Supporting Information

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SI Materials and Methods

Construction of the TS2-Neo Mouse. In this publication, we follow the nomenclature used by Splawski et al., wherein the more 5′-positioned, MQDAM-containing exon is called exon 8, and the more 3′-located VNDAV-containing exon is designated as 8A (1, 2). TS2-neo mice were generated by In-Genious Targeting Laboratory, Stony Brook, NY using homologous recombination in mouse embryonic stem cells. The targeting vector was constructed using Red/ET recombineering technology. A ∼10-kb region used to generate the targeting vector was first subcloned from a positively identified C57BL/6 BAC clone. The region was designed such that the long homology arm (LA) extended ∼7.6 kb 5′ to exon 8 and the short homology arm extended 1.9 kb 3′ to exon 8. A Neo cassette was inserted 301 bp 3′ to the G-A point mutation engineered into the end of exon 8, and thus caused the introduction of a stop codon in exon 8A. All vectors were constructed and introduced into mouse stem cells to produce heterozygous TS2-neo mice. The mutation was first introduced into a 50:50 C57BL/6J:129Sv strain and then backcrossed for at least three generations with pure C57BL/6J animals. For the final production of testing cohorts, heterozygous male TS2-neo mice were bred with pure C57BL/6J females.

Relative Levels of Exon 8 vs. Exon 8A Expression in TS2-Neo Mice. To quantify relative levels of expression of specific exon 8 isoforms, we used a variation of the technique used by Splawski et al. (3). Whole brain mRNA was probed using primers to exon 7 and exon 9. Individual PCR products were incorporated into plasmid DNA and individual clones were isolated and digested to show the specific isoform and the presence of the mutation. Selected colonies were periodically evaluated using sequencing to ensure quality control. As a control for brain, exon 8 vs. exon 8A distribution was first determined in uterine smooth muscle. Sixty-four out of 69 colonies were found to be exon 8A, consistent with the high expression of exon 8A relative to exon 8 in the uterus of humans (opposite to the expression pattern in brain) (3). In brain tissue of WT mice, 51 colonies were found to be exon 8 and 2 exon 8A. In brain tissue of TS2-neo mice, 83 colonies were found to be exon 8 and 1 exon 8A. These data indicate a high expression of exon 8 relative to exon 8A, once again consistent with the relative prevalence of the corresponding isoforms in humans (3). Sequencing analysis of exon 8 colonies from TS2-neo heterozygotes showed that only 21 out 83 (25%) contained the mutation, far less than the 50% expected, suggesting that the Neo cassette partially suppressed expression of exon 8 in the mutant allele.

General Experimental Conditions. Only male mice were tested in this study. Subject mice were kept in a 20 °C room on a 12-h light/dark cycle (lights off at 6:00 AM) with food and water available ad libidum. Mice were singly housed for at least 5 d before experiments. Unless otherwise specified, experiments were performed during the dark cycle, after acclimatization to the experimental room for at least 1 h. The experimenter was blind to the genotype of animals. To avoid order effects, less stressful tests were conducted before more stressful tests (maximum age, 200 d). Detailed order of testing of TS2-neo vs. WT mice was as follows:

Cohort 1: (*i*) activity chamber, (*ii*) three-chamber test, and (*iii*) Morris water maze.
Cohort 2: (*i*) six-trial social memory test.
Cohort 3: (*i*) activity chamber and (*ii*) fear conditioning.
Cohort 4: (*i*) home-cage activity, (*ii*) annex test, (*iii*) activity chamber, (*iv*) open field, (*v*) marble bury, and (*vi*) Morris water maze.
Cohort 5: (*i*) marble bury and (*ii*) water Y-maze.
Cohort 6: (*i*) home-cage activity, (*ii*) automated social home-cage assay, (*iii*) light/dark box, and (*iv*) SHIRPA.
Cohort 7: (*i*) elevated zero maze and (*ii*) hot plate test.

The order of tests of $Ca_V1.2+/\sim$ vs. WT mice was as follows:

Cohort 1: (*i*) home-cage activity, (*ii*) automated social home-cage assay, (*iii*) activity chamber, and (*iv*) marble bury.
Cohort 2: (*i*) home-cage activity, (*ii*) annex test, (*iii*) activity chamber, and (*iv*) marble bury.

All experiments were in accordance with protocols approved by the Institutional Animal Care and Use Committee of Stanford University and were performed on the basis of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

SHIRPA Test. A SmithKline Beecham, Harwell, Imperial College and Royal London Hospital Phenotype Assessment (SHIRPA) test was conducted for assessment of general physical characteristics, motor abilities, and reflexes (4). We used a protocol described elsewhere (http://www.har.mrc.ac.uk/services/phenotyping/neurology/shirpa.html). In brief, mice were weighed and then put in a viewing jar for 60 s and assessed for physical characteristics including coat condition, presence of whiskers, piloerection, lacrimation, and palpebral closure. Furthermore, level of activity (inactive, active, or excessively active), and any display of tremor or defecation were noted. An arena (44 cm long, 24 cm wide, 15 cm high; divided by floor marks into eight equal squares) was used to assess immediate transfer arousal, locomotor activity (numbers of squares entered with four paws during 30 s), movement fluidity, pelvic elevation, and tail elevation. Thereafter, the following reflexes were tested: corneal reflex, visual placing, righting reflex, vibrissae orienting, Preyer reflex, and contact reflex.

Home-Cage Activity. Home-cage activity was assessed in the PhenoTyper apparatus (Noldus Information Technology) in the holding room of animals under regular light/dark cycle. Each home-cage (30 × 30 cm) was enriched with floor bedding and a shelter. The mouse behavior was recorded by an infrared camera and tracked using Ethovision (Noldus Information Technology). Tracking was performed over a 7 d period, the first 2 d treated as habituation to the new environment (Fig. S1*A*) and the remaining 5 d as a steady-state period (Fig. S1*B*).

Activity Chamber. Activity in a novel environment was assessed using a commercially available chamber containing a square arena (43.2 × 43.2 cm) (Med Associates), placed within a sound attenuated chamber (66 × 55.9 × 55.9 cm). Animals were placed in the center of the arena and allowed to freely explore the arena for 5 min in the dark. Locomotion was detected by two planes of infrared beams and detectors that were connected to automated tracking software. The arena was cleaned with 70% alcohol between tests.

Open Field. To assess locomotor activity, open field tests were performed in a relatively large novel environment under low light conditions. The open field chamber consisted of a square arena (76 × 76 cm) with opaque white walls. The test was initiated by placing mice in the middle of the open field and allowing them to move freely for 10 min, while being tracked by the Ethovision automated tracking system. The chamber was cleaned with 70% alcohol between tests. Distance moved, velocity, and time spent in each predefined zone were analyzed.

Light/Dark Box. Anxiety was tested in a light/dark box test (5). This test was conducted in a square arena (43.2 × 43.2 cm) within a sound attenuated chamber (66 × 55.9 × 55.9 cm, Med Associates). Black plastic inserts were used to separate adjoining dark and well-lit areas (900 lx). Mice were placed in the center of the bright area and allowed to explore the arena for 10 min. Movements were detected by infrared beams and detectors that were connected to a computer-running automated tracking software. The arena was cleaned with 70% alcohol between tests. For validation in C57BL/6J mice, randomly chosen mice were exposed to either soiled bedding from a rat home-cage or clean bedding for 30 min before the test.

Elevated Zero Maze. To test for anxiety traits, we conducted an elevated zero maze test. The zero maze consisted of an annular platform (diameter 40 cm, lane width 5 cm), elevated 40 cm from the floor, and was divided into four equal quadrants. Two opposite quadrants were enclosed by gray plastic walls (height 15 cm). For validation, C57BL/6J mice were either injected with 1.5 mg/kg diazepam or vehicle 30 min before the start of the experiment. In the test, mice were put on an open quadrant facing a closed quadrant in 50 lx red light and tracked for 8 min. Tests were videotaped and subsequently analyzed for time in open and closed quadrants using Ethovision.

Annex Test. Restricted behavior was tested in a newly devised annex test for 15 min in low light conditions. The day after the home-cage activity test, we added a novel addition to the home-cage environment, a tube (length: 34.3 cm; diameter: 5.1 cm) connected to a small chamber (34.3 × 22.9 cm). Following the attachment of the novel environment, a video camera recorded the exploratory behavior of mice for a subsequent scoring by an experimenter using Annotation. Scored parameters included latencies to (*i*) first nose contact with the tube, (*ii*) entry into the tube with four paws, (*iii*) first nose contact with the novel chamber, and (*iv*) first entry into the novel chamber with four paws. Additionally, we traced (*v*) time in shelter, (*vi*) time sniffing the tube from the home-cage environment, (*vii*) time spent in tube, (*viii*) time spent between tube and novel chamber, (*ix*) time spent in novel chamber, and (*x*) total time spent in the novel environment. The tube and novel chamber were sprayed with 70% ethanol and cleaned with paper towels between testing sessions.

Marble Bury Test. Repetitive behavior was tested in the marble bury test. Individual mice were introduced into cages containing 20 black glass marbles (1.5 cm diameter, four equidistant rows of five marbles each) on top of bedding 5 cm deep. After 30 min under low-light conditions, mice were removed and the number of marbles that were at least half-covered was determined.

Morris Water Maze. Spatial reference memory and insistence on sameness was tested in a circular tank (diameter: 183 cm) filled with water and surrounded by uniform blinds and visual cues (6). The water was made opaque by adding a sufficient amount of Tempera paint. Animal movement was tracked using a ceiling camera and Ethovision tracking software. Mice were introduced into the pool at pseudorandomized drop locations outside of the target quadrant. During the "hidden platform learning task," an escape platform (15 cm) was placed in the middle of a designated target quadrant 1 cm below the water surface. Mice were trained to find the platform by four 60-s trials per day for 5 consecutive days. A trial ended either when a subject rested on the hidden platform for 3 s or the end of the trial was reached. Mice who had failed to find the platform were then manually guided to it. On day 6, a 45-s "probe trial" was conducted with the escape platform removed. On the same day, the "reverse hidden platform learning task" was started by moving the platform to the diagonally opposite quadrant using a platform with an increased diameter (17 cm). Mice were trained by four 60-s trials per day for 3 consecutive days.

Water Y-Maze. Place learning and insistence on sameness was tested in a water-based Y-maze. One day before the start of the test, mice were familiarized with a Y-maze (white color, height 20 cm, arms 32 cm long and 7.5 cm wide, water level 13 cm) filled with opaque water (Tempera paint) and surrounded by blinds without explicit visual cues for three 60-s sessions. On day 1 of the experiment, mice were given four blocks of 5 trials in which they were presented with a hidden platform in either the right or left target arm (randomized platform position). In each trial, mice were introduced into the start arm and given 20 s to make an arm choice. Correct arm choices were rewarded with the escape platform; mice that made wrong choices were confined in the wrong arm for 20 s. Mice not making a choice within 20 s were guided to the escape platform. On day 2, mice were tested for achievement of a minimal criterion (success in 4 out of 5 trials). Mice that met the criterion were then given reversal training on day 3 by switching the location of the escape platform to the other arm. Mice that never entered the correct arm during 25 trials of reversal learning were given 5 additional trials with the wrong arm blocked as an inducement to enter the correct arm.

Three-Chamber Test. Sociability was tested in a three-chamber test (each chamber 20 cm long, 40.5 cm wide, 22 cm high). The chambers were separated by two clear plastic walls, each with a connecting doorway (10.2 cm wide, 5.4 cm high). In the "habituation session," test mice were freely allowed to explore the three chambers for 10 min. In the subsequent "sociability session," an empty corral (inverted pencil cup, 11 cm high, 10 cm diameter solid bottom, with stainless steel bars spaced at 1-cm intervals) and a similar corral containing a C57BL/6J male object mouse were added to the flanking side chambers for 10 min. The location of the occupied corral and the empty corral were exchanged for half of the test mice. The object mouse was habituated to the corral for 10 min on 3 consecutive days before the test. The box and corrals were cleaned with 70% ethanol between subject testing. Time in chambers and time spent sniffing the corral were scored using the video recordings and Annotation scoring software.

Automated Social Home-Cage Assay. Social behavior was tested in a newly devised automated home-cage assay. Singly housed mice were acclimated to a home-cage (30 × 30 cm) over 4 d with access to a shelter in one corner and food and water in the diagonally opposite corner. During the dark phase of day 5, a C57BL/6J male mouse was placed under a corral (inverted pencil cup, stainless steel, 11 cm high, 10 cm diameter solid bottom, stainless steel bars spaced at 1-cm intervals) in one of the corners. An identical, unoccupied corral was placed in the remaining corner. Both corrals were weighed down with cone-shaped water bottles that also prevented test mice from climbing on top of the corrals. The locations of the occupied corral and the empty corral were exchanged for half of the animals. Activity of mice was monitored for 4 h using an infrared camera on the ceiling of the home cage and analyzed after the end of the experiment with Etho-

vision tracking software. Scored were time spent in proximity (<5 cm) of the empty or occupied corral. Intensity maps were generated using MATLAB (MathWorks).

Six-Trial Social Memory Test. Social memory was tested in an extended version of the five-trial social memory test. To extinguish sexual behavior, singly housed subject mice were exposed to single ovariectomized (OEF) females for 5 d. OEFs were exchanged daily. One hour before the start of the experiment, OEFs were removed from the home-cages of subject mice. In the test, subject mice were exposed four times to the same never-before-met OEF for 1 min with intertrial intervals (ITIs) of 10 min. In the fifth trial 10 min later, subject mice were exposed to a novel never-before-met OEF for 1 min. In the sixth trial 10 min later, mice were reexposed to the same OEF for a final 1-min meeting. Trials were videotaped and subsequently analyzed using Annotation. Scored was total investigation time (nose-to-body contact of the test animal vs. the intruder OEF).

Pup Separation Test. To test for potentially altered communication, ultrasonic vocalizations (USVs) of pups were recorded. On postnatal day (PND) 1, mice were tattooed with animal tattoo ink on their paws and tails (Green Paste Permanent Tattoo Ink, Ketchum Manufacturing). On PND 2, 4, 6, 8, 10, and 12, individual pups were separated from the dam and litter and placed into a clean cup inside a sound-attenuating Styrofoam box. Subsequently, USVs were recorded with an ultrasound microphone (Avisoft UltraSoundGate condenser microphone capsule CM16, Avisoft Bioacoustics) for 5 min. After the test, mice were weighed and then returned to their dam and litter. Data were scored with regard to duration of calls, number of calls, peak frequency, and peak amplitude using Avisoft SASLab Pro.

Fear Conditioning. To test for associative learning and memory, fear conditioning was performed in a Coulbourn Instruments chamber. On day 1 of the test, mice were introduced in context A (steel rod flooring, square arena, clear and gray plastic walls, white light, mint scent) for a 200-s baseline period. Thereafter, five tone-shock pairings were applied. An aversive shock (0.7 mA, 50 Hz, 2 s) was delivered 18 s after the tone (70 dB, 2 kHz, 20 s). An ITI was defined as the 80 s between the end of one tone and the start of the next tone (except the last ITI that ended 80 s after the last tone). On days 2, 8, and 18, mice were placed in context B (different room than used for the experiments on days 1, 3, 9, and 16; blue plastic floor and walls, round shaped arena, blue light, lemon scent). After a 200-s baseline period, three tones (70 dB, 2 kHz, 20 s) with ITIs of 80 s were presented. On days 3, 9, and 16, animals were placed in context A for 5 min without any shock or tone presentation. Freezing was assessed using FreezeFrame software (Actimetrics) and defined as lack of motion (except breathing) for 0.75 s. The % freezing was calculated as the time during which the animal froze, expressed as a percentage of total time.

Hot Plate Test. To test responsiveness to an aversive stimulus, mice were put on a hot plate apparatus (IITC; temperature set at 55 °C ± 0.1 °C) and covered by a glass transparent cylinder (height 25 cm, diameter 12 cm). The latency to the first hind paw licking or jumping was determined using a remote foot-switch pad connected to a timer. The apparatus was cleaned with 70% alcohol between subject testing.

Statistics. Data are presented as average ± SEM and reported as significant at $P < 0.05$. Statistical analysis was performed with GraphPad Prism version 5.0d and MATLAB. Pooled data were analyzed using Student's t test after passing the D'Agostino and Pearson omnibus normality test; otherwise, the nonparametric Mann–Whitney u Test was performed. See Table S2 for a summary of statistical data and tests used for each dataset.

1. Yazawa M, et al. (2011) Using induced pluripotent stem cells to investigate cardiac phenotypes in Timothy syndrome. *Nature* 471:230–234.
2. Splawski I, et al. (2004) Ca(V)1.2 calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. *Cell* 119:19–31.
3. Splawski I, et al. (2005) Severe arrhythmia disorder caused by cardiac L-type calcium channel mutations. *Proc Natl Acad Sci USA* 102:8089–8096, discussion 8086–8088.
4. Brooks SP, Dunnett SB (2009) Tests to assess motor phenotype in mice: A user's guide. *Nat Rev Neurosci* 10:519–529.
5. Bourin M, Hascoët M (2003) The mouse light/dark box test. *Eur J Pharmacol* 463:55–65.
6. Morris RG, Garrud P, Rawlins JN, O'Keefe J (1982) Place navigation impaired in rats with hippocampal lesions. *Nature* 297:681–683.

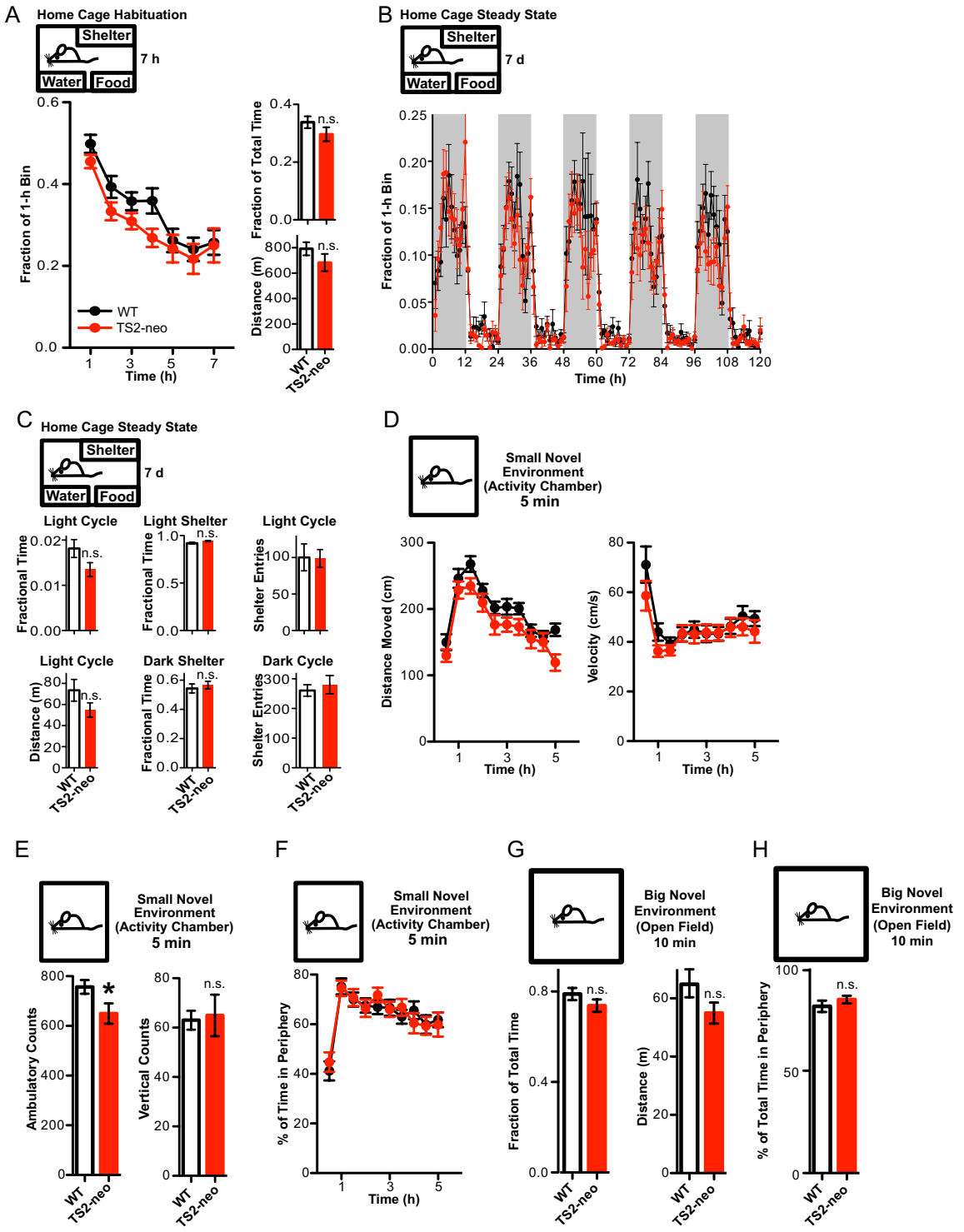

Fig. S1. Home-cage activity, activity chamber, open field test. (*A*) Home-cage activity habituation. An hour-to-hour comparison of the time spent moving during the initial 7 h after introduction into a novel home-cage environment showed no genotype difference (*Left*). The cumulative time spent moving (*Upper Right*) and the total distance moved (*Lower Right*) during this habituation period were not significantly different between genotypes. *n* (each genotype) = 9. (*B*) Steady-state home-cage activity. TS2-neo mice and WT mice displayed typically high activity during the dark periods (gray background segments) and low activity during light periods (white background segments) without differences between genotypes. (*C*) Microanalysis of home-cage activity of the 5-d steady-state phase revealed no significant differences for the following parameters: Fractional time spent moving and distance moved during the light cycles (*Left*), fractional time spent in the shelter during the light and dark cycles (*Center*), and number of shelter entries during the light and dark cycles (*Right*). *n* (each genotype) = 12. (*D*) Activity chamber. Monitoring of ambulatory activity in a relatively small, novel environment over 5 min in the dark. Time course of distance moved (*Left*) and velocity (*Right*) revealed no differences between TS2-neo and WT mice. (*E*) TS2-neo mice showed significantly fewer ambulatory counts ($P < 0.04$, Student's *t* test) but the same number of vertical counts (rearing) as WT mice. (*F*) Both genotypes spent the same amount of time in the periphery of the novel environment. *n* (each genotype) = 31. (*G*) Open field behavior. Monitoring of ambulatory activity in a relatively large, novel environment over 10 min in low white light. TS2-neo mice showed a nonsignificant trend for decreased time spent moving (*Left*) and distance moved (*Right*). (*H*) Both genotypes spent the same amount of time in the periphery of the novel environment. *n* (WT) = 12, *n* (TS2-neo) = 13.

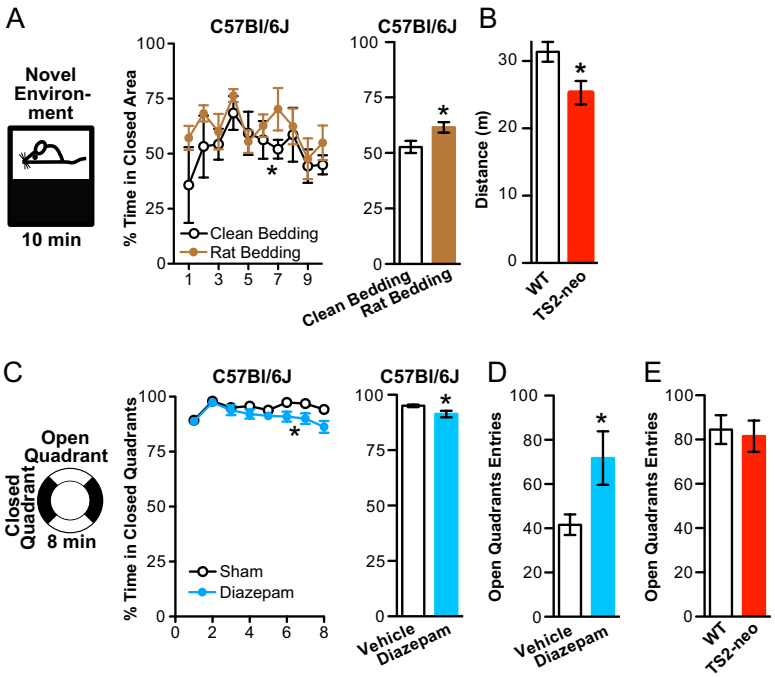

Fig. S2. Light/dark box and elevated zero maze. (*A*) Light/dark box validation using C57BL/6J mice. Randomly chosen subject mice were either exposed to clean bedding or predator odor (using soiled bedding from a rat home-cage) for 30 min. Subsequently, mice were tested in the light/dark box for 10 min. C57BL/6J mice that had been preexposed to rat bedding spent significantly more time in dark area of the box than mice exposed to clean bedding ($P < 0.05$ for genotype effect, ANOVA; $P < 0.04$, Mann–Whitney *u* test). *n* (each condition) = 5. (*B*) Light/dark box test conducted with TS2-neo vs. WT littermates. TS2-neo mice traveled a significantly shorter distance than WT littermates ($P < 0.05$, Student's *t*-test). *n* (WT) = 13, *n* (TS2-neo) = 12. (*C*) Elevated zero maze validation using C57BL/6J mice. Randomly chosen C57BL/6J mice were injected with either 1.5 mg/kg diazepam or vehicle without drug 30 min before the start of the 8-min experiment. Diazepam-injected mice spent significantly less time in the closed quadrants than sham-treated mice ($P < 0.03$ for drug effect, ANOVA; $P < 0.03$, Student's *t* test). (*D*) Drug-treated animals entered the open quadrants more often than vehicle-injected controls ($P < 0.03$, Student's *t* test) (*Right*). *n* (sham) = 10, *n* (diazepam) = 9. (*E*) Elevated zero maze testing of TS2-neo vs. WT littermates. Mutant mice entered the open quadrants as often as WT littermates (*Right*). *n* (WT) = 9, *n* (TS2-neo) = 13.

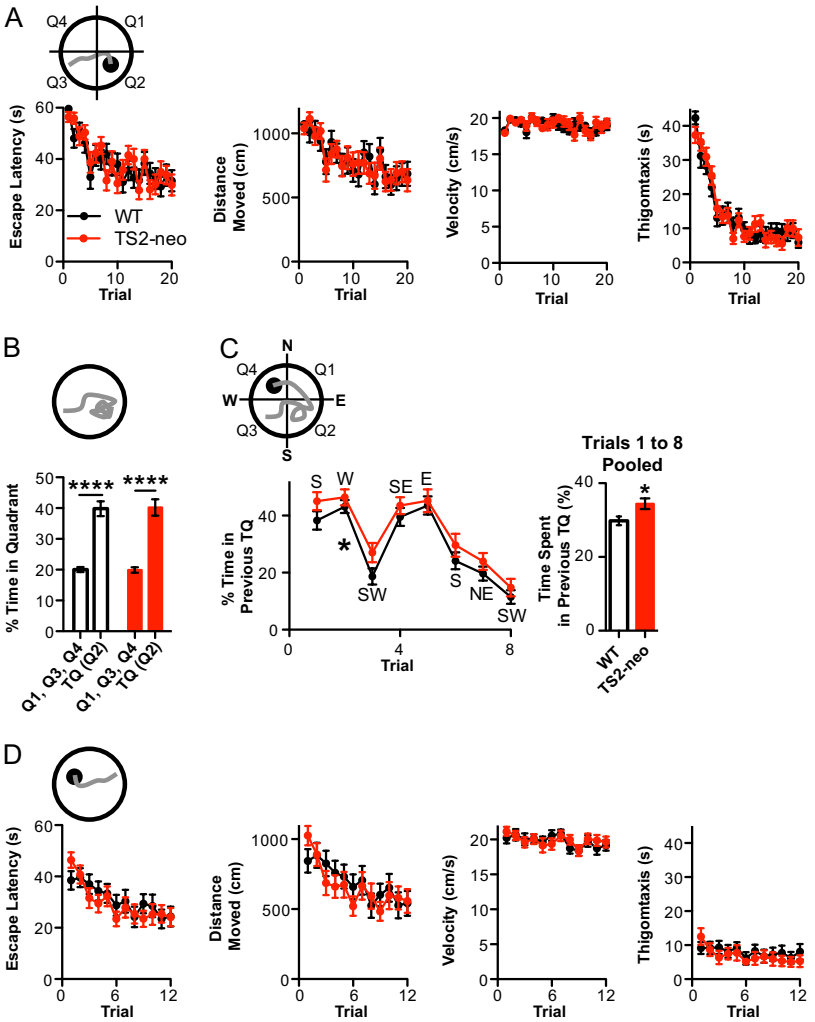

Fig. S3. Morris water maze. (*A*) During acquisition of the hidden platform location, TS2-neo and WT mice did not differ significantly in escape latency (*Left*), distance moved (*Center Left*), velocity (*Center Right*), and thigmotaxis (*Right*). (*B*) In the probe trial with the hidden escape platform missing, both genotypes spent significantly more time in the quadrant (Q2, TQ) where the platform was previously located ($P < 0.0001$, Bonferroni post hoc test). (*C*) During reversal learning, TS2-neo mice spent significantly more time in the previous TQ than WT littermates ($P < 0.02$ for genotype effect, ANOVA) (*Left*). Drop locations: S, south; E, east; N, north; W, west. The cumulative time spent in the previous TQ was also significantly increased for trials 1–8 ($P < 0.04$, Mann–Whitney *u* test). (*D*) During reversal learning, TS2-neo and WT mice did not differ significantly in escape latency (*Left*), distance moved (*Center Left*), velocity (*Center Right*), and thigmotaxis (*Right*). *n* (WT) = 27, *n* (TS2-neo) = 29.

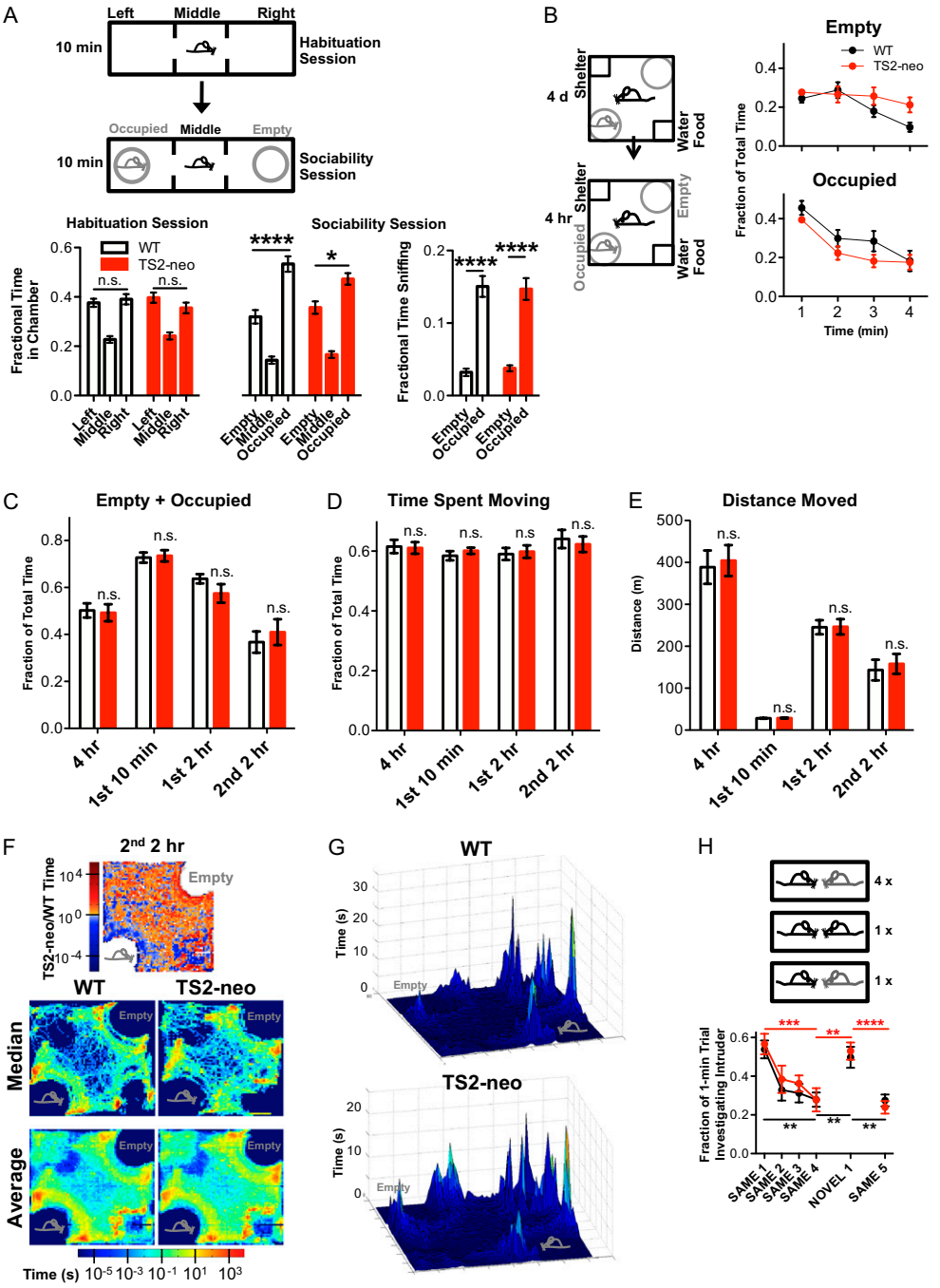

Fig. S4. Social behavior. (*A*) Three-chamber test. After a habituation of subject mice to the three-chamber apparatus for 10 min ("habituation session"), empty and occupied corrals were added to the side chambers ("sociability session") (*Upper*). No side preference was apparent during the habituation session for WT and TS2-neo mice (*Lower Left*). During the sociability session, both WT and TS2-neo mice spent significantly more time in the chamber with the occupied corral vs. the empty corral (P (WT) < 0.0001, P (TS2-neo) < 0.05, Bonferroni post hoc test) (*Lower Center*) and sniffed significantly longer at the occupied corral than at the empty corral (P < 0.0001, Bonferroni post hoc test) (*Lower Right*). n (each genotype) = 12. (*B*) Automated social home-cage assay. After a 4-d exposure to a home-cage environment with a shelter and ad libidum access to water/food, empty and occupied corrals were presented in opposite corners for 4 h. During the last 2 h of the experiment, TS2-neo mice showed more interest in the empty corral than WT mice (*Upper Right*). Except for the last hour, TS2-neo mice showed less interest in the occupied corral than WT mice (*Lower Right*). The total time spent close to the empty and occupied corrals (*C*), the time spent moving (*D*), and the distance moved (*E*), were not different between genotypes. (*F*, *Top*) Intensity map of the last 2 h shows the TS2-neo:WT time ratio in red where TS2-neo mice spent relatively more respectively in blue where they spent relatively less time than WT mice. Plot duplicated from main text for clarity. (*Middle*) Spatial distribution of median animals from WT and TS2-neo groups, showing relative time spent within the arena. (*Bottom*) Average intensity maps of the time spent in the arena. (*G*) 3D plot of data presented in *Bottom* panels of *F*. n (each genotype) = 12. (*H*) Six-trial social memory test. Both TS2-neo and WT littermates exhibited significant habituation (WT: P < 0.01, TS2-neo: P < 0.001, Bonferroni post hoc test) to the same OEF (trials 1–4) and significant dishabituation (P < 0.01, Bonferroni post hoc test) to the novel OEF (novel 1). For added stringency, the same OEF was presented in a sixth trial 10 min after trial 5. Both genotypes significantly decreased their investigation time during this last presentation (WT: P < 0.01, TS2-neo: P < 0.0001, Bonferroni post hoc test). n (each genotype) = 10.

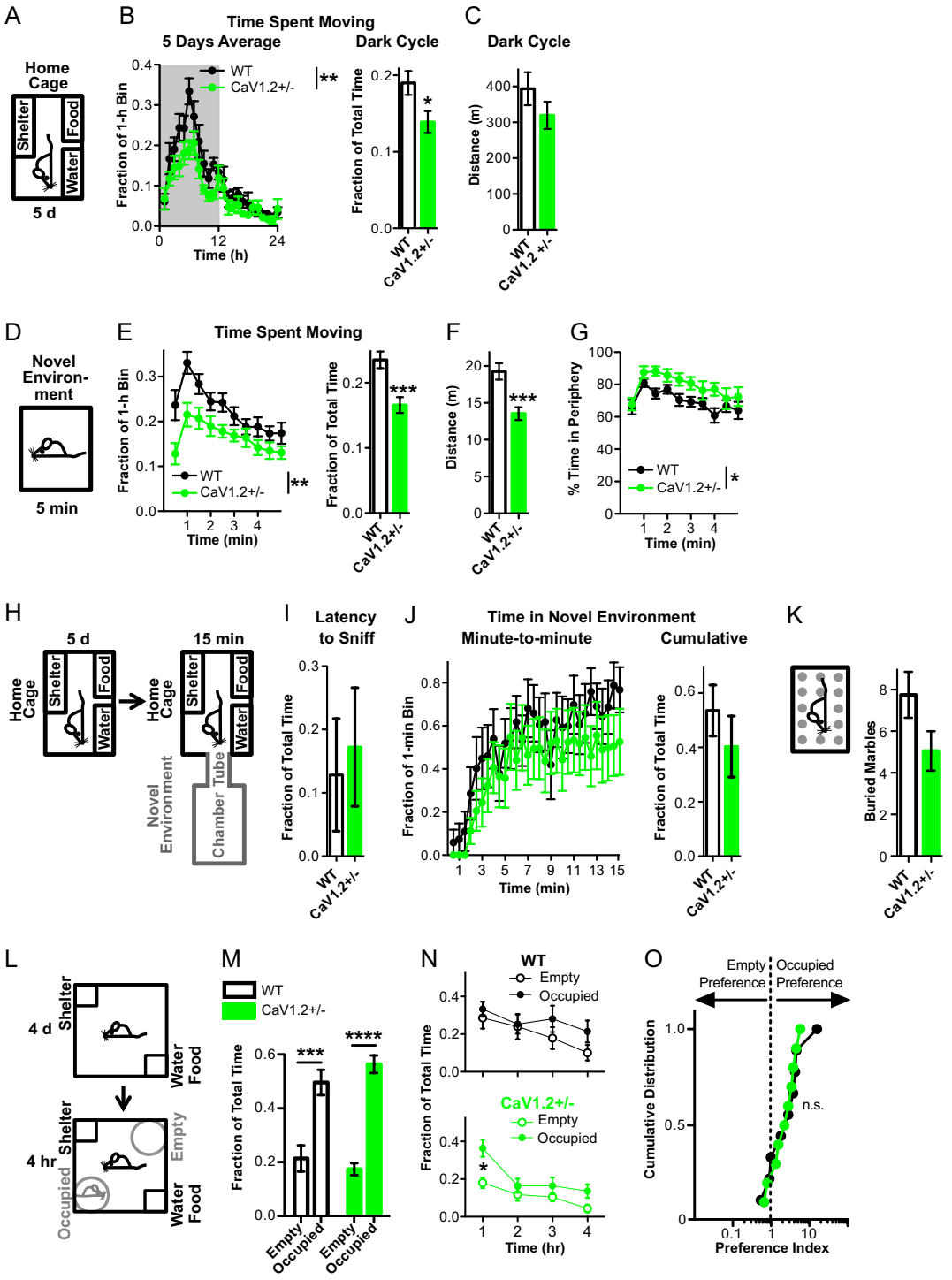

Fig. S5. $Ca_V1.2^{+/-}$ are hypoactive with increased anxiety but show no restricted/repetitive behavior and altered social behavior. (*A*) Basic activity monitored over 5 d in a home-cage with a shelter and ad libidum access to water/food. (*B*) Time spent moving. Average of five 24-h dark/light cycles (gray/white background, respectively) (*Left*) and pooled data of five dark periods (*Right*) revealed a significantly decreased activity in $Ca_V1.2^{+/-}$ mice compared with WT littermates (time course: $P < 0.01$ for genotype effect, ANOVA; pooled: $P < 0.05$, Mann–Whitney *u* Test). (*C*) Distance moved. During the five dark cycles, $Ca_V1.2^{+/-}$ mice traveled the same average distance as WT littermates. *n* (WT) = 9, *n* ($Ca_V1.2^{+/-}$) = 9. (*D*) Monitoring of ambulatory activity over 5 min in the activity chamber. (*E*) Time spent moving. Time course ($P < 0.01$ for genotype effect, ANOVA) (*Left*) and cumulative time spent moving ($P < 0.001$, Student's *t* test) (*Right*) showed significantly less movement in $Ca_V1.2^{+/-}$ mice than WT littermates. (*F*) Distance moved. $Ca_V1.2^{+/-}$ mice traveled a significantly shorter distance ($P < 0.001$, Student's *t* test). (*G*) $Ca_V1.2^{+/-}$ mice spent significantly more time in the periphery (thigmotaxis) of the novel environment ($P < 0.03$, for ANOVA genotype effect). *n* (WT) = 20, *n* ($Ca_V1.2^{+/-}$) = 19. (*H*) Annex test. One day after a 5-d exposure to a home-cage environment with a shelter and ad libidum access to water/food, a novel environment consisting of a tube leading to an additional chamber was attached for 15 min. (*I*) $Ca_V1.2^{+/-}$ mice and WT littermates did not differ in latency to approach the novel environment. (*J*) A minute-to-minute comparison of the time spent in the novel environment showed no significant genotype effect (*Left*) and the cumulative time spent in the novel environment was not significantly different (*Right*). *n* (WT) = 9, *n* ($Ca_V1.2^{+/-}$) = 10. (*K*) Marble bury test. $Ca_V1.2^{+/-}$ mice exhibited a nonsignificant trend to bury fewer marbles than WT littermates. *n* (WT) = 20, *n* ($Ca_V1.2^{+/-}$) = 19.

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(*L*) Social home-cage assay. After a 4-d exposure to a home-cage environment with a shelter and ad libidum access to water/food, empty and occupied corrals were presented in opposite corners. (*M*) During the initial 10 min of the presentation, both genotypes preferred to stay close to the occupied corral rather than close to the empty corral (WT: $P < 0.001$, $Ca_V1.2^{+/-}$: $P < 0.0001$, Bonferroni post hoc test). (*N*) Both genotypes kept a preference for the occupied vs. the empty corral during the 4 h of the experiment. (*O*) Cumulative distribution of the preference index (ration of time spent with the occupied corral vs. time with the empty corral) of the last 2 h of the experiment revealed no significant difference between genotypes. *n* (WT) = 9, *n* ($Ca_V1.2^{+/-}$) = 10. This panel duplicates Fig. 5*E* (*Inset*) for clarity.

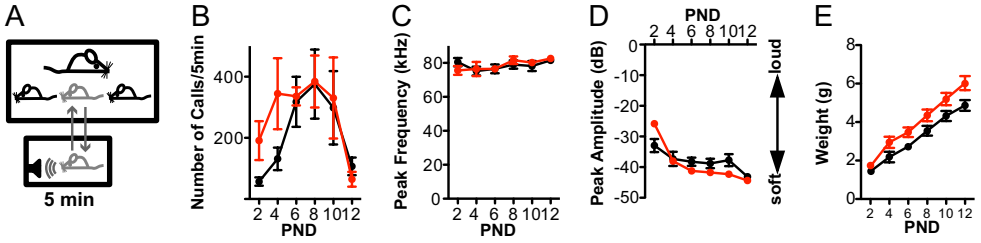


Fig. S6. Ultrasonic vocalization. (*A*) Starting at PND 2, pups were separated from their dam and litter and USVs were recorded for 5 min. TS2-neo pups did not differ significantly with respect to the number of calls (*B*), peak frequency (*C*), peak amplitude (*D*), or body weight (*E*). *n* (WT) = 6, *n* (TS2-neo) = 4.

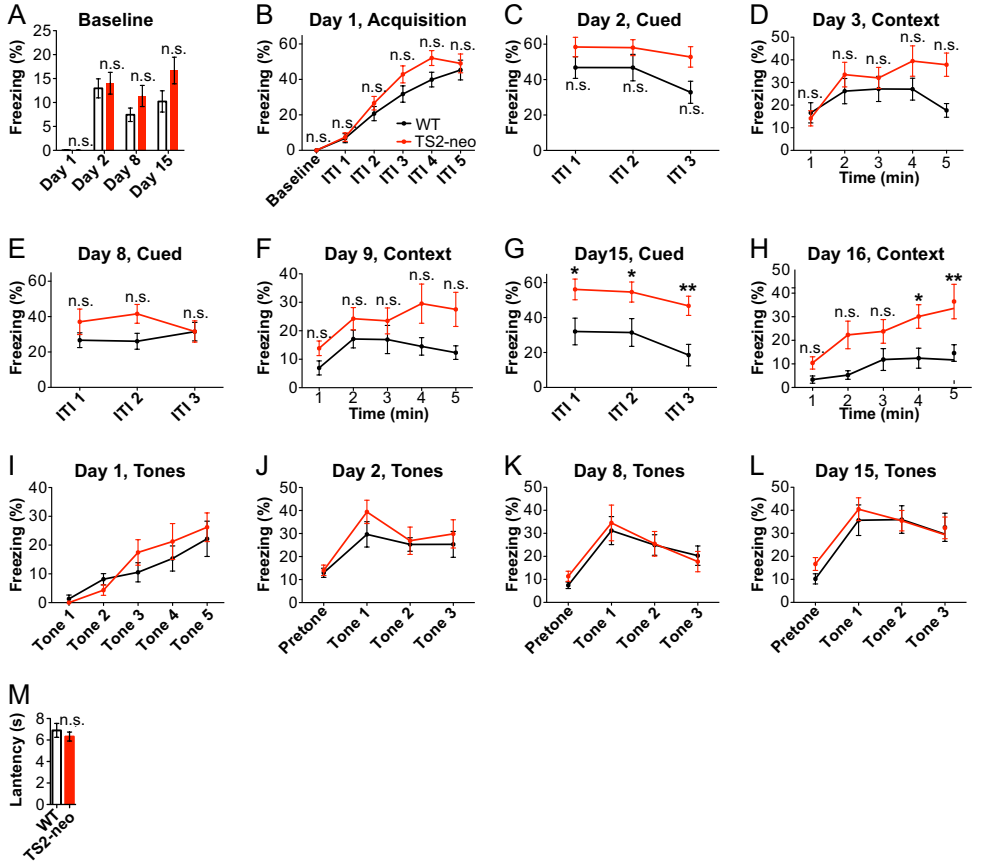
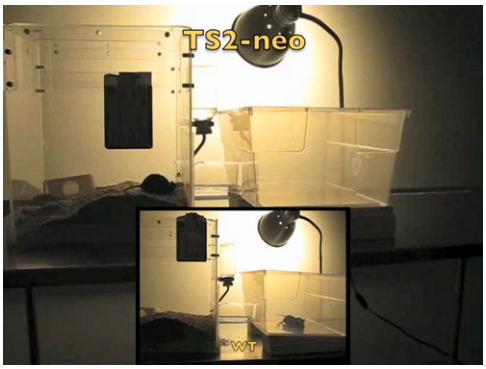
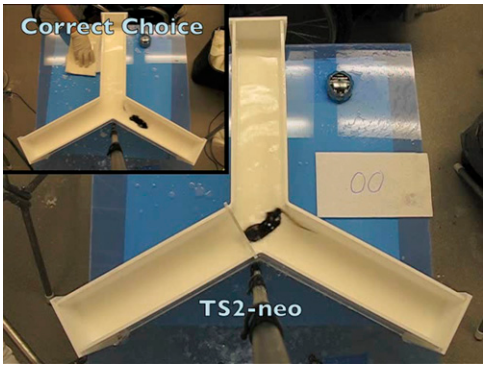


Fig. S7. Fear conditioning and hot plate test. (*A*) Fear conditioning. TS2-neo and WT mice did not differ significantly in baseline freezing (percentage of freezing before first tone presentation) on days 1, 2, 8, and 15. (*B–H*) Detailed presentation of analysis of percentage time of freezing. Over acquisition phase, and tests of cued memory, analysis performed during ITIs. Analysis of contextual memory carried out over 1-min time bins as indicated. (*I–L*) Percentage of freezing during tone presentations on days 1, 2, 8, and 15 showing no significant differences between genotypes. *n* (WT) = 10, *n* (TS2-neo) = 11. (*M*) The latency to hind paw licking or jumping when animals were put on a hot plate (55 °C ± 0.1 °C) was not significantly different between genotypes. *n* (WT) = 12, *n* (TS2-neo) = 13. ns, not significant; $*P < 0.05$; $**P < 0.01$.



Movie S1. Representative videos of annex test. A TS2-neo mouse and a WT mouse (*Inset*) were habituated to a home-cage environment (left side) for 5 d. The videos show the attachment of a NE consisting of a tube leading to a second chamber (right side) for 15 min, presented at 15× accelerated speed.

Movie S1



Movie S2. Representative video of a water Y-maze forced training trial of a TS2-neo mouse. A TS2-neo mouse is inserted in the start arm (*Top*), the hidden escape platform is located in the *Left* target arm, and the incorrect *Right* target arm is blocked. The mouse repeatedly pushes its nose against the partition blocking the incorrect arm before returning to the origin for another attempt. This form of behavior was observed in three out of four TS2-neo animals that progressed to this stage of testing (Fig. 4*D*). (*Inset*) Representative example of a WT mouse making a correct arm choice during a reversal trial.

Movie S2

Other Supporting Information Files

Table S1 (DOC)
Table S2 (DOC)