Chaves and Gadsby, http://www.jgp.org/cgi/content/full/jgp.201411347/DC1

Equivalent residues to target cysteine positions

	ABC signature		D loop		H loop	
CFTR-NBD1:	-- L **S549** G G Q	-----	G	-----	**S605**	--
CFTR-NBD2:	-- L **S1347** H G H	-----	**A1374**	-----	H	--
TM287-NBD1:	-- F **S471** G G Q	-----	S	-----	**Q526**	--
TM288-NBD2:	-- L **S493** Q G Q	-----	**S520**	-----	H	--
Sav1866-NBDs:	-- L **S479** G G Q	-----	**S506**	-----	**H534**	--

Figure S1. Sparse alignment relating the four CFTR residues (bold with gray shadow) that were replaced by target cysteines to their equivalents (bold) in heterodimeric TM287/288 and homodimeric Sav1866.

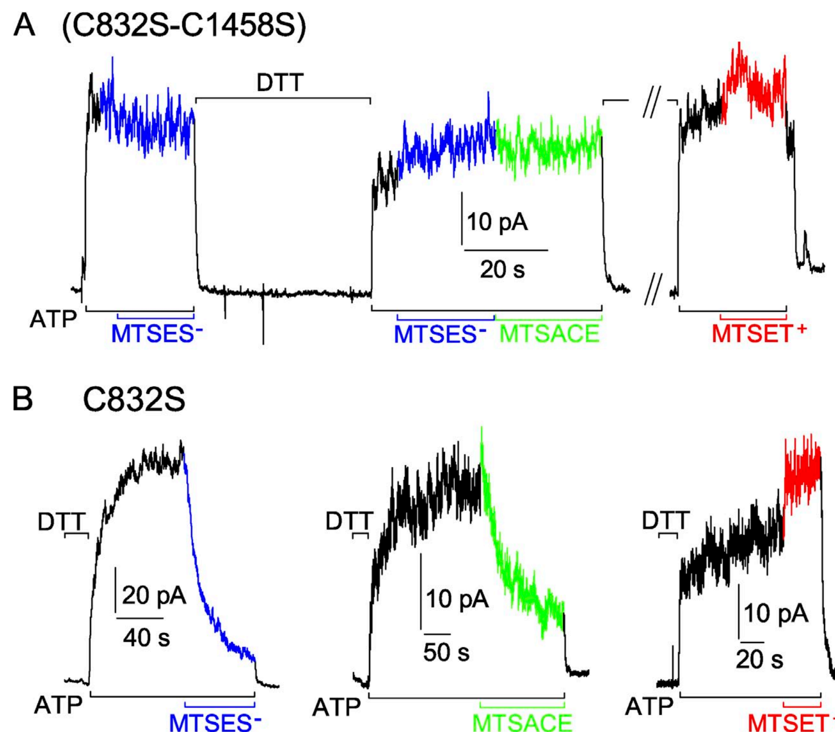

Figure S2. "Background" (C832S-C1458S) CFTR channels (lacking eight native cysteines), but not C832S CFTR channels (lacking a single native cysteine), are insensitive to high concentrations of MTSES$^-$, MTSACE, or MTSET$^+$. (A) ATP-activated (3 mM, black bars below record) current of (C832S-C1458S) CFTR channels was unaffected by 1 mM MTSES$^-$ (blue trace and bar), 1 mM MTSACE (green trace and bar), or 1 mM MTSET$^+$ (red trace and bar). Exposure to 20 mM DTT (black bars above record) ensured the 10 remaining native thiols would be reactive if accessible; PKA was reapplied for 25 s during the 85-s gap to rephosphorylate the channels. Similar results were obtained in a total of five patches. (B) ATP-activated (3 mM, black bars below record) current of prephosphorylated C832S CFTR channels in three separate patches slowly (note extended time scales compared with A) decreased during exposure to 1 mM MTSES$^-$ (left; blue trace and bar) and to 1 mM MTSACE (middle; green trace and bar), but showed a small increase during exposure to 1 mM MTSET$^+$ (right; red trace and bar). 20 mM DTT (black bars above records) kept thiols reactive. Ca^{2+}-dependent Cl^- current decay time constants were all 0.3 s in these patches; similar results were obtained in two, five, and six patches, respectively.

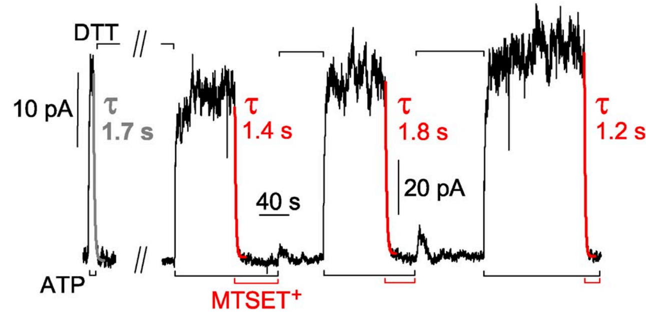

Figure S3. The target cysteine, S549C, in the active-site LSGGQ sequence of CFTR channels otherwise differing from wild type by substitution of only a single native cysteine (C832S) is rapidly modified by $MTSET^+$, just like the same S549C target in a more cysteine-depleted CFTR background (main text Fig. 3). ATP-activated currents (3 mM, black bars below record) of S549C-C832S CFTR channels decreased rapidly upon ATP washout or exposure to 50 µM $MTSET^+$ (red bars). 20 mM DTT (black bars above record) removed adducts between modifications. The gap marks omission of 5 min of recording; Ca^{2+}-dependent Cl^- current decay time constant was 0.1 s in this patch. Colored lines show single-exponential fits to current decay time courses on ATP removal (gray) or on $MTSET^+$ modification (red), and colored numbers give time constants. Their average ratio, $\tau_{MTSET}/\tau_{ATPw/o}$, was 0.9 for the S549C-C832S channels in this patch (similar results were obtained in a total of three patches), close to unity as found for S549C-(C832S-C1458S) CFTR channels.

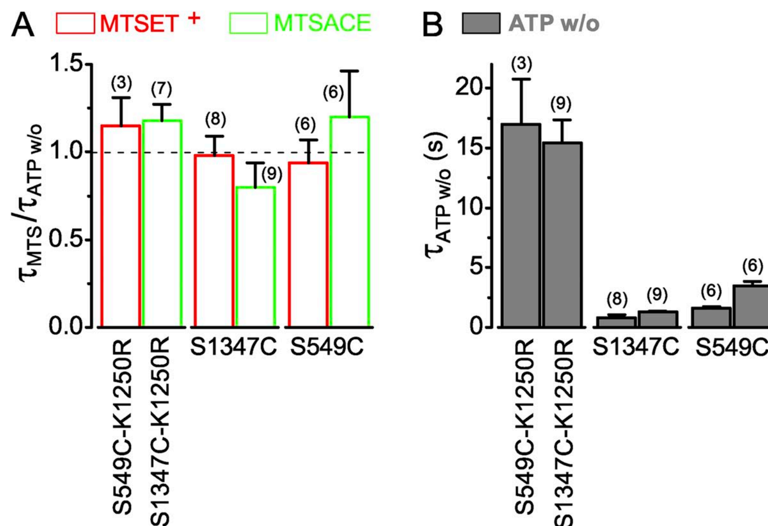

Figure S4. Constant unity ratio of current decay rates on MTS modification or ATP washout despite the fact that ~10-fold variation of the rates themselves indicates strict dependence of accessibility of both S549C and S1347C positions on the gating state of a CFTR channel. (A) Time constant ratios, $\tau_{MTS}/\tau_{ATPw/o}$, replotted from main text Figs. 3 D, 5 D, and 7 D, are all close to unity (dashed line). (B) Despite this uniformity, the absolute current decay time constants, $\tau_{ATPw/o}$, replotted for ATP washout from Figs. 3 C, 5 C, and 7 C, vary over an order of magnitude. The near unity ratios mean that modification cannot occur when a channel is open, but then occurs as soon as each channel closes, before it can reopen. In structural terms, tight NBD1–NBD2 heterodimerization precludes access of MTS reagents to either target while a channel is open, but separation of the heterodimer interface along its full length upon channel closure allows instantaneous MTS reagent access to both target sites. Error bars represent mean ± SEM.

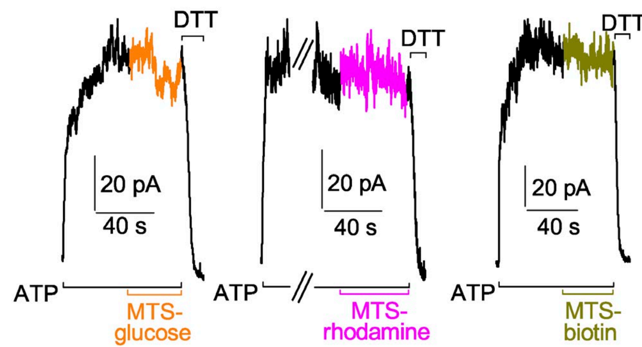

Figure S5. Background (C832S-C1458S) CFTR channels lacking engineered target cysteines are also insensitive to larger MTS reagents. ATP-activated (3 mM, black bars below records) current of (C832S-C1458S) CFTR channels was not altered by 50 µM MTS-glucose (left; orange trace and bar), 50 µM MTS-rhodamine (middle; magenta trace and bar), or 50 µM MTS-biotin (right; dark yellow trace and bar). The middle and right records are from the same patch; intervening exposures to 10 mM DTT (black bars above records) in the absence of ATP ensured that the 10 native thiols would be reactive if accessible; the gap in the middle record marks a 150-s period during which the channels were briefly rephosphorylated with PKA. Comparable results were obtained in a total of nine patches, in which the ratios of mean CFTR channel current during each 40-s MTS reagent exposure to that during the immediately preceding 20 s in ATP averaged 1.0 ± 0.08 (n = 5) for MTS-glucose, 1.1 ± 0.04 (n = 3) for MTS-rhodamine, and 1.0 ± 0.07 (n = 2) for MTS-biotin.

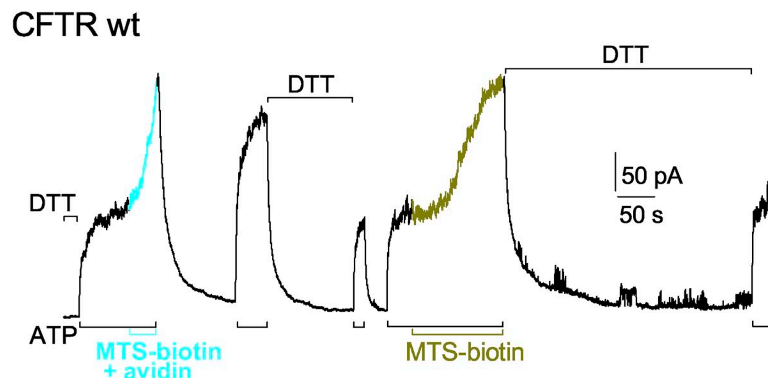

Figure S6. Even when bound tightly by avidin, MTS-biotin still retains its ability to modify accessible cysteine(s). ATP-activated current (3 mM, black bars below record) of prephosphorylated wild-type CFTR channels, containing all 18 native cysteines, was increased about twofold by MTS-biotin–avidin complex (cyan trace and bar; 5 µM MTS-biotin plus 25 µM avidin to ensure absence of unbound MTS-biotin). This reflected covalent modification because, after washout of ATP and unreacted complex, readdition of ATP alone activated current still twice the control amplitude. But treatment with 10 mM DTT (black bars above record) restored ATP-activated current to control size, whereupon exposure to MTS-biotin without 5 µM avidin (dark yellow trace and bar) similarly increased current about twofold, an effect also reversed by DTT. The modification was likely at native R-domain cysteine 832, as the neutral MTSACE decreased, rather than increased, ATP-activated current of C832S CFTR (Fig. S2 B, middle), which differs from wild-type CFTR by that single conservative substitution. Wild-type CFTR channel current was similarly augmented by irreversible modification by 100 µM NEM, an effect also attributed to reaction with cysteine 832 because of its prevention by the mutation C832A (Cotten, J.F., and M.J. Welsh. 1997. *J. Biol. Chem.* 272:25617–25622).