

## Supplemental Information

### **Wnt/ $\beta$ -catenin signaling alters distal lung epithelial progenitor cell phenotype and function in emphysema**

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#### **1. Supplemental Methods and Material**

##### **Lung epithelial cell isolation**

Lungs of TCF:GFP mice of minimum 8-16 weeks old were flushed through the right heart with PBS, instilled with low-melt agarose (Sigma Aldrich, 9414), and dispase (Corning, 354235) and incubated at room temperature for 45 minutes. Trachea and extrapulmonary airways were removed. The remaining lobes were homogenized in DMEM containing DNase I (PanReac Applichem, A3778) to generate a single-cell suspension that was passed through 70 $\mu$ m and 40 $\mu$ m nylon filters. The single cell suspension was incubated with Microbeads conjugated to antibodies for CD45 (Miltenyi Biotec, 130-052-301) and CD31 (Miltenyi Biotec, 130-097-418), and passed through LS columns (Miltenyi Biotec, 130-091-051) to deplete hematopoietic and endothelial cells. The resultant suspension (CD31-CD45-) was enriched for epithelial cells by positive selection using Epcam (CD326) Microbeads (Miltenyi Biotec, 130-105-958).

##### **Fluorescence-activated cell sorting (FACS) according to EpCAM and GFP fluorescence**

The EpCAM<sup>+</sup>/CD31<sup>-</sup>/CD45<sup>-</sup> single cell suspension from TCF:GFP mouse lungs was prepared as described above. The suspension was stained with APC-conjugated anti-mouse EpCAM antibodies for 15 minutes at room temperature in the dark, washed with PBS, and resuspended in PBS containing 1% FBS and 25mM HEPES. GFP reporter activity in the EpCAM<sup>+</sup> population was assessed based on fluorescence intensity using FACSDiva software (BD Bioscience). The EpCAM<sup>+</sup> Wnt<sup>neg/low/high</sup> populations were sorted using a FACSria Fusion cell sorter (BD Bioscience), washed and centrifuged at 300 g for 10 minutes at 15°C, prior to organoid assay, or RNA isolation.

##### **Flow cytometric analysis**

EpCAM<sup>+</sup>/CD31<sup>-</sup>/CD45<sup>-</sup> cells were stained by APC-conjugated anti mouse EpCAM, PE-conjugated anti mouse CD24 (BioLegend, 138504) and APC-Cy7 conjugated anti mouse Sca-1 antibodies (BioLegend, 108126) or their respective isotype controls, APC rat IgG2a isotype control for EpCAM (BioLegend 400511), PE Rat IgG2c isotype control for CD24 (BioLegend, 400707) and APC/Cy7 Rat IgG2a isotype control for Sca-1 (BioLegend, 400524) for 15 mins at room temperature in dark. The cells were measure by FACS Fortessa cell analyzer (BD Bioscience) and analyzed using FACSDiva software (BD Bioscience).

### **Organoid culture**

Mlg ([MLg2908, CCL206], ATCC) cells at 90% confluence were proliferation-inactivated by incubation in culture medium containing mitomycin C (10µg/ml, Sigma #M4287) for 2 hours, followed by 3 washes in warm PBS, and recovery in culture media for >1 hour. Mlg were trypsinized and resuspended with equal numbers of freshly sorted distal lung epithelial cells in ice-cold DMEM. The cell suspension was mixed in equal volume of growth factor reduced Matrigel (Corning, 354263, diluted to 10mg/ml with sterile PBS). 100 µl of the cell-matrigel mix was added to each 0.4µm permeable insert for 24-well plate (Falcon 353095). 7,000 sorted EpCAM+Wnt<sup>neg/low/high</sup> and 7,000 Mlg cells per well were used for treatments with Wnt inhibitor and activators. For quantification of Ki67 cells and IF for cell markers, 20,000 epithelial and 20,000 Mlg cells per well were used. The mixture was incubated at 37°C for 20 minutes to solidify. Cultures were maintained in DMEM/F12 (Gibco, 11330-032) containing 5% FBS, pen/strep (100 U/ml), 1% GlutaMax (Life Technologies, 35050-061), 1X amphotericin B (Gibco, 15290018), 1X insulin-transferrin-selenium (Gibco #51300-044), recombinant mouse EGF (0.025µg/ml, Sigma, SRP3196), Cholera toxin (0.1µg/ml, Sigma C8052), bovine pituitary extract (30µg/ml, Sigma, P1476), and freshly added all-trans retinoic acid (0.01µM, Sigma, R2625). Y-27632 (10 µM, Tocris, 1254) was added for the first 48 hours of culture to prevent anoikis. Media was refreshed every 2-3 days. iCRT14 (10µM, Tocris, 4299) and CHIR99021 (2µM, Tocris, 4423) were used to manipulate Wnt/β-catenin signaling *in vitro*. Stock solution in dimethyl sulfoxide (DMSO) were stored at -20°C. Prior to treatments, all compounds were further diluted in DMSO so that the volume added to culture was equal across treatment groups. The same volume of DMSO was added for vehicle control.

### **Immunofluorescence on organoids**

Inserts containing Matrigel-organoid cultures were transferred to fresh 24-well plates, washed once underneath with PBS, fixed with ice cold acetone/methanol (1:1) for 15 minutes at -20°C, then washed again with PBS and

blocked in 5% (w/v) bovine serum albumin (BSA, Sigma) in PBS. Whole-mount immunofluorescence was performed with antibodies diluted in PBS with 0.1% (w/v) BSA, 0.1% Triton-X100. Cultures were incubated with primary antibodies at 4°C overnight, washed 3x in PBS for 15 minutes each, incubated with secondary antibodies at 4°C overnight, then incubated with DAPI as counterstain. For results in Figure 3C-D and Figure 4C-D and G, organoids were imaged within the culture plate using a Cytation1 Cell Imaging Multi-Mode Reader (BioTek), and images were obtained and analyzed with Gen5 Image+ software (BioTek). For results in Figure S3A, 4E-F, S4A, organoid cultures were excised from the inserts and mounted on glass slides with fluorescent mounting media (Dako, S3023) and glass coverslips. Immunofluorescence was visualized using a Zeiss LSM710 confocal microscope (Carl Zeiss, Germany). Images were obtained with ZEN2009 software (Carl Zeiss) or imported into Imaris 7.6.5 or 8.0.0 software (Bitplane, Zurich, Switzerland) for analysis. Antibodies used, with concentrations, are summarized in the table below.

### **Immunofluorescence on tissue sections and cytopins**

To generate lung tissue sections, mice were anaesthetized and sacrificed, and lungs were flushed through the heart with PBS. Lungs were instilled with OCT/PBS (1:1) and frozen on liquid nitrogen. For staining, 10µm cryosections were fixed with ice cold acetone/methanol (1:1) for 15 minutes at -20°C, blocked for 2 hours with 5% BSA in PBS, then incubated with primary antibodies at 4°C overnight, washed 3x in PBS, and incubated with secondary antibodies at room temperature for 2 hours. Sections were mounted with fluorescent mounting media containing DAPI (Abcam, 104139) and glass coverslips.

To generate cytopins, glass slides were pre-coated with BSA, and lung EpCAM+ cells were centrifuged at 450 rpm for 6 minutes. Slides were processed for staining as for tissue sections. Immunofluorescence was visualized using a Zeiss LSM710 (Carl Zeiss) or Leica SP8 (Leica) confocal microscope. Images were obtained with ZEN2009 (Carl Zeiss) or LASX (Leica) software or imported into Imaris 7.6.5 or 8.0.0 software (Bitplane, Zurich, Switzerland) for analysis. Secondary antibody specificity was confirmed by absence of signal in control samples stained without primary antibody.

### **Primary antibodies**

<b>Antibody</b>	<b>Host species (Isotype)</b>	<b>Company, Catalog number</b>	<b>Concentration used</b>
SFTPC	Rabbit	Millipore, AB3786	1:200
CC10	Mouse (IgG1)	Santa Cruz, sc-365992	1:50

E-cadherin	Mouse	BD, 610182	1:200
Acetylated tubulin	Mouse (IgG2b)	Abcam, ab24610	1:2000
Ki67	Rat	Dako/Agilent, M7248	1:200
Zo-1	Rabbit	Thermo Fisher, 40-2200	1:100
GFP-Alexa fluor 488	Rabbit	Molecular Probes, A-21311	1:200

### Secondary antibodies

Antibody	Host species	Company, Catalog number	Concentration used
Anti-mouse-Alexa Fluor 647	Goat	Abcam, ab150107	1:200
Anti-mouse-Alexa Fluor 488	Goat	Thermo Fisher, A-11011	1:200
Anti-rabbit-Alexa Fluor 647	Goat	Abcam, ab150079	1:200
Anti-rabbit-Alexa Fluor 555	Goat	Thermo Fisher, A-21429	1:200
Anti-rabbit-Alexa Fluor 488	Goat	Thermo Fisher, A-11055	1:200

### Quantitative real-time polymerase chain reaction (qRT-PCR)

RNA was extracted directly from FACS sorted cells using a RNeasy Micro Kit (Qiagen, 74004) according to the manufacturer's instructions. cDNA synthesis was performed with 100ng of RNA using SuperScript II (Life Technologies). qRT-PCR was performed using SYBR green and the QuantStudio 7 Flex system (Applied Biosystems by Life Technologies). Hypoxanthine guanine phosphoribosyl transferase (Hprt) gene was used as a reference gene in all qRT-PCR reactions. Statistics were performed on  $\Delta Ct$  values ( $\Delta Ct = Ct^{\text{reference}} - Ct^{\text{target}}$ ).

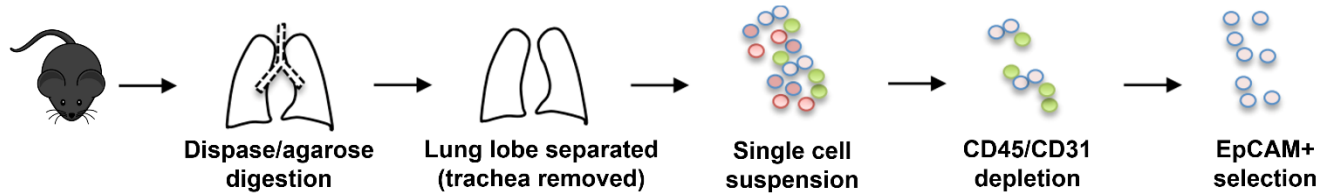
Primers used are listed in table below:

Gene name	Forward primer	Reverse primer
Hprt	CCTAAGATGAGCGCAAGTTGAA	CCACAGGACTAGAACACCTGCTAA
Egfp	ATGGCCGACAAGCAGAAGAA	CTCAGGTAGTGGTTGTCTGGG
Sftpb	ATCCCAAGGGTGTGCTG	GTGCGTCTAGCAGGAGAACT
sftpd	ATAGCCGAGCCTGACAAACA	GAAAGGGCAGCATGTCAGATTG
Scgb1a1	CAGACACCAAAGCCTCCAAC	CAGATGTCCGAAGAAGCTGAG
Foxj1	TTGACTGGGAGGCCATCTTT	GTAGCAGGGCAGTTGATGTG
Muc5ac	TTCGTGTGGAACGAGGATGA	TGGATTTTGGACTGCCGTTG

Muc5b	AGGGTCAGGCAGAGCTGA	TGTAAGGCGCTCATGCTAGG
Itgb4	CAAGTCCAACCTCAGCAACCC	CTCCTGTCCGTTTCATCGAG

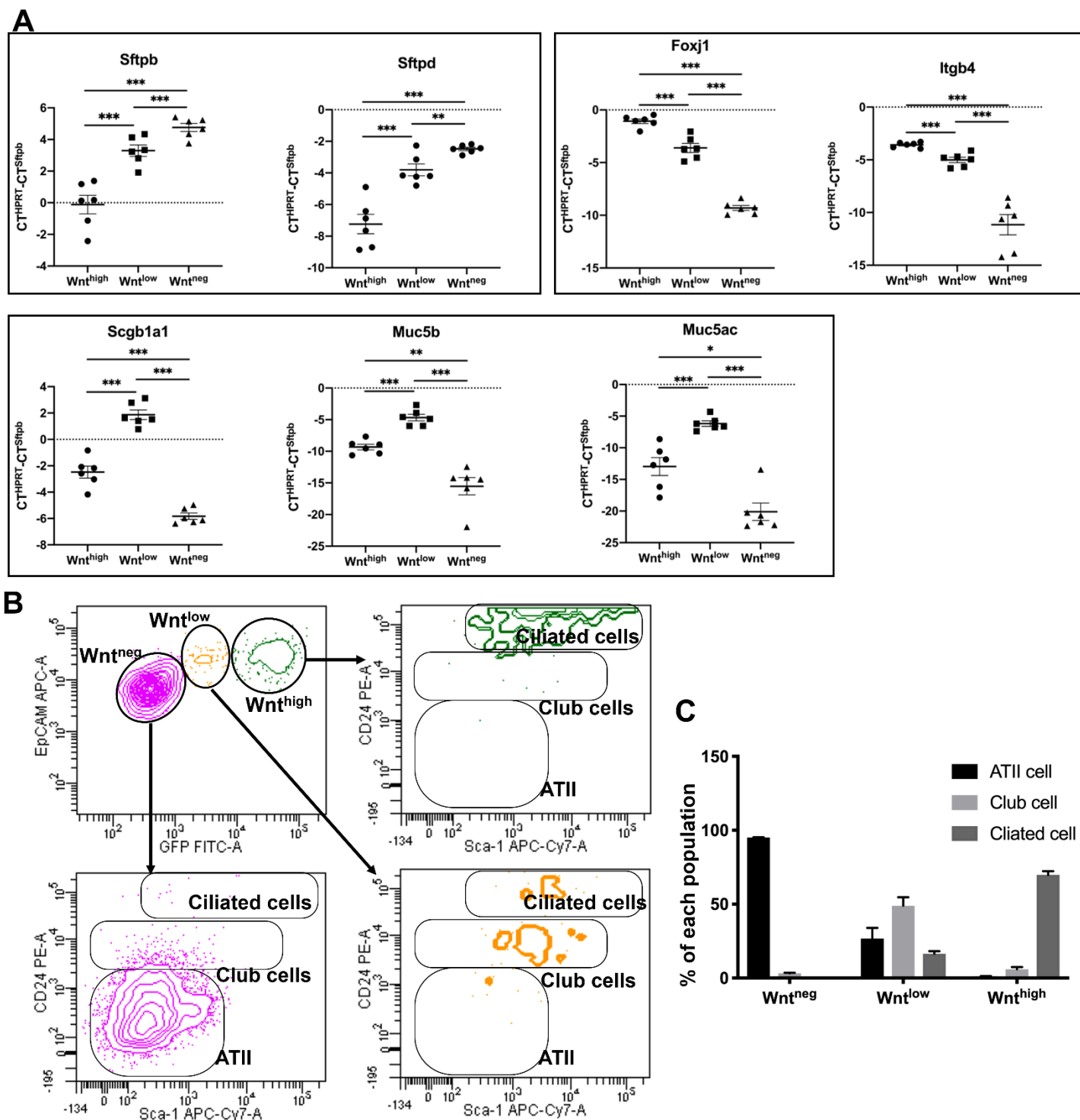
## 2. Supplemental Figures and Figure Legends

### A Magnetic bead isolation of lung epithelial cell



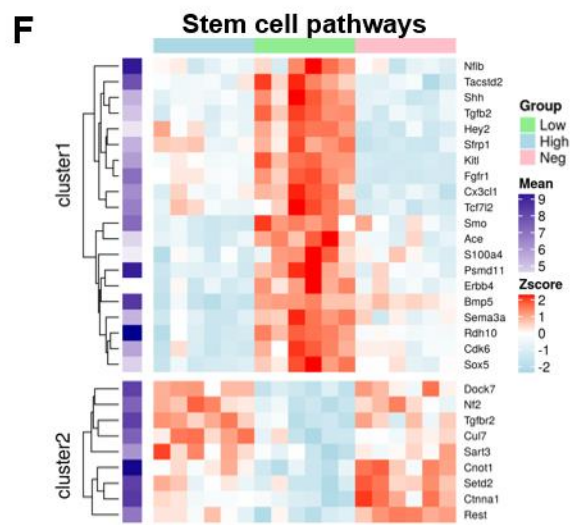
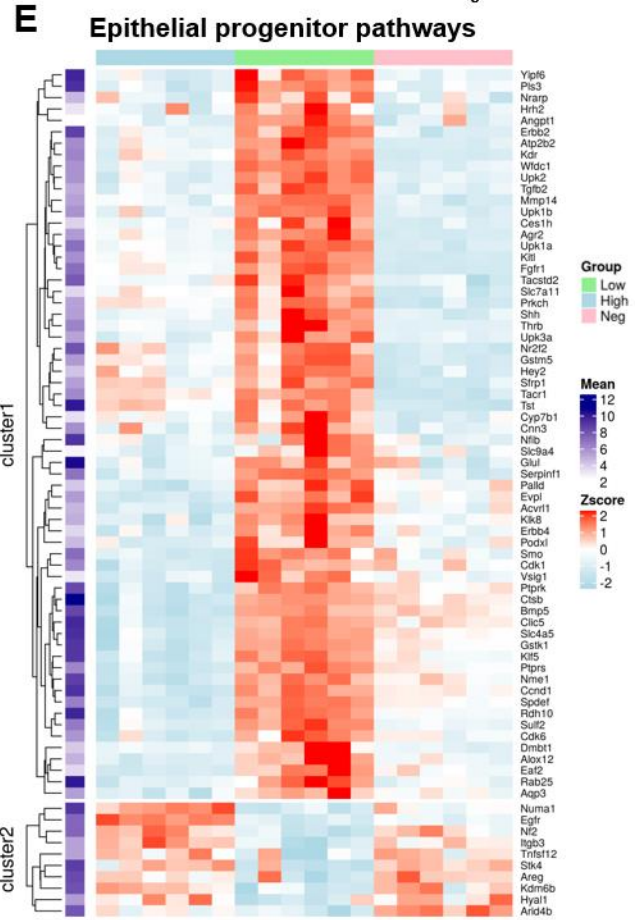
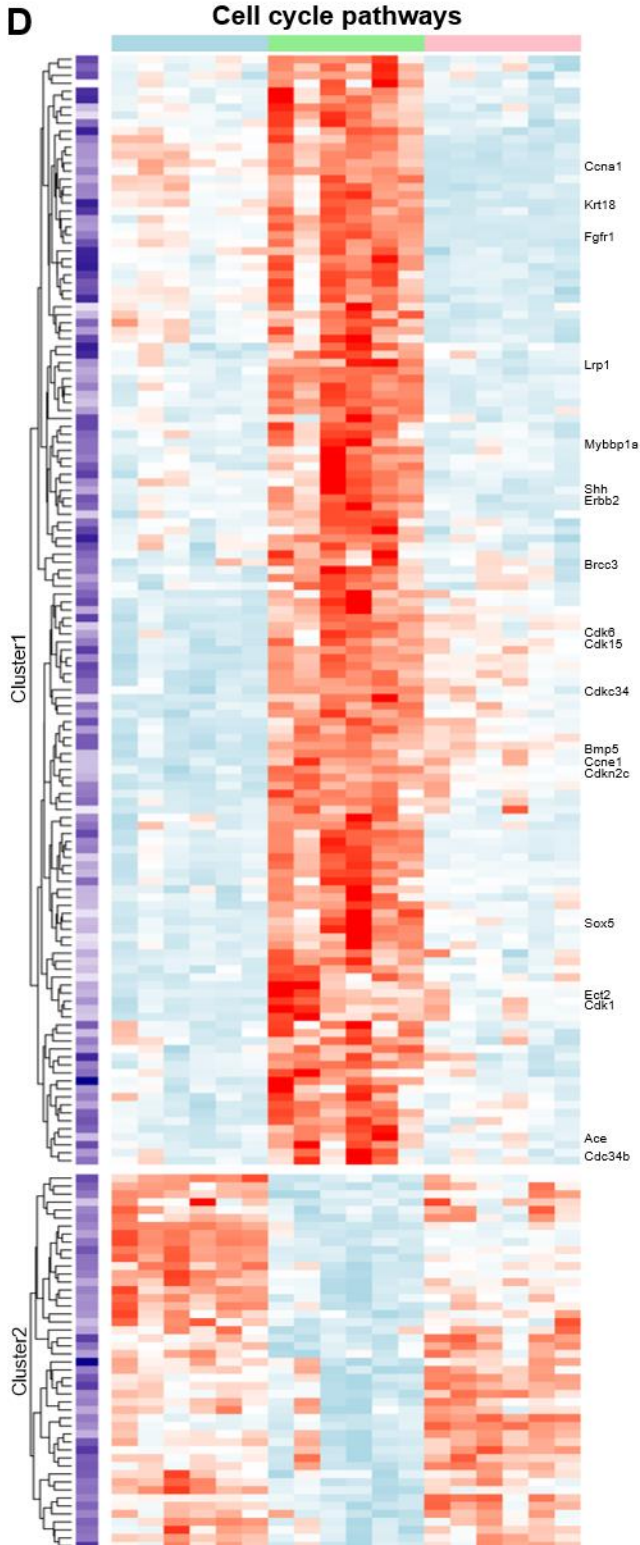
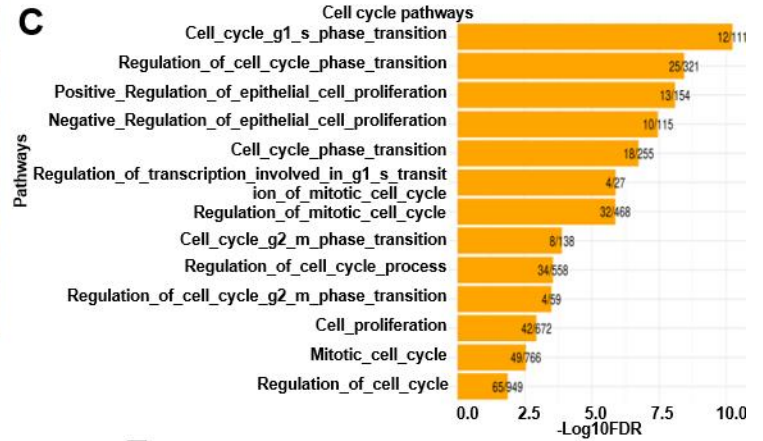
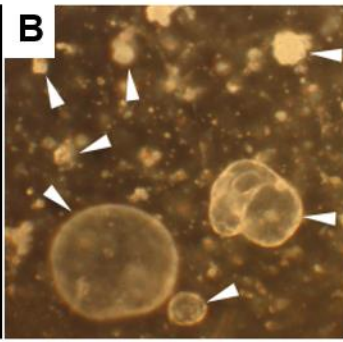
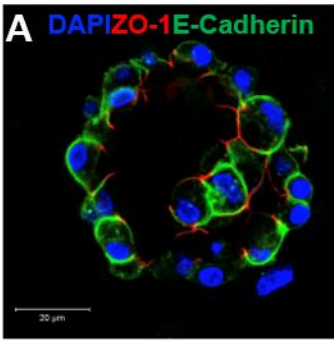
### Supplemental figures S1, related to Figure 1

A. Schematic of magnetic bead separation method to isolate EpCAM+ cells from adult distal mouse lung.



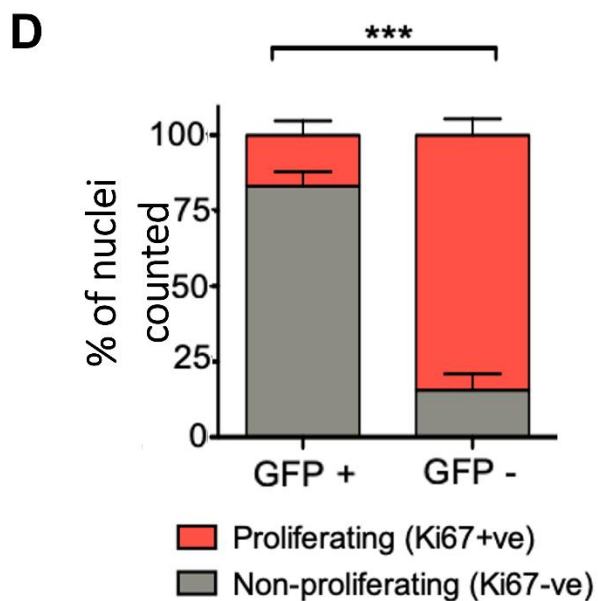
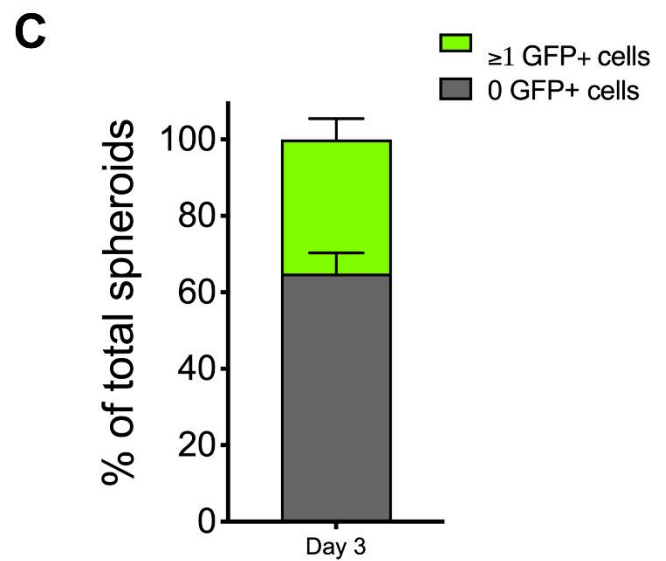
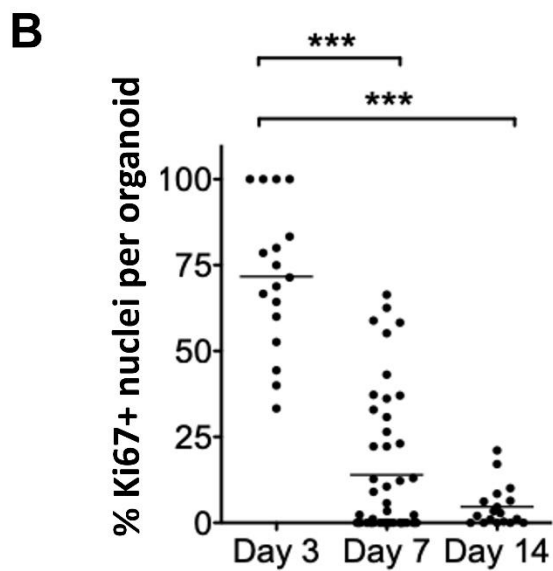
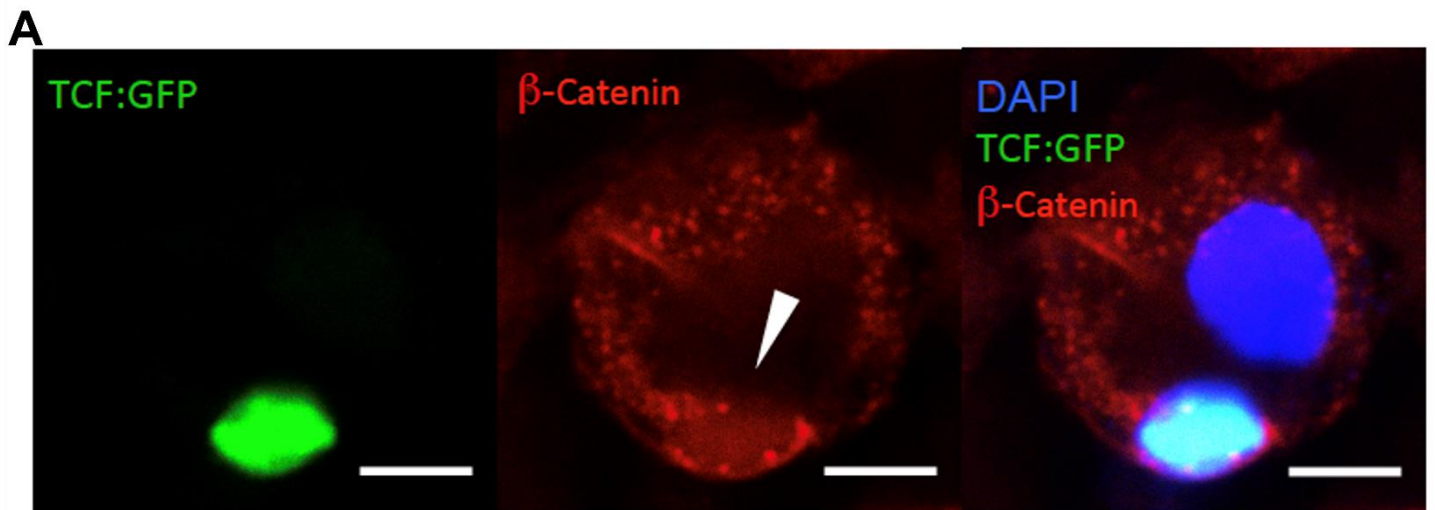
**Supplemental figures S2, related to Figure 2**

A) RT-PCR validation of epithelial cell markers in freshly sorted Wnt<sup>neg/low/high</sup> cells from the lung of TCF:GFP mice (N=6). Statistics was performed on  $\Delta CT$  values ( $\Delta CT = CT^{HPRT} - CT^{target}$ ). \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , Student T test. . B-C) Flow cytometry analysis of the expressions of CD24 and Sca-1 in Wnt<sup>neg/low/high</sup> cells and quantification (C) of the percentages of CD24<sup>neg</sup>/Sca-1<sup>neg</sup> ATII cells, the CD24<sup>low</sup>/Sca-1<sup>+</sup> club cells and CD24<sup>high</sup>/Sca-1<sup>+</sup> ciliated cells in the three populations. Data shown is Mean $\pm$ SEM. N=4 individual animals.



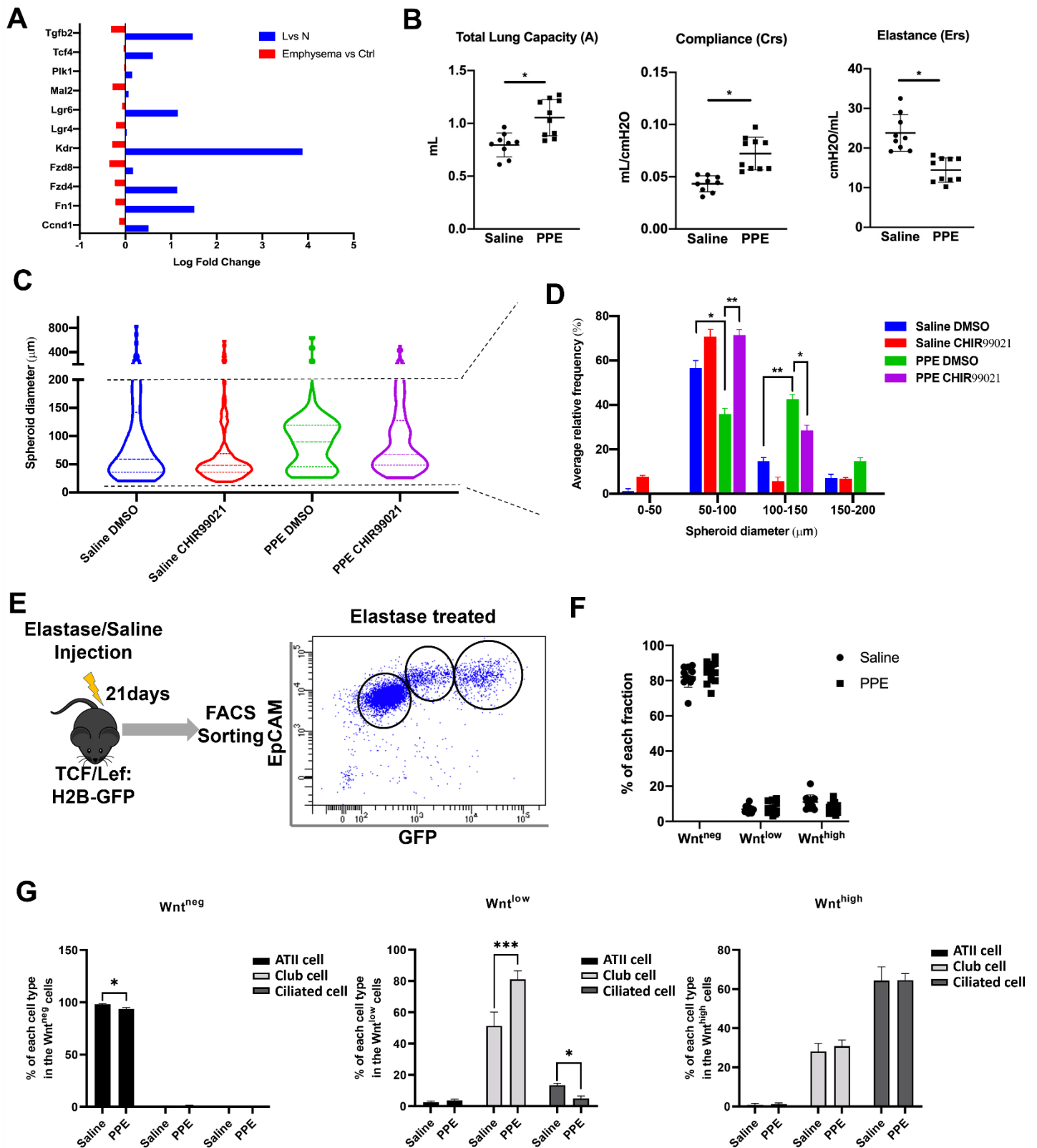
**Supplemental Figure S3, related to Figure 3** A) Whole-mount immunofluorescence staining of day 14 organoid showing a lumen enclosed by ZO-1<sup>+</sup> (in red) and E-Cadherin<sup>+</sup> (in green) epithelial cells. Nuclei are stained with DAPI in blue. Scale bar, 20  $\mu$ m. B) Representative image of organoids at day 28 indicating heterogeneity in size and shape (arrowheads). C) Pathways enriched in the Wnt<sup>low</sup> cells related to cell cycle. Plotted values are  $-\text{Log}_{10}\text{FDR}$ . Data shown on the right end of the bars: the numbers of significantly changed genes (both Wnt<sup>low</sup> vs Wnt<sup>neg</sup> and Wnt<sup>low</sup> vs Wnt<sup>high</sup>)/ total number of genes in each pathway. D-F) Heatmaps show enrichment of genes (FDR<0.05) in cell cycle (D), epithelial progenitor (E), and stem cell (F) pathways in the Wnt<sup>low</sup> cells.





**Supplemental figures S4, related to Figure 4** A) Whole-mount immunofluorescence (IF) for  $\beta$ -catenin (red) and GFP (green) in organoids at day 3. Nuclear  $\beta$ -catenin was observed in GFP<sup>+</sup> cells (white arrowhead). Scale

bar, 5 $\mu$ m. B) Percentage of Ki67<sup>+</sup> cells per organoid at days 3, 7 and 14. Data points represent individual organoids, horizontal lines represent median \*\*\*p<0.001, Kruskal-Wallis with Dunn's post-test (n=17-49 organoids, N=3). C) Quantification of GFP<sup>+</sup> cells in organoids at day 3 (N=3, >500 organoids counted in total). Mean  $\pm$  SEM. D) Quantification of Ki67 co-staining in GFP<sup>+</sup> cells (106 counted) or GFP<sup>-</sup> cells (720 counted) within organoids at day 3 (N=3). Mean  $\pm$  SEM. \*\*\*p<0.001, unpaired t-test.



**Supplemental Figure 5, related to Figure 5.** A) Genes related to Wnt/ $\beta$ -catenin signaling that are up-regulated in  $Wnt^{low}$  cells (compared to  $Wnt^{neg}$  cells,  $p > 0.05$ ) but down-regulated in emphysematous human lung (compared to healthy donor lungs,  $p > 0.05$ ). B) Lung function analysis of PPE and saline treated TCF:GFP mice showed

that the PPE treated lungs showed significant increase of the total lung capacity (A, ml) and compliance (Crs, ml/cmH<sub>2</sub>O) but reduced elastance (Ers, cmH<sub>2</sub>O/ml). C) Violin plot of organoid diameters (Feret's diameter,  $\mu$ m) measured at day 14 (n > 120 organoids, N=3 animals per treatment). D) Histogram of the average percentage of organoids with diameter from 0 to 200 $\mu$ m shown in F. Bin=50  $\mu$ m. Data presented as mean $\pm$ SEM. \*p<0.05, \*\*p<0.01, unpaired student's t test using the Holm-Sidak method. E) Schematic of PPE/saline treatment to the TCF/Lef:H2B-GFP mice and gating strategy for FACS sorting EpCAM+ Wnt<sup>neg/low/high</sup> fractions from elastase treated mouse lungs. F) The percentages of Wnt<sup>neg/low/high</sup> fractions in total epithelial population after PPE treatment were not changed. N=8, individual animals of each treatment.

### 3. Supplemental Tables

**Supplemental Table 1. Wnt<sup>low</sup> cells enriched genes that are down-regulated in COPD.**

	MGI symbol	HGNC symbol	logFC Emphysema vs Healthy	adj.P.Val	logFC. Low vs High	adj.P.Val. Low vs High	logFC.Low vs Neg	adj.P.Val. Low vs Neg
1	Ace2	ACE2	-0.52	3.70E-03	2.74	7.10E-06	2.17	1.10E-04
2	Acp5	ACP5	-0.43	3.10E-02	0.98	6.80E-07	0.71	1.30E-05
3	Adra2a	ADRA2A	-0.48	2.40E-04	0.26	7.10E-03	0.42	1.80E-04
4	Ak3	AK3	-0.21	2.20E-02	0.41	1.20E-04	0.55	9.50E-06
5	Aldh18a1	ALDH18A1	-0.57	5.90E-05	0.2	1.40E-02	0.49	7.10E-06
6	Amdhd1	AMDHD1	-0.53	1.70E-02	0.44	2.70E-04	0.34	3.30E-03
7	Aqp11	AQP11	-0.51	1.40E-03	0.82	5.70E-04	1.71	4.00E-07
8	Arf4	ARF4	-0.38	4.90E-02	0.47	6.20E-06	0.3	7.70E-04
9	Arhgap24	ARHGAP24	-0.58	6.40E-03	1.03	2.20E-05	0.97	6.40E-05
10	Atp6v0a2	ATP6V0A2	-0.28	3.00E-03	0.3	9.90E-05	0.2	4.10E-03
11	Brcc3	BRCC3	-0.27	4.60E-02	0.17	3.00E-02	0.15	4.60E-02
12	C1qbp	C1QBP	-0.2	2.80E-02	0.29	1.80E-04	0.32	9.50E-05
13	Ccdc115	CCDC115	-0.27	4.10E-03	0.45	2.90E-03	0.3	3.50E-02
14	Ccdc51	CCDC51	-0.35	7.30E-03	0.38	2.50E-05	0.24	2.10E-03
15	Ccne1	CCNE1	-0.39	2.10E-02	0.79	4.70E-06	0.25	4.80E-02
16	Cd14	CD14	-0.33	2.60E-02	1.32	8.70E-06	1.08	9.20E-05
17	Cdca7l	CDCA7L	-0.35	3.20E-02	0.32	6.40E-04	0.21	1.50E-02
18	Cdkn2c	CDKN2C	-0.47	2.10E-02	0.59	3.10E-06	0.29	2.80E-03
19	Cenpl	CENPL	-0.37	2.40E-02	0.33	9.40E-05	0.23	3.10E-03
20	Cfb	CFB	-0.39	2.20E-02	1.83	3.20E-04	3.02	3.00E-06
21	Chad	CHAD	-0.26	4.90E-02	3.11	1.10E-05	5.54	2.00E-08
22	Chn2	CHN2	-0.69	2.70E-04	0.58	3.50E-03	1.97	1.50E-08
23	Cisd1	CISD1	-0.2	3.00E-03	0.29	1.60E-02	1.16	3.20E-08
24	Cldn10	CLDN10	-0.67	1.10E-02	1.76	2.70E-05	3.56	1.30E-08
25	Clint1	CLINT1	-0.22	1.70E-02	0.65	7.80E-07	0.25	4.50E-03

26	Clptm1l	CLPTM1L	-0.23	1.70E-02	0.27	5.30E-05	0.19	2.00E-03
27	Clstn3	CLSTN3	-0.53	3.60E-03	0.51	1.70E-05	0.34	1.10E-03
28	Cmas	CMAS	-0.22	4.40E-03	0.29	1.60E-02	0.81	2.00E-06
29	Cmtm7	CMTM7	-0.37	3.40E-02	0.46	4.30E-03	1.25	3.00E-07
30	Copb2	COPB2	-0.15	3.20E-02	0.36	1.10E-04	0.25	3.20E-03
31	Cops4	COPS4	-0.16	2.00E-02	0.18	1.20E-02	0.34	7.20E-05
32	Creld1	CRELD1	-0.42	2.40E-02	0.31	8.60E-04	0.19	2.90E-02
33	Ctsb	CTSB	-0.57	2.70E-04	0.93	6.30E-07	0.28	1.70E-02
34	Ctsf	CTSF	-0.17	4.40E-02	0.49	4.80E-05	0.45	1.80E-04
35	Cyp1b1	CYP1B1	-0.83	4.90E-04	1.37	3.60E-05	1.75	4.30E-06
36	Ddi2	RSC1A1	-0.48	6.40E-03	0.49	1.60E-03	0.76	3.80E-05
37	Eaf2	EAF2	-0.54	3.70E-03	0.76	2.70E-06	0.41	1.10E-03
38	Ears2	EARS2	-0.29	8.70E-03	0.29	7.40E-03	0.31	5.80E-03
39	Eif2b1	EIF2B1	-0.32	8.50E-04	0.27	1.10E-03	0.18	2.00E-02
40	Eif3f	EIF3F	-0.2	2.10E-03	0.76	8.70E-07	0.21	4.00E-02
41	Eif3i	EIF3I	-0.21	1.50E-02	0.33	2.90E-05	0.21	2.30E-03
42	Ephb2	EPHB2	-0.5	2.70E-03	0.76	1.80E-06	0.69	5.50E-06
43	Eprs	EPRS	-0.22	2.50E-04	0.46	1.20E-05	0.17	4.00E-02
44	Fmo2	FMO2	-0.33	3.60E-02	2.47	9.90E-06	3.12	1.20E-06
45	Fntb	FNTB	-0.31	6.00E-04	0.21	7.50E-03	0.2	1.30E-02
46	Fundc2	FUNDC2	-0.46	1.10E-03	0.42	8.00E-05	0.24	9.70E-03
47	Fyco1	FYCO1	-0.21	5.10E-03	0.57	3.40E-05	0.31	8.10E-03
48	Ghitm	GHITM	-0.15	6.60E-03	0.19	1.20E-02	0.34	1.30E-04
49	Gjb2	GJB2	-0.71	4.20E-02	1.5	4.80E-07	0.61	1.40E-03
50	Gltp	GLTP	-0.14	1.30E-02	1.09	5.80E-07	0.5	6.70E-04
51	Gpi1	GPI	-0.35	1.00E-03	0.35	1.10E-04	0.23	5.80E-03
52	Gss	GSS	-0.27	1.50E-02	0.78	1.40E-04	1.2	2.60E-06
53	Gstk1	GSTK1	-0.26	7.30E-04	1.83	7.60E-07	0.83	1.30E-03
54	Gsto1	GSTO1	-0.31	1.90E-02	0.76	2.60E-04	3.66	9.10E-12
55	Gusb	GUSB	-0.28	3.30E-03	0.61	3.60E-06	0.43	1.70E-04
56	Hexb	HEXB	-0.22	2.50E-02	1.08	2.80E-06	0.55	2.10E-03
57	Hp	HP	-0.75	3.90E-02	0.73	6.00E-06	0.92	6.40E-07
58	Hspa9	HSPA9	-0.22	1.30E-03	0.39	5.60E-05	0.22	8.20E-03
59	Htra2	HTRA2	-0.27	8.60E-03	0.3	3.90E-03	0.3	4.20E-03
60	Hyls1	HYLS1	-0.5	1.00E-04	0.4	1.50E-02	0.41	1.20E-02
61	Ikzf3	IKZF3	-0.56	3.30E-03	0.29	6.30E-03	0.3	6.20E-03
62	Imp4	IMP4	-0.2	3.60E-02	0.24	5.80E-03	0.17	4.20E-02
63	Lipa	LIPA	-0.51	3.70E-03	0.75	1.20E-05	0.63	1.10E-04
64	Lrp1	LRP1	-0.39	2.90E-03	0.78	6.70E-05	0.92	2.10E-05
65	Lrrc28	LRRC28	-0.4	7.70E-03	0.49	4.30E-04	0.53	2.70E-04
66	Ltv1	LTV1	-0.25	1.80E-04	0.45	2.70E-05	0.29	2.00E-03
67	M6pr	M6PR	-0.27	4.20E-02	0.3	3.20E-04	0.17	2.20E-02
68	Man1c1	MAN1C1	-0.38	9.90E-03	1.58	7.70E-07	0.7	1.70E-03
69	Mcoln2	MCOLN2	-0.39	6.00E-03	0.28	9.90E-04	0.27	2.00E-03

70	Metap2	METAP2	-0.1	3.70E-02	0.39	2.60E-06	0.22	1.00E-03
71	Mettl1	METTL1	-0.29	3.80E-02	1.43	7.50E-07	0.43	2.20E-02
72	Mgst2	MGST2	-0.16	4.40E-02	1.08	1.60E-05	1.98	2.20E-08
73	Mia3	MIA3	-0.85	6.70E-05	0.24	2.10E-02	0.44	2.20E-04
74	Mid1	MID1	-0.33	3.90E-02	0.43	1.10E-03	0.46	7.90E-04
75	Mmachc	MMACHC	-0.53	2.20E-03	0.37	4.20E-04	0.36	7.00E-04
76	Mmp14	MMP14	-0.42	1.00E-02	0.46	5.60E-06	0.61	3.20E-07
77	Mpi	MPI	-0.38	2.10E-03	0.26	1.20E-03	0.31	3.20E-04
78	Mrpl12	MRPL12	-0.32	7.90E-03	0.26	1.90E-03	0.17	3.30E-02
79	Msc	MSC	-0.82	2.20E-02	0.42	3.40E-04	0.27	1.20E-02
80	Ndufa1	NDUFA1	-0.19	1.50E-02	0.42	1.10E-04	0.46	6.90E-05
81	Ndufa3	NDUFA3	-0.27	6.00E-03	0.34	1.10E-04	0.18	1.70E-02
82	Ndufa9	NDUFA9	-0.32	1.30E-03	0.25	6.40E-04	0.16	1.90E-02
83	Ndufv3	NDUFV3	-0.48	1.10E-03	0.37	6.50E-04	0.25	1.20E-02
84	Nfu1	NFU1	-0.19	1.90E-02	0.25	4.10E-03	0.2	1.70E-02
85	Noc2l	NOC2L	-0.31	3.10E-02	0.2	7.90E-03	0.22	5.20E-03
86	Nop10	NOP10	-0.2	3.10E-02	0.28	1.10E-03	0.23	5.90E-03
87	Nufip1	NUFIP1	-0.41	1.50E-02	0.25	3.20E-02	0.3	9.90E-03
88	Pcx	PC	-0.51	2.50E-02	1.72	1.80E-06	1.11	1.80E-04
89	Pdxdc1	PDXDC1	-0.22	3.30E-03	0.42	2.50E-05	0.27	2.00E-03
90	Phlpp1	PHLPP1	-0.36	4.90E-02	0.28	4.50E-03	0.42	2.00E-04
91	Pinx1	PINX1	-0.51	1.20E-03	0.65	4.60E-05	0.37	7.80E-03
92	Pmm2	PMM2	-0.33	6.00E-03	0.51	7.40E-06	0.38	2.10E-04
93	Pmpcb	PMPCB	-0.12	3.20E-02	0.16	9.20E-03	0.14	2.80E-02
94	Polr1e	POLR1E	-0.33	1.10E-03	0.74	1.10E-06	0.32	3.40E-03
95	Pou2af1	POU2AF1	-1.33	6.70E-05	0.55	5.10E-06	0.33	9.50E-04
96	Ppa1	PPA1	-0.22	6.10E-03	0.79	1.60E-06	0.58	4.30E-05
97	Psat1	PSAT1	-0.76	3.50E-03	0.3	6.30E-04	0.17	3.00E-02
98	Psmb1	PSMB1	-0.16	1.40E-03	0.3	4.50E-04	0.41	2.70E-05
99	Psmb10	PSMB10	-0.19	4.30E-02	0.75	2.80E-06	0.33	6.70E-03
100	Psmb3	PSMB3	-0.16	3.40E-02	0.39	1.20E-02	0.52	1.70E-03
101	Psmb4	PSMB4	-0.26	5.00E-04	0.25	1.60E-03	0.37	5.70E-05
102	Psmb7	PSMB7	-0.2	3.40E-02	0.25	1.90E-03	0.3	5.00E-04
103	Psmd1	PSMD1	-0.28	8.60E-04	0.45	1.80E-05	0.41	6.90E-05
104	Pusl1	PUSL1	-0.5	1.40E-03	0.62	7.30E-07	0.16	4.90E-02
105	Rasgrf1	RASGRF1	-0.73	2.90E-03	1.26	2.90E-06	1.05	2.50E-05
106	Rit1	RIT1	-0.41	3.10E-03	0.24	9.90E-03	0.34	8.70E-04
107	Rnf121	RNF121	-0.44	7.20E-04	0.29	4.80E-04	0.24	2.50E-03
108	Rps21	RPS21	-0.22	5.00E-02	0.62	7.10E-06	0.34	2.20E-03
109	Rspo1	RSPO1	-0.77	6.00E-03	0.59	3.50E-04	1.16	4.60E-07
110	Saa3	SAA1	-0.86	2.80E-02	1.48	1.90E-03	0.93	3.70E-02
111	Saal1	SAAL1	-0.21	1.10E-03	0.27	9.60E-04	0.15	4.40E-02
112	Samd4	SAMD4A	-0.66	6.60E-03	1.55	1.70E-06	1.68	7.20E-07
113	Shmt2	SHMT2	-0.26	6.90E-04	0.29	1.80E-03	0.38	2.30E-04

114	Sidt1	SIDT1	-0.42	4.10E-02	1.66	5.40E-06	2.11	5.10E-07
115	Sil1	SIL1	-0.3	3.80E-02	0.49	1.80E-06	0.25	1.30E-03
116	Slc22a18	SLC22A18	-0.33	1.80E-02	0.89	1.90E-05	1.09	3.40E-06
117	Slc45a3	SLC45A3	-0.61	1.90E-03	0.43	1.40E-03	0.74	1.10E-05
118	Slc46a3	SLC46A3	-0.56	1.40E-05	0.61	4.20E-04	1.32	1.90E-07
119	Smpdl3b	SMPDL3B	-0.66	9.10E-03	1.01	1.00E-05	1.11	5.30E-06
120	Sqstm1	SQSTM1	-0.27	7.30E-04	0.26	7.60E-03	0.26	7.90E-03
121	Srp68	SRP68	-0.2	2.60E-03	0.19	2.60E-02	0.32	7.80E-04
122	Ssr4	SSR4	-0.43	2.30E-04	0.35	9.00E-05	0.24	2.70E-03
123	St3gal5	ST3GAL5	-0.4	3.70E-03	0.92	1.40E-07	0.35	2.40E-04
124	Sytl4	SYTL4	-0.42	6.50E-03	0.49	3.70E-05	0.28	6.10E-03
125	Tef	TEF	-0.5	4.20E-02	0.24	2.70E-02	0.95	1.10E-07
126	Tlr5	TLR5	-0.24	1.60E-02	1.66	4.60E-06	1.28	8.90E-05
127	Tmem109	TMEM109	-0.27	2.60E-03	0.27	8.10E-04	0.2	8.90E-03
128	Tmem147	TMEM147	-0.22	2.40E-03	0.43	7.40E-06	0.27	1.00E-03
129	Tmem176b	TMEM176B	-0.77	1.20E-04	0.37	8.80E-04	0.61	9.00E-06
130	Tmem87a	TMEM87A	-0.13	3.60E-02	0.81	5.90E-07	0.19	4.90E-02
131	Tox3	TOX3	-0.58	3.40E-02	0.63	6.50E-03	1.36	8.30E-06
132	Tpd52l1	TPD52L1	-0.34	3.80E-02	0.44	2.20E-05	0.45	2.50E-05
133	Trappc1	TRAPPC1	-0.14	1.60E-02	0.47	2.10E-04	0.43	6.00E-04
134	Trp53i11	TP53I11	-0.51	1.80E-03	0.64	6.20E-06	0.25	1.90E-02
135	Tshz2	TSHZ2	-0.57	1.00E-03	0.45	5.60E-03	1.4	8.20E-08
136	Uchl3	UCHL3	-0.31	9.40E-04	0.24	1.20E-02	0.43	1.00E-04
137	Uchl4	UCHL3	-0.31	9.40E-04	0.21	3.00E-02	0.53	1.90E-05
138	Uck2	UCK2	-0.44	2.50E-02	0.55	7.10E-06	0.28	4.10E-03
139	Uqcrc1	UQCRC1	-0.2	2.00E-02	0.19	5.50E-03	0.27	4.60E-04
140	Wdr12	WDR12	-0.33	1.40E-03	0.54	3.50E-05	0.35	2.40E-03
141	Wdr75	WDR75	-0.15	3.60E-02	0.31	4.60E-03	0.25	2.30E-02
142	Yipf1	YIPF1	-0.29	1.90E-04	0.26	1.20E-03	0.32	2.10E-04
143	Zmat5	ZMAT5	-0.45	3.90E-03	0.35	6.50E-04	0.9	3.50E-08

**Supplemental Table 2. Wnt<sup>low</sup> cells enriched pathways and genes related to epithelial progenitor functions.**

Pathway	Set Size	Changed	P.val	Gene	FDR	-Log10 FDR
Epithelial_cell_development	186	18	8.36E-13	ATP2B2,CLIC5,HRH2,BMP5,GSTM3,SLC4A5,PTPRS,YIPF6,KLF5,FGFR1,PODXL,SPDEF,SLC9A4,CDK6,VSIG1,RAB25,PALLD,SMO	1.09E-11	10.963808
Epithelial_cell_differentiation	495	39	8.46E-11	ATP2B2,CDK1,CLIC5,UPK1A,EVPL,HRH2,BMP5,CES1,NFIB,CTSB,UPK1B,SLC7A11,THRB,PLS3,TST,GSTM3,SLC4A5,PTPRS,YIPF6,KLF5,UPK2,GSTK1,FGFR1,AGR2,DMBT1,PODXL,CNN3,ERBB4,SPDEF,SLC9A4,UPK3A,NR2F2,ACVRL1,CDK6,VSIG1,HEY2,RAB25,PALLD,SMO	8.46E-10	9.0726051
Epithelial_cell_fate_commitment	15	1	0.012479	NR2F2	0.037437	1.4266976
Epithelial_cell_morphogenesis	42	5	5.08E-08	HRH2,VSIG1,RAB25,PALLD,SMO	4.07E-07	6.390773
Epithelial_cell_proliferation	89	6	3.57E-06	NRARP,KLK8,MMP14,CCND1,ACVRL1,SHH	1.78E-05	4.7484511
Epithelial_tube_branching_involved_in_lung_morphogenesis	25	2	0.000522	RDH10,SHH	0.002089	2.6799801
Intestinal_epithelial_cell_development	11	3	4.04E-08	YIPF6,KLF5,SPDEF	3.63E-07	6.4397194
Intestinal_epithelial_cell_differentiation	17	3	5.60E-07	YIPF6,KLF5,SPDEF	3.92E-06	5.4067427
Regulation_of_epithelial_cell_differentiation	122	8	9.06E-07	PRKCH,AQP3,CCND1,ALOX12,ACVRL1,HEY2,KITLG,SMO	5.44E-06	5.2646676
Regulation_of_epithelial_cell_proliferation	285	24	6.71E-12	CYP7B1,SERPINF1,SULF2,KDR,NRARP,ERBB2,BMP5,NFIB,CCND1,NME1,EAF2,TACR1,WFDC1,SFRP1,ANGPT1,PTPRK,TGFB2,NR2F2,ACVRL1,CDK6,GLUL,TACSTD2,SHH,SMO	8.05E-11	10.094045
Columnar_cuboidal_epithelial_cell_development	48	9	1.02E-14	ATP2B2,CLIC5,BMP5,YIPF6,KLF5,FGFR1,SPDEF,CDK6,SMO	1.43E-13	12.843616
Columnar_cuboidal_epithelial_cell_differentiation	111	11	6.19E-11	ATP2B2,CLIC5,BMP5,PLS3,YIPF6,KLF5,FGFR1,AGR2,SPDEF,CDK6,SMO	6.81E-10	9.1671443



**Supplemental Table 3. Wnt<sup>low</sup> cells enriched pathways and genes related to stem cell functions.**

Pathway	Set Size	Changed	P.val	Gene	FDR	-Log10 FDR
Negative_regulation_of_stem_cell_differentiation	43	3	0.00013	BMP5,SFRP1,TGFB2	0.000393	3.4060145
Positive_regulation_of_stem_cell_differentiation	50	4	7.97E-06	TCF7L2,TGFB2,SOX5,TACSTD2	3.98E-05	4.3996156
Positive_regulation_of_stem_cell_proliferation	61	5	1.97E-06	FGFR1,CX3CL1,KITLG,SHH,SMO	1.38E-05	4.8609847
Regulation_of_stem_cell_differentiation	113	7	4.13E-06	BMP5,TCF7L2,SFRP1,TGFB2,SOX5,TACSTD2,SMO	2.48E-05	4.6063113
Regulation_of_stem_cell_population_maintenance	17	1	0.01501	SMO	0.015007	1.8236989
Regulation_of_stem_cell_proliferation	88	7	1.98E-07	NFIB,ACE,FGFR1,CX3CL1,KITLG,SHH,SMO	1.58E-06	5.8011762
Stem_cell_differentiation	190	14	8.02E-09	RDH10,ACE,FGFR1,SFRP1,ERBB4,PSMD11,S100A4,TGFB2,CDK6,SEMA3A,HEY2,KITLG,SHH,SMO	7.22E-08	7.1415693
Stem_cell_division	29	3	1.24E-05	FGFR1,TGFB2,SOX5	4.94E-05	4.3062697

**Supplemental Table 4. Wnt<sup>low</sup> cells enriched pathways and genes related to cell cycle.**

Pathway	Set Size	Changed	P.val	Gene	FDR	-Log10 FDR
Cell_cycle_g1_s_phase_transition	111	12	2.53E-12	CCNA1,CDC34,CDK1,CCNE1,E2F6,CCND1,CCNH,EIF4EBP1,DBF4,CDK6,CDKN2C,RHO	5.81E-11	10.235647
Cell_cycle_g2_m_phase_transition	138	8	8.09E-06	TERF1,AURKA,CDK1,PLK4,CEP70,CCNH,PLCB1,TPD52L1	0.000129	3.8881704
Cell_cycle_phase_transition	255	18	9.59E-09	CCNA1,TERF1,AURKA,CDC34,CDK1,CCNE1,PLK4,CEP70,E2F6,CCND1,CCNH,PLCB1,TPD52L1,EIF4EBP1,DBF4,CDK6,CDKN2C,RHO	1.82E-07	6.7395242
Mitotic_cell_cycle	766	49	0.00028	CCNA1,TERF1,AURKA,PSMD3,CDC34,CDK1,CCNE1,PLAGL1,PSMB7,NSUN2,CD2AP,PRDM5,ANAPC13,PSMA4,PSMA1,PLK4,PSMD12,FBXW5,CEP70,PSMB3,E2F6,BECN1,STIL,CCND1,PSMC3,PSMB10,CCNH,PLCB1,TPD52L1,EIF4EBP1,TOP2A,PINX1,CCNG1,PSMB2,DBF4,PSMC1,PSMD11,BRCC3,PSMD14,PSMB4,CDK6,PSMD1,CDKN2C,AAAS,WDR43,RHO,NCAPH,PSMB1,PSMD2	0.002831	2.5481083
Regulation_of_cell_cycle	949	65	0.00194	TERF1,RAD51B,AURKA,PSMD3,SGK1,CDK1,SIK1,MYBBP1A,GMNN,CCNE1,PLAGL1,CDKL5,PKHD1,PSMB7,PSMA4,BID,ILK,NUPR1,PSMA1,PLK4,PSMD12,PSMB3,SGSM3,BECN1,CCND1,PSMC3,PSMB10,FNTB,CCNH,MAPK12,TBRG1,PLCB1,CALR,EIF4EBP1,AURKAIP1,ECT2,BTC,TCF7L2,TOP2A,CLIC1,HPGD,TIMP2,FGFR1,SFRP1,CCNG1,PTPRK,PSMB2,NOTCH2,PSMC1,PSMD11,PBX1,PSMD14,PSMB4,TGFB2,NR2F2,CDK6,HTRA2,PSMD1,CDKN2C,PRKAB1,DTL,CKS1B,CAB39,PSMB1,PSMD2	0.013612	1.8660626
Regulation_of_cell_cycle_g2_m_phase_transition	59	4	2.28E-05	RAD51B,CDK1,CCND1,PBX1	0.000319	3.4957808
Regulation_of_cell_cycle_phase_transition	321	25	1.65E-10	TERF1,RAD51B,AURKA,PSMD3,CDK1,PLAGL1,PSMB7,PSMA4,BID,PSMA1,PSMD12,PSMB3,CCND1,PSMC3,PSMB10,PLCB1,PSMB2,PSMC1,PSMD11,PBX1,PSMD14,PSMB4,PSMD1,PSMB1,PSMD2	3.63E-09	8.4405141
Regulation_of_cell_cycle_process	558	34	1.85E-05	TERF1,RAD51B,AURKA,PSMD3,CDK1,MYBBP1A,PLAGL1,PKHD1,PSMB7,PSMA4,BID,PSMA1,PLK4,PSMD12,PSMB3,BECN1,CCND1,PSMC3,PSMB10,PLCB1,CALR,AURKAIP1,ECT2,BTC,SFRP1,PSMB2,PSMC1,PSMD11,PBX1,PSMD14,PSMB4,PSMD1,PSMB1,PSMD2	0.000278	3.555805
Regulation_of_mitotic_cell_cycle	468	32	7.84E-08	TERF1,RAD51B,AURKA,PSMD3,CDK1,SIK1,PLAGL1,PSMB7,PSMA4,BID,PSMA1,PSMD12,PSMB3,BECN1,CCND1,PSMC3,PSMB10,PLCB1,EIF4EBP1,AURKAIP1,BTC,TOP2A,TIMP2,PSMB2,PSMC1,PSMD11,PBX1,PSMD14,PSMB4,PSMD1,PSMB1,PSMD2	1.33E-06	5.8751059

Regulation_of_transcription_involved_in_g1_s_transition_of_mitotic_cell_cycle	27	4	7.28E-08	CCNA1,CDK1,CCNE1,E2F6	1.31E-06	5.8825438
Cell_proliferation	672	42	9.59E-05	SERPINF1,RBBP7,SRA1,ZFP36L1,KAT2A,NAA60,PSMG1,NRARP,ILK,ERBB2,LGI1,RASGRF1,MIA,KLK8,MMP14,FES,CLU,GMNC,STIL,CCND1,ACE,RETNLB,PSMB10,REG1B,LRP1,TCF7L2,FGFR1,DDR2,ERBB4,EMP1,TGFB2,ACVRL1,SOX5,HDGF,LIPA,DTYMK,WDR12,GLUL,TACSTD2,CKS1B,KITLG,SHH	0.001151	2.9389534
Negative_regulation_of_epithelial_cell_proliferation	115	10	1.74E-09	SERPINF1,NFIB, EAF2,WFDC1,SFRP1,PTPRK,TGFB2,NR2F2,ACVRL1,CDK6	3.47E-08	7.4596525
Positive_regulation_of_epithelial_cell_proliferation	154	13	3.80E-10	CYP7B1,KDR,NRARP,ERBB2,BMP5,CCND1,NME1,TACR1,SFRP1,ACVRL1,GLUL,SHH,SMO	7.99E-09	8.0976978