

Supplemental material

Gregorich et al., <https://doi.org/10.1085/jgp.201812214>

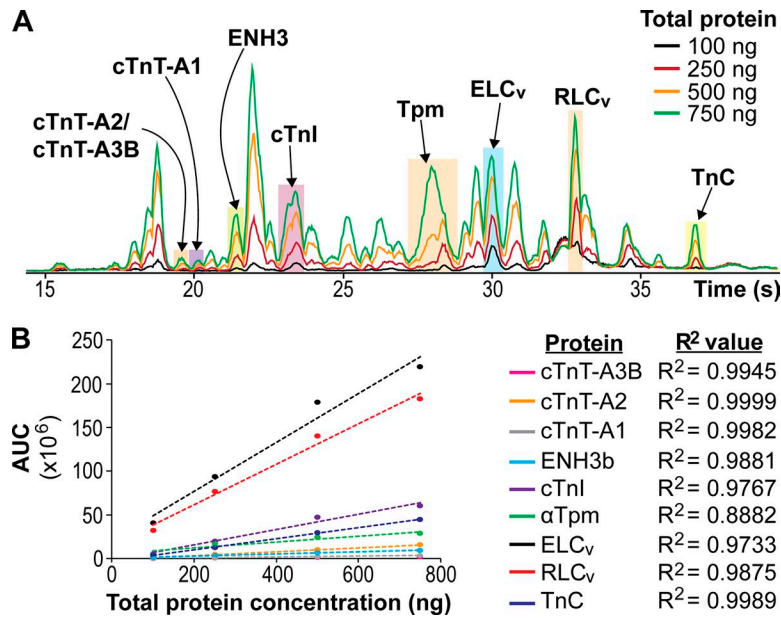


Figure S1. **Establishment of linear response range for individual sarcomeric proteins.** (A) Overlay of base peak chromatograms obtained from injection of a single *ENH*^{+/+} sarcomeric protein extract at four different total protein concentrations. Chromatographic peaks corresponding to specific sarcomeric proteins are highlighted. (B) Plot of the area under the curve (AUC) versus total protein concentration for individual sarcomeric proteins. The R² value for the best-fit lines of individual proteins is given.

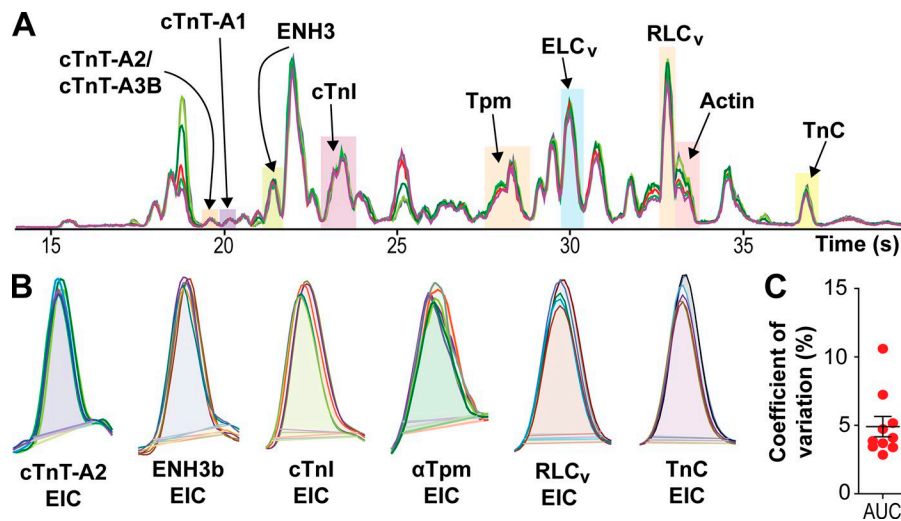


Figure S2. **Reproducibility of the chromatographic separation and sarcomeric protein signal intensities across multiple injections of the same sample.** (A) Overlay of chromatograms from six injections of a single *ENH*^{+/+} sample (injection replicates). Chromatographic peaks corresponding to specific sarcomeric proteins are highlighted. (B) Overlays of EICs for select sarcomeric proteins from six injection replicates. (C) Graph showing determined coefficient of variation values calculated for individual sarcomeric protein intensities (represented by the areas under the curve [AUCs]). Each dot corresponds to the coefficient of variation value computed for the intensity of a single sarcomeric protein across six injection replicates.

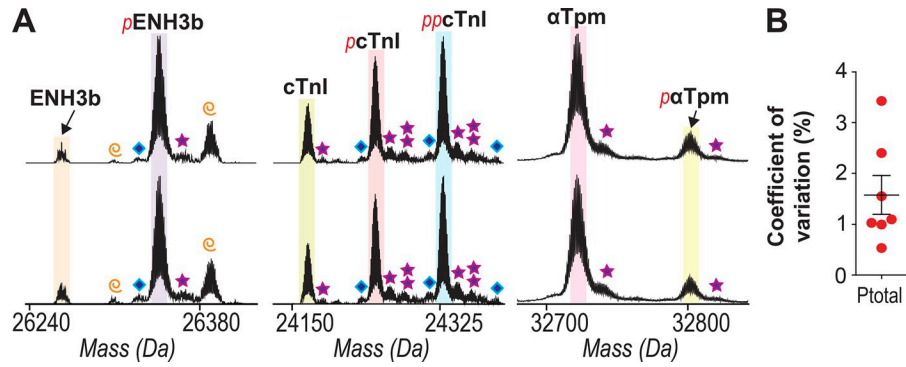


Figure S3. **Reproducibility of sarcomeric protein phosphorylation measured across multiple injections of the same sample.** (A) Zoomed-in deconvoluted mass spectra showing the relative intensities of unmodified and modified forms of ENH3b (left), cTnI (middle), and αTpm (right) in different injections of the same *ENH^{+/+}* sample. Swirls, diamonds, and stars denote peaks corresponding to potential acetylated forms of ENH3b and pENH3b, protein species with NH₃ loss, and oxidized protein species, respectively. (B) Calculated percent coefficient of variation values determined for the measured phosphorylation (Ptotal; in mol of Pi/mol of protein) of individual sarcomeric proteins. Each dot corresponds to the coefficient of variation value computed for the Ptotal of a single sarcomeric protein in six different injection replicates.

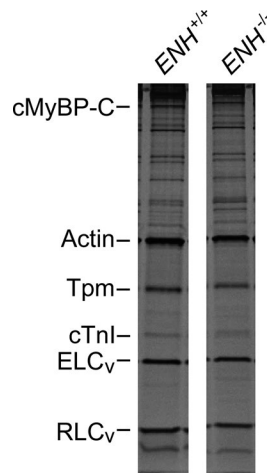


Figure S4. **Expression profile of thin and thick filament regulatory proteins in right ventricular trabeculae isolated from the hearts of *ENH^{+/+}* and *ENH^{-/-}* mice.** Representative silver stained 12.5% Tris-HCl Bio-Rad PreCast Criterion gel showing the expression profile of contractile proteins in *ENH^{+/+}* and *ENH^{-/-}* trabeculae.