

**Supplemental Figure 1. Single molecule force-SL simulations.** We calculated the force of a single N2B and a single N2BA titin molecule in WT and N2B KO mice (solid lines) and the average force of a single molecule in WT and KO sarcomere taking into account the N2BA: N2B expression ratio (inset), see broken lines. Expression of N2BA titin in a N2B dominant background reduces force, but the effect is small. The main effect of the N2B deletion is the large increase in force of the N2B titin isoform.

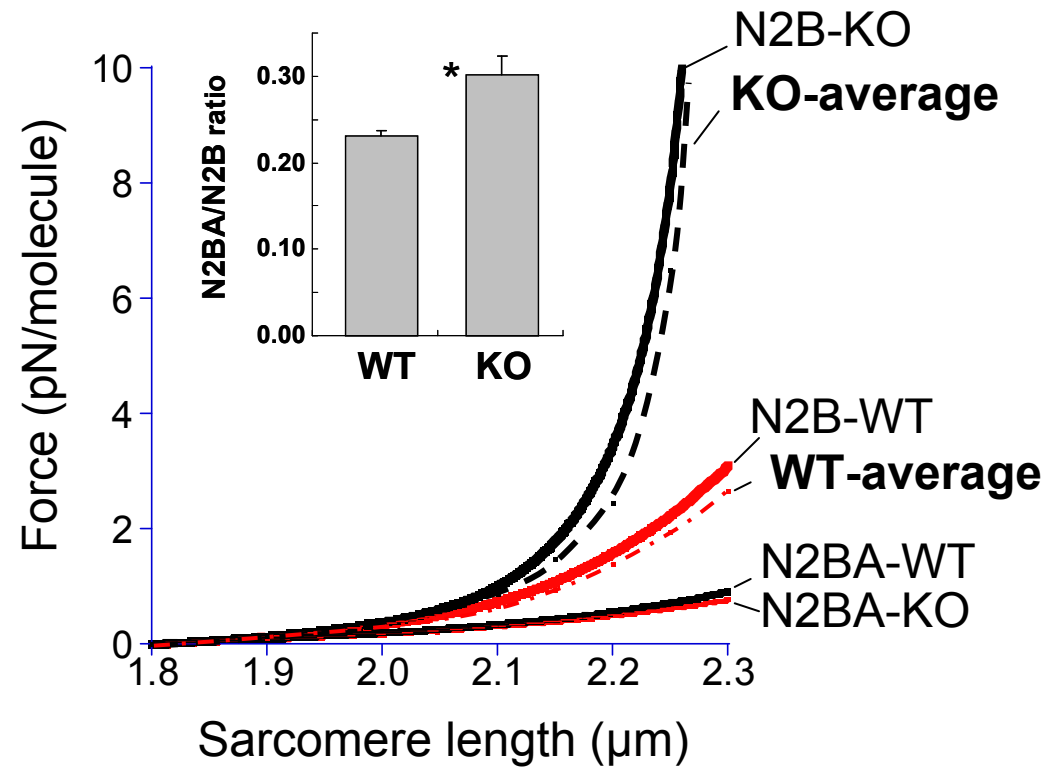
Details: We calculated the force—SL relations of single titin molecules using the wormlike chain WLC equation:

$$\frac{Fx(PL)}{k_B T} = \frac{z}{L} + \frac{1}{4(1-z/L)^2} - \frac{1}{4} \quad (1)$$

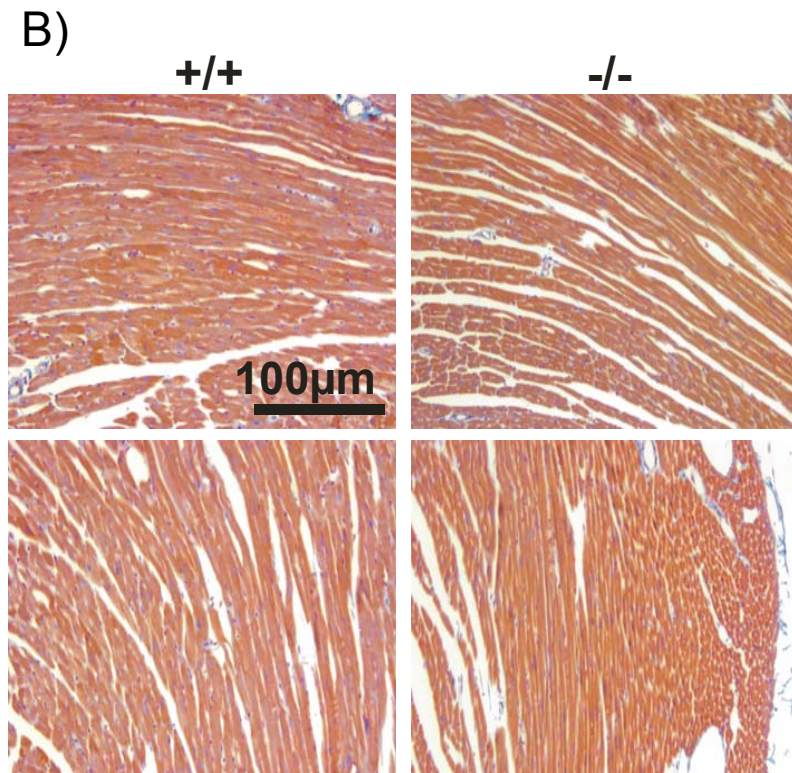
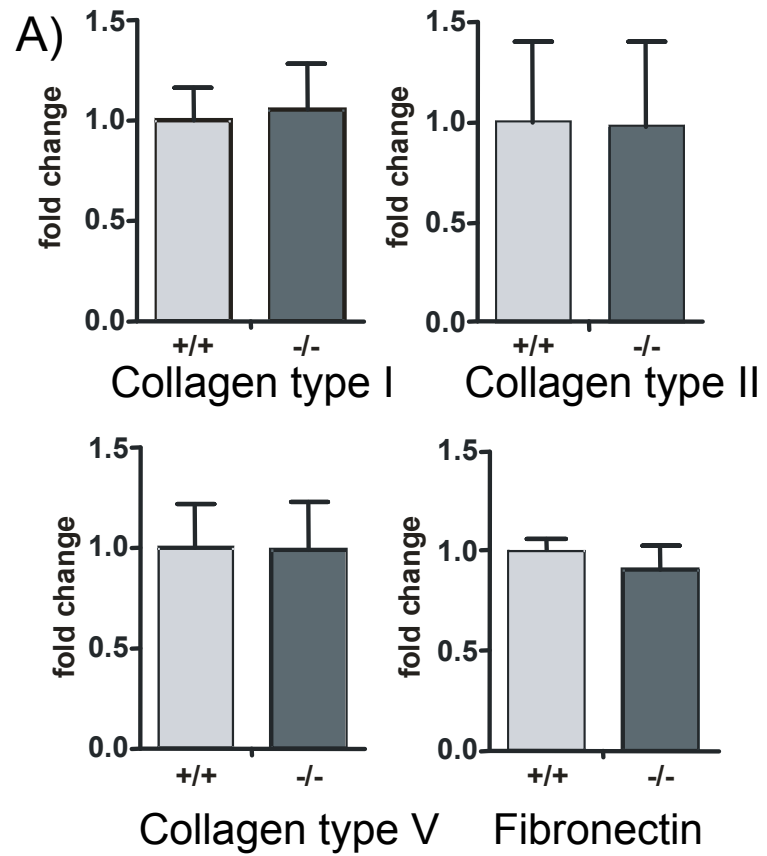
where F is force (in pN),  $k_B$  is Boltzmann's constant, T is absolute temperature, PL and L are the persistence and contour lengths. We serially-linked three WLCs, representing the combined tandem Ig segments, the PEVK, and N2B-Us spring elements. We assumed a PEVK contour length (CL) of 70 nm in N2B titin and 300 nm in N2BA titin; CL of tandem Ig segments were set at 200 nm in N2B titin and 300 nm in N2BA titin; CL of N2B Us was 200 nm for both isoforms. The assumed persistence lengths (PL) were 1.3 nm, 12 nm and 0.65 nm, respectively. We then calculated the force-SL relation of a single titin molecule and compared results for WT N2B titin, WT N2BA titin, KO N2B and KO N2BA titin and the average force-SL relation for sarcomeres that coexpress N2B and N2BA titins. For additional details on the assumed contour length, see (Trombitas et al. *Biophys J* 2000;79(6):3226-34) . For details on the assumed PL values, see Watanabe et al., *JCB*, 2002, 29;277(13):11549-58.

**Supplemental Figure 2. Assessment of extracellular matrix proteins in myocardium of WT and N2B KO mice.** **A)** Mean ( $\pm$ SEM) of collagen (type I, III, V) and fibronectin (FN) expression levels using qPCR (TaqMan) for N2B WT and KO mice (n=6 per genotype). Data was normalized first to 18S, and then to WT. No significant differences

were found between genotypes. **B)** Example of Trichrom staining of left ventricular myocardium from N2B WT and KO mice. N2B KO myocardium shows comparable staining of collagen (blue) with no signs of fibrosis.



Supplemental Fig. 1



Supplemental Fig. 2