

Supplementary figures

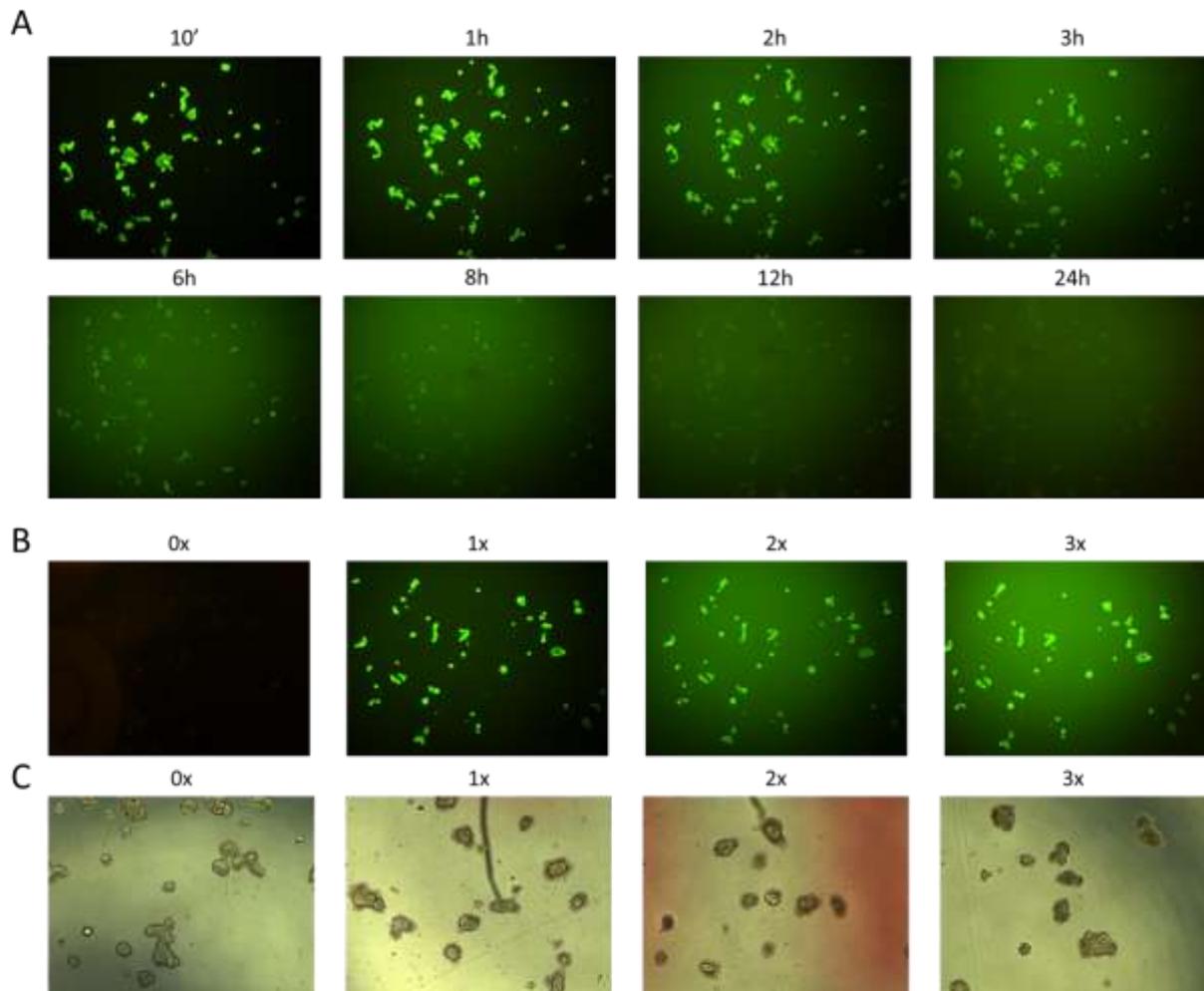


Figure S1: Calcein-labeled organoids do not allow for long term follow-up. Calcein AM (5 μ M) was added to rectal organoids which were then imaged for up to 24h. **(A)** Fluorescent images (4x) were taken at the mentioned time points, showing a decrease in organoid fluorescence compared to background fluorescence over time. **(B)** Calcein AM was re-added after 4 and 8 hours, but resulted in loss of a preferential signal-to-noise ratio. **(C)** Brightfield images (10x) showing rectal organoids 24h after receiving calcein AM (5 μ M) for the specified number of times. Increased organoid toxicity is apparent with increasing repeats of calcein addition.

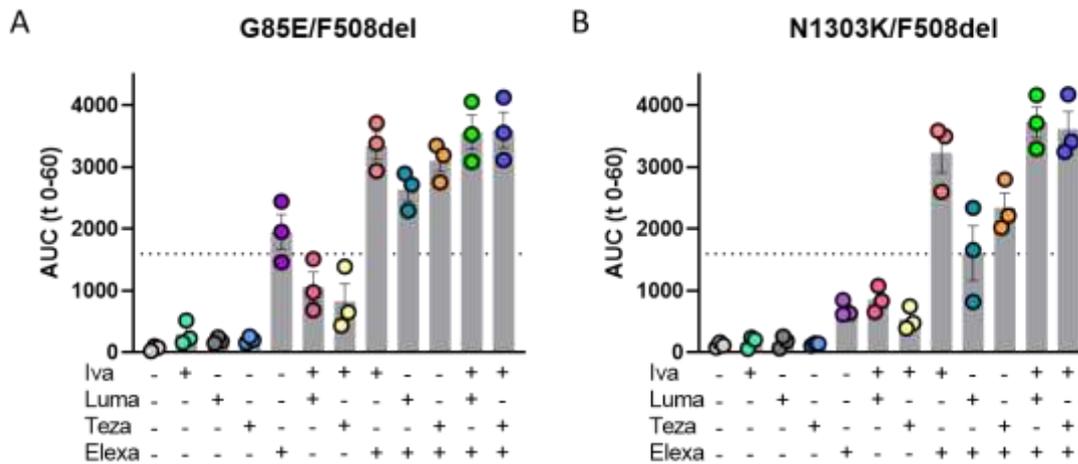


Figure S2: CFTR modulator responses of G85E/F508del and N1303K/F508del organoids. (A) G85E/F508del or (B) N1303K/F508del rectal organoids were treated with combinations of CFTR correctors (lumacaftor, tezacaftor, elexacaftor; all at 3 μ M) or DMSO 24h before analysis. At the start of the assay, forskolin (0.8 μ M) with or without the CFTR potentiator ivacaftor (3 μ M) was added and organoid swelling was assessed over the next 60 minutes using brightfield microscopy. Area-under-the-curve (AUC) of the relative increase in organoid area over time is shown. Abbreviations: FIS: forskolin induced swelling; AUC – area-under-the-curve; PM – plasma membrane; fsk – forskolin; Iva – ivacaftor; Luma – lumacaftor; Teza – tezacaftor; Elexa – elexacaftor. Each dot represents the result of an independent repeat, each performed in quadruplicate. Bars show mean+SEM. Dotted line represents the arbitrary threshold for relevant functional rescue (average response of F508del/F508del organoids to luma-ivacaftor).

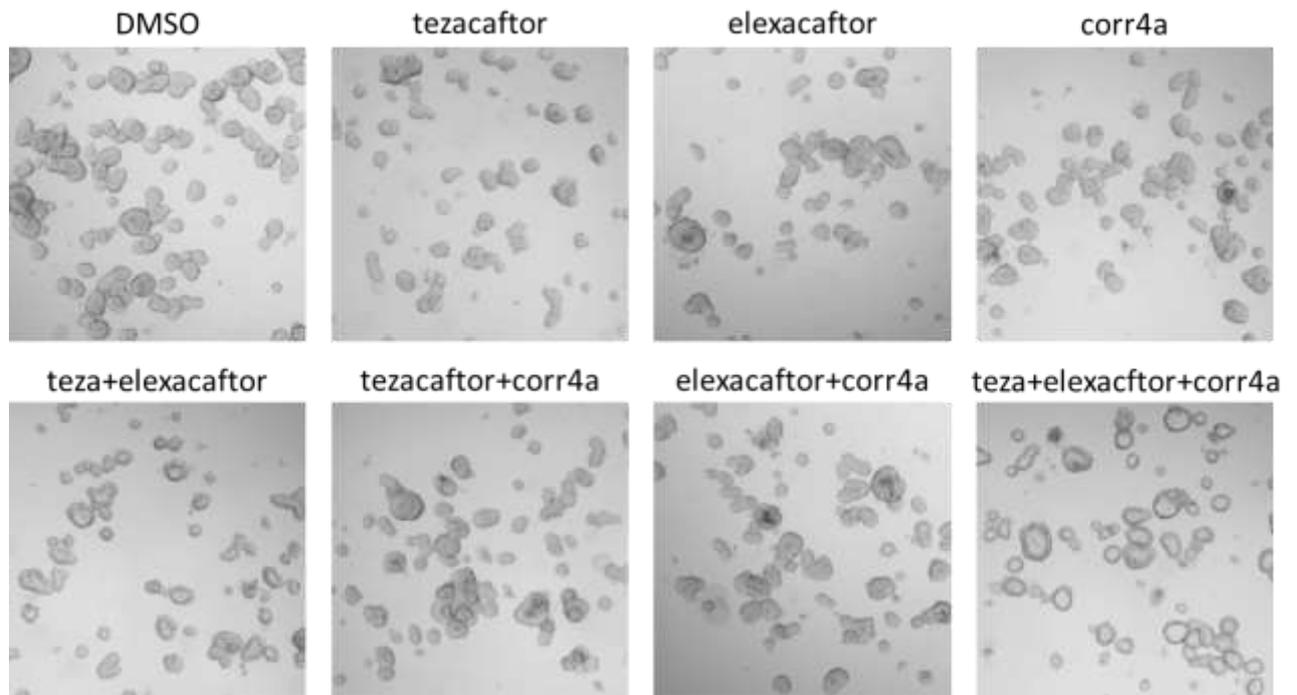


Figure S3: Evidence of a functional correction of F508del-CFTR in rectal organoids by specific CFTR corrector combinations in the absence of forskolin stimulation. F508del/F508del organoids were treated with the mentioned CFTR modulators (teza/elexacaftor 3 μ M; corr4a 5 μ M). Brightfield images were taken after 24h incubation with compounds or DMSO controls. The appearance of an organoid lumen for teza+elexacaftor and teza+elexacaftor+corr4a treated organoids in the absence of forskolin stimulation suggests a substantial functional correction of CFTR, resembling that of non-CF organoids (for example, see Dekkers et al., Nat. Med. 2013).

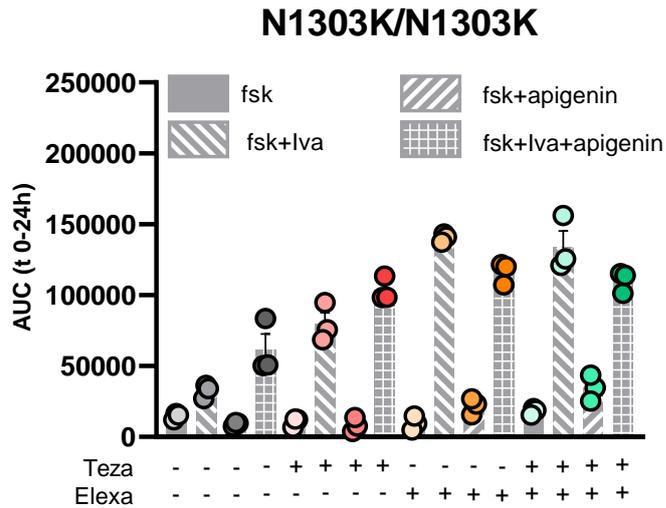


Figure S4: CFTR modulator responses determined by long term (24h) FIS in N1303K/N1303K organoids. This graph provides the corresponding data on which the Venn diagram shown in Figure 5H is based. N1303K/N1303K organoids were treated with teza/elexacaftor (3 μ M) or DMSO controls for 24h before analysis. At the start of FIS, forskolin (0.8 μ M) and ivacaftor (3 μ M)/apigenin (50 μ M) were added and organoids were followed up every 30 minutes for the next 24h. Increases in organoid area over time were calculated. AUC: area-under-the-curve; teza: tezacaftor; elixa: elexacaftor; fsk: forskolin; iva: ivacaftor. Each dot represents the result of an independent experiment, each performed in triplicate. Bars show mean+SEM.