

## Supplemental information

### **Wilms' Tumor 1 Drives Fibroproliferation and Myofibroblast Transformation in Severe Fibrotic Lung Disease**

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The authors have declared that no conflict of interest exists

**Running Title:** WT1 regulation of pulmonary fibrosis

## Supplemental figure legends:

### **Figure S1. WT1-positive cells are restricted to the pleura/subpleural fibrotic lesions in pulmonary fibrosis.**

(A) Schematic diagram of treatments with tamoxifen and Dox. Control or  $TGF\alpha$ /WT1<sup>CreERT2/mTmG</sup> mice were induced with tamoxifen and one week later pulmonary fibrosis was induced by administering Dox in food for four or six wk. (B) Immunofluorescence images show progressive accumulation of WT1-derived cells residing in subpleura but not adventitia in both pre-tamoxifen treated and pre/post-tamoxifen treated  $TGF\alpha$ /WT1<sup>CreERT2/mTmG</sup> mice placed on Dox for four or six wks. 10x low magnification, Scale bar, 200  $\mu$ m. Adventitial regions (dashed square boxes) were highlighted in the high magnification images taken at 40x, Scale bar, 50  $\mu$ m.

**Figure S2.** Gating strategy used for assessing the GFP positive population by flow cytometry in WT1 lineage tracing experiments.

### **Figure S3. Computational mapping of conserved WT1-binding sites in the promoter region of $\alpha$ SMA gene.**

Schematic representation of  $\alpha$ SMA gene with location of the putative WT1 binding sites that are conserved among mammals including humans and mice.

### **Figure S4. Genetic knockdown of WT1 alters lung resident fibroblast gene expression.**

CD45<sup>-</sup> Col<sup>+</sup> Lung resident fibroblast were isolated by negative selection using MACS CD45<sup>+</sup> magnetic beads from primary lung cultures of CCSP/ $TGF\alpha$  mice placed on doxycycline (Dox) food for 10days, RNA was isolated, and RNA-seq analysis was performed using next-generation sequencing. Heat map shows two clusters of differentially expressed genes up or down regulated (indicated with color key) by two fold or more upon genetic knockdown of WT1 compare to control siRNA. (N=3).

### **Figure S5. WT1-regulated gene networks in idiopathic pulmonary fibrosis (IPF).**

(A) Venn diagram depicting the comparison and overlap of differentially expressed genes in IPF lungs and WT1 siRNA-treated fibrotic fibroblasts (from RNA-seq data). The red color box indicates genes that were up- (107 genes) or down-regulated (DN, 63 genes) in IPF lungs compared with WT1 siRNA knockdown gene expression signatures. (B) WT1-driven genes activated in IPF were analyzed using ToppFun and visualized using Cytoscape. Red- and

green-colored circles represent genes that are up and downregulated respectively in IPF lungs. The blue-colored circle represents enriched biological processes for the inversely correlated genes between WT1 siRNA knockdown and IPF. (C) WT1 regulates pro-fibrotic ECM gene expression in lung-resident fibroblasts. The lung-resident fibroblasts were isolated from primary lung cultures of TGF $\alpha$  mice on doxycycline (Dox) food for 10 days and transfected with either control or WT1 specific siRNA for 72 hr. We observed a significant decrease in the transcript levels of Wt1, Col14, Col15, Itg7, Itg2 and Lum. Gene expression values were normalized using hypoxanthine guanine phosphoribosyl transferase as a control. Data are means + SEM. Results are representative of three independent experiments and the statistical significance between groups was measured using an unpaired Student's t-test. \*P<0.05, \*\*P<0.005, \*\*\*P<0.0005, \*\*\*\*P<0.00005.

**Figure S6. Increased proliferation of lung resident fibroblasts in IPF.** Lung resident fibroblasts were isolated from primary lung cultures of IPF or normal lungs by negative selection using ant-CD45 magnetic beads. Fibroblast proliferation was quantified by BrdU incorporation assay. Results are cumulative of two independent experiments with similar results (N=6).

**Figure S7. Decreased proliferation with the loss of WT1 in lung resident fibroblasts.** Lung resident fibroblasts were isolated from primary lung cultures of TGF $\alpha$  mice on Dox for 4 wk by negative selection using ant-CD45 magnetic beads. The proliferation was quantified by PCNA immunostaining of lung resident fibroblasts transfected with either control or WT1-specific siRNA. Quantification was performed post 72 hr of transection. Results are cumulative of two independent experiments with similar results (N=3). Unpaired student t test \*P<0.05, \*\*p<0.005.

**Figure S8. The loss of WT1 has no effect on lung morphology and collagen deposition in control mice.** CCSP/rtTA and tetO/TGF $\alpha$  transgenes were bred into WT1CreERT2 knock-in mice (WT1+/-) to generate CCSP/rtTA transgenic mice with wild type or mutant WT1 allele. Images are representative of Masson's Trichrome stained lung sections of CCSP/rtTA/WT1+/+ and CCSP/rtTA/WT1+/- mice on Dox for four wks (N=4-5/group); Image magnification x5; Scale bar, 400  $\mu$ m.

Figure S1.

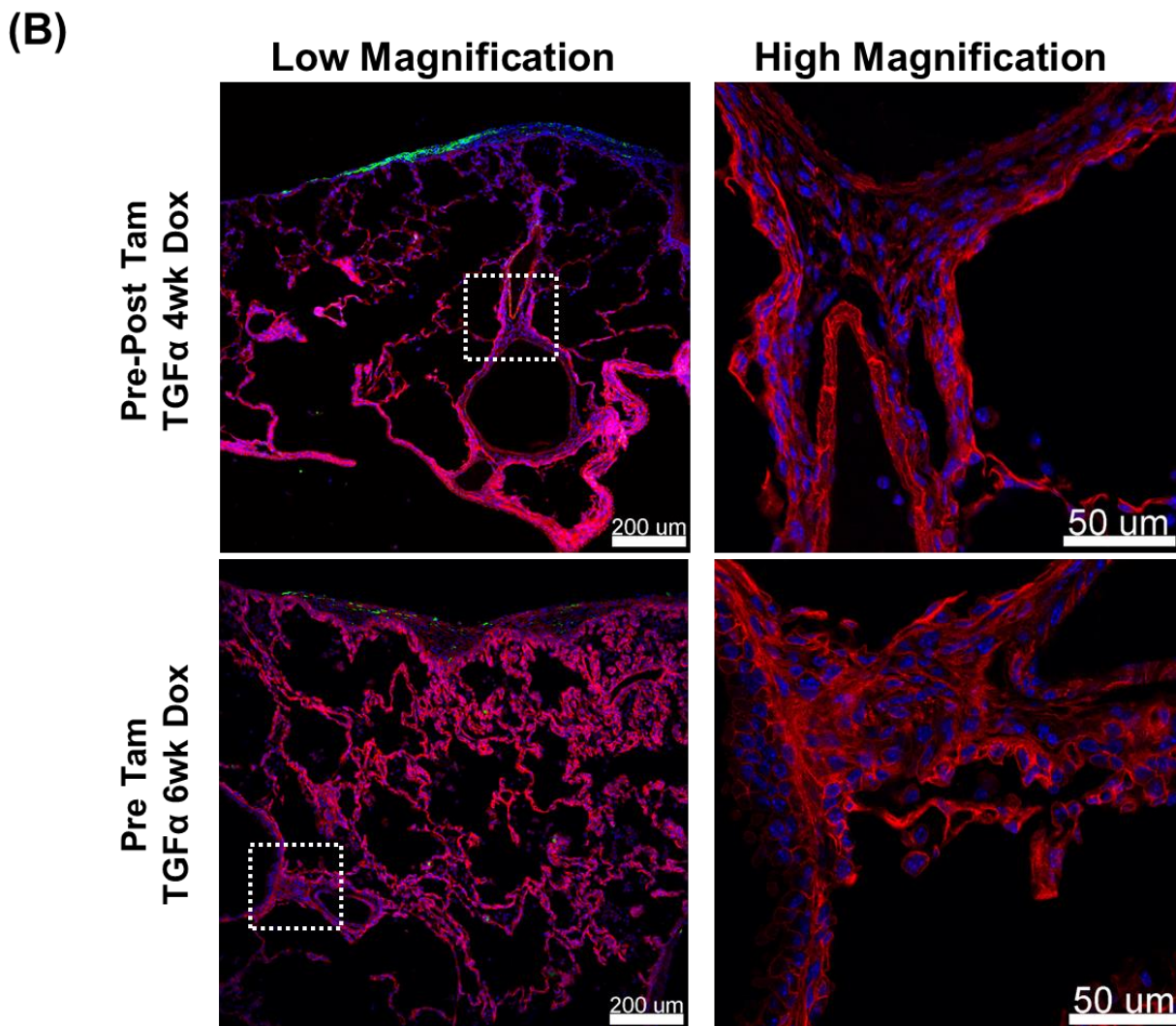
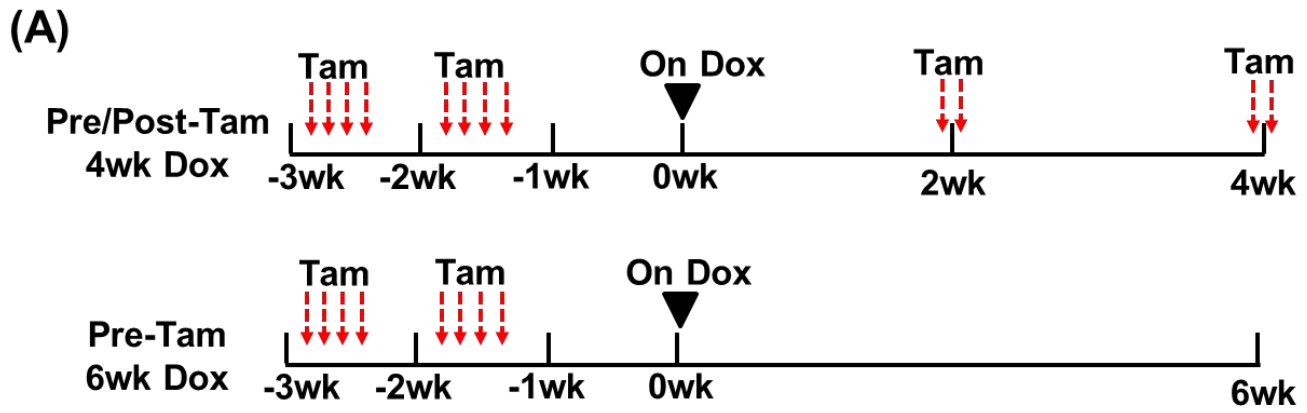


Figure S2.

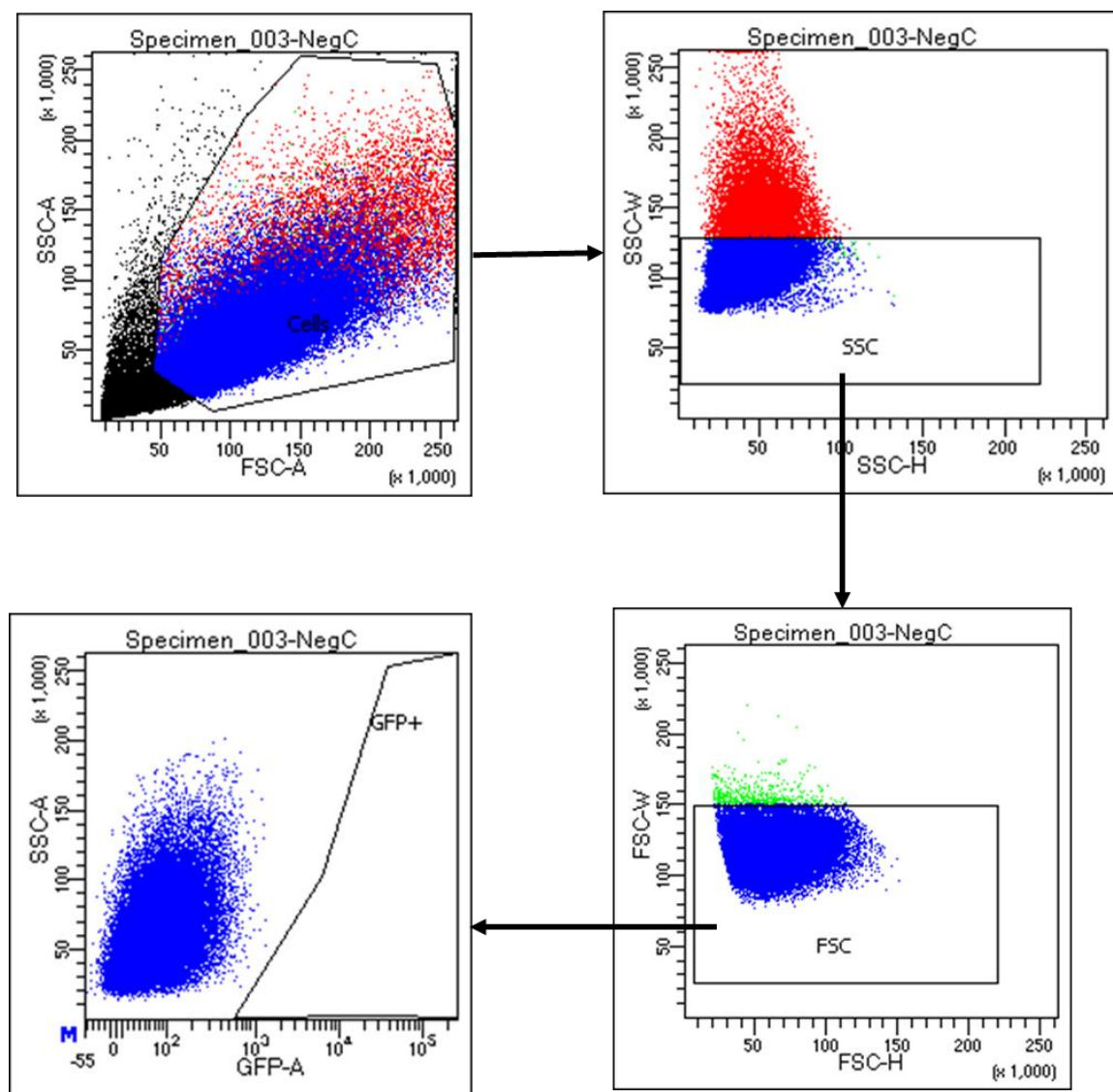


Figure S3.

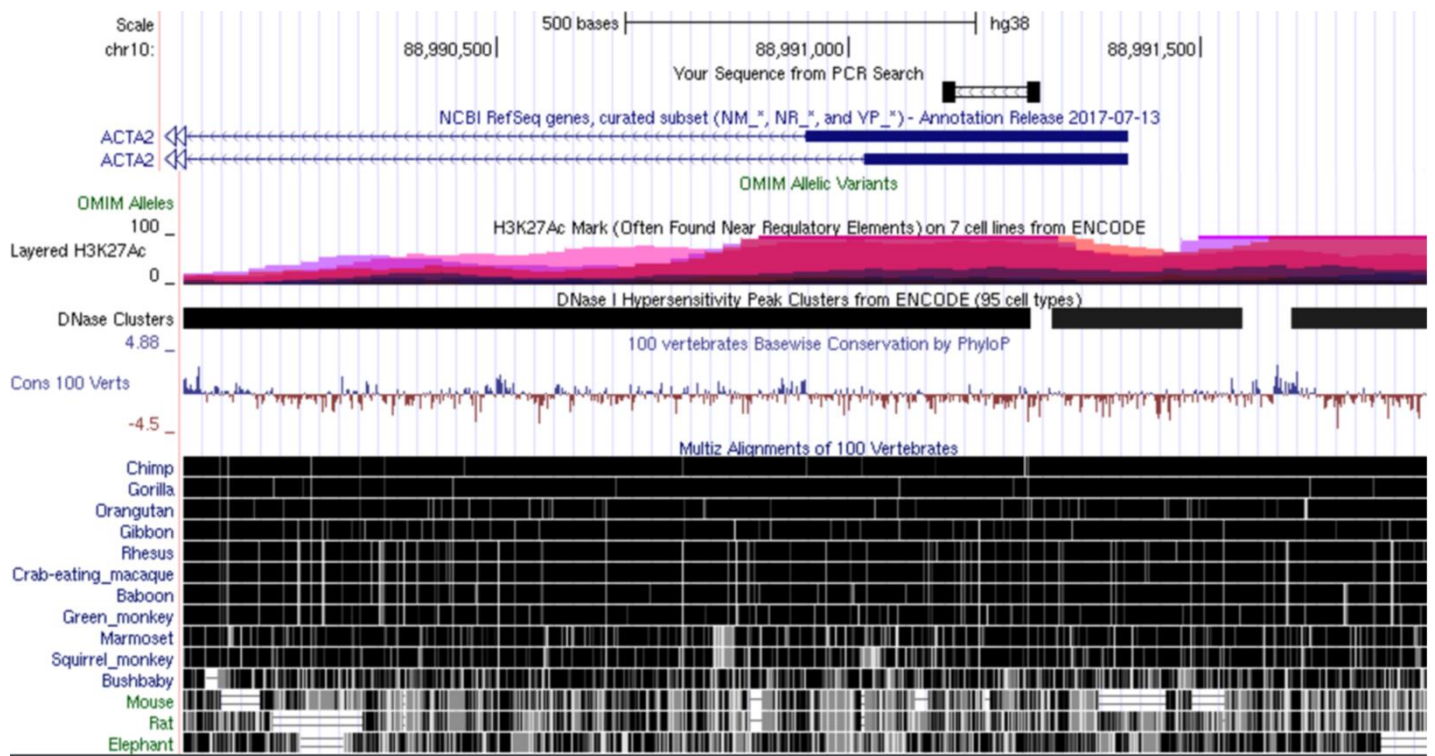


Figure S4.

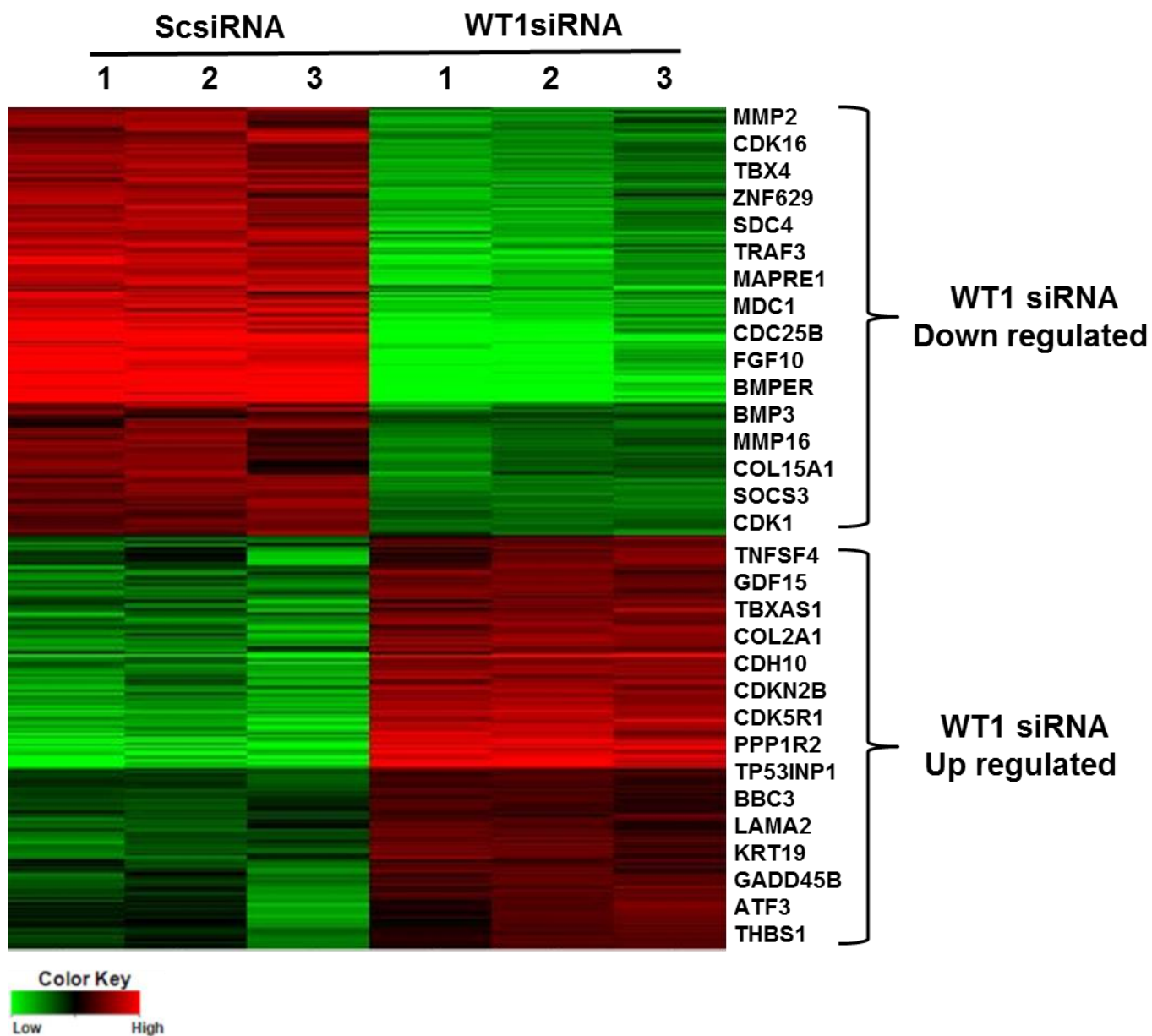


Figure S5.

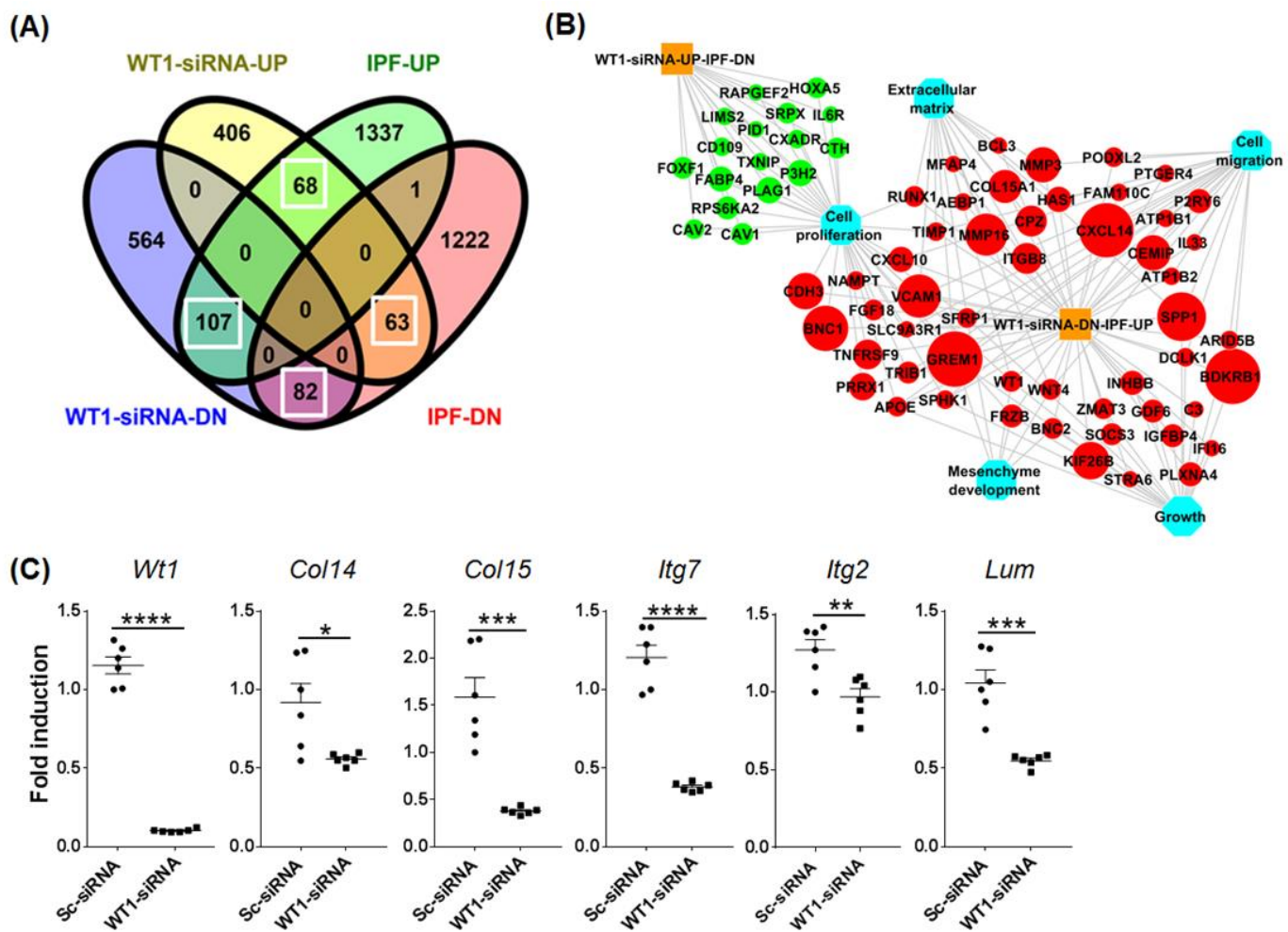




Figure S6.

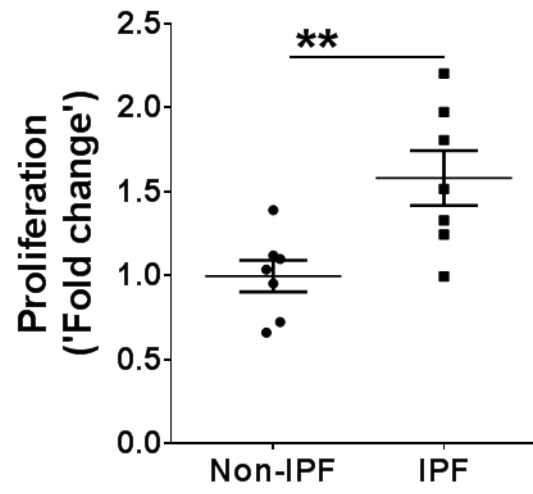


Figure S7.

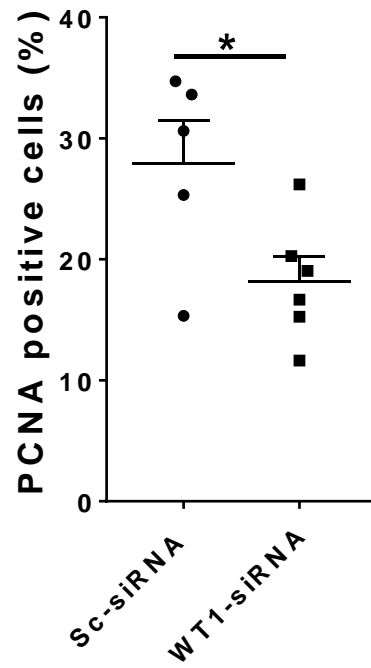
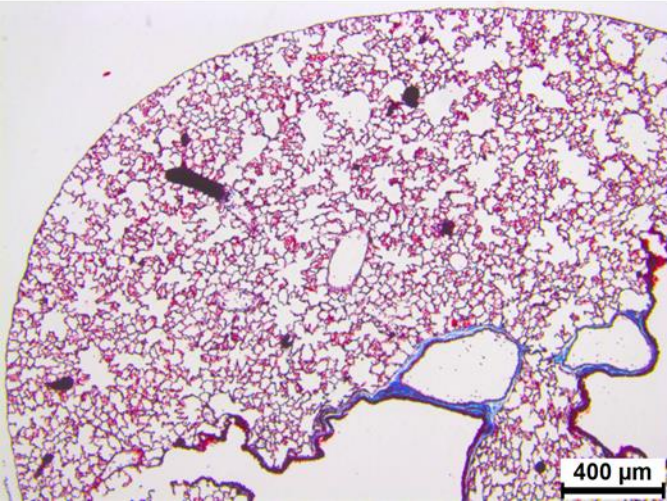


Figure S8.

**CCSP/- WT1<sup>+/+</sup>**



**CCSP/- WT1<sup>+/-</sup>**



**Supplemental Table 1.**

<b>Genes Upregulated in IPF and Downregulated by WT1 Knockdown</b>	<b>Genes Downregulated in IPF and Upregulated by WT1 Knockdown</b>
<b>Gene ID</b>	<b>Gene ID</b>
SLC29A3	OPN3
EFCAB11	RPS6KA2
CYGB	CD109
DACT1	FAM174B
MFAP4	PCDH12
AEBP1	FABP4
ARID5B	FABP5
C1QTNF2	PLIN2
SMPD3	HOXA5
MMP3	ADRB1
ARHGAP20	PLAG1
MMP16	SRPX
SFRP1	FOXF1
EMB	SH3BP5
ENC1	MIER2
PLEKHA4	FBLN5
CPZ	SORBS2
APOE	SNX24
BHLHE40	WFS1
AQP5	TMEM243
SBNO2	GBA2
SLC16A2	SLC40A1
FRZB	LIMS2
UHRF1	TXNIP
SNAPC1	PDE8B
ATP1B1	LGI3
ATP1B2	CTH
FIGNL1	RASL10B
SPP1	RAPGEF2
ADAM23	CTNND2
ADGRB2	IL6R
P4HA3	DKK2
SEC11C	CXADR
BCL3	SEMA3E

BDKRB1	FAM65B
FGF18	DOCK11
STAR	INPP5A
SEPT6	KLRG2
BNC1	SPIN4
GBP5	LIN7A
IER5L	PTPRB
SPHK1	FIBIN
C3	MAMDC2
C4B	SIGLEC10
CACNA1G	PID1
STRA6	ANKS1A
IL33	DMTF1
NT5E	TBXAS1
SOCS3	TSC22D3
CH25H	GRK5
RUNX1	LAMA2
ZMAT3	CAV1
TIMP1	CAV2
P2RY6	SLC7A7
CPXM1	OPHN1
HAS1	ADGRG6
CDH3	TMTC1
DCLK1	FADS3
FRMD5	SYTL5
SP140	P3H2
CFH	MAP3K13
ENPP3	AJUBA
GDF6	CDH10
WNT4	
CHN1	
ZBP1	
KCNE4	
SLC9A3R1	
FAM110C	
VCAM1	
PRRX1	
COL15A1	
WT1	

CXCL14	
CP	
PODXL2	
IFI16	
GDA	
IGFBP4	
SLFN13	
PLXNA4	
BNC2	
TNFRSF9	
TSHZ2	
INHBB	
CXCL10	
FAM46C	
PTGER4	
PMEPA1	
ITGB8	
LYPD1	
FAM83D	
TMEM45A	
KIF26B	
DUSP4	
STEAP4	
TSPAN5	
E2F8	
CEMIP	
SSPN	
NAMPT	
LGI2	
STEAP3	
CLMP	
GREM1	
CKAP2	
TRIB1	

**Supplemental Table 2.** Sequences of gene specific primers used for quantitative RT-PCR in murine samples.

<b>Gene</b>	<b>Forward</b>	<b>Reverse</b>
mHprt	GCCCTTGACTATAATGAGTACTTCAGG	TTCAACTTGCGCTCATCTTAGG
mWT1	CAGATGAACCTAGGAGCTACCTTAAA	TGCCCTTCTGTCCATTTC
m $\alpha$ Sma	TGACGCTGAAGTATCCGATAGA	CGAAGCTCGTTATAGAAAGAGTGG
mCol14 $\alpha$	ACGACGTGACTGAGAACAGC	AATGTCTGTGTGTGTCTCTCCAA
mCol15 $\alpha$	CCAGGGCTAAAAGGAGAACA	GGACGTCCCCAGTCAAGA
mItg $\alpha$ 7	CGTGCTCTGGACTCTGTGG	CCCAGCTCACACTCGACAT
mItg $\alpha$ 2	ACTTCCGGCATAACGAAAGAA	TCAGCCAGCAGGTGATGTTA
mLum	CAGCAACATTCCGGATGAG	TCATTGTGAGATAAACGCAGGT
mGrem1	GACCCACGGAAGTGACAGA	CCCTCAGCTGTTGGCAGTAG
mRunx1	CTCCGTGCTACCCACTCACT	ATGACGGTGACCAGAGTGC
mWnt4	ACTGGACTCCCTCCCTGTCT	TGCCCTTGTCACTGCAAA
mStat3	GTTCTGGCACCTTGGATT	CAACGTGGCATGTGACTCTT
mPrrx1	GGAGCAACCCATCGTACCT	AAGTAGCCATGGCGCTGTA
mIgf1	AGCAGCCTTCCAACCTCAATTAT	GAAGACGACATGATGTGTATCTTTATC
mCcnb1	GCGCTGAAAATTCTTGACAAC	TTCTTAGCCAGGTGCTGCAT
mE2f8	TCAGCCCAAACAACAGTGG	GCAGACTGCTCAGCCTCTAAG
mTgfa	TTGCTGCCACTCAGAAACAG	ATCTGCCACAGTCCACCTG
mCol1 $\alpha$	AGACATGTTTCAGCTTTGTGGAC	GCAGCTGACTTCAGGGATG
mCol5 $\alpha$	CTACATCCGTGCCCTGGT	CCAGCACCGTCTTCTGGTAG

**Supplemental Table 3.** Sequences of gene specific primers used for quantitative RT-PCR in human samples.

<b>Gene</b>	<b>Forward</b>	<b>Reverse</b>
h $\beta$ -actin	CCAACCGCGAGAAGATGA	CCAGAGGCGTACAGGGATAG
h $\alpha$ SMA	GCTTTCAGCTTCCCTGAACA	GGAGCTGCTTCACAGGATTC
hWT1	AGCTGTCCCACTTACAGATGC	CCTTGAAGTCACACTGGTATGG

**Supplemental Table 4.** Antibodies used for Immunostainings

<b>Antibody</b>	<b>Dilution</b>	<b>Catalog#.</b>	<b>Company</b>
WT1	1:50	ab89901	Abcam
Calretinin	1:250	AB1550	EMD Millipore
PCNA	1:1000	2586S	Cell signaling technology
GFP	1:1500	ab13970	Abcam
$\alpha$ SMA	1:2000	A5228	Sigma
Ki67	1:400	12202	Cell signaling technology
RFP	1:500	600-401-379	Rockland immunochemicals

**Supplemental Table 5.** Antibodies used for Western blot

<b>Antibody</b>	<b>Dilution</b>	<b>Catalog#.</b>	<b>Company</b>
$\alpha$ SMA	1:20000	A5228	Sigma
PCNA	1:1000	2586S	Cell signaling technology
WT1	1:500	12609-1-AP	Proteintech
$\beta$ -Actin	1:10000	sc-47778	Santa cruze biotechnology