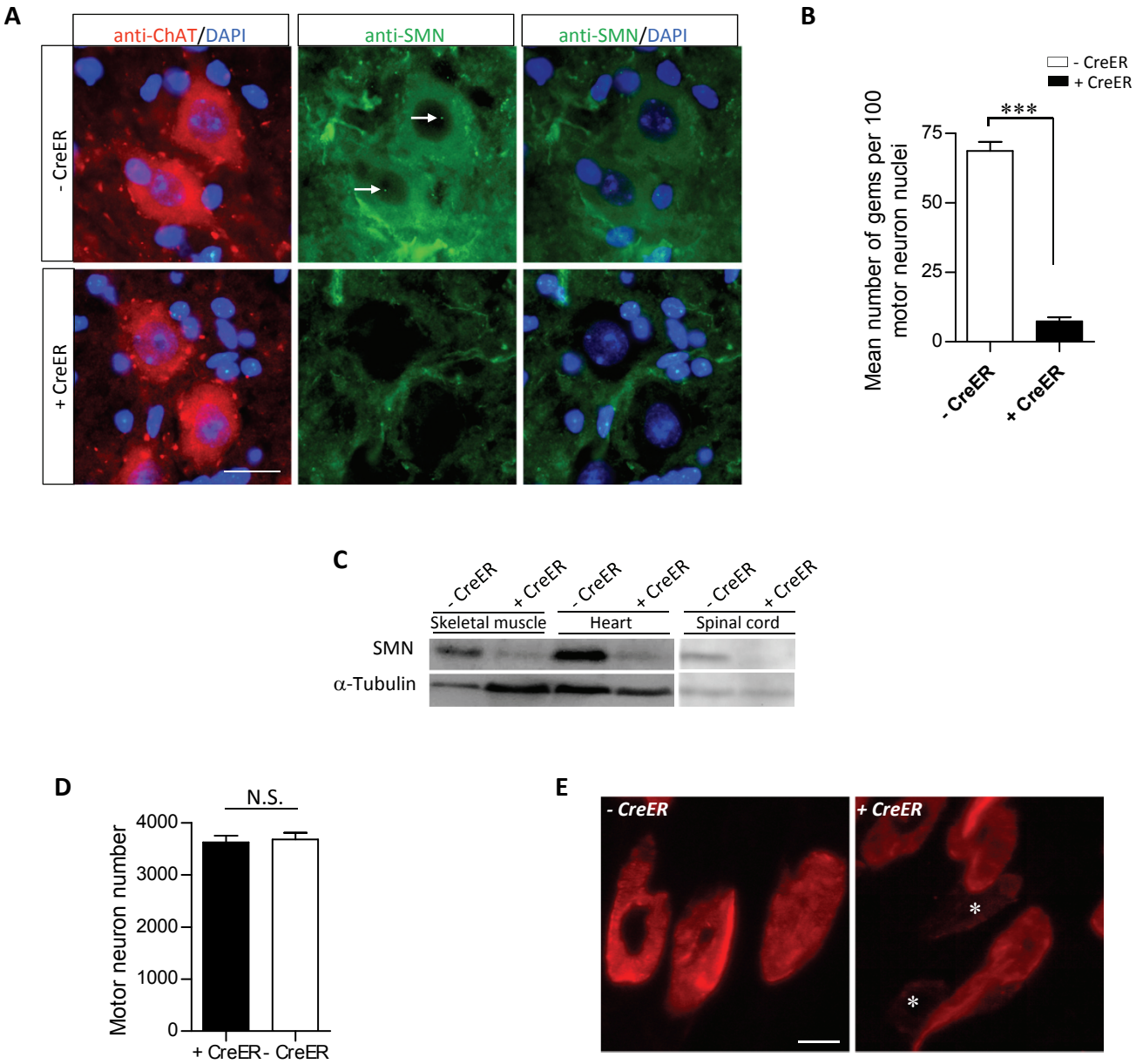
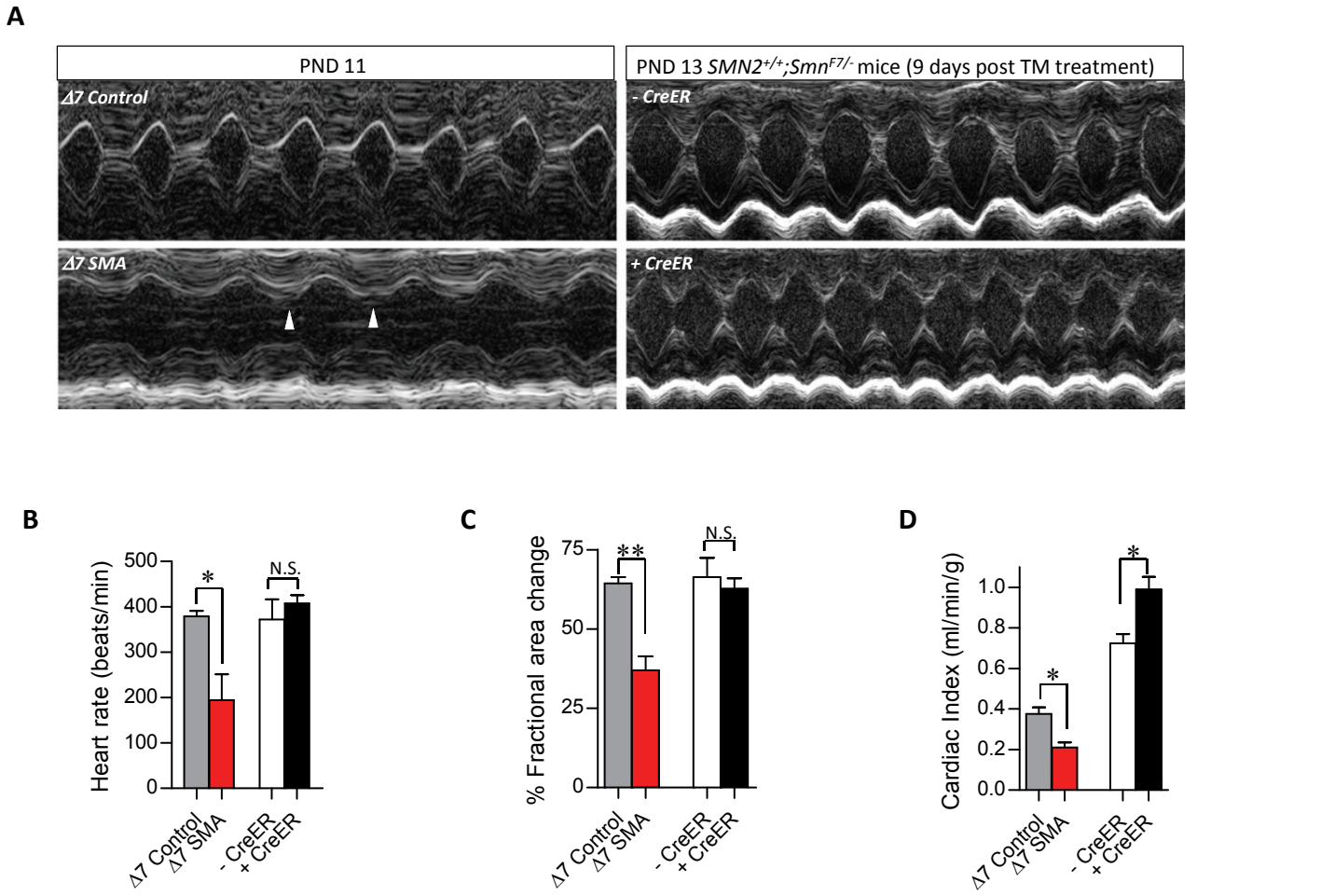
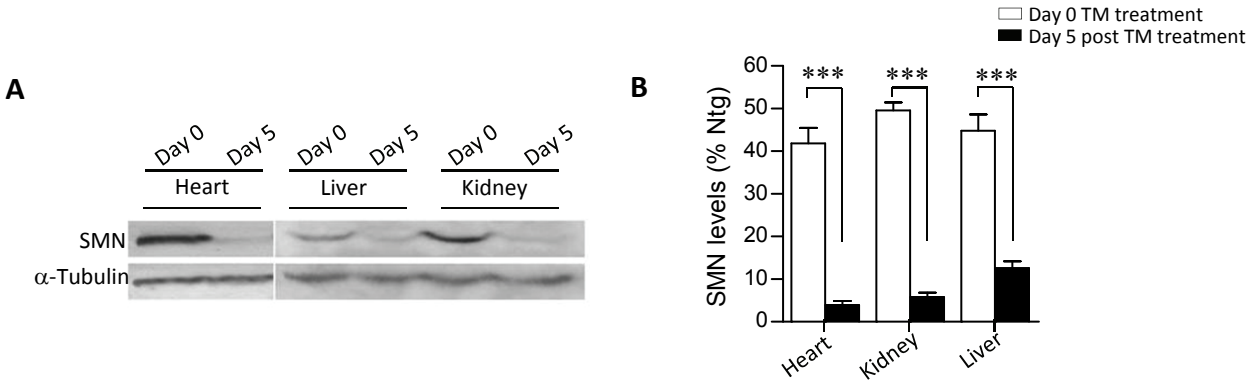


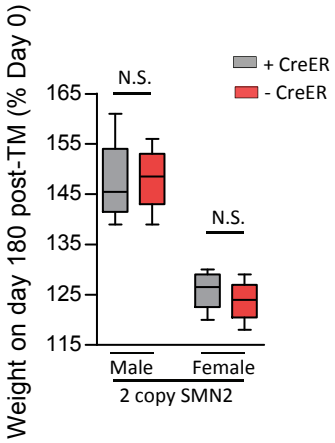
Supplemental Figure 1 – Cellular pathology in 11-12 day old *SMN2*$^{+/+}$*;CreER;Smn*$^{F7/-}$ mice following SMN depletion at 4 days of age. **(A)** Immunostaining of spinal motor neurons of mutant mice depleted of SMN reveals markedly reduced protein in the cell soma. Control motor neurons from mice without the *CreER* transgene exhibit robust cytoplasmic SMN and nuclear gems (arrows). Scale bar: 30μm. **(B)** Quantification of gems in the mice indicates marked loss in the mutants. ***, $P < 0.001$, *t* test, n=300 motor neurons from N=3 mice of each genotype. **(C)** Western blot analysis of SMN protein in three different tissues of mutants demonstrating general depletion of the protein relative to that in controls. **(D)** Yet, quantification of lumbar motor neurons of the SMN-depleted mutants failed to reveal cell loss ($n \geq 3$, $P > 0.05$, *t* test). **(E)** Examples of poorly staining AChR clusters (asterisks) at the synapses of the mutants are illustrative of NMJ defects. Scale bar: 10μm.



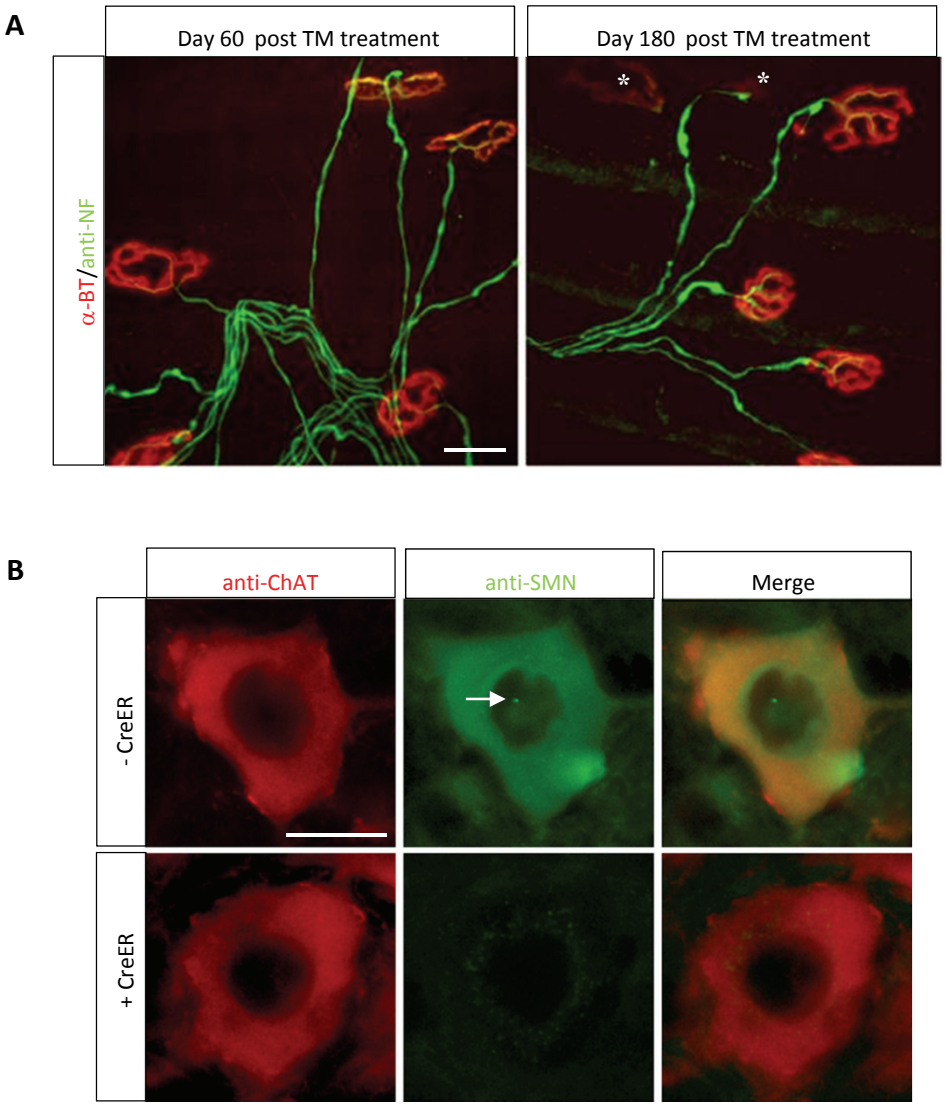
Supplemental Figure 2 – Neonatal depletion of SMN does not affect cardiac function. **(A)** M-mode echocardiographs depict normal ventricular wall movement in neonatal SMN depleted animals. In contrast, Δ7 SMA mice constitutively depleted for the protein from the earliest developmental time point did display evidence of cardiac dysfunction (arrowheads indicate poor wall movement). Quantification of **(B)** heart rate, **(C)** fractional area change and **(D)** cardiac index revealed related defects in the D7 SMA mice but not mutants in whom SMN was depleted post-natally. n ≥ 3 mice of each genotype, *, **, $P < 0.05$ and $P < 0.01$ respectively, *t* test.



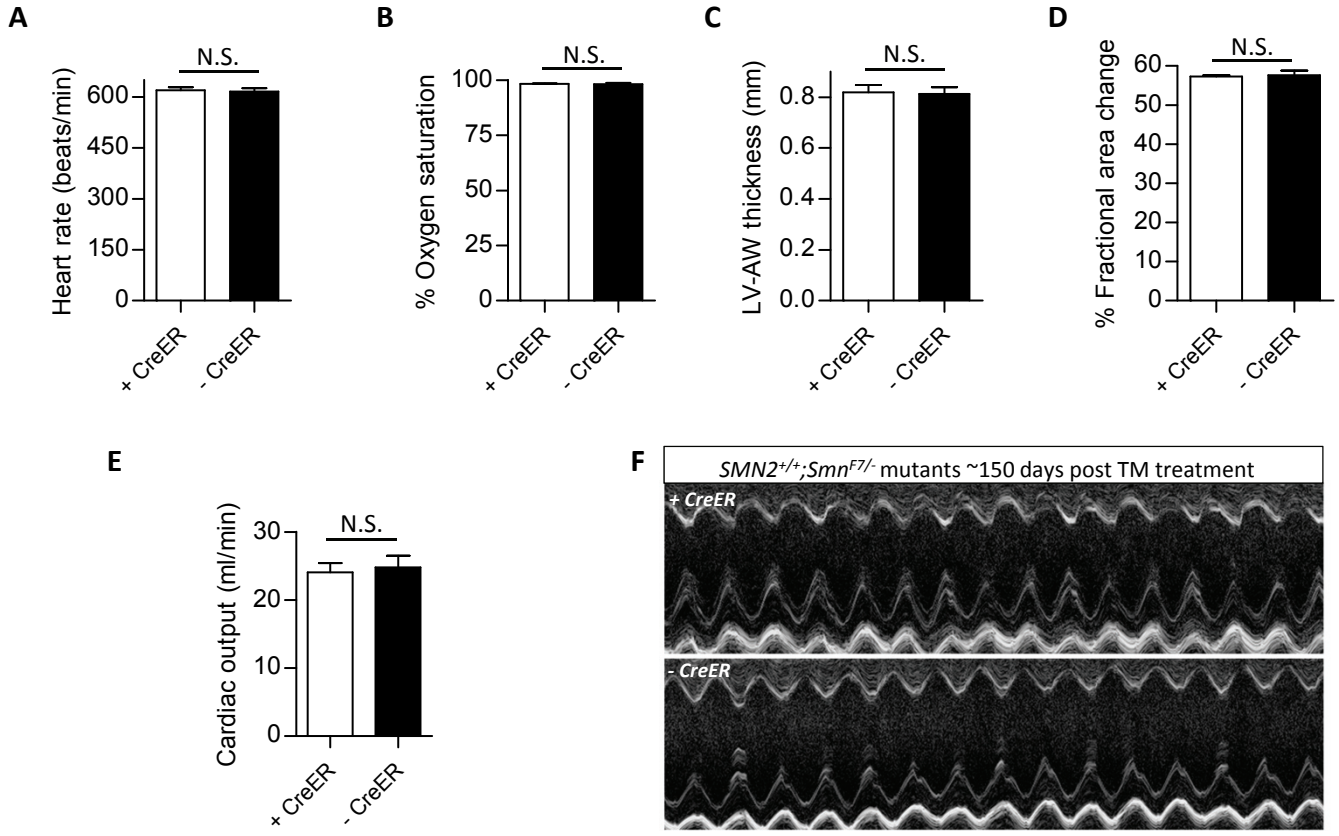
Supplemental Figure 3 – Tamoxifen induction effects efficient tissue-wide SMN depletion in inducible mutant mice. **(A)** Representative western blot analysis depicting knockdown of the SMN protein in non-nervous tissue at two time points following tamoxifen treatment of *CreER;Smn*$^{F7/-}$ mutant mice. **(B)** Quantification of SMN knockdown indicates robust and significant (≤ 15% of wild-type protein) depletion of the protein. ***, $P < 0.001$, one-way ANOVA, n ≥ 3 mice for each time point that was analyzed.



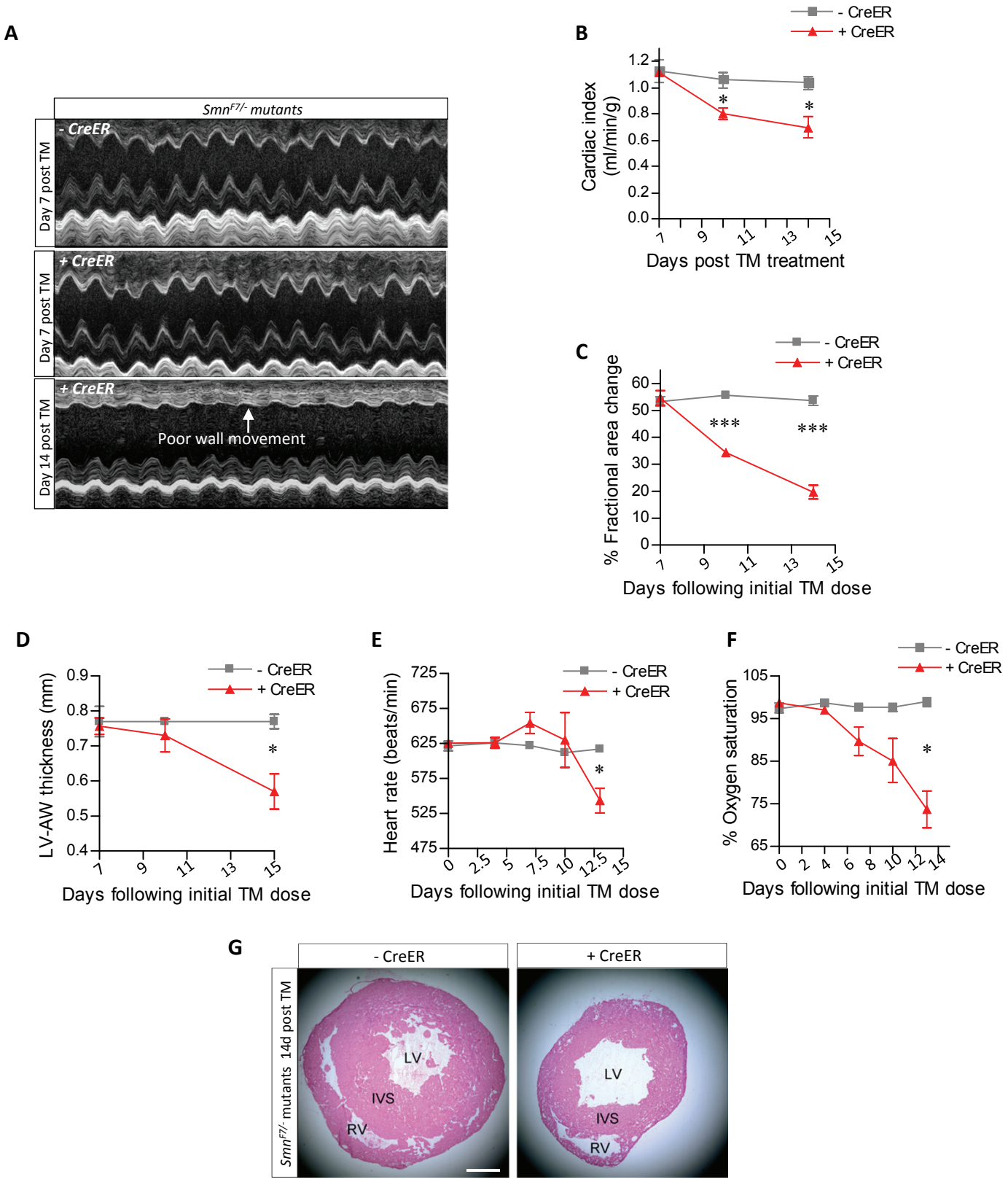
Supplemental Figure 4 – Disease mitigating effects of 2 copies of the SMN2 gene in mice depleted of the SMN protein as adults. Body weights of mutants with low SMN were equivalent to those of animals that continued to express normal levels of the protein. n ≥ 4 mice of each cohort, one-way ANOVA



Supplemental Figure 5 – Age-dependent emergence of NMJ defects in adult homozygous *SMN2* inducible mutants. **(A)** Immunostains of gastrocnemius muscle NMJs reveals normal synapse morphology 60 days following SMN depletion. However, occasional pathology in the form of poorly staining, denervated NMJs (asterisks) became evident in older (PND230) mutants. Scale bar: 25μm. **(B)** A lumbar motor neuron cell body from a treated *SMN2*$^{+/+}$*;CreER;Smn*$^{F7/-}$ mutant is illustrative of the striking loss of cytoplasmic and nuclear (arrow in control panel) SMN. Scale bar: 30μm.



Supplemental Figure 6 – Depletion of SMN in adult 2 *SMN2* copy mice does not affect cardiopulmonary function. Quantification of cardiac function parameters (A-E) in adult TM-treated *SMN2*$^{+/+}$;*Smn*$^{F7/-}$ mutants with or without *CreER* failed to provide evidence of pathology when SMN is depleted. **(A)** Heart rate, **(B)** Oxygen saturation, **(C)** Left ventricular anterior wall thickness, **(D)** Fractional area change, **(E)** Cardiac output. **(F)** Representative m-mode echocardiographs of an SMN depleted mouse and a control littermate depict similar movement of the ventricular walls confirming normal cardiac function despite SMN loss. Note n ≥ 3 mice of each genotype used for the echo analysis.

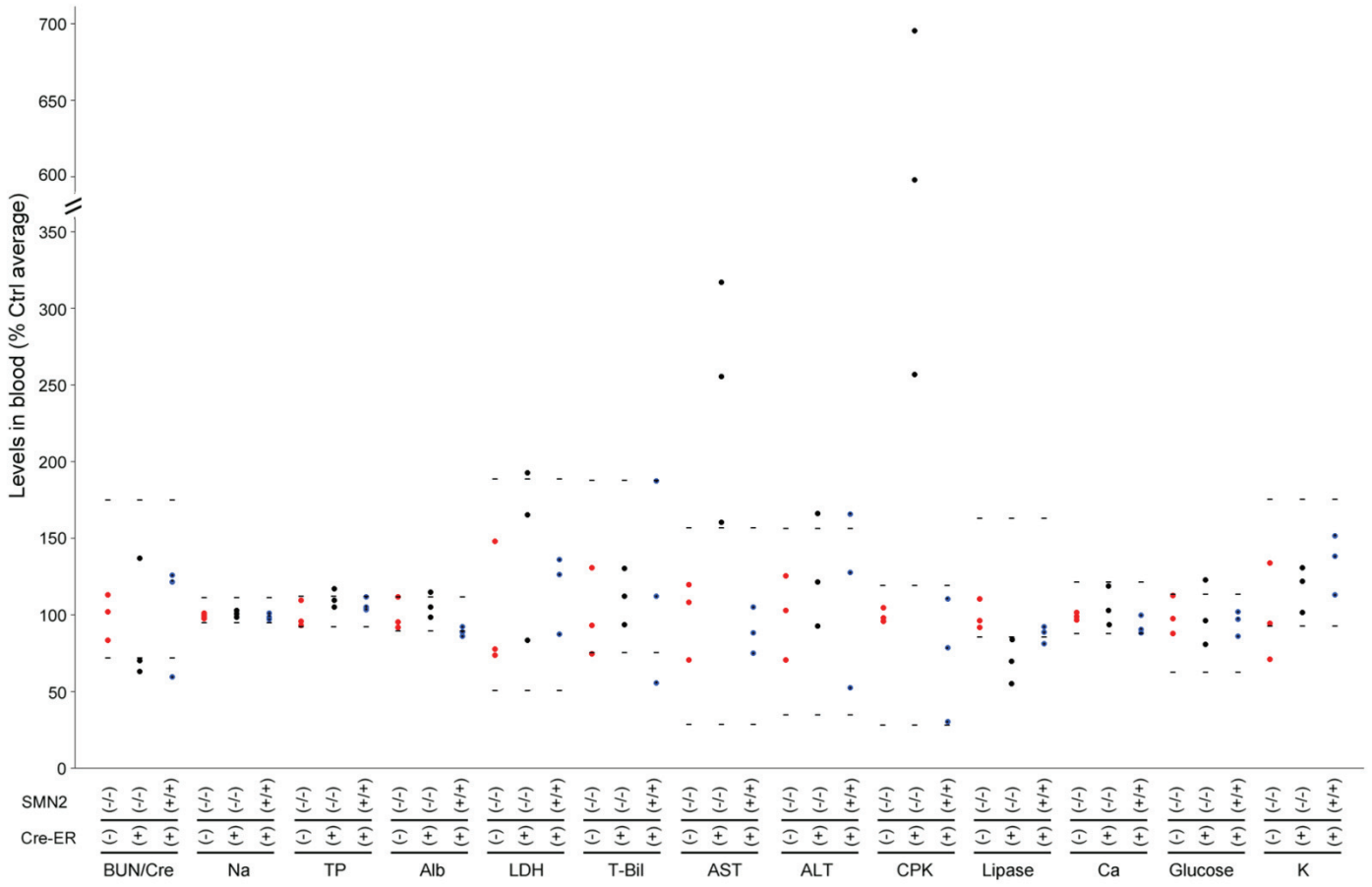


Supplemental Figure 7 – Cardiac dysfunction is a relatively late event following SMN loss. **(A)** Representative echocardiographs of *CreER;Smn*$^{F7/-}$ mutants is suggestive of normal heart function 7 but not 14 days post-TM induction (see poor wall movement). Quantification of **(B)** cardiac index, **(C)** fractional area change, **(D)** left ventricular anterior wall (LV-AW) thickness, **(E)** heart rate and, **(F)** oxygen saturation confirmed normal cardiac function 7 days following TM administration, when NMJ defects had already become apparent. Differences in the measurements became statistically significant 10 or more days after SMN depletion. *, ***, $P < 0.05$ and $P < 0.001$, *t* test, n ≥ 3 mice of each genotype. **(G)** H&E stains of transverse sections through the heart of treated *Smn*$^{F7/-}$ mice with or without *CreER* revealed dramatic thinning of the left ventricular wall and an enlarged inter-ventricular space. LV – left ventricle, IVS – inter-ventricular space, RV – right ventricle. Scale bar: 650μm.

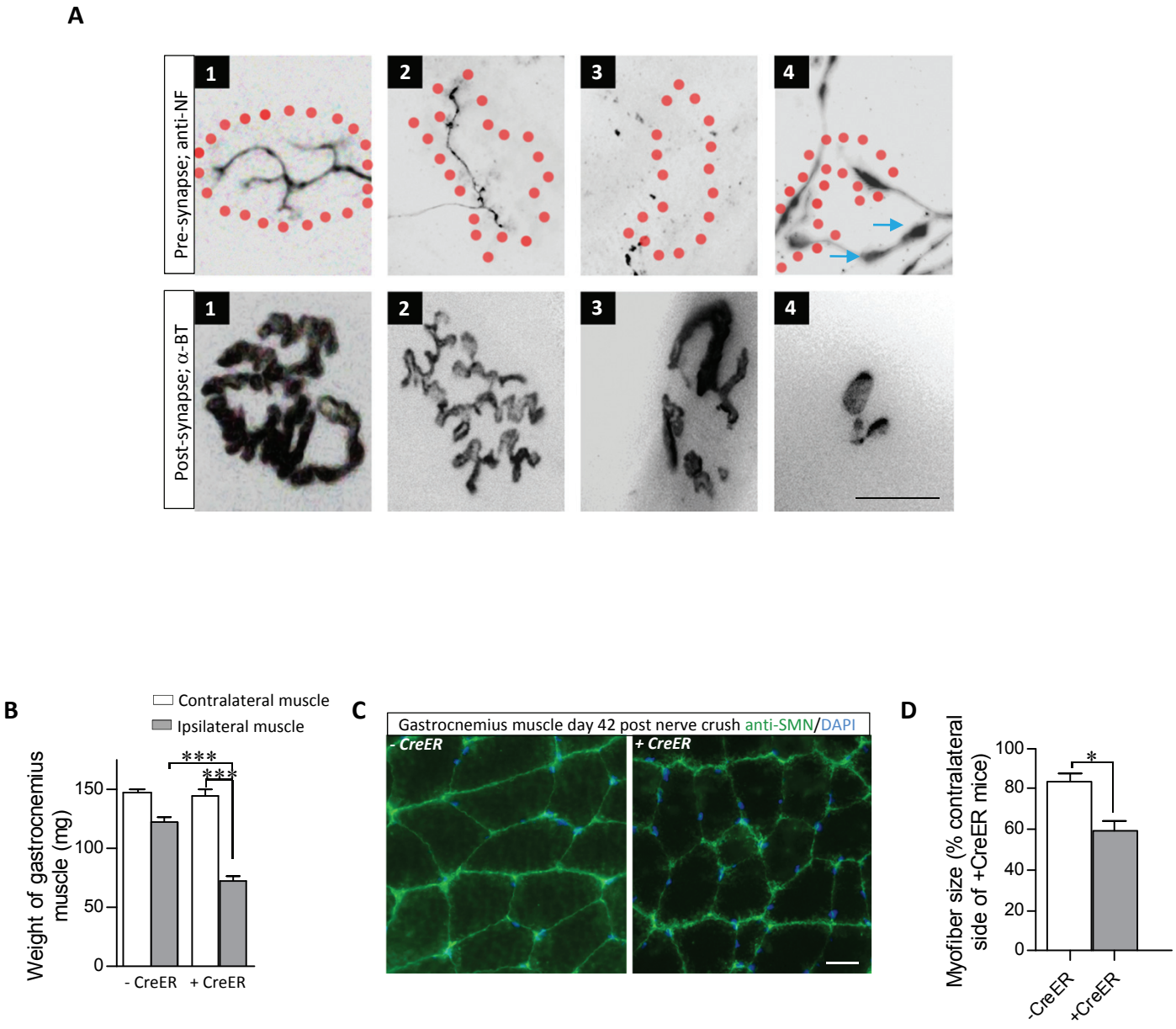
A

		Day 7								Day 120 <		
		CreER(-);SMN(F7/-);SMN2(-/-)			CreER(+);SMN(F7/-);SMN2(-/-)					CreER(+);SMN(F7/-);SMN2(+/+)		
	NR	1	2	3	1	2	3	4	5	1	2	3
RBC(x10^6/uL)	6.4-9.4	9.32	9.32	10.70	7.92	9.28	7.52	8.67	6.24	9.40	9.12	9.27
Hgb(g/dL)	11-15.1	12.0	12.4	13.8	10.0	12.4	10.0	10.7	8.1	12.8	12.8	12.0
Hct(%)	35.1-45.4	41.2	41.2	52.6	32.8	44.4	31.6	34.7	26.1	40.4	44.0	44.4
Ret%	0-5	1.00	1.11	1.50	1.09	2.62	1.29	0.42	1.23	0.56	1.73	2.36
WBC(x10^3/uL)	1.8-10.7	2.56	3.6	1.84	8.64	1.76	3.2	1.2	6.78	6.72	3.52	4.5
Proportion (%)												
Basophils	0-2	0	1.15	0.72	0.21	0	0	2.26	3.94	0.89	0	0
Eosinophils	0-3.9	0.35	3.58	1.13	0.38	6.80	0.55	1.65	5.08	1.88	0.25	0.91
Neutrophils	6.6-38.9	10.8	17.56	13.54	31.38	13.94	5.86	17.07	13.45	11.07	6.40	11.72
Lymphocytes	55.8-91.6	84.78	70.44	77.30	44.77	64.83	80.08	69.13	68.05	81.20	86.03	82.96
Monocytes	0-7.5	4.07	7.27	7.32	23.26	14.44	13.50	9.89	9.48	5.97	7.05	4.42
Platelets(x10^3/uL)	592-2972	1828	1720	2494	932	1308	1472	1065	1266	1508	1908	2106

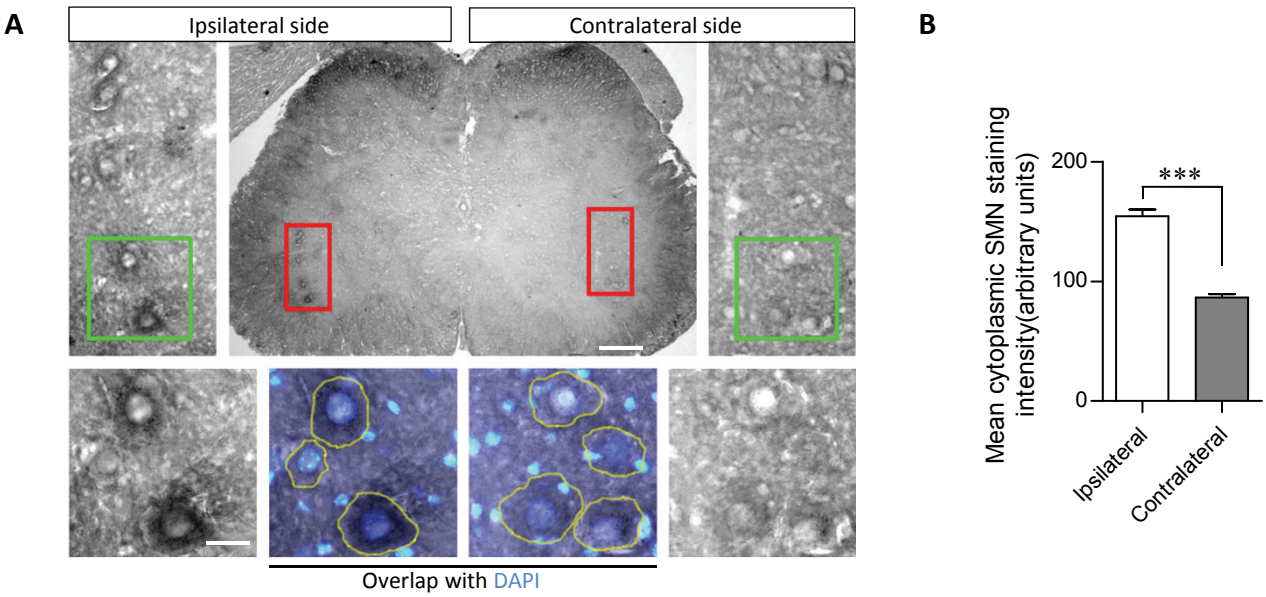
B



Supplemental Figure 8 – Blood chemistry of SMN depleted *CreER;Smn$^{F7/-}$* mutants is suggestive of normal organ function in the presence of 2 *SMN2* copies. Quantification of **(A)** circulating blood cells and **(B)** serum proteins in SMN depleted *CreER;Smn$^{F7/-}$* mutants with (2 copies) or without the *SMN2* gene.



Supplemental Figure 9 – Recovery of NMJ form and function is impaired in the absence of sufficient SMN. **(A)** Representative images of the four classes of NMJs (pre- and post-synapses) used to define and assess recovery in mice with or without sufficient SMN following nerve crush. Post-synaptic specialization classification: 1 – fully mature, normally staining AChR clusters; 2 – fragmented, poorly staining AChR clusters; 3 – partially fragmented but normally staining AChR clusters; 4 – disassembled, immature, plaque-like AChR clusters. Pre-synaptic specialization classification: 1 – elaborately branched nerve terminals fully apposed with AChR clusters; 2 – poorly branched terminals partially overlapping AChR clusters; 3 – complete absence of nerve terminals at NMJs, 4 – poorly branched terminals with numerous neurofilament filled varicosities (arrows). Scale bar: 25μm. Incomplete recovery of function in the ipsilateral (nerve crushed) muscle of SMN-depleted mutants as assessed by **(B)** weight of the gastrocnemius and **(C,D)** atrophied fibers. Scale bar: 25μm. Note; n ≥ 150 myofibers and n=3 mice of each genotype for the analysis in (B) and (D). ***, $P < 0.001$, one-way ANOVA and *, $P < 0.05$, *t* test respectively for the statistics in the two graphs.



Supplemental Figure 10 – NMJ maturation induces a transcriptional increase in SMN. **(A)** In situ hybridization on mouse spinal cord 30 days following sciatic nerve crush depicts and increase in signal in ipsilateral motor neurons. Scale bars: 200µm; 30µm (inset). **(B)** Quantification of signal indicates a significant increase in these motor neurons relative to contralateral ones. ***, $P < 0.001$, t test, n=96 motor neurons from 3 mice of each genotype.