Expanded View Figures

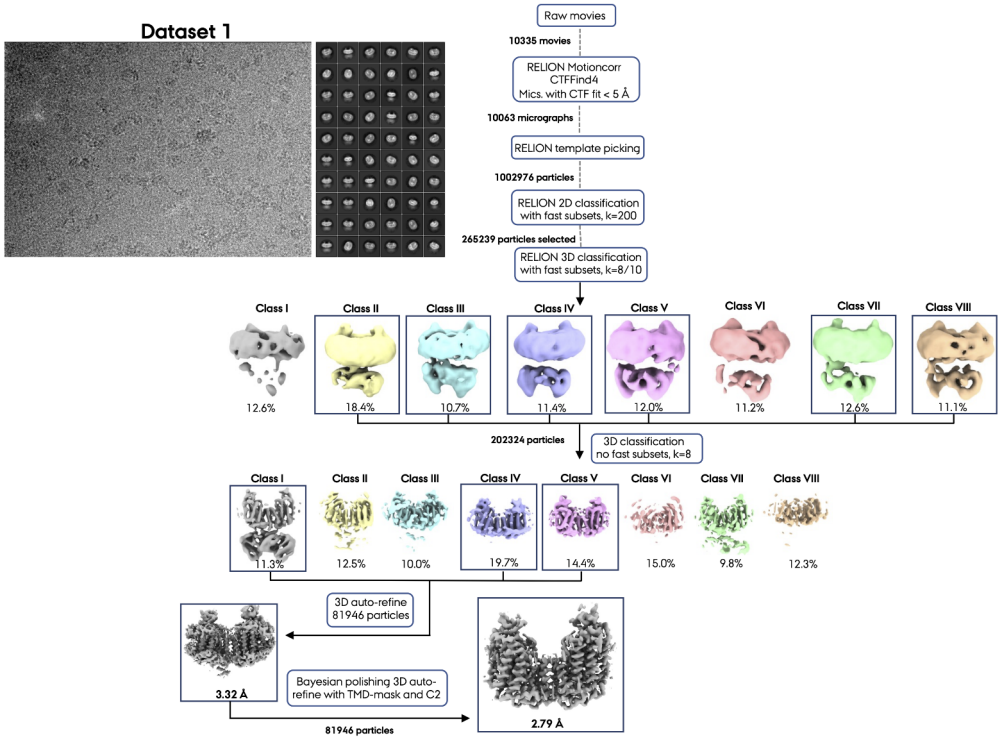

Figure EV1. Processing of cryo-EM data sets 1 Relion.

Representative cryo-EM micrographs and two-dimensional class averages are shown for both data sets as well as the processing strategies used to obtain a 2.6 Å structure of the transmembrane domain of hNKCC1.

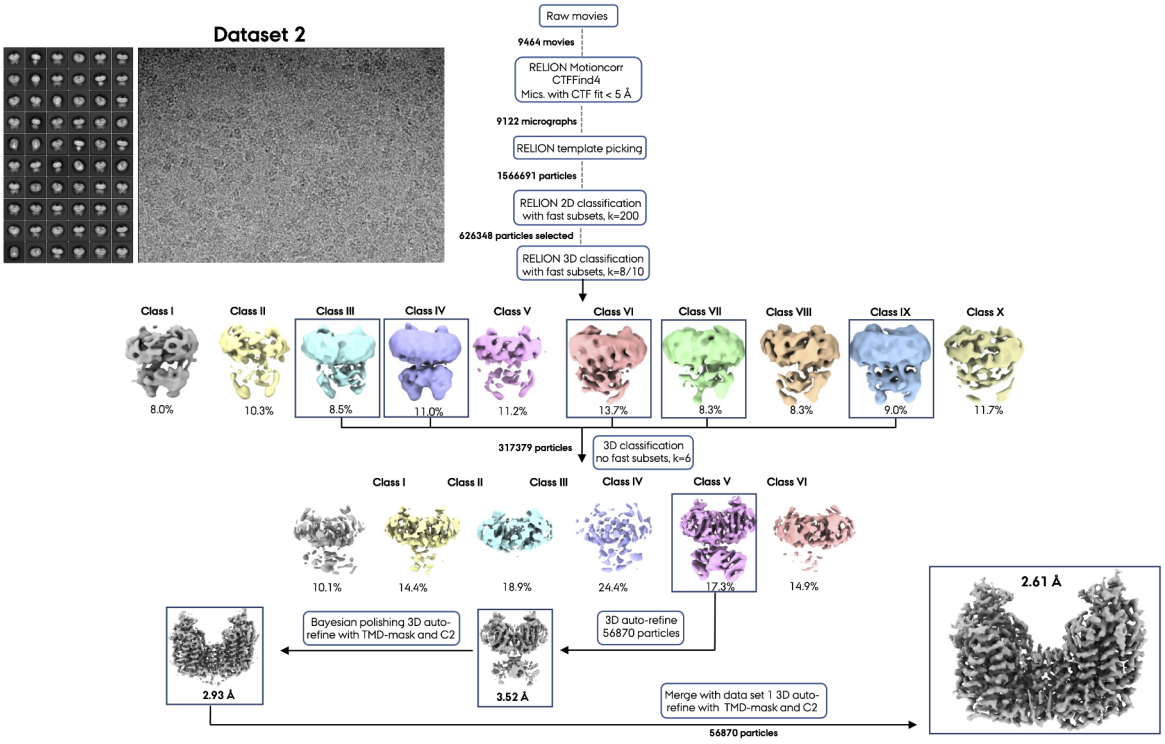

Figure EV2. Processing of cryo-EM data sets 2 in Relion.

Representative cryo-EM micrographs and two-dimensional class averages are shown for both data sets as well as the processing strategies used to obtain a 2.6 Å structure of the transmembrane domain of hNKCC1.

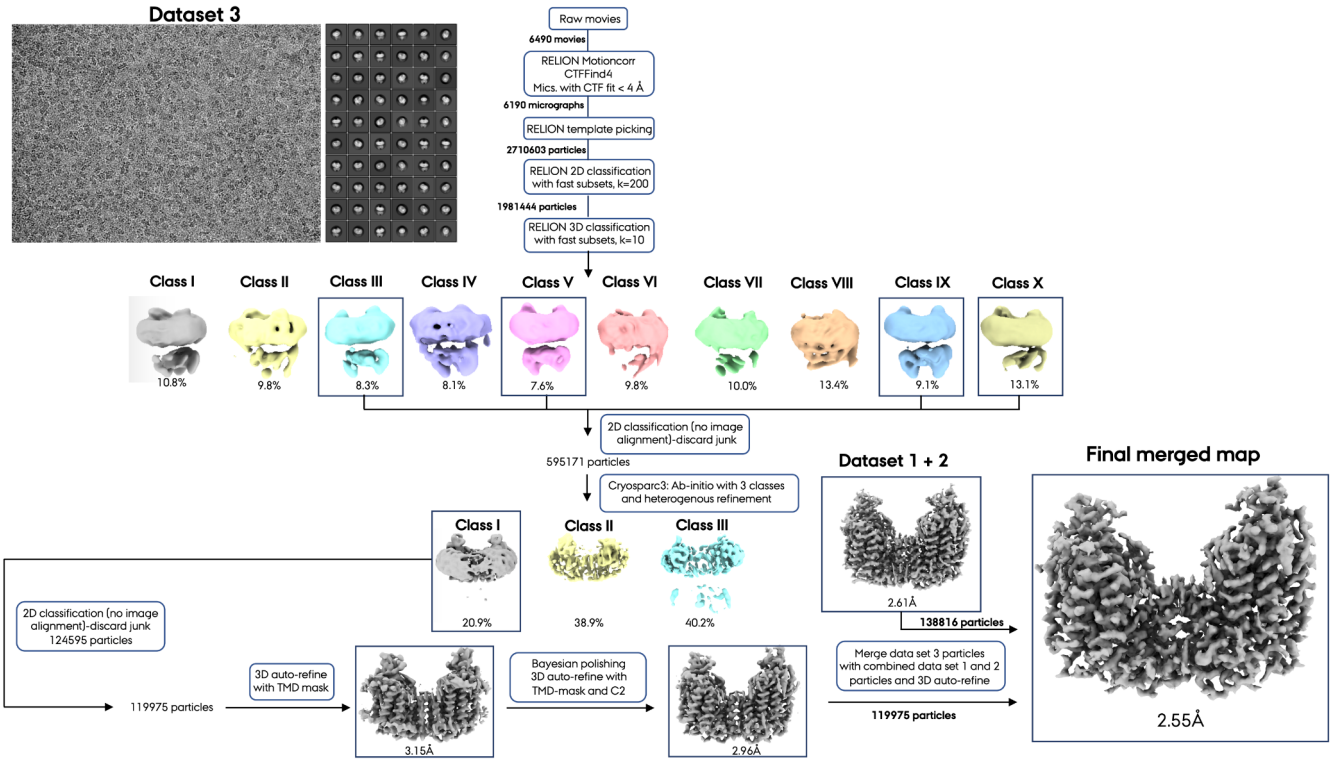

Figure EV3. Processing of the third cryo-EM data set of hNKCC1 in Relion and CryoSPARC3.

Representative cryo-EM micrographs, two-dimensional class averages, and the processing strategy used to obtain the final structure of the transmembrane domain of hNKCC1.

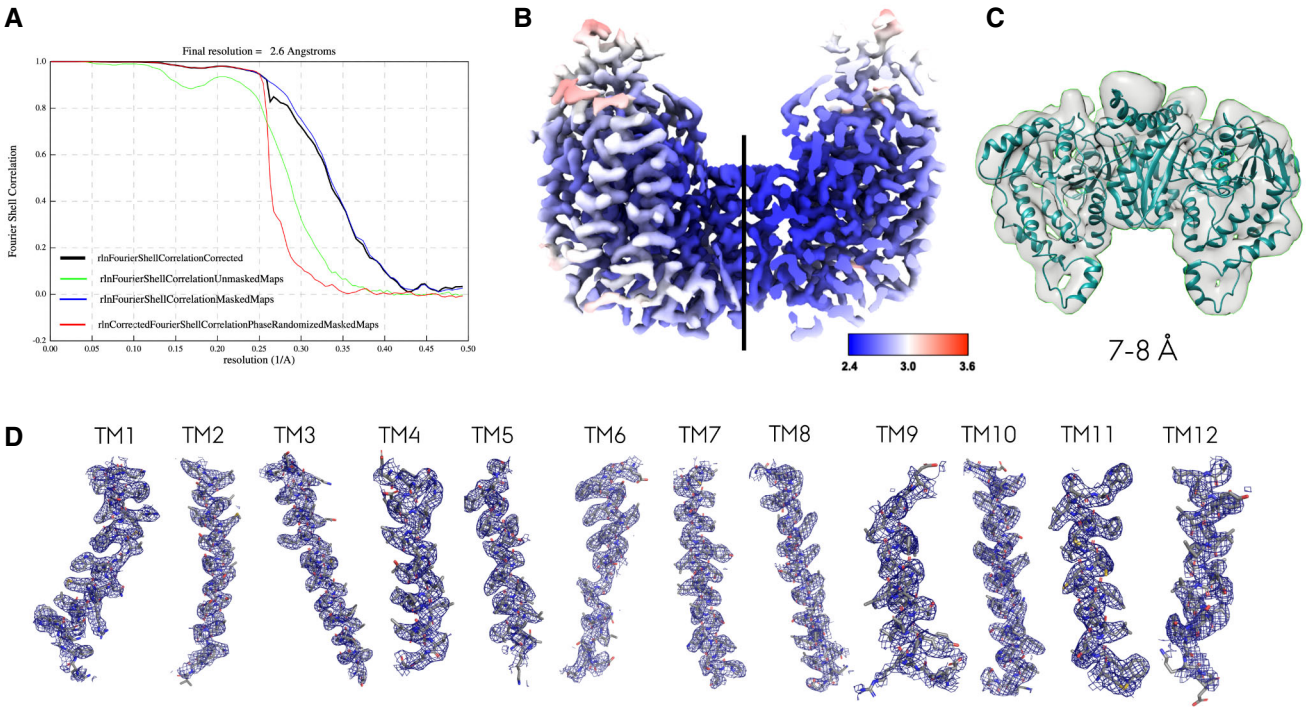

Figure EV4. Cryo-EM structure of hNKCC1 in complex with Na^+, K^+, and Cl^- ions.

A Gold standard Fourier shell correlation curves between the two half maps with the final resolution at FSC = 0.143.
B Local resolution estimations throughout the determined transmembrane domain of hNKCC1.
C Cryo-EM map of the cytoplasmic domain of hNKCC1 with the *Danio rerio* NKCC1 model docked (PDB: 6NPJ).
D Representative densities of the different transmembrane helices of molecule A of hNKCC1.

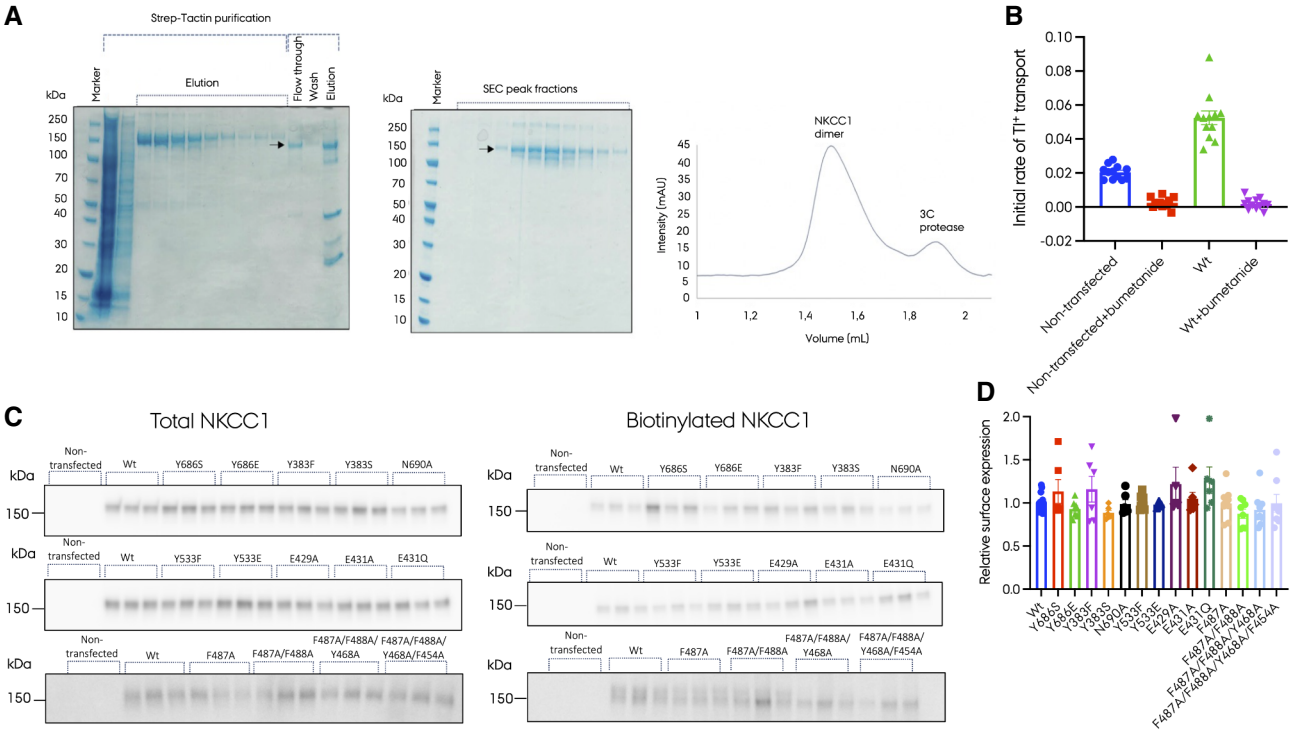

Figure EV5. Expression, purification, and functional studies of hNKCC1.

A Purification of hNKCC1 transporter expressed in HEK293 GnTI^{-} cells. The protein used for structure determination was purified by Strep-Tactin purification, Ni^{2+}-ion affinity chromatography (SDS–PAGE 1), followed by size exclusion chromatography (SDS–PAGE 2 and size exclusion chromatogram).

B Initial rate of Tl^{+} transport in non-transfected cells (with and without bumetanide) as well as in cells transfected with wild-type hNKCC1 (with and without bumetanide).

C Representative western blotting of NKCC1 in total and biotinylated (plasma membrane) fractions of wt NKCC1- and mutant NKCC1-expressing cells.

D Surface expression of NKCC1 mutants relative to wt NKCC1 quantified by densitometry of western blot with six replicas per construct.