Supplemental Information

SMN is essential for the biogenesis of U7 snRNP and 3'-end formation of histone mRNAs

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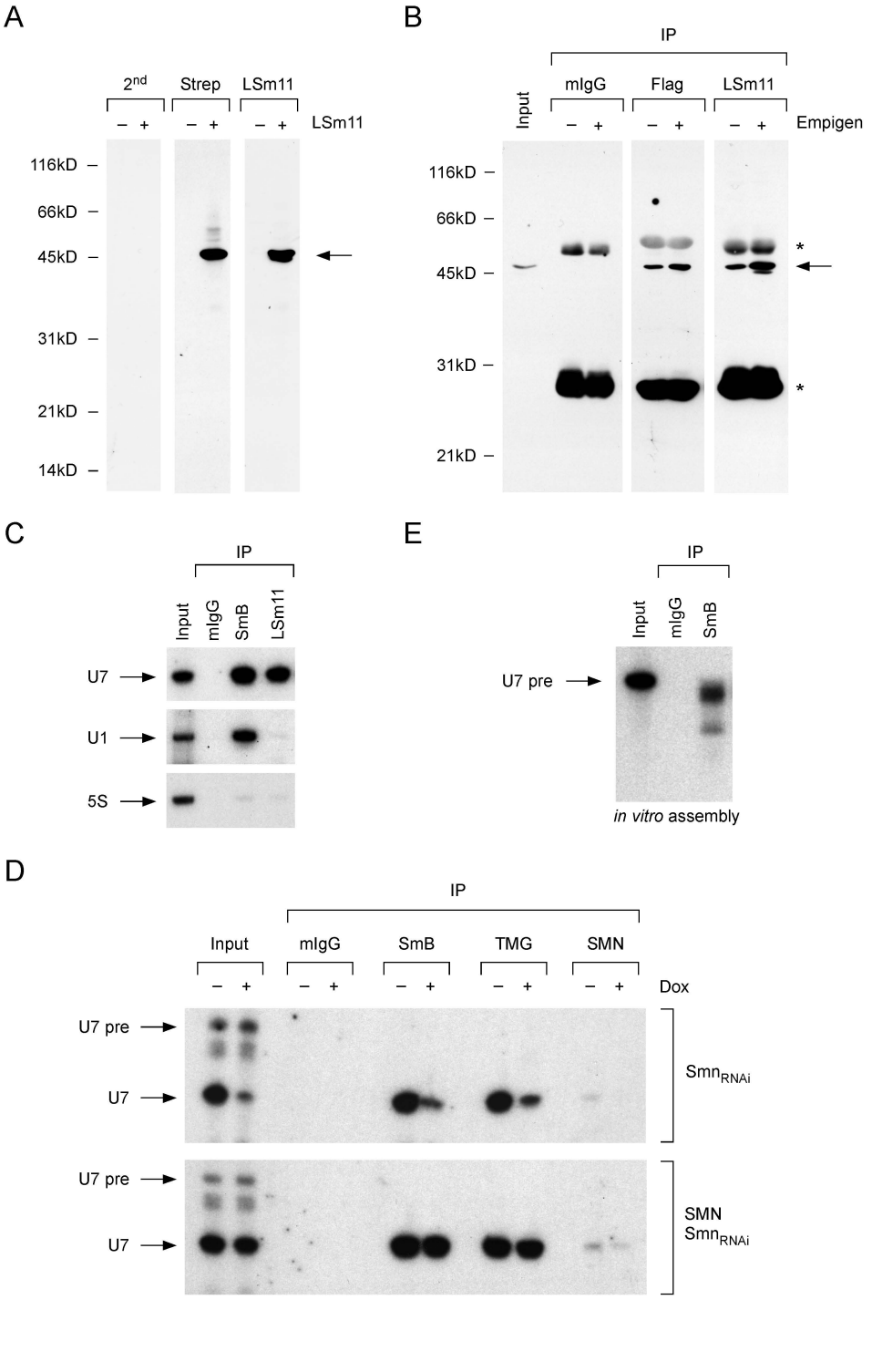
A
2nd
Strep
LSm11
LSm11
116kD
66kD
45kD
31kD
21kD
14kD
B
IP
Input
mIgG
Flag
LSm11
Empigen
116kD
66kD
45kD
31kD
21kD
C
IP
Input
mIgG
SmB
LSm11
U7
U1
5S
E
IP
Input
mIgG
SmB
U7 pre
in vitro assembly
D
IP
Input
mIgG
SmB
TMG
SMN
Dox
U7 pre
U7
Smn RNAi
U7 pre
U7
SMN
Smn RNAi

Figure S1

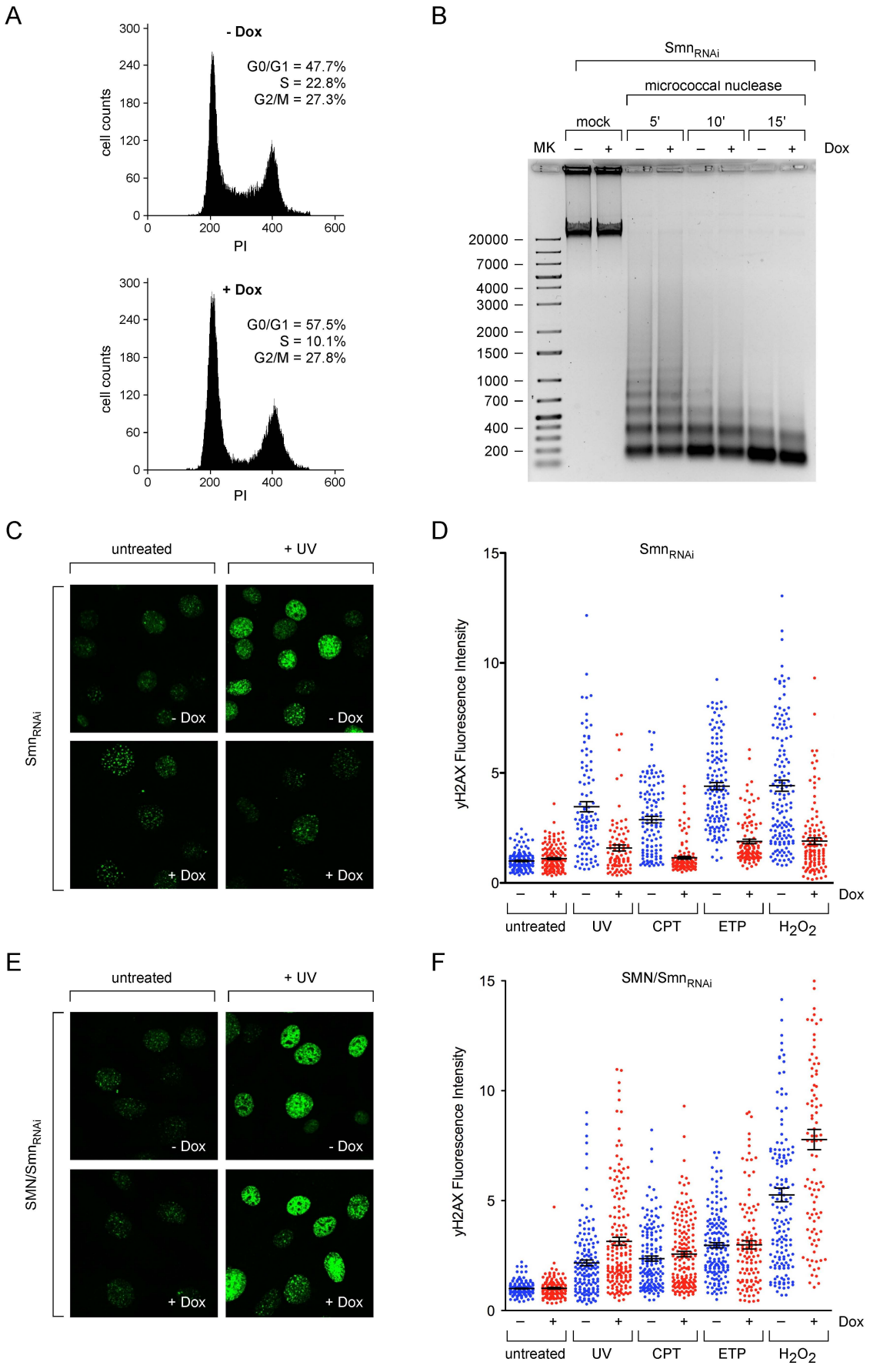
A
- Dox
G0/G1 = 47.7%
S = 22.8%
G2/M = 27.3%
cell counts
PI
+ Dox
G0/G1 = 57.5%
S = 10.1%
G2/M = 27.8%
B
Smn$_{RNAi}$
micrococcal nuclease
mock
5'
10'
15'
MK
Dox
20000
7000
4000
3000
2000
1500
1000
700
400
200
C
untreated
+ UV
Smn$_{RNAi}$
- Dox
+ Dox
D
Smn$_{RNAi}$
γH2AX Fluorescence Intensity
Dox
untreated
UV
CPT
ETP
H$_2$O$_2$
E
SMN/Smn$_{RNAi}$
F
SMN/Smn$_{RNAi}$

Figure S2

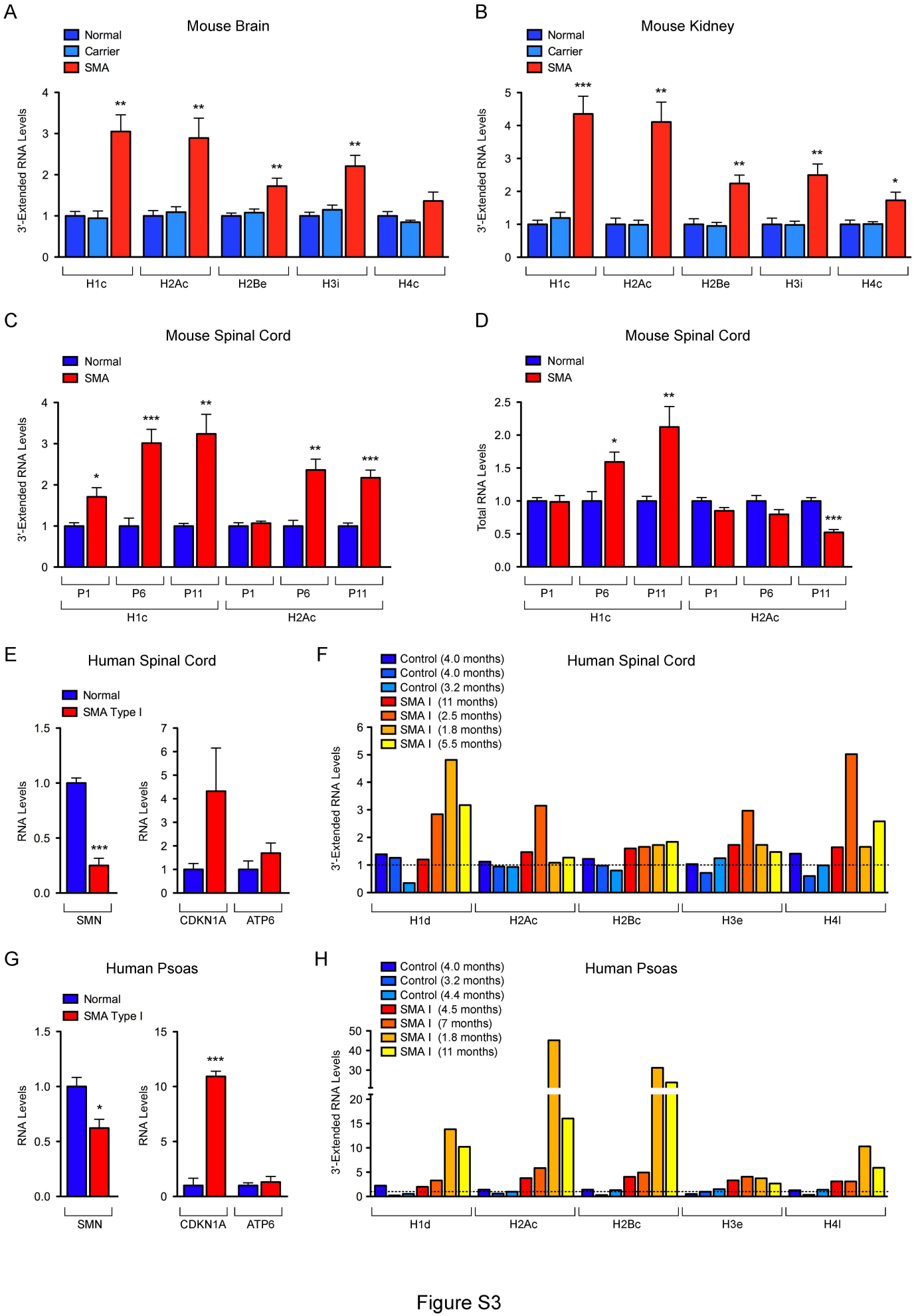
A
Mouse Brain
Normal
Carrier
SMA
3'-Extended RNA Levels
H1c
H2Ac
H2Be
H3i
H4c
B
Mouse Kidney
C
Mouse Spinal Cord
P1
P6
P11
D
Total RNA Levels
E
Human Spinal Cord
SMA Type I
RNA Levels
SMN
CDKN1A
ATP6
F
Control (4.0 months)
Control (3.2 months)
SMA I (11 months)
SMA I (2.5 months)
SMA I (1.8 months)
SMA I (5.5 months)
H1d
H2Bc
H3e
H4l
G
Human Psoas
H
Control (4.4 months)
SMA I (4.5 months)
SMA I (7 months)
SMA I (11 months)

Figure S3

Figure S1. Characterization of a novel mouse monoclonal antibody against LSm11, Related to Figure 1. (A) Western blot analysis of HEK293 cells transfected with or without epitope-tagged LSm11 cDNA with the indicated antibodies. (B) Extracts from HEK293 cells transfected with epitope-tagged LSm11 cDNA were immunoprecipitated with antibodies against Flag, LSm11 or control mouse immunoglobulins (mIgG). Immunoprecipitations were carried out in the absence or presence of 1% Empigen, which disrupts most protein-protein as well as RNA-protein interactions. Input and immunoprecipitates were analyzed by Western blot using anti-LSm11 antibodies. (C) Extracts from wild-type NIH3T3 cells were immunoprecipitated with antibodies against SmB, LSm11, or control mouse immunoglobulins (mIgG). Input and immunoprecipitated RNAs were analyzed by Northern blot. (D) Northern blot analysis of U7 snRNAs immunoprecipitated using the indicated antibodies from extracts of NIH3T3-Smn_{RNAi} and NIH3T3-SMN/Smn_{RNAi} cells cultured with and without Dox for 5 days. (E) *In vitro* snRNP assembly with radioactive U7 snRNA precursor. Reactions were immunoprecipitated with the indicated antibodies followed by denaturing gel electrophoresis and autoradiography.

Figure S2. SMN deficiency disrupts the cell cycle and alters the levels of phosphorylated H2AX in response to DNA damage in NIH3T3 cells, Related to Figure 3. (A) Cell cycle analysis of NIH3T3-Smn_{RNAi} cells cultured with and without Dox for 5 days. (B) Analysis of nucleosome repeat length by micrococcal nuclease digestion of nuclei isolated from NIH3T3-Smn_{RNAi} cells cultured with and without Dox for 5 days. (C) Immunofluorescence analysis of phosphorylated H2AX (γH2AX) in NIH3T3-Smn_{RNAi} cells cultured with and without Dox for 5 days followed by treatment with or without UV. (D) Quantification of the nuclear γH2AX fluorescence intensity (pixels/μm^2) in NIH3T3-

Smn_{RNAi} cells cultured with and without Dox for 5 days followed by treatment with UV, camptothecin (CPT), etoposide (ETP) or hydrogen peroxide (H_2O_2). Values were normalized to untreated cells. (E) Immunofluorescence analysis of phosphorylated H2AX (γH2AX) in NIH3T3-SMN/Smn_{RNAi} cells treated as in (C). (F) Quantification of the nuclear γH2AX fluorescence intensity (pixels/μm^2) in NIH3T3-SMN/Smn_{RNAi} treated as in (D). Values were normalized to untreated cells.

Figure S3. Disruption of histone mRNA 3'-end formation in SMA, Related to Figure 4. (A) RT-qPCR analysis of 3'-extended histone mRNA levels in brain from normal, carrier, and SMA mice at P6. Data are represented as mean and SEM. (B) RT-qPCR analysis of 3'-extended histone mRNA levels in kidney from normal, carrier, and SMA mice at P6. Data are represented as mean and SEM. (C) RT-qPCR analysis of 3'-extended histone mRNA leves in spinal cord from normal and SMA mice at P1, P6 and P11. Data are represented as mean and SEM. (D) RT-qPCR analysis of total histone mRNA levels in spinal cord from normal and SMA mice at P1, P6 and P11. Data are represented as mean and SEM. (E) RT-qPCR analysis of SMN, CDKN1A and ATP6 mRNA levels in the spinal cord from normal individuals (n=3) and SMA patients (n=4). Data were normalized to the average of values in controls and is represented as mean and SEM. (F) RT-qPCR analysis of 3'-extended histone mRNA levels in the spinal cord from normal individuals and SMA patients. Data represent the mean from triplicate reactions normalized to the average of the values in controls. (G) RT-qPCR analysis of SMN, CDKN1A and ATP6 mRNA levels in the psoas from normal individuals (n=3) and SMA patients (n=4). Data were normalized to the average of values in controls and is represented as mean and SEM. (H) RT-qPCR analysis of 3'-extended histone mRNA levels in the psoas from normal individuals and SMA patients. Data represent the mean from triplicate reactions normalized to the average of the values in controls.