Supporting Information

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SI Materials and Methods

Human Spinal Fluid Collection. The protocol and consent form were approved by the Institutional Review Boards of Baylor College of Medicine, University of Alabama–Birmingham, and the Children's Hospital of Eastern Ontario. Spinal fluid was collected from 64 girls with clinically defined RTT with pathogenic *MECP2* mutations using the consensus criteria (1). Spinal taps were performed using standard sterile technique under mild sedation (0.05 mg/kg midazolam). A 5-cc quantity of spinal fluid was collected into five predefined, prelabeled tubes provided by the examining laboratory at Baylor University Institute for Metabolic Studies, Dallas, TX, where the analysis for amine metabolites was performed using established HPLC techniques (2). A natural logarithm transformation was applied to the values of 5-HIAA and HVA to acquire a more normally distributed distribution for parametric statistical analysis. A general linear modeling technique was used to perform ANCOVA, comparing metabolite levels in groups (e.g., control vs. study, p.Arg168X vs. p.Arg133Cys) while controlling for age. Control data were restricted to the same age (2.5–28 years) as individuals with RTT.

HPLC Analysis of Biogenic Amine Levels. Monoamines in mouse brain tissue were measured after isocratic HPLC separation by electrochemical detection. Briefly, samples were homogenized (1:9 wt/vol) in ice-cold 0.4 M perchloric acid containing 1 mmol/L sodium bisulfite. Samples were centrifuged at 10,000 *g* for 10 min and 10 μl of the clear supernatant injected onto a SphereClone 3μ ODS C18 reversed phase column (100 × 4.6 mm, Phenomenex). The mobile phase consisted of 0.05M KH_2PO_4 containing 1 mM sodium octyl sulfate, 50 μM EDTA, and 9% methanol. The pH was adjusted to 3.0 using concentrated phosphoric acid. The flow rate was 1 ml/min and temperature was maintained at 35 °C. Analytes were detected and quantified using an ESA Coularray 8 electrode electrochemical detector (ESA). At least four animals per genotype were used. *Mecp2*$^{\mathrm{null/y}}$ animals and their respective wild-type littermates were 6–8 weeks of age, and aminergic-CKO animals and their respective littermates were at least 20 weeks of age.

Nonradioactive in Situ Hybridization. Probes were PCR amplified from wild-type mouse brain cDNA using the following primers, followed by digoxigenin labeling: *Th* (5′-GATTGCAGAGATTGCCTTCC-3′ and 5′-CCTGTGGGTGGTACCCTATG-3′); *Tph2* (5′-GTATTGAGAATGTGGTGCAGGA-3′ and 5′-CACTCAGTCTACATCCATCCCA-3′). ISH was performed on brain tissue obtained from *Mecp2*$^{\mathrm{null/y}}$ animals and their respective wild-type littermates at 6–8 weeks of age.

ChIP–PCR and ChIP-qPCR. ChIP using three *Mecp2*$^{\mathrm{null/y}}$ and three wild-type littermate brains was performed as previously described (3). DNA was PCR amplified using the following primers: *Th* (5′-GAAAGGTCCCCTCTCTGGTC-3′ and 5′-TTGAAGACACAGCCTGCAAC-3′, 60 °C anneal, 32 cycles, 347 bp product); *Tph2* (5′-CAAGCTTTCCTGTGGCTTTC-3′ and 5′-AACCCATGGTGTTTCCATGT-3′, 60 °C anneal, 32 cycles, 267 bp product). ChIP-qPCR was performed as previously described (3). The ddCT method was used to calculate the fold enrichment of chromatin fragments immunoprecipitated with anti-MeCP2 antibody (Millipore) compared with a control antibody (normal rabbit IgG, Millipore), relative to input samples. Statistical significance was determined using a paired *t* test.

Immunofluorescence. Coronal floating sections (50 μm) obtained from the brain of an adult (≈16- to 20-week old) mouse were processed and imaged as previously described (4). Primary antibodies used were anti-MeCP2 (1:100, Millipore) and anti-TH (1:1000, Sigma) for TH-CKO animals or anti-βgal (1:500, Abcam) for PET1-CKO animals that harbored an additional *ROSA*$^{\mathrm{R26R}}$ allele (5). Secondary antibodies used were goat anti-rabbit conjugated to Alexa 488 (MeCP2), and goat anti-mouse conjugated to Cy3 (TH) or chicken anti–βgal (Abcam).

Behavioral Analysis. TH-Cre animals were maintained on a pure FVB/N background. PET1-Cre animals were maintained on a pure C57BL/6 background. Mice were maintained on a 12 h light:12 h dark cycle with standard mouse chow and water ad libitum. All research and animal care procedures were approved by the Baylor College of Medicine Institutional Animal Care and Use Committee. Cre animals were bred to *Mecp2*$^{\mathrm{flox/+}}$ females on a pure 129S6/SvEv background. At least 15 animals per genotype were used for behavioral testing, except where indicated. All four subsequent male F1 progeny (wild-type, Cre, *Mecp2*$^{\mathrm{Flox/y}}$ and Cre; *Mecp2*$^{\mathrm{Flox/y}}$) were tested for motor function (dowel walking test at 16 weeks of age, and open-field arena at 15 weeks of age), motor learning (accelerating rotating rod at 13 weeks of age), anxiety-like behavior (light-dark box exploration task at 12–14 weeks of age), social behavior (partition test for social interest at 20 weeks of age), and learning and memory (fear conditioning at 21 weeks of age), as previously described (4, 6). For PET1-CKO animals, an additional cohort of at least 19 animals per group were generated for grooming, repetitive behavior, and breathing tests (see below). Marble burying was performed as previously described at 8 weeks of age (7). A resident intruder test was performed at 20 weeks of age on six to 10 animals per genotype as previously described (6). After whole-body plethysmography, a splash test for grooming was performed as previously described at ≈28 weeks of age (8).

Breathing Measurements. For TH-CKO animals, whole-body plethysmographic measurements of the frequency and depth of breathing were made from unrestrained male mice (16 weeks of age, 4 per genotype) as previously described (4). For PET1-CKO animals (≈27 weeks of age, 7–10 per genotype), mice were placed within unrestrained whole-body plethysmography chambers (Buxco), ≈500 ml in volume with a continuous flow rate of 1 L/min flushing the chambers with fresh air. Mice were allowed to acclimate for 20 min, and breathing was then recorded for 30 min. Breath waveforms and the instantaneous breathing rate were identified and calculated with Biosystem XA software (Buxco). Breathing rate distributions were constructed by determining the percentage of total accepted breaths spent at a particular breath rate with bins of 25 breaths/min.

Statistical Analysis. All data were analyzed using a commercially available statistical software package (SPSS, version 17.0). Analysis of HPLC, qPCR, ISH, and Western data were performed using a one-way ANOVA. The majority of behavior data were analyzed using two-way ANOVA (*Mecp2*-Flox allele and Cre allele), and one-way ANOVA followed by LSD post hoc comparisons ($P \leq 0.05$). Data related to accelerating rotating rod and partition test for social interest were analyzed using a three-way ANOVA with repeated measures (*Mecp2*-Flox allele, Cre allele, and day (for rotating rod) or encounter type (for social interest). Data related to dowel walking, resident intruder, and splash test were analyzed using Kruskal–Wallis with Mann–Whitney U post hoc comparisons made for significant differences ($P \leq 0.05$).

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5. Soriano P (1999) Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat Genet* 21:70–71.
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7. Thomas A, et al. (2009) Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. *Psychopharmacology (Berl)* 204:361–373.
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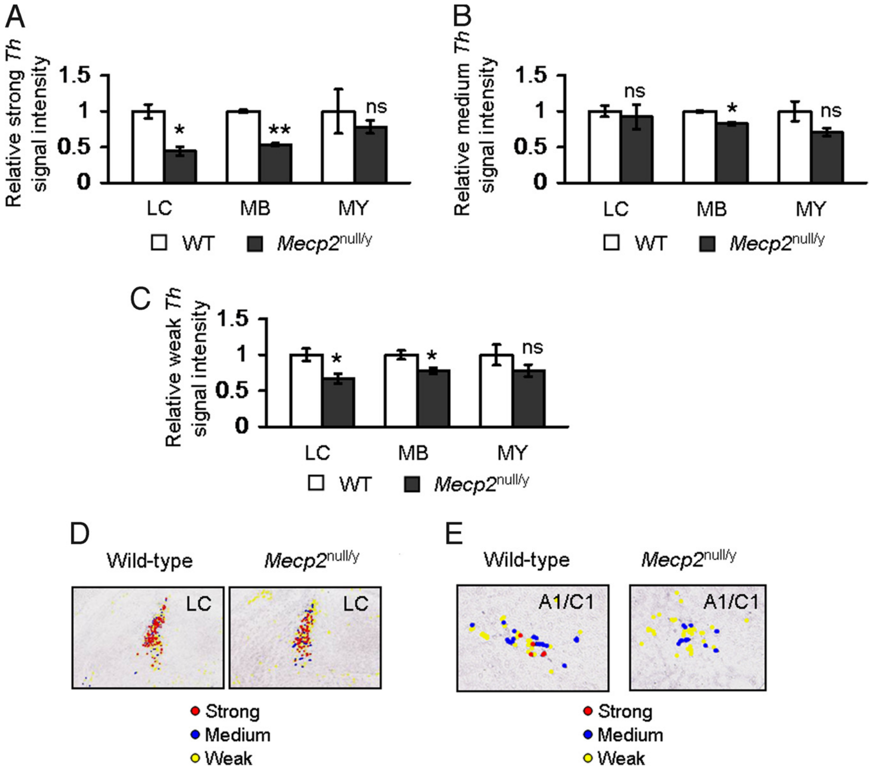

Fig. S1. Quantification of strong, medium, and weak *Th* ISH signal intensities in *Mecp2*$^{\mathrm{null/y}}$ animals. (*A*) Strong *Th* signal intensity is reduced in the locus ceruleus (LC) and midbrain (MB) but not the medulla (MY). (*B*) Medium *Th* signal intensity is reduced in MB but is otherwise not significantly decreased in LC and MY. (*C*) Weak *Th* signal intensity is reduced in the LC and MB but not the medulla. (*D*) Representative pseudocolored images of *Th* signal in the LC are shown. (*E*) Representative pseudocolored images of *Th* signal in the medullary A1/C1 nuclei are shown. *, $P \le 0.05$; **, $P \le 0.001$. ns, Not significant. Values were normalized to those of wild-type samples and represent mean ± SEM.

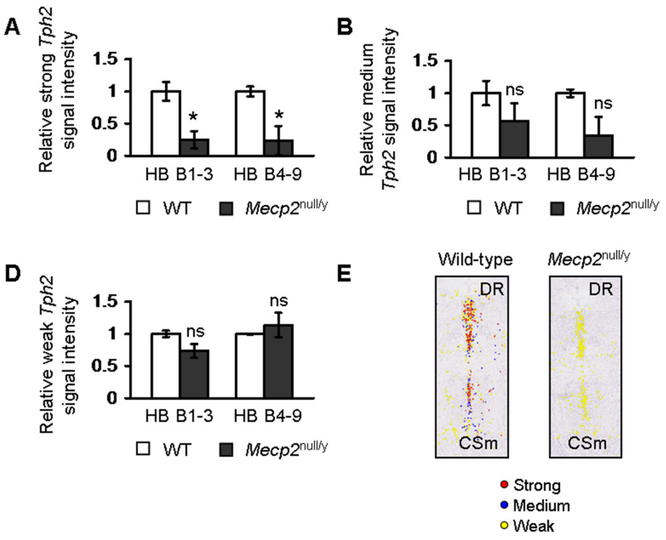

Fig. S2. Quantification of strong, medium, and weak *Tph2* ISH signal intensities in *Mecp2*$^{\text{null/y}}$ animals. (*A*) Strong signal intensity is reduced in all regions of the HB that include both raphe nuclei clusters (HB B1–3 and HB B4_9). (*B*) A nonsignificant trend in decreased medium *Tph2* signal intensity is observed in both HB B1–3 and HB B4–9 of *Mecp2*$^{\text{null/y}}$ animals. (*C*) Weak *Tph2* signal intensity is not significantly altered in *Mecp2*$^{\text{null/y}}$ animals. (*D*) Representative pseudocolored images of *Tph2* signal in HB4–9 are shown, the dorsal raphe nucleus (DR) and the medial part of the superior central raphe nucleus (CSm) are indicated. *, $P \leq 0.05$. ns, Not significant. Values were normalized to those of wild-type samples and represent mean ± SEM.

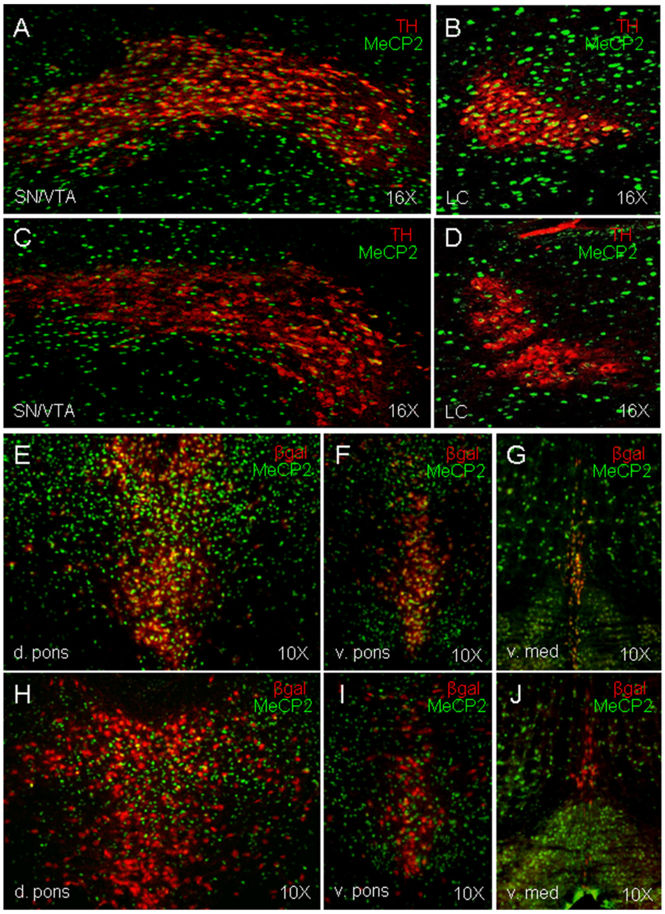

Fig. S3. Efficient recombination within Cre expression domains for both TH CKO and Pet1 CKO lines. Immunofluorescence analysis of coronal sections through various brain regions was performed to demonstrate the efficiency of recombination at the *Mecp2* locus when the conditional allele was exposed to either TH-Cre (*C* and *D*) or Pet1-Cre (*H*–*J*). All sections were stained with anti-Mecp2 and labeled with a green fluorophore. To identify the TH-expressing neurons, sections in panels *A*–*D* were colabeled with an anti-TH antibody (red) and anti-MeCP2 antibody (green). To identify the Pet1 expression lineage, sections in panels *E*–*J* also contained a transgenic reporter that expresses the lacZ gene product β-galactosidase (βgal) in a Cre-dependent fashion. These sections were colabeled with an antibody that recognizes βgal (red) and anti-MeCP2 antibody (green). MeCP2 is lost in the majority of substantia nigra/ventral tegmental area (SN/VTA) cells and locus ceruleus (LC) in TH CKO animals (*C* and *D*) compared with Flox alone animals (*A* and *B*). Likewise, Pet1-CKO animals (*H*–*J*) showed a significant reduction compared with wild-type animals (*E*–*G*) in cells that express MeCP2 in the major serotonin producing neurons of the dorsal pons (*E* and *H*), ventral pons (*F* and *I*), and ventral medulla (*G* and *J*). Magnification is indicated for each image in the lower right-hand corner.

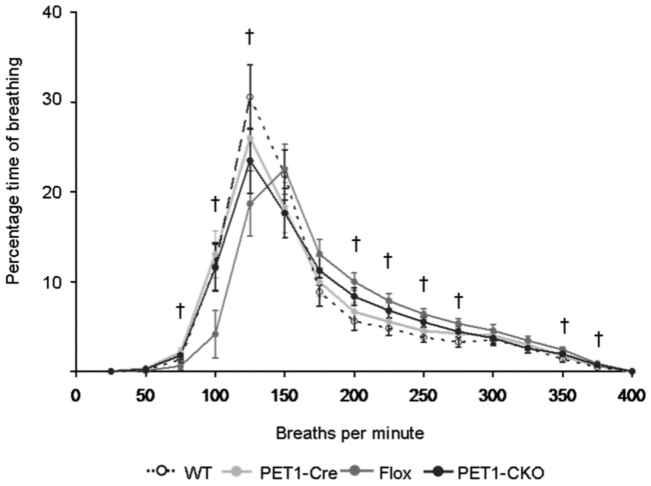

Fig. S4. Whole-body plethysmographic measurements indicated that Flox animals spent more time breathing faster compared with controls at 200 and 250 breaths/min; this was not worsened by deleting *Mecp2* in PET1-positive serotonergic neurons. †, Flox effect compared with WT or Cre, $P \leq .05$.

Table S1. Raw values for HPLC analyses

Human data (age-adjusted mean)	HVA	5-HIAA	
Control individuals	398.38 ± 1.02	130.75 ± 1.03	
Rett individuals	319.06 ± 1.04	101.42 ± 1.05	
R168X individuals	240.33 ± 24.69	76.78 ± 8.34	
R133C individuals	390.33 ± 55.42	129.54 ± 19.61	
Murine data	DA	NE	5HT
Wild-type	794.57 ± 12.85	284.36 ± 11.93	405.10 ± 3.16
Mecp2$^{\mathrm{null/y}}$	579.91 ± 10.28	173.68 ± 9.54	291.53 ± 2.53
Wild-type	796.51 ± 2.43	275.82 ± 14.65	464.13 ± 8.93
TH-Cre	854.59 ± 1.88	269.87 ± 11.30	461.57 ± 6.89
Flox	702.95 ± 1.88	217.71 ± 11.30	371.48 ± 6.89
TH-CKO	404.98 ± 2.43	159.59 ± 14.65	393.98 ± 8.93
Wild-type	940.89 ± 20.86	291.06 ± 17.34	486.49 ± 7.24
PET1-Cre	946.85 ± 24.36	291.00 ± 12.80	448.38 ± 7.36
Flox	734.08 ± 16.34	225.42 ± 17.74	392.17 ± 5.36
PET1-CKO	746.13 ± 27.21	226.76 ± 19.33	271.74 ± 12.65

Values are mean ± SEM.

Table S2. Statistical summary of behavioral data

Behavioral paradigm	Measurement	Statistical test	Comparison	Statistics	Degrees of freedom	*p*	Fig.
TH-CKO: Open field	Total distance traveled (cm)	Two-way ANOVA	Factor 1: *Mecp2*-Flox allele	F = 13.54	1, 57	0.001	4*A*
			Factor 2: TH-Cre allele	F <0.001	1, 57	0.99	
			Interaction (F1 × F2)	F = 8.71	1, 57	0.01	
		One-way ANOVA	Genotype	F = 7.55	3, 57	<0.001	
		Fisher's LSD	TH-CKO vs. WT			0.01	
			TH-CKO vs. Cre			<0.001	
			TH-CKO vs. Flox			0.05	
			Flox vs. WT			0.61	
			Flox vs. Cre			0.01	
			WT vs. Cre			0.15	
	Vertical activity (no. of beam breaks)	One-way ANOVA	Genotype	F = 5.53	3, 57	0.00	4*B*
		Two-way ANOVA	Factor 1: *Mecp2*-Flox allele	F = 6.40	1, 57	0.01	
			Factor 2: TH-Cre allele	F = 0.50	1, 57	0.48	
			Interaction (F1 × F2)	F = 9.54	1, 57	0.003	
		Fisher's LSD	TH-CKO vs. WT			0.02	
			TH-CKO vs. Cre			<0.001	
			TH-CKO vs. Flox			0.01	
			Flox vs. WT			0.70	
			Flox vs. Cre			0.21	
			WT vs. Cre			0.09	
TH-CKO: Dowel walking	Side touches (total no.)	Kruskal–Wallis		H = 17.54	3	<0.001	4C
		Mann–Whitney *U*	TH-CKO vs. WT			<0.001	
			TH-CKO vs. Cre			<0.001	
			TH-CKO vs. Flox			0.02	
			Flox vs. WT			0.22	
			Flox vs. Cre			0.07	
			WT vs. Cre			0.54	
TH-CKO: Plethysmography	Apneas per hour	Two-way ANOVA	Factor 1: *Mecp2*-Flox allele	F = 87.97	1, 12	<0.001	4D
			Factor 2: TH-Cre allele	F = 0.78	1, 12	0.40	
			Interaction (F1 × F2)	F = 0.36	1, 12	0.56	
		One-way ANOVA	Genotype	F = 29.70	3, 12	<0.001	
		Fisher's LSD	TH-CKO vs. WT			<0.001	
			TH-CKO vs. Cre			<0.001	
			TH-CKO vs. Flox			0.05	
			Flox vs. WT			0.32	
			Flox vs. Cre			<0.001	
			WT vs. .Cre			0.84	
TH-CKO: Rotating rod	Latency to fall (sec)	Three-way repeated-measures ANOVA	Factor 1: Mecp2-Flox allele	F = 3.88	1, 59	0.05	4*E*
			Factor 2: TH-Cre allele	F = 0.02	1, 59	0.88	
			Factor 3: Day	F = 53.71	3, 177	<0.001	
			Interaction (F1 × F3)	F = 1.01	3, 177	0.39	
			Interaction (F2 × F3)	F = 0.58	3, 177	0.63	
			Interaction (F1 × F2)	F = 0.98	1, 59	0.33	
			Interaction (F1 × F2 × F3)	F = 2.23	3, 177	0.09	
		One-way ANOVA: Day 1	Genotype	F = 0.51	3, 59	0.68	
		Fisher's LSD: Day 1	TH-CKO vs. WT			0.59	
			TH-CKO vs. Cre			0.37	
			TH-CKO vs. Flox			0.87	
			Flox vs. WT			0.47	
			Flox vs. Cre			0.28	
			WT vs. Cre			0.71	
		One-way ANOVA: Day 2	Genotype	F = 1.35	3, 59	0.27	
		Fisher's LSD: Day 2	TH-CKO vs. WT			0.40	

Behavioral paradigm	Measurement	Statistical test	Comparison	Statistics	Degrees of freedom	*p*	Fig.
TH-CKO: Rotating rod (cont'd)	Latency to fall (sec)	Fisher's LSD: Day 2	TH-CKO vs. Cre			0.50	4*E*
			TH-CKO vs. Flox			0.35	
			Flox vs. WT			0.08	
			Flox vs. Cre			0.11	
			WT vs. Cre			0.87	
		One-way ANOVA: Day 3	Genotype	F = 1.08	3, 59	0.18	
		Fisher's LSD: Day 3	TH-CKO vs. WT			0.23	
			TH-CKO vs. Cre			0.75	
			TH-CKO vs. Flox			0.33	
			Flox vs. WT			0.03	
			Flox vs. Cre			0.19	
			WT vs. Cre			0.36	
		One-way ANOVA: Day 4	Genotype	F = 2.62	3, 59	0.06	
		Fisher's LSD: Day 4	TH-CKO vs. WT			0.07	
			TH-CKO vs. Cre			0.59	
			TH-CKO vs. Flox			0.42	
			Flox vs. WT			0.01	
			Flox vs. Cre			0.17	
			WT vs. Cre			0.18	
TH-CKO: Light–dark exploration	Time in lit side (% time)	Two-way ANOVA	Factor 1: *Mecp2*-Flox allele	F = 0.58	1, 58	0.45	4*F*
			Factor 2: TH-Cre allele	F = 0.15	1, 58	0.70	
			Interaction (F1 × F2)	F = 1.02	1, 58	0.32	
		One-way ANOVA	Genotype	F = 0.58	3, 58	0.63	
		Fisher's LSD	TH-CKO vs. WT			0.79	
			TH-CKO vs. Cre			0.86	
			TH-CKO vs. Flox			0.34	
			Flox vs. WT			0.25	
			Flox vs. Cre			0.42	
			WT vs. Cre			0.66	
TH-CKO: Partition	Time social interest (% time)	Three-way repeated measures ANOVA	Factor 1: Mecp2-Flox allele	F = 6.05	1, 57	0.02	4*G*
			Factor 2: TH-Cre allele	F = 1.49	1, 57	0.23	
			Factor 3: Encounter	F = 121.58	2, 114	<0.001	
			Interaction (F1 × F3)	F = 2.62	2, 114	0.08	
			Interaction (F2 × F3)	F = 0.06	2, 114	0.94	
			Interaction (F1 × F2)	F = 3.78	1, 57	0.06	
			Interaction (F1 × F2 × F3)	F = 1.04	2, 114	0.36	
		One-way ANOVA: Familiar	Genotype	F = 1.31	3, 57	0.28	
		Fisher's LSD: Familiar	TH-CKO vs. WT			0.96	
			TH-CKO vs. Cre			0.93	
			TH-CKO vs. Flox			0.11	
			Flox vs. WT			0.11	
			Flox vs. Cre			0.13	
			WT vs. Cre			0.89	
		One-way ANOVA: Novel	Genotype	F = 3.44		0.02	
		Fisher's LSD: Novel	TH-CKO vs. WT			0.18	
			TH-CKO vs. Cre			0.25	
			TH-CKO vs. Flox			0.15	
			Flox vs. WT			0.01	
			Flox vs. Cre			0.01	
			WT vs. Cre			0.82	
		One-way ANOVA: Familiar, 2nd encounter	Genotype	F = 3.26		0.03	
		Fisher's LSD: Familiar, 2nd encounter	TH-CKO vs. WT			0.64	

Behavioral paradigm	Measurement	Statistical test	Comparison	Statistics	Degrees of freedom	*p*	Fig.
			TH-CKO vs. Cre			0.73	
			TH-CKO vs. Flox			0.02	
			Flox vs. WT			0.01	
			Flox vs. Cre			0.04	
			WT vs. Cre			0.41	
TH-CKO: Fear conditioning	Cue (% freezing)	Two-way ANOVA	Factor 1: *Mecp2*-Flox allele	F = 2.05	1, 59	0.16	4*H*
			Factor 2: TH-Cre allele	F = 0.57	1, 59	0.45	
			Interaction (F1 × F2)	F = 0.11	1, 59	0.74	
		One-way ANOVA	Genotype	F = 0.92	3, 59	0.44	
		Fisher's LSD	TH-CKO vs. WT			0.64	
			TH-CKO vs. Cre			0.22	
			TH-CKO vs. Flox			0.77	
			Flox vs. WT			0.44	
			Flox vs. Cre			0.12	
			WT vs. Cre			0.44	
		Two-way ANOVA	Factor 1: *Mecp2*-Flox allele	F = 18.39	1, 59	<0.001	
			Factor 2: TH-Cre allele	F = 2.04	1, 59	0.16	
			Interaction (F1 × F2)	F = 0.05	1, 59	0.82	
		One-way ANOVA	Genotype	F = 6.90	3, 59	<0.001	
	Context (% freezing)	Fisher's LSD	TH-CKO vs. WT			0.05	
			TH-CKO vs. Cre			0.01	
			TH-CKO vs. Flox			0.25	
			Flox vs. WT			0.00	
			Flox vs. Cre			0.00	
			WT vs. Cre			0.40	
PET1-CKO: Partition	Time social interest (% time)	Three-way repeated measures ANOVA	Factor 1: Mecp2-Flox allele	F = 24.47	1, 60	<0.001	5*A*
			Factor 2: PET1-Cre allele	F = 0.43	1, 60	0.52	
			Factor 3: Encounter	F = 124.27	2, 120	<0.001	
			Interaction (F1 × F3)	F = 2.40	2, 120	0.09	
			Interaction (F2 × F3)	F = 0.36	2, 120	0.70	
			Interaction (F1 × F2)	F = 0.14	1, 60	0.71	
			Interaction (F1 × F2 × F3)	F = 0.24	2, 120	0.79	
		One-way ANOVA: Familiar	Genotype	F = 3.36	3, 60	0.02	
		Fisher's LSD: Familiar	PET1-CKO vs. WT			0.04	
			PET1-CKO vs. Cre			0.13	
			PET1-CKO vs. Flox			0.51	
			Flox vs. WT			0.01	
			Flox vs. Cre			0.03	
			WT vs. Cre			0.52	
		One-way ANOVA: Novel	Genotype	F = 4.43	3, 60	0.01	
		Fisher's LSD: Novel	PET1-CKO vs. WT			<0.001	
			PET1-CKO vs. Cre			0.02	
			PET1-CKO vs. Flox			0.66	
			Flox vs. WT			0.01	
			Flox vs. Cre			0.05	
			WT vs. Cre			0.54	
		One-way ANOVA: Familiar, 2nd encounter	Genotype	F = 7.92	3, 60	0.01	
		Fisher's LSD: Familiar, 2nd encounter	PET1-CKO vs. WT			<0.001	
			PET1-CKO vs. Cre			<0.001	
			PET1-CKO vs. Flox			0.66	
			Flox vs. WT			<0.001	
			Flox vs. Cre			<0.001	
			WT vs. Cre			0.64	

Behavioral paradigm	Measurement	Statistical test	Comparison	Statistics	Degrees of freedom	p	Fig.
		Kruskal–Wallis		H = 16.75	3	0.00	
PET1-CKO: Resident intruder	Time aggressive (% time)	Mann–Whitney *U*	PET1-CKO vs. WT			<0.001	5*B*
			PET1-CKO vs. Cre			<0.001	
			PET1-CKO vs. Flox			<0.001	
			Flox vs. WT			<0.001	
			Flox vs. Cre			<0.001	
			WT vs. Cre			1.00	
PET1-CKO: Light–dark exploration	Time in lit side (% time)	Two-way ANOVA	Factor 1: *Mecp2*-Flox allele	F = 0.11	1, 60	0.75	5C
			Factor 2: PET1-Cre allele	F = 0.39	1, 60	0.53	
			Interaction (F1 × F2)	F = 0.002	1, 60	0.97	
		One-way ANOVA	Genotype	F = 0.17	3, 60	0.92	
		Fisher's LSD	PET1-CKO vs. WT			0.83	
			PET1-CKO vs. Cre			0.84	
			PET1-CKO vs. Flox			0.64	
			Flox vs. WT			0.80	
			Flox vs. Cre			0.50	
			WT vs. Cre			0.68	
		Kruskal–Wallis		H = 6.83	3	0.08	
PET1-CKO: Splash test	Grooming (% time)	Mann–Whitney *U*	PET1-CKO vs. WT			0.96	5*D*
			PET1-CKO vs. Cre			0.33	
			PET1-CKO vs. Flox			0.13	
			Flox vs. WT			0.11	
			Flox vs. Cre			0.02	
			WT vs. Cre			0.28	
PET-CKO: Marble burying	Marbles buried (total #)	Two-way ANOVA	Factor 1: *Mecp2*-Flox allele	F = 29.34	1, 76	<0.001	5*E*
			Factor 2: PET1-Cre allele	F = 0.07	1, 76	0.79	
			Interaction (F1 × F2)	F = 1.05	1, 76	0.31	
		One-way ANOVA	Genotype	F = 10.13	3, 76	<0.001	
		Fisher's LSD	PET1-CKO vs. WT			<0.001	
			PET1-CKO vs. Cre			<0.001	
			PET1-CKO vs. Flox			0.60	
			Flox vs. WT			<0.001	
			Flox vs. Cre			<0.001	
			WT vs. Cre			0.36	
PET1-CKO: Open field	Total distance traveled (cm)	Two-way ANOVA	Factor 1: *Mecp2*-Flox allele	F = 4.70	1, 60	0.03	5*F*
			Factor 2: PET1-Cre allele	F = 5.19	1, 60	0.03	
			Interaction (F1 × F2)	F = 0.30	1, 60	0.59	
		One-way ANOVA	Genotype	F = 3.40	3, 60	0.02	
		Fisher's LSD	PET1-CKO vs. WT			<0.001	
			PET1-CKO vs. Cre			0.06	
			PET1-CKO vs. Flox			0.05	
			Flox vs. WT			0.26	
			Flox vs. Cre			0.94	
			WT vs. Cre			0.22	
		Kruskal–Wallis		H = 1.41	3	0.07	5*G*
PET1-CKO: Dowel walking	Side touches (total #)	Mann–Whitney *U*	PET1-CKO vs. WT			0.48	
			PET1-CKO vs. Cre			0.85	
			PET1-CKO vs. Flox			0.71	
			Flox vs. WT			0.22	
			Flox vs. Cre			0.52	
			WT vs. Cre			0.60	
PET1-CKO: Rotating rod	Latency to fall (sec)	Three-way repeated measures ANOVA	Factor 1: Mecp2-Flox allele	F = 8.97	1, 76	<0.001	5*H*
			Factor 2: PET1-Cre allele	F = 10.32	1, 76	<0.001	

Behavioral paradigm	Measurement	Statistical test	Comparison	Statistics	Degrees of freedom	*p*	Fig.
			Factor 3: Day	F = 127.02	3, 228	<0.001	
			Interaction (F1 × F3)	F = 2.67	3, 228	0.05	
			Interaction (F2 × F3)	F = 6.46	3, 228	<0.001	
			Interaction (F1 × F2)	F = 1.01	1, 76	0.32	
			Interaction (F1 × F2 × F3)	F = 0.35	3, 228	0.79	
		One-way ANOVA: Day 1	Genotype	F = 3.65	3, 76	0.02	
		Fisher's LSD: Day 1	PET1-CKO vs. WT			0.66	
			PET1-CKO vs. Cre			0.01	
			PET1-CKO vs. Flox			0.76	
			Flox vs. WT			0.46	
			Flox vs. Cre			<0.001	
			WT vs. Cre			0.03	
		One-way ANOVA: Day 2	Genotype	F = 4.91	3, 76	<0.001	
PET1-CKO: Rotating rod (cont'd)	Latency to fall (sec)	Fisher's LSD: Day 2	PET1-CKO vs. WT			0.74	5*H*
			PET1-CKO vs. Cre			<0.001	
			PET1-CKO vs. Flox			0.56	
			Flox vs. WT			0.36	
			Flox vs. Cre			<0.001	
			WT vs. Cre			0.01	
		One-way ANOVA: Day 3	Genotype	F = 6.39	3, 76	<0.001	
		Fisher's LSD: Day 3	PET1-CKO vs. WT			0.96	
			PET1-CKO vs. Cre			<0.001	
			PET1-CKO vs. Flox			0.20	
			Flox vs. WT			0.21	
			Flox vs. Cre			<0.001	
			WT vs. Cre			<0.001	
		One-way ANOVA: Day 4	Genotype	F = 5.30	3, 76	<0.001	
		Fisher's LSD: Day 4	PET1-CKO vs. WT			0.02	
			PET1-CKO vs. Cre			0.14	
			PET1-CKO vs. Flox			0.55	
			Flox vs. WT			0.14	
			Flox vs. Cre			<0.001	
			WT vs. Cre			0.02	
PET1-CKO: Fear conditioning	Cue (% freezing)	Two-way ANOVA	Factor 1: *Mecp2*-Flox allele	F = 0.58	1, 60	<0.001	5*I*
			Factor 2: PET1-Cre allele	F = 0.15	1, 60	0.180	
			Interaction (F1 × F2)	F = 1.02	1, 60	0.91	
		One-way ANOVA	Genotype	F = 3.53	3, 60	0.020	
		Fisher's LSD	PET1-CKO vs. WT			<0.001	
			PET1-CKO vs. Cre			0.05	
			PET1-CKO vs. Flox			0.39	
			Flox vs. WT			0.04	
			Flox vs. Cre			0.26	
			WT vs. Cre			0.29	
		Two-way ANOVA	Factor 1: *Mecp2*-Flox allele	F = 32.54	1, 60	<0.001	
	Context (% freezing)		Factor 2: PET1-Cre allele	F = 0.26	1, 60	0.61	
			Interaction (F1 × F2)	F = 2.48	1, 60	0.12	
		One-way ANOVA	Genotype	F = 11.94	3, 60	<0.001	
		Fisher's LSD	PET1-CKO vs. WT			<0.001	
			PET1-CKO vs. Cre			<0.001	
			PET1-CKO vs. Flox			0.15	
			Flox vs. WT			0.01	
			Flox vs. Cre			<0.001	
			WT vs. Cre			0.45	

Behavioral paradigm	Measurement	Statistical test	Comparison	Statistics	Degrees of freedom	*p*	Fig.
PET1-CKO: Plethysmography	Breathing distribution (% time breathing × 25 breaths per minute intervals)	Two-way ANOVA: 25 breaths/min	Factor 1: *Mecp2*-Flox allele	F = 0.20	1, 32	0.20	S4
			Factor 2: PET1-Cre allele	F = 0.08	1, 32	0.08	
			Interaction (F1 × F2)	F = 0.65	1, 32	0.65	
		One-way ANOVA	Genotype	F = 1.73	3, 32	0.18	
		Fisher's LSD	PET1-CKO vs. WT			0.74	
			PET1-CKO vs. Cre			0.22	
			PET1-CKO vs. Flox			0.35	
			Flox vs. WT			0.55	
			Flox vs. Cre			0.03	
			WT vs. Cre			0.12	
		Two-way ANOVA: 50 breaths/min	Factor 1: *Mecp2*-Flox allele	F = 0.50	1, 32	0.50	
			Factor 2: PET1-Cre allele	F = 0.09	1, 32	0.09	
			Interaction (F1 × F2)	F = 0.94	1, 32	0.94	
		One-way ANOVA	Genotype	F = 1.15	3, 32	0.34	
		Fisher's LSD	PET1-CKO vs. WT			0.46	
			PET1-CKO vs. Cre			0.60	
			PET1-CKO vs. Flox			0.25	
			Flox vs. WT			0.67	
			Flox vs. Cre			0.10	
			WT vs. Cre			0.21	
PET1-CKO: Plethysmography (cont'd)	Breathing distribution (% time breathing × 25 breaths per minute intervals)	Two-way ANOVA: 75 breaths/min	Factor 1: *Mecp2*-Flox allele	F = 0.23	1, 32	0.23	S4
			Factor 2: PET1-Cre allele	F = 0.03	1, 32	0.03	
			Interaction (F1 × F2)	F = 0.67	1, 32	0.67	
		One-way ANOVA	Genotype	F = 2.25	3, 32	0.10	
		Fisher's LSD	PET1-CKO vs. WT			0.47	
			PET1-CKO vs. Cre			0.58	
			PET1-CKO vs. Flox			0.07	
			Flox vs. WT			0.25	
			Flox vs. Cre			0.02	
			WT vs. Cre			0.20	
		Two-way ANOVA: 100 breaths/min	Factor 1: *Mecp2*-Flox allele	F = 0.09	1, 32	0.09	
			Factor 2: PET1-Cre allele	F = 0.11	1, 32	0.11	
			Interaction (F1 × F2)	F = 0.26	1, 32	0.26	
		One-way ANOVA	Genotype	F = 2.36	3, 32	0.09	
		Fisher's LSD	PET1-CKO vs. WT			0.97	
			PET1-CKO vs. Cre			0.69	
			PET1-CKO vs. Flox			0.06	
			Flox vs. WT			0.05	
			Flox vs. Cre			0.02	
			WT vs. Cre			0.72	
		Two-way ANOVA: 125 breaths/min	Factor 1: *Mecp2*-Flox allele	F = 0.05	1, 32	0.05	
			Factor 2: PET1-Cre allele	F = 0.983197910566376	1, 32	0.98	
			Interaction (F1 × F2)	F = 0.20	1, 32	0.20	
		One-way ANOVA	Genotype	F = 1.89	3, 32	0.15	
		Fisher's LSD	PET1-CKO vs. WT			0.17	
			PET1-CKO vs. Cre			0.63	
			PET1-CKO vs. Flox			0.35	
			Flox vs. WT			0.03	
			Flox vs. Cre			0.16	
			WT vs. Cre			0.38	

Behavioral paradigm	Measurement	Statistical test	Comparison	Statistics	Degrees of freedom	p	Fig.
		Two-way ANOVA: 150 breaths/min	Factor 1: *Mecp2*-Flox allele	F = 1.00	1, 32	1.00	
			Factor 2: PET1-Cre allele	F = 0.13	1, 32	0.13	
			Interaction (F1 × F2)	F = 0.82	1, 32	0.82	
		One-way ANOVA	Genotype	F = 0.81	3, 32	0.50	
		Fisher's LSD	PET1-CKO vs. WT			0.29	
			PET1-CKO vs. Cre			0.88	
			PET1-CKO vs. Flox			0.22	
			Flox vs. WT			0.87	
			Flox vs. Cre			0.28	
			WT vs. Cre			0.36	
		Two-way ANOVA: 175 breaths/min	Factor 1: *Mecp2*-Flox allele	F = 0.10	1, 32	0.10	
			Factor 2: PET1-Cre allele	F = 0.82	1, 32	0.82	
			Interaction (F1 × F2)	F = 0.36	1, 32	0.36	
		One-way ANOVA	Genotype	F = 1.28	3, 32	0.30	
		Fisher's LSD	PET1-CKO vs. WT			0.30	
			PET1-CKO vs. Cre			0.58	
			PET1-CKO vs. Flox			0.42	
			Flox vs. WT			0.07	
			Flox vs. Cre			0.18	
			WT vs. Cre			0.63	
		Two-way ANOVA: 200 breaths/min	Factor 1: *Mecp2*-Flox allele	F = 0.004	1, 32	<0.001	
			Factor 2: PET1-Cre allele	F = 0.76	1, 32	0.76	
			Interaction (F1 × F2)	F = 0.18	1, 32	0.18	
		One-way ANOVA	Genotype	F = 3.95	3, 32	0.02	
		Fisher's LSD	PET1-CKO vs. WT			0.05	
			PET1-CKO vs. Cre			0.22	
			PET1-CKO vs. Flox			0.24	
			Flox vs. WT			<0.001	
			Flox vs. Cre			0.02	
			WT vs. Cre			0.45	
		Two-way ANOVA: 225 breaths/min	Factor 1: *Mecp2*-Flox allele	F = 0.02	1, 32	0.02	
			Factor 2: PET1-Cre allele	F = 0.84	1, 32	0.84	
			Interaction (F1 × F2)	F = 0.30	1, 32	0.30	
		One-way ANOVA	Genotype	F = 2.51	3, 32	0.08	
PET1-CKO: Plethysmography (cont'd)	Breathing distribution (% time breathing × 25 breaths per minute intervals)	Fisher's LSD	PET1-CKO vs. WT			0.11	S4
			PET1-CKO vs. Cre			0.31	
			PET1-CKO vs. Flox			0.38	
			Flox vs. WT			0.02	
			Flox vs. Cre			0.06	
			WT vs. Cre			0.55	
		Two-way ANOVA: 250 breaths/min	Factor 1: *Mecp2*-Flox allele	F = 0.005	1, 32	<0.001	
			Factor 2: PET1-Cre allele	F = 0.88	1, 32	0.88	
			Interaction (F1 × F2)	F = 0.18	1, 32	0.18	
		One-way ANOVA	Genotype	F = 3.73	3, 32	0.02	
		Fisher's LSD	PET1-CKO vs. WT			0.05	
			PET1-CKO vs. Cre			0.25	
			PET1-CKO vs. Flox			0.29	
			Flox vs. WT			<0.001	
			Flox vs. Cre			0.03	
			WT vs. Cre			0.40	

Behavioral paradigm	Measurement	Statistical test	Comparison	Statistics	Degrees of freedom	*p*	Fig.
		Two-way ANOVA: 275 breaths/min	Factor 1: *Mecp2*-Flox allele	F = 0.05	1, 32	0.05	
			Factor 2: PET1-Cre allele	F = 0.96	1, 32	0.96	
			Interaction (F1 × F2)	F = 0.12	1, 32	0.12	
		One-way ANOVA	Genotype	F = 2.22	3, 32	0.11	
		Fisher's LSD	PET1-CKO vs. WT			0.15	
			PET1-CKO vs. Cre			0.76	
			PET1-CKO vs. Flox			0.28	
			Flox vs. WT			0.02	
			Flox vs. Cre			0.17	
			WT vs. Cre			0.25	
		Two-way ANOVA: 300 breaths/min	Factor 1: *Mecp2*-Flox allele	F = 0.58	1, 32	0.58	
			Factor 2: PET1-Cre allele	F = 0.78	1, 32	0.78	
			Interaction (F1 × F2)	F = 0.34	1, 32	0.34	
		One-way ANOVA	Genotype	F = 0.44	3, 32	0.72	
		Fisher's LSD	PET1-CKO vs. WT			0.85	
			PET1-CKO vs. Cre			0.77	
			PET1-CKO vs. Flox			0.38	
			Flox vs. WT			0.29	
			Flox vs. Cre			0.56	
			WT vs. Cre			0.63	
		Two-way ANOVA: 325 breaths/min	Factor 1: *Mecp2*-Flox allele	F = 0.67	1, 32	0.67	
			Factor 2: PET1-Cre allele	F = 0.66	1, 32	0.66	
			Interaction (F1 × F2)	F = 0.22	1, 32	0.22	
		One-way ANOVA	Genotype	F = 0.65	3, 32	0.59	
		Fisher's LSD	PET1-CKO vs. WT			0.99	
			PET1-CKO vs. Cre			0.56	
			PET1-CKO vs. Flox			0.24	
			Flox vs. WT			0.24	
			Flox vs. Cre			0.54	
			WT vs. Cre			0.57	
		Two-way ANOVA: 350 breaths/min	Factor 1: *Mecp2*-Flox allele	F = 0.06	1, 32	0.06	
			Factor 2: PET1-Cre allele	F = 0.83	1, 32	0.83	
			Interaction (F1 × F2)	F = 0.21	1, 32	0.21	
		One-way ANOVA	Genotype	F = 1.88	3, 32	0.15	
		Fisher's LSD	PET1-CKO vs. WT			0.22	
			PET1-CKO vs. Cre			0.62	
			PET1-CKO vs. Flox			0.30	
			Flox vs. WT			0.03	
			Flox vs. Cre			0.13	
			WT vs. Cre			0.46	
PET1-CKO: Plethysmography (cont'd)	Breathing distribution (% time breathing × 25 breaths/min intervals)	Two-way ANOVA: 375 breaths/min	Factor 1: *Mecp2*-Flox allele	F = 0.03	1, 32	0.03	S4
			Factor 2: PET1-Cre allele	F = 0.98	1, 32	0.98	
			Interaction (F1 × F2)	F = 0.20	1, 32	0.20	
		One-way ANOVA	Genotype	F = 2.36	3, 32	0.09	
		Fisher's LSD	PET1-CKO vs. WT			0.11	
			PET1-CKO vs. Cre			0.48	
			PET1-CKO vs. Flox			0.36	
			Flox vs. WT			0.02	
			Flox vs. Cre			0.11	
			WT vs. Cre			0.37	
		Two-way ANOVA: 400 breaths/min	Factor 1: *Mecp2*-Flox allele	F = 0.45	1, 32	0.45	

Behavioral paradigm	Measurement	Statistical test	Comparison	Statistics	Degrees of freedom	*p*	Fig.
			Factor 2: PET1-Cre allele	F = 0.62	1, 32	0.62	
			Interaction (F1 × F2)	F = 0.65	1, 32	0.65	
		One-way ANOVA	Genotype	F = 0.35	3, 32	0.79	
		Fisher's LSD	PET1-CKO vs. WT			0.85	
			PET1-CKO vs. Cre			0.40	
			PET1-CKO vs. Flox			0.97	
			Flox vs. WT			0.83	
			Flox vs. Cre			0.38	
			WT vs. Cre			0.50	