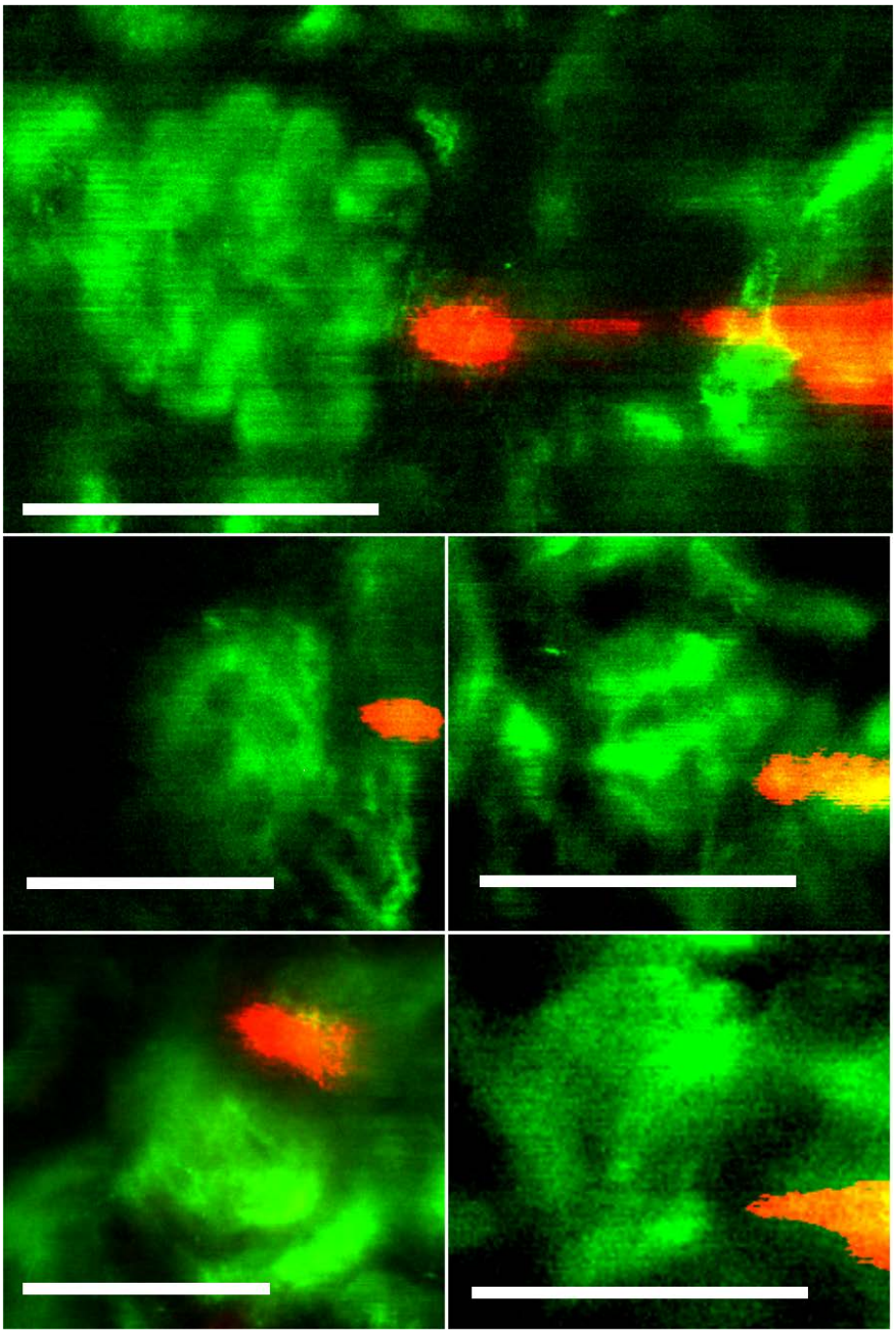
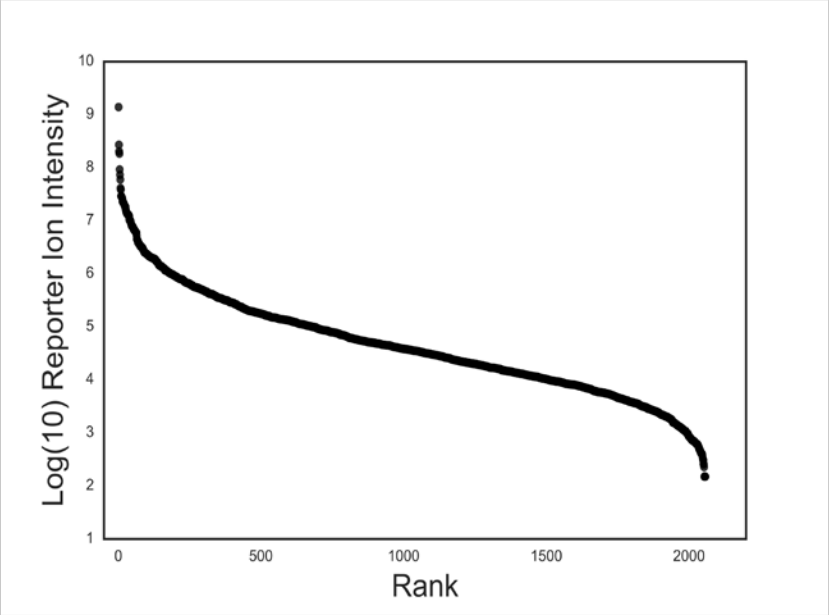
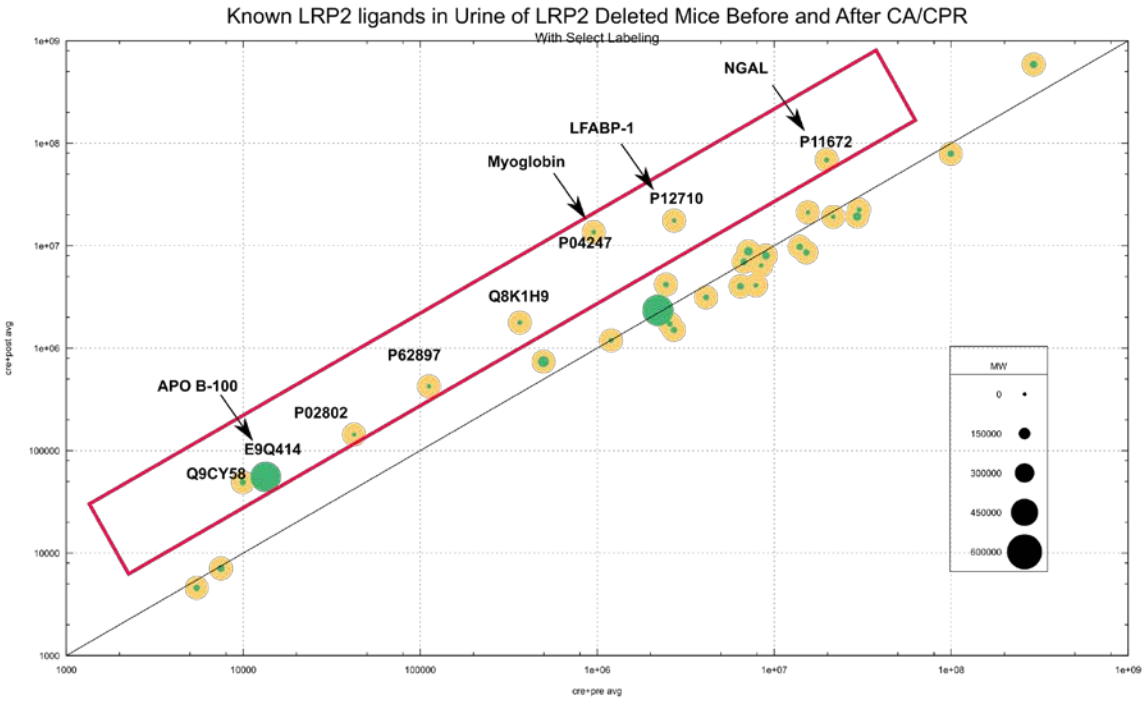
Wakasaki et al: Supplemental Figures



Supplemental Figure 1: Placement of fluorescent micropipettes in Bowman's space. Representative images of quantum dot-coated glass micropipette tips (red) positioned for sample acquisition within Bowman's space in a superficial glomerulus (top) and several glomeruli at the depth from which acquisition was performed in this study. The renal vasculature including glomerular capillaries is opacified with FITC-dextran 2000kD. Micropipettes were positioned under guidance with frequent, tip-focused imaging. The quantum dot fluorescence signal is brighter than the FITC signal; to image both simultaneously without saturating detectors, it is necessary to offset the red signal and image as little of the catheter tip as possible, leading to some visible scatter and drop-out artifact in the red signal. The loss of image quality at depth is evident in the difference between the superficial glomerulus and deeper glomeruli. Images have been appropriately adjusted to compensate for loss of time-, depth- and motion-perception in still imaging; acquisition procedures were performed using live imaging procedures.



Supplemental Figure 2: Urine mass spectrometry assay quality. Each identified protein from 10 urine samples from LRP2$^{fl/fl}$;apoE^{Cre} mice is plotted as log_{10} (reporter ion intensity) by rank in the total sample. The assay demonstrates wide dynamic range, with identifications spread across 7 orders of magnitude.



Supplemental figure 3: CA/CPR leads to increased identification of known megalin ligands in the urine of $LRP2^{fl/fl}$;$apoE^{Cre}$ mice, primarily of low molecular weight. Known megalin ligand identifications are plotted as Log_{10}(reporter ion intensity) in urine before (x axis) and 24h after (y axis) CA/CPR. The red box indicates false discovery ratio <0.01 and p<0.01, upregulated proteins are labeled with Uniprot accession numbers, and select proteins identified with an arrow by common name or symbol. The size of the inner (green) dot represents molecular weight.

Explanatory Note: Proteins with published megalin-ligand status identfied in the urine of LRP2fl/fl ApoE cre (positive and negative) mice in the normal (pre CA/CPR) state.

	Accession	Gene	MW	creneg1	creneg2	crepos1	crepos2	crepos3	negAvg	posAvg	PValue	FDR	Fold Change
0	P07724	ALBU	68693.51	84173269	2.26E+08	1.36E+08	4.09E+08	3.32E+08	1.55E+08	2.92E+08	0.8850887	0.9278638	1.886395
1	P21614	VTDB	53601.34	1367175	2101429	86890260	88787527	1.23E+08	1734302	99491536	0	6.00E-10	57.3669
2	P11672	NGAL	22876	947854.3	2151074	14062390	40741371	4581611	1549464	19795124	0.00604726	0.0271458	12.77546
3	P07309	TTHY	15776.93	895488.7	2676076	13369816	11666992	21501982	1785782	15512930	0.00023758	0.0020993	8.68691
4	P13020	GELS	85942.58	784241.7	1189901	26053818	27822467	34395162	987071.6	29423816	2.80E-09	1.42E-07	29.8092
5	Q00724	RET4	23207.08	583347.3	495635.7	30027940	21164212	39279246	539491.5	30157132	1.24E-08	5.25E-07	55.89918
6	P21460	CYTC	15531.83	681411.3	335912	21892576	20580588	22248402	508661.7	21573855	2.99E-06	4.97E-05	42.41298
7	Q06890	CLUS	51656.6	10232977	14650567	12953193	12552360	16320541	12441772	13942031	0.1536804	0.2955617	1.120582
8	O88968	TCO2	47586.77	1628335	6270248	13043126	12454336	20111246	3949292	15202903	0.04640636	0.1260489	3.849527
9	P20918	PLMN	90808.91	2614986	3643347	6937974	8084789	6426482	3129166	7149748	0.6748549	0.7854628	2.284873
10	P12710	FABPL	14246.55	307043.8	1128771	2619923	2692812	2847146	717907.4	2719960	0.04034956	0.1123035	3.788734
11	P00688	AMYP	57319.29	4874834	13764161	4774388	6220396	8348329	9319497	6447704	0.01654668	0.0570004	-1.4454
12	A2ARV4	LRP2	519209.1	7882606	13533188	1421036	2947185	2238755	10707897	2202325	7.83E-08	2.29E-06	-4.86209
13	P08071	TRFL	77838.76	214312.4	506506.7	6083295	13603253	7242981	360409.5	8976509	1.53E-07	3.93E-06	24.90641
14	P00687	AMY1	57644.71	444802.2	853668.9	6702009	7212010	6300900	649235.6	6738306	3.07E-05	0.0003691	10.37883
15	P08905	LYZ2	16689.98	199141.5	431219.9	5667071	13087980	6550062	315180.7	8435038	1.49E-07	3.92E-06	26.76254
16	P04247	MYG	17070.68	71064.16	338071	984462.9	1715769	166441.3	204567.6	955557.7	0.1284821	0.2604298	4.67111
17	P05017	IGF1	17094.03	1019556	2471371	7623607	5898728	10149690	1745463	7890675	0.0208443	0.0669627	4.520676
18	P06869	UROK	48268.88	3723996	5908715	2591817	2608299	2933508	4816355	2711208	0.00117174	0.0074064	-1.77646
19	P10605	CATB	37280.82	591704.9	839170.5	3199141	4012806	5162446	715437.7	4124798	0.00904286	0.0364047	5.765419
20	P29268	CTGF	37825.09	114984	130134.3	2193647	3044160	2108476	122559.2	2448761	2.16E-06	3.72E-05	19.98024
21	P08226	APOE	35867.59	63391.53	106928	2808559	1470769	3438913	85159.76	2572747	7.79E-08	2.29E-06	30.21083
22	Q8K1H9	OBP2A	19997.08	916033.3	439948.4	425882.1	261690	410039.2	677990.8	365870.5	0.00562382	0.0258161	-1.85309
23	Q9Z1R3	APOM	21274.3	117831.8	174169.5	1121872	1155821	1319645	146000.7	1199112	0.00050913	0.0038546	8.213062
24	Q01279	EGFR	134854.3	364022.9	468686.5	478911.6	458186.1	554187.5	416354.7	497095.1	0.1975392	0.351502	1.193922
25	P62897	CYC	11606.44	45874.14	92063.74	104135.9	132831	99360.22	68968.94	112109	0.7741226	0.86101	1.6255
26	P02802	MT1	6019.102	8079.857	11103.87	35578.09	68515.59	22783.96	9591.866	42292.54	0.09087591	0.2051026	4.409209
27	E9Q414	APOB	509433.1	2708.237	9719.883	9807.84	13633.13	16755.28	6214.06	13398.75	0.4746463	0.6310991	2.156199
28	Q9CY58	PAIRB	44715.12	1422.465	8971.724	5165.782	12626.42	12044.26	5197.095	9945.487	0.6648293	0.7783836	1.913663
29	P11214	TPA	63097.54	8322.63	13200.99	6802.022	4386.462	11289.45	10761.81	7492.644	0.00963386	0.0383113	-1.43632
30	P51569	AGAL	47643.66	6803.341	5816.266	4853.254	5060.474	6454.073	6309.804	5455.934	0.02545545	0.0781379	-1.1565

Proteins upregulated in urine of LRP2 fl/fl ApoE cre mice after CA/CPR identified by 2 or more unique peptides

Accession	Gene	MW	UniquePep	pre_27_M	pre_28_M	pre_29_OF	post_27_M	post_28_M	post_29_OF	pre_Average	post_Average	logFC	PValue	FDR	FoldChange	OrganStdSc	Organ
P13412	TNNI2	21358.52	2	1969.93	11800.34	5105.70	156439.74	113486.60	19447.46	6291.99	96457.93	3.33298	0.000317	0.010599	15.33	5.18	Muscle
P11404	FABPH	14819.85	10	167894.25	748352.58	1062981.69	4198101.71	22853344.02	3269680.78	659742.84	10107042.17	3.071688	0.000319	0.010599	15.32	4.89	Heart
P04247	MYG	17070.68	13	166441.35	984462.86	1715768.88	2114671.34	36787367.19	1793124.39	955557.69	13565054.31	2.831061	0.008641	0.048567	14.20	4.36	Heart
P32848	PRVA	11931.53	15	824683.70	1517982.69	3215254.16	6511043.32	41239565.73	27316843.37	1852640.18	25022484.14	3.058559	6.97E-05	0.005439	13.51	5.18	Muscle
Q8CI51	PDLI5	63300.23	5	26119.53	64007.49	75684.24	364382.89	1208958.29	252529.26	55270.42	608623.48	2.68173	0.000153	0.00777	11.01	4.36	Muscle
Q60604	ADSV	80254.69	3	2265.52	641.06	3167.64	1241.20	48432.41	14264.12	2024.74	21312.58	2.605894	0.006518	0.043218	10.53	4.42	Colon
P67984	RL22	14759.94	2	784.26	795.72	583.57	2530.48	16566.85	2342.44	721.18	7146.59	2.470043	0.00126	0.021394	9.91	4.90	Pancreas
P62245	RS15A	14840.51	3	7975.69	7307.61	6217.92	25421.73	142276.18	32271.91	7167.08	66656.61	2.43303	0.000123	0.007446	9.30	4.87	Pancreas
Q99MN9	PCCB	58410.02	2	958.78	319.99	719.23	12740.91	3846.17	1544.40	666.00	6043.83	2.84812	0.001611	0.024029	9.07	4.40	Brown
P97457	MLRS	18956.46	10	89159.36	355772.33	200133.14	3261082.50	1571904.06	1014821.82	215021.61	1949269.46	2.669122	0.000657	0.016453	9.07	5.19	Muscle
Q64669	NQO1	30960.68	2	10098.33	9348.11	15567.96	29137.69	245290.59	27775.26	11671.47	100734.52	2.245193	0.002483	0.028683	8.63	4.17	Stomach
Q5SX39	MYH4	222859.8	6	36332.38	72620.68	88948.06	419911.46	852104.78	422925.83	65967.04	564980.69	2.443217	9.40E-05	0.006358	8.56	5.15	Muscle
P45591	COF2	18710.59	6	32089.30	55574.56	67079.28	196388.80	1014728.31	78749.60	51581.04	429955.57	2.193135	0.005595	0.039419	8.34	4.57	Muscle
P14869	RLA0	34217.42	5	16551.31	10632.36	14660.30	79723.30	234234.48	32428.33	13947.99	115462.04	2.327077	0.000405	0.012262	8.28	4.93	Pancreas
P50462	CSRP3	20895.82	2	12260.23	20895.66	33000.75	73155.63	410194.84	62287.54	22052.21	181879.33	2.206258	0.002676	0.029666	8.25	4.49	Heart
P97447	FHL1	31889.71	7	17585.36	17495.18	25745.97	64885.67	368110.22	64786.41	20275.50	165927.43	2.225298	0.001109	0.020699	8.18	4.17	Diaphragm
P06801	MAOX	63954.75	24	133791.39	144549.11	172603.09	400394.18	2977596.34	227759.27	150314.53	1201916.60	2.117316	0.006222	0.042263	8.00	4.21	White
P62900	RL31	14463.86	5	35255.80	19693.55	30878.58	42297.08	513304.77	121736.83	28609.31	225779.56	2.178931	0.003077	0.031534	7.89	5.15	Pancreas
A2ASS6	TITIN	3906489	41	224368.75	366944.41	410179.43	2165774.84	4203626.58	1395151.49	333830.86	2588184.30	2.284582	0.000152	0.00777	7.75	5.12	Muscle
Q9D1A2	CNDP2	52768.29	20	161535.07	152751.64	180185.19	350704.51	3138802.48	327495.43	164823.97	1272334.14	2.073037	0.005662	0.039622	7.72	4.55	Jejunum
Q3TJD7	PDLI7	50120.01	2	5478.93	9594.51	15298.78	70309.38	135469.85	27776.59	10124.07	77851.94	2.252525	0.000747	0.017232	7.69	5.14	Muscle
O70209	PDLI3	34300.72	3	8947.57	27263.55	33737.21	103119.34	326309.80	91430.88	23316.11	173620.01	2.137103	0.002292	0.027845	7.45	4.11	Muscle
O09173	HGD	49960.92	19	328733.41	245528.79	320097.61	579495.73	4718580.39	1320906.48	298119.94	2206327.53	2.108362	0.001429	0.022827	7.40	4.11	Liver
P05977	MYL1	20595.51	5	9852.91	26323.84	29950.47	196764.66	230376.37	56284.94	22042.40	161141.99	2.242487	0.001964	0.026387	7.31	5.13	Muscle
Q9R0H0	ACOX1	74649.73	14	36000.79	50733.04	21688.98	472688.84	205386.39	107060.43	36140.94	261711.89	2.399598	0.001711	0.024399	7.24	4.36	Liver
Q9D8N0	EF1G	50061.77	8	59959.50	68891.68	109079.06	279421.62	1229181.45	185802.08	79310.08	564801.72	2.034394	0.003051	0.031534	7.12	4.31	Pancreas
P62908	RS3	26675.29	7	32461.25	19911.14	23801.46	77249.64	374791.17	85872.38	25391.28	179304.40	2.073213	0.001156	0.020699	7.06	4.96	Pancreas
P52760	UK114	14256.42	9	352889.55	312514.37	381971.06	1535800.58	4110920.78	1738320.21	349124.99	2461680.52	2.157965	0.000102	0.006644	7.05	4.10	Kidney
P16015	CAH3	29367.28	16	714182.38	1384468.07	1902109.54	8528885.48	17406102.99	1921986.65	1333586.66	9285658.37	2.073245	0.005212	0.038592	6.96	5.03	White
P37804	TAGL	22576.81	12	139617.18	236647.87	106483.15	1908383.50	1183441.72	160777.57	160916.07	1084200.93	2.222624	0.008288	0.047931	6.74	4.09	Stomach
Q8R0Y6	AL1L1	98710.17	39	377492.91	302435.60	423429.26	737007.32	5576015.61	1098355.73	367785.92	2470459.55	1.936808	0.003886	0.034671	6.72	4.56	Liver
P56375	ACYP2	11878.61	4	51748.20	76372.09	143890.82	270369.35	1061819.76	464804.81	90670.37	598997.97	2.012479	0.001978	0.026406	6.61	4.78	Muscle
Q8QZS1	HIBCH	43038.63	5	8950.84	7379.08	5567.60	67512.30	44198.16	20431.10	7299.17	44047.19	2.119435	0.00089	0.018622	6.03	4.65	Brown
P62889	RL30	12785.05	5	69335.74	41743.32	58536.77	166558.39	628367.57	215292.75	56538.61	336739.57	1.884602	0.001679	0.024399	5.96	4.93	Pancreas
Q8R4N0	CLYBL	37549.53	5	7204.52	2766.89	1373.86	32637.32	17252.26	17221.67	3781.76	22370.42	2.188116	0.002429	0.028491	5.92	4.45	Brown
P25444	RS2	31232.31	6	61726.55	38375.91	45774.68	96140.84	615256.43	144987.61	48625.71	285461.63	1.785029	0.006478	0.043218	5.87	4.94	Pancreas
P53026	RL10A	24917.42	3	45350.85	39922.55	70792.80	203137.85	558477.31	151881.05	52022.07	304498.74	1.85467	0.002486	0.028683	5.85	4.52	Pancreas
P32921	SYWC	54358.9	2	1293.65	1277.85	1265.12	5085.00	13087.26	4170.53	1278.87	7447.60	1.85823	0.003014	0.03152	5.82	4.67	Pancreas
P13707	GPDA	37573.71	15	258575.23	207965.67	263264.18	556210.05	2826623.58	673676.05	243268.36	1352169.89	1.710743	0.005387	0.038877	5.56	4.97	Brown
Q8BMF4	ODP2	67942.7	3	8136.04	6338.03	9448.01	16542.10	88084.66	28125.24	7974.03	44250.67	1.732334	0.003526	0.033436	5.55	4.84	Brown
P47911	RL6	33510.63	4	8766.50	5908.17	7367.84	19741.26	75043.97	26322.34	7347.50	40369.19	1.763501	0.00152	0.023726	5.49	4.04	Pancreas
Q61425	HCDH	34464.87	10	98076.38	89463.07	69332.80	796721.68	383266.84	220237.47	85624.09	466742.00	2.009379	0.003495	0.033436	5.45	4.57	Brown
Q9CQ62	DECR	36214.79	9	90257.76	36674.60	36966.23	423909.31	215918.29	248446.22	54632.86	296091.27	2.059198	0.002264	0.027676	5.42	4.69	Brown
Q9EPL9	ACOX3	78404.74	5	7130.35	6100.17	10196.32	58739.75	47625.21	19462.04	7808.94	41942.33	1.917444	0.001873	0.025685	5.37	4.12	Kidney
P62301	RS13	17223.31	2	8376.01	4576.99	5782.94	23956.54	56718.22	18858.86	6245.31	33177.88	1.77332	0.001139	0.020699	5.31	4.95	Pancreas
Q9D2G2	ODO2	48995.53	8	62678.79	42641.44	50672.57	325933.98	281397.57	201991.06	51997.60	269774.21	1.891042	0.001347	0.022401	5.19	4.58	Brown
P50247	SAHH	47689.16	16	218768.75	166436.75	193756.95	621863.82	1939395.74	427600.11	192987.48	996286.56	1.652732	0.005503	0.039037	5.16	4.56	Liver
P24270	CATA	59796.32	26	396509.36	341711.76	280657.13	2801437.22	1404789.55	1026740.84	339626.08	1744322.54	1.926098	0.002855	0.030555	5.14	4.13	Liver
P47962	RL5	34401.64	5	45975.86	25826.78	39830.24	96364.83	361153.44	114504.02	37210.96	190674.10	1.664898	0.005263	0.038592	5.12	5.02	Pancreas
P35486	ODPA	43232.63	5	3338.84	2331.70	4296.02	20463.47	20052.40	6335.34	3322.19	15617.07	1.700366	0.007912	0.046801	4.70	4.64	Brown
Q9QXE0	HACL1	63661.25	8	31253.77	12657.93	9706.42	92459.84	65676.31	89710.17	17872.71	82615.44	1.806923	0.004636	0.037625	4.62	4.47	Liver
P38647	GRP75	73462.25	8	43072.24	22827.36	31970.35	141167.26	202477.58	103023.79	32623.32	148889.54	1.642786	0.003648	0.033645	4.56	4.14	Brown
P63323	RS12	14525.96	6	60502.71	43417.96	52617.09	133217.43	419685.49	156803.23	52179.25	236568.72	1.506078	0.008488	0.048379	4.53	4.93	Pancreas
Q99L13	3HIDH	35440.91	10	58130.29	39677.22	34377.85	286470.04	135405.37	171157.50	44061.78	197677.64	1.762751	0.006249	0.042263	4.49	4.17	Brown
P58771	TPM1	32681.56	2	11002.57	12107.54	12412.16	55621.44	65352.71	32998.37	11840.76	51324.17	1.551125	0.004411	0.036729	4.33	5.17	Muscle
P56480	ATPB	56301.49	18	212981.13	132141.84	146891.57	919787.68	756670.48	414863.16	164004.85	697107.11	1.605502	0.006953	0.044787	4.25	4.08	Heart
P42125	ECI1	32251.45	2	20117.03	11097.42	14589.24	102344.68	43814.86	47267.79	15267.90	64475.77	1.693275	0.007828	0.046801	4.22	4.89	Brown

Cardiac-specific proteins (all) identified in the urine of LRP2 fl/fl ApoE cre mice, before and after CA/CPR. FoldChange compares the urine LFQ after/before CA/CPR, that is, the change induced by CA/CPR

Accession	Gene_UR	MW	pre_27_M	pre_28_M	pre_29_OF	post_27_M	post_28_M	post_29_OF	pre_Average	post_Average	logFC	PValue	FDR	FoldChange	OrganStdSc	Organ
P11404	FABPH	14819.85	167894.248	748352.584	1062981.69	4198101.71	22853344	3269680.78	659742.841	10107042.2	3.071688	0.000319	0.010599	15.31967	4.890869	Heart
P04247	MYG	17070.68	166441.345	984462.864	1715768.88	2114671.34	36787367.2	1793124.39	955557.695	13565054.3	2.831061	0.008641	0.048567	14.19596	4.35812	Heart
P50462	CSRP3	20895.82	12260.2298	20895.6589	33000.7524	73155.6295	410194.836	62287.5373	22052.2137	181879.334	2.206258	0.002676	0.029666	8.247668	4.494545	Heart
Q9CQR4	ACO13	15183.84	2993.06677	747.721128	880.737863	11417.7631	7332.92527	7177.13206	1540.50859	8642.60681	2.109427	0.003608	0.033645	5.61023	4.190266	Heart
Q99KR7	PPIF	21737.88	13078.3056	11957.1382	18928.3272	57405.6721	95431.9147	38510.4703	14654.5903	63782.6857	1.512358	0.005721	0.039891	4.352403	4.180693	Heart
P56480	ATPB	56301.49	212981.134	132141.843	146891.571	919787.68	756670.481	414863.156	164004.849	697107.105	1.605502	0.006953	0.044787	4.250527	4.075476	Heart
Q9D3D9	ATPD	17601.04	9418.77971	2581.2912	5670.96127	22507.7435	24884.8987	19924.6943	5890.34406	22439.1122	1.469627	0.013013	0.058546	3.809474	4.878829	Heart
Q9D855	QCR7	13528.47	108891.347	44023.8147	101732.76	240100.808	309413.385	232841.86	84882.6405	260785.351	1.117134	0.055228	0.13699	3.072305	4.096583	Heart
Q62425	NDUA4	9327.797	50188.4067	36595.6693	54317.1928	92015.1519	115480.737	102673.819	47033.7563	103389.902	0.618713	0.25943	0.384221	2.198206	4.368225	Heart
P97450	ATP5J	12497.44	26948.5086	8436.12896	32415.0295	34331.9571	33490.7271	65947.6891	22599.889	44590.1244	0.564793	0.383335	0.505057	1.973024	4.510335	Heart
Q03265	ATPA	59753.6	439451.792	367010.543	179118.179	646872.549	448740.159	355629.943	328526.838	483747.55	0.090479	0.883045	0.917879	1.472475	4.319983	Heart
Q06185	ATP5I	8236.605	172310.766	162745.077	116287.545	112689.498	122678.729	154256.639	150447.796	129874.955	-0.71089	0.219914	0.342708	-1.1584	4.847719	Heart
Q9WTR5	CAD13	78186.63	596707.782	681545.142	632477.455	212052.444	337648.909	469179.49	636910.126	339626.948	-1.43653	0.014023	0.060773	-1.87532	4.182679	Heart
Q02566	MYH6	223566.3	193284.049	193490.175	8494.0599	8573.70211	21004.0963	4942.06656	131756.095	11506.6217	-4.2196	3.30E-05	0.003715	-11.4505	4.118396	Heart