



**Figure S3.** The conformational changes of VSD segments relative to the S4-5 connecting helix. **(A)** The VSD segments undergo discordant motion relative to the S4-5 helices of Na<sub>v</sub>Rh and Na<sub>v</sub>Ab. **(B)** Dissection of the conformational changes of each VSD segment relative to the S4-5 helix. Distances of the C $\alpha$  atoms between the indicated residues are shown in unit Å. **(C)** The changes of R1-R4 (on the S4

segment) and the CTC (on the S2 segment) relative to the S4-5 helix. R1-R4 appear to remain at similar heights within the lipid bilayer when the S4-5 helices of Na<sub>v</sub>Rh and Na<sub>v</sub>Ab are superimposed. The CTC elements in Na<sub>v</sub>Rh are displaced by one helical turn toward the intracellular side compared to that in Na<sub>v</sub>Ab. S4 and S2 are shown in two opposite views to highlight the displacement of indicated residues. **(D)** The shift of the CTC on S2 segment results in the extracellular transfer of R4 across the occluding Phe when the two structures are superimposed relative to the S4-5 helices.