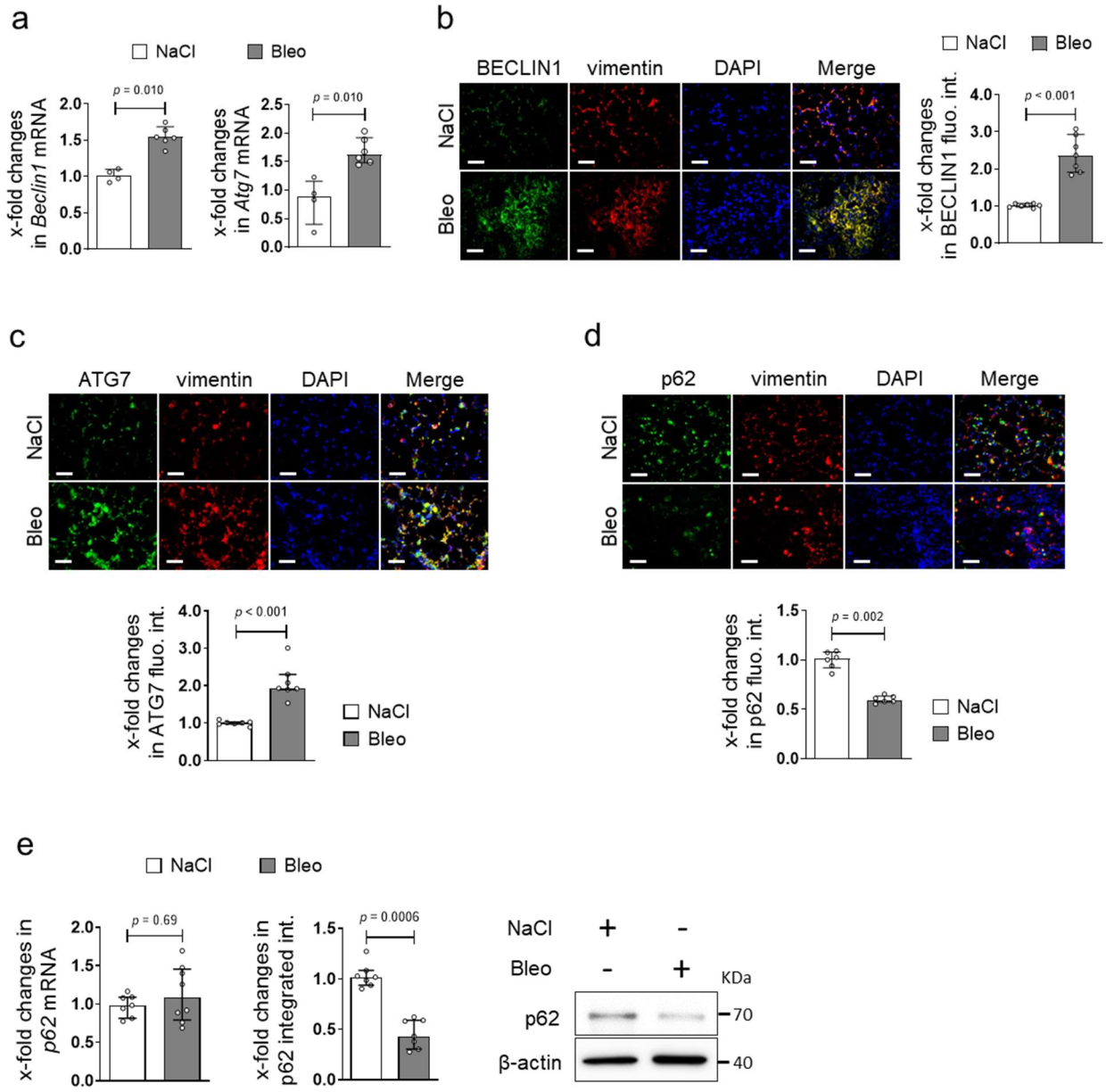
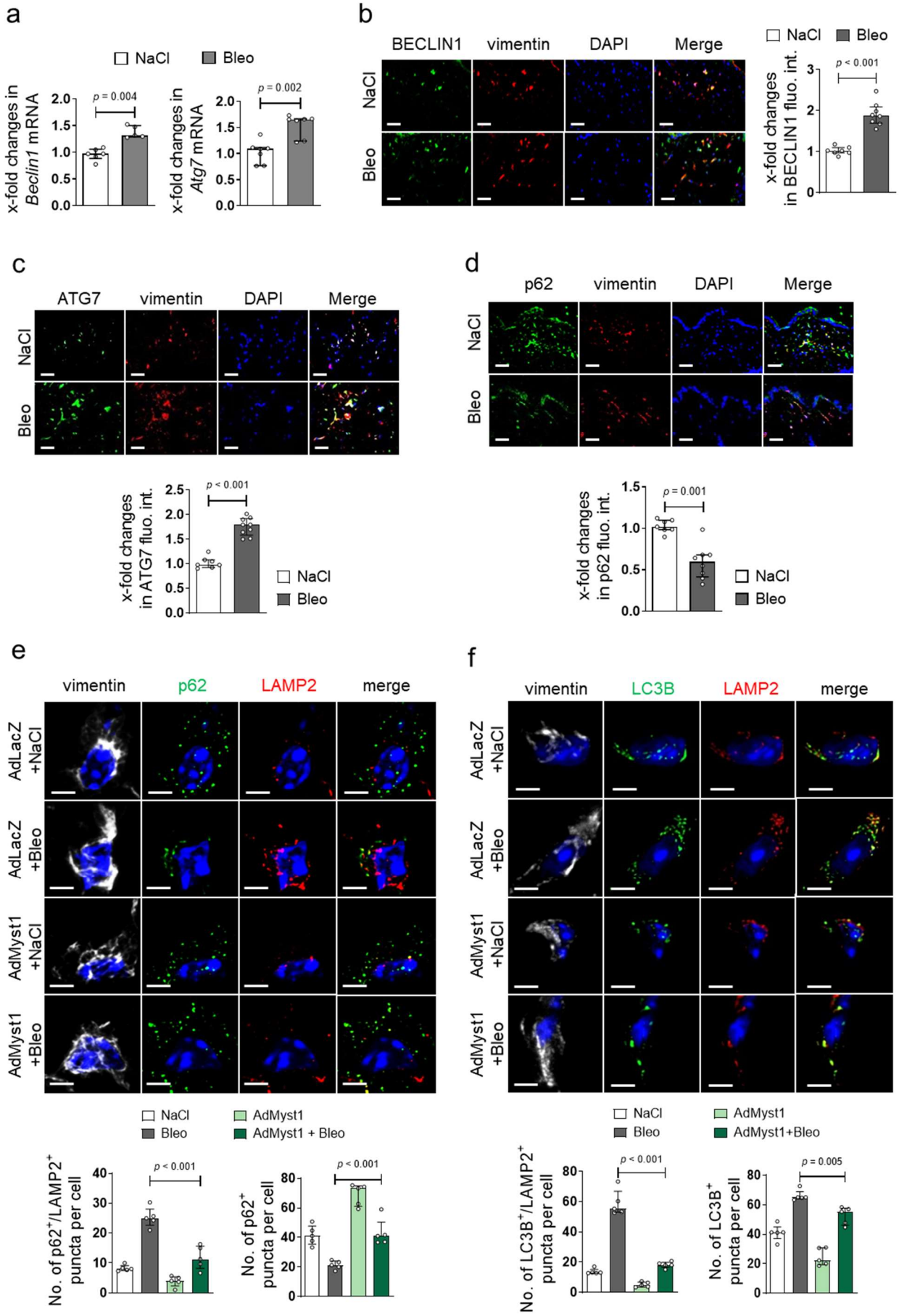
Supplementary Information:

TGFβ promotes fibrosis by MYST1-dependent epigenetic regulation of autophagy

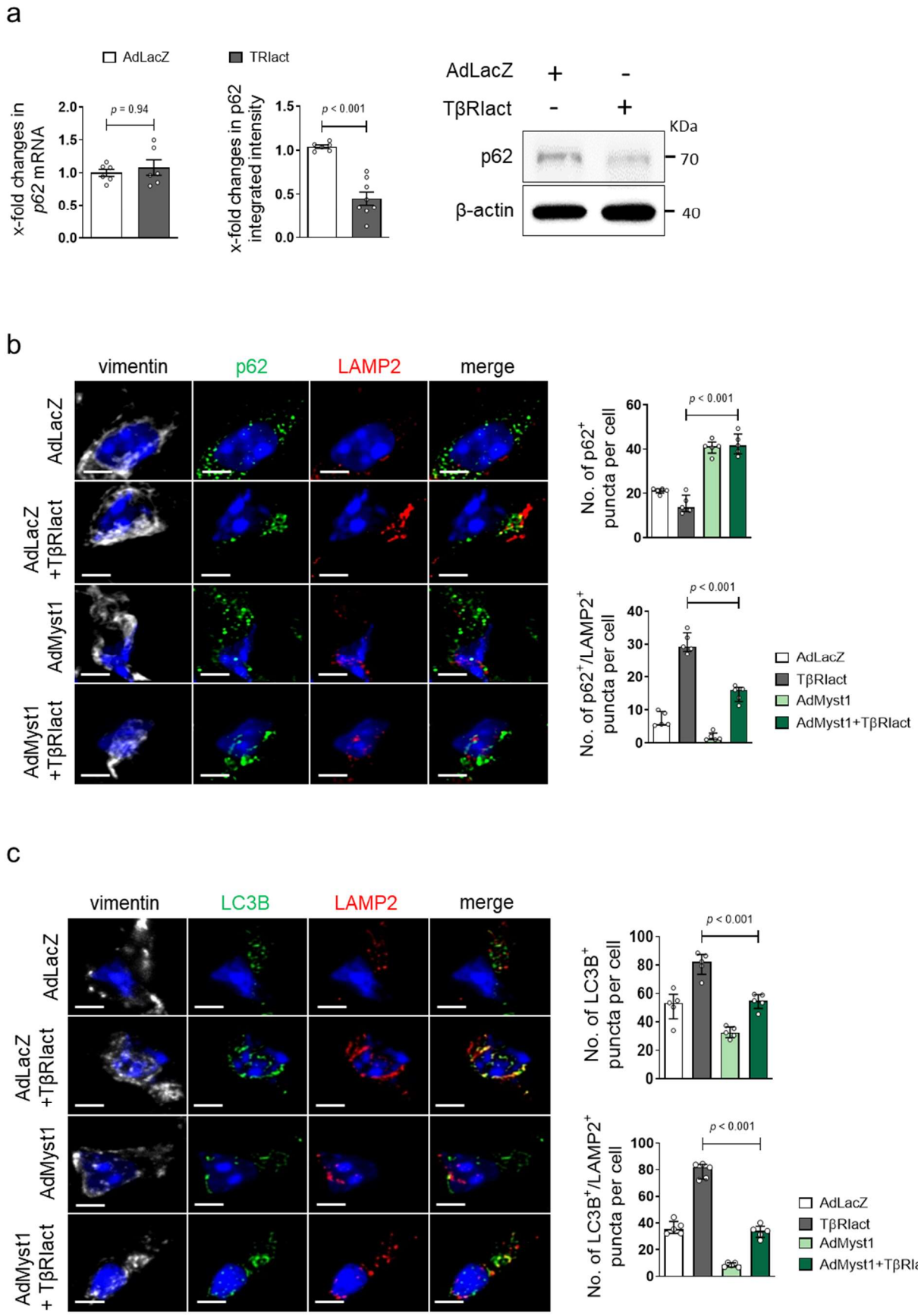
Zehender *et al*.



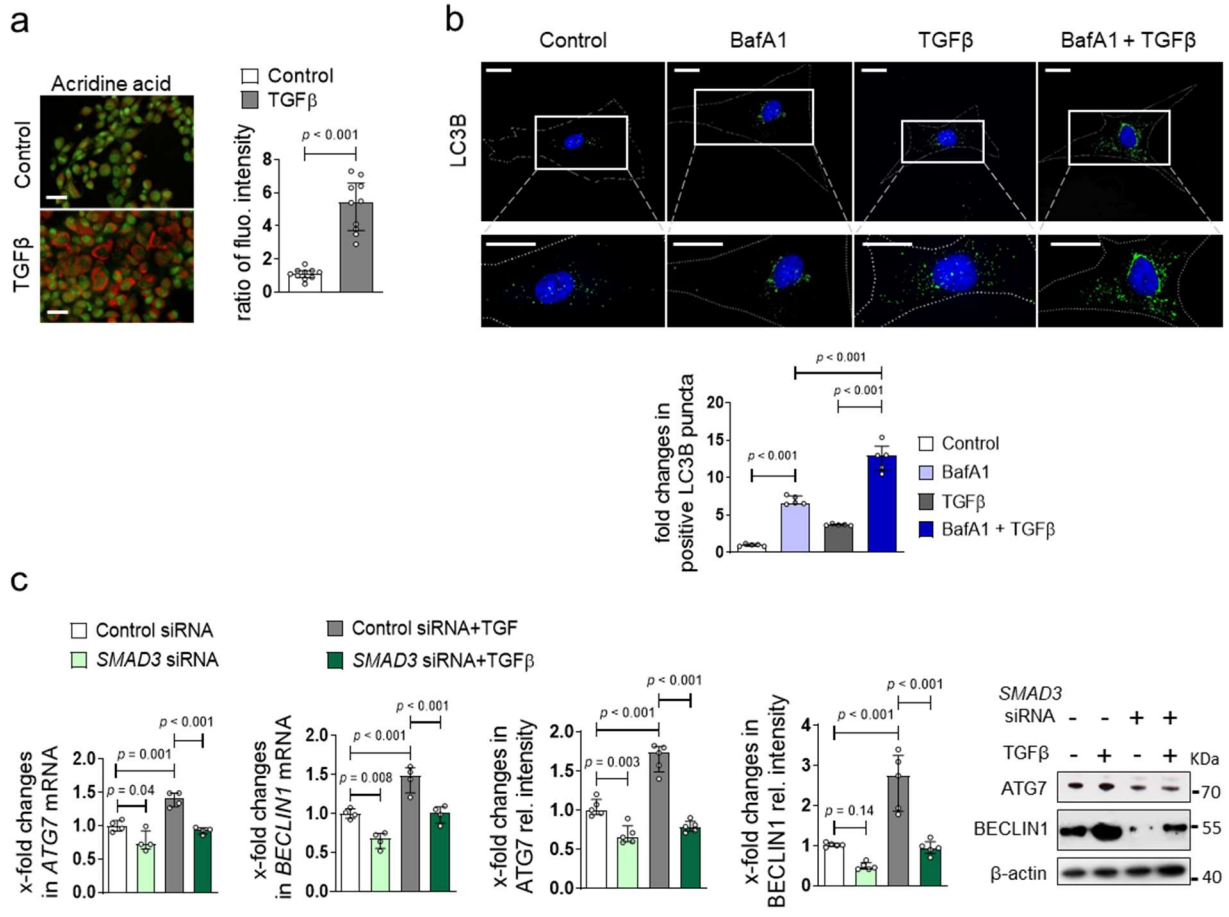
Supplementary Figure 1: Induction of autophagy related proteins in bleomycin-induced lung and skin fibrosis. A: mRNA levels of *Beclin1* and *Atg7* (n = 4 biological replicates for control and n = 6 for bleomycin group). **B-D:** Representative immunofluorescence staining of BECLIN1 (**B**), ATG7 (**C**) or p62 (**D**) (all green) in combination with DAPI (blue) and vimentin (red) and respective quantifications of BECLIN1, ATG7 (n = 7 biological replicates per group) and p62 (n= 6 biological replicates per group) expression. Horizontal scale bars, 50 µm. **E:** *p62* mRNA (n = 7 biological replicates for control and n = 8 for bleomycin group) and protein levels (n = 7 biological replicates per bleomycin group) in murine skin. Representative western blot and quantification. All data are presented as median ± IQR. *p*-values were determined by two-sided Mann-Whitney test and are indicated in the figure. See source data for more detailed information. int.: intensity, fluo.: fluorescence., Bleo: bleomycin.


a
NaCl
Bleo
x-fold changes in Beclin1 mRNA
p = 0.004
x-fold changes in Atg7 mRNA
p = 0.002
b
BECLIN1
vimentin
DAPI
Merge
NaCl
Bleo
x-fold changes in BECLIN1 fluo. int.
p < 0.001
c
ATG7
vimentin
DAPI
Merge
NaCl
Bleo
x-fold changes in ATG7 fluo. int.
p < 0.001
d
p62
vimentin
DAPI
Merge
NaCl
Bleo
x-fold changes in p62 fluo. int.
p = 0.001
e
vimentin
p62
LAMP2
merge
AdLacZ +NaCl
AdLacZ +Bleo
AdMyst1 +NaCl
AdMyst1 +Bleo
NaCl
Bleo
AdMyst1
AdMyst1 + Bleo
No. of p62+/LAMP2+ puncta per cell
p < 0.001
No. of p62+ puncta per cell
p < 0.001
f
vimentin
LC3B
LAMP2
merge
AdLacZ +NaCl
AdLacZ +Bleo
AdMyst1 +NaCl
AdMyst1 +Bleo
NaCl
Bleo
AdMyst1
AdMyst1+Bleo
No. of LC3B+/LAMP2+ puncta per cell
p < 0.001
No. of LC3B+ puncta per cell
p = 0.005

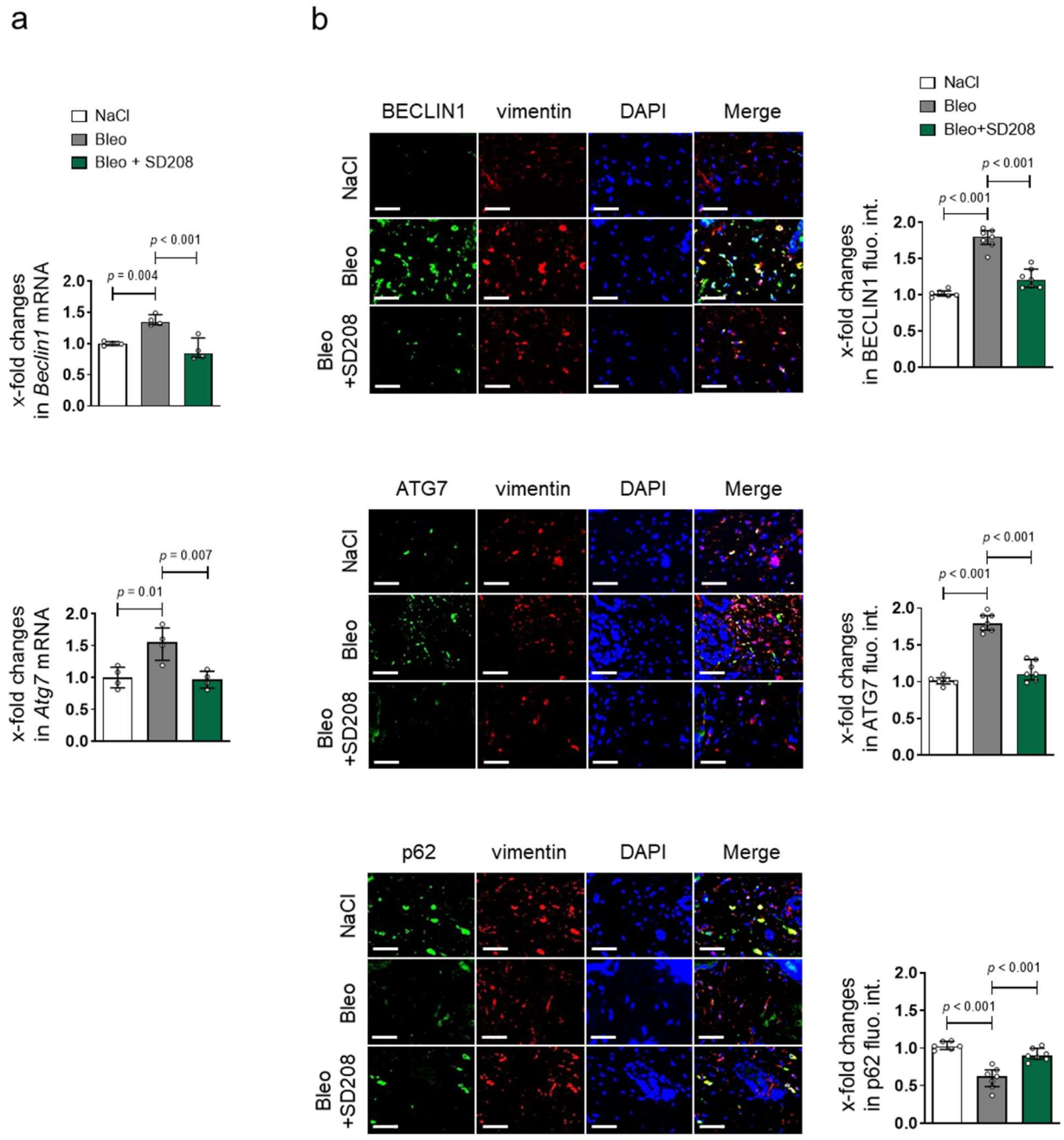
Supplementary Figure 2: Autophagy is activated in bleomycin-induced skin fibrosis. A: mRNA levels of *Beclin1* (n = 6 biological replicates for control and n = 5 for bleomycin group) and *Atg7* (n = 7 biological replicates per group) **B-D:** Representative immunofluorescence staining of BECLIN1 (**A**; n = 7 biological replicates for control and n = 8 for bleomycin group), ATG7 (**C;** n = 7 samples for control and n = 9 for bleomycin group) or p62 (**D;** n = 7 biological replicates for control and n = 8 for bleomycin group) (all green) in combination with DAPI (blue) and vimentin (red). Horizontal scale bars, 50 µm. **E-F: Autophagic flux in bleomycin-induced skin fibrosis. E:** Co-staining of p62 (green) and LAMP2 (red) in combination with DAPI (blue) and vimentin (gray) (n = 5 biological replicates per group). Representative confocal images and quantifications. **F:** Co-staining of LC3B (green) and LAMP2 (red) in Combination with DAPI (blue) and vimentin (gray) (n = n = 5 biological replicates per group): Representative confocal images and quantifications. Horizontal scale bars, 5 µm. All data are presented as median ± IQR. *p*-values were determined by two-sided Mann-Whitney test (**A-D**) or ANOVA one-way with Tukey's multiple comparison post hoc test (**E-F**) and are indicated in the figure. See source data for more detailed information. Fluo.: fluorescence, Int.: intensity, Bleo: bleomycin, Ad: adenovirus.


a
AdLacZ
TRIact
x-fold changes in p62 mRNA
p = 0.94
x-fold changes in p62 integrated intensity
p < 0.001
AdLacZ + -
TβRIact - +
KDa
p62
70
β-actin
40
b
vimentin
p62
LAMP2
merge
AdLacZ
AdLacZ +TβRIact
AdMyst1
AdMyst1 +TβRIact
No. of p62+ puncta per cell
p < 0.001
No. of p62+/LAMP2+ puncta per cell
p < 0.001
AdLacZ
TβRIact
AdMyst1
AdMyst1+TβRIact
c
vimentin
LC3B
LAMP2
merge
AdLacZ
AdLacZ +TβRIact
AdMyst1
AdMyst1 + TβRIact
No. of LC3B+ puncta per cell
p < 0.001
No. of LC3B+/LAMP2+ puncta per cell
p < 0.001
AdLacZ
TβRIact
AdMyst1
AdMyst1+TβRIact

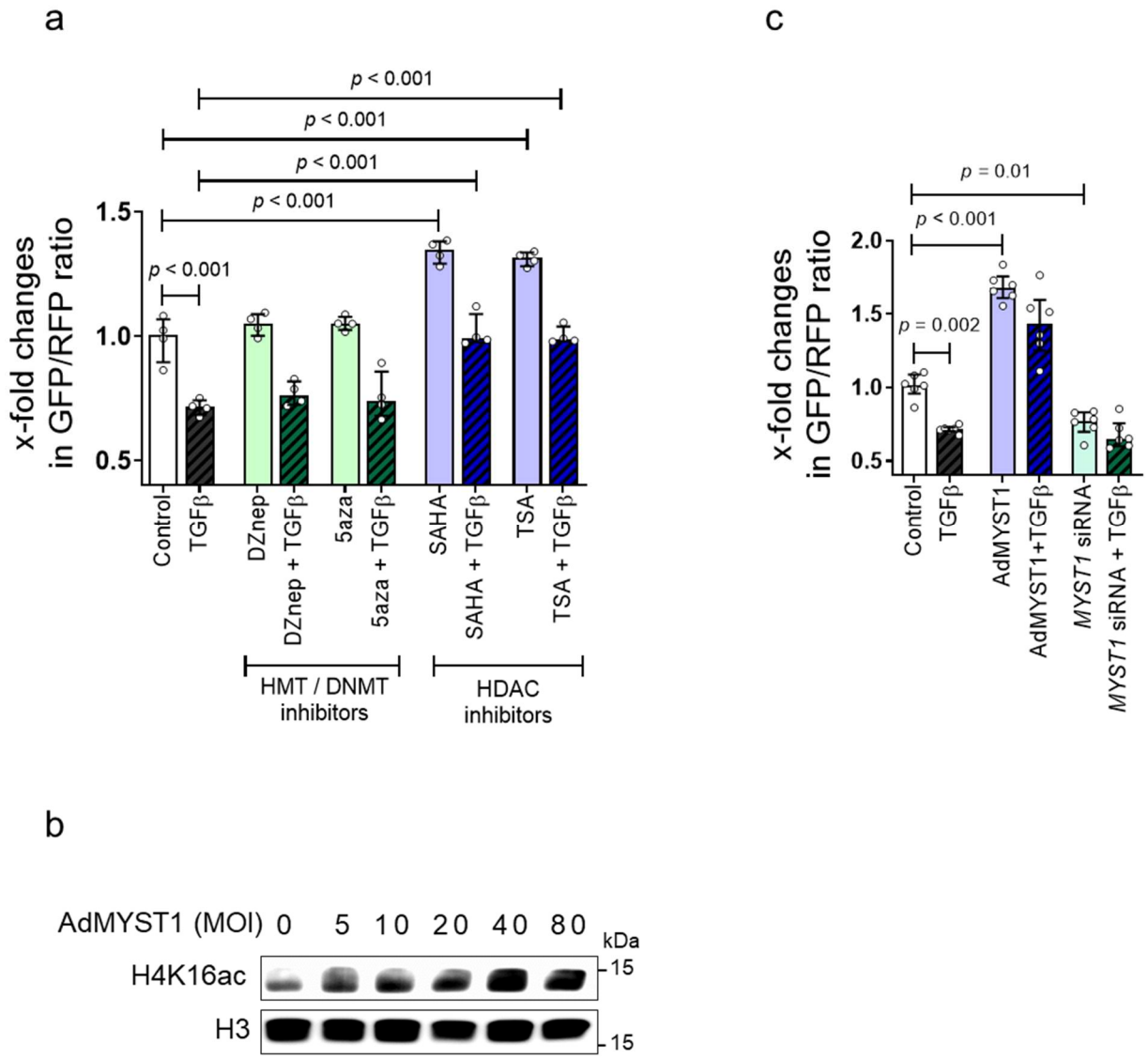
Supplementary Figure 3: Autophagy is activated in TβRIact-induced skin fibrosis. A: *p62* mRNA (n = 6 biological replicates per group) and protein levels (n = 6 biological replicates for control and n = 8 samples for TβRIact group). Representative western blot and quantification. **B:** Co-staining of p62 (green) and LAMP2 (red) in combination with DAPI (blue) and vimentin (gray). Representative confocal images and quantifications (n = 5 biological replicates per group). **C:** Co-staining of LC3B (green) and LAMP2 (red) in combination with DAPI (blue) and vimentin (gray): Representative confocal images and quantifications (n = 5 biological replicates per group). Horizontal scale bar, 5 µm. All data are presented as median ± IQR. *p*-values were determined by two-sided Mann-Whitney test (**A**) or ANOVA one-way with Tukey's multiple comparison post hoc test (**B-C**) and are indicated in the figures. See source data for more detailed information. Ad: Adenovirus, TβRIact: constitutively active TGFβ receptor type I.


a
Acridine acid
Control
TGFβ
Control
TGFβ
ratio of fluo. intensity
p < 0.001
b
Control
BafA1
TGFβ
BafA1 + TGFβ
LC3B
fold changes in positive LC3B puncta
p < 0.001
p < 0.001
p < 0.001
Control
BafA1
TGFβ
BafA1 + TGFβ
c
Control siRNA
SMAD3 siRNA
Control siRNA+TGF
SMAD3 siRNA+TGFβ
x-fold changes in ATG7 mRNA
x-fold changes in BECLIN1 mRNA
x-fold changes in ATG7 rel. intensity
x-fold changes in BECLIN1 rel. intensity
SMAD3 siRNA
TGFβ
KDa
ATG7
BECLIN1
β-actin
70
55
40
d
AdLacZ
TRIact
x-fold changes in Beclin1 mRNA
p = 0.003
x-fold changes in Atg7 mRNA
p < 0.001
e
AdLacZ
TRIact
x-fold changes in BECLIN1 fluo. int.
p < 0.001
x-fold changes in ATG7 fluo. int.
p < 0.001
x-fold changes in p62 fluo. int.
p = 0.004
BECLIN1
vimentin
DAPI
Merge
Control
TβRIact
ATG7
vimentin
DAPI
Merge
Control
TβRIact
p62
vimentin
DAPI
Merge
Control
TβRIact

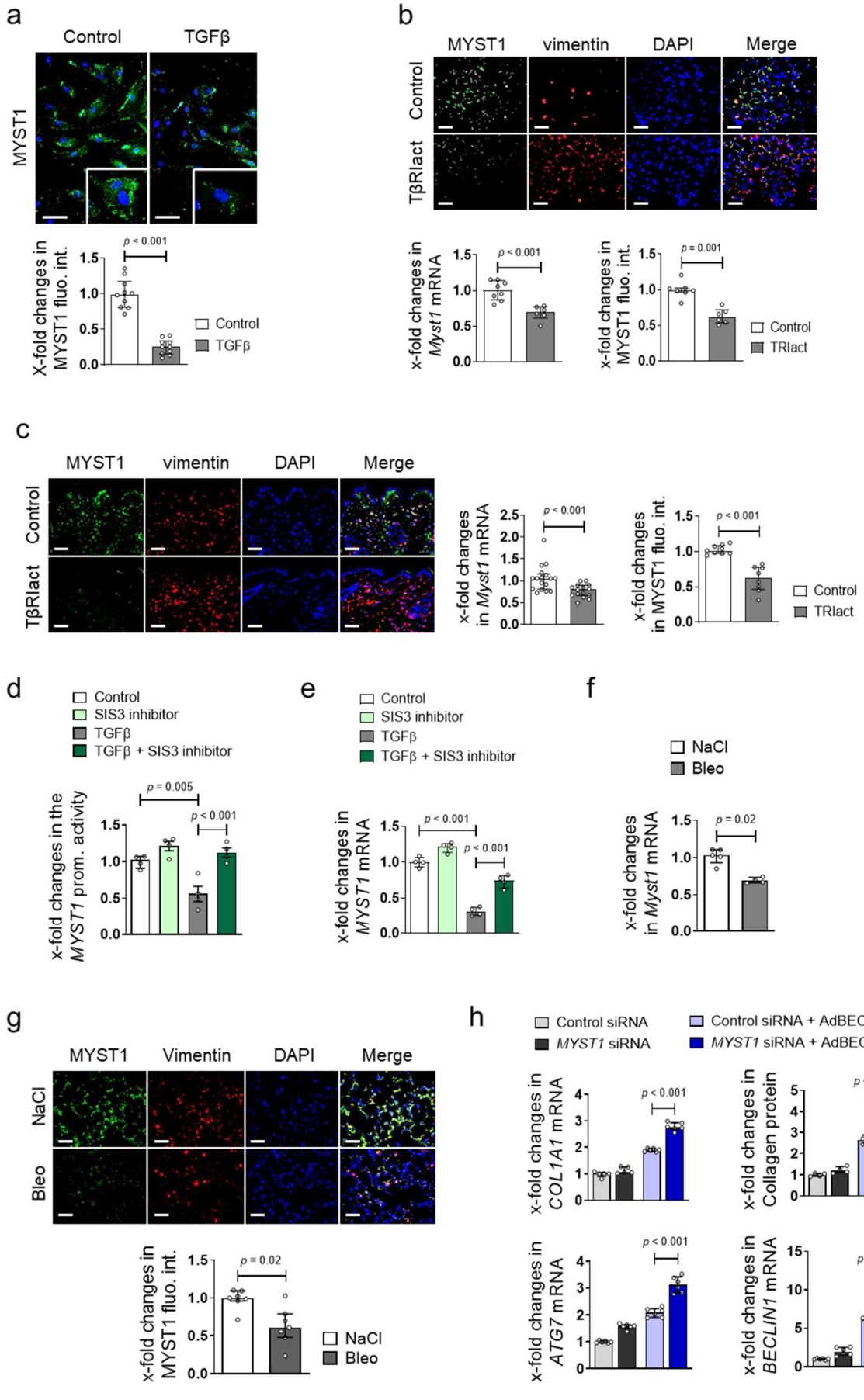
Supplementary Figure 4: TGFβ / Smad3-dependent regulation of autophagy-regulators in fibroblasts. A: Representative images and quantification of acridine staining in fibroblasts (n = 10 independent quantifications within 3 biological replicates per group). **B:** Representative images and quantification of LC3B fluorescence staining in fibroblasts treated with TGFβ in the presence or absence of BafA1 (n = 5 biological replicates per group). Horizontal scale bar, 5 µm. **C:** Effects of siRNA-mediated knockdown of SMAD3 on ATG7 and BECLIN1 mRNA (n = 4 biological replicates per group) and protein (n = 5 biological replicates per group) in TGFβ stimulated fibroblasts. **D-E:** Overexpression of TβRI in murine lungs: mRNA levels of *Beclin1* (**D**; n = 7 samples for AdLacZ and n = 5 for TβRIact) and *Atg7* (n = 8 biological replicates for AdLacZ and n = 6 for TβRIact) and representative stainings of BECLIN1, ATG7 and p62 (all green) in combination with DAPI (blue) and vimentin (red) and respective quantifications (**E**; n = 7 biological replicates per group). Horizontal scale bar, 50 µm. All data are presented as median ± IQR (**C-E**). *p*-values were determined by two-sided Mann-Whitney test and are indicated in the figure. See source data for more detailed information. rel.: relative, fluo.: fluorescence, int.: intensity, TβRIact: constitutively active TGFβ receptor type I, BafA1: Bafilomycin A1.



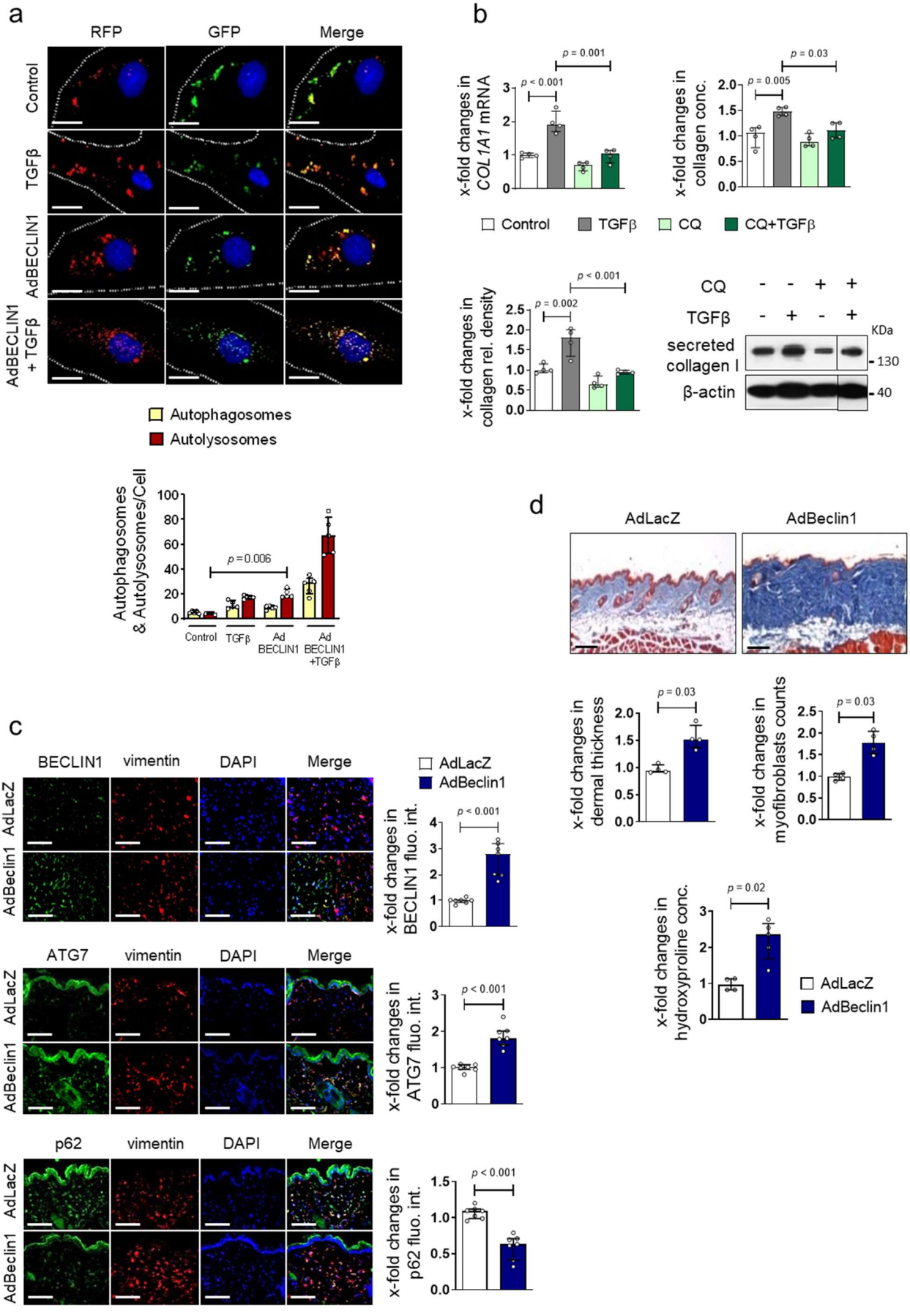
Supplementary Figure 5: Selective inhibition of TGFβ signaling by SD-208 decreases the expression of autophagy markers in bleomycin-induced fibrosis. A: mRNA levels of *Beclin1* and *Atg7* (n = 4 biological replicates per group). **B:** Representative immunofluorescence staining of markers of autophagy BECLIN1, ATG7 or p62 (all green; n = 6 biological replicates for control group and n = 7 for other groups) in combination with DAPI (blue) and vimentin (red). Horizontal scale bar, 50 µm. All data are presented as median ± IQR. *p*-values were determined by ANOVA one-way with Tukey's multiple comparison post hoc test and are indicated in the figure. See source data for more detailed information. int.: intensity, fluo.: fluorescence., Bleo: bleomycin.



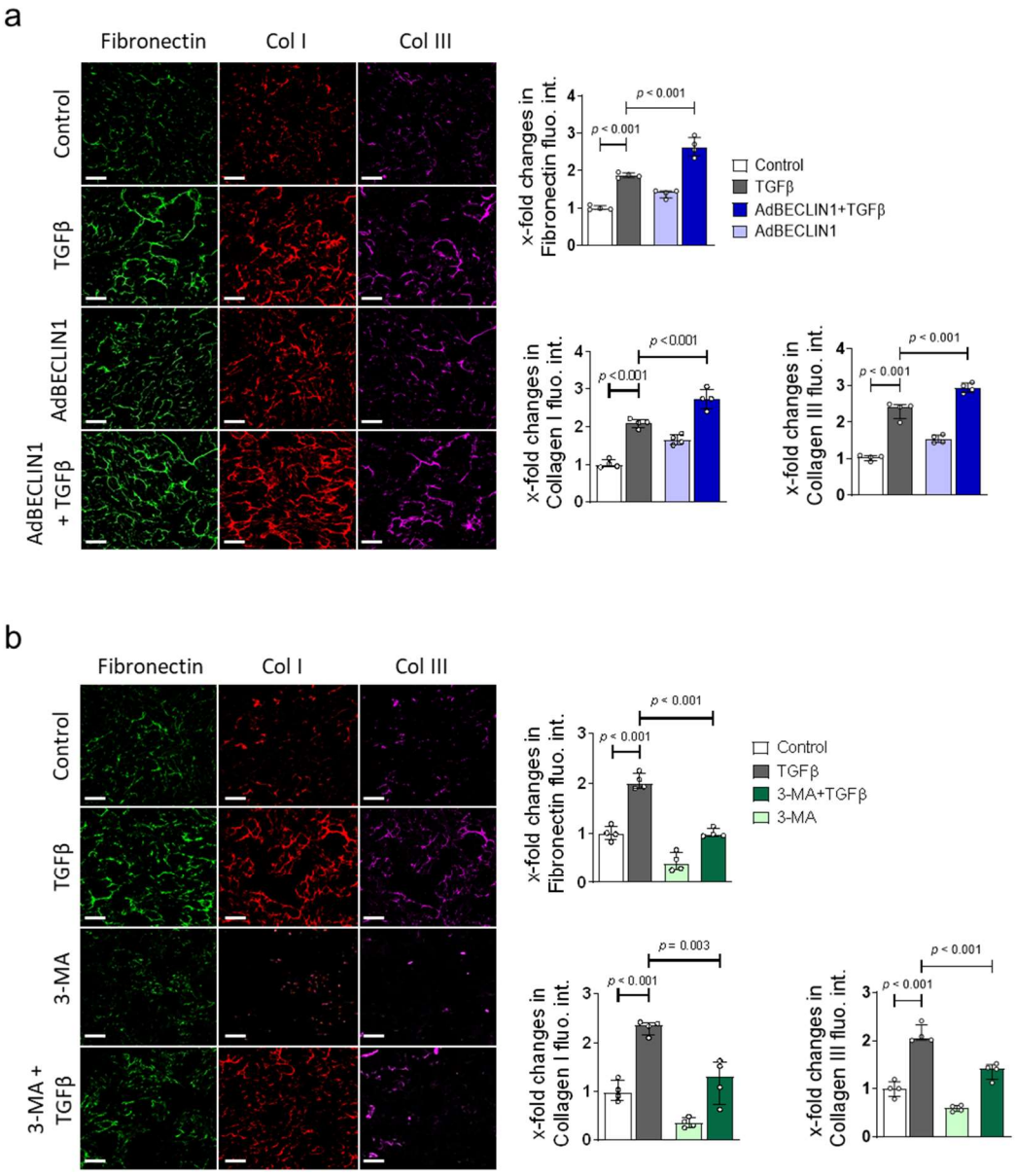
Supplementary Figure 6: MYST1 acetyltransferase modulates TGFβ-induced autophagy. EGFP/RFP-LC3 reporter activity, in which the GFP/RFP ratio correlates inversely with autophagy activity: **A:** Inhibition of different epigenetic mechanisms that have been linked to the pathogenesis of fibrosis including H3K27 histone methylation (DZnep), DNA methyltransferases (5aza) and HDACs (TSA and SAHA) in TGFβ-stimulated fibroblasts (n = 4 biological replicates per group). **B:** Histone acetylation levels in cultured fibroblasts upon adenoviral overexpression of MYST1. Representative Western blot images of fibroblasts infected with different concentrations of adenovirus encoding for *MYST1*. **C:** GFP/RFP ratio upon overexpression and knockdown of *MYST1* in fibroblasts (n = 6 biological replicates per group). All Data are presented as median ± IQR. *p*-values were determined by ANOVA one-way with Tukey's multiple comparison post hoc test and are indicated in the figure. See source data for more detailed information. Ad: Adenovirus. DZnep: 3-deazaneplanocin A, 5aza: 5-Aza-2'-deoxycytidine; TSA: Trichostatin A; SAHA: Vorinostat. The samples in panels A and C are part of a large set of experiments and share the same controls. Data are presented separately just for the purpose of this figure.


a
Control
TGFβ
MYST1
X-fold changes in MYST1 fluo. int.
p < 0.001
Control
TGFβ
b
MYST1
vimentin
DAPI
Merge
Control
TβRIact
x-fold changes in Myst1 mRNA
p < 0.001
x-fold changes in MYST1 fluo. int.
p = 0.001
Control
TRIact
c
MYST1
vimentin
DAPI
Merge
Control
TβRIact
x-fold changes in Myst1 mRNA
p < 0.001
x-fold changes in MYST1 fluo. int.
p < 0.001
Control
TRIact
d
Control
SIS3 inhibitor
TGFβ
TGFβ + SIS3 inhibitor
x-fold changes in the MYST1 prom. activity
p = 0.005
p < 0.001
e
Control
SIS3 inhibitor
TGFβ
TGFβ + SIS3 inhibitor
x-fold changes in MYST1 mRNA
p < 0.001
p < 0.001
f
NaCl
Bleo
x-fold changes in Myst1 mRNA
p = 0.02
g
MYST1
Vimentin
DAPI
Merge
NaCl
Bleo
x-fold changes in MYST1 fluo. int.
p = 0.02
NaCl
Bleo
h
Control siRNA
MYST1 siRNA
Control siRNA + AdBECLIN1
MYST1 siRNA + AdBECLIN1
x-fold changes in COL1A1 mRNA
p < 0.001
x-fold changes in Collagen protein
p < 0.001
x-fold changes in ATG7 mRNA
p < 0.001
x-fold changes in BECLIN1 mRNA
p < 0.001

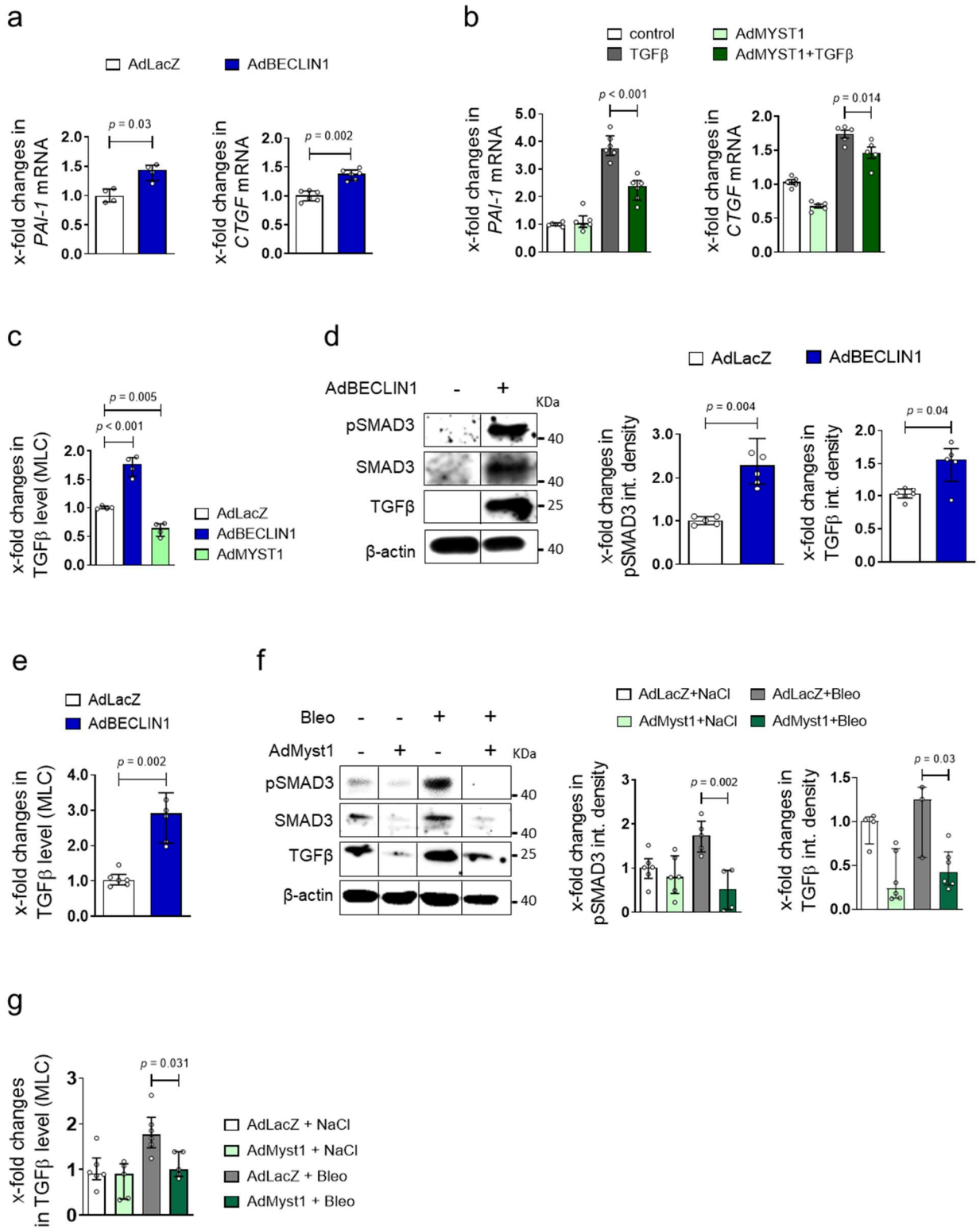
Supplementary Figure 7: TGFβ downregulates MYST1 in a SMAD3-dependent manner. A: Expression of MYST1 in human dermal fibroblasts stimulated with recombinant TGFβ. Representative immunofluorescence stainings of MYST1 (green) in combination with DAPI (blue) (400-fold magnification) and quantification of fluorescence intensity (n = 10 independent quantifications within 3 biological replicates per group). **B:** Levels of MYST1 upon adenoviral overexpression of TβRIact in murine lungs as analyzed by qRT-PCR (n = 8 biological replicates for control and n = 6 for TβRIact group) and immunofluorescence staining for MYST1 (green), vimentin (red) and DAPI (blue) at 400-fold magnification (n = 7 biological replicates for control and n = 6 for TβRIact group). **C:** Effect of TβRIact overexpression on MYST1 levels: mRNA (n = 17 biological replicates for control and n = 15 for TβRIact group) and immunofluorescence staining (n = 9 biological replicates for control and n = 8 for TβRIact group) for MYST1 in murine skin. **D-E:** Activity of the *MYST1* promoter (**D**) and mRNA (**E**) of *MYST1* in fibroblasts upon treatment with the SMAD3 inhibitor SIS3 (n = 4 biological replicates per group). **F-G: Bleomycin-induced pulmonary fibrosis:** mRNA levels of *Myst1* (**F**; n = 5 biological replicates for control and n = 4 for bleomycin group) and representative Immunofluorescence staining of MYST1 (green) in combination with DAPI (blue) and vimentin (red) at 400 fold magnification in murine lungs (**G**; n = 7 biological replicates per group). **H:** Knockdown of *MYST1* and overexpression of BECLIN1 in fibroblasts. mRNA levels of *COL1A1* (n = 6 biological replicates for groups overexpressing BECLIN1 and n = 5 for other groups) , *ATG7*, *BECLIN1* (n = 6 biological replicates per group) and secreted collagen protein (n = 4 biological replicates per group). All data are presented as median ± IQR. *p*-values were determined by two-sided Mann-Whitney test (**A**, **B**, **C, F, G**) or by ANOVA one-way with Tukey's multiple comparison post hoc test and are indicated in the figure (**D**, **E**, **H)**. See source data for more detailed information. Horizontal scale bars, 50 µm. fluo.: fluorescence, int.: intensity, rel.: relative, prom.: promoter.


a
RFP
GFP
Merge
Control
TGFβ
AdBECLIN1
AdBECLIN1 + TGFβ
Autophagosomes
Autolysosomes
Autophagosomes & Autolysosomes/Cell
p = 0.006
Control
TGFβ
Ad BECLIN1
Ad BECLIN1 +TGFβ
b
x-fold changes in COL1A1 mRNA
p = 0.001
p < 0.001
x-fold changes in collagen conc.
p = 0.03
p = 0.005
Control
TGFβ
CQ
CQ+TGFβ
x-fold changes in collagen rel. density
p < 0.001
p = 0.002
CQ
TGFβ
KDa
secreted collagen I
β-actin
130
40
c
BECLIN1
vimentin
DAPI
Merge
AdLacZ
AdBeclin1
x-fold changes in BECLIN1 fluo. int.
p < 0.001
ATG7
x-fold changes in ATG7 fluo. int.
p < 0.001
p62
x-fold changes in p62 fluo. int.
p < 0.001
d
AdLacZ
AdBeclin1
x-fold changes in dermal thickness
p = 0.03
x-fold changes in myofibroblasts counts
p = 0.03
x-fold changes in hydroxyproline conc.
p = 0.02

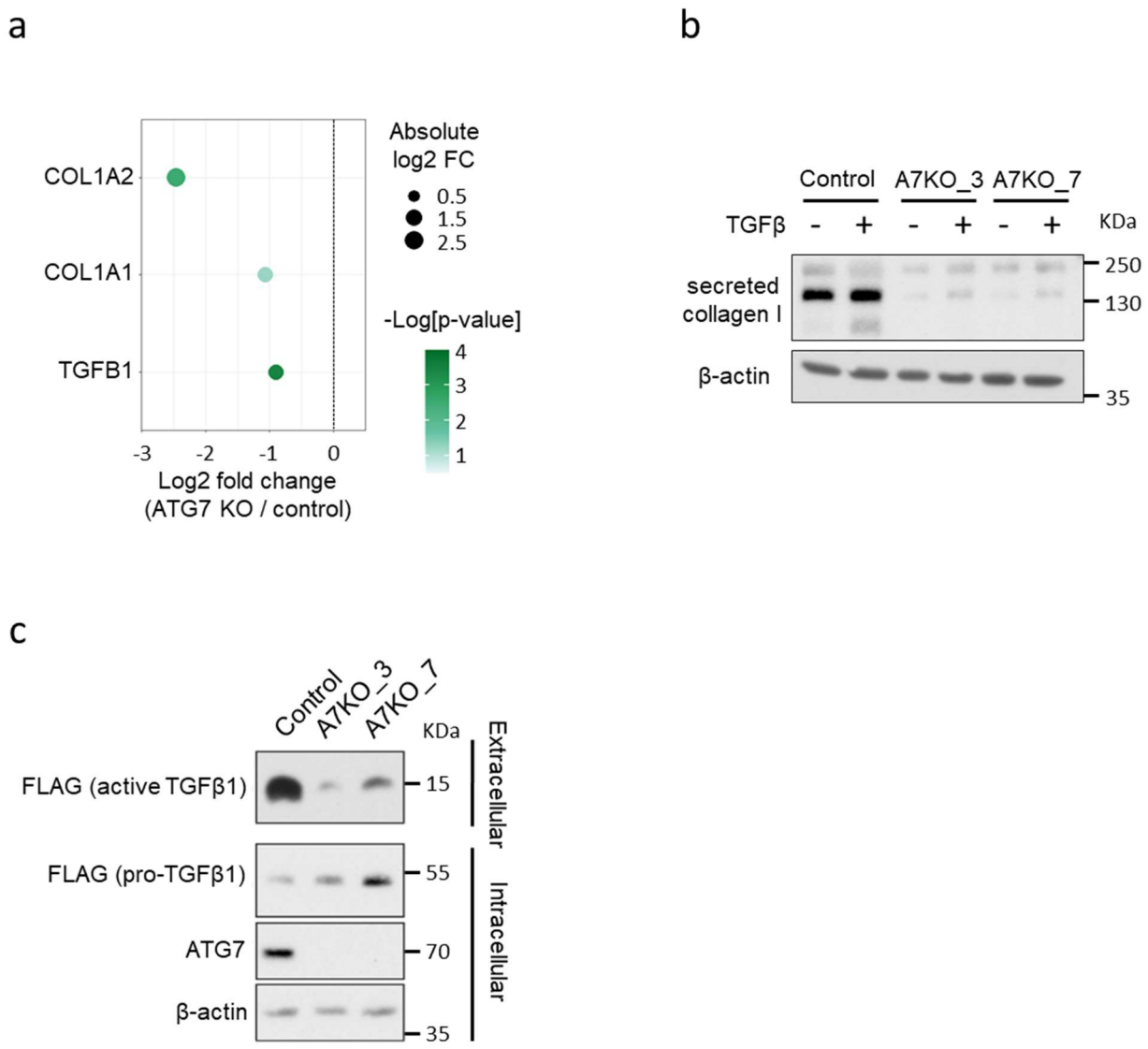
Supplementary Figure 8: Modulation of autophagy by overexpression of BECLIN1 or by the autophagy inhibitor chloroquine regulates fibroblast activation and tissue fibrosis. A: Effects of TGFβ and overexpression of BECLIN1 on EGFP/RFP-LC3 reporter activity; representative images and quantification (n = 5 biological replicates per group). Horizontal scale bars, 5 µm. **B:** Regulation of fibroblast activation by chloroquine (CQ): mRNA levels of *COL1A1*, levels of total collagen and levels of secreted type I collagen (n = 4 biological replicates per group) as analyzed by qRT-PCR, hydroxyproline assay and Western blot, respectively. Horizontal scale bar, 5 µm. **C-D: Adenoviral overexpression of BECLIN1 in murine skin. C:** Representative immunofluorescence staining of markers of autophagy BECLIN1, ATG7 or p62 (all green) in combination with DAPI (blue) and vimentin (red) at 400-fold magnification (n = 7 biological replicates per group). **D:** Representative trichrome stainings, dermal thickening, myofibroblast counts (n = 4 biological replicates per group) and hydroxyproline content (n = 4 biological replicates for control and n = 5 for Adbeclin1 group). Horizontal scale bars, 100 µm. Western blot samples in panel **B** were run on the same gel. Images were cropped at the lines only for the purpose of this figure. All data are presented as median ± IQR. *p*-values were determined by two-sided Mann-Whitney test (**C, D**) or by ANOVA one-way with Tukey's multiple comparison post hoc test (**A**, **B**) and are indicated in the figure. See source data for more detailed information. conc.: concentration, rel.: relative, fluo.: fluorescence, int.: intensity, CQ: chloroquine. The results in panel A are part of a large set of experiments and share the controls with figure 4c. Data are presented separately just for the purpose of this figure.



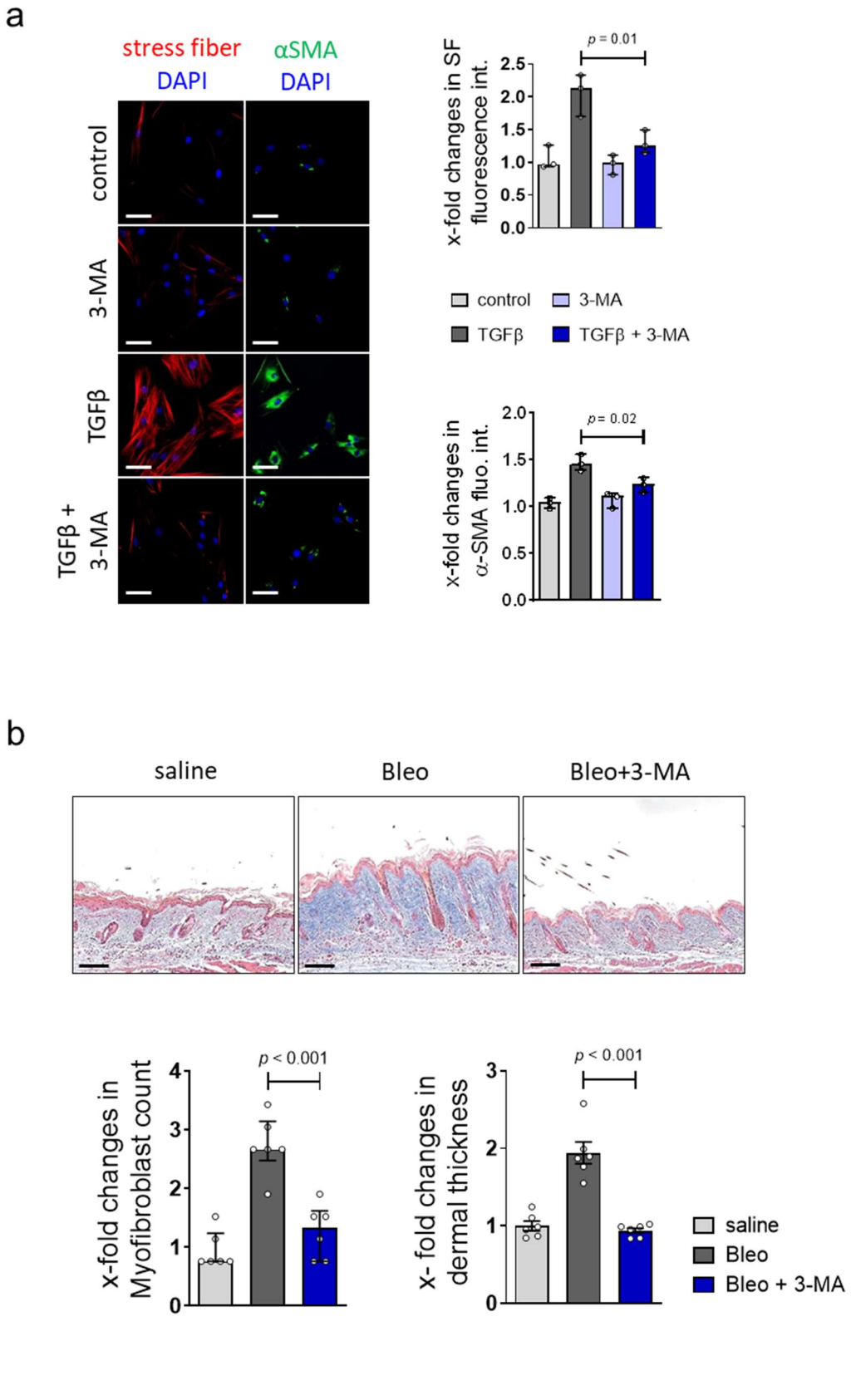
Supplementary Figure 9: Autophagy-dependent regulation of extracellular matrix deposition. A: Extracellular matrix fluorescence staining of human fibroblasts overexpressing BECLIN. Representative immunofluorescence staining of fibronectin (green), collagen I (red) and collagen III (magenta; n = 4 biological replicates per group) and respective quantifications. **B**: Extracellular matrix staining of human fibroblats treated with the autophagy inhibitor 3-methyladenine (3-MA). Representative images and quantifications of deposited fibronectin (green), collagen I (red) and collagen III (magenta; n = 4 biological replicates per group). Horizontal scale bars, 50 µm. All data are presented as median ± IQR. *p*-values were determined by ANOVA one-way with Tukey's multiple comparison post hoc test (**A**, **B**) and are indicated in the figure. See source data for more detailed information. Ad: Adenovirus, Fluo.: fluorescence, Int.: Intensity, Col I: collagen I, Col III: collagen III.


a
AdLacZ
AdBECLIN1
x-fold changes in PAI-1 mRNA
p = 0.03
x-fold changes in CTGF mRNA
p = 0.002
b
control
TGFβ
AdMYST1
AdMYST1+TGFβ
x-fold changes in PAI-1 mRNA
p < 0.001
x-fold changes in CTGF mRNA
p = 0.014
c
x-fold changes in TGFβ level (MLC)
p = 0.005
p < 0.001
AdLacZ
AdBECLIN1
AdMYST1
d
AdBECLIN1 - +
KDa
pSMAD3
SMAD3
TGFβ
β-actin
40
40
25
40
AdLacZ
AdBECLIN1
x-fold changes in pSMAD3 int. density
p = 0.004
x-fold changes in TGFβ int. density
p = 0.04
e
AdLacZ
AdBECLIN1
x-fold changes in TGFβ level (MLC)
p = 0.002
f
Bleo - - + +
AdMyst1 - + - +
KDa
pSMAD3
SMAD3
TGFβ
β-actin
40
40
25
40
AdLacZ+NaCl
AdLacZ+Bleo
AdMyst1+NaCl
AdMyst1+Bleo
x-fold changes in pSMAD3 int. density
p = 0.002
x-fold changes in TGFβ int. density
p = 0.03
g
x-fold changes in TGFβ level (MLC)
p = 0.031
AdLacZ + NaCl
AdMyst1 + NaCl
AdLacZ + Bleo
AdMyst1 + Bleo

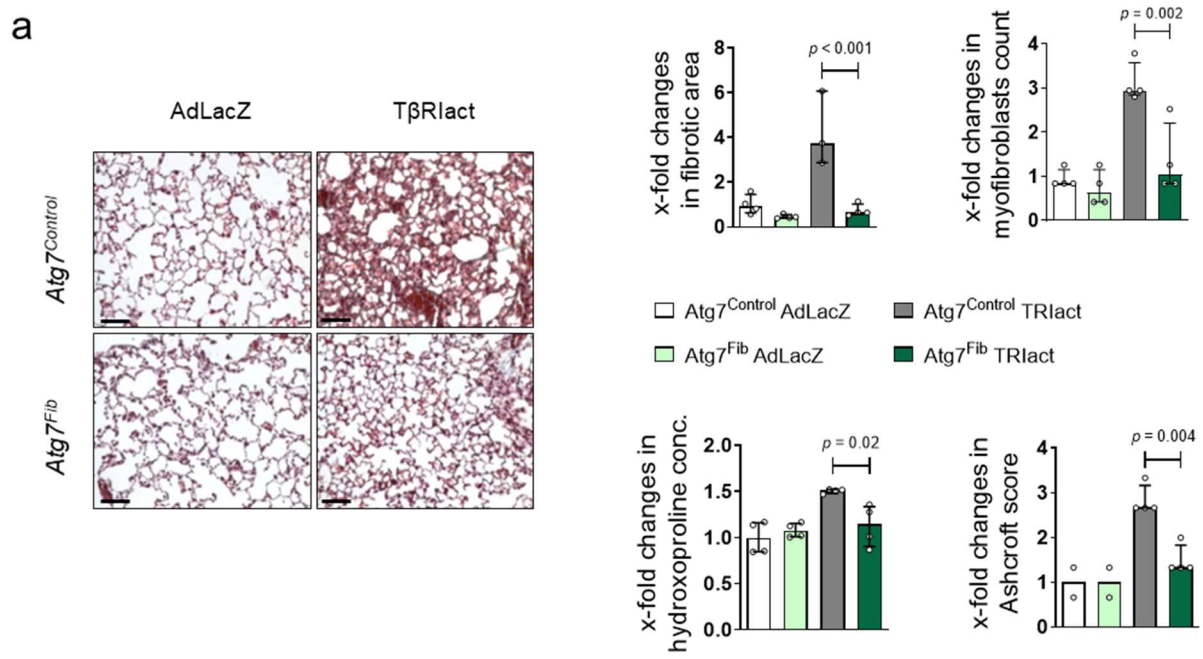
Supplementary Figure 10: Autophagy and MYST1 regulate TGFβ / SMAD signaling. A-C Human dermal fibroblasts: Changes in the mRNA levels of the prototypical TGFβ / SMAD target genes *PAI-1* (n = 4 biological replicates per group) and *CTGF* (n = 6 biological replicates per group) induced by overexpression of BECLIN1 in cultured human dermal fibroblasts. **B:** Effects of MYST1 overexpression on *PAI-1* (n = 6 biological replicates) and *CTGF*, (n = 5 biological replicates per group) mRNA levels in fibroblasts stimulated with TGFβ. **C:** Changes in the levels of active TGFβ released from human dermal fibroblasts upon overexpression of BECLIN1 or MYST1, respectively, as measured by mink lung cell assays (n = 4 biological replicates per group). **D-G: Murine skin:** Levels of pSMAD3 (n = 5 biological replicates for control and n = 6 for AdBECLIN1 group), of total TGFβ (**D**; n = 6 biological replicates for control and n = 5 for AdBECLIN1 group) and of active TGFβ in murine skin (**E**; n = 6 biological replicates per group) upon overexpression of BECLIN1. Effects of the overexpresssion of MYST1 on bleomycin-induced accumulation of pSMAD3 (n = 5 biological replicates for bleomycin, n = 4 for AdMyst1+Bleo group and n = 6 for other groups) of total TGFβ (**F;** n = 4 biological replicates for control, n = 3 for bleomycin group and n = 6 for other groups), and of active TGFβ (**G**) in mice challenged with bleomycin (n = 5 biological replicates for groups overexpressing MYST1 and n = 6 for other groups). Western blot samples in panel **D** and **F** were run on the same gel. Images were cropped at the lines only for the purpose of this figure. All data are presented as median ± IQR . *p*-values were determined two-sided Mann-Whitney test (**A, D, E**) or by ANOVA one-way with Tukey's multiple comparison post hoc test (**B, C, F, G**) and are indicated in the figure. See source data for more detailed information. Ad: Adenovirus, rel.: relative, Bleo: bleomycin, Int.: Intensity.



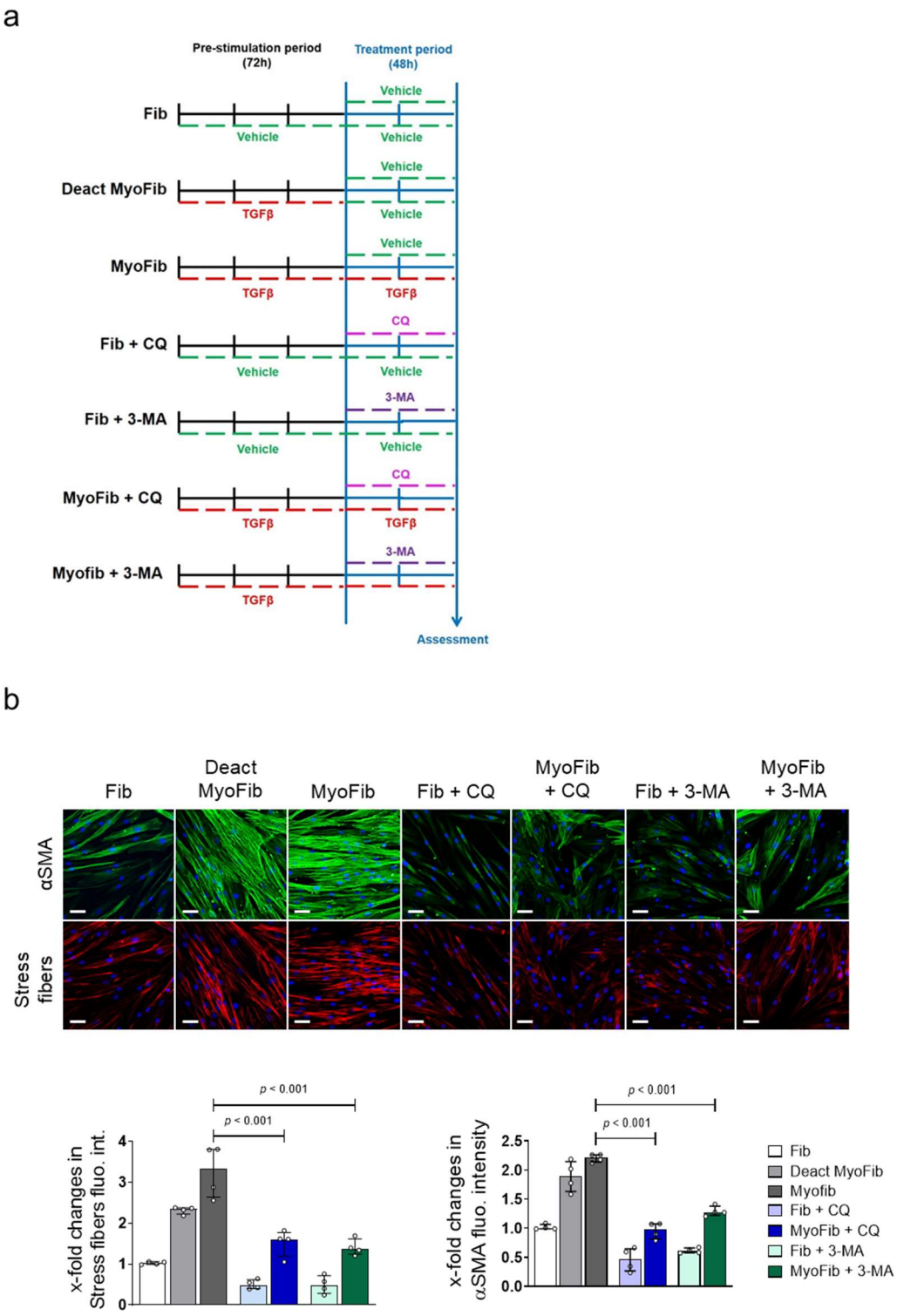
Supplementary Figure 11: *ATG7* stable knockout in human WI26/SV-40 fibroblasts ameliorates collagen release. A: Analysis of extracellular levels of COL1A1, COL1A2, and TGFβ1 using SILAC-based quantitative proteomics. COL1A2: -log [*p*-value] = 2,453; COL1A1: -log [*p*-value] = 1,233; TGFβ1: -log [*p*-value] = 3,458 (n = 4 biological replicates). **B**: Representative Western blot images of secreted collagen type I in *ATG7* knockout Wi-26 cell lines (n = 2 biological replicates). **C**: Representative Western blot images of intra- and extracellular TGFβ levels in *ATG7* knockout fibroblasts transfected with FLAG-(5A) TGFβ1 construct lines (n = 2 biological replicates). The SILAC data are presented as log2 fold changes (log2 FC) in *ATG7* knockout compared to control cells. The -log [*p*-values] were determined by one-sample t-test (Value = 0, S0 = 0.1, side = both). Control: Cells transfected with empty CRISPR/Cas9 vector, A7KO_3 and A7KO_7: *ATG7* knockout clones 3 and 7, FC: fold change. See source data for more detailed information.


a
stress fiber
DAPI
αSMA
DAPI
control
3-MA
TGFβ
TGFβ +
3-MA
x-fold changes in SF
fluorescence int.
p = 0.01
control
3-MA
TGFβ
TGFβ + 3-MA
x-fold changes in
α-SMA fluo. int.
p = 0.02
b
saline
Bleo
Bleo+3-MA
x-fold changes in
Myofibroblast count
p < 0.001
x- fold changes in
dermal thickness
p < 0.001
saline
Bleo
Bleo + 3-MA

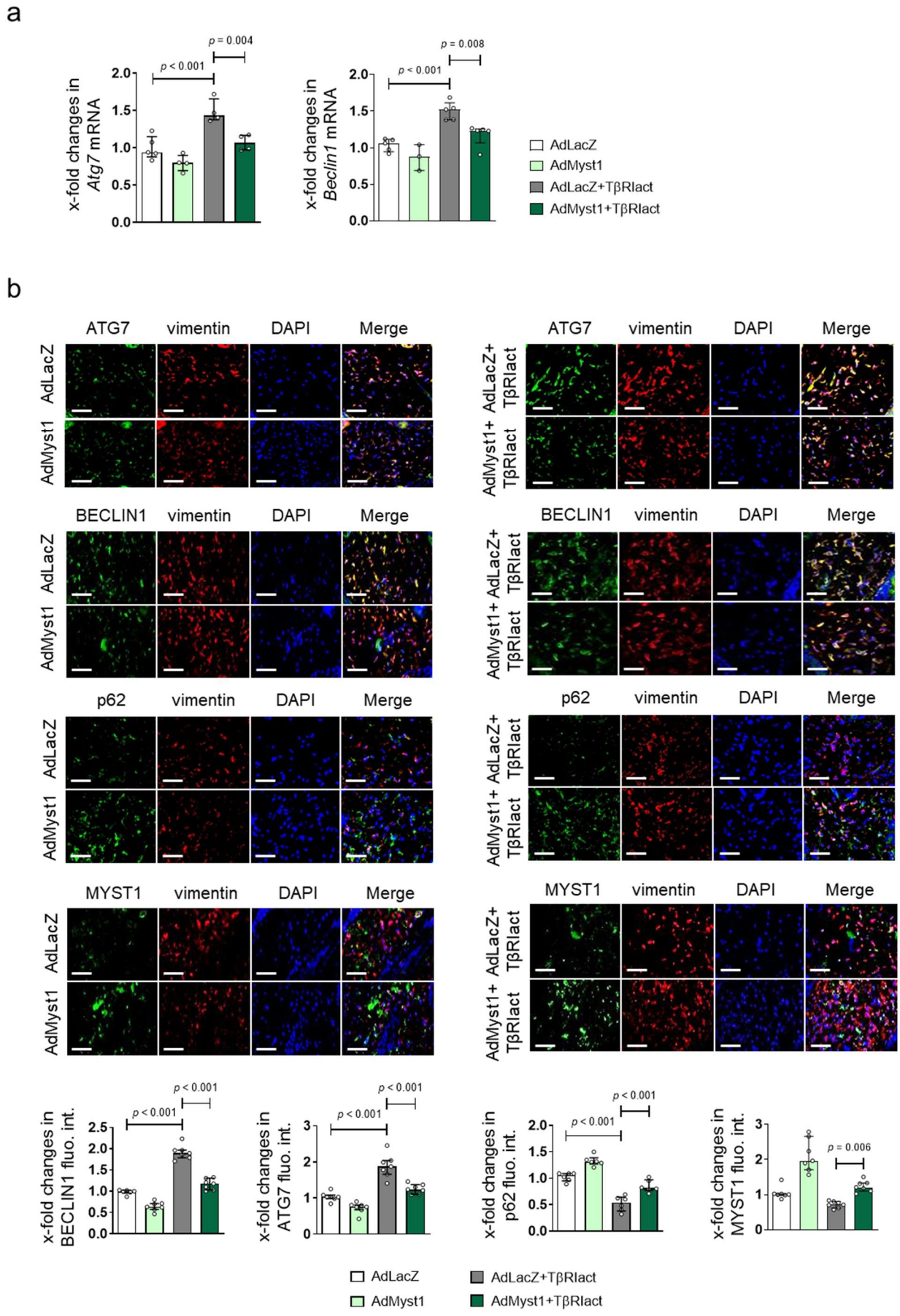
Supplementary Figure 12: Autophagy inhibition by 3-MA reduces myofibroblast differentiation and ameliorates bleomycin-induced skin fibrosis. A: Cultured human fibroblasts. Fibroblasts treated with with 3-methyladenine (3-MA) in the presence or absence of TGFβ. Representative images and quantifications of stress fibers (red) and αSMA (green; n = 3 biological replicates per group) costained with DAPI (blue). Horizontal scale bars, 50 µm. **B: Bleomycin-induced skin fibrosis**. Representative trichrome stainings, myofibroblast counts and dermal thickening (n = 6 biological replicates per group). Horizontal scale bars, 100 µm. All data are presented as median ± IQR. *p*-values were determined by ANOVA one-way with Tukey's multiple comparison post hoc test and are indicated in the figure. See source data for more detailed information. Bleo: bleomycin, int.: intensity, SF: stress fibers.



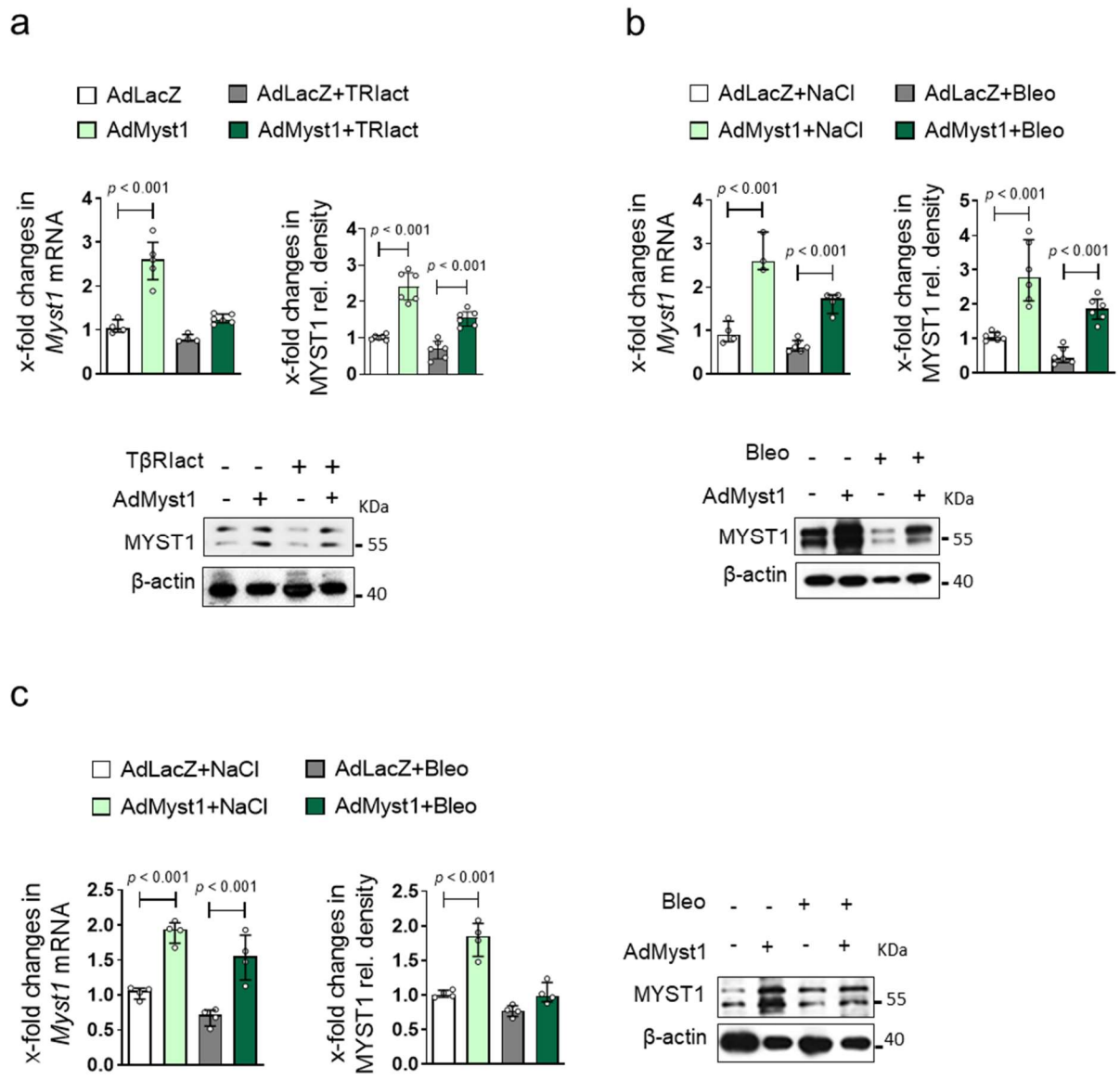
Supplementary Figure 13: Fibroblast-specific knockout of ATG7 ameliorates TβRIact-induced pulmonary fibrosis. Representative trichrome stainings shown, fibrotic area (n = 3 biological replicates for $Atg7^{Control}$ TβRIact, n = 4 for the other groups), myofibroblast counts (n= 4 biological replicates per group), hydroxyproline content (n = 4 biological replicates per group) and Ashcroft scores (n = 2 biological replicates for $Atg7^{Control}$ AdLacZ and $Atg7^{Fib}$ AdLacZ and n = 4 for other groups). Horizontal scale bar, 100 µm. All data are presented as median ± IQR. *p*-values were determined by ANOVA one-way with Tukey's multiple comparison post hoc test and are indicated in the figure. See source data for more detailed information. Ad: Adenovirus, TβRIact: constitutively active TGFβ receptor type I, conc.: concentration. *Atg7*^{control}: littermate control mice *Atg7*^{fib}: fibroblast-specific *Atg7* knockout mice.



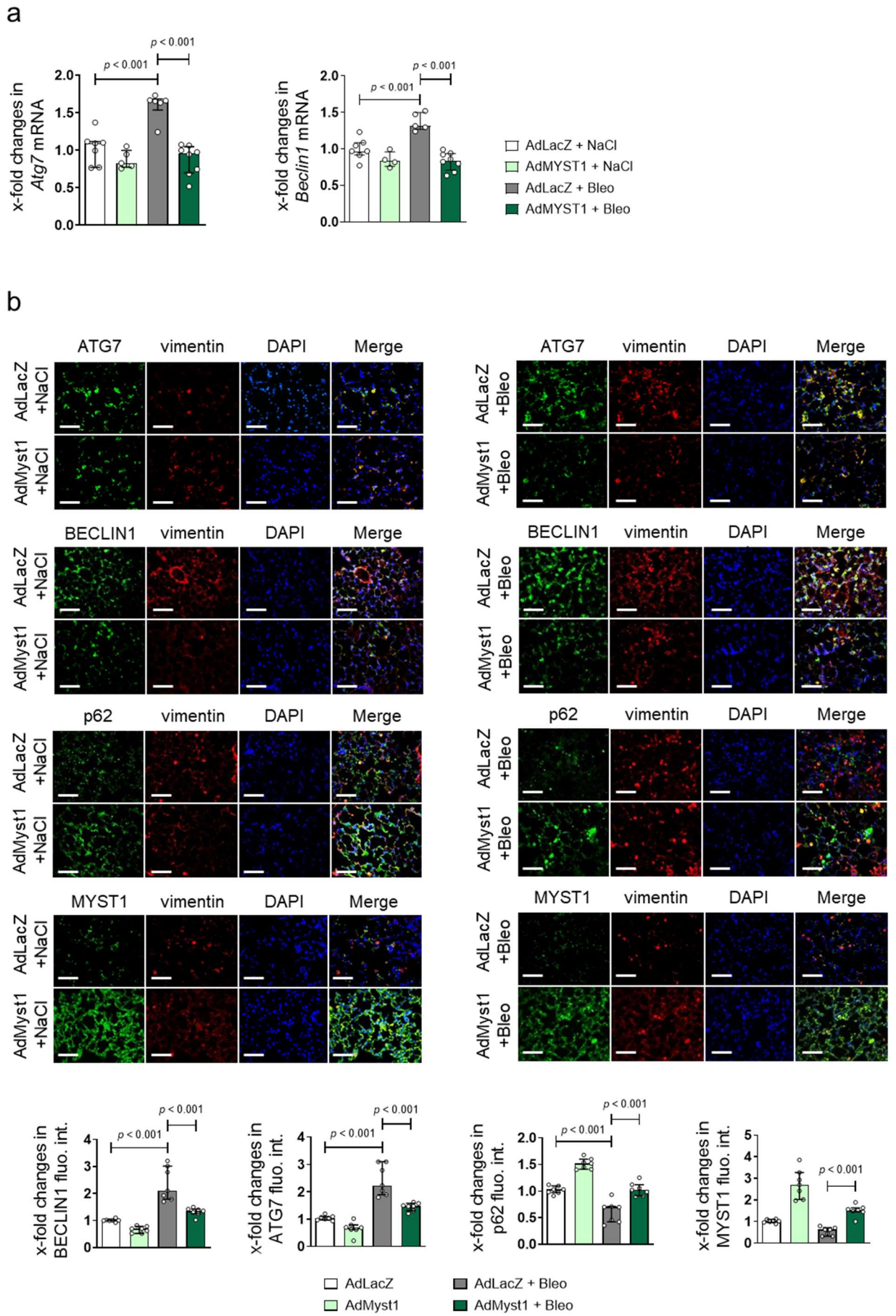
Supplementary Figure 14: Inhibition of autophagy can induce re-differentiation of myofibroblasts into fibroblasts. A: Schematic illustration of the experimental procedure. **B:** Representative images and quantification of αSMA (green) and stress fibers (red; n = 4 biological replicates) costained with DAPI (blue). Horizontal scale bars, 30 µm. All data are presented as median ± IQR. *p*-values were determined by ANOVA one-way with Tukey's multiple comparison post hoc test and are indicated in the figure. See source data for more detailed information. Fluo.: fluorescence, Fib: fibroblast, MyoFib: myofibroblast, deact MyoFib: deactivated myofibroblast, CQ: chloroquine.


a
x-fold changes in Atg7 mRNA
p = 0.004
p < 0.001
x-fold changes in Beclin1 mRNA
p = 0.008
p < 0.001
AdLacZ
AdMyst1
AdLacZ+TβRIact
AdMyst1+TβRIact
b
ATG7
vimentin
DAPI
Merge
AdLacZ
AdMyst1
AdLacZ+ TβRIact
AdMyst1+ TβRIact
BECLIN1
p62
MYST1
x-fold changes in BECLIN1 fluo. int.
x-fold changes in ATG7 fluo. int.
x-fold changes in p62 fluo. int.
x-fold changes in MYST1 fluo. int.
p = 0.006

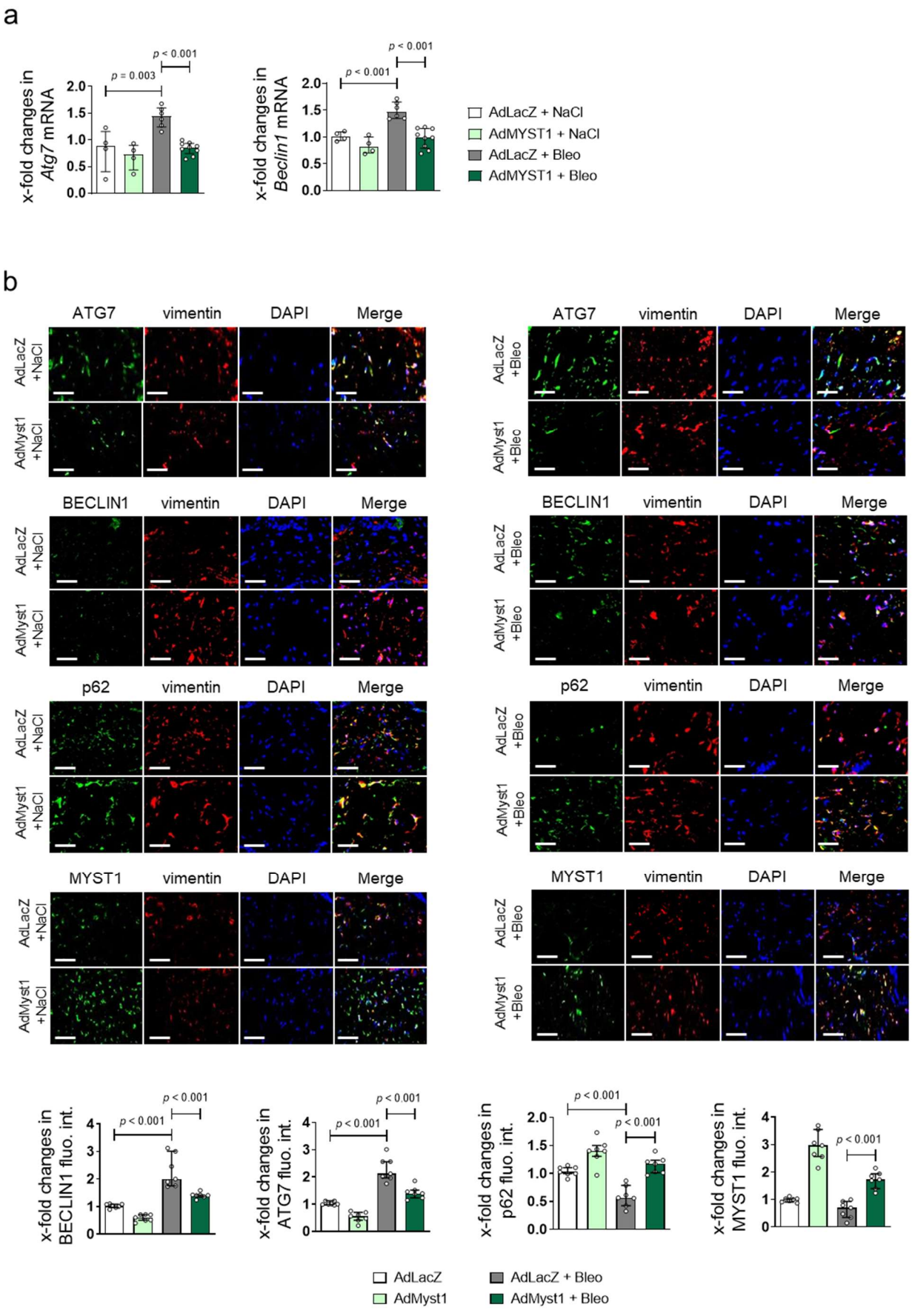
Supplementary Figure 15: Overexpression of MYST1 prevents the activation of autophagy upon overexpression of TβRIact in murine skin. A: mRNA levels of *Atg7* (n = 5 biological replicates for AdLacZ group and n = 4 for other groups) and *Beclin1* (n = 3 biological replicates for AdMyst1 group and n = 5 for other groups). **B:** Representative immunofluorescence staining for ATG7 (n = 7 biological replicates), BECLIN1 (n = 6 biological replicates for control and n = 7 for other groups), p62 (n = 6 biological replicates per group) and MYST1 (all green; n = 7 biological replicates per group) in combination with DAPI (blue) and vimentin (red) and respective quantification. Horizontal scale bar, 50 µm. All data are presented as median ± IQR. *p*-values were determined by ANOVA one-way with Tukey's multiple comparison post hoc test and are indicated in the figure. See source data for more detailed information. Ad: adenovirus, TβRIact: constitutively active TGFβ receptor type I, rel.: relative, fluo.: fluorescence, int.: intensity.



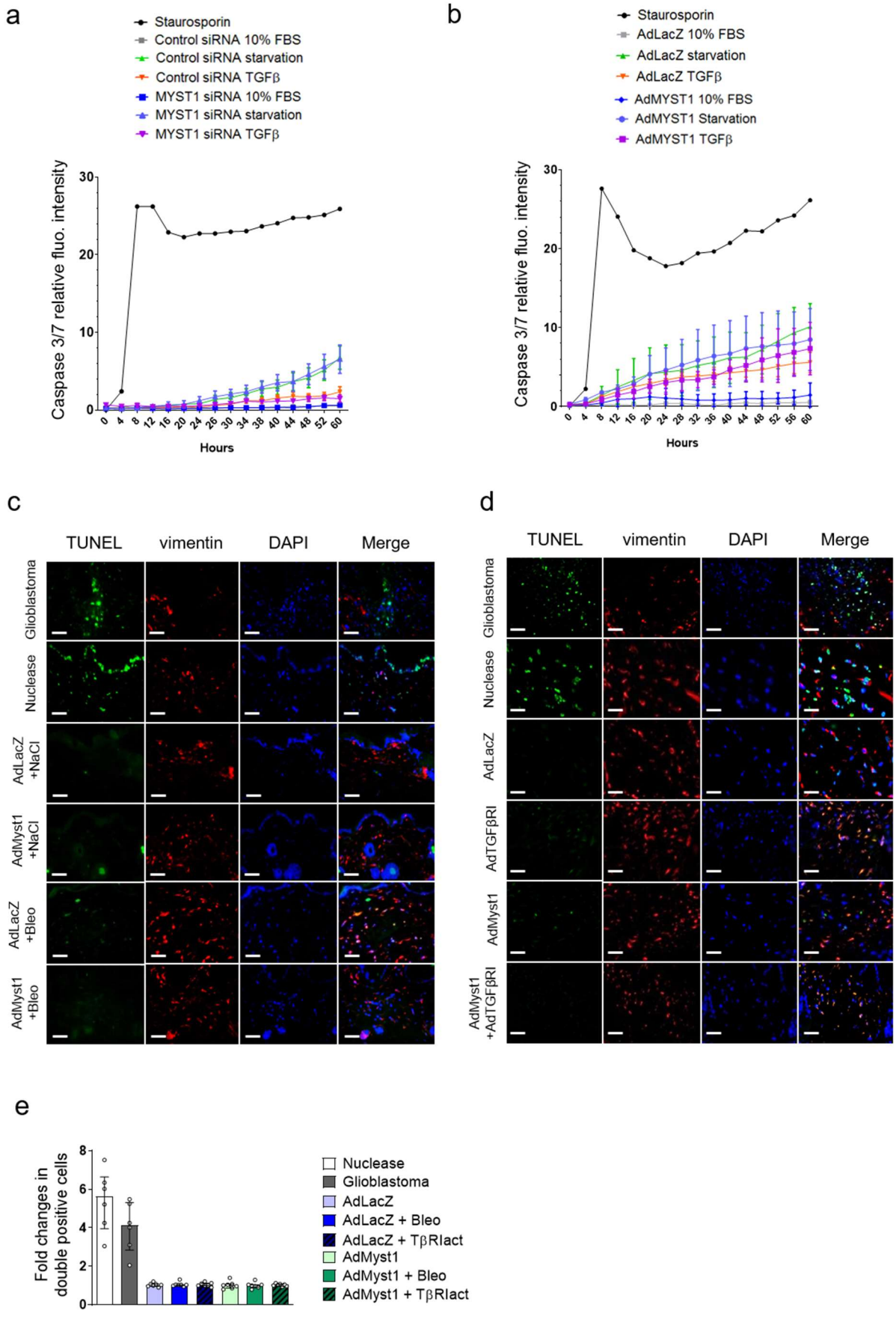
Supplementary Figure 16: Adenoviral overexpression of MYST1 in murine models of fibrosis. A: TβRIact-induced skin fibrosis: mRNA (n = 5 biological replicates for AdMyst1 group, n = 6 for TβRIact group overexpressing MYST1 and n = 4 for other groups) and protein levels of MYST1 (n = 6 biological replicates). **B: Bleomycin-induced pulmonary fibrosis**: mRNA (n = 3 biological replicates for AdMyst1 group, n = 6 for bleomycin group and n = 4 for other groups) and protein levels of MYST1 (n = 6 biological replicates). **C: Bleomycin-induced dermal fibrosis**: mRNA and protein levels of MYST1 (n = 4 biological replicates). All data are presented as median ± IQR. *p*-values were determined by ANOVA one-way with Tukey's multiple comparison post hoc test and are indicated in the figure. See source data for more detailed information. Ad: adenovirus, TβRIact: constitutively active TGFβ receptor type I, rel.: relative, Bleo: bleomycin.


a
x-fold changes in Atg7 mRNA
x-fold changes in Beclin1 mRNA
p < 0.001
AdLacZ + NaCl
AdMYST1 + NaCl
AdLacZ + Bleo
AdMYST1 + Bleo
b
ATG7
vimentin
DAPI
Merge
BECLIN1
p62
MYST1
AdLacZ +NaCl
AdMyst1 +NaCl
AdLacZ +Bleo
AdMyst1 +Bleo
x-fold changes in BECLIN1 fluo. int.
x-fold changes in ATG7 fluo. int.
x-fold changes in p62 fluo. int.
x-fold changes in MYST1 fluo. int.
AdLacZ
AdMyst1
AdLacZ + Bleo
AdMyst1 + Bleo

Supplementary Figure 17: Overexpression of MYST1 prevents the activation of autophagy in bleomycin-induced pulmonary fibrosis. A: mRNA levels of *Atg7* (n = 7 biological replicates for control, n = 5 for AdMyst1, n = 6 for bleomycin and n = 8 for AdMyst1 + bleomycin group) and *Beclin1* (n = 7 biological replicates for control, n = 4 for AdMyst1 group, n = 5 for bleomycin group and n = 8 for AdMyst1 + bleomycin group). **B:** Representative immunofluorescence staining for ATG7, BECLIN1, p62 and MYST1 (all green) in combination with DAPI (blue) and vimentin (red) and respective quantification (n = 7 biological replicates per group for all readouts). Horizontal scale bar, 50 µm. All data are presented as median ± IQR. *p*-values were determined by ANOVA one-way with Tukey's multiple comparison post hoc test and are indicated in the figure. See source data for more detailed information. Ad: adenovirus, Bleo: bleomycin, rel.: relative, fluo.: fluorescence, int.: intensity.


a
x-fold changes in Atg7 mRNA
p = 0.003
p < 0.001
x-fold changes in Beclin1 mRNA
p < 0.001
p < 0.001
AdLacZ + NaCl
AdMYST1 + NaCl
AdLacZ + Bleo
AdMYST1 + Bleo
b
ATG7
vimentin
DAPI
Merge
BECLIN1
p62
MYST1
AdLacZ +NaCl
AdMyst1 +NaCl
AdLacZ +Bleo
AdMyst1 +Bleo
x-fold changes in BECLIN1 fluo. int.
x-fold changes in ATG7 fluo. int.
x-fold changes in p62 fluo. int.
x-fold changes in MYST1 fluo. int.
p < 0.001
AdLacZ
AdMyst1
AdLacZ + Bleo
AdMyst1 + Bleo

Supplementary Figure 18: Overexpression of MYST1 prevents the activation of autophagy in bleomycin-induced dermal fibrosis. A: mRNA levels of *Atg7* and *Beclin1* (n = 4 biological replicates for NaCl treated groups, n = 6 for bleomycin and n = 9 for AdMyst1 + bleomycin). **B:** Representative immunofluorescence staining for ATG7, BECLIN1, and p62 (all green) in combination with DAPI (blue) and vimentin (red) and respective quantification (n = 7 biological replicates per group for all readouts). Horizontal scale bar, 50 µm. All data are presented as median ± IQR. *p*-values were determined by ANOVA one-way with Tukey's multiple comparison post hoc test and are indicated in the figure. See source data for more detailed information. Ad: adenovirus, Bleo: bleomycin, rel.: relative, fluo.: fluorescence, int.: intensity.


a
Staurosporin
Control siRNA 10% FBS
Control siRNA starvation
Control siRNA TGFβ
MYST1 siRNA 10% FBS
MYST1 siRNA starvation
MYST1 siRNA TGFβ
Caspase 3/7 relative fluo. intensity
Hours
b
Staurosporin
AdLacZ 10% FBS
AdLacZ starvation
AdLacZ TGFβ
AdMYST1 10% FBS
AdMYST1 Starvation
AdMYST1 TGFβ
Caspase 3/7 relative fluo. intensity
Hours
c
TUNEL
vimentin
DAPI
Merge
Glioblastoma
Nuclease
AdLacZ +NaCl
AdMyst1 +NaCl
AdLacZ +Bleo
AdMyst1 +Bleo
d
TUNEL
vimentin
DAPI
Merge
Glioblastoma
Nuclease
AdLacZ
AdTGFβRI
AdMyst1
AdMyst1 +AdTGFβRI
e
Fold changes in double positive cells
Nuclease
Glioblastoma
AdLacZ
AdLacZ + Bleo
AdLacZ + TβRIact
AdMyst1
AdMyst1 + Bleo
AdMyst1 + TβRIact

Supplementary Figure 19: Modulation of MYST1 expression does not induce apoptosis in fibroblasts. A-B: Quantification of the activity of Caspases 3 and 7 in fibroblasts with siRNA-mediated knockdown (**A**) or overexpression of MYST1 (**B;** n = 2 biological replicates for staurosporin group and n = 4 for other groups, for all readouts) **C-E:** TUNEL staining of skin sections from mice with or without overexpression of MYST1. **C:** Representative images of sections of bleomycin-induced skin fibrosis costained with vimentin and DAPI. **D:** Representative images of sections of TβRIact-induced skin fibrosis costained with vimentin and DAPI. Horizontal scale bars, 50 μm. **E:** Quantification of TUNEL-positive apoptotic fibroblast (n = 6 biological replicates for nuclease and glioblastoma group and n = 7 for other groups). Data are presented as mean or median ± IQR. See source data for more detailed information. Ad: adenovirus, Bleo: bleomycin, TβRIact: constitutively active TGFβ receptor type I, fluo: fluorescence.

Supplementary Table 1: List of primers used for the mutagenesis of SBEs in MYST1 promoter. Fwd: forward primer.

Primers	**Sequence reaction 5'-3'**
SBE1 fwd	GGATCACGAGGTCAGAAGATCGGCGTTATTCTGGCTAACACGATGAAACTC
SBE2 fwd	CCCGGCCTTTTTATTTTTTTTGAGATGGAACGCGGCTCTTGGTCCCCAGGC
SBE3 fwd	CGGTGTTAGCCAGGATGACGCCGATCTCCGCCCGCCTC

Supplementary Table 2: Primers for qRT-PCR.

Human primers	**Forward reaction 5'-3'**	**Reverse reaction 5'-3'**
ATG7	ATTGCTGCATCAAGAAACCC	GATGGAGAGCTCCTCAGCA
BECLIN1	TGGTAGTTCTGGAGGCCT	AGGCACTGTGGCCTCGGG
β-ACTIN	AGAAAATCTGGCACCACACC	TAGCACAGCCTGGATAGCAA
COL1A1	ACGAAGACATCCCACCAATC	ATGGTACCTGAGGCCGTTC
MYST1	ACCTCAAAAGTGCCCAGTATAAGA	GACGGAGTCCACTGTGATGG
CTGF	AACTCACACAACAACTCTTCCCCGC	GAGTCGCACTGGCTGTCTCCTCT
PAI-1	TCATTGCTGCCCCTTATGA	GTTGGTGAGGGCAGAGAGAG
P62	TGCCCAGACTACGACTTGTG	AGTGTCCGTGTTTCACCTTCC
Mouse primers	**Forward reaction 5'-3'**	**Reverse reaction 5'-3'**
Atg7	TGCCTATGATGATCTGTGTC	CACCAACTGTTATCTTTGTCC

Beclin1	GGCCAATAAGATGGGTCTGA	GCTGCACACAGTCCAGAAAA
β-actin	TCTTTGATGTCACGCACGAT	TACAGCTTCACCACCACA
Col1a1	GAAGCACGTCTGGTTTGGA	ACTCGAACGGGAATCCATC
Myst1	CGGCCGGATAGCACCTG	TTCACTCGAGACTGGATCACTTC
P62	GCTGCCCTATACCCACATCT	CGCCTTCATCCGAGAAAC

Supplementary Table 3: List of antibodies and dyes used in this study**.** WB: Western blot, IF: Immunofluorescence staining, ECM: Extracellular Matrix staining, ChIP: Chromatin Immunoprecipitation, Cat.: Catalog number. N/A: not applicable.

Reagent type/ Aplication	**Species**	**Designation**	**Source** (Catalog number, clone name, lot number)	**Dilution / Application**
Primary antibody	monoclonal mouse	αSMA (Alpha-smooth muscle)	Sigma-Aldrich / Cat. A5228, clone 1A4 or A2547 / clone 1A4	both 1:1000 IF
Primary antibody	monoclonal mouse	β-actin	Sigma-Aldrich / Cat. A5441 / clone AC-15	1:10000 WB
Primary antibody	polyclonal rabbit	ATG7	Abcam / Cat. ab133528 / clone EPR6251	1:1000 WB / 1:200 IF
Primary antibody	polyclonal rabbit	ATG7	AnaSpec / Cat. AS-54230	1:1000 WB / 1:200 IF
Primary antibody	polyclonal rabbit	BECLIN1	Abcam / Cat. ab62557	1:1000 WB / 1:200 IF
Primary antibody	polyclonal rabbit	Collagen type I (Col I)	Abcam / ab138492 / clone EPR7785	1:1000 WB
Primary antibody	polyclonal goat	Collagen type I (Col I)	Southern Biotech / Cat. 1310-01	1:2000 WB
Primary antibody	polyclonal rabbit	Collagen type I (Col I)	Merck Millipore / Cat. #AB745	1:100 ECM
Primary antibody	polyclonal rabbit	Collagen type III (Col III)	Merck Millipore / Cat. #AB747	1:100 ECM

Primary antibody	monoclonal mouse	anti- Fibronectin antibody conjugated with Alexa Fluor 488	eBiosciences / Cat. #53-9869-82 / clone FN-3	1:400 ECM
Primary antibody	polyclonal rabbit	FLAG tag	Proteintech / Cat. 80010-1-RR / clone 4K14	1:5000 WB
Primary antibody	polyclonal goat	GFP	Abcam / Cat. ab6673	1:200 IF
Primary antibody	polyclonal rabbit	Histone H3	Cell Signaling / Cat. #9715	1:1000 WB
Primary antibody	polyclonal rabbit	H4K16ac (acetyl-Histone H4 (Lys16))	Merck Millipore / Cat. #07-329/ Lot # 2506422	1:1000 WB
Primary antibody	monoclonal rat	LAMP2	Abcam /Cat. ab13524 / clone GL2A7	1:200 IF
Primary antibody	polyclonal goat	LAP (pro-domain of TGFβ1)	R&D systems / Cat. AF-246-NA	1:2000 WB
Primary antibody	polyclonal rabbit	LC3B / MAP1LC3B	Novus biologicals / Cat. NB 100-2220	1:1000 WB / 1:200 IF
Primary antibody	monoclonal mouse	MYST1 / MOF	Genetex / Cat. GTX83065 / clone 8C4C4	1: 1000 WB / 1:200 IF
Primary antibody	monoclonal mouse	MYST1 / MOF	Santa cruz biotechnology / Cat. Sc-271691	1: 1000 WB / 1:200 IF
Primary antibody	monoclonal rabbit	prolyl-4-hydroxylase-β (P4Hβ)	Acris antibodies / TA308403	1:200 IF
Primary antibody	monoclonal rabbit	pSMAD3 (SMAD3 phospho S423 + S425)	Abcam/ Cat. ab52903 / clone EP823Y	1:1000 WB
Primary antibody	monoclonal rabbit	RFP	Abcam / Cat. ab62341	1:200 IF
Primary antibody	polyclonal rabbit	SMAD3	Cell signaling / Cat. #9523S / clone C67H9	1:1000 WB / 1:50 ChIP
Primary antibody	monoclonal mouse	SQSTM1/p62	Abcam / Cat. ab56416	1:2000 WB / 1:200 IF

Primary antibody	polyclonal rabbit	TGFβ	Cell signaling / Cat. #3711S	1:1000 WB
Primary antibody	monoclonal rabbit	Vimentin	Abcam / Cat. ab92547 / clone EPR3776	1: 200 IF
IgG control	Rabbit	Normal rabbit IgG	Santa Cruz Biotechnology / sc-2027	IF and ChIP
IgG control	Mouse	Normal mouse IgG	Santa cruz biotechnology / sc-2025	IF
Secondary antibody	Polyclonal Goat Anti-Mouse	HRP-conjugated	Dako / Cat. P044701-2	1:5000 WB / 1:500 IHC
Secondary antibody	Polyclonal Goat Anti-Rabbit	HRP-conjugated	Dako / Cat. P044801-2	1:5000 WB
Secondary antibody	Polyclonal Rabbit Anti-Goat	HRP-conjugated	Dako / Cat. P044901-2	1:5000 WB
Secondary antibody	Goat anti-Rabbit IgG	Alexa fluor 488	Invitrogen / Cat. A-11008	1:200 IF / 1:250 ECM
Secondary antibody	Goat anti-Mouse IgG	Alexa fluor 488	Invitrogen / Cat. A-11001	1:200 IF
Secondary antibody	Goat anti-Rat IgG	Alexa fluor 488	Invitrogen / Cat. A-11006	1:200 IF
Secondary antibody	Donkey anti-Goat	Alexa fluor 488	Invitrogen / Cat. AA32814	1:200 IF
Secondary antibody	Goat anti-Mouse IgG	Alexa fluor 555	Invitrogen / Cat. A-21422	1:200 IF
Secondary antibody	Goat anti-Rabbit IgG	Alexa fluor 555	Invitrogen / Cat. A-21428	1:200 IF
Secondary antibody	Goat anti-Rat IgG	Alexa fluor 555	Invitrogen / Cat. A-21434	1:200 IF
Secondary antibody	Goat Anti-Rabbit	Alexa fluor 647	Invitrogen / Cat. #A21244	1:200 IF / 1:250 ECM
Dye	N/A	DAPI	Santa Cruz Biotechnology / CAS 28718-90-3	1.5 µg / ml IF

Dye	N/A	rhodamine-conjugated phalloidin	Sigma-Aldrich / Cat. 50556	1:250 IF
Dye	N/A	rhodamine-conjugated phalloidin	Thermo Fisher Scientific / Cat. R415	1:250 IF

Supplementary Table 4: Settings and parameters used for the mass spectrometry analysis.

HPLC gradients

Experiment(s)	Gradient length	Duration (min)	Solvent B (80 % acetonitrile, 0.1% formic acid)
Secretome	150 min	5	5 to 8%
		119	8 to 29%
		5	29 to 68%
		5	68 to 95%
		6	95%

MS settings

	MS1					
Experiment(s)	**Resolution**	**AGC Target**	**Max IT**	**Scan range**		
all	70000	3,00E+06	20 ms	300 - 1750 m/z		
	MS2					
Experiment(s)	**Resolution**	**AGC target**	**Max IT**	**Isolation window**	**TopN**	**NCE**
Secretome and Surfactome	17500	5,00E+05	60 ms	2.1 m/z	10	25

Supplementary Table 5: Primers for ChIP.

Primers	Forward reaction 5'-3'	Reverse reaction 5'-3'
*MYST1*promoter_SBE 1	ACACCATTCTCCTGCCTCAG	GGTGGATCACGAGGTCAGA
*MYST1*promoter_SBE 2	TCCCAAAGTGCTGGGATTAC	GGCAGGAGAATCGCTTGA
*MYST1*promoter_SBE 3	CTCCCGAGTAGCTGGGACTA	GTGGCTCACGCCTGTAATCT