

MOLECULAR PHARMACOLOGY

Supplemental Data

Synergy-based small-molecule screen using a human epithelial cell line ΔF508-CFTR correctors that augment VX-809 maximal efficacy

Puay-Wah Phuan, Guido Veit, Joseph Tan, Ariel Roldan, Walter E. Finkbeiner, Gergely Lukacs, and A.S. Verkman

Supplemental Table 1. Corrector activities in ΔF508-HRP and R1070W-HRP CFBE41o- cells measured with and without VX-809.

Supplemental Table 2. Corrector activities of selected class A, D and H analogs, with and without VX-809, in ΔF508-HRP and R1070W-HRP CFBE41o- cells.

Supplemental Table 3. Functional activities of correctors in A549 cells expressing ΔF508-CFTR and halide-sensitive YFP.

Supplemental Figure 1. Plasma membrane (PM) density of HRP-tagged ΔF508-CFTR.

Supplemental Figure 2. Correctors activities in R1070W and ΔF508 cell lines.

Supplemental Figure 3. Dose-dependent activities D and H analogs.

Supplemental Figure 4. Functional assays in primary cultures of human bronchial epithelial cells from homozygous ΔF508 CF patient.

Supplemental Figure 5. Immunoblot of ΔF508-CFTR.

Supplemental Table 1. Corrector activities in ΔF508-HRP and R1070W-HRP CFBE41o- cells measured with and without VX-809

Compound	ΔF508-HRP		R1070W-HRP		ΔF508-HRP + 2 μM VX-809		R1070W-HRP + 2 μM VX-809	
	EC ₅₀ (μM)	V _{max} [*] (%)	EC ₅₀ (μM)	V _{max} [*] (%)	EC ₅₀ (μM)	V _{max} [*] (%)	EC ₅₀ (μM)	V _{max} [*] (%)
A-01	6.0	23	2.1	25	3.0	148	9.4	123
B-01	8.3	8	10	68	16	148	7.1	138
C-01	16	55	15	49	33	297	12.4	148
D-01	inactive	n.a.	1.2	65	inactive	n.a.	2.9	165
E-01	inactive	n.a.	0.4	17	inactive	n.a.	3.7	102
F-01	inactive	n.a.	33	32	inactive	n.a.	9.8	146
H-01	1.6	17	0.4	25	1.3	334	3.1	139
J-01	0.7	16	inactive	n.a.	0.5	170	inactive	n.a.
K-01	12	21	1.5	25	9.5	169	10	122

* as percentage of V_{max} for VX-809

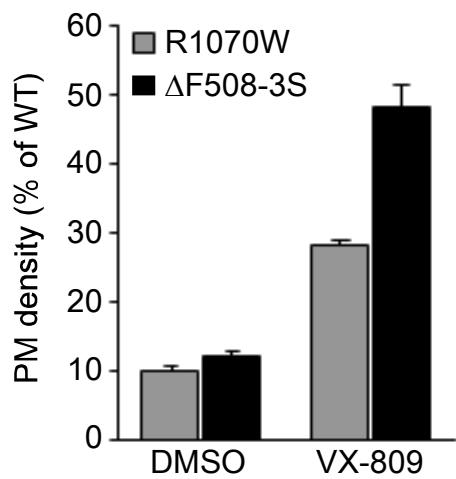
Supplemental Table 2. Corrector activities of selected class A, D and H analogs, with and without VX-809, in ΔF508-HRP and R1070W-HRP CFBE41o- cells

Compound	Structure	EC ₅₀ (μM)		EC ₅₀ (μM) + 2 μM VX-809	
		ΔF508	R1070W	ΔF508	R1070W
H-02		0.9	2.9	3.1	1.8
H-03		1.1	4.2	1.9	1.9
H-04		0.5	3.7	2.4	3.2
H-05		2.2	5.0	4.1	0.2
H-06		1.1	6.1	1.8	0.9
D-02		inactive	0.6	inactive	1.5
D-03		inactive	3.8	inactive	2.4
D-04		inactive	3.4	inactive	0.5
D-05		inactive	11	inactive	10

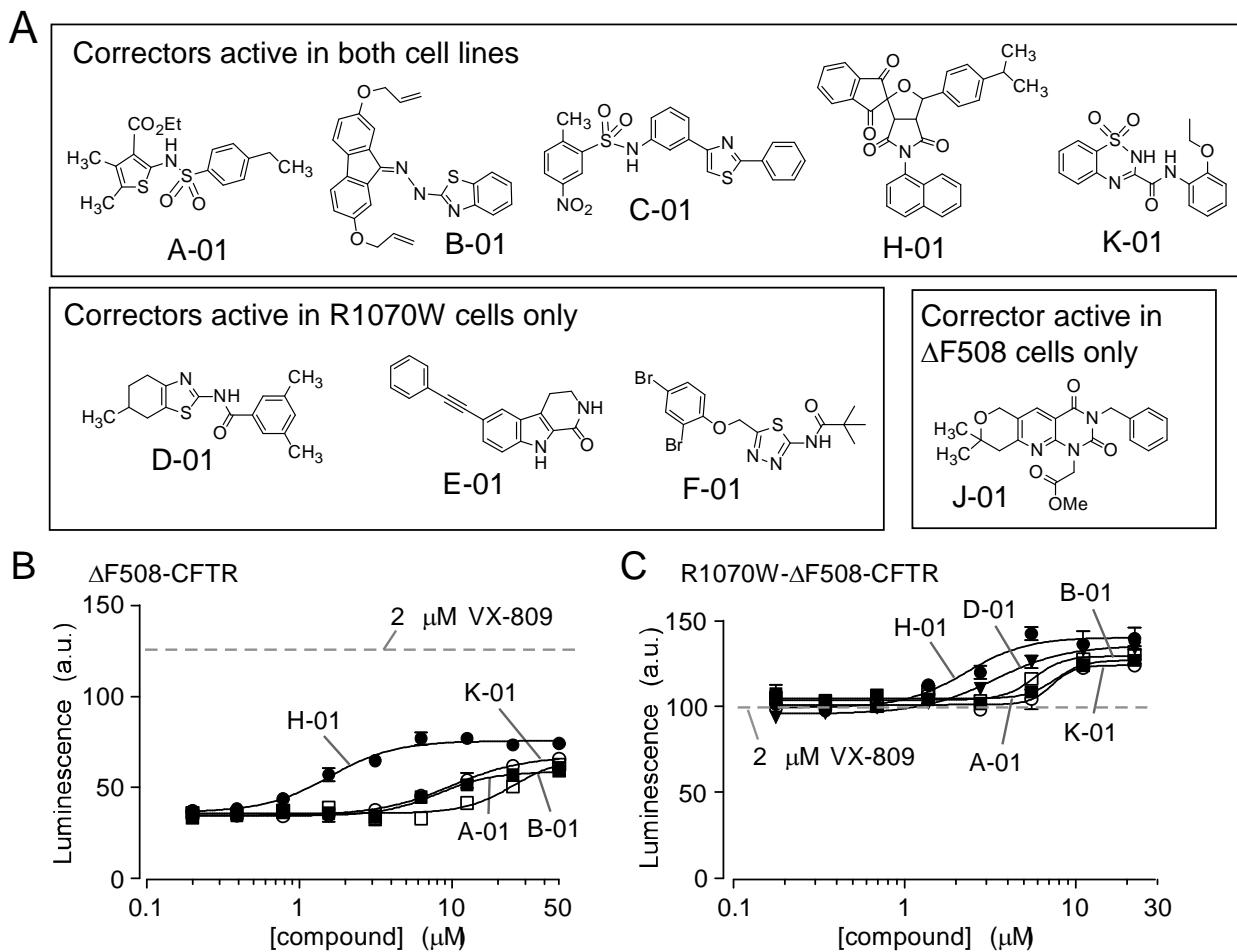
Supplemental Table 3. Functional activities of correctors in A549 cells expressing ΔF508-CFTR and halide-sensitive YFP.

Compound	ΔF508-A549		ΔF508-A549 + 2 μM VX-809	
	EC ₅₀ (μM)	V _{max} (%) [*]	EC ₅₀ (μM)	V _{max} (%) [*]
A-01	3.7	45	0.6	117
B-01	3.1	54	2.4	125
C-01	12.8	41	2.4	122
D-01	2.9	43	0.6	120
E-01	0.1	28	10.4	108
F-01	1.1	25	2.5	114
H-01	0.7	38	0.6	122
J-01	1.7	31	2.3	123
K-01	0.9	32	4.7	118

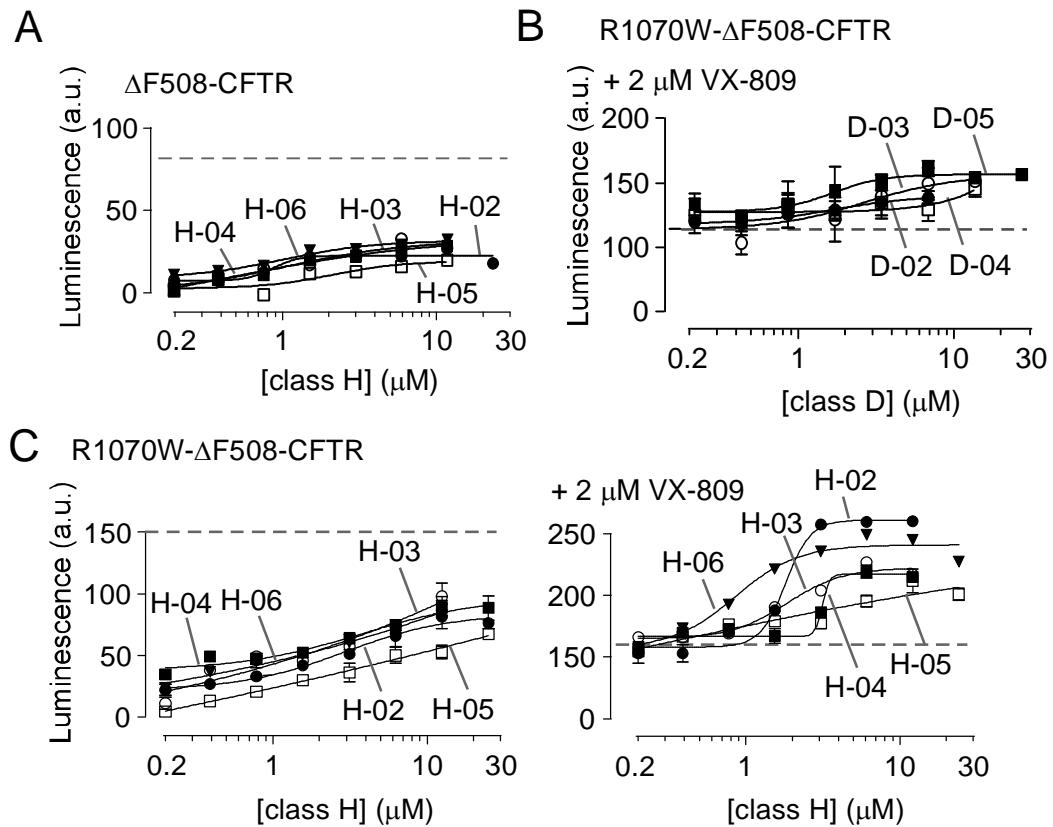
* as percentage of V_{max} for VX-809



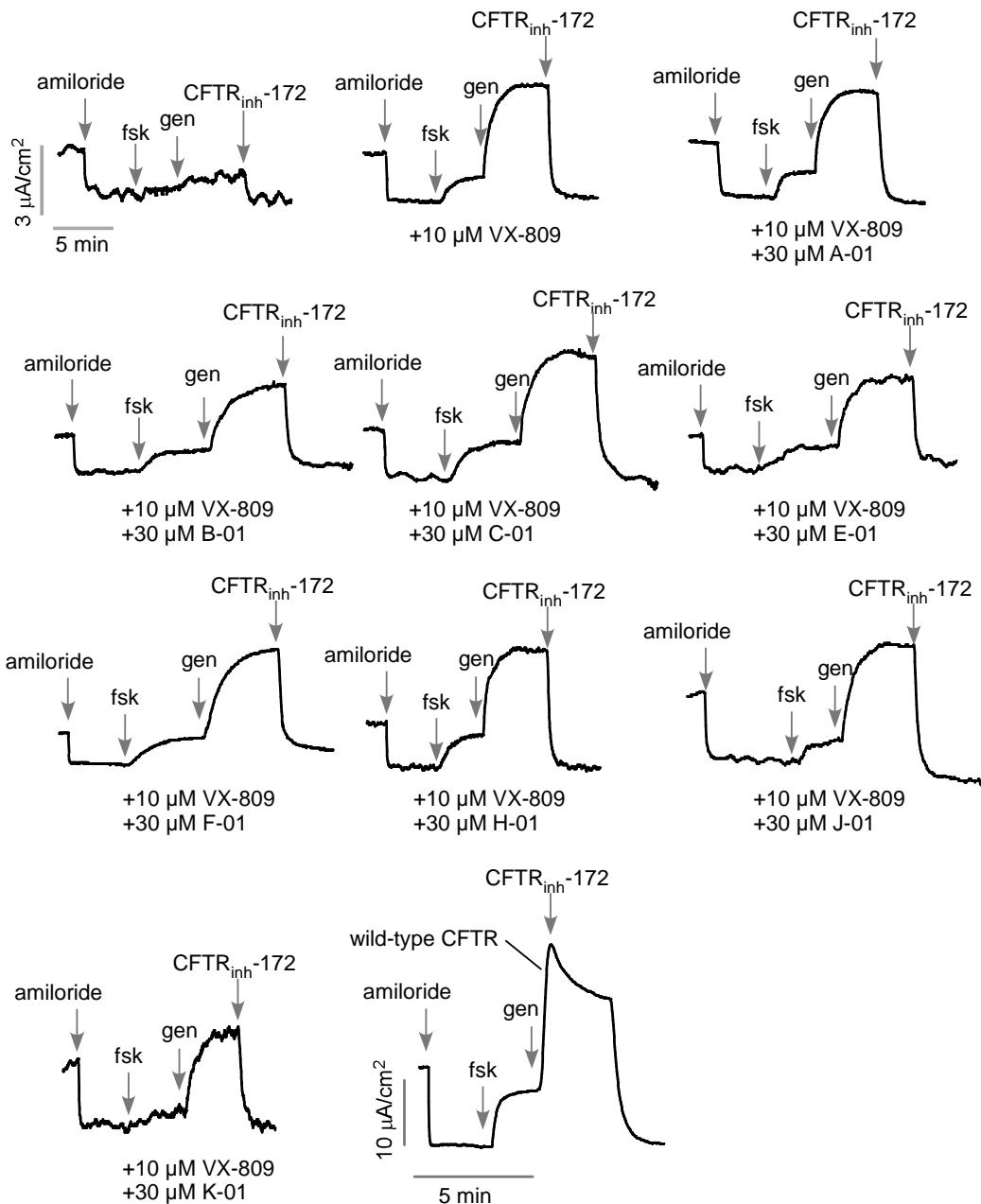
Supplemental Figure 1. Plasma membrane (PM) density of HRP-tagged Δ F508-CFTR with the suppressor mutations 3S or R1070W in presence or absence of VX-809 (3 μ M, 24 h, 37 °C) was measured by luminescence in CFBE41o- cells. Data shown as percent of wild-type CFTR PM density (S.E., n = 3).



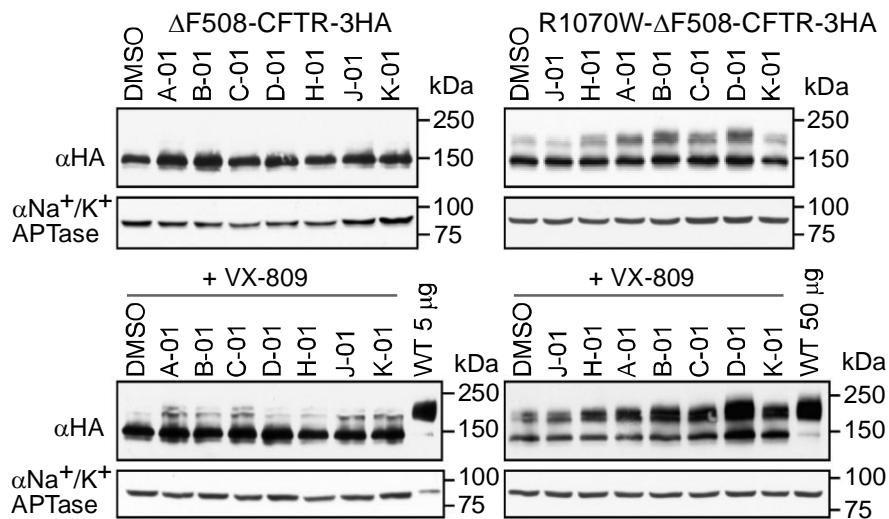
Supplemental Figure 2. Correctors activities in R1070W and $\Delta F508$ cell lines. A. Activities of correctors in cell lines. B. Dose-response data of class A-01, B-01, H-01 and K-01 in $\Delta F508$ -CFTR-HRP CFBE41o- cells (S.E., n = 3). C. Dose-response data of class A-01, B-01, D-01, H-01 and K-01, synergized with 2 μM VX-809, in R1070W- $\Delta F508$ -CFTR-HRP CFBE41o- cells (S.E., n = 3).



Supplemental Figure 3. Dose-dependent activities D and H analogs. A. Dose-response data of class H analogs without 2 μ M VX-809 in Δ F508-CFTR-HRP CFBE41o- cells (S.E., n = 3). B. Dose-response data of class D analogs, with 2 μ M VX-809, in R1070W-DF508-CFTR-HRP CFBE41o- cells (S.E., n = 3). C. Dose-response data of class H analogs, with (left) and without (right) 2 μ M VX809, in R1070W- Δ F508-CFTR-HRP CFBE41o- cells (S.E., n = 3).



Supplemental Figure 4. Functional assays in primary cultures of human bronchial epithelial cells from a homozygous $\Delta F 508$ CF patient. Representative short-circuit current recordings. Cells were incubated at 37 °C for 24 h with DMSO vehicle, 10 μM VX-809, 10 μM VX-809 + 30 μM compounds. Untreated wild-type CFTR from non-CF human bronchial epithelial cells were shown as further control. Concentrations were: amiloride, 10 μM ; forskolin, 20 μM ; genistein, 50 μM ; $\text{CFTR}_{\text{inh}}\text{-172}$, 10 μM .



Supplemental Figure 5. Immunoblot of $\Delta F508$ -CFTR. Effect of the indicated correctors (A-01 - D-01, J-01, K-01 25 μM , H-01 5 μM , 24 h, 37 °C) alone or in combination with 3 μM VX-809 on the expression pattern of $\Delta F508$ -CFTR-3HA and R1070W- $\Delta F508$ -CFTR-3HA in CFBE41o- cells. CFTR was visualized using anti-HA antibody, anti- Na^+/K^+ -ATPase antibody was used as loading control.