SUPPLEMENTAL MATERIAL

Heart failure related hyper-phosphorylation in the cardiac troponin I C-terminus has divergent effects on cardiac function *in vivo*

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First Author's surname and short title: Li, in vivo effect of cTnI C-terminus phosphorylation

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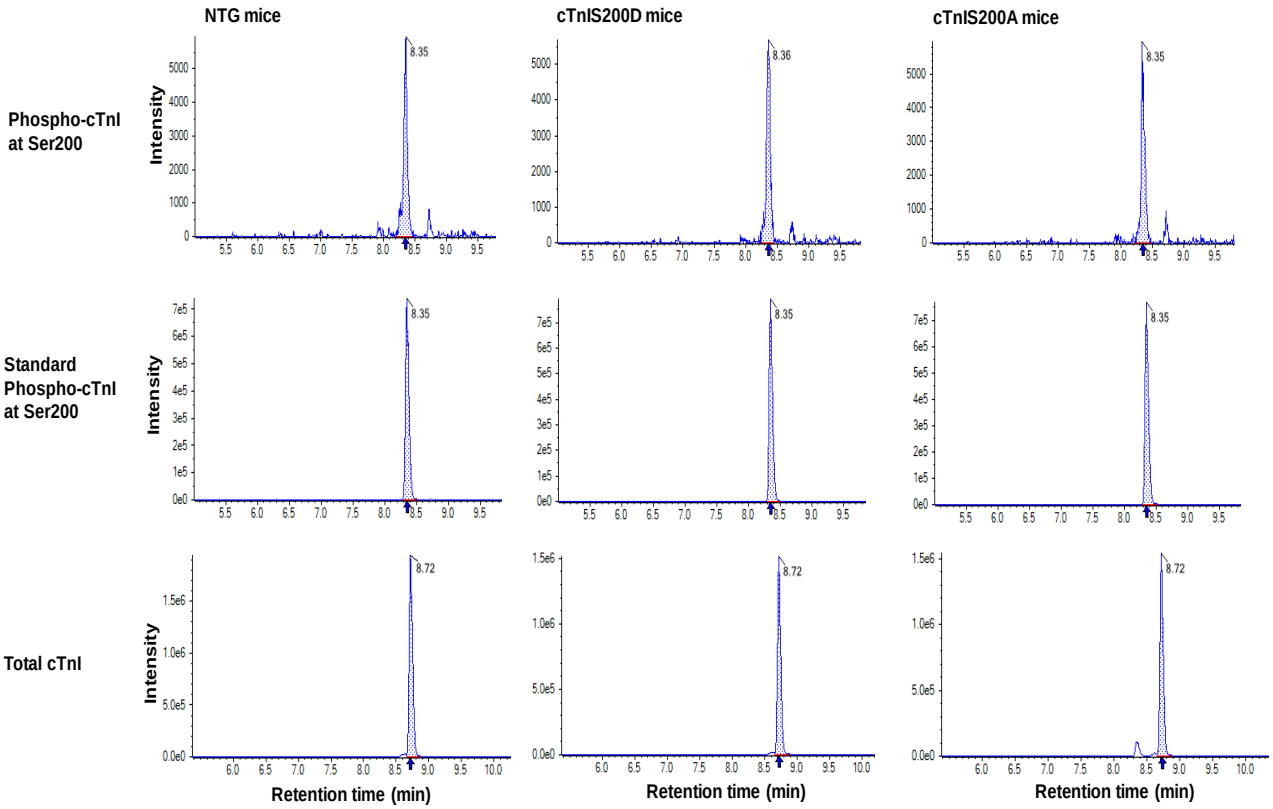
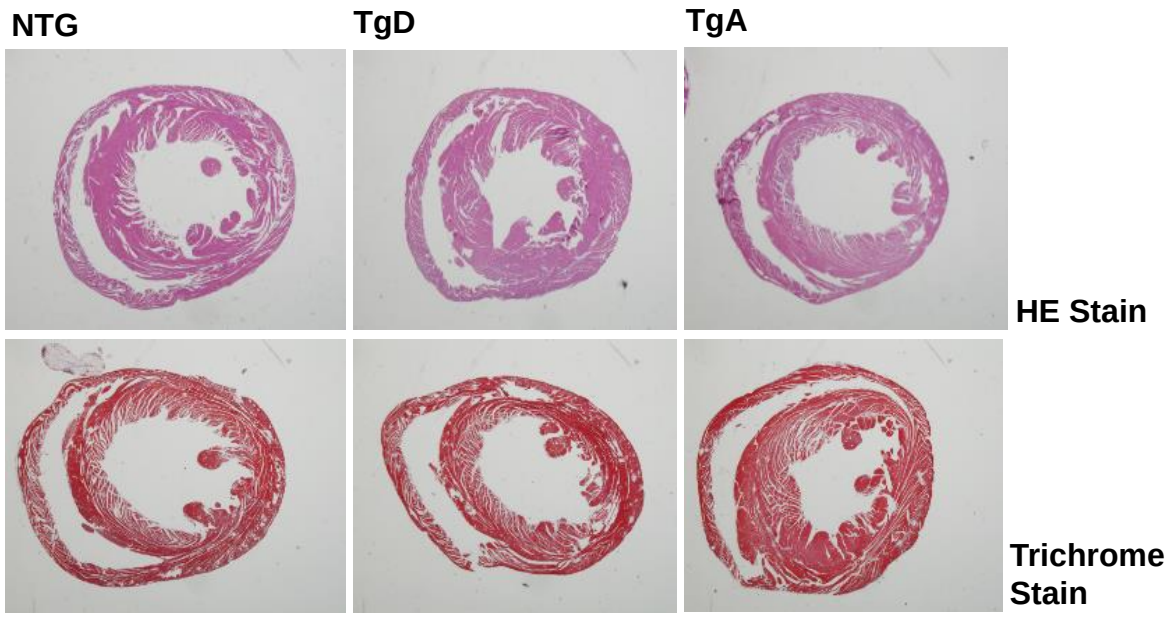
Figures and legends

Supplemental Figure 1.



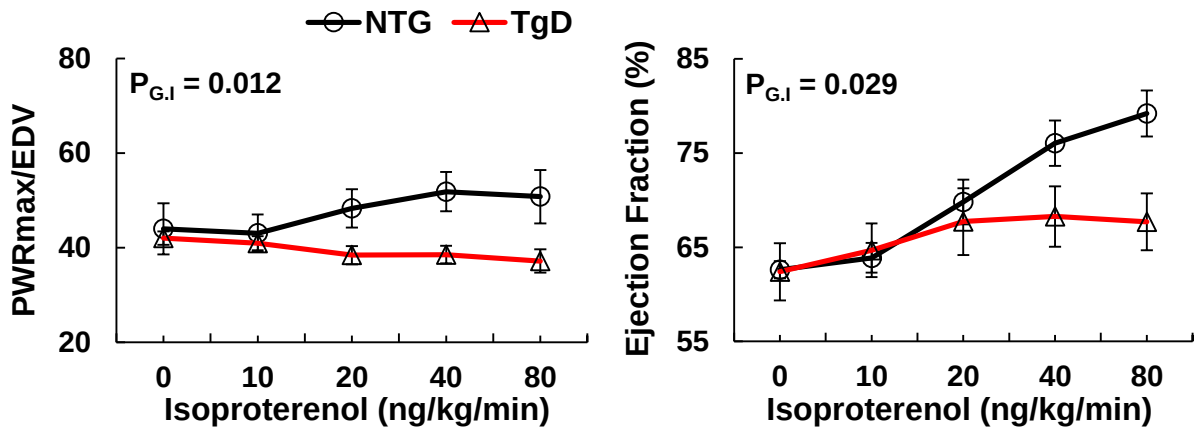
Supplemental Figure 1. Mass chromatograph of mouse cTnI. The retention time of the peptide fragment of cTnI containing phosphorylated Ser200, NIDAL(p)SGMEGR, is 8.35 minutes. Its representative transitions from NTG, cTnIS200D, and cTnIS200A are listed in the top row. An internal standard peptide NIDAL(p)SGMEGR* was used as positive control; its representative transitions are listed in the middle row. The peptide for total cTnI is NITEIADLTQK whose retention time is 8.71 minutes, representative transitions are list in the bottom row.

Supplemental Figure 2.



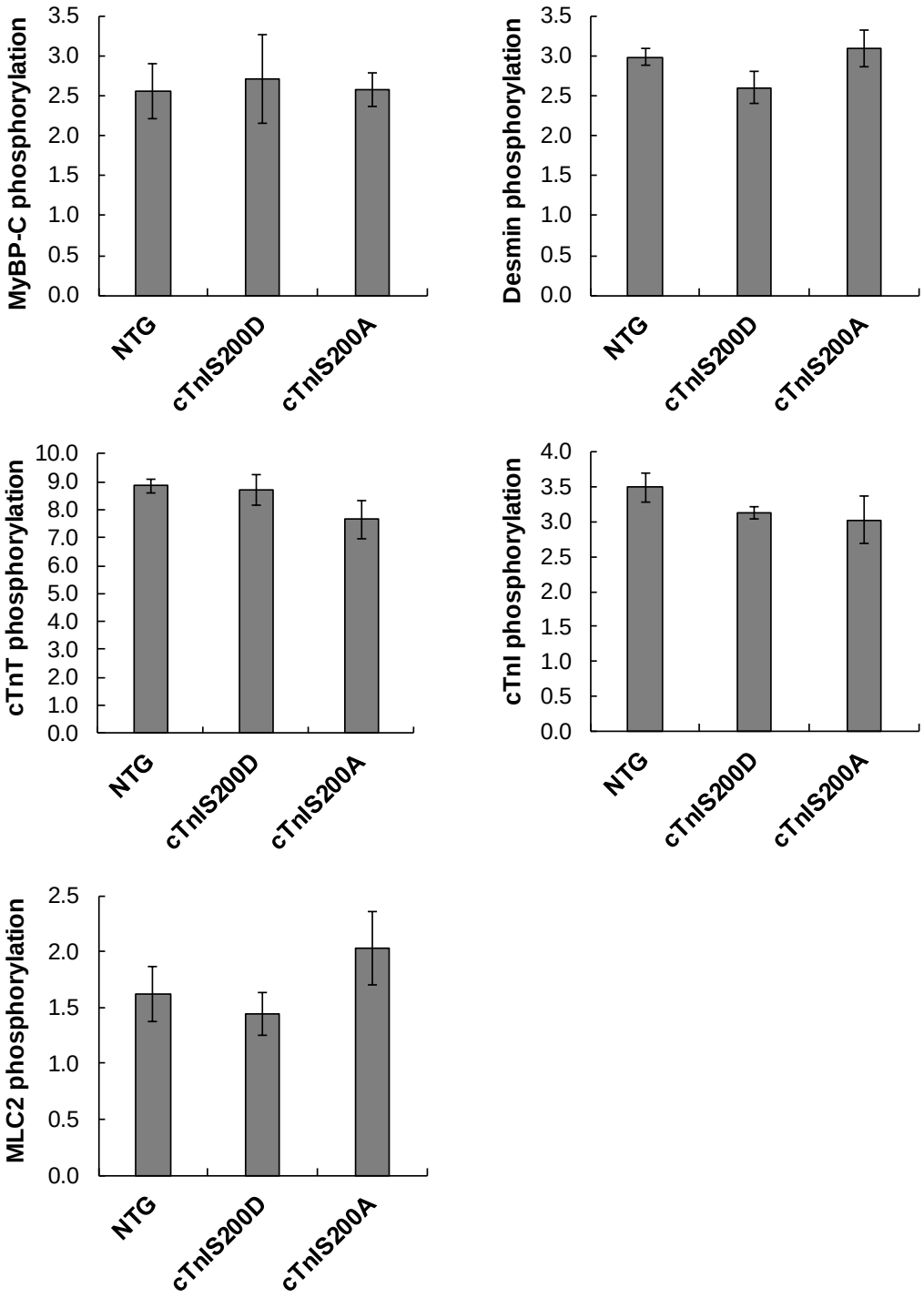
Supplemental Figure 2. Histological examination of cardiac structure. Hematoxylin and eosin (HE) stain showed no myocardium hypertrophy or chamber dilation in either type of transgenic hearts. No fibrosis was detected by trichrome stain.

Supplemental Figure 3.



Supplemental Figure 3. Cardiac responses to β-adrenergic stimulation with isoproterenol at fixed heart rate. n=4. $P_{G.I}$ <0.05 indicates significance among the interactions between genotype and the concentration of isoproterenol.

Supplemental Figure 4.



Supplemental Figure 4. The overall phosphorylation level of myofilament proteins in mouse hearts of NTG, cTnIS200D, and cTnIS200A, detected with ProQ Diamond staining. n=5. The quantitative data

were analyzed with one-way ANOVA followed by post hoc Tukey's test. The overall phosphorylation level of each protein detected is not significant different between the three types of mice. Abbreviations: cMyBP-C, cardiac myosin-binding protein-C; cTnT, cardiac troponin T; cTnI, cardiac troponin I, MLC2, myosin light chain 2.