SUPPLEMENTARY DATA

ML216 prevents DNA-damage induced senescence by modulating DBC1-BLM interaction
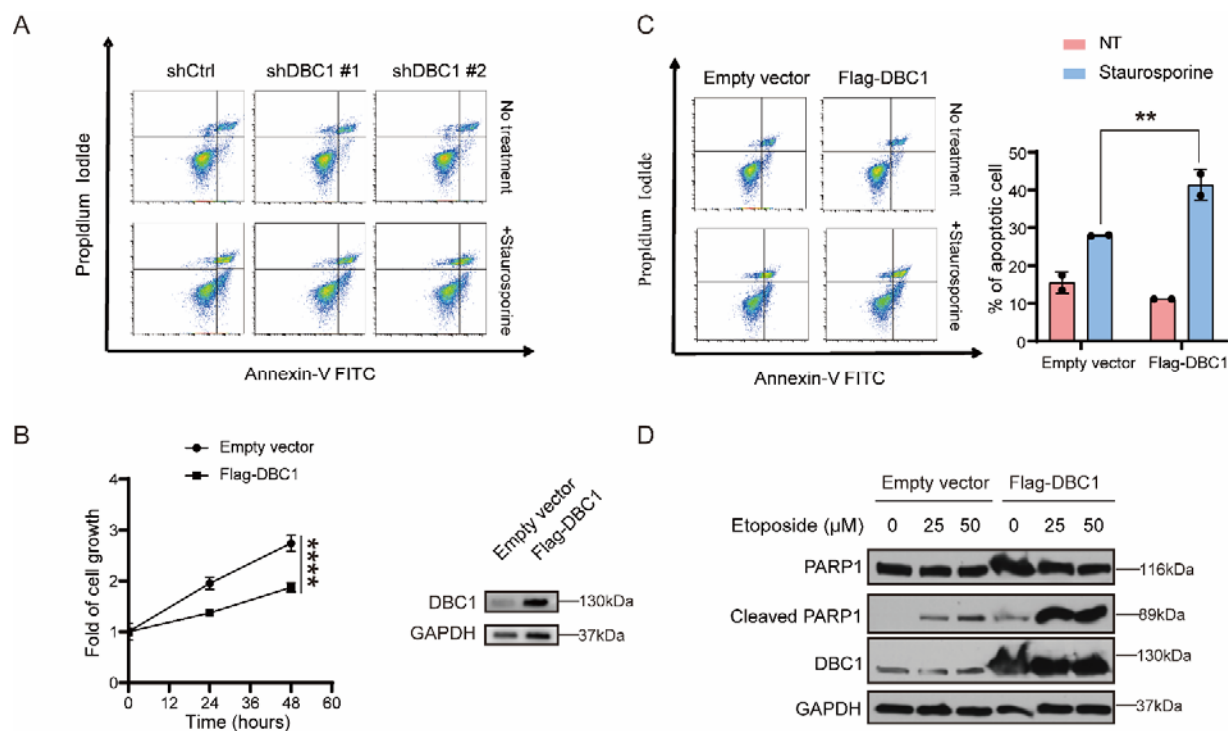
Feng Cui [#], Xueying Han [#], Xiaoqian Zhang [#], Siqi Wang, Na Liang, Qing Tan, Wuga Sha, and Jun Li *

*Correspondence: Jun Li@ibms.pumc.edu.cn

The PDF file includes:

Supplementary Figures S1–S10

Supplementary Tables S1 and S2

A

shCtrl   shDBC1 #1   shDBC1 #2

Propidium Iodide

No treatment

+Staurosporine

Annexin-V FITC

B

Fold of cell growth

- Empty vector
- Flag-DBC1

****

Time (hours)

Empty vector   Flag-DBC1

DBC1 ——130kDa
GAPDH ——37kDa

C

Empty vector   Flag-DBC1

Propidium Iodide

No treatment

+Staurosporine

Annexin-V FITC

■ NT
■ Staurosporine

% of apoptotic cell

**

Empty vector   Flag-DBC1

D

Empty vector   Flag-DBC1

Etoposide (μM)   0   25   50   0   25   50

PARP1 ——116kDa
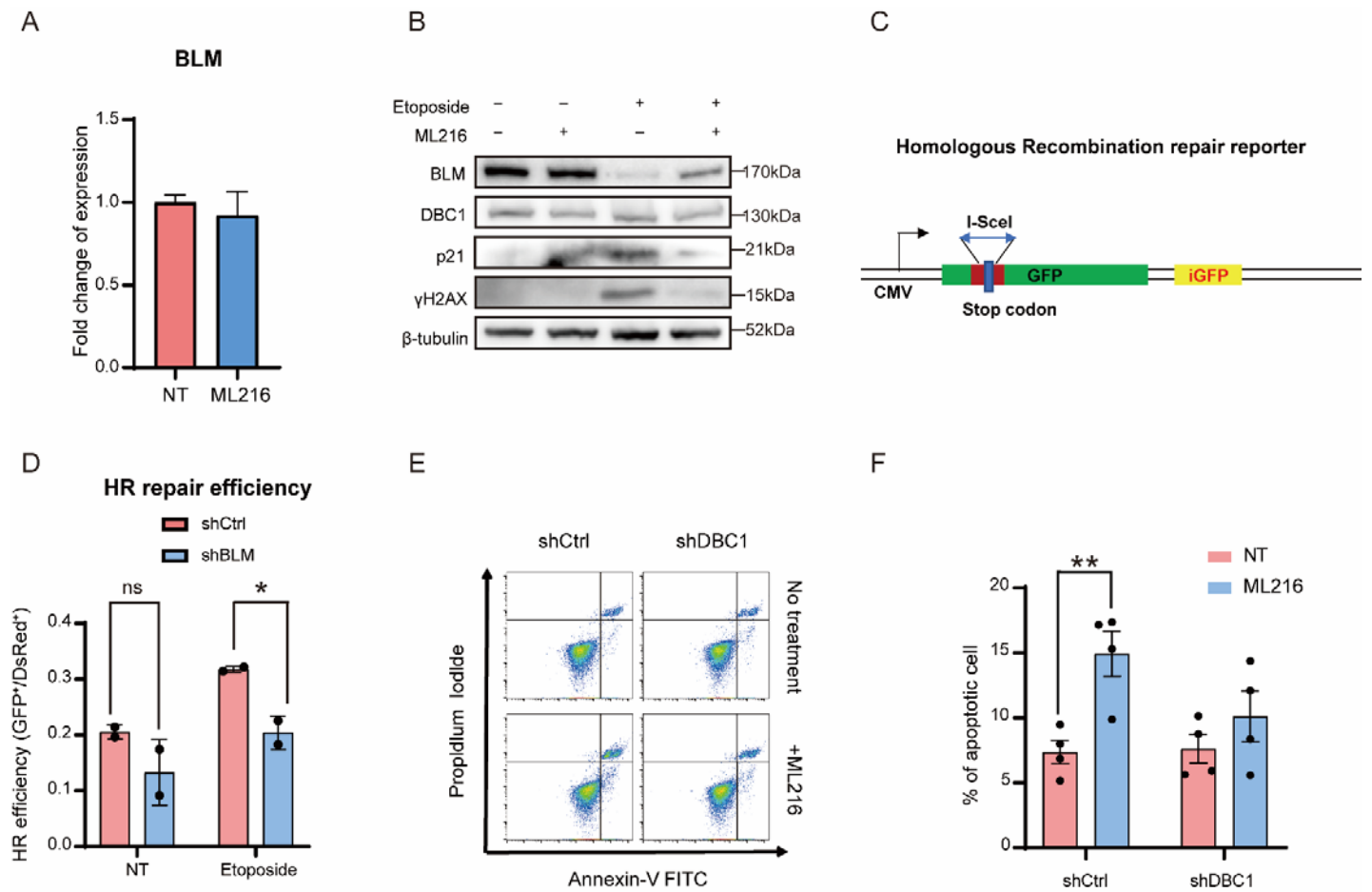Cleaved PARP1 ——89kDa
DBC1 ——130kDa
GAPDH ——37kDa

2

Supplementary Figure S1. Overexpressing DBC1 promotes apoptosis and decreases cell survival after DNA damage. (A) Representative scatter plots of PI (y-axis) vs. annexin V (x-axis) of the apoptosis analysis described in Fig. 1B. (B) Cell survival analysis of DBC1 overexpression cells with cisplatin (50 μM) treatment using cell counting kit 8, the overexpression level of DBC1 is shown in the right, two-way ANOVA test. (C) Representative scatter plots and quantification of apoptosis analysis on DBC1 overexpression cells treated with Staurosporine (1 μM, 2 hrs) using Annexin V-FITC staining and flow cytometry, n= 2 biological replicates. (D) Western blotting analysis of PARP1 and cleaved PARP1 protein levels in DBC1 overexpression cells treated with Etoposide (25 or 50 μM, 24hrs). All data were presented as mean ± SD.
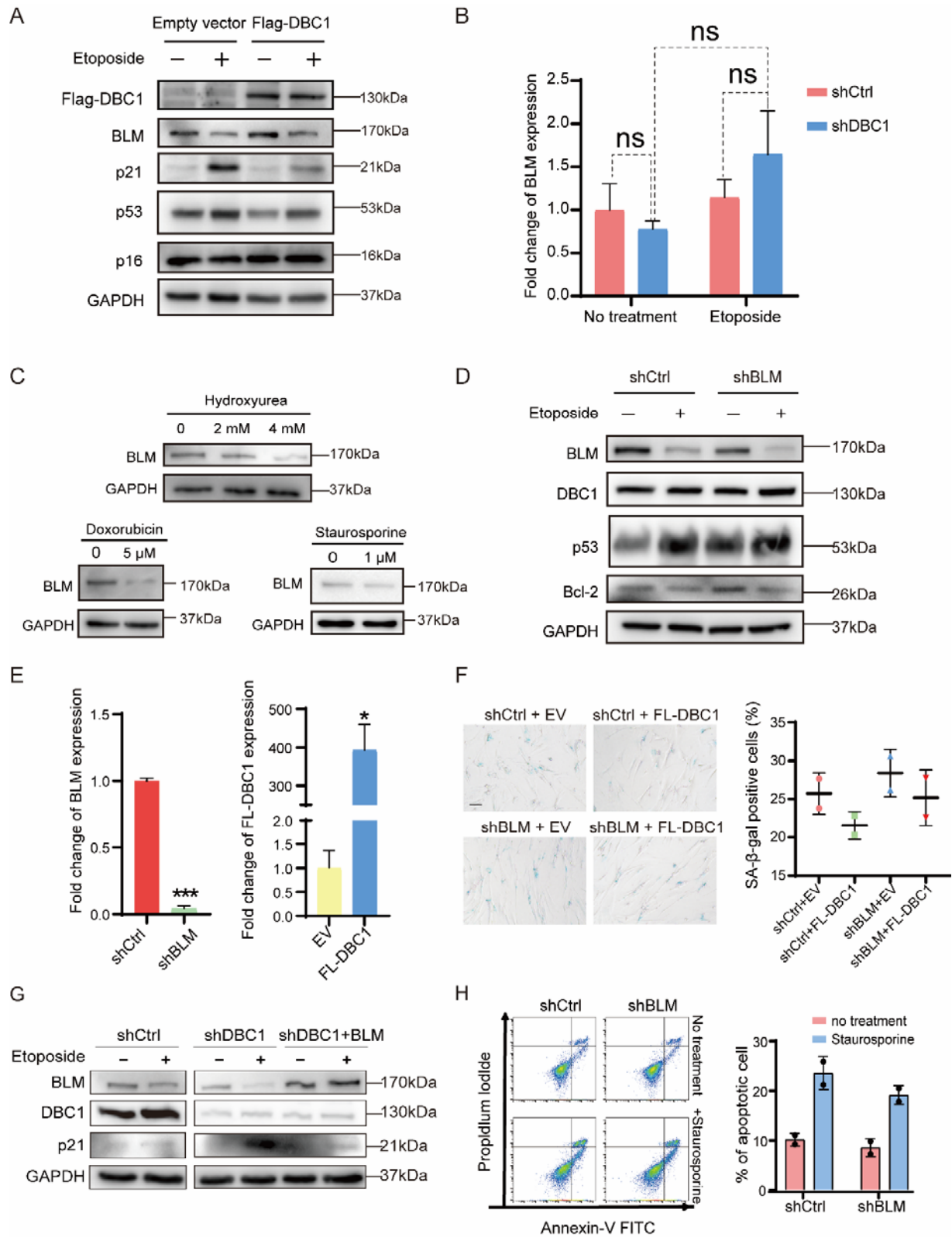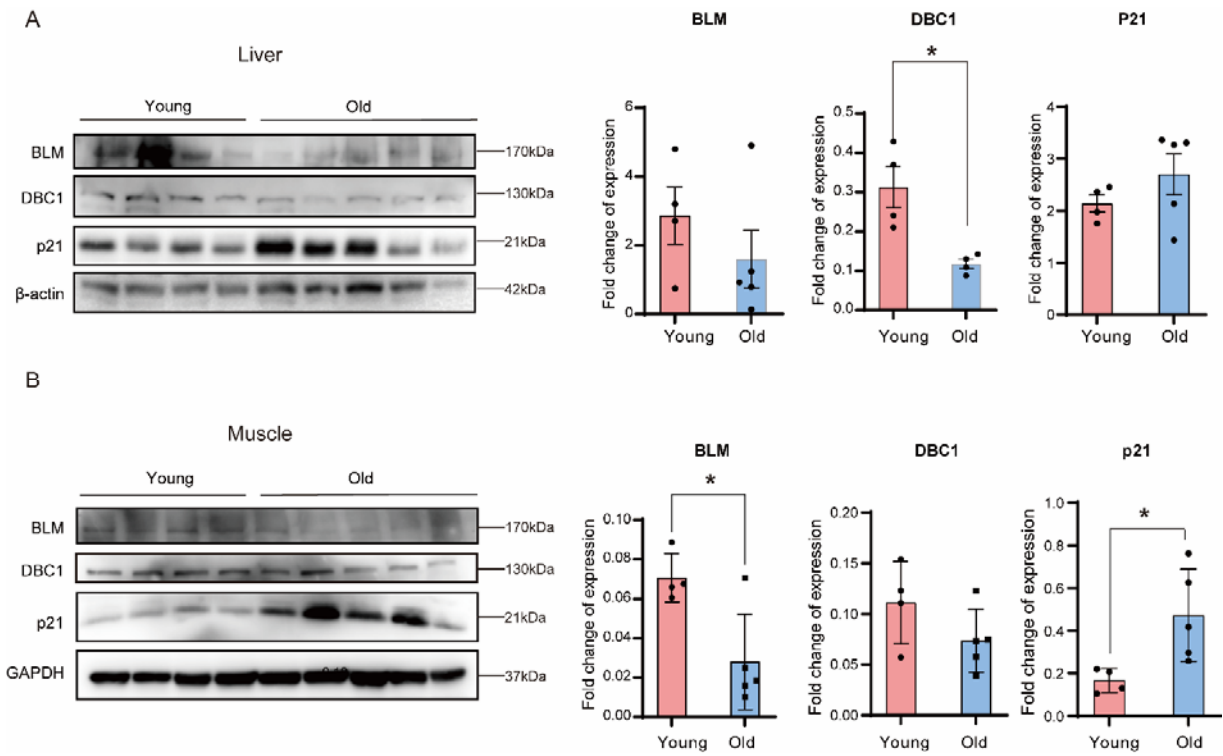
Supplementary Figure S2. DBC1 regulates cell cycle, apoptosis and senescence by preserving BLM integrity. **(A) Western blotting analysis of BLM, p53, p16 and p21 protein levels in DBC1 overexpression cells treated with Etoposide (50 μM, 24 hrs). (B) RT-PCR measurement of BLM expression with DBC1 knockdown upon Etoposide induced DNA damage in the IMR-90 cells. (C) Western blotting analysis of BLM protein levels in 293T cells treated with DNA damage reagents Hydroxyurea (48 hrs), Doxorubicin (24 hrs) and Staurosporine (6 hrs). (D) Western blotting analysis of BLM, p53, BCL-2 and DBC1 protein levels in BLM knockdown cells treated with Etoposide (100 μM, 24 hrs). (E) RT-PCR measurement of knockdown level of BLM and overexpression level of DBC1 in the IMR-90 cells used in Fig. 2F. (F) Representative images and quantification of SA-β-Gal staining of BLM knockdown IMR-90 cells transfected with DBC1, n = 2 biological replicates. Scale bar = 100 μm. (G) Western blotting analysis of BLM, DBC1, and p21 protein levels in DBC1 knockdown 293T cells after overexpressing BLM using lentivirus (Addgene, #127641). (H) Representative scatter plots of PI (y-axis) vs. annexin V (x-axis) in the apoptosis analysis of BLM knockdown cells treated staurosporine (1 μM, 2 hrs) using Annexin V-FITC staining and flow cytometry. The quantifications of apoptotic cells are**

shown in the right, n = 2 biological replicates. Except for [B] ±SEM, all data were

presented as mean ± SD.

A

BLM

B

Etoposide

ML216

BLM — 170kDa

DBC1 — 130kDa

p21 — 21kDa

γH2AX — 15kDa

β-tubulin — 52kDa

C

Homologous Recombination repair reporter

I-SceI

CMV GFP iGFP

Stop codon

D

HR repair efficiency

shCtrl

shBLM

ns

*

E

shCtrl shDBC1

No treatment

+ML216

Propidium Iodide

Annexin-V FITC

F
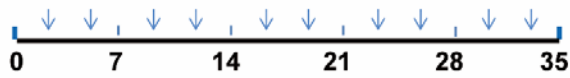
**

NT

ML216

shCtrl shDBC1

Supplementary Figure S3. ML216 reduces DNA damage response and promotes apoptosis in a DBC1-dependent fashion.**(A) The effect of ML216 on BLM mRNA expression in 293T cells, measured by RT-PCR analysis. (B) Western blotting analysis of BLM, p21, γH2AX and DBC1 protein levels in 293T cells treated with Etoposide (100 μM), ML216 (50 μM) or both for 24 hrs. (C) Schematic design of homologous recombination (HR) repair reporter construct. (D) HR repair efficiency in BLM knockdown cells treated with Etoposide (100 μM, 24 hrs), n = 2 biological replicates. (E) and (F), Representative scatter plots and quantification of apoptosis analysis in BLM knockdown cells treated with ML216 (10 μM, 24 hrs), n = 4 biological replicates. Data were presented as mean ± SD [A, D]; Data were presented as mean ± SEM [F]**

A

Liver

BLM   ——170kDa
DBC1   ——130kDa
p21   ——21kDa
β-actin ——42kDa

BLM   DBC1   P21

B

Muscle

BLM   ——170kDa
DBC1   ——130kDa
p21   ——21kDa
GAPDH   ——37kDa

BLM   DBC1   p21

.

Supplementary Figure S4 DBC1 or BLM decrease in liver and muscle with aging, while p21 increases. **(A-B) Western blotting analysis of BLM, DBC1 and p21 protein levels in liver and muscle of young (6 months, n=4) and old (20-22 months, n=5) C57BL/6 mice. All data were presented as mean ±SEM.**
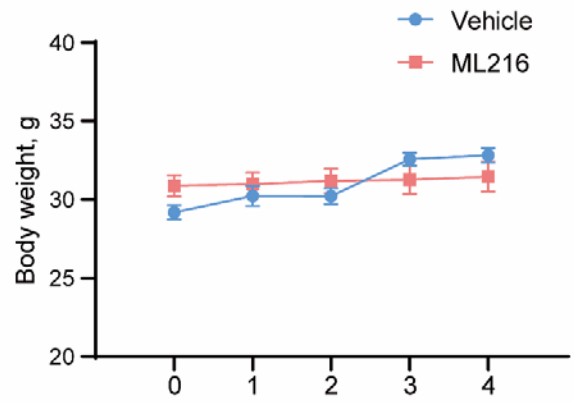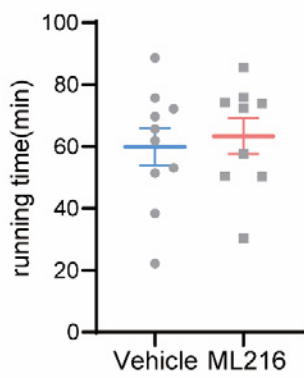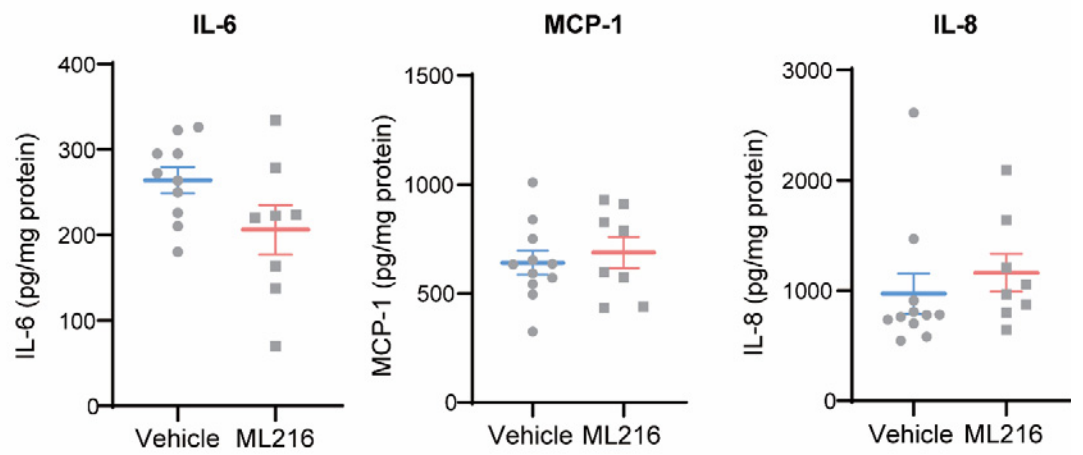
A

ML216, 1 mg/kg, twice a week, I.P Injection

0   7   14   21   28   35

Mouse: 22 months old

B

Body weight, g

Vehicle
ML216

0   1   2   3   4

C

running time(min)

Vehicle   ML216

D

IL-6

IL-6 (pg/mg protein)

Vehicle   ML216

MCP-1

MCP-1 (pg/mg protein)

Vehicle   ML216

IL-8

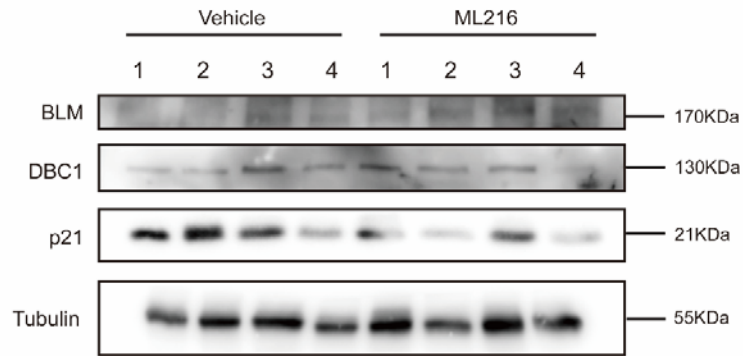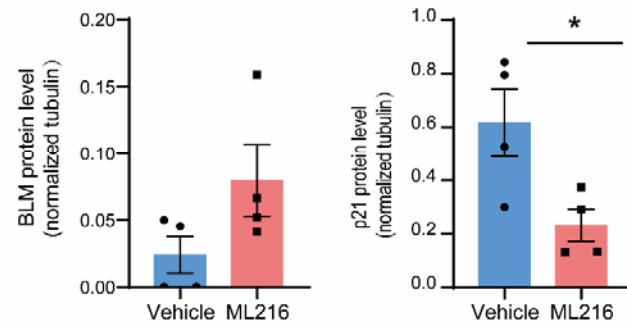IL-8 (pg/mg protein)

Vehicle   ML216

Supplementary Figure S5 ML216 reduces inflammation and improves physiological functions in old mice.**(A) Schematic to illustrate experimental design of naturally aging C57BL/6 J mice. (B) Body weight of vehicle (n = 11) or ML216 (n = 8) treated mice. (C) Quantification of maximal running time in the rotarod test for vehicle (n = 12) or ML216 (n = 14) treated mice. (D) Protein levels of SASP factors IL-6, MCP-1 and IL-8 in lung, measured by ELISA assay (Vehicle, n = 5-11; ML216, n = 7-8). All data were presented as mean ±SEM.**
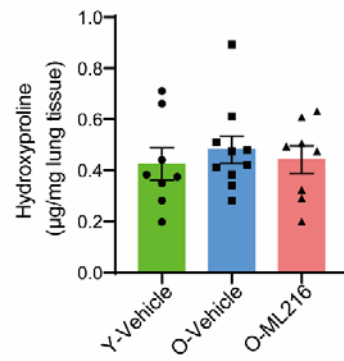
A

Vehicle   ML216
1   2   3   4   1   2   3   4

BLM ————————————————————— 170KDa

DBC1 ———————————————————— 130KDa

p21 ————————————————————— 21KDa

Tubulin —————————————————— 55KDa

B

BLM protein level
(normalized tubulin)

p21 protein level
(normalized tubulin)

*

Vehicle   ML216

C

Hydroxyproline
(µg/mg lung tissue)
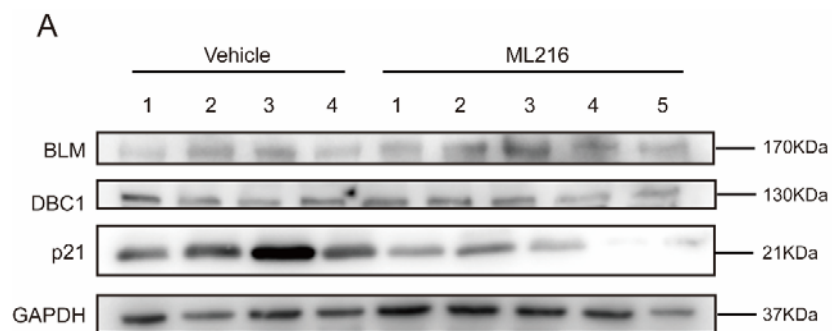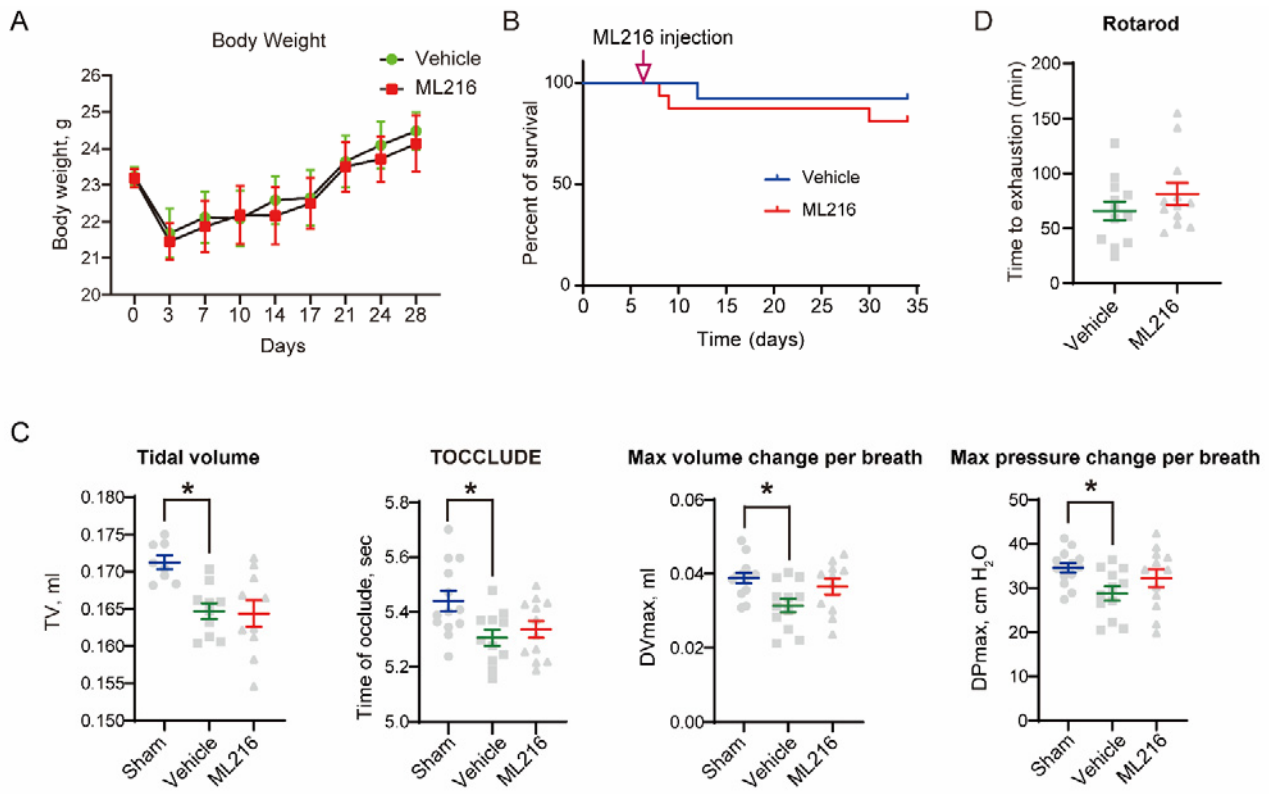
Y-Vehicle   O-Vehicle   O-ML216

Supplementary Figure S6 ML216 alleviates fibrosis in lung in old mice. **(A-B) Western blotting analysis of BLM, DBC1 and p21 protein levels in lung of vehicle-treated (n = 4) and ML216-treated (n = 4) aging C57BL/6 mice, the quantifications of BLM and p21 protein levels in mouse lungs by ImageJ. (C) The collagen contents in lungs measured by hydroxyproline assay of old mouse treated with vehicle groups (O-Vehicle, n = 11) or ML216 groups (O-ML216, n = 8), young mice (4 months) were used as control (Y-Vehicle, n = 8). All data were presented as mean ±SEM.**

A

B

C
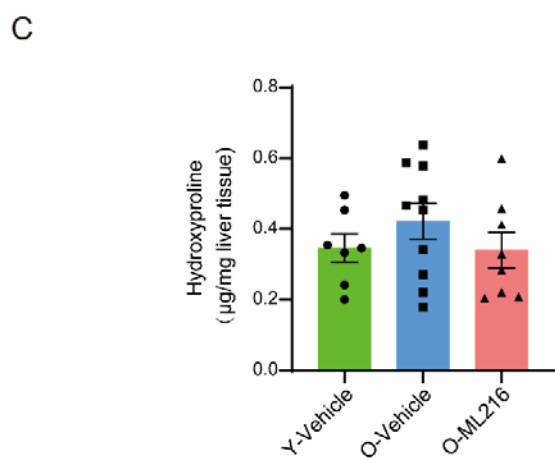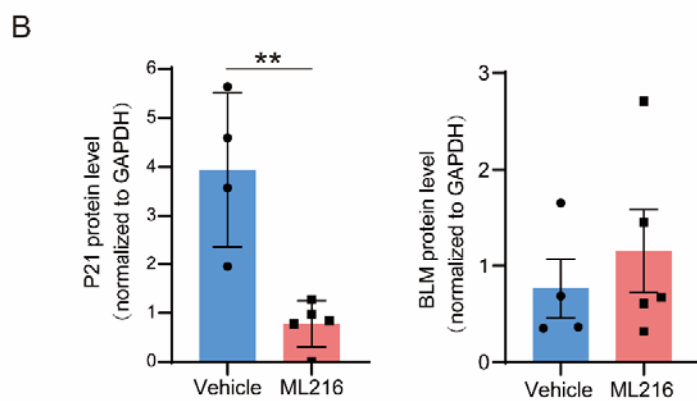
Supplementary Figure S7 ML216 alleviates fibrosis in livers of old mice. **(A-B) Western blotting analysis of BLM, DBC1 and p21 protein levels in liver of vehicle-treated (n = 4) and ML216-treated (n = 5) aging C57BL/6 mice, the quantifications of BLM and p21 protein levels in mouse livers by ImageJ. (C) The collagen contents in livers measured by hydroxyproline assay of old mouse treated with vehicle groups (O-Vehicle, n = 11) or ML216 groups (O-ML216, n = 8), young mice (4 months) were used as control (Y-Vehicle, n = 7). All data were presented as mean ±SEM.**
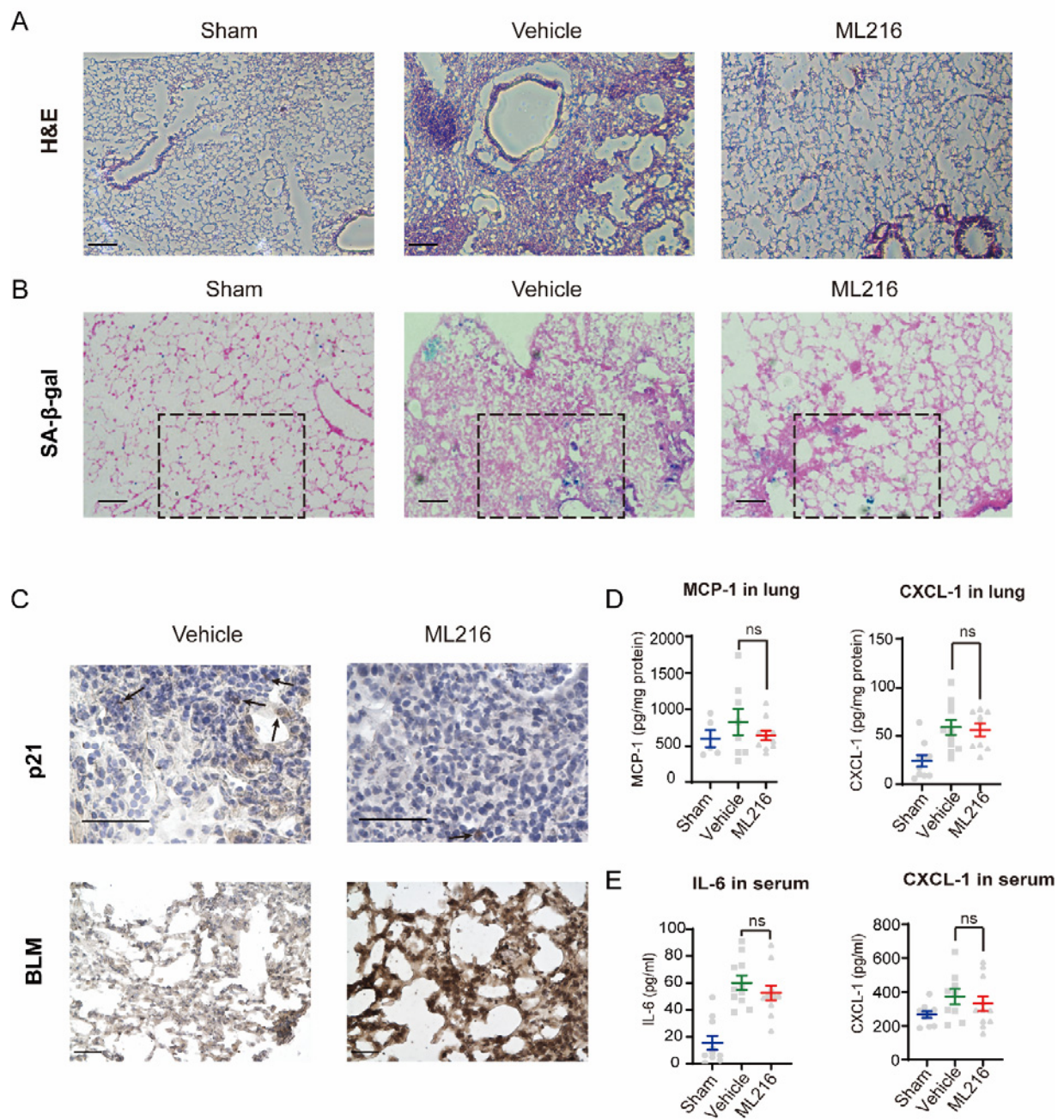
A Body Weight

B ML216 injection

D Rotarod

C Tidal volume TOCCLUDE Max volume change per breath Max pressure change per breath

Supplementary Figure S8. ML216 improves physiological functions in IPF mice.

(**A**) Body weight of vehicle (n = 12) or ML216 (n = 14) treated mice. (**B**) Survival curves of vehicle (n = 12) or ML216 (n = 14) treated mice, long-rank test p=0.3909. (**C**) Pulmonary function measurements of Tidal volume, Tocclude, max volume change per breath and max pressure change per breath in mice (Sham, n = 13-14; Vehicle, n = 11-12; ML216, n = 11-12). (**D**) Quantification of maximal running time in the rotarod test for vehicle (n = 12) or ML216 (n = 14) treated mice. All data were presented as mean ± SEM.

A

Sham | Vehicle | ML216

H&E

B

Sham | Vehicle | ML216

SA-β-gal

C

Vehicle | ML216

p21

BLM

D

MCP-1 in lung

MCP-1 (pg/mg protein)

ns

2000
1500
1000
500
0

Sham | Vehicle | ML216

CXCL-1 in lung

CXCL-1 (pg/mg protein)

ns

150
100
50
0

Sham | Vehicle | ML216

E

IL-6 in serum

IL-6 (pg/ml)

ns

100
80
60
40
20
0

Sham | Vehicle | ML216

CXCL-1 in serum

CXCL-1 (pg/ml)

ns

800
600
400
200
0

Sham | Vehicle | ML216

Supplementary Figure S9 ML216 reduces senescence and inflammation in IPF mice.

(**A**) Representative images of H&E staining on lung sections of mice. Scale bar = 100 μm. (**B**) Representative images of SA-β-gal staining positive cells in mouse lungs. Scale bar= 100 μm. (**C**) Representative images of immunohistochemistry (IHC) staining for p21 and BLM, Scale bar = 25 μm. (**D**) and (**E**) Protein levels of SASP factors IL-6, MCP-1 and CXCL-1 in lung [C] and serum [F], measured by ELISA assay (Sham, n = 5-13; Vehicle, n = 5-12; ML216, n = 7-13). All data are presented as mean ± SEM.
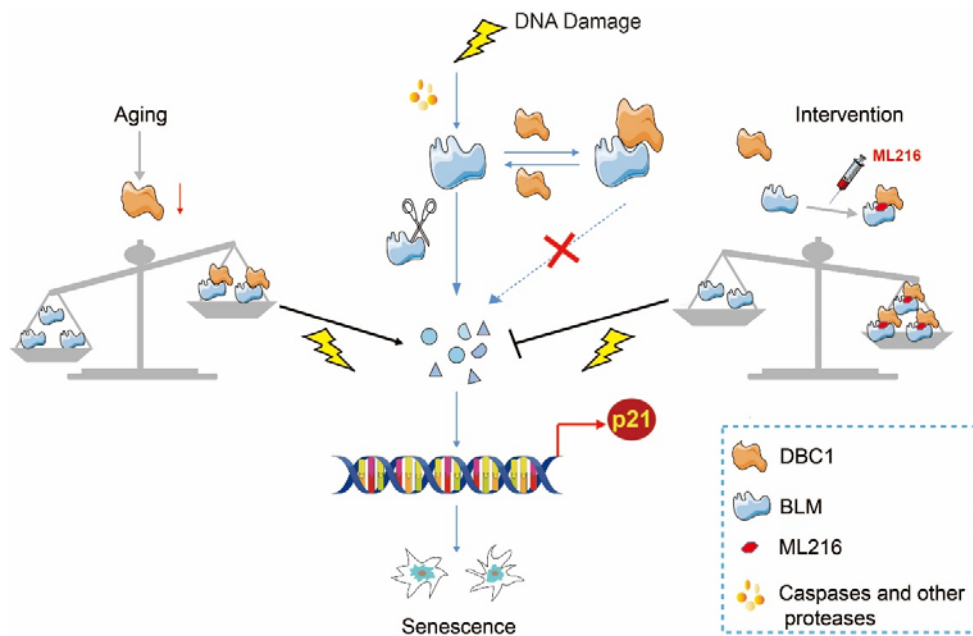
Supplementary Figure S10 A model for BLM-DBC1 interaction in regulating senescence induction.**This Fig. was made with Servier Medical Art templates, which are licensed under a Creative Commons Attribution 3.0 Unported Licence (www.smart.servier.com).**