Supplemental Figures and Tables

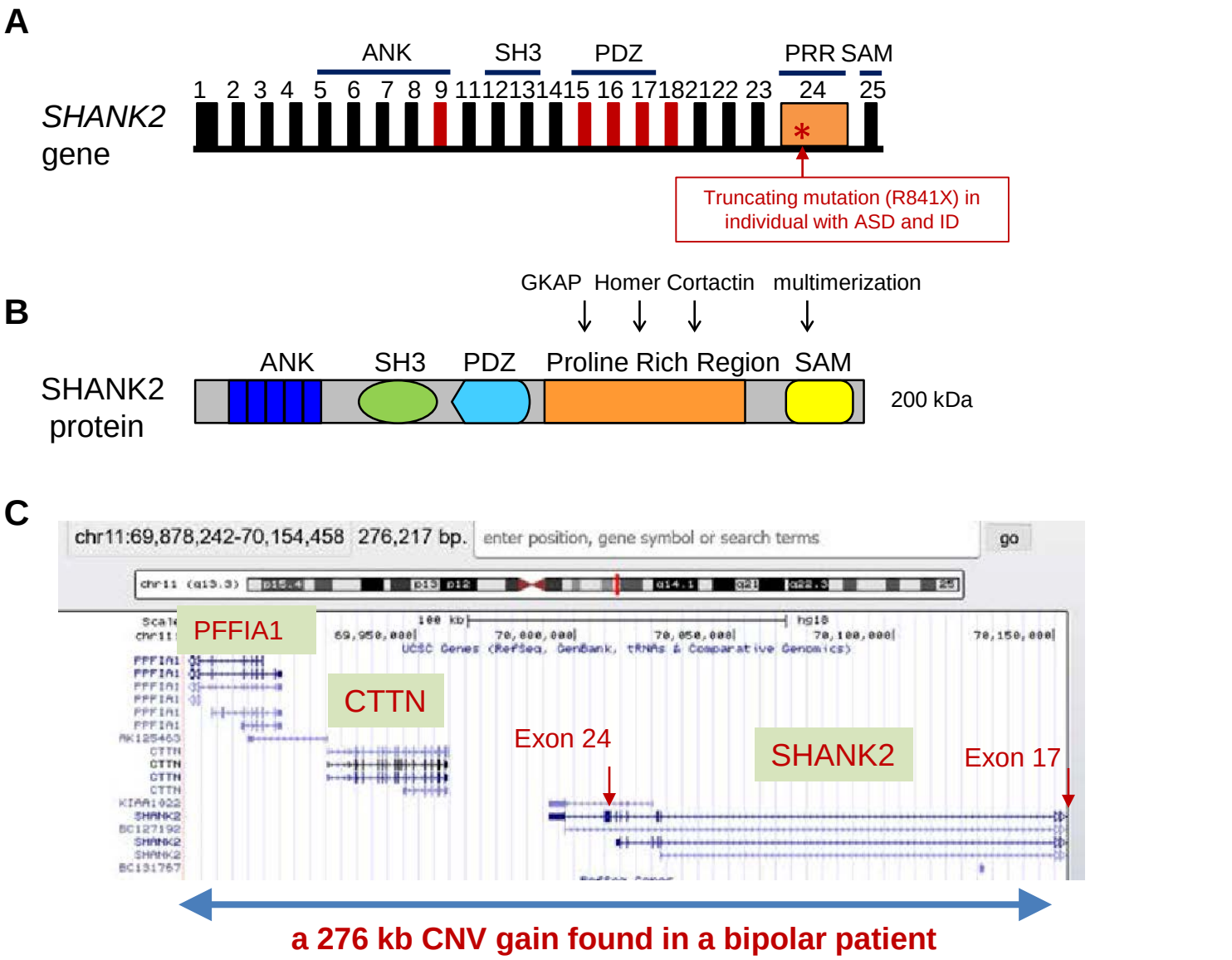
Deficiency of *Shank2* Causes Mania-like Behavior That Responds to Mood Stabilizers

Andrea L. Pappas[1], Alexandra L. Bey[1], Xiaoming Wang[2], Mark Rossi[1], Yong Ho Kim[5], Hai Dun Yan[2], Lara J. Duffney[2], Samantha M. Phillips[2], Fiona Porkka[3], Xinyu Cao[1], Jin-dong Ding[9], Ramona M. Rodriguiz[3], Henry Yin[1,4], Richard J. Weinberg[9], Ru-Rong Ji[1,5], William C. Wetsel[1,3,6,7], Yong-hui Jiang[1,2,6,7,8*].

Departments of [1]Neurobiology, [2]Pediatrics, [3]Psychiatry and Behavioral Sciences, [4]Psychology and Neuroscience, [5]Anesthesiology, [6]Cell Biology, [7]Duke Institute of Brain Science, [8]Genomics and Genetics Graduate Program, Duke University, Durham NC 27710, USA; [9]Department of Cell Biology and Physiology, the University of North Carolina at Chapel Hill, NC 27599, USA

*Correspondence:
Yong-hui Jiang, MD, PhD.
Departments of Pediatrics and Neurobiology
Duke University School of Medicine
Email:yong-hui.jiang@duke.edu
Office: GSRB1 4004
905 S. LaSalle ST, Durham. NC 27710
Phone:919-681-2789
Fax:919-684-8944

Supplemental Figure 1.

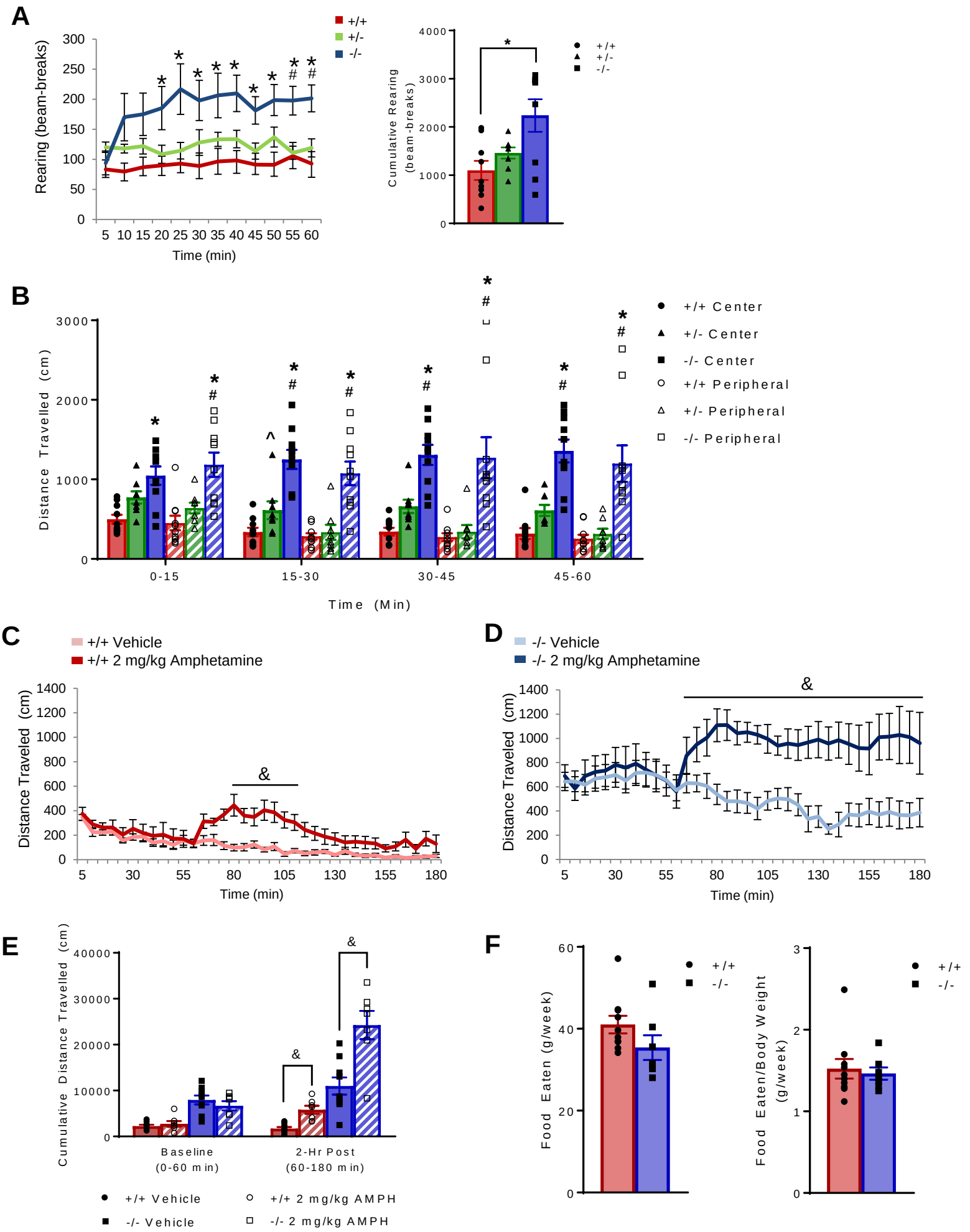


Supplemental Figure 1. *SHANK2* gene, protein, *SHANK2* genetic defects in human, and RNA analysis of Shank2 in Δe24 mutant mice.

A-B. Diagram of the structure of the human *SHANK2* gene and protein. (A) The nonsense mutation R841X is found in an individual with ASD and ID. (B) SHANK2 protein contains 5 conserved domains that include Ankrin repeats (ANK), Src homology 3 domain (SH3), *Drosophila* disk large tumor suppressor (DlgA), and Zonula occludens-1 protein domain (Zo-1) (PDZ), proline-rich region (PRR), and the sterile α-motif domain (SAM). The predicted protein structures and potential binding partners are shown. **C.** A 276 kb duplication (hg18: 6878242-70154458) is reported in an individual with bipolar disorder (Noor et al). The proximal breakpoint is within intron 17 of the *PFFIA1* gene and the distal breakpoint is within the intron 16 of the *SHANK2* gene. *In silico* analysis predicts the disruption of exons 17-25 of *SHANK2* in this patient. **D**. RT-PCR analysis showed that deletion of exon 24 in mice produced a mRNA product with e24 missing *(left panel*) when primers were anchoring exon 18 and exon 25. There is no WT product in Δe24$^{-/-}$ brain tissue (*right panel*) when the primer is located in exon 24 and exon 25. The WT has a 2.8 kb, HET has a 478 kb, and KO has no Shank2 product.

(Noor A, et al. Copy number variant study of bipolar disorder in Canadian and UK populations implicates synaptic genes. *Am J Med Genet B Neuropsychiatr Genet.* 2014;165B(4):303-13.)

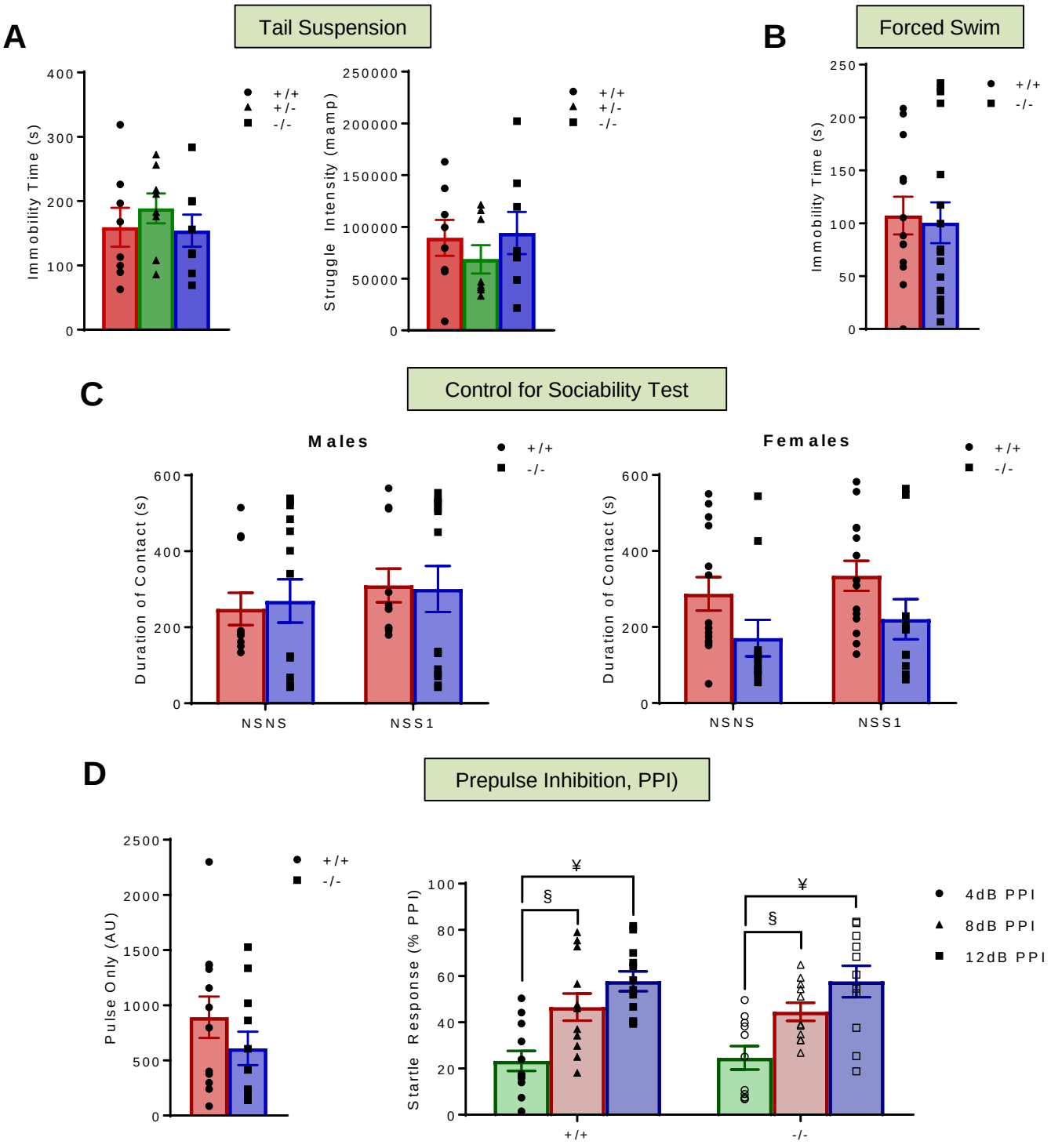
Supplemental Figure 2



Supplemental Figure 2. Shank2 Δe24 mice display increased rearing and enhanced locomotor activity in response to amphetamine.

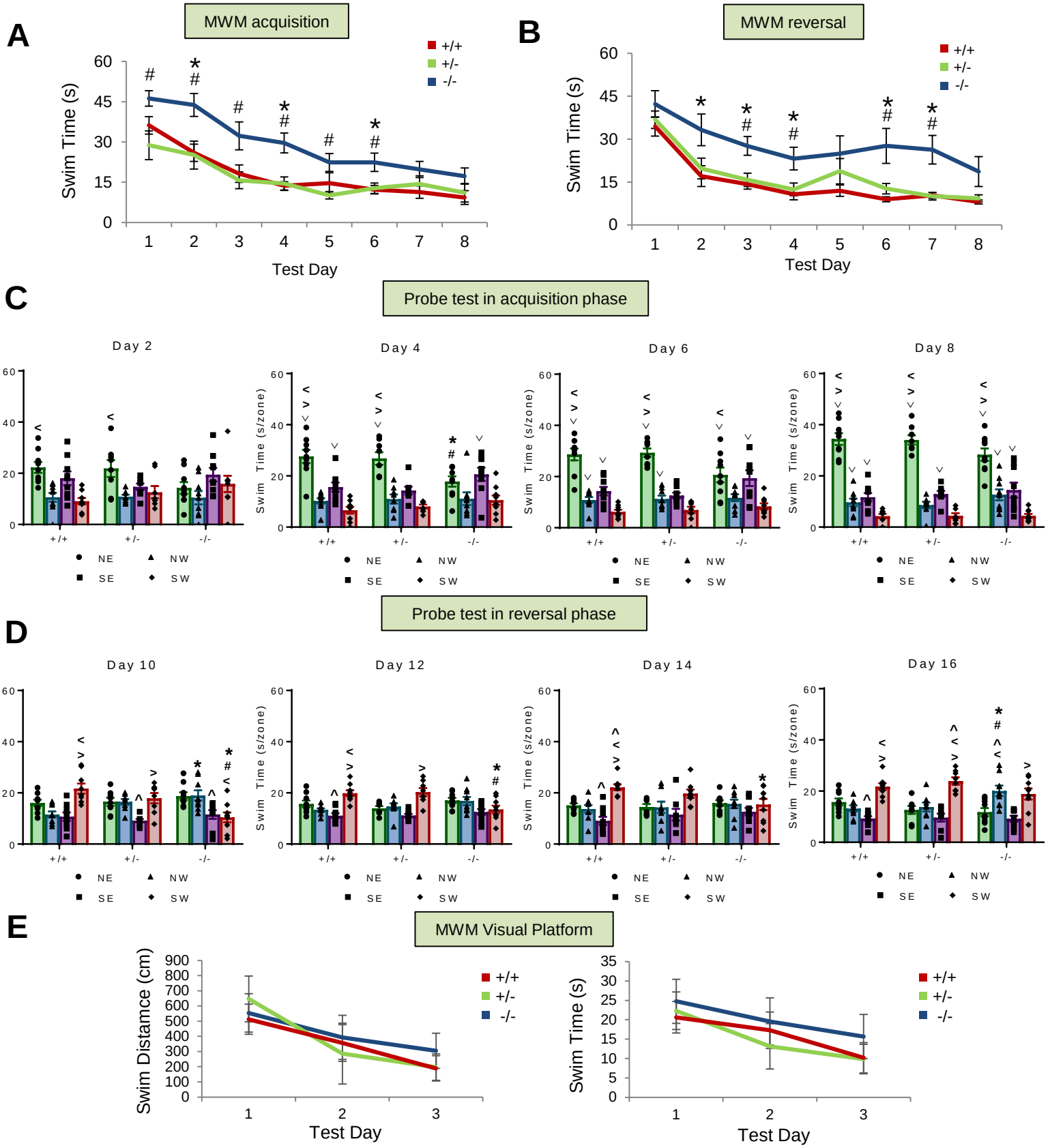
A. Rearing (vertical) activity was significantly increased in $\Delta e24^{-/-}$ mice. **B.** The distance traveled in the center and peripheral zones per 15 minute bin during the 60 minute open field was comparable between $\Delta e24^{-/-}$ and $e24^{+/+}$ mice (N=8-10 mice/genotype; **p*<0.05, +/+ vs -/-; #*p*<0.05, +/- vs -/-, +*p*<0.05, +/+ vs +/-, ^*p*<0.05, +/- Center vs +/- peripheral). **C-E.** Enhanced locomotor activity in response to amphetamine. As expected $e24^{+/+}$ mice responded to amphetamine (AMPH) treatment with increased locomotor activity. The $\Delta e24^{-/-}$ mice had a significantly enhanced response to AMPH. (N=7-9 mice/genotype; &*p*<0.05, for AMPH treated vs Vehicle). **F.** Food intake in $\Delta e24^{-/-}$ mice is similar to $e24^{+/+}$ mice**.** (Mean ± SEM)

Supplementary Figure 3



Supplemental Figure 3. Depressive-like behaviors, social interaction, and PPI were normal in Shank2 Δe24 mice. **A.** In the tail suspension test the Δe24$^{-/-}$ mice spent similar amounts of time immobile (*left*) and engaged in similar levels of struggle activity (*right*) as the other genotypes (N=8 mice/genotype). **B.** In the forced swim test the Δe24$^{-/-}$ mice spent similar times immobile as e24$^{+/+}$ controls (N=13-17 mice/genotype). **C.** Male and female Δe24$^{-/-}$ mice spent similar amounts of total time interacting with the social and non-social stimuli in two phases of the sociability test (N=11-14 mice/genotype/sex). **D**. Δe24$^{-/-}$ mice showed normal startle activity to the 120 dB acoustic stimulus (*right*) and normal PPI *(left*) (N=11-12 mice/genotype). (§ p<0.05, 4 vs 8 db; ¥p<0.05, 4b vs 12 db) (Mean ± SEM)

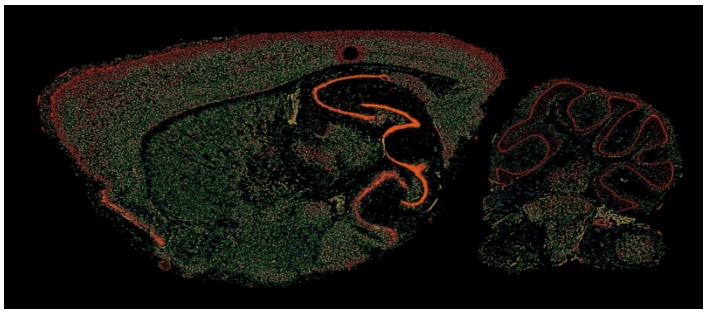
Supplemental Figure 4



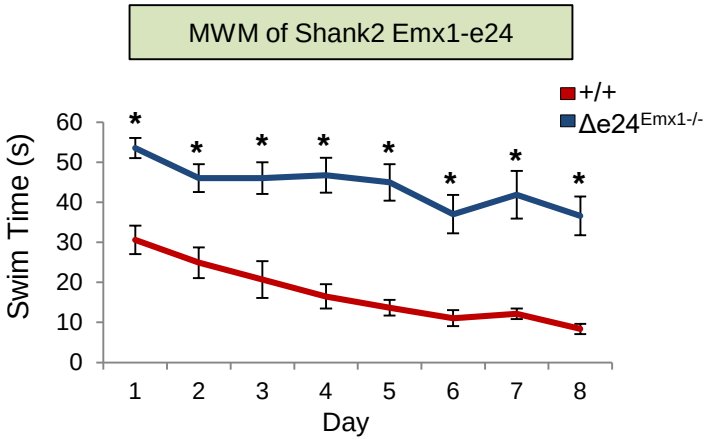
Supplemental Figure 4. Morris water maze performance of *Shank2* Δe24$^{-/-}$ mice. **A-B.** Δe24$^{-/-}$ mice were deficient in spatial learning in the MWM task. Δe24$^{-/-}$ mice took a significantly longer time to find the hidden platform than the Δe24$^{+/-}$ and e24$^{+/+}$ mice in both the acquisition (A) and reversal phases (B) of the test. (*p<0.05, +/+ vs -/-; #p<0.05, +/- vs -/-; N=8-9 mice/genotype). **C-D.** Probe tests in the acquisition phase (C) showed that Δe24$^{-/-}$ mice spent significantly less time in the Northeast (NE) quadrant (platform quadrant) than the e24$^{+/+}$ and e24$^{+/-}$ mice on probe day 4. In the probe test for the reversal phase (D), Δe24$^{-/-}$ mice spent significantly less time in the Southwest (SW) quadrant (platform quadrant) than the e24$^{+/+}$ mice on probe days 10, 12 and 14. Abbreviations: SE, south-east; NW, north-west. (*p<0.05, +/+ vs -/-; #p<0.05, +/- vs -/-; ^p<0.05 vs NE; <p<0.05 vs NW; >p<0.05 vs SE; vp<0.05 vs SW; N=8-9 mice/genotype) **E.** Mice from all three genotypes were able to locate the visible platform over similar distances (*left*) and time (*right*) (N=7-9 mice/genotype).

Supplemental Figure 5

A

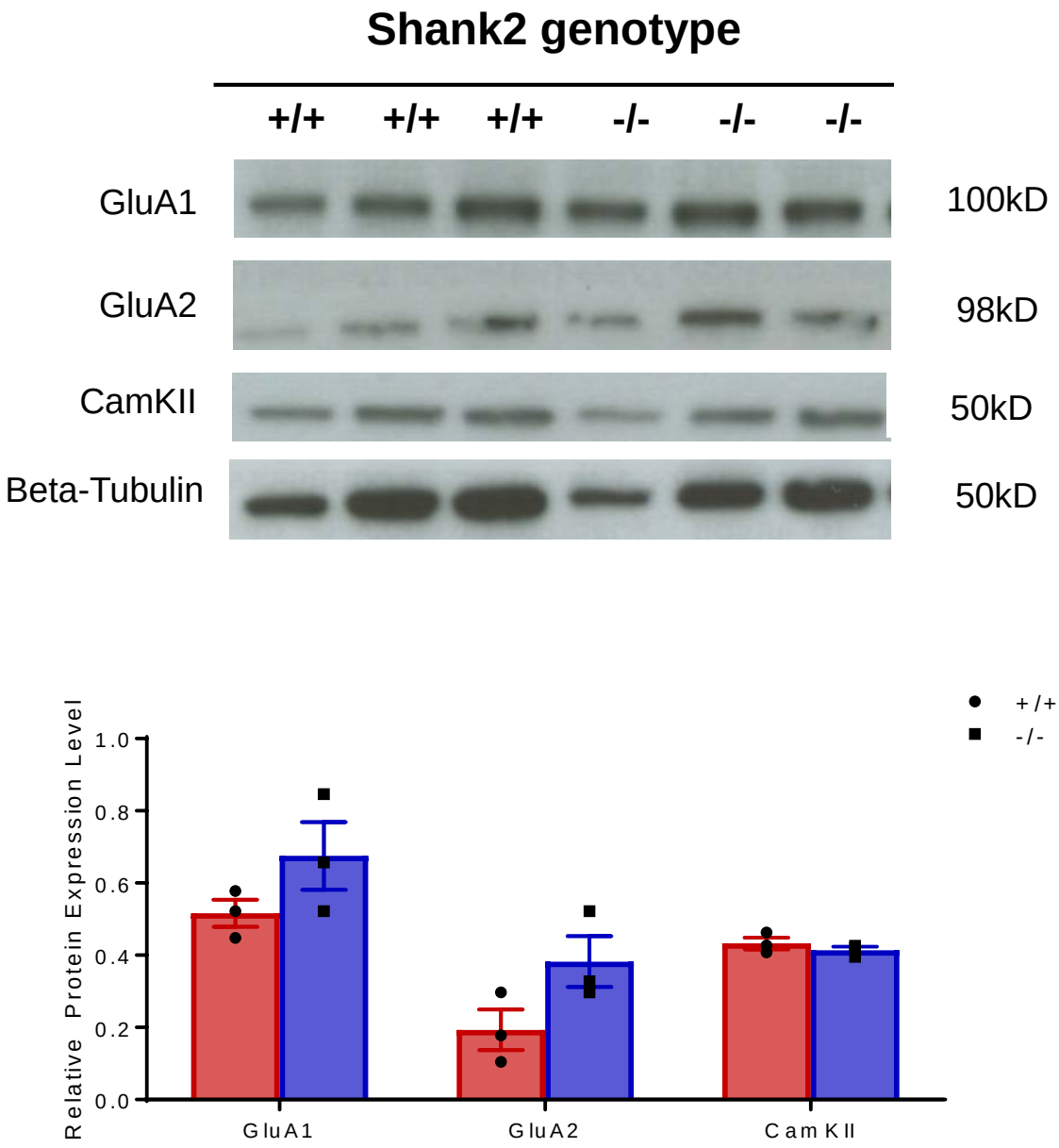


B



Supplemental Figure 5. **A.** Region specific expression of *Shank2* in mouse brain with *in situ* hybridization as displayed in the Allen Brain Atlas. **B.** The $\Delta e24^{Emx1-/-}$ mice were deficient in spatial learning and memory. In the MWM task, $\Delta e24^{Emx1-/-}$ mice took a significantly longer time to find the hidden platform on all 8 days of acquisition testing than the $e24^{+/+}$ mice (*p<0.05, +/+ vs -/-; N=13 mice/genotype).

Supplemental Figure 6.



Supplemental Figure 6. The expression of AMPAR subunits are not significantly different in PSD of hippocampus between Shank2+/+ and Shank2-/-. A. Images of immunoblot for GluA1, GluA2, and CamKII. B. Quantification of GluA1, GluA2, and CamKII normalized to Tubulin

Supplemental Table 1. Cohorts of Shank2 Δe24 mutant mice used for behavioral analyses

Shank2 e24 (M=male and F=female)								
	Cohort 1	Cohort 2	Cohort 3	Cohort 4	Cohort 5	Cohort 6	Cohort 7	Total/Results
Number of animals	-/- F =2 -/- M =7 +/+ F= 6 +/+ M =3 +/- F=5 +/- M=3	-/- F=4 -/- M=3 +/+F= 1 +/+M=6 +/- M=9 +/- F=0	-/- F= 13 -/- M= 11 +/+ F= 11 +/+M= 9	-/- F= 6 -/- M = 4 +/+ F= 9 +/+ M= 8	-/- F = 10 -/- M = 2 +/+ F= 7 +/+ M= 5	-/- F = 5 -/- M = 2 +/+ F= 4 +/+ M= 6	-/-F=14 -/- M=4 +/+ F=13 +/+M=13	-/-F=54 -/-M=32 +/+F=51 +/+M=50 +/- F=5 +/- M=12
PPI					X			No Difference
Elevated Zero Maze	X							Altered
Open field	X	X						Altered
Spray test	X				X			Altered
Hole-board	X	X	X		X			No Difference
Sociability	X		X					Altered
Morris water maze	X							Altered
Rotarod	X				X			Altered
48 hour open field	X	X						Altered
Tail suspension			X					No Difference
Forced swim			X	X				No Difference
Circadian activity				X				Altered
Dyadic social behavior	X	X						Altered
Open field -- amphetamine	X				X			Increased Difference
Open field -- valproic acid			X					Responded
Open field -- lithium			X					Responded
Circadian activity -- lithium			X					Responded
Lever Press						X		Altered
Food Consumption						X		No Difference
Anhedonia							X	Altered

Supplemental Table 2. Primers used for PCR and RT-PCR experiments

	Primer	Sequence	Annealing Temp.
Shank2 Genotyping			
WT band: 571 bp	X - SH2 – genomic forward	5’ AGAAGAGAGCCTCAGTCCCTTAG 3’	58º
FLOX band: 666 bp	Y - SH2 – genomic reverse	5’ GTCCTCAGAAGTGTCTGGCATG 3’	58º
KO band: 951 bp	Z - SH2 – transgenic reverse	5’ TGCCCAGCGGGAGAATAAAG 3’	58º
MIB2 Genotyping			
WT band: 640 bp	Mib2.7842:	5’ GCATCCAGAGCAGGCAAGTTAC 3’	60º
KO band: 450 bp	Neo66:	5’ ATGCTCCAGACTGCCTTG 3’	60º
	Mib2.8481:	5’ GCATTCTGCCATCTCTGCTGGT 3’	60º
Disc Genotyping			
	Disc – forward	5’ GCTGTGACCTGATGGCAGT 3’	58º
	Disc – reverse	5’ GCAAAGTCACCTCAATAACCA 3’	58º
Cre Genotyping			
	Cre – forward	5’ CAACGAGTGATGAGGTTCGCAAG 3'	58º
	Cre – reverse	5' ATATTTACATTGGTCCAGCCACCAGC 3'	58º
Yeast 2 Hybrid Sequencing	Y2H – SP6 forward	5’ TACGATTTAGGTGACACTATAG 3’	58º
	Y2H – M13 reverse	5’ CAGGAAACAGCTATGACCATG	58º
RNA analysis			
KO band 478 bp	RT-PCR Exon – 18 forward	5’ ATGACAGCGGAGTTGGAGGAAC 3’	60º
WT band 2.8 kb	RT-PCR Exon 25 – reverse	5’ GGCAGGATCTCTTTGCTCAATTC 3’	60º
KO band 0 bp	RT-PCR Exon 24 – forward	5’ GGATTGAGGAGGTGGACAGC 3’	58º
WT band 700 bp	RT-PCR Exon 25 – reverse	5’ GGCAGGATCTCTTTGCTCAATTC 3’	58º

Supplemental Table 3. Antibodies used in Western blot experiments

Antibody name	Application	Dilution	Manufacturer
GluA2(L21/32)	Western blot	1:2000	UC Davis/NIH NeuroMab, CA
NR2B(N59/36)	Western blot	1:500	UC Davis/NIH NeuroMab, CA
NR2A	Western blot	1:1000	Millipore, Billerica, MA
NR1	Western blot	1:500	Millipore, Billerica, MA
GluA1(C-terminal)	Western blot	1:1000	Abcam, Cambridge, MA
Actin	Western blot	1:3000	Santa cruz biotechnology, Santa Cruz, CA .
SHANK2 (H-150)	Western blot	1:250	Santa cruz biotechnology, Santa Cruz, CA.
NR2C	Western blot	1:500	ABCAM, Cambridge, MA
NR2D	Western Blot	1:3000	Millipore, Bilerica MA
CamKII	Western blot	1:500	UC Davis/NIH NeuroMab, CA

Supplemental Table 4: Comparison of Shank2 Δe24 and other Shank2 mutant mice

	Shank2 Δe24	Shank2 Δe6*	Shank2 Δe6-7#	Shank2 e6/floxed L7-Cre §	Shank2 e6-7/floxed Pcp2-Cre ¶
Behaviors					
Locomotor activity					
Open field 60min	Increased	Increased	Increased	NT	Not increased
Open field (48 hours)	Increased	NT	NT	NT	NT
Zero maze	Increased time in open arms	NT	NT	NT	NT
Elevated plus maze		Decreased time in open arms	NT	NT	NT
Hole board	Normal	NT	NT	NT	Abnormal
Grooming	Normal	Increased	Normal	NT	Normal
MWM: acquisition	Impaired	Impaired	NT	NT	NT
reversal	Impaired	NT	NT	NT	NT
Social Affiliation (3 chamber test)	Impaired in female	Impaired	Impaired	Impaired	Normal
Social dyadic	Impaired	NT	NT	NT	NT
Rotarod	Impaired	Normal	NT	NT	NT
Running wheel	Abnormal	NT	NT	NT	NT
Lever press	Increased food rewarding	NT	NT	NT	NT
USV	NT	Abnormal	Abnormal	NT	Normal
Light dark	NT	Abnormal	Normal	NT	Abnormal
Novel objective recognition	NT	Normal	Normal	NT	NT

Synaptic Protein	**Hippocampus**	**Hippocampus**	**Whole brain**	**Cerebellum**	**Cerebellum**
NR1	Reduced	Normal	Reduced	NT	Normal
NR2A	Reduced	Increased	Reduced	NT	Normal
NR2B	Increased	Increased	Reduced	NT	Normal
Synaptic function					
NMDAR function in CA1	Reduced NMDR Current	Enhanced NMDA/ AMPA ratio current	Reduced NMDA/AMPA ratio	Increased sIPSC	Reduced mEPSC, increased AMPA ePSC, reduced NMDA/AMPA ratio
Synaptic plasticity in CA1	NT	Enhanced LTP	Reduced LTP Reduced LTD	Reduced LTP	NT

*, Schmeisser., et al. (2012). Autistic-like behaviours and hyperactivity in mice lacking ProSAP1/Shank2. Nature 486, 256-260.
#Won, et al. (2012). Autistic-like social behaviour in Shank2-mutant mice improved by restoring NMDA receptor function. Nature 486, 261-265
§, Ha, S., Lee, D., Cho, Y.S., Chung, C., Yoo, Y.E., Kim, J., Lee, J., Kim, W., Kim, H., Bae, Y.C., et al. (2016). Cerebellar Shank2 Regulates Excitatory Synapse Density, Motor Coordination, and Specific Repetitive and Anxiety-Like Behaviors. J. Neurosci. 36, 12129-12143
¶, Peter, S., Ten Brinke, M.M., Stedehouder, J., Reinelt, C.M., Wu, B., Zhou, H., Zhou, K., Boele, H.J., Kushner, S.A., Lee, M.G., et al. (2016). Dysfunctional cerebellar Purkinje cells contribute to autism-like behaviour in Shank2-deficient mice. Nature communications 7, 12627
NT, not tested

Supplemental Methods

Open field activity and amphetamine induced hyperlocomotion

Spontaneous activity in the open field was conducted over 1 hour or over 48 hours in an automated Versamax Legacy open field apparatus for mice (Omnitech Electronics Incorporated, Columbus, OH). Distance traveled, vertical activity (beam-breaks), and center time were measured by infrared photobeams located around the perimeter of the arenas (see (1) and interfaced to a computer running Fusion 5.3 for Versamax software (Omnitech Electronics Incorporated) which monitored the location and activity of the mouse during testing . Activity plots were generated using the Fusion Locomotor Activity Plotter analyses module (Omnitech Electronics Incorporated). To determine whether amphetamine would modify locomotion, mice were placed into the open field for 60 min to assess baseline activity. Mice were removed from the open field, injected with 2 mg/kg amphetamine (i.p.), and immediately returned to the open field for 120 min. For all tests, mice were transferred to the testing room 18-24 hr prior to testing.

Rotarod performance

Rotarod performance was assessed using a steady-speed (24 rpm) protocol. Animals were tested on a Med-Associates (St. Albans, VT) rotarod for mice, based on methods previously described (2, 3). Mice were given 4 successive 5-min trials which were separated by 30 min intervals. Trials were terminated when the mouse fell from the rod or at 300 s.

Anhedonia

Mice were examined for anhedonia-like behavior based Fukui and colleagues(4). Mice were housed individually for the duration of testing with two bottles. For the first two days, both

bottles were filled with tap water. On day 3 the water bottles were replaced with one bottle filled with water and the other with 2% sucrose solution (Superfine Quick Dissolve Cane Sugar; Domino Foods Inc., Yonkers, NY). After 24 hr the positions of the bottles counterbalanced to preclude positional bias. At the beginning and end of each 24 hour period, the bottles were weighed and total volumes of water and/or sucrose consumed were noted. A preference score was calculated as the total volume of sucrose consumed minus the total volume of water consumed, divided by the total volume of both sucrose and water consumed. Positive preference scores indicated a preference for the sucrose solution across the two test days; scores approaching "0" (indicated no preference) were operationally defined as "anhedonic-like" responses.

Tail suspension

Mice were suspended by their tails in a Med Associates tail suspension apparatus (St. Albans, VT) for 6 min as described previously (4). Immobility time was scored automatically by a computer interfaced to the apparatus and running Tail Suspension software (MedAssociates).

Forced swim

In forced swim, mice were placed into a 4L beaker (18 cm diameter x 25 cm tall) of water held at 25°C with the water depth at 15 cm for 6 min (4). Behavior was recorded from a side-view and saved to digital video (Noldus Media Player; Noldus Information Technologies, Leesburg, VA) and scored subsequently for immobility with Ethovision 11.5 (Noldus Information Technologies). Immobility time was scored automatically by software calibrated by trained observers to equal the time that the animal spent floating or engaged in minimal activity for at least 1 sec.

Circadian activity and lithium treatment

Mice were individually housed in cages (32 x 14 x 12 cm) equipped with 10 cm diameter x 5 cm wide-track width running wheels (Coulburn Instruments, Whitehall, PA). The cages were housed in a Phenome Technologies (Lincolnshire, IL) ventilated cabinet for mice, equipped with 526 nm green wave-length LED for illumination during the light cycle, and infrared LED for the dark cycle. Water and rodent chow (Lab Diet 5001; Lab Diet, St. Louis, MO) were provided throughout the course of the study. Activity data were collected using the ClockLab software (Actimetrics, Wilmette, IL). Mice were placed on a 12:12 hr light:dark (LD) cycle (light onset 0800 hr) for 10 days. Following the entrainment of mice to the 12:12 hr LD cycle, they were placed into constant darkness (DD) for 32 days. On day 42, the mice were subjected to a 6 hr light pulse at CT 16 (circadian hr 16 within a projected 24 hr cycle, based on the individual "tau" for each animal) and returned to DD for another 20 days. On day 62, the animals were placed back on a 12:12 hr LD cycle for 14 days for a final entrainment. Actigrams were generated with Actimetrics Clock software and the data were exported to SPSS (IBM, Armonk, NY) for subsequent statistical analyses. Tau (τ), activity and the average wheel running counts (revolutions per minute for each hr in a single circadian day) were estimated for individual mouse using data from 7 consecutive days before the completion of each phase of testing (5) for entrainment (days 3-10), the free running period (days 36-42), and the final re-entrainment period (days 56-62).

For lithium treatment, mice were fed lithium carbonate chow prepared by Harlan Teklad (Madison, WI). The lithium diet was prepared in a base diet of 5001 for comparison to the untreated control mice. Lithium treatment was initiated with 0.2% lithium carbonate chow. After 2 weeks, mice were placed on 0.4% lithium chow for 4 weeks and then placed into the running wheel cages, using the same protocol as for the untreated mice. The lithium-treated mice remained on the 0.4% chow for the duration of the study. As a precaution to offset the possibility of hyponatremia induced by long-term consumption of the lithium, the drinking water for these animals was supplemented with 450 nM NaCl as described (6).

Effects of valproic acid (VPA) and lithium on locomotor activity

To assess the effects of valproic acid on locomotor activity, mice were injected with valproate (400 mg/kg) or saline (5 ml/kg, i.p.) 30 min before being placed into the open field. To examine the effects of chronic lithium treatment on open field activity, mice were fed a control diet of 5001 rodent chow or 0.4% lithium carbonate diet for 4 weeks prior to testing.

Sociability testing

Mice were examined for sociability as described (84) Sociability was operationally defined as a preference to interact with a social stimulus over a non-social stimulus. One week prior to testing, C3H female mice (Jackson Labs) were handled and trained to sit inside the wire-mesh cages. These animals served as the social stimuli during testing. There were two test phases: exploration of two identical non-social stimuli, and the pairing of a social stimulus (C3H mouse) with a non-social stimulus. Test phase 1 began when a Shank2 mouse was placed into the center chamber and was given free access to the entire apparatus with two wire mesh cages, each containing identical inanimate objects. After 10 min the Shank2 mouse was removed and one of the wire-mesh cages was replaced with an identical cage containing a novel C3H partner. Test phase 2 (social affiliation) began immediately with reintroduction of the target mouse into the center chamber. After 10 min, all animals were removed and the test arena, cages, and objects througly cleaned with LabSan. All tests were filmed and analyzed using Ethovision 9 (Noldus Information Technologies) for the duration of contacts with each cage. Preference scores were calculated, where time spent with one stimulus (non-social stimulus 1 or the social stimulus 1) was subtracted from the time spent with the the 2nd non-social stimulus, and divided by the total time spent exploring both stimuli. Positive scores indicated preference the novel social stimulus, whereas negative scores denoted preference for the non-social relative to the social stimulus. Scores approaching zero indicated no preference.

Water-spray induced grooming

Mice were placed into a clean mouse cage for 5 min and baseline grooming behavior was scored. After 5 min, mice were lightly misted with tap water and grooming was scored for 5 additional min. Behavior was recorded with Media Recorder 2 (Noldus Information Technologies) and the videos were scored for grooming behavior with automated behavioral recognition TopScan software (CleverSys, Reston, VA), which scored the number of grooming bouts and the duration of grooming for each 5-min interval before and after water misting. .

Hole-board test

Animals were examined in a hole-board test for 5 min as described (7). Mice were placed individually into a 42 x 42 x 30 cm arena with a hole-board containing 16 equally spaced holes (3 cm in diameter) arranged in 4 rows. Behaviors were filmed with Media Player 2 (Noldus Information Technologies) and scored with the Observer XT 10 program (Noldus Information Technologies) by trained observers blinded to the genotype and sex of the animals. The number of head-pokes and the frequency of holes used were scored for each animal. Data were reported as the total holes visited, percent of holes used of the 16 holes available, and the frequency of head-pokes for the number of holes used by each mouse.

Morris water maze

Spatial learning and memory, and plasticity were examined in the Morris water maze (MWM) as described in the mouse (2, 3, 7). Before testing, mice were handled and acclimated to standing in water for the week prior to testing. Two days before the start of testing, mice were tail-marked and moved to the test room. On the day before testing, mice were placed on the hidden platform in the NE quadrant and then allowed to swim freely for 15-20 sec before being returned to the platform for 5 sec for one trial. The next day, water-maze testing began and was divided

into 2 phases: acquisition (days 1 to 8) with the hidden platform in the NE quadrant and reversal (days 9 to 16) with the platform in the SW quadrant. Each day the mice received two sets of paired trials that were separated by 60 min, for a total of 4 trials per day. Release points were randomized across trials and days. On days 2, 4, 6, 8, 10, 12, 14, and 16, a single probe trial was given 1 hr after the 4 test trials. On probe trials, the platform was removed and the mice were released from the southern-most point on days 2, 4, 6 and 8, and from the northern-most point on days 10, 12, 14 and 16. In addition to acquisition and reversal training, a separate cohort of naïve mice was tested in the visible platform task over 3 consecutive days at 4 trials a day. Here, the mice were released from the northern-most point and given 60 sec to swim to the visible platform. Performance on all tests was scored by Ethovision XT 9 (Noldus Information Technologies) using a high-resolution camera suspended 1.8 m above the center of the pool. Except for probe trials that were preset for 60 sec, all trials ended when the animal located the platform or after 60 sec of swimming.

Reference:

1. Pogorelov VM, Rodriguiz RM, Insco ML, Caron MG, and Wetsel WC. Novelty Seeking and Stereotypic Activation of Behavior in Mice with Disruption of the Dat1 Gene. *Neuropsychopharmacology.* 2005;30(10):1818-31.

2. Wang X, McCoy PA, Rodriguiz RM, Pan Y, Je HS, Roberts AC, Kim CJ, Berrios J, Colvin JS, Bousquet-Moore D, et al. Synaptic dysfunction and abnormal behaviors in mice lacking major isoforms of Shank3. *Hum Mol Genet.* 2011;20(15):3093-108.

3. Taylor GA, Rodriguiz RM, Greene RI, Daniell X, Henry SC, Crooks KR, Kotloski R, Tessarollo L, Phillips LE, and Wetsel WC. Behavioral characterization of P311 knockout mice. *Genes, brain, and behavior.* 2008;7(7):786-95.

4. Fukui M, Rodriguiz RM, Zhou J, Jiang SX, Phillips LE, Caron MG, and Wetsel WC. Vmat2 heterozygous mutant mice display a depressive-like phenotype. *J Neurosci.* 2007;27(39):10520-9.

5. Jud C, Schmutz I, Hampp G, Oster H, and Albrecht U. A guideline for analyzing circadian wheel-running behavior in rodents under different lighting conditions. *Biol Proced Online.* 2005;7(101-16.

6. O'Brien WT, Harper AD, Jové F, Woodgett JR, Maretto S, Piccolo S, and Klein PS. Glycogen Synthase Kinase-3β Haploinsufficiency Mimics the Behavioral and Molecular Effects of Lithium. *The Journal of Neuroscience.* 2004;24(30):6791-8.

7. Porton B, Rodriguiz RM, Phillips LE, Gilbert JW, Feng J, Greengard P, Kao H-T, and Wetsel WC. Mice lacking synapsin III show abnormalities in explicit memory and conditioned fear. *Genes, brain, and behavior.* 2010;9(3):257-68.