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Supplemental Information

Angiocrine Sphingosine-1-Phosphate Activation of S1PR2-YAP Signaling Axis in Alveolar Type II Cells Is Essential for Lung Repair

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SUPPLEMENTAL FILES:

Figure S1 related to Figure 1

Figure S2 related to Figure 2

Figure S3 related to Figure 2

Figure S4 related to Figure 3

Figure S5 related to Figure 4

Figure S6 related to Figure 6

Figure S7 related to Figure 7

Table S1 related to Star Method

SUPPLEMENTAL FIGURES

Chen et al Supplemental Figure 1

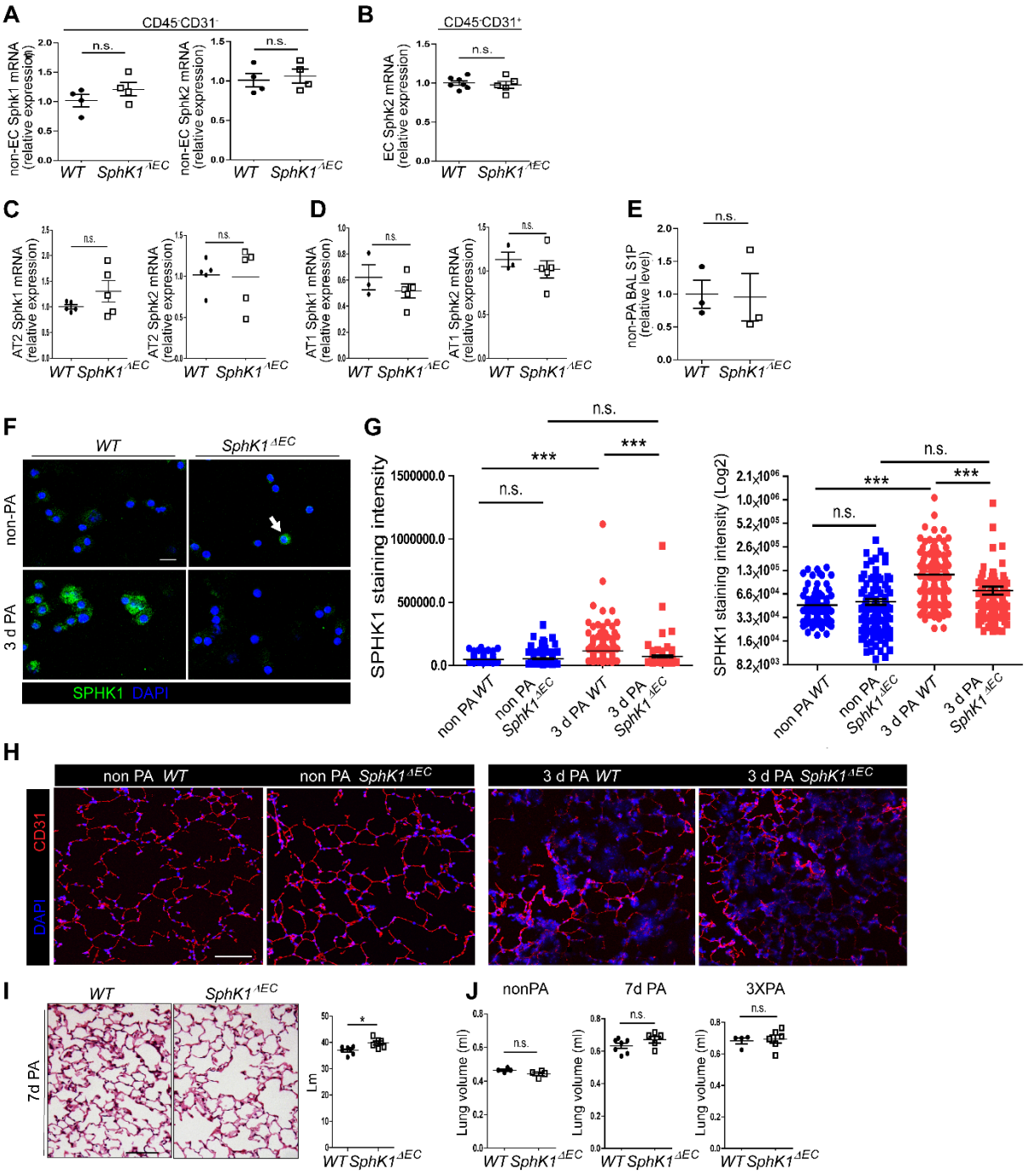

Figure S1 related to Figure 1. The specificity of endothelial *Sphk1* disruption in *SphK1$^{\Delta EC}$* mice. (A-D) qPCR analysis. (A) Similar expression levels of *Sphk1* and *Sphk2* in non-EC cells ($CD45^-CD31^-$) comparing *WT* and *SphK1$^{\Delta EC}$* mice. (B) Similar levels of *Sphk2* in ECs ($CD45^-CD31^+$) between *WT* and *SphK1$^{\Delta EC}$* mice. (C and D) The expression levels of *Sphk1* and *Sphk2* in AT2 (C) and AT1 (D) were similar between *WT* and *SphK1$^{\Delta EC}$* mice. (E) BAL S1P levels were measured for uninjured *WT* and *SphK1$^{\Delta EC}$* mice. (F) EC were isolated from uninjured and 3 d post injured lungs of *WT* and *SphK1$^{\Delta EC}$* mice and fixed on slides by cytospin. EC were stained for SPHK1. Scale bar = 10 μm. (G) The SPHK1 staining intensity in individual cells were quantified and both linear and log2 values were shown. >100 cells were quantified per sample, 3 independent experiments were conducted and showed similar results. (H) Sections of uninjured and 3 d post PA lungs of *WT* and *SphK1$^{\Delta EC}$* mice were stained for CD31. (I) Lungs were collected from *WT* and *SphK1$^{\Delta EC}$* mice at 7 d post a single PA injection, Lm was quantified on H/E stained sections. (J) Total lung volumes were measured from uninjured, 7 d post a single PA and 7 d post 3 repetitive PA injured lungs. Mean ± SEM. *: $p<0.05$; ***: $p<0.001$; n.s.: not significant.

Chen et al Supplemental Figure 2 ↑

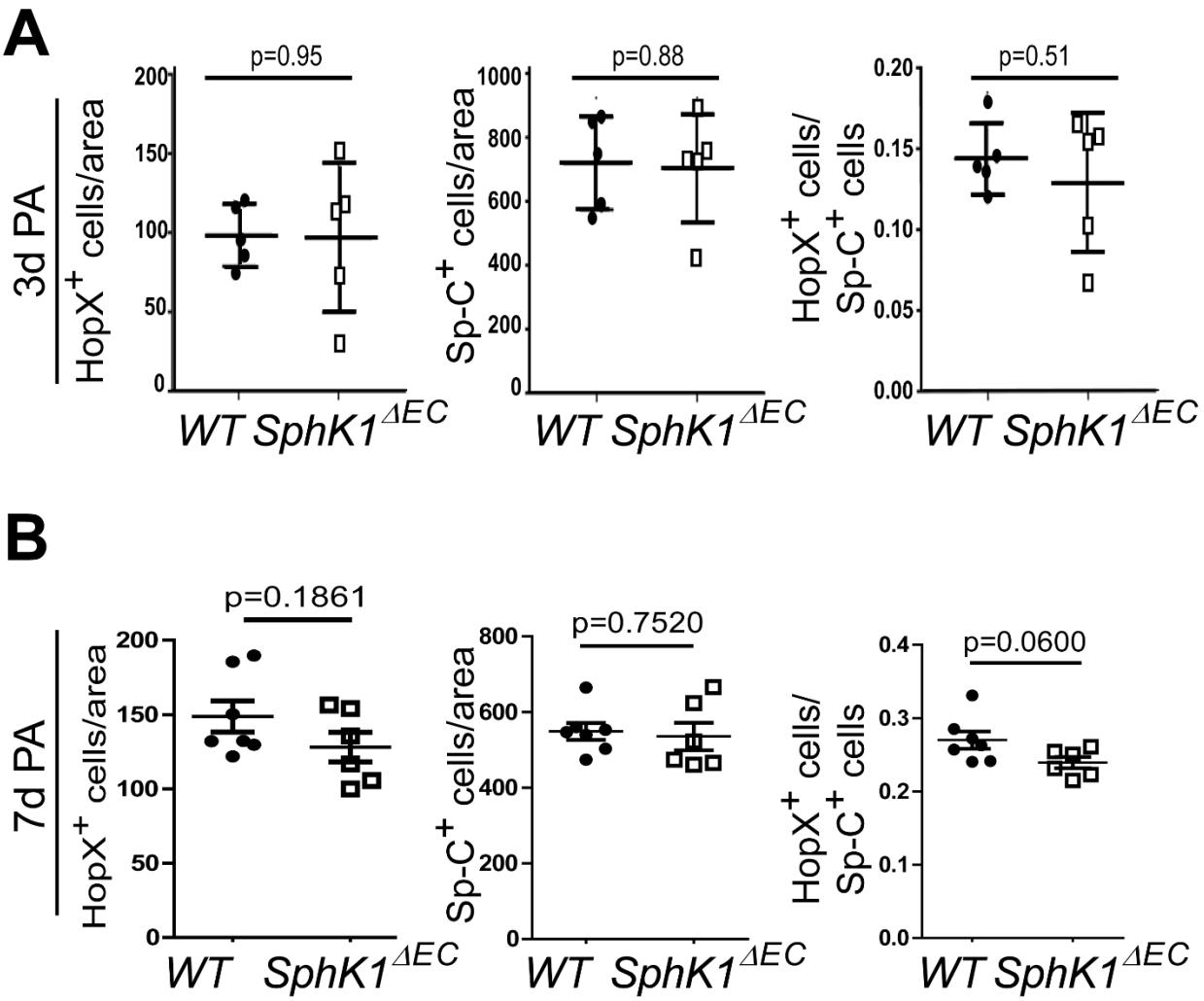

Figure S2 related to Figure 2. Quantification of AT1 and AT2 in *SphK1$^{\Delta EC}$* lungs post injury. Lung sections of *WT* and *SphK1$^{\Delta EC}$* mice at 3 d (A) and 7 d (B) post 1X PA were stained for HopX and Sp-C and the number of HopX$^+$ and Sp-C$^+$ cells per area and the ratio of HopX+/Sp-C+ cells were quantified. Mean ± SEM.

Chen et al Supplemental Figure 3 ↑

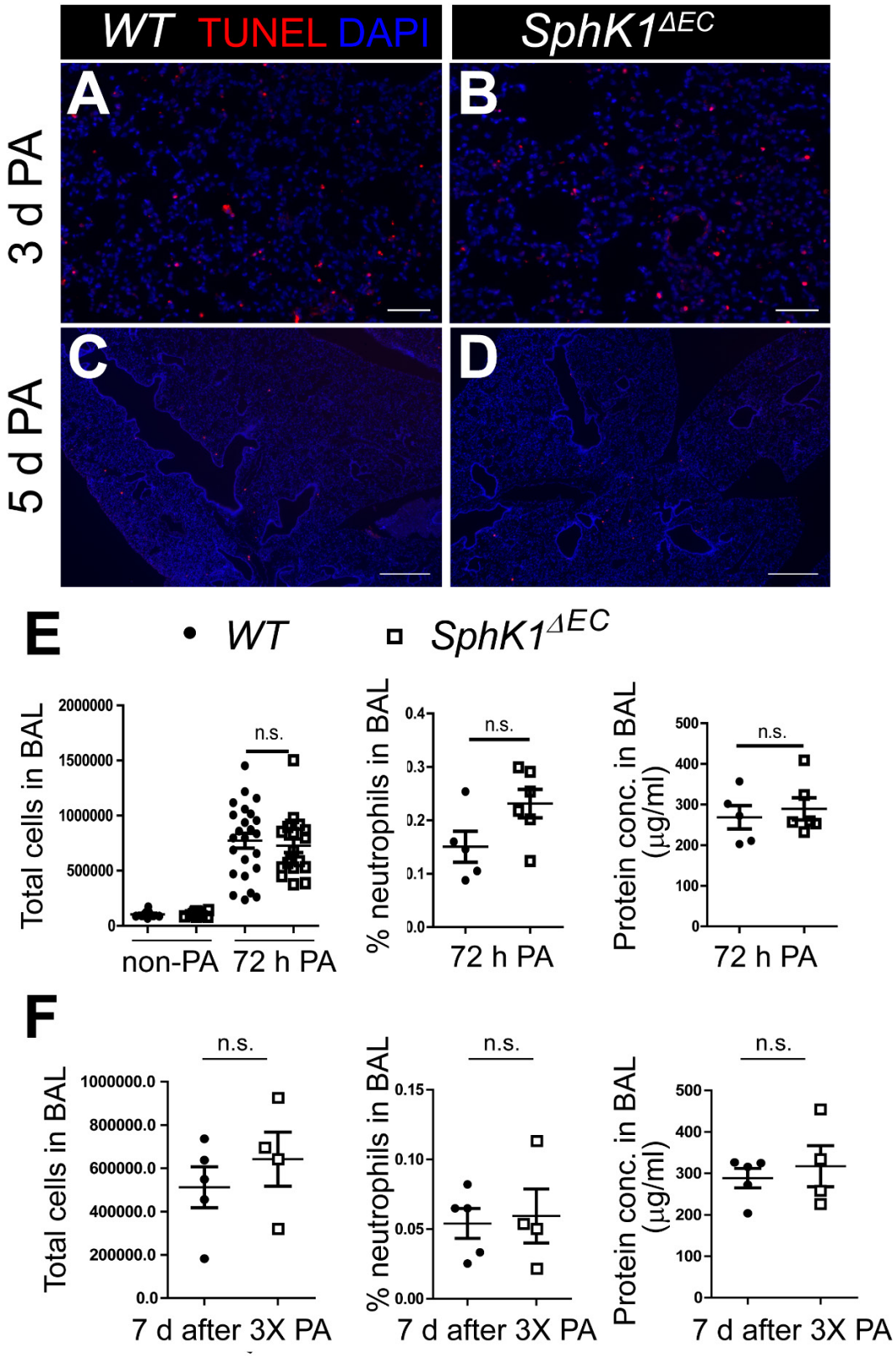

Figure S3 related to Figure 2. Apoptosis and inflammation in *WT* and *SphK1*$^{\Delta EC}$ lungs after PA injury. (A-D) TUNEL assay was performed on lung sections of 3 d and 5 d post PA *WT* and *SphK1*$^{\Delta EC}$ mice. Scale bar in 3d PA images = 50 μm. Scale bar in 5d PA images = 500 μm. (E, F) BAL fluids were collected from uninjured, 72h post injured (E) or 7 d post 3 repetitively injured (F) lungs of *WT* and *SphK1*$^{\Delta EC}$ mice. Total numbers of cells in BAL were counted. Percentage of neutrophils in BAL cells was scored by HEMA3 staining on cells fixed by cytospin. Protein concentration in BAL fluids was measured by BCA assays. Mean ± SEM. n.s.: not significant.

Chen et al Supplemental Figure 4

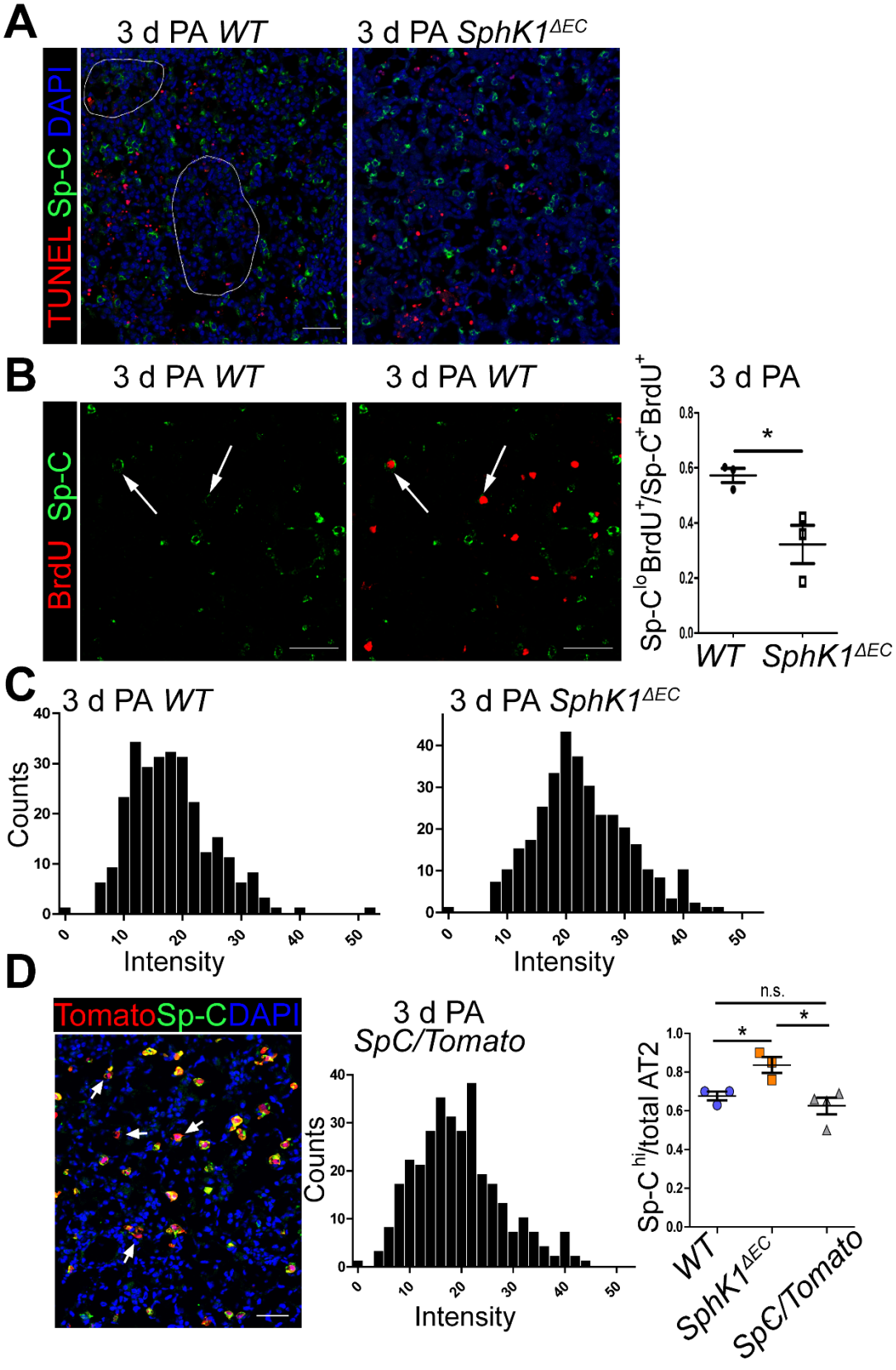

Figure S4 related to Figure 3. Reduction of Sp-C expression in *WT* AT2 after PA is not due to cell death. (A) Lung sections of *WT* and *SphK1*$^{\Delta EC}$ mice at 72 h post-PA were stained by TUNEL assay and anti-Sp-C antibodies. Images were taken at areas showing signs of pneumonia, and regions showing Sp-C reduction were circled. Cells in the circles did not show higher apoptotic levels than those in neighboring areas, and the overall apoptotic levels were similar between *WT* and *SphK1*$^{\Delta EC}$ mice. Scale bar = 50μm. (B) BrdU and Sp-C staining on 3d post PA lungs showed that some cells with low Sp-C expression (arrow) are BrdU$^+$ (Sp-C^{lo}BrdU$^+$). Scale bar = 50μm. The fraction of Sp-C^{lo}BrdU$^+$ cells among total BrdU$^+$ AT2 were quantified and compared between *WT* and *SphK1*$^{\Delta EC}$ mice. (C) Sp-C fluorescence staining intensity of Sp-C$^+$ cells were measured on lung sections of 3 d post PA *WT* and *SphK1*$^{\Delta EC}$ mice. > 100 cells were scored per animal and distribution of Sp-C fluorescence intensity was plotted. The measurements were performed with 3 mice of each genotype and similar results were obtained. (D) Cyrosections of *SpC/Tomato* mice lungs isolated at 3 d post PA were stained for Sp-C. Endogenous Tomato is shown. Arrows point to Tomato$^+$ AT2 that show reduced Sp-C levels. Scale bar = 25 μm. The staining was repeated for 4 mice and Sp-C fluorescence staining intensity was measured from >200 cells per animal. A representative plot of the distribution of Sp-C fluorescence is shown. Cells with Sp-C fluorescence staining intensity >15 were scored as Sp-C^{hi} cells and the percentage of Sp-C^{hi} cells over total Sp-C$^+$ cells were quantified in *WT, SphK1*$^{\Delta EC}$ and *SpC/Tomato* mice. Mean ± SEM. *: $p<0.05$; n.s.: not significant.

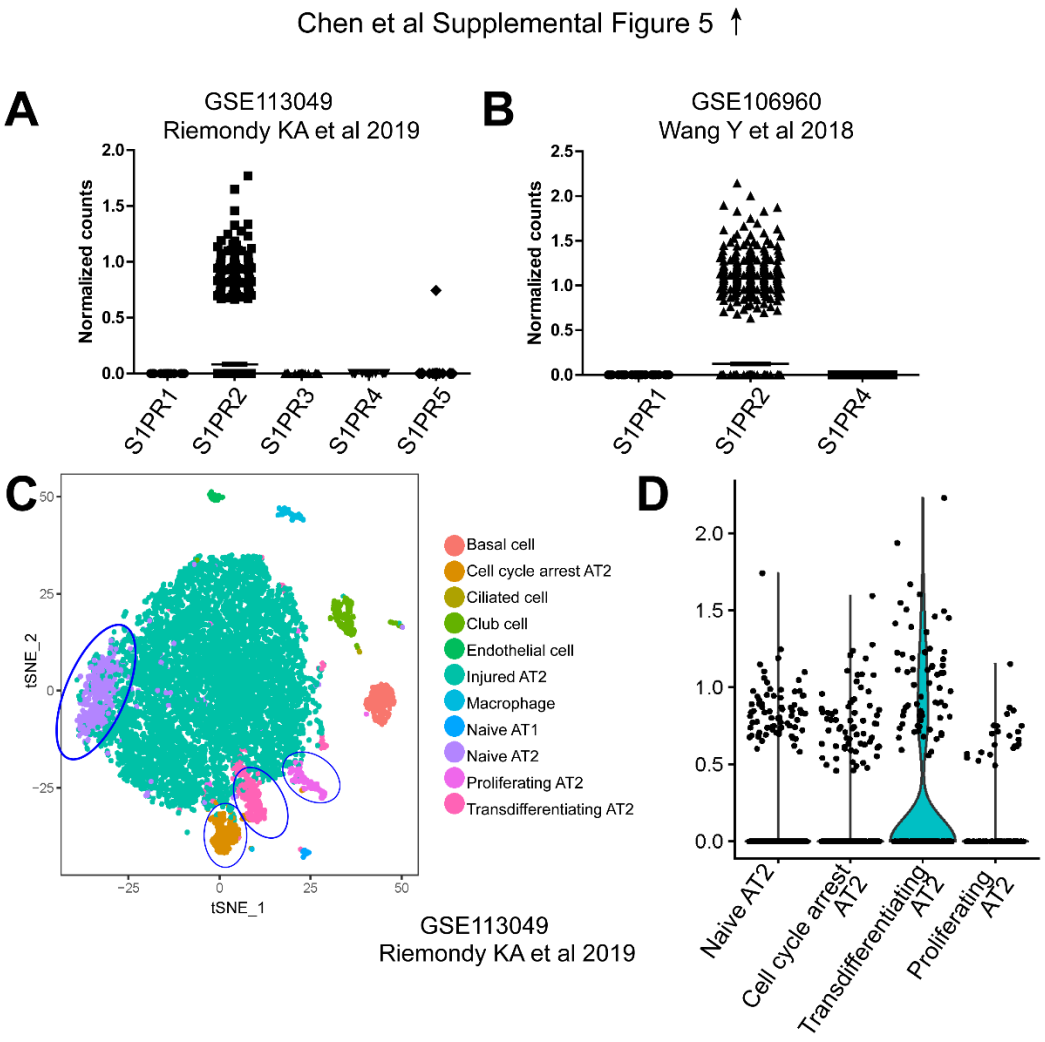

Figure S5 related to Figure 4. S1P receptor expression in AT2 analyzed from published scRNA-seq data. (A) scRNA-seq data of naïve AT2 cells were extracted from the GSE113049 dataset and normalized expression levels of the five S1P receptors were plotted. (B) *S1PR1*, *S1PR2* and *S1PR4* expression in postnatal day 60 AT2 cells (GSM2858342) in the GSE106960 dataset were plotted. *S1PR3* and *S1PR5* were not in this dataset. (C) Unsupervised clustering of the scRNA-seq data from GSE113049 revealed distinct clustering of AT2 cells at different reparative stages as similarly shown in Riemondy KA et.al. (D) Expression levels of *S1PR2* in different subpopulations of AT2 cells were plotted in a violin plot.

Chen et al Supplemental Figure 6↑

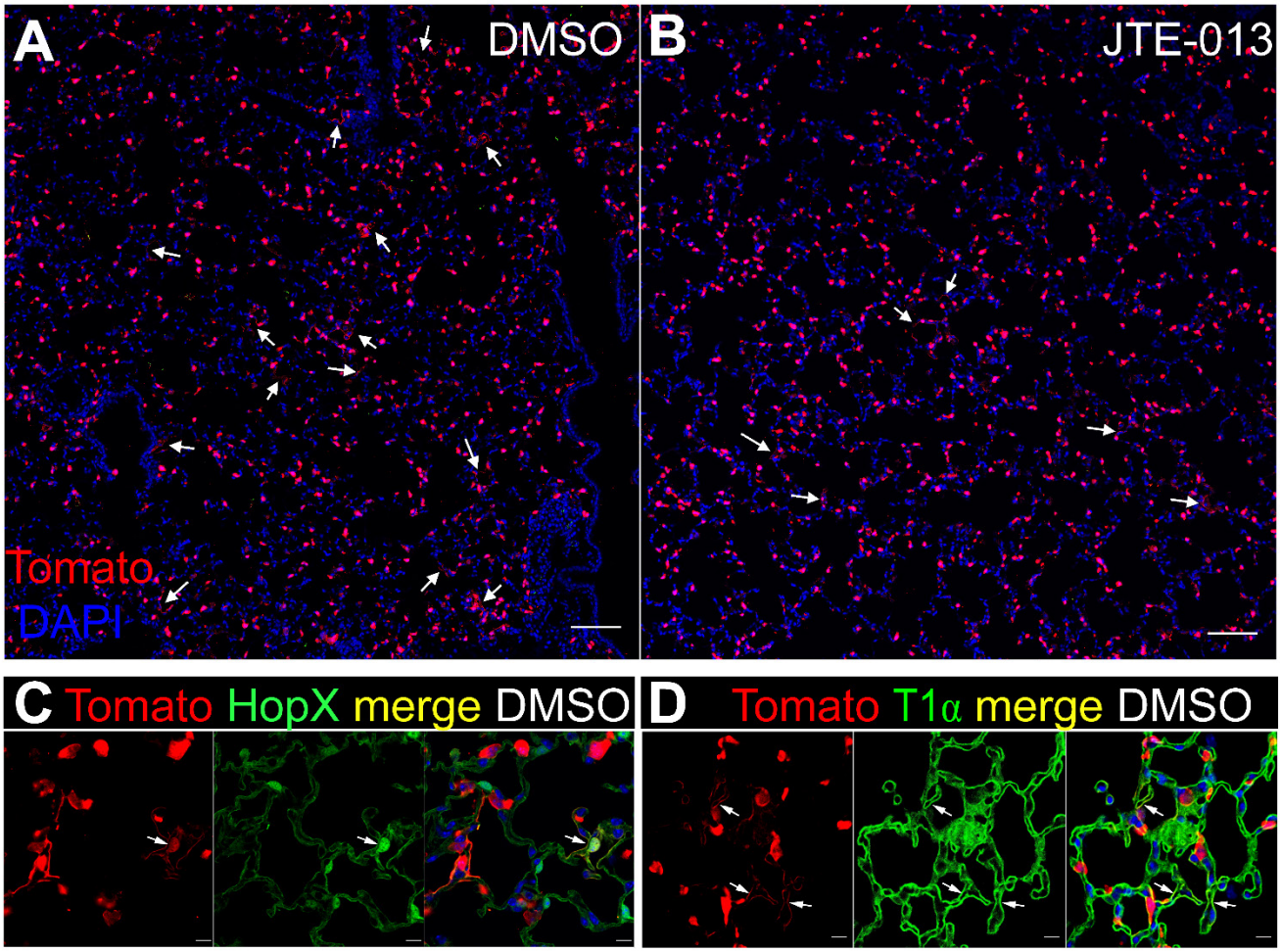

Figure S6 related to Figure 6. JTE-013 inhibits AT2 to AT1 cell transition *in vivo*. (A-B) Tiled images were taken to show larger areas of the lungs of *SpC/Tomato* mice that had been injected with DMSO (A) or JTE-013 (B) after PA injury. Arrows point to AT1-like squamous Tomato^{+} cells. Scale bar= 100µm. (C, D) Squamous Tomato^{+} cells (arrows) in DMSO treated lungs co-express AT1 markers, HopX (C) and T1α (D). Scale bar = 10µm.

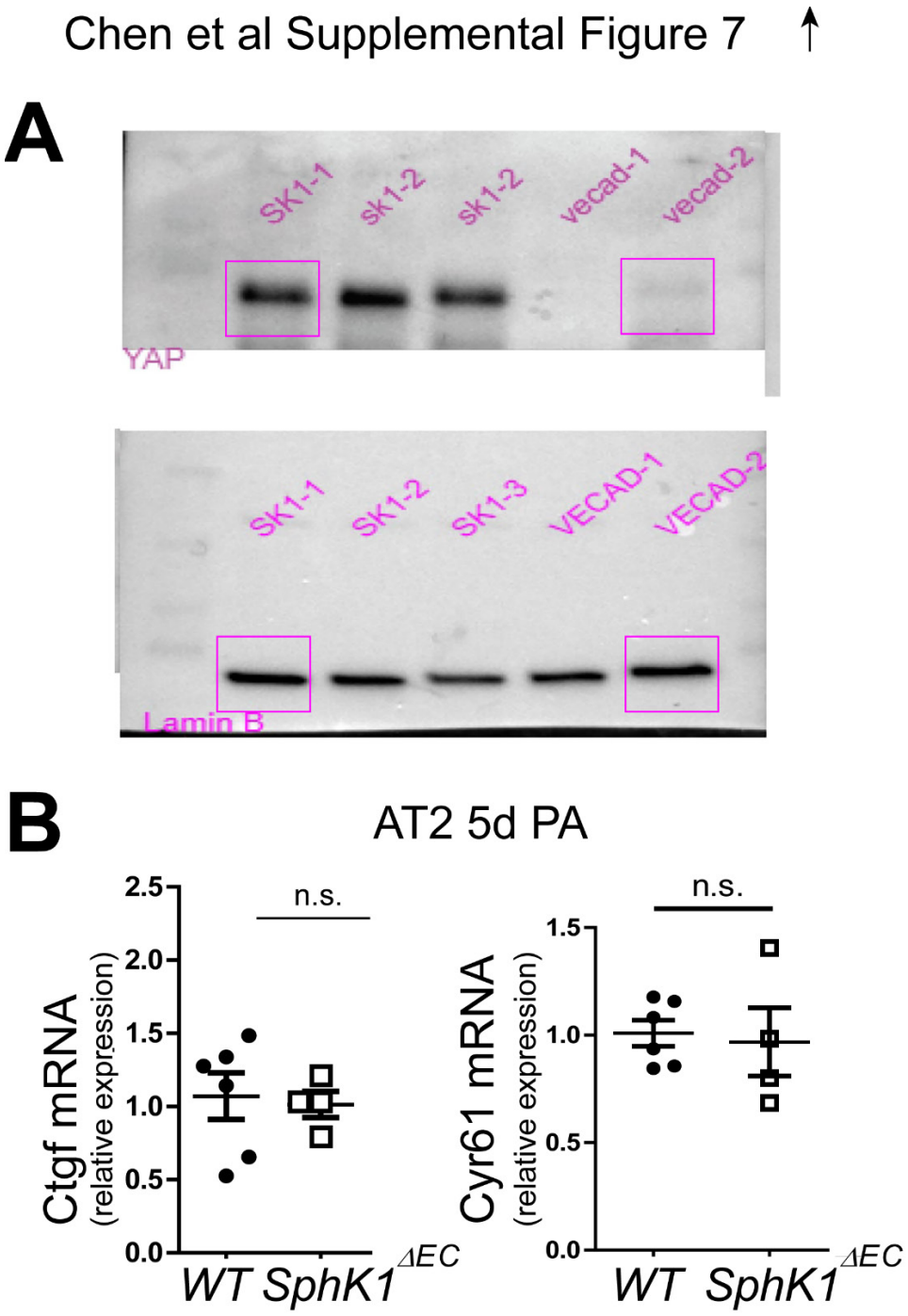

Figure S7 related to Figure 7. YAP activation and target gene expression. (A) Original blot of Figure 7H. (B) Expression levels of two YAP targets, *Ctgf* and *Cyr61*, in AT2 isolated from 5d post PA lungs of *WT* and *SphK1*$^{\Delta EC}$ mice were analyzed by qPCR. Mean ± SEM. n.s.: not significant.

Table S1 Related to Star Methods

Gene		Primer sequence (5'--3')
S1pr1	Forward	CTGTTAGATGTGGGCTGCAA
	Reverse	ATGATGGGGTTGGTACCTGA
S1pr2	Forward	CCGACATTTCTGGAGGGTAA
	Reverse	TGAGGTCACCTGGTCTCTCC
S1pr3	Forward	TGCCTTATGAACCCTGGAAG
	Reverse	AAGAGCAAATGCCATCAGGT
S1pr4	Forward	CTCCAAGGGCTATGTGCTCT
	Reverse	ATTGGCTCGGACCACTCTAA
S1pr5	Forward	ACACCAAATGCCCAGCTTAC
	Reverse	TGGAGCACTGTGCAAAAGTC
SphK1	Forward	GCTGTGAGGCTGGTGTTATG
	Reverse	ATATGCTTGCCCTTCTGCAT
SphK2	Forward	ACTGCTCGCTTCTTCTCTGC
	Reverse	GCCACTGACAGGAAGGAAAA
Sp-C	Forward	GCCTTCTCATCGTGGTTGT
	Reverse	CCAGTATCATGCCCTTCCT
Hopx	Forward	TCAACAAGGTCGACAAGCAC
	Reverse	AGGCGCTGCTTAAACCATT
Aqp5	Forward	AGCCTTATCCATTGGCTTGTC
	Reverse	TGAGAGGGGCTGAACCGAT
CTGF	Forward	CTCCACCCGAGTTACCAATG
	Reverse	TGGCGATTTTAGGTGTCCG
Cyr61	Forward	GGAGGTGGAGTTAACGAGAAAC
	Reverse	GTGGTCTGAACGATGCATTTC
Ppp1r3b	Forward	AGCCGTACAATGGACCAGAT
	Reverse	AGTAGTAGGGCCCCAGCTTT
Gadd45b	Forward	GCCCGAGACCTGCACTGCCT
	Reverse	CCATTGGTTATTGCCTCTGCTCTCTT
Sgk1	Forward	CGCCAAGTCCCTCTCAACAA
	Reverse	TGCCCTTTCCGATCACTTTC
CLO	Forward	CTTGTCCATGGCAAATGCTG
	Reverse	TGATCTTCTTGCTGGTCTTGC
CDC25C	QuantiTect Primer Assay (Qiagen)	
CCNB1	QuantiTect Primer Assay (Qiagen)	
Ager	Taqman assay	Assay ID: Mm01134790_g1, Cat#:4331182
Gapdh	Taqman assay	Assay ID: Mm99999915_g1, Cat#:4331182