Expanded View Figures

Figure EV1. Mapping the $Ca_V1.2$ IQ binding site in α-actinin and apoCaM.

A Overlay of ^{15}N-^{1}H HSQC spectra of ^{15}N-labeled α-actinin-1 CH1/CH2 domain (residues 19–192) by itself (black peaks) and after addition of saturating, unlabeled IQ peptide (red peaks).

B Overlay of ^{15}N-^{1}H HSQC spectra of ^{15}N-labeled α-actinin-1 EF-hand domain (residues 750–892) by itself (black peaks) and after addition of saturating, unlabeled IQ peptide (red peaks).

C Overlay of ^{15}N-^{1}H HSQC spectra of ^{15}N-labeled α-actinin-1 C-lobe (α-actinin-1 EF34; residues 822–892) by itself (black peaks) and after addition of saturating, unlabeled IQ peptide (red peaks).

D Overlay of ^{15}N-^{1}H HSQC spectra of ^{15}N-labeled apoCaM by itself (black peaks) and after addition of saturating, unlabeled IQ peptide (red peaks).

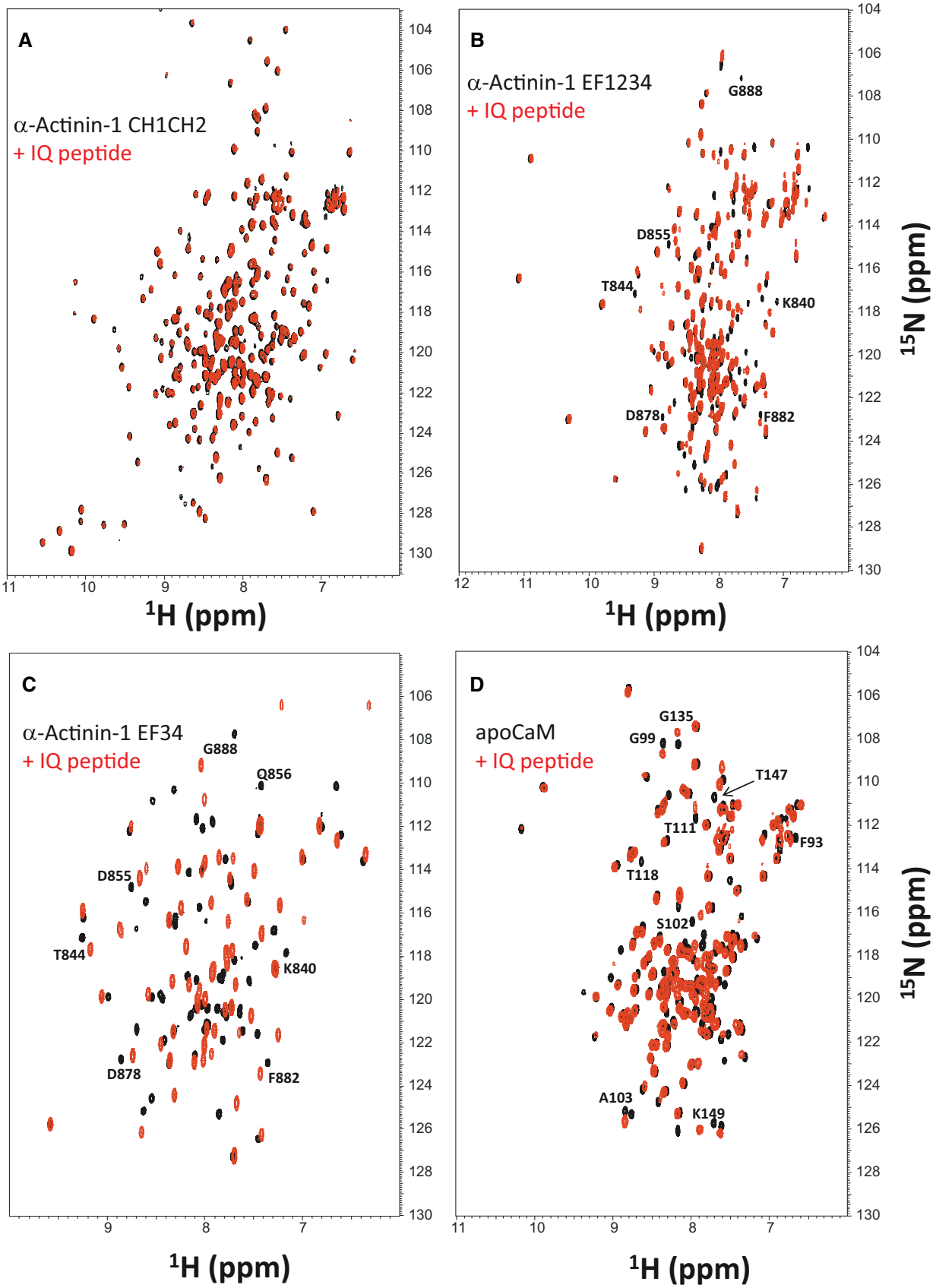
A
α-Actinin-1 CH1CH2
+ IQ peptide
B
α-Actinin-1 EF1234
+ IQ peptide
G888
D855
T844
K840
D878
F882
C
α-Actinin-1 EF34
+ IQ peptide
G888
Q856
D855
T844
K840
D878
F882
D
apoCaM
+ IQ peptide
G135
G99
T147
T111
F93
T118
S102
A103
K149
^{1}H (ppm)
^{15}N (ppm)

Figure EV1.

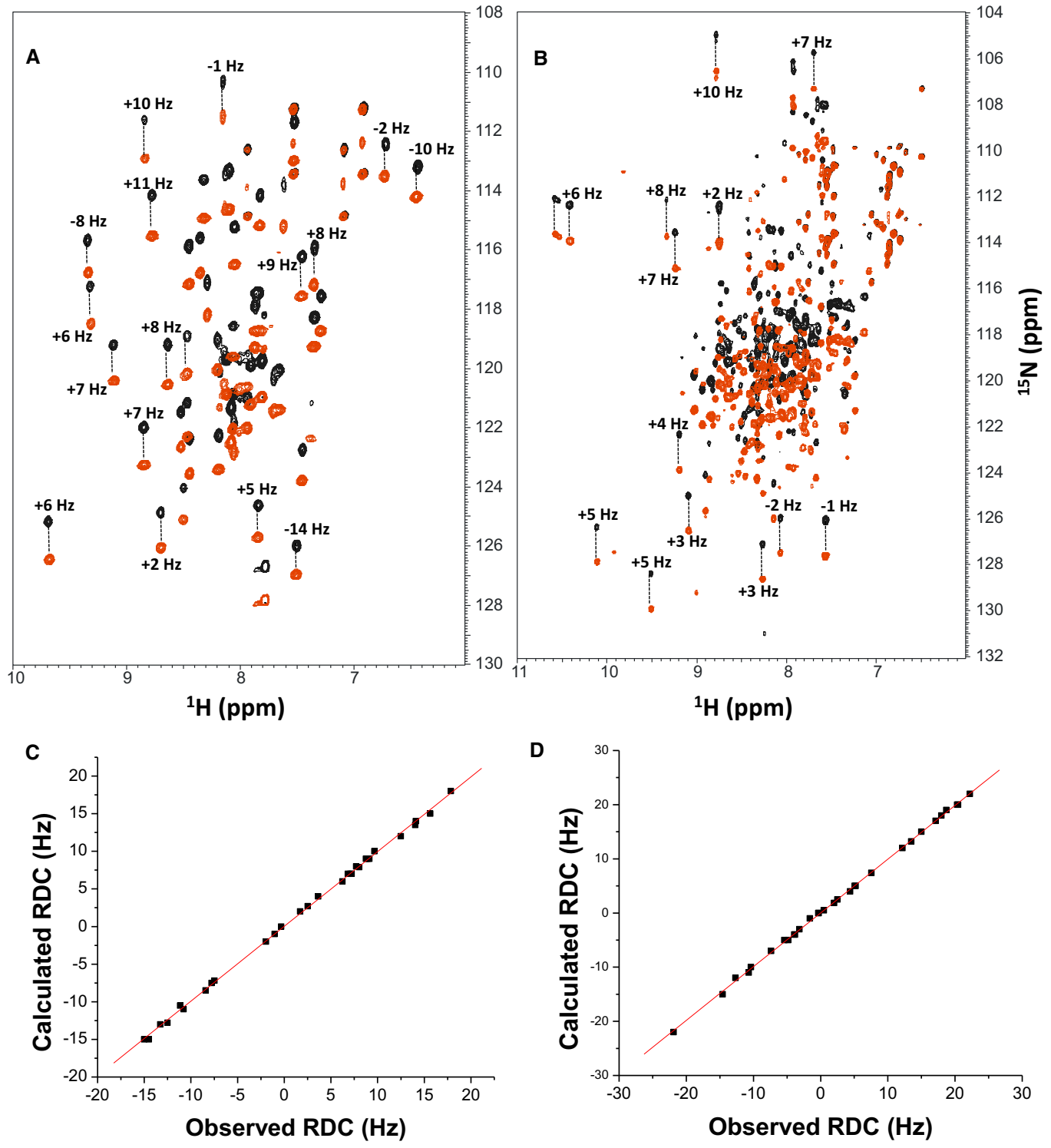

Figure EV2.

Figure EV2. NMR structural analysis using residual dipolar couplings (RDCs).

A, B ^{15}N-^{1}H HSQC-IPAP spectra (Tjandra & Bax, 1997) of ^{15}N-labeled α-actinin-1_EF34 (A) or apoCaM (B) in the presence of saturating unlabeled IQ peptide. Representative and measured RDC values are marked above-selected peaks. Samples used in HSQC-IPAP experiments were prepared by adding 5 mg of ^{15}N-labeled protein to 0.5 ml of NMR buffer containing 12 mg/ml of filamentous bacteriophage Pf1. HSQC-IPAP spectra were recorded in the presence of Pf1 (A, B) and absence of Pf1 (not shown). Residual dipolar couplings (RDCs) were measured (in Hz) as the difference in splitting for the ^{15}N-[^{1}H] doublet components relative to the isotropic $^{1}J_{NH}$ coupling. RDCs measured for 34 residues (α-actinin-1_EF34/IQ) and 36 residues (apoCaM/IQ) served as orientational structural restraints applied during the refinement phase of the structure calculation (Schwieters *et al*, 2003).

C, D Plots showing the correlation between observed versus calculated backbone RDCs predicted from the final NMR-derived structures for α-actinin-1_EF34/IQ and apoCaM/IQ, respectively. The correlation coefficient (r^2) equals 0.99, and Q is 0.095 and 0.089 for α-actinin-1_EF34/IQ and apoCaM/IQ, respectively.

Figure EV3. α-actinin-1 and apoCaM do not form a ternary complex with IQ.

A Overlay of ^{15}N-^{1}H HSQC spectra of ^{15}N-labeled α-actinin-1 EF-hand domain (residues 750–892) by itself (black peaks), after adding an equivalent amount of unlabeled IQ peptide (red peaks), and after adding fivefold excess of unlabeled apoCaM combined with the 1 equivalent of unlabeled IQ peptide (green peaks).

B Overlay of ^{15}N-^{1}H HSQC spectra of ^{15}N-labeled apoCaM by itself (black peaks), after adding an equivalent amount of unlabeled IQ peptide (red peaks), and after adding fivefold excess of unlabeled α-actinin-1 combined with the 1 equivalent of unlabeled IQ peptide (green peaks).

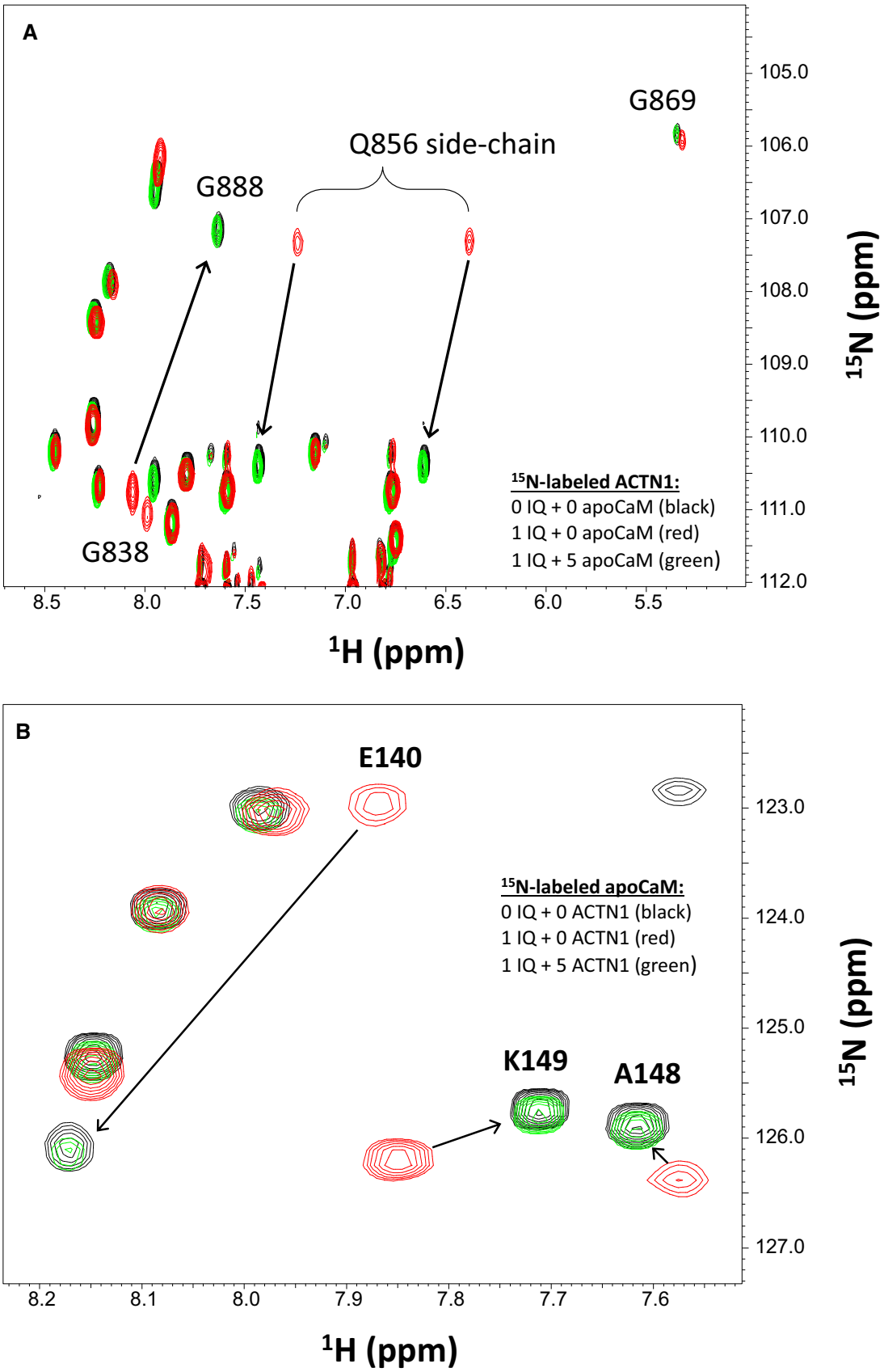

Figure EV3.

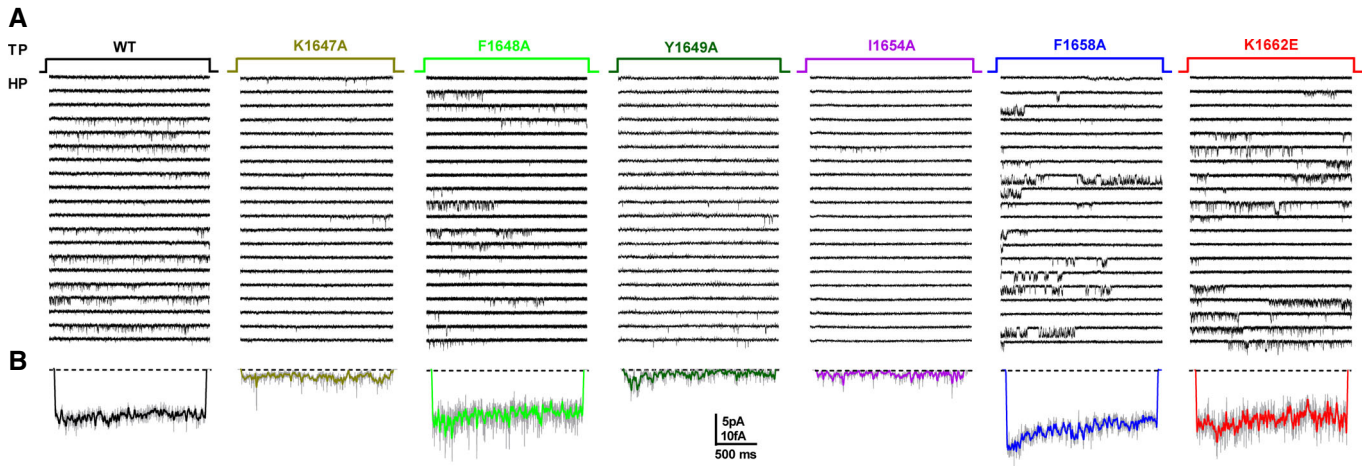

Figure EV4. Representative single-channel traces for $Ca_V1.2$ WT, K1647A, F1648A, Y1649A, I1654A, F1658A, and K1662E.
HEK293 cells were transfected with $\alpha_1$1.2, $\alpha_2\delta$-1, and β_{2A} before cell-attached patch recording.

A, B Holding potential (HP) was −80 mV and test potential (TP) 0 mV. Shown are 20 consecutive sweeps from representative experiments (A) and mean assemble average currents (B) for all experiments for $Ca_V1.2$ WT and each mutant.

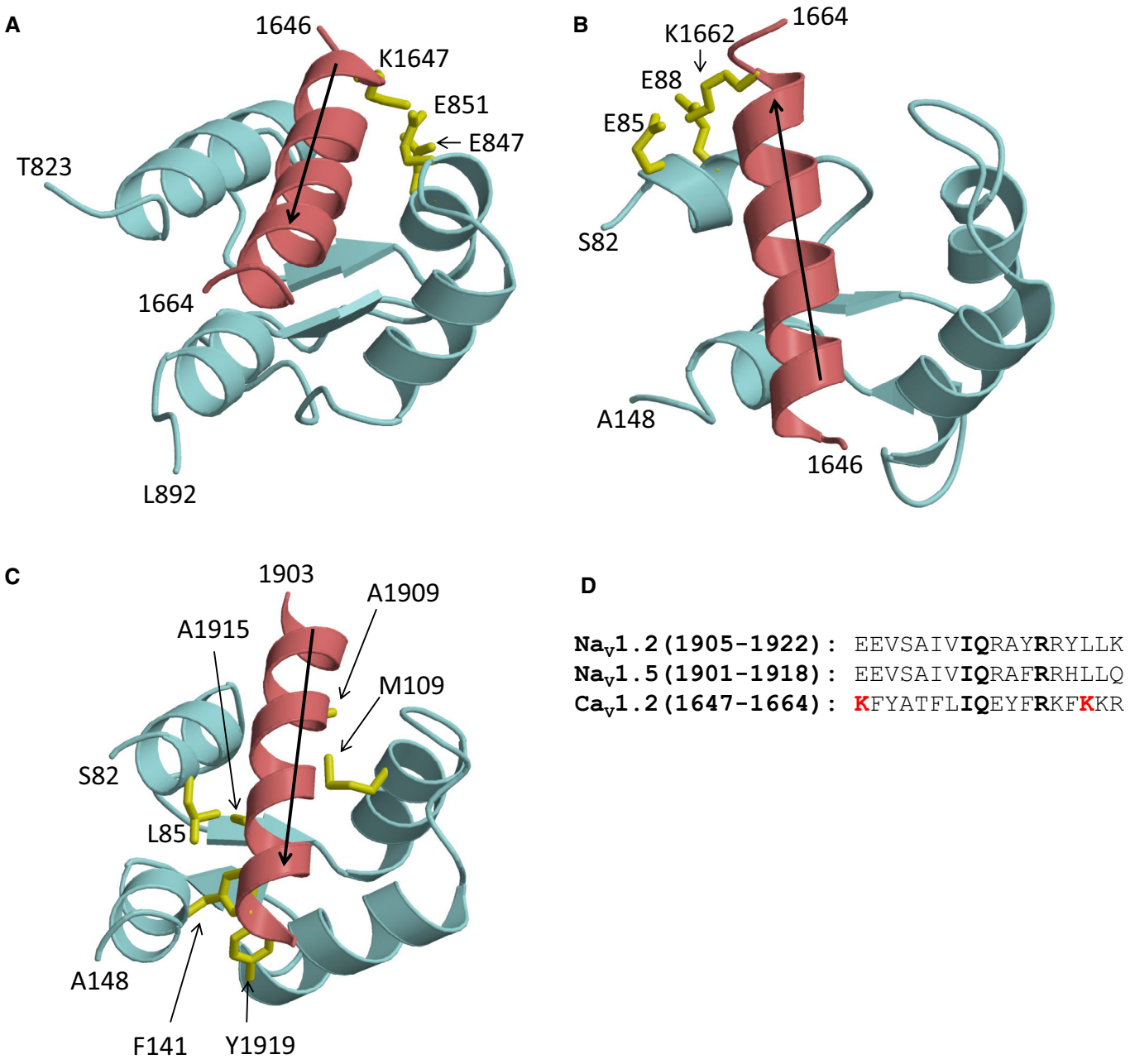

Figure EV5. IQ peptide binds with opposite polarity to α-actinin-1 versus apoCaM.

A NMR structure of α-actinin-1 (cyan) bound to $Ca_V1.2$ IQ motif (red).

B NMR structure of apoCaM (cyan) bound to $Ca_V1.2$ IQ motif (red).

C NMR structure apoCaM (cyan) bound to the $Na_V1.2$ IQ motif (red). PDB accession number is 2KXW (Feldkamp *et al*, 2011). Non-conserved intermolecular contacts between hydrophobic side-chain atoms are colored yellow. The β-methyl side-chain atoms of $Na_V1.2$ residues A1909 and A1915 are 2.5 Å away from side-chain methyl atoms of apoCaM residues M109 and L85, respectively. The aromatic side-chain atoms of $Na_V1.2$ residue Y1919 are 3.3 Å away from aromatic side-chain atoms of apoCaM residue F141.

D Amino acid sequence alignment of IQ motifs from $Na_V1.2$, $Na_V1.5$, and $Ca_V1.2$. Residues that are conserved between Na_V and Ca_V are shown in boldface font. Lysine residues in $Ca_V1.2$ that form intermolecular salt bridges in the $Ca_V1.2$ IQ peptide complexes with α-actinin-1_EF3/4 and apoCaM and are not conserved between the Ca^{2+} and Na^{2+} channels are colored red.

Data information: (A–C), Black arrows depict directionality of the IQ helix.