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Supplemental Information

Steric Inhibition of 5′ UTR Regulatory Elements Results in Upregulation of Human CFTR

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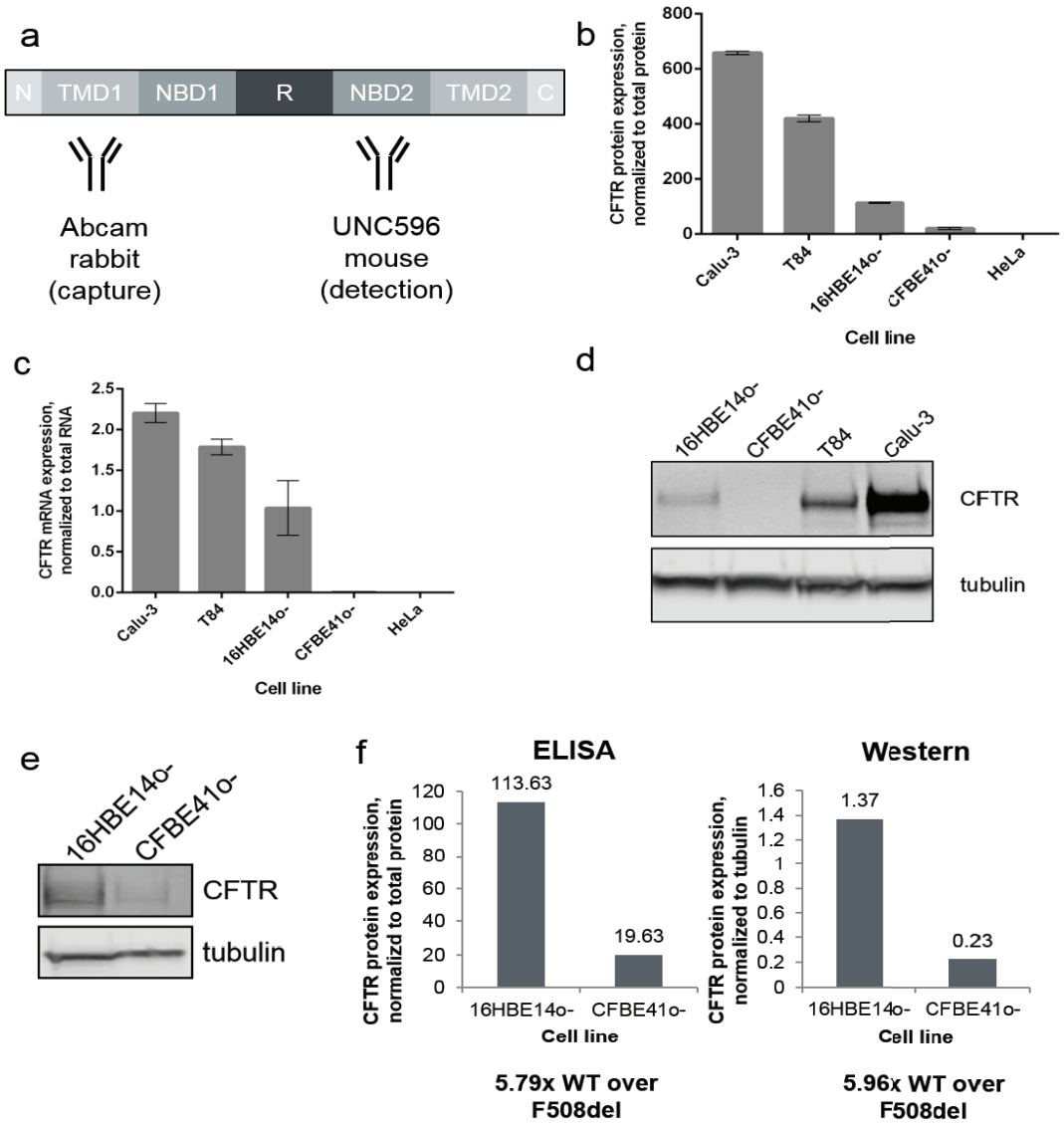

Figure S1. A sandwich immunoassay detects CFTR protein from cell lysates.
A. Schematic of CFTR protein and regions bound by antibodies used for ELISA. N and C termini are indicated. TMD: transmembrane domain; NBD: nucleotide binding domain; R: regulatory domain. B. Levels of CFTR protein detected by ELISA in various cell lines. Normalization is to total protein measured by BCA. C. *CFTR* mRNA levels detected by qPCR. D. CFTR and tubulin detected by western blot in HBE and immortalized cell lines. E. Longer exposure of blot for CFTR in HBE cell lines. F. Comparison between ELISA and western blot quantifications for HBE cell lines. Data are mean +SEM.

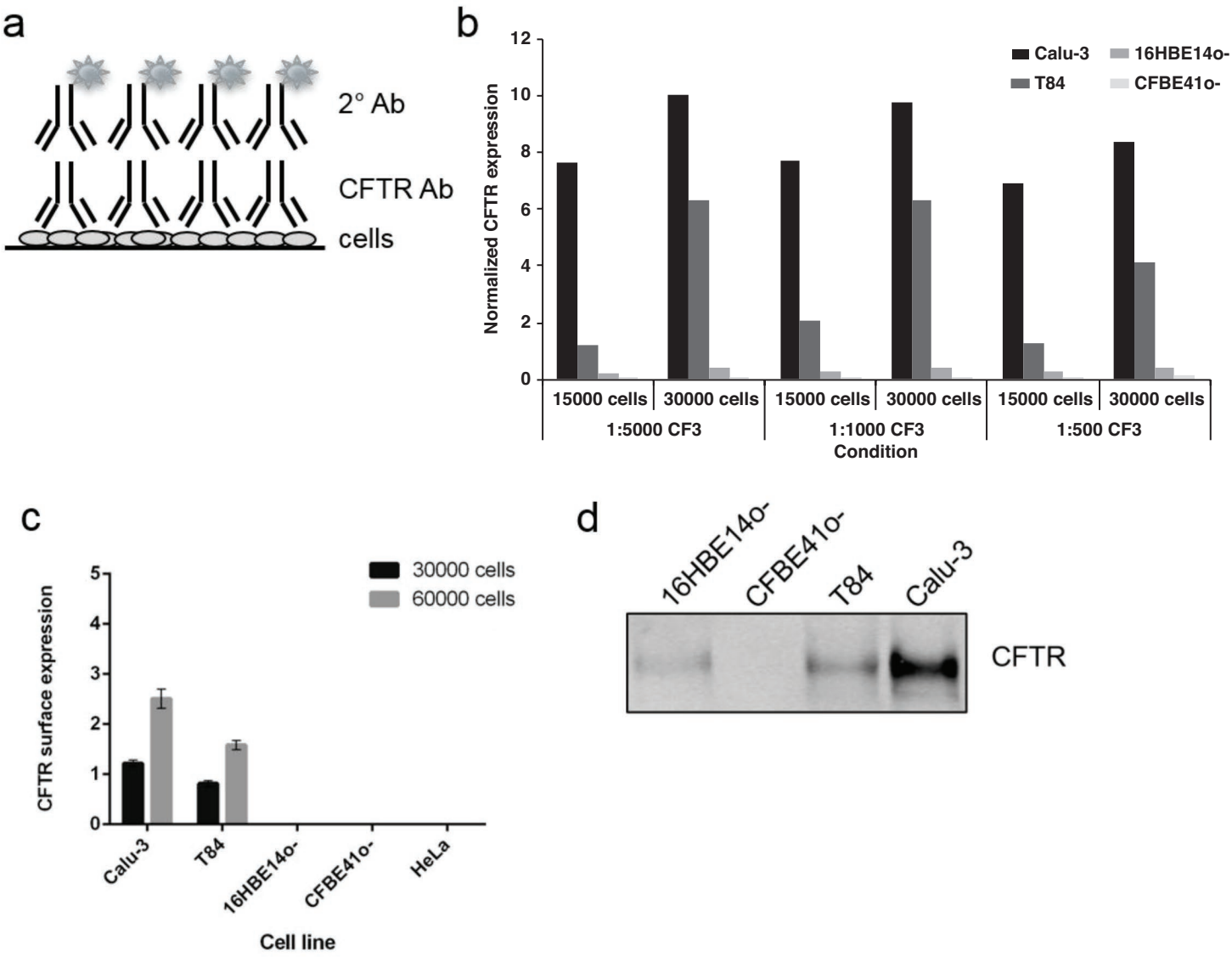

Figure S2. Cell-based assay for surface-localized CFTR.
A. Schematic of the assay. Ab, antibody. B. Detection of CFTR surface protein in the indicated cell lines. C. Optimized cell-based assay data at indicated seeding densities and cell lines. Data are means of 3 replicate wells, with error bars representing standard deviation. D. Surface-biotinylation followed by western blot for CFTR in the four indicated cell lines. Data are mean +/-SEM.

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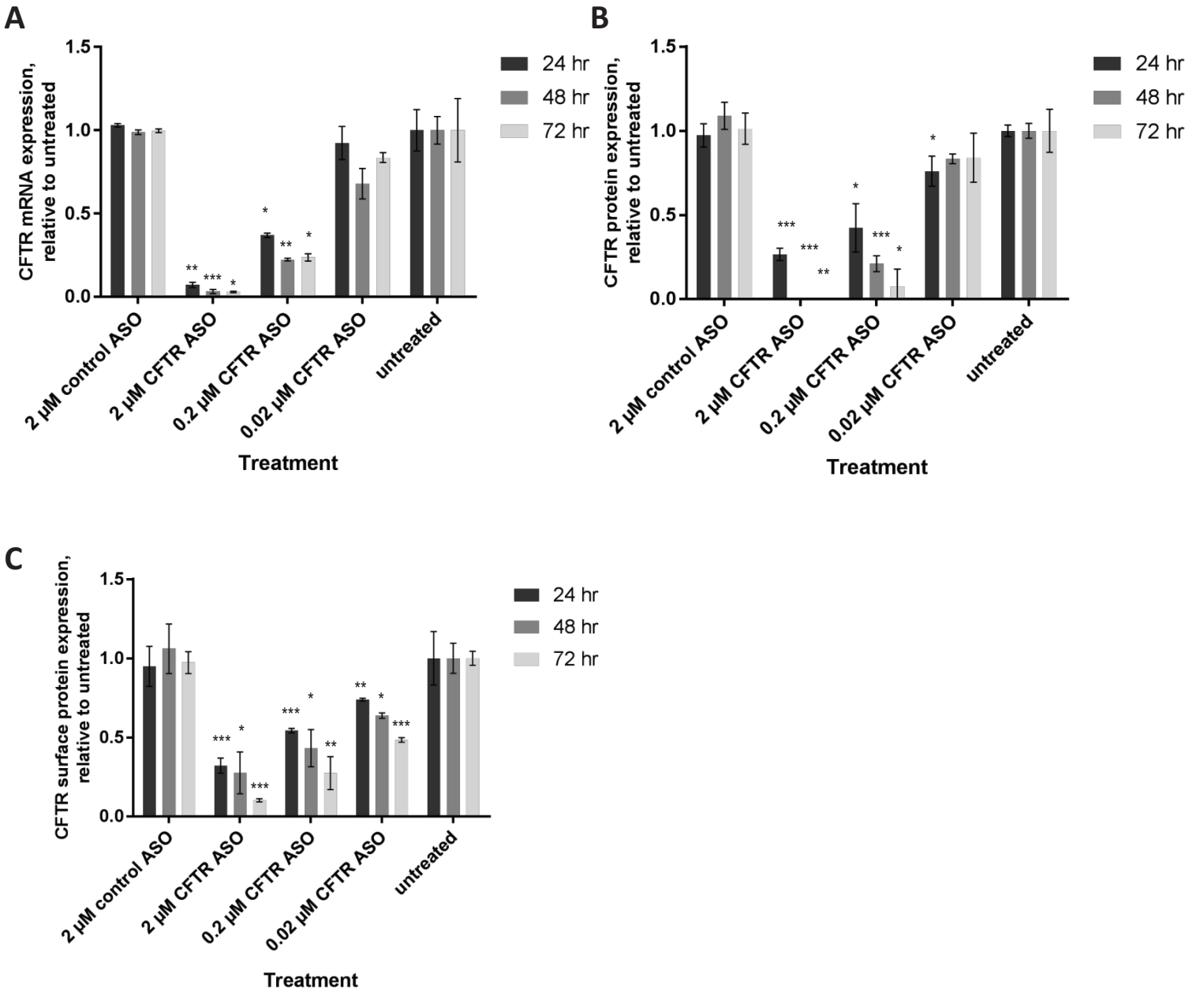

Figure S3. Treatment with ASOs results in reduction of CFTR mRNA, total protein, and surface-localized protein.

A. CFTR mRNA expression in 16HBE14o- cells after ASO treatment. B. CFTR protein expression, measured by ELISA, in 16HBE14o- cells after ASO treatment. C. CFTR surface expression in 16HBE14o- cells after ASO treatment. *p< 0.05, **p<0.005, ***p<0.0005 by Holm-Sidak method, α = 0.05. Data are mean +/-SEM.

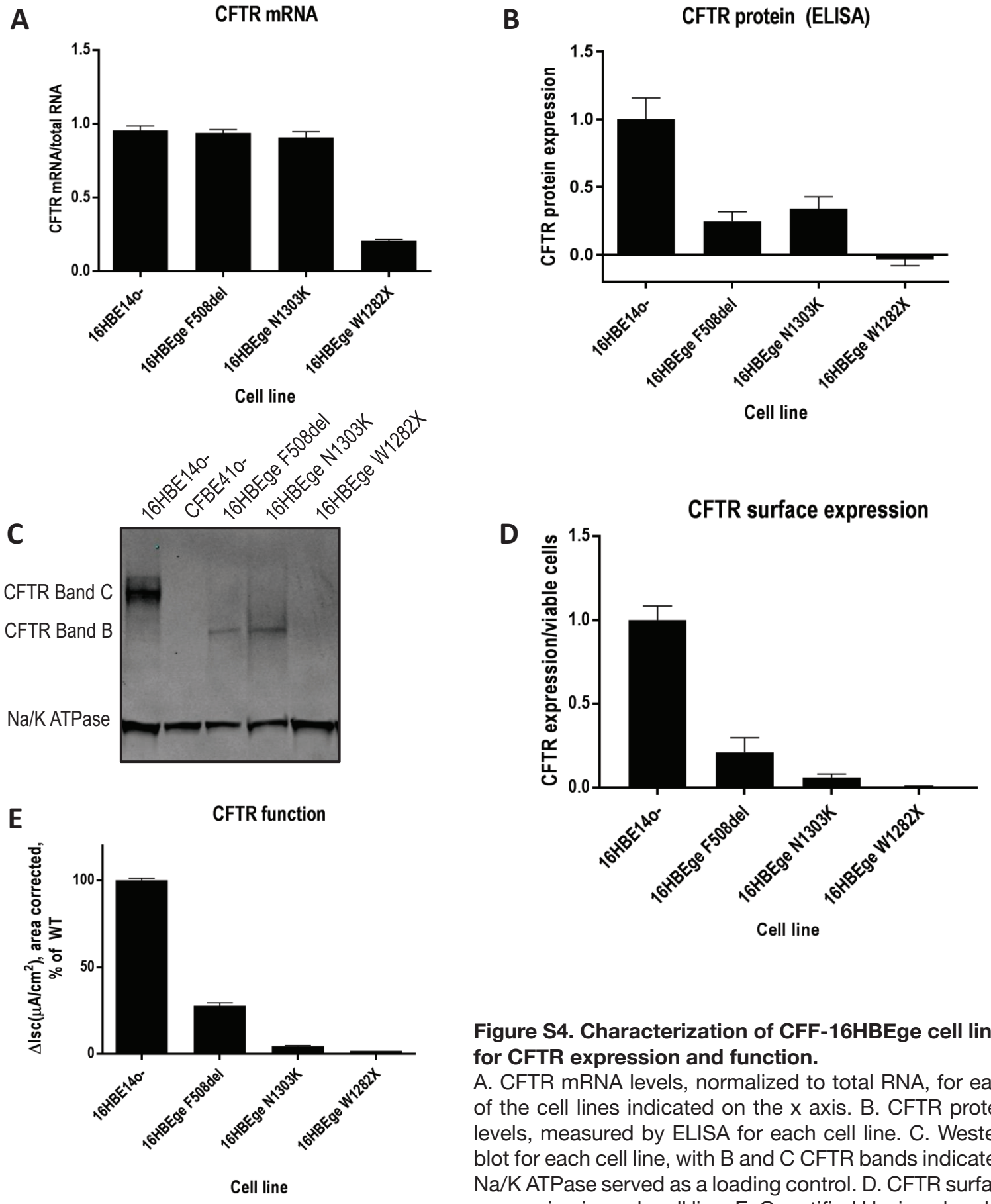

Figure S4. Characterization of CFF-16HBEge cell lines for CFTR expression and function.

A. CFTR mRNA levels, normalized to total RNA, for each of the cell lines indicated on the x axis. B. CFTR protein levels, measured by ELISA for each cell line. C. Western blot for each cell line, with B and C CFTR bands indicated. Na/K ATPase served as a loading control. D. CFTR surface expression in each cell line. E. Quantified Ussing chamber data for each cell line, represented as a percentage of CFTR-specific short circuit current in 16HBE14o- cells. Data are mean + SEM.

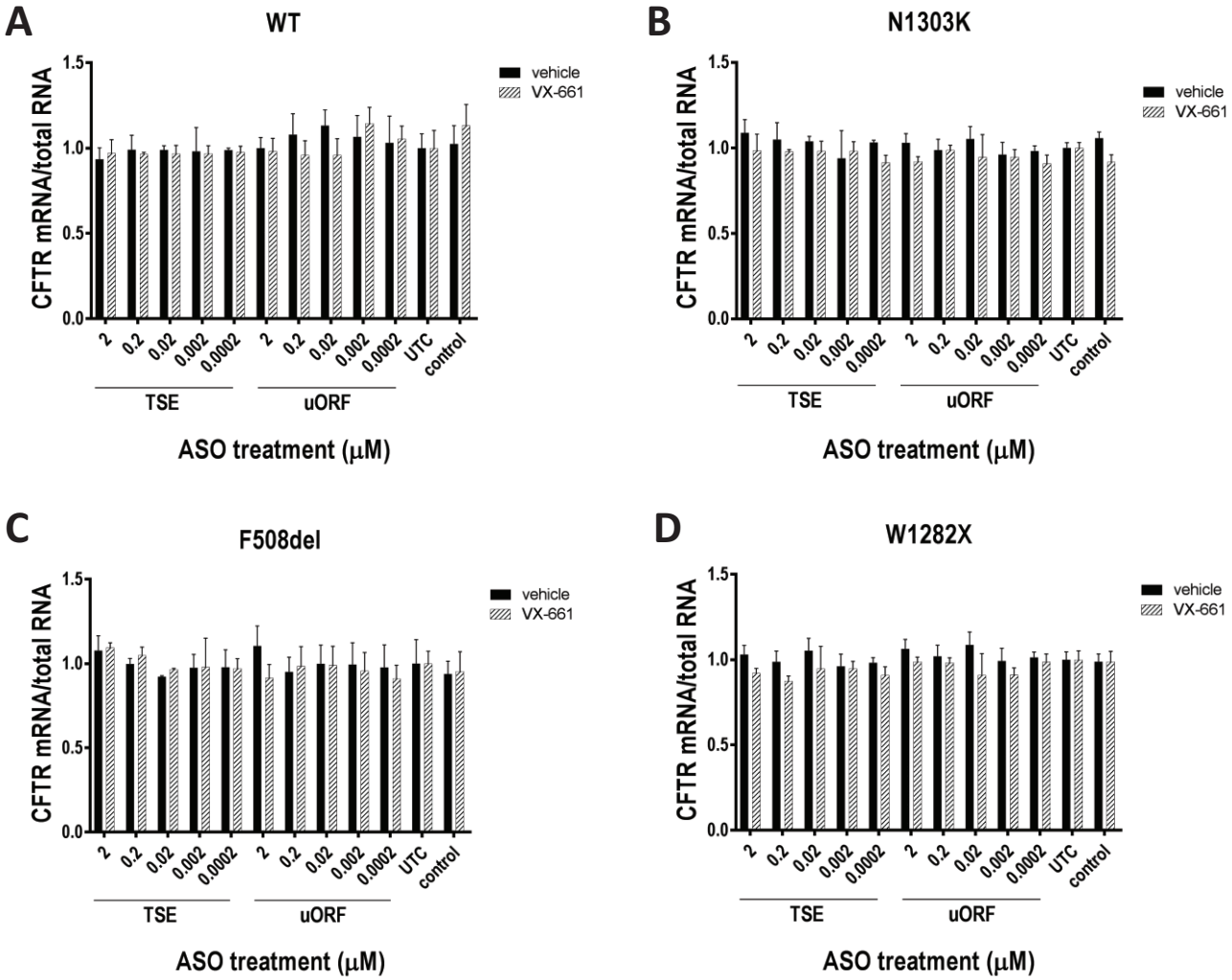

Figure S5. Effect of ASO combination with corrector compounds on CFTR mRNA levels in cell lines with defective CFTR.

CFTR mRNA levels, relative to total RNA and normalized to untreated control (UTC), for 16HBE14o- cells (WT, A), and the gene edited variant cell lines CFF-16HBEge N1303K (B), F508del (C), and W1282X (D). Data are mean + SEM.

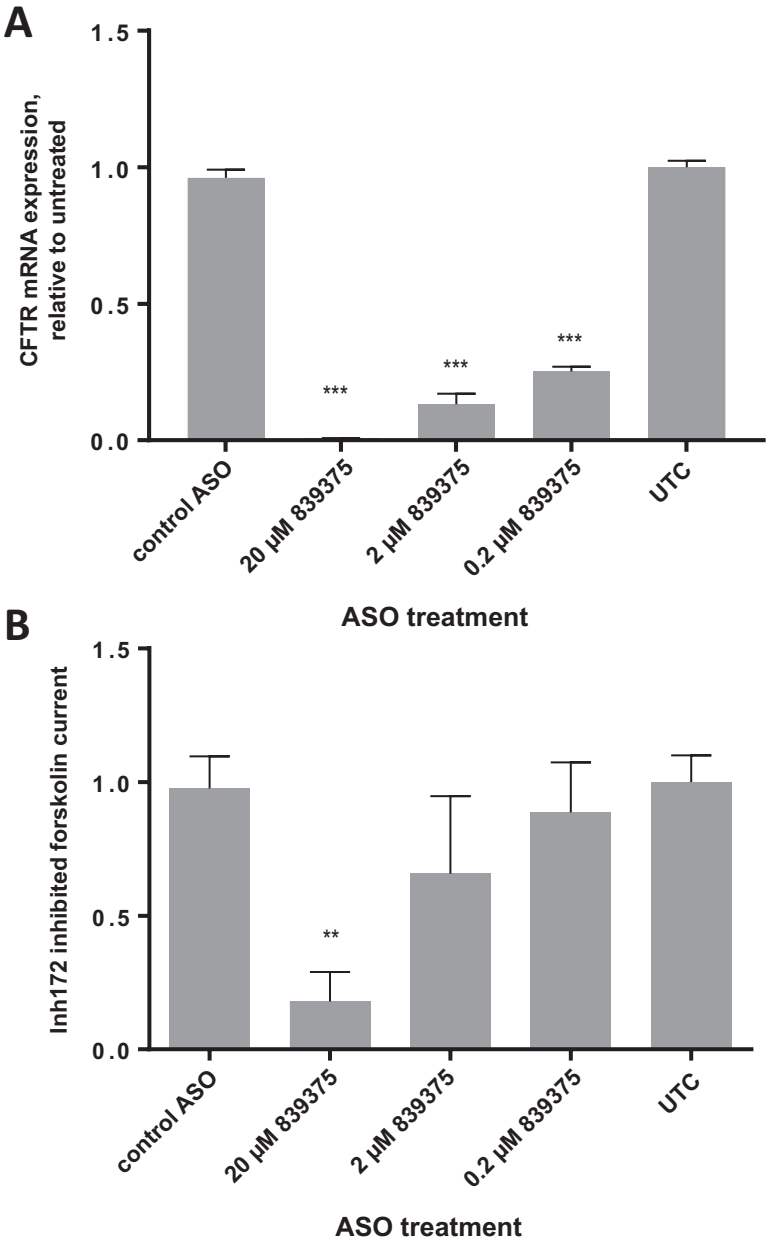

Figure S6. ASO delivery is confirmed in wildtype primary hBE cells.
A. CFTR mRNA levels, relative to total RNA and normalized to untreated control (UTC), after indicated ASO treatments. B. Quantified Ussing chamber data, normalized to untreated control (UTC) after the indicated ASO treatments. *p< 0.05, **p<0.005, ***p<0.0005 by Holm-Sidak method, α = 0.05. Data are mean + SEM.