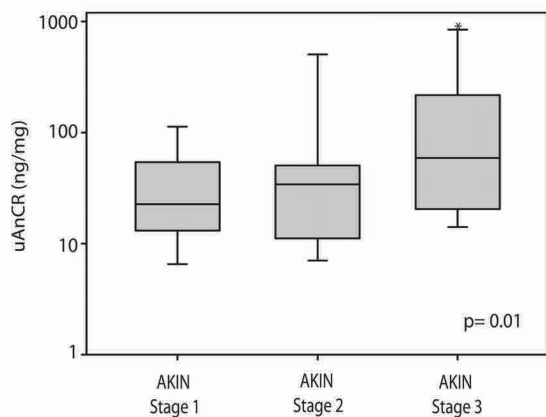
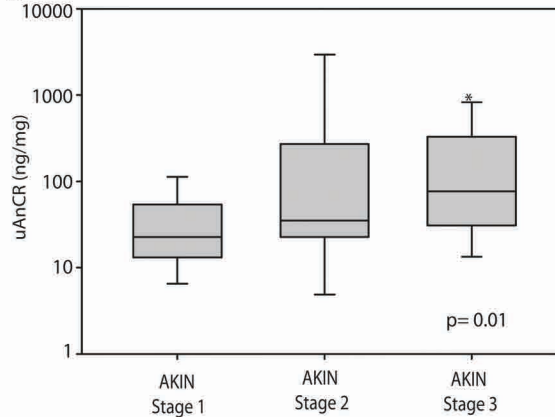


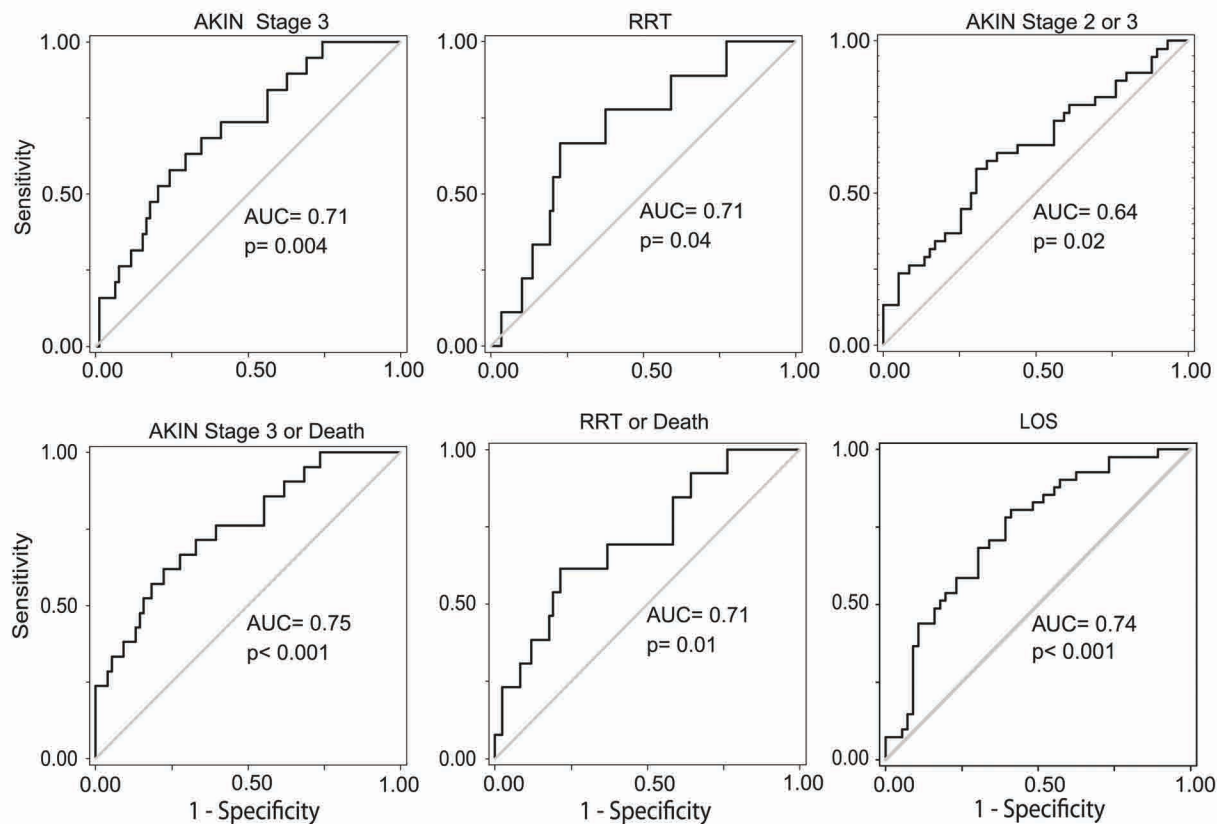
A



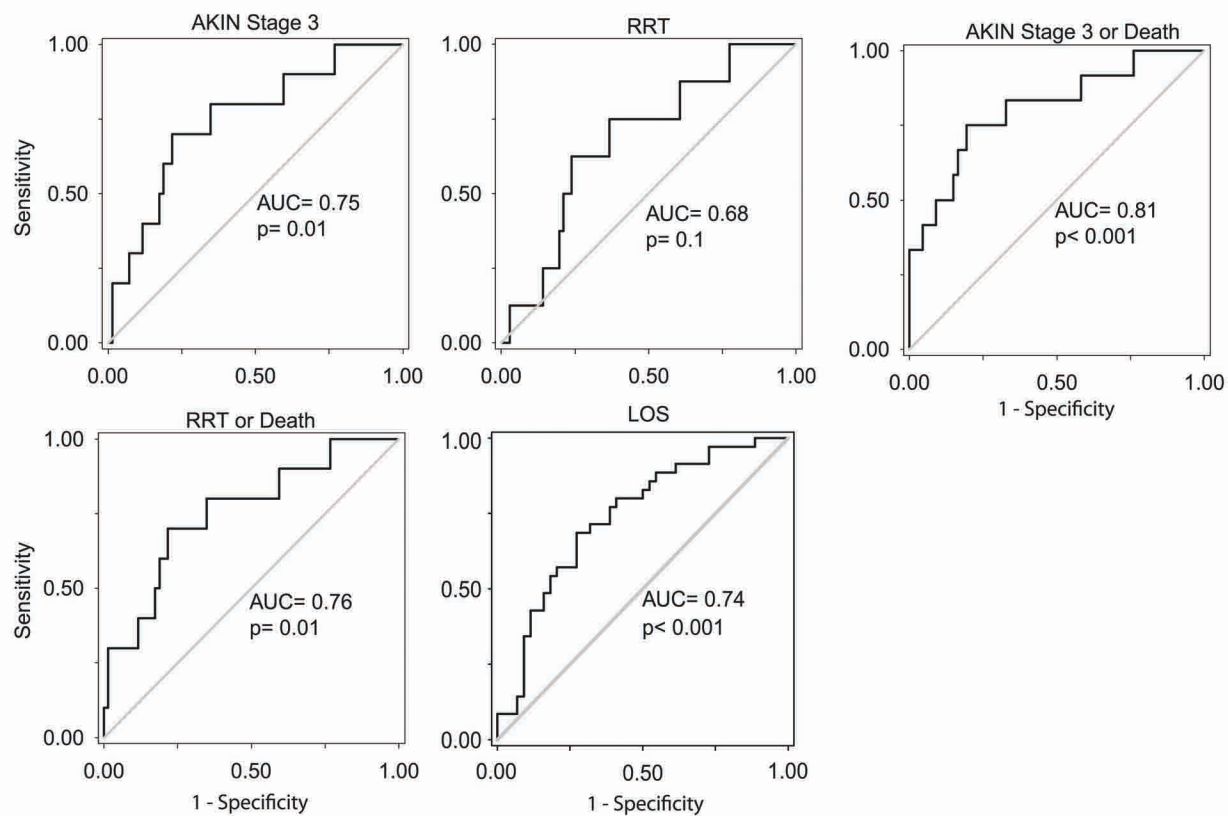
B



Supplemental Figure 1. Box and whisker plots showing the distribution of uAnCR by group in patients who developed AKI after cardiac surgery. (A) Among patients who had AKI of any AKIN stage at the time of sample collection (n=97), uAnCR increased in a graded manner with AKI severity (as determined by maximum AKIN Stage) (B) uAnCR increased in a graded manner with AKI severity in the subset of patients who were classified as AKIN Stage 1 AKI at the time that their urine samples were collected (n=79). Box plots show the median (solid line), 25th and 75th percentiles. Error bars represent the 5th and 95th percentiles. AKIN Stage groups were compared with the Kruskal-Wallis test (p-value shown in bottom right). The * represents a p-value <0.05 compared to AKIN Stage 1 in the post-hoc Dunn's test for pairwise comparison.



Supplemental Figure 2. ROC curves show the predictive power of uAnCR for multiple outcomes in patients who develop AKI after cardiac surgery (n=97). For each tested outcome, cut-offs are iteratively determined throughout the dataset, and the sensitivity (true positive rate) and 1-specificity (false positive rate) is calculated at each cut-off. These values are plotted against each other, and the area under the resulting ROC curve (AUC) is used to evaluate the predictive power of the biomarker. A perfect biomarker would have an AUC of 1.0, whereas random chance (having equal true positive and false positive rates) would have an AUC of 0.5 (shown as gray diagonal line).

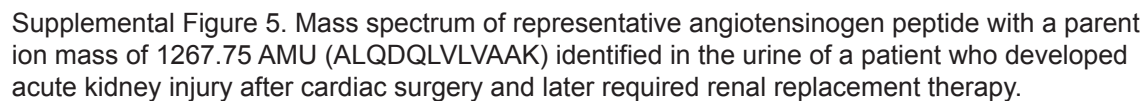


Supplemental Figure 3. ROC curves demonstrate the ability of uAnCR to predict outcomes in patients who had developed mild AKI (defined as AKIN stage 1) at the time of sample collection (n=79). With the exception of RRT, its predictive power was slightly improved for all outcomes in this subpopulation compared with the overall sample set.

Angiotensinogen (P01019) 53.1548 kDa

MRKRAPQSEM	10	20	30	40	50
	APAGVSLRAT	ILCLLAWAGL	AAGDRVYIHP	FHL*VIHNEST	
CEQLAKANAG	60	70	80	90	100
	KPKDPTFIPA	PIQAKTSPVD	EKALQDQLVL	VAAKLDTEDK	
LRAAMVGMLA	110	120	130	140	150
	NFLGFRIYGM	HSELWGVVHG	ATVLSPTAVF	GTLASLYLGA	
LDHTADRLQA	160	170	180	190	200
	ILGVPWKDKN	CTSRLDAHKV	LSALQAVQGL	LVAQGRADSQ	
AQLLLSTVVG	210	220	230	240	250
	VFTAPGLHLK	QPFVQGLALY	TPVVLPRSLD	FTELDVAEEK	
IDRFMQAVTG	260	270	280	290	300
	WKTGCSLMGA	SVDSTLAFNT	YVHFQGKMKMG	FSLLAEPQEF	
WVDNSTSVSV	310	320	330	340	350
	PMLSGMGTFQ	HWSDIQDNFS	VTQVPFTESA	CLLLIQPHYA	
SDLDKVEGLT	360	370	380	390	400
	FQQNSLNWMK	KLSPRTIHLT	MPQLVLQGSY	DLQDLLAQAE	
LPAILHTELN	410	420	430	440	450
	LQKLSNDRIR	VGEVLNSIFF	ELEADEREPT	ESTQQLNKPE	
VLEVTLNRPF	460	470	480		
	LFAVYDQSAT	ALHFLGRVAN	PLSTA		

Supplemental Figure 4. Sequence coverage of angiotensinogen identified by LC-MS/MS. 7 unique peptides, 63/485 amino acids (13% sequence coverage). Identified sequences are highlighted in yellow. Renin cleaves angiotensinogen at amino acid 43 (denoted by asterisk), to release angiotensin I.



Supplemental Figure 5. Mass spectrum of representative angiotensinogen peptide with a parent ion mass of 1267.75 AMU (ALQDQLVLA^{AK}) identified in the urine of a patient who developed acute kidney injury after cardiac surgery and later required renal replacement therapy.

Supplemental Table 1. Characteristics of patients used in a discovery phase proteomics study to identify candidate biomarkers of severe AKI

	No RRT	RRT	p-value
n	6	6	
Male	67% (4)	67% (4)	1
Caucasian	100% (6)	100% (6)	1
Age (yrs) ^a	63.8 +/- 7.9	72.5 +/- 17.1	0.29
Weight (kg) ^a	75.9 +/- 40.2	85.3 +/- 37.5	0.33
Sample Collection Time (hrs post-op) ^a	29.2 +/- 14.4	38.0 +/- 12.0	0.3
Operative Variables			
CABG	50% (3)	33% (2)	1
Valve Replacement	17 % (1)	17% (1)	1
CABG+Valve Replacement	33% (2)	17% (1)	1
Other Surgery	0% (0)	33% (2)	0.46
Bypass	67% (4)	67% (4)	1
Bypass Time (min) ^a	160.8 +/- 67.0	165.0 +/- 88.2	1
Serum Creatinine (mg/dL)			
Pre-op Value ^a	1.3 +/- 0.4	1.4 +/- 0.3	0.76
At Sample Collection ^a	1.9 +/- 0.4	2.6 +/- 0.6	0.37
Maximum Post-Op Value ^a	2.1 +/- 0.5	4.2 +/- 1.4	0.006
Outcomes			
Days to Max sCr (from surgery) ^a	1.7 +/- 1.0	4.8 +/- 2.4	0.02
RRT	0% (0)	100% (6)	0.002
Death	0% (0)	67% (4)	0.06

^aMean +/- SD

Supplemental Table 2. Complete list of protein identifications

Identified Proteins (349)		No RRT 1	No RRT 3	No RRT 7	No RRT 5	No RRT 9	No RRT 11	RRT 10	RRT 4	RRT 2	RRT 8	RRT 6	RRT 12	Wilcoxon Rank-Sum p-val	Up or Down	Mean Fold Change
Angiotensinogen	P01019	0.00	0.00	0.00	2.13	1.25	0.00	2.95	17.00	3.36	4.90	44.63	25.12	0.002	Up	9.67
Serum albumin	P02768	577	796	479	419	918	734	972	5874	1038	1167	5840	2465	0.002	Up	4.42
Apolipoprotein A-IV	P06727	0.00	0.00	0.00	0.00	0.00	2.36	1.18	19.13	6.71	1.63	80.76	19.32	0.006	Up	9.09
Complement C3	P01024	0.00	9.66	17.00	0.00	6.25	2.36	21.84	93.51	40.27	14.71	114.76	15.46	0.009	Up	5.68
Vitamin D-binding protein	P02774	1.77	0.00	4.25	0.00	7.50	0.00	21.84	91.38	10.07	3.27	148.76	52.16	0.009	Up	12.11
Complement C4-B	P0C0L5	0.00	0.00	0.85	6.38	1.25	2.36	41.91	36.13	16.78	4.90	34.00	5.80	0.009	Up	8.58
Superoxide dismutase [Cu-Zn]	P00441	0.00	0.00	0.43	14.88	15.00	0.00	4.13	42.50	19.01	16.35	38.25	23.18	0.009	Up	2.37
Epididymal secretory protein E1	P61916	1.77	0.00	1.28	2.13	7.50	0.00	3.54	8.50	10.07	14.71	4.25	21.25	0.009	Up	3.28
Phosphatidylethanolamine-binding protein 1	P30086	0.00	0.00	0.00	0.00	0.00	0.00	4.72	2.13	14.54	0.00	4.25	3.86	0.015	Up	N/A
Complement factor D	P00746	0.00	0.00	0.00	0.00	0.00	0.00	1.18	2.13	8.95	0.00	14.88	1.93	0.015	Up	N/A
Coactosin-like protein	Q14019	0.00	0.00	0.00	0.00	0.00	0.00	2.36	0.00	6.71	3.27	4.25	1.93	0.015	Up	N/A
Serotransferrin	P02787	20	100	59	81	161	14	106	1165	140	121	910	392	0.015	Up	6.50
Profilin-1	P07737	0.00	0.00	0.85	4.25	12.50	0.00	7.08	10.63	21.25	42.50	53.13	9.66	0.015	Up	4.10
Cystatin-B	P04080	0.00	0.00	0.43	0.00	0.00	0.00	3.54	2.13	2.24	0.00	14.88	1.93	0.015	Up	11.63
Fibrinogen alpha chain	P02671	4.43	7.73	5.95	8.50	15.00	2.36	23.02	25.50	44.74	11.44	99.88	7.73	0.017	Up	4.83
Brain acid soluble protein 1	P80723	0.89	0.00	0.85	0.00	0.00	0.00	2.95	0.00	2.24	3.27	14.88	1.93	0.022	Up	5.82
Zinc-alpha-2-glycoprotein	P25311	54.90	0.00	145.79	82.88	196.27	80.28	115	300	110	366	215	267	0.026	Up	2.04
Alpha-1-antitrypsin	P01009	1.77	1.93	48.45	2.13	53.75	21.25	65	578	18	52	576	147	0.026	Up	11.11
Alpha-1-acid glycoprotein 1	P02763	30.11	5.80	47.60	51.00	95.01	40.14	104.49	167.89	30.20	137.32	99.88	137.17	0.026	Up	2.51
Hemopexin	P02790	1.77	5.80	6.38	12.75	16.25	4.72	25.38	38.25	24.61	4.90	27.63	59.89	0.026	Up	3.79
Fibrinogen beta chain	P02675	1.77	3.86	4.25	2.13	7.50	2.36	24.20	6.38	6.71	6.54	36.13	5.80	0.026	Up	3.92
Pigment epithelium-derived factor	P36955	0.00	0.00	0.00	0.00	2.50	0.00	0.00	4.25	8.95	1.63	44.63	54.10	0.028	Up	9.08
Fatty acid-binding protein, adipocyte	P15090	0.00	0.00	0.00	0.00	6.25	0.00	0.00	6.38	15.66	1.63	12.75	13.52	0.028	Up	1.60
Alpha-1-acid glycoprotein 2	P19652	3.54	3.86	10.63	23.38	46.25	21.25	49.59	80.76	6.71	34.33	63.76	50.23	0.041	Up	2.62
Metallothionein-2	P02795	0.89	0.00	1.28	2.13	0.00	0.00	4.13	2.13	22.37	0.00	63.76	3.86	0.041	Up	13.47
Apolipoprotein A-I	P02647	0.89	0.00	1.28	0.00	5.00	0.00	5.90	6.38	8.95	0.00	78.63	1.93	0.043	Up	8.53
Thymosin beta-4-like protein 3	A8MW06	0.00	0.00	0.00	0.00	2.50	0.00	0.59	2.13	14.54	1.63	76.51	0.00	0.054	Up	7.63
Apolipoprotein C-III	P02656	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.50	40.27	3.27	25.50	0.00	0.061	Up	N/A
Dermcidin	P81605	0.00	0.00	0.00	0.00	0.00	0.00	0.59	0.00	3.36	53.95	14.88	0.00	0.061	Up	N/A
Neutrophil gelatinase-associated lipocalin	P80188	0.00	0.00	0.00	0.00	0.00	0.00	2.95	0.00	11.19	0.00	10.63	3.86	0.061	Up	N/A

Insulin-like growth factor-binding protein 1	P08833	0.00	0.00	0.00	0.00	0.00	0.00	0.59	0.00	8.95	8.17	12.75	0.00	0.061	Up	N/A
Carbonic anhydrase 3	P07451	0.00	0.00	0.00	0.00	0.00	0.00	0.00	17.00	0.00	3.27	17.00	3.86	0.061	Up	N/A
Ig heavy chain V-III region BUT	P01767	0.00	0.00	0.00	0.00	0.00	0.00	1.77	12.75	0.00	1.63	4.25	0.00	0.061	Up	N/A
Heme-binding protein 2	Q9Y5Z4	0.00	0.00	0.00	0.00	0.00	0.00	1.18	2.13	0.00	3.27	0.00	1.93	0.061	Up	N/A
Myoglobin	P02144	0.00	0.00	0.00	0.00	1.25	0.00	0.00	82.88	5.59	9.81	44.63	0.00	0.061	Up	28.58
Ig heavy chain V-III region BRO	P01766	0.00	0.00	0.85	0.00	0.00	0.00	16.53	38.25	0.00	1.63	8.50	0.00	0.061	Up	19.09
Ribosome-binding protein 1	Q9P2E9	0.00	0.00	0.00	0.00	1.25	0.00	6.49	34.00	0.00	0.00	2.13	3.86	0.061	Up	9.30
Ig lambda chain V-III region SH	P01714	0.00	0.00	1.28	0.00	0.00	0.00	2.95	8.50	0.00	0.00	12.75	7.73	0.061	Up	6.26
Retinol-binding protein 4	P02753	0.89	1.93	25.50	108.38	25.00	7.08	2.95	53.13	136.46	173.28	184.89	34.78	0.065	Up	3.47
Monocyte differentiation antigen CD14	P08571	1.77	0.00	4.25	17.00	22.50	11.81	41.91	21.25	11.19	19.62	19.13	30.91	0.065	Up	2.09
Ig mu chain C region	P01871	0.00	0.00	13.18	0.00	17.50	0.00	17.12	12.75	3.36	1.63	19.13	23.18	0.076	Up	1.19
Fatty acid-binding protein, heart	P05413	0.00	0.00	0.43	21.25	26.25	23.61	12.40	121.13	14.54	68.66	61.63	25.12	0.089	Up	2.83
Complement factor B	P00751	0.00	0.00	2.55	12.75	0.00	40.14	5.31	21.25	17.90	19.62	46.75	3.86	0.091	Up	1.03
Alpha-1B-glycoprotein	P04217	0.89	13.52	11.90	29.75	20.00	7.08	33.65	167.89	2.24	22.89	65.88	25.12	0.093	Up	3.82
Ceruloplasmin	P00450	0.89	3.86	8.08	4.25	7.50	0.00	23.61	240.14	1.12	6.54	95.63	7.73	0.093	Up	12.71
Clusterin	P10909	1.77	3.86	7.23	2.13	26.25	4.72	38.37	10.63	2.24	42.50	8.50	19.32	0.093	Up	2.64
Beta-2-glycoprotein 1	P02749	2.66	21.25	13.18	8.50	8.75	4.72	18.89	19.13	10.07	13.08	42.50	17.39	0.093	Up	2.05
Glutathione peroxidase 3	P22352	0.00	0.00	0.85	0.00	2.50	0.00	4.72	31.88	4.47	0.00	19.13	0.00	0.100	Up	8.98
Plasma protease C1 inhibitor	P05155	0.00	0.00	2.55	0.00	2.50	0.00	3.54	4.25	0.00	0.00	8.50	13.52	0.100	Up	2.95
Fatty acid-binding protein, liver	P07148	0.00	0.00	0.00	0.00	3.75	0.00	0.00	2.13	89.48	0.00	29.75	34.78	0.106	Up	10.41
SH3 domain-binding glutamic acid-rich-like protein	O75368	0.00	0.00	0.00	0.00	1.25	0.00	1.18	0.00	2.24	8.17	12.75	0.00	0.106	Up	4.87
Apolipoprotein A-II	P02652	0.00	0.00	0.00	0.00	1.25	0.00	1.18	4.25	3.36	0.00	14.88	0.00	0.106	Up	4.73
Ganglioside GM2 activator	P17900	4.43	0.00	12.75	0.00	8.75	2.36	21.25	6.38	11.19	0.00	14.88	17.39	0.128	Up	2.01
Alpha-2-HS-glycoprotein	P02765	0.89	5.80	11.05	63.76	52.50	49.59	59.62	65.88	19.01	112.80	53.13	36.71	0.132	Up	1.89
Afamin	P43652	0.00	0.00	0.00	0.00	5.00	0.00	0.59	53.13	0.00	0.00	21.25	1.93	0.152	Up	3.84
Serine protease inhibitor Kazal-type 5	Q9NQ38	0.00	0.00	0.00	0.00	1.25	0.00	1.18	0.00	1.12	4.90	2.13	0.00	0.152	Up	1.87
Carbonic anhydrase 1	P00915	48.70	0.00	1.70	199.77	312.53	0.00	83.24	99.88	162.18	119.34	306.02	473.33	0.169	Up	1.47
Ig lambda chain V-III region LOI	P80748	0.00	0.00	0.85	14.88	32.50	2.36	2.95	57.38	1.12	8.17	23.38	17.39	0.169	Up	1.45
Peptidyl-prolyl cis-trans isomerase A	P62937	0.00	0.00	0.00	2.13	3.75	0.00	0.59	0.00	2.24	3.27	2.13	3.86	0.177	Up	1.22
Ig kappa chain V-I region AU	P01594	10.63	0.00	7.65	0.00	33.75	0.00	22.43	40.38	3.36	4.90	12.75	15.46	0.177	Up	1.05
Ig gamma-2 chain C region	P01859	5.31	50.23	27.20	19.13	40.00	33.06	72.61	242.27	11.19	21.25	229.52	63.76	0.180	Up	3.66

N(G),N(G)-dimethylarginine dimethylaminohydrolase 2	Q95865	0.00	0.00	0.00	0.00	0.00	0.00	6.49	2.13	1.12	0.00	0.00	0.00	0.182	Up	N/A
Triosephosphate isomerase	P60174	0.00	0.00	0.00	0.00	0.00	0.00	1.18	0.00	0.00	3.27	0.00	11.59	0.182	Up	N/A
Fatty acid-binding protein, epidermal	Q01469	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.59	1.63	2.13	0.00	0.182	Up	N/A
Phosphatidylethanolamine-binding protein 4	Q96S96	0.00	0.00	0.00	0.00	0.00	0.00	1.18	0.00	2.24	0.00	2.13	0.00	0.182	Up	N/A
DNA repair protein REV1	Q9UBZ9	0.00	0.00	0.00	0.00	0.00	0.00	0.59	4.25	0.00	0.00	2.13	0.00	0.182	Up	N/A
Metalloproteinase inhibitor 1	P01033	0.00	0.00	0.00	0.00	0.00	0.00	0.59	0.00	2.24	0.00	2.13	0.00	0.182	Up	N/A
Complement component C8 gamma chain	P07360	0.00	0.00	0.43	0.00	0.00	0.00	0.00	0.00	1.12	8.17	34.00	0.00	0.182	Up	33.95
Transthyretin	P02766	0.00	0.00	0.00	0.00	1.25	0.00	0.00	0.00	5.59	0.00	10.63	1.93	0.182	Up	4.84
Vesicular integral-membrane protein VIP36	Q12907	0.89	1.93	8.50	21.25	28.75	11.81	30.70	12.75	3.36	16.35	21.25	28.98	0.193	Up	1.55
Alpha-1-antichymotrypsin	P01011	4.43	7.73	7.23	19.13	2.50	23.61	54.90	131.76	2.24	19.62	85.01	7.73	0.197	Up	4.66
N-acetylmuramoyl-L-alanine amidase	Q96PD5	0.00	0.00	0.00	0.00	7.50	0.00	2.95	23.38	0.00	0.00	6.38	3.86	0.197	Up	1.22
Abhydrolase domain-containing protein 14B	Q96IU4	0.00	0.00	0.00	2.13	0.00	0.00	5.90	0.00	1.12	0.00	2.13	1.93	0.197	Up	1.30
Neutrophil defensin 1	P59665	0.00	0.00	1.28	4.25	5.00	0.00	2.36	8.50	2.24	14.71	4.25	0.00	0.203	Up	1.83
Uteroglobin	P11684	0.00	0.00	1.28	0.00	5.00	0.00	17.71	2.13	3.36	0.00	6.38	0.00	0.210	Up	2.36
Trypsin-2	P07478	0.00	0.00	0.00	14.88	11.25	0.00	0.59	0.00	6.71	34.33	21.25	3.86	0.221	Up	1.02
Lysozyme C	P61626	0.89	0.00	0.43	0.00	2.50	0.00	2.36	6.38	23.49	0.00	31.88	0.00	0.221	Up	12.62
Prothymosin alpha	P06454	0.00	0.00	0.43	4.25	1.25	0.00	11.22	0.00	6.71	1.63	38.25	0.00	0.221	Up	7.32
Cathepsin Z	Q9UBR2	0.00	0.00	0.85	12.75	8.75	0.00	3.54	6.38	1.12	14.71	6.38	1.93	0.221	Up	1.31
Pancreatic secretory trypsin inhibitor	P00995	0.00	0.00	1.70	2.13	3.75	9.45	12.40	0.00	3.36	94.82	10.63	1.93	0.234	Up	5.79
Ig kappa chain V-I region Scw	P01609	13.28	0.00	7.23	0.00	35.00	0.00	25.97	59.50	3.36	4.90	12.75	15.46	0.234	Up	1.10
Ig kappa chain C region	P01834	294.87	142.97	230.37	507.92	461.29	453.37	322.91	733.18	278.51	315.51	558.92	562.20	0.240	Up	1.33
Ig alpha-1 chain C region	P01876	1.77	27.05	22.10	36.13	62.51	37.78	48.41	116.88	10.07	42.50	97.76	28.98	0.240	Up	1.84
Ig gamma-3 chain C region	P01860	13.28	44.44	29.33	25.50	50.00	25.97	68.48	221.02	21.25	19.62	284.77	79.21	0.240	Up	3.68
Cystatin-C	P01034	0.00	3.86	3.40	4.25	6.25	7.08	2.95	14.88	23.49	17.98	38.25	1.93	0.240	Up	3.34
Fibrinogen gamma chain	P02679	0.89	1.93	1.28	2.13	12.50	0.00	17.12	2.13	0.00	6.54	40.38	1.93	0.260	Up	3.64
N-acetylglucosamine-6-sulfatase	P15586	1.77	0.00	5.53	2.13	0.00	0.00	4.13	4.25	0.00	8.17	0.00	9.66	0.286	Up	2.09
Ubiquitin carboxyl-terminal hydrolase 36	Q9P275	0.89	5.80	0.85	2.13	1.25	0.00	1.77	12.75	1.12	0.00	6.38	5.80	0.290	Up	2.55
Apolipoprotein C-II	P02655	0.00	0.00	0.00	0.00	0.00	2.36	0.00	0.00	6.71	1.63	10.63	0.00	0.303	Up	2.68
Polyubiquitin-B	P0CG47	2.66	0.00	4.25	17.00	35.00	0.00	14.17	0.00	19.01	6.54	63.76	27.05	0.307	Up	1.77

Protein FAM3C	Q92520	0.00	0.00	0.00	2.13	6.25	0.00	2.36	0.00	3.36	8.17	2.13	0.00	0.307	Up	1.05
Ig gamma-1 chain C region	P01857	25.68	69.55	39.95	70.13	65.01	40.14	73.79	233.77	20.13	35.96	299.65	98.53	0.310	Up	2.45
Ig lambda-2 chain C regions	P0CG05	35.42	17.39	31.45	89.26	56.25	110.98	79.69	104.13	54.81	57.22	180.64	40.57	0.310	Up	1.52
SH3 domain-binding glutamic acid-rich-like protein 3	Q9H299	7.97	7.73	8.08	40.38	11.25	61.39	35.42	17.00	16.78	16.35	42.50	17.39	0.310	Up	1.06
Ig heavy chain V-III region GAL	P01781	0.00	0.00	0.43	0.00	1.25	0.00	2.36	10.63	0.00	0.00	6.38	0.00	0.318	Up	7.71
Chromogranin-A	P10645	0.89	0.00	0.43	0.00	0.00	0.00	1.18	0.00	4.47	0.00	4.25	0.00	0.318	Up	5.04
Ig kappa chain V-I region Wes	P01611	0.00	0.00	0.43	0.00	6.25	7.08	2.95	12.75	2.24	0.00	6.38	3.86	0.327	Up	1.23
Antithrombin-III	P01008	0.00	0.00	0.43	2.13	10.00	0.00	0.00	17.00	1.12	0.00	14.88	7.73	0.372	Up	2.43
Alpha-2-macroglobulin	P01023	0.00	5.80	2.98	2.13	8.75	4.72	19.48	40.38	0.00	8.17	38.25	0.00	0.387	Up	5.45
Protein AMBP	P02760	177.98	218.31	250.35	461.16	413.78	998.83	402.01	465.41	304.24	953.06	612.05	347.76	0.394	Up	1.22
Leucine-rich alpha-2-glycoprotein	P02750	15.94	0.00	59.08	48.88	77.51	54.31	107.44	80.76	33.56	168.38	34.00	30.91	0.394	Up	1.48
Immunoglobulin lambda-like polypeptide 5	B9A064	24.79	17.39	26.35	61.63	40.00	110.98	47.23	82.88	23.49	75.20	106.26	48.30	0.394	Up	1.36
Ig kappa chain V-III region VG (Fragment)	P04433	2.66	5.80	5.53	23.38	5.00	9.45	3.54	10.63	7.83	8.17	25.50	5.80	0.416	Up	1.19
Beta-2-microglobulin	P61769	16.82	3.86	8.50	74.38	18.75	37.78	24.20	19.13	30.20	191.27	40.38	3.86	0.418	Up	1.93
3-mercaptopyruvate sulfurtransferase	P25325	0.00	3.86	0.00	0.00	0.00	0.00	1.18	0.00	0.00	1.63	0.00	5.80	0.424	Up	1.35
Myc box-dependent-interacting protein 1	O00499	0.00	0.00	0.00	0.00	0.00	2.36	1.18	0.00	0.00	3.27	2.13	0.00	0.424	Up	1.08
Glutaredoxin-1	P35754	0.00	0.00	0.00	4.25	0.00	2.36	4.13	0.00	1.12	1.63	6.38	0.00	0.437	Up	1.00
Gastrotropin	P51161	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	17.90	0.00	0.00	1.93	0.455	Up	N/A
Fructose-bisphosphate aldolase B	P05062	0.00	0.00	0.00	0.00	1.25	0.00	7.67	0.00	0.00	0.00	4.25	0.00	0.455	Up	4.77
Thyroxine-binding globulin	P05543	0.00	0.00	0.43	0.00	0.00	0.00	1.18	12.75	0.00	0.00	0.00	0.00	0.455	Up	16.39
Complement component C9	P02748	0.00	0.00	0.00	0.00	0.00	0.00	0.59	0.00	0.00	0.00	12.75	0.00	0.455	Up	N/A
Phosphoglycerate kinase 1	P00558	0.00	0.00	0.00	0.00	1.25	0.00	1.77	0.00	0.00	0.00	0.00	5.80	0.455	Up	3.03
Thioredoxin	P10599	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.63	3.86	0.455	Up	N/A
Alpha-2-antiplasmin	P08697	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.13	0.00	0.00	12.75	0.00	0.455	Up	N/A
Junctional adhesion molecule A	Q9Y624	0.00	0.00	0.43	0.00	0.00	0.00	1.77	0.00	0.00	0.00	4.25	0.00	0.455	Up	7.08
C-reactive protein	P02741	0.00	0.00	0.43	0.00	0.00	0.00	1.18	0.00	0.00	0.00	6.38	0.00	0.455	Up	8.89
Complement factor H	P08603	0.00	0.00	0.00	0.00	0.00	0.00	2.95	0.00	0.00	0.00	2.13	0.00	0.455	Up	N/A
Dermokine	Q6E0U4	0.00	0.00	0.43	0.00	0.00	0.00	0.00	0.00	0.00	1.63	4.25	0.00	0.455	Up	6.92
Intercellular adhesion molecule 2	P13598	0.00	0.00	0.00	0.00	0.00	0.00	2.95	0.00	0.00	1.63	0.00	0.00	0.455	Up	N/A
Protein NOV homolog	P48745	0.00	0.00	0.43	0.00	0.00	0.00	0.59	0.00	0.00	4.90	0.00	0.00	0.455	Up	6.46

Connective tissue growth factor	P29279	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.47	0.00	2.13	0.00	0.455	Up	N/A
Inter-alpha-trypsin inhibitor heavy chain H2	P19823	0.00	0.00	0.00	0.00	0.00	0.00	0.59	0.00	0.00	0.00	8.50	0.00	0.455	Up	N/A
Protein S100-A6	P06703	0.00	0.00	0.43	0.00	0.00	0.00	1.18	0.00	0.00	0.00	0.00	1.93	0.455	Up	3.66
Alpha-hemoglobin-stabilizing protein	Q9NZD4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.12	0.00	6.38	0.00	0.455	Up	N/A
Complement factor H-related protein 2	P36980	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.90	2.13	0.00	0.455	Up	N/A
Insulin-like growth factor-binding protein 4	P22692	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.24	0.00	2.13	0.00	0.455	Up	N/A
Macrophage colony-stimulating factor 1	P09603	0.00	0.00	1.28	6.38	0.00	7.08	10.63	0.00	4.47	14.71	2.13	0.00	0.470	Up	1.63
Complement component C7	P10643	0.00	0.00	1.28	2.13	1.25	0.00	2.36	4.25	0.00	0.00	21.25	0.00	0.481	Up	5.99
Cadherin-13	P55290	0.89	0.00	3.83	0.00	10.00	2.36	4.13	4.25	0.00	3.27	8.50	1.93	0.483	Up	1.03
Ig kappa chain V-I region EU	P01598	0.89	0.00	4.68	4.25	8.75	7.08	12.40	17.00	2.24	1.63	8.50	3.86	0.485	Up	1.48
Ig alpha-2 chain C region	P01877	0.89	7.73	10.20	10.63	12.50	11.81	14.17	40.38	10.07	3.27	61.63	9.66	0.485	Up	2.59
Immunoglobulin J chain	P01591	0.00	0.00	2.98	8.50	2.50	0.00	4.72	2.13	3.36	3.27	0.00	1.93	0.498	Up	1.51
Guanylin	Q02747	2.66	0.00	0.43	12.75	18.75	16.53	2.36	6.38	17.90	27.79	12.75	9.66	0.509	Up	1.25
Thrombospondin-1	P07996	2.66	0.00	0.00	0.00	5.00	0.00	8.26	2.13	0.00	1.63	0.00	1.93	0.545	Up	1.10
Endothelial protein C receptor	Q9UNN8	1.77	0.00	2.55	0.00	1.25	0.00	9.45	0.00	0.00	0.00	0.00	0.00	0.545	Up	5.09
Fibulin-1	P23142	0.00	0.00	0.00	2.13	2.50	0.00	9.45	0.00	0.00	4.90	0.00	1.93	0.545	Up	2.35
SPARC-like protein 1	Q14515	0.00	3.86	0.00	2.13	0.00	0.00	6.49	0.00	1.12	0.00	10.63	0.00	0.545	Up	2.03
Nuclear transport factor 2	P61970	0.00	0.00	0.00	2.13	3.75	0.00	6.49	0.00	1.12	0.00	0.00	3.86	0.545	Up	1.30
L-lactate dehydrogenase B chain	P07195	0.00	0.00	0.43	0.00	2.50	0.00	2.36	0.00	0.00	3.27	0.00	1.93	0.545	Up	1.72
Lithostathine-1-beta	P48304	0.00	0.00	1.28	21.25	28.75	49.59	16.53	10.63	2.24	45.77	25.50	25.12	0.554	Up	1.20
Haptoglobin	P00738	0.00	7.73	21.25	14.88	13.75	11.81	16.53	2.13	20.13	17.98	6.38	17.39	0.589	Up	1.03
Nidogen-1	P14543	2.66	1.93	1.28	14.88	23.75	7.08	27.15	8.50	0.00	8.17	4.25	19.32	0.589	Up	1.57
Tumor necrosis factor receptor superfamily member 12A	Q9NP84	0.00	0.00	0.85	2.13	1.25	0.00	2.95	0.00	0.00	1.63	21.25	0.00	0.589	Up	6.12
Proactivator polypeptide	P07602	0.89	0.00	0.85	0.00	1.25	0.00	1.18	0.00	2.24	0.00	2.13	0.00	0.589	Up	1.86
6-phosphogluconolactonase	Q95336	0.00	0.00	0.00	4.25	3.75	2.36	15.94	0.00	1.12	1.63	2.13	3.86	0.606	Up	1.43
Ig lambda chain V-I region HA	P01700	0.00	1.93	0.00	8.50	8.75	0.00	2.36	6.38	0.00	4.90	2.13	3.86	0.606	Up	1.63
Fibulin-5	Q9UBX5	1.77	0.00	5.95	2.13	15.00	37.78	17.71	6.38	0.00	14.71	14.88	9.66	0.623	Up	1.01
Syndecan-1	P18827	0.00	0.00	0.00	2.13	1.25	0.00	4.72	0.00	0.00	4.90	0.00	0.00	0.697	Up	2.85
Cathepsin L1	P07711	0.00	0.00	0.43	0.00	2.50	0.00	1.18	0.00	1.12	1.63	0.00	0.00	0.697	Up	1.12
Apolipoprotein D	P05090	1.77	13.52	149.19	25.50	40.00	99.17	58.44	238.02	0.00	91.55	46.75	27.05	0.699	Up	1.68

Kininogen-1	P01042	15.94	77.28	30.18	68.01	72.51	61.39	64.94	76.51	43.62	39.23	97.76	61.82	0.699	Up	1.18
Prothrombin	P00734	0.89	67.62	23.80	34.00	12.50	40.14	42.50	14.88	24.61	53.95	38.25	17.39	0.699	Up	1.07
Ig kappa chain V-I region AG	P01593	12.40	0.00	13.60	19.13	37.50	7.08	27.15	40.38	3.36	6.54	14.88	17.39	0.699	Up	1.02
EGF-containing fibulin-like extracellular matrix protein 1	Q12805	0.89	9.66	11.48	12.75	16.25	14.17	28.34	6.38	3.36	45.77	38.25	3.86	0.699	Up	1.93
Liver-expressed antimicrobial peptide 2	Q969E1	7.97	9.66	2.55	6.38	6.25	28.34	7.08	2.13	5.59	13.08	38.25	1.93	0.699	Up	1.11
Peptidase inhibitor 16	Q6UXB8	0.00	0.00	0.85	2.13	1.25	0.00	4.72	0.00	1.12	0.00	4.25	0.00	0.708	Up	2.39
Vascular cell adhesion protein 1	P19320	0.00	0.00	2.98	2.13	15.00	0.00	9.45	0.00	0.00	19.62	2.13	1.93	0.719	Up	1.24
Carbonic anhydrase 2	P00918	0.00	0.00	0.00	0.00	7.50	0.00	0.59	0.00	0.00	0.00	0.00	52.16	0.727	Up	3.52
Prostate-specific antigen	P07288	0.00	0.00	0.00	0.00	10.00	0.00	0.00	0.00	11.19	9.81	0.00	0.00	0.727	Up	1.05
Acyl-CoA-binding protein	P07108	0.00	0.00	0.00	0.00	1.25	0.00	0.00	0.00	1.12	0.00	21.25	0.00	0.727	Up	8.95
V-set and immunoglobulin domain-containing protein 4	Q9Y279	0.00	0.00	0.00	0.00	0.00	2.36	1.18	0.00	0.00	0.00	14.88	0.00	0.727	Up	3.40
Lumican	P51884	0.00	0.00	0.00	0.00	2.50	0.00	2.36	0.00	0.00	0.00	0.00	3.86	0.727	Up	1.24
ADM	P35318	0.00	0.00	0.00	0.00	0.00	2.36	0.00	0.00	4.47	0.00	2.13	0.00	0.727	Up	1.40
Tyrosine-protein phosphatase non-receptor type substrate 1	P78324	0.00	1.93	0.00	0.00	0.00	0.00	2.36	0.00	0.00	1.63	0.00	0.00	0.727	Up	1.03
Lymphatic vessel endothelial hyaluronic acid receptor 1	Q9Y5Y7	0.00	0.00	11.05	8.50	16.25	2.36	13.58	8.50	1.12	1.63	12.75	5.80	0.729	Up	1.32
Actin, cytoplasmic 1	P60709	0.00	3.86	4.25	12.75	17.50	33.06	13.58	0.00	7.83	21.25	19.13	9.66	0.738	Up	1.00
Vitronectin	P04004	0.89	1.93	6.38	0.00	3.75	9.45	8.85	2.13	0.00	4.90	4.25	3.86	0.738	Up	1.07
Complement decay-accelerating factor	P08174	0.00	1.93	2.13	8.50	3.75	0.00	14.17	0.00	0.00	3.27	0.00	1.93	0.740	Up	1.58
Lithostathine-1-alpha	P05451	0.00	0.00	2.13	31.88	47.50	49.59	24.79	10.63	4.47	44.14	40.38	25.12	0.777	Up	1.32
Plasminogen	P00747	0.00	0.00	2.55	6.38	3.75	4.72	4.13	0.00	17.90	1.63	65.88	0.00	0.797	Up	5.15
Glyceraldehyde-3-phosphate dehydrogenase	P04406	0.00	0.00	0.43	0.00	2.50	0.00	8.85	0.00	0.00	0.00	0.00	1.93	0.848	Up	3.69
Tripeptidyl-peptidase 1	O14773	0.00	0.00	1.28	0.00	3.75	0.00	2.36	0.00	0.00	0.00	0.00	3.86	0.848	Up	1.24
Complement factor I	P05156	0.00	0.00	0.85	0.00	0.00	2.36	3.54	0.00	0.00	0.00	0.00	1.93	0.848	Up	1.70
Insulin-like growth factor-binding protein 3	P17936	0.00	0.00	0.43	0.00	1.25	0.00	2.36	0.00	1.12	0.00	0.00	0.00	0.848	Up	2.08
Hemoglobin subunit gamma-1	P69891	0.00	0.00	0.43	0.00	3.75	0.00	1.18	0.00	0.00	0.00	0.00	27.05	0.848	Up	6.76
WNT1-inducible-signaling pathway protein 2	Q76076	0.00	0.00	1.28	2.13	0.00	2.36	6.49	0.00	0.00	1.63	0.00	0.00	0.848	Up	2.12
Lactotransferrin	P02788	1.77	0.00	14.03	0.00	1.25	0.00	11.22	0.00	0.00	13.08	0.00	1.93	0.870	Up	1.54
Endonuclease domain-containing 1 protein	O94919	0.00	0.00	0.85	2.13	1.25	0.00	6.49	0.00	0.00	1.63	0.00	0.00	0.924	Up	2.89
Secretogranin-1	P05060	1.77	0.00	0.85	0.00	2.50	0.00	4.13	0.00	2.24	0.00	0.00	0.00	0.924	Up	1.87

Hemoglobin subunit beta	P68871	4.43	23.18	8.93	29.75	108.76	9.45	12.40	2.13	8.95	4.90	42.50	481.06	0.937	Up	2.99
Hemoglobin subunit alpha	P69905	1.77	21.25	3.40	51.00	85.01	7.08	9.45	0.00	4.47	6.54	25.50	548.68	0.937	Up	4.21
Gelsolin	P06396	4.43	3.86	10.20	48.88	25.00	59.03	36.60	12.75	3.36	58.85	44.63	21.25	0.937	Up	1.17
Cathepsin D	P07339	0.89	1.93	9.78	12.75	21.25	16.53	17.71	10.63	3.36	9.81	14.88	7.73	0.937	Up	1.02
Lysosomal alpha-glucosidase	P10253	1.77	9.66	2.98	0.00	5.00	4.72	7.08	2.13	2.24	6.54	4.25	1.93	0.937	Up	1.20
Latent-transforming growth factor beta-binding protein 2	Q14767	0.00	0.00	1.28	2.13	1.25	2.36	1.77	0.00	0.00	13.08	2.13	0.00	0.957	Up	3.23
Collagen alpha-3(VI) chain	P12111	0.00	0.00	1.70	4.25	2.50	2.36	2.36	0.00	1.12	9.81	2.13	1.93	0.972	Up	1.28
Collagen alpha-1(I) chain	P02452	3.54	0.00	4.68	0.00	6.25	4.72	4.72	4.25	3.36	4.90	2.13	0.00	0.974	Up	1.24
Hemoglobin subunit delta	P02042	1.77	3.86	3.40	8.50	61.25	0.00	2.95	0.00	7.83	0.00	8.50	260.82	0.974	Up	4.44
Latent-transforming growth factor beta-binding protein 1	Q14766	0.00	0.00	0.00	2.13	1.25	2.36	1.18	0.00	1.12	1.63	2.13	0.00	0.978	Up	1.26
Alpha-amylase 1	P04745	0.00	1.93	2.13	27.63	5.00	18.89	183.00	0.00	3.36	13.08	0.00	5.80	1.000	Up	4.62
Insulin-like growth factor-binding protein 7	Q16270	3.54	3.86	6.38	8.50	27.50	33.06	29.52	31.88	1.12	19.62	2.13	9.66	1.000	Up	1.13
Semenogelin-2	Q02383	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	147.13	0.00	0.00	1.000	Up	N/A
Granulins	P28799	0.89	5.80	4.68	4.25	1.25	21.25	27.15	6.38	0.00	8.17	0.00	1.93	1.000	Up	1.72
Fibronectin	P02751	0.00	0.00	2.13	0.00	1.25	7.08	17.71	0.00	0.00	14.71	0.00	0.00	1.000	Up	4.65
Vasorin	Q6EMK4	1.77	5.80	2.55	0.00	3.75	0.00	11.22	0.00	0.00	1.63	2.13	3.86	1.000	Up	1.36
Low-density lipoprotein receptor-related protein 2	P98164	0.89	0.00	2.98	4.25	0.00	2.36	7.08	2.13	0.00	0.00	0.00	7.73	1.000	Up	2.16
Cathelicidin antimicrobial peptide	P49913	0.00	0.00	0.00	4.25	0.00	7.08	12.40	0.00	0.00	3.27	0.00	0.00	1.000	Up	1.38
Mucin-like protein 1	Q96DR8	0.00	0.00	0.00	2.13	5.00	0.00	1.18	0.00	0.00	27.79	0.00	0.00	1.000	Up	4.07
Semenogelin-1	P04279	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	73.56	0.00	0.00	1.000	Up	N/A
Deleted in malignant brain tumors 1 protein	Q9UGM3	0.00	0.00	0.00	0.00	0.00	0.00	10.04	0.00	0.00	0.00	0.00	0.00	1.000	Up	N/A
Pyruvate kinase isozymes M1/M2	P14618	0.00	0.00	0.00	0.00	1.25	0.00	5.31	0.00	0.00	0.00	0.00	0.00	1.000	Up	4.25
Tumor necrosis factor receptor superfamily member 16	P08138	0.00	0.00	0.00	0.00	0.00	2.36	4.72	0.00	0.00	0.00	0.00	0.00	1.000	Up	2.00
Zymogen granule protein 16 homolog B	Q96DA0	0.00	0.00	0.00	0.00	2.50	0.00	1.77	0.00	1.12	0.00	0.00	0.00	1.000	Up	1.73
Calreticulin	P27797	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	14.88	0.00	1.000	Up	N/A
Flavin reductase	P30043	0.00	0.00	0.00	0.00	3.75	0.00	1.77	0.00	0.00	0.00	2.13	0.00	1.000	Up	1.93
Bisphosphoglycerate mutase	P07738	0.00	0.00	0.00	0.00	0.00	2.36	0.00	0.00	0.00	0.00	0.00	9.66	1.000	Up	4.09
Cadherin-related family member 2	Q9BYE9	0.00	0.00	0.43	0.00	1.25	0.00	1.77	0.00	0.00	0.00	0.00	0.00	1.000	Up	2.11
Cadherin-11	P55287	0.00	0.00	0.43	0.00	0.00	0.00	1.77	0.00	0.00	0.00	0.00	0.00	1.000	Up	4.17

Prostatic acid phosphatase	P15309	0.00	0.00	0.00	0.00	2.50	0.00	0.00	0.00	3.36	0.00	0.00	0.00	1.000	Up	1.34
Beta-microseminoprotein	P08118	0.00	0.00	0.00	0.00	2.50	0.00	0.00	0.00	4.47	0.00	0.00	0.00	1.000	Up	1.79
Ubiquitin carboxyl-terminal hydrolase 8	P40818	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.50	0.00	0.00	0.00	0.00	1.000	Up	N/A
Signal-regulatory protein beta-1	O00241	0.00	0.00	0.00	0.00	0.00	2.36	1.77	0.00	0.00	1.63	0.00	0.00	1.000	Up	1.39
Chitinase-3-like protein 1	P36222	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.24	0.00	0.00	0.00	1.000	Up	N/A
Heparin cofactor 2	P05546	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	8.50	0.00	1.000	Up	N/A
Complement C2	P06681	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.25	0.00	0.00	0.00	0.00	1.000	Up	N/A
Aminoacylase-1	Q03154	0.00	0.00	0.00	0.00	1.25	0.00	1.77	0.00	0.00	0.00	0.00	0.00	1.000	Up	1.42
Thioredoxin domain-containing protein 5	Q8NBS9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	6.38	0.00	1.000	Up	N/A
Mesothelin	Q13421	0.00	0.00	0.00	0.00	0.00	0.00	1.77	0.00	0.00	0.00	0.00	0.00	1.000	Up	N/A
Glutaminyl-peptide cyclotransferase	Q16769	0.00	0.00	0.00	0.00	0.00	0.00	2.36	0.00	0.00	0.00	0.00	0.00	1.000	Up	N/A
Cystatin-S	P01036	0.00	0.00	0.00	0.00	0.00	0.00	2.36	0.00	0.00	0.00	0.00	0.00	1.000	Up	N/A
Di-N-acetylchitobiase	Q01459	0.00	0.00	0.00	0.00	0.00	0.00	2.36	0.00	0.00	0.00	0.00	0.00	1.000	Up	N/A
Matrix-remodeling-associated protein 8	Q9BRK3	0.00	0.00	0.00	0.00	0.00	0.00	1.77	0.00	0.00	0.00	0.00	0.00	1.000	Up	N/A
Mucin-5B	Q9HC84	0.00	0.00	0.00	0.00	0.00	0.00	2.36	0.00	0.00	0.00	0.00	0.00	1.000	Up	N/A
Copper transport protein ATOX1	O00244	0.00	0.00	0.00	0.00	0.00	0.00	2.36	0.00	0.00	0.00	0.00	0.00	1.000	Up	N/A
CMRF35-like molecule 1	Q8TDQ1	0.00	0.00	0.00	0.00	0.00	0.00	2.36	0.00	0.00	0.00	0.00	0.00	1.000	Up	N/A
Neprilysin	P08473	0.00	0.00	0.00	0.00	0.00	0.00	1.18	0.00	0.00	0.00	0.00	0.00	1.000	Up	N/A
Cytosolic non-specific dipeptidase	Q96KP4	0.00	0.00	0.00	0.00	0.00	0.00	1.18	0.00	0.00	0.00	0.00	0.00	1.000	Up	N/A
Ephrin type-B receptor 4	P54760	0.00	0.00	0.00	0.00	0.00	0.00	1.18	0.00	0.00	0.00	0.00	0.00	1.000	Up	N/A
Fructose-1,6-bisphosphatase 1	P09467	0.00	0.00	0.00	0.00	0.00	0.00	1.77	0.00	0.00	0.00	0.00	0.00	1.000	Up	N/A
Peroxiredoxin-6	P30041	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.80	1.000	Up	N/A
5'(3')-deoxyribonucleotidase, cytosolic type	Q8TCD5	0.00	0.00	0.00	0.00	0.00	0.00	1.77	0.00	0.00	0.00	0.00	0.00	1.000	Up	N/A
Inter-alpha-trypsin inhibitor heavy chain H1	P19827	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.25	0.00	1.000	Up	N/A
D-dopachrome decarboxylase	P30046	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.25	0.00	1.000	Up	N/A
Collagen alpha-1(XV) chain	P39059	0.00	0.00	0.00	0.00	0.00	0.00	1.18	0.00	0.00	0.00	0.00	0.00	1.000	Up	N/A
Folate receptor gamma	P41439	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	4.25	0.00	1.000	Up	N/A
Elongation factor 1-alpha 1	P68104	0.00	0.00	0.00	0.00	0.00	0.00	1.18	0.00	0.00	0.00	0.00	0.00	1.000	Up	N/A
Gamma-glutamyl hydrolase	Q92820	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.86	1.000	Up	N/A
Heat shock protein beta-11	Q9Y547	0.00	0.00	0.00	0.00	0.00	0.00	1.18	0.00	0.00	0.00	0.00	0.00	1.000	Up	N/A

Trans-Golgi network integral membrane protein 2	Q43493	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.24	0.00	0.00	0.00	1.000	Up	N/A
Tetratricopeptide repeat protein 17	Q96AE7	0.00	0.00	0.00	0.00	0.00	0.00	1.18	0.00	0.00	0.00	0.00	0.00	1.000	Up	N/A
Keratin, type II cytoskeletal 5	P13647	2.66	1.93	7.65	4.25	1.25	4.72	1.18	0.00	3.36	0.00	0.00	1.93	0.028	Down	1.74
Secreted Ly-6/uPAR-related protein 1	P55000	1.77	1.93	3.83	6.38	1.25	11.81	4.72	0.00	0.00	1.63	0.00	0.00	0.035	Down	1.41
Non-secretory ribonuclease	P10153	7.08	17.39	11.48	27.63	6.25	33.06	21.84	6.38	3.36	4.90	2.13	0.00	0.041	Down	2.22
Keratin, type II cytoskeletal 1	P04264	17.71	17.39	36.98	31.88	23.75	87.37	15.35	10.63	10.07	49.04	10.63	17.39	0.048	Down	1.90
Leukocyte-associated immunoglobulin-like receptor 1	Q6GTX8	0.00	5.80	0.85	4.25	1.25	2.36	4.13	0.00	0.00	0.00	0.00	0.00	0.054	Down	1.42
Keratin, type I cytoskeletal 10	P13645	1.77	13.52	48.88	25.50	8.75	21.25	12.40	0.00	5.59	14.71	0.00	5.80	0.089	Down	2.07
Keratin, type I cytoskeletal 9	P35527	6.20	5.80	13.18	29.75	15.00	28.34	5.90	4.25	3.36	8.17	2.13	21.25	0.093	Down	2.18
Immunoglobulin superfamily member 8	Q969P0	0.00	3.86	0.43	6.38	2.50	40.14	3.54	0.00	0.00	3.27	0.00	0.00	0.113	Down	3.13
CD27 antigen	P26842	0.89	1.93	1.70	2.13	0.00	2.36	4.13	0.00	0.00	0.00	0.00	0.00	0.113	Down	2.29
CD44 antigen	P16070	3.54	13.52	5.95	4.25	6.25	11.81	9.45	0.00	0.00	4.90	0.00	5.80	0.128	Down	1.12
Extracellular sulfatase Sulf-2	Q8IWU5	3.54	19.32	5.10	17.00	21.25	61.39	6.49	0.00	1.12	27.79	2.13	3.86	0.132	Down	2.57
Keratin, type I cytoskeletal 14	P02533	0.00	0.00	5.10	4.25	0.00	4.72	0.00	0.00	1.12	0.00	0.00	0.00	0.182	Down	4.19
Kallikrein-1	P06870	0.00	11.59	0.85	0.00	13.75	11.81	11.22	0.00	0.00	1.63	0.00	0.00	0.210	Down	1.48
Pepsin A	P00790	17.71	3.86	8.08	8.50	10.00	54.31	7.67	2.13	4.47	19.62	2.13	9.66	0.225	Down	2.24
Keratin, type II cytoskeletal 8	P05787	0.89	3.86	2.13	14.88	2.50	2.36	3.54	0.00	3.36	1.63	0.00	1.93	0.225	Down	1.70
CMRF35-like molecule 9	Q6UXG3	0.00	5.80	0.43	4.25	0.00	7.08	1.18	4.25	0.00	0.00	0.00	0.00	0.242	Down	1.62
Cubilin	O60494	1.77	5.80	1.70	0.00	5.00	7.08	8.85	4.25	0.00	0.00	0.00	0.00	0.286	Down	1.53
Tumor necrosis factor receptor superfamily member 14	Q92956	0.89	0.00	0.00	2.13	1.25	0.00	1.18	0.00	0.00	0.00	0.00	0.00	0.303	Down	1.20
Secreted and transmembrane protein 1	Q8WVN6	2.66	0.00	0.00	2.13	3.75	4.72	2.95	0.00	1.12	0.00	2.13	0.00	0.307	Down	1.60
Uromodulin	P07911	80.58	177.74	51.43	463.29	207.52	443.93	183.59	144.51	34.67	349.84	78.63	54.10	0.310	Down	1.69
CD59 glycoprotein	P13987	18.60	40.57	40.38	61.63	32.50	181.82	45.46	27.63	11.19	94.82	14.88	27.05	0.310	Down	1.70
Ig kappa chain V-III region NG9 (Fragment)	P01621	1.77	5.80	5.10	17.00	8.75	16.53	6.49	4.25	5.59	4.90	14.88	0.00	0.310	Down	1.27
Protein shisa-5	Q8N114	0.00	0.00	2.13	14.88	3.75	14.17	3.54	0.00	1.12	9.81	0.00	0.00	0.372	Down	1.81
Carboxypeptidase N subunit 2	P22792	0.00	1.93	0.43	0.00	2.50	4.72	4.13	0.00	0.00	1.63	0.00	0.00	0.372	Down	1.20
Pro-epidermal growth factor	P01133	0.89	11.59	2.13	0.00	0.00	0.00	2.95	2.13	1.12	0.00	4.25	1.93	0.381	Down	1.97
Ig kappa chain V-IV region Len	P01625	3.54	5.80	2.98	2.13	16.25	4.72	12.99	0.00	10.07	3.27	0.00	0.00	0.387	Down	1.49
Keratin, type II cytoskeletal 2 epidermal	P35908	7.97	1.93	50.15	6.38	7.50	2.36	5.90	8.50	6.71	1.63	0.00	5.80	0.394	Down	2.23

Trefoil factor 1	P04155	0.00	0.00	0.43	0.00	2.50	25.97	8.26	2.13	1.12	3.27	2.13	0.00	0.411	Down	2.85
Phosphoinositide-3-kinase-interacting protein 1	Q96FE7	7.08	9.66	12.33	2.13	13.75	18.89	33.06	6.38	0.00	8.17	2.13	11.59	0.420	Down	1.15
Platelet glycoprotein VI	Q9HCN6	0.00	1.93	0.85	0.00	1.25	0.00	1.77	0.00	0.00	0.00	0.00	0.00	0.424	Down	1.32
Alpha-enolase	P06733	0.00	1.93	0.43	2.13	1.25	0.00	3.54	0.00	1.12	0.00	0.00	0.00	0.437	Down	1.63
Cathepsin B	P07858	0.00	1.93	0.43	6.38	2.50	2.36	2.36	2.13	0.00	3.27	0.00	0.00	0.450	Down	1.05
Src substrate cortactin	Q14247	0.00	3.86	0.00	6.38	0.00	0.00	1.77	0.00	0.00	0.00	0.00	0.00	0.455	Down	2.89
Matrix metalloproteinase-9	P14780	0.00	0.00	0.85	0.00	0.00	2.36	0.00	0.00	0.00	0.00	0.00	0.00	0.455	Down	N/A
Protein YIPF3	Q9GZM5	0.89	3.86	4.25	8.50	6.25	25.97	8.26	0.00	0.00	11.44	0.00	7.73	0.483	Down	1.10
Basement membrane-specific heparan sulfate proteoglycan core protein	P98160	10.63	21.25	27.20	80.76	42.50	247.94	46.64	40.38	26.84	170.01	99.88	44.44	0.485	Down	1.00
Trefoil factor 2	Q03403	0.00	3.86	3.83	4.25	1.25	18.89	6.49	2.13	0.00	9.81	0.00	0.00	0.498	Down	1.04
Ig kappa chain V-II region MIL	P01616	1.77	7.73	8.50	14.88	6.25	9.45	11.22	4.25	1.12	8.17	8.50	3.86	0.513	Down	1.31
Agrin	O00468	0.00	0.00	0.43	6.38	12.50	7.08	7.08	0.00	0.00	3.27	0.00	3.86	0.524	Down	1.39
Growth/differentiation factor 15	Q99988	0.00	0.00	0.00	0.00	0.00	7.08	2.95	0.00	0.00	0.00	2.13	1.93	0.545	Down	3.03
Biotinidase	P43251	0.00	1.93	0.00	2.13	2.50	0.00	4.72	0.00	0.00	0.00	0.00	0.00	0.545	Down	2.16
Amyloid beta A4 protein	P05067	0.89	0.00	0.00	2.13	0.00	2.36	2.95	0.00	0.00	0.00	0.00	0.00	0.545	Down	1.65
Ig kappa chain V-II region TEW	P01617	5.31	7.73	14.03	21.25	21.25	11.81	20.07	8.50	4.47	14.71	10.63	9.66	0.554	Down	1.20
Mannan-binding lectin serine protease 2	O00187	0.89	1.93	13.60	12.75	2.50	18.89	17.71	6.38	0.00	9.81	0.00	11.59	0.554	Down	1.35
Aminopeptidase N	P15144	0.89	0.00	1.70	4.25	3.75	9.45	5.31	4.25	0.00	0.00	0.00	1.93	0.567	Down	1.05
Beta-defensin 1	P60022	0.89	7.73	1.28	2.13	3.75	18.89	4.72	0.00	0.00	3.27	2.13	7.73	0.571	Down	1.29
Cadherin-1	P12830	2.66	1.93	7.23	2.13	2.50	18.89	21.25	0.00	0.00	3.27	0.00	3.86	0.582	Down	1.61
Acid ceramidase	Q13510	0.00	1.93	2.55	4.25	2.50	2.36	1.77	2.13	0.00	3.27	0.00	3.86	0.582	Down	1.01
Galectin-3-binding protein	Q08380	7.08	9.66	9.78	4.25	6.25	14.17	20.07	10.63	4.47	3.27	6.38	1.93	0.589	Down	1.09
Maltase-glucoamylase, intestinal	O43451	2.66	0.00	0.43	2.13	0.00	0.00	0.59	2.13	0.00	0.00	0.00	0.00	0.621	Down	1.28
Collagen alpha-1(VI) chain	P12109	0.00	3.86	0.85	0.00	0.00	2.36	2.95	0.00	0.00	1.63	0.00	0.00	0.697	Down	1.03
Peroxiredoxin-2	P32119	0.00	0.00	0.00	4.25	5.00	0.00	0.59	0.00	0.00	0.00	0.00	3.86	0.697	Down	2.08
Tyrosine-protein kinase receptor UFO	P30530	0.00	1.93	0.00	4.25	0.00	0.00	1.18	0.00	0.00	1.63	0.00	0.00	0.697	Down	2.20
Osteopontin	P10451	0.00	63.76	25.50	119.01	147.51	231.41	97.40	106.26	14.54	130.78	27.63	52.16	0.699	Down	1.64
WAP four-disulfide core domain protein 2	Q14508	47.82	9.66	19.55	53.13	42.50	61.39	67.89	40.38	15.66	37.60	31.88	28.98	0.699	Down	1.05
Interleukin-18-binding protein	O95998	0.00	0.00	1.28	0.00	0.00	21.25	2.36	0.00	0.00	0.00	0.00	0.00	0.727	Down	4.77
Colipase	P04118	0.00	0.00	0.00	23.38	0.00	2.36	0.00	0.00	0.00	0.00	6.38	0.00	0.727	Down	2.02

Hepatitis A virus cellular receptor 2	Q8TDQ0	0.00	0.00	1.70	0.00	0.00	7.08	3.54	0.00	0.00	0.00	0.00	0.00	0.727	Down	1.24
Low affinity immunoglobulin gamma Fc region receptor III-A	P08637	0.00	0.00	0.43	0.00	0.00	7.08	1.18	0.00	0.00	0.00	0.00	0.00	0.727	Down	3.18
Hemicentin-1	Q96RW7	0.00	1.93	0.43	0.00	0.00	0.00	1.77	0.00	0.00	0.00	0.00	0.00	0.727	Down	1.50
Pappalysin-2	Q9BXP8	0.00	0.00	0.43	0.00	0.00	2.36	1.77	0.00	0.00	0.00	0.00	0.00	0.727	Down	1.27
Titin	Q8WZ42	0.00	0.00	1.28	0.00	3.75	14.17	2.36	10.63	0.00	3.27	2.13	0.00	0.805	Down	1.39
Resistin	Q9HD89	0.00	1.93	0.85	14.88	2.50	14.17	5.31	8.50	0.00	9.81	2.13	0.00	0.814	Down	1.07
Polymeric immunoglobulin receptor	P01833	41.62	67.62	24.23	68.01	43.75	85.01	64.35	48.88	26.84	78.47	53.13	25.12	0.818	Down	1.11
Ribonuclease pancreatic	P07998	5.31	30.91	10.63	21.25	10.00	35.42	17.71	6.38	6.71	39.23	29.75	5.80	0.818	Down	1.08
Peptidoglycan recognition protein 1	O75594	1.77	9.66	2.98	29.75	10.00	30.70	16.53	0.00	3.36	31.06	6.38	9.66	0.848	Down	1.06
Plasma serine protease inhibitor	P05154	0.00	1.93	0.00	2.13	0.00	18.89	10.04	0.00	0.00	4.90	0.00	0.00	0.848	Down	1.02
Vitelline membrane outer layer protein 1 homolog	Q7Z5L0	0.00	5.80	2.55	2.13	0.00	35.42	18.30	2.13	0.00	9.81	0.00	1.93	0.857	Down	1.43
Desmocollin-2	Q02487	0.00	0.00	0.85	2.13	0.00	2.36	1.18	0.00	1.12	1.63	0.00	0.00	0.870	Down	1.36
Fibrillin-1	P35555	0.00	7.73	2.55	0.00	2.50	11.81	7.67	6.38	0.00	8.17	2.13	0.00	0.922	Down	1.01
Lipocalin-1	P31025	0.00	0.00	0.00	6.38	18.75	0.00	12.40	0.00	3.36	0.00	0.00	1.93	0.924	Down	2.13
Dipeptidyl peptidase 1	P53634	2.66	0.00	1.70	4.25	1.25	0.00	2.95	2.13	0.00	0.00	0.00	3.86	0.935	Down	1.21
Ig kappa chain V-III region CLL	P04207	1.77	3.86	8.50	21.25	5.00	16.53	2.95	6.38	5.59	6.54	12.75	5.80	0.937	Down	1.42
Ig kappa chain V-III region HAH	P18135	7.97	7.73	14.88	25.50	22.50	30.70	30.70	12.75	8.95	8.17	19.13	17.39	0.981	Down	1.13
Proteoglycan 4	Q92954	0.89	0.00	2.55	10.63	3.75	21.25	2.95	2.13	2.24	22.89	0.00	5.80	0.981	Down	1.09
Prostaglandin-H2 D-isomerase	P41222	64.64	81.14	40.38	99.88	108.76	328.22	115.11	89.26	33.56	219.06	48.88	104.33	1.000	Down	1.18
Cystatin-M	Q15828	2.66	5.80	8.93	42.50	22.50	56.67	11.81	2.13	25.73	52.31	10.63	17.39	1.000	Down	1.16
Inter-alpha-trypsin inhibitor heavy chain H4	Q14624	2.66	5.80	6.80	34.00	13.75	54.31	22.43	4.25	10.07	11.44	23.38	9.66	1.000	Down	1.44
Interleukin-1 receptor antagonist protein	P18510	0.00	0.00	0.00	8.50	13.75	0.00	15.35	0.00	0.00	6.54	0.00	0.00	1.000	Down	1.02
Trefoil factor 3	Q07654	0.00	0.00	0.43	0.00	3.75	23.61	4.13	0.00	0.00	0.00	6.38	1.93	1.000	Down	2.23
Hepcidin	P81172	0.00	1.93	1.70	0.00	6.25	0.00	3.54	0.00	0.00	1.63	0.00	3.86	1.000	Down	1.09
Dipeptidyl peptidase 4	P27487	0.00	0.00	6.80	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.000	Down	N/A
Submaxillary gland androgen-regulated protein 3B	P02814	0.00	3.86	0.00	0.00	2.50	0.00	5.90	0.00	0.00	0.00	0.00	0.00	1.000	Down	1.86
Olfactomedin-4	Q6UX06	0.00	0.00	2.55	0.00	2.50	0.00	3.54	0.00	0.00	0.00	0.00	0.00	1.000	Down	1.40
Platelet factor 4	P02776 (+1)	0.00	0.00	0.00	0.00	6.25	0.00	4.13	0.00	1.12	0.00	0.00	0.00	1.000	Down	2.38
Eukaryotic translation initiation factor 6	P56537	0.00	5.80	0.00	0.00	0.00	0.00	4.72	0.00	0.00	0.00	0.00	0.00	1.000	Down	1.23

Poliovirus receptor-related protein 2	Q92692	0.00	0.00	0.43	0.00	0.00	7.08	1.18	0.00	0.00	4.90	0.00	0.00	1.000	Down	1.23
Glutamyl aminopeptidase	Q07075	0.00	0.00	2.55	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.000	Down	N/A
Na(+)/H(+) exchange regulatory cofactor NHE-RF1	Q14745	0.89	0.00	0.00	0.00	5.00	0.00	1.18	0.00	1.12	0.00	0.00	0.00	1.000	Down	2.56
Lymphocyte antigen 6D	Q14210	0.00	0.00	0.00	0.00	0.00	7.08	0.59	0.00	0.00	4.90	0.00	0.00	1.000	Down	2.58
Collagen alpha-1(XII) chain	Q99715	0.00	0.00	1.70	0.00	0.00	0.00	0.59	0.00	0.00	0.00	0.00	0.00	1.000	Down	2.88
Extracellular superoxide dismutase [Cu-Zn]	P08294	0.00	0.00	0.00	12.75	0.00	0.00	0.59	0.00	0.00	0.00	0.00	0.00	1.000	Down	21.60
Bone marrow proteoglycan	P13727	0.00	0.00	0.43	4.25	0.00	0.00	1.18	0.00	0.00	0.00	2.13	0.00	1.000	Down	1.41
Arylsulfatase A	P15289	0.00	0.00	1.70	0.00	0.00	0.00	1.18	0.00	0.00	0.00	0.00	0.00	1.000	Down	1.44
Sodium/potassium-transporting ATPase subunit gamma	P54710	0.00	1.93	0.00	0.00	0.00	0.00	1.77	0.00	0.00	0.00	0.00	0.00	1.000	Down	1.09
Tenascin-X	P22105	0.00	0.00	0.00	0.00	0.00	2.36	1.18	0.00	1.12	0.00	0.00	0.00	1.000	Down	2.05
Folate receptor alpha	P15328	0.00	0.00	0.00	0.00	2.50	0.00	1.18	0.00	0.00	0.00	0.00	0.00	1.000	Down	2.12
Histone H2B type 1-K	O60814 (+13	0.00	0.00	0.00	2.13	0.00	0.00	1.77	0.00	0.00	0.00	0.00	0.00	1.000	Down	1.20
G-protein coupled receptor family C group 5 member C	Q9NQ84	0.00	0.00	0.00	0.00	1.25	0.00	1.18	0.00	0.00	0.00	0.00	0.00	1.000	Down	1.06
Gamma-interferon-inducible lysosomal thiol reductase	P13284	0.00	0.00	0.85	0.00	0.00	0.00	0.59	0.00	0.00	0.00	0.00	0.00	1.000	Down	1.44
Syntenin-1	O00560	0.00	0.00	0.00	0.00	1.25	0.00	1.18	0.00	0.00	0.00	0.00	0.00	1.000	Down	1.06
Myosin-Vb	Q9ULV0	0.00	0.00	0.85	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.000	Down	N/A
Lysosomal acid phosphatase	P11117	0.00	0.00	0.85	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.000	Down	N/A

Supplemental Table 3. Risk of Outcomes by uAnCR Quartile

Patients who were any stage AKI at the time of sample collection (n=97)										
	Worsening of AKI			AKIN stage 3			RRT			
	Yes	No	OR	Yes	No	OR	Yes	No	OR	
	1st Quartile	13% (3)	87% (21)	5.0	4% (1)	96% (21)	13.8	4% (1)	96% (23)	6.1
	2nd Quartile	16% (4)	84% (21)	(1.16-21.46)	16% (4)	84% (21)	(1.58-120.37)	4% (1)	96% (24)	(0.65-56.37)
	3rd Quartile	29% (7)	71% (17)		21% (5)	79% (19)		8% (2)	92% (22)	
	4th Quartile	42% (10)	58% (14)		38% (9)	62% (15)		21% (5)	79% (19)	
	LOS			RRT or Death			AKIN stage 3 or Death			
	Yes	No	OR	Yes	No	OR	Yes	No	OR	
	1st Quartile	25% (6)	75% (18)	21.0	4% (1)	96% (23)	9.5	4% (1)	96% (23)	13.8
	2nd Quartile	56% (14)	44% (11)	(4.58-96.23)	12% (3)	88% (22)	(1.06-84.37)	16% (4)	84% (21)	(1.58-120.38)
3rd Quartile	62% (15)	38% (9)		8% (2)	92% (22)		21% (5)	79% (19)		
4th Quartile	87% (21)	13% (3)		29% (7)	71% (17)		38% (9)	62% (15)		
AKIN stage 2 or 3										
Yes	No	OR								
1st Quartile	29% (7)	71% (17)								2.9
2nd Quartile	28% (7)	72% (18)								(0.87-9.45)
3rd Quartile	46% (11)	54% (13)								
4th Quartile	54% (13)	46% (11)								
Patients who were classified as AKIN stage 1 at the time of sample collection (n=79)										
	Worsening of AKI			AKIN stage 3			RRT			
	Yes	No	OR	Yes	No	OR	Yes	No	OR	
	1st Quartile	15% (3)	85% (17)	4.64	5% (1)	95% (19)	8.14	5% (1)	95% (19)	4.75
	2nd Quartile	10% (2)	90% (18)	(1.02-21.0)	5% (1)	95% (19)	(0.88-75.48)	5% (1)	95% (19)	(0.48-46.91)
	3rd Quartile	32% (6)	68% (13)		11%(2)	89% (17)		11% (2)	89% (17)	
4th Quartile	45% (9)	55% (11)		30% (6)	70% (14)		20% (4)	80% (16)		
	LOS			RRT or Death			AKIN stage 3 or Death			
	Yes	No	OR	Yes	No	OR	Yes	No	OR	
	1st Quartile	25% (5)	75% (15)	17.0	5% (1)	95% (19)	8.14	5% (1)	95% (19)	8.0
	2nd Quartile	50% (10)	50% (10)	(3.46-83.44)	5% (1)	95% (19)	(0.88-75.48)	5% (1)	95% (19)	(0.91-70.34)
	3rd Quartile	63% (12)	37% (7)		11% (2)	89% (17)		11% (2)	89 (17)	
4th Quartile	85% (17)	15% (3)		30% (6)	70% (14)		30% (8)	70% (12)		

The percentage and number of patients who met each outcome are shown by quartile of uAnCR. The odds ratio of 4th quartile to 1st quartile of patients and 95% CI are shown for each outcome.

Supplemental Table 4. Comparison of uAnCR in patients who developed AKI after cardiac surgery compared to those who did not

	AKI	No AKI	p-value
n	6	7	
Male	67% (4)	71% (5)	1
Caucasian	100% (6)	100% (7)	1
Age (yrs) ^a	75.8 +/- 9.5	62.3 +/- 15.3	0.09
Weight (kg) ^a	77.7 +/- 12.7	73 +/- 35.2	0.77
Sample Collection Time (hrs post-op) ^a	20.3 +/- 3.4	21.3 +/- 3.8	0.6
uAnCR ^b	66.6	20.1	0.21
Operative Variables			
Bypass	100% (6)	86% (6)	1
Bypass Time (min) ^a	94.9 +/- 48.5	148 +/- 68.9	0.13
Serum Creatinine (mg/dL)			
Pre-Op ^a	1.33 +/- 0.4	1.2 +/- 0.3	0.6
sCr at Collection ^a	1.45 +/- 0.4	1.1 +/- 0.4	0.18
Maximum Post-Op sCr ^a	1.70 +/- 0.4	1.29 +/- 0.3	0.07
Outcomes			
RRT	0% (0)	0% (0)	1
Death	0% (0)	0% (0)	1

^aMean +/- SD; ^bMedian

Comprehensive Methods

Urine Samples

The Southern Acute Kidney Injury Network (SAKInet) was formed in 2007 to collect samples from patients who developed AKI after cardiac surgery with the goal of testing the diagnostic and prognostic accuracy of previously described AKI biomarkers and identifying novel ones. Urine samples were obtained between August 1, 2008 and October 6, 2011 from patients who had cardiac surgery at one of the SAKInet institutions (the Medical University of South Carolina, Duke University, George Washington University or University of Tennessee College of Medicine in Chattanooga). Prior to collection, informed consent was obtained in accordance with the Institutional Review Board approved protocol at each member institution. Samples were collected and stored using a rigorous standard operating procedure (SOP). Most patients were catheterized and urine was collected preferentially from the Foley tube or the urometer and processed immediately. Urine specimens were treated with a reversible, serine and cysteine protease inhibitor cocktail tablet (Roche, Complete mini , EDTA-free) at a concentration of 1 tablet per 50 ml of urine. The urine was centrifuged for 10 min at $1,000 \times g$ and the supernatant was immediately stored at -80°C in polypropylene tubes that had been washed with 100% acetonitrile in order to minimize sample contamination with plastic polymer.

Patient Selection. The SAKInet SOP for urine collection is primarily focused on collection of urine samples from patients who have developed AKI after cardiac surgery. The goal is to collect urine samples as early as possible after AKIN serum creatinine criteria are met (increase in serum creatinine ≥ 0.3 mg/dL or $\geq 50\%$ from baseline). Collections are made in the surgical ICUs. Inclusion criteria are consent by the patient or appropriate surrogate, surgery of the heart or ascending aorta and development of AKI (defined by the AKIN serum creatinine criteria) within 2 days of surgery. The only exclusion criterion is a baseline serum creatinine greater than 3 mg/dL. Only subjects who had collection of urine within 48 hours were used in order to conform to the AKIN staging criteria and to attempt to eliminate confounding effects of events that were not directly related to the cardiac surgery. Twelve samples were used in the proteomic studies, 10 of which were also used in a validation set that included samples collected from the remaining 87 subjects in the study. Of the samples used in the validation set, 79 were from patients classified as AKIN stage 1 at the time of sample collection. Urine samples were stored at -80°C and shipped to MUSC on dry ice. Samples used in this study were collected within the first 48 post-operative hours and were selected from among the stored samples to fit the criteria described in the results section.

The discovery phase analysis compared the urine proteome of 12 patients who had AKIN stage 1 AKI at the time of urine collection. Six patients required renal replacement

therapy within 10 days and six did not. Patients for the discovery phase analysis were selected from among all patients in the cohort who had AKI within 48 hours of surgery. Subjects were matched first on bypass status, then on bypass time and preoperative serum creatinine. Finally, we attempted to match patients based on the type of surgery. Both groups had two patients who had off pump surgery and four that used cardiopulmonary bypass. Mean bypass time was 165 minutes in both groups and mean baseline serum creatinine was 1.3 mg/dl in both groups. The group that did not require RRT consisted of three patients with CABG alone, one with valve replacement and two with CABG/valve. The group that required RRT consisted of two patients with CABG alone, one septal myectomy, one valve replacement, one CABG/valve replacements and one CABG with repair of ascending aorta. Supplemental table 1 shows the characteristics of the subjects in the discovery analysis.

Patients in the follow-up study to confirm the ability of uAnCR to predict worsening of AKI were selected from all subjects in the cohort for whom data was available at the time the assay was run. Exclusion criteria were subjects who did not have AKI, samples that were collected greater than 48 hours after conclusion of the cardiac surgery, subjects with a baseline serum creatinine of 3.0 or greater, surgeries other than CABG and valve replacement. We excluded patients with samples collected more than 48 hours after surgery in order to conform to the AKIN criteria and to limit confounding effects of events that were not related to the surgery. All subjects with samples and data available at the time of analysis who met the inclusion and exclusion criteria were included in the analysis.

Proteomic Analyses

Trypsin Digestion. Urine (supernatant from the 1,000 x *g* centrifugation) was thawed in a 37°C water bath and digested in-solution with trypsin using the following protocol. One hundred µL of each sample was diluted with 100 µL of 0.2% Rapigest SF surfactant (Waters) in 100mM ammonium bicarbonate. To account for technical variability in the digestion and liquid chromatography-tandem mass spectrometry (LC-MS/MS) protocols, 200 ng of the internal standard recombinant HIV protein gp160 (Bioclone, Inc.) was spiked into each sample. Proteins were denatured by the addition of 5mM dithiothreitol and heated to 60°C for 30 min. After cooling to room temperature, proteins were alkylated by the addition 12mM iodoacetamide and incubation at room temperature in the dark for 30 min. Proteins were digested with 10 µg of trypsin (Applied Biosystems, TPCK treated with CaCl₂) overnight at 37°C.

LC-MS/MS. Each digested sample was pre-fractionated using offline reversed phase solid phase extraction (SPE). The Strata-X SPE cartridge (Phenomenex; 30 mg/mL) was activated and equilibrated by application of 1 mL methanol followed by 1 mL of 0.1% formic acid in water. The sample was loaded onto the SPE column, and a series of elutions containing progressively higher concentrations of acetonitrile (10%, 15%,

20%, 25%, 30%, 35%, 40%, 50%, and 60%) in 0.1% formic acid were performed to separate the sample into fractions of increasing hydrophobicity. The 10% and 15% eluates were combined, as were the 50% and 60% eluates. Sample fractions were completely dried in a centrifugal vacuum concentrator, and each fraction was reconstituted in 50 μ L of mobile phase A (98% H₂O, 0.1% formic acid; 2% acetonitrile). Fractions from each elution were analyzed by LC-MS/MS. Five μ L of each fraction was injected onto an Acclaim PepMap100 trap column (100 μ m ID x 2 cm, C18, 5 μ m, 100 Å; Thermo Scientific) and washed with 100% mobile phase A for 10 minutes at 2 μ L per minute. The fraction was then separated on an Acclaim PepMap100 analytical column (75 μ m ID x 15 cm, C18, 3 μ m, 100 Å; Thermo Scientific). The combined 50% and 60% elution fractions were separated using a 40 minute 2-step continuous gradient of increasing mobile phase B (MPB; 98% acetonitrile, 2% H₂O, 0.1% formic acid). The first step increased from 10% MPB to 40% MPB at 1.5% per minute. The second step increased from 40% MPB to 60% MPB at 1% per minute. All other elution fractions were separated using a 45 minute 2-step gradient. The first step increased from 10% to 40% MPB at 1% per minute, and the second step increased from 40% to 60% at 2% per minute. Tandem mass spectrometry was performed using an AB SCIEX Triple TOF 5600 mass spectrometer. This instrument was run in information dependent acquisition mode with the following parameters: 250 ms MS accumulation time, 50 ms MS/MS accumulation time, 20 ions selected per cycle, total cycle time of 1.3 s, 4 s dynamic exclusion time after one occurrence, and rolling collision energy. The scanning windows for the TOF-MS and MS/MS were 300 to 1250 and 55 to 2000 m/z, respectively.

Protein Identification and Quantification. Acquired spectra (.wiff files) were converted to the MGF format using an AB SCIEX converter (version 1.1 beta). MGF files from all the fractions of each sample were merged and searched against the 2011_6 release of the Human UniProtKB/Swiss-Prot database (20,127 entries) with addition of the common contaminants (112 entries) using the Mascot search engine with trypsin as the specified enzyme. Carbamidomethyl (C) was selected as a fixed modification, and oxidation (M) and deamidation (NQ) were selected as variable modifications. Monoisotopic masses were used, and the error tolerances were 10 ppm and 0.5 Da for peptides and MS/MS fragments, respectively. Mascot search results were loaded into Scaffold (Proteome Software, Inc), which used the Peptide Prophet and Protein Prophet algorithms to validate peptide and protein identifications. The Scaffold quantitative values of identified proteins were normalized to the internal standard recombinant HIV protein present in each biological sample, and the relative abundance of each protein is reported in normalized spectral counts.

Angiotensinogen ELISA. The Human Total Angiotensinogen Assay Kit (Immuno-Biological Laboratories Co., Ltd.), a solid phase sandwich ELISA, was used according the manufacturer's protocol to measure urinary angiotensinogen. Urine samples were

diluted 1:8 in EIA buffer. One hundred μL of diluted sample was added to the appropriate well and incubated for 60 min at 37°C . The plate was then washed 7 times by pipetting 250 μL of the provided wash buffer into each well using a multichannel, repeating pipet. After drying the plate, 100 μL of 30x diluted HRP-conjugated anti-angiotensinogen antibody was added to each well and incubated for 30 minutes at 37°C . The plate was washed 9 times as before and dried. 100 μL of chromogen (TMB) was added to each well, and the plate was incubated for 30 min in the dark at room temperature. One hundred μL of stop solution was added to each well, and the absorbance was measured at 450 nm using a SpectraMAX 340PC 96-well plate reader. The linear range of the assay is 20 to 0.31 ng/mL. Intra- and inter-assay variability (coefficient of variation) were calculated by measuring the standards and three selected biological samples in quadruplicate once, and in duplicate on all remaining plates. Values for intra- and interassay variability were 2.4% and 9.9%, respectively. Data were analyzed using Softmax Pro3.1.2. Samples whose values were above the upper limit of quantification for the assay were diluted 1:10 in EIA buffer and re-run on a separate plate.

Urine Creatinine Determination. Urine creatinine was used to correct the urine angiotensinogen concentration and values were reported as the ratio of angiotensinogen in ng/ml to creatinine in mg/ml (uAnCR, ng/mg). Urine creatinine was measured using the Jaffe assay. Three μL of sample was combined with 100 μL of 1% picric acid (Sigma-Aldrich), 100 μL of 0.75M NaOH (Genomic Solutions), and 300 μL distilled deionized H_2O . Samples were incubated at room temperature for 15 min and absorbance at 490 nm was measured using a SpectraMAX 340PC 96-well plate reader. Data were analyzed using Softmax Pro 3.1.2.

Outcomes. The primary outcome was worsening of AKI, defined as progression to a higher AKIN stage after the time of sample collection. Secondary outcomes were progression to AKIN stage 3, the need for renal replacement therapy (RRT) within 10 days of sample collection, progression to AKIN stage 2 or 3, progression to AKIN stage 3 or death, RRT or death, and discharge >7 days from the time of sample collection or in-hospital mortality. Outcomes were tested using the entire cohort and in the subset of patients classified as AKIN stage 1 at the time of sample collection.

Statistical Analysis. Differential abundance of proteins identified and quantified by LC-MS/MS were selected using the Wilcoxon Rank-Sum test with a significance threshold of $p \leq 0.05$. This test was used because it has been previously shown to be a robust test for the identification of candidate biomarkers in proteomics studies with small sample sizes. Candidate biomarkers were selected based upon the combination of p-value from the Wilcoxon Rank-Sum test and mean fold-change between the experimental groups. The relationship between these two measures was visualized by “volcano plot”. In verification studies, count data were analyzed using the χ^2 or Fisher’s exact test as

appropriate. Continuous variables were analyzed using the *t* test or Mann Whitney U test when comparing two groups. ANOVA or Kruskal-Wallis ANOVA on Ranks test and the *post-hoc* Dunn's test for pairwise comparison were used to evaluate continuous variables when more than two groups were compared. Odds ratios (OR) were used to test the association of uAnCR with selected outcomes. Patients were stratified by uAnCR into quartiles, the effect of uAnCR on the risk of developing an outcome was tested by calculating the OR of the upper and lower quartiles and estimating the 95% confidence interval of the OR. Receiver operator characteristic curves were constructed to determine the predictive power of uAnCR. The area under the ROC curve (AUC) was used as an estimate of an overall accuracy of the biomarker. An AUC of 1.0 represents 100% accuracy, whereas an AUC of 0.5 indicates 50% accuracy, which is no better than random chance. Univariate ROC curves were considered statistically significant if the AUC differed from 0.5, as determined by the z-test. Optimal cut-offs were determined by selecting the data point that minimized the geometric distance from 100% sensitivity and 100% specificity on the ROC curve.¹ To visualize the relationship between uAnCR and length of stay, patients were stratified into tertiles by uAnCR. Additionally, cut-offs that maximized the positive likelihood ratio and minimized the negative likelihood ratio were reported since they could be useful in assigning high or low risk to a patient. Likelihood ratios of positive and negative predictive value were used since they are insensitive to changes in prevalence (unlike PPV and NPV) and can be used to infer post-test probability. Kaplan-Meier curves were used to visualize the relationship between uAnCR and length of stay. Patients who died were censored. The log-rank test was used to compare the curves, and the Holm-Sidak test was used for *post-hoc* pairwise comparison. Category free net reclassification improvement was used to determine if addition of uAnCR to a multivariate logistic regression model for prediction of risk increased the ability of the model to predict worsening of AKI. First, multiple logistic regression model (reference model) was created using the variables percent change in serum creatinine from baseline and Cleveland Score, a perioperative risk score that has been demonstrated to predict AKI outcomes after cardiac surgery.² ³Then, a new model was created with included uAnCR, in addition to these two variables. Each patient's probability (risk) of experiencing worsening of AKI after sample collection was calculated with each model. The category free net reclassification index was calculated as previously described, and was used to quantify the improved prognostic predictive power gained by including uAnCR in the model.^{4, 5} Statistical tests were performed in either Matlab or SigmaPlot.

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