Inventory of Supplemental Items

Figure S1, Related to Figure 1, shows expression of MeCP2 is preserved in SOM+ cells of *PV-Mecp2*$^{-/y}$ mice and PV+ cells of *SOM-Mecp2*$^{-/y}$ mice.

Figure S2, Related to Figure 2, shows *PV-Mecp2*$^{-/y}$ mice develop splayed hindlimbs and impaired forelimb motor coordination.

Figure S3, Related to Table 1, shows *PV-Mecp2*$^{-/y}$ and *SOM-Mecp2*$^{-/y}$ mice did not display anxiety, increased body weight, altered pre-pulse inhibition, or reduced locomotion.

Movie S1, Related to Figure 4, shows a *SOM-Mecp2*$^{-/y}$ mouse with seizure.

Table S1, Related to Figure 2, 3, and 4, shows results of statistics for all the behavioral tests conducted in this study.

Supplemental Figures and Legends

Figure S1

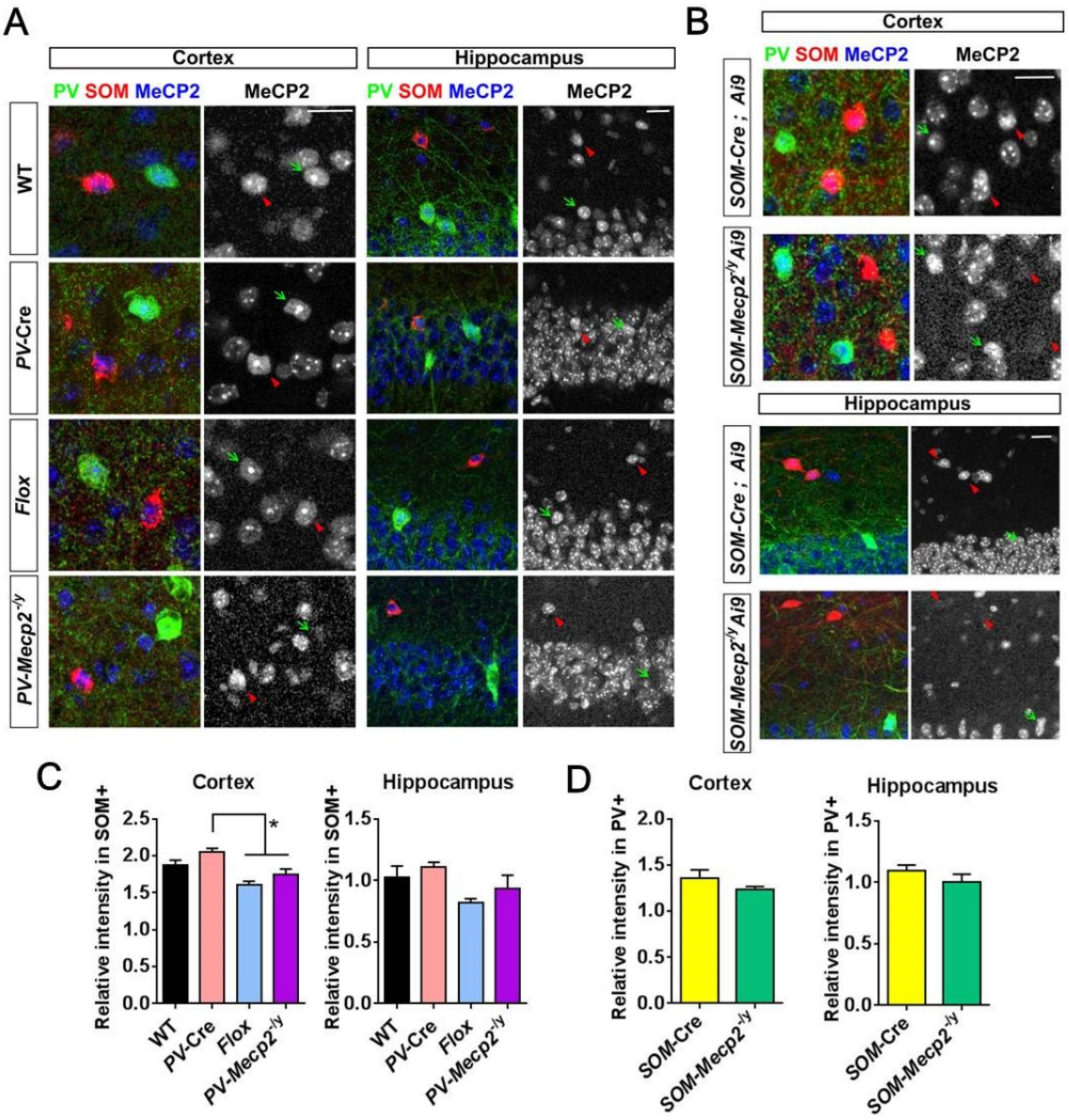

Figure S1, Related to Figure 1. MeCP2 expression is preserved in SOM+ cells of *PV-Mecp2*$^{-/y}$ mice and PV+ cells of *SOM-Mecp2*$^{-/y}$ mice.

A, B, Immunofluorescence images of cortex (layer 2/3) and hippocampus (CA1) in 4-month-old *PV-Mecp2*$^{-/y}$, *SOM-Mecp2*$^{-/y}$, and control mice. PV+ cells were stained with anti-parvalbumin antibody. SOM+ cells were visualized by either an anti-somatostatin antibody (**A**) or by crossing the

mice with a reporter line (Ai9) which induced tdTomato expression in SOM+ cells (**B**). MeCP2 was depleted from PV+ cells (green arrows) of *PV-Mecp2*$^{-/y}$ mouse brains, while it was preserved in SOM+ cells (red arrowheads, **A**). Conversely, MeCP2 was depleted from SOM+ cells in *SOM-Mecp2*$^{-/y}$ mice, while it was preserved in PV+ cells (**B**). Scale bars, 20 μm.

C, D, Quantitative analysis of relative expression levels of MeCP2 in SOM+ cells of *PV-Mecp2*$^{-/y}$ mice (**C**) and in PV+ cells of *SOM-Mecp2*$^{-/y}$ mice (**D**). Statistical analysis by one-way ANOVA (**C**) and t-test (**D**) showed no significant difference between either conditional knockout or control groups. MeCP2 expression of SOM+ cells in the cortex was lower in *PV-Mecp2*$^{-/y}$ compared to *PV*-Cre mice, but there was no significant difference between *PV-Mecp2*$^{-/y}$ and *Flox* mice. n = 3 mice per genotype.

Figure S2

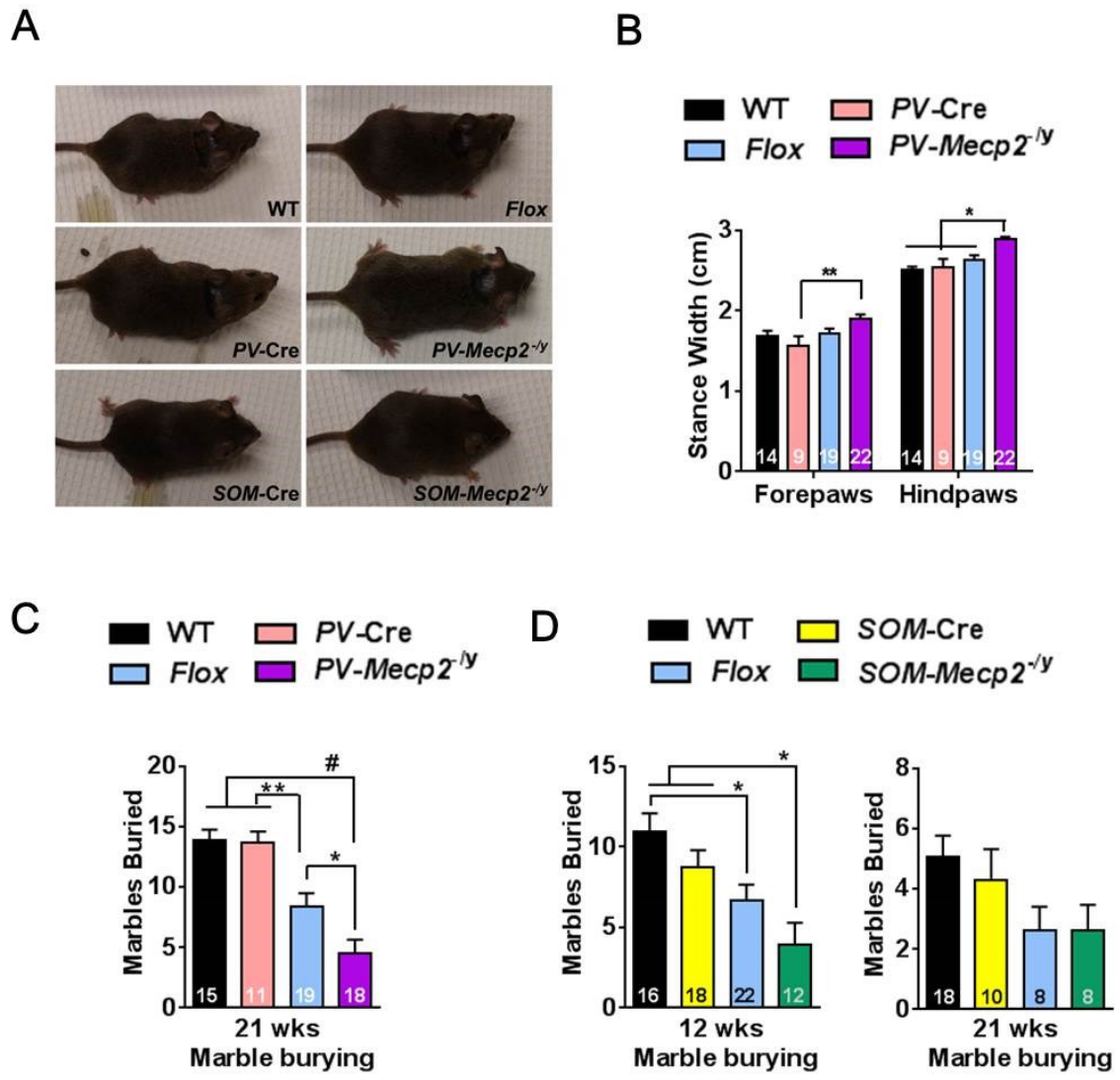

Figure S2, Related to Figure 2. ***PV-Mecp2*$^{-/y}$ mice develop splayed hindlimbs and impaired forelimb motor coordination.**

A, By 10 weeks of age, *PV-Mecp2*$^{-/y}$ mice developed splayed hindlimbs; *SOM-Mecp2*$^{-/y}$ mice remained grossly indistinguishable from control mice.

B, The distance between hindlimbs in *PV-Mecp2*$^{-/y}$ mice was statistically significant at 12 weeks of age compared to three control groups.

C, *PV-Mecp2*$^{-/y}$ mice buried fewer marbles than control mice, suggesting apraxia. While *Flox* mice buried fewer marbles compared to WT and *PV*-Cre, *PV-Mecp2*$^{-/y}$ mice buried fewer than all three control groups, including *Flox.*

D, While *SOM-Mecp2*$^{-/y}$ mice buried fewer marbles compared to WT and *SOM*-Cre mice at 12 weeks, the number of buried marbles was comparable to *Flox* mice at 12 weeks and 21 weeks. Data represent mean ± s.e.m. Numbers in the bar graphs represent number of mice (n) per genotype.* $p < 0.05$, ** $p < 0.01$, # $p < 0.0001$.

Figure S3

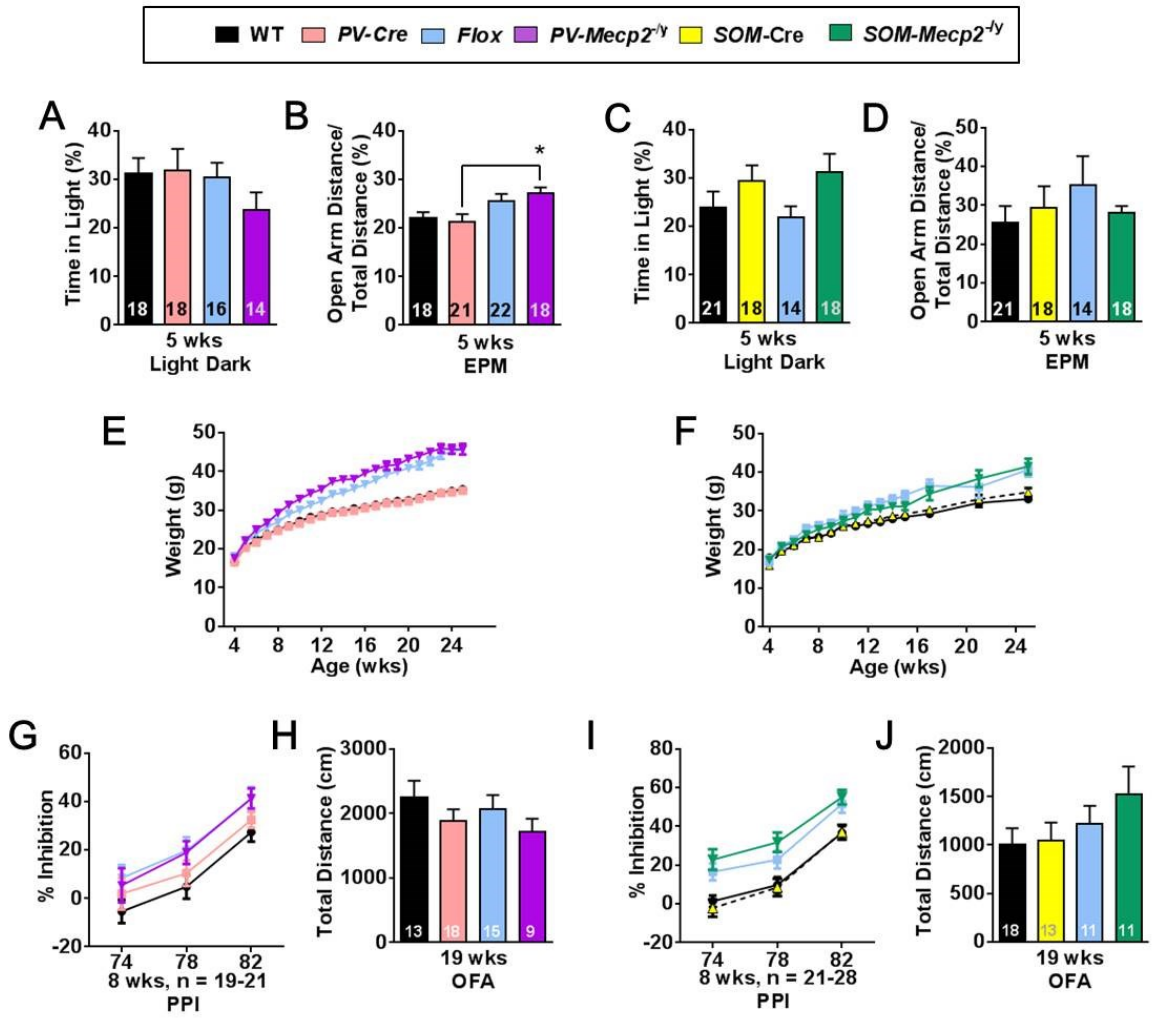

Figure S3, Related to Table 1. ***PV-Mecp2*$^{-/y}$ and *SOM-Mecp2*$^{-/y}$ mice did not display anxiety, increased body weight, altered pre-pulse inhibition, reduced locomotion, or defects in synaptic transmission and plasticity of hippocampal pyramidal cells.**

A-D, *PV-Mecp2*$^{-/y}$ and *SOM-Mecp2*$^{-/y}$ mice did not display anxiety-related behavior in the Light Dark test (**A,C**) or the Elevated Plus Maze (EPM) (**B,D**) test at 5 weeks of age.

E, F, The difference in body weight between *PV-Mecp2*$^{-/y}$ (**E**) and *SOM-Mecp2*$^{-/y}$ (**F**) and their *Flox* littermates was not significant. **E,** n = 44 (WT), 35 (*PV*-Cre), 49 (*Flox*), and 47 (*PV-Mecp2*$^{-/y}$). **F,** n = 25 (WT), 21 (*SOM*-Cre), 18 (*Flox*) and 17 (*SOM-Mecp2*$^{-/y}$).

G, I, *PV-Mecp2*$^{-/y}$ (**G**) and *SOM-Mecp2*$^{-/y}$ (**I**) did not differ in prepulse inhibition (PPI) ratio when

compared to *Flox* mice. **G,** n = 20 (WT), 19 (*PV*-Cre), 21 (*Flox*), and 19 (*PV-Mecp2*$^{-/y}$). **I,** n = 24 (WT), 21 (*PV*-Cre), 20 (*Flox*) and 28 (*SOM-Mecp2*$^{-/y}$).

H, J, Trends in locomotor activity of *PV-Mecp2*$^{-/y}$ (**H**) and *SOM-Mecp2*$^{-/y}$ (**J**) did not reach statistical significance. Data represent mean ± s.e.m. Numbers in the bar graphs represent number of mice (n) per genotype.

Movie S1, Related to Figure 4. *SOM-Mecp2*$^{-/y}$ mice develop seizures.

The movie shows a seizure event that occurred in a 20-week old *SOM-Mecp2*$^{-/y}$ mouse after it was transferred to a new cage.

Supplemental Experimental Procedures

Mouse husbandry and handling

Mice were housed in an AAALAS-certified Level 3 facility on a 14 hour light cycle. Male C57Bl/6 mice carrying either the Pvalb-2A-Cre (B6.Cg-Pvalb$^{tm1.1(cre)Aibs}$/J, JAX 012358, *PV*-Cre) or Sst-IRES-Cre (Sst$^{tm2.1(cre)Zjh}$/J, JAX 013044, *SOM*-Cre) allele were mated with 129S6SvEvTac females heterozygous for the *Mecp2*-flox allele (Guy et al., 2001), resulting in male and female F1 hybrid offspring. After weaning, all mice were group housed (3-5 mice per cage) without enrichment as a mix of genotypes. All mice included in the survival curve were weighed weekly and scored according to the 6-category disease scoring scale, as previously described (Guy et al., 2007). All husbandry and experimental procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Baylor College of Medicine.

Immunohistochemistry

Male mice carrying the *SOM*-Cre allele were mated with female mice carrying the Ai9 (*Gt(ROSA)26Sor*$^{tm9(CAG-tdTomato)Hze}$, JAX 007909) allele. Brain sections from male offspring carrying both *SOM*-Cre and Ai9 alleles were obtained as previously described (Chao et al., 2010). The brains were fixed by transcardial perfusion of PBS-buffered 4% paraformaldehyde (PFA). Brains were removed, kept in 4% PFA overnight, cryoprotected in 25% sucrose solution, and frozen in optimal cutting temperature medium (O.C.T). Sagittal sections were obtained using a Leica CM3050S cryostat at 45 μm thickness. The slices were incubated in a PBS-buffered blocking solution containing 2% normal goat serum and 0.3% Triton-X for 1 hour, followed by primary antibody solution containing anti-MeCP2 antibody (1:1000, rabbit monoclonal, D4F3, Cell Signaling, Cat #3456), anti-parvalbumin antibody (1:5000, mouse monoclonal, Swant #PV235), and anti-somatostatin antibody (1:250, rat monoclonal, Millipore, MAB354) overnight at 4°C. After 3 washes with PBS, the slices were incubated with secondary antibodies conjugated with Alexa 488,

555 and Alexa 633 (1:1000, Invitrogen) overnight at 4°C. In some experiments, SOM+ cells were identified by crossing SOM-Cre mice with Ai9 (*Gt(ROSA)26Sor*$^{tm9(CAG\text{-}tdTomato)Hze}$, JAX 007909) allele (Figure 1A, C).

Stained brain sections from 4 mice were imaged using a Leica LCS SP8 confocal microscope, with 20x lens and zoom 1.28 in 1024 x 1024 pixels. Representative images were obtained as z-stacks with 15-20 z-steps, and maximum projection images were obtained using Image J.

Quantitative analysis of MeCP2 intensity was performed on maximum projection images from 8 z-sections using Image-Pro Analyzer 7.0 (Media Cybernetics). Relative MeCP2 intensity in PV+ and SOM+ cells was analyzed by following steps: (1) background intensity was subtracted from MeCP2-channel, (2) images were binarized using the MeCP2 channel using three times the average background intensity as threshold, (3) MeCP2 intensity was measured in PV+, SOM+, and PV/SOM-negative cells, and (4) the average intensity of MeCP2 in PV+ or SOM+ cells was normalized to PV/SOM-negative cells. Average of normalized MeCP2 intensity was obtained from two areas in each region. The numbers of cells per mouse used for analysis were 8-22 in the cortex, 4-16 in the hippocampus, and 6-15 in the striatum. Small fractions of cells expressing both PV and SOM were excluded from the analysis.

The percentage of cells expressing MeCP2 in either PV+ or SOM+ cells in *PV-Mecp2*$^{-/y}$ or *SOM-Mecp2*$^{-/y}$ mice, respectively, was analyzed by following steps: (1) background intensity was subtracted from MeCP2-channel, (2) images were binarized using the DAPI channel, using three times the background intensity as threshold, (3) total intensity of MeCP2 within the nucleus of PV+ /SOM+ cells was measured, (4) total intensity of the surrounding cells negative for either PV or SOM was measured, (5) average intensity (A) and standard deviation (B) of total intensity among PV- or SOM-negative cells was calculated, (6) if the intensity in PV or SOM+ cells was less than "A-2B", the cell was considered as negative for MeCP2. The data were collected from two areas in each region. The numbers of cells per mouse used for analysis were 20-33 in the cortex, 10-17 in the hippocampus, and 7-21 in the striatum.

Behavioral tests

For the PV conditional knockout group, naïve male mice used for behavioral assays were divided into 2 cohorts, each being assessed by different assays at different time points. For Cohort 1, mice were tested at 12 weeks of age on holeboard, at 14 weeks for partition test, at 15 weeks for conditioned fear, at 20 weeks for rotarod, and at 21 weeks for marble burying. Cohort 2 was tested at 6 weeks for rotarod, at 8 weeks for PPI, at 9 weeks for dowel walk, and at 19 weeks for OFA. For the SOM conditional knockout mice, one cohort of naïve mice was subjected to the same tests as the PV group at the same timepoints except for the conditioned fear test. An additional two naïve cohorts were tested for conditioned fear at 10 and 15 wk. Each cohort consisted of at least 10 litters. All behavioral assessments were carried out during the light cycle, generally in the afternoon. The investigator was blinded to all genotypes until after completion of data collection.

Rotarod

Mice were habituated in the test room for 30 minutes. Mice were placed on the rotating cylinder of an accelerating rotarod apparatus (Ugo Basile) and allowed to move freely as the rotation increased from 5 rpm to 40 rpm over a five-minute period. "Latency to fall" was recorded either when the mouse fell from the rod or when the mouse had ridden the rotating rod for two revolutions without regaining control. Data are shown as mean ± standard error of mean. Latency to fall was analyzed by two-way ANOVA with Bonferroni's post hoc analysis.

Dowel Walk

Mice were habituated in the test room for 30 minutes. The mouse was placed on a 0.635 cm diameter dowel with all four paws allowed to grip the dowel. Latency to fall from the dowel was recorded during the two-minute test. Data are shown as mean ± standard error of mean and was analyzed by Kruskal-Wallis test followed by Dunn's post hoc analysis.

Digigait Analysis of Gait

To increase contrast between the feet and fur, the soles of each test mouse's paws were painted with red food coloring. The mice were then placed in the test room to habituate for 30 minutes. Each mouse was placed on the imaging surface of a MSI Digigait™ Imaging System apparatus and recorded for at least 5 seconds of continuous walking using the Digigait™ Imager software. Gait was analyzed using the MSI Digigait™ Imaging Analyses software (version 10.0). Data are shown as mean ± standard error of mean and was analyzed by one-way ANOVA with Tukey's post hoc analysis.

Marble Burying Test

Mice were habituated for 30 minutes in the test room. A standard mouse housing cage was 50% filled with clean bedding material and 20 black glass marbles were placed in a 4x5 grid pattern on the surface of the bedding. Mice were placed individually into the prepared cage for 30 minutes. After the mouse was removed, the number of buried marbles were counted, with a marble considered buried if 75% of its surface was covered with bedding. Data are shown as mean ± standard error of mean and were analyzed by one-way ANOVA with Tukey's post hoc analysis.

Acoustic Startle and Prepulse Inhibition (PPI)

Mice were habituated for 30 minutes outside the test room. Each mouse was placed individually in a SR-LAB PPI apparatus (San Diego Instruments), which consists of a Plexiglass tube-shaped holder in a sound-insulated lighted box with 70dB white noise, and allowed to habituate for 5 minutes. The mouse was presented with eight types of stimulus, each presented six times in pseudo-random order with a 10-20 sec intertrial period: no sound; a 40ms 120db startle burst; three 20ms prepulse sounds of 74, 78, and 82dB, each presented alone; and a combination of each of the three prepulse intensities presented 100ms before the 120dB startle burst. After the test, mice were returned to their home cage. The acoustic startle response was recorded every 1ms during the 65ms period following the onset of the startle stimulus and was calculated as the average response to the 120db startle burst normalized to body weight. Percent PPI was calculated using the following formula: (1-(averaged startle response to prepulse before startle stimulus/averaged response to startle stimulus)) x 100.

Data is shown as mean ± standard error of mean. Percent PPI was analyzed by two-way ANOVA with Bonferroni's post hoc analysis, and acoustic startle response was analyzed by one-way ANOVA with Tukey's post hoc analysis.

Partition Test

Mice were single-housed for 48 hours on one side of a standard mouse housing cage. The cage was divided across its width by a divider with holes small enough to allow scent but no physical interaction. The test mouse was provided with a KimWipe folded in fourths as nesting material, food, and a water bottle. At least 16 hours before the partition tests, a novel age- and gender-matched C57Bl/6 partner mouse was placed on the opposite side of the partition. On the day of the test, the cage was placed on a well-lit flat surface. All nesting material and water bottles were removed from both sides of the cage, and the test mice were observed for 5 minutes while interaction time with the now-familiar partner mouse was recorded. Interactions involved the test mouse smelling, chewing, or actively exploring the partition. At the end of the first test (Familiar 1), the familiar partner mouse was replaced by a novel mouse of the same age, gender, and strain, and test mouse interactions were recorded for five minutes (Novel). The novel mouse was then removed and the familiar partner mouse returned to the cage, followed by observation for another 5 minutes (Familiar 2). At the completion of the partition test, test mice were returned to their original home cage. Data are shown as mean ± standard error of mean. Interaction times were analyzed by two-way ANOVA with Holm-Sidak's post hoc analysis.

Conditioned Fear

Mice were habituated for 30 minutes outside the test room. Mice were placed singly into the conditioned fear apparatus (Coulbourn Instruments) that consisted of a lighted box with a grated floor. On the training day, mice were placed in the chamber and subjected to two rounds of training, each of which consisted of 180 seconds of silence followed by a 30 second-long 80-85dB tone and 2 seconds of a 0.72 mA shock. 25 hours after training, the grated floor of the test chamber was covered and the

shape changed with plastic panels and vanilla scent was added to the chamber. Mice were returned to the apparatus and subjected to a cue test consisting of 180 seconds of silence followed by 180 seconds of the original 80-85dB tone. Freezing behavior for training and cue tests was scored using Freeze Frame 3 software (Actimetrics) with a threshold of 5.0. Data is shown as mean ± standard error of mean. Cue tests were analyzed by two-way ANOVA with Bonferroni's post hoc analysis.

Holeboard

Mice were habituated for 30 minutes in the test room with full room light and 60db white noise. The apparatus was the same as that used in the OFA test but with a modified floor consisting of a white plastic board punctured with 16 identical holes. Mice were placed in the middle of the cage and allowed to explore for five minutes while the experimenter recorded each nose poke into a hole. After five minutes, the mouse was returned to its home cage. Data were analyzed for the number of repeated and uninterrupted nosepokes into the same hole. Data are shown as mean ± standard error of mean. Data were analyzed by two-way ANOVA with Bonferroni's post hoc analysis.

Light Dark

Mice were habituated for 30 minutes in the test room lit at 200 lux with white noise playing at 60dB. Mice were placed singly in the light side of the light dark apparatus (OmniTech Electronics) and allowed to move freely for 10 minutes. Locomotion parameters and zones were recorded using Fusion activity monitoring software. Data is shown as mean ± standard error of mean. Time in Light was analyzed by one-way ANOVA with Tukey's post hoc analysis.

Elevated Plus Maze

Mice were habituated for 30 minutes in the test room lit at 200 lux with white noise playing at 60dB. The elevated plus maze is a plus sign-shaped maze with two opposite arm enclosed by walls and two opposite arms open without walls. The entire maze is elevated above the floor. Mice were placed singly at the intersection of the four arms and allowed to move freely for 10 minutes. Activity was recorded by a suspended digital camera and recorded by the ANY-maze software (Stoelting Co.).

Data is shown as mean ± standard error of mean. Time and distance in the open arm were each analyzed by one-way ANOVA with Tukey's post hoc analysis.

Open Field Assay

Mice were habituated for 30 minutes in the test room lit at 200 lux with white noise playing at 60dB. Each mouse was placed individually in the open field apparatus (OmniTech Electronics) and allowed to move freely for 30 minutes. Locomotion parameters and zones were recorded using Fusion activity monitoring software. Data are shown as mean ± standard error of mean and was analyzed by one-way ANOVA with Tukey's post hoc analysis.

Statistics Summary

Statistical tests for each behavioral assay were chosen based on their appropriateness for the assay. All statistical calculations were carried out using Graphpad Prism software. Variances were assessed using Bartlett's test for equal variances. Normalcy was determined using the D'Agostino and Pearson omnibus normality test. Statistical values for the behavioral tests, including all post hoc test p values, are shown in Table S1.

Supplemental References

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Guy, J., Gan, J., Selfridge, J., Cobb, S., and Bird, A. (2007). Reversal of Neurological Defects in a Mouse Model of Rett Syndrome. Science *315*, 1143-1147.

Guy, J., Hendrich, B., Holmes, M., Martin, J.E., and Bird, A. (2001). A mouse Mecp2-null mutation causes neurological symptoms that mimic Rett syndrome. Nat Genet *27*, 322-326.