

Supplemental Figures Sun et al.

Supplemental figure 1. Verification of hippocampal deletion of Smad4. (A) Upon expression of Cre recombinase in the hippocampus, Smad4 protein levels were undetectable in adult stage. (B) mRNA of Smad4 was reduced to 50% of the level in the wildtype hippocampus with Cre expression at 3 weeks. Only 20% Smad4 mRNA left at 3 month old hippocampal CA1. Remaining Smad4 is presumably from cell populations other than pyramidal neurons in CA1. (C) Cre antibody staining showed the pattern of transgene expression in adult mouse hippocampal section.

Supplemental figure 2. 24 hour continuous homecage behavior of Smad4 mutants. (A, B) Smad4 mutants showed normal circadian behavior, less sleep during the nocturnal portion of the light-dark cycle as control littermates did. (C, D) Smad4 KO mice showed hyperactive during light cycle. (E, F) control mice showed less grooming activity during light cycle but Smad4 KO mice did not.

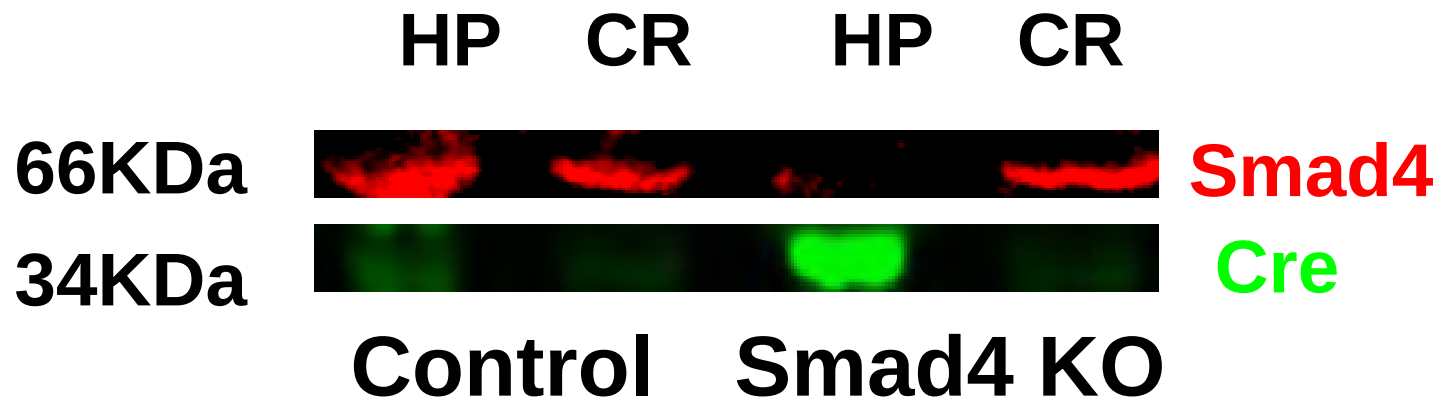
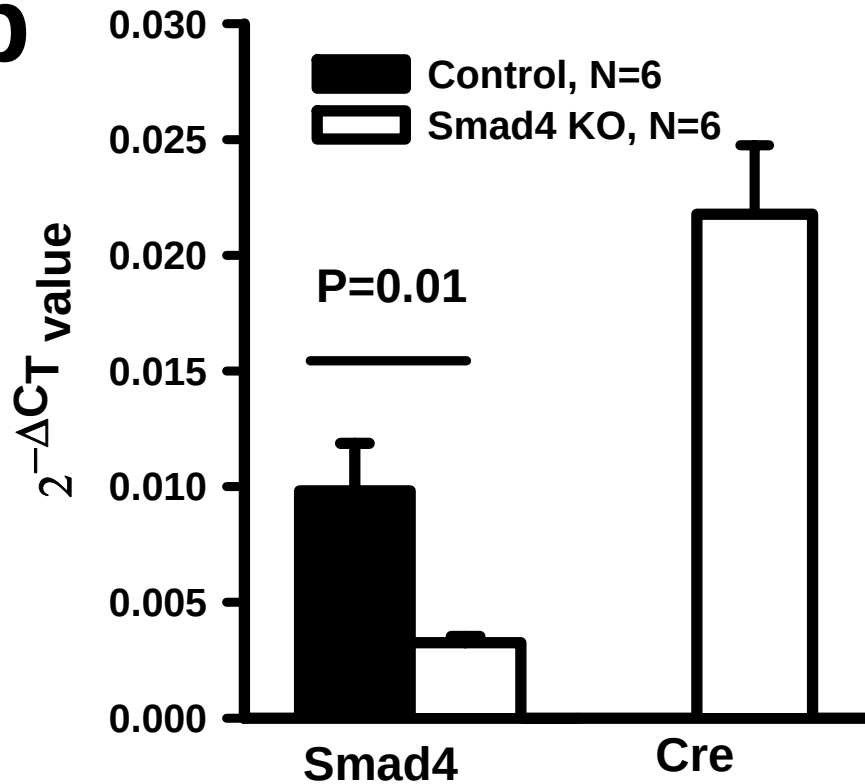
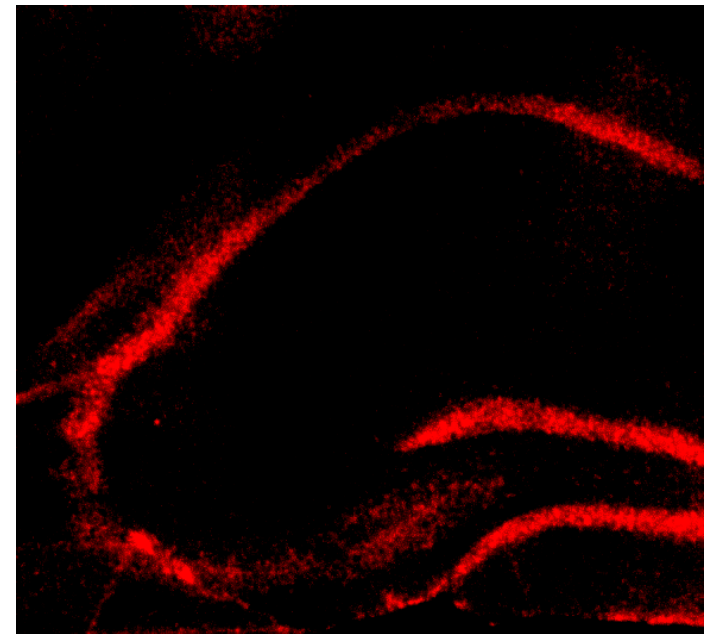
Supplemental figure 3. The profile of New Homecage Behavior from Smad4 mutants. Smad4 mutants showed indistinguishable levels of walking distance and time (A, B), sniffing (D), remain low (E), eating (F), hang cuddled (H) and digging and foraging (I) compared to control littermates. (J) Undetectable behaviors from both groups accounted for <5% percent of recording time. (C) Smad4 KO mice showed less vertical behavior in new homecages. (G) Smad4 KO mice showed more grooming behavior in a new homecage, while control mice showed less grooming upon exposure to a novel environment.

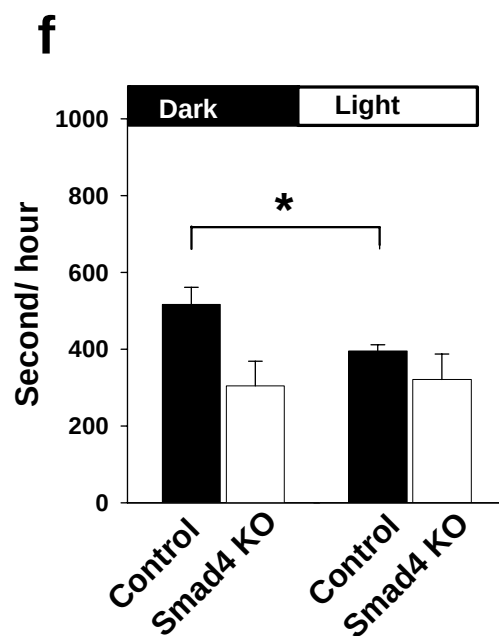
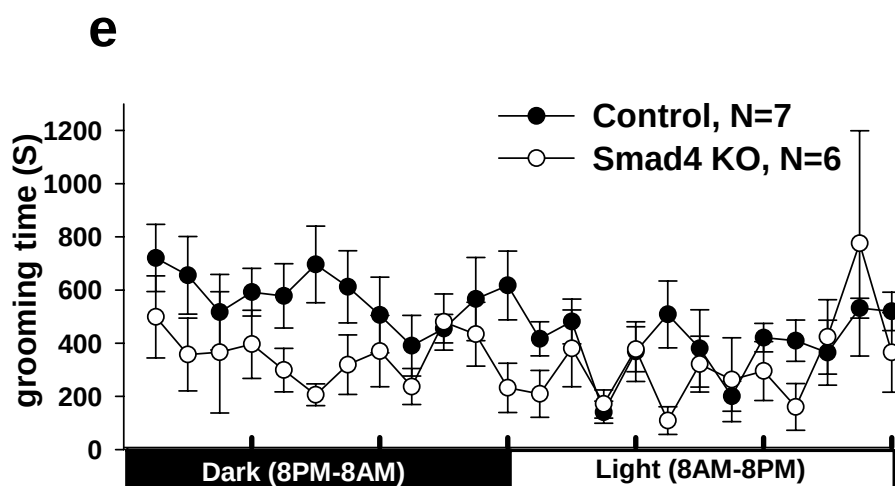
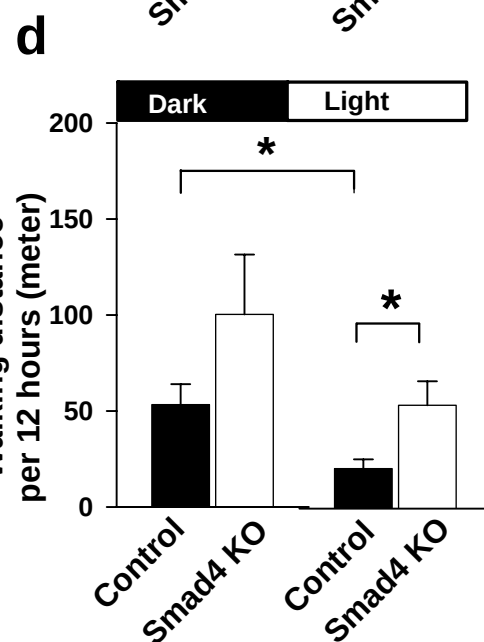
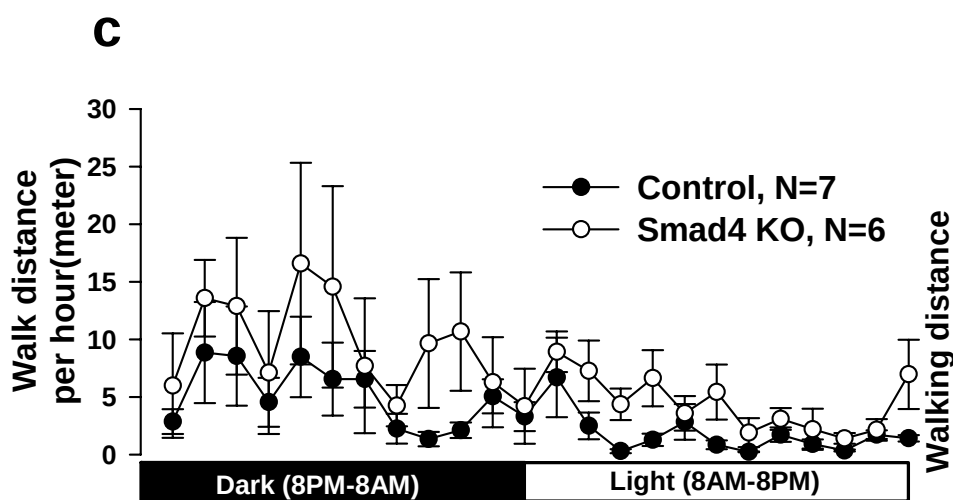
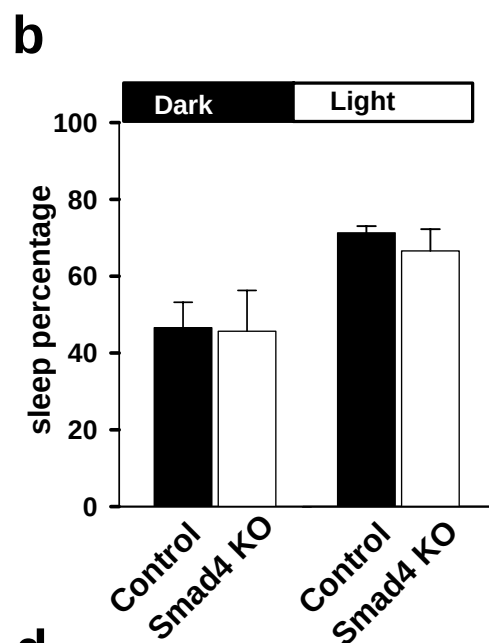
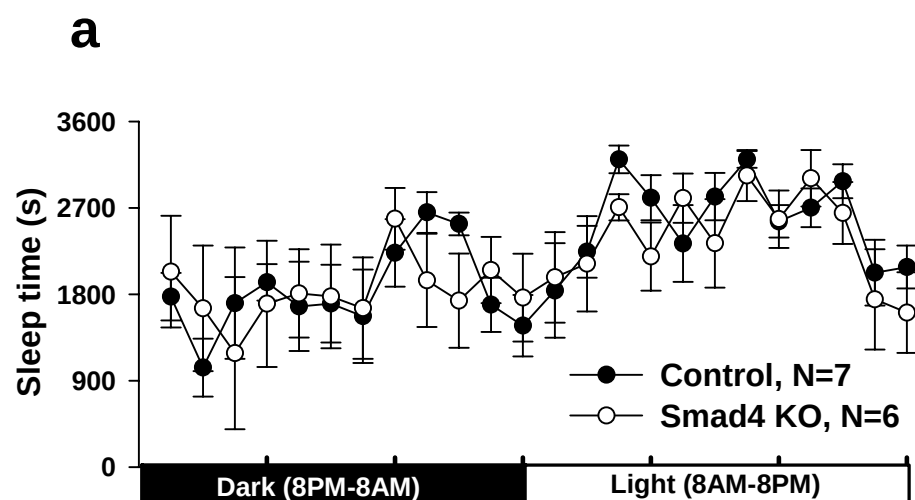
Supplemental figure 4. Smad4 KO mice did not show less anxiety in elevated plus maze. (A, B) No differences in times to enter an arm or time spent in an arm were detected between two genotypes. (C) moving speed of Smad4 KO mice was normal in the center area and open arms. (D) Subtle increase of head-dip bouts were detected in Smad4 KO mice. (E) Smad4 KO mice showed no significant differences compared to littermates in SAP bouts.

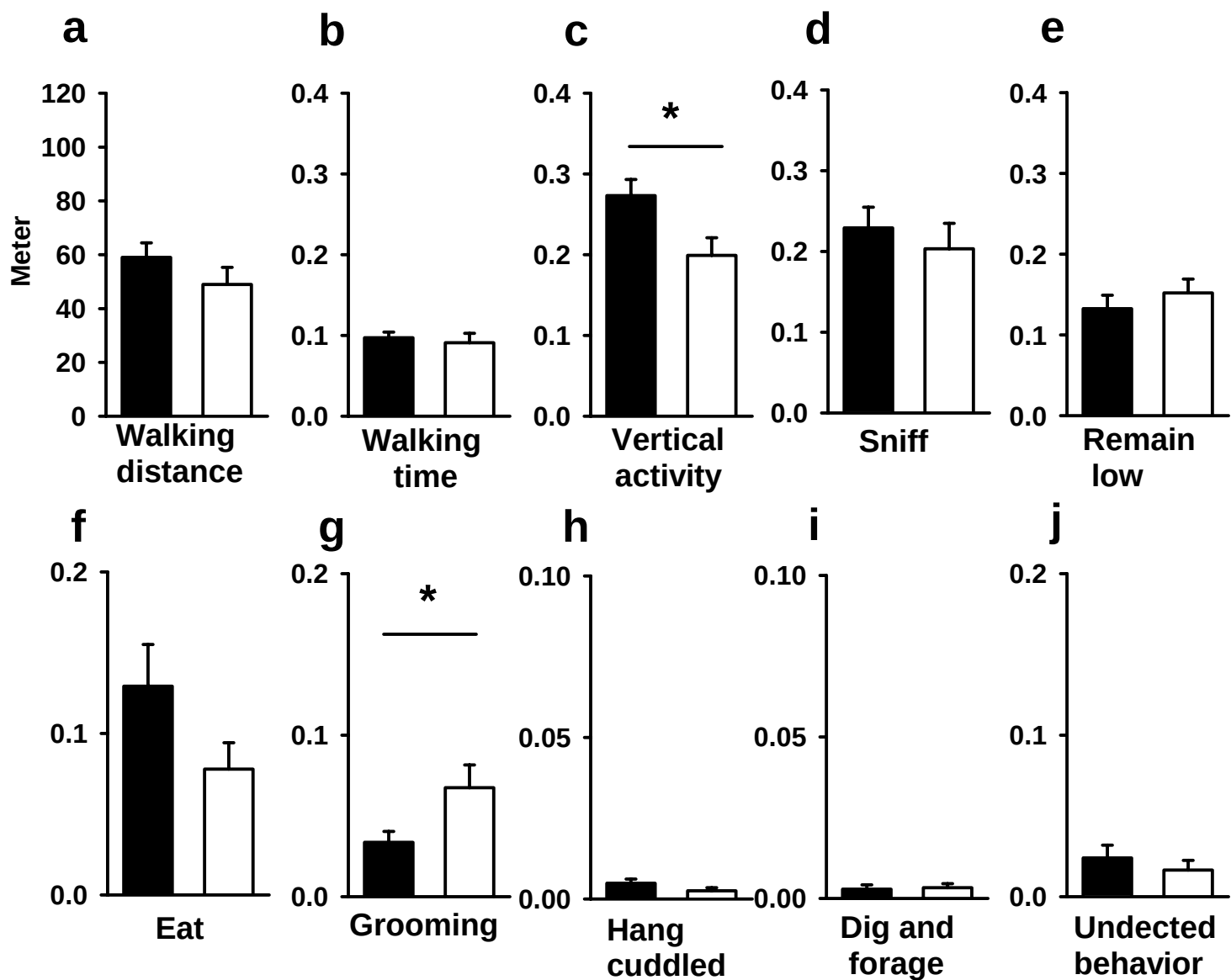
Supplemental figure 5. QPCR array of GABA_A subunits from hippocampal CA1 total mRNA of Smad4 KO and control mice. The mRNA level of individual subunits was stable after Smad4 deletion in the hippocampus. (A) Relative value with reference gene, cyclophilin A. (B) Normalized with control mice and represented as fold change.

Supplemental table 1. Results of QPCR array of TGF- β signaling pathway in Smad4 KO and control hippocampi.

Supplemental table 2. Primers used for QPCR assays.

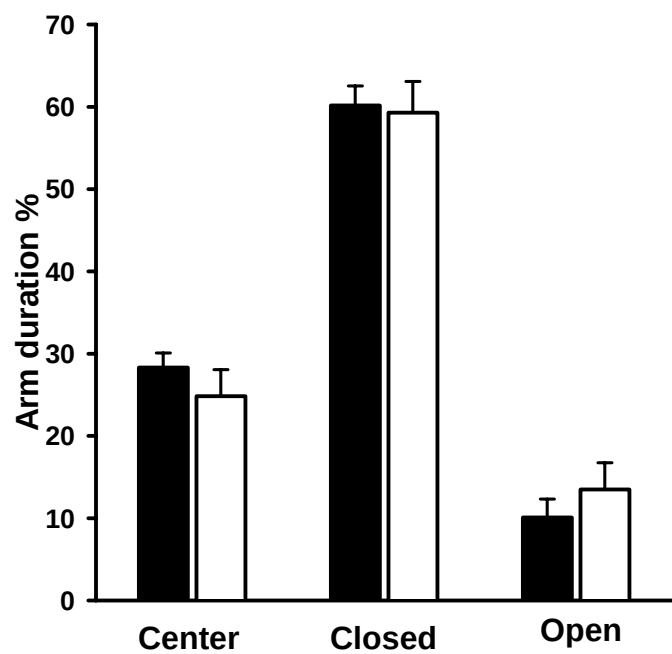
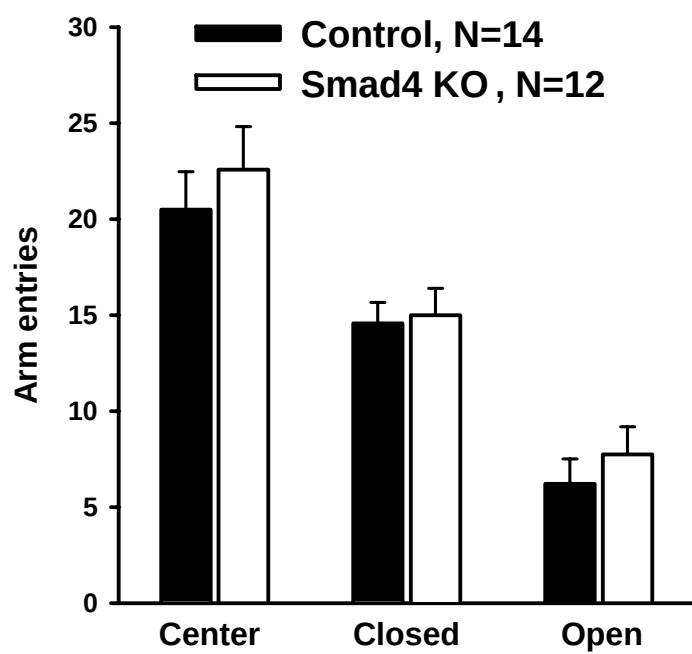
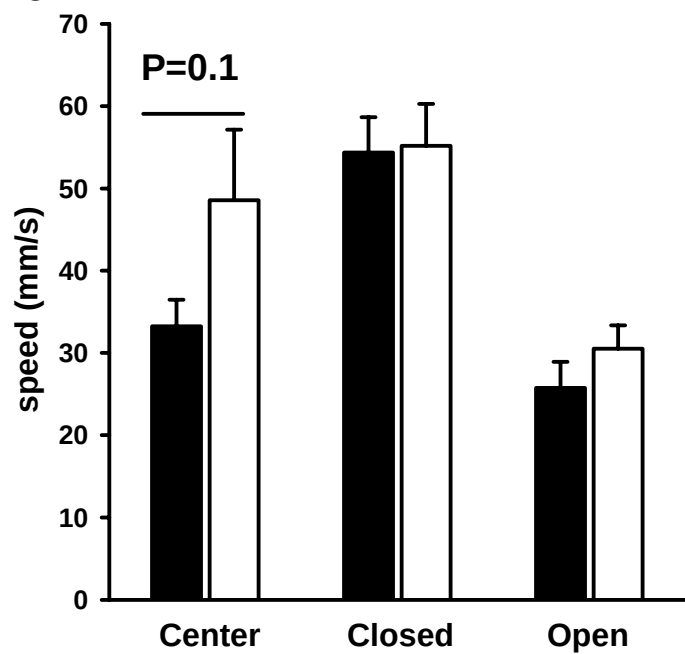
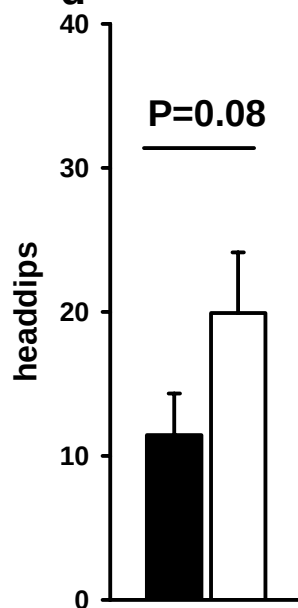
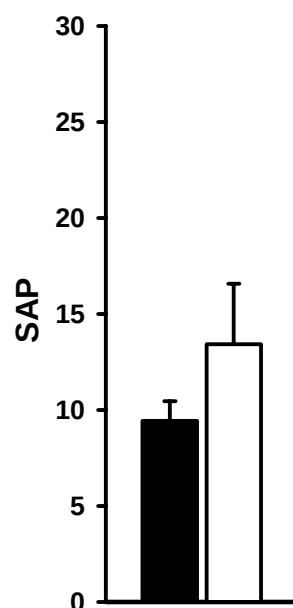
a**b****c**

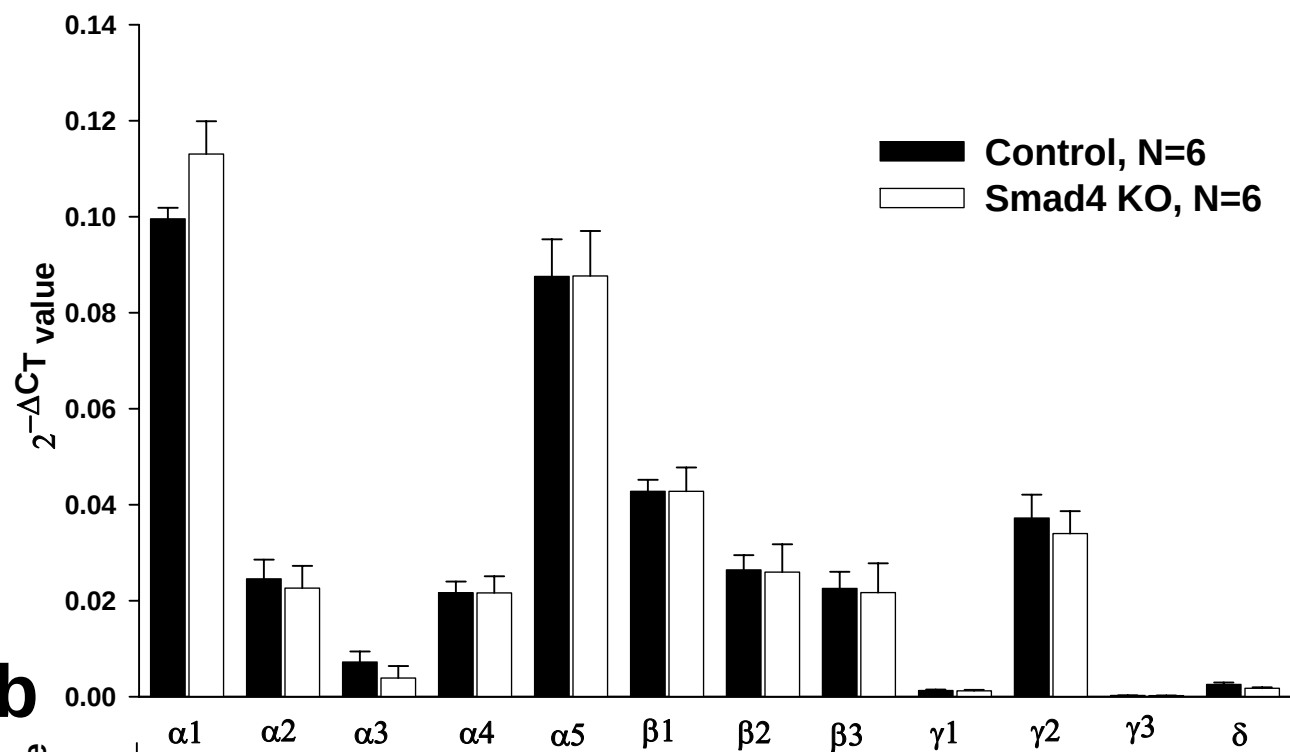
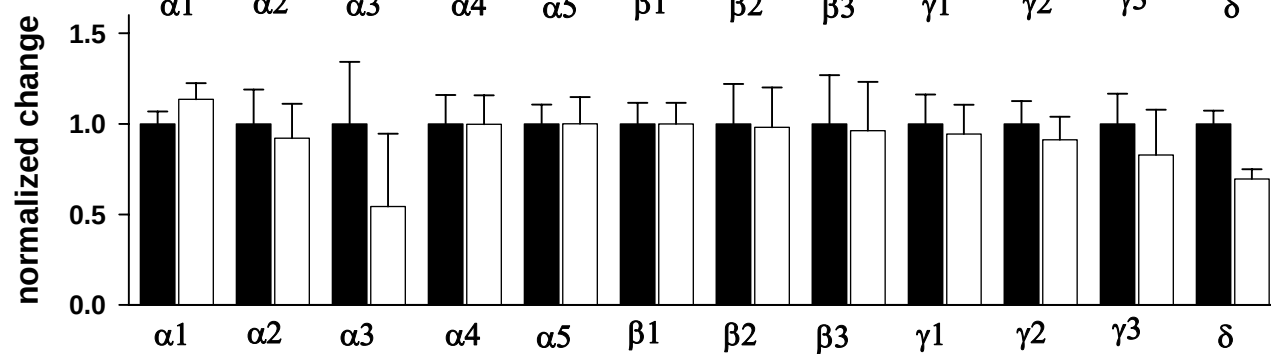




All parameters above are time percentage except for Walking distance.

Control, N=13
Smad4 KO, N=14

a**b****c****d****e**

a**b**

		p value	
	fold change		Description
Acvr1	1.0507	0.815007	Activin A receptor, type 1
Acvr2a	1.4621	0.172843	Activin receptor IIA
Acvr11	1.0903	0.771714	Activin A receptor, type II-like 1
Amh	1.1903	0.388533	Anti-Mullerian hormone
Amhr2	1.2757	0.04506	Anti-Mullerian hormone type 2 receptor
Bambi	1.2153	0.677855	BMP and activin membrane-bound inhibitor, homolog (Xenopus laevis)
Bglap2	1.2995	0.280377	Bone gamma-carboxyglutamate protein 2
Bmp1	1.0507	0.727081	Bone morphogenetic protein 1
Bmp2	1.2125	0.625887	Bone morphogenetic protein 2
Bmp3	1.4419	0.486338	Bone morphogenetic protein 3
Bmp4	1.1157	0.804611	Bone morphogenetic protein 4
Bmp5	1.0338	0.962306	Bone morphogenetic protein 5
Bmp6	1.1903	0.795613	Bone morphogenetic protein 6
Bmp7	1.238	0.742184	Bone morphogenetic protein 7
Bmper	1.1631	0.595549	BMP-binding endothelial regulator
Bmpr1a	1.1712	0.370262	Bone morphogenetic protein receptor, type 1A
Bmpr1b	1.1366	0.435955	Bone morphogenetic protein receptor, type 1B
Bmpr2	1.2995	0.448923	Bone morphogenetic protein receptor, type II (serine/threonine kinase)
Cd79a	1.5312	0.089938	CD79A antigen (immunoglobulin-associated alpha)
Cdc25a	1.3993	0.213942	Cell division cycle 25 homolog A (S. pombe)
Cdkn1a	1.2409	0.576637	Cyclin-dependent kinase inhibitor 1A (P21)
Cdkn2b	1.0703	0.857791	Cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)
Chrd	1.1132	0.602629	Chordin
Col1a1	-1.1111	0.837029	Collagen, type I, alpha 1
Col1a2	-1.3062	0.605272	Collagen, type I, alpha 2
Col3a1	-1.1854	0.784615	Collagen, type III, alpha 1
Dlx2	1.4688	0.115321	Distal-less homeobox 2
Eng	1.2935	0.314024	Endoglin
Evi1	1.3116	0.451146	Ecotropic viral integration site 1
Fkbp1b	1.1794	0.416752	FK506 binding protein 1b
Fos	1.7148	0.215322	FBJ osteosarcoma oncogene
Fst	6.1674	0.00531	Follistatin
Gdf1	1.3299	0.23152	Growth differentiation factor 1
Gdf2	1.5706	0.448	Growth differentiation factor 2
Gdf3	1.1418	0.690113	Growth differentiation factor 3
Gdf5	2.0965	0.272994	Growth differentiation factor 5
Gdf6	-2.3707	0.011137	Growth differentiation factor 6
Gdf7	1.1794	0.38899	Growth differentiation factor 7
Gsc	1.0629	0.964815	Goosecoid homeobox
Id1	-1.0683	0.821869	Inhibitor of DNA binding 1
Id2	-1.0958	0.583183	Inhibitor of DNA binding 2
Igf1	-1.0634	0.802254	Insulin-like growth factor 1
Igfbp3	1.4287	0.377871	Insulin-like growth factor binding protein 3
Il6	-1.0487	0.907694	Interleukin 6
Inha	1.1931	0.544957	Inhibin alpha
Inhba	-1.3062	0.454282	Inhibin beta-A
Inhbb	-1.0536	0.793577	Inhibin beta-B
Itgb5	1.2699	0.293384	Integrin beta 5
Itgb7	-1.1637	0.562206	Integrin beta 7
Jun	1.2757	0.350994	Jun oncogene

Junb	1.0978	0.757496	Jun-B oncogene
Lefty1	1.0629	0.791021	Left right determination factor 1
Ltbp1	1.022	0.70273	Latent transforming growth factor beta binding protein 1
Ltbp2	-1.0634	0.780145	Latent transforming growth factor beta binding protein 2
Ltbp4	1.1261	0.427676	Latent transforming growth factor beta binding protein 4
Myc	1.1106	0.486236	Myelocytomatosis oncogene
Nbl1	1.0678	0.62227	Neuroblastoma, suppression of tumorigenicity 1
Nodal	1.2757	0.412572	Nodal
Nog	1.396	0.318223	Noggin
Nr0b1	1.1658	0.734966	Nuclear receptor subfamily 0, group B, member 1
Pdgfb	1.1157	0.269828	Platelet derived growth factor, B polypeptide
Plat	-1.0343	0.686702	Plasminogen activator, tissue
Plau	1.5032	0.135994	Plasminogen activator, urokinase
Runx1	-1.0248	0.846508	Runt related transcription factor 1
Serpine1	1.2876	0.349961	Serine (or cysteine) peptidase inhibitor, clade E, member 1
Smad1	-1.0908	0.422119	MAD homolog 1 (Drosophila)
Smad2	1.0531	0.570383	MAD homolog 2 (Drosophila)
Smad3	-1.4393	0.032219	MAD homolog 3 (Drosophila)
Smad4	-2.6549	0.016343	MAD homolog 4 (Drosophila)
Smad5	1.0604	0.74845	MAD homolog 5 (Drosophila)
Smurf1	-1.2215	0.081209	SMAD specific E3 ubiquitin protein ligase 1
Sox4	-1.1503	0.502659	SRY-box containing gene 4
Stat1	-1.1663	0.181908	Signal transducer and activator of transcription 1
Tdgf1	-1.253	0.34575	Teratocarcinoma-derived growth factor
Tgfb1	-1.1663	0.50573	Transforming growth factor, beta 1
Tgfbli1	1.4893	0.008978	Transforming growth factor beta 1 induced transcript 1
Tsc22d1	1.2728	0.270199	TSC22 domain family, member 1
Tgfb2	1.0483	0.790564	Transforming growth factor, beta 2
Tgfb3	1.2266	0.134832	Transforming growth factor, beta 3
Tgfb1	-1.1111	0.765427	Transforming growth factor, beta induced
Tgfb1	-1.1663	0.013702	Transforming growth factor, beta receptor I
Tgfb2	1.0196	0.916745	Transforming growth factor, beta receptor II
Tgfb3	-1.1718	0.584506	Transforming growth factor, beta receptor III
Tgfb1	-1.1345	0.10701	Transforming growth factor, beta receptor associated protein 1
Gusb	1.022	0.819388	Glucuronidase, beta
Hprt1	1.022	0.691567	Hypoxanthine guanine phosphoribosyl transferase 1
Hsp90ab1	-1.0154	0.83326	Heat shock protein 90kDa alpha (cytosolic), class B member 1
Gapdh	-1.0367	0.378059	Glyceraldehyde-3-phosphate dehydrogenase
Actb	1.0079	0.941939	Actin, beta, cytoplasmic
MGDC	1.0507	0.765904	Mouse Genomic DNA Contamination
RTC	1.1418	0.690113	Reverse Transcription Control
RTC	1.2466	0.491687	Reverse Transcription Control
RTC	1.1418	0.690113	Reverse Transcription Control
PPC	1.1029	0.772758	Positive PCR Control
PPC	1.0728	0.856173	Positive PCR Control
PPC	1.1157	0.762343	Positive PCR Control
Smad4 KO N=3, Control N=3, samples were from hippocampal total RNA.			

Name of Gene	forward primer	reverse primer
Cyclonphlin A	TGGTCAACCCCACCGTGTTCTT	GCCATCCAGCCACTCAGTCTT
Smad4	CCATCATAACAGCACTACCAC	AGCAGGATGATTGGAAATGG
Cre recombinase	CCCTGTTACGTATAGCCGA	GCTCGACCAGTTTAGTTACC
Gabra1	ACTGTCTTTGGAGTGACGA	ATAGCTGGTTGCTGTAGGA
Gabra2	CTGTGTTTGGAGTAACAACCTG	TTGACTACACTCTTCCCGT
Gabra3	GGACCAATGAAGATCCTTCC	TGGTATAGGCATAGCTTCCA
Gabra4	AATAACCACAGTCCTCACGA	GGCATGTGTATTCTGAAGGG
Gabra5	CAGCACAGGTGAATATACGA	GGCAAATTCAATCAGAGCAG
Gabra6	TTGTTGTGCATTCTGACTCC	AGTACACAAGGTTGAATCCTG
Gabrb1	CAAAGTCCAAGTTGATGCC	GGGACCACTTGTCTATGGA
Gabrb2	TCTCCACAGGTTCTTATCCC	TAAAGACGAAGACAAAGCACC
Gabrb3	AATAAACCGGGTGATGCT	TTGAGCTGTGAAGACCTCC
Gabrg1	TTTCAAGCTATGGATACCCT	GGAATGTATGTCTGAATCGTG
Gabrg2	AACTTCTGGTGACTATGTGG	GGTGCTTAAAGTTGTCATGG
Gabrg3	AAGATGAGAATGAAGACTCCC	CTTTGCTGAGCATGTTTATGG
Gabrd	AGTATCTCTAGGCATCACCA	GTTCTCCTCACATCCATCTCTG
Smad3	AGAGTACAGGAGACAGACAG	CCTTAGTTGAGTGGAATCGG
Amhr2	GTTTCTCCCAGGTAATCCAG	AGTGATAAAGCGAACGATATGG
Fst	TCCCTTGTAAGAAACGTGTG	TTACATTTGCCCTGGTACTG
L1cam	CTTACCAGAAGGGAAAGTGAG	TTTGATGAAGCAGAGGATGAG
Chl1	GTTGATAAAGACACTGCCAC	ATACCTTTACTCCCTTCTCCT
Ncam	CAGTGAACCGTATTGGACAG	CATACCACTTGAAATGCCAG
Gdf6	ACGATCACTAGCTTTGTAGAC	GGATAAACAAGGGAAGAGCTG