

Disease Models & Mechanisms | Supplementary Material

**Fig. S1. The Cp-GFP transgenic mouse is a faithful reporter of Notch signaling in satellite cells.** (A) GFP signal in a putative satellite cell on a freshly isolated live EDL myofiber. (B) Double labeling with Pax7 (Red, satellite cell marker) and GFP (Green) indicates that 83% satellite cells (Pax7<sup>+</sup>) are GFP<sup>-</sup> (B1), but 17% are GFP+ (B2, n=102 cells counted from 3 mice). (C) Isolation of GFP<sup>+</sup> and GFP<sup>-</sup> satellite cells from the Cp-GFP mouse by fluorescent activated cell sorting using a7-integrin as a positive selection marker. (D) Analysis of sorted a7-integrin<sup>+</sup> cells indicates that 8% of cells are GFP<sup>+</sup>. (E) Realtime PCR analysis indicate that sorted GFP<sup>+</sup> satellite cells expressed higher levels of *Hes1*, a canonical target of Notch signaling. (F-H) Activation of Notch signaling turns on Cp-GFP in satellite cells in vivo. Shown are images of Pax7 and GFP staining in control (F), Jag1 overexpressing (G), and RAMIC (NICD) overexpressing (H) TA muscle sections 7 days after electroporation of plasmids encoding empty vector, Jag1 cDNA and NICD cDNA, respectively. Arrows point to Pax7<sup>+</sup>/GFP<sup>+</sup> satellite cells.



**Fig. S2.** *mdx* **satellite cells have a higher tendency for terminal differentiation**. (A) Representative images of satellite cell clusters from 2 and 12-months-old WT and *mdx* mice labeled with MyoG (red) and MyoD (green). Nuclei were counterstained with DAPI (blue). (B) Percentages of MyoG<sup>+</sup> cells per cluster at the indicated ages. Data were from 3 independent experiments with at least 20 clusters analyzed in each experiment. Error bars represent s.e.m. \**P*<0.05.

12m

0

2m



**Fig. S3. Constitutive activation of Notch in** *mdx* **satellite cells fail to improve muscle pathology.** (A) Representative images showing Notch pathway was activated in the *mdx*/NICD+ satellite cells after Tamoxifen injection. Muscle fibers from MDX/NICD- (Pax7<sup>CreER/+</sup>/*mdx*) and MDX/NICD+ (Pax7<sup>CreER/+</sup>/Rosa26<sup>NICD</sup>/mdx) mice were labeled with Pax7 (red) and GFP (green). Nuclei were counterstained with DAPI (blue). GFP signal is turned on by Cre in the Rosa26<sup>NICD-ires-nGFP</sup> mice (called Rosa26<sup>NICD</sup> here). (B) Representative H&E staining images showing muscle morphology of resting and CTX-injured *mdx*/NICD- and *mdx*/NICD+ mice (refer to Fig. 4). (C) Quantitative analysis of number of myofibers (>500 µm<sup>2</sup>) per unit field (1 mm<sup>2</sup>) in resting (-CTX) and CTX-treated (+CTX) muscles. n=3.