FIGURE 1

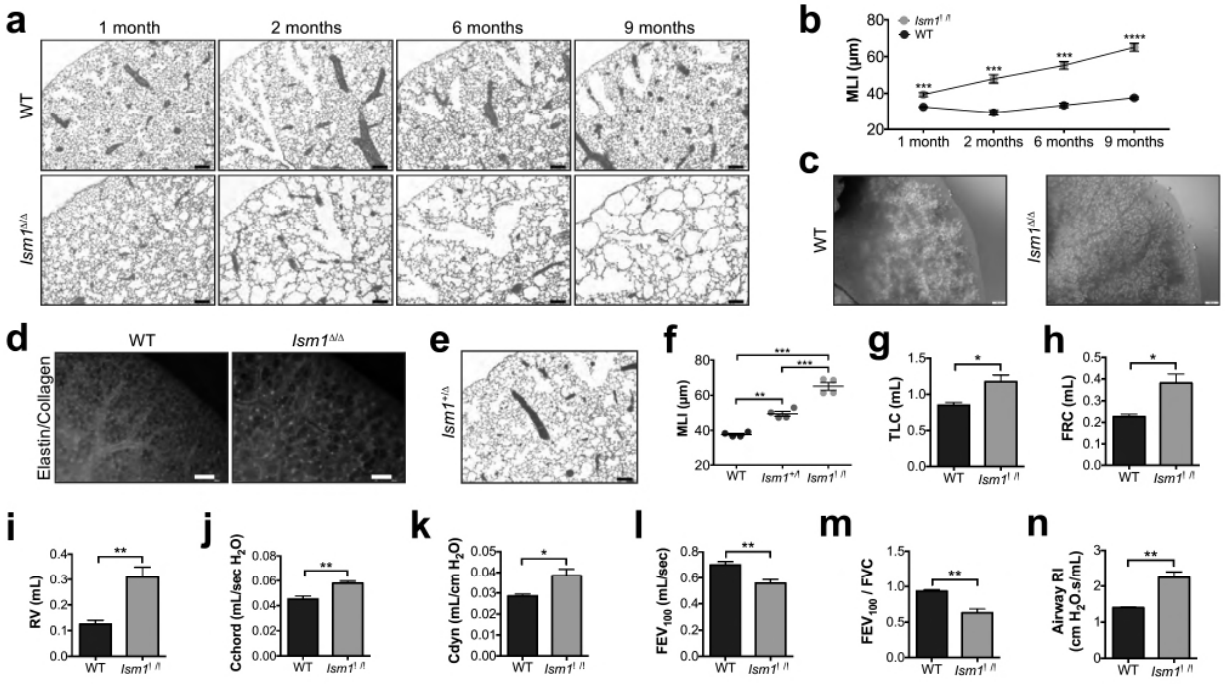

a
1 month
2 months
6 months
9 months
WT
lsm1Δ/Δ
b
lsm1f/f
WT
MLI (µm)
1 month
2 months
6 months
9 months
c
WT
lsm1Δ/Δ
d
WT
lsm1Δ/Δ
Elastin/Collagen
e
lsm1+/Δ
f
MLI (µm)
WT
lsm1+/f
lsm1f/f
g
TLC (mL)
h
FRC (mL)
i
RV (mL)
j
Cchord (mL/sec H2O)
k
Cdyn (mL/cm H2O)
l
FEV100 (mL/sec)
m
FEV100 / FVC
n
Airway RI (cm H2O.s/mL)

FIGURE 2

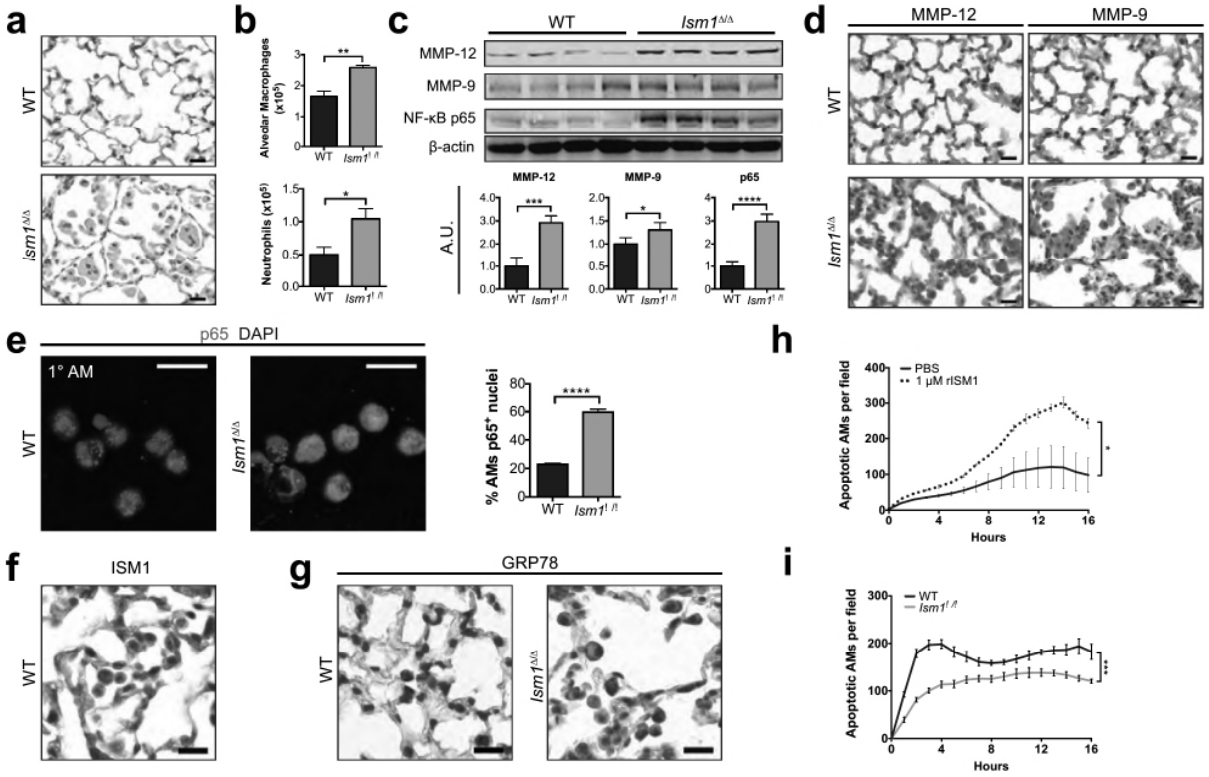

a
WT
Ism1ΔΔ
b
Alveolar Macrophages (x10^5)
Neutrophils (x10^5)
WT
Ism1 fl/fl
c
WT
Ism1ΔΔ
MMP-12
MMP-9
NF-κB p65
β-actin
A.U.
d
MMP-12
MMP-9
e
p65 DAPI
1° AM
% AMs p65+ nuclei
f
ISM1
g
GRP78
h
PBS
1 μM rISM1
Apoptotic AMs per field
Hours
i
Apoptotic AMs per field
Hours

FIGURE 3

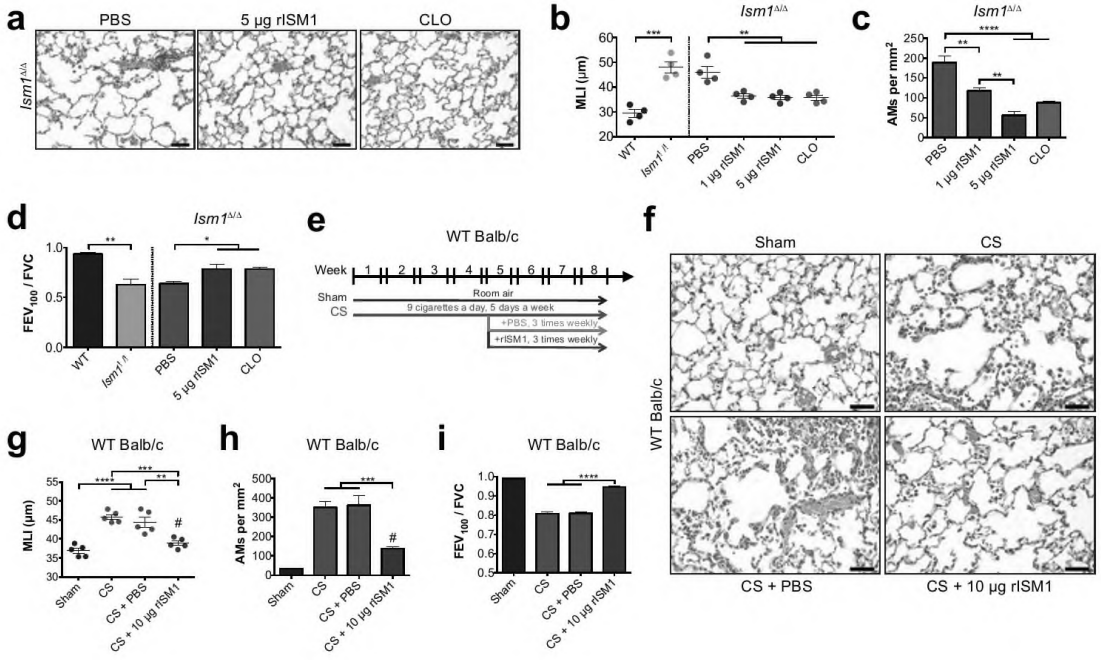

a
PBS
5 µg rISM1
CLO
Ism1Δ/Δ
b
Ism1Δ/Δ
MLI (µm)
WT
Ism1 f/f
PBS
1 µg rISM1
5 µg rISM1
CLO
c
Ism1Δ/Δ
AMs per mm2
PBS
1 µg rISM1
5 µg rISM1
CLO
d
Ism1Δ/Δ
FEV100 / FVC
WT
Ism1 f/f
PBS
5 µg rISM1
CLO
e
WT Balb/c
Week
1 2 3 4 5 6 7 8
Sham
CS
Room air
9 cigarettes a day, 5 days a week
+PBS, 3 times weekly
+rISM1, 3 times weekly
f
Sham
CS
WT Balb/c
CS + PBS
CS + 10 µg rISM1
g
WT Balb/c
MLI (µm)
Sham
CS
CS + PBS
CS + 10 µg rISM1
h
WT Balb/c
AMs per mm2
Sham
CS
CS + PBS
CS + 10 µg rISM1
i
WT Balb/c
FEV100 / FVC
Sham
CS
CS + PBS
CS + 10 µg rISM1

FIGURE 4

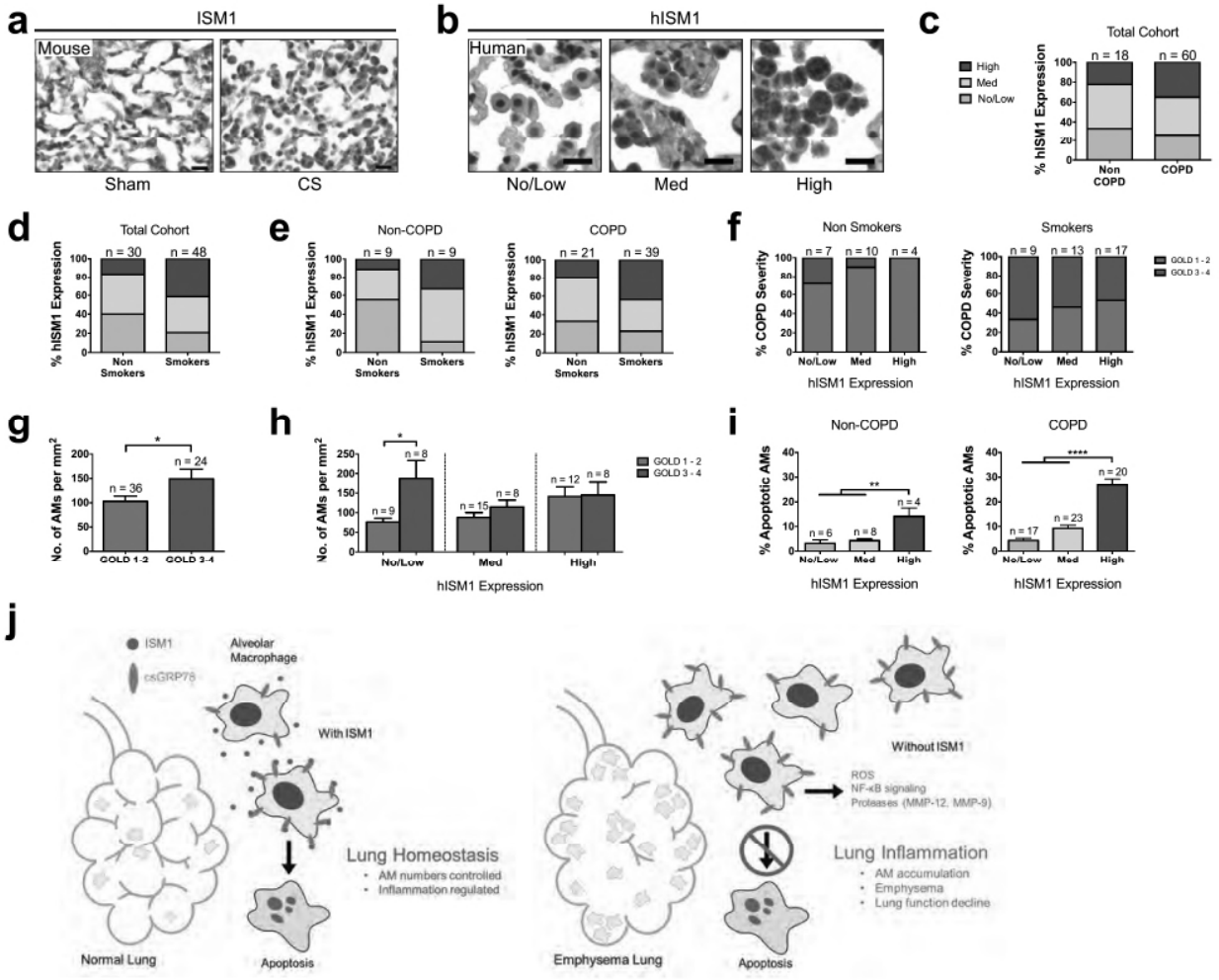

a
ISM1
Mouse
Sham
CS
b
hISM1
Human
No/Low
Med
High
c
Total Cohort
High
Med
No/Low
% hISM1 Expression
n = 18
n = 60
Non COPD
COPD
d
Total Cohort
% hISM1 Expression
n = 30
n = 48
Non Smokers
Smokers
e
Non-COPD
COPD
% hISM1 Expression
n = 9
n = 9
n = 21
n = 39
Non Smokers
Smokers
f
Non Smokers
Smokers
% COPD Severity
n = 7
n = 10
n = 4
n = 9
n = 13
n = 17
No/Low
Med
High
hISM1 Expression
GOLD 1 - 2
GOLD 3 - 4
g
No. of AMs per mm²
n = 36
n = 24
GOLD 1-2
GOLD 3-4
h
No. of AMs per mm²
n = 9
n = 8
n = 15
n = 8
n = 12
n = 8
No/Low
Med
High
hISM1 Expression
GOLD 1 - 2
GOLD 3 - 4
i
Non-COPD
COPD
% Apoptotic AMs
n = 6
n = 8
n = 4
n = 17
n = 23
n = 20
No/Low
Med
High
hISM1 Expression
j
ISM1
csGRP78
Alveolar Macrophage
With ISM1
Lung Homeostasis
AM numbers controlled
Inflammation regulated
Normal Lung
Apoptosis
Without ISM1
ROS
NF-κB signaling
Proteases (MMP-12, MMP-9)
Lung Inflammation
AM accumulation
Emphysema
Lung function decline
Emphysema Lung
Apoptosis

FIGURE 5

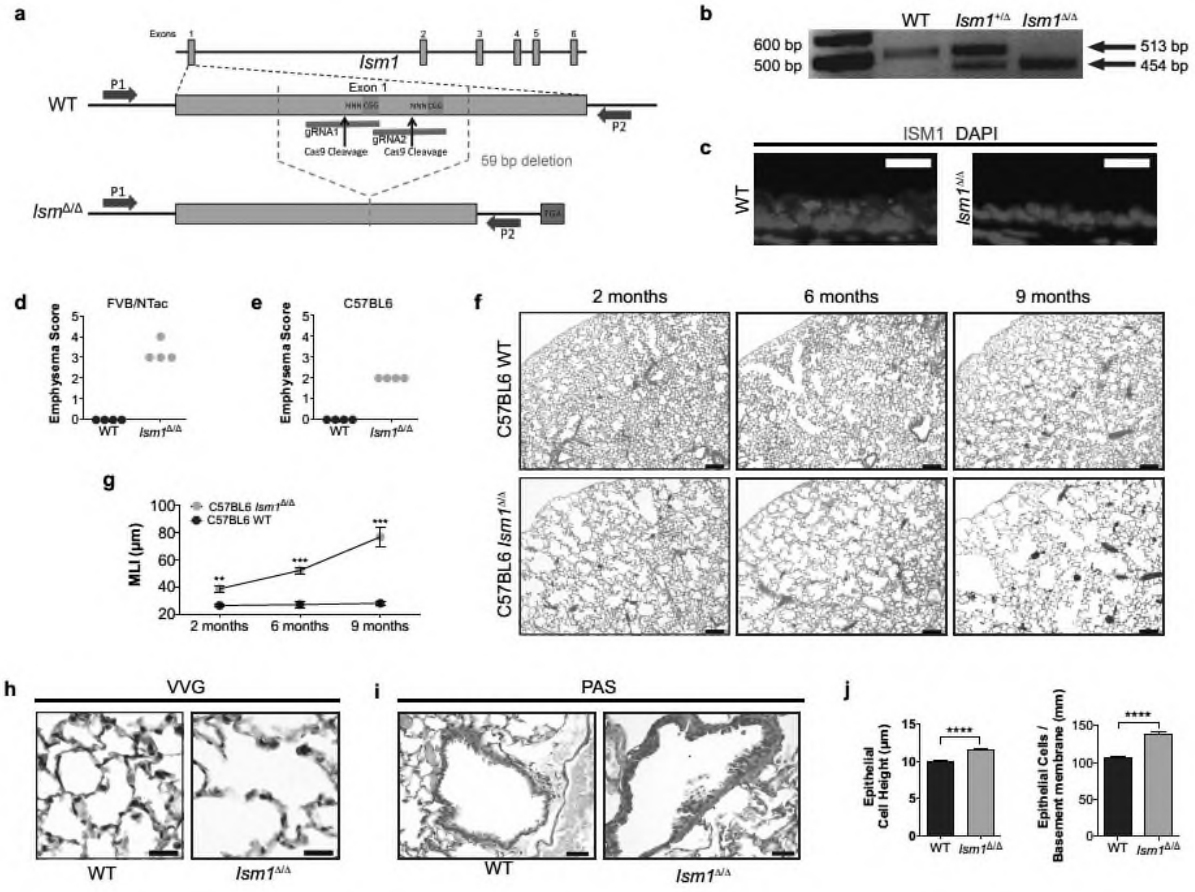

a
Exons
lsm1
Exon 1
WT
P1
P2
gRNA1
gRNA2
Cas9 Cleavage
Cas9 Cleavage
59 bp deletion
lsm1Δ/Δ
b
WT
lsm1+/Δ
lsm1Δ/Δ
600 bp
500 bp
513 bp
454 bp
c
ISM1 DAPI
WT
lsm1Δ/Δ
d
FVB/NTac
Emphysema Score
e
C57BL6
f
2 months
6 months
9 months
C57BL6 WT
C57BL6 lsm1Δ/Δ
g
C57BL6 lsm1Δ/Δ
C57BL6 WT
MLI (µm)
h
VVG
i
PAS
j
Epithelial Cell Height (µm)
Epithelial Cells / Basement membrane (mm)

FIGURE 6

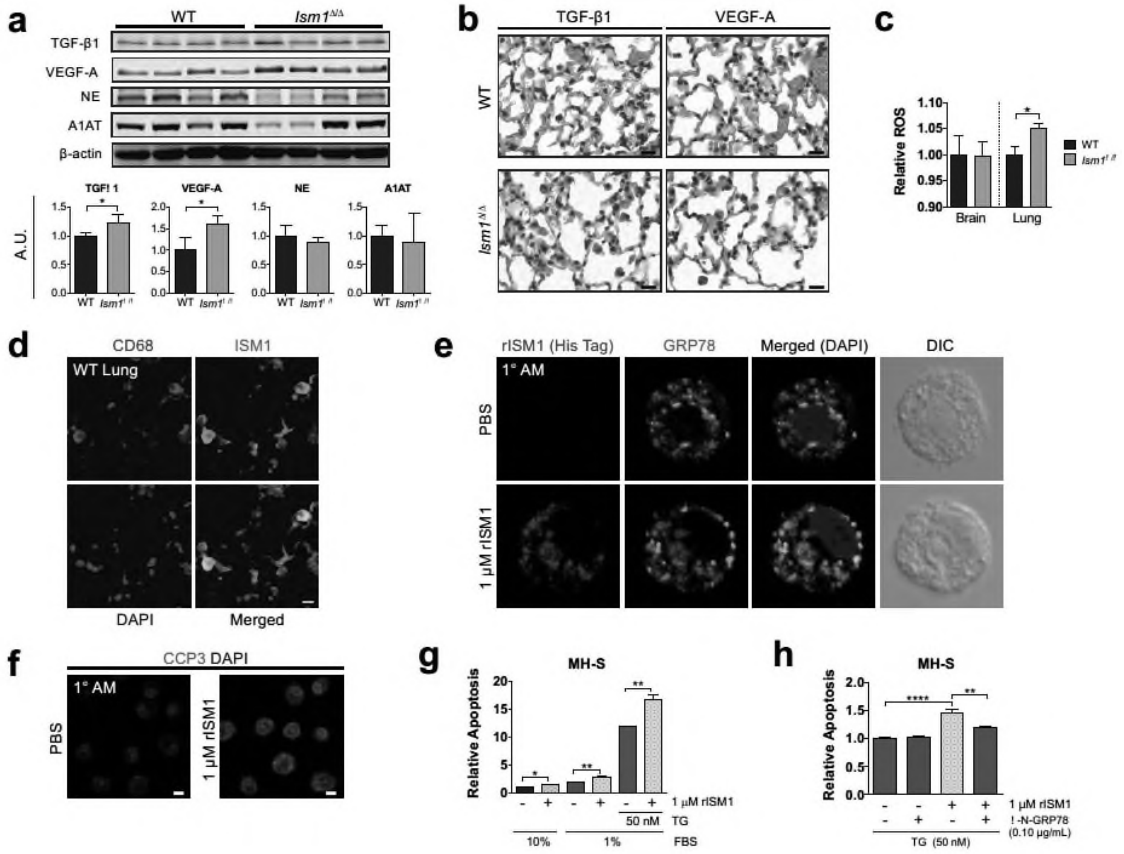

a
WT
Ism1Δ/Δ
TGF-β1
VEGF-A
NE
A1AT
β-actin
A.U.
b
TGF-β1
VEGF-A
WT
Ism1Δ/Δ
c
Relative ROS
Brain
Lung
WT
Ism1-/-
d
CD68
ISM1
WT Lung
DAPI
Merged
e
rISM1 (His Tag)
GRP78
Merged (DAPI)
DIC
1° AM
PBS
1 μM rISM1
f
CCP3 DAPI
1° AM
PBS
1 μM rISM1
g
MH-S
Relative Apoptosis
1 μM rISM1
50 nM TG
10%
1%
FBS
h
MH-S
Relative Apoptosis
1 μM rISM1
! -N-GRP78 (0.10 μg/mL)
TG (50 nM)

FIGURE 7

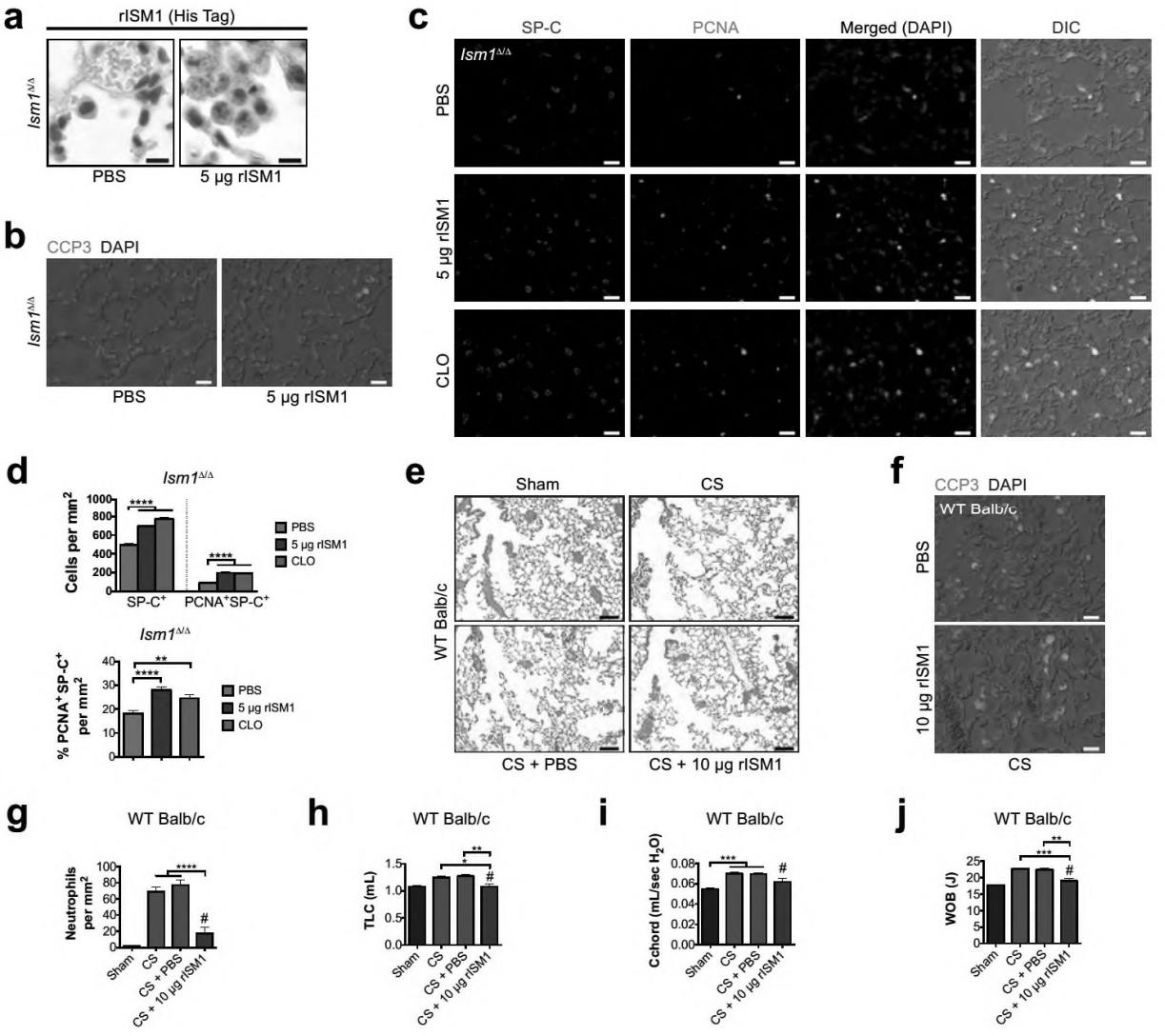

a
rISM1 (His Tag)
Ism1Δ/Δ
PBS
5 μg rISM1
b
CCP3 DAPI
Ism1Δ/Δ
PBS
5 μg rISM1
c
SP-C
PCNA
Merged (DAPI)
DIC
Ism1Δ/Δ
PBS
5 μg rISM1
CLO
d
Ism1Δ/Δ
Cells per mm2
SP-C+
PCNA+SP-C+
PBS
5 μg rISM1
CLO
Ism1Δ/Δ
% PCNA+SP-C+ per mm2
PBS
5 μg rISM1
CLO
e
Sham
CS
WT Balb/c
CS + PBS
CS + 10 μg rISM1
f
CCP3 DAPI
WT Balb/c
PBS
10 μg rISM1
CS
g
WT Balb/c
Neutrophils per mm2
Sham
CS
CS + PBS
CS + 10 μg rISM1
h
WT Balb/c
TLC (mL)
i
WT Balb/c
Cchord (mL/sec H2O)
j
WT Balb/c
WOB (J)

FIGURE 8

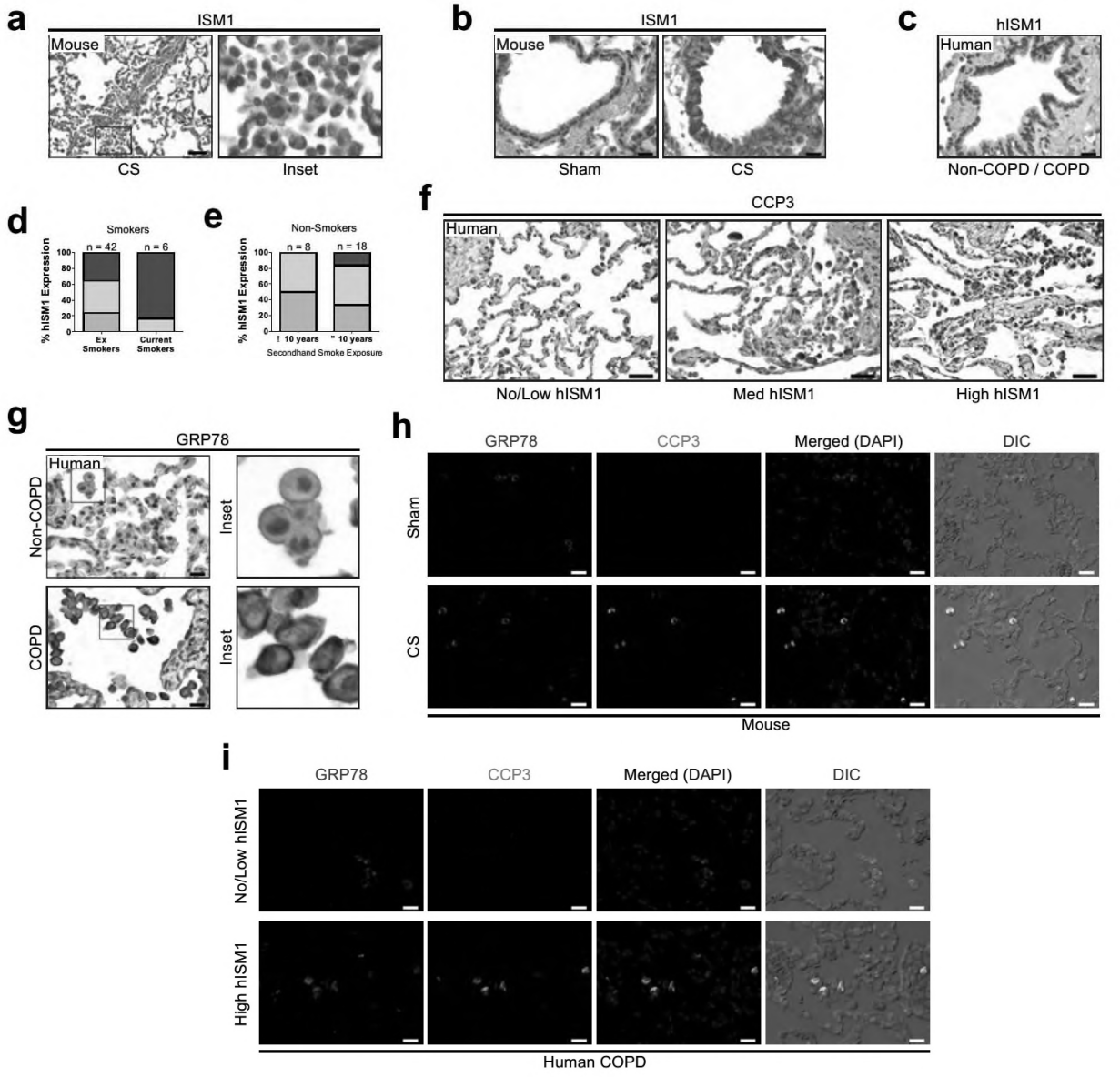

a
ISM1
Mouse
CS
Inset
b
ISM1
Mouse
Sham
CS
c
hISM1
Human
Non-COPD / COPD
d
Smokers
% hISM1 Expression
n = 42
n = 6
Ex Smokers
Current Smokers
e
Non-Smokers
% hISM1 Expression
n = 8
n = 18
! 10 years
" 10 years
Secondhand Smoke Exposure
f
CCP3
Human
No/Low hISM1
Med hISM1
High hISM1
g
GRP78
Human
Non-COPD
COPD
Inset
Inset
h
GRP78
CCP3
Merged (DAPI)
DIC
Sham
CS
Mouse
i
GRP78
CCP3
Merged (DAPI)
DIC
No/Low hISM1
High hISM1
Human COPD

FIGURE 9

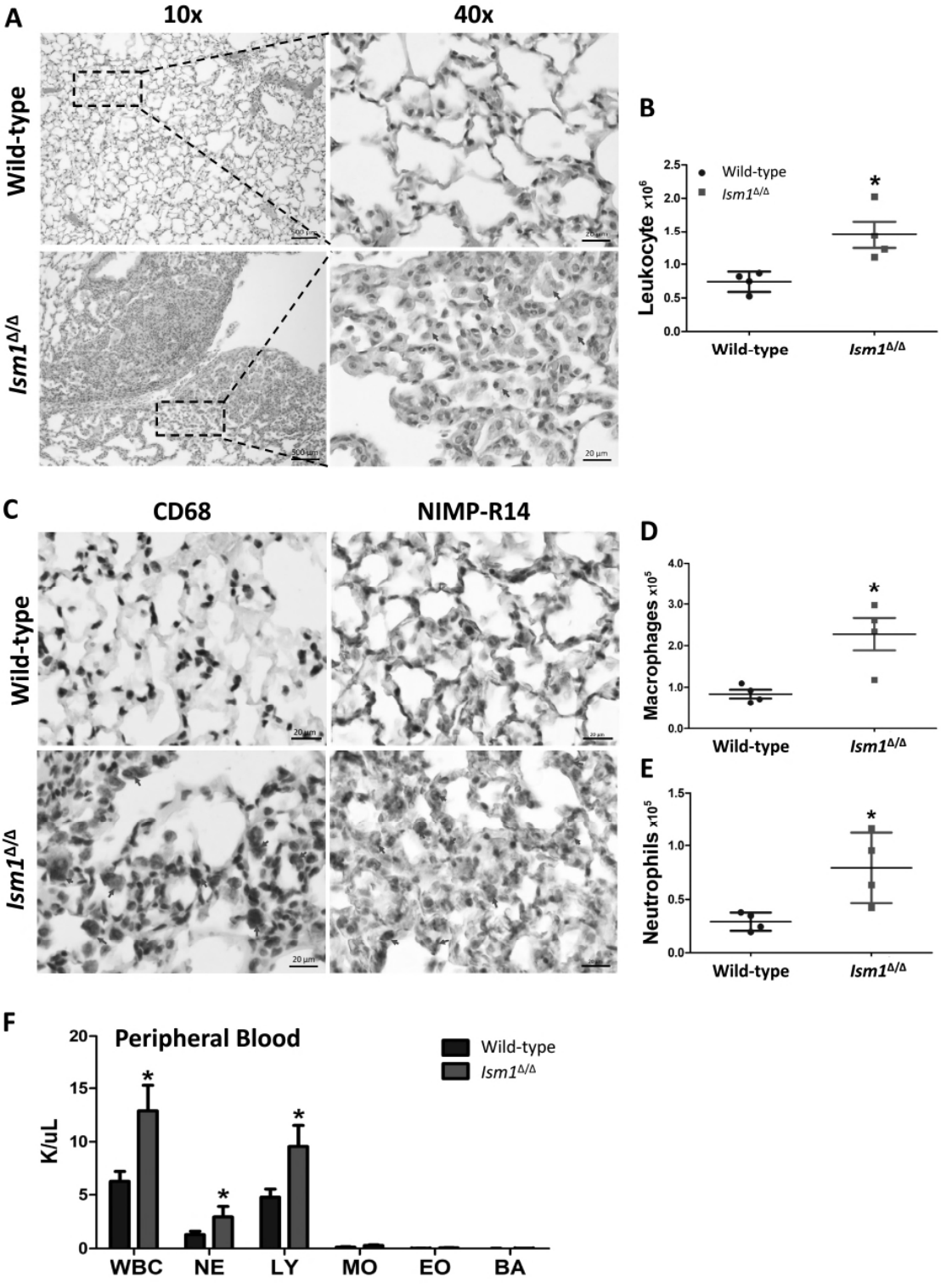

A
10x
40x
Wild-type
Ism1Δ/Δ
B
Wild-type
Ism1Δ/Δ
Leukocyte x10^6
C
CD68
NIMP-R14
D
Macrophages x10^5
E
Neutrophils x10^5
F
Peripheral Blood
K/uL
WBC
NE
LY
MO
EO
BA

FIGURE 10

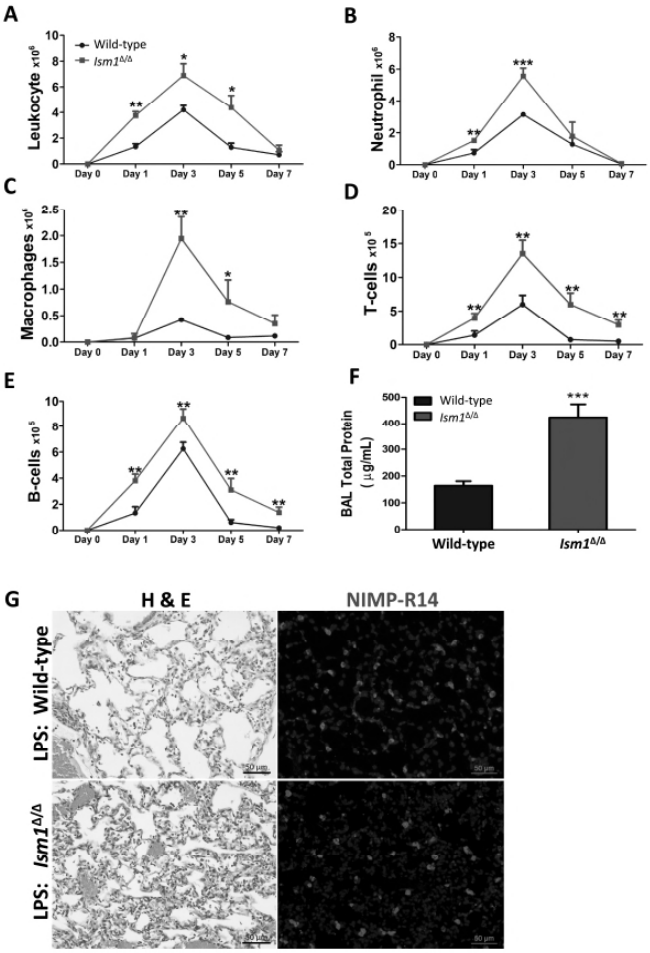

A
Leukocyte x10^6
Wild-type
lsm1Δ/Δ
Day 0
Day 1
Day 3
Day 5
Day 7
B
Neutrophil x10^6
C
Macrophages x10^6
D
T-cells x10^5
E
B-cells x10^5
F
BAL Total Protein (μg/mL)
Wild-type
lsm1Δ/Δ
G
H & E
NIMP-R14
LPS: Wild-type
LPS: lsm1Δ/Δ

FIGURE 11

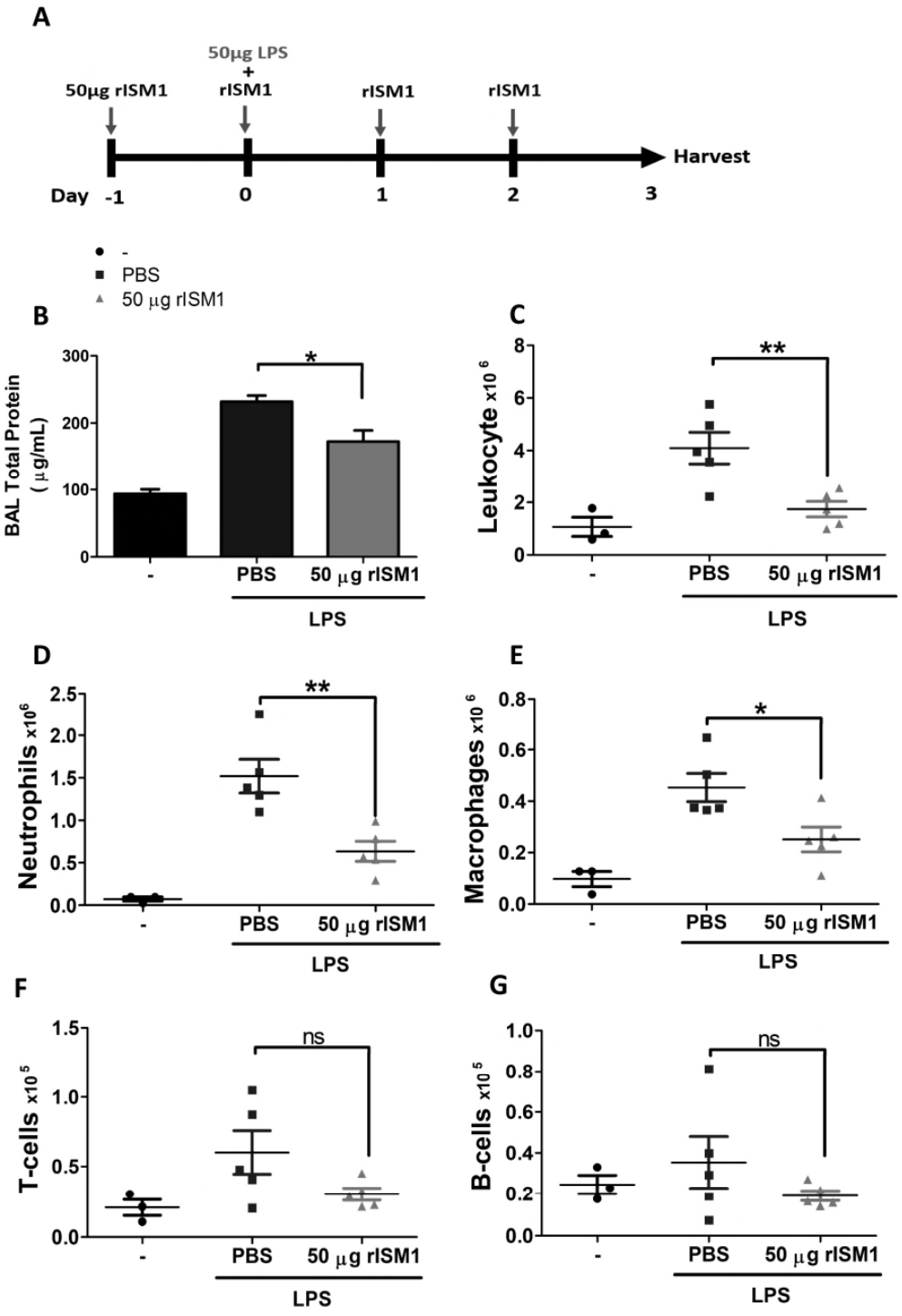

A
50µg LPS
+
rISM1
50µg rISM1
rISM1
rISM1
Harvest
Day -1
0
1
2
3
-
PBS
50 µg rISM1
B
BAL Total Protein (µg/mL)
300
200
100
0
*
-
PBS
50 µg rISM1
LPS
C
Leukocyte x10 6
8
6
4
2
0
**
-
PBS
50 µg rISM1
LPS
D
Neutrophils x10⁶
2.5
2.0
1.5
1.0
0.5
0.0
**
-
PBS
50 µg rISM1
LPS
E
Macrophages x10 6
0.8
0.6
0.4
0.2
0.0
*
-
PBS
50 µg rISM1
LPS
F
T-cells x10 5
1.5
1.0
0.5
0.0
ns
-
PBS
50 µg rISM1
LPS
G
B-cells x10 5
1.0
0.8
0.6
0.4
0.2
0.0
ns
-
PBS
50 µg rISM1
LPS

FIGURE 12

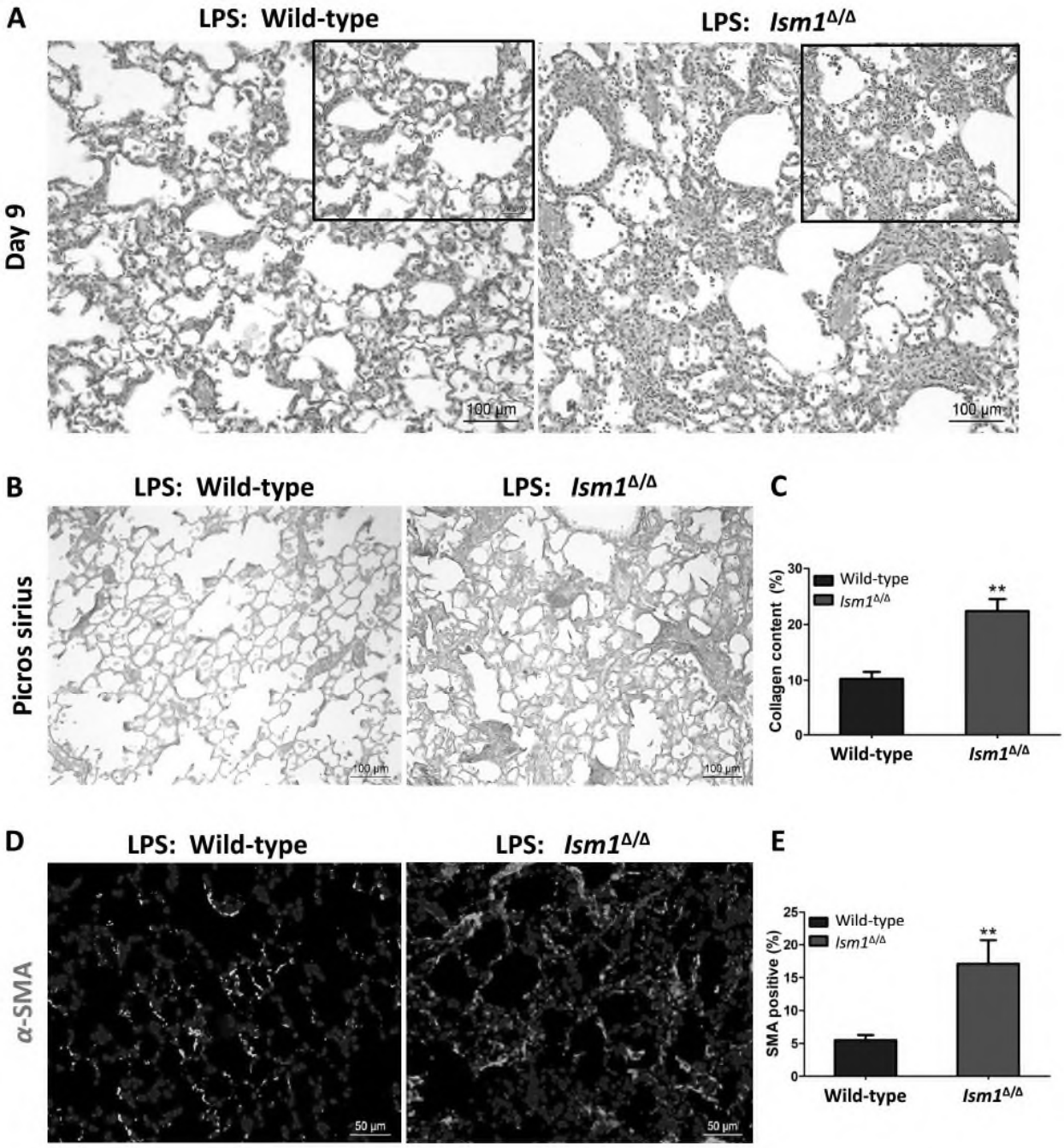

A
LPS: Wild-type
LPS: Ism1Δ/Δ
Day 9
100 µm
100 µm
B
LPS: Wild-type
LPS: Ism1Δ/Δ
Picros sirius
100 µm
100 µm
C
Collagen content (%)
Wild-type
Ism1Δ/Δ
**
Wild-type
Ism1Δ/Δ
D
LPS: Wild-type
LPS: Ism1Δ/Δ
α-SMA
50 µm
50 µm
E
SMA positive (%)
Wild-type
Ism1Δ/Δ
**
Wild-type
Ism1Δ/Δ

FIGURE 13

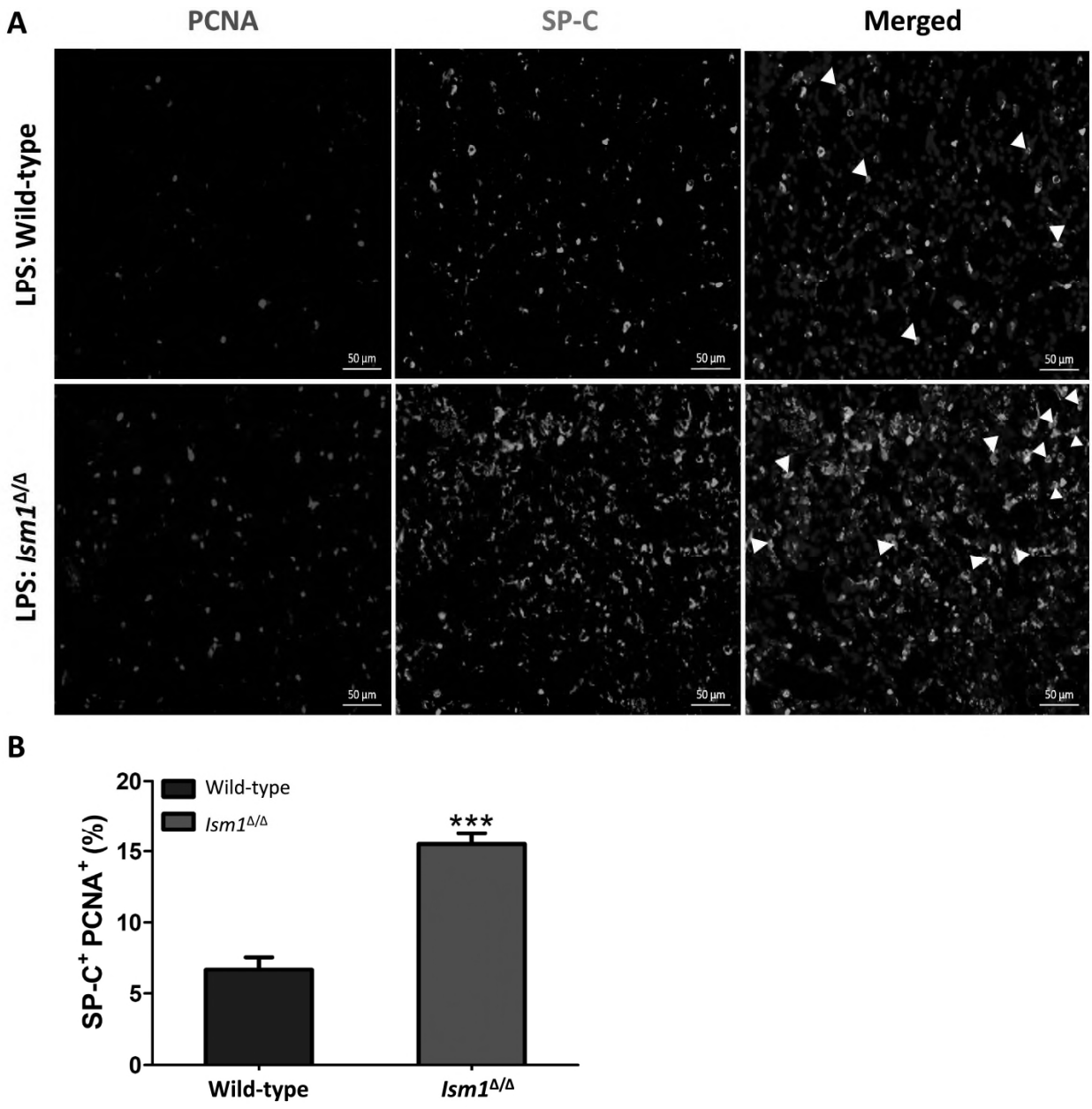

A
PCNA
SP-C
Merged
LPS: Wild-type
LPS: Ism1Δ/Δ
50 µm
B
Wild-type
Ism1Δ/Δ
***
SP-C+ PCNA+ (%)
20
15
10
5
0
Wild-type
Ism1Δ/Δ

FIGURE 14

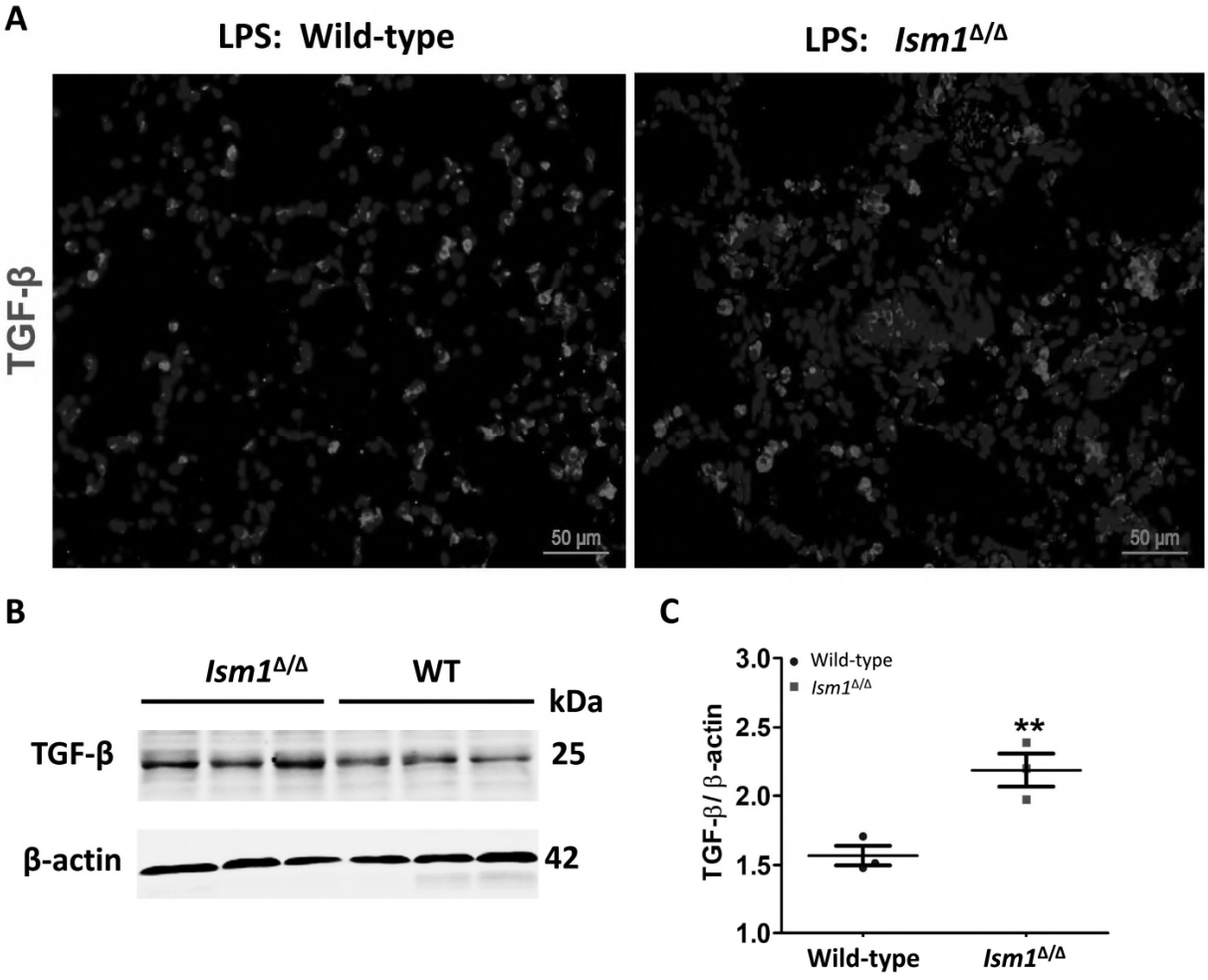

A
LPS: Wild-type
LPS: Ism1Δ/Δ
TGF-β
50 μm
50 μm
B
Ism1Δ/Δ
WT
kDa
TGF-β
25
β-actin
42
C
Wild-type
Ism1Δ/Δ
TGF-β/ β-actin
3.0
2.5
2.0
1.5
1.0
**
Wild-type
Ism1Δ/Δ

FIGURE 15

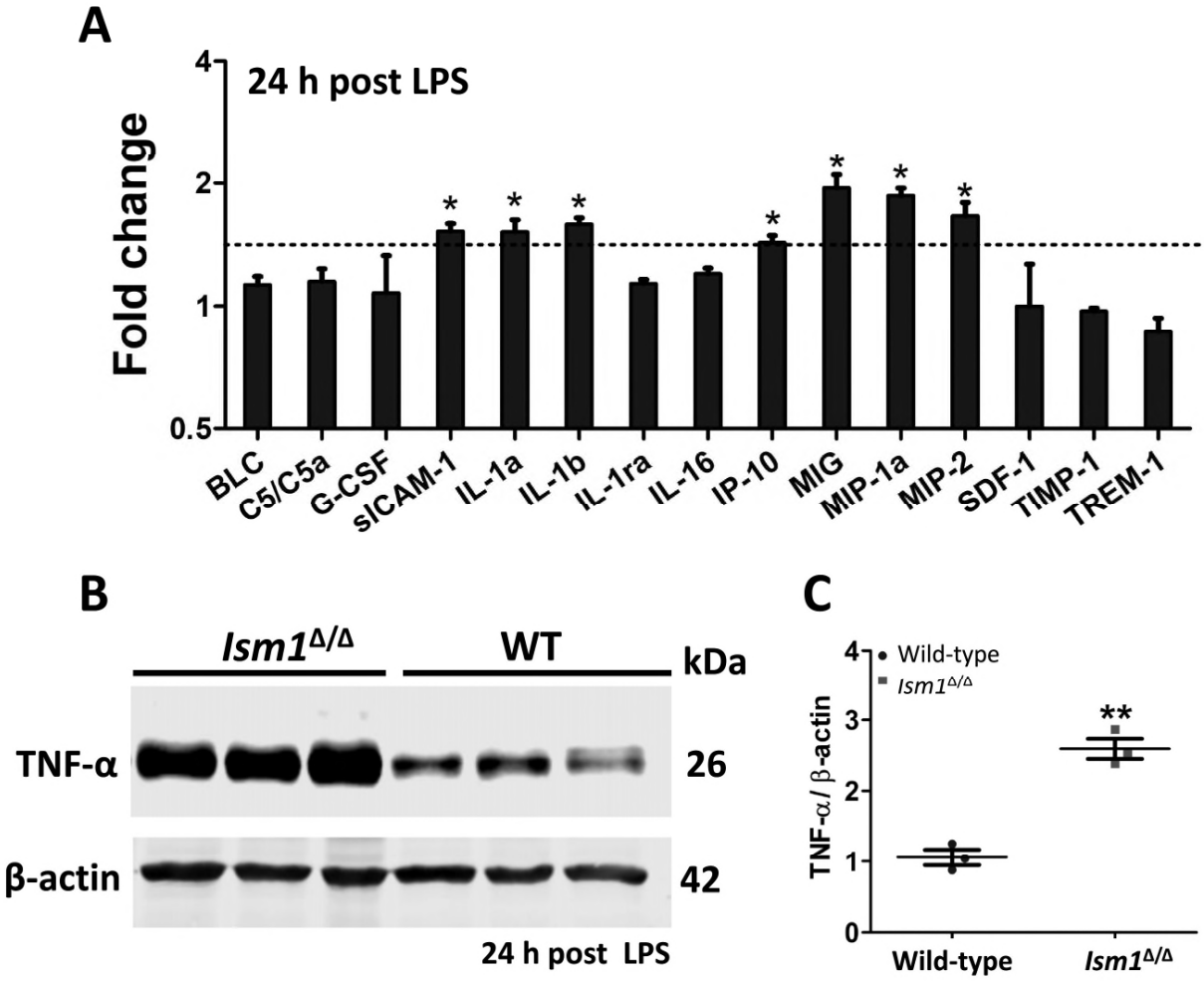

A
24 h post LPS
Fold change
4
2
1
0.5
BLC
C5/C5a
G-CSF
sICAM-1
IL-1a
IL-1b
IL-1ra
IL-16
IP-10
MIG
MIP-1a
MIP-2
SDF-1
TIMP-1
TREM-1
B
Ism1Δ/Δ
WT
kDa
TNF-α
26
β-actin
42
24 h post LPS
C
Wild-type
Ism1Δ/Δ
TNF-α / β-actin
**
Wild-type
Ism1Δ/Δ

FIGURE 16

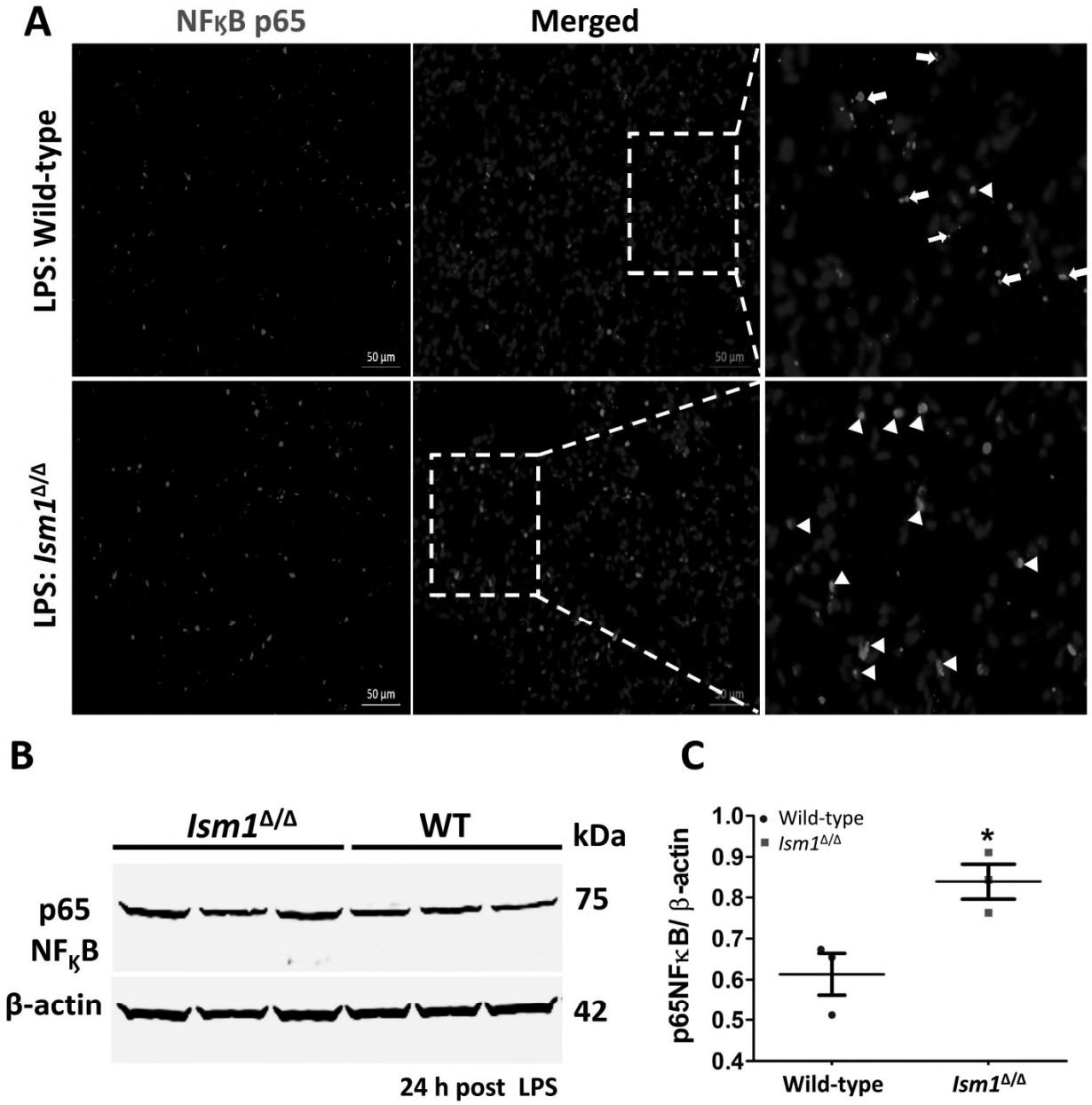

A
NFκB p65
Merged
LPS: Wild-type
LPS: Ism1Δ/Δ
50 µm
B
Ism1Δ/Δ
WT
kDa
p65 NFκB
β-actin
75
42
24 h post LPS
C
Wild-type
Ism1Δ/Δ
p65NFκB/ β-actin
1.0
0.9
0.8
0.7
0.6
0.5
0.4
*
Wild-type
Ism1Δ/Δ

FIGURE 17

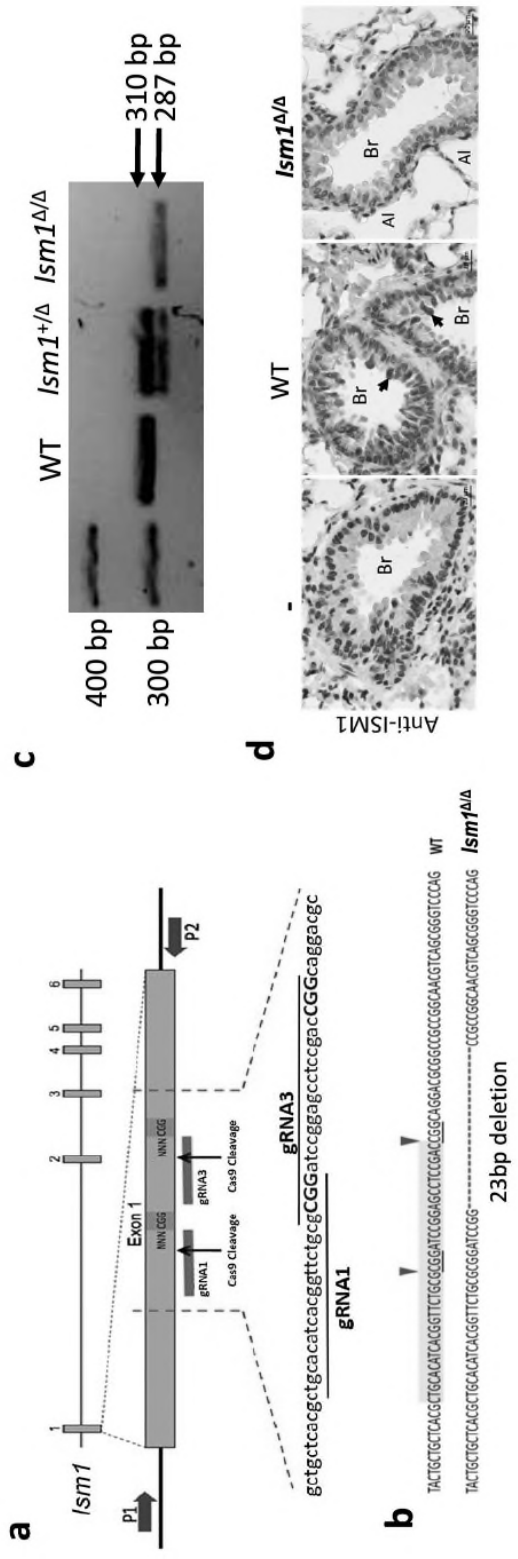

a
Ism1
P1
P2
Exon 1
NNN CGG
gRNA1
Cas9 Cleavage
gRNA3
gctgctcacgctgcacatcacggttctgcgCGGatccggagcctccgacCGGcaggacgc
gRNA3
gRNA1
b
TACTGCTGCTCACGCTGCACATCACGGTTCTGCGGGATCCGGAGCCTCCGACCGGCAGGACGGGCCGCCGGCAACGTCAGCGGGTCCCAG
TACTGCTGCTCACGCTGCACATCACGGTTCTGCGCGGATCCGG----------CCGCCGGCAACGTCAGCGGGTCCCAG
WT
Ism1Δ/Δ
23bp deletion
c
WT
Ism1+/Δ
Ism1Δ/Δ
310 bp
287 bp
400 bp
300 bp
d
WT
Ism1Δ/Δ
Br
Al
-
Anti-ISM1

FIGURE 18

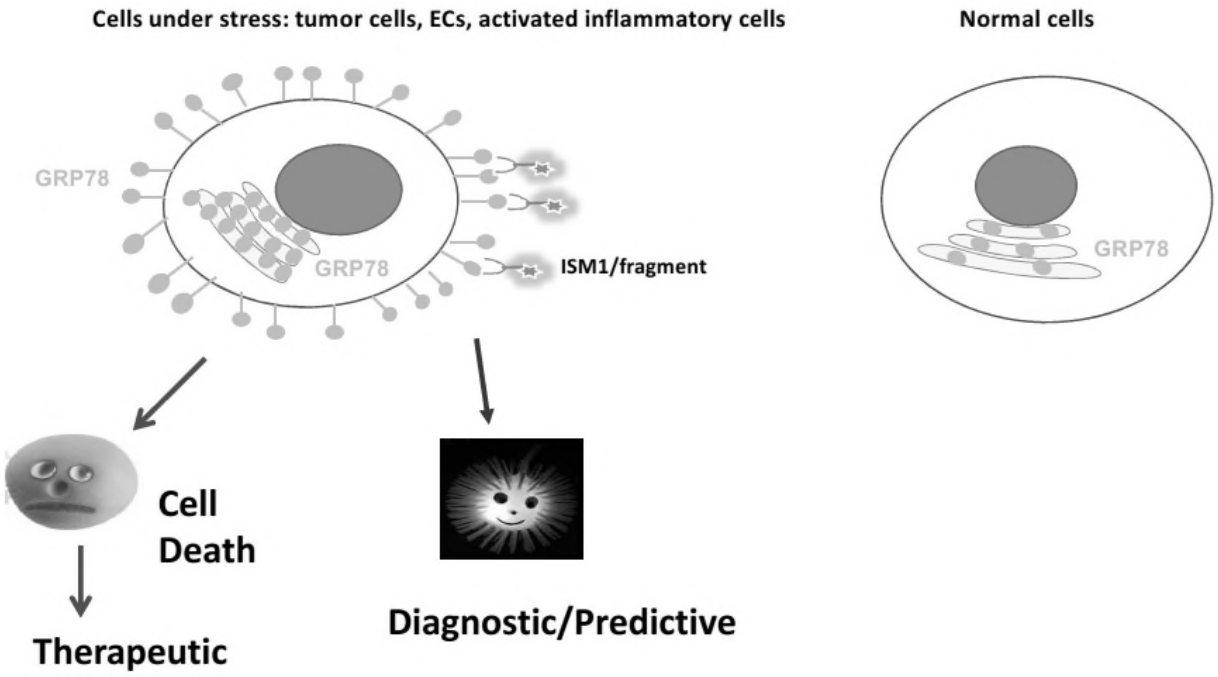

Cells under stress: tumor cells, ECs, activated inflammatory cells
Normal cells
GRP78
GRP78
ISM1/fragment
GRP78
Cell
Death
Therapeutic
Diagnostic/Predictive

FIGURE 19

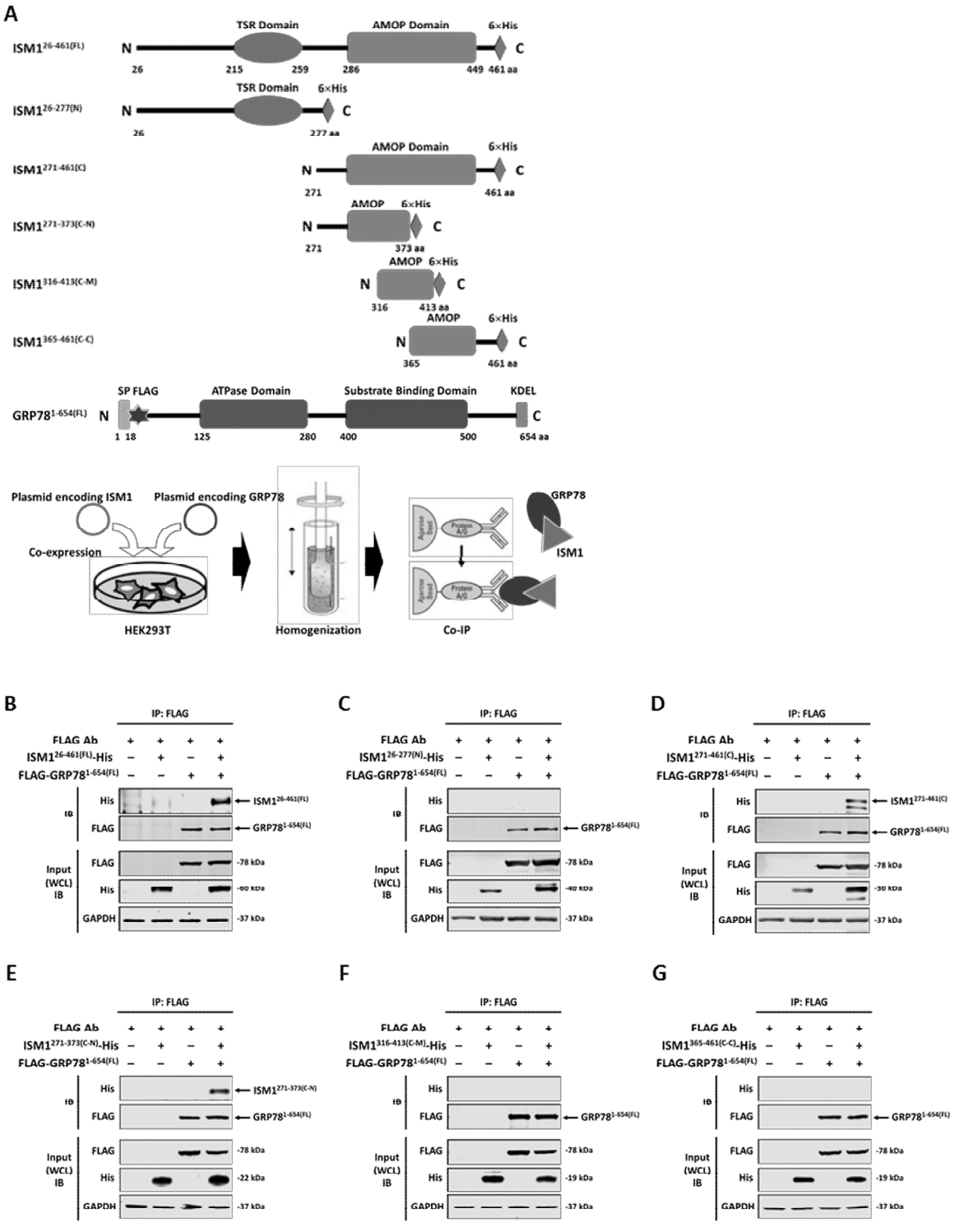

A
ISM1 26-461(FL)
TSR Domain
AMOP Domain
6×His
N
C
26
215
259
286
449
461 aa
ISM1 26-277(N)
277 aa
ISM1 271-461(C)
271
461 aa
ISM1 271-373(C-N)
AMOP
373 aa
ISM1 316-413(C-M)
316
413 aa
ISM1 365-461(C-C)
365
GRP78 1-654(FL)
SP FLAG
ATPase Domain
Substrate Binding Domain
KDEL
1
18
125
280
400
500
654 aa
Plasmid encoding ISM1
Plasmid encoding GRP78
Co-expression
HEK293T
Homogenization
Co-IP
GRP78
ISM1
B
C
D
E
F
G
IP: FLAG
FLAG Ab
ISM1 26-461(FL)-His
ISM1 26-277(N)-His
ISM1 271-461(C)-His
ISM1 271-373(C-N)-His
ISM1 316-413(C-M)-His
ISM1 365-461(C-C)-His
FLAG-GRP78 1-654(FL)
IB
Input (WCL) IB
His
FLAG
GAPDH
-78 kDa
-37 kDa
-22 kDa
-19 kDa

FIGURE 20

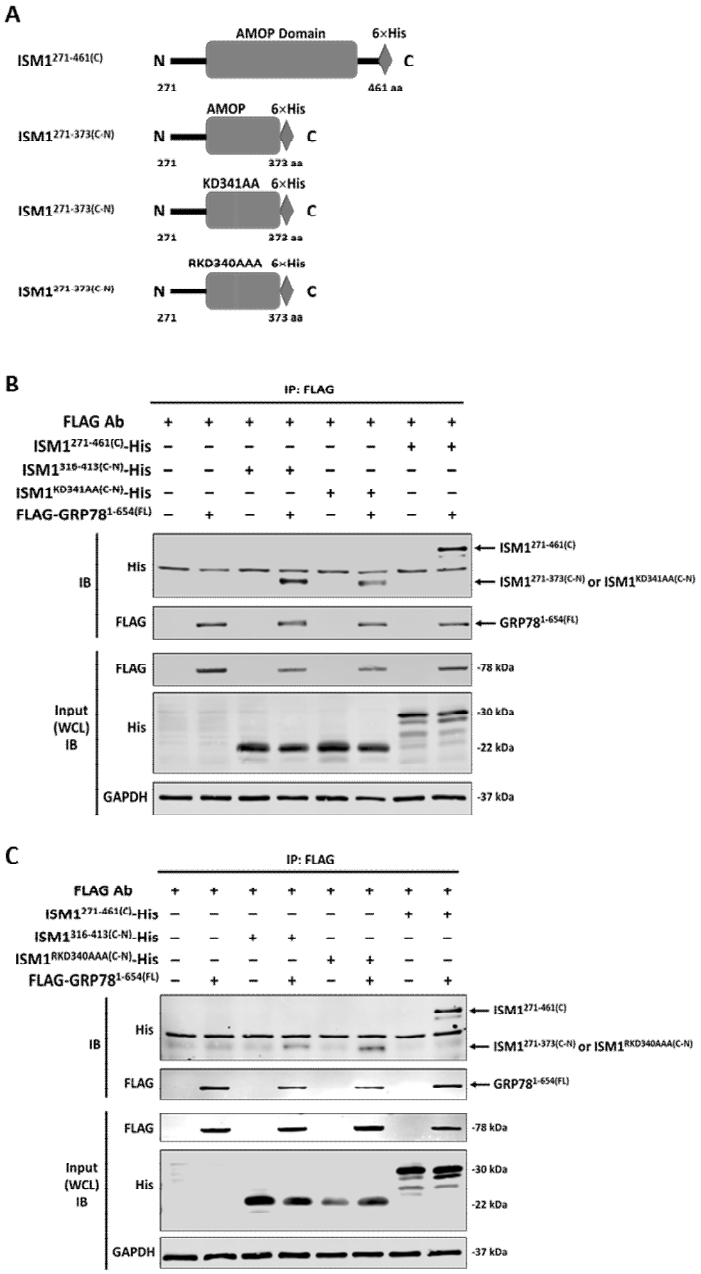

A
AMOP Domain
6×His
ISM1271-461(C)
N
C
271
461 aa
AMOP
6×His
ISM1271-373(C-N)
373 aa
KD341AA
RKD340AAA
B
IP: FLAG
FLAG Ab
ISM1271-461(C)-His
ISM1316-413(C-N)-His
ISM1KD341AA(C-N)-His
FLAG-GRP781-654(FL)
IB
His
FLAG
Input (WCL) IB
GAPDH
ISM1271-461(C)
ISM1271-373(C-N) or ISM1KD341AA(C-N)
GRP781-654(FL)
-78 kDa
-30 kDa
-22 kDa
-37 kDa
C
ISM1RKD340AAA(C-N)-His
ISM1271-373(C-N) or ISM1RKD340AAA(C-N)

FIGURE 21

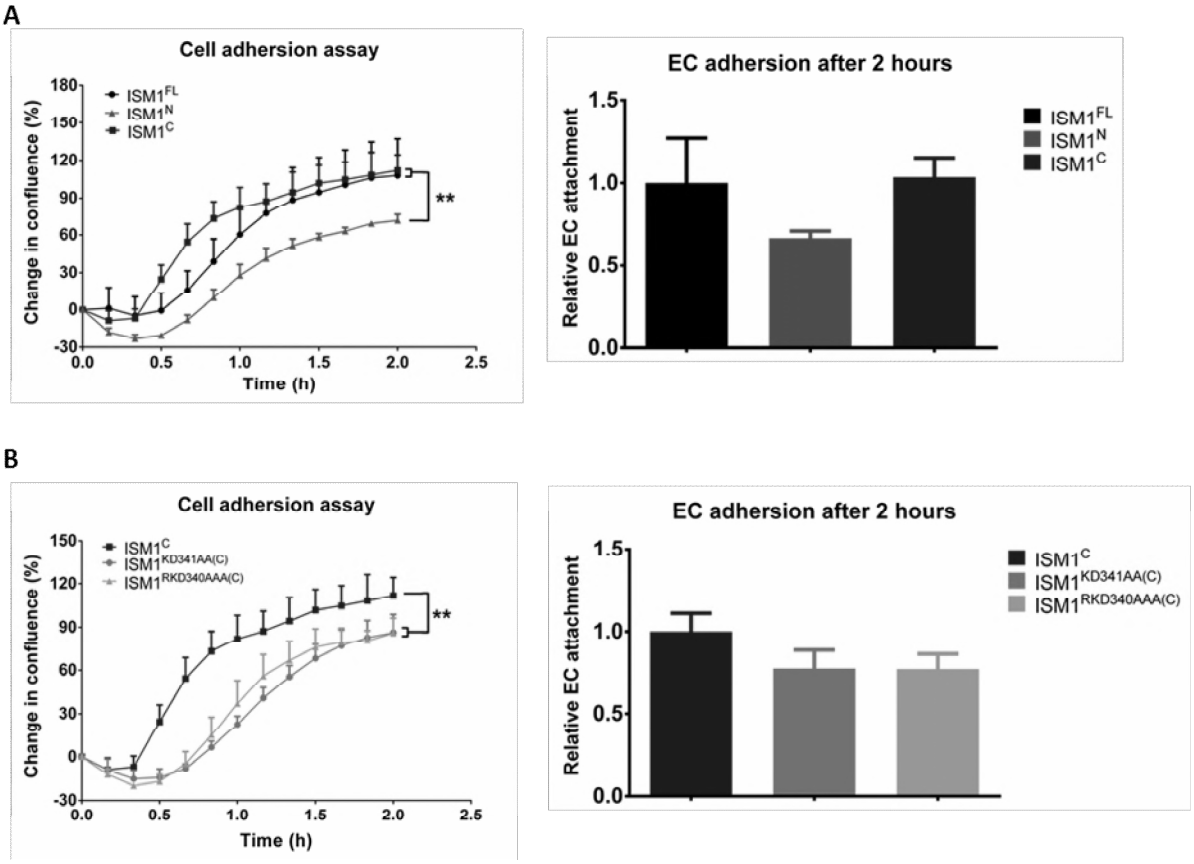

A
Cell adhersion assay
Change in confluence (%)
Time (h)
ISM1FL
ISM1N
ISM1C
**
EC adhersion after 2 hours
Relative EC attachment
B
Cell adhersion assay
ISM1C
ISM1KD341AA(C)
ISM1RKD340AAA(C)
EC adhersion after 2 hours

FIGURE 22

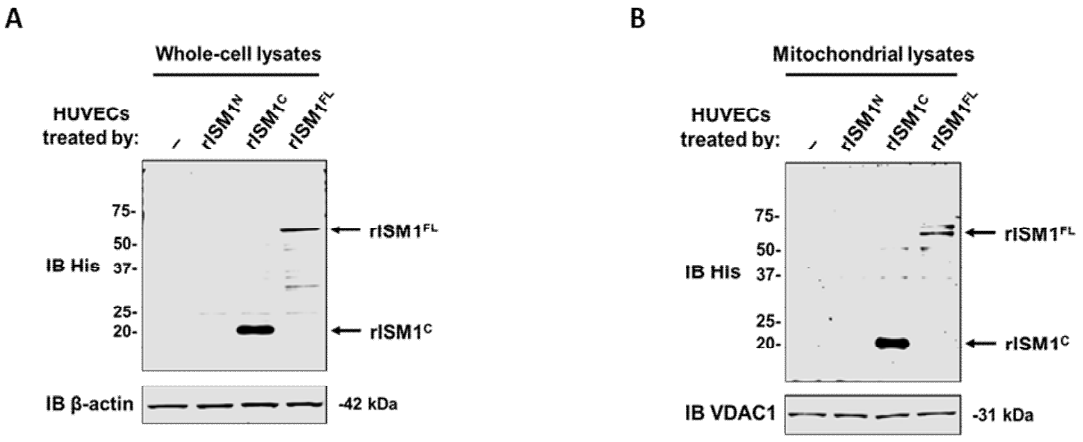

A
Whole-cell lysates
HUVECs treated by:
-
rISM1N
rISM1C
rISM1FL
75-
50-
37-
25-
20-
IB His
rISM1FL
rISM1C
IB β-actin
-42 kDa
B
Mitochondrial lysates
HUVECs treated by:
-
rISM1N
rISM1C
rISM1FL
75-
50-
37-
25-
20-
IB His
rISM1FL
rISM1C
IB VDAC1
-31 kDa

FIGURE 23

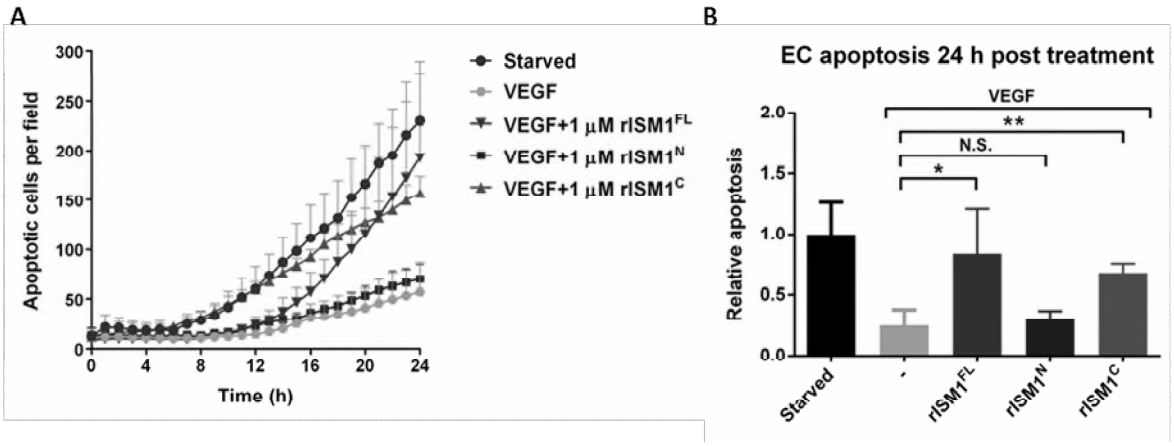

A
Apoptotic cells per field
300
250
200
150
100
50
0
0
4
8
12
16
20
24
Time (h)
Starved
VEGF
VEGF+1 μM rISM1FL
VEGF+1 μM rISM1N
VEGF+1 μM rISM1C
B
EC apoptosis 24 h post treatment
Relative apoptosis
2.0
1.5
1.0
0.5
0.0
VEGF
**
N.S.
*
Starved
-
rISM1FL
rISM1N
rISM1C

FIGURE 24

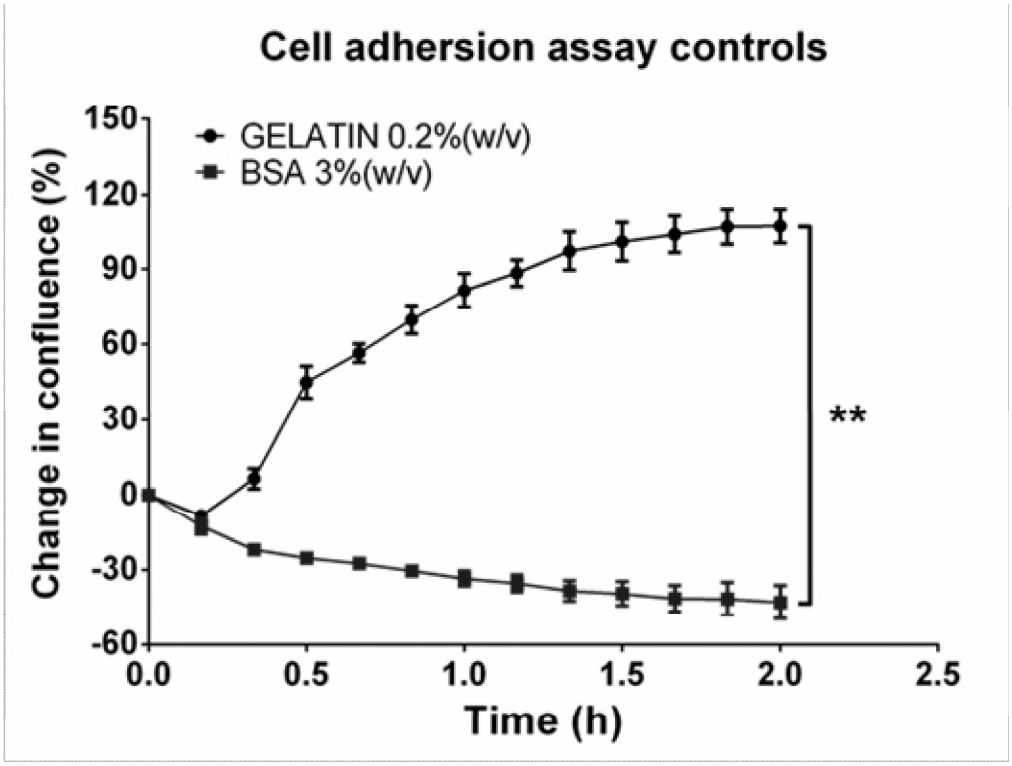
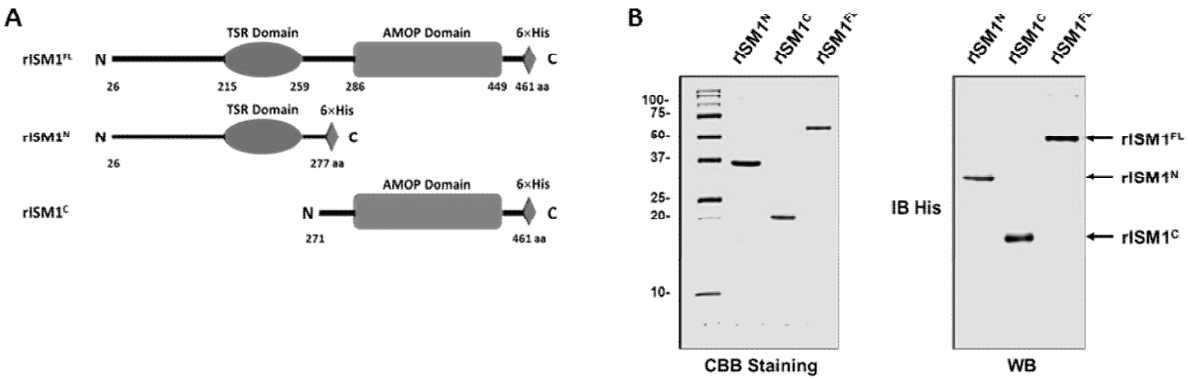

Cell adhersion assay controls
GELATIN 0.2%(w/v)
BSA 3%(w/v)
Change in confluence (%)
150
120
90
60
30
0
-30
-60
0.0
0.5
1.0
1.5
2.0
2.5
Time (h)
**

FIGURE 25


A
rISM1FL
TSR Domain
AMOP Domain
6×His
N
C
26
215
259
286
449
461 aa
rISM1N
277 aa
rISM1C
271
B
100-
75-
60-
37-
25-
20-
10-
CBB Staining
IB His
WB
rISM1FL
rISM1N
rISM1C