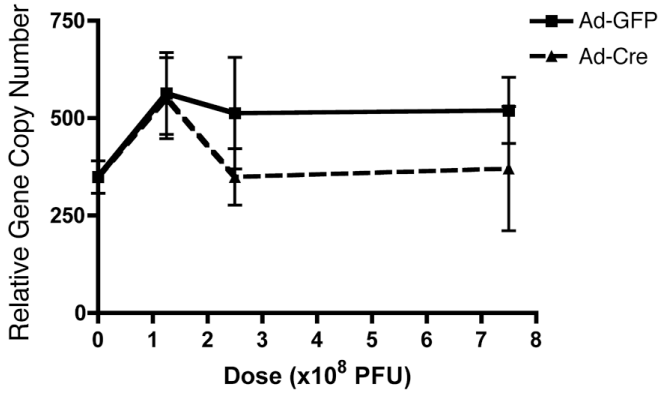
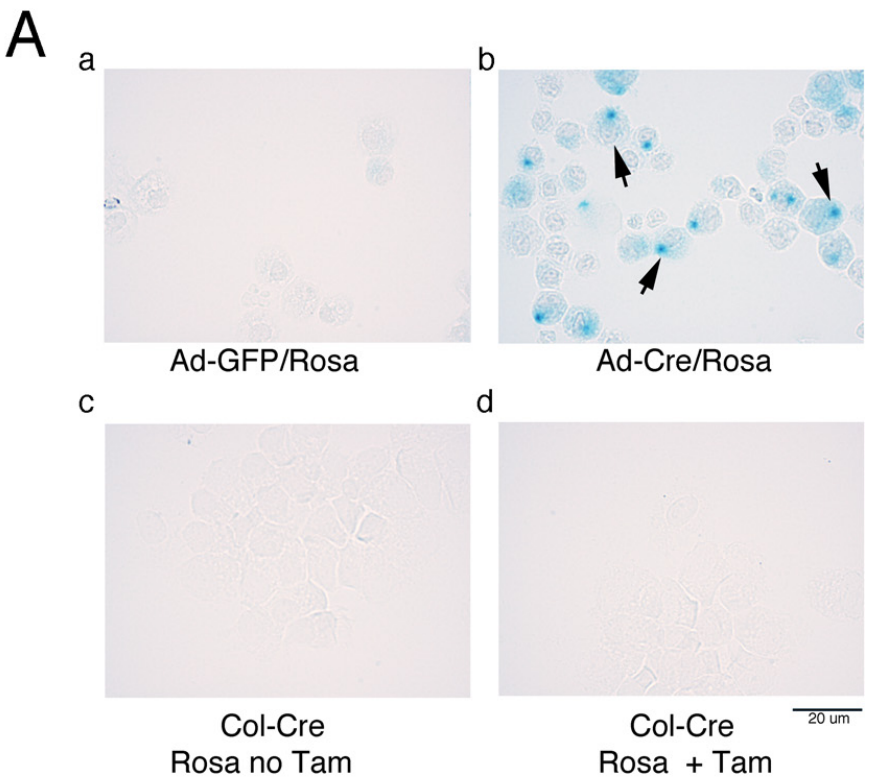
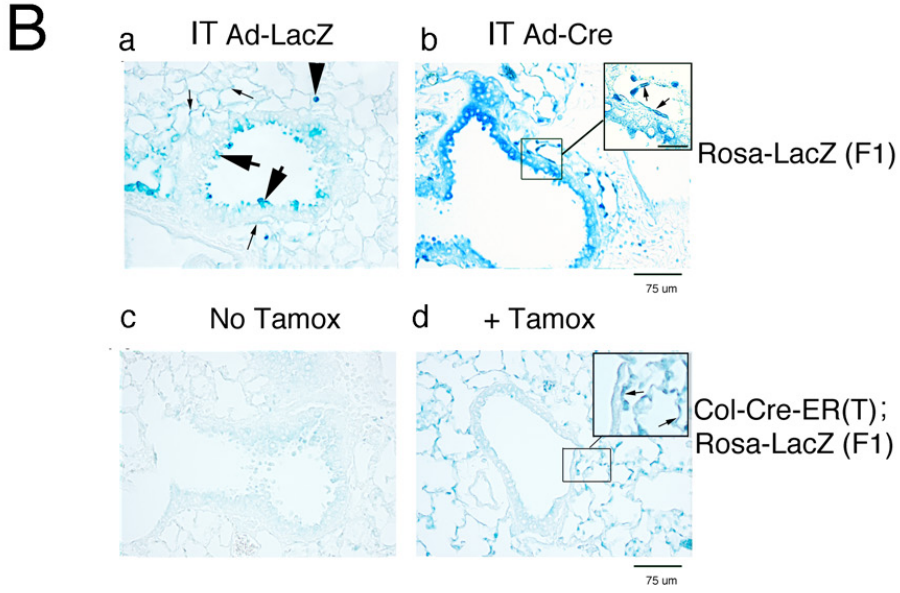
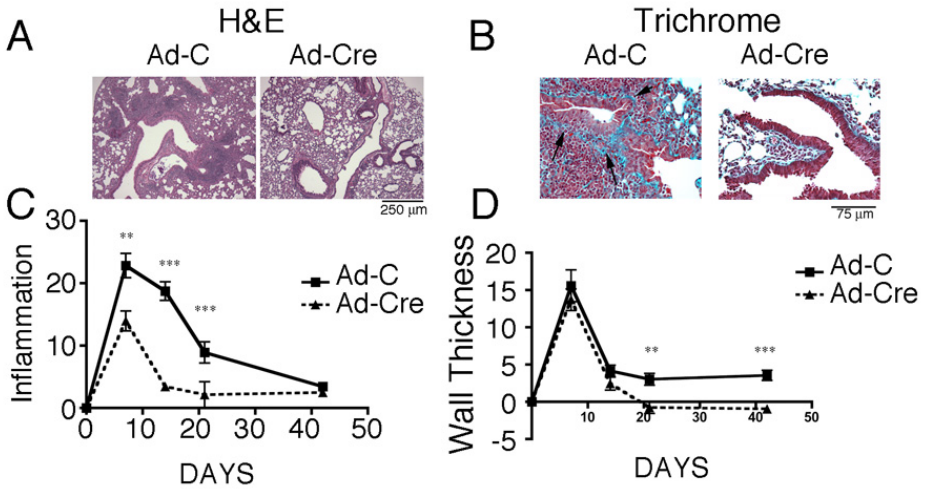


Supplemental Figure 1: Increased *itgb8* expression mediated by Ad-IL-1β is decreased by Ad-Cre. The levels of *itgb8* mRNA in whole lung homogenates were assessed 7d with or without intratracheal (IT) Ad-IL-1β. IT-Ad-IL-1β treated *itgb8* F/- mice were treated with increasing doses of Ad-Cre or Ad-GFP. Shown are the *itgb8* gene copy numbers relative to *gapdh* and *actb*. Note that the effects of IT-Ad-Cre are restricted to field of biodistribution, which is the airway and immediately surrounding cells and not the distal lung parenchyma (see **Supplemental figure 2**). Therefore, changes in expression of *itgb8* from whole lung homogenates represent only the airway and *itgb8* expression in the distal lung is unaffected.

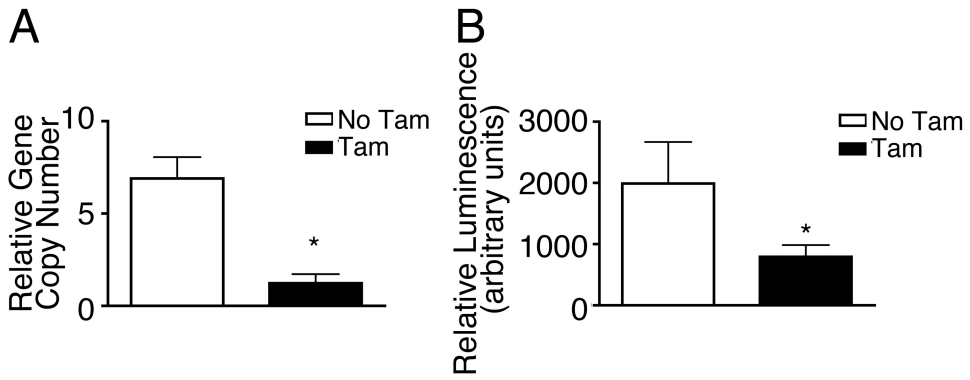

A
a
b
Ad-GFP/Rosa
Ad-Cre/Rosa
c
d
Col-Cre
Rosa no Tam
Col-Cre
Rosa + Tam
20 um


B
a IT Ad-LacZ
b IT Ad-Cre
Rosa-LacZ (F1)
75 um
c No Tamox
d + Tamox
Col-Cre-ER(T);
Rosa-LacZ (F1)
75 um

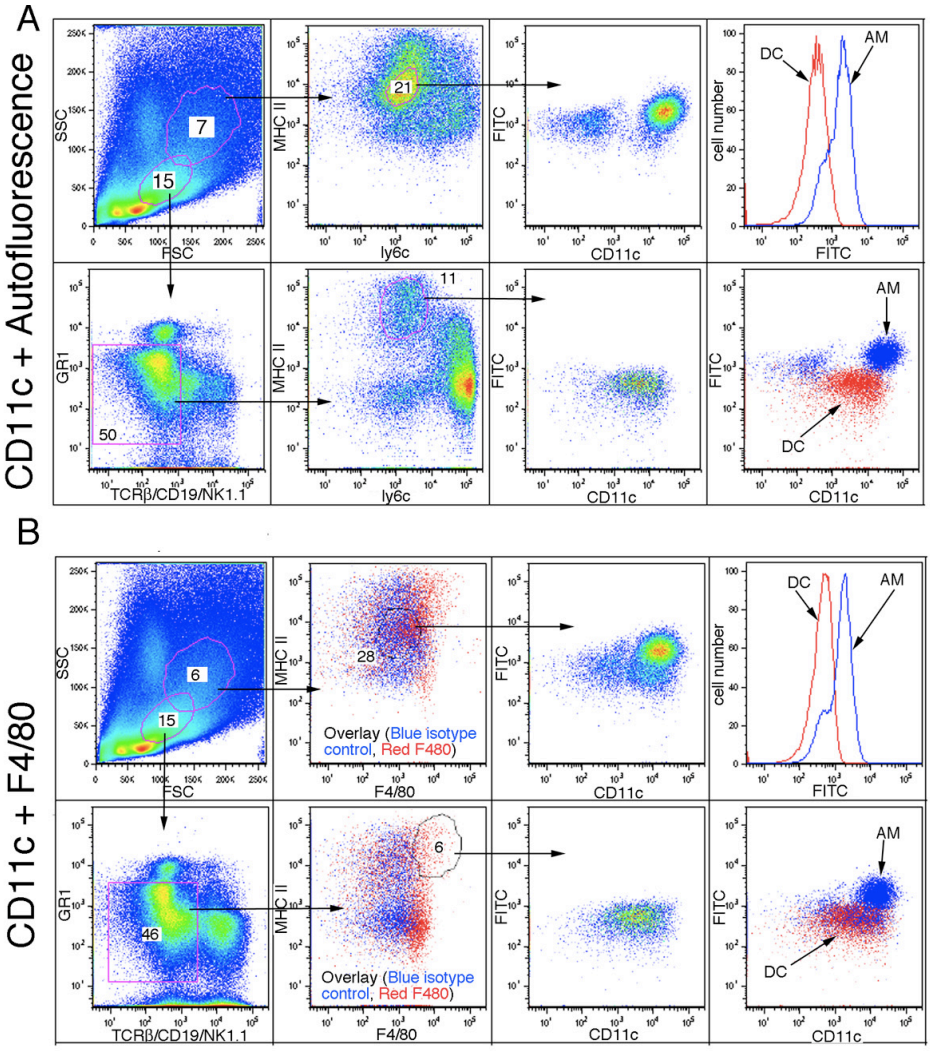
Supplemental Figure 2: Intratracheal Ad-Cre-mediated recombination in multiple airway cell types, and Tamoxifen-dependent recombination only in airway and lung fibroblasts. A) The airway cell-types expressing Ad-Cre following IT-Ad-Cre injection or by Col1a2-Cre-ER(T) mice were assessed using R26-LacZ reporter mice. LacZ expression in Cre-expressing cells and their progeny is activated by Cre-mediated excision of a loxP-flanked polyadenylation sequence in the R26 reporter gene [72]. Col1a2-Cre-ER(T) mice express, only in fibroblasts, a fusion protein consisting of the catalytic domain of Cre-recombinase with a mutated ligand-binding domain of the estrogen receptor which only binds tamoxifen or 4-hydroxy Tamoxifen, but not estradiol [67]. **a**) IT-Ad- GFP treatment of R26-LacZ mice did not reveal recombination in cells from cytospin preparations of BAL fluid, while **b**) IT-Ad-Cre treatment revealed recombination in macrophages (arrows). **C,d**) Col1a2-Cre-ER(T) mice were crossed to R26-LacZ reporter mice in the absence (**c**) or presence (**d**) of tamoxifen (Tamox). Cytospin preparations were stained to detect β-galactosidase activity. Bar=20 μm. No cells in the bronchoalveolar lavage (BAL) fluid or airway epithelial cells showed evidence of recombination in tamoxifen treated mice. **B**) IT-Ad-Cre-mediates recombination in airway epithelial cells, airway mesenchymal cells and macrophages; Tamoxifen-dependent recombination in Col-Cre-ER(T) mice is fibroblast specific. **a**) IT Ad-LacZ reveals the field of adenoviral infection in airway epithelial cells (large arrow), mesenchymal cells (small arrows) and macrophages (arrowhead). **b**) IT Ad-Cre mediated recombination in R26-LacZ mice reveals recombination in all cell types within the adenoviral field of infection. Inset shows close-up of mesenchymal cells surrounding airways (small arrows). **c,d**) Adult Col1a2-Cre-ER(T) mice crossed to R26-LacZ reporter mice reveal tamoxifen-dependent recombination in spindle cells resembling fibroblasts surrounding the airways and in parenchymal interstitial fibroblasts (small arrows). Recombination is not seen in the absence of tamoxifen (**c**). Inset shows high magnification image of mesenchymal cell staining surrounding airways (small arrows). Bar=75 μm, These data demonstrate the fibroblast specificity and efficiency of deletion of adult mouse lung airway and parenchymal fibroblasts using transgenic Col1a2-Cre-ER(T) mice.



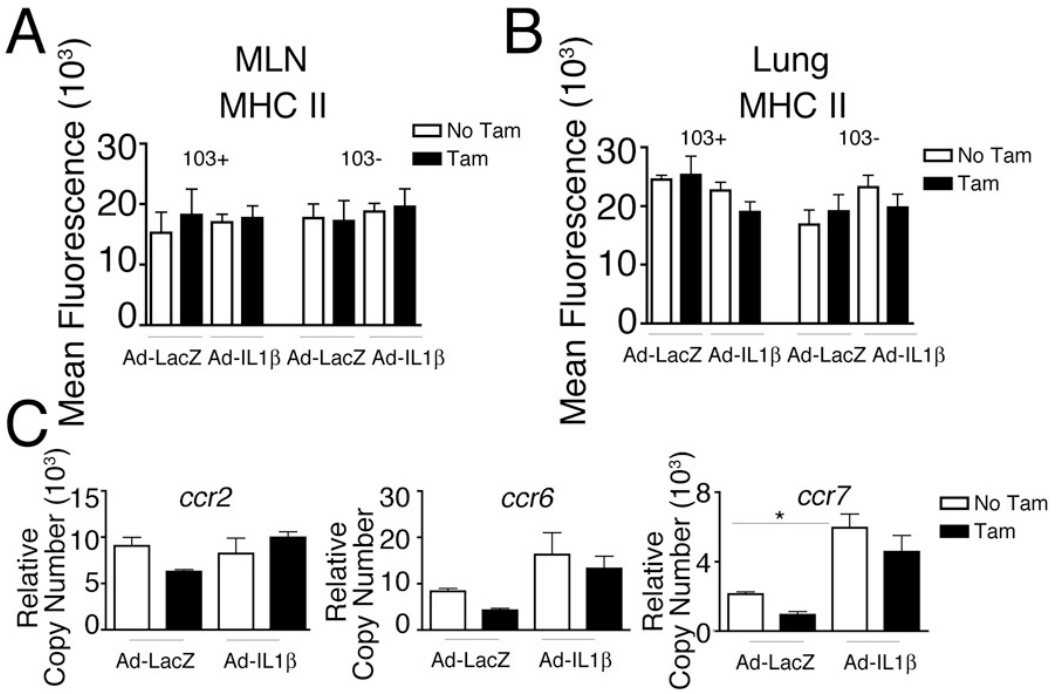
Supplemental Figure 3. Deletion of *itgb8* in all airway cell types inhibits Ad-IL-1β-induced airway remodeling. A-D: Histologic (micrographs represent 14 days post treatment) and morphometric analysis of inflammation (**C**, H&E) or fibrosis (**D**, trichrome), 7, 14, 21 or 42 days after mice with a single floxed *itgb8* allele, and a single knock-out *itgb8* allele (*itgb8* F/-) were treated with IT Ad-IL1-β with (solid lines) or without Ad-Cre (dotted lines). In **B**, Arrows point to thick collagen fibers. Shown are values minus the non-IT-Ad-IL-1β treated controls.



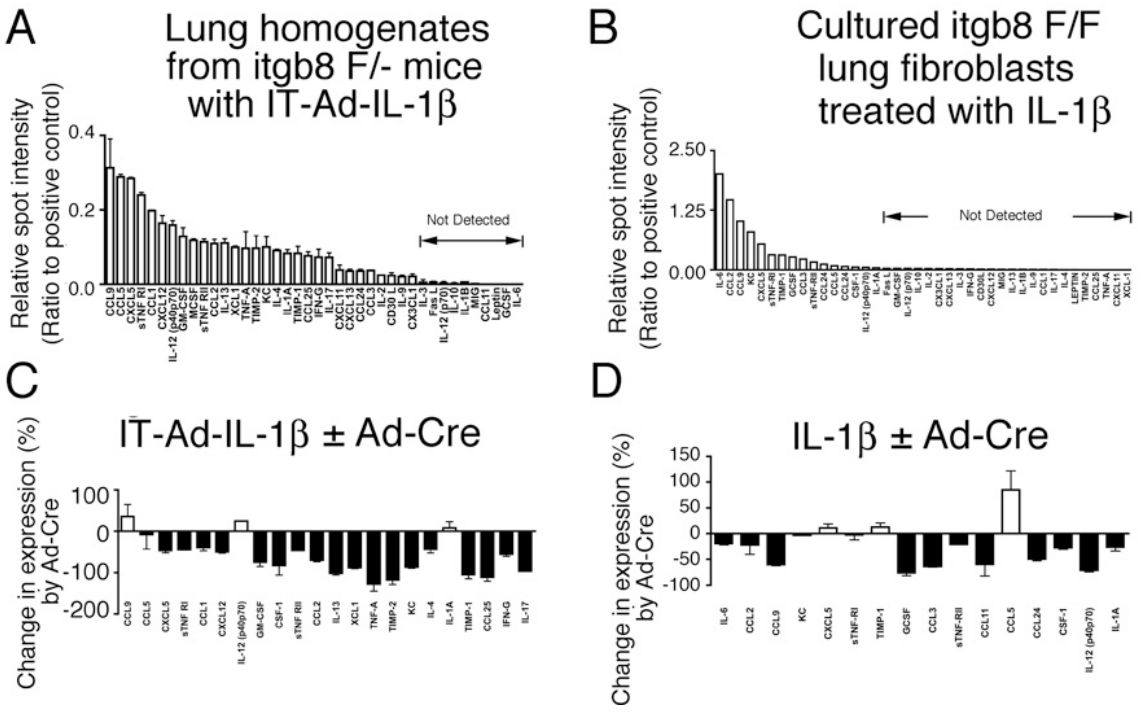
Supplemental Figure 4. Ligand-dependent recombination of *itgb8* in fibroblasts decreases *itgb8* expression and αvβ8-mediated TGF-β activation in cultured lung fibroblasts. Lung fibroblasts enriched above 40/60% Percoll interface from whole lung homogenates of Col-Cre-ER(T);itgb8 F/- mice treated without or with Tamoxifen, were cultured until confluent and tested for *itgb8* expression using **A:** qPCR or **B:** TGF-β activation using a TMLC TGF-β bioassay. *p<0.05



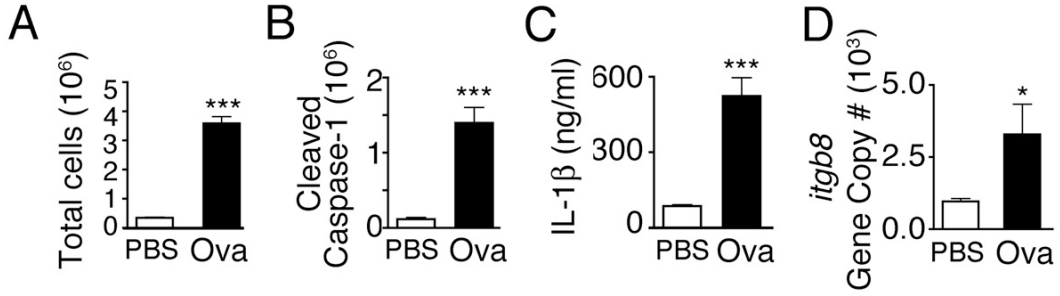
Supplemental Figure 5: Lung DCs can be separated from alveolar macrophages based on forward and side scatter, CD11c, MHC II and Ly6c staining: Flow cytometric separation of lung DCs from alveolar macrophages using CD11c and MHC II in combination with either Ly6c (**A**) or F4/80 (**B**). Forward and side scatter gates were based on "back-gating" on CD11c hi, autofluorescent, or CD11c high, non-autofluorescent cells. **A:** High side scatter, high forward scatter, MHC II intermediate, Ly6c intermediate cells were mostly autofluorescent (AF) alveolar macrophages (**AM**). Low side scatter, low forward scatter, Gr1, TCRβ, NK1.1, CD19 negative, MHCII high, Ly6c intermediate cells were non-AF lung DCs. **B:** High side scatter, high forward scatter, MHC II intermediate, F4/80 negative cells were mostly autofluorescent (AF) alveolar macrophages (**AM**). Low side scatter, low forward scatter, Gr1, TCRβ, NK1.1, CD19 negative, MHCII high, F4/80 positive cells were non-AF lung DCs. Shown in **B**, second column are overlays (isotype control in blue vs. F4/80 in red). Histograms in the right upper panel of both **A** and **B** show autofluorescent separation of AM vs. DCs, and in the lower right panels, overlays of AM (blue) and DC (red) using AF vs. CD11c staining.



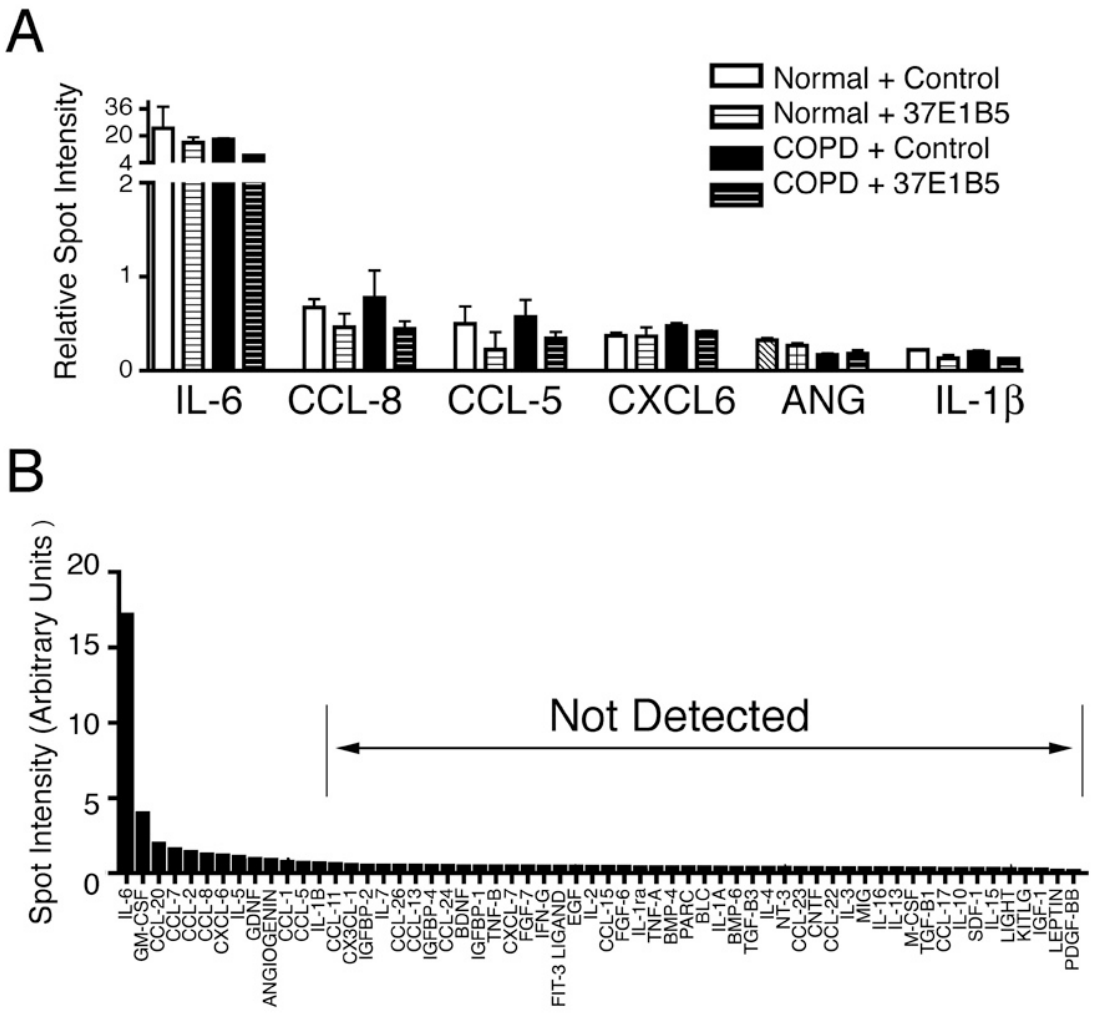
Supplemental Figure 6: Loss of *itgb8* on fibroblasts does not affect lung or MLN DC maturation. A,B: Lungs or MLN were harvested from Col-Cre-ER(T);*itgb8*F/- mice treated with IT- Ad-LacZ or IT-Ad-IL-1β, with or without Tamoxifen. Shown is the mean fluorescence intensity in arbitrary units for MHC II expressed by 103+ or 103- DCs from the MLN (**A**) or lung (**B**). Data represents a minimum of 6 mice for Ad-LacZ controls and a minimum of 12 mice for Ad-IL-1β groups. **C:** Lung CD11c+ DCs from Col-Cre-ER(T);*itgb8*F/- mice treated with IT- Ad-LacZ or IT-Ad-IL-1β, with or without Tamoxifen were purified using CD11c microbeads (Miltenyi Biotech, Auburn, CA) and were assessed using qPCR for *ccr2*, *ccr6*, and *ccr7* (left to right). Shown are relative gene copy numbers. *p<0.05



Supplemental Figure 7: Cytokine array analysis of *itgb8*-dependent changes in cytokine expression from IL-1β treated lungs and cultured lung fibroblasts. Cytokine antibody array of pooled mouse lung homogenates (n=6) harvested 14 d after IT-Ad-IL-1β (**A**) or of pooled mouse fibroblast (n=5) supernatants 48 hrs after IL-1β treatment (**B**). Shown is the spot intensity relative to internal positive control for each cytokine listed from highest to lowest expression, left to right. **C,D:** Change in expression of each cytokine detected on the array after treatment with Ad-Cre from whole lung homogenates (**C**) or primary cultures of *itgb8* F/F lung fibroblasts (**D**). Shown is percent change in expression of IL-1β treated vs. IL-1β treated + Ad-Cre.



Supplemental Figure 8: Ovalbumin challenge stimulates inflammasome activation, IL-1β secretion, and increased *itgb8* expression. *Col-cre-ER(T);itgb8* F/-mice underwent a mock (PBS, open bars) or acute ovalbumin (Ova, filled bars) sensitization and challenge protocol. Eight hrs after the final Ova challenge, the mice were sacrificed, BAL harvested, and **A**) total cells counted. **B**) Pelleted BAL cells were stained with a fluorescent cleaved caspase-1 detection kit and numbers of labeled cells determined using flow cytometry. **C**) Lung homogenates were made from the right lung of each mouse and IL-1β ELISA was performed from an equal volume of lung homogenate. **D**) Gene copy number for *itgb8* was determined from the left lung of each mouse using qPCR. Results were normalized to β-actin and *gapdh.*n=8. *p<0.05, ***p<0.001.



Supplemental Figure 9: IL-1β treated human normal and COPD fibroblasts secrete a limited repertoire of cytokines. Normal (n=5) or COPD (n=5) fibroblasts were treated with recombinant IL-1β for 24 hr in the presence or absence of isotype-matched control or anti-β8 (37E1B5). Pooled supernatants were applied to cytokine antibody arrays (Ray Biotech, Inc., Norcross GA) and detected by chemiluminescence (Chemidoc, BioRad, Hercules, CA). Shown is data not represented in **Figure 6D. A**) Relative spot intensity (minus negative control) was expressed as a ratio to the positive control. **B**) Relative spot intensity of each detected cytokine from IL-1β treated COPD fibroblast supernatants are plotted from the most highly expressed to the lowest. Cytokines that were not detected are indicated.

Supplemental Table 1: Summary of Airway Morphometry

Mouse group	Genotype	n	Time (d)	Airways examined	Inflammation	Fibrosis
Ad-LacZ Control	WT	2	14	29	3.1±0.4	4.4±0.4
Ad-Lac Z 3G9	WT	2	14	40	2.7±0.4	4.1±0.4
Ad-Lac Z 1D11	WT	2	14	42	2.9±0.3	2.8±0.2
Ad-IL-1β Control	WT	3	14	57	33.3±2.4	10.0±0.8
Ad-IL1β 1D11	WT	3	14	48	17.0±1.8	6.9±0.7
Ad-IL1β 3G9	WT	3	14	55	28.9±3.1	9.7±0.9
Ad-LacZ no Tam	Col-Cre ER(T); itgb8 F/-	3	14	46	2.9+/-0.3	7.6+/0.6
Ad-LacZ Tam	Col-Cre ER(T); itgb8 F/-	3	14	67	3.6+/-0.3	9.9+/0.6
Ad-IL-1β no Tam	Col-Cre ER(T); itgb8 F/-	4	14	95	36.5+/3.3	24.2+/-1.4
Ad-IL-1β Tam	Col-Cre ER(T); itgb8 F/-	6	14	148	10.5+/1.0	13.1+/-0.7
Ad-LacZ no Tam	Col-Cre ER(T); itgb8 F/-	3	21	62	4.4+/-0.5	9.3+/-0.8
Ad-LacZ Tam	Col-Cre ER(T); itgb8 F/-	3	21	59	3.2+/-0.3	8.0+/-0.7
Ad-IL-1β no Tam	Col-Cre ER(T); itgb8 F/-	5	21	104	17.3+/1.5	13.0+/0.9
Ad-IL-1β Tam	Col-Cre ER(T); itgb8 F/-	5	21	122	11.4+/1.1	12.1+/0.9
Ad-LacZ no Tam	Col-Cre ER(T); itgb8 F/-	1	42	15	2.7+/-0.3	4.4+/-0.4
Ad-LacZ Tam	Col-Cre ER(T); itgb8 F/-	1	42	20	3.1+/-0.4	4.1+/-0.4
Ad-IL-1β no Tam	Col-Cre ER(T); itgb8 F/-	2	42	35	5.9+/-0.8	7.3+/-0.7
Ad-IL-1β Tam	Col-Cre ER(T); itgb8 F/-	2	42	39	3.1+/-0.3	4.2+/-0.5
Ad-LacZ no Tam	K14 Cre-ER(T); itgb8 F/-	7	14	80	3.1+/-0.2	11.2+/-1.0
Ad-LacZ Tam	K14-Cre ER(T); itgb8 F/-	6	14	72	3.2+/-0.2	8.7+/-0.8
Ad-IL-1β no Tam	K14-Cre ER(T); itgb8 F/-	9	14	111	24.3+/-1.7	17.1+/-1.6
Ad-IL-1β Tam	K14-Cre ER(T); itgb8 F/-	9	14	114	20.5+/-1.4	15.6+/-1.7
Ad-LacZ no Tam	WT	2	14	41	3.2±0.3	ND
Ad-LacZ Tam	WT	2	14	43	3.1±0.2	ND
Ad-IL-1β no Tam	WT	3	14	53	38.8±2.5	ND
Ad-IL-1β Tam	WT	3	14	58	43.3±3.1	ND
PBS no Tam	Col-Cre ER(T); itgb8 F/-	2	70	34	3.9+/-0.4	4.8+/-2.7
PBS Tam	Col-Cre ER(T); itgb8 F/-	2	70	42	3.4+/-0.3	5.5+/- 3.3
Ova no Tam	Col-Cre ER(T); itgb8 F/-	7	70	142	15.3+/1.2	14.0+/-1.0
Ova Tam	Col-Cre ER(T); itgb8 F/-	6	70	110	6.1+/-0.7	7.6+/-0.7
Ad-LacZ/Ad-GFP	Itgb8 F/-	6	7	76	5.4+/-0.6	12.6+/-1.0
Ad-LacZ/Ad-Cre	Itgb8 F/-	6	7	77	6.9+/-0.9	7.1+/-0.6
Ad-IL-1β/Ad-GFP	Itgb8 F/-	8	7	96	28.2+/-1.8	28.2+/-1.9
Ad-IL1β/Ad-Cre	Itgb8 F/-	8	7	103	20.9+/-1.3	20.8+/-1.3
Ad-LacZ/Ad-GFP	Itgb8 F/-	6	14	112	5.9+/-0.8	9.5+/-0.6
Ad-LacZ/Ad-Cre	Itgb8 F/-	4	14	74	5.4+/-0.6	9.1+/-0.8
Ad-IL-1β/Ad-GFP	Itgb8 F/-	8	14	145	25.1+/-1.8	14.8+/-0.8
Ad-IL1β/Ad-Cre	Itgb8 F/-	8	14	135	12.3+/-1.1	12.8+/-0.7
Ad-LacZ/Ad-GFP	Itgb8 F/-	8	21	176	13.1+/-1.0	9.3+/-0.5
Ad-LacZ/Ad-Cre	Itgb8 F/-	8	21	165	20.6+/-1.5	12.6+/-0.6
Ad-IL-1β/Ad-GFP	Itgb8 F/-	11	21	217	22.3+/-1.4	12.4+/-0.6
Ad-IL1β/Ad-Cre	Itgb8 F/-	11	21	229	22.7+/-1.4	11.8+/-0.5
Ad-LacZ/Ad-GFP	Itgb8 F/-	8	42	131	6.4+/-0.4	8.9+/-0.3
Ad-LacZ/Ad-Cre	Itgb8 F/-	9	42	201	5.6+/-0.3	8.6+/-0.3
Ad-IL-1β/Ad-GFP	Itgb8 F/-	14	42	245	9.8+/-0.5	12.4+/-0.6
Ad-IL-1β/Ad-Cre	Itgb8 F/-	13	42	276	8.1+/-0.4	7.7+/-0.3
Ad-LacZ/Ad-GFP	WT	1	14	12	7.2±1.6	ND[1]
Ad-LacZ/Ad-Cre	WT	1	14	16	4.1±1.1	ND
Ad-IL-1β/Ad-GFP	WT	3	14	54	20.6±2.5	ND
Ad-IL1β/Ad-Cre	WT	3	14	43	21.2±2.5	ND

1. ND, Not Done

Supplemental Table 2: Primers

gene name	Species	application	5' primer name	Sequence (5' to 3')	3' primer name	Sequence (5' to 3')
itgb8	Mouse	genotyping	mutant F	AGAGGCCACTTGTGTAGCGGCCAAG	mutant R	GGAGGCATACAGTCTAAATTGT
itgb8	Mouse	genotyping	Flox F	GAGATGCAAGAGTGTTTACC	Flox R	CACTTTAGTATGCTAATGATGG
cre	Mouse	genotyping	colcre F	ATCCGAAAAGAAAACGTTGA	colcre R	ATCCAGGTTACGGATATAGT
itgb8	Mouse	recombination	B8 intron 3 F	GTGGTTAAGAGCACCGATTG	exon 4-5 R	CTAAATTGTAGTCACTGCACTGATTG
itgb8	Mouse	SYBR Green	exon 3-4 F	CCAACTGCATCCAGGAGCTGAAGCT	exon 4-5 R	CTAAATTGTAGTCACTGCACTGATTG
itgb8	Mouse	SYBR Green	B8.2 F	GGGAGTGTGAAGGTGGCAGA	B8.2 R	CTGAGGCTGATGGGCACTGA
serpine1	Mouse	SYBR Green	Pai1(2) F	AGTGATGGAGCCTTGACAG	Pai1(2) R	AGGAGGAGTTGCCTTCTCTT
col1a2	Mouse	SYBR Green	coll1a F	AAGGTGCTGATGGTTCTCC	coll 1a R	TCTTTCTCCTCTCTGACCG
tgfb1	Mouse	SYBR Green	tgfb F	GCAACATGTGGAACTCTACCAGAA	TGFB R	GACGTCAAAAGACAGCCACTC
ifng	Mouse	SYBR Green	infg F	ATGAACGCTACACACTGCATC	inf g R	CGACTCCTTTTCCGCTTCCTG
il4	Mouse	SYBR Green	il-4(2) F	AGGTCACAGGAGAAGGGACGCC	il-4(2) R	TGCGAAGCACCTTGGAAGCCC
il5	Mouse	SYBR Green	il-5 F	CTCTGTTGACAAGCAATGAGACG	il-5 R	TCTTCAGTATGTCTAGCCCCTG
il6	Mouse	SYBR Green	il-6 F	AGTTGCCTTCTTGGGACTGA	il-6 R	TCCACGATTTCCCAGAGAAC
il13	Mouse	SYBR Green	il-13(1) F	CTTGCTTGCCTTGGTGGTCTCGC	il-13(1) R	GCAGTTTTGTTATAAAGTGGGCT
il17a	Mouse	SYBR Green	il-17A(2) F	ACCCTGGACTCTCCACCGCA	il-17A(2) R	CAGGTGCAGCCCACACCCAC
il17f	Mouse	SYBR Green	il-17F(2) F	TCGAGACCCCCACCGGTTCC	il-17F(2) R	AGCGTTGTCAGGCCGCTTGG
ccl2	Mouse	SYBR Green	ccl2(2) F	GAGGAAGGCCAGCCCAGCAC	ccl2(2) R	TGGGGCGTTAACTGCATCTGGC
ccl20	Mouse	SYBR Green	ccl20(2) F	GCAGCCAGGCAGAAGCAGCA	ccl20(2) R	CGCCCCCATGGATTGTGGGA
ITGB8	Human	SYBR Green	B8 JM F	AGGATCTTCTACCCCTCTTGC	B8 JM R	ATCTGGACAGATGGCGGTAATG
ITGB8	Human	SYBR Green	ITGB8 ja F	TGGTCGAGGAGTTTGTGTTTG	ITGB8 ja R	AGCCACTGAAGCATTGGCA
COL1A2	Human	SYBR Green	Type1coll F	TCTGGATGGATTGAAGGGACA	Type1coll R	CCAACACGTCCTCTCTCACC

Cells and reagents: Cell culture media and antibiotics were prepared by the University of California, San Francisco Cell Culture Facility using deionized water and analytical grade reagents. Fetal calf serum was obtained from Invitrogen (Carlsbad, CA), human recombinant IL-1β and TGF-β1 were obtained from R&D Systems (Minneapolis, MN). Hybridoma clone 1D11 (American Tissue Type Collection (ATCC), Manassus, VA), is a pan-TGF-β isoform monoclonal antibody producer which generates an antibody cross reactive to TGF-β1, 2, 3 of human, mink and mouse origin. Anti-SV5 (1) and anti-human MHC class I (IgG2a, ATCC) hybridomas were grown and purified using FPLC, as previously described (2). Antibodies were tested for endotoxin to confirm endotoxin levels <0.2 EU/μg as determined by LAL method, (Genscript, Piscataway, NJ). Anti-mouse CCL2 (MAB479) and CCL20 (MAB760) were purchased (R&D systems). ELISA kits human CCL2 (Cat#DY279), human CCL20 (Cat#DY360), mouse CCL2 (Cat#DY479), mouse CCL20 (Cat#DY760) and mouse IL-1β (Cat#MLB00B) were from R&D systems. Human Cytokine antibody array-6 (cat#AAH-CYT-6) and mouse inflammatory antibody array-1 (cat#AAM-INF-1) were from RayBiotech, (Norcross, GA). TMLC TGF-β reporter cells were maintained in 10% FCS in DMEM (gift of John Munger, New York University Medical Center, NYC, NY).

Antibody engineering and affinity maturation of clone 37E1: The variable regions of anti-human integrin β8 (clone 37E148) were sequenced. Mutations were introduced into the variable regions by error-prone PCR, the mutant repertoire displayed as scFv antibodies on the surface of Saccharomyces Cerevisiae and higher affinity scFv selected by using flow cytometry 90. An scFv with increased affinity over the parental scFv was identified (37E1B5) and cloned into a mouse IgG2a backbone Details of 37E1B5 antibody engineering and production are to be published in a separate manuscript.The parental mouse antibody 37E1 was affinity maturated to 37E1B5, using antibody yeast display technology. Briefly, total RNA was extracted from 5×10^7 cells of 37E1(3), and the heavy and light chain variable domain genes (VH and VL) were amplified separately using using 5'-RACE kit (Invitrogen Catalog Number 18374-058) and mouse V-gene family specific primers (4). The VH & VL genes were subcloned into pYD2 vectors and expressed as single chain variable domain (scFv) on the surface of yeast strain EBY100 as described (1). The binding specificity of the parental 37E1 scFv was confirmed using soluble truncated human integrin αvβ8 in FACS analysis (5). A scFv yeast display library at the size of 2×10^7 was created via error-prone PCR (GeneMorph® II Random Mutagenesis Kit, Strategene) amplification of both VH and VL genes and was displayed as scFv on the surface of yeast (1). This yeast displayed scFv library was sorted 6 times with decreased concentration of soluble truncated human integrin αvβ8, and the binding of any scFv was detected with a non-overlapping monoclonal antibody directed against the αv integrin subunit (clone 8B8(3)) during the FACS sorting and analysis process. Twenty-four randomly picked best binders after the last sorting were affinity compared and sequenced. Clone 37E1B5 was chosen to convert into full length immunoglobulin. The full-length immunoglobulin 37E1B5 was produced from stable transformed CHO cell lines as chimeric IgG1 and mouse

IgG2a after subcloning the V-genes into two different mammalian cell expression vectors (6)(U.S. Patent Application No. 61/305,749).

Ovalbumin sensitization and challenge: Six- to 12 wk-old sex-matched and littermate control Col-Cre-ER(T);*itgb8* F/- mice were sensitized with 50 μg Ovalbumin (Sigma) in a total volume of 200 μl saline intraperitoneally (IP) on days 0, 4, and 7. Starting on day 10, mice were injected IP for 5 consecutive days with 1 mg of tamoxifen emulsified in corn oil or controls injected with corn oil alone. Intranasal challenge with ovalbumin (20 μg in 30 μl of saline) or saline alone was performed once a week for 9 weeks with tamoxifen or corn oil injected IP on the same day. Bronchoalveolar lavage (BAL) was performed and lung harvested 10 wks after sensitization and used for ELISA. To determine the role of *itgb8* in DC migration, 6 to 12 wk-old sex-matched and littermate control Col-Cre-ER(T);*itgb8* F/- mice were sensitized on days 0, 7, and 14 by IP injection of 50 μg ovalbumin (Ova) (Sigma-Aldrich) emulsified in 1 mg of aluminum potassium sulfate in a total volume of 200 μl in PBS. Control animals received an equal volume of PBS/aluminum potassium sulfate. On day 16, mice were injected IP for 5 consecutive days with 1 mg of tamoxifen emulsified in corn oil or controls injected with corn oil alone. Intranasal challenge with ovalbumin (100 μg Ova/40 μl of PBS) or with PBS alone was given on days 21, 22, and 23 to lightly anesthetized mice (isoflurane inhalation). In some experiments, Ova-FITC (Invitrogen, Carlsbad, CA) delivered intranasally (30 μl of a 5mg/ml stock) was co-administered with the day 23 challenge, 24 h prior to organ harvest for DC analysis.

Recombinant Adenovirus: The recombinant E1-E3 deleted type 5 adenovirus, either empty (Ad-C) or expressing human active IL-1β (Ad-IL-1β), has been described in detail elsewhere (7). The replication-deficient virus was commercially amplified and purified by cesium chloride gradient centrifugation and PD-10 Sephadex chromatography, plaque titered on 293 cells and checked for wild-type contamination (ViraQuest Inc., North Liberty, IA). Recombinant type 5 Adenoviral vectors expressing Cre-eGFP fusion protein, eGFP, or LacZ were obtained from the Gene Transfer Vector Core (University of Iowa, Iowa City, IA)

Induction of Tamoxifen-dependent recombination: 4-hydroxy Tamoxifen (Sigma) was dissolved in ethanol (100 mg/ml) and suspended in 4 parts corn oil (20mg/ml) and stored a -20°C. For intraperitoneal (IP) injections, the tamoxifen solution was diluted twice in corn oil, sonicated for 2 min (2 x 1 min by using 3-s pulses). Six to 12 week old mice received IP injection of 1 mg of tamoxifen per day or corn oil alone for 5 consecutive days prior to IT-Ad treatment.

Intratracheal injections: Mice were anesthetized with IP injection of Avertin (250 mg/kg, IP). Then Ad-hIL-1β or Ad-LacZ (2.5 x 10^8 pfu in 75μl sterile PBS) was instilled intratracheally with a needle(Popper® 24G-1' Straight 1.25mm ball) using the direct visualized instillation (DVI) technique (8). For Ad-Cre dose-response experiments, the following doses of Ad-Cre were premixed with Ad-IL-1β prior to

intratracheal instillation into *itgb8* F/- mice: 1.25, 2.5 or 7.5x10^8 pfu. All other experiments with combinations of viruses were performed with 2.5 x10^8 pfu of each. Pairs of viruses and their appropriate controls were Ad-IL-1β/Ad-LacZ, or Ad-IL-1β/Ad-C and Ad-Cre-eGFP/Ad-eGFP. For anti-β6 or anti-TGF-β systemic treatment, mice were injected IP with 3G9 (neutralizing anti-β6, gift of Shiela Violette, Stromedix, Cambridge, MA), 1D11 antibody (4mg/kg/mouse) for 3 consecutive days prior to IT-Ad-IL-1β treatment (day 0) and then injected 3 times/wk (4mg/kg/mouse) with 1D11 until the organ harvest on day 14.

Field of recombination of Ad-Cre and Col-Cre-ER(T) and activation of the Cre-ER(T) fusion protein: ROSA26-STOP-LacZ (R26-LacZ) reporter mice have the LacZ reporter with an upstream STOP cassette flanked by LoxP sequences. In the presence of functional Cre-recombinase this STOP cassette is excised and the LacZ gene is expressed (9). Thus IT-Ad-Cre (2.5 x10^8 pfu) or IT-Ad-eGFP (negative control) was administered to R26-LacZ mice to determine the field of recombination in the lung and compared to WT mice infected with IT-Ad-LacZ (2.5 x10^8 pfu) to determine the field of adenoviral infection in the lung. Rosa-LacZ ; Col-Cre-ER(T) mice were injected IP for 5 consecutive days with 1 mg of tamoxifen or with corn oil alone, as above, prior to IT-Ad-IL-1β or Ad-C. These mice were sacrificed at 7 d after injection.

Staining for β-Galactosidase: 7 days after injection, the lungs were fixed by intratracheal perfusion with 1% glutaraldehyde in PBS (pH 7.3) at 4°C for 1 h. The lungs were rinsed twice by PBS and X-Gal staining solution (5 mM $K_4Fe(CN)_6$, 5 mM $K_3Fe(CN)_6$, 2 mM magnesium chloride, 1 mg/ml X-Gal in PBS) was instilled. After tracheal ligation, harvested lungs were incubated in X-Gal solution overnight, washed with PBS, and the lungs were post-fixed in 10% formalin and paraffin embedded. Cytospin preparations of BAL were air dried for 1 h, and stored at -80°C until use. These slides were fixed with 0.5% glutaraldehyde for 10 min at RT, washed twice in PBS, and stained for 3 h in X-Gal staining solution.

Mouse organ harvests and bronchoalveolar lavage (BAL): For BAL, the trachea was cannulated and the lungs were lavaged 5 times using 0.8 ml of sterile PBS with 5mM EDTA. The recovery of the total lavage exceeded 90%. The fractions were centrifuged (600 *g* for 10 minutes), and the supernatant from the first fraction collected and kept at –80°C for ELISA assays or TGF-β bioassays, performed as described (3); the cell pellets from all fractions were pooled and resuspended in 1.0 ml sterile PBS and the total cell count was determined using a hemocytometer. Differential cell counts were performed using cytospin preparations (Cytospin 3; Thermo Shandon), which were prepared by centrifuging at 800 rpm for 6 min. Differential cell counts were made by counting 200 cells using standard morphological criteria. Caspase-1 activation was determined in BAL cells using a Green FLICA™ Caspase 1 Assay Kit (ImmunoChemistry Technologies, Bloomington, MN).
The heart was punctured and the lungs perfused with 10 ml sterile PBS containing

50 U of heparin per ml (Sigma-Aldrich). The right lung was isolated and divided into its lobes, placed in 20 ml of 10% formalin and shaken vigorously for 30 s to inflate. The left lung was divided into 5 portions, the left upper and the right lower portion used for DNA harvest, the middle frozen for protein analysis and the right upper and the left lower placed in RNA later (Applied Biosystems/Ambion, Austin,TX).

Airway morphometry and immunohistochemistry: Measurements of airway inflammation were estimated using Hematoxylin and Eosin (H&E) stained slides and wall fibrosis was assessed by the presence of thick collagen bundles stained by the trichrome method essentially as described by Hogg(10), which expresses wall thickness as a function of area of the airway wall/basement membrane length determined using image analysis software (Image J, v1.36b). Microtome sections from H&E or trichrome stained sections of paraffin embedded mouse lungs were digitally imaged at 200X magnification (QCapture v2.68.2, Surry, BC, Canada). The slides were coded by an investigator (H.K.) while another investigator (S.L.N) who was blinded to the experimental groups acquired 5 digital images representing each lung lobe (and two images from the largest lobe) and the images coded and catalogued. Airway inflammation was defined as the inflammatory infiltrate extending from the airway basement membrane towards the lung parenchyma. Airway fibrosis was defined as thick collagen bundles (stained blue in trichrome stains) below the airway basement membrane. Digital images were analyzed by a third investigator (S.C) who was blinded to the experimental groups. A learning data set was acquired on a test group using this methodology and based on an experimentally determined standardized width of the confidence interval (W/S), the number of airway measurements/mouse required for 90, 95 and 99% confidence intervals was determined to be 9, 12 and 21, respectively. A minimum of 12 airways was examined/mouse.

Microtome sections from 22 (15 COPD and 7 normal) lung samples from a cohort of COPD patients were immunostained with a monoclonal rabbit anti-human CD11c (Epitomics) or with goat anti-β8 (G17, Santa Cruz Biotechnology, Santa Cruz, CA). The slides were coded and blinded prior to pathologic assessment and digital imaging, and β8 immunostaining assessed as previously described (2). Digital images were assessed for CD11c immunostaining based on a 0-3 scale, with 0 being absent staining; grade 1, 1-3 positive cells/airway; grade 2, 4-10 positive cells/airway, grade 3, >10 positive cells/airway. Correlation between β8 and CD11c was performed using the Pearson correlation test. The degree of pulmonary disability (stages 0 to 4, with 4 being the worst) was determined using the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria (www.goldcopd.com). The number of patients in each stage were; Normal n=7; Stage 1-2, n=12 Stage 3, n=3; Stage 4, n=0.

Bone marrow dendritic cell culture and CFSE labeling: Non-adherent granulocytic lineages were removed at day 2-7 of culture, at which point non-adherent immature BMDC were removed and subcultured for an additional 2-3 d in DC culture medium (RPMI 1640 supplemented with 5% FCS, 50 μM 2-

mercaptoethanol [Sigma-Aldrich, St Louis, MO], 50 μg/ml gentamicin [Invitrogen, Carlsbad, CA], and 20 ng/ml of murine GM-CSF (PeproTech, Rocky Hill, NJ)]. At the end of the subculture period, nearly 95% of the cells were CD11c hi, CD11b hi. BMDC were CFSE-labeled according to manufacturer's instructions (Invitrogen) and used for adoptive transfer. Briefly, cells were resuspended in prewarmed PBS with 0.1% BSA at 1 x10^6 cells/ml. CFSE was added to a final concentration of 10μM and cells were incubated at 37°C for 10 min. Label was quenched by addition of 5 volumes of ice-cold 10% FCS/RPMI, cells were washed 3x and then resuspended in PBS at a concentration of 2x10^6/ 500 μl. Cells (2x106/500 μl/mouse) were injected into the lateral tail-vein. The chronic ovalbumin model has been previously described (11).

Preparation of cell suspensions: Single cell suspensions were prepared from the lungs by mincing and digesting with 0.28 U/ml Liberase TM, (Roche, Indianapolis, IN), 0.1% hyaluronidase and 30 μg/ml DNAse I (Sigma Aldrich, St. Louis, MO) in RPMI 1640 for 90 min in a shaking 37 °C waterbath. Digested lungs were mechanically disrupted by passing through a 70 μM nylon cell strainer (BD-Biosciences, Bedford, MA). Live mononuclear leuokocytes were enriched from lung suspensions by density gradient centrifugation in Percoll (GE Heathcare, Piscataway, NJ) and harvested at the 40/60% interface. CD11c + lung DC cells were purified using CD11c MicroBeads (Miltenyi Biotech, Auburn, CA). Single cell suspensions were obtained from MLNs and spleens by mashing through 70 μM nylon cell strainers (BD-Biosciences) without enzymatic digestion. RBCs were lysed using Red Blood Cell Lysis Solution (Sigma).

Cell staining and flow cytometry: For cell surface staining, Fc receptors were blocked with 10μg/ml (2.4G2, BD Biosciences,), followed by fluorochrome-labeled antibodies from BD Biosciences (San Jose, CA) [Ly6c (AL21), CD103 (M290), F4/80(BM8), GR1 (RB6-8C5), CD62L (MEL-14), CD11b (M1/70), TCRb (H57-597), CD19 (ID3), NK1.1 (PK136)] eBiosciences (San Diego, CA) [CD103 (2E7), CD40 (1C10), CD80 (16-10A1), CD86 (GL1), MHCII (M5/114.14.2), CD11c (N418), CD4 (GK1.5), B220 (RA3-6B2)], BioLegend (San Diego, CA) [CCR6 (29-2L17)], and Invitrogen [CD8a (5H10)]. Cells were washed twice after a 15 min incubation at 4°C and washed once after 15 min at 4°C with streptavidin-Qdot 605 (Invitrogen). For intracellular staining, cells were treated with PMA (Sigma Aldrich) and Ionomycin (Sigma Aldrich) for 1hr at 37°C, prior to adding Golgiplug (BD Biosciences) followed by 4 hrs at 37°C. Fc receptors were blocked with 10 μg/ml (2.4G2, BD Biosciences), followed by fluorochrome-labeled antibodies to TCRβ (H57-59, eBiosciences), NK1.1(PK136, BD Biosciences), CD4 (GK1.5, eBiosciences), CD8α (Invitrogen). Cells were washed after 15 min incubation at 4°C. Cells were then permeabilized with Cytofix/Cytoperm (BD Biosciences) at 4°C for 20 minutes and Fc receptors blocked again with 10 μg/ml 2.4G2, followed by staining in Perm Wash (BD Biosciences) with IL-17-PE (BD Biosciences, TC11-18H10) and IFN-γ-APC (eBiosciences, XMG1.2). Cells were washed twice with Perm Wash and once with staining buffer. FoxP3 was stained using a commercial kit (BD Biosciences). For ELISpot assays, cells were counted and immediately plated in an anti-cytokine mAb-

coated 96-well microplate (ELISpot mouse IFN-γ and IL-17 kits; BD). Eight serial 2- or 3-fold dilutions were done in duplicate, per condition. Spots were counted automatically by using an AID ELISpot Reader.
Flow cytometry acquisition was performed on a LSR II Flow Cytometer (BD-Biosciences) available through the Liver Center Flow Cytometry Core Facility at the University of California, San Francisco and analysis was performed with Flowjo software (Treestar, Ashland, OR).

Human and mouse lung fibroblast cell culture: Left-over human explant lung samples from pulmonary pneumonectomies performed at Moffit-Long Hospital, University of California San Francisco, for severe emphysema (Global Initiative for Chronic Obstructive Lung Disease Criteria, stages III or IV) were gathered during the study period (2006-2010). Control lung tissues were obtained from donor lungs not utilized for lung transplantation, usually for reasons of size mismatching. Adult lung parenchyma were collected from resections performed for primary lung cancer or from normal lungs not used for transplantation. Informed consent was obtained from all surgical participants as part of an approved ongoing research protocol by the University of California San Francisco Committee on Human Research in full accordance with the declaration of Helsinki principles. Airway fibroblasts were cultured from the lung parenchyma by the explant technique and used P1 to P4, as previously described (2). Adult mouse lung fibroblasts were harvested from mouse lung, using an identical explant technique as used for human lung fibroblasts, and used at P1 to P4. Some fibroblasts were treated with human recombinant IL-1β (1 ng/ml) from R&D Systems. Sircol assay of cell lysates was performed to determine collagen concentration according to the manufacturer's instructions (Acurate Chemicals, Westbury, NY).

Genomic DNA isolation, genotyping, RNA isolation, cDNA synthesis and qPCR: Genotyping was performed using tail-tip DNA, as described (3). Genomic DNA was isolated using the Wizard Genomic purification kit (Promega, Madison, WI). PCR to detect recombination of LoxP sites was performed using primers spanning the Exon 4 junctions. RNA isolation, cDNA synthesis and SYBR green PCR (qPCR) was performed, as previously described (2). Details on primers used for amplifications are listed in **Supplemental Table 2.**

Migration Assays: Human adult lung fibroblasts (n=4 Normal and n=4 COPD) or mouse itgb8 F/F fibroblasts were plated on 6-well plates (2.5x105/well) on day 1. On day 2 humans cells were treated with or without IL-1β (1 ng/ml), anti-TGF-β (Clone 1D11, 40μg/ml) anti-β8 (37E1B5, 20μg/ml) or control Mab (W6/32, 40μg/ml) for 24h. Mouse fibroblasts were treated as above with the exception that they were treated with Ad-Cre or Ad-GFP (2.5 x 108 pfu/ml) vectors on day 2, then antibodies and IL-1β on day 3. Ad-Cre was used since neutralizing anti-mouse β8 antibodies do not exist. On day 3 human/day 4 mouse, the media was removed, cells washed in PBS and serum-free DMEM media added. After 24 h the media was collected, pooled, sterile filtered (0.2μm) and 750μl of the pooled sample was added

to the bottom chamber of a transwell plate, 5.0 μm pore size (Costar). BMDCs or live human dendritic cells [derived from CD34+ cord blood cells obtained from MatTrek Corporation (Lot# 1118DC) (Ashland, MA)] were added (1.5x105 cells) to the upper chamber in 200μl of RPMI 1640 and incubated at 37°C in a humidified CO2 incubator. After 4h, the media in the bottom chamber was collected, spun down and the cells counted using a hemocytometer.

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