Supplementary Figures.

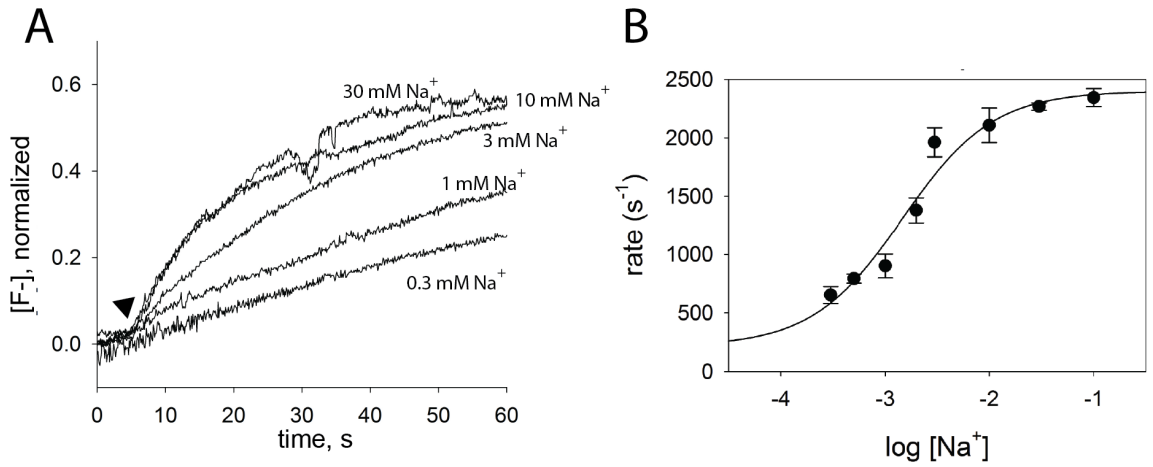

Figure S1. Na^+ dependence of fluoride transport by Bpe-N43D. A. Fluoride efflux measured with a fluoride-specific electrode in the presence of varying Na^+-isethionate, as indicated. Transport is initiated by addition of valinomycin (closed triangle). Traces are normalized with respect to total encapsulated fluoride. B. Rate of fluoride transport as a function of Na^+ concentration. Solid line shows fit to a single-site binding model with a K_d of 1.5 mM, and limiting rates of 210 s^{-1} and 2400 s^{-1} for Na^+-free and saturating Na^+ conditions, respectively. Error bars represent the standard error of measurement (n=3-5).

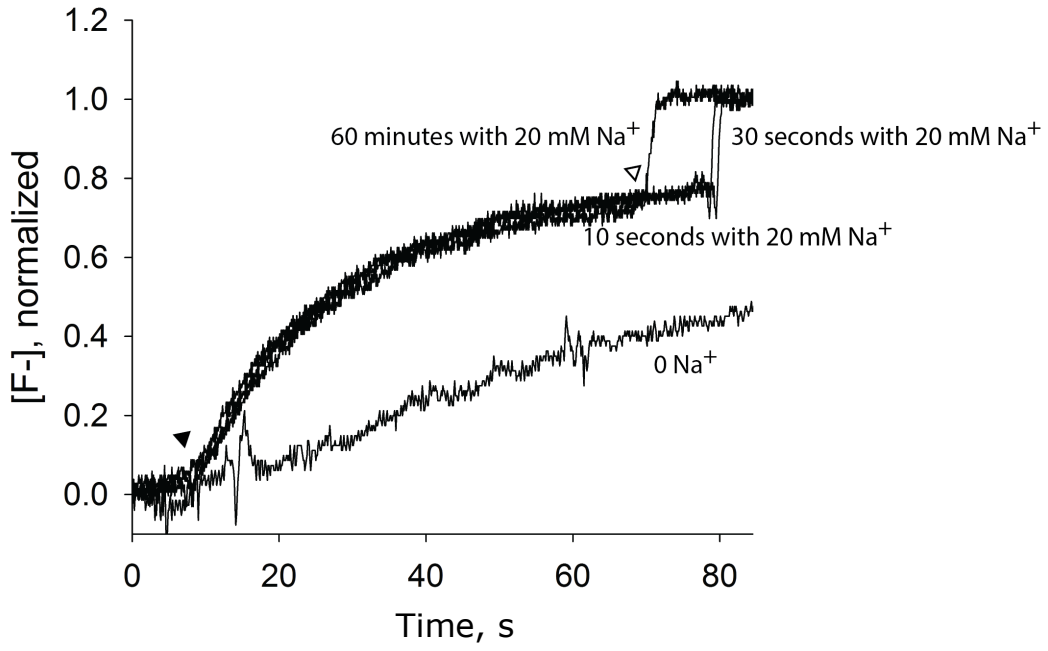

Figure S2. Fast kinetics of Na^+ exchange for Bpe-N43S. Fluoride efflux measured with a fluoride-specific electrode. Proteoliposomes were incubated with 20 mM Na^+-isethionate for the indicated time before transport is initiated by addition of valinomycin (closed triangle). After steady state is reached, remaining fluoride is released from the liposomes by addition of detergent (e.g., white triangle). Traces are normalized with respect to total encapsulated fluoride.

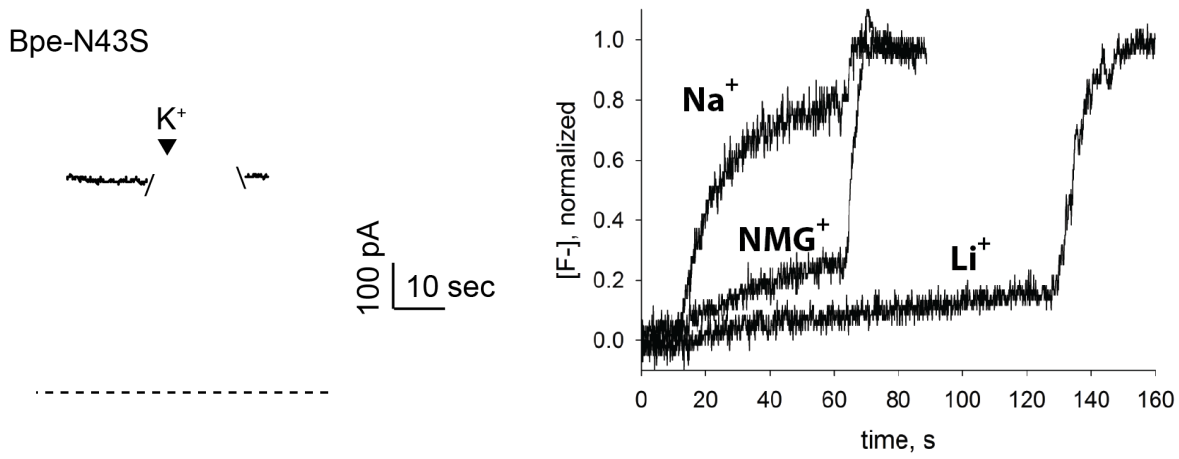

Figure S3. Fluoride transport by N43 mutants in the presence of different cations. A. Fluoride currents mediated by Fluc-N43S in response to addition of 10 mM K^+ isethionate at the indicated time. Recording solution contains 10 mM Na^+ and 290 mM NMG^+ to maintain ionic strength. Zero current level indicated by the dashed line. B. Fluoride efflux from Fluc-N43D proteoliposomes with 10 mM Na^+, NMG^+, or Li^+ isethionate, as indicated. Efflux monitored with a fluoride-specific electrode. 300 mM K^+ is present in all experiments.

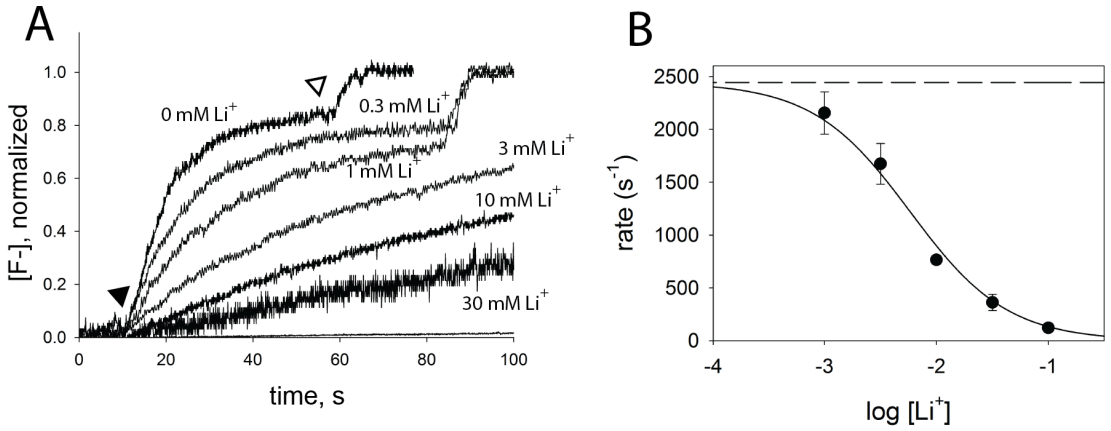

Figure S4. Li^+ dependence of fluoride transport by Bpe-N43D. A. Fluoride efflux measured with a fluoride-specific electrode in the presence of 20 mM Na^+-isethionate and indicated concentrations of Li^+ isethionate. Transport is initiated by addition of valinomycin (closed triangle), and after steady state is reached, remaining fluoride is released from the liposomes by addition of detergent (e.g., white triangle). Traces are normalized with respect to total encapsulated fluoride. B. Rate of fluoride transport as a function of Li^+ concentration. Solid line shows fit to a single-site binding model, with an apparent K_d of 5.8 mM. The maximum rate (dashed line) was defined according to the rate of fluoride efflux with 0 mM Li^+ (2440 s^{-1}). Error bars represent the standard error of measurement (n=3).

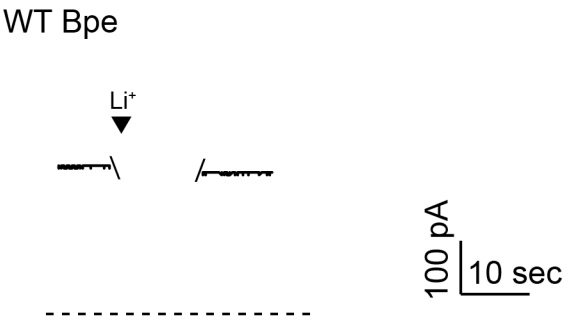

Figure S5. Electrophysiological recording of WT Fluc macroscopic currents. Currents recorded in 300 mM $NMG^{+}F^{-}$ 10 mM Li^{+} isethionate added at the indicated time. Dashed line indicates zero current level.