Figure 1S. Binding curve determined from the $\Delta \delta_{total}$ of 2D $^{1}H,^{15}N$-HSQC NMR spectral changes of E41A-sNTnC•2Ca$^{2+}$ upon addition of sTnI$_{115-131}$. The curve is an average of 82 residues, which exclude Ser$^{15}$, Asp$^{30}$, Asp$^{56}$, Val$^{65}$, and Glu$^{76}$ due to spectral overlap, Pro$^{53}$ due to the lack of an amide hydrogen resonance, Ala$^{1}$ and Ser$^{2}$ presumably due to rapid exchange with solvent, and residues involved in site I. The determined dissociation constant was 300 ± 100 μM.

Figure 2S. (A) Contour plot of 2D $^{1}H,^{15}N$-HSQC NMR spectra of apo E41A-sNTnC showing the effect of sTnI$_{115-131}$ addition. The apo E41A-sNTnC spectrum before the addition of peptide is shown with multiple contours while each addition of sTnI$_{115-131}$ are indicated by a single contour. (B) 1D-trace of Gly$^{43}$ throughout the titration of sTnI$_{115-131}$, showing the progressive line broadening effect.

Figure 3S. The total change in chemical shift ($\Delta \delta_{total}$ in Hz) for each backbone amide pair in E41A-sNTnC (A,D), cNTnC (B,E) and sNTnC (C,F) upon addition of calcium (A-C), or TnI peptide (D-F). The thin dashed lines indicate one standard deviation above the average.