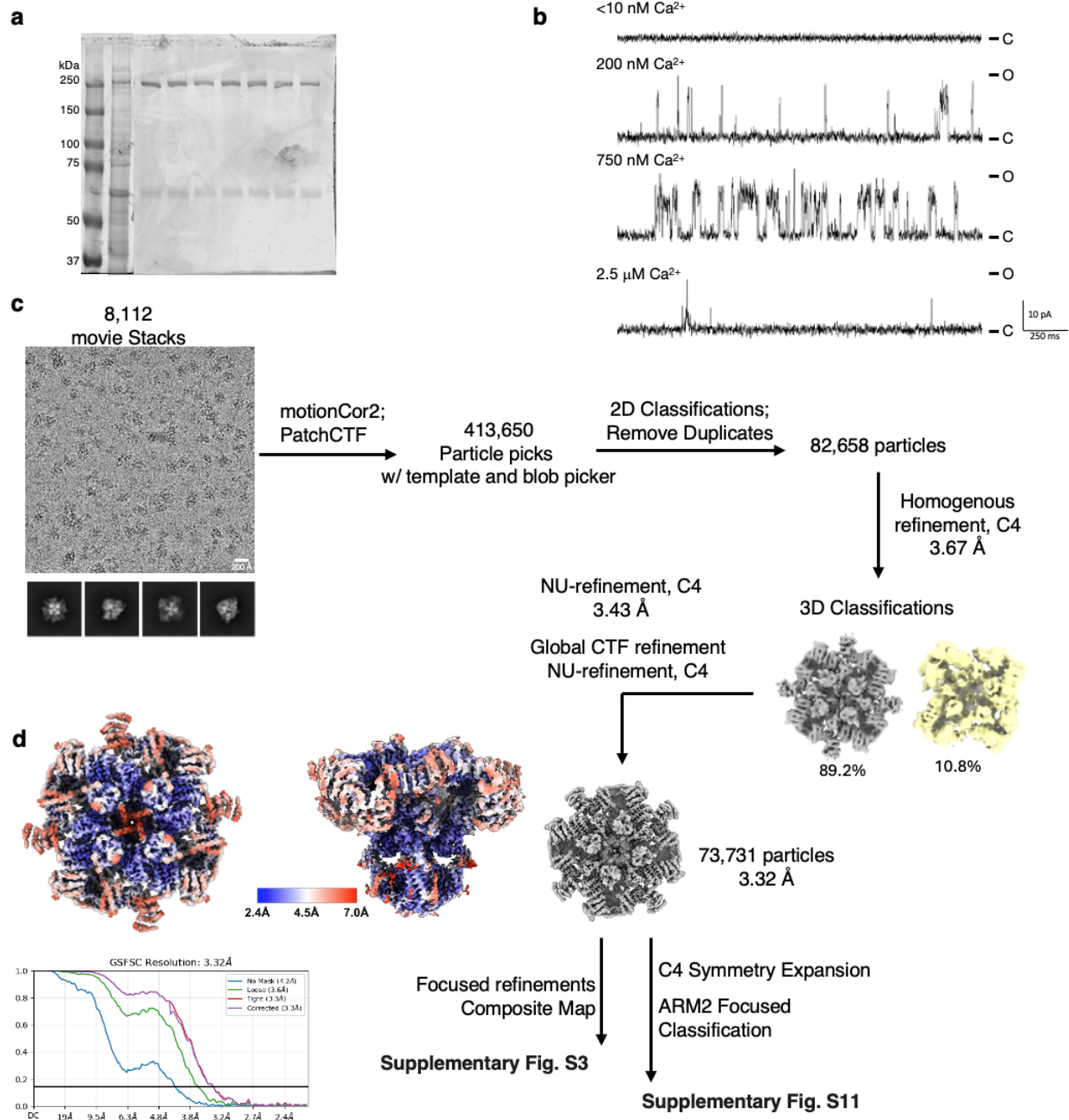
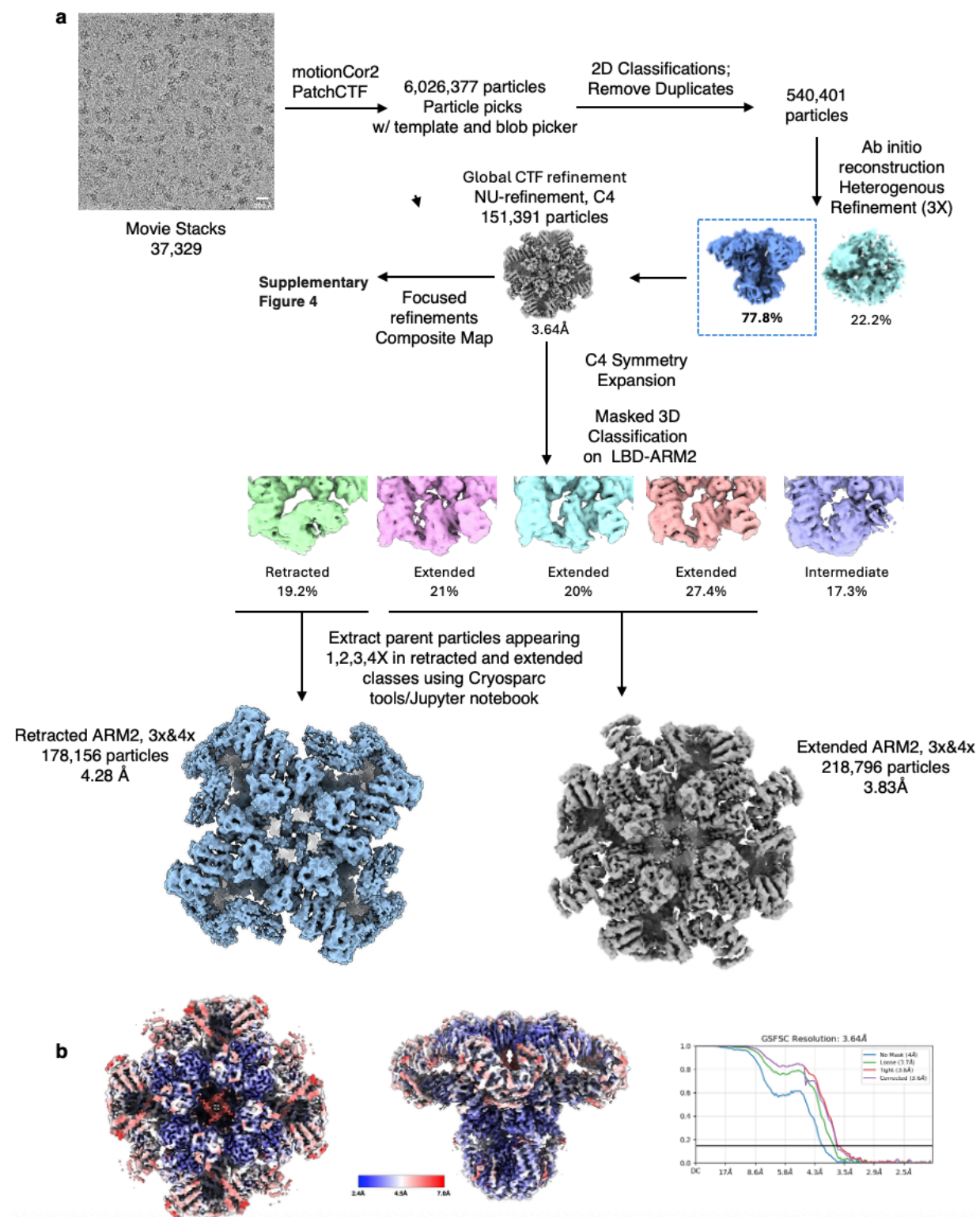


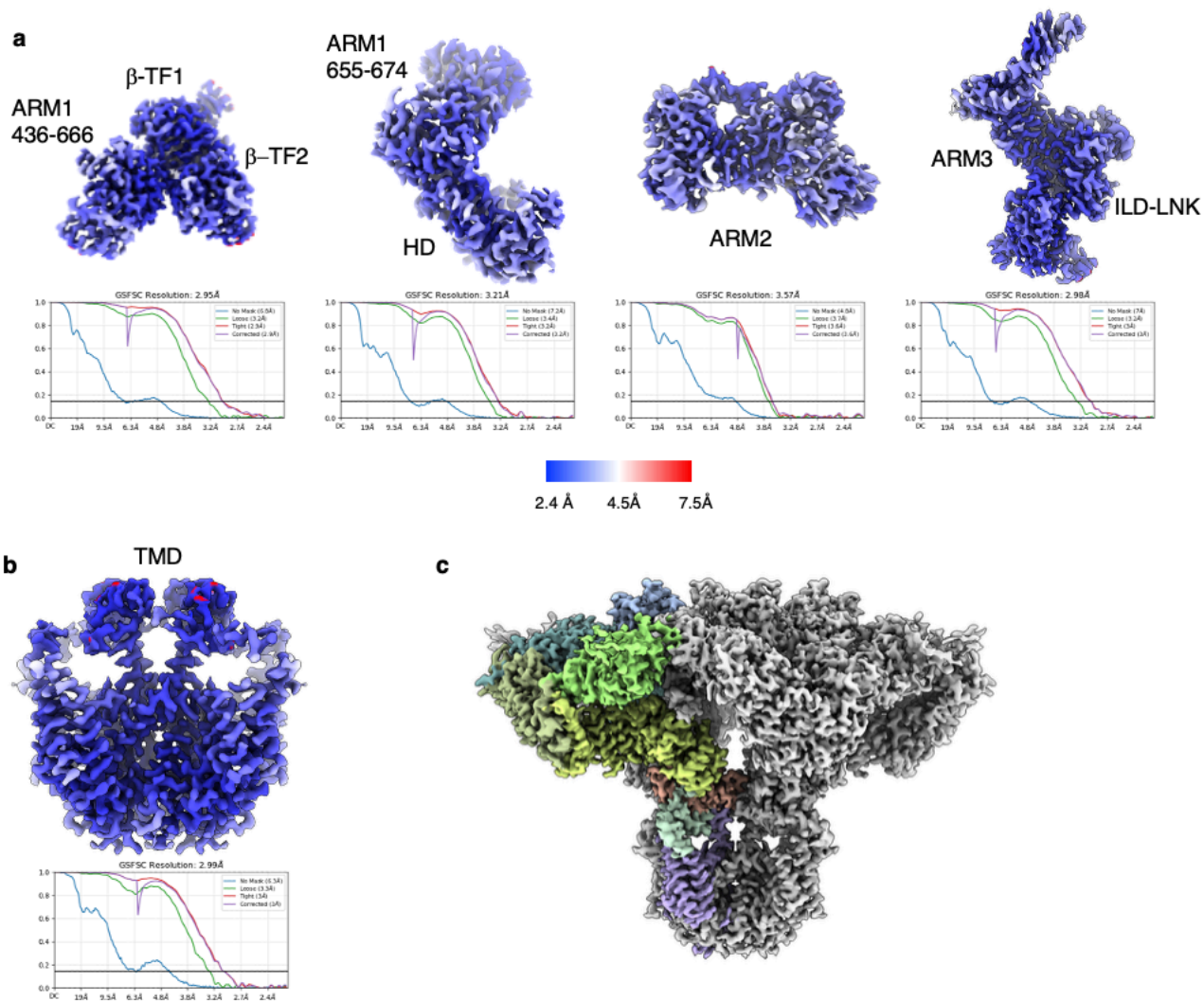


Supplementary Figure S1. Cryo-EM workflow for IP₃R2 in the apo state.

a, SDS-PAGE, lane 1-molecular weight marker, lane 2 – cell lysate, lane 3-10 – elution profile of mIP₃R2. **b**, Bilayer recordings of mIP₃R2-ND. **c**, Cryo-EM processing scheme for Apo-mIP₃R2 **d**, Cryo-EM map colored based on local resolution.

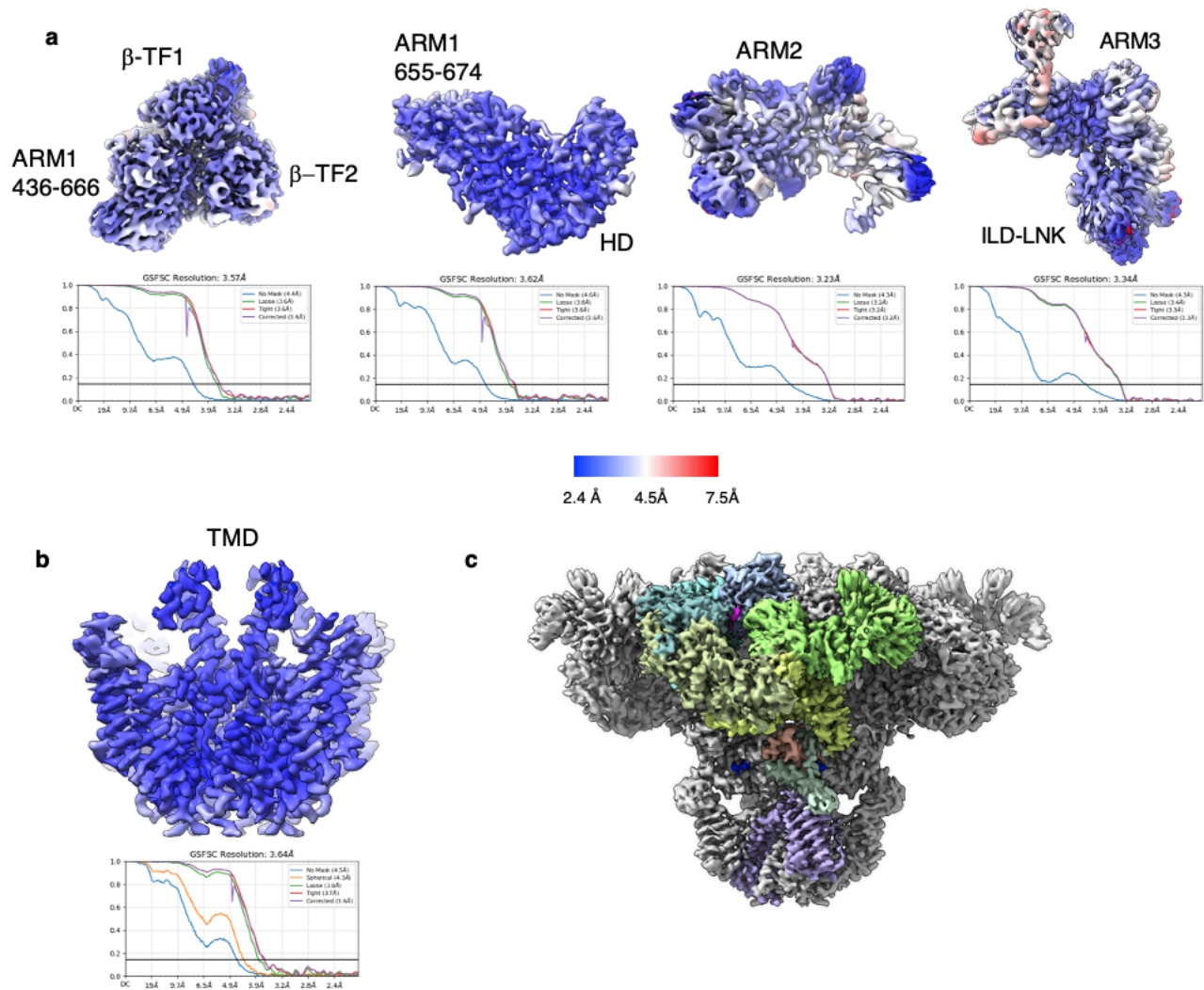


Supplementary Figure S2. Cryo-EM workflow mIP₃R2 in the ligand-bound. a, Cryo-EM processing scheme for CIA-mIP₃R2; **b**, Cryo-EM map colored based on local resolution.



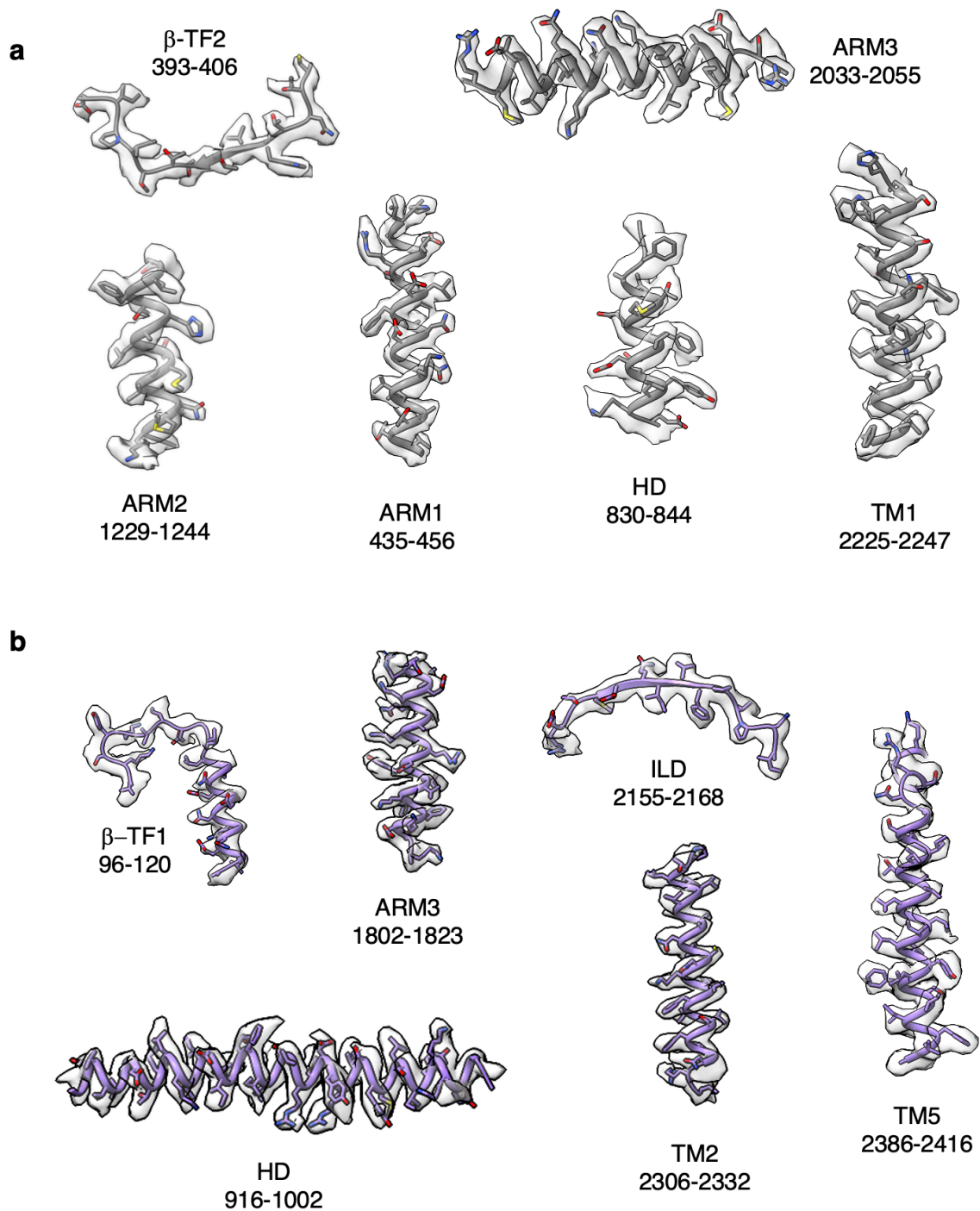
Supplementary Figure S3. Local refinement and assembly of the composite map for apo-IP₃R2.

a, Particles contributing to the consensus reconstruction were symmetry-expanded (C4), followed by focused local refinements on masked regions (C1) as labeled. **b**, The transmembrane domain (TMD) was locally refined without symmetry expansion and with C4 symmetry applied. Densities are colored according to local resolution estimates, and corresponding gold-standard FSC curves are shown. **c**, Composite map for apo-IP₃R2 with one subunit colored by domain.



Supplementary Figure S4. Local refinement and assembly of the composite map for CIA-IP₃R2.

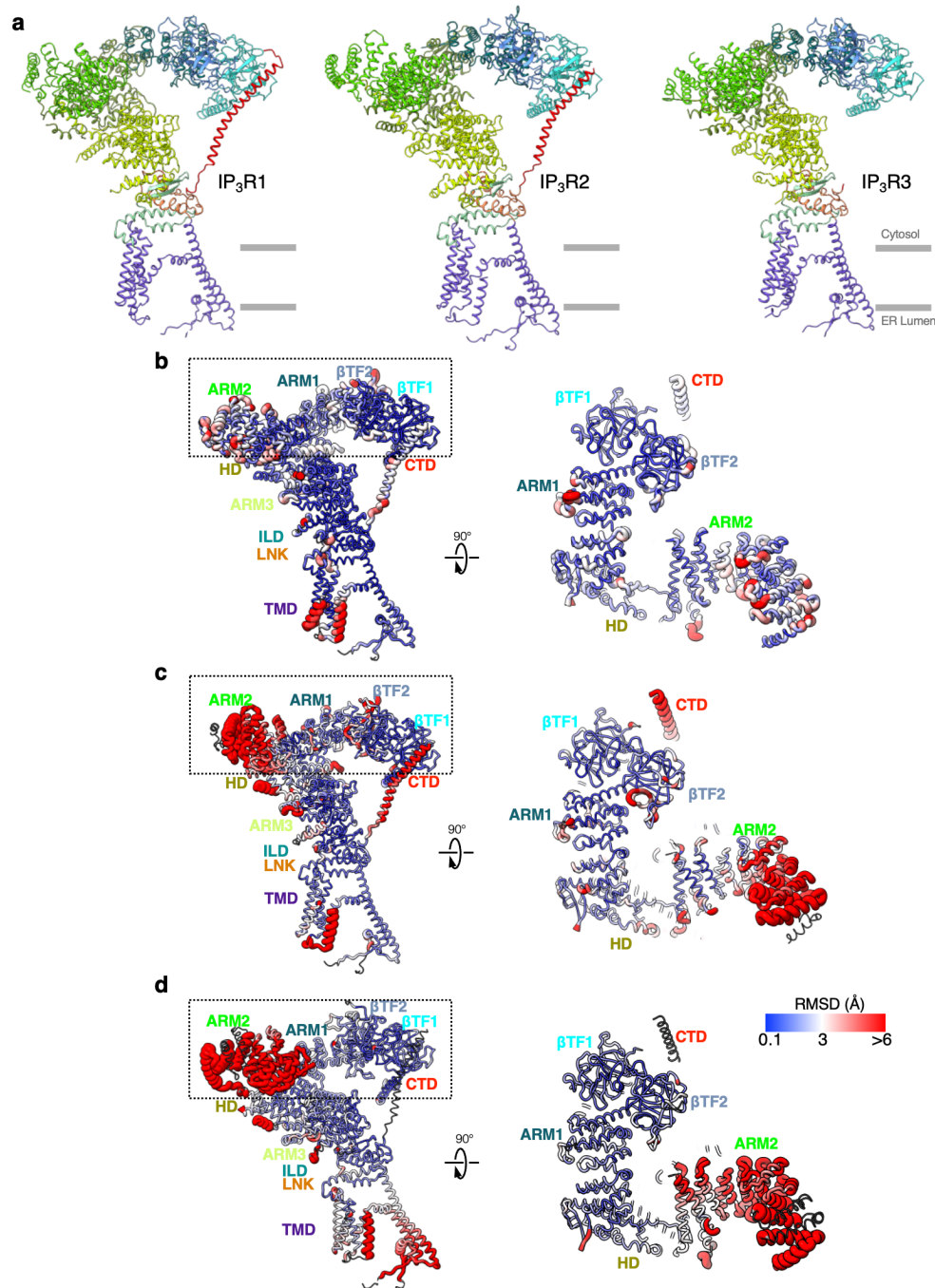
a, Particles contributing to the consensus reconstruction were symmetry-expanded (C4), followed by focused local refinements on masked regions (C1) as labeled. **b**, The transmembrane domain (TMD) was locally refined without symmetry expansion and with C4 symmetry applied. Densities are colored according to local resolution estimates, and corresponding gold-standard FSC curves are shown. **c**, Composite map for apo-IP₃R2 with one subunit colored by domain.



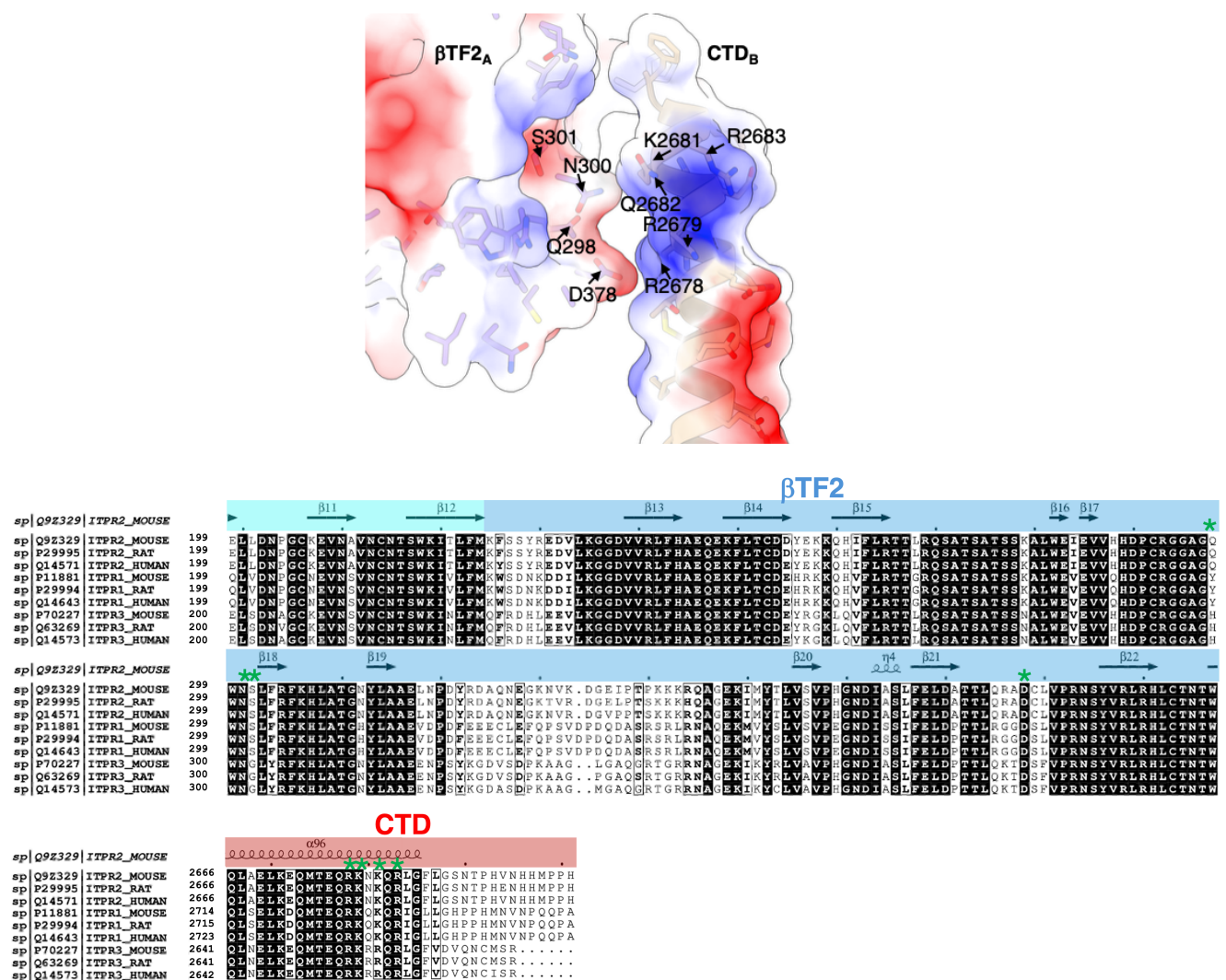
Supplementary Figure S5. Representative map-to-model fits for locally refined reconstructions of apo-IP₃R2 (a) and CIA-IP₃R2 (b).

9 HD

ARM3

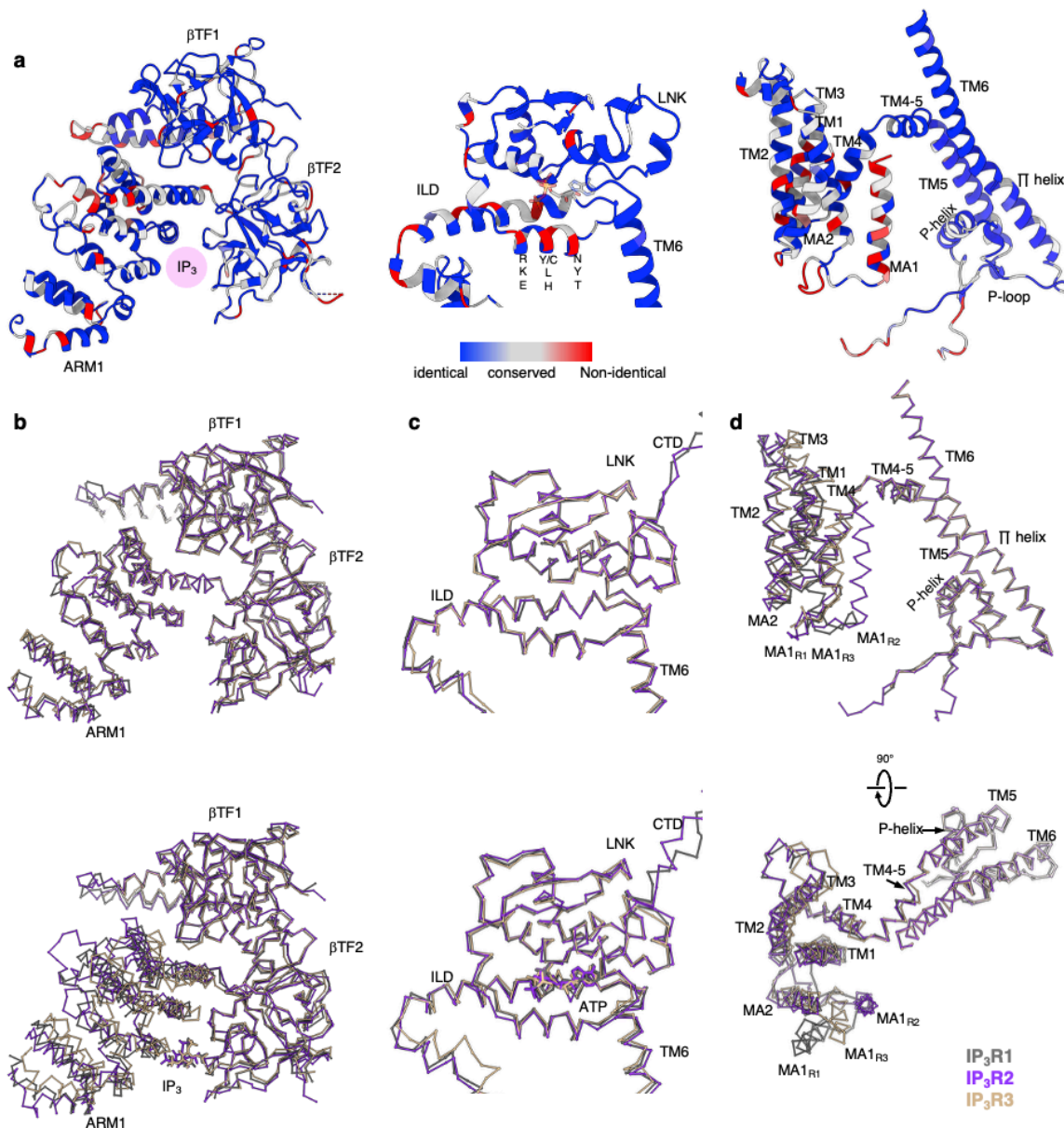


Supplementary Figure S7. Domain Structure of the IP₃R channel family. **a**, Structural comparison of the three IP₃R channel subtypes determined by cryo-EM in ligand-free state colored by domain boundaries. One subunit is shown for each, IP₃R1 (8EAR; 3.5 Å), IP₃R2 (12AD; 3.3 Å), and IP₃R3 (6DQJ; 3.5 Å). **b-d**, RMS deviations calculated between apo-IP₃R2 (12AD; 3.3 Å) and: **b**, CIA-IP₃R2 (12AI; 3.6 Å), **c**, apo-IP₃R1 (7LHF; 3.0 Å), **d**, apo-IP₃R3 (6DQJ; 3.5 Å), with an overall 2 Å, 4 Å, and 7 Å RMSD, respectively. Per-residue deviations are mapped onto one apo-IP₃R2 subunit and color-coded based on deviation - lowest RMSD (blue/thinnest) to highest RMSD (red/thickest), gray indicates no corresponding structure.

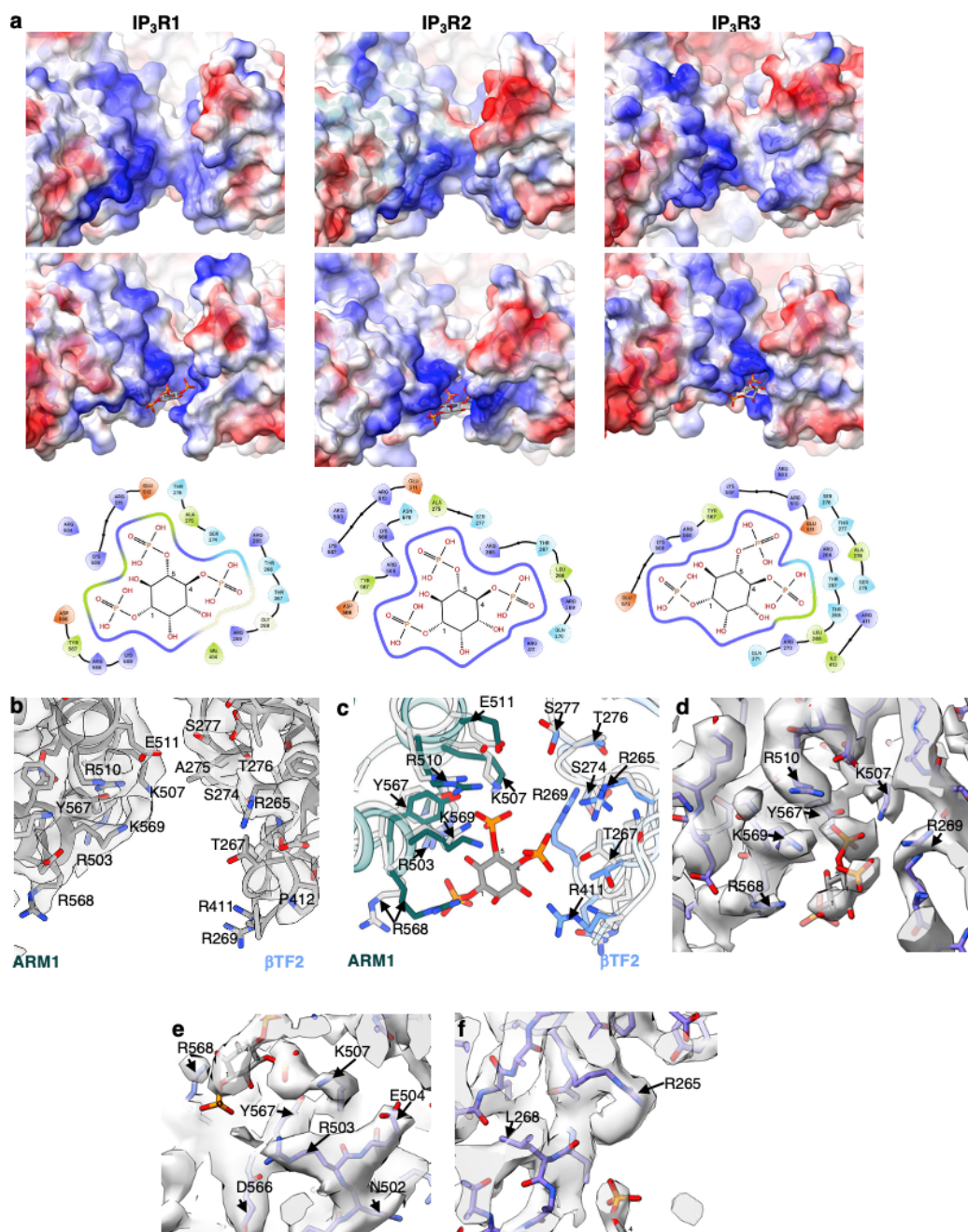


Supplementary Figure S8. Sequence and structural comparison of the CTD-βTF2 interface across IP₃R isoforms.

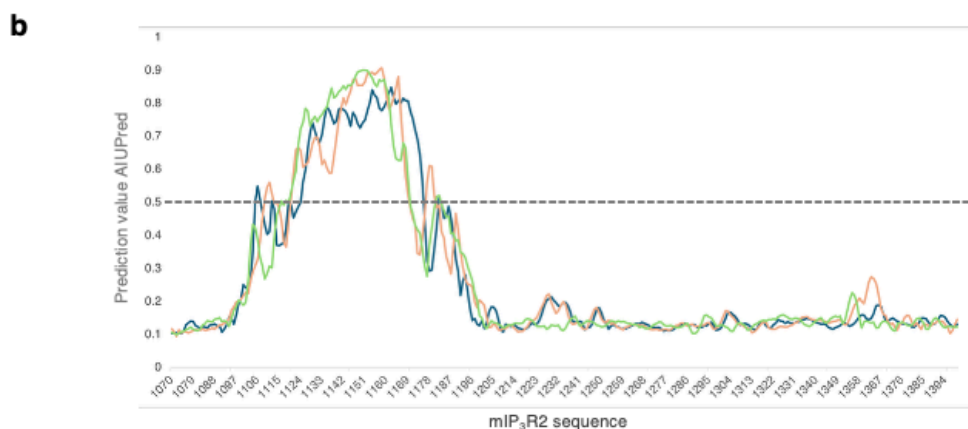
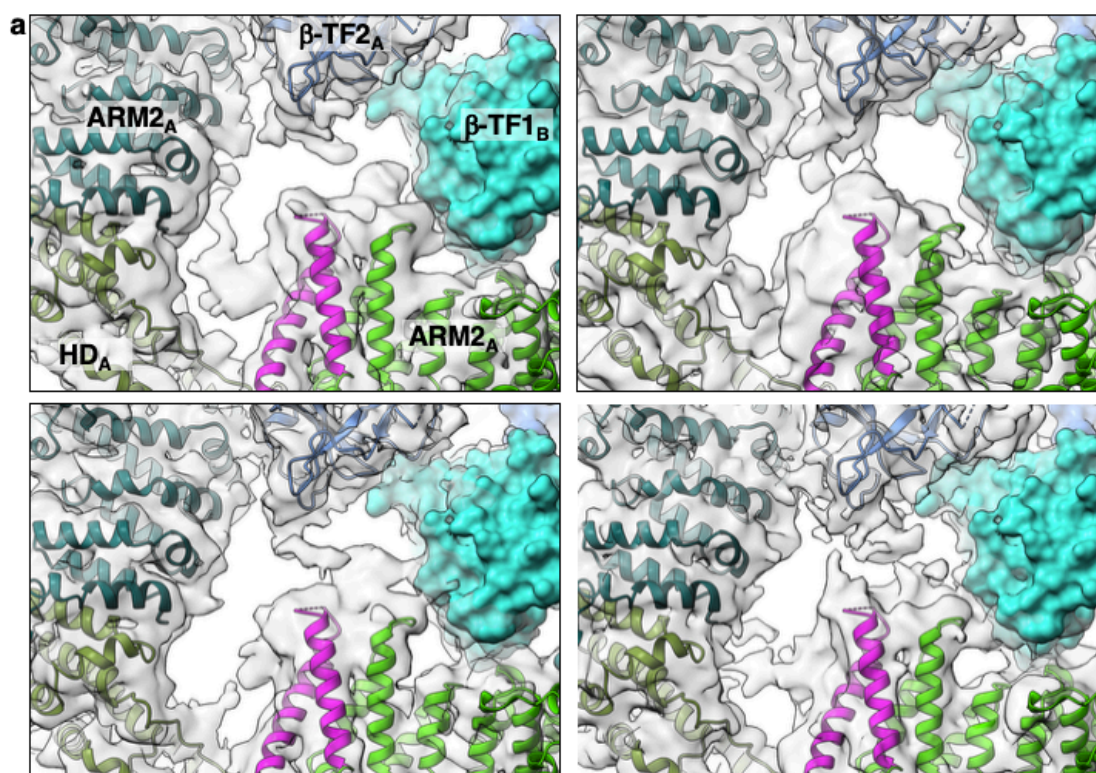
Upper panel displays the surface electrostatic properties at the inter-subunit interface between CTD and βTF2 domains with corresponding residues labeled. Surface coloring: Blue - positive charges; red - negative charges. Lower panel shows the sequence alignment for IP₃R1/2/3 for human, rat and mouse species for βTF2 and CTD with interface lining residues marked with a green asterisk. Non-conserved residues at this interaction surface may lead to subtle isoform-specific differences.



Supplementary Figure S9. Sequence and domain conservation among IP₃Rs. **a**, Sequence conservation mapped onto the IP₃R2 structure for the LBDs, ATP binding domains, and TMDs for one subunit. The ribbon is colored according to sequence conservation from a multiple sequence alignment of IP₃R1/2/3 from human, rat, and mouse (Supplementary Fig. S6): identical residues - blue; conservative substitutions – light gray; non-identical residues - red. Aligned structures of IP₃R1/2/3 showing IP₃ binding (**b**) and ATP binding (**c**) domains: top panels – apo-IP₃Rs, bottom panels CIA-IP₃Rs for IP₃R1 (gray; PDB: 7LHF/8EAR), IP₃R2 (purple; PDB: 12AD/12AI) and IP₃R3 (tan; PDB: 6DQJ/7T3T). **d**, Transmembrane domains from one subunit from apo-IP₃R1/2/3 aligned and shown along the membrane plane (top panel) and from the cytosolic side (bottom panel).



Supplemental Figure S10. Properties of the IP₃ binding pocket. **a**, Surface electrostatic potential in the IP₃ binding pocket of IP₃R1 (PDB: 7LHF/8EAR), IP₃R2 (PDB: 12AD/12AI), and IP₃R3 (PDB: 6DQJ/7T3T) in the absence (top panels) or presence (middle panels) of IP₃. Blue - positive charges; red - negative charges. Bottom panels show the 2D ligand plot of the IP₃ molecule with surrounding residues (7 Å) within the corresponding ligand-bound structure. **b**, IP₃ binding pocket for apo-IP₃R2 displayed with cryo-EM densities. **c**, IP₃ binding pocket structures from apo- (gray) and CIA-IP₃R2 (colored by domain). **d-f**, Additional zoomed-in views of the IP₃ binding pocket IP₃R2 overlaid with the ligand-bound density map. Several residues are labeled.

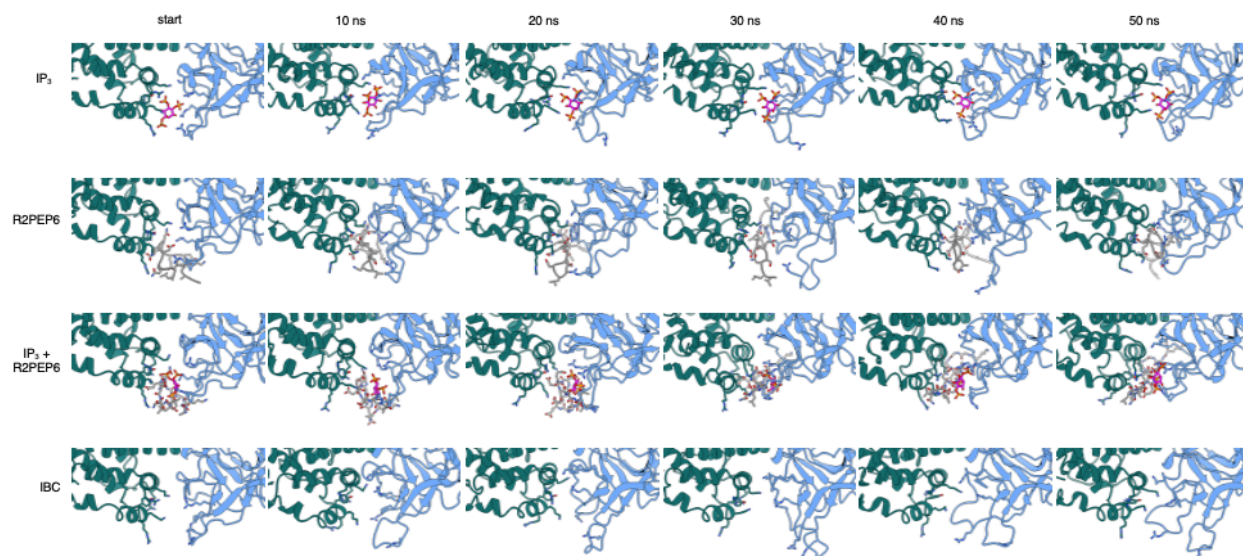


Supplementary Figure S11. Representative densities observed between ARM2 and surrounding LBDs, likely reflecting conformational flexibility of the connecting loop.

a, Apo-mIP₃R2 density maps (filtered to 5Å) after symmetry expansion, focused 3D classification and local refinement, overlaid with the molecular model. The helices flanking the unmodeled ARM2 loop at E1124-K1175 are indicated in magenta. **b**, Prediction of intrinsic disorder for the region encompassing the ARM2 loop for IP₃R1/2/3 determined by AIUPred. Predicted values above 0.5 indicate protein disorder.

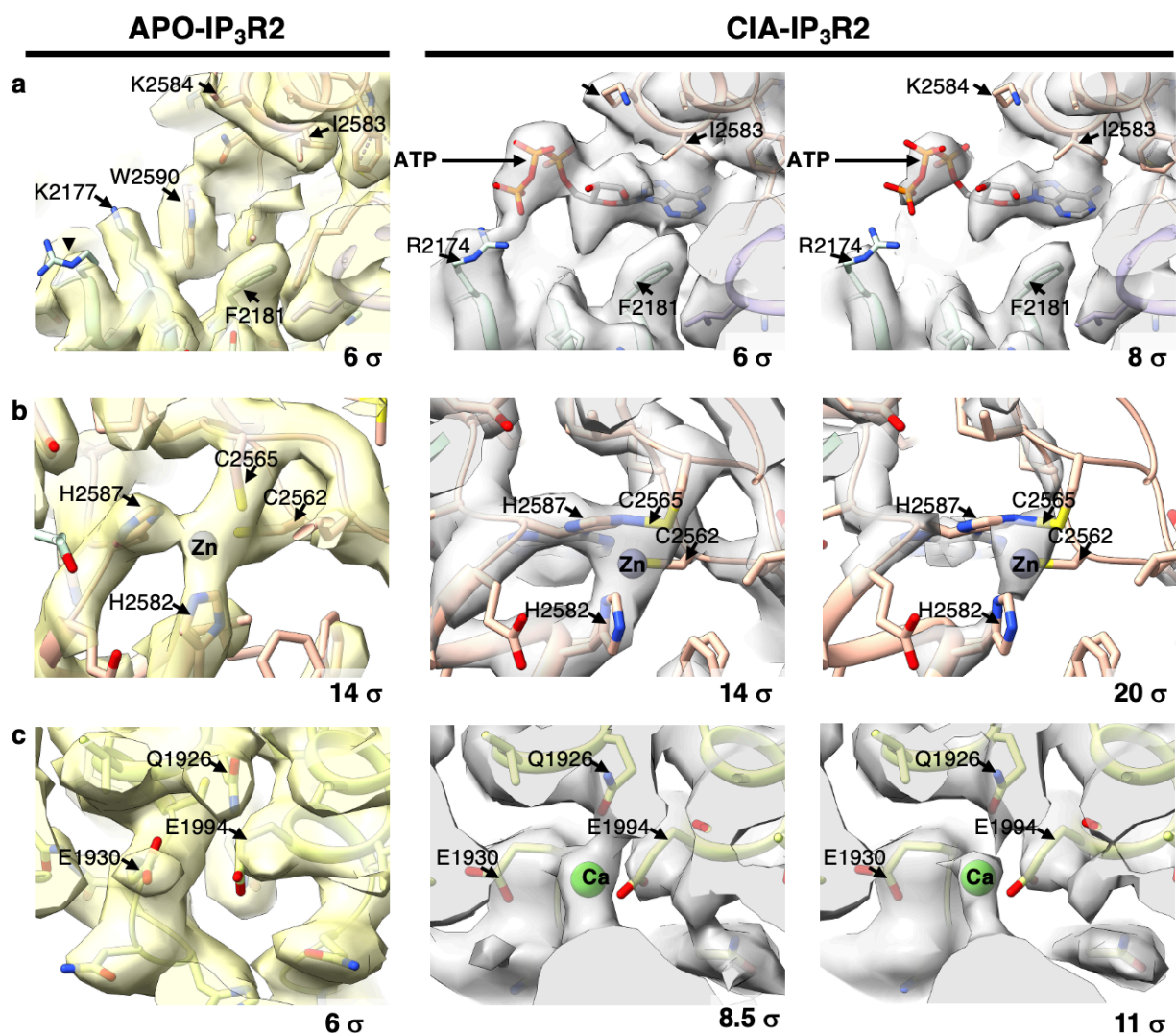
a

	Length	Acidic (D+E)	Basic (K+R+H)	Net charge per residue ($\approx$)	Polar uncharged	Hydrophobic	Gly+Pro
IP₃R1	46	0.22	0.17	-0.083	0.28	0.20	0.17
IP₃R2	54	0.23	0.08	-0.154	0.29	0.25	0.17
IP₃R3	47	0.28	0.21	-0.083	0.11	0.21	0.19

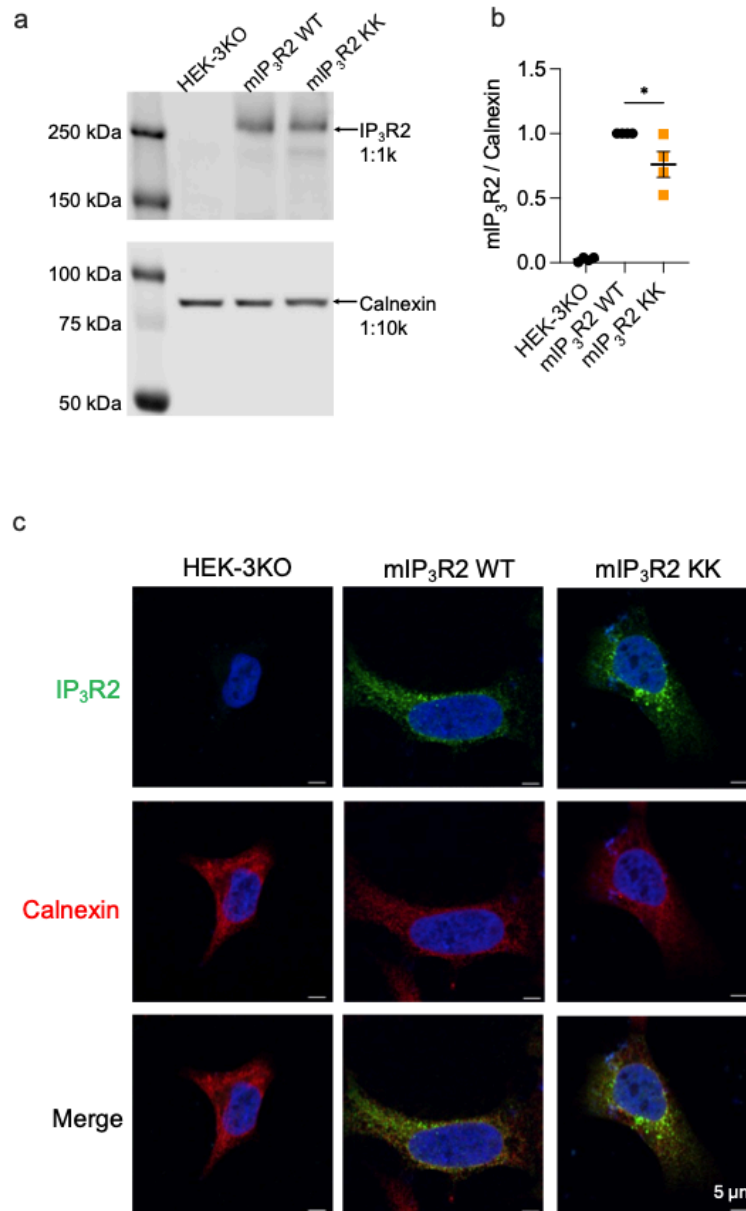
b

Supplementary Figure S12. Sequence properties of the R2PEP6 peptide and molecular dynamics analysis of its interaction with the IP₃-binding core (IBC).

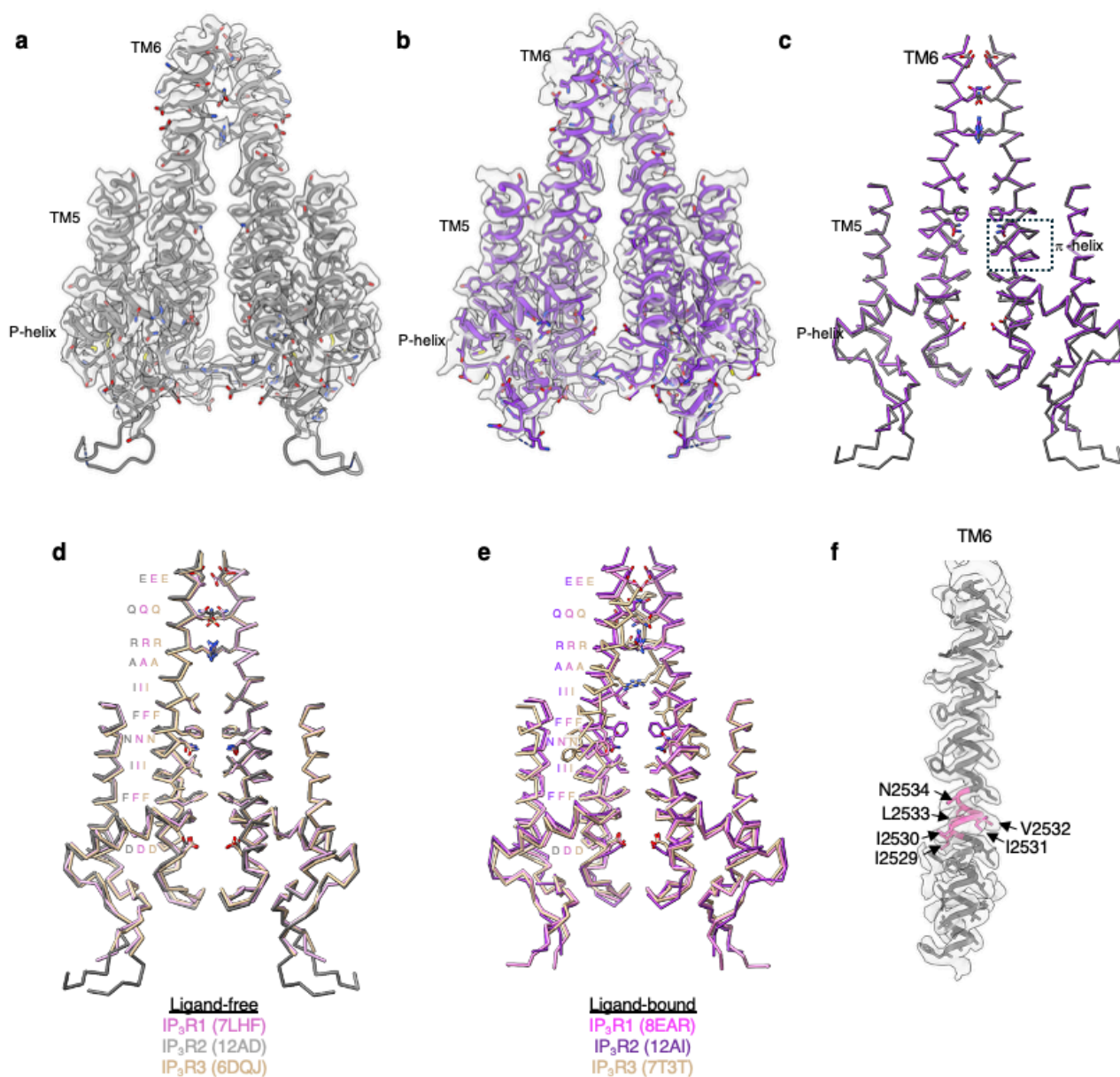
a, Comparative sequence analysis of the R2PEP6 peptide derived from IP₃R1, IP₃R2, and IP₃R3, showing peptide length, amino-acid composition, net charge per residue, and relative content of polar uncharged, hydrophobic, and Gly/Pro residues calculated with EMBOSS ⁷⁵. **b**, Representative snapshots from all-atom molecular dynamics simulations of the IBC in complex with IP₃ alone, R2PEP6 alone, IP₃ + R2PEP6, or ligand-free IBC. Snapshots are shown at the indicated time points (0–50 ns). The IBC lobes are shown in teal (ARM1) and light blue (βTF2), IP₃ is depicted as sticks, and R2PEP6 is shown in gray.



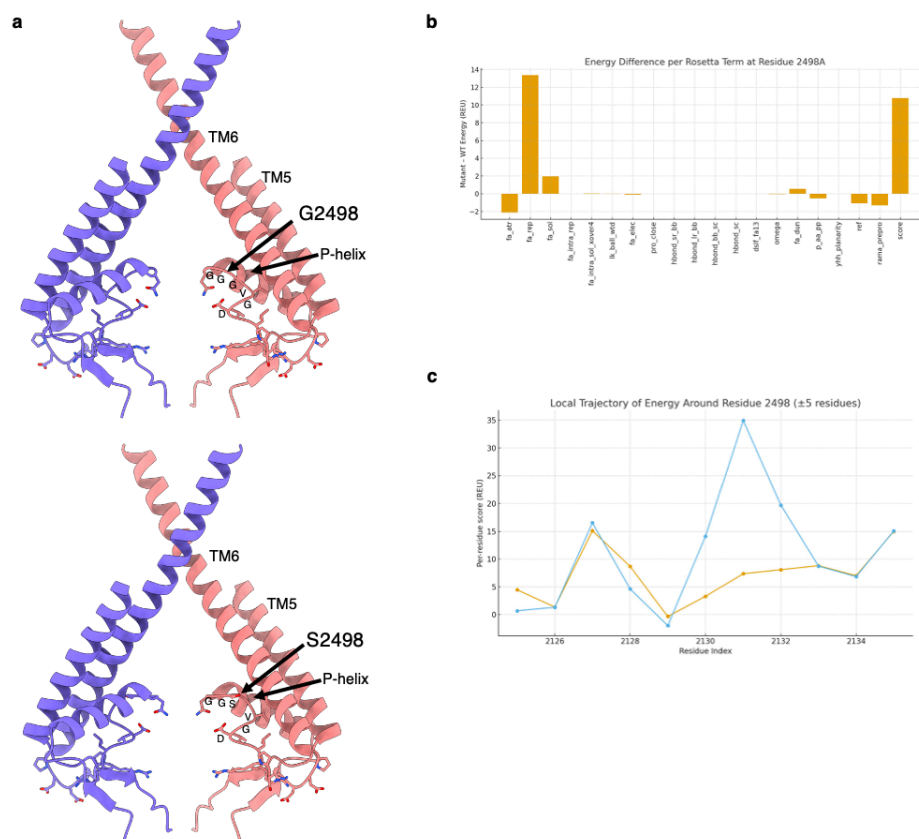
Supplementary Figure S13. Effect of contour level on visualization of the ATP, Zn²⁺, and Ca²⁺ binding sites. Representative views of the ATP (a), Zn²⁺ (b), and Ca²⁺ (c) -binding pockets in cryo-EM maps of IP₃R2 are shown for apo- and CIA-states. The apo state is shown at a single lower contour threshold, whereas the CIA state is shown at two contour thresholds (low and high). Within each panel, σ cutoff values are indicated, and several residues are labeled. At lower contour thresholds, ligand-associated density appears more continuous with surrounding coordinating residues, whereas higher thresholds emphasize the most prominent local density features. Zn²⁺- associated density is observed in the apo- state, consistent with other IP₃R isoforms^{23,24,26-28}, where this site is proposed to play a structural role and is not readily removed under the chelation conditions used in this study.



Supplementary Figure S14. **a**, Representative western blots of IP₃R2 and calnexin from HEK-3KO cells, expressing mIP₃R2 and mIP₃R2-KK. **b**, Scatterplot depicting quantification of IP₃R2 protein level normalized to calnexin from three independent western blots. Data are means ± SDs of three (n = 3) independent experiments. **c**, Representative immunocytochemistry images of HEK-3KO, mIP₃R2 WT, and mIP₃R2 KK cells. IP₃R2 is shown in green, the ER marker calnexin in red, and nuclei are stained with DAPI (blue). Merged images illustrate subcellular localization and colocalization of IP₃R2 with the ER. Scale bar, 5 μm.



Supplementary Figure S15. IP₃R channel TM domains. Two opposing subunits of apo-IP₃R2 (**a**) and CIA-IP₃R2 (**b**) cryo-EM density maps with corresponding models shown for the ion conduction pathway TM5-TM6 viewed parallel to the membrane plane. **c**, Overlay of Apo-IP₃R2 and CIA-IP₃R2 models shown as main-chain trace with pore-lining side chains and location of π -helix denoted. **d-e**, Overlay of 2-opposing subunits of the ion conduction pathway for ligand-free (**d**) and ligand-bound (**e**) IP₃R structures. **f**, One TM6 helix shown overlaid with cryo-EM densities (apo-IP₃R2), indicating in pink the residues comprising the π -helix denoted in **c**.



Supplementary Figure S16. Rosetta energy breakdown analysis of the severe anhidrosis IP₃R2 G2498S mutation.

a, Two subunits of the ion conduction pathway of the cryo-EM structure of WT-IP₃R2 (upper panel) and the *in silico* mutation of IP₃R2-G2498S (lower panel). Arrows indicate the location of the substitution site. **b**, Per-Term Rosetta Energy Difference (ΔE) at residue G2498S shows a quantitative breakdown of the change in the Rosetta energy score (REU) between the mutant and WT-IP₃R2 protein after the G2498S substitution. The energy change is partitioned into major Rosetta terms indicating the underlying physical forces altered by the G to S substitution are mainly destabilizing. **c**, Local trajectory of the energy difference (ΔE) across residues 2493-2503 is plotted for each residue within a ± 5 residue window surrounding the mutation site: orange line – WT-IP₃R2, blue line – IP₃R2-G2498S. The energy change propagates to neighboring residues, indicating a structural or energetic ripple effect on the local environment.