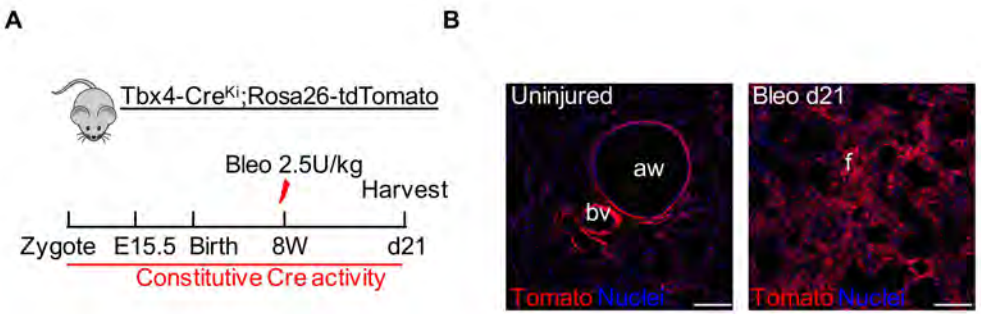
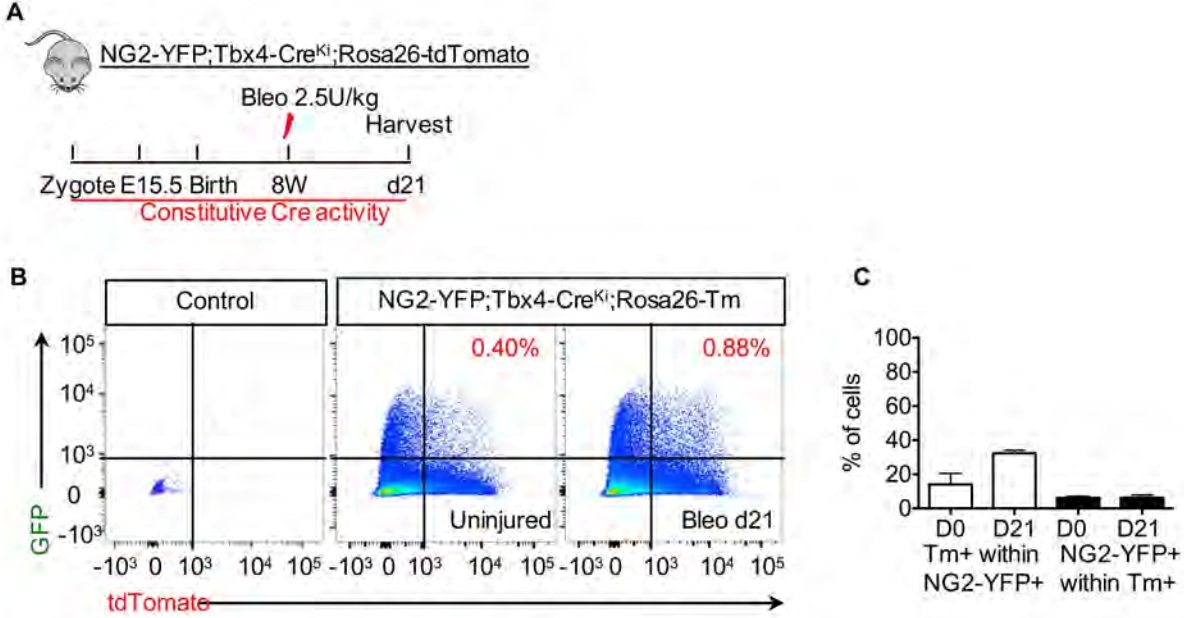
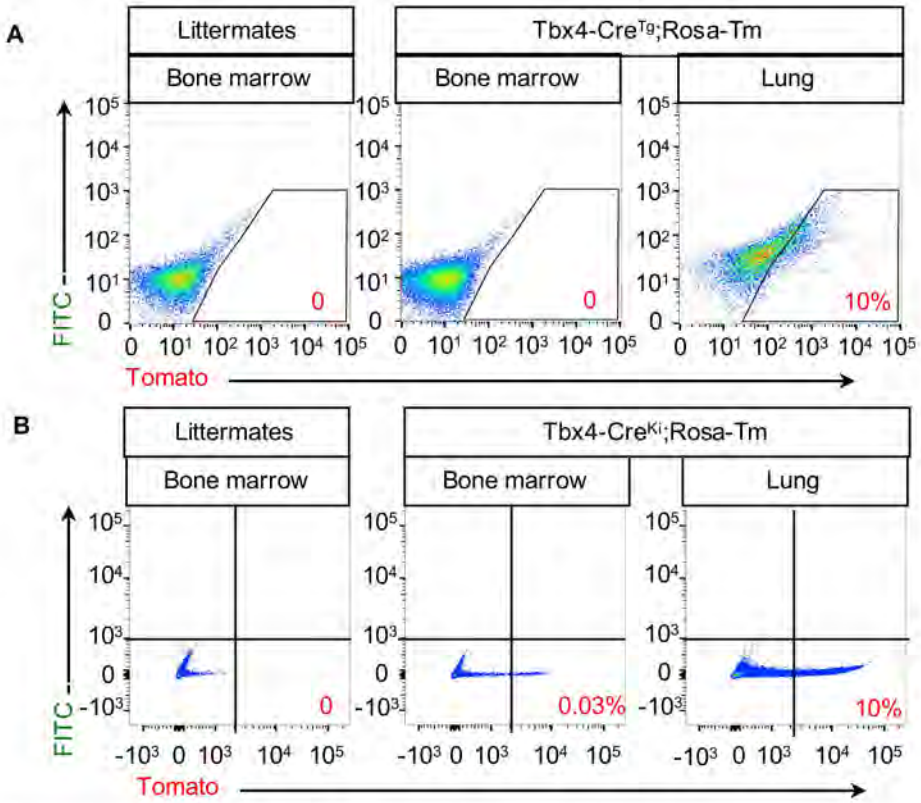


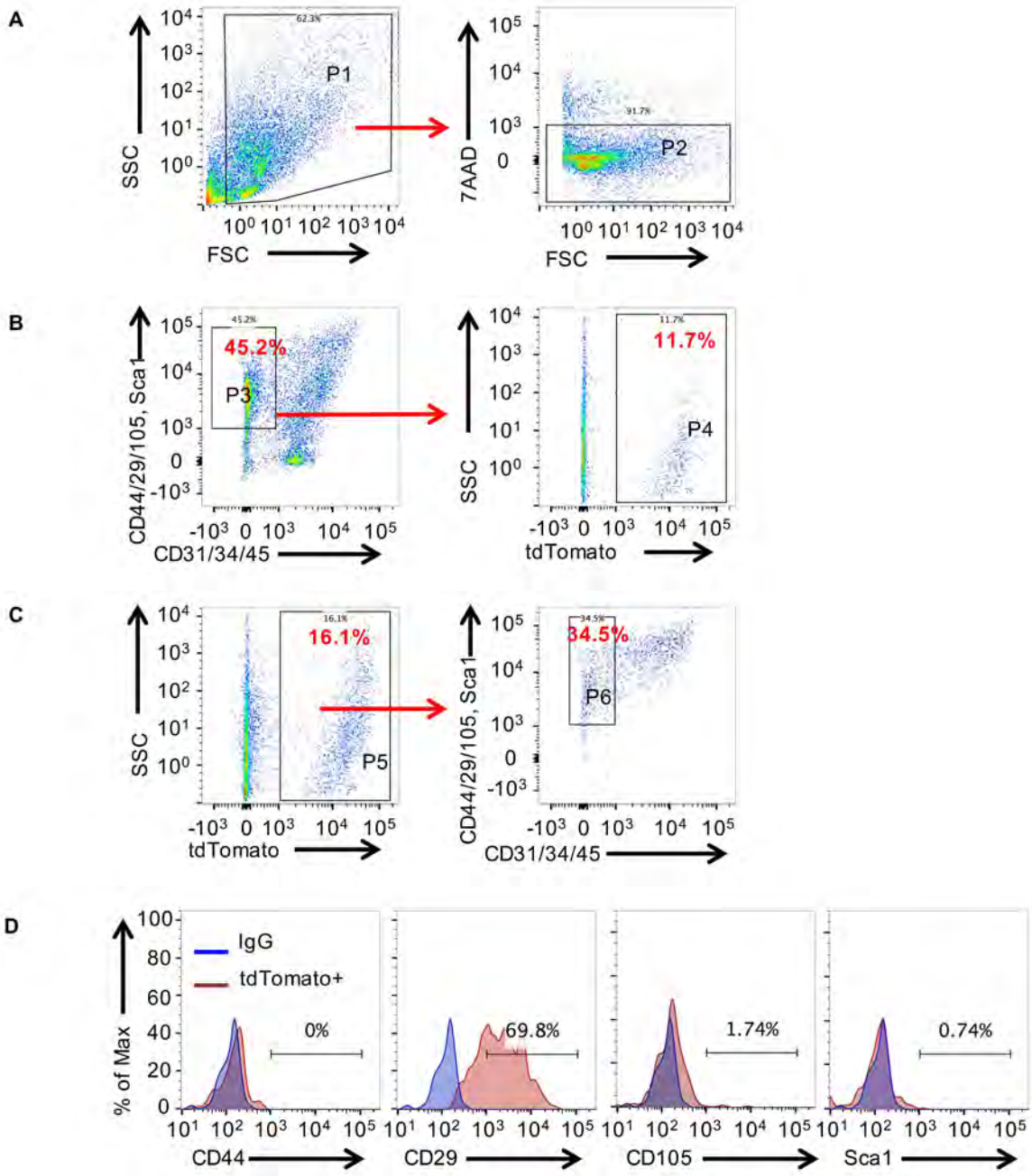
Supplementary Figure 1 Tbx4 lineage cells are immensely expanded during fibrosis . (**A**) Schematic depicting lineage analysis methodology. Tbx4-Cre^{Ki};Rosa26-Tm mice were used for experiments in this figure. (**B**) Representative histological sections of Tbx4-Cre^{Ki};Rosa26-Tm mouse lungs at uninjured 8 weeks-old, and bleomycin treated (d21) adult Tbx4-Cre;Rosa26-Tm with Tbx4 lineage cells in red, and nuclei in blue. *n* = 9 lungs examined. Scale bars, 100 μm. aw = airway, bv = blood vessel, f = fibrotic foci.



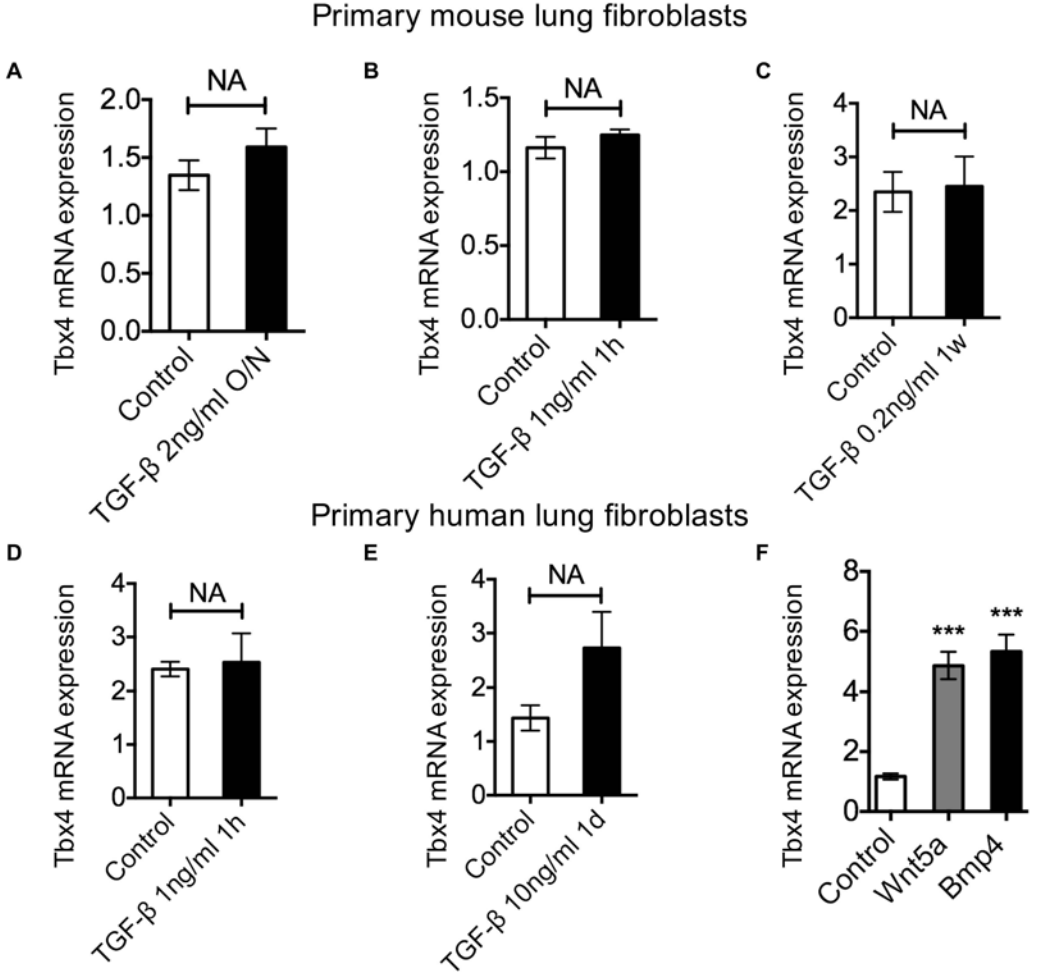
Supplementary Figure 2 Tbx4 lineage cells in the lung include a portion of the NG2+ pericytes. **(A)** Representative FACS plot of mouse lung single cells isolated from bleomycin treated (d21) NG2-YFP;Tbx4-Cre^{Tg};Rosa26-Tm mice. (**C**) Bar graph showing percentages of the NG2-YFP+ and tdTomato double positive cells within tdTomato labeled Tbx4 lineage cells and within NG2-YFP+ cells. *n* = 3 mice analyzed, mean ± SEM.



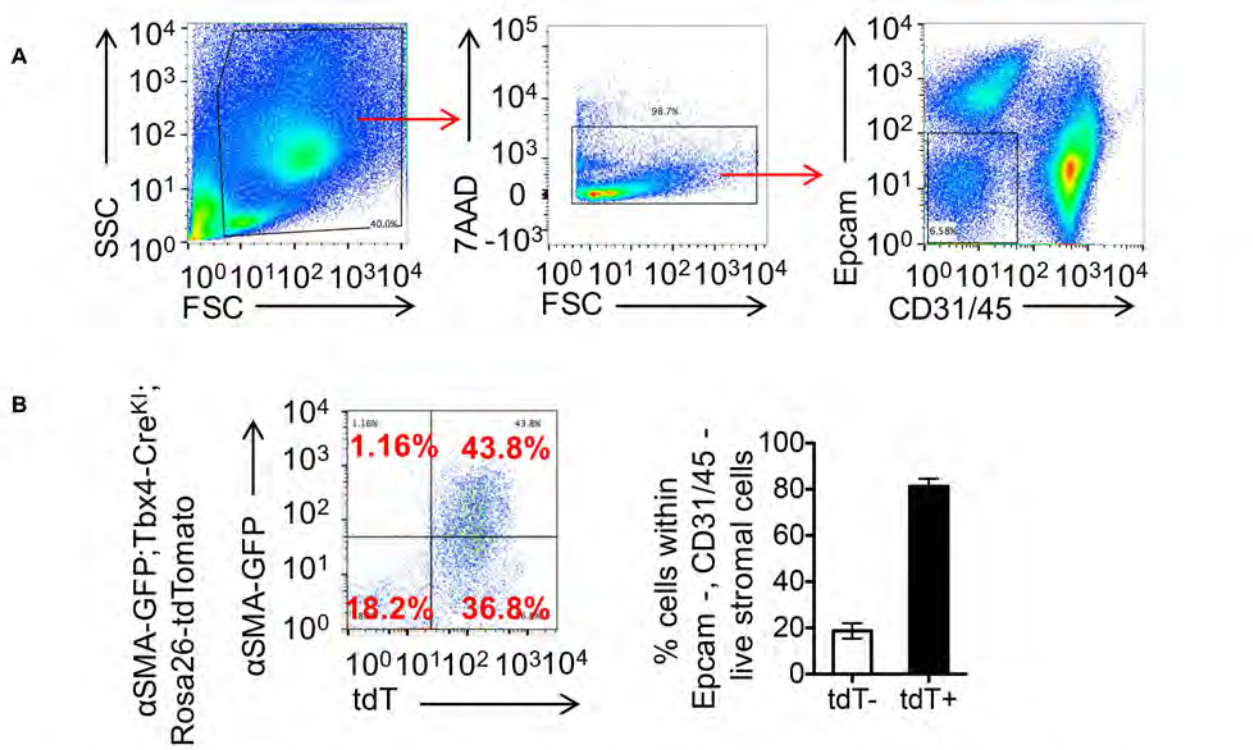
Supplementary Figure 3 Tbx4 lineage in bone marrow and the lung. (**A-B**) FACS analysis of bone marrow and lung single cells from Tbx4-Cre^{Tg};Rosa26-Tm (**A**) or Tbx4-Cre^{Ki};Rosa26-Tm mice (**B**). There were around 10% of Tbx4+ cells within the lung single cells from Tbx4-Cre^{Tg};Rosa26-Tm or Tbx4-Cre^{Ki};Rosa26-Tm mice. Bone marrow from Tbx4-Cre^{Tg};Rosa26-Tm and littermate control mice showed no labeling of tdTomato, while bone marrow from Tbx4cre^{Ki};Rosa26-Tm mice included very small amount (around 0.03%) of tdTomato labeled cells compared with littermate control mice. n = 3 mice analyzed.



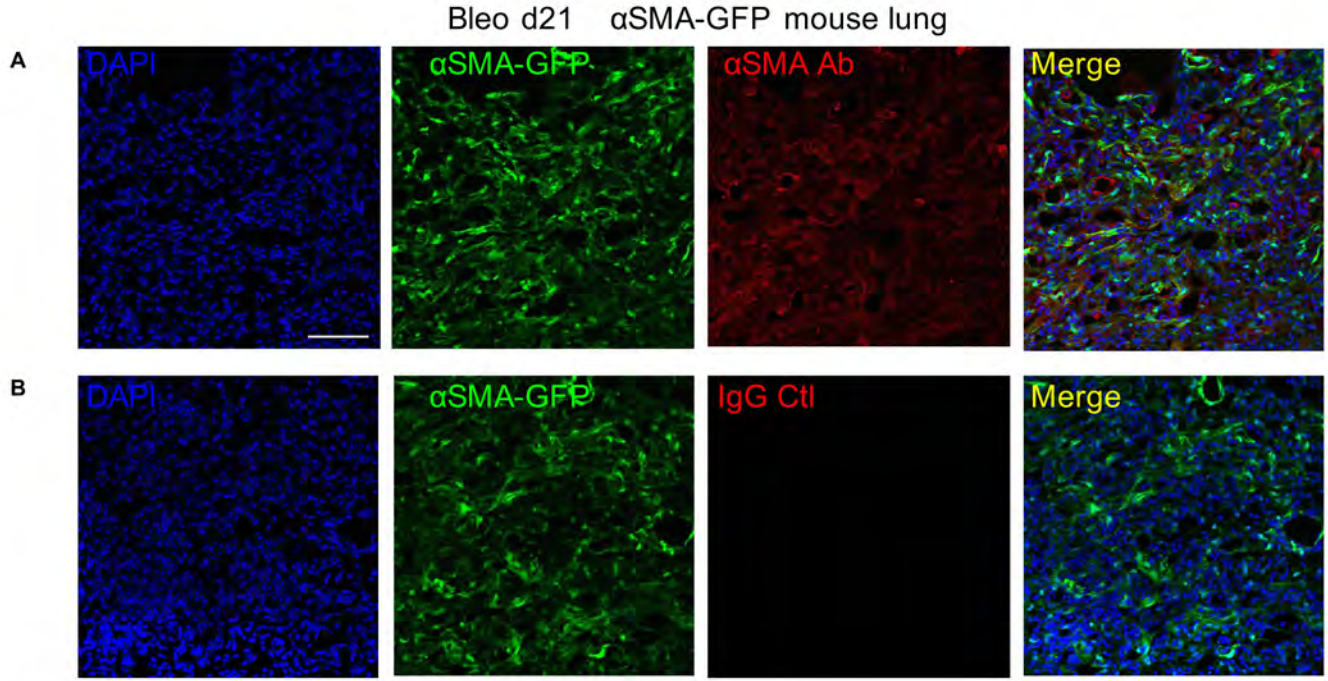
Supplementary Figure 4 Non classic MSC pattern for Tbx4-lineage cells. (A) FACS analysis for single cell suspension of Tbx4-Cre^{Tg};Rosa26-tdTomato mouse lung. (B) Within 7AAD negative total single lung cells (P2), around 45% cells are CD44,CD29,CD105,and Sca1 positive and CD31,CD34,and CD45 negative. Within CD44/29/105,Sca1+ & CD31/34/45- cells (P3), around 11% cells (P4) are tdTomato positive. (C) Within 7AAD- total single lung cells, around 16% cells are tdTomato positive. Within tdTomato positive cells (P5), around 34% cells are CD44/29/105,Sca1+ & CD31/34/45- (P6). (D) The above-mentioned surface antigens were further analyzed individually. Within Lin-Tbx4+ cells, around 69% of tdT+ cells are stained with CD29, and other markers (CD44, CD105 and Sca1) are minimal or not detectable. $n = 3$ mice analyzed.



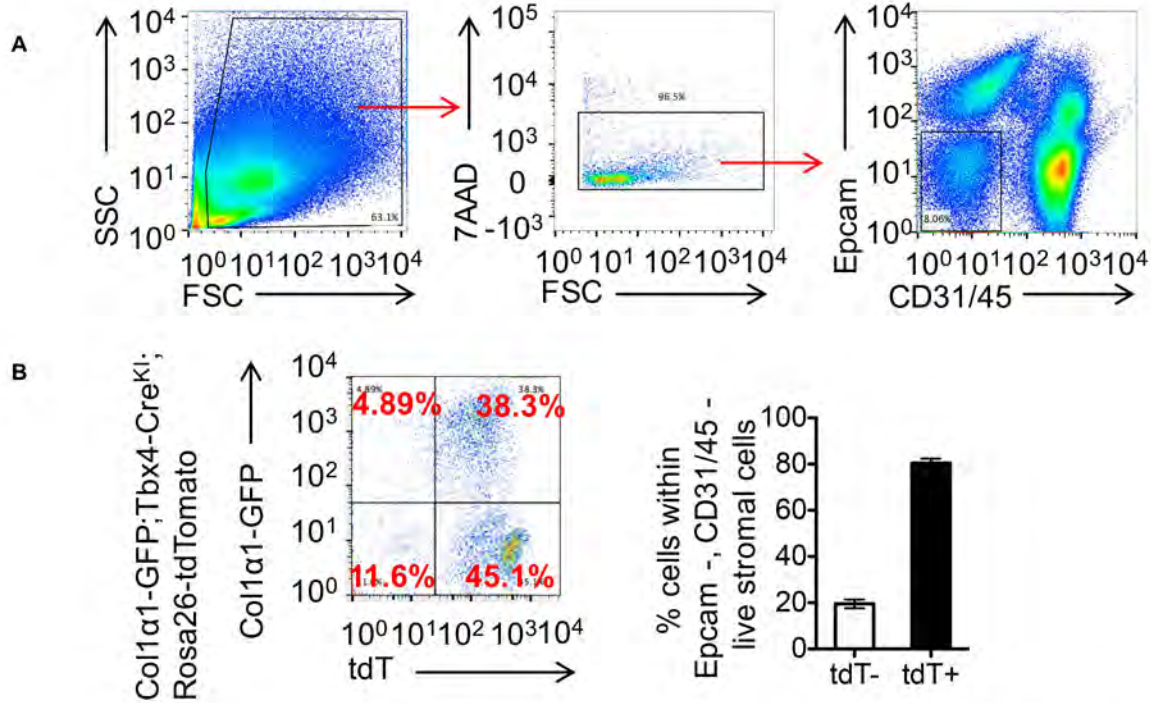
Supplementary Figure 5 Tbx4 upstream regulation in fibroblasts in culture. **(A-C)** Lung fibroblasts were isolated from uninjured WT mouse lung and and cultured for 3 passages. Tbx4 mRNA expression was analyzed by qRT-PCR upon different doses and time of TGF-β treatment. **(D-E)** Tbx4 expression did not respond to TGF-β treatment in primary human lung fibroblasts. **(F)** Treatment with Wnt5a (250ng/ml) and Bmp4 (100ng/ml) in human lung fibroblasts for 4 hrs significantly upregulated Tbx4 expression. $n \geq 3$ in each group. All results are the mean of triplicate experiments ± SEM, NA no significant difference, $^{***}P \leq 0.001$ analyzed by Student's *t*-test **(A-E)** or one-way ANOVA with Bonferroni test **(F).**



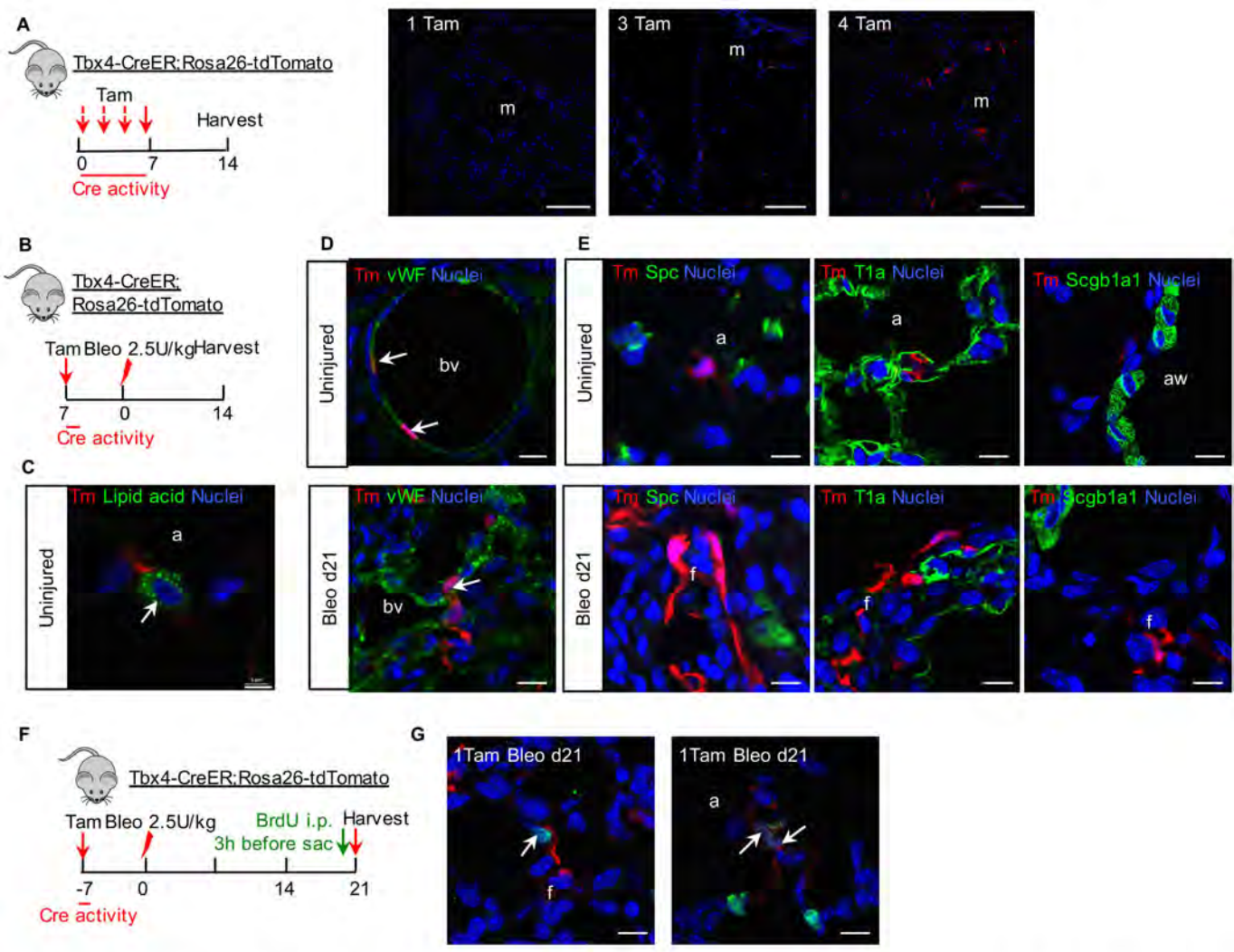
Supplementary Figure 6 FACS analysis of Tbx4-lineage cells in the lungs of αSMA-GFP;Tbx4-Cre^{KI}; Rosa26-tdTomato mouse strains. **(A)** FACS analysis of lung single cells gate for 7AAD-, Epcam-, and CD31/45-. **(B)** FACS plot and percentage of 7AAD-, Epcam-, and CD31/45- stromal cells expressing tdTomato or GFP in uninjured αSMA-GFP;Tbx4-Cre^{KI}; Rosa26-tdTomato mouse lung. *n* = 3 mice analyzed, mean ± SEM.



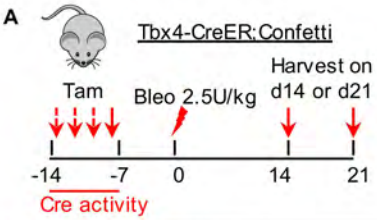
Supplementary Figure 7 αSMA-GPF expression pattern is comparable with antibody staining. **(A)** αSMA antibody staining for bleo d21 αSMA-GFP mouse lung. **(B)** IgG control. *n* = 3 lungs examined, Scale bars, 100 μm.

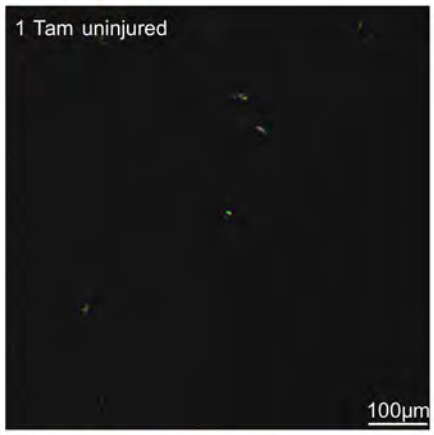


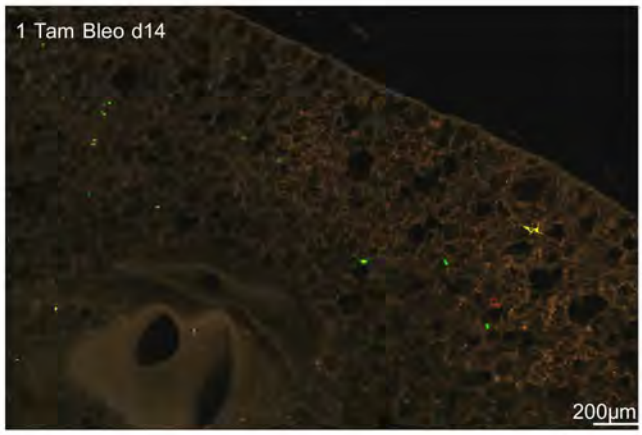
Supplementary Figure 8 FACS analysis of Tbx4-lineage cells in the lungs of Col1α1-GFP;Tbx4-Cre^{KI}; Rosa26-tdTomato mouse strains. **(A)** FACS analysis of lung single cells gate for 7AAD-, Epcam-, and CD31/45-. **(B)** FACS plot and percentage of 7AAD-, Epcam-, and CD31/45- stromal cells expressing tdTomato or GFP in uninjured Col1α1-GFP;Tbx4-Cre^{KI}; Rosa26-tdTomato mouse lung. *n* = 3 mice analyzed, mean ± SEM.

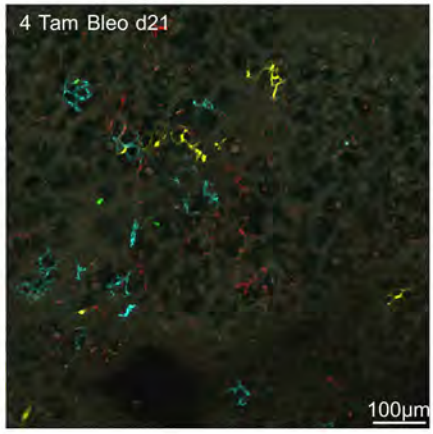


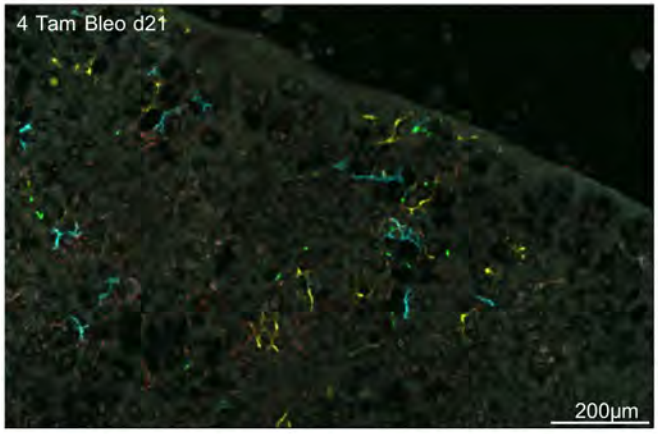
Supplementary Figure 9 Adult tdTomato+ cells are proliferative, and include lipofibroblasts as well as endothelial cells, but not epithelial cells. **(A)** Adult Tbx4-CreER;Rosa26-Tm mice were injected with 1, 3, or 4 doses of tamoxifen to label Tbx4 expressing cells. Tbx4 cell labeling was increased in a dose dependent manner with the Tamoxifen injection times (n=6 lungs examined). **(B-E)** Tbx4-CreER;Rosa26-Tm mice were injected with 1 dose of tamoxifen, followed with bleomycin injury, and lungs were harvested on d14 after bleomycin injury. The schematic **(B)** was used for the following experiments in this figure. Representative histological section stained for lipid acid **(C)**, von Willebrand Factor (vWF) **(D)**, CC10, SPC, and T1a **(E)** (n=6 lungs examined). **(F)** BrdU labeling experiment scheme: Tbx4+ cells were marked in Tbx4-CreER;Rosa-Tm mice using one dose of tamoxifen (20 mg/g/dose). Bleomycin injury was performed intratracheally one week after tamoxifen administration. On d21 after bleomycin, BrdU was injected intraperitoneally 3 hrs before lung harvesting. Frozen section and confocal imaging were performed thereafter. **(G)** Representative images showing the tdT+ cells were incorporated with BrdU (arrowheads, n = 3 mice examined). Scale bars, 5 μm **(C)**, 10 μm **(D,E,F)**, and 100μm **(A)**. a = alveoli, aw = airway, bv = blood vessel, f = fibrotic foci, and m = mesenchyme.

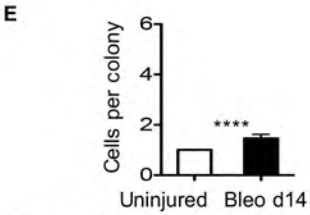


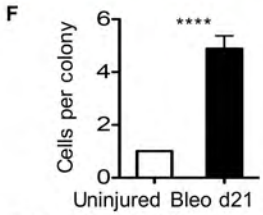




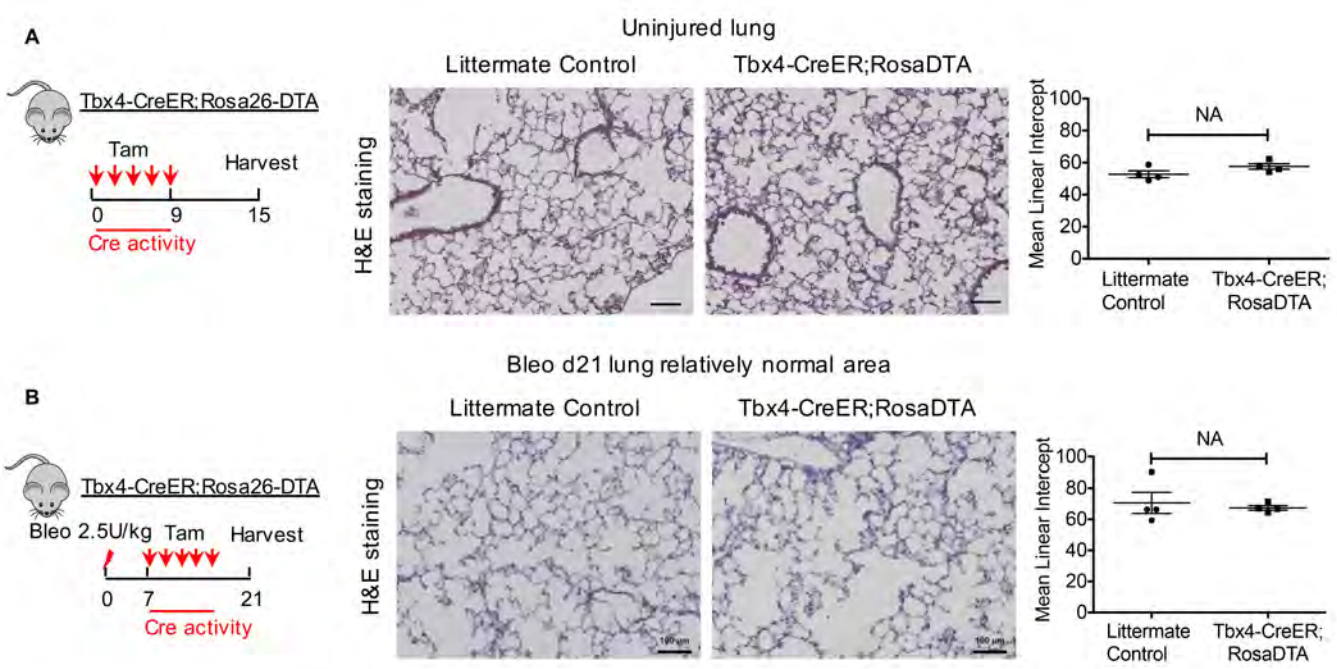




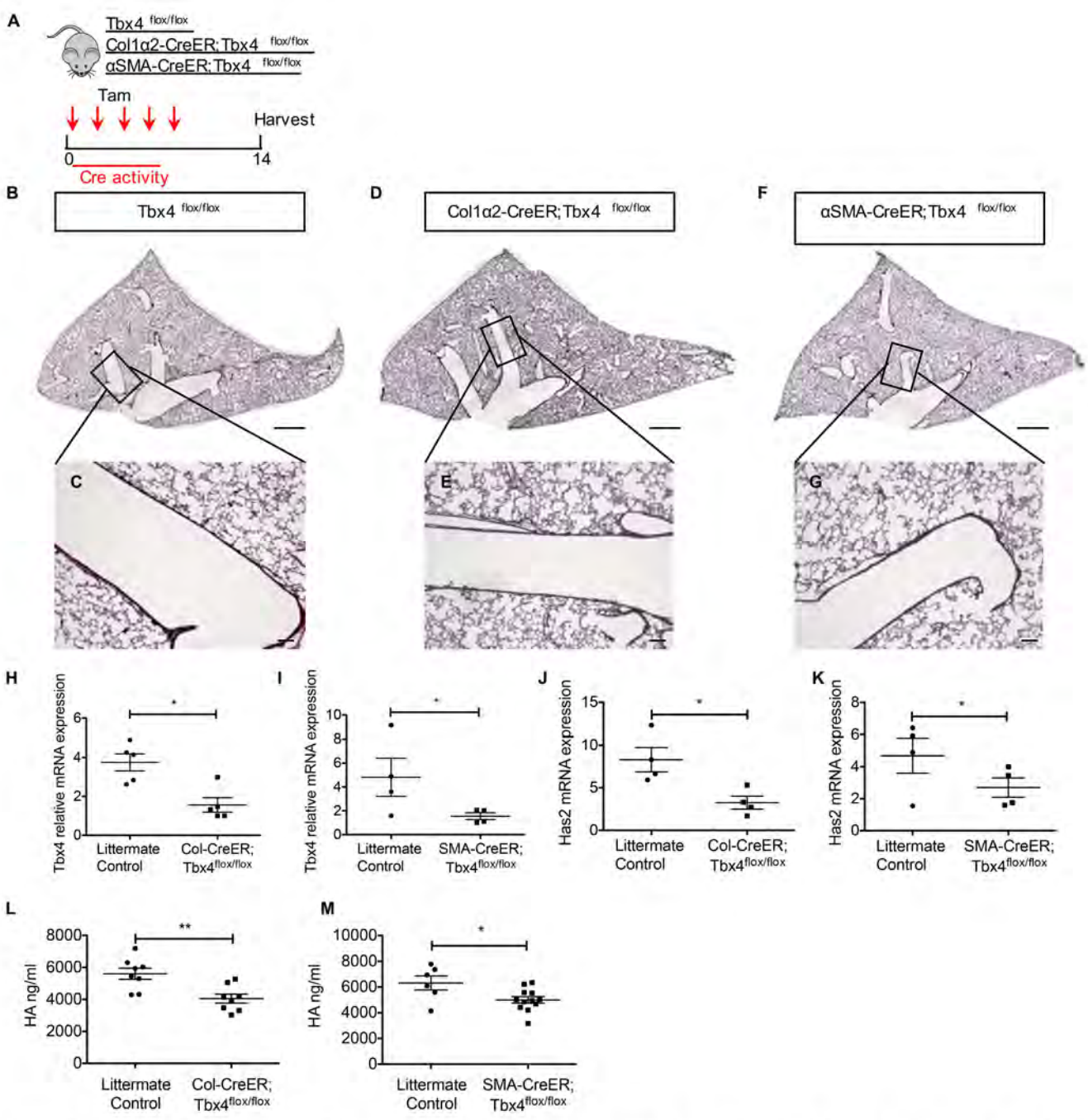




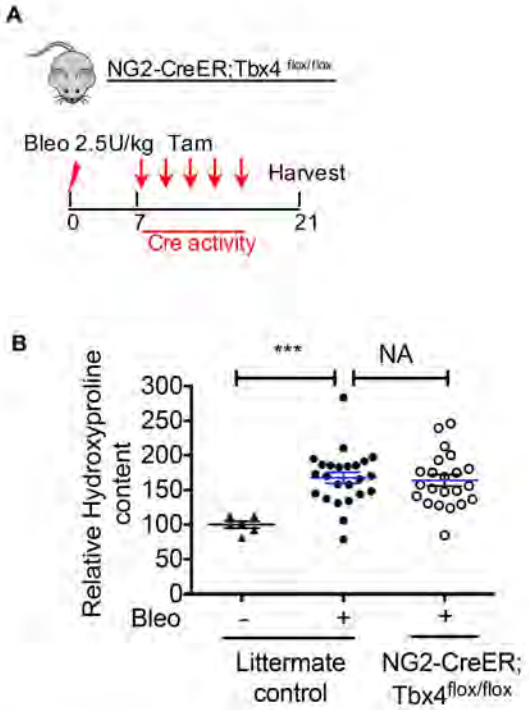
Supplementary Figure 10 Clonal-like expansion of Tbx4+ fibroblasts during fibrosis. **(A-F)** Experimental scheme **(A)**: Tbx4+ cells were marked in Tbx4-CreER;Confetti mice using one or four doses of tamoxifen (12.5 μg/g/dose). Bleomycin injury was performed intratracheally one week after the last dose of tamoxifen. Mouse lungs were harvested at d14 and d21. Whole mount mouse lungs were subject to SCALE clearing and confocal imaging. **(B)** Prior to bleomycin injury, single Tbx4+ cell was marked. **(C)** Bleomycin injury induced Tbx4+ cell replication, producing clones of one or two identically marked cells. **(D)** Bleomycin induced Tbx4+ cells self-renewal or clonal-like expansion. **(E)** Histogram of Tbx4+ colony size in **(B)** and **(C)**. **(F)** Histogram of Tbx4+ colony size in **(D)**. n=9 lungs examined in all experiment group, scale bars as indicated in figures. ****$P \leq 0.0001$ by Student's t-test; mean ± SEM).



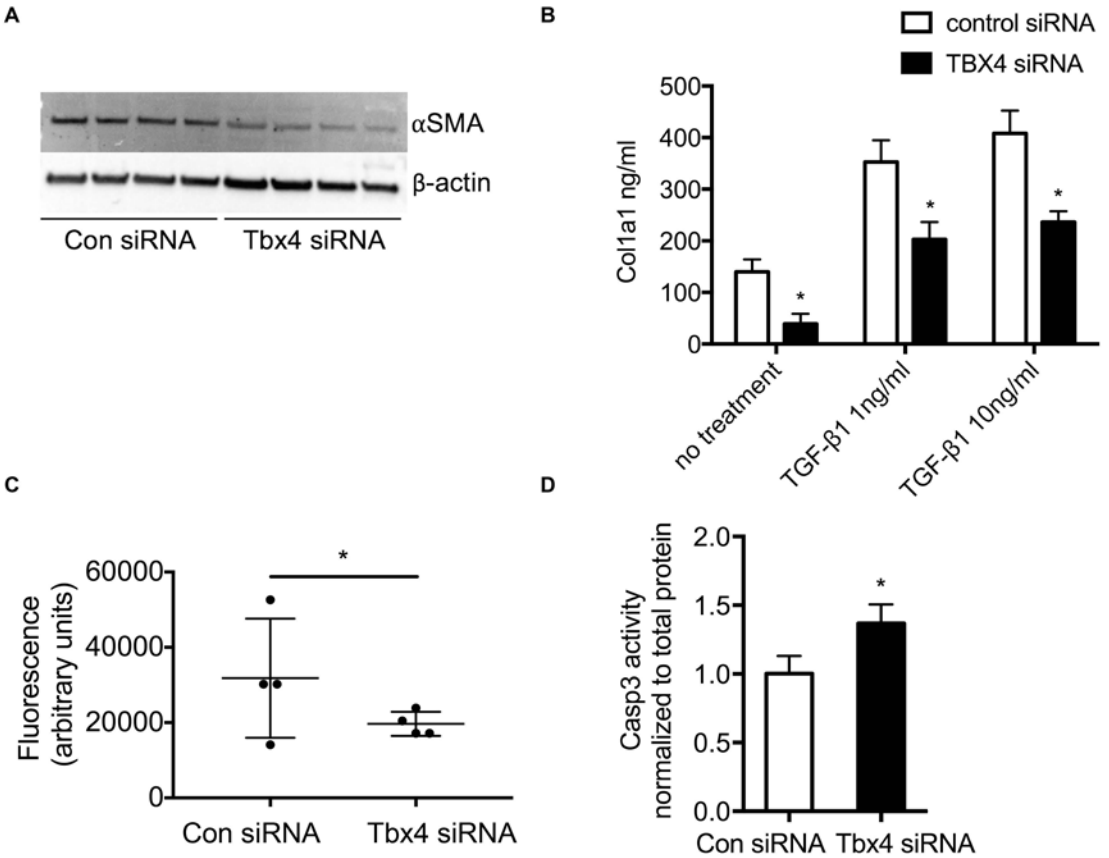
Supplementary Figure 11 Short-term ablation of Tbx4 cells do not change lung structure in both uninjured **(A)** or relative normal area of injured mouse lung **(B)** by means of H&E staining and mean linear intercept calculation. $n \geq 3$ mice / group examined. NA no significant difference, as analyzed by Student's *t*-test. Scale bars, 100μm.



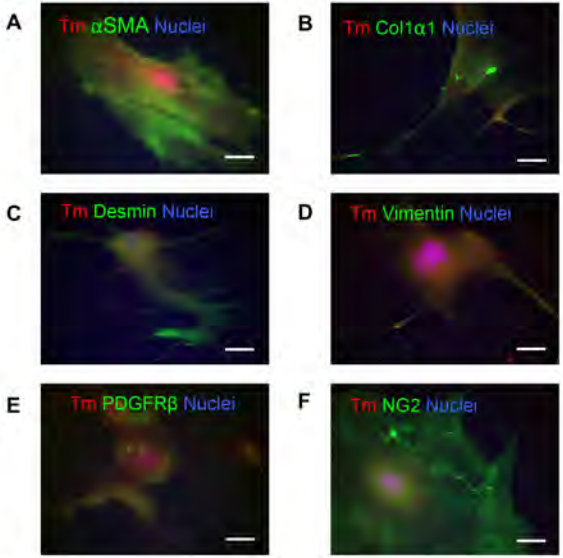
Supplementary Figure 12 Normal lung in Col1α2-CreER;Tbx4$^{flox/flox}$ and αSMA-CreER;Tbx4$^{flox/flox}$ mice. Mouse lung histology (H&E staining) of littermates (Tbx4$^{flox/flox}$) **(B-C),** Col1α2-CreER;Tbx4$^{flox/flox}$ **(D-E)**, and αSMA-CreER;Tbx4$^{flox/flox}$ **(F-G)** at 9 weeks of age after 5 doses of Tamoxifen injection (20 mg/g/injection). n= 4 in each group. **(H-K)** Tbx4 and Has2 expression of lung fibroblasts Col1α2-CreER;Tbx4$^{flox/flox}$ and αSMA-CreER;Tbx4$^{flox/flox}$ were analyzed by quantitative RT-PCR, and normalized by GAPDH. **(L-M)** Lung fibroblasts from Col1α2-CreER;Tbx4$^{flox/flox}$ and αSMA-CreER;Tbx4$^{flox/flox}$ were cultured. HA production from supernatants were analyzed by HA ELISA. $n \geq 4$ mice / group examined. *$p \leq 0.05$, **$p \leq 0.01$, as analyzed by Student's t-test. Scale bars, 1mm **(B, D, F)**, 100μm **(C, E, G)**.



Supplementary Figure 13 Deletion of Tbx4 in NG2 expressing cells has no impact on fibrosis. **(A)** Strategy for inducible deletion of Tbx4 expression in NG2+ cells. NG2-CreER;Tbx4$^{flox/flox}$ mice were used in these experiments. The above mentioned transgenic mice and their WT littermates (8 - 16 weeks old) were treated with bleomycin (2.5U/kg), followed by 5 doses of tamoxifen (20 μg/g/injection) every other day starting on d7. The lungs were collected for hydroxyproline content determination on d21. **(B)** Targeting Tbx4 expression in NG2+ cells have no effect on lung fibrosis (means ± SEM, $^{***}p \leq 0.001$, One-way ANOVA with Bonferroni test; uninjured littermate control, $n = 6$, bleo littermate control, $n = 22$, NG2-CreER;Tbx4$^{flox/flox}$ bleo, $n = 20$). Hydroxyproline content values were expressed as percentage of control, which is set to 100%.



Supplementary Figure 14 Knockdown of Tbx4 affected IPF lung fibroblast function. **(A)** αSMA protein expression was reduced in IPF lung fibroblast upon Tbx4 siRNA transfection. **(B)** Tbx4 knockdown decreased the secreted Col1a1 production in IPF lung fibroblast supernatants in the presence and absence of TGF-β1 treatment. **(C)** Calcein AM Cell viability assay revealed a reduction of viability for Tbx4 deficient IPF lung fibroblasts. **(D)** Casp3 activity was increased in Tbx4 knockdown IPF lung fibroblasts. *n* = 4 IPF lung lung fibroblasts examined, representative result of 3 experiments.



Supplementary Figure 15 Tbx4 lineage cells express stromal cell markers in culture**. (A-F)** Lung fibroblasts were isolated from uninjured Tbx4-Cre;Rosa26-Tm mice and cultured for 3 passages. TdTomato positive cells were sorted by flow cytometry and cultured for immunofluorescence staining. Representative images for αSMA **(A)**, Col1α1 **(B)**, Desmin **(C)**, Vimentin **(D)**, Pdgfrβ **(E)**, and NG2 **(F).** Primary cells from *n* = 3 mice examined. Scale bars, 10 μm.

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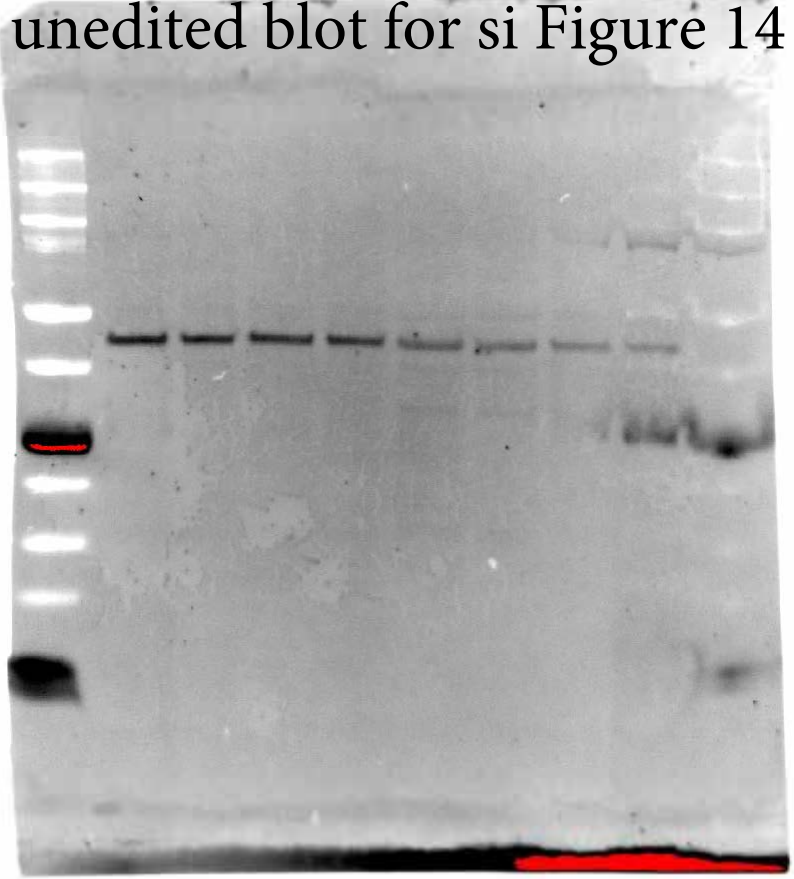

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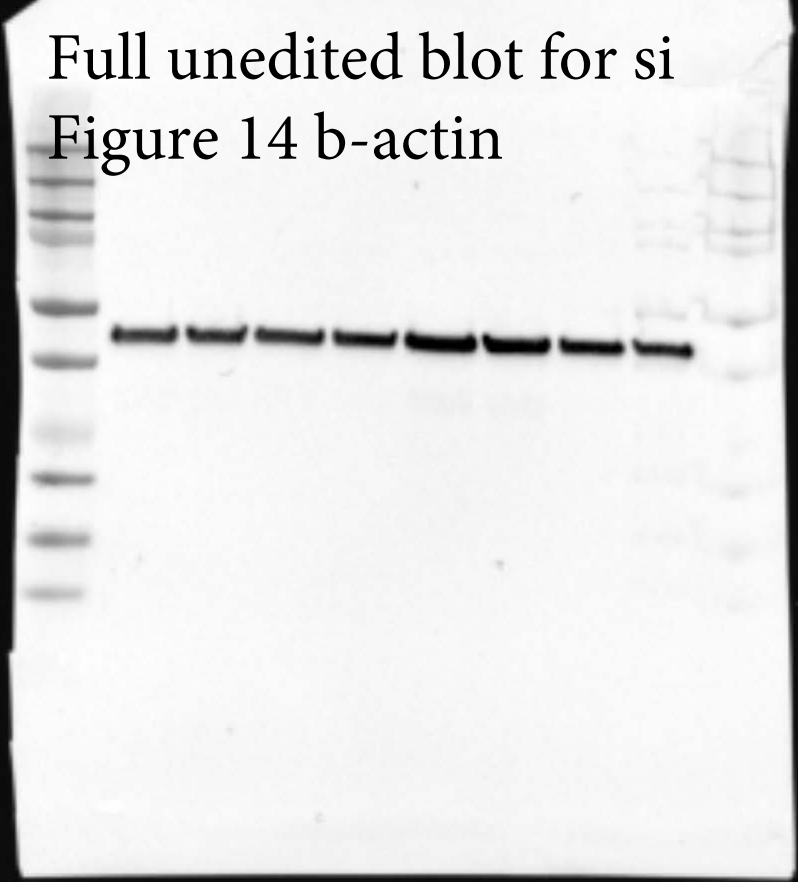
Figure 14 b-actin



Supplementary Table 1 Tbx4 response mesenchymal genes

Gene symbol	Fold Change
Acta2	1.34497
Acta2	2.60271
Acta2	5.88333
Adam12	3.10439
Adam12	2.72716
Col1a2	1.90932
Col3a1	1.92797
Col4a1	2.04113
Col4a1	1.64705
Col4a2	1.90476
Col4a5	3.9369
Col4a5	2.98105
Col4a6	1.54582
Col5a1	1.87582
Col5a1	1.83045
Col5a2	2.08097
Col5a2	1.54845
Col6a1	1.74991
Col6a2	2.23893
Col6a2	1.83583
Col6a3	1.98669
Des	2.09765
Fgf10	2.92405
Fgf7	3.07114
Fgf7	2.97177
Has2	3.39511
Has2	2.7951
Mmp10	2.41523
Mmp11	1.55259
Mmp8	2.87054
Pdgfra	7.36035
Pdgfra	2.79731
Pdgfra	2.44939

Selected up-regulated mesenchymal genes in response to Tbx4. This table was extracted from the Affymetrix array data which Tbx4+ and Tbx4- cells were sorted from Tbx4-Cre^{Tg};RosaTm uninjured mouse lung fibroblasts in culture. The Tbx4+ and Tbx4- cells were pooled from 4 – 5 mouse lungs. The data were expressed as fold changes (Tbx4+ / Tbx4-).

Supplementary Table 2 Tbx4 response mesenchymal transcription factors

Gene symbol	Fold Change
Ebf1	2.16171
Ebf1	2.05805
Ebf1	1.60298
Gata2	2.29148
Gata2	2.03642
Gata3	1.75008
Gata4	1.63537
Gata4	1.53031
Gata5	1.51172
Hmga1 / mga1-rs1	1.67734
Hmga2	1.74949
Hmga2	1.51195
Pax3	2.91327
Pax3	1.68831
Pax3	1.53283
Smad1	1.94011
Smad2	1.62282
Smad3	2.52619
Smad3	1.78529
Smad5	1.82761
Smad5	1.68113
Smad7	2.28324
Snai1	4.75153
Snai2	4.07267
Snai2	2.70444
Sox11	9.35873
Sox11	3.36148
Sox11	2.66943
Sox11	1.77601
Sox11	1.73816
Sox13	1.58862
Sox4	3.20981
Sox4	1.81122
Sox5	2.44708
Sox5	1.93192
Sox9	1.66698
Tbx18	3.53064
Tbx18	1.89916

Tbx2	2.59443
Tbx3	4.81164
Tbx3	3.41996
Tbx3	2.56435
Tbx3	2.18837
Tbx4	3.0827
Tbx4	1.97045
Tbx5	5.52309
Tbx5	2.17176

Relative expression of selected up-regulated mesenchymal transcription factors in response to Tbx4. This table was extracted from the same Affymetrix array data from table 1. The data were expressed as fold changes (Tbx4+ / Tbx4).

Supplementary Table 3 Tbx4 response TGF-β family members

TGF-β and TGFβR expression in Tbx4+ vs Tbx4-	
Tgfb1	-1.57093
Tgfb1	-1.11491
Tgfbr1	-2.24829
Tgfbr1	-2.02172
Tgfbr1	-1.49873
Tgfb2	1.29553
Tgfb2	1.62409
Tgfb2	2.11194
Tgfbr2	1.13563
Tgfbr2	1.97274

Relative expression of selected TGF-β family members in response to Tbx4. This table was extracted from the same Affymetrix array data from table 1. The data were expressed as fold changes (Tbx4+ / Tbx4).