## Supplement Material

## Materials and Methods

***Fusion proteins and pulldowns*** — Rat brain and rabbit cardiac cDNAs were used to engineer GST-fusion proteins spanning the intracellular domains of $\alpha_1$1.2. The C-terminus was further subdivided into shorter overlapping fragments and designated alphabetically (CT-B through CT-E) for clones expressing the rat brain $\alpha_1$1.2 isoform (Genbank M67515) and numerically (CT-1 through CT-23) for clones expressing the rabbit cardiac isoform (Genbank CAA33546). GST-fusion constructs containing intracellular loops I, II, and III were expressed from rabbit cardiac $\alpha_1$1.2 (**Online Table I**). The sequences were amplified by polymerase chain reaction (PCR) and cloned into pGEX-4T1 vector (Amersham Biosciences) in frame with GST, at the BamH I and Not I sites as described previously[1]. Clones were transformed in *E. coli* BL-21 strain, and fusion-protein expression was induced by isopropyl-$\beta$-D-thiogalactoside (IPTG). The proteins were solubilized in buffer containing (mM) 15 Tris-HCl, pH 7.4, 0.1mg/mL lysozyme, 1.5% N-lauroyl-sarcosine, 150 NaCl, 15 DTT, 10 EDTA, and protease inhibitors, and purified on Glutathione-Sepharose beads (Amersham Biosciences). The immobilized fusion proteins were then incubated with extracts from rat ventricle in the presence of 10 mM EGTA or 10 μM cyclosporine A, where indicated. Pulldowns were also performed from purified, recombinant calcineurin A$\alpha$ and B subunits, prepared and purified as described[2], or from HEK 293 cell lysates over-expressing constitutively active (CnA*) or full-length (F.L.) calcineurin tagged with HA (see *Cell transfections* below). For peptide competition assays, recombinant calcineurin was pre-incubated with peptide for up to 1 hr before pulldowns were performed with the GST fusion proteins. For affinity studies, 15 nM recombinant calcineurin was mixed with 7.5, 22.5 and 45 nM of immobilized and purified CT-8 in a final volume of 100 $\mu$L. Protein concentration was estimated using a standard curve of recombinant carbonic anhydrase and albumin (Sigma). The mixtures were incubated at 4°C for 2 hours and centrifuged at 5,000 rpm for 2 min. The supernatant fractions were aliquoted and pellets washed twice in the same buffer. Pellet and supernatant fractions were resolved by SDS-PAGE and analyzed by western blot.

Myc-tagged fusion proteins encoding the rabbit cardiac $\alpha_1$1.2 C-terminus of various lengths were sub-cloned into a pCMV-Tag1 (Stratagene) mammalian expression vector. Clones were confirmed by sequencing and then expressed in HEK 293 cells (see *Cell*

*transfections*). Cell lysates were incubated with anti-myc agarose conjugate (Sigma) for 2 hrs. at 4°C, washed in lysis buffer, and resolved by SDS-PAGE followed by western blot.

***Adenoviral infection and primary culture of neonatal rat ventricular myocytes*** — Cardiomyocytes were isolated from the ventricles of 1-2 day old rat pups (Sprague Dawley) and plated (1250 cells/mm$^2$) in medium containing 10% fetal calf serum as described[3]. Myocyte cultures obtained from this differential plating method contained less than 5% non-cardiomyocytes as determined microscopically using a myocyte-specific $\alpha$-actinin antibody (data not shown). Cells were transferred to serum-free medium containing the serum supplement Nutridoma (Roche) at 0.5x concentration 24 hours before treatment or infection with adenovirus. For expression of constitutively-active calcineurin and RCAN1, cells were infected with either AdCMVCnA*iresGFP or AdCMVMCIP, respectively, which contained the cDNAs for human calcineurin or RCAN1. Expression of each gene was driven by the CMV promoter, and AdCMVCnA*iresGFP co-expressed GFP from the bicistronic IRES transcription unit to ascertain infection efficiency and localize infected cells for voltage-clamp experiments. Experiments were performed at a multiplicity-of-infection (MOI) of 100 (≥95% infection efficiency), and voltage-clamp experiments (see *Electrophysiology*) were performed on plated cells 48 hours after infection.

For Western blot time course studies using a phospho-specific Ser-1928 antibody, cultured cardiomyocytes were treated with Isoproterenol (Iso; 1 $\mu$M) for time points as indicated – 1, 2, 4, 6 and 12 hrs. After each time point, cells were washed in PBS, harvested with membrane extraction buffer (see *Co-immunoprecipitation*) supplemented with phosphatase inhibitor cocktails (Sigma), and analyzed by SDS-PAGE. Where indicated, cells were pre-treated with Cyclosporine A (CsA; 10 $\mu$M) for 30 mins., and then supplemented with Iso for 4 hrs.

***Cell transfections*** — HEK 293 cells were cultured (37°C) in standard DMEM supplemented with 10% FBS. Cells were transfected at 60-70% confluency using Lipofectamine Plus reagent (Invitrogen) with constructs encoding the murine calcineurin A$\alpha$ subunit (full-length; tagged with HA or GFP) and constitutively active mutant (CnA*; tagged with HA) truncated at amino acid

398 (**Figure 3C**). When using the HA-tagged constructs, cells were co-transfected with pEGFP-C1 (BD Biosciences Clontech) to localize and quantify transfection efficiency. Twenty-four hours after transfection, cells were harvested and lyzed in buffer containing (mM) 10 Tris, pH 7.4, 150 NaCl, 1 DTT, 20% glycerol, 0.1% Triton-X 100, protease inhibitors (Roche) and phosphatase inhibitor cocktails I and II (Sigma), and used for experiments.

***Site-directed mutagenesis*** — PCR was performed on cloned plasmid encoding the GST fusion protein CT-8 ($\alpha_1$1.2 aa. 1909-2029), using the forward primer 5'-CTGGGTCGAAGGGCTGCCTTCCACCTGGAG-3' and reverse primer 5'-CTCCAGGTGGAAGGCAGCCCTTCGACCCAG-3' to replace serine-1928 with alanine. The template vector was digested with Dpn I, and the remaining mutant plasmid was directly transformed into *E. coli* XL1-Blue Supercompetent cells (Stratagene). Mutant plasmid was purified and sequenced to confirm mutation. A similar PCR approach was also used to mutate the putative PxIxIT motif in GST fusion protein CT-4 (PsI → AAA; $\alpha_1$1.2 aa. 2018-2020), using the forward primer 5'-CAACAGCAGCTTCGCGGCCGCCCACTGCGGC-3' and reverse primer 5'-GCCGCAGTGGGCGGCCGCGAAGCTGCTGTTG-3'. Two C-terminal truncations of CT-8 (aa. 1909-1946 and 1909-1971) were made via substitution of Gln1946 and Arg1971, respectively, into stop codons. The N-terminal truncations of CT-8 (1943-2029 and 1969-2029) were made via PCR using primers 5'-CGAGGATCCGACATCTCTCAGAAGACAGTCC-3' and 5'-TTGGATCCCTGCAGAGAAGCCATTCCC-3' along with the same reverse primer 5'-TCCGGGAGCTGCATGTGTCAGAGG-3', respectively. Finally, an alanine to proline mutation was introduced at residue 1959 within the $\alpha$ helix region ($\alpha_1$1.2 aa. 1952-1966) using the mutagenic oligonucleotide forward primer 5'- GTCCACCACCAGCCATTGGCAGTGGCG-3' and reverse primer 5'- CGCCACTGCCAATGGCTGGTGGTGGAC-3' to generate "CT-8 helix-Pro".

***In vitro kinase/phosphatase assay*** — *In vitro* phosphorylation and dephosphorylation assays on select GST fusion proteins were performed using recombinant PKA and calcineurin. Briefly, immobilized GST fusion proteins were phosphorylated with the recombinant murine PKA catalytic subunit ($\alpha$ isoform; Calbiochem) in the presence of $^{32}\gamma$P-ATP (30 min, 30°C) in kinase buffer containing (mM) 50 Tris, pH 7.2, 10 $MgCl_2$, 0.5 DTT, protease inhibitors. The kinase and unincorporated radiolabeled ATP were removed by thorough washing, and the proteins were divided into 2 aliquots, one of which was put aside for subsequent gel analysis. The other

aliquot was treated with a recombinant heterodimer of human calcineurin Aα + calcineurin B subunits (60 min, 30°C) in calcineurin buffer containing (mM) 100 Tris, pH 7.5, 200 NaCl, 12 $MgCl_2$, 1 DTT, 1 $CaCl_2$, 0.05% NP-40, and 2 μM calmodulin. Reactions were stopped in SDS buffer followed by PAGE and autoradiography. For PKC phosphorylation reactions, GST fusion proteins were incubated as described above with recombinant PKC (ξ isoform; Invitrogen) in kinase buffer containing (mM) 20 HEPES, pH 7.4, 10 $MgCl_2$, 0.1 EGTA, supplemented with 200 μg/mL DAG (Sigma), 1 mg/mL phosphatidylserine (Sigma), and 50 μM ATP.

***Cardiomyocyte Isolation*** — Adult mouse ventricular cardiomyocytes were isolated after enzymatic dissociation as described[4] with slight modification. Briefly, after retrograde perfusion with Krebs-Ringer solution at (2 mL/min, 5 min), the heart was perfused with fresh solution containing 0.8 mg/mL collagenase (Worthington type II) for another 12-15 min. The LV was removed and cut into small pieces in "KB" solution [(mM) taurine 10, glutamic acid 70, KCl 25, $KH_2PO_4$ 10, Glucose 22, EGTA 0.5, pH 7.2]. After trituration, cells were studied within 4 to 6 hours. All isolation steps were carried out at 36°C with continuous gassing with 95% $O_2$ + 5% $CO_2$. Only $Ca^{2+}$-tolerant, quiescent and rod-shaped cells, showing clear cross striations were used.

***Electrophysiology*** — Cultured neonatal rat cardiomyocytes or acutely isolated adult cardiomyocytes were studied in a continuously superfused (1.5 mL/min) recording chamber fixed to an inverted microscope. For recordings of L-type $Ca^{2+}$ current ($I_{Ca,L}$), the whole-cell voltage clamp configuration was used on cells bathed in (mM) 135 tetraethylammonium (TEA) Cl, 0.53 $MgCl_2$, 1.8 $CaCl_2$, 20 CsCl, 5 HEPES. The pipette solution for $I_{Ca,L}$ recording contained (mM): 110 CsOH, 90 aspartic acid, 20 CsCl, 10 TEACl, 10 HEPES, 10 EGTA, 5 Mg-ATP, 5 $Na_2$ creatine phosphate, 0.4 GTP (Tris), 0.1 leupeptin, pH 7.2 with CsOH. 5 mM EGTA or 10 mM BAPTA were substituted for 10 mM EGTA in this internal solution in some experiments. For certain studies, the amphotericin patch clamp technique was used. Here, patch clamp pipettes were prepared and the tips filled by dipping into internal solution containing (mM): 120 K-aspartate, 20 KCl, 5 $MgCl_2$, 10 HEPES, 1.8 $CaCl_2$, pH adjusted to 7.2 with KOH. The remainder of the pipette was back-filled with the same solution plus amphotericin B (200 μg/mL). Where indicated, bath solution was complemented with 10 μM CsA or FK-506. For peptide inhibitory

studies, peptides were dissolved in pipette solution and dialyzed into the cell during whole-cell configuration.

$I_{Ca,L}$ run-down, when present, typically occurs within the first 5 minutes of recording. In order to minimize the impact of run-down, we added 5 mmol/L Mg-ATP to the pipette solution (see above) and commenced data acquisition after 5-10 minutes of equilibration between pipette solution and intracellular contents[5]. Cells showing continuous current run-down (≈ 5%) were excluded from the analysis.

***Immunohistochemistry –*** Mice were anesthetized with intraperitoneal injection of pentobarbital (Sleepaway, Fort Dodge Animal Health, Fort Dodge, IA) and sacrificed by cervical dislocation in accordance with institutional and NIH animal use guidelines. A midline thoracotomy was performed and hearts excised while still beating. Hearts were grossly trimmed in the coronal plane and blotted free of excess blood prior to cryoembedding. Dorsal and ventral halves of heart specimens were placed in tissue freezing medium (TFM, Triangle BioScience, Raleigh, NC) and oriented for coronal sectioning in peelaway cryomolds (Polysciences, Warrington, PA). Cryoembedments were flash frozen by partial immersion in liquid-nitrogen-supercooled-isopentane and stored at -80°C until time of sectioning. Subsequently, samples were equilibrated to -22°C and eight-micron frozen sections were made on a Leica CM3000 cryostat (Wetzlar, Germany). Sections were air-dried and returned to frozen storage at -80°C until time of immunohistochemical staining.

For immunostaining, slides were thawed and sections fixed for 10 minutes in ice-cold methanol. Sections were rinsed in phosphate-buffered saline, pH7.3 (PBS) and permeabilized in 0.1% Triton X-100/PBS. Residual Triton surfactant was removed with a PBS rinse and sections were blocked with Mouse IgG Blocking Reagent according to kit manufacturer's instructions (Mouse on Mouse Kit, Vector Laboratories, Burlingame, CA) Sections were incubated with primary antibody (diluted in PBS) against $\alpha_1$1.2, calcineurin and $\alpha$-actinin (overnight, 4°C). After rinsing with PBS, bound primary antibody was detected with Alexa 488 or 555-labeled secondary antibody (1 hr, RT). Sections were rinsed in PBS and mounted with Vectashield (Vector Laboratories, Burlingame, CA). A Nikon (Eclipse TE2000-U) confocal microscope was used to scan the tissue sections viewed at 60X objective-lens magnification, and the images were obtained with the EZ-C1 version 2.0 software (Nikon Instruments).

***Peptides –*** All but one peptide used were synthesized (>70% purity) and HPLC analyzed by the Protein Chemistry Technology Center at UT Southwestern Medical Center. The peptides were designed based on the primary amino acid sequence of the C-terminus of the cardiac L-type $Ca^{2+}$ channel $\alpha_1$1.2 subunit (and corresponding to CT-8 GST fusion protein). The peptides overlapped by three residues as follows:

peptide 1976: TSLPRPCATPPATPGSRGWP (aa. 1976-1995 of $\alpha_1$1.2)

peptide 1993: GWPPPQPIPTLRLEGADSSEK (aa. 1993-2012 of $\alpha_1$1.2)

peptide 2010: SEKLNSSFPSIHCGSWSGEN (aa. 2010-2029 of $\alpha_1$1.2)

Calcineurin-inhibitory peptide was purchased from Sigma (#C-3937) and Santa Cruz (#sc-3055).

peptide AID: ITSFEEAKGLDRINERMPPRR (aa. 457-482 derived from autoinhibitory domain of rat brain calcineurin A$\alpha$)

***Reagents*** — Antibodies were purchased from Alomone Labs ($\alpha_1$1.2 #ACC003), BD Biosciences (CnA #610259), Santa Cruz (PP2B-A #9070, AKAP79 #10764), Roche (HA #12CA5), Sigma (GST #A7340, $\alpha$-actinin #A7811), Invitrogen Molecular Probes (GFP #A11122), and Cell Signaling Technology (myc #2276). The phospho-specific Ser-1928 antibody was prepared as previously described[6].

## Figure Legends

**Online Figure I: CnA-$\alpha_1$1.2 interaction mapping. A.** Potential PxIxIT motifs on $\alpha_1$1.2 CT-4. Alignment of primary amino acid sequences of murine NFAT1 (mNFAT1), human NFAT3 (hNFAT3), rat brain $\alpha_1$1.2, rabbit cardiac $\alpha_1$1.2, and the mutated CT-4 construct. GST-pulldowns of recombinant CnA using CT-4 or CT-4 PSI→AAA revealed comparable binding of CnA to each fusion protein. The membrane was re-blotted with anti-GST to confirm equivalence of fusion protein concentrations. **B.** Competition-binding assays were performed with peptides spanning CT-8. Peptides were incorporated in molar excess (10 μM) relative to CnA/B (20 nM) and CT-8 (100 nM). **C.** Prediction of CT-8 secondary structure based on Robson-Garnier algorithm analysis of primary amino acid sequence. CT-8 comprises of aa. 1909-2029, with two distinct halves – aa. 1909-1968 comprised of one alpha-helix (cylinder), and aa. 1969-2029 containing no definitive secondary structure but containing potential PxIxIT motifs (underlined). Overlapping peptides (1976, 1993 and 2010) of 20 aa. length comprised of the $\alpha_1$1.2 primary amino acid sequence are shown, with their respective PxIxIT motifs underlined. **D.** Schematic of CT-8 topology predicted by Robson-Garnier algorithm. CT-8 contains one alpha-helix (within 1943-1971) and an unstructured globular region (1969-2029). CT-8 truncations were used in pulldowns (**E**) from ventricular extracts. **F.** Immunoprecipitation experiments of myc-tagged $\alpha_1$1.2 C-terminus constructs co-expressed in HEK 293 cells with GFP-CnA$\alpha$. Immunoblots for GFP show CnA binds $\alpha_1$1.2 only in the presence of aa. 1909-1969, consistent with GST pulldown results.

**Online Figure II: CnA binds $\alpha_1$1.2 with high affinity.** Representative GST-pulldown experiment with recombinant CnA/B (15 nM) and increasing concentrations (7.5, 22.5 and 45 nM) of CT-8. After each pulldown, supernatant (S) was saved to compare unbound to bound CnA co-sedimenting with bead-immobilized GST-CT-8 pellet (P). CT-8:CnA = molar ratio of CT-8 to CnA.

**Online Figure III: Isoproterenol-induced phosphorylation of endogenous$\alpha_1$1.2.** To test the efficacy and specificity of the anti-Ser-1928 phospho-specific antibody, we treated NRCMs with Iso (1 μM), a potent β-adrenergic receptor agonist. As expected, endogenous Ser-1928 was

minimally phosphorylated under basal conditions (0 hr), but robustly phosphorylated at all time points on exposure to Iso.

**Online Figure IV: Acute suppression of $I_{Ca,L}$ by CsA. A.** I-V relation depicting mean $I_{Ca,L}$ in the presence-vs-absence of CsA. CsA-induced suppression of $I_{Ca,L}$ elicited no shift in the steady-state I-V relation (n=8 myocytes from 3 hearts in each group). **B.** Mean data demonstrating that CsA-induced suppression of $I_{Ca,L}$ did not elicit changes in fast ($\tau$1) or slow ($\tau$2) time-constants of $I_{Ca,L}$ inactivation.

# Tables

**Online Table I. Amino acid residues in $\alpha_1$1.2 GST-fusion proteins.**

| Fusion | Rabbit cardiac $\alpha_1$1.2 | Rat brain $\alpha_1$1.2 |
|---|---|---|
| **N-Term** | 1-154 | **1-124** |
| **Loop I** | **437-554** | 409-526 |
| **Loop II** | **785-930** | 754-901 |
| **Loop III** | **1196-1248** | 1165-1219 |
| C-Term | 1507-2171 | 1477-2140 |
| **CT-1** | **1507-1733** | 1477-1703 |
| **CT-4** | **1909-2171** | 1879-2140 |
| **CT-7** | **2030-2171** | 2000-2140 |
| **CT-8** | **1909-2029** | 1879-1999 |
| **CT-23** | **1622-1905** | 1592-1875 |
| **CT-B** | 1724-1847 | **1694-1817** |
| **CT-C** | 1834-1957 | **1804-1927** |
| **CT-D** | 1944-2067 | **1914-2037** |
| **CT-E** | 2054-2171 | **2024-2140** |
| **PKA/PKC/PKG site** | **Ser 1928** | Ser 1898 |

Sequences listed in bold font were synthesized and studied here. Sequences not listed in bold are provided to facilitate comparison between rabbit cardiac and rat brain $\alpha_1$1.2.

## Online Table II. Summary of reports in the literature on the effects of calcineurin on $I_{Ca,L}$.

| Author | Journal | Compound/Method | Effect | Species | Cell/Tissue |
|---|---|---|---|---|---|
| **Studies that report inhibitory effects of calcineurin on $I_{Ca,L}$** | | | | | |
| Chad JE and Eckert R | *J. Physiol.* (1986) **378**:31-51. | 40 µg/mL PP2B dialysis | "small" ↓ $I_{Ca,L}$ | *Helix* | neuron |
| Schuhmann K et al. | *J. Gen. Physiol.* (1997) **110**:503-513. | 1 µg/mL PP2B diffusion (inside-out patch) | ~50% ↓ $I_{Ca,L}$ | Human | smooth muscle |
| Lulkyanetz EA et al. | *J. Physiol.* (1998) **510**:371-385. | PP2B over-expression | ~60% ↓ $I_{Ca,N}$<br>No effect on $I_{Ca,L}$ | Rat/mouse hybrid | NG108-15 cells |
| Burley JR et al. | *Eur. J. Neurosci.* (2000) **12**:2881-2891. | PP2B over-expression | ~30% ↓ $I_{Ca}$ (L, T, N-type) | Rat/mouse hybrid | NG108-15 cells |
| Santana LF et al. | *J. Physiol.* (2002) **544**:57-69. | 100 nM CsA | ~33% ↑ $I_{Ca,L}$ | Mouse | Ventriculocyte |
| **Studies that report no effect of calcineurin on $I_{Ca,L}$** | | | | | |
| Frace AM et al. | *J. Physiol.* (1993) **472**:305-326. | µM inhibitory peptide | No effect on $I_{Ca,L}$ | Frog | Cardiomyocyte |
| McCall E et al. | *Circ. Res.* (1996) **79**:1110-1121 | 5 µM FK-506 | No effect on $I_{Ca,L}$ | Rat | Ventriculocyte |
| duBell WH et al. | *J. Physiol.* (1997) **501**:509-516 | 25 µM FK-506 | No effect on $I_{Ca,L}$ | Rat | Ventriculocyte |
| Zeilhofer HU et al. | *Neurosci.* (2000) **95**:235-241. | 10 µM FK-506 | No effect on $I_{Ca,L}$ | Rat | GH3 cells |
| Yatani A et al. | *J. Mol. Cell Cardiol.* (2001) **33**:249-259. | 1 µM CsA | No effect on $I_{Ca,L}$ | Mouse | Ventriculocyte |
| Su Z et al. | *J. Pharmacol. Exp. Ther.* (2003) **304**:334-341. | 10 µM FK-506 | No effect on $I_{Ca,L}$ | Rabbit/Mouse | Ventriculocyte |
| **Studies that report activating effects of calcineurin on $I_{Ca,L}$** | | | | | |
| Mijares A et al. | *J. Mol. Cell Cardiol.* (1997) **29**:2067-2076. | 15 mg/kg/day CsA subcutaneous injection<br>100 pM – 80 µM CsA | ~25% ↓ $I_{Ca,L}$<br>~11 – 98% ↓ $I_{Ca,L}$ | Guinea pig | Cardiomyocyte |
| Yasutsune T et al. | *Br. J. Pharmacol.* (1999) **126**:717-729. | 10 µM FK-506<br>30 µM FK-506 | No effect on $I_{Ca,L}$<br>~22% ↓ $I_{Ca,L}$ | Pig | Coronary artery strips |
| Norris CM et al. | *Neurosci.* (2002) **110**:213-225. | 0.5 – 50 µM FK-506<br>20 µM CsA | ~10 – 50% ↓ $I_{Ca,L}$<br>~25% ↓ $I_{Ca,L}$ | Rat | hippocampal cells |
| Fauconnier J et al. | *Am. J. Physiol. Heart Circ. Physiol.* (2005) **288**:778-786 | 25 µM FK-506 | ~25% ↓ $I_{Ca,L}$ | Rat | Ventriculocyte |

## References


**1.** Gao T, Cuadra AE, Ma H, Bunemann M, Gerhardstein BL, Cheng T, Eick RT, Hosey MM. C-terminal fragments of the alpha 1C (CaV1.2) subunit associate with and regulate L-type calcium channels containing C-terminal-truncated alpha 1C subunits. *J Biol Chem.* 2001;276:21089-21097.

**2.** Mondragon A, Griffith EC, Sun L, Xiong F, Armstrong C, Liu JO. Overexpression and purification of human calcineurin alpha from Escherichia coli and assessment of catalytic functions of residues surrounding the binuclear metal center. *Biochemistry.* 1997;36:4934-4942.

**3.** Ni YG, Berenji K, Wang N, Oh M, Sachan N, Dey A, Cheng J, Lu G, Morris DJ, Castrillon DH, Gerard RD, Rothermel BA, Hill JA. Foxo Transcription Factors Blunt Cardiac Hypertrophy by Inhibiting Calcineurin Signaling. *Circulation.* 2006;114:1159-1168.

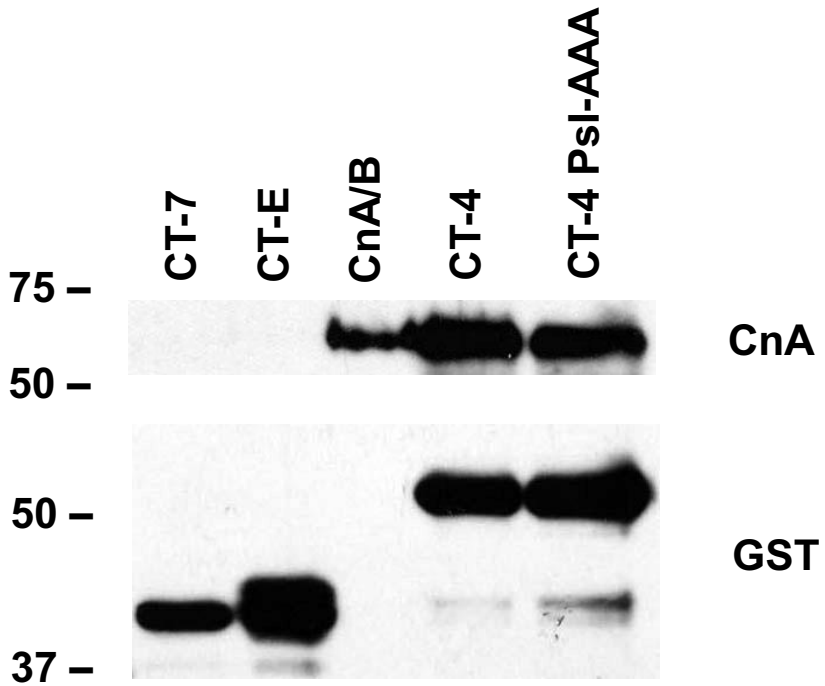
**4.** Wang Z, Kutschke W, Richardson KE, Karimi M, Hill JA. Electrical remodeling in pressure-overload cardiac hypertrophy: Role of calcineurin. *Circulation.* 2001;104:1657-1663.

**5.** Wang Y, Wagner MB, Joyner RW, Kumar R. cGMP-dependent protein kinase mediates stimulation of L-type calcium current by cGMP in rabbit atrial cells. *Cardiovasc Res.* 2000;48:310-322.

**6.** Hall DD, Davare MA, Shi M, Allen ML, Weisenhaus M, McKnight GS, Hell JW. Critical role of cAMP-dependent protein kinase anchoring to the L-type calcium channel Cav1.2 via A-kinase anchor protein 150 in neurons. *Biochemistry.* 2007;46:1635-1646.

A

| | |
|---|---|
| | P x I x I T |
| mNFAT1 | S P R I E I T P S H E |
| hNFAT3 | C P S I R I T S I S P |
| Brain $\alpha_1$1.2 | F P S I H C S S W S E |
| Cardiac $\alpha_1$1.2 (CT-4) | F P S I H C G S W S G |
| CT-4 PsI-AAA | F A A A H C G S W S G |

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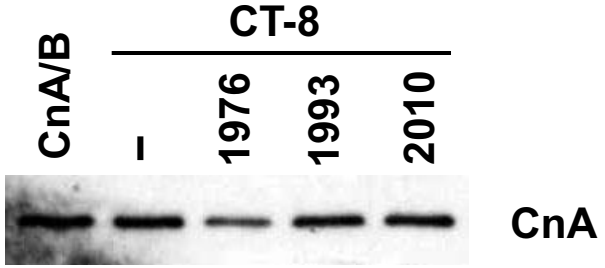
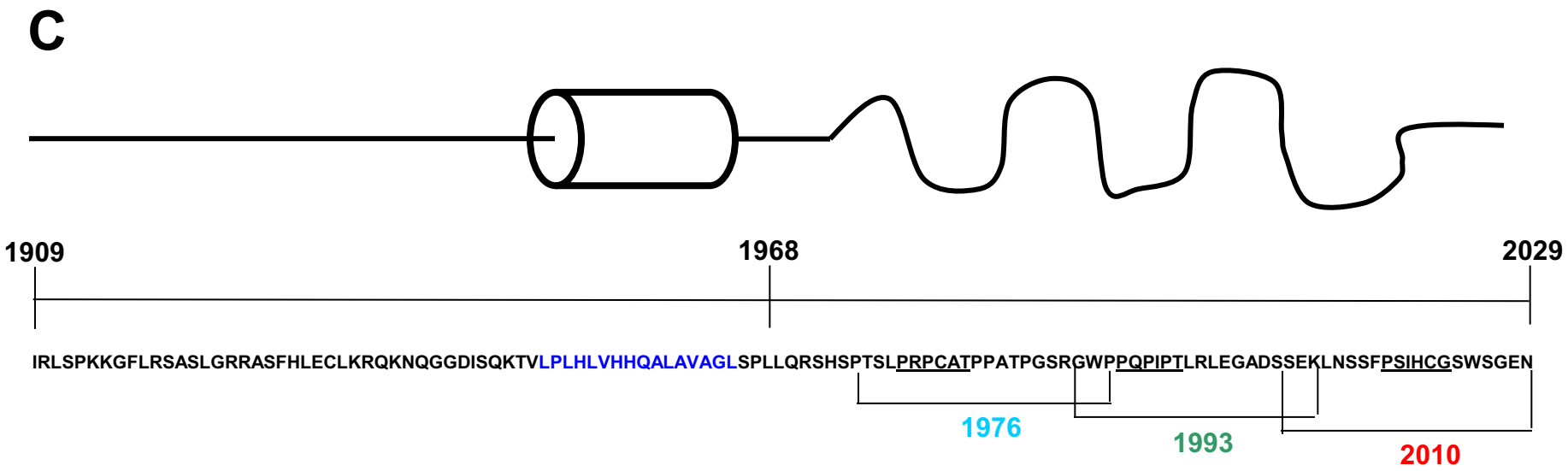



Online Figure I




**Peptides**

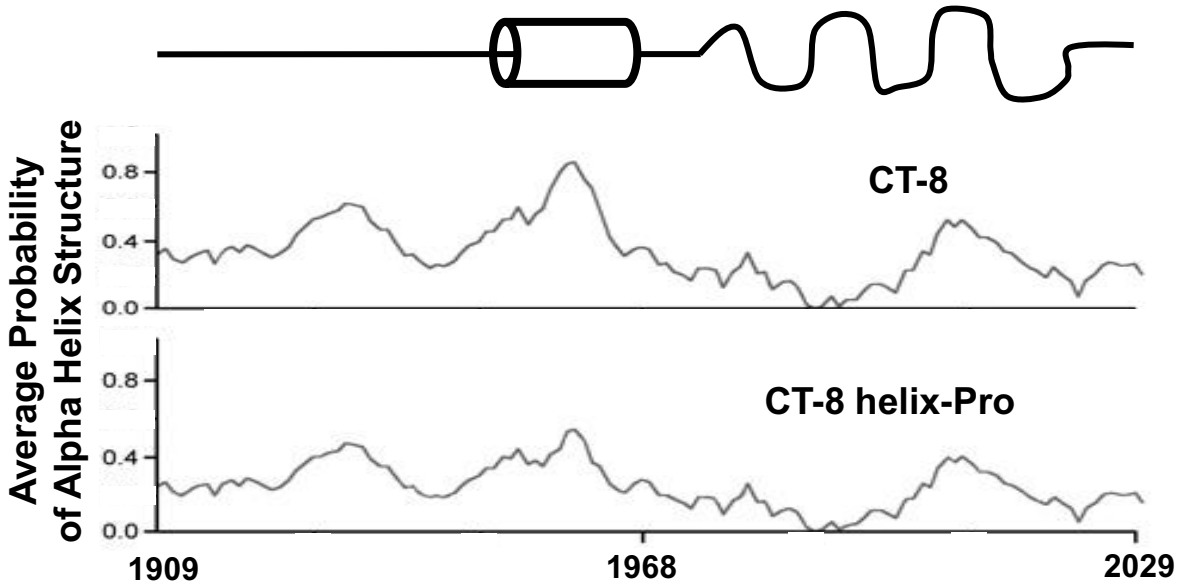
**1976**-1995: **TSLPRPCATPPATPGSRGWP**
**1993**-2012: **GWPPQPIPTLRLEGADSSEK**
**2010**-2029: **SEKLNSSFPSIHCGSWSGEN**

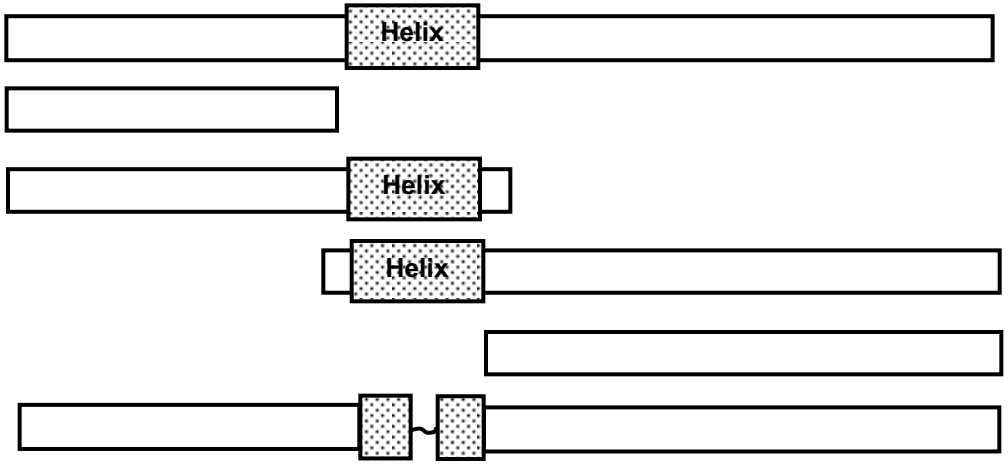
**alpha-helix shown in blue**
Potential PxIxIT motifs are underlined



**Online Figure I**

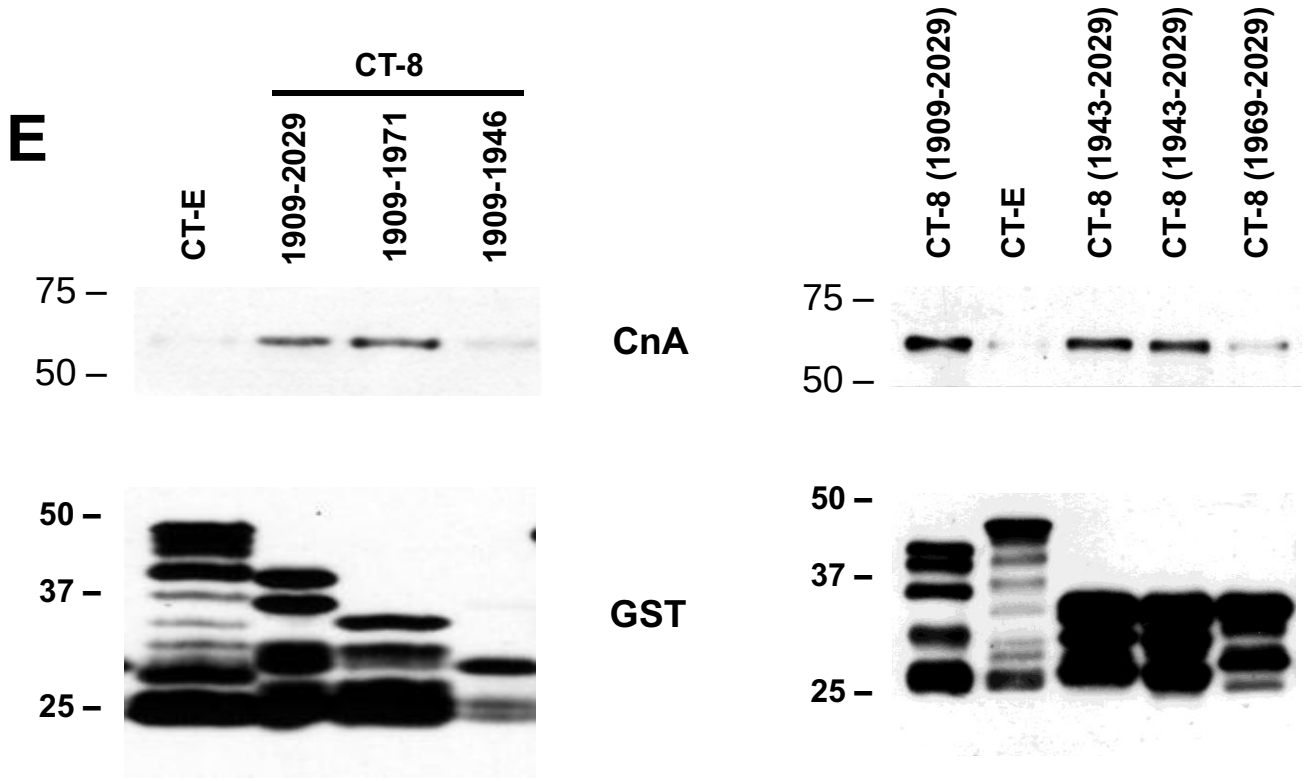
D




| Residue | Calcineurin Binding |
|---|---|
| 1909-2029 | + |
| 1909-1946 | – |
| 1909-1971 | + |
| 1943-2029 | + |
| 1969-2029 | – |
| CT-8 helix-Pro | – |

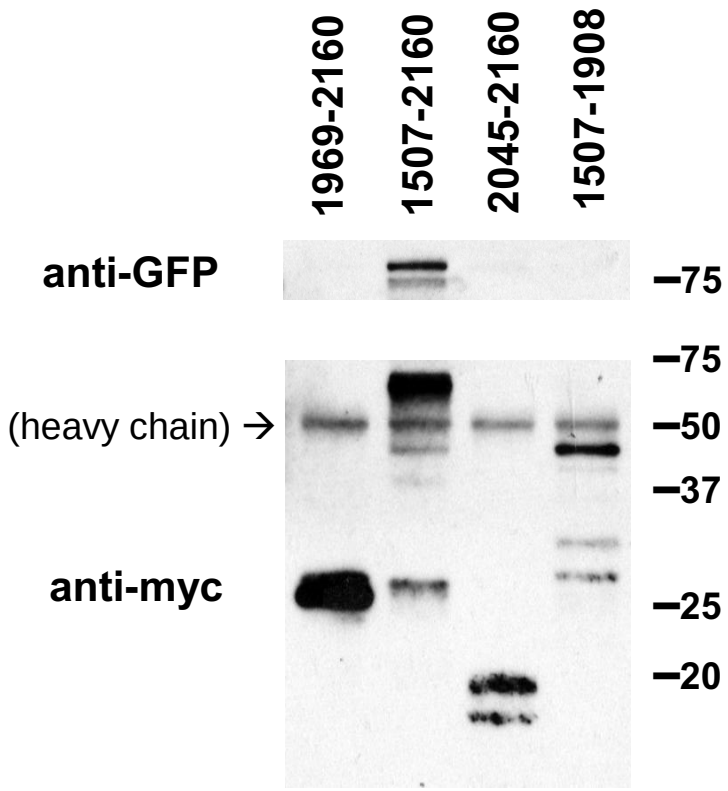
**Online Figure I**




**Online Figure I**

**F**

| Myc-tagged construct | a.a | Description |
|---|---|---|
| CT-8 | 1507-2160 | Entire $\alpha_1$1.2 C-terminus |
| | 1507-1908 | |
| | 2045-2160 | |
| -8 | 1969-2160 | Encoding half of CT-8; lacking alpha-helix |

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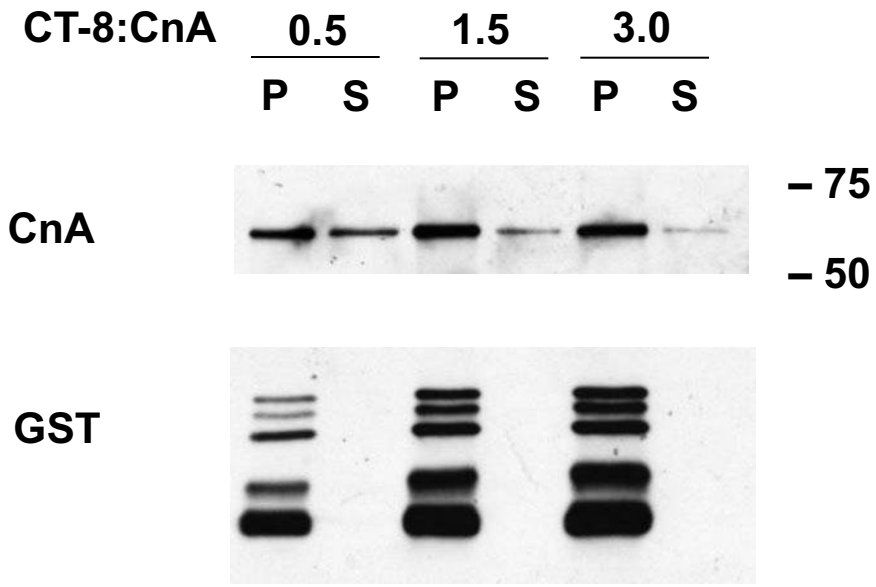


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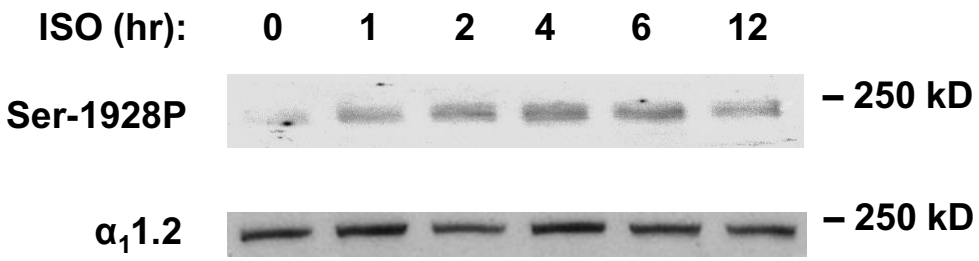
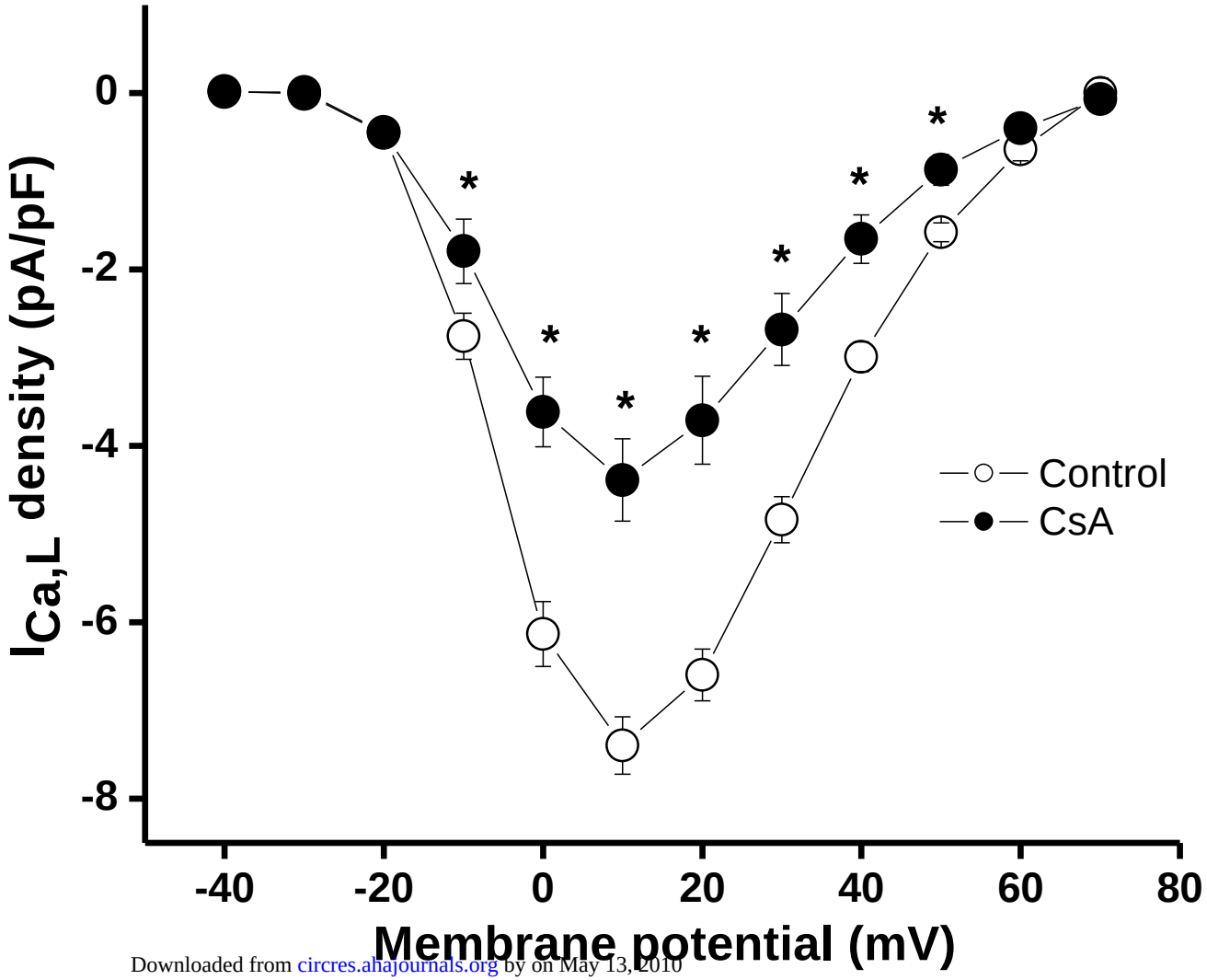


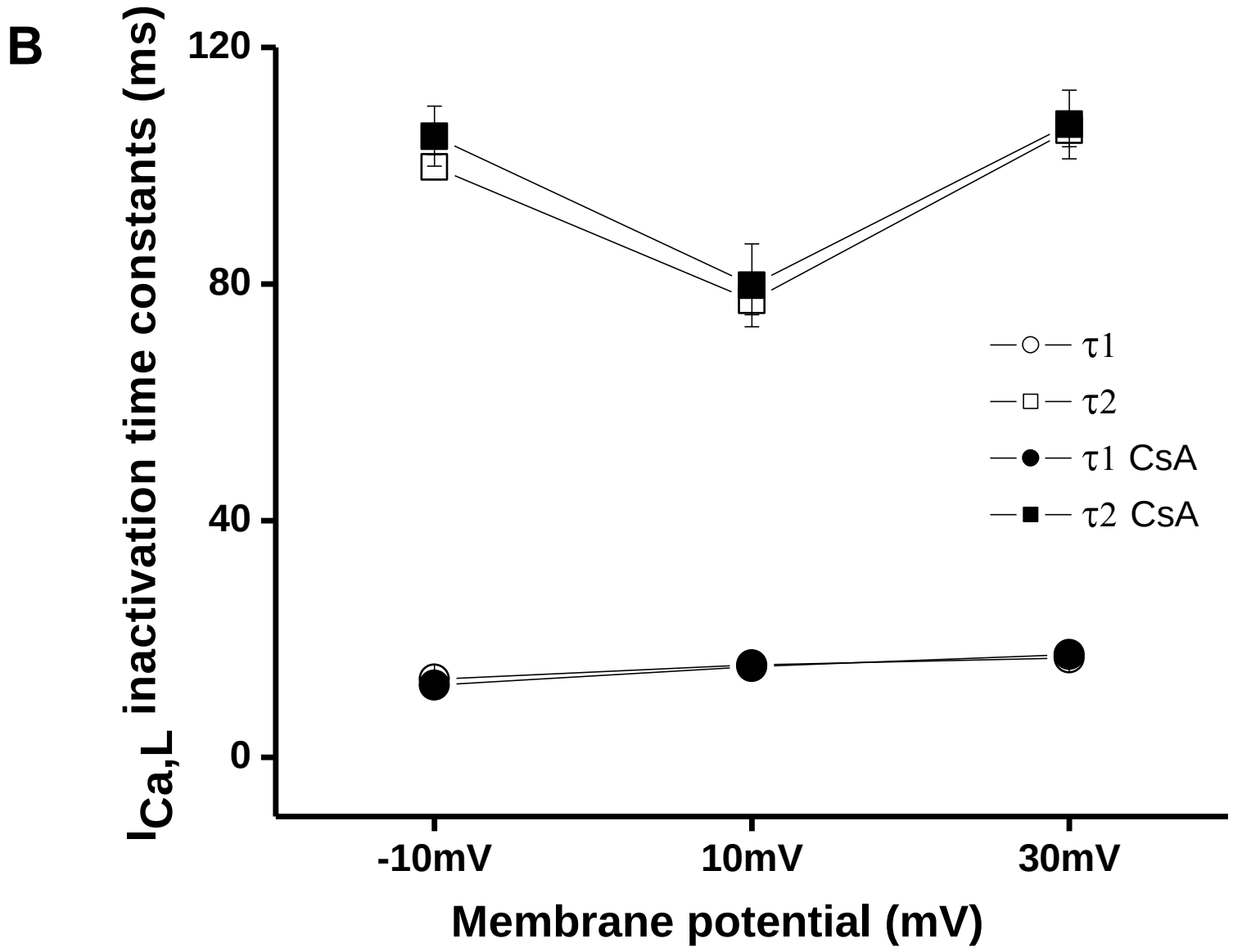

**Online Figure III**

A






**Online Figure IV**




**Online Figure IV**