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Supplementary Materials for

Antisense Oligonucleotides Delivered to the Mouse CNS Ameliorate Symptoms of Severe Spinal Muscular Atrophy

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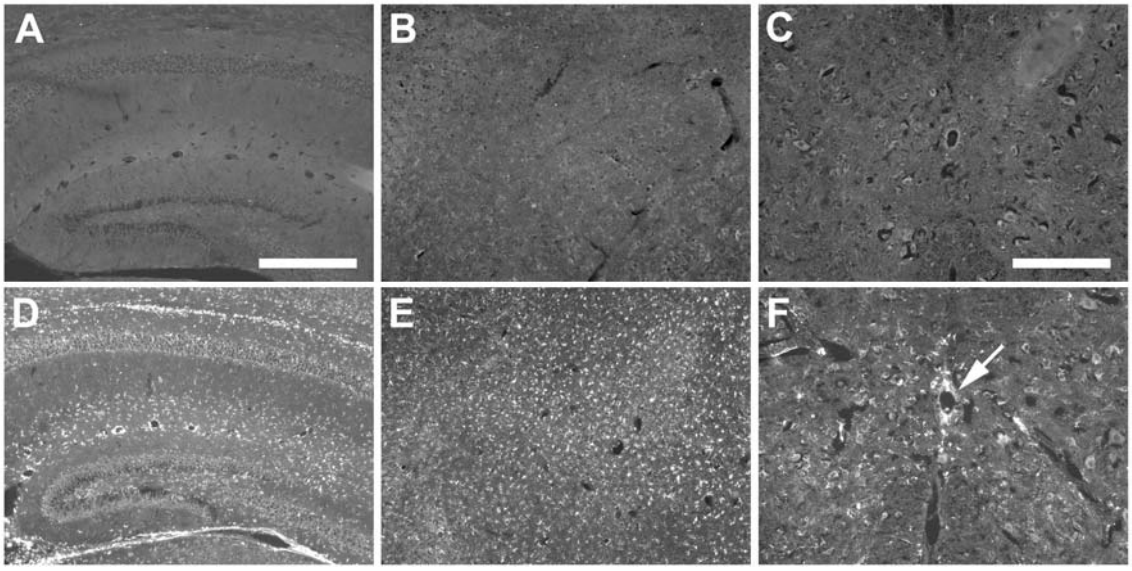
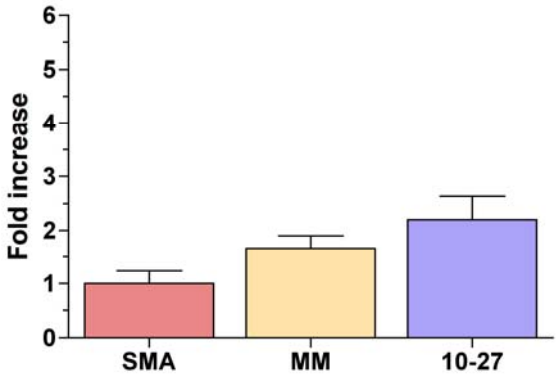
The PDF file includes:

Figure S1.



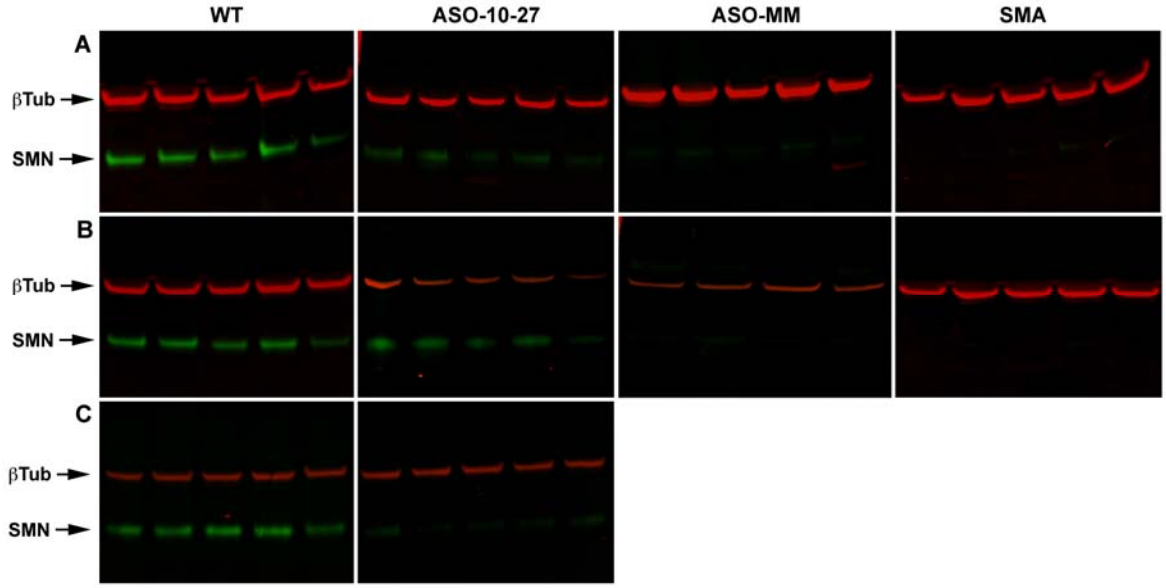
Distribution of ASO-10-27 in the brain after intracerebroventricular administration. Injection of ASO-10-27 resulted in widespread distribution of the oligonucleotide in the brain and spinal cord of 14-day old heterozygote mice. Immuno-staining against the phosphorothioate backbone of the oligonucleotide (see Methods) in saline-treated (A-C) and ASO-10-27-treated (D-F) mice. Shown are the hippocampus (A, D), brain stem (B, E), and lumbar spinal cord (C, F). The efficient delivery to the spinal cord may have been due in part to the unimpeded flow of the cerebrospinal fluid into the patent central canal of neonatal mice, as evidenced by the positive staining of ependymal cells along the rostrocaudal extent of the spinal cord (arrow, F). Scale bars: 400 μm (A, B, D, E), 200 μm (C, F).

Figure S2.



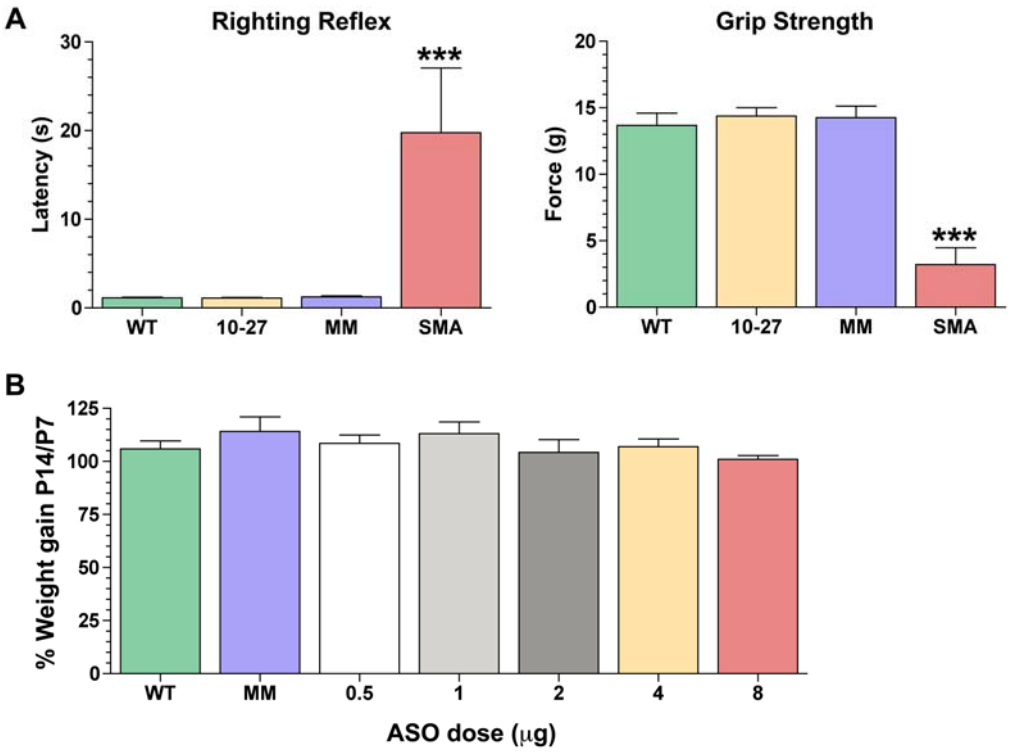
Splicing of SMN2 in the liver at 16 days. ICV injection of 4 ug of ASO-10-27 or ASO-mismatch (MM) in P0 SMA mice did not result in a signficant increase ($p>0.05$) in exon-7-containing SMN2 transcripts, compared to untreated SMA mice. The data was normalized to untreated SMA, and analyzed using one-way ANOVA and Bonferroni multiple *post hoc* comparisons. As a reference, there was a 6-fold increase in SMN2 transcripts that contained exon 7 in the spinal cord of ASO-10-27-treated SMA mice at 16 days (see Figure 2).

Figure S3.



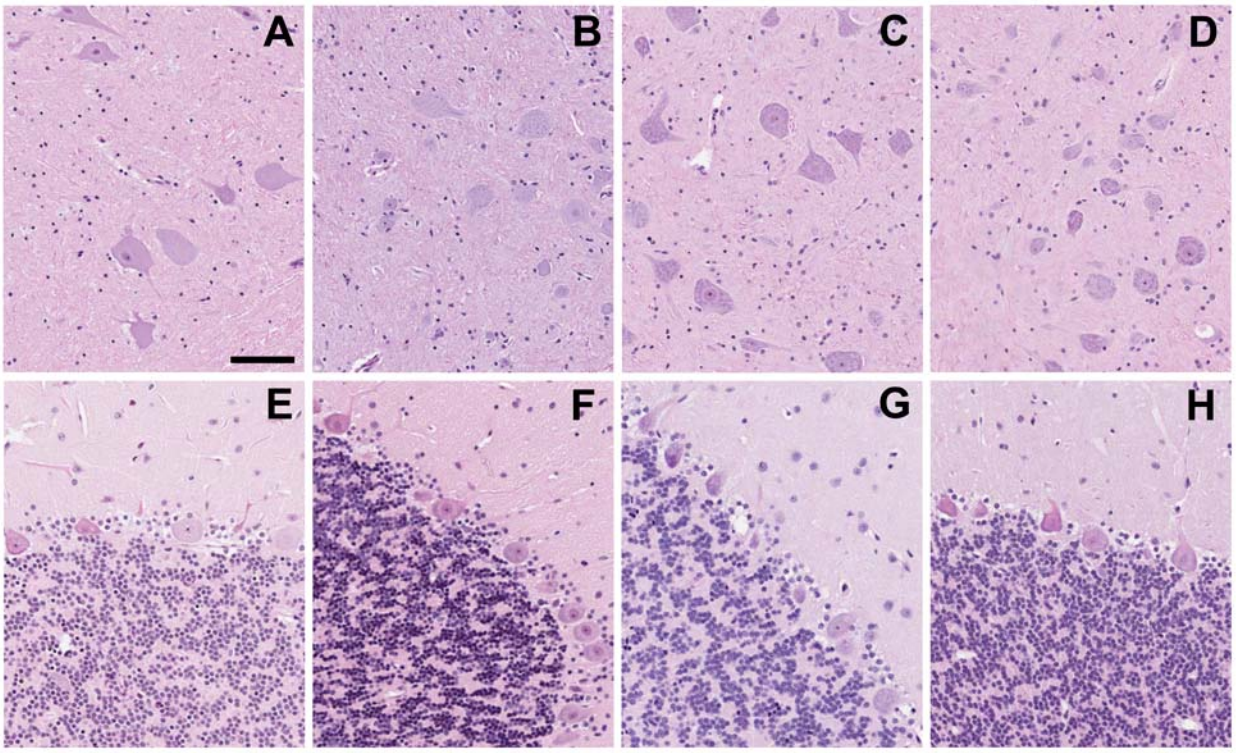
SMN Western blots of the thoracic region at 3 (A), 16 (B), and 30 (C) days post-injection. The highest level of SMN protein following ASO-10-27 treatment was detected at 16 days post-injection. Each lane represents a different animal, and SMN (green) and β-tubulin (red) were detected as reported (12). Western blotting was not performed on ASO-mismatch-treated and untreated SMA control mice at 30 days because none of the mice survived to this age. Abbreviations: WT, untreated WT; 10-27, ASO-10-27-treated SMA, ASO-MM, ASO-mismatch-treated SMA; SMA, untreated SMA.

Figure S4.



Behavioral performances and weight changes in treated wild-type mice. ICV administration of 4 ug of ASO-10-27 or ASO-mismatch in P0 WT mice did not produce deleterious performances on the righting reflex and grip strength tests at 14 days post-injection (A). There was no signficant difference ($p>0.05$) on either test as verified by one-way ANOVA and Bonferroni multiple *post hoc* analyses. However, untreated SMA mice performed significantly worse ($p<0.001$) than all the WT groups using the same statistical analyses (A). The percent weight gain between P14 and P7 with the different doses of ASO-10-27 in wild-type mice following ICV injection at P0 (B). There was no statistical difference in weight gain between the different groups ($p>0.05$) with one-way ANOVA and Bonferroni multiple *post hoc* analyses. Abbreviations in panel A (n=5 per group): WT, untreated WT; 10-27, ASO-10-27-treated WT, MM, ASO-mismatch-treated WT; SMA, untreated SMA. Abbreviations and n-values in panel B: untreated WT (n=20); MM, WT mice treated with 4 µg of ASO-mismatch (n=8); WT mice treated with 0.5 µg (n=8), 1 µg (n=8), 2 µg (n=8), 4 µg (n=20), or 8 µg (n=8) of ASO-10-27.

Figure S5.



H&E staining of the cynomolgus brain and spinal cord. There was no overt pathology in the brain and spinal cord of ASO-10-27-treated cynomolgus monkeys. Shown are representative tissue sections of the lumbar ventral horn (A-D) and cerebellum (E-H) from animals that received 14-day ICV infusion of saline (A, E), and from animals that received 1-day (B, F), 3-day (C, G) and 14-day (D, H) intrathecal infusions of ASO-10-27. Scale bar: 100 μm.