

1 Pyk2 suppresses contextual fear memory

2 in a kinase-independent manner

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8 Supporting information

9 S1 Fig. Expression patterns of *Pyk2* gene in mouse brain at different developmental 10 stages

11 (A and B) Confocal images of brain sections from mice at E18.5 and P0 stages were
12 immunostained with antibodies against Pyk2 (A, B), DAPI (A', B') and merged (A'', B'').
13 Bar in B'' represents 1,000 μm for A and B. (C) Expression of the *Pyk2* gene in the
14 hippocampus of mice at different developmental stages using Western blot.

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16 S2 Fig. *Pyk2* kinase site mutant mice production and its behavior test

17 (A and B) Schematic of the Pyk2 protein and the sgRNA-targeting sequence of the *Pyk2*
18 gene. (B) The mutant single-stranded donor DNA sequence with mutations at the codon
19 of tyrosine residue 402 and PAM region was illustrated. (C) Confirmation of genotypes by
20 Sanger sequencing. (D) Trace fear response in *Pyk2*^{Y402F} (n = 22) and WT (n = 18) mice
21 were significantly enhanced during training but without significant difference between
22 genotypes ($F_{\text{times}}(21,798) = 157.46$, $p < 0.0001$; $F_{\text{genotype}}(1,798) = 0.04$, $p = 0.84$). (E and
23 F) There was no significantly difference in training context 24 hours post training
24 (*Pyk2*^{Y402F} = 57.95%, WT = 49.39%, $p > 0.05$) (E), in novel context (*Pyk2*^{Y402F} = 18.86%,
25 WT = 17.06%, $p > 0.05$) as well as in auditory fear conditioning ($F_{\text{sessions}}(3,114) = 29.01$,
26 $p < 0.0001$; $F_{\text{genotype}}(1,114) = 0.05$, $p = 0.82$) between genotypes (F). (G-I) In Morris water
27 maze test, escape latencies were similar between *Pyk2*^{Y402F} and wild-type mice (WT: n =
28 11; *Pyk2*^{Y402F}: n = 11) in both the acquired sessions ($F_{\text{times}}(3,60) = 8.74$, $p < 0.0001$;
29 $F_{\text{genotype}}(1,60) = 3.42$, $p = 0.08$) (G) and reversal sessions ($F_{\text{times}}(4,80) = 7.72$, $p < 0.0001$;
30 $F_{\text{genotype}}(1,80) = 1.42$, $p = 0.25$) (I) as well as the percentage of time spent in each quadrant

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31 during reverse trials on test Day 5 (H) ($F_{\text{quadrants}}(3,60) = 12.9, p < 0.0001$; $F_{\text{genotype}}(1,60) =$
32 $0.00, p = 1.00$). Data are represented as mean \pm SEM.

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34 **S1 Table. Differentially expressed genes in the hippocampus of *Pyk2*-KO mice**

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36 **S2 Table. Differentially expressed genes in the hippocampus of *Pyk2*^{Y402F} mice**

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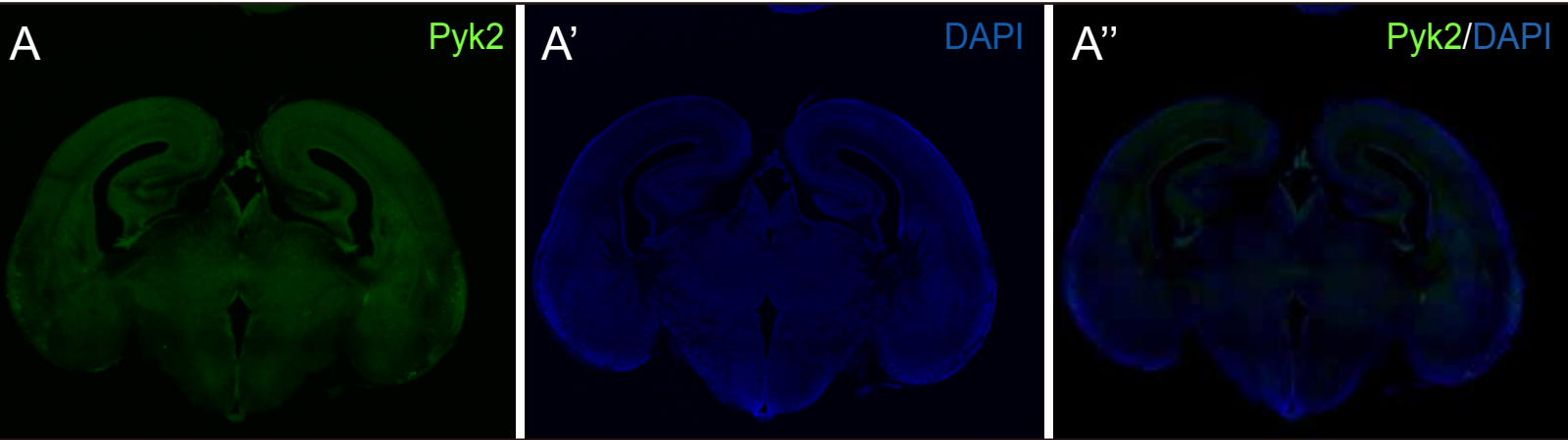
38 **S1 Movie. Time-lapse of hippocampal culture neurons from wild-type mice after**
39 **culturing for 14 days *in vitro***

40

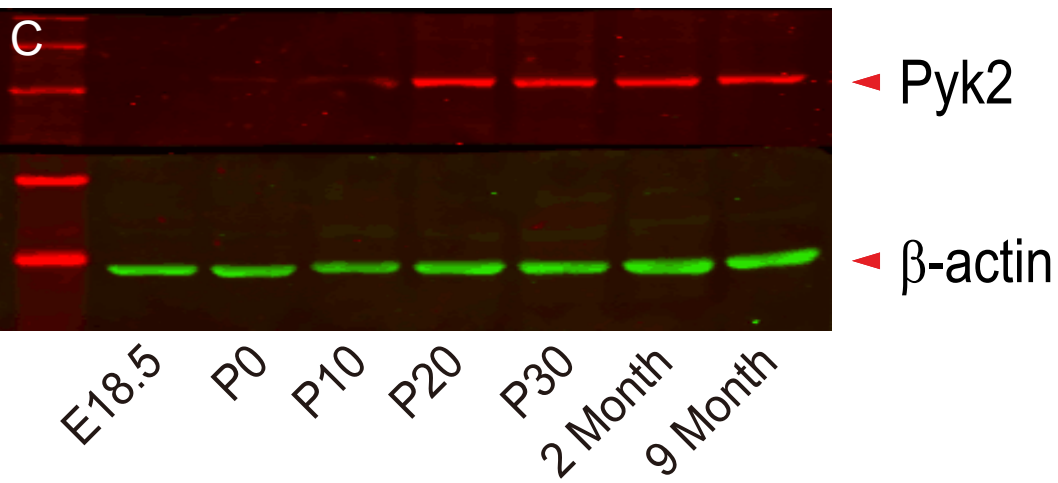
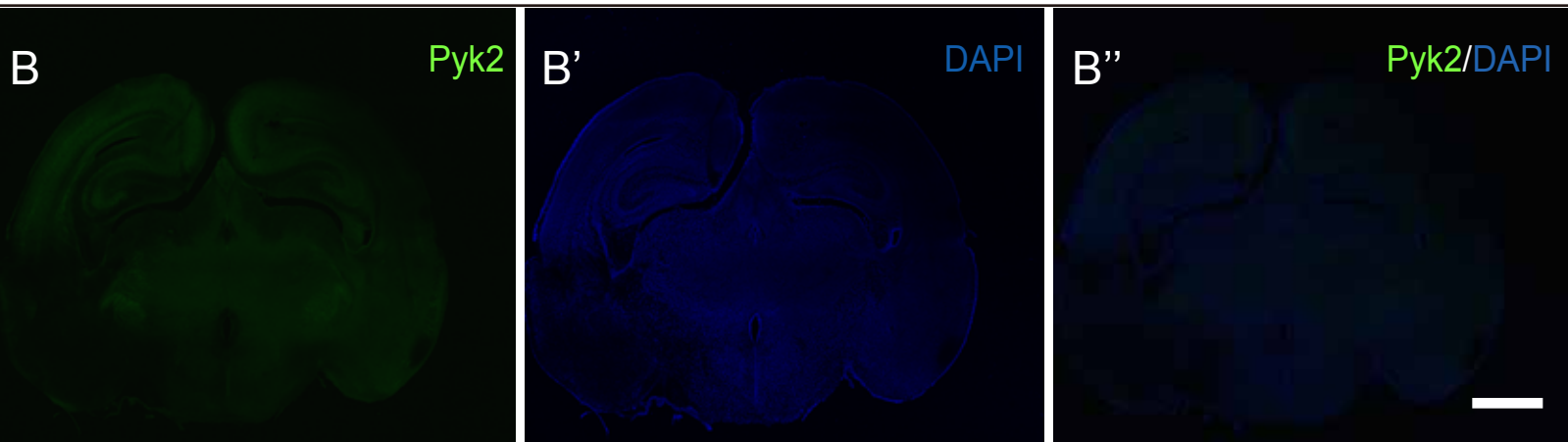
41 **S2 Movie. Time-lapse of hippocampal culture neurons from *Pyk2*-KO mice after**
42 **culturing for 14 days *in vitro***

S1 Fig.

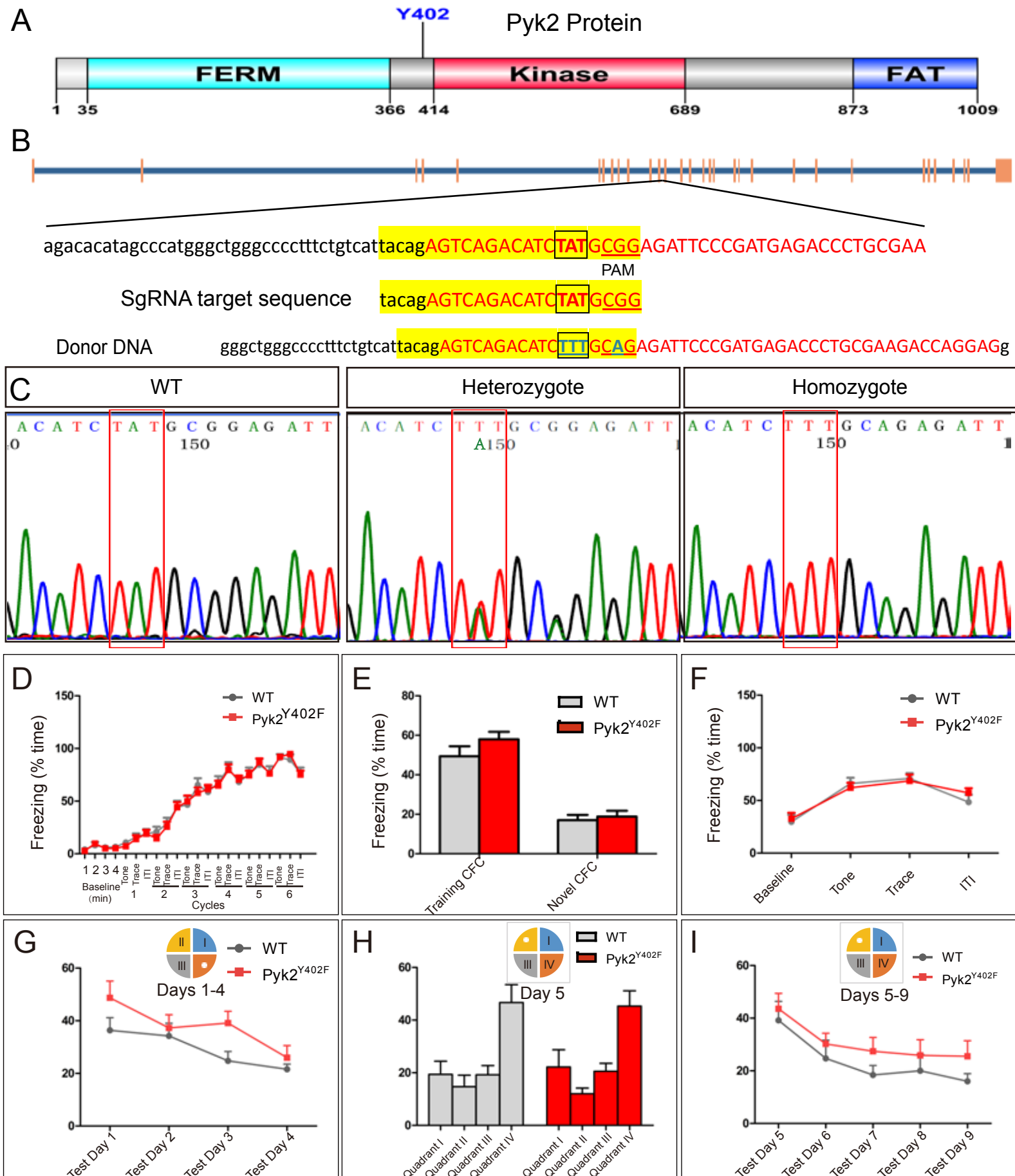
E18.5



P0



S2 Fig.



S1 Table. Differentially Expressed Genes in the Hippocampus of *Pyk2*-KO Mice

Gene ID	Symbol	log ₂ RNA fold change(<i>Pyk2</i> KO/WT)	FDR	Description
73385	Fam177a	9.691743519	2.98E-54	family with sequence similarity 177, member A
627914	Gm14430	7.417852515	1.41E-12	predicted gene 14430
100039257	Tmem254b	5.591569888	1.15E-60	transmembrane protein 254b
16353	lpw	3.732450113	1.71E-05	imprinted gene in the Prader-Willi syndrome region
13654	Egr2	3.023332504	1.15E-05	early growth response 2
319317	Shhg11	2.944143532	0.00E+00	small nucleolar RNA host gene 11
329096	Gm17644	2.62058641	5.04E-07	predicted gene, 17644
72289	Malat1	2.438710328	2.42E-90	metastasis associated lung adenocarcinoma transcript 1 (non-coding RNA)
268755	Mir124a-1hg	2.3249542	1.43E-56	RIKEN cDNA A930011O12 gene
17263	Meg3	2.289386727	0.00E+00	maternally expressed 3
225872	Npas4	2.220048481	1.58E-09	neuronal PAS domain protein 4
14281	cFos	2.070773074	3.64E-10	FBJ osteosarcoma oncogene
330166	Miat	2.028278776	3.57E-75	myocardial infarction associated transcript (non-protein coding)
67120	Ttc14	1.96906814	3.94E-32	tetratricopeptide repeat domain 14
72472	Slc16a10	1.909635972	1.14E-04	solute carrier family 16 (monocarboxylic acid transporters), member 10
78889	Wsb1	1.87883431	1.04E-13	WD repeat and SOCS box-containing 1
21933	Tnfrsf10b	1.853158612	1.75E-04	tumor necrosis factor receptor superfamily, member 10b
547127	Tmem181b-ps	1.760977716	1.10E-34	transmembrane protein 181B, pseudogene
667118	Zbed6	1.709998547	8.13E-07	zinc finger, BED domain containing 6
353236	Pcdhac1	1.5360529	7.65E-07	protocadherin alpha subfamily C, 1
320429	Trank1	1.503914055	8.82E-44	tetratricopeptide repeat and ankyrin repeat containing 1
13653	Egr1/Zif268	1.498170999	6.36E-78	early growth response 1
11838	Arc	1.411986095	2.09E-65	activity regulated cytoskeletal-associated protein
329575	Gm14325	1.408911396	5.17E-08	predicted gene 14325
214133	Tet2	1.335867286	1.96E-08	tet methylcytosine dioxygenase 2
20190	Ryr1	1.330148602	5.09E-05	ryanodine receptor 1, skeletal muscle
68750	Rreb1	1.321928095	3.18E-10	ras responsive element binding protein 1
103012	Firre	1.250283704	4.61E-07	functional intergenic repeating RNA element
236790	Ddx26b	1.230780207	2.24E-04	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 26B
208968	Zfp280c	1.209750982	5.80E-04	zinc finger protein 280C
231470	Fras1	1.207324973	3.85E-06	Fraser syndrome 1 homolog (human)

15370	Nr4a1	1.104637342	2.96E-21	nuclear receptor subfamily 4, group A, member 1
260305	Nphp4	1.0538227	1.23E-04	nephronophthisis 4 (juvenile) homolog (human)
12941	Pcdha5	1.026311277	2.55E-04	protocadherin alpha 5
213742	Xist	3.23349013	0.00E+00	inactive X specific transcripts
100039060	0610010B08Ri k	-8.781359714	2.42E-33	RIKEN cDNA 0610010B08 gene
433406	Gm13363	-8.519636253	7.98E-17	predicted gene 13363
619441	Tnfsfm13	-8.312882955	2.05E-11	tumor necrosis factor (ligand) superfamily, membrane-bound member 13
14264	Fmod	-2.958314097	9.84E-17	fibromodulin
68371	Pbld1	-2.644490202	2.04E-09	phenazine biosynthesis-like protein domain containing 1
19229	Pyk2	-2.562313762	0.00E+00	PTK2 protein tyrosine kinase 2 beta
100039192	Tmem254c	-2.283432103	9.70E-57	transmembrane protein 254c
232987	B9d2	-1.623046002	2.41E-07	B9 protein domain 2
22320	Vamp8	-1.59626515	2.74E-09	vesicle-associated membrane protein 8
100568459	Bc1	-1.30519994	0.00E+00	brain cytoplasmic RNA 1
100040563	Dynl1c	-1.2410081	9.82E-04	dynein light chain Tctex-type 1C
15267	Hist2h2aa1	-1.154033629	2.92E-09	histone cluster 2, H2aa1
245424	Gpr101	-1.152003093	4.94E-04	G protein-coupled receptor 101
17313	Mgp	-1.06436554	4.32E-05	matrix Gla protein
66915	Myeov2	-1.043658757	6.60E-18	myeloma overexpressed 2
68436	Rpl34	-1.004799129	3.03E-25	ribosomal protein L34
68195	Rnaset2b	-8.77807713	6.16E-07	ribonuclease T2B

S2 Table. Differentially Expressed Genes in the Hippocampus of Pyk2^{Y402F} Mice

Gene ID	Symbol	log ₂ RNA fold change(Pyk2 ^{Y402F} / WT)	FDR	Description
97114	Hist2h3c2	10.02928723	1.07E-09	histone cluster 2, H3c2
69994	Rsc1a1	8.214319121	1.07E-09	regulatory solute carrier protein, family 1, member 1
574404	Gm14685	6.965784285	4.95E-05	predicted gene 14685
102238433	Gm28042	6.554588852	3.57E-07	predicted gene, 28042
668039	Gm14434	4.99699125	8.57E-30	predicted gene 14434
100043915	Gm4724	4.99699125	8.18E-30	predicted gene 4724
100043381	Gm14308	4.99699125	7.83E-30	predicted gene 14308
12939	Pcdha7	1.983759359	5.85E-08	protocadherin alpha 7
319370	Ubald2	1.761342524	3.16E-16	UBA-like domain containing 2
15077	Hist2h3c1	-9.975847968	1.27E-09	histone cluster 2, H3c1
100039060	0610010B08Rik	-9.321928095	2.15E-48	RIKEN cDNA 0610010B08 gene
100041874	Gm3558	-8.038918989	3.54E-08	predicted gene 3558
574405	DXBay18	-7.159871337	5.96E-06	DNA segment, Chr X, Baylor 18
100040591	Kcnj13	-5.591881915	1.04E-11	potassium inwardly-rectifying channel, subfamily J, member 13
211429	Pla2g4b	-5.491853096	3.90E-05	phospholipase A2, group IVB (cytosolic)
107885	Mthfs	-2.340523777	6.94E-05	5, 10-methenyltetrahydrofolate synthetase
13386	Dlk1	-2.196538994	1.12E-22	delta-like 1 homolog (Drosophila)
574437	Xlr3b	-1.833890645	2.27E-05	X-linked lymphocyte-regulated 3B
664849	Gm7367	-1.790670896	1.04E-21	1110014K08Rik pseudogene
627914	Gm14430	-1.73470962	0.00016	predicted gene 14430
13106	Cyp2e1	-1.485264279	6.81E-12	cytochrome P450, family 2, subfamily e, polypeptide 1
71950	Nanog	-1.239223445	0.00066	Nanog homeobox
192163	Pcdha3	-1.113714609	2.22E-05	protocadherin alpha 3