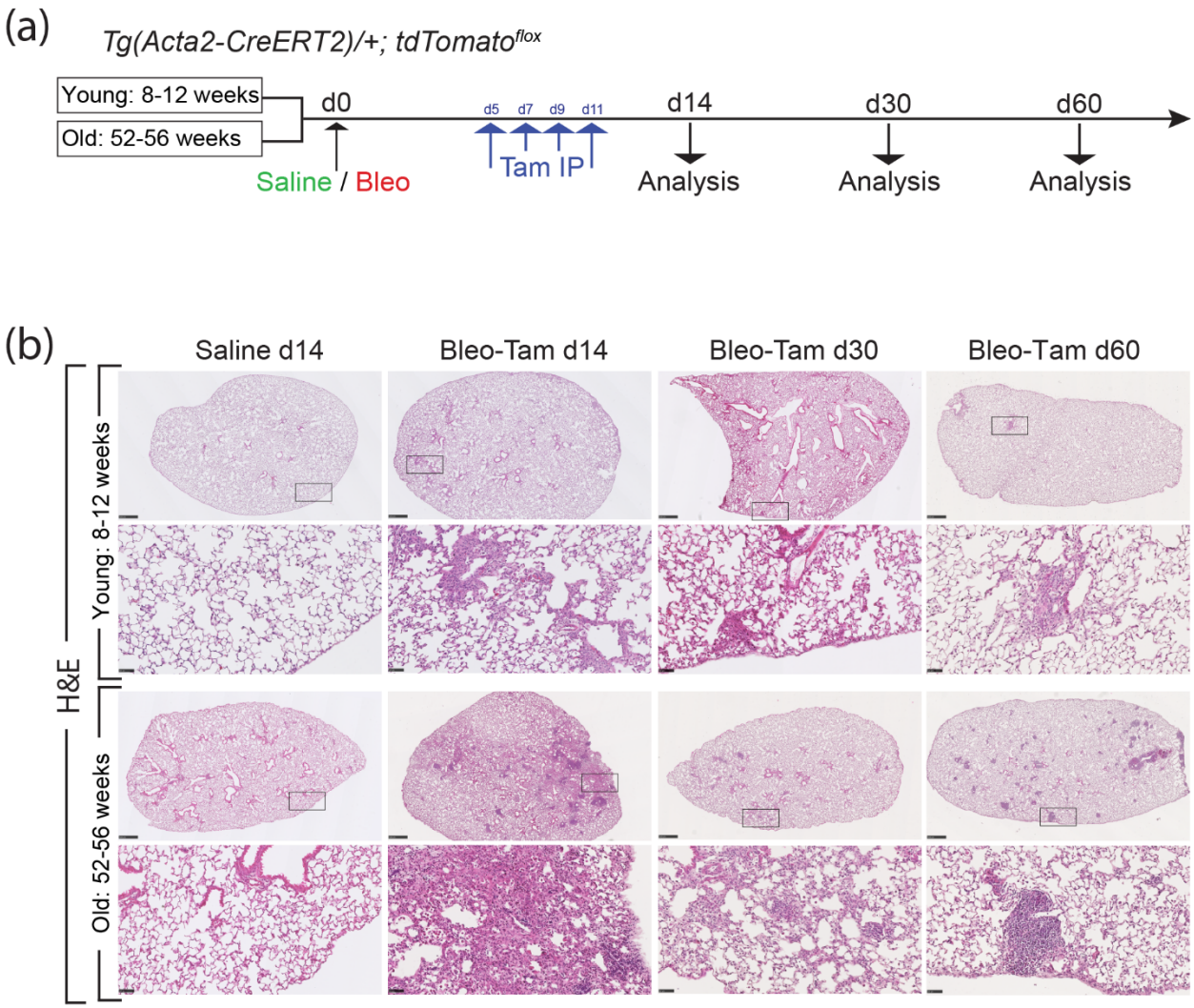
(a)
Tg(Acta2-CreERT2)/+; tdTomatoflox
Young: 8-12 weeks
Old: 52-56 weeks
d0
d5 d7 d9 d11
d14
d30
d60
Saline / Bleo
Tam IP
Analysis
Analysis
Analysis
(b)
Saline d14
Bleo-Tam d14
Bleo-Tam d30
Bleo-Tam d60
H&E
Young: 8-12 weeks
Old: 52-56 weeks
(c)
Fibrosis score
ns
*
ns
**
0
2
4
6
8
Young
Old
Saline d14
Bleo-Tam d14
Bleo-Tam d30
Bleo-Tam d60

Figure S1**: Comparison of fibrosis in young versus old mice. (a)** Female *Tg(Acta2-CreERT2)/+;tdTomato^{flox}* young (8-12-week-old) and old (52-56-week-old) mice are either subjected to saline or 2 U.kg^{-1} bleomycin (bleo). Control lungs are collected at d14 following saline administration, and experimental lungs (Bleo-Tam) are collected at d14, d30, and d60 following bleomycin injury. **(b)** Corresponding low and high magnification of H&E staining showing massive fibrosis formation in old mice lungs compared to young at Bleo-Tam d14. Additionally, fibrotic lesions/regions are still present at resolution Bleo-Tam d30 and Bleo-Tam d60 in young and old mice lungs. **(c)** Comparison of the fibrotic scores between young and old mice at different time points and conditions. Scale bars: b: Low magnification- 500 μm, High magnification- 50 μm; Statistical analysis was performed using (c) 2-way ANOVA with Šídák's *post hoc* test for multiple comparisons. *p<0.05; **p<0.01.

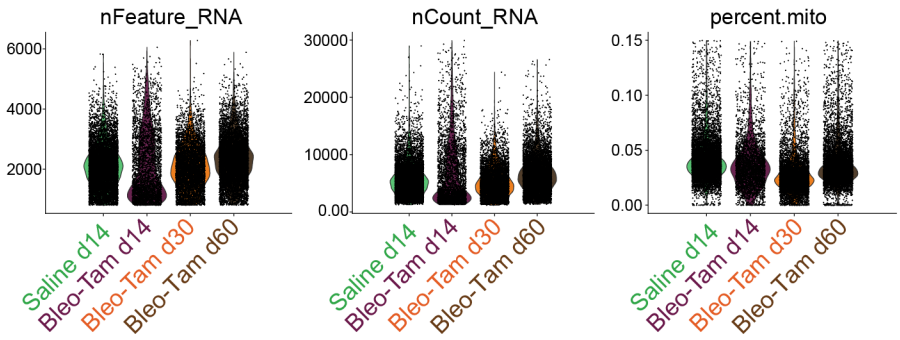

Figure S2**: Quality control of scRNA-seq datasets from lineage-labeled *Acta2*+ (tdTom+) cells sorted from saline d14 and Bleo-Tam lungs at d14, d30 and d60 (52-56-week-old mice).** nFeature_RNA (the number of genes detected in each cell), nCount_RNA (the total number of RNA molecules detected within a cell), and the percentage of mitochondrial genes are quantified for each condition.

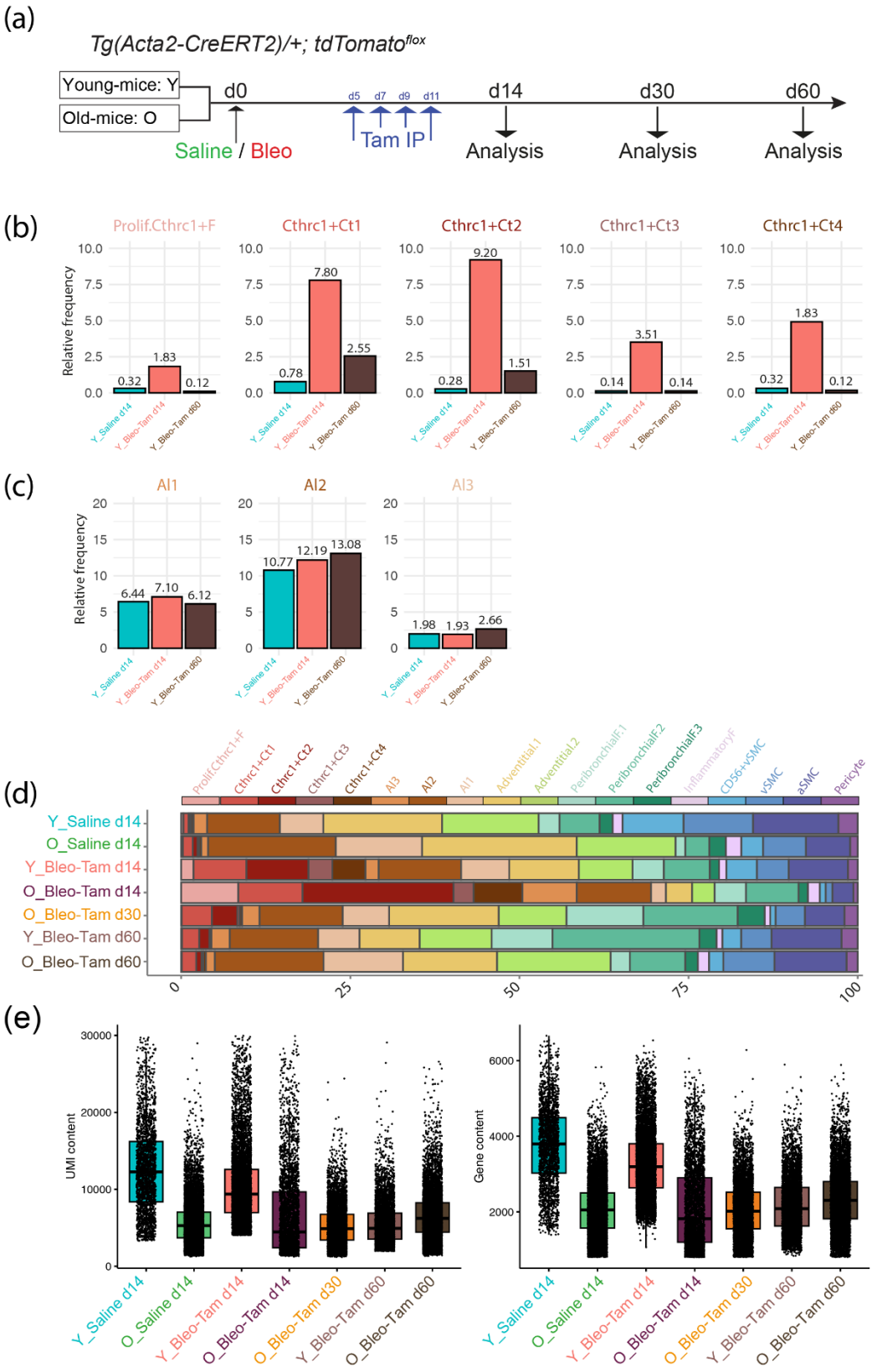
(a)
Tg(Acta2-CreERT2)/+; tdTomatoflox
Young-mice: Y
Old-mice: O
d0
d5 d7 d9 d11
d14
d30
d60
Saline / Bleo
Tam IP
Analysis
Analysis
Analysis
(b)
Prolif.Cthrc1+F
Cthrc1+Ct1
Cthrc1+Ct2
Cthrc1+Ct3
Cthrc1+Ct4
Relative frequency
0.32 1.83 0.12
0.78 7.80 2.55
0.28 9.20 1.51
0.14 3.51 0.14
0.32 1.83 0.12
Y_Saline d14
Y_Bleo-Tam d14
Y_Bleo-Tam d60
(c)
AI1
AI2
AI3
Relative frequency
6.44 7.10 6.12
10.77 12.19 13.08
1.98 1.93 2.66
(d)
Prolif.Cthrc1+F
Cthrc1+Ct1
Cthrc1+Ct2
Cthrc1+Ct3
Cthrc1+Ct4
AI3
AI2
AI1
Adventitial1
Adventitial2
PeribronchialF1
PeribronchialF2
PeribronchialF3
InflammatoryF
CD36+vSMC
vSMC
aSMC
Pericyte
Y_Saline d14
O_Saline d14
Y_Bleo-Tam d14
O_Bleo-Tam d14
O_Bleo-Tam d30
Y_Bleo-Tam d60
O_Bleo-Tam d60
0
25
50
75
100
(e)
UMI content
Gene content
Y_Saline d14
O_Saline d14
Y_Bleo-Tam d14
O_Bleo-Tam d14
O_Bleo-Tam d30
Y_Bleo-Tam d60
O_Bleo-Tam d60

Figure S3**: Comparison of Cthrc1 and alveolar fibroblast subclusters in young versus old mice. (a)** Female *Tg(Acta2-CreERT2)/+;tdTomato*^{flox} young (8-12-week-old) and old (52-56-week-old) mice are either subjected to saline or 2 U.kg^{-1} bleomycin (bleo). Saline lungs are collected at d14 and experimental lungs (Bleo-Tam) are collected at d14, d30, and d60 following bleomycin injury. **(b)** Relative frequencies of each *Cthrc1*-expressing subpopulation at each time-point in young mice. **(c)** Relative frequencies of each alveolar fibroblast subpopulation at each time point in young mice. **(d)** The relative frequencies of each subpopulation in each sample from the integrated dataset of young and old mice. **(e)** UMI and gene content in each sample from the integrated young and old mice dataset. O, old mice; Y, young mice

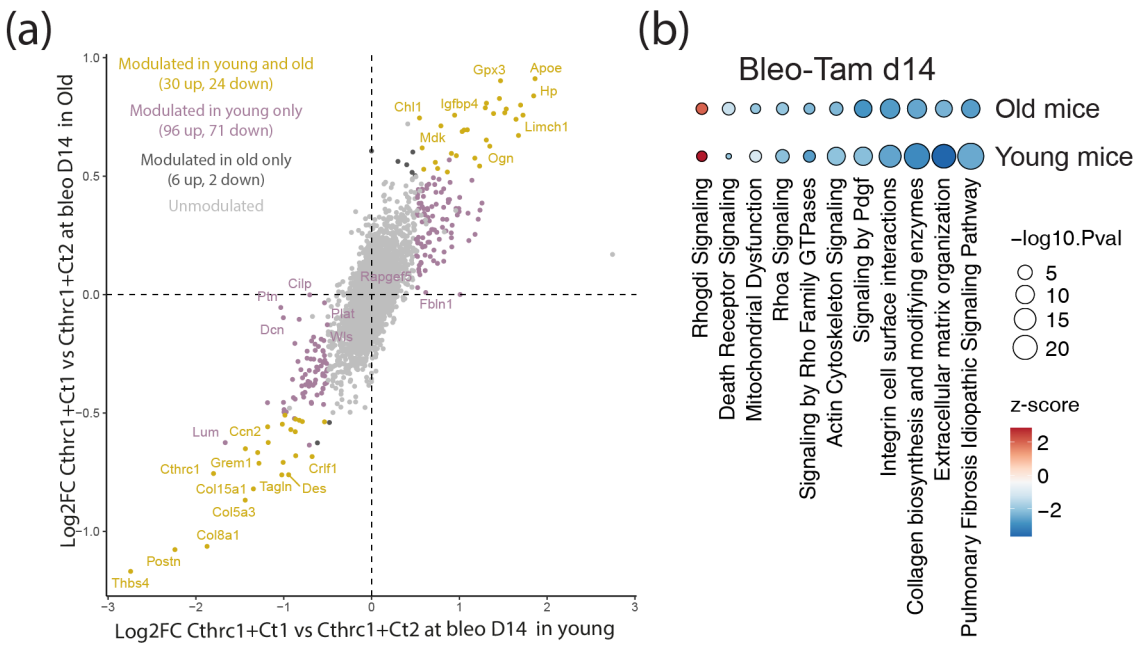

Figure S4: **Comparison of *Cthrc1*+ myofibroblast cells (Ct1 versus Ct2) during fibrosis formation (d14) in young and old mice. (a)** Correlation of the two Log2 Fold change obtained by comparing *Cthrc1*+ Ct1 to Ct2 of the Bleo-Tam d14 sample from young (8-12-week-old) and old (52-56-week-old) mice. Golden dots correspond to genes significantly modulated (|log2FC| > 0.5 and p_{adj} < 0.05) in both young and old. Mauve and dark gray dots correspond to genes only significantly modulated in young or old mice, respectively. **(b)** IPA canonical pathways enrichment obtained from the differential analyses of Ct1 compared to Ct2 cells of the Bleo-Tam d14 sample in both young and old mice.

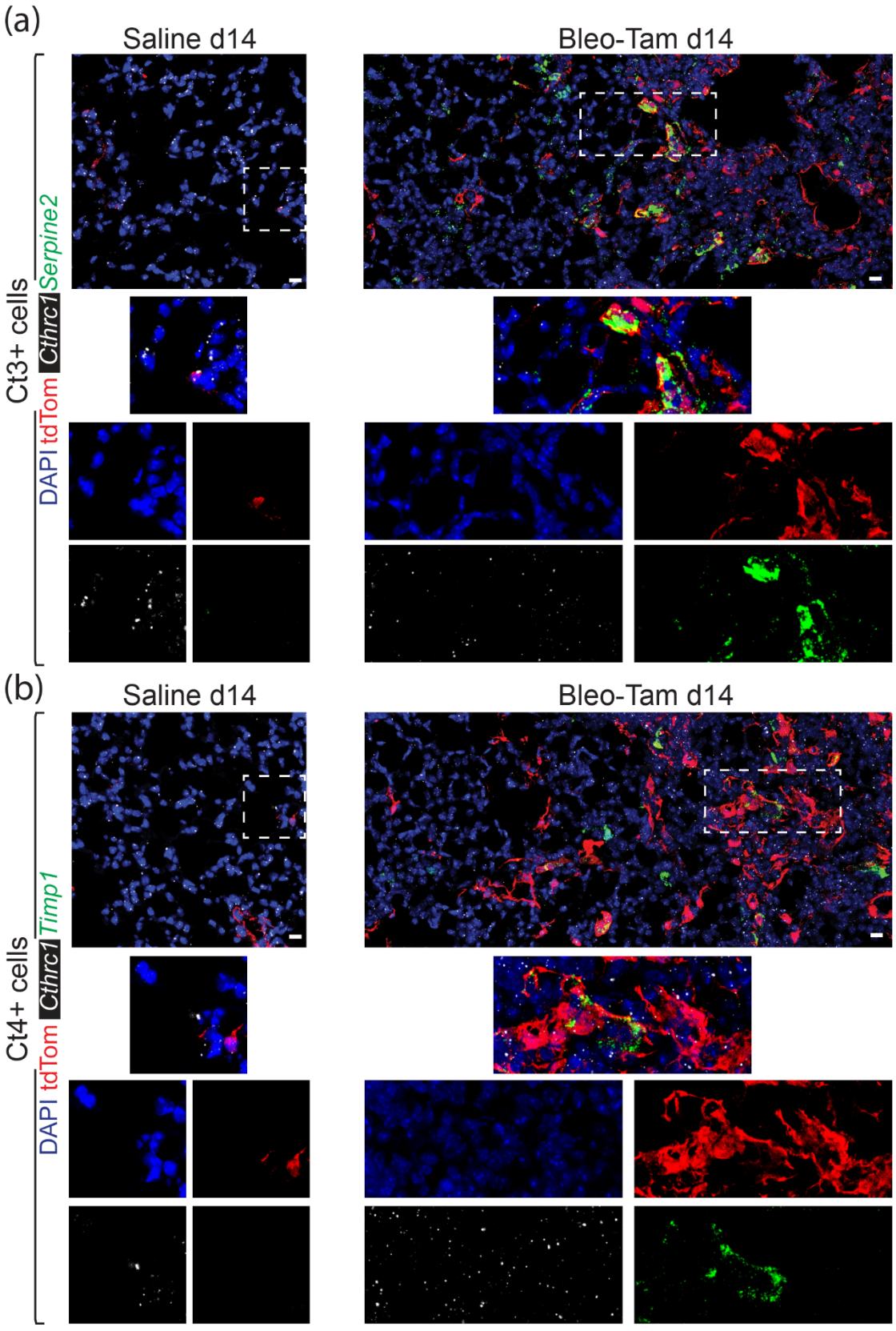
(a)
Saline d14
Bleo-Tam d14
Ct3+ cells
Serpine2
Cthrc1
tdTom
DAPI
(b)
Saline d14
Bleo-Tam d14
Ct4+ cells
Timp1
Cthrc1
tdTom
DAPI

Figure S5**: Characterization of Ct3 and Ct4 cells in saline and Bleo-Tam d14 lungs. (a)** RNA-scope of *Cthrc1*+aMYF marker *Cthrc1* and Ct3-cluster marker *Serpine2* showing colocalization with tdTom+ cells. **(b)** RNA-scope of *Cthrc1*+aMYF marker *Cthrc1* and Ct4-cluster marker *Timp1* showing colocalization with tdTom+ cells. Scale bars: (a, b): 150 µm.

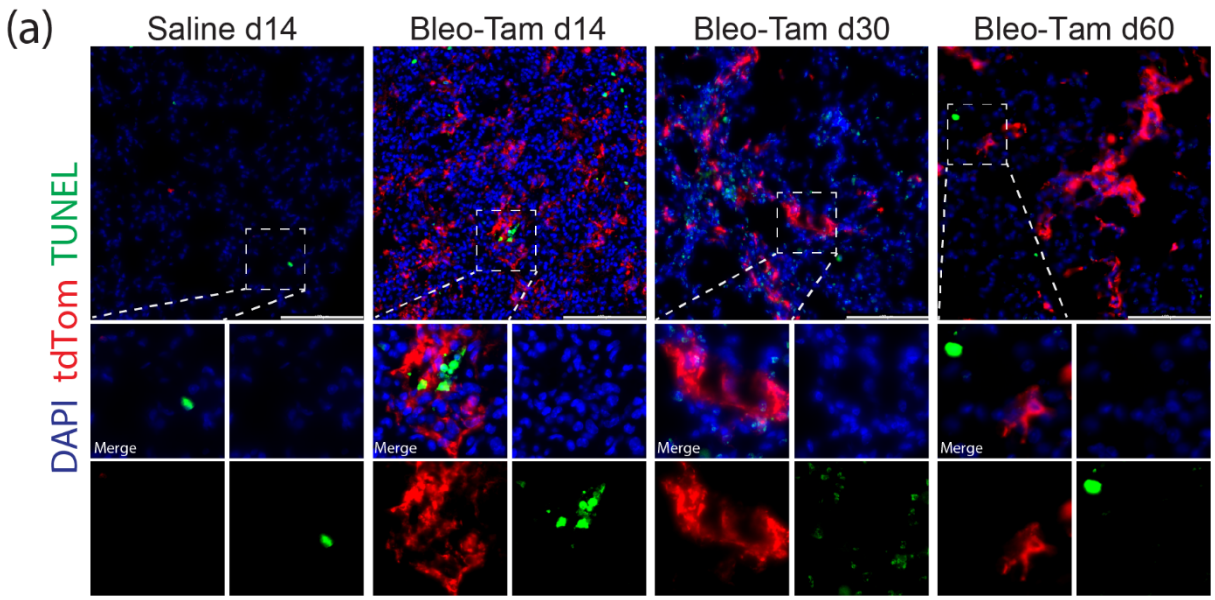

Figure S6**: TUNEL assay showing the tdTom+ cells undergo apoptosis during fibrosis formation (d14) and early fibrosis resolution (d30)**. Many tdTom+ cells persist during late fibrosis resolution (d60). Scale bar: 100 μm.