Supplementary Fig. 1 Intracellular application of charged MTS reagents affected $NP_o$ of wildtype but not cysless CFTR channels. A. Both 100 μM $MTSET^+$ ($ET^+$) and $MTSEA^+$ ($EA^+$) increased $NP_o$ of WT-CFTR. 100 μM $MTSES^-$ ($ES^-$) decreased $NP_o$ of WT-CFTR. -$ET^+$, $EA^+$, $ES^-$: ATP+PKA alone; +$ET^-$, $EA^-$, $ES^-$: ATP+PKA+MTS reagents; Washout: wash out ATP+PKA+MTS reagents with control solution, then wash on ATP+PKA alone. B. Recorded in the same condition as A, the three charged MTS reagents had no effects on $NP_o$ of cysless-V510A-CFTR.

Supplementary Fig. 2 $MTSES^-$ failed to rescue R352C-CFTR (A) and $MTSET^+$ failed to rescue the stable open state in R352A-CFTR (B). A. R352C-CFTR maintained multiple open states in the absence of $MTSES^-$ (*upper trace*, solution containing ATP + PKA alone) and in the presence of 100 μM $MTSES^-$ (*lower trace*, solution containing ATP + PKA + $MTSES^-$), recorded from the same patch. Bath and pipette solutions contained 150 mM $Cl^-$, and membrane potential was held at $V_M$=-100mV (n=3). B. R352A-CFTR exhibited multiple open states in the absence of $MTSET^+$ (*upper trace*, solution containing ATP + PKA alone) and in the presence of 100 μM $MTSET^+$ (*lower trace*, solution containing ATP + PKA + $MTSET^+$), recorded from the same patch (n=5).

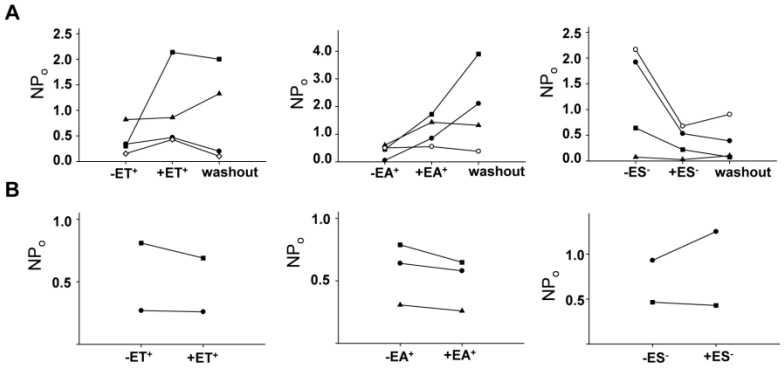
Supplementary Fig. 3 Deposition of negative charge at D993C improved stability of the open state. D993C-CFTR was modified by 100 μM $MTSES^-$ (A) but not by 100 μM $MTSEA^+$ (C). All traces were recorded in the presence of Mg-ATP and PKA from excised inside-out patches with symmetrical 150 mM $Cl^-$ solution at $V_M$=-100 mV. B. Comparison of single channel amplitudes of the f open state of D993C-CFTR and mean burst duration of D993C-CFTR in the absence (-MTSES) and presence of 100 μM $MTSES^-$ (+MTSES) condition. Single channel amplitude was

significantly decreased while mean burst duration was significantly increased with $MTSES^-$ compared to the pre-$MTSES^-$ condition (*n*=5).

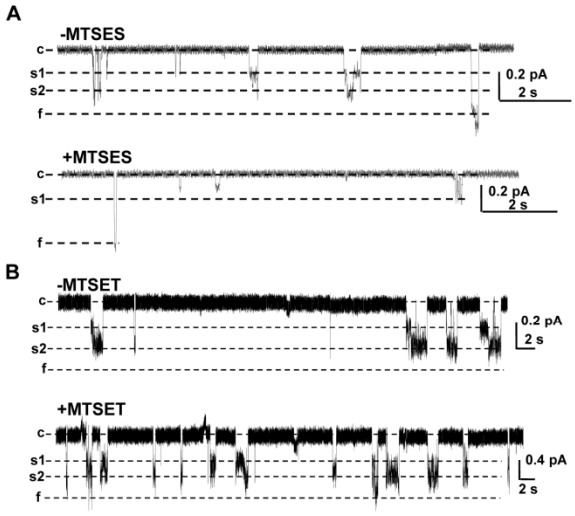
Supplementary Fig. 4 Charge recovery at R352C and D993C on the cysless background repaired channel behavior. R352C-cysless-V510A-CFTR (A) and D993C-cysless-V510A-CFTR (B) in the absence (-MTSEA or –MTSES, with ATP+PKA) and in the presence of 100 μM MTS reagents (+MTSEA or +MTSES, with ATP+PKA+$MTSEA^+$ or $MTSES^-$) in excised inside-out patches with similar conditions as Fig. S2 (*n*=3 each).

Supplementary Fig. 5 Energetic mutant cycle analysis supports the interaction between R352 and D993 of CFTR. For each single mutation, such as R352C and D993C, the change in free energy relative to that of the wild-type is expressed by the equation: $\Delta G=-RT\ln(\theta_{mutant}/\theta_{ww})$, where $\theta$ is the equilibrium gating ratio (see Methods). $\Delta\Delta G_{int}$ is calculated as described in Methods. $\Delta\Delta G_{int}$= -1.508 $kcal.mol^{-1}$.

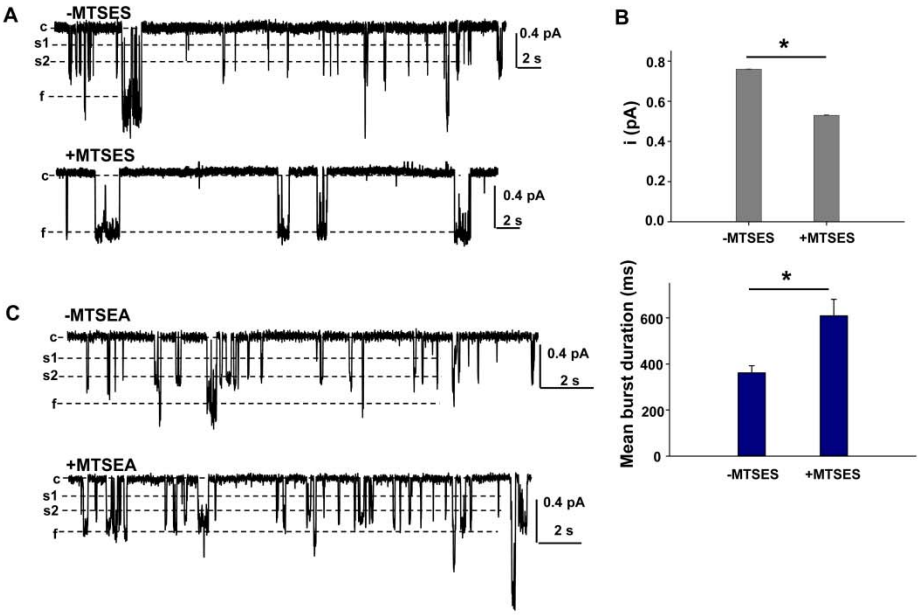
Supplementary Fig. 1



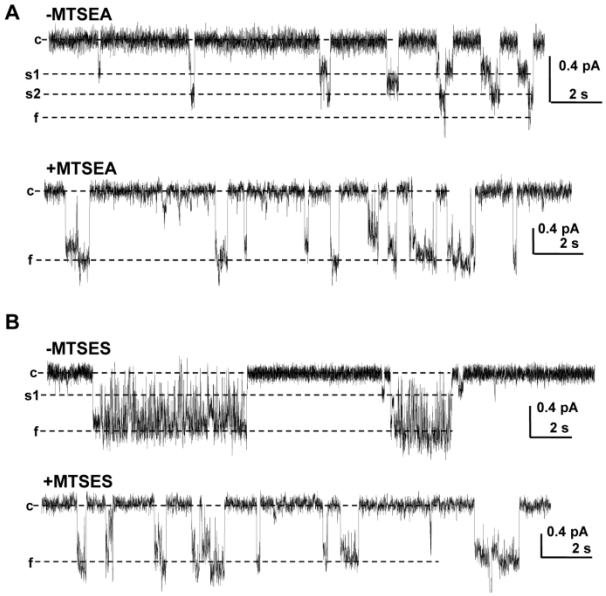
Supplementary Fig. 2



Supplementary Fig. 3



Supplementary Fig. 4



Supplementary Fig. 5