

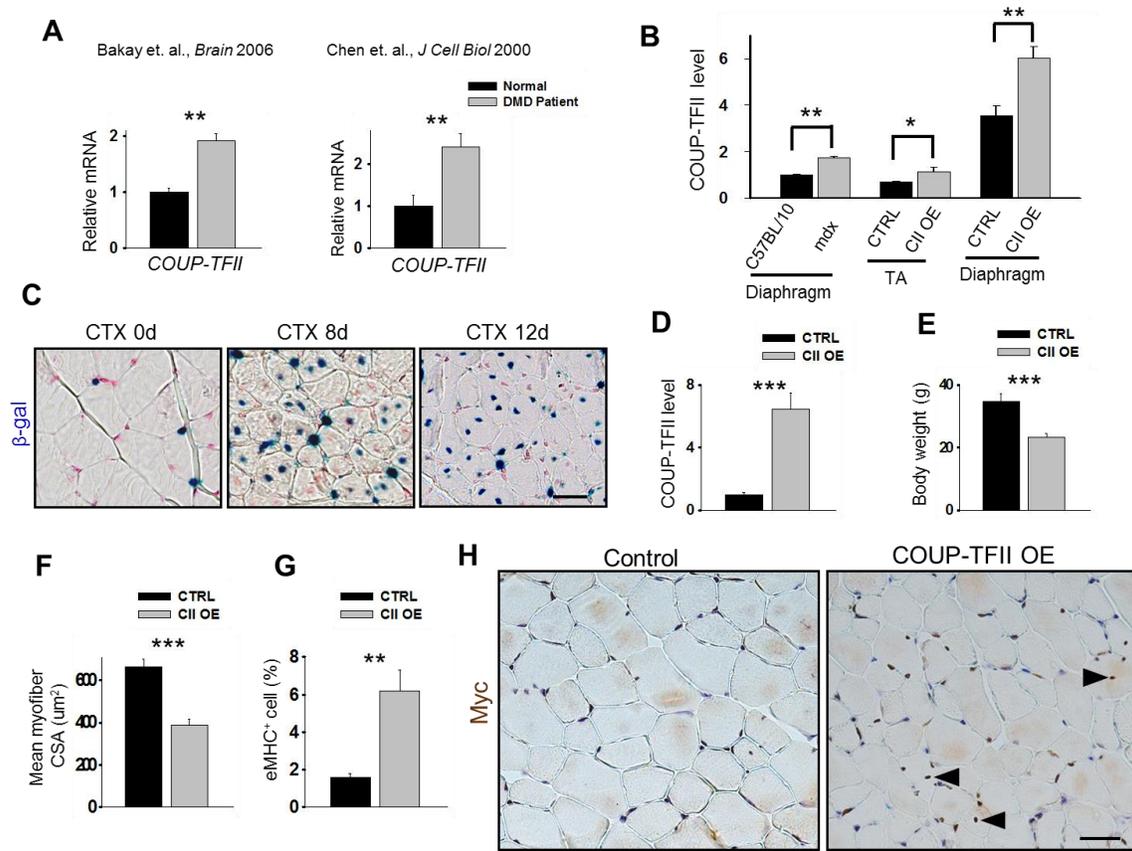
## **COUP-TFII regulates satellite cell function and muscular dystrophy**

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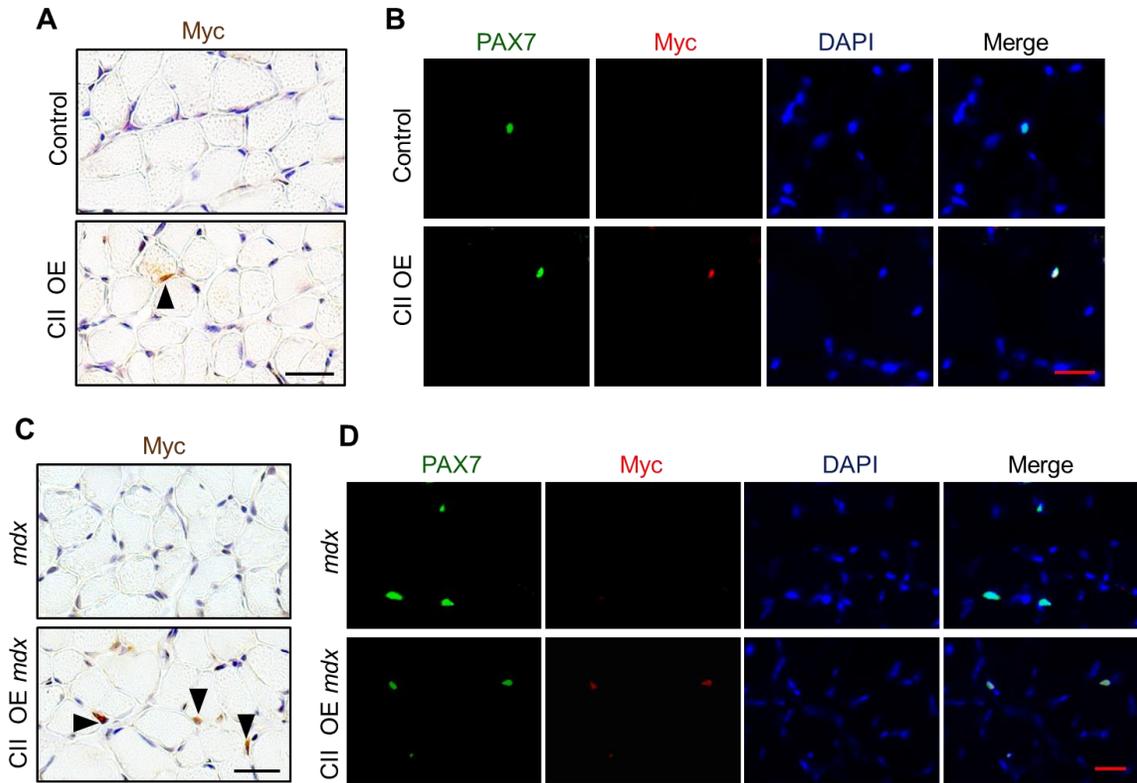
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**SUPPLEMENTAL INFORMATION:**

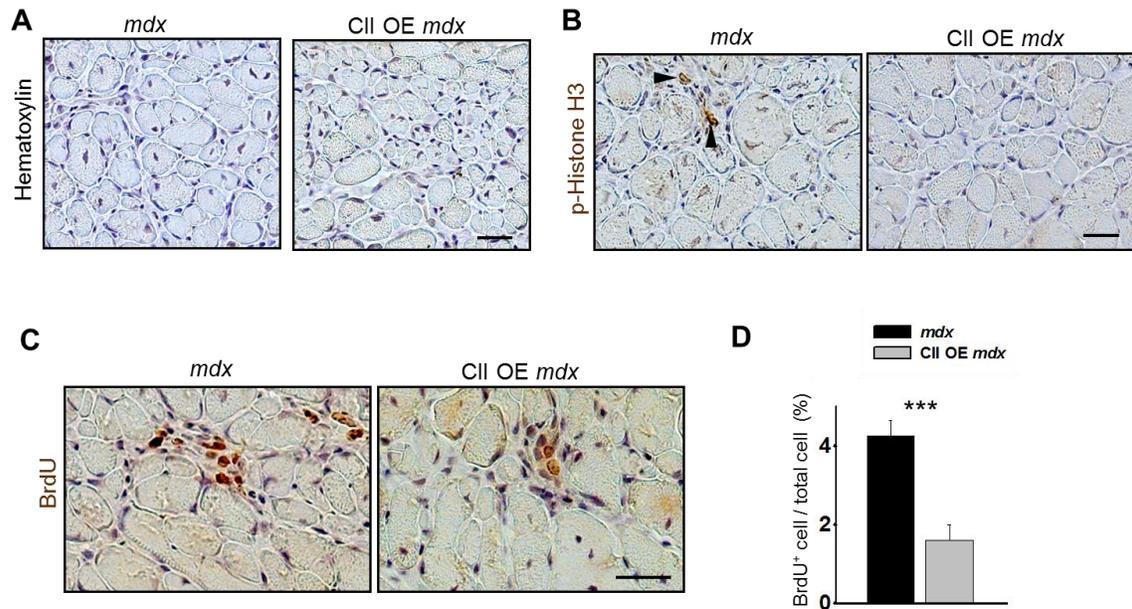
**SUPPLEMENTAL FIGURES.**



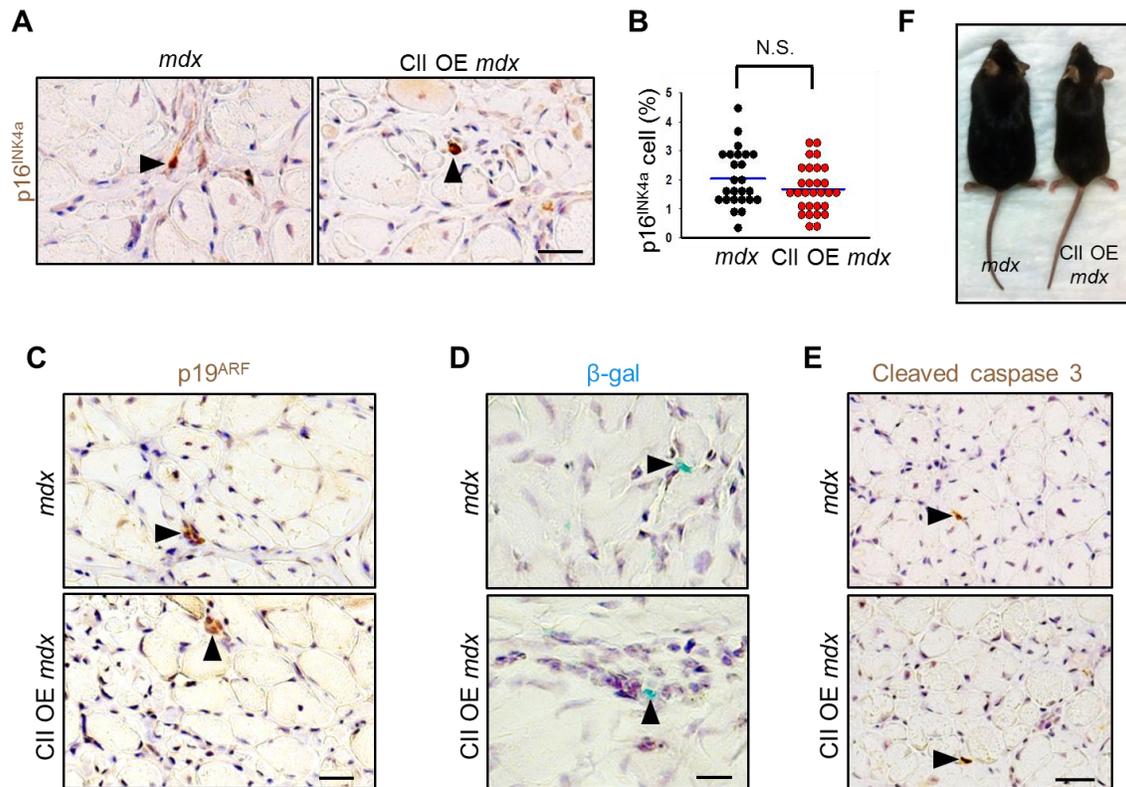
**Supplemental Figure 1.** COUP-TFII expression in satellite cells and characterization of COUP-TFII-expressing mice. (A) COUP-TFII expression in DMD patients by querying public dataset. (B) COUP-TFII level in 1-month-old *mdx* and its control C57BL/10ScSnJ mice ( $n=6$ ), as well as 2-month-old control and COUP-TFII transgenic animals ( $n=6$ ). (C) Representative  $\beta$ -gal staining of TA muscles collected on day 0, 8, and 12 after injury. Images are representative of three different animals at each time point. (D) Freshly FACS-isolated SCs from control and COUP-TFII OE mice were examined for COUP-TFII levels one week after tamoxifen injection. (E) Body weight of control ( $n=5$ ) and COUP-TFII OE mice ( $n=8$ ) at 20 months of age. (F) Mean fiber CSA of soleus muscles in control ( $n=6$ ) and COUP-TFII OE mice ( $n=5$ ). (G) Percentage of eMHC<sup>+</sup> cells in soleus muscles in control ( $n=6$ ) and COUP-TFII OE mice ( $n=5$ ). (H) Representative Myc photomicrograph of soleus muscles from 5 animals of each genotype. Arrowheads indicate the central-nucleated myc positive fibers. \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$  by Student's *t*-test. Data represent mean  $\pm$  SEM. Scale bars: 25  $\mu\text{m}$  (C,H).



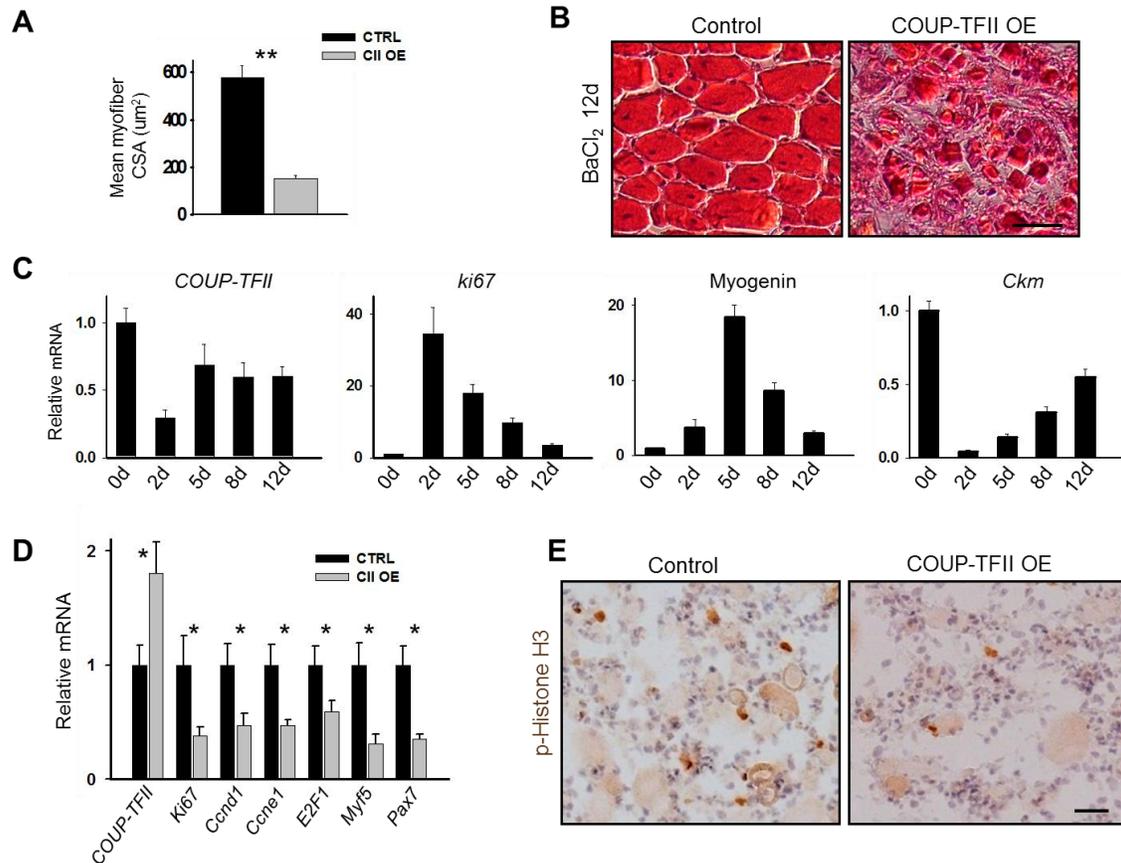
**Supplemental Figure 2.** Satellite cell specific COUP-TFII overexpression in COUP-TFII OE and COUP-TFII OE *mdx* animals. (A,B) Myc and PAX7 staining indicates myc-tagged COUP-TFII-expressing SCs in diaphragm muscles in COUP-TFII OE mice one week after tamoxifen treatment. (C,D) Representative images of Myc and PAX7 stained diaphragm in *mdx* and COUP-TFII OE *mdx* mice. Arrowhead indicates myc positive cells (A,C). Data are representative of three independent experiments. Scare bars: 25 μm (A,B,C,D).



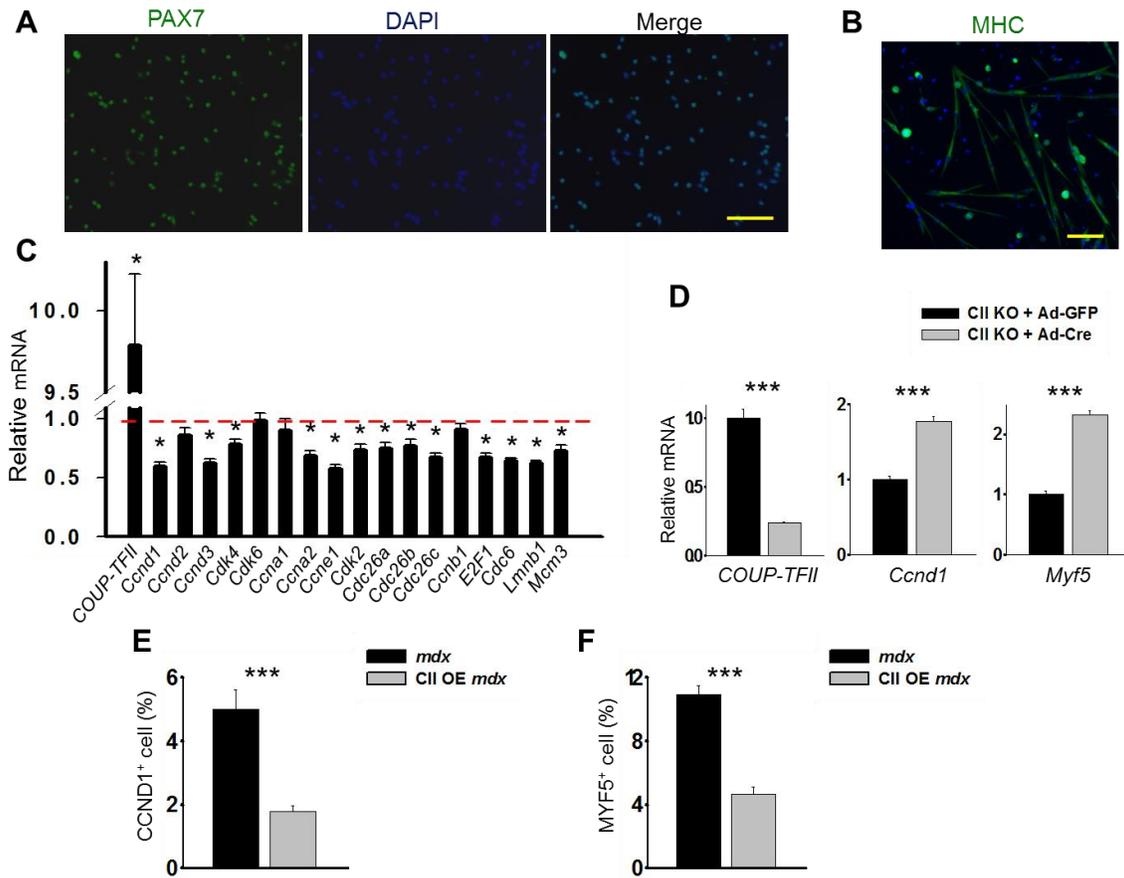
**Supplemental Figure 3.** Characterization of *mdx* and COUP-TFII OE *mdx* mice. (A) Hematoxylin-stained diaphragm muscles indicate centrally positioned myonuclei. (B-D) The diaphragm muscles were stained with p-Histone H3 and BrdU antibodies. Quantification of the number of BrdU<sup>+</sup> cell per 100 nuclei in *mdx* and COUP-TFII OE *mdx* mice ( $n=6$ ). Arrowheads indicate the cycling SCs with p-Histone H3 signal. Scare bars: 25  $\mu$ m (A,B,C). \*\*\* $P<0.001$  by Student's  $t$ -test. Results are the mean  $\pm$  SEM.



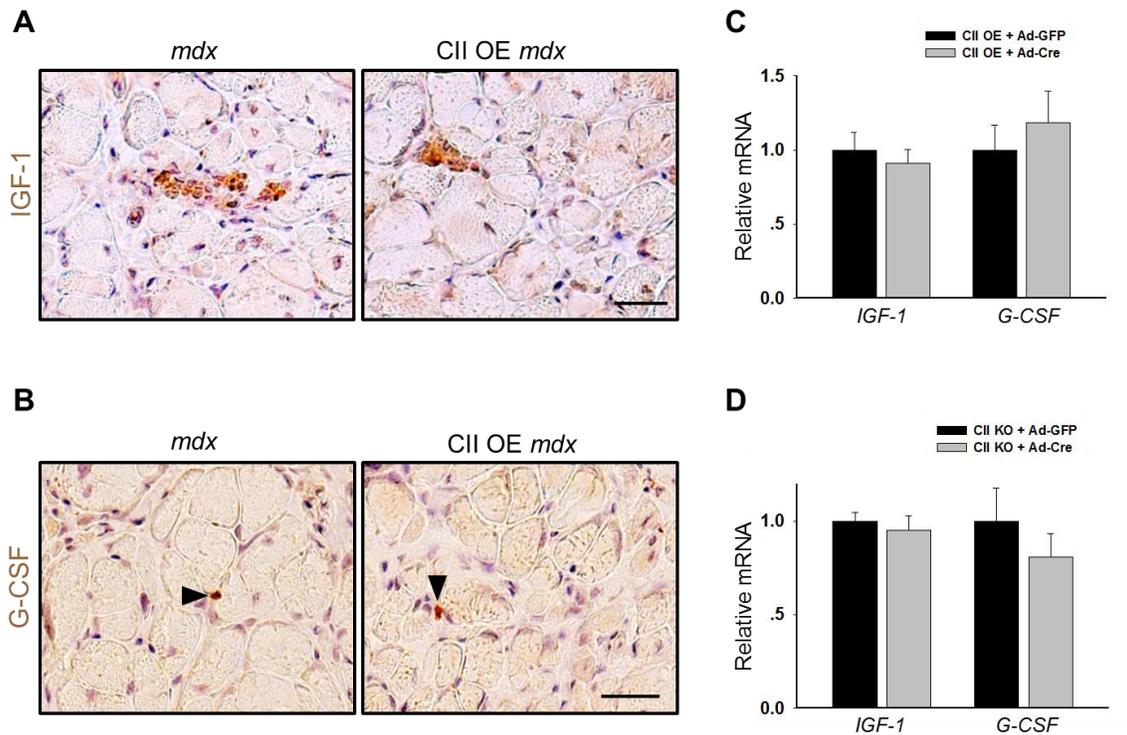
**Supplemental Figure 4.** Examination of cellular senescence and apoptosis in COUP-TFII OE *mdx* animals. (A,B) Representative photomicrograph (A) and percentage (B) of P16<sup>INK4a</sup> positive cells in *mdx* ( $n=25$  images from 5 animals) and COUP-TFII OE *mdx* ( $n=29$  images from 6 animals) mice. (C-E) Diaphragm muscles from 4-month-old *mdx* and COUP-TFII OE *mdx* animals were assayed for p19<sup>ARF</sup> (C), SA-β-gal activity (D) and cleaved caspase 3 (E). Images are representative of four animals for indicated genotypes. (F) Gross appearance of *mdx* and COUP-TFII OE *mdx* mice at 8 months of age. Arrowhead indicated immunoreactive signals in A,C,D,E. Scale bars: 25 μm (A,C,D,E). Statistical significance was determined by Student's *t*-test



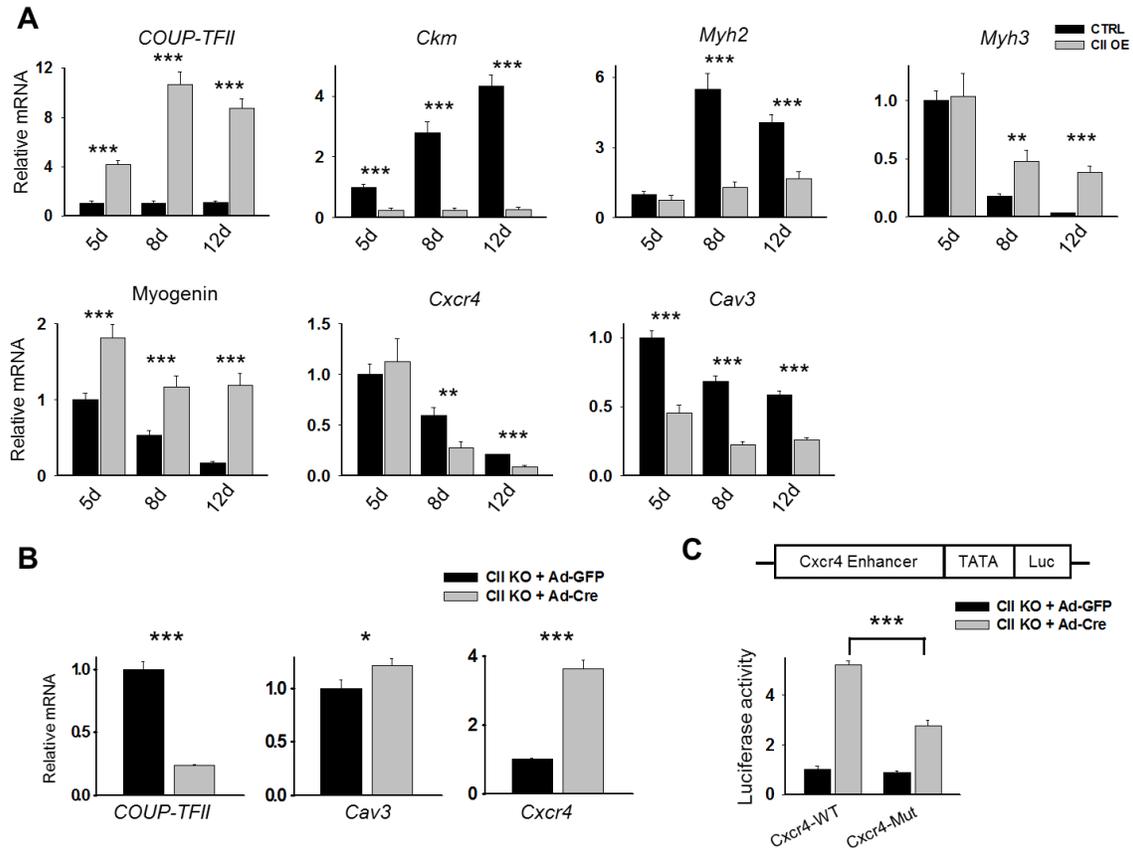
**Supplemental Figure 5.** Defective regenerative response in COUP-TFII overexpression mice following transient injury. (A) Quantification of myofiber size as evaluated by cross-sectional area in control ( $n=6$ ) and COUP-TFII OE mice ( $n=6$ ) on day 12 post-injury. (B) H&E staining of TA muscles 12 days after BaCl<sub>2</sub> injection. Results are representative of 3 independent experiments. (C) Real-time PCR shows the expression of *COUP-TFII*, *ki67*, myogenin and *ckm* in regenerating skeletal myocytes ( $n=6$ ). (D) Transcripts of denoted genes in TA muscles from control ( $n=6$ ) and COUP-TFII OE ( $n=8$ ) mice on day 2 after CTX injury. (E) p-Histone H3 stained proliferating SCs in control and COUP-TFII OE mice 2 days after damage. Scale bars: 25 µm (B,E). \* $P<0.05$ , \*\* $P<0.01$  by Student's *t*-test. Results are the mean  $\pm$  SEM.



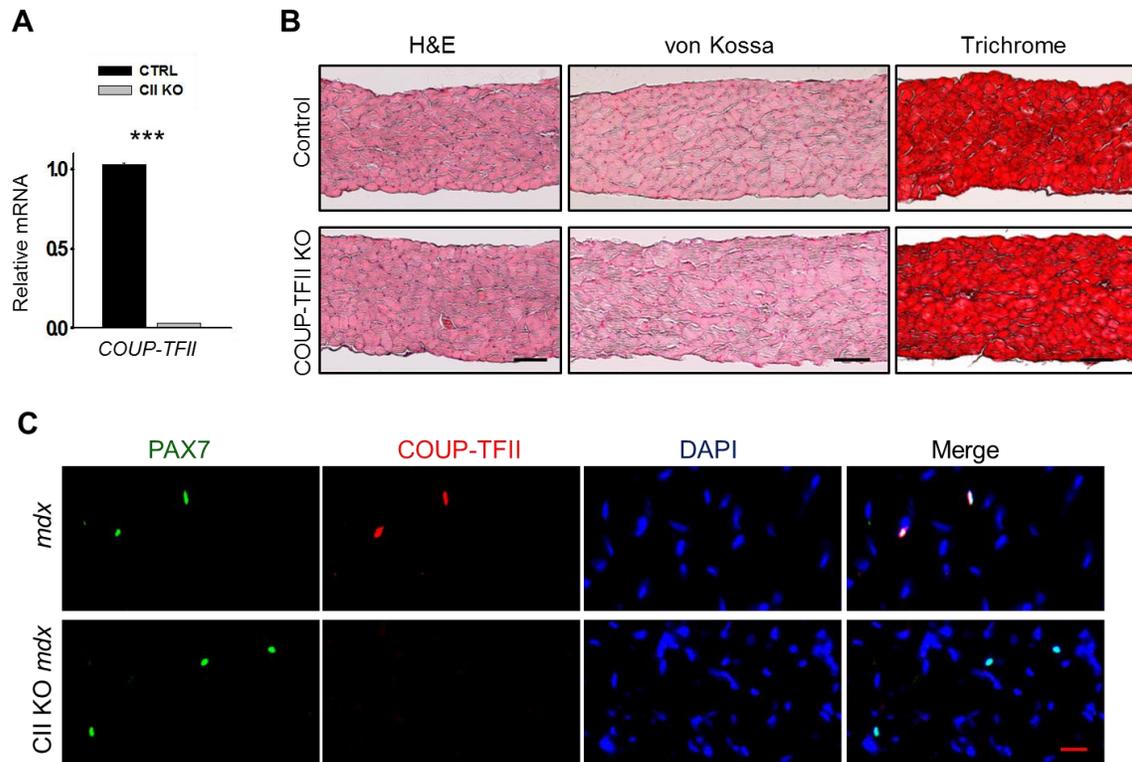
**Supplemental Figure 6.** COUP-TFII overexpression inhibits satellite cell proliferation. (A) Immunofluorescence staining of PAX7 in FACS-sorted satellite cells. (B) Differentiation of isolated satellite cells shown by MHC staining 5 days after induction. (C) The mRNA levels of cell cycle-associated genes in COUP-TFII OE myoblasts 3 days after COUP-TFII induction. Results are representative of 3 independent experiments. (D) COUP-TFII represses *Ccnd1* and *Myf5* transcription in primary myoblasts. (E) Quantification of CCND1<sup>+</sup> cell evaluated as a fraction of CCND1<sup>+</sup> cells in total nuclei in *mdx* ( $n=10$ ) and COUP-TFII OE *mdx* ( $n=8$ ) mice. (F) Measurement of MYF5<sup>+</sup> cell in *mdx* ( $n=10$ ) and COUP-TFII OE *mdx* ( $n=9$ ) mice. Scale bars: 50  $\mu$ m (A,B). \* $P<0.05$ , \*\*\* $P<0.001$  by Student's *t*-test. Data are presented as mean  $\pm$  SEM.



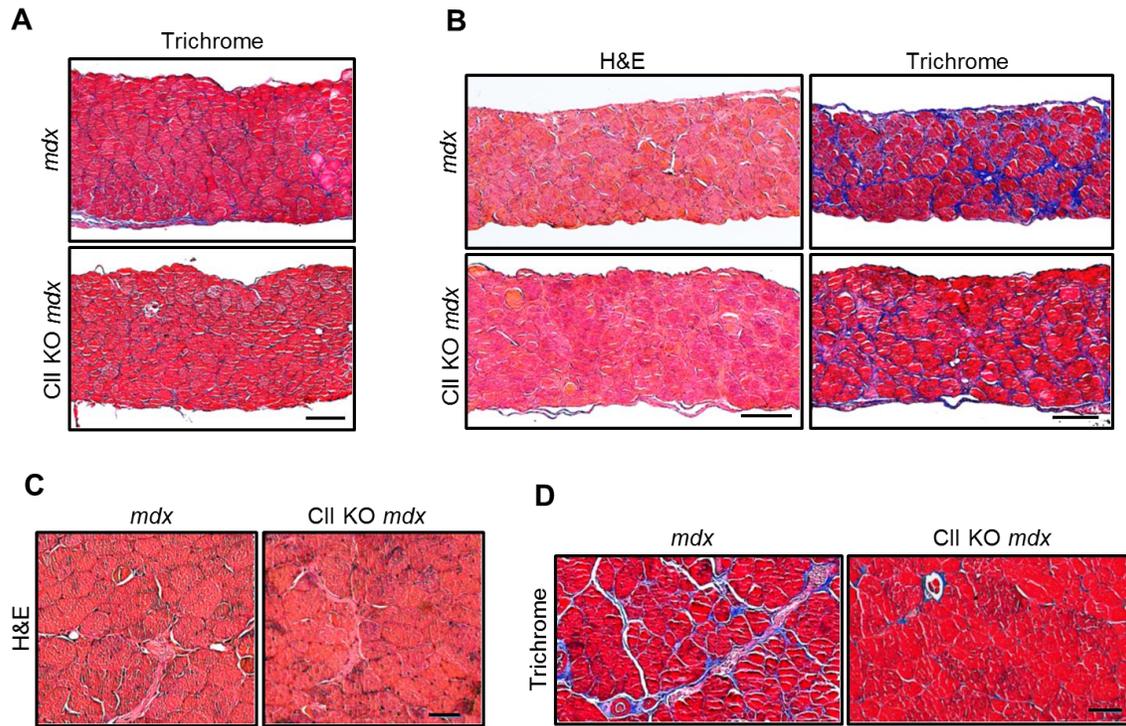
**Supplemental Figure 7.** IGF-1 and G-CSF expression in COUP-TFII OE *mdx* mice. (A,B) Representative images of IGF-1 (A) and G-CSF (B) staining of 4-month-old diaphragm from *mdx* and COUP-TFII OE *mdx* mice. (C,D) Expression of *IGF-1* and *G-CSF* genes in cultured COUP-TFII over- (C) and under-expression (D) myoblasts 3 days after virus infection. Results are representative of 3 independent experiments. Scare bars: 25  $\mu$ m (A,B). Statistical significance was determined by Student's *t*-test



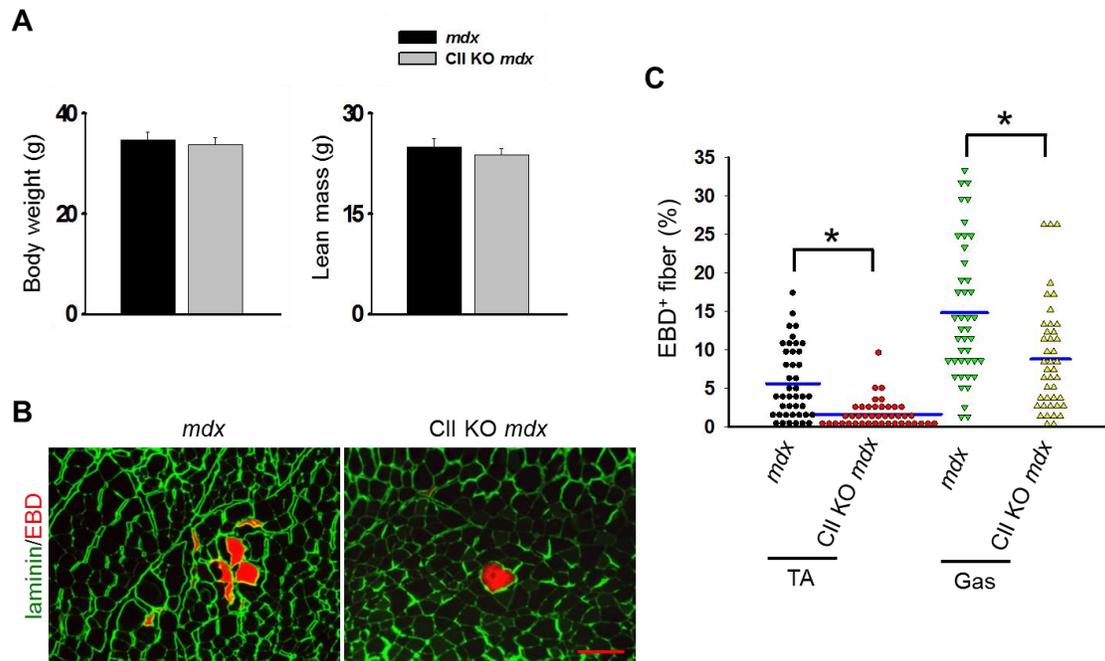
**Supplemental Figure 8.** Fusion defects in COUP-TFII transgenic mice. (A) Expression profile of the indicated genes in control ( $n=6$ ) and COUP-TFII OE ( $n=6$ ) muscles from day 5 to day 12 after CTX injection. (B) Activation of *Cav3* and *Cxcr4* expression in COUP-TFII deficient myoblasts. Data are representative of 3 independent experiments. (C) Luciferase assay of wild type and mutant *Cxcr4* enhancer in the presence and absence of COUP-TFII in primary SCs. *Cxcr4* enhancer fragment was placed in front of the TATA basic promoter that drives the luciferase reporter. Data are representative of 3 independent experiments. \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$  by Student's *t*-test. Results are the mean  $\pm$  SEM.



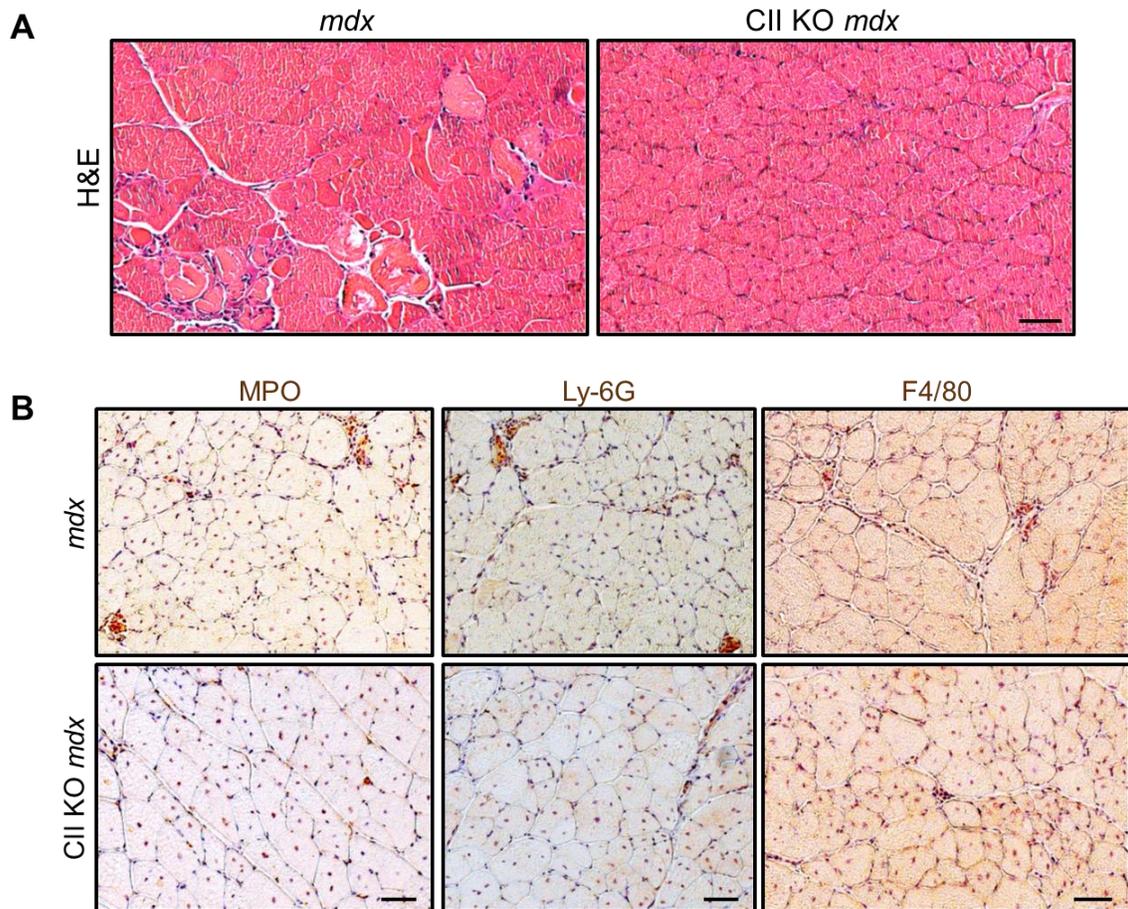
**Supplemental Figure 9.** Characterization of COUP-TFII KO and COUP-TFII KO *mdx* mice. (A) COUP-TFII expression in FACS-sorted satellite cells from control and COUP-TFII KO mice. Results are representative of 3 independent experiments. (B) Histological analyses of diaphragm muscles in control and COUP-TFII KO mice. Interstitial fibrosis and mineralization were assessed by Masson's trichrome and von Kossa staining, respectively. (C) Immunofluorescence staining of PAX7 and COUP-TFII in diaphragm muscles showed the depletion of COUP-TFII protein in PAX7<sup>+</sup> cells one week after tamoxifen injection. Images are representative of four different animals of indicated genotypes. Scale bars: 100  $\mu$ m (B), 25  $\mu$ m (C). \*\*\* $P$ <0.001 by Student's *t*-test. Results are the mean  $\pm$  SEM.



**Supplemental Figure 10.** Histological examination of *mdx* and COUP-TFII KO *mdx* mice. (A) Representative photograph of trichrome stained diaphragm at 4 months of age. (B-D) 11-month-old diaphragm (B) and tibialis anterior muscles (C,D) were stained with H&E and trichrome. Images are representative of four animals for each genotype. Scare bars: 100  $\mu$ m (A,B), 50  $\mu$ m (C,D).



**Supplemental Figure 11.** EBD uptake in *mdx* and COUP-TFII KO *mdx* mice. (A) Body weight and lean mass of 4-month-old *mdx* ( $n=8$ ) and COUP-TFII KO *mdx* mice ( $n=7$ ). (B) Representative images for EBD infiltration in 4-month-old TA muscles. (C) Quantified mean data for EBD uptake in *mdx* and COUP-TFII KO *mdx* mice ( $n=42$  images from 7 animals). For every mice, six images taken from three different muscle sections were counted for EBD absorption. TA, tibialis anterior, Gas, gastrocnemius. Scale bars: 200  $\mu\text{m}$  (B). \* $P < 0.05$  by Student's *t*-test. Results are shown as mean  $\pm$  SEM.



**Supplemental Figure 12.** Representative photomicrographs of H&E staining, macrophage and neutrophil infiltration. (A) Images of H&E-stained 4-month-old tibialis anterior muscles. (B) TA muscles were stained with neutrophil (MPO and Ly-6G) and macrophage (F4/80) markers. Data are representative of four independent experiments. Scare bars: 100  $\mu\text{m}$  (A), 50  $\mu\text{m}$  (B).