Figure 1a

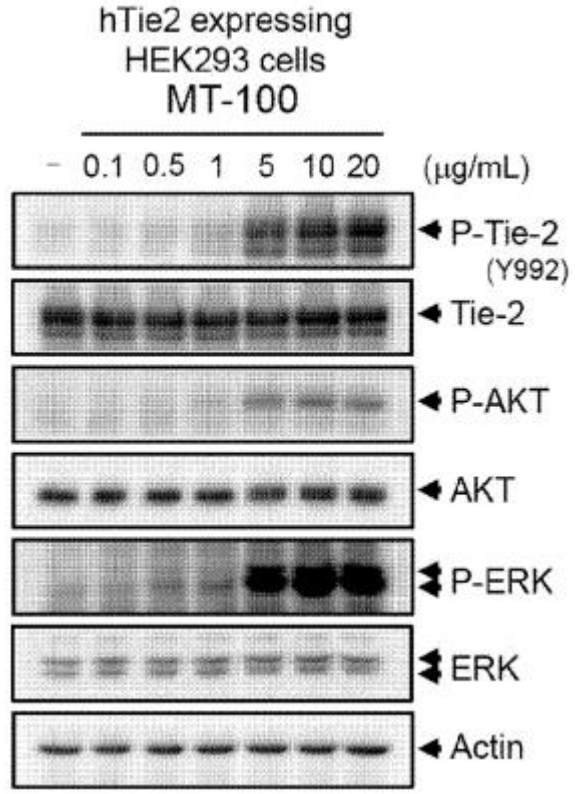


Figure 1b

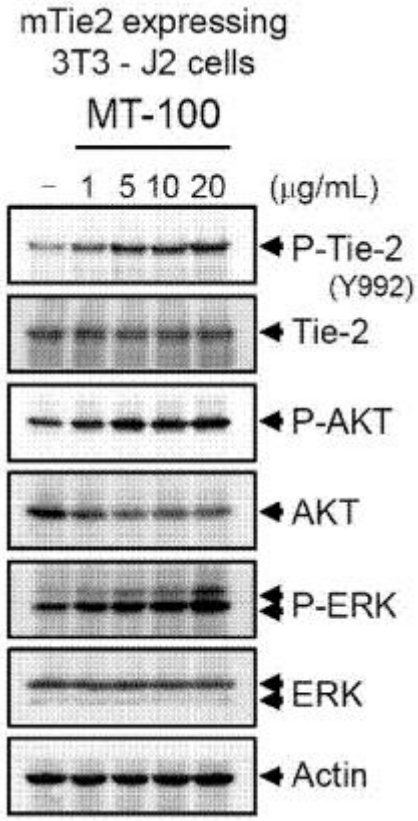


Figure 1c

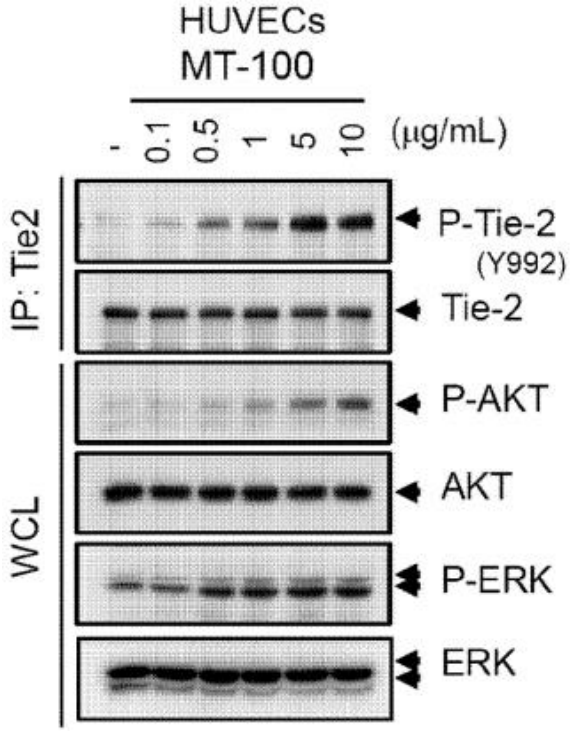


Figure 2a

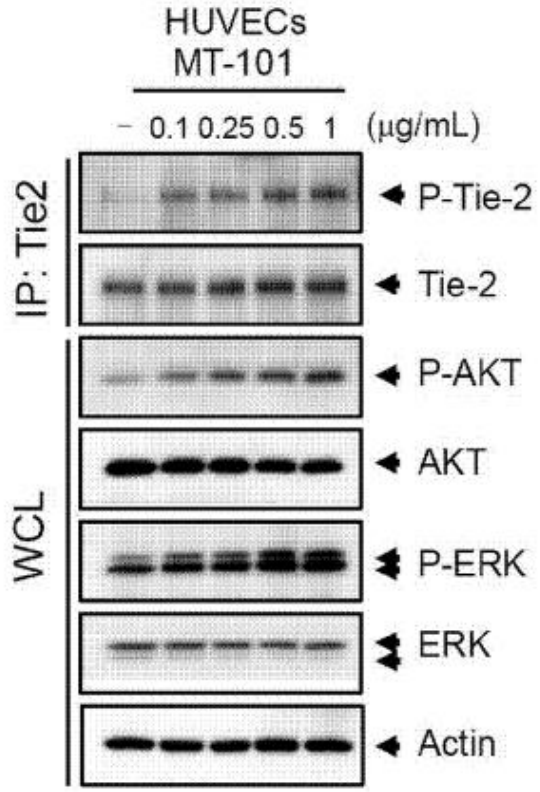


Figure 2b

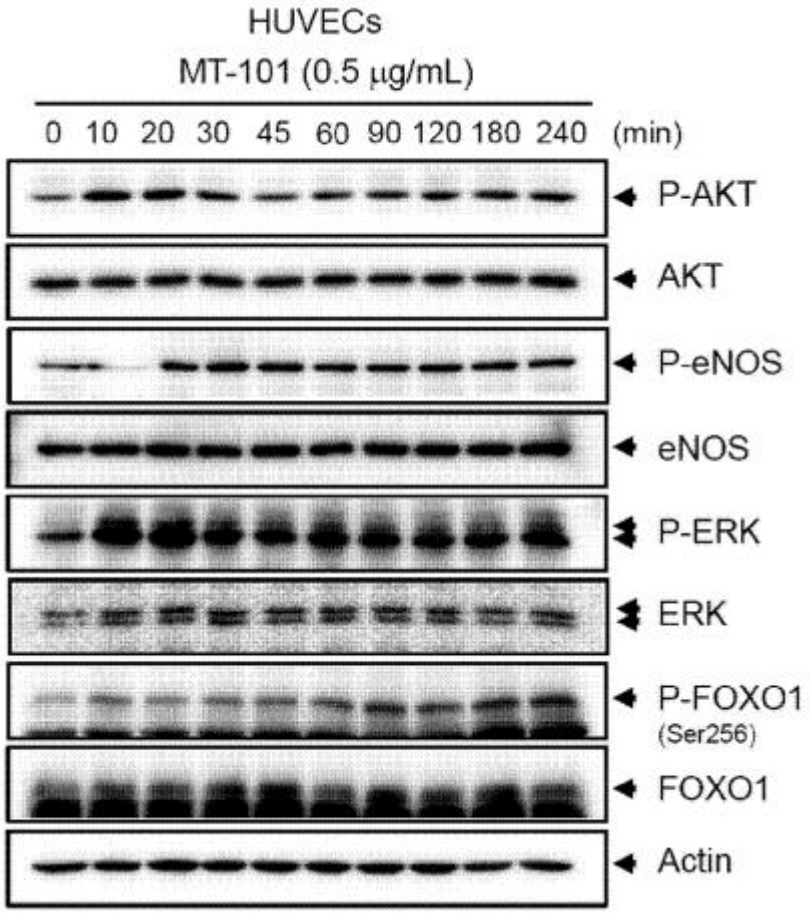


Figure 2c

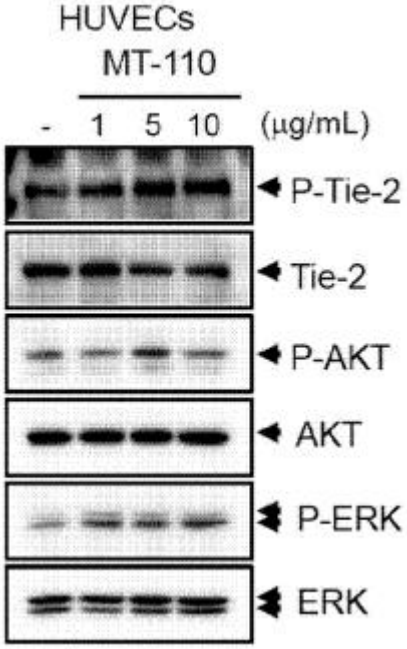


Figure 2d

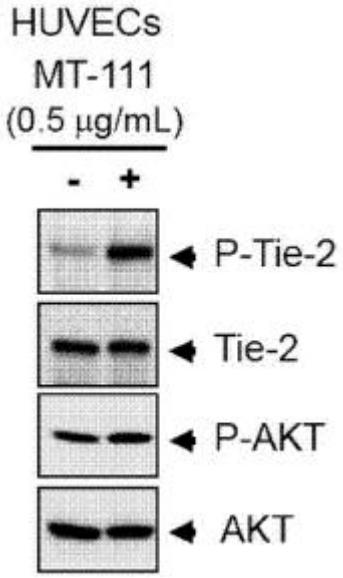


Figure 3

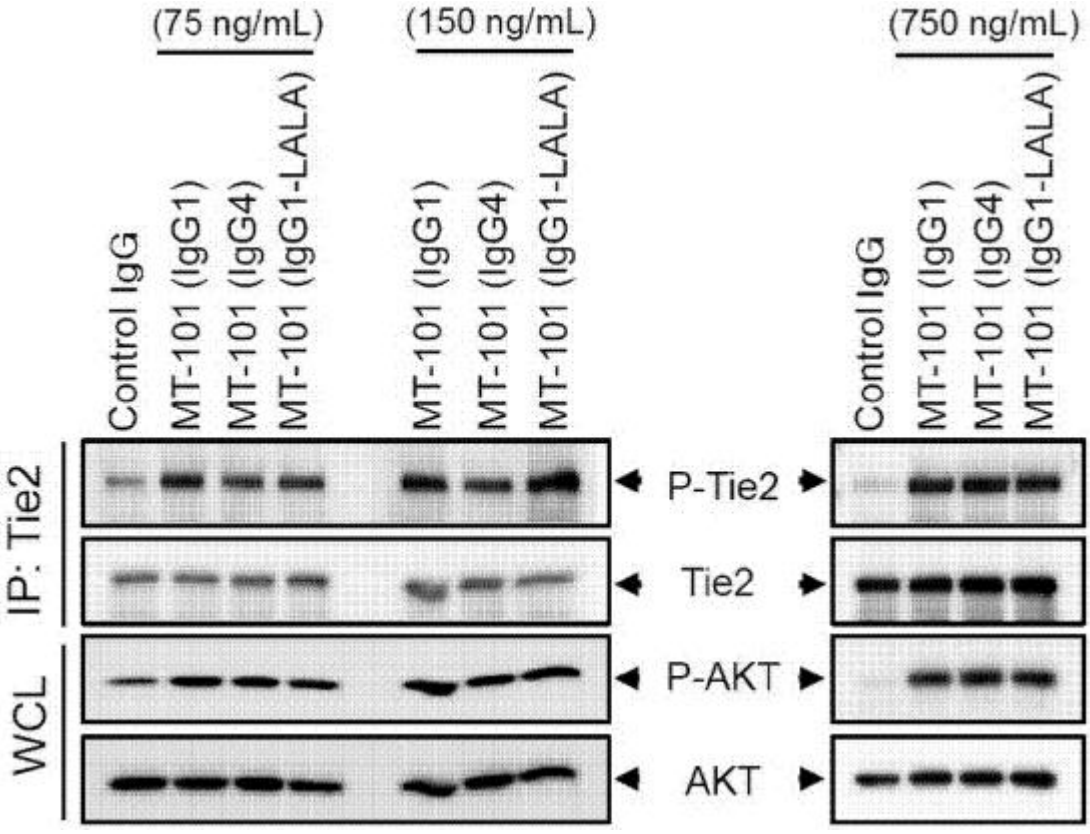


Figure 4

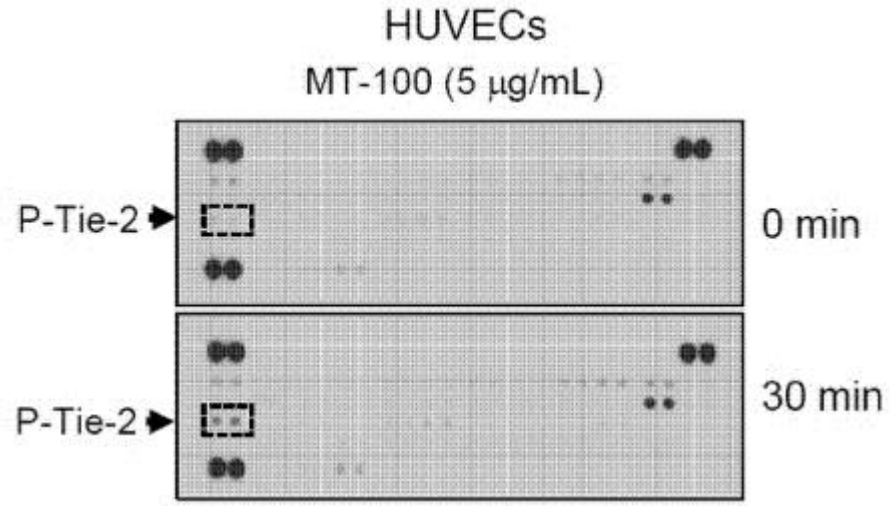


Figure 5a

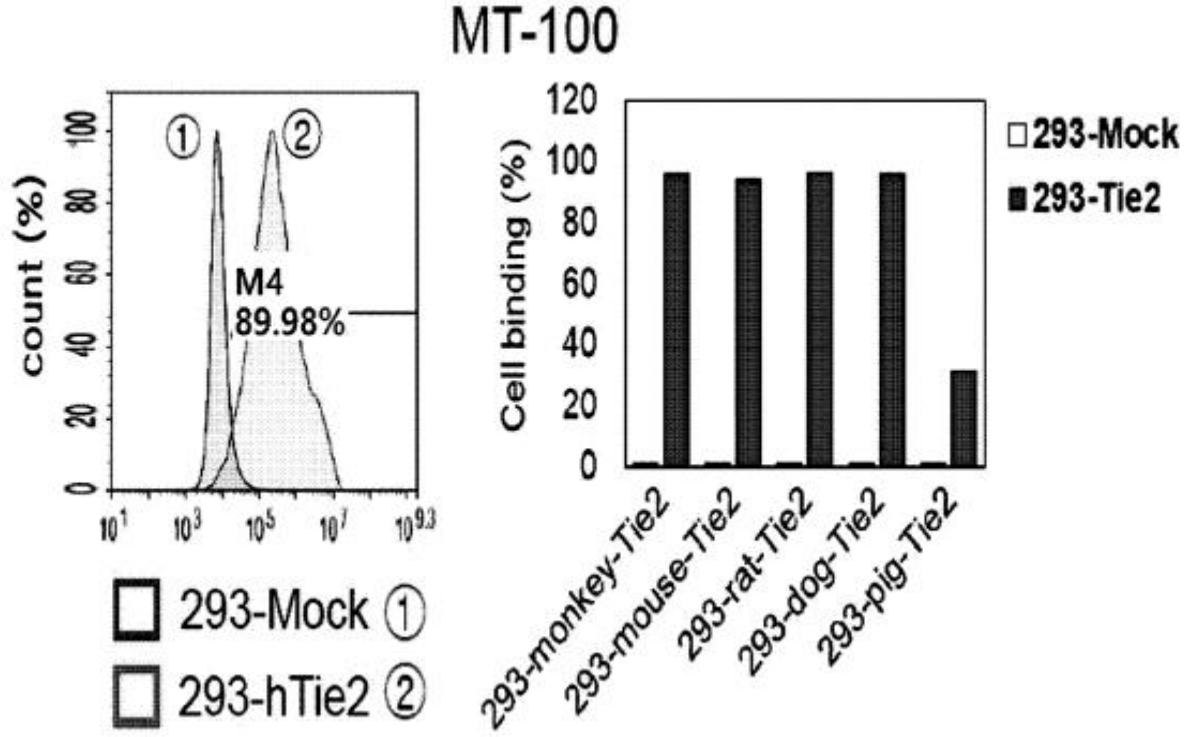


Figure 5b

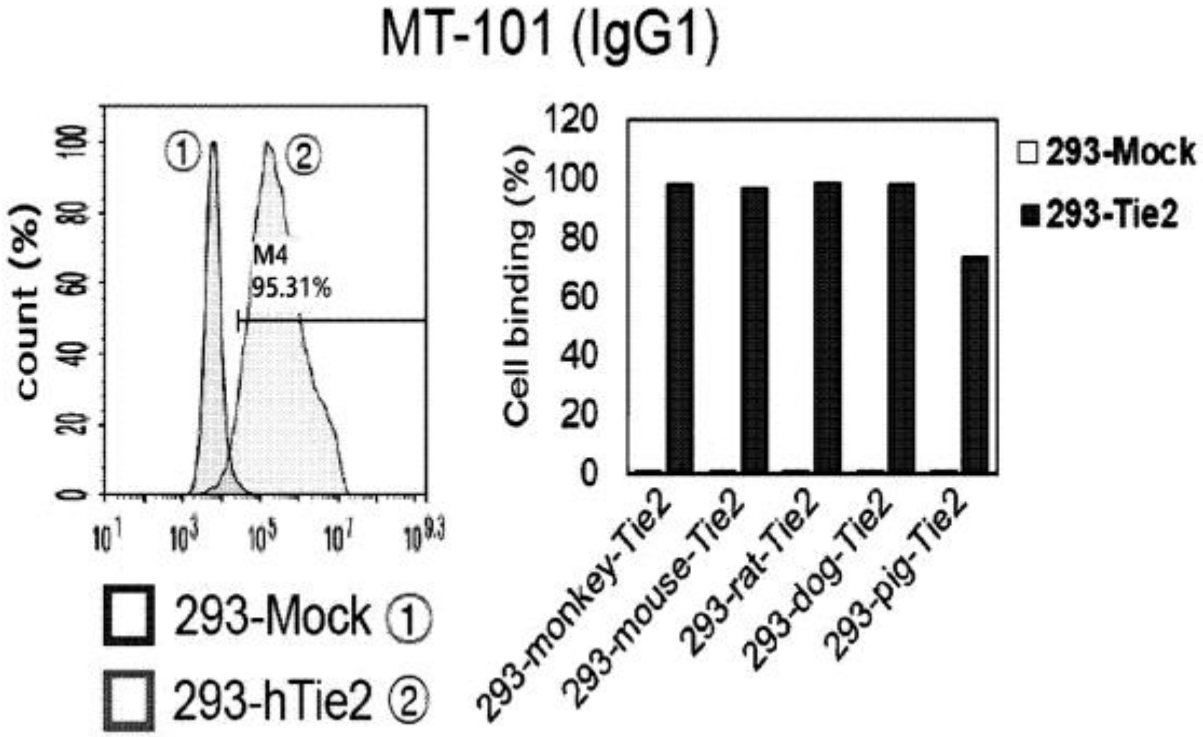


Figure 5c

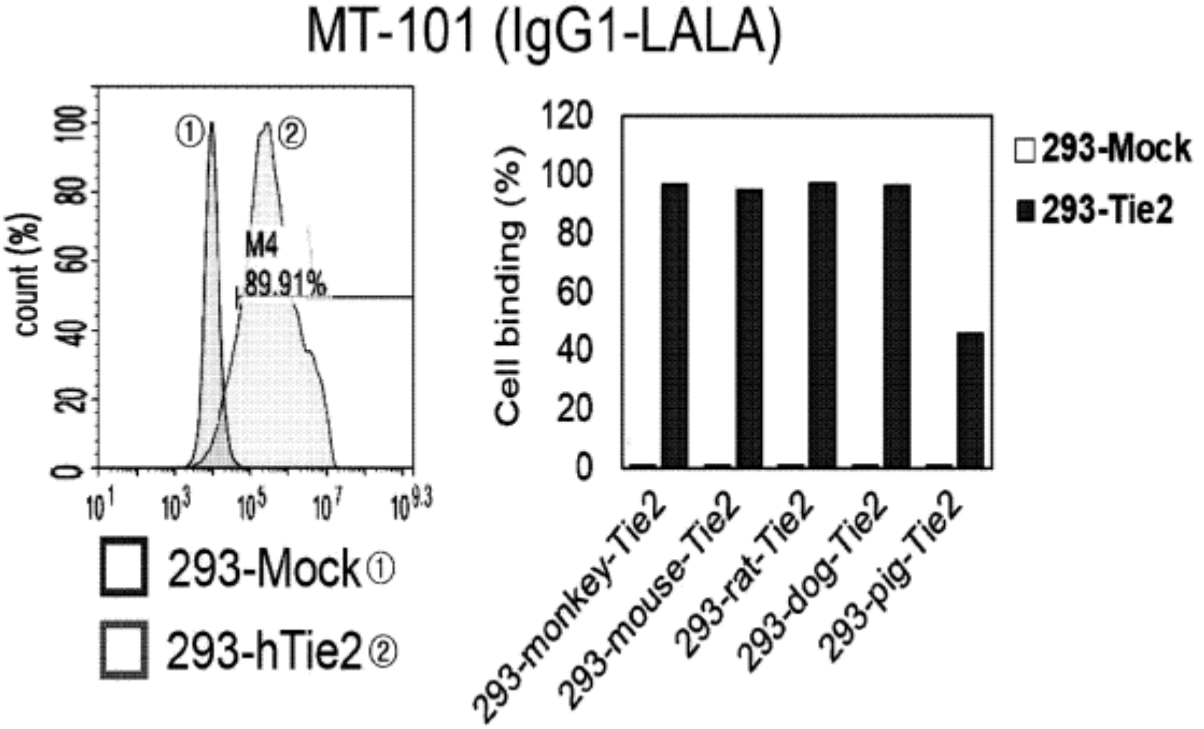


Figure 5d

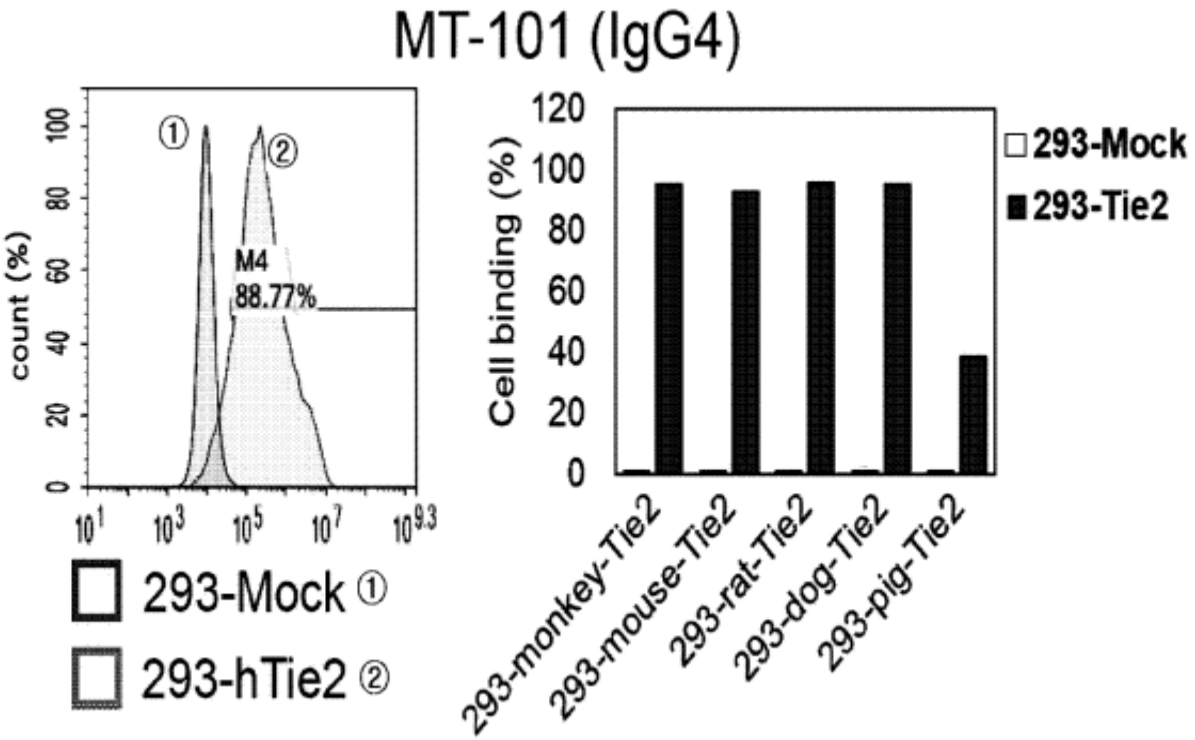

MT-101 (IgG4)
count (%)
①
②
M4
88.77%
293-Mock ①
293-hTie2 ②
Cell binding (%)
293-Mock
293-Tie2
293-monkey-Tie2
293-mouse-Tie2
293-rat-Tie2
293-dog-Tie2
293-pig-Tie2

Figure 6

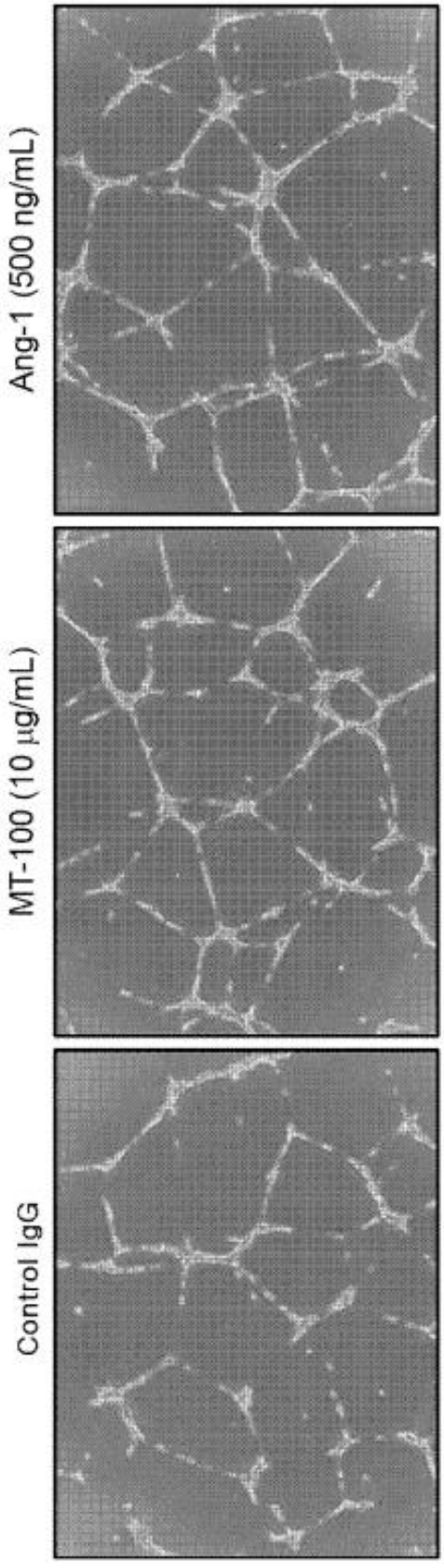


Figure 7a

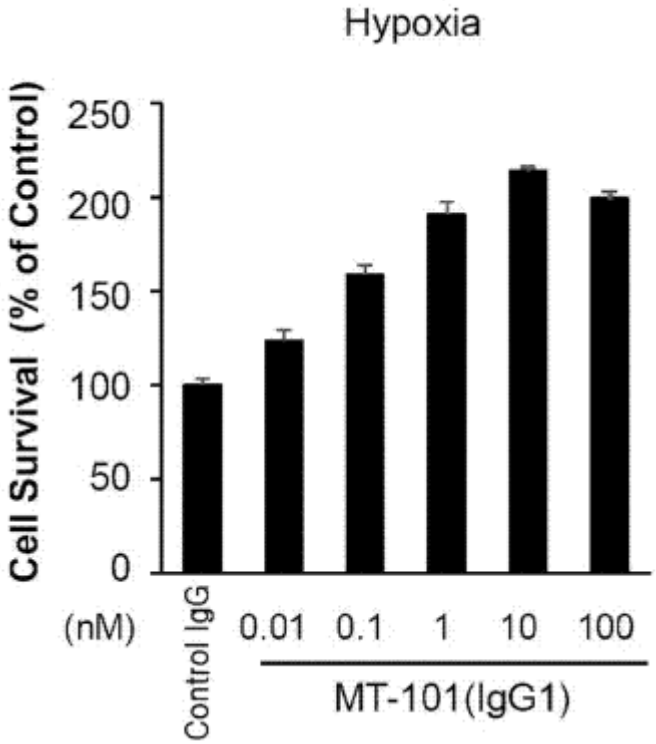


Figure 7b

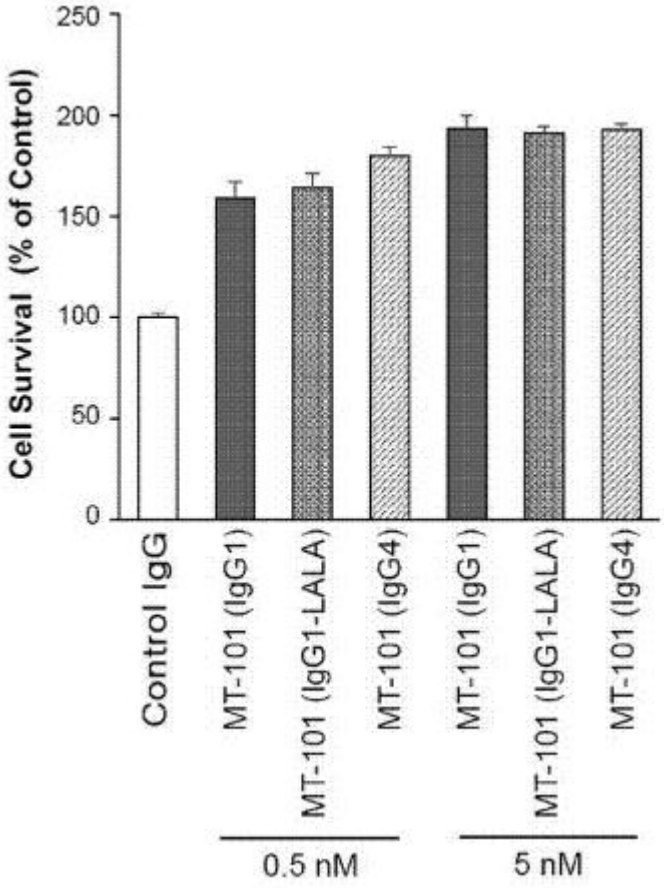


Figure 8a

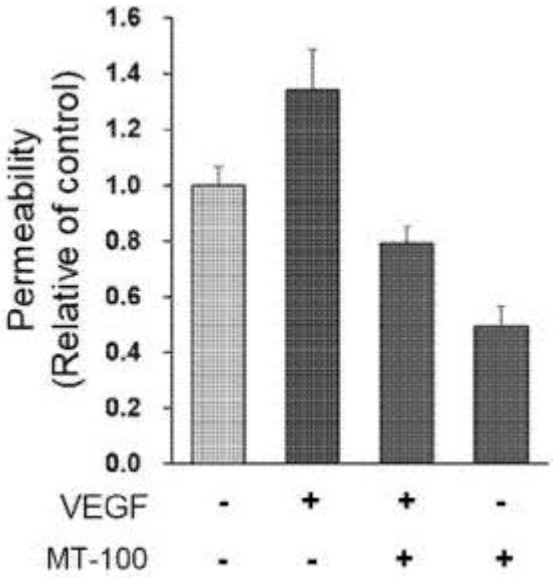
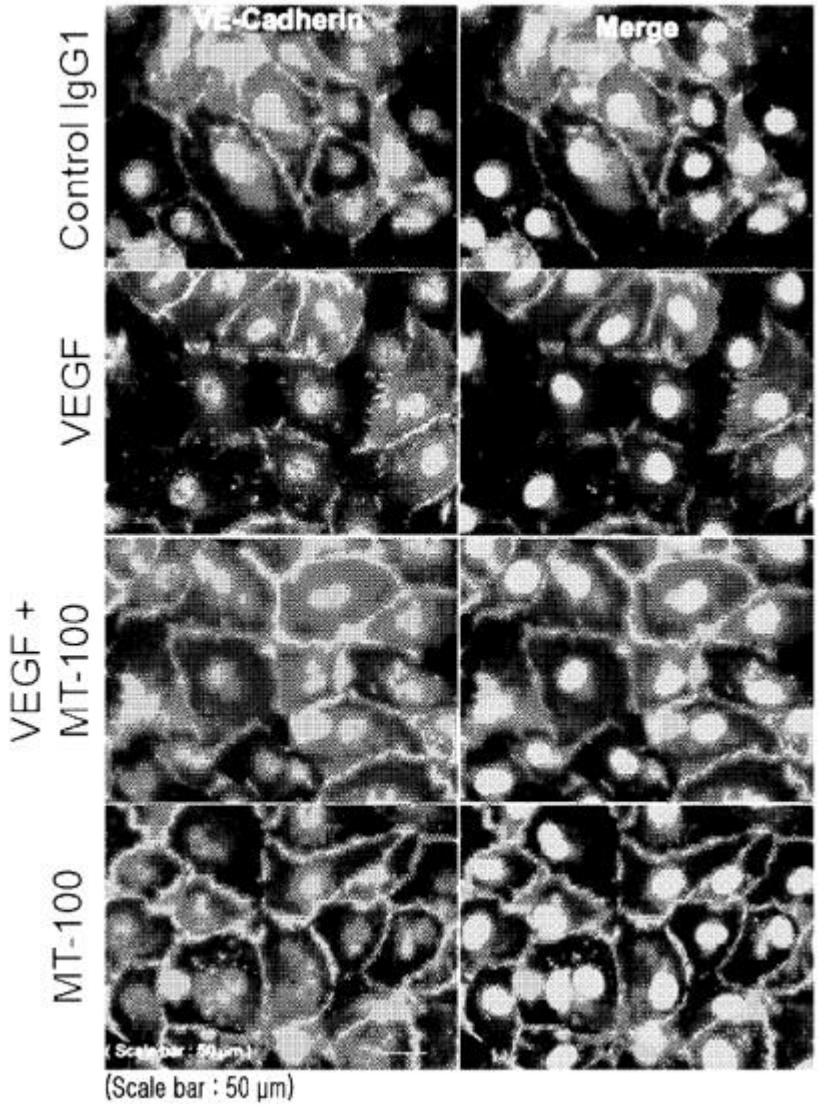


Figure 8b



(Scale bar : 50 μm)

Figure 8c

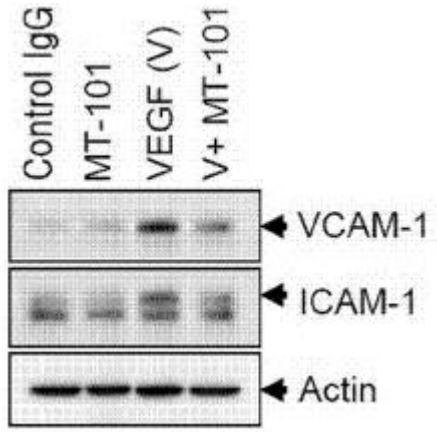


Figure 9a

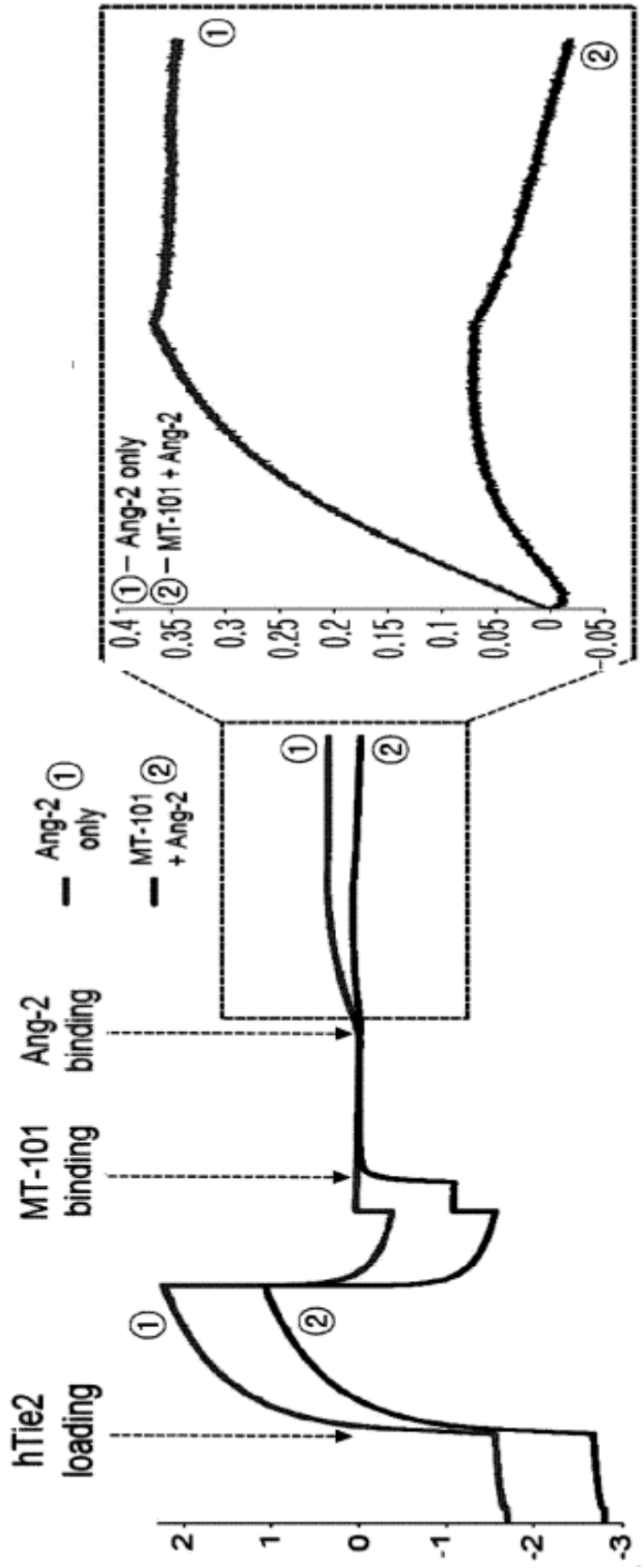

hTie2 loading
MT-101 binding
Ang-2 binding
Ang-2 only
MT-101 + Ang-2
2
1
0
-1
-2
-3
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
-0.05
① — Ang-2 only
② — MT-101 + Ang-2

Figure 9b

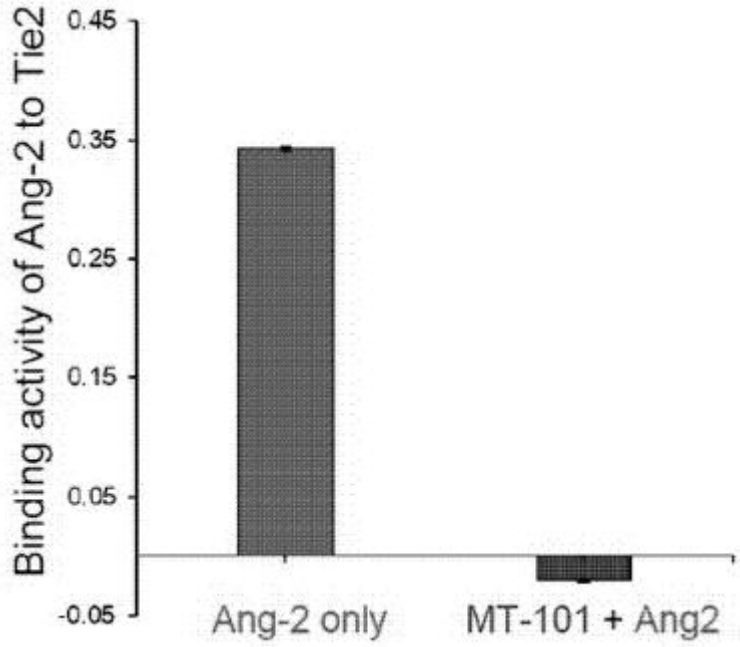

Binding activity of Ang-2 to Tie2
0.45
0.35
0.25
0.15
0.05
-0.05
Ang-2 only
MT-101 + Ang2

Figure 10

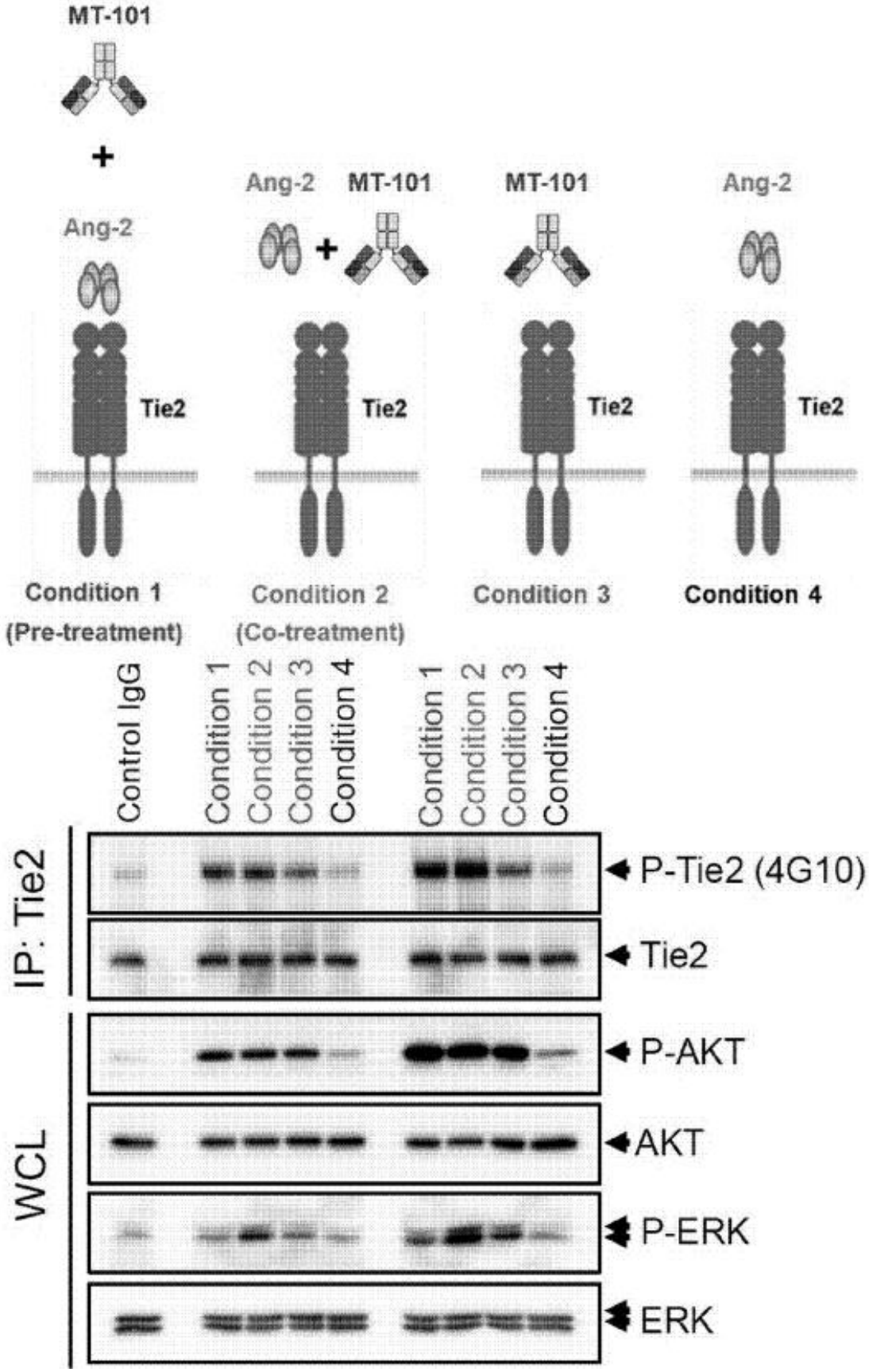

MT-101
+
Ang-2
Tie2
Condition 1
(Pre-treatment)
Ang-2
MT-101
+
Tie2
Condition 2
(Co-treatment)
MT-101
Tie2
Condition 3
Ang-2
Tie2
Condition 4
Control IgG
Condition 1
Condition 2
Condition 3
Condition 4
Condition 1
Condition 2
Condition 3
Condition 4
IP: Tie2
P-Tie2 (4G10)
Tie2
WCL
P-AKT
AKT
P-ERK
ERK

Figure 11a

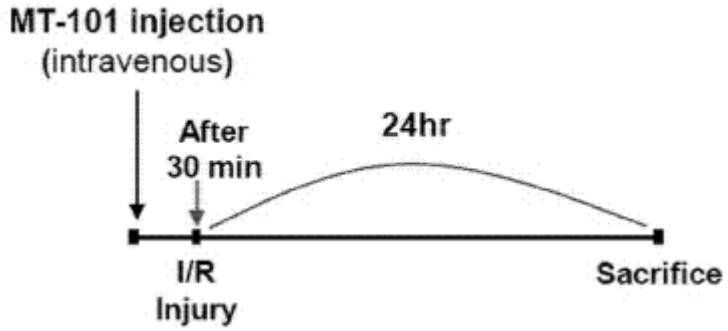


Figure 11b

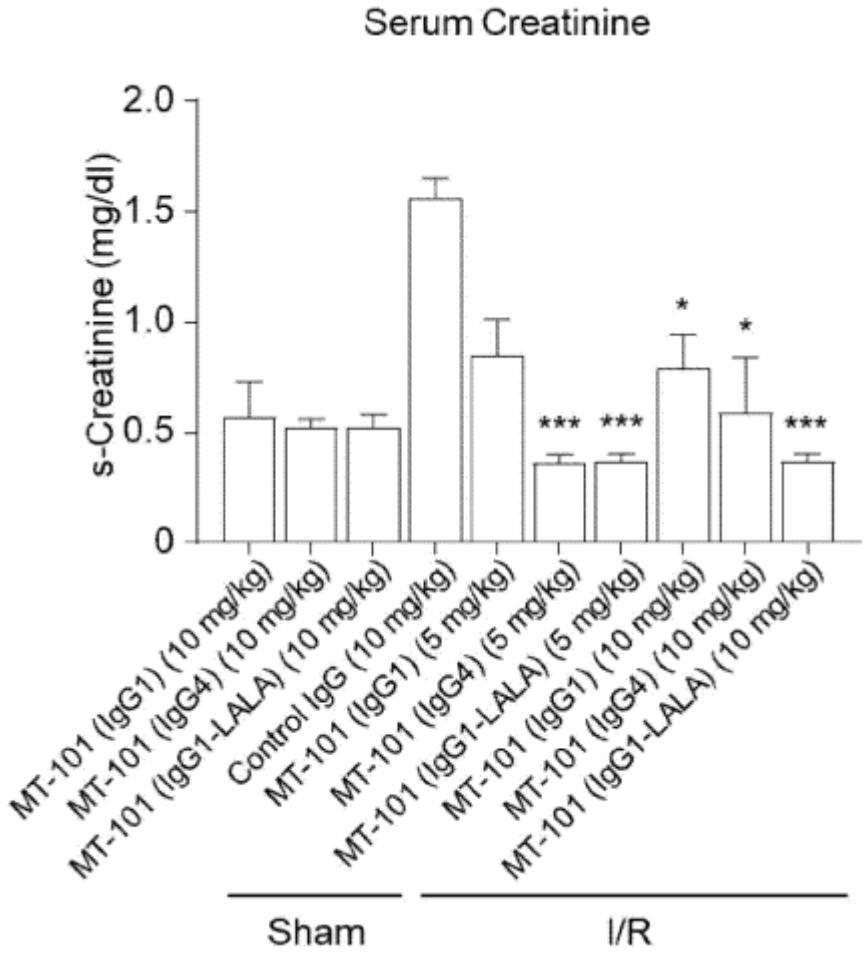


Figure 11c

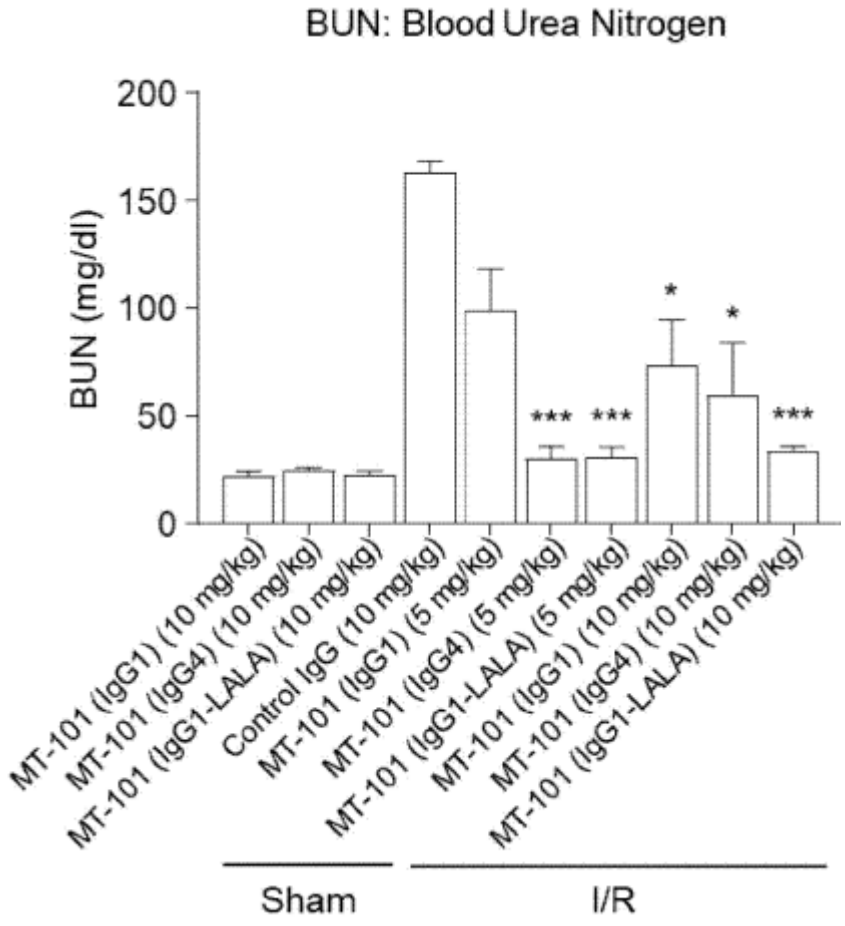

BUN: Blood Urea Nitrogen
BUN (mg/dl)
200
150
100
50
0
***
***
*
*
***
MT-101 (IgG1) (10 mg/kg)
MT-101 (IgG4) (10 mg/kg)
MT-101 (IgG1-LALA) (10 mg/kg)
Control IgG (10 mg/kg)
MT-101 (IgG1) (5 mg/kg)
MT-101 (IgG4) (5 mg/kg)
MT-101 (IgG1-LALA) (5 mg/kg)
MT-101 (IgG1) (10 mg/kg)
MT-101 (IgG4) (10 mg/kg)
MT-101 (IgG1-LALA) (10 mg/kg)
Sham
I/R

Figure 12a

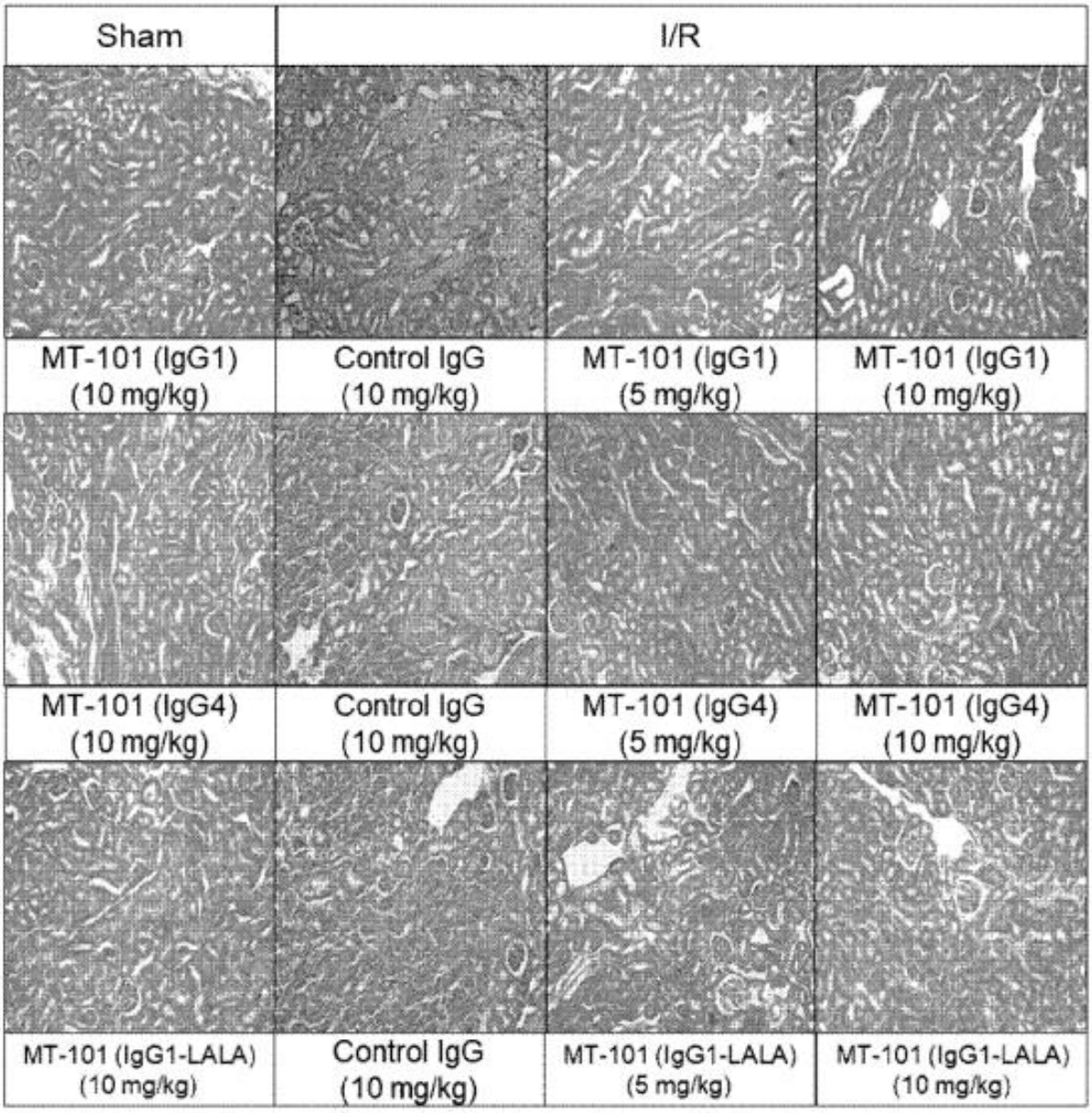

Sham
I/R
MT-101 (IgG1) (10 mg/kg)
Control IgG (10 mg/kg)
MT-101 (IgG1) (5 mg/kg)
MT-101 (IgG1) (10 mg/kg)
MT-101 (IgG4) (10 mg/kg)
Control IgG (10 mg/kg)
MT-101 (IgG4) (5 mg/kg)
MT-101 (IgG4) (10 mg/kg)
MT-101 (IgG1-LALA) (10 mg/kg)
Control IgG (10 mg/kg)
MT-101 (IgG1-LALA) (5 mg/kg)
MT-101 (IgG1-LALA) (10 mg/kg)

Figure 12b

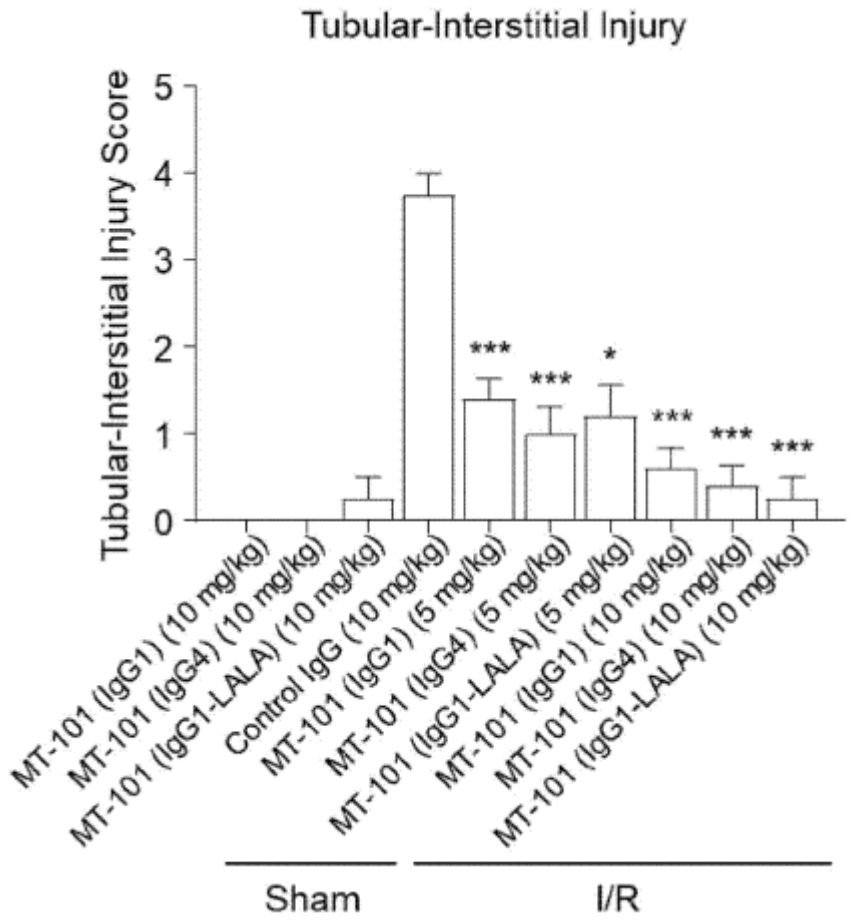

Tubular-Interstitial Injury
Tubular-Interstitial Injury Score
0
1
2
3
4
5
***
***
*
***
***
***
MT-101 (IgG1) (10 mg/kg)
MT-101 (IgG4) (10 mg/kg)
MT-101 (IgG1-LALA) (10 mg/kg)
Control IgG (10 mg/kg)
MT-101 (IgG1) (5 mg/kg)
MT-101 (IgG4) (5 mg/kg)
MT-101 (IgG1-LALA) (5 mg/kg)
MT-101 (IgG1) (10 mg/kg)
MT-101 (IgG4) (10 mg/kg)
MT-101 (IgG1-LALA) (10 mg/kg)
Sham
I/R

Figure 13a

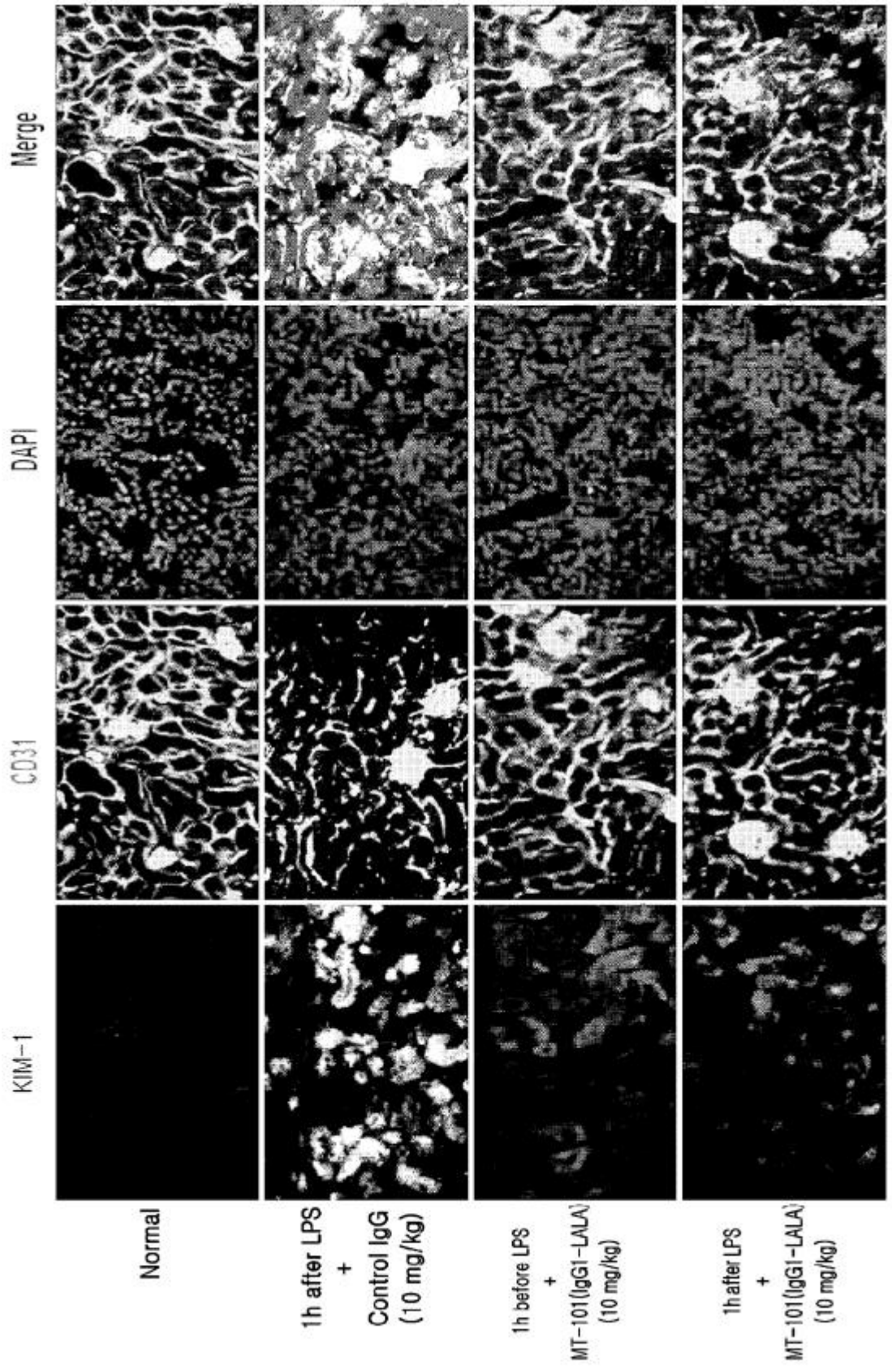

KIM-1
CD31
DAPI
Merge
Normal
1h after LPS + Control IgG (10 mg/kg)
1h before LPS + MT-101(IgG1-LALA) (10 mg/kg)
1h after LPS + MT-101(IgG1-LALA) (10 mg/kg)

Figure 13b

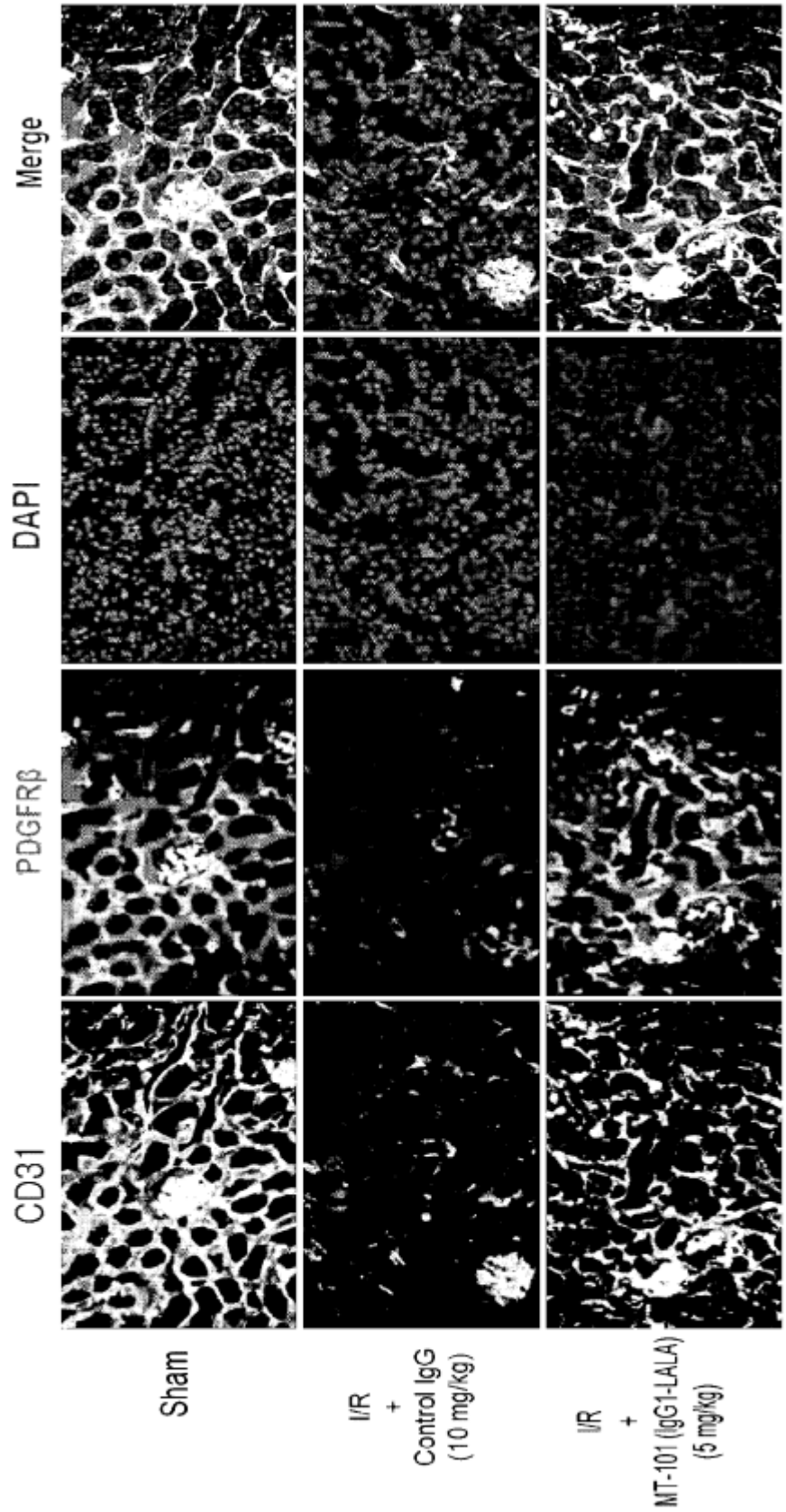

CD31
PDGFRβ
DAPI
Merge
Sham
I/R + Control IgG (10 mg/kg)
I/R + MT-101 (IgG1-LALA) (5 mg/kg)

Figure 14a

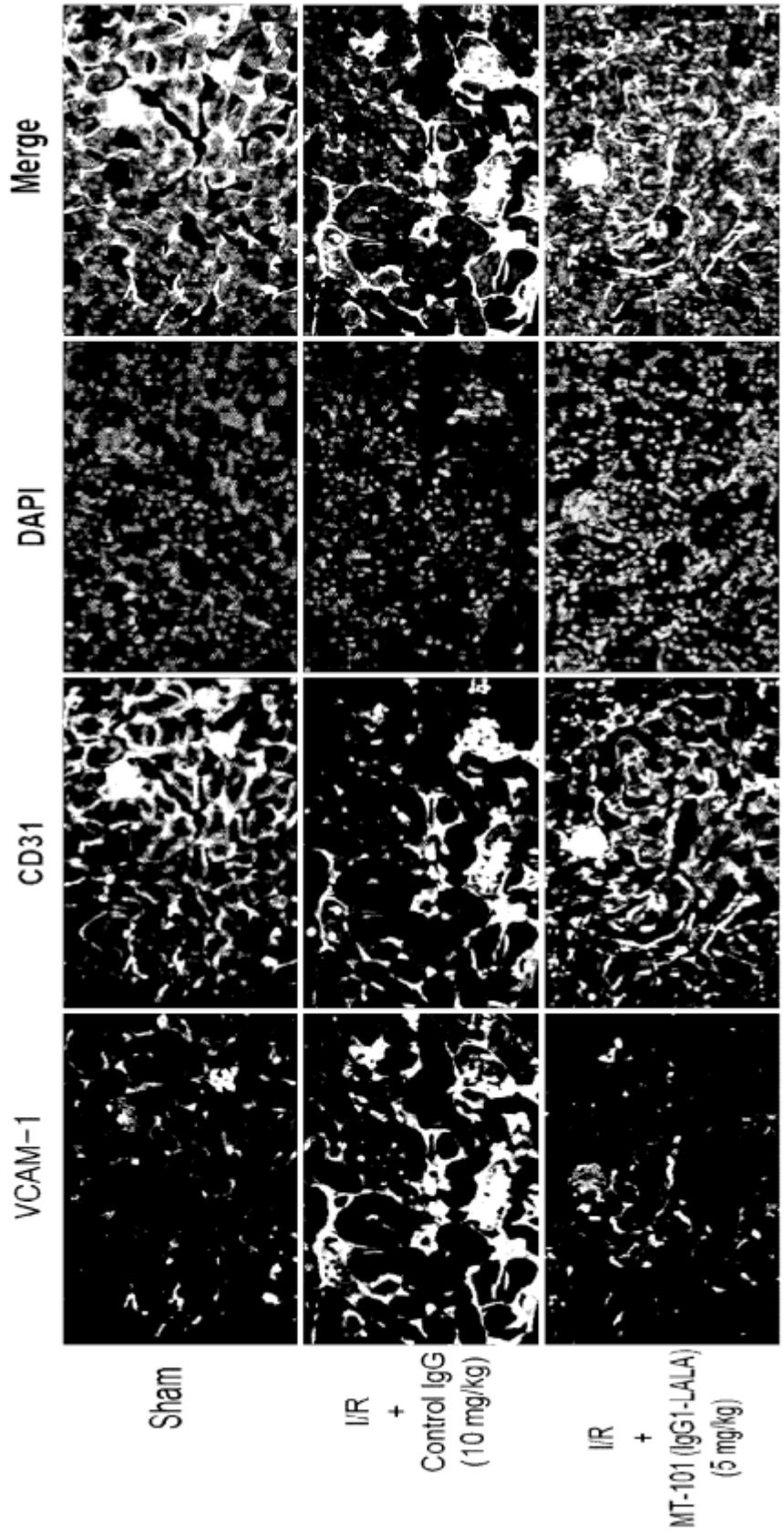

VCAM-1
CD31
DAPI
Merge
Sham
I/R
+
Control IgG
(10 mg/kg)
I/R
+
MT-101 (IgG1-LALA)
(5 mg/kg)

Figure 14b

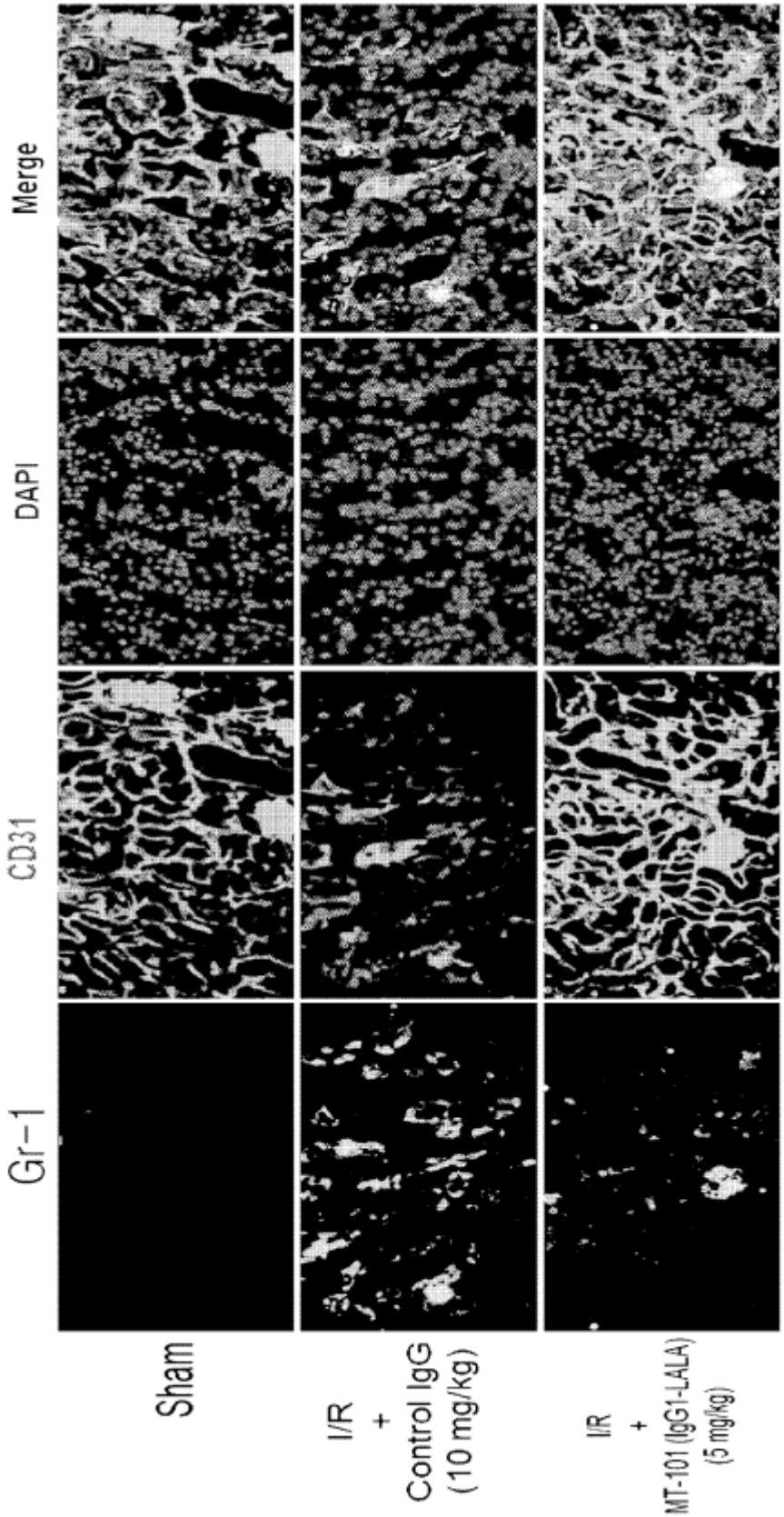

Gr-1
CD31
DAPI
Merge
Sham
I/R
+
Control IgG
(10 mg/kg)
I/R
+
MT-101 (IgG1-LALA)
(5 mg/kg)

Figure 14c

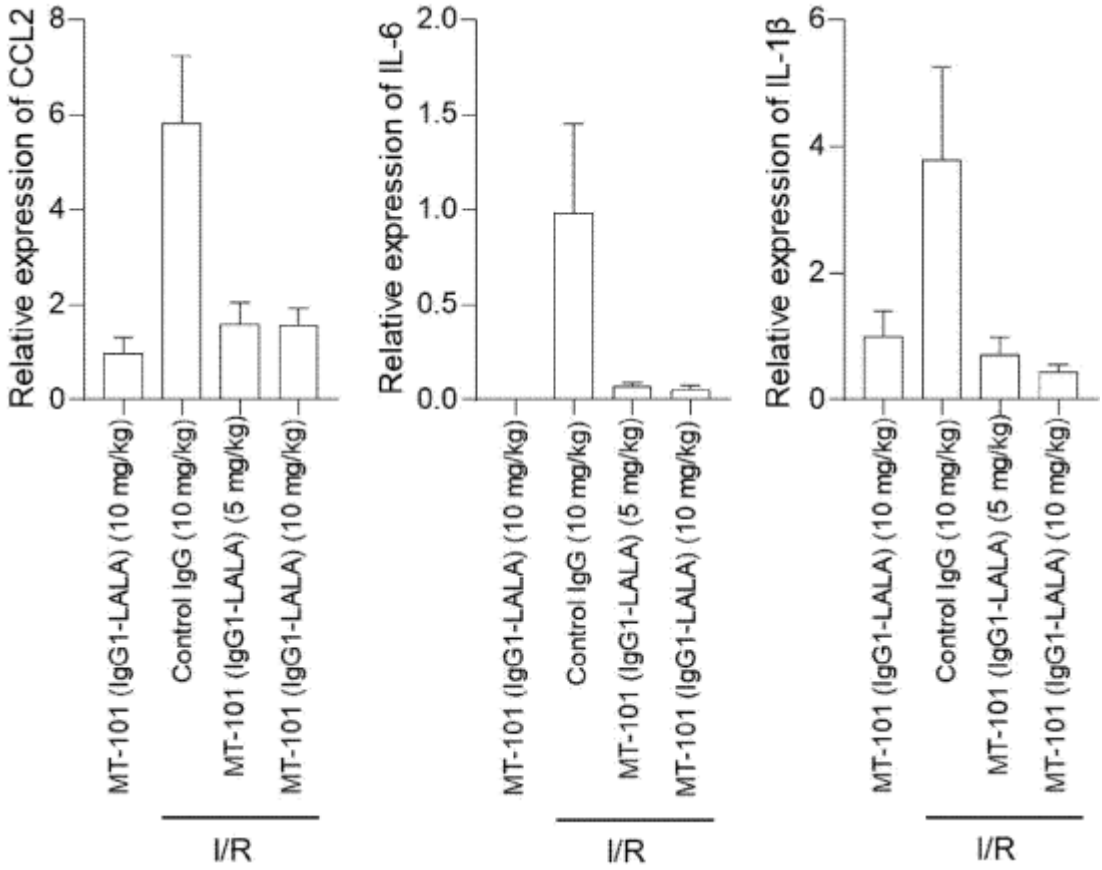


Figure 15a

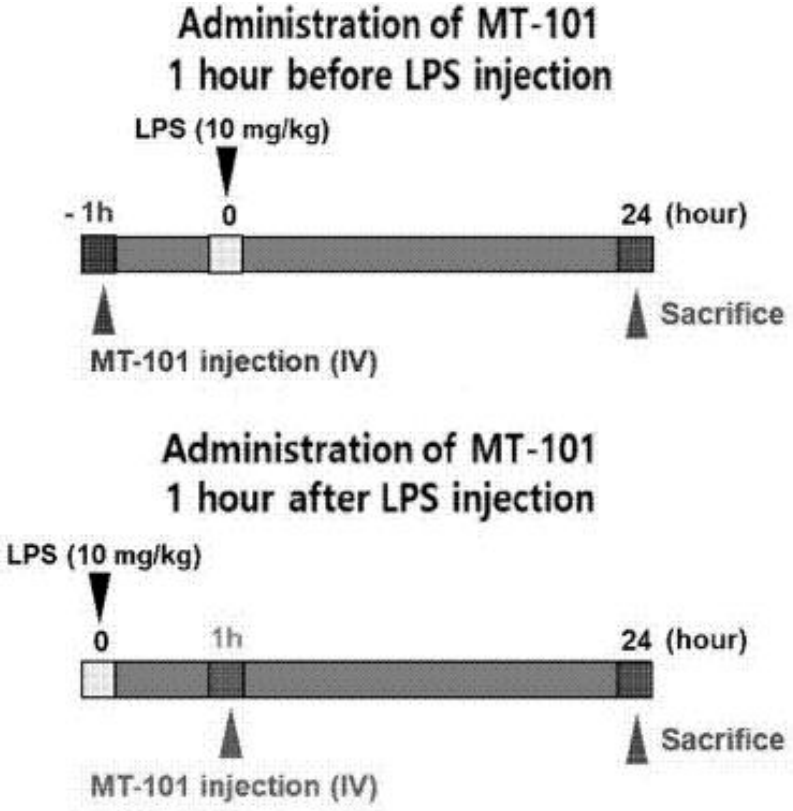


Figure 15b

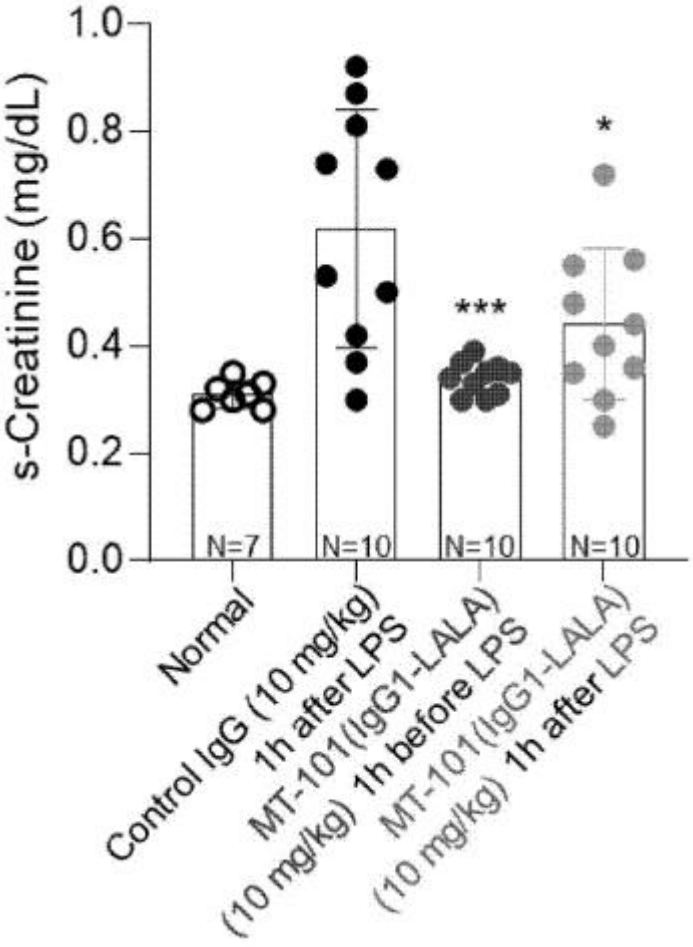

s-Creatinine (mg/dL)
1.0
0.8
0.6
0.4
0.2
0.0
N=7
N=10
N=10
N=10
***
*
Normal
Control IgG (10 mg/kg) 1h after LPS
MT-101(IgG1-LALA) (10 mg/kg) 1h before LPS
MT-101(IgG1-LALA) (10 mg/kg) 1h after LPS

Figure 15c

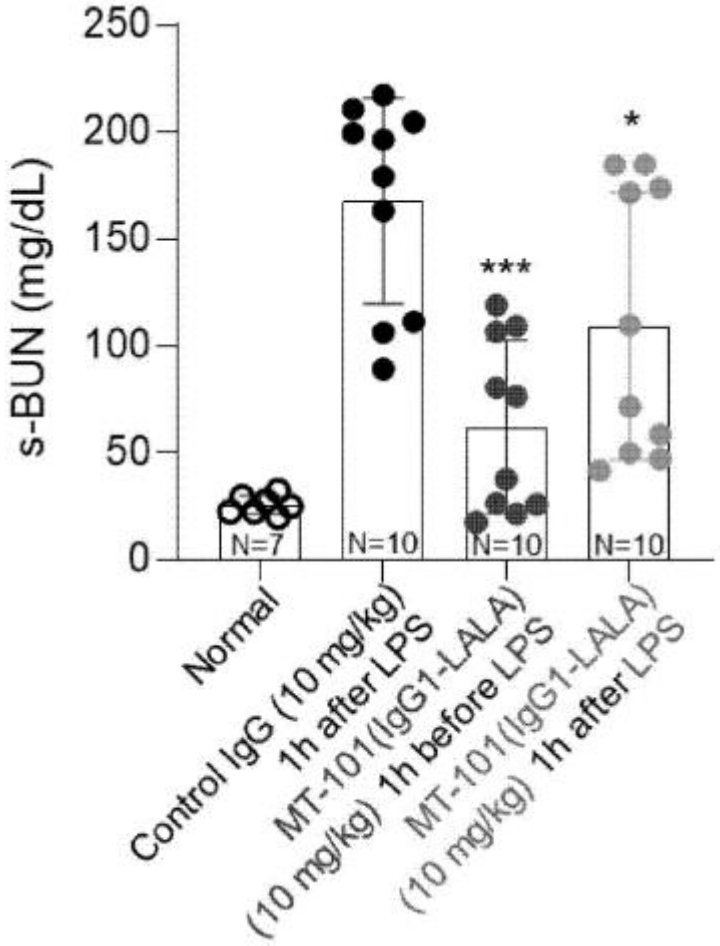

250
200
150
100
50
0
s-BUN (mg/dL)
N=7
N=10
N=10
N=10
***
*
Normal
Control IgG (10 mg/kg) 1h after LPS
MT-101(IgG1-LALA) (10 mg/kg) 1h before LPS
MT-101(IgG1-LALA) (10 mg/kg) 1h after LPS

Figure 15d

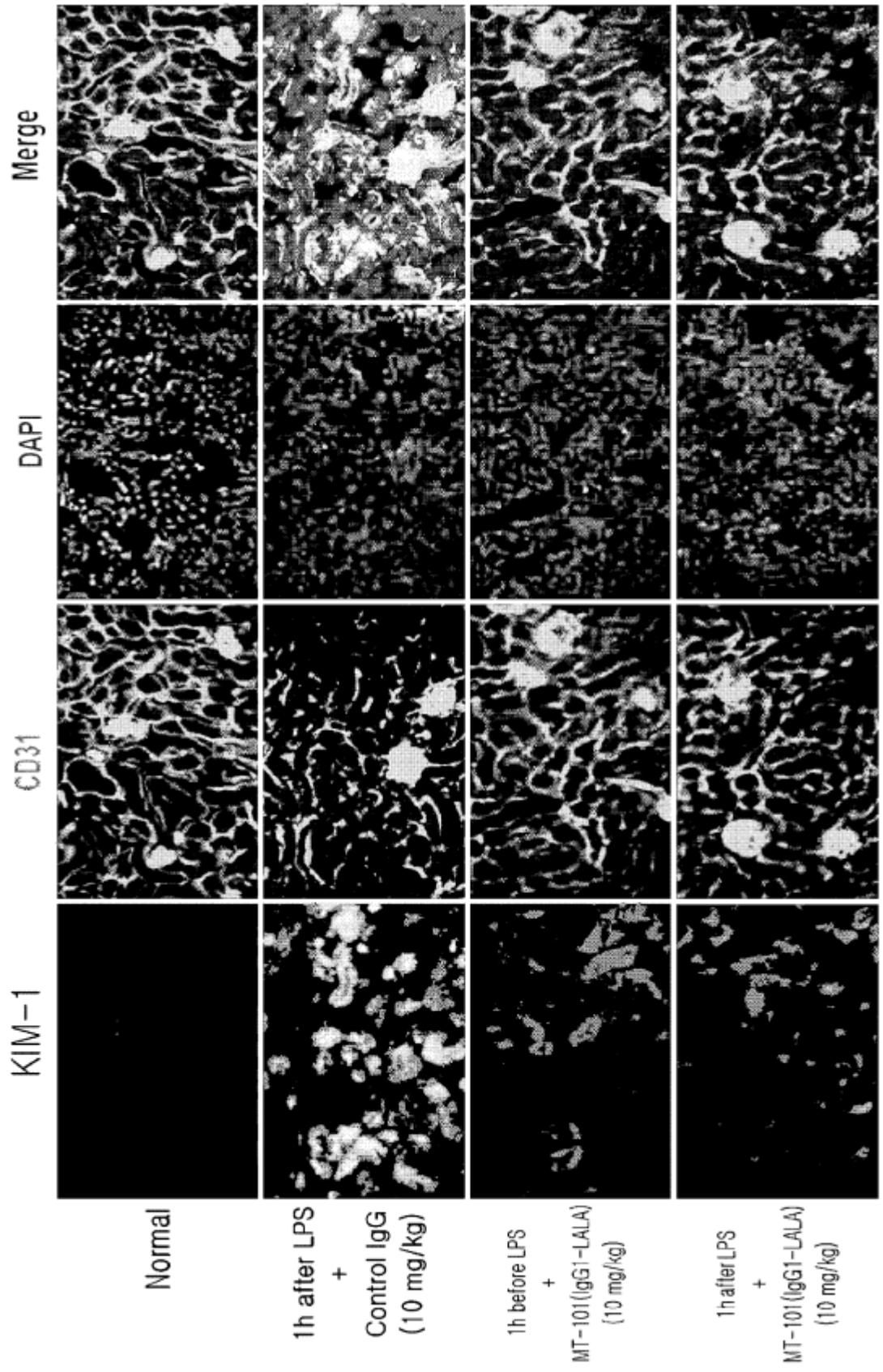

KIM-1
CD31
DAPI
Merge
Normal
1h after LPS + Control IgG (10 mg/kg)
1h before LPS + MT-101(IgG1-LALA) (10 mg/kg)
1h after LPS + MT-101(IgG1-LALA) (10 mg/kg)

Figure 16a

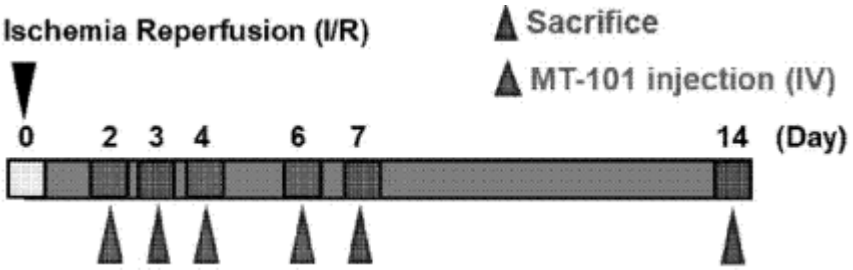


Figure 16b

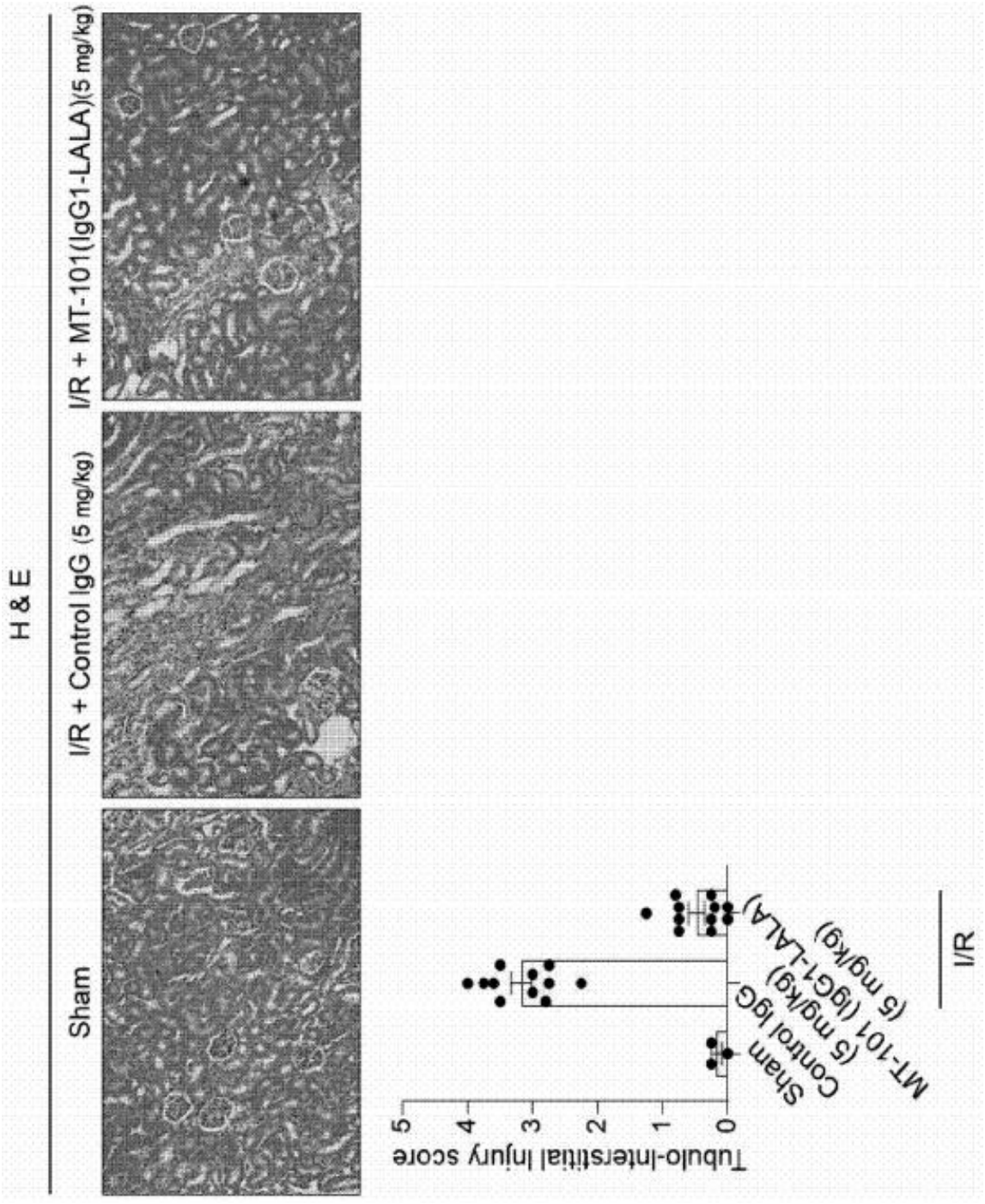


Figure 16c

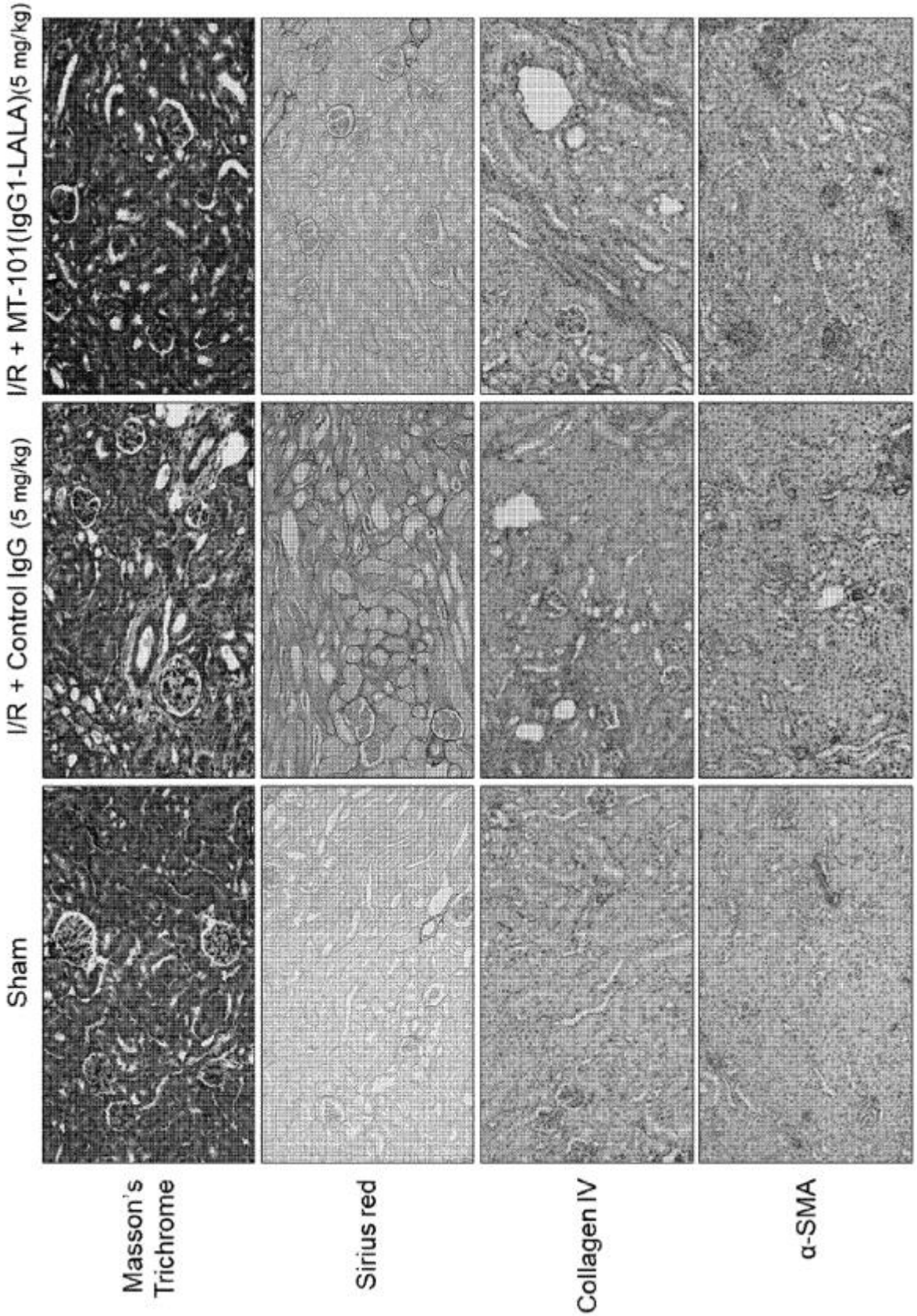

Sham
I/R + Control IgG (5 mg/kg)
I/R + MT-101(IgG1-LALA)(5 mg/kg)
Masson's Trichrome
Sirius red
Collagen IV
α-SMA

Figure 17a

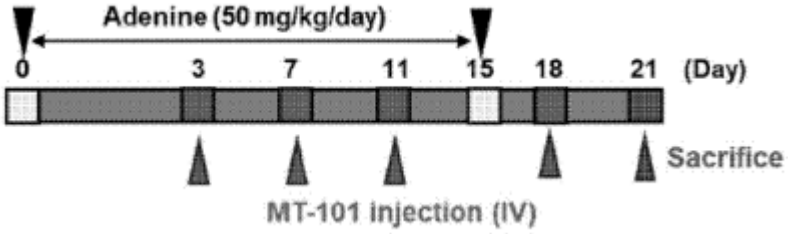


Figure 17b

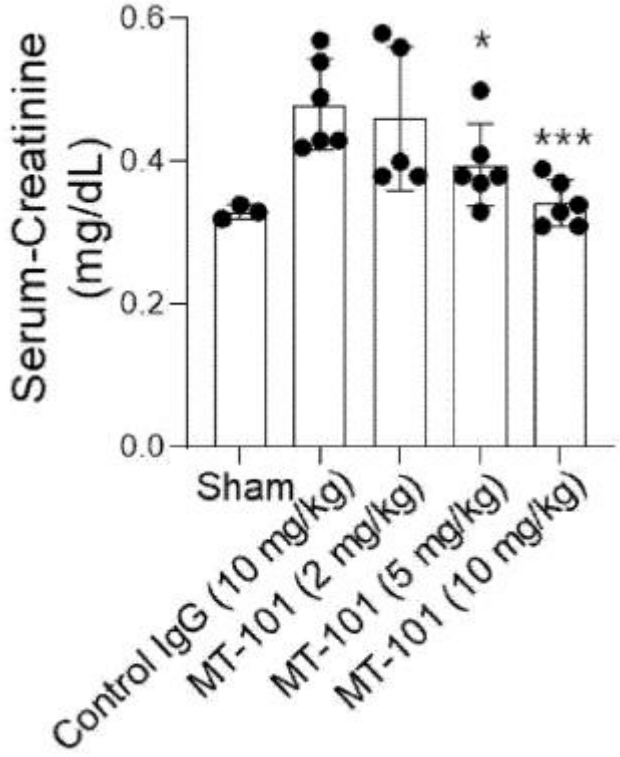


Figure 17c

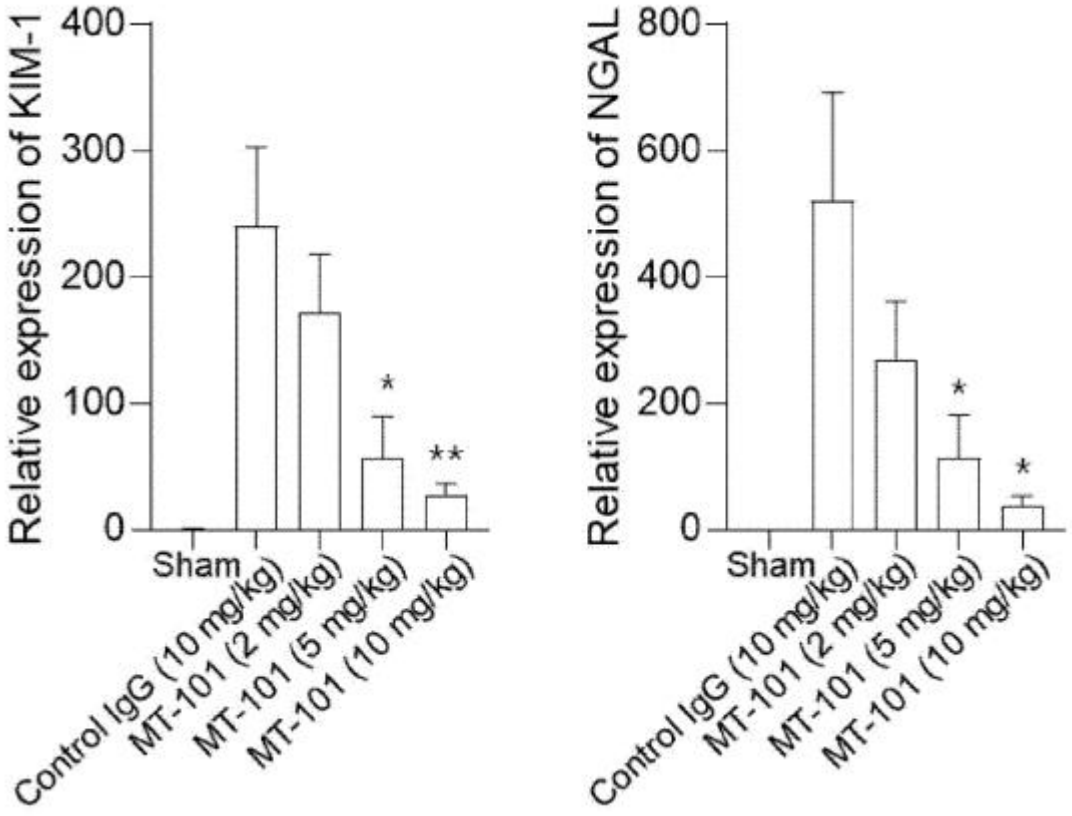

Relative expression of KIM-1
400
300
200
100
0
*
**
Sham
Control IgG (10 mg/kg)
MT-101 (2 mg/kg)
MT-101 (5 mg/kg)
MT-101 (10 mg/kg)
Relative expression of NGAL
800
600
400
200
0
*
*
Sham
Control IgG (10 mg/kg)
MT-101 (2 mg/kg)
MT-101 (5 mg/kg)
MT-101 (10 mg/kg)

Figure 17d

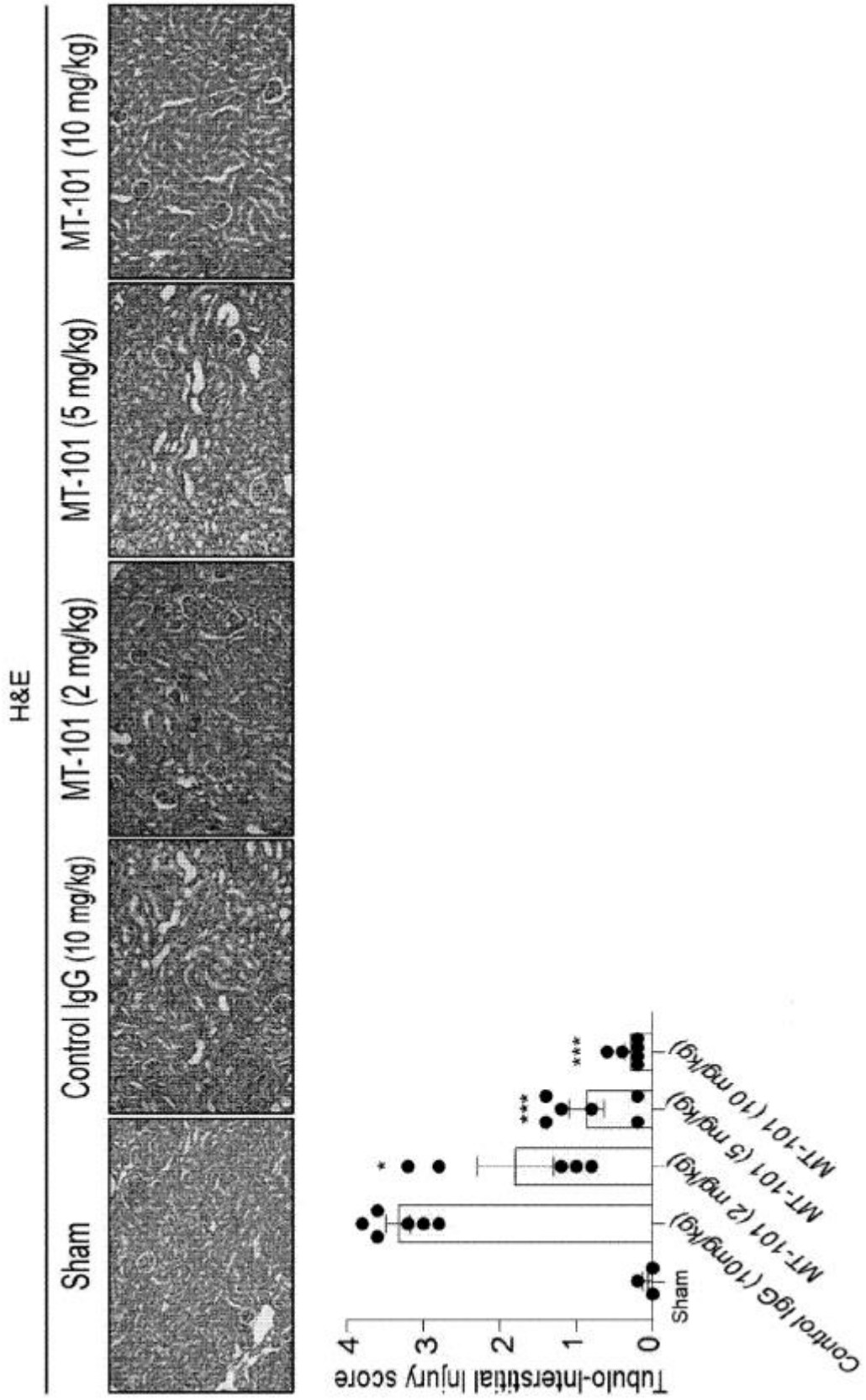

H&E
Sham
Control IgG (10 mg/kg)
MT-101 (2 mg/kg)
MT-101 (5 mg/kg)
MT-101 (10 mg/kg)
Tubulo-interstitial injury score
0
1
2
3
4
Sham
Control IgG (10mg/kg)
MT-101 (2 mg/kg)
MT-101 (5 mg/kg)
MT-101 (10 mg/kg)
*
***
***

Figure 17e

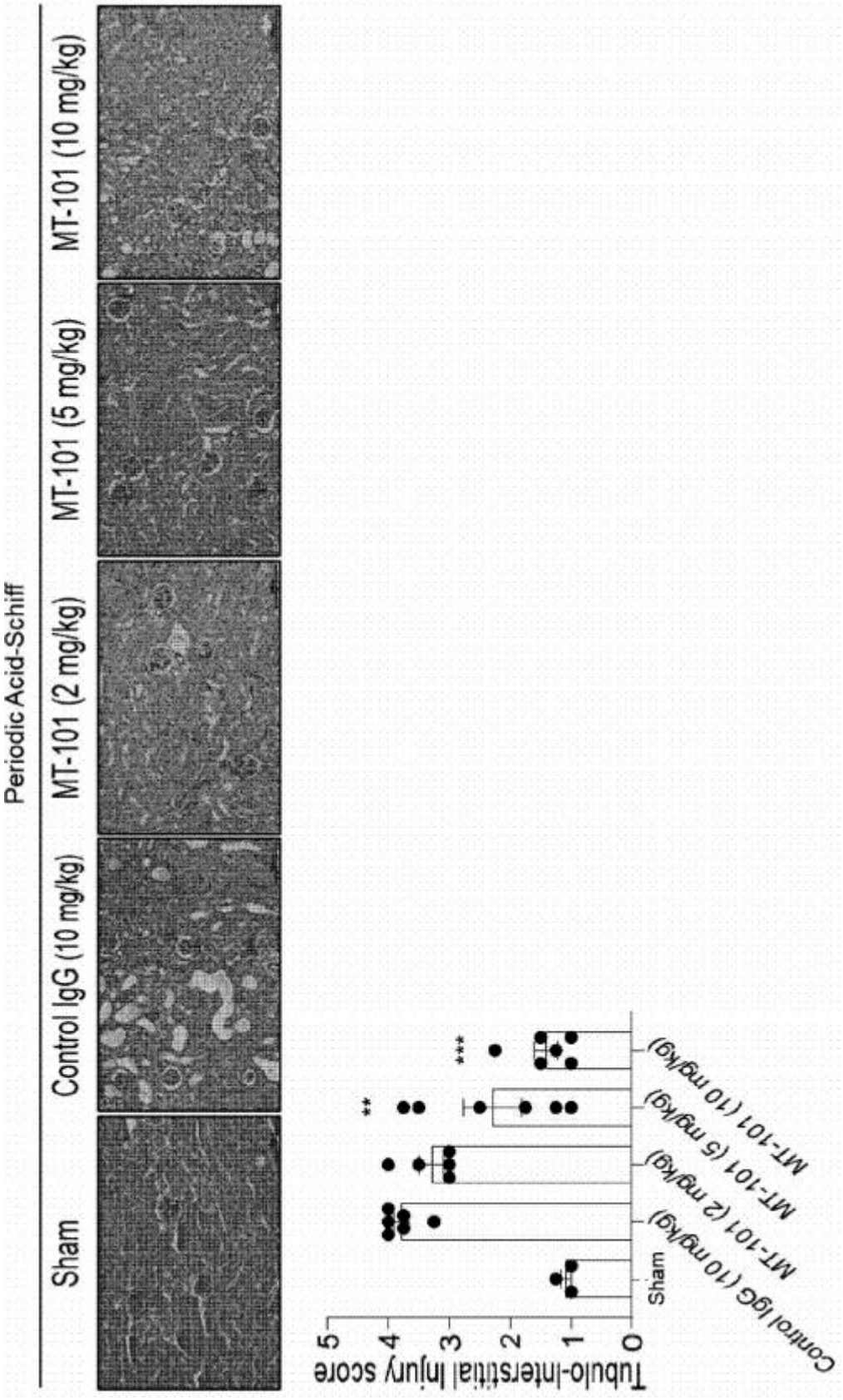

Periodic Acid-Schiff
Sham
Control IgG (10 mg/kg)
MT-101 (2 mg/kg)
MT-101 (5 mg/kg)
MT-101 (10 mg/kg)
Tubulo-interstitial injury score
0
1
2
3
4
5
**
***
Sham
Control IgG (10 mg/kg)
MT-101 (2 mg/kg)
MT-101 (5 mg/kg)
MT-101 (10 mg/kg)

Figure 18a

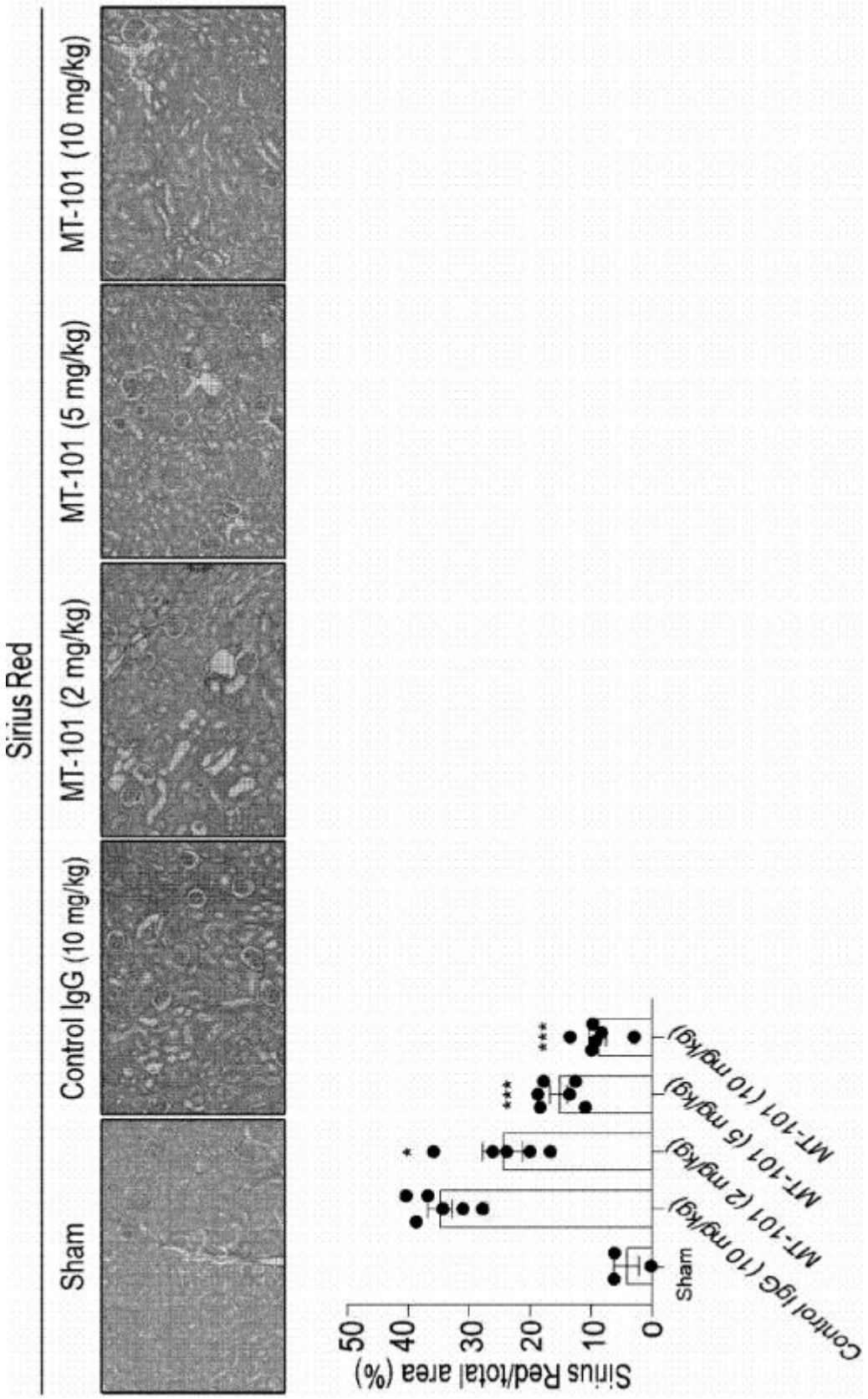

Sirius Red
Sham
Control IgG (10 mg/kg)
MT-101 (2 mg/kg)
MT-101 (5 mg/kg)
MT-101 (10 mg/kg)
Sirius Red/total area (%)
0
10
20
30
40
50
Sham
Control IgG (10 mg/kg)
MT-101 (2 mg/kg)
MT-101 (5 mg/kg)
MT-101 (10 mg/kg)
*
***
***

Figure 18b

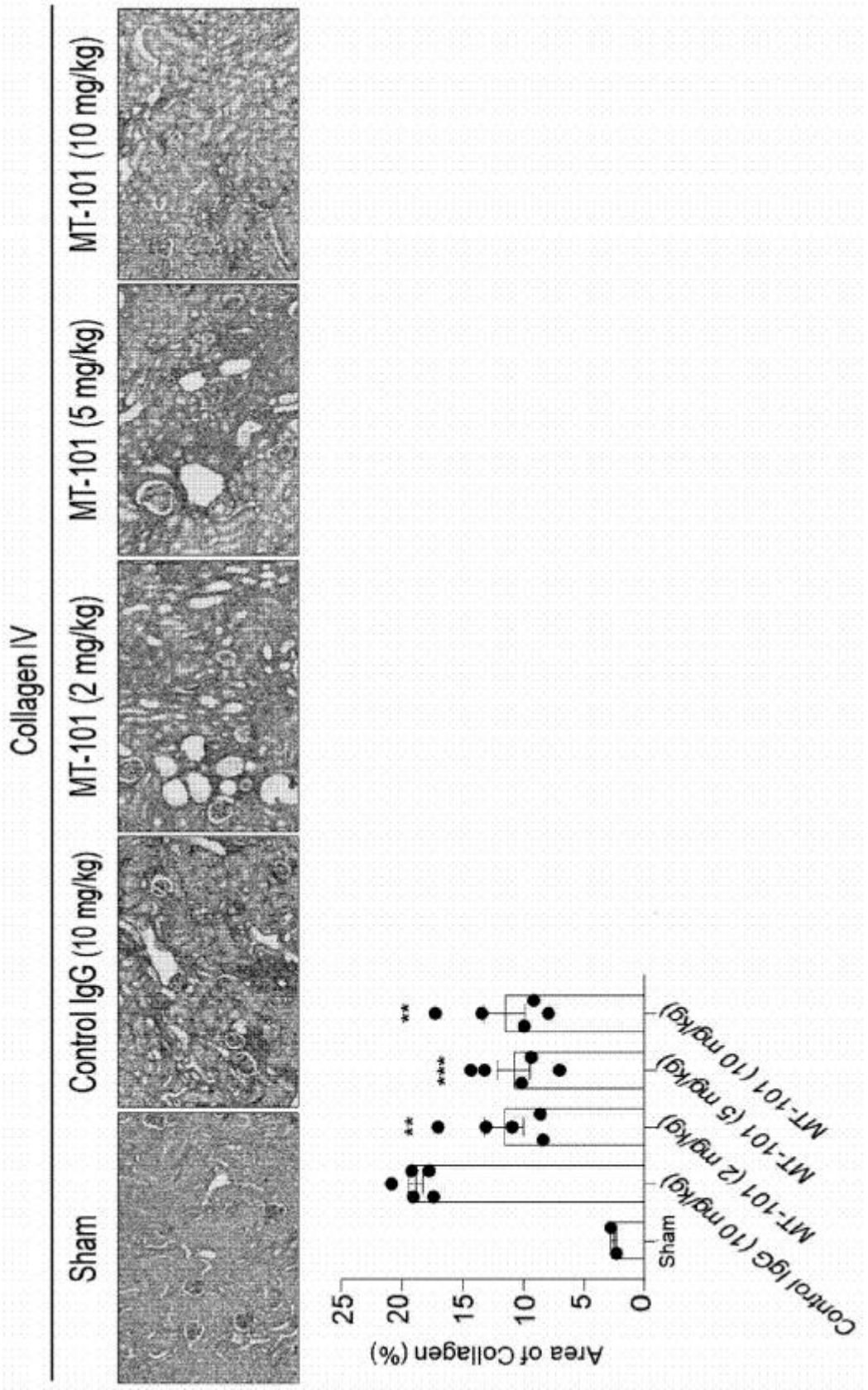

Collagen IV
Sham
Control IgG (10 mg/kg)
MT-101 (2 mg/kg)
MT-101 (5 mg/kg)
MT-101 (10 mg/kg)
Area of Collagen (%)
25
20
15
10
5
0
**
**
***
**
Sham
Control IgG (10 mg/kg)
MT-101 (2 mg/kg)
MT-101 (5 mg/kg)
MT-101 (10 mg/kg)

Figure 18c

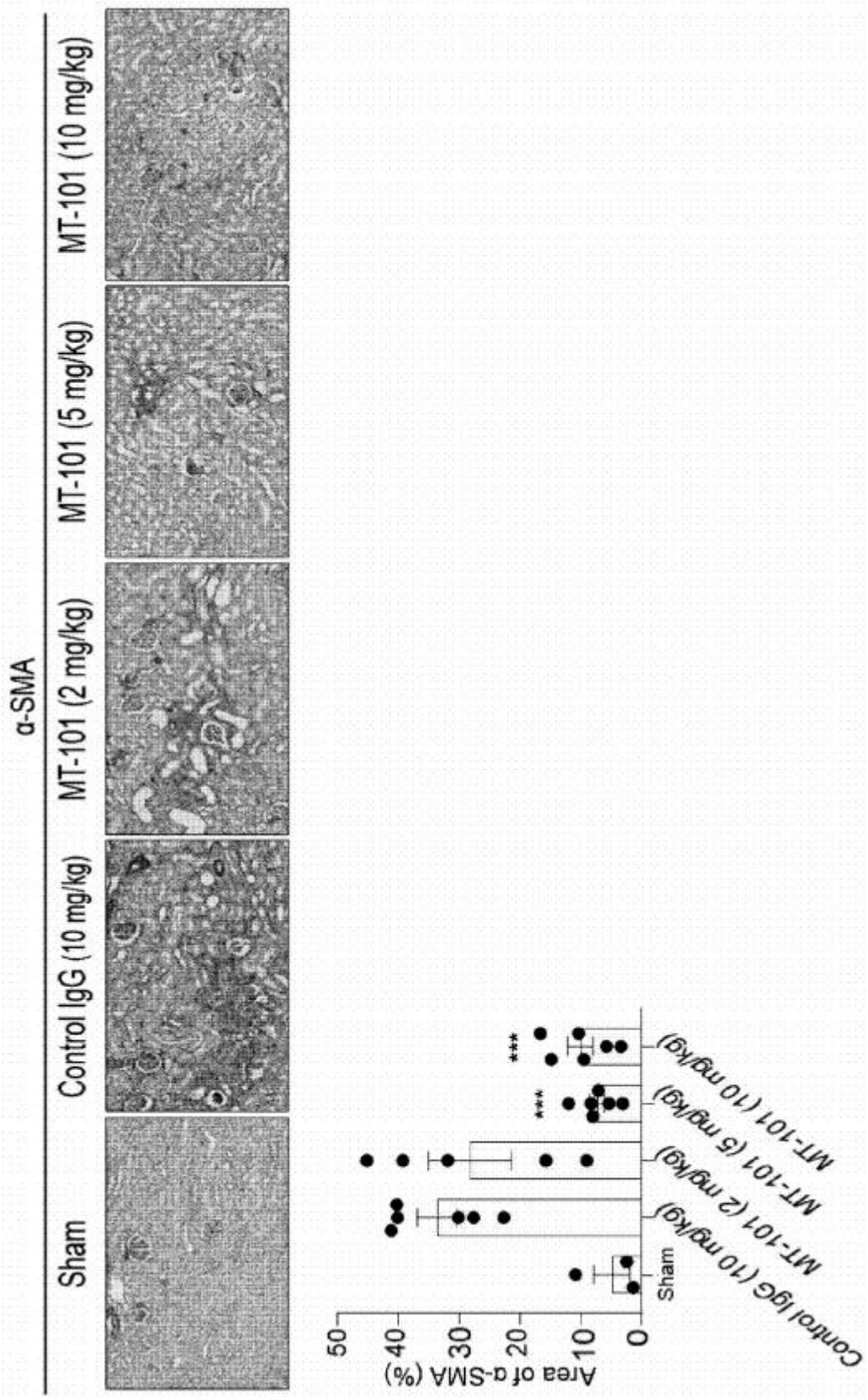

α-SMA
Sham
Control IgG (10 mg/kg)
MT-101 (2 mg/kg)
MT-101 (5 mg/kg)
MT-101 (10 mg/kg)
Area of α-SMA (%)
0
10
20
30
40
50
Sham
Control IgG (10 mg/kg)
MT-101 (2 mg/kg)
MT-101 (5 mg/kg)
MT-101 (10 mg/kg)
***
***

Figure 18d

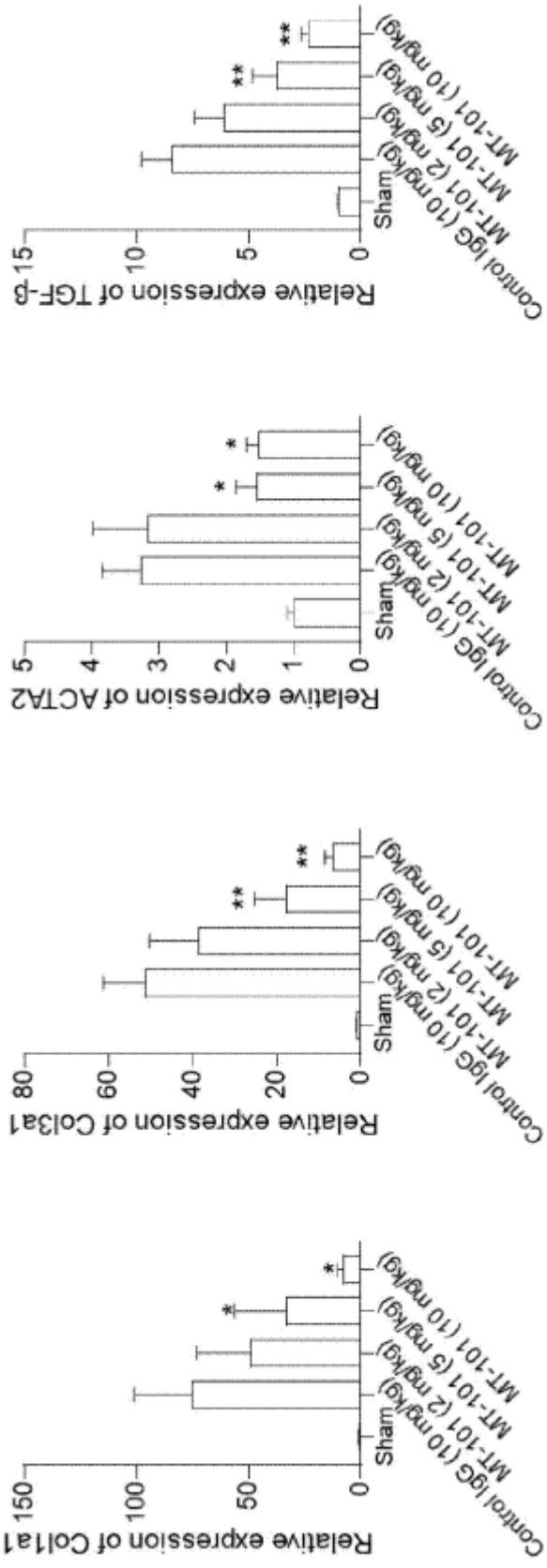

Relative expression of Col1a1
Relative expression of Col3a1
Relative expression of ACTA2
Relative expression of TGF-β
Sham
MT-101 (2 mg/kg)
MT-101 (5 mg/kg)
MT-101 (10 mg/kg)
Control IgG (10 mg/kg)

Figure 19a

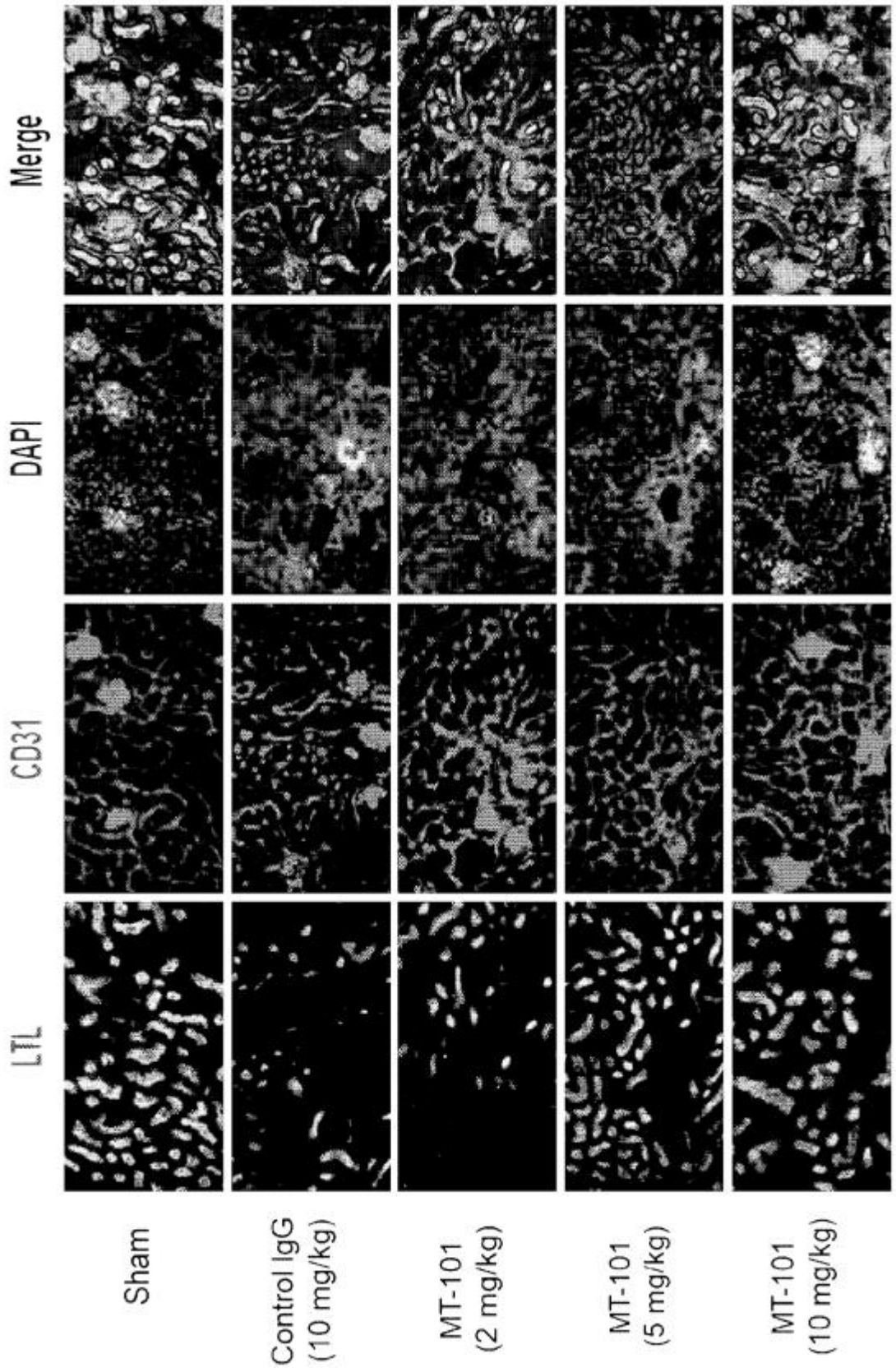

LTL
CD31
DAPI
Merge
Sham
Control IgG (10 mg/kg)
MT-101 (2 mg/kg)
MT-101 (5 mg/kg)
MT-101 (10 mg/kg)

Figure 19b

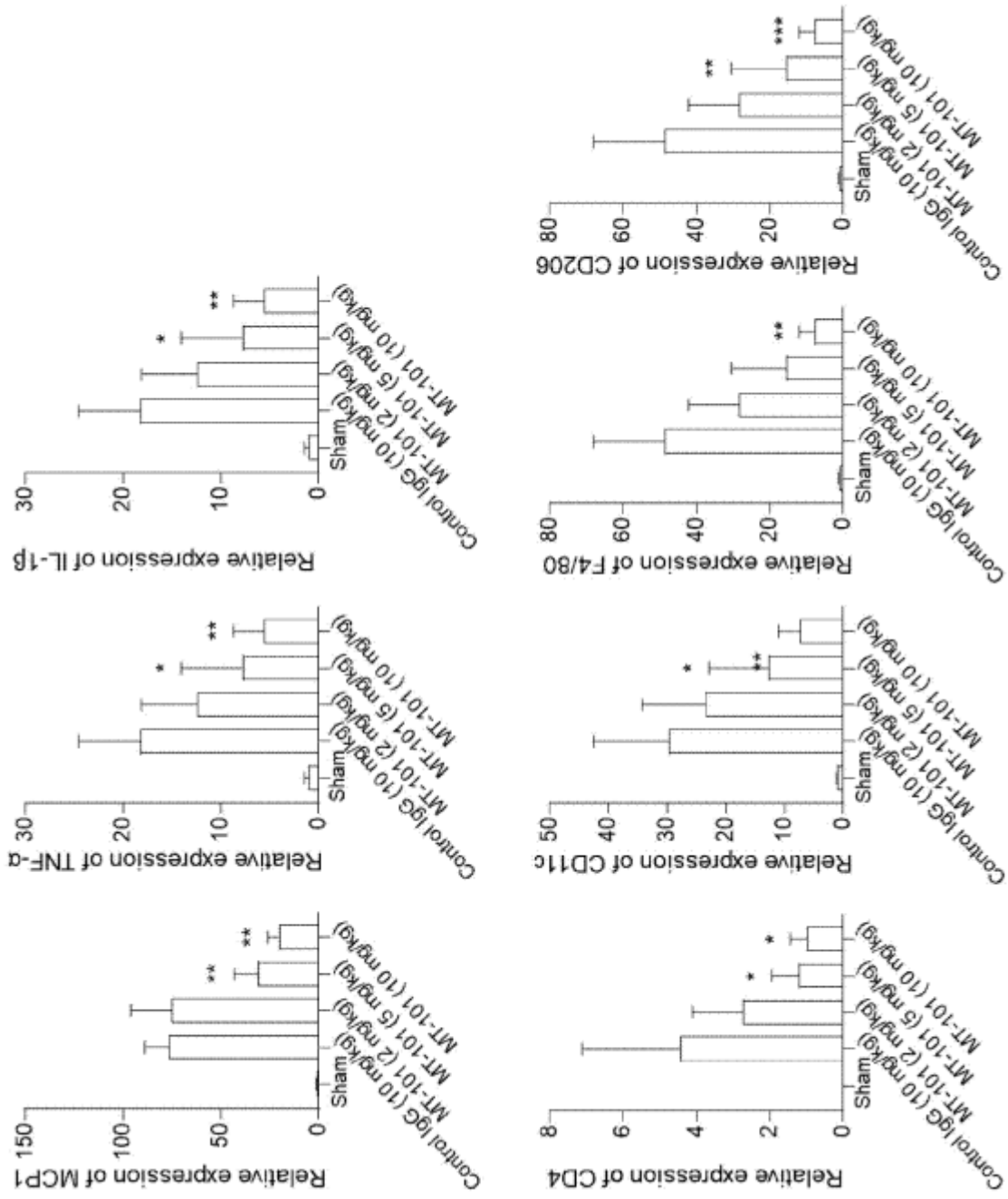

Relative expression of MCP1
Relative expression of TNF-α
Relative expression of IL-1β
Relative expression of CD4
Relative expression of CD11c
Relative expression of F4/80
Relative expression of CD206
Control IgG (10 mg/kg)
Sham
MT-101 (2 mg/kg)
MT-101 (5 mg/kg)
MT-101 (10 mg/kg)

Figure 20a

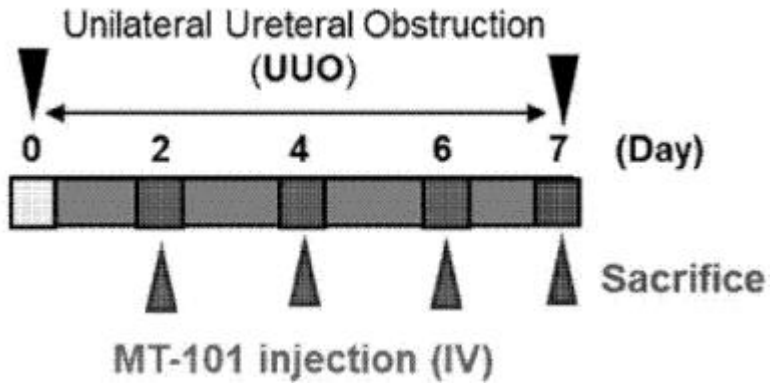


Figure 20b

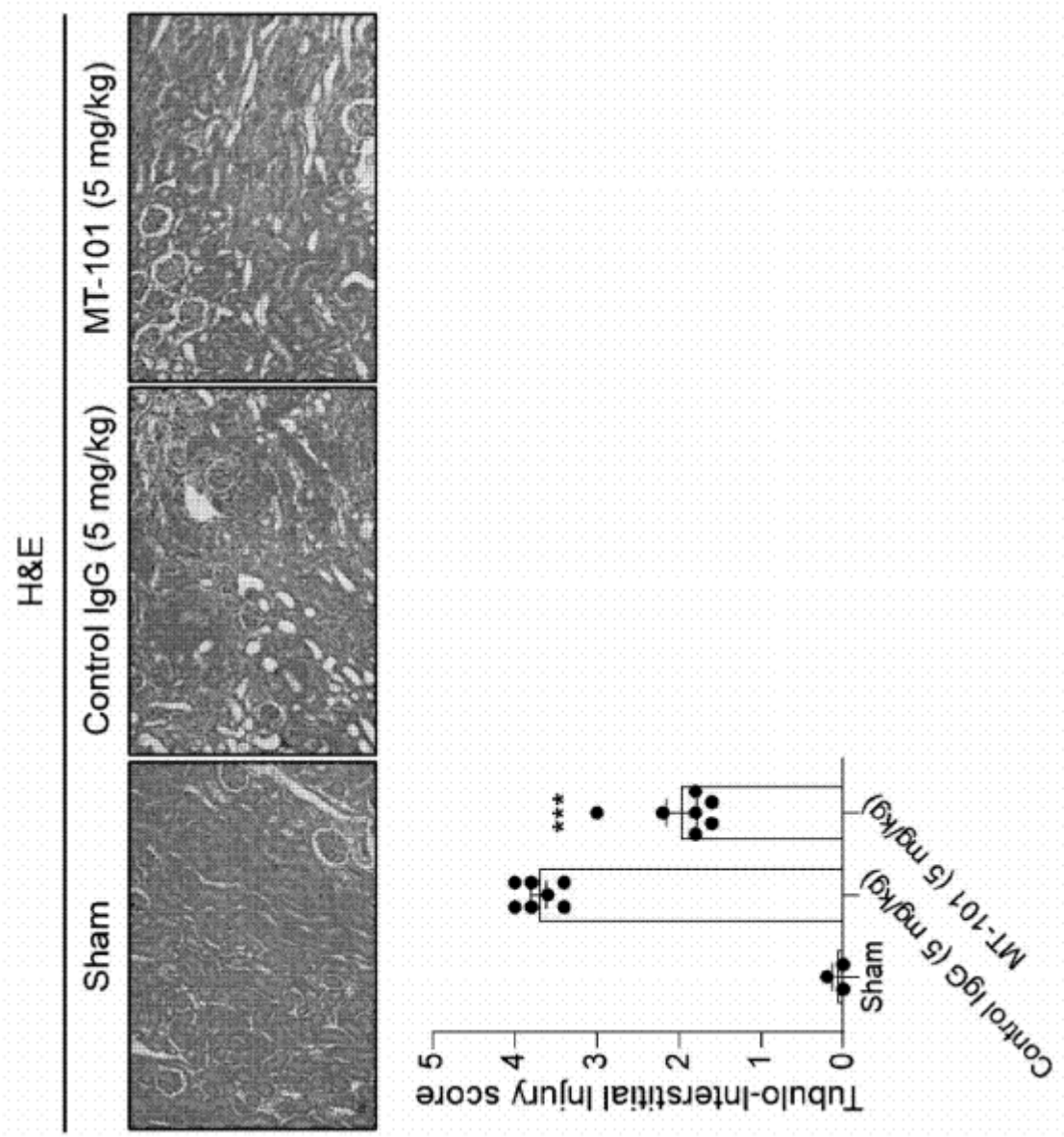


Figure 20c

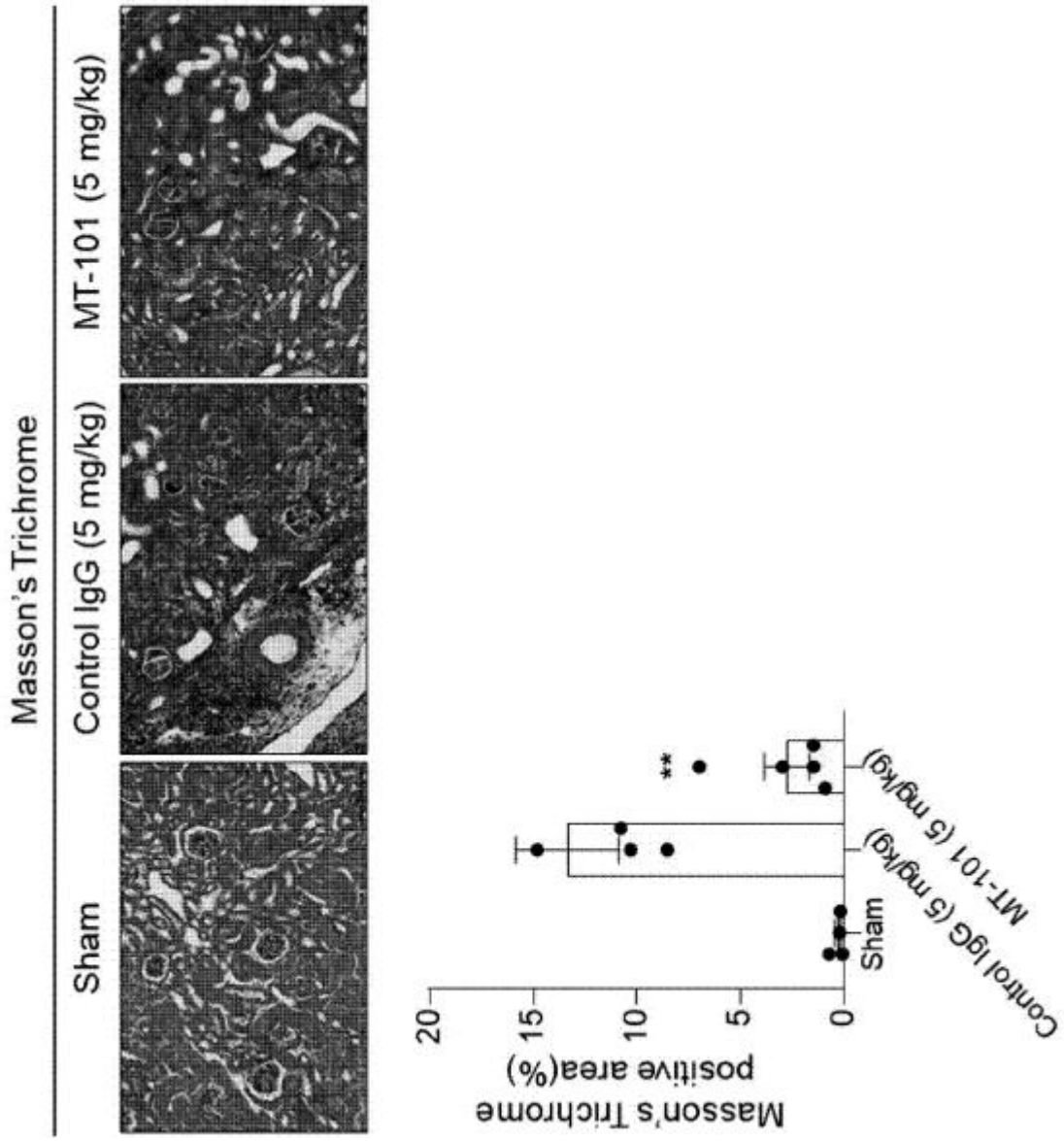

Masson's Trichrome
Sham
Control IgG (5 mg/kg)
MT-101 (5 mg/kg)
Masson's Trichrome positive area(%)
20
15
10
5
0
**
Sham
Control IgG (5 mg/kg)
MT-101 (5 mg/kg)

Figure 20d

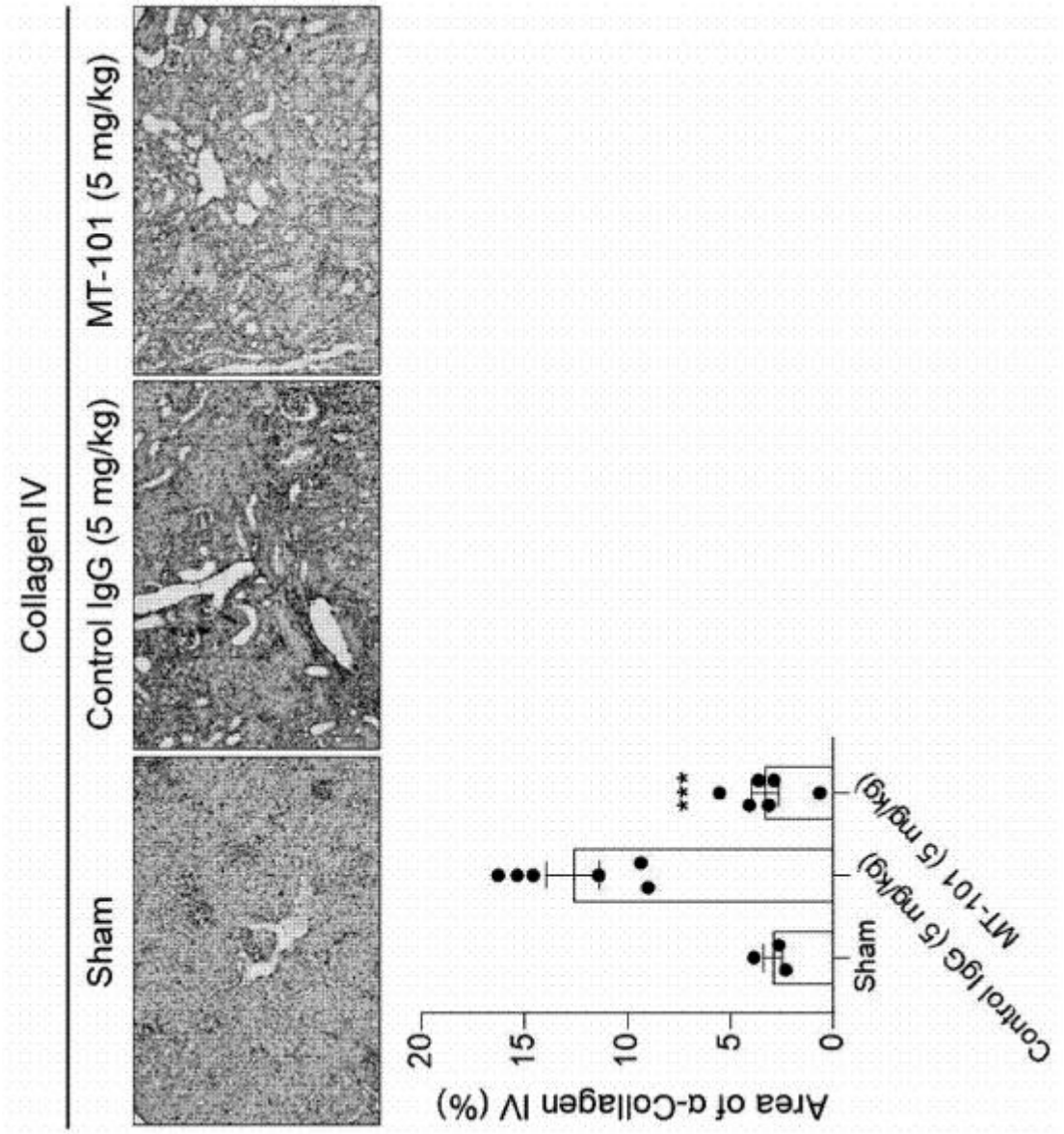

Collagen IV
Sham
Control IgG (5 mg/kg)
MT-101 (5 mg/kg)
Area of α-Collagen IV (%)
20
15
10
5
0
***
Sham
Control IgG (5 mg/kg)
MT-101 (5 mg/kg)

Figure 20e

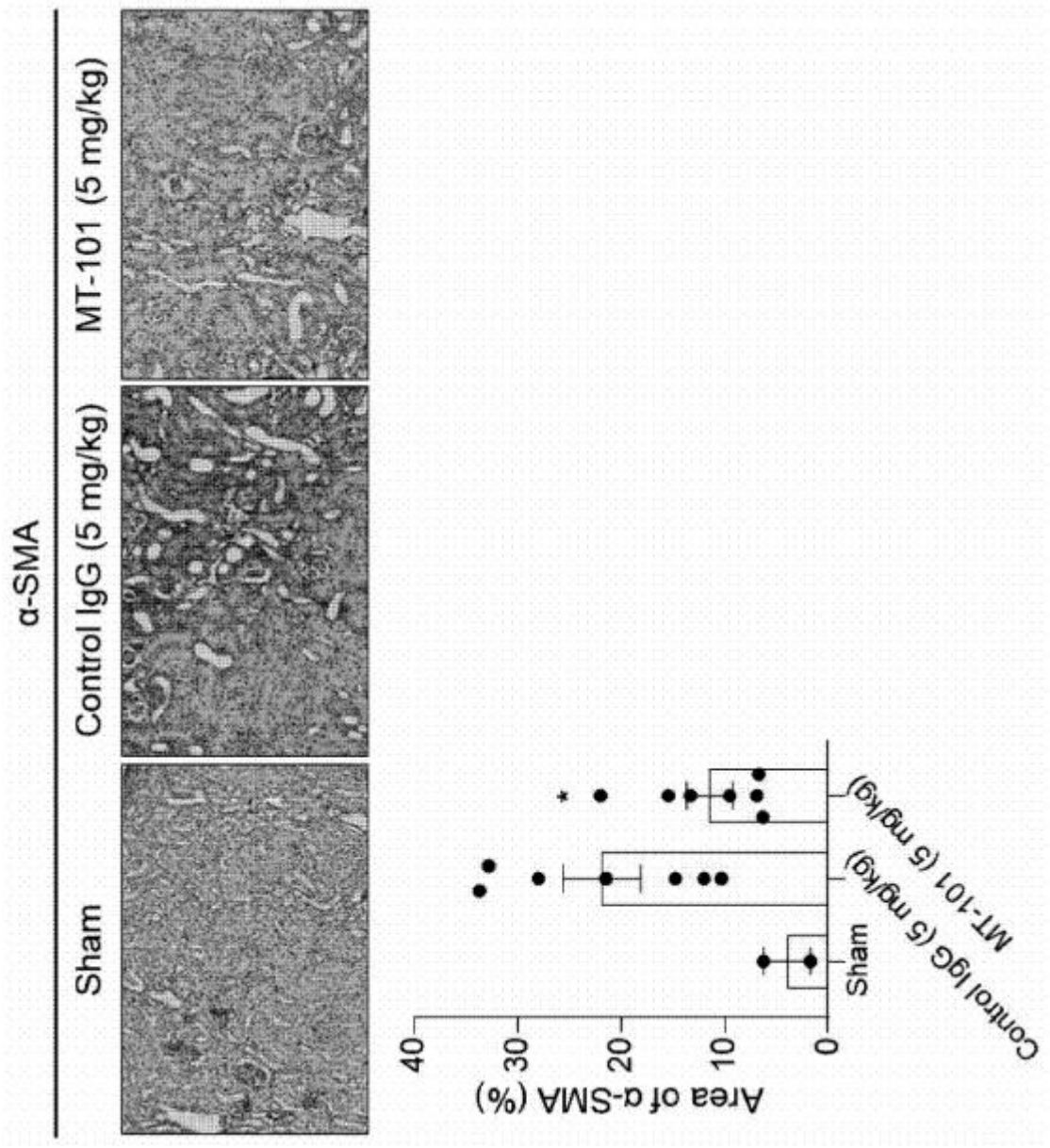

α-SMA
Sham
Control IgG (5 mg/kg)
MT-101 (5 mg/kg)
Area of α-SMA (%)
40
30
20
10
0
Sham
Control IgG (5 mg/kg)
MT-101 (5 mg/kg)

Figure 21a

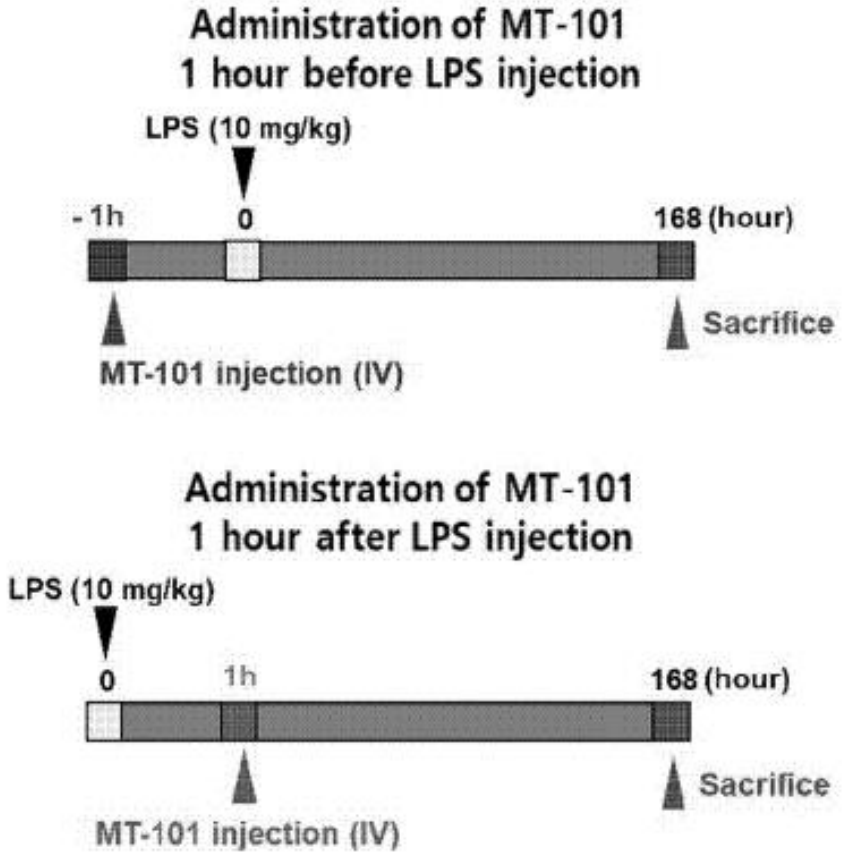

Administration of MT-101
1 hour before LPS injection
LPS (10 mg/kg)
- 1h
0
168 (hour)
Sacrifice
MT-101 injection (IV)
Administration of MT-101
1 hour after LPS injection
LPS (10 mg/kg)
0
1h
168 (hour)
Sacrifice
MT-101 injection (IV)

Figure 21b

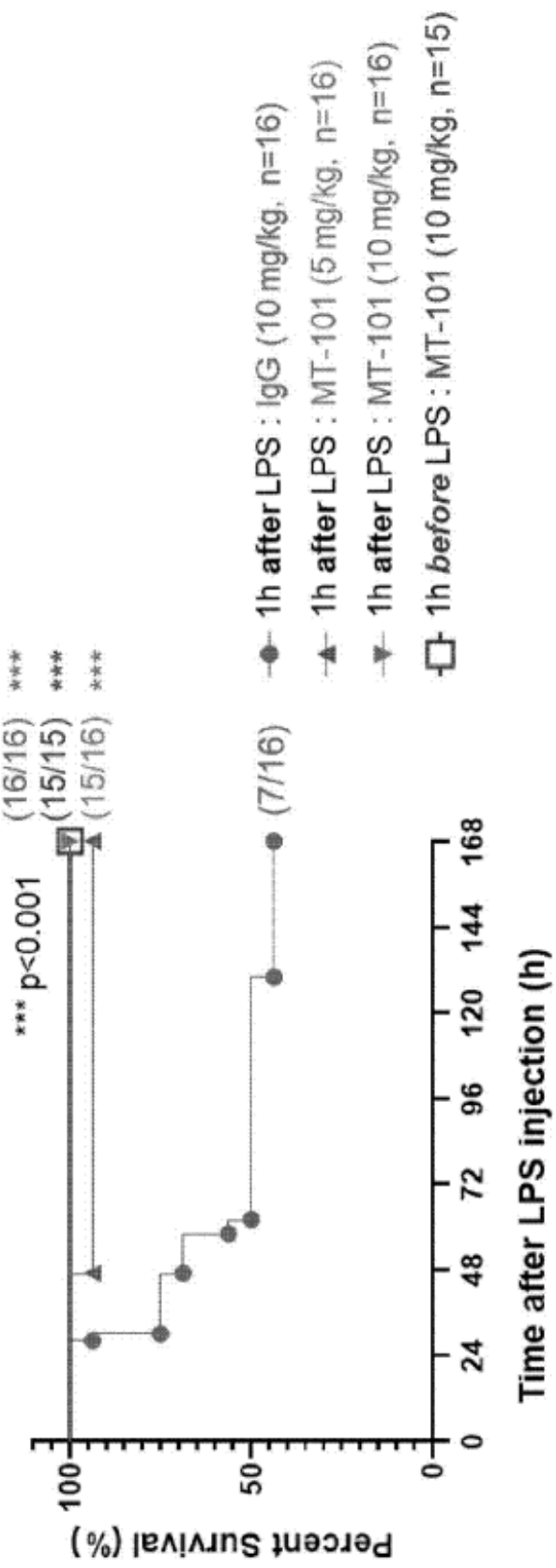

Percent Survival (%)
100
50
0
0
24
48
72
96
120
144
168
Time after LPS injection (h)
*** p<0.001
(16/16) ***
(15/15) ***
(15/16) ***
(7/16)
1h after LPS : IgG (10 mg/kg, n=16)
1h after LPS : MT-101 (5 mg/kg, n=16)
1h after LPS : MT-101 (10 mg/kg, n=16)
1h before LPS : MT-101 (10 mg/kg, n=15)

Figure 22a

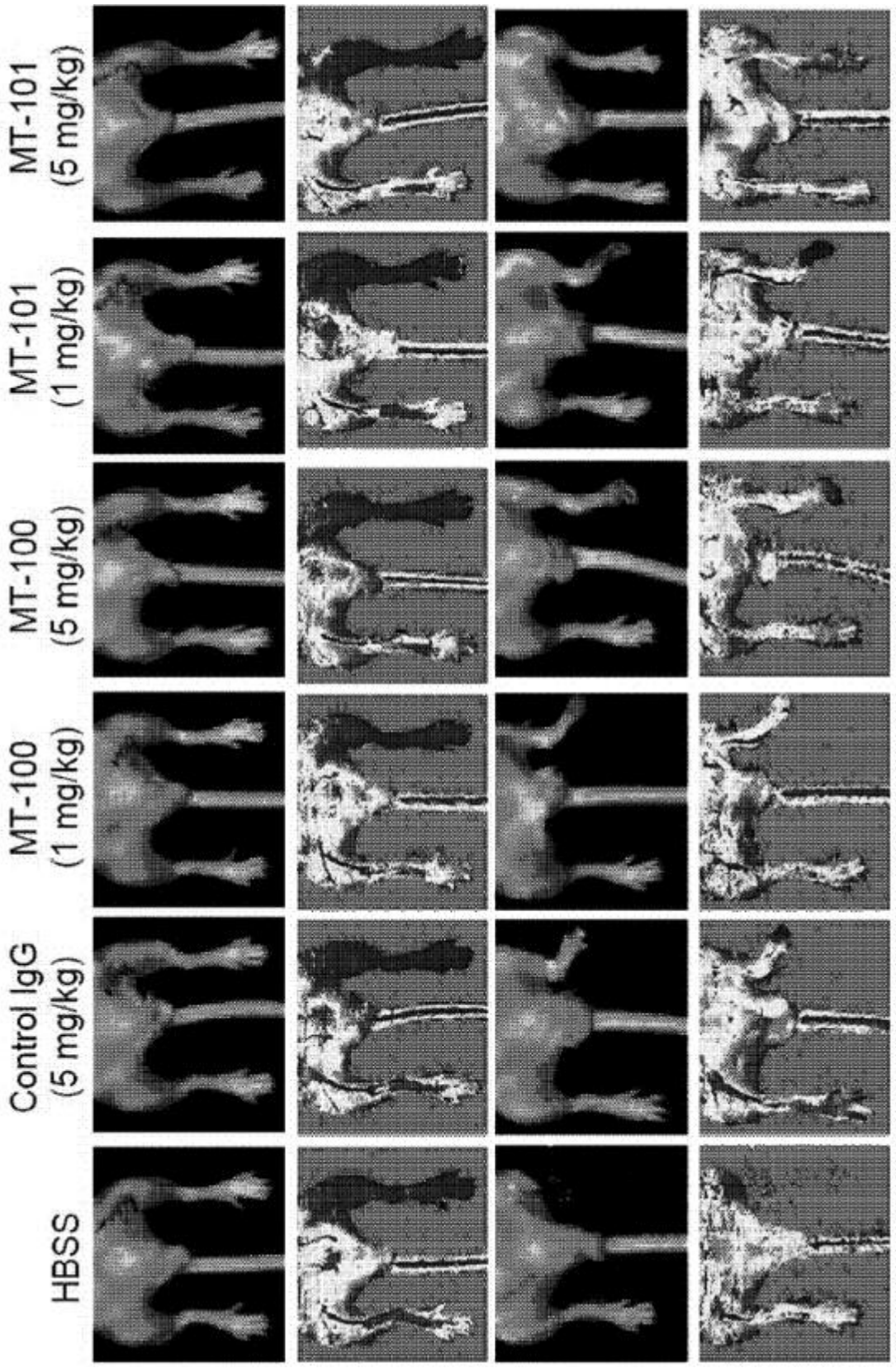

HBSS
Control IgG (5 mg/kg)
MT-100 (1 mg/kg)
MT-100 (5 mg/kg)
MT-101 (1 mg/kg)
MT-101 (5 mg/kg)

Figure 22b

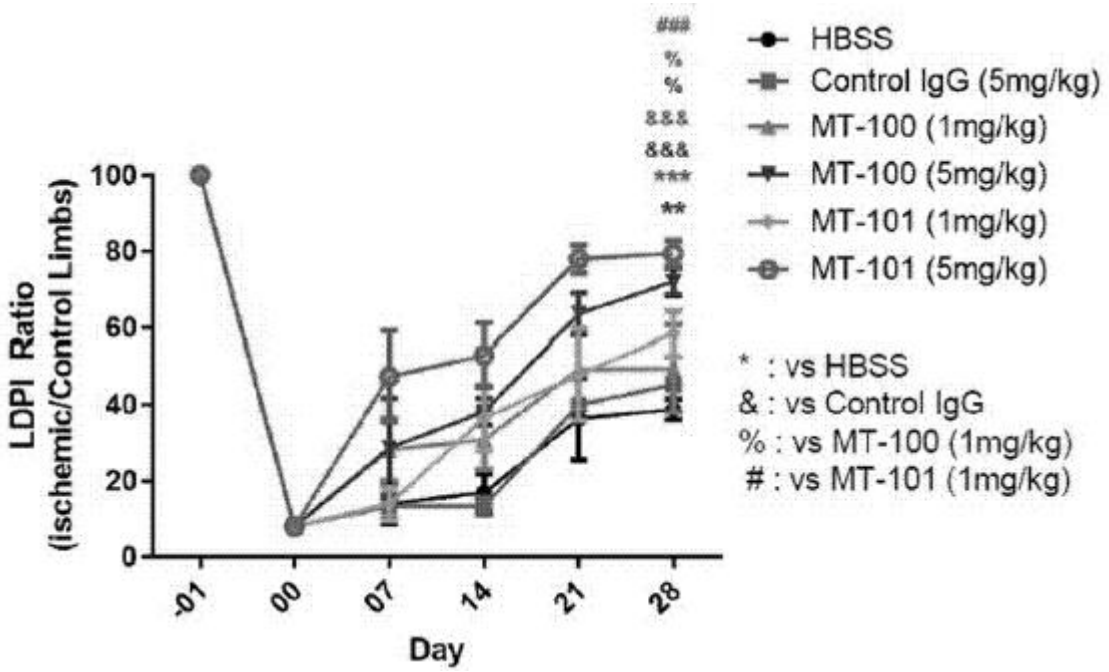


Figure 22c

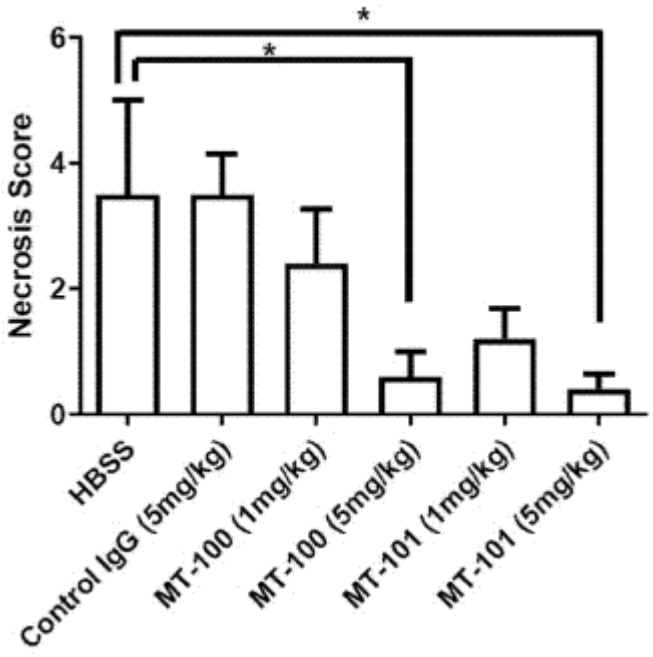


Figure 22d

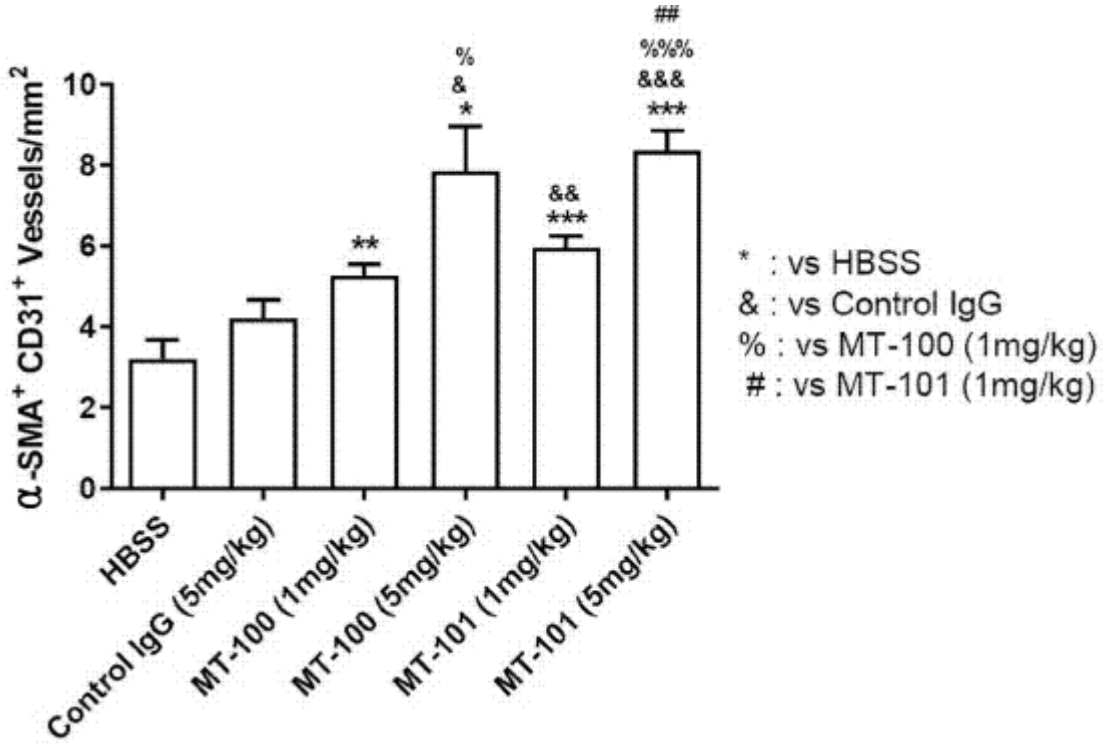

α-SMA+ CD31+ Vessels/mm2
10
8
6
4
2
0
**
%
&
*
&&
***
##
%%%
&&&
***
HBSS
Control IgG (5mg/kg)
MT-100 (1mg/kg)
MT-100 (5mg/kg)
MT-101 (1mg/kg)
MT-101 (5mg/kg)
* : vs HBSS
& : vs Control IgG
% : vs MT-100 (1mg/kg)
# : vs MT-101 (1mg/kg)

Figure 23a

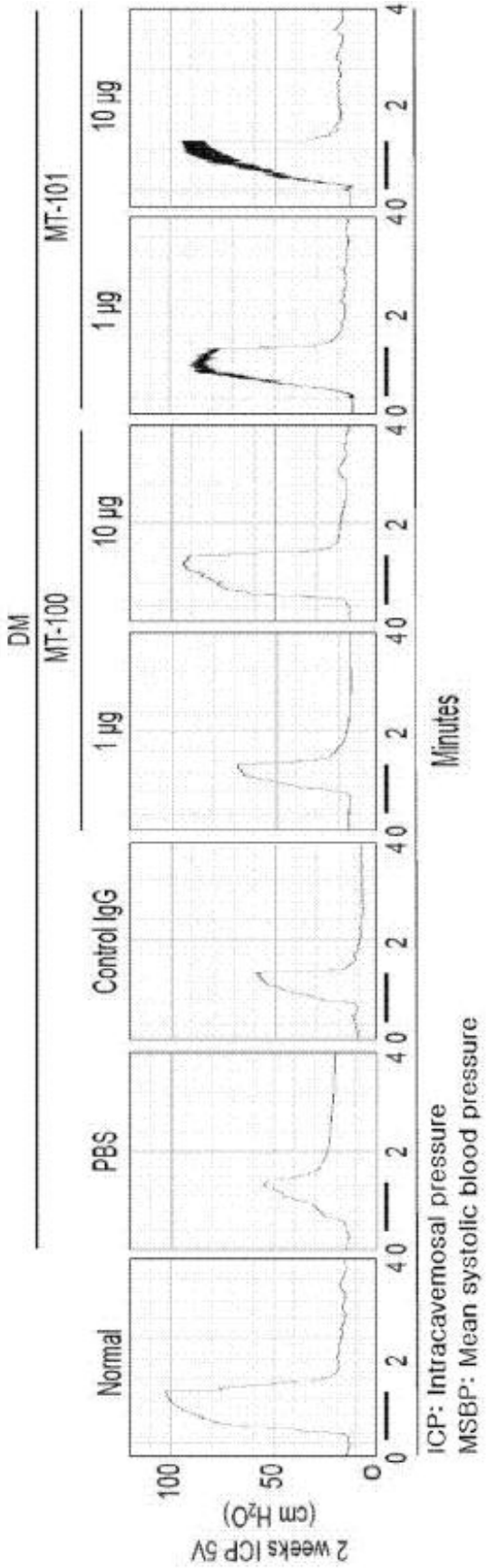

2 weeks ICP 5V
(cm H2O)
100
50
0
Normal
PBS
Control IgG
DM
MT-100
MT-101
1 μg
10 μg
1 μg
10 μg
0
2
4
Minutes
ICP: Intracavemosal pressure
MSBP: Mean systolic blood pressure

Figure 23b

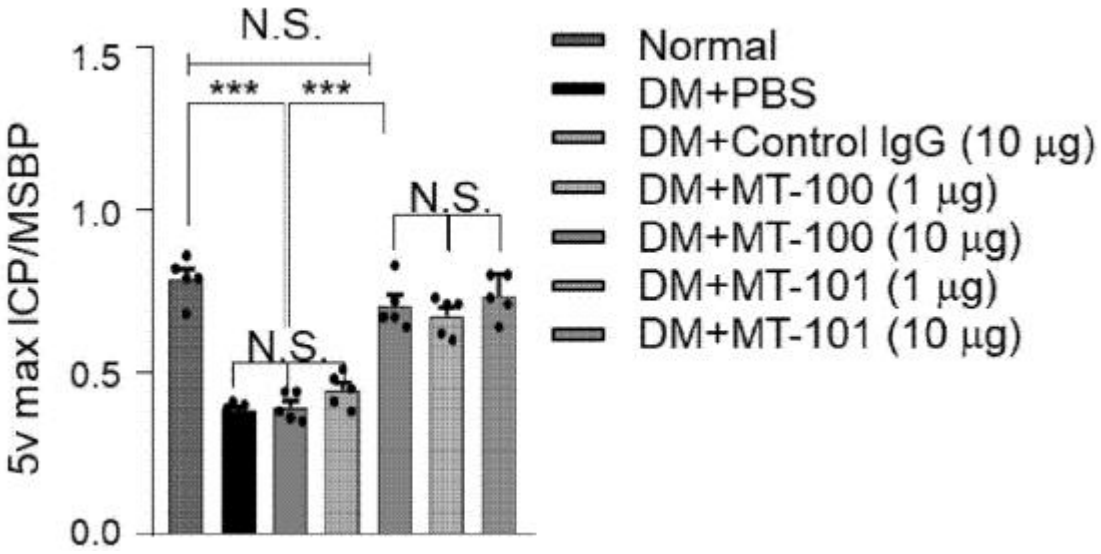


Figure 23c

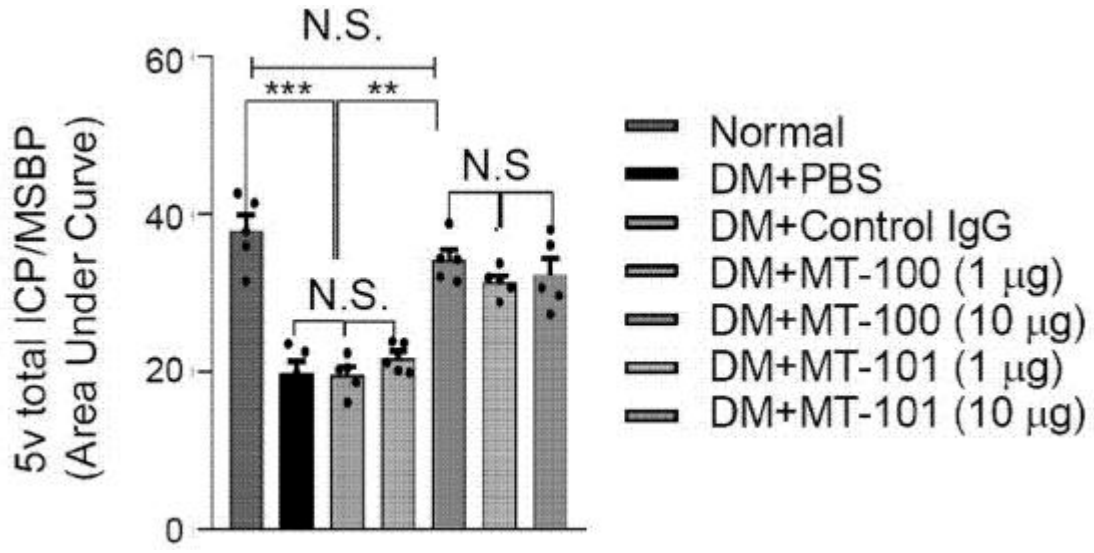


Figure 24a

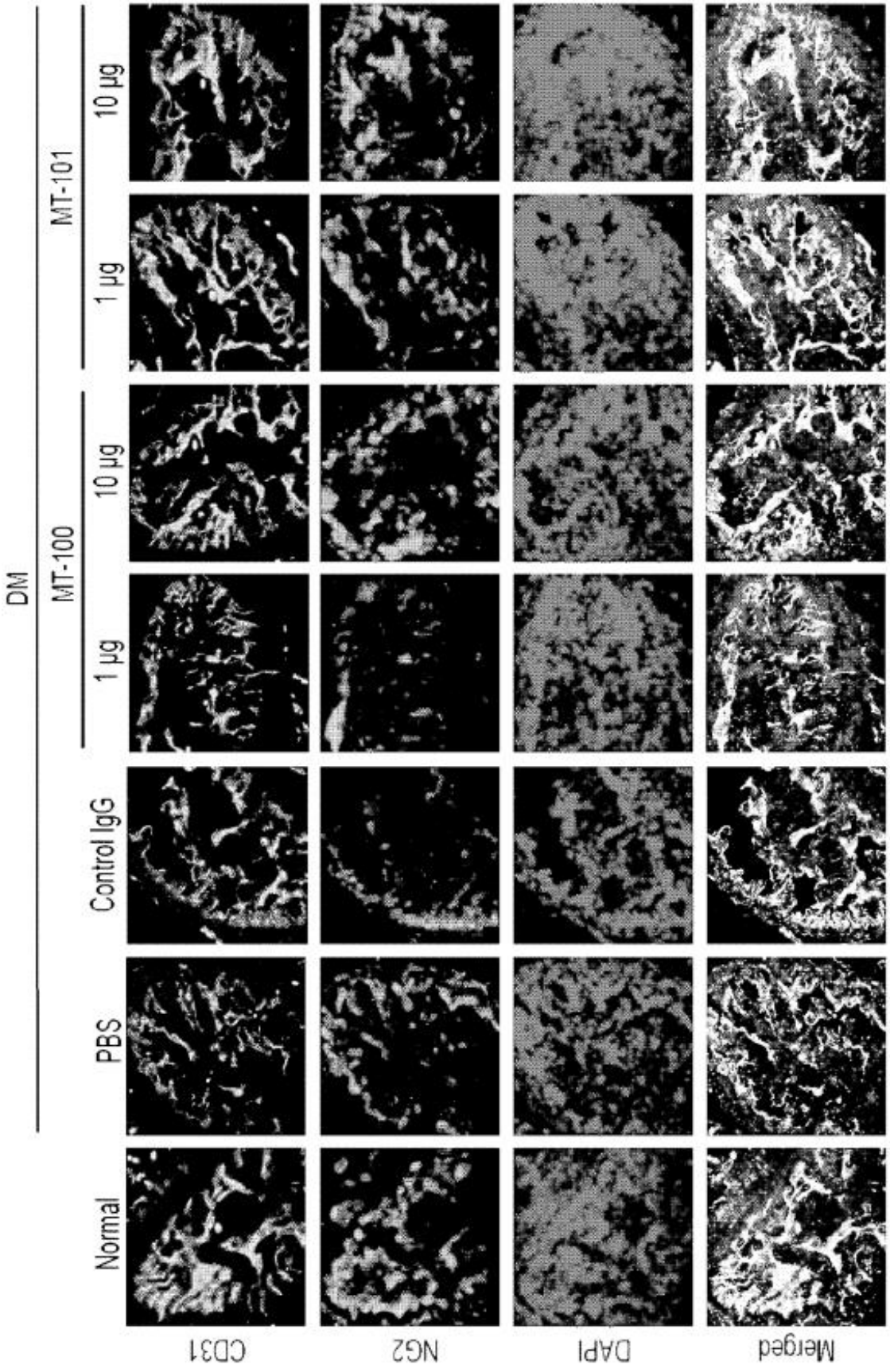

DM
Normal
PBS
Control IgG
MT-100
1 μg
10 μg
MT-101
1 μg
10 μg
CD31
NG2
DAPI
Merged

Figure 24b

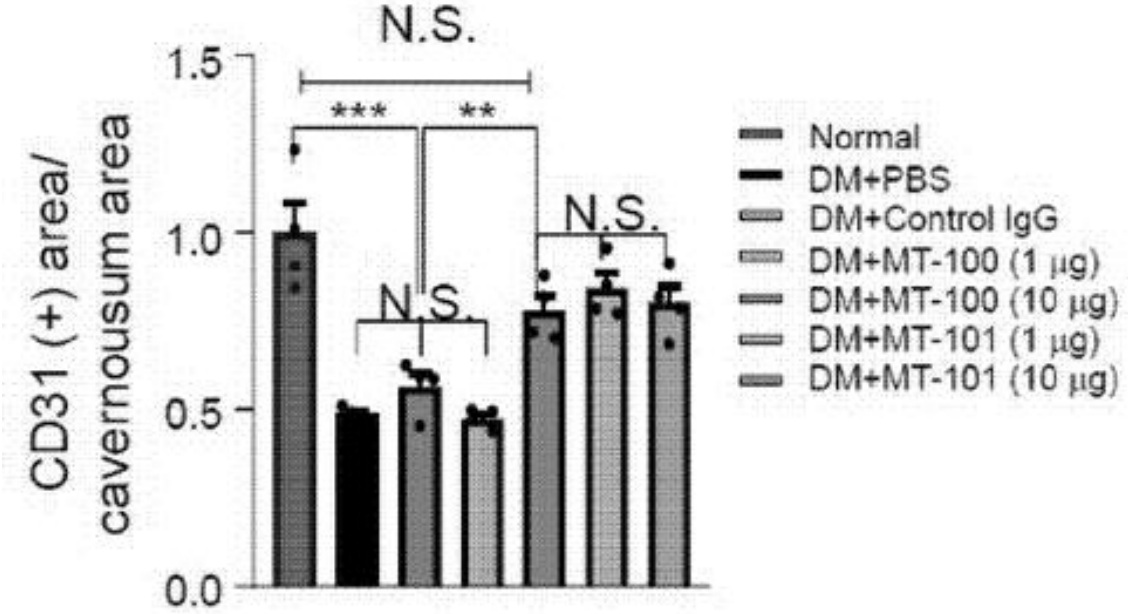


Figure 24c

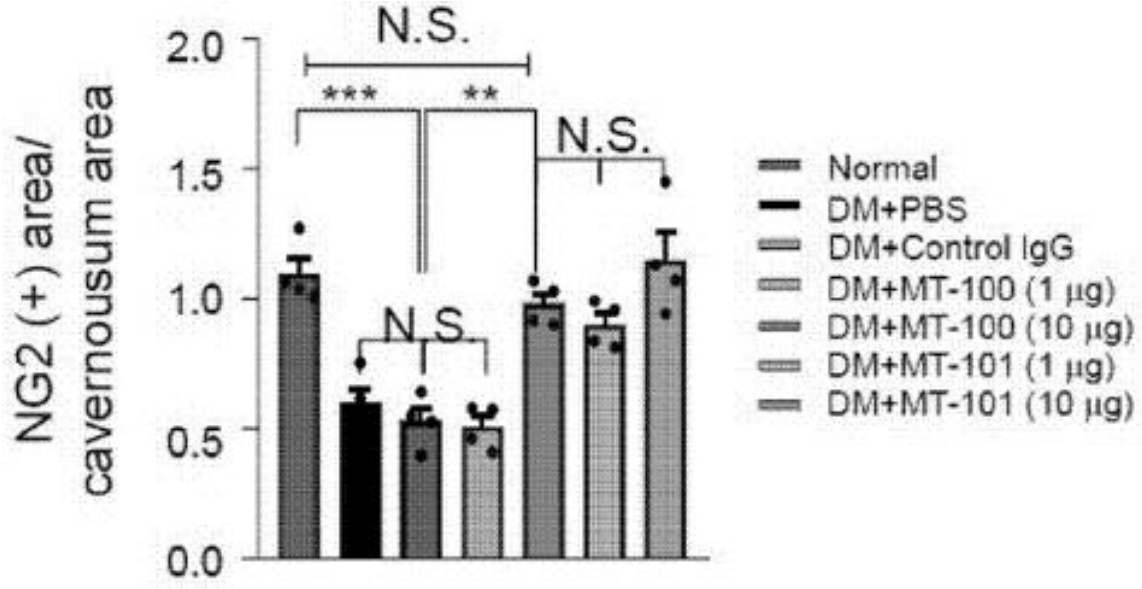


Figure 25a

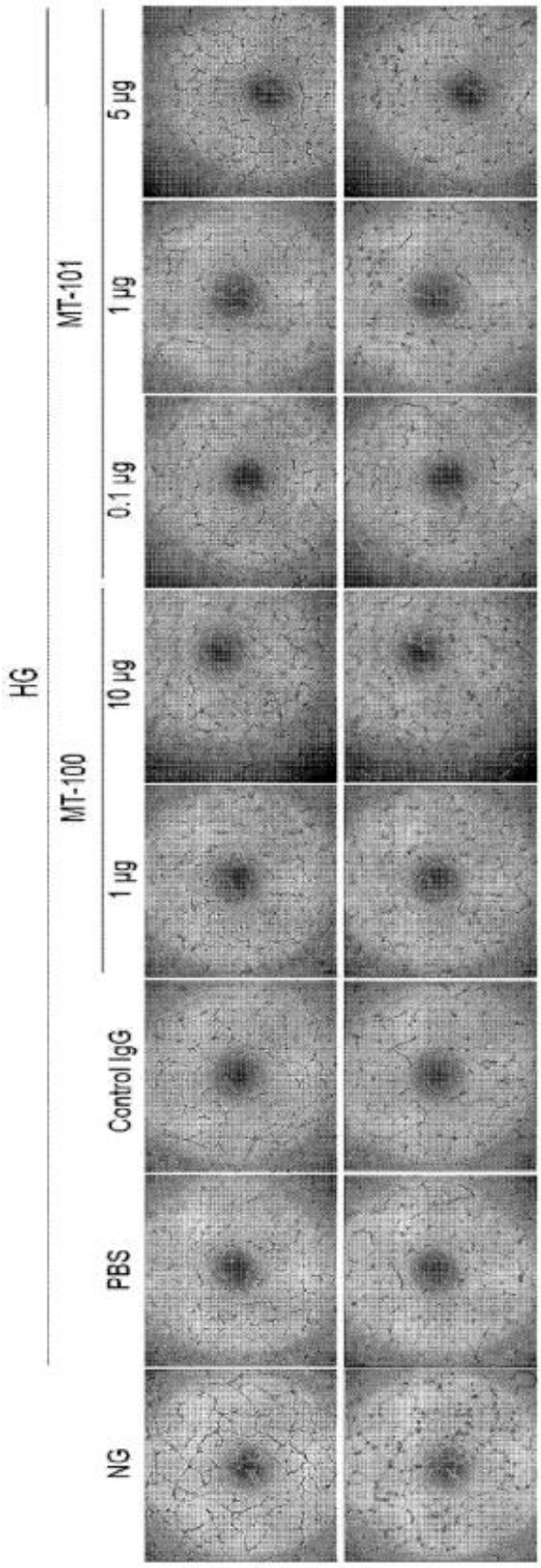


Figure 25b

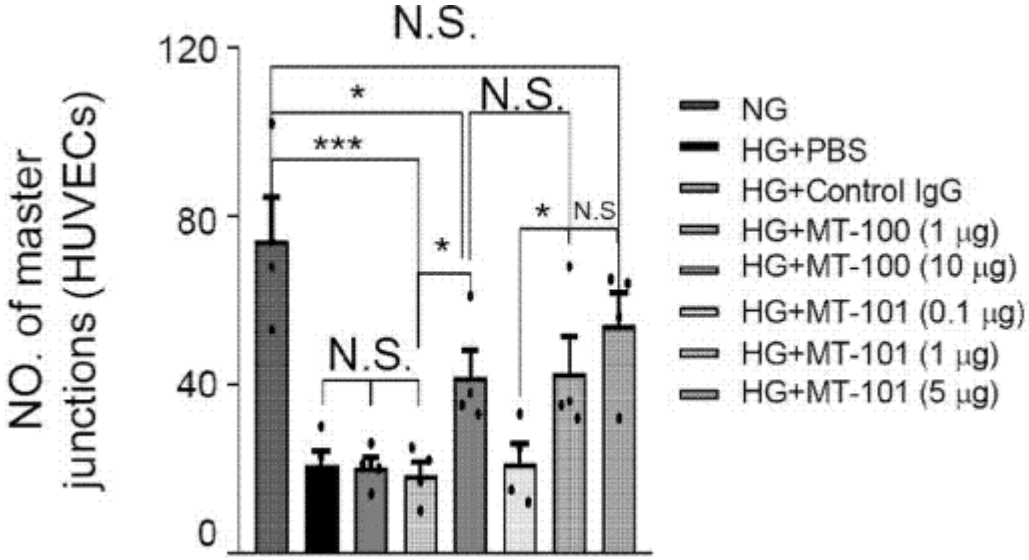


Figure 26a

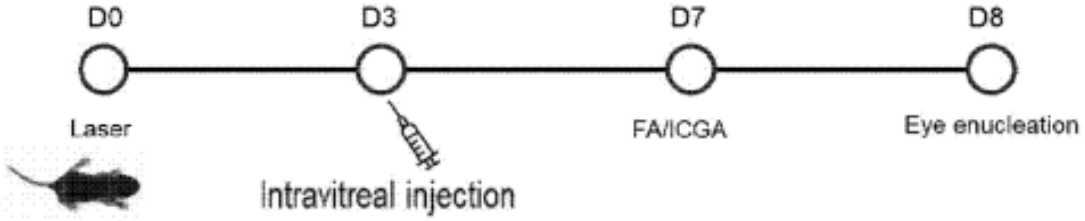


Figure 26b

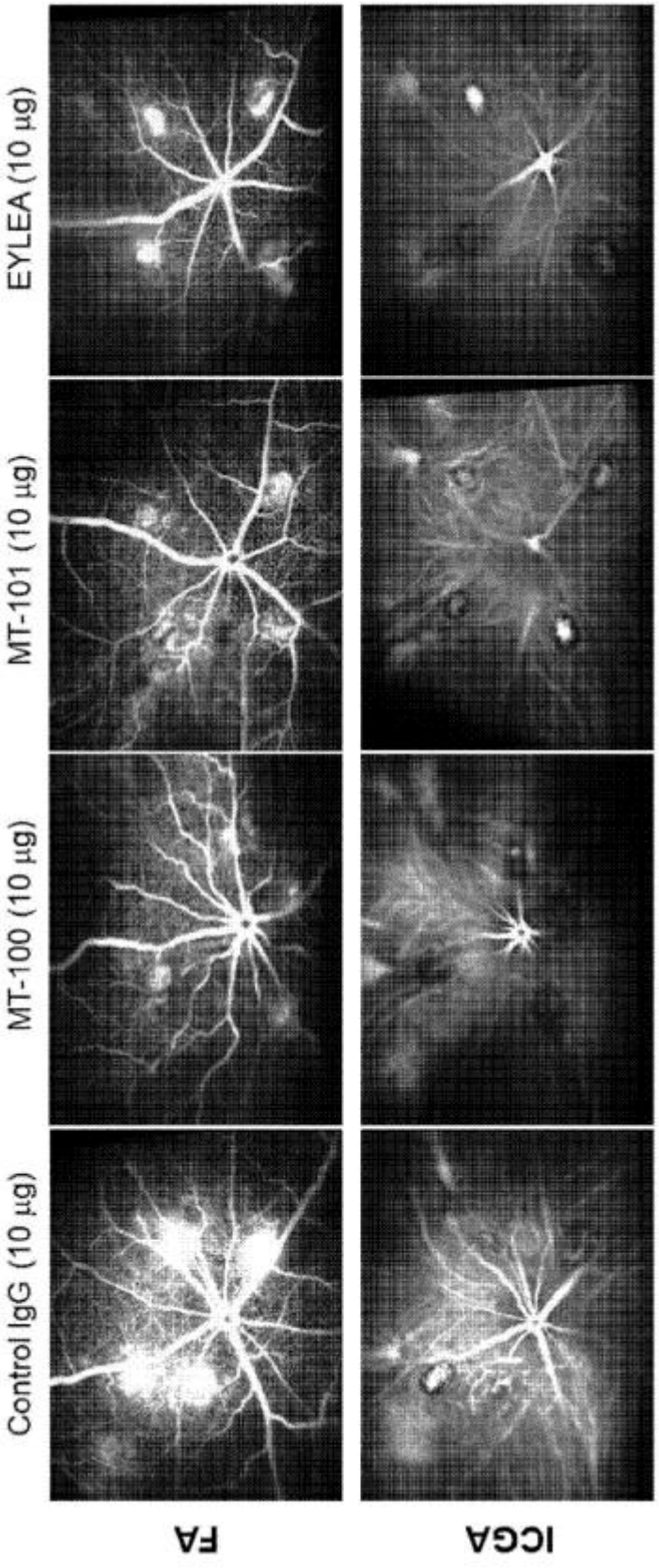


Figure 26c

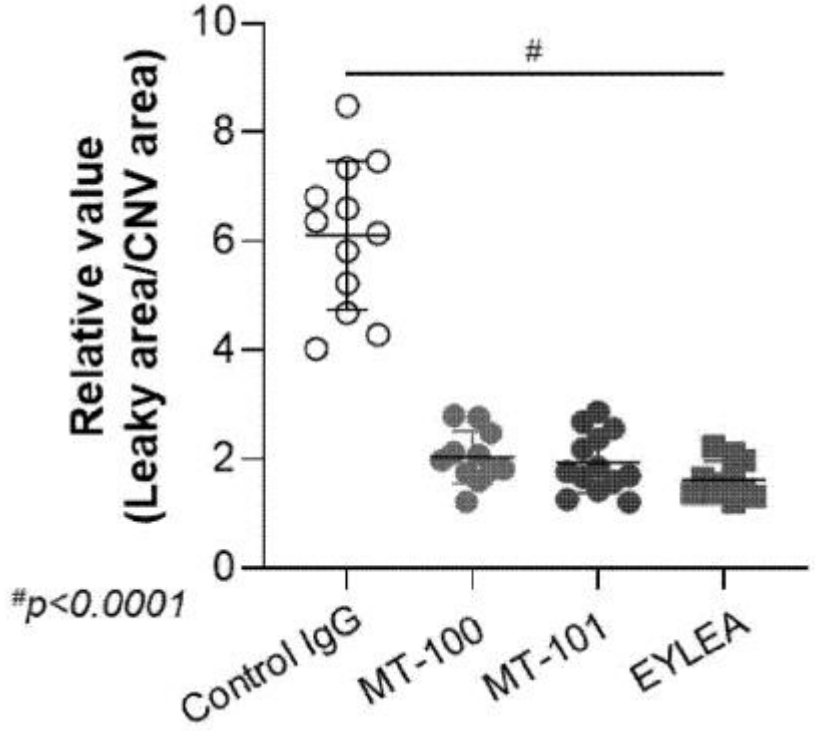

Relative value
(Leaky area/CNV area)
10
8
6
4
2
0
#
#p<0.0001
Control IgG
MT-100
MT-101
EYLEA

Figure 27a

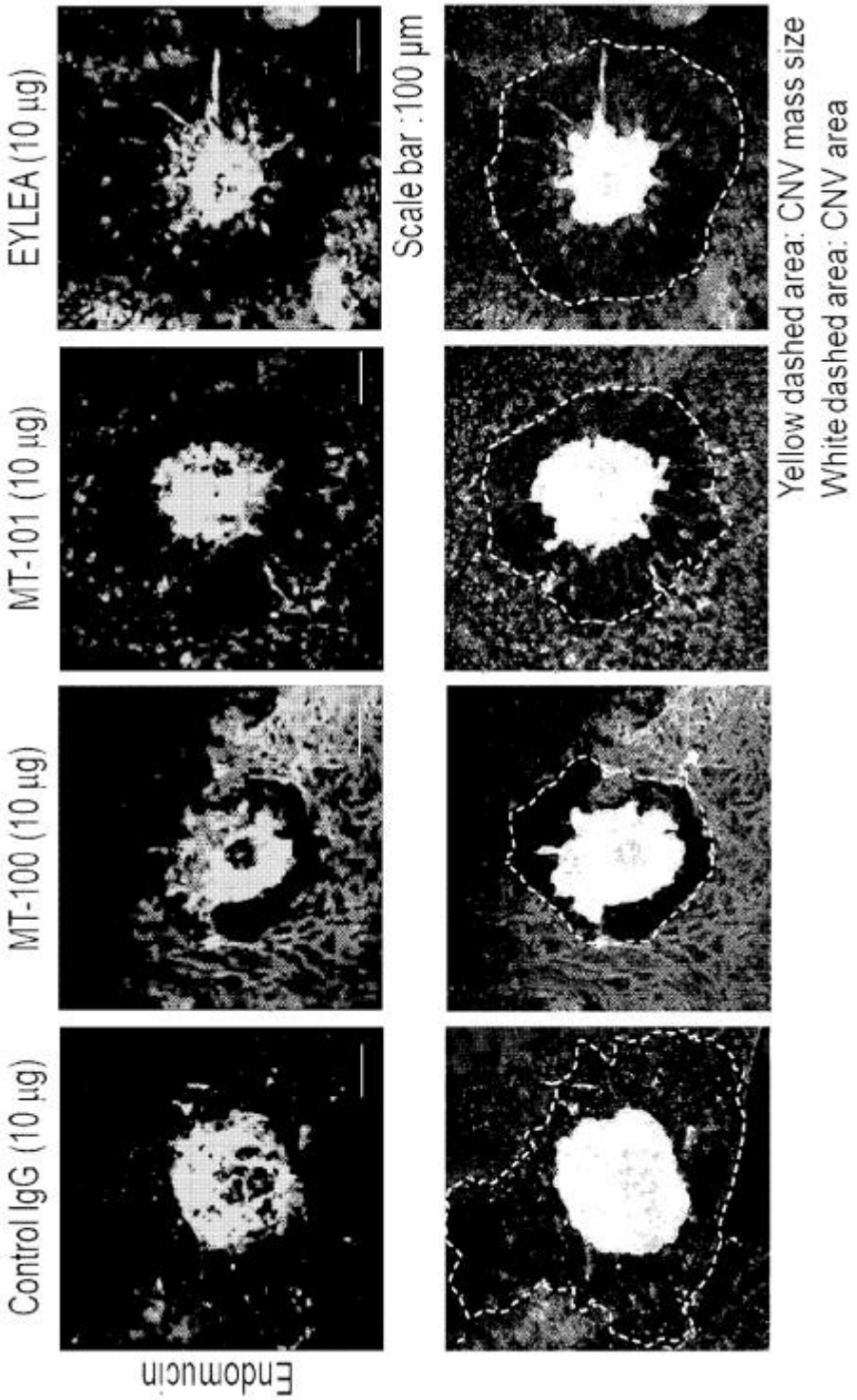

Control IgG (10 μg)
MT-100 (10 μg)
MT-101 (10 μg)
EYLEA (10 μg)
Endomucin
Scale bar :100 μm
Yellow dashed area: CNV mass size
White dashed area: CNV area

Figure 27b

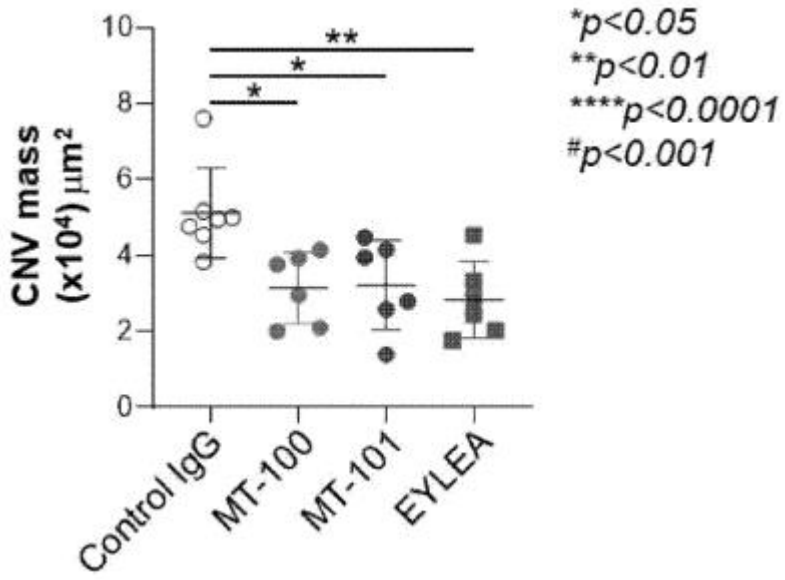


Figure 27c

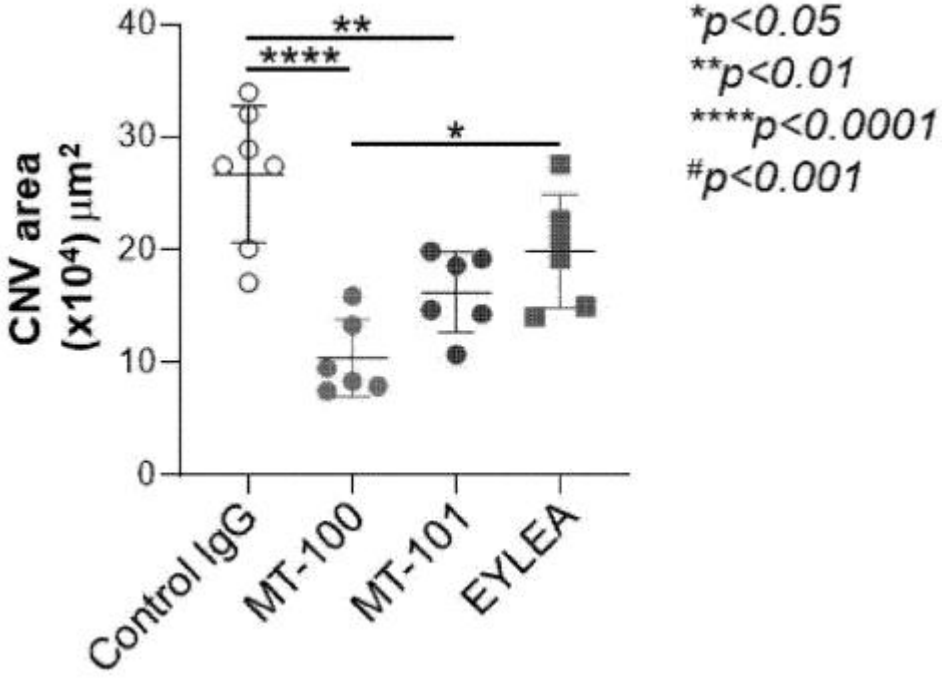


Figure 27d

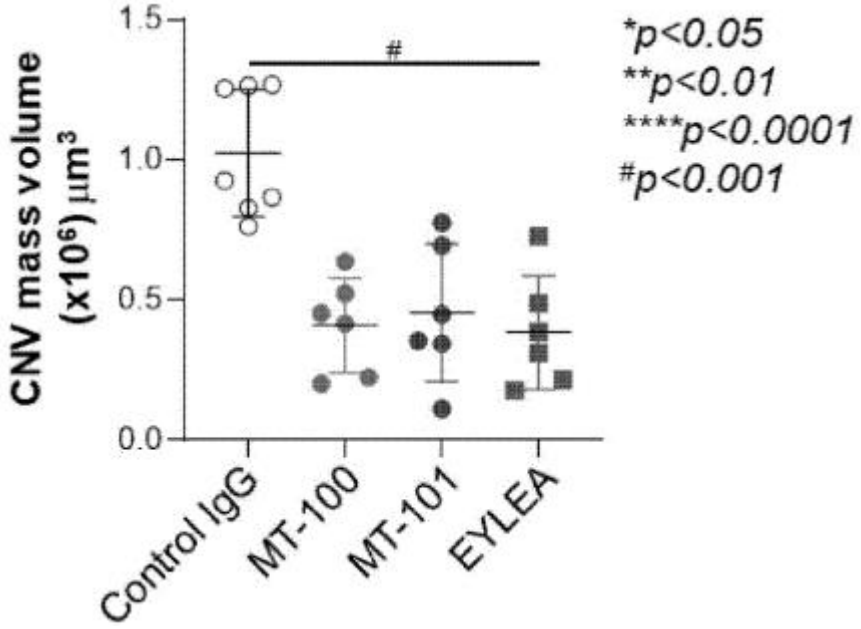


Figure 28a

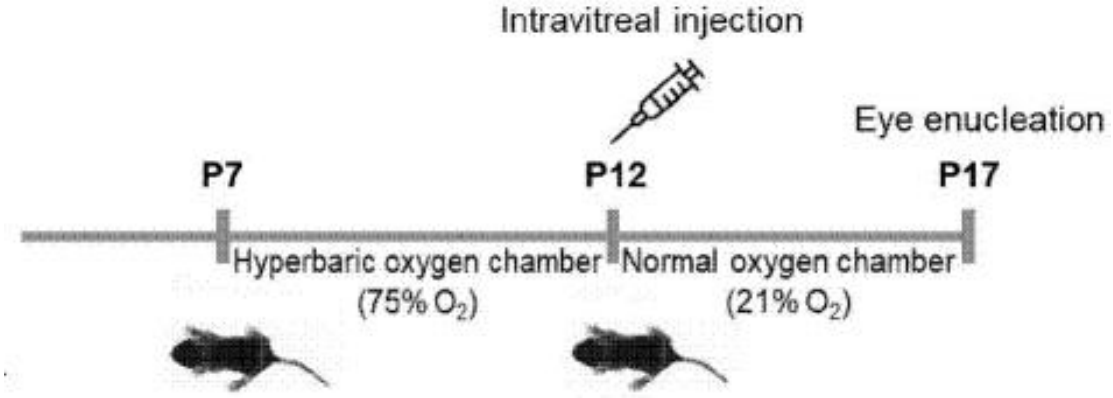


Figure 28b

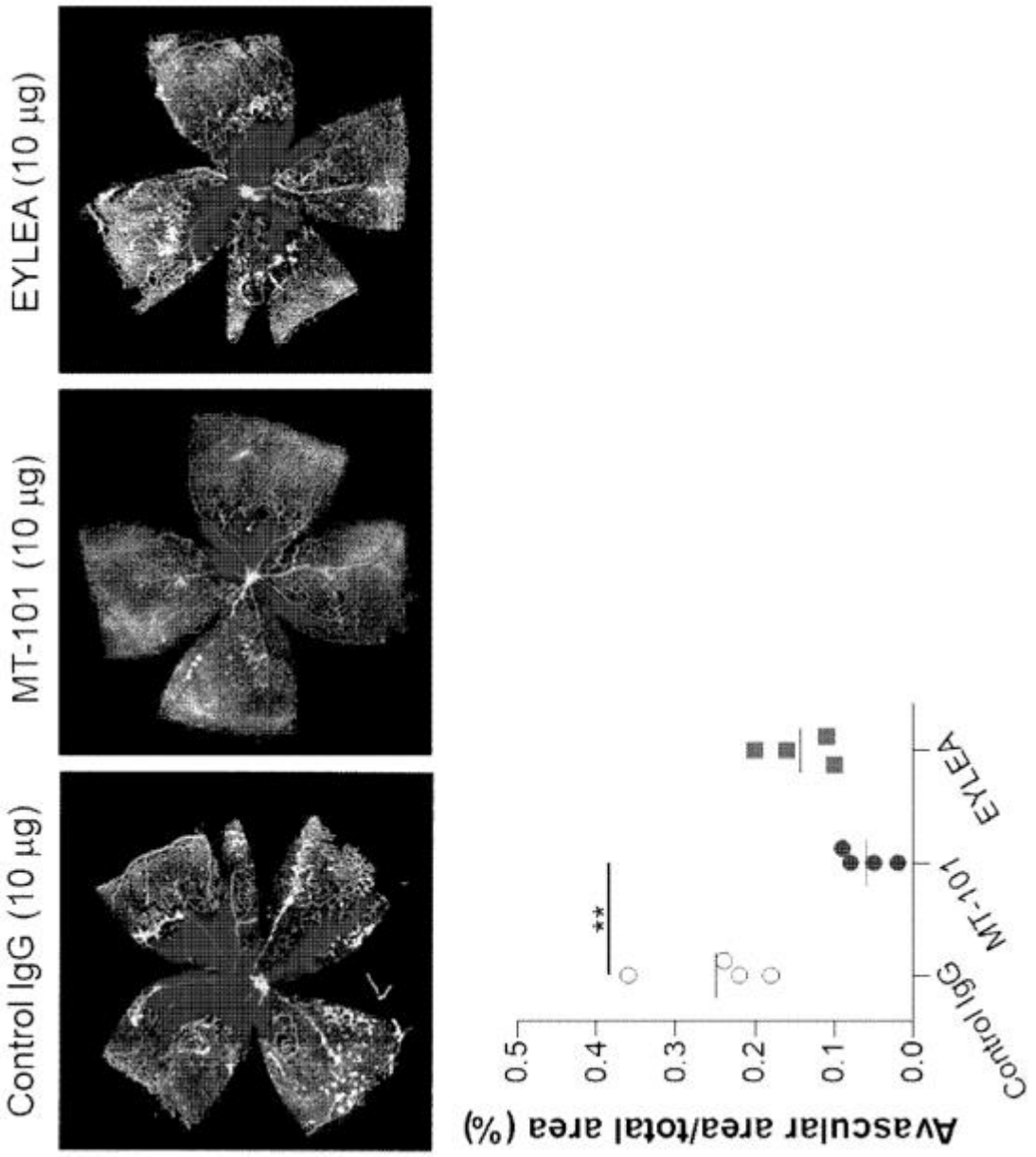

Control IgG (10 µg)
MT-101 (10 µg)
EYLEA (10 µg)
Avascular area/total area (%)
0.5
0.4
0.3
0.2
0.1
0.0
**
Control IgG
MT-101
EYLEA

Figure 28c

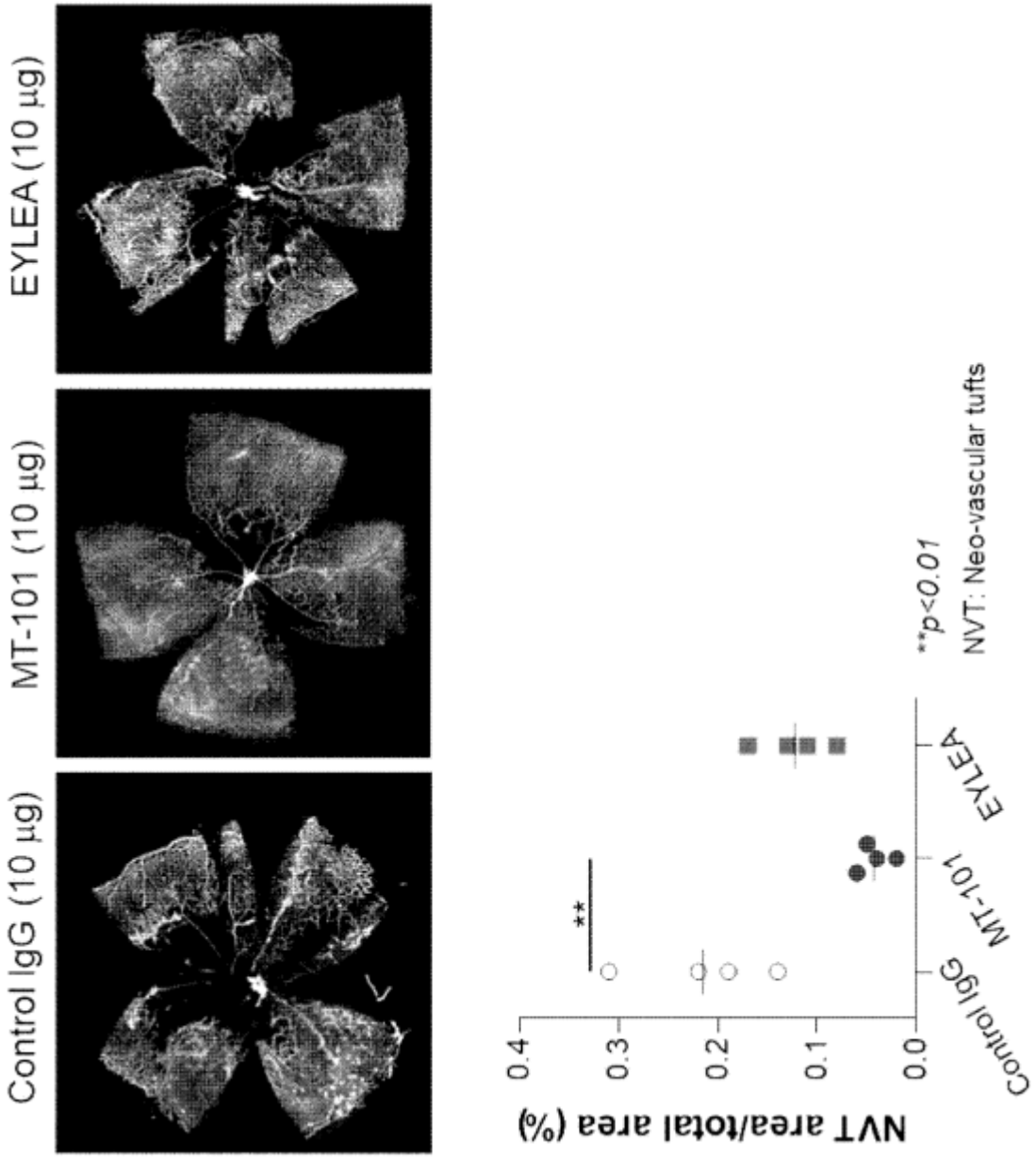

Control IgG (10 µg)
MT-101 (10 µg)
EYLEA (10 µg)
NVT area/total area (%)
0.4
0.3
0.2
0.1
0.0
**
Control IgG
MT-101
EYLEA
**p<0.01
NVT: Neo-vascular tufts

Figure 29a

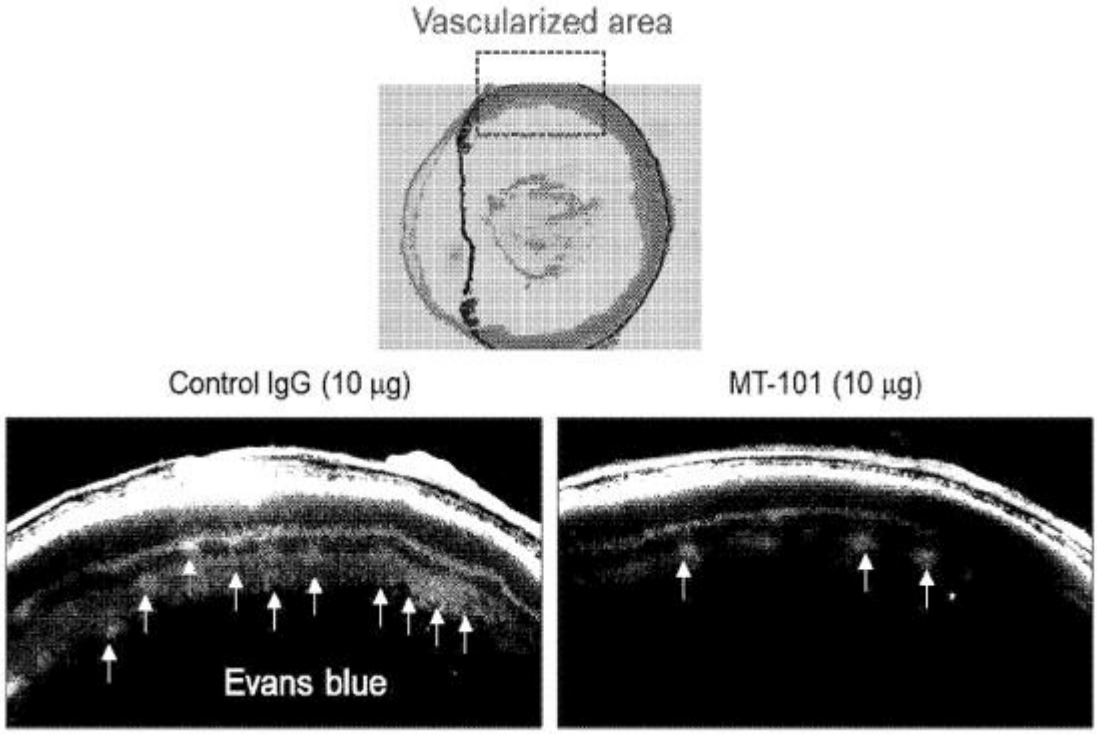


Figure 29b

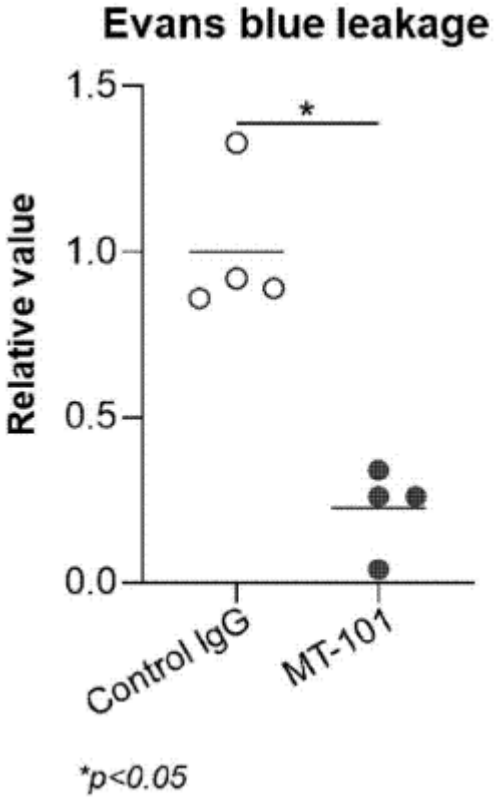


Figure 30

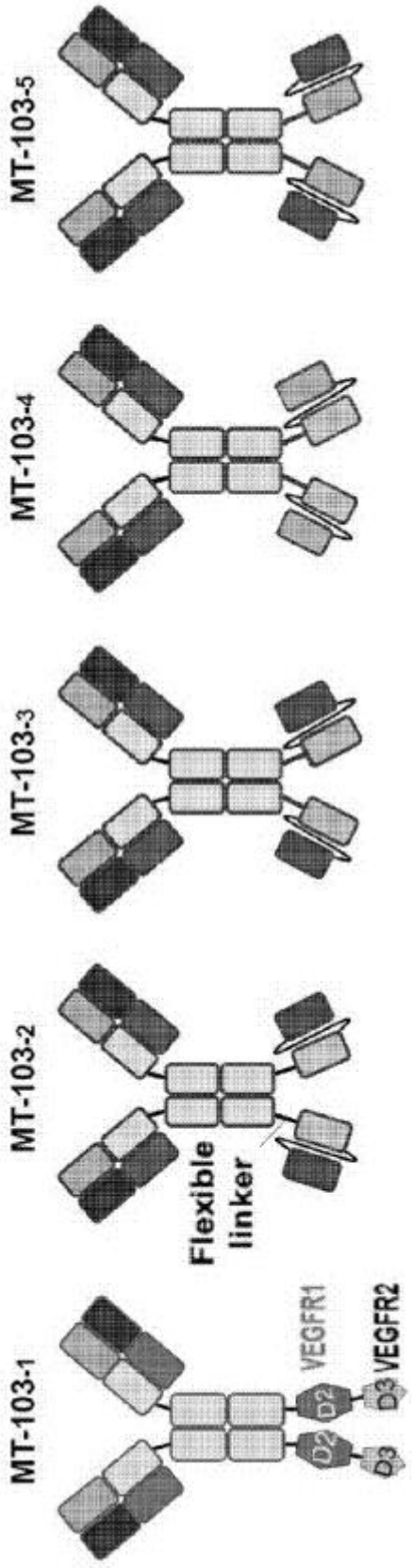

MT-103-1
MT-103-2
MT-103-3
MT-103-4
MT-103-5
Flexible linker
VEGFR1
D2
D3
VEGFR2
D2
D3

Figure 31a

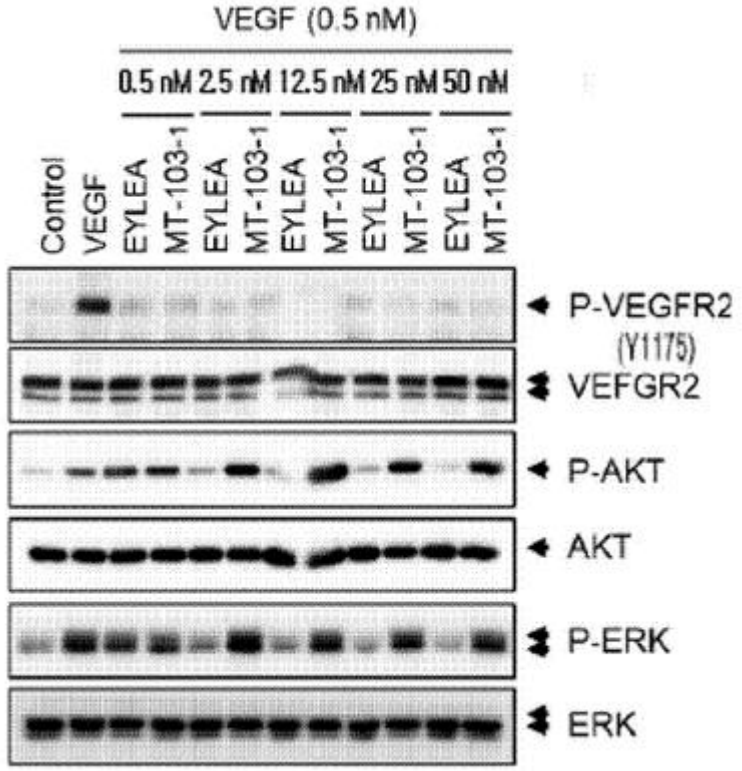


Figure 31b

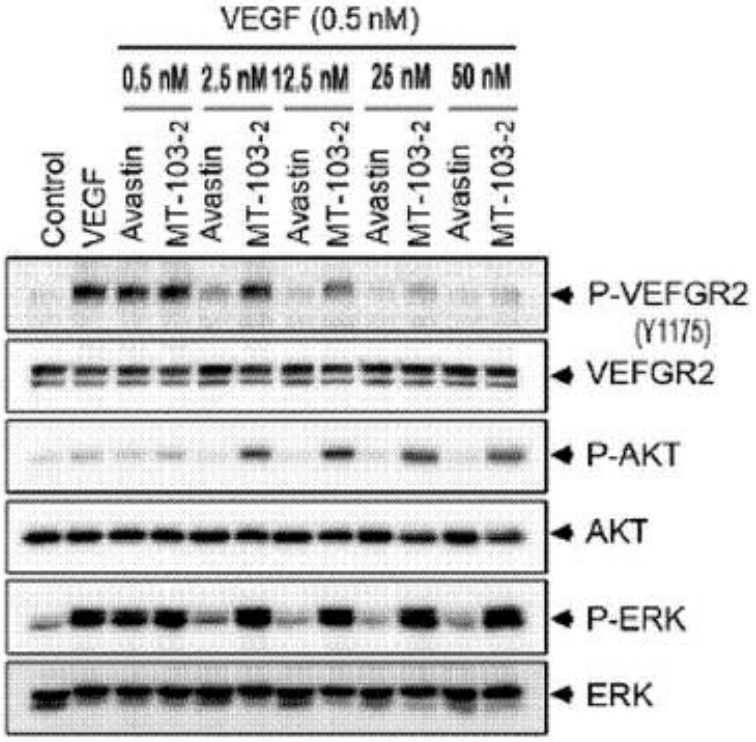


Figure 31c

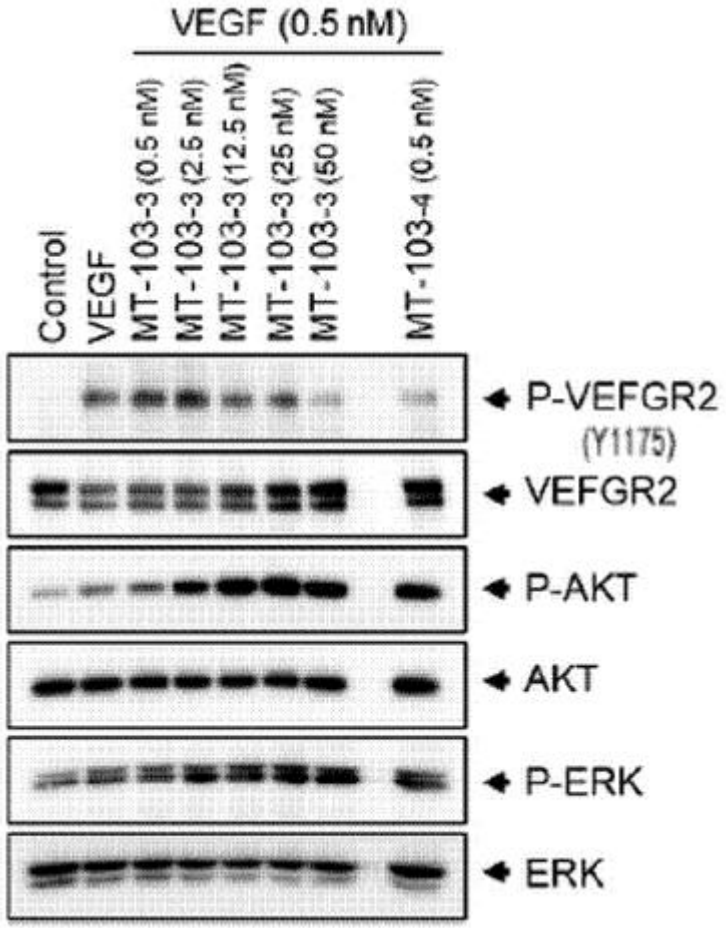


Figure 31d

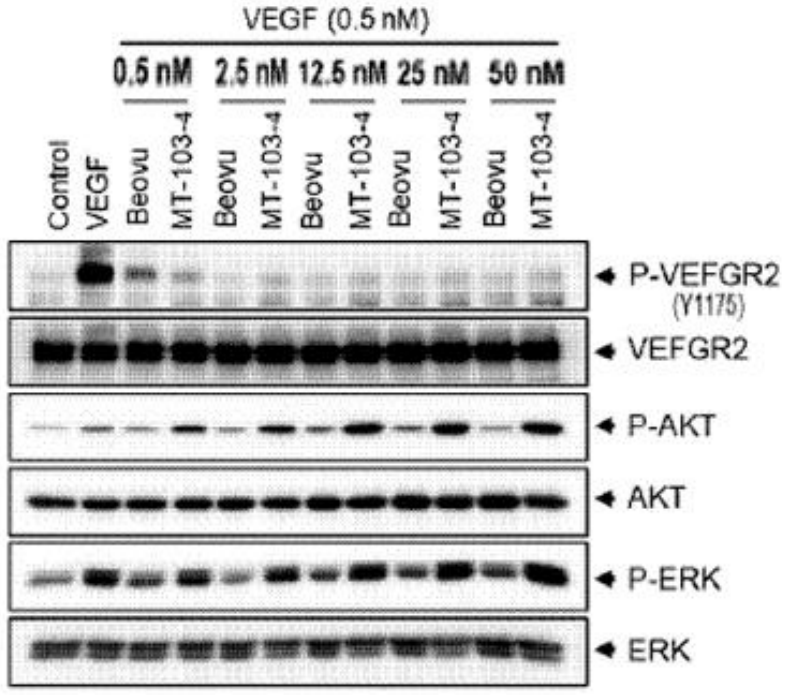


Figure 31e

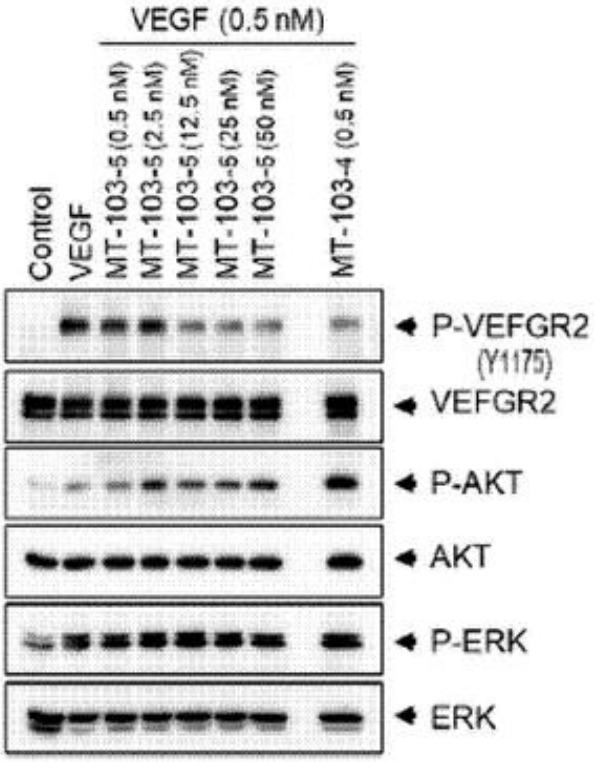


Figure 32a

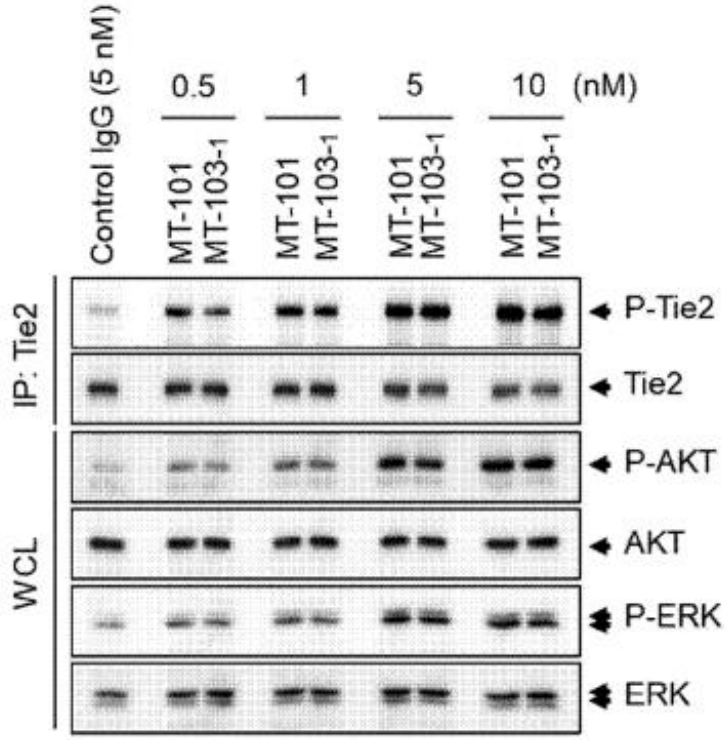


Figure 32b

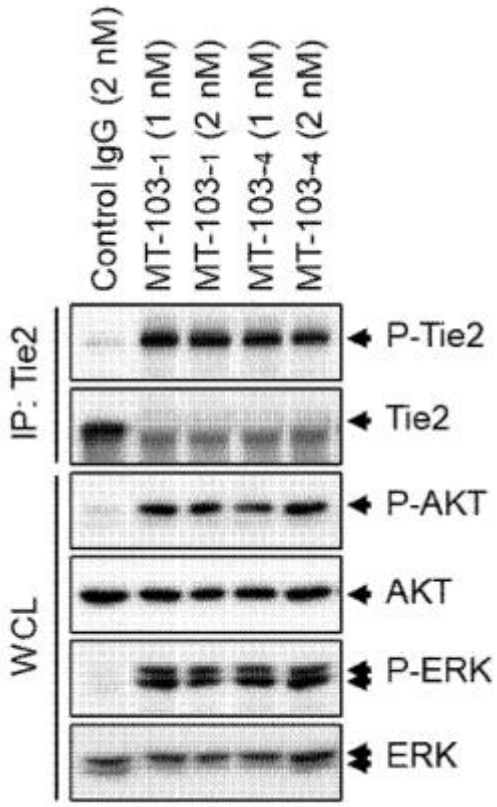


Figure 33a

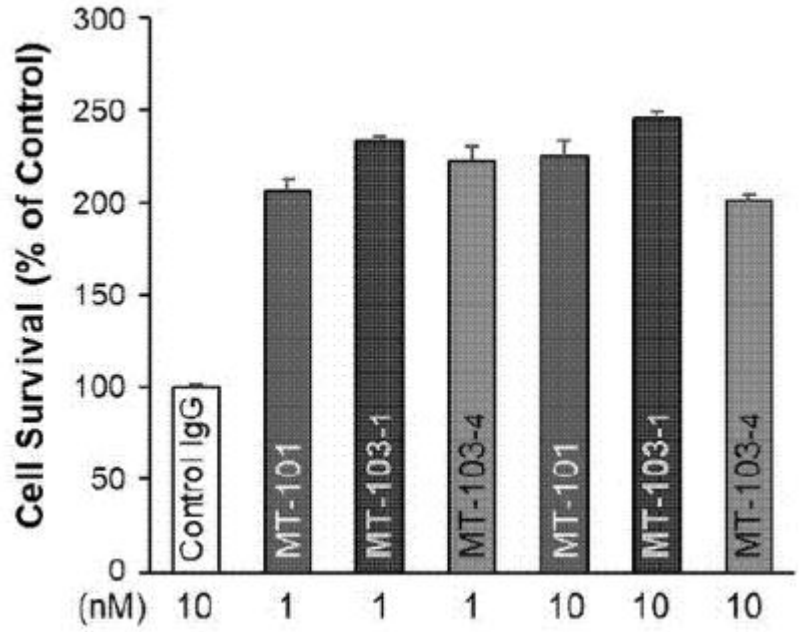


Figure 33b

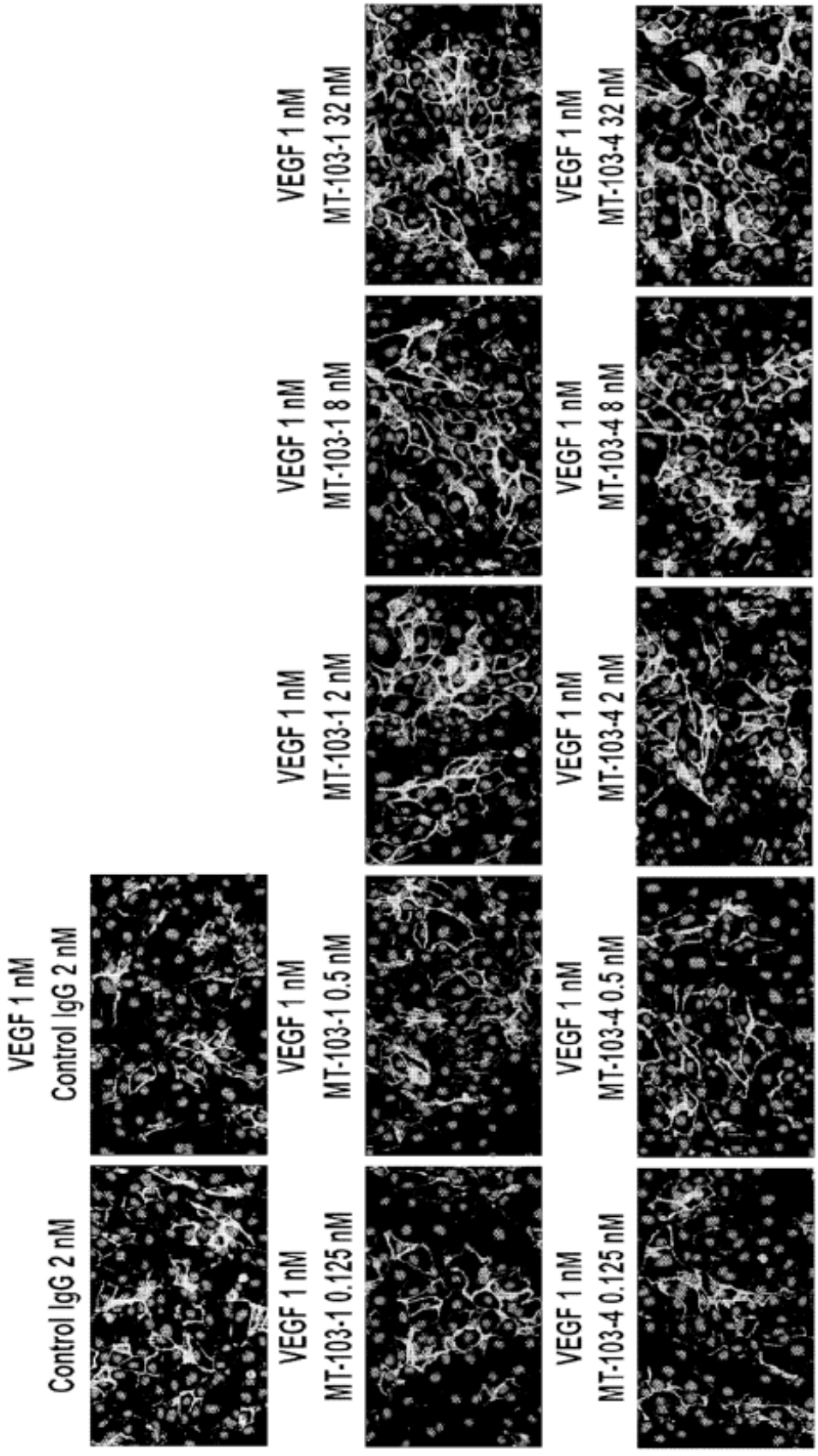

VEGF 1 nM
Control IgG 2 nM
VEGF 1 nM
MT-103-1 0.125 nM
VEGF 1 nM
MT-103-1 0.5 nM
VEGF 1 nM
MT-103-1 2 nM
VEGF 1 nM
MT-103-1 8 nM
VEGF 1 nM
MT-103-1 32 nM
Control IgG 2 nM
VEGF 1 nM
MT-103-4 0.125 nM
VEGF 1 nM
MT-103-4 0.5 nM
VEGF 1 nM
MT-103-4 2 nM
VEGF 1 nM
MT-103-4 8 nM
VEGF 1 nM
MT-103-4 32 nM

Figure 33c

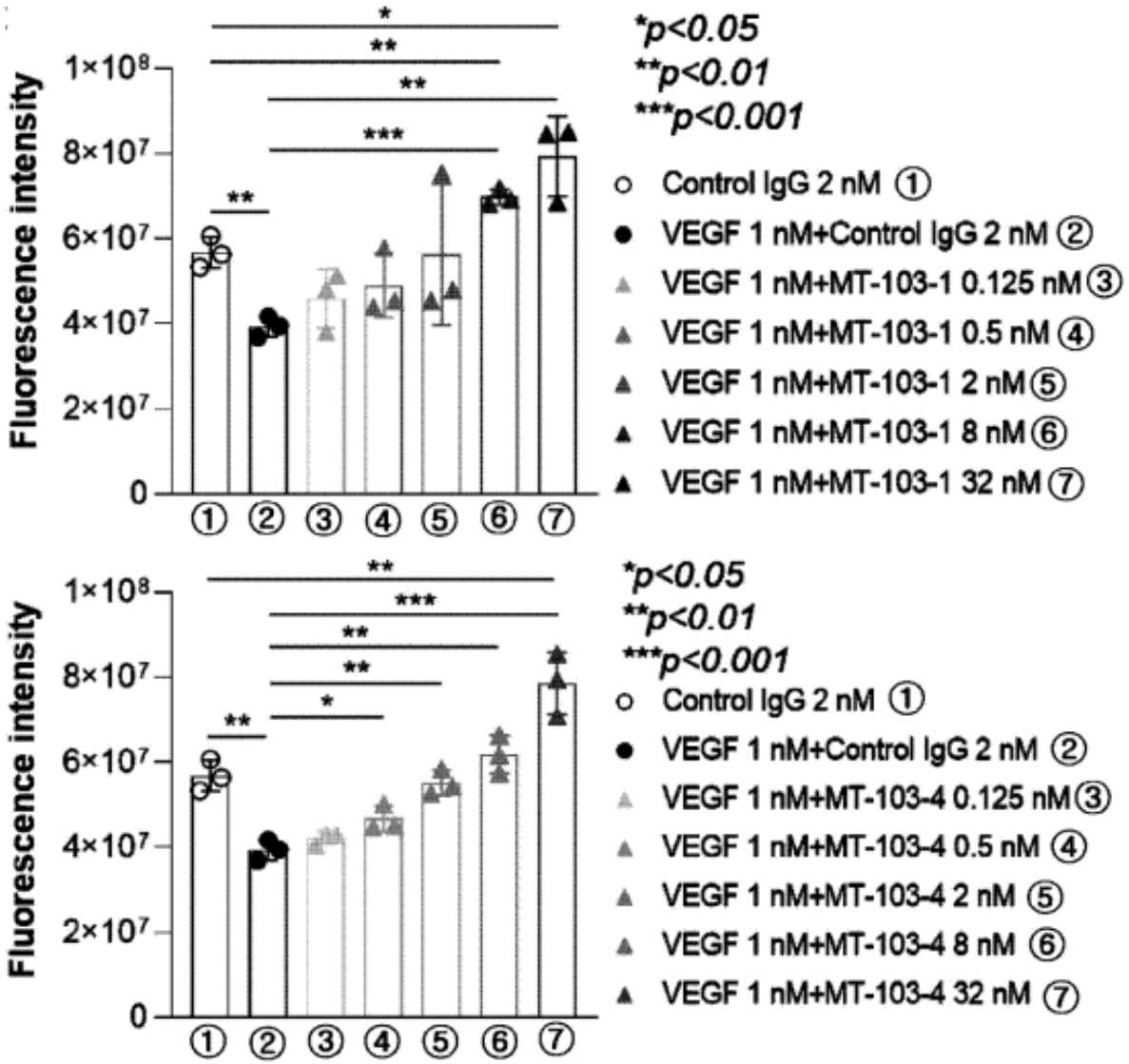

Fluorescence intensity
1×10⁸
8×10⁷
6×10⁷
4×10⁷
2×10⁷
0
①②③④⑤⑥⑦
*p<0.05
**p<0.01
***p<0.001
Control IgG 2 nM ①
VEGF 1 nM+Control IgG 2 nM ②
VEGF 1 nM+MT-103-1 0.125 nM ③
VEGF 1 nM+MT-103-1 0.5 nM ④
VEGF 1 nM+MT-103-1 2 nM ⑤
VEGF 1 nM+MT-103-1 8 nM ⑥
VEGF 1 nM+MT-103-1 32 nM ⑦
Fluorescence intensity
1×10⁸
8×10⁷
6×10⁷
4×10⁷
2×10⁷
0
①②③④⑤⑥⑦
*p<0.05
**p<0.01
***p<0.001
Control IgG 2 nM ①
VEGF 1 nM+Control IgG 2 nM ②
VEGF 1 nM+MT-103-4 0.125 nM ③
VEGF 1 nM+MT-103-4 0.5 nM ④
VEGF 1 nM+MT-103-4 2 nM ⑤
VEGF 1 nM+MT-103-4 8 nM ⑥
VEGF 1 nM+MT-103-4 32 nM ⑦

Figure 33d

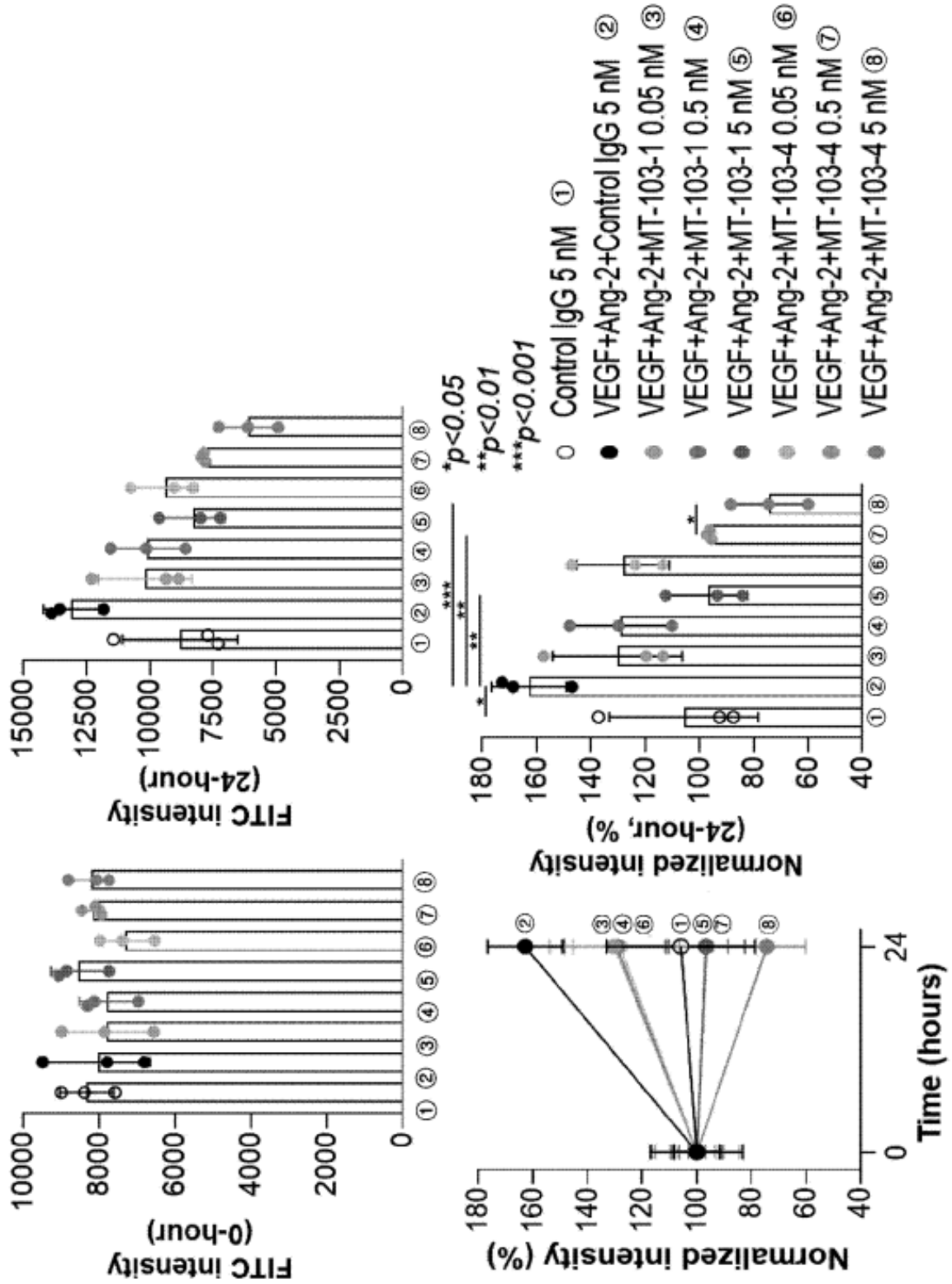

FITC intensity (0-hour)
FITC intensity (24-hour)
Normalized intensity (%)
Normalized intensity (24-hour, %)
Time (hours)
*p<0.05
**p<0.01
***p<0.001
Control IgG 5 nM ①
VEGF+Ang-2+Control IgG 5 nM ②
VEGF+Ang-2+MT-103-1 0.05 nM ③
VEGF+Ang-2+MT-103-1 0.5 nM ④
VEGF+Ang-2+MT-103-1 5 nM ⑤
VEGF+Ang-2+MT-103-4 0.05 nM ⑥
VEGF+Ang-2+MT-103-4 0.5 nM ⑦
VEGF+Ang-2+MT-103-4 5 nM ⑧

Figure 34

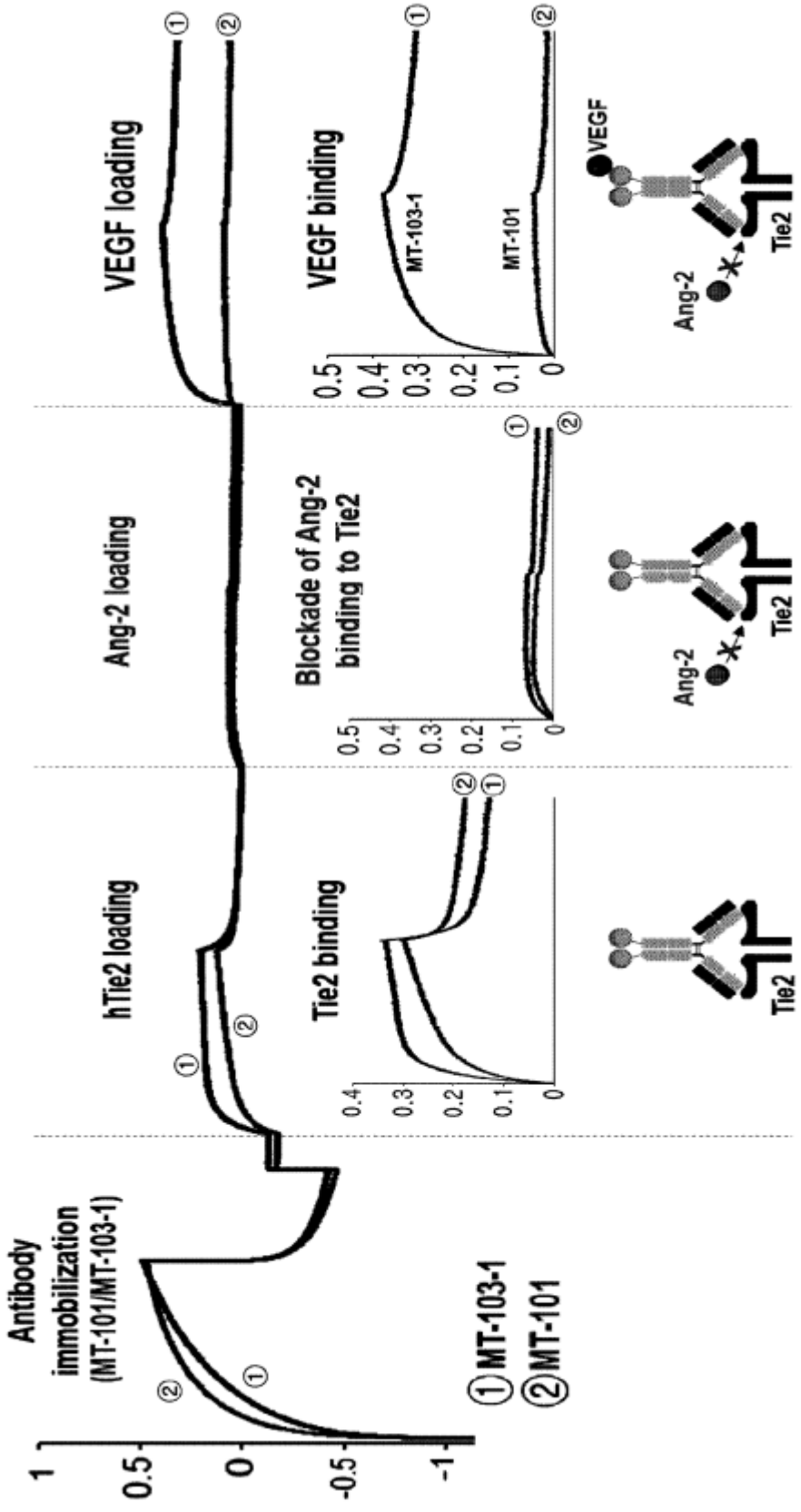

Antibody immobilization (MT-101/MT-103-1)
1
0.5
0
-0.5
-1
① MT-103-1
② MT-101
hTie2 loading
Tie2 binding
Tie2
Ang-2 loading
Blockade of Ang-2 binding to Tie2
Ang-2
Tie2
VEGF loading
VEGF binding
MT-103-1
MT-101
VEGF
Ang-2
Tie2

Figure 35a

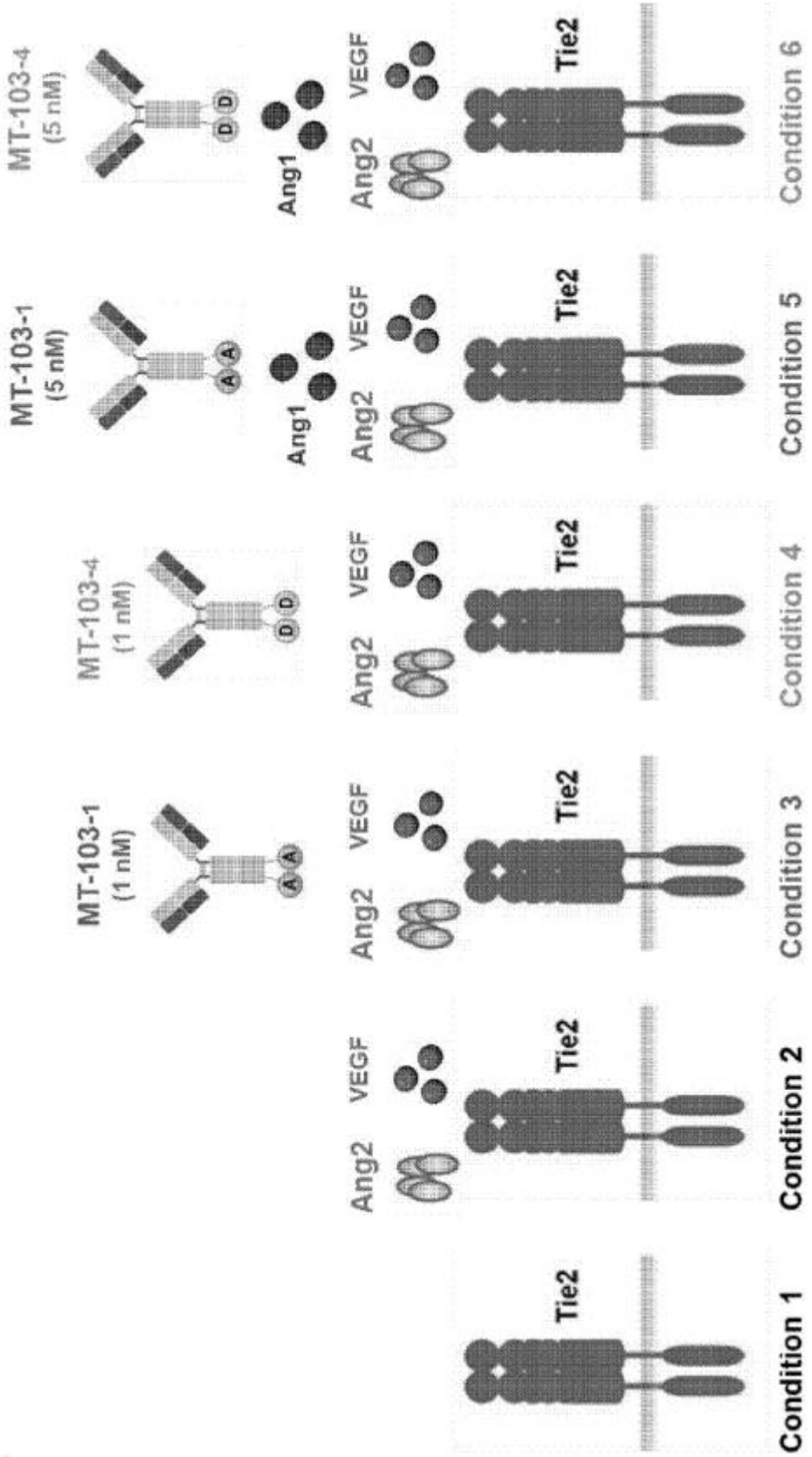

MT-103-4 (5 nM)
MT-103-1 (5 nM)
MT-103-4 (1 nM)
MT-103-1 (1 nM)
Ang1
Ang2
VEGF
Tie2
Condition 1
Condition 2
Condition 3
Condition 4
Condition 5
Condition 6

Figure 35b

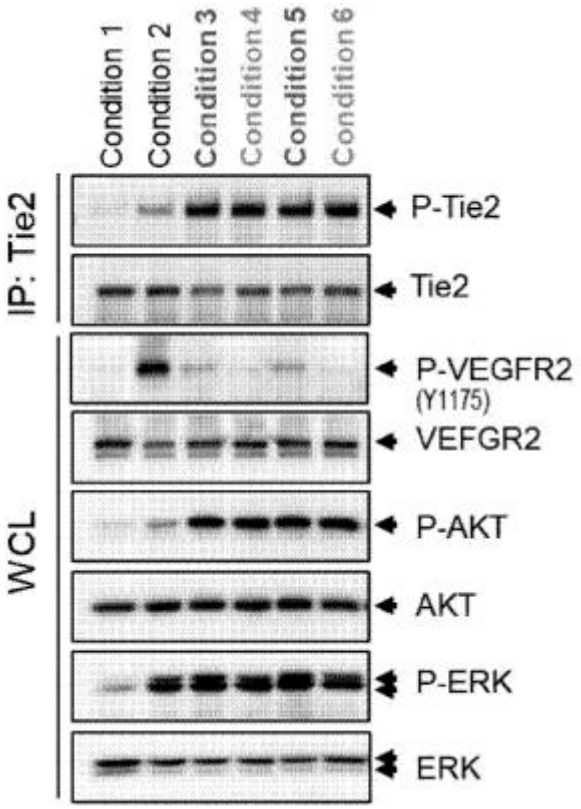


Figure 36a

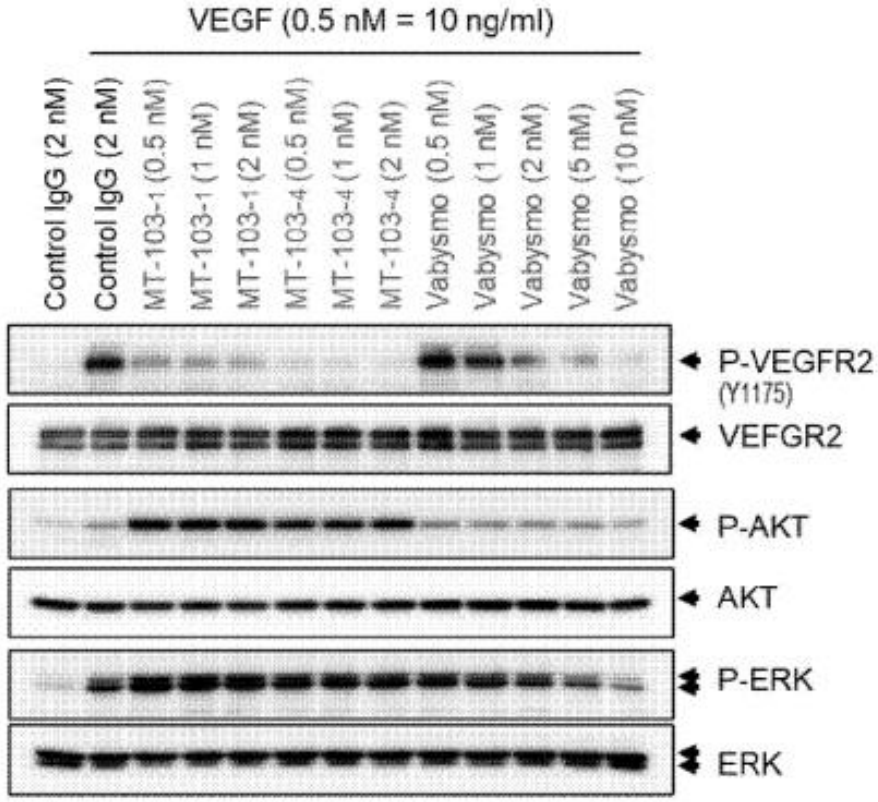


Figure 36b

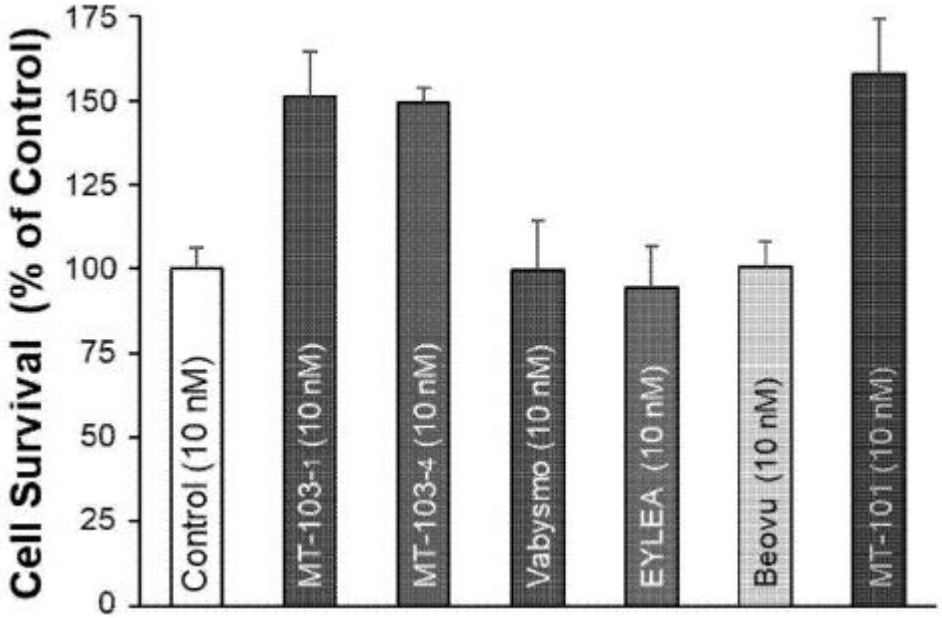


Figure 37a

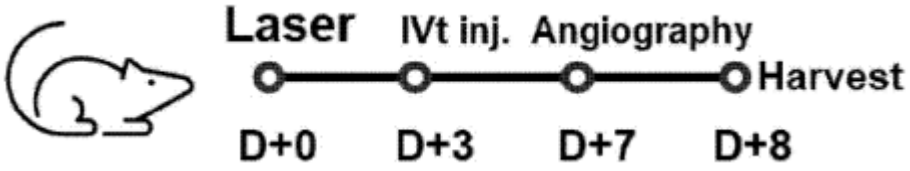


Figure 37b

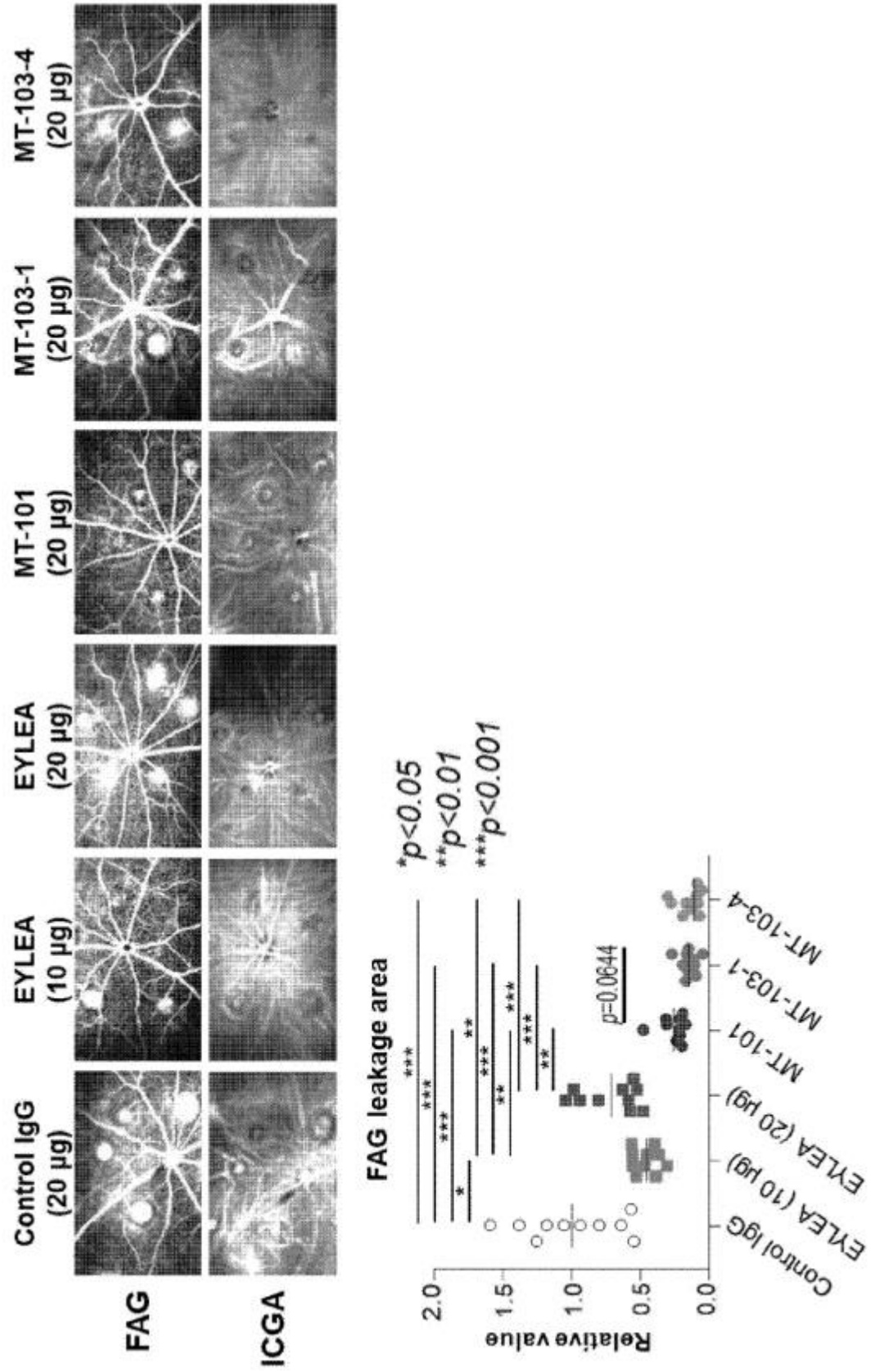

Control IgG (20 μg)
EYLEA (10 μg)
EYLEA (20 μg)
MT-101 (20 μg)
MT-103-1 (20 μg)
MT-103-4 (20 μg)
FAG
ICGA
FAG leakage area
*p<0.05
**p<0.01
***p<0.001
p=0.0644
Relative value
2.0
1.5
1.0
0.5
0.0
Control IgG
EYLEA (10 μg)
EYLEA (20 μg)
MT-101
MT-103-1
MT-103-4

Figure 37c

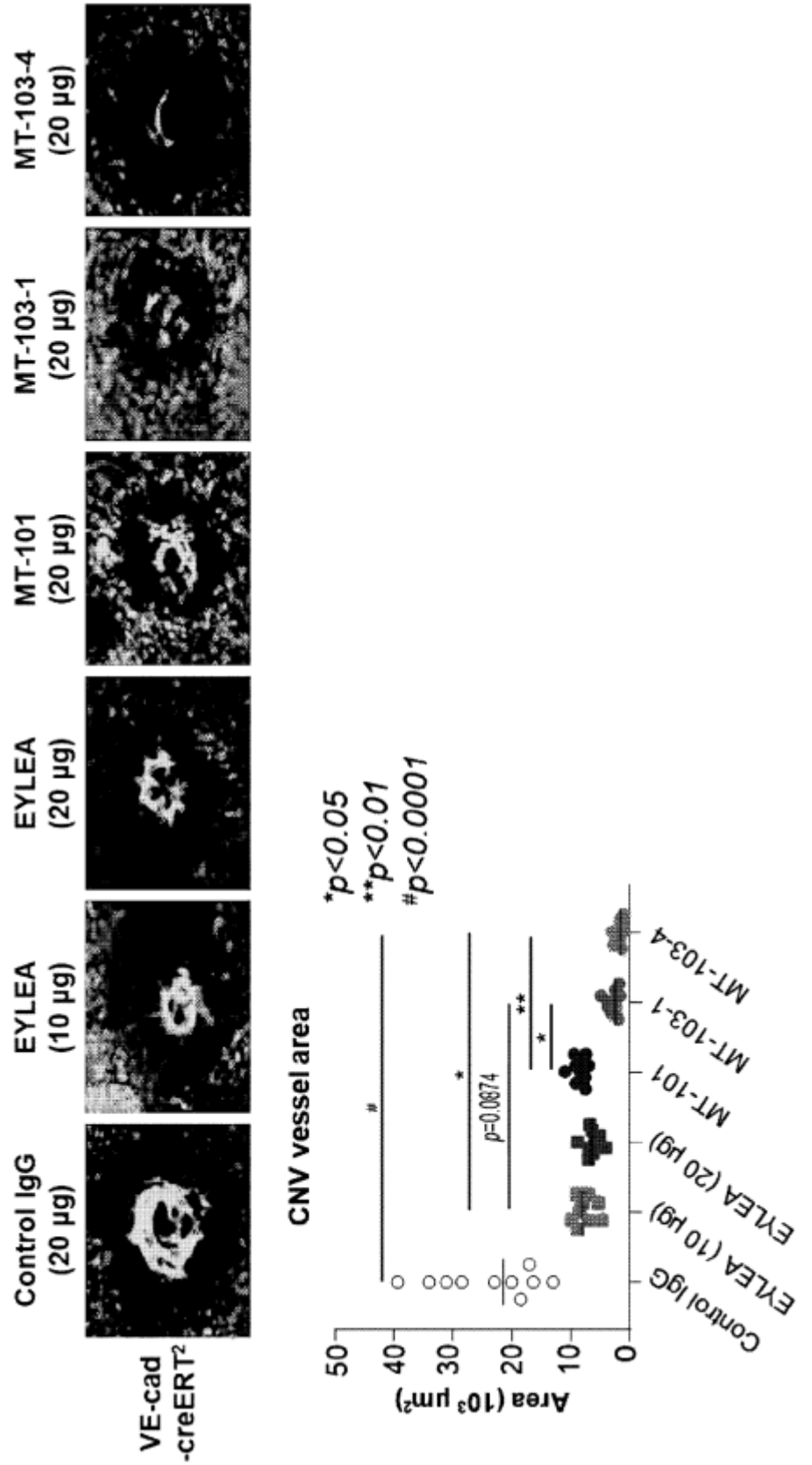

Control IgG (20 µg)
EYLEA (10 µg)
EYLEA (20 µg)
MT-101 (20 µg)
MT-103-1 (20 µg)
MT-103-4 (20 µg)
VE-cad -creERT2
CNV vessel area
*p<0.05
**p<0.01
#p<0.0001
p=0.0874
Area (10³ µm²)
0
10
20
30
40
50
Control IgG
EYLEA (10 µg)
EYLEA (20 µg)
MT-101
MT-103-1
MT-103-4

Figure 38a

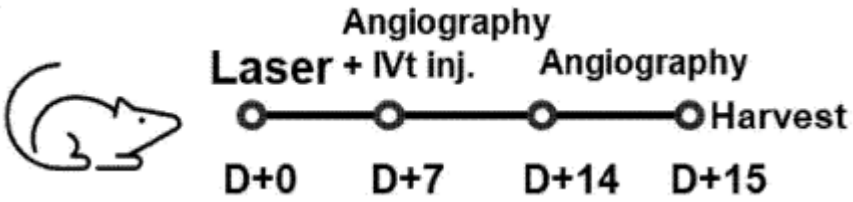


Figure 38b

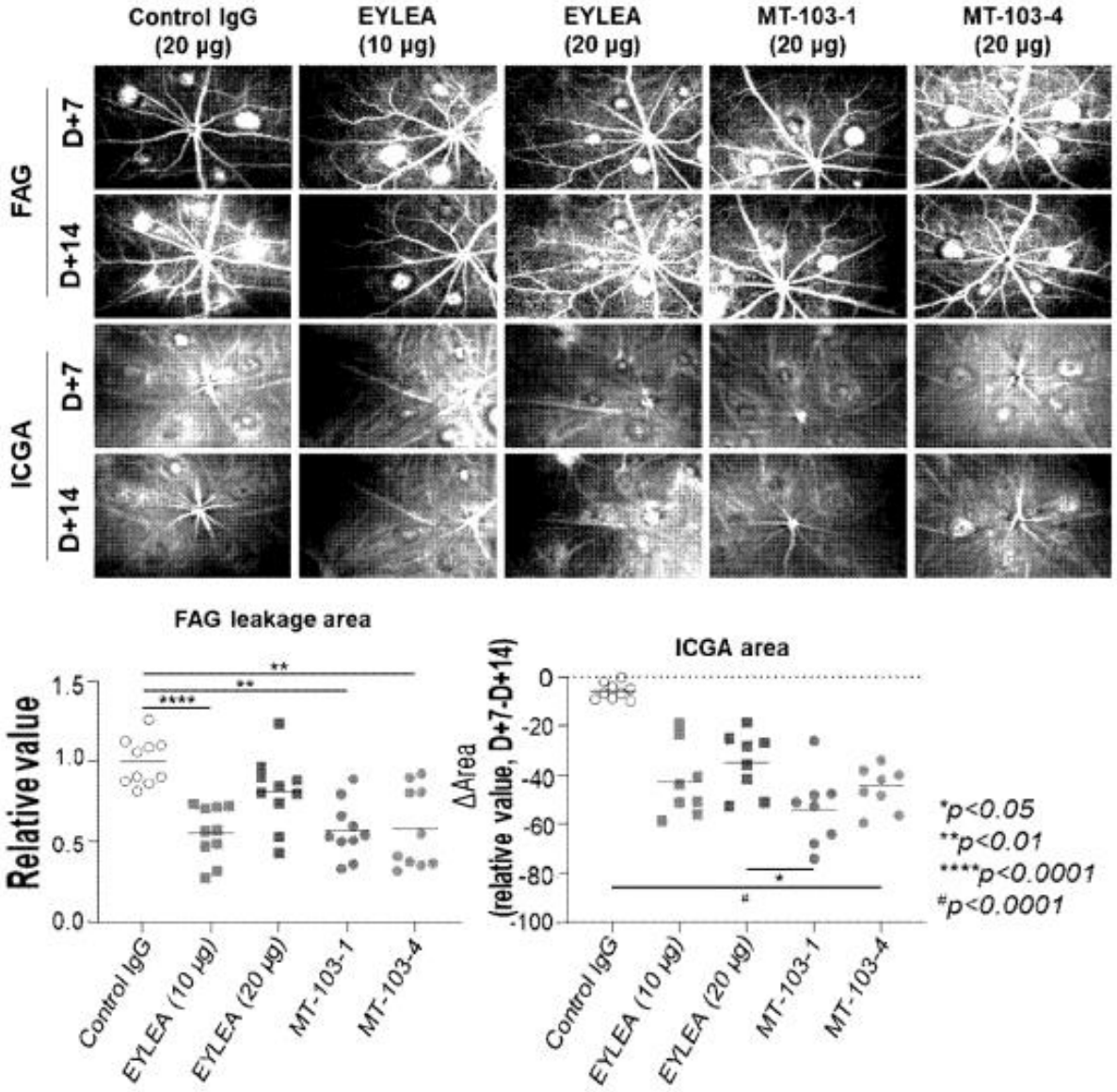


Figure 38c

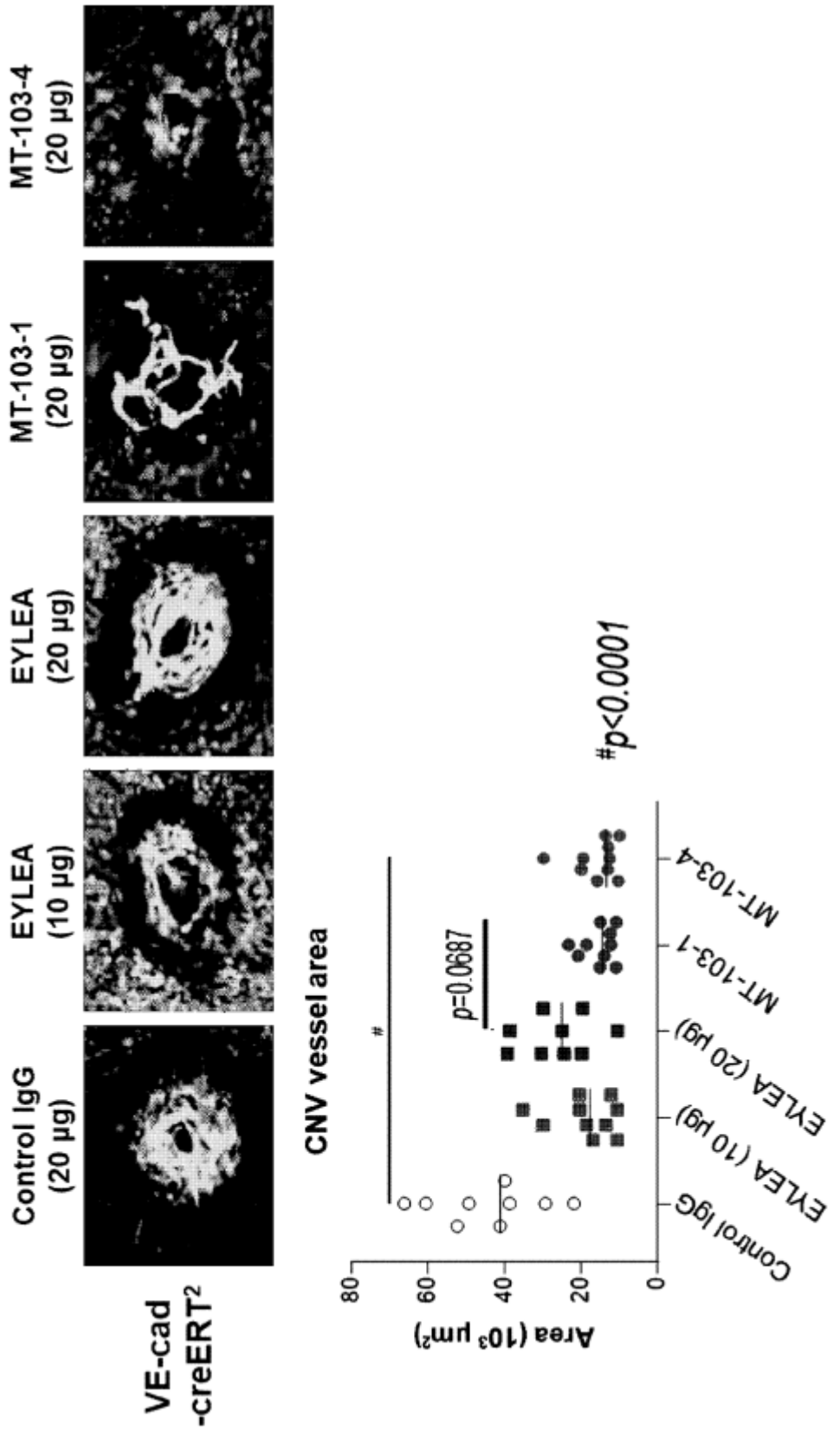

Control IgG (20 μg)
EYLEA (10 μg)
EYLEA (20 μg)
MT-103-1 (20 μg)
MT-103-4 (20 μg)
VE-cad -creERT2
CNV vessel area
Area (10³ μm²)
80
60
40
20
0
#
p=0.0687
#p<0.0001
Control IgG
EYLEA (10 μg)
EYLEA (20 μg)
MT-103-1
MT-103-4

Figure 39a

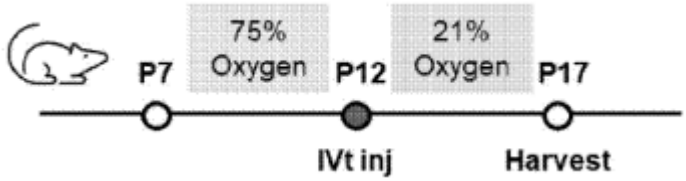


Figure 39b

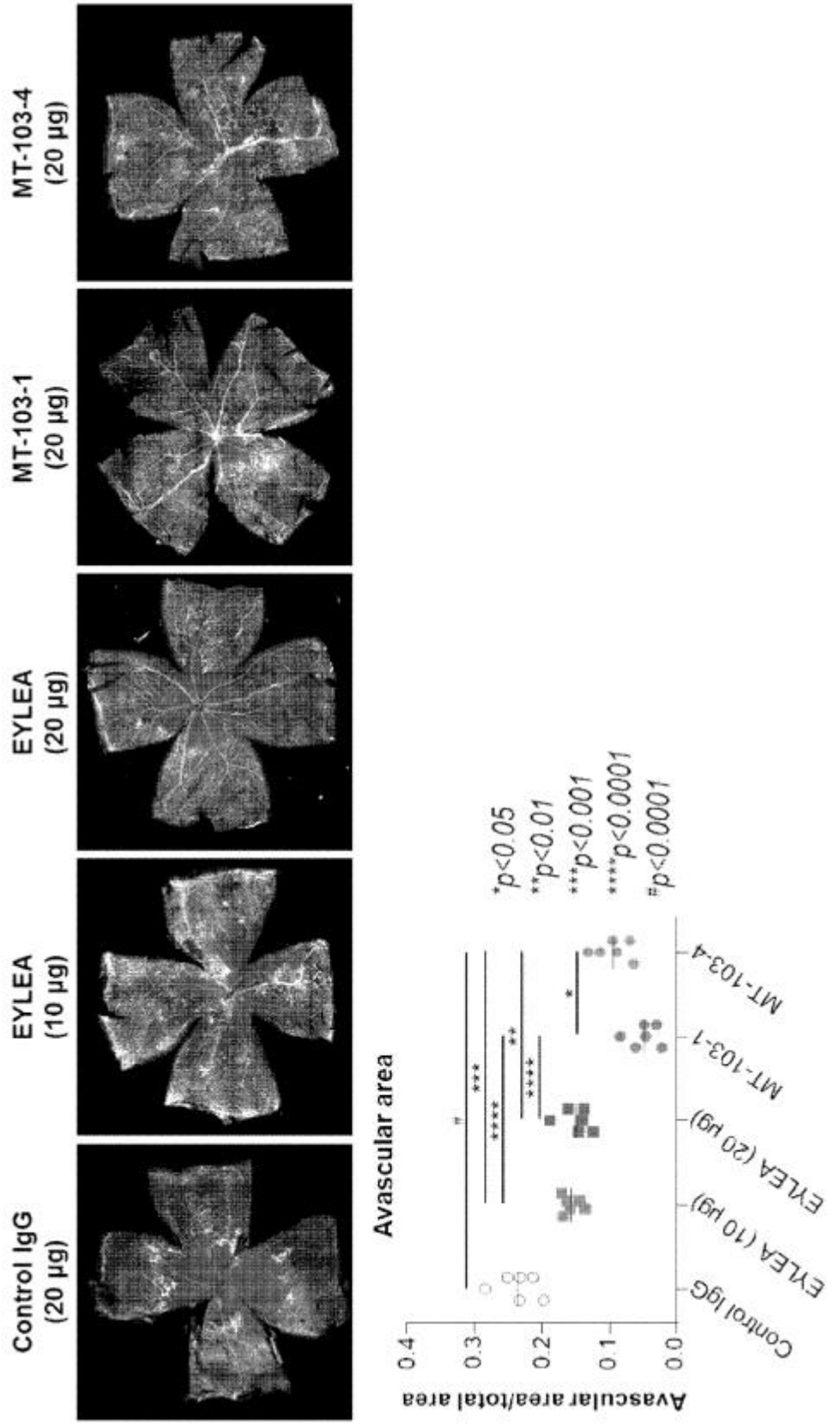
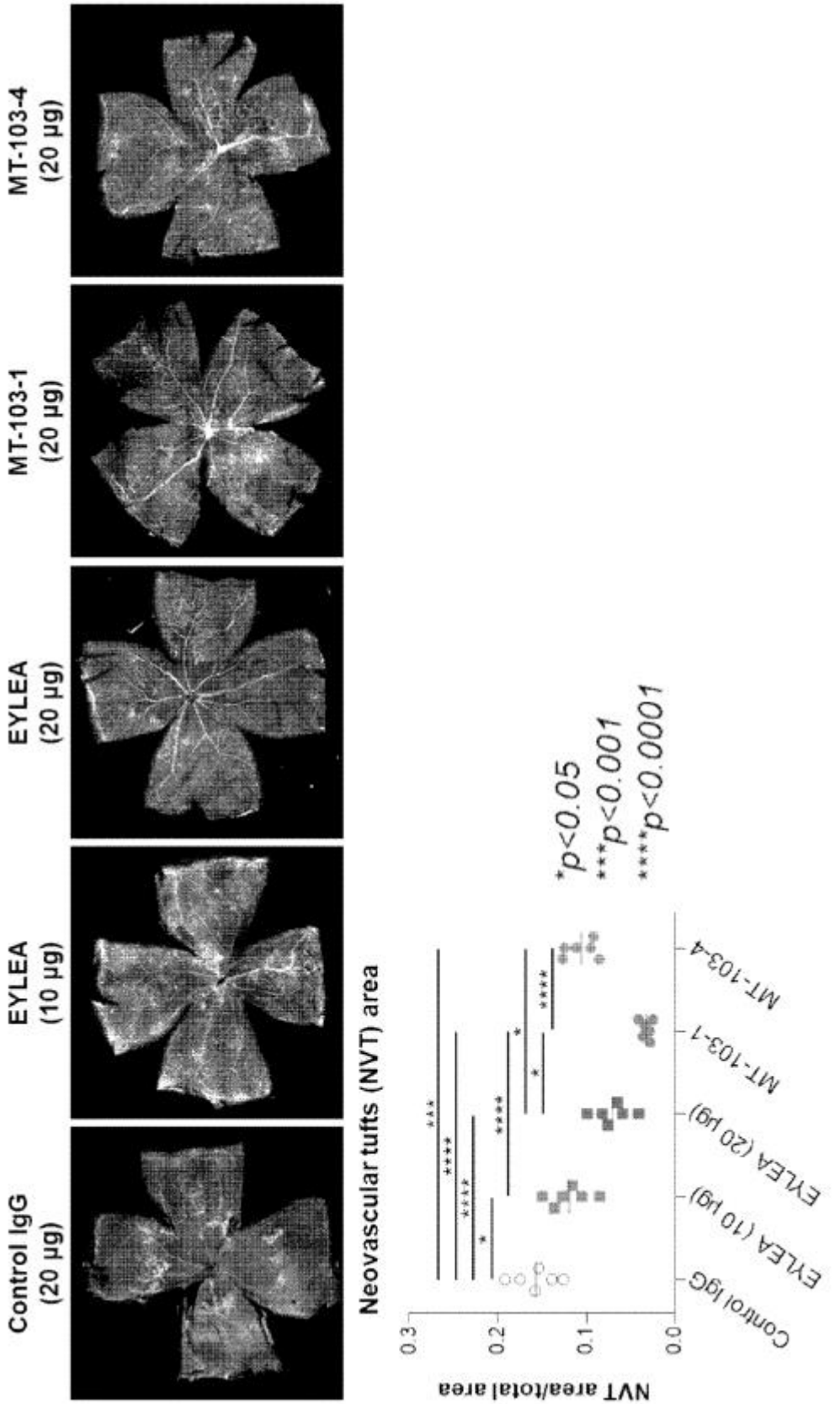

Control IgG (20 µg)
EYLEA (10 µg)
EYLEA (20 µg)
MT-103-1 (20 µg)
MT-103-4 (20 µg)
Avascular area
Avascular area/total area
0.4
0.3
0.2
0.1
0.0
Control IgG
EYLEA (10 µg)
EYLEA (20 µg)
MT-103-1
MT-103-4
*p<0.05
**p<0.01
***p<0.001
****p<0.0001
#p<0.0001

Figure 39c


Control IgG (20 µg)
EYLEA (10 µg)
EYLEA (20 µg)
MT-103-1 (20 µg)
MT-103-4 (20 µg)
Neovascular tufts (NVT) area
NVT area/total area
0.3
0.2
0.1
0.0
Control IgG
EYLEA (10 µg)
EYLEA (20 µg)
MT-103-1
MT-103-4
*p<0.05
***p<0.001
****p<0.0001