

Tsai et al., <http://www.jgp.org/cgi/content/full/jgp.201010399/DC1>

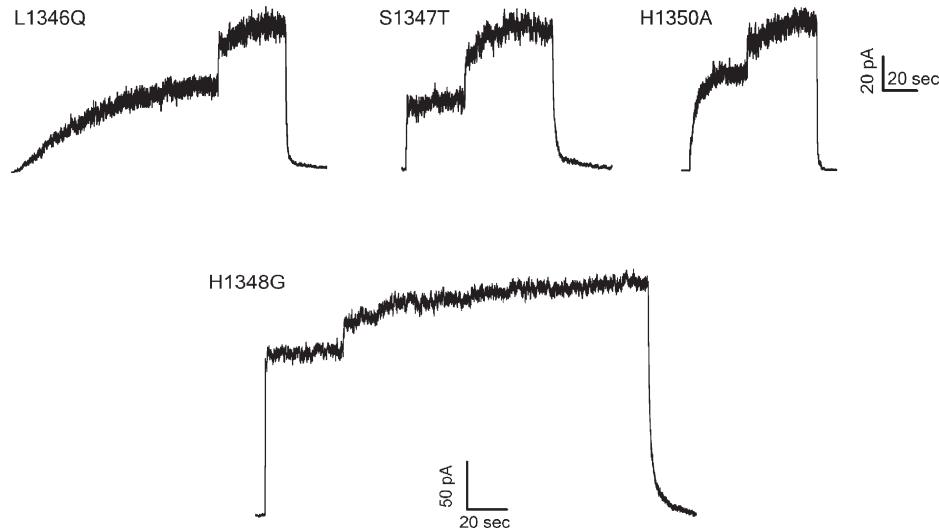


Figure S1. Ligand exchange experiments conducted with macroscopic current recordings for mutations in the signature sequence of NBD2. The mutant channels were first opened by 2.75 mM ATP. Solution was then changed to 50 μ M PATP until the current reached a steady state. PKA was present in all solutions to maintain a maximal phosphorylation level. Current traces for L1346Q-CFTR, S1347T-CFTR, and H1350A-CFTR share the same time and amplitude scales.

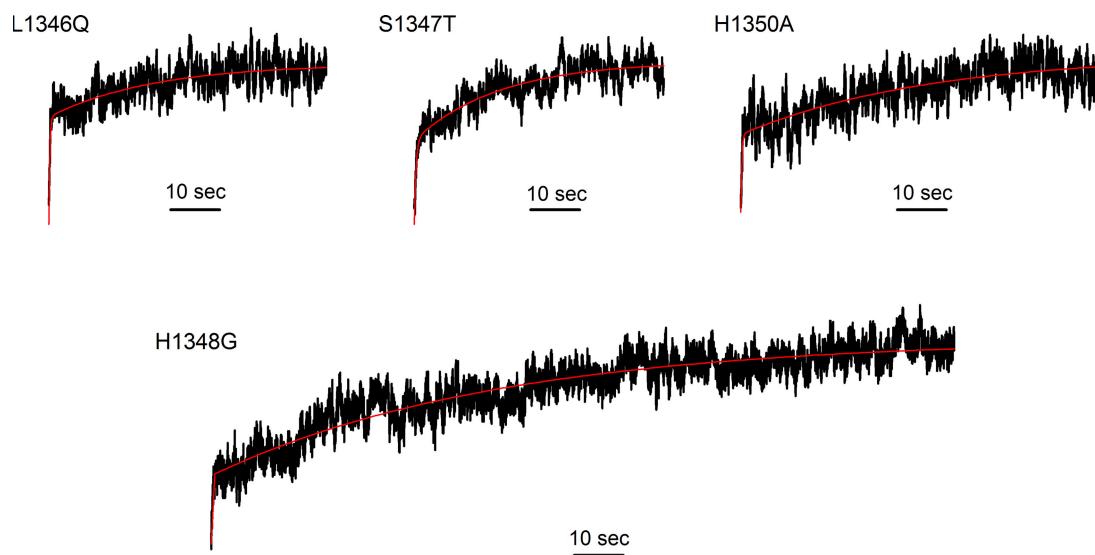


Figure S2. The time courses of PATP-induced two-step current rise from Fig. S1. All traces were fitted with a double-exponential function (red lines).

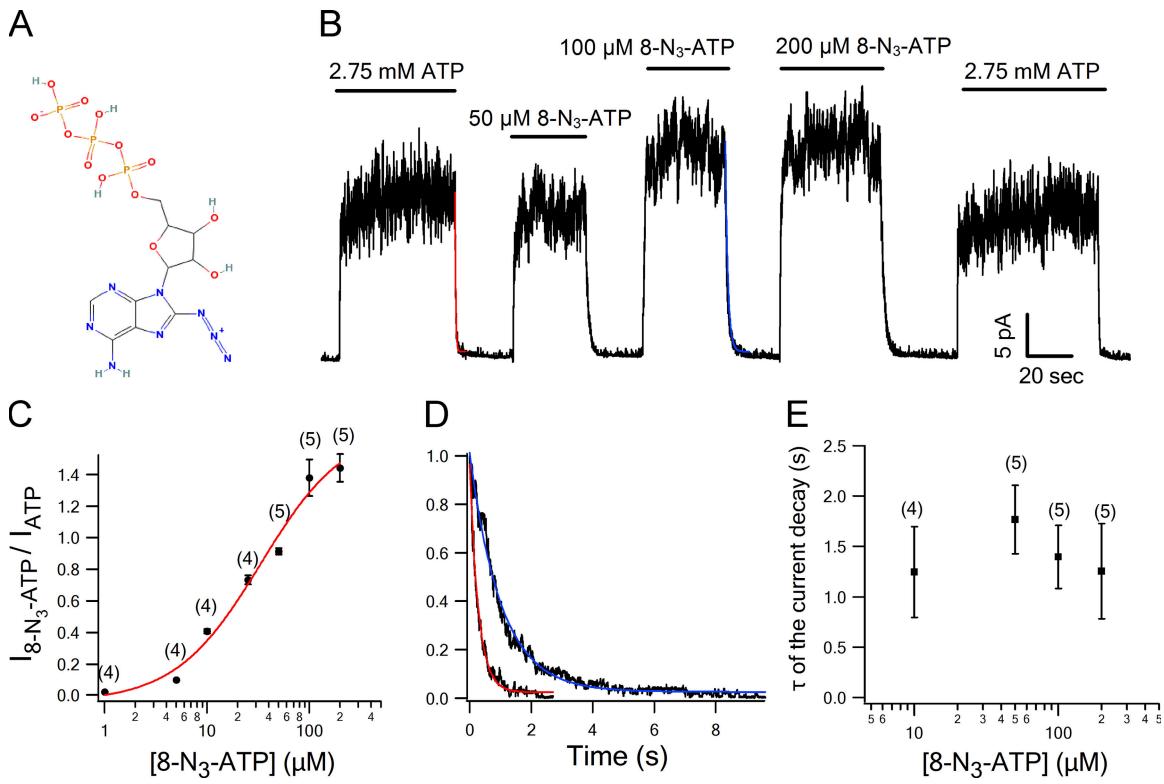


Figure S3. Gating of WT-CFTR by 8-N₃-ATP. (A) The structural formula of 8-N₃-ATP downloaded from National Center for Biotechnology Information. (B) WT-CFTR channels were exposed to different concentrations of 8-N₃-ATP. 100 μM seemed to be close to the maximally effective concentration. The Po induced by 100 μM 8-N₃-ATP is ~1.4-fold higher than that induced by 2.75 mM ATP. (C) The dose-response relationship of 8-N₃-ATP on WT-CFTR. The amplitude of macroscopic current induced by 8-N₃-ATP was normalized to that by 2.75 mM ATP in the same patch. The data were then fitted with the Hill equation (red curve) with a $K_{1/2}$ of $33.44 \pm 13.4 \mu\text{M}$ and the Hill coefficient of 1.04 ± 0.39 . (D) The current decay trace (from B) after washing out of 2.75 mM ATP or 100 μM 8-N₃-ATP. The red and blue curves represent single-exponential fits. 8-N₃-ATP apparently elicited longer openings than ATP. (E) The relaxation time constant after the removal of 8-N₃-ATP at different concentrations was approximately constant and approximately fourfold longer than that of ATP. Because the mean macroscopic current in the presence of a maximal concentration of 8-N₃-ATP was only 1.4-fold higher than that with 2.75 mM ATP, the opening rate of 8-N₃-ATP-gated WT-CFTR was estimated to be approximately two- to threefold lower than that of 2.75 mM ATP.

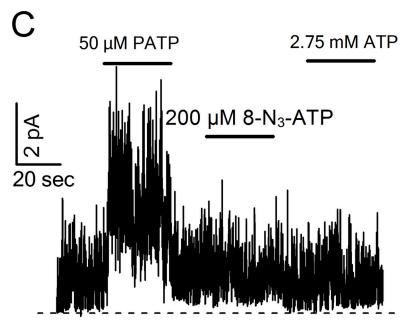
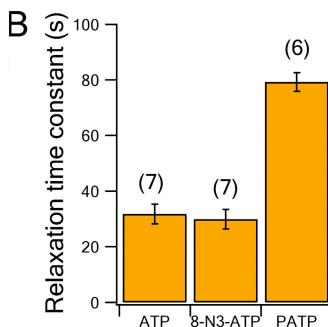
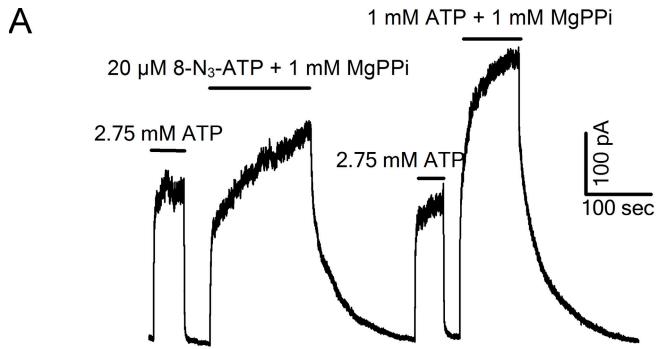


Figure S4. The idea that 8-N₃-ATP and ATP exert similar effects on CFTR gating kinetics when binding to NBD1 predicts two functional consequences. First, the burst duration for channels locked open by ATP plus MgPPi should be similar to that with 8-N₃-ATP plus MgPPi because in the lock-open state, NBD2 is occupied by MgPPi while the nucleotide (ATP or 8-N₃-ATP) is bound in NBD1. This was indeed the case as shown in A and B. In contrast, PATP, which prolongs channel open time by binding to NBD1, greatly enhance the stability of the lock-open state (B). Second, 8-N³-ATP, like ATP, should pose little effect on G551D-CFTR, as the G551D mutation eliminates the nucleotide's effects on NBD2. This prediction is again valid. G551D-CFTR channels responded to neither ATP nor 8-N³-ATP ($n = 3$). PATP, on the other hand, potentiated G551D channels (C).

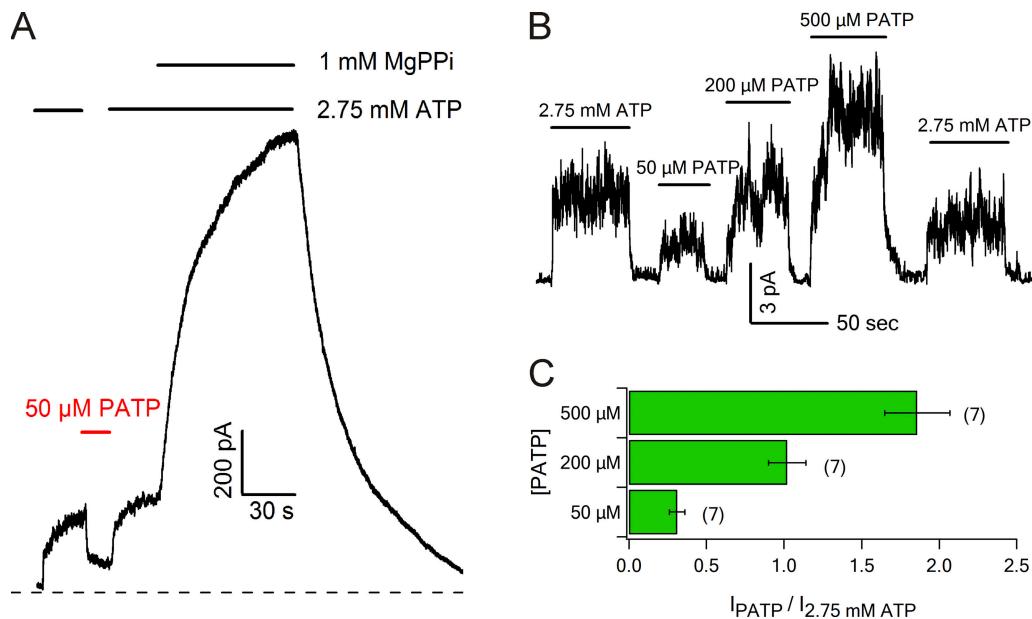


Figure S5. Effect of the Y1219G mutation on the apparent affinity for ATP. (A) In this macroscopic current trace (a similar result was seen in three other patches), 50 μM PATP only opened a very small portion (< 10%) of the Y1219G channels, as revealed by a much larger current elicited by the subsequent addition of ATP plus MgPPi. Thus, the P_o induced by 50 μM PATP in Y1219G-CFTR channels is lower than that observed for WT-CFTR, as shown in Fig. 1 A. (B and C) The effects of Y1219G mutation can be overcome by higher concentrations (200 and 500 μM) of PATP, supporting the idea that this mutation does reduce the apparent affinity for PATP in NBD2.

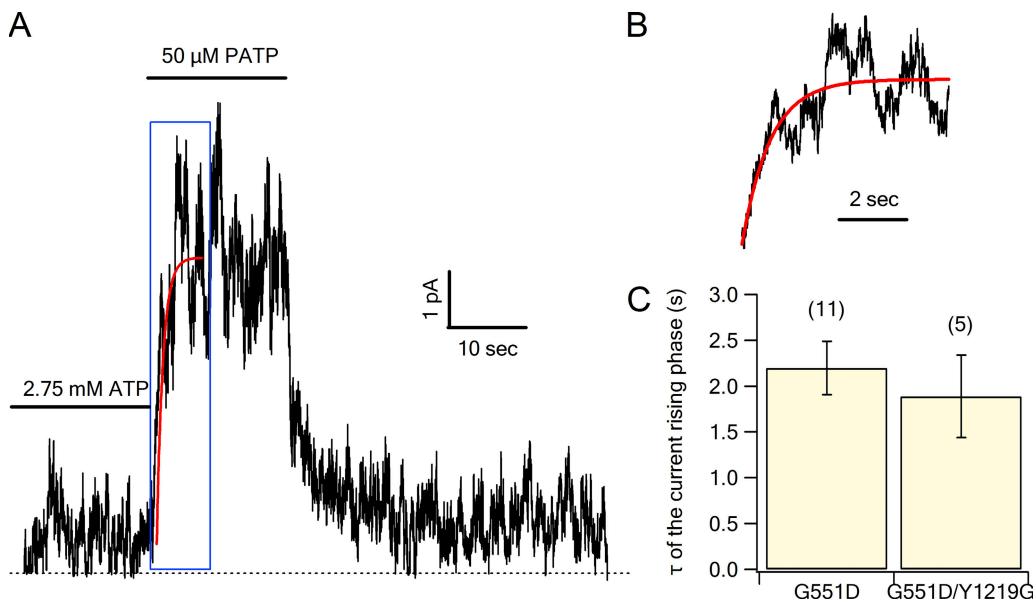


Figure S6. Ligand exchange experiments performed with G551D/Y1219G-CFTR channels. (A) Macroscopic current recordings from G551D/Y1219G double-mutant channels. PATP induced rapid current increase upon solution changes. (B) The time course of current increase can be reasonably fitted with a single-exponential function (red line). (C) Time constants of the current rise upon ATP/PATP exchange as shown in A. There is little difference ($P = 0.78$) between the time constant for G551D and that for G551D/Y1219G channels. These results suggest that G551D channels fail to trap ATP in NBD1 not because of the electrostatic repulsion between the aspartate side chain at position 551 and the ATP or PATP molecule bound in the NBD2 site.