Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplemental Information

Estimated Dimensions (width x spacing x height (µm))	**Measured Dimensions (µm)**		
	width	**spacing**	**height**
3x3x1	3.1 ± 0.2	2.5 ± 0.1	0.9 ± 0.1
3x3x3	2.9 ± 0.1	2.6 ± 0.1	2.7 ± 0.1
3x3x5	2.9 ± 0.1	2.6 ± 0.1	4.7 ± 0.1
5x5x1	4.9 ± 0.1	4.6 ± 0.1	0.9 ± 0.1
5x5x3	4.7 ± 0.2	4.4 ± 0.1	3.4 ± 0.7
5x5x5	4.7 ± 0.2	4.7 ± 0.3	4.7 ± 0.1
10x10x1	9.4 ± 0.1	9.4 ± 0.4	0.9 ± 0.1
10x10x3	9.6 ± 0.3	8.5 ± 0.3	3.2 ± 0.2
10x10x5	9.7 ± 0.1	9.1 ± 0.3	4.6 ± 0.2

Table S1. Estimated and measured dimensions of groove topography features

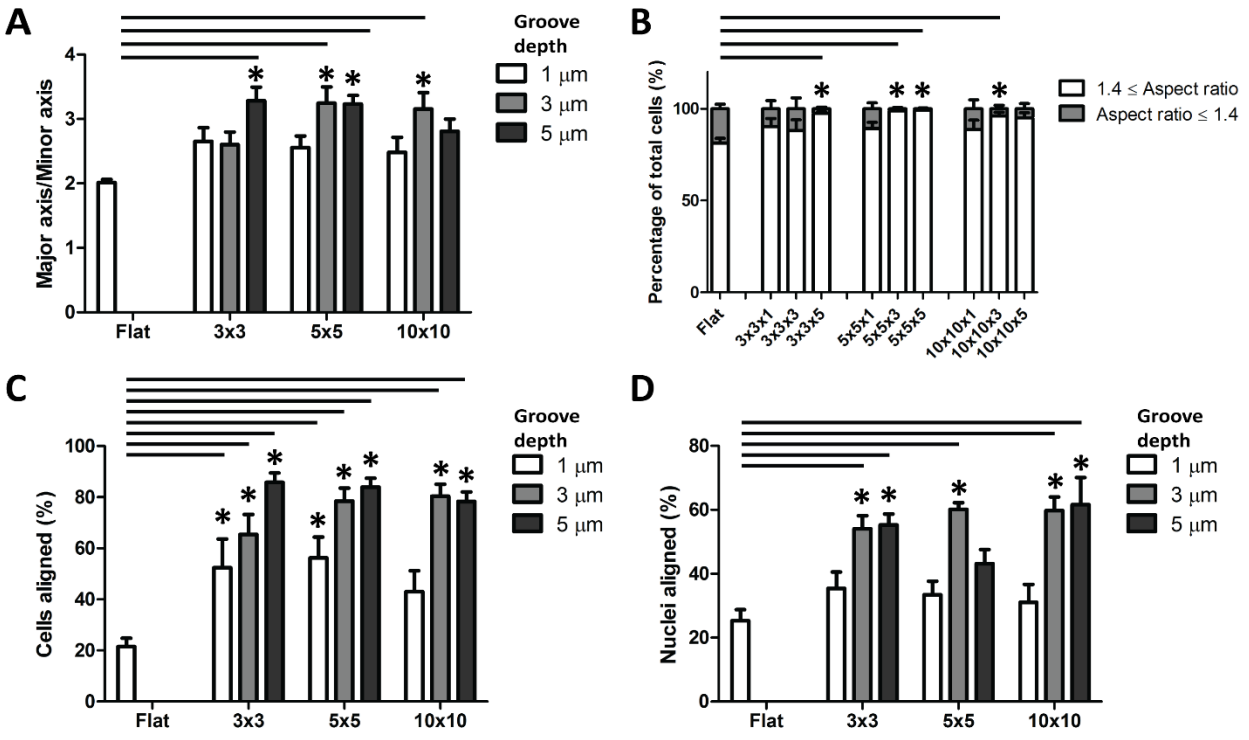

Figure S1. BEAS-2Bs robustly elongate and align on all tested groove topographies. Cell images were analyzed to assess BEAS-2B elongation (aspect ratio) on grooves of varying pitches and depths (A). Proportion of BEAS-2Bs with an elongated phenotype (defined as major axis/minor axis ≥ 1.4) (B). Cellular (C) and nuclear (D) alignment on flat versus different groove dimensions. Alignment was defined as the percentage of cells or nuclei oriented within 15° of the groove direction. Error bars are standard error of the mean and * indicates $p<0.05$ as analyzed by one-way ANOVA and Tukey's post hoc test. (n = 3).

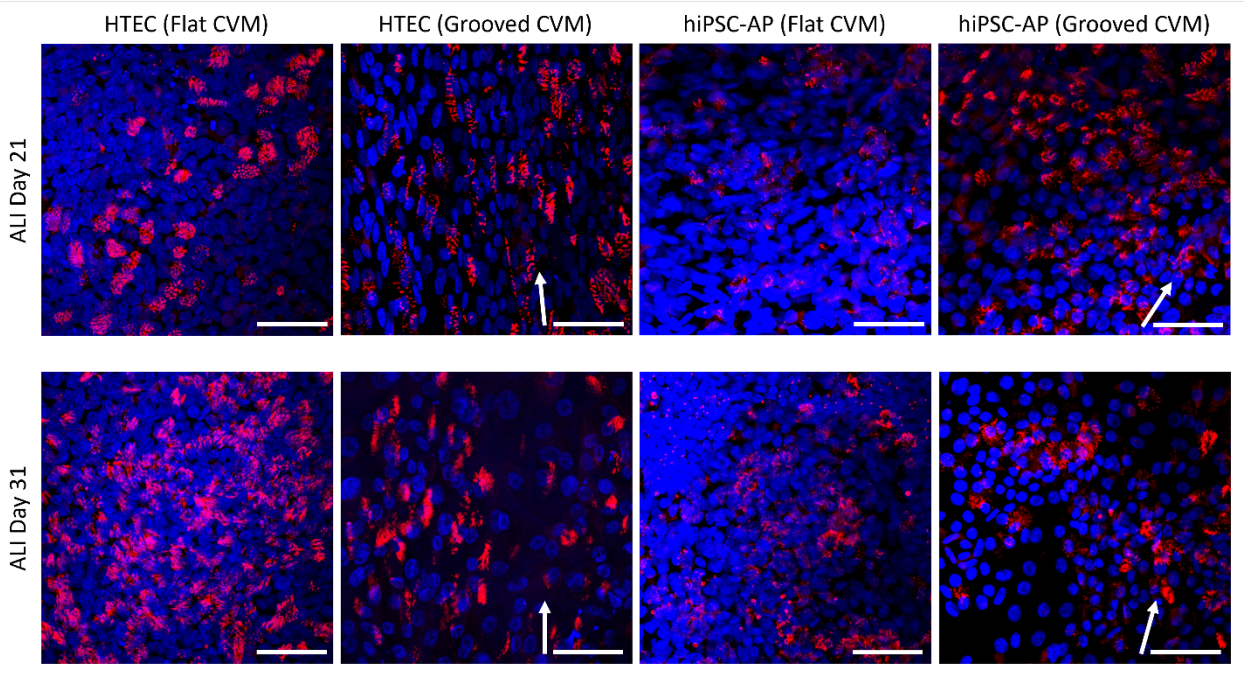

Figure S2. Both HTECs and hiPSC-APs differentiate towards multi-ciliated cells on flat and grooved CVM. HTECs and hiPSC-APs were seeded on flat CVM substrates within transwells and differentiated under ALI culture conditions across 21 and 31 days. Stains are acetylated α-tubulin (red) and Hoechst (blue). White arrows indicate groove direction. Scale bar = 50 µm. (n = 3).

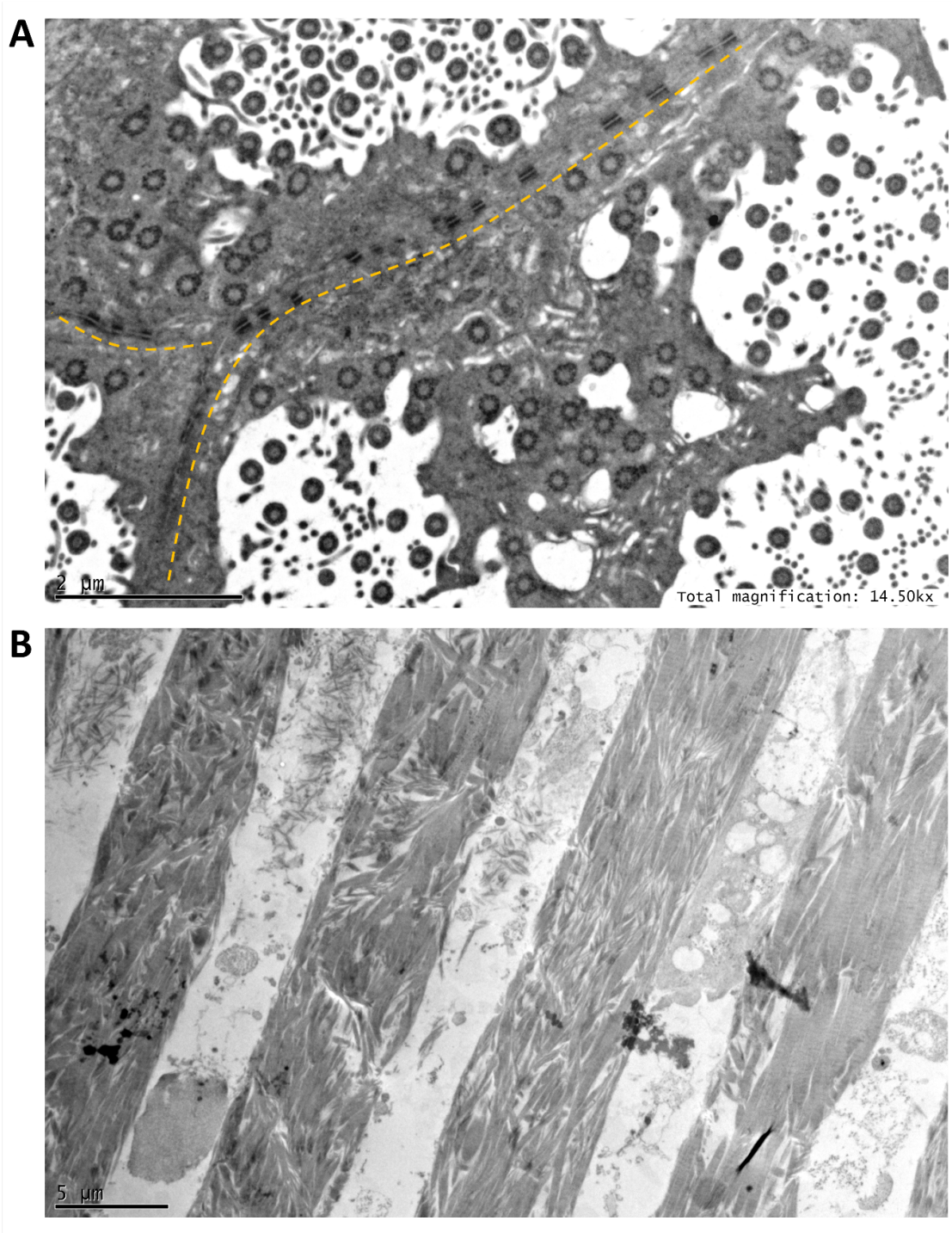

Figure S3. TEM imaging after terminal differentiation of airway epithelia. (A) Representative TEM image of two neighbouring multi-ciliated cells with basal body and basal foot structures. Orange dashed lines indicate tight junctions (cell boundaries). (B) Microgroove topography of CVM substrates underneath the epithelial monolayer could be observed during TEM analysis suggesting that the topography is maintained throughout the ALI culture time period.