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Supplemental Information

Comparing the Effects of Low-Protein and High-Carbohydrate Diets and Caloric Restriction on Brain Aging in Mice

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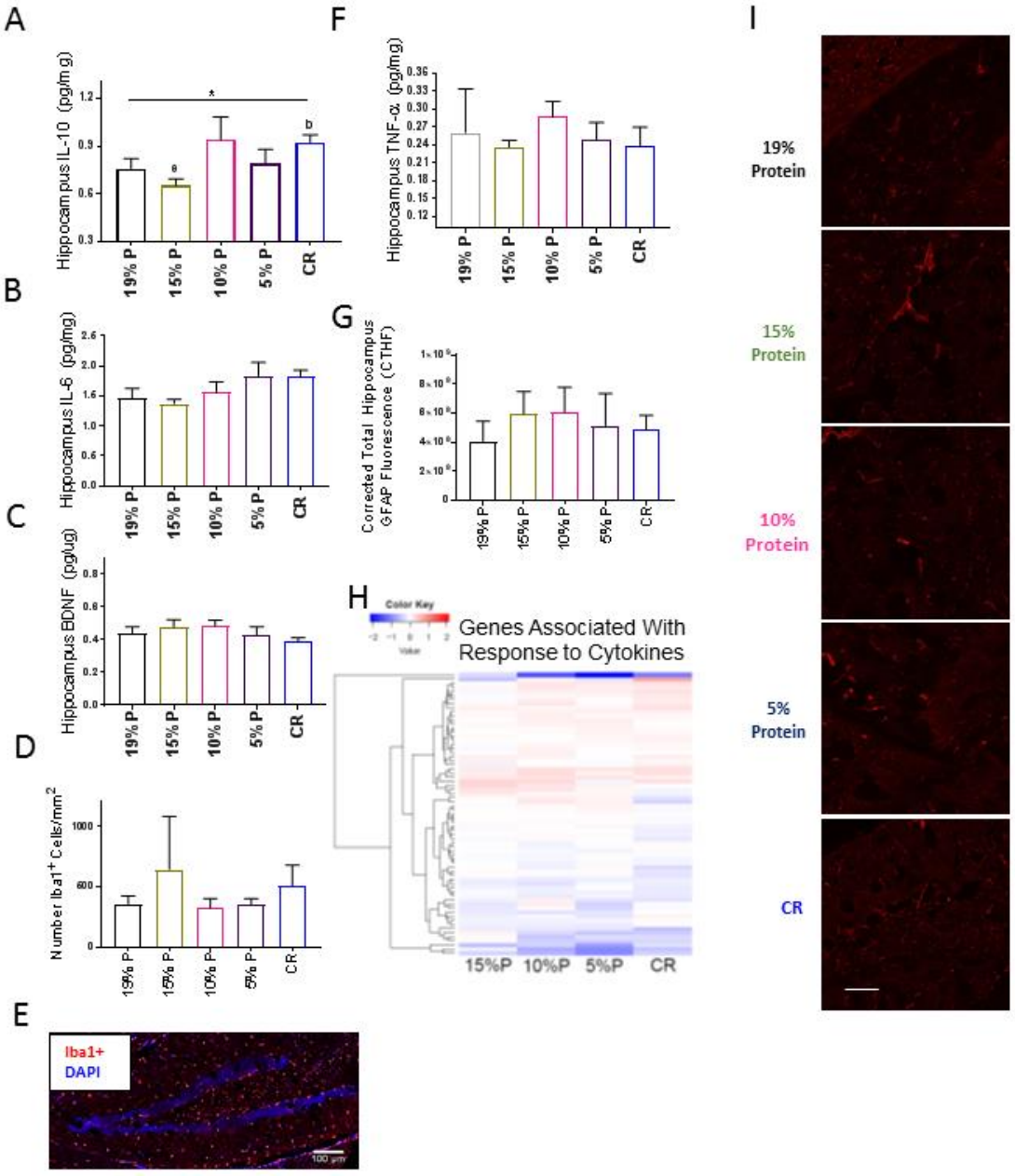

Figure S1. Hippocampal changes in markers of inflammation and associated gene changes in response to CR of LPHC diets, Related to Figure 1.

(A) IL-10 concentrations as measured by ELISA. n=8 biological replicates (4 male, 4 female) per experimental group, 15 months of age. *p < 0.05, **p < 0.01, ***p < 0.001, ****p<0.0001 Kruskal-Wallis ANOVA. a = significantly different to 19% P; b= significantly different to 15% P; c=significantly different to 10% P; d=significantly different to 5% P; e=significantly different to CR as determined by a Dunn's post-hoc analysis.

(B) IL-6 concentrations as measured by ELISA. n=8 biological replicates (4 male, 4 female) per experimental group, 15 months of age.

(C) BDNF concentrations as measured by ELISA. n=8 biological replicates (4 male, 4 female) per experimental group, 15 months of age.
(D) Number of $Iba1^+$ cells per mm^2 of hippocampus and representative image, 15 months of age, n=3-7 biological replicates per experimental group. All data are represented by the mean ±SEM of the biological replicates.
(E) Representative image of Iba1 immunofluorescence staining. Scale bar=100 μm.
(F) TNF-α concentrations as measured by ELISA. n=8 biological replicates (4 male, 4 female) per experimental group, 15 months of age.
(G) Corrected total hippocampus GFAP immunofluorescence in the hippocampus, 15 months of age, 3 – 6 biological replicates per experimental group. All data are represented by the mean ±SEM of the biological replicates. Scale bar = 100 μm.
(H) Heatmap of significantly upregulated or downregulated genes involved to some degree in response to cytokines as revealed by the AmiGO gene ontology online database. Each experimental group is compared to 19% P, and the degree of relatedness among the genes is shown on the y-axis. n=6 biological replicates per experimental group. See also Table S9.
(I) Representative images of GFAP immunofluorescence staining in the hippocampus of each group. Scale bar = 100 μm.

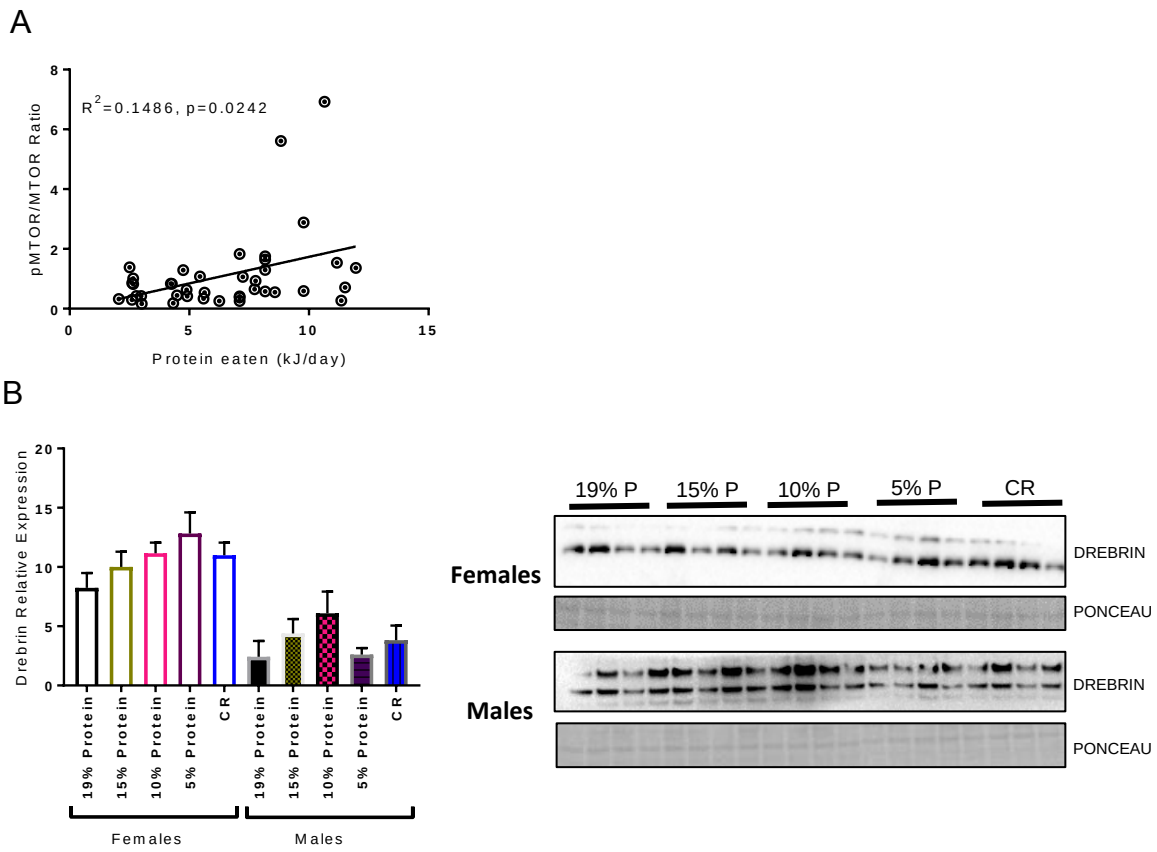

Figure S2. (A) Correlation of average protein intake measured from 12 – 15 months of age and p-mTOR/mTOR as measured by immunoblotting. 15 months of age, 14 months on diet, n=40 (B) Drebrin protein expression as measured by immunoblotting, n=4 mice per experimental group, Related to Figures 2 and 3.

Table S1. Systemic and cardiometabolic measurements related to mouse health and the differences among the groups as determined by ANOVA, Related to Figure 1.

	19% Protein F	**15% Protein F**	**10% Protein F**	**5% Protein F**	**CR F**	
Number of values	12	12	11	12	12	
Urea (mmol/L)	8.13	5.99	4.51	3.12	5.9	****
Std. Error of Mean	0.52	0.37	0.13	0.19	0.29	
	19% Protein M	**15% Protein M**	**10% Protein M**	**5% Protein M**	**CR M**	
Number of values	12	12	12	12	12	
Urea (mmol/L)	7.31	5.64	5.43	3.02	5.88	****
Std. Error of Mean	0.5	0.33	0.29	0.16	0.27	
	19% Protein F	**15% Protein F**	**10% Protein F**	**5% Protein F**	**CR F**	
Number of values	12	11	10	11	11	
Albumin (g/L)	31.67	24.91	27.7	25.45	26.64	p=0.07
Std. Error of Mean	0.86	1.95	2.1	2.57	1.33	
	19% Protein M	**15% Protein M**	**10% Protein M**	**5% Protein M**	**CR M**	
Number of values	12	10	12	12	12	
Albumin (g/L)	30.25	24.9	30.42	27.08	25.17	p=0.06
Std. Error of Mean	0.89	3.31	1.19	1.35	1.49	
	19% Protein F	**15% Protein F**	**10% Protein F**	**5% Protein F**	**CR F**	
Number of values	11	11	9	11	11	
Alanine Transaminase (U/L)	60.45	59.18	77.44	31.91	31.27	**
Std. Error of Mean	12.34	9.375	10.55	8.201	3.063	

	19% Protein M	15% Protein M	10% Protein M	5% Protein M	CR M	
Number of values	12	10	12	12	12	
Alanine Transaminase (U/L)	109.6	100	78.33	61.67	48.08	**n.s**
Std. Error of Mean	20.71	26.43	11.68	35.16	18.18	
	19% Protein F	**15% Protein F**	**10% Protein F**	**5% Protein F**	**CR F**	
Number of values	8	8	8	8	8	
RQ (CO_2 eliminated/O_2 consumed)	0.85	0.86	0.85	0.88	0.85	**n.s**
Std. Error of Mean	0.03	0.02	0.02	0.02	0.02	
	19% Protein M	**15% Protein M**	**10% Protein M**	**5% Protein M**	**CR M**	
Number of values	8	8	8	8	8	
RQ (CO_2 eliminated/O_2 consumed)	0.86	0.78	0.86	0.87	0.88	**n.s**
Std. Error of Mean	0.03	0.07	0.03	0.02	0.01	

Table S2. The top 10 differentially regulated genes when comparing each group to 19% protein as determined by whole-hippocampus RNA sequencing, Related to Figure 2.

CR	log2Fold	padj	5% protein	log2Fold	padj	10% protein	log2Fold	padj	15% protein	log2Fold	padj
Pcmtd1	-0.3	9.63E-08	***Gpr17***	0.45	0.0001624	***Gpr17***	0.55	1.35E-07	***Gpr17***	0.59	4.70E-09
Ogt	-0.22	5.91E-06	***Banp***	0.35110019	0.07741306	***Dpysl5***	0.6	0.000512442	***Hspa5***	0.43	5.43E-05
Mgea5	-0.23	6.65E-06	***Hps4***	0.33538882	0.07741306	***Celf6***	0.48	0.000512442	***Zkscan2***	-0.48	5.43E-05
Dbp	0.66	1.41E-05	***Tbkbp1***	0.27080488	0.07741306	***Gabrb2***	-0.36	0.000512442	***Hif3a***	1.5	7.52E-05
Krit1	-0.28	1.41E-05	***C1qb***	0.25910273	0.07741306	***Hif3a***	1.37	0.000558493	***Hyou1***	0.27	0.00018658
Nr2f6	0.58	4.94E-05	***Adam15***	0.22450936	0.07741306	***Sin3b***	0.27	0.000558493	***Zfp46***	-0.39	0.00018658
Zfp46	0.37	5.15E-05	***Bicd2***	0.20701693	0.07741306	***Htra1***	0.27	0.000558493	***Sdf2l1***	0.65	0.00027147
Dpp8	-0.19	5.15E-05	***Nr1d2***	-0.2183401	0.07741306	***Adam15***	0.28	0.000645404	***Zbtb16***	0.82	0.00032782
Dchs1	0.47	6.31E-05	***Ppm1k***	-0.2571362	0.07741306	***Pik3r3***	-0.32	0.000645404	***Xbp1***	0.36	0.00064116
Cited2	0.53	6.48E-05	***Rab26***	0.33478248	0.08287597	***Sema4b***	0.43	0.000708531	***Plin4***	2.22	0.00067156

Table S3. The top 20 genes positively and negatively correlated with daily protein intake (kJ/day) averaged during 12 – 15 months of age, Related to Figure 2.

Positive Correlation	**pearsonR**	**pearsonP**
Gabrb2	0.784133581	5.79E-06
9330159M07Rik	0.736824362	4.02E-05
Gm26782	0.706759482	0.00011304
Tox	0.696467344	0.00015653
Wwp1	0.695299857	0.00016228
Nr3c1	0.692538917	0.00017662
4930570G19Rik	0.691203312	0.00018395
Kcnj2	0.689924315	0.00019121
Rbms1	0.689492841	0.00019372
Ackr2	0.684763554	0.00022312
Rfc4	0.681916376	0.00024264
Klf7	0.675662426	0.00029079
1190002N15Rik	0.670712936	0.00033458
Abca5	0.670653265	0.00033514
Pou2f1	0.66813019	0.00035963
Gm37893	0.667798783	0.00036296
Ankrd45	0.666294702	0.00037841
Gpr21	0.66539544	0.00038791
Fgf22	0.6609034	0.00043855
Adam22	0.655440583	0.00050778
Negative Correlation	**pearsonR**	**pearsonP**
Zc3h13	-0.7260193	5.92E-05
Tnfsf9	-0.7213022	6.97E-05
Nkd2	-0.7098425	0.00010227
Rasip1	-0.7000014	0.00014018
Sema4f	-0.691355	0.0001831
Scfd2	-0.6886966	0.00019842
Gm12397	-0.6885625	0.00019922
Bicd2	-0.6873154	0.00020681
Tubb2b	-0.6673107	0.00036791
Dchs1	-0.6662718	0.00037865
Sap30	-0.6600511	0.00044878
Chrd	-0.6575432	0.00048009
AI846148	-0.6540868	0.00052632
Syt3	-0.6501643	0.00058342
Tm6sf2	-0.6486166	0.00060737
AI429214	-0.647457	0.00062587
Sin3b	-0.6473848	0.00062704
H2afy	-0.6416336	0.00072632
Cys1	-0.6414134	0.00073037
Pald1	-0.6399919	0.00075703

Table S4. All shared genes between CR and lower protein, higher carbohydrate intake groups, Related to Figure 2.

Shared gene upregulated with CR and overexpressed with lower protein intake (indicated by a negative Pearson correlation)	**CR log2fold Change compared to 19% protein**	**padj**	**pearsonR**	**pearsonP**
sSema4b	0.35405266	0.00395479	-0.4564001	0.02497717
Dll1	0.39680323	0.04350238	-0.4584617	0.02424797
Comtd1	0.43191836	0.03282046	-0.460656	0.02349074
Cpt1c	0.23754724	0.01157335	-0.4608663	0.02341919
Gpc1	0.28894253	0.04392112	-0.4672625	0.02132536
Csf1r	0.27288127	0.0434855	-0.4713588	0.0200659
Caskin1	0.26486868	0.00202484	-0.4716514	0.01997831
Gal3st3	0.3972901	0.00603562	-0.4787775	0.01793871
Samd14	0.50928462	0.0079495	-0.4797691	0.01766886
Nop56	0.19101881	0.01455354	-0.4827977	0.01686502
Tspyl2	0.20810608	0.00687638	-0.4839497	0.01656722
Trim11	0.22123632	0.04413688	-0.4906383	0.01492168
Gm13375	0.64435866	0.00016257	-0.4990689	0.01304064
Hspa2	0.4416678	0.00193604	-0.499491	0.01295185
Tbkbp1	0.28845956	0.00181942	-0.5070009	0.01145318
Gm7367	0.55333569	0.0271947	-0.5116942	0.01059142
Irs2	0.39567919	0.0192398	-0.5123711	0.01047167
Nup214	0.22864657	0.03726844	-0.5149816	0.01002029
Foxo6	0.73404501	0.00828187	-0.5246487	0.00848648
Ap2a2	0.25068819	0.00241388	-0.5321432	0.00743631
Nr2f6	0.57619746	4.94E-05	-0.5367311	0.00684858
Pcdh8	0.59059	0.01599156	-0.5578539	0.00461804
Ebf4	0.34335844	0.01988292	-0.5632839	0.00415579
Cpe	0.19651479	0.0028257	-0.5673905	0.00383267
1700017B05Rik	0.55912801	0.00017238	-0.5676455	0.00381332
Dmwd	0.20250142	0.00301955	-0.5689788	0.00371352
Icam5	0.35340368	0.01668618	-0.5703367	0.00361414
Wscd1	0.29228139	0.00395479	-0.57126	0.00354786
Fbxo21	0.22978884	0.00301955	-0.5771505	0.00314871
2510039O18Rik	0.29879598	0.00121498	-0.5787825	0.0030451
Bcar1	0.18898261	0.0492433	-0.5892797	0.00244525
Kcng2	0.68077627	0.02018061	-0.5944876	0.00218705
Prkcsh	0.16888627	0.01603777	-0.5965451	0.00209164
Prdm8	0.44193864	0.04848226	-0.604109	0.00177072
Grasp	0.28017977	0.02323257	-0.6107547	0.00152441
Aldoa	0.20282323	0.00208295	-0.6107806	0.00152351

Adam11	0.25321804	0.04350238	-0.6317069	0.0009297
Sin3b	0.18626064	0.01860923	-0.6473848	0.00062704
Syt3	0.25567563	0.00422911	-0.6501643	0.00058342
Dchs1	0.46948691	6.31E-05	-0.6662718	0.00037865
Shared gene downregulated in CR and under expressed with lower protein intake (indicated by a positive Pearson correlation).	**CR log2fold Change compared to 19% protein**	**padj**	**pearsonR**	**pearsonP**
Gabrb2	-0.3508222	0.00018875	0.78413358	0.00000579
Klf7	-0.2217289	0.03548333	0.67566243	0.00029079
Ankrd45	-0.3521763	0.00123508	0.6662947	0.00037841
Gabra1	-0.310818	0.04736295	0.63382655	0.00088259
Kcnh7	-0.6226457	0.03486339	0.6330891	0.00089874
Ptpn4	-0.3417165	0.00235884	0.61488654	0.00138655
Ppm1k	-0.2359459	0.01519108	0.61390032	0.00141845
Il1rapl1	-0.3754498	0.04413688	0.60033979	0.00192494
Kcnh5	-0.9108005	0.03642204	0.59523422	0.00215201
Pik3r3	-0.2398985	0.0081861	0.59402245	0.00220912
Tab3	-0.2474935	0.00681953	0.59386447	0.00221666
Pgr	-0.4488183	0.01157335	0.58760374	0.00253367
Zfyve9	-0.2056544	0.00470079	0.58703924	0.00256405
Fbxl3	-0.125008	0.0492433	0.58524524	0.00266268
Pde7b	-0.7022167	0.01988292	0.57891453	0.00303685
Trim37	-0.2730551	0.00808642	0.57806273	0.00309043
Abcd2	-0.4812836	0.01830501	0.56309238	0.00417141
Cacnb4	-0.404557	0.04698135	0.55710299	0.00468523
Ccng1	-0.3696455	0.00076862	0.55567729	0.00481509
Ptar1	-0.4602855	0.00321787	0.54848416	0.00551729
Plag1	-0.4717523	0.01772261	0.54474836	0.0059146
Nxt2	-0.2335756	0.0271947	0.54411182	0.00598463
Zfp106	-0.1500603	0.00016078	0.54147036	0.00628269
Pvalb	-0.5989511	0.02198946	0.53723473	0.0067865
Spcs3	-0.2220862	0.04736295	0.53280558	0.00734894
Spag9	-0.1668287	0.00029442	0.52601692	0.00828607
Atrnl1	-0.3261077	0.0286946	0.52594548	0.00829644
Tmem56	-0.3539449	0.01730313	0.52466698	0.00848378
Tmem14a	-0.3502767	0.04223044	0.52126277	0.0089998
Gprin3	-0.5619832	0.02821759	0.49492424	0.01393944
Cdh12	-0.5120774	0.01988292	0.48018983	0.01755535
Rgs7bp	-0.2796904	0.00192408	0.47954087	0.01773067
Creb1	-0.1782215	0.0167314	0.47816274	0.01810769
Klf6	-0.2859272	0.02748043	0.46765283	0.02120265

Table S6. The log2fold values of each gene involved in *mus-musculus* anti-aging when comparing each group to 19%P as revealed by *GenAge*, the online aging gene database, Related to Figure 2.

Anti-longevity	**15% protein**	**10% protein**	**5% protein**	**CR**
Adcy5	0.00101141	0.05771784	-0.0523274	-0.0534537
Cdkn1a	0.7377191	0.35277723	0.54534961	-0.7218583
Coq7	-0.0737427	0.00790468	-0.1330624	0.04306016
Ghr	-0.0975083	-0.0622679	-0.1283798	-0.0774089
Gpx4	-0.0120894	0.02557822	-0.0212931	0.03566496
Igf1r	0.10137465	-0.0513397	-0.0435251	-0.0809981
Insr	-0.0826957	-0.0620446	-0.1249071	0.00858514
Irs1	0.01163419	-0.0842415	-0.0278227	0.1763891
Irs2	0.415006	0.38797032	0.33342455	0.39567919
Pappa	0.12777558	0.14983361	0.06923623	0.32701302
Shc1	-0.0085756	0.05193752	-0.1203029	0.05694044
Surf1	-0.041281	0.0327547	-0.0322512	0.03530401
Terf2	0.01977974	-0.0201558	-0.0127406	0.05504929
Agtr1a	0.29542411	0.40157403	-0.0768968	0.6068944
Eef1e1	0.10322327	0.00036954	0.10715376	0.02357882
Eps8	0.22462836	0.20697937	0.11479969	0.07668302
Htt	0.0247453	-0.0075698	0.05237501	0.00793609
Kcna3	-0.0507403	0.01600972	0.02044149	-0.0960018
Trp53bp1	0.08334146	0.08299112	0.04582947	0.04969909
Rps6kb1	-0.0358813	-0.1126248	0.01474385	0.01180968
Prkar2b	-0.066228	-0.1862829	-0.1960429	-0.229938
Eif5a2	-0.105278	-0.1477355	-0.0895156	-0.2019709
Mif	0.03320796	0.04232219	-0.0038491	0.06173697
Dgat1	0.02517592	0.17927008	0.08849546	0.21041512
Gsta4	-0.0276925	0.04205657	-0.0100078	0.02963213
Mtor	0.07834324	0.0802618	0.05700023	0.13390206
Akt1	-0.0098096	0.03854792	0.0135716	0.04650128
Ikbkb	0.01562326	0.02150382	0.08282916	-0.0622174
Serpine1	0.56801856	0.45845617	0.11176432	0.38427494
Myc	0.35789686	0.37327365	0.26443569	0.34441771
Ctf1	0.04690428	0.18322558	-0.0154515	0.60451893
Trpv1	-0.8993551	0.33012914	-0.3504407	-0.2146092
Adra1b	-0.1498984	-0.648872	-0.5868867	-0.5445847
Mtbp	-0.1725916	0.00078446	-0.1709641	0.03955118
GMFB	-0.0162379	-0.0598283	0.04766998	-0.1754922
Per2	0.30984155	0.2393255	0.22066461	0.16328089

Table S7. The log2fold values of each gene involved in *mus-musculus* pro-aging when comparing each group to 19%P as revealed by *GenAge*, the online aging gene database, Related to Figure 2.

Pro-longevity	**15% protein**	**10% protein**	**5% protein**	**CR**
Arhgap1	0.01472539	0.02169014	0.0283409	0.04870202
Arntl	-0.0235492	-0.0922206	-0.0117955	-0.296538
Atm	-0.1350654	-0.1323911	-0.0631488	-0.1103872
Atr	-0.157324	-0.1734812	-0.1028854	-0.1585894
Brca1	-0.1799138	-0.4354884	0.0697703	-0.2901321
Bub1b	-0.5832571	-0.2441502	0.17637462	-0.1148866
Bub3	-0.0140228	-0.0550891	-0.0429496	-0.0357142
Casp2	-0.2311755	-0.034555	-0.0794455	0.04008864
Cat	-0.0019598	-0.044525	-0.046639	0.06908542
Cav1	-0.0506545	0.03932483	0.02036309	-0.1862541
Chek2	0.3034059	0.14290546	0.2064488	0.22604372
Efemp1	-0.0010094	0.12383775	-0.299932	-0.0501378
Ercc2	0.11967058	0.21557756	0.09748366	0.23052638
Ercc4	0.01829984	-0.0393227	0.03393852	0.04402606
Foxm1	0.39617119	0.3546454	0.41792158	0.26177433
Fxn	0.30521288	0.40300464	0.30338086	0.21960896
Hells	-0.0551892	-0.4533302	-0.2362269	-0.2805961
Htr1b	0.11440774	-0.1981081	-0.2953036	-0.3121809
Kl	-0.1645951	-0.724464	-1.2783803	-0.1869482
Mcm2	-0.1288324	-0.0379488	-0.0364486	0.17960689
Mgat5	0.05205893	-0.0962008	-0.0287829	-0.0610701
Msh2	-0.184829	-0.1546783	-0.1923001	-0.2006773
Msra	-0.1441285	0.00817381	-0.0222092	0.07818727
Neil1	-0.0442839	0.06125585	0.05353876	-0.0482191
Nos3	0.09270389	0.16102161	0.07008146	-0.0127572
Pawr	0.48891806	0.30262196	0.12309857	0.30313031
Plau	0.12046579	-0.1699962	-0.2446204	-0.146323
Polg	-0.0507667	-0.0414472	-0.0575781	0.00198663
Ppm1d	-0.0921606	-0.066257	-0.1730867	0.06070633
Prdx1	-0.0632609	-0.1009401	-0.0722758	-0.0465243
Rae1	-0.0646415	0.05941629	0.04225784	0.15557218
Sirt6	0.0747384	0.10655685	0.08336311	0.1374625
Slc25a4	-0.0493511	-0.0492517	-0.0291346	-0.0188985
Stub1	0.04627869	0.05517608	0.05667367	0.08146132
Tert	-0.220479	0.09297109	0.19679318	-0.1721455
Top3b	-0.0132511	-0.0420395	0.00995746	0.04664054
Trp53	-0.1050617	0.02056071	-0.0228488	0.14547218

Trp63	-0.0237196	-0.0220774	-0.159791	-0.4238329
Txn1	-0.1197964	0.0342771	0.0503297	-0.0382848
Ucp2	0.22380362	0.16068458	-0.1717167	0.24193137
Xpa	-0.2123533	-0.1424581	0.00965685	-0.0536911
Xrcc5	0.04207824	-0.1571704	-0.1187839	-0.0298264
Xrcc6	-0.1114411	-0.1234526	-0.1345597	-0.0366144
Zmpste24	0.01297913	-0.0204502	-0.0648816	-0.1187497
Apoe	0.1288558	0.23588882	0.06155151	0.23889281
Cisd2	-0.0575493	-0.1531616	-0.068797	-0.1075128
Clock	-0.0254955	-0.1162018	-0.0642864	-0.2036687
Dmd	-0.1094382	-0.1136045	-0.0881478	-0.1333848
Fn1	0.07591546	0.34096519	0.0811186	0.16981677
Hnrnpd	0.00353037	-0.0079354	-0.019962	0.03498714
Jund	0.25449322	0.39133029	0.21424433	0.41596706
Pparg	-0.2879742	-0.6457382	-0.3525091	-0.6113942
Sirt7	0.0655924	0.20156253	0.09263095	0.11525205
Socs2	0.00789526	0.11893311	0.14228172	0.01562922
Sod2	0.00589075	-0.0768286	-0.0098854	-0.1439157
Topors	-0.0865081	-0.15807	-0.1448736	-0.0843207
Tpp2	-0.0242168	-0.0684694	0.02095572	-0.0437661
Parp1	0.02931385	0.08868345	0.15697886	0.0770911
Sirt1	0.02226846	-0.1053712	-0.0144651	0.06436107
Pten	0.00963482	-0.1002418	-0.0707925	-0.0758108
Cdc14b	-0.0121771	-0.227941	-0.2742356	-0.2442611
Mt1	0.04160547	0.15355501	0.05442407	0.13364061
Trp73	0.28424114	-0.1068311	-0.4463664	0.27153949
Htra2	-0.0664208	-0.0143931	-0.031976	0.02341684
Gsk3a	-0.0090046	-0.037634	0.0007846	0.02773572
NUDT1	0.12563543	0.1936328	0.00911153	-0.1758967
Sqstm1	0.00416845	0.03353688	0.02217693	0.11893822
Cdk7	-0.0619699	-0.1046035	-0.0776116	-0.0936238
Grn	-0.0116829	0.11031105	0.03003236	0.11047344
Ncor2	0.21859183	0.17958035	0.14104844	0.16339809
Ercc1	0.01312657	-0.0136704	0.06253632	-0.0139188
Rictor	-0.0395646	-0.0869194	-0.0081099	-0.1273791
Atg5	-0.062396	-0.0215193	-0.0368581	-0.0897468
Adra1a	0.02744343	-0.0380839	0.02020799	-0.1409094
Nfkb1	0.07013187	0.07207794	0.09139244	0.06806671
Rbm38	0.20968059	0.23496542	0.2971683	0.64836596
Col1a1	-0.1680529	0.13717079	-0.6490787	0.08997952
Siglece	-0.4827507	-0.4301152	-0.2071509	-0.3080146
SOD3	0.10686177	0.18017767	-0.0393098	0.07608005

Table S8. Genes associated with CR and the corresponding log2fold values when comparing each group to 19%P, Related to Figure 3.

Overexpressed with CR	**15% protein**	**10% protein**	**5% protein**	**CR**
Acot12	-0.4510922	-0.0282806	0.09478237	0.15608275
Inmt	-0.4455502	-0.3770715	-0.287243	0.71539539
Mat1a	-0.3018161	-1.3844592	-0.5723038	-0.2298032
Tmem218	-0.2754751	-0.3216527	-0.3111763	-0.3017594
Klf10	-0.2689117	-0.235222	-0.0516133	0.01296802
Epb4.1	-0.2574814	-0.3401682	-0.2811228	-0.2815561
Dbp	-0.2161203	-0.1110509	-0.3084841	0.65835636
Gpr146	-0.2093064	-0.1425984	-0.1245682	-0.0451288
Aldh1a1	-0.2072118	-0.3537867	-0.1784337	-0.2910412
St3gal5	-0.2047768	-0.2017289	-0.0694903	-0.1745451
Sun2	-0.1815075	-0.1281606	-0.1103428	-0.0930545
Rhobtb1	-0.1795693	-0.0471919	-0.0988085	-0.0753131
Mgp	-0.1453807	0.14646765	-0.4430167	0.11290186
Ehhadh	-0.141166	-0.2217855	-0.0391253	0.2352127
Bnip3	-0.1386123	-0.1119989	0.03939532	-0.0804203
Igfbp2	-0.1231492	-0.3704185	-1.0580601	-0.1243689
Usp2	-0.1208957	-0.1807186	-0.2043066	0.12193633
Cyp2j6	-0.1093863	-0.1228871	-0.1889964	-0.0842898
Dusp1	-0.0902481	-0.1788821	-0.3117596	-0.0070359
Ntf3	-0.0834746	0.06064372	0.05948092	0.32655401
Hacl1	-0.083386	-0.2291783	-0.0286324	0.03363977
Ppara	-0.0689816	0.05138249	0.09588951	0.10866857
Slc37a4	-0.0641777	0.04351523	0.0249397	0.04270592
Adcy1	-0.0570892	-0.1947714	-0.029705	-0.1771128
Decr2	-0.0509821	0.02704746	-0.0543016	-0.0726938
Decr1	-0.0508268	-0.1072088	-0.1334743	-0.1762638
Tob1	-0.044159	-0.1874523	-0.2183286	0.07368644
Cbr1	-0.0408525	0.04875346	0.02661066	0.0731318
Per1	-0.0224094	-0.0334725	-0.0285945	0.22766577
Wee1	-0.0216056	-0.0744612	-0.1935011	-0.2043732
Acot4	-0.009239	0.22999321	0.13312325	-0.0117283
Cpt1a	-0.0087565	-0.0543521	-0.2633878	0.1174669
Lpin1	-0.0079843	0.05961562	-0.0030391	0.04540081
Pla2g12a	0.00973676	-0.0053818	-0.022337	0.05238588
Ablim3	0.02178646	-0.0781005	-0.0303703	0.02870046
Rhbdd2	0.02956889	0.04586255	0.00088878	0.03823837
Klf9	0.04064265	-0.0255918	-0.0227936	-0.1127542
Klf9	0.04064265	-0.0255918	-0.0227936	-0.1127542

Mt1	0.04160547	0.15355501	0.05442407	0.13364061
Ifrd1	0.04205248	0.01948604	0.03144177	-0.0418738
Crym	0.04682847	0.37502488	0.35962182	0.31178074
Slc25a25	0.04813346	0.03294377	-0.0181616	-0.0818371
Zfp354a	0.0647907	-0.1386225	0.24452415	0.00846424
Enpep	0.07847471	-0.1889923	-0.1513999	-0.4677518
Nfkbia	0.09375226	0.43106597	0.15339755	-0.0178166
Por	0.10008187	0.06311304	-0.0043761	0.21481561
Sall1	0.11153679	0.025667	-0.0045194	0.14597973
Slc25a42	0.11350385	0.11584435	0.19290471	0.02084894
Fam195a	0.11976624	0.1781308	0.21054772	0.0635081
Pim3	0.13951806	0.2070903	0.09276525	0.16127446
Mt2	0.14890754	0.2766307	0.17300344	0.1476554
Cobll1	0.14983442	0.00249694	0.00184324	0.18703689
Plcxd3	0.15690057	0.10954026	0.121864	-0.090086
Fam107a	0.17038021	0.15551367	0.01805516	-0.2562289
Ctgf	0.17194899	0.24490704	0.17929895	-0.016758
Nat8	0.19793868	0.0250599	-0.1664148	-0.0016687
Cry1	0.20938696	0.02570787	0.01403533	0.06276243
Herpud1	0.26219829	0.04185022	0.07672109	0.05425907
Trp53i13	0.26322852	0.37215079	0.1450828	0.39032931
Smoc1	0.29468753	0.34363698	0.02806185	0.27279036
Per2	0.30984155	0.2393255	0.22066461	0.16328089
Fkbp5	0.33165152	0.31806022	0.30150165	0.09886719
Klf15	0.33594585	0.34486323	0.17454914	0.05100368
Tsc22d3	0.35975863	0.39717674	0.12507946	-0.0896571
Arrdc2	0.38448986	0.39440052	0.29753779	-0.177259
Fzd1	0.39085794	0.44413288	0.13652337	0.55863088
Irs2	0.415006	0.38797032	0.33342455	0.39567919
Sult1a1	0.42825862	0.46386113	0.07635109	-0.0791534
Rgs16	0.43144714	-0.3308686	-0.300976	-0.1803528
Angptl4	0.49113968	0.73679064	0.27201196	0.15678084
Cd163	0.52539695	0.51771548	-0.4282355	-0.1997675
Map3k6	0.56449422	0.58182212	0.39503114	0.16610049
Gys2	0.70771346	-0.2968843	0.63632324	1.52193882
Zbtb16	0.81555612	0.6481624	0.46377654	0.20752908
Plin5	1.11831391	0.29213504	0.18592859	0.81500252
Plin4	2.21956688	1.38281782	1.0719565	0.69817751
Underexpressed with CR	**15% protein**	**10% protein**	**5% protein**	**CR**
Casc5	-1.1915579	-0.9115165	-0.3733886	-0.4002452
Ifi27l2a	-1.0223006	-0.3363049	-1.1486906	0.15412521
Phf19	-0.8146298	-0.2609268	-0.2056072	-0.4251833

Tnfsf10	-0.6984303	-0.4869579	-0.0826616	-0.2735283
Ifih1	-0.5987212	-0.4574485	-0.6186532	-0.4272112
Tmem132d	-0.3745109	-0.4973567	-0.4804528	-0.5382282
Alas2	-0.3558136	-0.257527	-0.0886628	0.54501254
Insig1	-0.342859	-0.3188008	-0.2644325	-0.0074479
Gck	-0.2855682	-0.1010476	-0.1877749	0.53371548
Col15a1	-0.2471893	-0.2388577	-0.2817814	-0.0562077
C4bp	-0.1898903	-0.9261569	0.28982861	0.61588131
Irf7	-0.1737809	-0.0369168	-0.3389087	-0.0266234
Irgm1	-0.1620699	-0.2569813	-0.0610272	-0.1470485
Scrt1	-0.1618382	-0.2410631	-0.2066196	-0.1956099
Extl1	-0.1421953	-0.1540141	-0.056344	-0.0396413
Sc5d	-0.1219082	-0.0894164	-0.057975	-0.1168206
Cdc42ep2	-0.1177925	-0.2260356	-0.0870679	0.13019473
G6pdx	-0.1095388	-0.0764554	-0.1118923	-0.0250055
Ghr	-0.0975083	-0.0622679	-0.1283798	-0.0774089
Slc6a6	-0.0931028	-0.0806533	-0.0540442	0.09558094
Zfp64	-0.0785603	0.00598083	0.0294814	0.11536721
Ly6e	-0.0646478	-0.0214757	0.0685652	-0.0744331
Nr1d1	-0.0329025	-0.106986	-0.2160847	0.45646414
Srebf1	-0.0257264	0.12923937	-0.0652295	0.3449339
Fabp5	-0.0254908	0.00881646	-0.01323	0.06591191
Arntl	-0.0235492	-0.0922206	-0.0117955	-0.296538
1110051M20Rik	-0.0068467	0.05837647	0.05276045	0.02034748
Dhcr7	-0.0062799	-0.0128339	0.0843046	-0.0362128
Acly	-0.002286	-0.016171	-0.0683946	-0.0665175
Psmb8	0.00410806	-0.0244623	0.08102317	-0.0097197
Scly	0.01006253	-0.0055203	-0.106322	-0.0494183
R3hdm2	0.02121147	-0.1549211	-0.0949987	-0.2178529
Gtf2ird1	0.02962381	-0.0835866	-0.0947068	0.08469086
Litaf	0.05273628	-0.0769627	-0.0290641	0.12214575
Dpp9	0.08204702	0.06567618	0.07891555	-0.0107709
Stac3	0.08858554	-0.0270473	0.11122527	0.13789108
Actg1	0.10932333	0.17951554	0.08617046	0.34357994
Pdia3	0.13168417	0.03701201	0.01424799	-0.1994026
Ptprj	0.13291944	-0.0315285	0.06656098	-0.0084613
G0s2	0.14072088	0.17985659	0.30948104	0.13054967
Hipk2	0.14756454	0.00044062	-0.0055519	-0.1040742
Phlda1	0.16046551	-0.019217	-0.0082457	0.10433809
Serpinh1	0.16663189	0.10050277	-0.1119897	-0.0313443
Cldn1	0.18630663	-0.5353095	-0.8608371	-0.0642067
Mmp15	0.19812773	0.12577564	-0.0740732	-0.0370251

Ttll12	0.22451206	0.18176083	0.1011936	0.15092252
Dnase1l2	0.2358725	0.24456262	0.25622468	-0.0177012
Hspa5	0.43436974	0.20979303	0.15726423	-0.2931173

Table S9. The top differentially genes associated with cytokine response as determined by and the corresponding log2fold values when comparing each group to 19%P, Related to Figure 1.

GO:0034097: Response to cytokine	**15% protein**	**10% protein**	**5% protein**	**CR**
Prlr	-0.3124379	-1.4854024	-2.1264179	-1.0987817
Il2ra	-0.5127679	0.09017402	-0.2243204	0.76074868
Lepr	-0.380699	-0.6124417	-0.9759275	-0.380013
Il20ra	-0.5622562	-0.3511454	-0.8281828	-0.2487755
Socs1	0.08811141	-0.3144046	-0.4770619	-0.4164325
Tnfrsf11a	0.0362551	-0.4523289	-0.266672	-0.2844594
Sigirr	-0.1517293	-0.1743266	-0.1923981	-0.5195826
Grem2	0.03539414	0.14672447	0.08904538	-0.2392744
Mkks	-0.0148601	-0.1399518	0.06324986	-0.3196247
Stat6	-0.0483035	-0.3804103	-0.3950694	-0.3218779
Parp9	-0.1261586	0.12127626	-0.2192537	-0.2226355
Irf7	-0.1737809	-0.0369168	-0.3389087	-0.0266234
Irf3	0.21830031	0.21247326	0.06261317	-0.0830612
Adipor2	0.34075238	0.20656695	0.10639012	0.00965924
Il10rb	-0.1297183	-0.063788	-0.2116667	0.09160023
Ripk2	-0.1681861	-0.2638534	-0.2004819	0.00583
Ptpn2	0.19040233	0.0610843	0.06838353	-0.0904268
Tnip2	-0.0517848	0.13181735	0.11696048	0.22270761
Jak2	0.03915672	-0.0240166	0.01805368	-0.1995238
Tnfrsf1a	0.05299786	0.20940839	-0.017096	0.17760221
Trp53	-0.1050617	0.02056071	-0.0228488	0.14547218
Cav1	-0.0506545	0.03932483	0.02036309	-0.1862541
Parp14	-0.0112184	-0.0505822	-0.0618727	-0.2309541
Slc27a1	0.16101155	0.29478276	0.09296984	0.25022265
Ptk2b	-0.0263274	0.0623601	0.09468539	0.18805537
Nol3	-0.0294703	0.12527003	0.03103341	0.15922547
Pias4	0.12979144	0.06255174	0.08790193	0.25625004
Kit	0.03252986	0.18885826	0.17344145	0.22149999
Acsl1	-0.1222602	-0.1462795	-0.1658713	0.0163453
Irgm1	-0.1620699	-0.2569813	-0.0610272	-0.1470485
Peli3	-0.0567931	0.11092357	-0.0281605	0.0637812
Sirt1	0.02226846	-0.1053712	-0.0144651	0.06436107
Stat2	-0.042218	-0.0124125	0.07106699	-0.1024895
Gab1	0.12752407	0.10163881	-0.0151442	0.14445643
Fer	-0.0972999	-0.1281971	-0.0033358	-0.1728492
Il1r1	-0.0206728	0.03637892	0.10896669	-0.0483269
Tjp2	0.12938579	0.00751226	-0.028789	0.06469961

Flt3	-0.0569778	-0.1759885	-0.0786975	-0.1942215
Cx3cr1	0.00476811	0.12471401	0.15210297	0.04912149
Jagn1	0.0095568	0.04512287	-0.0216618	-0.1091516
Bbs4	-0.0249573	-0.0140786	0.00858946	-0.1366441
Traf3	0.1727386	0.22543352	0.24589551	0.10329988
Mt3	0.05115107	0.10850541	0.12232796	-0.0133889
Stat1	0.00326868	0.12226689	0.00690766	-0.0089325
Ikbkb	0.01562326	0.02150382	0.08282916	-0.0622174
Cib1	0.07730703	0.20710862	0.12418305	0.1798827
Otulin	-0.0037782	-0.0922348	-0.0454188	0.03129271
Trim32	0.00896249	0.01671982	0.05668749	0.11179068
Traf6	0.061785	-0.0056239	0.05390657	0.10552657
Irf1	-0.030716	0.07553137	0.03442714	0.05023534
Adar	0.04747298	0.03806305	-0.0098541	-0.0495348
Fkbp1a	-0.003495	0.03866164	0.10328484	0.06589078
Crebrf	-0.0458969	-0.1190454	-0.1163381	-0.1505748
Csf1r	0.18230967	0.26893674	0.22037066	0.27288127
Stat3	0.04760643	0.13692599	0.04511536	0.06878485
Bbs2	-0.038754	-0.0809681	0.02165674	-0.0313752
Adipor1	-0.009471	-0.0388892	-0.0997059	-0.0173026
Cx3cl1	-0.0159395	-0.1087117	-0.0386722	-0.0736198
Ifnar1	-0.0897248	-0.1162311	-0.0788426	-0.0319161
Rabgef1	-0.016632	-0.067883	-0.0263551	-0.0846645
Zcchc11	-0.1418487	-0.1923305	-0.1438413	-0.1178958
Sharpin	0.05408174	0.10035881	0.12055046	0.11199024
Il1rap	0.04642549	0.04047231	0.10001194	0.04719802
Ctr9	0.00306432	0.01857674	0.0185325	-0.0401354
Med1	0.03173762	-0.0023999	0.0292154	-0.0215872

Table S10. The top differentiated genes associated with dendrite morphogenesis and the corresponding log2fold values when comparing each group to 19% protein, Related to Figure 4.

GO:0048813: Dendrite morphogenesis	**15% protein**	**10% protein**	**5% protein**	**CR**
Cacna1f	-0.0183999	-0.3600848	-0.5399677	0.37805278
Sema3a	-0.1114775	-0.9538858	-0.5631468	-0.8613153
Xlr3b	0.37932445	-0.0122672	0.20431643	-0.304775
Nfatc4	-0.3097113	-0.0687018	-0.3471485	0.14849524
Ephb3	-0.3315877	-0.3066718	-0.0838266	0.14847308
Atp7a	0.06392776	-0.1854235	-0.2475899	-0.3367102
Cux2	-0.1512067	-0.5112572	-0.4520998	-0.4175882
Chrna7	-0.1929339	0.02888425	0.1099817	0.03311738
Cux1	-0.1900122	-0.4923989	-0.3885139	-0.3632077
Ephb1	0.04139052	-0.1638925	0.01568811	0.08827617
Zfp365	-0.0917968	-0.176813	-0.0257962	-0.270881
Dact1	0.00614278	0.0783615	-0.0075147	0.21958902
Cdkl3	-0.1659417	-0.1299049	-0.0340576	0.06355596
Caprin2	-0.1937324	0.00236138	-0.0325774	-0.0271619
Prex2	0.08877366	0.04804348	0.03671918	-0.1112024
Epha4	-0.1314543	-0.0892548	0.05932841	0.00579808
Nlgn3	0.13263807	0.06230024	0.0891848	0.24459838
Ptprd	-0.037442	-0.1844852	-0.0924727	-0.2093489
Rbfox2	0.06520249	0.04149948	0.03738559	-0.1095126
Kalrn	-0.1863182	-0.2214488	-0.0915808	-0.0617582
Il1rapl1	-0.2054591	-0.3204012	-0.2665911	-0.3754498
Slc11a2	-0.0974959	0.00430504	0.00288069	0.07924403
Slitrk5	-0.0916813	-0.0835903	-0.0174982	0.06277435
Ankrd27	-0.0923923	-0.0315642	0.07105617	0.02286226
Elavl4	-0.0840633	-0.2331131	-0.1408282	-0.2119659
Ephb2	0.15298138	0.13131575	0.1707556	0.28418091
Arhgap44	-0.0158529	-0.0929735	-0.007467	0.07149749
Caprin1	0.03663942	0.0020223	0.01197248	-0.1106857
Dock10	0.26257051	0.12765267	0.14990567	0.13176725
Ppp3ca	-0.1477279	-0.1695103	-0.0473339	-0.0518907
Ss18l1	-0.032182	-0.0450651	0.02646264	0.0929691
Vldlr	-0.0603936	-0.0627664	-0.0572817	0.06552959
Pak3	0.08804301	0.04058181	0.0644234	-0.0540939
Fmn1	-0.2406328	-0.3392874	-0.1949128	-0.2751251
Nlgn1	-0.0935203	-0.0840151	0.03405325	-0.0277452
Rab21	0.0510943	-0.0577027	0.00156715	-0.0706992
Reln	0.20969432	0.27236936	0.14353411	0.18055388

Lrp8	0.06803703	0.10847874	0.10751869	-0.005584
Camk2a	0.01643545	0.06209554	0.1198379	0.13090484
Mapk8	-0.065824	-0.1015729	-0.0046997	-0.1267613
Itgb1	0.00413143	-0.0857656	-0.1152058	-0.0955679
Dscam	-0.0637295	-0.0532056	-0.0829103	0.03477883
Pten	0.00963482	-0.1002418	-0.0707925	-0.0758108
Dtnbp1	-0.0845296	-0.0844208	0.00741362	-0.0278588
Picalm	0.0466801	-0.0161881	0.03264976	-0.0508746
Mapk8ip2	0.04528685	0.09765425	0.06541195	0.14744807
Fyn	0.0098059	0.08234397	0.10107542	0.08454398
Cdk5r1	-0.0209863	-0.0693114	-0.0540815	0.02016562
Hprt	-0.1327103	-0.150682	-0.0644663	-0.1115482
Kidins220	-0.0144046	-0.0188056	-0.0079433	-0.0871573
Pafah1b1	-0.0839754	-0.1043807	-0.0177611	-0.0683855
Hdac6	0.05399071	0.11111336	0.08960867	0.13716647
Abl2	0.0728928	0.07181999	0.08803355	0.01756956
Dvl1	0.08825457	0.08302734	0.08207024	0.0257026
Cdk5	-0.032159	0.02545157	0.02843784	-0.0105184
Rere	0.15253974	0.171391	0.11536209	0.11597048
Ctnna2	-0.0800337	-0.121171	-0.0583412	-0.0964205
Wasl	0.06028392	0.00485558	0.04359949	0.04627474
Rapgef2	0.08429412	0.10281787	0.09172912	0.13554566
Cacna1a	-0.0363766	-0.0801176	-0.0436606	-0.0391737
Shank3	0.11053102	0.14201556	0.1398349	0.1051724
Cdc42	-0.0917285	-0.0933177	-0.0686672	-0.0744869
Klf7	-0.2077166	-0.2206282	-0.223446	-0.2217289