

Supplementary Online Content

Middeldorp J, Lehallier B, Villeda SA, et al. Preclinical assessment of young blood plasma for Alzheimer disease. *JAMA Neurol*. Published online September 6, 2016. doi:10.1001/jamaneurol.2016.3185

eFigure 1 Female APP MICE SHOW MORE HIPPOCAMPAL A β Deposits at Middle-Age Than Male APP Mice

eFigure 2 Heterochronic Parabiosis Does Not Affect A β Levels in the Hippocampus

eFigure 3 Heterochronic Parabiosis and Administration of Young Blood Plasma Does Not Affect CD68 Immunoreactivity in the Hippocampus of APP Mice

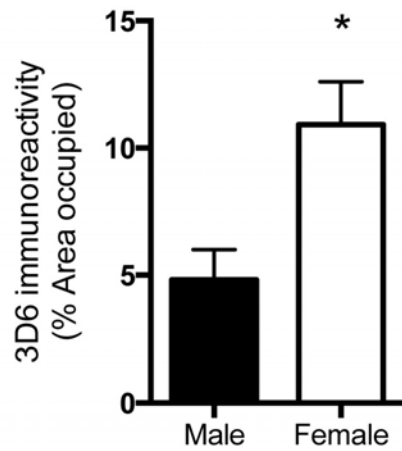
eFigure 4 Whole Genome Microarray Analysis on the Hippocampi of Middle-aged Female WT iso (n = 6), APP iso (n = 7) and APP het (n = 7) Parabionts

eFigure 5 Top Gene Ontology Terms of the Top 100 Differentially Expressed Genes

eFigure 6 Administration of Young Blood Plasma Reverses Neocortical Synaptophysin Depletion

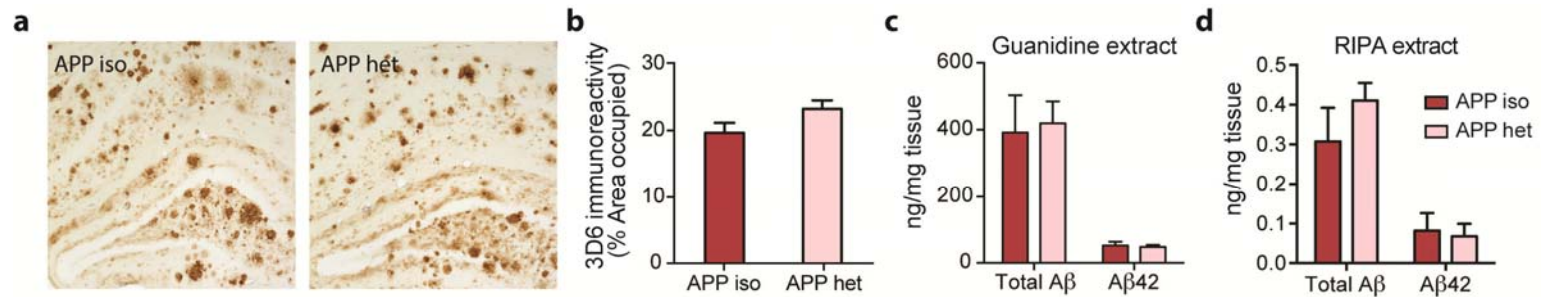
eTable 1 Top 100 Metaranked Genes

This supplementary material has been provided by the authors to give readers additional information about their work.



eFigure 1 Female APP mice show more hippocampal A β deposits at middle-age than male APP mice

Quantification of 3D6 immunoreactivity in the hippocampi of 10-12 month old male and female APP mice. Data are shown as the mean + s.e.m., * P < 0.05, Student's t test.



eFigure 2 Heterochronic parabiosis does not affect Aβ levels in the hippocampus

Representative images (a) and quantification (b) of immunohistochemical detection of amyloid plaques (3D6 staining) in the hippocampus of APP iso and APP het parabionts. (c-d) ELISA measurements of insoluble (c) and soluble (d) total Aβ and Aβ42 levels in the hippocampus of old male APP iso and APP het parabionts. All data are shown as the mean + s.e.m. Student's t test (b), two-way ANOVA, Sidak's post hoc test (d-e), one-way ANOVA, Tukey's post hoc test (g-h,j). Scale bars, 100μm (f), 25μm (i).

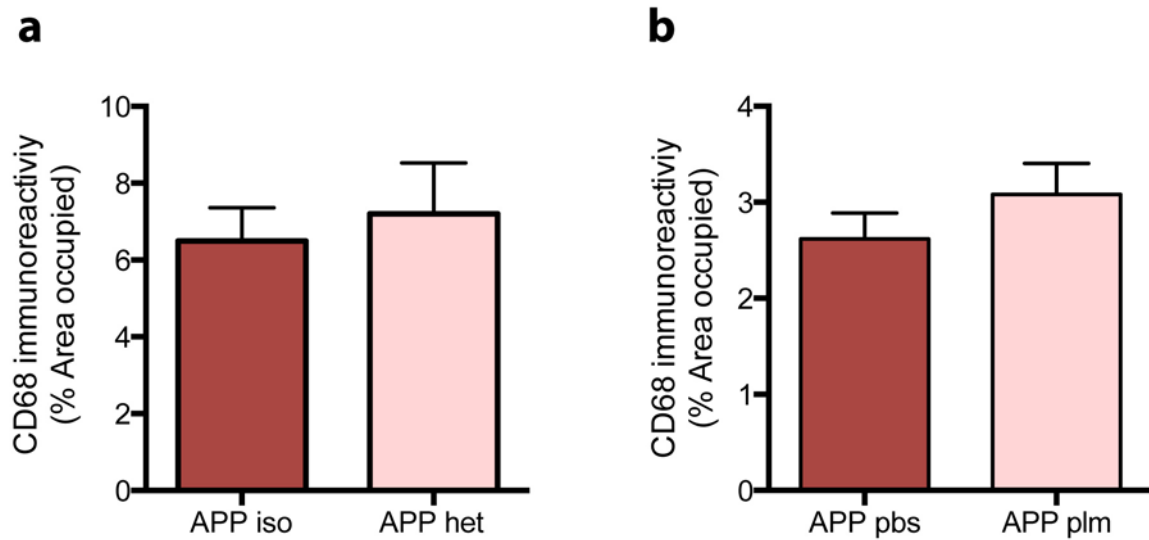


Figure 3 Heterochronic parabiosis and administration of young blood plasma does not affect CD68 immunoreactivity in the hippocampus of APP mice

Quantification of CD68 immunoreactivity in the hippocampi of old APP iso and APP het parabionts (a) and middle-aged APP mice treated with either PBS or young plasma (b); APP iso (n = 5), APP het (n = 4), APP pbs (n=12), APP plm (n=12). Data are shown as the mean + s.e.m., Student's t test.

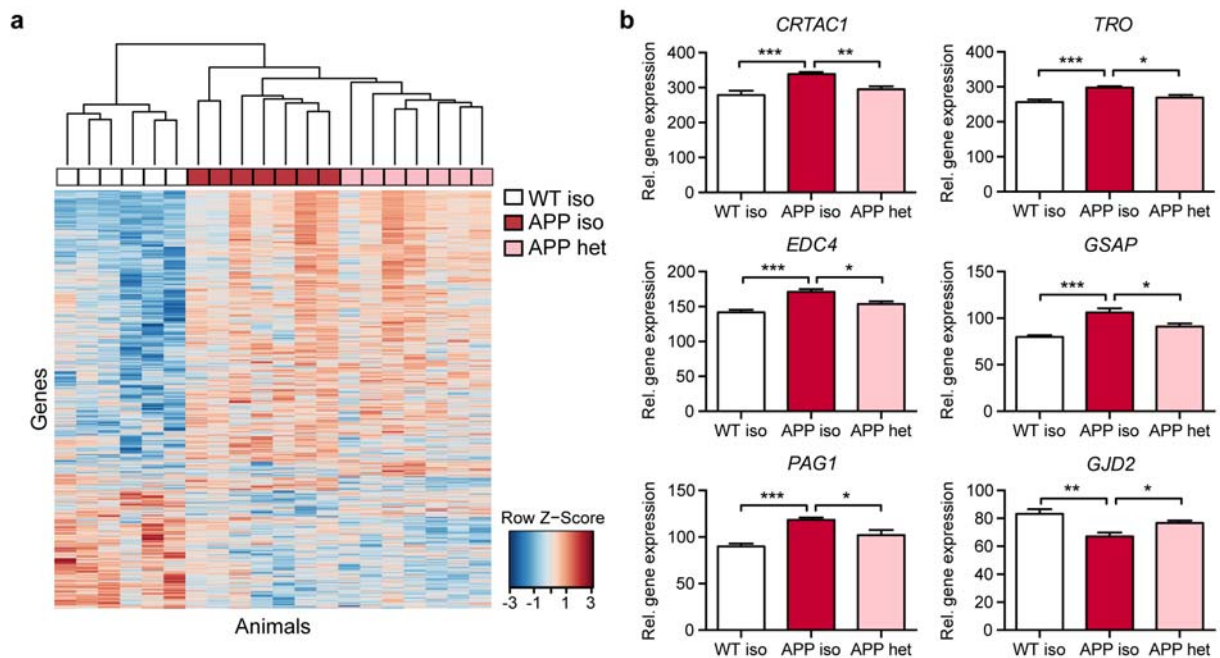
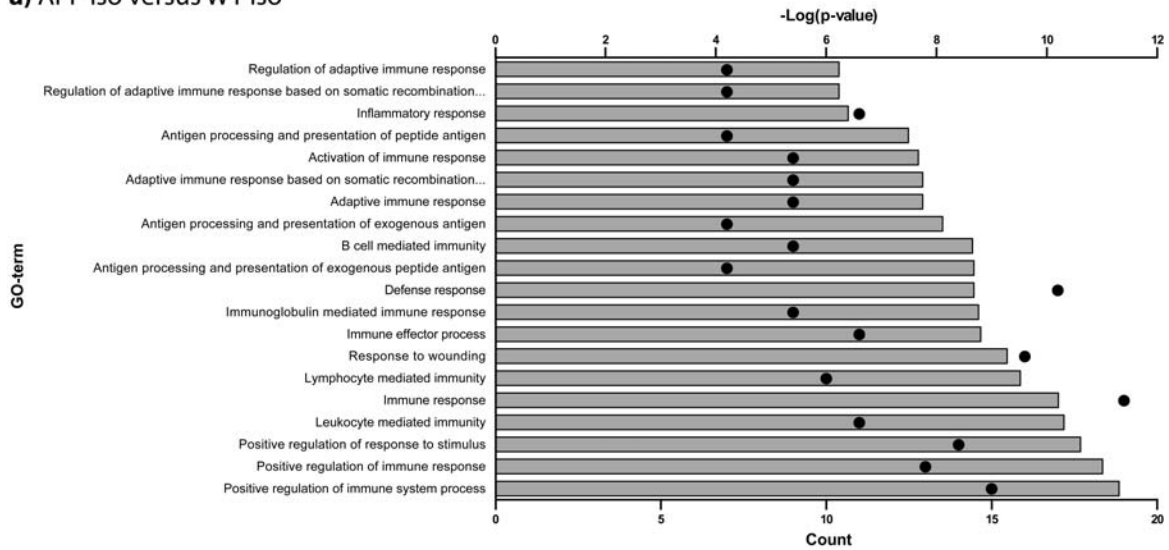


Figure 4 Whole genome microarray analysis on the hippocampi of middle-aged female WT iso (n = 6), APP iso (n = 7) and APP het (n = 7) parabionts

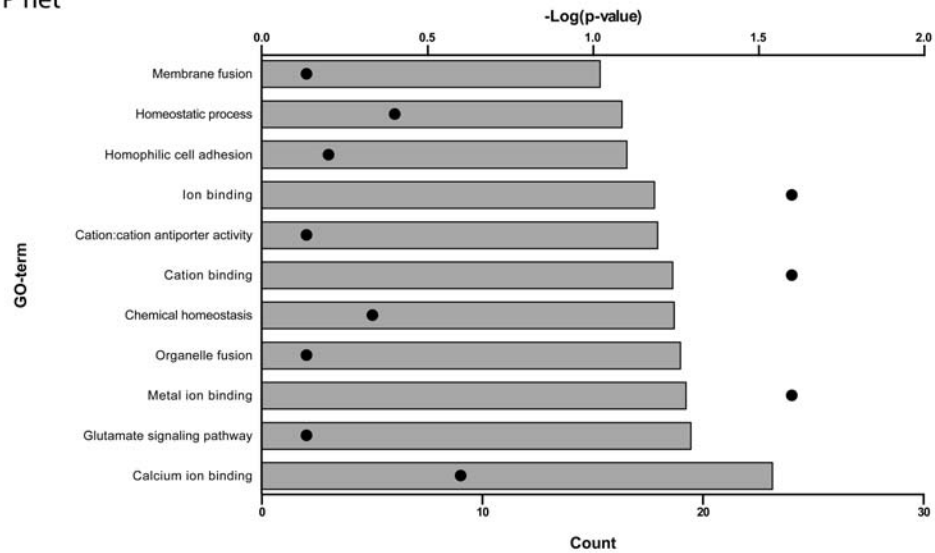
(a) Heat map generated by unsupervised hierarchical clustering based on differentially expressed genes using Significant Analysis of Microarray (SAM, $q < 0.05$). This illustrates that the three groups are perfectly separated, and differences in gene expression are most evident between WT iso parabionts and both APP groups and less noticeable between APP iso and APP het parabionts. Color bars reflect the z scores with downregulated genes shown in shades of blue, and upregulated genes shown in shades of red. (b) 6 out of 8 genes that were significantly different when comparing all 3 groups in Significance Analysis of Microarrays (SAM, $q < 0.05$) followed by Tukey Honestly Significant Difference (HSD, $P < 0.05$) showed a pattern in which expression levels of APP het parabionts were reversed to WT iso levels. These genes were cartilage acidic protein 1 (Crtac1), trophinin (Tro), enhancer of mRNA decapping 4 (Edc4), γ -secretase activating protein (GSAP), phosphoprotein associated with glycosphingolipid microdomains 1 (PAG1), and gap junction protein, delta 2, 36kDa (Gjd2) for which relative gene expression is presented here. The values represent normalized values using robust multi-array average method (RMA)(Yamin 2009; Irizarry et al. 2003). All data are shown as the mean + s.e.m. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, one-way ANOVA, Tukey's post hoc test (b).

eFigure 5

a) APP iso versus WT iso

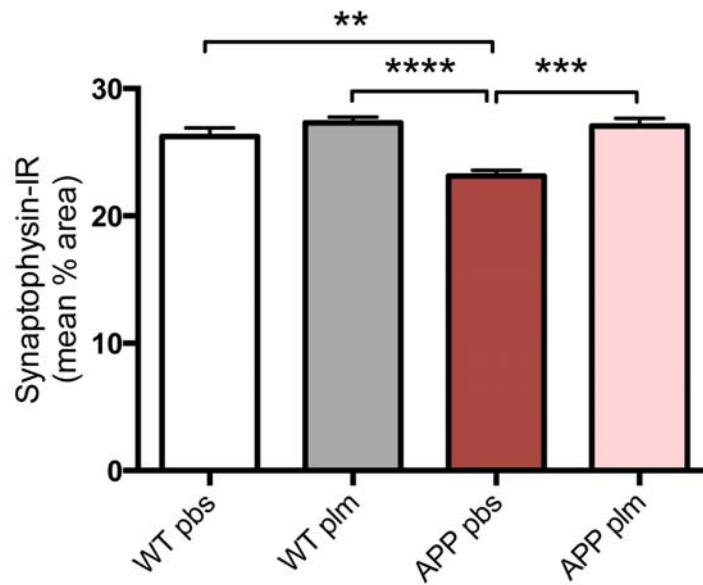


b) APP iso versus APP het



eFigure 5 Top gene ontology terms of the top 100 differentially expressed genes

The top 100 differentially expressed genes between hippocampi of APP iso and WT iso (a), and of APP iso and APP het (b) parabionts according to SAM ($P < 0.05$) were analyzed using the online gene ontology tool DAVID for gene ontology term (GO-term) annotation categories 'Biological Process', and 'Molecular Function'. (a) This graph represents the top 20 all GO-terms with $P < 0.05$ (195 total) for differentially expressed genes between APP iso and WT iso hippocampi. (b) This graph represents all GO-terms with $P < 0.1$ (only calcium ion binding is < 0.05) for differentially expressed genes between APP iso and APP het hippocampi. Gray bars represent the p-values and the black dots represent the number of genes in each category.



eFigure 6 Administration of young blood plasma reverses neocortical synaptophysin depletion

Quantification of synaptophysin immunoreactivity in the neocortex of WT pbs (n = 14), WT plm (n = 13), APP pbs (n = 11) and APP plm (n = 13) mice. Data are shown as the mean + s.e.m., ** P < 0.01, *** P < 0.001, **** P < 0.0001, one-way ANOVA.

eTable 1

eTable 1. Top 100 metaranked genes

Metarank	GeneSymbol	Score	Rank t-test	Rank Anova	Rank Enets	Rank Lasso	Rank Log fold change	P-value t-test	log fold change	P-value Anova
1	Atp6v1g2	5,93E-08	1	na	1	1	510	7,08E-05	-0.151456796	na
2	Vps37b	2,84E-07	2	na	2	na	165	0,000290503	-0.209316853	na
3	Zkscan14	5,25E-07	3	na	3	2	234	0,000433717	-0.192731666	na
4	Mir763	5,41E-07	24	na	20	na	2	0,002538997	-0.533156486	na
5	Rbmw2	1,53E-06	4	na	3	4	154	0,000598608	-0.211876671	na
6	Foxq1	4,32E-06	16	na	14	na	41	0,001720412	-0.304623385	na
7	Ccdc117	4,38E-06	5	na	5	3	571	0,000729108	-0.146072907	na
8	H2afb2-ps	6,44E-06	17	na	32	na	28	0,00194143	0,33413137	na
9	Lrrc51	6,81E-06	7	na	7	na	273	0,000835865	0,184288884	na
10	Lamp2	1,01E-05	11	na	8	na	219	0,001210884	0,196651683	na
11	Tnip2	1,10E-05	23	na	na	na	44	0,002390539	-0.296207029	na
12	Foxl1	1,12E-05	29	na	20	na	46	0,002961311	0,294086598	na
13	Pcdh8	1,20E-05	30	na	na	na	42	0,002968683	-0.300864394	na
14	Tmem242	1,31E-05	8	na	12	na	253	0,001118232	-0.187706159	na
15	Crtac1	1,55E-05	15	na	10	na	195	0,001561958	-0.201500627	na
16	1110038F14Rik	1,74E-05	13	na	9	5	199	0,001504491	-0.199913435	na
17	Fam76b	1,94E-05	14	na	14	na	149	0,001517477	-0.213929238	na
18	Ppnr	2,22E-05	51	na	na	na	55	0,00500356	0,280181426	na
19	Cln3	2,79E-05	25	na	28	na	74	0,002597457	-0.255403031	na
20	Mir187	3,33E-05	82	na	na	na	35	0,007660818	-0.315093131	na
21	Clic6	3,40E-05	2303	na	na	na	1	0,185989679	0,59441925	na
22	Susd1	3,85E-05	9	na	43	na	171	0,00112143	-0.207457242	na
23	Ncapd3	3,89E-05	6	na	5	6	1573	0,000751553	-0.100169237	na
24	Snord83b	3,97E-05	124	na	na	na	24	0,01005379	-0.364963695	na
25	Plscr3	5,05E-05	56	na	na	na	89	0,005182506	0,243098951	na
26	BC002059	5,07E-05	84	na	na	na	70	0,007836585	-0.258433344	na
27	Gpn3	5,41E-05	10	na	10	8	410	0,001124974	-0.163302783	na
28	Cngb1	5,66E-05	38	na	34	na	81	0,003799683	0,247202594	na
29	Cited4	6,47E-05	85	na	na	na	94	0,007845531	0,238436812	na
30	Rab40b	6,72E-05	22	na	18	na	316	0,002271841	-0.176741493	na
31	Btg2	6,84E-05	122	na	na	na	47	0,0097588	-0.293544323	na
32	Mir1963	7,28E-05	498	na	na	na	10	0,039498656	-0.43077331	na
33	Hscb	7,38E-05	100	na	na	na	97	0,008455852	-0.235652124	na
34	Ccdc3	7,57E-05	110	na	na	na	67	0,009005486	0,259932846	na
35	Cdh7	7,58E-05	168	na	na	na	34	0,013301289	0,320656746	na
36	Ccnj	7,63E-05	33	na	24	na	177	0,003179812	-0.205373839	na
37	Homer3	8,07E-05	52	na	26	na	129	0,005032139	-0.218352782	na
38	1700013F07Rik	8,39E-05	111	na	na	na	80	0,009009831	0,247528904	na
39	Tmem175	8,56E-05	18	na	14	na	580	0,001986084	-0.145490337	na
40	Hs3t4	8,66E-05	12	na	18	na	742	0,001434307	-0.134441858	na
41	Slc9a2	8,98E-05	118	na	na	na	76	0,009358834	-0.252648278	na
42	Rps12l2	9,02E-05	2038	na	na	na	3	0,161918391	-0.51623016	na
43	Smoc2	9,31E-05	50	na	na	na	151	0,004905336	-0.213099953	na
44	Mir297a-4	9,61E-05	817	na	na	na	8	0,064471492	0,443412054	na
45	Aagab	0,000104511	21	na	20	na	399	0,002129926	-0.164701325	na
46	Rabggtb	0,00010744	206	na	na	na	39	0,016525481	-0.306240698	na
47	Ptchd4	0,000108755	125	na	na	na	95	0,010081287	-0.238387003	na
48	Syne1	0,000113068	81	na	na	na	135	0,007633158	-0.217181591	na
49	Rpp40	0,000119117	148	na	na	na	72	0,011787889	0,257095381	na
50	Gpr88	0,000122485	697	na	na	na	12	0,054245087	0,427453899	na
51	Usp50	0,000122898	162	na	na	na	64	0,012914113	0,263657949	na
52	Lrrc48	0,000131348	36	na	na	na	265	0,003623412	0,186078748	na
53	Irf2bp2	0,000134715	137	na	na	na	114	0,01087471	0,224553952	na
54	Syne1	0,000135513	66	na	na	na	172	0,006514335	-0.207159618	na
55	Sfil1	0,000140565	78	na	na	na	161	0,007533184	0,210329959	na
56	Dusp1	0,00014072	167	na	na	na	73	0,01329043	-0.256226984	na
57	Zic1	0,000147123	1663	na	na	na	6	0,132137928	0,477710014	na
58	Arsj	0,000152892	145	na	na	na	127	0,011528094	-0.218417115	na
59	Irga8	0,000159851	42	na	43	na	173	0,0040835	-0.20644528	na
60	Mrp110	0,000160477	112	na	na	na	153	0,009079667	0,212072592	na
61	Vat1l	0,000161105	997	na	na	na	11	0,0782966	0,429069158	na
62	Myog	0,000166976	332	na	na	na	36	0,027506038	0,314075462	na
63	1700011M02Rik	0,000171075	562	na	na	na	21	0,043761334	-0.378260253	na
64	Gpld1	0,000174746	156	na	na	na	130	0,012435193	-0.2181668	na
65	Fut11	0,000177516	642	na	na	na	19	0,050437153	-0.387937388	na
66	Pcdhb7	0,000181689	173	na	na	na	100	0,013787554	-0.234250363	na
67	Wdr78	0,000182597	67	na	na	na	218	0,006648805	0,196782266	na
68	Prss35	0,000192767	254	na	na	na	58	0,02115949	-0.275687823	na
69	Chchd4	0,000193706	20	na	12	7	843	0,002054614	-0.128620067	na
70	Sinx22	0,000195389	143	na	na	na	164	0,011420846	-0.209569052	na
71	Kcnk13	0,000196187	101	na	na	na	182	0,008461553	0,204601511	na
72	Mir3091	0,000204437	366	na	na	na	40	0,030272566	0,305931603	na
73	Mir574	0,000205441	385	na	na	na	38	0,032246633	0,309816266	na
74	Zfp14	0,000205788	149	na	na	na	168	0,011861308	0,208510881	na
75	Zfp160	0,000210877	86	na	na	na	209	0,007850148	-0.198319728	na
76	Apbb3	0,000211342	97	na	na	na	196	0,008225952	-0.200848101	na
77	Mfsd3	0,000218049	26	na	71	na	215	0,00262774	0,196908952	na
78	Cdr1	0,000218056	682	na	na	na	22	0,052745042	-0.372974122	na
79	Pdcd2l	0,000224083	74	na	na	na	242	0,007322885	-0.190394131	na
80	Chsy3	0,000233336	54	na	34	na	235	0,005074929	0,192158164	na
81	Cutc	0,0002385	186	na	na	na	138	0,014693656	-0.216500237	na
82	Mettl7a3	0,000242724	645	na	na	na	26	0,050590867	-0.346991409	na
83	Prrm3	0,00024485	39	na	50	na	227	0,00391332	0,195039072	na
84	Syne1	0,000255014	105	na	50	na	134	0,008820386	-0.217590508	na
85	Mettl24	0,000257909	180	na	na	na	187	0,014437626	-0.203576161	na
86	Gpc4	0,000268168	197	na	na	na	147	0,015818613	-0.214603104	na
87	1500015O10Rik	0,000280894	4756	na	na	na	4	0,39466748	0,51224752	na
88	Rras2	0,00028142	27	na	50	na	318	0,002780031	-0.176354371	na
89	Vav3	0,000294247	226	na	na	na	120	0,018666935	-0.223402171	na
90	Gpr50	0,000294712	212	na	na	na	141	0,017077958	0,215837763	na
91	Hist3h2a	0,000295008	368	na	na	na	59	0,030664681	-0.273666752	na
92	Zfp263	0,000300768	202	na	na	na	193	0,016188525	-0.202832455	na
93	Gjd2	0,000304882	172	na	na	na	206	0,013758512	0,198953068	na
94	Rnaseh2a	0,000306507	166	na	na	na	208	0,013223318	-0.198655931	na
95	1700003M02Rik	0,000328849	903	na	na	na	25	0,07049866	0,356428675	na
96	Lhp	0,000334551	3239	na	na	na	7	0,268385546	0,446704304	na
97	Ccnj1	0,000341279	152	na	na	na	228	0,012023102	-0.194786317	na
98	6030419C18Rik	0,000347599	37	na	20	na	846	0,003729345	-0.12854863	na
99	Syne1	0,000353562	220	na	na	na	197	0,017879848	-0.200262091	na
100	Rassf5	0,000356957	32	na	28	na	756	0,00308856	0,134038637	na