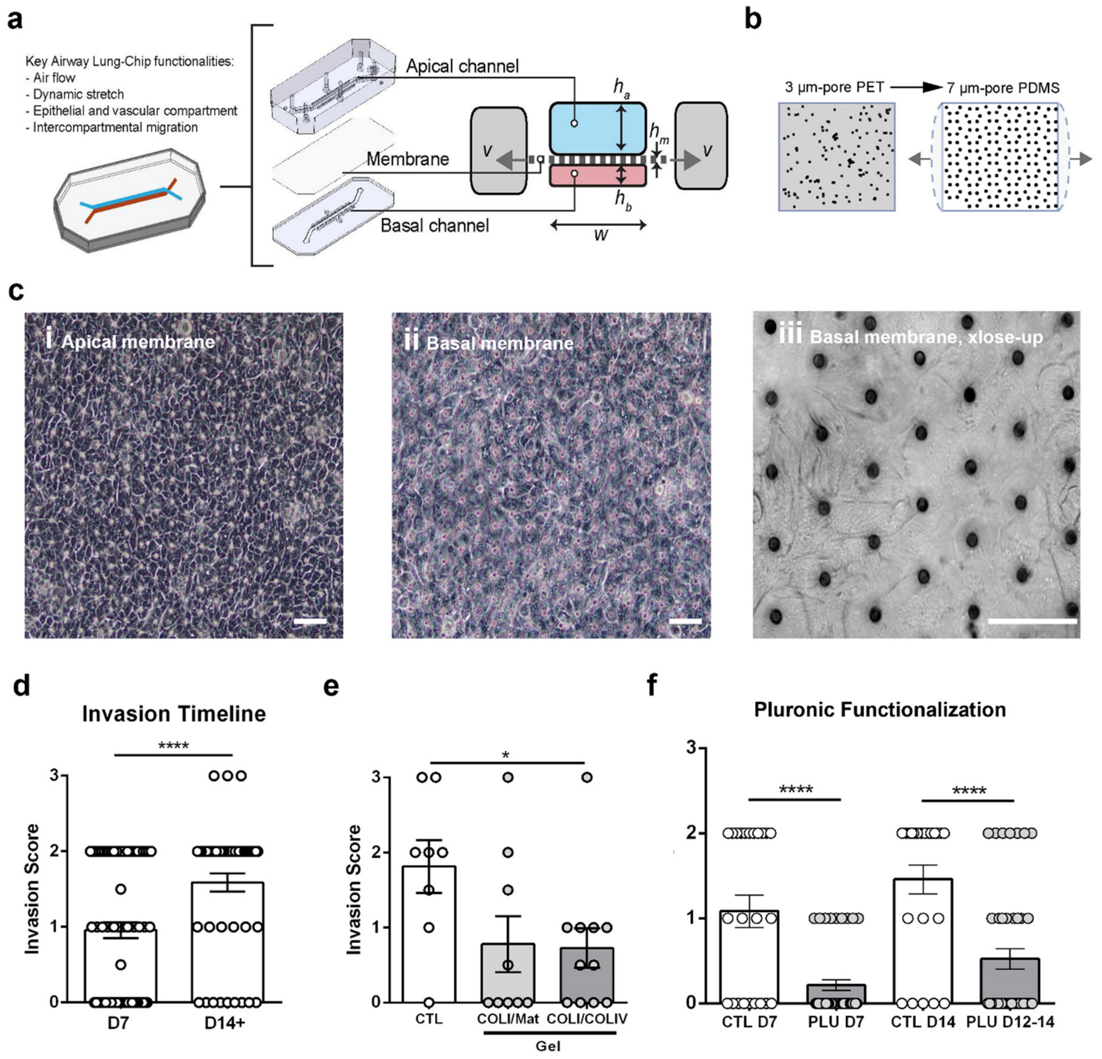
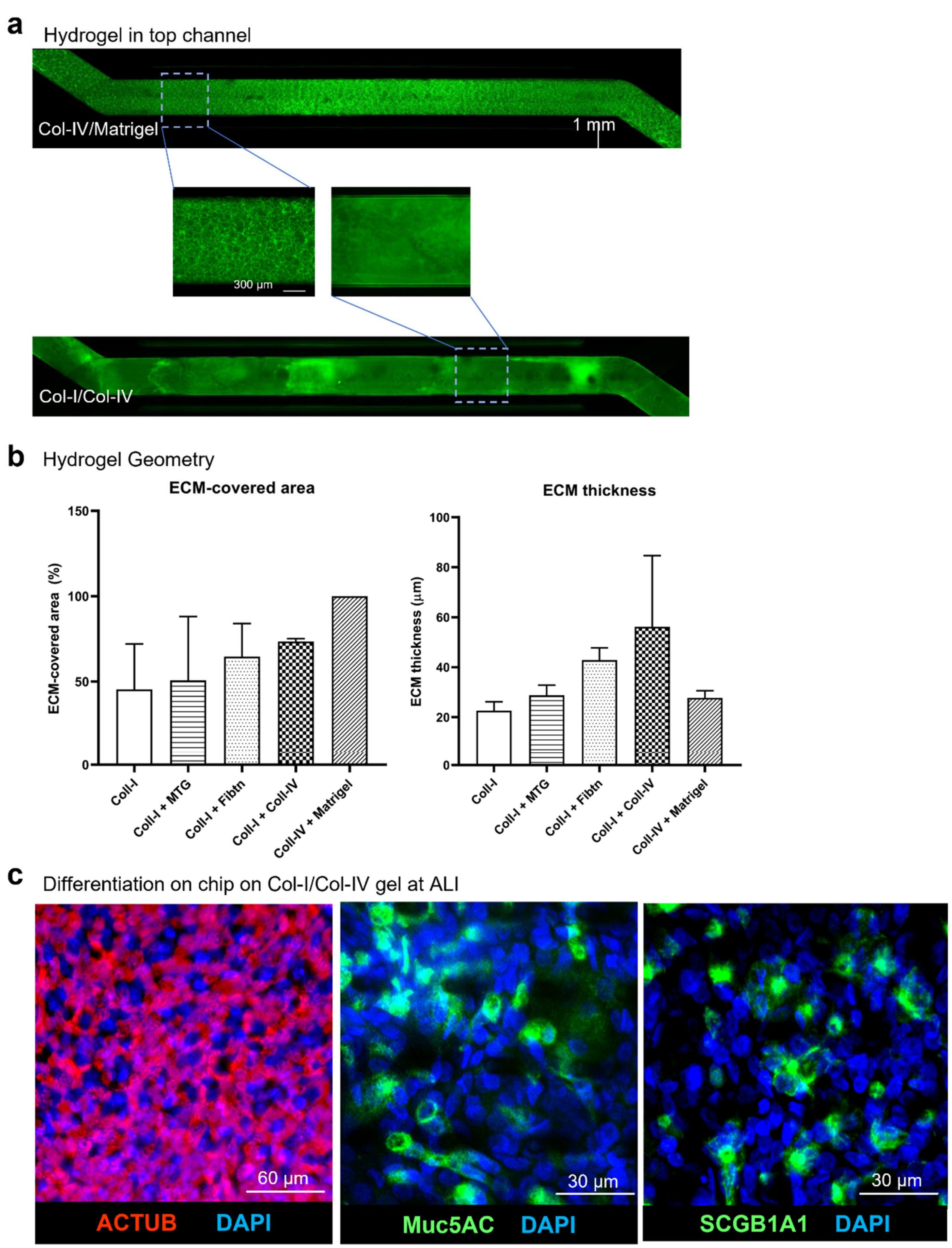


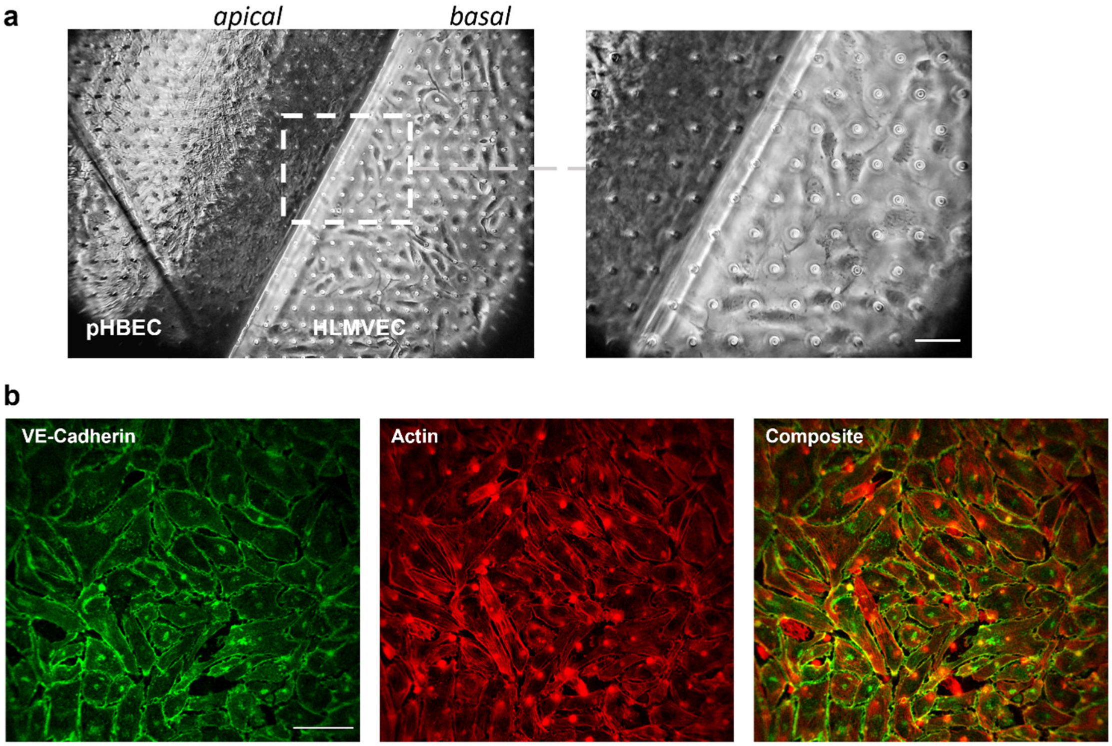
Supplementary Figure 1: Benefits and challenges of the Chip S1 design for pHBEC culture.

a, The Chip-S1 supports key functionalities for in vitro airway studies through the perfusable dual-compartment design and porous vacuum(v)-actuatable membrane. w, channel width = 1000 µm; ha, top channel height = 1000 µm; hb, bottom channel height = 200 µm; hm, membrane thickness = 25 µm. **b**, Previous airway chip studies commonly used a rigid and translucent PET membrane. To enable the current study, we switched to the Chip-S1's flexible PDMS membrane with optical transparency. **c**, Phase contrast microscopy of top of membrane showing pHBEC at ALI (left) and bottom of membrane showing a confluent layer of pHBEC that have migrated through the 7-µm pores to the bottom channel (center and right). Scalebars: 50 µm. **d**, Quantification of bottom channel invasion by hPBEC on day 7 and after day 14 of ALI using a score of 0 (no invasion) to 3 (basal channel fully lined). Data pooled from multiple media and membrane coating conditions. N=4 donors with 2-6 chips per donor. Each data point is from 1 chip. Columns and error bars represent mean +/- SEM, ****p<0.0001, Mann-Whitney test. **e**, Reduced invasion at day 14 ALI in chips with collagen I/Matrigel (COLI/Mat) or collagen I/collagen IV (COLI/COLIV) hydrogels. N=1 donor; each data point is from 1 chip. Data are depicted as mean +/- SEM. *p<0.05, ANOVA, followed by Dunnett's multiple comparison test. **f**, Strongly reduced invasion in chips treated with Pluronic. N=3 donors, 12 chips per donor, each data point is from 1 chip. CTL data are the same as in Figure 1d. Columns and error bars represent mean +/- SEM. ****, p<0.001, Mann-Whitney test.



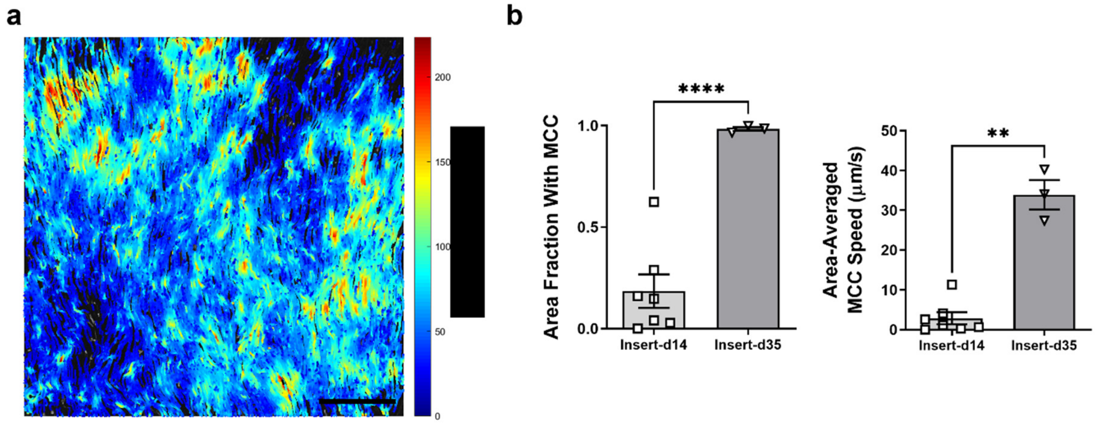
Supplementary Figure 2: Hydrogel barrier supports pHBEC differentiation at ALI.

a, Representative images of hydrogels (green) consisting of collagen I/Matrigel or collagen I/collagen IV lining the entire membrane in the top channel of the chip. **b**, Compared to other gel formulations, the gel compositions in (A) achieved superior percentage of membrane coverage (left) combined with robust gel thicknesses above 20 µm (right). Columns and error bars represent mean +/- SEM. **c**, Immunofluorescent (IF) staining of hydrogel chips at day 14 ALI shows presence of 3 major differentiated airway epithelial cell types: acetylated α-tubulin (ciliated cells), Muc5AC (goblet cells), SCGB1A1 (club cells). Nuclei are stained with DAPI.



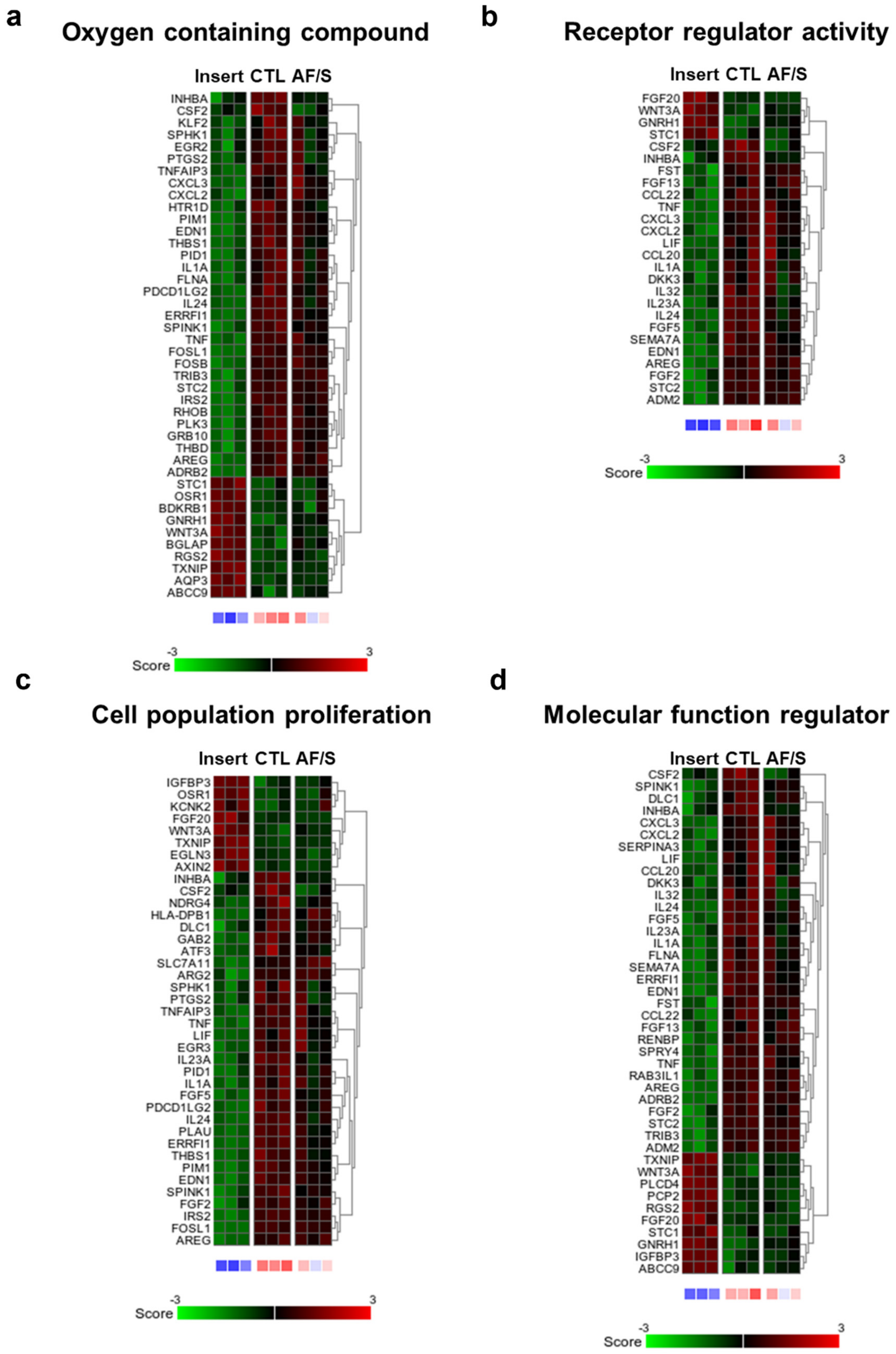
Supplementary Figure 3: Endothelial co-culture on invasion-free airway epithelial chips.

a, Left: Phase contrast image of co-culture of HPBEC in apical (top) channel and HLMVEC in basal (bottom) channel, on opposing sides of the PDMS membrane (shown here is junction where the channels diverge), 3h after seeding of the endothelial cells at day 12 ALI. Right: inset showing typical endothelial morphology and successful adhesion to the fibronectin-coated PDMS membrane. Scale bar: 50 µm. **b,** IF stain showing that HLMVEC lining the basal channel membrane express the endothelial-specific cell junction marker vascular endothelial (VE)-cadherin at day 14 ALI, i.e., 48h post-seeding of the endothelial cells. Note that the regularly spaced, circular structures are the auto-fluorescent membrane pores. Scale bar: 100 µm



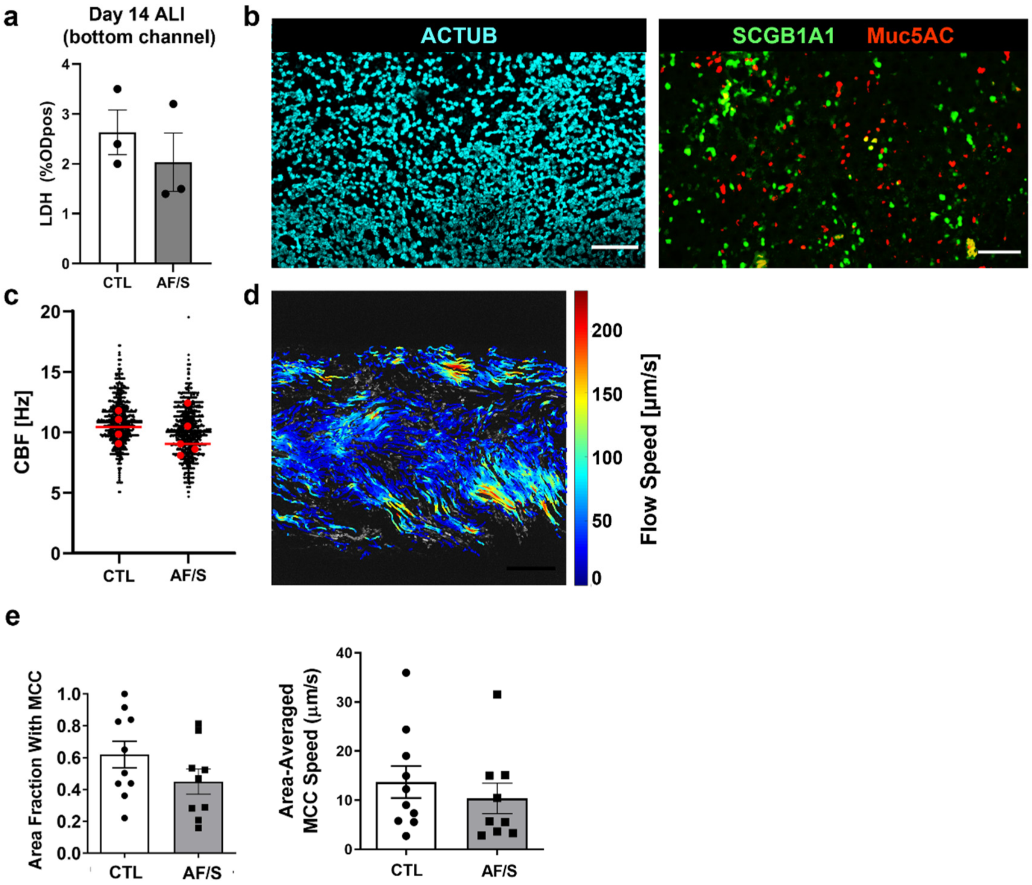
Supplementary Figure 4: Mucociliary differentiation in inserts is improved by day 35 ALI.

a, Example of fluorescent bead flow speeds in insert at day 35 ALI. Scale bar: 200 µm. **b**, Quantification of area fraction covered by MCC (left) and area-averaged MCC speed (right). Depicted are mean +/- SEM of 7 inserts at day 14 ALI from N=7 donors (one insert per donor) and 3 inserts from N=3 donors (one insert per donor) at day 35 ALI. **p<0.01, ****p<0.0001; two-tailed Welch's t-test.

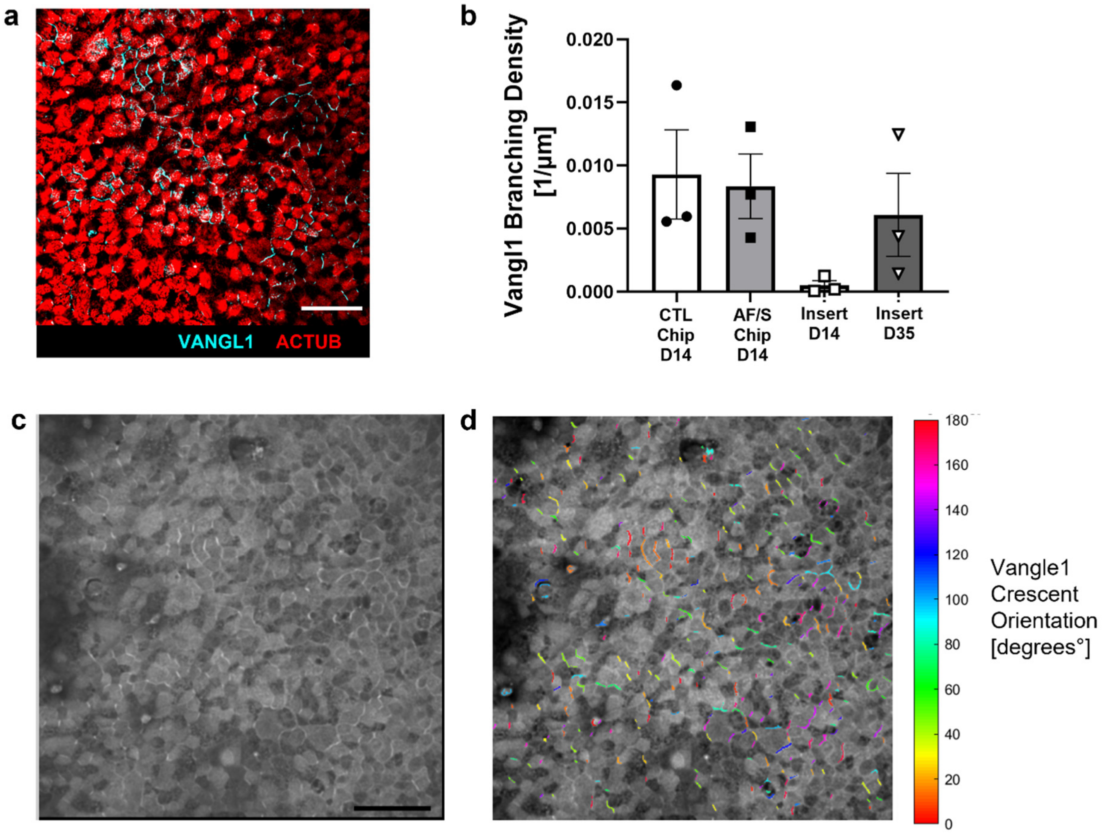


Supplementary Figure 5: Changes in gene expression related to top pathways identified by comparison of insert with chip cultures.

a, Heat maps displaying the Z score of DEGs related to the oxygen-containing compound pathway identified in pathway analysis of the DEGs identified between insert and chip cultures, which are depicted in Table 2. **b,** Heat maps displaying the Z score of DEGs related to the receptor regulator pathway. **c,** Heat maps displaying the Z score of DEGs related to the cell population proliferation pathway. **d,** Heat maps displaying the Z score of DEGs related to the molecular function regulator pathway. Expression was compared between insert, CTL chip and AF/S chip cultures with N=3 donors that are paired (one donor per column).

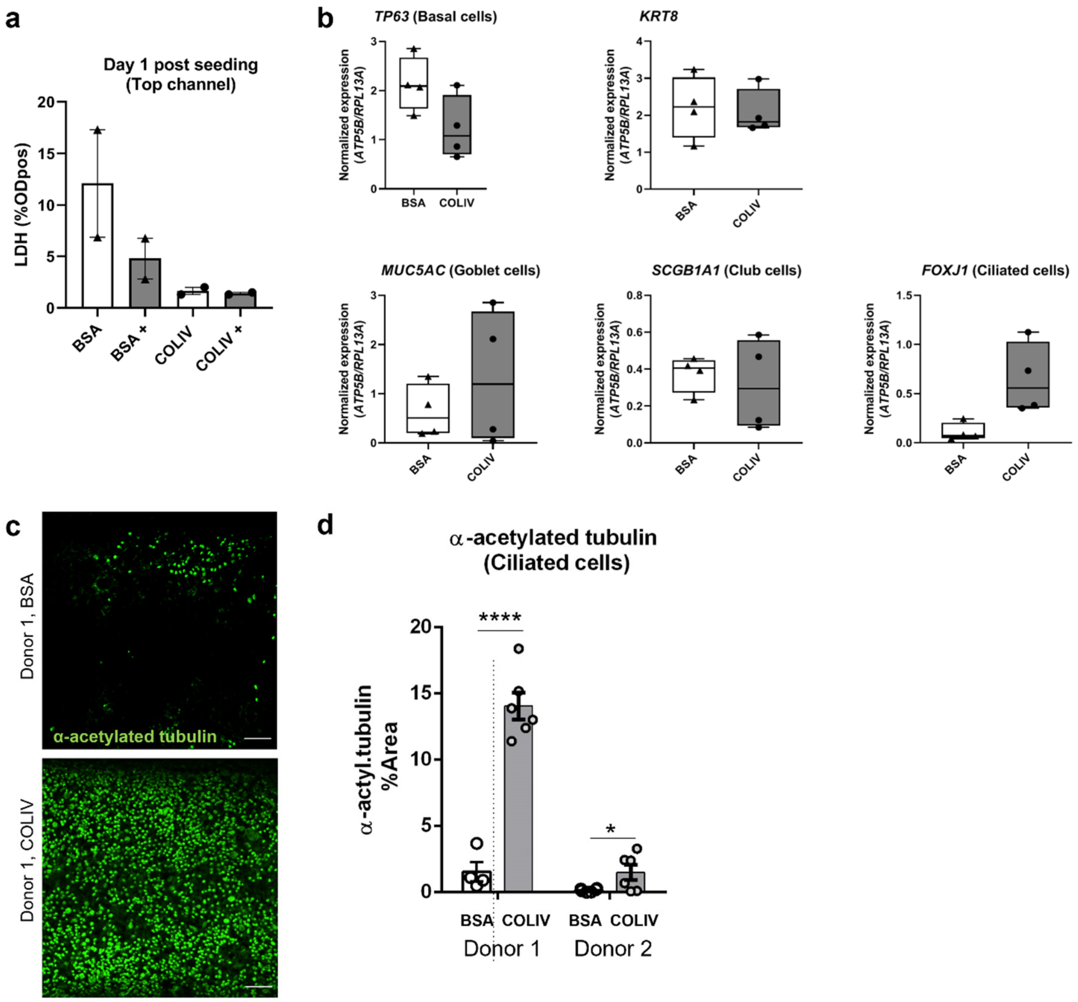


Supplementary Figure 6: Mucociliary differentiation is comparable between CTL and AF/S chips.
a, LDH analysis on medium collected from the bottom channel reservoir after 24 h of flowing between day 13 and 14 of ALI. Data are from N=3 different donors, 1 chip each. **b,** Representative IF staining of club (SCGB1A1), goblet (muc5ac), and ciliated cells (acetylated alpha-tubulin, ACTUB) in AF/S chip Scalebar: 100 µm. **c,** CBF analysis. Data from 4 biological replicates per condition from N=2 donors each. Each black dot represents approximately 1 ciliated cell (CTL Chip: 2481; AF/S Chip: 2046). Red dots indicate individual means of biological replicates and line indicates their median. **d,** Example of fluorescent bead flow speeds in AF/S chip. Scale bar: 200 µm. **e**, Quantification of area fraction covered by MCC (left) and area-averaged MCC speed (right). Depicted are mean +/- SEM of 8 AF/S chips from N=8 donors (one chip per donor) and 9 CTL chips from N=9 donors (one chip per donor). *, p<0.05; **, p<0.01; two-tailed Welch's t-test.



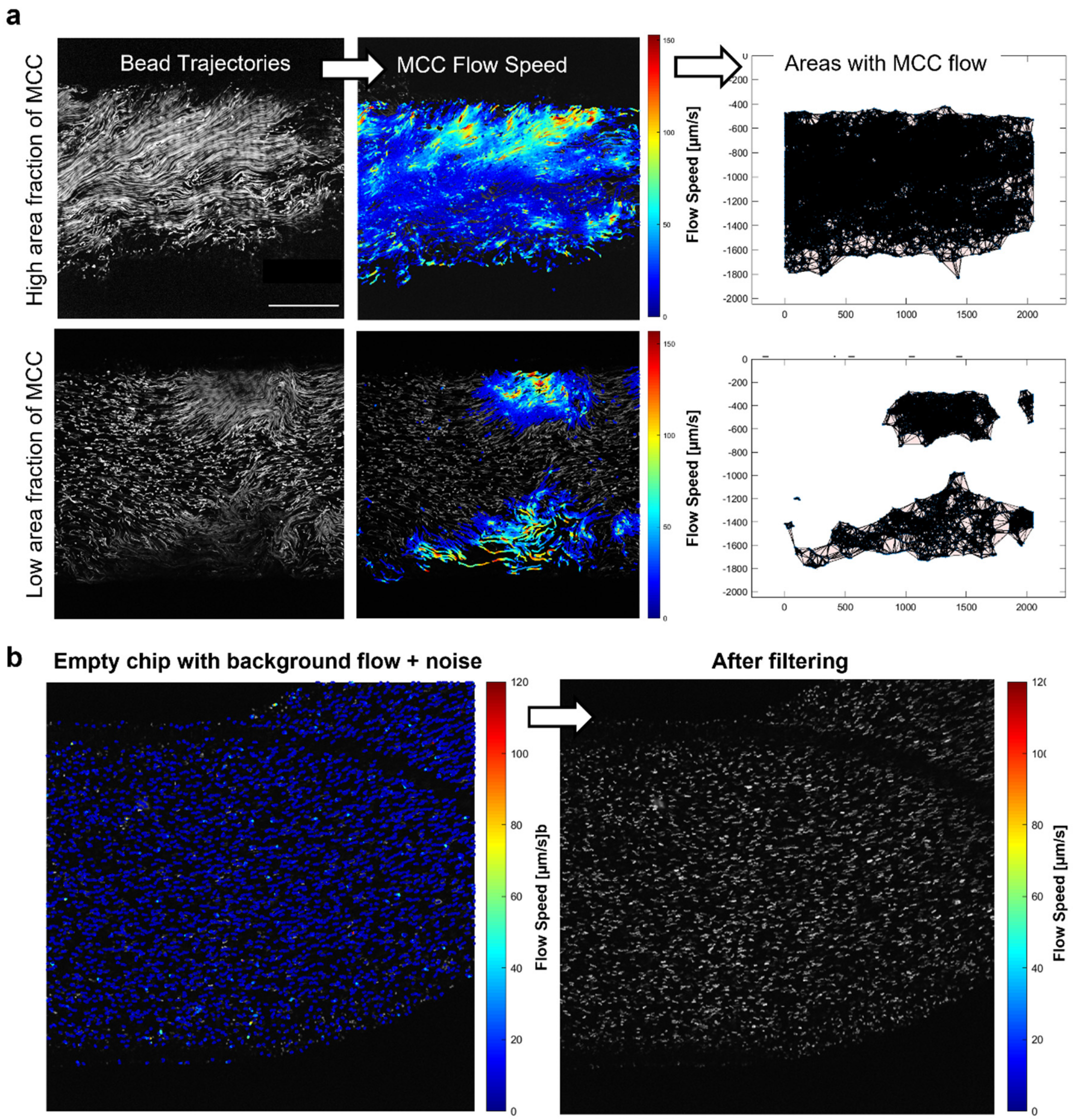
Supplementary Figure 7: VANGL1 crescent density is comparable between chips at day 14 of ALI and inserts at day 35 of ALI.

a, Representative staining of VANGL1 at day 35 ALI in same donor as in Fig.3 at day 14 ALI. Scale bar: 50 µm. **b,** Quantification of VANGL1 crescent density in chips at day 14 ALI compared to inserts at day 14 ALI and day 35 ALI. N=3 donors, one chip or insert each; each point is mean value from 1 insert or chip; columns and error bars represent mean +/- SEM. .**c,** Raw intensity image of VANGL1 IF stain. Scale bar: 50 µm. **d,** Detected VANGL1 crescents using Hessian matrix based multiscale analysis of curvature in Matlab.



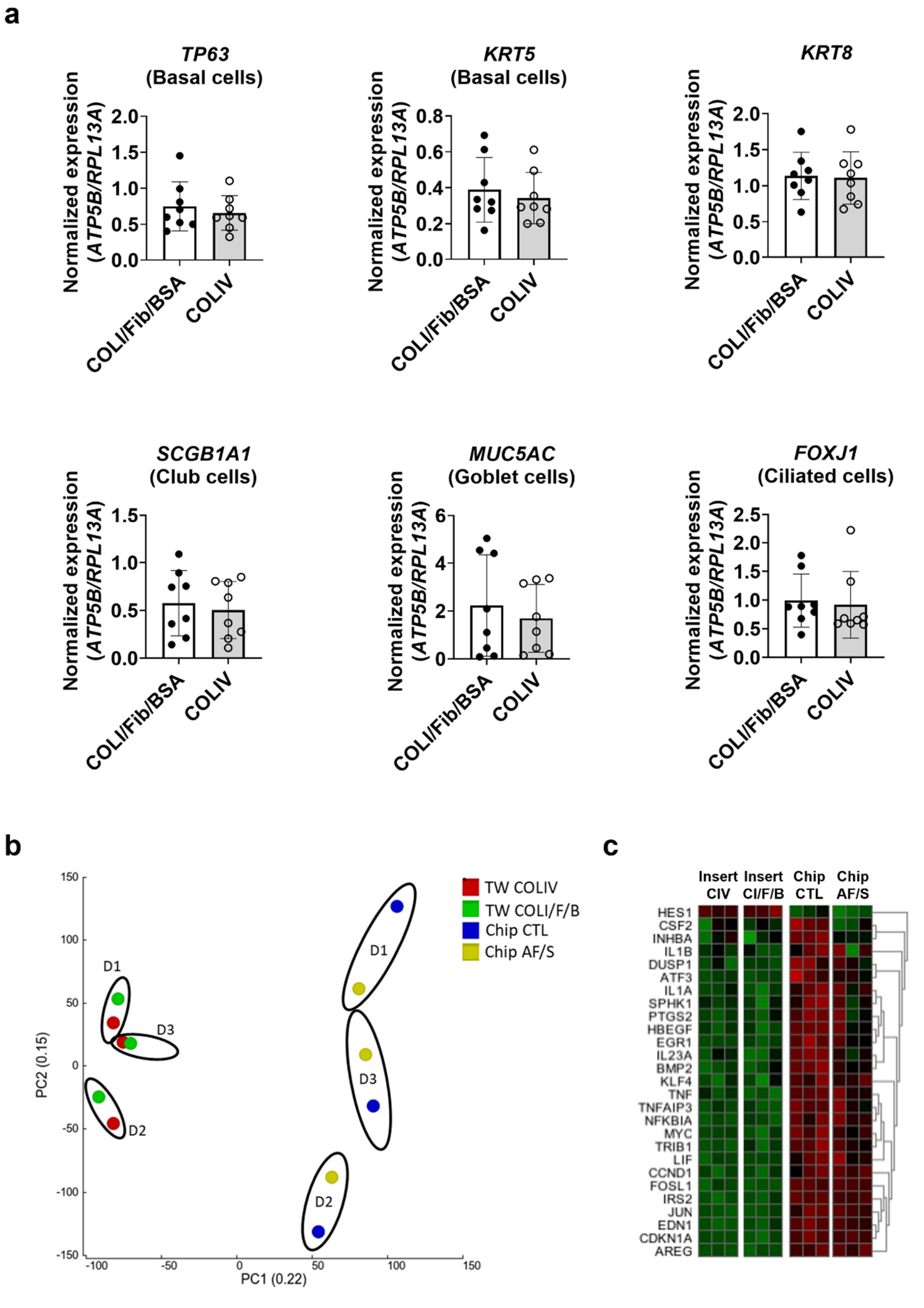
Supplementary Figure 8: Beneficial effect of Collagen IV coating on pHBEC viability and differentiation on Chip.

a, LDH analysis on medium collected from the top channel reservoir after 24 h of flowing, 24 h after seeding from chips coated with BSA, BSA and pluronic treatment (+) or collagen 4 (COLIV) with and without pluronic treatment. Data are from N=2 donors, 1 chip per donor. Data are depicted as mean +/- SEM. **b,** At 14 days post air-liquid interface (ALI) cells were lysed and RNA was isolated followed by cDNA synthesis to assess gene expression of *TP63* (basal cells), *KRT8* (intermediate cells), *MUC5AC* (goblet cells), *SCGB1A1* (club cells) and *FOXJ1* (ciliated cells). Triangles: BSA-coated chips, black circles: COLIV-coated chips, data are shown as target gene expression normalized for the geometric mean expression of the reference genes ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide (*ATP5B*), β2-microglobulin (B2M) and Ribosomal Protein L13a (*RPL13A*); N=4 different donors, one chip/donor. **c,** Representative IF stains of cilia in BSA- and COLIV- coated chips. **d,** Quantification of surface area fraction positive for cilia staining for N= 2 donors, 1 chip per donor. Each data point represents one field of view. Scalebar: 100 µm. Column and error bars represent mean +/- SEM. *p<0.05, ****p<0.0001, t-test with Holm-Sidak multiple comparison correction.



Supplementary Figure 9: MCC quantification and noise removal.

a, MCC flow speed along each bead trajectory is determined from bead displacement (left and center column), then averaged for each coordinate in order to get the Eulerian flow field that allows estimating the continuous areas with MCC flow (right column). Trajectories due to background flow or noise are filtered out based on trajectory speed, length, and directness, as seen in comparison of movies with high versus low fraction of MCC (top and bottom row). Scalebar 500 µm. **b,** Detected flow speeds in empty chip (left, colored lines overlaid on bead trajectories) are ignored (right) after filtering with parameters determined by machine learning approach.



Supplementary Figure 10: comparison of insert cultures with different coatings.

a, Gene expression of *TP63* (basal cells), *KRT5* (basal cells), *KRT8* (differentiated non-basal cells), *SCGB1A1* (club cells), *MUC5AC* (goblet cells) and *FOXJ1* (ciliated cells) in collagen IV (COLIV)-coated or Collagen 1 (COLI), fibronectin and BSA-coated inserts at day 14 ALI. Data are shown as target gene expression normalized for the geometric mean expression of the reference genes *ATP5B* and *RPL13A*; N=8 donors, one chip or insert per donor. Data are depicted as mean +/- SEM. **b**, PCA on all transcriptomes showing distinct clusters of airway epithelial cells from N=3 donors in CTL chips, AF/S chips, or cell culture inserts coated with collagen IV or collagen I, fibronectin and BSA. **c,** Heat maps displaying the Z score of selected DEGs between insert, in CTL chips, AF/S chips, or cell culture inserts coated with collagen IV or collagen I, fibronectin and BSA, that were most frequently expressed in the top related pathways when comparing COLIV insert and CTL Chip with N=3 donors that are paired between insert, CTL chip and AF/S chip cultures (one donor per column).

Supplementary Table 1. Differentially expressed genes (*n*=182) between airway epithelial cells cultured on chip compared to cultures on inserts.

S.No	ID	Base Mean	log2 Fold Change	q value	Gene Name
1	ENSG00000138166	2554.40	2.71	2.21E-56	DUSP5
2	ENSG00000175592	1178.69	2.54	9.51E-56	FOSL1
3	ENSG00000142871	5839.09	2.92	2.65E-42	CYR61
4	ENSG00000067082	6509.70	1.94	6.32E-39	KLF6
5	ENSG00000005108	316.95	3.50	8.53E-33	THSD7A
6	ENSG00000173706	2357.66	1.86	1.21E-31	HEG1
7	ENSG00000116285	6509.04	1.74	6.55E-31	ERRFI1
8	ENSG00000112182	197.20	2.76	2.03E-26	BACH2
9	ENSG00000125740	214.15	2.80	6.3E-26	FOSB
10	ENSG00000117289	26962.15	-1.60	1.27E-25	TXNIP
11	ENSG00000185950	2895.95	1.88	1.75E-25	IRS2
12	ENSG00000118503	5539.40	2.32	2.42E-24	TNFAIP3
13	ENSG00000122861	10509.12	1.56	2.89E-24	PLAU
14	ENSG00000232810	72.60	3.45	5.27E-24	TNF
15	ENSG00000078401	1044.29	3.02	6.7E-24	EDN1
16	ENSG00000135069	3214.49	2.41	8.52E-24	PSAT1
17	ENSG00000128965	829.91	1.87	5.34E-22	CHAC1
18	ENSG00000129521	1306.88	-1.65	3.64E-19	EGLN3
19	ENSG00000137193	2099.09	1.82	3.67E-19	PIM1
20	ENSG00000169252	1196.70	1.53	8.47E-19	ADRB2
21	ENSG00000168003	10338.71	1.60	1.57E-18	SLC3A2
22	ENSG00000078081	885.06	2.10	1.52E-17	LAMP3
23	ENSG00000179148	78.37	3.03	4.5E-17	ALOXE3
24	ENSG00000137801	13490.22	2.15	1.03E-16	THBS1
25	ENSG00000151012	2458.37	1.85	1.91E-16	SLC7A11
26	ENSG00000143878	3339.03	1.71	2.19E-16	RHOB

27	ENSG00000128591	111.52	2.83	2.2E-16	FLNC
28	ENSG00000122254	314.28	3.08	4.69E-16	HS3ST2
29	ENSG00000187840	1340.47	1.53	4.98E-16	EIF4EBP1
30	ENSG00000101255	3540.07	2.90	3.88E-15	TRIB3
31	ENSG00000165272	27793.94	-1.52	9.26E-15	AQP3
32	ENSG00000116741	526.16	-1.74	1.02E-14	RGS2
33	ENSG00000139289	3564.85	1.57	1.22E-14	PHLDA1
34	ENSG00000168646	840.17	-1.84	5.62E-14	AXIN2
35	ENSG00000221968	873.33	2.06	1.08E-13	FADS3
36	ENSG00000179388	298.44	2.27	1.79E-13	EGR3
37	ENSG00000212724	38.40	2.93	2.96E-13	KRTAP2-3
38	ENSG00000163734	705.93	2.24	3.99E-13	CXCL3
39	ENSG00000078018	1155.01	2.23	8.65E-13	MAP2
40	ENSG00000130598	266.37	-1.83	1.07E-12	TNNI2
41	ENSG00000138675	106.24	2.27	1.78E-12	FGF5
42	ENSG00000102032	92.33	2.64	2.22E-12	RENBP
43	ENSG00000103257	13838.64	1.87	2.79E-12	SLC7A5
44	ENSG00000109321	198.64	1.86	4.12E-12	AREG
45	ENSG00000033327	513.55	1.64	1.03E-11	GAB2
46	ENSG00000173846	973.85	1.68	1.2E-11	PLK3
47	ENSG00000074416	1878.28	1.55	1.24E-11	MGLL
48	ENSG00000163638	877.13	2.64	1.92E-11	ADAMTS9
49	ENSG00000153132	855.93	1.84	2.09E-11	CLGN
50	ENSG00000196167	5387.56	-1.51	4.03E-11	COLCA1
51	ENSG00000115556	112.75	-1.87	6.15E-11	PLCD4
52	ENSG00000106070	1612.77	1.59	7.16E-11	GRB10
53	ENSG00000070669	3431.99	1.52	7.92E-11	ASNS
54	ENSG00000128165	650.51	2.10	8.87E-11	ADM2
55	ENSG00000138623	1978.41	2.46	9.91E-11	SEMA7A
56	ENSG00000079308	5281.32	-1.60	1.81E-10	TNS1
57	ENSG00000178726	1449.10	1.75	2.06E-10	THBD
58	ENSG00000130294	34.29	2.61	2.09E-10	KIF1A
59	ENSG00000113739	2337.69	1.96	2.12E-10	STC2
60	ENSG00000233539	305.89	-1.55	2.14E-10	AC011294.3
61	ENSG00000115008	807.50	2.22	2.51E-10	IL1A
62	ENSG00000143867	402.76	-1.63	6.89E-10	OSR1
63	ENSG00000073756	5561.02	2.07	1.1E-09	PTGS2
64	ENSG00000011422	1160.57	1.68	1.23E-09	PLAUR
65	ENSG00000244242	2970.49	-1.51	1.4E-09	IFITM10
66	ENSG00000167995	115.69	2.09	1.5E-09	BEST1
67	ENSG00000092621	3482.85	1.58	2.34E-09	PHGDH
68	ENSG00000189410	241.52	2.07	2.43E-09	SH2D5
69	ENSG00000187678	136.97	1.70	2.57E-09	SPRY4
70	ENSG00000153446	366.64	-1.62	2.82E-09	C16orf89
71	ENSG00000197446	5021.70	-1.88	2.82E-09	CYP2F1

72	ENSG00000147437	77.08	-1.98	2.99E-09	GNRH1
73	ENSG00000153823	168.52	2.25	3.52E-09	PID1
74	ENSG00000115112	22267.74	-1.55	3.82E-09	TFCP2L1
75	ENSG00000178031	108.60	-2.47	7.05E-09	ADAMTSL1
76	ENSG00000172548	154.30	2.31	9.9E-09	NIPAL4
77	ENSG00000110944	106.43	1.98	9.9E-09	IL23A
78	ENSG00000111981	122.02	2.32	1.03E-08	ULBP1
79	ENSG00000136404	110.39	1.81	1.04E-08	TM6SF1
80	ENSG00000162892	18.15	2.45	1.05E-08	IL24
81	ENSG00000177614	215.91	1.88	1.75E-08	PGBD5
82	ENSG00000162772	2355.44	1.84	1.79E-08	ATF3
83	ENSG00000169429	11410.93	1.69	1.82E-08	IL8
84	ENSG00000008517	631.02	1.97	1.85E-08	IL32
85	ENSG00000156510	399.76	1.94	1.92E-08	HKDC1
86	ENSG00000196924	40824.80	1.53	2.1E-08	FLNA
87	ENSG00000103034	410.59	2.02	2.38E-08	NDRG4
88	ENSG00000174788	31.96	-2.18	2.67E-08	PCP2
89	ENSG00000198759	247.00	-1.52	3.36E-08	EGFL6
90	ENSG00000128342	1554.74	1.61	3.44E-08	LIF
91	ENSG00000076706	407.26	2.15	5.34E-08	MCAM
92	ENSG00000105499	65.05	2.17	6.95E-08	PLA2G4C
93	ENSG00000197408	343.49	-1.86	7.39E-08	CYP2B6
94	ENSG00000146674	67497.38	-1.51	8.89E-08	IGFBP3
95	ENSG00000060982	825.50	1.82	1.59E-07	BCAT1
96	ENSG00000154342	60.52	-1.87	1.62E-07	WNT3A
97	ENSG00000164266	41.20	2.26	1.66E-07	SPINK1
98	ENSG00000223865	402.46	1.57	1.66E-07	HLA-DPB1
99	ENSG00000233930	108.61	1.53	1.81E-07	KRTAP5-AS1
100	ENSG00000081041	1159.03	2.03	1.96E-07	CXCL2
101	ENSG00000026559	405.12	1.83	2.07E-07	KCNG1
102	ENSG00000153294	1127.03	1.58	2.86E-07	GPR115
103	ENSG00000210195	105.15	1.56	3.86E-07	MT-TT
104	ENSG00000206538	697.11	1.90	4.57E-07	VGLL3
105	ENSG00000127561	68.27	1.58	4.78E-07	SYNGR3
106	ENSG00000183837	39.15	-1.83	5.82E-07	PNMA3
107	ENSG00000172602	470.02	1.78	9.54E-07	RND1
108	ENSG00000136010	473.92	2.02	1.5E-06	ALDH1L2
109	ENSG00000165868	150.33	1.54	1.52E-06	HSPA12A
110	ENSG00000072041	215.36	1.85	1.71E-06	SLC6A15
111	ENSG00000164400	58.04	2.03	1.89E-06	CSF2
112	ENSG00000069431	770.80	-1.82	2.32E-06	ABCC9
113	ENSG00000128422	9823.81	1.83	2.63E-06	KRT17
114	ENSG00000167994	20.90	2.07	2.8E-06	RAB3IL1
115	ENSG00000100739	41.71	-1.67	2.88E-06	BDKRB1
116	ENSG00000197646	29.46	2.00	3.07E-06	PDCD1LG2

117	ENSG00000081181	337.51	1.61	4.73E-06	ARG2
118	ENSG00000134363	545.84	1.52	4.84E-06	FST
119	ENSG00000162576	40.16	2.02	5.39E-06	MXRA8
120	ENSG00000122786	3998.71	1.54	5.71E-06	CALD1
121	ENSG00000130775	159.39	1.73	8E-06	THEMIS2
122	ENSG00000049323	1263.59	1.54	8.16E-06	LTBP1
123	ENSG00000249601	50.20	-1.97	8.43E-06	CTB-27N1.1
124	ENSG00000112149	47.89	1.85	9.63E-06	CD83
125	ENSG00000179546	29.07	1.95	1.32E-05	HTR1D
126	ENSG00000122641	472.75	1.80	1.63E-05	INHBA
127	ENSG00000101335	1440.40	1.79	1.67E-05	MYL9
128	ENSG00000101670	637.25	1.57	1.73E-05	LIPG
129	ENSG00000162614	144.05	1.81	1.83E-05	NEXN
130	ENSG00000119938	147.15	-1.52	1.97E-05	PPP1R3C
131	ENSG00000128655	755.38	-1.55	1.98E-05	PDE11A
132	ENSG00000269962	28.06	1.85	2.25E-05	RP13-238F13.5
133	ENSG00000108551	139.51	1.87	2.43E-05	RASD1
134	ENSG00000152463	115.11	1.83	2.56E-05	OLAH
135	ENSG00000135960	73.81	-1.64	2.92E-05	EDAR
136	ENSG00000158014	77.78	1.81	3.19E-05	SLC30A2
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138	ENSG00000167767	4907.05	1.70	3.85E-05	KRT80
139	ENSG00000139278	774.67	1.68	3.89E-05	GLIPR1
140	ENSG00000184908	44.61	-1.85	4.14E-05	CLCNKB
141	ENSG00000166073	249.18	1.64	5.05E-05	GPR176
142	ENSG00000138685	170.59	1.81	5.45E-05	FGF2
143	ENSG00000123405	41.29	-1.67	5.47E-05	NFE2
144	ENSG00000176170	1093.97	1.50	5.86E-05	SPHK1
145	ENSG00000251323	27.71	1.61	5.87E-05	RP11-452H21.4
146	ENSG00000139211	1824.88	1.60	6.25E-05	AMIGO2
147	ENSG00000101210	115.19	1.70	6.32E-05	EEF1A2
148	ENSG00000129682	77.01	1.66	6.32E-05	FGF13
149	ENSG00000169035	747.43	-1.55	6.75E-05	KLK7
150	ENSG00000115009	852.75	1.60	6.77E-05	CCL20
151	ENSG00000168269	49.99	-1.78	7.92E-05	FOXI1
152	ENSG00000127528	143.27	1.69	8.24E-05	KLF2
153	ENSG00000050165	1027.22	1.58	8.25E-05	DKK3
154	ENSG00000203632	33.22	-1.55	0.000101	AC007690.1
155	ENSG00000196136	1211.05	1.51	0.000111	SERPINA3
156	ENSG00000146592	162.97	1.67	0.000119	CREB5
157	ENSG00000102962	63.74	1.62	0.00013	CCL22
158	ENSG00000242252	20.07	-1.67	0.000143	BGLAP
159	ENSG00000272666	34.66	1.73	0.00015	CTA-384D8.35
160	ENSG00000134668	769.22	1.54	0.000155	SPOCD1
161	ENSG00000203727	42.47	1.64	0.000185	SAMD5

162	ENSG00000268852	12.70	1.71	0.000199	AC132872.2
163	ENSG00000255400	13.20	1.66	0.000215	RP13-631K18.5
164	ENSG00000188175	28.09	-1.70	0.000221	HEPACAM2
165	ENSG00000260953	11.85	1.68	0.000236	RP11-426C22.6
166	ENSG00000182168	40.46	-1.62	0.000239	UNC5C
167	ENSG00000109758	23.27	1.68	0.000253	HGFAC
168	ENSG00000087128	57.30	-1.55	0.000319	TMPRSS11E
169	ENSG00000269959	11.58	-1.64	0.000391	SPACA6P-AS
170	ENSG00000139209	28.34	1.64	0.0004	SLC38A4
171	ENSG00000164741	282.16	1.53	0.000471	DLC1
172	ENSG00000078579	12.20	-1.60	0.000488	FGF20
173	ENSG00000184551	30.21	1.57	0.000512	AC132872.1
174	ENSG00000124191	97.10	1.59	0.000653	TOX2
175	ENSG00000178150	39.68	1.54	0.000696	ZNF114
176	ENSG00000260604	286.44	1.52	0.000723	RP1-140K8.5
177	ENSG00000082482	23.00	-1.58	0.000743	KCNK2
178	ENSG00000163395	16.45	1.57	0.000783	IGFN1
179	ENSG00000122877	99.60	1.50	0.001139	EGR2
180	ENSG00000149591	4655.50	1.52	0.001196	TAGLN
181	ENSG00000081138	10.55	-1.52	0.001329	CDH7
182	ENSG00000067445	20.64	-1.50	0.001395	TRO

Supplementary Table 2. Details of top 10 pathways associated with DEGs between cultures on inserts and control chips.

S.No	**Pathway description [# genes]**	**Pathway Description**	**#DEGs**	**ID**	**p value**	**q value**
1	GOBP_RESPONSE_TO_OXYGEN_CONTAINING_COMPOUND [1653]	Any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of an oxygen-containing compound stimulus. [GOC:pr, GOC:TermGenie]	43	GO:1901700	$2.76\ e^{-22}$	$2.87\ e^{-18}$
2	GOMF_SIGNALING_RECEPTOR_REGULATOR_ACTIVITY [543]	Binds to and modulates the activity of a receptor. [GOC:ceb]	27	GO:0030545	$4.15\ e^{-21}$	$2.16\ e^{-17}$
3	GOBP_TISSUE_DEVELOPMENT [1916]	The process whose specific outcome is the progression of a tissue over time, from its formation to the mature structure. [ISBN:0471245208]	44	GO:0009888	$1.03\ e^{-20}$	$3.56\ e^{-17}$

4	GOBP_REGULATION_OF_CELL_POPULATION_PROLIFERATION [1745]	Any process that modulates the frequency, rate or extent of cell proliferation. [GOC:jl]	41	GO:0042127	$1.23\ e^{-19}$	$3.12\ e^{-16}$
5	GOMF_MOLECULAR_FUNCTION_REGULATOR [1953]	A molecular function regulator regulates the activity of its target via non-covalent binding that does not result in covalent modification to the target. Examples of molecular function regulators include regulatory subunits of multimeric enzymes and channels. Mechanisms of regulation include allosteric changes in the target and competitive inhibition. [GOC:dos, GOC:pt]	43	GO:0098772	$1.5\ e^{-19}$	$3.12\ e^{-16}$
6	GOBP_LOCOMOTION [1921]	Self-propelled movement of a cell or organism from one location to another. [GOC:dgh]	42	GO:0040011	$5.59\ e^{-19}$	$9.7\ e^{-16}$
7	GOMF_SIGNALING_RECEPTOR_BINDING [1550]	Binding to one or more specific sites on a receptor molecule, a macromolecule that undergoes combination with a hormone, neurotransmitter, drug or intracellular messenger to initiate a change in cell function. [GOC:bf, GOC:ceb, ISBN:0198506732]	38	GO:0005102	$8.29\ e^{-19}$	$1.23\ e^{-15}$
8	GOBP_REGULATION_OF_MULTICELLULAR_ORGANGANISMAL_DEVELOPMENT [1394]	Any process that modulates the frequency, rate or extent of multicellular organismal development. [GOC:obol]	36	GO:2000026	$1.53\ e^{-18}$	$1.99\ e^{-15}$
9	GOBP_REGULATION_OF_CELL_DEATH [1643]	Any process that modulates the rate or frequency of cell death. Cell death is the specific activation or halting of processes within a cell so that its vital functions markedly cease, rather than simply deteriorating gradually over time, which culminates in cell death. [GOC:dph, GOC:tb]	38	GO:0010941	$5.73\ e^{-18}$	$6.62\ e^{-15}$
10	GOBP_CELL_MIGRATION [1556]	The controlled self-propelled movement of a cell from one site to a destination guided by molecular cues. Cell migration is a central process in the development and maintenance of multicellular organisms. [GOC:cjm, GOC:dph, GOC:ems,	37	GO:0016477	$6.92\ e^{-18}$	$7.19\ e^{-15}$

		GOC:pf, Wikipedia:Cell_migration]				

Supplementary Table 3. Differentially expressed genes (*n*=20) between airway epithelial cells cultured in airflow and stretch (AF/S)-exposed chips and control chip cultures.

S.No	ID	Base Mean	log2 Fold Change	q value	Gene Name
1	ENSG00000122641	523.795	-1.587	1.38E-19	INHBA
2	ENSG00000177614	235.737	-1.124	3.50E-06	PGBD5
3	ENSG00000100311	564.862	-0.993	0.000189	PDGFB
4	ENSG00000212724	46.661	-0.891	0.001414	KRTAP2-3
5	ENSG00000179362	59.103	-0.851	0.004539	HMGN2P46
6	ENSG00000084731	1352.044	-0.737	0.018856	KIF3C
7	ENSG00000163216	29.640	-0.705	0.033372	SPRR2D
8	ENSG00000139926	3125.493	-0.684	0.005365	FRMD6
9	ENSG00000125845	1002.406	-0.669	0.034607	BMP2
10	ENSG00000206538	959.505	-0.666	0.026932	VGLL3
11	ENSG00000173281	1044.277	-0.626	0.047517	PPP1R3B
12	ENSG00000180573	2059.068	-0.621	0.000833	HIST1H2AC
13	ENSG00000167535	1438.138	-0.568	0.017703	CACNB3
14	ENSG00000105855	4780.501	-0.482	0.025469	ITGB8
15	ENSG00000185650	13894.804	-0.466	0.000771	ZFP36L1
16	ENSG00000151726	9698.947	0.427	0.026932	ACSL1
17	ENSG00000144908	2473.898	0.529	0.026932	ALDH1L1
18	ENSG00000168497	1020.886	0.576	0.004539	SDPR
19	ENSG00000060762	1069.072	0.588	6.24E-05	MPC1
20	ENSG00000119711	731.348	0.622	0.000724	ALDH6A1

Supplementary Table 4. Donor characteristics.

Number of donors	13
Male/Female	6/6*
Age (years) mean [SD]	61.7 [9.4]*
BMI mean [SD]	26.0 [4.7]*
Smoking Status (non-/ex-/smokers)	5/5/1**

Abbreviations: SD (standard deviation); BMI (body mass index)

*Data available from 12 donors; ** Data available from 11 donors

Supplementary Table 5. Media/inhibitor strategies for reducing epithelial migration to bottom channel.

Media types	
PneumaCult ALI	StemCell 05001
B/D complete using supplements without hydrocortisone	ScienCell custom order
PneumaCult ALI w/o hydrocortisone	StemCell 05001
Vertex ALI medium	Neuberger et al, 2011[1]
Airway organoid medium	Sachs et al, 2019[2]
Cell culture compounds	
Retinoic acid	15; 150; 1500 ng/ml; Sigma R2625
Dexamethasone	0.1; 1; 10 µM; Sigma D4902
Inhibitors	
AG-1478 Epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor	30; 150; 300 nM; Sigma T4182
PD098059 Mitogen-Activated Protein Kinase inhibitor	10 µM; Sigma P215
Y-27632 Rho-associated, coiled-coil containing protein kinase (ROCK) inhibitor	5 µM; Cayman Chemical 10005583
GM6001 Matrix metalloprotease (MMP) inhibitor	0.2; 0.5; 25 nM; Sigma CC1000
SB202190 + A83-01 P38 MAPK inhibitor + TGFβ kinase/activin receptor-like kinase (ALK 5) inhibitor	500 nM; 500 nM; Sigma S7067; Tocris 2939
SB202190 + A83-01 + noggin	500 nM; 500 nM; 100 ng/ml Sigma S7067; Tocris 2939; Peprotech 120-10c

P38 MAPK inhibitor + TGFβ ALK inhibitor + Bone Morphogenetic Protein (BMP) Inhibitor	

References:

1. Neuberger, T., Burton, B., Clark, H. & Van Goor, F. Use of primary cultures of human bronchial epithelial cells isolated from cystic fibrosis patients for the pre-clinical testing of CFTR modulators. *Methods Mol Biol* **741**, 39–54 (2011).

2. Sachs, N. *et al.* Long-term expanding human airway organoids for disease modeling. *EMBO J* **38**, (2019).

Supplementary Table 6. Primer sequences.

Gene	forward sequence (5' to 3')	reverse sequence (5' to 3')
ATP5B	TCACCCAGGCTGGTTCAGA	AGTGGCCAGGGTAGGCTGAT
RPL13A	AAGGTGGTGGTCGTACGCTGTG	CGGGAAGGGTTGGTGTTCATCC
KRT5	AGGAGTTGGACCAGTCAACAT	TGGAGTAGTAGCTTCCACTGC
TP63	CCACCTGGACGTATTCCACTG	TCGAATCAAATGACTAGGAGGGG
KRT8	TCCTCAGGCAGCTATATGAAGAG	GGTTGGCAATATCCTCGTACTGT
FOXJ1	GGAGGGGACGTAAATCCCTA	TTGGTCCCAGTAGTTCCAGC
SCGB1A1	ACATGAGGGAGGCAGGGGCTC	ACTCAAAGCATGGCAGCGGCA
MUC5AC	CCTTCGACGGACAGAGCTAC	TCTCGGTGACAACACGAAAG
RSPH4A	GAAGGGACGTGAGCTATAACAAC	GCAGGTAAGCCTTAGCATTCTGA
DNAH11	CAACAGCTTACCTTTCACCTGA	TTCTTCCCATAAAGTAGCTTGCC
VANGL1	CCGATCCTGTGGAGGGATGA	AAACACCCGTGGCATGTCA
PTGS2	TAAGTGCGATTGTACCCGGAC	TTTGTAGCCATAGTCAGCATTGT
MMP9	ACCTCGAACTTTGACAGCGAC	GAGGAATGATCTAAGCCCAGC
FN1	TGGAGGAAGCCGAGGTTT	CAGCGGTTTGCGATGGTA

Supplementary Table 7. Antibodies used for confocal imaging.

Antibody	Supplier	Catalog #	species	Antibody dilution
CK-8	Millipore	MABT329	rat	1:100
p63	Abcam	ab124762	rabbit	1:100
Mucin 5AC	Labvision Neomarkers	MS-145-P1	mouse	1:200
Mucin 5AC	Abcam	218363	rabbit	1:200
CC16	Hycult Biotech	HM2178	mouse	1:50
Acetylated α-Tubulin	Sigma Aldrich	T6793	mouse	1:200
CD144	Thermo Fisher Scientific	14-1449-82	mouse	1:200
Vangl1	Sigma Aldrich	HPA025235	rabbit	1:100

Supplementary Video 1: 3D imaging from top to bottom of the Airway Lung-Chip with primary differentiated bronchial epithelial cells cultured in the top channel and microvascular endothelial cells in the bottom channel at 14 days after introduction of the air-liquid interface. Endothelial cells are stained with phalloidin (red) and the epithelial cells with ZO-1 (green) and nuclei with dapi (blue).

Supplementary Video 2: 3D imaging of the Airway Lung-Chip with focus on the bottom channel compartment with primary differentiated bronchial epithelial cells cultured in the top channel and microvascular endothelial cells in the bottom channel at 14 days after introduction of the air-liquid interface. Endothelial cells are stained with phalloidin (red) and the epithelial cells with ZO-1 (green) and nuclei with dapi (blue).

Supplementary Video 3: Ciliary beat was recorded via phase contrast videomicroscopy. Comparison between chip and insert cultures of the same donor shows no obvious difference at day 14 ALI.

Supplementary Video 4: Mucus clearance was visualized by the motion of suspended fluorescent microparticles (here shown as accumulative timelapse to show the trajectories). Chip cultures show more pronounced clearance than inserts of the same donor at day 14 ALI, but at d35 ALI the inserts exhibit well developed clearance. This shows that mucociliary maturation is accelerated on chip compared to insert cultures.