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Correctors and Potentiators Rescue Function of the Truncated W1282X-Cystic Fibrosis Transmembrane Regulator (CFTR) Translation Product*[♦]

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W1282X is the fifth most common cystic fibrosis transmembrane regulator (CFTR) mutation that causes cystic fibrosis. Here, we investigated the utility of a small molecule corrector/potentiator strategy, as used for $\Delta F508$ -CFTR, to produce functional rescue of the truncated translation product of the W1282X mutation, CFTR₁₂₈₁, without the need for read-through. In transfected cell systems, certain potentiators and correctors, including VX-809 and VX-770, increased CFTR₁₂₈₁ activity. To identify novel correctors and potentiators with potentially greater efficacy on CFTR₁₂₈₁, functional screens were done of ~30,000 synthetic small molecules and drugs/nutraceuticals in CFTR₁₂₈₁-transfected cells. Corrector scaffolds of 1-arylpyrazole-4-arylsulfonyl-piperazine and spiro-piperidine-quinazolinone classes were identified with up to ~5-fold greater efficacy than VX-809, some of which were selective for CFTR₁₂₈₁, whereas others also corrected $\Delta F508$ -CFTR. Several novel potentiator scaffolds were identified with efficacy comparable with VX-770; remarkably, a phenylsulfonamide-pyrrolopyridine acted synergistically with VX-770 to increase CFTR₁₂₈₁ function ~8-fold over that of VX-770 alone, normalizing CFTR₁₂₈₁ channel activity to that of wild type CFTR. Corrector and potentiator combinations were tested in primary cultures

and conditionally reprogrammed cells generated from nasal brushings from one W1282X homozygous subject. Although robust chloride conductance was seen with correctors and potentiators in homozygous $\Delta F508$ cells, increased chloride conductance was not found in W1282X cells despite the presence of adequate transcript levels. Notwithstanding the negative data in W1282X cells from one human subject, we speculate that corrector and potentiator combinations may have therapeutic efficacy in cystic fibrosis caused by the W1282X mutation, although additional studies are needed on human cells from W1282X subjects.

Premature termination codons (PTCs,⁵ or nonsense mutations) in the cystic fibrosis transmembrane conductance regulator (CFTR) gene are found in nearly 7% of patients with CF (1). PTCs result in synthesis of truncated protein, generally without normal function, as well as reduced transcript levels caused by nonsense-mediated mRNA decay (NMD) (2, 3). NMD suppressors that restore gene function by promoting incorporation of the correct or a near-cognate aminoacyl tRNA into nascent polypeptides represent attractive drug development candidates (2, 4, 5). The read-through candidate drug Ataluren (PTC124) is under evaluation for CF caused by PTCs, although initial phase III clinical trial data did not show efficacy (6, 7), and some laboratories were unable to demonstrate Ataluren-mediated translation read-through in various systems (8). One limitation of therapeutics causing NMD suppression is insertion of incorrect amino acids at the PTC site.

The W1282X mutation, which truncates CFTR to remove ~60% of nucleotide binding domain 2 (NBD2; Fig. 1A, top), is the fifth most common CF-causing mutation worldwide with a prevalence of ~50% in Ashkenazi Jewish subjects with CF (1). Motivated by the fact that the W1282X mutation is predicted to generate a truncated protein (CFTR₁₂₈₁) constituting most of the full-length wild type CFTR (1480 amino acids), and postulating that CFTR₁₂₈₁ has defective cellular processing and gat-

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⁵ The abbreviations used are: PTC, premature termination codon; CFTR, cystic fibrosis transmembrane regulator; endo H, endoglycosidase H; PNGase F, peptide:N-glycosidase F; fsk, forskolin; ANOVA, analysis of variance; 3HA, triplet hemagglutinin epitope tag; CF, cystic fibrosis; CRC, conditionally reprogrammed cell; NMD, nonsense-mediated mRNA decay.

Pharmacological Correction of W1282X-CFTR

ing, we investigated whether functional rescue of CFTR₁₂₈₁ is possible using correctors and/or potentiators, a strategy used for CF caused by the Δ F508 mutation as well as G551D and other CFTR missense mutations (9–11).

Several lines of evidence suggest multiple defects in W1282X-CFTR and support corrector, potentiator, and read-through strategies (12). Transcript encoding W1282X-CFTR was seen in nasal epithelial cells of homozygous W1282X CF subjects with 30–80% abundance compared with that of wild type CFTR in non-CF subjects (13). In bronchial epithelial cells virally infected to express W1282X-CFTR cDNA, butyrate-induced up-regulation of truncated CFTR expression produced cAMP/genistein-stimulated chloride conductance (14), and in the same cell model, VX-770 produced an ~4-fold increase in CFTR activity despite the apparent absence of full-length protein (15). Patch clamp experiments showed that VX-770 in combination with curcumin produced significant activation of W1282X-CFTR despite evidence of impaired ATP-dependent NBD1-NBD2 dimerization (16). Biochemical and functional approaches using heterologous expression systems have demonstrated that NBD2 is not required for efficient cell-surface presentation or CFTR activity, albeit with reduced open probability (17, 18). Partial deletion of NBD2 reduces channel biosynthetic processing and post-Golgi stability (18–21), probably by interfering with NBD2 folding and cooperative CFTR domain assembly (18).

In this study, we demonstrate the activity of available correctors and potentiators in transfected cells expressing the truncated protein product of the W1282X CFTR gene (CFTR₁₂₈₁), identify improved correctors and potentiators by high throughput screening, and test compounds in human nasal epithelial cell cultures from a W1282X-homozygous CF subject. These studies were presented in part in abstract form at the 29th North American CF Conference in October, 2015 (22).

Results

CFTR Correctors Stabilize W1282X-CFTR Translational Products in Respiratory Epithelial Cells—To test whether the W1282X-CFTR gene product is susceptible to pharmacological modulation, initial biochemical assessment was done in a CF bronchial epithelial cell line (CFBE41o–, designated as CFBE) lacking endogenous CFTR expression (23). Lentiviral vectors were generated to express W1282X-CFTR translational products stably under the control of the tetracycline transactivator as described (24). Two expression cassettes were used. The first contained a stop codon inserted into the full-length cDNA (W1282X-CFTR), permitting expression of both the full-length channel and truncated protein product (CFTR₁₂₈₁). The second encoded CFTR₁₂₈₁ (Fig. 1A). Both constructs were engineered with a triplet hemagglutinin epitope tag (3HA) in the fourth extracellular loop of CFTR to monitor cell-surface expression in a live cell enzyme-linked immunosorbent assay format (25).

Immunoblot analysis in combination with endo H and PNGase F digestion showed the expression of core-glycosylated (band B, endo H-sensitive; *black arrowhead*) and complex-glycosylated (band C, PNGase F-sensitive; *white arrowhead*) forms of both W1282X-CFTR and CFTR₁₂₈₁ (Fig. 1B). The apparent

molecular mass of the core- and complex-glycosylated forms of CFTR₁₂₈₁ and W1282X-CFTR was reduced compared with wild type CFTR. Assuming comparable transcript levels, these observations suggest that the truncated CFTR₁₂₈₁ has inefficient biosynthetic processing and/or impaired stability. Supporting inefficient processing, the known Δ F508-CFTR correctors VX-809 and corr4a (C4) increased the complex-glycosylated forms of CFTR₁₂₈₁ and W1282X-CFTR, as well as their plasma membrane expression as assessed by immunoblot analysis and live-cell ELISA (Fig. 1, C and D). As evidence for improved CFTR₁₂₈₁ processing in the presence of VX-809, metabolic pulse-chase labeling with [³⁵S]methionine and [³⁵S]cysteine in polarized CFBE cell cultures showed ~75% increased incorporation of radiolabel into complex-glycosylated CFTR₁₂₈₁ with VX-809 (Fig. 1E).

High Throughput Assay Development for W1282X-CFTR Correctors and Potentiators—Motivated by the observations above, we developed a high throughput screen to identify correctors and potentiators of W1282X-CFTR. FRT cell lines were generated that stably express YFP-H148Q/I152L/F46L (YFP) and CFTR containing either the W1282X mutation (FRT-YFP-W1282X-CFTR) or CFTR₁₂₈₁ (FRT-YFP-CFTR₁₂₈₁); FRT cells were used because of their low intrinsic permeability to iodide and other CFTR-permeable ions and prior experience with these cells in various CFTR modulator screens (26, 27). The corrector assay was done by 24-h incubation of cells with test compounds followed by brief (10 min) incubation with the cAMP agonist forskolin (fsk) and a potentiator prior to assay (Fig. 2A, *left*). For potentiator assays, cells were incubated with a corrector to increase cell-surface expression of W1282X-CFTR and then briefly incubated (10 min) with test compounds and forskolin prior to assay (Fig. 2A, *right*). CFTR activity was deduced from the initial rate of YFP fluorescence quenching in response to iodide addition to the extracellular solution.

Known Correctors and Potentiators Rescue W1282X-CFTR Function in FRT Cells—Incubation of FRT-YFP-W1282X-CFTR cells with forskolin and genistein (50 μ M) produced limited fluorescence quenching in response to an iodide gradient, although 24-h culture with sodium butyrate, a pharmacological chaperone (28), increased this response, as found previously (Fig. 2B) (14). A similar pattern of CFTR activation was observed in cells incubated with VX-809, and combined VX-809 plus butyrate produced an additive response. In cells incubated with VX-809 alone or VX-809 plus butyrate, VX-770 (5 μ M) had greater efficacy than genistein (Fig. 2, B and C). CFTR activation required both cAMP elevation and a potentiator (VX-770 or genistein; Fig. 2C and data not shown).

Fig. 2C (*left*) summarizes the experiments above and additional measurements for a panel of known CFTR modulators. Correctors VX-661, corr4a (C4), C3, and C18 each increased forskolin/VX-770-stimulated CFTR activity by ~2–3-fold over that in uncorrected cells, whereas CoPo-22 and C7 had minimal activity. Potentiators PG01 (P2) (29) and A04 (27) showed activity in VX-809-corrected/forskolin-stimulated cells, whereas P3, P5, P8, P9, P12, and CoPo-22 were inactive (Fig. 2C, *right*).

Further experiments were done in FRT-YFP-CFTR₁₂₈₁ cells to investigate whether CFTR modulators that correct or poten-

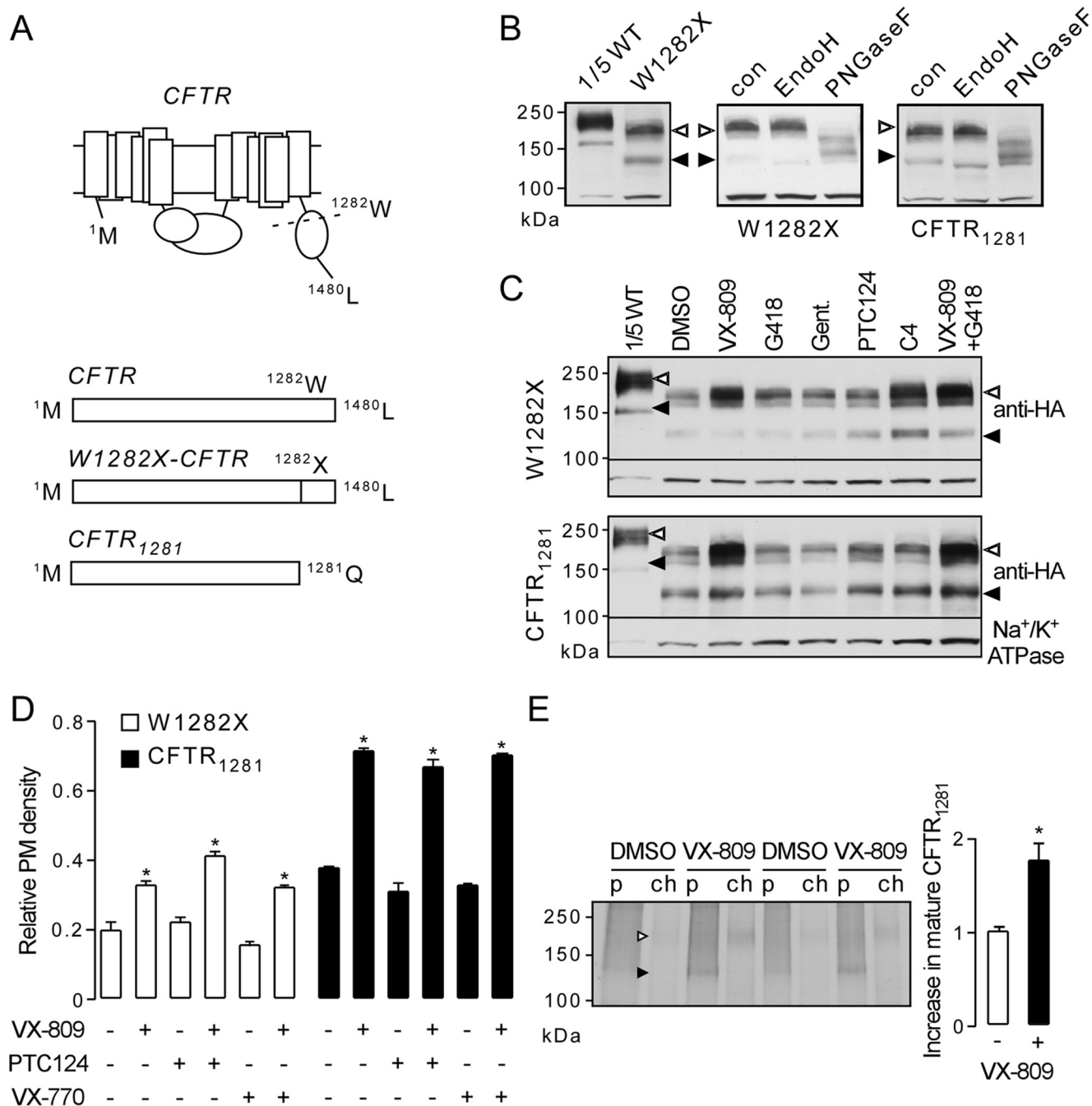


FIGURE 1. Biochemical rescue of CFTR₁₂₈₁ by correctors in CFBE cells. *A*, schematic of CFTR showing site of W1282X premature termination codon in nucleotide binding domain 2 (top). Wild type, mutated (W1282X-CFTR), and truncated (CFTR₁₂₈₁) expression constructs were used in this study (bottom). *B*, immunoblot of wild type CFTR (WT, one-fifth the amount of protein loaded), W1282X-CFTR, and CFTR₁₂₈₁ in CFBE cells. Arrowheads represent core-glycosylated (black arrowheads) and complex-glycosylated (open arrowheads) CFTR. *C*, immunoblot of wild type CFTR, W1282X-CFTR, and CFTR₁₂₈₁ in CFBE cells in response to Δ F508-CFTR correctors (3 μ M VX-809 and 10 μ M C4) and putative modulators of read-through (200 μ g/ml G418, 200 μ g/ml gentamycin, and 10 μ M PTC124). Arrowheads represent core-glycosylated (black) and complex-glycosylated (open) CFTR. *D*, surface presentation of W1282X-CFTR and CFTR₁₂₈₁ in response to CFTR modulators and PTC124 measured by live cell ELISA (mean \pm S.E., ANOVA with Dunnett's post hoc test compared with control (DMSO-treated) cells, *, $p < 0.01$). *E*, metabolic pulse-chase analysis of CFTR₁₂₈₁ maturation in response to VX-809. Right, quantification of VX-809 effect on CFTR₁₂₈₁ maturation (mean \pm S.E., t test, *, $p < 0.01$). Data shown in *B–E* are representative of at least triplicate experiments. *p*, pulse; *ch*, chase.

tiating CFTR activity in W1282X-CFTR-expressing cells act on the truncated protein product produced by the W1282X mutation. Correctors (VX-809, VX-661, corr4a, C3, and C18) and potentiators (VX-770, PG01, and A04) that were active in FRT-YFP-W1282X-CFTR cells were comparably active in FRT-YFP-CFTR₁₂₈₁ cells, and compounds that were inactive in FRT-YFP-W1282X-CFTR cells (C7, P3, P8, P9, P12, and

CoPo-22) were also inactive in FRT-YFP-CFTR₁₂₈₁ cells (Fig. 2D and data not shown).

Novel Small Molecule CFTR₁₂₈₁ Correctors Identified by High Throughput Screening—Screening was done to identify novel CFTR₁₂₈₁ correctors with improved efficacy over known Δ F508-CFTR correctors. Screening of \sim 30,000 synthetic small molecules identified six compound classes that at 25 μ M nor-

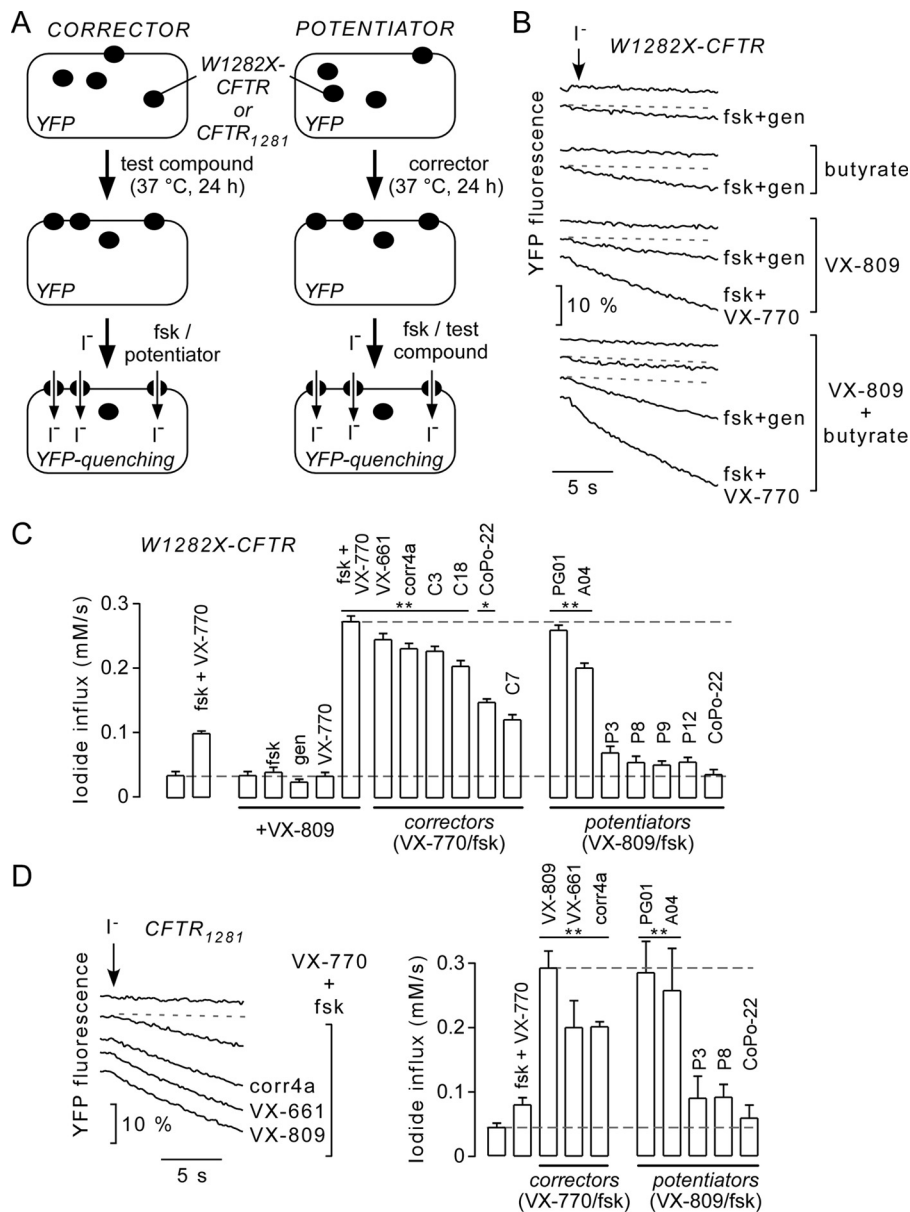


FIGURE 2. Functional rescue of CFTR₁₂₈₁ by known correctors and potentiators. *A*, design of assays to identify correctors (*left*) and potentiators (*right*). *B*, original YFP fluorescence quenching data from W1282X-CFTR-expressing FRT cells treated with the indicated CFTR modulators. Concentrations used are as follows: sodium butyrate (3 mM), VX-809 (3 μ M), fsk (10 μ M), genistein (*gen*, 50 μ M) and VX-770 (5 μ M). *C*, summary (mean \pm S.E., $n = 3-6$) for experiments as in *B* in response to a panel of known CFTR correctors (at 3 μ M) and potentiators (at 5 μ M). Cells were treated with forskolin plus VX-770 for corrector assays, and cells were corrected with VX-809 and treated with forskolin for potentiator assays. Statistical analysis by ANOVA with Dunnett's post hoc test: for correctors, data were compared with cells treated with fsk/VX-770; for potentiators, data were compared with cells treated with fsk/VX-809 (*, $p < 0.05$; **, $p < 0.001$). *D*, original data (*left*) and summary (*right*; mean \pm S.E., $n = 3-6$) for CFTR₁₂₈₁-mediated YFP quenching in FRT cells that are uncorrected or treated with correctors and/or potentiators (as in *C*). Statistical analysis as in *C*. Dashed lines in *C* and *D* represent unstimulated and maximal responses, respectively.

malized forskolin/VX-770-stimulated CFTR₁₂₈₁ activity to >50% of that produced by 3 μ M VX-809. Fig. 3A shows chemical structures of the two most active compounds, W1282X_{corr}-A23 and W1282X_{corr}-B09, which are chemically distinct from VX-809. In YFP quenching assays, these compounds increased CFTR₁₂₈₁ activity in a concentration-dependent manner producing ~5-fold greater maximal activity than VX-809 (Fig. 3B).

To quantify cell-surface expression, an FRT cell line expressing W1282X-CFTR containing a 3HA epitope tag in the fourth extracellular loop was generated. Cell-surface expression, as assayed in a live cell ELISA format, showed that the correctors

VX-809, W1282X_{corr}-A23, and W1282X_{corr}-B09 (at 3 μ M) increased surface presentation of W1282X-CFTR in FRT cells with efficacy similar to that seen in the functional assays (Fig. 3C, top). To investigate whether CFTR₁₂₈₁ or full-length (produced by read-through) CFTR was present at the cell surface in W1282X-CFTR-3HA-expressing cells, a luciferase-based W1282X read-through reporter was generated and expressed in FRT cells. As a positive control, the read-through compound geneticin (G418) produced a luciferase-dependent response, whereas W1282X_{corr}-A23, W1282X_{corr}-B09, and VX-809 did not (Fig. 3C, bottom). Together, these studies indicate that correctors identified by the screen, as well as VX-809, increase

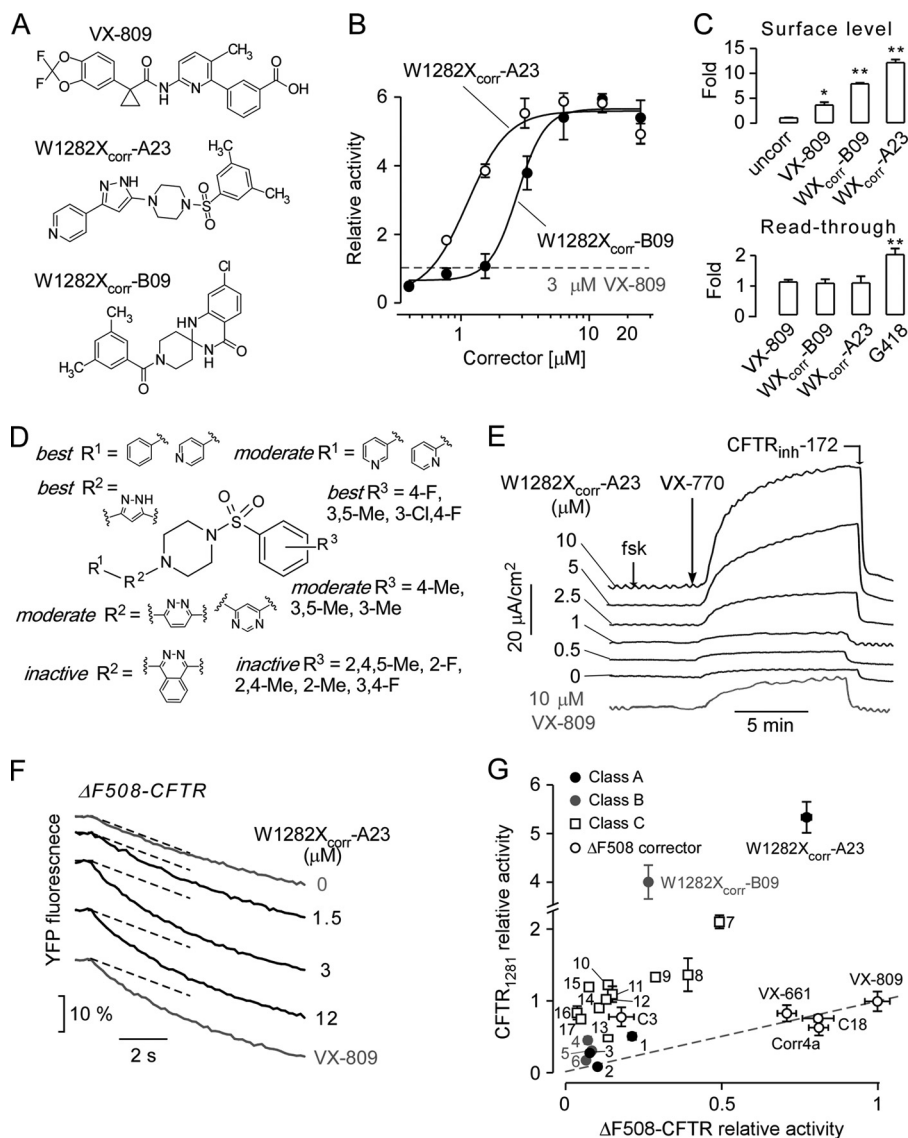


FIGURE 3. Novel CFTR₁₂₈₁ correctors with greater efficacy than VX-809. *A*, chemical structures of novel correctors identified by screening and that of VX-809. *B*, concentration-dependent CFTR correction in FRT cells expressing CFTR₁₂₈₁ by W1282X_{corr}-A23 and W1282X_{corr}-B09 (mean \pm S.E., $n = 3-4$). *Dashed line* represents response to VX-809 (3 μ M). *C*, *top*, CFTR cell-surface presentation in FRT cells expressing W1282X-CFTR-3HA measured by chemiluminescence using a horseradish peroxidase-coupled antibody labeling assay (mean \pm S.E., $n = 10-24$). *Bottom*, W1282X read-through in response to correctors (3 μ M) and G418 (1 mg/ml) measured using a luciferase-based reporter in FRT cells (mean \pm S.E., $n = 6-30$, ANOVA with Dunnett's post hoc test compared with control data, *, $p < 0.05$; **, $p < 0.001$). *D*, structure-activity analysis of class A correctors. *E*, short-circuit current measurement of corrector action in CFTR₁₂₈₁-expressing FRT cells. Data are shown for W1282X_{corr}-A23 (black traces) and VX-809 (gray trace; 10 μ M). Data are representative of triplicate experiments. *F*, efficacy of correctors identified in the W1282X screen on Δ F508-CFTR. YFP fluorescence quenching data for Δ F508-CFTR-expressing FRT cells treated with W1282X_{corr}-A23 or VX-809 (3 μ M). *G*, relative efficacy of correctors from classes A, B, and C (at 3 μ M, 24 h) in CFTR₁₂₈₁ and Δ F508-CFTR-expressing FRT cells (mean \pm S.E., $n = 4-12$). Data were normalized to VX-809 efficacy. Compound structure provided in Table 1. In *B*, *E*, *F*, and *G* cells were stimulated with forskolin (10 μ M) and VX-770 (5 μ M). CFTR_{inh}-172 was used at 10 μ M in all studies.

cell-surface CFTR₁₂₈₁ in FRT cells rather than promoting read-through.

Further studies were done with the most potent corrector identified, W1282X_{corr}-A23, a 1-arylpyrazole-4-arylsulfonyl-piperazine. Structure-activity studies of 100 commercially available analogs of W1282X_{corr}-A23 revealed that pyrazoles substituted with benzene and 4-pyridine (R¹) gave good activity, whereas 2- and 3-pyridine reduced activity (Fig. 3D). Changing the five-member pyrazole (R²) ring to 6-member pyrimidine or pyridazine rings reduced activity. The substituent (R³) on the arylsulfonyl group also affected activity, with 3,5- and 3,4-disubstitution giving the best activity, whereas

unsubstituted and 2-substitution reduced activity. Methyl and halide substituents were well tolerated. Short-circuit current (I_{sc}) measurements using CFTR₁₂₈₁-expressing cells and forskolin/VX-770 stimulation confirmed the concentration-dependent correction efficacy of W1282X_{corr}-A23; CFTR₁₂₈₁ correction by W1282X_{corr}-A23 produced \sim 4-fold more current than VX-809, similar to values determined by YFP quenching assays, and current was fully blocked by CFTR_{inh}-172 (Fig. 3E).

The correctors identified in the CFTR₁₂₈₁ screen were also tested for corrector activity on Δ F508-CFTR using FRT-YFP- Δ F508-CFTR cells. Fig. 3F shows YFP fluorescence quenching in Δ F508-CFTR-expressing FRT cells following correction by

Pharmacological Correction of W1282X-CFTR

TABLE 1

Chemical structures and corrector activities of selected Class A, B and C compounds

Class	Structure	Name	R ¹	R ²	R ³	%Correction	
						W1282X	ΔF508
A		1	phenyl		3-F,4-Me	51.4±4.8	21.2±0.6
		2	4-Me-phenyl		3,4-Me	8.9±0.5	10.1±0.4
		3	4-F-phenyl		4-Et	28.3±2.8	7.6±0.2
B		4	3,5-Me	H	2-Cl	46.0±3.8	7.3±1.0
		5	3,5-Me	Me	4-Cl	31.6±3.4	8.5±0.8
		6	5-Me	Me	4-Cl	18.2±1.4	6.7±0.4
C		7	4-Me	4-Et		210±9	49.4±1.2
		8	4-Me	4-MeO		137±23	39.3±0.8
		9	4-Me	3-Me,4-F		134±6	28.8±1.1
		10	-	3-Cl,4-Me		122±4	13.4±0.9
		11	4-Me	3-Me,4-Me		110±11	15.1±1.4
		12	4-F	4-Et		103±5	13.1±0.8
		13	-	3-Me,4-F		48.4±2.1	13.6±0.9
		14	-	4-EtO		91.5±2.6	10.8±1.1
		15	-	4-MeO		120±6	7.7±0.7
		16	-	2-F		84.1±9.4	3.9±0.2
17		-	2-F,5-F		75.2±4.8	5.1±0.4	

W1282X_{corr}-A23, with near maximal activity at ~3 μM. To investigate specificity of CFTR₁₂₈₁ correctors, active analogs from three compound classes were compared at 3 μM in CFTR₁₂₈₁ and ΔF508-CFTR-expressing cells. Whereas most known ΔF508-CFTR correctors (VX-809, VX-661, C18, and Corr4a) were comparably active on both CFTR mutants, many of the CFTR₁₂₈₁ correctors identified in the screen here were

substantially more effective in CFTR₁₂₈₁-expressing cells (Fig. 3G and Table 1). Although correctors with improved efficacy over VX-809 were identified by screening, subsequent assays were done with VX-809 as it is an approved drug.

Novel Small Molecule CFTR₁₂₈₁ Potentiators Identified by High Throughput Screening—Initial experiments were done to characterize VX-770 as a potentiator of VX-809-

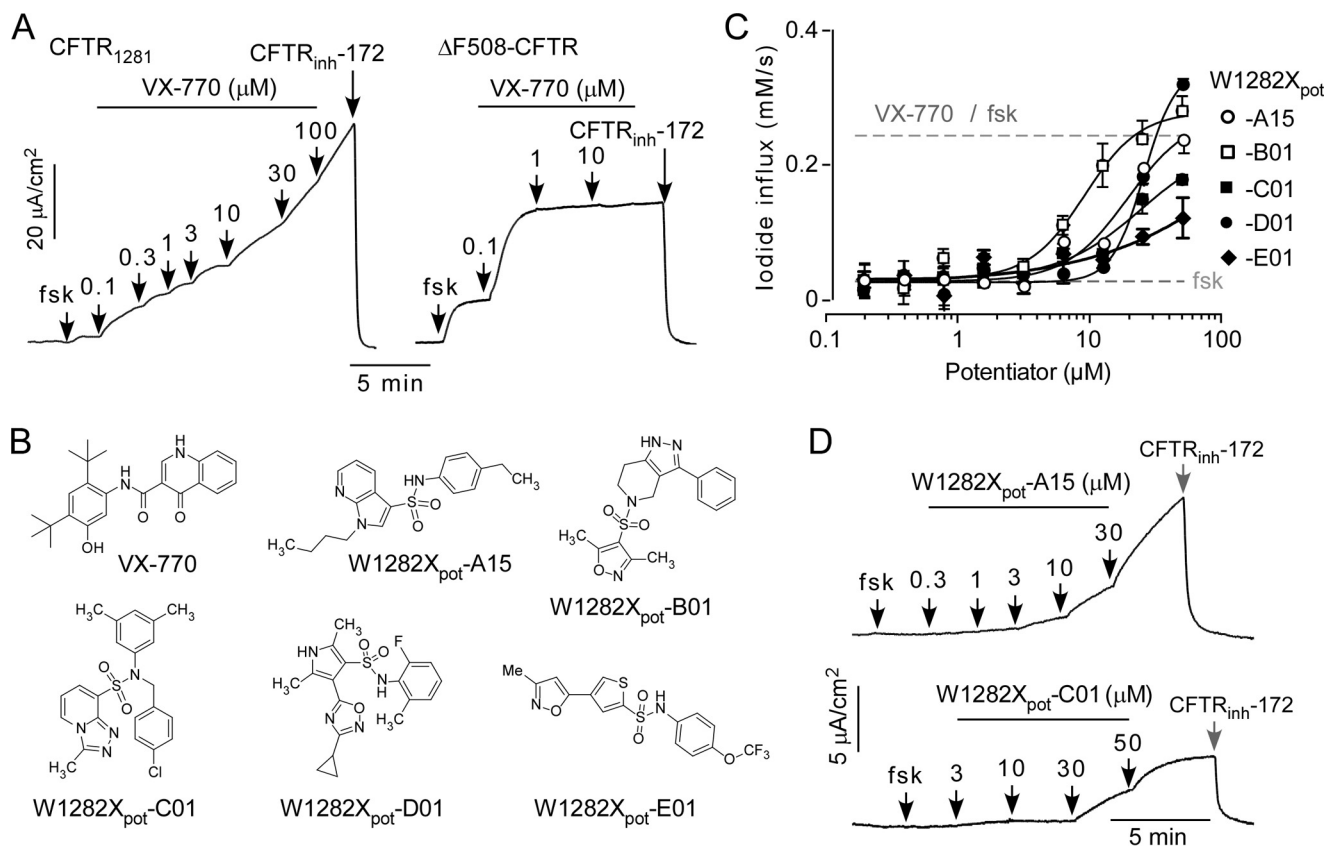


FIGURE 4. Novel CFTR₁₂₈₁ potentiators. *A, left*, concentration dependence of VX-770 in VX-809-corrected (3 μM, 24 h) CFTR₁₂₈₁ (left) and ΔF508-CFTR (right)-expressing FRT cells. *B*, chemical structures of potentiators identified in the screen, compared with VX-770. *C*, concentration-dependent CFTR₁₂₈₁ activity in FRT cells for indicated potentiators (mean ± S.E., $n = 3-4$). Dashed line indicates CFTR₁₂₈₁ activation produced by VX-770 (5 μM). Cells were corrected with VX-809 (3 μM, 24 h). *D*, short-circuit current in CFTR₁₂₈₁-expressing FRT cells in response to W1282X_{pot}-A15 (top) or W1282X_{pot}-C01 (bottom). In all studies forskolin was used at 10 μM, and cells were corrected with VX-809 (3 μM, 24 h). Data in *A* and *D* are representative of 3–4 experiments.

corrected CFTR₁₂₈₁. Interestingly, increasing VX-770 increased CFTR₁₂₈₁ activity without saturation at up to 200 μM, as seen in both plate reader and short-circuit current assays (Fig. 4A, left, and data not shown). In contrast, ΔF508-CFTR activity in VX-809-corrected cells was saturable, as expected (Fig. 4A, right). CFTR₁₂₈₁ and ΔF508-CFTR activities were fully blocked by CFTR_{inh}-172. These data suggest that CFTR₁₂₈₁ has very low open probability at clinically achievable concentrations of VX-770 but has the potential to be greatly activated.

A small molecule screen was done to identify novel CFTR₁₂₈₁ potentiators as diagrammed in Fig. 2A (right), in which cells were incubated with VX-809 to increase cell-surface CFTR₁₂₈₁ expression, and test compounds with forskolin were added 10 min prior to assay. Fig. 4B shows structures of five active compounds emerging from the screen, each of which is chemically distinct from VX-770. Concentration dependence measurements in VX-809-corrected CFTR₁₂₈₁-expressing cells showed that the new CFTR₁₂₈₁ potentiators had similar or lower activity than VX-770 (Fig. 4C). Control experiments confirmed that the potentiators did not activate CFTR₁₂₈₁ in the absence of forskolin and that YFP quenching did not occur in cells without CFTR₁₂₈₁. CFTR₁₂₈₁ activity was confirmed by short-circuit current measurements in VX-809-treated cells, with data shown for W1282X_{pot}-A15 and W1282X_{pot}-C01 (Fig. 4D).

Potentiator Combinations Restore CFTR₁₂₈₁ Activity to Wild Type Levels—Because of the relatively low activity of CFTR₁₂₈₁ in response to individual potentiators, we postulated that combinations of potentiators might show additive or perhaps synergistic effects. Initial experiments were done with VX-770 and the potentiators that were identified by screening. Remarkably, combination of VX-770 with W1282X_{pot}-A15, -C01, -D01, or -E01 significantly increased CFTR₁₂₈₁ activity, whereas W1282X_{pot}-B01 did not increase the VX-770 response (Fig. 5A, top). In cells expressing ΔF508-CFTR, each of the potentiators (except W1282X_{pot}-E01) increased channel activity; however, none of the potentiators further increased channel activity in the presence of VX-770 (Fig. 5A, bottom). Short-circuit current measurements showed W1282X_{pot}-A15 activation of VX-809-corrected CFTR₁₂₈₁ with $EC_{50} \sim 5 \mu\text{M}$ (Fig. 5B, left), which was independent of the order of addition (Fig. 5B, right). Activation by VX-770 and W1282X_{pot}-A15 ($100 \pm 8 \mu\text{A}/\text{cm}^2$) ($p < 0.0001$ by ANOVA) was remarkably greater than by forskolin alone ($2 \pm 1 \mu\text{A}/\text{cm}^2$) or forskolin in combination with W1282X_{pot}-A15 ($4 \pm 1 \mu\text{A}/\text{cm}^2$) or with VX-770 ($9 \pm 2 \mu\text{A}/\text{cm}^2$) (mean ± S.E., $n = 3-10$), indicating a synergistic response.

To estimate the CFTR₁₂₈₁ channel activity in response to potentiators, cell-surface expression and channel activity were measured in FRT cells expressing epitope (3HA)-tagged wild type or W1282X-CFTR. Incubation of cells expressing CFTR-

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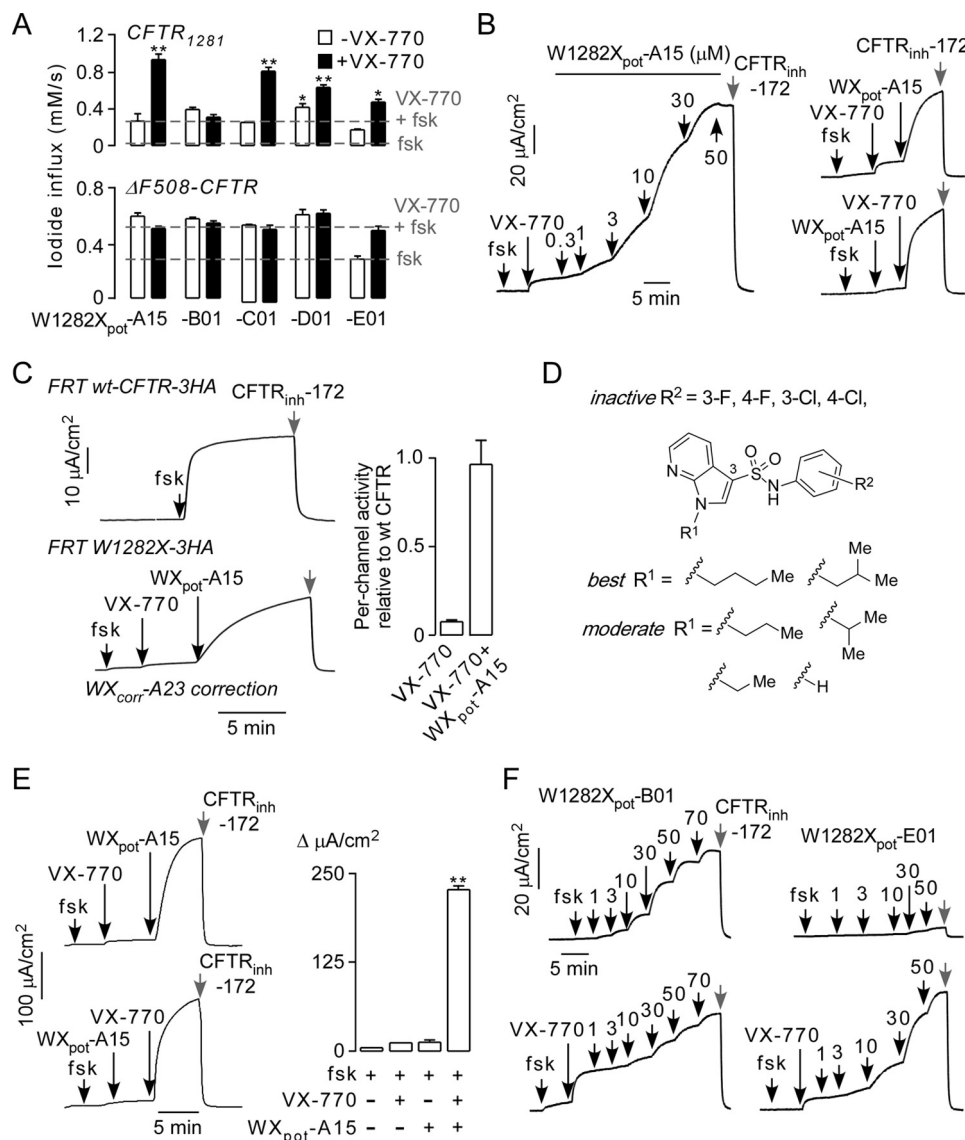


FIGURE 5. Marked synergy of CFTR₁₂₈₁ potentiators with VX-770. *A*, potentiator (25 μM) activity in the absence and presence of VX-770 (5 μM) in CFTR₁₂₈₁ (top) and $\Delta\text{F508-CFTR}$ (bottom)-expressing FRT cells measured by YFP quenching (mean \pm S.E., $n = 6-8$, ANOVA with Dunnett's post hoc test compared with cells treated with fsk/VX-770. *, $p < 0.01$; **, $p < 0.0001$). *B*, left, short-circuit current of CFTR₁₂₈₁ responses in FRT cells to VX-770 and W1282X_{pot}-A15; right, synergy of VX-770 (5 μM) and W1282X_{pot}-A15 (10 μM) is not dependent on order of addition. *C*, per-channel activity of CFTR₁₂₈₁ relative to wild type CFTR. CFTR current was measured in FRT cells expressing wild type CFTR-3HA (top left) or W1282X-CFTR-3HA (bottom left). W1282X-CFTR-3HA cells were corrected with W1282X_{corr}-A23 (3 μM) and stimulated with VX-770 (5 μM) and W1282X_{pot}-A15 (30 μM). Right, normalized per-channel activity of CFTR₁₂₈₁ in response to VX-770 without or with W1282X_{pot}-A15 relative to wild type CFTR (assigned a value of 1). *D*, structure-activity analysis of class A potentiators. *E*, I_{sc} of CFTR₁₂₈₁ responses to potentiator combination in CFBE cells. Representative I_{sc} recording (left) and average data (right; mean \pm S.E., ANOVA with Dunnett's post hoc test, **, $p < 0.0001$). *F*, short-circuit current of CFTR₁₂₈₁ in FRT cells in response to W1282X_{pot}-B01 (left) or W1282X_{pot}-E01 (right) in the absence (top) and presence (bottom) of VX-770 (5 μM). In *A*, *B*, *E*, and *F* cells were corrected with VX-809 (3 μM , 24 h). Forskolin was used at 10 μM in *A-C*, and *F* and 20 μM in *E*. Data in *B*, *C*, and *E* are representative of 3–4 experiments. Data in *F* are representative of 2–3 experiments.

3HA with forskolin produced robust current that was inhibited by CFTR_{inh}-172 (Fig. 5C, top left). W1282X-CFTR-3HA-expressing cells corrected with W1282X_{corr}-A23 produced CFTR channel activity in response to forskolin, VX-770, and W1282X_{pot}-A15 that was inhibited by CFTR_{inh}-172 (Fig. 5C, bottom left). Following normalization by cell-surface expression, deduced per-channel activity for CFTR₁₂₈₁ relative to wild type CFTR (normalized to 1) is shown (Fig. 5C, right). Remarkably, the potentiator combination increased CFTR₁₂₈₁ channel activity to that of wild type CFTR.

Structure-activity studies were done on 120 commercially available analogs of W1282X_{pot}-A15 (Fig. 5D). Class A poten-

tiators are composed of pyrrolo[2,3-*b*]pyridine with a phenylsulfonamide linked at the 3-position. Several alkyl groups (R¹) on the pyrrolo[2,3-*b*]pyridine were studied, and *n*-butyl and isobutyl groups gave active potentiators showing synergy with VX-770. Shorter alkyl groups such as propyl and ethyl reduced activity. The R² substituent on the phenylsulfonamide ring also affected activity, with electron-neutral or donating groups such as ethyl, methyl, and methoxy giving the best activity, although halides such as fluoro and chloro reduced activity. The position of the substituent also affected activity, with *para* and *ortho* being more active than *meta* substitution, suggesting that electronic properties of the phenylsulfonamide ring affect activity.

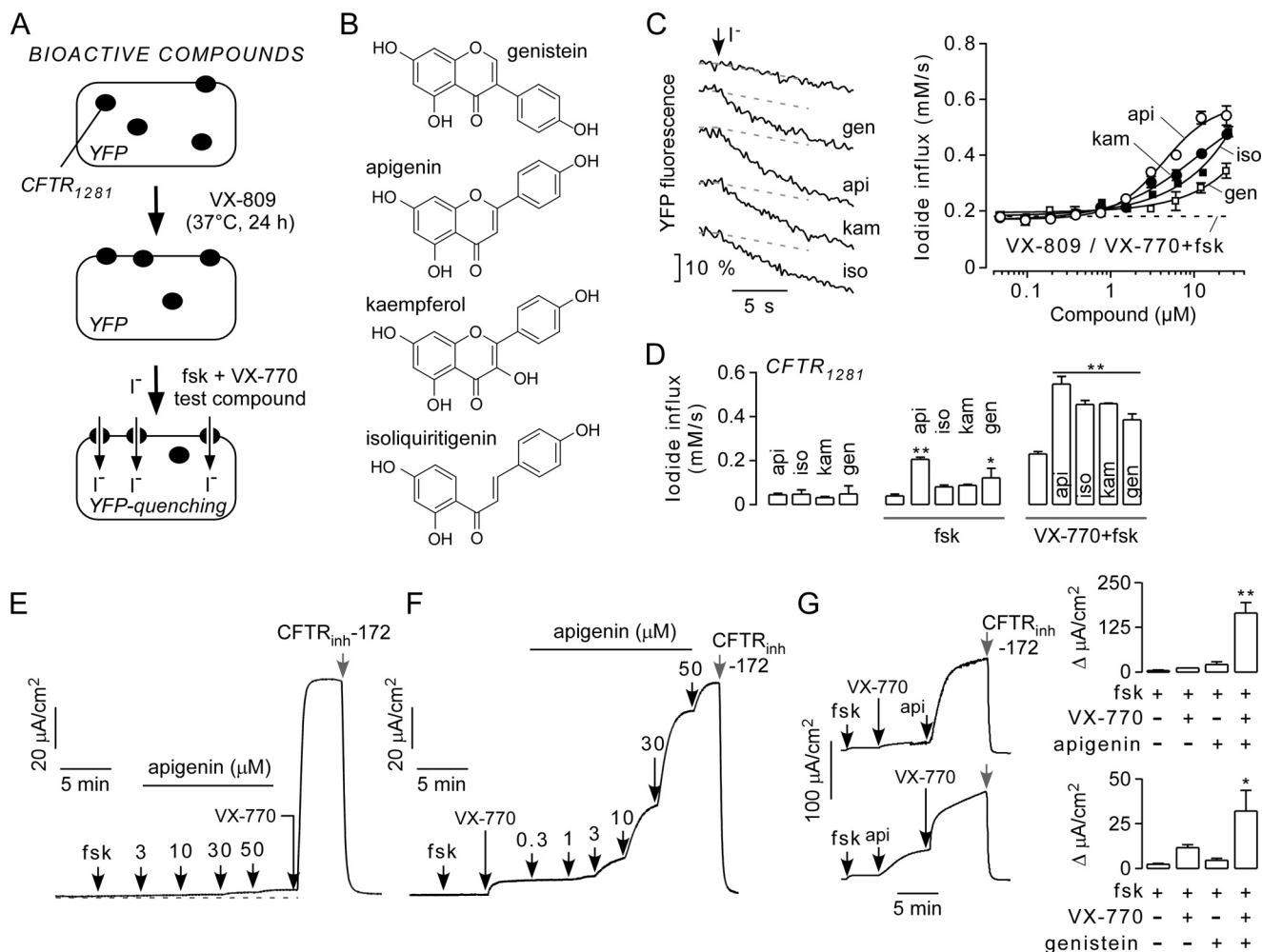


FIGURE 6. Synergy with VX-770 of bioactive small molecules identified in a synergy screen. *A*, schematic of screen used to identify bioactive molecules that act in synergy with VX-770. CFTR₁₂₈₁-expressing FRT cells were corrected with VX-809, and test compounds were added with forskolin and VX-770. *B*, structures of bioactive compounds that act in synergy with VX-770. *C*, left, YFP fluorescence quenching in CFTR₁₂₈₁-expressing FRT cells in response to VX-770 alone (top trace) and with indicated compounds. Right, concentration dependence of CFTR₁₂₈₁ activity in response to VX-770 and indicated compounds (mean \pm S.E., $n = 3-6$). gen, genistein; api, apigenin; kam, kaempferol; iso, isoliquiritigenin. *D*, compounds do not activate CFTR₁₂₈₁ in FRT cells alone and produce limited activity in the presence of forskolin (mean \pm S.E., $n = 3-6$). Statistical analysis was by ANOVA with Dunnett's post hoc test. For treatment of cells with compounds alone, or with fsk and compounds, data are compared with fsk-alone treatment; for cells treated with fsk/VX-770 and compound, data are compared with cells treated with fsk/VX-770 (*, $p < 0.05$; **, $p < 0.0001$). *E*, short-circuit current in FRT cells expressing CFTR₁₂₈₁ in response to VX-770 (5 μ M) and apigenin. *F*, dose-response of CFTR₁₂₈₁ activation in FRT cells by VX-770 and apigenin. *G*, left, CFTR₁₂₈₁ activation in CFBE cells by apigenin and VX-770 is independent of addition order and (right) is more efficacious than genistein. Statistical analysis was by ANOVA with Dunnett's post hoc test (*, $p < 0.05$; **, $p < 0.001$). In *C-G* cells were corrected with VX-809 (3 μ M, 24 h) and stimulated with forskolin (10 μ M). Data in *E-G* are representative of 3-5 experiments.

Short-circuit current measurements were also done in VX-809-treated CFBE cells to confirm results from FRT cells in a human airway epithelial cell model (Fig. 5E). Little activation of CFTR₁₂₈₁ was seen with VX-770 or W1282X_{pot}-A15 alone; however, in combination these potentiators strongly increased channel activity (Fig. 5E).

Interestingly, we found that some of the new potentiators showed activity alone but limited synergy with VX-770, although others were relatively inactive alone but showed synergy with VX-770. Fig. 5F shows examples of each, with W1282X_{pot}-B01 in the former category and W1282X_{pot}-E01 in the latter.

Screen of Approved and Investigational Drugs for Synergy with VX-770—Motivated by the marked synergy of VX-770 with some potentiators, we carried out a “synergy screen,” reasoning that repurposing of an existing drug or bioactive molecule for use in combination with VX-809 and VX-770 might

accelerate drug therapy for CF caused by the W1282X mutation. The screen might also identify bioactive compounds that are effective as potentiators when used alone. Screening was done using a collection of \sim 2600 bioactive compounds, natural products, and approved drugs that were added acutely with forskolin and VX-770 to VX-809-corrected CFTR₁₂₈₁-expressing cells (Fig. 6A). Screening identified several compounds that increased CFTR₁₂₈₁ activity above that produced by VX-770 and forskolin, including the flavones apigenin, genistein, and kaempferol, and the flavone-like anti-inflammatory compound isoliquiritigenin (Fig. 6B). Fig. 6C shows original YFP fluorescence quenching data and concentration-activity curves. Compounds did not increase CFTR₁₂₈₁ function when used alone (Fig. 6D). Apigenin and genistein had limited potentiator activity with forskolin, whereas isoliquiritigenin and kaempferol were inactive as potentiators.

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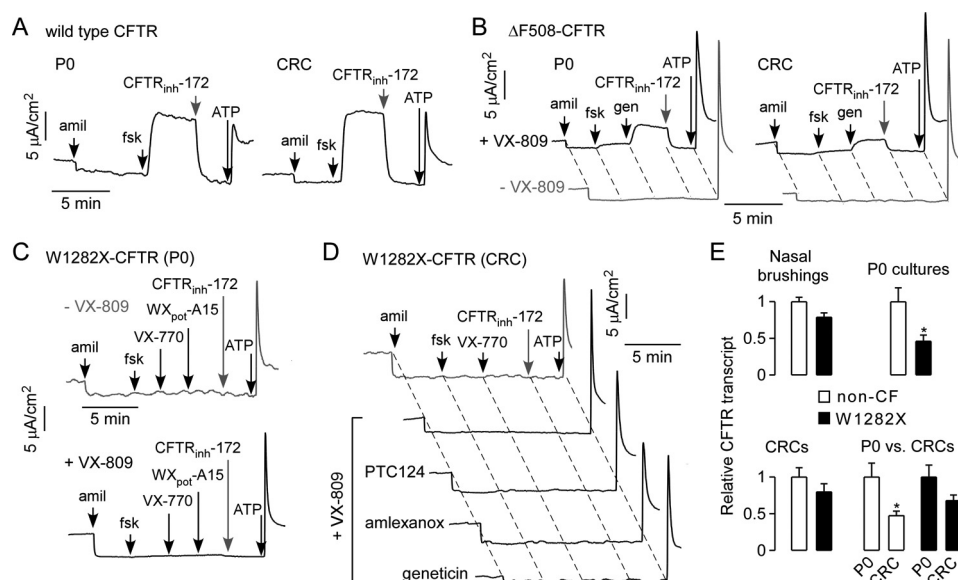


FIGURE 7. Testing of correctors and potentiators in human nasal epithelial cells from a single homozygous W1282X CF subject. *A*, short-circuit current in primary cultures (*left*) and CRCs (*right*) from a non-CF subject. *amil*, amiloride. *B*, short-circuit current in primary cultures (*left*) and CRCs (*right*) from a homozygous $\Delta F508$ -CFTR subject with (*black traces*) or without (*gray traces*) VX-809 correction. *gen*, genistein. *C*, short-circuit current in primary cultures from a homozygous W1282X-CFTR subject without (*gray trace*) or with (*black trace*) VX-809 correction. In some experiments, cells were treated for 24 h with PTC124 (ataluren; 25 μM), amlexanox (25 μM), or genistein (0.5 mg/ml). Concentrations used were amiloride (20 μM), forskolin (20 μM), genistein (50 μM), ATP (100 μM), W1282X_{pot}-A15 (10 μM), and VX-809 (3 μM , 24 h). *E*, CFTR transcript levels (mean \pm S.E., $n = 3$) in nasal brushings (*top left*), and P0 cultures (*top right*), or CRCs (*bottom left*) from a non-CF subject (*open bars*) and a homozygous W1282X-CFTR subject (*black bars*) (*bottom right*). Comparison of CFTR transcript levels in P0 cultures and CRCs. Statistical analysis was by *t* test (*, $p < 0.05$). Data in *A–D* are representative of 3–4 experiments.

I_{sc} data for apigenin, the most potent compound identified in the synergy screen, revealed limited CFTR₁₂₈₁ activity when used alone but robust CFTR₁₂₈₁ activity in the presence of VX-770 (Fig. 6E). CFTR₁₂₈₁ activation by apigenin in the presence of VX-770 had an EC_{50} of $\sim 20 \mu M$ (Fig. 6F), ~ 4 -fold less potent than W1282X_{pot}-A15. As found for W1282X_{pot}-A15, activation of CFTR₁₂₈₁ by VX-770 and apigenin was independent of the order of addition (data not shown). Comparing with cells incubated with W1282X_{pot}-A15, the per-channel activity of CFTR₁₂₈₁ relative to wild type CFTR in cells incubated with VX-770/apigenin is ~ 0.6 and with VX-770/genistein was ~ 0.2 . Experiments in VX-809-treated CFBE cells confirmed synergy of VX-770 with apigenin (Fig. 6G).

Corrector and Potentiator Testing in W1282X Homozygous Human Airway Epithelial Cells—Compounds were tested in airway epithelial cells from a homozygous W1282X-CFTR subject. Initial experiments were done with airway epithelial cells from non-CF and homozygous $\Delta F508$ -CFTR subjects to confirm the utility of conditionally reprogrammed cells (CRCs) for these studies. Primary and CRCs from a non-CF subject showed similar responses when treated with amiloride (ENaC channel blocker), forskolin, CFTR_{inh}-172, and ATP (activator of calcium-activated chloride channels) (Fig. 7A). Also, primary and conditionally reprogrammed human bronchial epithelial cells from a homozygous $\Delta F508$ -CFTR CF subject showed similar responses following correction with VX-809, with no activity in the absence of corrector (Fig. 7B). These studies support the use of CRCs to study pharmacology of CFTR modulators, which allows considerable expansion of cell cultures derived from rare patient specimens, as needed for the W1282X studies here.

Nasal epithelial cells were obtained by brushings from a single W1282X-CFTR homozygous CF subject. In primary cells, VX-770 and W1282X_{pot}-A15 produced no significant CFTR current without or with VX-809 incubation (Fig. 7C). The amiloride and ATP responses were similar in these cells to those in non-CF and $\Delta F508$ -CFTR P0 cultures and CRCs. No CFTR current was observed under the same experimental conditions using CRCs generated from nasal epithelial cells from the same CF subject (data not shown). Further experiments tested the activity of read-through compounds in CRCs generated from the W1282X-CFTR P0 cultures. As with P0 cultures, CRCs without and with VX-809 showed no response to CFTR modulators (Fig. 7D). VX-809-corrected W1282X-CFTR CRCs treated with the read-through agents PTC124/ataluren (6), amlexanox (30), and genistein (14) produced no significant CFTR current in response to correctors and potentiators but showed robust amiloride and ATP responses.

Quantitative PCR was done to determine CFTR transcript levels in the human airway epithelial cell models (Fig. 7E). In nasal brushings, CFTR transcript was reduced only by $\sim 20\%$ in CF cells as compared with a non-CF subject (Fig. 7E, *top left*). In P0 cultures, non-CF nasal and bronchial airway epithelial cells had similar CFTR transcript levels (data not shown), and cells from the W1282X-CFTR subject had $\sim 50\%$ less CFTR transcript (Fig. 7E, *top right*). CRCs from non-CF and W1282X-CFTR subjects had similar levels of CFTR transcript (Fig. 7E, *bottom left*). Finally, in P0 cultures and CRCs from non-CF or W1282X-CFTR subjects, CFTR transcript levels were similar, suggesting that reprogramming does not greatly reduce CFTR transcript. First passage CRC cultures were morphologically

indistinguishable from PO cultures with both showing pseudostratified and ciliated differentiation (data not shown).

Discussion

Therapeutic approaches for CF caused by the W1282X mutation and other PTC mutations such as G542X have focused on promoting read-through to generate full-length CFTR protein (5). The limited efficiency of read-through and function of the translated full-length proteins, which generally contain non-native amino acids at the PTC, are major challenges in this approach. We demonstrate here that in the absence of read-through the truncated protein product generated by the W1282X mutation (CFTR₁₂₈₁) has the potential to be efficiently processed and gated using combinations of small molecules. The major novel findings of this work include the application of correctors to facilitate processing of CFTR₁₂₈₁, the identification of correctors and potentiators with much greater efficacy than existing compounds, and the discovery of potentiator combinations that restore CFTR₁₂₈₁ channel conductance to that of wild type CFTR. However, whether the findings from cell cultures models will translate to native human airway epithelial cells remains uncertain at this time, as corrector and potentiator combinations did not show efficacy in airway epithelial cell cultures from a single homozygous W1282X CF subject.

The results here suggest that pharmacological activation of CFTR₁₂₈₁ may require triple drug combination therapy with a corrector and two potentiators acting in synergy. Of note, therapies for Δ F508-CFTR in development include a single potentiator and two correctors, supporting the viability of triple drug CFTR modulator therapy for CF (25, 31–33). High throughput screening in this study produced novel chemical scaffolds that corrected CFTR₁₂₈₁ trafficking with greater efficacy than existing Δ F508-CFTR correctors. Perhaps second and third generation correctors under development for Δ F508-CFTR may be active as CFTR₁₂₈₁ correctors; and VX-770 together with newer potentiators with improved activity may act in synergy on CFTR₁₂₈₁.

Although this study was focused on the W1282X-CFTR mutation, the findings may have relevance to CF associated with a variety of different CF-causing CFTR mutations. Several PTC mutations, including W1274X, Q1281X, Q1291X, Y1307X, Q1313X, Q1412X and S1455X, and frameshift mutations, such as c.3855delC, c.3884_3885insT, c.3890_3891insT, c.3908dupA, c.4139delC, and c.4147_4148insA, result in deletion of various lengths of the carboxyl terminus of CFTR within NBD2. Corrector/potentiator strategies may be useful for CF patients with these CFTR mutations. Also, the activity of Δ F508-CFTR modulators such as VX-809 and VX-770 on CFTR₁₂₈₁ shown here may be of benefit in compound heterozygous CF patients having W1282X along with Δ F508 or various gating mutations.

An important finding of this study is that potentiator combinations can activate CFTR₁₂₈₁ gating to near-wild type levels, whereas single potentiators only produce limited channel opening. Although beyond the scope of this study, investigation of the mechanisms responsible for this observation will benefit from information on the structure of CFTR₁₂₈₁ in the presence

of potentiators. We speculate that binding of both potentiators is sufficient to induce CFTR₁₂₈₁ conformational changes such that the channel assumes a structure with wild type characteristics. During completion of this manuscript, Wang *et al.* (16) reported synergistic stimulation of CFTR₁₂₈₁ by VX-770 and the herbal supplement curcumin; patch clamp of FRT cells expressing W1282X-CFTR showed an increase in P_o from <0.01 to ~ 0.2 with VX-770 or curcumin and both compounds increased P_o to ~ 0.9 (16). Our study here identified flavones as additional bioactive compounds that activate CFTR₁₂₈₁ in synergy with VX-770. Curcumin is unlikely to be useful clinically as a CFTR₁₂₈₁ potentiator because of its low potency and poor bioavailability (34). In contrast, flavones represent potential candidates for drug development because of their slow metabolism, good bioavailability, and predominantly renal excretion (35, 36).

A disappointing finding was that various combinations of potentiators and correctors, even together with read-through drugs, did not produce measurable chloride currents in primary and conditionally reprogrammed nasal epithelial cell cultures generated from a single homozygous W1282X CF subject. Quantitative PCR showed that transcript levels in CF cell models used in this study were not much lower than those in non-CF cells, suggesting that very low levels of CFTR transcript in the human cells tested here are not responsible for the absence of channel activity. Perhaps patient variability and subject-specific phenotypic factors might modulate drug responses, as seen for Δ F508, mandating the need for drug testing in cells from other W1282X CF patients. It is well recognized that the efficacy of Δ F508-CFTR correctors can be cell type-dependent (37, 38), as correctors identified in transfected cells often are not effective in patient-derived human airway epithelial cells. However, potentiators tend to be cell context-independent as they likely target mutant CFTRs at the plasma membrane directly.

There are limited prior reports on the biological properties of the 1-arylpyrazole-4-arylsulfonyl-piperazine corrector scaffold identified in this study. Some naphthalene arylsulfonyl-piperazines were identified as inhibitors of activated coagulation factor X (39) and human 11β -hydroxysteroid dehydrogenase type 1 (40). Limited biological data have been reported for the phenylsulfonamide-pyrrolopyridine class of W1282X potentiators. Some phenylsulfonamide-pyrrolopyridines were found as cannabinoid agonists for therapy for pain and nausea (41). Virtual screening also identified phenylsulfonamide-pyrrolopyridines as potential inhibitors of heat shock protein Hsp90 (42). The arylpyrazole-arylsulfonyl-piperazine and phenylsulfonamide-pyrrolopyridine scaffolds have favorable drug-like properties, including the presence of multiple hydrogen bond acceptors, molecular masses of 396 and 357 Da, aLogP values of 3.2 and 3.8, and topological polar surface areas of 79.4 and 61.8 Å², respectively (43, 44). In addition, these scaffolds do not belong to promiscuous binder families known as pan-assay interference compounds (45). Further medicinal chemistry to generate targeted analogs may give compounds with improved potency.

In summary, the studies here support the potential utility of small molecule correctors and potentiators as a therapeutic approach for CF caused by the W1282X mutation and perhaps

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for other CF-causing mutations in the carboxyl-terminal region of CFTR. Although compounds did not produce measurable current in nasal epithelial cells from a single homozygous W1282X CF subject, further testing of CFTR₁₂₈₁ correctors and potentiators in cells from additional W1282X homozygous subjects may show benefit, as might testing in subjects with appropriate compound mutations such as W1282X/G542X.

Experimental Procedures

Chemicals—VX-809, VX-661, VX-770, and CFTR_{inh}-172 were purchased from Selleck Chemicals (Boston, MA). The previously described potentiators and correctors (prefixed with P and C, respectively) P3, P8, P9, C3, C7, and C18 were obtained from the CFTR Compound Program, The Cystic Fibrosis Foundation Therapeutics Inc., which is administered at Rosalind Franklin University of Medicine and Science; Corr4a (C4), CoPo-22, PG01, A04, and P12 were obtained from an in-house repository of CFTR modulators. Geneticin (G418) was purchased from Gibco-Life Technologies, Inc. Screening was done using a collection of ~30,000 drug-like synthetic small molecules (ChemDiv, San Diego) and collections of approved and investigational drugs (~2500 molecules; Microsource Spectrum Library, Gaylordsville, CT; the National Institutes of Health Clinical Collection, Biofocus, South San Francisco, CA). All other chemicals were purchased from Sigma unless otherwise stated.

Complementary DNA Constructs—Expression cassettes containing CFTR cDNA were generated in pcDNA3.1/Zeo(+) (Invitrogen) that contains a cytomegalovirus intermediate-early (CMV) promoter. Constructs were generated in two steps. The 5'-region of CFTR (~3.8 kb) was excised as a KpnI-BstXI fragment from a plasmid encoding enhanced GFP-CFTR (46). Subsequently, gBlock gene fragments (Integrated DNA Technology, Coralville, IA) were synthesized to regenerate the 3'-region of wild type and mutant CFTR cDNAs (Fig. 1A, bottom). Gene fragments were designed to contain an endogenous BstXI site and an engineered XhoI site for cloning, together with the following: CFTR cDNA with its endogenous stop codon for wild type CFTR (~600 bp); CFTR cDNA, including the W1282X mutation and its endogenous stop codon for W1282X-CFTR (~600 bp); or CFTR cDNA up to Gln¹²⁸¹ and tandem stop codons (TGA TAG TAA) for CFTR₁₂₈₁ (~120 bp). To generate engineered CFTR constructs containing 3HA in the fourth extracellular loop, a cDNA region encompassing the 3HA tag was excised as an EcoRI-EcoRI fragment from a previously described CFTR-3HA construct (47) and ligated into wild type- and W1282X-CFTR cDNAs contained in pcDNA3.1/Zeo(+). To measure W1282X read-through, a reporter (GFP-W1282X-luc) was generated in pcDNA3.1/Zeo(+) using gBlock gene fragments consisting of GFP, CFTR cDNA from residue Asp¹²⁷⁰ to Phe¹²⁸⁶, including the W1282X PTC, and *Photinus pyralis* luciferase. Constructs were fully sequenced. Lentiviral particles expressing W1282X-CFTR and CFTR₁₂₈₁ were generated using the Lenti-X TenON Advanced Inducible Expression System (Clontech) and the pLVX-tight-puro transfer vector as described (24).

Cell Culture Models—Fischer rat thyroid (FRT) cells were cultured in Kaig's modified Ham's F-12 medium supplemented with 10% FBS, 2 mM L-glutamine, 100 units/ml penicil-

lin, 100 µg/ml streptomycin, 18 µg/ml myoinositol, and 45 µg/ml ascorbic acid. For screening, clonal FRT cell lines were generated in two steps. Cells expressing EYFP-H148Q/I152L/F46L (YFP) were generated using the FELIX third generation feline immunodeficiency lentiviral system (deposited at Addgene as a gift by Garry Nolan, plasmid 1728); subsequently, cells were transfected with pcDNA3.1/Zeo(+) plasmids encoding W1282X-CFTR or CFTR₁₂₈₁, and clonal cell lines were selected using 0.25 µg/ml Zeocin. To study CFTR cell-surface presentation, cell lines expressing CFTR and W1282X-CFTR containing extracellular 3HA tags were generated in a similar manner. A cell line expressing ΔF508-CFTR was described previously (27). To generate an FRT cell model to probe W1282X read-through, cells were transfected with plasmid encoding GFP-W1282X-luc and enriched for reporter expression by culture with 0.25 µg/ml Zeocin. CFBE41o- (CFBE) cells expressing tetracycline-inducible W1282X-CFTR and CFTR₁₂₈₁ with 3HA tags were generated and cultured as described (24).

Biochemistry—Protein expression of CFTR-3HA variants was monitored by Western blotting of CFBE cell lysates using anti-hemagglutinin (HA) antibody (clone 16B12; Biolegend, San Diego) and enhanced chemiluminescence detection, as described (48). To monitor N-linked glycans as an indicator of CFTR biosynthetic processing, high molecular weight mannose N-glycans or all N-glycans were cleaved by incubating cell lysates with 10 µg/ml endo H or 40 µg/ml PNGase F (New England Biolabs, Ipswich, MA), respectively, for 2 h at 35 °C, followed by immunoblotting (49). CFTR₁₂₈₁ maturation efficiency in CFBE cells was measured by metabolic pulse-chase as described (50). Briefly, CFBE cells expressing CFTR₁₂₈₁ were pulse-labeled with 0.2 mCi/ml [³⁵S]methionine and [³⁵S]cysteine (EasyTag Express Protein Labeling Mix, PerkinElmer Life Sciences) in cysteine- and methionine-free medium for 1 h at 37 °C and then chased for 2 h at 37 °C in normal medium. Radioactivity incorporated into the core- and complex-glycosylated glycoproteins was visualized by fluorography and quantified by phosphorimage analysis using a Typhoon imaging platform (GE Healthcare). Relative maturation efficiency of CFTR₁₂₈₁ in the presence of VX-809 was determined by comparison of radioactivity incorporated into the complex- and core-glycosylated forms of CFTR, with efficiency expressed as fold change relative to control measured in the presence of DMSO.

Primary and Conditionally Reprogrammed Cultures of Human Nasal Epithelia—Human tissues were acquired and used with approval from the University of California, San Francisco Committee on Human Research. Nasal brushings were obtained on two occasions from a single CF subject homozygous for the W1282X mutation in CFTR, with no additional mutations identified by sequencing. Tissue isolated by brushing, consisting primarily of small aggregates of nasal mucosa and mucus, was rinsed three times in PBS with antibiotics (penicillin, 100 units/ml; streptomycin, 100 µg/ml; gentamicin, 100 µg/ml; amphotericin B, 2.5 µg/ml) and 5 mM dithiothreitol and one time in PBS with antibiotics prior to overnight incubation in PBS with antibiotics and 0.025% type XIV protease at 4 °C. A 1:1 mixture of DMEM and Ham's F-12 (DF12) containing 5% FBS and antibiotics was used to neutralize proteases and, as needed, trypsin (0.05%), or agitation was used to disperse

remaining cell clumps. For some experiments, isolated nasal epithelial cells were plated on permeable tissue culture inserts (12 mm diameter, 0.4- μm polyester membrane; Corning Costar Transwell) for polarized growth (termed P0 cultures) as described (25). Conditional reprogramming of nasal epithelial cells was performed using a modification of reported methods (51, 52). Briefly, 3T3-L1 fibroblasts (obtained from the UCSF Cell Culture Facility) were suspended in F-medium (3:1 Ham's F-12/DMEM, supplemented with 5% FBS, 5 $\mu\text{g}/\text{ml}$ bovine insulin, 8.4 ng/ml cholera toxin, 10 ng/ml recombinant human EGF (Atlanta Biologicals, Norcross, GA), 25 ng/ml hydrocortisone, 1 μM Y27632-ROCK inhibitor (Enzo Life Sciences, Farmingdale, NY), penicillin/streptomycin (as above), 250 $\mu\text{g}/\text{ml}$ amphotericin B, and 10 $\mu\text{g}/\text{ml}$ gentamicin) and irradiated in a Gammacell 3000 Elan (Best Theratronics, Springfield, VA) at 30 gray. Irradiated cells were then plated (3×10^6 per 10-cm tissue culture dish) and allowed to attach for 2 h. Nasal cells (1.5×10^6) were added to each dish and allowed to proliferate for 4–7 days and were subsequently harvested by differential trypsinization when they reached $\sim 80\%$ confluence. Conditionally reprogrammed cells were subsequently plated (5×10^5 cells/ cm^2) on tissue culture inserts coated with human placental collagen (20 $\mu\text{g}/\text{cm}^2$) in DF12 containing 5% FBS and antibiotics (penicillin, 100 units/ml; streptomycin, 100 $\mu\text{g}/\text{ml}$) to generate differentiated nasal epithelial cell sheets (termed P1 CRCs). After 24 h, the inserts were rinsed with PBS containing antibiotics and cultured with UNC-ALI media as described (25). CFTR transcript levels in human cells were measured by TaqMan quantitative RT-PCR using the Hs00357011_m1 probe set (Thermo Fisher Scientific, Waltham, MA) with data normalized to GAPDH transcript levels. Experiments were done at the UCSF Helen Diller Cancer Center Genome Analysis Core.

Screening Procedures—High throughput screening used a semi-automated screening platform (Beckman, Fullerton, CA) configured as described (53). FRT cells were plated in 96-well black-walled, clear-bottom tissue culture plates (Corning) at a density of 20,000 cells/well and cultured to confluence over 48 h prior to assays. For the corrector screen (Fig. 2A, left), cells expressing W1282X-CFTR and YFP were treated with test compounds in culture medium for 1 day, then washed twice with PBS, and incubated for 10 min in 100 μl of PBS containing forskolin (10 μM) and VX-770 (5 μM) prior to assay of CFTR activity. All plates in the corrector screen contained wells with positive (3 μM VX-809, 5 μM VX-770, and 10 μM forskolin) and negative (5 μM VX-770 and 10 μM forskolin) controls. For the potentiator screen (Fig. 2A, right), cells expressing CFTR₁₂₈₁ were cultured in medium containing VX-809 (3 μM) for 1 day, washed twice with PBS, and then incubated for 10 min in 100 μl of PBS containing forskolin (10 μM) and test compounds prior to assay. All plates in the potentiator screen contained positive (3 μM VX-809, 5 μM VX-770, and 10 μM forskolin) and negative (3 μM VX-809 and 10 μM forskolin) controls. A “synergy” screen to investigate combined effects of VX-770 with bioactive molecules and approved drugs was done using cells expressing CFTR₁₂₈₁ in a similar manner as the potentiator screen, with the exception that test wells contained VX-770 (5 μM). Assays were performed using a BMG Labtech FLUOstar OMEGA (Cary, NC) over 12 s with initial fluorescence intensity recorded

for 1 s prior to additional of 100 μl of NaI-substituted PBS (137 mM NaCl replaced with NaI). Initial I^- influx was computed from fluorescence intensity by single exponential regression (26). Control experiments were done using FRT cells expressing EYFP-H148Q/I152L/F46L alone.

I_{sc} Measurements—Measurements of I_{sc} were done with cells cultured on permeable supports as described (27). For FRT cells, the basolateral membrane was permeabilized with 250 $\mu\text{g}/\text{ml}$ amphotericin B, and experiments were done using a HCO_3^- -buffered system (in mM: 120 NaCl, 5 KCl, 1 MgCl_2 , 1 CaCl_2 , 5 HEPES, 25 NaHCO_3 , pH 7.4) with a basolateral to apical chloride gradient (generated by replacing 60 mM NaCl with sodium gluconate in the apical solution). For nasal epithelial cells, experiments were done using symmetrical HCO_3^- -buffered solutions (containing 120 mM NaCl). Modulators of ion conductance were added to both apical and basolateral bathing solutions, and cells were equilibrated with 95% O_2 , 5% CO_2 and maintained at 37 $^\circ\text{C}$.

CFTR Cell Surface Assays—FRT cells expressing 3HA-tagged CFTR constructs were washed in PBS (supplemented with 0.5% BSA) and incubated at 4 $^\circ\text{C}$ for 10 min prior to incubation with anti-hemagglutinin antibody (2 $\mu\text{g}/\text{ml}$, 1 h, 4 $^\circ\text{C}$; clone 16B12, Biologend, San Diego, CA), HRP-conjugated secondary antibody (0.16 $\mu\text{g}/\text{ml}$, 1 h, 4 $^\circ\text{C}$; goat anti-mouse IgG antibody, Thermo Scientific, Waltham, MA), and Western Bright Sirius luminescence detection reagent (Advansta Inc., Menlo Park, CA). Luminescence representing surface CFTR was detected using a Tecan Infinite F500 plate reader (San Jose, CA). CFTR activity was measured by I_{sc} for computation of relative channel activity. A similar approach was used for CFBE cells but with Amplex-Red (Molecular Probes, Waltham, MA) HRP substrate to measure CFTR cell-surface density (50).

Author Contributions—P. M. H. conceived the study, designed experiments, generated cDNA constructs and cell models used in the study, conducted read-through and surface presentation assays, analyzed results, and wrote the paper. P. W. P. conducted structure-activity studies and electrophysiological experiments using FRT and reprogrammed cell models, analyzed results, and wrote aspects of the paper. J. A. T. conducted HTS and structure-activity studies, and analyzed data. H. X. participated in generation of lentiviral expression constructs and performed biochemical analysis. R. G. A. participated in generation of lentiviral expression constructs, performed electrophysiological studies on CFBE cultures, and was responsible for the initial observation of combined VX-770/genistein potentiation. D. P. performed pulse-chase studies. L. Z. cultured primary and conditionally reprogrammed human cell cultures. D. W. N. acquired patient samples. W. E. F. conceived certain studies, designed experiments, and wrote aspects of the paper. G. L. L. and A. S. V. conceived the study, designed experiments, and wrote the paper.

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References

- Watson, M. S., Cutting, G. R., Desnick, R. J., Driscoll, D. A., Klinger, K., Mennuti, M., Palomaki, G. E., Popovich, B. W., Pratt, V. M., Rohlf, E. M., Strom, C. M., Richards, C. S., Witt, D. R., and Grody, W. W. (2004) Cystic fibrosis population carrier screening: 2004 revision of American College of Medical Genetics mutation panel. *Genet. Med.* **6**, 387–391
- Kervestin, S., and Jacobson, A. (2012) NMD: a multifaceted response to premature translation termination. *Nat. Rev. Mol. Cell Biol.* **13**, 700–712
- Popp, M. W., and Maquat, L. E. (2013) Organizing principles of mammalian nonsense-mediated mRNA decay. *Annu. Rev. Genet.* **47**, 139–165
- Kuzmiak, H. A., and Maquat, L. E. (2006) Applying nonsense-mediated mRNA decay research to the clinic: progress and challenges. *Trends Mol. Med.* **12**, 306–316
- Keeling, K. M., Xue, X., Gunn, G., and Bedwell, D. M. (2014) Therapeutics based on stop codon readthrough. *Annu. Rev. Genomics Hum. Genet.* **15**, 371–394
- Du, M., Liu, X., Welch, E. M., Hirawat, S., Peltz, S. W., and Bedwell, D. M. (2008) PTC124 is an orally bioavailable compound that promotes suppression of the human CFTR-G542X nonsense allele in a CF mouse model. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 2064–2069
- Kerem, E., Konstan, M. W., De Boeck, K., Accurso, F. J., Sermet-Gaudelus, I., Wilschanski, M., Elborn, J. S., Melotti, P., Bronsveld, I., Fajac, I., Malfroot, A., Rosenbluth, D. B., Walker, P. A., McColley, S. A., Knoop, C., et al. (2014) Ataluren for the treatment of nonsense-mutation cystic fibrosis: a randomized, double-blind, placebo-controlled phase 3 trial. *Lancet Respir. Med.* **2**, 539–547
- McElroy, S. P., Nomura, T., Torrie, L. S., Warbrick, E., Gartner, U., Wood, G., and McLean, W. H. (2013) A lack of premature termination codon read-through efficacy of PTC124 (Ataluren) in a diverse array of reporter assays. *Plos Biol.* **11**, e1001593
- Van Goor, F., Yu, H., Burton, B., and Hoffman, B. J. (2014) Effect of ivacaftor on CFTR forms with missense mutations associated with defects in protein processing or function. *J. Cyst. Fibros.* **13**, 29–36
- Davies, J. C. (2015) The future of CFTR modulating therapies for cystic fibrosis. *Curr. Opin. Pulm. Med.* **21**, 579–584
- Elborn, J. S. (2016) Cystic fibrosis. *Lancet* **388**, 2519–2531
- Veit, G., Avramescu, R. G., Chiang, A. N., Houck, S. A., Cai, Z., Peters, K. W., Hong, J. S., Pollard, H. B., Guggino, W. B., Balch, W. E., Skach, W. R., Cutting, G. R., Frizzell, R. A., Sheppard, D. N., Cyr, D. M., Sorscher, E. J., Brodsky, J. L., and Lukacs, G. L. (2016) From CFTR biology toward combination pharmacotherapy: expanded classification of cystic fibrosis mutations. *Mol. Biol. Cell* **27**, 424–433
- Linde, L., Boelz, S., Nissim-Rafinia, M., Oren, Y. S., Wilschanski, M., Yacov, Y., Virgilis, D., Neu-Yilik, G., Kulozik, A. E., Kerem, E., and Kerem, B. (2007) Nonsense-mediated mRNA decay affects nonsense transcript levels and governs response of cystic fibrosis patients to gentamicin. *J. Clin. Invest.* **117**, 683–692
- Rowe, S. M., Varga, K., Rab, A., Bebok, Z., Byram, K., Li, Y., Sorscher, E. J., and Clancy, J. P. (2007) Restoration of W1282X CFTR activity by enhanced expression. *Am. J. Respir. Cell Mol. Biol.* **37**, 347–356
- Xue, X., Mutyam, V., Tang, L., Biswas, S., Du, M., Jackson, L. A., Dai, Y., Belakhov, V., Shalev, M., Chen, F., Schacht, J., J. Bridges, R., Baasov, T., Hong, J., Bedwell, D. M., and Rowe, S. M. (2014) Synthetic aminoglycosides efficiently suppress cystic fibrosis transmembrane conductance regulator nonsense mutations and are enhanced by ivacaftor. *Am. J. Respir. Cell Mol. Biol.* **50**, 805–816
- Wang, W., Hong, J. S., Rab, A., Sorscher, E. J., and Kirk, K. L. (2016) Robust stimulation of W1282X-CFTR channel activity by a combination of allosteric modulators. *PLoS One* **11**, e0152232
- Cui, L., Aleksandrov, L., Chang, X.-B., Hou, Y.-X., He, L., Hegedus, T., Gentsch, M., Aleksandrov, A., Balch, W. E., and Riordan, J. R. (2007) Domain interdependence in the biosynthetic assembly of CFTR. *J. Mol. Biol.* **365**, 981–994
- Du, K., and Lukacs, G. L. (2009) Cooperative assembly and misfolding of CFTR domains *in vivo*. *Mol. Biol. Cell* **20**, 1903–1915
- Gentsch, M., and Riordan, J. R. (2001) Localization of sequence within the C-terminal domain of the cystic fibrosis transmembrane conductance regulator which impact maturation and stability. *J. Biol. Chem.* **276**, 1291–1298
- Gentsch, M., Aleksandrov, A., Aleksandrov, L., and Riordan, J. R. (2002) Functional analysis of the C-terminal boundary of the second nucleotide binding domain of the cystic fibrosis transmembrane conductance regulator and structural implications. *Biochem. J.* **366**, 541–548
- Benharouga, M., Sharma, M., So, J., Haardt, M., Drzymala, L., Popov, M., Schwapach, B., Grinstein, S., Du, K., and Lukacs, G. L. (2003) The role of the C terminus and Na⁺/H⁺ exchanger regulatory factor in the functional expression of cystic fibrosis transmembrane conductance regulator in nonpolarized cells and epithelia. *J. Biol. Chem.* **278**, 22079–22089
- Haggie, P. M., Tan, J. A., Phuan, P.-W., Nielson, D. W., Finkbeiner, W. E., and Verkman, A. S. (2015) Novel therapeutic approach for cystic fibrosis caused by the W1282X premature termination codon. *Pediatr. Pulm.* **50**, S264
- Ehrhardt, C., Collnot, E. M., Baldes, C., Becker, U., Laue, M., Kim, K. J., and Lehr, C. M. (2006) Towards an *in vitro* model of cystic fibrosis small airway epithelium: characterization of the human bronchial epithelial cell line CFB410-. *Cell Tissue Res.* **323**, 405–415
- Veit, G., Bossard, F., Goepp, J., Verkman, A. S., Galletta, L. J., Hanrahan, J. W., and Lukacs, G. L. (2012) Proinflammatory cytokine secretion is suppressed by TMEM16A or CFTR channel activity in human cystic fibrosis bronchial epithelial. *Mol. Biol. Cell* **23**, 4188–4202
- Phuan, P. W., Veit, G., Tan, J., Roldan, A., Finkbeiner, W. E., Lukacs, G. L., and Verkman, A. S. (2014) Synergy-based small-molecule screen using a human lung epithelial cell line yields ΔF508-CFTR correctors that augment VX-809 maximal efficacy. *Mol. Pharmacol.* **86**, 42–51
- Pedemonte, N., Lukacs, G. L., Du, K., Caci, E., Zegarra-Moran, O., Galletta, L. J., and Verkman, A. S. (2005) Small-molecule correctors of defective ΔF508-CFTR cellular processing identified by high throughput screening. *J. Clin. Invest.* **115**, 2564–2571
- Phuan, P. W., Veit, G., Tan, J. A., Finkbeiner, W. E., Lukacs, G. L., and Verkman, A. S. (2015) Potentiators of defective ΔF508-CFTR gating that do not interfere with corrector action. *Mol. Pharmacol.* **88**, 791–799
- Rubenstein, R. C., Egan, M. E., and Zeitlin, P. L. (1997) *In vitro* pharmacologic restoration of CFTR-mediated chloride transport with sodium 4-phenylbutyrate in cystic fibrosis epithelial cells containing ΔF508-CFTR. *J. Clin. Invest.* **100**, 2457–2465
- Pedemonte, N., Sonawane, N. D., Taddei, A., Hu, J., Zegarra-Moran, O., Suen, Y. F., Robins, L. I., Dicus, C. W., Willenbring, D., Nantz, M. H., Kurth, M. J., Galletta, L. J., and Verkman, A. S. (2005) Phenylglycine and sulfonamide correctors of defective ΔF508 and G551D cystic fibrosis transmembrane conductance regulator chloride-channel gating. *Mol. Pharmacol.* **67**, 1797–1807
- Gonzalez-Hilarion, S., Beghyn, T., Jia, J., Debreuck, N., Berte, G., Mamchaoui, K., Mouly, V., Gruenert, D. C., Déprez, B., and Lejeune, F. (2012) Rescue of nonsense mutations by amlexanox in human cells. *Orphanet. J. Rare Dis.* **7**, 58
- Okiyonedo, T., Veit, G., Dekkers, J. F., Bagdany, M., Soya, N., Xu, H., Roldan, A., Verkman, A. S., Kurth, M., Simon, A., Hegedus, T., Beekman, J. M., and Lukacs, G. L. (2013) Mechanism-based corrector combination restores ΔF508-CFTR folding and function. *Nat. Chem. Biol.* **9**, 444–454
- Cutting, G. R. (2015) Cystic fibrosis genetics: from molecular understanding to clinical application. *Nat. Rev. Genet.* **16**, 45–56
- Quon, B. S., and Rowe, S. M. (2016) New and emerging targeted therapies for cystic fibrosis. *BMJ* **352**, i859
- Anand, P., Kunnumakkara, A. B., Newman, R. A., and Aggarwal, B. B. (2007) Bioavailability of curcumin: problems and promises. *Mol. Pharm.* **4**, 807–818
- Meyer, H., Bolarinwa, A., Wolfram, G., and Linseisen, J. (2006) Bioavailability of apigenin from apiin-rich parsley in humans. *Ann. Nutr. Metab.* **50**, 167–172
- Gradolatto, A., Basly, J.-P., Berges, R., Teyssier, C., Chagnon, M.-C., Siess, M.-H., and Canivenc-Lavier, M.-C. (2005) Pharmacokinetics and metabolism of apigenin in female and male rats after a single oral administration. *Drug Metab. Dispos.* **33**, 49–54
- Rowe, S. M., Pyle, L. C., Jurkevante, A., Varga, K., Collawn, J., Sloane, P. A., Woodworth, B., Mazur, M., Fulton, J., Fan, L., Li, Y., Fortenberry, J.,

- Sorscher, E. J., and Clancy, J. P. (2010) Δ F508 CFTR processing correction and activity in polarized and non-airway cell monolayers. *Pulm. Pharmacol. Ther.* **23**, 268–278
38. Pedemonte, N., Tomati, V., Sondo, E., and Galiotta, L. J. (2010) Influence of cell background on pharmacological response of mutant CFTR. *Am. J. Physiol. Cell Physiol.* **298**, C866–C874
 39. Tawada, H., Ito, F., Moriya, N., and Terashita, Z. (December 3, 1998) WO Patent 9854164 A1 19981203
 40. Sun, D., Wang, Z., Di, Y., Jaen, J. C., Labelle, M., Ma, J., Miao, S., Sodom, A., Tang, L., Tomooka, C. S., Tu, H., Ursu, S., Walker, N., Yan, X., Ye, Q., and Powers, J. P. (2008) Discovery and initial SAR of arylsulfonylpiperazine inhibitors of 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1). *Bioorg. Med. Chem. Lett.* **18**, 3513–3516
 41. Bowden, M. J., and Williamson, J. P. (October 16, 2014) WO Patent 2014167530 A1 20141016
 42. Sun, H.-P., Jia, J.-M., Jiang, F., Xu, X.-L., Liu, F., Guo, X.-K., Cherfaoui, B., Huang, H.-Z., Pan, Y., and You, Q.-D. (2014) Identification and optimization of novel Hsp90 inhibitors with tetrahydropyrido[4,3-d]pyrimidines core through shape-based screening. *Eur. J. Med. Chem.* **79**, 399–412
 43. Lipinski, C. A., Lombardo, F., Dominy, B. W., and Feeney, P. J. (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Deliv. Rev.* **46**, 3–26
 44. Veber, D. F., Johnson, S. R., Cheng, H. Y., Smith, B. R., Ward, K. W., and Kopple, K. D. (2002) Molecular properties that influence the oral bioavailability of drug candidates. *J. Med. Chem.* **45**, 2615–2623
 45. Baell, J. B., and Holloway, G. A. (2010) New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays. *J. Med. Chem.* **53**, 2719–2740
 46. Moyer, B. D., Loffing, J., Schwiebert, E. M., Loffing-Cueni, D., Halpin, P. A., Karlson, K. H., Ismailov, I. I., Guggino, W. B., Langford, G. M., and Stanton, B. A. (1998) Membrane trafficking of the cystic fibrosis gene product, cystic fibrosis transmembrane conductance regulator, tagged with green fluorescent protein in Madin-Darby canine kidney cells. *J. Biol. Chem.* **273**, 21759–21768
 47. Valentine, C. D., Lukacs, G. L., Verkman, A. S., and Haggie, P. M. (2012) Reduced PDZ interactions of rescued Δ F508CFTR increases its cell surface mobility. *J. Biol. Chem.* **287**, 43630–43638
 48. Veit, G., Avramescu, R. G., Perdomo, D., Phuan, P.-W., Bagdany, M., Apaja, P. M., Borot, F., Szollosi, D., Wu, Y. S., Finkbeiner, W. E., Hegedus, T., Verkman, A. S., and Lukacs, G. L. (2014) Some gating potentiators, including VX-770, diminishes Δ F508-CFTR functional expression. *Sci. Transl. Med.* **6**, 246ra97
 49. Gluzman, R., Okiyoneda, T., Mulvihill, C. M., Rini, J. M., Barriere, H., and Lukacs, G. L. (2009) N-Glycans are direct determinants of CFTR folding and stability in secretory and endocytic membrane traffic. *J. Cell Biol.* **184**, 847–862
 50. Veit, G., Oliver, K., Apaja, P. M., Perdomo, D., Bidaud-Meynard, A., Lin, S. T., Guo, J., Icyuz, M., Sorscher, E. J., Hartman Iv, J. L., and Lukacs, G. L. (2016) Ribosomal stalk protein silencing partially corrects the Δ F508-CFTR functional expression defect. *PLoS Biol.* **14**, e1002462
 51. Palechor-Ceron, N., Suprynowicz, F. A., Upadhyay, G., Dakic, A., Minas, T., Simic, V., Johnson, M., Albanese, C., Schlegel, R., and Liu, X. (2013) Radiation induces diffusible feeder cell factors (s) that cooperate with ROCK inhibitor to conditionally reprogram and immortalize epithelial cells. *Am. J. Pathol.* **183**, 1862–1870
 52. Liu, X., Ory, V., Chapman, S., Yuan, H., Albanese, C., Kallakury, B., Timofeeva, O. A., Nealon, C., Dakic, A., Simic, V., Haddad, B. R., Rhim, J. S., Dritschilo, A., Riegel, A., McBride, A., and Schlegel, R. (2012) ROCK inhibitor and feeder cells induce the conditional reprogramming of epithelial cells. *Am. J. Pathol.* **180**, 599–607
 53. Ma, T., Thiagarajah, J. R., Yang, H., Sonawane, N. D., Folli, C., Galiotta, L. J., and Verkman, A. S. (2002) Thiazolidinone CFTR inhibitor identified by high throughput screening blocks cholera toxin-induced intestinal fluid secretion. *J. Clin. Invest.* **110**, 1651–1658