

Supplementary Information to:

Full Rescue of F508del-CFTR Processing and Function by VX-809 can be achieved by Removal of Two Regulatory Regions.

Inna Uliyakina¹, Ana C Da Paula¹, Sara Afonso¹, Miguel J Lobo¹, Verónica Felício, Hugo M Botelho, Carlos M Farinha, Margarida D Amaral*

University of Lisboa, Faculty of Sciences, BioISI – Biosystems & Integrative Sciences Institute, Lisboa, Portugal.

Short title: Role of regulatory extension on CFTR stability.

¹Current addresses: Department of Physiology, Anatomy and Genetics, University of Oxford, UK (IU and MJL); Novartis Pharmaceuticals, Montreal, Quebec, Canada (ACDP); Department of Physiology, University of Regensburg, Germany (SA).

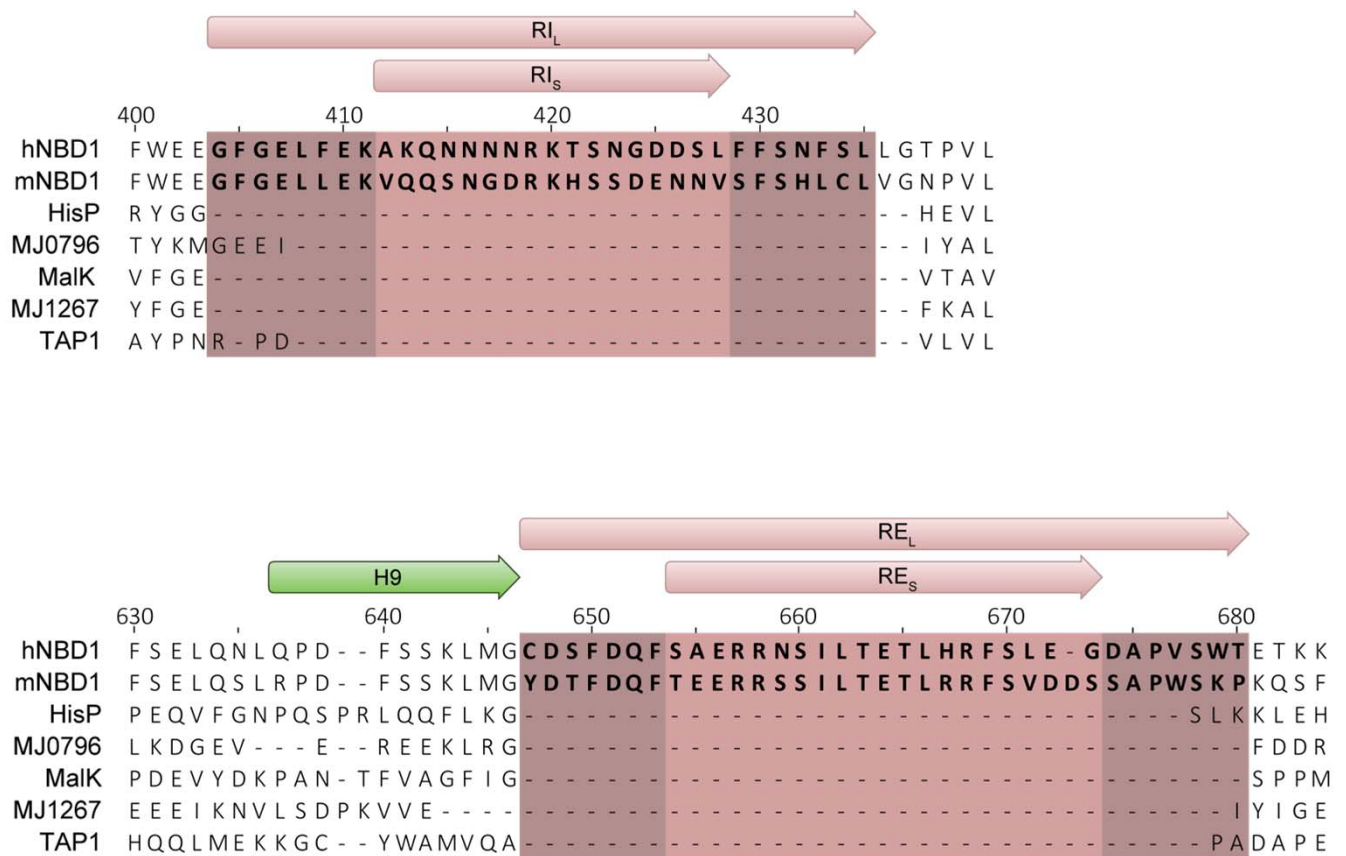


Figure S1-ABC transporters alignment. Sequence alignment of NBD sequences from ABC transporters. Sequences correspond to NBD domains with experimentally determined 3D structures: human CFTR (hNBD1) and Tap1, mouse CFTR (mNBD1), HisP from *S. typhimurium*, MJ0796 and MJ1267 from *M. jannaschii* and MalK from *T. litoralis*. Only RI and RE regions are shown. Numbering is the one of human CFTR. The localization of the RI, RE and H9 are shown as arrows and shades.

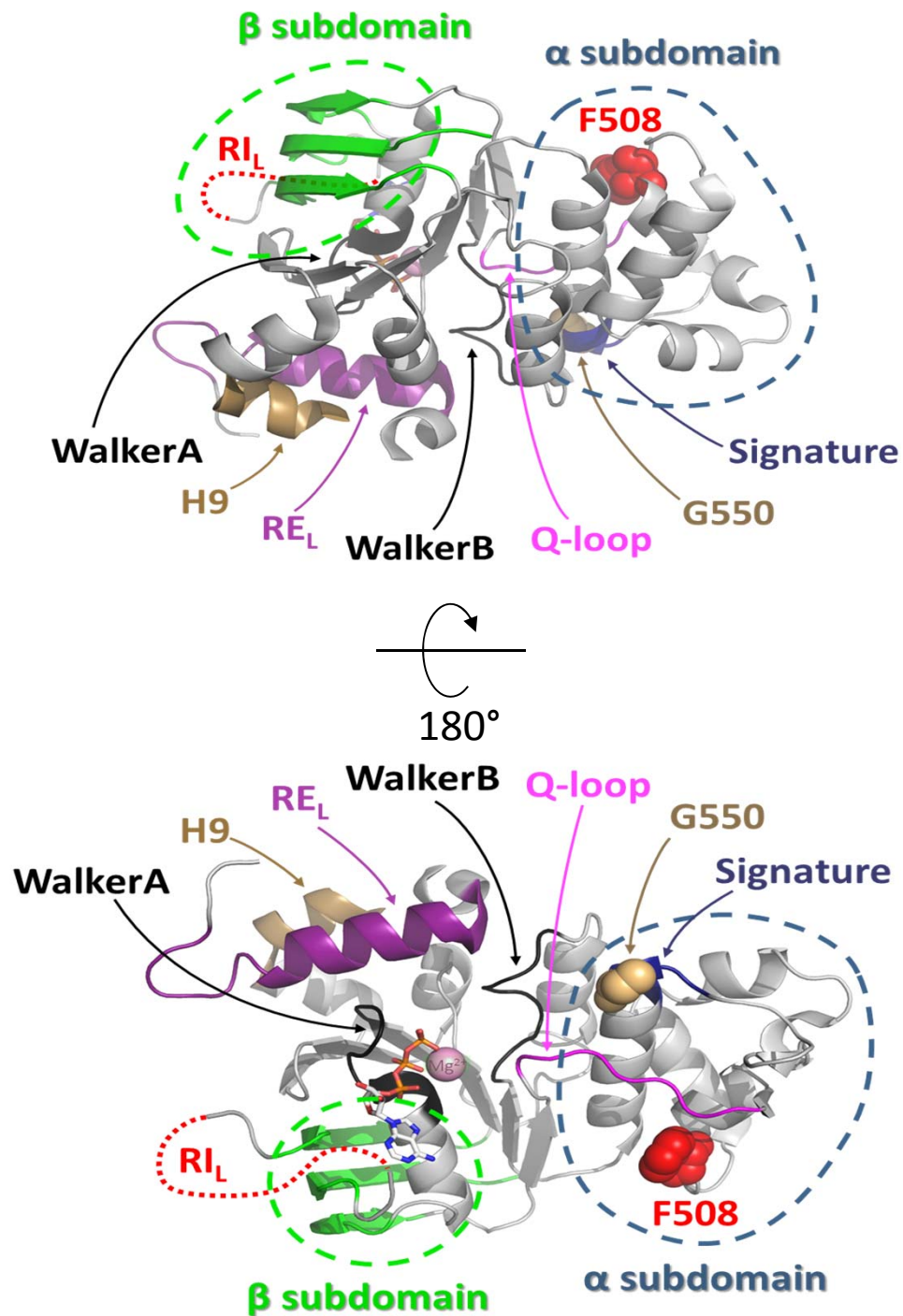


Figure S2-NBD1 structural details. Structural features of the CFTR NBD1 domain (PDB ID: 2BBO). The main structural regions are color coded. F508 is represented as red spheres. G550 is represented as brown spheres. ATP is shown as sticks and Mg^{2+} as a pink sphere. RI is unstructured in the crystal structure and represented as a dotted red line. The G550E mutation in this structure was reverted *in silico*.

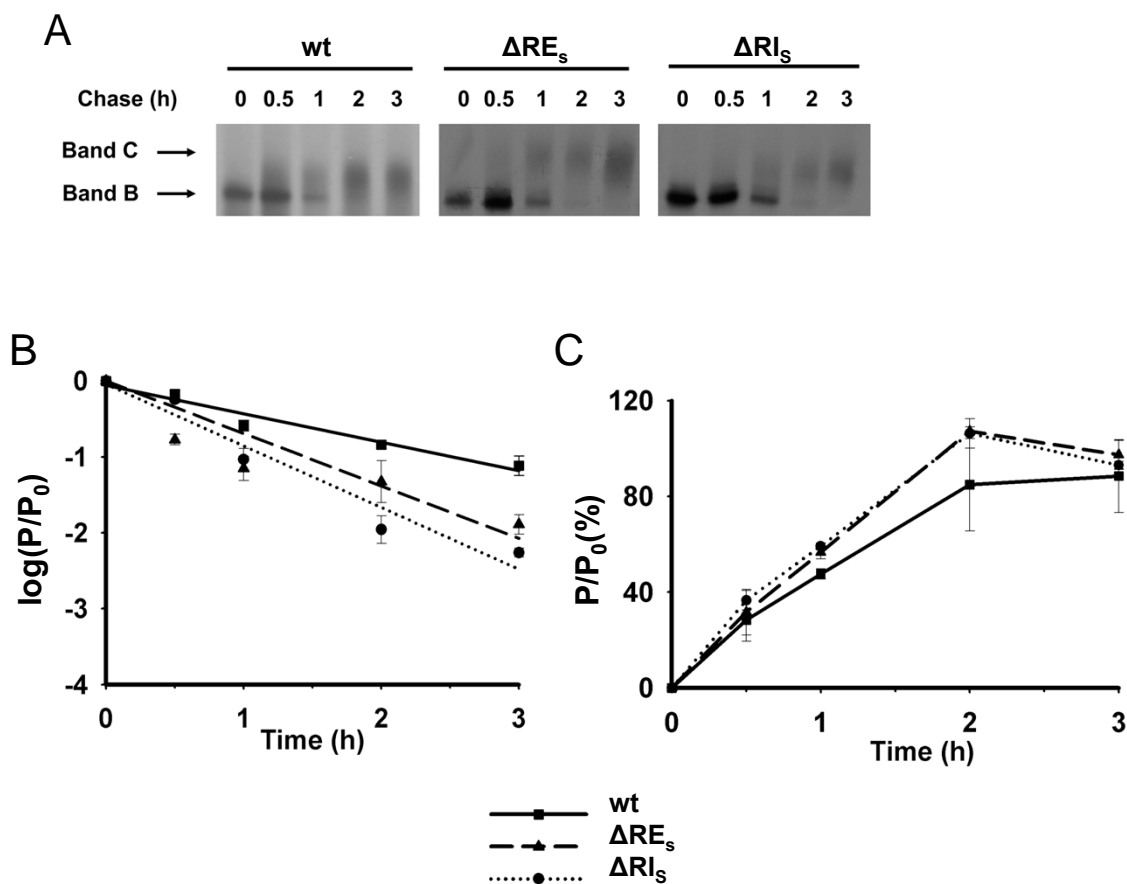


Figure S3-Turnover and processing of wt-CFTR, alone or in *cis* with ΔRE_s and ΔRI_s . (A) BHK cells expressing wt, ΔRE_s and ΔRI_s were labelled with ^{35}S -methionine for 30 min and then chased for the indicated times (0, 0.5, 1, 2, 3h) before lysis. IP was performed with the anti-CFTR 596 antibody. After electrophoresis and fluorography, images were analysed by densitometry. (B) Turnover of immature (band B) CFTR for different CFTR variants is shown as the percentage of immature protein at a given time point of chase (P_t) relative to the amount at $t=0$ (P_0). (C) Efficiency of processing of band B into band C is shown as the percentage of band C at a given time of chase relative to the amount of band B at $t=0$. Data are mean \pm SEM at each point ($n=3$).

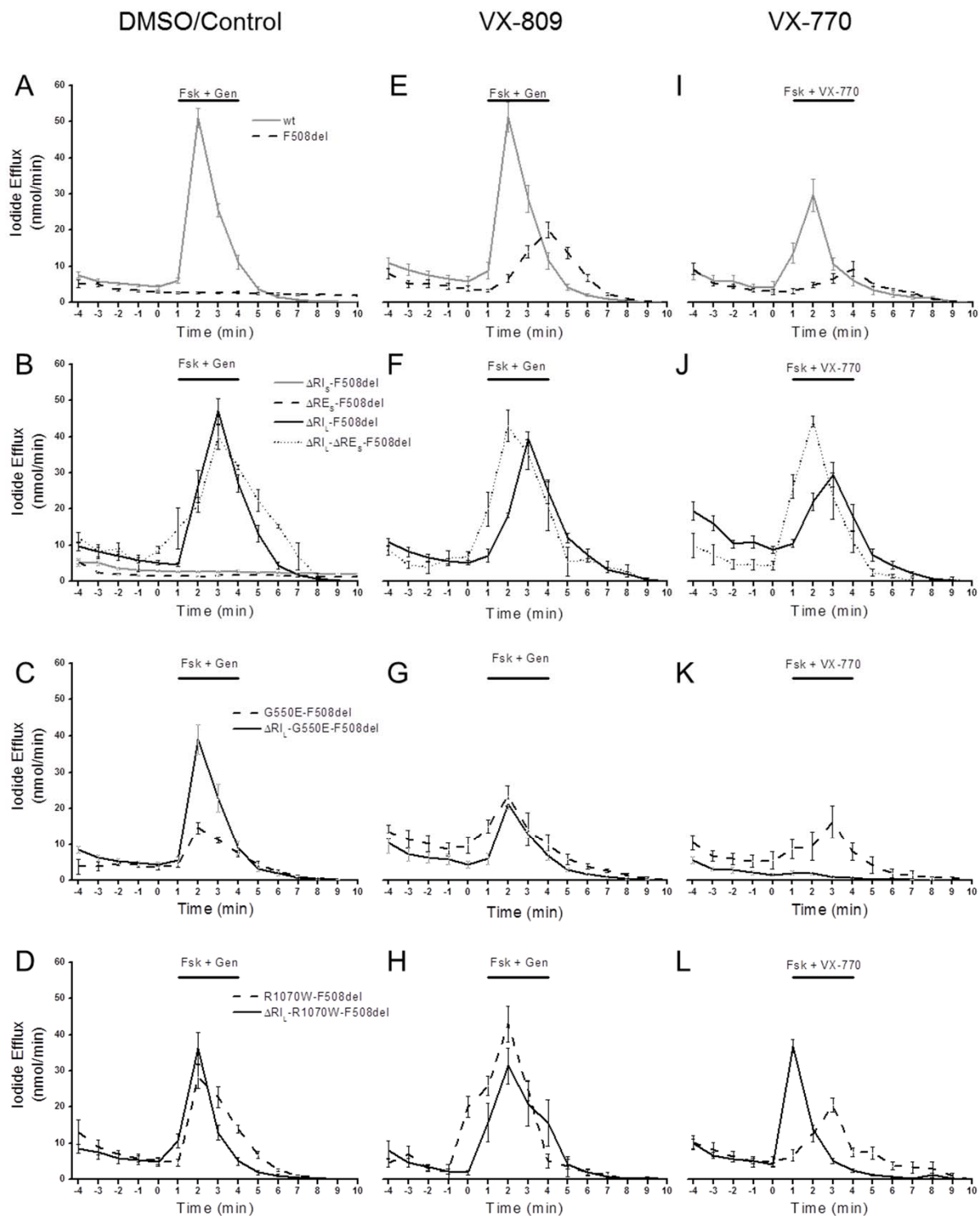
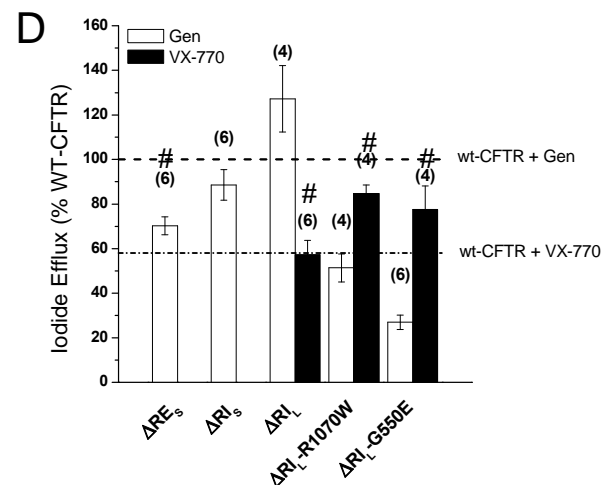
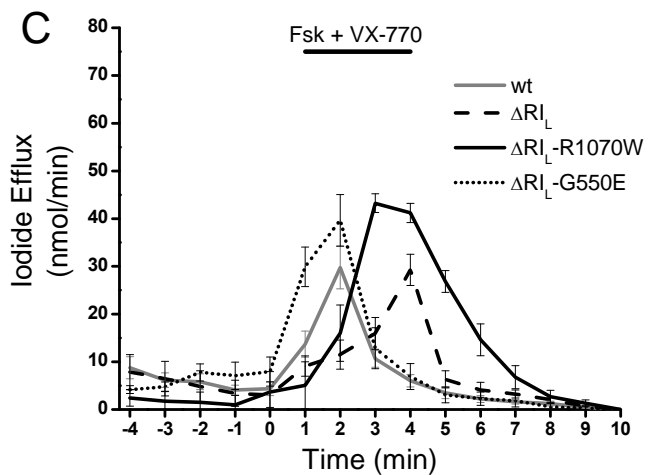
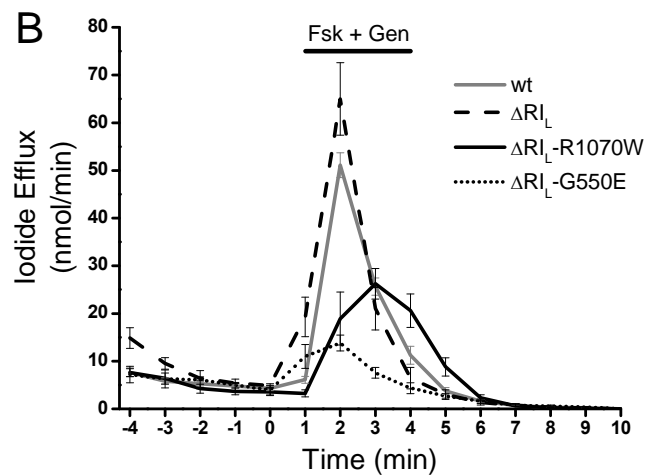
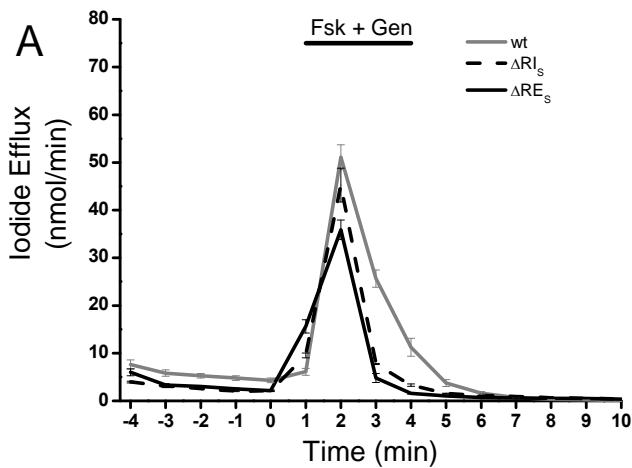


Figure S4-Functional characterization of the ΔRE_S and ΔRI_L variants of F508del-CFTR with and without VX-809 or VX-770 treatments. (A-L) Iodide efflux from BHK cells stably expressing ΔRE_S , ΔRI_S - and ΔRI_L -F508del-CFTR variants. Cells were incubated either with 3 μ M VX-809 (E-H) or equivalent concentration of DMSO (control) for 48h at 37°C (A-D; I-L) and for the assay stimulated with Fsk/Gen (A-H) or Fsk/VX-770 (I-L), as indicated above the bar and for the period indicated by the bar.



E

CFTR variant	Fsk + Gen			Fsk + VX-770		
	peak (min)	value	% to control wt-CFTR	peak (min)	value	% to control wt-CFTR
wt	2	51 ± 3	100 ± 5	2	30 ± 4	58 ± 9
ΔRI _S	2	45 ± 4	89 ± 7	-	-	-
ΔRE _S	2	36 ± 2	70 ± 4	-	-	-
ΔRI _L	2	65 ± 8	127 ± 15	4	29 ± 2	57 ± 6
ΔRI _L -R1070W	3	26 ± 3	51 ± 6	3	43 ± 2	85 ± 4
ΔRI _L -G550E	2	14 ± 2	27 ± 3	2	40 ± 5	78 ± 11

Figure S5-Functional characterization of the ΔRE_S and ΔRI_L variants of wt-CFTR. (A-C) Iodide efflux from BHK cells stably expressing ΔRE_S- or ΔRI-wt-CFTR alone (A) or jointly with revertants R1070W and G550E (B,C). Cells were stimulated during the assay with Fsk/Gen (A,B) or with Fsk/VX-770 (C), as indicated above the bar and for the period indicated by the bar. (D) Graph summarizing data from the iodide efflux peak magnitude generated by different BHK cells stably expressing the various CFTR variants. Data are shown as a percentage of wt-CFTR activity and as mean±SEM. (n) indicates n^o of independent experiments. "#" indicates significantly different from the wt-CFTR stimulated with Fsk/Gen (p<0.05). (E) Quantification of the iodide efflux data, showing peak response (time and value) and percentage of wt-CFTR activity.