Supplemental Material

Long-term IL-33 producing epithelial progenitor cells in chronic obstructive lung disease

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Supplemental Table 1. Gene expression in lungs from mice at SeV versus SeV-UV post-inoculation (p.i.) Day 49. This list includes the 20 genes with the greatest fold-change as well as interleukin genes and basal cell markers (*Krt5*, *Ngfr*, and *Itga6*). Values correspond to those in Figure 1A.

Gene Symbol	Gene Description	Gene Expression (Log2, mouse lung, d49 PI)		Fold Change (SeV vs SeV-UV)	Rank (by fold-change)
		SeV	SeV-UV		
Clca3	chloride channel calcium activated 3	13.79	6.90	128.88	1
Krt14	keratin 14	12.96	6.85	69.15	2
Ear11	eosinophil-associated, ribonuclease A family, member 11	11.68	6.45	37.50	3
Retnla	resistin like alpha	14.67	9.65	32.35	4
Krt17	keratin 17	11.46	6.56	29.97	5
Slc26a4	solute carrier family 26, member 4	12.32	7.54	27.34	6
Arg1	arginase, liver	11.33	7.02	23.48	7
Chi3l4	chitinase 3-like 4	15.02	11.42	17.90	8
Saa3	Serum amyloid A 3	11.35	7.21	17.59	9
Spp1	Secreted phosphoprotein 1	12.28	8.38	14.98	10
Nmes1/Mir147	normal mucosa esophagus specific 1	12.25	8.48	13.59	11
Plunc	palate, lung, and nasal epithelium associated	13.49	9.74	13.48	12
Alox12e	arachidonate lipoxygenase, epidermal	10.21	6.53	12.84	13
Gp2	glycoprotein 2 (zymogen granule membrane)	10.51	6.96	11.69	14
Scgb3a1	secretoglobin, family 3A, member 1	11.88	8.59	10.86	15
Ltf	lactotransferrin	9.94	6.74	10.71	16
Dmbt1	deleted in malignant brain tumors 1	9.94	6.55	10.45	17
Aqp3	aquaporin 3	10.26	6.96	9.84	18
Krt15	keratin 15	10.14	6.87	9.65	19
Ctsk	cathepsin K	13.00	9.76	9.45	20
Trp63	transformation related protein 63	9.79	6.78	8.07	24
Il33	interleukin 33	12.03	9.65	5.22	49
Krt5	keratin 5	8.95	6.68	4.82	58
Il1rn	interleukin 1 receptor antagonist	9.28	7.77	2.89	155
Ngfr	nerve growth factor receptor (TNFR superfamily, member 16)	8.17	6.94	2.34	258
Il8ra	chemokine (C-X-C motif) receptor 1	9.59	8.39	2.30	273
Il20rb	interleukin 20 receptor beta	8.17	7.51	1.57	946
Il4	interleukin 4	7.75	7.27	1.39	1522
Il31ra	interleukin 31 receptor A	7.23	6.81	1.34	1838
Il17rb	interleukin 17 receptor B	7.06	6.71	1.28	2333

Supplemental Table 1. (continued)

Gene Symbol	Gene Description	Gene Expression (Log2, mouse lung, d49 PI)		Fold Change (SeV vs UV-SeV)	Rank (by absolute fold-change)
		SeV	UV-SeV		
Il1rl1	interleukin 1 receptor-like 1	7.05	6.76	1.22	3050
Il13ra1	interleukin 13 receptor, alpha 1	10.25	9.98	1.21	3298
Il7r	interleukin 7 receptor	6.78	6.78	1.17	3869
Il25	interleukin 25	6.60	6.55	1.04	12264
Il1rap	interleukin 1 receptor accessory protein	7.21	7.15	-1.01	17853
Tslp	thymic stromal lymphopoietin	6.43	6.50	-1.05	23868
Itga6	integrin alpha 6	7.07	7.31	-1.10	28041
Il17ra	interleukin 17 receptor A	7.47	7.87	-1.32	33588
Il10ra	interleukin 10 receptor, alpha	8.75	9.16	-1.33	33736
Il6ra	interleukin 6 receptor, alpha	7.56	8.00	-1.48	34717
Il12a	interleukin 12a	7.60	8.17	-1.49	34763
Il8rb	chemokine (C-X-C motif) receptor 2	7.17	8.13	-1.94	35785

Supplemental Table 2. Clinical characteristics of lung transplant donors without COPD (Non-COPD) and recipients with severe (GOLD Stage IV) COPD.

Characteristics	Non-COPD	COPD
Number per group	18	35
Mean age (range)	43 (11-56)	59 (44-69)
Male:Female	9:9	19:16
FVC (L)	–	2.03 ± 0.64
FVC (% predicted)	–	54.9%
FEV1 (L)	–	0.55 ± 0.16
FEV_1 (% predicted)	–	18.5%
Pack-years (range)	3.8 (0-20)	47 (25-60+)

Supplemental Table 3. Sources and sequences for primers and probes for real-time PCR assays.

Human		**ID/Sequence**
IL33	*	Hs01125943_m1
IL25	*	Hs00224471_m1
TSLP	*	Hs01572933_m1
IL33R	*	Hs0249384_m1
IL13Ra1	F	5'-TCTACATACTGGGCATGGCT-3'
	R	5'-AAAGCTGAGCACGTCACG-3'
	P	5'-TCTGTCCCTCGTTGAATTCAGGC-3'
MUC5AC	F	5'-AGGCCAGCTACCGGGCCGGCCAGACCAT-3'
	R	5'-GTCCCCGTACACGGCGCAGGTGGCCAGGCA-3'
	P	5'-TGCAACACCTGCACCTGTGACAGCAGGAT-3'
KRT5[#]	F	5'-AGAGCCACCTCCTGCGTCCT-3'
	R	5'-CTGAAGCTACGACTGCCCCCG-3'
KRT14[#]	F	5'-CAGAGATGTGACCTCCAGCC-3'
	R	5'-GGACCTGCTCGTGGGTGGACA-3'
TRP63	*	Hs00609815_m1
GAPDH	*	Hs00266705_g1
Mouse		
Il33	*	Mm00505403_m1
Il33r	*	Mm00516117_m1
Tslp	*	Mm00498739_m1
Arg1	*	Mm00475988_m1
Il25	F	5'-CACACTGCGTCAGCCTACAGA-3'
	R	5'-TGTGGTAAAGTGGGACGGAGTT-3'
	P	5'-CTCCCACATGGACCCGCTGGG-3'
Il13	F	5'-GGAGCTGAGCAACATCACACA-3'
	R	5'-CACACTCCATACCATGCTGCC-3'
	P	5'-CCAGACTCCCCTGTGCA-3'
Chi3l3	F	5'-CTTGTCACAGGTCTGGCAATTC -3'
	R	5'-GTAGCACATCAGCTGGTAGGAAGA -3'
	P	5'-TCTGAACGTACAGCTGGG -3'
Muc5ac	F	5'-TACCACTCCCTGCTTCTGCAGCGTGTCA-3'
	R	5'-ATAGTAACAGTGGCCATCAAGGTCTGTCT-3'
	P	5'-TATACCCCTTGGGATCCATCATCTACA-3'
Gapdh	F	5'-TGCAGTGCCAGCCTCGT-3'
	R	5'-CCAATACGGCCAAATCCG-3'
	P	5'-ACACCGACCTTCACCATTTTGTCTACGG-3'
SeV	F	5'-GGCGGTGGTGCAATTGAG-3'
	R	5'-CATGAGCTTCTGTTTCTAGGTCGAT-3'
	P	5'-AGCTCTAGACAATGCC-3'

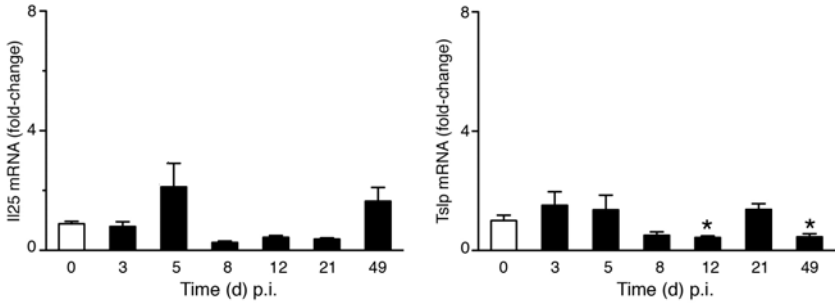
[*]Purchased from Applied Biosystems.
[#]KRT5 and KRT14 were detected with SYBR-Green based assays.

Supplemental Table 4. Specifications and sources of antibodies used for immunostaining.

Antibody	Type/Clone	Source
Anti-mouse		
Acetylated α-tubulin	mAb, 6-11B-1	Sigma
β-galactosidase	pAb[1]	Abcam
F4/80	mAb CL:A3-1	Abcam
IL-13	pAb	R&D Systems
Krt5	pAb	Abcam
Mac-3	mAb, M3/84	BD Pharmingen
Scgb1a1	pAb	Santa Cruz
Scgb3a1	pAb	R&D Systems
Sftpc	pAb	Abcam
Anti-human		
FOXJ1	mAb, 3D4	Washington U.
IL-33	mAb, Nessy-1	Enzo Life Sciences
IL-33	pAb	Sigma
ITGA6	mAb, GoH3	eBioscience
KRT5	pAb	Abcam
KRT8	mAb, TROMA-I	DSHB, U. Iowa
KRT14	pAb	Covance
MUC5AC	mAb, 45M1[2]	Thermo Scientific
NGFR	pAb	Abcam
SCGB1A1	mAb, 394324	R&D Systems
SCGB3A1	pAb	R&D Systems
TRP63	mAb, 4B1E12	LifeSpan Biosciences

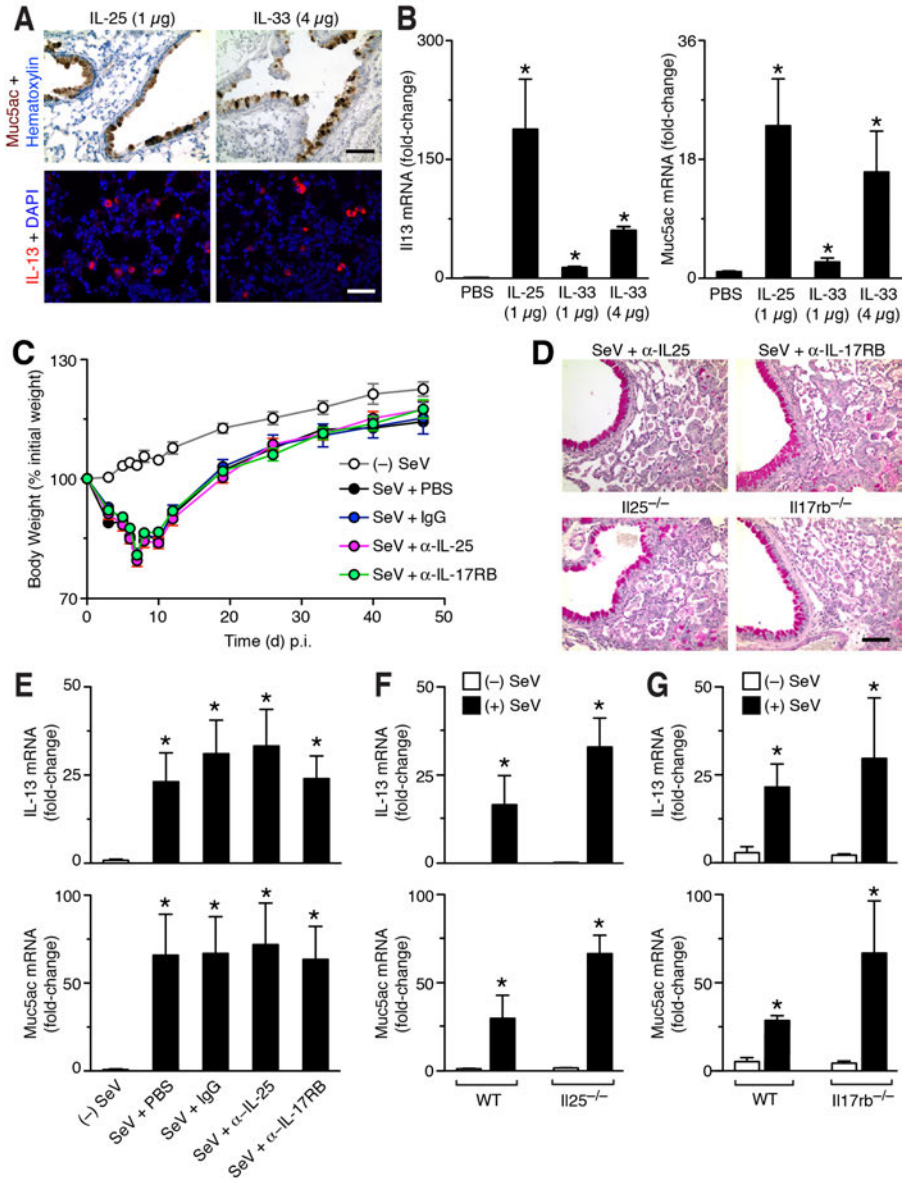
[1]pAb, polyclonal antibody.
[2]Cross-reactive with mouse Muc5ac.

Supplemental Figure 1



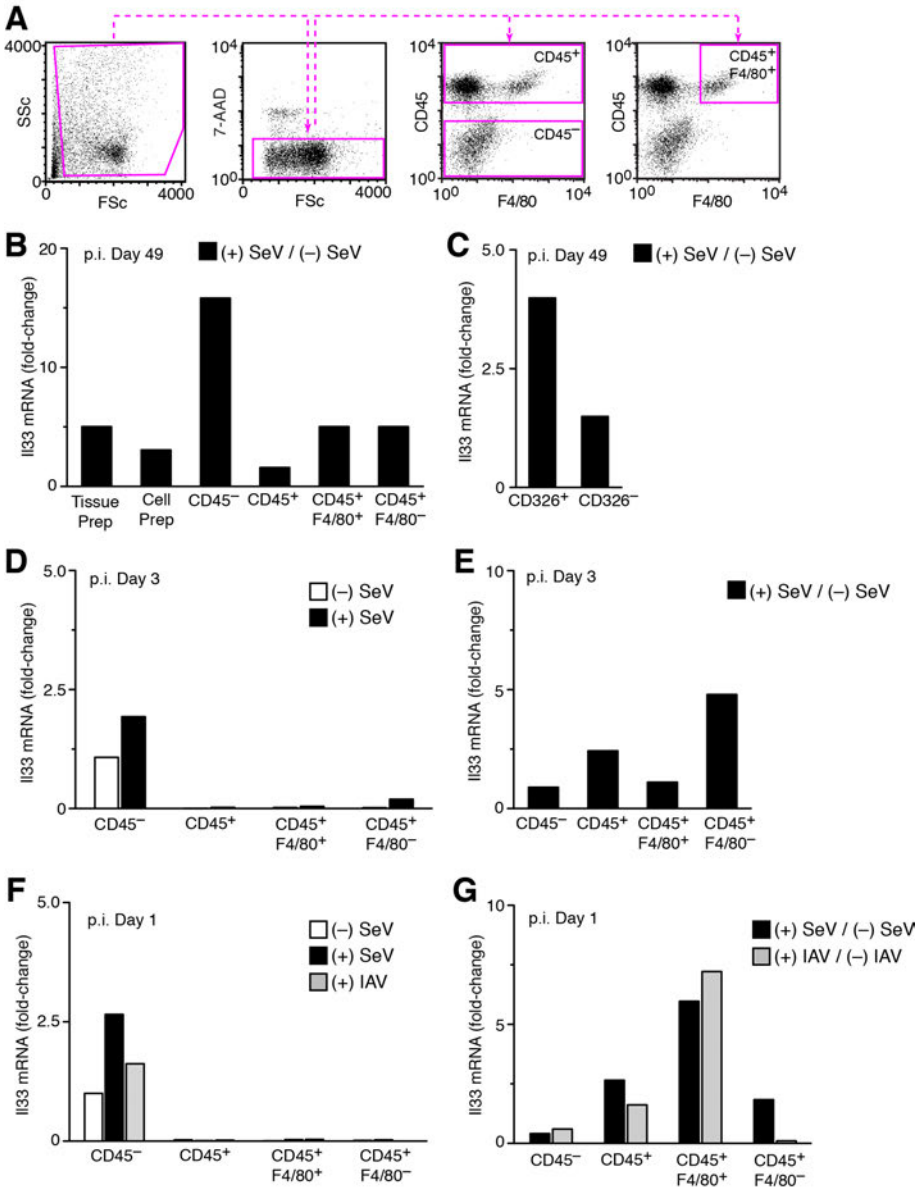
Supplemental Figure 1. Time course of IL-25 and TSLP expression levels in the postviral mouse model. Levels of *Il25* and *Tslp* mRNA in lung tissue obtained on the indicated SeV p.i. Day. Bars represent mean ± SEM (n=5 mice per condition). We found no significant differences from corresponding values for SeV p.i. Day 0.

Supplemental Figure 2



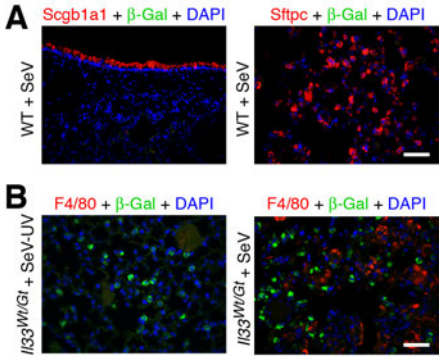
Supplemental Figure 2. Effect of IL-25–IL-25R signaling blockade on postviral chronic lung disease. (**A**) Representative photomicrographs of lung sections from mice given IL-25 or IL-33 (1 or 4 μg intranasally on Day 1 and Day 3) and then immunostained for Muc5ac (at Day 7). Bar=50 μm. (**B**) Level of *Il13* and *Muc5ac* mRNA in lungs from mice given IL-25 or IL-33 as in (A). (**C**) Body weight loss for the indicated groups of mice with or without SeV inoculation. (**D**) Representative photomicrographs of lung sections from SeV p.i. Day 49 in wild-type mice treated with anti-IL-25 or anti-IL-17RB mAb from p.i. Day 12 to 49 and in *Il25*$^{-/-}$ and *Il17rb*$^{-/-}$ mice. (**E**) Levels of *Il13* and *Muc5ac* mRNA in lungs at p.i. Day 49 in WT mice treated with control IgG_1 mAb (IgG), anti-IL25 mAb, or anti-IL17RB mAb from p.i. Day 12 to 49. (**F**) Lung levels of *Il13* and *Muc5ac* at p.i. Day 49 in *Il25*$^{-/-}$ and WT mice. (**G**) Corresponding values for conditions in (F) for *Il17rb*$^{-/-}$ and WT mice. For (B,C,E,F,G), bars represent mean ± SEM (n=3-7 mice per condition), and * represents a significant increase from untreated condition.

Supplemental Figure 3



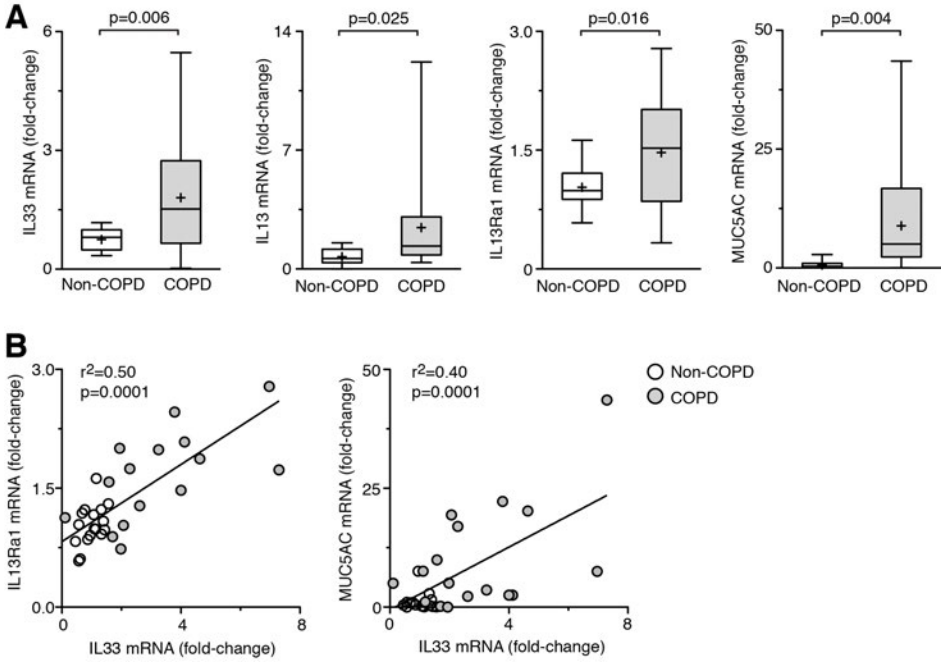
Supplemental Figure 3. Expression levels of *Il33* during the acute phase of respiratory virus infection. (**A**) Representative cytograms from FACS analysis of lung immune cells using forward and side scatter and then 7-AAD, CD45, and F4/80 detection. (**B**) Levels of *Il33* mRNA from lung tissue preparation (tissue prep), lung cell preparation before FACS (cell prep), and the indicated cell populations purified by FACS from lungs obtained at SeV p.i. Day 49. Values are calculated as the fold-change within each cell population and are representative of 3 experiments. (**C**) Levels of *Il33* mRNA in CD45$^-$ lung cells sorted for EpCAM (CD326) at SeV p.i. Day 49. (**D**) Levels of *Il33* mRNA for the indicated populations of lung cells at SeV p.i. Day 3. (**E**) For (D), corresponding values for *Il33* mRNA expressed as fold-change within each cell population. (**F**) Levels of *Il33* mRNA for the indicated populations of lung cells at SeV or IAV p.i. Day 1. (**G**) For (F), corresponding values for *Il33* mRNA expressed as fold-change within each cell population. For (A-F), values are representative of three experiments.

Supplemental Figure 4



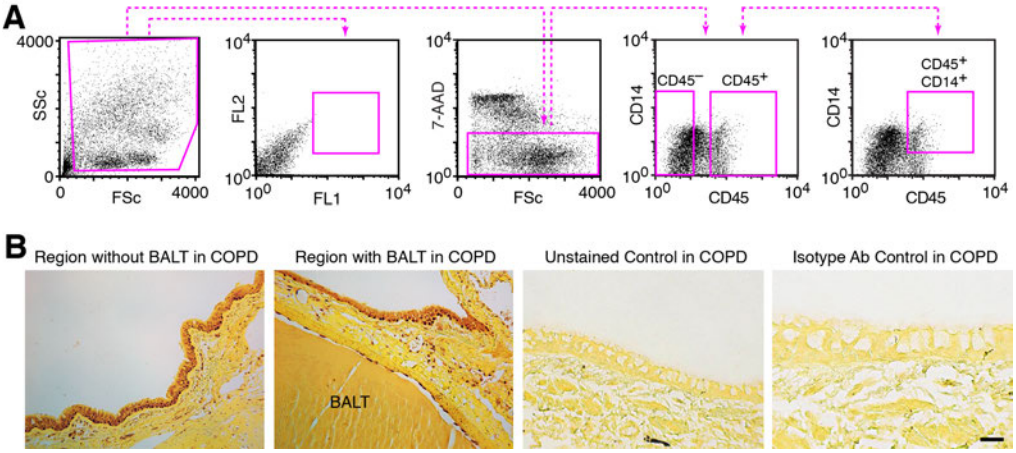
Supplemental Figure 4. Cell sources of IL-33 production in the postviral mouse model. (**A**) Representative photomicrographs for epithelial Scgb1a1 and Sftpc immunostaining and DAPI counterstaining at SeV p.i. Day 49 in lung sections from WT mice. Bar=100 µm. (**B**) Representative photomicrographs for IL-33 immunostaining (detected with anti-β-galactosidase Ab) and F4/80 co-staining and DAPI counterstaining at SeV and SeV-UV p.i. Day 49 in lung sections from *Il33*$^{Wt/Gt}$ mice. Bar=100 µm.

Supplemental Figure 5



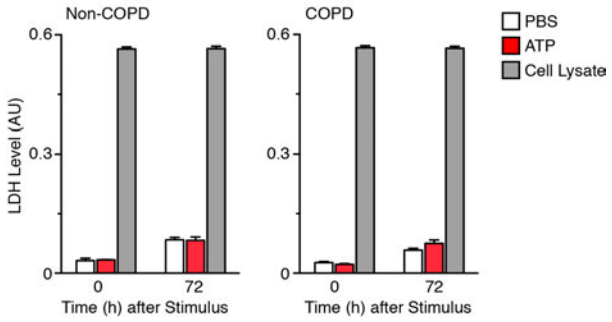
Supplemental Figure 5. Analysis of gene expression in lung tissue from non-COPD and very severe COPD subjects. (**A**) Levels of *IL33*, *IL13, IL13Ra1,* and *MUC5AC* mRNA in lung tissue samples containing airways from non-COPD (n = 17 airway samples from 7 subjects) and very severe COPD (n = 24 airway samples from 16 subjects). (**B**) For data in (A), correlations of *IL33* with *IL13Ra1* and *MUC5AC* mRNA levels (n=41 airway samples) based on Pearson correlations.

Supplemental Figure 6



Supplemental Figure 6. Analysis of human lung samples using FACS and immunostaining. (**A**) Representative cytograms from FACS analysis of tissue cells from human lung samples using forward scatter (FSc) and side scatter (SSc) and then 7-AAD, CD45, and CD14 detection along with unstained control (FL1/FL2). (**B**) Representative photomicrographs of sections of airway epithelium (with or without associated BALT) that was obtained from a subject with very severe COPD and then immunostained for IL-33 with DAB (brown) reporter and counterstained with tartrazine (yellow) as well as unstained and isotype Ab stained controls. Bar=50 µm.

Supplemental Figure 7



Supplemental Figure 7. Lack of effect of ATP on cell viability. Primary-culture hTECs were maintained under 2-D submerged culture conditions and incubated with ATP (10 μM) or PBS vehicle control for the indicated times at 37 °C as also described in Figure 8H. Levels of LDH release into cell medium for these conditions and for control cell lysate were determined by activity assay. Values represent mean ± SEM (n=4 per group and are representative of at least 3 subjects). No significant difference was found for ATP compared to corresponding PBS treatment condition.