Supplemental information

SMN controls neuromuscular junction integrity through U7 snRNP

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Figure S1. Co-expression of Lsm10 and Lsm11 enhances U7 snRNP assembly, related to Figure 1.

(A) Schematic representation of the lentiviral constructs used to generate stable NIH3T3 cell lines with mouse Lsm10 and/or Lsm11 overexpression.

(B) RT-qPCR analysis of Lsm10 and Lsm11 mRNA levels in NIH3T3-SmN RNAi cells with and without (control) overexpression of Lsm10 and Lsm11 either individually or in combination. Data are mean and SEM (n=3 independent experiments) normalized to Gapdh mRNA and expressed relative to control NIH3T3-SmN RNAi cells set as 1. Statistics were performed with one-way ANOVA with Tukey’s post hoc test. ****P<0.0001; ***P<0.001; **P<0.01.
(C) Northern blot analysis of U1 and U7 snRNAs immunoprecipitated with the indicated antibodies from extracts of NIH3T3-Smnrna cell lines with and without overexpression of Lsm10 and Lsm11 either individually or in combination.

(D) Schematic representation of the vector expressing both Lsm10 and Lsm11 with an intervening 2A self-cleaving peptide.

(E) Western blot analysis with anti-FLAG antibodies of HEK293T cells with or without transient transfection of the Lsm10-2A-Lsm11 vector depicted in (D).

(F) In vitro snRNP assembly for U7 and U1 snRNAs (top panels) and Western blot analysis of SMN levels (bottom panels) using the same extracts as in (E). Quantification of snRNP assembly for each snRNA is shown as a percentage of control at the bottom of the corresponding panel.
Figure S2. Co-expression of Lsm10 and Lsm11 does not increase SMN expression and enhances U7 snRNP biogenesis in SMA mice, related to Figure 2.

(A) Schematic representation of the AAV9 viral vector and workflow to achieve Lsm10 and Lsm11 co-expression in vivo.

(B) In vitro snRNP assembly of U1 and U7 snRNAs in brain extracts from uninjected and AAV9-Lsm10-2A-Lsm11 injected WT mice at P11. Quantification of snRNP assembly for each snRNA is shown as a percentage of control at the bottom of the corresponding panel.

(C) Western blot analysis of SMN levels in brain extracts from the same samples used in (B).

(D) RT-qPCR analysis of Lsm10 (left), Lsm11 (middle), and full-length human SMN2 (right) mRNA levels in the spinal cord of uninjected WT mice and SMA mice injected with AAV9-GFP or AAV9-Lsm10/11 at P11. Data are mean and SEM (n=6 mice) normalized to Gapdh mRNA and expressed relative to WT set as 1. Statistics were performed with the Kruskal-Wallis test followed by Dunn’s multiple comparisons test. ***P<0.001; **P<0.01; ns = not significant.

(E) Western blot analysis of SMN levels in spinal cord and liver from the same groups as in (D). Flag-Lsm10 and Flag-Lsm11 could only be detected with anti-FLAG antibodies from liver but not spinal cord of SMA mice injected with AAV9-Lsm10/11. In the Lsm11 panel, asterisks mark non-specific bands detected with anti-FLAG antibodies.

(F) In vitro U7 snRNP assembly (top panel) and Western blot analysis (bottom panels) of brain extracts from uninjected WT mice and SMA mice injected with AAV9-GFP or AAV9-Lsm10/11 at P11.

(G) Percentage of U7 snRNP assembly relative to uninjected WT mice from the experiment in (F). Data are mean and SEM (n=3 mice). Statistics were performed with two-tailed Student’s t test. **P<0.01.

(H) Northern blot analysis of U7 snRNA and 5S rRNA in spinal cord (top panels) and liver (bottom panels) from the same groups as in (F).

(I) Percentage of U7 snRNA levels relative to uninjected WT mice from the experiment in (H). Data are mean and SEM (n=3 mice). Statistics were performed with two-tailed Student’s t test. **P<0.01; ns = not significant.

(J) Immunostaining of L1 spinal cord, cerebellum, and liver from P11 SMA mice injected with AAV9-GFP using GFP, DAPI, and ChAT (spinal cord only). Scale bars=100µm.

(K) Quantification of the percentage of GFP+ motor neurons (MNs) from the L1 spinal cord of P11 SMA mice injected with AAV9-GFP as in (J). Data are mean and SEM (n=5 mice).
Figure S3. Co-expression of Lsm10 and Lsm11 improves histone mRNA processing, but not U7-independent RNA processing defects induced by SMN deficiency, related to Figure 2.

(A) RT-qPCR analysis of the ratio of 3'-extended and total histone mRNA levels in NIH3T3-SmnRNAi and NIH3T3-Lsm10/11/SmnRNAi cells. Schematic representation of a histone pre-mRNA and the 3'-end cleavage is shown at the top. For each cell line, fold change values of the ratio of 3'-extended (pre) and total (tot) histone mRNAs in Dox treated cells relative to untreated cells set as 1 are shown. Data are mean and SEM (n=3 independent experiments). Statistics were performed with two-tailed Student’s t test. *P<0.05; **P<0.01.

(B) RT-qPCR analysis of aberrant U12 splicing and U12 intron retention in the Stasimon (Stas) mRNA induced by SMN deficiency in the same cell lines as in (A). Schematic representation of the monitored RNA processing events is shown at the top. For each cell line, fold change values in Dox treated cells relative to untreated cells set as 1 are shown. Data are mean and SEM (n=3 independent experiments) normalized to Gapdh mRNA. Statistics were performed with two-tailed Student’s t test. ns = not significant.

(C) RT-qPCR analysis of exon skipping and U12 intron retention in the Clcn7 mRNA in the same cell lines as in (A). Schematic representation of the monitored RNA processing events is shown at the top. Data are mean and SEM (n=3 independent experiments) normalized to Gapdh mRNA. Statistics were performed with two-tailed Student’s t test. ns = not significant.

(D) RT-qPCR analysis of Cdkn1a mRNA levels in the same cell lines as in (A). Data are mean and SEM (n=3 independent experiments) normalized to Gapdh mRNA. Statistics were performed with two-tailed Student’s t test. ns = not significant.

(E) RT-qPCR analysis of 3'-extended histone mRNAs in liver from uninjected WT mice and SMA mice injected with AAV9-GFP or AAV9-Lsm10/11 at P11. Schematic representation of a histone pre-mRNA and the 3'-end cleavage is shown at the top. Data are mean and SEM (n=3 mice for WT and SMA+GFP; n=6 mice for SMA+Lsm10/11) normalized to Gapdh mRNA. Statistics were performed with one-way ANOVA with Tukey’s post hoc test. **P<0.01.

(F-H), RT-qPCR analysis of aberrant U12 splicing of Stas mRNA (F) as well as Chodl (G) and Cdkn1a (H) total mRNA levels in spinal cord from the same groups as in (E). Schematic representation of the monitored RNA processing events is shown at the top. Data are mean and SEM (n=6 mice) normalized to Gapdh mRNA. Statistics were performed with one-way ANOVA with Tukey’s post hoc test. ***P<0.001; ns = not significant.
Figure S4. Co-expression of Lsm10 and Lsm11 does not correct cell proliferation defects in SMN-deficient mammalian cells, related to Figure 2.

(A-B) Analysis of cell proliferation in NIH3T3-SmnRNAi (A) and NIH3T3-Lsm10/11/SmnRNAi (B) cell lines. Equal numbers of each cell line were cultured with or without Dox for the indicated number of days and cell number was determined at each time point.

(C) Western blot analysis of histone protein levels in spinal cord from WT and SMA mice at P11. A two-fold serial dilution of WT extract is shown on the left.
Figure S5. Co-expression of Lsm10 and Lsm11 improves motor function and skeletal muscle atrophy in SMA mice, related to Figure 3.

(A-B) Righting time (A) and body weight (B) of WT mice either uninjected or injected with AAV9-Lsm10/11 and SMA mice injected with AAV9-GFP, AAV9-Lsm10/11 or AAV9-SMN. Data are mean and SEM from the following number of mice per group (n=16 for WT, SMA+GFP, SMA+SMN, and SMA+Lsm10/11; n=10 for WT+Lsm10/11).

(C) Kaplan-Meier plot of mouse survival from the same treatment groups and number of mice as in (A-B).

(D) Frequency distribution of myofiber sizes in the triceps muscle from uninjected WT mice and SMA mice injected with AAV9-GFP, AAV9-Lsm10/11 or AAV9-SMN at P11. Data are mean and SEM (n=5 mice for WT, SMA+Lsm10/11 and SMA+SMN; n=6 mice for SMA+GFP).
Figure S6. Loss of SV2B expression at vulnerable NMJs in SMA mice is not U7-dependent, related to Figure 5.

(A) NMJ immunostaining with SV2B, Synaptophysin (SYP) and α-bungarotoxin (BTX) of quadratus lumborum muscles from untreated WT mice and SMA mice injected with AAV9-GFP, AAV9-Lsm10/11 or AAV9-SMN at P11. Denervated NMJs lacking pre-synaptic SYP staining are indicated by arrowheads and SYP staining is only shown for one representative innervated NMJ (dotted box) in the bottom insets. Scale bar=20µm.

(B) NMJ immunostaining with SV2B, Synaptophysin (SYP) and α-bungarotoxin (BTX) of tibialis anterior muscles from untreated WT mice and SMA mice injected with AAV9-GFP at P11. SYP staining is only shown for one representative innervated NMJ (dotted box) in the bottom insets. Scale bar=20µm.

(C) Percentage of innervated NMJs that are SV2B+ from experiments as in (A). Data are mean and SEM (n=3 mice). Statistics were performed with one-way ANOVA with Tukey’s post hoc test. **P<0.01; ***P<0.001; ns = not significant.

(D) Percentage of innervated NMJs that are SV2B+ from experiments as in (B). Data are mean and SEM (n=3 mice). Statistics were performed with two-sided Student’s t test. ns = not significant.
Table S1. List of primers and probes used in this study, related to Figure 1 and STAR Methods.

### Northern blot probes

| Name       | Reference            | Sense Sequence (5’ to 3’)                        |
|------------|----------------------|--------------------------------------------------|
| mouse U7   | Tisdale et al., 2013 | GCAGGTTTTCTGACTCCGGTGGAA                        |
| mouse U1   | Tisdale et al., 2013 | AATTGTGAGTGGGGACACTGGGT                        |
| mouse U12  | Tisdale et al., 2013 | AATAACGATGGGGTGACGCCGA                        |
| mouse 5S   | Tisdale et al., 2013 | CCGCCCATACACCCTGAAGCAGGCCC                    |
| mouse 5.8S | Tisdale et al., 2013 | GGTGGATCAGCGTCGTC                        |

### PCR primers for mouse genotyping

| Name       | Reference            | Forward Sequence (5’ to 3’) | Reverse Sequence (5’ to 3’) |
|------------|----------------------|-----------------------------|-----------------------------|
| SmnWT      | Simon et al., 2019   | GATGATTCTGACATTTGGGATG      | TGGCTTATCTGAAGTTCAGA       |
| SmnKO      | Simon et al., 2019   | GATGATTCTGACATTTGGGATG      | GAGTAACAGCGGGTCGGATCC      |

### qPCR primers for AAV titration

| Name       | Reference            | Forward Sequence (5’ to 3’) | Reverse Sequence (5’ to 3’) |
|------------|----------------------|-----------------------------|-----------------------------|
| AAV-5’     | Simon et al., 2019   | GTGCTTTTTATTCTACGCTGAGGAGT  | TCGGCCCCAACCGGCGTGGGAT      |

### RT-qPCR primers

| Name            | Reference            | Forward Sequence (5’ to 3’) | Reverse Sequence (5’ to 3’) |
|-----------------|----------------------|-----------------------------|-----------------------------|
| mouse H1c-pre   | Tisdale et al., 2013 | GAGCCACCACTCCCATGAGGAG      | GATGTGCTTCTACGCCATC         |
| mouse H2Ac-pre  | Tisdale et al., 2013 | GAGCCACCACTCCCATGAGGAG      | GATGTGCTTCTACGCCATC         |
| mouse H2Bepre   | Tisdale et al., 2013 | CTCACACTCTAAGCCAAAGGC       | CCAATAGGTTGTACTGCCC         |
| mouse H3-pre    | Tisdale et al., 2013 | CCTACTCGTAAATACAGAAAAGCT    | CATGATAGATGTTGAGACCAGA     |
| mouse H4c-pre   | Tisdale et al., 2013 | GATTTCCACTGTCAAGAAGAGG      | CACCTAAACTACTAGCAGAGC      |
| mouse H1c-tot   | Tisdale et al., 2013 | GCCGGGCTAAAGGGAGCGAAAG      | CCTTGGATTTTAAATACCGAGGAG    |
| mouse H2Ac-tot  | Tisdale et al., 2013 | CACCGAGCAGAGGAGCCACACAGAAG  | GAAGTTTCCGAGATTCTGCTG      |
| mouse H2Be-tot  | Tisdale et al., 2013 | GCTGTCACCAGTACAGCAGCA       | GCCCTTGTTGAGGAGTCTGA        |
| mouse H3-tot    | Tisdale et al., 2013 | CAAGGCGTGCAACATCATG         | GAAGCTTCTTGTATTAACAGGAGGAG |
| mouse H4c-tot   | Tisdale et al., 2013 | CAAGGCCAAGGAGCCGTCA         | CCTTTGTTGAGCAGGAAATTCA     |
| mouse Gapdh     | Ruggeri et al., 2012 | AATGTGCTGCTGCTGGGATCTQA     | GAAGCTGCTTCTACGAGCCATC     |
| mouse Smm      | Ruggeri et al., 2012 | TGCTGCCTGACCCGCAATTCTTTT    | TGGCTTCTTGTGGGCATCTGA       |
| human SMN2 FL   | Ruggeri et al., 2012 | CACCACTCTCCTATGCTGCAATG    | GAATGTGACAGCCTTCTCTTTT     |
| mouse Lsm10    | This paper           | CCAATGCTACCTATACCGACC       | GTGACGATGATGCTGACCAT       |
| mouse Lsm11    | This paper           | GCTCCAGAGAGGGGCCACACACAGAAG | ACCGATGAGAGGCGCACATC       |
| mouse Cdkn1a   | Ruggiu et al., 2012  | GACATTTGAGGAGCAGGAGGCC      | GAGGCGGATGACACGGCAAAGC    |
| mouse Chodl    | Van Alstyne et al., 2018 | CCAATTTCTACGAGGAGCAGA       | TGGTGGCCTCTGAGGAGGTGATGG    |
| mouse Stas U12 intron | Lotti et al., 2012 | GTCAACTTTACCTCTGATAGGGAAG   | GAGGAAGATGCGAAGCCGGTGAGC   |
| mouse Stas aberrant | Lotti et al., 2012 | TGAAGATGAGAGGCAAGTTGGGAAA   | CCAAGTGCCGGAGACTATGACATTAAGAG |
| mouse Clcn7 exon skipping | Lotti et al., 2012 | ATACACACACCACTATGCTG       | ATCTCCACCACTGAGGAGACAGC    |
| mouse Clcn7 U12 intron | Lotti et al., 2012 | CACCAAGGACAGATCCTGCAAGACAGAC | GCTGTCGTTCTTACTAGCCTGACT    |
| mouse Agrin TOT | This paper           | CGTGGTGGATGCTTCTGGC         | CATGACGCTACACTCAGT         |
| mouse Agrin ΔZ  | This paper           | TGCTGCTGGGCGCTCTCTG         | TGCAGGGCGGTCTGACTCAGG      |
Table S2. List of antibodies used in this study, related to Figure 1.

| Name                  | Source                      | Cat #   | Host   | Application | Dilution |
|-----------------------|-----------------------------|---------|--------|-------------|----------|
| SMN (clone 8)         | BD Transduction Lab         | 610646  | Mouse  | WB          | 1:10,000 |
| Tubulin (DM1A)        | Sigma                       | T9026   | Mouse  | WB          | 1:10,000 |
| H2A                   | Millipore                   | 07-146  | Rabbit | WB          | 1:2,000  |
| H2AX                  | Cell Signaling              | 7631S   | Rabbit | WB / IHC    | 1:1,000 / 1:200 |
| H2B                   | Abcam                       | ab1790  | Rabbit | WB          | 1:40,000 |
| Histones (pan)        | Millipore                   | MAB052  | Mouse  | WB          | 1:10,000 |
| SmB (18F6)            | Custom made                 | N/A     | Mouse  | IP          | N/A      |
| FLAG M2               | Sigma                       | F3165   | Mouse  | WB / IP     | 1:2,000  |
| ChAT                  | Millipore                   | AB144P  | Goat   | IHC         | 1:100    |
| Neurofilament M       | Millipore                   | AB1987  | Rabbit | IHC         | 1:500    |
| VGlut1                | Custom made                 | N/A     | Guinea pig | IHC        | 1:5,000 |
| Synaptophysin         | Synaptic Systems            | 101-004 | Guinea pig | IHC        | 1:500    |
| GFP                   | Sigma                       | G1544   | Rabbit | WB          | 1:5,000  |
| GFP                   | Aves                        | GFP-1020| Chicken| IHC         | 1:500    |
| Agrin                 | Custom made                 | N/A     | Rabbit | IHC         | 1:1,000  |
| SV2B                  | Synaptic Systems            | 119-102 | Rabbit | IHC         | 1:200    |
| Mouse immunoglobulin (IgG) | Sigma                   | I8765   | Mouse  | IP          | N/A      |
| Alexa-Fluor 488 donkey anti-rabbit | Jackson     | 711-545-152 | Donkey | IHC         | 1:250    |
| Alexa-Fluor 488 donkey anti-goat | Jackson    | 705-545-147 | Donkey | IHC         | 1:250    |
| Alexa-Fluor 488 donkey anti-chicken | Jackson   | 703-545-155 | Donkey | IHC         | 1:250    |
| Cy3 donkey anti-rabbit | Jackson                  | 711-165-152 | Donkey | IHC         | 1:250    |
| Cy3 donkey anti-mouse | Jackson                  | 715-165-150 | Donkey | IHC         | 1:250    |
| Cy3 donkey anti-goat  | Jackson                  | 705-165-147 | Donkey | IHC         | 1:250    |
| Cy5 donkey anti-goat  | Jackson                  | 705-175-147 | Donkey | IHC         | 1:250    |
| Cy5 donkey anti-guinea pig | Jackson    | 706-175-148 | Donkey | IHC         | 1:250    |
| HRP goat anti-mouse   | Jackson                  | 115-035-044 | Goat  | WB          | 1:10,000 |
| HRP goat anti-rabbit  | Jackson                  | 111-035-003 | Goat  | WB          | 1:10,000 |