

Supplemental Figures

Figure 1.

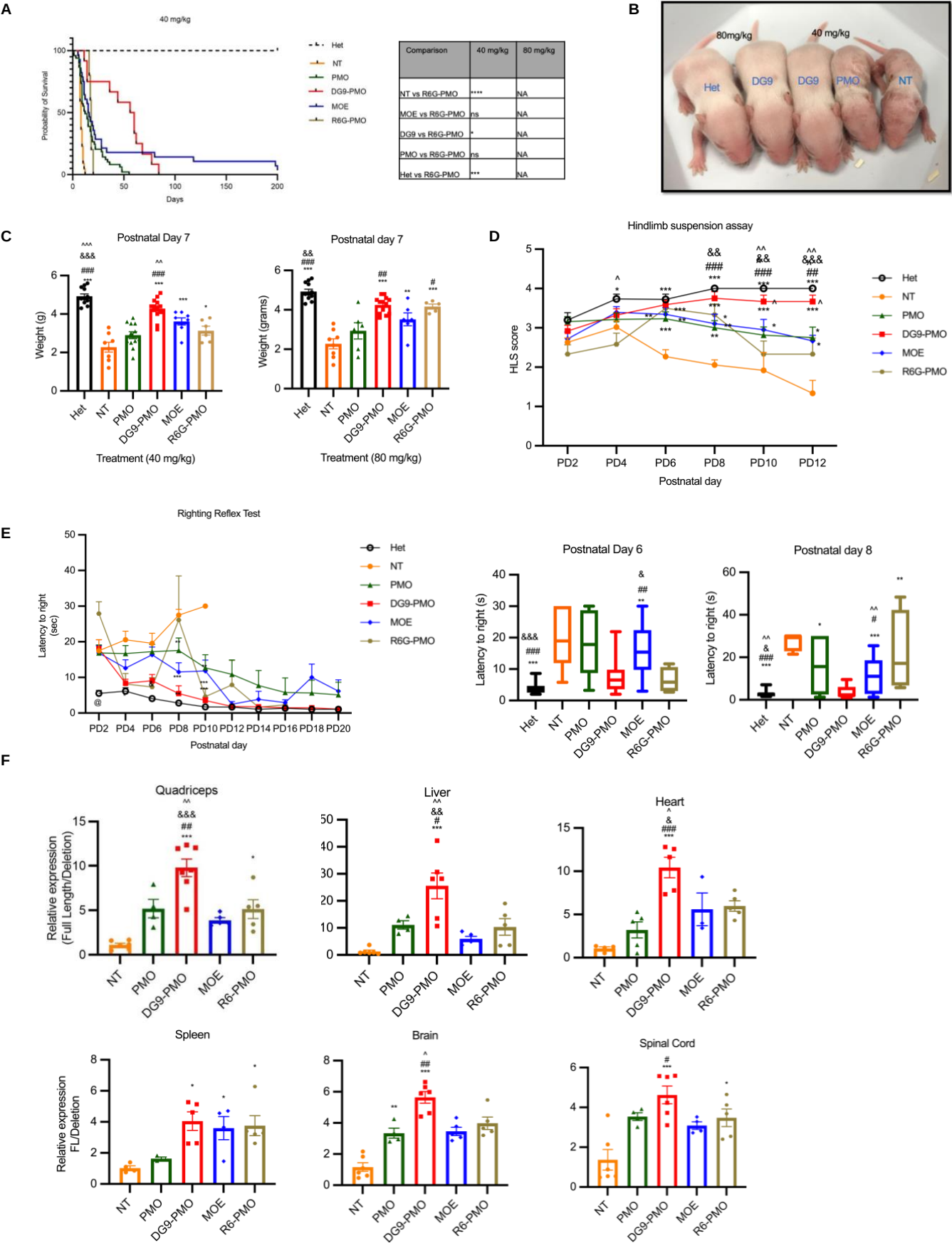


Figure 1. Improved efficacy following DG9-PMO treatment compared to R6-PMO in SMA mice. (A) Survival curves of heterozygous mice (Het), non-treated (NT), unconjugated-PMO (PMO), DG9-PMO, MOE and R6G-PMO injected at PD0 at a dose of 40 mg/kg. n=15 (Hets), n=22 (NT), n=49 (unconjugated PMO), n=14 (DG9-PMO), n=29 (MOE), and n=6 (R6G-PMO), ($p \leq 0.0001$, log-rank Mantel Cox test). (B) A representative image of heterozygous mouse, mice treated with either 80 mg/kg or 40 mg/kg of DG9-PMO, mouse injected with unconjugated- PMO (40 mg/kg), and saline-treated NT mouse at PD7 (left-to-right). (C) Weight of mice at PD7 administered with either 40 or 80 mg/kg doses. Each dot (symbol) indicates a neonatal pup. (D) Hindlimb suspension assay (HLS). Mice were treated with 40 mg/kg AOs at PD0. Scored is based on the position of the hindlimbs when suspended from a falcon tube. (E) Righting reflex test. Mice were treated with 40 mg/kg AOs at PD0. The ability of mice to right themselves on their paws was measured every alternate day between PD2 to PD20 (left). The mean righting reflex time at PD6 and PD8 was also indicated (right: box whiskers plots). Box edges, 25th and 75th percentiles; central line, median; whiskers, range. (F) Relative expression levels of full length *SMN2* (*FL-SMN2*) compared to deleted *SMN2* transcripts ($\Delta 7$ *SMN2*) measured by qPCR following 40 mg/kg ASO or PBS treatments. In C, E (box whisker plots), and F, One-way ANOVA followed by post hoc Tukey's test was performed. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$. In D and E, Two-way ANOVA followed by Sidak's multiple comparison was performed. * $p < 0.03$; ** $p < 0.002$; *** $p < 0.0002$. *NT, #PMO, @DG9-PMO, &MOE, ^R6-PMO. Error bars: SEM

Figure 2.

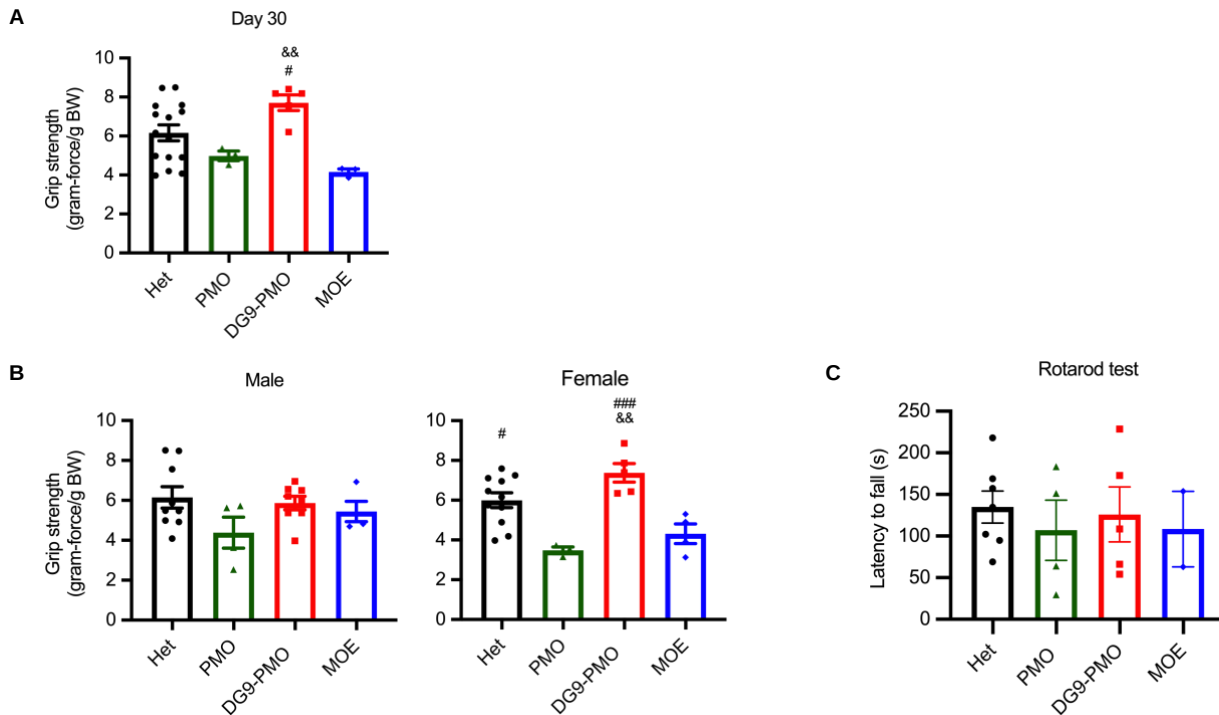
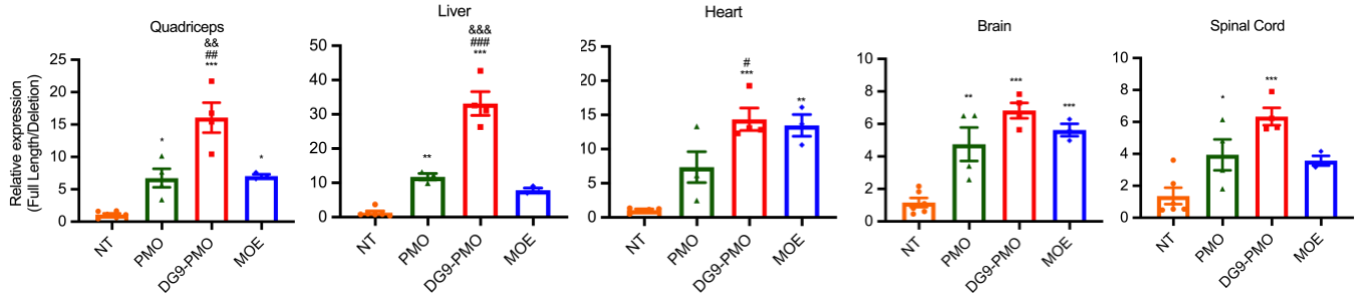


Figure 2. Muscle strength and motor function tests in DG9-PMO treated adult SMA mice. (A) Forelimb grip strength normalized to the body weight measured at PD30. 80 mg/kg of AOs were injected at PD0. (B) Forelimb grip strength measured in adult males and females at PD30. 40 mg/kg of AOs were injected at PD0. (C) Rotarod test with an acceleration profile. Measured at PD 30-35. Each mouse was subjected to three trials spaced 20 minutes from one other. The maximum time on the beam was noted down. 40 mg/kg of AOs were injected at PD0. One-way ANOVA followed by post hoc Tukey's test was performed *NT, #PMO, @DG9-PMO, &MOE. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$. Error bars: SEM.

Figure 3.

A



B

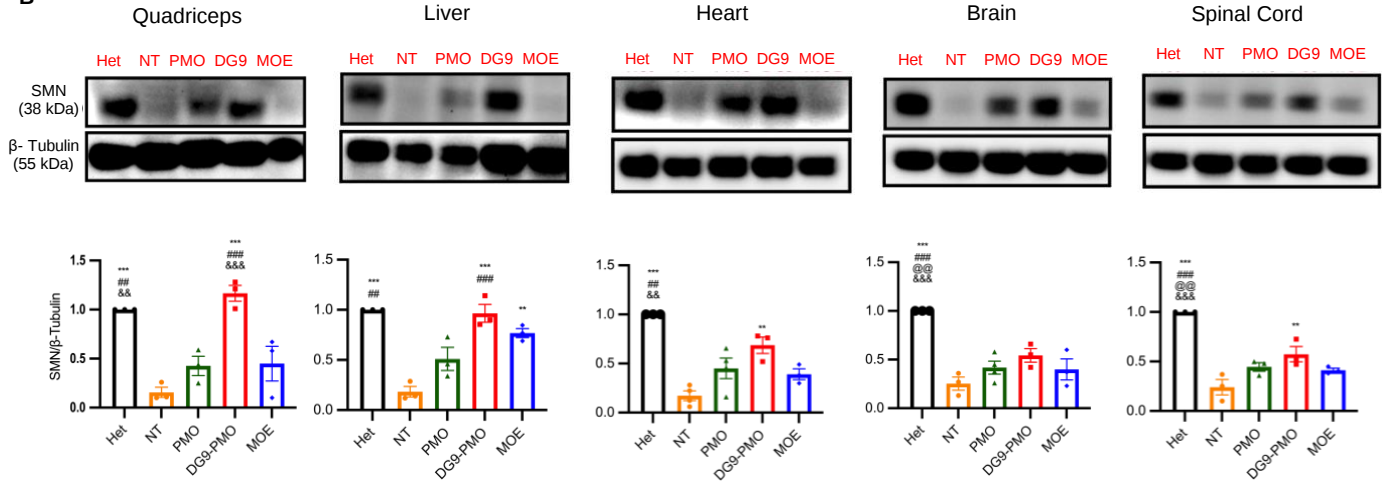


Figure 3. DG9-PMO treatment induces SMN expression in a dose-dependent manner in SMA mice. (A) Relative expression levels of full length *SMN2* (FL-*SMN2*) compared to deleted *SMN2* transcripts ($\Delta 7$ *SMN2*) measured by qPCR in NT and treated mice in the quadriceps muscle, liver, heart, brain, and spinal cord following 80 mg/kg AO treatment. **(B)** Representative images from western blotting and the quantification of SMN levels relative to β -Tubulin. The heterozygous mice were used as a control relative SMN expression set to 1. The tissues from the 80 mg/kg treated mice were collected at PD7. In **A** and **B**, one-way ANOVA followed by post hoc Tukey's test was performed *NT, #PMO, @DG9-PMO, &MOE. *p < 0.05, **p < 0.01, ***p < 0.005. Error bars: SEM.

Figure 4.

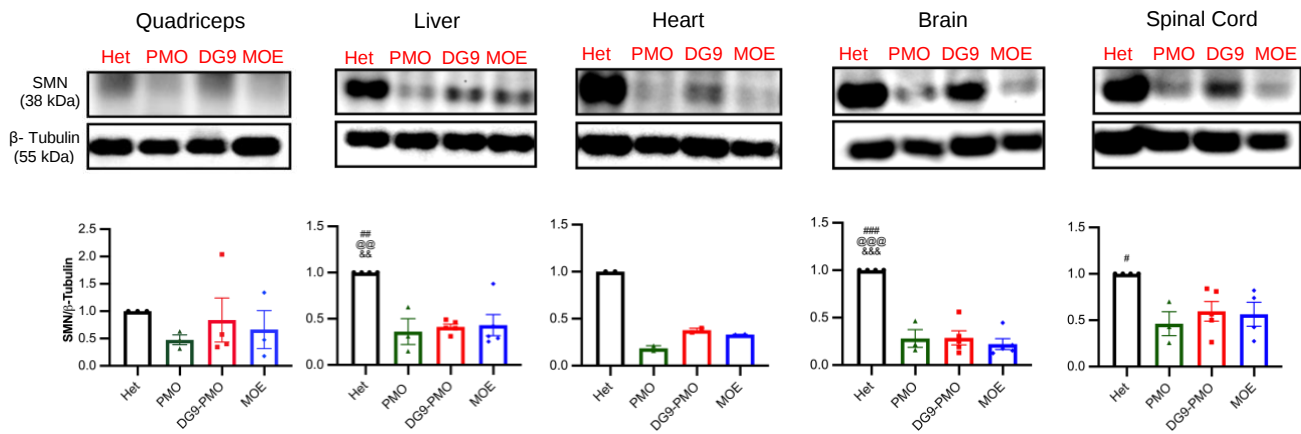


Figure 4. DG9-PMO maintains SMN levels in SMA mice at postnatal day 30. Representative images from western blotting and the quantification of SMN levels. The tissues were collected at PD30 from 40 mg/kg groups, relative to β -Tubulin. The heterozygous mice were used as a control with the relative SMN expression set to 1. Statistics performed using one-way ANOVA followed by post hoc Tukey's test. #PMO, @DG9-PMO, &MOE. *p<0.05, **p<0.01, ***p<0.005. Error bars: SEM.

Figure 5.

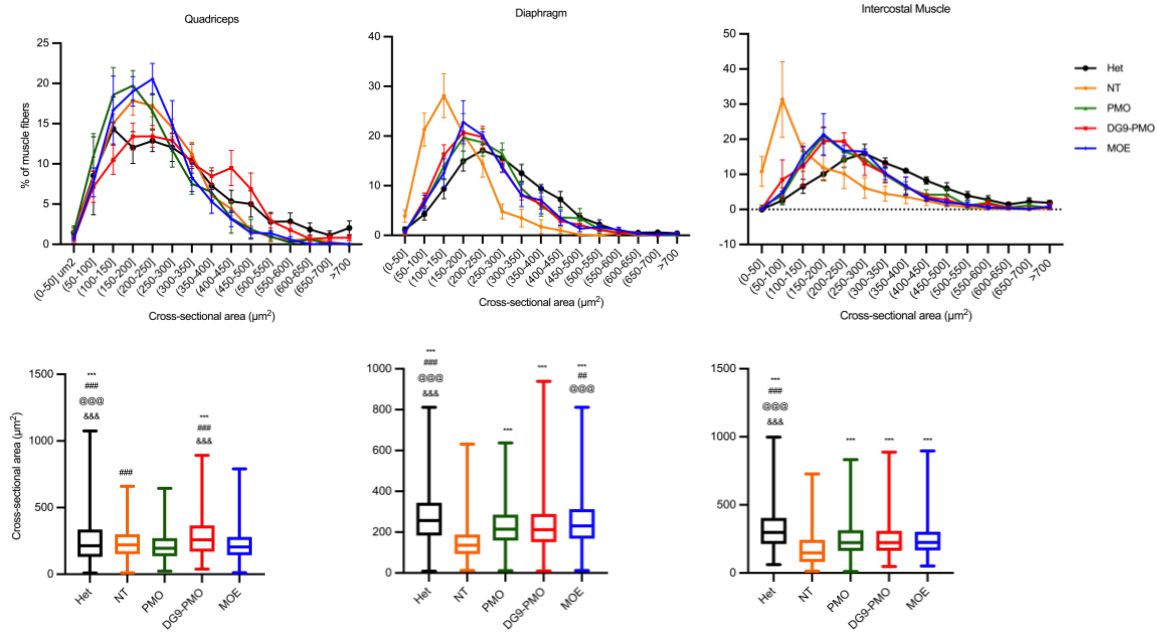
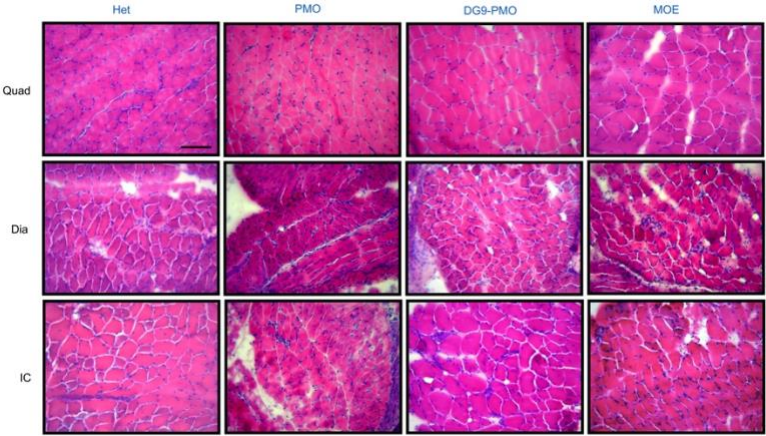


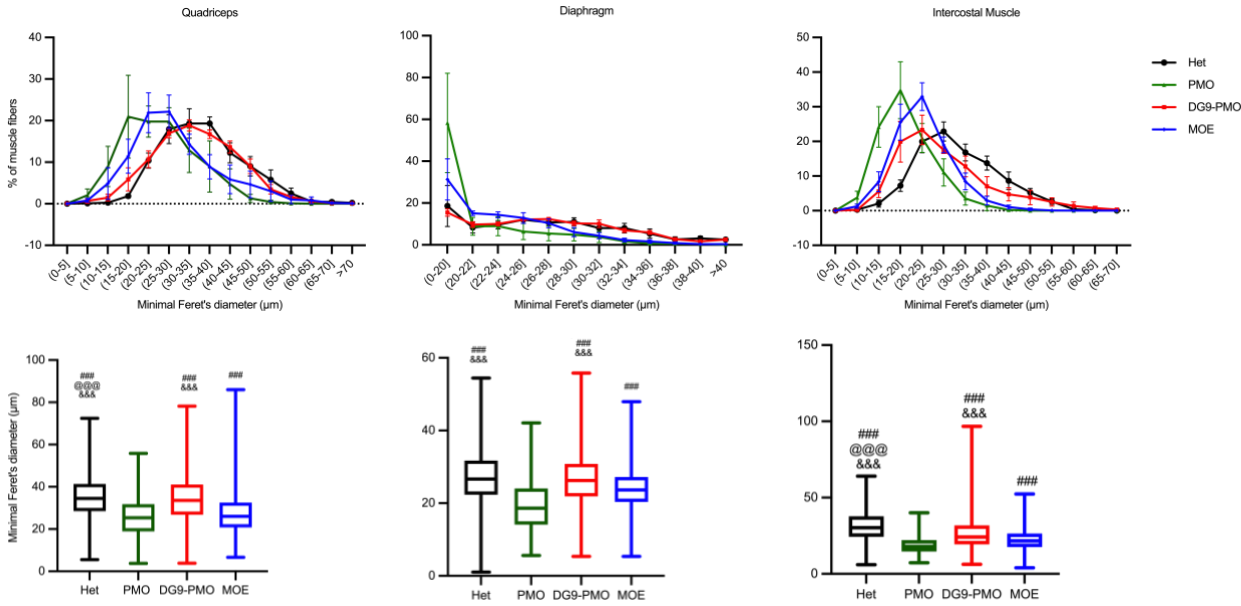
Figure 5. DG9-PMO improves the muscle pathology in SMA mice at postnatal day 7. Frequency distribution of cross-sectional area (CSA) (μm^2) and the quantification of individual myofibers from the quadriceps muscle, diaphragm and intercostal muscle harvested at PD7. Box edges, 25th and 75th percentiles; central line, median; whiskers, range (n=3-7 per group), with around 1292-1653 fibers for the quadriceps, 917-1746 fibers for the diaphragm and 642-1127 for the intercostal muscle. 40 mg/kg of AOs were injected at PD0. Statistics was performed using one-way ANOVA followed by post hoc Tukey's test. *NT, #PMO, @DG9-PMO, &MOE. *p<0.05, **p<0.01, ***p<0.005. Error bars: SEM.

Figure 6.

A



B



C

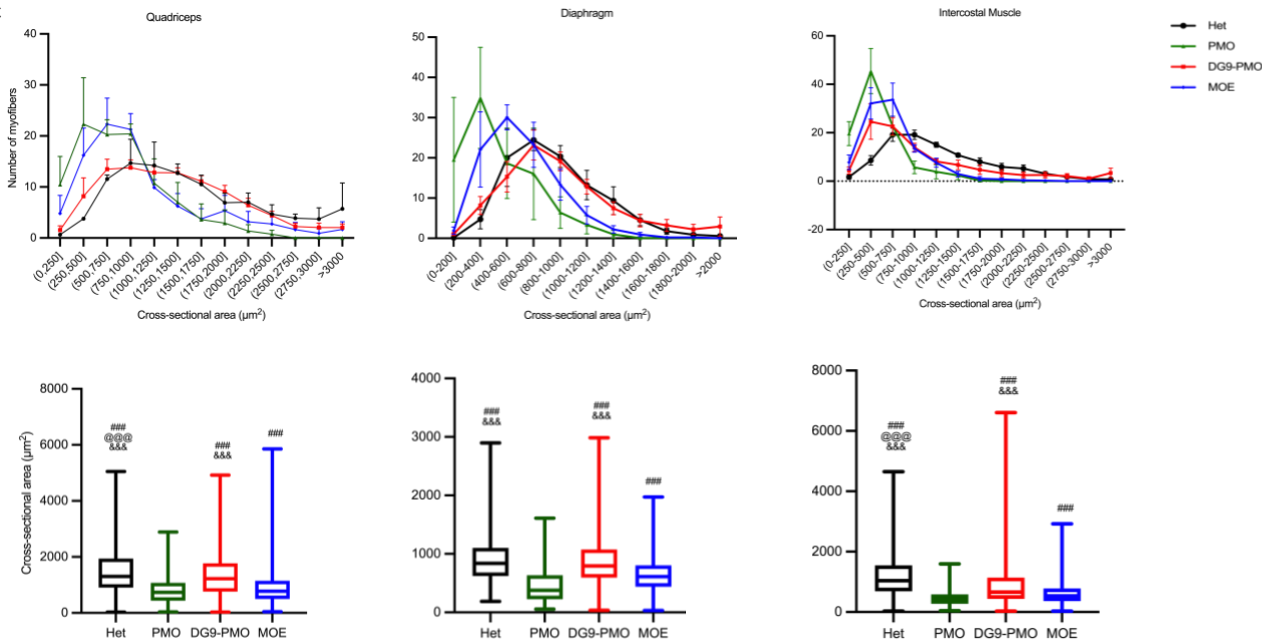


Figure 6. DG9-PMO improves the muscle pathology in adult SMA mice at postnatal day 30. (A) Representative images from H & E staining of the quadriceps muscle (top row), diaphragm (middle row) and the intercostal muscle (bottom row) at PD30 in the heterozygous, NT control and treated groups. 40 mg/kg of AOs were injected at PD0. Scale bar: 100 μ m. (B) Frequency distribution of the minimal Feret's diameter (μ m) and the quantification of individual myofibers from tissues collected at PD30 shown below. (C) Frequency distribution of cross-sectional area (CSA) (μ m²) and the quantification of individual myofibers shown below. Box edges, 25th and 75th percentiles; central line, median; whiskers, range (n=3-6 per group), with around 960-1868 fibers for the quadriceps, 983-1436 fibers for the diaphragm and 800-1802 for the intercostal muscle. 40 mg/kg of AOs were injected at PD0. Statistics was performed using one-way ANOVA followed by post hoc Tukey's test. #PMO, @DG9-PMO, &MOE. *p<0.05, **p<0.01, ***p<0.005. Error bars: SEM.

Figure 7.

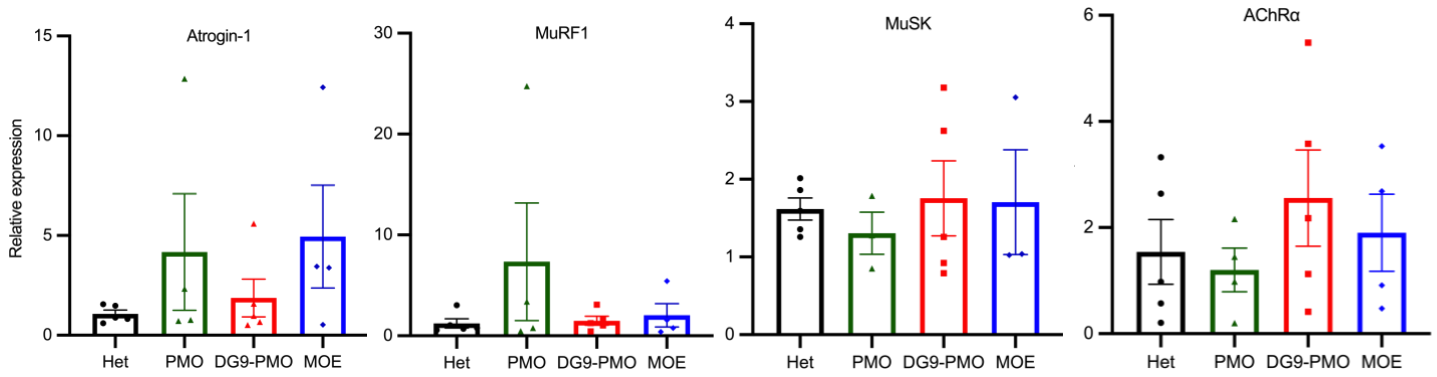


Figure 7. Evaluation of the expression of denervation markers at PD30. Expression of denervation markers Atrogin-1 and MuRF-1 as well as markers MuSK and AChRα necessary for NMJ maintenance relative to 18S ribosomal RNA in the quadriceps muscle at PD30 following treatment. N=4-5 per group with both males and females included. One-way ANOVA followed by post hoc Tukey's test was performed. * $p < 0.05$, ** $p < 0.01$. Error bars: SEM.

Figure 8.

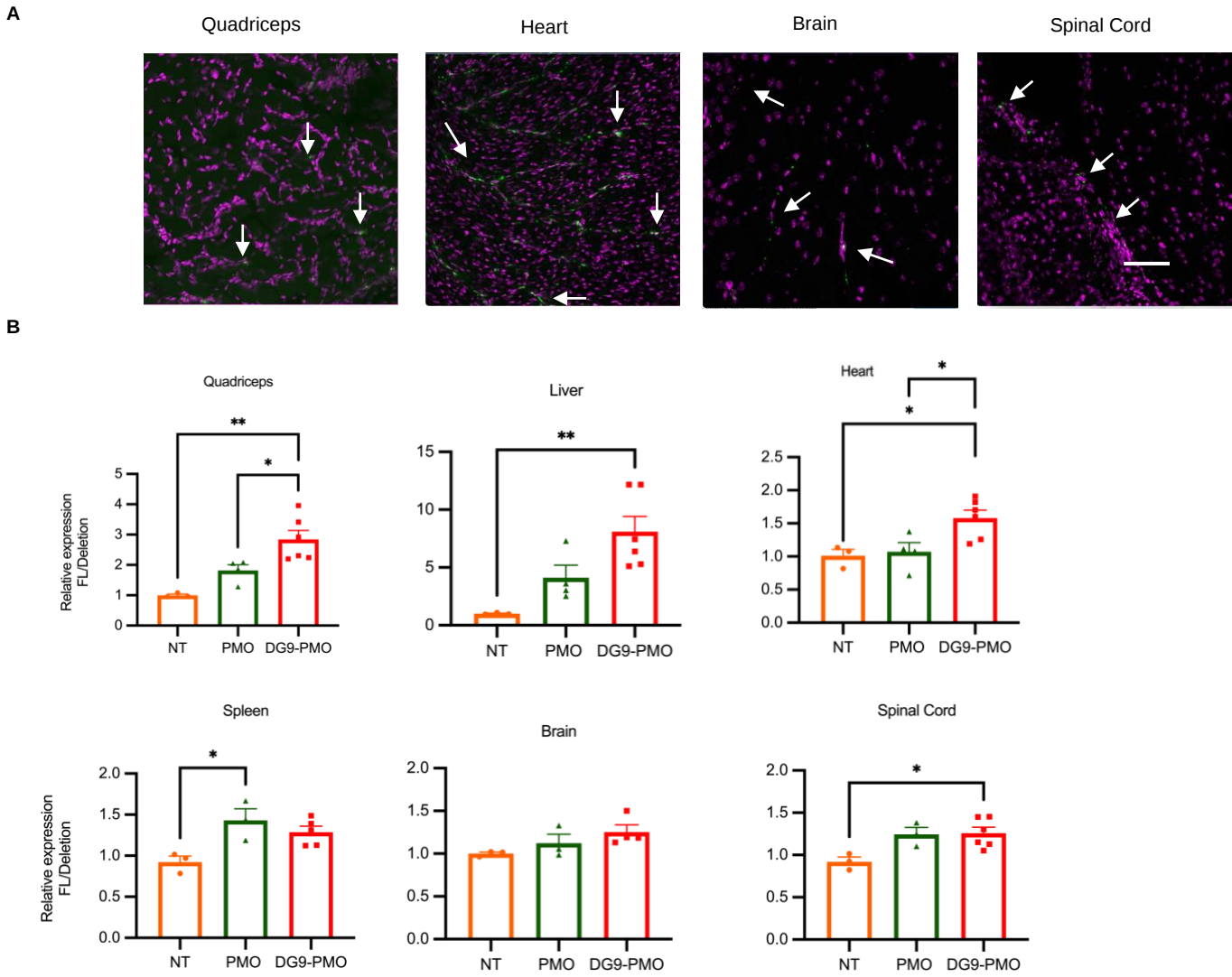


Figure 8. DG9-PMO bypasses the blood-brain barrier in a mild SMA model. (A) Representative immunohistochemistry images at PD13 from the quadriceps muscle, heart, brain, and the spinal cord, following fluorescently tagged DG9-PMO (green) subcutaneous administration at PD5 in F0 mice (*Smn*^{-/-} *SMN2*^{+/+}). Magenta: DAPI. n=3 per group. White arrows indicate DG9-PMO overlapped with nuclei (DAPI). Scale bar: 50 μ m. (B) Relative expression levels of full length *SMN2* (*FL-SMN2*) compared to deleted *SMN2* transcripts ($\Delta 7$ *SMN2*) in the quadriceps muscle, liver, heart, spleen, brain, and spinal cord. Saline, unconjugated- PMO, and DG9-PMO were injected into PD5 mice subcutaneously (n=3-6 per group). The tissues were collected at PD7. One-way ANOVA followed by post hoc Tukey's test was performed. *p<0.05, **p<0.01, ***p<0.005. Error bars: SEM.

Figure 9.

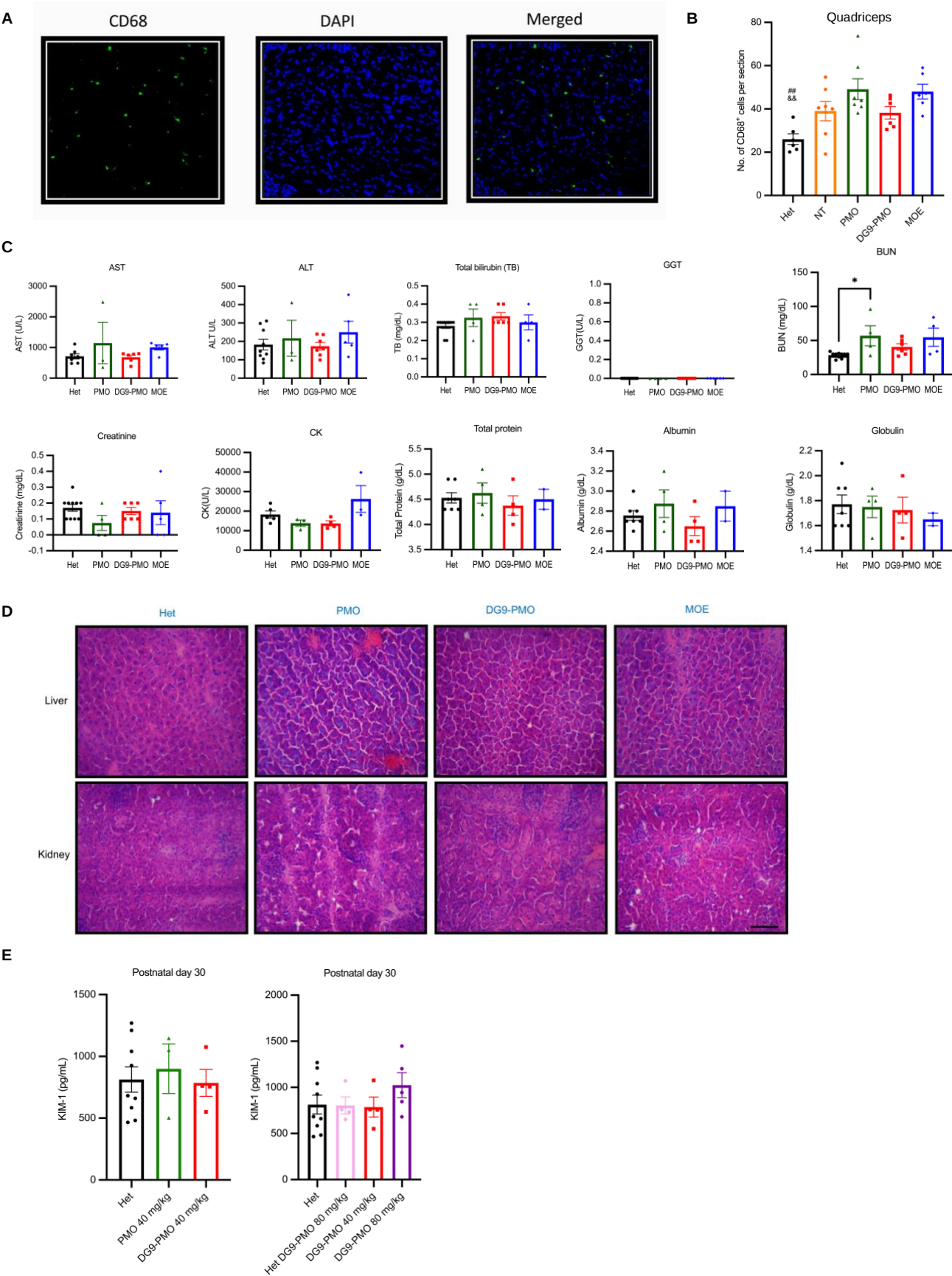


Figure 9. DG9-PMO treatment does not lead to an apparent immune response and toxicity. (A) Representative images from immunostaining of CD68⁺ macrophages (green) and DAPI (blue) in the quadriceps muscle collected at PD7. (B) Average CD68⁺ cells per section. 3-5 sections from the quadriceps muscle from each mouse were used for analysis. (n=3-5 per group). scale bar: 100 μ m. (C) Serum analysis for ALP, ALT, AST, total bilirubin, GGT, BUN, creatinine, CK, total protein, albumin, and globulin. Serum was collected at PD30. (n=2-7 per group). (D) Representative images from H and E staining of the liver (top row) and kidney (bottom row) at PD30. No apparent morphological differences were observed between the healthy heterozygous control and treated groups. scale bar: 100 μ m. 40 mg/kg of AOs were injected at PD0 in A-D. (E) Urinary KIM-1 analysis from mice treated with 40 or 80 mg/kg of DG9-PMO (DG9-PMO 40, DG9-PMO 80) or 40 mg/kg of unconjugated PMO (PMO 40) at PD30. Heterozygous healthy control mice (Het) treated with PBS alone (Het) or DG9-PMO 80 mg/kg (Het DG9-PMO 80). One-way ANOVA followed by post hoc Tukey's test was performed. *p<0.05, **p<0.01, ***p<0.005. Error bars: SEM

Supplemental Tables

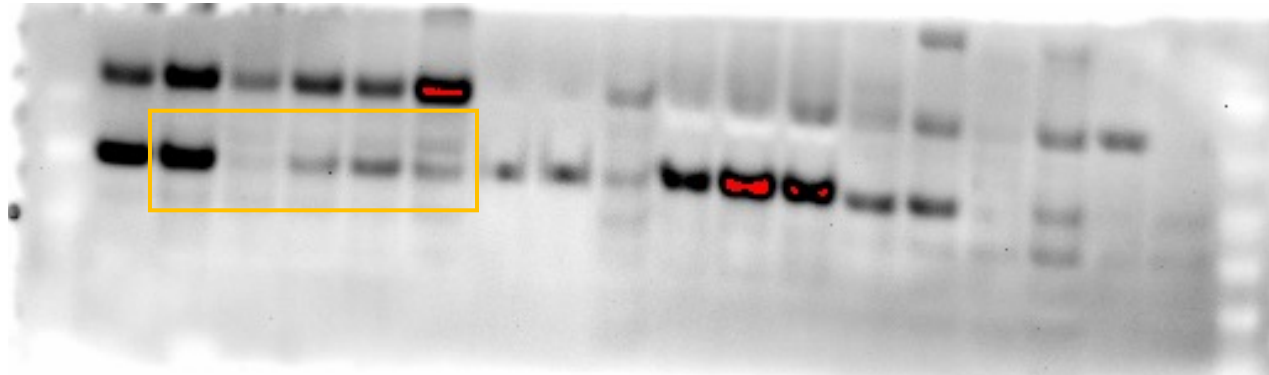
Table 1: List of primers used in this study.

Target	Sequence (5'-3')
<i>Smn-S1</i>	ATAACACCACCACTCTTACTC
<i>Smn-S2</i>	GTAGCCGTGATGCCATTGTCA
<i>Smn-H1</i>	AGCCTGAAGAACGAGATCAGC
FL-SMN2	F: GCTATCATACTGGCTATTATATGGGTTTT R: CTCTATGCCAGCATTTCTCCTTAAT
Δ SMN2	F: TCTGGACCACCAATAATTCCCC R: ATGCCAGCATTTCCATATAATAGCC
mGAPDH	F: GAGAAACCTGCCAAGTA R: CAGTGTAGCCCAAGATG
Atrogin-1 (50)	F: GCAGAGAGTCGGCAAGTC R: CAGGTCGGTGATCGTGAG
MuRF-1 (50)	F: AGTGTCCATGTCTGGAGGTCGTTT R: ACTGGAGCACTCCTGCTTGTAGAT
MuSK (49)	F: GCCTTCAGCGGGACTGAG R: GAGGCGTGGTGACAGG
AChR α (49)	F: GCCATTAACCCGAAAGTGAC R: CCCCCTCTCCATGAAGTT
18S (50)	F: GGGGAGTATGGTTGCAAAGC R: CGCTCCACCAACTAAGAACG

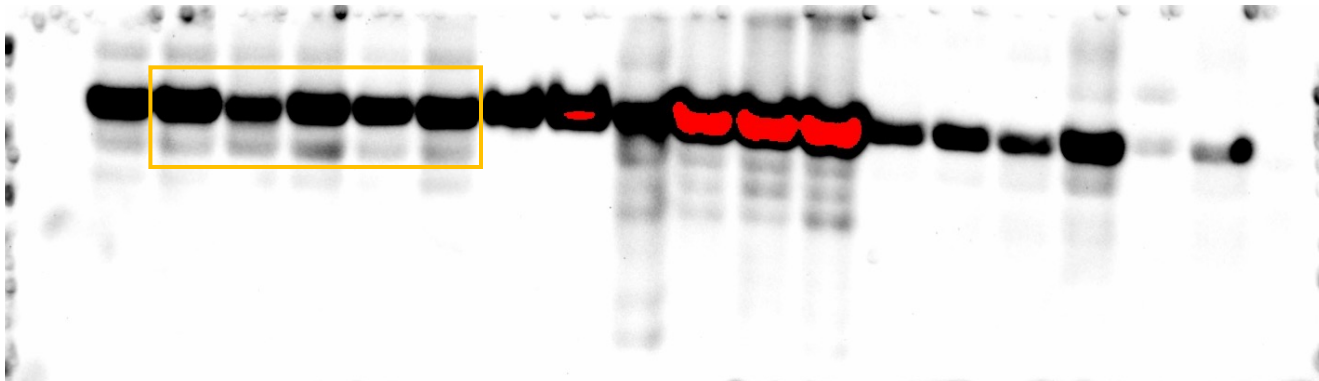
Unedited and uncut
blots/gels

Full unedited gel for Figure 2B (Quadriceps)

SMN

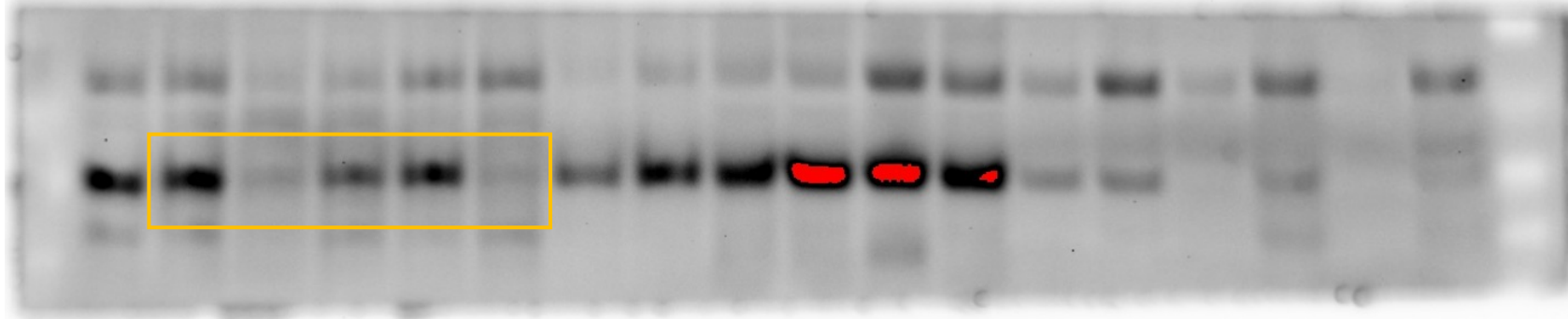


Tubulin

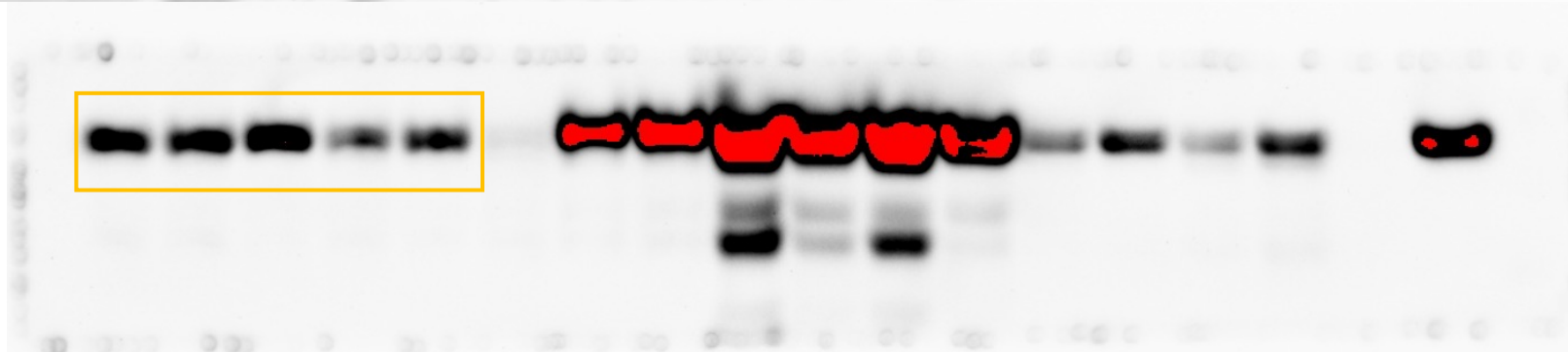


Full unedited gel for Figure 2B (Liver)

SMN



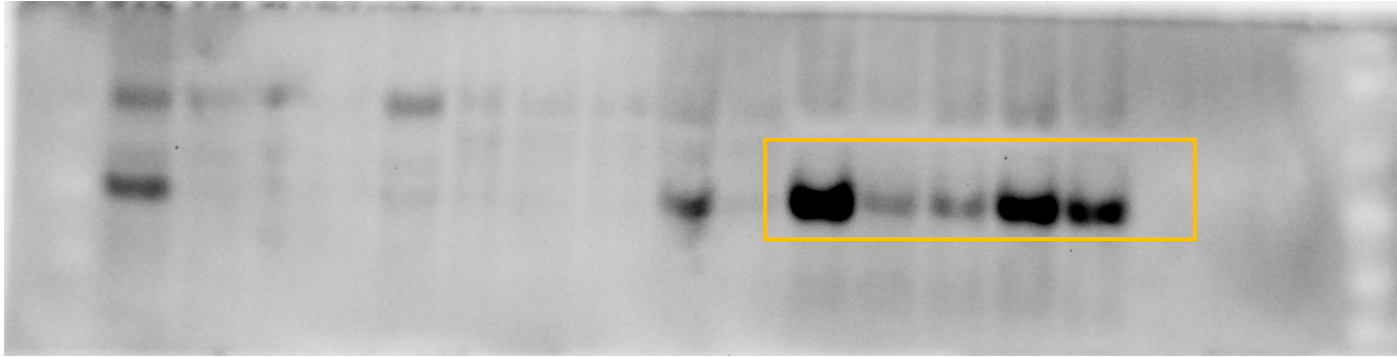
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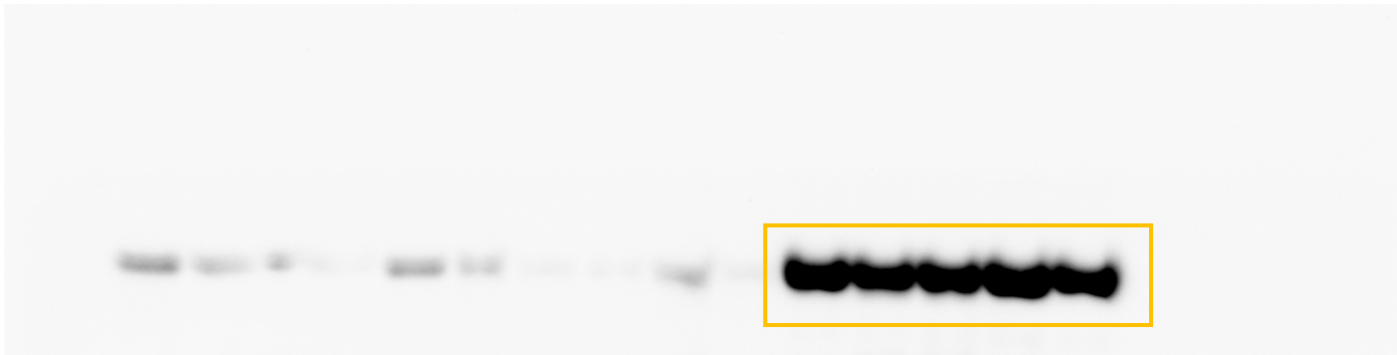
A black and white photograph of a gel electrophoresis result. The gel has multiple lanes. The first lane on the left contains a single, prominent dark band. This band is enclosed within a yellow rectangular box. The other lanes to the right show various patterns of bands, including some with multiple bands and others with a single band.

Full unedited gel for Figure 2B (Brain)

SMN

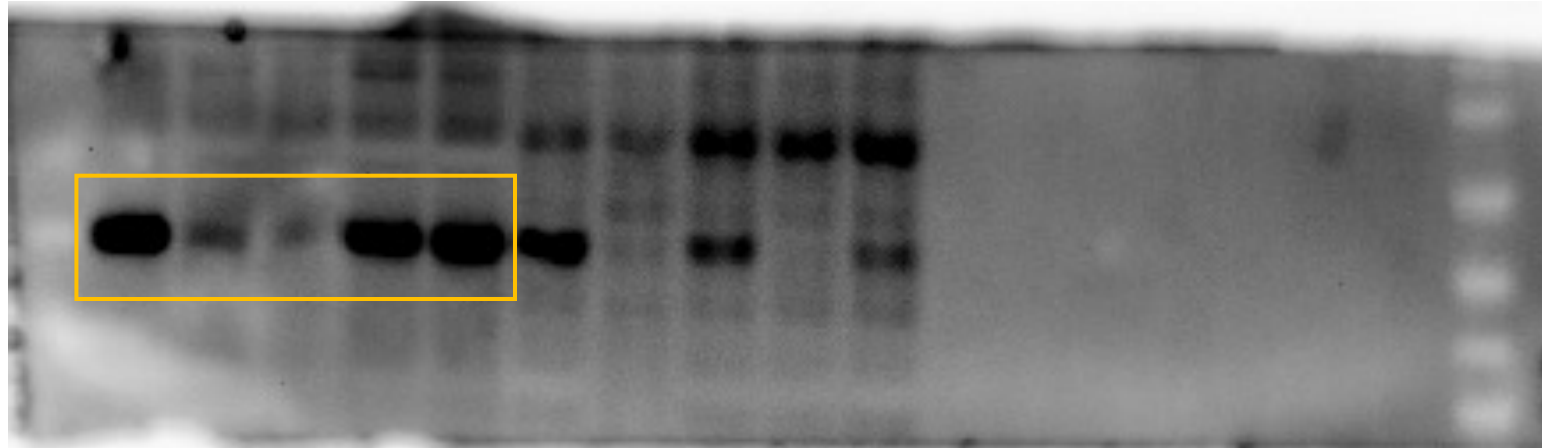


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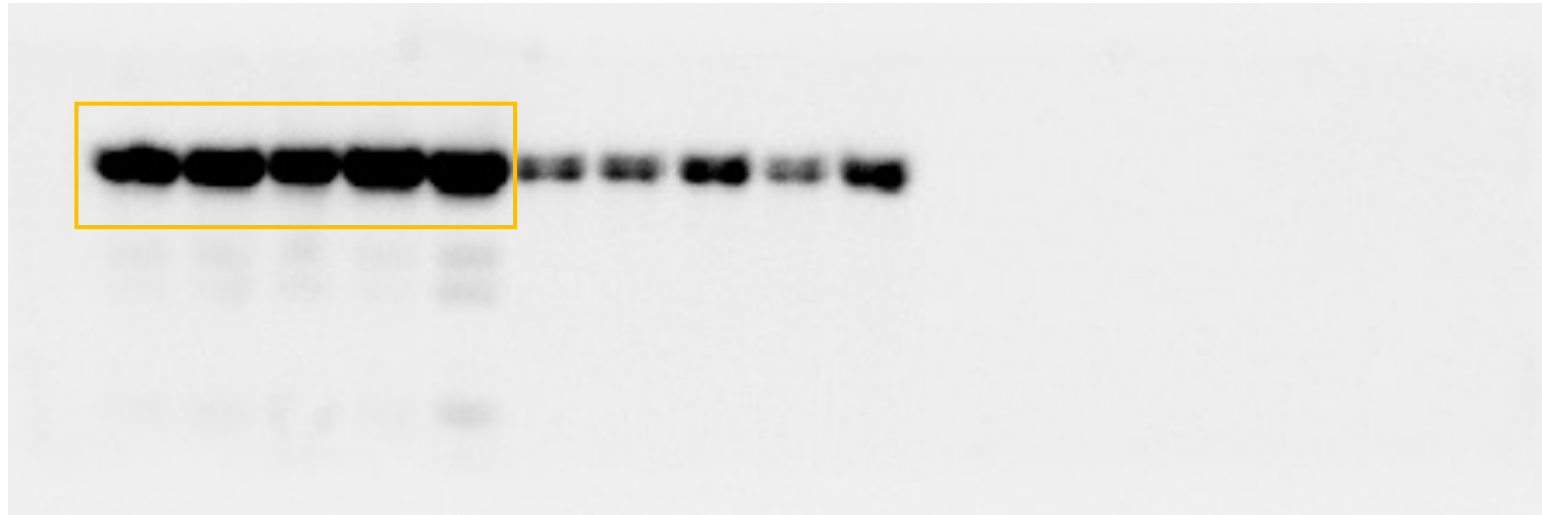


Full unedited gel for Figure 2B (Spinal Cord)

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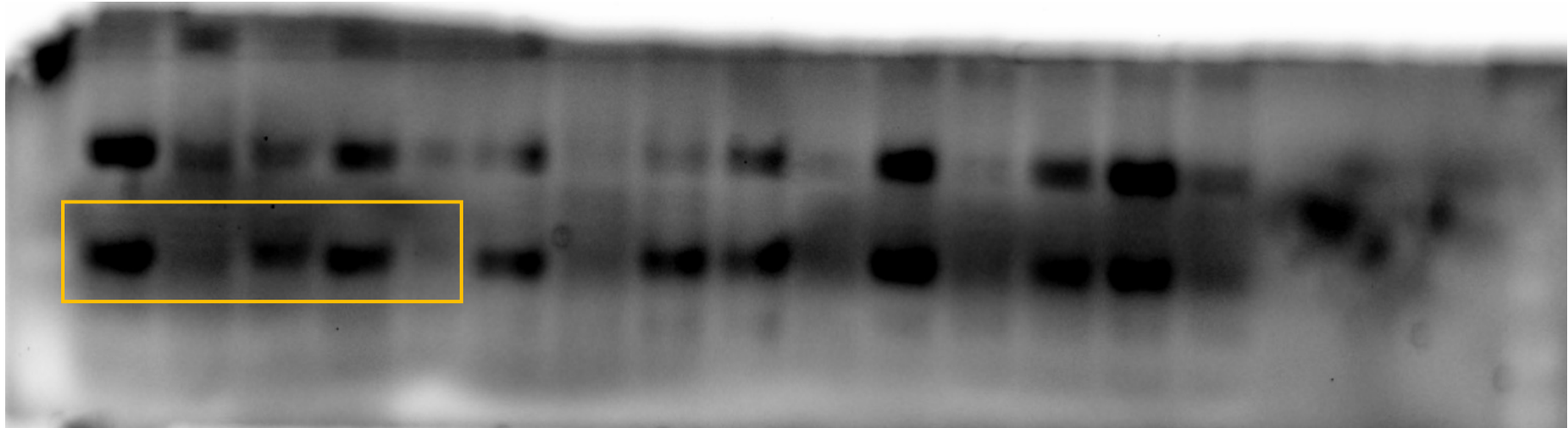


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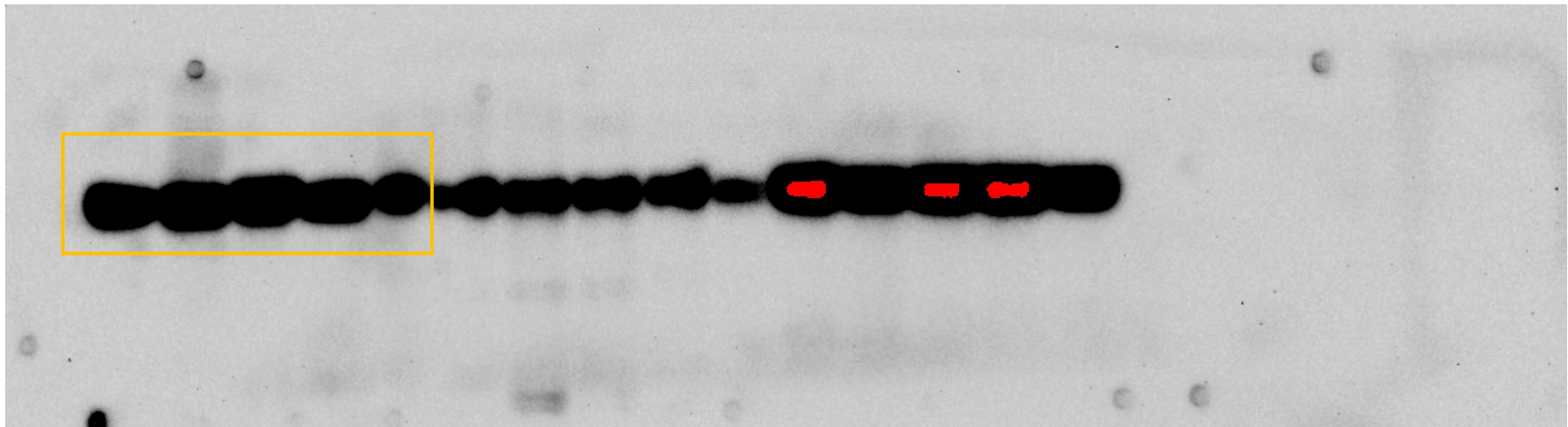


Full unedited gel for Supplemental Figure 3B (Quadriceps)

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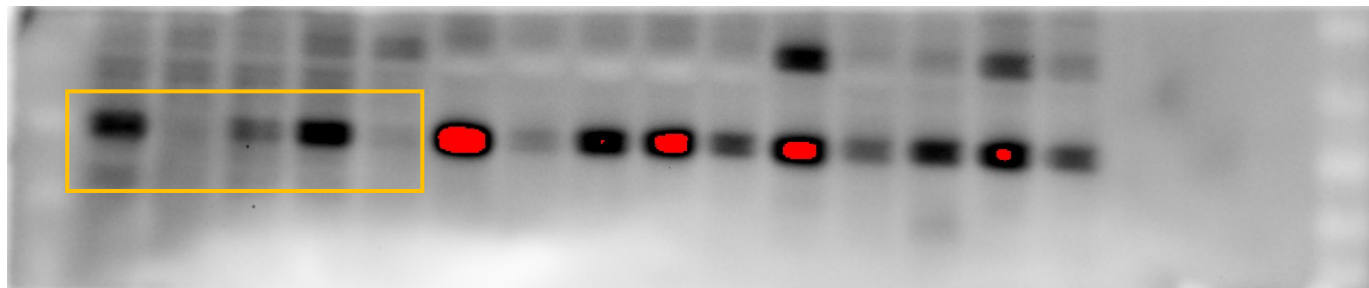


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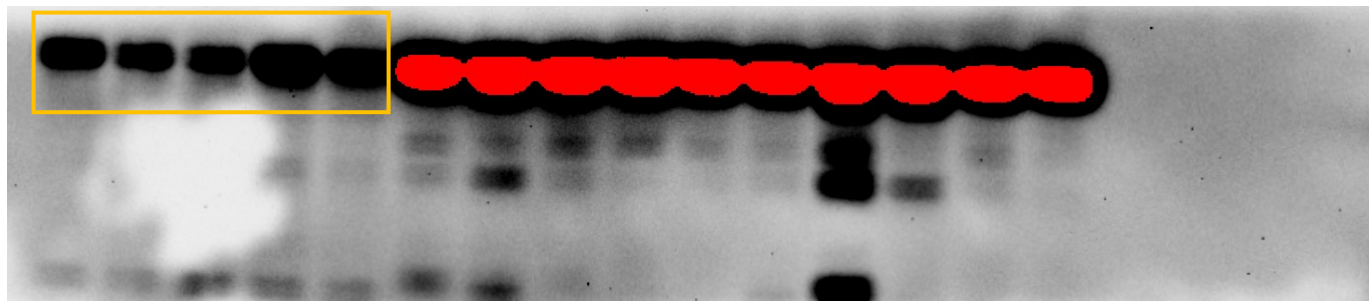


Full unedited gel for Supplemental Figure 3B (Liver)

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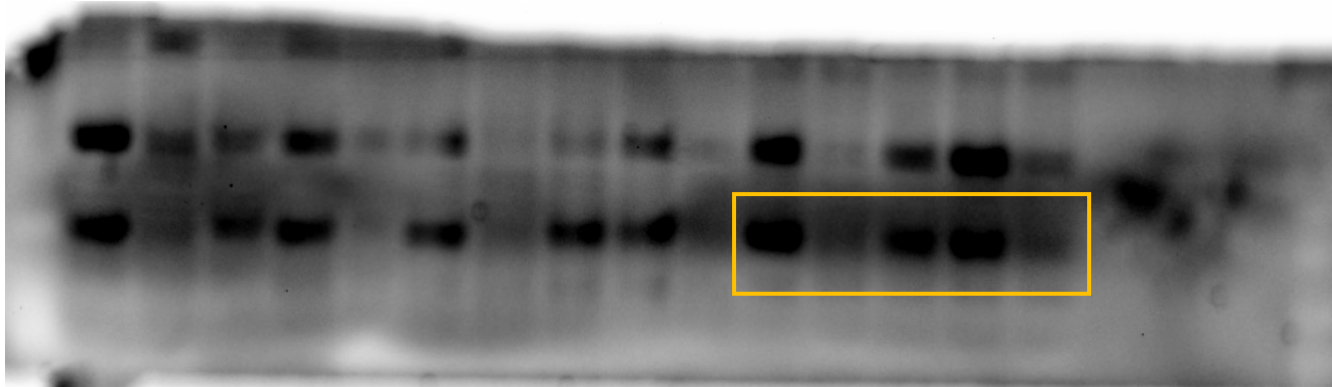


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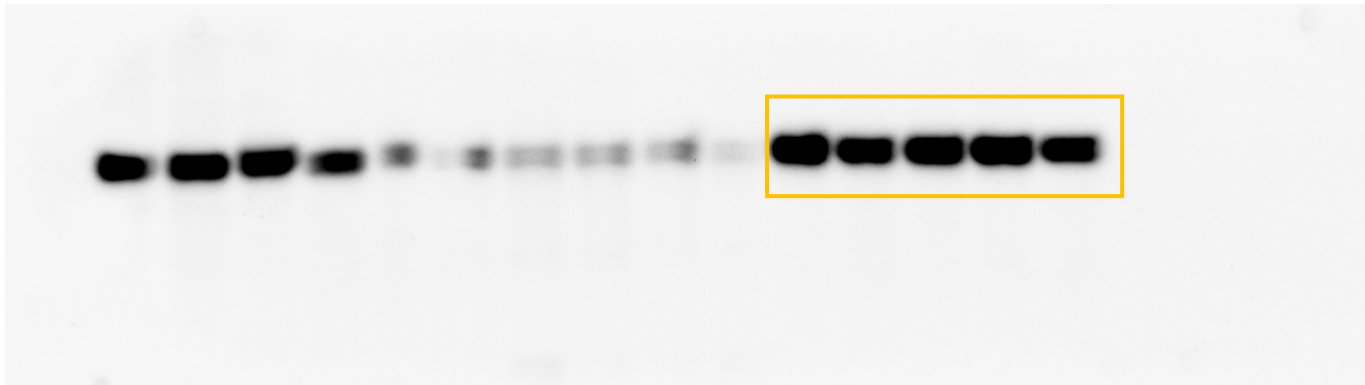


Full unedited gel for Supplemental Figure 3B (Heart)

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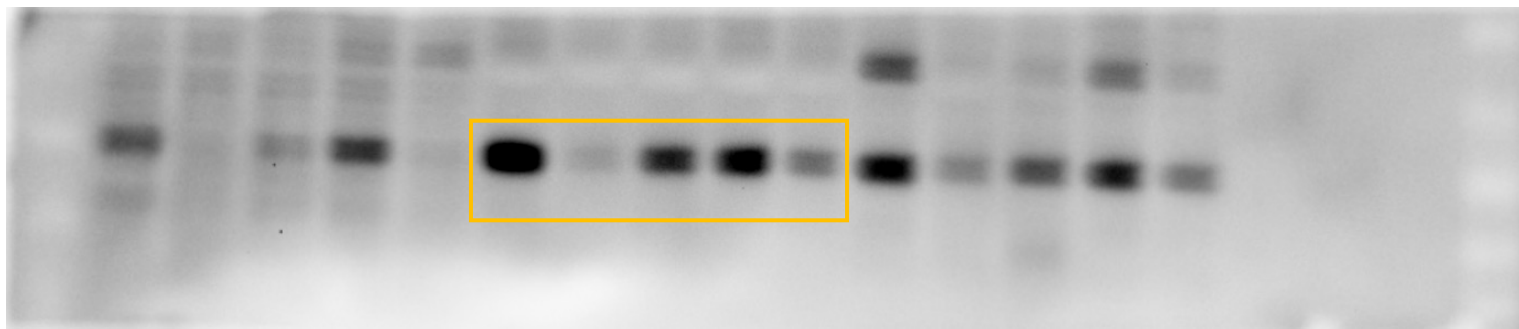


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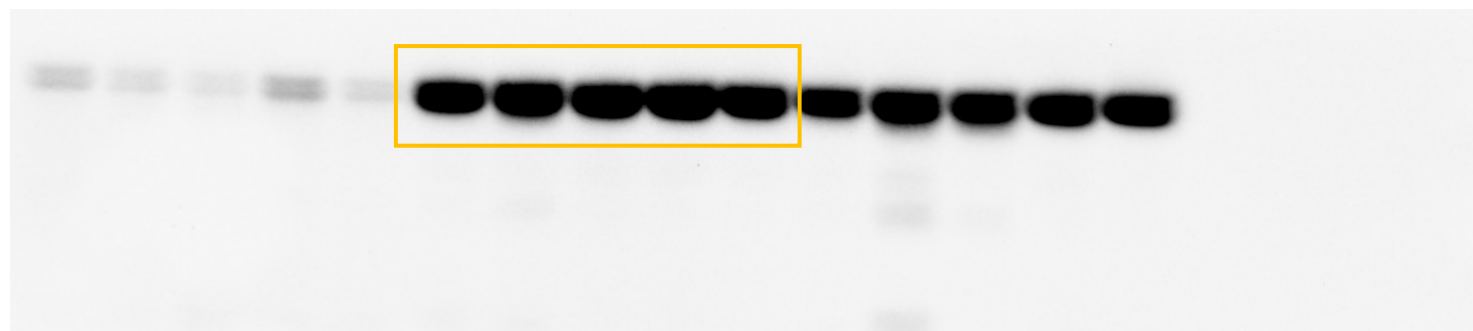


Full unedited gel for Supplemental Figure 3B (Brain)

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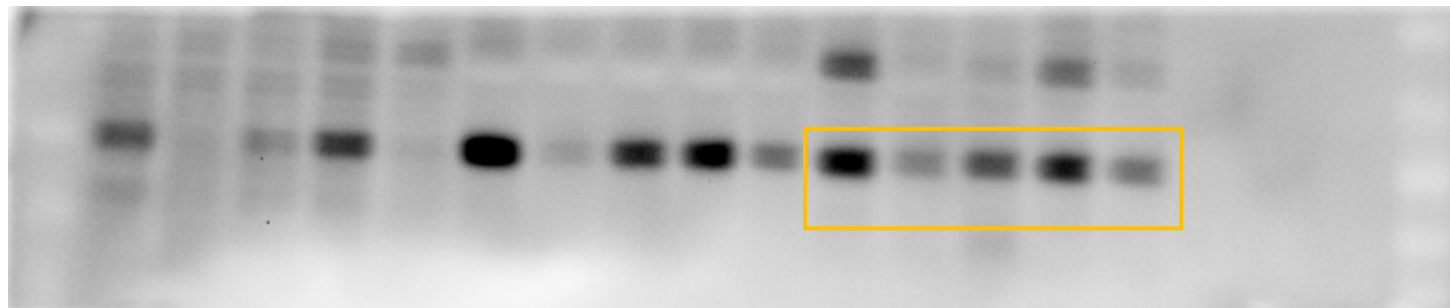


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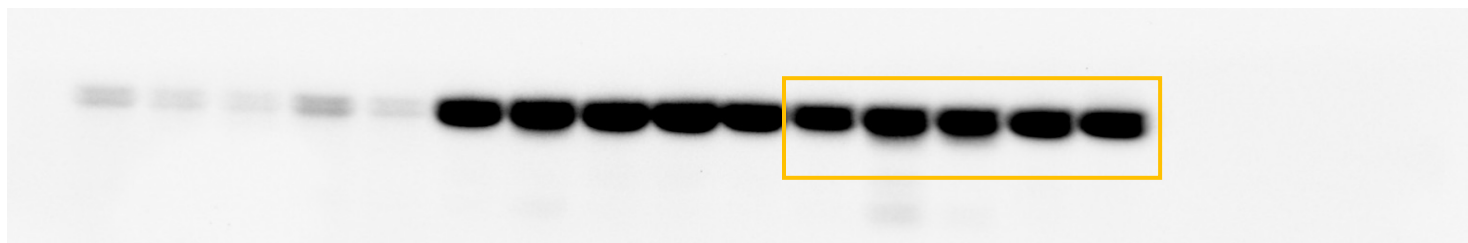


Full unedited gel for Supplemental Figure 3B (Spinal Cord)

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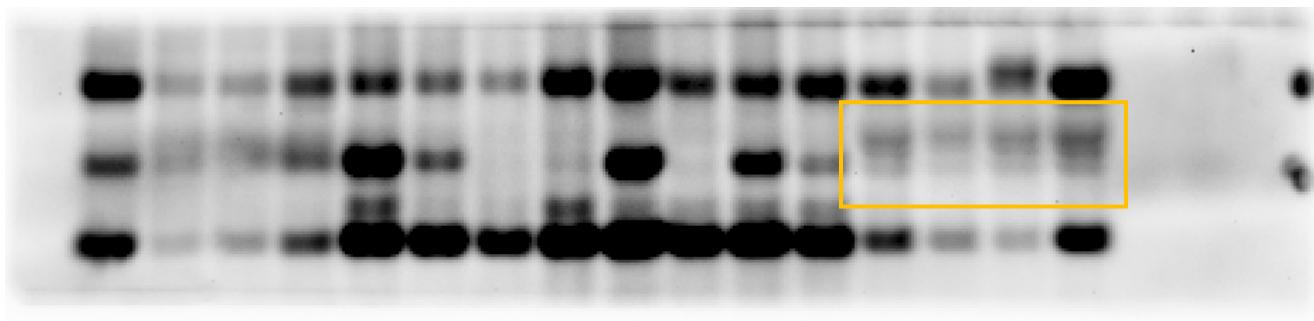


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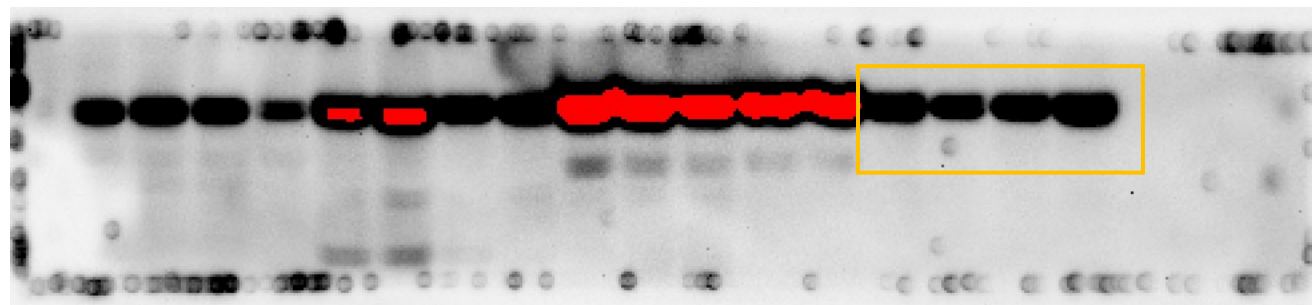


Full unedited gel for Supplemental Figure 4 (Quadriceps)

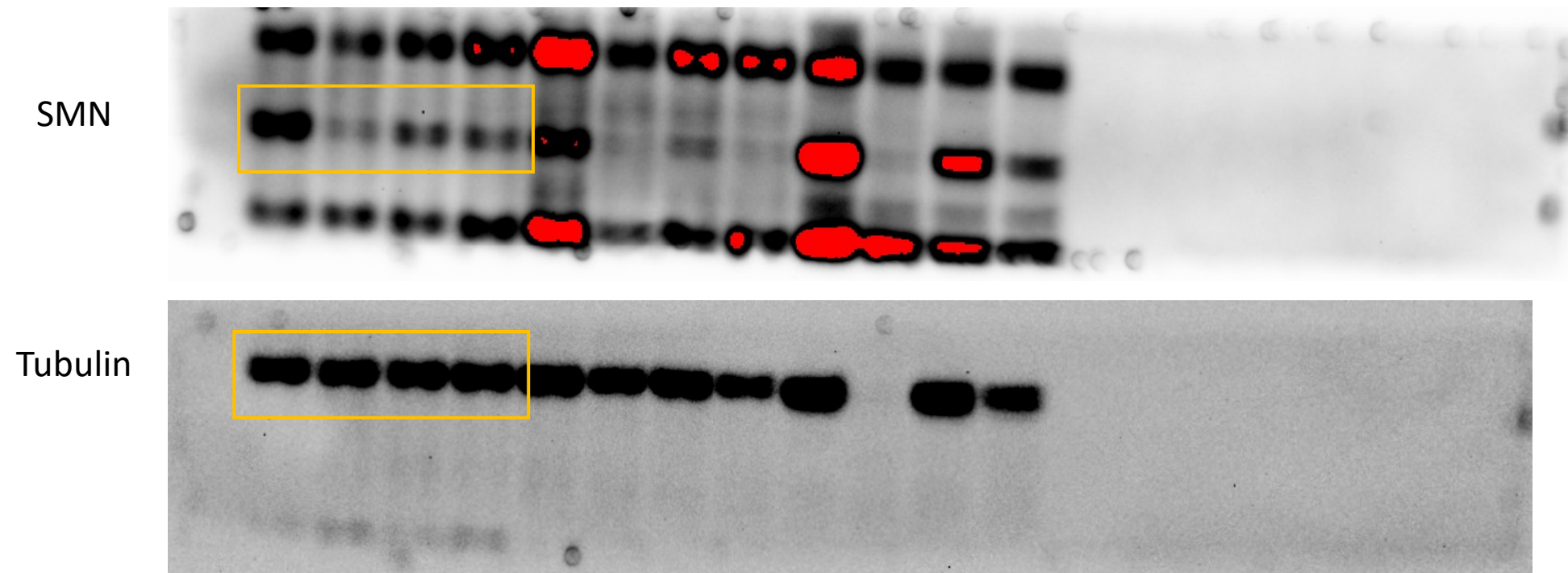
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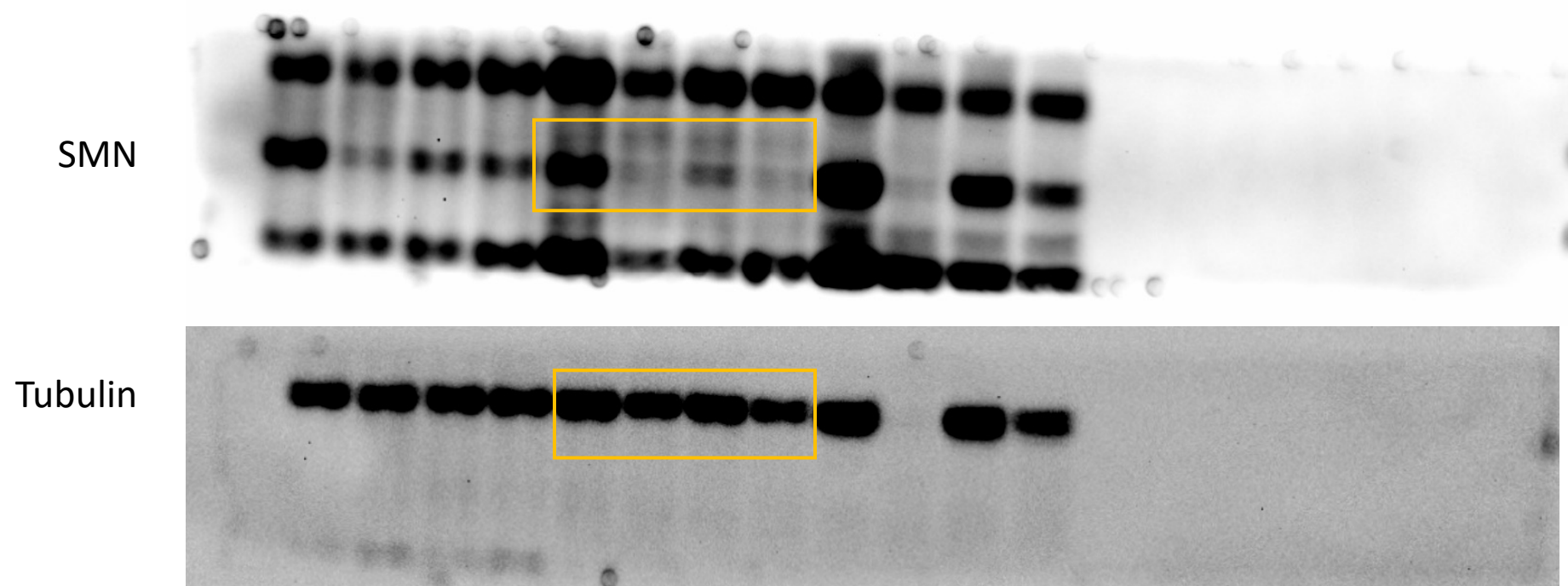
Tubulin



Full unedited gel for Supplemental Figure 4 (Liver)

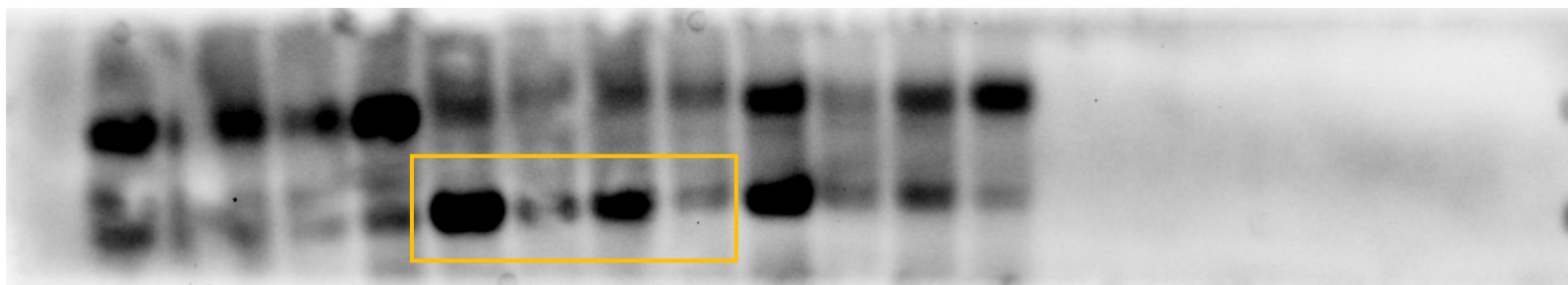


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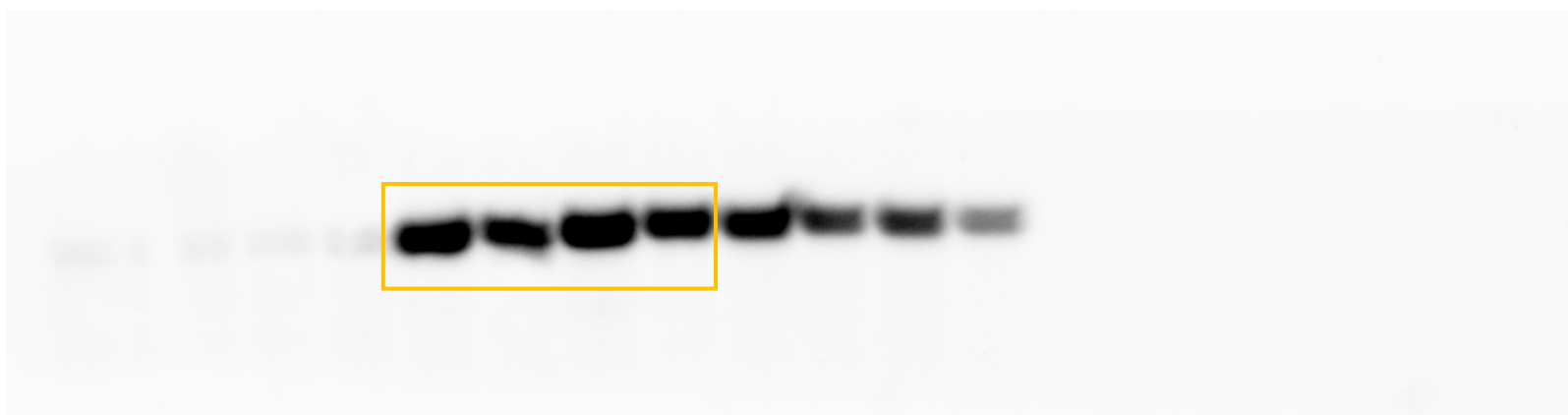


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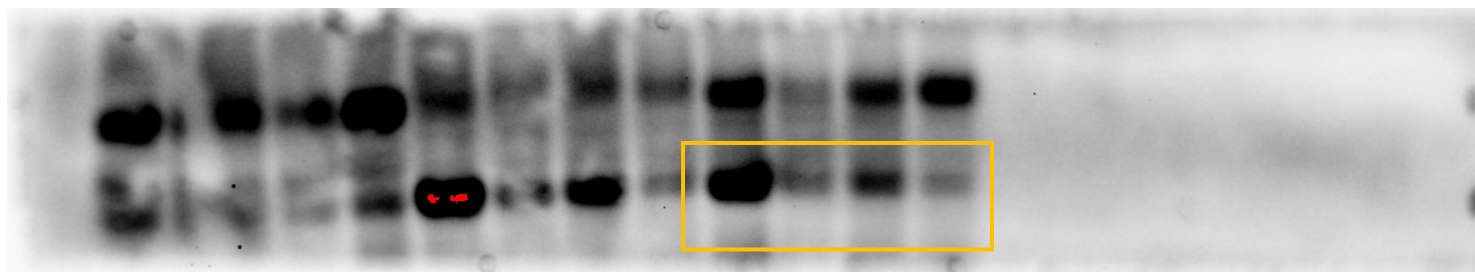


Tubulin



Full unedited gel for Supplemental Figure 4 (Spinal Cord)

SMN



Tubulin

