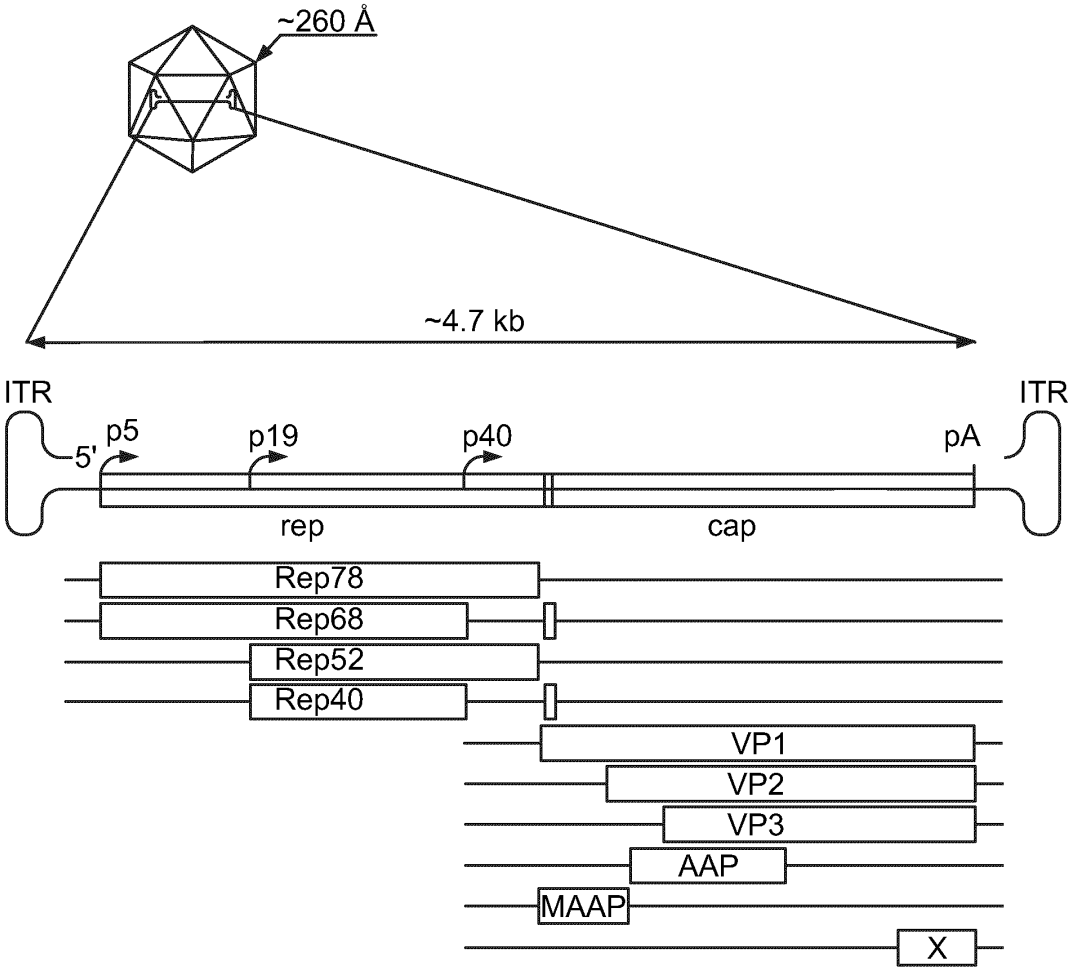

~260 Å
~4.7 kb
ITR
ITR
5'
p5
p19
p40
pA
rep
cap
Rep78
Rep68
Rep52
Rep40
VP1
VP2
VP3
AAP
MAAP
X

FIG. 1

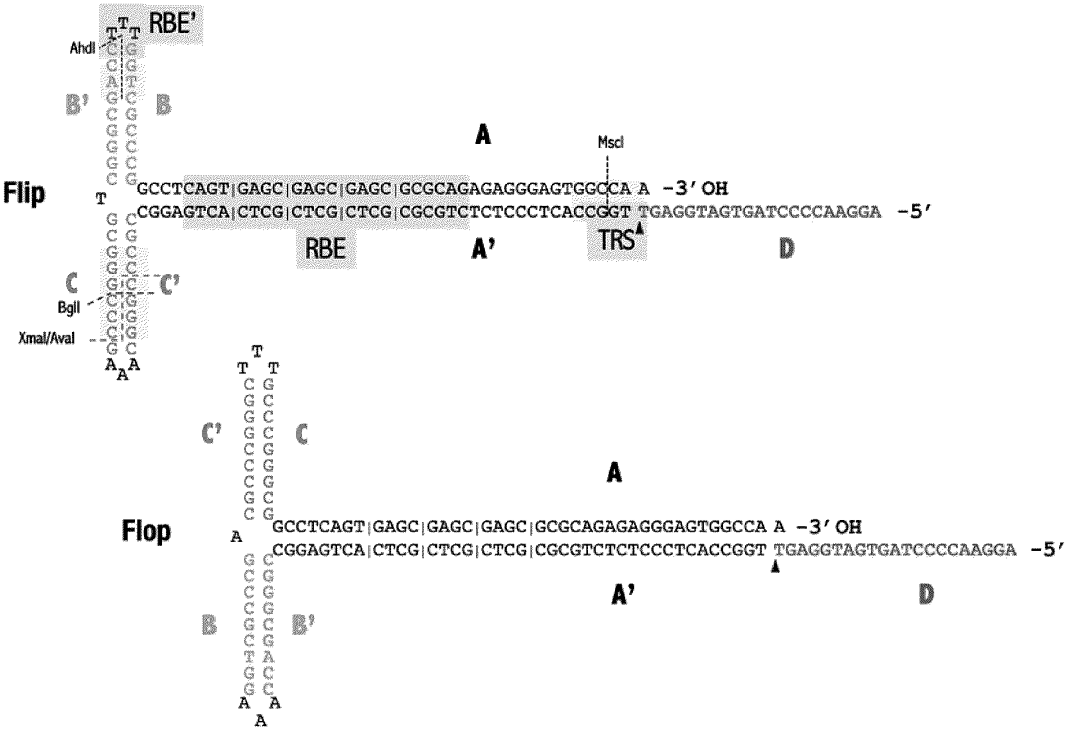

RBE'
AhdI
B'
B
A
MscI
Flip
GCCTCAGT|GAGC|GAGC|GAGC|GCGCAGAGAGGGAGTGGCCA A -3′OH
CGGAGTCA|CTCG|CTCG|CTCG|CGCGTCTCTCCCTCACCGGT TGAGGTAGTGATCCCCAAGGA -5′
RBE
A'
TRS
D
C
C'
BglI
XmaI/AvaI
C'
C
A
Flop
GCCTCAGT|GAGC|GAGC|GAGC|GCGCAGAGAGGGAGTGGCCA A -3′OH
CGGAGTCA|CTCG|CTCG|CTCG|CGCGTCTCTCCCTCACCGGT TGAGGTAGTGATCCCCAAGGA -5′
A'
D
B
B'

FIG. 2

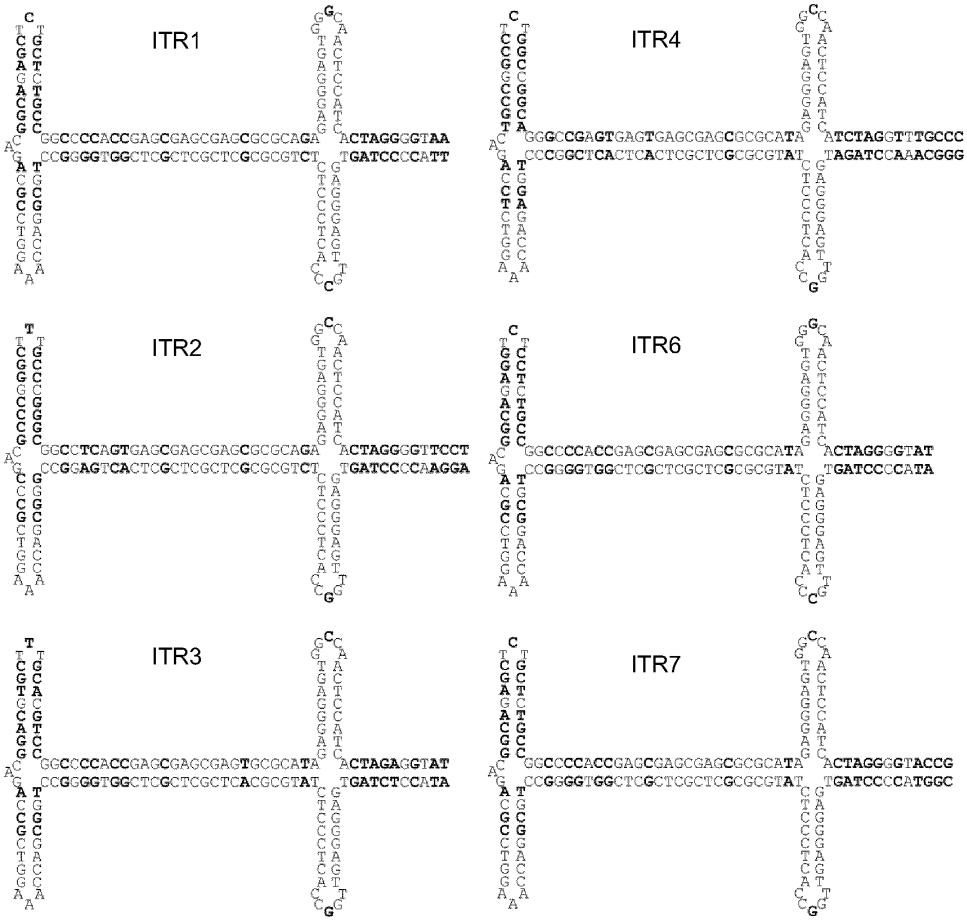


FIG. 3

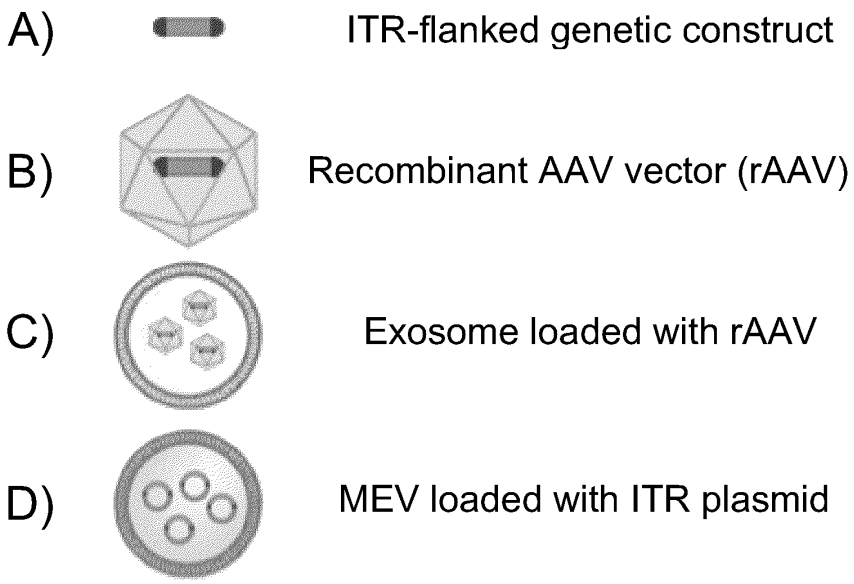

A)
ITR-flanked genetic construct
B)
Recombinant AAV vector (rAAV)
C)
Exosome loaded with rAAV
D)
MEV loaded with ITR plasmid

FIG. 4

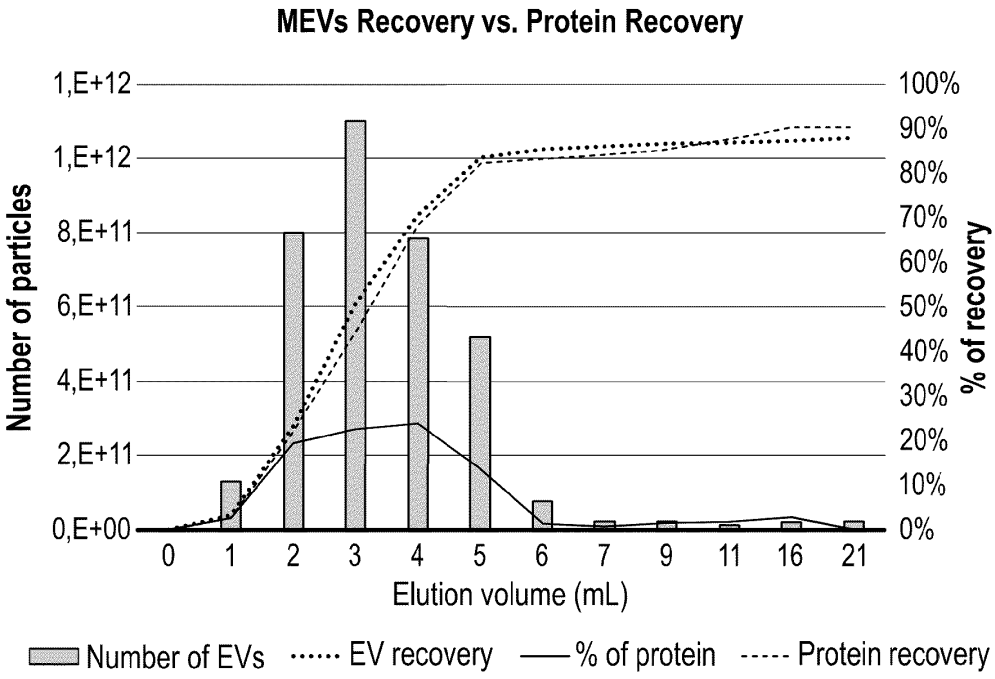

MEVs Recovery vs. Protein Recovery
Number of particles
1,E+12
1,E+12
8,E+11
6,E+11
4,E+11
2,E+11
0,E+00
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
% of recovery
0 1 2 3 4 5 6 7 9 11 16 21
Elution volume (mL)
Number of EVs
EV recovery
% of protein
Protein recovery

FIG. 5

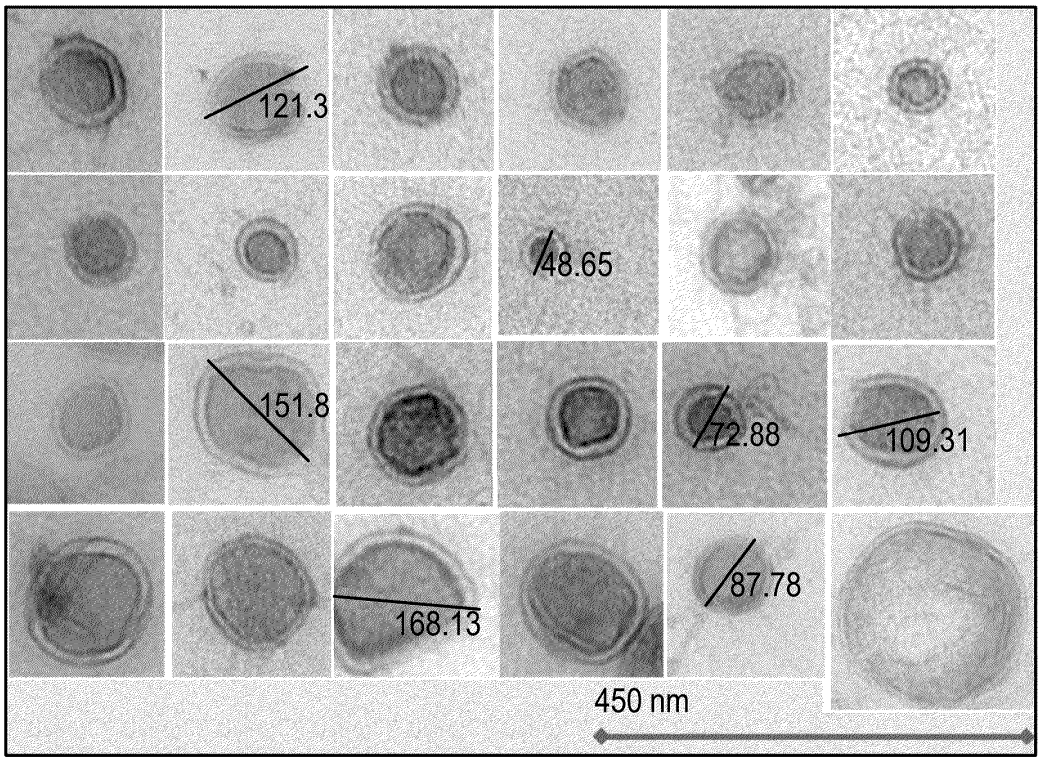

121.3
48.65
151.8
72.88
109.31
87.78
168.13
450 nm
* all measurements in nm

FIG. 6

FIG. 7A

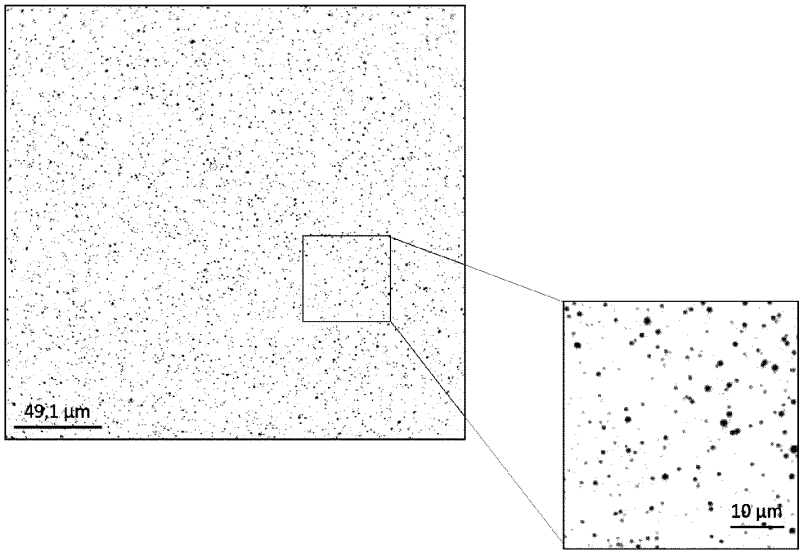


FIG. 7B

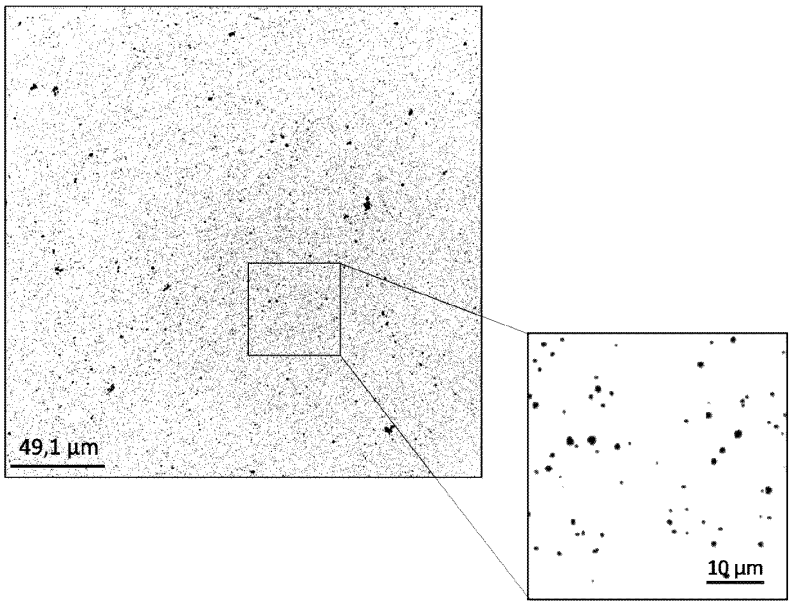


FIG. 8B



FIG. 8C

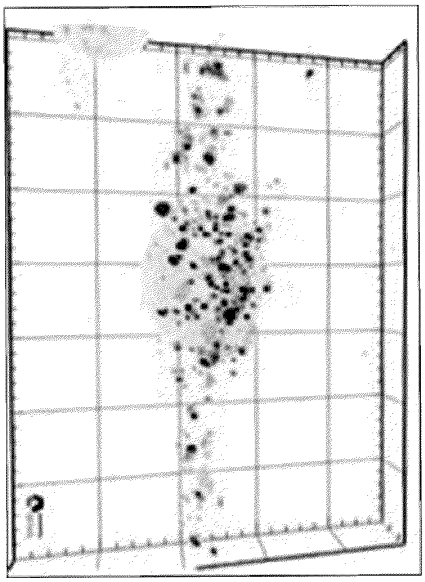
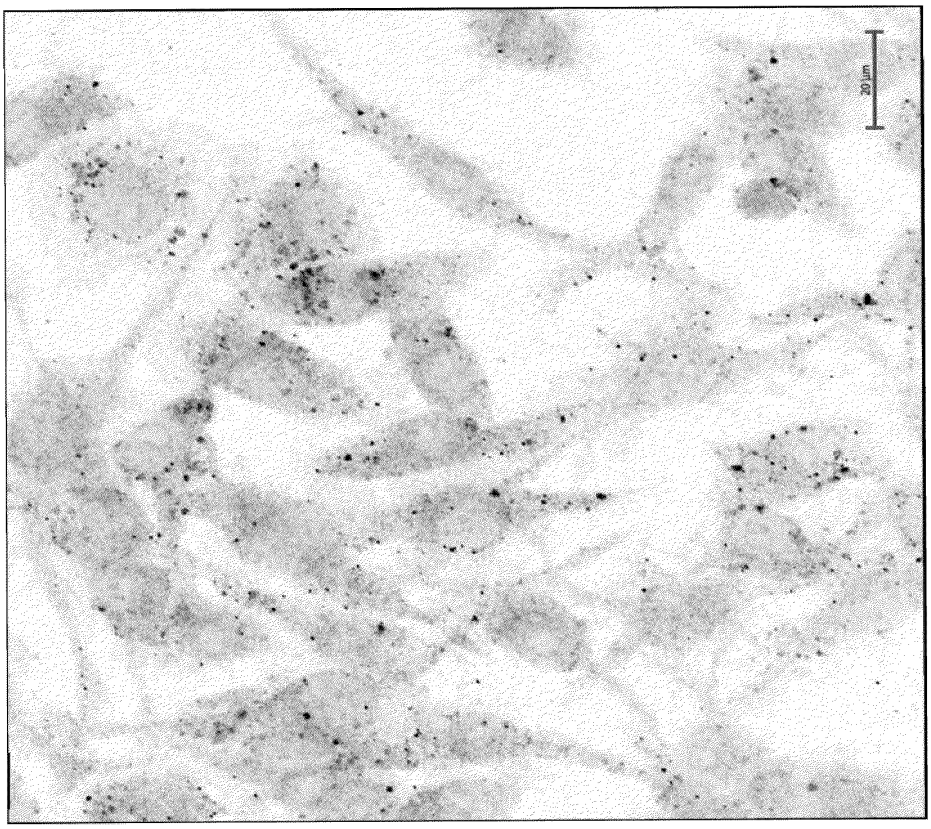


FIG. 8A



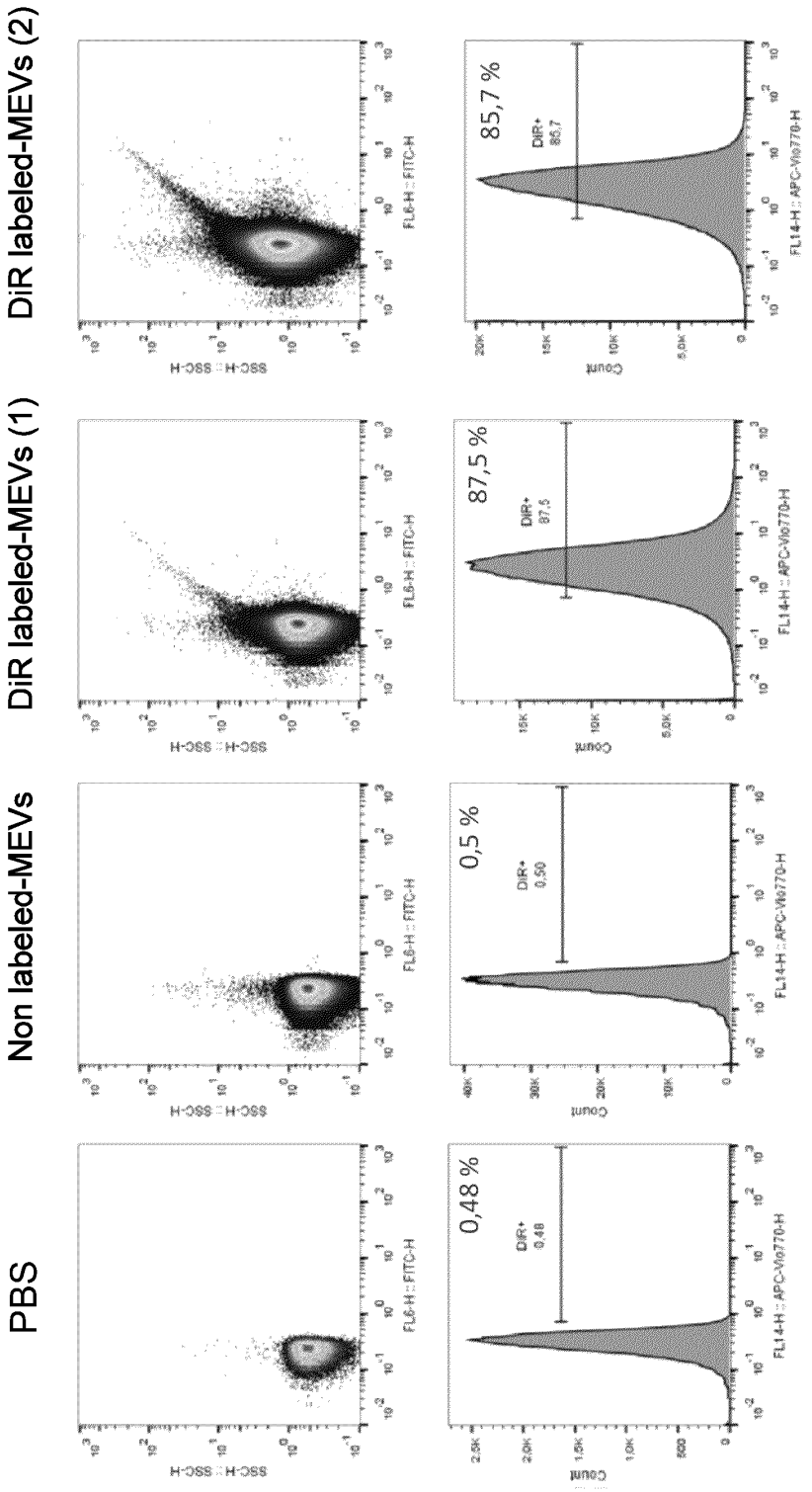

PBS
Non labeled-MEVs
DiR labeled-MEVs (1)
DiR labeled-MEVs (2)
0,48 %
0,5 %
87,5 %
85,7 %
DiR+
SSC-H :: SSC-H
FL6-H :: FITC-H
FL14-H :: APC-Vio770-H
Count

FIG. 9

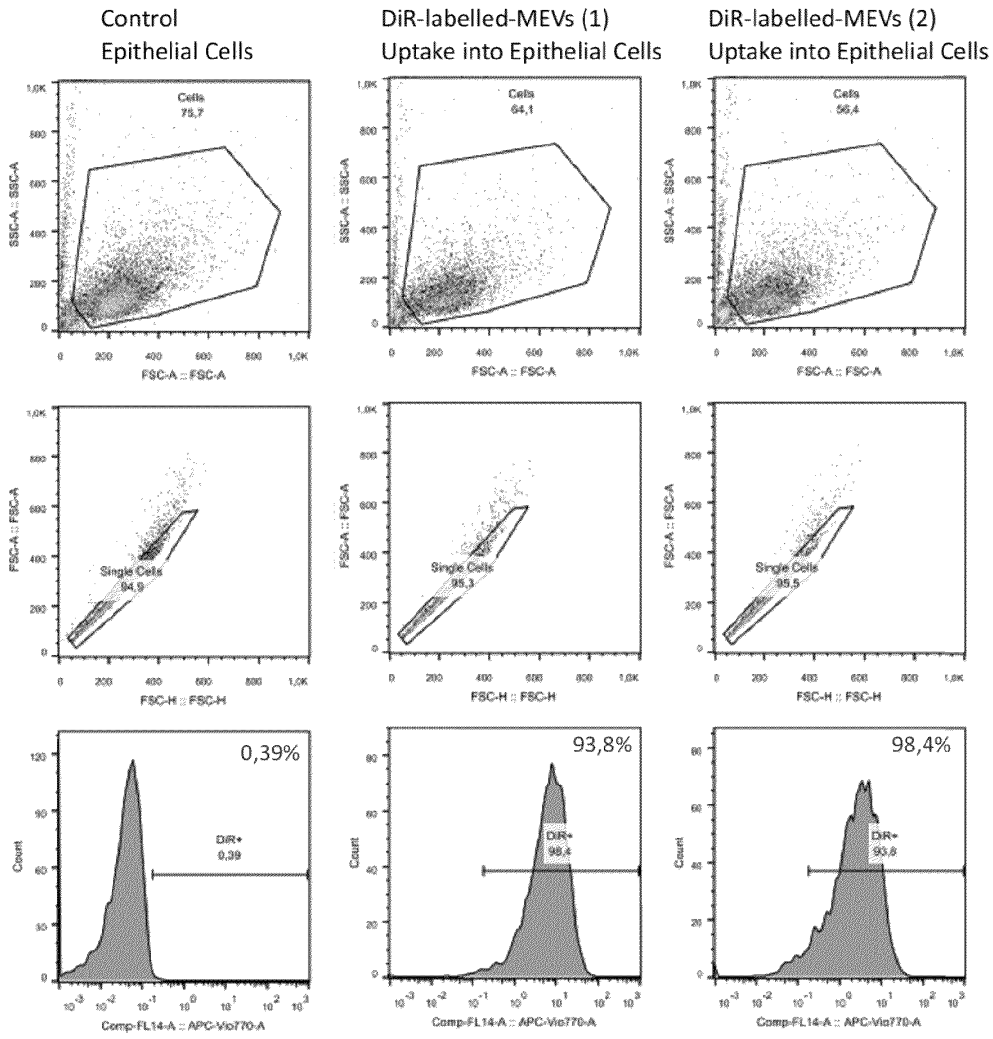

Control
Epithelial Cells
DiR-labelled-MEVs (1)
Uptake into Epithelial Cells
DiR-labelled-MEVs (2)
Uptake into Epithelial Cells
Cells
75,7
64,1
56,4
SSC-A :: SSC-A
FSC-A :: FSC-A
Single Cells
94,9
95,3
95,5
FSC-H :: FSC-H
0,39%
93,8%
98,4%
DiR+
0,39
98,4
93,8
Count
Comp-FL14-A :: APC-Vio770-A

FIG. 10A

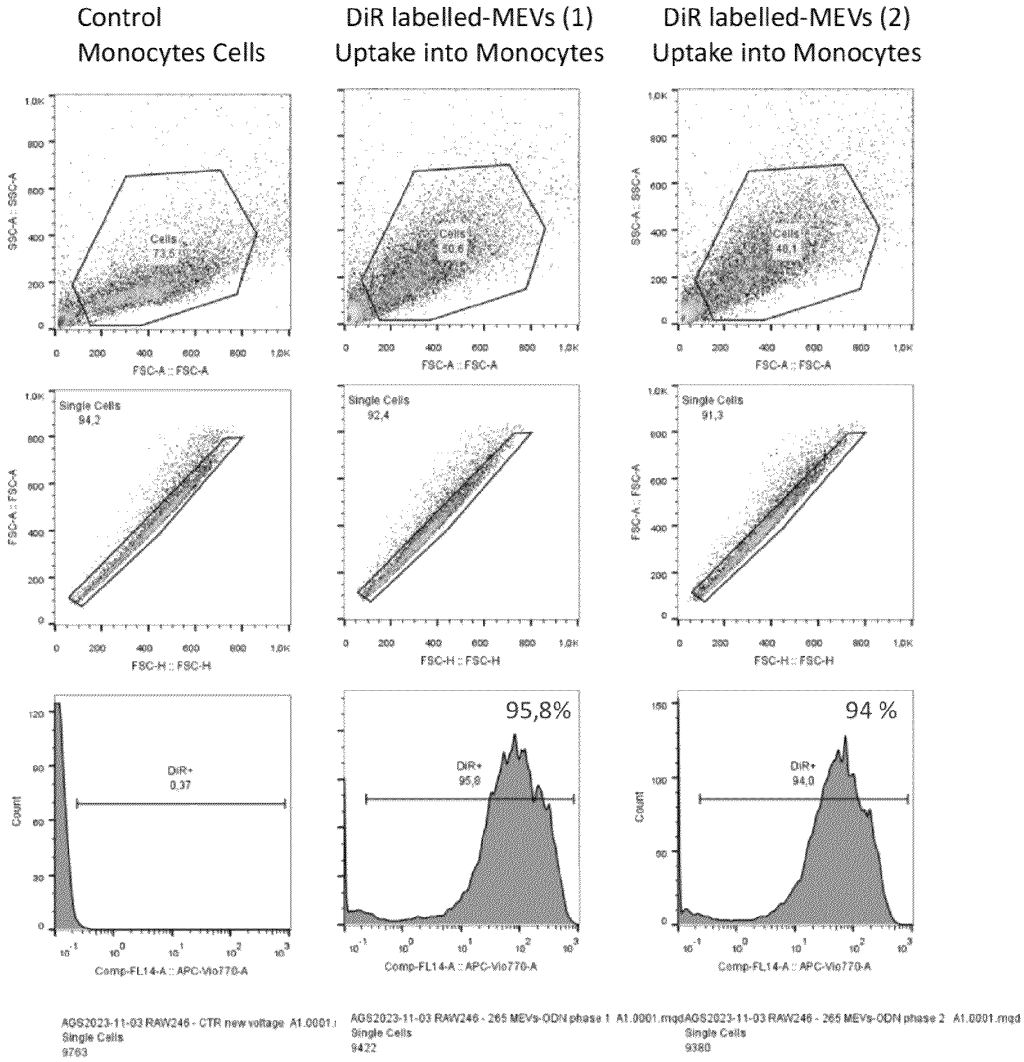

Control
Monocytes Cells
DiR labelled-MEVs (1)
Uptake into Monocytes
DiR labelled-MEVs (2)
Uptake into Monocytes
SSC-A :: SSC-A
FSC-A :: FSC-A
Cells
73,6
Cells
50,6
Cells
48,1
FSC-A :: FSC-A
FSC-H :: FSC-H
Single Cells
94,2
Single Cells
92,4
Single Cells
91,3
Count
DiR+
0,37
95,8%
DiR+
95,8
94 %
DiR+
94,0
Comp-FL14-A :: APC-Vio770-A
AGS2023-11-03 RAW246 - CTR new voltage A1.0001.
Single Cells
9763
AGS2023-11-03 RAW246 - 265 MEVs-ODN phase 1 A1.0001.mqd
Single Cells
9422
AGS2023-11-03 RAW246 - 265 MEVs-ODN phase 2 A1.0001.mqd
Single Cells
9380

FIG. 10B

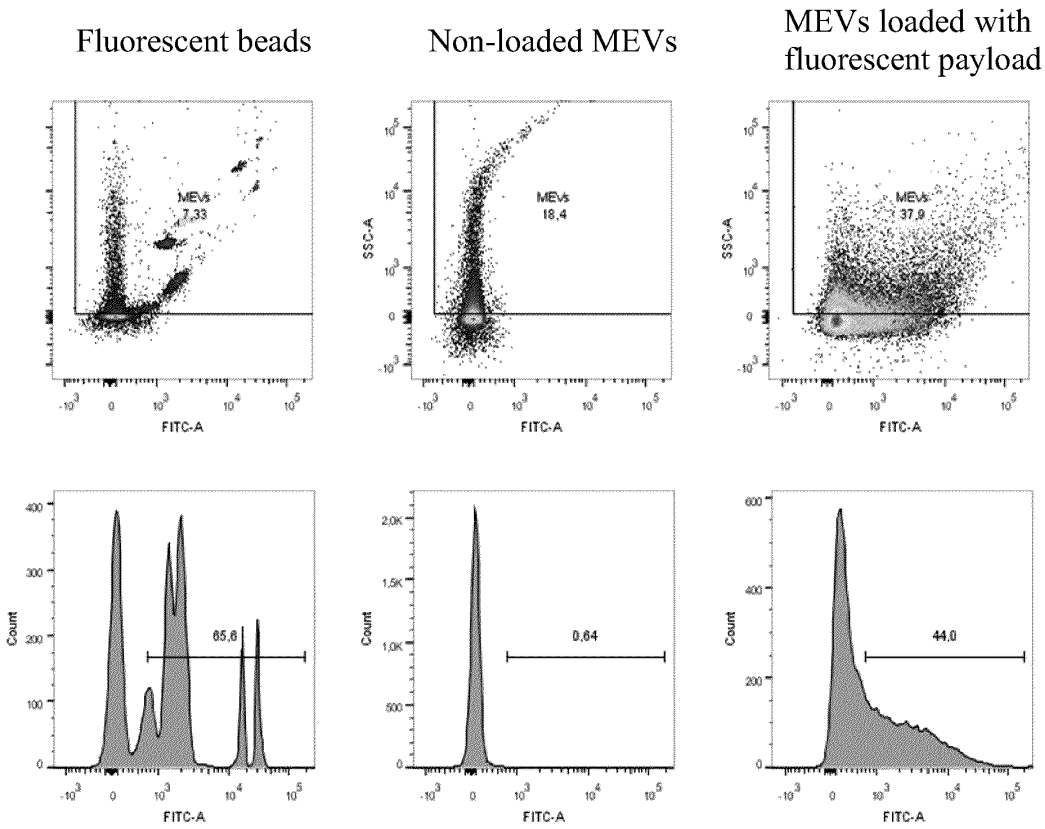

Fluorescent beads
Non-loaded MEVs
MEVs loaded with fluorescent payload
MEVs 7,33
MEVs 18,4
MEVs 37,9
SSC-A
FITC-A
Count
65,6
0,64
44,0

FIG. 11

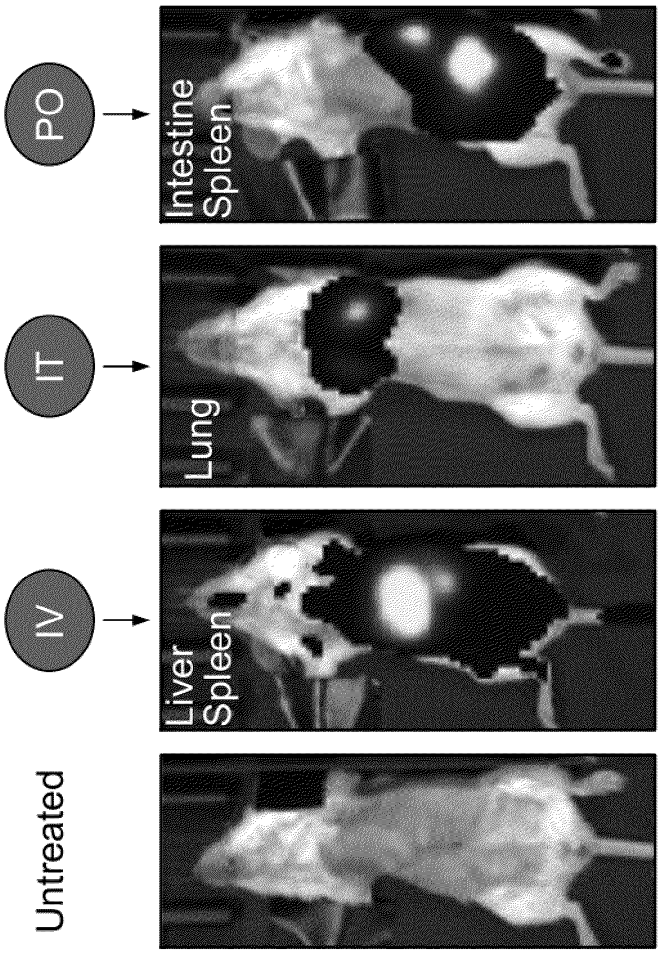

Untreated
IV
Liver
Spleen
IT
Lung
PO
Intestine
Spleen
PO -PER OS
IT -INTRATHRACHEAL
IV -INTRAVENOUS
Each delivery route
allows to target a specific
set of organs

FIG. 12

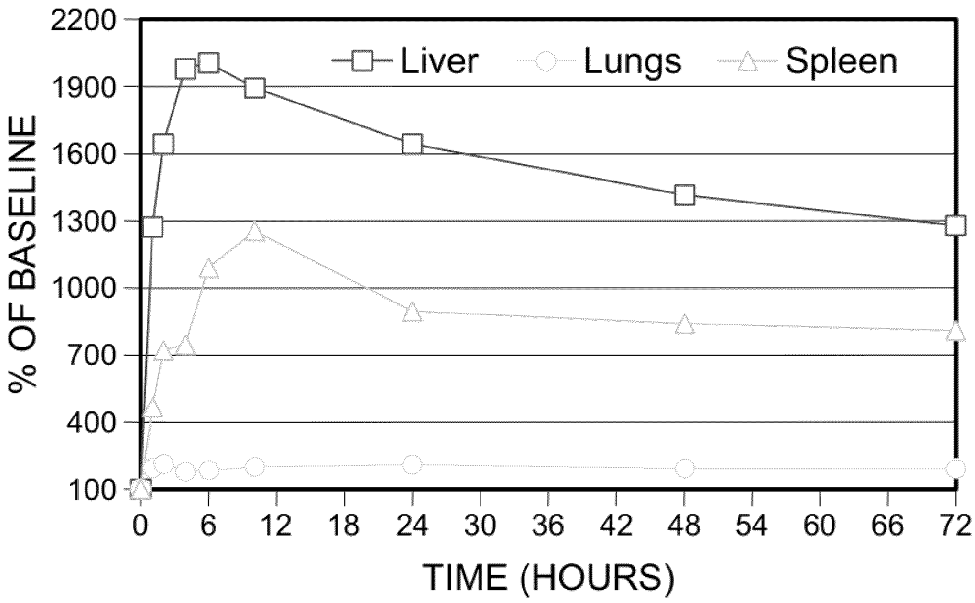

2200
1900
1600
1300
1000
700
400
100
% OF BASELINE
Liver
Lungs
Spleen
0
6
12
18
24
30
36
42
48
54
60
66
72
TIME (HOURS)

FIG. 13

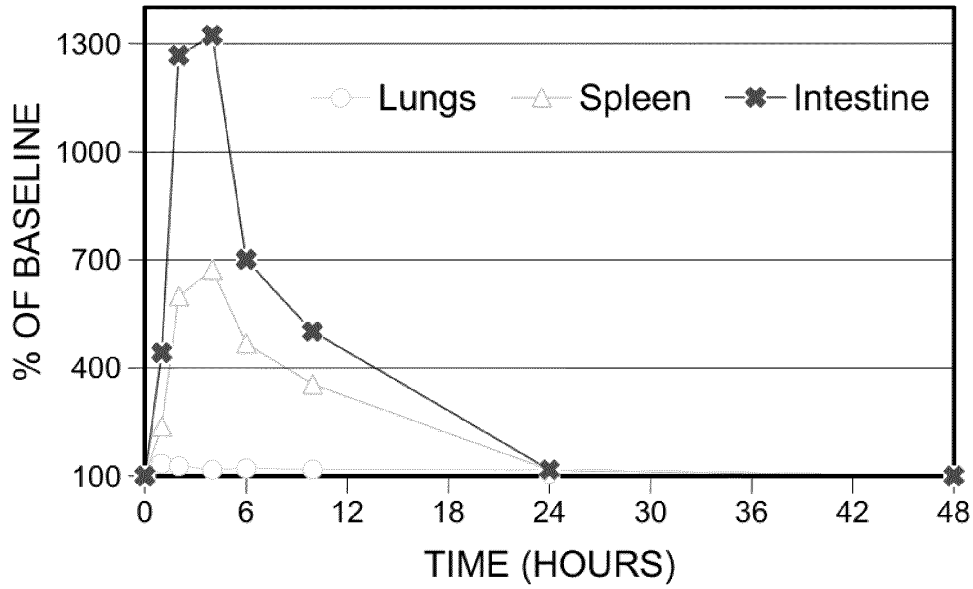

1300
1000
700
400
100
% OF BASELINE
Lungs
Spleen
Intestine
0
6
12
18
24
30
36
42
48
TIME (HOURS)

FIG. 14

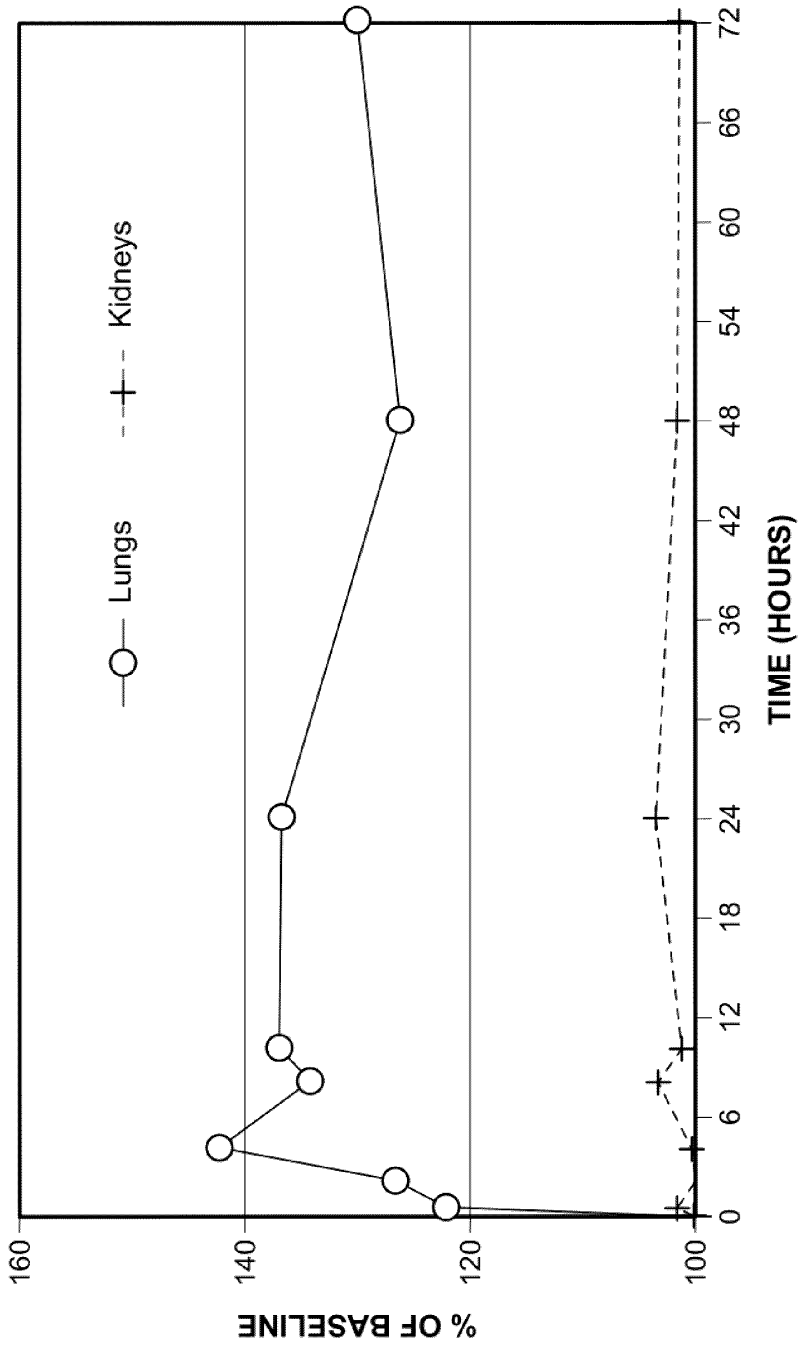

Lungs
Kidneys
% OF BASELINE
160
140
120
100
0
6
12
18
24
30
36
42
48
54
60
66
72
TIME (HOURS)

FIG. 15

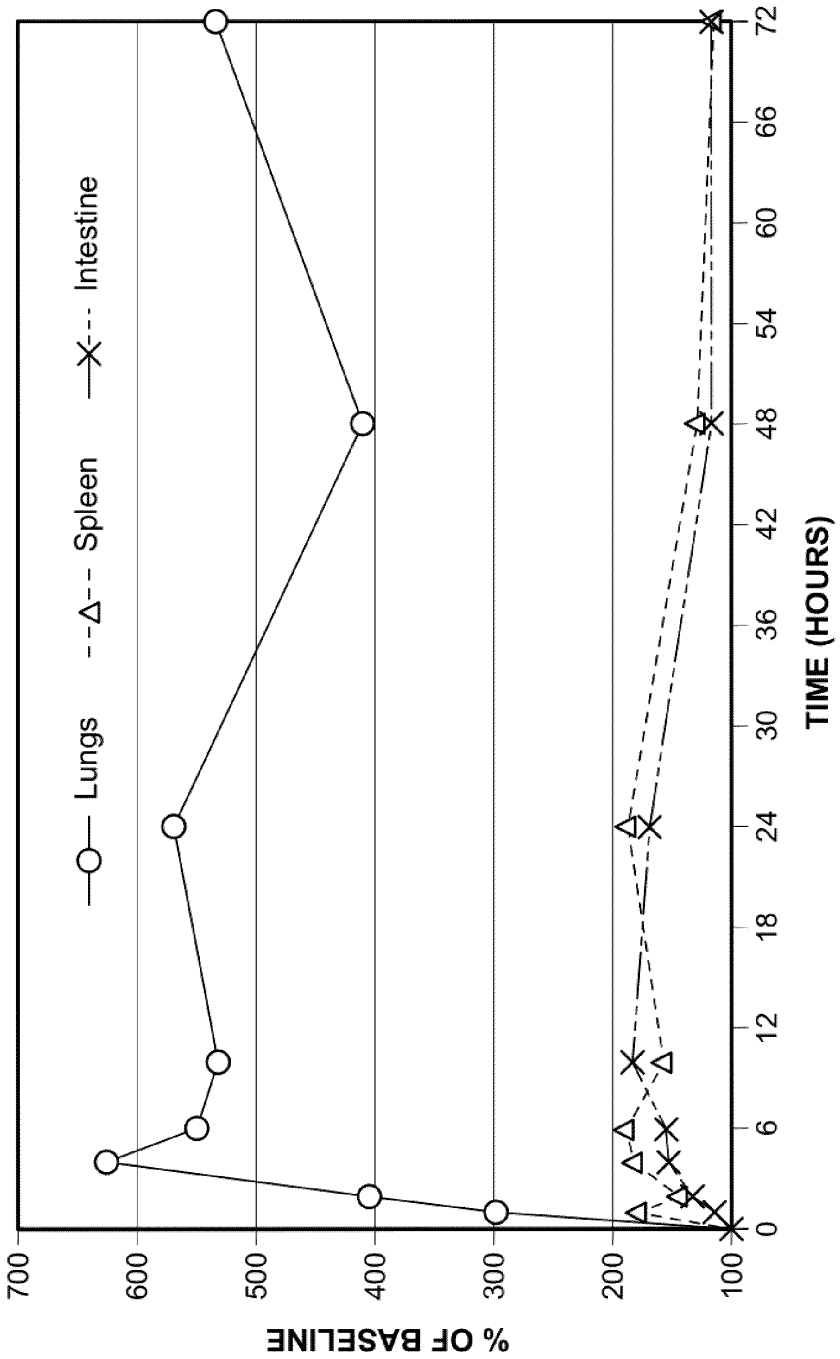

Lungs
Spleen
Intestine
% OF BASELINE
TIME (HOURS)
700
600
500
400
300
200
100
0
6
12
18
24
30
36
42
48
54
60
66
72

FIG. 16

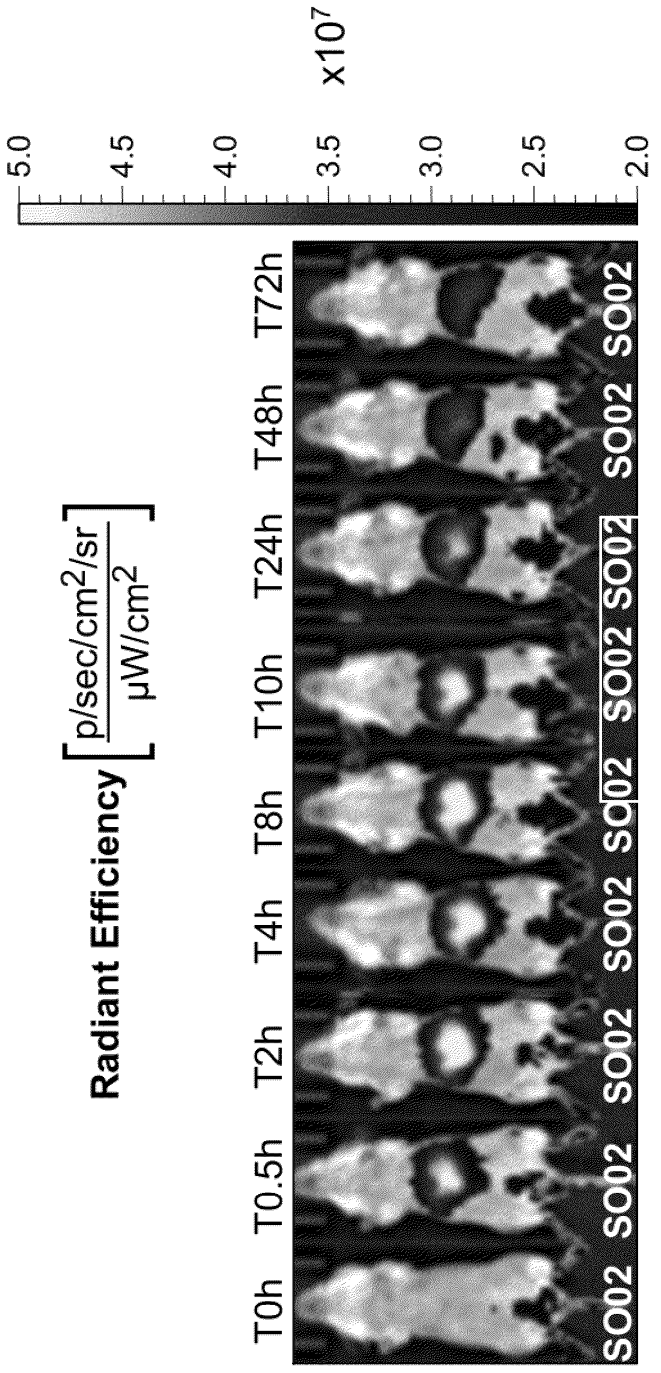

Radiant Efficiency [p/sec/cm²/sr / µW/cm²]
x10⁷
5.0
4.5
4.0
3.5
3.0
2.5
2.0
T0h
T0.5h
T2h
T4h
T8h
T10h
T24h
T48h
T72h
SO2

FIG. 17

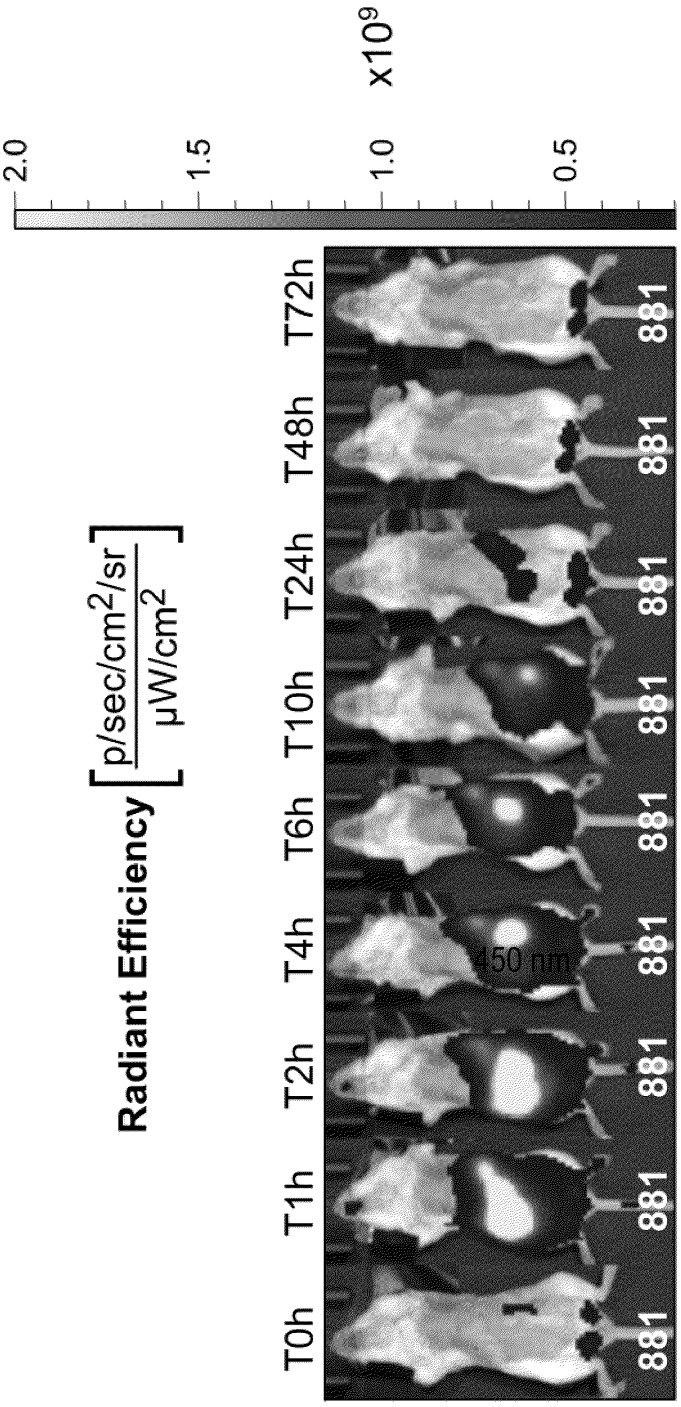

2.0
1.5
1.0
0.5
x10^9
Radiant Efficiency [p/sec/cm²/sr / µW/cm²]
T0h
T1h
T2h
T4h
T6h
T10h
T24h
T48h
T72h
881
450 nm

FIG. 18

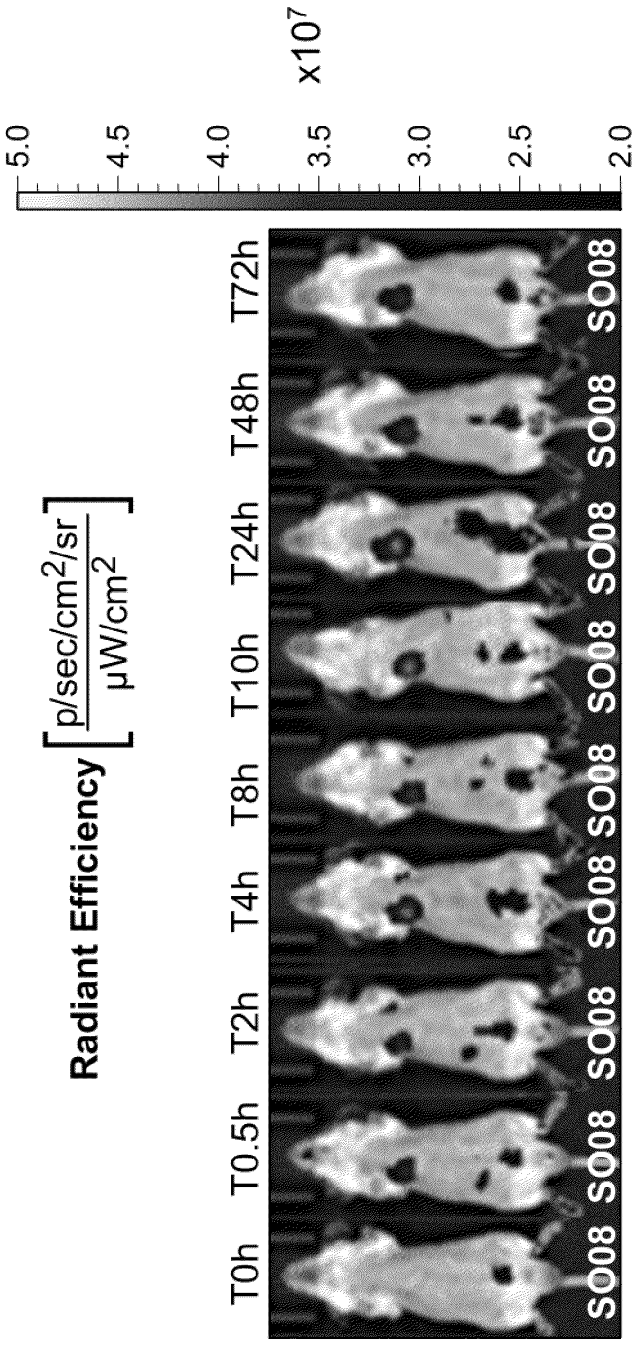

Radiant Efficiency [p/sec/cm²/sr / µW/cm²]
x10⁷
5.0
4.5
4.0
3.5
3.0
2.5
2.0
T0h
T0.5h
T2h
T4h
T8h
T10h
T24h
T48h
T72h
SO08

FIG. 19

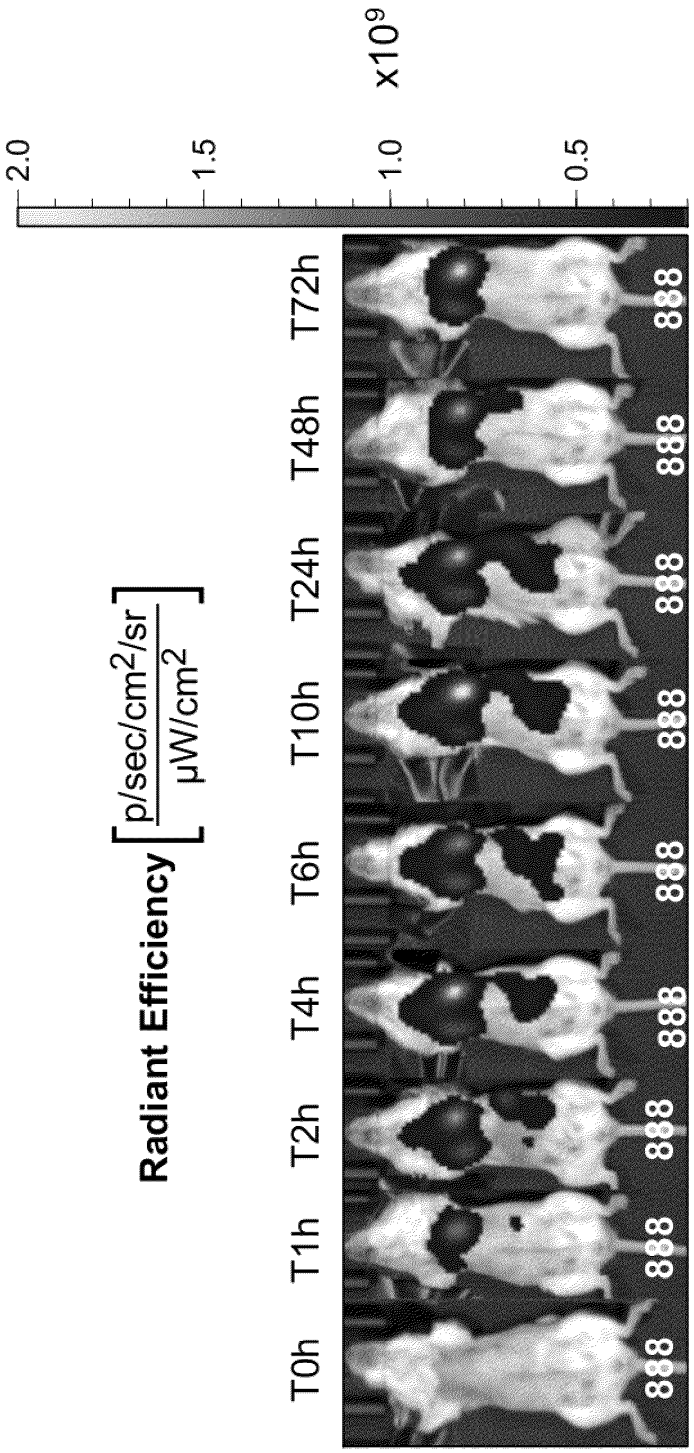

Radiant Efficiency [p/sec/cm²/sr / µW/cm²]
x10⁹
2.0
1.5
1.0
0.5
T0h
T1h
T2h
T4h
T6h
T10h
T24h
T48h
T72h

FIG. 20

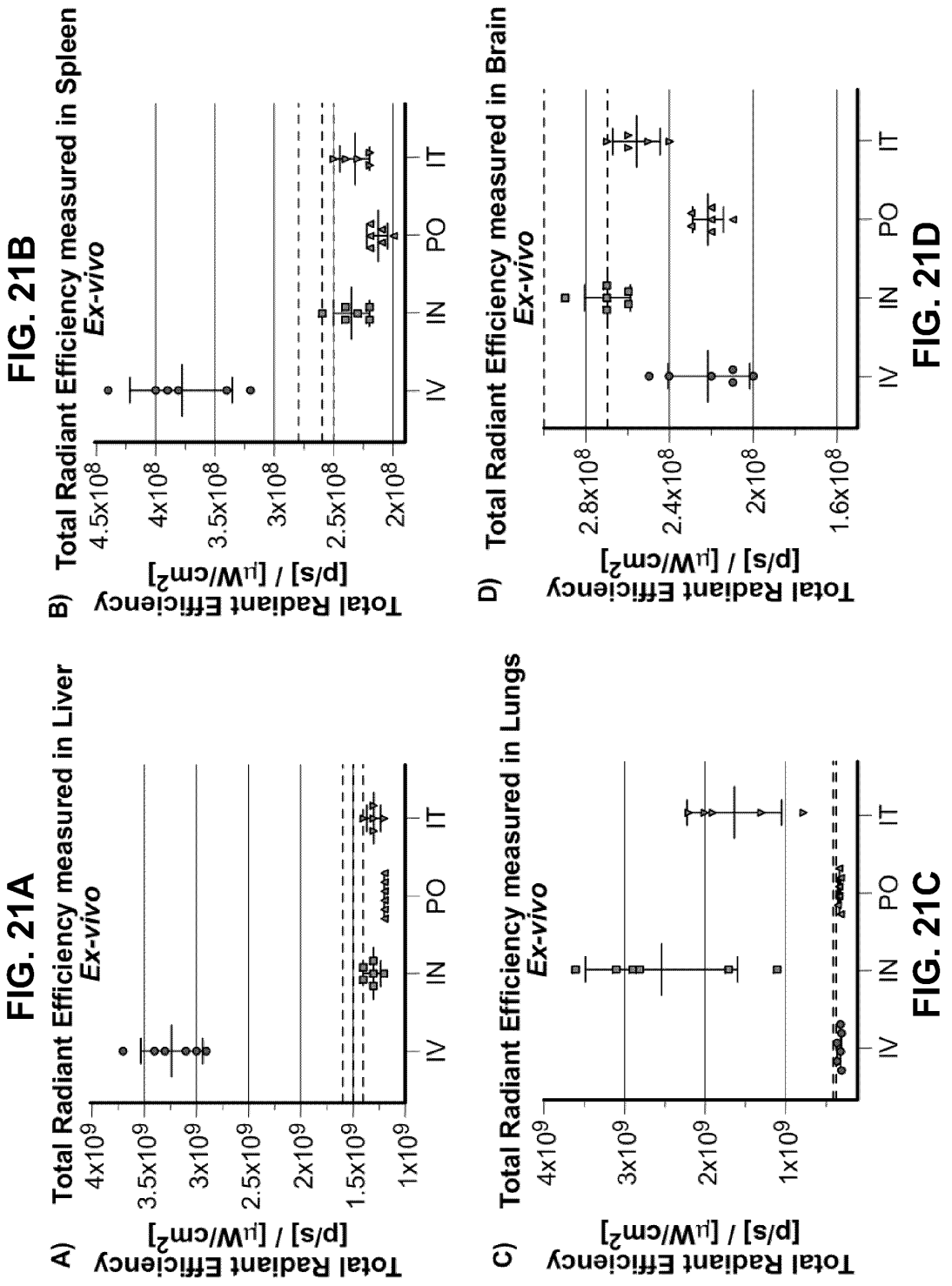


FIG. 21A

FIG. 21B

FIG. 21C

FIG. 21D

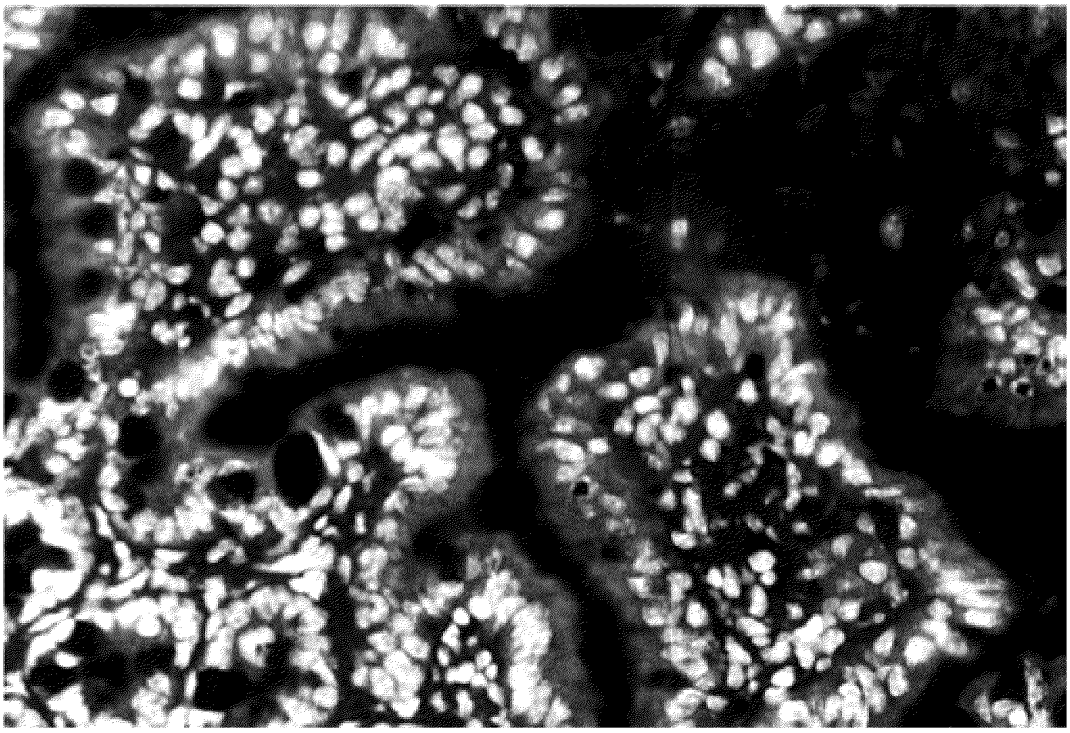


FIG. 22

FIG. 23A

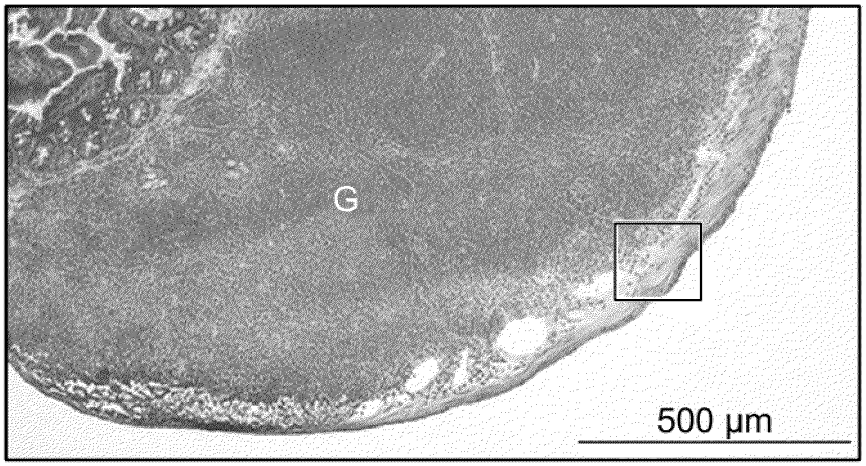
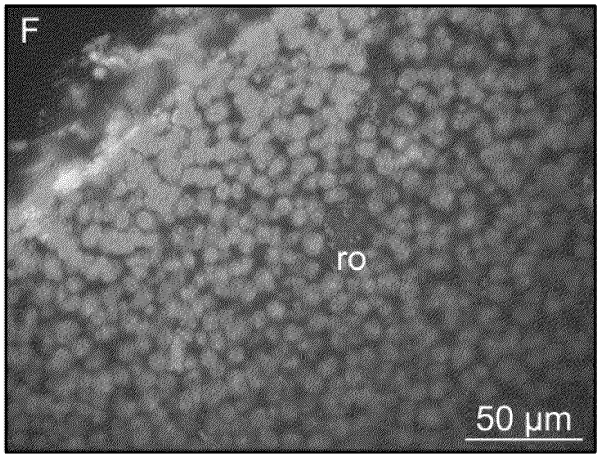


FIG. 23B



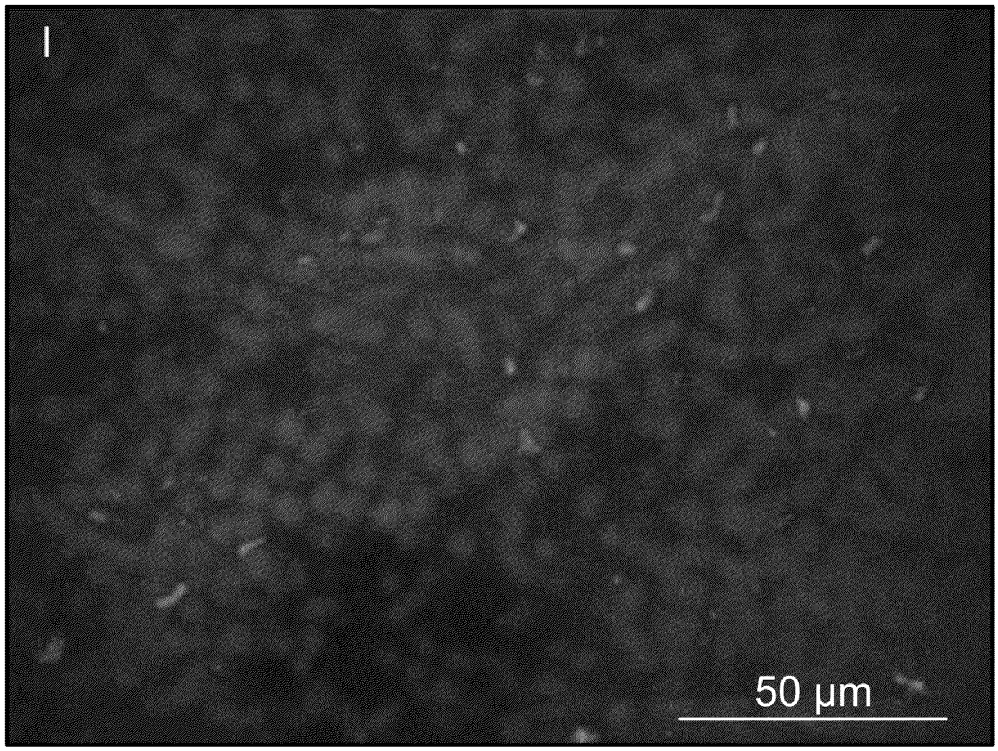


FIG. 24

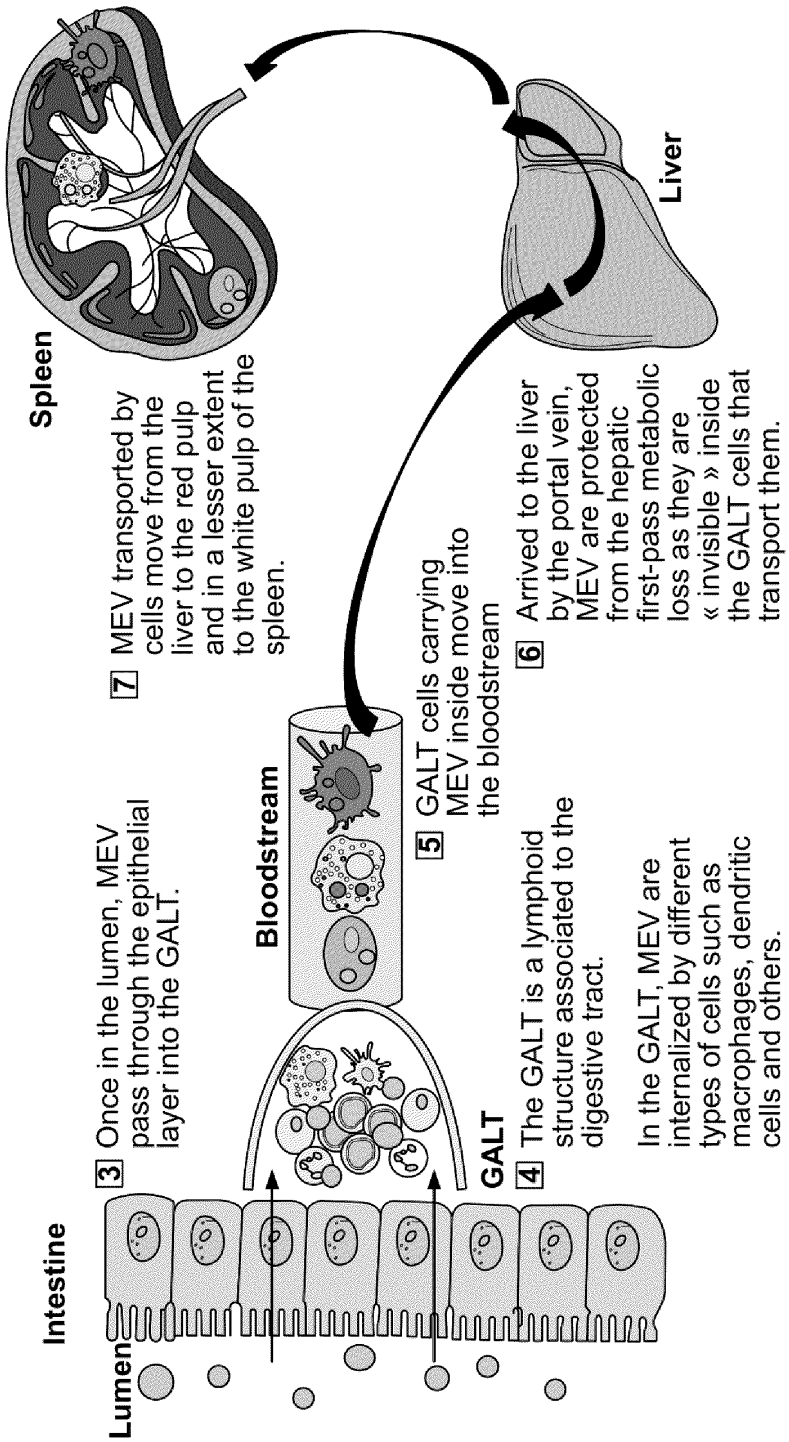

Spleen
Liver
Intestine
Lumen
Bloodstream
GALT
3 Once in the lumen, MEV pass through the epithelial layer into the GALT.
4 The GALT is a lymphoid structure associated to the digestive tract.
In the GALT, MEV are internalized by different types of cells such as macrophages, dendritic cells and others.
5 GALT cells carrying MEV inside move into the bloodstream
6 Arrived to the liver by the portal vein, MEV are protected from the hepatic first-pass metabolic loss as they are « invisible » inside the GALT cells that transport them.
7 MEV transported by cells move from the liver to the red pulp and in a lesser extent to the white pulp of the spleen.

FIG. 25

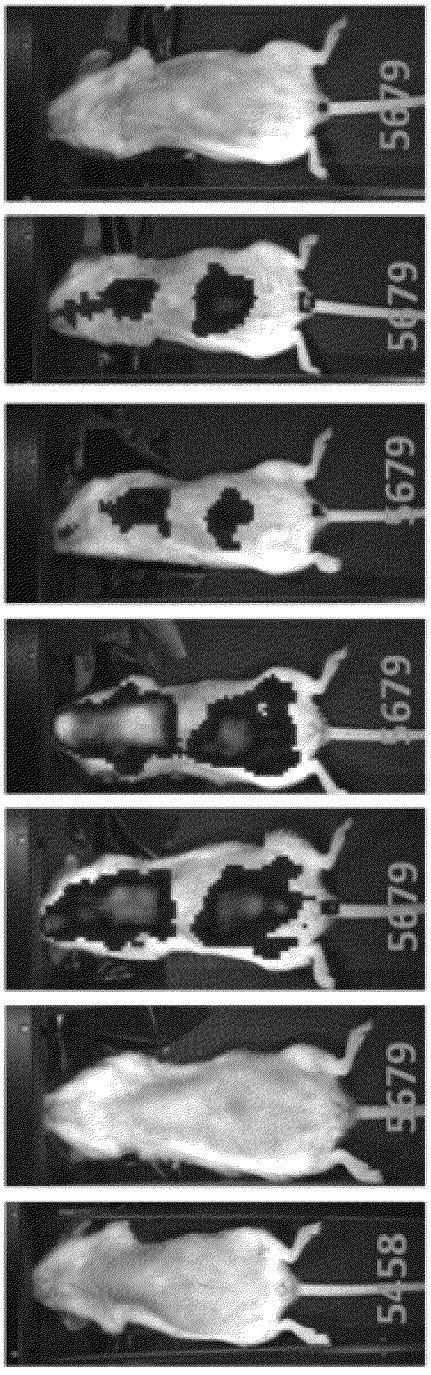


FIG. 26A

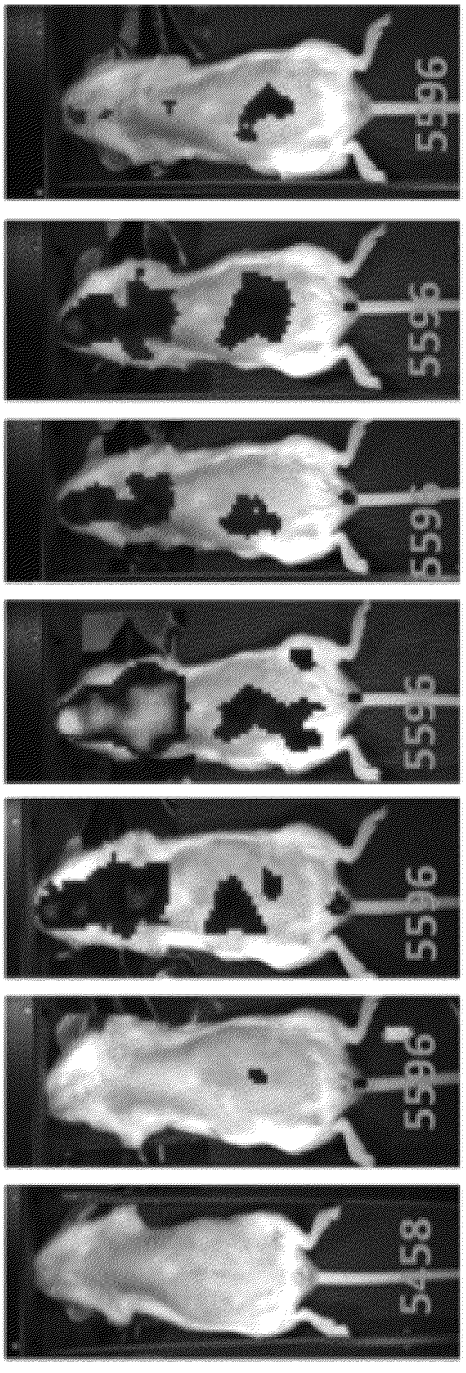


FIG. 26B

FIG. 27A

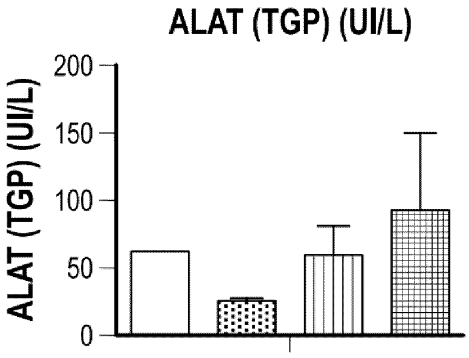


FIG. 27B

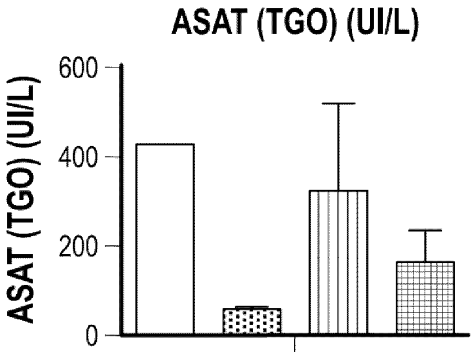


FIG. 27C

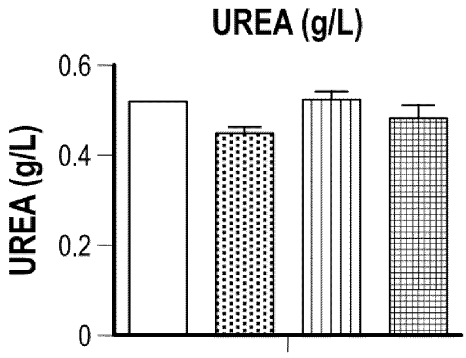
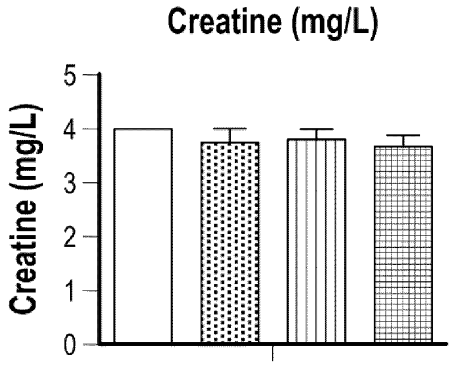


FIG. 27D



Group 1: PO PBS 100 µl

Group 2: PO, MEV 100 µl $4*10^{11}$

Group 3: PO, MEV 100 µl $1*10^{12}$

Group 4: IT, MEV 50 µl $4*10^{11}$

FIG. 27E

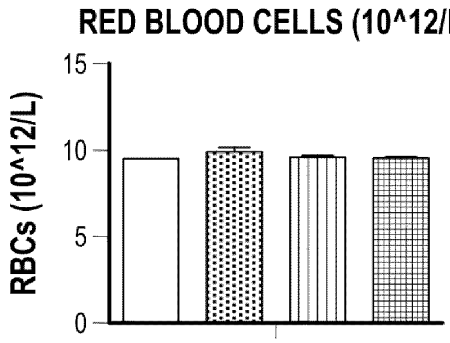


FIG. 27F

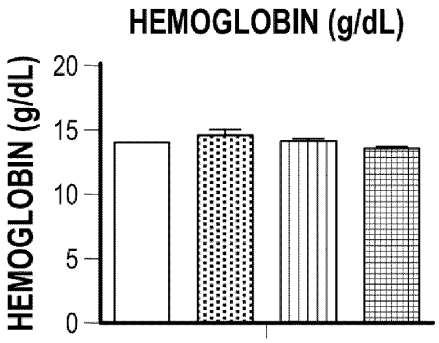


FIG. 27G

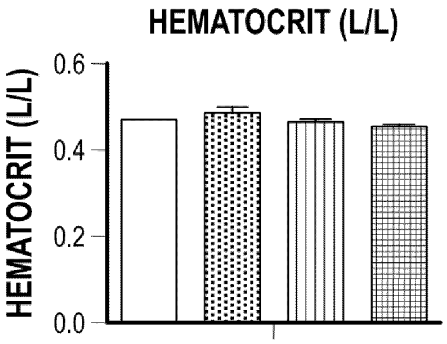


FIG. 27H

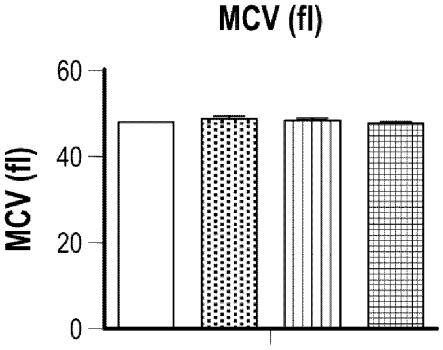
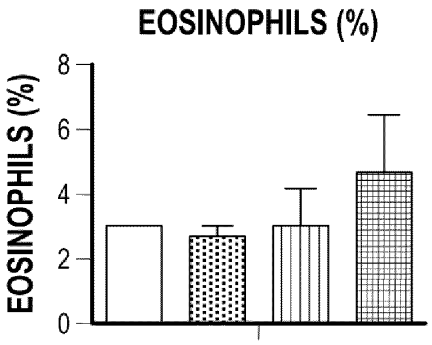


FIG. 27I



Group 1: PO PBS 100 µl
Group 2: PO, MEV 100 µl $4*10^{11}$
Group 3: PO, MEV 100 µl $1*10^{12}$
Group 4: IT, MEV 50 µl $4*10^{11}$

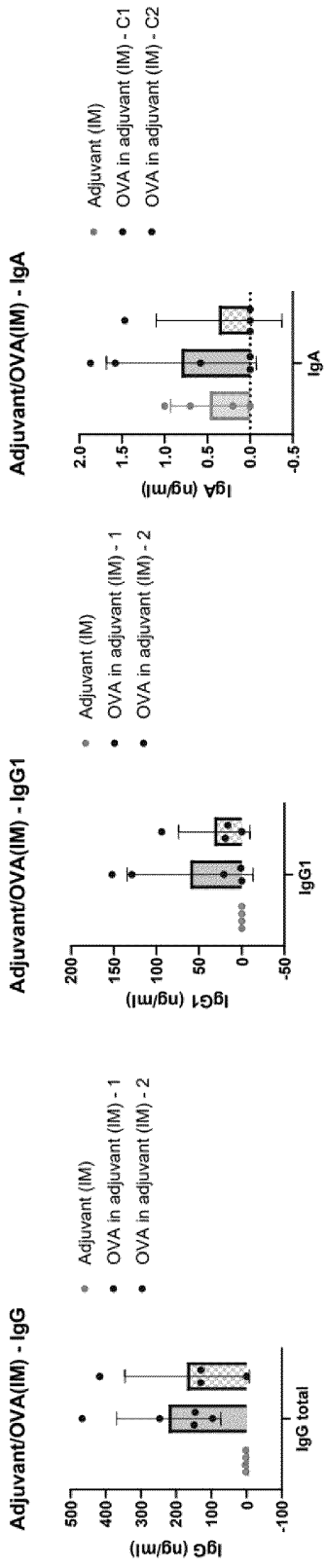

Adjuvant/OVA(IM) - IgG
IgG (ng/ml)
500
400
300
200
100
0
-100
IgG total
Adjuvant (IM)
OVA in adjuvant (IM) - 1
OVA in adjuvant (IM) - 2
Adjuvant/OVA(IM) - IgG1
IgG1 (ng/ml)
200
150
100
50
0
-50
IgG1
Adjuvant (IM)
OVA in adjuvant (IM) - 1
OVA in adjuvant (IM) - 2
Adjuvant/OVA(IM) - IgA
IgA (ng/ml)
2.0
1.5
1.0
0.5
0.0
-0.5
IgA
Adjuvant (IM)
OVA in adjuvant (IM) - C1
OVA in adjuvant (IM) - C2

FIG. 28A

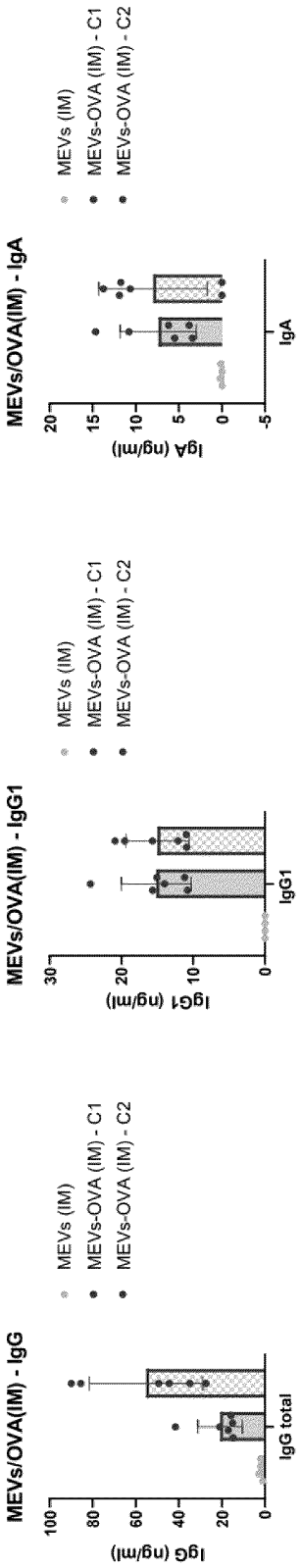

MEVs/OVA(IM) - IgG
IgG (ng/ml)
100
80
60
40
20
0
IgG total
MEVs (IM)
MEVs-OVA (IM) - C1
MEVs-OVA (IM) - C2
MEVs/OVA(IM) - IgG1
IgG1 (ng/ml)
30
20
10
0
IgG1
MEVs (IM)
MEVs-OVA (IM) - C1
MEVs-OVA (IM) - C2
MEVs/OVA(IM) - IgA
IgA (ng/ml)
20
15
10
5
0
-5
IgA
MEVs (IM)
MEVs-OVA (IM) - C1
MEVs-OVA (IM) - C2

FIG. 28B

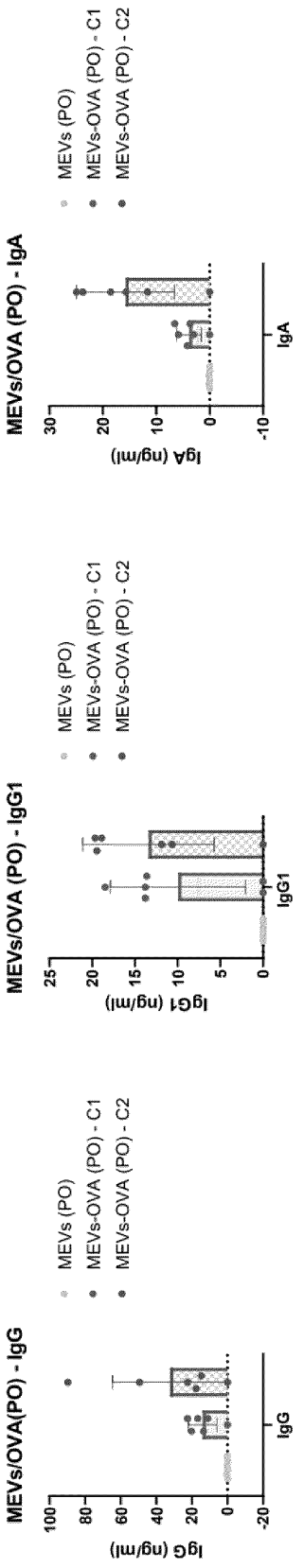

MEVs/OVA(PO) - IgG
IgG (ng/ml)
100
80
60
40
20
0
-20
IgG
MEVs (PO)
MEVs-OVA (PO) - C1
MEVs-OVA (PO) - C2
MEVs/OVA (PO) - IgG1
IgG1 (ng/ml)
25
20
15
10
5
0
IgG1
MEVs (PO)
MEVs-OVA (PO) - C1
MEVs-OVA (PO) - C2
MEVs/OVA (PO) - IgA
IgA (ng/ml)
30
20
10
0
-10
IgA
MEVs (PO)
MEVs-OVA (PO) - C1
MEVs-OVA (PO) - C2

FIG. 28C

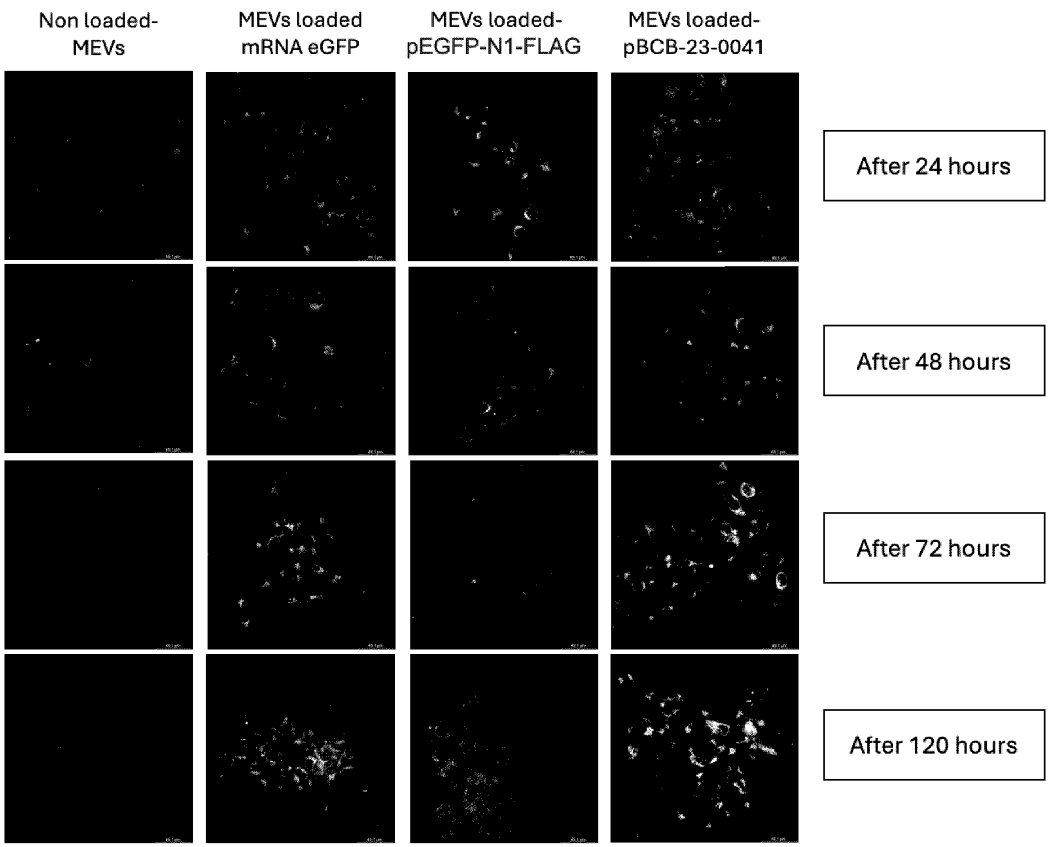

Non loaded-MEVs
MEVs loaded mRNA eGFP
MEVs loaded-pEGFP-N1-FLAG
MEVs loaded-pBCB-23-0041
After 24 hours
After 48 hours
After 72 hours
After 120 hours

FIG. 29

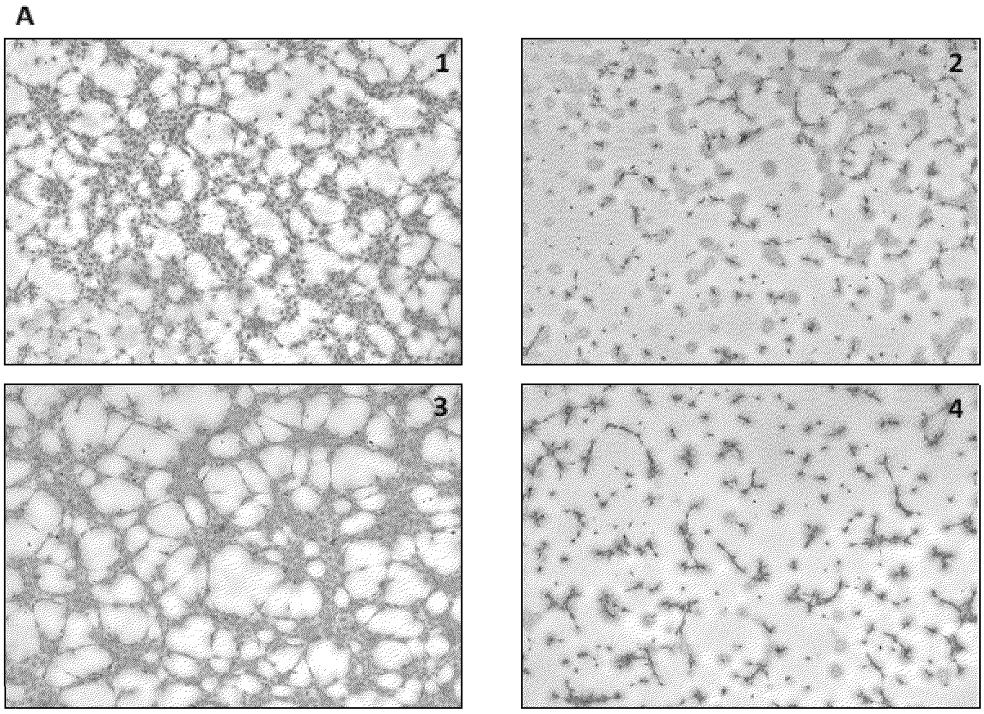


FIG. 30A

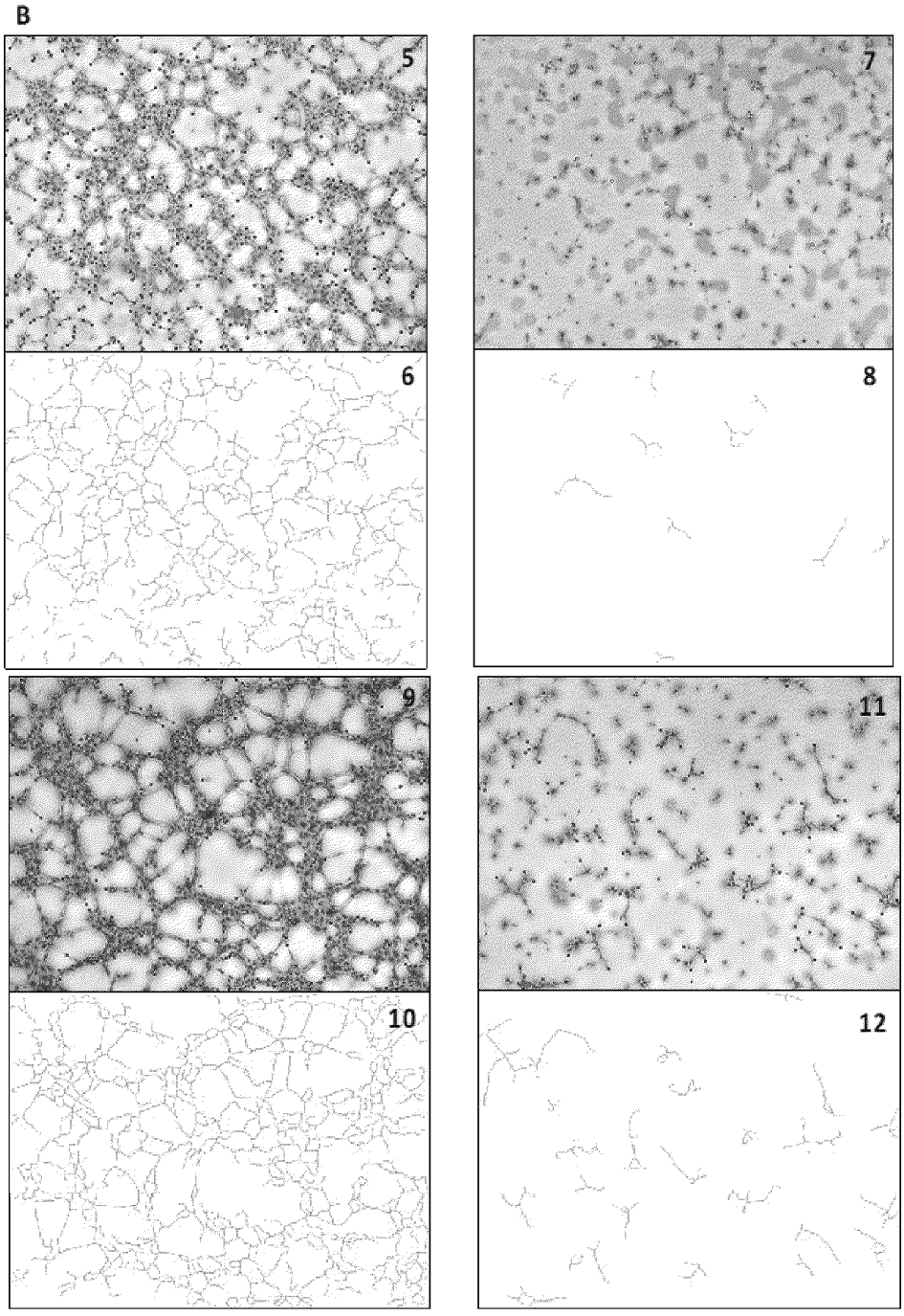


FIG. 30B

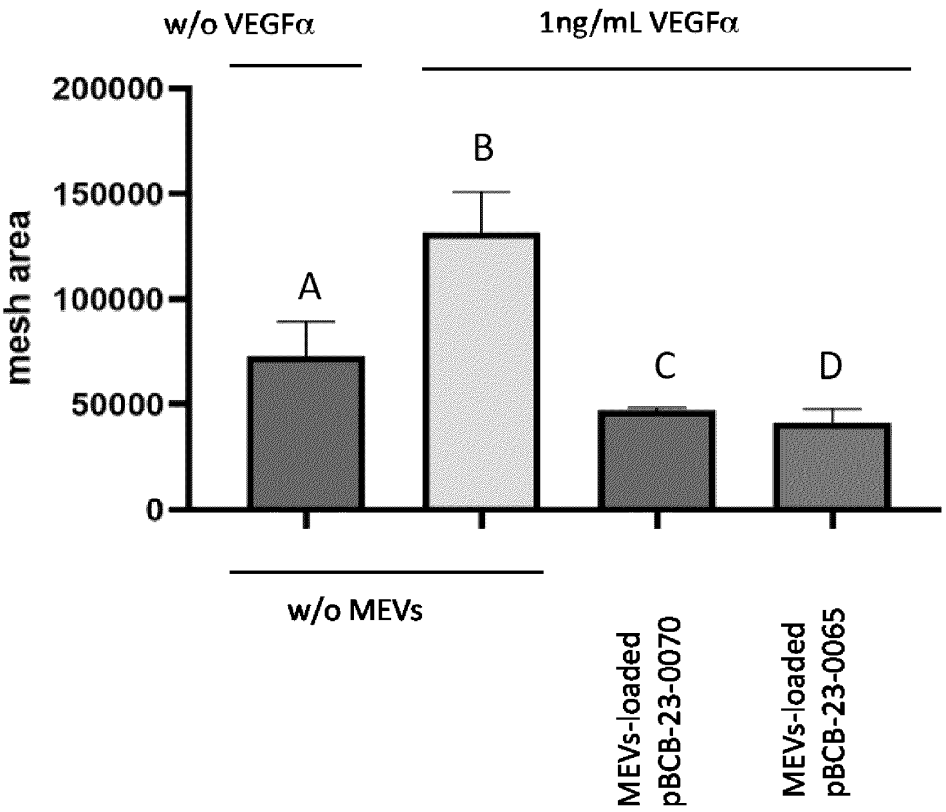

w/o VEGFα
1ng/mL VEGFα
200000
150000
100000
50000
0
mesh area
A
B
C
D
w/o MEVs
MEVs-loaded pBCB-23-0070
MEVs-loaded pBCB-23-0065

FIG. 31

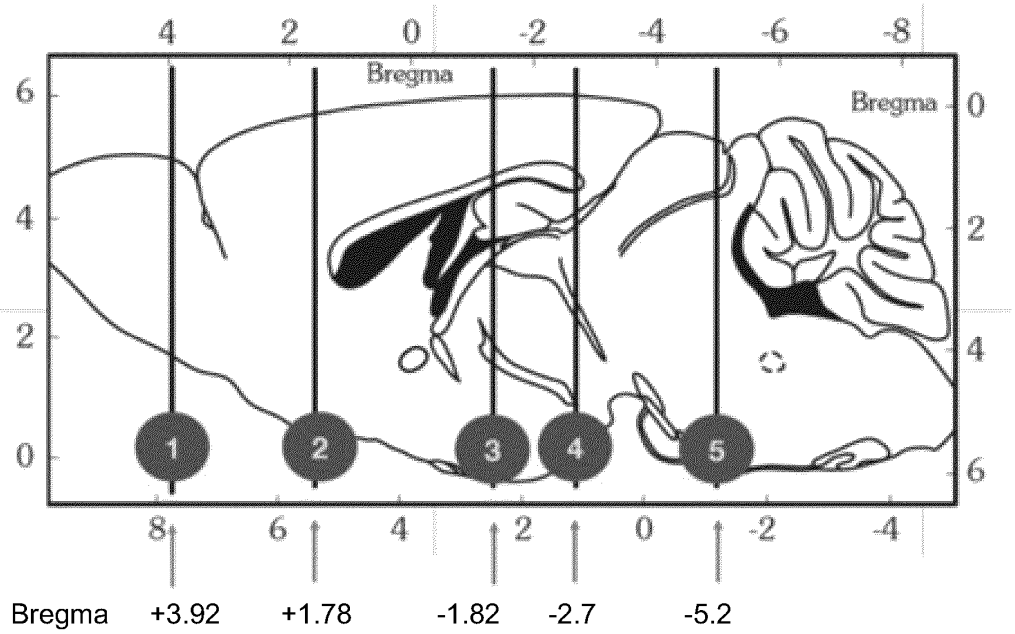
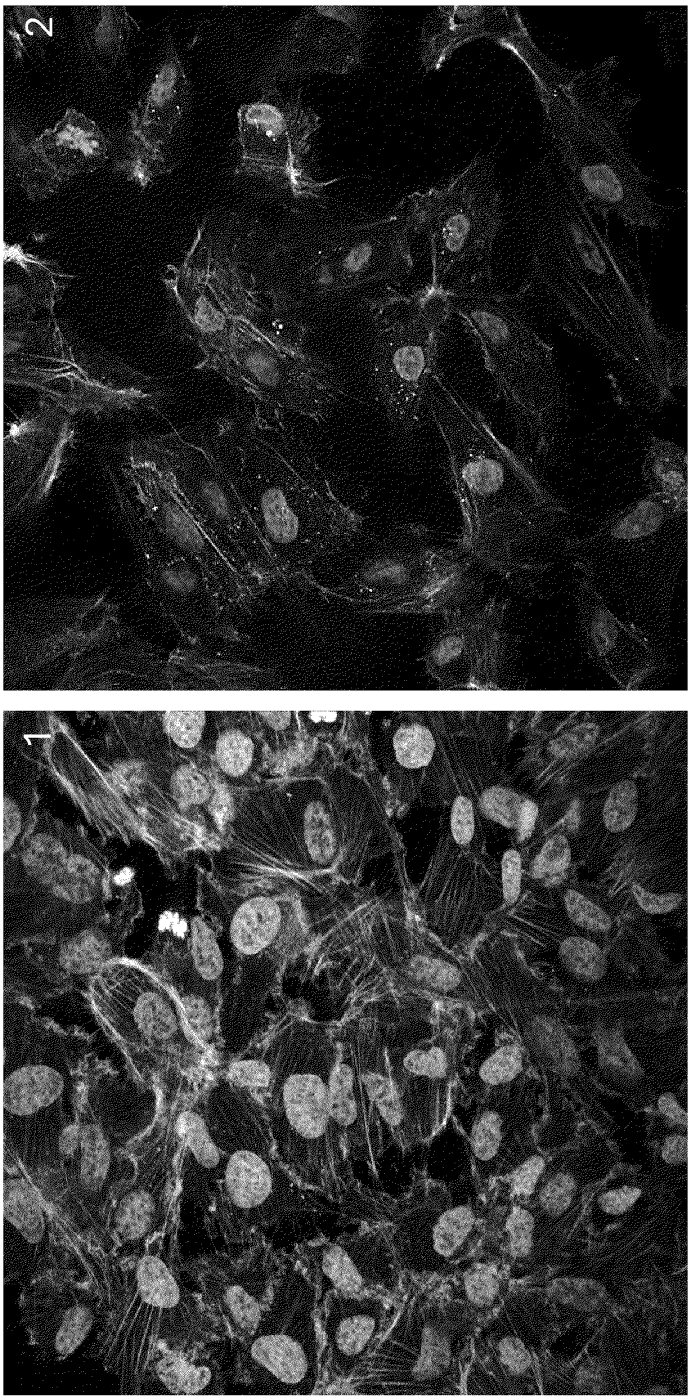

4
2
0
-2
-4
-6
-8
Bregma
Bregma
6
4
2
0
0
2
4
6
1
2
3
4
5
8
6
4
2
0
-2
-4
Bregma +3.92 +1.78 -1.82 -2.7 -5.2

FIG. 32



1- T=0h, ARPE-19 cells treated with MEVs labelled with PKH26 (merge DAPI/ Actin (Alexa 488) / MEVs labelled with PKH26 (Alexa 546))

2- T=24h, ARPE-19 cells treated with MEVs labelled with PKH26 (merge DAPI/ Actin (Alexa 488) / MEVs labelled with PKH26 (Alexa 546))

FIG. 33

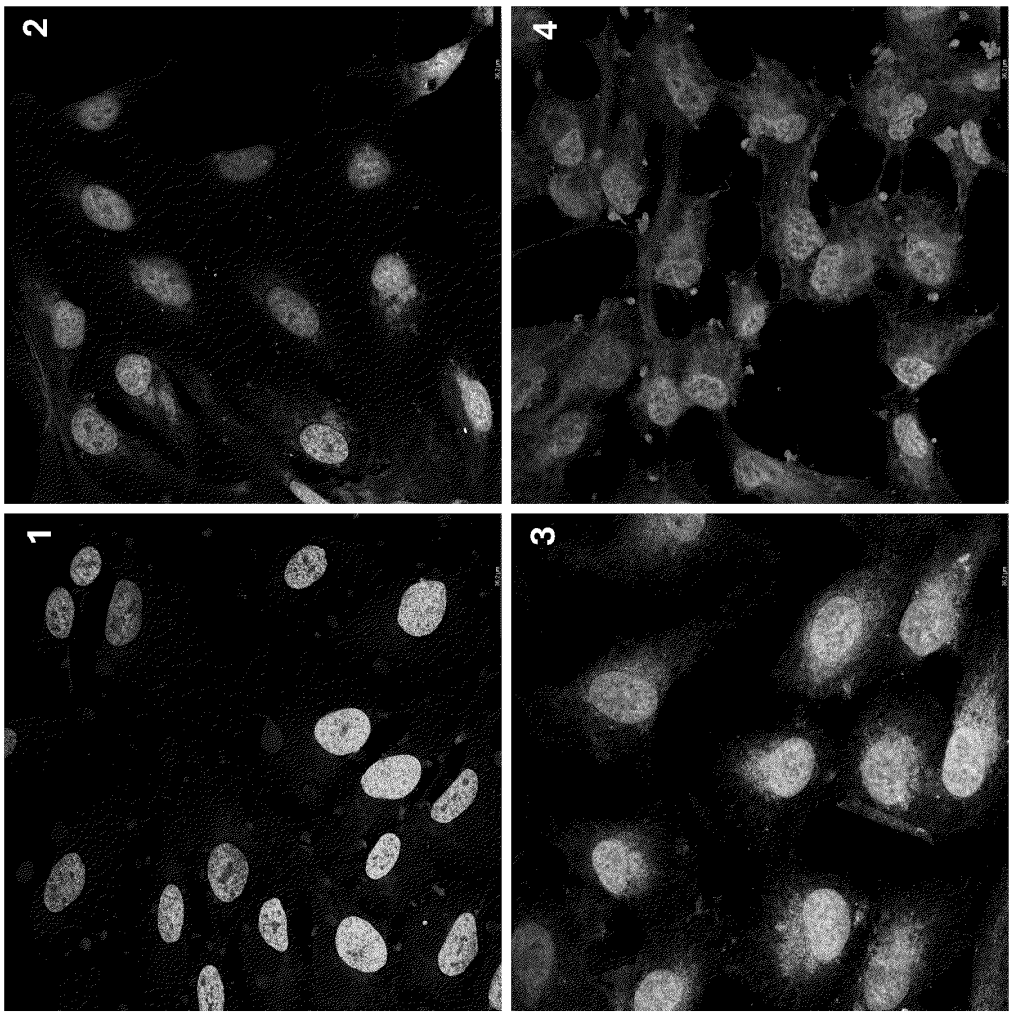

1
2
3
4
1- T=0h ARPE-19 cells treated with MEVs loaded with plasmid carrying AAV's ITRs and coding for GFP (merge DAPI / GFP protein (Alexa 488))
2- T=72h ARPE-19 cells treated with MEVs loaded with plasmid carrying AAV's ITRs and coding for GFP (merge DAPI / GFP protein (Alexa 488))
3- T= 96h ARPE-19 cells treated with MEVs loaded with plasmid carrying AAV's ITRs and coding for GFP (merge DAPI / GFP protein (Alexa 488))
4- T=144h ARPE-19 cells treated with MEVs loaded with plasmid carrying AAV's ITRs and coding for GFP (merge DAPI / GFP protein (Alexa 488))

FIG. 34

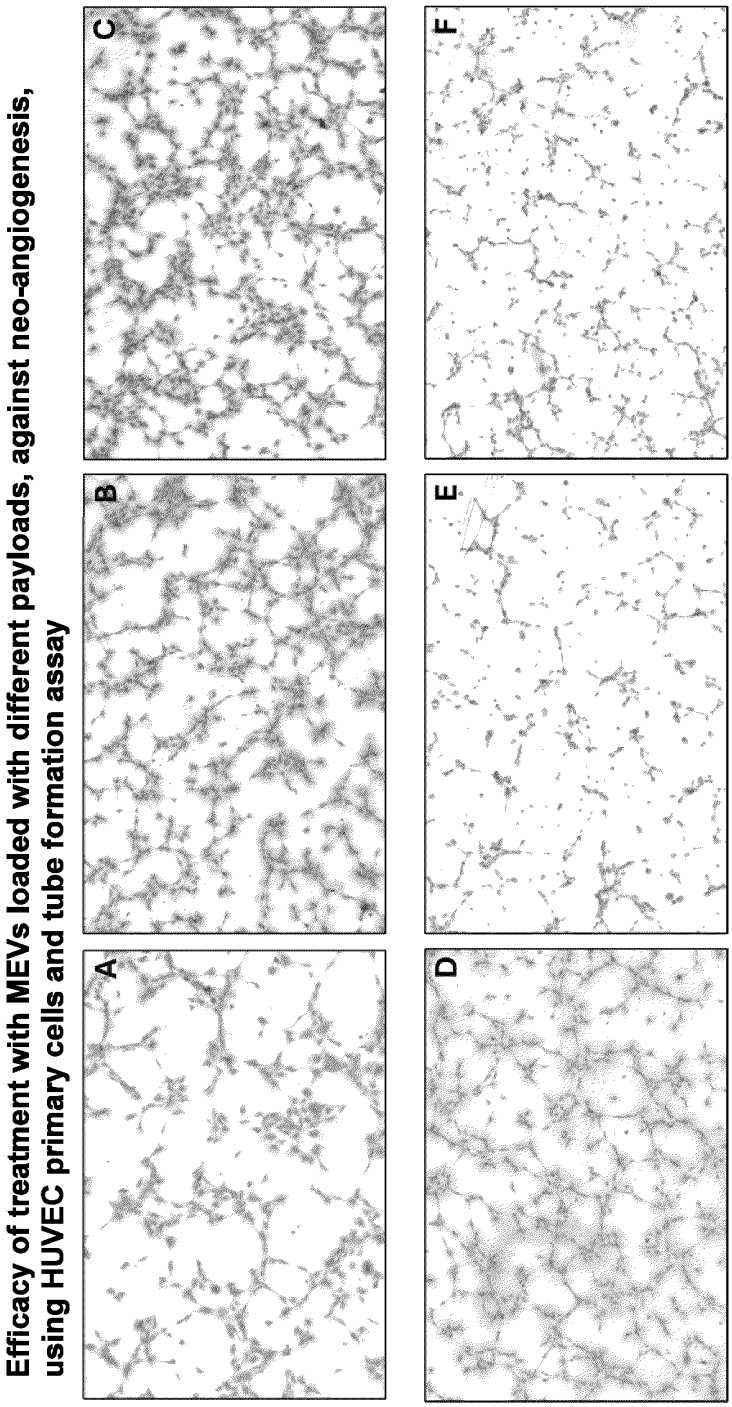

Efficacy of treatment with MEVs loaded with different payloads, against neo-angiogenesis, using HUVEC primary cells and tube formation assay
A
B
C
D
E
F

FIG. 35

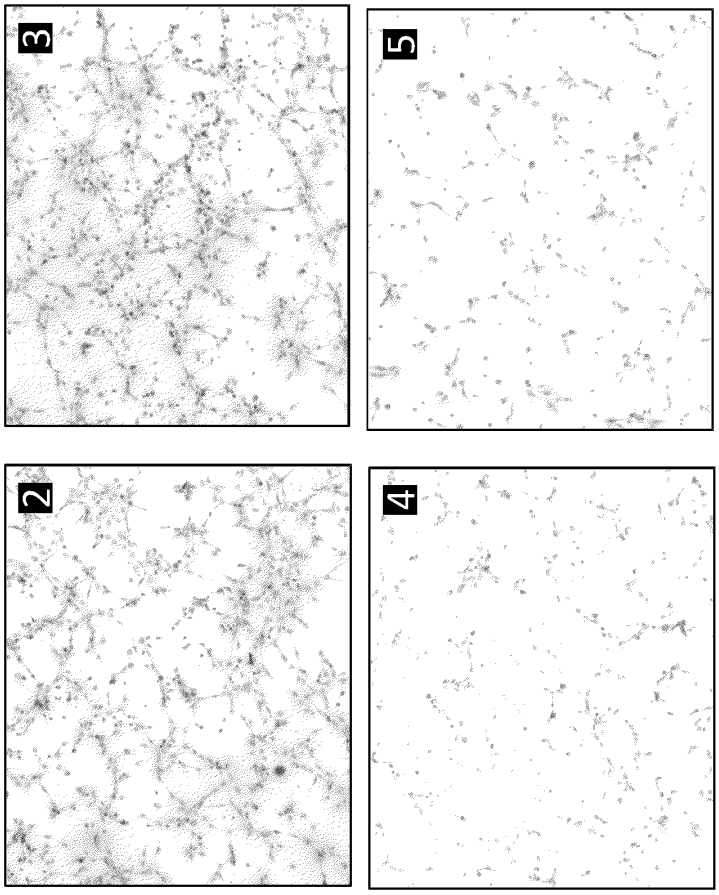

Efficacy of treatment against neo-angiogenesis, using HUVEC primary cells and the tube formation assay, using MEVs loaded with an ITR-plasmid (pITR) coding for Aflibercept.
1
2
3
4
5
1- Cells (Negative control (w/o VEGFa))
2- Cells (Positive control (1ng/mL VEGFa))
3- Cells (Positive control (2ng/mL VEGFa))
4- Cells treated with MEVs loaded with pITR (coding for aflibercept)+ VEGFa 1ng/mL
5- Cells treated with MEVs loaded with pITR' (coding for aflibercept) +VEGFa 1ng/mL

FIG. 36A

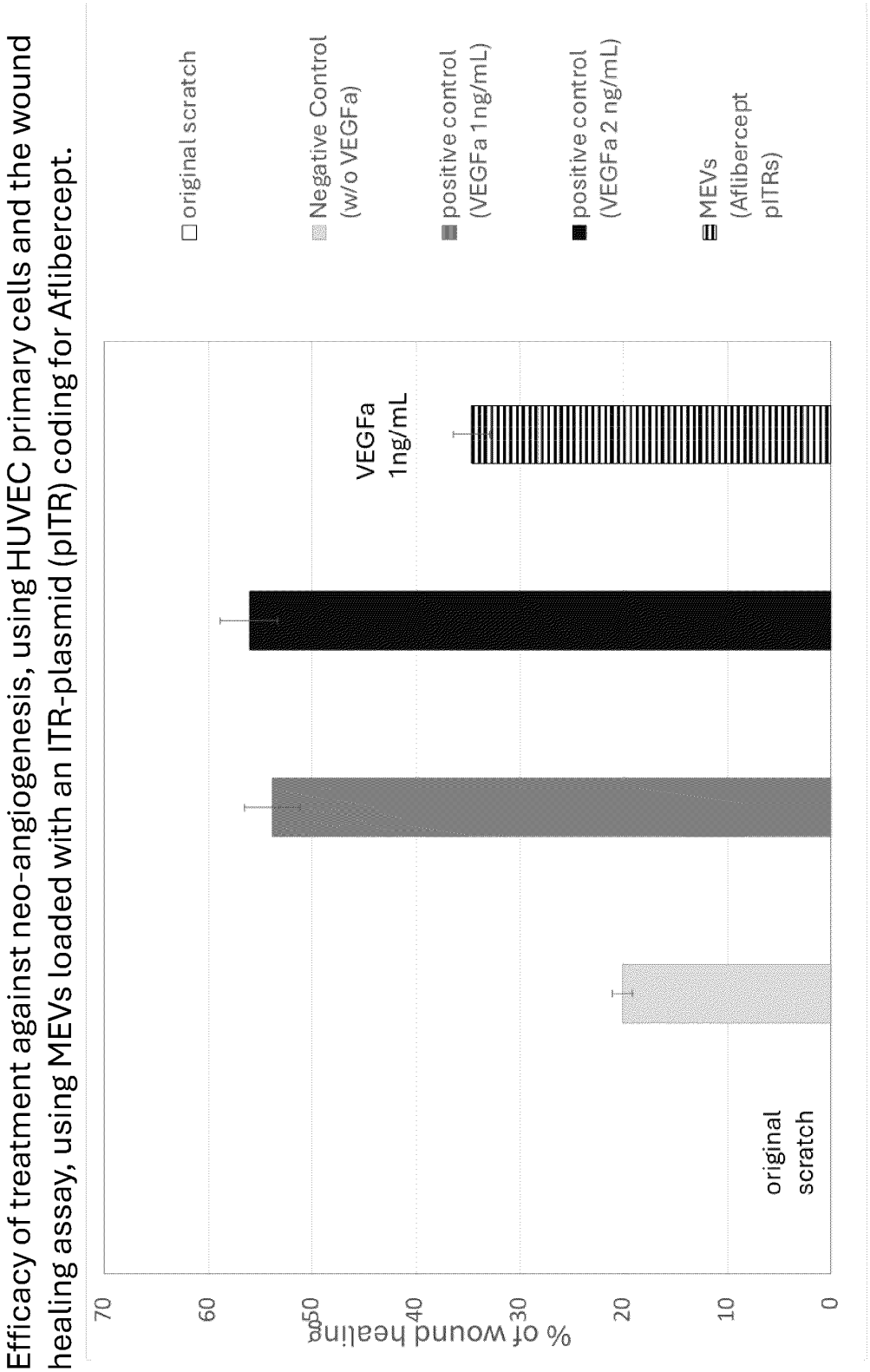

Efficacy of treatment against neo-angiogenesis, using HUVEC primary cells and the wound healing assay, using MEVs loaded with an ITR-plasmid (pITR) coding for Aflibercept.
% of wound healing
70
60
50
40
30
20
10
0
original scratch
VEGFa 1ng/mL
original scratch
Negative Control (w/o VEGFa)
positive control (VEGFa 1ng/mL)
positive control (VEGFa 2 ng/mL)
MEVs (Aflibercept pITRs)

FIG. 36B