Supplementary Figure Legends

Figure S1: Simplified schematic of CFTR domain structure. MSD, membrane spanning domain; ICD, intracellular domain; NBD, nucleotide binding domain; P, phosphorylation site; RI, regulatory insertion; RE, regulatory extension. The residue boundaries of NBD1, the RI and RE are indicated.

Figure S2: Chemical structures of compounds used in this study. (left) CFFT-001 (N-cyclohexyl-4-(6-methyl-3-pyridinyl) pyrimidine-2-amine) was derived from a pharmacophore screening hit and synthesized by EPIX Pharmaceuticals. The compound was an early member of a series of near 150 compounds and was selected for the described studies due to its solubility of ~30 μM in PBS. **(right)** The positive control compound, C18 originally identified by EPIX Pharmaceuticals in PCT Int. Appl. (2007) WO 2007/021982 A2 and synthesized after the compound exemplified in paragraph [182].

Figure S3: NH chemical shift differences in F508del NBD1 ΔRI ΔRE. The combined chemical shift differences (in Hz, for N and H) generated upon **(A)** mutation of H620, **(B)** addition of CFFT-001, **(C)** deletion of H9 and **(D)** addition of CFFT-001 to the ΔH9 protein are plotted as a function of residue number. Unassigned residues are shown as "zero". The dotted line at 7 Hz, in (B), represents 1.6x the standard deviation for all the chemical shift differences shown in A, B, C and D and represents the starting value for the color gradients in Figures 2, 4 and 5.

Figure S4: Secondary structure propensity in F508del NBD1 ΔRI ΔRE. The amide proton chemical shift differences (in ppm) generated upon **(A)** H620Q mutation and **(B)** addition of CFFT-001, plotted as a function of residue number. Positive bars indicate a shift upfield (to lower ppm values), while negative bars indicate a shift downfield (to higher ppm values). Many of the H8 and H9 residues are initially upfield of random coil values (indicative of helical character) and negative bars indicate that they shift towards the center of the spectrum, reflecting a loss of helicity. **(C)** Secondary structure propensity (SSP) score is plotted as a function of residue for F508del NBD1 ΔRI ΔRE. The SSP score (59) is based on HN, N, CO and Cα chemical shifts assignments. Values of 1 indicate fully α-helical, values of -1 indicate fully β-strand. The boxes above the graph represent α-helices (magenta) and β-strands (blue) as observed in the 2PZE crystal structure. Note the marginal α-helical propensities for H9. **(D)** Root-mean-squared-deviations of atomic positions as a function of residue number for all 25 chains of CFTR NBD1 in the PDB (human and murine in blue; human only in red).

Figure S5: CFFT-001 compound reduces helicity of H8 and H9 in WT NBD1 ΔRI ΔRE. (A) ^{15}N-^{1}H correlation spectra at 500 MHz for WT NBD1 ΔRI ΔRE. Apo spectra are black (background) and are overlaid with spectra obtained upon addition of the final titration point (3:1) of CFFT-001 (red). CFFT-001 was added in 250, 500 and 750 μM apparent concentrations to a 250 μM sample of WT NBD1 ΔRI ΔRE. **(B)** Close-up of boxed area of (A). Arrows in (A) and (B) indicate peaks that shift upon addition of compound.

Figure S6: Multiple binding modes of CFFT-001 for NBD1 (ΔRI ΔH9). Binding modes for CFFT-001 were generated as described in Fig. 6. **(A)** Ribbon diagram of NBD1 with compounds in the multiple binding modes and ATP shown as stick models. Compounds in different binding modes are shown as different colors, while ATP and the side chain of H620 are shown in cyan and orange, respectively. **(B)** Electrostatic surface representation of NBD1 (red: negative potential; blue: positive potential; white: hydrophobic) with the compounds docked as in (A).

Supplementary Table 1: Sequence Alignment of CFTR NBD1 with NBDs of ABC family C proteins (All_Sequences.named.NBD1.fa, FASTA format).

Figure S1

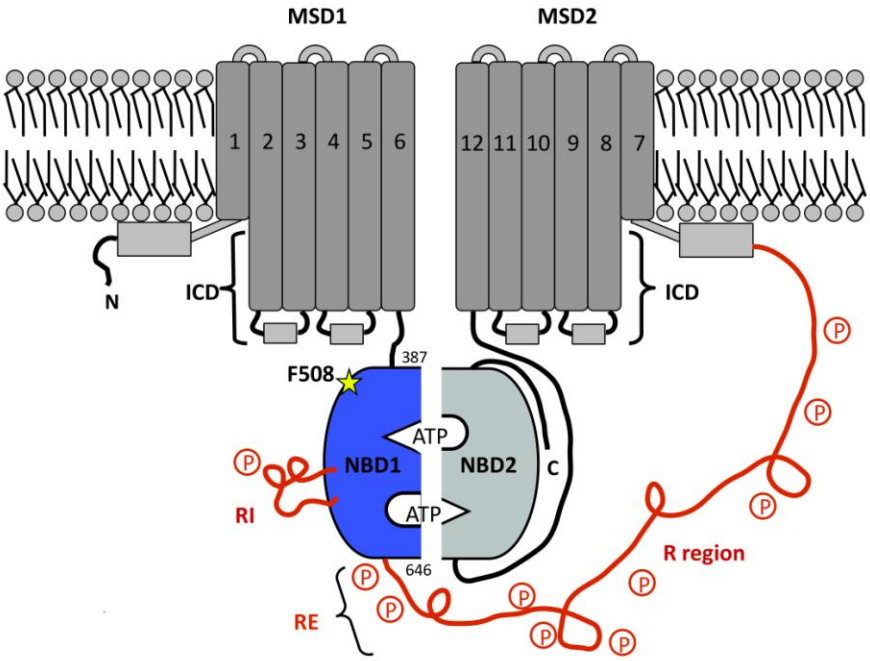
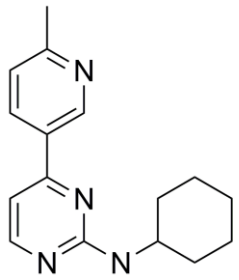
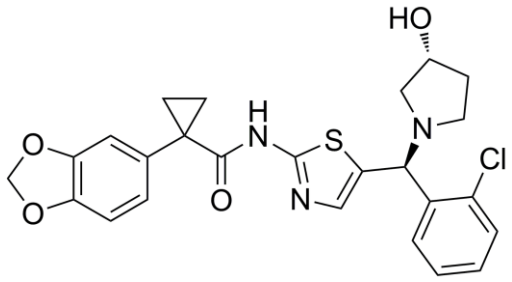

MSD1
MSD2
1
2
3
4
5
6
12
11
10
9
8
7
N
ICD
ICD
387
F508
ATP
NBD1
NBD2
C
ATP
RI
646
RE
R region
P

Figure S2



CFFT-001



C18

Figure S3

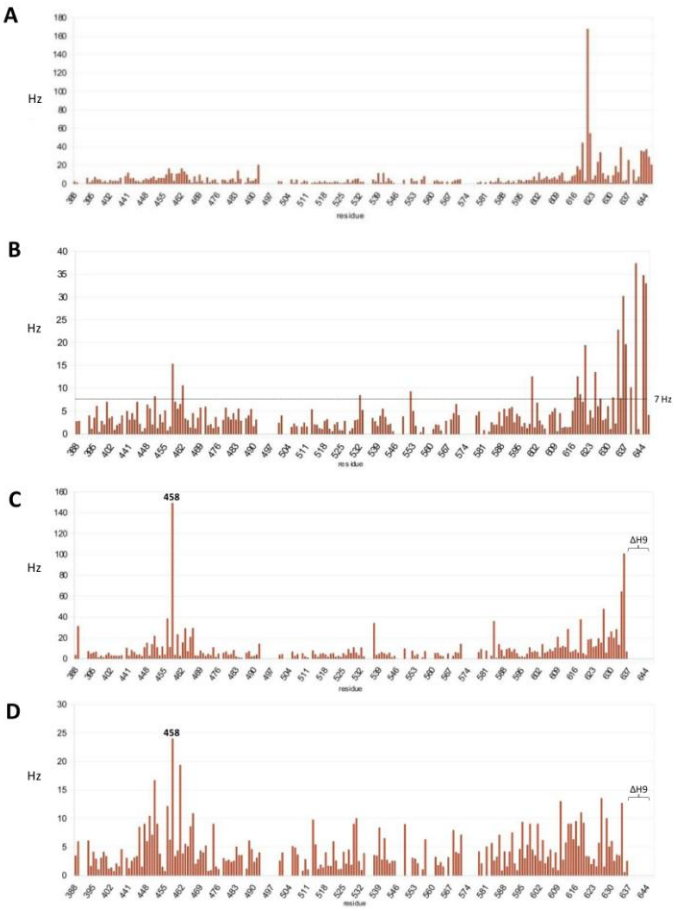

A
Hz
residue
B
Hz
7 Hz
residue
C
458
ΔH9
Hz
residue
D
458
ΔH9
Hz
residue

Figure S4

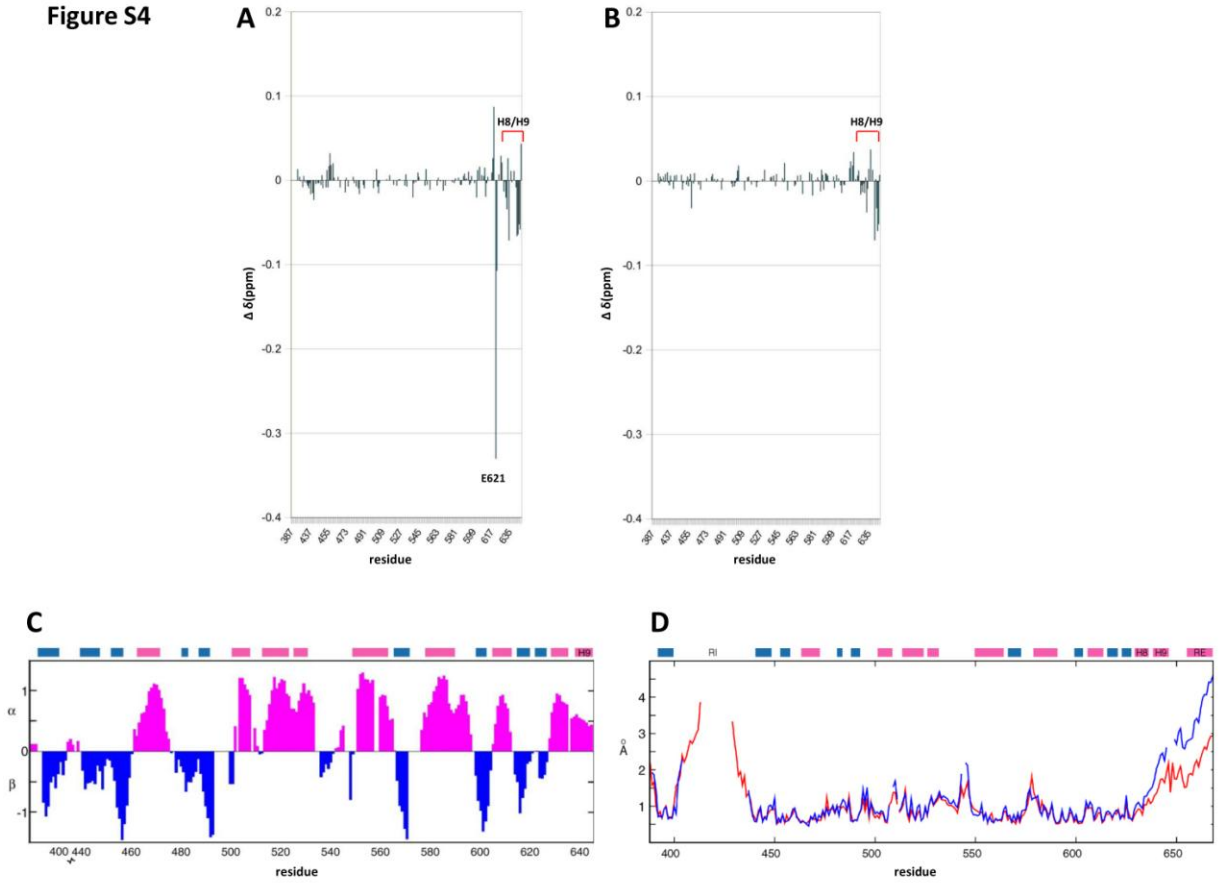


Figure S5

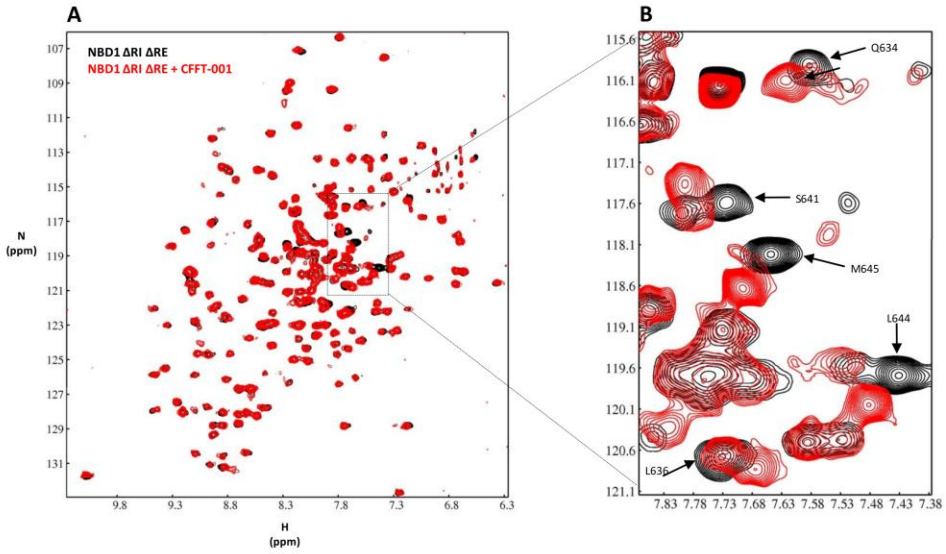

A
NBD1 ΔRI ΔRE
NBD1 ΔRI ΔRE + CFFT-001
N (ppm)
H (ppm)
B
Q634
S641
M645
L644
L635

Figure S6