SUPPORTING INFORMATION

In Silico and In Vitro Evaluation of the Mechanism of Action of Three VX809-Based Hybrid Derivatives as Correctors of the F508del CFTR Protein

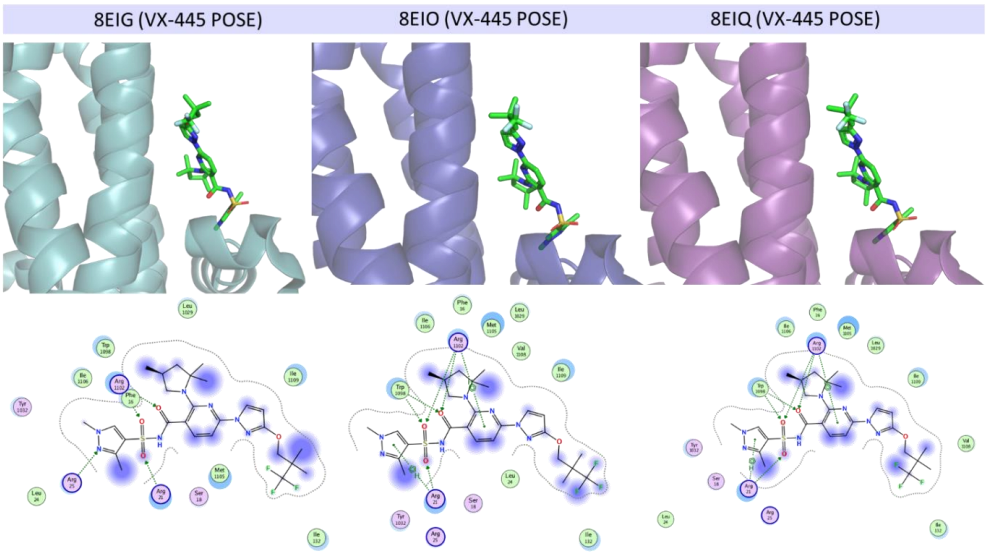

Figure S1. Comparison of the ligplot schemes concerning the 8EIG, 8EIO and 8EIQ experimental data. Polar and hydrophobic residues are colored in pink and green, respectively. The ligand portion to be projected outside the protein surface are highlighted in blue.

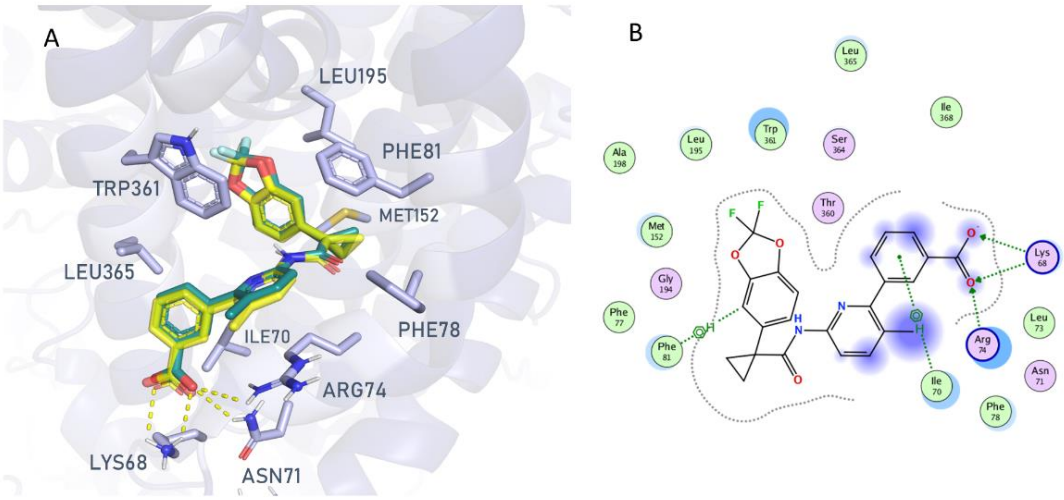

Figure S2. Docking pose of the corrector **VX809** (C atom; green) at the 8EIO PDB code, with respect to the experimental positioning (C atom; yellow) (A) Ligplot as a schematic representation of the main protein-ligand contacts is reported (B). Polar and hydrophobic residues are colored in pink and green, respectively. The ligand portion to be projected outside the protein surface are highlighted in blue.

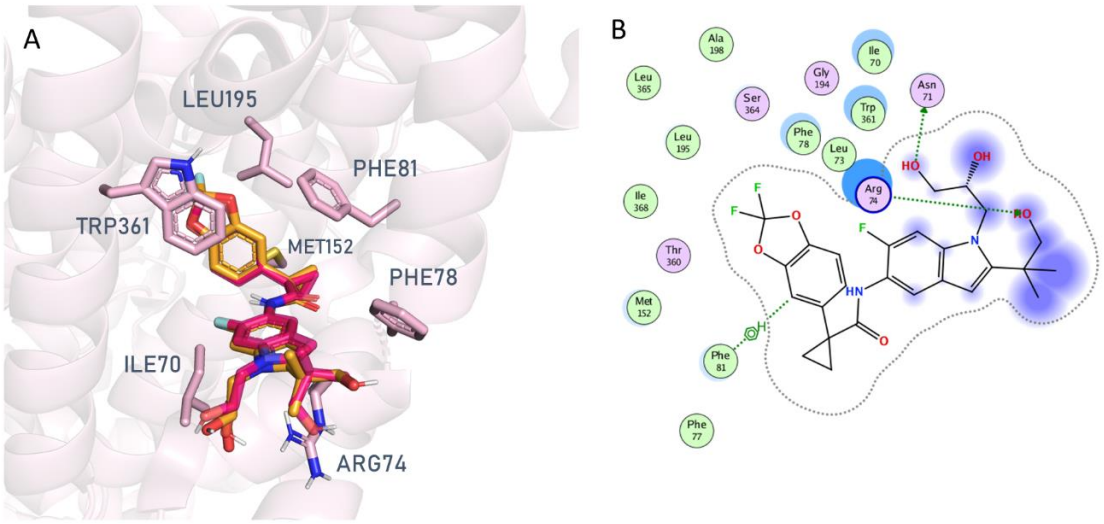

Figure S3. Docking pose of corrector **VX661** (C atom; orange) at the PDB code 8EIQ, with respect to the experimental positioning (C atom; magenta) (A) Ligplot as a schematic representation of the main protein-ligand contacts is reported (B). Polar and hydrophobic residues are coloured in pink and green, respectively. The ligand portion to be projected outside the protein surface is highlighted in blue.

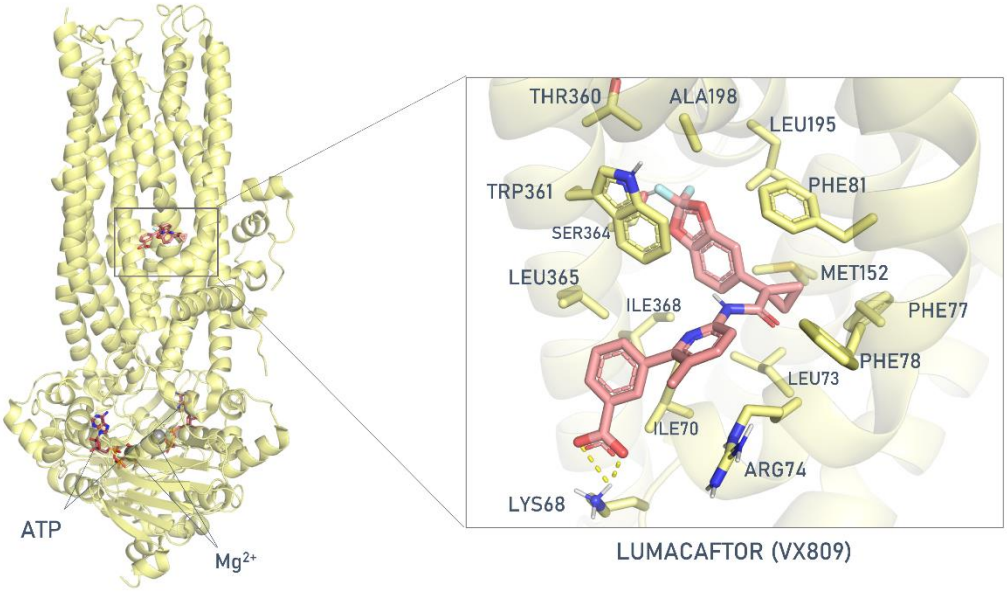

Figure S4. Experimental pose of **VX809** (C atom; pink) at the 7SVD PDB code. The whole protein is depicted (left side) being the corresponding protein-ligand binding mode also shown (right side).

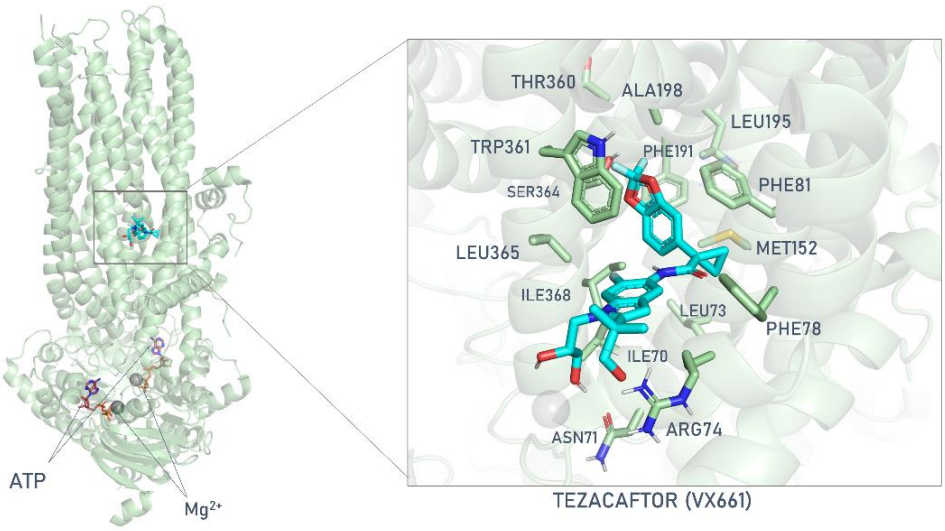

Figure S5. Experimental pose of **VX661** (C atom; cyan) at the 7SV7 PDB code. The whole protein is depicted (left side) being the corresponding protein-ligand binding mode also shown (right side).

Table S1. Ten top scored docking positioning at the PDB code 8EIG of the herein investigated hybrids **2a**, **7a**, **7m** and of the reference CFTR modulators **VX809**, **VX661** and **VX445**. The predicted ΔG value of each protein-ligand complex has been reported, as calculated in terms of final scoring function (S, as Kcal/mol).

Compound	S	E_conf	E_place	E_score1	E_refine	E_score2
2a	-4.4228	56.9634	-18.3158	-9.1392	-15.5229	-4.4228
2a	-4.4099	58.6776	-9.1591	-8.4493	-22.1654	-4.4099
2a	-4.2118	51.1288	-11.6429	-8.4974	-16.7278	-4.2118
2a	-4.1333	50.2729	-25.3931	-8.3503	-18.8067	-4.1333
2a	-4.1037	62.2578	-22.7305	-8.3799	-17.4027	-4.1037
2a	-3.7519	57.0222	-14.8068	-8.7094	-17.8910	-3.7519
2a	-3.7480	60.4186	-10.9114	-8.5670	-15.8403	-3.7480
2a	-3.6337	57.0071	-11.2084	-8.5839	-14.3311	-3.6337
2a	-3.6122	67.5482	-11.7842	-8.3229	-14.6793	-3.6122
2a	-3.5785	57.8832	-18.8391	-8.6674	-14.7912	-3.5785
7a	-4.6130	54.7758	-10.3368	-7.5923	-15.6945	-4.6130
7a	-4.3594	52.4895	-14.9376	-8.2332	-15.0947	-4.3594
7a	-4.2645	56.3198	-19.4090	-8.3653	-17.9217	-4.2645
7a	-4.2626	56.8435	-12.6205	-8.1601	-18.5669	-4.2626
7a	-4.2530	54.0131	-17.4000	-9.0553	-15.2420	-4.2530
7a	-4.2305	51.2142	-11.8824	-7.5602	-14.6812	-4.2305
7a	-4.1679	65.9758	-20.9108	-7.6579	-24.8061	-4.1679
7a	-4.0571	52.7103	-13.9638	-7.4941	-17.4643	-4.0571
7a	-4.0371	56.4814	-15.2284	-8.5400	-18.2004	-4.0371
7a	-3.8821	57.4821	-16.6414	-7.6769	-20.0048	-3.8821
7m	-5.5739	82.5771	-19.3154	-8.5506	-17.8229	-5.5739
7m	-5.5070	78.7220	-17.4841	-9.1314	-20.4701	-5.5070

7m	-5.3241	77.8900	-23.2073	-7.7716	-21.0670	-5.3241
7m	-5.2947	78.3826	-13.3029	-7.7725	-17.6208	-5.2947
7m	-5.1294	90.1531	-14.4151	-8.2315	-26.2099	-5.1294
7m	-5.0461	77.9617	-23.0444	-7.7261	-16.4003	-5.0461
7m	-4.9924	72.2097	-24.1816	-7.8483	-18.7974	-4.9924
7m	-4.9701	71.7269	-22.4046	-8.3210	-21.5056	-4.9701
7m	-4.9392	77.5923	-28.5912	-10.4936	-21.4183	-4.9392
7m	-4.7844	73.6395	-13.6819	-7.8326	-19.9741	-4.7844
VX809	-5.4008	-44.3477	-16.3145	-10.1315	-19.4386	-5.4008
VX809	-4.5365	-45.6606	-18.7366	-10.3997	-21.9572	-4.5365
VX809	-4.4755	-45.5444	-18.4278	-10.8512	-21.6917	-4.4755
VX809	-4.2128	-37.8443	-19.8072	-9.9893	-22.2434	-4.2128
VX809	-4.1660	-46.2295	-16.2937	-9.7170	-16.9632	-4.1660
VX809	-4.1471	-45.9700	-18.3068	-9.8728	-17.5237	-4.1471
VX809	-3.8710	-45.1056	-13.0989	-9.3610	-15.3866	-3.8710
VX809	-3.8554	-44.7563	-16.8875	-10.6660	-14.4862	-3.8554
VX809	-3.8307	-46.3563	-21.9392	-9.4455	-13.2429	-3.8307
VX809	-3.8199	-42.0960	-16.3085	-9.4833	-12.0893	-3.8199
VX445	-6.5860	24.2476	-37.9831	-9.5865	-26.7786	-6.5860
VX445	-6.5409	22.8789	-20.5021	-8.4063	-34.7520	-6.5409
VX445	-6.3788	24.4139	-31.8946	-8.1334	-27.9866	-6.3788
VX445	-6.1390	30.1084	-30.8127	-8.5172	-23.8528	-6.1390
VX445	-6.0727	20.3749	-19.7641	-8.7947	-31.7252	-6.0727
VX445	-5.6625	26.6032	-19.7742	-9.5048	-24.8288	-5.6625
VX445	-5.5202	24.7748	-17.7726	-8.8854	-29.8918	-5.5202
VX445	-5.1298	25.9926	-19.8374	-9.1308	-26.7523	-5.1298
VX445	-4.9616	32.9035	-30.3498	-9.1653	-24.7015	-4.9616
VX445	-4.9341	20.3617	-17.2918	-8.6630	-18.7333	-4.9341
VX661	-5.8536	89.1681	-25.2313	-9.5894	-19.1907	-5.8536
VX661	-4.8274	88.3569	-25.0704	-8.7452	-17.9080	-4.8274
VX661	-4.6785	88.5221	-16.6701	-9.9013	-17.8667	-4.6785
VX661	-4.6338	87.5115	-19.4357	-9.3553	-12.6473	-4.6338
VX661	-4.5808	90.1363	-16.8477	-8.8757	-18.8349	-4.5808
VX661	-4.5602	94.0658	-13.0931	-10.1650	-15.3349	-4.5602
VX661	-4.5362	93.9468	-29.7441	-9.6855	-14.4733	-4.5362
VX661	-4.3610	99.6229	-22.1399	-9.4511	-9.2065	-4.3610
VX661	-4.3421	88.8984	-14.4665	-9.0677	-13.8675	-4.3421
VX661	-4.3036	87.4641	-17.5855	-8.7127	-13.8601	-4.3036
VX770	-4.3525	67.6797	-16.9569	-8.5330	-16.8782	-4.3525
VX770	-4.3219	67.2216	-17.0488	-8.6016	-14.2117	-4.3219
VX770	-4.2726	79.7864	-20.7953	-8.9535	-13.2258	-4.2726
VX770	-4.1813	67.3965	-24.6563	-8.7471	-17.5856	-4.1813
VX770	-4.1799	69.7318	-21.6881	-8.5389	-18.2055	-4.1799
VX770	-4.1006	70.8510	-21.0288	-8.8552	-16.7869	-4.1006
VX770	-4.0835	69.3895	-23.4633	-8.9818	-15.5897	-4.0835
VX770	-4.0317	69.4344	-16.6557	-8.6268	-15.7996	-4.0317

VX770	-3.9886	70.6607	-19.9442	-9.0446	-16.0518	-3.9886
VX770	-3.5944	68.6958	-14.3132	-10.4784	-12.4640	-3.5944

Table S2. Ten top scored docking positioning at the PDB code 8EIO of the herein investigated hybrids **2a**, **7a**, **7m** and of the reference CFTR modulators **VX809**, **VX661** and **VX445**. The predicted ΔG value of each protein-ligand complex has been reported, as calculated in terms of final scoring function (S, as Kcal/mol).

Compound	S	E_conf	E_place	E_score1	E_refine	E_score2
2a	-7.8760	116.0195	-23.5390	-7.7545	-35.7797	-7.8760
2a	-7.6524	112.8703	-30.9584	-7.5365	-45.9355	-7.6524
2a	-7.4779	110.3908	-32.1541	-8.0422	-39.3087	-7.4779
2a	-7.2521	113.1609	-31.9264	-8.0629	-34.7592	-7.2521
2a	-7.0345	115.1808	-16.2659	-7.5219	-28.0563	-7.0345
2a	-6.8592	109.4064	-40.8402	-9.3119	-44.8716	-6.8592
2a	-6.8227	133.6937	-32.7090	-8.1424	-17.2157	-6.8227
2a	-6.7879	117.7266	-15.2858	-7.5962	-23.2209	-6.7879
2a	-6.7514	135.2815	-33.9475	-9.1055	-16.0762	-6.7514
2a	-6.6382	133.0922	-25.4548	-8.6742	-20.3167	-6.6382
7a	-8.5604	106.8645	-36.0003	-9.4109	-44.9491	-8.5604
7a	-8.3779	108.8403	-29.9209	-7.7165	-46.9429	-8.3779
7a	-8.1756	121.9859	-27.2932	-7.8331	-6.8057	-8.1756
7a	-8.1714	108.2967	-21.0676	-7.2808	-49.7397	-8.1714
7a	-8.0876	105.4895	-16.4576	-7.2870	-46.7500	-8.0876
7a	-8.0775	107.9158	-23.6461	-8.3844	-48.0181	-8.0775
7a	-8.0243	108.0966	-20.5120	-7.4902	-30.5772	-8.0243
7a	-7.9423	111.4310	-36.4321	-7.8171	-42.0793	-7.9423
7a	-7.6852	130.5894	-27.2734	-7.4069	-10.2409	-7.6852
7a	-7.3963	110.1238	-27.9648	-7.3559	-31.1471	-7.3963
7m	-10.3685	122.2545	-16.0101	-7.6162	-39.7925	-10.3685
7m	-10.2723	116.8313	-18.1172	-8.9993	-37.2291	-10.2723
7m	-9.7114	121.0419	-19.3945	-8.4804	-38.2188	-9.7114
7m	-9.5662	117.8600	-28.0876	-7.6774	-42.9933	-9.5662
7m	-9.3946	123.1918	-21.0130	-9.3477	-22.5081	-9.3946
7m	-8.8715	120.6809	-33.0147	-8.6818	-48.4946	-8.8715
7m	-8.6043	117.8894	-21.0437	-8.7636	-31.7588	-8.6043
7m	-8.3714	120.1070	-21.5628	-7.9919	-35.7030	-8.3714
7m	-8.1470	119.9670	-16.3607	-8.2047	-41.6722	-8.1470
7m	-8.0678	116.2293	-23.4112	-7.9267	-49.6466	-8.0678
VX809	-8.7534	0.5060	-31.5240	-11.3629	-59.9973	-8.7534
VX809	-8.6601	4.0513	-25.4632	-9.2755	-41.7578	-8.6601
VX809	-8.5667	10.5073	-27.5585	-9.2885	-50.9956	-8.5667
VX809	-8.4386	7.0309	-27.4009	-10.1334	-46.6416	-8.4386
VX809	-8.2508	9.4274	-27.4480	-9.3830	-30.5361	-8.2508
VX809	-8.0274	2.8064	-31.0662	-12.9882	-49.5065	-8.0274
VX809	-7.9213	3.5523	-32.9489	-10.8953	-48.5747	-7.9213

VX809	-7.6858	3.8581	-48.0437	-11.1632	-44.3771	-7.6858
VX809	-7.6615	7.3910	-31.7413	-11.8847	-37.2209	-7.6615
VX809	-7.5336	4.8728	-34.3204	-10.2162	-38.0646	-7.5336
VX445	-8.8879	-11.4383	-22.1244	-7.4005	-37.4239	-8.8879
VX445	-8.6380	0.0770	-19.2888	-7.9345	-13.6260	-8.6380
VX445	-8.2631	8.3296	-15.3546	-7.0670	-29.4215	-8.2631
VX445	-8.0541	-10.6323	-27.2839	-8.1029	-37.0600	-8.0541
VX445	-8.0460	14.5755	-30.9905	-8.1649	-19.9075	-8.0460
VX445	-8.0439	-0.7737	-26.1166	-7.7290	-37.7692	-8.0439
VX445	-8.0269	-2.5834	-26.1817	-7.3417	-15.0698	-8.0269
VX445	-7.9643	14.4722	-17.3904	-7.6905	-14.5522	-7.9643
VX445	-7.4496	36.0684	-20.7688	-7.3530	-26.0562	-7.4496
VX445	-7.3970	-10.1096	-27.4240	-8.6182	-34.4424	-7.3970
VX661	-9.7202	146.6608	-27.9352	-9.3063	-45.3188	-9.7202
VX661	-9.2703	147.3472	-41.3997	-9.8668	-45.8342	-9.2703
VX661	-8.9756	148.8089	-28.1827	-9.2730	-38.5385	-8.9756
VX661	-8.9596	145.6962	-27.9636	-8.9126	-43.4190	-8.9596
VX661	-8.8838	150.9577	-39.9014	-9.6556	-48.2573	-8.8838
VX661	-8.6724	152.3157	-34.5883	-9.1694	-43.5967	-8.6724
VX661	-8.5945	156.8173	-33.7225	-9.5907	-46.9538	-8.5945
VX661	-8.2392	150.3769	-27.9199	-9.1352	-38.6396	-8.2392
VX661	-8.0958	151.7892	-30.4774	-8.8291	-37.3454	-8.0958
VX661	-7.9088	150.6406	-26.6473	-8.5276	-35.8240	-7.9088
VX770	-7.8347	-33.9518	-17.3765	-8.0524	-3.3834	-7.8347
VX770	-7.0203	-34.2862	-21.8797	-8.1031	-5.9308	-7.0203
VX770	-6.0445	-47.5928	-21.2219	-7.9032	-17.9329	-6.0445
VX770	-5.8875	-48.1808	-21.8978	-9.7227	-19.8896	-5.8875
VX770	-5.7702	-50.7476	-30.4604	-8.0315	-18.0749	-5.7702
VX770	-5.3705	-62.9103	-18.8723	-9.0320	-21.2336	-5.3705
VX770	-5.3632	-64.4498	-17.8983	-8.3774	-19.6438	-5.3632
VX770	-5.2586	-64.6745	-15.4652	-8.2368	-17.4958	-5.2586
VX770	-5.2044	7.8535	-20.2484	-7.8782	25.8940	-5.2044
VX770	-5.1928	-60.1908	-20.1268	-8.4886	-18.3459	-5.1928

Table S3. Ten top scored docking positioning at the PDB code 8EIQ of the herein investigated hybrids **2a**, **7a**, **7m** and of the reference CFTR modulators **VX809**, **VX661** and **VX445**. The predicted ΔG value of each protein-ligand complex has been reported, as calculated in terms of final scoring function (S, as Kcal/mol).

Compound	S	E_conf	E_place	E_score1	E_refine	E_score2
2a	-8.3534	111.5993	-16.3119	-7.5907	-41.5406	-8.3534
2a	-8.2510	111.5280	-23.0820	-8.4811	-44.2405	-8.2510
2a	-8.2464	116.2800	-23.4254	-7.7754	-24.6866	-8.2464
2a	-8.0835	130.9052	-29.5426	-8.4959	7.4078	-8.0835
2a	-8.0270	114.0715	-34.0841	-8.1648	-44.2953	-8.0270
2a	-7.9737	123.2587	-28.1440	-8.2535	-23.4828	-7.9737
2a	-7.8299	109.9773	-29.4982	-7.9108	-39.1331	-7.8299

2a	-7.7772	112.0942	-21.5021	-7.4838	-30.2245	-7.7772
2a	-7.6574	111.6609	-34.8657	-7.5754	-45.2768	-7.6574
2a	-7.5278	109.2766	-29.3082	-8.0900	-41.5135	-7.5278
7a	-8.5864	111.6986	-19.6166	-8.4301	-38.3909	-8.5864
7a	-8.5337	117.9189	-24.3834	-7.9638	-12.3044	-8.5337
7a	-8.4784	108.7375	-22.2785	-8.9836	-34.5096	-8.4784
7a	-8.3238	110.5111	-20.6715	-9.0377	-32.9410	-8.3238
7a	-8.2339	108.4298	-30.4429	-8.6403	-41.1974	-8.2339
7a	-8.2304	107.4838	-30.5902	-8.4808	-48.6479	-8.2304
7a	-8.2076	106.3604	-33.8590	-8.0271	-44.9589	-8.2076
7a	-8.1941	106.1082	-17.3324	-8.1055	-32.2960	-8.1941
7a	-7.9427	114.3804	-24.8996	-8.3510	-22.4051	-7.9427
7a	-7.8847	104.8456	-32.1567	-8.4561	-44.4188	-7.8847
7m	-8.8892	114.9206	-22.2109	-8.2412	-37.0986	-8.8892
7m	-8.7612	118.6234	-25.7998	-9.2205	-48.4386	-8.7612
7m	-8.6524	115.9813	-20.9816	-7.9018	-44.3264	-8.6524
7m	-8.5723	126.6447	-31.3310	-9.4196	-2.9413	-8.5723
7m	-8.5250	119.3560	-30.7696	-9.5449	-45.7629	-8.5250
7m	-8.4863	115.9541	-30.6398	-9.5423	-47.6476	-8.4863
7m	-8.4188	118.3709	-31.1672	-7.8734	-45.2175	-8.4188
7m	-8.2799	118.8927	-35.1535	-8.5589	-39.5497	-8.2799
7m	-7.9816	120.7942	-28.5167	-7.9869	-41.9371	-7.9816
7m	-7.9016	117.7015	-30.4226	-7.8978	-44.8099	-7.9016
VX809	-9.0052	2.2213	-22.5744	-9.1115	-42.8242	-9.0052
VX809	-8.6360	1.0109	-21.6897	-9.2643	-47.0053	-8.6360
VX809	-8.1825	3.2027	-26.6370	-10.4513	-39.0939	-8.1825
VX809	-8.1520	0.4786	-30.8486	-9.6245	-43.6163	-8.1520
VX809	-8.1012	2.3734	-30.9284	-8.4550	-39.7959	-8.1012
VX809	-8.0947	1.9820	-33.7781	-8.0779	-41.0626	-8.0947
VX809	-7.7879	3.2906	-26.0511	-9.4302	-39.7564	-7.7879
VX809	-7.6487	8.6508	-20.1510	-8.0931	-26.9189	-7.6487
VX809	-7.6227	2.6937	-29.6413	-8.3318	-41.8047	-7.6227
VX809	-7.3294	1.2867	-32.2579	-10.4705	-30.3578	-7.3294
VX445	-9.5207	0.0342	-32.3420	-9.0708	-33.0028	-9.5207
VX445	-8.9537	-4.1180	-13.9611	-9.2306	-32.9748	-8.9537
VX445	-8.3979	-1.2488	-24.5129	-9.4438	-32.1247	-8.3979
VX445	-8.0046	-5.2436	-33.4995	-9.8872	-36.5432	-8.0046
VX445	-7.9867	-11.3411	-29.3282	-9.3418	-28.5844	-7.9867
VX445	-7.9020	-6.4645	-25.1217	-9.6491	-34.7645	-7.9020
VX445	-7.8158	-13.0721	-28.5840	-9.2695	-40.7140	-7.8158
VX445	-7.7172	-10.0348	-30.3656	-10.0866	-29.3394	-7.7172
VX445	-7.4673	-15.5061	-25.7903	-8.7002	-34.3133	-7.4673
VX445	-7.4092	-15.0419	-26.6748	-8.7892	-35.2276	-7.4092
VX661	-9.9823	150.1244	-40.2205	-10.9117	-45.3552	-9.9823
VX661	-9.7939	146.0527	-38.7318	-8.3753	-43.7035	-9.7939
VX661	-8.8051	147.8234	-44.9402	-9.7806	-42.0440	-8.8051

VX661	-8.7077	151.3457	-43.8284	-12.2488	-43.6721	-8.7077
VX661	-8.4495	148.1792	-47.2124	-11.3354	-43.9642	-8.4495
VX661	-8.4122	151.4115	-26.0129	-8.3495	-34.9538	-8.4122
VX661	-8.3407	144.5504	-33.5227	-9.3875	-41.6859	-8.3407
VX661	-8.1902	147.4272	-45.3528	-8.4205	-43.0460	-8.1902
VX661	-8.1781	153.5230	-31.7654	-8.2606	-41.7077	-8.1781
VX661	-8.0712	149.3826	-42.7571	-8.8094	-41.3579	-8.0712
VX770	-7.5505	-46.9035	-22.9151	-8.0302	-20.9402	-7.5505
VX770	-7.1309	-51.1266	-24.9403	-8.7437	-18.5165	-7.1309
VX770	-6.5369	-49.9515	-19.3523	-9.5596	-2.6425	-6.5369
VX770	-6.2985	-63.0451	-21.3399	-7.8532	-27.7993	-6.2985
VX770	-6.2882	-49.1486	-24.8200	-8.8010	-0.1246	-6.2882
VX770	-6.2698	-63.6828	-22.9188	-8.1095	-19.7896	-6.2698
VX770	-5.6648	-64.4831	-25.3537	-7.9872	-27.3219	-5.6648
VX770	-5.6163	-66.9415	-21.9140	-8.5708	-24.6298	-5.6163
VX770	-5.6062	-63.0726	-27.4657	-8.5783	-23.4933	-5.6062
VX770	-5.3798	-65.7533	-32.7362	-8.1598	-27.1794	-5.3798

Table S4. Evaluation of WT and F508del CFTR protein expression in HEK-t cells whole-cell lysates after treatment with 3 μM VX661, 3 μM VX445 and 2 μM **2a**, 2μM **7m**, 2 μM **7a**. Quantification either of total protein (C + B bands) and maturation rate (C/(C+B) bands) was obtained analyzing the band intensity with the software imageJ. Retrieved values were normalized to the intensity of the protein actin used as housekeeper protein and to the expression value of untreated F508del protein. Data represent the mean ± sem (standard error of the mean). For each condition, samples were run at least in quadruplicate. Comparisons of values with those of control, untreated samples were made with the Dunnett's test. Probability p values are reported. Asterisks indicate a significant difference ($p < 0.05$).

WT CFTR	**DMSO (Control)**	**VX661**	**VX445**	**2a**	**7m**	**7a**
C+B	1.00 ± 0.05	0.97 ± 0.09 P = 0.238	0.94± 0.05 P = 0.314	1.01± 0.04 P = 0.244	1.01± 0.07 P = 0.741	0.98 ± 0.07 P = 0.463
C/(C +B)	1.00 ± 0.01	0.99 ± 0.03 P = 0.298	1.02± 0.03 P = 0.237	0.98 ± 0.11 P = 0.453	1.02 ± 0.13 P = 0.747	1.01 ± 0.12 P = 0.224
F508del CFTR	**DMSO (Control)**	**VX661**	**VX445**	**2a**	**7m**	**7a**
C+B	1.01± 0.07	2.61 ± 0.27 P = 0.004 (*)	3.11± 0.37 P = 0.024 (*)	2.09 ± 0.25 P = 0.016 (*)	2.37 ± 0.19 P = 0.017 (*)	2.48± 0.16 P = 0.014 (*)
C/(C +B)	1.00 ± 0.11	2.10 ± 0.08 P = 0.014 (*)	2.50± 0.11 P = 0.005 (*)	1.42 ± 0.14 P = 0.011 (*)	1.68 ± 0.13 P = 0.016(*)	1.87 ± 0.08 P = 0.007 (*)

Table S5. Evaluation of CFTR single domain expression in HEK-t cells whole-cell lysates after treatment with 3 μM VX661, 3 μM VX445 and 2 μM **2a**, 2μM **7m**, 2 μM **7a**. Quantification protein expression was obtained analyzing the band intensity with the software imageJ. Retrieved values were normalized to the intensity of the protein actin used as housekeeper protein and to the expression value of each untreated single domain. Data represent the mean ± sem (standard error of the mean). For each condition, samples were run at least in quadruplicate. Comparisons of values with those of control, untreated samples were made with the Dunnett's test. Probability p values are reported. Asterisks indicate a significant difference ($p < 0.05$).

CFTR domain	DMSO (Control)	VX661	VX445	2a	7m	7a
MSD1	1.00 ± 0.05	2.19 ± 0.17 P = 0.012(*)	1.03 ± 0.07 P = 0.508	1.04 ± 0.05 P = 0.69	1.04 ± 0.12 P = 0.42	1.06± 0.08 P = 0.32
WT NBD1	1.00 ± 0.12	1.00± 0.15 P = 0.235	1.02 ± 0.18 P = 0.443	1.03 ± 0.09 P = 0.629	0.99 ± 0.04 P = 0.354	0.98 ± 0.08 P = 0.472
F508del NBD1	1.00 ± 0.04	1.02 ± 0.05 P = 0.507	1.01± 0.13 P = 0.464	2.06 ± 0.24 P = 0.013 (*)	2.19 ± 0.15 P = 0.004 (*)	2.32 ± 0.32 P = 0.035 (*)
R domain	1.02 ± 0.08	1.06± 0.09 P = 0.238	1.01 ± 0.04 P = 0.437	1.05 ± 0.08 P = 0.345	1.00 ± 0.05 P = 0.352	1.02 ± 0.05 P = 0.412
NBD2	1.01 ± 0.03	0.99 ± 0.04 P = 0.243	0.97 ± 0.11 P = 0.296	1.01 ± 0.05 P = 0.472	1.03 ± 0.11 P = 0.296	1.04 ± 0.15 P = 0.348
MSD2	1.01 ± 0.06	1.03 ± 0.05 P = 0.116	2.51 ± 0.15 P = 0.002 (*)	0.98 ± 0.13 P = 0.506	0.98± 0.06 P = 0.116	0.97± 0.16 P = 0.822

Table S6. Evaluation of F508del NBD1 protein expression in HEK-t cells whole-cell lysates after treatment with the compounds under investigation and protein new synthesis blockage with cycloheximide. Quantification of protein expression was obtained analyzing the band intensity with the software imageJ. Retrieved values were normalized to the intensity of the protein actin used as housekeeper protein and to the value of F508del NBD1 protein expression at time 0. Data represent the mean ± sem (standard error of the mean). For each condition, samples were run at least in triplicate. Comparisons of values with those of control, untreated samples were made with the Dunnett's test. Probability p values are reported. Asterisks indicate a significant difference ($p < 0.05$).

time (hours)	DMSO (Control)	VX661	VX445	2a	7m	7a
0	1.00 ± 0.01	1.00 ± 0.02 P = 0.411	1.00 ± 0.04 P = 0.232	1.00 ± 0.08 P = 0.681	1.00 ± 0.06 P = 0.331	1.00 ± 0.03 P = 0.145
1	0.72 ± 0.02	0.73 ± 0.12 P = 0.356	0.75 ± 0.12 P = 0.342	0.87 ± 0.05 P = 0.291	0.89 ± 0.03 P = 0.059	0.93 ± 0.06 P = 0.023 (*)
2	0.63± 0.08	0.62 ± 0.13 P = 0.741	0.65 ± 0.07 P = 0.624	0.78 ± 0.08 P = 0.027	0.81 ± 0.04 P = 0.114	0.85 ± 0.04 P = 0.022 (*)
4	0.43 ± 0.04	0.45 ± 0.11 P = 0.407	0.42 ± 0.09 P = 0.562	0.63 ± 0.03 P = 0.049 (*)	0.67 ± 0.04 P = 0.049 (*)	0.70 ± 0.03 P = 0.235 (*)
6	0.23 ± 0.13	0.26 ± 0.08 P = 0.176	0.27 ± 0.05 P = 0.405	0.45± 0.06 P = 0.019 (*)	0.50 ± 0.12 P = 0.035 (*)	0.53 ± 0.01 P = 0.023 (*)
8	0.07 ± 0.06	0.09 ± 0.03 P = 0.416	0.14 ± 0.03 P = 0.233	0.34 ± 0.03 P = 0.518	0.40 ± 0.09 P = 0.044 (*)	0.41 ± 0.02 P = 0.021(*)

Table S7. Primary antibodies used to detect whole length WT and F508del CFTR and CFTR single domains used in this work. For each antibody it is indicated the vendor, the clonality, the diluition (in PBS tween20 + 5% albumin) used to perform the western blot experiments, the epitope (when available in the data sheet) and the CFTR domain detected in our experiments

Antibody identifier	Vendors	Clonality	Diluition	Epitope	Target identified:
MM13-4	Millipore	Mouse monoclonal	1:200	N-terminus; residues 25-36	Full-lenght CFTR and MSD1
L12B4	Millipore	Mouse monoclonal	1:200	Residues 386-412	WT/F508del NBD1
Clone # 13-1 MAB1660	R&D system	Mouse monoclonal	1:100	R domain	R domain
ABIN350208	Antibodies on line	Rabbit polyclonal	1:100	Residues1150–1200	MSD2
clone M3A7	Millipore	Mouse monoclonal	1:200	C-terminus; residues 1365-1395	NBD2