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Supplemental Information

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Supplemental Results

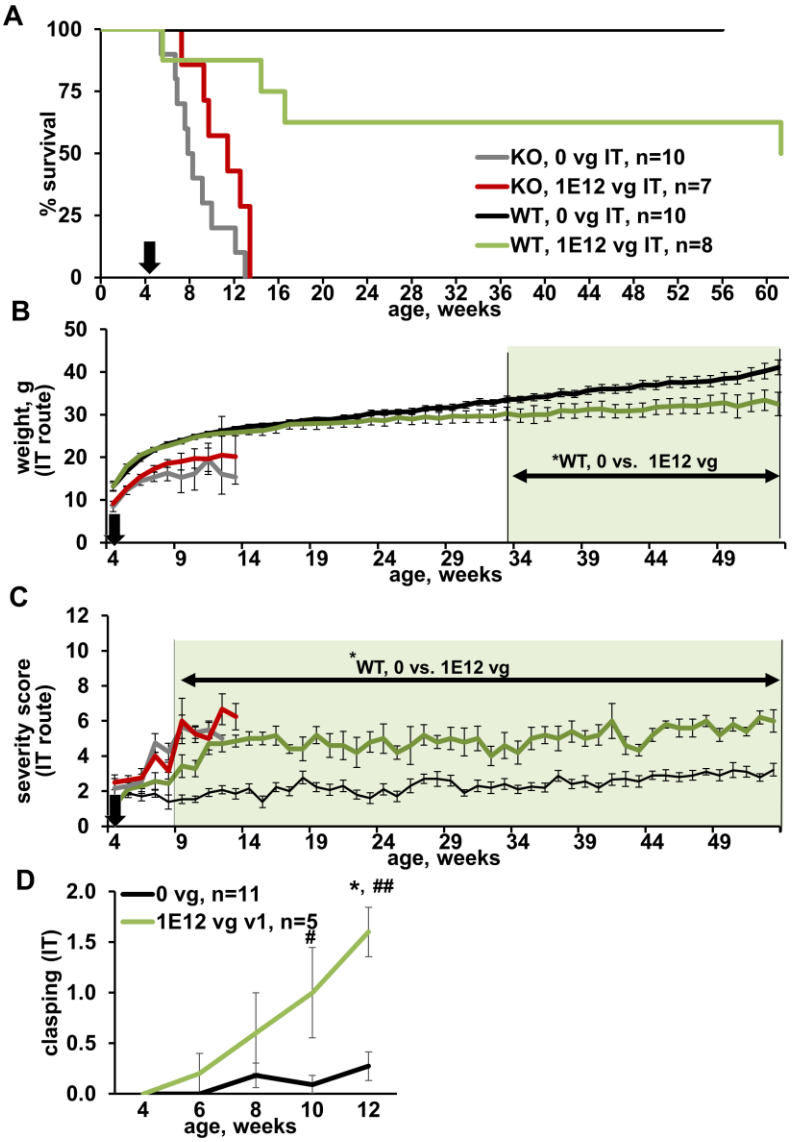

Fig. S1, related to Figs. 1 and 3: Percutaneous IT delivery of AAV9/*hMECP*2(v1) induces significant and persistent increases in phenotype severity scores for treated WT mice. Compare to **Figs. 1** and **3**, which present a parallel data set for ICM delivery of AAV9/*hMECP*2(v1). (**A**) Survival curves for saline- and virus-treated *Mecp2*$^{-/y}$ and WT mice. We did not observe a significant extension in survival for virus-treated *Mecp2*$^{-/y}$ mice injected intrathecally ($p = 0.06$). However, the lack of significance may be due to low power, as the median survivals of *Mecp2*$^{-/y}$ mice injected IT or ICM with 1 x 10^{12} vg AAV9/*hMECP2*(v1) are similar (80 and 84 days, respectively). (**B**) The shaded area indicates ages at which virus-treated WT mice had a significantly lower mean body weight compared to that of saline-treated WT mice ($p \leq 0.05$). (**C**) The shaded area indicates ages at which virus-treated WT mice had significantly higher aggregate phenotype severity scores compared to those of saline-treated WT mice ($p \leq 0.05$). (**D**) A significant increase in severity scores for hindlimb clasping contributed to the overall increase in aggregate severity score. For limb clasping, *$p = 0.003$ (4 vs. 12 weeks of age for virus-treated mice, pair-wise t-test); ##$p = 0.0002$ at 12 weeks of age; #$p = 0.01$ at 10 weeks of age, saline- vs. virus-treated mice. (**B**-**D**) Data points are mean ± SEM.

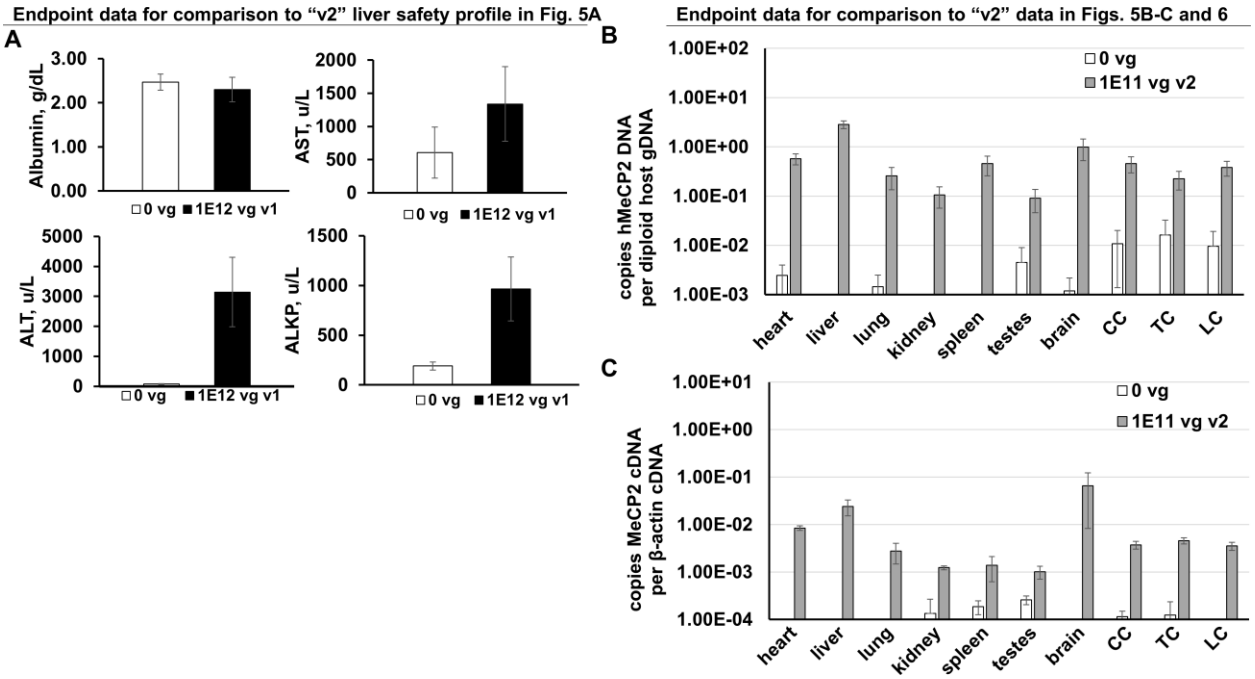

Fig. S2. Supplemental endpoint data for comparison to Figs. 5-6. (A) Blood serum levels of liver toxicity indicators after treatment with 1 x 10^{12} vg AAV9/*hMECP2*(v1) (ICM). $p = 0.7$ for albumin; $p = 0.1$ for ALT; $p = 0.4$ for AST; $p = 0.1$ for ALKP. The y-axis is plotted on the same scale as that shown in **Figs. 5A**. Data for 1 x 10^{12} vg AAV9/*hMECP2*(v1) can be compared to that of 1 x 10^{12} AAV9/*hMECP2*(v2), which is shown in **Fig. 5A**. (**B-C**) Biodistribution and gene expression data used to calculate normalized gene expression in mice treated with 1 x 10^{11} vg AAV9/*hMECP2*(v2) (see **Fig. 6**). Tissue samples for **Figs. 2** and **S2B-C** were processed in parallel. Therefore, the same saline control data appears in **Figs. 2** and **S2B-C**. (**B**) As expected, viral genome copies are about 10-fold less than those of observed for mice treated with 1 x 10^{12} vg AAV9/*hMECP2*(v2) (see **Fig. 5B**). (**A-C**) $n = 3$ saline-treated mice and 5 virus-treated *Mecp2*$^{-/y}$ mice. Data are mean ± SEM. CC, cervical spinal cord; g/dL, grams/deciliter; LC, lumbar spinal cord; TC, thoracic spinal cord; u/L, units/liter.

MeP426 promoter

ATAGGCGCCAAGAGCCTAGACTTCCTTAAGCGCCAGAGTCCACAAGGGCCCAGTT
AATCCTCAACATTCAAATGCTGCCCACAAAACCAGCCCCTCTGTGCCCTAGCCGC
CTCTTTTTTCCAAGTGACAGTAGAACTCCACCAATCCGCAGCTGAATGGGGTCCG
CCTCTTTTCCCTGCCTAAACAGACAGGAACTCCTGCCAATTGAGGGCGTCACCGC
TAAGGCTCCGCCCCAGCCTGGGCTCCACAACCAATGAAGGGTAATCTCGACAAAG
AGCAAGGGGTGGGGCGCGGGCGCGCAGGTGCAGCAGCACACAGGCTGGTCGG
GAGGGCGGGGCGCGACGTCTGCCGTGCGGGGTCCCGGCATCGGTTGCGCGCG
CGCTCCCTCCTCTCGGAGAGAGGGCTGTGGTAAAACCCGTCCGGAAA

RDH1pA

AGCTCGCTGATCAGCCTCACAAGAATAAAGGCAGCTGTTGTCTCTTCAGAAGTAG
CTTTGCACTTTTCTAAACTAGGAATATCACCAGGACTGTTACTCAATGTGTGCTGCA
GGAAAGCACTGATATATTTAAAAACAAAAGGTGTAACCTATTTATTATATAAAGAGTTT
GCCTTATAAATTTACATAAAAATGTCCGTTTGTGTCTTTTGTTGTAAAAATC

Fig. S3. Sequences of MeP426 and RDH1pA, related to Methods

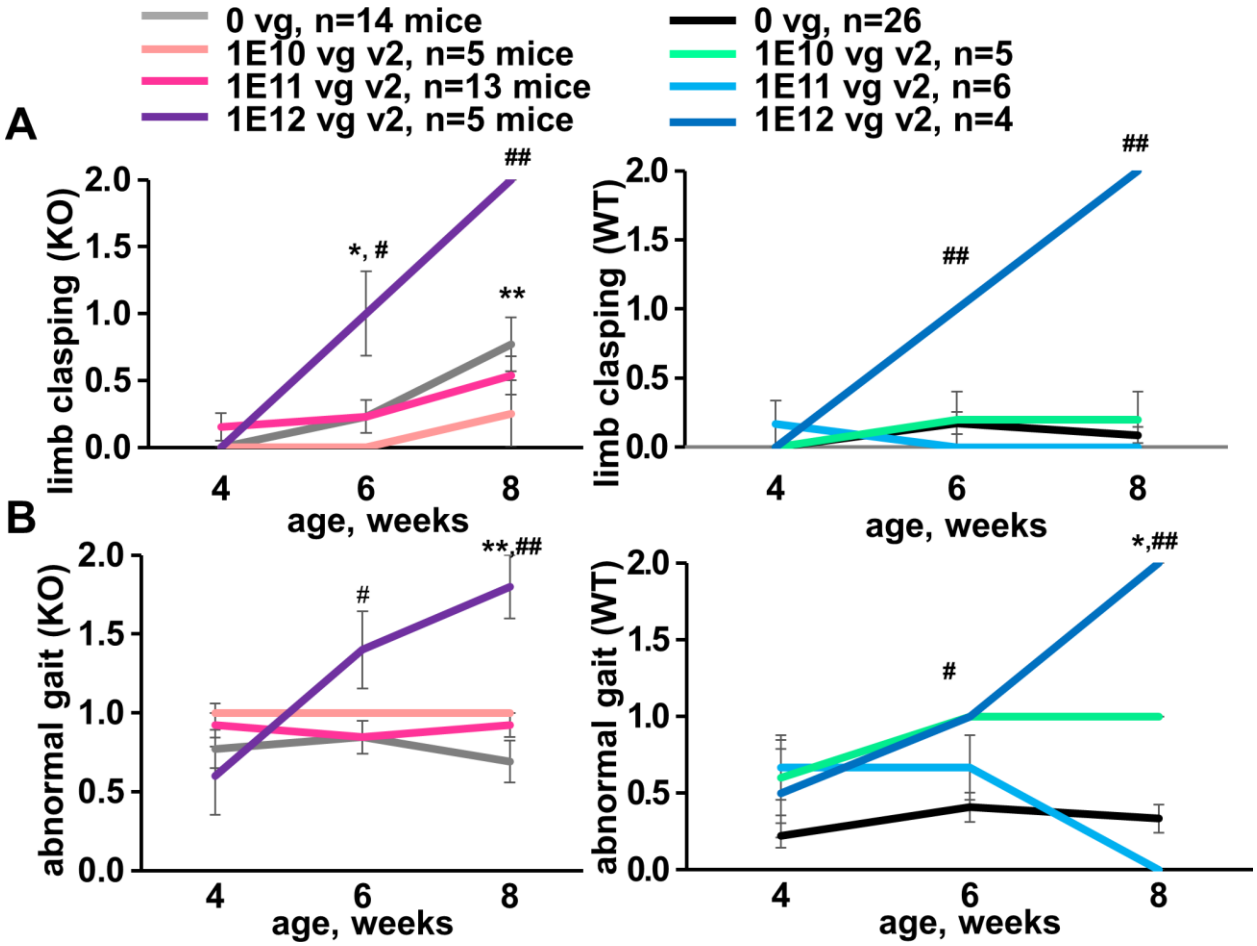

Fig. S4, related to Fig. 4. 1 x 10^{12} vg AAV9/*hMECP2*(v2) significantly increases clasping and abnormal gait scores in *Mecp2*$^{-/y}$ (left) and WT mice (right) after ICM injection at 4-5 weeks old. Compare to **Fig. 4D** for aggregate phenotype severity scores. Pair-wise analyses (pre- vs. post-ICM): *$p \leq 0.03$; **$p \leq 0.004$. Unpaired analyses (saline vs. virus treatment): #$p \leq 0.02$; ##$p \leq 0.002$. For clarity, unpaired p values are listed only for comparisons between saline and 1 x 10^{12} vg AAV9/*hMECP2*(v2)-treated mice; paired p values are listed for only saline and 1 x 10^{12} vg AAV9/*hMECP2*(v2)-treated mice. Data for saline-treated *Mecp2*$^{-/y}$ mice also appears in **Fig. 1F-G**. Data for saline-treated WT mice also appears in **Fig. 3D-E**. (**A-B**) Data points are mean ± SEM.

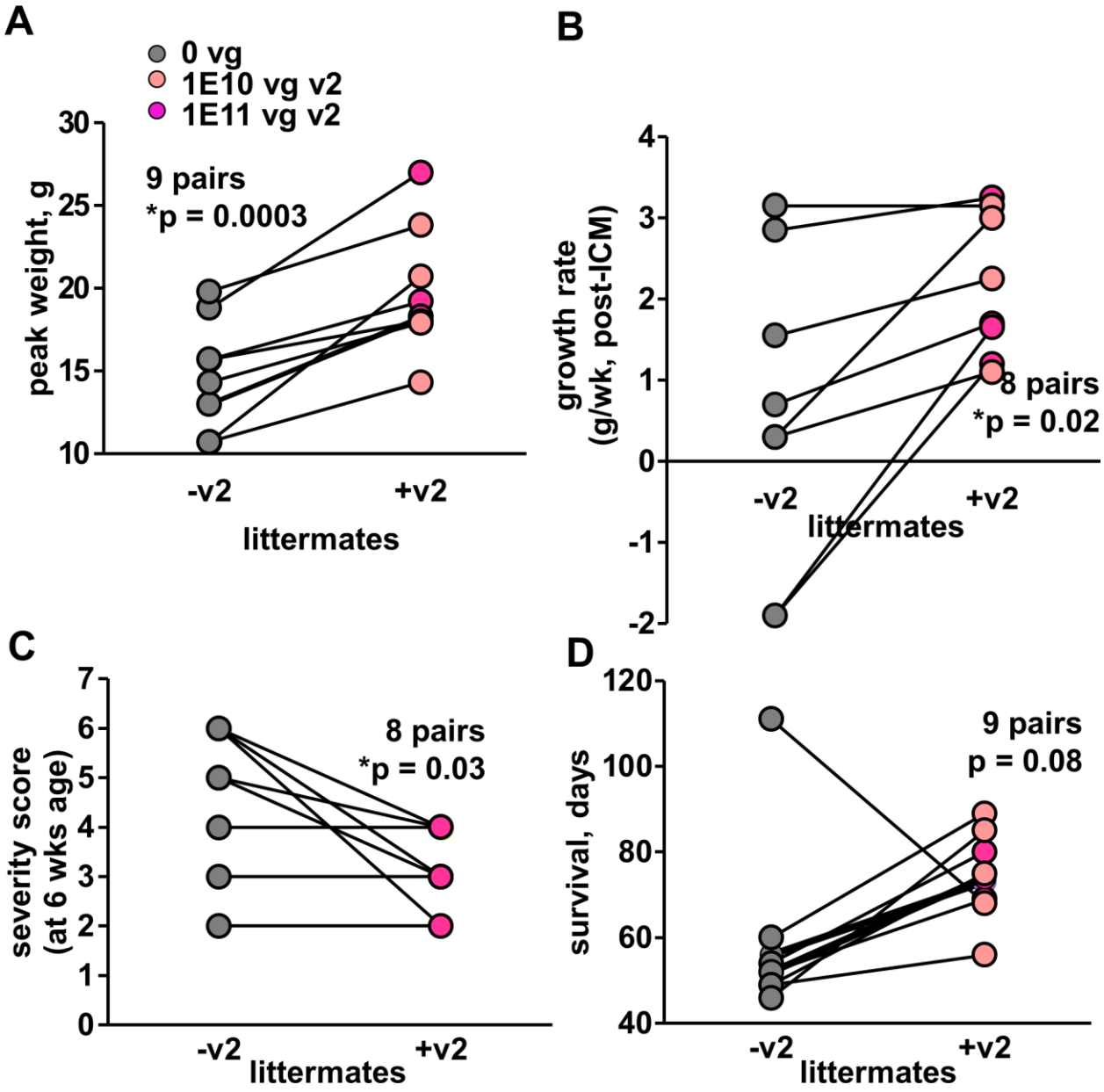

Fig. S5. Paired littermate analyses, related to Fig. 4. Note: Mean data for cohorts is presented in **Fig. 4**. For paired littermate analyses, vehicle and virus treatments were randomly assigned without any prior knowledge regarding each sibling's body weight or general physical condition. **(A)** AAV9/*hMECP2*(v2)-treated *Mecp2*$^{-/y}$ mice obtain greater peak body weights during their lifetime than their saline-treated littermates. (**A**-**D**) Each line connects a pair of littermates. **(B)** Compared to saline-treated littermates, AAV9/*hMECP2*(v2)-treated *Mecp2*$^{-/y}$ mice grow at a faster rate immediately after treatment (data shown for 5-7 weeks of age). **(C)** AAV9/*hMECP2*(v2) delays the deterioration in overall health shortly after treatment. Aggregate severity scores are shown for mice at 6 weeks of age. (**B**-**C**) One littermate pair was excluded from data analyses because the saline-treated littermate died prior to data collection at approximately 6 weeks of age. **(D)** Most *Mecp2*$^{-/y}$ mice treated with 1 x 10^{10} – 1 x 10^{11} vg AAV9/*hMECP2*(v2) live longer than their saline-treated *Mecp2*$^{-/y}$ littermates. In 8 of 9 pairs, the virus-treated mice outsurvived their vehicle-treated littermates.

Video S1 for Fig. 3. Representative WT mice treated with saline, 1E12 vg AAV9/*hMECP2*(v1), or 1 x 10^{12} vg AAV9/*EGFP*. WT mice were injected ICM between 4-5 weeks of age. At 6 months age, the saline-treated mouse in this video appears alert and has good mobility and gait. At 6 months age, the AAV9/*hMECP2*(v1)-treated mouse in this video appears alert but has difficulty using his hind legs correctly. At 6 months age the AAV9/*EGFP*-treated mouse has normal mobility and gait. The absence of hindlimb clasping in the AAV9/*EGFP*-treated mouse was photographed at this age and is featured at the end of the video.

Video S2 for Fig. 4. *Mecp2*$^{-/y}$ littermates treated with saline or 1 x 10^{11} vg AAV9/*hMECP2*(v2). Both littermates were injected on PND30 and were video-recorded on PND52. The saline-treated mouse has poor mobility and shows little spontaneous movement during the duration of the video. The virus-treated sibling exhibits more spontaneous movement than his saline-treated *Mecp2*$^{-/y}$ littermate. Note that the virus-treated littermate approaches the camera. This behavior could be an indicator of improved sociability.

Video S3 for Fig. 4. Second pair of video-recorded *Mecp2*$^{-/y}$ littermates treated with saline or 1 x 10^{11} vg AAV9/*hMECP2*(v2). These siblings were injected on PND31 and video-recorded on PND51. The saline-treated mouse walks slowly and explores in response to prompting. Note that he does not explore the camera. His virus-treated *Mecp2*$^{-/y}$ littermate walks, climbs, explores, and approaches camera without prompting.

Video S4 for Fig. 4. Third pair of video-recorded *Mecp2*$^{-/y}$ littermates treated with saline or 1 x 10^{10} vg AAV9/*hMECP2*(v2). These siblings were injected on PND28 and video-recorded on PND40. The saline-treated mouse immediately walks to his hut. The reason for this behavior (*i.e.*, aversion to light or aversion to open spaces) is unclear. His virus-treated *Mecp2*$^{-/y}$ littermate is more active.