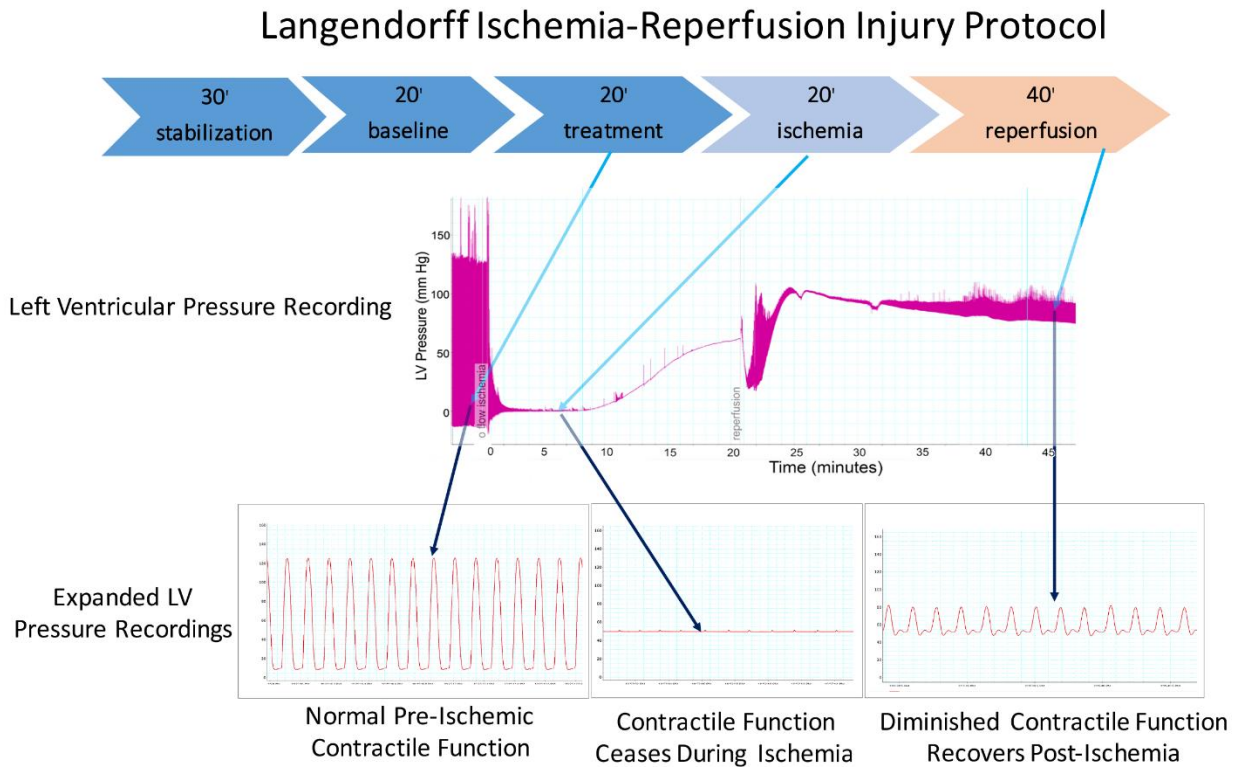
SUPPLEMENTAL MATERIAL

Table S1. ΔG of binding after a Molecular Mechanics Energy combined with Generalized Born and Surface Area continuum solvation (MM-GBSA) minimization of the protein:peptide complex using a 5 Å distance threshold between docked peptide and protein.

Peptide	ΔG (MM-GBSA)	RMSD Y313-A348
aCT11	-54.58	1.950
aCT11-I	-41.84	1.959
M1	-30.26	1.842
M2	-26.73	1.885
M3	-40.26	1.865

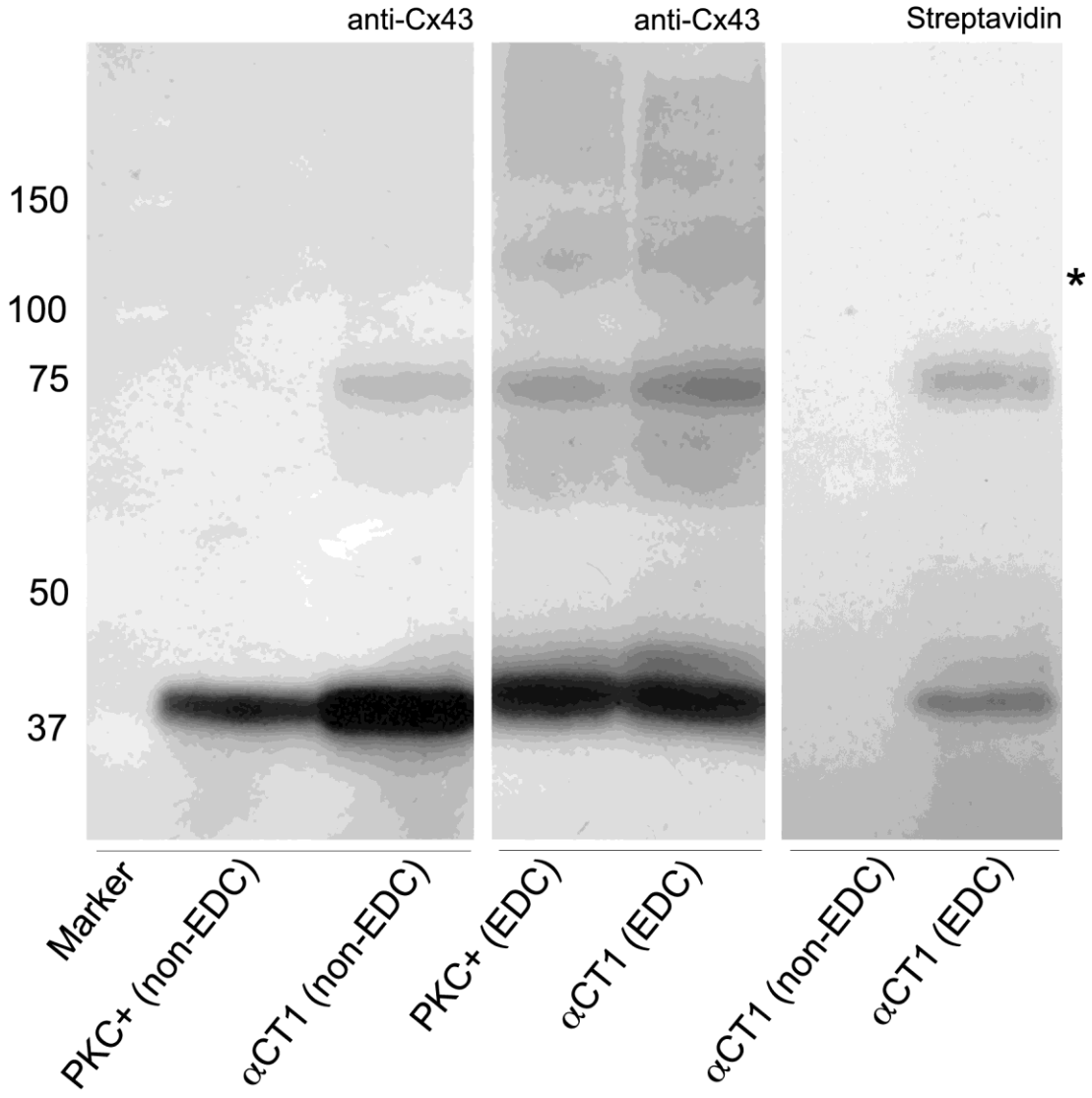
Root-Mean-Square Deviation of atomic positions (RMSD) changes after minimization around the ligand in the Y313-A348 region, relative to the input structure from PDB 1R5S.

Figure S1. The Ischemia Reperfusion (I/R) injury model/protocol used in this study involved a 20-minute period of no flow ischemia period followed by 40 minutes of reperfusion, left ventricular (LV) contractile function was monitored throughout the whole process.



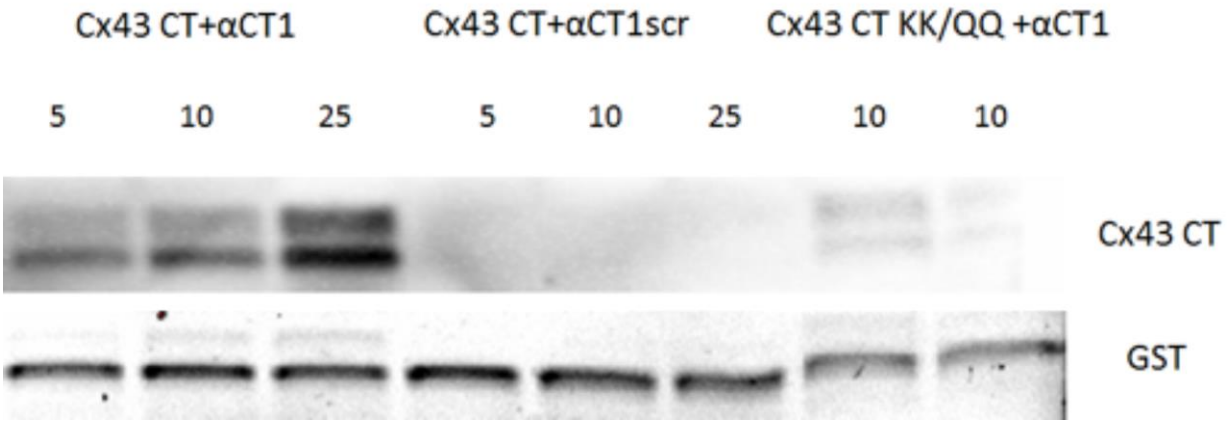
For treatment, peptides were infused into hearts over a 20-minute period just prior to the ischemic episode. Expanded representative pressure traces for each of these phases are shown below.

Figure S2. Blots of products of kinase reaction mixtures containing glutathione S-transferase (GST)-Connexin 43 (Cx43) carboxyl terminus (CT), GST-Protein Kinase C (PKC)ε and biotinylated αCT1 (20 μM).



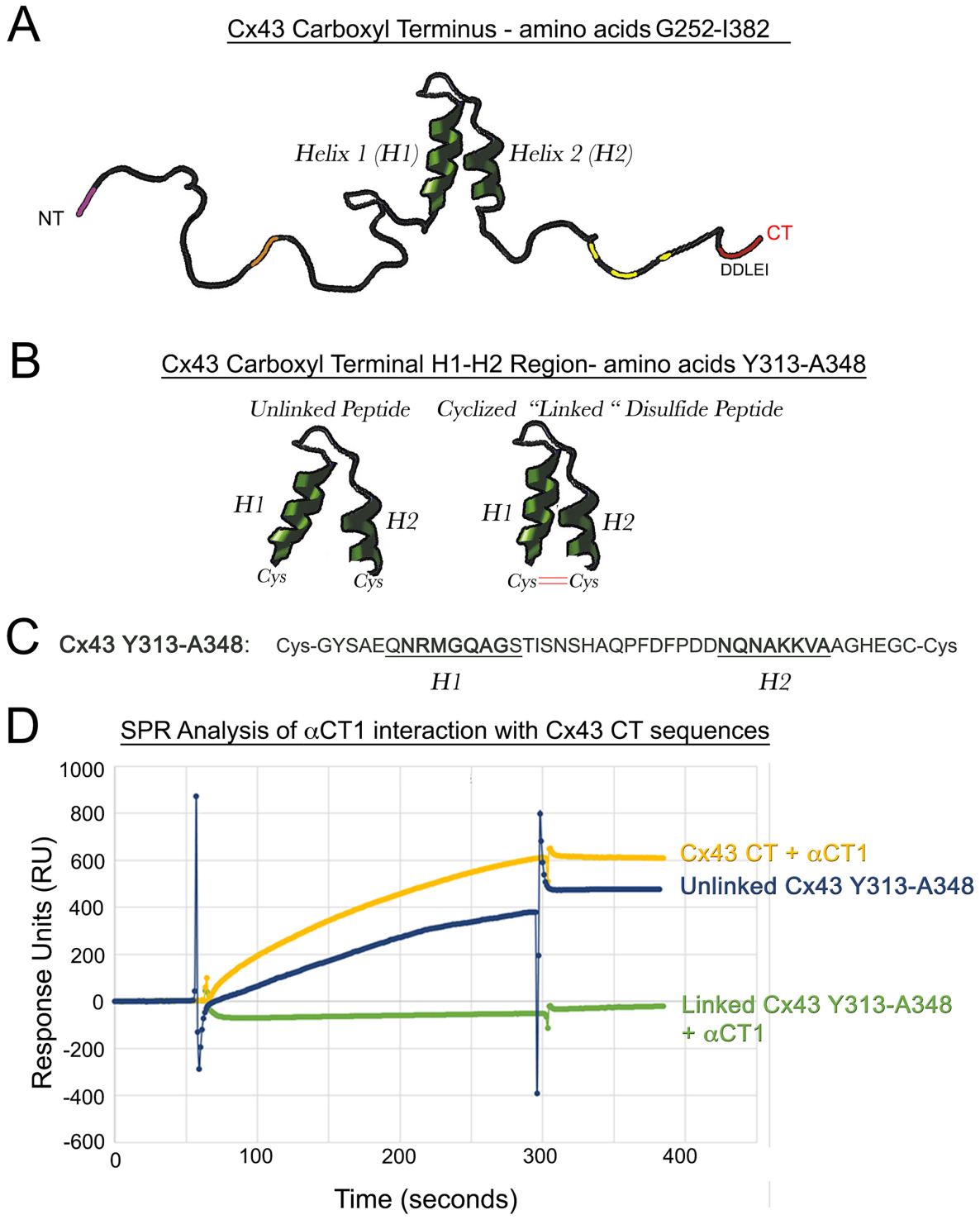
Left hand blot shows anti-Cx43 blot of products kinase reaction that have not been cross-linked. Middle blot shows anti-Cx43 blot of products kinase reactions that have been cross-linked. Cx43 antibodies detect bands at molecular masses consistent with monomers and dimers of the Cx43 CT construct. The dimer band appears to be more intense in the cross-linked reactions. Right hand blot shows streptavidin detection of biotinylated αCT1 for non-crosslinked (first lane) and cross-linked (second lane) reaction products. Biotin is detected only at bands consistent with the molecular mass of Cx43 monomers and dimers. No signal is detected in the region of the gel corresponding to the molecular mass of GST-PKCε (asterisk, 110 kDa).

Figure S3. Blots of cross-linked products of kinase reaction mixtures containing glutathione S-transferase (GST)-Connexin 43 (Cx43) carboxyl terminus (CT), GST-Cx43 CT QQ/KK in which the lysine (K) residues were mutated to neutral glutamines (Q), Protein Kinase C (PKC)ε and αCT1 (at 5, 10 and 25 μM) and a scrambled αCT1 (M4 scr) variant at the same concentrations.



Only αCT1 is seen to be covalently cross-linked to Cx43 CT in a concentration-dependent manner.

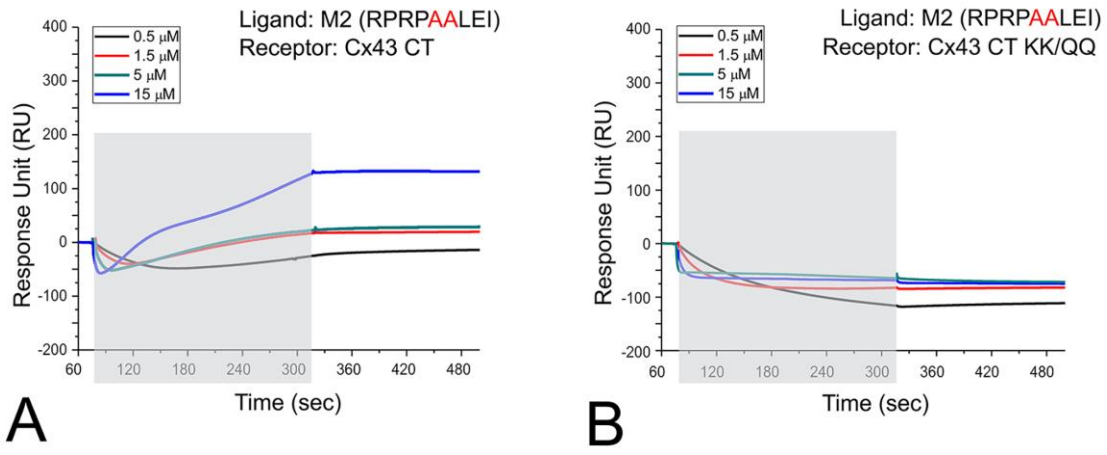
Figure S4. A. Cartoon depiction of the two alpha helical regions of the Connexin 43 (Cx43) carboxyl terminus (CT), H1 and H2 from Sorgen et al 2004 [28].



B. Schematic representation of the Cx43 Y313-A348 peptide synthesized for a binding surface surrogate with linkable cysteine (Cys) on the amino terminus and CT. **C.** Single letter amino acid

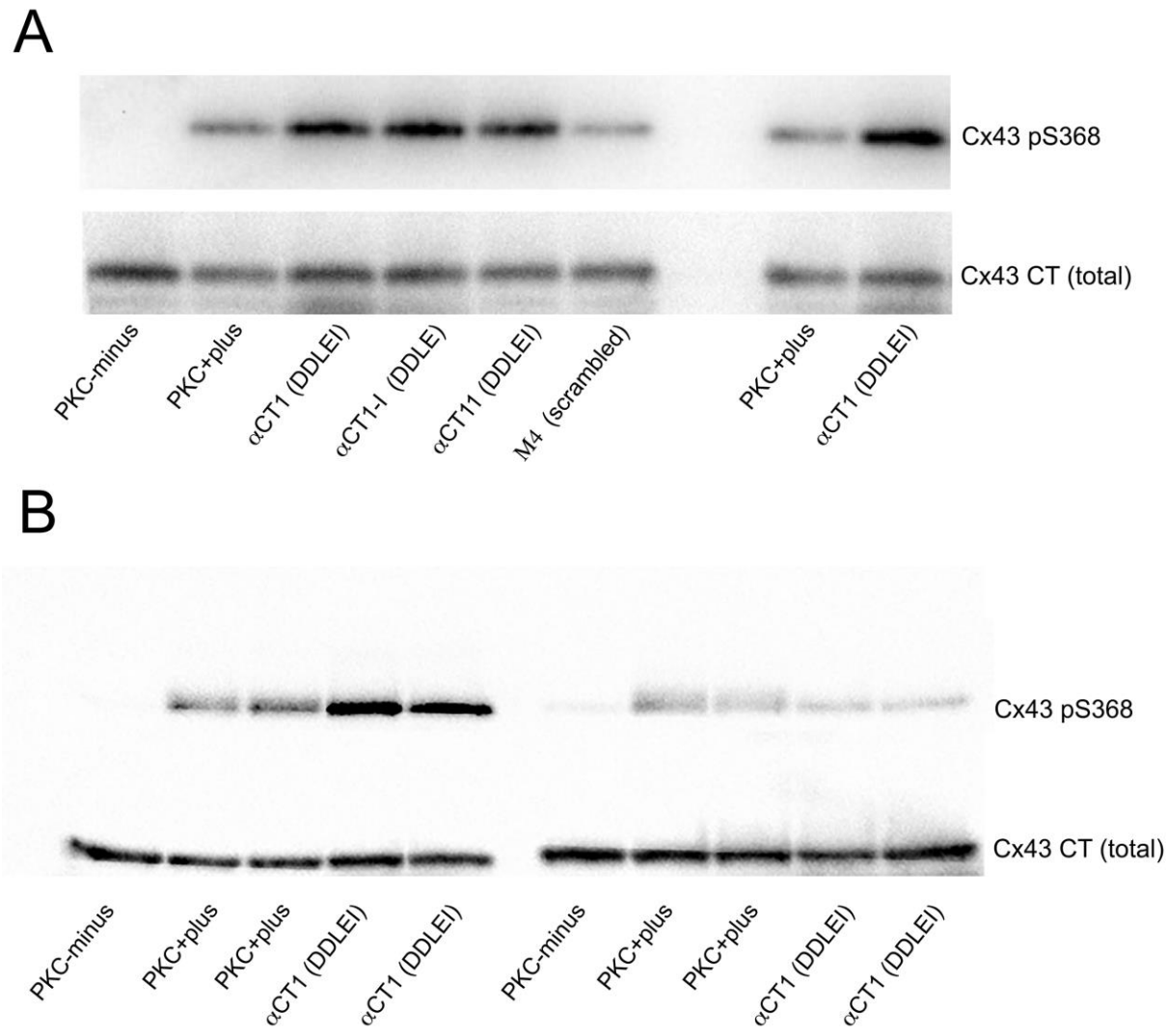
sequence of Cx43 Y313-A348 peptide with predicted helix secondary structure underlined. **D.** Surface Plasmon Resonance (SPR) analysis of substrate captured αCT1 (700-1000 RUs) binding recombinant Cx43 CT (100 μM, yellow), unlinked Cx43 Y313-A348 peptide (25 μM, Blue), and disulfide linked Cx43 Y313-A348 (25 μM, green). SPR indicates that non-disulfide linked Cx43 Y313-A348 peptide shows levels of interaction with αCT1 comparable to the full Cx43 CT polypeptide sequence (~150 amino acids). Disulfide cross-linking Cx43 Y313-A348 into a looped conformation results in a loss of αCT1 binding, suggesting that αCT1 interaction with this peptide requires a degree conformational flexibility.

Figure S5. The αCT1 variant peptide M2 AALEI shows limited ability to bind Connexin 43 (Cx43) carboxyl terminus (CT). Surface Plasmon Resonance (SPR) was used to analyze interactions of biotin-M2 AALEI with the Cx43 CT (A) and Cx43 CT-KK/QQ (B) as respective analytes.



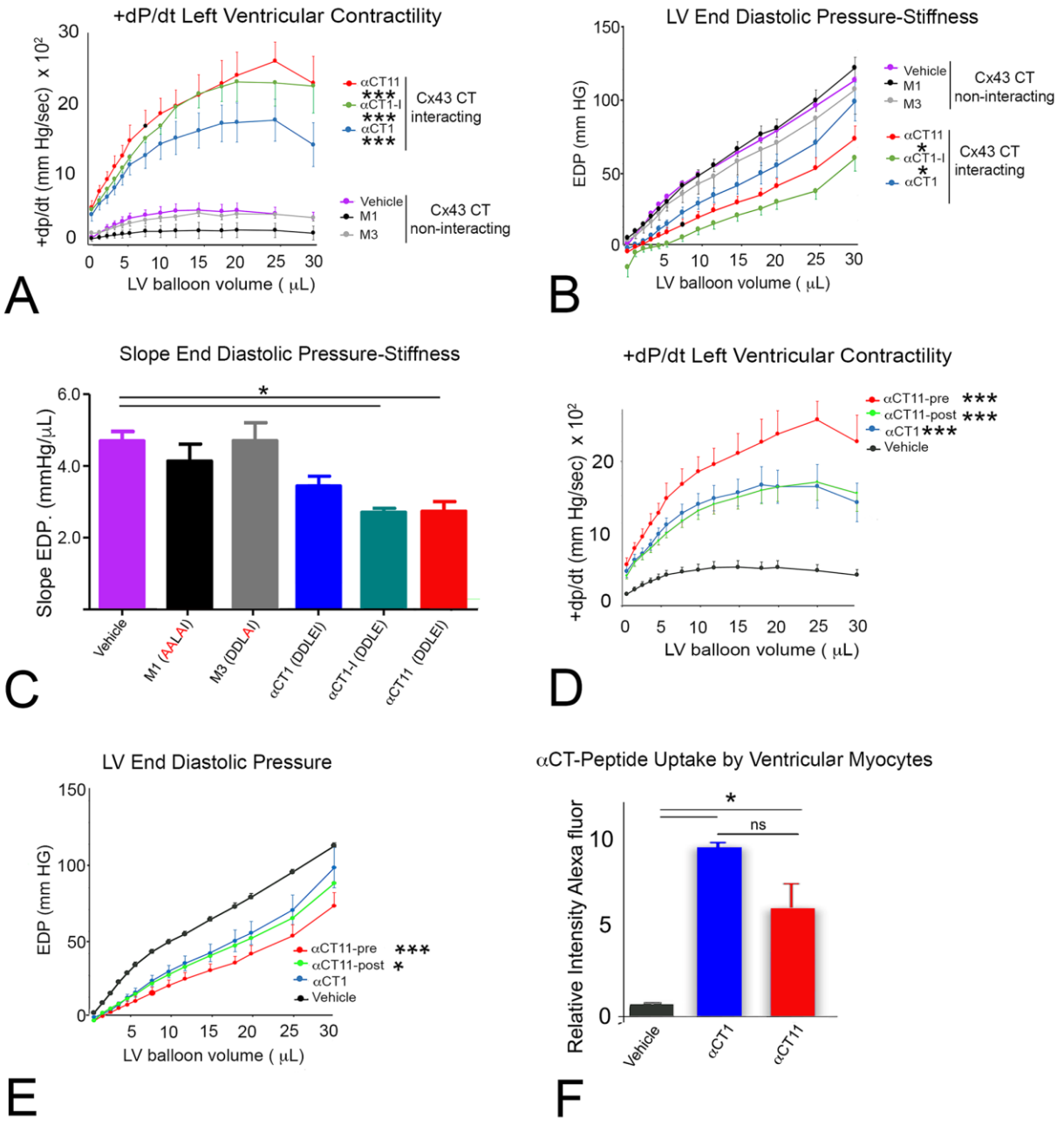
The mean of three runs is plotted for each analyte concentration. The exposure of the sensor chip to the specific analyte is indicated by the gray area.

Figure S6. A) Blots of Connexin 43 (Cx43) phosphorylated at S368 (pS368-top) and total Cx43 (bottom) in kinase reactions mixtures including no-kinase controls with Cx43 carboxyl terminus (CT) substrate, but no Protein Kinase C (PKC)ε (PKC-minus); Cx43-CT substrate with PKC-ε (PKC-plus); and mixtures containing PKC-ε, Cx43 CT, and biotin-αCT1, biotin-αCT1- I or biotin-αCT11 (RPRPDDLEI with no antennapedia sequence at peptide amino terminus) and biotin-M4 scrambled peptide.



B) Blots of Cx43-pS368 (top) and total Cx43 (bottom) in kinase reactions mixtures including no-kinase controls with Cx43 CT and Cx43 CT KK/QQ substrates, but no PKC-ε (PKC-minus); Cx43-CT and Cx43 CT KK/QQ substrates with PKC-ε (PKC-plus); and in mixtures containing CT substrates, PKC-ε, and biotin-αCT1(20 μM).

Figure S7. (A-C) Langendorff Ischemia-Reperfusion (I/R) injury protocols were performed on adult mouse hearts pre-treated with peptides and instrumented to monitor left ventricular (LV) contractility as shown in Figure 7.



Plots of **A)** Maximal systolic elastance (E_{max}) – i.e., the slope from plots shown in Figure 7A in the manuscript**; B)** LV end diastolic pressure (EDP) against balloon volume; **C)** Maximal systolic elastance (E_{max}), the slopes from supplemental figure 7B. **(D, E)** Langendorff I/R injury protocols were performed on adult mouse hearts pre-and post-treated with peptides and instrumented to monitor LV contractility as shown in Figure 8. Plots of **(D)** Maximal systolic elastance (E_{max}) –

i.e., the slope from plots shown in Figure 7A in the manuscript**;** and **(E)** LV end diastolic pressure (EDP) against balloon volume; Data shown in supplemental figures 7A-E are mean ± S.E. *p<0.05, ***p<0.001, N hearts/group: N hearts/group: Vehicle pre ischemia=8; Vehicle post ischemia=4; αCT1 pre ischemia =7; αCT1-I pre ischemia =6; αCT11 pre and post ischemia=4; M1 pre ischemia =5; and M3 pre ischemia =5 hearts. **F)** Average intensities of biotinylated peptide (indicated by streptavidin Alexa647 fluorescence intensity in ventricular myocytes level relative to background) in frozen sections from ventricles in the Vehicle control, αCT1, and αCT11 treated groups. * p<0.05; not significant (ns) N=5 hearts/group.