Supporting Information

Replacement of Lys-300 with a glutamine in the NhaA Na^+/H^+ antiporter of *Escherichia coli* yields a functional electrogenic transporter

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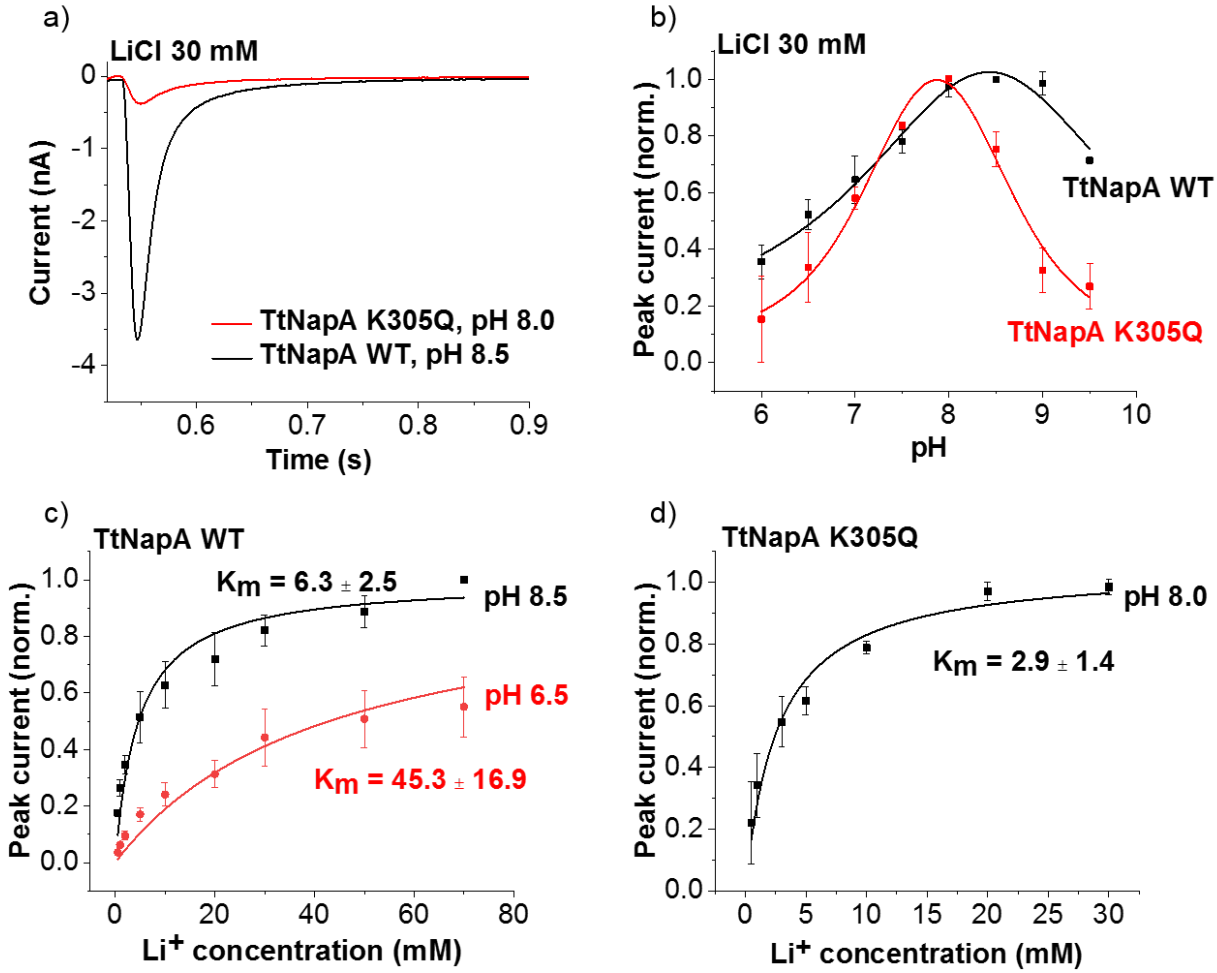

Figure S1. SSM-based electrophysiology of LiCl jumps in proteoliposomes of TtNapA WT and K305Q. a).Current traces generated on TtNapA WT and K305Q upon LiCl 30 mM jumps at their respective optimal pHs (8.5 for WT and 8.0 for K305Q). b). pH dependence of the transient current magnitude recorded after LiCl 30 mM jumps at pHs between 6.0 and 9.5 for TtNapA WT (black) and K305Q (red). Data from at least three different experiments were normalized to the maximum determined amplitude averaged and presented as mean ± s.d. Curves correspond to Voigt fits of the experimental data. c). Li^+ dependence of the transient current magnitude recorded after LiCl concentration jumps at pH 8.5 (black) and pH 6.5 (red) for TtNapA WT. Data were normalized to the extrapolated maximum of the hyperbolic fit, averaged and presented as mean ± s.d. d). Li^+ dependence of the transient current magnitude recorded after LiCl concentration jumps at pH 8.0 for TtNapA K305Q. Data were normalized to the extrapolated maximum of the hyperbolic fit, averaged and presented as mean ± s.d.

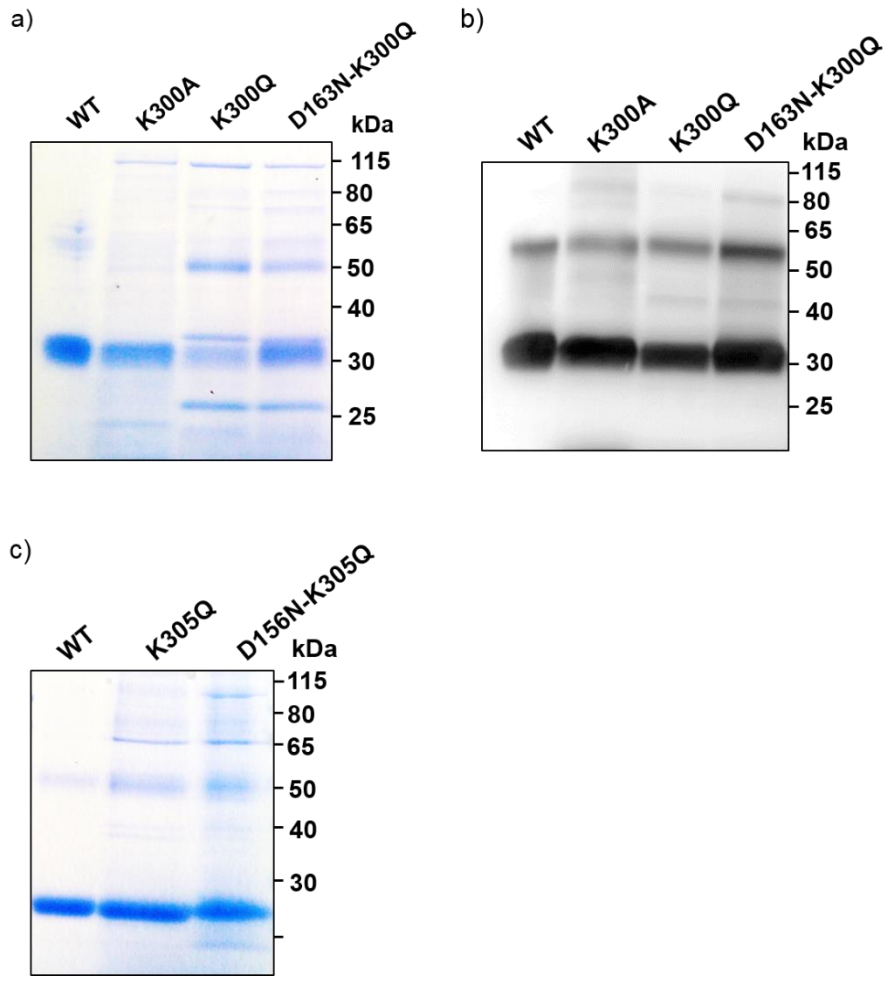

Figure S2. Assessment of the reconstitution of EcNhaA and TtNapA variants in proteoliposomes. a) SDS-PAGE and b) Western blot of EcNhaA WT, K300A, K300Q, D163N-K300Q variants in LPR10 proteoliposomes. c) SDS-PAGE of TtNapA WT, K305Q and D156N-K305Q variants in LPR10 proteoliposomes. The presence of EcNhaA proteins in comparable amounts in proteoliposomes is confirmed by the detection of a band slightly above 30 kDa with similar intensity between the variants. The same can be concluded for TtNapA variants which were detected between 25-30 kDa by SDS-PAGE. The predicted molecular weights for EcNhaA and TtNapA are 43 kDa and 41 kDa, respectively. Samples of proteoliposomes were prepared as follows: 40µL of proteoliposomes were pelleted by ultracentrifugation at 100000 g during 30 min at 4°C, washed with reconstitution buffer and collected by ultracentrifugation. Pelleted proteoliposomes were resuspended in sample buffer and loaded into a Bis-Tris 10 % polyacrylamide gel under denaturating conditions. Gels in a) and c) were stained with Coomassie Brilliant Blue. For Western blot in b), after the SDS-PAGE run was completed, proteins were electroblotted to a nitrocellulose membrane and incubated with mouse Penta-His primary antibody (Qiagen, 34660). As secondary antibody goat anti-mouse immunoglobulins conjugated to HRP (Dako, P0447) was used. Detection was performed by chemiluminescence.