



**Figure S1.** Overview of the structural changes between Na<sub>v</sub>Rh and Na<sub>v</sub>Ab. **(A)** Primary sequence alignment of Na<sub>v</sub>Rh, Na<sub>v</sub>Ab, and NaChBac. The secondary structural elements of Na<sub>v</sub>Rh are shown above the sequence alignment. Conserved amino acids are colored blue. The functionally important residues discussed in the manuscript are colored red, magenta, and green. The sequences were aligned with ClustalW. **(B)** R4 transfer observed in Na<sub>v</sub>Rh and Na<sub>v</sub>Ab. When the charge transfer center (CTC) elements of the two structures are superimposed, there is one extra charge (R4) transferred toward the extracellular side in each VSD of Na<sub>v</sub>Rh. All structure figures were prepared with PyMol.