FIG. 1A-1E

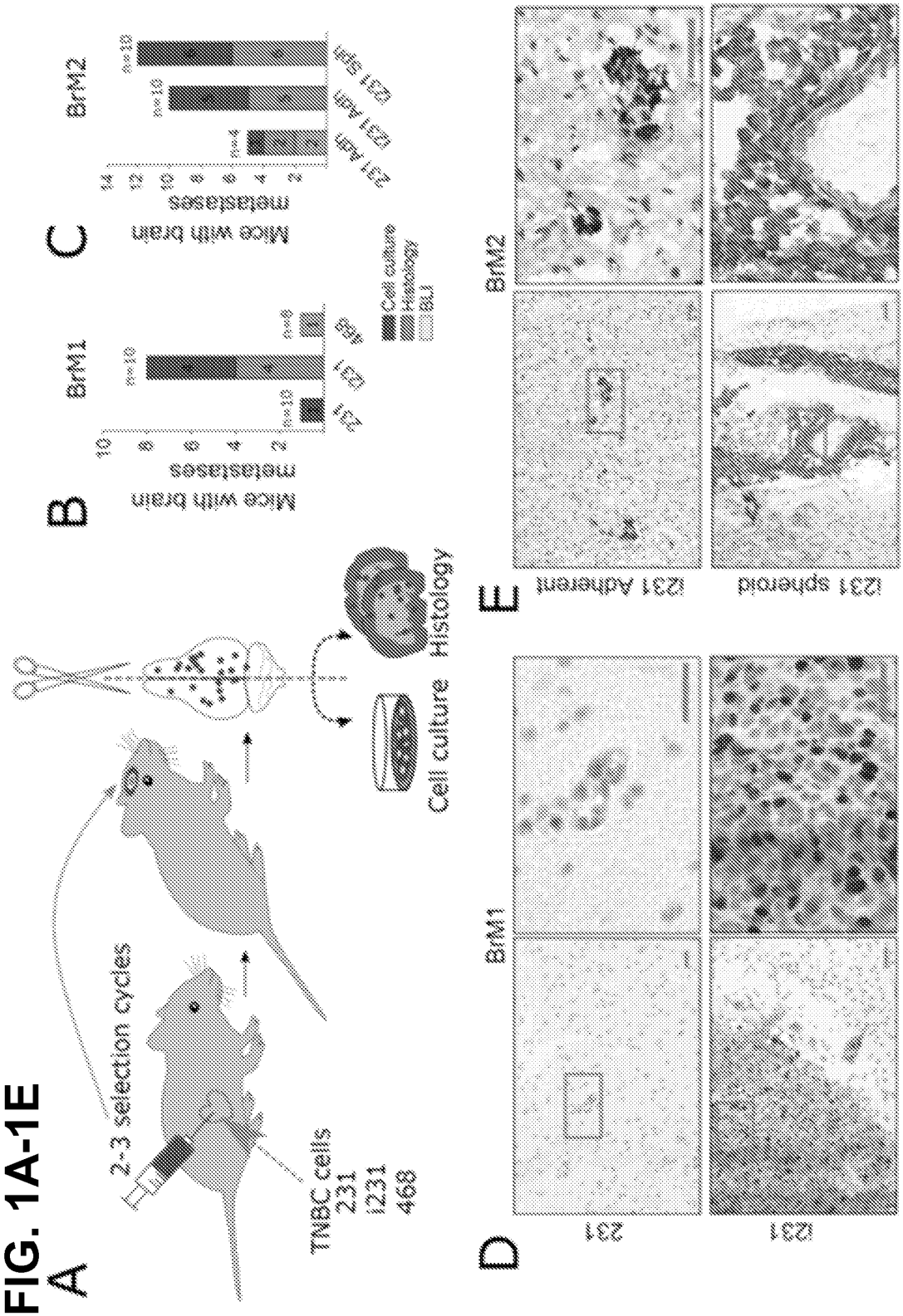
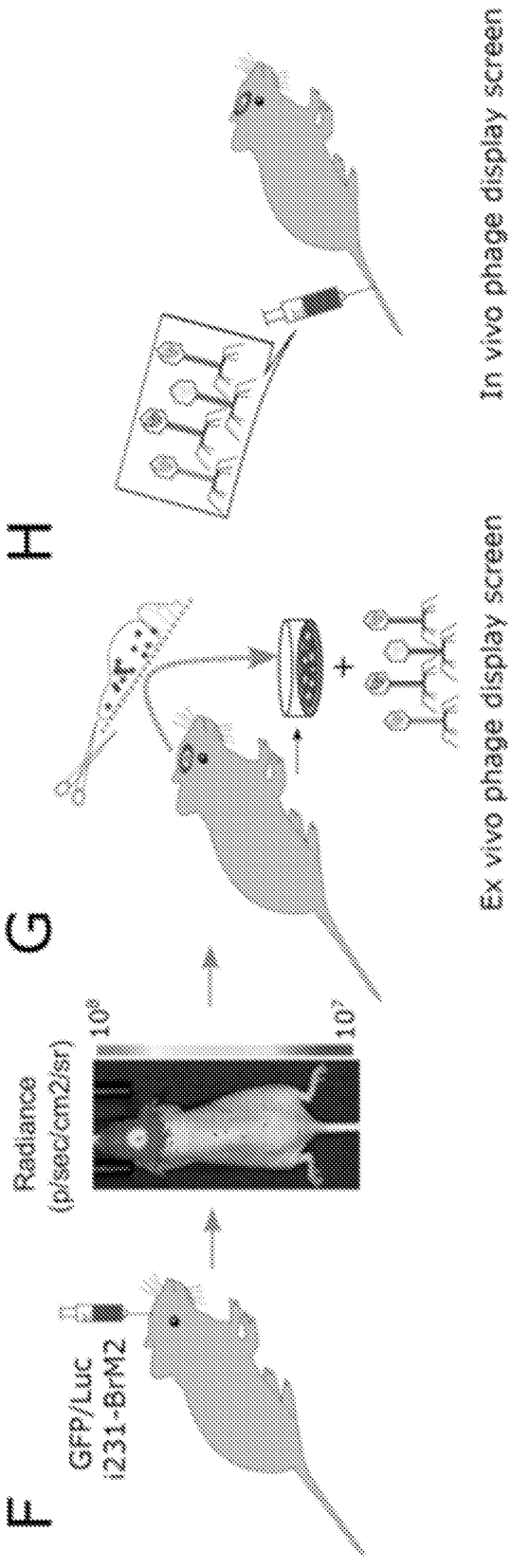
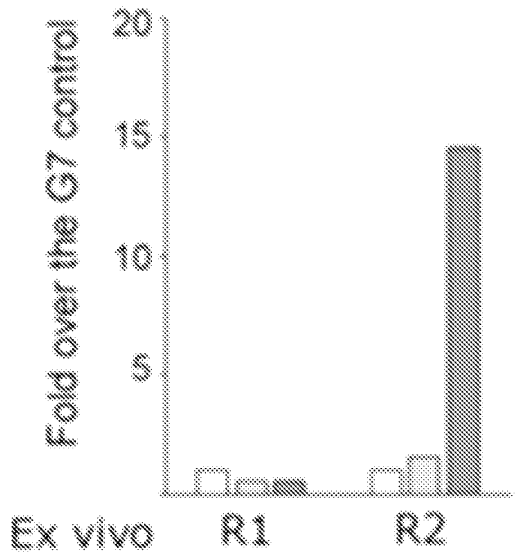

A
2-3 selection cycles
TNBC cells
231
i231
468
Cell culture
Histology
B
Mice with brain metastases
BrM1
231
i231
468
Cell culture
Histology
BLI
C
Mice with brain metastases
BrM2
231 Adh
i231 Adh
i231 Sph
D
BrM1
231
i231
E
BrM2
231 Adherent
i231 spheroid

FIG. 1F-1H


F
GFP/Luc
231-BrM2
Radiance
(p/sec/cm2/sr)
10⁸
10⁷
G
Ex vivo phage display screen
H
In vivo phage display screen

I


Fold over the G7 control
20
15
10
5
Ex vivo
R1
R2

J

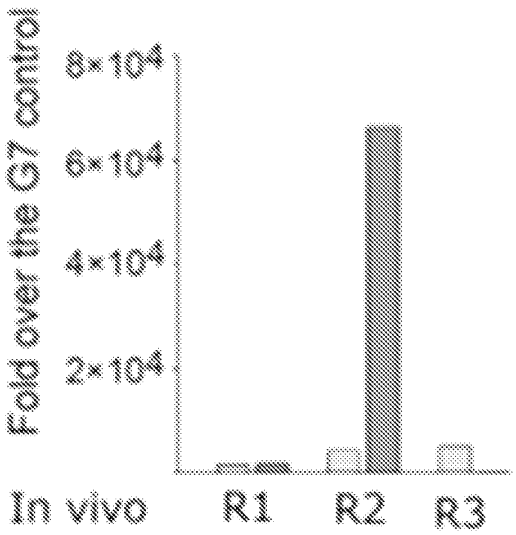
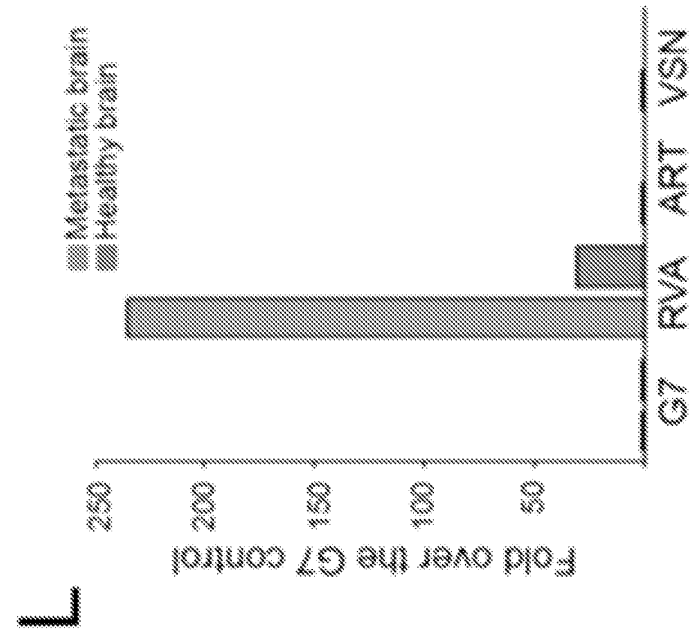
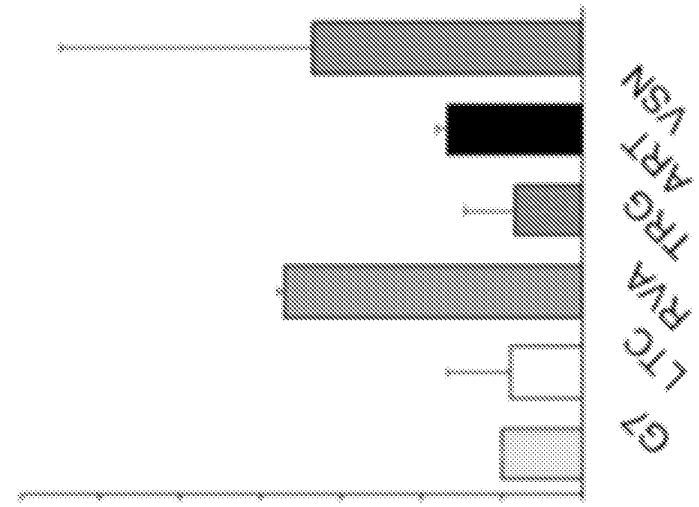

Fold over the G7 control
8×10⁴
6×10⁴
4×10⁴
2×10⁴
In vivo
R1
R2
R3

FIG. 1I-1J


Metastatic brain
Healthy brain
250
200
150
100
50
Fold over the G7 control
G7
RVA
ART
VSN

L


7
6
5
4
3
2
1
Ex vivo binding to brain
metastasis over healthy brain
G7
LTG
RVA
TRG
ART
VSN

K

FIG. 1K-1L

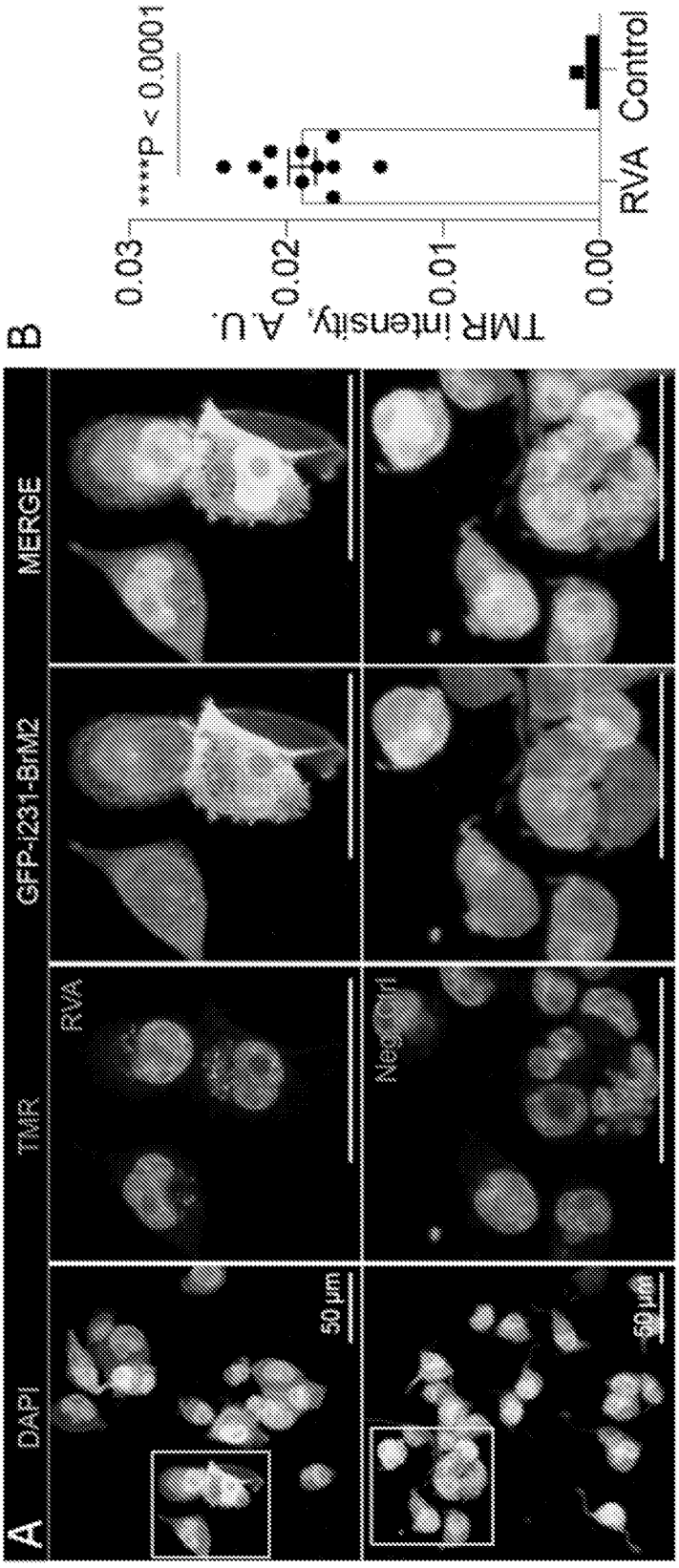

A
DAPI
TMR
GFP-i231-BrM2
MERGE
RVA
Neg. Ctl
50 µm
50 µm
B
TMR intensity, A.U.
0.03
0.02
0.01
0.00
****P < 0.0001
RVA
Control

FIG. 2A-2B

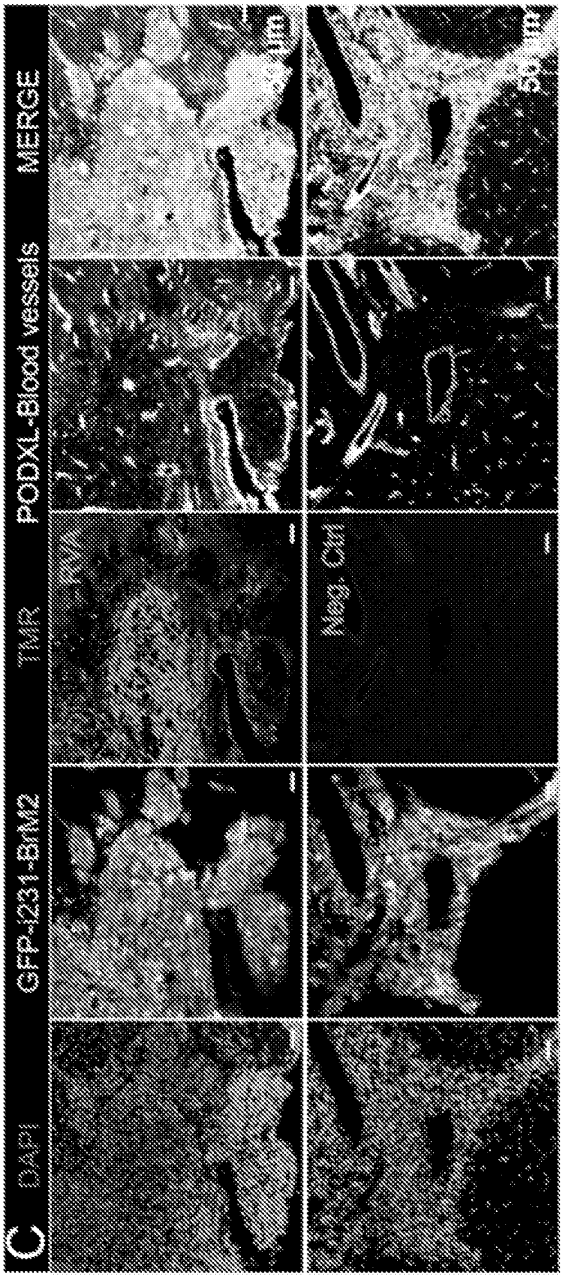

C
DAPI
GFP-231-BrM2
TMR
PODXL-Blood vessels
MERGE
Neg. Ctrl
RVA
30 min.
60 min.
D
Norm. TMR mean intensity
400
300
200
100
0
Neg. Ctrl
RVA
****p < 0.0001
****p < 0.0001
30 min.
60 min.

FIG. 2C-2D

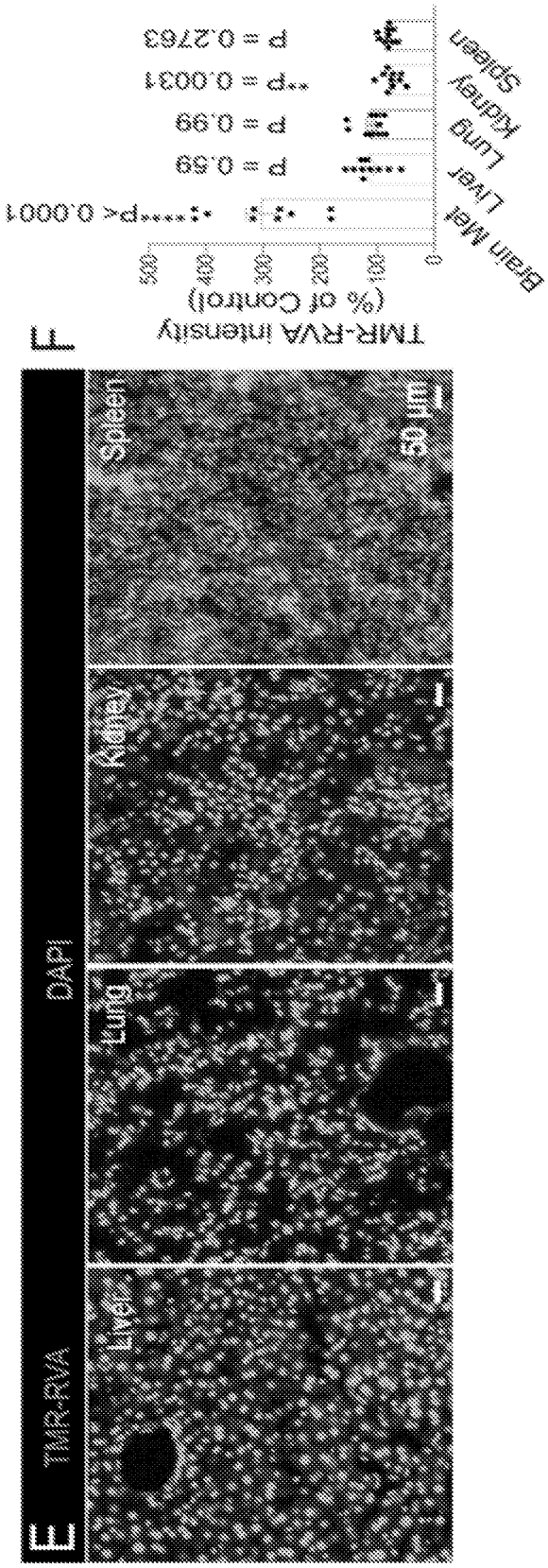

F
TMR-RVA intensity
(% of Control)
600%
400%
300%
200%
100%
0
Brain Met
Liver
Lung
Kidney
Spleen
****P< 0.0001
P = 0.59
P = 0.99
**P = 0.0031
P = 0.2763
E
TMR-RVA
DAPI
Liver
Lung
Kidney
Spleen
50 µm

FIG. 2E-2F

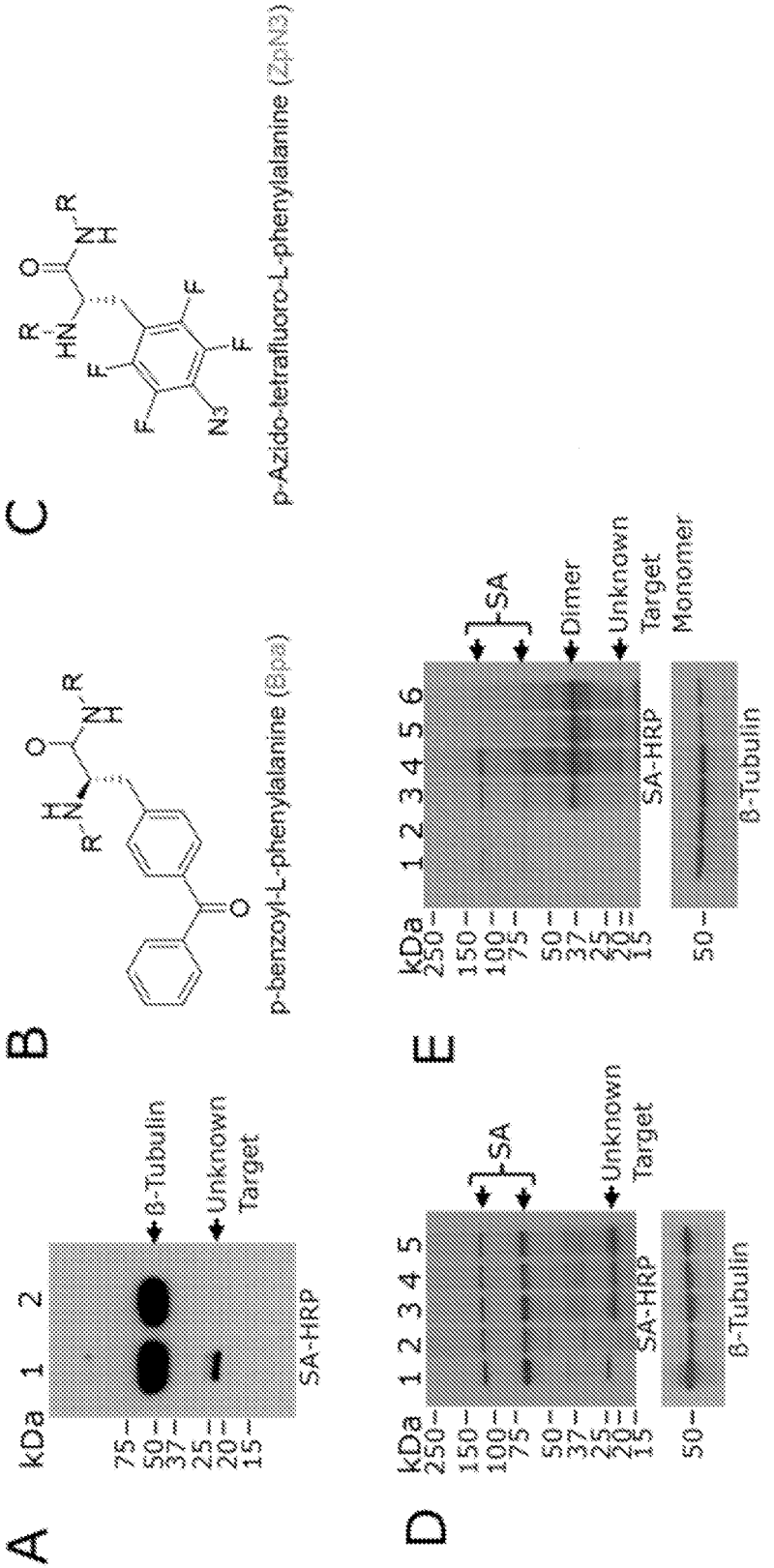
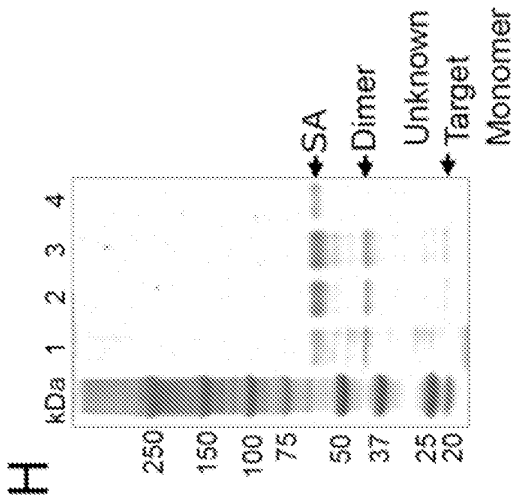
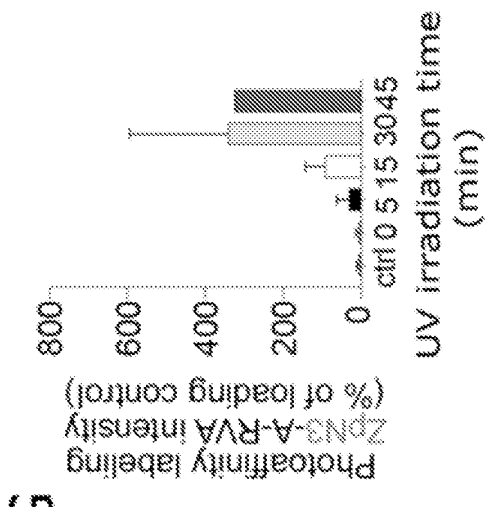

A
kDa
1
2
β-Tubulin
Unknown Target
SA-HRP
75
50
37
25
20
15
B
p-benzoyl-L-phenylalanine (Bpa)
C
p-Azido-tetrafluoro-L-phenylalanine (ZpNB)
D
kDa
1 2 3 4 5
SA
Unknown Target
SA-HRP
β-Tubulin
250
150
100
75
50
37
25
20
15
E
kDa
1 2 3 4 5 6
SA
Dimer
Unknown Target
Monomer
SA-HRP
β-Tubulin

FIG. 3A-3E


H
kDa
1
2
3
4
250
150
100
75
50
37
25
20
SA
Dimer
Unknown
Target
Monomer


G
Photoaffinity labeling
ZpNs-A-RVA intensity
(% of loading control)
800
600
400
200
0
ctrl 0 5 15 30 45
UV irradiation time
(min)

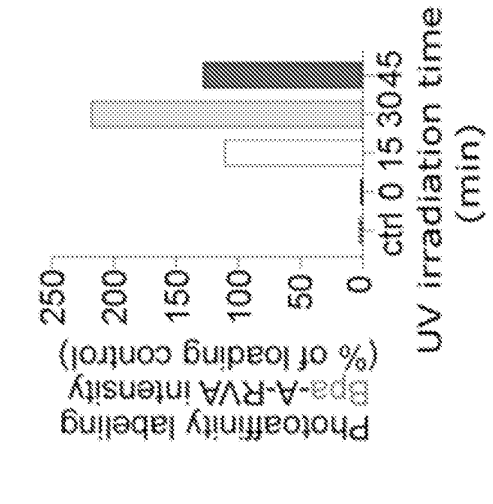

F
Photoaffinity labeling
Bpa-A-RVA intensity
(% of loading control)
250
200
150
100
50
0
ctrl 0 15 30 45
UV irradiation time
(min)

FIG. 3F-3H

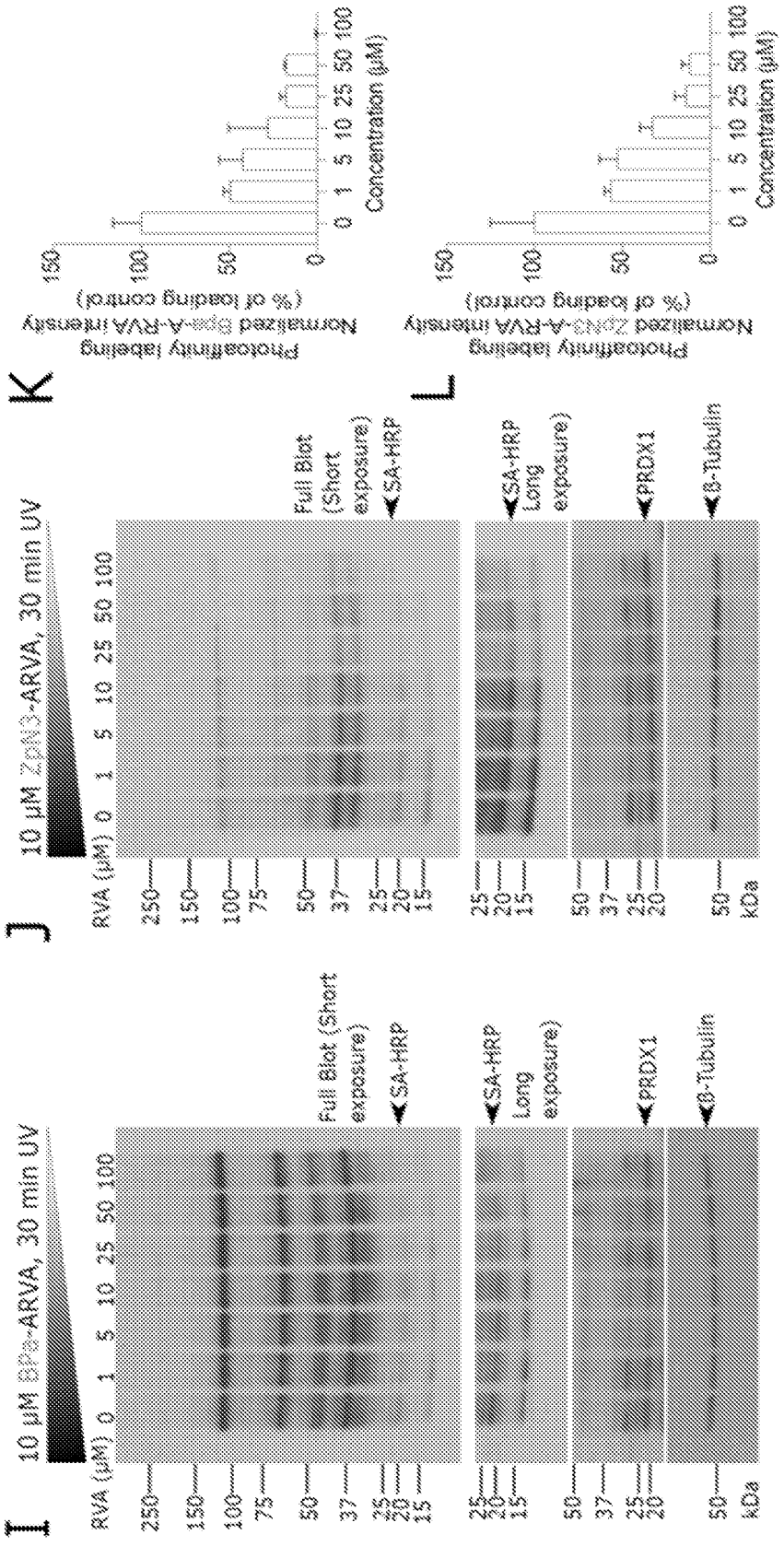
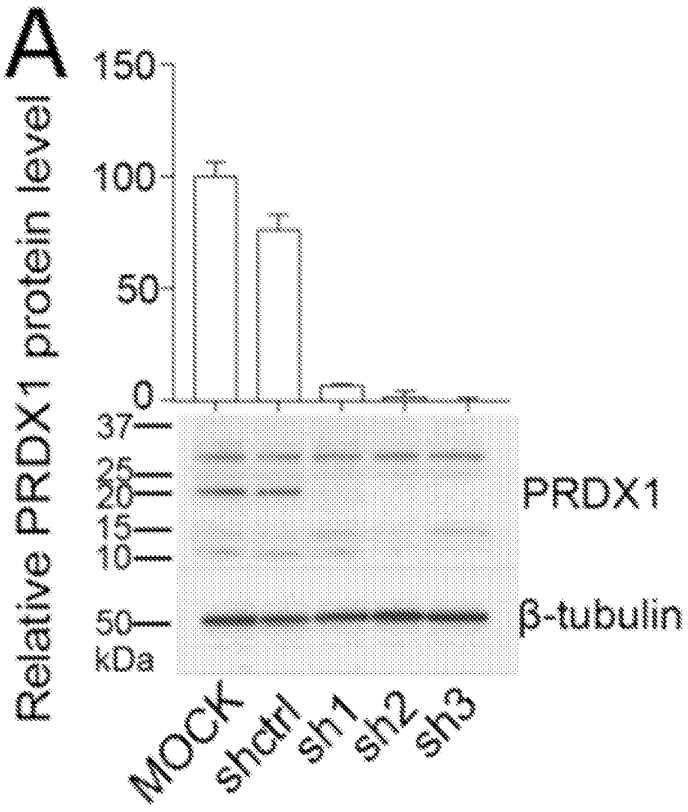

I
10 μM BPa-ARVA, 30 min UV
RVA (μM) 0 1 5 10 25 50 100
Full Blot (Short exposure)
SA-HRP
SA-HRP Long exposure
PRDX1
β-Tubulin
kDa
J
10 μM ZsN3-ARVA, 30 min UV
K
Photoaffinity labeling
Normalized Bpa-A-RVA intensity
(% of loading control)
Concentration (μM)
L
Photoaffinity labeling
Normalized ZsN3-A-RVA intensity
(% of loading control)
Concentration (μM)

FIG. 3I-3L


A
Relative PRDX1 protein level
150
100
50
0
37
25
20
15
10
50
kDa
PRDX1
β-tubulin
MOCK
shctrl
sh1
sh2
sh3

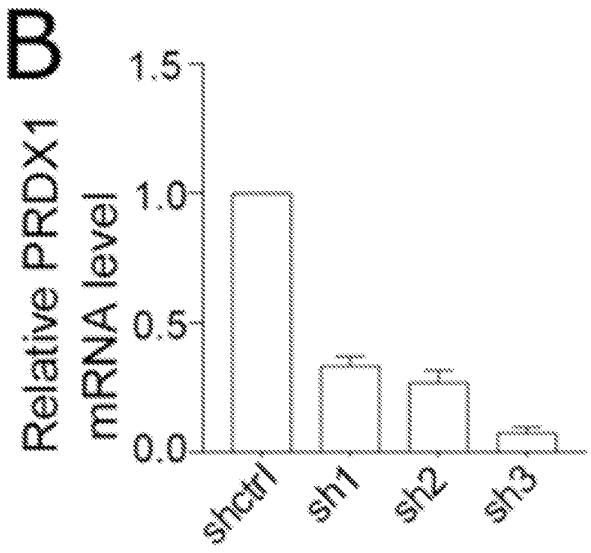

B
Relative PRDX1 mRNA level
1.5
1.0
0.5
0.0
shctrl
sh1
sh2
sh3

FIG. 4A-4B

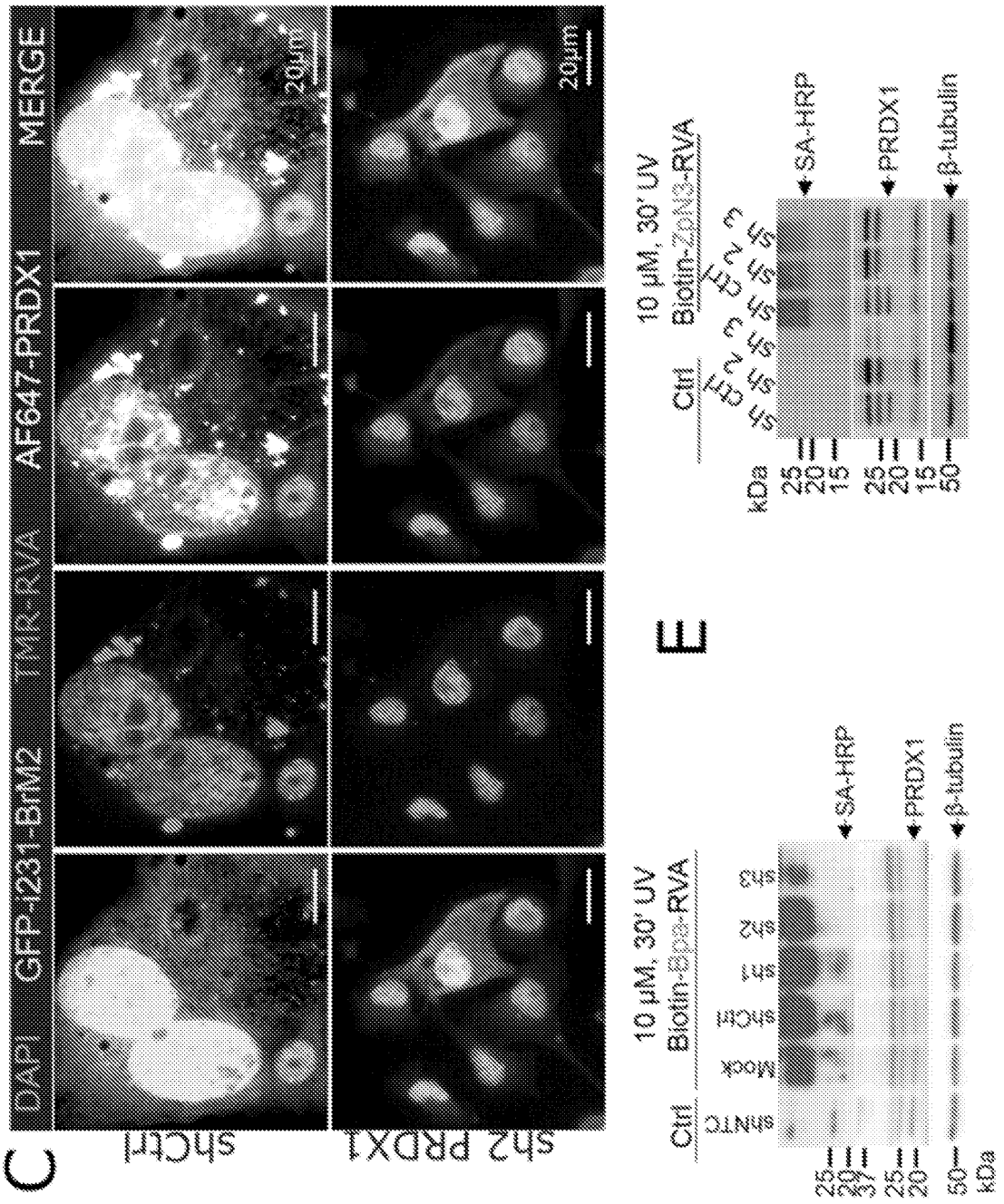

C
DAPI
GFP-231-BrM2
TMR-RVA
AF647-PRDX1
MERGE
shCtrl
sh2 PRDX1
20μm
D
10 μM, 30' UV
Biotin-Bpa-RVA
Ctrl
shNTC
Mock
shCtl
sh1
sh2
sh3
SA-HRP
PRDX1
β-tubulin
kDa
E
10 μM, 30' UV
Biotin-ZpNS-RVA
Ctrl
SA-HRP
PRDX1
β-tubulin
kDa

FIG. 4C-4E

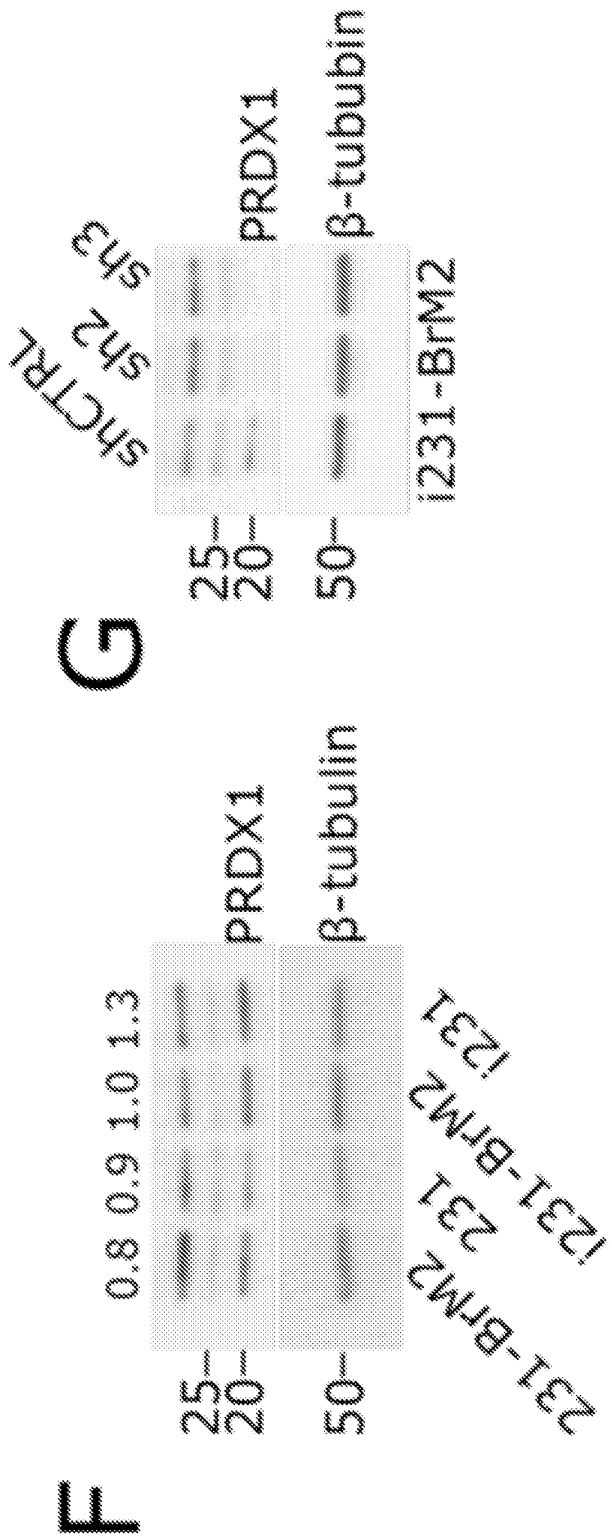
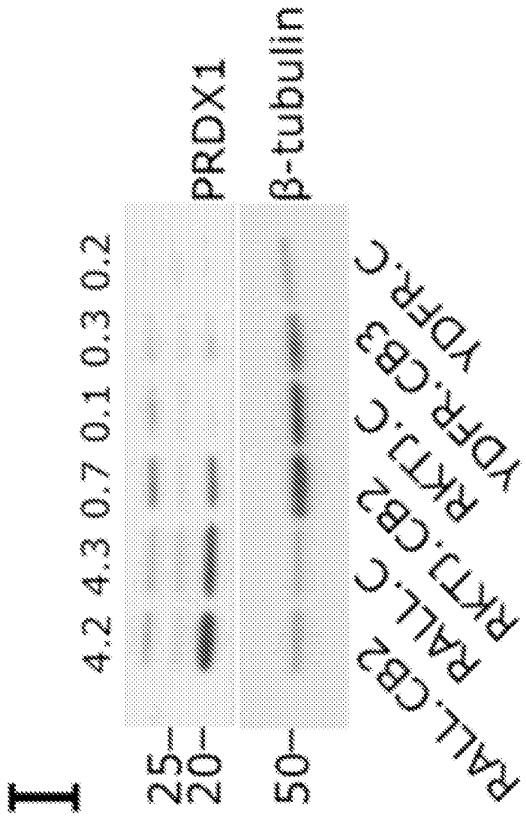

G
shCTRL
sh2
sh3
PRDX1
β-tububin
i231-BrM2
25
20
50
F
0.8 0.9 1.0 1.3
PRDX1
β-tubulin
231-BrM2
231
i231-BrM2
i231
25
20
50

FIG. 4F-4G


I
PRDX1
β-tubulin
4.2 4.3 0.7 0.1 0.3 0.2
25
20
50
RALL.CB2
RALL.C
RKTJ.CB2
RKTJ.C
VDFR.CB3
VDFR.C

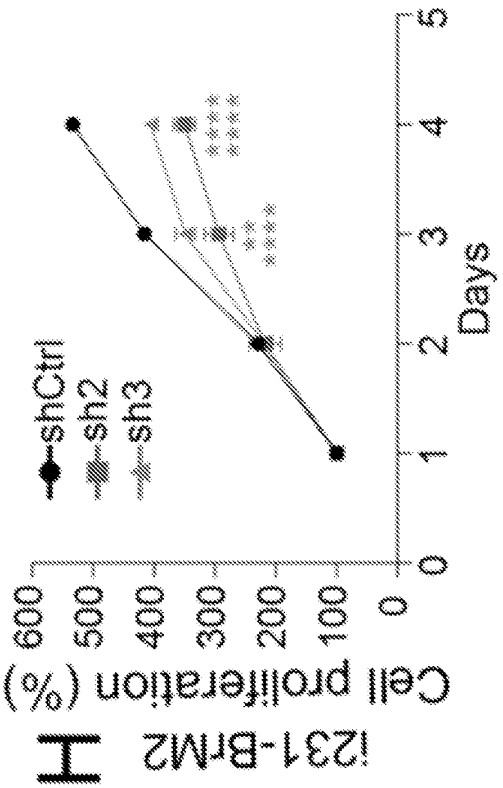
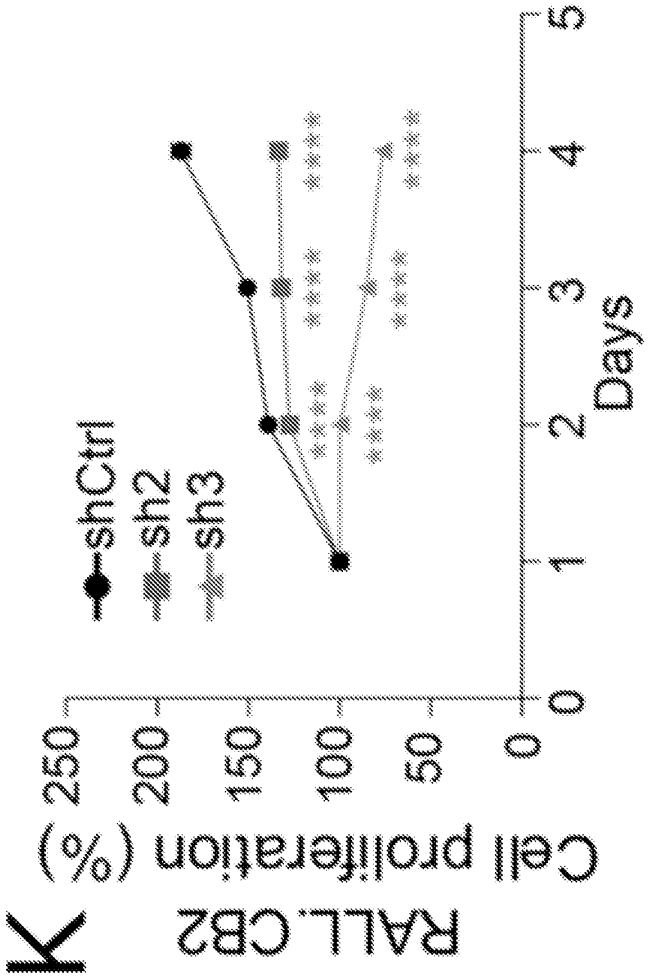

H
i231-BrM2
Cell proliferation (%)
600
500
400
300
200
100
0
shCtrl
sh2
sh3
0
1
2
3
4
5
Days

FIG. 4H-4I


K
RALL.CB2
Cell proliferation (%)
250
200
150
100
50
0
0
1
2
3
4
5
Days
shCtrl
sh2
sh3
****

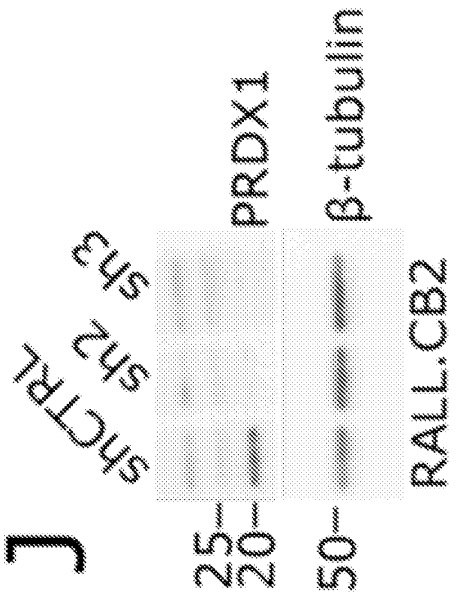

J
shCTRL
sh2
sh3
PRDX1
β-tubulin
RALL.CB2
25
20
50

FIG. 4J-4K

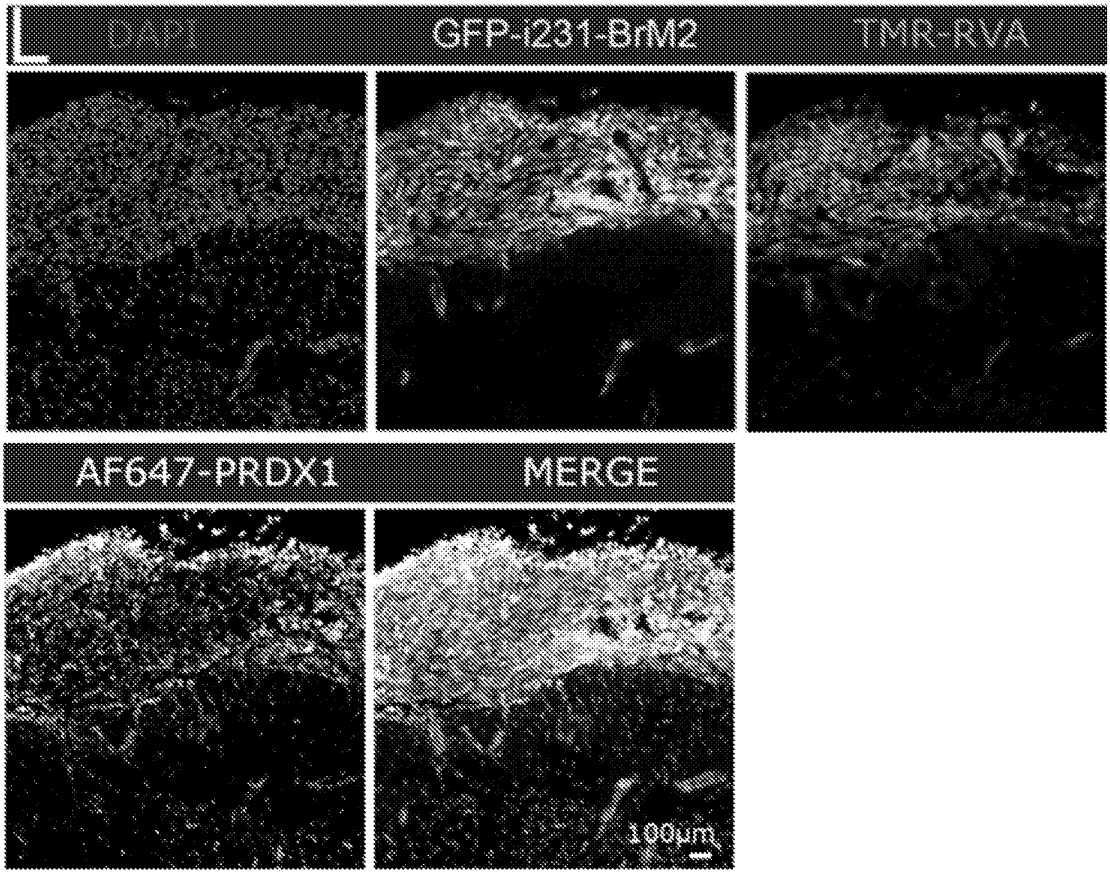


FIG. 4L

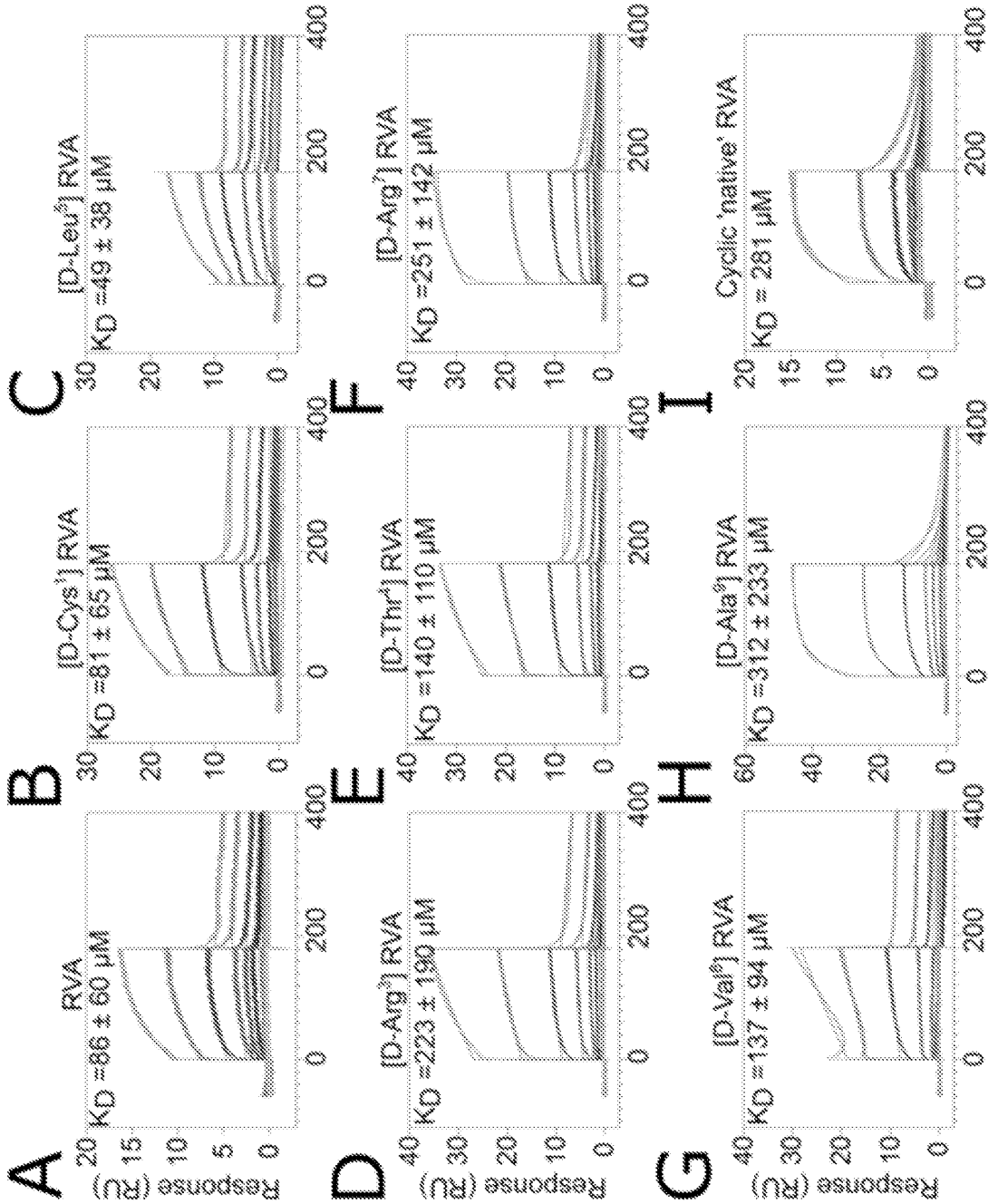

A
RVA
KD =86 ± 60 µM
B
[D-Cys1] RVA
KD =81 ± 65 µM
C
[D-Leu5] RVA
KD =49 ± 38 µM
D
[D-Arg3] RVA
KD =223 ± 190 µM
E
[D-Thr4] RVA
KD =140 ± 110 µM
F
[D-Arg7] RVA
KD =251 ± 142 µM
G
[D-Val6] RVA
KD =137 ± 94 µM
H
[D-Ala8] RVA
KD =312 ± 233 µM
I
Cyclic 'native' RVA
KD = 281 µM
Response (RU)
Time (s)

FIG. 5A-5I

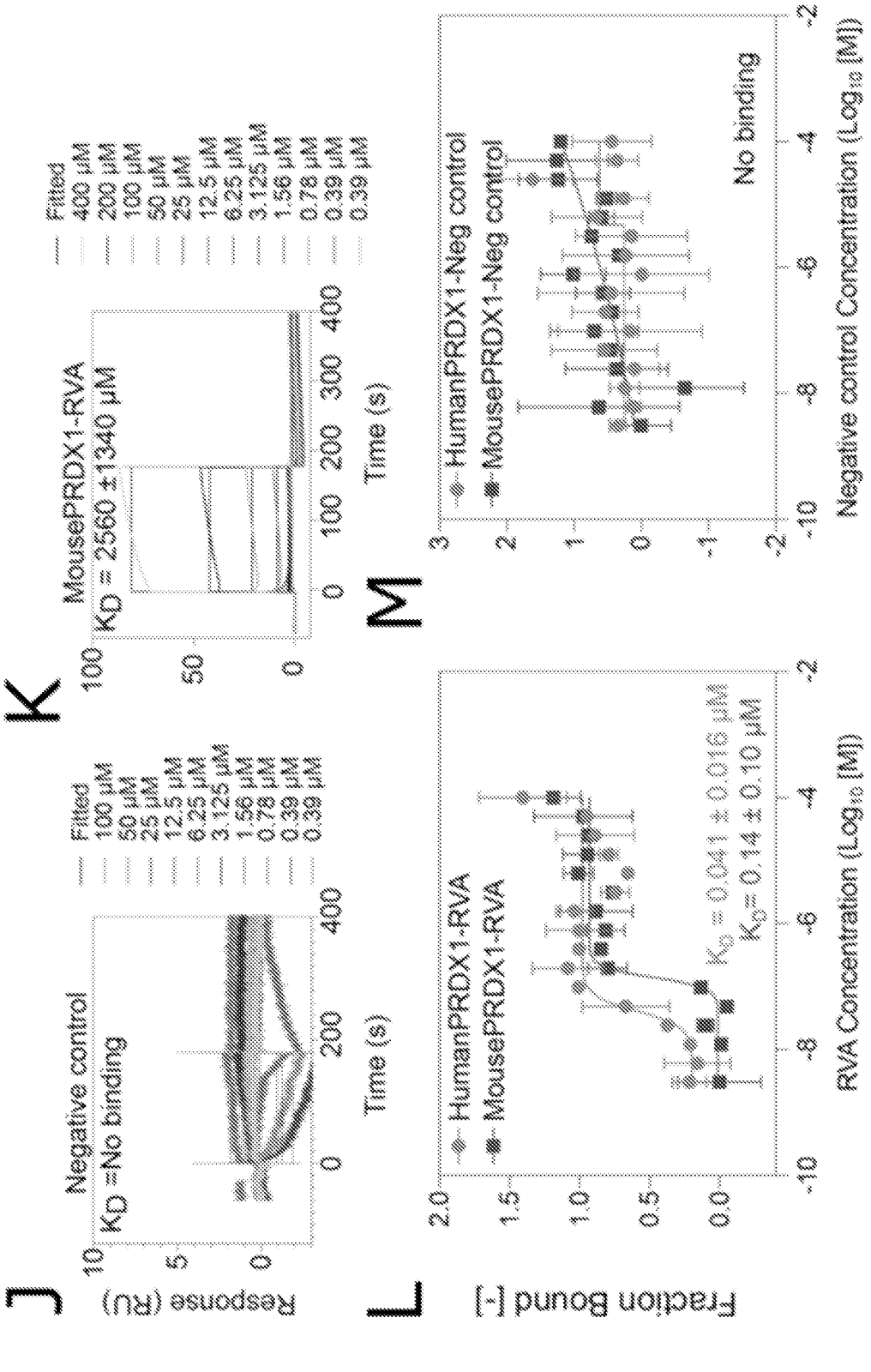

J
Negative control
KD =No binding
Response (RU)
Time (s)
Fitted
100 µM
50 µM
25 µM
12.5 µM
6.25 µM
3.125 µM
1.56 µM
0.78 µM
0.39 µM
0.39 µM
K
MousePRDX1-RVA
KD = 2560 ±1340 µM
Time (s)
Fitted
400 µM
200 µM
100 µM
50 µM
25 µM
12.5 µM
6.25 µM
3.125 µM
1.56 µM
0.78 µM
0.39 µM
0.39 µM
L
HumanPRDX1-RVA
MousePRDX1-RVA
KD = 0.041 ± 0.016 µM
KD= 0.14 ± 0.10 µM
Fraction Bound [-]
RVA Concentration (Log10 [M])
M
HumanPRDX1-Neg control
MousePRDX1-Neg control
No binding
Negative control Concentration (Log10 [M])

FIG. 5J-5M

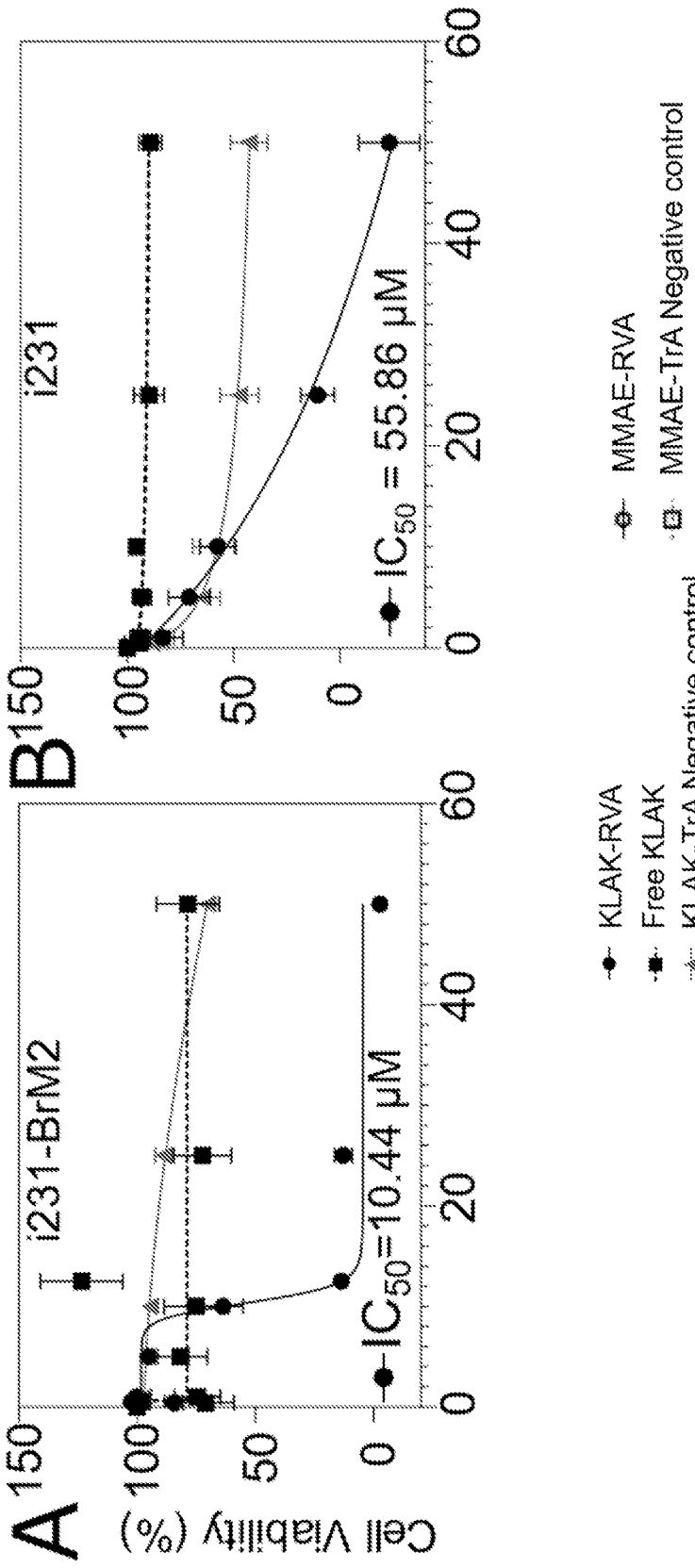

A
B
Cell Viability (%)
150
100
50
0
0
20
40
60
i231-BrM2
i231
IC50=10.44 µM
IC50 = 55.86 µM
KLAK-RVA
Free KLAK
KLAK-TrA Negative control
MMAE-RVA
MMAE-TrA Negative control

FIG. 6A-6B

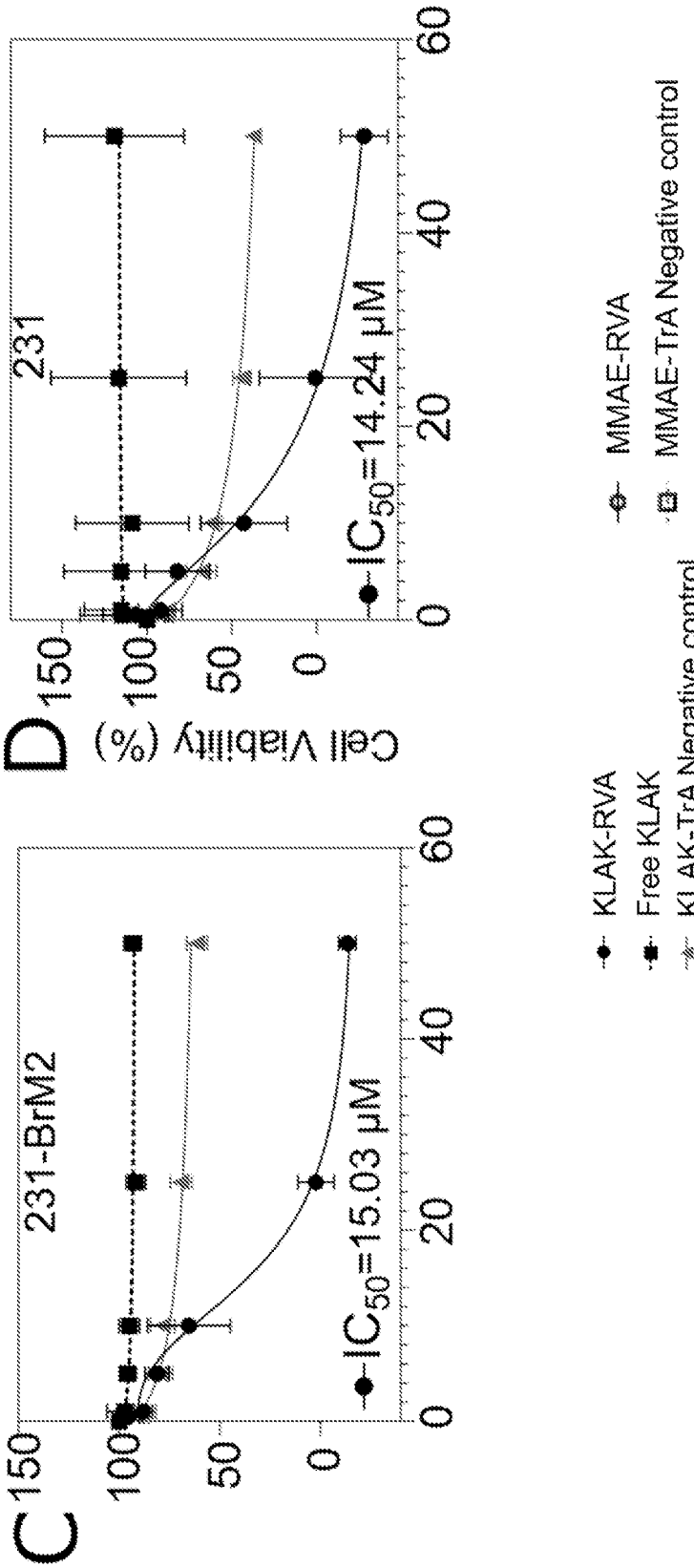

C
231-BrM2
Cell Viability (%)
150
100
50
0
0
20
40
60
IC50=15.03 µM
D
231
IC50=14.24 µM
KLAK-RVA
Free KLAK
KLAK-TrA Negative control
MMAE-RVA
MMAE-TrA Negative control

FIG. 6C-6D

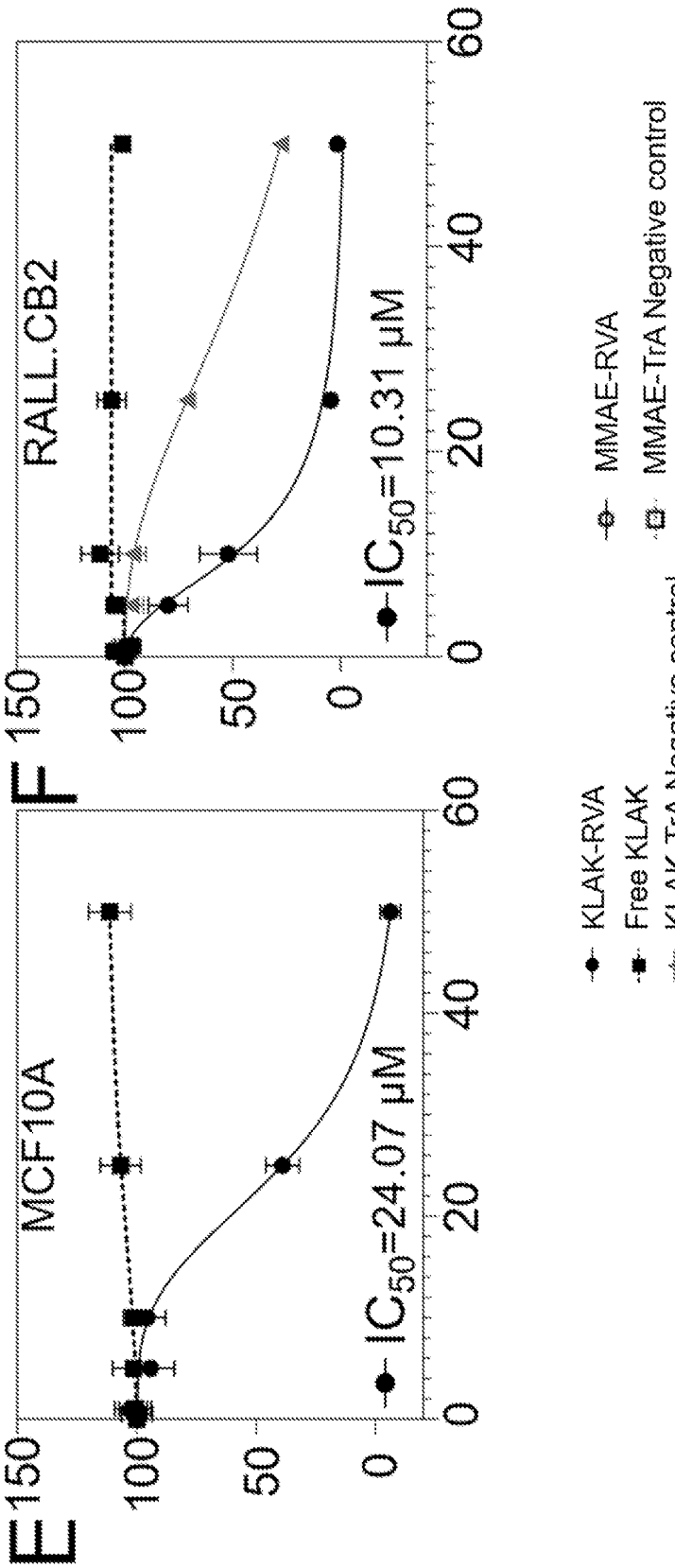

E
MCF10A
150
100
50
0
0
20
40
60
IC50=24.07 µM
F
RALL.CB2
150
100
50
0
0
20
40
60
IC50=10.31 µM
KLAK-RVA
Free KLAK
KLAK-TrA Negative control
MMAE-RVA
MMAE-TrA Negative control

FIG. 6E-6F

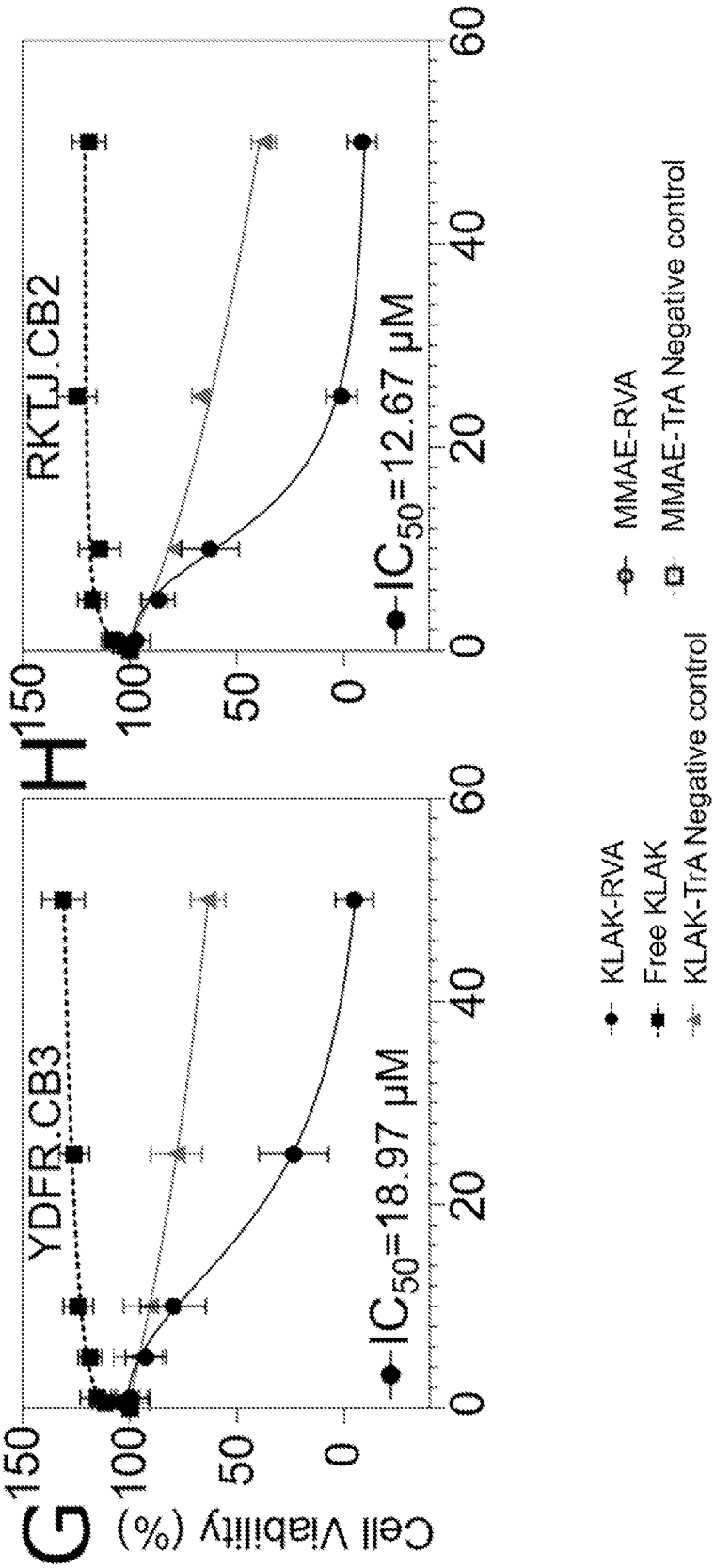

G
H
Cell Viability (%)
150
100
50
0
0
20
40
60
YDFR.CB3
RKTJ.CB2
IC50=18.97 μM
IC50=12.67 μM
KLAK-RVA
Free KLAK
KLAK-TrA Negative control
MMAE-RVA
MMAE-TrA Negative control

FIG. 6G-6H

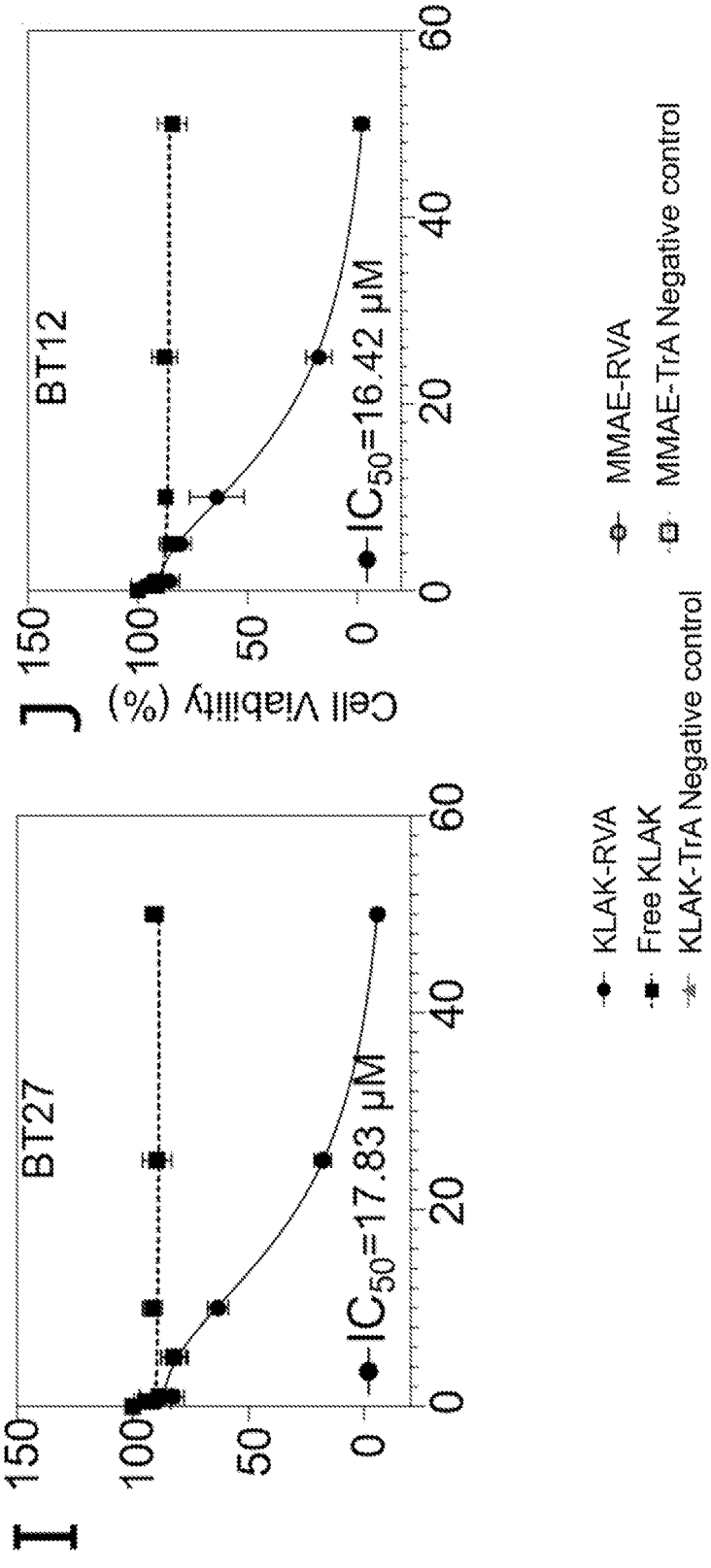


FIG. 6I-6J

FIG. 6I-6J

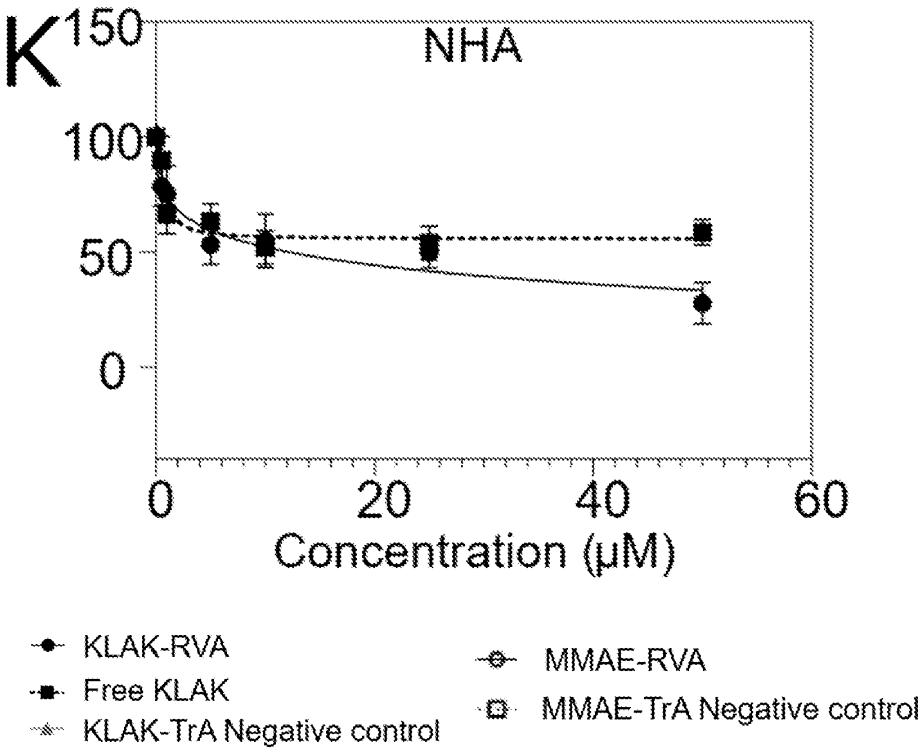

K
150
100
50
0
NHA
0
20
40
60
Concentration (μM)
KLAK-RVA
Free KLAK
KLAK-TrA Negative control
MMAE-RVA
MMAE-TrA Negative control

FIG. 6K

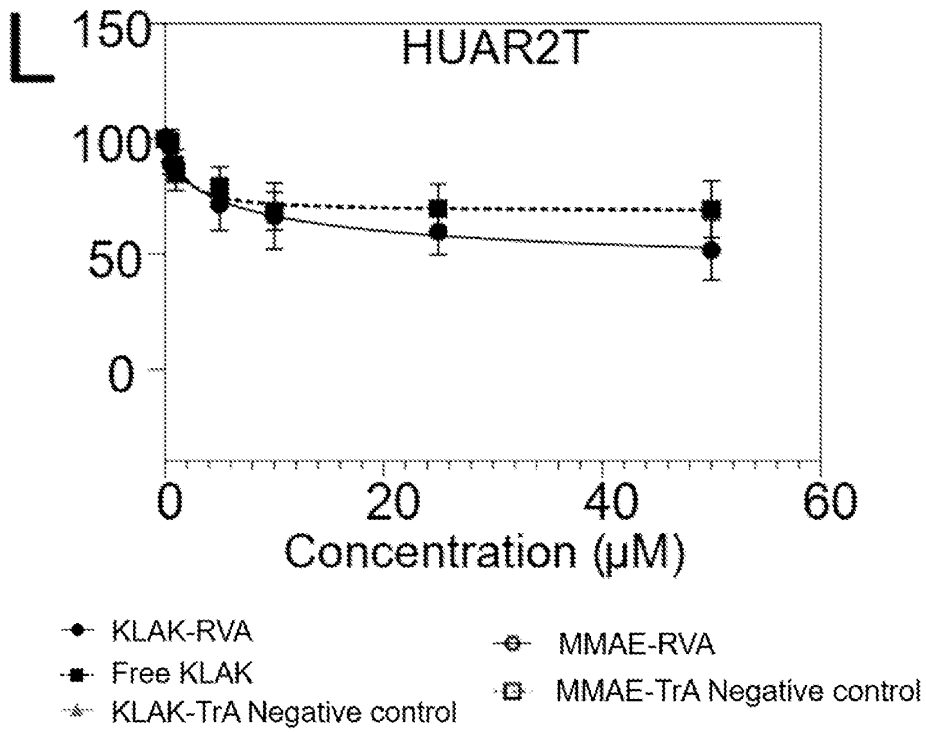

L
150
100
50
0
HUAR2T
0
20
40
60
Concentration (μM)
KLAK-RVA
Free KLAK
KLAK-TrA Negative control
MMAE-RVA
MMAE-TrA Negative control

FIG. 6L

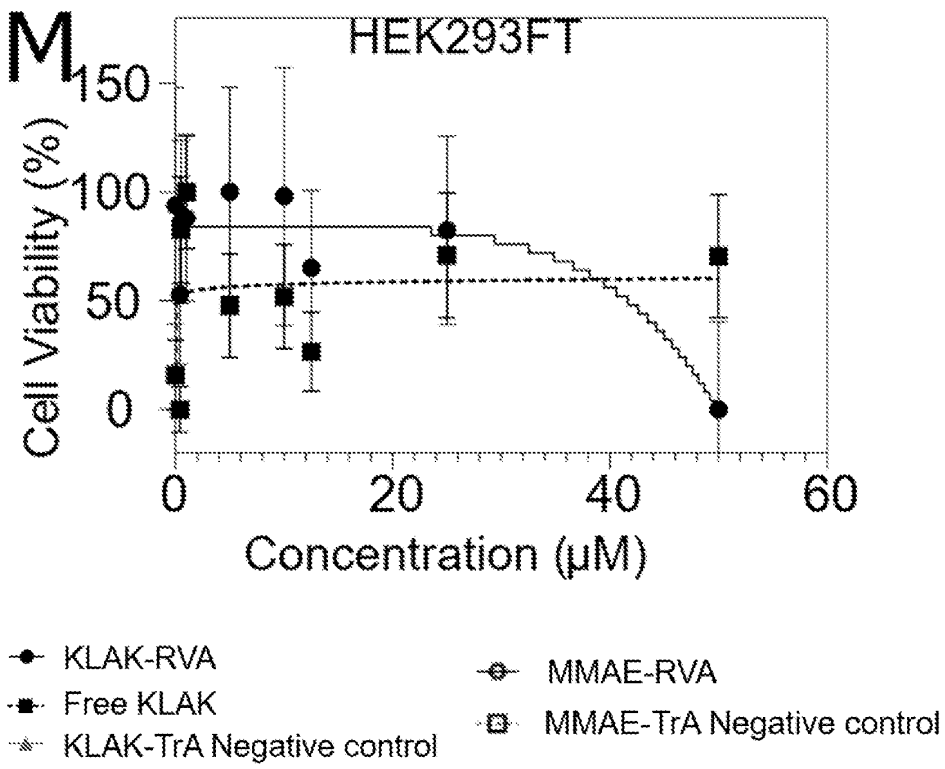

M
HEK293FT
150
100
50
0
Cell Viability (%)
0
20
40
60
Concentration (μM)
KLAK-RVA
Free KLAK
KLAK-TrA Negative control
MMAE-RVA
MMAE-TrA Negative control

FIG. 6M

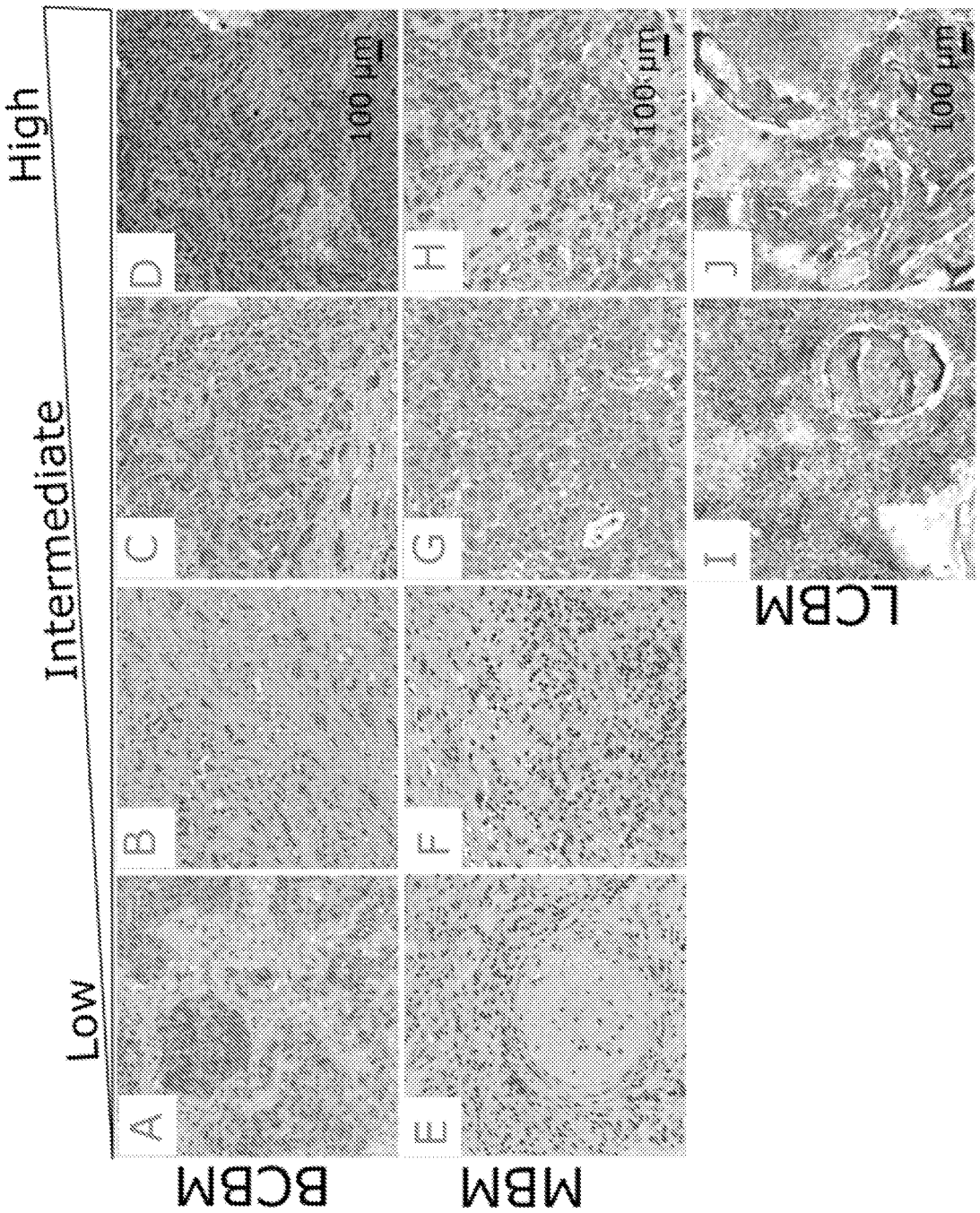

High
Intermediate
Low
BCBM
MBM
LCBM
A
B
C
D
E
F
G
H
I
J
100 µm
100 µm
100 µm

FIG. 7

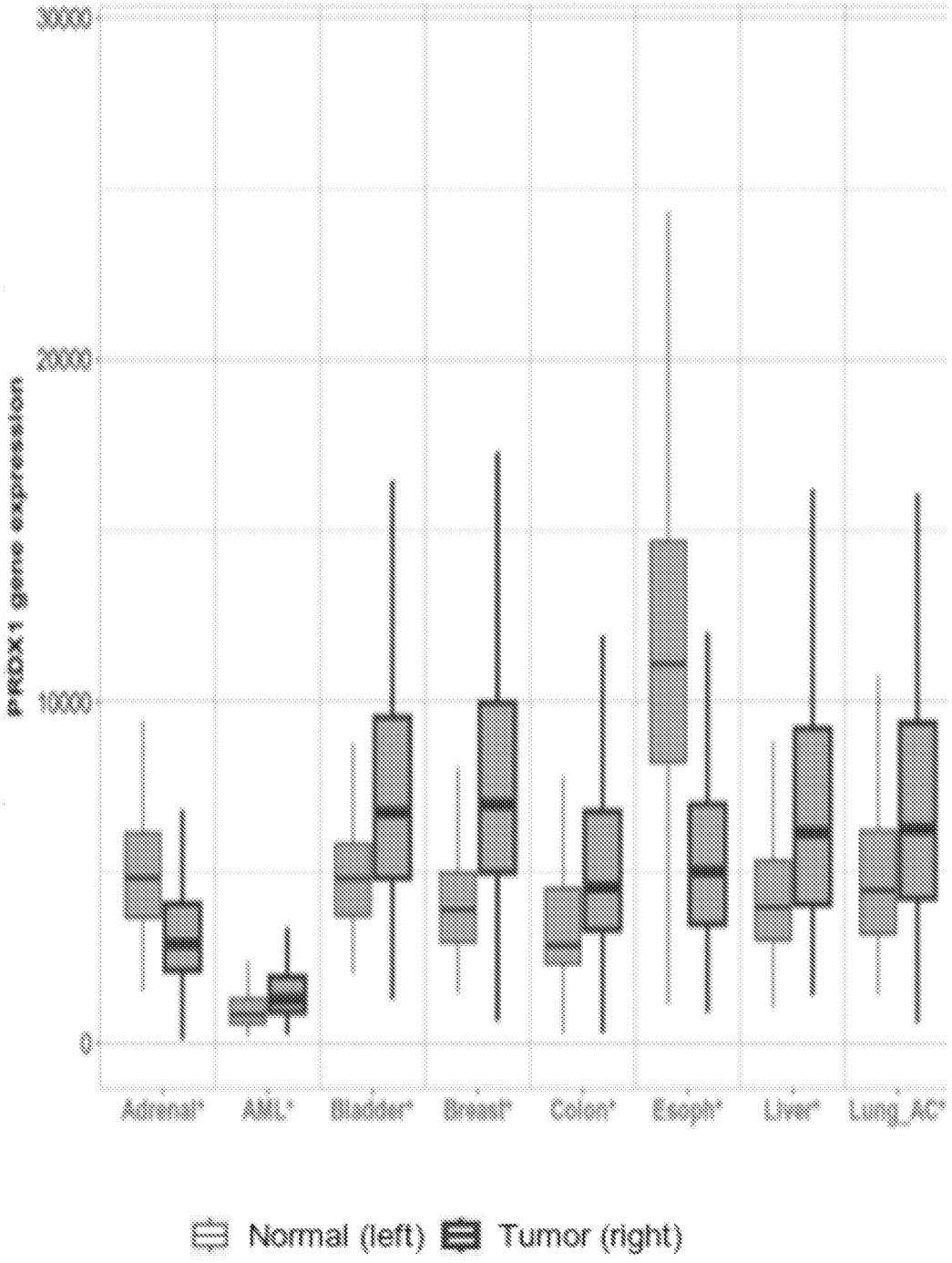

3000
2000
1000
0
PRDX1 gene expression
Adrenal*
AML*
Bladder*
Breast*
Colon*
Esoph*
Liver*
Lung_AC*
Normal (left)
Tumor (right)

FIG. 8

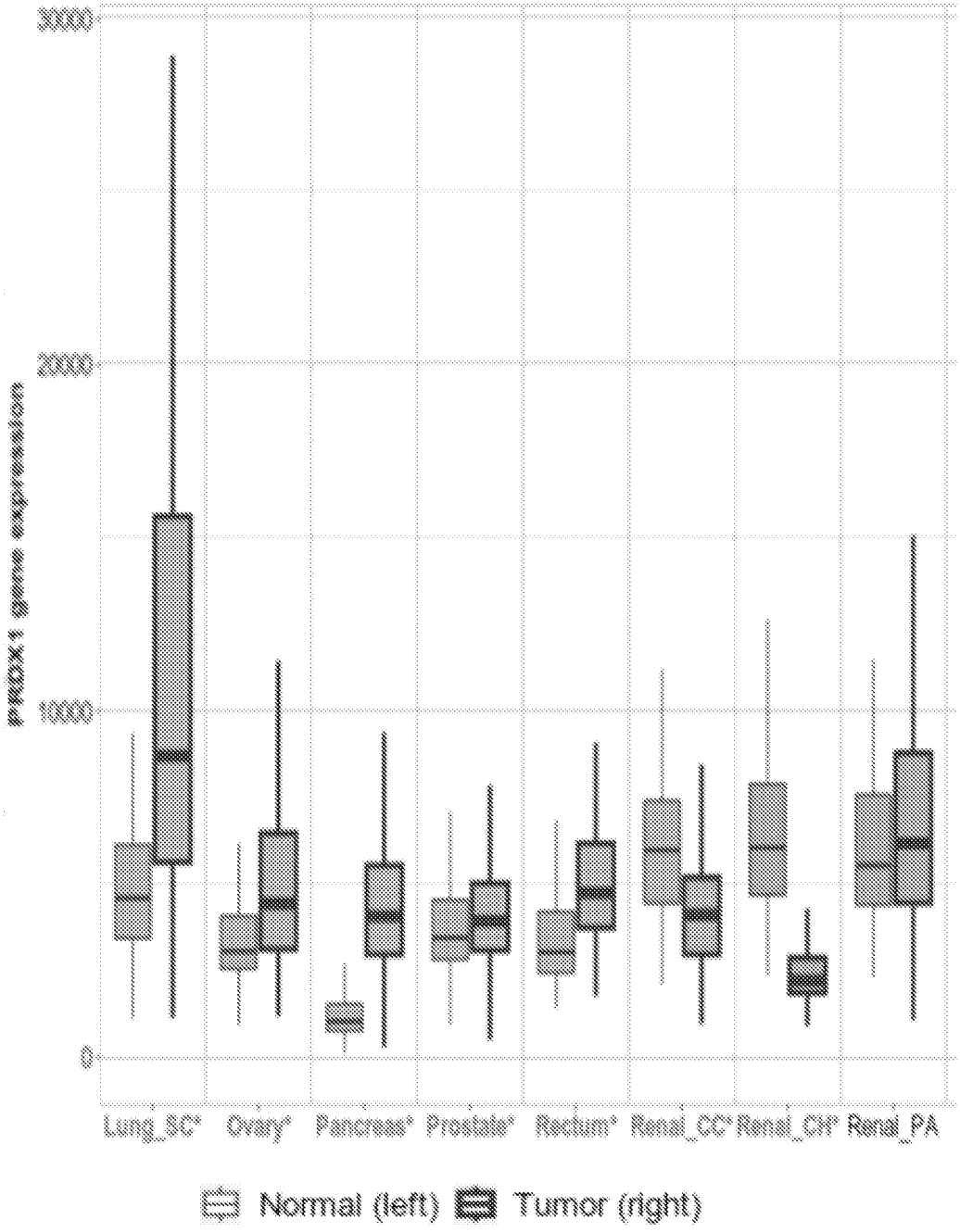


FIG. 8 (continued)

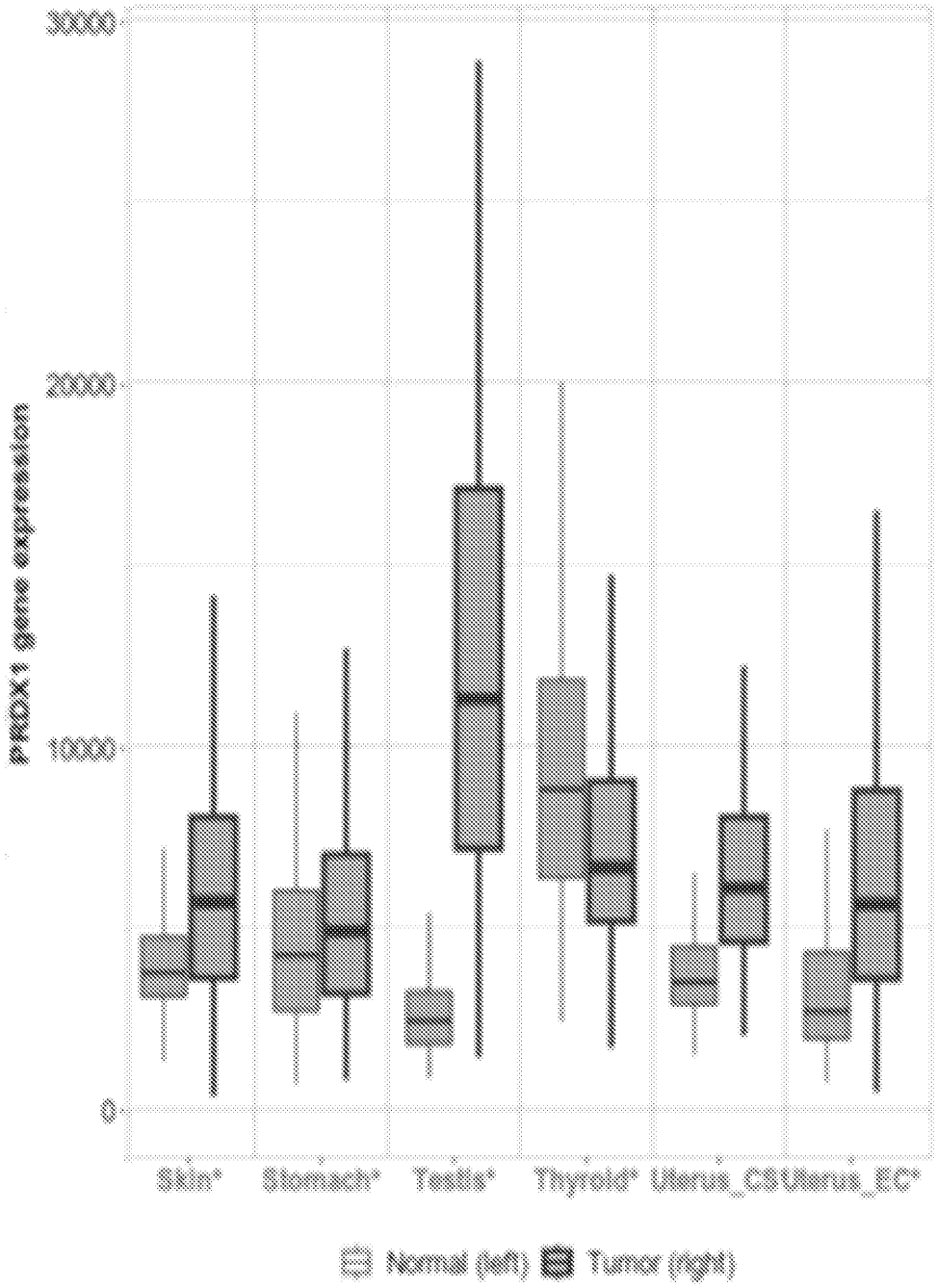


FIG. 8 (continued)

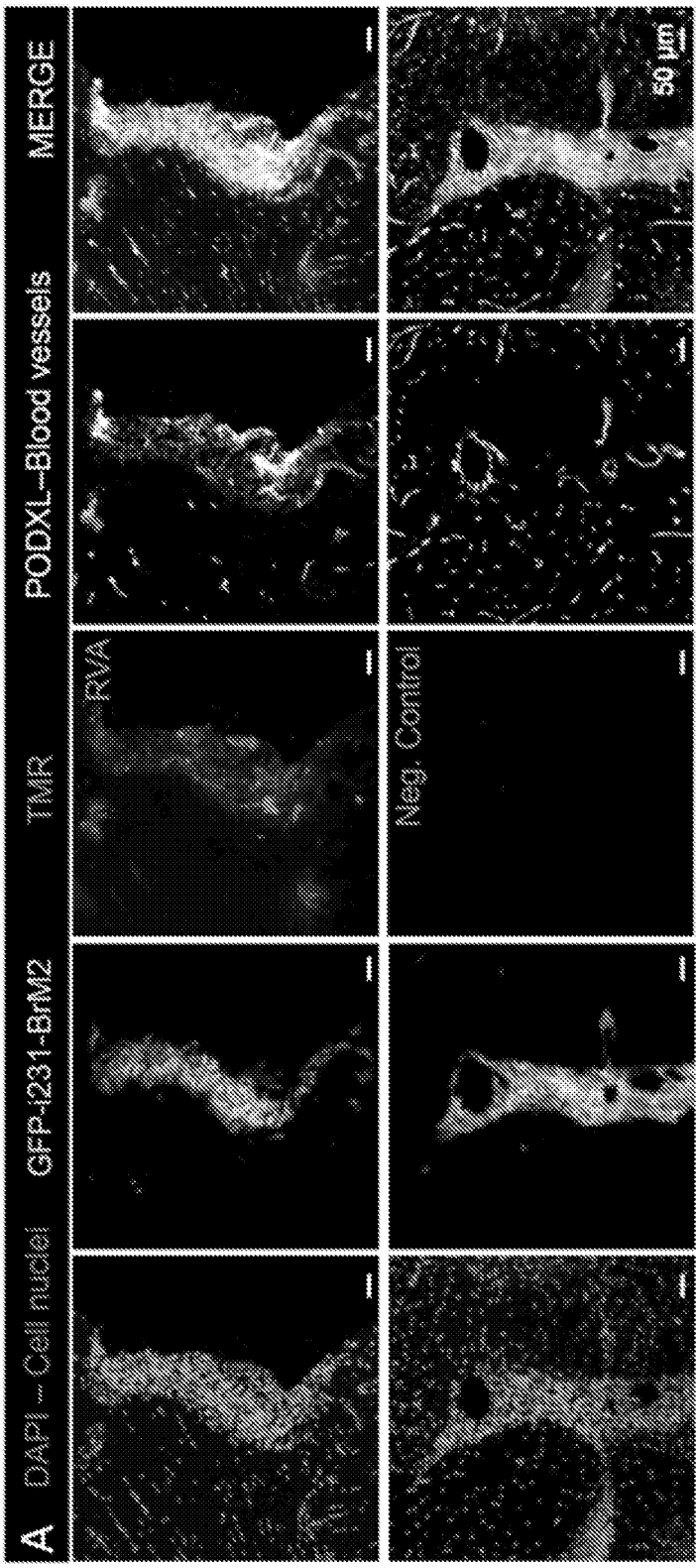

A
DAPI – Cell nuclei
GFP-i231-BrM2
TMR
PODXL–Blood vessels
MERGE
RVA
Neg. Control
50 µm

FIG. 9A

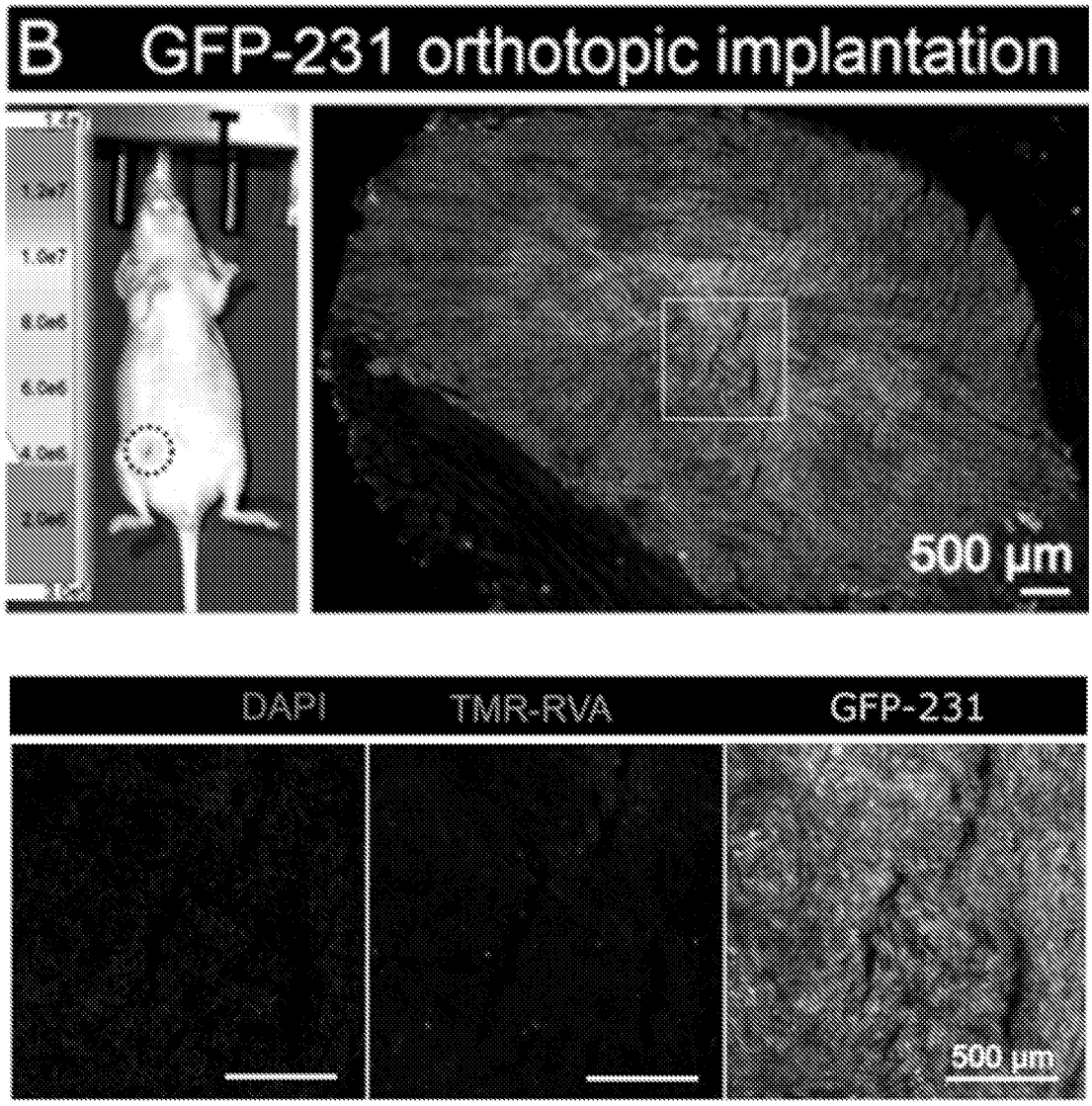
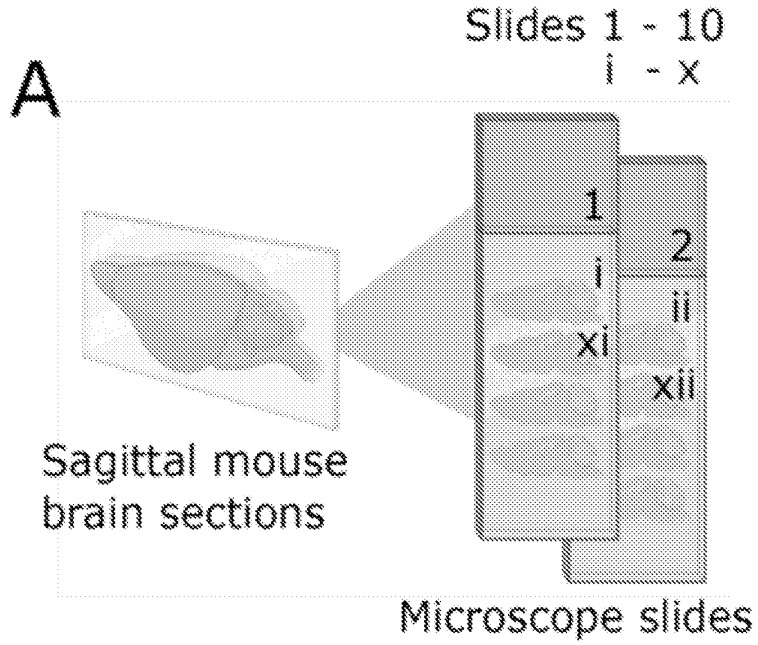

B GFP-231 orthotopic implantation
500 µm
DAPI
TMR-RVA
GFP-231
500 µm

FIG. 9B


A
Slides 1 - 10
i - x
1
2
i
ii
xi
xii
Sagittal mouse
brain sections
Microscope slides

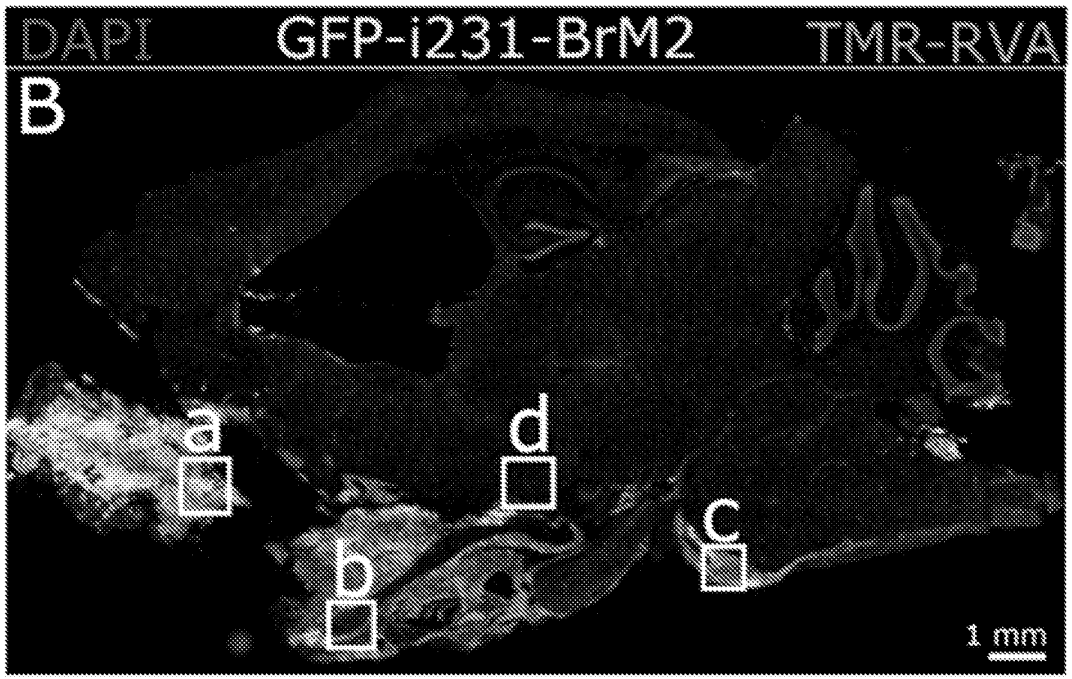

DAPI
GFP-i231-BrM2
TMR-RVA
B
a
b
c
d
1 mm

FIG. 10A-10B

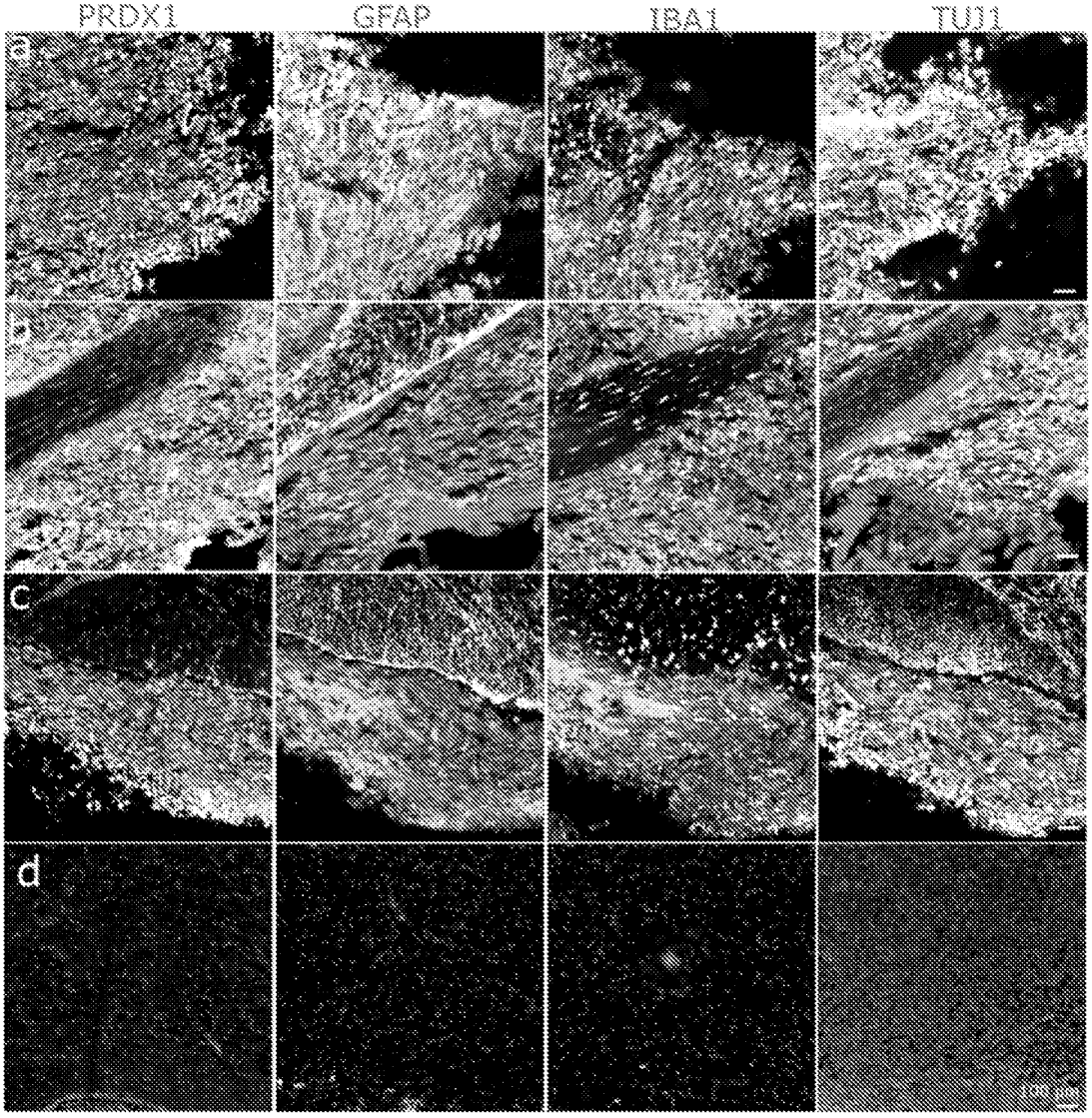


FIG. 10a-10d

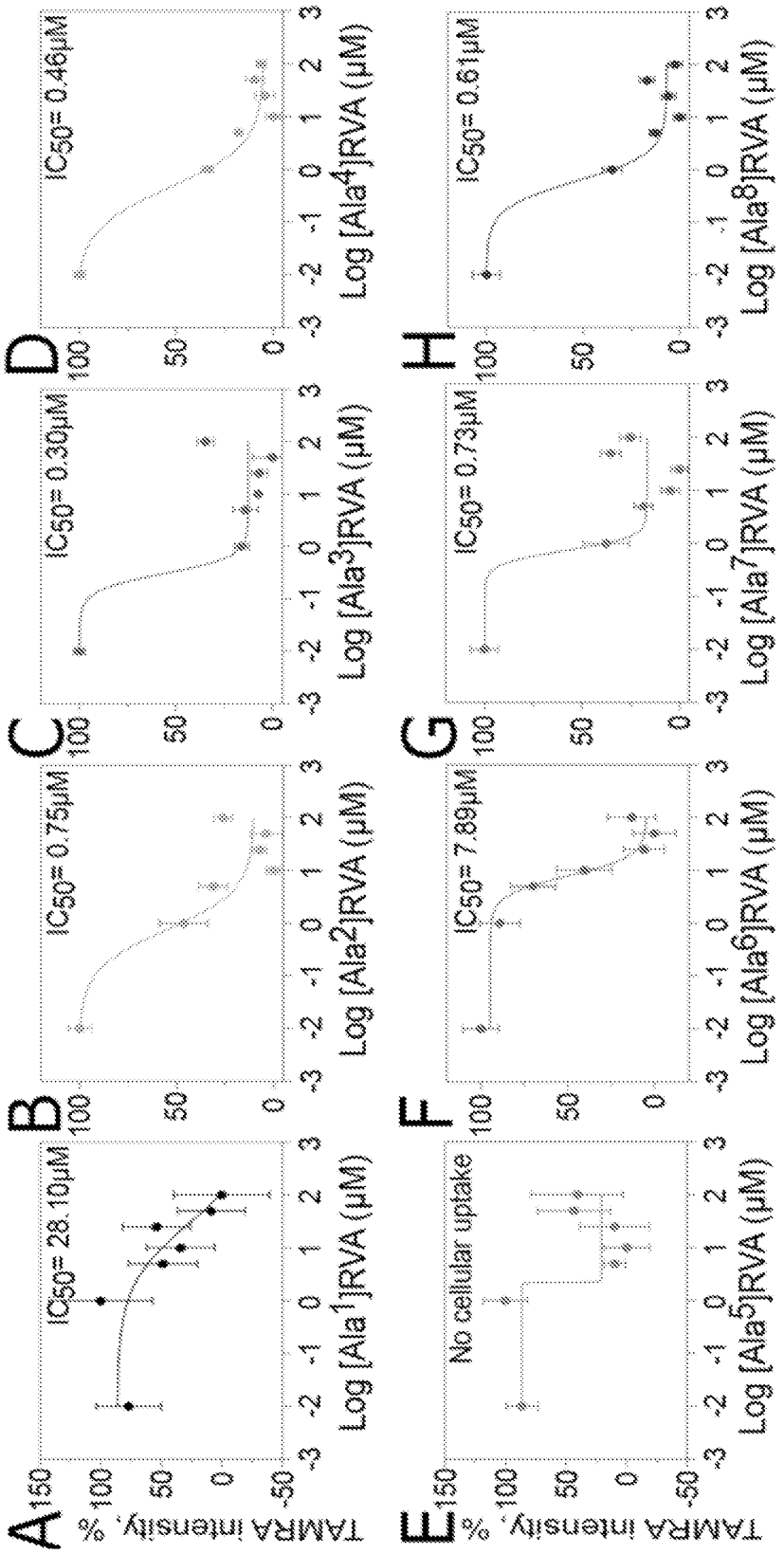

A
B
C
D
E
F
G
H
TAMRA intensity, %
IC50= 28.10μM
IC50= 0.75μM
IC50= 0.30μM
IC50= 0.46μM
No cellular uptake
IC50= 7.89μM
IC50= 0.73μM
IC50= 0.61μM
Log [Ala1]RVA (μM)
Log [Ala2]RVA (μM)
Log [Ala3]RVA (μM)
Log [Ala4]RVA (μM)
Log [Ala5]RVA (μM)
Log [Ala6]RVA (μM)
Log [Ala7]RVA (μM)
Log [Ala8]RVA (μM)

FIG. 11A-11H

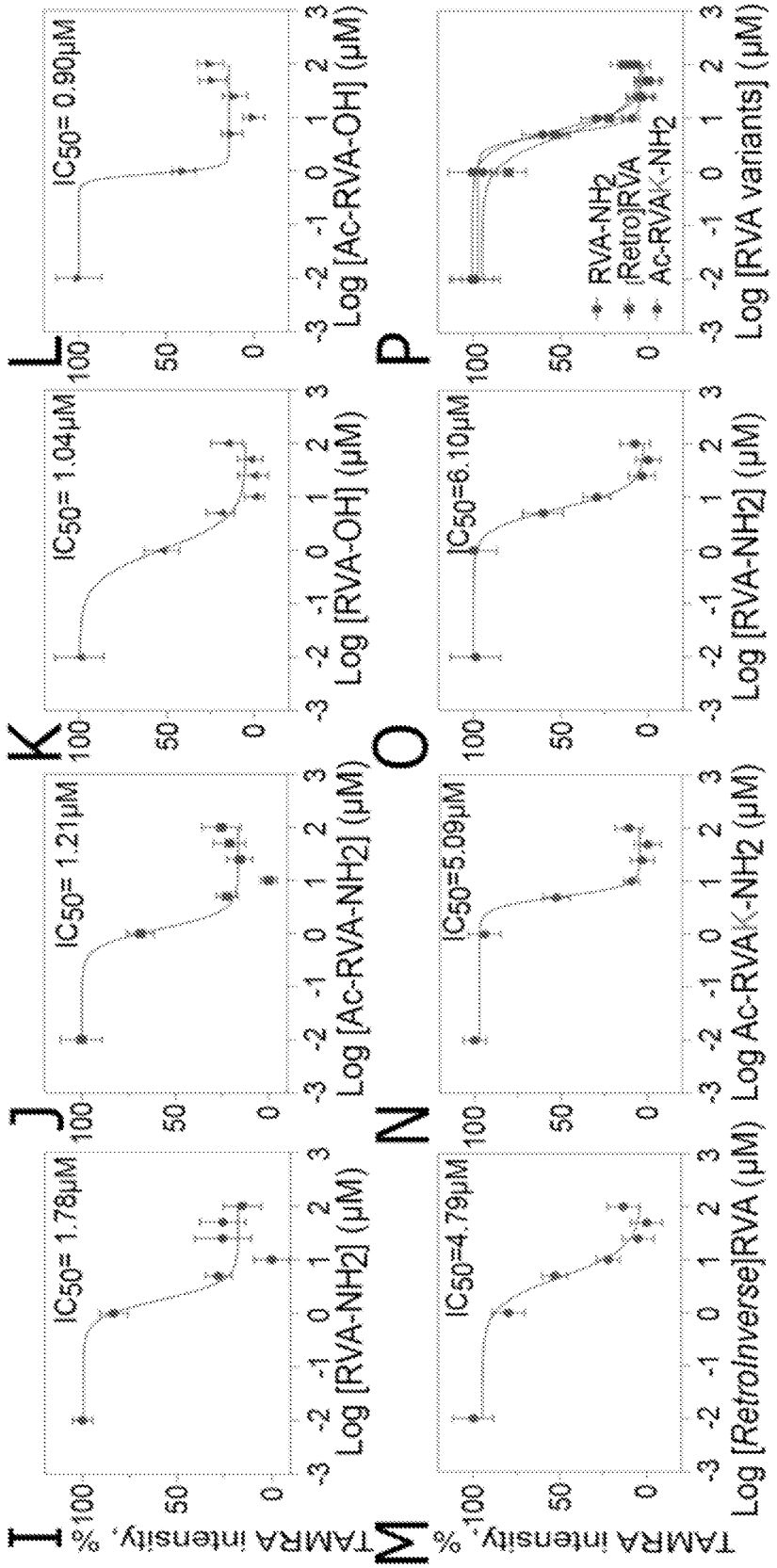

I
J
K
L
M
N
O
P
IC50= 1.78μM
IC50= 1.21μM
IC50= 1.04μM
IC50= 0.90μM
IC50=4.79μM
IC50=5.09μM
IC50=6.10μM
TAMRA intensity, %
Log [RVA-NH2] (μM)
Log [Ac-RVA-NH2] (μM)
Log [RVA-OH] (μM)
Log [Ac-RVA-OH] (μM)
Log [Retro/Inverse]RVA (μM)
Log Ac-RVA(K-NH2 (μM)
Log [RVA-NH2] (μM)
Log [RVA variants] (μM)
RVA-NH2
(Retro)RVA
Ac-RVA(K-NH2

FIG. 11I-11P

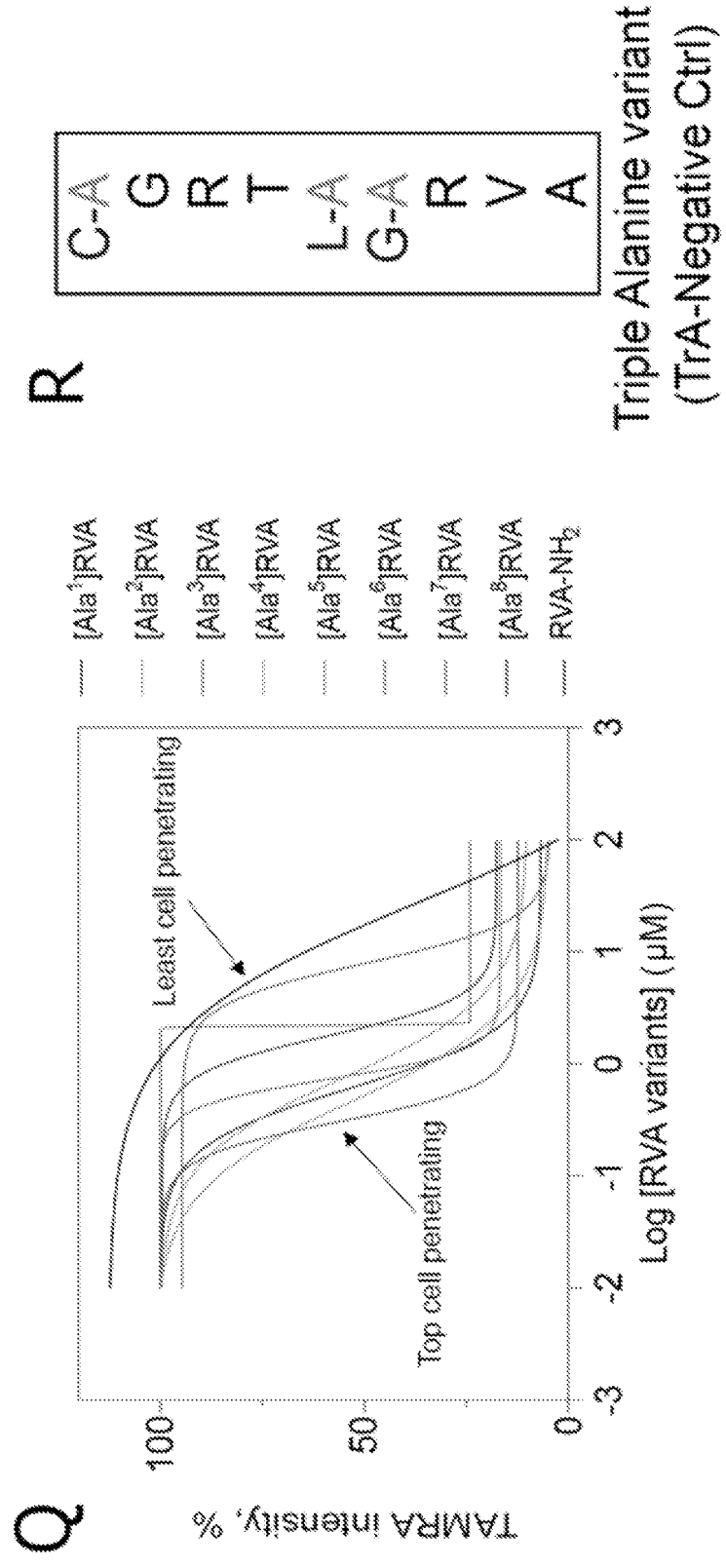

Q
TAMRA intensity, %
100
50
0
-3
-2
-1
0
1
2
3
Log [RVA variants] (μM)
Least cell penetrating
Top cell penetrating
[Ala1]RVA
[Ala2]RVA
[Ala3]RVA
[Ala4]RVA
[Ala5]RVA
[Ala6]RVA
[Ala7]RVA
[Ala8]RVA
RVA-NH2
R
C-A G R T L-A G-A R V A
Triple Alanine variant
(TrA-Negative Ctrl)

FIG. 11Q-11R

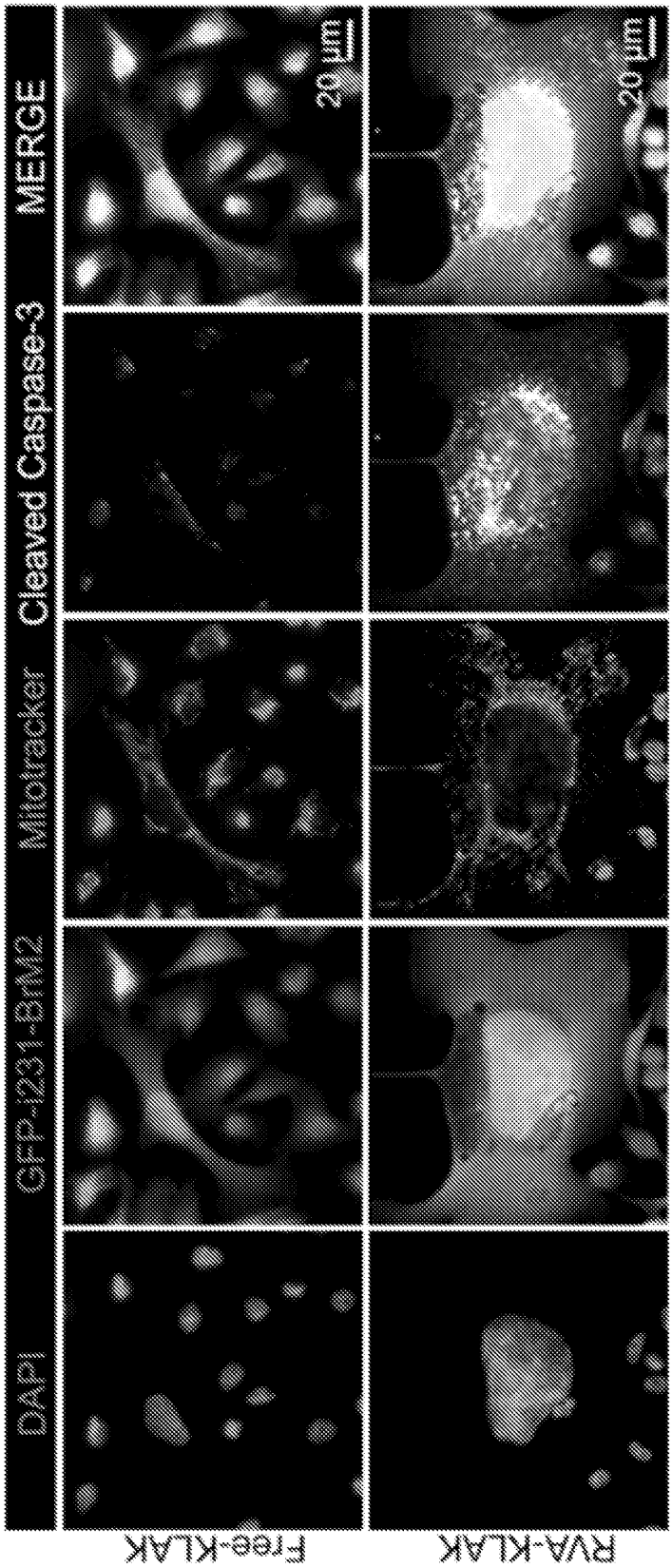

DAPI
GFP-i231-BrM2
Mitotracker
Cleaved Caspase-3
MERGE
20 µm
20 µm
Free-KLAK
RVA-KLAK

FIG. 12

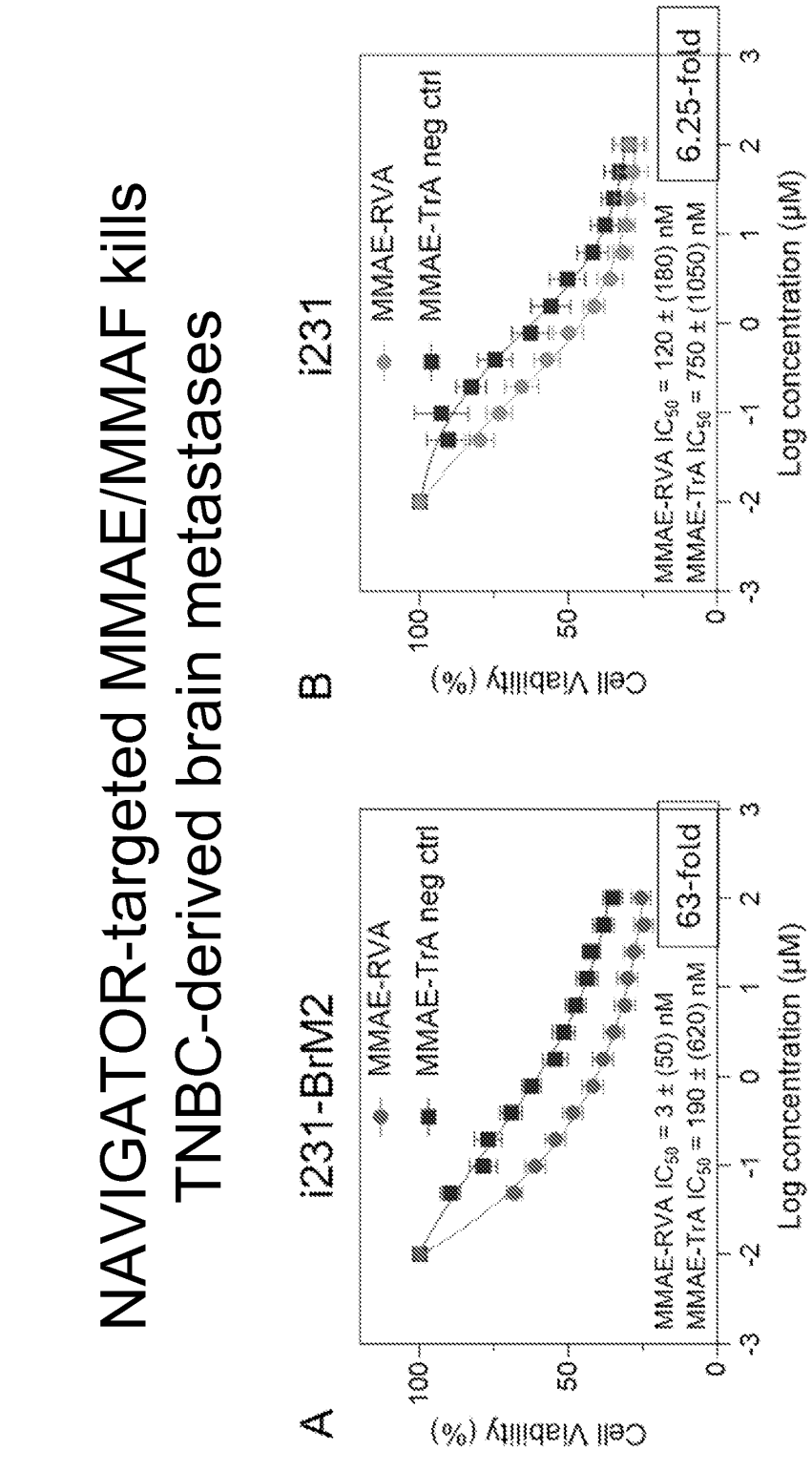

NAVIGATOR-targeted MMAE/MMAF kills
TNBC-derived brain metastases
A
i231-BrM2
MMAE-RVA
MMAE-TrA neg ctrl
MMAE-RVA IC50 = 3 ± (50) nM
MMAE-TrA IC50 = 190 ± (620) nM
63-fold
Cell Viability (%)
Log concentration (µM)
B
i231
MMAE-RVA
MMAE-TrA neg ctrl
MMAE-RVA IC50 = 120 ± (180) nM
MMAE-TrA IC50 = 750 ± (1050) nM
6.25-fold
Cell Viability (%)
Log concentration (µM)

FIG. 13A

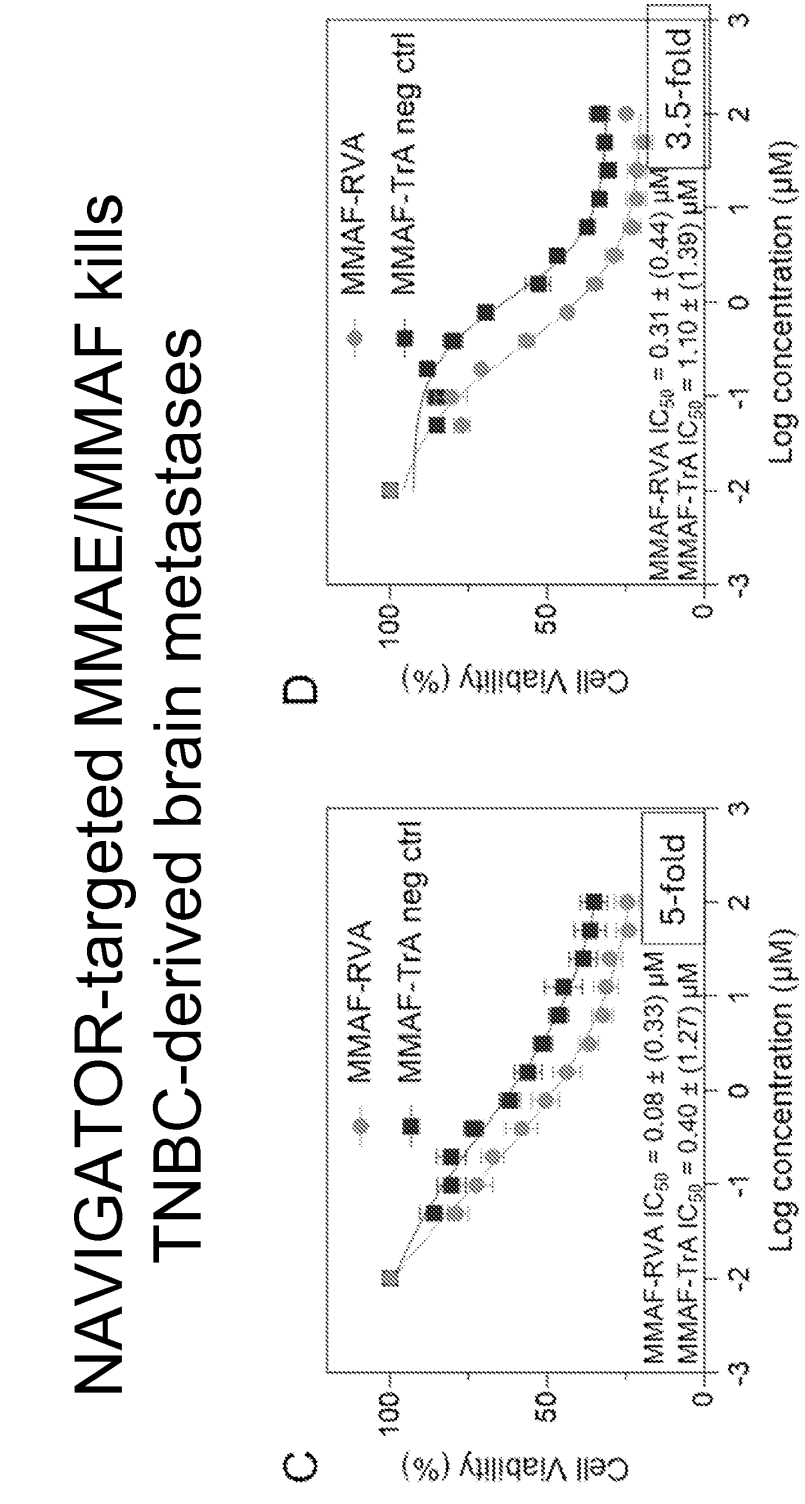

NAVIGATOR-targeted MMAE/MMAF kills
TNBC-derived brain metastases
C
D
Cell Viability (%)
Log concentration (μM)
MMAF-RVA
MMAF-TrA neg ctrl
MMAF-RVA IC50 = 0.08 ± (0.33) μM
MMAF-TrA IC50 = 0.40 ± (1.27) μM
5-fold
MMAF-RVA IC50 = 0.31 ± (0.44) μM
MMAF-TrA IC50 = 1.10 ± (1.39) μM
3.5-fold

FIG. 13B

FIG. 14A

NAVIGATOR-targeted KLAK inhibits brain tumor progression

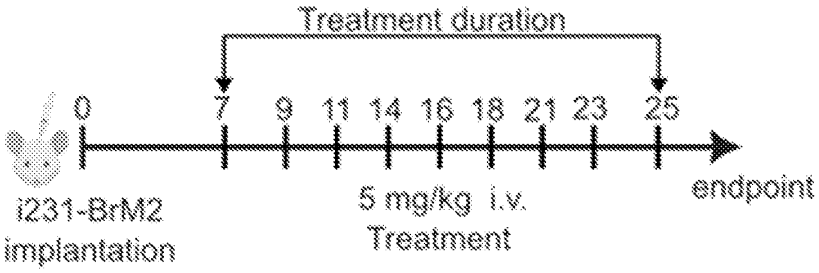


FIG. 14B

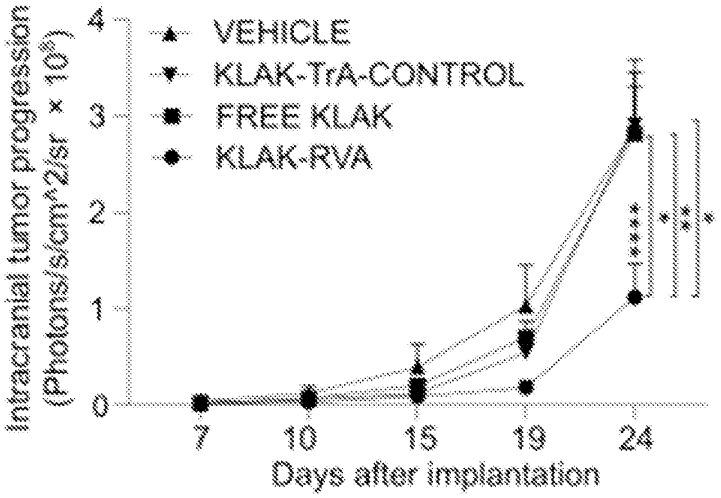


FIG. 14C, 14D

NAVIGATOR-targeted KLAK inhibits brain tumor progression

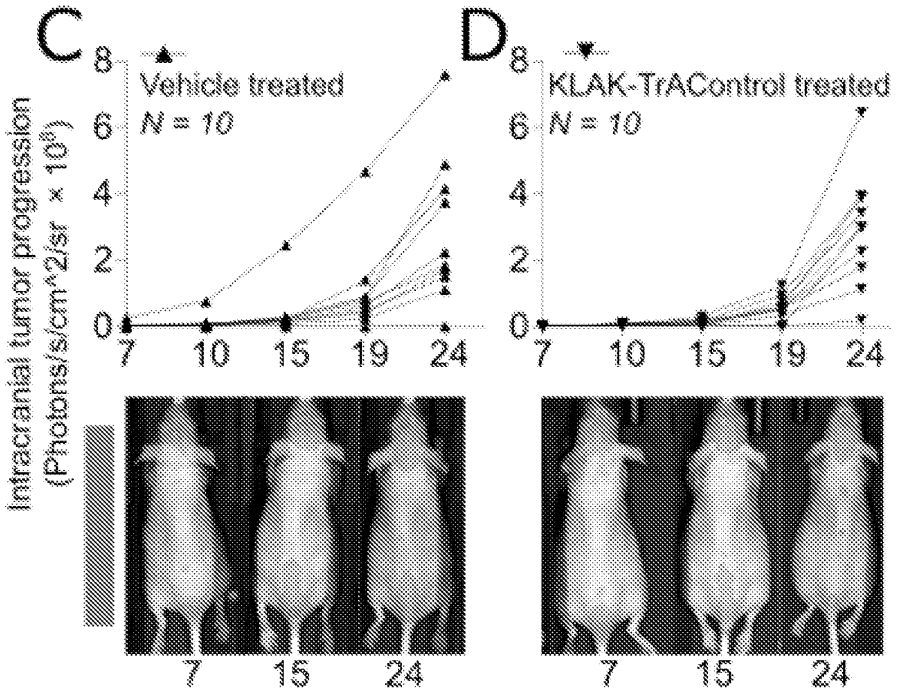


FIG. 14E, 14F

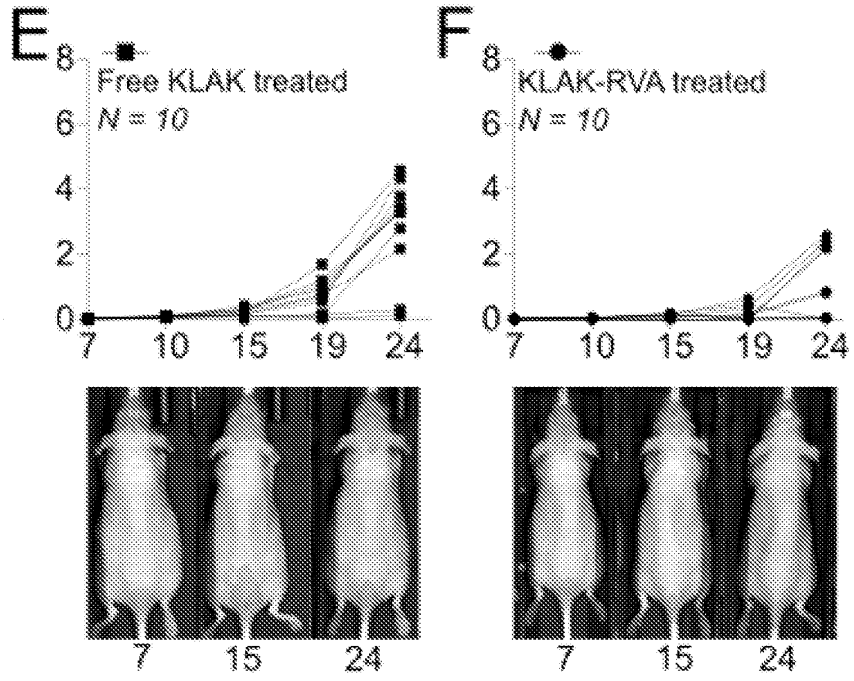
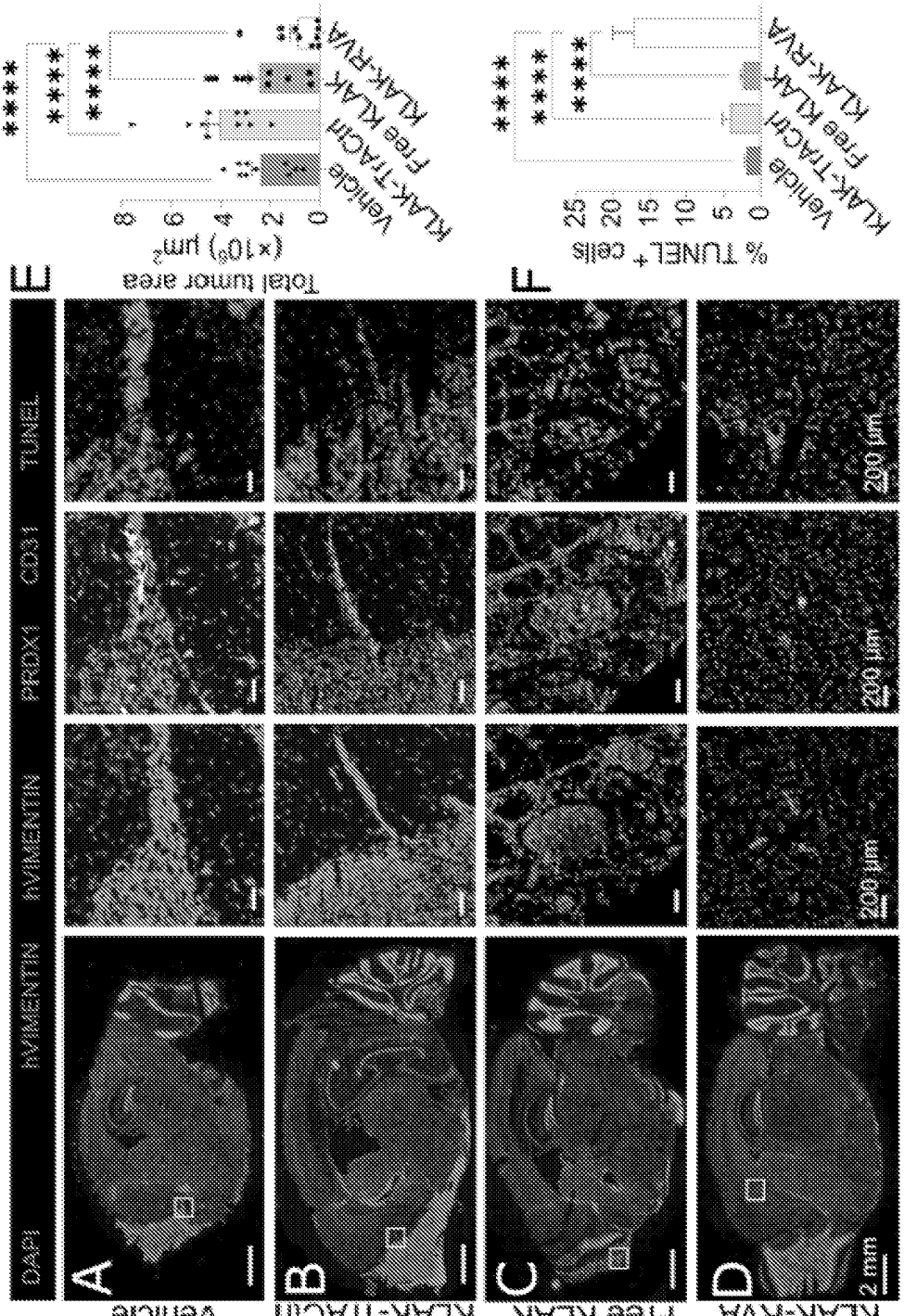


FIG. 15 NAVIGATOR-targeted KLAK show anti-tumor effect



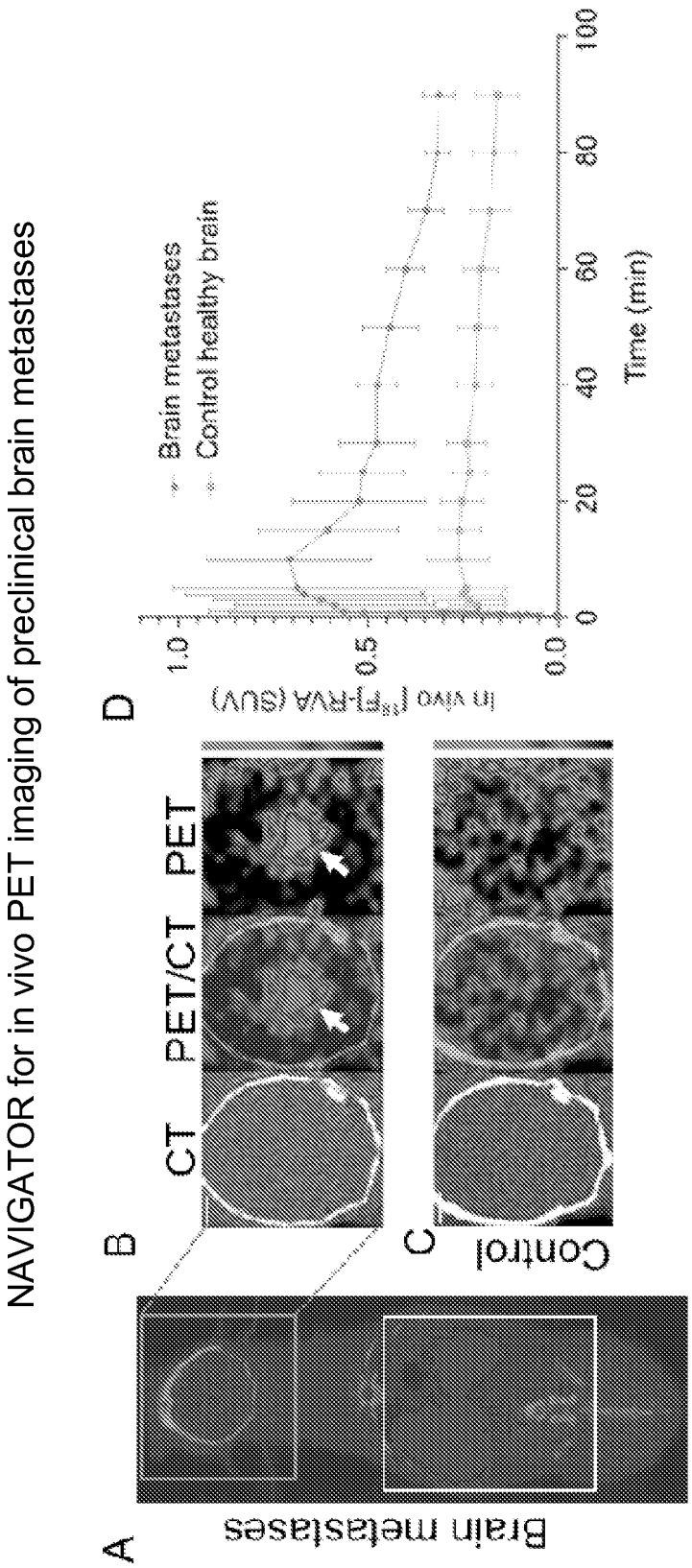

NAVIGATOR for in vivo PET imaging of preclinical brain metastases
A
Brain metastases
B
CT PET/CT PET
C
Control
D
In vivo [18F]-RVA (SUV)
1.0
0.5
0.0
0
20
40
60
80
100
Time (min)
Brain metastases
Control healthy brain

FIG. 16

FIG. 17A

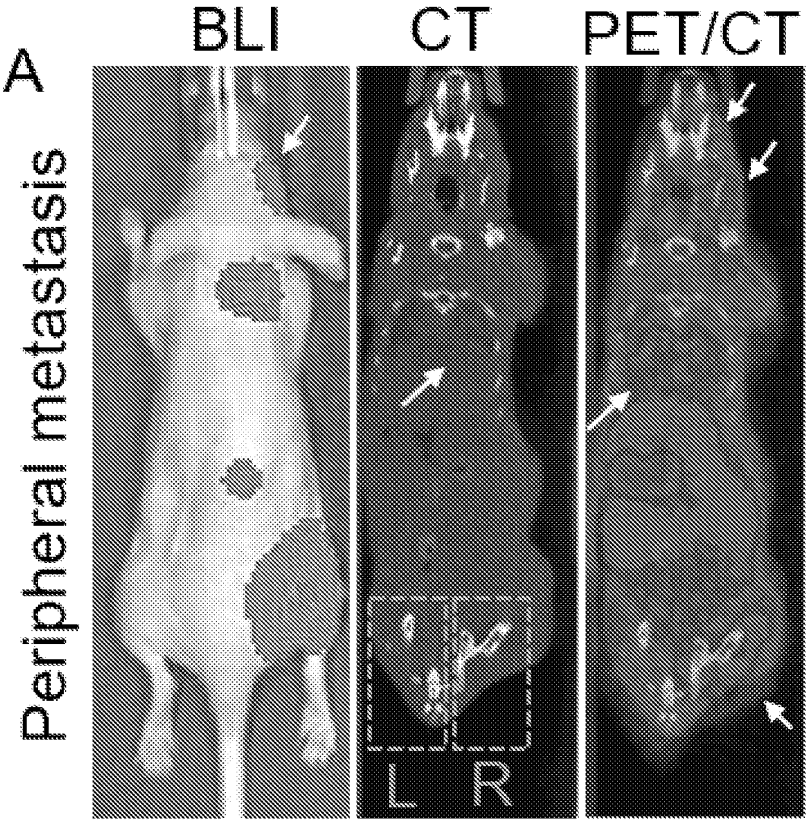


FIG. 17B

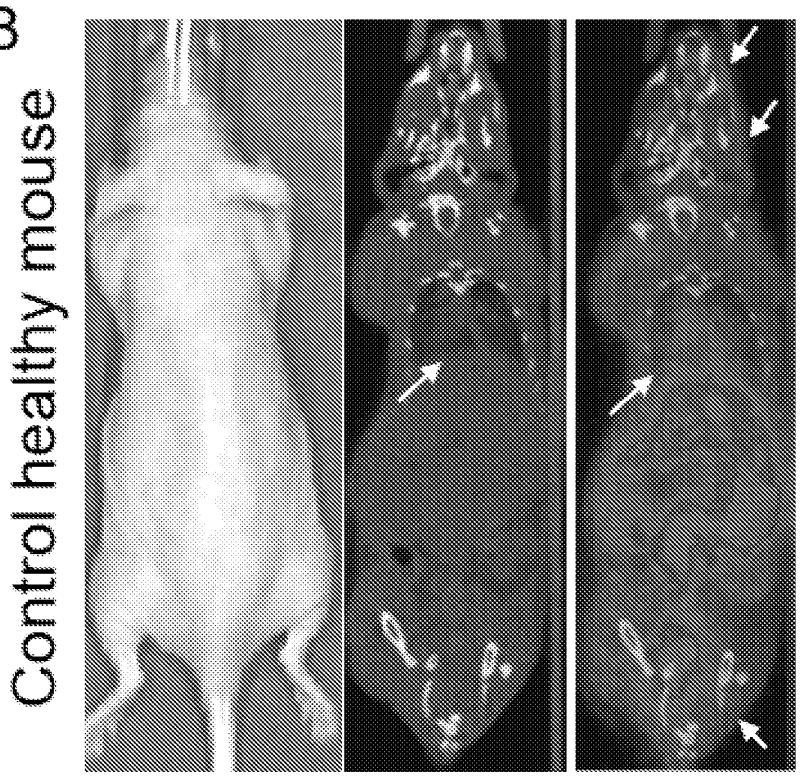
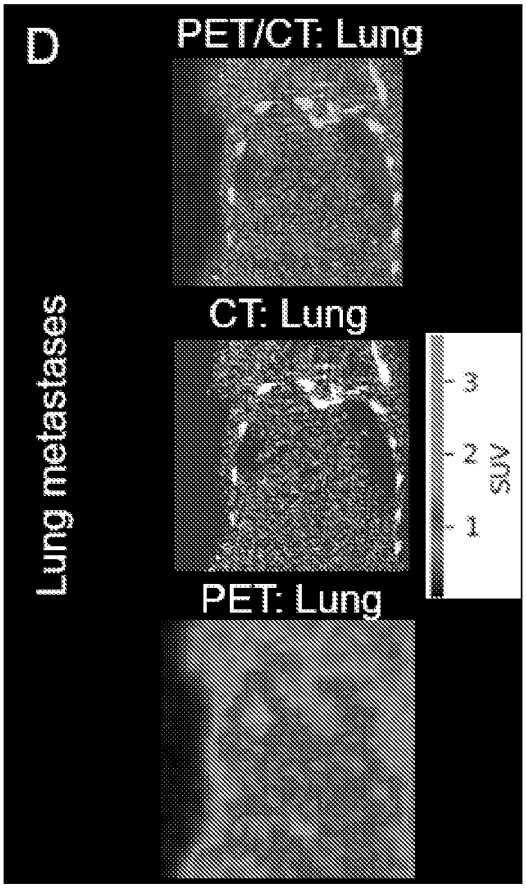


FIG. 17C



FIG. 17D



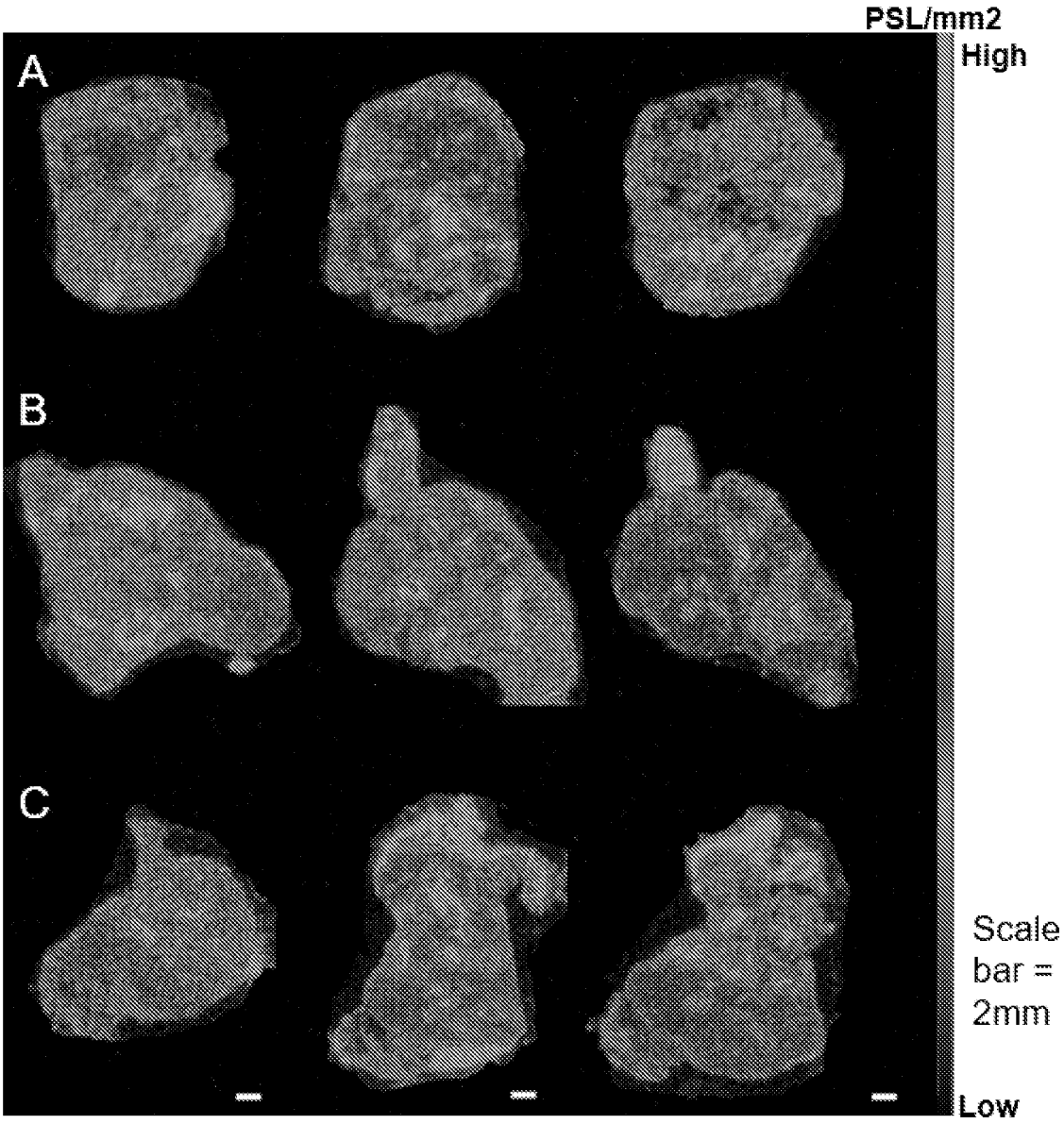


FIG. 18

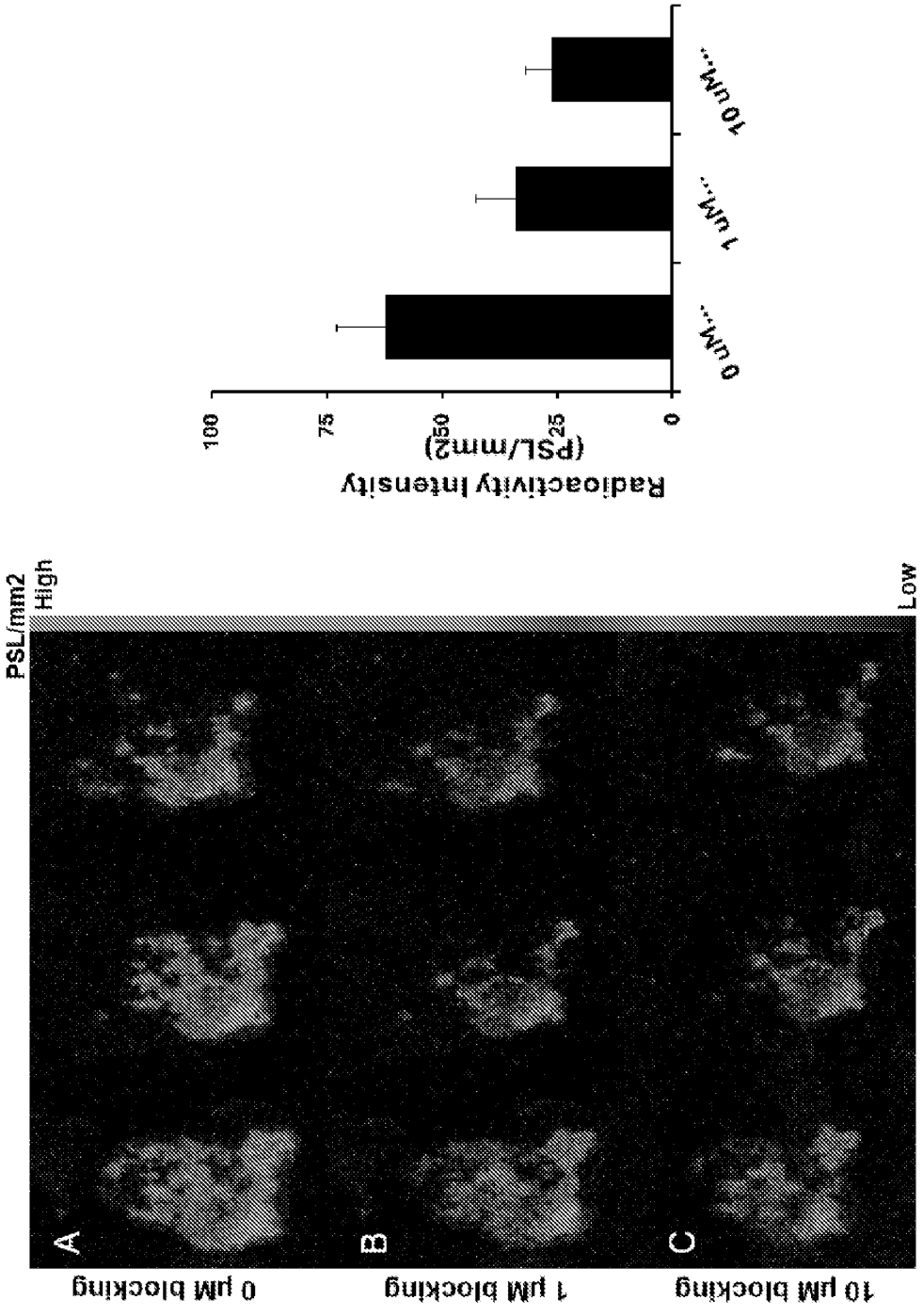

Radioactivity Intensity
(PSL/mm2)
100
75
50
25
0
0 uM...
1 uM...
10 uM...
PSL/mm2
High
Low
A
B
C
0 µM blocking
1 µM blocking
10 µM blocking

FIG. 19

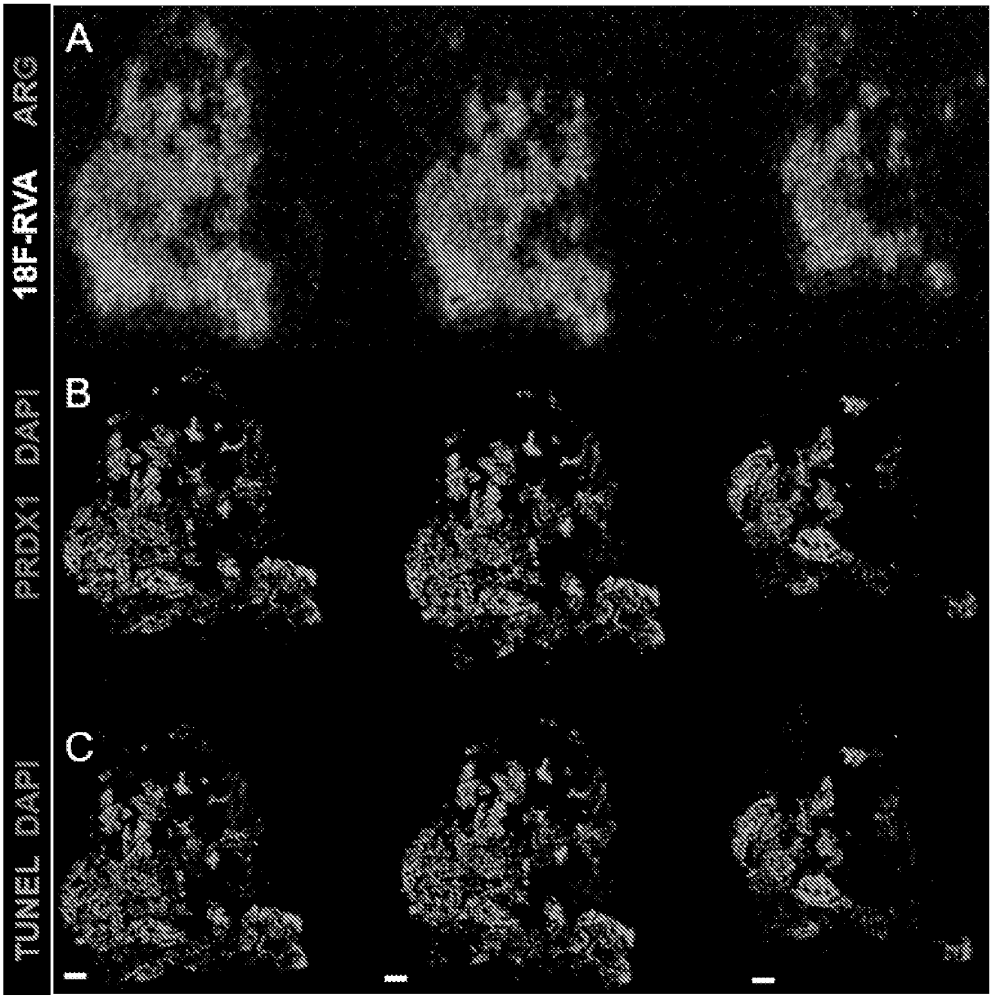


FIG. 20