# Ferroptosis activate retinoic acid inflammation ignite the development of Silicosis

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## Abstract

Lung cells damage caused by inhalation of silica and silicon crystals leads to Silicosis. Retinoic acid is a mitogen in the development of lung organs and exerts pleiotropic effects on immune reactions. How retinoic acid signaling engaged in Silicosis remains unknown. We report here that retinoic acid signaling in dendritic cells was activated in silicosis lesions. SiO2 activates the retinoic acid signaling by provoking ferroptosis. Ferroptosis trigger a downstream "retinoic acid inflammation" characterized by upregulating cGAS-STING signaling genes and inflammasome associated IL-1βand IL-1α. However, knockdown of retinoic acid receptor α slightly mitigates ferroptosis-induced cell death. Inhibition of ferroptosis in mice relieves silica-induced lung inflammation and fibrosis. This cellular retinoic acid response may act as a cytosolic adjuvant to promote extended and upgraded inflammation. Our work unveils a mechanism by which retinoic acid reaction integrates cGAS-STING and inflammasome signaling to sustain silica-induced inflammation.

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Figure 1

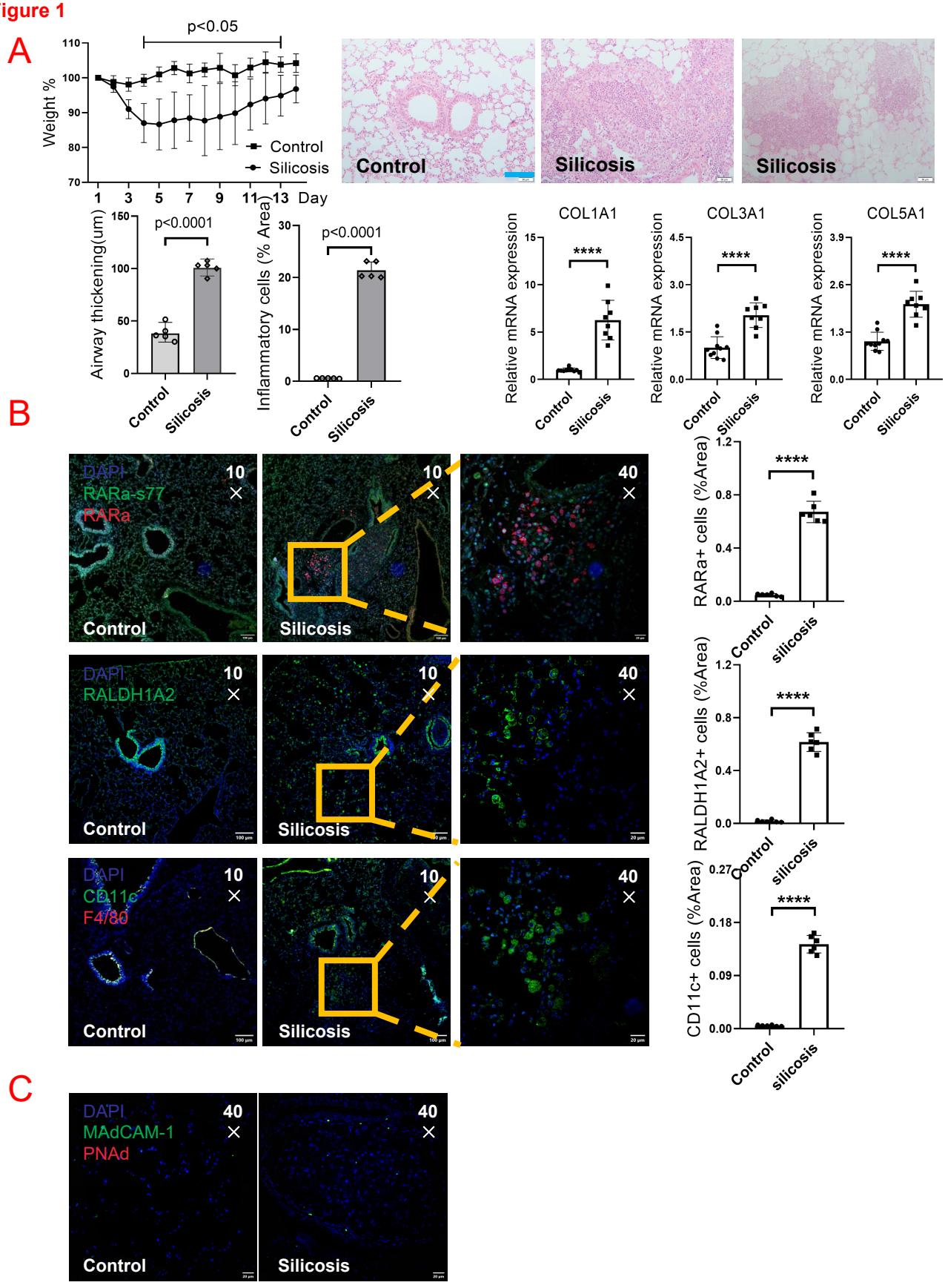
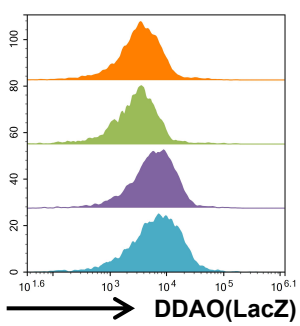
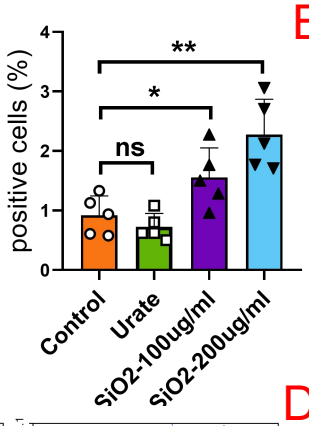

A
p<0.05
Weight %
Control
Silicosis
Day
Control
Silicosis
Silicosis
p<0.0001
Airway thickening(um)
p<0.0001
Inflammatory cells (% Area)
COL1A1
COL3A1
COL5A1
Relative mRNA expression
****
B
DAPI
RARa-s77
RARa
RALDH1A2
CD11c
F4/80
10
40
RARa+ cells (%Area)
RALDH1A2+ cells (%Area)
CD11c+ cells (%Area)
C
MAdCAM-1
PNAd

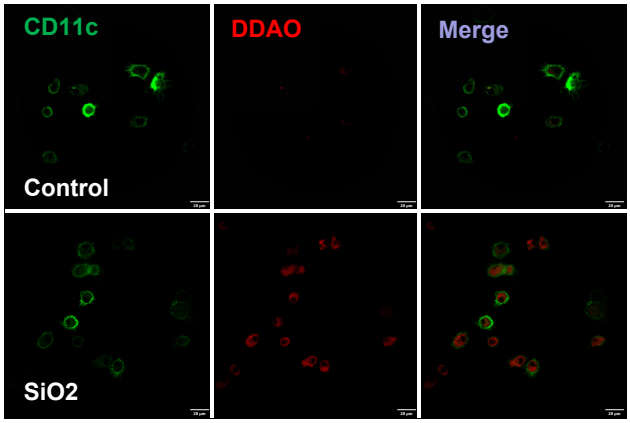
Figure 2

A

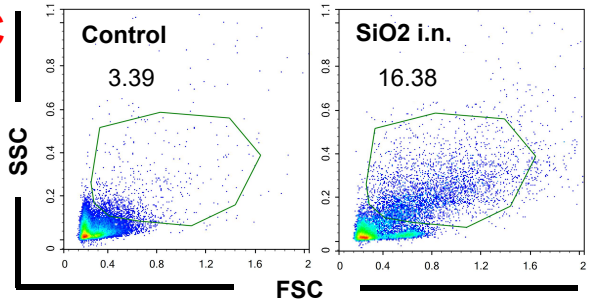


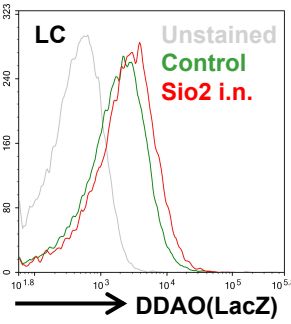



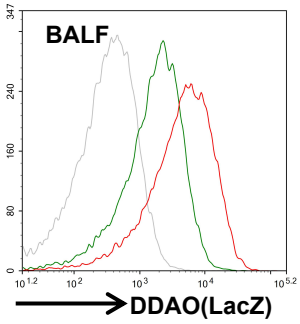

B

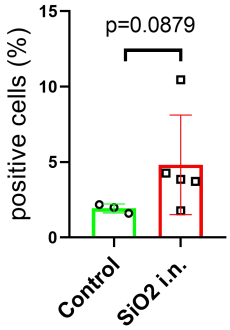


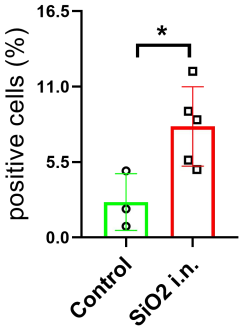

C

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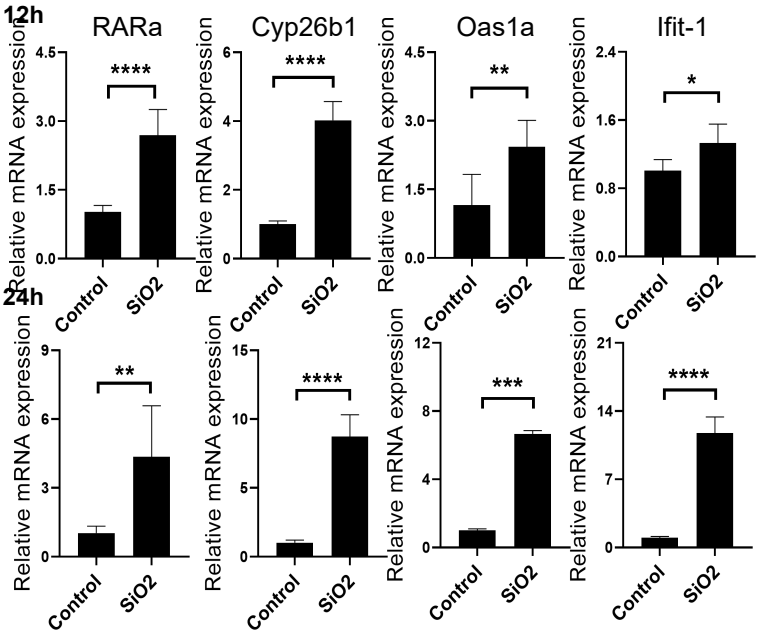


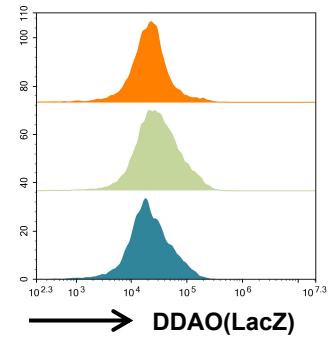


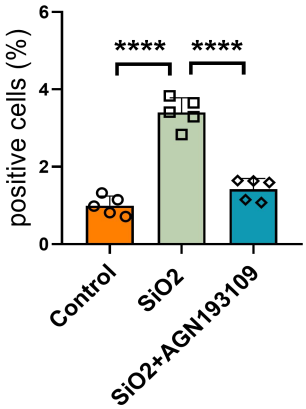


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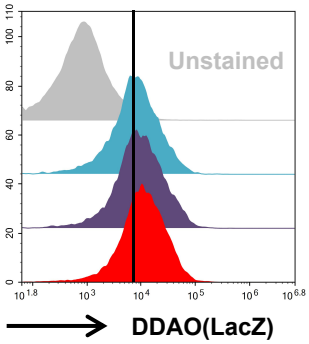


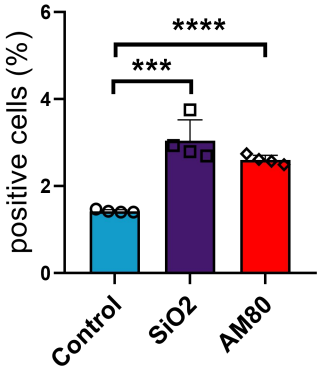



Figure 3

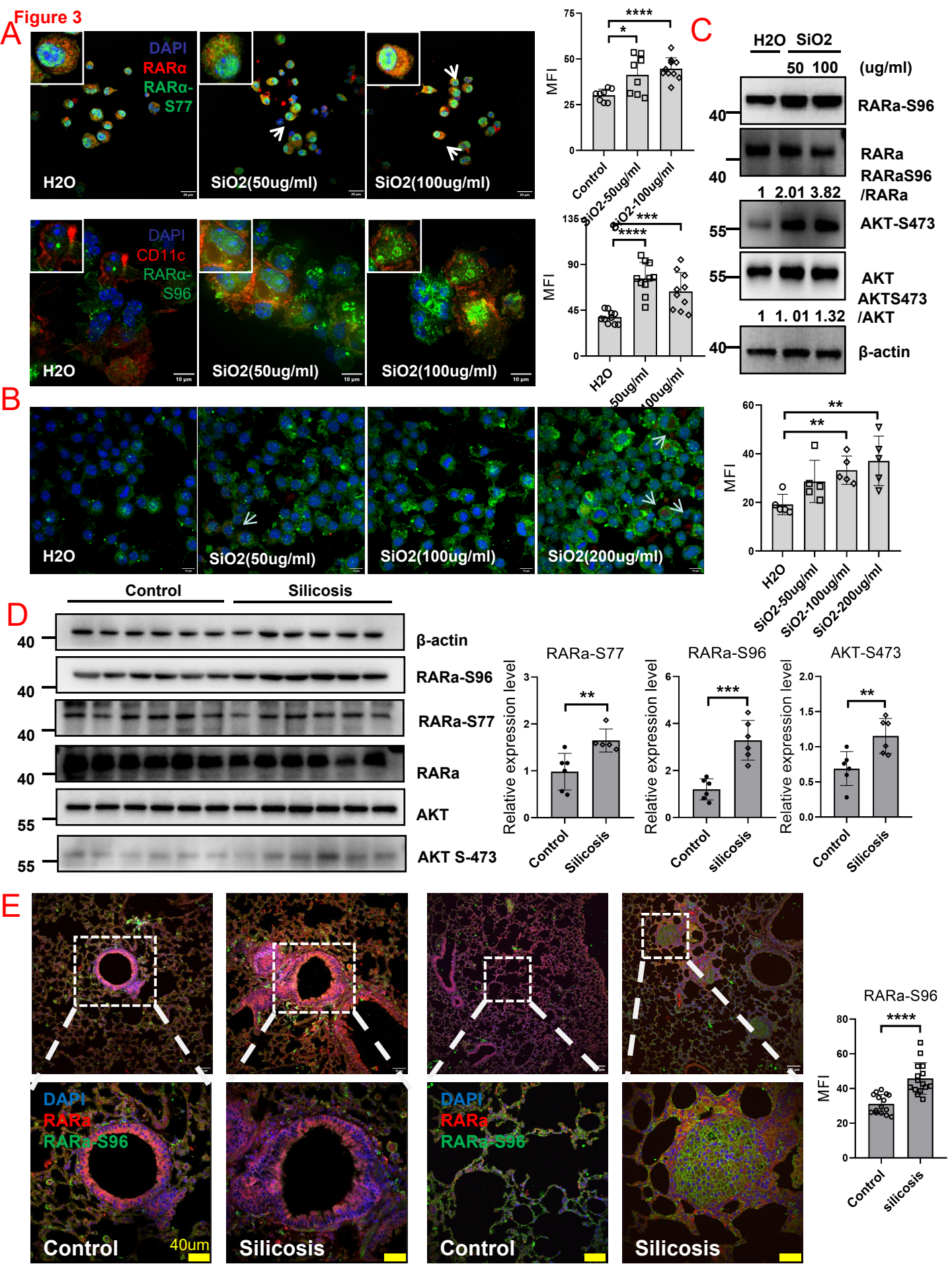

A
DAPI
RARα
RARα-S77
H2O
SiO2(50ug/ml)
SiO2(100ug/ml)
MFI
Control
SiO2-50ug/ml
SiO2-100ug/ml
DAPI
CD11c
RARα-S96
H2O
SiO2(50ug/ml)
SiO2(100ug/ml)
MFI
H2O
50ug/ml
100ug/ml
C
H2O SiO2
50 100 (ug/ml)
RARa-S96
RARa
RARaS96 /RARa
1 2.01 3.82
AKT-S473
AKT
AKTS473 /AKT
1 1. 01 1.32
β-actin
B
H2O
SiO2(50ug/ml)
SiO2(100ug/ml)
SiO2(200ug/ml)
MFI
H2O
SiO2-50ug/ml
SiO2-100ug/ml
SiO2-200ug/ml
D
Control
Silicosis
β-actin
RARa-S96
RARa-S77
RARa
AKT
AKT S-473
RARa-S77
RARa-S96
AKT-S473
Relative expression level
Control
Silicosis
E
DAPI
RARa
RARa-S96
Control
40um
Silicosis
Control
Silicosis
RARa-S96
MFI
Control
silicosis

Figure 4

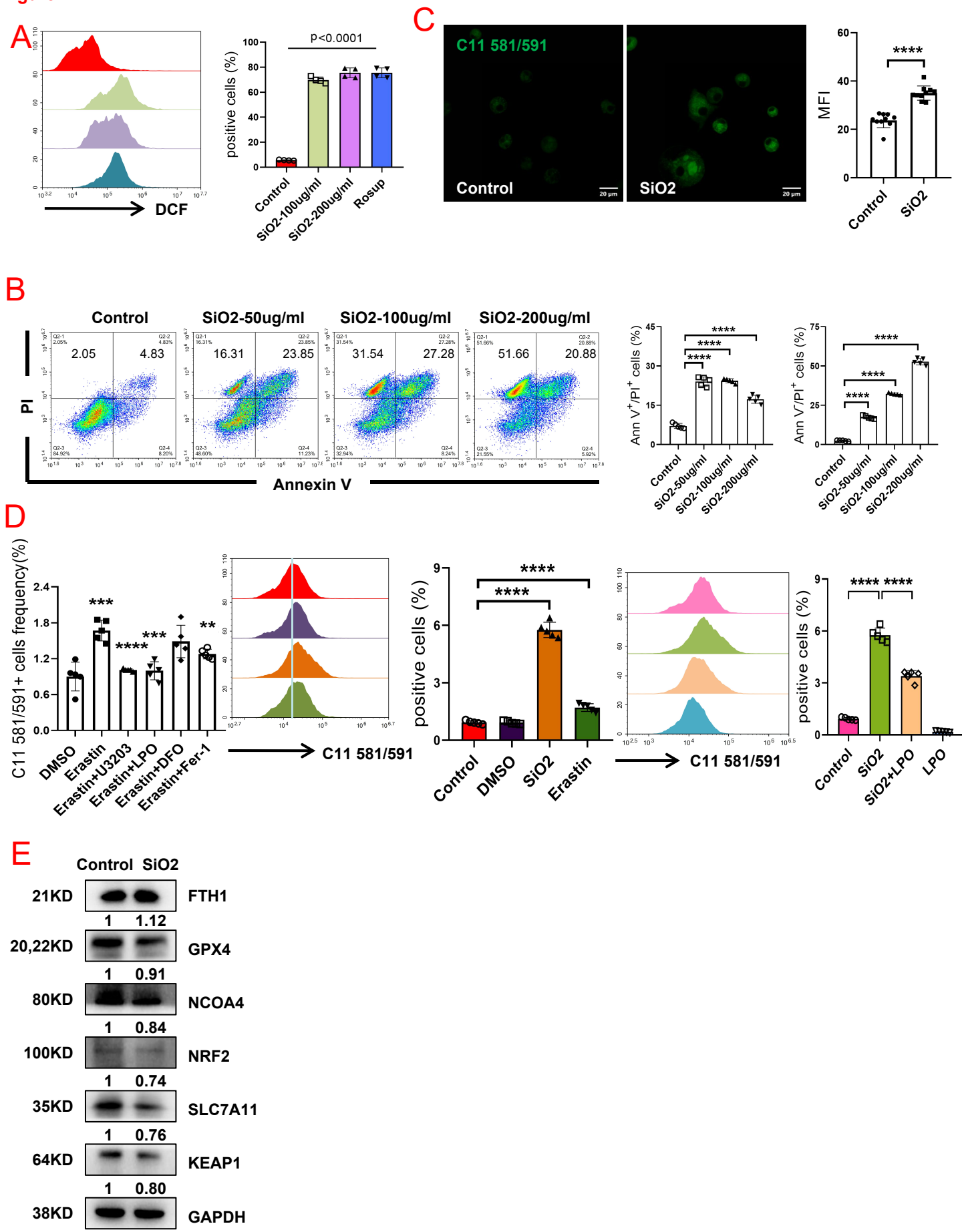

A
DCF
p<0.0001
positive cells (%)
Control
SiO2-100ug/ml
SiO2-200ug/ml
Rosup
C
C11 581/591
Control
SiO2
MFI
****
B
Control
SiO2-50ug/ml
SiO2-100ug/ml
SiO2-200ug/ml
2.05
4.83
16.31
23.85
31.54
27.28
51.66
20.88
PI
Annexin V
Ann V+/PI+ cells (%)
Ann V-/PI+ cells (%)
D
C11 581/591+ cells frequency(%)
DMSO
Erastin
Erastin+U3203
Erastin+LPO
Erastin+DFO
Erastin+Fer-1
C11 581/591
positive cells (%)
Control
DMSO
SiO2
Erastin
SiO2+LPO
LPO
E
Control
SiO2
21KD
FTH1
1
1.12
20,22KD
GPX4
1
0.91
80KD
NCOA4
1
0.84
100KD
NRF2
1
0.74
35KD
SLC7A11
1
0.76
64KD
KEAP1
1
0.80
38KD
GAPDH

Figure 5

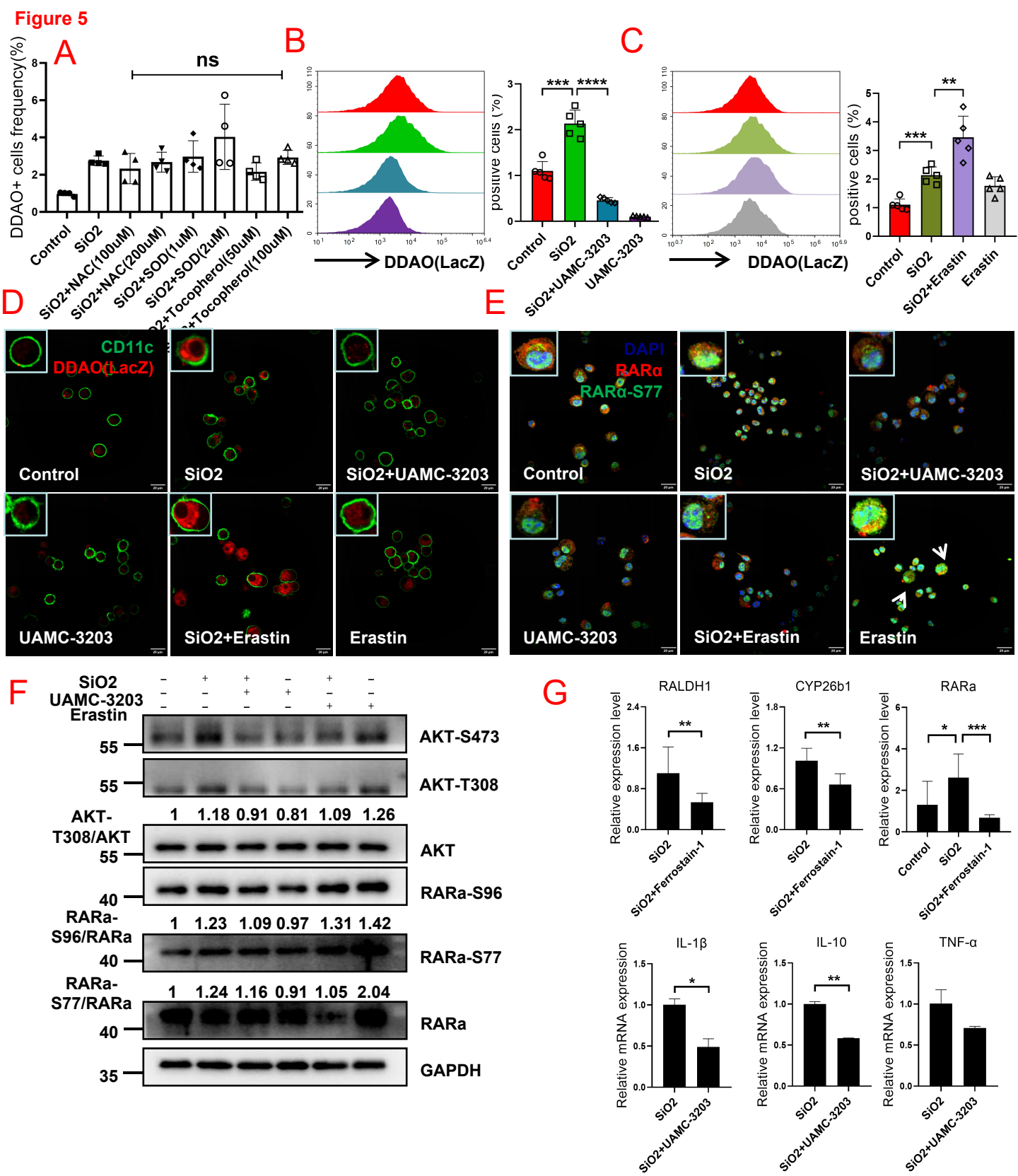

A
ns
DDAO+ cells frequency(%)
Control
SiO2
SiO2+NAC(100uM)
SiO2+NAC(200uM)
SiO2+SOD(1uM)
SiO2+SOD(2uM)
SiO2+Tocopherol(50uM)
SiO2+Tocopherol(100uM)
B
DDAO(LacZ)
positive cells (%)
***
****
Control
SiO2
SiO2+UAMC-3203
UAMC-3203
C
DDAO(LacZ)
positive cells (%)
***
**
Control
SiO2
SiO2+Erastin
Erastin
D
CD11c
DDAO(LacZ)
Control
SiO2
SiO2+UAMC-3203
UAMC-3203
SiO2+Erastin
Erastin
E
DAPI
RARα
RARα-S77
Control
SiO2
SiO2+UAMC-3203
UAMC-3203
SiO2+Erastin
Erastin
F
SiO2
UAMC-3203
Erastin
55
AKT-S473
55
AKT-T308
AKT-T308/AKT
1 1.18 0.91 0.81 1.09 1.26
55
AKT
40
RARa-S96
RARa-S96/RARa
1 1.23 1.09 0.97 1.31 1.42
40
RARa-S77
RARa-S77/RARa
1 1.24 1.16 0.91 1.05 2.04
40
RARa
35
GAPDH
G
RALDH1
CYP26b1
RARa
Relative expression level
SiO2
SiO2+Ferrostain-1
Control
IL-1β
IL-10
TNF-α
Relative mRNA expression
SiO2
SiO2+UAMC-3203

Figure 6

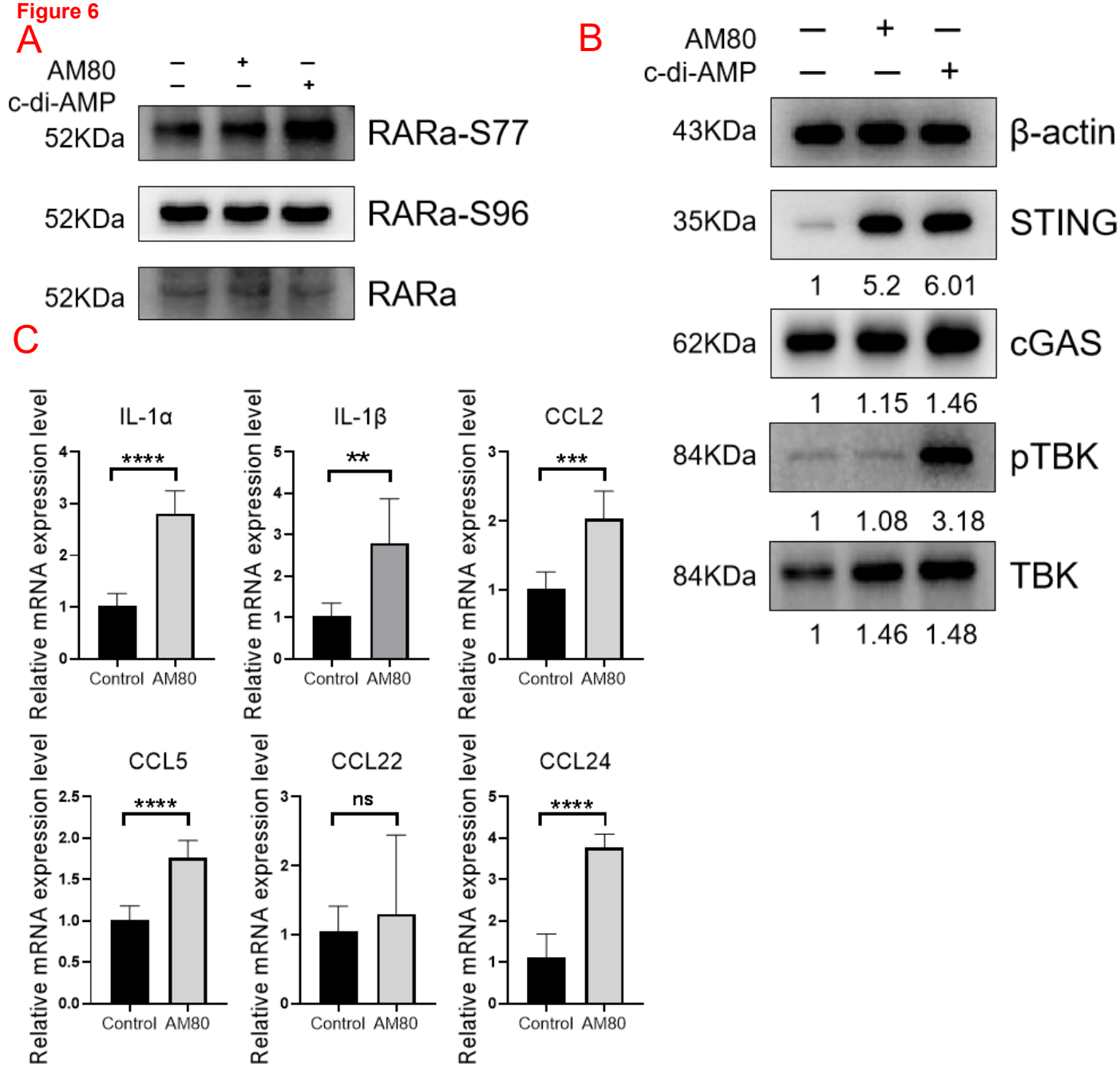

A
AM80
c-di-AMP
52KDa
RARa-S77
52KDa
RARa-S96
52KDa
RARa
B
AM80
c-di-AMP
43KDa
β-actin
35KDa
STING
1 5.2 6.01
62KDa
cGAS
1 1.15 1.46
84KDa
pTBK
1 1.08 3.18
84KDa
TBK
1 1.46 1.48
C
IL-1α
IL-1β
CCL2
CCL5
CCL22
CCL24
Relative mRNA expression level
Control
AM80
****
**
***
ns

Figure 7

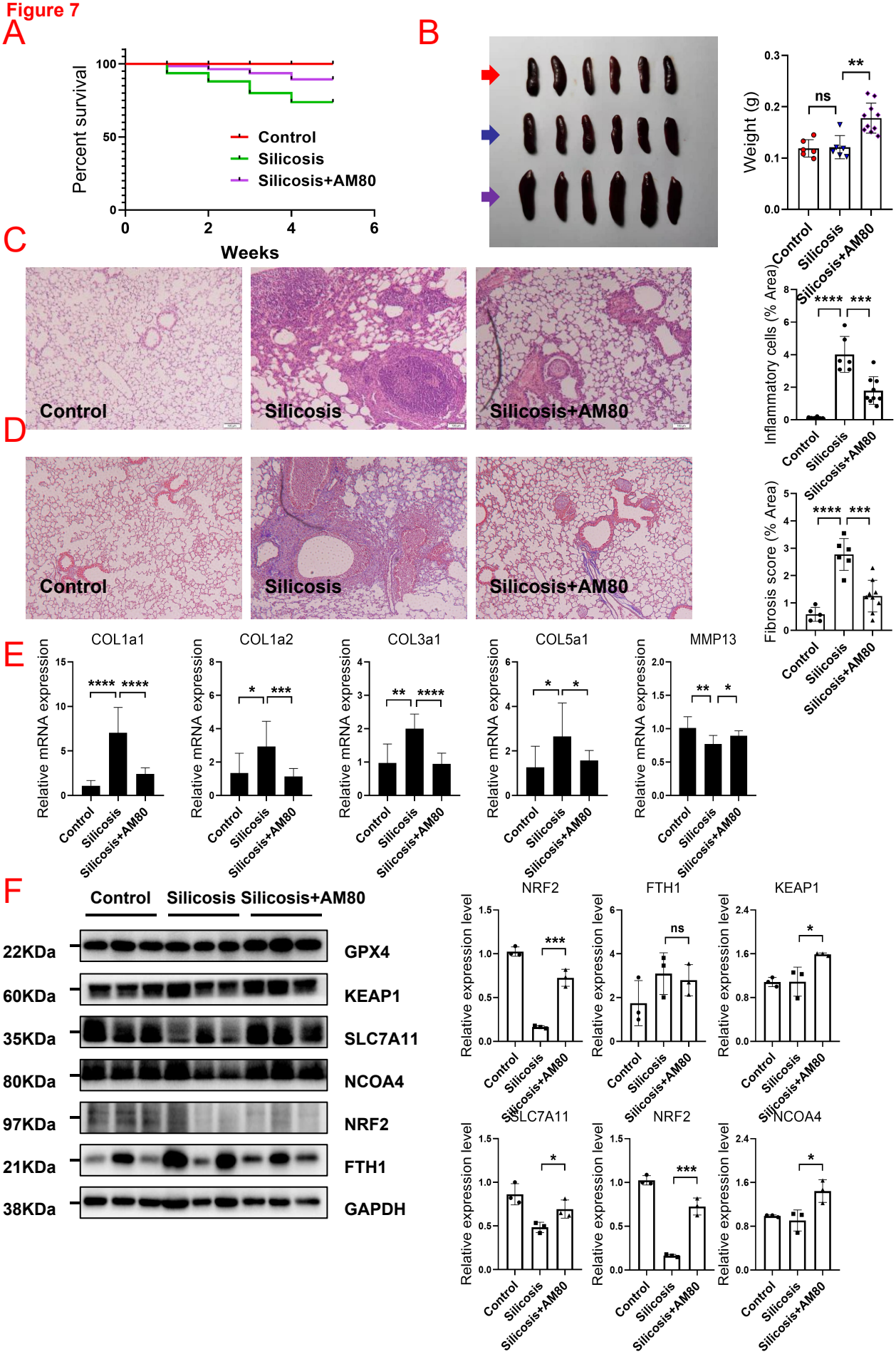
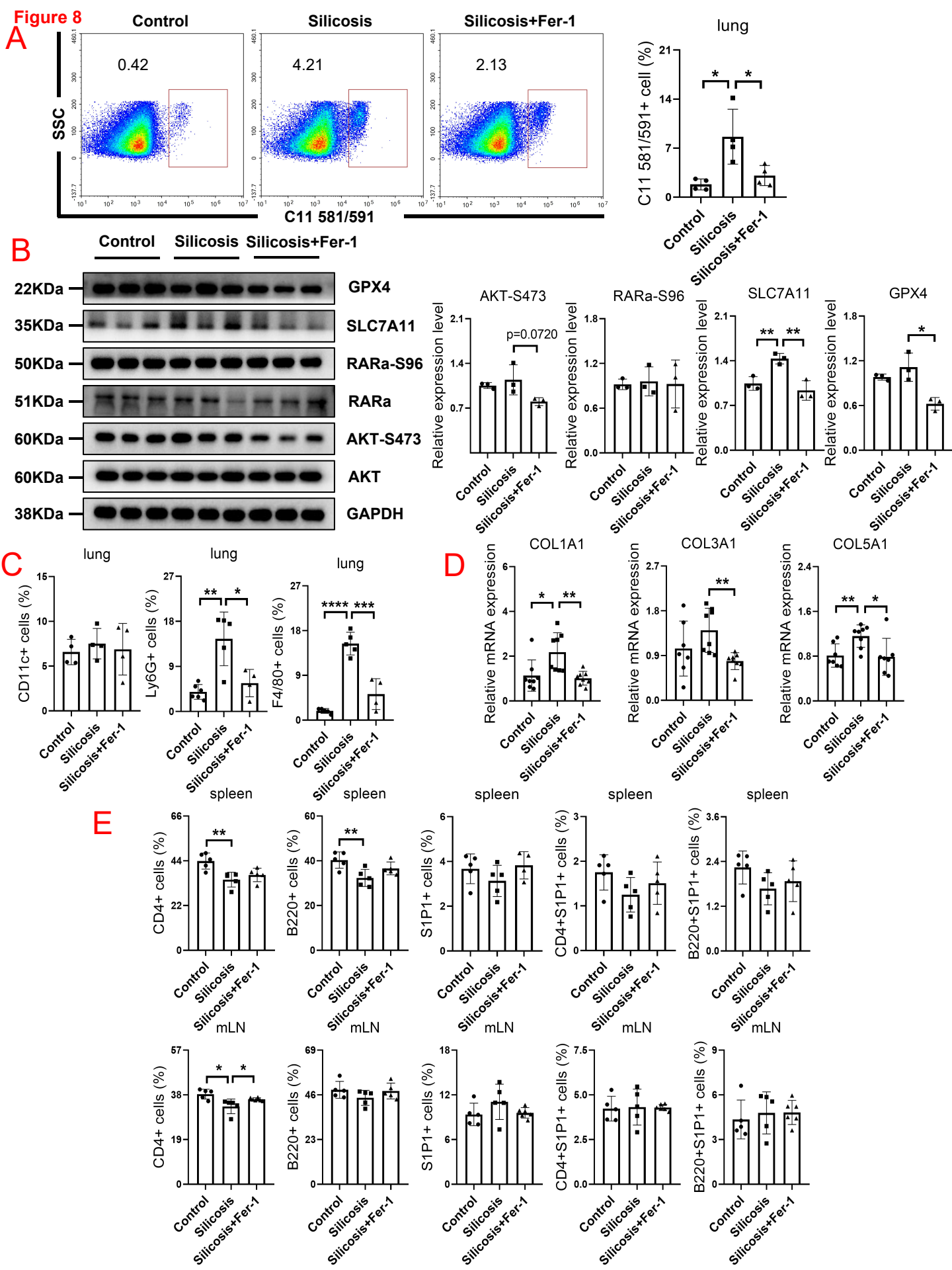
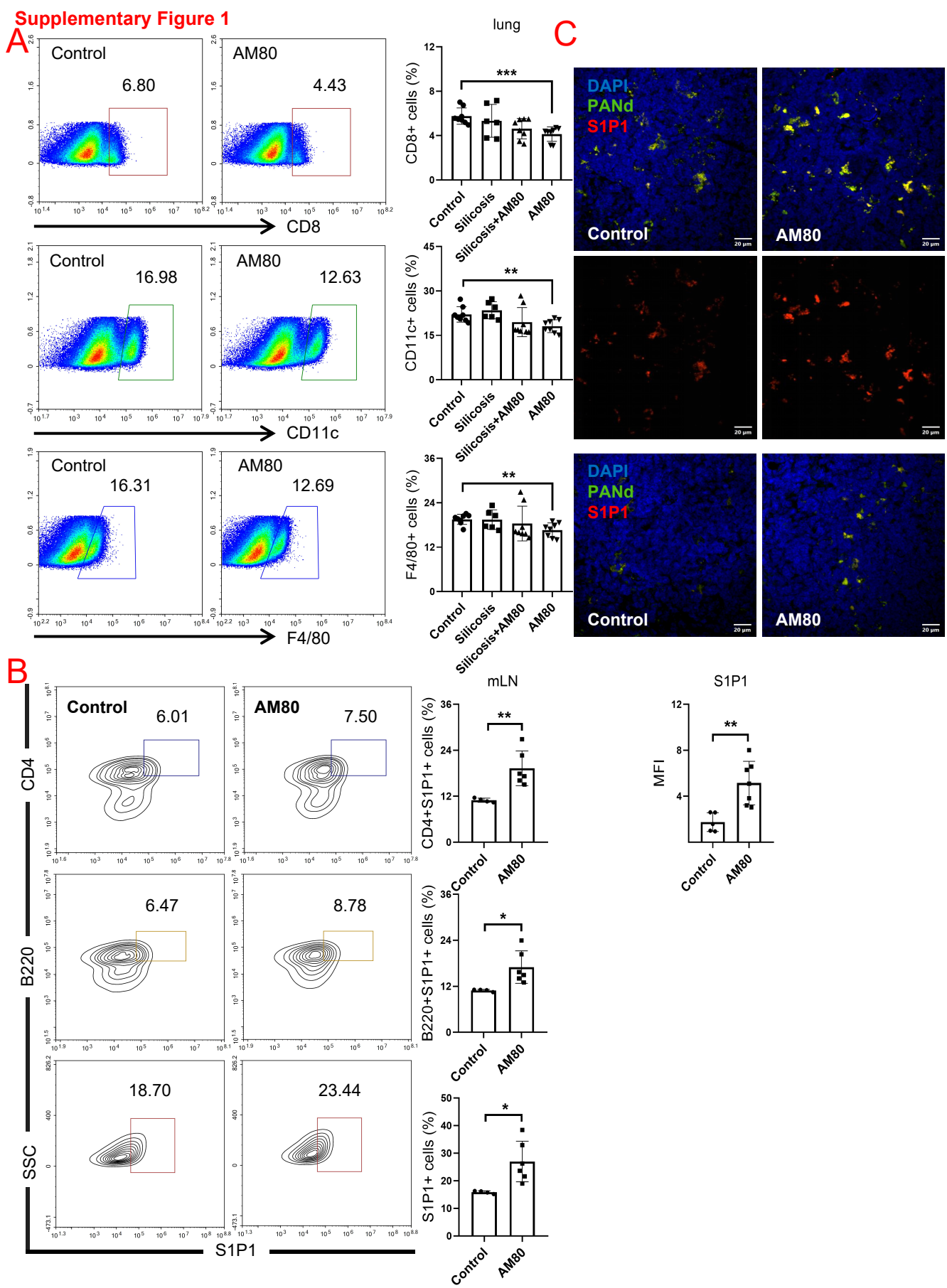

A
Percent survival
Weeks
Control
Silicosis
Silicosis+AM80
B
Weight (g)
ns
**
C
Control
Silicosis
Silicosis+AM80
Inflammatory cells (% Area)
****
***
D
Fibrosis score (% Area)
E
COL1a1
COL1a2
COL3a1
COL5a1
MMP13
Relative mRNA expression
F
22KDa
60KDa
35KDa
80KDa
97KDa
21KDa
38KDa
GPX4
KEAP1
SLC7A11
NCOA4
NRF2
FTH1
GAPDH
Relative expression level
ns

Figure 8

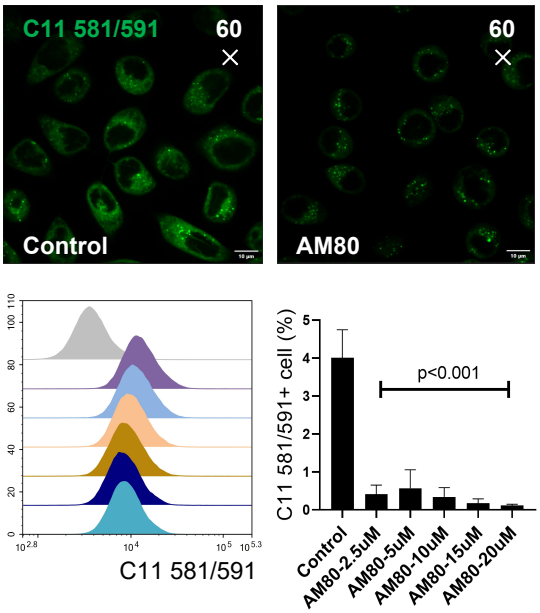

A
Control
Silicosis
Silicosis+Fer-1
0.42
4.21
2.13
SSC
C11 581/591
lung
C11 581/591+ cell (%)
B
22KDa
35KDa
50KDa
51KDa
60KDa
60KDa
38KDa
GPX4
SLC7A11
RARa-S96
RARa
AKT-S473
AKT
GAPDH
AKT-S473
RARa-S96
SLC7A11
GPX4
Relative expression level
p=0.0720
C
lung
CD11c+ cells (%)
Ly6G+ cells (%)
F4/80+ cells (%)
D
COL1A1
COL3A1
COL5A1
Relative mRNA expression
E
spleen
mLN
CD4+ cells (%)
B220+ cells (%)
S1P1+ cells (%)
CD4+S1P1+ cells (%)
B220+S1P1+ cells (%)
Control
Silicosis
Silicosis+Fer-1

Supplementary Figure 1

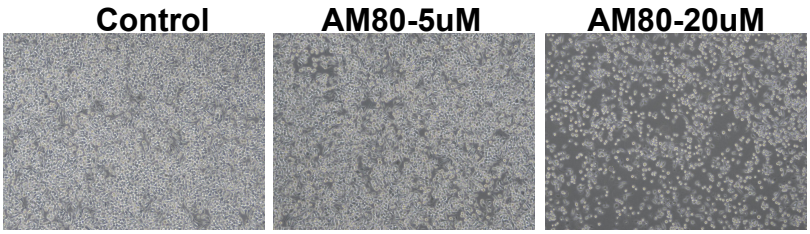


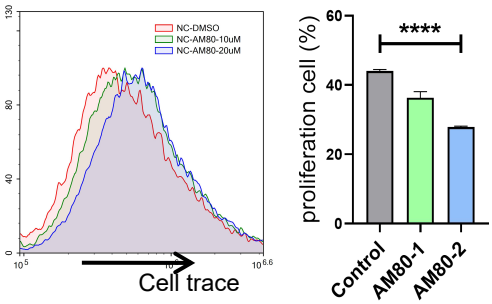
Supplementary Figure 2

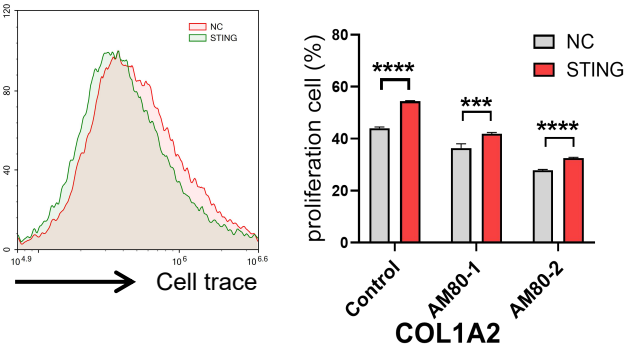
A



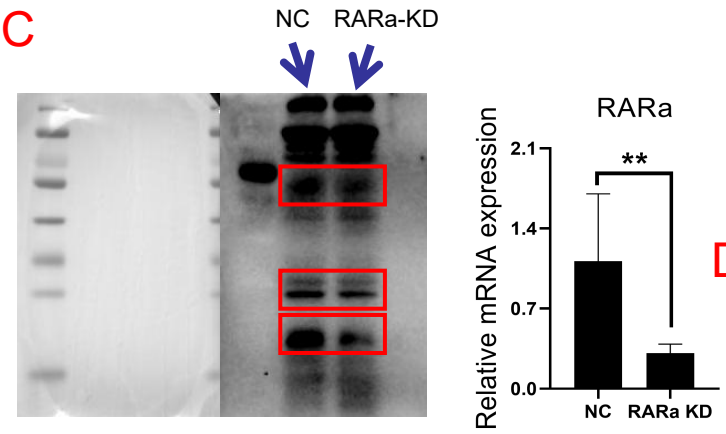

B

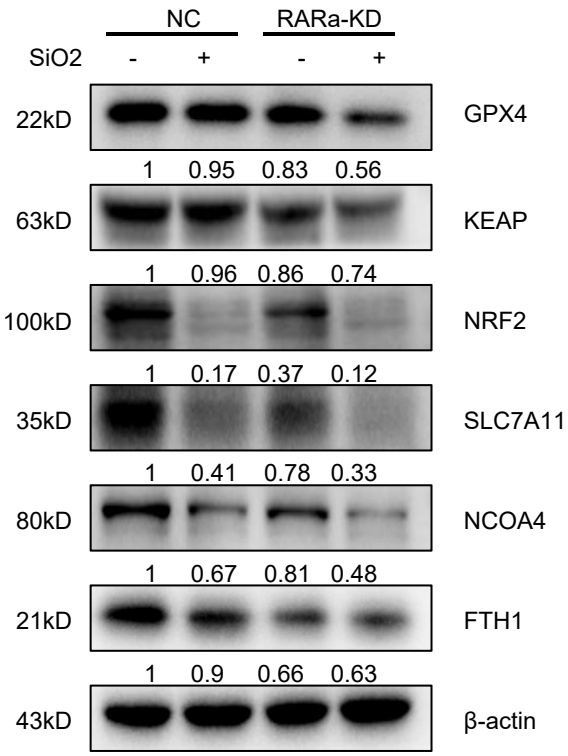



C

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