Supplementary Data

Shank3 mutant mice display autistic-like behaviours and striatal dysfunction

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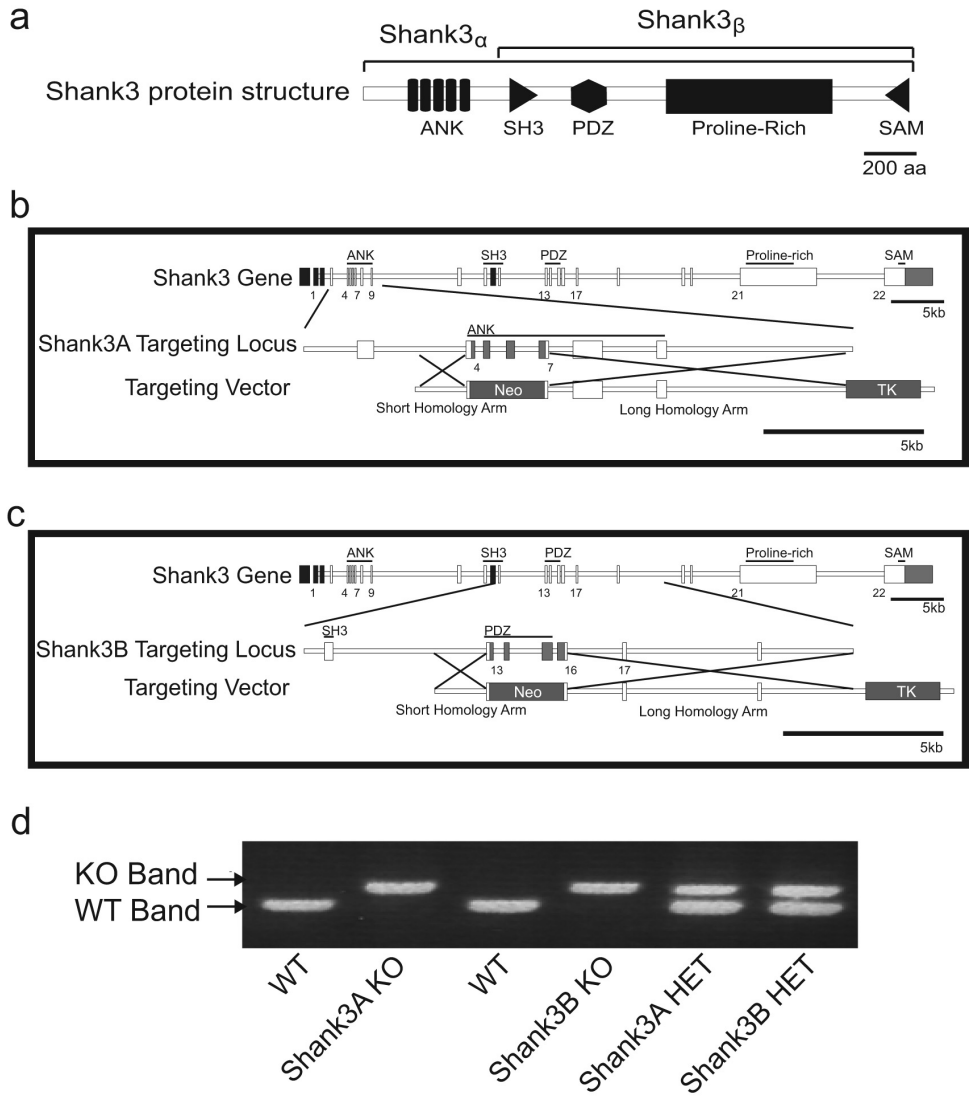
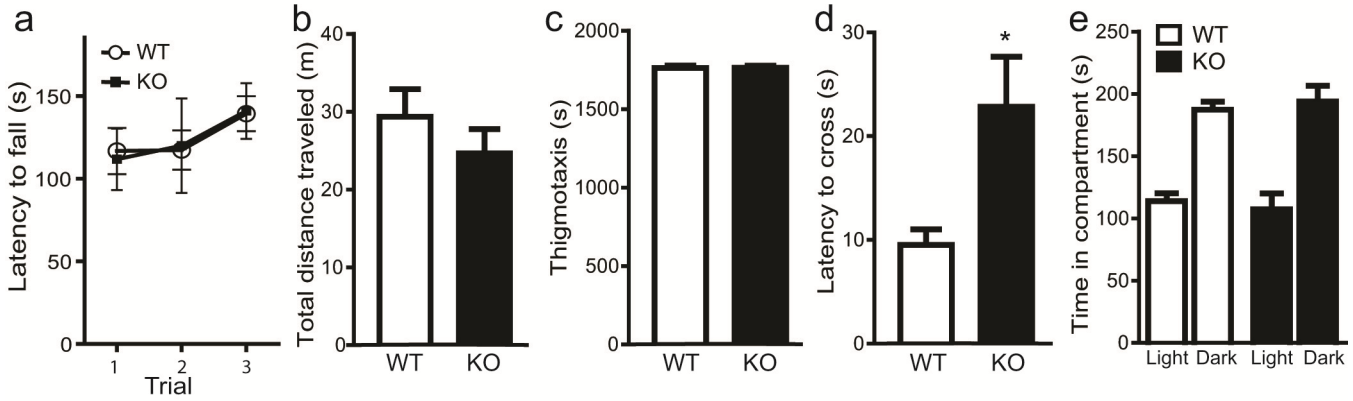
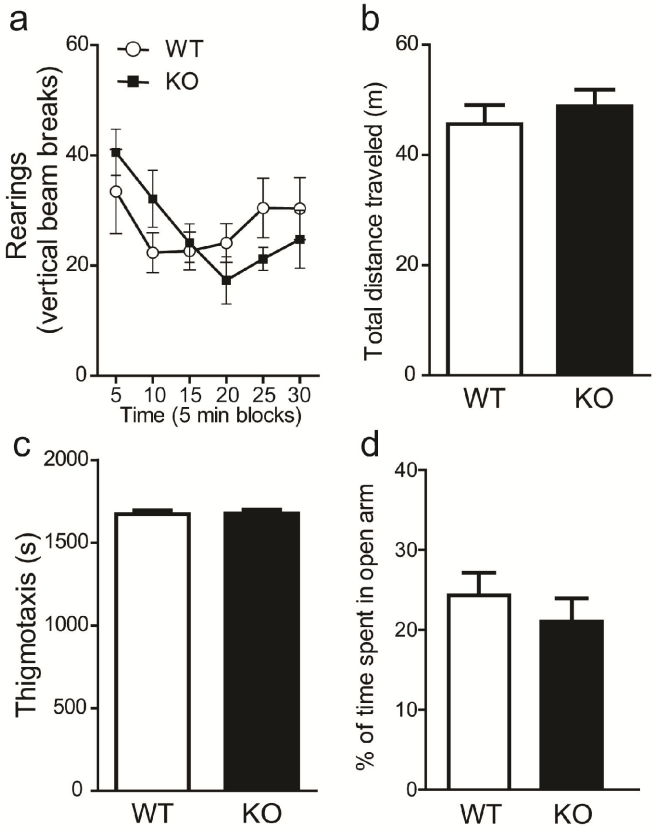


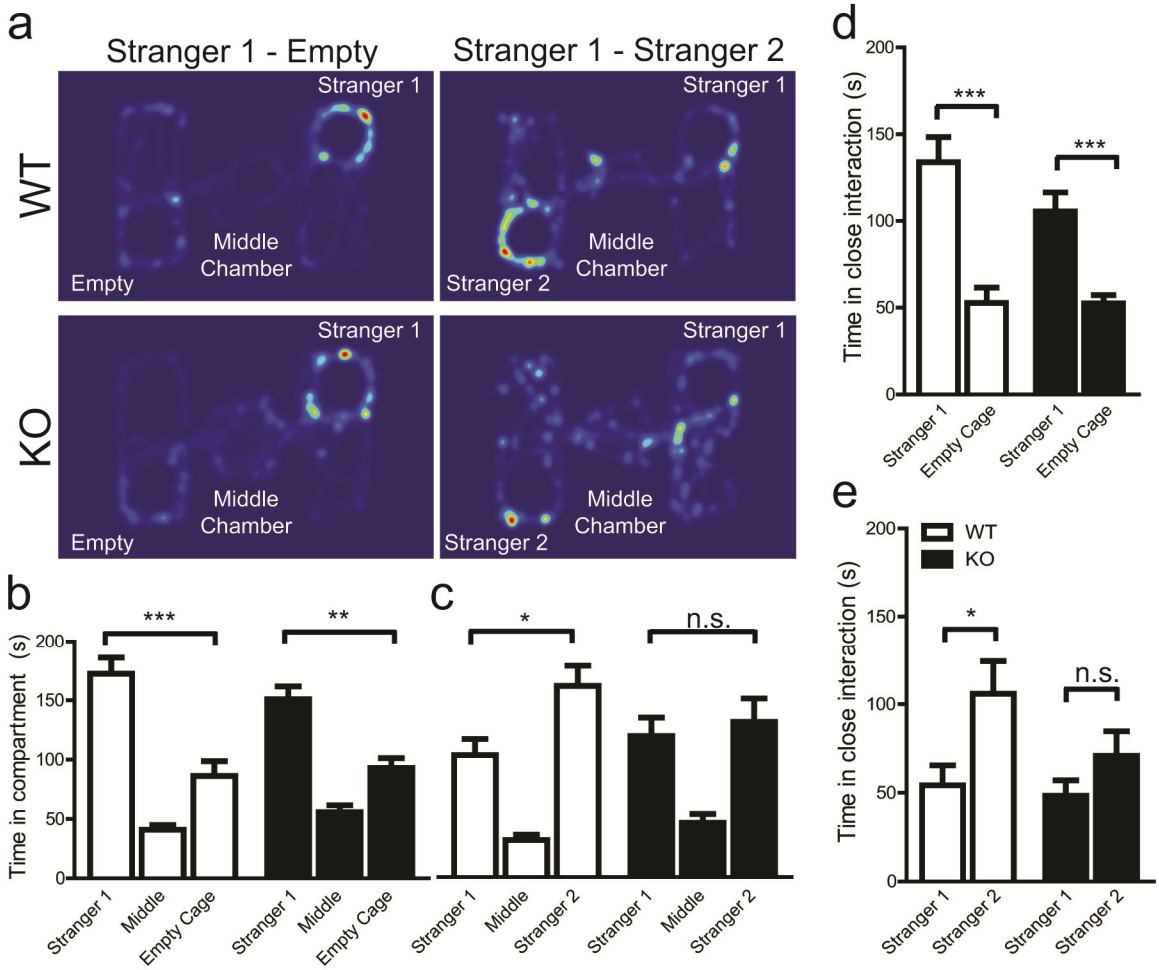
Supplementary Figure 1 | Shank3 protein structure, gene structure and targeted disruptions.a,Shank3 protein structure, with known isoforms highlighted.**b**, **c**, Targeting strategy and targeting vector for Shank3A-mutant mice (**b**), and Shank3B-mutant mice (**c**). **d**, PCR genotyping for $Shank3A^{+/+}$, $Shank3A^{+/-}$, $Shank3A^{-/-}$, $Shank3B^{+/+}$, $Shank3B^{+/-}$ and $Shank3B^{-/-}$ mice.



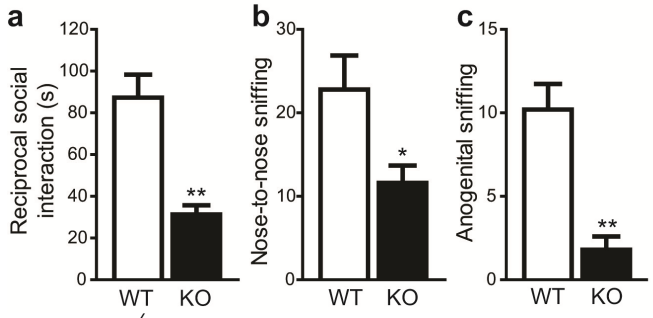
Supplementary Figure 2 | Motor learning and anxiety tests in *Shank3B*$^{-/-}$ *mice.* a, In the rotarod test, *Shank3B*$^{-/-}$mice and controls display similar levels of motor coordination. **b, c,** In the open field test, *Shank3B*$^{-/-}$ mice, when compared to controls, display similar levels of total distance traveled (**b**) and thigmotaxis (**c**). **d, e,** In the dark-light box emergence test, *Shank3B*$^{-/-}$ mice display an increased latency to initially cross from the dark into the brightly lit chamber (**d**), but spend a similar amount of time in either chamber when compared to controls (**e**). * $p<0.05$, two-tailed *t*-test for **d**; all data are presented as means ± s.e.m; n=5 mice per genotype for **a,** and 8-9 mice per genotype for **b-e**.



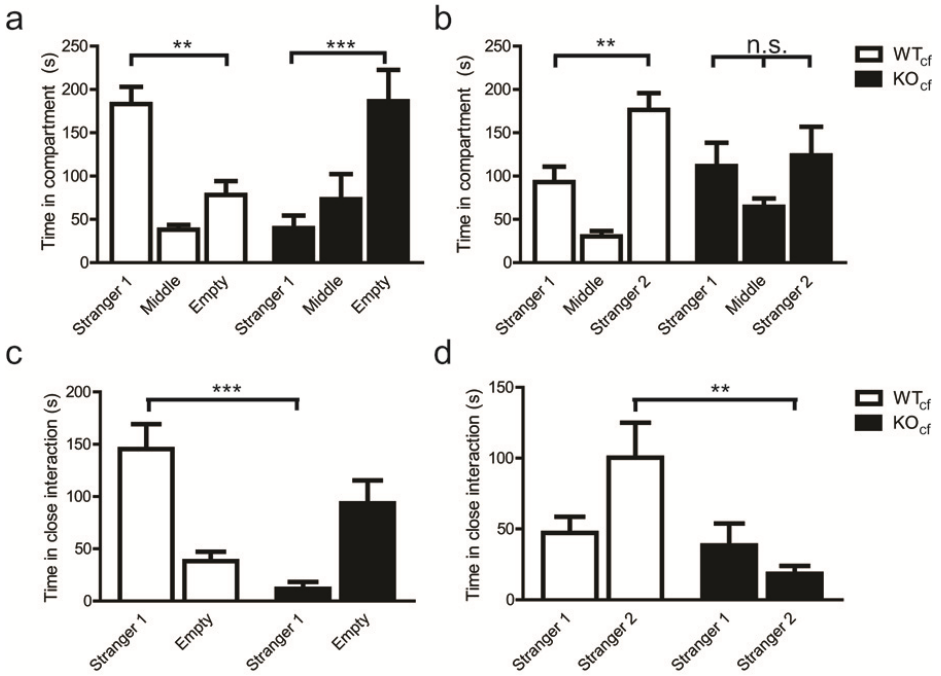
Supplementary Figure 3 | Anxiety levels and exploratory behaviours in *Shank3A*$^{-/-}$ *mice.* a-c, In the open field test *Shank3A*$^{-/-}$rearing (**a**), total distance traveled (**b**), and time spent close to the chamber walls (thigmotaxis) (**c**) is not significantly different from controls. **d,** In the zero maze test, *Shank3A*$^{-/-}$mice spent a similar amount of time in the open area when compared to wildtype controls. Two-tailed *t*-test for **b-d**;Two-way repeated measures ANOVA for **a**; all data presented as means ± s.e.m. n = 8 mice per genotype.



Supplementary Figure 4 | Social interaction and social novelty recognition tests in *Shank3A*$^{-/-}$mice.a, Representative heat map analysis from "Stranger 1 – Empty" and "Stranger 1 – Stranger 2" trials from *Shank3A*$^{-/-}$ mice and controls. **b,** In the social interaction test, both *Shank3A*$^{-/-}$ mice (KO – black bars) and wildtype controls (WT – white bars) spent more time in the chamber containing the social partner (Stranger 1) than in the chamber containing the empty wire cage (Empty Cage).**c,** In the social novelty test, *Shank3A*$^{-/-}$ mice do not display preference for the novel social partner (Stranger 2). **d, e,** When analyzing social interaction by close proximity (within 5cm) to either "Stranger 1", "Empty Cage" (**d**), or "Stranger 1", "Stranger 2" (**e**), *Shank3A*$^{-/-}$ mice again display a preference for close social interaction with "Stranger 1"than "Empty cage" (**d**); however, under a social novelty paradigm (**e**), *Shank3A* mutantsdisplay abnormal social novelty recognition, as indicated by reduced preference for the novel social partner (Stranger 2). * $p<0.05$, ** $p<0.01$, *** $p<0.0001$, One-way ANOVA, with Bonferroni *post hoc t*-test; data are presented as means ± s.e.m. from 12-13 mice per group.



Supplementary Figure 5 | *Shank3B*$^{-/-}$ mice display social deficits during dyadic interaction in an open arena test. a, Unfamiliar pairs ofwildtype-*Shank3B*$^{-/-}$ mice (black bars) spend less time in reciprocal social interaction when compared to unfamiliar pairs of wildtype-wildtype mice (white bars). **b, c,** Quantification of nose-to-nose sniffing (**b**) and anogenital sniffing (**c**) by the target animal (wildtype or *Shank3B*$^{-/-}$) towards a stimulus animal (wildtype) demonstrates that the *Shank3B*$^{-/-}$mice (black bars) display a reduction in the frequency of social exploratory events when compared to wildtype controls (white bars). * $p<0.05$, ** $p<0.01$, two-tailed *t*-test;all data presented as means ± s.e.m;n=5 mice per group.



Supplementary Figure 6 | Cross-fostering of Shank3B mutants and wildtype mice does not rescue the sociability phenotype in *Shank3B*$^{-/-}$mice and does not impact social interaction and social recognition in wildtype mice.Wildtype and *Shank3B*$^{-/-}$ neonatal pups from homozygous x homozygous mating were cross-fostered at postnatal day 1 and reared by dams of the opposite genotype (wildtype mice with *Shank3B*$^{-/-}$ dams "WT_{cf}" and *Shank3B*$^{-/-}$ mice with wildtype dams "KO_{cf}"). **a,** In the three-chamber social interaction test, cross-fostered *Shank3B*$^{-/-}$ mice (KO_{cf} – black bars) spent less time in the chamber containing the social partner (Stranger 1) and more time in the chamber containing the empty wire cage (Empty Cage), cross-fostered wildtype mice display normal sociability levels (WT_{cf} – white bars). **b,** In the social novelty test, cross-fostered *Shank3B*$^{-/-}$ mice do not display a preference for the novel social partner (Stranger 2). **c, d,** When analyzing social interaction by close proximity (within 5cm) to either "Stranger 1", "Empty Cage" (**c**), or "Stranger 1", "Stranger 2" (**d**), cross-fostered *Shank3B*$^{-/-}$ mice displayed a clear reduction in social interaction(**c**); while under a social novelty paradigm (**d**), cross-fostered *Shank3B*$^{-/-}$ mice displayed a clear reduction in time spent with "Stranger 2". ** $p<0.01$, *** $p<0.001$, One-Way ANOVA, with Bonferroni *post hoct*-test; all data are presented as means ± s.e.m. from 7 mice per group.

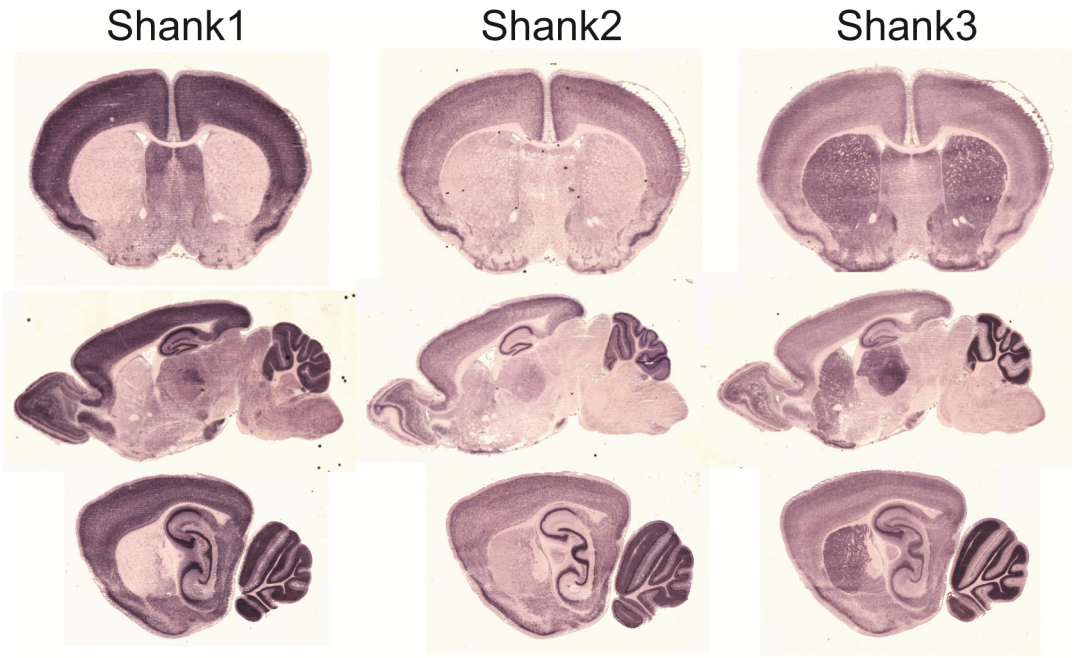


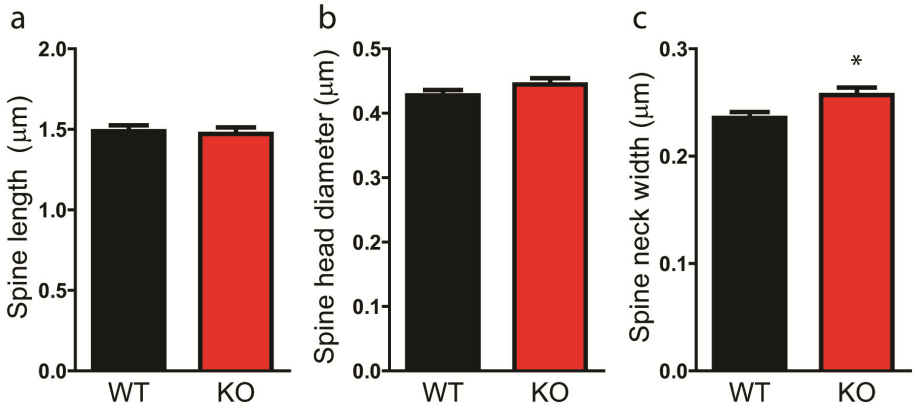
Table 1

Expression of Shank family of proteins in the CNS

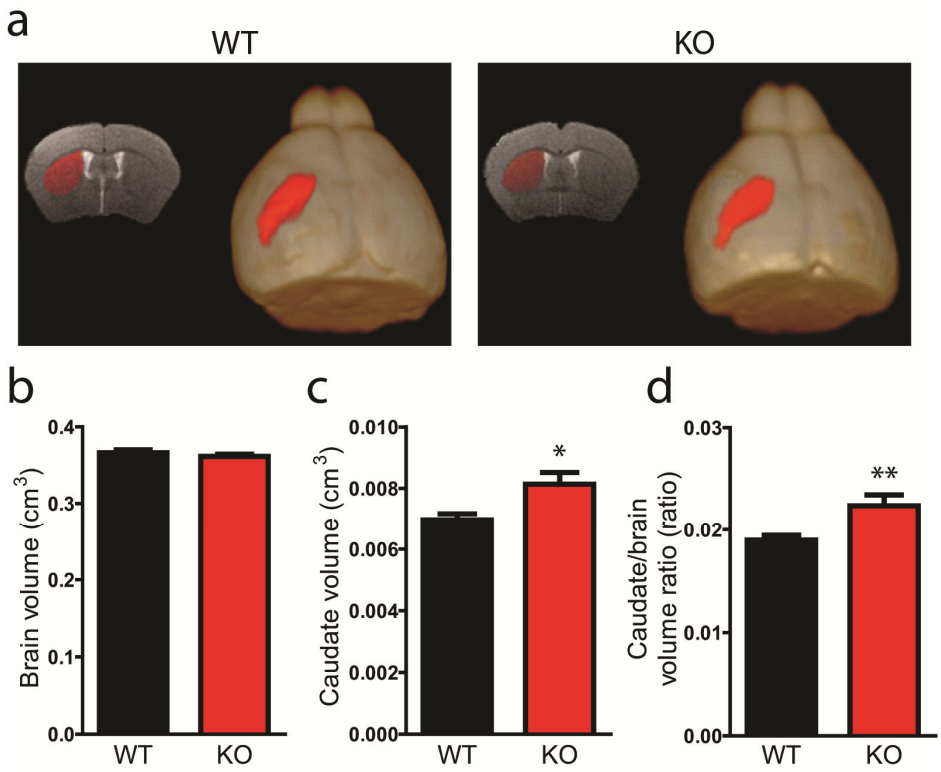
	Shank1	Shank2	Shank3
Cortex	++++++	+++	+++
Thalamus	+++	+	+++++
Striatum	-	-	+++++
Amygdala	+++	+	+
Hippocampus	+++++	+++	+++
CA1	+++++	+++	++
CA3	++++	+++	++++
Dentate Gyrus	+++	+++	+
Cerebellum	+++	+++	+++
Purkinje cells	++++	+++++	-
Granule cells	++	-	++++
Colliculus	++	-	+
Brain Stem	++	-	+
Dendritically targeted	Yes	No	Yes

Supplementary Figure 7 | mRNA *in situ* characterization of *Shank* family of genes. a, *Shank1*, *Shank2* and *Shank3* mRNA *in situ* hybridization of coronal, medial parasagittal and lateral parasagittal mouse brain section from 5 week old mice. *Shank3* is the only Shank family member highly expressed in the striatum.

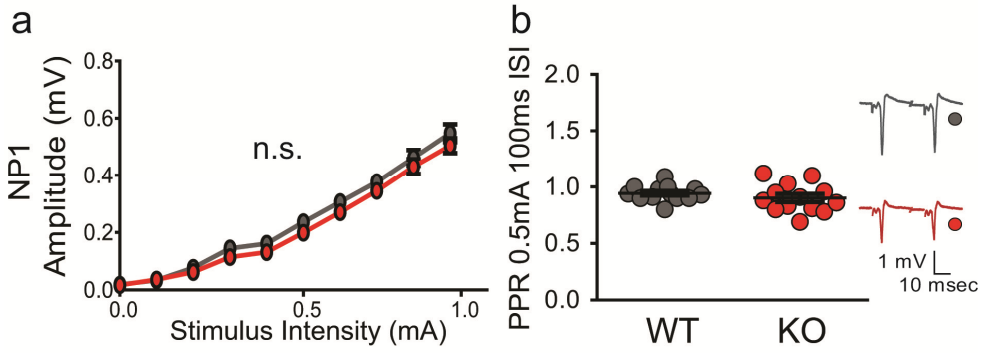
Supplementary Table 1 | Expression of *Shank* family of proteins in the CNS.Relative expression levels of *Shank1, Shank2* and *Shank3* in different areas of the central nervous system in serial sections from 5 week old mice (++++++ highest expression; - no detectable expression).



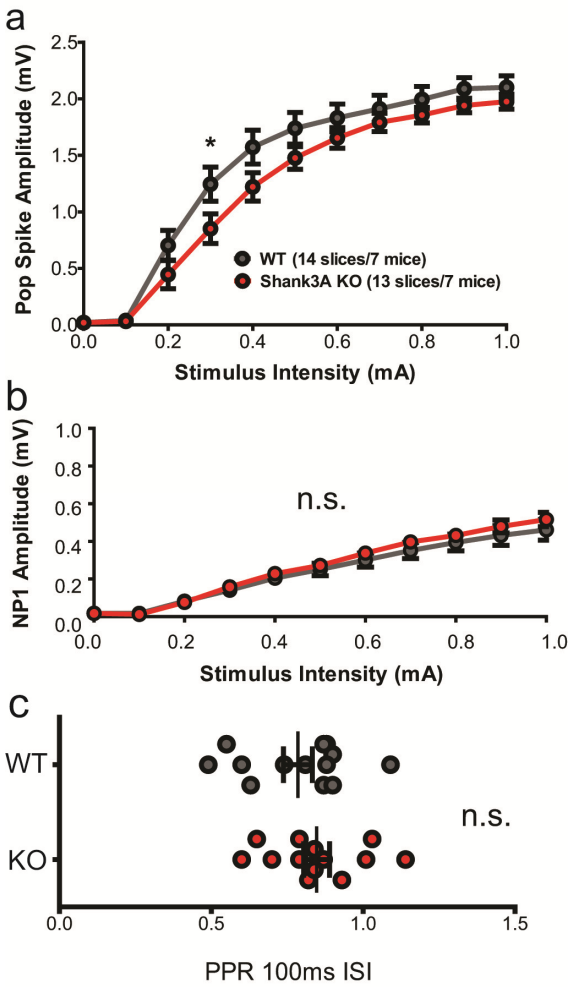
Supplementary Figure 8 | Morphological spine metrics from *Shank3B*$^{-/-}$ and control MSNs. a-c, Spine length and spine head diameter are not significantly different between *Shank3B*$^{-/-}$and wildtype medium spiny neurons (**a, b)**, whereas spine neck width is larger in *Shank3B*$^{-/-}$spines (**c**). * p<0.05, two-tailed *t*-test; all data are presented as means ± s.e.m; n = 140 spines per genotype.



Supplementary Figure 9 | Increased striatal volume in *Shank3B*$^{-/-}$mice.a-c, Striatum and whole brain volumes measured by MRI. **a,** A 3D reconstruction of the brain is used for striatal and whole brain volumetric analysis as depicted for both WT and KO. **b,** Whole brain volume is not significantly different between control (WT) and *Shank3B*$^{-/-}$ mice (KO). **c,** Striatal volume is significantly larger in the *Shank3B*$^{-/-}$ mice. **d**, Striatum/brain volume ratio is increased in the *Shank3B*$^{-/-}$ mice.* p<0.05, ** p<0.01, two-tailed *t*-test. Data are presented as means ± s.e.m, n = 12 mice per genotype.

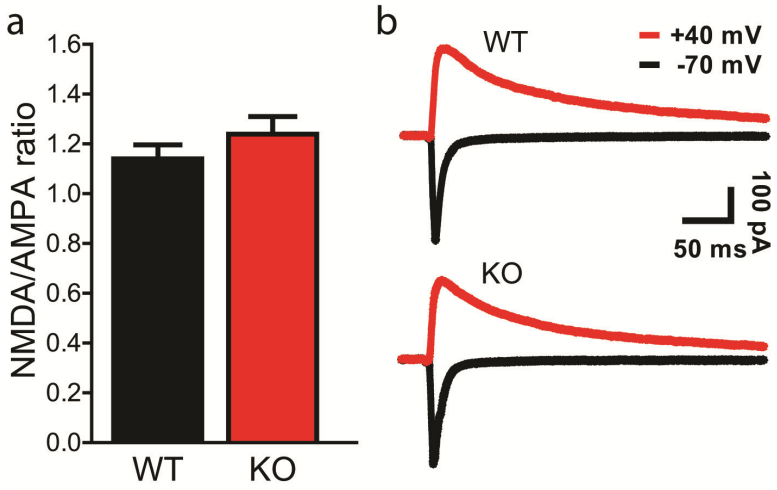


Supplementary Figure 10 | Normal presynaptic function in*Shank3B*$^{-/-}$ mice. Presynaptic function was measured by the relationship of stimulation intensity to the amplitude of the action potential component of the response termed negative peak 1 (NP1, **a**), and the paired-pulse ratios (PPR, **b**). NP1 amplitude (**a**) and PPR (**b**) are not significantly different between *Shank3B*$^{-/-}$ and control mice; example traces are shown as an inset for *Shank3B*$^{-/-}$ mice (red) and wildtype (grey). All data are presented as means ± s.e.m; n=13 slices from 4 mice per group.

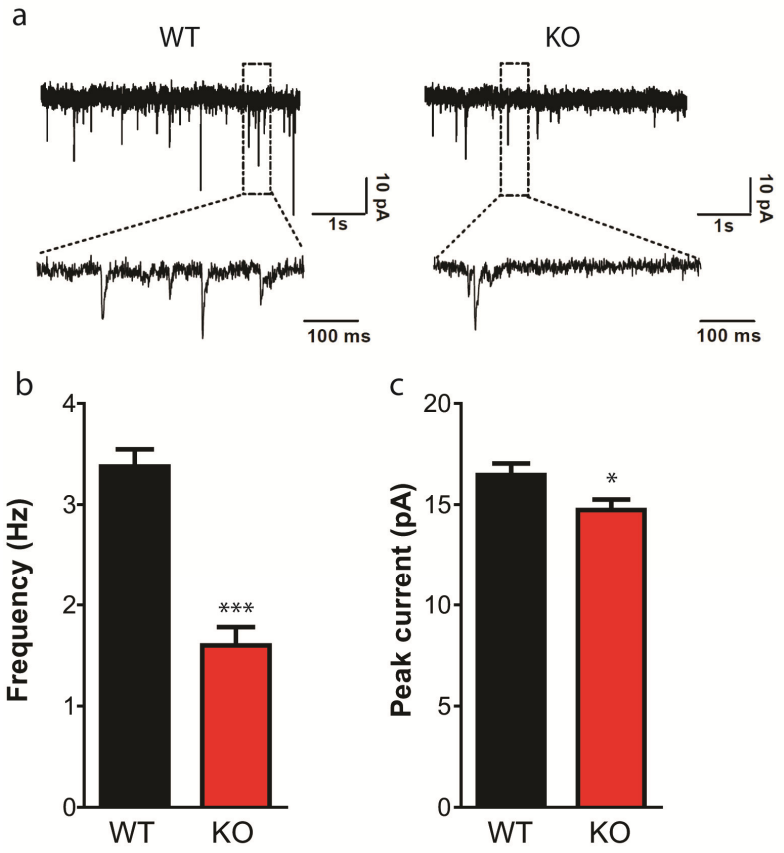


Supplementary Figure 11 | Slight reduction in cortico-striatal synaptic transmission in *Shank3A*$^{-/-}$ mice.a, Cortico-striatal pop spike amplitude is only statistically significantly decreased at one point in the stimulus intensity range for the *Shank3A*$^{-/-}$ mice (red trace) when compared to controls. **b**, **c**, NP1 amplitude (**b**) and PPR (**c**) are not significantly altered between *Shank3A*-mutant and controls. * $p<0.05$; Two-way

repeated measures ANOVA (p>0.05, not statistically significant), with Bonferroni *post hoc* test for **a** and**b;** two-tailed *t*-test for **c**; all data presented as means ± s.e.m; field recordings n=13-14 from 7 mice per group.

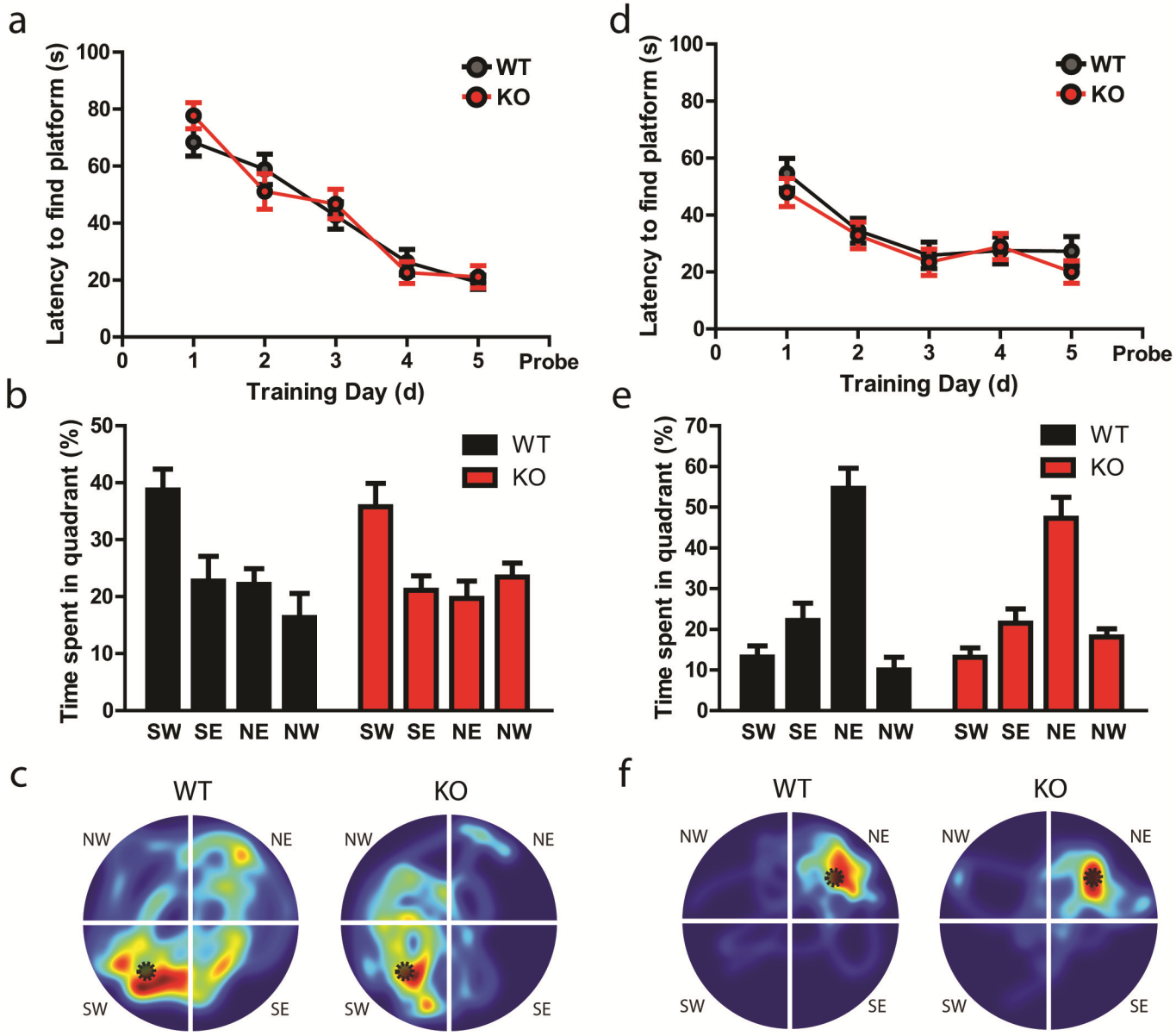


Supplementary Figure 12 | NMDA/AMPA ratio is not changed in *Shank3B*$^{-/-}$ MSNs. a, NMDA/AMPA ratio from MSNs is not changed when comparing *Shank3B*$^{-/-}$to controls. **b,** Example trace recordings from wildtype and *Shank3B*$^{-/-}$ MSNs at a stimulus intensity that evokes a ~300pA AMPA mediated response. Two-tailed *t*-test, data are presented as means ± s.e.m; n=15 from wildtype, n=13 from *Shank3B*$^{-/-}$ mice.

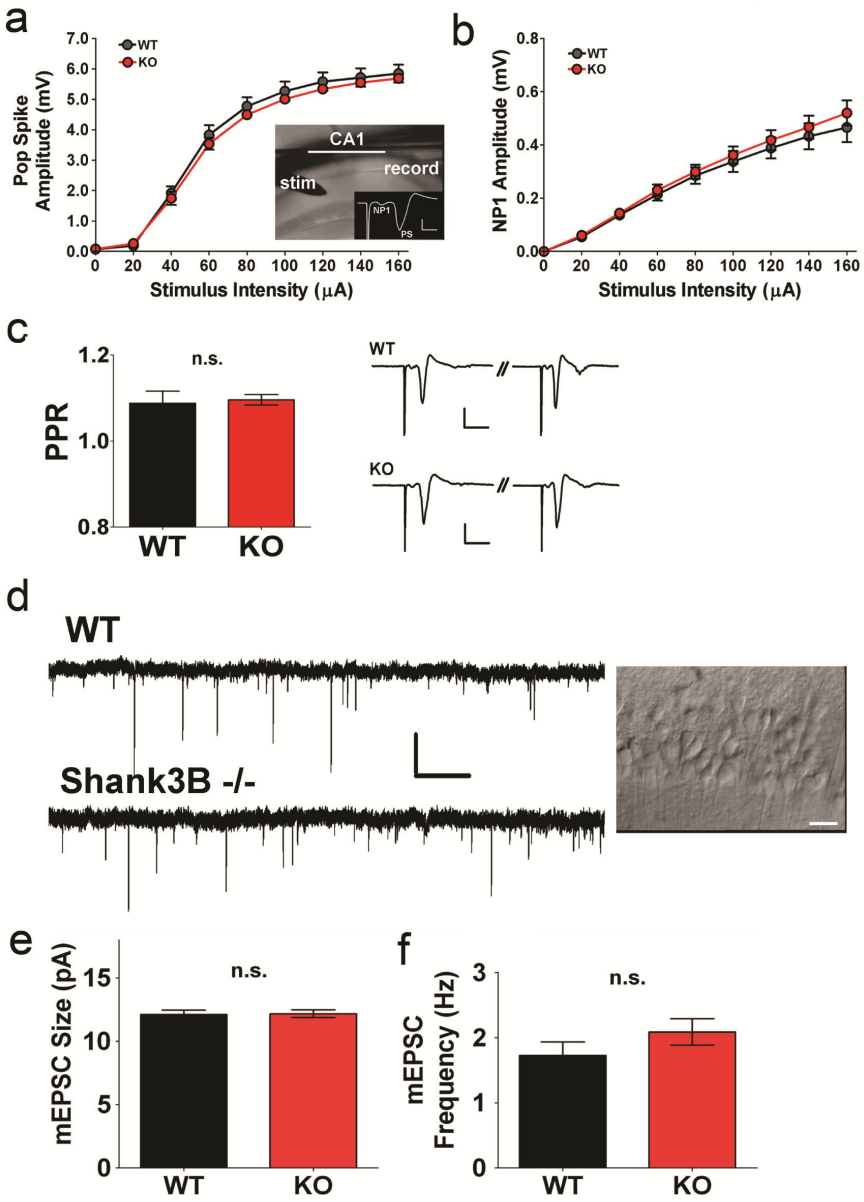


Supplementary Figure 13 | Reduced synaptic transmission inMSNs of *Shank3B*$^{-/-}$mice from heterozygous matings. a, mEPSC example traces fromwildtypeand *Shank3B*$^{-/-}$ MSNs recorded with whole cell voltage clamp. **b**, mEPSC event frequency in *Shank3B*$^{-/-}$ MSNs (red) is lower when compared to

wildtype (black).**c**, Peak current from mEPSC is smaller in*Shank3B*$^{-/-}$ than in wildtype MSNs. * p<0.05, *** p<0.001; unpaired two-tailed *t*-test;n=15cells per genotype; all data presented as means ± s.e.m.



Supplementary Figure 14 | Morris Water Maze learning and memory test in *Shank3B*$^{-/-}$mice.a-b, Latency to find the platform decreases significantly across 5 days of training for both wildtype and *Shank3B*$^{-/-}$mice (**a**). In the probe trial, when compared to controls, *Shank3B*$^{-/-}$ mice spent a similar amount of time in target quadrant (SW) (**b**). **c**, Representative example heat maps from control (WT) and *Shank3B*$^{-/-}$mice (KO) during the probe trial. **d-e,** Latency to find the platform decreases significantly across subsequent 5 days of reversal training (platform switched to NE) for both wildtype and *Shank3B*$^{-/-}$mice (**d**). In the reversal probe trial, when compared to controls, *Shank3B*$^{-/-}$ mice spent a similar amount of time in target quadrant (NE) (**e**). **f**, Representative example heat maps from control (WT) and *Shank3B*$^{-/-}$mice (KO) during the reversal probe trial. Two-way repeated measures ANOVA (effect of genotype not statistically significant, p>0.05), all data are presented as means ± s.e.m; n=9 for wildtype, n=8 for *Shank3B*$^{-/-}$mice.



Supplementary Figure 15 | Hippocampal CA1 pyramidal neuron synaptic function is not significantly altered in *Shank3B*$^{-/-}$ mice. a, No significant difference in input-output functions of hippocampal CA1 population spikes measured from acute brain slices from wildtype (WT – black trace) and *Shank3B*$^{-/-}$(KO – red trace) mice. Inset, example DIC image of an acute hippocampal slice showing stimulation electrode and recordings electrode positioning. Lower right, example trace showing fiber volley component (NP1) and population spike (PS). Scale bars, 2 mV/3 msec. **b**, Fiber volley (NP1) amplitude is not significantly different between *Shank3B*$^{-/-}$and WT slices. $p>0.05$, Two-way repeated measures ANOVA, with Bonferroni *post hoc* test. **c**, Summary data for 100 msec interstimulus interval PPR (100 μA stimulus) of CA1 population spikes. Right, example traces of population spike paired pulses. Scale bars: 2 mV / 10 msec PPR is not significantly altered between *Shank3B*$^{-/-}$ and WT slices. $p>0.05$, two-tailed *t*-test. Data from **a-c** are presented as means ± s.e.m; field recordings n=10 slices from 4 mice per group. **d**, Example traces of mEPSCs recorded from CA1 pyramidal neurons (WT – top trace, *Shank3B*$^{-/-}$ – bottom trace). Scale bars: 20 pA / 2 sec. Right inset, 40X IR-DIC image of a recorded CA1 pyramidal neuron. Scale bar: 20 μm. **e**, Summary data for mEPSC size. **f**, summary data for mEPSC frequency. Whole-cell recordings, n=20 neurons for WT and n=20 neurons for *Shank3B*$^{-/-}$. n.s., not significant, two-tailed *t*-test; data are presented as means ± s.e.m.

Supplementary Table 2

Shank3B — Summary of behavioural statistical data

Test	Duration	Measurement	# of animals	Values	Statistical Test	p value	*Post hoc* test	p value	Figure
Lesion Score	-	Presence/Absence of skin lesions	WT=38 KO=29	WT=0/38 KO=10/19	Fisher's exact test	< 0.0001***	-	-	-
Grooming Score	2 hours	Time spent grooming (% of total)	WT=6 KO=6	WT = 16.24 ± 1.252 KO = 26.83 ± 1.142	Unpaired two-tailed t-test	< 0.0001***	-	-	1d
Open Field	30 minutes (5 minute bins)	Vertical beam-breaks	WT = 9 KO = 9	5min - WT = 46.78 ± 12.92 KO = 8.333 ± 2.646	Two-way repeated measure ANOVA	Genotype < 0.05* Time bin <0.001***	Unpaired two-tailed t-test (WT v KO)	< 0.05*	1e
		Vertical beam-breaks		10min - WT = 35.78 ± 11.12 KO = 4.556 ± 1.709				< 0.05*	1e
		Vertical beam-breaks		15min - WT = 33.00 ± 9.814 KO = 7.667 ± 2.522				< 0.05*	1e
		Vertical beam-breaks		20 min - WT = 22.33 ± 6.451 KO = 5.333 ± 2.147				< 0.05*	1e
		Vertical beam-breaks		25 min - WT = 22.89 ± 11.03 KO = 3.889 ± 1.679				> 0.05	1e
		Vertical beam-breaks		30 - WT = 18.89 ± 8.448 KO = 4.889 ± 2.214				> 0.05	1e
	30 minutes	Total distance travelled (m)		WT = 29.34 ± 3.552 KO = 24.67 ± 3.111	Unpaired two-tailed t-test	> 0.05	-	-	Supplementary Fig 2b
		Thigmotaxis (s)		WT = 1758 ± 17.22 KO = 1770 ± 12.17	Unpaired two-tailed t-test	> 0.05	-	-	Supplementary Fig 2c
Zero Maze	5 minutes	Time spent in open arm (% of total)	WT = 9 KO = 9	WT = 31.46 ± 6.802 KO = 14.66 ± 2.740	Unpaired two-tailed t-test	< 0.05*	-	-	1f
Light-Dark box	5 minutes	Duration in Light (s)	WT = 8 KO = 8	WT = 113.8 ± 6.401 KO = 107.3 ± 12.72	Unpaired two-tailed t-test	> 0.05	-	-	Supplementary Fig 2e
		Duration in Dark (s)		WT = 187.3 ± 6.396 KO = 193.8 ± 12.69	Unpaired two-tailed t-test	> 0.05	-	-	Supplementary Fig 2e
		Latency to Light (s)		WT = 9.513 ± 1.494 KO = 22.83 ± 4.837	Unpaired two-tailed t-test	< 0.05*	-	-	Supplementary Fig 2d

Social Interaction	5 minutes	Time in compartment (s)	WT=12 KO=14	WT - Stranger 1 = 167 ± 16.65 Middle = 59.15 ± 6.77 Empty = 73.74 ± 15.27 KO - Stranger 1 = 88.01 ± 18.42 Middle 65.98 ± 7.47 Empty = 145.9 ± 16.63	One-way ANOVA	< 0.0001***	Bonferroni's Multiple Comparison Test (Strg1 v Empty)	< 0.0001*** < 0.01**	2b 2b
Social Novelty	5 minutes	Time in compartment (s)		WT - Stranger 1 = 100.3 ± 16.85 Middle 42.83 ± 4.765 Stranger 2 = 156.7 ± 15.51 KO - Stranger 1 = 96.8 ± 23.15 Middle = 88 ± 19.8 Stranger 2 = 115 ± 22.49	One-way ANOVA	< 0.01**	Bonferroni's Multiple Comparison Test (Strg1 v Strg 2)	< 0.05* > 0.05	2c 2c
Social Interaction	5 minutes	Time spent in close proximity (s)		Stranger 1 - WT = 132.7 ± 15.46 KO = 62.06 ± 11.72 Empty Cage WT = 47.95 ± 8.743 KO = 100.5 ± 14.53	One-way ANOVA	< 0.0001***	Bonferroni's Multiple Comparison Test (Strg 1 v Strg 1) (Empty v Empty)	< 0.0001*** < 0.05*	2d 2d
Social Novelty	5 minutes	Time spent in close proximity (s)		Stranger 1 - WT = 60.39 ± 12.27 KO = 35.59 ± 11.43 Stranger 2 - WT = 83.21 ± 16.46 KO = 40.68 ± 10.52	One-way ANOVA	< 0.05*	Bonferroni's Multiple Comparison Test (Strg1 v Strg 1) (Strg2 v Strg 2)	> 0.05 < 0.05*	2e 2e
Dyadic Test	5 minutes	Time spent in reciprocal social interaction (s)	WT = 5 KO = 5	WT = 87.28 ± 11.02 KO = 31.30 ± 4.389	Unpaired two-tailed t-test	< 0.01**	-	-	Supplementary Fig 5a
		Frequency of nose-to-nose sniffing		WT = 22.80 ± 4.05 KO = 11.60 ± 2.09	Unpaired two-tailed t-test	< 0.05*	-	-	Supplementary Fig 5b
		Frequency of anogenital sniffing initiated by the test animal		WT = 10.20 ± 1.53 KO = 1.80 ± 0.8	Unpaired two-tailed t-test	< 0.01**	-	-	Supplementary Fig 5c

Social Interaction (Cross Fostering)	5 minutes	Time in compartment (s)	WT=7 KO=7	WT - Stranger 1 = 183.3 ± 19.61 Middle = 38.24 ± 5.38 Empty = 78.31 ± 15.86 KO - Stranger 1 = 40.00 ± 14.50 Middle = 73.29 ± 29.01 Empty = 186.6 ± 36.07	One-way ANOVA	< 0.01**	Bonferroni's Multiple Comparison Test (Strg1 v Empty)	< 0.01** < 0.001***	Supplementary Figure 6a Supplementary Figure 6a
Social Novelty (Cross Fostering)	5 minutes	Time in compartment (s)		WT - Stranger 1 = 93.06 ± 17.81 Middle = 30.32 ± 6.31 Stranger 2 = 176.40 ± 19.19 KO - Stranger 1 = 111.7 ± 26.69 Middle = 64.45 ± 9.59 Stranger 2 = 123.8 ± 33.20	One-way ANOVA	< 0.001***	Bonferroni's Multiple Comparison Test (Strg1 v Strg 2)	< 0.01** > 0.05	Supplementary Figure 6b Supplementary Figure 6b
Social Interaction (Cross Fostering)	5 minutes	Time spent in close proximity (s)		Stranger 1 - WT = 145.4 ± 23.84 KO = 11.70 ± 6.53 Empty Cage WT = 38.20 ± 9.11 KO = 93.67 ± 21.75	One-way ANOVA	< 0.0001***	Bonferroni's Multiple Comparison Test (Strg 1 v Strg 1) (Empty v Empty)	< 0.001*** > 0.05	Supplementary Figure 6c Supplementary Figure 6c
Social Novelty (Cross Fostering)	5 minutes	Time spent in close proximity (s)		Stranger 1 - WT = 47.23 ± 11.50 KO = 38.40 ± 15.43 Stranger 2 - WT = 100.3 ± 24.77 KO = 18.32 ± 5.59	One-way ANOVA	< 0.05*	Bonferroni's Multiple Comparison Test (Strg1 v Strg 1) (Strg2 v Strg 2)	> 0.05 < 0.01**	Supplementary Figure 6d Supplementary Figure 6d
Morris Water Maze - Training	Maximum duration of 90 seconds per trial	Latency to Platform (Day 1; 4 trials per day) Latency to Platform (Day 2; 4 trials per day) Latency to Platform (Day 3; 4 trials per day) Latency to Platform (Day 4; 4 trials per day) Latency to Platform (Day 5; 4 trials per day)		WT = 65.07 ± 5.314 KO = 77.69 ± 4.598 WT =58.85 ± 5.339 KO = 51.11 ± 6.253 WT =42.65 ± 4.795 KO = 46.7 ± 5.143 WT =26.3 ± 4.49 KO = 22.66 ± 3.825 WT =19.22 ± 2.492 KO = 21.12 ± 3.885	Two-way repeated measure ANOVA	Effect of Training p < 0.0001*** Effect of Genotype p > 0.05	Bonferroni's Multiple Comparison Test (WT v KO)	> 0.05 > 0.05 > 0.05 > 0.05 > 0.05	Supplementary 14a

Morris Water Maze - Reversal Training	Maximum duration of 90 seconds per trial	Latency to Platform (Day 1; 4 trials per day)	WT=9 KO=8	WT =54.62 ± 5.228 KO = 47.95 ± 4.954	Two-way repeated measure ANOVA	Effect of Training p < 0.0001*** Effect of Genotype p > 0.05	Bonferroni's Multiple Comparison Test (WT v KO)	> 0.05	Supplementary 14b
		Latency to Platform (Day 2; 4 trials per day)		WT =34.52 ± 4.376 KO =32.87 ± 4.662				> 0.05	
		Latency to Platform (Day 3; 4 trials per day)		WT =25.85 ± 4.683 KO = 23.43 ± 4.614				> 0.05	
		Latency to Platform (Day 4; 4 trials per day)		WT =27.56 ± 4.697 KO = 28.96 ± 4.588				> 0.05	
		Latency to Platform (Day 5; 4 trials per day)		WT =27.3 ± 5.131 KO = 20 ± 3.959				> 0.05	
Morris Water Maze - Probe Trial	60 seconds	Percent of Time in SW Quadrant		WT = 38.74 ± 3.65 KO = 35.76 ± 4119	Two-way repeated measure ANOVA	Effect of Quadrant p < 0.001*** Effect of Genotype p > 0.05	Bonferroni's Multiple Comparison Test (WT v KO)	> 0.05	Supplementary 14c
		Percent of Time in SE Quadrant		WT = 22.71 ± 4.364 KO = 21.12 ± 2.515				> 0.05	
		Percent of Time in NE Quadrant		WT = 22.16 ± 2.733 KO = 19.69 ± 3.022				> 0.05	
		Percent of Time in NW Quadrant		WT = 16.37 ± 2.733 KO = 19.69 ± 3.022				> 0.05	
Morris Water Maze - Reversal Probe Trial	60 seconds	Percent of Time in SW Quadrant		WT = 13.17 ± 2.732 KO = 13.08 ± 2.334		Effect of Training p < 0.0001*** Effect of Genotype p > 0.05	Bonferroni's Multiple Comparison Test (WT v KO)	> 0.05	Supplementary 14d
		Percent of Time in SE Quadrant		WT = 22.16 ± 4.225 KO = 21.52 ± 3.483				> 0.05	
		Percent of Time in NE Quadrant		WT = 54.65 ± 4.897 KO = 47.3 ± 5.13				> 0.05	
		Percent of Time in NW Quadrant		WT = 10.01 ± 3.081 KO = 18.09 ± 2.024				> 0.05	
Rotorod	Trial 1	Latency to fall (s)	WT=5 KO=5	WT = 116.8 ± 13.92 KO = 111.8 ± 18.59	Two-way repeated measure ANOVA	Effect of Trial p < 0.05* Effect of Genotype p > 0.05	Bonferroni's Multiple Comparison Test (WT v KO)	> 0.05	Supplementary Fig 2a
	Trial 2	Latency to fall (s)		WT = 117.4 ± 11.86 KO = 120 ± 28.6				> 0.05	
	Trial 3	Latency to fall (s)		WT = 139.4 ± 10.51 KO = 141 ± 16.83				> 0.05	
Swim Speed	Trial 1 of MWM	Velocity (cm/s)	WT=9 KO=8	WT = 17.94 ± 0.8249 KO = 18.47 ± 1.268	Unpaired two-tailed t test	> 0.05	-	-	-

Shank3A

Test	Duration	Measurement	# of animals	Values	Statistical Test	p	*Post hoc* test	p	Figure
Open Field	30 minutes (5 minute bins)	Vertical beam-breaks	WT = 8 KO = 8	5min - WT = 33.44 ± 7.627 KO = 40.56 ± 4.187	Two-way repeated measure ANOVA	Time bin <0.05* Genotype p > 0.05	Unpaired two-tailed t-test (WT v KO)	> 0.05	Suplementary 3a
				10min - WT = 22.33 ± 3.603 KO = 32.11 ± 5.16				> 0.05	
				15min - WT = 22.67 ± 3.403 KO = 24.11 ± 3.486				> 0.05	
				20min - WT = 24.11 ± 3.506 KO = 17.33 ± 4.282				> 0.05	
				25min - WT = 30.44 ± 5.409 KO = 21.22 ± 2.093				> 0.05	
				30min - WT = 30.33 ± 5.6 KO = 24.78 ± 5.23				> 0.05	
	30 minutes	Total distance travelled (m)		WT = 45.62 ± 3.459 KO = 48.82 ± 3.02	Unpaired two-tailed t-test	> 0.05	-	-	Suplementary 3b
		Thigmotaxis (s)		WT = 1673 ± 23.58 KO = 1676 ± 23.6	Unpaired two-tailed t-test	> 0.05	-	-	Suplementary 3c
Zero Maze	5 minutes	Time spent in open arm (% of total)	WT = 8 KO = 8	WT = 24.35 ± 2.809 KO = 21.02 ± 2.951	Unpaired two-tailed t-test	> 0.05	-	-	Suplementary 3d
Social Interaction	5 minutes	Time in compartment		WT - Stranger 1 = 172 ± 13.72 Middle = 41.12 ± 3.781 Empty = 86.32 ± 12.43	One-way ANOVA	< 0.0001***	Bonferroni's Multiple Comparison Test (Strg 1 v Empty)	< 0.0001***	Suplementary 4b
				KO - Stranger 1 = 151 ± 10.73 Middle 55.69 ± 5.847 Empty = 93 ± 8.178				< 0.01**	
Social Novelty	5 minutes	Time in compartment	WT=12 KO=13	WT - Stranger 1 = 104.5 ± 13.57 Middle 31.7 ± 4.527 Stranger 2 = 163.6 ± 17.31	One-way ANOVA	< 0.0001***	Bonferroni's Multiple Comparison Test (Strg 1 v Strg 2)	< 0.05*	Suplementary 4c
				KO - Stranger 1 =120.6 ± 15.79 Middle = 46.36 ± 7.503 Stranger 2 = 132.7 ± 20.03				> 0.05	

Social Interaction	5 minutes	Time Spent in close proximity	WT - Stranger 1 = 133.8 ± 14.52 Empty = 52.77 ± 8.891 KO - Stranger 1 = 105.6 ± 10.97 Empty = 52.52 ± 4.811	One-way ANOVA	< 0.0001***	Bonferroni's Multiple Comparison Test (Strg 1 v Empty)	< 0.0001*** < 0.001***	Suplementary 4d
Social Novelty	5 minutes	Time Spent in close proximity	WT - Stranger 1 = 54.25 ± 11.22 Stranger 2 = 105.9 ± 18.4 KO - Stranger 1 = 48.39 ± 8.624 Stranger 2 = 70.87 ± 13.83	One-way ANOVA	< 0.05*	Bonferroni's Multiple Comparison Test (Strg 1 v Strg 2)	< 0.05* > 0.05	Suplementary 4e