Supplemental figure legends

Fig. S1. Related to Figure 1

A. Survival curve of mice infected with a range of viral titers (10^5 -10^3 pfu) and the mock infection.
B. Survival curve of mice infected with a range of viral titers (500 -125 pfu)
C. Body weight over time of mice infected with 125, 250 and 500 pfu of virus. Error bars, SD of mean.
D. Control panel shows normal distribution of Clara cells (anti-CC10, red) in bronchioles, AT2 cells (SPC, red) in lung parenchyma, and ciliated cells (acetylated alpha tubulin, green; TAp73, red) in trachea (top panel). At 7dpi Clara cells, AT2 cells, and ciliated cells are disorganized and largely depleted (bottom panel). Scale bar, 50μm.
E. Immunofluorescence detection of viral M2 (red) protein in cells expressing CC10 (green) and SPC (green) at 7dpi. Scale bar, 20μm.
F. Detection of common leukocyte antigen CD45 (green) in normal lung (0dpi), 11dpi lung, and 21dpi lung. Scale bar, 100μm.
G. Masson trichrome staining in murine lung at 15dpi. Keratin and muscle fibers stain red, collagen and bone stain blue, cytoplasm stains light red or pink and nuclei stain brown or black. Scale bar, 100μm.

Fig. S2. Related to Figure 1

A. Immunofluorescence on sections of bronchiolar epithelium at progressive times of infection showing distribution of Clara cells (CC10, green) and basal cells (p63, red). Scale bar, 20mm.
B. Distribution of p63+ cells in the bronchioles and lung parenchyma before and 11 days after infection. Error bars, SD of mean.
C. Western blot of whole lung with antibodies to p63 at different times of H1N1 influenza infection. Lower panel graph depicts p63 expression normalized with respect to actin expression.
D. Left, Immunofluorescence image of BrdU (green) incorporation in the bronchioles at 11dpi and quantification of BrdU-positive Krt5+ (red) cells

versus total Krt5+ cells over time. Right, Immunofluorescence image of BrdU (green) incorporation in lung parenchyma at 15dpi and quantification of BrdU-positive Krt5+ cells versus total Krt5+(red) cells over time. Scale bar, 20μm. Error bars, SD of mean.

E. Krt5 pods are not seen in normal lung parenchyma nor in lung damaged by bleomycin. Left panel, control and bleomycin treated lungs stained with antibodies to Pdpn (green) and SPC (red). Right panel, control and bleomycin treated lungs stained with antibodies to Krt5 (green) and p63 (red). Scale bar, 50μm.

Fig. S3. Related to Figure 3.

A. Left panel, immunofluorescence comparison of anti-CC10 staining (green) in DASC and TASC- ALI cultures. Right panel, histogram reflecting direct counting of CC10-positive cells in DASC and TASC ALI cultures.

B. Upper panel, immunofluorescence labeling of p63-positive cells (red) in 10 day TASC Matrigel cultures. Lower panel, absence of anti-activated capase 3 staining in squamous metaplasia structures formed in Matrigel by TASCs. DNA is counterstained with DAPI (blue).

C. Upper panel, absence of p63 staining in 10 day DASC Matrigel cultures. Lower panel, immunofluroescence detection of anti-activated caspase 3 antibodies (green) in DASC Matrigel culture at day 7. DNA is counterstained DAPI (blue).

Fig. S4. Related to Figure 4.

44 gene provisional signature of TASCs versus DASCs derived from three whole genome microarray analyses of each. P values and fold-changes are presented and those genes validated by qPCR are shown in red.

Fig. S5. Related to Figure 5

Expression heatmaps of microarray data of three control and infected lung colonies covering related GO categories of Wound Healing (top), Tissue Development (middle), and Regulation of Growth (bottom).

Fig. S6. Related to Figure 6

A. Immunofluorescence imaging of 11B6 (red) and CC10 (green) in normal lung. Scale bar, 50μm.
B. Immunofluorescence imaging of PDPN (red) and CC10 (green) in normal lung.
C. Low power view of 21dpi lung after IHC staining of Krt5. Scale bar, 200μm.
D. Merged and isolated images of anti-Krt5 (red) and anti-CD45 (green) staining in lung parenchyma at 15dpi.
E. Merged and isolated images of anti-Krt5 (red) and anti-smooth muscle actin (SMA, green) staining in 15dpi lung.
F. Merged of isolated images of anti-Krt5 (red) and anti-pan-keratin (green) in lung parenchyma of 15dpi lung. Scale bar, 50μm.

Fig. S7. Related to Figure 7

A. Staining of histologically normal appearing 25dpi lung with antibodies to SPC (red) and 11B6 (green).
B. Staining of Krt5+ regions of 25dpi lung stained with antibodies to Krt5 (red) and with 11B6 (green).
C. Staining of Krt5-/SPC+ regions of 25dpi lung with antibodies to SPC (red) and with 11B6 (green).
D. Staining of Krt5-/SPC- regions of 25dpi lung with antibodies to SPC (red) and with 11B6 (green). Scale bar, 20μm.

Fig. S8. Related to Figure 8

A. Merged and isolated images of anti-Krt5 (red) and anti-Krt14 (green) staining in lung parenchyma at 13dpi.

B. Histogram reflecting counting of Krt14-positive cells in bronchioles and deep lung at indicated dpi's.

Table S1. Related to Figure 3: Literature analysis of genes activated in DASC Matrigel cultures.

Table S2. Related to Figure 6: Gene Set Enrichment Analysis of Infected Lung

Supplemental Methods:

Cloning and Differentiating Human and Rat Airway Stem Cells

Airway stem cell culture

Human nasal epithelial cells were isolated from human inferior turbinate. The biopsy specimens of inferior turbinate were obtained from patients who underwent surgery for nasal septal deviation under National University Health Services (Singapore) IRB approval. Normal human tracheobronchial epithelial cells and human small airway epithelial cells were purchase from Lonza. Primary rat lung epithelial cells were isolated from three-week-old Wistar rats. The airway cells were cultivated onto a feeder layer of lethally irradiated 3T3-J2 cells and clonal analysis was based on previously described methods for epidermal stem cells (Barrandon and Green, 1987).

Expression Microarrays and Bioinformatics

All samples were prepared according to manufacturer's instructions (WT-Ovation™ Pico RNA Amplification System WT-Ovation™ Exon Module, Encore™ Biotin Module, NuGEN technologies). Hybridization was performed with human exon 1.0 ST array chips (Affymetrix). All log2-transformed expression data was normalized, and a 1-way ANOVA was done to identify differentially expressed genes using Partek Genomics Suite 6.4. PCA was produced with

whole transcriptome, and heatmaps were generated with sorted datasets by Euclidean distance based on average linkage methods. Gene Set Enrichment Analysis (version 2.0) software was used to identify gene sets and pathways. (Subramanian et al., 2005)

Histology and Immunofluorescence

Histology, immunohistochemistry, and immunofluorescence were performed using standard techniques, processed at the Histology Core at the Institute of Molecular and Cellular Biology at A-STAR and imaged at the Institute of Medical Biology, A-STAR.

Immunofluorescence staining was performed on 4% paraformaldehyde-fixed, paraffin-embedded sections or frozen sections. The sections were stained with antibodies to mucin 5AC (Santa Cruz Biotechnology), acetylated α-tubulin (Sigma) and CC10 (US-Bio). p63 (clone 4A4), K5 (Neomarkers), K10 (Covance), Sp-C (Santa Cruz Biotechnology), podoplanin (PDPN) (Santa Cruz Biotechnology), involucrin (Abcam), and loricrin (Abcam). Alexa Fluor-coupled secondary antibodies (Invitrogen) were used for all immunofluorescence staining. All images for section slides were captured by using Alxo Observer.Z1 fluorescence microscope (Zeiss) with monochrome MR Rev3 and color ICc1 (Zeiss) cameras and Axiovision 4.8 software (Zeiss) or LSM 510 confocal microscope (Zeiss) with LSM software. Bright field cell culture images were obtained on an Eclipse TS100 microscope (Nikon) with Digital Sight DS-Fi1camera (Nikon) and NIS-Elements F3.0 software (Nikon).

Monoclonal Antibodies to Rat Lung

Six-week-old Balb/c mice were immunized with 1mg of homogenized rat lung by five intraperitoneal injections every three weeks and then B cells from the spleen were fused with NSO myeloma cells at a ratio of 4:1 (Kohler and Milstein, 1975). Hybridomas were obtained after 10-20 days on HAT selection, and antibodies from these hybridomas were screened directly on sections of adult rat lung using immunofluorescence detection. All positive clones were then cloned by limiting dilution and expanded for antibody production.

In vitro differentiation assays

Air-liquid interface culture of nasal epithelial cells was performed as described (Schmidt et al., 1996). Briefly, cells were cultured on Transwell plates (Corning). At confluence, the medium on the inserts was removed and the medium outside the insert was changed to differentiation medium (DMEM/F12 1:1, 50µg/ml penicillin; 50µg/ml streptomycin; fungizone 2.5µg/ml (Gibco); 10ng/ml cholera toxin, retinoic acid 10^{-7} M; 10% Knockout SR serum replacement (Gibco)). At day10, the membranes with cells were fixed for immunofluorescence staining or paraffin embedding.

Self-assembly sphere culture was performed as described (Ulrich et al., 1998). The cells were cultured in differentiation medium (DMEM/F12 1:1, 50µg/ml penicillin; 50µg/ml streptomycin; fungizone 2.5µg/ml (Gibco); 10ng/ml cholera toxin, retinoic acid 10^{-7} M; 10% Knockout SR serum replacement (Gibco)).The culture dishes were placed on an orbital shaker in an incubator at 37°C and 5% CO_2. On day 8, the self-assembly spheres were fixed for immunofluorescence staining.

3-D Matrigel assay was performed on chambered glass slides as describe in mammary cell 3-D culture (Xian et al., 2009). Briefly, the cell suspensions were placed on Matrigel at $3x10^4$ cells/chamber. After 20 days culturing in the differentiation medium CnT-23(Cellntec) + 1mM$CaCl_2$ with 1% Matrigel, the 3-D structures fixed for sectioning and staining.

Supplemental Figure 1

Figure S1. Related to Figure 1

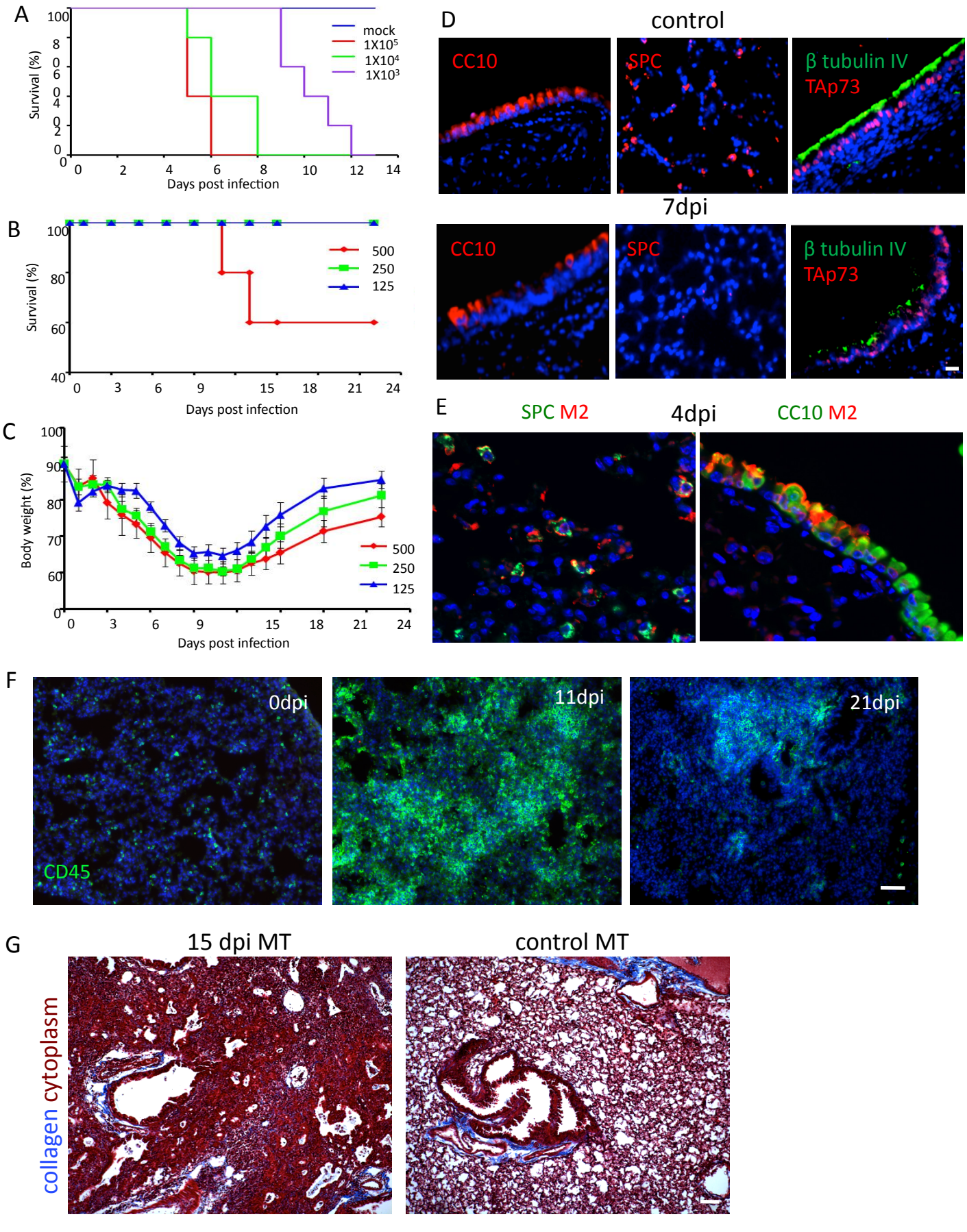

Figure S2. Related to Figure 1

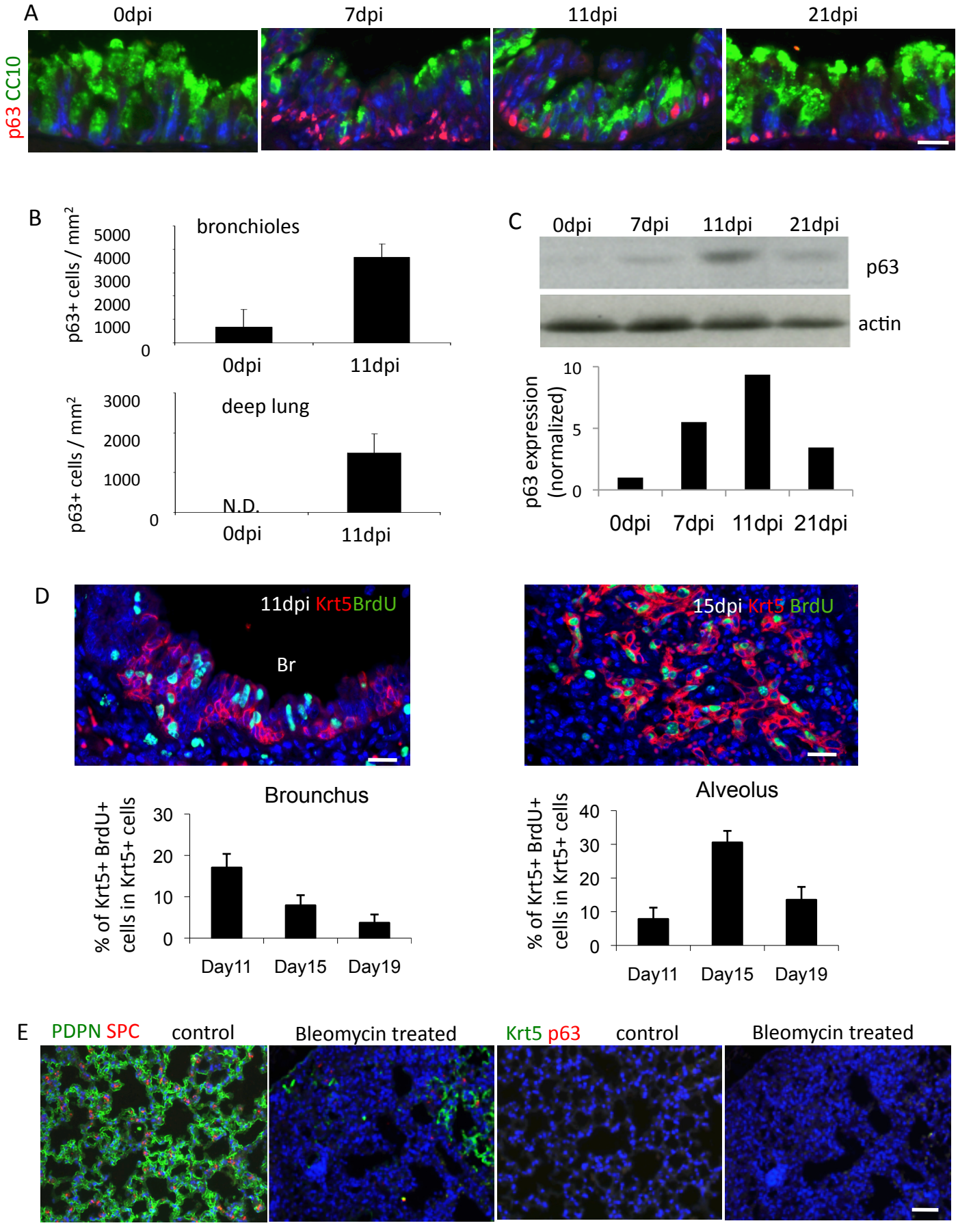

Figure S3. Related to Figure 3

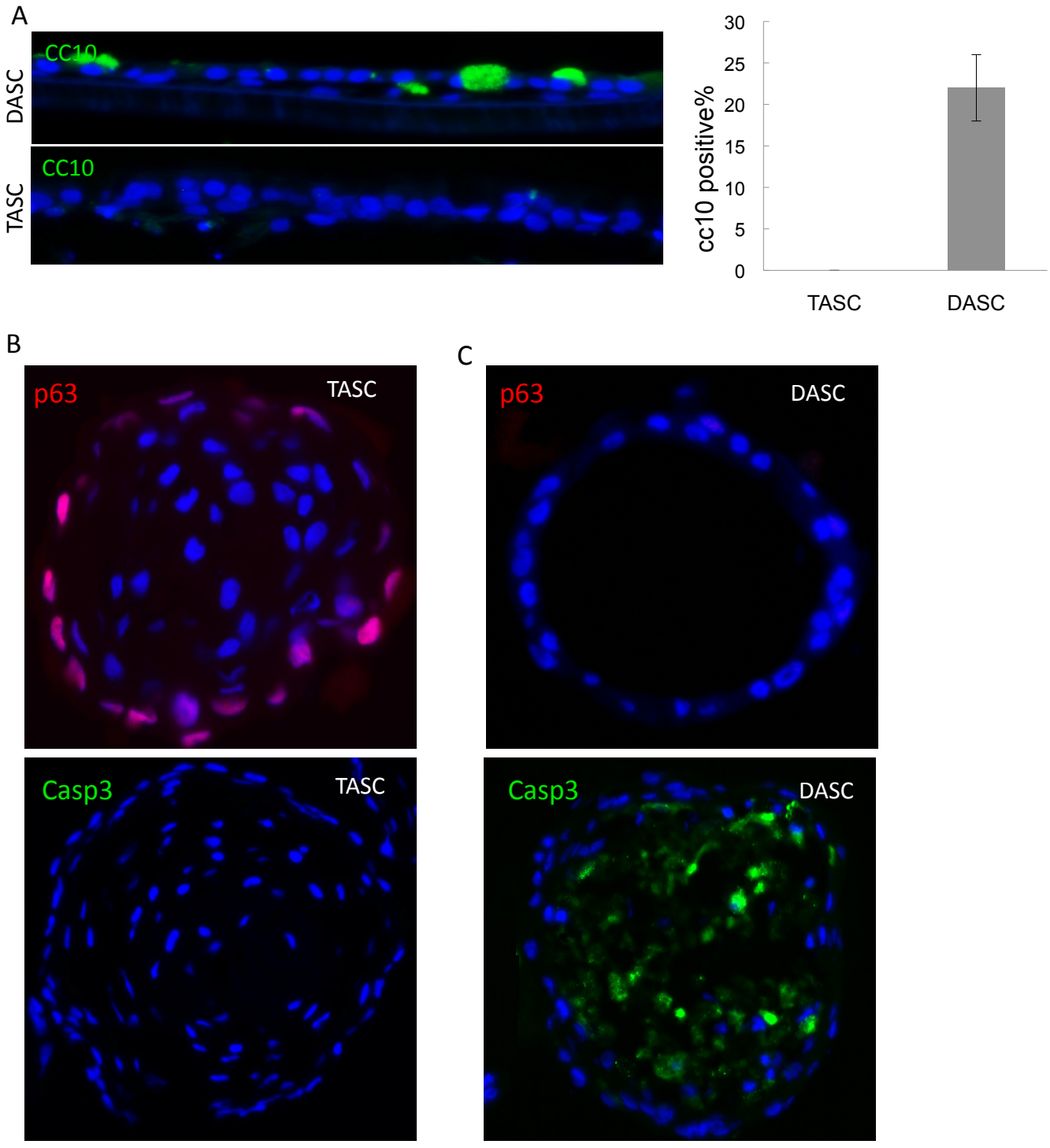

Figure S4. Related to Figure 4

TASC signature

Gene Symbol	p-value	Fold-Change(TASC vs. DASC)
*TMPRSS11D	7.03E-07	29.7703
SPRR1A	1.44E-06	24.4047
SPRR2C	6.17E-07	20.5046
*KRTDAP	5.78E-07	16.3986
*TMPRSS11B	3.87E-07	14.9493
CRNN	2.80E-05	13.0657
MT1L	0.000489486	12.6329
USP6	0.000121694	11.7649
RGS2	2.55E-06	10.9295
SPINK7	8.58E-05	10.7646
STARD4	1.42E-06	9.95315
RAET1L	0.000287793	9.88988
*TMPRSS11A	1.37E-05	9.73362
*DSG1	1.59E-08	9.00731
ZBED2	9.47E-05	7.9196
*ERAP2	7.17E-07	7.69252
HMGCS1	9.72E-06	7.63513
HLA-C	1.79E-05	7.6308
*POF1B	6.88E-07	7.57532
*GLIPR1	1.74E-06	7.47008
POPDC3	0.000584655	6.98849
*PRAC	0.000111292	6.84123
CCDC144A	0.000825603	6.78438
KLK6	3.49E-05	6.52463
LASS3	1.60E-06	6.21085
BCAT1	1.67E-06	6.07267
MMP10	1.03E-05	5.81306
GJB6	3.32E-07	5.77245
A2ML1	0.000667931	5.69143
DNAJC15	4.15E-06	5.59529
*IFI44	4.60E-07	5.17531
SLFN13	4.06E-06	5.08375
TMTC1	2.44E-05	4.9664
COL12A1	8.00E-08	4.93866
PTHLH	1.28E-05	4.9038
ECM1	8.40E-05	4.83907
SLFN11	1.26E-06	4.79127
KRT14	3.19E-07	4.79094
SCG5	1.73E-05	4.70702
TMPRSS11E	0.000821669	4.67233
*FBN2	2.72E-05	4.15269
*ECM1	8.40E-05	4.83907
*KRT13	0.00745423	4.07872

DASC signature

Gene Symbol	p-value	Fold-Change(DASC vs TASC)
GSTA2	6.70E-06	130.893
GSTA1	9.81E-07	42.6899
*LMO3	3.75E-08	40.8243
PPARGC1A	2.78E-08	21.4615
RPS15A	0.0244778	18.9258
ALDH1A1	5.59E-08	18.7274
*SCGB1A1	5.28E-05	18.1001
TF	2.63E-06	10.1834
GOLGA8A	8.42E-06	9.14632
ATP5L	0.0190024	8.39835
LSM3	0.00283319	7.84288
CP	0.000148531	7.56011
SLC34A2	0.000137024	7.47741
KRT15	9.61E-06	6.88251
RPL18	0.00405378	6.84999
RPL14	0.01627	6.69458
*SAA1	1.70E-06	6.0007
TMEM14C	0.00600444	5.80962
HINT1	0.00592648	5.64451
BNIP3	0.0122902	5.62118
COMMD6	0.0367675	5.51012
*SERPINF1	4.07E-07	5.20435
CYP4B1	8.70E-07	5.06866
LYPLA1	0.000328677	5.01599
TXNIP	1.81E-05	5.00165
RAP1B	0.0280893	4.98122
TRIM22	2.79E-06	4.88373
CHCHD3	0.0144078	4.7621
RPL17	0.0204879	4.69287
S100P	0.00456373	4.64782
CCDC146	4.50E-05	4.63141
SERPINB3	1.44E-05	4.53137
SH3PXD2A	0.0314038	4.35029
CIAPIN1	0.00286368	4.33304
CYP1B1	0.000900554	4.27465
SF3B5	0.000387497	4.19474
FAM46C	0.00065419	4.18709
STEAP4	0.00381719	4.15361
WFDC2	0.000125701	4.1281
*C4orf18	0.000166752	3.783
*SPRY1	0.000583069	3.60757
*DHRS3	8.46E-05	3.27823
*ADAM28	0.000389122	2.96755
*KLK7	0.00547097	2.90156

* qPCR verified

Figure S5. Related to Figure 5

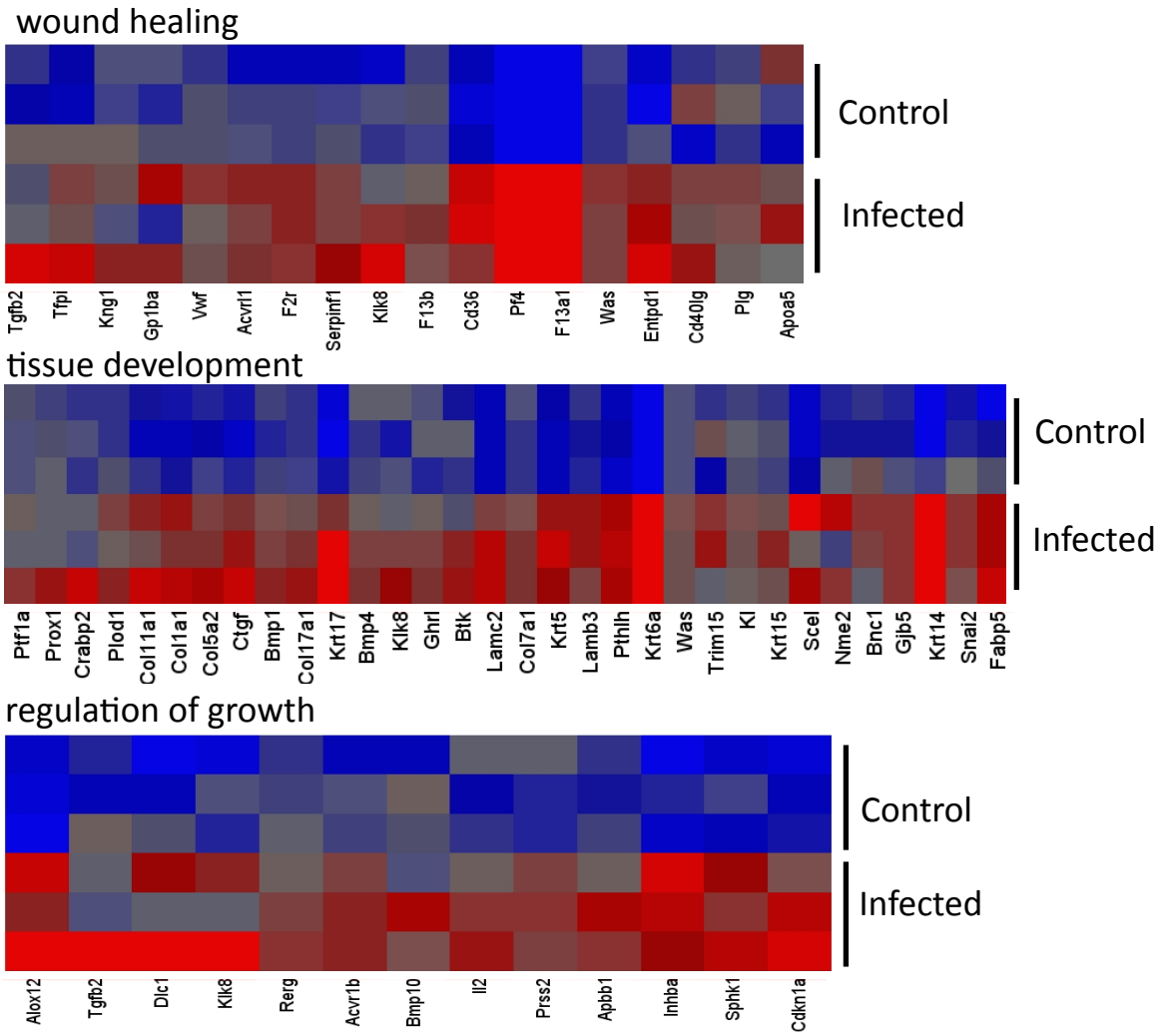

Figure S6. Related to Figure 6

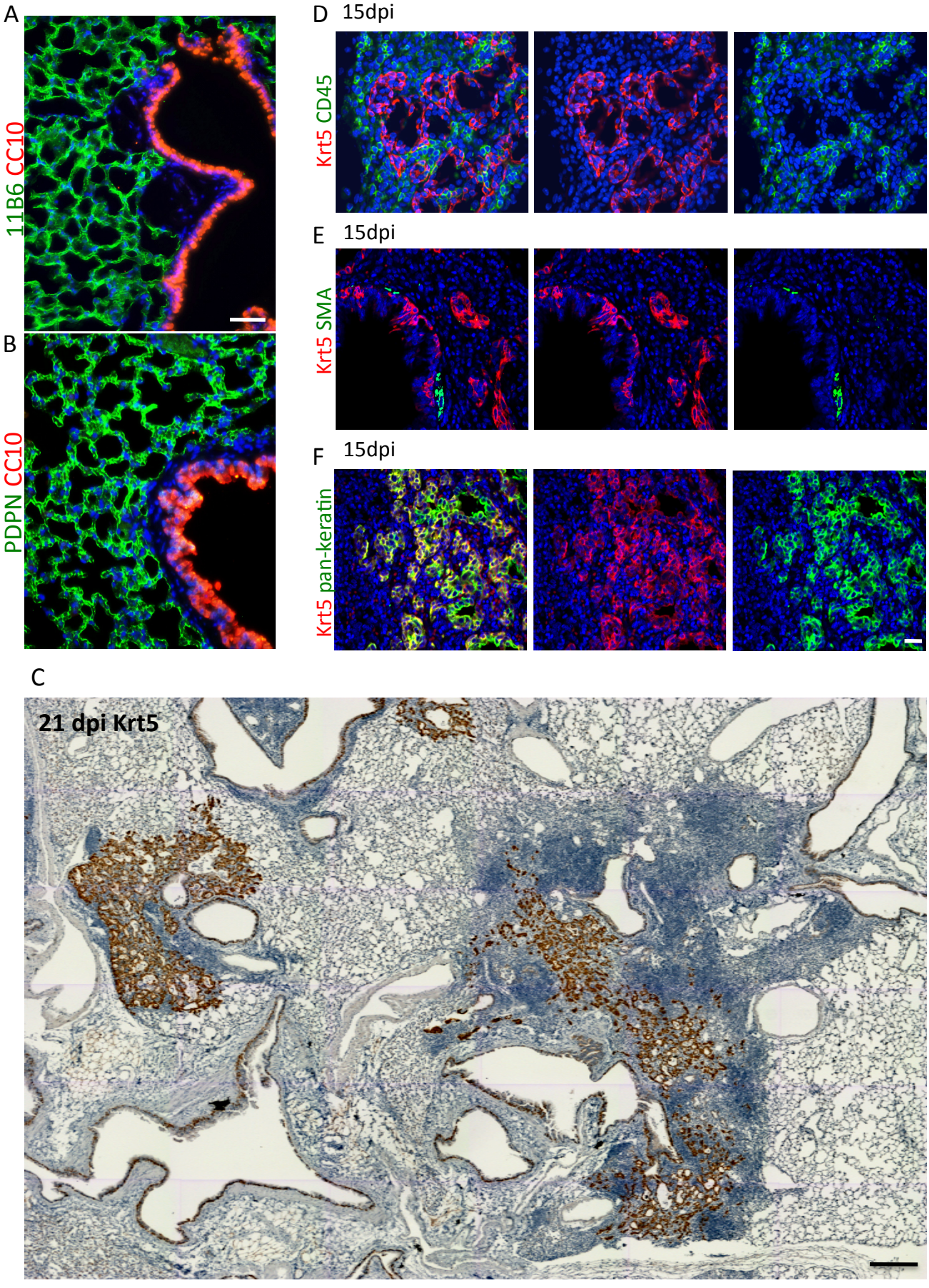

Figure S7. Related to Figure 7

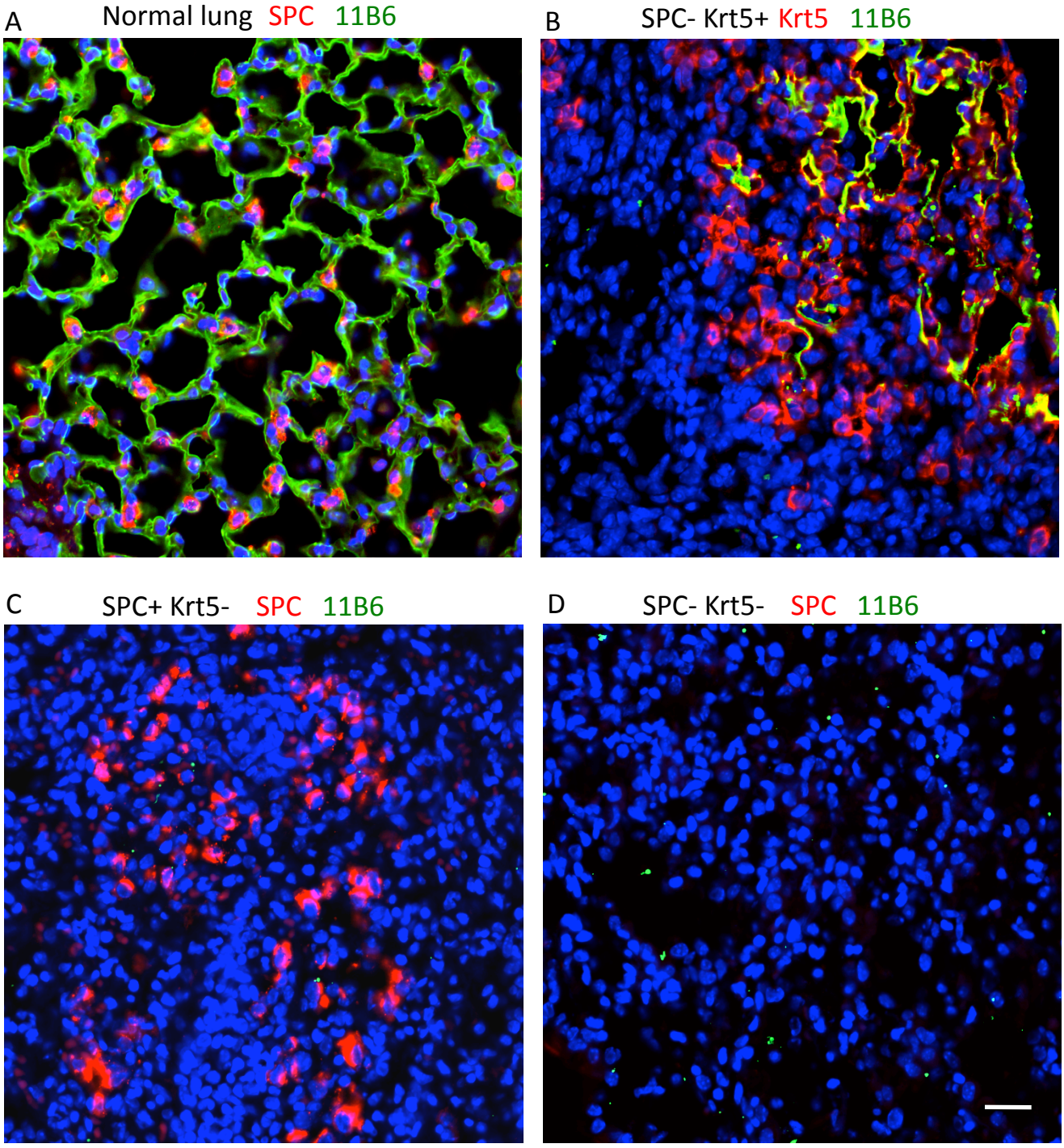

Figure S8. Related to Figure 8