Supporting Information

Serysheva *et al*. 10.1073/pnas.0803189105

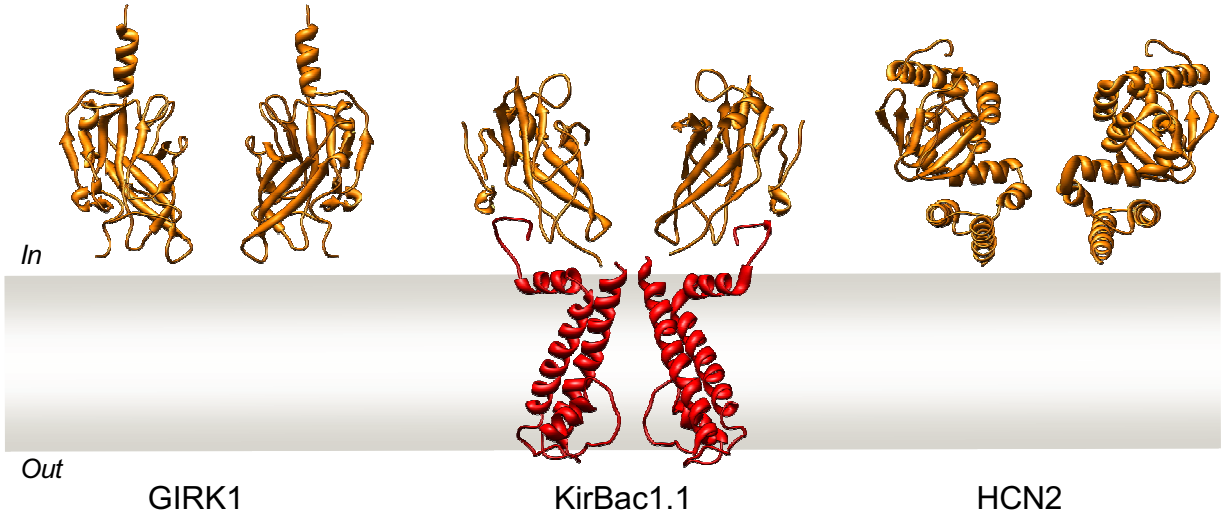


Fig. S1. X-ray structures of the cytoplasmic pore of the inward rectifier GIRK1 (1), the KirBac 1.1 channel (2), and the HCN2 channel (3). Only two subunits of channel tetrameric structures are shown for clarity.

1. Nishida M, MacKinnon R (2002) Structural basis of inward rectification: Cytoplasmic pore of the G protein-gated inward rectifier GIRK1 at 1.8 A resolution. *Cell* 111:957–965.
2. Kuo A, *et al.* (2003) Crystal structure of the potassium channel KirBac1.1 in the closed state. *Science* 300:1922–1926.
3. Zagotta WN, *et al.* (2003) Structural basis for modulation and agonist specificity of HCN pacemaker channels. *Nature* 425:200–205.

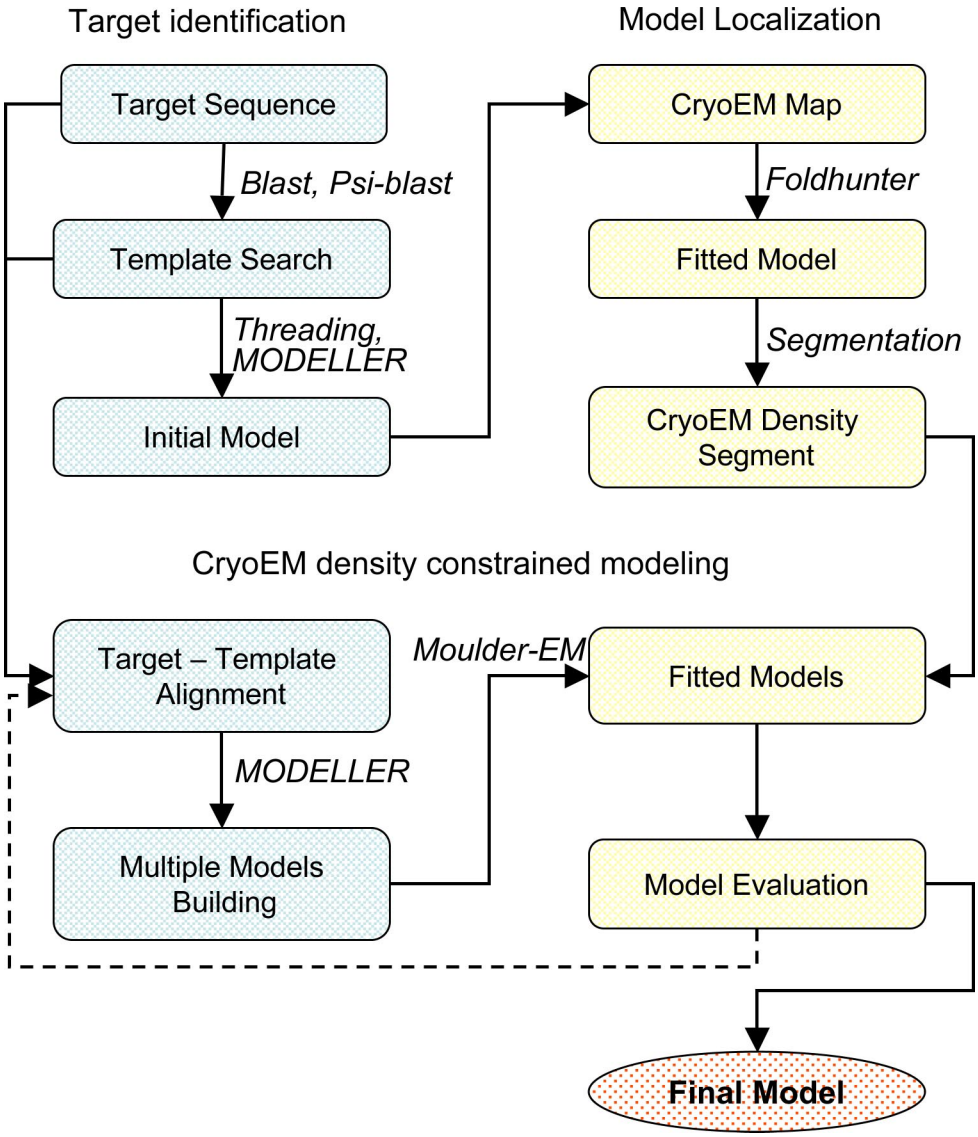


Fig. S2. Cryo-EM restrained comparative modeling protocol. To produce a pseudoatomic structure of this part of the protein, a combined comparative-protein-structure-modeling and cryo-EM-density-fitting procedure was used (1).

1. Topf M, Baker ML, Marti-Renom MA, Chiu W, Sali A (2006) Refinement of protein structures by iterative comparative modeling and CryoEM density fitting. *J Mol Biol* 357:1655–1668.

A

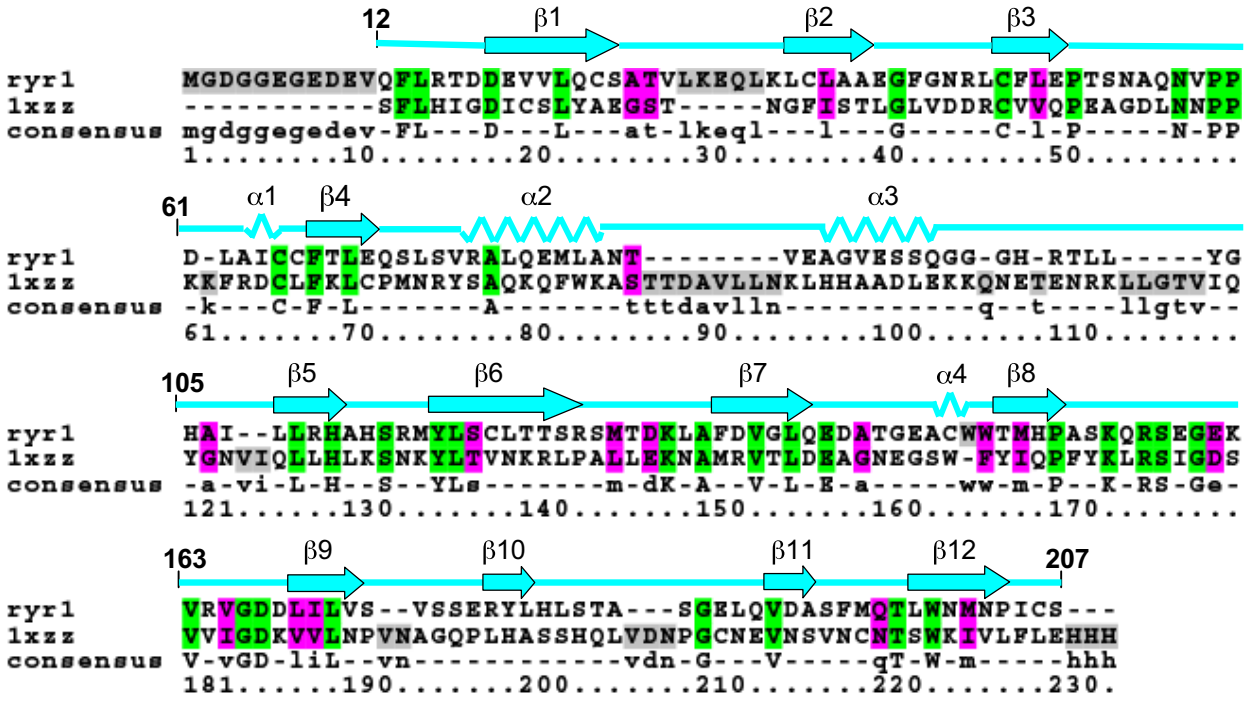


Fig. S3. (*A*) Sequence alignment of the RyR1 N-terminal region (residues Q12–S207) and type 1 inositol 1,4,5-trisphosphate receptor (IP_3R1) sequence including residues 2–223 (the ligand binding suppressor domain, PDB ID code 1XZZ). Secondary structure elements predicted in RyR1 using Moulder-EM (4) are indicated in the above sequences. (*B*) Sequence alignment of the RyR1 N-terminal region (G216–Y565) and IP_3R1 sequence encompassing residues 224–575 (the ligand binding core region, PDB ID code 1N4K). Secondary structure elements predicted in Ryr1 using Moulder-EM (1) are indicated in the above sequences.

1. Topf M, Baker ML, Marti-Renom MA, Chiu W, Sali A (2006) Refinement of protein structures by iterative comparative modeling and CryoEM density fitting. *J Mol Biol* 357:1655–1668.

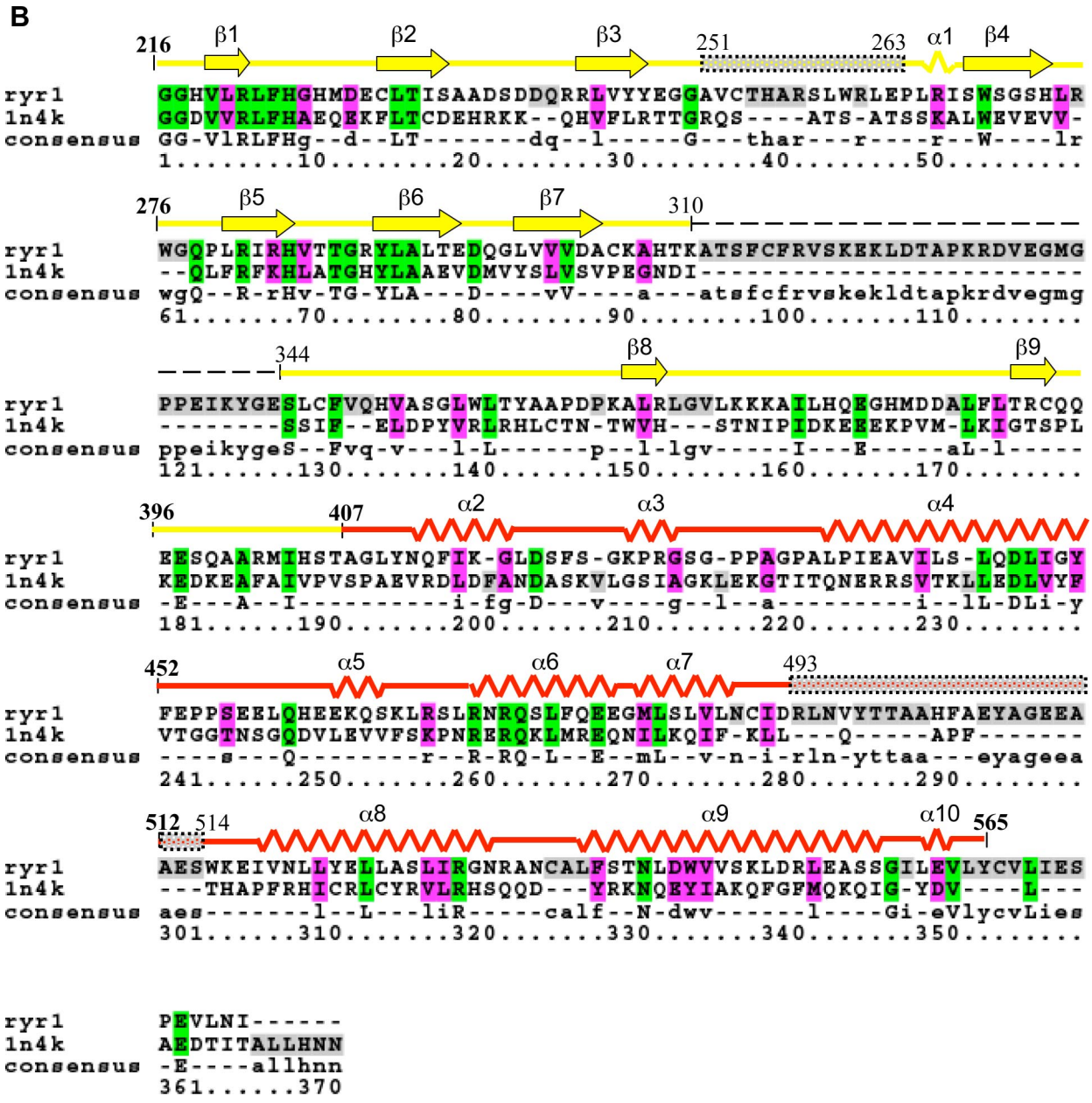


Fig. S3. Continued.

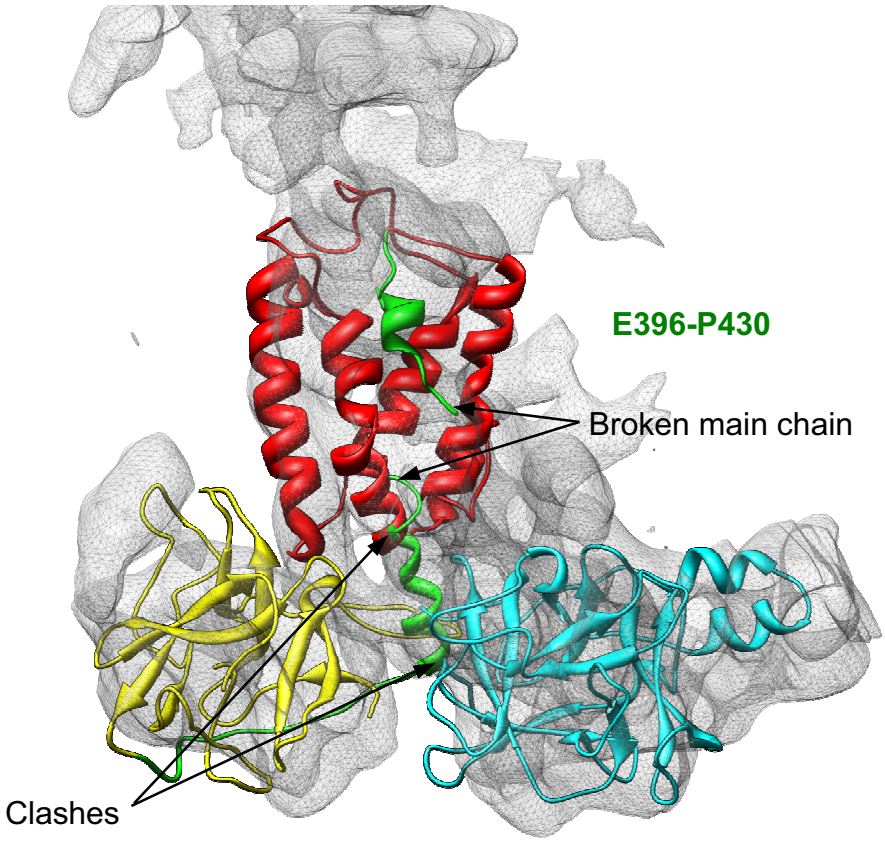


Fig. S4. Illustration of cryo-EM restrained comparative models of the N-terminal region of RyR1 before energy minimization. Model 1 (residues Q12–S207) is shown in cyan. Two parts of model 2 were built independently: the N-terminal portion (residues G216–T407) is shown in yellow, and the C-terminal portion (residues A408–Y565) is shown in red. Green (residues E396–P430) indicates the region that was later energy-minimized and consists of broken helix as well as steric clashes in the restrained comparative model.

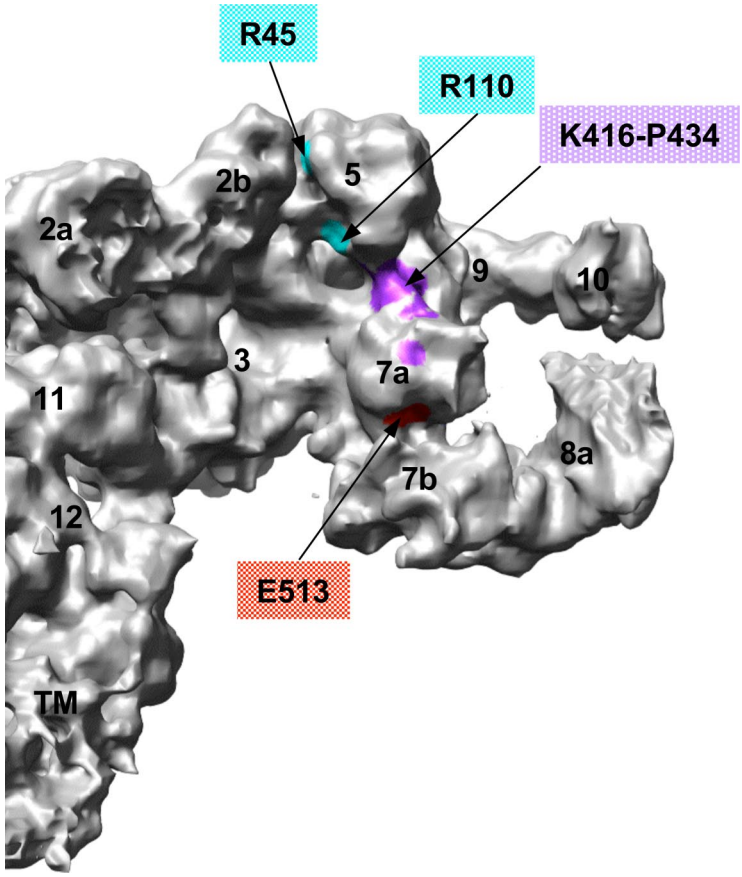
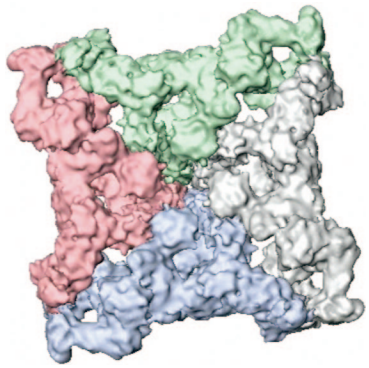


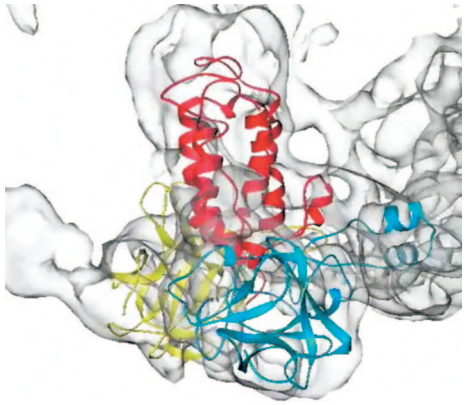
Fig. S5. One subunit of RyR1 with the ''clamp'' color-colored based on docking of the N-terminal models. The subunit is shown from the side facing inside the channel tetramer. Surface-exposed disease-linked residues are indicated. Surface shown in purple corresponds to the position of residues K416–P434 localized by using anti-peptide antibody (1).

1. Baker ML, *et al.* (2002) The skeletal muscle Ca^{2+} release channel has an oxidoreductase-like domain. *Proc Natl Acad Sci USA* 99:12155–12160.



Movie S1. From Figs. 1 and 2. A 9.6-Å resolution 3D map of the RyR1 Ca^{2+} release channel in its closed conformation is seen in three different views: from cytoplasm, side view, and from SR lumen. Segmented individual RyR1 subunits are shown in different colors. The map is displayed initially at the threshold level corresponding to channel molecular mass of ≈2.3 MDa. Two opposing RyR1 subunits are displayed as semitransparent surfaces contoured at higher threshold level than initially. Localized α-helices are annotated with cylinders (red, TM region; cyan, column region; green, central part of the CY region; magenta, clamp region). β-Sheets are shown as yellow surfaces.

Movie S1



Movie S2. From Fig. 3 *B* and *C*. A close-up view of homology models for the N-terminal region of the RyR1 fitted into the 9.6-Å cryo-EM density map of RyR1: model 1 (residues Q12–S207) is shown with cyan ribbons; model 2 is composed of two parts, shown with yellow (residues G216–T407) and red (residues A408–Y565) ribbons. SSEHunter-identified α-helices and β-sheets are shown with magenta cylinders and yellow solid densities, respectively.

Movie S2