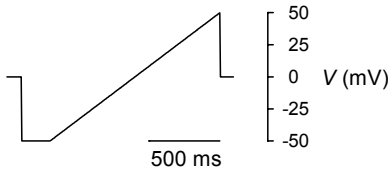
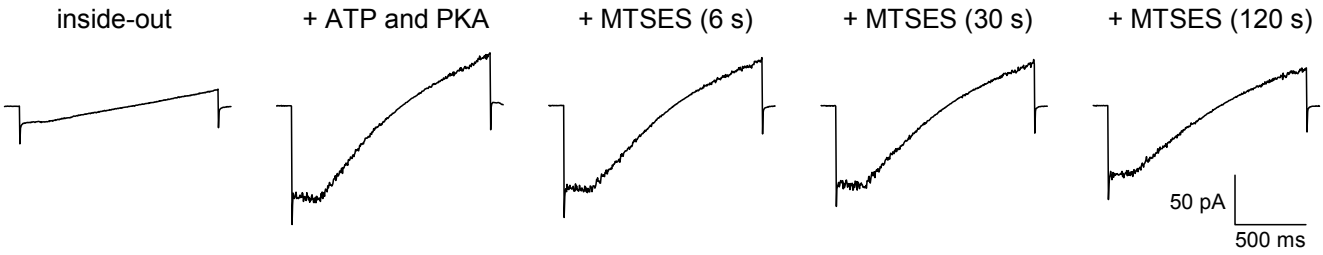
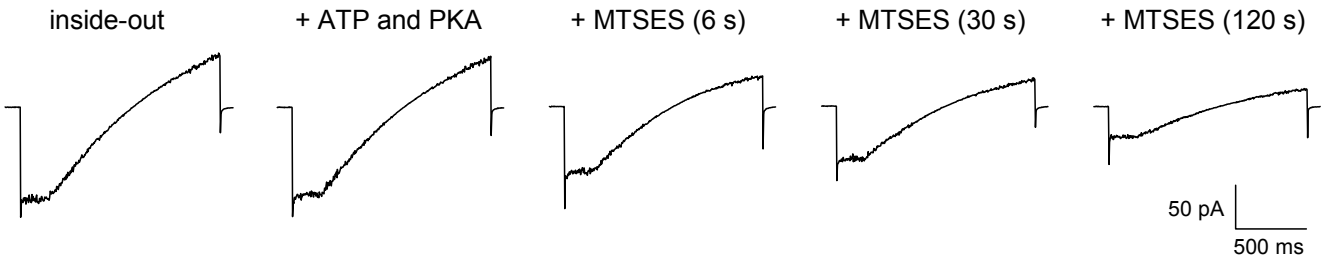
A. Voltage Protocol



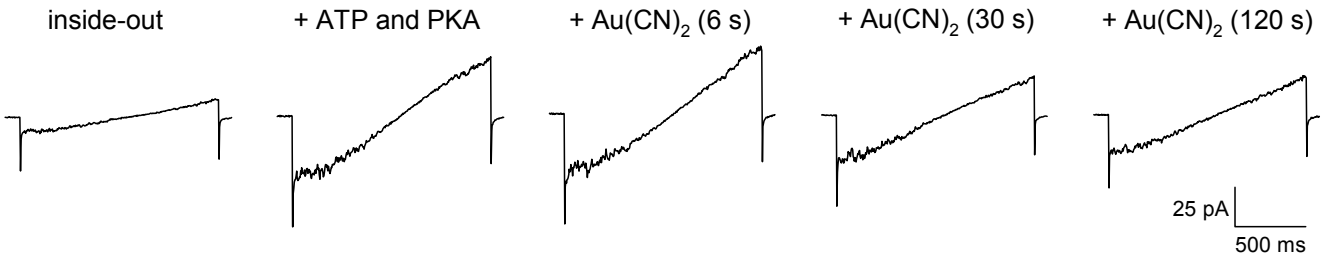
B. T338C



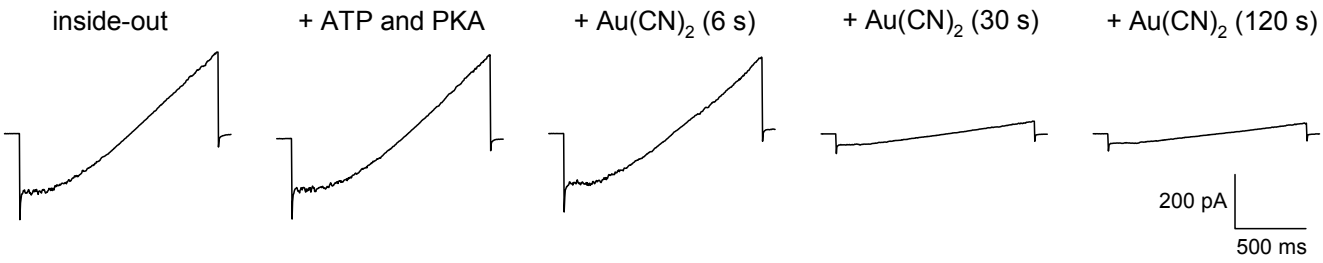
C. T338C-E1371Q



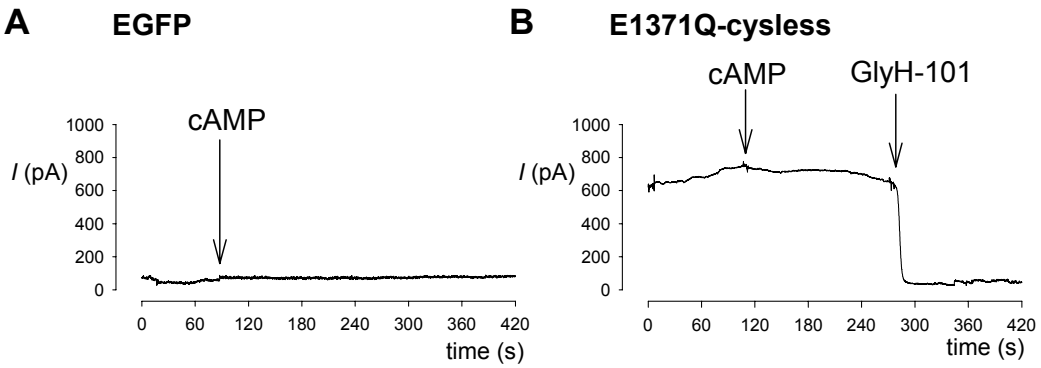
D. L102C



E. L102C-E1371Q



Supplemental Figure S1



Supplemental Figure S2

Supplemental Figure S1 Examples of currents in inside-out membrane patches during voltage ramps. *A*, depolarizing voltage ramp protocol used. The voltage ramp was applied every 6 s from a holding membrane potential of 0 mV. *B-D*, raw currents carried by the channel variants named following patch excision from the cell (inside-out), following channel activation with ATP and PKA, and and different times after application of MTSES (200 μM) or $Au(CN)_2^-$ (2 μM) to the cytoplasmic face of the membrane patch. Note that background (leak) currents are small in T338C and L102C prior to channel activation with ATP and PKA, whereas T338C/E1371Q and L102C/E1371Q carry constitutively active currents that are not further increased in amplitude by ATP and PKA.

Supplemental Figure S2 Controls for CFTR whole cell current recording. *A*, expression of EGFP alone (empty vector control) does not lead to the appearance of cAMP-activated whole cell current in visibly green fluorescent cells. *B*, cys-less E1371Q-CFTR expresses a constitutively active whole cell current that is not further increased in amplitude by addition of cAMP stimulatory cocktail. Identity of this constitutive current as CFTR is confirmed by inhibition by the CFTR-selective inhibitor GlyH-101 (50 μM).