Title

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

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Edited by Thomas Söllner

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 ΔF508-CFTR, to produce functional rescue of the truncated translation product of the W1282X mutation, $CFTR_{1281}$, without the need for read-through. In transfected cell systems, certain potentiators and correctors, including VX-809 and VX-770, increased $CFTR_{1281}$ activity. To identify novel correctors and potentiators with potentially greater efficacy on $CFTR_{1281}$, functional screens were done of ~30,000 synthetic small molecules and drugs/nutraceuticals in $CFTR_{1281}$-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 $CFTR_{1281}$, whereas others also corrected ΔF508-CFTR. Several novel potentiator scaffolds were identified with efficacy comparable with VX-770; remarkably, a phenylsulfonamide-pyrrolopyridine acted synergistically with VX-770 to increase $CFTR_{1281}$ function ~8-fold over that of VX-770 alone, normalizing $CFTR_{1281}$ 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 ΔF508 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. 1*A*, *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 ($CFTR_{1281}$) constituting most of the full-length wild type CFTR (1480 amino acids), and postulating that $CFTR_{1281}$ has defective cellular processing and gat-

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◆ This article was selected as one of our Editors' Picks.

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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.

ing, we investigated whether functional rescue of $CFTR_{1281}$ is possible using correctors and/or potentiators, a strategy used for CF caused by the ΔF508 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-though 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 ($CFTR_{1281}$), 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 ($CFTR_{1281}$). The second encoded $CFTR_{1281}$ (Fig. 1*A*). 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 $CFTR_{1281}$ (Fig. 1*B*). The apparent molecular mass of the core- and complex-glycosylated forms of $CFTR_{1281}$ and W1282X-CFTR was reduced compared with wild type CFTR. Assuming comparable transcript levels, these observations suggest that that the truncated $CFTR_{1281}$ has inefficient biosynthetic processing and/or impaired stability. Supporting inefficient processing, the known ΔF508-CFTR correctors VX-809 and corr4a (C4) increased the complex-glycosylated forms of $CFTR_{1281}$ 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 $CFTR_{1281}$ processing in the presence of VX-809, metabolic pulse-chase labeling with [^{35}S]methionine and [^{35}S]cysteine in polarized CFBE cell cultures showed ~75% increased incorporation of radiolabel into complex-glycosylated $CFTR_{1281}$ with VX-809 (Fig. 1*E*).

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 $CFTR_{1281}$ (FRT-YFP-$CFTR_{1281}$); 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. 2*A*, *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. 2*A*, *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 μM) 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. 2*B*) (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 μM) had greater efficacy than genistein (Fig. 2, *B* and *C*). CFTR activation required both cAMP elevation and a potentiator (VX-770 or genistein; Fig. 2*C* and data not shown).

Fig. 2*C* (*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. 2*C, right*).

Further experiments were done in FRT-YFP-$CFTR_{1281}$ cells to investigate whether CFTR modulators that correct or poten-

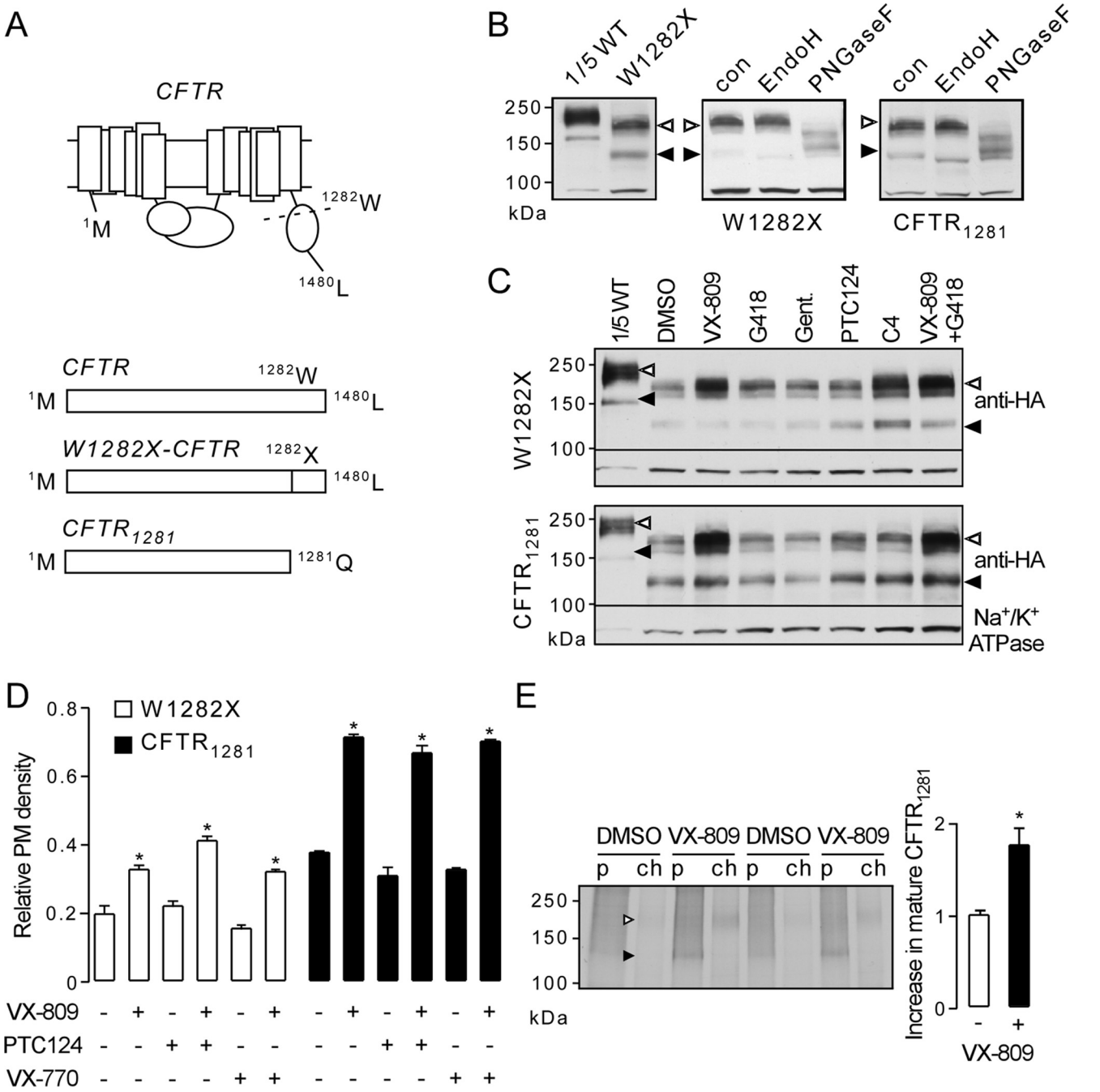


FIGURE 1. **Biochemical rescue of $CFTR_{1281}$ by correctors in CFBE cells.** *A*, schematic of CFTR showing site of W1282X premature termination codon in nucleotide binding domain 2 (*top*). Wild type, mutated (W1282X-CFTR), and truncated ($CFTR_{1281}$) expression constructs were used in this study (*bottom*). *B*, immunoblot of wild type CFTR (WT, one-fifth the amount of protein loaded), W1282X-CFTR, and $CFTR_{1281}$ in CFBE cells. *Arrowheads* represent core-glycosylated (*black arrowheads*) and complex-glycosylated (*open arrowheads*) CFTR. *C*, immunoblot of wild type CFTR, W1282X-CFTR, and $CFTR_{1281}$ in CFBE cells in response to ΔF508-CFTR correctors (3 μM VX-809 and 10 μM C4) and putative modulators of read-through (200 μg/ml G418, 200 μg/ml gentamycin, and 10 μM PTC124). *Arrowheads* represent core-glycosylated (*black*) and complex-glycosylated (*open*) CFTR. *D*, surface presentation of W1282X-CFTR and $CFTR_{1281}$ in response to CFTR modulators and PTC124 measured by live cell ELISA (mean ± S.E., ANOVA with Dunnett's post hoc test compared with control (DMSO-treated) cells, *, $p <$ 0.01). *E*, metabolic pulse-chase analysis of $CFTR_{1281}$ maturation in response to VX-809. *Right,* quantification of VX-809 effect on $CFTR_{1281}$ maturation (mean ± S.E., *t* test, *, $p < 0.01$). Data shown in *B–E* are representative of at least triplicate experiments. *p*, pulse; *ch*, chase.

tiate 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-$CFTR_{1281}$ 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-$CFTR_{1281}$ cells (Fig. 2*D* and data not shown).

Novel Small Molecule $CFTR_{1281}$ Correctors Identified by High Throughput Screening—Screening was done to identify novel $CFTR_{1281}$ correctors with improved efficacy over known ΔF508-CFTR correctors. Screening of ~30,000 synthetic small molecules identified six compound classes that at 25 μM nor-

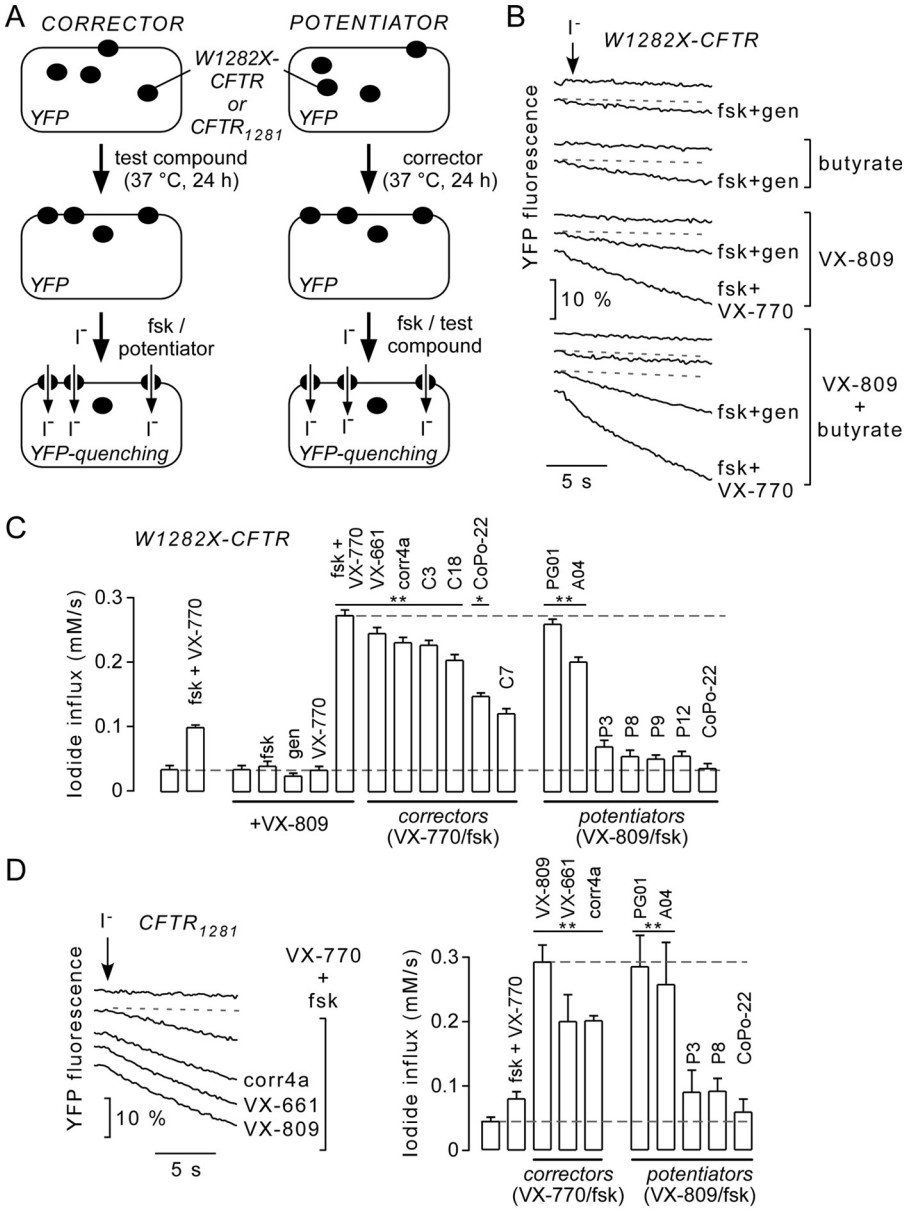


FIGURE 2. **Functional rescue of $CFTR_{1281}$ by known correctors and potentiators.** *A*, design of assays to identify correctors (*left*) and potentiators (*right*). *B*, original YFP fluorescence quenching data from W1282X-CFTR-expressing FRT cells treated with the indicated CFTR modulators. Concentrations used are as follows: sodium butyrate (3 mM), VX-809 (3 μM), fsk (10 μM), genistein (*gen*, 50 μM) and VX-770 (5 μM). *C*, summary (mean ± S.E., $n = 3–6$) for experiments as in *B* in response to a panel of known CFTR correctors (at 3 μM) and potentiators (at 5 μM). 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 ± S.E., $n = 3–6$) for $CFTR_{1281}$-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 $CFTR_{1281}$ activity to >50% of that produced by 3 μM VX-809. Fig. 3*A* 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 $CFTR_{1281}$ activity in a concentration-dependent manner producing ~5-fold greater maximal activity than VX-809 (Fig. 3*B*).

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 μM) increased surface presentation of W1282X-CFTR in FRT cells with efficacy similar to that seen in the functional assays (Fig. 3*C*, *top*). To investigate whether $CFTR_{1281}$ 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. 3*C*, *bottom*). Together, these studies indicate that correctors identified by the screen, as well as VX-809, increase

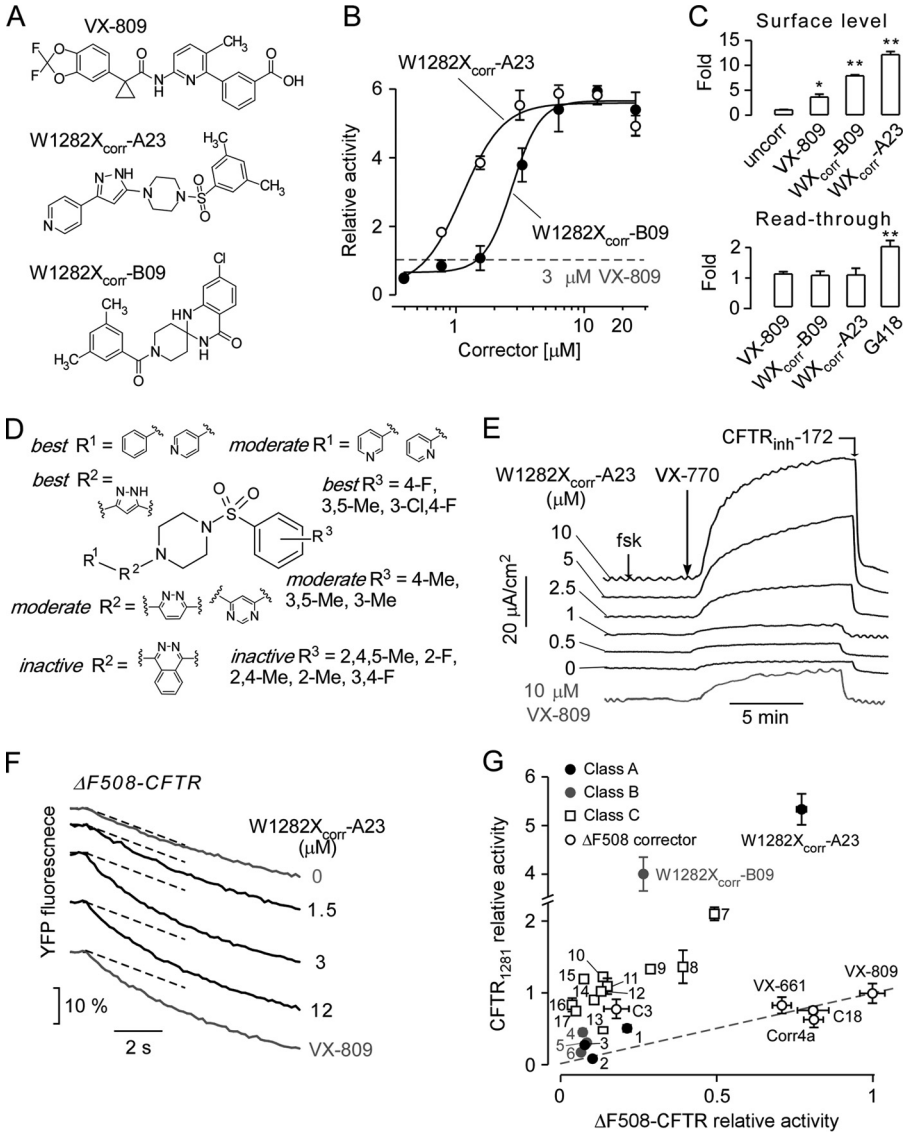


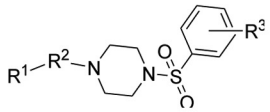
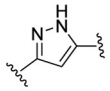
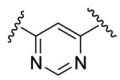
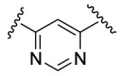
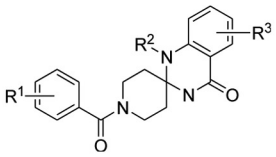
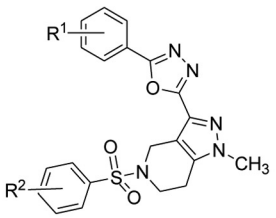
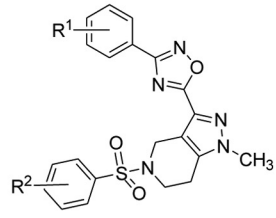
FIGURE 3. **Novel CFTR$_{1281}$ correctors with greater efficacy than VX-809.** *A*, chemical structures of novel correctors identified by screening and that of VX-809. *B*, concentration-dependent CFTR correction in FRT cells expressing CFTR$_{1281}$ by W1282X$_{corr}$-A23 and W1282X$_{corr}$-B09 (mean ± S.E., $n = 3–4$). *Dashed line* represents response to VX-809 (3 μM). *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 ± S.E., $n = 10–24$). *Bottom,* W1282X read-through in response to correctors (3 μM) and G418 (1 mg/ml) measured using a luciferase-based reporter in FRT cells (mean ± 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 CFTR$_{1281}$-expressing FRT cells. Data are shown for W1282X$_{corr}$-A23 (*black traces*) and VX-809 (*gray trace*; 10 μM). Data are representative of triplicate experiments. *F*, efficacy of correctors identified in the W1282X screen on ΔF508-CFTR. YFP fluorescence quenching data for ΔF508-CFTR-expressing FRT cells treated with W1282X$_{corr}$-A23 or VX-809 (3 μM). *G*, relative efficacy of correctors from classes A, B, and C (at 3 μM, 24 h) in CFTR$_{1281}$ and ΔF508-CFTR-expressing FRT cells (mean ± 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 μM) and VX-770 (5 μM). CFTR$_{inh}$-172 was used at 10 μM in all studies.

cell-surface CFTR$_{1281}$ 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-arylsulfonylpiperazine. Structure-activity studies of 100 commercially available analogs of W1282X$_{corr}$-A23 revealed that pyrazoles substituted with benzene and 4-pyridine (R^1) gave good activity, whereas 2- and 3-pyridine reduced activity (Fig. 3*D*). Changing the five-member pyrazole (R^2) ring to 6-member pyrimidine or pyridazine rings reduced activity. The substituent (R^3) 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 CFTR$_{1281}$-expressing cells and forskolin/VX-770 stimulation confirmed the concentration-dependent correction efficacy of W1282X$_{corr}$-A23; CFTR$_{1281}$ correction by W1282X$_{corr}$-A23 produced ~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. 3*E*).

The correctors identified in the CFTR$_{1281}$ screen were also tested for corrector activity on ΔF508-CFTR using FRT-YFP-ΔF508-CFTR cells. Fig. 3*F* shows YFP fluorescence quenching in ΔF508-CFTR-expressing FRT cells following correction by

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 $CFTR_{1281}$ correctors, active analogs from three compound classes were compared at 3 μM in $CFTR_{1281}$ 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 $CFTR_{1281}$ correctors identified in the screen here were substantially more effective in $CFTR_{1281}$-expressing cells (Fig. 3*G* 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 $CFTR_{1281}$ Potentiators Identified by High Throughput Screening—Initial experiments were done to characterize VX-770 as a potentiator of VX-809-

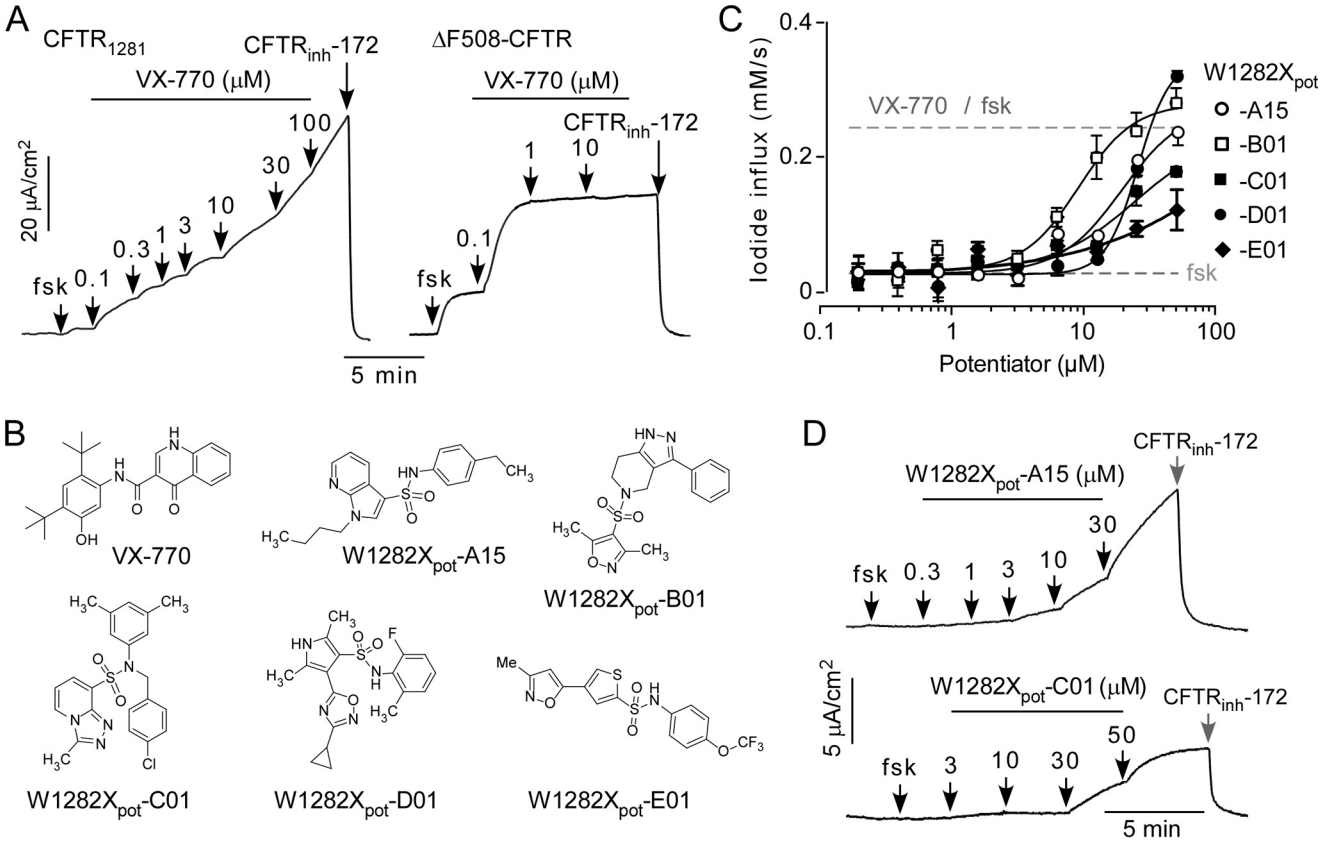


FIGURE 4. **Novel $CFTR_{1281}$ potentiators.** *A*, *left,* concentration dependence of VX-770 in VX-809-corrected (3 μM, 24 h) $CFTR_{1281}$ (*left*) and ΔF508-CFTR (*right*)-expressing FRT cells. *B*, chemical structures of potentiators identified in the screen, compared with VX-770. *C*, concentration-dependent $CFTR_{1281}$ activity in FRT cells for indicated potentiators (mean ± S.E., $n = 3–4$). *Dashed line* indicates $CFTR_{1281}$ activation produced by VX-770 (5 μM). Cells were corrected with VX-809 (3 μM, 24 h). *D*, short-circuit current in $CFTR_{1281}$-expressing FRT cells in response $W1282X_{pot}$-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 $CFTR_{1281}$. Interestingly, increasing VX-770 increased $CFTR_{1281}$ activity without saturation at up to 200 μM, as seen in both plate reader and short-circuit current assays (Fig. 4*A*, *left,* and data not shown). In contrast, ΔF508-CFTR activity in VX-809-corrected cells was saturable, as expected (Fig. 4*A*, *right*). $CFTR_{1281}$ and ΔF508-CFTR activities were fully blocked by $CFTR_{inh}$-172. These data suggest that $CFTR_{1281}$ has very low open probably at clinically achievable concentrations of VX-770 but has the potential to be greatly activated.

A small molecule screen was done to identify novel $CFTR_{1281}$ potentiators as diagrammed in Fig. 2*A* (*right*), in which cells were incubated with VX-809 to increase cell-surface $CFTR_{1281}$ expression, and test compounds with forskolin were added 10 min prior to assay. Fig. 4*B* 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 $CFTR_{1281}$-expressing cells showed that the new $CFTR_{1281}$ potentiators had similar or lower activity than VX-770 (Fig. 4*C*). Control experiments confirmed that the potentiators did not activate $CFTR_{1281}$ in the absence of forskolin and that YFP quenching did not occur in cells without $CFTR_{1281}$. $CFTR_{1281}$ 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. 4*D*).

Potentiator Combinations Restore $CFTR_{1281}$ Activity to Wild Type Levels—Because of the relatively low activity of $CFTR_{1281}$ 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 $CFTR_{1281}$ activity, whereas $W1282X_{pot}$-B01 did not increase the VX-770 response (Fig. 5*A*, *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. 5*A*, *bottom*). Short-circuit current measurements showed $W1282X_{pot}$-A15 activation of VX-809-corrected $CFTR_{1281}$ with EC_{50} ~5 μM (Fig. 5*B*, *left*), which was independent of the order of addition (Fig. 5*B*, *right*). Activation by VX-770 and $W1282X_{pot}$-A15 (100 ± 8 $\mu A/cm^2$) ($p < 0.0001$ by ANOVA) was remarkably greater than by forskolin alone (2 ± 1 $\mu A/cm^2$) or forskolin in combination with $W1282X_{pot}$-A15 (4 ± 1 $\mu A/cm^2$) or with VX-770 (9 ± 2 $\mu A/cm^2$) (mean ± S.E., $n = 3–10$), indicating a synergistic response.

To estimate the $CFTR_{1281}$ 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-

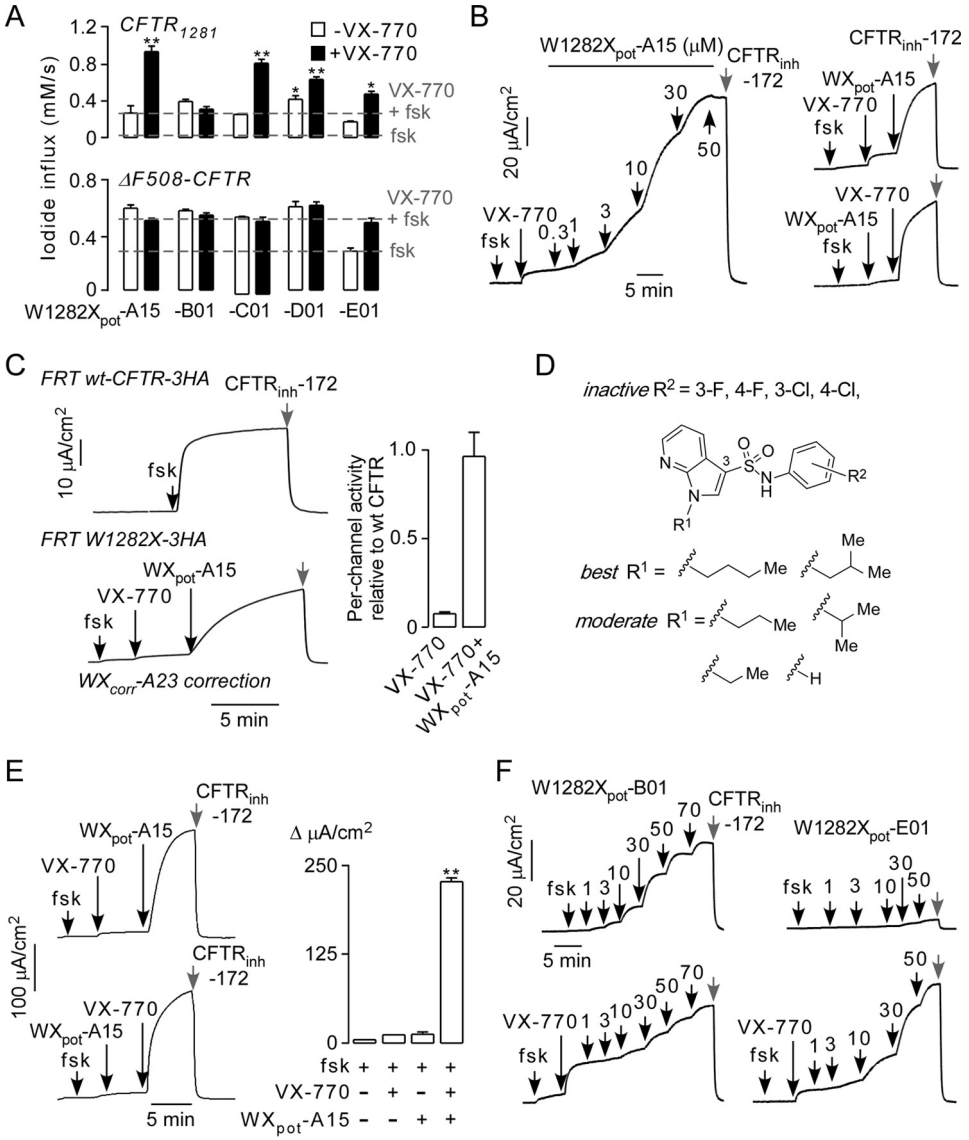


FIGURE 5. **Marked synergy of $CFTR_{1281}$ potentiators with VX-770.** *A*, potentiator (25 μM) activity in the absence and presence of VX-770 (5 μM) in $CFTR_{1281}$ (*top*) and ΔF508-CFTR (*bottom*)-expressing FRT cells measured by YFP quenching (mean ± S.E., $n = 6–8$, ANOVA with Dunnett's post hoc test compared with cells treated with fsk/VX-770. *, $p < 0.01$; **, $p < 0.0001$). *B*, *left,* short-circuit current of $CFTR_{1281}$ 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 $CFTR_{1281}$ 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 $CFTR_{1281}$ 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 $CFTR_{1281}$ responses to potentiator combination in CFBE cells. Representative I_{sc} recording (*left*) and average data (*right*; mean ± S.E., ANOVA with Dunnett's post hoc test, **, $p <$ 0.0001). *F*, short-circuit current of $CFTR_{1281}$ 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. 5*C*, *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. 5*C*, *bottom left*). Following normalization by cell-surface expression, deduced per-channel activity for $CFTR_{1281}$ relative to wild type CFTR (normalized to 1) is shown (Fig. 5*C*, *right*). Remarkably, the potentiator combination increased $CFTR_{1281}$ channel activity to that of wild type CFTR.

Structure-activity studies were done on 120 commercially available analogs of $W1282X_{pot}$-A15 (Fig. 5*D*). Class A potentiators are composed of pyrrolo[2,3-*b*]pyridine with a phenylsulfonamide linked at the 3-position. Several alkyl groups (R^1) 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 group such as propyl and ethyl reduced activity. The R^2 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.

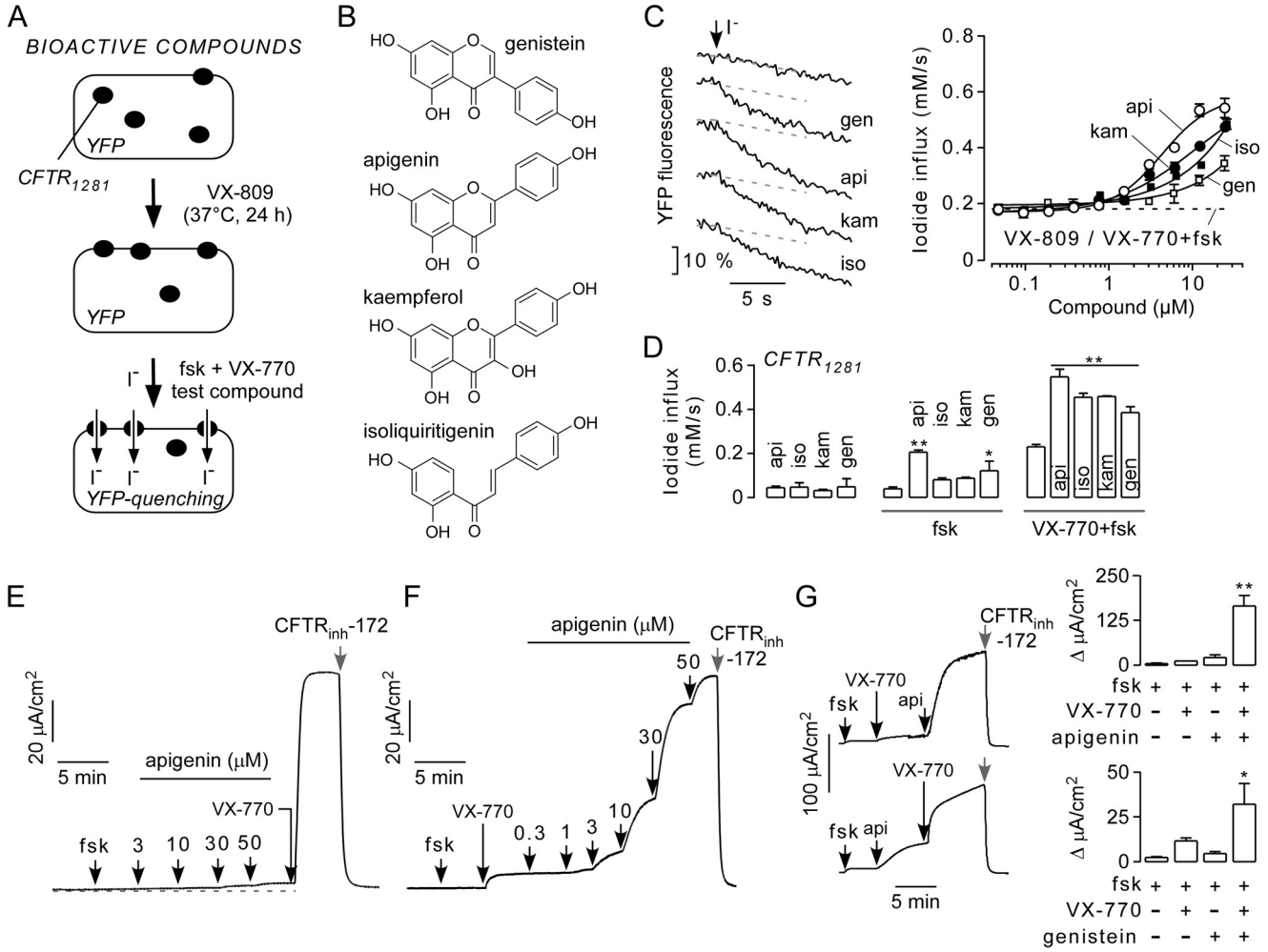


FIGURE 6. **Synergy with VX-770 of bioactive small molecules identified in a synergy screen.** *A,* schematic of screen used to identify bioactive molecules that act in synergy with VX-770. $CFTR_{1281}$-expressing FRT cells were corrected with VX-809, and test compounds were added with forskolin and VX-770. *B*, structures of bioactive compounds that act in synergy with VX-770. *C*, *left,* YFP fluorescence quenching in $CFTR_{1281}$-expressing FRT cells in response to VX-770 alone (*top trace*) and with indicated compounds. *Right,* concentration dependence of $CFTR_{1281}$ activity in response to VX-770 and indicated compounds (mean ± S.E., $n = 3–6$). *gen*, genistein; *api*, apigenin; *kam*, kaempferol; *iso*, isoliquiritigenin. *D*, compounds do not activate $CFTR_{1281}$ in FRT cells alone and produce limited activity in the presence of forskolin (mean ± 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 $CFTR_{1281}$ in response to VX-770 (5 μM) and apigenin. *F*, dose-response of $CFTR_{1281}$ activation in FRT cells by VX-770 and apigenin. *G*, *left,* $CFTR_{1281}$ 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 μM, 24 h) and stimulated with forskolin (10 μM). 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. 5*E*). Little activation of $CFTR_{1281}$ was seen with VX-770 or $W1282X_{pot}$-A15 alone; however, in combination these potentiators strongly increased channel activity (Fig. 5*E*).

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. 5*F* 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 ~2600 bioactive compounds, natural products, and approved drugs that were added acutely with forskolin and VX-770 to VX-809-corrected $CFTR_{1281}$-expressing cells (Fig. 6*A*). Screening identified several compounds that increased $CFTR_{1281}$ 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. 6*B*). Fig. 6*C* shows original YFP fluorescence quenching data and concentration-activity curves. Compounds did not increase $CFTR_{1281}$ function when used alone (Fig. 6*D*). Apigenin and genistein had limited potentiator activity with forskolin, whereas isoliquiritigenin and kaempferol were inactive as potentiators.

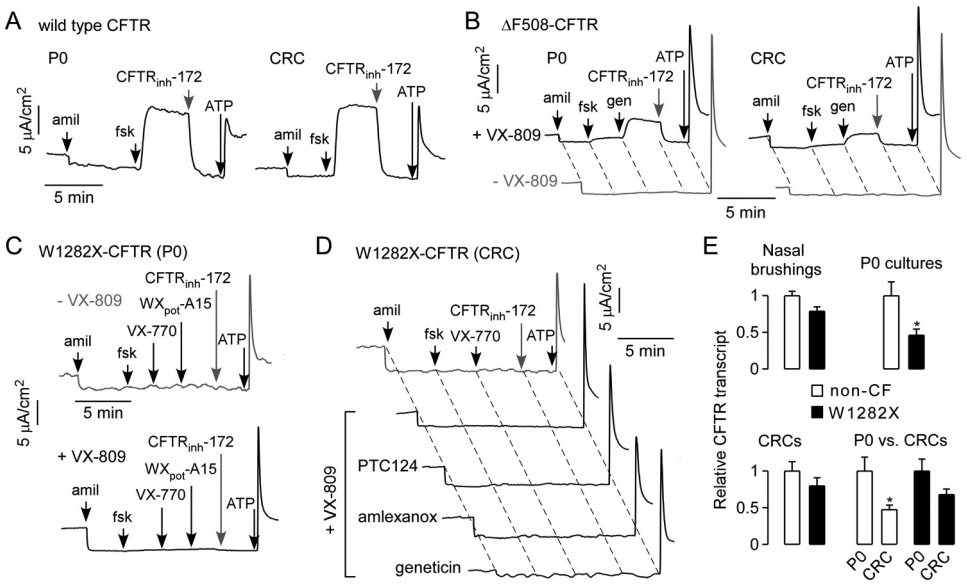


FIGURE 7. **Testing of correctors and potentiators in human nasal epithelial cells from a single homozygous W1282X CF subject.** *A*, short-circuit current in primary cultures (*left*) and CRCs (*right*) from a non-CF subject. *amil,* amiloride. *B*, short-circuit current in primary cultures (*left*) and CRCs (*right*) from a homozygous ΔF508-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. *D*, short-circuit current in W1282X-CFTR CRCs without (*gray trace*) or with (*black traces*) VX-809 correction. In some experiments, cells were treated for 24 h with PTC124 (ataluren; 25 μM), amlexanox (25 μM), or geneticin (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 ± 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 CFTR$_{1281}$ activity when used alone but robust CFTR$_{1281}$ activity in the presence of VX-770 (Fig. 6*E*). CFTR$_{1281}$ activation by apigenin in the presence of VX-770 had an EC$_{50}$ of ~20 μM (Fig. 6*F*), ~4-fold less potent than W1282X$_{pot}$-A15. As found for W1282X$_{pot}$-A15, activation of CFTR$_{1281}$ 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 CFTR$_{1281}$ 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. 6*G*).

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 ΔF508-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. 7*A*). Also, primary and conditionally reprogrammed human bronchial epithelial cells from a homozygous ΔF508-CFTR CF subject showed similar responses following correction with VX-809, with no activity in the absence of corrector (Fig. 7*B*). 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. 7*C*). The amiloride and ATP responses were similar in these cells to those in non-CF and ΔF508-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. 7*D*). VX-809-corrected W1282X-CFTR CRCs treated with the read-through agents PTC124/ataluren (6), amlexanox (30), and geneticin (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. 7*E*). In nasal brushings, CFTR transcript was reduced only by ~20% in CF cells as compared with a non-CF subject (Fig. 7*E*, *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 ~50% less CFTR transcript (Fig. 7*E*, *top right*). CRCs from non-CF and W1282X-CFTR subjects had similar levels of CFTR transcript (Fig. 7*E*, *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 P0 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 ($CFTR_{1281}$) 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 $CFTR_{1281}$, the identification of correctors and potentiators with much greater efficacy than existing compounds, and the discovery of potentiator combinations that restore $CFTR_{1281}$ 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 $CFTR_{1281}$ may require triple drug combination therapy with a corrector and two potentiators acting in synergy. Of note, therapies for ΔF508-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 $CFTR_{1281}$ trafficking with greater efficacy than existing ΔF508-CFTR correctors. Perhaps second and third generation correctors under development for ΔF508-CFTR may be active as $CFTR_{1281}$ correctors; and VX-770 together with newer potentiators with improved activity may act in synergy on $CFTR_{1281}$.

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, c3890_3891insT, c3908dupA, 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 ΔF508-CFTR modulators such as VX-809 and VX-770 on $CFTR_{1281}$ shown here may be of benefit in compound heterozygous CF patients having W1282X along with ΔF508 or various gating mutations.

An important finding of this study is that potentiator combinations can activate $CFTR_{1281}$ 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 $CFTR_{1281}$ in the presence of potentiators. We speculate that binding of both potentiators is sufficient to induce $CFTR_{1281}$ 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 $CFTR_{1281}$ 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 $CFTR_{1281}$ in synergy with VX-770. Curcumin is unlikely to be useful clinically as a $CFTR_{1281}$ 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 ΔF508, mandating the need for drug testing in cells from other W1282X CF patients. It is well recognized that the efficacy of ΔF508-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 $Å^2$, 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

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 $CFTR_{1281}$ 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. 1*A*, *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 Gln^{1281} and tandem stop codons (TGA TAG TAA) for $CFTR_{1281}$ (~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 Asp^{1270} to Phe^{1286}, including the W1282X PTC, and *Photinus pyralis* luciferase. Constructs were fully sequenced. Lentiviral particles expressing W1282X-CFTR and $CFTR_{1281}$ 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 Kaign's modified Ham's F-12 medium supplemented with 10% FBS, 2 mM L-glutamine, 100 units/ml penicillin, 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 $CFTR_{1281}$, 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 $CFTR_{1281}$ 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). $CFTR_{1281}$ maturation efficiency in CFBE cells was measured by metabolic pulse-chase as described (50). Briefly, CFBE cells expressing $CFTR_{1281}$ were pulse-labeled with 0.2 mCi/ml [^{35}S]methionine and [^{35}S]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 $CFTR_{1281}$ 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 μg/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 μg/ml amphotericin B, and 10 μg/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 ~80% confluence. Conditionally reprogrammed cells were subsequently plated (5×10^5 cells/cm^2) on tissue culture inserts coated with human placental collagen (20 μg/cm^2) in DF12 containing 5% FBS and antibiotics (penicillin, 100 units/ml; streptomycin, 100 μg/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. 2*A*, *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. 2*A*, *right*), cells expressing $CFTR_{1281}$ 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 $CFTR_{1281}$ 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 μg/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 °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 °C for 10 min prior to incubation with anti-hemagglutinin antibody (2 μg/ml, 1 h, 4 °C; clone 16B12, Biolegend, San Diego, CA), HRP-conjugated secondary antibody (0.16 μg/ml, 1 h, 4 °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

1. 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., Rohlfs, 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
2. Kervestin, S., and Jacobson, A. (2012) NMD: a multifaceted response to premature translation termination. *Nat. Rev. Mol. Cell Biol.* **13,** 700–712
3. Popp, M. W., and Maquat, L. E. (2013) Organizing principles of mammalian nonsense-mediated mRNA decay. *Annu. Rev. Genet.* **47,** 139–165
4. 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
5. 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
6. 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
7. 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
8. 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 assasy. *Plos Biol.* **11,** e1001593
9. 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
10. Davies, J. C. (2015) The future of CFTR modulating therapies for cystic fibrosis. *Curr. Opin. Pulm. Med.* **21,** 579–584
11. Elborn, J. S. (2016) Cystic fibrosis. *Lancet* **388,** 2519–2531
12. Veit, G., Avramescu, R. G., Chiang, A. N., Houck, S. A., Cai, Z., Peters, K. W., Hong, J. S., Pollard HB, 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 combinotorial pharmacotherapy: expanded classification of cystic fibrosis mutations. *Mol. Biol. Cell* **27,** 424–433
13. Linde, L., Boelz, S., Nissim-Rafinia, M., Oren, Y. S., Wilschanski, M., Yaacov, 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
14. 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
15. 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
16. 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
17. Cui, L., Aleksandrov, L., Chang, X.-B., Hou, Y.-X., He, L., Hegedus, T., Gentzsch, 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
18. Du, K., and Lukacs, G. L. (2009) Cooperative assembly and misfolding of CFTR domains *in vivo*. *Mol. Biol. Cell* **20,** 1903–1915
19. Gentzsch, 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
20. Gentzsch, 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
21. 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
22. 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
23. 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 CFBE41o−. *Cell Tissue Res.* **323,** 405–415
24. Veit, G., Bossard, F., Goepp, J., Verkman, A. S., Galietta, L. J., Hanrahan, J. W., and Lukacs, G. L. (2012) Proinflammatory cytokine secretion is supressed by TMEM16A or CFTR channel activity in human cystic fibrosis bronchial epithelial. *Mol. Biol. Cell* **23,** 4188–4202
25. 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
26. Pedemonte, N., Lukacs, G. L., Du, K., Caci, E., Zegarra-Moran, O., Galietta, 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
27. 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
28. 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
29. 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., Galietta, 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 6
30. 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
31. Okiyoneda, 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 commbination restores ΔF508-CFTR folding and function. *Nat. Chem. Biol.* **9,** 444–454
32. Cutting, G. R. (2015) Cystic fibrosis genetics: from molecular understanding to clinical application. *Nat. Rev. Genet.* **16,** 45–56
33. Quon, B. S., and Rowe, S. M. (2016) New and emerging targeted therapies for cystic fibrosis. *BMJ* **352,** i859
34. Anand, P., Kunnumakkara, A. B., Newman, R. A., and Aggarwal, B. B. (2007) Bioavailability of curcumin: problems and promises. *Mol. Pharm.* **4,** 807–818
35. 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
36. 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
37. 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) ΔF508 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 Galietta, L. J. (2010) Influence of cell background on pharmacological resource 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., Sudom, 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 11β-hydroxysteroid dehydrogenase type 1 (11β-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 ΔF508CFTR 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 ΔF508-CFTR functional expression. *Sci. Transl. Med.* **6,** 246ra97
49. Glozman, 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 ΔF508-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., Galietta, 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