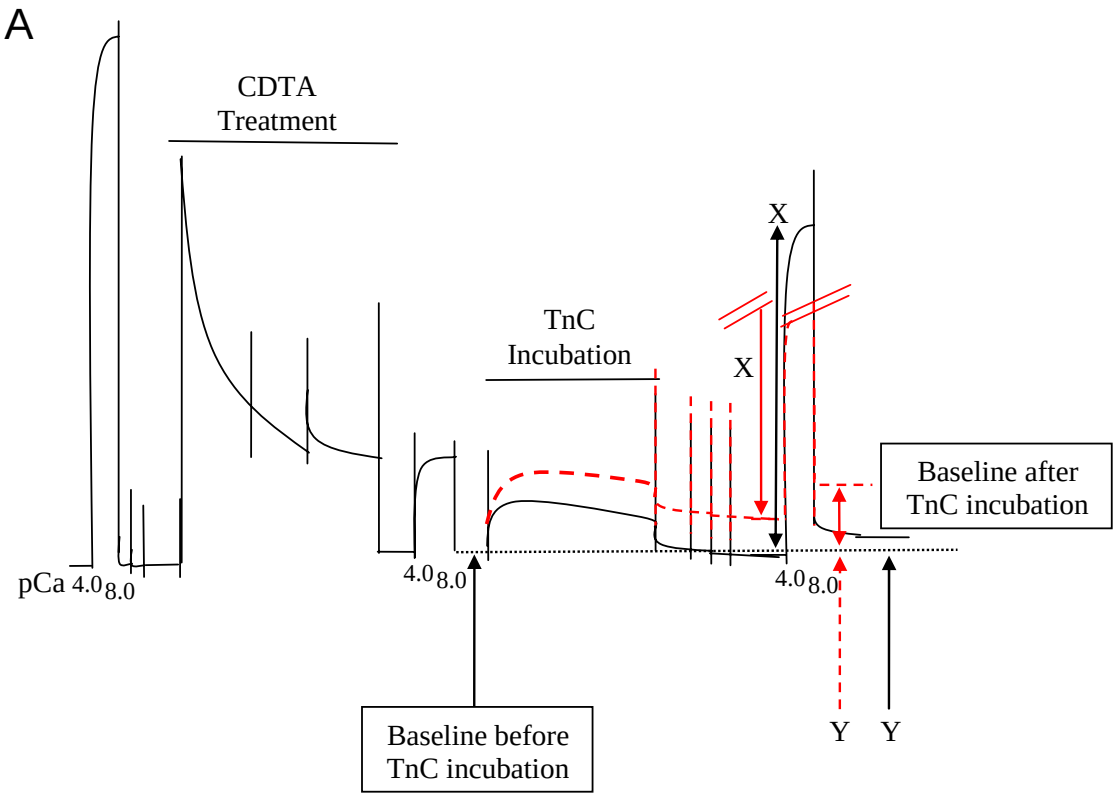
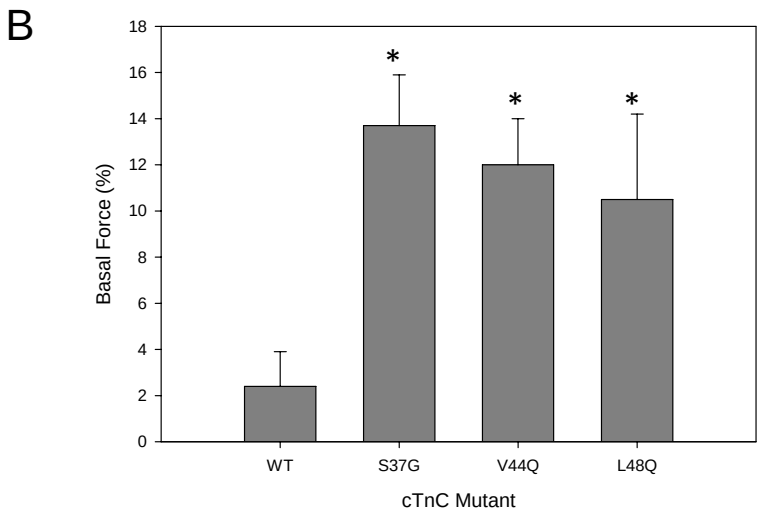
Suppl. Figure 1**.** Schematic of basal force measurements in cardiac skinned fiber preparations.

A) After the fibers were maximally activated in pCa 4 and the initial force recorded, the endogenous porcine cTnC was extracted with 5 mM CDTA, pH 8.2. The fibers are then maximally activated (in pCa 4) to determine the residual force (due to the remaining cTnC). The extracted fibers were incubated with cTnC for 1 hr. The cTnC reconstitution was performed in pCa 8. The dotted line represents the increased basal force seen with some of the cTnC mutants after cTnC reconstitution and values were calculated according to the following equation: (Y/X) x 100. The variable X was derived from the maximal force obtained after TnC reconstitution and Y was derived from differences in the baseline before and after reconstitution with mutant cTnCs. This indicates the amount of tension generated in the fibers after cTnC reconstitution at low [Ca^{2+}]. B) The basal force data obtained from cTnC(+F27W) "RCM-like" mutants in contrast to data shown for cTnC(-F27W) mutants in Figure 1D.





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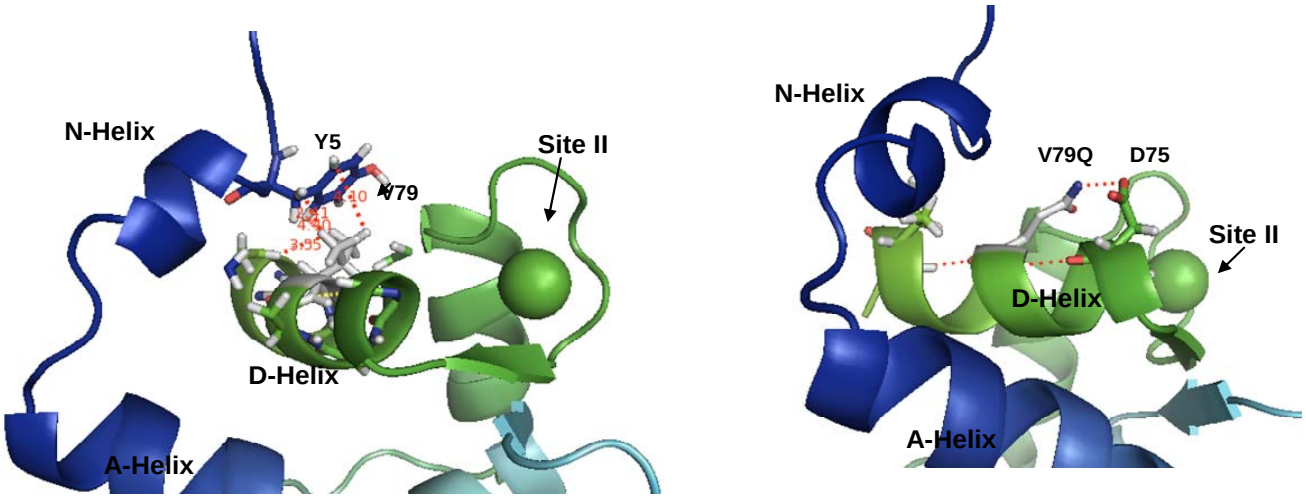
Suppl. Figure 2**.** Modeling of the V79 and V79Q mutation in the PDB file 1AJ4. A) V79, distances are shown with dotted red lines and reported in Angstroms B) V79Q and dotted red lines show potential for Hydrogen bonding between the mutant and adjacent residue D75. The high affinity Ca^{2+} binding site II is indicated by the arrow.



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Suppl. Figure 3**.** The E40A mutation modeled into PDB 1J1E. A) E40 and B) E40A is shown below. The red dotted lines indicate putative Hydrogen bonds. C) The location of I61 and closely interacting residues modeled into PDB 1AJ4. Red dotted lines with measurements indicate the distances in Angstroms D) I61Q, Red dotted lines show potential Hydrogen bonds that may form in the presence of the mutation. The high affinity Ca^{2+} binding site II is indicated by arrows.