

Supplementary Material

Female *Mecp2*^{+/-} mice display robust behavioral deficits on two different genetic backgrounds providing a framework for pre-clinical studies

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Methods and references2
Figure Legends.....4
Figures.....7
Table Legends.....13
Tables.....14

METHODS

Animal husbandry for 129.FVBF1 mice. Mice were maintained on a 12 h light:12 h dark cycle with standard mouse chow and water ad libitum. F1 hybrid 129S6/SvEv x FVB/N (129.FVBF1) animals were generated by mating female *Mecp2*^{+/-} mice of a pure 129SvEv background to wild-type male mice of a pure FVB/N background. Animals were housed 4-5 animals per cage, all research and animal care procedures were approved by the Baylor College of Medicine Institutional Animal Care and Use Committee.

Neurobehavioral assays. A select number of behavioral tests were performed with 129.FVBF1 *Mecp2*^{+/-} and wild-type littermate mice (n=12 and 15, respectively). These include elevated plus maze at 5 weeks of life, open field, acoustic startle response and prepulse inhibition at 7 weeks of life, fear conditioning at 8 weeks of life, and three chamber at 12 weeks of life. Neurobehavioral assays were performed as previously described (1–6) and as described in the main text.

Statistical analysis of behavioral data. Statistical analysis of data was performed using SPSS (version 19) as described in the main text.

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FIGURE LEGENDS

Figure S1. *Mecp2*^{+/-} mice are less anxious during the first five minutes of the elevated plus maze test, similar to the findings observed in a ten minute elevated plus maze test. FVB.129F1 *Mecp2*^{+/-} mice spend more time in the open arms of the elevated plus maze compared with wild-type littermates (**A**), but do not show differences in the number of open arm entries (**B**), or in the distance traveled in the maze (**C**). 129.B6F1 *Mecp2*^{+/-} mice show significant differences in all three test parameters, spending more time in the open arms (**D**), making more open arm entries (**E**) and traveling further in the elevated plus maze (**F**) compared with wild-type littermates. At seven weeks of life, FVB.129F1 *Mecp2*^{+/-} mice have an acoustic startle response (ASR) similar to their wild-type littermates when exposed to sound in the range of 70 to 102 dB, but have a decreased ASR when exposed to sound in the range of 106 to 118 dB (**G**). In contrast, 129.B6F1 *Mecp2*^{+/-} mice have an ASR similar to their respective littermates when exposed to this range of sound (**H**). An analysis of ASR at a 20 weeks of life revealed that both FVB.129F1 and 129.B6F1 *Mecp2*^{+/-} mice showed a reduction in the ASR when presented with sound ranging from 102 dB to 118 dB (**I, J**). * $p < 0.05$; ** $p < 0.001$; ns, not significant.

Figure S2. *Mecp2*^{+/-} mice have normal olfaction. *Mecp2*^{+/-} mice of both backgrounds spend a similar amount of time sniffing a novel odor (vanilla) and a familiar odor (water) compared with wild-type littermates, and both groups spend more time overall investigating the novel odor (**A, B**). ns, not significant.

Figure S3. In control measurements for social approach behavior at 12 weeks of life, *Mecp2*^{+/-} mice performed normally compared with wild-type mice during the habituation phase (time spent in each chamber, **A, B**; time spent investigating empty cages, **C, E**; and distance traveled, **D, F**), and during the test phase (time spent in each chamber **G, I**, and distance traveled **H, J**).

Figure S4. In control measurements for social approach behavior at 22 weeks of life, FVB.129F1 *Mecp2*^{+/-} mice spend slightly more time in the center compartment compared with wild-type littermates, but do not show a preference for either the left or right chambers during the habituation phase (**A**). In addition, no differences are observed in the time spent investigating the empty test cages (**C**), and in the total distance traveled (**D**). 129.B6 *Mecp2*^{+/-} mice explore the chambers and empty cages normally (**B**, **E**), but travel slightly less than wild-type littermates (**F**). During the test for social approach toward a novel mouse versus a novel object, FVB.129F1 *Mecp2*^{+/-} mice spend less time in the compartment with the novel object compared with wild-type littermates, but spend a similar amount of time in the center and in the compartment with the novel partner (**G**). The distance traveled during the test is also similar between mutant and wild-type animals (**H**). 129.B6F1, *Mecp2*^{+/-} mice spend a similar amount of time in each chamber compared with wild-type littermates (**I**), but travel less during the test (**J**). * $p < 0.05$; ns, not significant.

Figure S5. As a control for fear conditioning, animals were exposed to shock intensities of various levels and their response was recorded. The percentage of animals that flinch, vocalize or run/jump in response to shock intensities of different levels are similar between *Mecp2*^{+/-} mice and wild-type littermates of both backgrounds (**A**, **B**). During the second day of passive avoidance learning, FVB.129F1 *Mecp2*^{+/-} mice that remained in the lit compartment froze less compared with littermates (**C**) and made a greater number of approaches toward the dark compartment (**D**). In contrast, 129.B6F1 *Mecp2*^{+/-} mice showed normal freezing behavior (**E**) and equally approached the dark compartment compared with wild-type littermates (**F**). * $p < 0.05$; ns, not significant.

Figure S6. 129.FVBF1 *Mecp2*^{+/-} mice representing the reciprocal cross of FVB.129F1 mice also display behavioral phenotypes in a select number of assays. 129.FVBF1 *Mecp2*^{+/-} mice compared with wild-type littermate mice spend more time in the open arm of the elevated plus maze (**A**), but do not show differences in the number of open arm entries (**B**), or in the distance traveled in the maze (**C**). 129.FVBF1 *Mecp2*^{+/-} mice compared with wild-type littermates travel the same distance in an open field (**D**), and display decreased acoustic startle response and enhanced prepulse inhibition at 7 weeks of life (**E, F**). 129.FVBF1 *Mecp2*^{+/-} mice also have impaired contextual fear memory but normal cued fear memory at 8 weeks of life (**G**). At 12 weeks of life, 129.FVBF1 *Mecp2*^{+/-} mice showed a reduction in social approach similar to the defects observed in FVB.129F1 *Mecp2*^{+/-} mice. Both 129.FVBF1 *Mecp2*^{+/-} mice and wild-type littermates spend more time investigating a novel mouse compared with a novel object. Across genotypes, 129.FVBF1 *Mecp2*^{+/-} mice compared with wild-type littermates spend less time investigating a novel mouse. No difference was observed in the time spent investigating a novel object (**H**). In control measurements for social approach behavior, 129.FVBF1 *Mecp2*^{+/-} mice performed normally during the habituation phase (time spent in each chamber, **I**; time spent investigating empty cages, **J**; and distance traveled, **K**), and during the test phase (time spent in each chamber **L**, and distance traveled **M**). * $p < 0.05$; ** $p < 0.001$; ns, not significant.

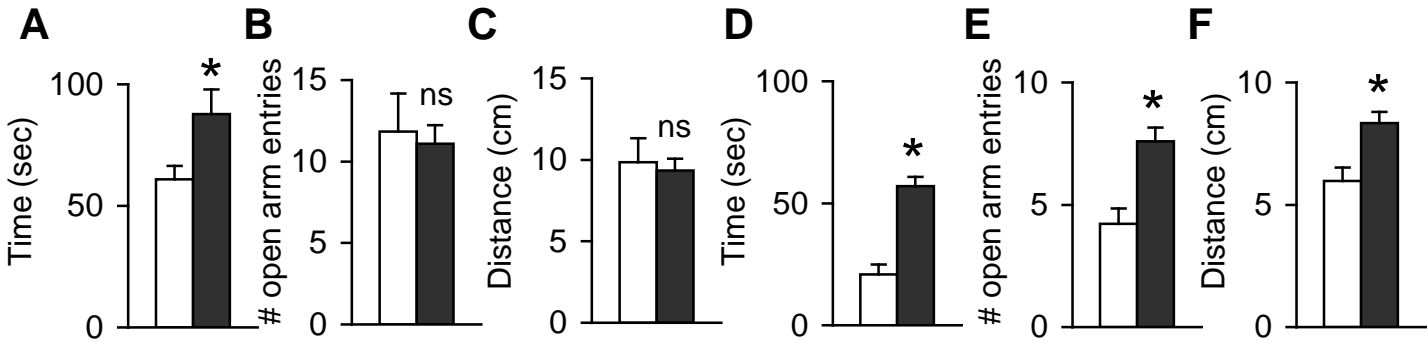
Supplementary Figure 1

FVB/N x 129S6/SvEv

129S6/SvEv x C57BL/6

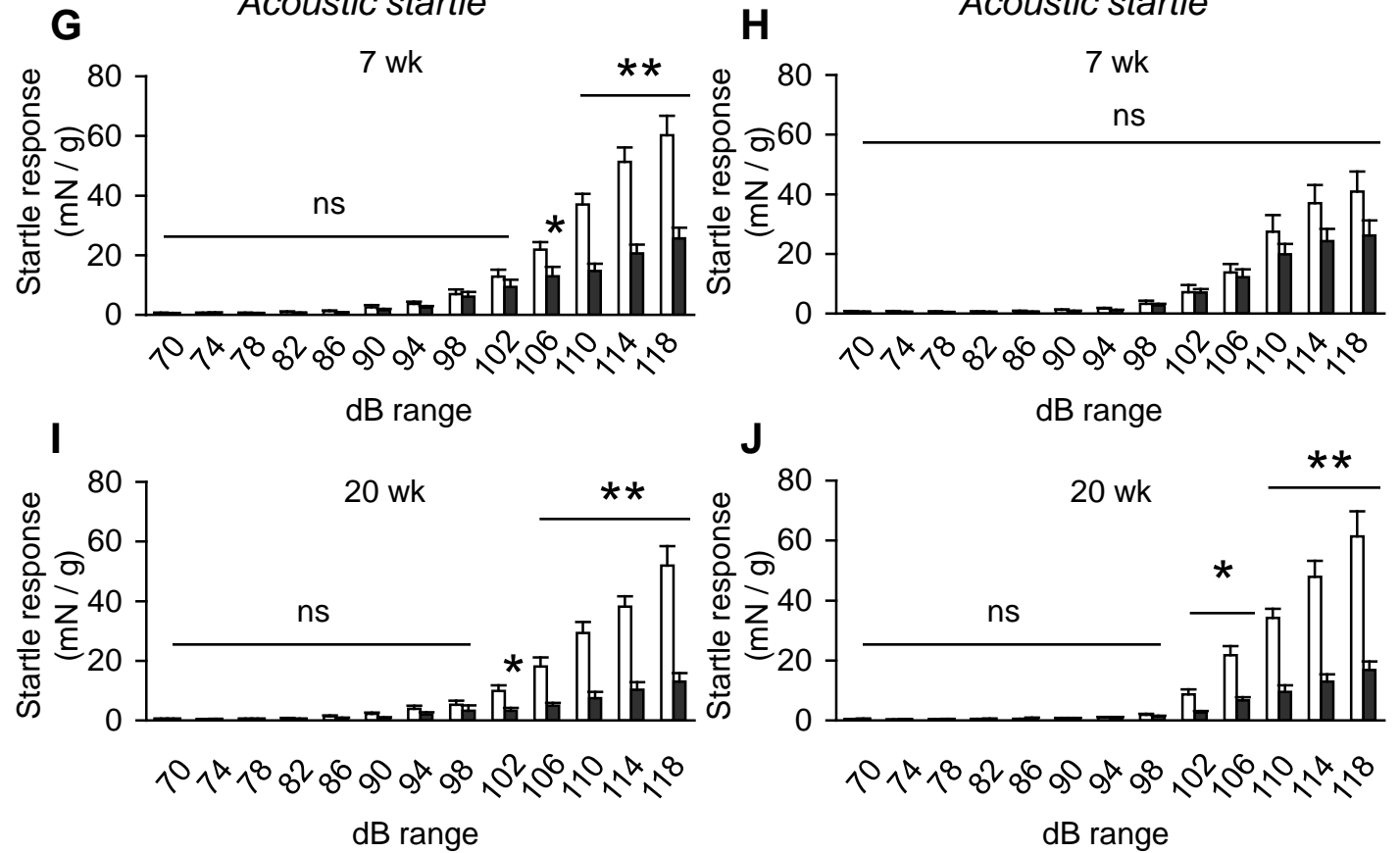
Elevated plus maze – first 5 minutes

Elevated plus maze – first 5 minutes



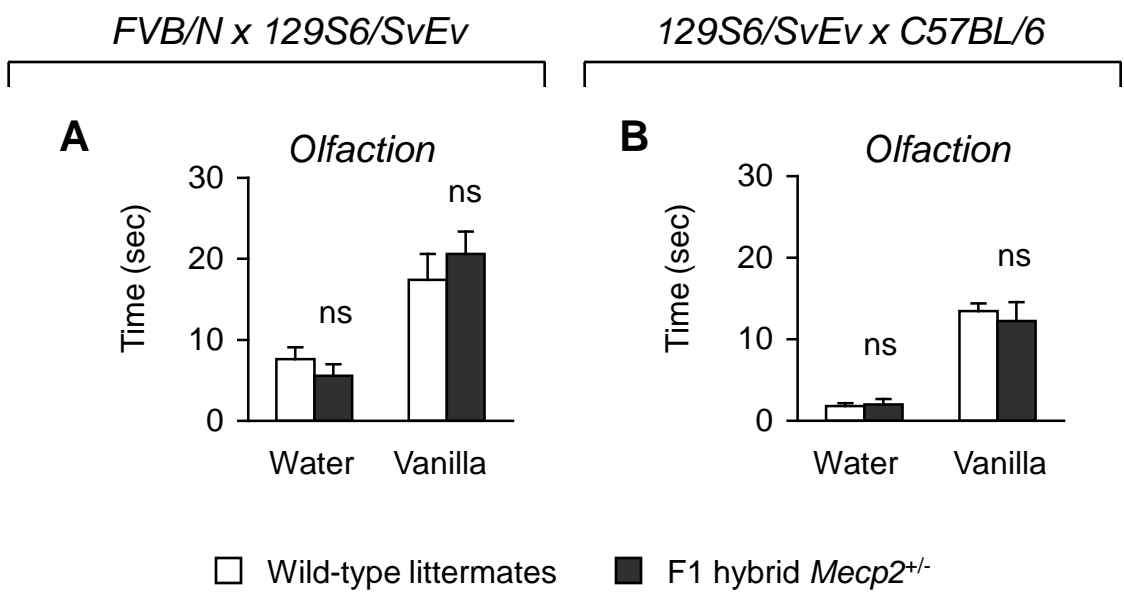
Acoustic startle

Acoustic startle



□ Wild-type littermates ■ F1 hybrid *Mecp2*^{+/-}

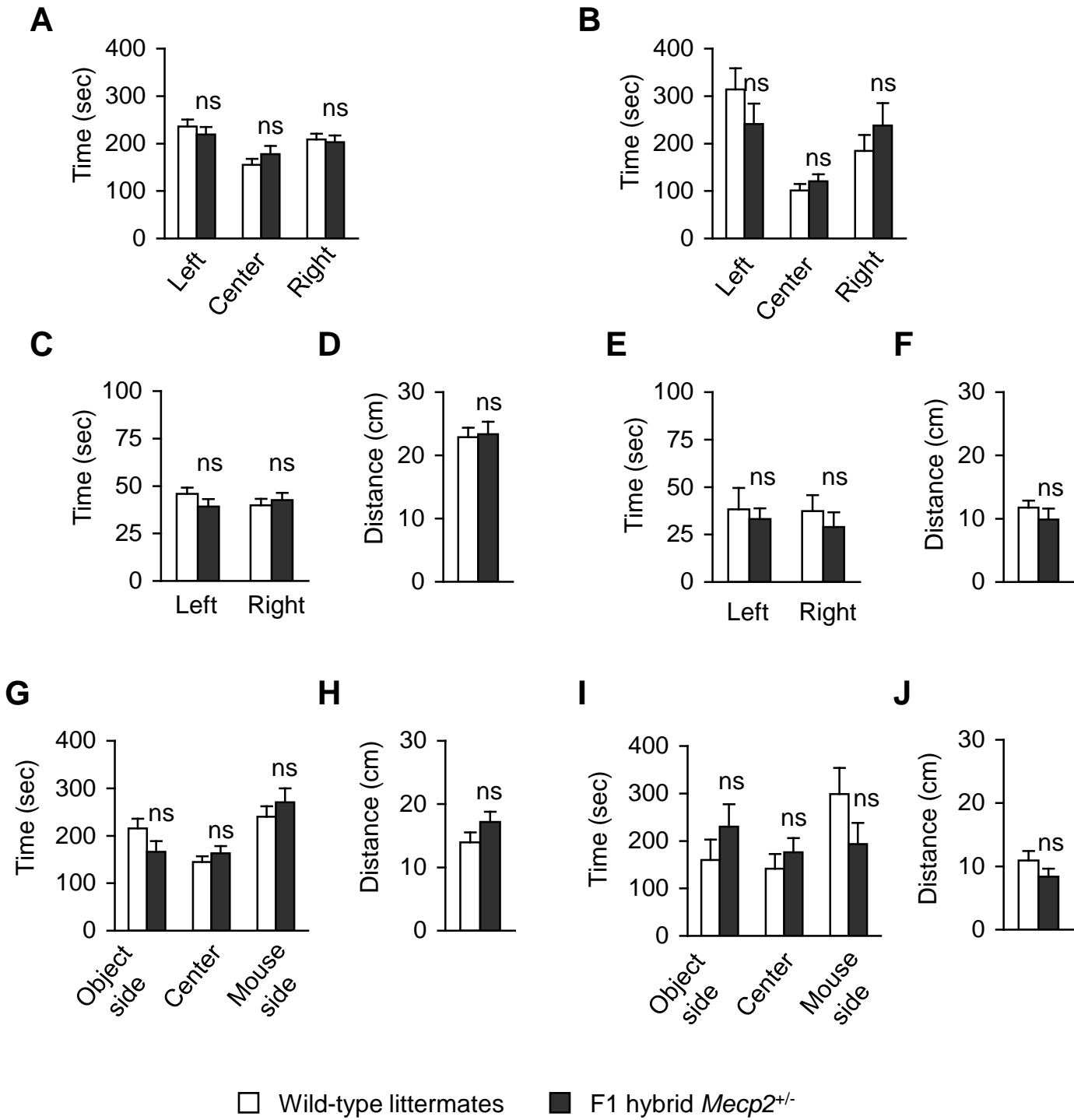
Supplementary Figure 2



Supplementary Figure 3

FVB/N x 129S6/SvEv

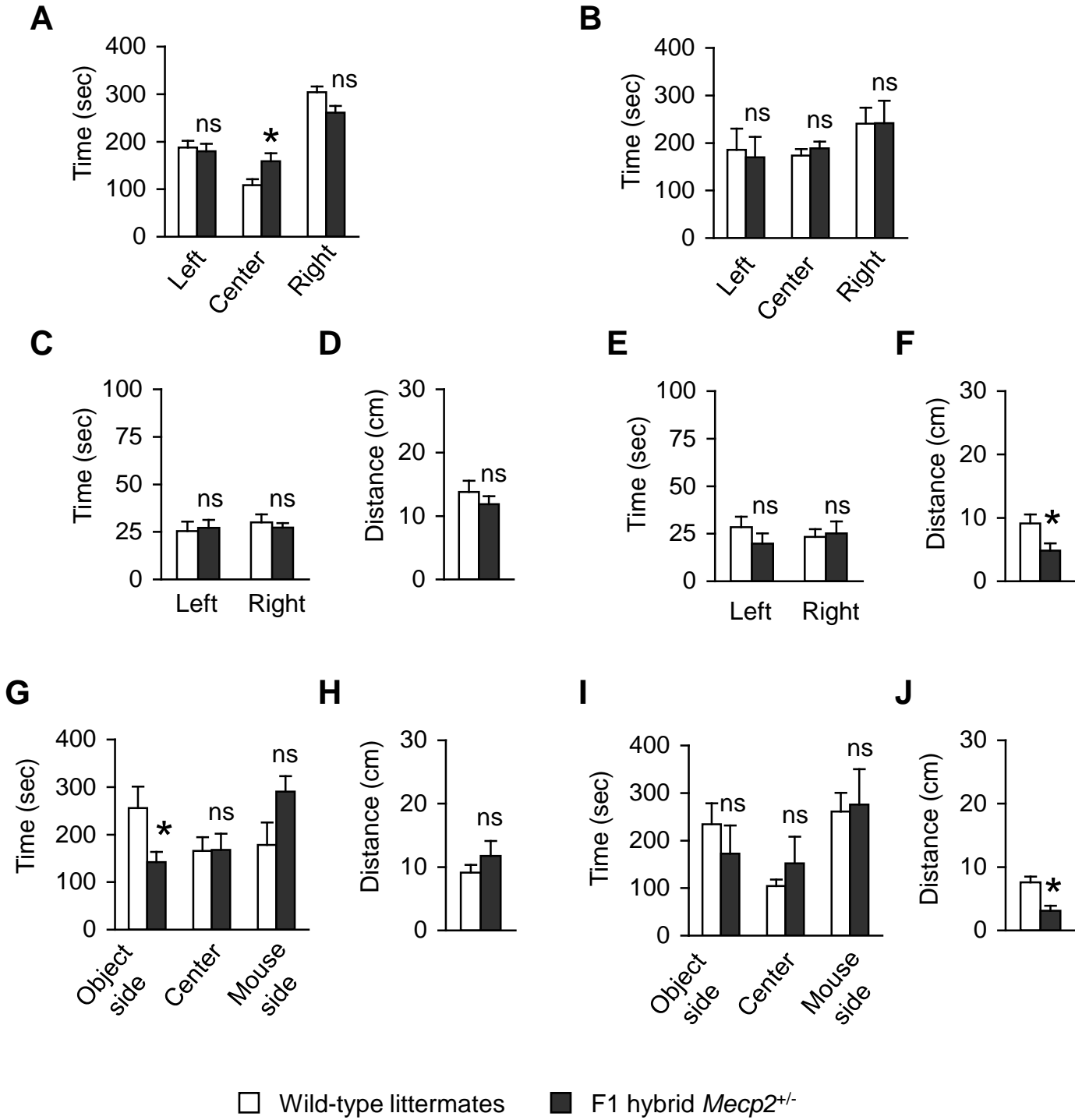
129S6/SvEv x C57BL/6



Supplementary Figure 4

FVB/N x 129S6/SvEv

129S6/SvEv x C57BL/6



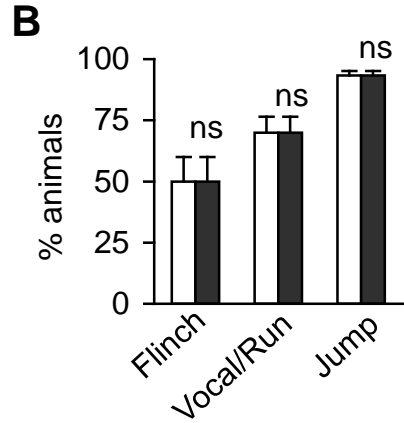
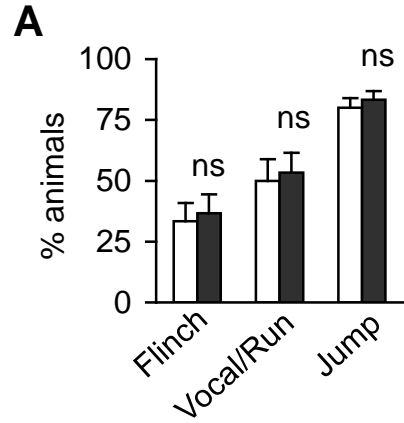
Supplementary Figure 5

FVB/N x 129S6/SvEv

129S6/SvEv x C57BL/6

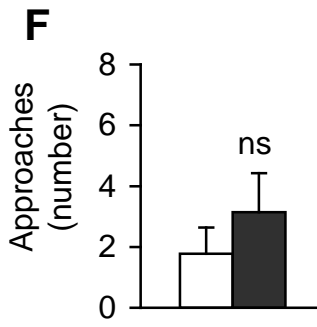
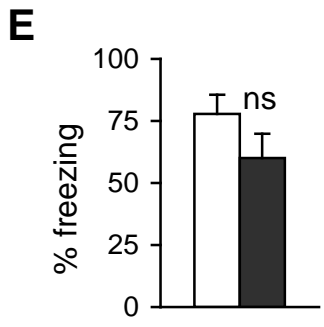
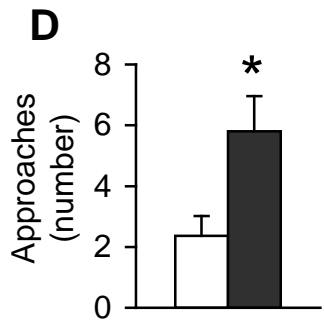
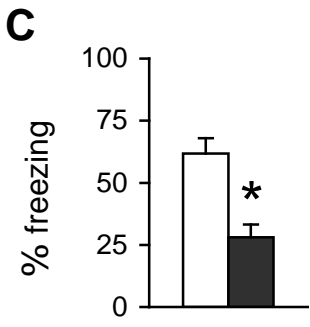
Fear conditioning

Fear conditioning



Passive avoidance

Passive avoidance



□ Wild-type littermates ■ F1 hybrid *Mecp2*^{+/-}

Supplementary Figure 6

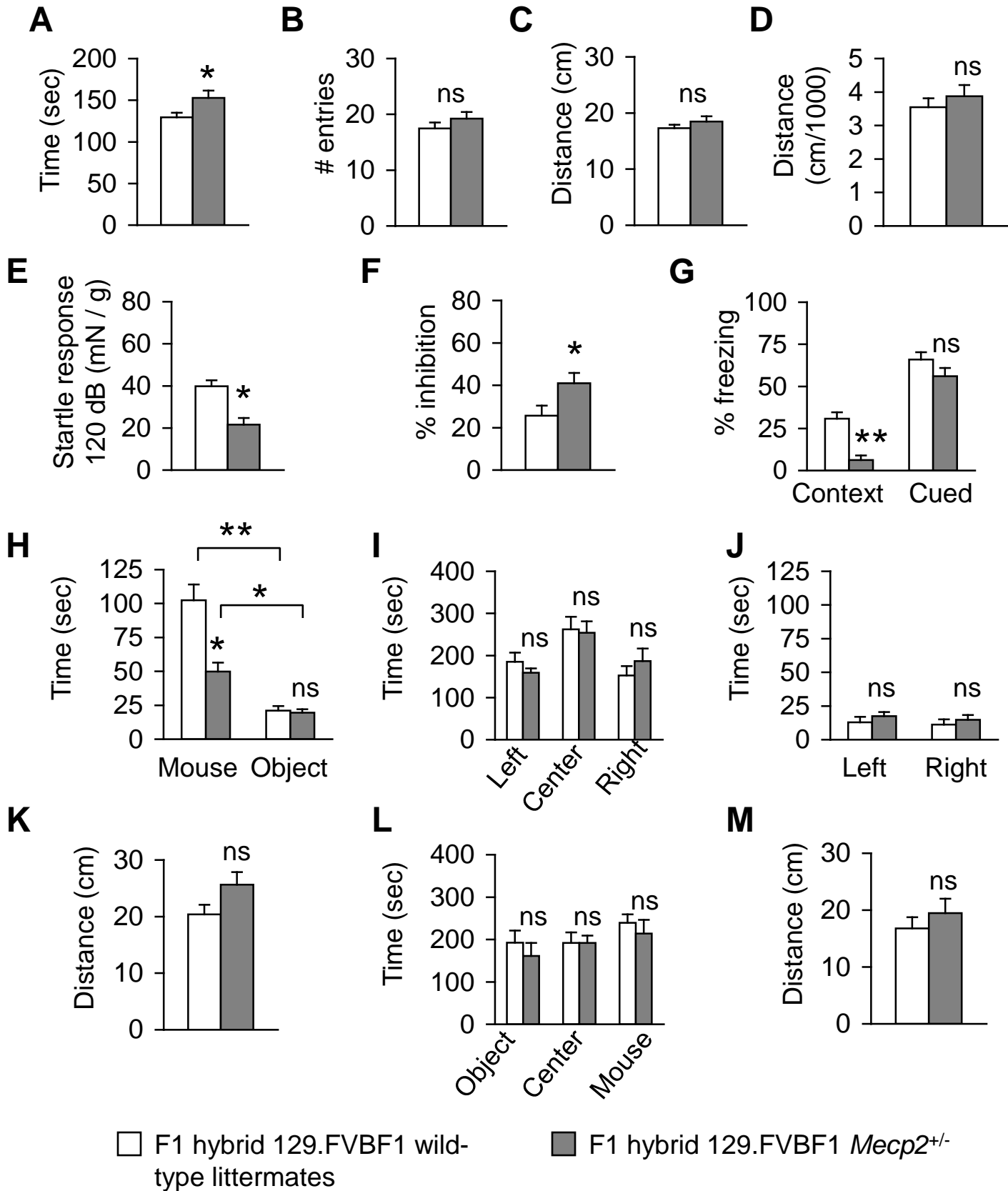


TABLE LEGENDS

Table S1. Summary of neurobehavioral tests and physiological measurements.

In this study, the most reliable and robust phenotypes present in *Mecp2*^{+/-} mice of two F1 hybrid backgrounds phenotypes that may be used as useful readouts in pre-clinical studies are decreased anxiety-like behavior at 5 weeks of life, decreased exploratory behavior in an open field and decreased motor function at 17 weeks of life, enhanced prepulse inhibition and decreased acoustic startle response at 20 weeks of life, decreased social approach behavior at 22 weeks of life, decreased contextual fear memory at 8 weeks of life, decreased passive avoidance learning at 14 weeks of life, decreased pain sensitivity at 22 weeks of life, increased apneas at 7 weeks of life.

Table S2. Summary of documented phenotypes in female *Mecp2*^{tm1.1Bird} mice.

The reported phenotypes from previous studies of female *Mecp2*^{tm1.1Bird} mice is shown in a side-by-side comparison. The majority of published work has focused on motor dysfunction, breathing abnormalities and general health.

Table 1. Summary of neurobehavioral tests and physiological measurements.

Phenotype	Age (wks)	FVB.129F1 <i>Mecp2</i> ^{+/-}	129.B6F1 <i>Mecp2</i> ^{+/-}
Anxiety-like behavior	5	↓	↓
Motor function	7	n.s.	n.s.
	17	↓	↓
Pre-pulse inhibition	7	↑	n.s.
	20	↑	n.s.
	52	n.d.	↑
Acoustic startle response	7	↓	n.s.
	20	↓	↓
	52	n.d.	↓
Social behavior	12	↓	n.s.
	22	↓	↓
Olfaction	12	n.s.	n.s.
Contextual fear memory	8	↓	↓
Cued fear memory	8	n.s.	n.s.
Passive avoidance learning	14	↓	↓
Pain recognition	22	↓	↓
Corticosterone levels - basal	6	n.s.	n.s.
Corticosterone levels - stress	6	↓	n.s.
Apneas	7	↑	↑
Breathing rate	7	n.s.	n.s.
Weight	5 to 22	↑ (8 to 22)	n.s.
	52	n.d.	↑

n.d., not determined

n.s., not significant compared with wild-type littermates

Table 2. Summary of documented phenotypes in female *Mecp2*^{tm1.1Bird} mice.

	Guy et al., 2001	Bissonnette et al., 2006	Bissonnette et al., 2007	Guy et al., 2007
Motor function			n.d.	n.d.
Age(s)	9 mo.	2.6 mo and 4 mo.	-	-
Strain	mixed C57BL/6, 129	C57BL/6	-	-
Findings	Decreased	n.s. 2.6 mo., decreased 4 mo.	-	-
Breathing			n.d.	n.d.
Age	9 mo.	2.6 - 5 mo.	-	-
Strain	mixed C57BL/6, 129	C57BL/6	-	-
Findings	Irregular	Abnormal	-	-
General health evaluation				
Age	> 3 mo.	2.6, 3.7, and 12. 5mo.	8.7 mo.	4 mo.
Strain	mixed C57BL/6, 129	C57BL/6	C57BL/6	C57BL/6
Findings	Poor health	Hind limb clasping starting at 3.7 mo., worsen by 12.5 mo.	Hind limb clasping	High symptom score
Weight	n.d.			
Age	-	2 mo. and 1 yr	8.7 mo.	> 4 mo.
Strain	-	C57BL/6	C57BL/6	C57BL/6
Findings	-	n.s.	n.s.	Increased
Autonomic cardiovascular regulation	n.d.	n.d.		n.d.
Age	-	-	8.7 mo.	-
Strain	-	-	C57BL/6	-
Findings	-	-	n.s.	-
Postnatal neurological reflexes	n.d.	n.d.	n.d.	n.d.
Age	-	-	-	-
Strain	-	-	-	-
Findings	-	-	-	-
Anxiety-like behavior	n.d.	n.d.	n.d.	n.d.
Age	-	-	-	-
Strain	-	-	-	-
Findings	-	-	-	-
Other neurobehavioral	n.d.	n.d.	n.d.	n.d.

n.d., not determined or reported

n.s., not significant compared with wild-type littermates

Table 2 (continued). Summary of documented phenotypes in female *Mecp2*^{tm1.1Bird} mice.

	Santos et al., 2007	Bissonnette et al., 2008	Jugloff et al., 2008	Abdala et al., 2010
Motor function		n.d.		
Age(s)	5 wks	-	3-6 mo. and 9-12 mo.	6.4 - 7.8 mo.
Strain	C57BL/6	-	C57BL/6	C57BL/6
Findings	Decreased (rotarod)	-	n.s. 3-6 mo., decreased 9-12 mo.	Decreased
Breathing	n.d.		n.d.	
Age	-	5 - 15 mo.	-	4 - 14 mo.
Strain	-	C57BL/6	-	C57BL/6
Findings	-	Abnormal	-	Abnormal
General health evaluation	n.d.		n.d.	n.d.
Age	-	n.d.	-	-
Strain	-	C57BL/6	-	-
Findings	-	Hind limb clasping	-	-
Weight			n.d.	n.d.
Age	PND4 - 21	n.d.	-	-
Strain	C57BL/6	C57BL/6	-	-
Findings	Decreased	n.s.	-	-
Autonomic cardiovascular regulation	n.d.	n.d.	n.d.	n.d.
Age	-	-	-	-
Strain	-	-	-	-
Findings	-	-	-	-
Postnatal neurological reflexes		n.d.	n.d.	n.d.
Age	PND4 - 21	-	-	-
Strain	C57BL/6	-	-	-
Findings	Abnormal	-	-	-
Anxiety-like behavior		n.d.	n.d.	n.d.
Age	4 wks	-	-	-
Strain	C57BL/6	-	-	-
Findings	n.s.	-	-	-
Other neurobehavioral	n.d.	n.d.	n.d.	n.d.

n.d., not determined or reported

n.s., not significant compared with wild-type littermates