S3. Intestinal current measurements (ICM)

Rectal biopsies (4 per individual; ~12 months of age) were collected with a suction biopsy device (Model Meekers Medical 790166, Utrecht, Netherlands). The electrogenic transport of ions across the intestinal epithelium was measured as short circuit current (Isc) by a standardized protocol used by the CF centers in Rotterdam/Utrecht and Hannover [Derichs et al. Thorax 2010 and De Jonge et al. JCF 2004]. In brief, the biopsies were mounted in tissue sliders (aperture 1.13 mm2), inserted in recirculating Ussing chambers, and incubated at 37°C with Meyler buffer solution gassed with 95% O2, 5% CO2. After equilibration for 20 min, the basal potential difference, transepithelial resistance and short-circuit current (Isc) were determined by a voltage clamp-amplifier (DVC-1000, WPI) and a PowerLab digitalizer (AD Instruments). Next, compounds were added in a standardized order to either the mucosal (M) or the serosal (S) side of the epithelial tissue and Isc response to these additions was registered: amiloride (100 μM, M); carbachol (100 μM, S); DIDS (200 μM, M); histamine (500 μM, S), and 8-bromo-cAMP (1 mM, M+S)/forskolin (10μM, S). Finally, the transepithelial resistance and potential difference were measured to verify tissue viability. Crude Isc values (μA) were converted to $\mu A/cm^2$ on the basis of the surface area of the aperture, and the maximal individual ΔIsc responses after stimulation with specific substances were averaged from all biopsies without technical problems (3-4 per subject). The cumulative value of the average current increase provoked by the secretagogues carbachol, cAMP/forskolin, and histamine (ΔIsc, carb+cAMP+hista), mainly reflecting electrogenic, CFTR mediated chloride secretion, rather than the response to cAMP/forskolin alone (as monitored in the FIS assay), was used for

analyses in this study. As described in the paper of Derichs et al. (19) we used a cut-off value for subjects with CF-PI of <10 $\mu A/cm^2$.