

S4. Organoid culture from rectal biopsies and forskolin-induced swelling assay (FIS)

Crypts were isolated from rectal biopsies and seeded in 50% Matrigel (growth factor-reduced and phenol-free; Corning) in 24-well plates (~10 to 30 crypts in three 10- μ l Matrigel droplets per well). Growth medium [Sato et al, Gastroenterology 2011; Dekkers et al, Nature Med 2013] was further supplemented with Primocin (1:500; Invivogen). Vancomycin and gentamycin (Sigma) were added during the first week of culture to prevent infection. The medium was refreshed every 2 to 3 days, and organoids were passaged ~1:5 every 7 to 10 days. In short, intestinal organoids (passages 1 to 20) from a 7- to 10-day old culture were seeded in 96-well culture plates (Greiner) in 50% Matrigel containing 20 to 80 organoid structures and immersed overnight in 50 μ l organoid culture medium. One day after seeding, organoids were incubated for 30 minutes with 3 μ M calcein green (Invitrogen) and stimulated with forskolin (Sigma). To assess individual level of residual CFTR function in intestinal organoids, organoid swelling was induced by stimulating organoids with various forskolin concentrations. The 96-well plates were directly analyzed by confocal live cell microscopy (LSM 800, Zeiss) (all experiments were performed at 37°C, 5% CO₂). Per well, the total organoid area (xy plane in μ m²) increase relative to t = 0 of forskolin treatment was quantified using Zen Image analysis software module (Zeiss). Area under the curve (AUC) (t = 60 min; baseline, 100%) was calculated using Excel.

All forskolin concentrations were measured in duplicate. 21 out of 34 organoid cultures were measured at three independent culture time points, 9 cultures were measured at two culture timepoints and 4 cultures were measured at four independent time points. Sporadically, we

measured only 6 instead of 8 forskolin concentrations due to cell availability. For the 34 organoid lines, we generated 1552 data points and censored 9 data points as they were qualified as extreme outliers ($>6SD$ as compared to the average of all replicates) due to technical reasons related to imaging.