Table S1. Primers and probes used in this study, Related to Figure 2.

Northern blot probes

Name	Sense Sequence (5' to 3')
mouse U7	GCAGGTTTTCTGACTTCGGTCGGAA
mouse 5S	CGGCCATACCACCCTGAACGCGCCC
mouse 5.8S	GGTGGATCACTCGGCTCGTGCGTCG

RT-qPCR primers for 3' end-extended histone mRNAs

Name	Forward Sequence (5' to 3')	Reverse Sequence (5' to 3')
mouse H1c	GAGCCACCACTCCCACTTAAG	GGATCGAGTCCCTTGCAAC
mouse H2Ac	GGAAACTTCAAACCCAAAGG	GAGCCCACCAGATTAGGGTAG
mouse H2Be	CTCACACTCCTAAACCAAAGGC	CCATATAAAGGTGGTACTGCGG
mouse H3i	CCTACCTGTTAATAACAAAAGGCT	CATGATAGATGTTGGACCTGAG
mouse H4c	GATTTCCACTGTCAACAAAAGG	CACTTAAACTACCTAGCAGGCTC
mouse H2AX	TCCCCACACCTCCACAAAG	GGAAAGAGAAAGGATGGGGACG
human H1d	AGTGGCCAAGAGTGCGAAAA	CTTCGGCTTCCCCGACTTAG
human H2Ac	GCAACGACGAGGAACTGAAC	GGCTTTGTGGCTTTCGGTTT
human H2Bc	ATCACCTCCAGGGAGATCCA	GAGCCTTTGGGGTTAGGTGT
human H3e	CTATTCATGCCAAACGCGTG	GAGCCTTTGGATTTAAGGTTTG
human H4l	CAGCCATGGACGTGGTTTAC	TGAGAAGGGCCTTTGAGGAAC

RT-qPCR primers for total histone mRNAs

Name	Forward Sequence (5' to 3')	Reverse Sequence (5' to 3')
mouse H1c	GCGCGTCTAAAGCCGTAAAG	CTTTTGGATTGTTAGGGGAGGC
mouse H2Ac	CAACGACGAGGAGCTCAACAAG	GAAGTTTCCGCAGATTCTGTTGC
mouse H2Be	GCTGTCACCAAGTACACCAGC	GCCTTTGGTTTAGGAGTGTGAG
mouse H3i	CAAGCGTGTCACCATCATG	GAGCCTTTTGTTATTAACAGGTAGG
mouse H4c	CAAGCGCAAGACCGTCA	CCTTTTGTTGACAGTGGAAATC
mouse H2AX	TCCTGCCCAACATCCAGG	TCAGTACTCCTGAGAGGCCTGC
mouse H2A.Z	CGCTGATCGGGAAGAAAGG	CACAGAGATACAGTCCACTGG
mouse H3.3	CCGTCGTTACCAGAAATCG	CTTCGCTAGCCTCCTGAAG

RT-qPCR primers for other mRNAs

Name	Forward Sequence (5' to 3')	Reverse Sequence (5' to 3')
mouse Gapdh	AATGTGTCCGTCGTGGATCTGA	GATGCCTGCTTCACCACCTTCT
human GAPDH	CTCAACGACCACTTTGTCAAGCTC	TCTTACTCCTTGGAGGCCATGT
human SMN	CACCACCTCCCATATGTCCAGATT	GAATGTGAGCACCTTCCTTCTTT
human CDKN1A	AGACTCTCAGGGTCGAAAACGG	GGCGTTTGGAGTGGTAGAAATCTGTC
human ATP6	GAAGCGCCACCCTAGCAATA	GCTTGGATTAAGGCGACAGC

Supplemental Experimental Procedures

Antibodies

The following antibodies were used in this study: anti-SMN clone 8 (BD Transduction Laboratories), anti-SMN 7F3 (Carissimi et al., 2005), anti-Tubulin DM 1A (Sigma), anti-H2A (Millipore), anti-H2B (Abcam), anti-H3 (Abcam), anti-H4 (Millipore), anti-H1 and core histones (Chemicon), anti-H2AX (Cell Signaling), anti-γH2AX (Abcam), anti-H2AZ (Cell Signaling), anti-mouse IgG (Sigma), anti-SmB 18F6 (Carissimi et al., 2006), anti-TMG (Oncogene), anti-Sm Y12 (NeoMarkers), anti-Strep (Qiagen), anti-FLAG M2 (Sigma) and mouse IgG immunoglobulins (Sigma). The anti-LSm11 3K4 monoclonal antibody was custom generated by Abmart using recombinant histidine-tagged mouse LSm11 protein.

DNA constructs

Tagged mouse LSm11 was generated by PCR amplification from a cDNA clone (clone ID 5294386; Thermo Scientific) and cloned downstream of FLAG and Strep tag sequences into a pcDNA3 vector (Invitrogen). Mature and precursor U7 cDNAs were generated by PCR amplification from genomic DNA of wild-type NIH3T3 cells using specific primers. The PCR products contained a T7 promoter immediately upstream of each U7 sequence for use *in vitro* transcription and were cloned into the pCR8/GW/TOPO vector using the TOPO TA cloning kit. All constructs were confirmed by DNA sequencing.

Cell culture

NIH3T3 cells were cultured in Dulbecco modified Eagle medium (DMEM) with high glucose (Gibco) containing 10% fetal bovine serum (HyClone), 2 mM glutamine

(Gibco), and 0.1 mg/ml gentamicin (Gibco). Induction of RNAi was achieved through addition of doxycyline (Fisher) to the growth medium at a final concentration of 100 ng/ml. Cells were treated with doxycyline for 5 days, unless otherwise indicated.

HEK293 cells were cultured in Dulbecco modified Eagle medium (DMEM) with high glucose (Gibco) containing 10% fetal bovine serum (HyClone), 2 mM glutamine (Gibco), 100 units/ml penicillin (Gibco) and 100 μg/ml streptomycin (Gibco). For transient transfection, 6 x 10^5 cells were seeded in individual wells of a 6-well tissue culture plate. Approximately 24 hours later, cells were transfected with 2 μg of pcDNA3-FLAG-LSm11 using the CalPhos transfection kit (Clontech) according to the manufacturer's instructions. The media was changed the following morning, and cells were harvested into ice-cold PBS 60 hours post-transfection.

For protein labeling experiments, NIH3T3 cells were grown in 10 cm^2 dishes for 4.5 days in the presence or absence of doxycycline. Cells were washed once in PBS and incubated for 30 minutes in methionine- and cysteine-free DMEM (Gibco) containing 10% dialyzed fetal bovine serum (Gibco), 2 mM glutamine (Gibco), and 0.1 mg/ml gentamicin (Gibco). Cells were then incubated overnight in the same medium supplemented with 250 μCi of a [^{35}S]-methionine and [^{35}S]-cysteine mixture using EXPRE^{35}S^{35}S Protein Labeling "Easy Tag" Mix (Perkin Elmer). The following morning cells were washed twice in ice cold PBS, harvested, and pelleted by centrifugation at 4,000 rpm for 3 minutes. Cells were fractionated into soluble proteins and insoluble chromatin pellet, and equal amounts of extract from each fraction were resolved on a denaturing 12% SDS-PAGE gel. Total protein was detected using the EZBlue™ Gel Staining Reagent (Sigma-Aldrich) and [^{35}S]-labeled protein was detected by autoradiography. Quantification was done using a Typhoon PhosphorImager (Molecular Dynamics).

For DNA damage experiments, NIH3T3 cells were grown in the presence or absence of doxycyline for 5 days and then subjected to the following treatments prior to processing for immunofluorescence. For UV treatment, cells were exposed to 20 J/m^2 UV light using a SpectroLinker XL-1000 UV Crosslinker (Spectronics Corporation), followed by 1 hour of recovery at 37ºC. For drug treatments, cells were treated at 37ºC with 60 μg/ml of phleomycin (Sigma) for 2 hours, 1 μM camptothecin (Tocris) for 1 hour, 10 μM etoposide (Tocris) for 1 hour, and 50 μM hydrogen peroxide (Sigma) for 1 hour.

SMA mice and LCM

Mouse genotyping was carried out as previously described (Gabanella et al., 2007). Mice were sacrificed at the indicated times and tissues were harvested by manual dissection, immediately frozen in liquid nitrogen and stored at -80ºC until use.

For isolation of motor neurons by LCM, spinal cords from control and SMA mice injected in the iliopsoas muscle with CTb-488 were embedded in OCT, cryo-sectioned (10 μm) and adhered to UV treated PEN membrane slides (Leica). Tissue sections from L1-2 spinal segments of control and SMA mice were immediately fixed in ethanol for 15 seconds prior to collection of CTb-488^+ motor neurons with a Leica DM6000B instrument under 40X magnification as previously reported (Lotti et al., 2012).

Human SMA tissue

Human tissues were first cut into small pieces using a razor blade and then homogenized in 750 μl TRIzol Reagent using a Kinematica Polytron at 17,000 rpm for 20 seconds. An additional 250 μl TRIzol was added to each sample and mixed by pipetting. Samples were incubated at room temperature for 5 minutes. 200 μl of chloroform was added to each sample and the samples were vortexed until they became opaque (>10 seconds). Samples were then incubated at room temperature for 2 minutes and

centrifuged at 12,000 g for 10 minutes at 4ºC. The aqueous phase was transferred to a fresh tube containing 500 µl of isopropyl alcohol and 50 µl of 3 M sodium acetate. Samples were incubated at room temperature for 10 minutes and then centrifuged at 12000 g for 10 minutes at 4ºC. The supernatant was removed and the pellets were washed once in 70% ethanol, vortexed, and centrifuged at 7,500 g for 5 minutes at 4ºC. The supernatant was removed and the pellet was air-dried for 10 minutes at room temperature and then resuspended in 100 µl of DNAse-free, RNAse-free water. RNA samples were purified using the RNA Cleanup protocol from Qiagen (RNeasy Mini Kit) and RNA concentrations were measured using a NanoDrop 1000 (Fisher Scientific).

Extract preparation and immunoprecipitation

Total protein extracts were generated by cell lysis in SDS sample buffer (2% SDS, 10% glycerol, 5% β-mercaptoethanol, 60 mM Tris-HCl pH 6.8, bromophenol blue) followed by brief sonication and boiling. Protein was quantified using the RC DC Protein Assay (Bio-Rad).

For immunoprecipitation experiments, extracts were prepared in ice cold RSB-100 buffer (100 mM NaCl, 10 mM Tris-HCl pH 7.4, 2.5 mM $MgCl_2$) containing 0.1% NP40, protease inhibitors, and phosphatase inhibitors, by passing five times through a 27 gauge needle followed by centrifugation at 10,000 x g for 15 minutes at 4ºC. Extract supernatant was quantified by Bradford assay using Bradford Quick Dye (Bio-Rad). Antibodies were bound to protein G-Sepharose (Sigma) in RSB-100 buffer containing 0.1% NP40, protease inhibitors, and phosphatase inhibitors for 2 hours at 4ºC. Following five washes with the same buffer, antibody-bound beads were incubated with 200 µg of cell extract for 2 hours at 4ºC with tumbling. Following five washes with the same buffer, samples were processed for either RNA or protein analysis. Bound RNAs were extracted by treatment with proteinase K (200 µg) for 20 minutes at room temperature followed by

phenol-chloroform extraction and ethanol precipitation. Immunoprecipitated RNA was analyzed by electrophoresis on a denaturing 8% polyacrylamide gel containing 8 M urea followed by Northern blotting. Proteins were eluted from beads by boiling in SDS sample buffer and analyzed by SDS/PAGE on 12% polyacrylamide gels followed by Western blotting.

RNA analysis

For RT-qPCR experiments, RNA was reverse transcribed using RevertAid First Strand cDNA Kit (Fermentas) and triplicate reactions were performed using Power SYBR Green PCR master mix (Applied Biosystem) in a Realplex^4 Mastercycler (Eppendorf).

For Northern blot analysis, total RNA (2 μg) was analyzed by electrophoresis on a denaturing 8% polyacrylamide gel containing 8 M urea followed by transfer to a Hybond+ nylon membrane (GE Healthcare). Radioactive antisense RNA probes were *in vitro* transcribed from DNA oligonucleotide templates in the presence of [α^{32}P]-UTP (3000 Ci/mmol; Perkin Elmer) using the MEGAshortscript T7 kit (Ambion). RNA probes were denatured at 70ºC for 5 minutes and transferred to ice. Hybridization was carried out overnight at 60ºC in ULTRAhyb-Oligo hybridization buffer (Ambion). Quantification was carried out using a Typhoon PhosphorImager (Molecular Dynamics).

For cloning the U7 snRNA precursor, total RNA (5 μg) in formamide loading buffer (95% formamide, 18 mM EDTA, 0.025% SDS, 0.025% xylene cyanol, 0.025% bromophenol blue) from wild-type NIH3T3 cells was separated by electrophoresis on an 8% denaturing polyacrylamide gel containing 8 M urea. Following electrophoresis, the gel was washed once in 1X TBE buffer for 15 minutes and then incubated in 1X TBE containing ethidium bromide (0.5 μg/ml) for 15 minutes followed by visualization with UV

light using a BioDoc-It LSM 26E (UVP). A band corresponding to the size of U7 snRNA precursor was excised from the gel and RNA eluted by tumbling overnight in 400 μl of elution buffer (0.3 M sodium acetate pH 5.5, 1 mM EDTA, 0.1% SDS) with 40 μl of phenol. Eluted RNA was phenol-chloroform extracted once and ethanol precipitated. A 3'-linker was ligated to the precipitated RNA by incubation of the resuspended RNA with linker oligonucleotide (5'–rAppTGGAATTCTCGGGTGCCAAGG/ddC/-3'; 50 μM), 1X RNA ligase buffer (Promega), 12% PEG 8000, T4 RNA ligase 2 truncated (200 U; Promega) in a final volume of 20 μl with incubation at 22ºC for 2 hours. RNA ligase was heat inactivated at 65ºC for 15 minutes, and excess oligonucleotide was removed by passing ligation reaction through a Chroma Spin TE 30 column. Eluted RNA was precipitated and resuspended in sterile water. The RNA was then reverse transcribed using a primer complementary to the 3' linker (5'-CCTTGGCACCCGAGAATT-3') together with SuperScript II (200 U), RNasin (20 U), 0.5 mM dNTPs, 5X Super Script II buffer (Invitrogen), and 10 mM DTT. The RT reaction was incubated at 42ºC for 45 minutes, after which a primer complementary to the 5' linker was added and incubated for an additional 15 minutes. The reaction was then purified through a Chroma Spin TE-30 column followed by ethanol precipitation. U7 snRNA was then amplified by PCR using one U7 snRNA specific primer (forward; 5'-CAGCTCTTTTAGAATTTGTCTAGCAGG-3') and a primer complementary to the 3' linker (reverse; 5'-CCTTGGCACCCGAGAATT-3'). PCR reactions were separated on a 1.5% agarose gel containing GelRed (Biotium) and bands were excised and gel purified using Qiagen Gel Extraction kit (Qiagen). Purified DNA was cloned using the TOPO TA Cloning kit and unique clones were DNA sequenced.

***In vitro* snRNP assembly**

For *in vitro* snRNP assembly experiments, NIH3T3 cell extracts were prepared in ice-cold reconstitution buffer (20 mM HEPES-KOH, pH 7.9, 50 mM KCl, 5 mM $MgCl_2$, 0.2 mM EDTA, 5% glycerol) containing 0.01% NP-40 as previously described (Gabanella et al., 2007). Radioactive U1, U7, and U7-pre snRNAs were generated by run-off transcription with T7 polymerase from template DNA in the presence of [α-^{32}P]UTP (3,000 Ci/mmol; Perkin Elmer) and m7G cap analogue (Promega) using the MEGAshortscript T7 kit (Ambion) followed by DNase-treatment and purification after denaturing polyacrylamide gel electrophoresis according to standard procedures. *In vitro* snRNP assembly reactions were carried out using freshly prepared NIH3T3 cell extract (25 μg for U1 reactions; 60 μg for U7 and U7-pre reactions) in a final volume of 20 μl of reconstitution buffer containing 0.01% NP-40, 2.5 mM ATP, 10 μM Escherichia coli tRNA, RNasin (Promega), and either 10,000 cpm of radioactive U1 snRNA or 50,000 cpm of radioactive U7 or U7-pre snRNA. Reactions were incubated for 1 hour at 30ºC followed by treatment with heparin (5 mg/ml) and 2 M urea for 15 minutes at room temperature. Reactions were then immunoprecipitated with either mouse IgG, anti-SmB 18F6 or anti-LSm11 3K4 antibodies in RSB-500 buffer (500 mM NaCl, 10 mM Tris-HCl, pH 7.4, 2.5 mM $MgCl_2$) containing 0.1% NP40, protease inhibitors, and phosphatase inhibitors. Following five washes with the same buffer, bound RNAs were extracted by proteinase K treatment followed by phenol-chloroform extraction and ethanol precipitation. Immunoprecipitated RNA was analyzed by electrophoresis on a denaturing 8% polyacrylamide gel containing 8 M urea followed by autoradiography. Quantification was done using a Typhoon PhosphorImager (Molecular Dynamics).

Nucleosome repeat length analysis

NIH3T3 cells were washed twice with ice cold PBS and then harvested by scraping into ice cold PBS and centrifugation at 4,000 rpm for 2 minutes at 4ºC. Cell pellets were resuspended gently in 500 μl of cytoplasmic buffer (1 mM $MgCl_2$, 10 mM Tris-HCl pH 7.5, 10 mM KCl, 1 mM DTT, 0.01% NP40) containing protease inhibitors and phosphatase inhibitors, and passed five times through a 25 G needle. Samples were centrifuged at 4,000 rpm for 1 minute at 4ºC and pelleted nuclei were washed once in 500 ul of micrococcal nuclease digestion buffer (10 mM NaCl, 3 mM $MgCl_2$, 10 mM Tris-HCl pH 7.5, 1 mM $CaCl_2$) by gentle tapping followed by centrifugation at 4,000 rpm for 5 minutes at 4ºC. Washed nuclei were treated with micrococcal nuclease (100 U/ml; Fermentas) diluted in micrococcal nuclease digestion buffer at 37ºC for the indicated times. Reactions were terminated by addition of 10 mM EDTA. Nuclei were pelleted at 10,000 rpm for 5 minutes and resuspended in 50 μl TE buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA) and treated with 1% SDS, 0.2% proteinase K for 1 hour at 37ºC. DNA was phenol-chloroform extracted twice, followed by treatment with RNaseA/T1 (Fermentas) at 37ºC for 15 minutes. DNA was phenol-chloroform extracted again and ethanol precipitated. DNA fragments were analyzed by electrophoresis on a 1% agarose gel followed by ethidium bromide staining and visualization with UV light using a BioDoc-It LSM 26E (UVP).

Cell cycle analysis

NIH3T3 cells cultured with and without doxycycline for 5 days were harvested by trypsinization, washed in PBS, and fixed in 70% ethanol on ice for 30 minutes. Fixed cells were washed twice in PBS, treated with RNase A/T1 (100 ug/ml; Fermentas), and then stained with propidium iodide (50 ug/ml; Invitrogen). Samples were analyzed by

flow cytometry using a FACSCalibur Cell Analyzer (BD Biosciences) followed by data analysis with CellQuest Pro software (BD Biosciences).

Immunofluorescence and confocal microscopy

NIH3T3 cells were grown on glass coverslips in 24-well plates. Cells were washed once with PBS and then fixed in 4% PFA-PBS for 15 minutes at room temperature. Following fixation, cells were permeabilized in 0.5% Triton-X in PBS for 5 minutes at room temperature. Blocking and both primary and secondary antibody incubations were performed with 3% BSA in PBS. Images were collected with a SP5 confocal microscope (Leica). Fluorescent intensity was quantified using the LASAF software (Leica) to measure pixels/μm^2 within individual nuclei.

Statistical analysis

Statistical analysis was carried out with the Prism 5 (GraphPad) software using unpaired Student's t-test and one-way or two-way ANOVA followed by the Bonferroni post-hoc test as applicable. Data are represented as mean and SEM from at least three independent experiments and P values are indicated as follows: * = $p<0.05$; ** = $p<0.01$; *** = $p<0.001$.

Supplemental References

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