

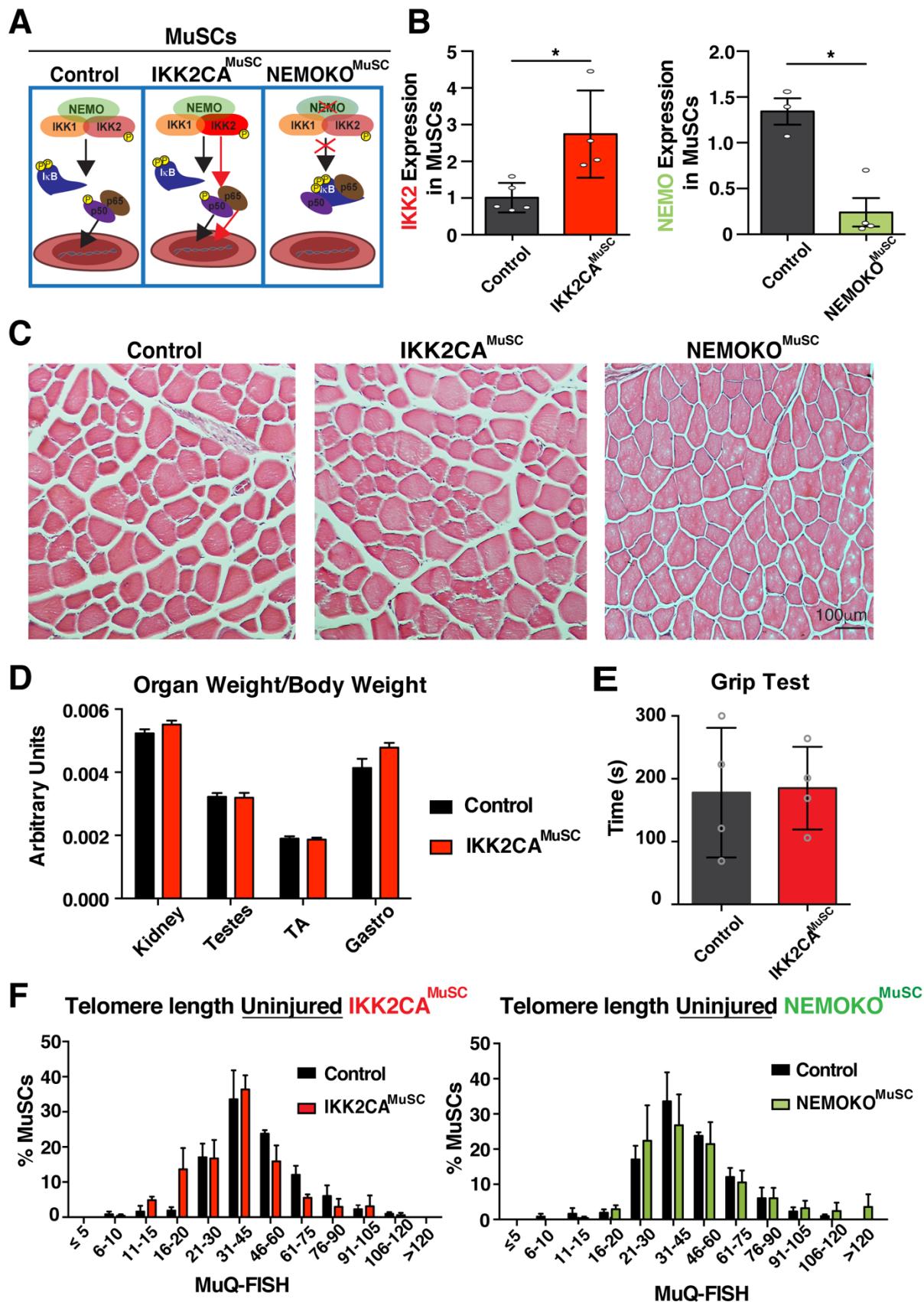
**Supplemental information**

**Persistent NF-κB activation  
in muscle stem cells induces  
proliferation-independent telomere shortening**

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SUPPLEMENTAL DATA

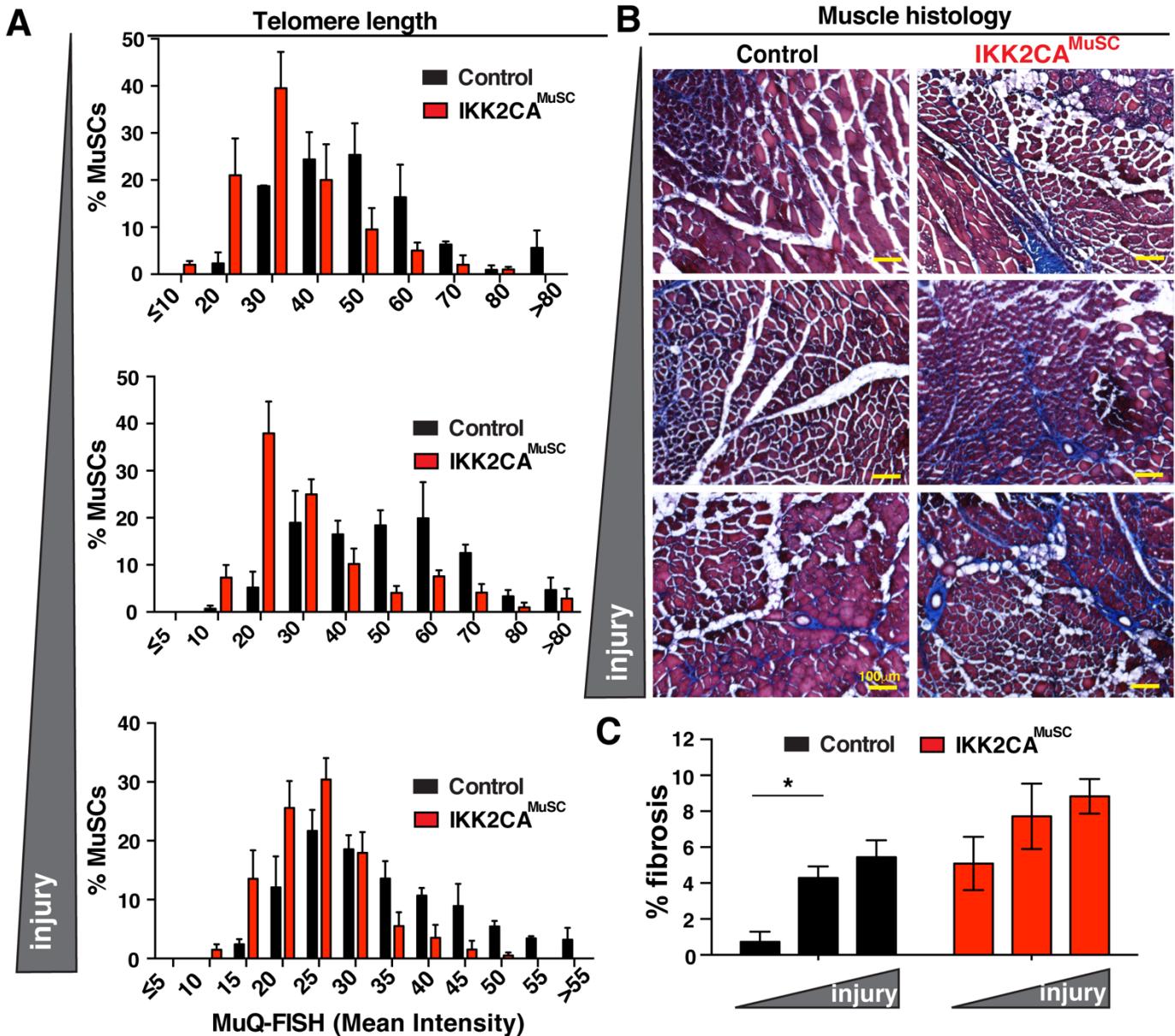
**Figure S1**



**Figure S1. Uninjured IKK2CA<sup>MuSC</sup> or NEMOKO<sup>MuSC</sup> mice display no defects in muscle metrics in steady-state conditions. Related to Figure 2.**

**(A)** Cartoon of the effect of NF-κB signaling in IKK2CA<sup>MuSC</sup> and NEMOKO<sup>MuSC</sup> mice. **(B)** Quantitative Real-Time PCR of IKK2 gene in IKK2CA<sup>MuSC</sup> MuSCs (red) and IKBKG (NEMO, green) expression in NEMOKO<sup>MuSC</sup> MuSCs, following tamoxifen administration shows significant upregulation or downregulation, respectively. **(C)** Hematoxylin and Eosin staining of uninjured control, IKK2CA<sup>MuSC</sup> and NEMOKO<sup>MuSC</sup> Tibialis Anterior muscles shows normal morphology. n=3 mice/group **(D)** Similar organ weights of control and IKK2CA<sup>MuSC</sup> mice, post-tamoxifen treatment. **(E)** 2-month-old mice were subjected to hanging grip test and their performance was measured as the time they were able to hold onto the grid until falling. As shown, uninjured IKK2CA<sup>MuSC</sup> mice had no difference to control mice in this test (n=4 per genotype). **(F)** MuQ-FISH telomere length histogram from uninjured Control and IKK2CA<sup>MuSC</sup> MuSCs (left) and Control and NEMOKO<sup>MuSC</sup> MuSCs. Note the lack of appreciable telomere shortening from either model in uninjured conditions. n≥3 mice per genotype per condition. Results are shown as mean ± SEM; Statistical analyses in **(B)**, **(D)** and **(E)** were performed using unpaired student t-test with Welch's correction, \*p≤0.05.

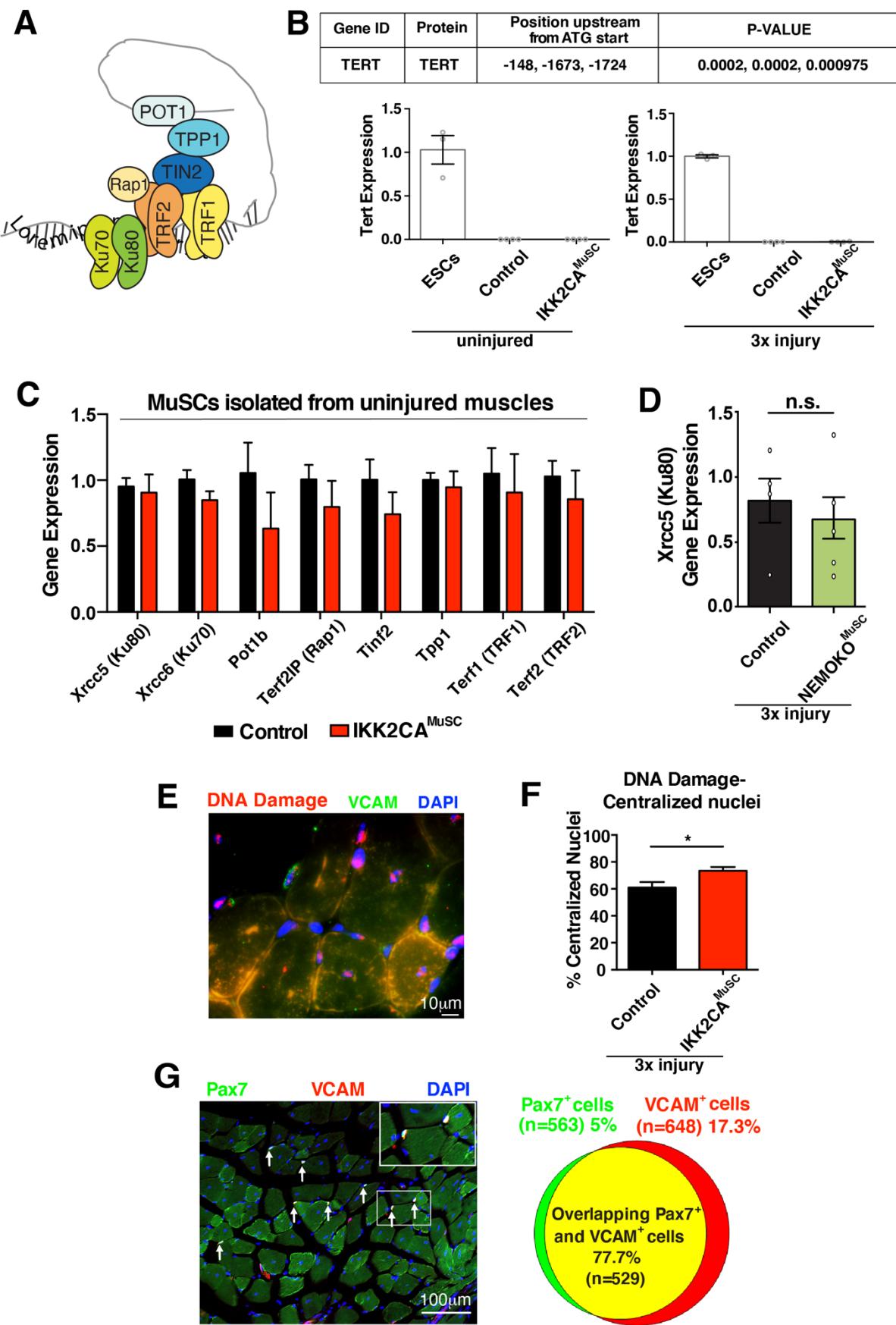
## Figure S2



**Figure S2.** Telomere length and fibrotic analysis of IKK2CA<sup>MuSC</sup> muscles compared to controls with increased number of injuries. Related to Figure 2.

(A) Distribution of telomere lengths (mean intensity) of MuSCs following 3x, (D), 6x (E) or 20x (F) injuries. Telomeres start to shorten after 3 injuries in IKK2CA<sup>MuSC</sup> cells (red bars) but this reduction becomes more severe with increasing number of injuries. n=3 mice/condition. N>100 cells. (B) Representative Trichrome staining of Control and IKK2CA<sup>MuSC</sup> muscles after 3x, (A), 6x (B) or 14x (C) injuries. Scale bar: 100μm. (C) Quantification analysis of fibrotic tissue. n=3-4 mice per genotype per condition. Results are shown as mean ± SEM; Statistical analysis was performed using two-way ANOVA with multiple comparisons, \*p≤0.05.

## Figure S3



**Figure S3. Gene regulation and effects of MuSC-specific NF-κB activation. Related to Figure 4.**

**(A)** Schematic of resident telomeric proteins binding on (capping) telomeric DNA. **(B)** Top: TERT κB-binding sites. Bottom: qRT-PCR of *Tert* expression. Since *Tert* transcripts are detectable in ESCs within the same reaction (technical control), TERT is either present at very low (undetectable) levels or expressed transiently in MuSCs. **(C)** qRT-PCR of telomeric genes. Note that NF-κB activation without injury has no effect on Ku80 levels. n=4-7 mice per genotype. **(D)** Unchanged Ku80 levels in injured NEMOKO<sup>MuSC</sup> MuSCs. n=4-5 mice per genotype. **(E)** Representative muscle section stained for DNA damage (53BP1, red), muscle stem cell marker (VCAM, green) and DAPI (blue). **(F)** Quantification of centralized nuclei shows higher DNA damage. n=3-4 mice per genotype. **(G)** Left: Pax7EGFP muscle with Pax7 cells (EGFP, green) stained for VCAM (red) and DAPI (blue). Right: overlap between Pax7 and VCAM validate VCAM as a reliable stem cell marker. Results are shown as mean ± SEM.

## Figure S4

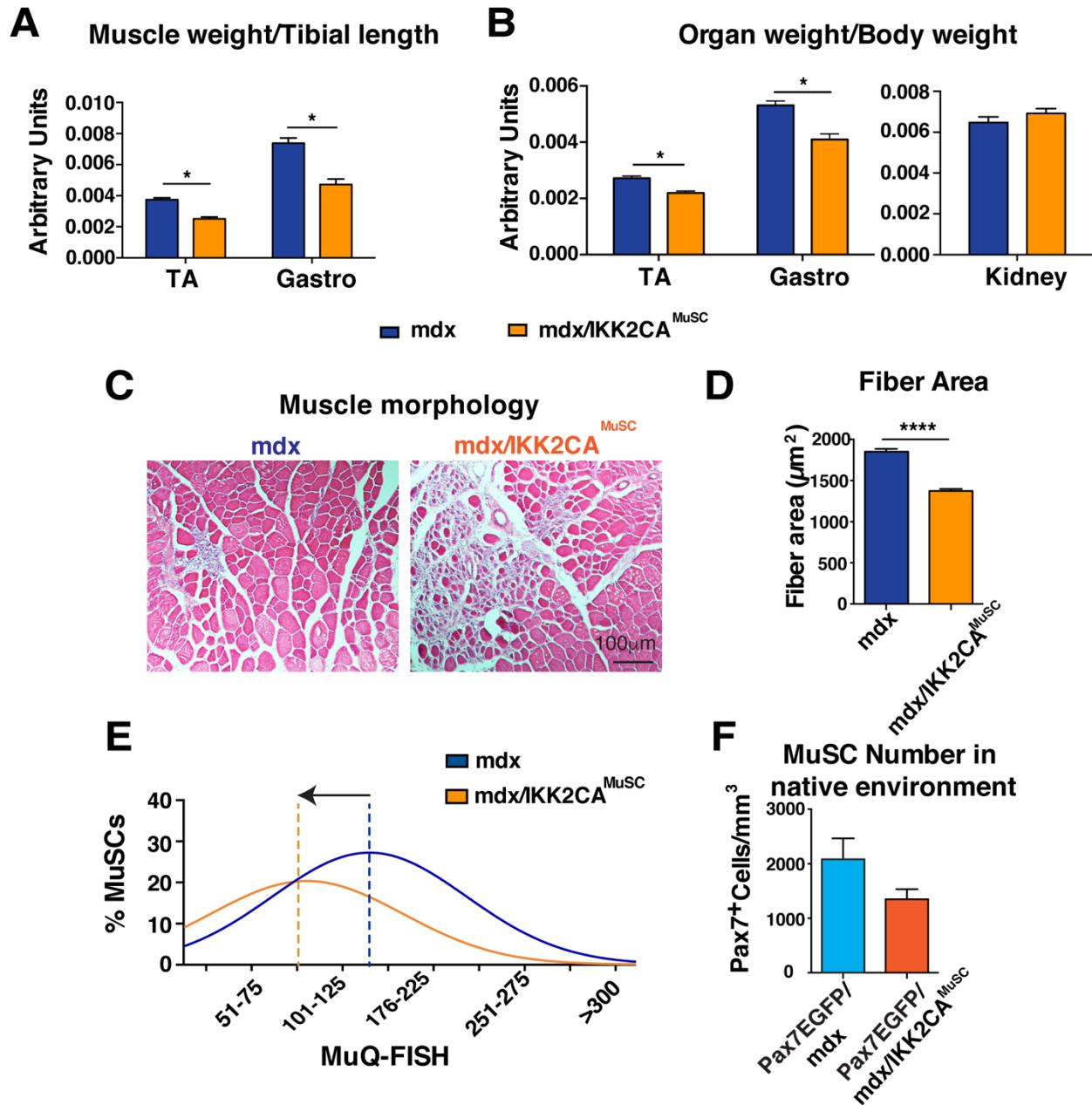


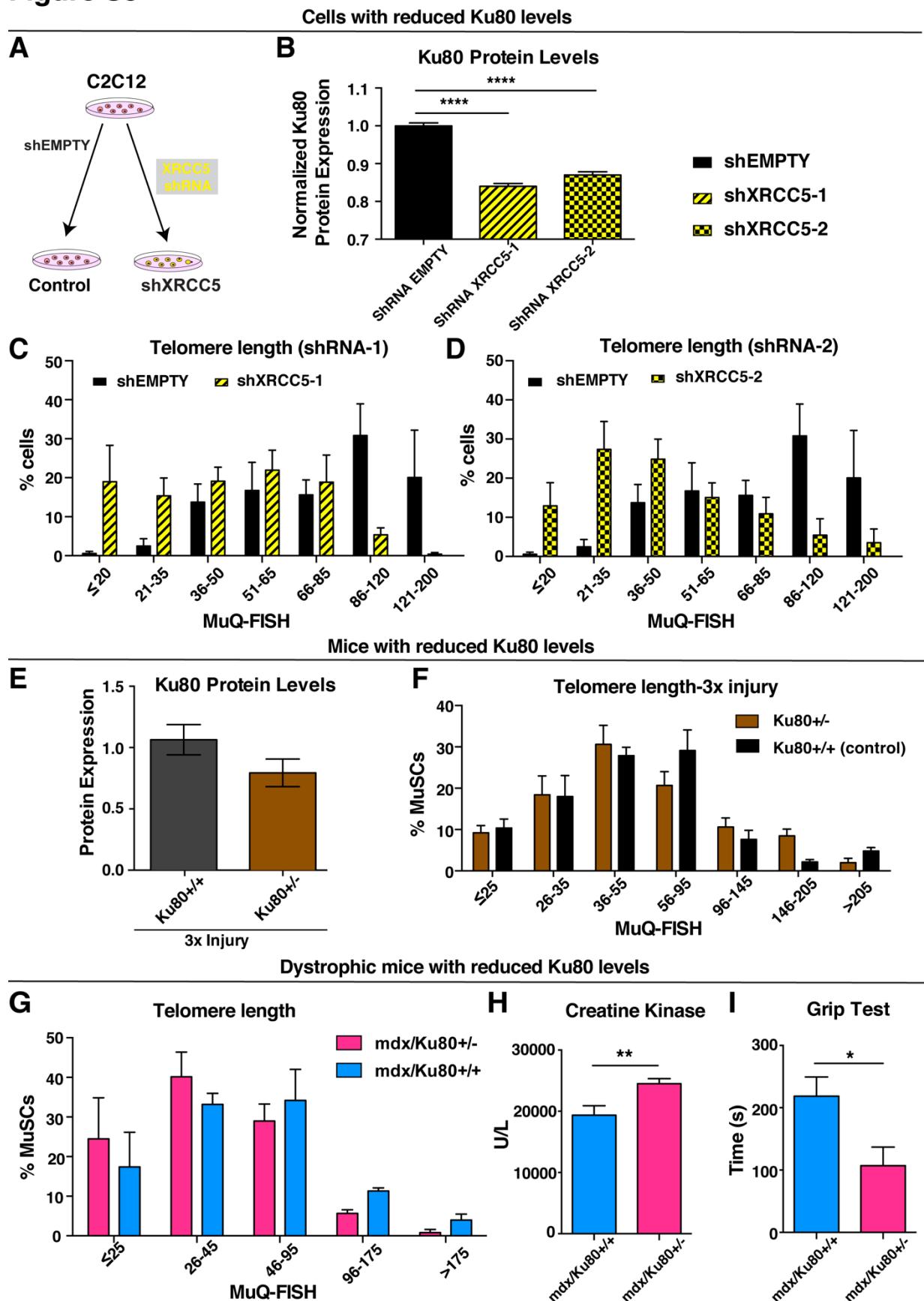
Figure S4. Activation of NF- $\kappa$ B in MuSCs deteriorates the dystrophic phenotype. Related to Figure 5.

(A) Reduced muscle weight/tibial length of skeletal muscles in mdx/IKK2CA<sup>MuSC</sup> mice compared to mdx mice. (B) smaller muscles in mdx/IKK2CA<sup>MuSC</sup> mice, while other organs, such as kidney have similar weights. (C) Worsened histology in mdx/IKK2CA<sup>MuSC</sup> compared to mdx muscles. Scale bar:100 $\mu\text{m}$ . (D) average muscle diameter is significantly decreased in mdx/IKK2CA<sup>MuSC</sup> mice, as shown by the smaller area of centralized nuclei fibers. n $\geq$ 4 mice per genotype. (E) Alternative representation of telomere length (MuQ-FISH) by Gaussian curves shows a distribution shift towards shorter telomeres in mdx/IKK2CA<sup>MuSC</sup> compared to mdx cells.. n=3-4 mice per genotype. n>70 cells per condition. (F) fewer MuSCs in

$\text{mdx}/\text{IKK2CA}^{\text{MuSC}}$  compared to  $\text{mdx}$  mice, suggesting stem cell exhaustion over time.  $n=3$  mice per genotype.

mean  $\pm$  SEM; Statistical analyses were performed unpaired student t-test with Welch's correction, \* $p \leq 0.05$ ; \*\*\*  $p < 0.001$ .

**Figure S5**

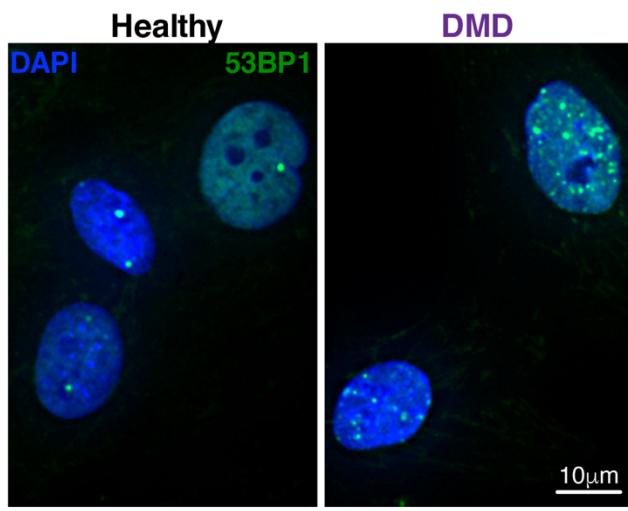


**Figure S5. Downregulation of Ku80 by itself or in the context of dystrophy reduces telomere length. Related to Figure 5.**

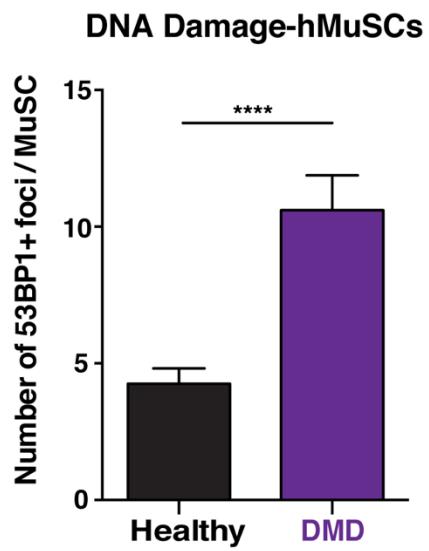
**(A)** Scheme of C2C12 cells transfected with either empty shRNA backbone vector or with two different shRNAs (shXRCC5-1 and shXRCC5-2) for the *xrcc5* gene (encodes for Ku80 protein). **(B)** Reduced Ku80 protein levels in C2C12 cells transfected with two different shRNAs compared to control (sh-empty) transfected cells. n=3 wells/condition, n≥35 cells per well. mean ± SEM; Statistical analyses were performed unpaired student t-test with Welch's correction\*\*\*\* p<0.0001. **(C)** Telomere length assessment by MuQ-FISH of C2C12 cells with the shXRCC5-1 shRNA. n=3 wells/condition, n≥35 cells per well. **(D)** Telomere length assessment by MuQ-FISH of C2C12 cells with the shXRCC5-2 shRNA. n=3 wells/condition, n≥35 cells per well. **(E)** 20% reduction of Ku80 protein levels in Ku80<sup>+/−</sup> mice after 3x injury **(F)** Shift towards smaller telomere lengths in MuSCs isolated from Ku80<sup>+/−</sup> mice after 3x injury. For (E) and (F): n=3-4 mice per genotype per condition. n>40 cells per mouse analyzed. **(G)** To explore whether Ku80 downregulation affects the dystrophic phenotype, mdx mice were bred to Ku80<sup>+/−</sup> mice. Telomere length assessment by MuQ-FISH in MuSCs shows reduced telomere length in mdx/Ku80<sup>+/−</sup> mice compared to controls (mdx/Ku80<sup>+/+</sup>). n=3-4 mice per genotype. n>45 cells per condition. **(H)** mdx/Ku80<sup>+/−</sup> mice exhibited higher serum creatine kinase activity, indicative of skeletal muscle damage. n≥4 mice per genotype. **(I)** mdx/Ku80<sup>+/−</sup> mice are significantly impaired in the grip test. n=4-5 mice per genotype. Mean ± SEM; All statistical analyses were performed using unpaired student t-test with Welch's correction. \*p≤0.05; \*\*p≤0.01.

## Figure S6

**A**



**B**



**Figure S6. Increased DNA damage in human diseased MuSCs. Related to Figure 6.**

(A) Representative images of human MuSCs isolated from Healthy (left) and DMD samples (right) were stained for the DNA damage marker 53BP1 (green) and nuclei (DAPI, blue). (B) Quantification analysis shows that human DMD-diseased MuSCs exhibit higher levels of DNA damage compared to healthy MuSCs. n=3 human samples per conditions. n>35 cells per sample. mean ± SEM; Statistical analysis in (B) was performed unpaired student t-test with Welch's correction, \*\*\* p<0.0001.