Supplemental Information

Post-translational regulation of PGC-1α modulates fibrotic repair

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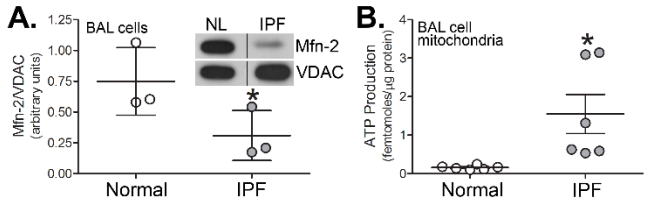

Figure S1. IPF BAL cells have reduced mitochondrial fusion. (**A**) Mitochondrial immunoblot analysis and quantification from normal ($n = 3$) or IPF subjects ($n = 3$). (**B**) Mitochondrial ATP production was determined in BAL cells from normal ($n = 6$) or IPF subjects ($n = 6$). *, $p < 0.05$. Values shown as mean ± S.E.M. Two-tailed *t*-test statistical analysis was utilized.

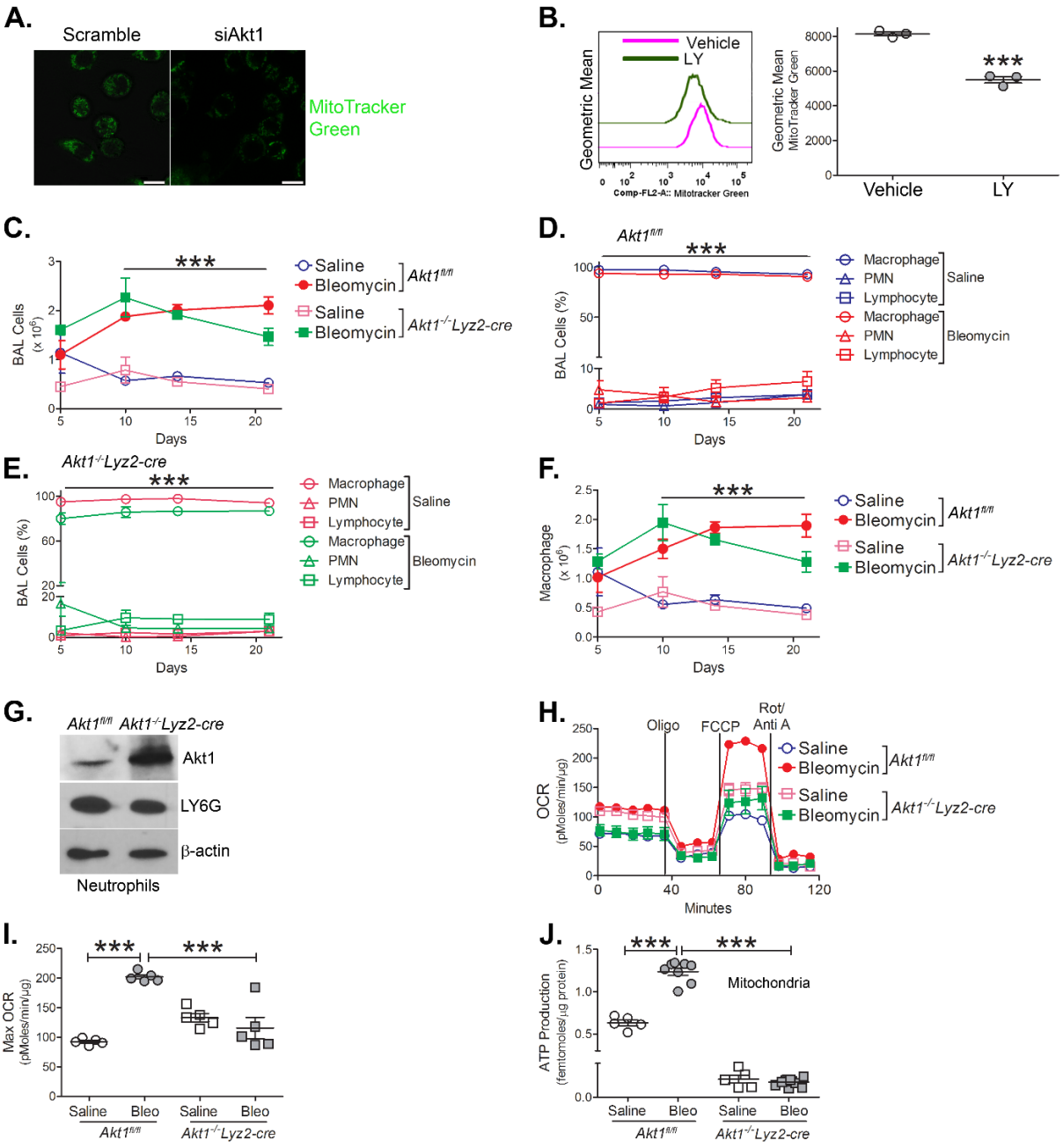

Figure S2. Monocytic cells are predominant in BAL from *Akt1$^{fl/fl}$* and *Akt1$^{-/-}$Lyz2-cre* mice. (**A**) MitoTracker green staining of macrophages transfected with scramble or Akt1 siRNA ($n = 4$). Scale bars represent 10 μm. (**B**) Flow cytometry of THP-1 cells treated with vehicle vehicle, or LY294002 (LY) ($n = 3$). BAL cells were isolated at indicated days after saline or bleomycin exposure from *Akt1$^{fl/fl}$* or *Akt1$^{-/-}$Lyz2-cre* mice. (**C**) Total number of BAL cells ($n = 4$-5). Cell differential of (**D**) *Akt1$^{fl/fl}$* ($n = 4$-5) and (**E**) *Akt1$^{-/-}$Lyz2-cre* mice ($n = 4$-5). (**F**) Number of macrophages from exposed *Akt1$^{fl/fl}$* ($n = 4$-5) and *Akt1$^{-/-}$Lyz2-cre* mice ($n = 4$-5). Akt1 immunoblot analysis in (**G**) neutrophils isolated from *Akt1$^{fl/fl}$* and *Akt1$^{-/-}$Lyz2-cre* mice. (**H**) OCR and (**I**) maximal OCR in BAL cells 21 days after exposure from *Akt1$^{fl/fl}$* and *Akt1$^{-/-}$Lyz2-cre* mice ($n = 5$). (**J**) Mitochondrial ATP production was measured in BAL cells 21 days after exposure from *Akt1$^{fl/fl}$* and *Akt1$^{-/-}$Lyz2-cre* mice. ($n = 5$-10). Mac = macrophage, PMN = polymorphonuclear, Lymph = lymphocyte. ***, $p < 0.0001$. Values shown as mean ± S.E.M. Two-tailed *t*-test statistical analysis was utilized for B. Two-way ANOVA followed by Bonferroni post-test was utilized for C-F. One-way ANOVA followed by Tukey's multiple comparison test was utilized for I, J. *** in C and F refer to *Akt1$^{fl/fl}$* bleomycin vs *Akt1$^{fl/fl}$* saline at 10, 14, and 21 days and *Akt1$^{-/-}$Lyz2-cre* bleomycin vs *Akt1$^{-/-}$Lyz2-cre* saline at 10, 14, and 21 days. *** in D and E refer to macrophage saline vs PMN saline and lymphocyte saline at 5, 10, 14, and 21 days and macrophage bleomycin vs PMN bleomycin and lymphocyte bleomycin at 5, 10, 14, and 21 days.

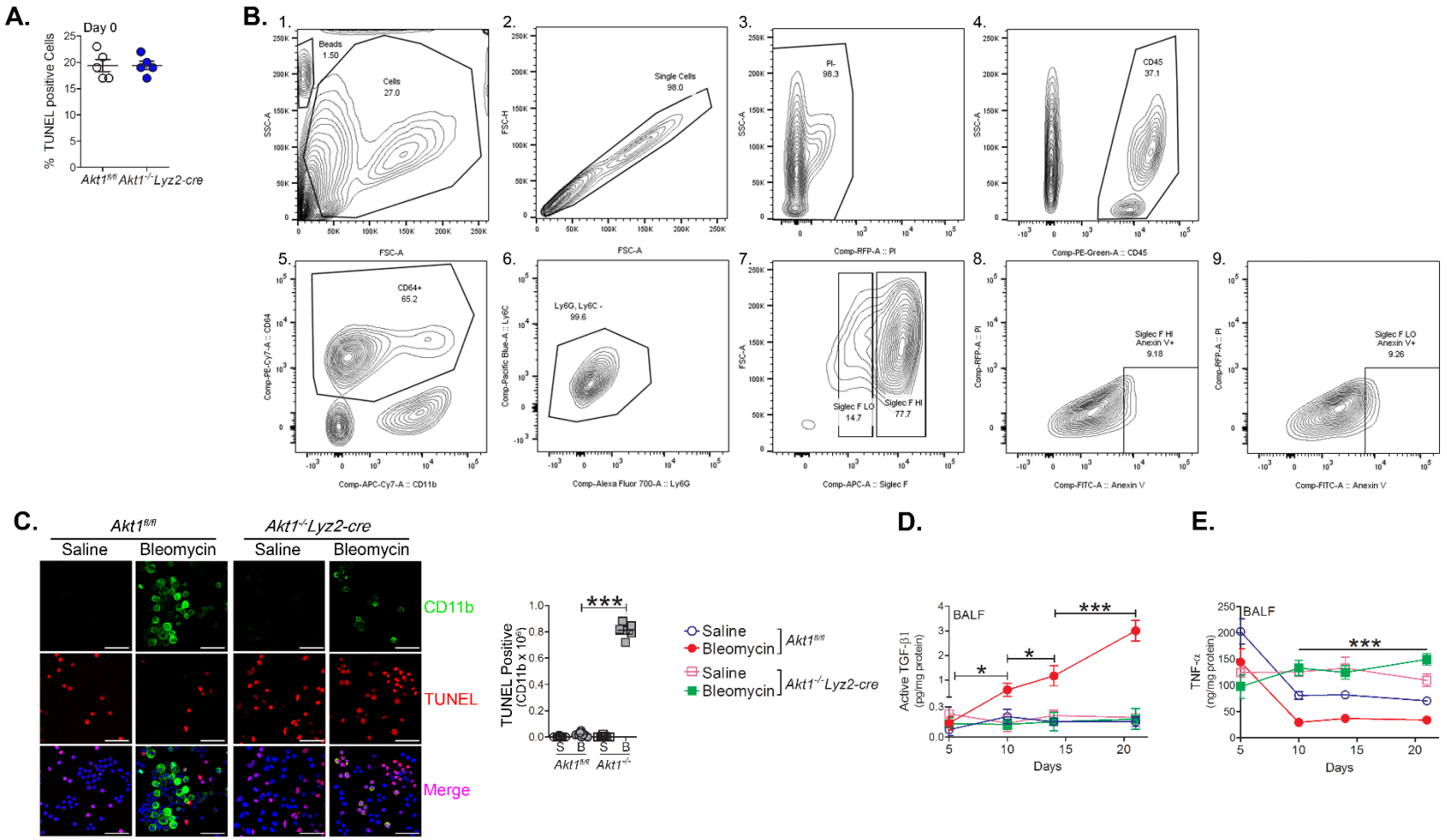

Figure S3. ***Akt1*$^{-/-}$*Lyz2-cre*** **BAL cells undergo apoptosis.** (**A**) Percentage of TUNEL positive cells at day 0 ($n = 5$) from *Akt1*$^{fl/fl}$ or *Akt1*$^{-/-}$*Lyz2-cre* mice. (**B**) Representative flow gating strategy from isolated BAL after bleomycin exposure from *Akt1*$^{fl/fl}$ or *Akt1*$^{-/-}$*Lyz2-cre* mice. (**C**) Confocal imaging and number of BAL cells from *Akt1*$^{fl/fl}$or *Akt1*$^{-/-}$*Lyz2-cre* (*Akt1*$^{-/-}$) mice 21 days in after saline (S) or bleomycin (B) exposure stained with CD11b ($n = 9$) together with TUNEL and DAPI. Scale bars represent 50 µm. (**D**) Active TGF-β1 ($n = 5$) and (**E**) TNF-α levels ($n = 5$) in BALF. *, $p < 0.05$; ***, $p < 0.0001$. Two-tailed *t*-test statistical analysis was utilized for A. Values shown as mean ± S.E.M. One-way ANOVA followed by Tukey's multiple comparison test was utilized for C, and two-way ANOVA followed by Bonferroni post-test was utilized for D and E. Bracketed lines in D are comparing *Akt1*$^{fl/fl}$ bleomycin at indicated timepoints.*** in E refer to *Akt1*$^{fl/fl}$ bleomycin vs *Akt1*$^{-/-}$*Lyz2-cre* bleomycin at 10, 14, and 21 days.

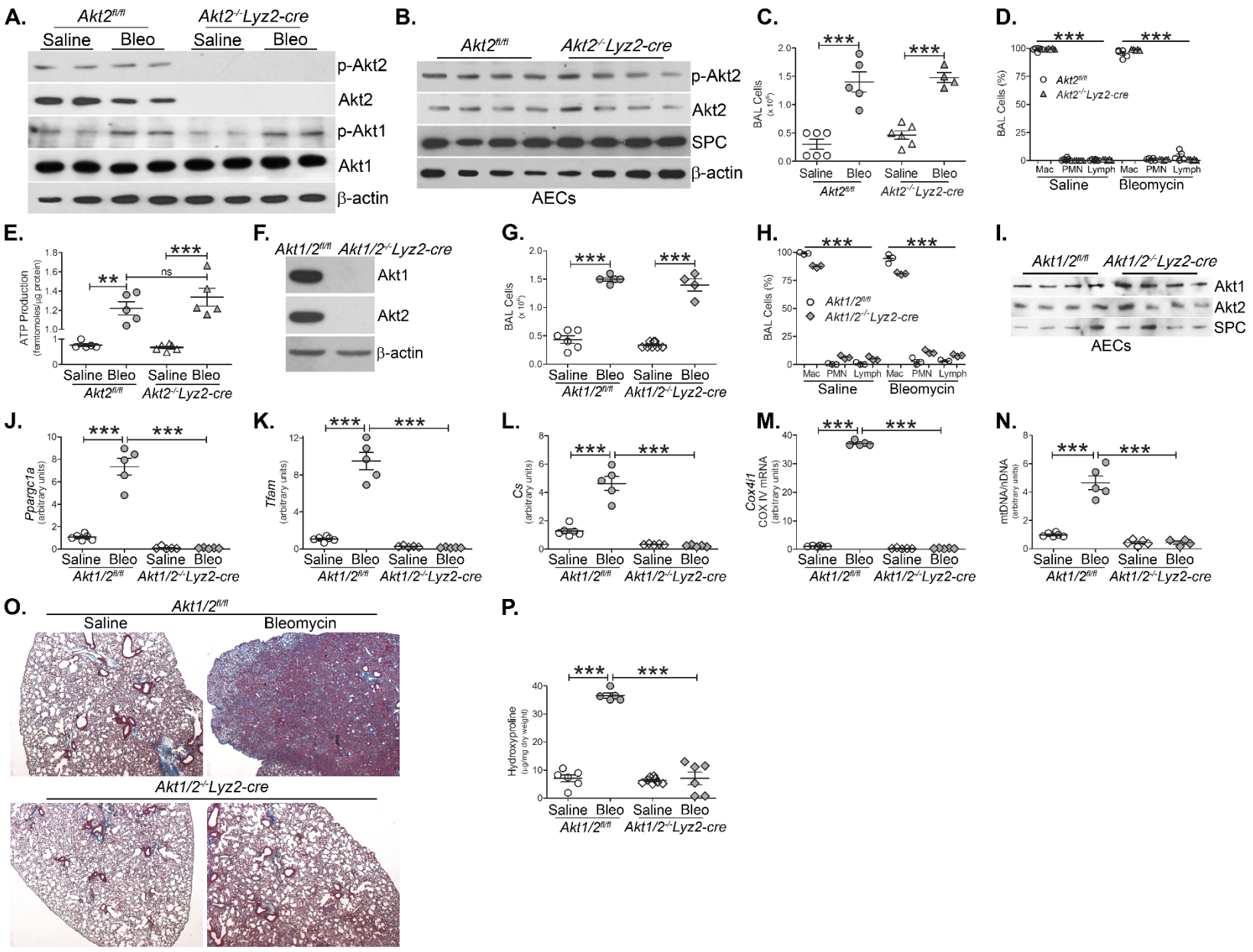
A.
Akt2fl/fl
Akt2-/-Lyz2-cre
Saline
Bleo
Saline
Bleo
p-Akt2
Akt2
p-Akt1
Akt1
β-actin
B.
Akt2fl/fl
Akt2-/-Lyz2-cre
p-Akt2
Akt2
SPC
β-actin
AECs
C.
BAL Cells (x 10⁶)
***
***
Saline
Bleo
Saline
Bleo
Akt2fl/fl
Akt2-/-Lyz2-cre
D.
BAL Cells (%)
***
***
Akt2fl/fl
Akt2-/-Lyz2-cre
Mac
PMN
Lymph
Mac
PMN
Lymph
Saline
Bleomycin
E.
ATP Production (femtomoles/ug protein)
**
ns
***
Saline
Bleo
Saline
Bleo
Akt2fl/fl
Akt2-/-Lyz2-cre
F.
Akt1/2fl/fl
Akt1/2-/-Lyz2-cre
Akt1
Akt2
β-actin
G.
BAL Cells (x 10⁶)
***
***
Saline
Bleo
Saline
Bleo
Akt1/2fl/fl
Akt1/2-/-Lyz2-cre
H.
BAL Cells (%)
***
***
Akt1/2fl/fl
Akt1/2-/-Lyz2-cre
Mac
PMN
Lymph
Mac
PMN
Lymph
Saline
Bleomycin
I.
Akt1/2fl/fl
Akt1/2-/-Lyz2-cre
Akt1
Akt2
SPC
AECs
J.
Ppargc1a (arbitrary units)
***
***
Saline
Bleo
Saline
Bleo
Akt1/2fl/fl
Akt1/2-/-Lyz2-cre
K.
Tfam (arbitrary units)
***
***
Saline
Bleo
Saline
Bleo
Akt1/2fl/fl
Akt1/2-/-Lyz2-cre
L.
Cs (arbitrary units)
***
***
Saline
Bleo
Saline
Bleo
Akt1/2fl/fl
Akt1/2-/-Lyz2-cre
M.
Cox4i1 COX IV mRNA (arbitrary units)
***
***
Saline
Bleo
Saline
Bleo
Akt1/2fl/fl
Akt1/2-/-Lyz2-cre
N.
mtDNA/nDNA (arbitrary units)
***
***
Saline
Bleo
Saline
Bleo
Akt1/2fl/fl
Akt1/2-/-Lyz2-cre
O.
Akt1/2fl/fl
Saline
Bleomycin
Akt1/2-/-Lyz2-cre
P.
Hydroxyproline (μg/mg dry weight)
***
***
Saline
Bleo
Saline
Bleo
Akt1/2fl/fl
Akt1/2-/-Lyz2-cre

Figure S4. Conditional deletion of Akt1 and Akt2 protects mice against bleomycin-induced fibrosis. (**A**) BAL cells were isolated 21 days after saline or bleomycin (Bleo) exposure from *Akt2*$^{fl/fl}$ or *Akt2*$^{-/-}$*Lyz2-cre* mice and immunoblot analysis was performed. (**B**) Immunoblot analysis of type II alveolar epithelial cells (AECs) isolated from *Akt2*$^{fl/fl}$or *Akt2*$^{-/-}$*Lyz2-cre* mice. (**C**) Number of BAL cells (n = 4-6) and (**D**) BAL cell differential *Akt2*$^{fl/fl}$ or *Akt2*$^{-/-}$*Lyz2-cre* mice isolated 21 days after exposure (n = 5). (**E**) ATP production measured in BAL cells (n = 5-6). *Akt1/2*$^{fl/fl}$ or *Akt1/2*$^{-/-}$*Lyz2-cre* mice were exposed to saline or bleomycin. BAL cells were isolated 21 days later. (**F**) Immunoblot analysis in isolated BAL cells. (**G**) Total number of BAL cells (n = 4-8). (**H**) Cell differential (n = 3). (**I**) Immunoblot analysis of type II alveolar epithelial cells (AECs) isolated from *Akt1/2*$^{fl/fl}$ or *Akt1/2*$^{-/-}$*Lyz2-cre* mice. mRNA analysis of (**J**) *Ppargc1a* (n = 5-6), (**K**) *Tfam* (n = 5-6), (**L**) *Cs* (n = 5-6), and (**M**) *Cox4i1* expression (n = 5-6). (**N**) mtDNA/nDNA in BAL cells (n = 5-6). (**O**) Masson's trichrome staining of lung sections (n = 5-6) and (**P**) hydroxyproline analysis (n = 5-6). **, $p < 0.001$; ***, $p < 0.0001$. Values shown as mean ± S.E.M. One-way ANOVA followed by Tukey's multiple comparison test was utilized.

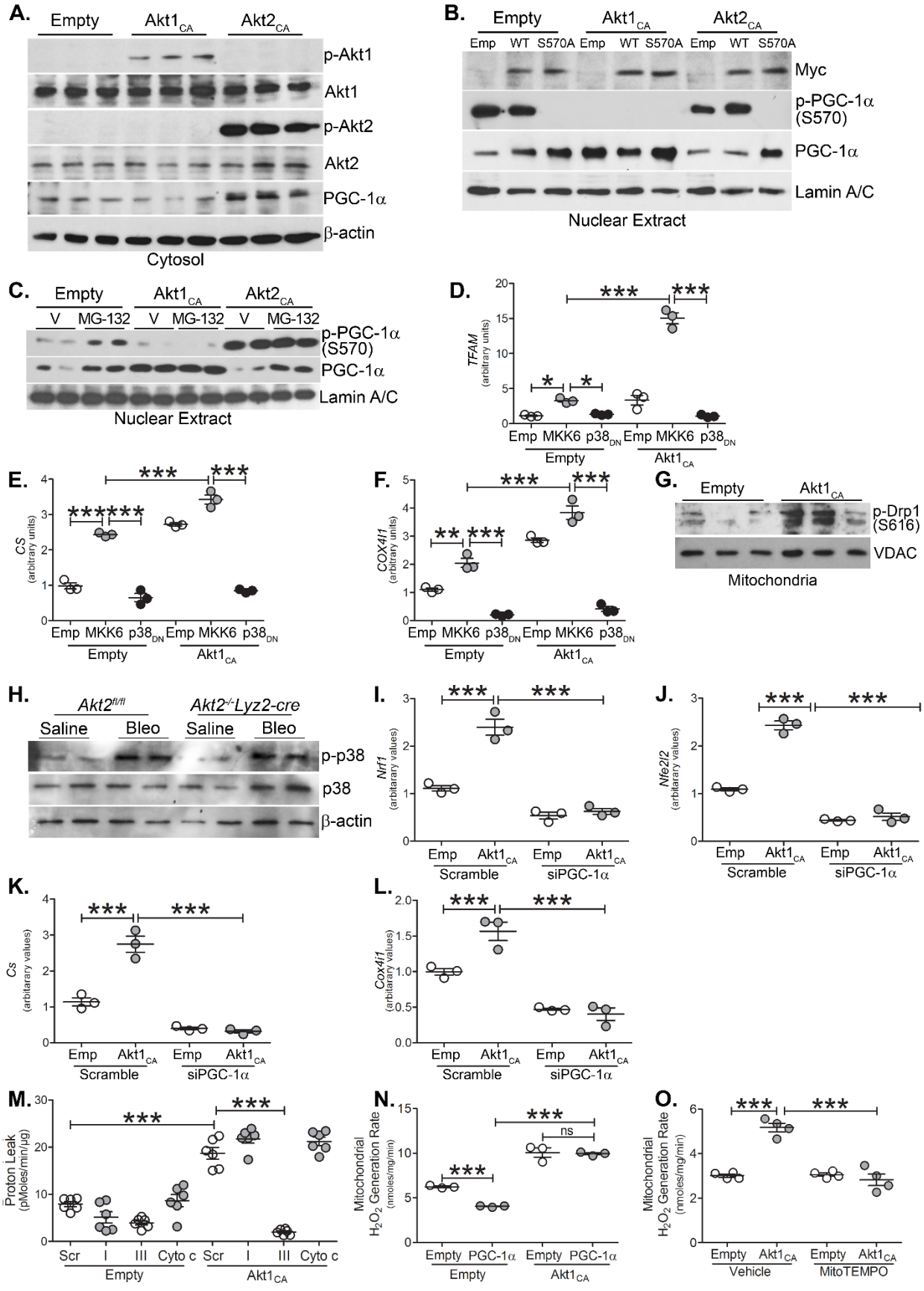
A.
Empty
Akt1CA
Akt2CA
p-Akt1
Akt1
p-Akt2
Akt2
PGC-1α
β-actin
Cytosol
B.
Empty
Akt1CA
Akt2CA
Emp WT S570A Emp WT S570A Emp WT S570A
Myc
p-PGC-1α (S570)
PGC-1α
Lamin A/C
Nuclear Extract
C.
Empty
Akt1CA
Akt2CA
V MG-132 V MG-132 V MG-132
p-PGC-1α (S570)
PGC-1α
Lamin A/C
Nuclear Extract
D.
TFAM (arbitrary units)
Emp MKK6 p38DN Emp MKK6 p38DN
Empty
Akt1CA
E.
CS (arbitrary units)
Emp MKK6 p38DN Emp MKK6 p38DN
Empty
Akt1CA
F.
COX4i1 (arbitrary units)
Emp MKK6 p38DN Emp MKK6 p38DN
Empty
Akt1CA
G.
Empty
Akt1CA
p-Drp1 (S616)
VDAC
Mitochondria
H.
Akt2fl/fl
Akt2-/-Lyz2-cre
Saline Bleo Saline Bleo
p-p38
p38
β-actin
I.
Nrf1 (arbitrary values)
Emp Akt1CA Emp Akt1CA
Scramble
siPGC-1α
J.
Nfe2l2 (arbitrary values)
Emp Akt1CA Emp Akt1CA
Scramble
siPGC-1α
K.
Cs (arbitrary values)
Emp Akt1CA Emp Akt1CA
Scramble
siPGC-1α
L.
Cox4i1 (arbitrary values)
Emp Akt1CA Emp Akt1CA
Scramble
siPGC-1α
M.
Proton Leak (pMoles/min/μg)
Scr I III Cyto c Scr I III Cyto c
Empty
Akt1CA
N.
Mitochondrial H2O2 Generation Rate (nmoles/mg/min)
ns
Empty PGC-1α Empty PGC-1α
Empty
Akt1CA
O.
Mitochondrial H2O2 Generation Rate (nmoles/mg/min)
Empty Akt1CA Empty Akt1CA
Vehicle
MitoTEMPO

Figure S5. Akt1 mediates p38 activation of PGC-1α. (**A**) Macrophage expressing empty, $Akt1_{CA}$, or $Akt2_{CA}$ were analyzed by immunoblot analysis in isolated cytosol fractions. (**B**) Nuclear immunoblot analysis of macrophages expressing empty, $Akt1_{CA}$, or $Akt2_{CA}$ together with $PGC\text{-}1\alpha_{WT}$ (WT) or $PGC\text{-}1\alpha_{S570A}$ (S570A). (**C**) Macrophages were treated with vehicle (V) or MG-132 and immunoblot analysis performed in isolated nuclear factions. mRNA expression of macrophages transfected with empty or $Akt1_{CA}$ together with MKK6(Glu) or $p38_{DN}$ for (**D**) *Tfam* ($n = 3$), (**E**) *Cs* ($n = 3$), and (**F**) *Cox4i1* ($n = 3$) expression. (**G**) Immunoblot analysis of p-Drp1 (S616) in mitochondrial fractions of macrophages expressing empty or $Akt1_{CA}$. (**H**) Immunoblot analysis of p-p38 in BAL cells from $Akt2^{fl/fl}$ or $Akt2^{-/-}$*Lyz2-cre* mice exposed to saline or bleomycin. mRNA analysis of macrophages transfected with scramble or PGC-1α siRNA and empty or $Akt1_{CA}$ for (**I**) *Nrf1* ($n = 3$), (**J**) *Nfe2l2* ($n = 3$), (**K**) *Cs* ($n = 3$), and (**L**) *Cox4i1* expression ($n = 3$). (**M**) Proton leak determined by OCR in macrophages with NDUFB8 (complex I, I), Rieske (complex III), or cytochrome *c* (cyto c) silenced ($n = 6$). mtROS generation in macrophages expressing (**N**) empty or PGC-1α and $Akt1_{CA}$ ($n = 3$) and (**O**) expressing empty or $Akt1_{CA}$ and treated with vehicle or MitoTEMPO (10 μM, overnight, $n = 4$). *, $p < 0.05$; **, $p < 0.001$; ***, $p < 0.0001$. Values shown as mean ± S.E.M. One-way ANOVA followed by Tukey's multiple comparison test was utilized.

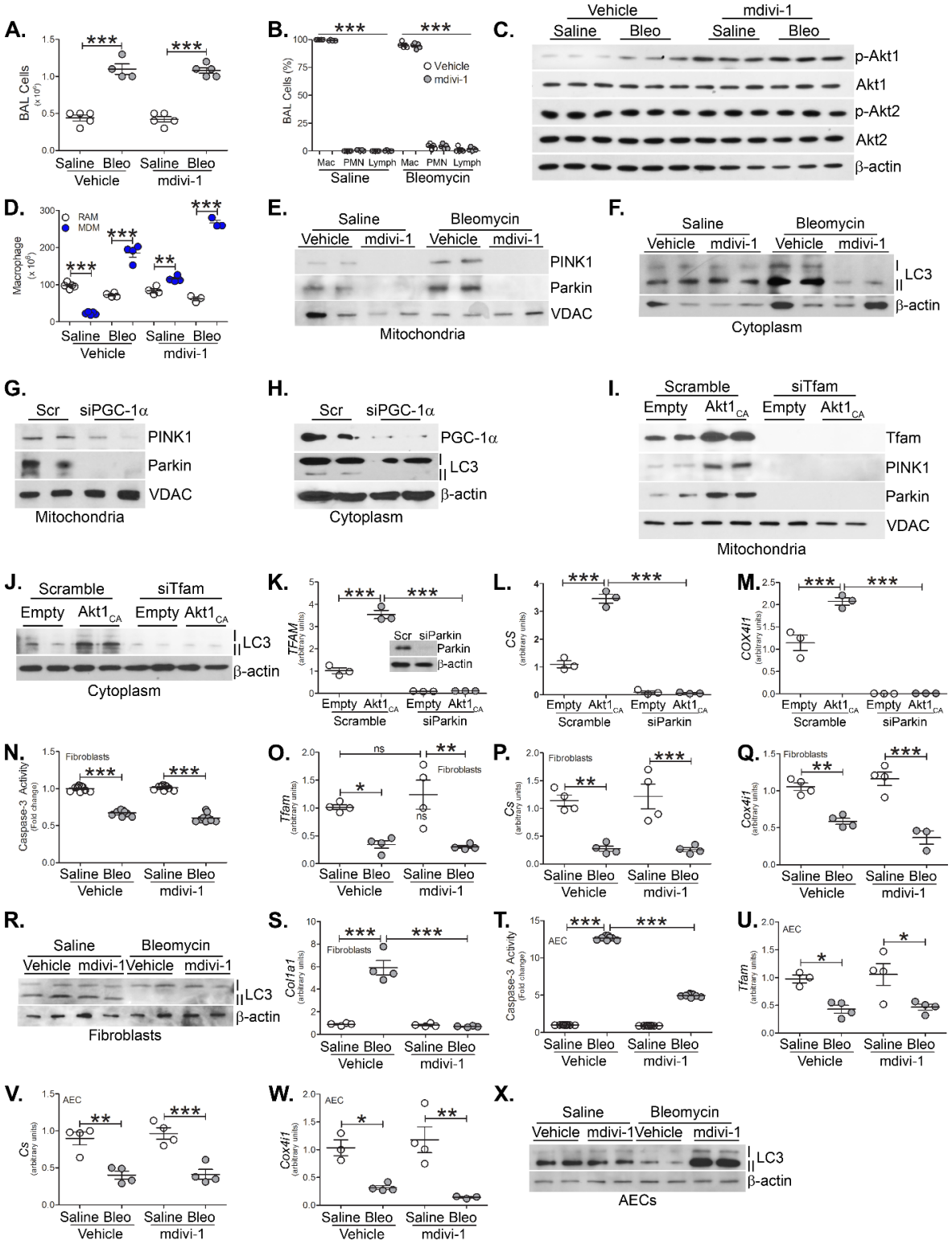

Figure S6. Mitochondrial division inhibitor prevents bleomycin-induced pulmonary fibrosis. C57BL/6J WT mice were exposed to saline or bleomycin (bleo), 10 days after exposure mice were administered daily i.p. injections of vehicle or mdivi-1. BAL cells, fibroblasts, and AECs were isolated 21 days later. (**A**) Total number of BAL cells (n = 4-5). (**B**) BAL cell differential (n = 4-5). ATP production in BAL cells (n = 5). (**C**) Immunoblot analysis of BAL cells from exposed mice treated with vehicle or mdivi-1 i.p. (**D**) Number of MDM or RAM in exposed mice treated with vehicle or mdivi-1 i.p (n = 3-4). Immunoblot analysis of BAL cells in isolated (**E**) mitochondria or (**F**) cytosol fractions. Immunoblot analysis in macrophages expressing scramble or

PGC-1α siRNA in isolated (**G**) mitochondria or (**H**) cytosol fractions. Immunoblot analysis in macrophages expressing scramble or Tfam siRNA and empty or $Akt1_{CA}$ in isolated (**I**) mitochondria or (**J**) cytosol fractions. mRNA analysis of (**K**) *TFAM* ($n = 3$), (**L**) *CS* ($n = 3$), and (**M**) *COX4I1* ($n = 3$) expression in macrophages expressing scramble or Parkin siRNA and empty or $Akt1_{CA}$. Inset in I, immunoblot analysis of Parkin. (**N**) Caspase-3 activity in isolated lung fibroblasts from exposed mice ($n = 9$). mRNA analysis of (**N**) *Tfam* ($n = 4$), (**P**) *Cs* ($n = 4$), (**Q**) *Cox4i1* ($n = 4$), (**R**) immunoblot analysis, and (**S**) *Col1a1* expression ($n = 4$) in isolated fibroblasts. (**T**) Caspase-3 activity in isolated AECs from exposed mice ($n = 8$). mRNA analysis of (**U**) *Tfam* ($n = 3$-4), (**V**) *Cs* ($n = 4$), and (**W**) *Cox4i1* expression ($n = 3$-4) in isolated AECs. (**X**) Immunoblot analysis of AECs. *, $p < 0.05$; **, $p < 0.001$; ***, $p < 0.0001$. Values shown as mean ± S.E.M. One-way ANOVA followed by Tukey's multiple comparison test was utilized.

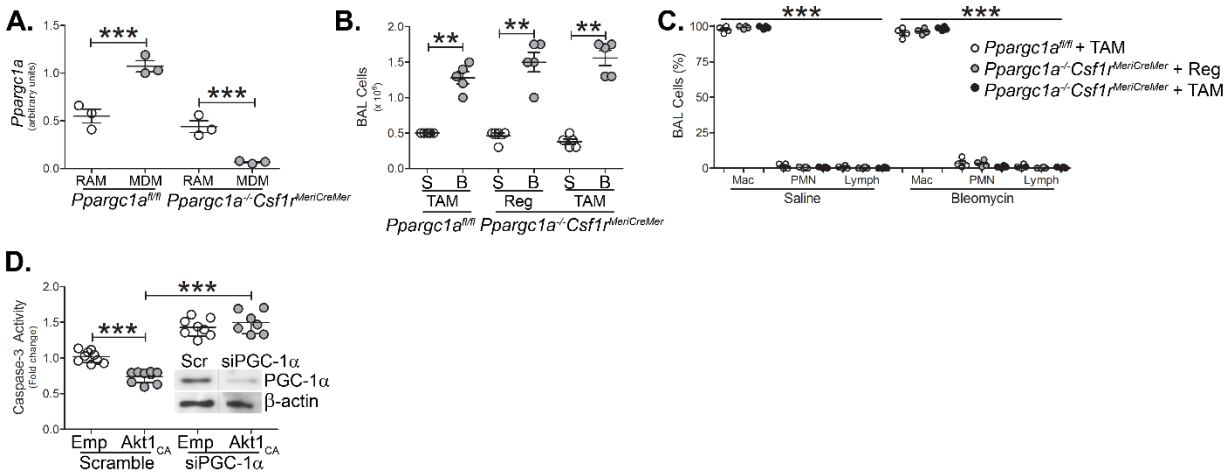

Figure S7. *Ppargc1a*$^{-/-}$*Csf1r*^{MeriCreMer} **mice are protected from bleomycin-induced fibrosis.** *Ppargc1a*$^{fl/fl}$ or *Ppargc1a*$^{-/-}$ *Csf1r*^{MeriCreMer} mice were administered tamoxifen (TAM) or regular (Reg) chow. Mice were exposed to saline (S) or bleomycin (B). BAL cells were isolated 21 days (**A**) *Ppargc1a* mRNA analysis in FACS-sorted tissue resident alveolar macrophages (RAM) and monocyte-derived macrophages (MDM) from chrysotile exposed mice isolated by BAL (n = 3). (**B**) Total number of BAL cells (n = 5-6). (**C**) Cell differential (n = 4). (**D**) Caspase-3 activity in transfected macrophages (n = 8-9). **, $p < 0.001$; ***, $p < 0.0001$. Values shown as mean ± S.E.M. One-way ANOVA followed by Tukey's multiple comparison test was utilized. *** in C refer to macrophage saline vs PMN saline and lymphocyte saline within each strain and macrophage bleomycin vs PMN bleomycin and lymphocyte bleomycin within each strain.