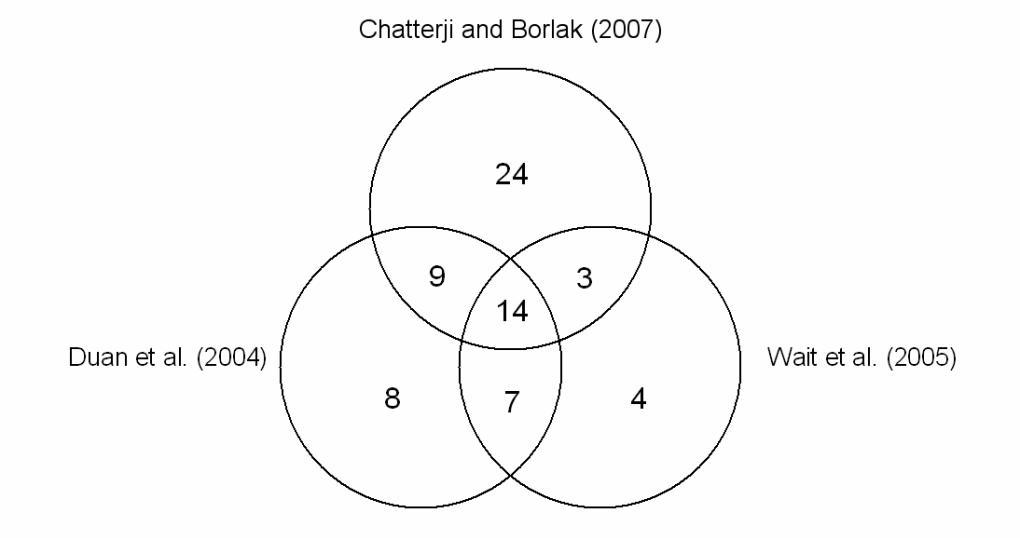
Figure 2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Duan et al. (2004)
Wait et al. (2005)
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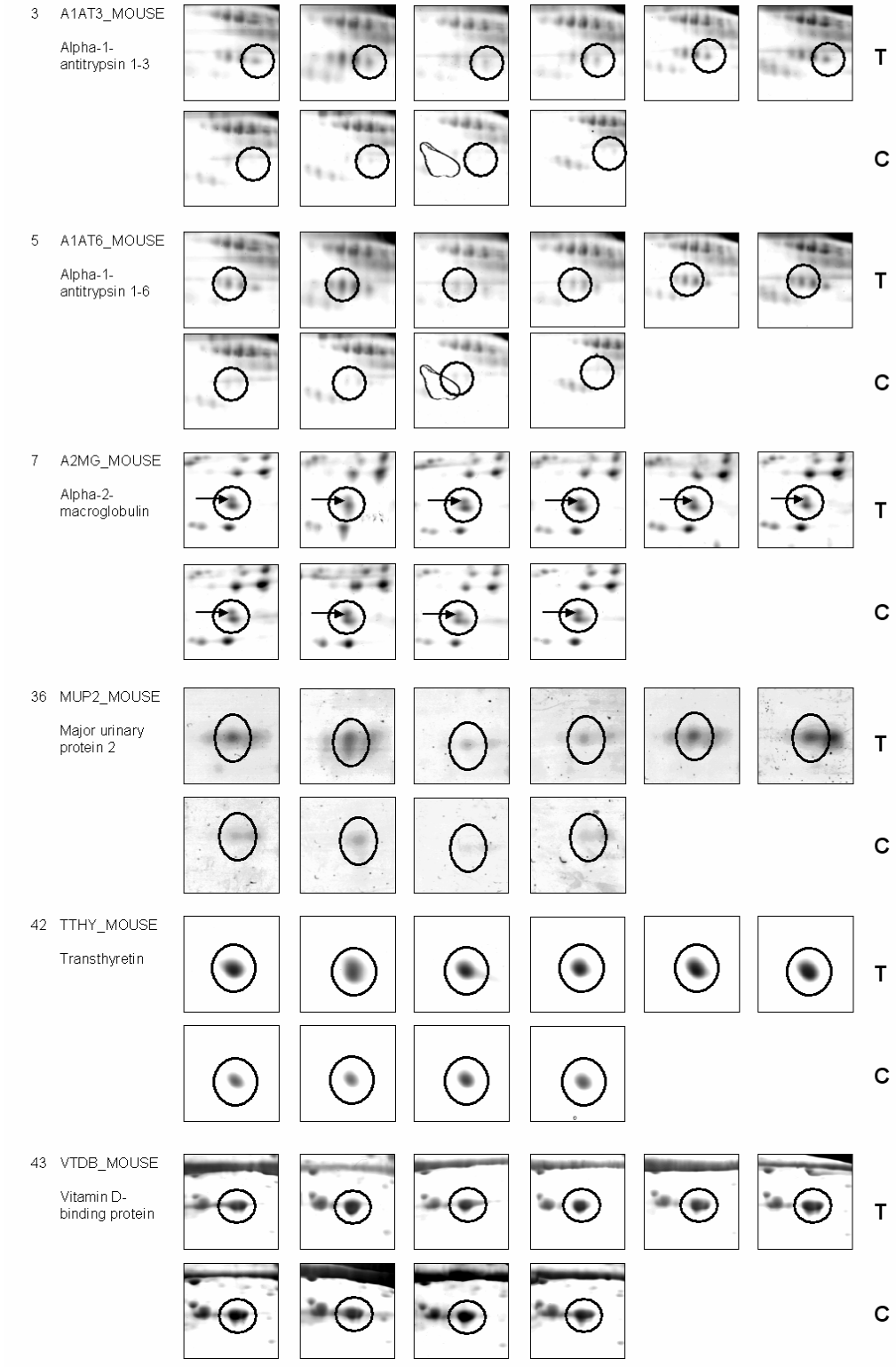
Figure 3a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T
C
5 A1AT6_MOUSE
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C
7 A2MG_MOUSE
Alpha-2-macroglobulin
T
C
36 MUP2_MOUSE
Major urinary protein 2
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Transthyretin
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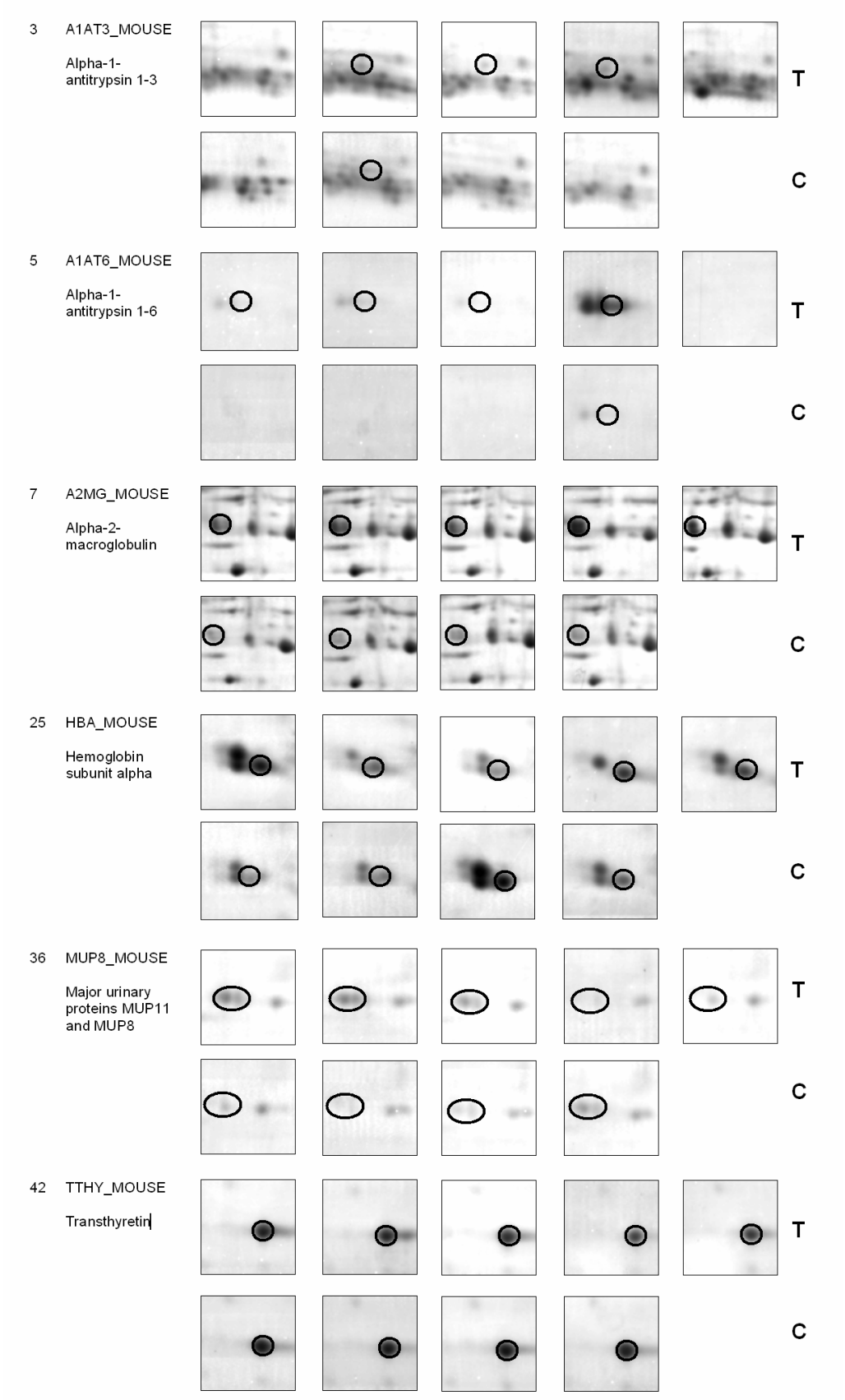
Vitamin D-binding protein
T
C

Figure 3b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T
C
7
A2MG_MOUSE
Alpha-2-macroglobulin
T
C
25
HBA_MOUSE
Hemoglobin subunit alpha
T
C
36
MUP8_MOUSE
Major urinary proteins MUP11 and MUP8
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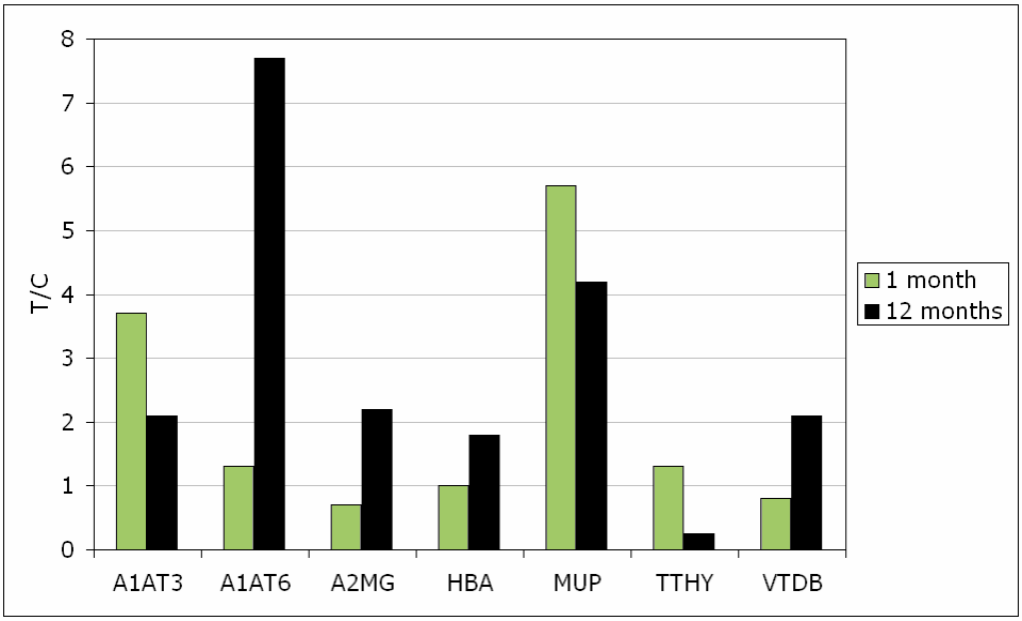
42
TTHY_MOUSE
Transthyretin
T
C

Figure 4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A2MG
HBA
MUP
TTHY
VTDB
1 month
12 months

Figure 5

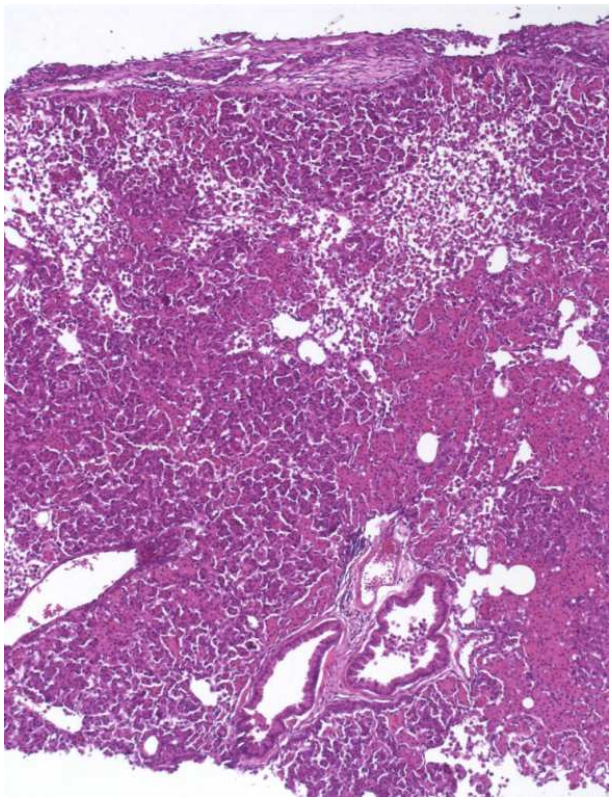

Table 1

No.	Protein identification		Protein superfamily
1	A1AG1_MOUSE	Alpha-1-acid glycoprotein 1 (Orosomucoid-1)	Lipocalin
2	A1AT2_MOUSE	Alpha-1-antitrypsin 1-2	Serpin
3	A1AT3_MOUSE	Alpha-1-antitrypsin 1-3	Serpin
4	A1AT4_MOUSE	Alpha-1-antitrypsin 1-4	Serpin
5	A1AT6_MOUSE	Alpha-1-antitrypsin 1-6	Serpin
6	A2AP_MOUSE	Alpha-2-antiplasmin	Serpin
7	A2MG_MOUSE	Alpha-2-macroglobulin	Protease inhibitor I39
8	ACTG_MOUSE	Actin, cytoplasmic 2 Gamma-actin	Actin
9	AFAM_MOUSE	Afamin (Alpha-albumin)	ALB/AFP/VDB
10	ALBU_MOUSE	Serum albumin	ALB/AFP/VDB
11	APOA1_MOUSE	Apolipoprotein A-I	Apolipoprotein A1/A4/E
12	APOA4_MOUSE	Apolipoprotein A-IV	Apolipoprotein A1/A4/E
13	APOE_MOUSE	Apolipoprotein E	Apolipoprotein A1/A4/E
14	APOH_MOUSE	Apolipoprotein H (Beta-2-glycoprotein 1)	Complement control module/SCR domain
15	CFAB_MOUSE	Complement factor B (C3/C5 convertase)	Peptidase S1
16	CLUS_MOUSE	Apolipoprotein J (Clusterin)	Clusterin
17	CPN2_MOUSE	Carboxypeptidase N subunit 2	
18	ESTN_MOUSE	Liver carboxylesterase N (Lung surfactant convertase)	Type-B carboxylesterase/ lipase
19	FETUA_MOUSE	Fetuin A (Alpha-2-HS-glycoprotein)	Fetuin
20	FETUB_MOUSE	Fetuin B	Fetuin
21	GCB_MOUSE	Ig gamma-2B chain C region secreted form	Immunglobulin
22	GELS_MOUSE	Gelsolin (Brevin)	Villin/gelsolin
23	GPX3_MOUSE	Glutathione peroxidase 3	Glutathione peroxidase
24	HA10_MOUSE	H-2 class I histocompatibility antigen, Q10 alpha chain	MHC class 1
25	HBA_MOUSE	Hemoglobin subunit alpha (Alpha-globin)	Globin
26	HEMO_MOUSE	Hemopexin	Hemopexin-like domain
27	HV51_MOUSE	Ig heavy chain V region AC38 205.12	Immunglobulin
28	IGHG1_MOUSE	Ig gamma-1 chain C region secreted form	Immunglobulin
29	KAC_MOUSE	Ig kappa chain C region	Immunglobulin
30	KNG1_MOUSE	Kininogen-1	
31	KV3D_MOUSE	Ig kappa chain V-III region 50S10.1	Immunglobulin
32	KV5K_MOUSE	Ig kappa chain V-V region HP R16.7	Immunglobulin
33	KV5L_MOUSE	Ig kappa chain V-V region HP 93G7	Immunglobulin
34	MBL1_MOUSE	Mannose-binding protein A	C-type lectin like
35	MUC_MOUSE	Ig mu chain C region secreted form	Immunglobulin
36	MUP8_MOUSE	Major urinary proteins 11 and 8 (MUP8)	Lipocalin
37	PLMN_MOUSE	Plasminogen	plasminogen/peptidase S1
38	RETBP_MOUSE	Plasma retinol-binding protein	Lipocalin
39	S6A11_MOUSE	Sodium- and chloride-dependent GABA transporter 4	SNF (sodium neurotransmitter symporter)
40	SAA4_MOUSE	Serum amyloid A-4 protein	SAA
41	TRFE_MOUSE	Serotransferrin	Transferrin
42	TTHY_MOUSE	Transthyretin (Prealbumin)	Transthyretin
43	VTDB_MOUSE	Vitamin D-binding protein (Gc-globulin)	ALB/AFP/VDB
44	VTNC_MOUSE	Vitronectin	Somatomedin B domain
45	ZA2G_MOUSE	Zinc-alpha-2-glycoprotein	MHC class 1

Table 2a

No.	Protein ID	Tumor (T1)	Tumor (T2)	Tumor (T3)	Tumor (T4)	Tumor (T5)	Tumor (T6)	Control (C1)	Control (C2)	Control (C3)	Control (C4)	Ratio (T/C)
3	A1AT3_MOUSE	3825	3948	3698	3641	2769	2488	1562	643	281	1211	**3.7**
5	A1AT6_MOUSE	2477	4772	1325	2462	2742	2644	1700	1240	2067	3606	**1.3**
7	A2MG_MOUSE	2683	319	3362	3404	1452	1345	2253	5181	1723	3635	**0.7**
25	HBA_MOUSE	39568	18081	4104	27938	24936	20095	32241	12354	21703	21248	**1.0**
36	MUP2_MOUSE	1499	1240	47	309	1933	1326	327	300	77	41	**5.7**
42	TTHY_MOUSE	36920	27522	28645	33670	34577	30648	19699	23241	28600	26460	**1.3**
43	VTDB_MOUSE	12981	19652	22967	24802	19831	16896	21342	25219	26614	29599	**0.8**

Table 2b

No.	Protein ID	Tumor (T1)	Tumor (T2)	Tumor (T3)	Tumor (T4)	Tumor (T5)	Control (C1)	Control (C2)	Control (C3)	Control (C4)	Ratio (T/C)
3	A1AT3_MOUSE	-	538	234	186	-	-	152	-	-	**2.1**
5	A1AT6_MOUSE	100	68	6	654	-	-	-	-	27	**7.67**
7	A2MG_MOUSE	506	1124	610	872	655	536	219	155	469	**2.18**
25	HBA_MOUSE	4990	2161	627	3176	3938	479	365	3608	2228	**1.79**
29	KAC_MOUSE	1146 375 461 478	897 281 1465 1509	- 393 1972 1482	566 635 1365 2660	1420 786 1176 2911	214 347 1009 2194	176 51 138 247	703 297 244 167	751 104 223 144	**2.15** **2.47** **3.19** **2.63**
36	MUP8_MOUSE	2198 1134	2333 786	1882 298	34 111	93 481	114 176	25 57	136 62	1852 236	**2.46** **4.23**
39	S6A11_MOUSE	203 600	154 1763	56 2439	742 1095	503 5878	- 1294	- 103	- 649	- 206	**T** **4.18**
42	TTHY_MOUSE	111	479	84	21	50	276	417	1324	335	**0.25**
43	VTDB_MOUSE	3096	2466	4026	459	4149	2375	1124	1088	878	**2.08**
46	EGFR_MOUSE (Epidermal growth factor receptor)	-	-	-	-	-	365	695	846	232	**C**
47	GCBM_MOUSE (Ig gamma-2B chain C region, membrane-bound form)	-	-	-	-	-	1548	1904	302	1509	**C**
48	IGJ_MOUSE (Ig J chain)	1156	1428	1853	766	5384	-	-	-	-	**T**
49	KV3C_MOUSE (Ig kappa chain V-III region MOPC 70)	478	1509	1482	2660	2911	-	-	-	-	**T**
50	PROP_MOUSE (Properdin)	-	-	-	-	-	400	509	887	334	**C**

Supplementary Table 1:
A summary of serum proteins from SP-C/c-raf transgenic mice

No. (internal no.)	Swissprot ID	Swissprot Accession no.	Protein aliases	Mascot score No. of matched peptides Percentual coverage	Peptide sequences	Gene	Subcellular location	Protein function / notes
1 (418)	A1AG1_MOUSE	Q63593	Alpha-1-acid glycoprotein 1 [Precursor] AGP 1 Orosomucoid-1 OMD 1	84.5 7 85.7	AVTHVGMDESEIFVDWKK + OXPM HGAFMLAFDLK.DEK HGAFMLAFDLK.DEK + OXPM RPDITPELR RPDITPELREVFQK YEGGYETFAHLVLR YEGGYETFAHLVLRK	Orm1 Agp1		Appears to function in modulating the activity of the immune system during the acute-phase reaction.
2 (1621)	A1AT2_MOUSE	P22599	Alpha-1-antitrypsin 1-2 [Precursor] Serine protease inhibitor 1-2 Alpha-1 protease inhibitor 2 Alpha-1-antiproteinase AAT	183.3 24 46.5	AVHKAVLTIDETGTEAAAATVTEAVPMSMPPILR AVLTIDETGTEAAAATVTEAVPMSMPPILR AVLTIDETGTEAAAATVTEAVPMSMPPILR + 2 OXPM AVLTIDETGTEAAAATVTEAVPMSMPPILR + OXPM DQSPASHEIATNLGDFAISLYR FDHPFLFIFEEHTQSPIFVGK FLEEAKNHYQAEVFSVNFAESEEAK IFNNIGADLSGITEENAPLK IFNNIGADLSGITEENAPLK.LSK IYEAVKELDQDTVFALAIYLEFK KPFDPENTEEAEFHVDK KPFDPENTEEAEFHVDKSTTVK LSISGDYNLK LSISGDYNLKTLMSPLGTR LSISGDYNLKTLMSPLGTR + OXPM LVQIHPR LVQIHPRLSISGDYNLK MQHLEQTLNKELSK RLVQIHPR SFQHLLQTLNRPDSELQLST GNGLFV NNDLK TLMSPLGTFR TLMSPLGTFR + OXPM VINDFVEK.GTQGK VKKPFDPENTEEAEFHVDK	Spi1-2 Aat2 Dom2	Secreted	Inhibitor of serine proteases. Its primary target is elastase, but it also has a moderate affinity for plasmin and thrombin.
3 (2632)	A1AT3_MOUSE	Q00896	Alpha-1-antitrypsin 1-3 [Precursor] Serine protease inhibitor 1-3 Alpha-1 protease inhibitor 3	223.0 23 73.3	AVLTIDETGTEAAAATVLLAVPYSMPPIVR + 2 OXPM AVLTIDETGTEAAAATVLLAVPYSMPPIVR + OXPM DQSPASHEIATNLGDFAISLYR FDHPFLFIFEEHTQSPLFVGK FLEEAKNHYQAEVFSVNFAESEEAK FLLKRPR IFNNIGADLSGITEENAPLK IFNNIGADLSGITEENAPLKLSQAVHK KLDQDTVFALANYLEFK KPFDPENTEEAEFHVDESTTVK LAQHFPR LSISGEYNLK MQHLEQTLSK NHYQAEVFSVNFAESEEAK NHYQAEVFSVNFAESEEAKK RLAQHFPR SFQHLLQTLNRPDSELQLST GNGLFV NNDLK SFQHLLQTLNRPDSELQLST GNGLFV NNDLKLVEK TLMSPLGTFR TLMSPLGTFR + OXPM TLMSPLGTFRFNNIGADLSGITEENAPLK + OXPM VINDFVEK VINDFVEK.GTQGK VKQPFDPENTEEAEFHVDESTTVK	Spi1-3 Dom3	Secreted	Inhibitor of serine proteases. Its primary target is elastase, but it also has a moderate affinity for plasmin and thrombin.
4 (1613)	A1AT4_MOUSE	Q00897	Alpha-1-antitrypsin 1-4 [Precursor] Serine protease inhibitor 1-4 Alpha-1 protease inhibitor 4	233.0 23 53.3	AVLTIDETGTEAAAATVLQVATYSMPPIVR + OXPM DQSPASHEIATNLGDFALR ELISQFLLNR ELISQFLLNRR FDHPFLFIFEEHTQSPIFVGK IFNNIGADLSGITEENAPLK IFNNIGADLSGITEENAPLK.LSK KLDQDTVFALANYLEFK LDQDTVFALANYLEFK LSISGNYNLK NHYQAEVFSVNFAESEEAK NHYQAEVFSVNFAESEEAKK QPFDPENTEEAEFHVDESTTVK RSDAQIHPR SDAQIHPR SFQHLLQTLNRPDSELQLST GNGLFV NNDLK SFQHLLQTLNRPDSELQLST GNGLFV NNDLKLVEK TLMSPLGTFR TLMSPLGTFR + OXPM TLMSPLGTFRFNNIGADLSGITEENAPLK + OXPM VINDFVEK VINDFVEKGTQGK VKQPFDPENTEEAEFHVDESTTVK	Spi1-4 Dom4	Secreted	Inhibitor of serine proteases. Its primary target is elastase, but it also has a moderate affinity for plasmin and thrombin.
5 (1612)	A1AT6_MOUSE	P81135	Alpha-1-antitrypsin 1-6 [Precursor] Serine protease inhibitor 1-6 Alpha-1 protease inhibitor 6	145.3 12 78.4	DQSPASHEIATNLGDFAISLYR ELISKFLLNR FDHPFLFIFEEHTQSPLFVGK IFNNIGADLSGITEENAPLK KLDQDTVFALANYLEFK LAQHFPR NHYQAEVFSVNFAESEEAKK RLAQHFPR SFQHLLQTLNRPDSELQLST GNGLFV NNDLK TLMSPLGTFR TLMSPLGTFR + OXPM TLMSPLGTFRFNNIGADLSGITEENAPLK + OXPM	Spi1-6 Dom6	Secreted	Inhibitor of serine proteases. Its primary target is elastase, but it also has a moderate affinity for plasmin and thrombin.
6 (2633)	A2AP_MOUSE	Q61247	Alpha-2-antiplasmin [Precursor] Alpha-2-plasmin inhibitor Alpha-2-PI Alpha-2-AP	93.4 8 63.3	GFPIKDDFLEQSER LAPNEEDFYFSSPK + OXPM LDHQDFGDHATLK LDHQDFGDHATLKR LIQQHIDKADFANIGGK NPNPSALPQLQEQR SVPTAEETRR TFGPDLKLAPR	Pi	Secreted	The major targets of this inhibitor are plasmin and trypsin, but it also inactivates chymotrypsin.
7 (4853)	A2MG_MOUSE	Q61838	Alpha-2-macroglobulin [Precursor] Alpha-2-M Pregnancy zone protein	133.3 12 77.3	AAPLSLCALTAVDQSVLLLKPEAK ABYLISGYQR ALLAYAFALAGNK ALSFYQPR EEGTGIELTGKGSCEIANALSKLK HSLGDNDAHSIFQSVGNIFTNSK KTVSAVATPK LSPDSYNLLPGK LTEVPALVHKDTVVK SKAIHYLSGYQR VKALSFYQPR VNITNYRPGLPFSGQVLLVDEK	A2m Pzp	Secreted	Is able to inhibit all four classes of proteinases by a unique 'trapping' mechanism. This protein has a peptide stretch, called the 'bait region' which contains specific cleavage sites for different proteinases. When a proteinase cleaves the bait region, a conformational change is induced in the protein which traps the proteinase. The entrapped enzyme remains active against low molecular weight substrates (activity against high molecular weight substrates is greatly reduced). Following cleavage in the bait region a thioester bond is hydrolyzed and mediates the covalent binding of the protein to the proteinase.
8 (4533)	ACTG_MOUSE	P63713	Actin, cytoplasmic 2 Gamma-actin	94.9 6 97.7	AVFPSIVGRPR GYSFTTTAEREIVR LDLAGRDLTDYLMK + OXPM QEYDESGPSIVHR SVELPDGQVITIGNER VAPEEHPVLLTEAPLNPK	Actg1 Actg	Cytoplasma	Cell structure and mobility proteins: actins are highly conserved proteins that are involved in various types of cell motility and are ubiquitously expressed in all eukaryotic cells.
9 (2838)	AFAM_MOUSE	O89023	Afamin [Precursor] Alpha-albumin Alpha-Alb	189.3 22 53.8	AAPEQYLK AAPQLPMEELVSLSK AAPQLPMEELVSLSK + OXPM AHVGFLPPFPTLDPEEK CVADHTLPECSK DSDPDKFFAEFIVEYSR EACFMANIKDDRPEGLSLR EAKFTESEIHVCQER EDLQNKK ENPAGCYR FTESEIHVCQER HCFEHLK HFLVKFTK RLCFFYNK TDFAFRR TINPAVDHCCK TINPAVDHCCKTDFAFR VLNSINVAVFSK VMLDYRDR VMLDYRDR + OXPM VYMDFLEDCCSR VYMDFLEDCCSR + OXPM	Afm	Secreted	Possible role in the transport of yet unknown ligand.

13 (1732)	ALBU_MOUSE	P07724	Serum albumin [Precursor]	455.0 39 95.7	AADKDTCFSTEGPNLVTR AETFTFHSDICTLPEKEK AFKAWAVAR APQVSTPTLVEAAR CCAEANPPACYGTVLAEFQPLVEEPK CCSQSLVER CCTLPEDQRLPCVEDYLSAILNR CSYDEHAK CSYDEHAKLVQEVTDFAK DVFLGTFLYEYSR EAHKSEIAHR EHPTTFMGHYLHEVAR EHPTTFMGHYLHEVAR + OXPM HPDYSVSLLLR KYEATLEK LCAIPHLR LCAIPHLRENYGELADCCTK LGEYGFQNALIVR LPCVEDYLSAILNR LQTCCDKPLLK LQTCCDKPLLKK LSQTFPNADFAEITK LSQTFPNADFAEITKLATDLTK LVQEVTDFAK QTALAELVK RHPDYSVSLLLR RPCFSALTVDETYVPK RPCFSALTVDETYVPKEFK SLHTLFGDKLCAIPHLR TNCDLVEKLGEYGFQNALIVR TPVSEHVTK TVMDDFAQFLDTCCK TVMDDFAQFLDTCCK + OXPM VCLLHEK VCLLHEKTPVSEHVTK YNAETEKQATESK + OXPM YNDLQEQHFK YTQKAPQVSTPTLVEAAR	Alb Alb1 Alb-1	Secreted	Serum albumin, the main protein of plasma, has a good binding capacity for water, Ca(2+), Na(+), K(+), fatty acids, hormones, bilirubin and drugs. Its main function is the regulation of the colloidal osmotic pressure of blood.
11 (2312)	APOA1_MOUSE	Q03623	Apolipoprotein A-I [Precursor] Apo-AI ApoA-I	238.0 19 87.3	ARPALEDLR DFANVYVDAVKDSGR DFNVDNLEK DFNVDNLEKETDWVR HSLMPMLETLK LAELKSNPTLNEYHTR LSPVAEEFR LSPVAEEFRDR QKLQELQGR SNPTLNEYHTR TQLAPHSEQMR TQLAPHSEQMR + OXPM TQLAPHSEQMRESLAQR + OXPM VAPLGAELQESAR VAPLGAELQESARQK VKDFANVYVDAVK VQPYLDEFQK VQPYLDEFQKK VWKEDVELYR	Apoa1	Secreted	APOA1 participates in the reverse transport of cholesterol from tissues to the liver for excretion by promoting cholesterol efflux from tissues and by acting as a cofactor for the lecithin cholesterol acyltransferase.
12 (2311)	APOA4_MOUSE	P06728	Apolipoprotein A-IV [Precursor] Apo-AIV ApoA-IV	296.0 24 83.4	ALVQQLEQFR ALVQQLEQFRQQLGPHSGEVESHLSFLEK ATEQVILEDLRR EAVEQFQKTDVTQQLSTLFQDK EKVNEFMSTLEK + OXPM KGSPDQPQALPLPGAQEQAQEQAQEQVQPKPLES LGDASTYADGVHNK LNHQMEGLAFQMKK + 2 OXPM LNHQMEGLAFQMKK + 2 OXPM LQEHLKPYAVDLQDQINTQTQEMK + OXPM LQLTPVQR LVPFVVQLSGHLAKETER MMPMANKVTQFFGENMQK + 3 OXPM NLALVEDVQSK NMEELKGHLTPR + OXPM QLEQQVEEFRR QQLGPHSGEVESHLSFLEK SLALTVGVQEK TDVTQQLSTLFQDKLGDASTYADGVHNK TVEPMQEMLTPK + 2 OXPM VKGNTEGLQK VNEFMSTLEK + OXPM VNEFMSTLEKK + OXPM VSAKIDQLQK	Apoa4	Secreted	May have a role in chylomicrons and VLDL secretion and catabolism. Required for efficient activation of lipoprotein lipase by ApoC-II; potent activator of LCAT. Apoa-IV is a major component of HDL and chylomicrons.

13 (3429)	APOE_MOUSE	P38226	Apolipoprotein E [Precursor] Apo-E	198.0 15 95.3	AQAFQDRIR ELEEQLQPVAEETR ELEEQLQPVAEETRAR FWDYLR GRLEEYQKHQAR GVSAIRER LGADMEDLRNR + OXPM LGKEYQAAQAAR LGPLVEQGR LGPLVEQGRQR LKGWEPVFEDMHR + OXPM LQAEFQAR QWAAILMEK SKMEEQTQQIR + OXPM TANLGAGAAQPLRDR	Apoe	Secreted	Mediates the binding, internalization, and catabolism of lipoprotein particles. It can serve as a ligand for the LDL (apo B/E) receptor and for the specific apo-E receptor (chylomicron remnant) of hepatic tissues.
14 (6736)	APOH_MOUSE	Q31339	Beta-2-glycoprotein 1 [Precursor] Beta-2-glycoprotein I Apolipoprotein H Apo-H B2GPI Beta(2)GPI Activated protein C-binding protein APC inhibitor	223.0 23 64.5	ATFGCHETYKLDGPEEAECTK ATVLYQGMR ATVLYQGMR + OXPM CPFPPRPENGYVNYPAKPVLLYK CPFPPRPENGYVNYPAKPVLLYKDK CSYTVEAHCR CTEEGKVVSPDIPACAR DGTIEIPSCFK DGTEIEIPSCFKEHSSLAFWK EHSSLAFWK FTCPLTGMVPFNTLR FTCPLTGMVPFNTLR + OXPM ICPKPDDLPFATVVVPLK IFCPPPVPK KATVLYQGMR + OXPM KCSYTVEAHCR LPECLEVK NGMVHHQDKIHFYCK + 2 OXPM TGTWSFLPTCR TSYDPGEQIVYSCKPGYVSR VCPFAGILENGIVR VKLQEQFK WSPDIPACAR	Apoh B2gp1	Secreted	Binds to various kinds of negatively charged substances such as heparin, phospholipids, and dextran sulfate. May prevent activation of the intrinsic blood coagulation cascade by binding to phospholipids on the surface of damaged cells.
15 (6832)	CFAB_MOUSE	P34186	Complement factor B [Precursor] EC 3.4.21.47 C3/C5 convertase	181.0 18 71.0	ALLDIGRDPK ALRJPQTATSEAK CLTNLEKVASYGVRPCR CPRPQDFEINGEFVKPR DLEIEEVLFHPK GNDYHKQPWQAK KAEGPEFYDYDVALVFK KDNEHHVFK LEDVTYHCSR QQLVPSYAR RDLEEEVLFHPK RQQLVPSYAR STGSWSDLQTR VASYGVRPR VGSQVRLEDNTYHCSR VKDASEVVTPR YGGTLRPICLPCTEGTTR	Cfb Bf H2-Bf	Secreted	Factor B which is part of the alternate pathway of the complement system is cleaved by factor D into 2 fragments: Ba and Bb. Bb, a serine protease, then combines with complement factor 3b to generate the C3 or C5 convertase.
16 (1138)	CLUS_MOUSE	Q06890	Clusterin [Precursor] Sulfated glycoprotein 2 SGP-2 Clustrin Apolipoprotein J Apo-J	73.5 7 43.0	ASGIDTLFQDR ASGIDTLFQDRFFAR ELHDPMVFSPRDFPHRPHFLYPK HTCMKFYAR + OXPM QQSQVLDAMQDSFAR + OXPM RPHFLYPK YINKEIQNAVQGVK	Clu Apoj Mfgp-2	Secreted	Not yet clear. It is known to be expressed in a variety of tissues and it seems to be able to bind to cells, membranes and hydrophobic proteins. It has been associated with programmed cell death.
17 (1813)	CPN2_MOUSE	Q9DBB9	Carboxypeptidase N subunit 2 [Precursor] Carboxypeptidase N polypeptide 2 Carboxypeptidase N 83 kDa chain Carboxypeptidase N regulatory subunit Carboxypeptidase N large subunit	137.0 12 62.7	AAHSQCAVSNPEGTVLACEESR ALGLDEGEPAGSWDLTVEGR CRVLNRQLSSR GAFQSLTGLQMLK + OXPM GQLVPHLQEQLQCPVNPGHLSFR HLEPDAFGGLPR LQLLNLSR LSNNMLAR + OXPM TLNLAQNLLTQLPK TLPGRLFQSLR VVFLHTQVR WLNRQLSSR	Cpn2	Secreted	The B3 kDa subunit binds and stabilizes the catalytic subunit at 37 degrees Celsius and keeps it in circulation. Under some circumstances it may be an allosteric modifier of the catalytic subunit.

18 (1739)	EST N_MOUSE	P23953	Liver carboxylesterase N [Precursor] EC 3.1.1.1 PES-N Lung surfactant convertase	146.0 2.0 73.1	AISESGVYIHFTNVGKK APEELAEK DAGFSTYMYEFR DAGFSTYMYEFR + OXIM EILPLKISEDCLYLNIYSPADLTK FAPPQPAEPWSFVK FVVANIFAR IGEDCLYLNIYSPADLTK MPEETASLLLR MPEETASLLLR + OXIM MPEETASLLLRR MPEETASLLLRR + OXIM MPVMPVVANIFAR + OXIM NGNPNGEGLPHWPEYDEKEGYLQIGATTQQAQR QKTESELLEISGK SLRDAGFSTYMYEFR SLRDAGFSTYMYEFR + OXIM VLGKYISLEGFEQPVAVFLGFPFAKPPLGSLR YISLEGFEQPVAVFLGFPFAKPPLGSLR YRPSFVSDK	Es1	Endo-plasmatic reticulum	Involved in the detoxification of xenobiotics and in the activation of ester and amide prodrugs. Involved in the extracellular metabolism of lung surfactant.
19 (2714)	FETUA_MOUSE	P29699	Alpha-2-HS-glycoprotein [Precursor] Fetuin-A Countertrypin	133.0 1.0 39.4	AHLMHNLGGEEVSVACK AHLMHNLGGEEVSVACK + OXIM AQFVFLPVSTLVEFVIAATDCTAK AQFVFLPVSTLVEFVIAATDCTAKEVTDPAK GLSDHRTVHDLR HAFSPVASVESASGETLHSPK QLTEHAVEGDCDFHILK QLTEHAVEGDCDFHILKQDGQFR VGQPQAAGPVSPMCPGR VGQPQAAGPVSPMCPGR + OXIM	Ahsg Fetua	Secreted	Probably involved in differentiation.
23 (3737)	FETUB_MOUSE	Q9QXC1	Fetuin-B [Precursor] IRL685	114.0 1.0 74.5	AMFHNIKPR AMFHNIKPR + OXIM AMFHNIKPRR + OXIM DGYMLSLNR + OXIM DQKDGYMLSLNR + OXIM HVPLQPVEK LVVLPPPGKEQR MFYESVYGQCK + OXIM SAECPGPEKENNPLVLPP SQASCSLQMGDSEPVGECQGSTVQSSLR	Fetub	Secreted	
21 (7632)	GCB_MOUSE	P31866	Ig gamma-2B chain C region secreted form	116.0 1.2 42.0	APQVYLPPPAEQLSR APQVYLPPPAEQLSRK DLPSPIER GLVRAPQVYLPPPAEQLSR HEGLKNYYLK LEPSGPRSTIPPCPPCK LEPSGPRSTIPPCPPCKECHK TDSPSCNVFR TDSPSCNVFRHEGLK TTPPSVYPLAPGCGDTTGSSVTLGCLVK VNHKDLPSPIER WEKTDSPSCNVFR		Secreted	
22 (4825)	GELS_MOUSE	P13020	Gelsolin [Precursor] Actin-depolymerizing factor ADF Brevin	236.0 1.9 91.5	AGKEPGLQIWR AQERAPQSR DGDQTAPASIR EPGLQIWR EVQGFESSTFSGYFK GGVASGFKHVVPNEVVVQR HVVPNEVVVQR QTQVSVLPEGGETPLFK RTPITVFR SEDCFILDHGR SEDCFILDHGRDGK SGALNSNDAFVLK SQHVQVEEGEPDAFWEALGGK TPSAAYLWVGAGAEEADK VSEARPSTMVVEHPEFLK VSEARPSTMVVEHPEFLK + OXIM VSNGAGSMSVSLVADENPFAQGALR + OXIM YIETDPANR YIETDPANRDR	Gsn Gsb	Secreted (Isoform 1) Cytoplasma (Isoform 2)	Calcium-regulated, actin-modulating protein that binds to the plus (or barbed) ends of actin monomers or filaments, preventing monomer exchange (end-blocking or capping). It can promote the assembly of monomers into filaments (nucleation) as well as sever filaments already formed.
23 (3139)	GPX3_MOUSE	P46412	Glutathione peroxidase 3 [Precursor] EC 1.11.1.9 GSHPx-3 GPx-3 Plasma glutathione peroxidase GSHPx-P	76.5 5 57.7	FLVGPDGIPVMR + OXIM MDILSVMRR + 2 OXIM NSCPPTAELGSGPGR TTVSNVKMDLSVMR + 2 OXIM YVRPGGGIFVPNFQLTEK	Gpx3	Secreted	Protects cells and enzymes from oxidative damage, by catalyzing the reduction of hydrogen peroxide, lipid peroxides and organic hydroperoxide, by glutathione.

24 (2533)	HA13_MOUSE	P01898	H-2 class I histocompatibility antigen, Q13 alpha chain [Precursor]	273.0 1.9 93.6	AKGHEQSFHVSLR APWMEQEGPEYWER + OXIM APWMEQEGPEYWERETQR + OXIM ATLEAECYBVGLR FDSDAETPRMEPR + OXIM FIVGYVDDTQFVR FIVGYVDDTQFVRFDSDAETPR GHEQSFHVSLR GYLQYAYDGR GYLQYAYDGRDYIALNEDLK KWEQAGAAEYYR TDPPKTHVTHHPISDHEGDVTLR THVTHHPGSEGDVTLR TWTAADVAAIITR VDSDGRFLR WASVVVPLGK WEQAGAAEYYR YRTSVSRPGLGEPR YLELGKETLLR	H2-Q13	Membrane	Involved in the presentation of foreign antigens to the immune system.
25 (7311)	HBA_MOUSE	P01942	Hemoglobin subunit alpha Hemoglobin alpha chain Alpha-globin	116.0 1.0 76.0	AAWGKVGGHGAEYGAEALER IGGHGAEYGAEALER IGGHGAEYGAEALERMFASFPTTK IGGHGAEYGAEALERMFASFPTTK + OXIM LRVDPVNFK MFASFPTTK MFASFPTTK + OXIM MFASFPTTKTYFPHFDVSHGSAQVK + OXIM TYFPHFDVSHGSAQVK TYFPHFDVSHGSAQVKGHGK	Hba Hba-a1	Extracellular	Involved in oxygen transport from the lung to the various peripheral tissues.
26 (2722)	HEMO_MOUSE	Q91X72	Hemopexin [Precursor]	225.0 2.2 76.7	CSPDPGLTALLSDHR DYFVSCPGR DYFVSCPGRGHGRPR ELGSPDGLETDQAAFSCPGSSR FNPVTGEVPPR FNPVTGEVPPRYPLDAR GATYAFTGDHYWR GECQSEGVLFFQGNRK GGNNLVSGYPK GPDSYFLRLDLK KVFWDFATR LFQEEPGIPYPPDAAVECHR LWWLDLK LYVSSGRR RLVWLDLK SGAQATWTEVSWPHEKVDGALCLDK SLPQPQKVFISILGCSQ VWVYPPEVK VFFWDFATR WKNPTSVDAAFR YYCFQGNK YYCFQGNKFLR	Hpx Hpxn	Secreted	Binds heme and transports it to the liver for breakdown and iron recovery, after which the free hemopexin returns to the circulation.
27 (7614)	HVS1_MOUSE	P36333	Ig heavy chain V region AC38.205.12	56.3 4 17.4	ASGYTFTDYYMNWVK EVQLQQSGPELVKPGASVK SLEWIGDINPNNGGTSYNQK SLTSEDSAVYYCAR			
28 (7555)	IGHG1_MOUSE	P01868	Ig gamma-1 chain C region secreted form	79.8 6 42.7	APQVYTIPPKEQMAK + OXIM CPVTSAAPAPEK SNWEAGNTFTCSVLHEGLHNHHTEK SVSELPIMHQDWLHGKEFK + OXIM TTPPSVYPLAPGSAAQTNSMVTLGCLVK + OXIM VNSAAPAPEK		Secreted	
29 (5239)	KAC_MOUSE	P01837	Ig kappa chain C region	91.6 6 29.5	DSTYSMSSTLTLTKDEYER + OXIM HNSYTCEATHK HNSYTCEATHKTSTSPIVK QHQVLTSWTDQDSK SFNRNEC WKIDGSER			

33 (2717)	KNG1_MOUSE	O38677	Kininogen-1 [Precursor]	97.9 17 88.6	ATSQVVAGTKYVIEFIAR AVFPCGCVNAVSTDSPDLEP/LK CQALDMTEMAR CQALDMTEMAR + 2 OxPM CQALDMTEMAR + OxPM DAEEAATGECTATVGK EHEFFIVTQTCK ESNTEAEDCEIK FPSLHGDCVALPHGDDGECR HLGQSLDCNANVYMRPVENIK + OxPM RPPGFSPFR SGNQYMLHR SGNQYMLHR + OxPM SVTYQETK TDGSPTFYSFK VIEGFKTDGSPTFYSFK YVIEFIAR	Kng1 Kng	Secreted Extracellular	(1) Kininogens are inhibitors of thiol proteases; (2) HMW-kininogen plays an important role in blood coagulation by helping to position optimally prekallikrein and factor XI next to factor XII; (3) HMW-kininogen inhibits the thrombin- and plasmin-induced aggregation of thrombocytes; (4) the active peptide bradykinin that is released from HMW-kininogen shows a variety of physiological effects: (4A) influence in smooth muscle contraction, (4B) induction of hypotension, (4C) natriuresis and diuresis, (4D) decrease in blood glucose level, (4E) it is a mediator of inflammation and causes (4E1) increase in vascular permeability, (4E2) stimulation of nociceptors (4E3) release of other mediators of inflammation (e.g. prostaglandins), (4F) it has a cardioprotective effect (directly via bradykinin action, indirectly via endothelium-derived relaxing factor action); (5) LMW-kininogen inhibits the aggregation of thrombocytes; (6) LMW-kininogen is in contrast to HMW-kininogen not involved in blood clotting.
31 (4339)	KV3A4_MOUSE	P33977	Ig kappa chain V-III region 53S13.1	85.7 7 65.7	DIVLTQSPASLAVSLGQR DIVLTQSPASLAVSLGQRATISCR EVPVTFGGGTKLEIK FSGSGSGTDFSLNIHPMEEDDTAMYFCQQSK FSGSGSGTDFSLNIHPMEEDDTAMYFCQQSK + 2 OxPM FSGSGSGTDFSLNIHPMEEDDTAMYFCQQSK + OxPM LLIYAASNQGSGVPAR			
32 (4333)	KV5K_MOUSE	P31644	Ig kappa chain V-V region HP R16.7	84.3 5 59.4	ASQDISNYLNWYQQKPDGTVK DIQMTQTTSSLSASLGDR + OxPM DIQMTQTTSSLSASLGDRVTISCR + OxPM LLIYYTSR TFGGGTKLEIK			Anti-arsonate hybridoma protein.
33 (5131)	KV5L_MOUSE	P31645	Ig kappa chain V-V region HP 93G7	81.3 8 33.6	ASQDISNYLNWYQQKPDGTVK DIQMTQTTSSLSASLGDR DIQMTQTTSSLSASLGDR + OxPM DIQMTQTTSSLSASLGDRVTISCR DIQMTQTTSSLSASLGDRVTISCR + OxPM LLIYYTSR LLIYTSRLHSGVPSR TFGGGTKLEIK			Anti-arsonate hybridoma protein.
34 (8231)	MBL1_MOUSE	P39339	Mannose-binding protein A [Precursor] MBP-A Mannan-binding protein Ra-reactive factor polysaccharide-binding component p28B polypeptide RaRF p28B	96.3 6 74.6	AIEEKLANIMEAEIR + OxPM LANIMEAEIR + OxPM NAEENKAIQEVATGIAPLGITDEATEGQFMYVTGGR + OxPM SLCTELQGTVAIPR SLCTELQGTVAIPRNAEENK VKSLCTELQGTVAIPR	Mbl1	Membrane	Binds mannose and N-acetylglucosamine in a calcium-dependent manner. Is capable of host defense against pathogens, by activating the classical complement pathway independently of the antibody.
35 (4738)	MUC_MOUSE	P31872	Ig mu chain C region secreted form	232.3 18 83.3	DGFSGPAPR DGFSGPAPRK DGKLVESGFTDPYTIENK EFVCTVTHR EQLNLRESATVTCLVK FSKPNEVHKHPAVYLLPPAR GFSPADISVQWLQR GVASVCVEDWNNRK GVASVCVEDWNNRK HPPAVYLLPPAR KEFVCTVTHR LICEATNTPKPTVSWLK LVESGFTDPYTIENK NLVAMGCLAR NLVAMGCLAR + OxPM SLEGSDEVLVCK TGGKVLATSQVLLSPK YLATSQVLLSPK	Igh-6	Secreted	
36 (1117)	MUP8_MOUSE	P24938	Major urinary proteins 11 and 8 [Fragment] MUP11 and MUP8	91.7 7 21.5	AGEYSVTYDGFNTFTIPK EKIEDNGNIFR ENIDLSNANR FAQLCEEHGILR FAQLCEEHGILRENIDLSNANR INGEWHTILASDKR LFLEQNYLEHSLVLK	Mup8 Mup11	Secreted	Binds pheromones that are released from drying urine of males. These pheromones affect the sexual behavior of females.

37 (5827)	PLMN_MOUSE	P22918	Plasminogen [Precursor] EC 3.4.21.7	456.3 39 88.4	AGLEKNYCR CEGETDFVCR CGNVAAAFPNR + OxPM CTTPPPPSPTYQCCL DVILEKR EQDCVIMAENSK FVDWER FVDWEREMR + OxPM GEIVPGSTVSVTVSGK GKTAVTAAGTPCQGWAAQEPHR HSFTPQTNPR KCQSVAAAMFPHR + OxPM LILEPNNR LILEPNNRDALLK LKEAQLPVEPK LSRPATFDK LYDYCDPLCASASSFECGKPQVEPKK MRDVILFEK + OxPM NLEENYCR NPDGDPWGPWCYTTNPR RVYLSECK SFQYHSK SFQYHSKEQQCVIMAENSK + OxPM SSRPEFYK TAVTAAGTPCQGWAAQEPHR TGKDIQDYRGTMSR + OxPM TICYIFQGWGETQGTFGAGR TPENFPCK TPENFPDAGLEMNYCR + OxPM VEYLNNR VILGAHEEYIR VIPACLPSPNYMVADR + OxPM VSRFVDWIER VVGGCVANPHSWPWQISLR YYLSECKTGKGNKGYR WEYCDIPR WEYCNLKR WSEQTPHR WSEQTPHRHNR	Plg	Secreted	Plasmin dissolves the fibrin of blood clots and acts as a proteolytic factor in a variety of other processes including embryonic development, tissue remodeling, tumor invasion, and inflammation; in ovulation it weakens the walls of the Graafian follicle. It activates the urokinase-type plasminogen activator, collagenases and several complement zymogens, such as C1 and C5. It cleaves fibrin, fibronectin, thrombospondin, laminin and von Willebrand factor. Its role in tissue remodeling and tumor invasion may be modulated by CSPG4.
38 (5215)	RETBP_MOUSE	Q03724	Plasma retinol-binding protein [Precursor] PRBP RBP	182.3 13 66.5	ARFSGLWYAIAK DPINGLSETFR FSGLWYAIAK LQNLDGTCADSYSFVFSR LQNLDGTCADSYSFVFSRDPNGLSPETR MKYWGVASFLQR MKYWGVASFLQR + OxPM QEELCLER QRQEELCLER QYRWEHNGYCQSRPSR WEHNGYCQSRPSR WEHNGYCQSRPSRNSL YWGVASFLQR	Rbp4	Secreted	Delivers retinol from the liver stores to the peripheral tissues. In plasma, the RBP-retinol complex interacts with transthyretin, this prevents its loss by filtration through the kidney glomeruli.
39 (5334)	S6A11_MOUSE	P31653	Sodium- and chloride-dependent GABA transporter 4 GAT4	59.2 5 67.2	AVHERGHWVNIK FPVLCVK LGASPRTVTVNIDCEAK VKQDGTSAITEK VLASDGIEHIGNLR	Gabt4 Gat4 Gat-4	Membrane	Terminates the action of GABA by its high affinity sodium-dependent reuptake into presynaptic terminals. Can also transport beta-alanine and taurine.
40 (8129)	SAA4_MOUSE	P31532	Serum amyloid A-4 protein [Precursor] Amyloid A-S protein	137.3 8 48.3	AVRDHLEAVNQNADQYFYAR EAVQGFWDDLWR GRYEAQQR KVFQGLLNR NHGLETLQATQK NHGLETLQATQKAEEWGR NPIHFRPEGLPEKF YFQGLLNR	Saa4 Saa5	Secreted	Major acute phase reactant. Apolipoprotein of the HDL complex.

41 (7722)	TRFE_MOUSE	Q92111	Serotransferrin [Precursor] Transferrin Siderophilin Beta-1-metal-binding globulin	473.0 42 95.8	A\SSFFSQSCV/PCADP/AFPK CAPHNKEEYNGVTGAFR CPVKLPEGTTPEK CLVDKGDVAFVK DFASCHLAQAPNHVVVSR DLFKDSAFGLLR DLFRDDTK DQYELLCLDNTR DSAFGLLR GTDFQLNQLEGK GTDFQLNQLEGKK GVYAVAVVVK HQFVLDNTEGKNPAEWAK IPSHAVVAR KPVDQYEDCYLAR KPVKDFASCHLAQAPNHVVVSR KSCHTGLGR KSCHTGVDR KTSVPDCIK LCQLCPGCGCSSTQPFFGYVGAFK LLEACTFHKH LPEGTTPEK LVLGHNYVTAIR NILQEDFELLCPDGTR NQQEGYCPEGSIDNSP/K SAGWVIPIGLLFCK SCHTGLGR SCHTGVDR SCHTGVDRTAGWNIPMGMLYNR + OXPM SKDFQLFSSPLGK TAGWNIPMGMLYNR TAGWNIPMGMLYNR + OXPM TSVPDCIK TVLPPDGPR VAQEHFGK VKAVLTSQETLFGGSDCTGNFCLFK VPPRMDYR + OXPM VVCALSHLER VVCAVSEHEATK YLGAEYMQSVGNMR YLGAEYMQSVGNMR + OXPM YLGAEYMQSVGNMRK + OXPM	Tf Trf	Secreted	Transferrins are iron binding transport proteins which can bind two atoms of ferric iron in association with the binding of an anion, usually bicarbonate. It is responsible for the transport of iron from sites of absorption and heme degradation to those of storage and utilization. Serum transferrin may also have a further role in stimulating cell proliferation.
42 (5131)	TTHY_MOUSE	P07309	Transthyretin [Precursor] Prealbumin	136.0 13 63.5	FVEGVYR FVEGVYRVELDTK GSPAVDVAVK HVTIAALSPYSYSTTAVVSNPQN KTSEQSWEPFASGK TAESGELHGLTTDEK TAESGELHGLTTDEKFVEGVYR TLGIGPFHEFADVVFTANDSGHR TSEGSWEPFASGK VLDAVRGSPAVDVAVK	Ttr	Secreted	Thyroid hormone-binding protein. Probably transports thyroxine from the bloodstream to the brain.
43 (3736)	VTDB_MOUSE	P21614	Vitamin D-binding protein [Precursor] DBP Group-specific component Gc-globulin VDB	239.0 24 86.7	CCESTSEDCMASELPEHTIK + OXPM DLCQGSTTQAMDQVTPELSR DLCQGSTTQAMDQVTPELSR + OXPM ECCDTQDSVACFSTQSPLLKR EVVSLTECCAEGADPTCYDTR FSSSTFEQVTNQLVK HLSLLTTMSNR HLSLLTTMSNR + OXPM KFSSTFEQVTNQLVK LAQKVPTADLEDVLPLAEDITEILSR LQMVKHLSLLTTMSNR + 2 OXPM LQMVKHLSLLTTMSNR + OXPM RTQVPEVFLSK SCESDAPFPVHPGTPECCTK SCESDAPFPVHPGTPECCTKEGLER SLSLLVSR SLSLLYSRK SRLSHLIK TQVPEVFLSK VCNELAMLGKEDFR VCNELAMLGKEDFR + OXPM VCSQYAAYGK VCSQYAAYGKEK VLEPTLKTLR	Gc	Secreted	Multifunctional protein found in plasma, ascitic fluid, cerebrospinal fluid, and urine and on the surface of many cell types. In plasma, it carries the vitamin D sterols and prevents polymerization of actin by binding its monomers. DBP associates with membrane-bound immunoglobulin on the surface of B-lymphocytes and with IgG Fc receptor on the membranes of T-lymphocytes.
44 (1714)	VTNC_MOUSE	P29788	Vitronectin [Precursor] Serum-spreading factor S-protein	85.0 13 52.1	EPQFISR FEGVFLDPDYPR GQYCVELDETAVRPGYPK IVVTGSLSHSAQAK LIQDVWGIEGPIDAAFTR NGSLEAFR NWHGVPGKVDAAMAGR NWHGVPGKVDAAMAGR + OXPM SIDGAREPQFISR TVLFKGSQVWR	Vtn	Secreted Extracellular	Vitronectin is a cell adhesion and spreading factor found in serum and tissues. Vitronectin interact with glycosaminoglycans and proteoglycans is recognized by certain members of the integrin family and serves as a cell-to-substrate adhesion molecule. Inhibitor of the membrane-damaging effect of the terminal cytolytic complement pathway.
45 (2535)	ZA2G_MOUSE	Q64726	Zinc-alpha-2-glycoprotein [Precursor] Zn-alpha-2-glycoprotein Zn-alpha-2-GP	93.7 11 60.3	AREEIFLVTLK AVLEECPECPEMLKLR AVLEEECPEMLKLR + OXPM CLAYGFYPQR DTTGSHTFQQMFGCEITNNIR + OXPM GFSQSLSYQWDR IDPPTVTITSR ISLPNVNK KLAFEPER SHLDRIDPPTVTITSR VIPDGRNFK	Azgp1	Secreted	Stimulates lipid degradation in adipocytes and causes the extensive fat losses associated with some advanced cancers
46 (2844)	EGFR_MOUSE	Q01279	Epidermal growth factor receptor	186.0 20 93.9	ACGPDYYEVEEDGIRK ACGPDYYEVEEDGIRK AVNHVCNPLCSSEGCWGPEPR CNLEGEPR GPDNCIQCAHYIDGPHCVK GRSPSDCCHNQCAAGCTGPR IKKAQDCSHR IPLENLQIR NLCYANTINWK NLQELGSAVR NVDLSFLK NYVVTDHGSCVR SLKEDDGDYISGNR SPSDCCHNQCAAGCTGPR TGLRELPMR TPPLDPRELEILK VCTAISGDLHILPVAFK VCTAISGDLHILPVAFKGDSFTR YSFGATCVK YSFGATCVKK	Egfr	Membrane	The EGF receptor mediates the biological signal of EGF, and also of TGF-alpha, amphiregulin, heparin-binding EGF, CRP39 and vaccinia virus growth factor.
47 (7636)	GCBM_MOUSE	P01867	Ig gamma-2B chain C region, membrane-bound form	109.0 11 57.8	APQVYILPPPAEQLSR CPAPNLEGGPSVFIFPPNIKDVLMISLTPK + OXPM HEDLKNYVLK LEPSGPRSTIIPCPPCK LEPSGPRSTIIPCPPCKECHK TDSFSCNVR TDSFSCNVRHEGLK TSRSPGLDLDDICAEAK TTPPSVYPLAPGCGDTTGSSVTLGCLVK VNHKDLPSPIER VNEKTDSFSCNVR		Membrane (Isoform 1) Secreted (Isoform 2)	
48 (1237)	IGJ_MOUSE	P01592	Ig J chain	88.7 6 62.6	CVTTMVPLR + OXPM CVTTMVPLRYHGETK + OXPM IPSTEDPIEDVERR IPSTEDPIEDVERNIR NPVHKLSDVCK NPVYHLSDVCKK	Igj		Serves to link two monomer units of either IgM or IgA. In the case of IgM, the J chain-joined dimer is a nucleating unit for the IgM pentamer, and in the case of IgA it induces larger polymers. It also help to bind these immunoglobulins to secretory component.
49 (2188)	KV3A3_MOUSE	P01656	Ig kappa chain V-III region MOPC 73	73.5 5 61.6	DIVLTQSPASLAVSLGQR DIVLTQSPASLAVSLGQRATISCR EVPWTFGGGTKLEIK FSGSGSDTDFSLNIHPMEEDDTAMYFCQQSK + 2 OXPM LLIYAASNQGSGVPAR			Bence-Jones protein
50 (7733)	PROP_MOUSE	P11683	Properdin	92.2 9 73.4	CGGHCPGEAQQSQACDTQK HGGPFCAGDATR LVVEEKR MSINCEGTPQQQSR MSINCEGTPQQQSR + OXPM QRLCTPLVPK QRYCDNPAPK SCSAPAPSHQPPGKPCSGPAYEHK TCDHPAPR	Cfp Pfc	Secreted	A positive regulator of the alternate pathway of complement. It binds to and stabilizes the C3- and C5-convertase enzyme complexes