Supplemental Data

Mutational Stabilization of the Second Nucleotide-binding Domain (NBD2) of the Cystic Fibrosis Transmembrane Conductance Regulator Yields Soluble Protein and Insight into NBD2 Disease-Causing Mutations

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Supplemental data included:

This document:

Figure S1

Figure S2

Table S1

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Additional files:

TableS2.xls

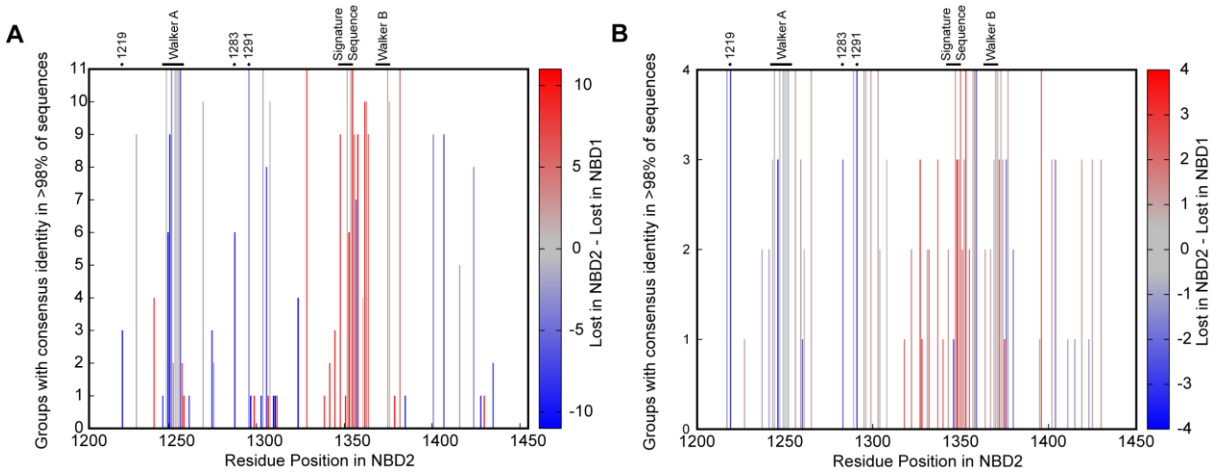

Figure S1. Asymmetric conservation of the ABC(C/B) consensus sequence in full channels.

For each residue position, aligned to NBD2, the number of individual human ABC(C/B) full channels with sequence conservation matching the overall consensus was determined. The Y axis shows the number of distinct human full channels with ≥98% of their sequence homologs having at least one NBD matching the consensus identity for that position, with panel **A** showing the number out of the 11 non-CFTR ABCC transporters and panel **B** showing the number of the 4 ABCB full-channels. Bars are coloured by asymmetric conservation statistics, showing the number of transporters which have dropped the consensus in NBD2 minus the number which have dropped the consensus in NBD1.

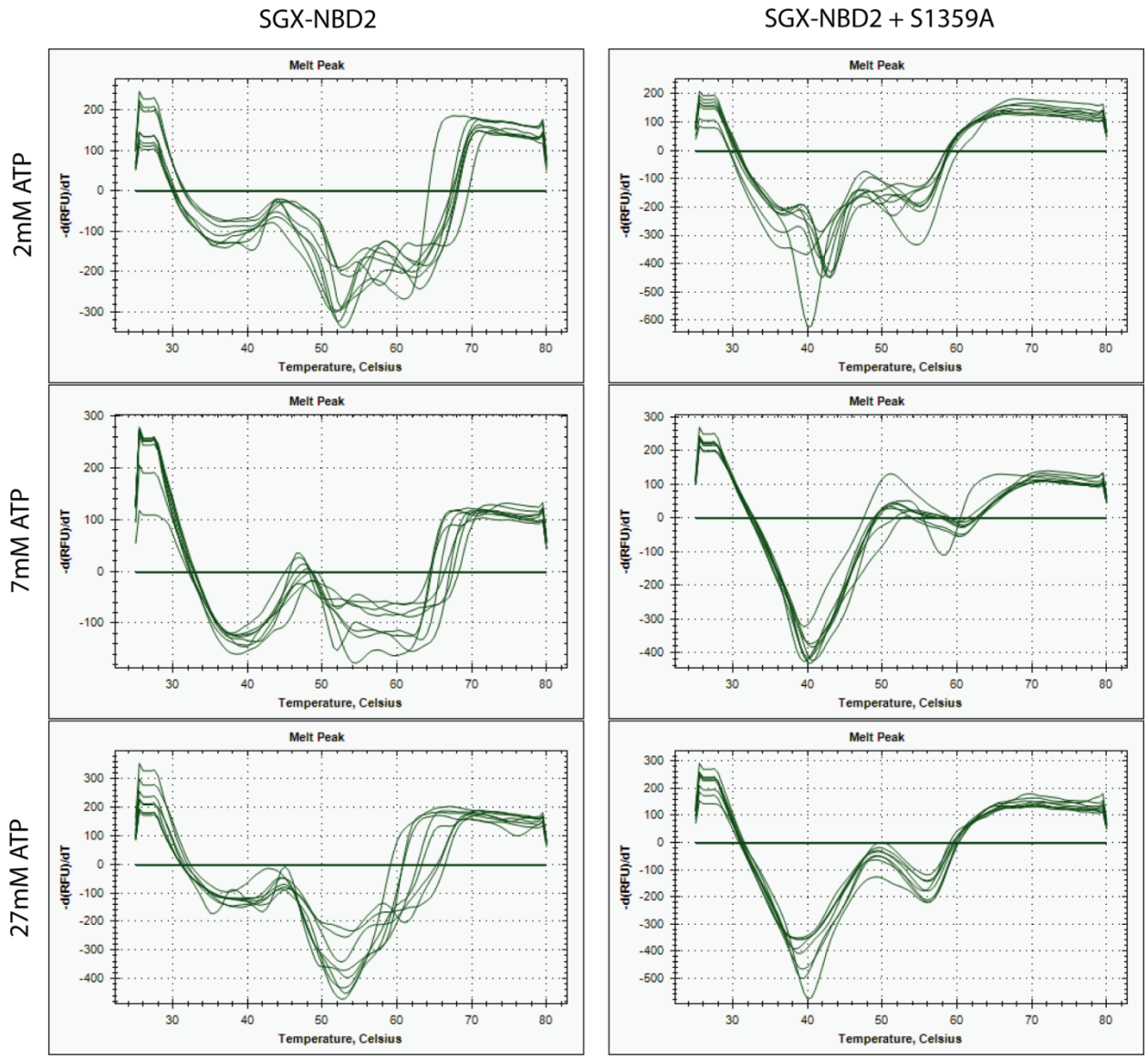

Figure S2. SYPRO-Orange melt data at the low temperature boundary.

SYPRO-Orange melt curve data showing changes in relative fluorescence for the SGX-NBD2 and SGX-NBD2 + S1359A constructs during the first round of mutagenesis, each tested with 8 replicates and three ATP/Mg^{+2} concentrations during the melt. Replicate curves for SGX-NBD2 show erratic behavior, with varying and inconsistent drops in fluorescence at all ATP concentrations, and an initial signal consistently below 40°C. At low ATP concentration, the SGX-NBD2 + S1359A construct showed similar inconsistency, but on increasing the ATP concentration replicates converge on a single initial peak at 40±0.5°C.

Table S1. Substitution Overview.

Back-to-consensus scores, predicted ΔΔG values, and relative stability and yield observations for point mutations tested, with observations categorized by round of mutagenesis and Δ values calculated by comparison to the internal control for each round.

Point Mutation[1]	Back-to-Consensus Score	DDG Prediction (Kcal)[2]	Round 2 SYPRO ΔT_m vs Control (°C)	Round 3 SYPRO ΔT_m vs Control (°C)	Round 4 SYPRO ΔT_m vs Control (°C)	Round 5 SYPRO ΔT_m vs Control (°C)	Round 6 SYPRO ΔT_m vs Control (°C)	Round 2 Yield Relative to Control	Round 3 Yield Relative to Control	Round 5 Yield Relative to Control	Round 6 Yield Relative to Control
Stabilizing Mutants											
S1255L	7	-2.15	4.0					3.7			
K1292D	47	-0.15	2.5	2.0				3.4	1.2		
K1334G	62	-1.58	0.0	2.0	2.5	1.5		2.8	1.2	0.8	
S1359A	175	-1.51									
SGX Mutants											
Q1280E	7	0.19			-1.0	-2.0				1.0	
Y1307N	-27	-0.34			0.5		-1.0				1.0
H1402A	-92	0.32			-2.0	-3.0	-5.0			0.5	0.7
Q1411D	185	0.89		*	*				0.3		
L1436D	68	1.77	-1.0		-3.0			0.8			
CFTR2 Mutants											
I1234V	8	1.35					-1.5				1.0
S1235R	8	0.50					-1.0				1.0
G1244E	-100	13.14					-6.5				0.5
S1251N	-77	1.37					-7.0				0.3
S1255P	-18	13.40					-6.0				0.5
D1270N	-59	-0.49					*				0.1
N1303K	-98	5.23					-5.0				0.2
G1349D	-99	1.90					-3.0				0.8
Other Tested											
F1294A	-11	2.25					-0.5				
F1294K	-11	1.14					-0.5				
F1296A	-67	2.16					-2.5				
F1296K	-67	2.55					-2.0				

[1] Colored blue for stabilizing and red for destabilizing, as defined in the text.
[2] Rosetta predicted ΔΔG of folding, where negative numbers predict stabilization and positive destabilization.
[3] Reversion of SGX background mutations, where negative SYPRO-ΔT_m indicates a stabilizing mutation.
*SYPRO-Tm too low to measure (i.e., inconsistent traces).

Table S2. Full construct overview.

Full construct details and SYPRO-Orange T_m measurements are shown for each round of mutagenesis. Construct # shows order of appearance, with constructs that were retested as the internal control of a subsequent round being noted with their original number in square brackets. Constructs are also listed using the full list of substitutions from wild type human CFTR, with substitutions above the round's internal control in red.

[external data file "TableS2.xls"]

Table S3. PROSS server results. Constructs and individual mutations predicted for stabilizing SGX background sequence and NBD2 structure (PDB ID 3GD7). Mutations in each suggested construct are described by shading, with untested mutations in black, and mutations tested during this study in blue for stabilizing effect and red for destabilizing effect. The automated approach suggests more mutations in general, with few that can be directly compared.

Mutation Suggested	Construct 1	Construct 2	Construct 3	Construct 4	Construct 5	Construct 6	Construct 7
M1210I			■	■	■	■	■
T1211E							■
K1213R							■
D1214N							■
A1217V		■	■		■		
K1218R				■	■	■	■
T1220R						■	■
E1221P					■	■	■
N1224P				■	■	■	■
A1225P			■	■	■	■	■
I1226V				■	■	■	■
E1228K	■	■	■	■	■	■	■
S1233E							■
S1235K			■	■	■	■	■
R1239F					■	■	■
L1243V				■			■
R1245P	■	■	■	■	■	■	■
S1248A							■
S1255D						■	
S1255Q					■		■
D1275N	■	■	■	■	■	■	■
S1276D							■
T1278P	■	■	■	■	■	■	■

Mutation Suggested	Construct 1	Construct 2	Construct 3	Construct 4	Construct 5	Construct 6	Construct 7
S1276D							■
T1278P	■	■	■	■	■	■	■
L1279P						■	■
K1292D							
N1307F				■	■	■	■
A1308N	■	■	■	■	■	■	■
A1309N	■	■	■	■	■	■	■
V1318A	■	■	■	■	■	■	■
E1321K					■	■	■
S1326D							■
V1340T				■	■	■	■
V1345N		■	■	■	■	■	■
H1350Q					■		
M1354I						■	
M1354V					■		■
S1359A							
S1362R							■
H1375N		■	■	■	■	■	■
V1379E					■	■	■
Q1382E							■
A1402H							
N1419G		■	■	■	■	■	■