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Supplementary Materials for

Potentiator ivacaftor abrogates pharmacological correction of ΔF508 CFTR in cystic fibrosis

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Published 23 July 2014, *Sci. Transl. Med.* **6**, 246ra96 (2014)
DOI: 10.1126/scitranslmed.3008680

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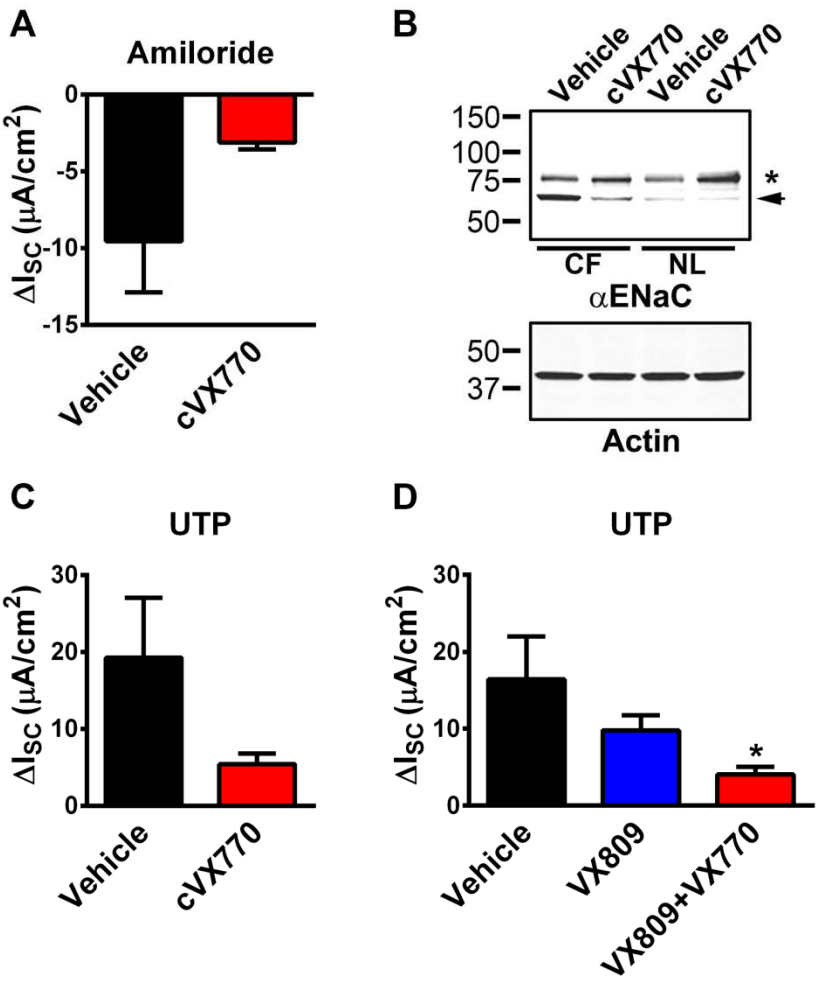

Fig. S1. Chronic VX-770 treatment alters responses to amiloride and UTP in CF HBE cells. (**A**) ΔI_{SC} responses to amiloride in CF HBE cultures (*G551D/ΔF508*) that were either treated with VX-770 (48 hrs, 5 μM) or vehicle (0.1% DMSO). Primary CF HBE cultures (*G551D/ΔF508*) were derived from 3 different patients and 3-4 replicates were performed for a total of 10 measurements per condition. (**B**) α-ENaC expression was not reduced by chronic VX-770 treatment in wild-type (NL) cultures, however cleavage of α-ENaC was diminished when CF cultures (*G551D/ΔF508*) were chronically treated with VX-770 (4 days, 10 μM). Full length α-ENaC is indicated by a star, cleaved α-ENaC fragment by an arrow. (**C**) ΔI_{SC} responses to UTP in CF HBE cultures (*G551D/ΔF508*) that were either treated with VX-770 (48 hrs, 5 μM) or vehicle. Primary CF HBE cultures (*G551D/ΔF508*) were derived from 3 different patients and 3-4 replicates per patient were performed. (**D**) ΔI_{SC} responses to UTP in CF HBE cultures (*ΔF508/ΔF508*) chronically treated with VX-770. ΔI_{SC} after addition of UTP did not change significantly in cells treated with VX-809, but was significantly different in the VX-809+VX-770-treated group when compared to vehicle (**P* = 0.0435). CF HBE cultures (*ΔF508/ΔF508*) were derived from 10 different patients, 2-4 replicates were performed per patient for a total of 32 measurements per condition.

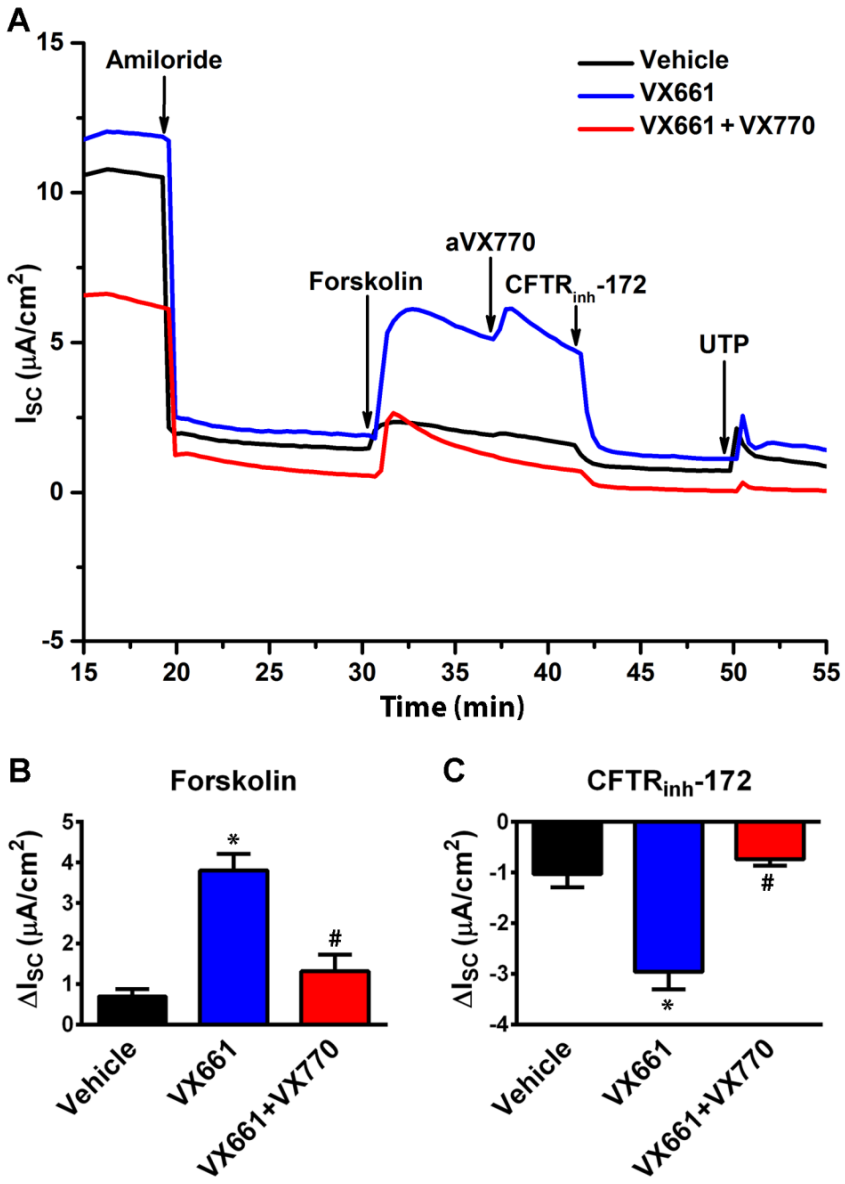

Fig. S2. Chronic VX-770 treatment inhibits functional rescue of ΔF508 by VX-661.
(**A**) Representative I_{SC} traces of CF HBE cells recorded in Ussing chambers in KBR/KBR. Primary CF HBE cells (Δ*F508*Δ*F508*) at passage 2 were treated with vehicle or VX-661 +/-VX-770 for 48 hrs at 5 μM each. (**B**) ΔI_{SC} response to forskolin observed in VX-661-treated CF HBE cells (**P* = 0.0021, VX661 vs. vehicle) was prevented by chronic VX-770 treatment and significantly different from VX-661-treated cells (#*P* = 0.0126, VX661 vs. VX661+VX770). (**C**) The response to $CFTR_{inh}$-172 that was observed in VX-661-treated cells (**P* = 0.0111, VX661 vs. vehicle) was significantly decreased in VX809+VX770 treated cells (#*P* = 0.0038, VX661 vs. VX661+VX770). Primary CF HBE cultures (*ΔF508/ΔF508*) were derived from 3 different patients, and 3-4 replicates were performed per patient.

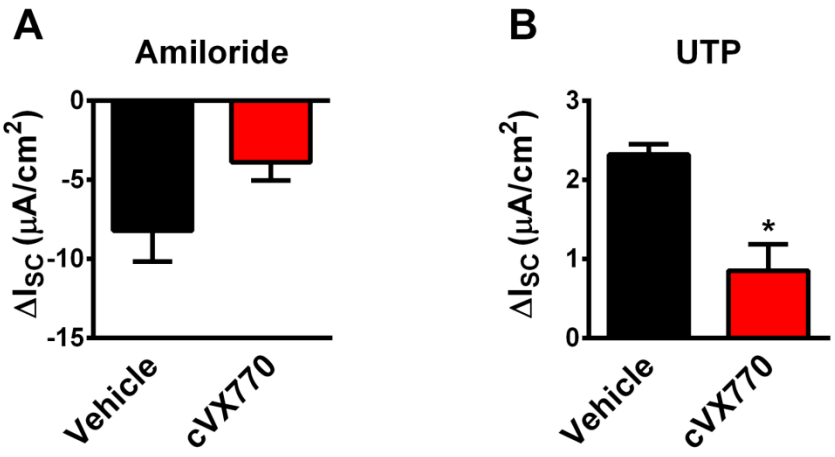

Fig. S3. Chronic VX-770 treatment alters responses to amiloride and UTP in normal HBE cells. ΔI_{SC} responses to (**A**) amiloride and (**B**) UTP of NL HBE cultures that were treated with either VX-770 (48 hrs, 5 μM) or vehicle and measured in KBR/KBR. VX-770-treated cells showed a significantly lowered response to UTP (*P = 0.0034, cultures were derived from 5 individuals, 2-4 replicates were performed per individual for a total of 15 measurements per condition).

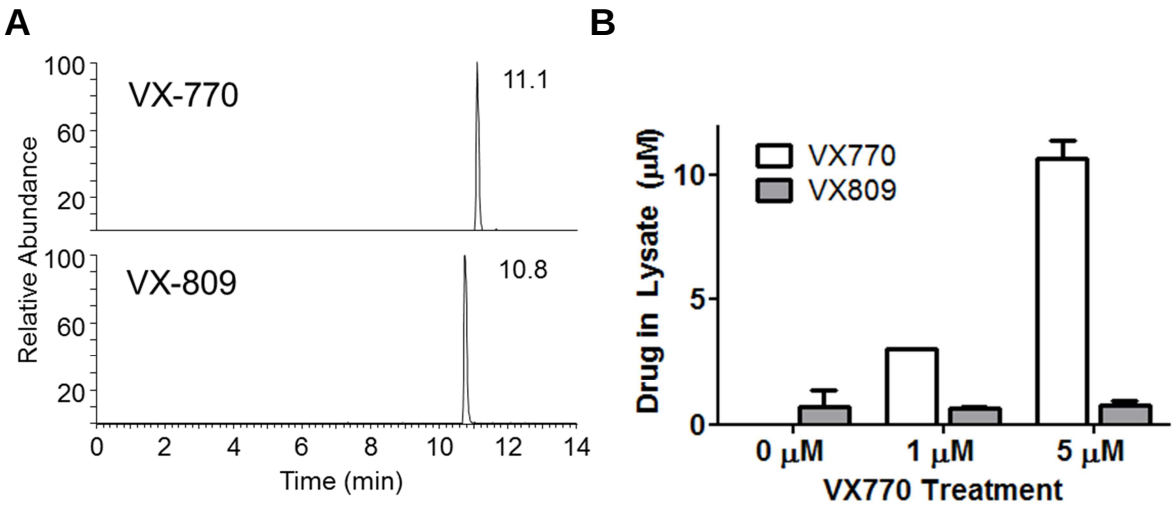

Fig. S4. VX-770 and VX-809 concentrations were measured in treated HBE cells. (A) MS chromatogram of 5 µl injection of 50 nM standard of VX-770 and VX-809. Single peaks for VX-770 and VX-809 were generated at run time of 11.1 and 10.8 minutes, respectively. **(B)** VX-770 and VX-809 concentrations in lysates of epithelial cells treated with 5 µM VX-809 and varying (0 - 5 µM) concentrations of VX-770 (n = 4; reported as median with interquartile range). Treating cells with different concentrations of VX-770 led to an alteration in VX-770 concentrations in the lysate but not VX-809 concentrations. Of note, lysate concentrations were estimated to be ~100-fold dilution of intracellular concentrations, based on an extraction lysis buffer volume of 180 µl and total estimated cell volume 1.7 ± 0.1 mm^3 (average cell height 14.7 ± 0.6 µm x 133 mm^2 surface area), suggesting intracellular accumulation of both compounds.

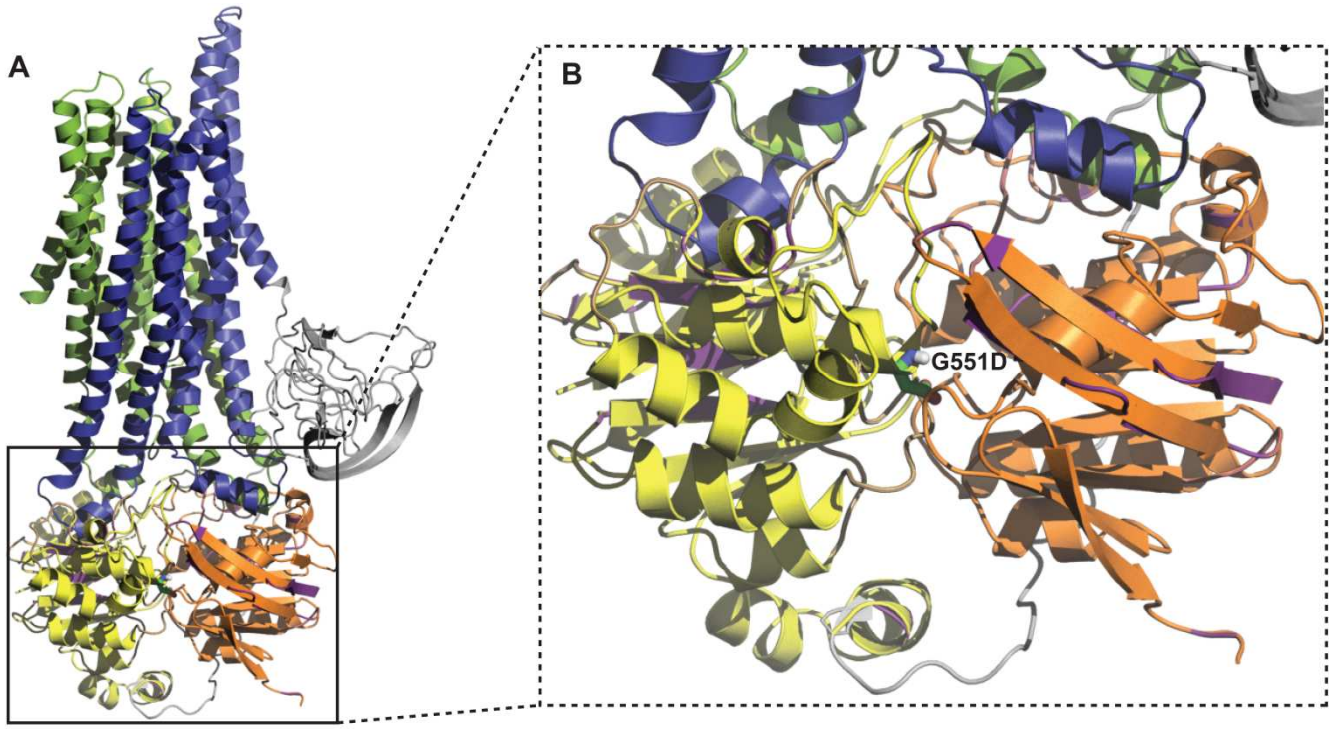

Fig. S5. *G551D* mutation in the NBD1 stabilizes CFTR protein. (**A**) Structure of CFTR-G551D model (transmembrane domain 1 (TMD1): green, TMD2: blue, NBD1: yellow and NBD2: orange and R domain: gray) with G551D labeled in stick representation. (**B**) Side view of CFTR model with G551D in stick representation. Structure of wild-type CFTR (purple) is superimposed onto the G551D CFTR model to demonstrate the conformational restructuring of the protein due to the presence of the *G551D* mutation.

Chronic:	Vehicle	VX770	Vehicle	VX770	Vehicle	VX770	Vehicle	VX770
Acute:	Forskolin	Forskolin	VX770	VX770	FSK + VX770	FSK + VX770	CFTRinh	CFTRinh
1	5.2154	14.5050	7.8530	1.5119	13.0604	15.9695	-8.7486	-7.4889
1	3.5394	16.2328	9.6607	2.5259	13.1489	18.7551	-10.4788	-8.1629
1	2.9258	16.1472	7.9160	1.1501	10.8064	17.2726	-8.8376	-10.3421
2	8.6693	15.8107	18.6967	0.7912	26.4886	15.4216	-26.1971	-15.5979
2	8.3923	15.5734	16.0575	0.4465	22.7547	14.6021	-20.8283	-15.8663
2	6.3684	13.0717	14.6484	0.0636	20.5082	10.9842	-18.1770	-18.8255
2	5.5186	15.5216	4.1835	0.7248	7.1420	14.9155	-9.0535	-18.8828
3	5.5026	10.9562	6.2149	0.5200	11.4971	8.0985	-8.0250	-11.2444
3	7.8969	17.9093	11.9690	-0.1121	18.0951	16.7498	-15.9238	-22.6904
3	6.4012	16.0180	7.3906	-0.0396	13.3867	14.9216	-12.0582	-18.4368
Average	6.0430	15.1746	10.4590	0.7582	15.6888	14.7690	-13.8328	-14.7538
1 (n=3)	3.8935	15.6283	8.4766	1.7293	12.3386	17.3324	-9.3550	-8.6646
2 (n=4)	7.2372	14.9944	13.3965	0.5065	19.2234	13.9808	-18.5640	-17.2931
3 (n=3)	6.6002	14.9612	8.5248	0.1228	14.3263	13.2566	-12.0023	-17.4572
Average	**5.9103**	**15.1946**	**10.1326**	**0.7862**	**15.2961**	**14.8566**	**-13.3071**	**-14.4717**

Table S1. VX-770 treatment restores G551D function. Primary CF HBE cells were derived from 3 CF patients (*G551D/ΔF508*). I_{SC} measurements in Ussing chambers of 3-4 replicates per patient were performed. Measured ΔI_{SC} ($\mu A/cm^2$) responses are shown. Forskolin (FSK)+VX770 is the measured response after addition of forskolin and VX-770.

G551D/ΔF508:

Chronic:	Vehicle	VX770	Vehicle	VX770
Acute:	Amiloride	Amiloride	UTP	UTP
1	-15.8465	-3.2891	27.9142	7.0134
1	-16.7290	-3.8082	37.9285	7.9619
1	-15.5623	-4.6713	33.5722	9.5170
2	-7.0123	-2.1498	23.2329	4.0860
2	-7.8498	-1.2716	16.6716	3.5830
2	-6.9067	-3.4459	25.1579	3.7766
2	-8.7780	-2.6300	8.4241	4.4759
3	-3.6497	-3.2270	6.3092	2.0899
3	-7.1400	-2.6053	5.7430	5.2694
3	-4.2058	-3.3400	6.8246	5.1021
Average	-9.3680	-3.0438	19.1778	5.2875
1 (n=3)	-16.0459	-3.9229	33.1383	8.1641
2 (n=4)	-7.6367	-2.3743	18.3717	3.9804
3 (n=3)	-4.9985	-3.0574	6.2923	4.1538
Average	**-9.5604**	**-3.1182**	**19.2674**	**5.4327**

NL:

Chronic:	Vehicle	VX770	Vehicle	VX770
Acute:	Amiloride	Amiloride	UTP	UTP
1	-10.2705	-5.4480	1.3902	1.0964
1	-10.1593	-4.4759	3.2552	1.2659
1	-13.6707	-4.8531	1.6615	1.5315
1	-13.9830	-6.4850	2.3114	0.8929
2	-5.7626	-3.7167	3.8175	1.3705
2	-7.0605	-3.3814	1.6869	1.2546
2	-8.4639	-1.5259	1.4298	2.3199
2	-7.1434	-1.4637	2.4527	1.9836
3	-1.6468	-0.9506	2.6720	1.0173
3	-1.4242	-1.1065	2.9229	0.8647
4	-9.4981	-3.4832	2.0458	-0.1865
4	-8.2360	-3.4643	3.3682	-0.9720
4	-6.7314	-2.3047	1.2906	0.2861
5	-10.1951	-6.9738	1.6220	0.4691
5	-14.3263	-8.1286	2.5092	0.8873
Average	-8.5714	-3.8508	2.2957	0.9387
1 (n=4)	-12.0209	-5.3155	2.1546	1.1967
2 (n=4)	-7.1076	-2.5219	2.3467	1.7322
3 (n=2)	-1.5355	-1.0286	2.7974	0.9410
4 (n=3)	-8.1551	-3.0841	2.2349	-0.2908
5 (n=2)	-12.2607	-7.5512	2.0656	0.6782
Average	**-8.2160**	**-3.9003**	**2.3199**	**0.8514**

ΔF508/ΔF508:

Chronic:	Vehicle	VX809	VX809 VX770
Acute:	UTP	UTP	UTP
1	6.2297	5.6458	3.3908
1	6.3133	6.1385	3.2627
2	15.8880	18.3124	5.2596
2	22.8945	14.8996	5.5327
2	10.2070	7.1890	4.4632
2	15.9934	9.3199	5.9732
3	3.5225	3.5225	2.0017
3	3.5058	3.5058	2.6043
3	2.3559	2.3559	1.6888
4	5.6585	3.3379	1.0808
4	3.7511	2.7847	0.7502
4	3.4577	2.5916	2.2757
4	2.0616	4.5244	0.8375
5	44.8411	19.1809	15.1090
5	73.2055	23.1510	9.6243
6	4.2951	4.5889	2.1815
6	13.8251	5.0894	2.2888
7	6.1893	4.6094	2.3746
7	13.0152	4.6511	1.9423
7	48.8408	23.6829	2.3425
7	73.8589	15.3224	4.2470
8	4.7048	10.8592	1.3083
8	12.7854	15.7960	3.7243
8	7.5305	21.0515	4.3201
8	4.8602	15.7279	1.2970
9	4.6511	21.0006	5.1936
9	27.4870	17.9799	5.9566
9	10.8526	4.2065	5.2452
9	19.7799	13.9382	6.0696
10	6.7007	6.2071	3.1780
10	10.1340	5.0142	2.2358
10	7.2187	3.2477	3.1667
Average	15.5192	9.9823	3.7790
1 (n=2)	6.2715	5.8921	3.3268
2 (n=4)	16.2457	12.4302	5.3072
3 (n=3)	3.1281	3.1281	2.0983
4 (n=4)	3.7322	3.3096	1.2361
5 (n=2)	59.0233	21.1659	12.3667
6 (n=2)	9.0601	4.8392	2.2351
7 (n=4)	35.4761	12.0665	2.7266
8 (n=4)	7.4702	15.8586	2.6624
9 (n=4)	15.6927	14.2813	5.6163
10 (n=3)	8.0178	4.8230	2.8602
Average	**16.4118**	**9.7795**	**4.0436**

Table S2. Chronic VX-770 treatment alters responses to amiloride and UTP. ΔI_{SC} ($\mu A/cm^2$) responses to amiloride and UTP are shown. CF HBE cultures (*G551D/ΔF508*) were derived from 3 different patients and 3-4 replicates per patient were performed. CF HBE cultures (Δ*F508*/Δ*F508*) were derived from 10 different patients, 2-4 replicates per patient were performed. NL HBE cultures were derived from 5 individuals, 2-4 replicates per individual were performed.

Chronic:			VX809			VX809			VX809
	Vehicle	VX809	VX770	Vehicle	VX809	VX770	Vehicle	VX809	VX770
Acute:	Forskolin	Forskolin	Forskolin	VX770	VX770	VX770	CFTRinh	CFTRinh	CFTRinh
1	2.6576	19.2940	7.4683	0.9862	2.2678	0.4620	-0.0326	-5.3151	-1.2603
1	4.8357	19.4235	8.6749	0.9529	1.8707	0.0127	-1.5895	-6.0209	-2.3001
2	3.4172	10.2629	2.8398	0.6905	1.2019	0.2435	-0.1174	-2.0390	0.0102
2	3.4173	6.9014	3.3598	1.0751	1.3395	0.3546	-0.4620	-1.1232	-0.2968
2	2.1866	6.7374	2.0758	0.8040	2.2796	0.3385	-0.3987	-2.2589	0.0127
2	3.7353	7.1092	2.1203	0.7251	2.3423	0.0229	-0.7429	-2.4379	-0.0127
3	4.6747	16.7739	11.2495	0.0347	1.1564	-0.0123	-4.3706	-4.3706	-1.1931
3	5.1780	25.8563	12.1641	0.4733	1.7394	0.0302	-6.8903	-6.8903	-1.6218
4	2.8716	10.6790	5.6797	0.6896	2.1801	0.2411	-0.1621	-3.8401	-0.0827
4	2.9967	9.0896	3.6028	0.7540	2.0778	0.1444	-0.0477	-1.3351	-0.4896
4	3.3914	11.6970	7.5925	0.5372	2.3860	-0.0887	-0.1529	-3.7357	-0.0109
5	1.7830	13.1932	2.4866	1.2603	5.1089	-0.0254	-1.4496	-8.0674	-0.5086
5	2.1673	7.5220	2.2097	0.6047	3.5378	0.2628	-1.0625	-4.3685	-0.5482
6	4.3770	9.2231	6.2420	1.7378	3.8062	0.8279	-0.0480	-4.2273	-0.1385
6	5.0227	7.5658	5.0481	2.3302	6.4627	1.6435	-0.0191	-4.0245	-0.0064
Average	3.5141	12.0886	5.5209	0.9104	2.6505	0.2972	-1.1697	-4.0036	-0.5631
1 (n=2)	3.7466	19.3588	8.0716	0.9695	2.0692	0.2374	-0.8110	-5.6680	-1.7802
2 (n=4)	4.1264	14.8432	5.7574	0.8237	1.7908	0.2399	-0.4303	-1.9648	-0.0716
3 (n=2)	3.4173	8.5822	3.0998	0.2540	1.4479	0.0089	-5.6304	-5.6304	-1.4074
4 (n=3)	2.8020	6.8194	2.7178	0.6603	2.2146	0.0989	-0.1209	-2.9703	-0.1944
5 (n=2)	2.9610	6.9233	2.0981	0.9325	4.3233	0.1187	-1.2560	-6.2180	-0.5284
6 (n=2)	4.2050	11.9416	6.6849	2.0340	5.1345	1.2357	-0.0336	-4.1259	-0.0724
Average	**3.5430**	**11.4114**	**4.7383**	**0.9456**	**2.8301**	**0.3232**	**-1.3804**	**-4.4296**	**-0.6757**

Table S3. Chronic VX-770 treatment inhibits functional rescue of ΔF508 by VX-809. Measured ΔI_{SC} ($\mu A/cm^2$) responses are shown. Primary CF HBE cells were derived from 6 CF patients (Δ*F508/*Δ*F508*). I_{SC} measurements of 2-4 replicates per patient were performed in Ussing chambers.

Chronic:	Vehicle	VX661	VX661 VX770	Vehicle	VX661	VX661 VX770	Vehicle	VX661	VX661 VX770
Acute:	**Amiloride**	**Amiloride**	**Amiloride**	**Forskolin**	**Forskolin**	**Forskolin**	**CFTRinh**	**CFTRinh**	**CFTRinh**
1	-9.7527	-11.0671	-3.1365	0.4537	4.2555	0.6435	-0.4531	-2.8445	-0.6565
1	-11.4715	-10.4930	-3.4054	0.8251	3.2689	0.6572	-1.2631	-3.2853	-0.6772
1	-10.3188	-11.6645	-4.9008	0.1780	1.9402	0.7036	-0.1801	-0.6729	-0.3741
1	-10.9641			0.0699			-0.2480		
2	-10.0425	-9.8071	-5.8172	0.7121	3.8232	1.8028	-1.7331	-3.1271	-1.1246
2	-9.1256	-9.0441	-6.1713	0.8965	2.9161	2.0684	-0.9282	-3.9202	-0.9947
2	-11.6645	-9.1680	-6.0654	0.6075	3.9355	2.4064	-0.7347	-2.4414	-1.0724
2	-11.4328	-10.0221	-5.6818	0.8534	4.2025	2.0599	-1.2377	-3.8507	-0.7354
3	-8.6975	-6.7944	-2.8681	0.9918	4.9958	1.5768	-1.4750	-3.2590	-0.5180
3	-7.9713	-8.8204	-3.4954	0.7940	4.5550	1.4383	-1.5127	-3.3362	-0.7479
3	-7.1674	-6.3684	-4.1453	0.8901	4.7143	0.9092	-1.4411	-3.4036	-0.7757
3	-7.2543	-8.1465	-3.7660	1.2143	3.9291	0.8774	-1.1698	-3.1069	-0.6273
Average	-9.6553	-9.2178	-4.4957	0.7072	3.8669	1.3767	-1.0314	-3.0225	-0.7549
1 (n=3-4)	-10.6268	-11.0749	-3.8142	0.3817	3.1549	0.6681	-0.5361	-2.2676	-0.5693
2 (n=4)	-10.5664	-9.5103	-5.9339	0.7674	3.7193	2.0844	-1.1584	-3.3349	-0.9818
3 (n=4)	-7.7726	-7.5324	-3.5687	0.9726	4.5486	1.2004	-1.3997	-3.2764	-0.6672
Average	**-9.6553**	**-9.3725**	**-4.4389**	**0.7072**	**3.8076**	**1.3176**	**-1.0314**	**-2.9596**	**-0.7394**

Table S4. Chronic VX-770 treatment inhibits functional rescue of ΔF508 by VX-661. ΔI_{SC} ($\mu A/cm^2$) response to forskolin and $CFTR_{inh}$-172 observed in VX-661-treated CF HBE cells measured in KBR/KBR. Primary CF HBE cells (*ΔF508/ΔF508*) were treated with vehicle or VX-661 +/-VX-770 for 48 hrs at 5 μM each. Primary CF HBE cultures (*ΔF508/ΔF508*) were derived from 3 different patients, 3-4 replicates were performed per patient.

Hours	VX809			VX809/VX770		
	1	2	3	1	2	3
0	108.88	94.50	96.62	112.28	85.98	101.74
3	92.79	82.49	89.27	75.43	69.82	85.23
6	80.61	73.74	86.00	47.36	55.70	71.72
Average:						
Hours	**VX809**			**VX809/VX770**		
0		100.00			100.00	
3		88.18			76.83	
6		80.12			58.26	

Table S5. VX-770 diminishes biochemical correction by increasing turnover of corrected ΔF508 CFTR. Experiments were performed in triplicate in BHK-21 cells. ΔF508 was rescued at 27°C in the presence of VX-809 +/- VX-770 for 24 hours. After adding cycloheximide (200 µg/ml, 37°C) cells were lysed at the indicated times and analyzed by Western blotting. The remaining mature CFTR band (normalized to actin) was quantified at 0, 3 and 6 hrs. The average signals at 0 hrs were defined as 100%.

Chronic:	Vehicle	VX809 (5 μM)	VX809 (5 μM) VX770 (50 nM)	VX809 (5 μM) VX770 (1 μM)	VX809 (5 μM) VX770 (5 μM)
	AUC/min	AUC/min	AUC/min	AUC/min	AUC/min
1	0.9922	6.4051	2.8535		1.7067
1	2.2581	9.2103	3.6011		2.4756
1	2.0762	6.2552	5.1001		2.4378
1	2.0865	6.1039	8.6730		2.7722
2	0.8074	7.2228	5.8794		2.5088
2	0.9744	5.9973	2.3708		3.4026
2	1.2671	7.7788	3.3887		2.0491
2	2.2310	7.5340	3.9408		2.4093
3	0.8619	9.8201	7.0898		4.9013
3	2.2871	2.5139	9.7221		4.2177
3	2.1262	12.2901	9.5334		3.2267
3	1.7391	12.2901	10.2775		4.5151
4	2.9047	12.0974	7.5825	5.9380	4.2577
4	3.3717	14.3373	8.5579	5.7560	3.7862
4	3.6142	11.5248	5.7516	5.4098	4.2487
4			9.9829	5.9591	
4			8.0165	7.4815	
5	2.5572	9.2139			3.6074
5	2.4335	9.2139			4.6079
6	1.5129	10.6359		3.8029	1.2428
6	1.5097	6.2733		5.0300	1.0865
6	1.6367	6.8059		4.2970	1.6391
6	1.0962	6.7837		3.1684	0.8488
7	1.4709	8.4935		10.7604	3.8473
7	1.5437	12.4298		4.8968	2.8789
7	2.3871	8.6484		5.1332	1.7803
8	1.2435	6.4275			1.4587
8	1.2209	4.5872			1.0767
8	1.8020	4.9294			4.5182
8	1.2695	6.7484			1.9652
9	1.1133	6.1621		3.2271	1.9689
9	1.2732	5.7404		5.3095	0.8661
Average	1.7889	8.1491	6.6072	5.4407	2.7436
1 (n=4)	1.8532	6.9936	5.0569		2.3481
2 (n=4)	1.3200	7.1332	3.8949		2.5924
3 (n=4)	1.7536	9.2285	9.1557		4.2152
4 (n=3-5)	3.2969	12.6532	7.9783	6.1089	4.0976
5 (n=2)	2.4954	9.2139			4.1077
6 (n=4)	1.4389	7.6247		4.0745	1.2043
7 (n=3)	1.8005	9.8572		6.9302	2.8355
8 (n=4)	1.3840	5.6731			2.2547
9 (n=2)	1.1933	5.9512		4.2683	1.4175
Average	**1.8373**	**8.2587**	**6.5215**	**5.3455**	**2.7859**

Table S6. VX-770–induced hindrance of ΔF508 correction is dose-dependent. Primary CF HBE cells were derived from at least 4 CF patients (Δ*F508/*Δ*F508*) and 2-5 replicates were performed per patient. The area under curve (AUC) was determined for the time interval from CFTR stimulation by forskolin to inhibition by $CFTR_{inh}$-172. Values are expressed as AUC/minute to yield the average CFTR-mediated ΔI_{SC} ($\mu A/cm^2$).

Chronic:	Vehicle	VX809 (5 μM)	VX809 (5 μM) VX770 (50 nM)
	Slope	Slope	Slope
1	0.0083	-0.1430	0.0387
1	0.1503	0.0628	-0.5160
1	-0.0945	-0.3167	-0.1977
1	-0.0343	-0.3103	-0.2980
2	-0.0308	-0.0585	-0.3928
2	-0.0126	-0.3027	-0.4453
2	-0.1187	-0.0931	-0.5404
2	0.1137	-0.2025	-0.5077
3	-0.0615	-0.0074	-0.9116
3	-0.0869	-0.2923	-0.4515
3	0.0337	-0.6233	-0.6327
3	-0.0199	-0.0383	-0.7600
4	0.0780	0.3073	-0.1196
4	-0.0745	-0.1382	-0.3496
4	0.0356	-0.0380	-0.5064
4			-0.4265
4			-0.3924
Average	-0.0076	-0.1463	-0.4359
1 (n=4)	0.0074	-0.1768	-0.2433
2 (n=4)	-0.0121	-0.1642	-0.4716
3 (n=4)	-0.0336	-0.2403	-0.6889
4 (n=3-5)	0.0130	0.0437	-0.3589
Average	**-0.0063**	**-0.1344**	**-0.4407**

Table S7. VX-770 at low dose (50 nM) affects I_{SC} of VX-809corrected CF HBE cells (Δ*F508*/Δ*F508*). Slope values after peak forskolin response were determined in vehicle, VX-809, and VX-809 plus VX-770 (50 nM)-treated cells. Primary CF HBE cultures were derived from 4 different patients; 3-5 replicates were performed per patient.

Chronic:	Vehicle	VX809 (5 μM)	VX809 (5 μM) VX770 (1 μM)	VX809 (5 μM) VX770 (5 μM)
	C/B Band	C/B Band	C/B Band	C/B Band
1	0.00	0.73	0.29	0.15
2	0.00	0.80	0.49	0.18
3	0.44	1.04	0.45	0.11
4	0.13	1.15	0.74	0.23
Average	**0.14**	**0.93**	**0.49**	**0.17**

Table S8. VX-770 affects C/B band ratio in CF HBE (Δ*F508*/Δ*F508*). CFTR C:B band ratio decreased in CF HBE cells as chronic VX-770 concentrations were increased. Primary CF HBE cultures (Δ*F508*/Δ*F508*) from 4 different patients were analyzed by Western blotting.

Chronic:	Vehicle	VX770	Vehicle	VX770	Vehicle	VX770
Acute:	**FSK Peak**	**FSK Peak**	**FSK PL**	**FSK PL**	**CFTRinh**	**CFTRinh**
1	28.1157	26.6068	13.7762	9.0008	-11.4554	-9.9747
1	28.3135	18.7288	14.3866	5.9321	-16.0302	-6.7902
1	28.3531	20.1303	17.6173	23.0135	-9.6168	-2.8540
1	32.1876	23.3262	17.3438	12.9201	-13.4635	-7.2451
2	48.8225	19.7093	13.8365	7.7914	-12.5913	-10.5625
2	63.9569	15.9878	19.0358	4.9582	-16.3608	-9.3079
2	47.4266	2.7409	14.3489	-1.3356	-15.2720	-9.8429
2	45.5786	5.7927	14.4638	1.8499	-16.9523	-11.3348
3	15.6578	7.4259	10.9784	6.0933	-14.0743	-7.6181
3	15.9607	7.2869	11.2788	6.1111	-13.5487	-7.6441
4	45.0050	25.6856	15.8673	6.3541	-16.7564	-8.1267
4	39.6813	33.8180	17.7115	14.1134	-19.5426	-16.8525
4	32.7789	14.7777	10.4570	0.5500	-14.2585	-6.6906
5	34.9257	17.7379	9.3266	7.2267	-15.0045	-9.0404
5	46.0910	20.1077	22.0972	10.7116	-26.3092	-15.3417
6	50.4030	31.6403	14.6527	3.5095	-15.0177	-7.0134
6	51.9986	32.2394	14.5792	12.3497	-21.0176	-15.2230
Average	36.8570	17.3242	14.8351	7.6860	-15.4158	-9.2817
1 (n=4)	29.2425	22.1980	15.7810	12.7166	-12.6415	-6.7160
2 (n=4)	51.4461	11.0577	15.4213	3.3160	-15.2941	-10.2620
3 (n=2)	15.8092	7.3564	11.1286	6.1022	-13.8115	-7.6311
4 (n=3)	39.1550	24.7604	14.6786	7.0058	-16.8525	-10.5566
5 (n=2)	40.5083	18.9228	15.7119	8.9691	-20.6569	-12.1910
6 (n=2)	51.2008	31.9398	14.6159	7.9296	-18.0176	-11.1182
Average	**37.8937**	**19.3725**	**14.5562**	**7.6732**	**-16.2123**	**-9.7458**

Table S9. Chronic VX-770 treatment decreases the function of wild-type CFTR. Measured ΔI_{SC} ($\mu A/cm^2$) responses are shown. Primary NL HBE cells were derived from 6 individuals. I_{SC} measurements of 2-4 replicates per individual were performed in Ussing chambers. PL = plateau.

Chronic:	Vehicle	VX770
	Rt	Rt
1	323	339
1	351	372
1	305	109
1	278	327
2	302	317
2	300	395
2	223	309
2	276	303
3	685	612
3	571	574
4	341	325
4	333	364
4	361	349
4	350	353
5	442	374
5	430	359
Average	367	361
1 (n=4)	314	287
2 (n=4)	275	331
3 (n=2)	628	593
4 (n=4)	346	348
5 (n=2)	436	367
Average	**400**	**385**

Chronic:	Vehicle	VX770
	Nystatin	Nystatin
1	6.3472	5.0820
1	8.2546	8.9349
1	5.5377	9.4011
2	4.8184	9.1086
2	7.5090	5.3075
2	8.2262	3.5595
2	7.5438	8.8543
3	12.1371	12.6839
3	12.8269	6.9841
4	5.4042	6.3776
4	9.4647	7.4997
5	6.0845	4.9286
5	6.1646	5.7945
Average	7.7168	7.2705
1 (n=3)	6.7132	7.8060
2 (n=4)	7.0243	6.7075
3 (n=2)	12.4820	9.8340
4 (n=2)	7.4344	6.9386
5 (n=2)	6.1245	5.3616
Average	**7.9557**	**7.3295**

Table S10. Transepithelial resistance and nystatin responses were not altered in HBE cultures chronically treated with VX-770. Transepithelial resistance (Rt (Ohm • cm^2)) and nystatin responses (ΔI_{SC} ($\mu A/cm^2$)) of primary HBE cultures were not altered after chronic treatment with VX-770 (48 hrs, 5 μM). Nystatin was added to the apical side in Ussing chambers. Primary HBE cultures were derived from 5 individuals, and 2-4 replicates were performed per individual.

Chronic:	VX770			VX809		
	0 µM	1 µM	5 µM	0 µM	1 µM	5 µM
1	0	3.05	10.59	0.5	0.62	0.94
2	0	3.02	11.59	0.64	0.71	0.62
3	0	2.06	9.44	3.07	0.39	0.48
4			10.75	0.59		0.63
5			10.62	0.77		0.91
6			11.3	0.8		0.92

Table S11. VX-770 and VX-809 concentrations were measured in HBE cell lysates. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was used to determine VX-770 and VX-809 concentrations in lysates of HBE cells treated with 5 µM VX-809 and varying (0-5 µM) concentrations of VX-770 (n=3-6; reported as median with interquartile range).

NL

Chronic:	Vehicle	VX770 (5 µM)
	C/B Band	C/B Band
1	20.27	7.34
2	22.02	9.69
3	15.05	7.17
Average	**19.11**	**8.07**

G551D/ΔF508

Chronic:	Vehicle	VX770 (5 µM)
	C/B Band	C/B Band
1	19.39	21.28
2	18.85	16.39
3	26.76	12.21
Average	**21.67**	**16.63**

Table S12. VX-770 reduces stability of CFTR. C:B band ratio in NL HBE cells and *G551D* CF patients (*G551D/ΔF508*) chronically treated for 48 hrs with vehicle or 5 µM VX-770. Cultures from 3 different NL individuals and three different CF patients were analyzed by immunoprecipitation and Western blotting.