The Resting State of a Human Proton Channel Dimer in the Lipid Environment

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Supplementary Materials

Supplementary figures: Figure S1-S7

Supplementary table: Table S1

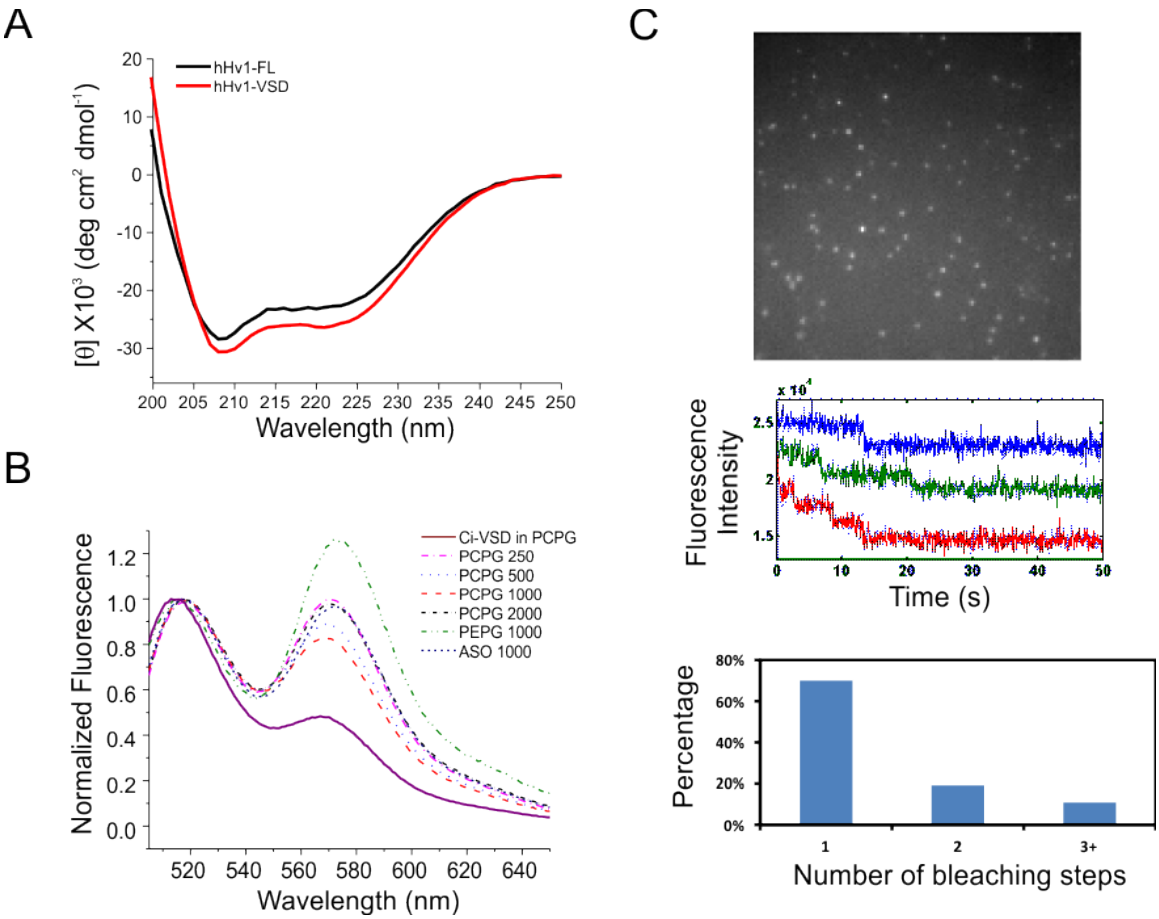

Figure S1. **hHv1 protein characterization.** (A) Circular dichroism spectra of hHv1-FL (black) and hHv1-VSD (red). The helical content calculated from the molar ellipicity was 75% for hHv1-FL and 82% for hHv1-VSD, which are consistent with the expected four transmembrane alpha helices. The hHv1 proteins are well folded in solution. (B) FRET signal of hHv1-VSD-203C individually labeled with fluorescein and TMRM in reconstituted proteoliposome. The significant energy transfer in around 575 nm indicates the hHv1-VSD protein is oligomeric at tested lipid compositions and reconstitution ratio. The FRET signal from a monomeric system CiVSD was plotted (purple) as the reference. (C) (top) Representative image of the hHv1-VSD-193C labeled with Bodipy-FL maleimide. (middle) Time courses of three different single spots from (top). Each trace is the average of a 3x3 pixel area centered on the single molecule. Traces have been offset vertically to avoid overlap. (bottom) Fraction of spots with different bleaching steps.

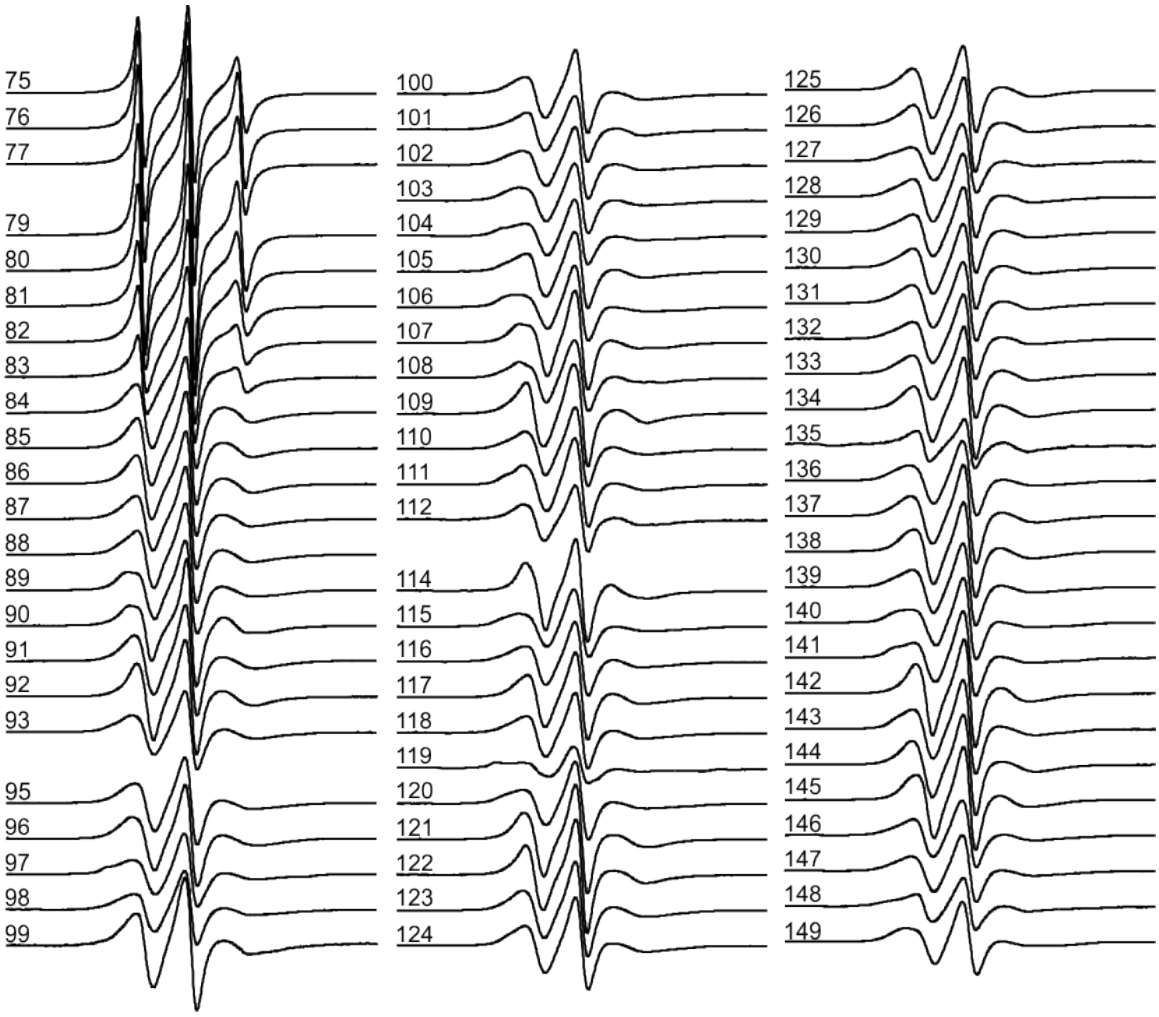

Figure S2. **EPR spectra.** Continuous wave EPR spectra of spin labeled single cysteine hHv1-VSD-75-223 mutants scanning the region 75-149. The spectra were normalized by double integration representing same number of spins. Single cysteine mutants at positions 78, 94 and 113 didn't yield enough protein samples with satisfactory quality for spectroscopic studies. The overall spectra for spin labled hHv1-VSD are isotropic with relatively high mobility, and the most mobile region is at the N-termnal such as residues 75-38.

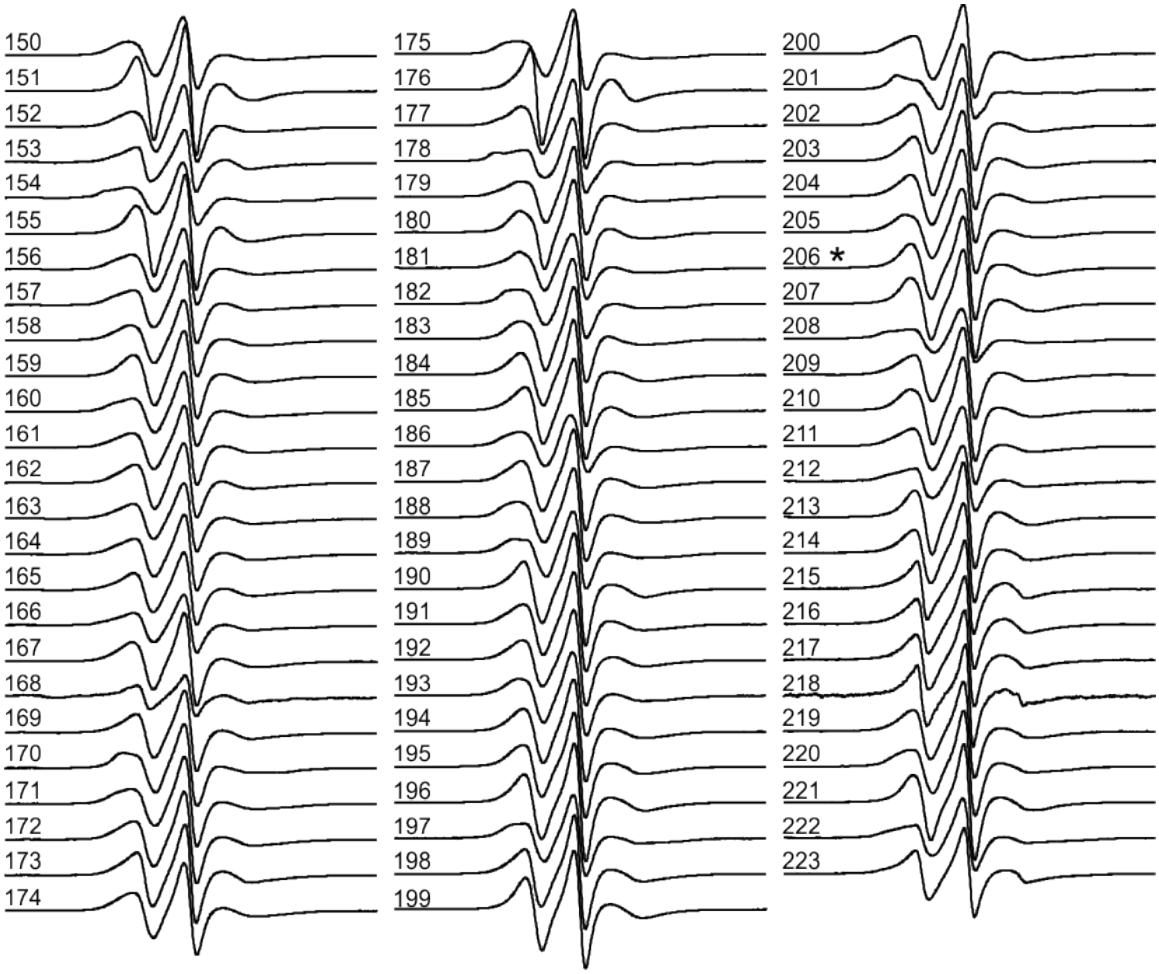

Figure S3. **EPR spectra (continued).** Continuous wave EPR spectra of spin labeled single cysteine hHv1-VSD-75-223 mutants scanning the region 150-223. The spectrum for spin labeled hHv1-VSD-206C (*) was acquired with under-labeling sample to avoid the dipolar coupling effects.

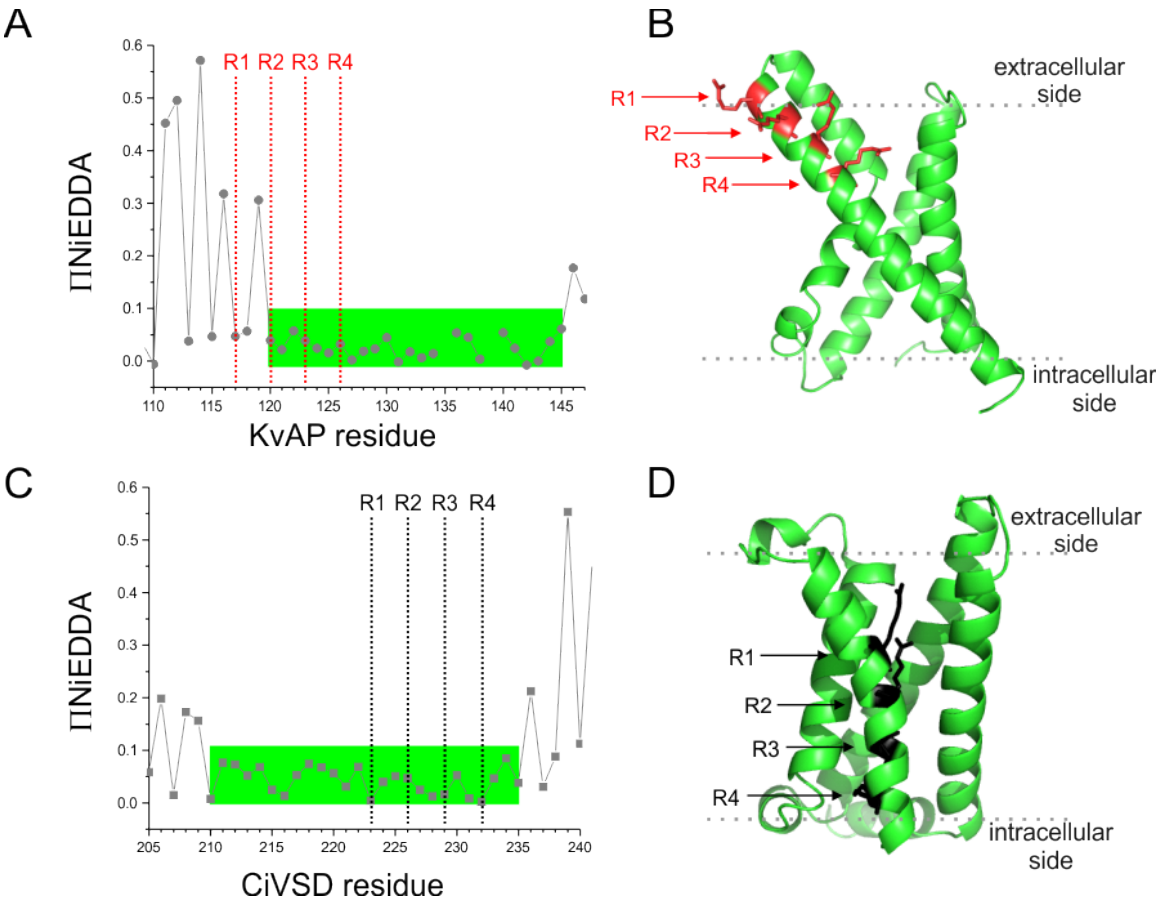

Figure S4. **Boundaries of transmembrane segments defined by solvent accessibility are in agreement with crystal structures for KvAP-VSD (A, B) and CiVSD-R217E (C, D).** Exposed loops experience significantly higher ΠNiEdda which unambiguously defines the boundary of S4 (highlighted with green rectangle in (A) and (C)). The gating charges (R1-R4) are on the top of S4 in KvAP-VSD, but on the lower part of S4 in CiVSD-R217E. The decent agreement between ΠNiEdda data with crystal structure (KvAP-VSD (A) and (B); CiVSD-R217E (C) and (D)) demonstrates not only the heterogeneity of existing VSD templates, but also the sensitivity and resolution of ΠNiEdda.

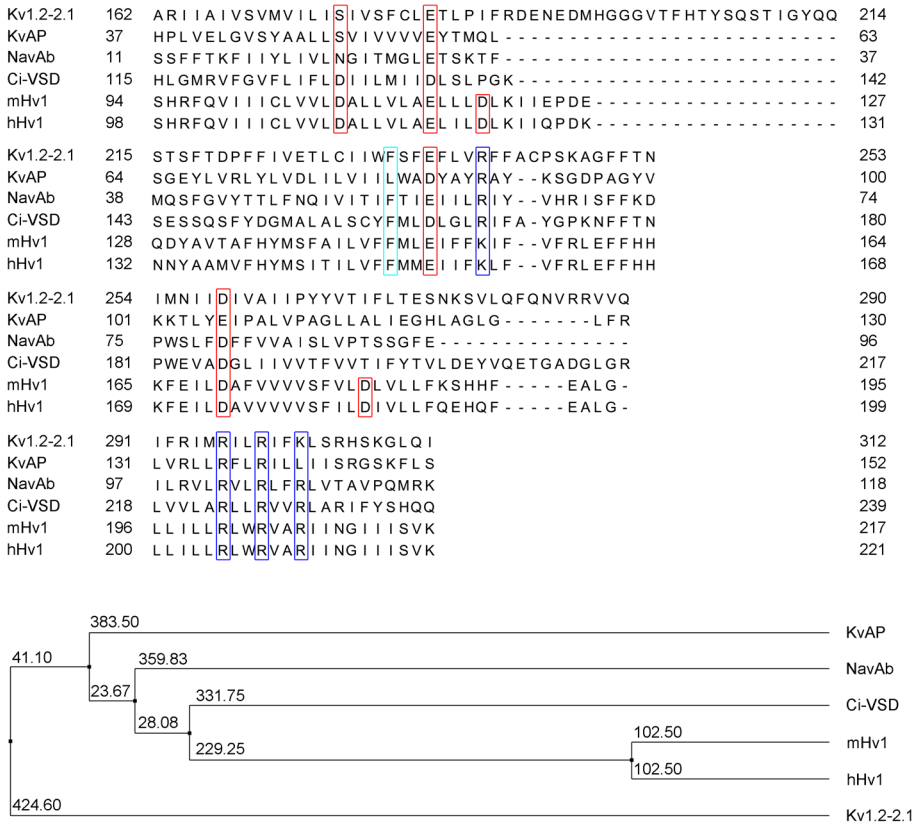

Figure S5. **Sequence alignment for hHv1 with VSD systems with explicit crystal structures.** (Top) Sequence of hHv1-VSD was aligned with KvAP, Ci-VSD, Kv1.2 chimera and NavAb, and this alignment was used to build the four homology models for hHv1 from the individual structural template. (Bottom) CiVSD is the closest to hHv1 among the four structural templates according to the sequence homology.

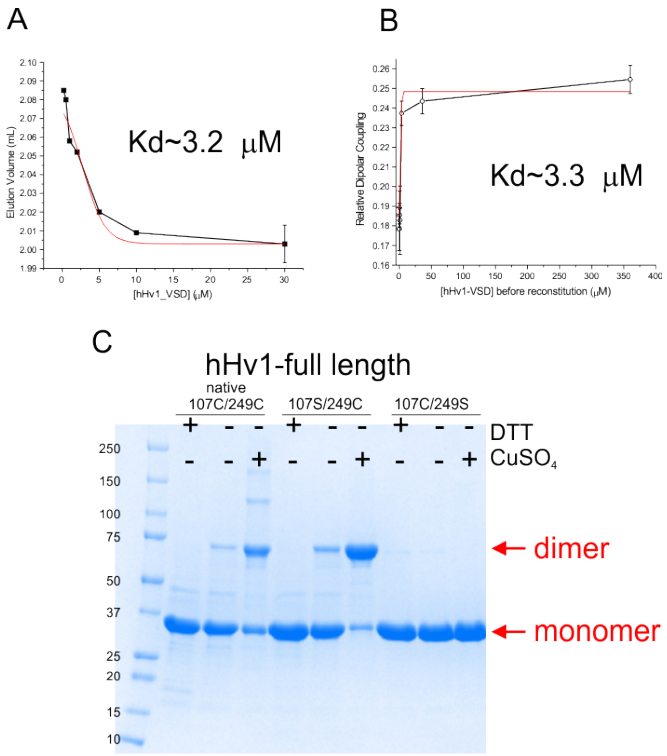

Figure S6. hHv1 dimerization. (A) Concentration dependent volume shift of hHv1-VSD on the analytical superdex 200 column in the buffer 20 mM Tris pH 8.0, 150 mM NaCl and 4 mM FosCholine 12. The right volume shift upon decreasing concentration indicates the dissociation of the hHv1-VSD dimer. The apparent Kd is 3.2 μM. (B) The dipolar coupling between 206 in hHv1-VSD dimer strongly depends on the protein concentration prior reconstitution. The apparent Kd of hHv1-VSD in lipids is 3.3 μM. It suggests the reconstituted hHv1-VSD directly reflect the oligomeric state in the solution. (C) Native hHv1-FL can be cross linked by disulfide bond, and this linkage is sensitive to the redox environment (5 mM DTT, air and 5 mM $CuSO_4$). Mutation of individual native cysteine into serine showed that 249C is responsible for the cross linkage between hHv1-FL dimer.

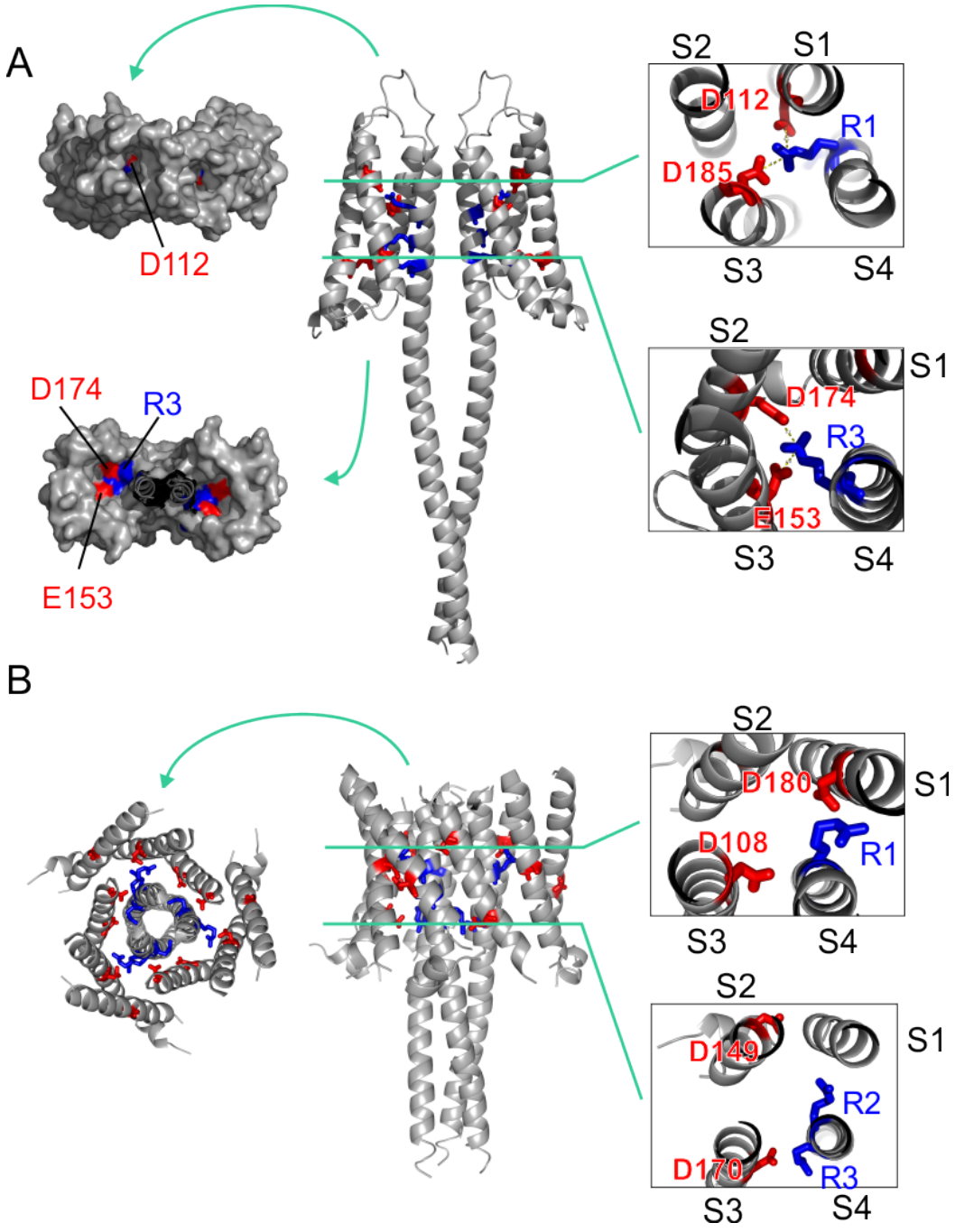

Figure S7. Model comparisons. (A) Homology model of hHv1-89-226 dimer. The two electrostatic clusters D112-R1-D185 and E153-R3-D174 are illustrated in detail on the right. The cluster of E153-R3-D174 is accessible to solvent from the intracellular side. (B) The trimer of mHv-cc structure. It is not apparent how the interactions around the critical residues (D108, D149, D170, D180, R1, R2 and R3) are linked to their functional importance.

CiHv			hHv1 homology model		mHv-cc structure	
residue pairs	**energy kcal/mol**	**interaction**	**homology pair**	**homology model**	**homology pair**	**mHv-cc structure**
201C: 255C	-2.44	Y	153-205	N	149-201	N
201C: 258C	-3.74	Y	153-208	Y	149-204	N
222A: 255C	-2.61	Y	174-205	N	170-201	N
222A: 258C	-5.61	Y	174-208	Y	170-204	N
167A: 255C	1.49	Y	119-205	N	115-201	N
167A: 258C	1.05	Y	119-208	N	115-204	N
233C: 258C	1.38	Y	185-208	N	181-204	N
201C: 261C	-0.19	N	153-211	N	149-207	N
171A: 255N	0.11	N	123-205	Y	119-201	N

Table S1. Predicted side chain interactions by thermocycle analysis using 0.89 kcal/mol (>1.5 KT) as the threshold of significant interactions. Most of them are not present in both our hHv1 homology model and mHv-cc structure.