

Expanded View Figures

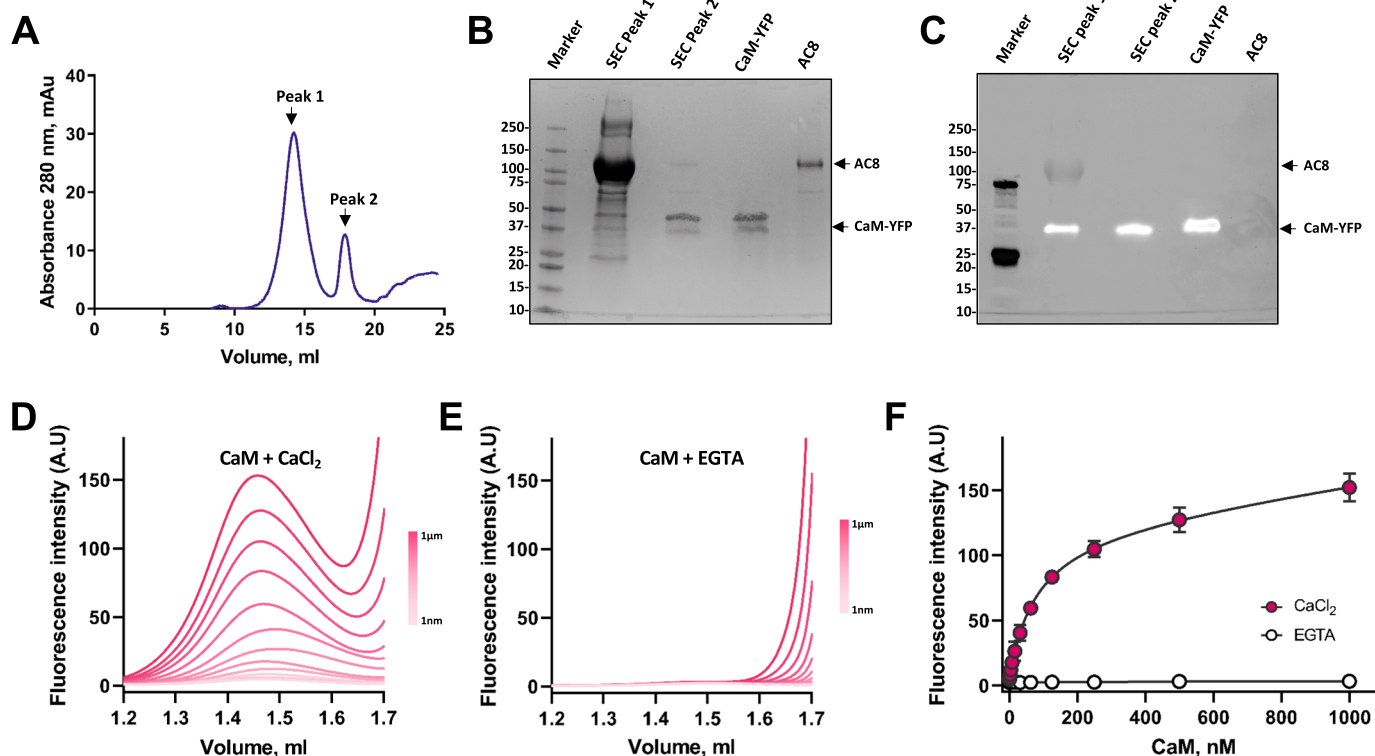
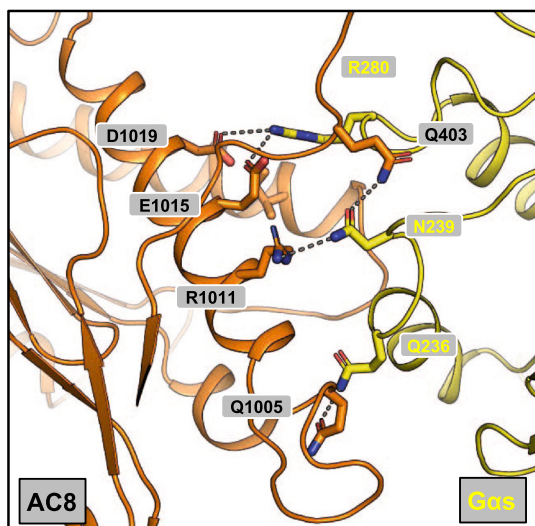
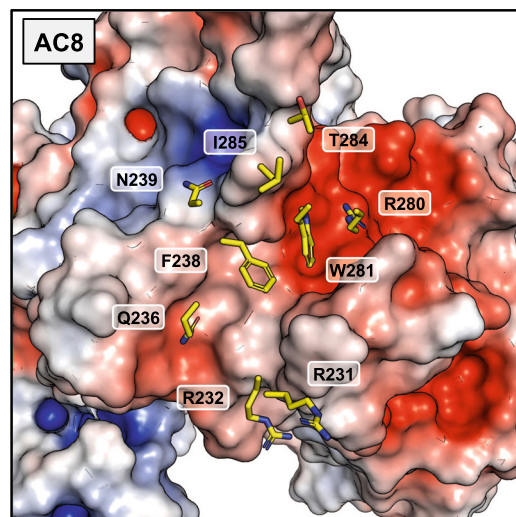
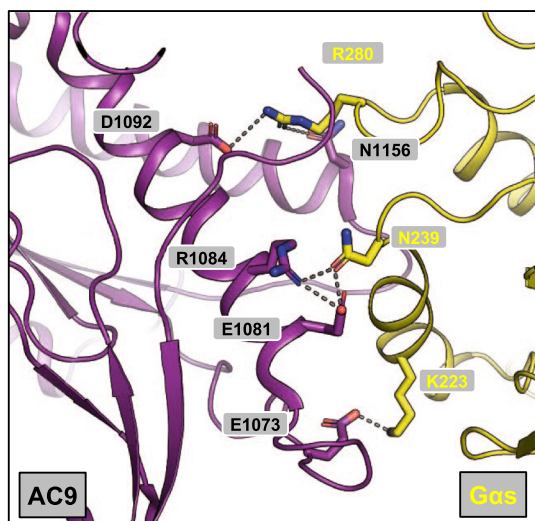
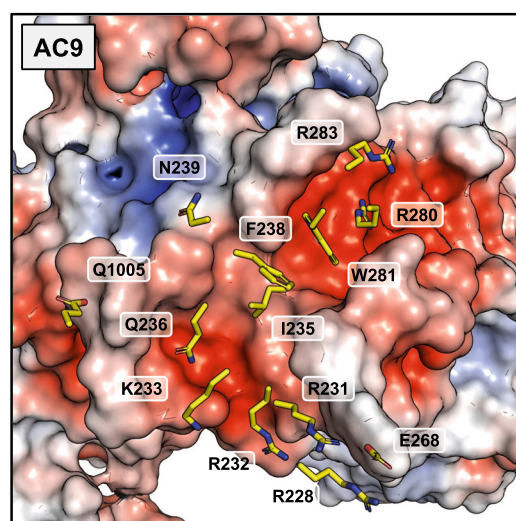
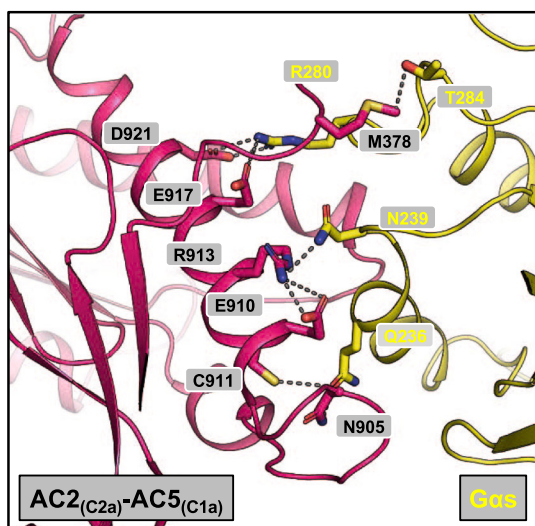
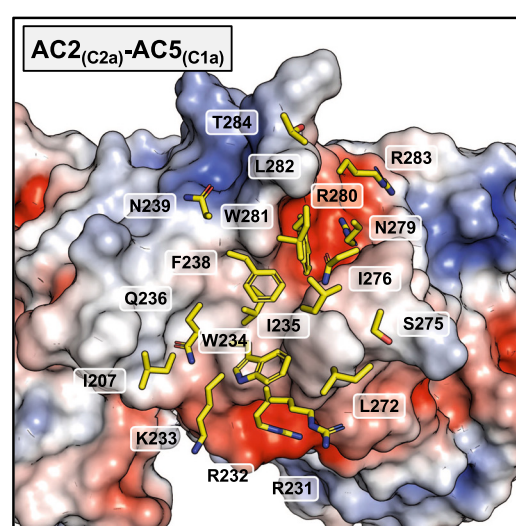


Figure EV1. Purified AC8 binds CaM with high affinity in the presence of Ca^{2+} .

(A) The size exclusion chromatography (SEC) profile of the AC8- Ca^{2+} /CaM-YFP complex. (B, C) SDS-PAGE analysis of AC8- Ca^{2+} /CaM-YFP complex. The complex was prepared by mixing purified recombinant AC8 and CaM-YFP in 1:2 ratio, respectively. The panel (B) shows the Coomassie stained SDS-PAGE of AC8- Ca^{2+} /CaM complex after SEC. Panel (C) shows overlaid prestained and YFP-fluorescence images of the SDS-PAGE displayed in panel (B). (D, E) Representative FSEC profiles an increase in fluorescence intensity of the AC8 elution peak with increasing concentration of CaM. (F) The saturation-binding curves show nanomolar affinity (77.7 ± 10.9 nM, $n = 4$, technical replicates) of AC8 for CaM in presence of calcium and no AC8-CaM interaction in absence of calcium. For the experiments in panel (F), the data are shown as mean $\pm$ S.E.M. ($n = 4$). Source data are available online for this figure.

A**D****B****E****C****F**

**Figure EV2. Gas binding interfaces in ACs.**

(A–C) Depiction of AC-Gas interfacial residues observed in the cryo-EM structure of AC8-CaM-Gas (top), AC9-Gas (middle), and crystal structure of chimeric AC2_(C2a)-AC5_(C1a)-Gas complex (bottom). (D–F) Electrostatic surface representation of AC8 (top), AC9 (middle), and chimeric AC2_(C2a)-AC5_(C1a) (bottom) showing interfacial residues of Gas (yellow colour) within 4 Å of AC.

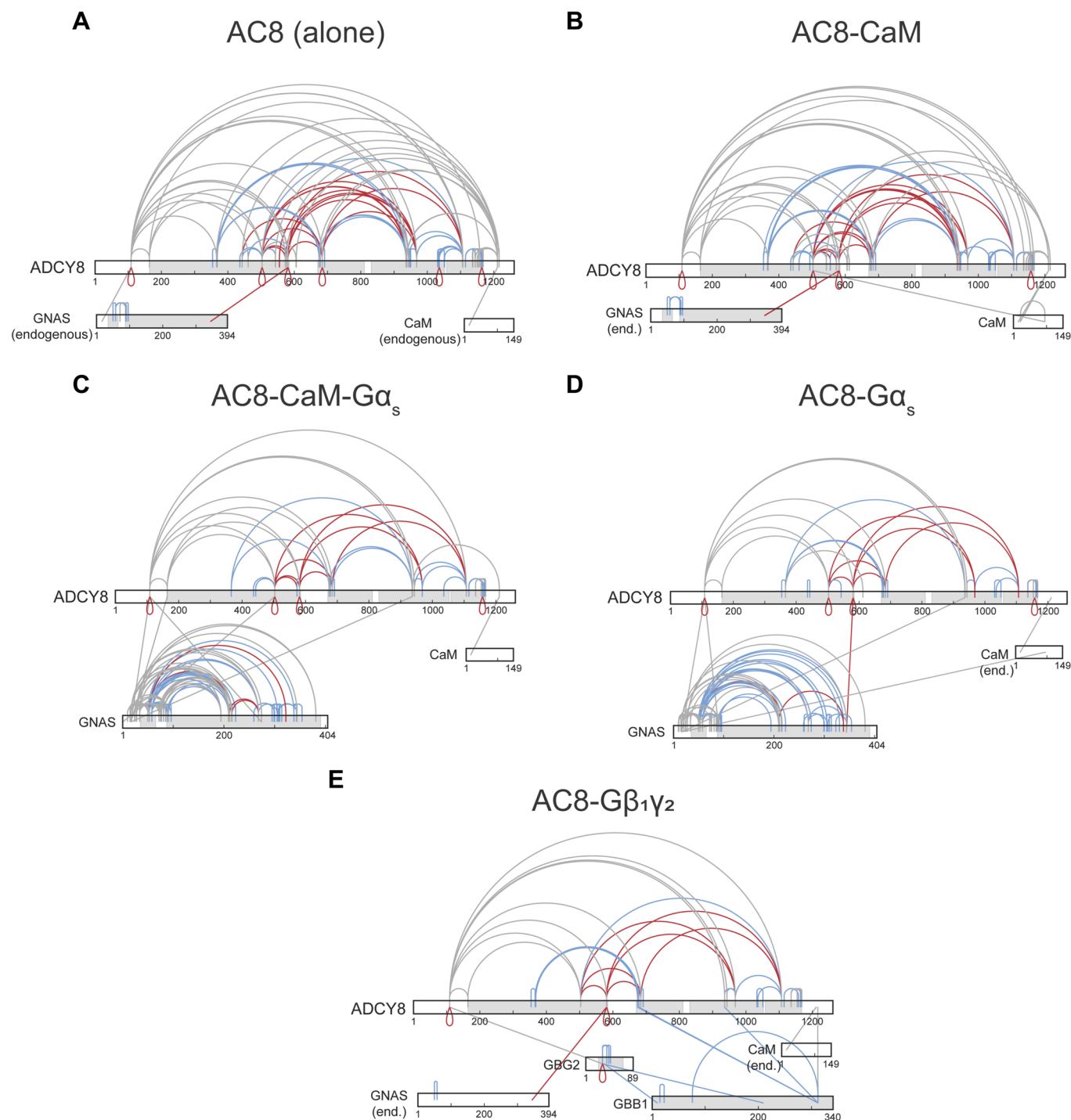


Figure EV3. All detected crosslinks (DSS and PDH/DMTMM).

(A) Crosslinked AC8 with copurified Gas and CaM. Violated crosslinks (>30 Å) are coloured in red, satisfied crosslinks between structured/resolved regions are coloured in blue. Crosslinks that involve flexible regions and regions that are not resolved in the cryo-EM or in the structures used for protein-protein docking are coloured in grey. Homomultimeric links (oligomerisation links) are indicated with red drops. Regions with available structure are highlighted in grey on the respective protein sequence. (B) Crosslinked AC8 and CaM with copurified Gas. (C) Crosslinked AC8, CaM and Gas. (D) crosslinked AC8 and Gas with copurified CaM (E) crosslinked AC8 and G $\beta\gamma$ with copurified Gas and CaM.

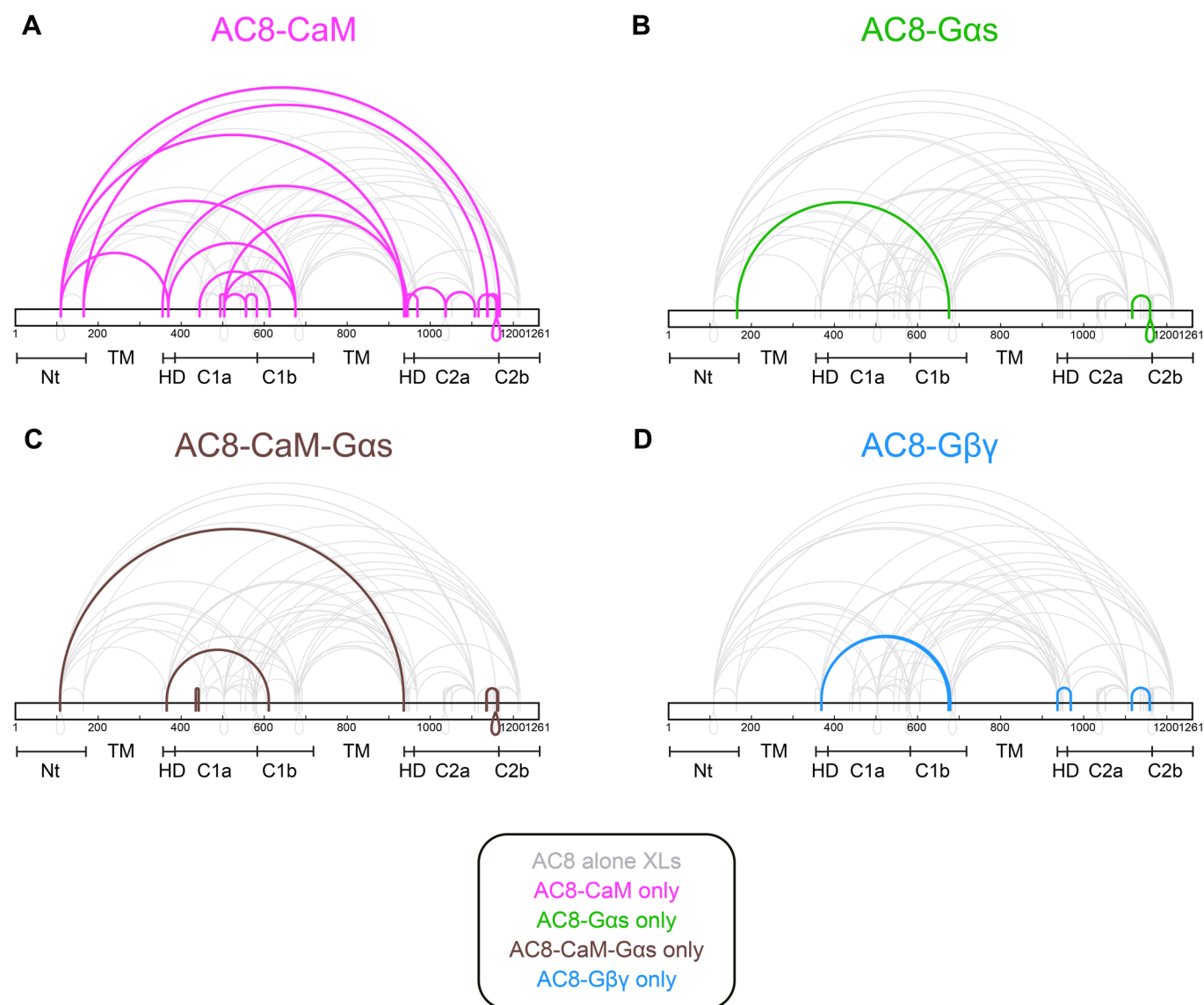


Figure EV4. XL-MS comparison of identified crosslinks between AC8 by itself and interactors.

(A) Crosslinks detected in the AC8 only sample (no added interactor) are coloured in light grey, positioned in the background. Crosslinks only found in the AC8-CaM sample (not in the AC8 alone sample) are coloured in pink. AC8 domains and their respective locations are indicated below. (B) Crosslinks only found in the AC8-G α s sample are coloured in green. (C) Crosslinks identified only in the AC8-CaM-G α s sample are highlighted in brown. (D) Crosslinks unique to the AC8-G $\beta\gamma$ sample are coloured in blue.

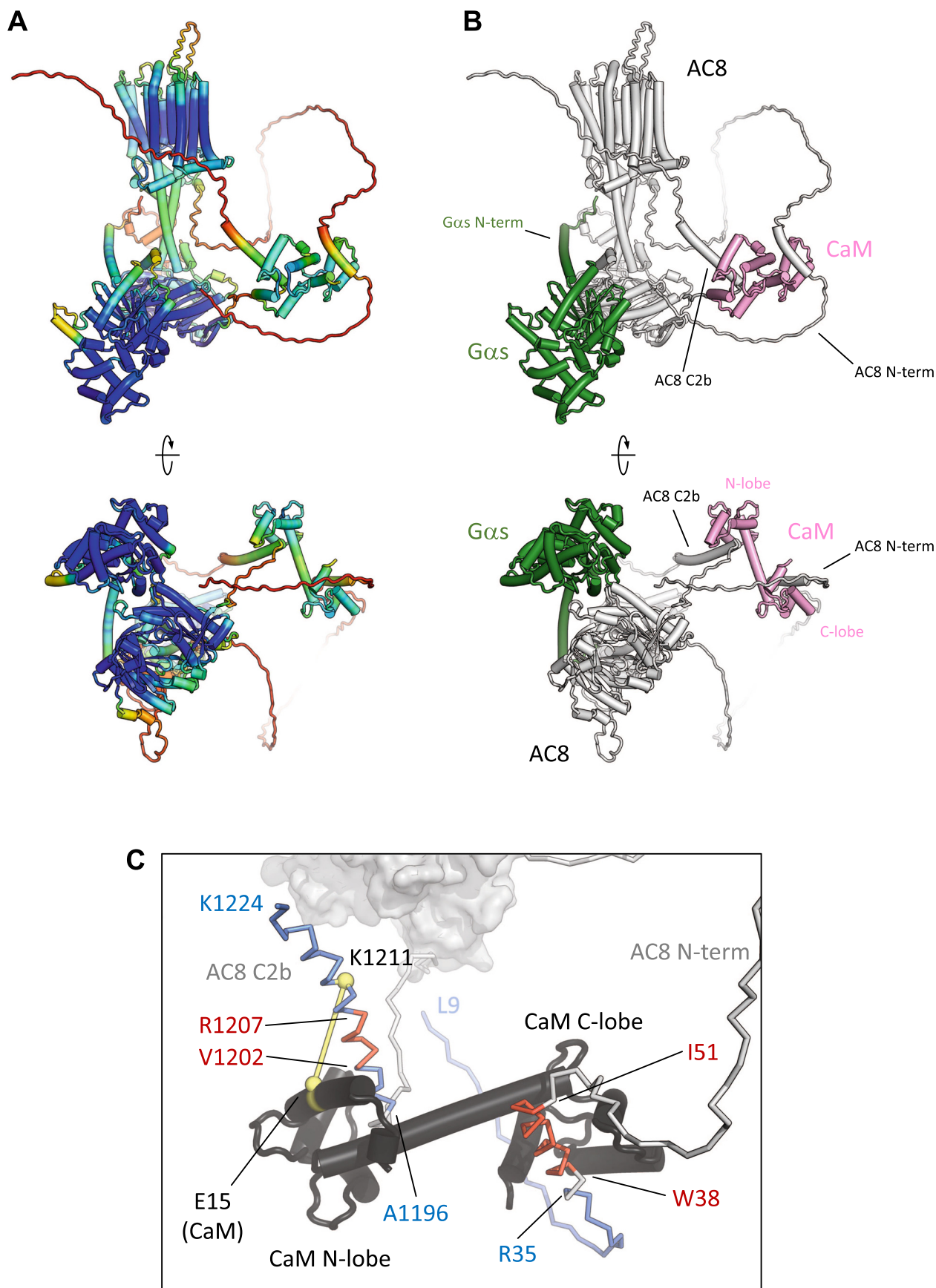


Figure EV5. AlphaFold2 model of AC8-CaM-Gαs.

(A) The views of the model of the complex generated using AlphaFold2 as described in Materials and Methods, coloured according to the pLDDT scores (blue – high pLDDT / confidence; red – low pLDDT / confidence). (B) The same views as in a, with key elements of the predicted structure labelled, and individual proteins coloured white (AC8), pink (CaM) and green (Gαs). (C) The AlphaFold2 prediction matches well the known CaM binding sites (the 1-5-8-14 motif (WXXXVXXIXXXXI) residues 38–51; the IQ-like motif (VQXXRR) 1202–1207, red (MacDougall et al, [2009](#))) and the LiP-MS-detected CaM binding peptides (9–35, 1196–1224, blue), respectively, in the N-terminus and C2b domain of AC8. CaM is coloured dark grey. The crosslink between E15 (CaM) and K1211 (AC8) detected in our XL-MS analysis is indicated as a yellow line. The distance between the Ca atoms of these residues (yellow spheres) is 17.5 Å.