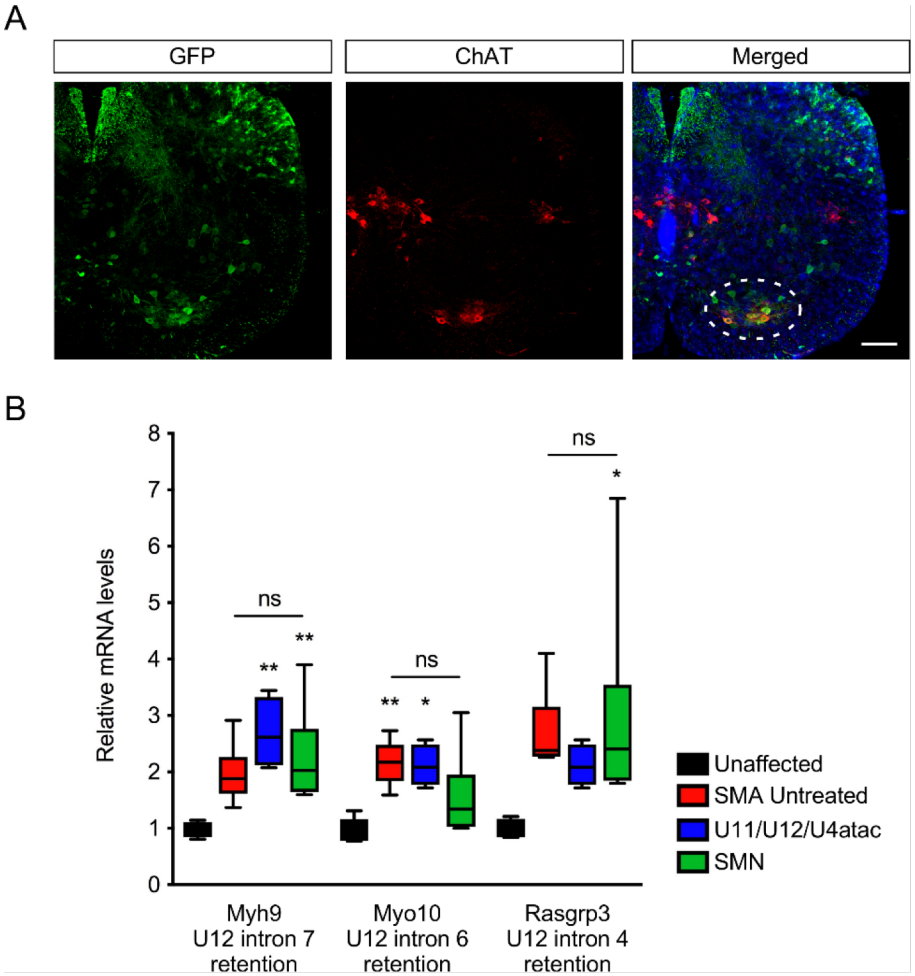
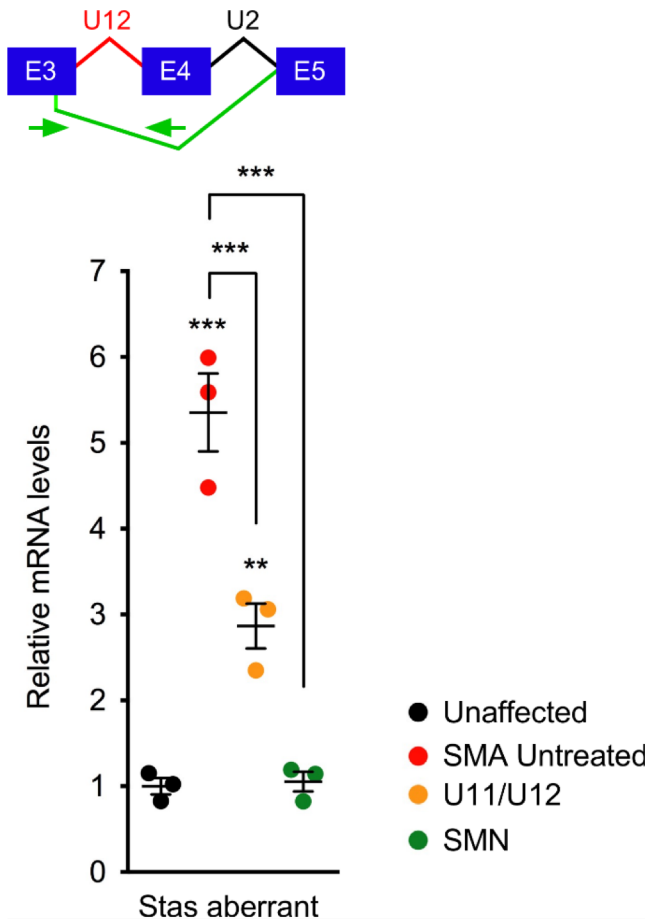


Supplementary Figure 1. Analysis of other U12 intron-containing genes dysregulated in SMA.
(**A**) Immunostaining of the L1 spinal cord at P11 from an SMA mouse ICV-injected with AAV9-GFP with antibodies against GFP (green) and ChAT (red). In the merged image, nuclei are counterstained with DAPI (blue) and a dotted circle highlights the motor neuron nucleus. Scale bar = 100mm. (**B**) RT-qPCR analysis of U12 intron retention in Myh9, Myo10 and Rasgrp3 mRNAs in the spinal cord of unaffected control mice (n=6) and SMA mice that were either untreated (n=6) or ICV-injected with AAV9-U11/U12/U4atac (n=4) and AAV9-SMN (n=6) at P9. The box-and-whiskers graph shows the median, interquartile range, minimum and maximum. Statistics were performed with one-way ANOVA with Tukey's *post hoc* test. * $P < 0.05$; ** $P < 0.01$; ns = no significance.

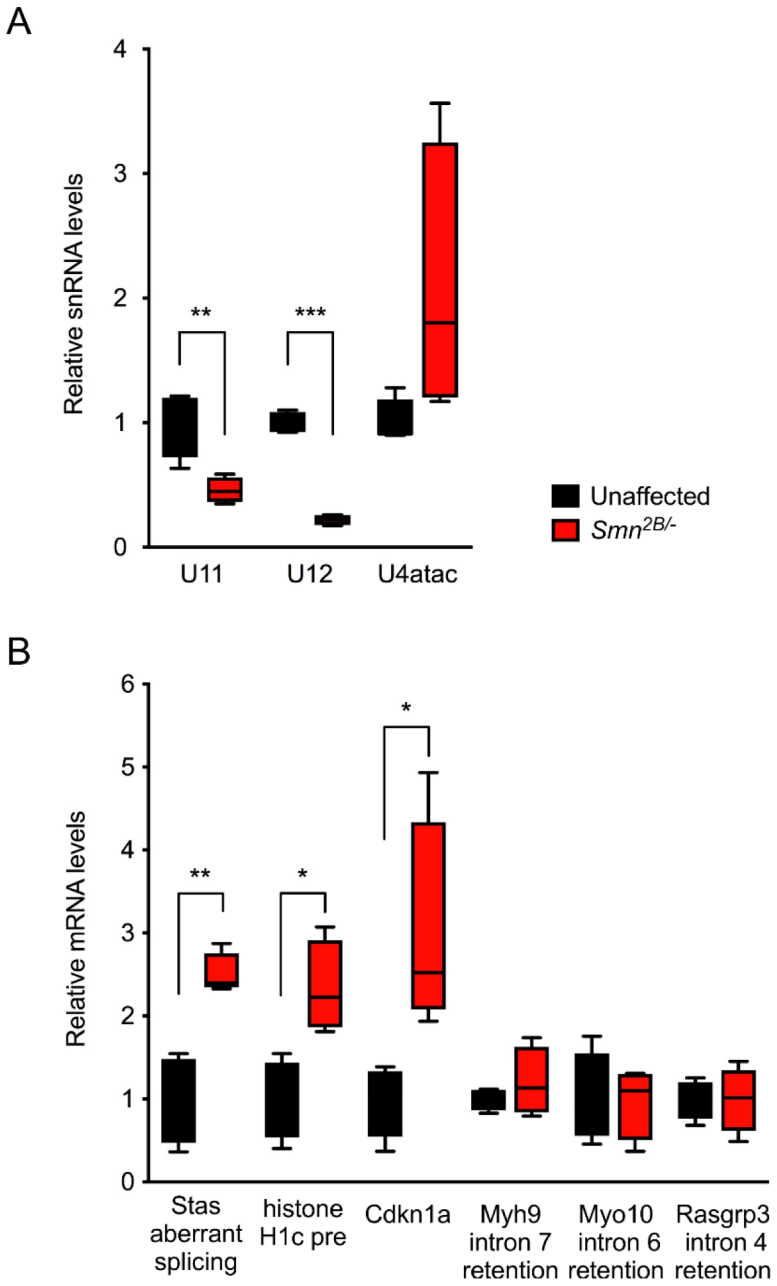


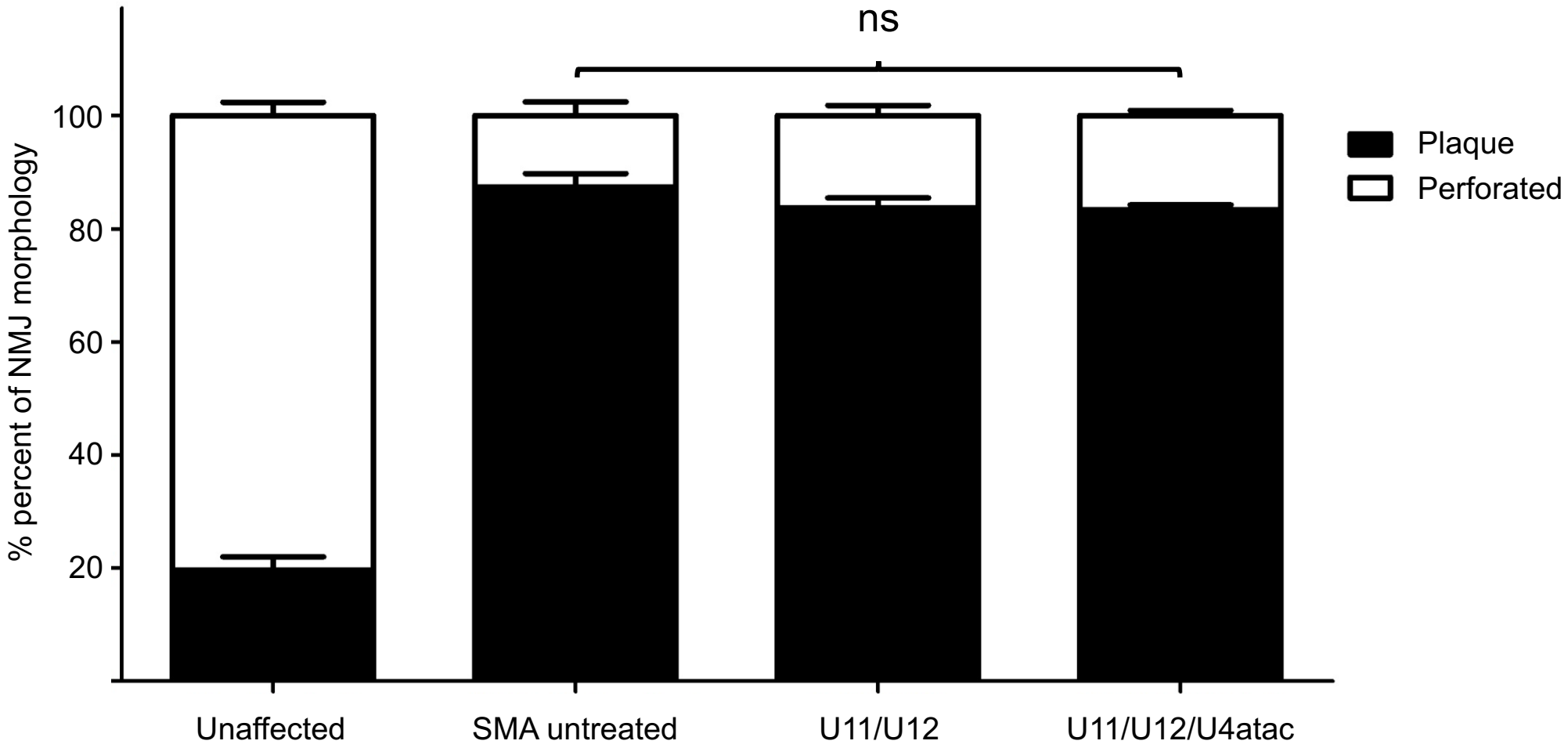
Supplementary Figure 2. AAV9-mediated correction of Stasimon U12 splicing defects in lumbar DRGs of SMA mice.

RT-qPCR analysis of the levels of aberrantly spliced Stasimon mRNA in lumbar DRGs from unaffected control mice and SMND7 SMA mice that were either untreated or ICV-injected with AAV9-U11/U12 and AAV9-SMN at P10. A schematic of the RNA processing event monitored in the assay is shown at the top. The scatter plot shows individual data points, mean and SEM from three mice per group. Statistics were performed with one-way ANOVA with Tukey's *post hoc* test. ** $P < 0.01$; *** $P < 0.001$.

Supplementary Figure 3. Analysis of minor snRNA expression and mRNA processing defects in the spinal cord of $Smn^{2B/-}$ SMA mice.

(**A**) RT-qPCR analysis of minor snRNA levels in the spinal cord of unaffected control mice (n=4) and $Smn^{2B/-}$ mice (n=4) at P18. The box-and-whiskers graph shows the median, interquartile range, minimum and maximum. Statistics were performed with two-tailed unpaired Student's *t*-test. ** P < 0.01; *** P < 0.001. (**B**) RT-qPCR analysis of the levels of aberrantly spliced Stasimon mRNA, 3' end-extended histone H1c precursor mRNA, Cdkn1a mRNA expression, and U12 intron retention in Myh9, Myo10 and Rasgrp3 mRNAs in the spinal cord from the same groups as in (A) at P18. The box-and-whiskers graph shows the median, interquartile range, minimum and maximum. Statistics were performed with two-tailed unpaired Student's *t*-test. * P < 0.05; ** P < 0.01.





Supplementary Figure 4

Percentage of mature endplate morphology of the NMJs over total number of endplates in the sternomastoid muscle. Samples were collected at P12 from unaffected control mice and SMA mice either untreated or injected with scAAV9-U11/U12 and scAAV9-U11/U12/U4atac. No statistical significance was observed for endplate maturity between SMA mice that were untreated or treated with either scAAV9-U11/U12 or scAAV9-U11/U12/U4atac. (Student *t*-test, P = 0.16; plaque vs. perforated)

RT-PCR Primers	Primer Sequences
Human U11 snRNA Forward	**5'-AAAAAGGGCTTCTGTCGTGA-3'**
Human U11 snRNA Reverse	**5'-AAAGGGCGCCGGGACCA-A-3'**
Human U12 snRNA Forward	**5'-ATGCCTTAAACTTATGAGTAAG-3'**
Human U12 snRNA Reverse	**5'-CGGGCAGATCGCAACTCC-3'**
Human U4atac snRNA Forward	**5'-AACCATCCTTTTCTTGGGGTT-3'**
Human U4atac snRNA Reverse	**5'-GCTCTAGTTGATGCGGGTG-3'**
Human 5S rRNA Forward	**5'-GTCTACGGCCATACCACCC-3'**
Human 5S rRNA Reverse	**5'-CGGTATTCCCAGGCGGTC-3'**
Human U1 snRNA Forward	**5'-CTTACCTGGCAGGGGAGATAC-3'**
Human U1 snRNA Reverse	**5'-CCCACATTTGGGGAAATCGC-3'**
Human U2 snRNA Forward	**5'-ATCGCTTCTCGGCCTTTTGG-3'**
Human U2 snRNA Reverse	**5'-AAGCTCCTATTCCATCTCCC-3'**

Supplementary Table 1
Primer pairs used to detect different human snRNAs and 5S rRNA.

Genotyping Primers	Primer Sequences
mSmn -WT Forward	**5'-TCTGTGTTCGTGCGTGGTGACTTT-3'**
mSmn -WT Reverse	**5'-CCCACCACCTAAGAAAGCCTCAAT-3'**
SMN1-KO Forward	**5'-CCAACTTAATCGCCTTGCAGCACA-3'**
SMN1-KO Reverse	**5'-AAGCGAGTGGCAACATGGAAATCG-3'**

Supplementary Table 2
List of primer sets used for animal genotyping.

Name	Forward Sequence (5’ to 3’)	Reverse Sequence (5’ to 3’)
Tspan31 U12 intron	CTGCTCTTCTTTGTATCCTGGCCTTAG	ACCGTCAACTTGTGTGGGTTACAG
Stas aberrant splicing	TGACGCCAAGGCTCTAGGAAAA	CCAAGTCCGGAGCATTGTACATAAAAGG
Clcn7 exon skipped	ATCAACCACACGCCTGTTGC	ATCTTCACCCCATTGAGGAAGCAC
Myh9 intron 7 retention	AAGAAGGACCAGGGGGAGTT	GATTCCATCAGCCATAAGGATAC
Myo10 intron 6 retention	GCCACCAGTATCTGTTTTCTTCC	AATTCTTCTCGCTCTCCCTGGT
Rasgrp3 intron 4 retention	GAGATGTTTGATGACAATGGAG	TGACCAAAAGCATTACACAGCCT
H1c-pre mRNA	GAGCCACCACTCCCACTTAAG	GGATCGAGTCCCTTGCAAC
Cdkn1a mRNA	GACATTCAGAGCCACAGGCACC	GAGCGCATCGCAATCACGGCGC
Smn mRNA	TGCTCCGTGGACCTCATTTCTT	TGGCTTTCCTGGTCCTAATCCTGA
Gapdh mRNA	AATGTGTCCGTCGTGGATCTGA	GATGCCTGCTTCACCACCTTCT
U11 snRNA	GTGCGGAATCGACATCAAGAG	CGCCGGGACCAACGAT
U12 snRNA	GAAAATAACGATTCGGGGTGACGC	CCCAGGCATCCCGCAAAGTA
U4atac snRNA	CTTTTCTTGGGGTTGCGCTACTGT	GGCGTTAGCAGTACTGCCCT
5.8S rRNA	TTCGCTTGTGGCAGATCACTC	GGGCTGCAAGTGCATTTGAA

Supplementary Table 3
List of RT-qPCR primers used in this study.