Mutation Prediction method	WT	G85E	G91R	G85A	G91A
HMMTOP	76-94	76-94	76-94	76-94	76-94
TMPred	76-97	69-90	69-90	76-97	76-97
G insertion	73-94	72-90	73-91	73-94	73-94
TopPred KD	76-96	86-106	73-93	76-96	76-96
TopPred GES	76-96	No TM	No TM	76-96	76-96
PHDhtm	75-92	76-93	75-92	75-93	76-93

Supplemental Table 1. Predicted TM1 spans for CF-causing and non-charged mutants.

Primer (5'-3' sense primers)	Mutations	Purpose
CCTCTTCAAGACAAAGGGGATAGTACTCATAGTAGAAATAACAG	N894D	Remove native glycosylation site
GGGATAGTACTCATAGTAGAGATAACAGCTATGCAGTGATTATC	N900D	Remove native glycosylation site
CCTCTTCAAGACAAAGGGGATAGTACTCATAGTAGAGATAACAGCTATGCAGTGATTATC	N894D, N900D	Remove both native glycosylation sites
CTCTTACTGGGAAGAATCATAGCTTCCTATAACGAATTTGATCAGAATAGTACTGGCCAAGGATTCGACCCGGATAACAAGGAGGAACGC		Introduce artificial glycosylation site into ECL1
GGGAAGAATCATAGCTTCCTATAACGAATTTAATAGTACTGGCCAAGGATTCGACCCGG	-2N	Remove residues from CFTR containing the artificial glycosylation site in ECL1
GGGAAGAATCATAGCTTCCTATAACAATAGTACTGGCCAAGGATTCGACC	-4N	
GGGAAGAATCATAGCTTCCTATAATAGTACTGGCCAAGGATTCG	-5N	
CAGCCTCTCTTACTGGGAAGAATCATAGCTTCCAATAGTACTGGCCAAGGATTCGACCCGGATAAC	-6N	
CCTCTCTTACTGGGAAGAATCATAAATAGTACTGGCCAAGGATTCG	-8N	
CAGCCTCTCTTACTGGGAAGAAATAGTACTGGCCAAGGATTCG	-10N	
GCAGTACAGCCTCTCTTACTGAATAGTACTGGCCAAGGATTCG	-12N	
CCAAAGCAGTACAGCCTCTCAATAGTACTGGCCAAGGATTCG	-14N	
GGAAGTCACCAAAGCAGTACAGAATAGTACTGGCCAAGGATTCG	-16N	
GGGGAAGTCACCAAAGCAAATAGTACTGGCCAAGGATTCG	-18N	
CATAGCTTCCTATAACGAATTTGATCAGAATAGTACTGGATTCGACCCGGATAACAAGGAGGAACGC	-2C	
CATAGCTTCCTATAACGAATTTGATCAGAATAGTACTGACCCGGATAACAAGGAGGAACGCTCTA	-4C	
GCTTCCTATAACGAATTTGATCAGGCCGCCAATAGTACTGGCCAAGGATTCG	+2N	Add residues to CFTR containing the artificial glycosylation site in ECL1
TCTGGAGATTTATGTTCTATGAAATCTTTTTATATTTAGGGGAAG	G85E	G85E
GGAGATTTATGTTCTATGCCATCTTTTTATATTTAGGGG	G85A	G85A
CTATGGAATCTTTTTATATTTAAGGGAAGTCACCAAAGCAGTACAGC	G91R	G91R
GGAATCTTTTTATATTTAGCCGAAGTCACCAAAGC	G91A	G91A

Supplemental Table 2. Sense primers used to generate DNA constructs.

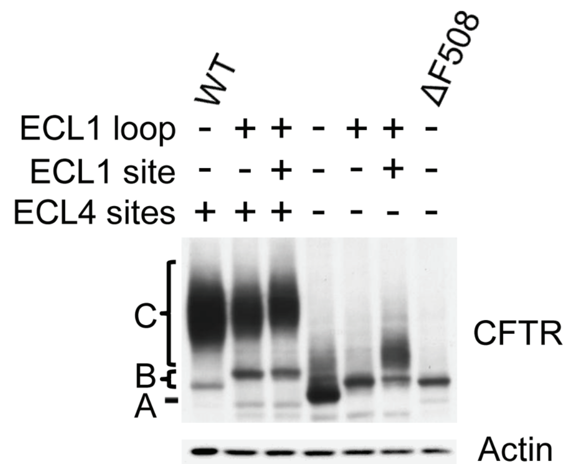
ΔF508

N894 site + + + - - - + + + - - - | +
N900 site + + + + + + - - - - - - | +
PNGaseF - - + - - + - - + - - + | -
EndoH - + - - + - - + - - + - | -

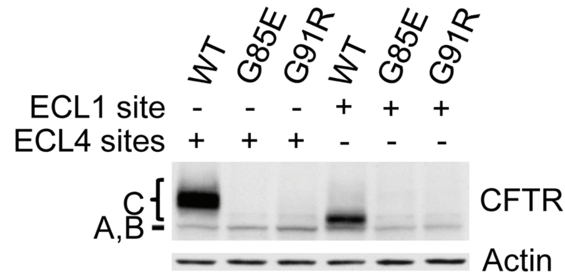
C
B
A

CFTR

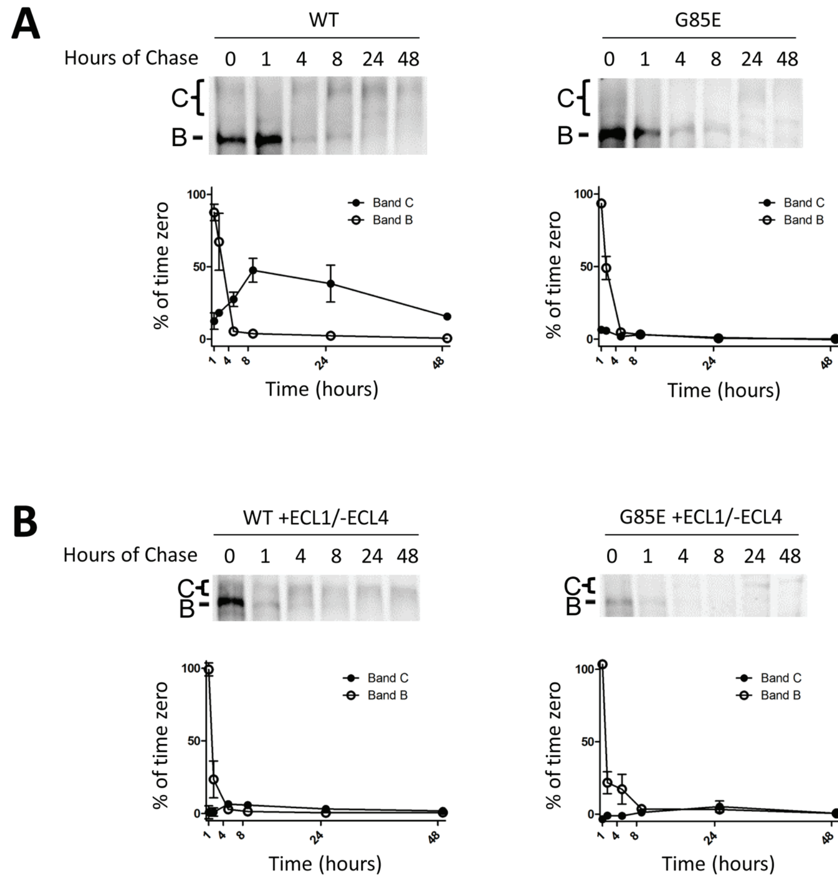
Supplemental Figure 1. Glycosidase treatment of CFTR natural ECL4 (N894 and N900) glycosylation sites. HeLa cell trafficking of CFTR containing both, individual, or no natural glycosylation sites monitored by Western blot analysis. Core and complex glycosylation were verified by digestion with glycosidase selective for core and complex glycosylation (PNGaseF) or core glycosylation (Endo H). The positions of CFTR with no glycosylation (Band A), core (Band B) and complex (Band C) glycosylation, WT and ΔF508 CFTR are marked.



Supplemental Figure 2. The artificial ECL1 glycosylation site is glycosylated. HeLa cell trafficking of CFTR containing combinations of the natural ECL4 sites, the ECL1 loop without a site, and the ECL1 loop with a site were monitored by Western blot analysis. The positions of CFTR with no glycosylation (Band A), core (Band B) and complex glycosylation (Band C), WT and ΔF508 are marked.



Supplemental Figure 3. WT, G85E, or G91R CFTR containing the natural ECL4 sites or the artificial ECL1 site was monitored for cellular trafficking in HeLa cells using Western blot analysis. The positions of CFTR with no glycosylation (Band A) or core (Band B) and complex (Band C) glycosylation are marked. Band A and B are not resolved in this image.



Supplemental Figure 4. Pulse-chase analysis of WT and G85E constructs containing glycosylation sites in ECL4 or in ECL1. HEK293 cells were transiently transfected with CFTR constructs with WT or G85E mutant and the natural glycosylation sites in ECL4 (A) or the artificial glycosylation site in ECL1 and no sites in ECL4 (B). Pulse-chase protocol was performed to label the proteins with ^{35}S methionine and collect cells after 0, 1, 4, 8, 24, or 48 hours of chase. Lysates were immunoprecipitated with M3A7 and analyzed by SDS-PAGE and phosphorimage analysis. Beneath each image, a graph representing the amount of radiolabeled Band B or Band C is shown with respect to time zero. Analysis was performed on 4 experiments with error bars representing SEM.