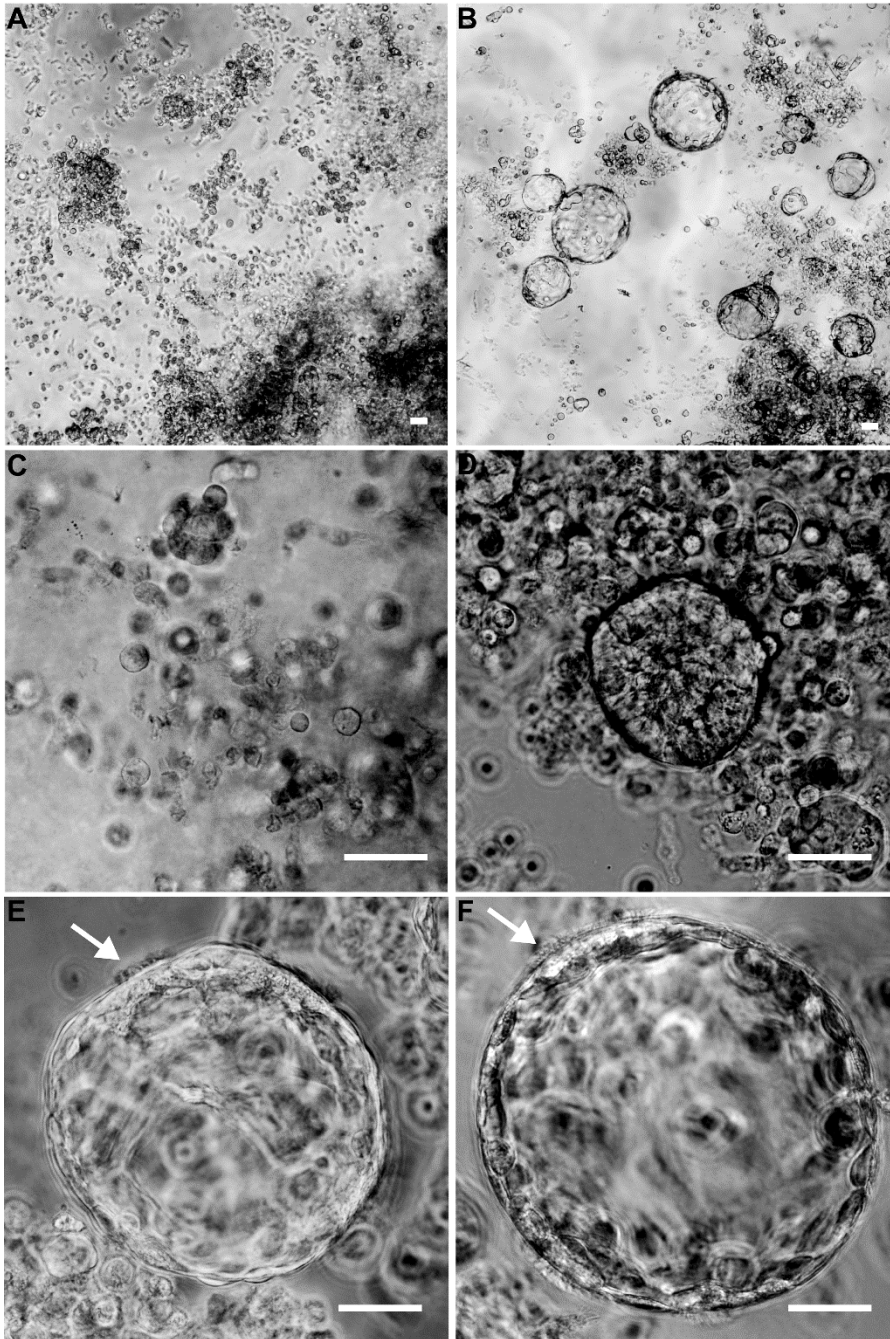
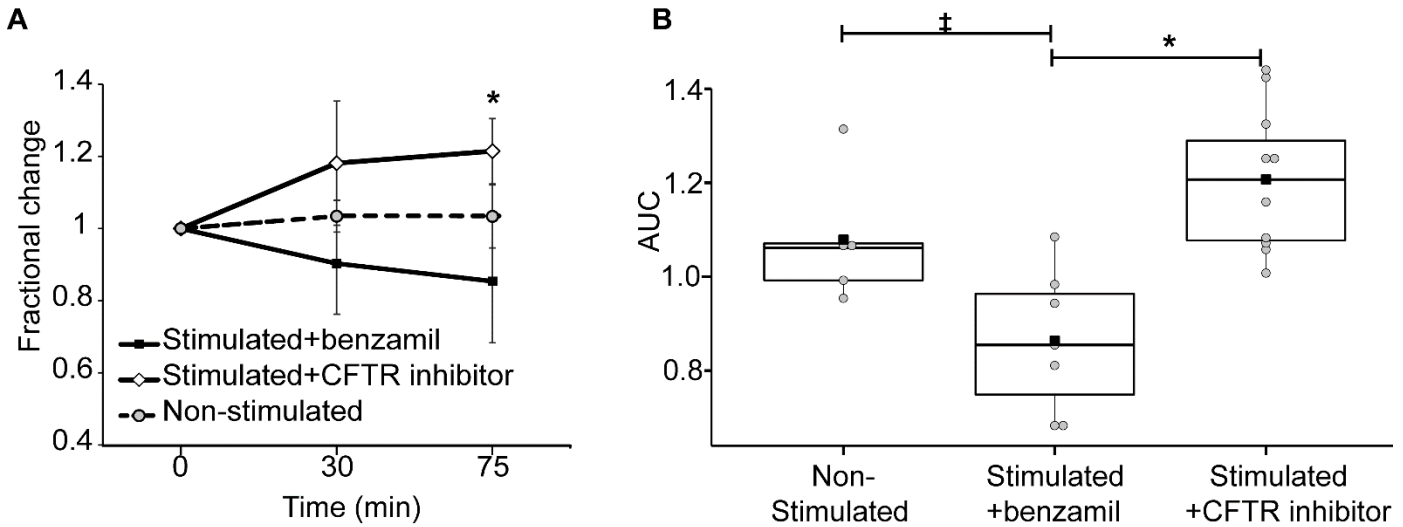


Supplemental Figures.

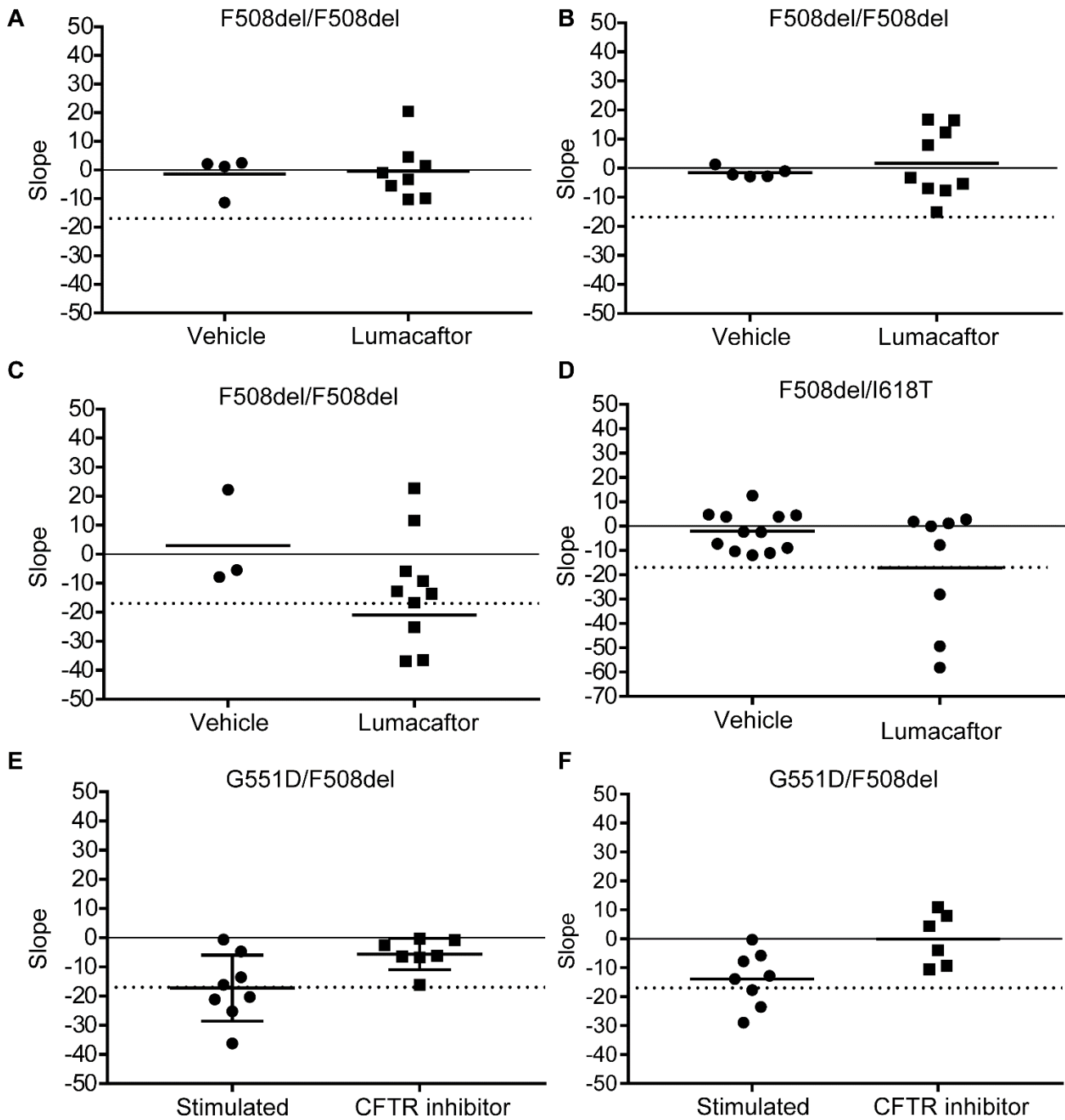


Supplemental Figure 1. Nasospheroids form over 2-5 days with apical membrane oriented to the bath.

A single subject's nasal brushings are shown at 4x magnification at day 0 (A) and day 4 (B). These structures appear to form from epithelial sheets and fill with fluid over time (C-F, 20x). Once fully formed, size stabilizes as these structures do not continue to grow, but remain intact. These structures are stable in media for at least three months. Arrow points to cilia. Scale bars=25 μ M.



Supplemental Figure 2. Epithelial sodium channel (ENaC) activity may be isolated and measured using the nasospheroid model. (A). The starting size of each nasospheroid was set at 1 and the fractional change calculated at all time points. The average fractional change was plotted at each time point. When CFTR is inhibited and nasospheroids are exposed to forskolin 10 μ M, the nasospheroid swells in size, suggesting ENaC activation, which we would expect would result in the influx of fluid into the interior of cells. Analysis of variance was calculated for the groups. Mean values \pm STD are shown. * $p < 0.0001$; † $p = 0.0035$. $n = 1$ subject; 5-11 nasospheroids per condition were analyzed. As shown in Figure 2, inhibition of ENaC with amiloride or benzamil is needed to isolate the activity of CFTR and evaluate shrinking behavior.



Supplemental Figure 3. Nasospheroids may be a useful tool for n-of-1 analysis. Each panel represents a single individual; each dot represents a single nasospheroid. Solid horizontal line at slope=0, indicating no change. Dotted horizontal line at slope=-17, the overall non-CF slope in the cohort of non-CF subjects. Panels A-C, show lumacaftor monotherapy response in F508del homozygote subjects in comparison to vehicle control (DMSO). Panel D, F508del/I618D nasospheroids in response to lumacaftor monotherapy in comparison to vehicle control. Panels E-F, shows G551D/F508del subject responses after subjects have taken ivacaftor. After stimulation with 10 μ M forskolin and inhibition of ENaC, slope is negative, which is inhibited by CFTR_{inh}-172.