1 **SUPPLEMENTARY FIGURES**

2 **Title:** Profiling beneficial and adverse effects of MeCP2 overexpression in a hypomorphic Rett syndrome mouse

3 model

4 **Running Title:** MeCP2 overexpression in a hypomorphic Rett syndrome mouse

5 **Keywords:** Rett syndrome; MeCP2; *MECP2* Duplication syndrome; Neurodevelopment; Mouse model

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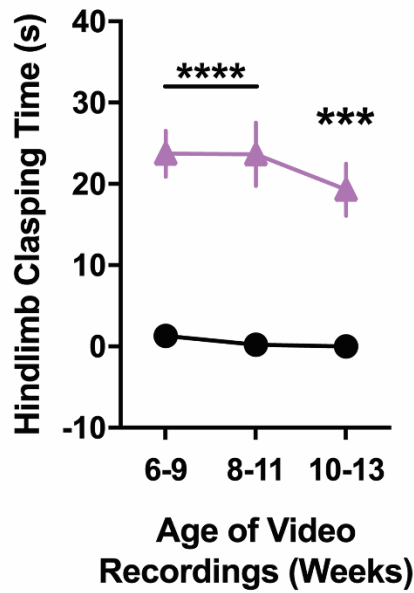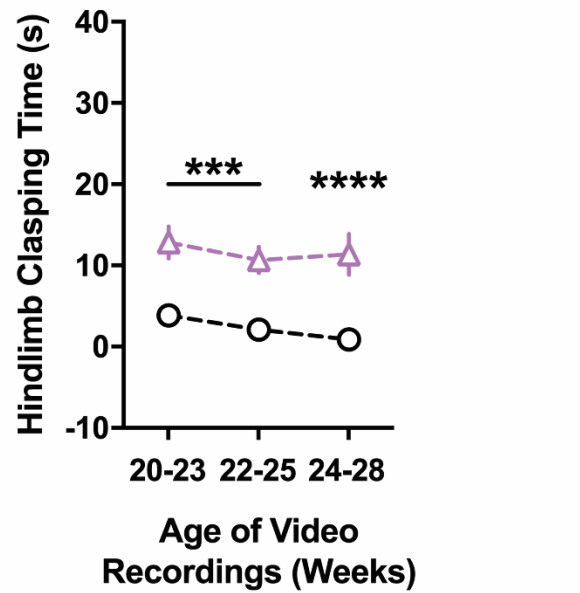18 Vanderbilt University

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A

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Supplementary Figure 1: Hindlimb clasping is maintained with age in *Mecp2 R133C* mice. (A-C) Hindlimb clasping was measured across age for male *Mecp2$^{R133C/y}$* (6-13 weeks old) and female *Mecp2$^{R133C/+}$* (20-28 weeks old). n=16-30 per genotype in males, n=31-32 per genotype in females. 2-way ANOVA with Sidak's post-hoc. *within-genotype comparison. ns (not significant), ***p<0.001, ****p<0.0001. WT = closed or open black circles. *Mecp2 R133C* = closed or open purple triangles.
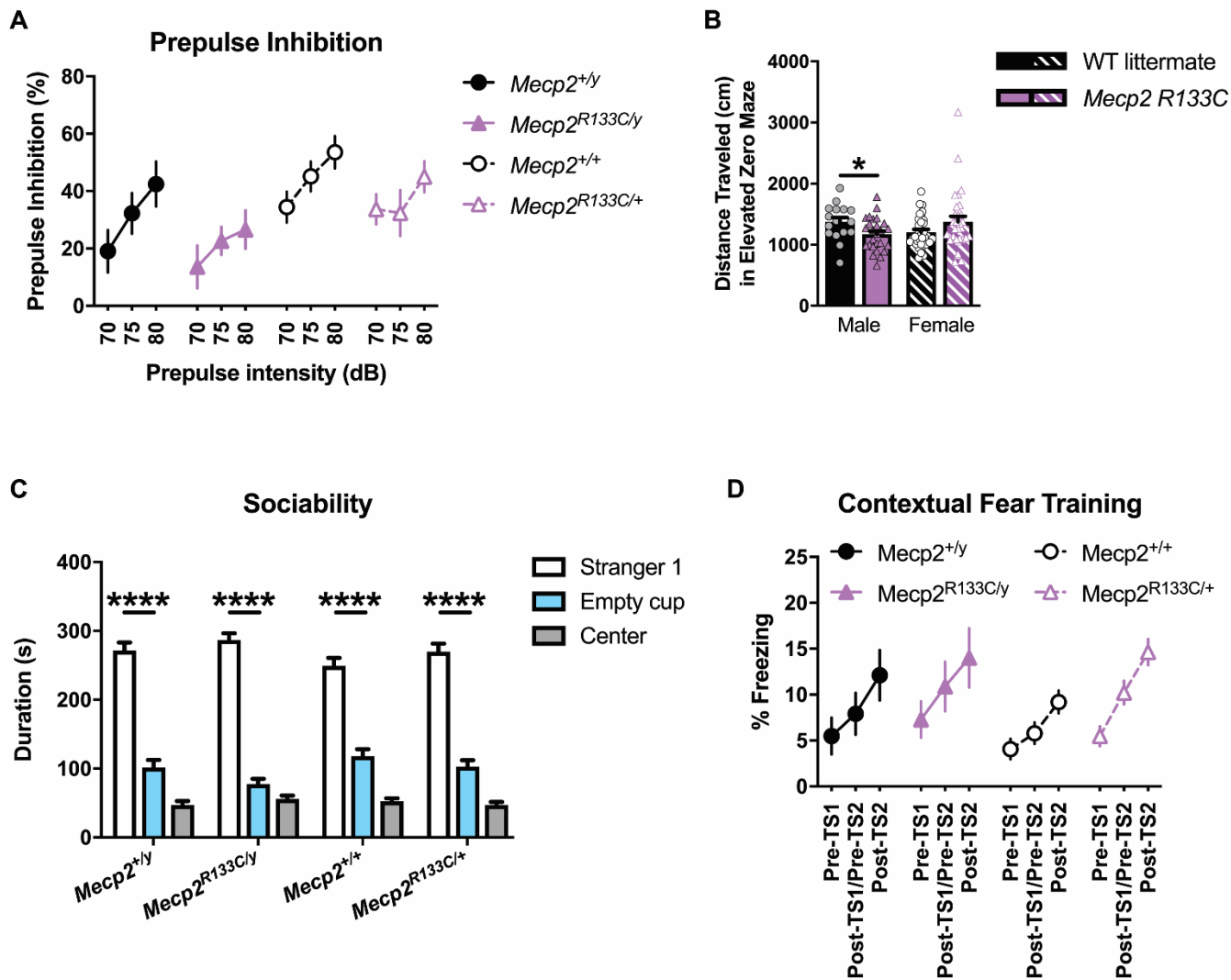
Supplementary Figure 2: Normal behaviors observed in *Mecp2 R133C* mice. (A-C) *Mecp2 R133C* mice displayed normal sensorimotor gating in pre-pulse inhibition (PPI) across several pre-pulse intensities (A), distance traveled in the elevated zero maze (B) and social behavior (C) assays, and spent more time with Stranger 1 mouse than the empty cup in the 3-chamber task. (D) On training day of contextual fear conditioning, male *Mecp2^{R133C/y}* and female *Mecp2^{R133C/+}* responded normally to the aversive stimuli. n=13-29 per genotype in males, n=18-32 per genotype in females. 2-way ANOVA with t-test post-hoc, or unpaired t-test. *within-genotype comparison. ****p<0.0001. WT = filled or patterned black bars or closed or open black circles. *Mecp2 R133C* = filled or patterned purple bars or closed or open purple triangles. Male = filled, closed. Female = patterned, open.
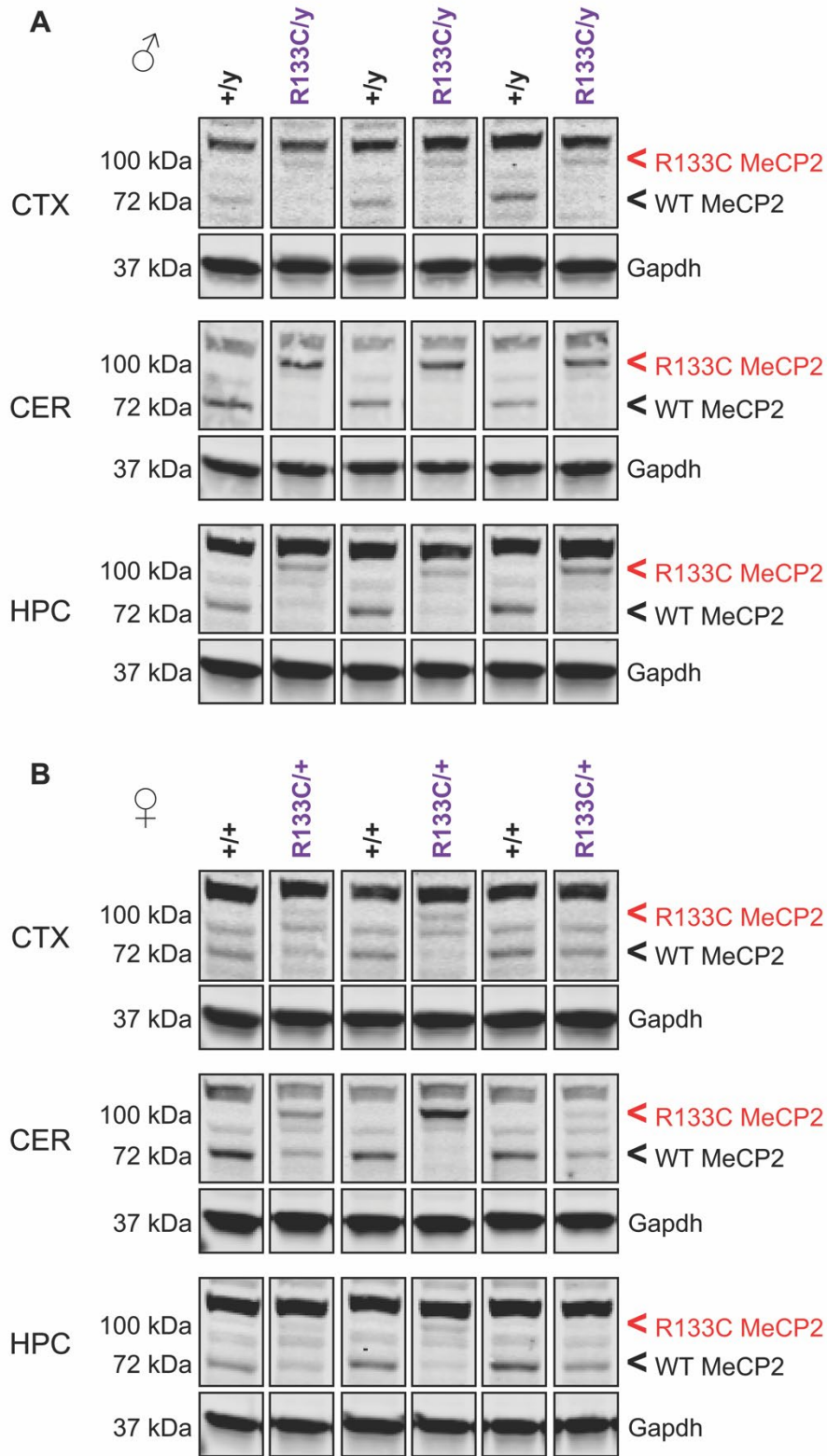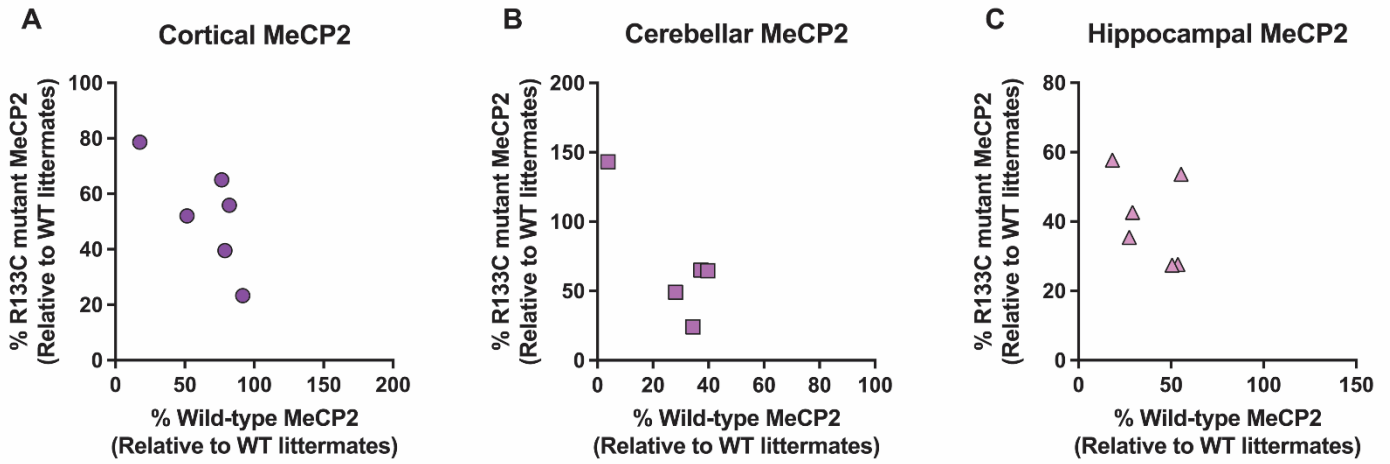
Supplementary Figure 3: WT and *R133C* mutant MeCP2 proteins are detected in brain samples via Western Blotting. (A-B) Representative images of Western blots and distinct bands are indicated for the *R133C* mutant (red arrow) and WT MeCP2 (black arrow), as well as the Gapdh control, in male (♂) and female (♀)

42   *Mecp2 R133C* mice and WT littermates across several brain regions. +/y or +/+ (WT), R133C/y or R133C/+

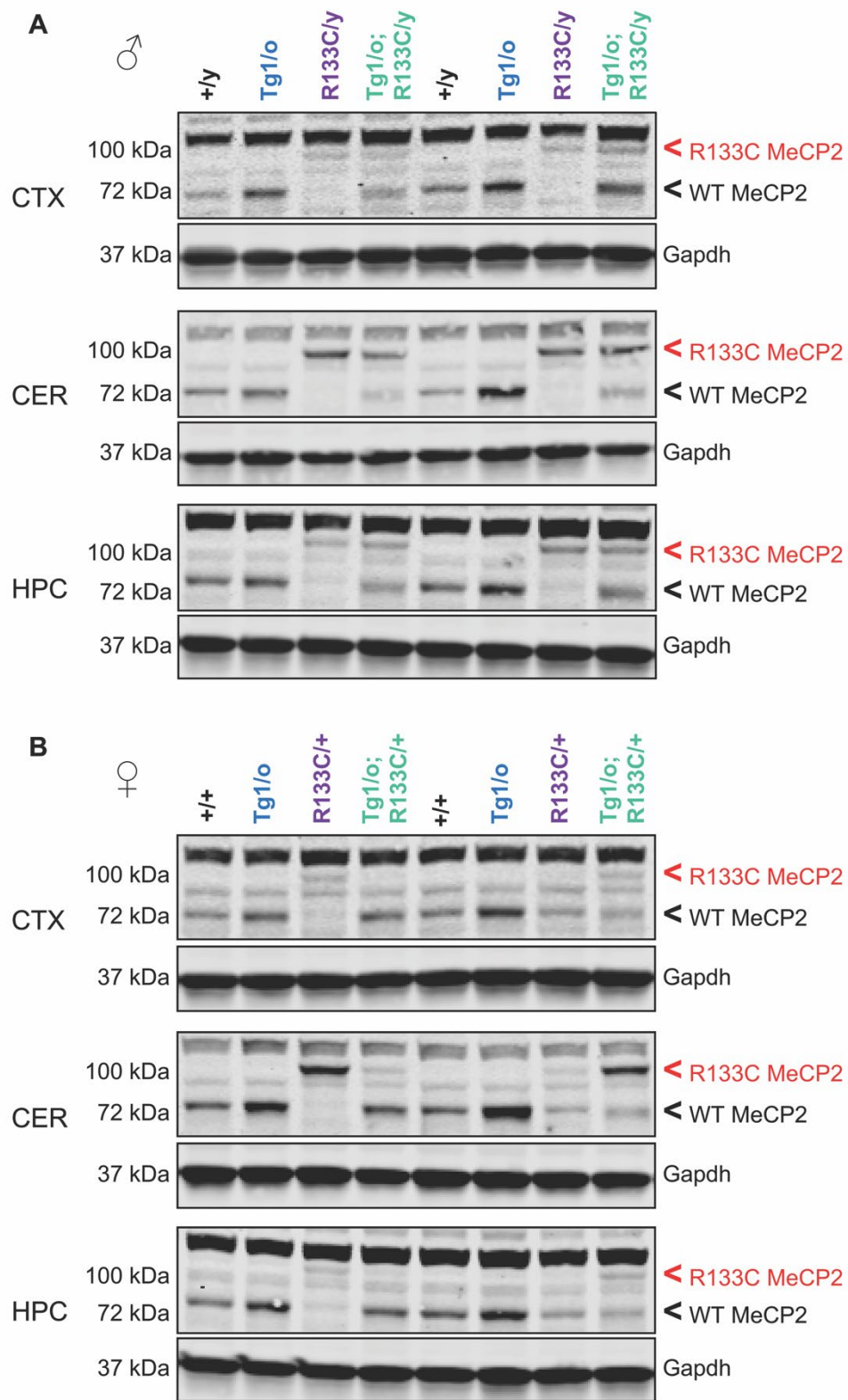43   (*Mecp2 R133C*). CTX (cortex), CER (cerebellum), HPC (hippocampus).

Supplementary Figure 4: Variability of WT and *R133C* mutant MeCP2 expression in *Mecp2^R133C/+* mice. The relationship of WT MeCP2 and the *R133C* mutant MeCP2 expression in the cortex, cerebellum and hippocampus of *Mecp2^R133C/+* mice is illustrated. Each point corresponds to a mouse subject, and the expression of both forms of MeCP2 is normalized to the Gapdh control and presented as percentage expression relative to MeCP2 expression in WT littermates.
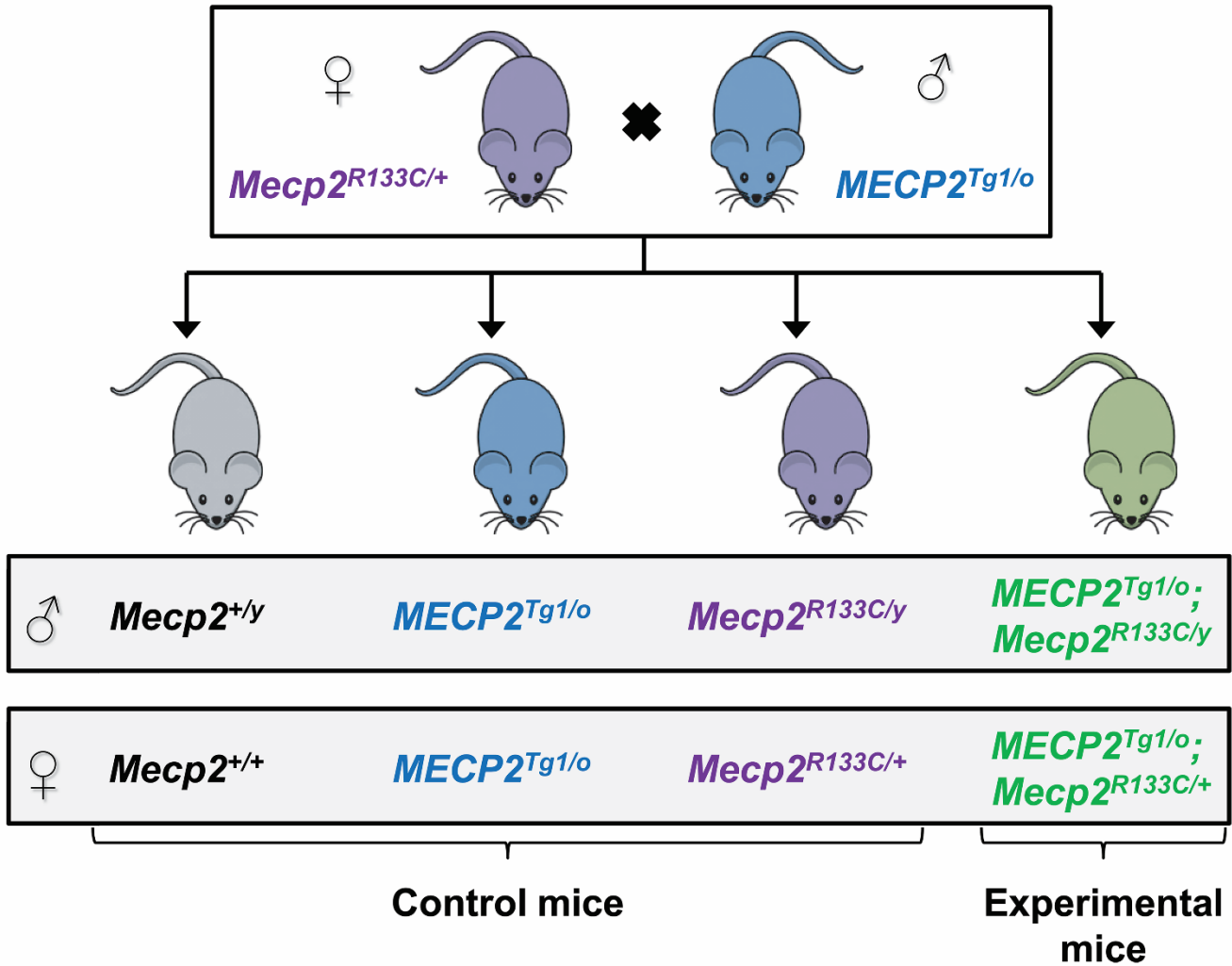
Supplementary Figure 5: Breeding scheme to generate *Mecp2 R133C* mice expressing an *MECP2* transgene. Male $MECP2^{Tg1/o}$ mice (blue) were bred with female $Mecp2^{R133C/+}$ (purple) animals to introduce the human *MECP2* transgene and generate the experimental mice $MECP2^{Tg1/o}$; $Mecp2^{R133C/y}$ and $MECP2^{Tg1/o}$; $Mecp2^{R133C/+}$ (green). Wild-type (gray), $MECP2^{Tg1/o}$, and $Mecp2^{R133C/y}$ or $Mecp2^{R133C/+}$ littermates were used as controls.
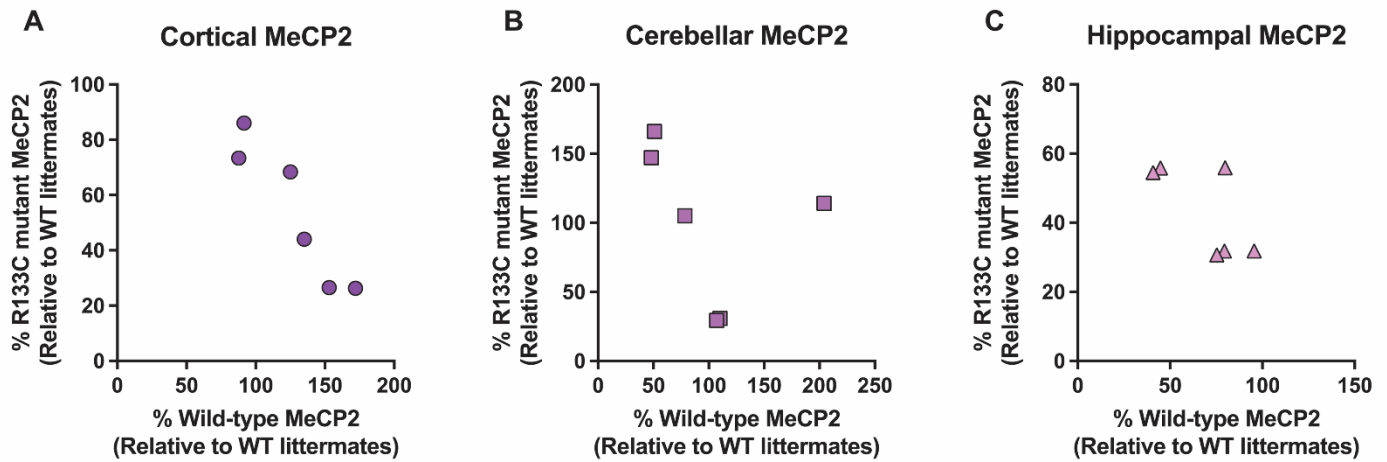
**Supplementary Figure 6: MeCP2 protein expression in *Mecp2 R133C* mice with *MECP2* transgene.** (A-B) Representative images of Western blots showing distinct bands for the *R133C* mutant (red arrow) and WT MeCP2 (black arrow), as well as the Gapdh control, in male (♂) and female (♀) mice across several brain regions.

57   +/y or +/+ (WT), Tg1/o (*MECP2*$^{Tg1/o}$), R133C/y or R133C/+ (*Mecp2 R133C*), Tg1; R133C/y or Tg1; R133C/+

58   (*Mecp2 R133C* with *MECP2* transgene). CTX (cortex), CER (cerebellum), HPC (hippocampus).

Supplementary Figure 7: Variability of WT and *R133C* mutant MeCP2 expression in *MECP2^Tg1/o^*; *Mecp2^R133C/+^* mice. The relationship of WT MeCP2 and the *R133C* mutant MeCP2 expression in the cortex, cerebellum and hippocampus of *Mecp2^R133C/+^* mice with the *MECP2* transgene is illustrated. Each point corresponds to a mouse subject, and the expression of both forms of MeCP2 is normalized to the Gapdh control and presented as percentage expression relative to MeCP2 expression in WT littermates.
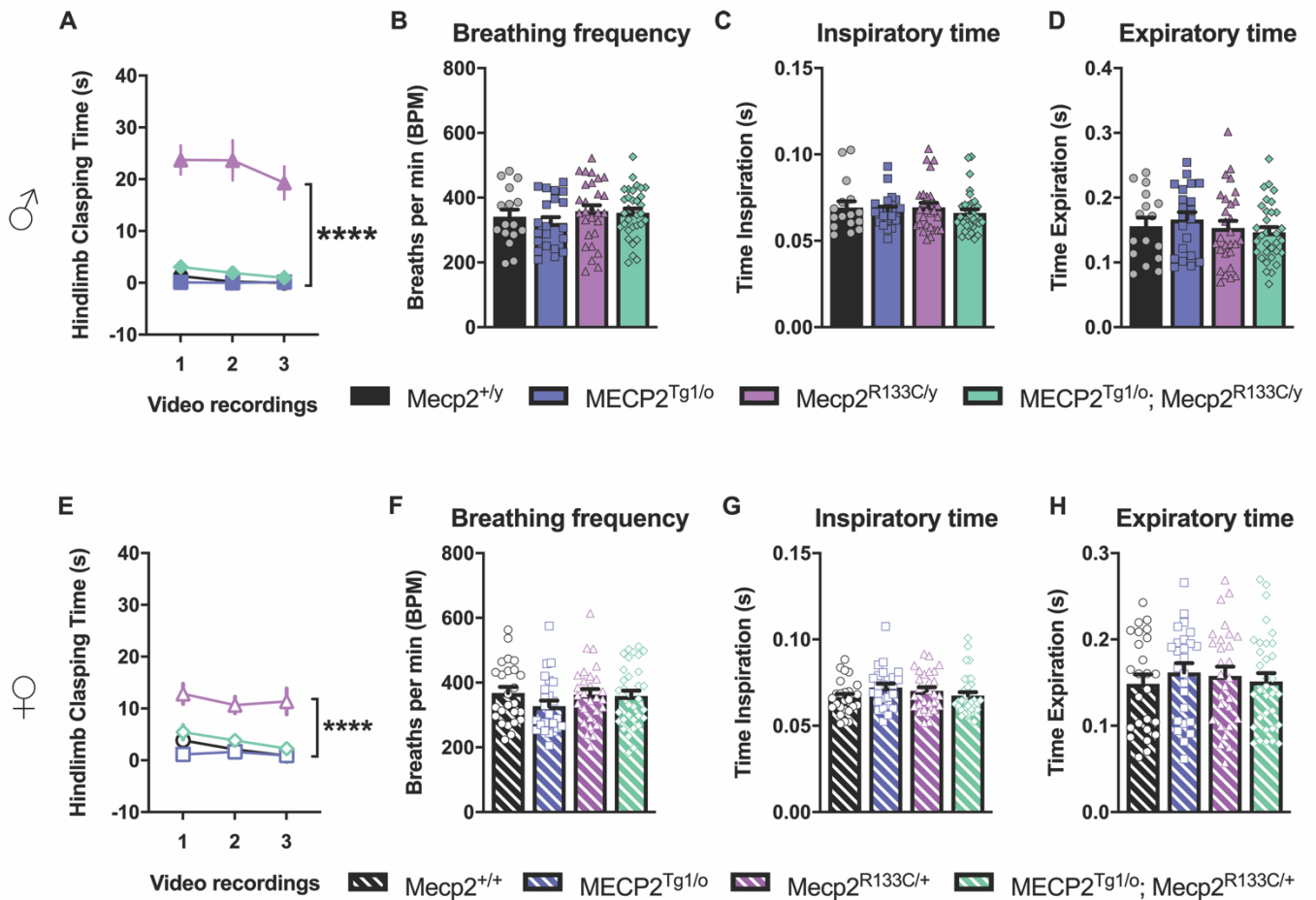
Supplementary Figure 8: Hindlimb clasping and respiratory function parameters in *Mecp2 R133C* mice with *MECP2* transgene. (A, E) Hindlimb clasping is reversed across all ages in $MECP2^{Tg1/o}$; $Mecp2^{R133C/y}$ and $MECP2^{Tg1/o}$; $Mecp2^{R133C/+}$ mice. Video recording numbers represent different ages: 6-9, 8-11 or 10-13 weeks old in males; 20-23, 22-25 or 24-28 weeks old in females. (B-D, F-H) No differences in other respiratory parameters between genotypes in males or females, including breathing frequency, and times of inspiration or expiration, were observed. n=16-32 per genotype in males, n=25-40 per genotype in females. 1- or 2-way ANOVA with Tukey's post-hoc tests. ****$p<0.0001$. WT = black bars or circles. $MECP2^{Tg1/o}$ = blue bars or squares. *Mecp2 R133C* = purple bars or triangles. $MECP2^{Tg1/o}$; *Mecp2 R133C* = green bars or diamonds. Male (♂) = filled bars or closed circles. Female (♀) = patterned bars or open circles.