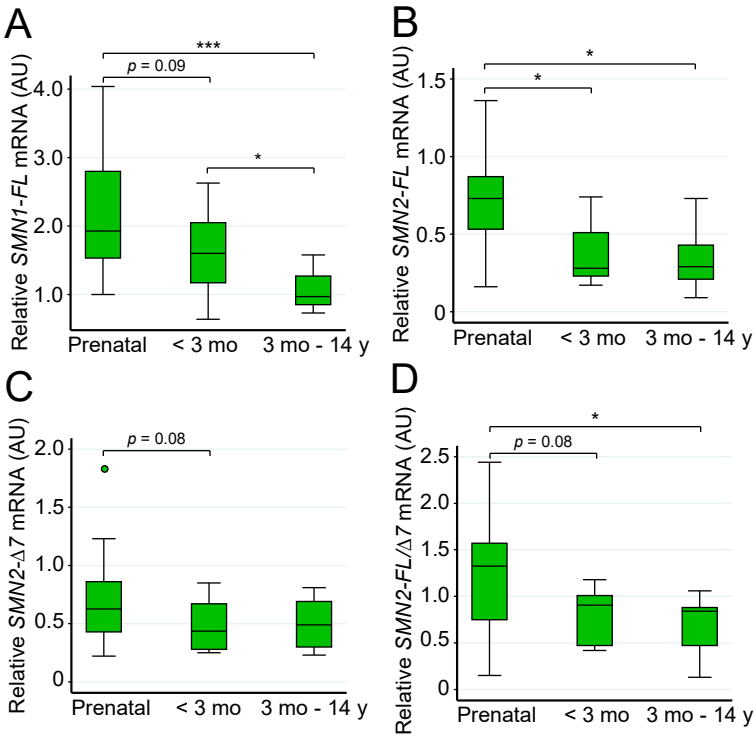
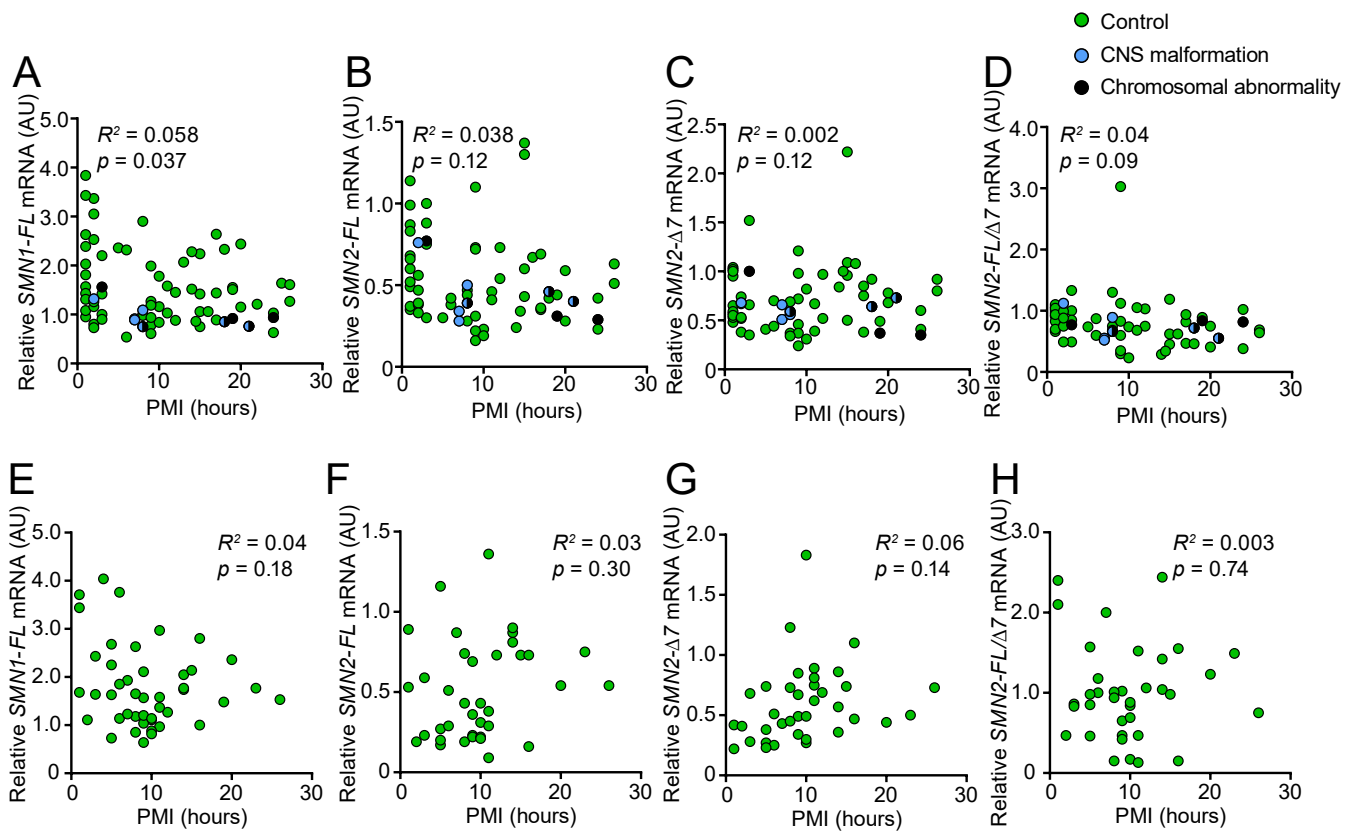
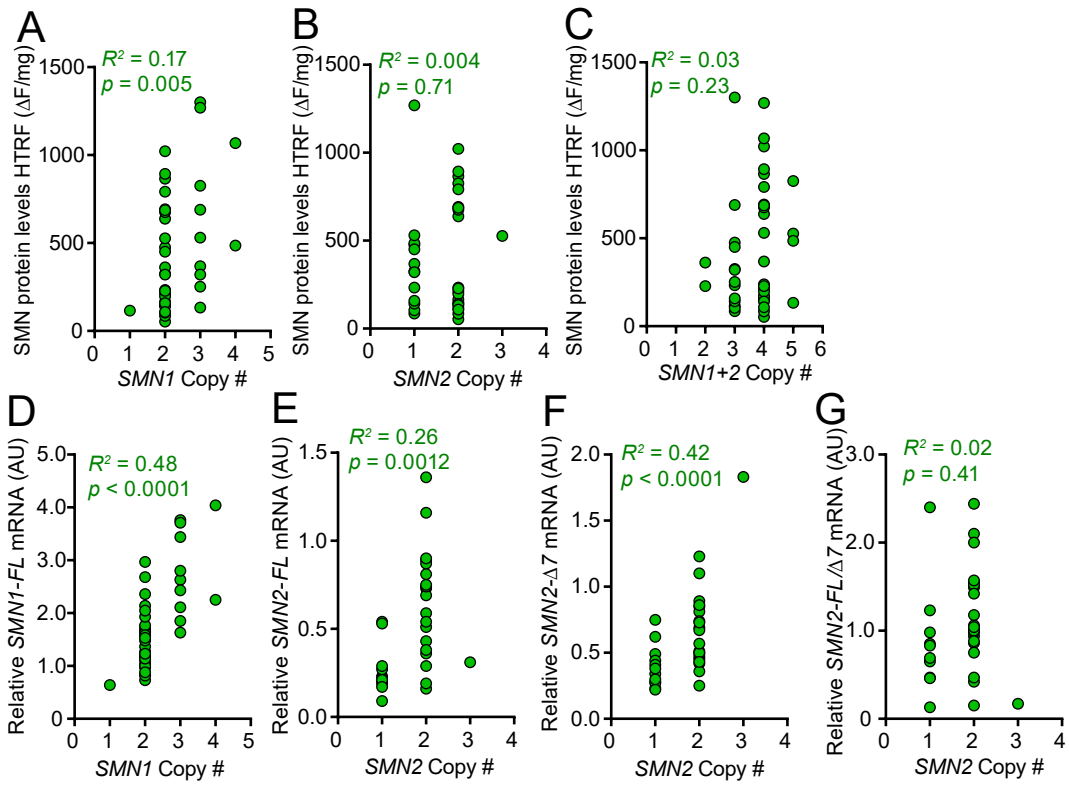


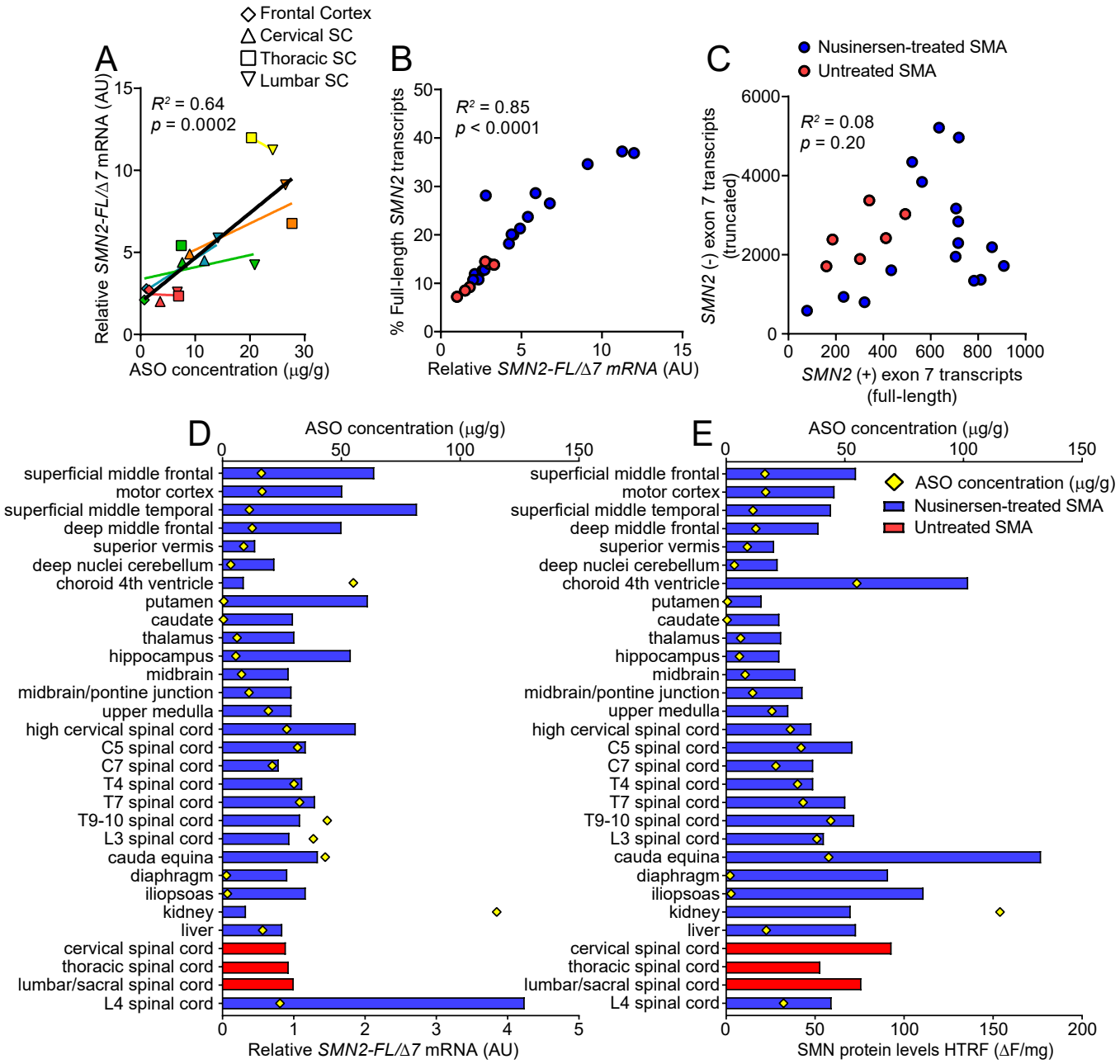
Supplemental Figure S1: *SMN* mRNA levels in human cortex at different ages. **(A-D) (A)** Full-length *SMN1* (*SMN1-FL*) mRNA, **(B)** full-length *SMN2* (*SMN2-FL*) mRNA, **(C)** truncated *SMN2*-Δ*7* mRNA, and **(D)** the ratio of *SMN2-FL*/Δ*7* mRNA. Data presented as medians, interquartile range (box), and 95% percentiles (whiskers). * $p < 0.05$, *** $p < 0.001$ and represent statistics before multiple comparisons (n=44 control). *P*-values of pairwise comparison of medians were calculated using a Wilcoxon Rank-sum test. Adjustment for multiple comparisons was applied to control groups between time points. α level was set to 0.0167 (0.05/3) using Bonferroni adjustment.



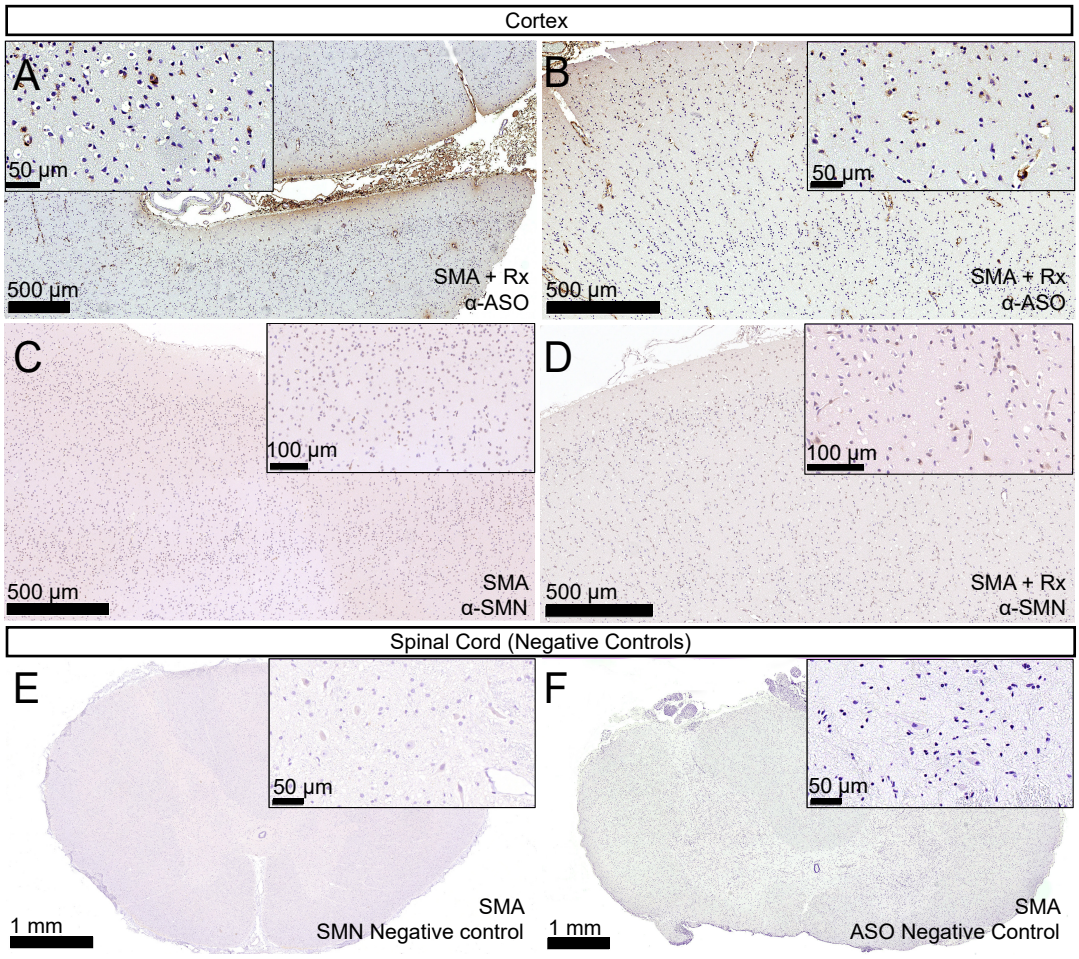
Supplemental Figure S2: PMI does not correlate with *SMN* transcript levels in human spinal cord or cortex. **(A-D)** Correlation between PMI and **(A)** full-length *SMN1* (*SMN1-FL*) mRNA, **(B)** full-length *SMN2* (*SMN2-FL*) mRNA, **(C)** truncated *SMN2* (*SMN2-*Δ*7*) mRNA, and **(D)** the ratio of *SMN2-FL/*Δ*7* mRNA in control spinal cord (n=75). **(E-H)** Correlation between PMI and **(E)** *SMN1-FL*, **(F)** *SMN2-FL*, **(G)** *SMN2-*Δ*7*, and **(H)** *SMN2-FL/*Δ*7* in control cortex (n=44). Linear regression analysis was performed to achieve R^2 and *p*-values.



Supplemental Figure S3: *SMN1* and *SMN2* copy number correlate poorly with SMN protein expression in control cortex. **(A-C)** Correlation between SMN protein levels in control cortex (n=44) measured by HTRF and **(A)** *SMN1* copy number, **(B)** *SMN2* copy number, or **(C)** the sum of *SMN1*+*SMN2* copy number. **(D)** *SMN1* copy number correlation with *SMN1-FL* mRNA. **(E-G)** *SMN2* copy # correlation with **(E)** *SMN2-FL*, **(F)** *SMN2*-Δ7, or **(G)** ratio of *SMN2-FL*/Δ7 mRNA. Linear regression analysis was performed to achieve R^2 and *p*-values.



Supplemental Figure S4: **(A)** ASO concentration correlates with *SMN2-FL/*Δ7 mRNA ratio in nusinersen-treated SMA patients. Shapes indicate tissue type. Different colors depict individual cases. Colored lines indicate line of best fit for each treated SMA patient. Black line indicates line of best fit for all tissues combined. **(B-C)** Correlations between relative and absolute *SMN2* mRNA measurements in nusinersen-treated (n=16 tissues from 5 patients) and untreated (n=6 tissues from 2 patients) SMA patients. **(B)** Scatter plot of relative *SMN2-FL/*Δ7 mRNA against percentage of absolute *SMN2* transcript levels including exon 7. **(C)** Scatter plot of absolute *SMN2* transcripts including exon 7 (*SMN2* (+) exon 7) against absolute *SMN2* transcripts excluding exon 7 (*SMN2* (-) exon 7). Linear regression analysis was performed to achieve R^2 and *p*-values. **(D-E)** Single case with poor final injection **(D)** *SMN2-FL/*Δ7 and **(E)** SMN protein expression in in multiple tissues. Untreated SMA controls in red. L4 spinal cord of a treated SMA case with robust *SMN2-FL/*Δ7 induction at bottom of each graph. ASO concentration in each tissue indicated with a yellow diamond.



Supplemental Figure S5: ASO and SMN IHC in nusinersen-treated patient cortex and spinal cord. **(A-B)** Representative immunostaining for ASO in **(A)** frontal, and **(B)** temporal cortex of 2 nusinersen-treated cases. **(C-D)** Representative immunostaining for SMN in cortex of **(C)** untreated, or **(D)** nusinersen-treated SMA patients. **(E-F)** Negative controls for **(E)** SMN and **(F)** ASO in spinal cord. High power images in insets. Scale bars indicated on each image.

SMN1 and SMN2 gene copy number analysis

DNA was extracted using the Blood & Tissue kit (Qiagen) using the manufacturer's protocol, including optional RNase-A step. Semi-quantitative PCR was performed to determine *SMN1* and *SMN2* gene copy numbers using 100 ng of template. The PCR reaction includes two internal standards (IS) with internal deletions that are amplified with the same primer set as the genomic counterpart, but can be distinguished by size and are used to monitor the efficiency of the reaction at the tube level (61). An amplicon of the cystic fibrosis gene *CFTR* is used in the competitive reaction as a standard for calculating the ratio of *SMN* (1 or 2) amplification compared to *CFTR* amplification. Restriction digestion with DraI differentiated between *SMN1* and *SMN2*, utilizing the C>T variation, c.840C>T. The digestion products were subjected to capillary electrophoresis and analyzed with GeneMarker software (Softgenetics). The ratio is calculated by using the areas of the gene-specific peaks. Copy numbers were assigned by dividing the ratio of *SMN* to *CFTR* amplification by the y value determined from the linearity of control samples. All samples are amplified in replicate. Amplification was performed in a 25 μl reaction using 1X Tris/ammonium sulfate buffer, 1mM dNTPs, 0.85U of Amplitaq buffer (Life Technologies), 1 μl of *SMN* and *CFTR* internal standard (IS) and 2.5, 10.0, 0.5 and 5.6 pM of SMA forward, SMA reverse, CFTR forward and CFTR reverse primers respectively (61). The reaction was denatured for 5 minutes at 94°C followed by 19 cycles of 1 min at 95°C, 2 min at 55°C and 3 min at 72°C with a final extension of 72°C for 7 minutes. For a subset of samples acquired later in our analysis, copy number was determined using the BioRad ddPCR *SMN1* and *SMN2* Copy Number Determination Kits. For DNA from these samples, restriction digest with HaeIII and ddPCR reactions were performed in a single reaction as outlined in Copy Number Determination protocol. RPP30 (known copy number of 2) was used as a reference target to determine *SMN1/2* copy number. At least 3 control samples of known *SMN1/2* copy number were used to confirm ability of the assay to call correct copy number.

Electrochemiluminescence assay

The SMN electrochemiluminescence (ECL) assay was performed as previously described (20). Meso Scale Discovery (MSD) standard plates were coated overnight at 4 °C with a mouse monoclonal anti-SMN antibody (2B1, Enzo Life Sciences) at a concentration of 1 μg/ml followed by a 1 hour blocking step with 5% (w/v) bovine serum albumin (BSA) in PBS. Samples were diluted in sample buffer composed of 50 mM Tris-HCl pH 7.5, 500 mM NaCl, 1% (w/v) BSA, 1% (v/v) Triton X-100, 0.05% (w/v) protease inhibitor cocktail (Sigma-Aldrich). A serial dilution of recombinant human SMN (Enzo Life Sciences)

was used to calibrate the assay. Samples and recombinant SMN were incubated for 2 hours at room temperature with shaking at 650 rpm. Primary and secondary antibodies were diluted in antibody dilution buffer composed of 50 mM Tris-HCl pH 7.5, 137.5 mM NaCl, 1%(w/v) BSA, 0.05% (v/v) Tween-20, 0.2% (w/v) mouse gamma globulin fraction (Rockland). Rabbit anti-SMN antibody (Protein Tech) was labeled with 150 nmol SULFO-TAG NHS ester (MSD) and caged ruthenium at a 20:1 challenge ratio following manufacturer's recommendations. SULFO-TAG labeled rabbit anti-SMN antibody (2 µg/ml) was incubated for 1 hour at room temperature followed by incubation with SULFO-TAG anti-rabbit IgG antibody (0.5 µg/ml; MSD) to amplify the signal. Finally, read buffer T (MSD) was added to the wells, and plates were read using a Meso Scale Discovery Imager 6000 instrument (MSD) and MSD software was used to determine SMN protein levels using a 4-parameter logistic fit equation based on the serial dilution of recombinant human SMN. Total soluble protein was determined using the BCA protein assay (ThermoFisher) per manufacturer's recommendations. SMN data were normalized to total soluble protein and expressed as ng SMN per mg total protein (ng/mg).

Western Blot analysis

SMN western blot analysis was performed as previously described (51). Equal amounts of total soluble protein were denatured at 95 °C for 10 minutes under reducing conditions by the addition of sampling buffer consisting of 250 mM Tris-HCl pH 6.8, 10% (w/v) sodium dodecyl sulfate (SDS), 30% (v/v) glycerol, 5% (v/v) β-mercaptoethanol and 0.02% (w/v) bromophenol blue. Soluble proteins were separated by size on a pre-poured 4-15% (w/v) gradient SDS polyacrylamide gel (BioRad) at a constant potential difference of 200 V for 35 minutes at room temperature. The separated proteins were subsequently transferred onto a polyvinyl difluoride (PVDF) membrane (0.2 µm pore size) (ThermoFisher) for 10 minutes in Tris-glycine transfer buffer (BioRad) using the Trans-Blot Turbo Blotting system (Biorad). Membranes were blocked in Tris-buffered saline, pH 7.4 (TBS) supplemented with 0.1% (v/v) Tween-20 and 5% (w/v) BSA for 1 hour at room temperature. Membranes were incubated overnight at 4 °C with primary antibodies diluted in TBS supplemented with 0.1% (v/v) Tween-20 and 2.5% (w/v) BSA. A mouse anti-SMN antibody (BD Transduction Laboratories, 610646) was used to detect SMN. Mouse anti-GAPDH (ThermoFisher, AM4300) was used as a loading control. Membranes were subsequently incubated for 1 hour at room temperature with a secondary anti-mouse IgG antibody coupled to alkaline phosphatase (Sigma-Aldrich, A3562) diluted 1:5,000 in TBS supplemented with 0.1% (v/v) Tween-20 and 2.5% (w/v) BSA. Finally, membranes were incubated for 1 minute at room temperature with enhanced chemifluorescence (ECF) substrate

(GE Healthcare) and fluorescence was measured using an ImageQuant LAS4000 Imager (GE Healthcare) equipped with a Cy2 filter and a CCD camera. Fluorescence signal intensity was quantified using the gel analysis tool of the Image J (NIH) software package. SMN data were normalized to the average signal intensity of the three loading controls.

Supplemental Table 1: Human samples used in study organized by age

Case ID	Age (GA weeks/months)	PMI (hours)	*SMN1* copy #	*SMN2* copy #	Tissue	Cause of death
NBB_1075	15w(GA)	3	1	2	TSC	Control
NBB_182	16w(GA)	1	2	1	TSC	Control
NBB_308	16w(GA)	1	2	1	I,D	Control
NBB_M1190	16w(GA)	4	4	0	C	Spontaneous Rupture of Membrane
NBB_4604	17w(GA)	1	3	0	C	Control
NBB_1072	18w(GA)	1	3	2	TSC	Control
NBB_1090	18w(GA)	2	3	0	TSC	Control
NBB_113	18w(GA)	1	2	2	I,D	Control
NBB_1621	18w(GA)	1	2	1	TSC	Control
NBB_19	18w(GA)	10	2	0	TSC	Control
NBB_278	18w(GA)	1	2	1	I,D	Control
NBB_4711	18w(GA)	1	3	1	C	Control
NBB_79	18w(GA)	21	2	2	TSC	Iniencephaly, trisomy 18
SMA_17_06	18w(GA)	0	0	3	SC	Type I SMA
NBB_1086	19w(GA)	1	3	1	TSC	Control
NBB_1566	19w(GA)	6	3	0	C	Control
NBB_1756	19w(GA)	1	1	2	TSC	Control
NBB_1879	19w(GA)	1	2	2	TSC,C	Control
NBB_205	19w(GA)	1	2	1	I,D	Control
NBB_319	19w(GA)	1	3	0	I,D	Control
NBB_393	19w(GA)	14	2	2	C	Control
NBB_400	19w(GA)	9	2	2	TSC	Control
NBB_412	19w(GA)	1	2	1	I,D	Control
NBB_46	19w(GA)	12	2	2	TSC	Control
NBB_7	19w(GA)	2	3	1	TSC	Control
NBB_97	19w(GA)	1	2	1	I,D	Meningomyelocele
NBB_1876	20w(GA)	1	3	1	TSC	Control
NBB_220	20w(GA)	13	2	1	I,D	Control
NBB_4311	20w(GA)	16	2	2	C	Retroplacental hematoma
NBB_126	21w(GA)	2	2	2	I,D	Turner Syndrome
NBB_1932	21w(GA)	20	2	1	TSC,C	Hypoplastic left heart syndrome
NBB_218	21w(GA)	16	2	0	I,D	Trisomy 12
NBB_314	21w(GA)	12	3	1	I,D	Trisomy 1
NBB_67	21w(GA)	5	2	1	I,D	Anencephaly
NBB_M1078	21w(GA)	11	2	2	C	Respiratory Insufficiency
NBB_M1081	21w(GA)	7	2	2	C	Infection
NBB_M1102	21w(GA)	14	2	2	C	Stillborn
NBB_1089	22w(GA)	3	3	3	TSC	Trisomy 21
NBB_262	22w(GA)	1	2	1	TSC	Control
NBB_487	22w(GA)	24	2	1	TSC	Trisomy 21
NBB_684	22w(GA)	5	2	2	I,D	Tuberous Sclerosis
NBB_M1085	22w(GA)	5	2	2	C	Respiratory Insufficiency
NBB_M3575	22w(GA)	14	2	2	C	Intrauterine Infection
NBB_111	23w(GA)	18	2	2	TSC,I,D	Holoprosencephaly, trisomy 13
NBB_130	23w(GA)	19	2	1	TSC	Ventriculomegaly, trisomy 13

NBB_4320	23w(GA)	9	3	0	C	Hyaline membrane disease
NBB_908	23w(GA)	3	2	2	TSC	Diaphragmatic hernia
NBB_1117	24w(GA)	2	2	0	TSC	Control
NBB_311	24w(GA)	1	2	2	TSC	Control
NBB_331	24w(GA)	12	2	1	I,D	Control
NBB_4310	24w(GA)	10	2	3	C	Pulmonary hemorrhage
NBB_447	24w(GA)	3	2	2	TSC,C	Prematurity
NBB_4293	25w(GA)	15	2	2	TSC	Premature (25w), *died at birth
NBB_4313	25w(GA)	8	2	2	C	Respiratory insufficiency
NBB_4942	25w(GA)	16	3	2	C	Prematurity
NBB_475	26w(GA)	7	2	1	TSC	Orbital encephalocele
NBB_4283	27w(GA)	19	2	0	TSC,C	Respiratory insufficiency
NBB_361	28w(GA)	1	2	1	TSC,I,D	Hydrops fetalis
NBB_70	33w(GA)	8	2	2	TSC	Dandy-Walker syndrome, trisomy 22
NBB_245	34w(GA)	2	3	1	I,D	Trisomy 18
NBB_5900	34w(GA)	23	2	2	C	Hypoplastic Left Heart Syndrome
NBB_1057	35w(GA)	1	3	1	TSC	Control
CTL_95_04	37w(GA)	8	4	3	TSC	Congenital heart disease
NBB_637	39w(GA)	2	2	1	C	Hypoplastic Left Heart
NBB_M2147	39w(GA)	26	2	2	C	Stillborn
NBB_780	0.00	13	5	0	TSC	Stillborn
CTL_12_02	0.03	26	2	2	TSC,I,D	Unknown
CTL_96_14	0.03	5	2	2	I	Diaphragmatic hernia
NBB_35	0.03	22	2	0	TSC	Control
NBB_5457	0.06	25	2	0	TSC	Cardiac arrhythmia
NBB_5019	0.07	6	2	2	C	Control
NBB_432	0.12	2	2	1	TSC	Hydrops fetalis
NBB_779	0.17	5	3	1	C	Congenital Heart Defect
NBB_M3250	0.33	10	2	1	TSC	Prematurity
NBB_1069	0.40	2	2	1	TSC	Malformation of brainstem, cerebellum
NBB_M1056	0.40	17	3	1	TSC	Prematurity
SMA_17_03	0.50	22	0	2	LSC,TSC,CSC,I,D,FC,TC,Cb,BS,T,L	Type I SMA
NBB_398	0.53	3	3	1	C	Prematurity
NBB_106	0.70	9	2	1	TSC,C,D	Premature (2 weeks)
NBB_199	0.7	15	2	2	TSC	SIDS
NBB_1184	1.00	26	2	1	TSC	SIDS
NBB_1537	1.03	9	2	2	C	Cardiac arrythmia
NBB_4353	1.13	5	4	1	CSC,C	SIDS
NBB_759	1.17	7	2	0	C	Idiopathic Pulmonary Hemorrhage
CTL_90_08	1.30	8	2	2	TSC	Arthrogryposis
CTL_10_17	1.40	15	3	2	I,D	Unknown
NBB_167	1.50	9	2	1	TSC,C	SIDS
NBB_25	1.56	24	2	1	TSC	SIDS, premature (1 month)
NBB_5700	1.80	6	3	2	TSC,C	SUDEP
SMA_11_01	1.80	7	0	2	TSC,I,D	Type I SMA
NBB_657	1.86	15	2	2	TSC	SIDS
NBB_18	1.90	20	2	1	TSC	SIDS
NBB_20	1.90	8	3	0	TSC,C	SIDS

NBB_1132	2.17	9	1	2	CSC,C	SIDS
NBB_36	2.40	10	2	1	TSC	SIDS, premature (1 month)
SMA_12_01	2.50	7	0	2	TSC,I,D,L	Type I SMA
NBB_4383	2.53	8	2	2	C	SIDS
CTL_02_02	3.00	15	2	2	TSC	Nemaline myopathy
NBB_4	3.06	14	3	1	TSC	SIDS
NBB_1055	3.2	12	2	2	TSC,C	Pneumonia
NBB_647	3.20	10	2	1	C	SIDS
CTL_08_01	4.00	14.5	2	2	TSC	Unknown
SMA_08_01	4.00	14.5	0	2	TSC,I,D	Type I SMA
SMA_09_02	4.00	4	0	2	TSC,I,D	Type I SMA
NBB_569	4.40	16	2	1	I	Unknown
NBB_166	4.53	11	2	1	C	Pneumonia
NBB_193	4.63	9	2	2	TSC	SIDS
NBB_169	4.80	14	3	0	TSC	SIDS
SMA_92_01	5.50	14	0	2	TSC,L	Type I SMA
SMARD_10_18	5.50	2	2	1	I,D	SMARD
NBB_134	5.83	18	3	1	TSC	SIDS
NBB_5947	5.97	11	2	1	CSC,C	Asphyxia
SMA_10_19	6.00	6	0	2	TSC,I,D	Type I SMA
SMA_10_20	6.00	12	0	2	D	Type I SMA
NBB_282	6.40	11	2	2	C	SIDS
NBB_947	6.70	7	2	2	TSC	Peters plus syndrome, hydrocephalus
SMA_10_14	7.00	25	0	2	LSC,TSC,CSC,D,BS	Type I SMA
SMA_99_17	8.80	11	0	2	TSC	Type I SMA
NBB_774	9.10	10	2	1	C	SIDS
NBB_383	10.5	8	3	2	TSC	Cerebral palsy
SMA_07_01	11.00	24	0	2	TSC,I,D	Type I SMA
SMA_94_06	12.80	10	0	2	TSC,L	Type I SMA
CTL_93_07	14.00	9	2	1	TSC	Liver disease
SMA_10_01	14.80	2	0	2	I,D	Type I SMA
CTL_10_16	15.00	16	2	1	I,D	Unknown
SMA_08_02	16.00	14	0	2	LSC,TSC,CSC,I,D	Type I SMA
NBB_1425	17.13	2	2	2	TSC	Zellweger syndrome
CTL_12_05	19.00	24	2	1	I,D	Unknown
NBB_103	26.50	11	2	1	TSC	Meningitis, seizures
NBB_1864	29.97	8	2	2	C	Laryngitis and bronchiolitis
NBB_5282	34.16	16	2	2	TSC	Asphyxia
SMA_10_21	35.00	10	0	2	I,D	Type I SMA
CTL_12_06	36.00	2	2	2	I,D	Transverse myelitis
NBB_1624	36.00	3	2	1	TSC	Batten Disease
SMA_10_12	36.00	3	0	2	I,D	Type I SMA
SMA_14_02	42.00	7	0	2	D	Type I SMA
SMA_14_01	48.00	23	N/A	N/A	TSC	Type I SMA
NBB_3	64.80	17	2	2	TSC	Cardiac arrest
NBB_561	72.00	2	2	1	TSC	Hurler syndrome
SMA_10_16	72.0	24	0	2	LSC,TSC,CSC,D,FC,TC,Cb,BS	Type II SMA
NBB_1181	96.00	8	2	1	TSC	Lennox-Gastaut syndrome

NBB_24	108.00	17	2	1	TSC	Motor vehicle accident
NBB_5173	129.40	10	2	2	C	Asthma
NBB_1144	144.00	6	2	1	TSC	Metachromatic leukodystrophy
NBB_1943	144.00	24	2	2	TSC	SUDEP
SMA_10_02	144.00	N/A	0	2	TSC	Type II SMA
NBB_5376	159.36	19	2	1	TSC	Suicide
NBB_1670	159.4	5	2	1	C	Asphyxia
CTL_13_01	168.00	2	2	1	TSC,I,D	Cardiac arrest
Rx_SMA_14_05	8.00	6	0	2	LSC,TSC,CSC,I,D,FC,TC,Cb,BS,T,L	Type I SMA | Rx=5d | doses=1
Rx_SMA_17_02	12.00	22	0	2	LSC,TSC,CSC,I,D	Type I SMA | Rx=3.5m | doses=4
Rx_SMA_17_04	24.00	2	0	2	LSC,TSC,D	Type I SMA | Rx=8m | doses=11
Rx_SMA_17_05	48.00	2	0	2	LSC,TSC,CSC,I,D,FC,TC,L	Type I SMA | Rx=12m | doses=10
Rx_SMA_18_01	162.00	48	0	3	LSC,TSC,CSC,D,FC,TC,Cb,L	Type II SMA | Rx=10m | doses=4
Rx_SMA_19_01	11.00	13.5	N/A	N/A	LSC,TSC,CSC,I,D,FC,TC,Cb,BS,T,L	Type I SMA | Rx=6m | doses=5

GA=gestational age, PMI=post-mortem interval, NBB=NIH NeuroBioBank, CTL=non-SMA control subject, SMA=SMA subject, SC=spinal cord, I=iliopsoas muscle, D=diaphragm muscle, C=cortex, FC=frontal cortex, TC=temporal cortex, Cb=cerebellum, BS=brainstem, T=thalamus, L=liver, Rx=Duration of nusinersen treatment (months), doses=Total number of nusinersen doses, N/A=not available

Supplemental Table 2: RT-qPCR Taqman assays and SYBR Green primers used in study

Gene name	DNA oligo	Sequence (5' to 3')	Type
SMN1-FL	Forward	TAT CAT ACT GGC TAT TAT ATG GGT TTC	Taqman
	Probe	AAG GAG AAA TGC TGG CAT AGA GCA GC	
	Reverse	TCG TTT CTT TAG TGG TGT CAT TTA G	
SMN2-FL	Forward	TAT CAT ACT GGC TAT TAT ATG GGT TTT	Taqman
	Probe	AAG GAG AAA TGC TGG CAT AGA GCA GC	
	Reverse	TCG TTT CTT TAG TGG TGT CAT TTA G	
SMN2-Δ7	Forward	TGG CTA TCA TAC TGG CTA TTA TAT GGA A	Taqman
	Probe	CTG GCA TAG AGC AGC ACT AAA TGA CAC CAC	
	Reverse	TCC AGA TCT GTC TGA TCG TTT CTT	
SMN2-Δ5	Forward	AGA CTG GGA CCA GGA AAG ATA A	SYBR Green
	Reverse	TGC TCT ATG CCA GCA TTT CCA TAT	
ACTB	Forward	GAC GAC ATG GAG AAA ATC TG	SYBR Green
	Reverse	ATG ATC TGG GTC ATC TTC TC	
ATP5B	Forward	TAC CAC CAA TTC TAA ATG CC	SYBR Green
	Reverse	GTG CTC TCA CCCC AAA TG	
B2M	Forward	AAG GAC TGG TCT TTC TAT CTC	SYBR Green
	Reverse	GAT CCC ACT TAA CTA TCT TGG	
EIF4A2	Forward	AGA GAG ATG TTA TCA TGA GGG	SYBR Green
	Reverse	TAA CCA AAG ACA CTT GTT GC	
FBXO38	Forward	AAA GAA GAT GCC AGA TGT TG	SYBR Green
	Reverse	AAA TGA GAA GTT TCC ACA CC	
GAPDH	Forward	ACA GTT GCC ATG TAG ACC	SYBR Green
	Reverse	TTT TTG GTT GAG CAC AGG	
UBC	Forward	CGT CAC TTG ACA ATG CAG	SYBR Green
	Reverse	TGT TTC CAG CAA AGA TCA G	
YWHAZ	Forward	AAC TTG ACA TTG TGG ACA TC	SYBR Green
	Reverse	AAA ACT ATT TGT GGG ACA CGC	

Supplemental Table 3: Comparisons of SMN Protein and RNA in control and SMA spinal cord samples

Spinal Cord	Control vs SMA				Comparison between age groups		
	Age	Median (IQR)		p-value	Comparison	p-value	
		Control	SMA	CTL vs SMA		Control	SMA
SMN Protein	Prenatal	748 (302–1188)	198 (N/A)	N/A	Prenatal vs <3 Months	<0.001***	N/A
	<3 Months	326 (154–437)	53 (41–128)	0.02*	Prenatal vs 3 Mo–14 Years	<0.001***	0.11
	3 Mo–14 Yr.	115 (63–165)	67 (63–102)	0.09	<3 Mo. vs 3 Mo–14 Years	0.01*	0.56
SMN1-FL	Prenatal	1.55 (1.0–2.39)	0 N/A	N/A	Prenatal vs <3 Months	0.53	N/A
	<3 Months	1.27 (1.15–2.07)	0 N/A	N/A	Prenatal vs 3 Mo–14 Years	0.003**	N/A
	3 Mo–14 Yr.	0.94 (0.80–1.44)	0.01 N/A	N/A	<3 Mo. vs 3 Mo–14 Years	0.02*	N/A
SMN2-FL	Prenatal	0.59 (0.39–0.83)	1.44 N/A	N/A	Prenatal vs <3 Months	0.047*	N/A
	<3 Months	0.38 (0.23–0.63)	0.58 (0.32–0.63)	0.49	Prenatal vs 3 Mo–14 Years	0.02*	0.11
	3 Mo–14 Yr.	0.42 (0.34–0.50)	0.47 (0.41–0.56)	0.40	<3 Mo. vs 3 Mo–14 Years	0.47	0.66
SMN-Δ7	Prenatal	0.66 (0.53–0.98)	0.64 N/A	N/A	Prenatal vs <3 Months	0.91	N/A
	<3 Months	0.69 (0.46–0.82)	0.65 (0.38–0.87)	0.60	Prenatal vs 3 Mo–14 Years	0.05*	0.79
	3 Mo–14 Yr.	0.53 (0.39–0.70)	0.79 (0.49–0.98)	0.07	<3 Mo. vs 3 Mo–14 Years	0.13	0.35
SMN-2FL/Δ7	Prenatal	0.82 (0.70–1.01)	2.23 N/A	N/A	Prenatal vs <3 Months	0.01*	N/A
	<3 Months	0.64 (0.41–0.74)	0.67 (0.49–1.79)	0.60	Prenatal vs 3 Mo–14 Years	0.74	0.11
	3 Mo–14 Yr.	0.84 (0.60–1.02)	0.65 (0.41–1.03)	0.07	<3 Mo. vs 3 Mo–14 Years	0.09	0.47

Median and inter-quartile range (IQR) of SMN protein and RNA levels in human thoracic spinal cord. P-values of pairwise comparison of medians between 1) control and SMA, or 2) age groups were calculated using a Wilcoxon Rank-sum test. Prenatal SMA group excluded in all statistical analyses. *p <0.05, **p <0.01, ***p <0.001, and represent statistics before multiple comparisons. N/A=not applicable.

Supplemental Table 4: Comparisons of SMN Protein and RNA in control cortex samples

Cortex	Comparison between age groups			
	Age	Median (IQR)	Comparison	p-value
		Control		Control
SMN Protein	Prenatal	638 (233–865)	Prenatal vs <3 Months	0.02*
	<3 Months	240 (165–427)	Prenatal vs 3 Mo–14 Years	<0.001***
	3 Mo–14 Yr.	148 (85–233)	<3 Mo. vs 3 Mo–14 Years	0.06
SMN1-FL	Prenatal	1.93 (1.53–2.80)	Prenatal vs <3 Months	0.09
	<3 Months	1.6 (1.17–2.05)	Prenatal vs 3 Mo–14 Years	<0.001***
	3 Mo–14 Yr.	0.97 (0.85–1.27)	<3 Mo. vs 3 Mo–14 Years	0.03*
SMN2-FL	Prenatal	0.73 (0.53–0.87)	Prenatal vs <3 Months	0.02*
	<3 Months	0.28 (0.23–0.51)	Prenatal vs 3 Mo–14 Years	0.01*
	3 Mo–14 Yr.	0.29 (0.21–0.43)	<3 Mo. vs 3 Mo–14 Years	0.62
SMN-Δ7	Prenatal	0.63 (0.43–0.86)	Prenatal vs <3 Months	0.08
	<3 Months	0.44 (0.28–0.67)	Prenatal vs 3 Mo–14 Years	0.3
	3 Mo–14 Yr.	0.49 (0.30–0.69)	<3 Mo. vs 3 Mo–14 Years	0.81
SMN-2FL/Δ7	Prenatal	1.33 (0.75–1.57)	Prenatal vs <3 Months	0.08
	<3 Months	0.91 (0.47–1.01)	Prenatal vs 3 Mo–14 Years	0.04*
	3 Mo–14 Yr.	0.85 (0.47–0.88)	<3 Mo. vs 3 Mo–14 Years	0.57

Median and inter-quartile range (IQR) of SMN protein and RNA levels in human cortex. P-values of pairwise comparison of medians between age groups were calculated using a Wilcoxon Rank-sum test. *p <0.05, ***p <0.001, and represent statistics before multiple comparisons. N/A=not applicable

Supplemental Table 5: Comparisons of SMN Protein and RNA in all tissues

		Median (IQR)			p-value	
		Prenatal Control	**Postnatal Control**	**Postnatal SMA**	**Prenatal Control vs Postnatal Control**	**Postnatal Control vs Postnatal SMA**
Spinal Cord	SMN Protein	740 (305.7–1170.2)	156 (79–341.9)	66 (53–107)	<0.001***	<0.001***
	SMN-1FL	1.56 (1.0–2.30)	1.16 (0.88–1.52)	0.01 (0–0.01)	0.01*	<0.001***
	SMN-2FL	0.59 (0.39–0.83)	0.42 (0.31–0.54)	0.48 (0.41–0.59)	0.01*	0.27
	SMN2-Δ7	0.66 (0.53–0.98)	0.66 (0.41–0.82)	0.69 (0.43–0.93)	0.18	0.28
	SMN2-FL/Δ7	0.82 (0.70–1.01)	0.74 (0.49–0.94)	0.67 (0.45–1.11)	0.12	0.82
Diaphragm		**Prenatal Control**	**Postnatal Control**	**Postnatal SMA**	**Prenatal Control vs Postnatal Control**	**Postnatal Control vs Postnatal SMA**
	SMN Protein	460.7 (373.5–555.0)	282.3 (245.1–392)	112.6 (86.8–133.5)	0.02*	0.01*
	SMN-1FL	3.92 (3.09–5.14)	2.37 (1.98–3.16)	0 N/A	0.08	<0.001***
	SMN-2FL	0.66 (0.56–0.74)	0.5 (0.30–0.69)	0.71 (0.62–0.82)	0.15	0.08
	SMN2-Δ7	0.82 (0.74–1.0)	0.7 (0.54–0.98)	0.79 (0.56–1.03)	0.12	0.78
	SMN2-FL/Δ7	0.7 (0.55–0.90)	0.57 (0.46–1.28)	1.11 (0.64–1.29)	0.74	0.16
Iliopsoas		**Prenatal Control**	**Postnatal Control**	**Postnatal SMA**	**Prenatal Control vs Postnatal Control**	**Postnatal Control vs Postnatal SMA**
	SMN Protein	882.1 (701.1–1241.2)	261.7 (174.4–401.9)	136 (94.2–191.7)	<0.001***	0.05*
	SMN-1FL	3.32 (2.78–4.52)	2.83 (2.23–3.38)	0 N/A	0.21	<0.001***
	SMN-2FL	0.33 (0.28–0.57)	0.34 (0.32–0.40)	0.49 (0.38–0.61)	0.94	0.02*
	SMN2-Δ7	0.68 (0.40–1.10)	0.53 (0.42–0.87)	0.84 (0.77–1.04)	0.43	0.045*
	SMN2-FL/Δ7	0.54 (0.39–0.78)	0.71 (0.38–1.17)	0.73 (0.37–0.76)	0.29	0.62
Cortex		**Prenatal Control**	**Postnatal Control**	**Postnatal SMA**	**Prenatal Control vs Postnatal Control**	**Postnatal Control vs Postnatal SMA**
	SMN Protein	638 (233–865)	213 (133–321)	N/A N/A	<0.001***	N/A
	SMN-1FL	1.93 (1.53–2.80)	1.23 (0.97–1.63)	N/A N/A	0.002**	N/A
	SMN-2FL	0.73 (0.53–0.87)	0.29 (0.22–0.43)	N/A N/A	0.004**	N/A
	SMN2-Δ7	0.63 (0.43–0.86)	0.49 (0.28–0.69)	N/A N/A	0.09	N/A
	SMN2-FL/Δ7	1.33 (0.75–1.57)	0.84 (0.47–1.0)	N/A N/A	0.02*	N/A

Median and inter-quartile range (IQR) of SMN protein and RNA levels in human spinal cord, diaphragm, iliopsoas, and cortex. P-values of pairwise comparison of medians between 1) prenatal and postnatal control, and 2) control and SMA were calculated using a Wilcoxon Rank-sum test. * p <0.05, ** p <0.05, *** p <0.001, N/A=not applicable

Supplemental Table 6: Cross-tissue comparisons of SMN Protein and RNA

		p-value					
		SC vs Dia	**SC vs Iliop**	**SC vs Cortex**	**Dia vs Iliop**	**Dia vs Cortex**	**Iliop vs Cortex**
Prenatal control	SMN Protein	0.09	0.4	0.16	0.003**	0.57	0.02*
	SMN1-FL	<0.001***	<0.001***	0.05*	0.39	<0.001***	<0.001***
	SMN2-FL	0.34	0.02*	0.54	0.004**	0.82	0.06
	SMN2-Δ7	0.047*	0.87	0.33	0.2	0.02*	0.77
	SMN2-FL/Δ7	0.05*	0.004**	0.03*	0.12	0.02*	0.01*
Postnatal control	SMN Protein	0.29	0.29	0.53	0.86	0.29	0.40
	SMN1-FL	<0.001***	<0.001***	0.45	0.64	0.002**	<0.001***
	SMN2-FL	0.46	0.19	0.04*	0.19	0.06	0.51
	SMN2-Δ7	0.29	0.63	0.03*	0.24	0.048*	0.40
	SMN2-FL/Δ7	0.91	0.78	0.68	0.91	0.85	0.88
Postnatal SMA	SMN Protein	0.06	0.006**	N/A	0.15	N/A	N/A
	SMN1-FL	N/A	N/A	N/A	N/A	N/A	N/A
	SMN2-FL	0.002**	0.76	N/A	0.01*	N/A	N/A
	SMN2-Δ7	0.46	0.20	N/A	0.41	N/A	N/A
	SMN2-FL/Δ7	0.13	0.32	N/A	0.047*	N/A	N/A

P-values of pairwise comparisons of SMN protein and RNA levels between indicated tissues were calculated using a Wilcoxon Rank-sum test. *p <0.05, **p <0.05, ***p <0.001. SC=spinal cord, Dia=diaphragm, Iliop=iliopsoas, N/A=not applicable

Supplemental Table 7: ASO concentration following nusinersen treatment

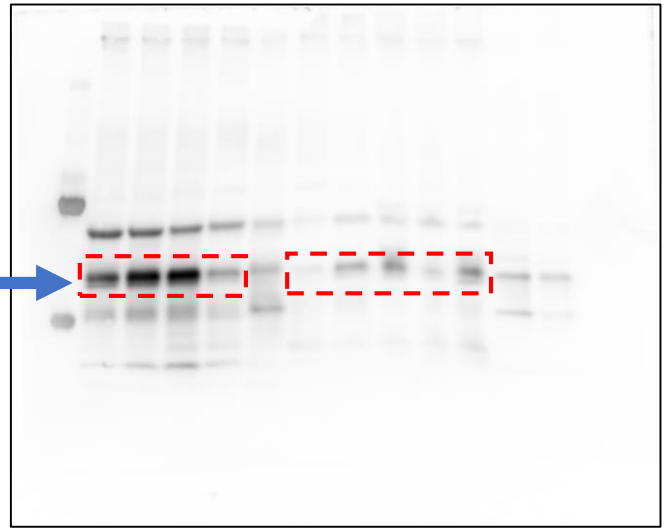
	Observed nusinersen concentration (μg/g)						
Tissue	**SMA_10_16**	**SMA_14_05**	**SMA_17_02**	**SMA_17_04**	**SMA_17_05**	**SMA_18_01**	**SMA_19_01**
Frontal Cortex	0.00	1.54	N/A	N/A	0.69	1.06	16.38
Temporal Cortex	N/A	0.70	N/A	N/A	0.05	8.22	11.3
Cerebellum	0.00	0.77	N/A	N/A	N/A	6.41	8.91
Thalamus	N/A	0.73	N/A	N/A	N/A	N/A	6.14
Brainstem	0.00	1.48	N/A	N/A	N/A	N/A	11.16
Cervical SC	0.00	3.58	8.97	N/A	7.62	11.68	31.57
Thoracic SC	0.00	6.96	27.69	20.26	7.46	N/A	32.43
Lumbar SC	0.00	6.74	26.50	24.18	20.88	14.15	38.26

ASO concentration measured in tissues from treated SMA patients. SMA_10_16 is an untreated SMA control. SC=spinal cord, N/A=Not available

Full unedited gel for Figure 2G

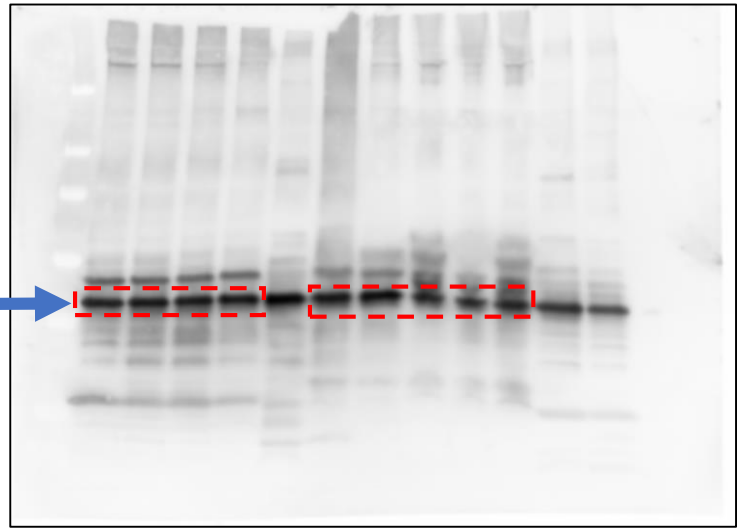
SMN

Quantified band



GAPDH

Quantified band



Lanes used in study