

## **Supplementary Information for**

### **Structural Identification of a Selectivity Filter in CFTR**

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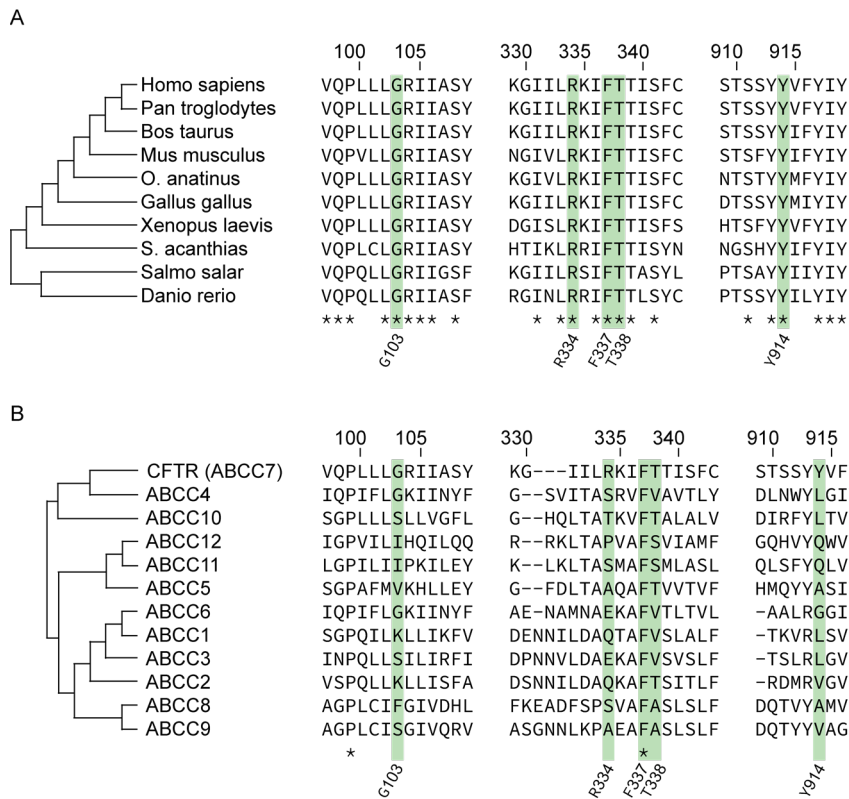
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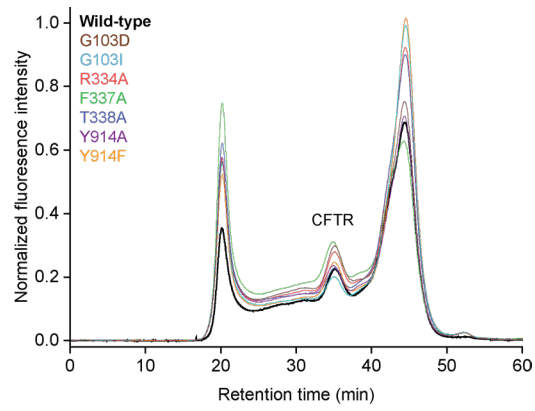
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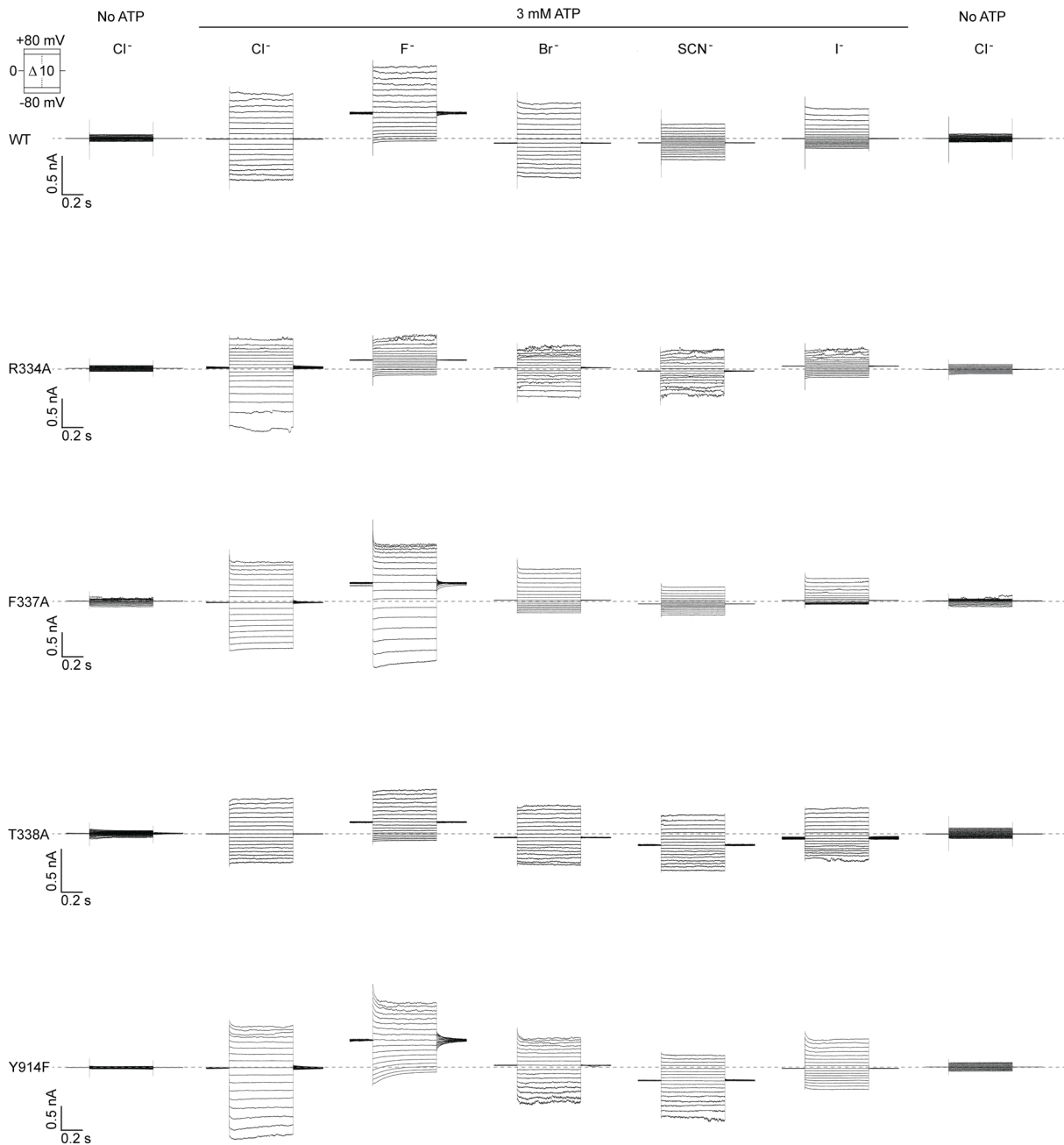
Figures S1 to S4



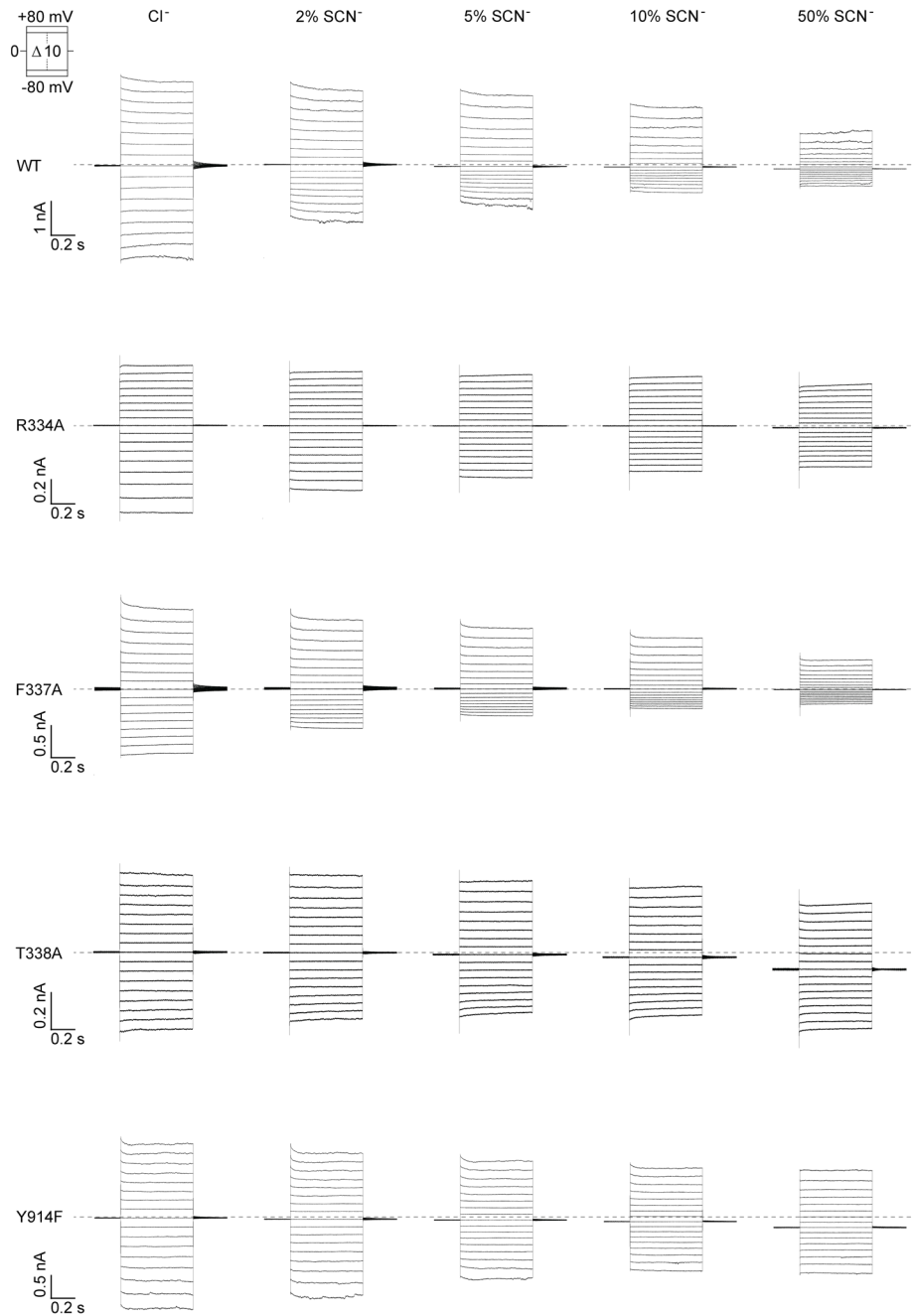
**Figure S1 | Sequence alignment of regions contributing to chloride coordination. (A)** Alignment of selected CFTR orthologues. **(B)** Alignment of human ABCC paralogues. Residues that coordinate chloride in CFTR are highlighted in green. Asterisks indicate invariance across the aligned sequences.



**Figure S2 | Fluorescence size-exclusion chromatograms of C-terminally GFP-fused CFTR Sfilter variants.**



**Figure S3 | Biionic potential measurements for selectivity filter variants.** Example voltage families for PKA-phosphorylated CFTR variants under biionic conditions in inside-out excised patches. The patch pipette contained 150 mM chloride, and the perfusion solution contained 150 mM of the indicated anion. 3 mM ATP was included in the perfusion solution where indicated. ATP-free measurements were performed at the beginning and end of each patch to ensure absence of CFTR-independent anion leak.



**Figure S4 | Open channel block of selectivity filter variants by thiocyanate.** Example voltage families for PKA-phosphorylated CFTR variants with the indicated mole fractions of thiocyanate. The patch pipette contained 150 mM chloride, and the perfusion solution contained a mixture of chloride and thiocyanate. 3 mM ATP was used in the perfusion solution for all experiments.