PROTEOMIC COMPARISON BETWEEN MRP4 KNOCKOUT AND WILD TYPE MOUSE BRAIN, LIVER, KIDNEY AND SERUM

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PROTEOMIC COMPARISON BETWEEN MRP4 KNOCKOUT AND WILD TYPE MOUSE BRAIN, LIVER, KIDNEY AND SERUM

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ABSTRACT

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Multidrug resistance protein 4 (MRP4) is a transmembrane efflux protein capable of substrate-specific transport of endogenous and xenobiotic molecules across the cell membrane, including several drugs used in disease and cancer treatment. Changes in expression of MRP4 affect bioavailability and efficacy of treatment drugs. Expression changes also affect intracellular and extracellular levels of substrate molecules that participate in secondary messenger pathways (e.g. cAMP). The *MRP4 (ABCC4)* gene is highly polymorphic in the human population, and polymorphisms alter the function and expression of the protein. The aim of this study was to conduct a large-scale proteomic

analysis of *Mrp4* deficient mice to test if levels of other proteins and small molecules are altered in the absence of MRP4. This study focuses on protein expression in liver, kidney, brain and serum by utilizing proprietary multi-analyte profiling platforms at MyriadRBM (Austin, TX). Eight analytes in kidney, two in serum and one in liver were found to be significantly different in six-month old C57BL/6-*Mrp4* knockout mice. The changes are suggestive of tissue repair and inflammation in the kidney. These data suggest potential adverse effects due to the absence of MRP4.

I. INTRODUCTION

Multidrug Resistance Protein 4 (MRP4)

Multidrug resistance protein 4 (MRP4/ABCC4) is 1 of 12 members within the C subfamily of the ATP-binding cassette (ABC) transporter superfamily. Since genetic nomenclature varies from species to species, multidrug resistance protein 4 will be referred to henceforth using the conventions applied to human genes, *MRP4,* and proteins, MRP4. This transmembrane organic anion efflux transporter uses energy derived from ATP hydrolysis to transport endogenous and xenobiotic molecules as well as some of their metabolites up their concentration gradients [1].

Over the years, a number of substrates have been identified for MRP4, each with a different affinity and kinetic parameters compared to other members in the ABCC family [2]. MRP4 has been found to transport cyclic nucleotides, cAMP and cGMP, which are best known for their function as secondary, or intracellular, messengers [3]. Other physiological substrates of MRP4 include conjugated steroids, prostanoids, bile acids, folates, purine analogs, and proinflammatory mediators [3-10]. Transport of some substrates may be regulated by conjugation with glutathione (GSH), sulfates, and glucuronide as well as co-transport of GSH, particularly in the case of bile acids [8, 10-11]. MRP4's ability to transport molecules out of the cell extends to antiviral, antibiotic, cardiovascular, and cytotoxic drugs and some of their metabolites. Among antiviral drugs MRP4 has been found to transport nucleoside monophosphate analogs adefovir,

tenofovir, and glanciclovir [3, 5, 12-16] and antibiotics, including the cephalosporins ceftizoxime, cefazolin, cefotaxime, and cefmetazole [17-18]. Cardiovascular drug substrates include furosemide and thiazide as well as the angiotensin II receptor antagonist omesartan [19, 20]. Cytotoxic anticancer and chemotherapy drug substrates include methotrexate, topotecan, leucovorin, 6-mercaptopurine, and 6-thioguanine [3- 5, 14, 21-22]. Anti-retroviral drugs azidothymidine monophosphate (AZT), 9-(2-phosphonylmethoxyethyl)adenine (PMEA), and lamivudine are also substrates for MRP4 [5, 16, 23]. As the search for new substrates expands and new drugs are created, this list of substrates is bound to increase.

MRP4 expression and localization has been determined in mouse, rat, and human tissue. Within human tissue, MRP4 is found in all normal tissue at low levels and has been localized to the kidney, liver, erythrocytes, ovaries, testes, lung, bladder, intestine, adrenal gland, platelets, hematopoietic stem cells, macrophages, brain, and pancreas [3-4, 6-7, 10, 14, 21-22, 24-34]. Highest mRNA levels are found in the prostate [33]. In mice, tissue levels of mRNA have been determined to be highest in kidney while MRP4 is also expressed in liver, lung, stomach, intestine, brain, gonads, and placenta [35]. In rat, mRNA is also found highest in the kidney with expression in lung, liver, cerebral cortex, cerebellum, prostate, intestine, and stomach also observed [36].

MRP4: Implications of Drug Resistance and Efficacy

Upon entering the body, a drug has multiple barriers to face in order to provide its therapeutic effects. MRP4 has been localized to multiple organs that may prevent certain drugs from entering target tissue or may enhance their elimination. Within the liver, MRP4 is found on the basolateral membrane of hepatocytes, enabling excretion of

chemicals toward blood for elimination by the kidney [6]. In the kidney, MRP4 is localized to the apical membrane of renal epithelial cells [22]. This cellular location allows the export of drugs from the kidney to urine. In the intestine, a major barrier to drug absorption and bioavailability, MRP4 has been found on the apical membrane of enterocytes, which may restrict the absorption of drugs into the bloodstream [31, 34]. At the blood-brain barrier, MRP4 is found at the apical membrane of brain capillary endothelial cells, where its activity may inhibit drug absorption into the central nervous system [21, 29, 37]. It has also been localized to the basolateral membrane of the choroid plexus epithelium, which allows transport of substrates from cerebral spinal fluid toward blood capillaries [21]. Within the prostate, MRP4 is localized to the basolateral membrane of prostate tubuloacinar cells [14].

Researchers have found evidence that MRP4 over-expression can lead to lower net absorption of drugs by cells. The export of certain drugs and their metabolites decreases the efficacy of these drugs in tissues that may be the intended target of the drug and can lead to increased elimination from the systemic circulation. Studies have found that a human T-lymphoid cell line (CEMr-1) and human erythroleukemia cell line (K562) over-express MRP4. These cells are capable of the increased efflux of PMEA used in antiviral treatment of HIV, herpes virus, and hepatitis B virus [12, 16].

Using the MRP4 over-expressing cell line CEMr-1, researchers found that accumulation of ganciclovir was reduced [12]. MRP4 has been found to be induced in macrophages following AZT treatment [38]. Observations of reduced absorption of anti-cancer drugs have also been made in other MRP4 over-expressing cell lines and MRP4 transfected cells [3-4, 11, 14, 21, 39]. Topotecan, an anticancer drug, and adefovir, an

antiretroviral drug, have been found at higher concentrations in brain tissue and cerebral spinal fluid of MRP4 knockout mice [21]. Knockout mice have similarly shown reduced excretion of diuretics, antiviral drugs, and cephalosporins in urine, which can be attributed to the lack of MRP4 for eliminating these drugs by way of the renal proximal tubules [13, 17, 19].

MRP4 Polymorphism

Single nucleotide polymorphisms (SNP) within a gene's coding region can sometimes result in changes that disrupt the protein's function. *MRP4* has been found previously to be highly polymorphic with many SNPs within the coding region of the gene. *MRP4* exhibits high polymorphism compared to other members of the *ABCC* family [40]. In the coding region, 22 SNPs are non-synonymous with two found to decrease function of MRP4. These SNPs are prevalent and highly conserved in the population [41]. Other researchers have found that different variants may either increase or decrease MRP4's ability to transport substrates [42, 43]. Some variants have been uncovered that have been linked to adverse drug reactions and poor prognosis in patients with esophageal adenocarcinoma [43-46]. Human polymorphisms may account for varying efficacies of treatment among patients. Exploring the proteomic effect of *MRP4* knockout mice may help elucidate the underlying causes of poor patient prognosis among patients with variants in *MRP4* with reduced or elevated function.

MRP4 Knockout Phenotype Studies

Knockout mice have been developed to help uncover the physiological roles of particular proteins as well as create models of human disease and pathogenesis. By removing a particular gene from the genome of an organism, one can elucidate the

function of the gene as well as determine the beneficial or detrimental effects the gene knockout has on other small molecules and proteins. MRP4 knockout mice and transgenic cell lines lacking MRP4 have been helpful in identifying substrates exported by the transmembrane protein and the effects that increasing or decreasing those substrates intracellularly or extracellularly as a result of the absence of MRP4 has on those organisms or cells. MRP4 knockout mice have been found to show no overt phenotypic difference from wild type mice. A mouse embryonic fibroblast transgenic cell line deficient in MRP4 has demonstrated increased intracellular cAMP levels, decreased extracellular cAMP levels, decreased protein kinase A (PKA) activity, decreased extracellular prostaglandin levels, as well as decreased expression of Cox-2 protein [47]. In vivo, many cAMP-, cAMP responsive element binding protein (CREB)-, or PKA-activated genes have been found to be expressed at reduced levels in the absence of MRP4 in Leydig cells of the testes. Additionally, MRP4 deficient testes show decreased testosterone concentrations [48]. Another study has indicated a lack of phenotype for MRP4 knockout mice at 3 months of age, but reported cardiac hypertrophy at 9 months. The authors attribute this phenomenon in cardiac myocytes to a compensatory increase in phosphodiesterase (PDE) production at 3 months that is lost at 9 months. Taken together, MRP4's role in cAMP homeostasis may be ameliorated by increased PDE activity in young mice due to the reduction of cAMP extrusion [49].

It has previously been shown that MRP4 and MRP4/5 knockout mice generate a sufficient immune response. Researchers discovered that MRP4 plays a role in dendritic cell migration for human immune response generation, but appears to not be important in

mice for eliciting an immune response, raising the possibility of different substrate specificity between the species [50-51].

Knockout mice of the ABC transporter protein family have also demonstrated adaptive regulation of other members of the MRPs in a compensatory role. MRP4 has been found to be up-regulated in MRP2 knockout mice kidneys [52-53]. MRP5 has exhibited induction following RNA interference of MRP4 in cultured Langerhans cells [50]. This compensation by other members due to shared substrates may facilitate a sufficient phenotypic response to prevent cytotoxicity and pathogenesis.

Proteomic Analysis

One way to evaluate the consequences of *MRP4* knockout on a wide variety of potential targets is through proteomic analysis. Proteomic analysis between knockout and wild type mice creates a vast array of information showing the changes resulting either directly or indirectly from gene-knockout. These differences can provide insight into functions previously unattributed to the gene of interest. Although microarrays have contributed to determining changes at the transcriptional level, not all changes in mRNA expression result in modifications of protein expression [54-57].

Many different techniques have been developed for proteomic analysis with varying sensitivity and specificity. Several limitations have been overcome with the use of multiplexed suspension arrays and sandwich techniques. By using a suspended bead based immunoassay, problems of antibody denaturation by the surface used to bind antibodies and high background binding can be prevented [58]. Using two different antibodies directed to different epitopes on the protein of interest, as in a sandwich technique, allows for higher specificity for the assay. Multiplexing antibodies with

suspended beads permits the detection of several different analytes within a single sample. One limitation to this technique is the amount of time and quality control necessary to evaluate cross reactivity between antibodies as the number of antibodies used increases.

To date, effects of *MRP4* knockout on protein expression has been directed toward a limited number of proteins per study. Microarray analysis has previously been conducted between *MRP4* knockout and wild type mouse liver and Leydig cells. The authors of the study focused primarily on steroid and cholesterol biosynthesis genes and cAMP-, PKA-, and CREB-regulated genes [48]. A large scale proteomic analysis has not been conducted comparing *MRP4* knockout mice to their wild type counterparts in different tissues.

II. STUDY AIM

This proteomic study aims to demonstrate expression of a wide variety of proteins which are either increased or decreased in the absence of MRP4. The data collected may provide insight into direct or indirect functions of MRP4 previously unidentified. This study focuses on protein expression of the liver, kidney, brain and serum. If changes in protein expression are discovered between wild type and knockout mice, the mechanism by which those changes are brought about could be investigated in future studies.

III. METHODS

Animals

MRP4 knockout mice were kindly provided by Dr. John Schuetz at St. Jude Research Hospital (Memphis, Tennessee). Generation of the specific knockout mice used in this study has been described elsewhere [21]. *MRP4* knockout mice were compared to age- and sex-matched mice of the same strain, C57B/6J, graciously provided by principal investigators at MD Anderson. Mice were housed in an Association for Assessment and Accreditation of Laboratory Animal Care accredited facility at MD Anderson Cancer Center, Science Park Research Division (Smithville, Texas) in a temperature- and humidity-controlled environment. Commercial rodent pelleted food and water were available ad libitum. All procedures were in compliance with the Public Health Service Guide for the Care and Use of Laboratory Animals. Mice arrived at MD Anderson and were acclimated for at least one month prior to tissue collection. Tissue collection from post mortem mice followed eye collection in accordance with the Institutional Animal Care and Use Committee (IACUC) approved protocol for a separate study; the IACUC protocol approval number for that study is 0913_1001_22.

Tissue Homogenization

Brain, liver and kidney from two male and four female knockout and wild type mice (total of twelve mice) were removed and snap-frozen immediately in liquid nitrogen prior to being stored at -80°C. Tissue homogenates were prepared using an IKA Ultra-

Turrax T8 variable speed homogenizer (Fisher Scientific) in lysis buffer containing 50 mM Tri-HCl and 2 mM EDTA, pH 7.4. Halt Protease Inhibitor Single-Use Cocktail (Thermo Scientific) was added shortly prior to tissue homogenization. Halt Protease Inhibitor contained 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (AEBSF; 1 mM), aprotinin (800 nM), bestatin (50 µM), (1S,2S)-2-(((S)-1-((4-guanidinobutyl)amino)-4-methyl-1-oxopentan-2-yl) carbamoyl)cyclopropanecarboxylic acid (E64; 15 µM), leupeptin (20 µM) and pepstatin A (10 µM) at final concentration. Tissue samples were added to 9X lysis buffer and protease inhibitor was added prior to homogenization. Homogenized samples were then centrifuged for five minutes at 3700x*g* in an Allegra X-15R centrifuge (Beckman Coulter). Supernatant was collected and aliquoted into vials which were subsequently stored at -80°C. Samples were centrifuged at 3700x*g* for five minutes prior to analysis.

Proteomic Analysis

This study utilized the proprietary multi-analyte profiling (MAP) platforms in a Clinical Laboratory Improved Amendments (CLIA)-certified laboratory at MyriadRBM (Austin, TX). The platforms on which the samples were tested were the RodentMAP version 3.0, Rat KidneyMAP version 1.0, Rodent MetabolicMAP version 1.0, collectively known as Rodent Discovery, and Human DiscoveryMAP 250+ version 2.0. All samples were stored at -80°C until tested. The samples were thawed at room temperature, vortexed, centrifuged at 3700x*g* for five minutes for clarification, and supernatant was removed for MAP analysis into a master microtiter plate. Using automated pipetting, an aliquot of each sample was introduced into one of the capture microsphere multiplexes of the CustomMAP. The mixture of sample, blocker and capture

microspheres were incubated without agitation at room temperature for one hour. Multiplexed cocktails of biotinylated, reporter antibodies for each multiplex were then added robotically and after thorough mixing, were incubated for an additional hour at room temperature. Multiplexes were developed using an excess of streptavidin-phycoerythrin solution which was thoroughly mixed into each multiplex and incubated for one hour at room temperature. The volume of each multiplexed reaction was reduced by vacuum filtration and then increased by dilution into matrix buffer for analysis. Analysis was performed in Luminex 100 or 200 instruments, and the resulting data stream was interpreted using proprietary data analysis software developed at MyriadRBM. For each multiplex, both calibrators and positive controls were included on each microtiter plate. The eight-point calibrators were run in the first and last column of each plate and assay performance controls were included in duplicate. Testing results were determined first for the high, medium and low controls for each multiplex to ensure proper assay performance. Unknown values for each of the analytes localized in a specific multiplex were determined using four- and five-parameter, weighted and non-weighted curve fitting algorithms included in the data analysis package.

The least detectable dose (LDD) for each analyte is determined as the mean ± three standard deviations of twenty readings of assay blocker only. The lower limit of quantitation (LLOQ) is determined as the concentration of an analyte at which the coefficient of variation of replicate standard samples is 30%.

Protein Sequence Alignment Analysis

Human and homologous mouse protein sequences were identified with the use of the online UniProt Knowledgebase (http://www.uniprot.org) [59]. A search was

performed using the analyte name, and the human protein was selected. Alignment of the human and mouse protein amino acid sequences were performed using BLAST integrated into the UniProt knowledgebase. Information on the length of the two proteins was documented. The identity score, the percentage of exact amino acid matches in the alignment generated from the two proteins, and the positives score, the percent of amino acids that score positive based on the BLOSUM62 matrix algorithm, was documented with the positives score being used to evaluate conservation between the proteins.

Statistical Analysis

Data are presented as mean ± standard error of the mean. Analytes for which quantification data was obtained were analyzed using the R computational environment software (http://www.R-project.org) [60]. Results were analyzed using the Mann-Whitney test. Statistical significance was inferred at p values of < 0.01. A p value of 0.01 was chosen as a conservative approach to reduce the probability of making a type I error by falsely rejecting the null hypothesis. One wild type kidney sample was excluded from statistical analysis due to an error in over-diluting the sample in lysis buffer during tissue homogenization. Analytes reported as “low” were replaced by the LLOQ for that particular analyte as a conservative approach for statistical analysis of the data. Fold changes in analyte concentrations were calculated by taking the ratio of the MRP4 knockout mean value to the wild type mean value.

IV. RESULTS

Results from the Rodent Discovery Product

The wild type and MRP4 knockout mouse brain, liver and kidney samples were analyzed using the Rodent Discovery product. The product generated results from seventy-two analytes for quantitative analysis. Results for two analytes, beta-2-microglobulin and adiponectin, were reported as below the LLOQ for the assay in all tissues tested. This result indicates that the concentration for these analytes was too low to accurately report and suggests the analytes are either extremely low or not present in the tissues tested.

Based on statistical analysis, five analytes were found to be differentially expressed between the wild type and knockout samples (see Table 1). The kidney demonstrated the most differentially expressed analytes with four analytes showing statistically significant differences with a p value < 0.01. These analytes were monocyte chemotactic protein 1 (MCP-1) ($U = 0$, $p = 0.006$), myoglobin ($U = 29$, $p = 0.009$), vascular endothelial growth factor A (VEGF-A) ($U = 0$, $p = 0.004$) and testosterone ($U = 30$, $p = 0.006$). MCP-1 and VEGF-A concentrations were found to be increased in the MRP4 knockout mouse kidney while myoglobin and testosterone concentrations were decreased. In liver samples from knockout mice, cortisol was found to be statistically significantly higher relative to wild type controls ($U = 0$, $p = 0.002$). None of the analytes tested were found to be statistically different in the brain.

Table 1. Analytes Tested from Rodent Discovery Product with *p* Value < 0.01

Tissue	Analyte	Units	*MRP4* (+/+) Mean ± SE	*MRP4* (-/-) Mean ± SE	*p* Value	Increase / Decrease
Kidney	Monocyte Chemotactic Protein 1 (MCP-1)	pg/ml	2.8 ± 0.5	6.8 ± 0.5	0.006	Increase
Kidney	Myoglobin	ng/ml	18 ± 3	4 ± 2	0.009	Decrease
Kidney	Vascular Endothelial Growth Factor A (VEGF-A)	pg/ml	490 ± 30	640 ± 20	0.004	Increase
Kidney	Testosterone	ng/ml	31 ± 2	22 ± 1	0.006	Decrease
Liver	Cortisol	ng/ml	4.2 ± 0.5	7.8 ± 0.3	0.002	Increase

Serum samples from the wild type mice and *MRP4* knockout mice were analyzed using the Rodent Discovery product. The product provided data for seventy-nine different analytes for quantitative analysis. Values for calbindin, epidermal growth factor rat (EGF Rat), glutathione S-transferase alpha (GST-alpha) and kidney injury molecule-1 rat (KIM-1 Rat) were not reported due to insufficient sample volume. Results for nineteen analytes, glucagon, peptide YY (PYY), adiponectin, fibrinogen, fibroblast growth factor 9 (FGF-9), granulocyte-macrophage colony-stimulating factor (GM-CSF), insulin, interferon gamma (IFN-γ), interleukin-2 (IL-2), interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-10 (IL-10), interleukin-12 subunit p70 (IL-12p70), interleukin-17A (IL-17A), tumor necrosis factor alpha (TNF-alpha), glutathione S-transferase mu (GST-Mu), neutrophil gelatinase-associated lipocalin (NGAL) and tissue inhibitor of metalloproteinases 1 rat (TIMP-1 Rat), were reported as below the LLOQ for the assay for all samples. The antibody pair used in the assay for detection of TIMP-1 Rat was found to not cross-react with the mouse protein based on testing by the manufacturer

documented on the antibody specification sheet. Otherwise, the other analyte concentrations were too low to accurately detect using the multi-analyte bead assay specific for the analyte.

Following statistical analysis, two analytes were found to be differentially expressed between the wild type and knockout serum samples (see Table 2). These analytes are macrophage inflammatory protein-1 gamma (MIP-1 γ) ($U = 2$, $p = 0.009$) and vascular cell adhesion molecule-1 (VCAM-1) ($U = 2$, $p = 0.004$). The mean concentration of both of the analytes was found to be increased in the MRP4 knockout serum.

Table 2. Serum Analytes Tested from Rodent Discovery Product with *p* Value < 0.01.

Analyte	Units	*MRP4* (+/+) Mean ± SE	*MRP4* (-/-) Mean ± SE	*p* Value	Increase / Decrease
Macrophage Inflammatory Protein-1 gamma (MIP-1 γ)	ng/ml	12 ± 1	17 ± 1	0.009	Increase
Vascular Cell Adhesion Molecule-1 (VCAM-1)	ng/ml	1100 ± 100	1660 ± 50	0.004	Increase

Results from the Human Discovery Product

The Human Discovery product allows for data to be collected on two hundred and forty-three analytes; however, for this study, results for only one hundred and eighty-four analytes were obtained. The discrepancy is due to duplicated analytes previously obtained from the Rodent Discovery product and analytes dropped to conserve sample volume since certain MAPs were known to only cross-react with human proteins. Analysis of brain, liver and kidney tissue homogenate samples from the wild type and *MRP4* knockout mice indicated seven analytes were differentially expressed with a *p* value of <

0.01 (see Table 3). These analytes include cancer antigen 15-3 (CA-15-3) ($U = 29$, $p = 0.009$) in liver; epiregulin (EPR) ($U = 0$, $p = 0.006$) , hepatocyte growth factor (HGF) ($U = 0$, $p = 0.005$), interleukin-15 (IL-15) ($U = 0$, $p = 0.006$), matrix metalloproteinase-9 (MMP-9) ($U = 0$, $p = 0.009$), and tenascin-C (TN-C) ($U = 0.5$, $p = 0.008$) in kidney; and T lymphocyte-secreted protein I-309 (I-309) ($U = 35$, $p = 0.006$) in brain.

Table 3. Analytes Tested from Human Discovery Product with *p* Value < 0.01.

Tissue	Analyte	Units	*MRP4* (+/+) Mean ± SE	*MRP4* (-/-) Mean ± SE	*p* Value	Increase / Decrease
Brain	T Lymphocyte-Secreted Protein I-309 (I-309)	pg/ml	76 ± 7	45 ± 3	0.006	Decrease
Kidney	Epiregulin (EPR)	pg/ml	26 ± 1	32 ± 1	0.006	Increase
Kidney	Hepatocyte Growth Factor (HGF)	ng/ml	0.86 ± 0.08	1.23 ± 0.02	0.005	Increase
Kidney	Interleukin-15 (IL-15)	ng/ml	0.23 ± 0.01	0.34 ± 0.02	0.006	Increase
Kidney	Matrix Metalloproteinase-9 (MMP-9)	ng/ml	0.9 ± 0.1	1.23 ± 0.06	0.009	Increase
Kidney	Tenascin-C (TN-C)	ng/ml	16 ± 2	23 ± 1	0.008	Increase
Liver	Cancer Antigen 15-3 (CA-15-3)	U/ml	8 ± 4	0.7 ± 0.3	0.009	Decrease

Results for sixteen analytes, alpha-1-antichymotrypsin, apolipoprotein B, apolipoprotein E, cellular fibronectin, endoglin, eotaxin-2, fibroblast growth factor 4, glucose-6-phosphate isomerase, hepatocyte growth factor receptor, human epidermal growth factor receptor, immunoglobulin E, platelet-derived growth factor BB, prostate-specific antigen, tissue plasminogen activator, vascular endothelial growth factor receptor

1 and vascular endothelial growth factor receptor 2 were reported as below the LLOQ for the assay in all tissues tested. These analyte concentrations were too low to accurately detect using the multi-analyte bead assay specific for the analyte, suggesting little or no analyte was present in the specific tissue.

Since the inability to detect the aforementioned analytes reliably may also have been a result of the antibodies used in the Human Discovery product assay not recognizing the mouse protein, the cross-reactivity of the select antibody pairs used in the assay as well as protein conservation between the human and mouse protein was evaluated. Analysis was conducted on analytes in which differences were detected with a $p < 0.01$ or analytes with $p < 0.05$ that also had a fold-change greater than ±1.5. Fifteen analytes were reviewed for cross-reactivity: CA-15-3, EPR, HGF, IL-15, interleukin-17 (IL-17), matrix metalloproteinase-7 (MMP-7), MMP-9, nerve growth factor beta (NGF-beta), neuron-specific enolase (NSE), osteoprotegerin (OPG), superoxide dismutase 1 (SOD-1), T lymphocyte-secreted protein I-309 (I-309), TN-C, vascular endothelial growth factor C (VEGF-C) and vitronectin (see Table 4). The analyte assay was considered invalid for use with rodent samples based on the following criteria: (1) the specification sheet and/or manufacturer of either primary or reporter antibody indicated through testing that the antibody does not cross-react with the mouse protein; or (2) the protein alignment in BLAST provided a positives score of < 80% unless the specification sheet and/or manufacturer indicated through testing that the antibody does cross-react with the mouse protein.

Table 4. Alignment Comparison between Human and Mouse Protein Analytes Tested from Human Discovery Product. * indicates proteins that were significantly different ($p < 0.01$) between *MRP4* knockout and wild type mice. Light grey shading indicates analytes with < 80% positives score compiled from BLAST.

Protein	Amino Acid Length (Human)	Amino Acid Length (Mouse)	Identity Score	Positives Score
I-309 *	96	92	42%	61%
CA-15-3 *	1255	630	50%	62%
IL-17	155	158	62%	74%
TN-C *	2110	2110	69%	79%
MMP-7	267	264	70%	82%
MMP-9 *	707	730	72%	82%
Vitronectin	478	478	75%	82%
IL-15 *	162	162	72%	84%
EPR *	169	162	80%	87%
SOD-1	154	154	83%	88%
NGF-beta	241	241	85%	91%
OPG	401	401	85%	91%
VEGF-C	419	415	85%	92%
HGF *	728	728	90%	96%
NSE	434	434	98%	99%

I-309, CA-15-3 and IL-17 alignments generated low positives scores. IL-15 was detected by an antibody, the supplier of which indicated that the antibody did not react with mouse proteins. TN-C was detected by antibodies, the manufacturer of which provided specification sheets for both antibodies indicating that they do cross-react with the mouse protein. The cross-reactivity analysis excluded three of the analytes previously determined to be statistically significantly different. These analytes are CA-15-3, IL-15 and I-309. Therefore, only four analytes were verified as statistically significantly different using the Human Discovery product. These analytes are EPR, HGF, MMP-9 and TN-C in the kidney. The mean values for the *MRP4* knockout mice kidney concentration was found to be elevated for each of the analytes. Liver and brain samples

from the mice provided no verifiably statistically significantly different results among the analytes tested using this product.

Summary of Results

Collectively, out of two hundred and sixty-three analytes tested, eleven analytes were found at significantly different levels in the *MRP4* knockout mice, following exclusion of analytes for which the antibodies used in the multi-analyte assay were not considered specific for the mouse protein. Eight analytes were found to be differentially expressed in the kidney, one in the liver and two in the serum without any overlap of statistically significant analytes across tissues and serum (see Table 5).

Table 5. Analytes Tested from all Products with *p* Value < 0.01 following Exclusion of Assays Not Specific for Mouse Protein.

Specimen Tested	Analyte	Units	MRP4 (+/+) Mean ± SE	MRP4 (-/-) Mean ± SE	*p* Value	Increase / Decrease
Kidney	Monocyte Chemotactic Protein 1 (MCP-1)	pg/ml	2.8 ± 0.5	6.8 ± 0.5	0.006	Increase
Kidney	Myoglobin	ng/ml	18 ± 3	4 ± 2	0.009	Decrease
Kidney	Vascular Endothelial Growth Factor A (VEGF-A)	pg/ml	490 ± 30	640 ± 20	0.004	Increase
Kidney	Testosterone	ng/ml	31 ± 2	22 ± 1	0.006	Decrease
Kidney	Epiregulin (EPR)	pg/ml	26 ± 1	32 ± 2	0.006	Increase
Kidney	Hepatocyte Growth Factor (HGF)	ng/ml	0.86 ± 0.08	1.23 ± 0.02	0.005	Increase
Kidney	Matrix Metalloproteinase-9 (MMP-9)	ng/ml	0.9 ± 0.1	1.23 ± 0.06	0.009	Increase

Table 5 Continued

Specimen Tested	Analyte	Units	MRP4 (+/+) Mean ± SE	MRP4 (-/-) Mean ± SE	*p* Value	Increase / Decrease
Kidney	Tenascin-C (TN-C)	ng/ml	16 ± 2	23 ± 1	0.008	Increase
Serum	Macrophage Inflammatory Protein-1 gamma (MIP-1 γ)	ng/ml	12 ± 1	17 ± 1	0.009	Increase
Serum	Vascular Cell Adhesion Molecule-1 (VCAM-1)	ng/ml	1100 ± 100	1660 ± 50	0.004	Increase
Liver	Cortisol	ng/ml	4.2 ± 0.5	7.8 ± 0.3	0.002	Increase

Changes varied across analytes with seven analytes having elevated expression and two analytes having decreased expression in the *MRP4* knockout mice compared to wild type mice (see Figure 1 and Figure 2).

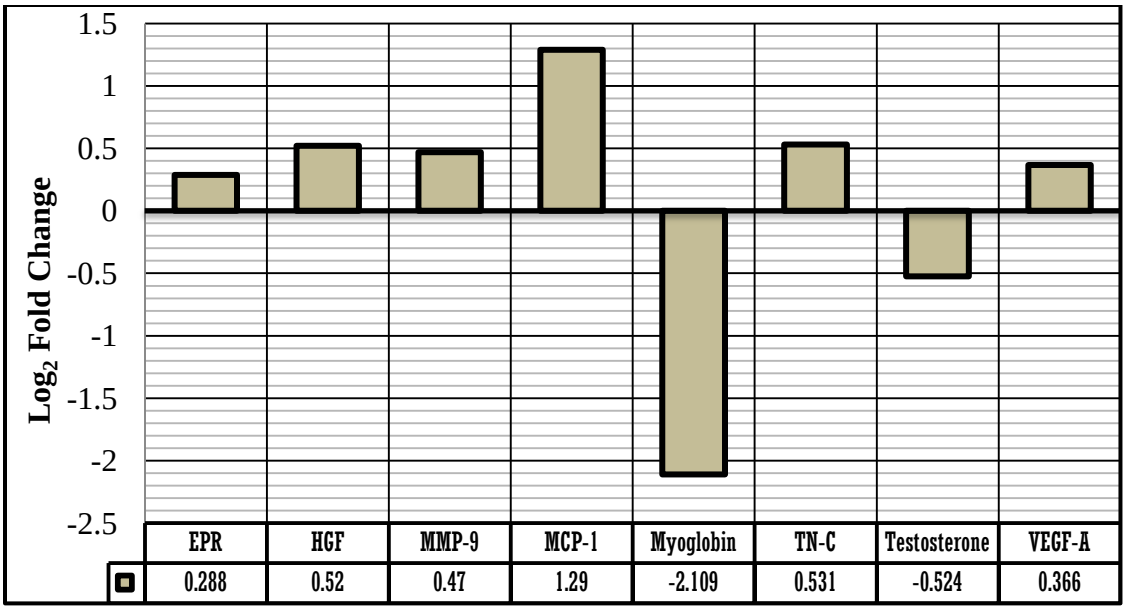


Figure 1. Log_2 Fold Change among Analytes with *p* Value < 0.01 in the Kidney. Fold changes in analyte concentrations were calculated by taking the ratio of the *MRP4* knockout mean value to the wild type mean value. The log base 2 of the fold-change is plotted above. A value of 1 indicates double while a value of -1 indicates half the mean protein concentration in the *MRP4* knockout relative to the wild type mice.

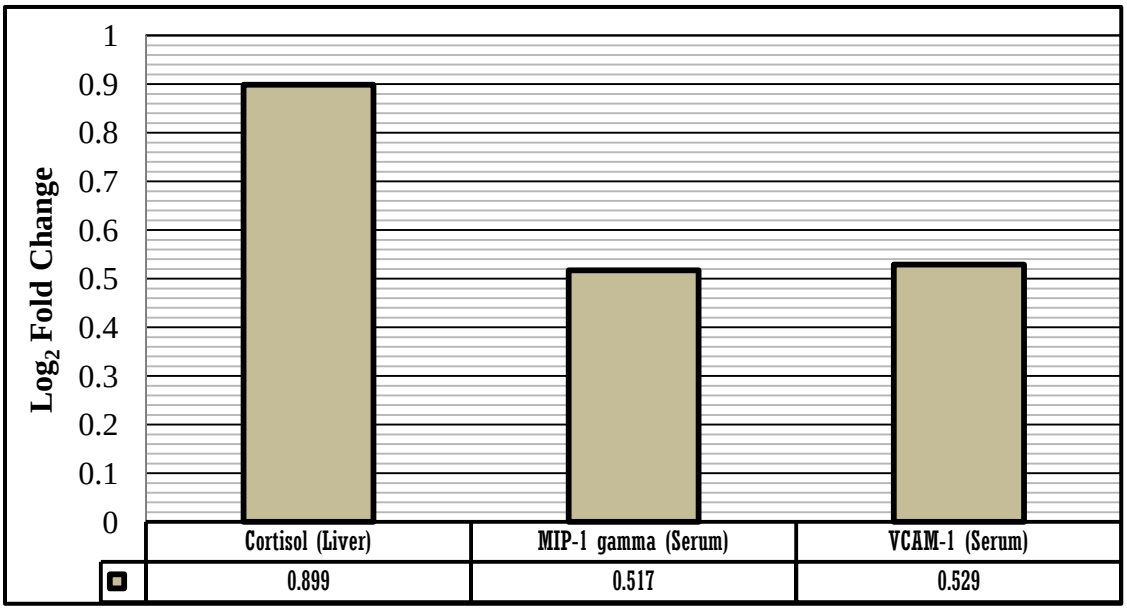


Figure 2. Log_2 Fold Change among Analytes with *p* Value < 0.01 in the Liver and Serum. Fold changes in analyte concentrations were calculated by taking the log base 2 of the ratio of the MRP4 knockout mean value to the wild type mean value. A value of 1 indicates double the mean protein concentration in the MRP4 knockout relative to the wild type mice.

V. DISCUSSION

Changes Observed in the Liver of MRP4 Knockout Mice

Cortisol is a major glucocorticoid which regulates protein, fat, and carbohydrate metabolism. One possible reason for a localized increase in this endocrine hormone could be the local expression of the enzyme 11-beta hydroxysteroid dehydrogenase type 1 (11ß-HSD1). 11ß-HSD1 regulates the conversion of circulating inactive cortisone to cortisol [61-64] and has been localized to liver and adipose tissue where it regulates the local concentration of cortisol with the highest concentration of the enzyme found in hepatocytes [63, 65-66]. Transgenic mice over expressing 11ß-HSD1 in liver and adipose tissue have exhibited insulin resistance, hyperlipidemia, hyperglycemia and hypertension [67-70]. The liver is the primary tissue for the production of cortisol through the conversion of cortisone to cortisol. 11ß -HSD1 modulates the local action of glucocorticoids independent of systemic changes in the concentration of circulating cortisol [65, 71-72]. This is consistent with our finding that the MRP4 knockout mice used in this study had an increase in cortisol concentration in the liver while the other tissues tested as well as serum concentration was unchanged relative to wild type mice. The increase in cortisol production may in time produce cumulative negative effects in the *MRP4* knockout mouse.

Changes in Protein Expression Observed in the Kidney of MRP4 Knockout Mice

Epiregulin (EPR) is a growth factor that plays a role in cell signaling by serving as a ligand for epidermal growth factor receptor (EGFR), a tyrosine kinase receptor [73]. EPR is a membrane protein that undergoes proteolysis and exits the plasma membrane, releasing growth factors [74-75]. EPR is one of the strongest activators of EGFR [73, 75]. In vitro studies have shown that EPR promotes proliferation of embryonic tissues, hepatocytes, smooth muscle cells and renal cells. In the kidney, EPR plays an important role in tissue regeneration in response to renal injury. After injury, kidney epithelial cells become undifferentiated, relocate to the injury site and differentiate into cells specific for the locale [75]. The increase in EPR levels in *MRP4* knockout mice kidney may suggest the cells increased the expression of EPR in response to renal injury.

Hepatocyte growth factor (HGF) is a mitogen involved in cell signaling by activating c-Met receptor tyrosine kinase, causing c-Met dimerization and autophosphorylation [76-78]. This activation triggers a cascade of events that results in cell migration and proliferation [77, 79]. HGF promotes angiogenesis and repairs damaged proximal renal tubules by inducing mitogenic events [80- 82]. HGF increases DNA synthesis in renal epithelial cells and up-regulates c-Met, allowing for more c-Met triggered events [82].

Additionally, HGF exhibits anti-fibrotic properties in the kidney by combating the buildup of excess extracellular matrix, which is characteristic of end-stage renal failure [80-81]. Specifically, HGF promotes the degradation of excess extracellular matrix by regulating MMP-9 in the kidney. Researchers have shown that the presence of HGF

increases the expression of MMP-9 and decreases the expression of tissue inhibitor of metalloproteinases 1 and 2 (TIMP-1 and TIMP-2) [80]. MMP-9 and HGF were found to be elevated in the MRP4 knockout mice kidney; however, TIMP-1 was not found to be statistically significantly changed in the kidney.

Studies have investigated the potential of using HGF as a therapeutic agent to combat renal failure. In addition to the benefits of HGF's role in renal cell proliferation, HGF has also shown to participate in the prevention of renal cell apoptosis [83]. Murine models with induced acute renal failure exhibited a significant increase in circulating HGF in plasma, which underscores HGF's importance as a defense mechanism in the kidney [82]. HGF concentration was not tested in *MRP4* knockout mice serum, so it is unknown if there is an increase corresponding with the increase observed in kidney tissue. HGF expression has been found to increase NF-E2 related factor-2 (Nrf2) expression in cultured liver cells [84]; however, Nrf2 expression was not evaluated in this study. Nrf2 is expressed in several tissues at low levels, but it is expressed at elevated levels upon oxidative stress [85]. Under oxidative stress, cells activate the transcription factor Nrf2 which then dissociates from Kelch-like ECH-associated protein 1 (Keap-1) [86]. Nrf2 then translocates to the nucleus where it binds to antioxidant response element (ARE) regions of genes responsible for the prevention of cell cytotoxicity [85-90]. These genes include drug metabolizing enzymes, GST, and antioxidant genes [91].

Increased cortisol levels in the liver could lead to high levels of systemic glucose as a result of gluconeogenesis. Elevated systemic glucose or other unknown mechanisms may increase oxidative stress in renal cells. In rat mesangial cells treated with high concentrations of glucose, HGF has been shown to suppress reactive oxygen species

(ROS) and could therefore work as an antioxidant mechanism in diabetic nephropathy [92]. The elevated concentration of HGF observed in *MRP4* knockout mice could be explained based on past studies of HGF: up-regulation of HGF compensates for the lack of MRP4 to defend renal cells.

Indeed, Nrf2 activators have been shown to induce expression of MRP4 [93-96]. An ARE region has been found in the regulatory sequence of *MRP4* and has been shown to bind to Nrf2 [95]. MRP4 has been observed to be elevated in the kidney of type 2 diabetic rats [97]. Presumably both MRP4 and HGF expression would increase under ROS conditions to combat cell toxicity.

The MMP family comprises zinc-dependent proteinases that engineer tissue remodeling and repair by degrading extracellular matrix (ECM) molecules, specifically proteoglycans and collagen [98-99]. MMP-9 in particular is well known for its ability to promote angiogenesis, cell invasion and cell migration by degrading gelatin, collagen and elastin in ECMs [100-104].

In the kidney, MMP-9 is integral to the prevention of renal fibrosis, which involves excess buildup of ECM. Murine studies have shown that a decrease in MMP-9 leads to apoptosis and a decrease in renal tissue repair [105]. As mentioned earlier, HGF induces MMP-9 and normally protects renal cells from fibrosis [80]. Thus an up-regulation of HGF would be expected to also be accompanied by an up-regulation of MMP-9. The increase in MMP-9 in *MRP4* knockout mice could therefore help maintain the integrity of the renal tissue in the absence of MRP4's protection.

Tenascin-C (TN-C) is another ECM protein with the ability to modulate the ECM through its interactions with fibronectin [106-108]. It has been observed to be up-

regulated during early inflammation within the kidney during immunoglobulin A (IgA)-induced nephropathy [109]. VEGF-A expression, which was found to be elevated in the *MRP4* knockout mice kidney, correlates with increased expression of TN-C in mammary epithelium [110].

TN-C is also able to induce expression of MMPs, including MMP-9, which are capable of directing deposition of TN-C into matrices [111-112]. TN-C has been found to co-localize with inflammatory areas of immune cell infiltration and chronically inflamed tissues, and it promotes lymphocyte migration [113-118]. TN-C knockout mice displayed a reduced concentration of MCP-1 and MMP-9 [119]. If absence of TN-C leads to reduced MCP-1 and MMP-9 levels, perhaps elevated TN-C leads to increased MCP-1 and MMP-9 levels, explaining their concomitant increase of expression observed in the *MRP4* knockout mice kidney. TN-C has been observed to be up-regulated under conditions of oxidative stress through a pathway involving NF-kB interaction with its promoter region [120-121]. It is interesting to speculate that in the absence of MRP4 to help detoxify the cells, a buildup of ROS is occurring.

Monocyte chemotactic protein (MCP-1) is an important chemokine in the immune response and inflammatory process [122]. MCP-1 is one of the most bioactive ligands for CC chemokine receptor 2 (CCR2) and, when complexed with CCR2, triggers a sequence of events leading to monocyte/macrophage recruitment in the inflammatory response [122-124]. Elevated MCP-1 expression has been observed in patients with diabetic nephropathy despite relatively normal systemic MCP-1 concentrations [125]. MCP-1 expression was statistically significantly elevated in the *MRP4* knockout mouse kidney. This increase may be a result of increased ROS. A high level of glucose has been found

to induce MCP-1 expression in a variety of different cell types with some induction attributable to increased ROS [126-129]. Increase of MCP-1 and other chemoattractant protein expression has been found in a mouse model of crescentic nephritis responsible for inflammatory responses in the kidney [130].

Vascular endothelial growth factor A (VEGF-A) is the primary promoter and regulator of angiogenesis as well as a factor regulating the vascular permeability of endothelial cells [131-133]. VEGF-A is primarily produced by podocytes in Bowman's capsule in the kidney [134-136]. It has been found to be an important factor for podocyte survival [137]. Glucose induces VEGF-A expression in podocytes [138-140]. The increase in VEGF-A expression in *MRP4* knockout mice may be a consequence of the increased cortisol levels observed in the liver. This elevated cortisol could lead to gluconeogenesis as mentioned previously. The resulting rise in blood glucose may drive enhanced expression of VEGF-A within the kidney. Indeed, hyper-filtration and increased VEGF-A expression has been observed in the kidney in diabetic models [134, 138, 141]. Over-expression of VEGF-A in the kidney has been observed in different disorders including diabetic nephropathy and hypertension [142-143]. HGF in combination with its receptor induces expression of VEGF-A [144]. Therefore, VEGF-A expression may result from high-glucose/ROS induced HGF expression proposed earlier. MCP-1 has been found to stimulate angiogenesis by inducing VEGF-A [145]. MCP-1 and HGF are both elevated in the *MRP4* knockout mouse kidney.

An alternative hypothesis is that increased intracellular cAMP level in *MRP4* knockout mice may account for the elevated VEGF-A expression since it has been found induction of VEGF-A can occur through cAMP-mediated pathways [146-147]. However,

elevated VEGF-A expression was not observed in the liver or brain of the *MRP4* knockout mice where presumably cAMP levels were also elevated. That said, MRP4 expression is highest in the rodent kidney which may account for this discrepancy. Yet another hypothesis arises from evidence that androgen deprivation increases expression of VEGF-A in endothelial cells [148]. Since testosterone was found to be decreased in the kidneys of *MRP4* knockout mice, it is possible that low testosterone may underlie the observed increase in VEGF-A expression.

Myoglobin is a protein responsible for oxygen binding in smooth, cardiac and skeletal muscles. Myoglobin plays a role in cellular respiration by delivering oxygen to myocyte mitochondria [149]. Myoglobin clearance is mediated by renal excretion with elevated systemic myoglobin levels typically resulting in renal damage by forming deposits that obstruct and damage tubular cells and decrease glomerular filtration rate [150-152]. In vitro studies have shown that myoglobin also promotes endoplasmic reticulum stress-induced apoptosis, though the mechanism is still unclear [153]. Myoglobin's cytotoxicity in renal cells continues even after initial myoglobin exposure: renal cell proliferation is suppressed by myoglobin, which prevents tubule reparation [154]. In the *MRP4* knockout mice, myoglobin levels were decreased in the kidney. This result does not lead to a clear conclusion, but it can be postulated that the lack of MRP4 proves beneficial in terms of myoglobin since elevated levels of myoglobin can prove detrimental to the kidney.

Testosterone binds to androgen receptors, which assist in the initiation of transcription. Studies on mice have shown that the binding of testosterone to its receptors regulates transcription in kidney cells by stimulating RNA polymerase activity [155-156].

Testosterone has been found to stimulate the production of cAMP; researchers have shown that testosterone increases fluid and solute secretion in canine kidney cells [157]. The decrease in testosterone levels in *MRP4* knockout mouse kidney may suggest that elevation of intracellular cAMP consequent to *MRP4* knockout obviates the need for testosterone-mediated generation of cAMP.

Testosterone production is impaired in *MRP4* knockout mice Leydig cells, and its metabolism in the liver is up-regulated in three week old *MRP4* knockout mice. However, this up-regulation in metabolism is absent in adult *MRP4* knockout mice which returns systemic testosterone concentrations to normal [48]. It remains unclear why testosterone concentration in the kidney of *MRP4* knockout mice observed in this study is decreased. As mentioned earlier, VEGF-A expression is induced by a decrease in testosterone [148]. Due to its participation in increasing induction of VEGF-A, reduced testosterone in the kidney may play a role in helping to alleviate any potential pathogenesis occurring within the kidney.

Protein Expression Changes Observed in the *MRP4* Knockout Mouse Serum

Vascular cell adhesion molecule-1 (VCAM-1) is a cellular adhesion molecule which is a member of the immunoglobulin-like superfamily. It facilitates the adhesion of leukocytes to endothelium at sites of inflammation and activation, and it is a marker for angiogenesis [158-162]. VCAM-1 is expressed on vascular endothelial cells but is also functional in soluble form in the blood through proteolytic cleavage [162]. Different potential roles of soluble VCAM-1 (sVCAM-1) have been proposed. One potential role is that cleavage provides a mechanism to decrease its expression at cell surfaces. Another potential role is to facilitate the binding of leukocytes in the circulation to prevent binding

and infiltration at endothelial cells [163]. No statistically significant differences in VCAM-1 expression were observed in the tissues of *MRP4* knockout mice, but VCAM-1 expression was statistically significantly elevated in the serum. This difference may indicate a mechanism to regulate leukocyte binding at tissue surfaces.

Up-regulation of VCAM-1 has been found in a number of different diseases and cancers [164-174]. A high level of glucose has been found to induce both sVCAM-1 and MCP-1 [175-177]. Hyperglycemia increases oxidative stress in endothelial cells [178-180]. ROS have been shown to increase activation of chemoattractant and cellular adhesion molecule expression [177, 181]. Thus, the observation of elevated sVCAM and MCP-1 may be attributed to the increased cortisol expression observed in the *MRP4* knockout mice potentially contributing to gluconeogenesis.

Macrophage inflammatory protein-1 gamma (MIP-1 γ) is a chemokine also known by three other names: MIP-related protein-2, CCF18, and CCL9 [182-184]. It has been localized to several different tissues and is found at relatively high concentrations in the normal mouse circulatory system compared to other chemokines [183]. MIP-1 γ has been found to be secreted by Langerhans' cells, dendritic cells, follicle-associated epithelium, macrophages and myeloid cell lines [182, 184-186]. It has been found to induce chemotaxis of monocytes, CD4+ and CD8+ T cells. MIP-1 γ is also found to activate calcium release in neutrophils and demonstrate pyrogenic properties [182, 185]. Little is known of MIP-1 γ's role in vivo; however, one study has implicated elevated serum levels of MIP-1 γ and VCAM-1 as potential biomarkers of a mouse model for inflammatory bowel disease [187]. Both MIP-1 γ and VCAM-1 were elevated in the *MRP4* knockout mice serum. Elevated MIP-1 γ and MCP-1 has also been implicated in

late stage atherosclerosis, indicating chronic inflammation [188]. MCP-1 was elevated in *MRP4* knockout mouse serum although not statistically significantly. It is unclear at this time how the lack of MRP4 may be either directly or indirectly leading to the elevated response of MIP-1 γ in serum. However, with elevated levels of VCAM-1 and MIP-1 γ in the serum of *MRP4* knockout mice, it is tempting to speculate that the absence of MRP4 is causing an inflammatory response somewhere in the body, presumably in the kidney.

VI. CONCLUSION

The *MRP4* knockout mice at six months of age show changes in protein expression that could reflect detrimental effects in the kidney that in turn could lead to disease if pathology is not already present. Of the four tissues examined, the kidney manifested the most statistically significant differences in analytes tested. The kidney was expected to reveal the most changes in analyte expression in the absence of MRP4, because in rodent MRP4 expression is known to be highest in the kidney.

Although some analytes were not found to differ statistically significantly, their low *p* value in combination with prior research implicating their involvement in regulation, correlation, or induction of analytes that were statistically different supports the observed results from this study. Other studies have observed compensatory mechanisms such as elevated PDE within the cell or up-regulation of other members of the MRP family that may help to modulate signaling and alleviate potential cytotoxicity in the absence of MRP4. Additionally, at least one study has documented that this compensation may diminish with age and that noticeable morphological changes may not be apparent till much later in life, beyond six months of age. One may postulate that many of the analytes with a low, but not statistically significant *p* values at six months of age may in fact fall below $p < 0.01$ at nine or twelve months. Similarly, the expression levels of many of the analytes that were found to be significantly differentially expressed in the knockout mice at six months of age may display more robust divergence from the

wild type expression levels with increasing age if their regulation is mediated by these compensatory mechanisms.

The analytes detected by the multi-analyte bead assays integrated into the Rodent and Human Discovery platforms are hormones, small molecules, metabolic proteins, angiogenic proteins, inflammatory proteins and potential biomarkers of toxicity. After comparing the wild type and knockout mouse liver, kidney, brain and serum concentrations on this wide array of analytes, potential mechanisms by which an absence of MRP4 is directly or indirectly influencing the differentially expressed analytes remains merely speculative. However, increased ROS was a reoccurring putative mechanism discovered while reviewing several of the proteins which may have influenced the observed expression changes. The analyte changes observed in this study as well as speculative pathways and analyte interactions are summarized in Figure 3.

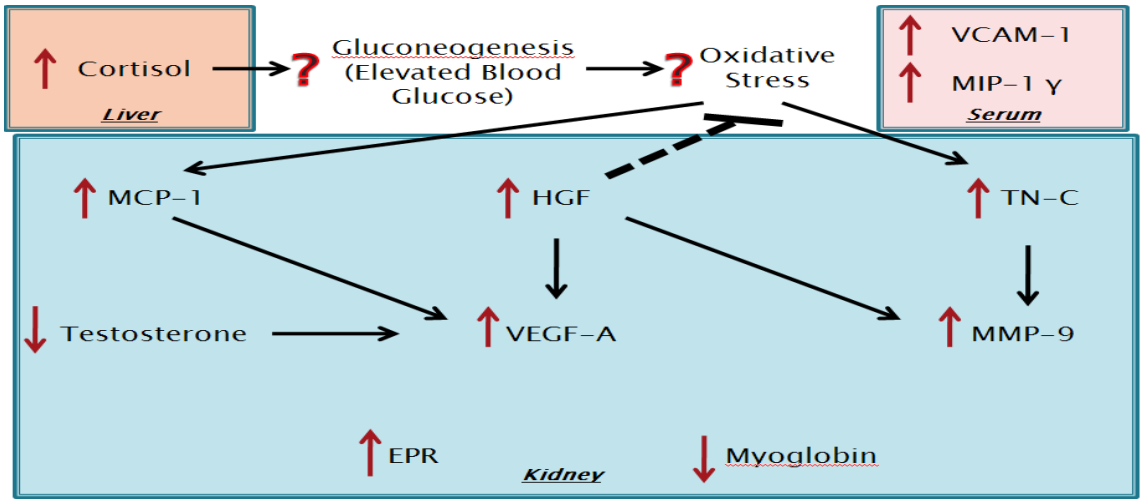


Figure 3. Speculative Pathways and Analyte Interactions. Analyte expression changes observed in the knockout mice are illustrated by red arrow. Interactions between analytes based on the literature review are illustrated by black arrows. Question marks represent events speculated to lead to the observed changes in analyte levels.

The elevated levels of cortisol in the liver may lead to elevated blood glucose levels in the *MRP4* knockout mouse, contributing to oxidative stress in renal cells. There are other mechanisms that are known to generate oxidative stress in cells such as deficiencies in antioxidant enzymes and impaired metabolism of antioxidants. Elevated glucose might be a more probable explanation since glucose is reabsorbed in the proximal tubules until a threshold is reached which no longer allows its absorption back into the blood stream. This potentially allows the effects from high levels of glucose to be exerted at the kidney. It is interesting to speculate that observed changes of expression levels found in the kidney would also be observed in other tissues with alternative methods of ROS generation. If cortisol and glucose are the primary initiators of the possible inflammation and nephropathy in the absence of MRP4, the factor inducing cortisol production remains unclear. However, I speculate that up-regulation of 11ß-HSD1 participates in this pathway to increase the local concentration of cortisol in the liver.

ROS and HGF are capable of inducing the expression of Nrf-2 which facilitates the increased transcription of genes responsible to help combat cellular toxicity. *MRP4* contains an ARE region in its promoter which Nrf-2 binds, indicating in wild type tissue HGF may indirectly elicit the help of MRP4 during situations of renal inflammation and repair.

Elevated TN-C and HGF are each found to induce MMP-9 expression in the kidney, which helps to exert their anti-fibrotic properties. These elevations observed in the *MRP4* knockout kidney might indicate that a lack of MRP4 is eliciting fibrosis of the kidney. These proteins also participate in ECM remodeling allowing for leukocyte

migration. Speculation that inflammation is occurring in the kidney of the knockout mice is further supported by the elevated expression of MCP-1 and EPR in concert with decreased expression of myoglobin.

MCP-1 is responsible for chemotaxis of leukocytes, which help guide inflammatory response molecules to the site they are needed. Leukocytes recruited by MCP-1 take advantage of the breakdown of the ECM facilitated by increased expression of MMP-9. Although a mechanism for elevated EPR and decreased myoglobin expression in the kidney was not derived from the literature review, the presence of EPR suggests that the kidney may be undergoing repair. The reduced presence of myoglobin may be indirectly helping to prevent oxidative stress-induced apoptosis and support renal tubule repair.

VEGF-A in the kidney was found to participate in the most interactions among all of the statistically significant analytes. VEGF-A is inducible by decreased testosterone levels and elevated MCP-1 and HGF expression. Its ability to promote and regulate angiogenesis as well as increase cell permeability is consistent with a model in which the *MRP4* knockout kidney is undergoing proliferation and repair accompanied by inflammation.

Collectively, these observations from this comparative analysis support the ability of MRP4 to prevent detrimental effects, specifically in the kidney, which may be indirectly mediated in part by systemic blood glucose levels. If this conjecture is accurate, the *MRP4* knockout mice could potentially develop hyperglycemia leading to more extreme diseases of the kidney with age.

The results of this study has potential implications for individuals who are carriers of SNPs of *MRP4* which lead to truncatation of the protein, rendering it nonfunctional, or decrease the expression of MRP4 in tissue. Care should be taken, however, before extrapolating these results to humans for several reasons, including that rodent proteins do not always perform the same functions as they do in humans. One should also be cautious when interpreting the results from the Human Discovery platform. Many of the antibodies used in the assay have not been independently tested for cross-reactivity with the mouse protein. The analysis performed on all of the statistically significantly different analytes in addition to some analytes not statistically significantly different carefully excluded antibodies which were known to not cross-react or proteins which were poorly conserved between human and mouse species. However, the exclusion criteria based on the comparison of the protein alignments were arbitrarily set, and it remains possible that the antibodies recognized a mouse protein not homologous to the human protein for which the assay has been characterized. It is reasonable to assume that the epitopes the monoclonal or polyclonal antibodies bind to may potentially be positioned in a region of low conservation between the human and mouse protein despite a high positives score generated by the alignment and vice versa.

Other limitations of this study includes the sample size and that the mice were not littermates. The small sample size used reduces the power of the statistical analysis and increases the probability of type 2 errors, otherwise known as false negatives. Therefore, using a small sample size reduced our ability to observe an effect that may indeed be present. Since the wild type mice and knockout mice were not siblings, unknown variables, such as epigenetic defects, were introduced that could account for some of the

difference observed. However, the strain of mouse used to generate the MRP4 knockout mice was identical to the strain of the wild type mice in the study. In addition, the mice were allowed to acclimate to identical settings in a controlled laboratory environment for a minimum of one month prior to obtaining samples for analysis.

In conclusion, this study uncovers the effects an absence of MRP4 has on other proteins and small molecules in the body. Many of the results observed in this study were in agreement or compatible with research conducted elsewhere. Future research is needed to further test several of the proposed mechanisms leading to the changes observed and unanswered questions resulting from this study. In addition, the observations made at six months of age as well as the literature review of the analytes has provided other interesting leads that can be investigated in the *MRP4* knockout mouse, including blood glucose levels and kidney histology. This study is soon to be followed up with an analysis of the *MRP4* knockout mice at 12 months of age to incorporate these observations. Considering the impact MRP4 has on intracellular/extracellular signaling and drug efficacy, more research is needed to fully understand the role of MRP4 in different tissues as well as the potential outcomes resulting from changes in its expression.

APPENDIX

Appendix Table 1: Serum Raw Data Results from Rodent Discovery Product.

The least detectable dose (LDD) is determined as the mean ± three standard deviations of twenty blank readings. The LLOQ (Lower Limit of Quantitation) is the lowest concentration of an analyte in a sample that can be reliably detected and at which the total error meets the laboratory's requirements for accuracy. MyriadRBM laboratory's requirement for accuracy is the concentration of an analyte at which the coefficient of variation of replicate standard samples is 30%. NR indicates unable to report data meeting quality standards. QNS indicates sample quantity not sufficient for testing.

ALT ID	Samples	Apolipoprotein A-I (Apo A-I)	C-Reactive Protein Mouse (CRP Mouse)	CD40 (CD40)	CD40 Ligand (CD40-L)	Eotaxin	Epidermal Growth Factor Mouse (EGF Mouse)	Factor VII	Fibrinogen	Fibroblast Growth Factor 9 (FGF-9)	Fibroblast Growth Factor basic (FGF-basic)	Granulocyte Chemotactic Protein-2 Mouse (GCP-2 Mouse)	Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)	Growth Hormone (GH)	Growth-Regulated Alpha Protein (KC/GRO)	Haptoglobin	Immunoglobulin A (IgA)	Insulin
Myriad RBM LLOQ		20	2.1	13	428	6.2	15	19	510	2.9	15	0.49	6.8	1.6	0.026	121	19	6.3
Myriad RBM LDD		2.3	0.75	6.7	370	3.5	18	25	231	2.4	12	0.16	4.1	1.9	0.012	118	18	4.1
Units		ug/mL	ug/mL	pg/mL	pg/mL	pg/mL	pg/mL	ng/mL	ug/mL	ng/mL	ng/mL	ng/mL	pg/mL	ng/mL	ng/mL	ug/mL	ug/mL	uIU/mL
13	Mouse 1 WT Serum	383	3.7	18	3280	570	23	35	<510	<2.9	<15	21	<6.8	5.2	0.082	127	296	<6.3
14	Mouse 2 WT Serum	228	3.6	24	2140	875	34	37	<510	<2.9	<15	34	<6.8	14	<0.026	123	230	<6.3
15	Mouse 3 WT Serum	156	4.7	22	3430	877	36	42	<510	<2.9	<15	36	<6.8	<1.6	0.074	142	273	<6.3
16	Mouse 4 WT Serum	259	3.6	56	1920	1140	53	59	<510	<2.9	<15	23	<6.8	4.6	<0.026	123	135	<6.3
17	Mouse 5 WT Serum	609	3.4	1210	4610	2300	71	79	<510	<2.9	70	17	<6.8	6.2	0.079	130	262	<6.3
18	Mouse 6 WT Serum	1000	3.4	1240	4370	1580	48	69	<510	<2.9	268	25	<6.8	19	<0.026	128	672	<6.3
19	Mouse 1 KO Serum	188	4.0	44	3740	496	45	45	<510	<2.9	<15	25	<6.8	31	<0.026	129	301	<6.3
20	Mouse 2 KO Serum	252	4.6	39	3590	660	46	45	<510	<2.9	<15	23	<6.8	<1.6	<0.026	149	283	<6.3
21	Mouse 3 KO Serum	171	4.6	30	3280	626	34	39	<510	<2.9	<15	22	<6.8	12	0.051	136	435	<6.3
22	Mouse 4 KO Serum	168	4.8	39	2900	883	39	45	<510	<2.9	<15	28	<6.8	4.7	0.051	127	244	<6.3
23	Mouse 5 KO Serum	202	QNS	38	4690	950	49	67	QNS	<2.9	<15	27	<6.8	27	0.043	146	392	<6.3
24	Mouse 6 KO Serum	118	4.6	27	3740	761	56	48	<510	<2.9	<15	26	<6.8	6.9	0.051	138	605	<6.3

Appendix Table 1 Continued

ALT ID	Samples	Interferon gamma (IFN-gamma)	Interferon gamma Induced Protein 10 (IP-10)	Interleukin-1 alpha (IL-1 alpha)	Interleukin-1 beta (IL-1 beta)	Interleukin-2 (IL-2)	Interleukin-3 (IL-3)	Interleukin-4 (IL-4)	Interleukin-5 (IL-5)	Interleukin-6 (IL-6)	Interleukin-7 (IL-7)	Interleukin-10 (IL-10)	Interleukin-11 (IL-11)	Interleukin-12 Subunit p70 (IL-12p70)	Interleukin-17A (IL-17A)	Interleukin-18 (IL-18)	Leptin	Leukemia Inhibitory Factor (LIF)
	Units	pg/mL	pg/mL	pg/mL	ng/mL	pg/mL	pg/mL	pg/mL	ng/mL	pg/mL	ng/mL	pg/mL	pg/mL	ng/mL	ng/mL	ng/mL	ng/mL	pg/mL
	Myriad RBM LDD	1.5	6.6	108	6.5	25	2.9	52	0.47	2.6	0.086	91	40	0.12	0.0071	6.2	0.026	218
	Myriad RBM LLOQ	38	7.1	132	3.5	49	4.3	68	0.75	3.8	0.12	220	70	0.14	0.0073	8.0	0.053	463
13	Mouse 1 WT Serum	<38	21	156	<3.5	<49	<4.3	<68	<0.75	<3.8	<0.12	<220	<70	<0.14	<0.0073	11	1.1	844
14	Mouse 2 WT Serum	<38	47	<132	<3.5	<49	<4.3	<68	<0.75	<3.8	<0.12	<220	<70	<0.14	<0.0073	22	0.51	937
15	Mouse 3 WT Serum	<38	54	<132	<3.5	<49	<4.3	<68	<0.75	3.9	<0.12	<220	<70	<0.14	<0.0073	21	0.92	1120
16	Mouse 4 WT Serum	<38	63	<132	<3.5	<49	<4.3	<68	<0.75	<3.8	<0.12	<220	<70	<0.14	<0.0073	22	0.18	1030
17	Mouse 5 WT Serum	<38	35	269	3.6	<49	<4.3	<68	<0.75	<3.8	<0.12	<220	<70	<0.14	<0.0073	28	0.49	2170
18	Mouse 6 WT Serum	<38	39	239	<3.5	<49	<4.3	<68	<0.75	<3.8	<0.12	<220	<70	<0.14	<0.0073	22	0.74	2260
19	Mouse 1 KO Serum	<38	57	141	<3.5	<49	<4.3	<68	<0.75	<3.8	<0.12	<220	<70	<0.14	<0.0073	27	0.71	1220
20	Mouse 2 KO Serum	<38	39	<132	<3.5	<49	<4.3	<68	<0.75	<3.8	<0.12	<220	<70	<0.14	<0.0073	25	1.2	1120
21	Mouse 3 KO Serum	<38	49	<132	<3.5	<49	<4.3	<68	<0.75	<3.8	<0.12	<220	<70	<0.14	<0.0073	25	0.68	1120
22	Mouse 4 KO Serum	<38	67	<132	<3.5	<49	<4.3	<68	<0.75	<3.8	0.29	<220	<70	<0.14	<0.0073	18	1.1	1220
23	Mouse 5 KO Serum	<38	77	217	<3.5	<49	<4.3	<68	<0.75	<3.8	<0.12	<220	105	<0.14	<0.0073	24	1.2	1550
24	Mouse 6 KO Serum	<38	55	<132	<3.5	<49	<4.3	<68	<0.75	<3.8	<0.12	<220	<70	<0.14	<0.0073	24	0.65	1080

Appendix Table 1 Continued

ALT ID	Samples	Lymphotactin	Macrophage Colony-Stimulating Factor-1 (M-CSF-1)	Macrophage-Derived Chemokine (MDC)	Macrophage Inflammatory Protein-1alpha (MIP-1 alpha)	Macrophage Inflammatory Protein-1 beta (MIP-1 beta)	Macrophage Inflammatory Protein-1 gamma (MIP-1 gamma)	Macrophage Inflammatory Protein-2 (MIP-2)	Macrophage Inflammatory Protein-3 beta (MIP-3 beta)	Matrix Metalloproteinase-9 (MMP-9)	Monocyte Chemotactic Protein 1 (MCP-1)	Monocyte Chemotactic Protein 3 (MCP-3)	Monocyte Chemotactic Protein-5 (MCP-5)	Myeloperoxidase (MPO)	Myoglobin	Oncostatin-M (OSM)	Plasminogen Activator Inhibitor 1 (PAI-1)	Resistin
	Myriad RBM LLOQ	21	0.051	55	3.3	53	0.67	11	1.2	14	7.9	4.4	2.3	0.66	6.0	0.21	0.034	0.039
	Myriad RBM LDD	19	0.033	34	4.1	47	0.45	5.3	0.36	9.9	4.9	4.2	1.7	0.48	5.7	0.097	0.015	0.013
	Units	pg/mL	ng/mL	pg/mL	ng/mL	pg/mL	ng/mL	pg/mL	ng/mL	ng/mL	pg/mL	pg/mL	pg/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL
13	Mouse 1 WT Serum	34	5.9	474	3.4	80	5.2	22	2.7	102	17	91	7.0	66	388	<0.21	0.28	<0.039
14	Mouse 2 WT Serum	75	5.8	995	7.1	105	11	16	3.3	124	29	93	4.5	53	80	<0.21	0.42	<0.039
15	Mouse 3 WT Serum	79	5.2	1100	8.4	105	14	<11	3.2	209	31	117	21	91	56	<0.21	0.39	<0.039
16	Mouse 4 WT Serum	79	5.2	1150	7.1	118	13	16	3.9	124	22	69	12	59	8900	<0.21	0.36	<0.039
17	Mouse 5 WT Serum	85	6.9	2740	9.2	114	12	42	5.7	93	51	192	9.1	107	1300	<0.21	0.39	0.19
18	Mouse 6 WT Serum	104	6.7	3300	7.6	151	14	37	5.8	149	37	118	9.8	60	>12600	<0.21	0.21	<0.039
19	Mouse 1 KO Serum	87	7.7	1110	8.8	114	16	11	3.6	142	57	115	5.4	29	1660	<0.21	0.36	<0.039
20	Mouse 2 KO Serum	87	5.5	1010	7.3	127	14	16	3.7	129	37	82	14	78	822	<0.21	0.23	0.083
21	Mouse 3 KO Serum	83	5.6	995	7.3	114	15	16	3.3	156	49	82	12	92	1150	<0.21	0.21	<0.039
22	Mouse 4 KO Serum	79	5.8	1150	8.0	92	21	14	3.3	153	48	122	17	76	209	<0.21	0.56	<0.039
23	Mouse 5 KO Serum	138	7.1	1260	6.7	313	14	41	4.6	169	83	148	16	76	5570	0.29	0.50	0.074
24	Mouse 6 KO Serum	62	5.5	963	7.6	64	19	<11	3.3	137	42	104	14	65	465	<0.21	0.33	<0.039

Appendix Table 1 Continued

ALT ID	Samples	Analytes	Units	Myriad RBM LDD	Myriad RBM LLOQ
13	Mouse 1 WT Serum	Serum Amyloid P-Component (SAP)	ug/mL	6.4	9.0
14	Mouse 2 WT Serum	Stem Cell Factor (SCF)	pg/mL	153	290
15	Mouse 3 WT Serum	T-Cell-Specific Protein RANTES (RANTES)	pg/mL	0.026	0.028
16	Mouse 4 WT Serum	Thrombopoietin	ng/mL	30	60
17	Mouse 5 WT Serum	Tissue Inhibitor of Metalloproteinases 1 Mouse (TIMP-1 Mouse)	ng/mL	0.021	0.084
18	Mouse 6 WT Serum	Tumor Necrosis Factor alpha (TNF-alpha)	ng/mL	0.029	0.11
19	Mouse 1 KO Serum	Vascular Cell Adhesion Molecule-1 (VCAM-1)	ng/mL	3.3	5.4
20	Mouse 2 KO Serum	Vascular Endothelial Growth Factor A (VEGF-A)	pg/mL	222	158
21	Mouse 3 KO Serum	von Willebrand factor (vWF)	ng/mL	27	45
22	Mouse 4 KO Serum	Cortisol (Cortisol)	ng/mL	12	20
23	Mouse 5 KO Serum	Glucagon	pg/mL	157	320
24	Mouse 6 KO Serum	Glucagon-like Peptide 1, total (GLP-1 total)	pg/mL	5.7	7.3
		Peptide YY (PYY)	pg/mL	73	101
		Progesterone	ng/mL	4.5	6.5
		Testosterone, Total	ng/mL	0.61	1.0
		Adiponectin	ug/mL	0.070	0.15
		Insulin-Like Growth Factor I (IGF-I)	ng/mL	4.7	16

26	469	<0.028	103	0.76	<0.11	1780	324	410	98	<320	<7.3	<101	33	5.1	<0.15	187
18	309	<0.028	84	0.48	<0.11	1490	277	335	85	<320	<7.3	<101	33	3.7	<0.15	175
29	371	<0.028	91	0.63	<0.11	1600	217	363	85	<320	<7.3	<101	32	4.5	<0.15	240
30	<290	<0.028	91	0.58	<0.11	1790	232	447	95	<320	<7.3	<101	28	3.9	<0.15	293
QNS	922	<0.028	78	0.86	<0.11	1650	570	401	53	<320	<7.3	<101	26	13	<0.15	232
30	309	<0.028	97	0.56	<0.11	1630	262	377	61	<320	<7.3	<101	43	2.4	<0.15	266
19	778	0.082	64	0.93	<0.11	1090	903	419	87	<320	18	<101	26	6.1	<0.15	187
21	569	0.031	78	0.84	<0.11	1050	293	354	46	<320	<7.3	NR	31	8.0	<0.15	293
13	603	0.051	109	0.70	<0.11	687	1750	211	102	<320	<7.3	<101	14	1.7	<0.15	293
26	603	<0.028	91	0.66	<0.11	1500	232	391	59	<320	<7.3	<101	24	2.0	<0.15	293
20	403	<0.028	81	0.51	<0.11	1250	262	335	75	<320	<7.3	<101	14	2.7	<0.15	260
20	796	<0.028	<60	0.33	<0.11	1310	262	387	37	<320	<7.3	<101	13	1.9	<0.15	154

Appendix Table 1 Continued

ALT ID	Samples	Beta-2-Microglobulin (B2M)	Calbindin	Clusterin (CLU)	Cystatin-C	Epidermal Growth Factor Rat (EGF Rat)	Glutathione S-Transferase alpha (GST-alpha)	Glutathione S-Transferase Mu (GST-Mu)	Kidney Injury Molecule-1 Rat (KIM-1 Rat)	Neutrophil Gelatinase-Associated Lipocalin (NGAL)	Osteopontin	Tissue Inhibitor of Metalloproteinases 1 Rat (TIMP-1 Rat)
	Myriad RBM LLOQ	0.82	0.15	0.78	46	4.7	13	17	0.16	3.9	3.7	0.069
	Myriad RBM LDD	0.35	0.10	0.36	18	6.3	10	4.9	0.042	1.8	1.6	0.062
	Units	ug/mL	ng/mL	ug/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL
13	Mouse 1 WT Serum	QNS	NR	QNS	QNS	NR	NR	QNS	NR	QNS	QNS	QNS
14	Mouse 2 WT Serum	<0.82	NR	299	789	NR	NR	<17	NR	NR	53	<0.069
15	Mouse 3 WT Serum	<0.82	NR	564	987	NR	NR	<17	NR	<3.9	87	<0.069
16	Mouse 4 WT Serum	QNS	NR	QNS	QNS	NR	NR	QNS	NR	QNS	QNS	QNS
17	Mouse 5 WT Serum	QNS	NR	QNS	QNS	NR	NR	QNS	NR	QNS	QNS	QNS
18	Mouse 6 WT Serum	QNS	NR	QNS	QNS	NR	NR	QNS	NR	QNS	QNS	QNS
19	Mouse 1 KO Serum	<0.82	NR	268	847	NR	NR	<17	NR	<3.9	67	<0.069
20	Mouse 2 KO Serum	<0.82	NR	431	706	NR	NR	<17	NR	<3.9	77	<0.069
21	Mouse 3 KO Serum	<0.82	NR	278	858	NR	NR	<17	NR	<3.9	57	<0.069
22	Mouse 4 KO Serum	0.90	NR	283	864	NR	NR	<17	NR	<3.9	74	<0.069
23	Mouse 5 KO Serum	QNS	NR	QNS	QNS	NR	NR	QNS	NR	QNS	QNS	QNS
24	Mouse 6 KO Serum	<0.82	NR	304	964	NR	NR	<17	NR	<3.9	71	<0.069

Appendix Table 2: Tissue Raw Data Results from Rodent Discovery Product.

The single wild type kidney sample with red font was excluded from data analysis due to incorrect dilution of the tissue with lysis buffer during tissue homogenization. LDD and LLOQ are explained in the caption for appendix table 1.

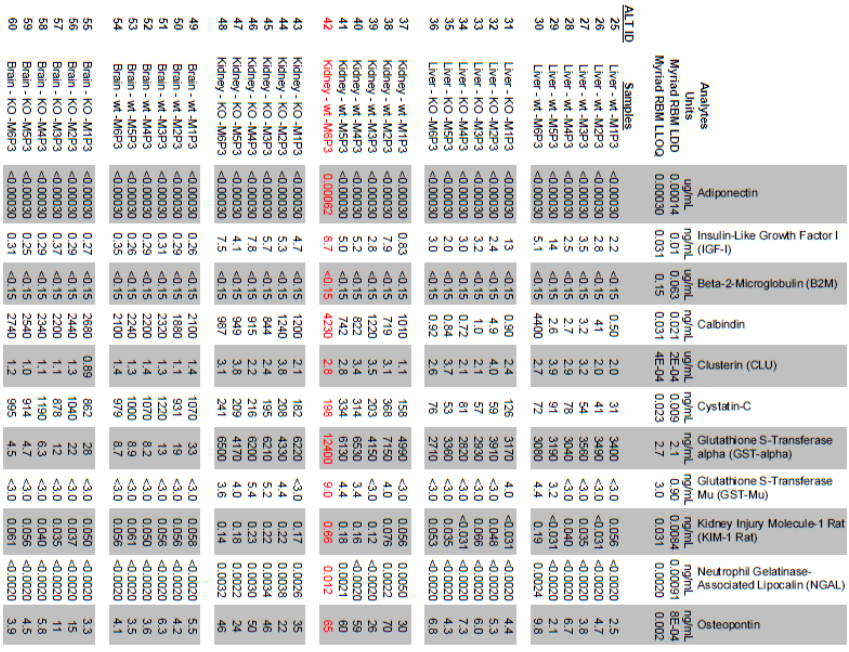
ALT ID	Samples	Analytes	Units	Myriad RBM LDD	Myriad RBM LLOQ
26	Liver - wt - M1P3	Apolipoprotein A-I (Apo A-I)	ug/mL	0.046	0.41
27	Liver - wt - M2P3	C-Reactive Protein Mouse (CRP Mouse)	ug/mL	0.004	0.011
28	Liver - wt - M3P3	CD40 (CD40)	pg/mL	1.3	2.5
29	Liver - wt - M4P3	CD40 Ligand (CD40-L)	pg/mL	74	86
30	Liver - wt - M5P3	Eotaxin	pg/mL	0.70	1.2
31	Liver - KO - M1P3	Epidermal Growth Factor Mouse (EGF Mouse)	pg/mL	3.6	3.1
32	Liver - KO - M2P3	Factor VII	ng/mL	4.9	3.8
33	Liver - KO - M3P3	Fibrinogen	ug/mL	1.2	2.5
34	Liver - KO - M4P3	Fibroblast Growth Factor 9 (FGF-9)	ng/mL	0.48	0.57
35	Liver - KO - M5P3	Fibroblast Growth Factor basic (FGF-basic)	pg/mL	2.4	3.1
36	Liver - KO - M6P3	Granulocyte Chemotactic Protein-2 Mouse (GCP-2 Mouse)	ng/mL	0.0016	0.0049
37	Kidney - wt - M1P3	Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)	pg/mL	0.81	1.4
38	Kidney - wt - M2P3	Growth-Regulated Alpha Protein (KC/GRO)	ng/mL	0.0024	0.0053
39	Kidney - wt - M3P3	Haptoglobin	ug/mL	2.4	2.4
40	Kidney - wt - M4P3	Immunoglobulin A (IgA)	ug/mL	0.36	0.37
41	Kidney - wt - M5P3	Interferon gamma (IFN-gamma)	pg/mL	3.0	7.6
42	Kidney - wt - M6P3	Interferon gamma Induced Protein 10 (IP-10)	pg/mL	1.3	1.4
43	Kidney - KO - M1P3	Interleukin-1 alpha (IL-1 alpha)	pg/mL	22	26
44	Kidney - KO - M2P3	Interleukin-1 beta (IL-1 beta)	pg/mL	1.3	0.69
45	Kidney - KO - M3P3	Interleukin-2 (IL-2)	pg/mL	4.9	9.9
46	Kidney - KO - M4P3	Interleukin-3 (IL-3)	pg/mL	0.57	0.85
47	Kidney - KO - M5P3	Interleukin-4 (IL-4)	pg/mL	10	14
48	Kidney - KO - M6P3	Interleukin-5 (IL-5)	ng/mL	0.094	0.15
49	Brain - wt - M1P3	Interleukin-6 (IL-6)	pg/mL	0.51	0.75
50	Brain - wt - M2P3	Interleukin-7 (IL-7)	ng/mL	0.017	0.024
51	Brain - wt - M3P3	Interleukin-10 (IL-10)	pg/mL	16	44
52	Brain - wt - M4P3	Interleukin-11 (IL-11)	pg/mL	8.0	14
53	Brain - wt - M5P3	Interleukin-12 Subunit p70 (IL-12p70)	ng/mL	0.023	0.027
54	Brain - wt - M6P3	Interleukin-17A (IL-17A)	ng/mL	0.0014	0.0015
55	Brain - KO - M1P3	Interleukin-18 (IL-18)	ng/mL	1.2	1.6
56	Brain - KO - M2P3	Leukemia Inhibitory Factor (LIF)	pg/mL	44	93
57	Brain - KO - M3P3				
58	Brain - KO - M4P3				
59	Brain - KO - M5P3				
60	Brain - KO - M6P3				

Samples	Apolipoprotein A-I (Apo A-I)	C-Reactive Protein Mouse (CRP Mouse)	CD40 (CD40)	CD40 Ligand (CD40-L)	Eotaxin	Epidermal Growth Factor Mouse (EGF Mouse)	Factor VII	Fibrinogen	Fibroblast Growth Factor 9 (FGF-9)	Fibroblast Growth Factor basic (FGF-basic)	Granulocyte Chemotactic Protein-2 Mouse (GCP-2 Mouse)	Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)	Growth-Regulated Alpha Protein (KC/GRO)	Haptoglobin	Immunoglobulin A (IgA)	Interferon gamma (IFN-gamma)	Interferon gamma Induced Protein 10 (IP-10)	Interleukin-1 alpha (IL-1 alpha)	Interleukin-1 beta (IL-1 beta)	Interleukin-2 (IL-2)	Interleukin-3 (IL-3)	Interleukin-4 (IL-4)	Interleukin-5 (IL-5)	Interleukin-6 (IL-6)	Interleukin-7 (IL-7)	Interleukin-10 (IL-10)	Interleukin-11 (IL-11)	Interleukin-12 Subunit p70 (IL-12p70)	Interleukin-17A (IL-17A)	Interleukin-18 (IL-18)	Leukemia Inhibitory Factor (LIF)
Liver - wt - M1P3	2.7	0.058	11	1010	61	7.5	16	267	2.7	7.6	0.28	<1.4	<0.0053	2.8	9.0	18	21	177	1.4	13	<0.85	18	<0.15	2.3	0.046	335	19	0.049	0.0047	6.3	210
Liver - wt - M2P3	2.9	0.078	13	683	84	7.1	13	441	2.0	13	0.28	<1.4	<0.0053	2.8	10	13	23	293	1.1	21	<0.85	17	<0.15	<0.75	0.026	94	<14	<0.027	0.0026	4.8	154
Liver - wt - M3P3	4.6	0.090	13	715	94	8.3	14	557	2.7	18	0.33	<1.4	0.0089	3.1	15	11	22	134	1.0	<9.9	<0.85	<14	<0.15	0.96	0.036	127	<14	0.034	0.0031	4.9	253
Liver - wt - M4P3	4.1	0.15	24	590	126	13	18	658	2.4	18	0.44	<1.4	<0.0053	2.9	11	27	37	557	1.7	13	<0.85	17	<0.15	1.6	0.039	168	<14	0.044	0.0041	7.8	341
Liver - wt - M5P3	13	0.11	36	652	55	9.7	9.5	890	1.6	85	0.41	<1.4	<0.0053	2.9	18	17	30	709	1.8	<9.9	<0.85	<14	<0.15	2.9	<0.024	152	<14	<0.027	0.0026	13	168
Liver - wt - M6P3	5.9	0.16	41	1340	93	17	30	580	3.3	34	0.34	1.8	<0.0053	3.0	15	21	37	335	3.7	24	<0.85	30	0.35	2.2	0.091	210	25	0.087	0.0073	9.0	621
Liver - KO - M1P3	14	0.17	37	1010	66	8.5	16	267	2.7	7.6	0.28	<1.4	<0.0053	2.8	9.0	18	21	177	1.4	13	<0.85	18	<0.15	2.3	0.046	335	19	0.049	0.0047	6.3	210
Liver - KO - M2P3	5.4	0.12	15	683	79	9.3	11	628	3.1	13	0.25	<1.4	<0.0053	3.1	11	17	24	313	1.9	21	<0.85	17	0.18	2.1	0.050	202	<14	0.054	0.0049	5.3	253
Liver - KO - M3P3	3.9	0.10	32	877	79	11	20	482	2.4	22	0.31	<1.4	<0.0053	2.9	15	23	29	603	2.3	15	<0.85	17	0.18	1.8	0.039	143	<14	0.054	0.0031	6.7	341
Liver - KO - M4P3	6.3	0.14	21	441	78	9.5	13	733	2.3	25	0.26	<1.4	<0.0053	3.1	13	20	48	409	1.4	11	<0.85	<14	0.16	1.6	0.036	156	<14	0.044	0.0041	6.7	239
Liver - KO - M5P3	5.6	0.12	16	590	85	12	17	701	2.4	14	0.28	<1.4	0.0091	3.2	19	14	29	204	1.5	11	1.2	17	<0.15	1.4	0.046	143	<14	0.049	0.0047	6.3	224
Liver - KO - M6P3	4.7	0.16	24	877	100	12	17	683	2.4	21	0.32	<1.4	0.0085	3.2	35	22	50	556	2.0	16	1.2	20	0.16	1.6	0.051	168	<14	0.056	0.0057	7.3	319
Kidney - wt - M1P3	8.8	0.079	117	977	4.4	26	28	833	4.2	19	0.048	<1.4	<0.0053	2.6	5.4	35	12	92	1.4	34	2.8	16	<0.15	<0.75	0.10	1160	15	0.35	0.033	8.2	182
Kidney - wt - M2P3	4.5	0.12	562	715	54	15	25	904	2.2	26	0.24	<1.4	<0.0053	2.6	5.9	11	48	33	2.2	13	<0.85	20	0.18	<0.75	0.053	119	15	0.064	0.0052	5.5	370
Kidney - wt - M3P3	4.8	0.090	332	715	32	15	23	896	2.0	21	0.25	<1.4	<0.0053	3.2	6.5	12	22	42	2.2	16	<0.85	23	0.17	1.2	0.061	202	19	0.069	0.0036	4.7	429
Kidney - wt - M4P3	6.2	0.11	379	927	66	18	30	837	3.1	29	0.33	<1.4	0.0062	2.6	5.1	18	48	65	3.0	23	<0.85	29	0.25	1.1	0.10	231	31	0.099	0.0067	6.5	592
Kidney - wt - M5P3	4.2	0.082	446	1330	69	20	34	779	3.4	32	0.27	2.0	0.0066	2.7	4.3	27	38	144	3.4	30	1.4	31	0.37	2.3	0.13	319	37	0.11	0.010	7.7	710
Kidney - wt - M6P3	7.2	0.10	217	3180	81	36	76	429	11	41	0.24	5.0	0.012	7.4	10	78	84	498	8.3	115	2.8	91	0.73	3.4	0.36	1710	100	0.35	0.026	23	1250
Kidney - KO - M1P3	4.6	0.083	264	1660	45	18	28	498	4.2	24	0.29	1.8	<0.0053	2.7	6.3	23	52	85	3.2	29	1.4	28	0.26	1.5	0.12	427	39	0.11	0.0078	6.7	548
Kidney - KO - M2P3	7.3	0.086	197	1470	48	19	38	737	4.3	35	0.32	2.6	0.0070	3.2	11	31	40	75	4.0	33	1.7	38	0.47	2.2	0.12	319	44	0.14	0.011	8.2	754
Kidney - KO - M3P3	4.8	0.070	329	1430	43	20	35	600	4.3	27	0.23	2.6	0.0078	2.8	11	28	51	79	4.0	35	2.1	35	0.35	2.1	0.14	352	47	0.14	0.011	8.2	651
Kidney - KO - M4P3	5.1	0.070	277	1620	51	21	37	448	3.2	31	0.19	3.3	0.012	2.7	8.5	27	60	95	4.0	37	1.9	36	0.43	2.3	0.14	402	52	0.16	0.0080	7.7	695
Kidney - KO - M5P3	6.1	0.094	202	1150	56	18	30	682	3.2	51	0.35	2.3	0.0089	3.1	12	18	60	61	3.5	40	1.4	31	0.35	1.3	0.098	218	34	0.10	0.0067	7.7	629
Kidney - KO - M6P3	4.8	0.089	244	1010	51	15	22	783	4.8	33	0.30	2.0	0.0078	2.9	20	22	42	58	2.7	24	1.4	24	0.30	1.2	0.084	277	28	0.087	0.0067	7.1	444
Brain - wt - M1P3	0.74	0.018	7.9	328	6.0	4.4	5.0	62	2.9	14	0.067	<1.4	<0.0053	2.5	1.0	<7.6	28	29	0.91	<9.9	<0.85	<14	<0.15	<0.75	<0.024	47	<14	<0.027	0.0052	4.3	94
Brain - wt - M2P3	0.57	0.015	6.8	301	5.0	5.0	6.0	52	3.6	16	0.067	<1.4	<0.0053	2.6	0.75	<7.6	14	38	0.95	<9.9	<0.85	<14	<0.15	<0.75	<0.024	54	<14	<0.027	0.0031	5.3	100
Brain - wt - M3P3	1.0	0.020	5.4	301	6.3	5.0	5.2	71	3.4	15	0.086	<1.4	<0.0053	2.7	1.3	<7.6	16	<26	0.81	<9.9	<0.85	<14	<0.15	<0.75	<0.024	54	<14	0.034	0.0054	4.3	<93
Brain - wt - M4P3	0.58	0.015	7.0	186	6.6	3.4	5.0	48	4.4	13	0.071	<1.4	<0.0053	2.5	0.68	<7.6	15	40	0.81	10	<0.85	<14	<0.15	<0.75	<0.024	70	<14	0.029	0.0026	4.6	113
Brain - wt - M5P3	0.42	0.015	4.7	275	7.5	4.6	5.5	69	3.2	12	0.058	<1.4	<0.0053	2.5	0.55	<7.6	15	33	0.91	<9.9	<0.85	<14	<0.15	<0.75	<0.024	86	<14	0.029	0.0026	4.6	113
Brain - wt - M6P3	0.66	0.021	5.9	223	5.6	4.2	5.5	40	3.9	12	0.065	<1.4	<0.0053	2.5	1.1	<7.6	21	35	0.70	<9.9	<0.85	14	<0.15	<0.75	<0.024	62	<14	<0.027	0.0036	4.3	113
Brain - KO - M1P3	0.49	0.014	5.4	288	5.5	3.6	6.5	41	2.6	12	0.049	<1.4	<0.0053	2.5	1.0	<7.6	14	<26	0.91	<9.9	<0.85	<14	<0.15	<0.75	<0.024	62	<14	<0.027	0.0021	4.5	140
Brain - KO - M2P3	0.81	0.018	5.4	301	6.0	<3.1	6.0	53	3.2	15	0.071	<1.4	<0.0053	2.7	1.0	<7.6	11	<26	0.91	<9.9	<0.85	<14	<0.15	<0.75	<0.024	47	<14	<0.027	0.0021	5.3	100
Brain - KO - M3P3	<0.41	0.016	4.4	223	5.4	3.4	5.0	50	3.0	13	0.071	<1.4	<0.0053	2.6	0.93	<7.6	16	<26	<0.69	<9.9	<0.85	<14	<0.15	<0.75	<0.024	<44	<14	<0.027	0.0023	4.1	<93
Brain - KO - M4P3	0.53	0.017	3.6	301	6.4	4.2	7.0	59	3.5	14	0.080	<1.4	<0.0053	2.5	0.99	<7.6	46	31	0.95	<9.9	<0.85	16	<0.15	<0.75	<0.024	54	<14	0.039	0.0026	4.9	<93
Brain - KO - M5P3	0.62	0.018	4.7	223	5.8	4.6	6.8	55	3.4	12	0.074	<1.4	<0.0053	2.7	0.99	<7.6	13	<26	0.70	<9.9	<0.85	<14	<0.15	<0.75	<0.024	62	<14	<0.027	0.0041	3.6	<93
Brain - KO - M6P3	0.60	0.019	4.0	301	6.6	5.0	7.5	68	3.6	15	0.11	<1.4	<0.0053	2.6	1.7	<7.6	20	27	0.81	<9.9	<0.85	<14	<0.15	<0.75	<0.024	<44	<14	0.034	0.0021	5.1	113

Appendix Table 2 Continued

ALT ID	Samples	Analytes	Lymphotactin	Macrophage Colony-Stimulating Factor-1 (M-CSF-1)	Macrophage-Derived Chemokine (MDC)	Macrophage Inflammatory Protein-1alpha (MIP-1 alpha)	Macrophage Inflammatory Protein-1 beta (MIP-1 beta)	Macrophage Inflammatory Protein-1 gamma (MIP-1 gamma)	Macrophage Inflammatory Protein-2 (MIP-2)	Macrophage Inflammatory Protein-3 beta (MIP-3 beta)	Matrix Metalloproteinase-9 (MMP-9)	Monocyte Chemotactic Protein 1 (MCP-1)	Monocyte Chemotactic Protein 3 (MCP-3)	Monocyte Chemotactic Protein-5 (MCP-5)	Myeloperoxidase (MPO)	Myoglobin	Oncostatin-M (OSM)	Serum Amyloid P-Component (SAP)	Stem Cell Factor (SCF)	T-Cell-Specific Protein RANTES (RANTES)	Thrombopoietin	Tissue Inhibitor of Metalloproteinases 1 Mouse (TIMP-1 Mouse)	Tumor Necrosis Factor alpha (TNF-alpha)	Vascular Cell Adhesion Molecule-1 (VCAM-1)	Vascular Endothelial Growth Factor A (VEGF-A)	von Willebrand factor (vWF)	Cortisol (Cortisol)	Glucagon	Glucagon-like Peptide 1, total (GLP-1 total)	Peptide YY (PYY)	Progesterone	Testosterone, Total
		Units	pg/mL	ng/mL	pg/mL	ng/mL	pg/mL	ng/mL	pg/mL	ng/mL	ng/mL	pg/mL	pg/mL	pg/mL	ng/mL	ng/mL	ng/mL	ug/mL	pg/mL	pg/mL	ng/mL	ng/mL	ng/mL	ng/mL	pg/mL	pg/mL	pg/mL	pg/mL	ng/mL	ng/mL	ng/mL
		Myriad RBM LLOQ	4.3	0.010	11	0.65	11	0.0067	2.2	0.23	0.14	1.6	0.88	0.47	0.13	0.060	0.043	0.045	58	0.0057	12	0.017	0.022	0.054	32	0.45	2.0	64	1.5	20	0.65	0.10
		Myriad RBM LLOD	3.7	0.0065	6.9	0.82	9.4	0.0045	1.1	0.073	0.099	0.98	0.83	0.33	0.096	0.057	0.019	0.032	31	0.0052	6.0	0.0043	0.0059	0.033	44	0.27	1.2	31	1.1	15	0.45	0.061
25	Liver - wt-M1P3		38	0.10	115	0.80	56	1.6	3.5	0.58	1.9	<1.6	5.8	1.6	5.0	3800	<0.043	0.63	195	0.053	<12	0.049	0.054	8.0	425	5.5	<2.0	124	1.9	68	0.91	6.9
26	Liver - wt-M2P3		49	0.14	322	1.1	31	2.9	<2.2	0.57	2.4	<1.6	5.8	1.2	1.8	238	0.051	0.35	102	0.068	<12	0.053	0.030	11	379	6.7	4.2	<64	<1.5	54	1.0	7.4
27	Liver - wt-M3P3		39	0.15	307	1.2	40	3.4	3.5	0.60	6.2	2.3	7.5	2.1	1.8	5870	0.056	0.32	125	0.046	<12	0.062	0.033	13	519	8.0	5.2	<64	<1.5	57	0.91	10
28	Liver - wt-M4P3		57	0.19	384	1.6	39	5.1	3.6	0.82	4.0	4.4	8.8	1.8	1.8	3810	0.080	0.61	148	0.060	16	0.11	0.047	12	592	9.9	3.8	85	2.0	72	0.90	7.4
29	Liver - wt-M5P3		32	0.20	351	1.1	139	3.7	3.2	0.53	7.9	3.3	8.7	2.7	4.9	4680	<0.043	0.45	218	0.12	<12	0.076	0.023	16	764	11	4.5	67	13	92	0.98	5.9
30	Liver - wt-M6P3		75	0.24	452	1.6	155	4.1	8.2	1.1	3.4	3.9	11	3.0	4.9	6110	0.13	0.51	329	0.098	16	0.10	0.12	20	784	13	5.5	162	3.3	145	1.4	7.1
31	Liver - KO-M1P3		20	0.18	228	0.95	140	3.4	2.4	0.34	9.2	18	13	2.0	5.0	3800	<0.043	0.63	195	0.053	<12	0.085	<0.022	17	1070	10	7.5	112	31	68	0.91	6.9
32	Liver - KO-M2P3		53	0.14	286	1.2	69	3.2	5.2	0.62	2.8	3.3	7.4	2.1	1.8	116	0.11	0.37	163	0.042	<12	0.062	0.056	9.6	689	6.2	8.2	85	1.7	71	1.1	6.1
33	Liver - KO-M3P3		51	0.18	408	1.3	69	3.7	4.3	0.74	4.1	4.6	8.5	2.0	139	12	0.084	0.44	132	0.064	14	0.066	0.053	12	494	9.4	7.1	67	2.1	98	1.4	11
34	Liver - KO-M4P3		46	0.15	486	1.1	56	4.9	3.6	0.63	5.7	4.9	10	2.4	145	3780	0.068	0.61	117	0.077	<12	0.075	0.035	9.8	543	5.4	7.9	67	2.1	60	1.3	10
35	Liver - KO-M5P3		44	0.16	295	1.4	55	3.8	4.9	0.70	4.4	6.8	9.8	2.0	105	78	0.089	0.34	152	0.038	<12	0.072	0.049	12	527	7.3	7.0	85	2.5	87	0.91	13
36	Liver - KO-M6P3		60	0.16	484	1.4	73	5.3	5.4	0.82	5.9	8.8	12	2.6	137	2.2	0.077	0.59	179	0.089	13	0.085	0.066	13	720	8.1	9.3	112	2.4	65	1.9	12
37	Kidney - wt-M1P3		45	0.48	26	<0.65	45	0.16	3.3	0.41	3.7	<1.6	4.2	0.66	0.67	24	0.24	0.082	853	0.0082	<12	0.043	0.025	16	503	5.9	2.1	92	7.0	170	<0.65	29
38	Kidney - wt-M2P3		64	1.1	163	1.4	115	3.6	4.6	0.84	1.8	3.1	23	2.9	1.6	23	0.082	0.47	1080	0.058	19	0.10	0.072	20	404	12	3.1	112	2.5	68	2.4	34
39	Kidney - wt-M3P3		56	0.62	99	0.95	81	1.7	5.6	0.82	4.4	<1.6	14	3.9	1.7	15	0.089	0.30	1140	0.037	17	0.083	0.074	21	437	13	<2.0	135	2.1	41	0.68	37
40	Kidney - wt-M4P3		69	0.68	147	1.7	130	3.8	9.0	1.1	2.4	3.4	17	2.9	2.2	8.8	0.13	0.43	1130	0.041	28	0.12	0.097	20	552	16	<2.0	154	3.3	84	3.6	29
41	Kidney - wt-M5P3		78	0.73	181	1.9	165	3.3	12	1.3	2.5	4.2	28	4.1	2.9	18	0.17	0.30	1110	0.041	28	0.13	0.14	20	568	16	<2.0	199	3.6	103	3.3	28
42	Kidney - wt-M6P3		165	0.79	279	3.6	379	2.7	22	2.2	3.4	3.8	37	5.9	5.0	32	0.51	0.50	1610	0.061	61	0.27	0.34	22	1440	23	<2.0	576	8.7	326	7.7	20
43	Kidney - KO-M1P3		75	0.60	179	1.9	157	2.9	8.8	1.1	2.3	6.5	21	4.6	3.7	13	0.18	0.34	918	0.065	28	0.12	0.11	22	624	13	<2.0	185	3.3	100	3.3	18
44	Kidney - KO-M2P3		87	0.52	195	1.8	192	2.7	12	1.3	2.9	6.2	19	4.3	2.8	3.5	0.20	0.30	1110	0.061	28	0.097	0.16	19	584	17	<2.0	217	4.1	148	1.6	21
45	Kidney - KO-M3P3		81	0.60	155	1.7	188	2.8	14	1.3	2.0	5.3	19	4.5	3.3	2.3	0.19	0.33	1220	0.055	23	0.10	0.14	18	653	15	2.1	199	4.0	136	2.2	21
46	Kidney - KO-M4P3		81	0.65	155	1.7	130	3.8	9.0	1.1	2.4	3.4	17	2.9	2.2	8.8	0.13	0.43	1130	0.041	28	0.12	0.097	20	552	16	<2.0	154	3.3	84	3.6	29
47	Kidney - KO-M5P3		72	0.49	148	1.9	162	2.9	9.8	1.1	5.4	6.2	25	3.6	4.9	1.0	0.19	0.28	1190	0.077	29	0.13	0.15	19	697	14	<2.0	217	3.6	138	2.3	20
48	Kidney - KO-M6P3		66	0.69	158	1.7	133	3.9	9.1	1.1	3.0	8.6	23	4.0	2.5	3.6	0.13	0.37	1200	0.060	22	0.12	0.094	21	632	13	2.9	170	2.3	72	8.1	24
49	Brain - wt-M1P3		12	0.43	19	<0.65	26	0.18	2.4	0.37	0.54	<1.6	6.7	0.57	0.57	22	<0.043	0.070	195	<0.0057	<12	<0.017	0.026	7.4	190	4.1	<2.0	76	<1.5	<20	1.5	11
50	Brain - wt-M2P3		16	0.52	21	<0.65	21	0.15	3.3	0.38	0.47	<1.6	8.5	2.0	0.33	16	<0.043	0.090	218	<0.0057	<12	0.021	0.028	7.3	280	4.3	<2.0	100	<1.5	<20	1.0	11
51	Brain - wt-M3P3		13	0.61	22	<0.65	11	0.16	2.2	0.40	2.6	<1.6	8.2	2.1	2.5	124	<0.043	0.091	195	<0.0057	<12	0.017	0.027	9.3	158	4.4	<2.0	85	<1.5	<20	1.9	11
52	Brain - wt-M4P3		12	0.83	23	<0.65	14	0.17	<2.2	0.33	0.72	<1.6	8.3	1.7	0.59	24	<0.043	0.092	278	<0.0057	<12	0.022	0.035	7.4	235	3.8	<2.0	76	<1.5	<20	1.8	9.9
53	Brain - wt-M5P3		17	0.51	19	<0.65	26	0.17	<2.2	0.38	0.98	<1.6	6.6	1.6	0.78	7.6	<0.043	0.080	242	<0.0057	<12	<0.017	0.030	6.7	178	4.7	<2.0	85	<1.5	<20	0.96	10
54	Brain - wt-M6P3		14	0.48	21	<0.65	19	0.16	2.4	0.33	0.68	<1.6	8.4	2.0	0.47	24	<0.043	0.11	199	<0.0057	<12	0.017	0.035	9.9	190	4.2	<2.0	92	<1.5	<20	1.1	12
55	Brain - KO-M1P3		19	0.34	24	<0.65	31	0.13	2.5	0.33	0.63	<1.6	7.1	1.9	0.71	27	<0.043	0.084	179	<0.0057	<12	<0.017	0.038	5.9	174	4.3	<2.0	100	<1.5	<20	1.1	9.2
56	Brain - KO-M2P3		12	0.55	22	<0.65	29	0.14	<2.2	0.36	3.7	<1.6	6.9	2.0	3.9	14	<0.043	0.080	179	<0.0057	<12	0.020	0.032	9.2	190	5.1	<2.0	67	<1.5	<20	1.4	12
57	Brain - KO-M3P3		14	0.44	21	<0.65	20	0.16	<2.2	0.36	2.4	<1.6	7.4	2.2	3.5	294	<0.043	0.089	175	<0.0057	<12	0.021	0.023	7.7	223	4.9	<2.0	85	<1.5	<20	1.7	12
58	Brain - KO-M4P3		14	0.49	26	<0.65	18	0.20	2.4	0.38	1.6	<1.6	7.6	3.2	1.9	31	<0.043	0.096	230	<0.0057	<12	0.020	0.034	9.8	239	4.6	<2.0	100	<1.5	<20	1.3	11
59	Brain - KO-M5P3		15	0.53	20	<0.65	24	0.13	2.4	0.41	1.5	<1.6	7.4	2.0	1.2	44	<0.043	0.075	195	<0.0057	<12	<0.017	0.030	8.3	198	5.9	<2.0	85	<1.5	<20	1.1	11
60	Brain - KO-M6P3		19	0.61	29	<0.65	31	0.18	2.7	0.43	1.1	<1.6	6.9	2.4	1.2	36	<0.043	0.11	230	<0.0057	<12	0.022	0.038	11	264	6.8	<2.0	100	<1.5	<20	5.4	9.5

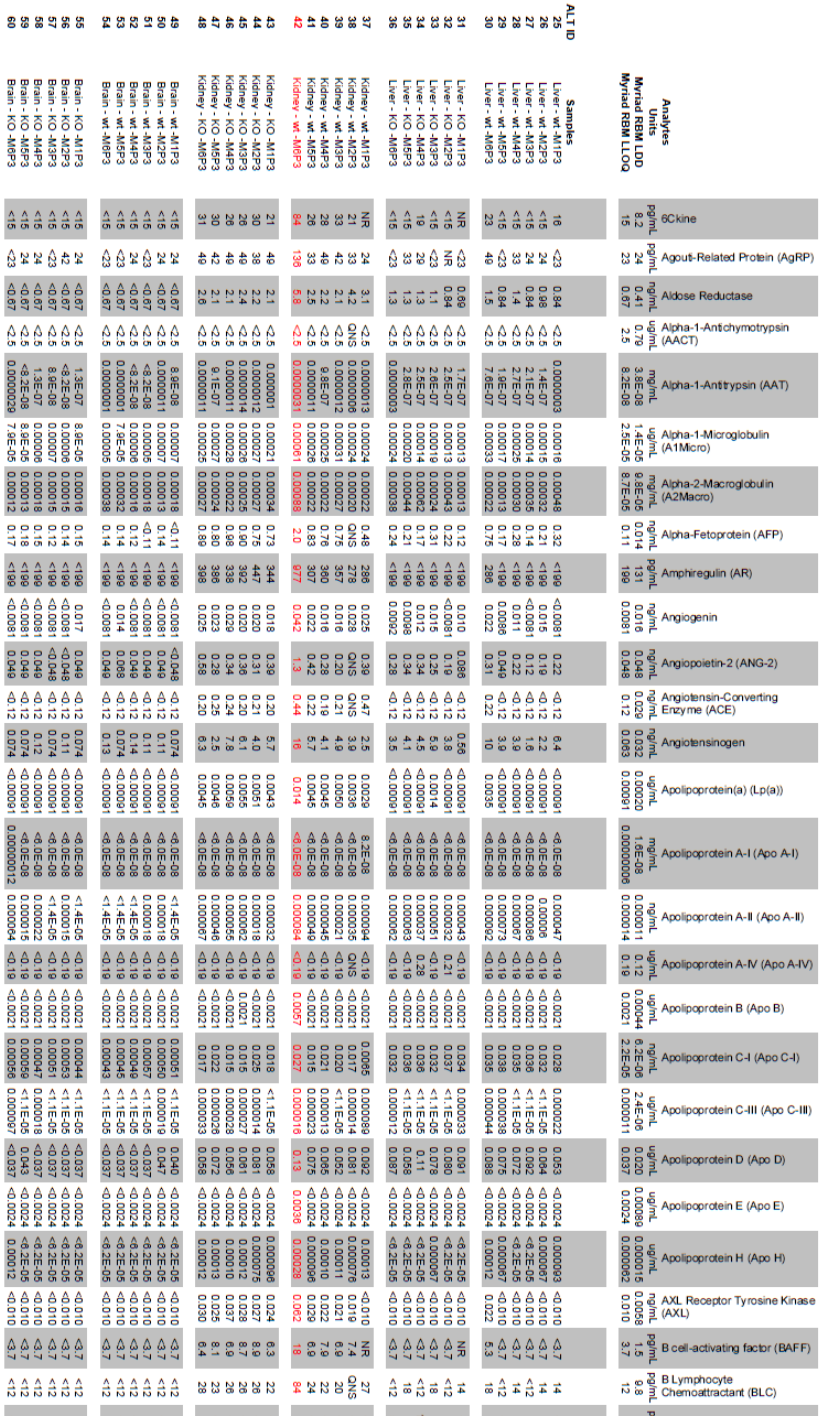
Appendix Table 2 Continued



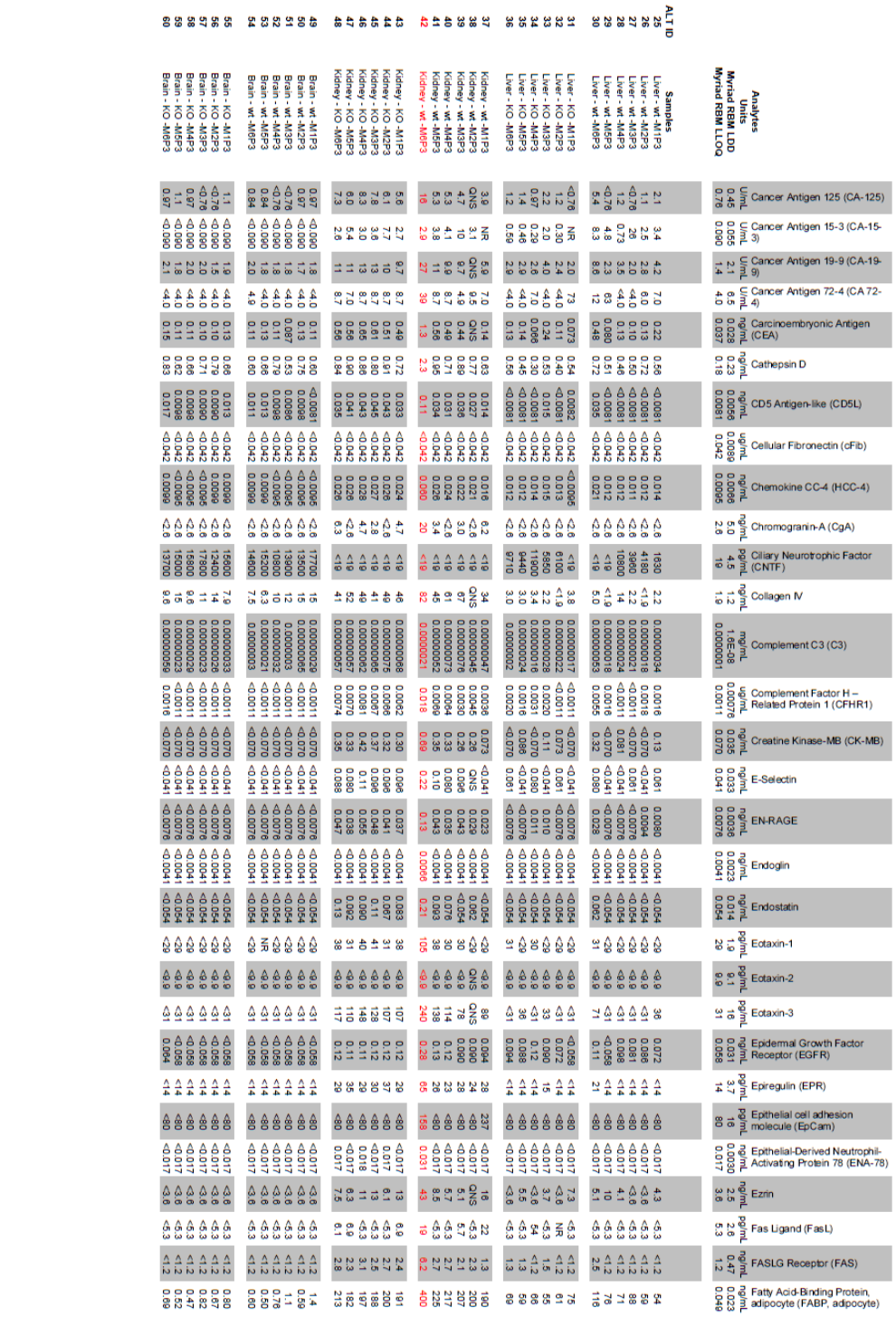
ALT ID	Samples	Adiponectin	Insulin-Like Growth Factor I (IGF-I)	Beta-2-Microglobulin (B2M)	Calbindin	Clusterin (CLU)	Cystatin-C	Glutathione S-Transferase alpha (GST-alpha)	Glutathione S-Transferase Mu (GST-Mu)	Kidney Injury Molecule-1 Rat (KIM-1 Rat)	Neutrophil Gelatinase-Associated Lipocalin (NGAL)	Osteopontin
	Analytes											
	Units	ug/mL	ng/mL	ug/mL	ng/mL	ug/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL	ng/mL
	Myriad RBM LDD	0.0014	0.031	0.063	0.021	2E-04	0.009	2.1	0.90	0.0034	0.00091	8E-04
	Myriad RBM LLOQ	0.00030	0.031	0.15	0.031	4E-04	0.023	2.7	3.0	0.031	0.0020	0.002
25	Liver - wt -M1P3	<0.00030	2.2	<0.15	0.50	2.0	31	3400	<3.0	0.056	<0.0020	2.5
26	Liver - wt -M2P3	<0.00030	2.8	<0.15	41	2.0	41	3490	<3.0	<0.031	<0.0020	4.7
27	Liver - wt -M3P3	<0.00030	3.5	<0.15	3.2	3.2	54	3560	<3.0	0.035	<0.0020	3.8
28	Liver - wt -M4P3	<0.00030	2.5	<0.15	2.7	2.9	78	3040	<3.0	0.040	<0.0020	6.7
29	Liver - wt -M5P3	<0.00030	14	<0.15	2.6	3.9	91	3190	3.2	<0.031	<0.0020	2.1
30	Liver - wt -M6P3	<0.00030	5.1	<0.15	4400	2.7	72	3080	4.4	0.19	0.0024	9.8
31	Liver - KO -M1P3	<0.00030	13	<0.15	0.90	2.4	126	3170	4.0	<0.031	<0.0020	4.4
32	Liver - KO -M2P3	<0.00030	2.4	<0.15	4.9	4.0	59	3910	<3.0	0.048	<0.0020	5.3
33	Liver - KO -M3P3	<0.00030	3.2	<0.15	1.0	2.1	57	2890	<3.0	0.066	<0.0020	6.0
34	Liver - KO -M4P3	<0.00030	3.0	<0.15	0.72	2.1	81	2820	<3.0	<0.031	<0.0020	7.3
35	Liver - KO -M5P3	<0.00030	2.0	<0.15	0.84	3.7	53	3360	<3.0	0.035	<0.0020	4.3
36	Liver - KO -M6P3	<0.00030	3.0	<0.15	0.92	2.6	76	2710	<3.0	0.053	<0.0020	6.8
37	Kidney - wt -M1P3	<0.00030	0.83	<0.15	1010	1.1	158	4990	<3.0	0.056	0.0050	30
38	Kidney - wt -M2P3	<0.00030	7.9	<0.15	719	3.1	368	7150	4.0	0.076	0.0022	70
39	Kidney - wt -M3P3	<0.00030	2.8	<0.15	1220	3.5	203	4150	<3.0	0.12	<0.0020	26
40	Kidney - wt -M4P3	<0.00030	5.2	<0.15	822	3.4	314	6530	3.4	0.16	<0.0020	59
41	Kidney - wt -M5P3	<0.00030	5.0	<0.15	742	2.8	334	6130	4.4	0.18	0.0021	60
42	Kidney - wt -M6P3	0.00062	8.7	<0.15	4230	2.8	198	12400	9.0	0.66	0.012	65
43	Kidney - KO -M1P3	<0.00030	4.7	<0.15	1200	2.1	162	6220	<3.0	0.17	0.0026	35
44	Kidney - KO -M2P3	<0.00030	5.3	<0.15	1240	3.8	208	4830	4.4	0.22	0.0038	22
45	Kidney - KO -M3P3	<0.00030	5.7	<0.15	844	2.4	195	6210	5.2	0.22	0.0034	46
46	Kidney - KO -M4P3	<0.00030	7.8	<0.15	915	2.2	216	6200	5.4	0.23	0.0030	50
47	Kidney - KO -M5P3	<0.00030	4.1	<0.15	949	3.8	209	4170	4.0	0.18	0.0022	24
48	Kidney - KO -M6P3	<0.00030	7.5	<0.15	987	3.1	241	6500	3.6	0.14	0.0032	46
49	Brain - wt -M1P3	<0.00030	0.26	<0.15	2100	1.4	1070	33	<3.0	0.058	<0.0020	5.5
50	Brain - wt -M2P3	<0.00030	0.29	<0.15	1880	1.1	931	19	<3.0	0.056	<0.0020	4.2
51	Brain - wt -M3P3	<0.00030	0.31	<0.15	2320	1.3	1220	13	<3.0	0.056	<0.0020	6.3
52	Brain - wt -M4P3	<0.00030	0.29	<0.15	2200	1.4	1070	8.2	<3.0	0.050	<0.0020	3.6
53	Brain - wt -M5P3	<0.00030	0.26	<0.15	2240	1.3	1000	8.9	<3.0	0.061	<0.0020	3.5
54	Brain - wt -M6P3	<0.00030	0.35	<0.15	2100	1.4	979	8.7	<3.0	0.056	<0.0020	4.1
55	Brain - KO -M1P3	<0.00030	0.27	<0.15	2680	0.89	862	28	<3.0	0.050	<0.0020	3.3
56	Brain - KO -M2P3	<0.00030	0.29	<0.15	2440	1.3	1040	22	<3.0	0.037	<0.0020	15
57	Brain - KO -M3P3	<0.00030	0.37	<0.15	2200	1.1	878	12	<3.0	0.035	<0.0020	11
58	Brain - KO -M4P3	<0.00030	0.29	<0.15	2340	1.1	1190	6.3	<3.0	0.040	<0.0020	5.8
59	Brain - KO -M5P3	<0.00030	0.25	<0.15	2540	1.0	914	4.7	<3.0	0.056	<0.0020	4.5
60	Brain - KO -M6P3	<0.00030	0.31	<0.15	2740	1.2	995	4.5	<3.0	0.061	<0.0020	3.9

Appendix Table 3: Tissue Results from Human Discovery Product.

The single wild type kidney sample with red font was excluded from data analysis due to incorrect dilution of the tissue with lysis buffer during tissue homogenization. LDD, LLOQ, QNS and NR are explained in the caption for appendix table 1. Care should be taken prior to interpreting results from the Human Discovery product since the assays are designed to detect human protein and may not cross-react specifically with rodent protein.

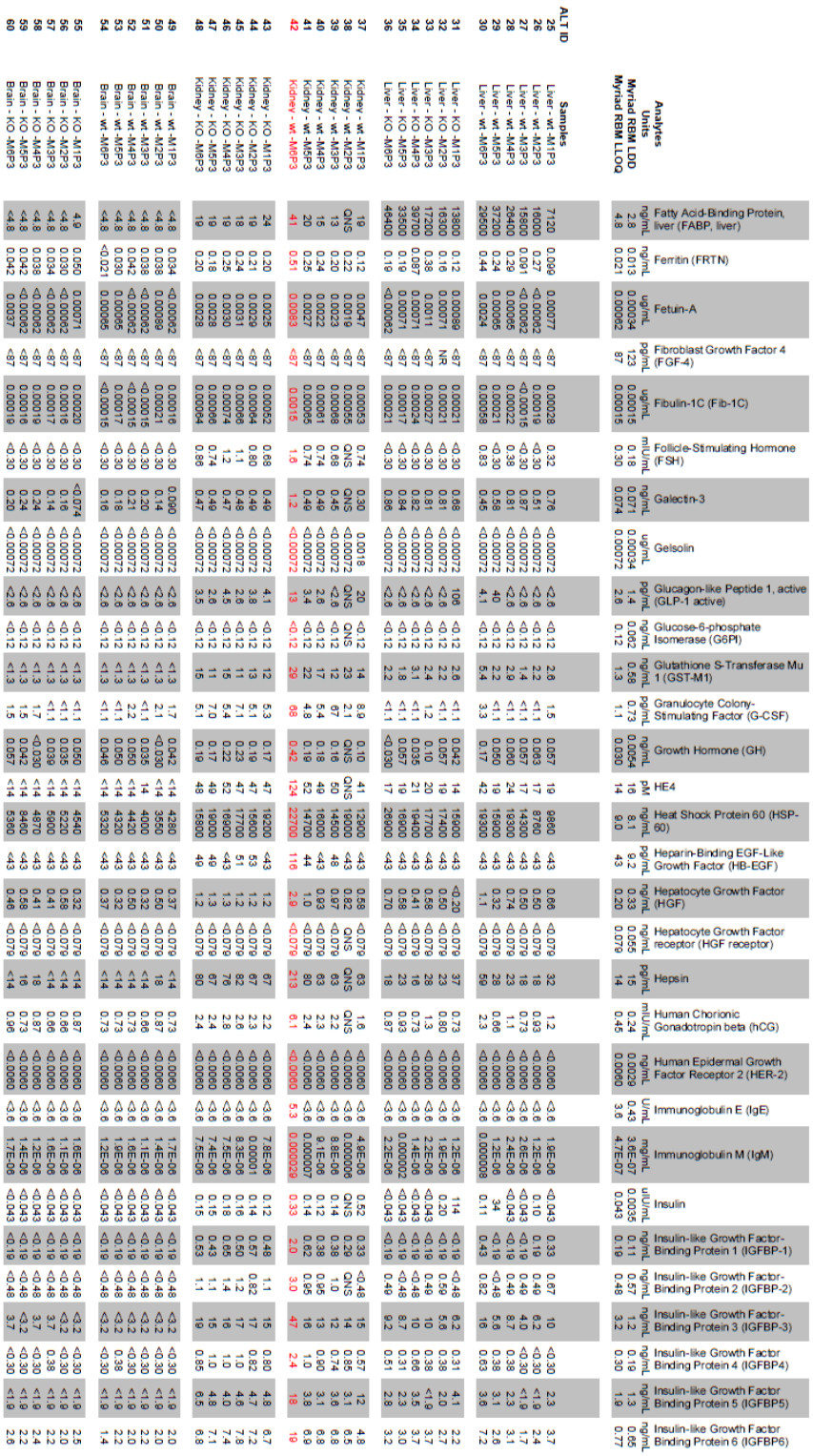


Appendix Table 3 Continued

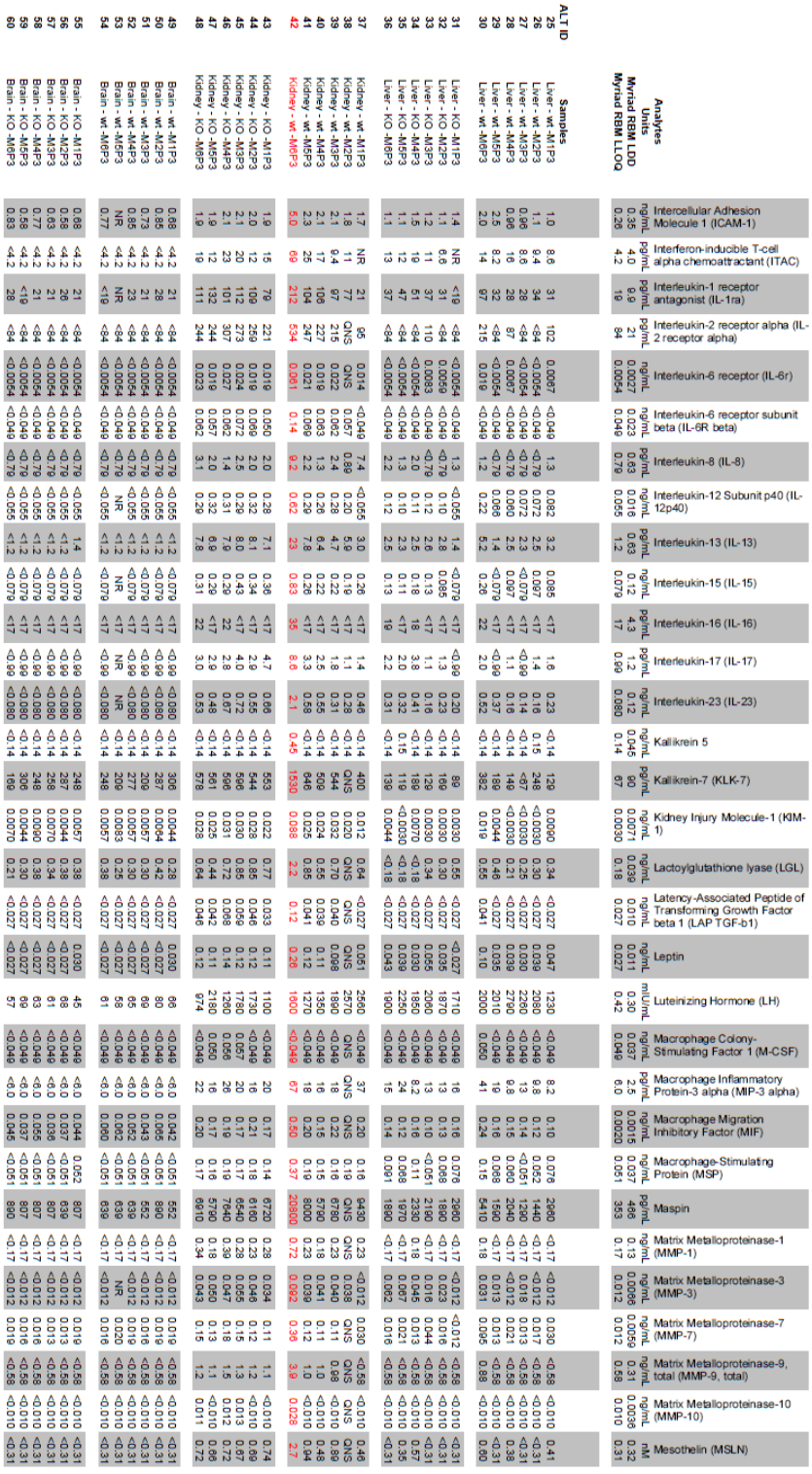


ALT ID	Samples	Cancer Antigen 125 (CA-125)	Cancer Antigen 15-3 (CA-15-3)	Cancer Antigen 19-9 (CA-19-9)	Cancer Antigen 72-4 (CA 72-4)	Carcinoembryonic Antigen (CEA)	Cathepsin D	CD5 Antigen-like (CD5L)	Cellular Fibronectin (cFib)	Chemokine CC-4 (HCC-4)	Chromogranin-A (CgA)	Ciliary Neurotrophic Factor (CNTF)	Collagen IV	Complement C3 (C3)	Complement Factor H – Related Protein 1 (CFHR1)	Creatine Kinase-MB (CK-MB)	E-Selectin	EN-RAGE	Endoglin	Endostatin	Eotaxin-1	Eotaxin-2	Eotaxin-3	Epidermal Growth Factor Receptor (EGFR)	Epiregulin (EPR)	Epithelial cell adhesion molecule (EpCam)	Epithelial-Derived Neutrophil-Activating Protein 78 (ENA-78)	Ezrin	Fas Ligand (FasL)	FASLG Receptor (FAS)	Fatty Acid-Binding Protein, adipocyte (FABP, adipocyte)
	Myriad RBM LLOQ	0.78	0.090	1.4	4.0	0.037	0.18	0.0081	0.042	0.0085	2.8	19	1.9	0.0000001	0.0011	0.070	0.041	0.0076	0.0041	0.064	29	8.9	31	0.088	14	80	<0.017	3.6	5.3	1.2	0.049
	Myriad RBM LLOD	0.45	0.055	2.1	9.5	0.023	0.13	0.0059	0.0089	0.0066	0.0	4.5	1.2	1.8E-08	0.00076	0.035	0.033	0.0036	0.0023	0.014	19	8.9	10	0.071	3.7	18	0.0020	2.5	2.8	0.47	0.023
	Analytes																														
	Units	U/mL	U/mL	U/mL	U/mL	ng/mL	ng/mL	ng/mL	ug/mL	ng/mL	ng/mL	pg/mL	ng/mL	mg/mL	ug/mL	ng/mL	ng/mL	ng/mL	pg/mL	ng/mL	pg/mL	pg/mL	pg/mL	ng/mL	pg/mL	pg/mL	ng/mL	pg/mL	pg/mL	pg/mL	ng/mL
25	Liver - wt-M1F3	2.1	3.4	4.2	7.0	0.22	0.56	<0.0081	<0.042	0.014	<2.6	1630	2.2	0.00000034	0.0016	0.13	0.061	0.0080	<0.0041	<0.054	<29	<8.9	36	0.072	<14	<80	<0.017	4.3	<5.3	<1.2	54
26	Liver - wt-M2F3	1.1	2.5	2.8	6.0	0.13	0.72	<0.0081	<0.042	0.012	<2.6	4180	<1.9	0.00000018	0.0018	<0.070	<0.041	0.0094	<0.0041	<0.054	<29	<8.9	<31	0.086	<14	<80	<0.017	<3.6	<5.3	<1.2	59
27	Liver - wt-M3F3	<0.78	26	2.0	<4.0	0.10	0.50	<0.0081	<0.042	0.011	<2.6	3960	2.2	0.00000021	<0.0011	<0.070	<0.041	<0.0076	<0.0041	<0.054	<29	<8.9	<31	0.081	<14	<80	<0.017	4.1	<5.3	<1.2	88
28	Liver - wt-M4F3	1.2	0.73	3.5	<4.0	0.13	0.46	<0.0081	<0.042	0.012	<2.6	1080	14	0.00000024	0.0016	0.081	<0.041	<0.0076	<0.0041	<0.054	<29	<8.9	<31	0.098	<14	<80	<0.017	10	<5.3	<1.2	71
29	Liver - wt-M5F3	<0.78	4.8	2.3	63	0.080	0.51	<0.0081	<0.042	0.012	<2.6	<19	<1.9	0.00000018	0.0016	<0.070	<0.041	<0.0076	<0.0041	<0.054	<29	<8.9	<31	<0.058	<14	<80	<0.017	5.1	<5.3	<1.2	76
30	Liver - wt-M6F3	5.4	8.3	8.6	12	0.48	0.72	0.035	<0.042	0.021	<2.6	<19	<1.9	0.00000053	0.0055	0.32	0.080	0.028	<0.0041	0.062	31	<8.9	71	0.11	21	<80	<0.017	5.1	<5.3	2.5	116
31	Liver - KO-M1P3	<0.78	NR	2.0	73	0.073	0.54	0.0082	<0.042	<0.0095	<2.6	1630	3.8	0.00000017	<0.0011	<0.070	<0.041	<0.0076	<0.0041	<0.054	<29	<8.9	<31	0.072	<14	<80	<0.017	7.3	<5.3	<1.2	75
32	Liver - KO-M2P3	1.2	0.30	2.4	<4.0	0.11	0.40	<0.0081	<0.042	0.013	<2.6	6100	<1.9	0.00000022	<0.0011	0.073	<0.041	<0.0076	<0.0041	<0.054	<29	<8.9	<31	0.090	<14	<80	<0.017	<3.6	NR	<1.2	61
33	Liver - KO-M3P3	2.2	2.0	4.0	<4.0	0.24	0.53	0.015	<0.042	0.015	<2.6	5850	2.2	0.00000028	0.0020	0.11	<0.041	0.010	<0.0041	<0.054	<29	<8.9	33	0.090	15	<80	<0.017	3.7	<5.3	1.5	55
34	Liver - KO-M4F3	0.97	0.29	2.6	7.0	0.066	0.30	<0.0081	<0.042	0.014	<2.6	11900	3.4	0.00000016	0.0031	<0.070	0.080	0.011	<0.0041	<0.054	30	<8.9	<31	0.12	<14	<80	<0.017	<3.6	54	<1.2	66
35	Liver - KO-M5F3	1.4	0.46	2.9	<4.0	0.14	0.45	<0.0081	<0.042	0.012	<2.6	9440	3.0	0.00000024	0.0016	0.088	0.061	<0.0076	<0.0041	<0.054	<29	<8.9	36	0.088	<14	<80	<0.017	5.5	<5.3	1.3	59
36	Liver - KO-M6P3	1.2	0.59	2.9	<4.0	0.13	0.56	<0.0081	<0.042	0.012	<2.6	9710	3.0	0.00000002	0.0020	<0.070	0.061	<0.0076	<0.0041	<0.054	31	<8.9	<31	0.094	<14	<80	<0.017	16	<5.3	2.2	190
37	Kidney - wt-M1P3	3.9	NR	5.9	7.0	0.14	0.83	0.014	<0.042	0.016	6.2	<19	34	0.00000047	0.0038	0.073	<0.041	0.023	<0.0041	0.023	<29	<8.9	<31	0.094	28	237	<0.017	22	<5.3	1.3	190
38	Kidney - wt-M2P3	QNS	NR	QNS	9.5	QNS	0.77	0.027	<0.042	0.021	<2.6	<19	QNS	0.00000045	0.0045	0.28	QNS	0.043	<0.0041	0.062	<29	QNS	<31	0.090	24	<80	<0.017	16	<5.3	2.3	200
39	Kidney - wt-M3P3	4.7	3.1	9.7	4.9	0.44	0.89	0.038	<0.042	0.022	3.0	<19	67	0.00000076	0.0030	0.28	0.090	0.043	<0.0041	<0.054	<29	<8.9	<31	0.090	28	<80	<0.017	QNS	<5.3	2.1	207
40	Kidney - wt-M4P3	5.3	4.1	9.9	8.7	0.49	0.71	0.031	<0.042	0.024	<2.6	<19	61	0.00000073	0.0064	0.33	0.080	0.035	<0.0041	0.076	30	<8.9	30	0.12	23	<80	<0.017	5.7	<5.3	2.7	217
41	Kidney - wt-M5P3	5.3	3.8	11	8.7	0.56	0.95	0.034	<0.042	0.028	3.4	<19	46	0.00000062	0.0069	0.35	0.10	0.043	<0.0041	0.083	38	<8.9	40	0.13	26	<80	<0.017	8.5	<5.3	2.7	225
42	Kidney - wt-M6P3	16	2.9	27	39	1.3	2.3	0.11	<0.042	0.060	20	<19	82	0.00000021	0.018	0.69	0.22	0.13	0.0068	0.21	105	<8.9	138	0.28	85	158	0.031	43	19	6.2	400
43	Kidney - KO-M1P3	5.6	2.7	9.7	8.7	0.49	0.72	0.033	<0.042	0.024	4.7	<19	46	0.00000088	0.0062	0.30	0.098	0.037	<0.0041	0.083	38	<8.9	107	0.12	29	<80	<0.017	13	<5.3	2.4	191
44	Kidney - KO-M2P3	7.1	7.7	10	8.7	0.61	0.91	0.043	<0.042	0.028	<2.6	<19	49	0.00000076	0.0066	0.32	0.098	0.041	<0.0041	0.067	31	<8.9	107	0.12	37	<80	<0.017	16	<5.3	2.7	200
45	Kidney - KO-M3P3	7.8	3.6	13	8.7	0.85	0.80	0.045	<0.042	0.027	2.9	<19	40	0.00000065	0.0087	0.37	0.098	0.048	<0.0041	0.11	41	<8.9	128	0.11	30	<80	<0.017	11	<5.3	3.0	189
46	Kidney - KO-M4P3	8.3	5.4	11	7.0	0.90	0.84	0.041	<0.042	0.028	4.9	<19	41	0.00000057	0.0091	0.32	0.10	0.035	<0.0041	0.092	40	<8.9	118	0.12	36	<80	<0.017	8.3	<5.3	2.3	187
47	Kidney - KO-M5P3	8.0	5.4	11	8.7	0.86	0.84	0.035	<0.042	0.028	6.3	<19	52	0.00000057	0.0074	0.35	0.088	0.047	<0.0041	0.13	38	<8.9	117	0.13	26	<80	<0.017	8.5	<5.3	2.8	213
48	Kidney - KO-M6P3	7.3	2.6	11	8.7	0.86	0.84	0.035	<0.042	0.028	6.3	<19	41	0.00000057	0.0074	0.35	0.088	0.047	<0.0041	0.13	38	<8.9	110	0.12	29	<80	0.017	7.5	<5.3	<1.2	0.60
49	Brain - wt-M1P3	0.97	<0.000	1.8	<4.0	0.11	0.60	<0.0081	<0.042	<0.0095	<2.6	17700	15	0.00000039	<0.0011	<0.070	<0.041	<0.0076	<0.0041	<0.054	<29	<8.9	<31	<0.058	<14	<80	<0.017	<3.6	<5.3	<1.2	1.4
50	Brain - wt-M2P3	0.97	<0.000	1.7	<4.0	0.13	0.75	0.0098	<0.042	<0.0095	<2.6	13500	15	0.00000076	<0.0011	<0.070	<0.041	<0.0076	<0.0041	<0.054	<29	<8.9	<31	<0.058	<14	<80	<0.017	<3.6	<5.3	<1.2	0.59
51	Brain - wt-M3P3	<0.78	<0.000	1.8	<4.0	0.087	0.53	0.0086	<0.042	<0.0095	<2.6	13900	12	0.00000032	<0.0011	<0.070	<0.041	<0.0076	<0.0041	<0.054	<29	<8.9	<31	<0.058	<14	<80	<0.017	<3.6	<5.3	<1.2	1.1
52	Brain - wt-M4P3	<0.78	<0.000	1.8	<4.0	0.11	0.79	0.0098	<0.042	<0.0095	<2.6	15200	10	0.00000021	<0.0011	<0.070	<0.041	<0.0076	<0.0041	<0.054	<29	<8.9	<31	<0.058	<14	<80	<0.017	<3.6	<5.3	<1.2	0.76
53	Brain - wt-M5P3	0.84	<0.000	1.8	<4.0	0.13	0.66	0.013	<0.042	0.0089	<2.6	14800	6.3	0.00000021	<0.0011	<0.070	<0.041	<0.0076	<0.0041	<0.054	<29	<8.9	<31	<0.058	<14	<80	<0.017	<3.6	<5.3	<1.2	0.50
54	Brain - wt-M6P3	0.84	<0.000	2.0	4.9	0.11	0.60	0.011	<0.042	0.0089	<2.6	14800	7.5	0.00000003	<0.0011	<0.070	<0.041	<0.0076	<0.0041	<0.054	<29	<8.9	<31	<0.058	<14	<80	<0.017	<3.6	<5.3	<1.2	0.60
55	Brain - KO-M1P3	1.1	<0.000	1.9	<4.0	0.13	0.66	0.013	<0.042	0.0089	<2.6	15800	7.9	0.00000033	<0.0011	<0.070	<0.041	<0.0076	<0.0041	<0.054	<29	<8.9	<31	<0.058	<14	<80	<0.017	<3.6	<5.3	<1.2	0.80
56	Brain - KO-M2P3	<0.78	<0.000	1.5	<4.0	0.10	0.79	0.0090	<0.042	0.0089	<2.6	12400	14	0.00000026	<0.0011	<0.070	<0.041	<0.0076	<0.0041	<0.054	<29	<8.9	<31	<0.058	<14	<80	<0.017	<3.6	<5.3	<1.2	0.67
57	Brain - KO-M3P3	<0.78	<0.000	2.0	<4.0	0.10	0.71	0.0090	<0.042	0.0096	<2.6	17800	11	0.00000023	<0.0011	<0.070	<0.041	<0.0076	<0.0041	<0.054	<29	<8.9	<31	<0.058	<14	<80	<0.017	<3.6	<5.3	<1.2	0.82
58	Brain - KO-M4P3	0.97	<0.000	2.0	<4.0	0.11	0.66	0.0098	<0.042	0.0096	<2.6	16800	9.6	0.00000020	<0.0011	<0.070	<0.041	<0.0076	<0.0041	<0.054	<29	<8.9	<31	<0.058	<14	<80	<0.017	<3.6	<5.3	<1.2	0.47
59	Brain - KO-M5P3	1.1	<0.000	1.8	<4.0	0.11	0.62	0.0098	<0.042	0.0096	<2.6	16000	15	0.00000023	<0.0011	<0.070	<0.041	<0.0076	<0.0041	<0.054	<29	<8.9	<31	<0.058	<14	<80	<0.017	<3.6	<5.3	<1.2	0.52
60	Brain - KO-M6P3	0.97	<0.000	2.1	<4.0	0.15	0.83	0.017	<0.042	0.0099	<2.6	13700	9.6	0.00000050	0.0016	<0.070	<0.041	<0.0076	<0.0041	<0.054	<29	<8.9	<31	0.094	<14	<80	<0.017	<3.6	<5.3	<1.2	0.69

Appendix Table 3 Continued

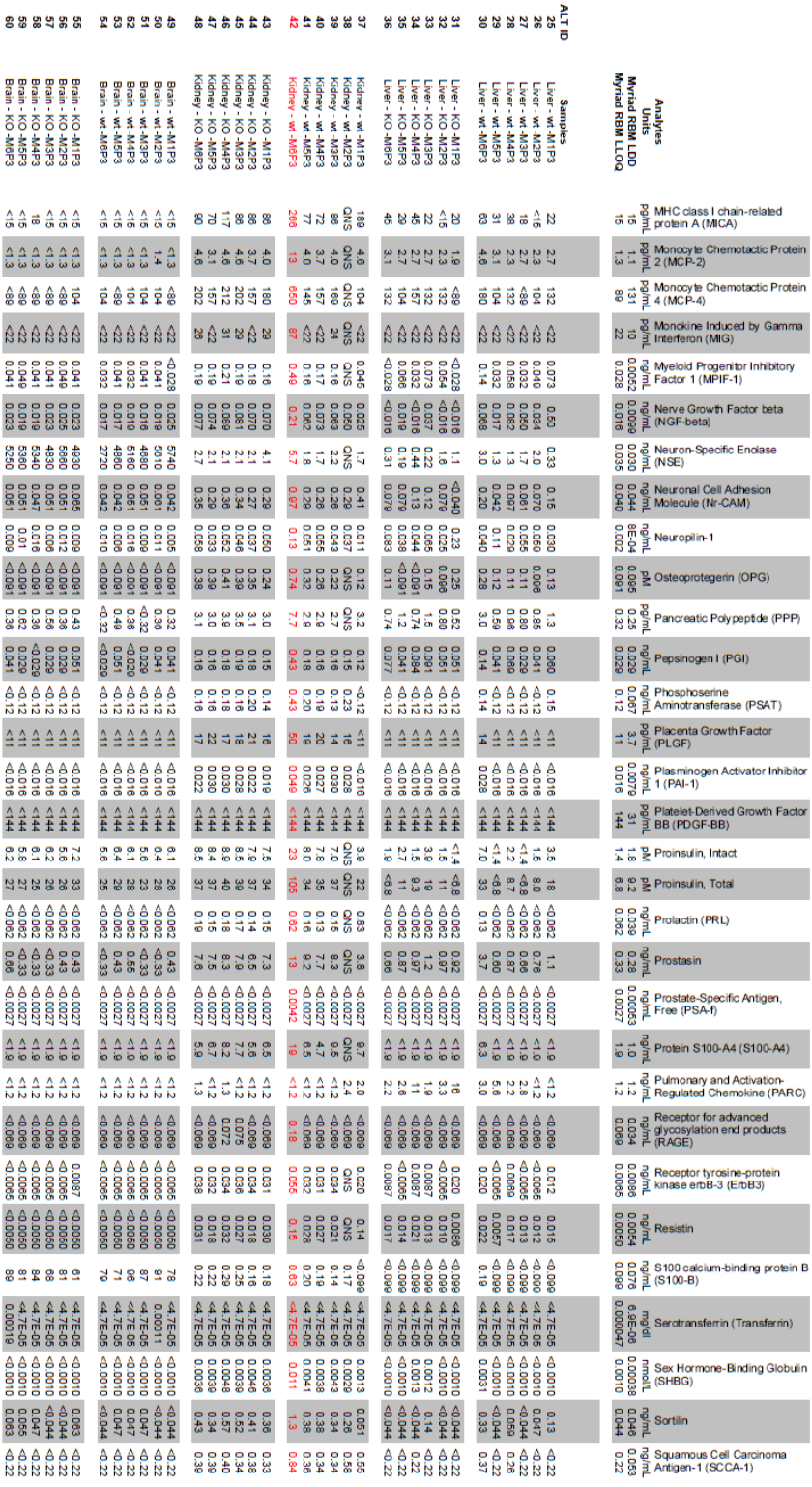


Appendix Table 3 Continued

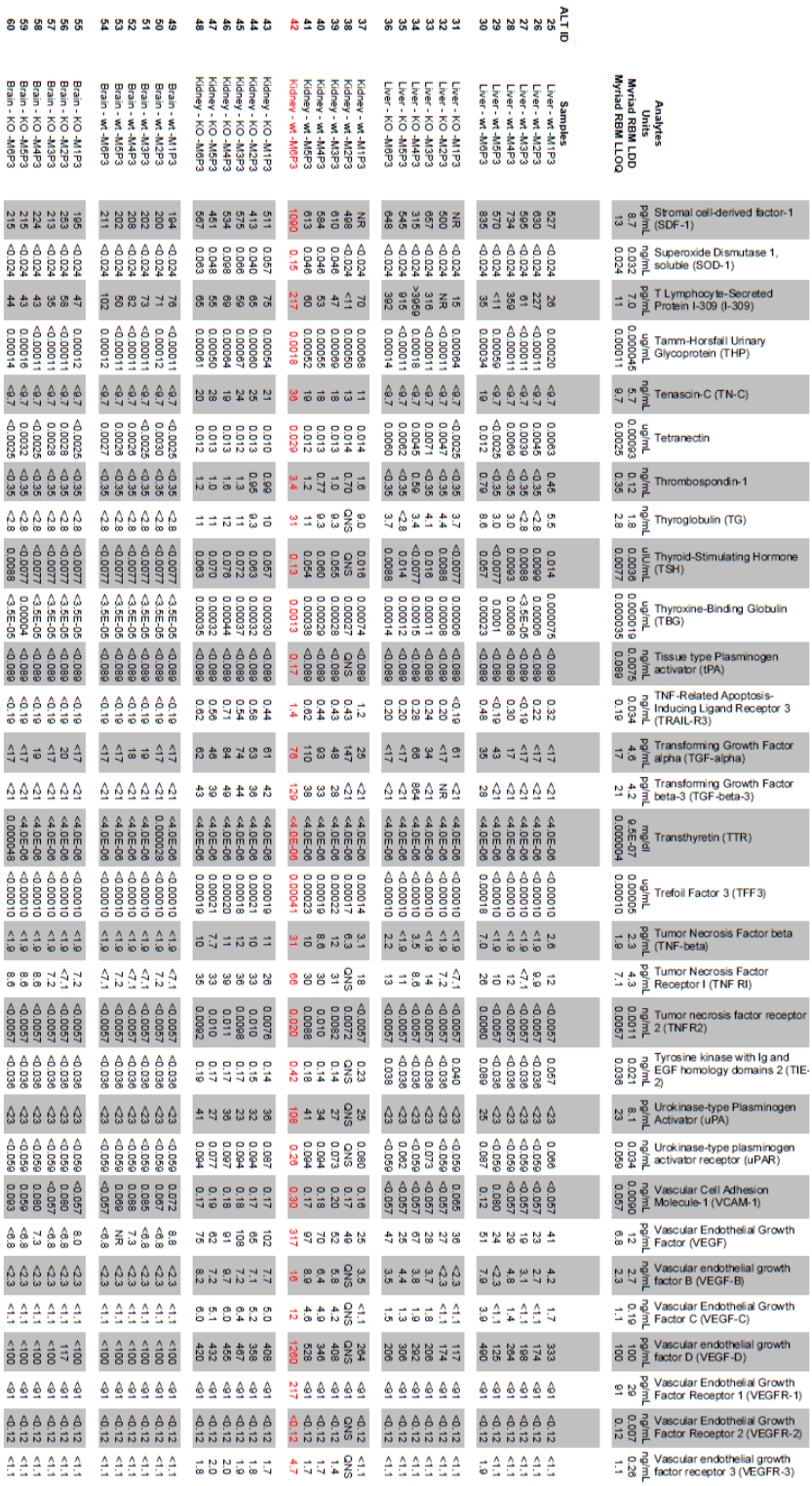


ALT ID	Samples	Analytes	Intercellular Adhesion Molecule 1 (ICAM-1)	Interferon-inducible T-cell alpha chemoattractant (ITAC)	Interleukin-1 receptor antagonist (IL-1ra)	Interleukin-2 receptor alpha (IL-2 receptor alpha)	Interleukin-6 receptor (IL-6r)	Interleukin-6 receptor subunit beta (IL-6R beta)	Interleukin-8 (IL-8)	Interleukin-12 Subunit p40 (IL-12p40)	Interleukin-13 (IL-13)	Interleukin-15 (IL-15)	Interleukin-16 (IL-16)	Interleukin-17 (IL-17)	Interleukin-23 (IL-23)	Kallikrein 5	Kallikrein-7 (KLK-7)	Kidney Injury Molecule-1 (KIM-1)	Lactoylglutathione lyase (LGL)	Latency-Associated Peptide of Transforming Growth Factor beta 1 (LAP TGF-b1)	Leptin	Luteinizing Hormone (LH)	Macrophage Colony-Stimulating Factor 1 (M-CSF)	Macrophage Inflammatory Protein-3 alpha (MIP-3 alpha)	Macrophage Migration Inhibitory Factor (MIF)	Macrophage-Stimulating Protein (MSP)	Maspin	Matrix Metalloproteinase-1 (MMP-1)	Matrix Metalloproteinase-3 (MMP-3)	Matrix Metalloproteinase-7 (MMP-7)	Matrix Metalloproteinase-9, total (MMP-9, total)	Matrix Metalloproteinase-10 (MMP-10)	Mesothelin (MSLN)
		Units	ng/mL	pg/mL	pg/mL	pg/mL	ng/mL	ng/mL	pg/mL	ng/mL	pg/mL	ng/mL	pg/mL	pg/mL	ng/mL	ng/mL	pg/mL	ng/mL	ng/mL	ng/mL	ng/mL	mIU/mL	ng/mL	pg/mL	ng/mL	ng/mL	pg/mL	ng/mL	ng/mL	ng/mL	ng/mL	nM
		Myriad RBM LDD	0.35	4.0	9.9	21	0.0027	0.023	0.83	0.016	1.2	0.12	4.3	1.2	0.12	0.045	90	0.0071	0.039	0.010	0.011	0.30	0.037	2.5	0.0015	0.037	486	0.13	0.0066	0.0056	0.31	0.0036	0.32
		Myriad RBM LLOQ	0.26	4.2	19	84	0.0054	0.049	0.79	0.055	1.2	0.079	17	0.99	0.080	0.14	67	0.0030	0.18	0.027	0.027	0.42	0.049	6.0	0.0020	0.051	393	0.17	0.012	0.012	0.58	0.010	0.31
25	Liver - wt -M1P3		1.0	8.6	31	102	0.0067	<0.049	1.3	0.082	3.2	0.085	<17	1.6	0.23	<0.14	129	0.0090	0.34	<0.027	0.047	1230	<0.049	8.2	0.10	0.078	2960	<0.17	<0.012	0.030	<0.58	<0.010	0.41
26	Liver - wt -M2P3		1.1	9.4	34	<84	<0.0054	<0.049	<0.79	0.072	2.5	0.097	<17	1.4	0.16	0.15	248	<0.0030	0.30	<0.027	0.039	2080	<0.049	9.8	0.12	0.052	1440	<0.17	<0.012	0.017	<0.58	<0.010	<0.31
27	Liver - wt -M3P3		0.96	8.6	28	<84	<0.0054	<0.049	<0.79	0.072	2.3	<0.079	<17	<0.99	0.14	<0.14	167	<0.0030	0.26	<0.027	0.030	2260	<0.049	13	0.14	<0.051	1290	<0.17	0.018	0.013	<0.58	<0.010	<0.31
28	Liver - wt -M4P3		0.96	16	28	87	0.0067	<0.049	<0.79	0.060	2.5	0.097	<17	1.1	0.16	<0.14	149	<0.0030	0.21	<0.027	0.039	2790	<0.049	9.8	0.15	0.060	2040	<0.17	<0.012	0.021	<0.58	<0.010	0.38
29	Liver - wt -M5P3		2.5	8.2	32	<84	<0.0054	<0.049	<0.79	0.068	1.4	<0.079	<17	<0.99	0.37	<0.14	189	0.0044	0.48	<0.027	0.035	2010	<0.049	19	0.16	0.068	1590	<0.17	0.013	0.013	<0.58	<0.010	<0.31
30	Liver - wt -M6P3		2.0	14	97	215	0.019	<0.049	1.2	0.22	5.2	0.26	22	2.0	0.52	<0.14	382	0.019	0.56	0.041	0.10	2000	0.050	41	0.24	0.15	5410	0.18	0.031	0.096	0.98	<0.010	0.80
31	Liver - KO -M1P3		1.4	NR	<19	<84	<0.0054	<0.049	1.3	<0.065	1.4	<0.079	<17	<0.99	0.20	<0.14	89	0.0030	0.55	<0.027	0.047	1710	<0.049	16	0.16	0.078	2960	<0.17	<0.012	<0.012	<0.58	<0.010	<0.31
32	Liver - KO -M2P3		1.1	6.6	31	<84	0.0059	<0.049	<0.79	0.10	2.8	0.085	<17	1.3	0.23	0.16	169	0.0030	0.30	<0.027	0.035	1870	<0.049	13	0.13	0.068	1890	<0.17	0.023	0.016	<0.58	<0.010	<0.31
33	Liver - KO -M3P3		1.2	11	37	110	0.0083	<0.049	<0.79	0.12	2.6	0.13	<17	1.1	0.16	<0.14	129	0.0030	0.34	<0.027	0.065	2080	<0.049	13	0.10	<0.051	2190	<0.17	0.016	0.044	<0.58	<0.010	<0.31
34	Liver - KO -M4P3		1.5	19	51	<84	<0.0054	<0.049	2.0	0.11	2.5	0.18	18	3.8	0.41	<0.14	189	0.0070	<0.18	<0.027	0.030	1850	<0.049	8.2	0.11	0.069	2330	0.18	0.045	0.013	<0.58	<0.010	0.57
35	Liver - KO -M5P3		1.1	12	47	<84	<0.0054	<0.049	1.3	0.10	2.3	0.11	<17	2.0	0.32	0.15	119	<0.0030	<0.18	<0.027	0.039	2250	<0.049	24	0.12	0.069	1970	<0.17	0.067	0.021	<0.58	<0.010	0.35
36	Liver - KO -M6P3			13	37	<84	<0.0054	<0.049	2.2	0.12	2.6	0.13	19	2.2	0.31	<0.14	139	0.0044	<0.18	<0.027	0.043	1900	<0.049	15	0.14	0.091	1890	<0.17	0.062	0.016	<0.58	<0.010	<0.31
37	Kidney - wt -M1P3		1.7	NR	21	95	0.014	<0.049	7.4	<0.065	3.0	0.26	<17	1.4	0.46	<0.14	400	0.012	0.64	<0.027	0.061	2560	<0.049	37	0.20	0.16	9430	0.23	<0.012	0.030	<0.58	<0.010	0.46
38	Kidney - wt -M2P3		1.8	1.1	77	QNS	0.022	0.057	0.89	0.20	4.9	0.19	<17	1.1	0.28	<0.14	QNS	0.020	QNS	QNS	QNS	2370	QNS	QNS	QNS	6780	QNS	0.038	0.040	0.11	QNS	QNS	QNS
39	Kidney - wt -M3P3		2.1	9.4	67	215	0.022	0.062	2.4	0.28	4.7	0.22	<17	1.8	0.31	<0.14	544	0.032	0.70	0.040	0.098	1890	<0.049	18	0.22	0.16	5780	0.23	0.041	0.040	0.11	0.99	<0.010	0.89
40	Kidney - wt -M4P3		2.1	17	106	227	0.019	0.063	1.3	0.26	4.4	0.22	<17	2.8	0.55	<0.14	509	0.024	0.55	0.039	0.11	1950	<0.049	18	0.16	0.15	6000	0.18	0.039	0.041	0.11	1.0	<0.010	0.48
41	Kidney - wt -M5P3		2.1	25	104	247	0.021	0.069	2.2	0.29	7.8	0.26	<17	3.3	0.98	<0.14	646	0.029	0.89	0.041	0.12	1270	<0.049	16	0.20	0.19	8000	0.23	0.039	0.12	0.36	3.9	0.028	2.7
42	Kidney - wt -M6P3		5.0	69	212	534	0.061	0.14	9.2	0.62	23	0.83	38	8.6	2.1	0.45	1530	0.088	2.2	0.12	0.23	1600	<0.049	67	0.50	0.37	20800	0.72	0.092				
43	Kidney - KO -M1P3		1.9	15	79	221	0.019	0.050	2.0	0.28	7.1	0.36	<17	4.7	0.86	<0.14	563	0.022	0.77	0.033	0.11	1100	<0.049	20	0.17	0.14	8720	0.29	0.034	0.030	<0.58	<0.010	0.74
44	Kidney - KO -M2P3		2.0	15	109	261	0.019	0.059	2.0	0.32	8.1	0.34	<17	4.9	0.95	<0.14	544	0.026	0.85	0.040	0.12	1730	0.046	20	0.17	0.14	8160	0.29	0.046	0.015	1.1	<0.010	0.89
45	Kidney - KO -M3P3		2.0	12	102	279	0.021	0.062	2.6	0.29	8.1	0.29	<17	4.9	0.97	<0.14	547	0.028	0.86	0.040	0.12	1730	0.047	20	0.17	0.14	8160	0.29	0.047	0.015	1.1	<0.010	0.89
46	Kidney - KO -M4P3		2.1	23	102	307	0.021	0.062	1.4	0.31	7.0	0.29	22	4.9	0.97	<0.14	560	0.031	0.75	0.039	0.11	1280	0.055	28	0.17	0.19	7840	0.29	0.047	0.016	1.3	0.012	0.72
47	Kidney - KO -M5P3		1.9	12	132	244	0.019	0.057	2.4	0.32	6.9	0.29	<17	2.9	0.42	<0.14	561	0.025	0.42	0.040	0.14	1260	0.050	16	0.17	0.18	5790	0.19	0.050	0.013	1.5	<0.010	0.82
48	Kidney - KO -M6P3		1.9	19	111	244	0.023	0.062	3.1	0.29	7.8	0.31	22	3.0	0.93	<0.14	578	0.028	0.64	0.046	0.12	2180	0.060	22	0.17	0.34	8610	0.34	0.043	0.015	1.2	0.011	0.72
49	Brain - wt -M1P3		0.88	<4.2	21	<84	<0.0054	<0.049	<0.79	<0.055	1.4	<0.079	<17	<0.99	<0.080	<0.14	308	0.0044	0.28	<0.027	0.030	974	<0.049	<8.0	0.042	0.053	562	<0.17	<0.012	0.019	<0.58	<0.010	<0.31
50	Brain - wt -M2P3		0.88	<4.2	28	<84	<0.0054	<0.049	<0.79	<0.055	<1.2	<0.079	<17	<0.99	<0.080	<0.14	327	0.0064	0.42	<0.027	<0.027	2180	<0.049	<8.0	0.065	<0.051	890	<0.17	<0.012	0.019	<0.58	<0.010	<0.31
51	Brain - wt -M3P3		0.73	<4.2	21	<84	<0.0054	<0.049	<0.79	<0.055	<1.2	<0.079	<17	<0.99	<0.080	<0.14	287	0.0057	0.30	<0.027	<0.027	1760	<0.049	<8.0	0.049	<0.051	562	<0.17	<0.012	0.016	<0.58	<0.010	<0.31
52	Brain - wt -M4P3		0.83	<4.2	23	<84	<0.0054	<0.049	<0.79	<0.055	<1.2	<0.079	<17	<0.99	<0.080	<0.14	277	0.0057	0.30	<0.027	<0.027	1280	<0.049	<8.0	0.053	<0.051	639	<0.17	<0.012	0.016	<0.58	<0.010	<0.31
53	Brain - wt -M5P3		0.77	NR	NR	<84	<0.0054	<0.049	<0.79	NR	<1.2	<0.079	<17	NR	<0.080	<0.14	209	0.0083	0.25	<0.027	<0.027	1280	<0.049	<8.0	0.062	<0.051	639	<0.17	NR	0.020	<0.58	<0.010	<0.31
54	Brain - wt -M6P3		0.77	<4.2	<19	<84	<0.0054	<0.049	<0.79	<0.055	<1.2	<0.079	<17	<0.99	<0.080	<0.14	248	0.0057	0.38	<0.027	<0.027	1610	<0.049	<8.0	0.060	<0.051	639	<0.17	<0.012	0.016	<0.58	<0.010	<0.31
55	Brain - KO -M1P3		0.68	<4.2	21	<84	<0.0054	<0.049	<0.79	<0.055	1.4	<0.079	<17	<0.99	<0.080	<0.14	248	0.0057	0.38	<0.027	0.030	974	<0.049	<8.0	0.044	0.053	807	<0.17	<0.012	0.019	<0.58	<0.010	<0.31
56	Brain - KO -M2P3		0.68	<4.2	28	<84	<0.0054	<0.049	<0.79	<0.055	<1.2	<0.079	<17	<0.99	<0.080	<0.14	287	0.0044	0.38	<0.027	<0.027	2180	<0.049	<8.0	0.037	<0.051	639	<0.17	<0.012	0.019	<0.58	<0.010	<0.31
57	Brain - KO -M3P3		0.63	<4.2	21	<84	<0.0054	<0.049	<0.79	<0.055	<1.2	<0.079	<17	<0.99	<0.080	<0.14	258	0.0070	0.34	<0.027	<0.027	1760	<0.049	<8.0	0.038	<0.051	807	<0.17	<0.012	0.013	<0.58	<0.010	<0.31
58	Brain - KO -M4P3		0.77	<4.2	21	<84	<0.0054	<0.049	<0.79	<0.055	<1.2	<0.079	<17	<0.99	<0.080	<0.14	248	0.0090	0.38	<0.027	<0.027	1610	<0.049	<8.0	0.045	<0.051	807	<0.17	<0.012	0.016	<0.58	<0.010	<0.31
59	Brain - KO -M5P3		0.58	<4.2	<19	<84	<0.0054	<0.049	<0.79	<0.055	<1.2	<0.079	<17	<0.99	<0.080	<0.14	306	0.0044	0.30	<0.027	<0.027	2180	<0.049	<8.0	0.063	<0.051	807	<0.17	<0.012	0.016	<0.58	<0.010	<0.31
60	Brain - KO -M6P3		0.83	<4.2	28	<84	<0.0054	<0.049	<0.79	<0.055	<1.2	<0.079	<17	<0.99	<0.080	<0.14	169	0.0070	0.21	<0.027	<0.027	974	<0.049	<8.0	0.046	<0.051	890	<0.17	<0.012	0.019	<0.58	<0.010	<0.31

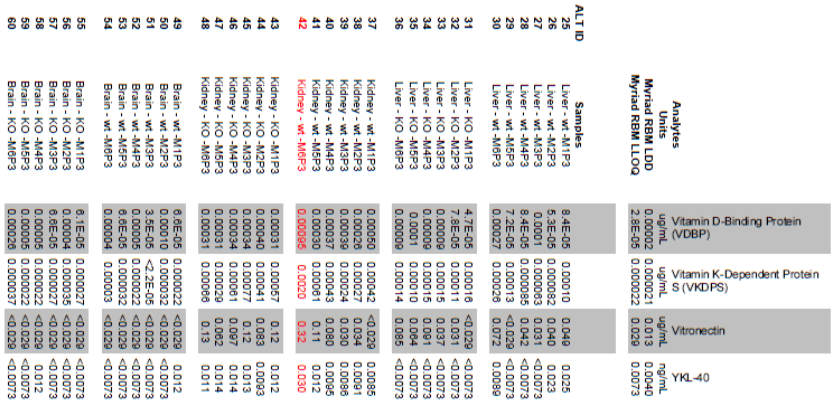
Appendix Table 3 Continued



Appendix Table 3 Continued



Appendix Table 3 Continued



Analytes				
Myriad RBM LDD		ug/mL	0.0013	0.0040
Myriad RBM LLOQ		ug/mL	0.029	0.0073
ALT ID	Samples			
26	Liver - wt-M1P3	8.4E-05	0.00010	0.049	0.025
27	Liver - wt-M2P3	5.3E-05	0.000082	0.040	0.023
28	Liver - wt-M3P3	0.0001	0.000083	0.031	<0.0073
29	Liver - wt-M4P3	8.4E-05	0.000085	0.042	<0.0073
30	Liver - wt-M5P3	7.2E-05	0.00013	<0.029	<0.0073
	Liver - wt-M6P3	0.00027	0.00028	0.072	0.0089
31	Liver - KO-M1P3	4.7E-05	0.00016	<0.029	<0.0073
32	Liver - KO-M2P3	7.8E-05	0.00011	0.031	<0.0073
33	Liver - KO-M3P3	0.00009	0.00015	0.037	<0.0073
34	Liver - KO-M4P3	0.00009	0.00015	0.091	<0.0073
35	Liver - KO-M5P3	0.0001	0.00010	0.064	<0.0073
36	Liver - KO-M6P3	0.00009	0.00014	0.085	<0.0073
37	Kidney - wt-M1P3	0.00050	0.0042	<0.029	0.0085
38	Kidney - wt-M2P3	0.00026	0.00027	0.034	0.0091
39	Kidney - wt-M3P3	0.00039	0.00024	0.030	0.0088
40	Kidney - wt-M4P3	0.00037	0.00043	0.080	0.0095
41	Kidney - wt-M5P3	0.00030	0.00061	0.11	0.012
42	Kidney - wt-M6P3	0.00095	0.0020	0.32	0.030
43	Kidney - KO-M1P3	0.00031	0.00057	0.12	0.012
44	Kidney - KO-M2P3	0.00040	0.00041	0.083	0.0093
45	Kidney - KO-M3P3	0.00034	0.00077	0.12	0.013
46	Kidney - KO-M4P3	0.00034	0.00061	0.097	0.014
47	Kidney - KO-M5P3	0.00031	0.00029	0.062	0.014
48	Kidney - KO-M6P3	0.00031	0.00086	0.13	0.011
49	Brain - wt-M1P3	6.6E-05	0.000022	<0.029	0.012
50	Brain - wt-M2P3	0.00010	0.000032	<0.029	<0.0073
51	Brain - wt-M3P3	3.5E-05	<2.2E-05	<0.029	<0.0073
52	Brain - wt-M4P3	0.00005	0.000022	<0.029	<0.0073
53	Brain - wt-M5P3	6.6E-05	0.000032	<0.029	<0.0073
54	Brain - wt-M6P3	0.00004	0.00003	<0.029	<0.0073
55	Brain - KO-M1P3	6.1E-05	0.000027	<0.029	<0.0073
56	Brain - KO-M2P3	0.00004	0.000035	<0.029	<0.0073
57	Brain - KO-M3P3	6.6E-05	0.000027	<0.029	<0.0073
58	Brain - KO-M4P3	0.00005	0.000022	<0.029	0.012
59	Brain - KO-M5P3	0.00005	0.000022	<0.029	<0.0073
60	Brain - KO-M6P3	0.00026	0.000037	<0.029	<0.0073

Appendix Table 4: Statistical Analysis of Results from Rodent Discovery Product.
The table contains analytes with p value < 0.05 following statistical analysis.

Specimen Tested	Analyte	*MRP4* (+/+) Mean ± SE	*MRP4* (-/-) Mean ± SE	*p* Value	Increase / Decrease
Brain	Calbindin	2140 ± 60 ng/mL	2490 ± 80 ng/mL	0.01	Increase
Brain	CD40	6.3 ± 0.5 pg/mL	4.6 ± 0.3 pg/mL	0.02	Decrease
Brain	von Willebrand factor (vWF)	4.3 ± 0.1 ng/mL	5.3 ± 0.4 ng/mL	0.02	Increase
Brain	Clusterin (CLU)	1.32 ± 0.05 ug/mL	1.10 ± 0.06 ug/mL	0.02	Decrease
Brain	Interleukin-1 alpha (IL-1 alpha)	34 ± 2 pg/mL	27 ± 1 pg/mL	0.03	Decrease
Brain	Myeloperoxidase (MPO)	0.9 ± 0.3 ng/mL	2.1 ± 0.5 ng/mL	0.04	Increase
Brain	Factor VII	5.4 ± 0.2 ng/mL	6.5 ± 0.4 ng/mL	0.04	Increase
Kidney	Vascular Endothelial Growth Factor A (VEGF-A)	490 ± 30 pg/mL	640 ± 20 pg/mL	0.004	Increase
Kidney	Monocyte Chemotactic Protein 1 (MCP-1)	2.8 ± 0.5 pg/mL	6.8 ± 0.5 pg/mL	0.006	Increase
Kidney	Testosterone, Total	31 ± 2 ng/mL	22 ± 1 ng/mL	0.006	Decrease
Kidney	Myoglobin	18 ± 3 ng/mL	4 ± 2 ng/mL	0.009	Decrease
Kidney	Immunoglobulin A (IgA)	5.4 ± 0.4 ug/mL	11 ± 2 ug/mL	0.02	Increase
Kidney	Myeloperoxidase (MPO)	1.8 ± 0.4 ng/mL	3.1 ± 0.2 ng/mL	0.02	Increase
Kidney	CD40 Ligand (CD40-L)	900 ± 100 pg/mL	1400 ± 100 pg/mL	0.02	Increase
Kidney	T-Cell-Specific Protein RANTES (RANTES)	0.037 ± 0.008 pg/mL	0.062 ± 0.003 pg/mL	0.02	Increase
Kidney	Fibroblast Growth Factor 9 (FGF-9)	3.0 ± 0.4 ng/mL	4.3 ± 0.3 ng/mL	0.02	Increase
Kidney	Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF)	1.6 ± 0.1 pg/mL	2.4 ± 0.2 pg/mL	0.03	Increase
Kidney	Interleukin-1 beta (IL-1 beta)	2.4 ± 0.3 ng/mL	3.6 ± 0.2 ng/mL	0.03	Increase
Kidney	Interleukin-11 (IL-11)	23 ± 4 pg/mL	41 ± 4 pg/mL	0.03	Increase

Appendix Table 4 Continued

Specimen Tested	Analyte	*MRP4* (+/+) Mean ± SE	*MRP4* (-/-) Mean ± SE	*p* Value	Increase / Decrease
Kidney	Monocyte Chemotactic Protein-5 (MCP-5)	2.9 ± 0.6 pg/mL	4.3 ± 0.2 pg/mL	0.03	Increase
Kidney	Macrophage Inflammatory Protein-1 beta (MIP-1 beta)	110 ± 20 pg/mL	170 ± 10 pg/mL	0.03	Increase
Kidney	Growth-Regulated Alpha Protein (KC/GRO)	0.0057 ± 0.0003 ng/mL	0.0081 ± 0.0009 ng/mL	0.03	Increase
Kidney	Glucagon	140 ± 20 pg/mL	190 ± 10 pg/mL	0.03	Increase
Kidney	Kidney Injury Molecule-1	0.12 ± 0.02 ng/mL	0.19 ± 0.01 ng/mL	0.03	Increase
Kidney	Interleukin-5 (IL-5)	0.22 ± 0.04 ng/mL	0.36 ± 0.03 ng/mL	0.04	Increase
Kidney	Lymphotactin	62 ± 6 pg/mL	78 ± 3 pg/mL	0.04	Increase
Liver	Cortisol	4.2 ± 0.5 ng/mL	7.8 ± 0.3 ng/mL	0.002	Increase
Liver	Monocyte Chemotactic Protein 1 (MCP-1)	2.9 ± 0.5 pg/mL	8 ± 2 pg/mL	0.01	Increase
Liver	Myoglobin	3800 ± 900 ng/mL	1300 ± 800 ng/mL	0.03	Decrease
Serum	Progesterone	20 ± 3 ng/mL	33 ± 2 ng/mL	0.01	Increase
Serum	Macrophage Inflammatory Protein-1 gamma (MIP-1 γ)	12 ± 1 ng/mL	17 ± 1 ng/mL	0.009	Increase
Serum	Monocyte Chemotactic Protein 1 (MCP-1)	31 ± 5 pg/mL	53 ± 7 pg/mL	0.03	Increase
Serum	Vascular Cell Adhesion Molecule-1 (VCAM-1)	1100 ± 100 ng/mL	1660 ± 50 ng/mL	0.004	Increase

Appendix Table 5: Statistical Analysis of Results from Human Discovery Product.
The table contains analytes with p value < 0.05 following statistical analysis. Care should be taken prior to interpreting results from the Human Discovery product since the assays are designed to detect human protein and may not cross-react specifically with rodent protein.

Tissue	Analyte	*MRP4* (+/+) Mean ± SE	*MRP4* (-/-) Mean ± SE	*p* Value	Increase / Decrease
Brain	T Lymphocyte-Secreted Protein I-309 (I-309)	76 ± 7 pg/mL	45 ± 3 pg/mL	0.006	Decrease
Brain	Heat Shock Protein 60 (HSP-60)	4300 ± 200 ng/mL	5700 ± 600 ng/mL	0.02	Increase
Brain	Alpha-Fetoprotein (AFP)	0.127 ± 0.006 ng/mL	0.152 ± 0.009 ng/mL	0.03	Increase
Brain	Myeloid Progenitor Inhibitory Factor 1 (MPIF-1)	0.036 ± 0.002 ng/mL	0.044 ± 0.002 ng/mL	0.03	Increase
Brain	Insulin-like Growth Factor Binding Protein 6 (IGFBP6)	2.0 ± 0.1 ng/mL	2.32 ± 0.09 ng/mL	0.04	Increase
Brain	Stromal cell-derived factor-1 (SDF-1)	203 ± 2 pg/mL	219 ± 8 pg/mL	0.04	Increase
Brain	Tumor Necrosis Factor Receptor I (TNF RI)	7.13 ± 0.02 pg/mL	7.9 ± 0.3 pg/mL	0.04	Increase
Kidney	Hepatocyte Growth Factor (HGF)	0.86 ± 0.08 ng/mL	1.23 ± 0.02 ng/mL	0.005	Increase
Kidney	Epiregulin (EPR)	26 ± 1 pg/mL	32 ± 1 pg/mL	0.006	Increase
Kidney	Interleukin-15 (IL-15)	0.23 ± 0.01 ng/mL	0.34 ± 0.02 ng/mL	0.006	Increase
Kidney	Tenascin-C (TN-C)	16 ± 2 ng/mL	23 ± 1 ng/mL	0.008	Increase
Kidney	Cancer Antigen 125 (CA-125)	4.8 ± 0.3 U/mL	6.9 ± 0.4 U/mL	0.009	Increase
Kidney	Matrix Metalloproteinase-9, total (MMP-9, total)	0.9 ± 0.1 ng/mL	1.23 ± 0.06 ng/mL	0.009	Increase
Kidney	Transforming Growth Factor beta-3 (TGF-beta-3)	28 ± 3 pg/mL	42 ± 2 pg/mL	0.01	Increase
Kidney	Vascular Endothelial Growth Factor C (VEGF-C)	3.7 ± 0.9 ng/mL	5.6 ± 0.2 ng/mL	0.01	Increase
Kidney	Chemokine CC-4 (HCC-4)	0.022 ± 0.002 ng/mL	0.0262 ± 0.0005 ng/mL	0.02	Increase

Appendix Table 5 Continued

Tissue	Analyte	*MRP4* (+/+) Mean ± SE	*MRP4* (-/-) Mean ± SE	*p* Value	Increase / Decrease
Kidney	Interleukin-12 Subunit p40 (IL-12p40)	0.22 ± 0.04 ng/mL	0.302 ± 0.007 ng/mL	0.02	Increase
Kidney	Interleukin-13 (IL-13)	5.6 ± 0.8 pg/mL	7.6 ± 0.2 pg/mL	0.02	Increase
Kidney	Nerve Growth Factor beta (NGF-beta)	0.055 ± 0.008 ng/mL	0.077 ± 0.003 ng/mL	0.02	Increase
Kidney	Thyroid-Stimulating Hormone (TSH)	0.05 ± 0.01 uIU/mL	0.067 ± 0.003 uIU/mL	0.02	Increase
Kidney	Vascular endothelial growth factor receptor 3 (VEGFR-3)	1.5 ± 0.1 ng/mL	1.87 ± 0.05 ng/mL	0.02	Increase
Kidney	CD5 Antigen-like (CD5L)	0.028 ± 0.004 ng/mL	0.040 ± 0.002 ng/mL	0.03	Increase
Kidney	Eotaxin-1	31 ± 2 pg/mL	37 ± 2 pg/mL	0.03	Increase
Kidney	Insulin-like Growth Factor-Binding Protein 3 (IGFBP-3)	14.0 ± 0.7 ng/mL	16.5 ± 0.6 ng/mL	0.03	Increase
Kidney	Myeloid Progenitor Inhibitory Factor 1 (MPIF-1)	0.13 ± 0.03 ng/mL	0.187 ± 0.007 ng/mL	0.03	Increase
Kidney	Osteoprotegerin (OPG)	0.23 ± 0.04 pM	0.36 ± 0.03 pM	0.03	Increase
Kidney	Superoxide Dismutase 1, soluble (SOD-1)	0.037 ± 0.005 ng/mL	0.062 ± 0.008 ng/mL	0.03	Increase
Kidney	YKL-40	0.0095 ± 0.0006 ng/mL	0.0122 ± 0.0008 ng/mL	0.03	Increase
Kidney	Interleukin-17 (IL-17)	2.0 ± 0.4 pg/mL	3.4 ± 0.3 pg/mL	0.04	Increase
Kidney	Matrix Metalloproteinase-7 (MMP-7)	0.09 ± 0.02 ng/mL	0.14 ± 0.01 ng/mL	0.04	Increase
Kidney	Vitronectin	0.06 ± 0.02 ug/mL	0.10 ± 0.01 ug/mL	0.04	Increase
Liver	Cancer Antigen 15-3 (CA-15-3)	8 ± 4 U/mL	0.7 ± 0.3 U/mL	0.009	Decrease

Appendix Table 5 Continued

Tissue	Analyte	*MRP4* (+/+) Mean ± SE	*MRP4* (-/-) Mean ± SE	*p* Value	Increase / Decrease
Liver	Nerve Growth Factor beta (NGF-beta)	0.13 ± 0.08 ng/mL	0.020 ± 0.003 ng/mL	0.01	Decrease
Liver	Apolipoprotein A-II (Apo A-II)	0.000071 ± 0.000007 ng/mL	0.000048 ± 0.000005 ng/mL	0.04	Decrease
Liver	Neuron-Specific Enolase (NSE)	1.6 ± 0.4 ng/mL	0.6 ± 0.2 ng/mL	0.04	Decrease

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