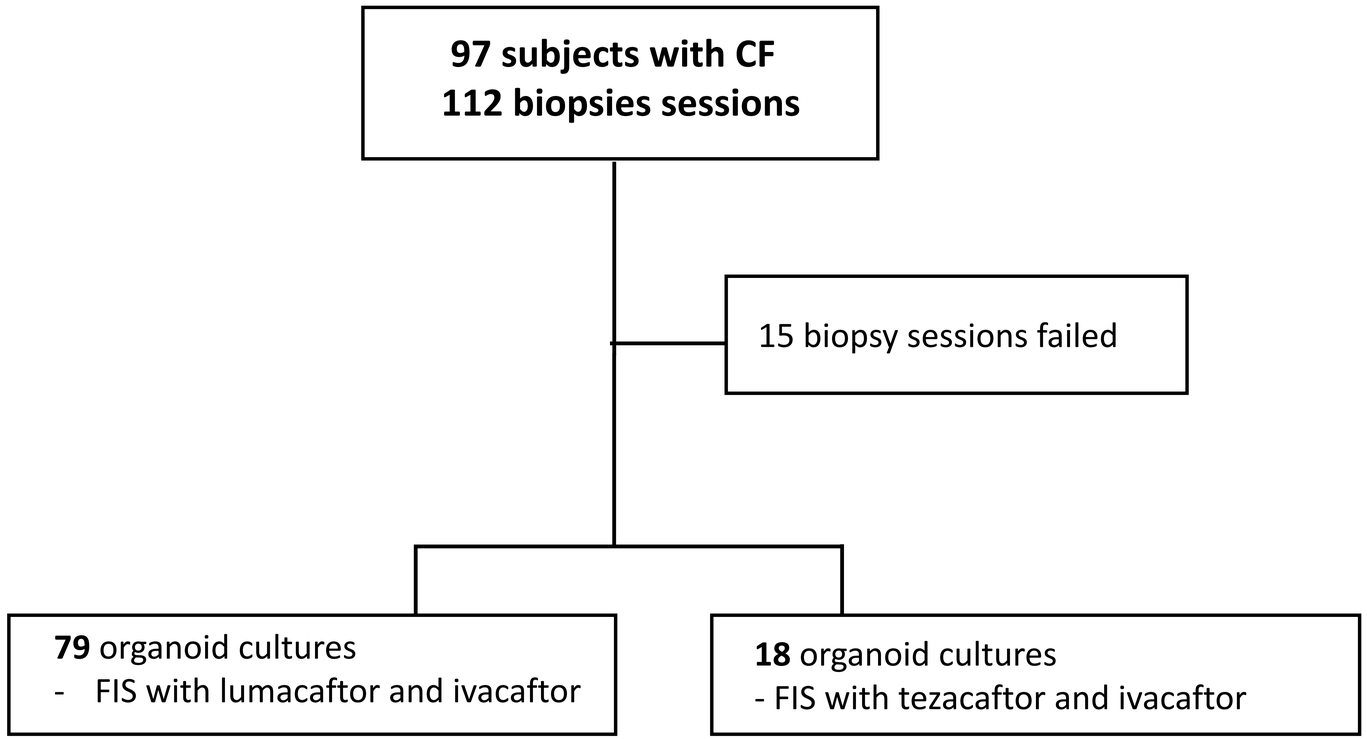
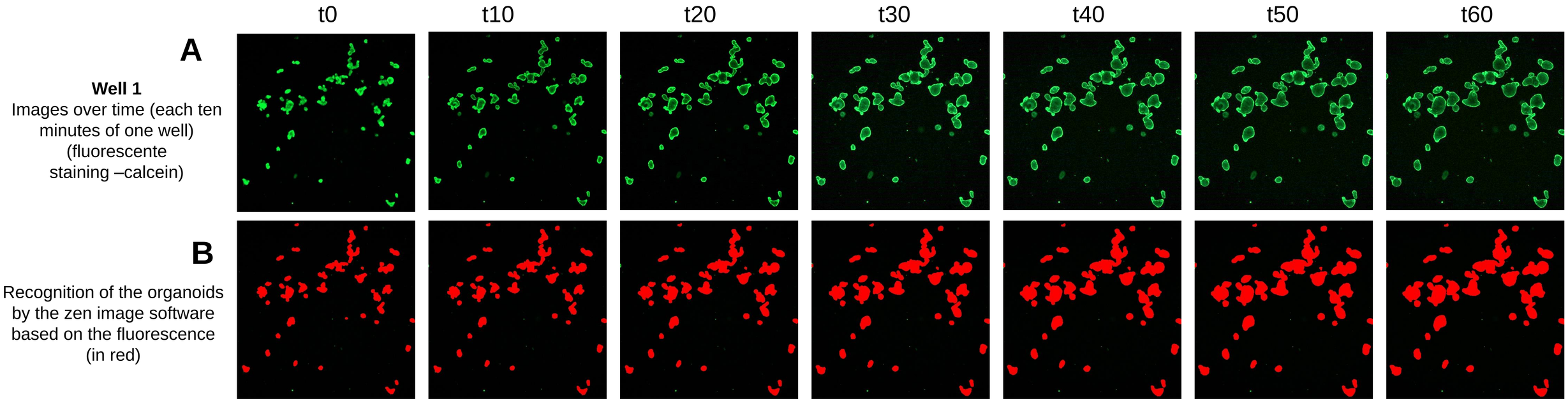
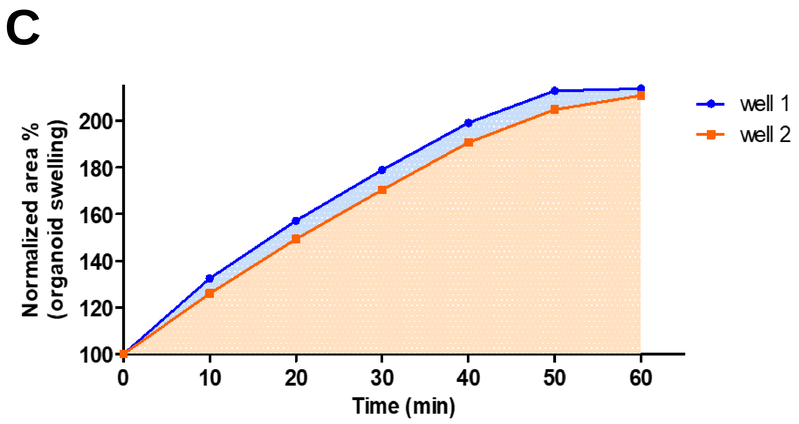
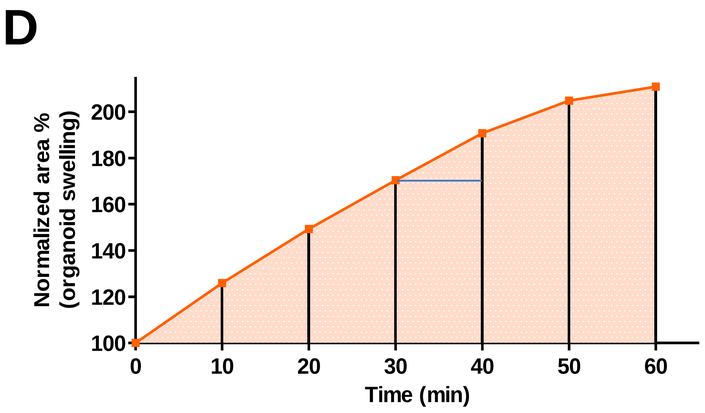
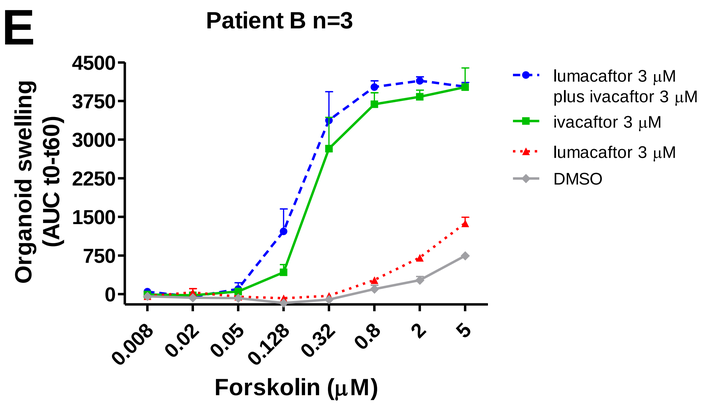



***Supplementary Figure S1-*** *Consort diagram of the organoids included in the study*



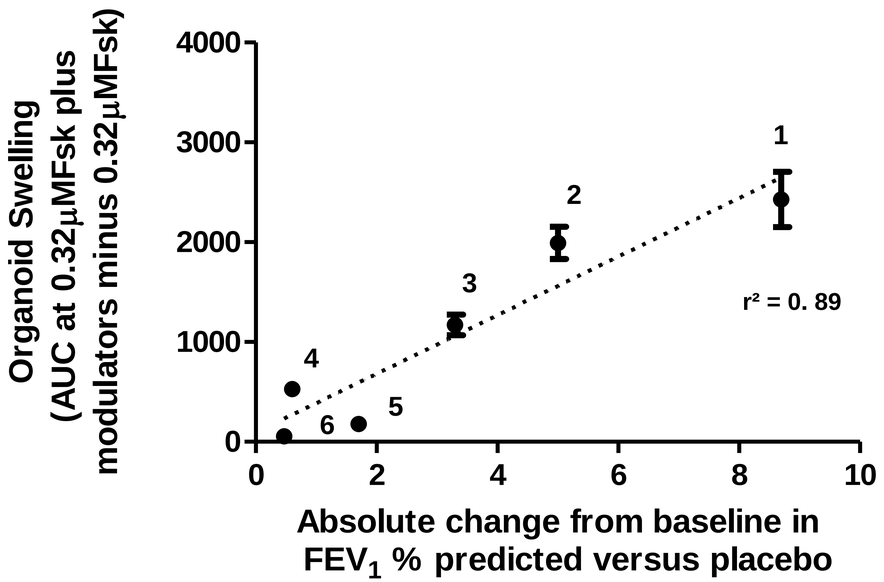

***Supplementary Figure S2 – Calculation and reporting of AUC A) Raw images.*** *Images of one well each 10 minutes from time zero (t0) to 1hour (t60) using a confocal microscope* ***B) Automated analysis****. After acquisition, images are analyzed with the ZEN blue software (Zeiss) to recognize organoid structures based on calcein fluorescence. For each well, the total organoid area is calculated at each time point and normalized to the total area of the organoids at t0 (considered to be 100%).* ***C) Graph of normalized area of organoids per time points.*** *Two wells are imaged for each condition (different forskolin concentration with or without the modulator(s)), from which respective results are shown in figure C.* ***D) AUC calculation.*** *To calculate AUC we divide the graph C in sections of 10 minutes, then calculate the area of each section and AUC of the graph correspond to the sum of the areas of these 6 section. For the first section t0-t10 the area is a triangle, so the area will be 1/2b*h (being b the base, that is 10 and the h is the height that is the Y value at t10 (that corresponds to the normalized area of the organoids at t10). For the others sections, the area corresponds area of a trapezoid=****1/2*10(y30+y40****): The sum of the areas of the sections in figure D corresponds to AUC of well 2. The AUC of both wells is averaged for each condition.* ***E) Results of a FIS assay with lumacaftor and ivacaftor in a subject with a gating mutation.*** *Each experiment is repeated on three different days, and the mean and SEM of the three AUCs is reported for each condition in a graph such as the example in E*

A

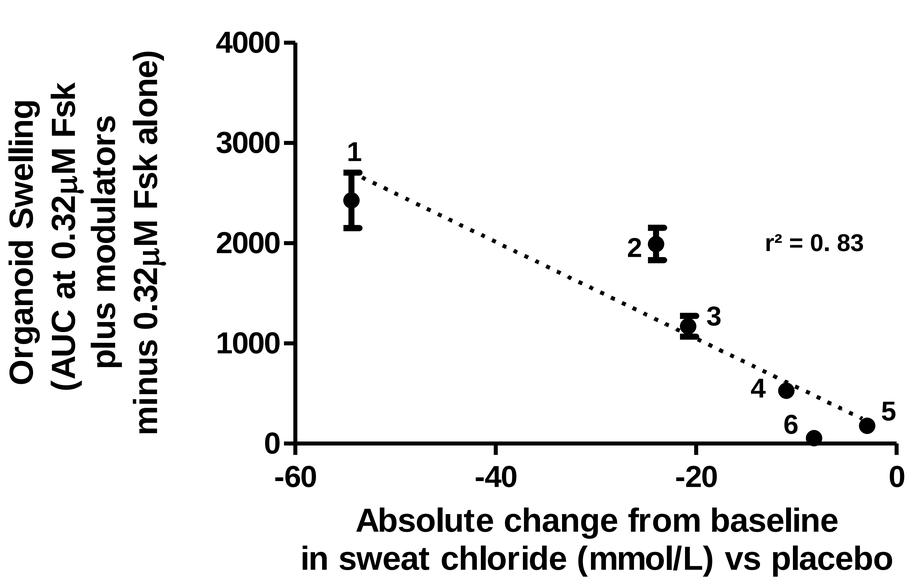
| | | | Absolute changes from baseline in FEV1 % predicted versus placebo | Absolute change from baseline in sweat chloride (mmol/L) | Organoid Swelling (AUC at **0.32 μM** Fsk plus modulators minus response 0.32 μM Fsk alone) | SEM | References |
|---|---|---|---|---|---|---|---|
| 1 | VX-770 | S1251N n=4 | 8.7 | − 54 | 2428 | 276 | 8 |
| 2 | VX-770 | R117H n=2 | 5.0 | −24 | 1993 | 162 | 9 |
| 3 | VX-809+ VX-770 | F508del/F508del n=35 | 3.3 | -21 | 1172 | 103 | 4[a], 22[b] |
| 4 | VX-809+ VX-770 | F508del/MF n=10 | 0.6 | -11 | 530 | 60 | 19 |
| 5 | VX-770 | F508del/F508del n=35 | 1.7 | -3 | 179 | 25 | 20 |
| 6 | VX-809 | F508del/F508del n=35 | 0.5 | −8 | 57 | 13 | 21 |

[a] reference for $FEV_1$ value; [b] reference for sweat chloride value

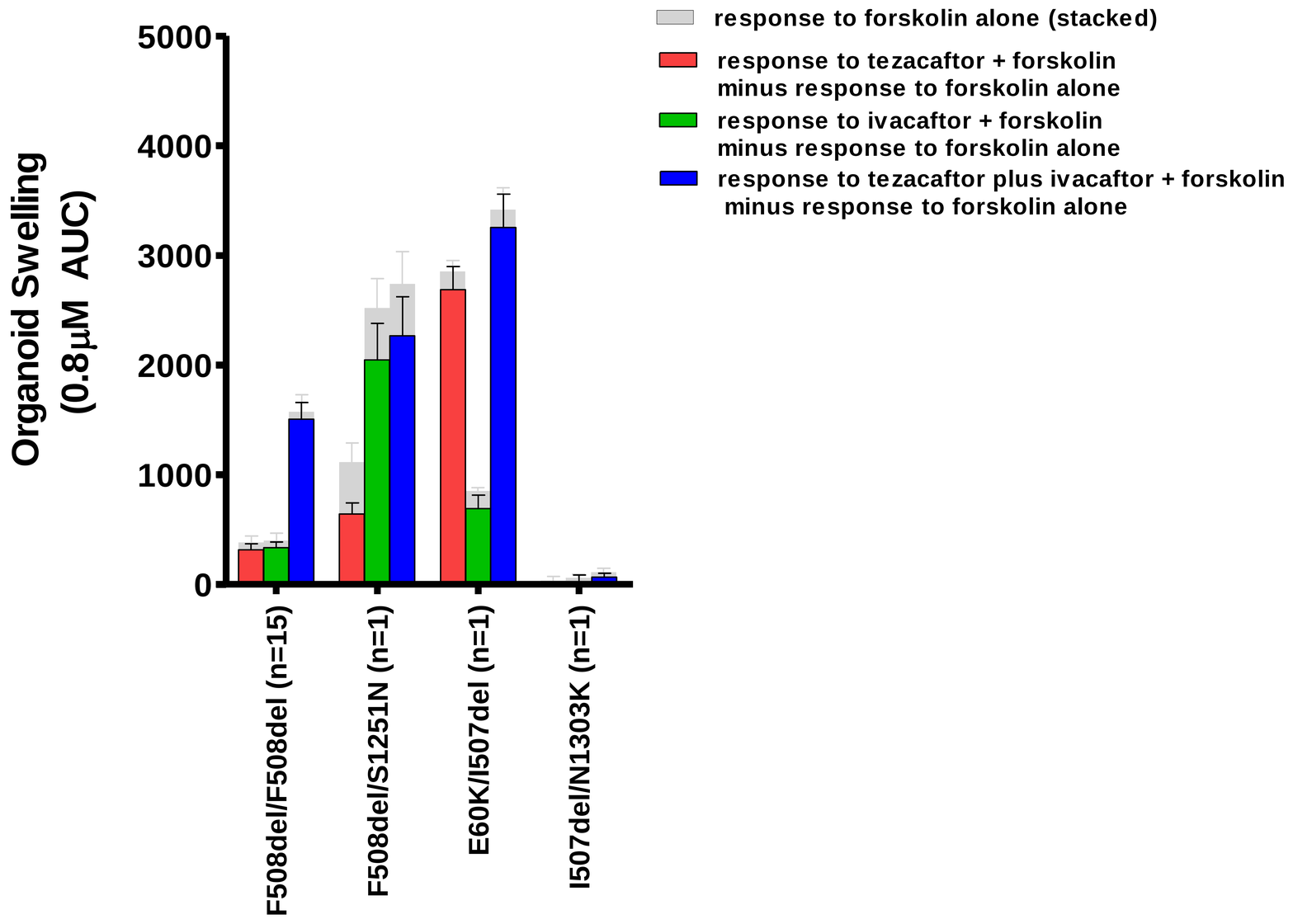
B




C




***Supplementary Figure S3 – Correlation between responses to modulators in vitro in the FIS assay and in vivo from published clinical trials. A)*** *Responses to lumacaftor and/or ivacaftor in organoids with the F508del/F508del, F508del/S1251N, F508del/R117H and F508del/minimal function (MF) were compared to the clinical responses (FEV1 and sweat chloride) obtained in published clinical trials with patients having the same genotypes* ***B)*** *Correlation between the absolute change in FEV1 in clinical trials and the organoid responses in our cohort at 0.32μM forskolin with the same modulators.* ***C)*** *Correlation between the absolute change in sweat chloride in clinical trials and the organoid responses in our cohort at 0.32μM forskolin with the same modulators.*




***Supplementary Figure S4 –CFTR modulator responses in organoids from subjects with rare CFTR mutations tested with tezacaftor and ivacaftor.*** *Mean and standard error of the AUC at 0.8mM forskolin without modulators and with tezacaftor, ivacaftor alone and their combination corrected for the residual function in the organoids from subjects with genotype F508del/F508del, F508del/S1251N, E60K/I507del (from the patient treated with Symkevi) and I507del/N1303K. Characteristic of the subjects are described in supplementary Table S1.*