Supplemental Figures

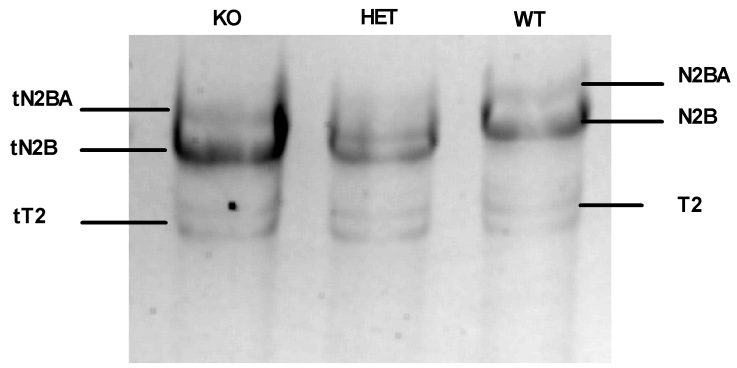

Fig. S1 Effect of N2B deletion on the structural stability of titin in myofibrils. A representative 1% SDS-agarose gel electrophoresis of myofibrils isolated from left ventricles of wild-type (WT), heterozygous (HET) and knockout (KO) mice. tN2BA and tN2B indicate the truncated N2BA and N2B isoforms of titin, respectively. T2 and tT2 indicate the degradation products.

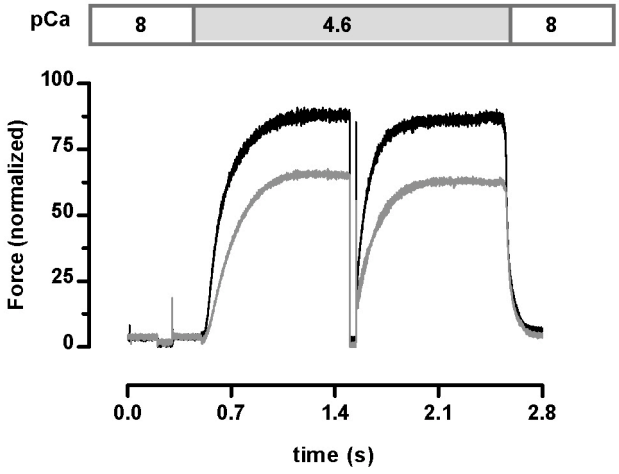

Fig. S2 Force transients from cardiac myofibrils from wild-type (grey lines) and knockout mice (black lines). Force is related to the cross-sectional area (*CSA*) of the respective myofibril bundle (wild type: 3.1 μm^2, knockout: 7.0 μm^2) to illustrate the difference in maximum tension.

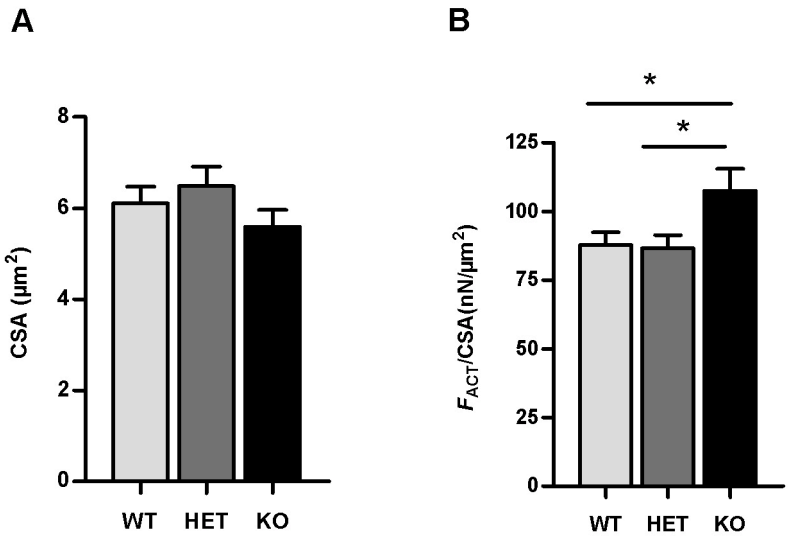

Fig. S3 Cross-sectional area and the maximum active tension of the myofibril bundles. (A) The cross-sectional area (*CSA*) was computed from the diameter of the myofibril bundles by assuming a circular shape. No statistically significant effect of the genotype on the *CSA* was observed. **(B)** Maximum active force (F_{ACT}) was determined by subtracting the passive tension (F_{pass}) measured prior activation from maximum tension (F_{max}). * indicate significant difference ($p < 0.05$) of knockout (KO) compared to wild-type (WT) and heterozygous (HET) revealed by Tukey´s multiple comparison tests. Bars show means ± SEM based on $n = 44$ (WT), $n = 31$ (HET) and $n = 32$ (KO) myofibrils.

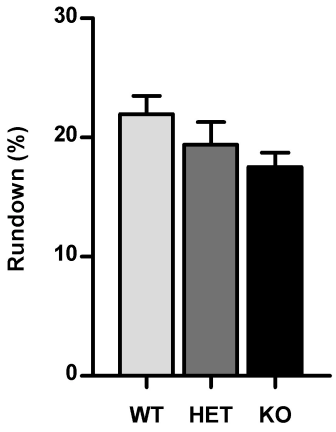

Fig. S4 Effects of N2B deletion on the stability of contractile function. The myofibrils were subjected to several contraction-relaxation cycles. After the first activation at maximum [Ca^{2+}] (pCa 4.6) for determining F_{MAX}, five partial Ca^{2+} activations ranging from pCa 6.16 to 5.02 were applied followed by a final control activation at pCa 4.6. The percentage reduction of force produced in the final compared to the first activation is expressed as rundown of maximum force. Rundown of myofibrils from knockout (KO) mice is smaller than those from heterozygous (HET) and wild-type (WT) mice but differences are not significant.