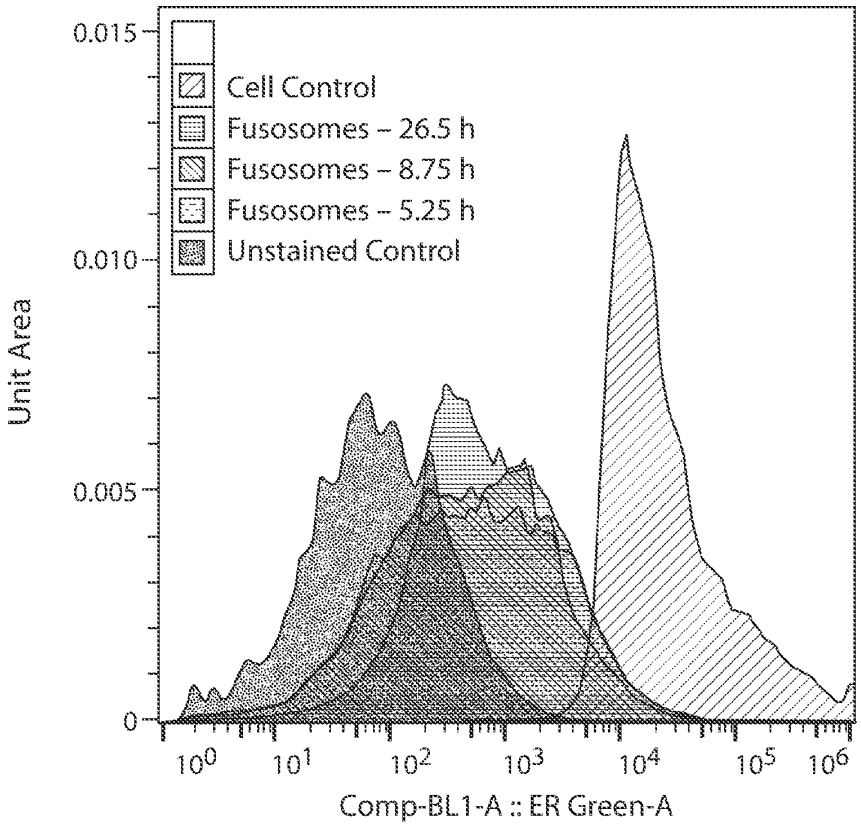

0.015
0.010
0.005
0
Unit Area
Cell Control
Fusosomes – 26.5 h
Fusosomes – 8.75 h
Fusosomes – 5.25 h
Unstained Control
10^0
10^1
10^2
10^3
10^4
10^5
10^6
Comp-BL1-A :: ER Green-A

Fig. 1

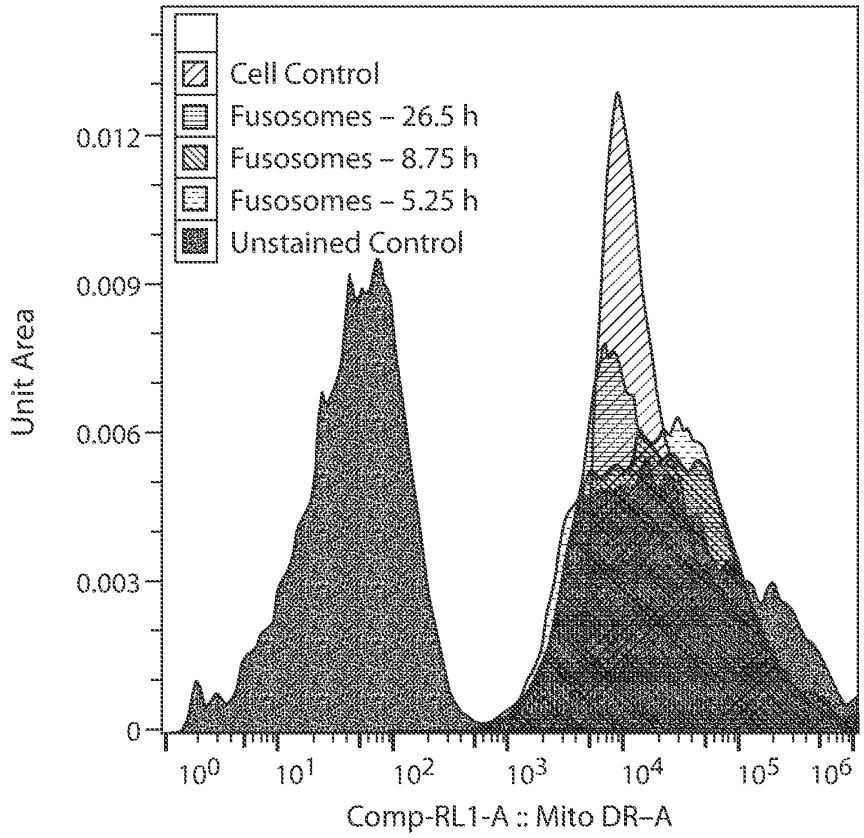

Cell Control
Fusosomes – 26.5 h
Fusosomes – 8.75 h
Fusosomes – 5.25 h
Unstained Control
Unit Area
0.012
0.009
0.006
0.003
0
10^0
10^1
10^2
10^3
10^4
10^5
10^6
Comp-RL1-A :: Mito DR–A

Fig. 2

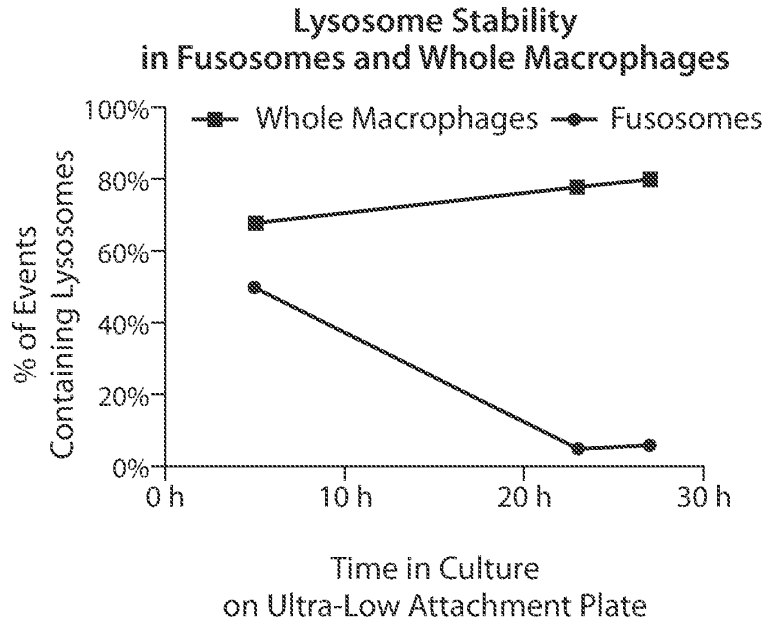

Lysosome Stability
in Fusosomes and Whole Macrophages
Whole Macrophages
Fusosomes
% of Events
Containing Lysosomes
100%
80%
60%
40%
20%
0%
0 h
10 h
20 h
30 h
Time in Culture
on Ultra-Low Attachment Plate

Fig. 3

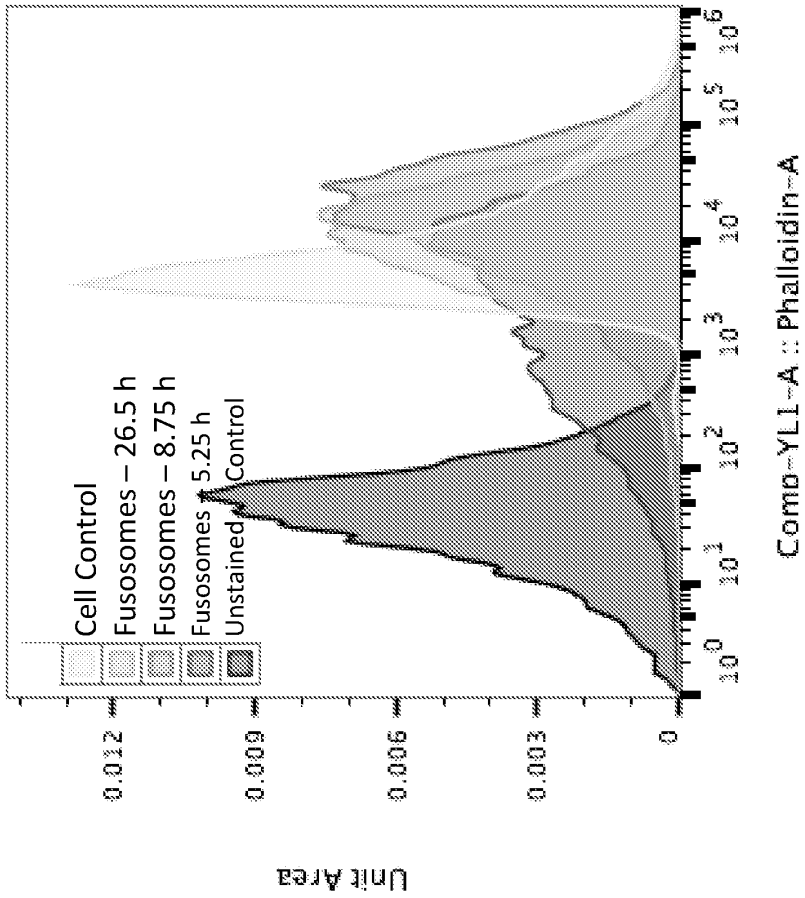

Unit Area
0.012
0.009
0.006
0.003
0
Cell Control
Fusosomes – 26.5 h
Fusosomes – 8.75 h
Fusosomes – 5.25 h
Unstained Control
10^0
10^1
10^2
10^3
10^4
10^5
10^6
Comp-YL1-A :: Phalloidin-A

Fig. 4

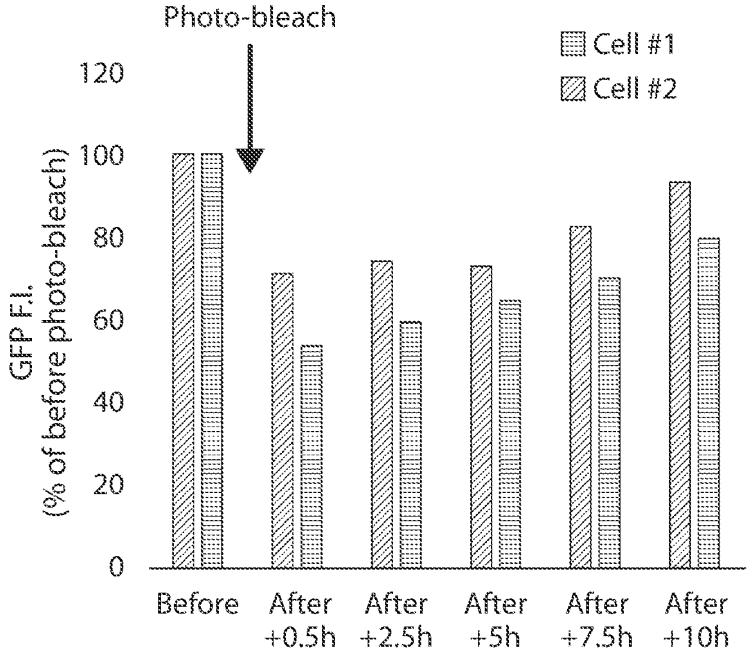
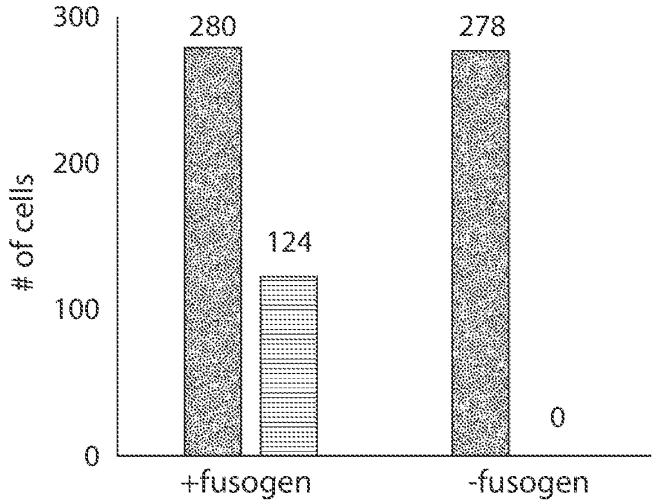

Photo-bleach
Cell #1
Cell #2
GFP F.I.
(% of before photo-bleach)
120
100
80
60
40
20
0
Before
After +0.5h
After +2.5h
After +5h
After +7.5h
After +10h

Fig. 5



Fraction fusosome delivery: 0.44 0.00

Fig. 6

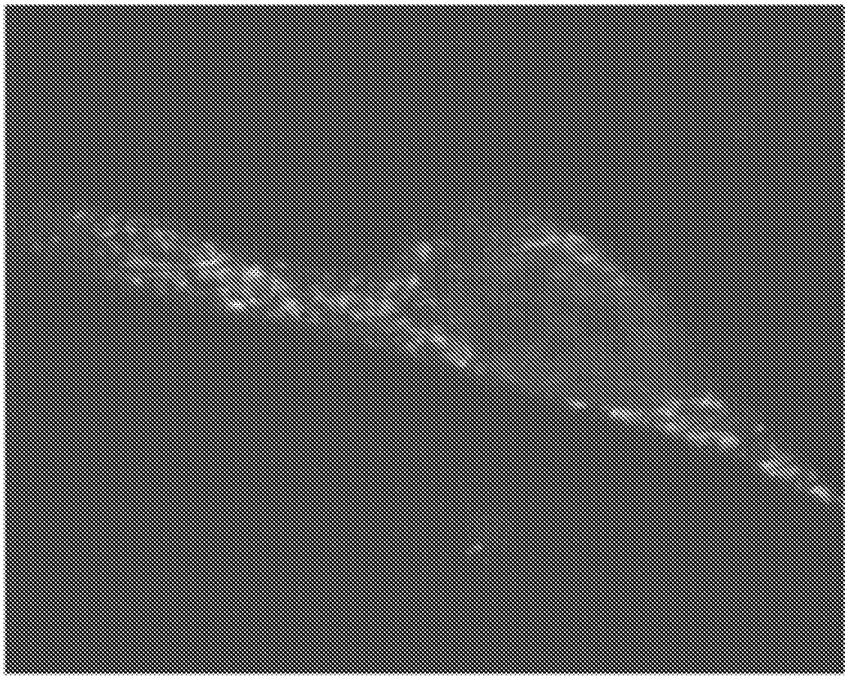


Fig. 7

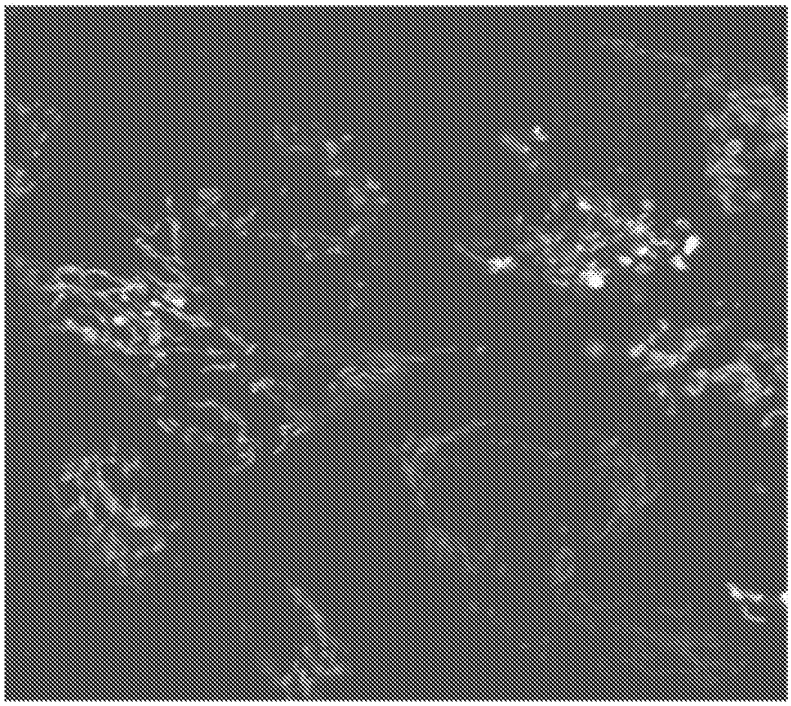


Fig. 8

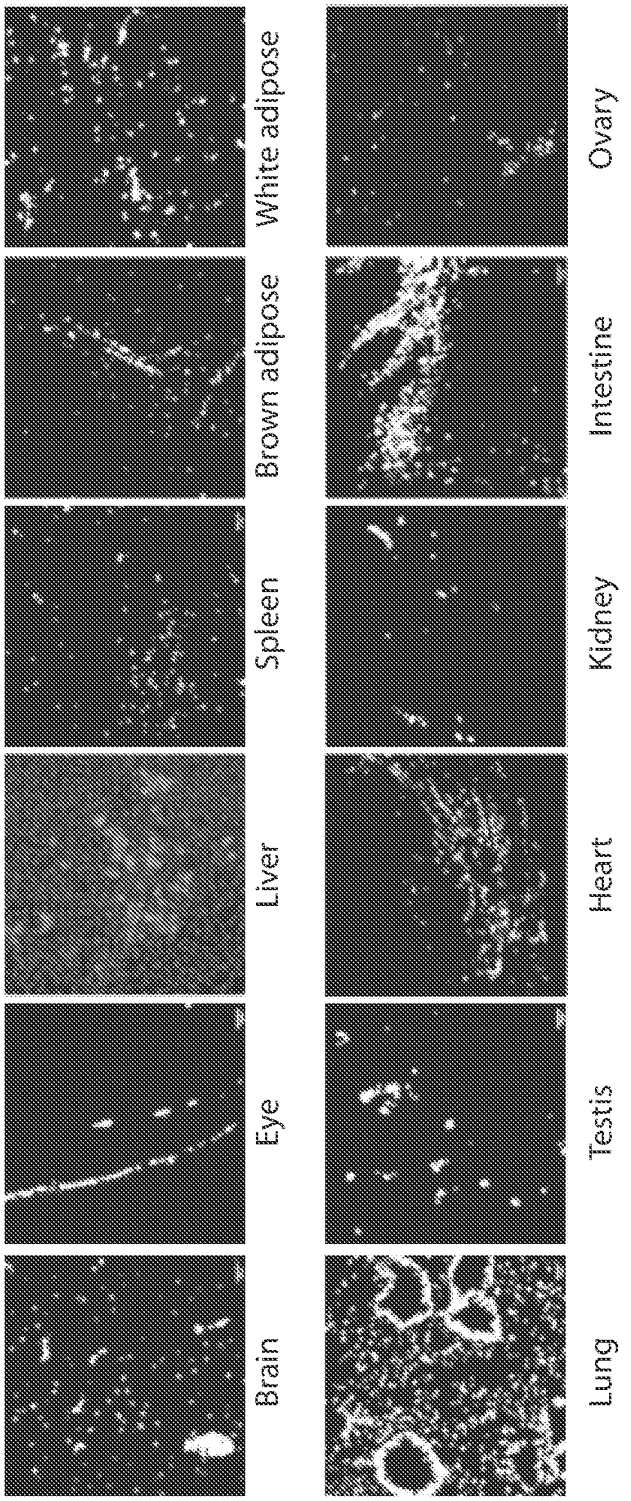

Brain
Eye
Liver
Spleen
Brown adipose
White adipose
Lung
Testis
Heart
Kidney
Intestine
Ovary

Fig. 9

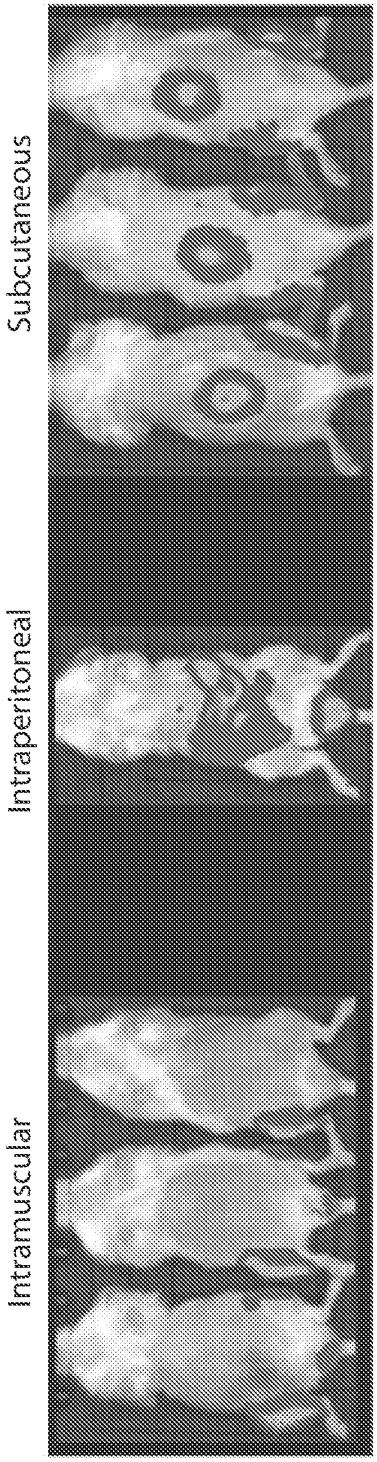


Fig. 10

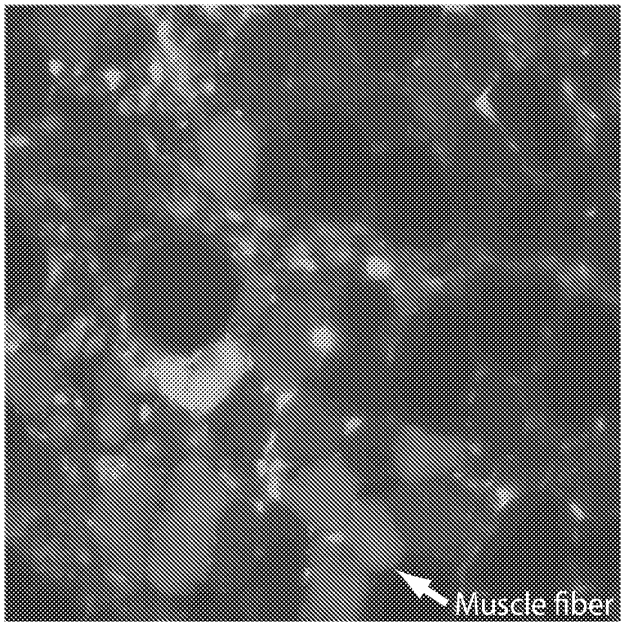


Fig.11

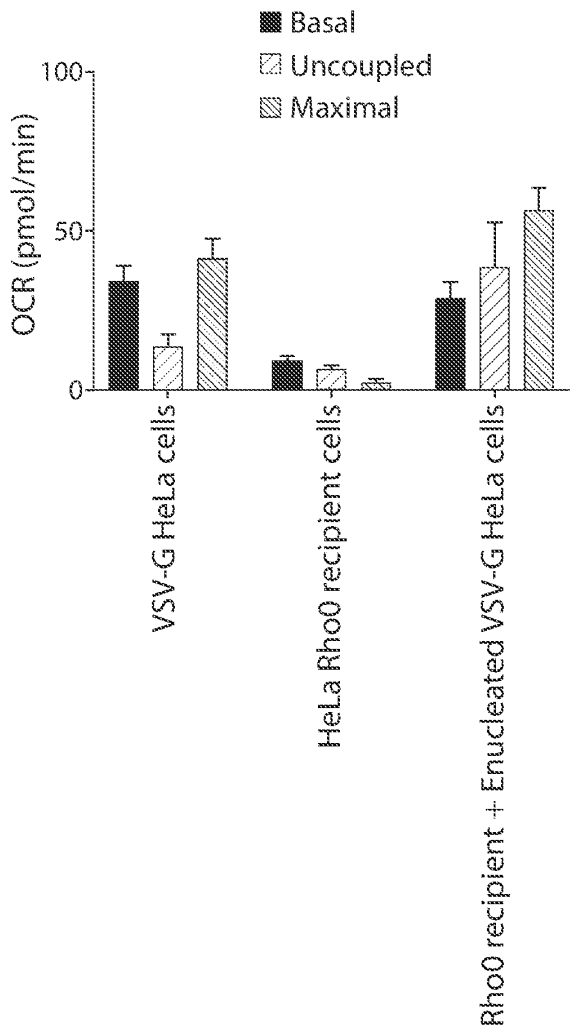

Basal
Uncoupled
Maximal
OCR (pmol/min)
100
50
0
VSV-G HeLa cells
HeLa Rho0 recipient cells
Rho0 recipient + Enucleated VSV-G HeLa cells

Fig. 12

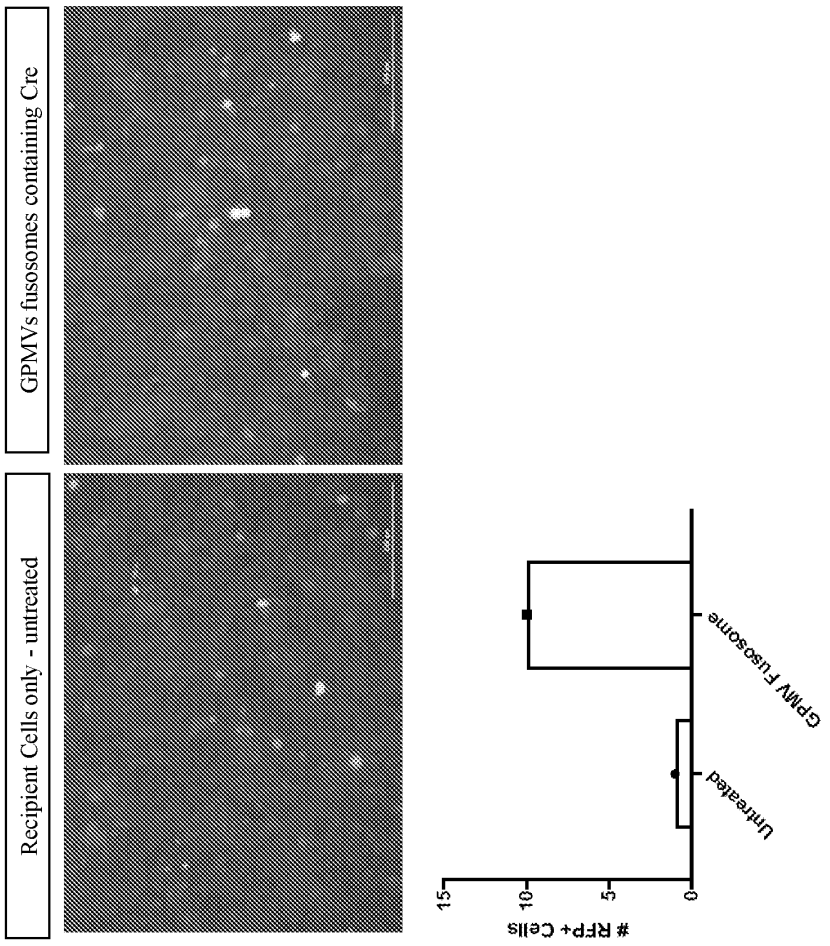

Recipient Cells only - untreated
GPMVs fusosomes containing Cre
RFP+ Cells
15
10
5
0
Untreated
GPMV Fusosome

Fig. 13

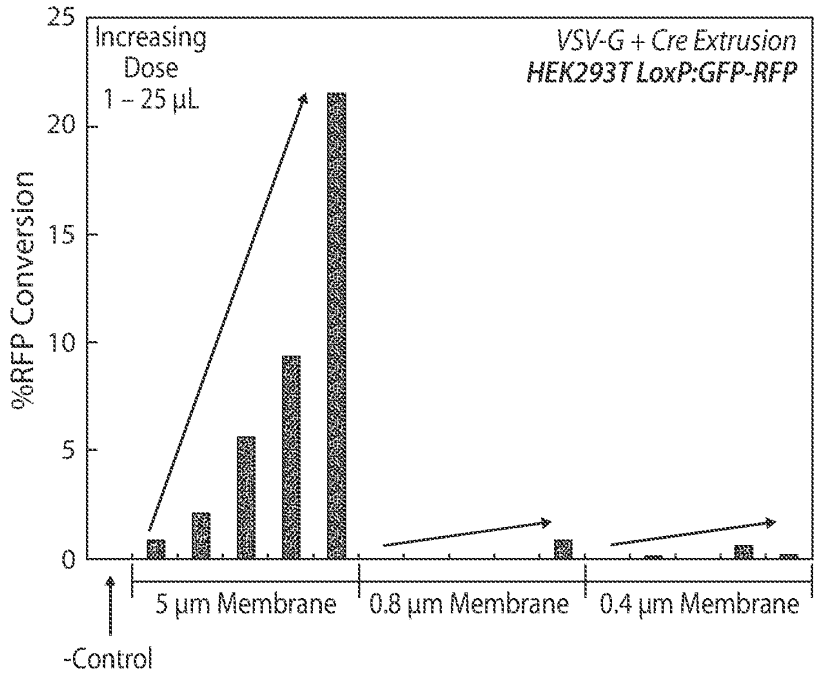

25
20
15
10
5
0
%RFP Conversion
Increasing
Dose
1 – 25 µL
VSV-G + Cre Extrusion
HEK293T LoxP:GFP-RFP
5 µm Membrane
0.8 µm Membrane
0.4 µm Membrane
-Control

Fig.14A

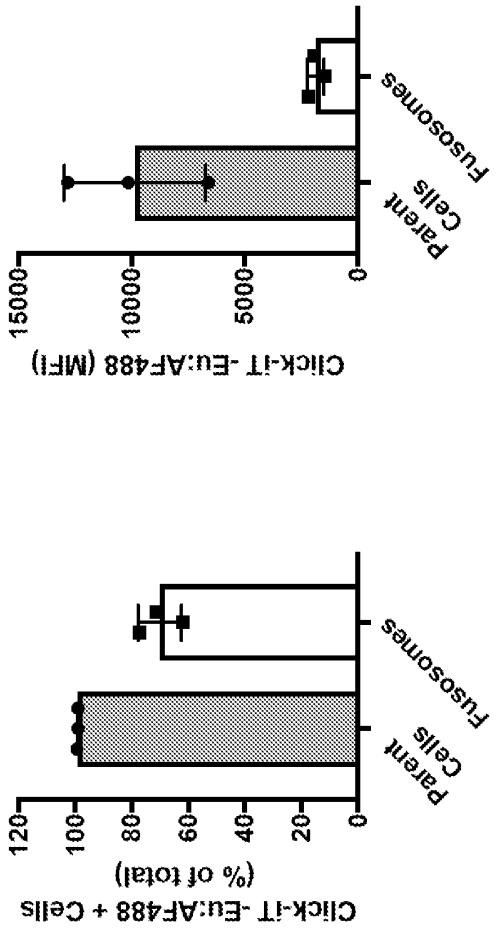

Click-iT -Eu:AF488 (MFI)
15000
10000
5000
0
Parent Cells
Fusosomes
Click-iT -Eu:AF488 + Cells
(% of total)
120
100
80
60
40
20
0
Parent Cells
Fusosomes

Fig. 14B

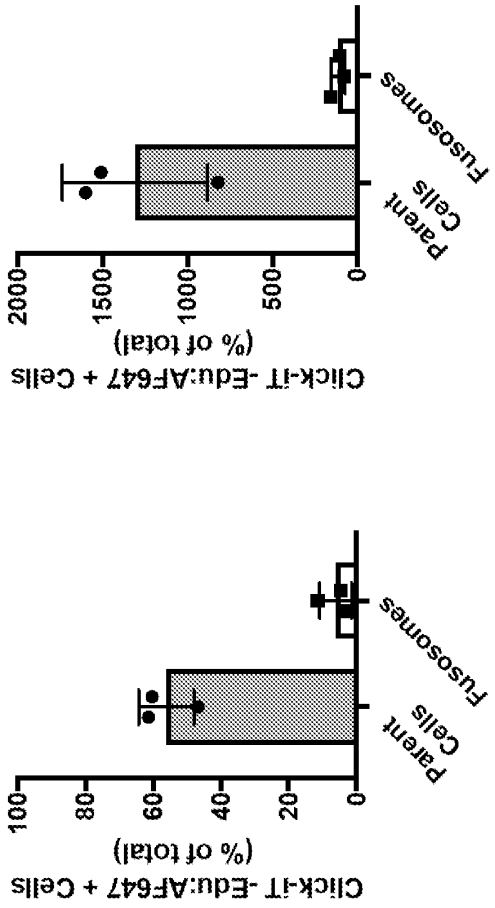

Click-iT -Edu:AF647 + Cells
(% of total)
2000
1500
1000
500
0
Parent Cells
Fusosomes
Click-iT -Edu:AF647 + Cells
(% of total)
100
80
60
40
20
0
Parent Cells
Fusosomes

Fig. 14C

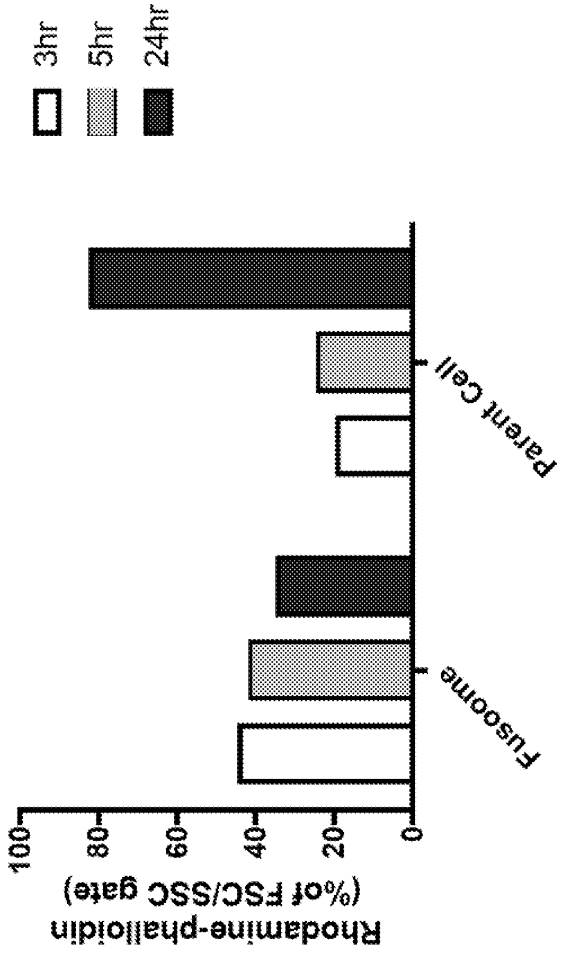

3hr
5hr
24hr
Rhodamine-phalloidin
(%of FSC/SSC gate)
100
80
60
40
20
0
Fusosome
Parent Cell

Fig. 14D

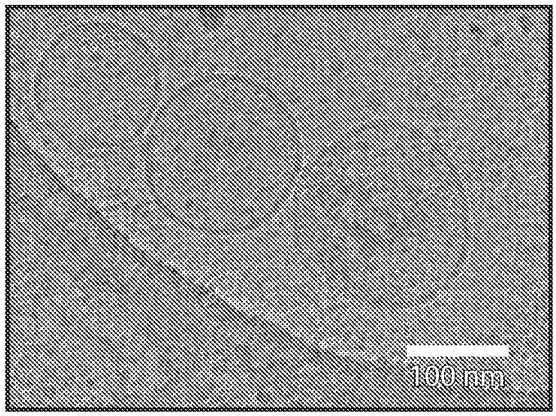


Fig. 15

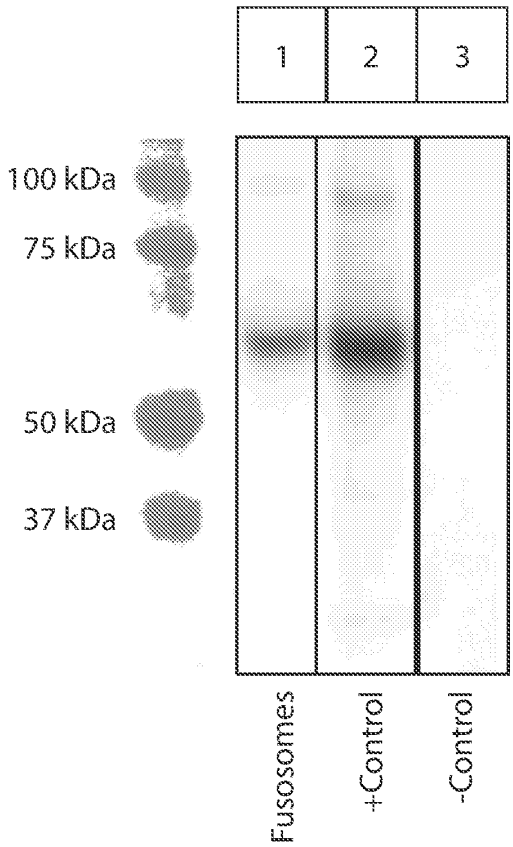


Fig. 16

Nanosight NS300 Conditions		Nanosight NS300 Settings	
Temperature/C	24.089	Detection Threshold	17
Viscosity/cP	0.907452	Max Jump Mode	Auto
Camera Type	sCMOS	Max Jump Distance	24.9209
Laser Type	Green	Blur	Auto
Camera Level	15	Min Track Length	Auto
Slider shutter	1206	First frame	0
Slider Gain	366	Total frames analysed	1498
Shutter/ms	30.15		
Camera Histogram Upper Limit	3294		
Camera Histogram Lower Limit	0		
Frame rate/fps	24.9825		
Syringe Pump Speed/AU	50		

Fig. 17A

Confocal Microscope Settings	
Objective	63X
Excitation (nm)	488
Emission (nm)	556

Fig. 17B

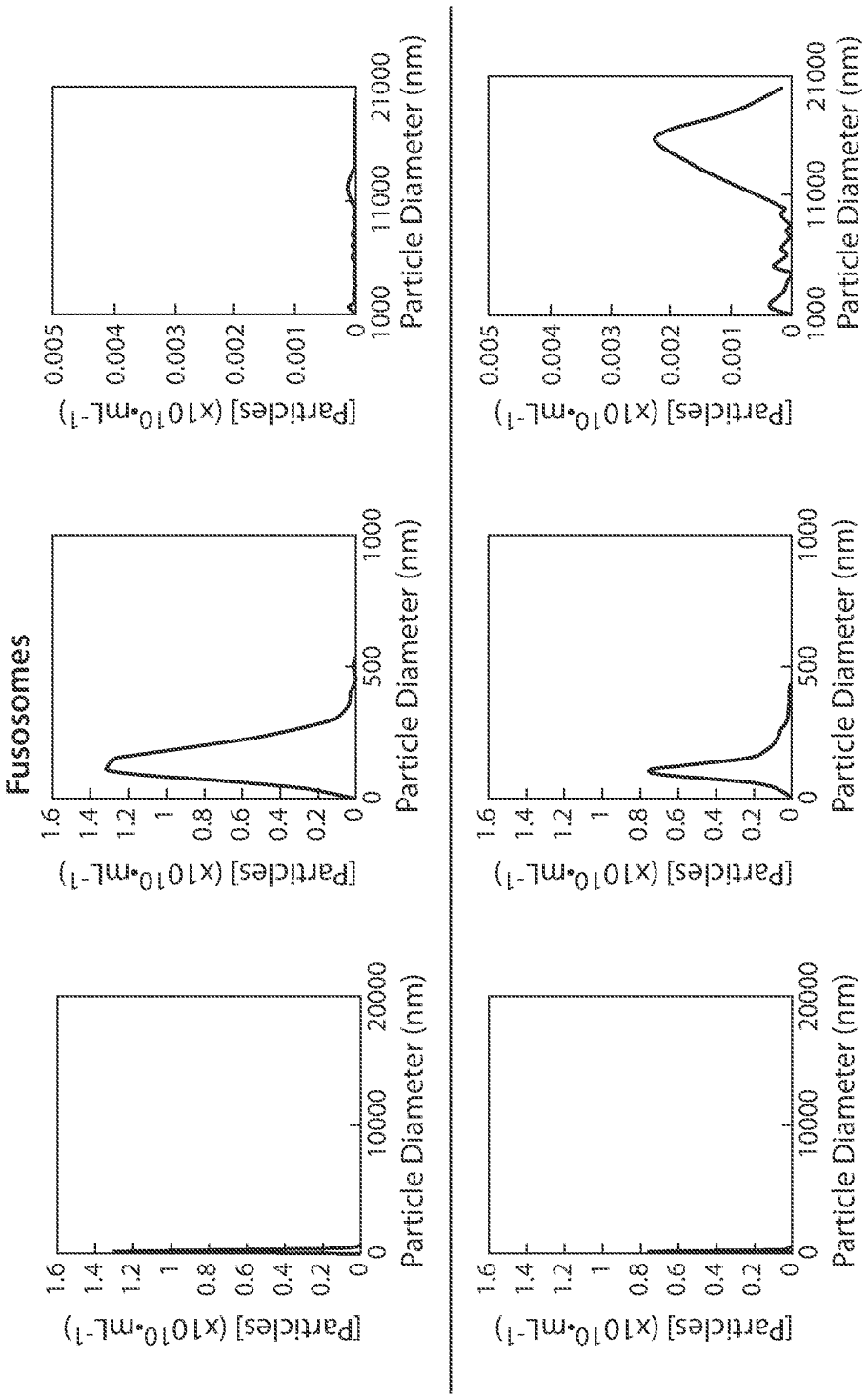

Fusosomes
Parental Cells
[Particles] (x10^10•mL^-1)
Particle Diameter (nm)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
0
500
1000
10000
20000
0.001
0.002
0.003
0.004
0.005
11000
21000

Fig. 17C

	Fusosomes	Parental Cells
Average diameter (nm)	128.7	175.4
Average volume (μm^3)	0.067	7.421

Fig.17D

	Fusosomes	Parental Cells
Average diameter (nm)	128.7	175.4
Min Diameter (nm)	14	29
Max Diameter (nm)	18720	19802
Median Diameter (nm)	134	99
10% Quantile (nm)	53	52
25% Quantile (nm)	88	66
75% Quantile (nm)	226	241
90% Quantile (nm)	4450	10649
Average volume (μm^3)	0.067	7.421

Fig. 18

	Fusosomes	Parental Cells
Average diameter (nm)	128.7	175.4
Average volume (μm^3)	0.067	7.421

Fig. 19

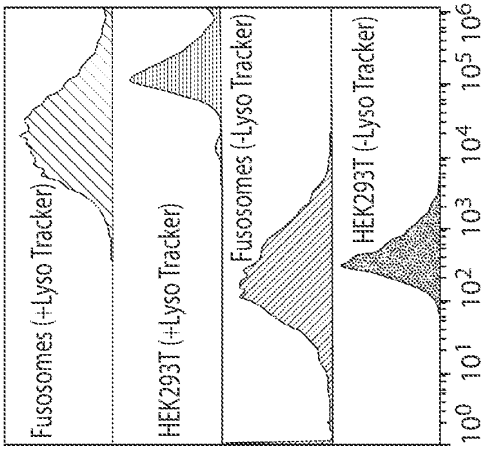


Fig. 20C

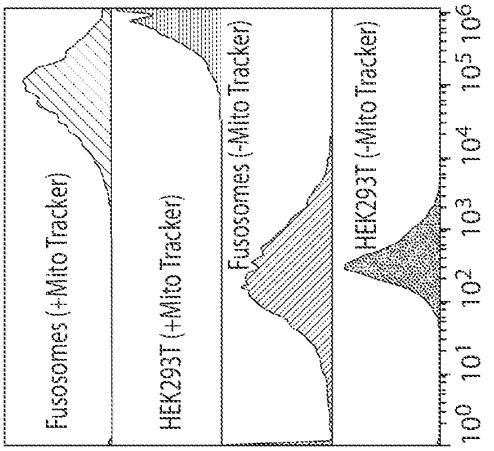


Fig. 20B

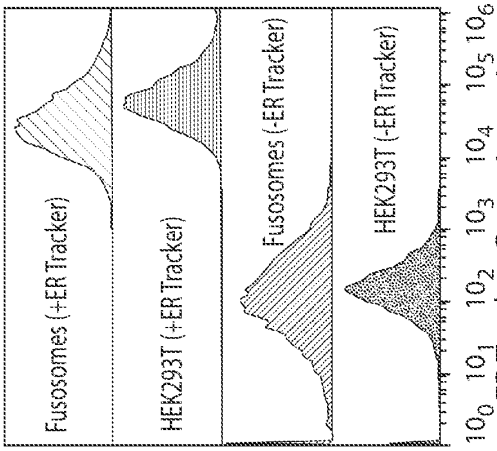


Fig. 20A

	[Protein] (mg/mL)		Ratio
	Insoluble	Soluble	
Cells	2.12	0.06	0.028
Fusosomes	1.28	0.72	0.563

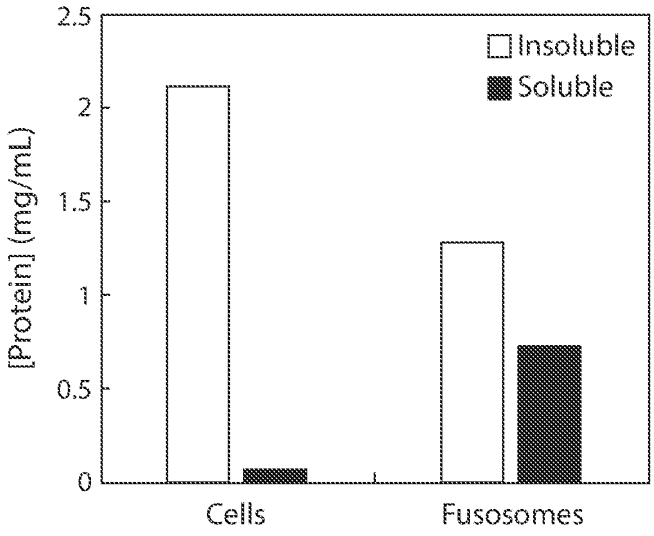
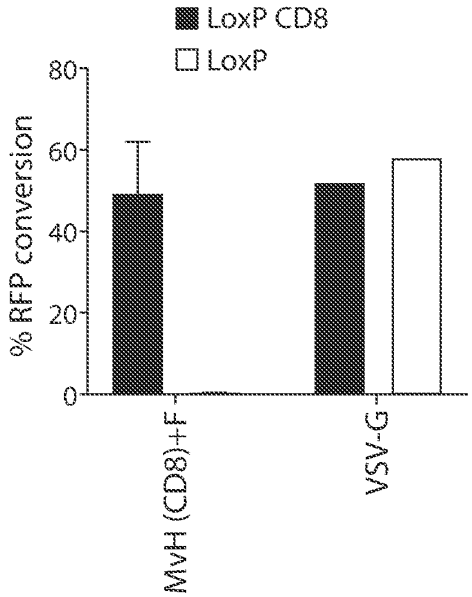


Fig. 21



Fusosome	Absolute amount of targeted fusion
MvH(CD8)+F	48.8%
VSV-G	0%

Fig. 22

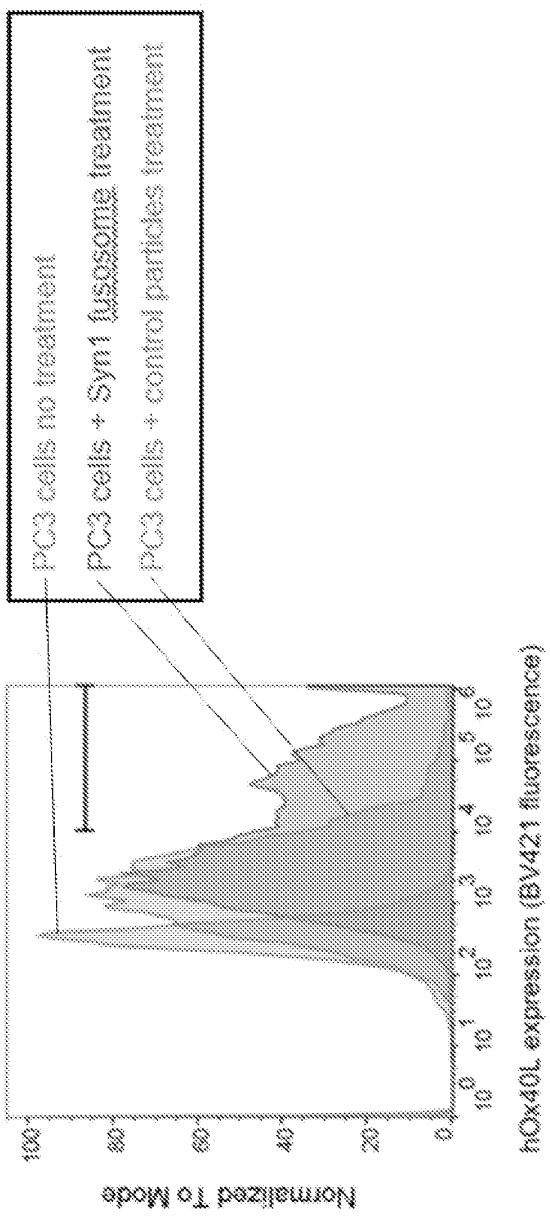

PC3 cells no treatment
PC3 cells + Sym1 liposome treatment
PC3 cells + control particles treatment
Normalized To Mode
100
80
60
40
20
0
10^0
10^1
10^2
10^3
10^4
10^5
10^6
hOx40L expression (BV421 fluorescence)

Fig. 23

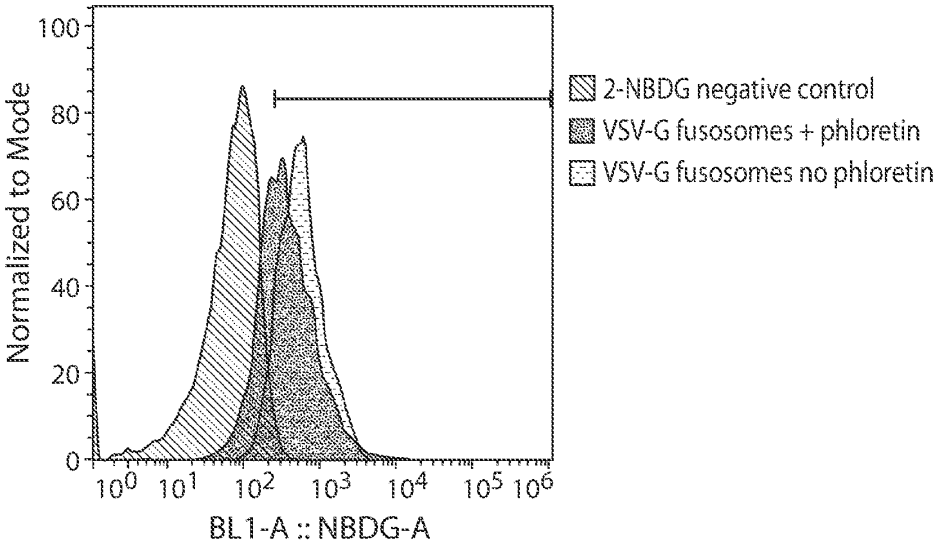

100
80
60
40
20
0
Normalized to Mode
10^0 10^1 10^2 10^3 10^4 10^5 10^6
BL1-A :: NBDG-A
2-NBDG negative control
VSV-G fusosomes + phloretin
VSV-G fusosomes no phloretin

Fig. 24

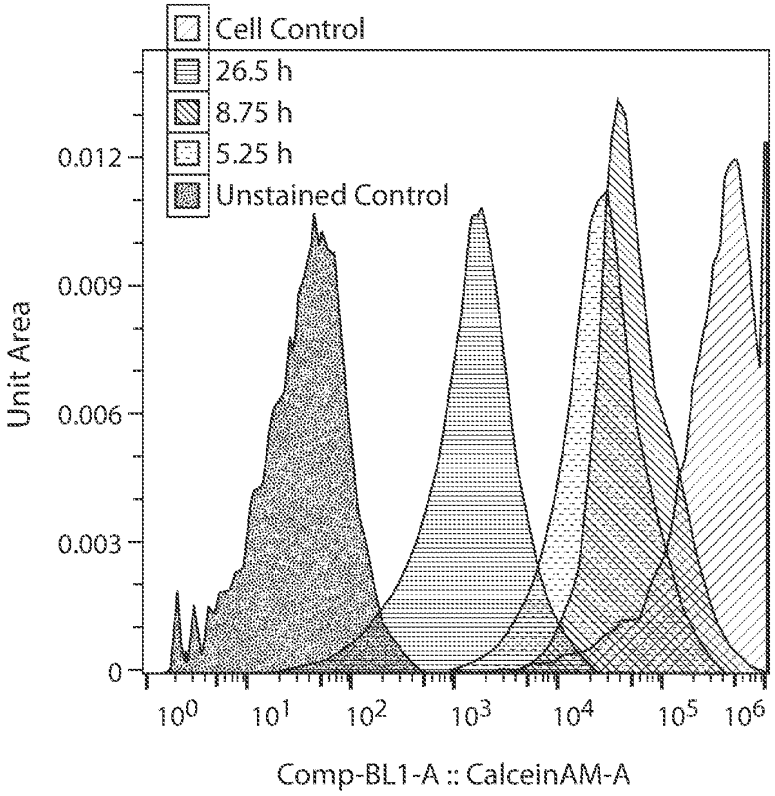

Cell Control
26.5 h
8.75 h
5.25 h
Unstained Control
Unit Area
0.012
0.009
0.006
0.003
0
10^0
10^1
10^2
10^3
10^4
10^5
10^6
Comp-BL1-A :: CalceinAM-A

Fig. 25

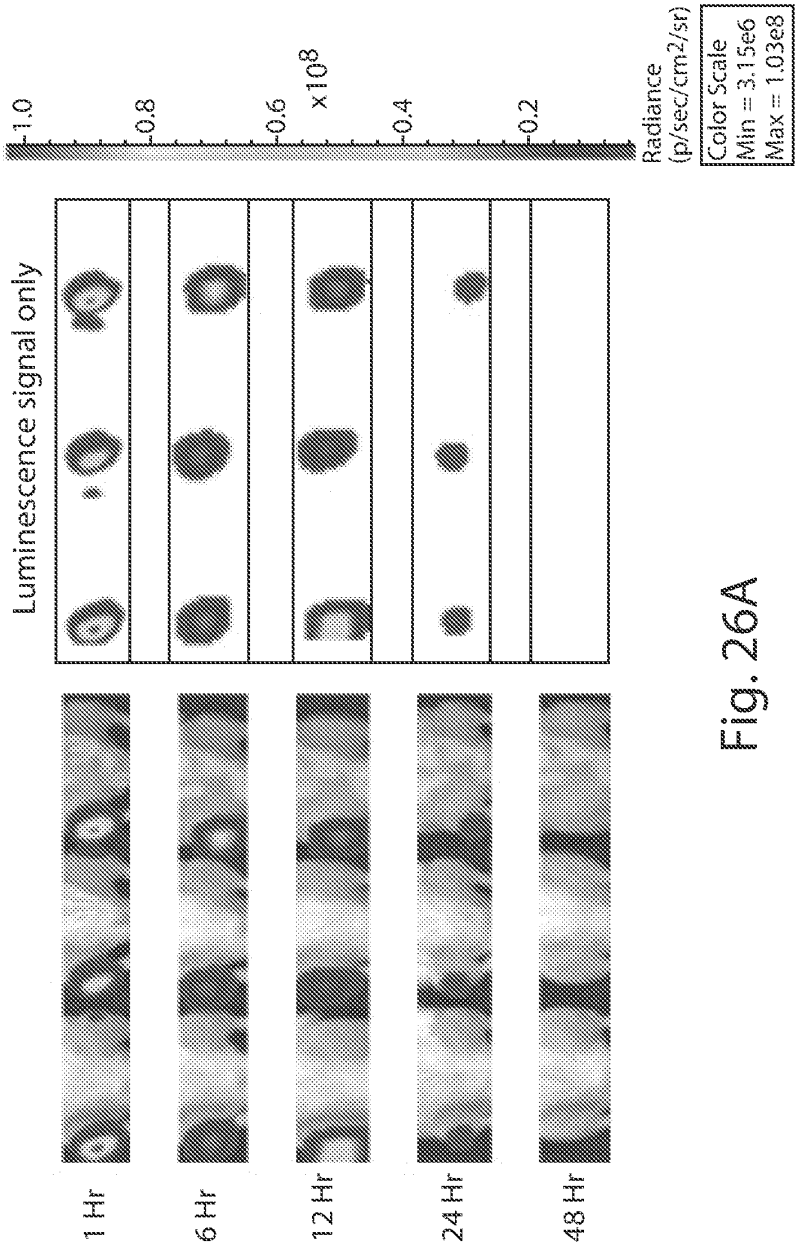

Luminescence signal only
1 Hr
6 Hr
12 Hr
24 Hr
48 Hr
1.0
0.8
0.6
0.4
0.2
x10^8
Radiance (p/sec/cm2/sr)
Color Scale
Min = 3.15e6
Max = 1.03e8

Fig. 26A

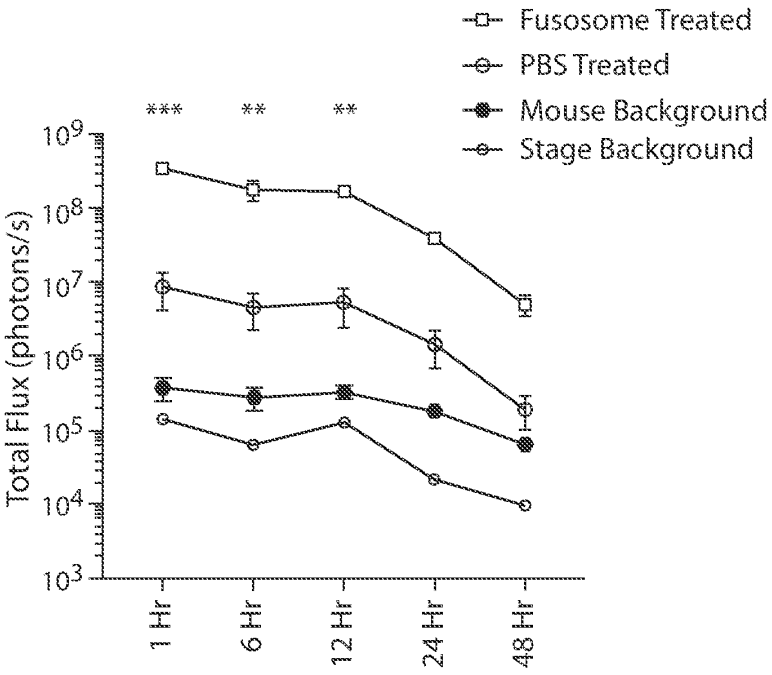

Fusosome Treated
PBS Treated
Mouse Background
Stage Background
***
**
**
Total Flux (photons/s)
10^9
10^8
10^7
10^6
10^5
10^4
10^3
1 Hr
6 Hr
12 Hr
24 Hr
48 Hr

Fig. 26B

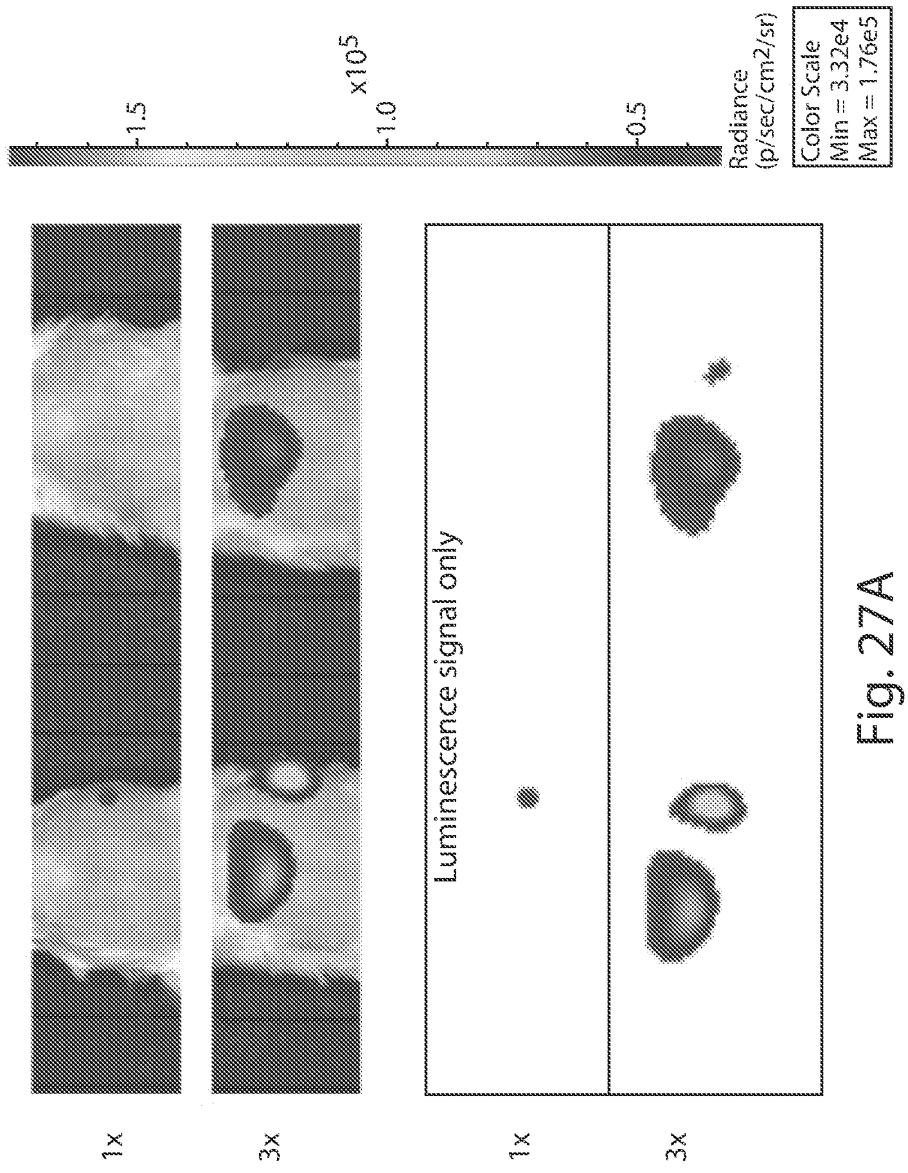

1.5
x10^5
1.0
0.5
Radiance (p/sec/cm2/sr)
Color Scale
Min = 3.32e4
Max = 1.76e5
Luminescence signal only
1x
3x
1x
3x

Fig. 27A

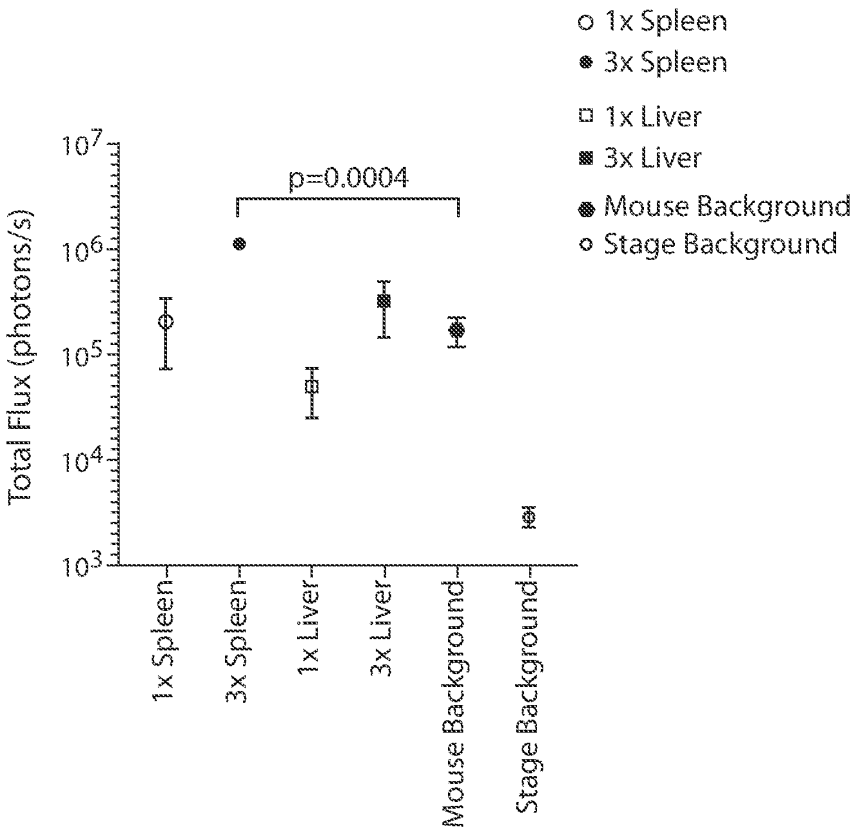

1x Spleen
3x Spleen
1x Liver
3x Liver
Mouse Background
Stage Background
p=0.0004
Total Flux (photons/s)
10^7
10^6
10^5
10^4
10^3
1x Spleen
3x Spleen
1x Liver
3x Liver
Mouse Background
Stage Background

Fig. 27B

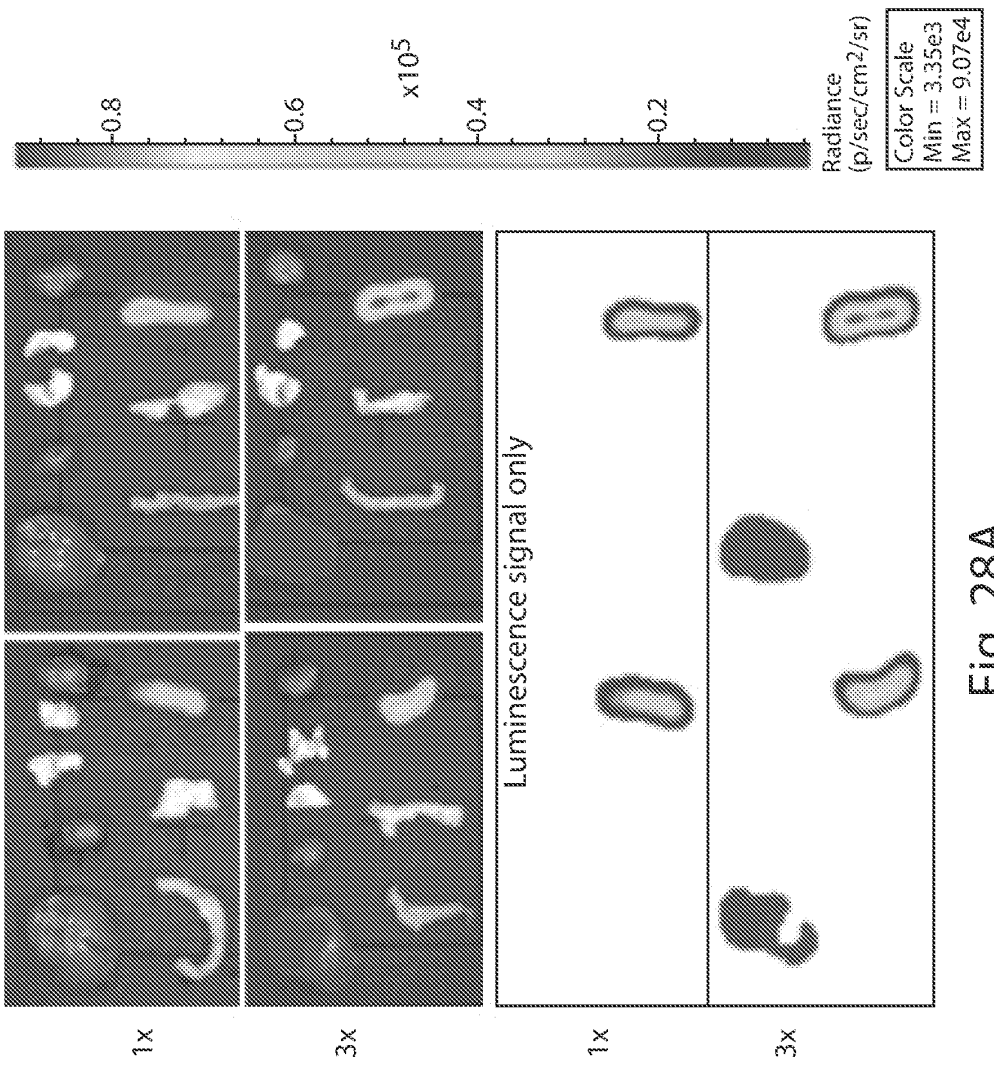

0.8
0.6
x10^5
0.4
0.2
Radiance (p/sec/cm2/sr)
Color Scale
Min = 3.35e3
Max = 9.07e4
Luminescence signal only
1x
3x
1x
3x

Fig. 28A

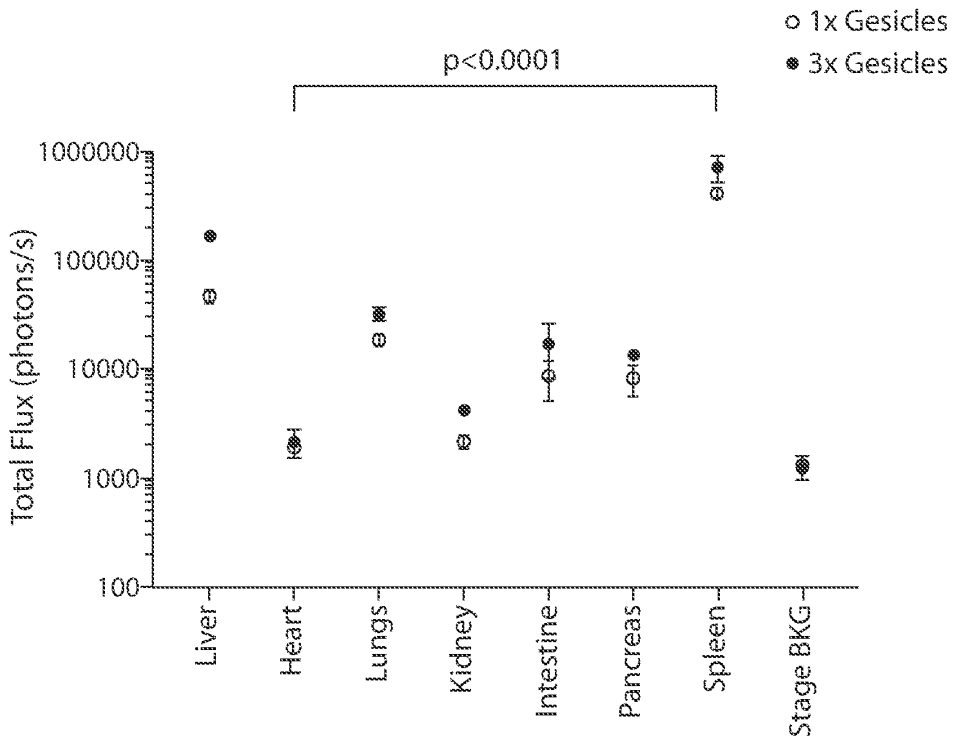
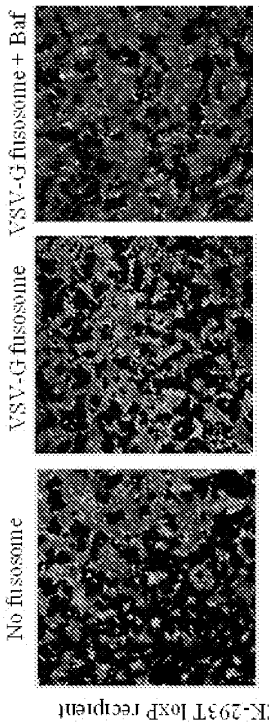

1x Gesicles
3x Gesicles
p<0.0001
Total Flux (photons/s)
1000000
100000
10000
1000
100
Liver
Heart
Lungs
Kidney
Intestine
Pancreas
Spleen
Stage BKG

Fig. 28B

Group	% RFP conversion (± SD)
Recipient + no fusosome	0.4 ± 0.2%
Recipient + NivG+F fusosome	88.9 ± 3.4%
Recipient + NivG+F fusosome + Baf	68.1 ± 2.7%

Fig. 29



Group	% RFP conversion (± SD)
Recipient + no fusosome	0.6 ± 0.2%
Recipient + VSV-G fusosome	53.2 ± 0.7%
Recipient + VSV-G fusosome + Baf	2.3 ± 1.2%

Fig. 30

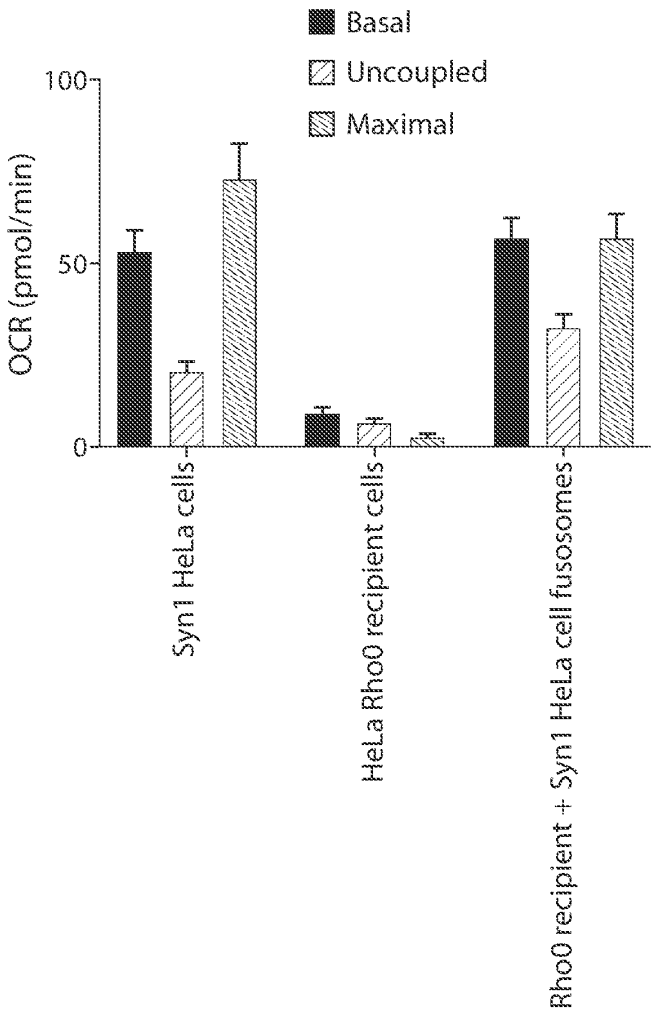

Basal
Uncoupled
Maximal
OCR (pmol/min)
100
50
0
Syn1 HeLa cells
HeLa Rho0 recipient cells
Rho0 recipient + Syn1 HeLa cell fusosomes

Fig. 31

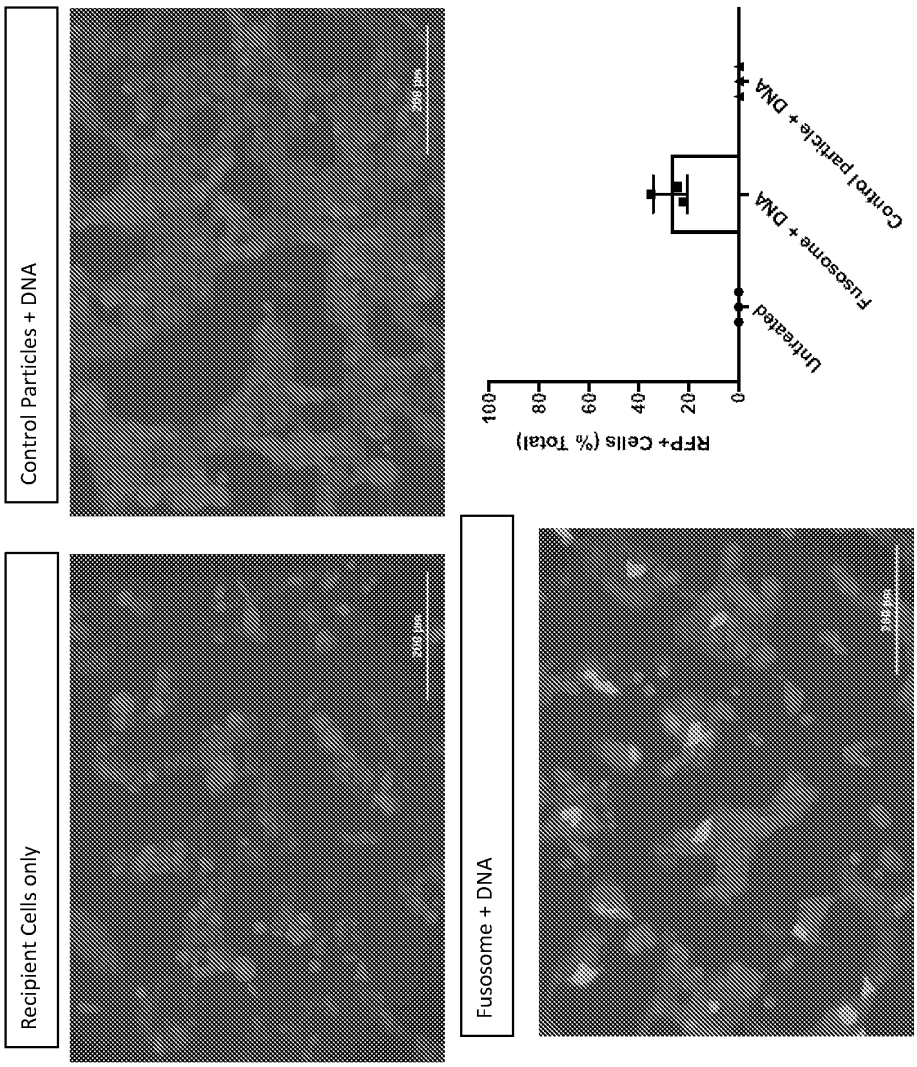

Recipient Cells only
Control Particles + DNA
Fusosome + DNA
RFP+ Cells (% Total)
100
80
60
40
20
0
Untreated
Fusosome + DNA
Control particle + DNA

Fig. 32

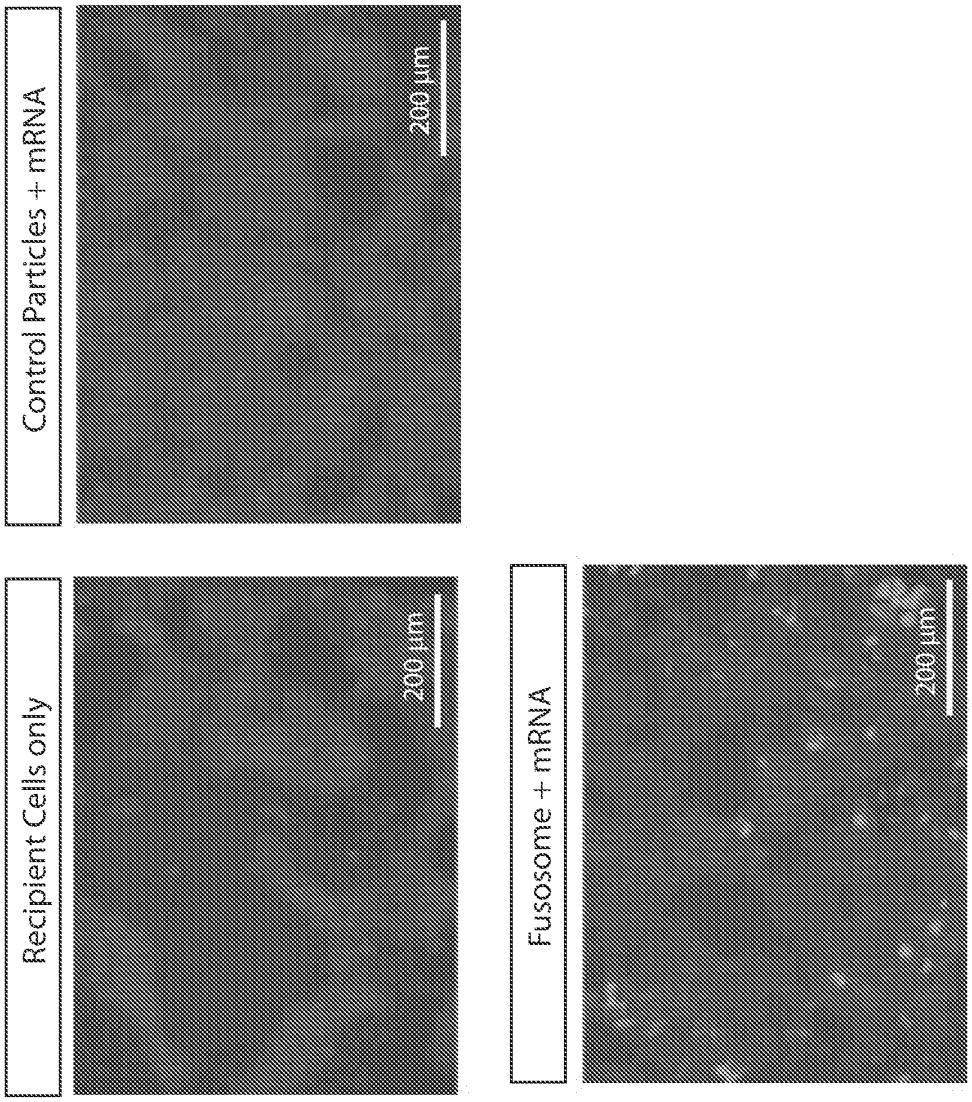

Control Particles + mRNA
200 µm
Recipient Cells only
200 µm
Fusosome + mRNA
200 µm

Fig. 33

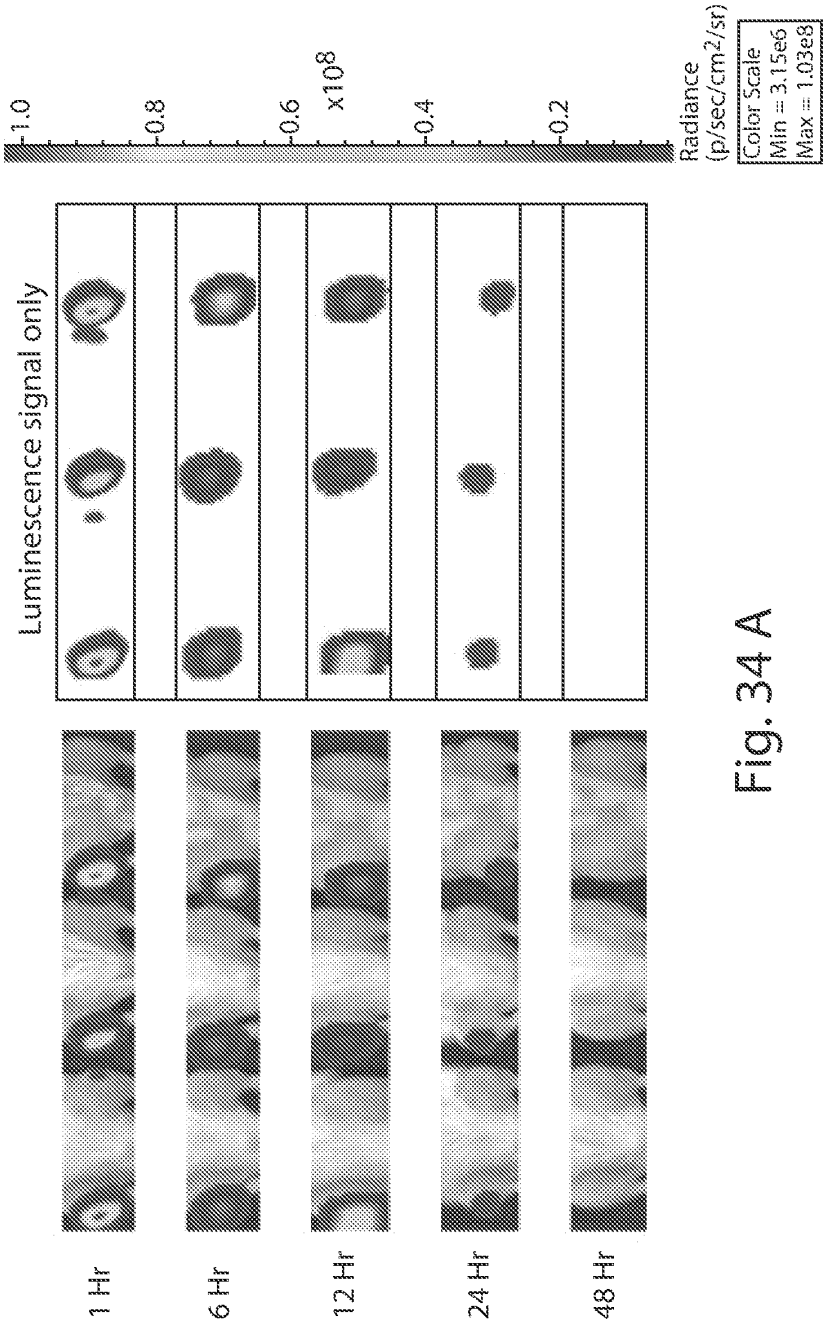

1.0
0.8
0.6
x10^8
0.4
0.2
Radiance (p/sec/cm²/sr)
Color Scale
Min = 3.15e6
Max = 1.03e8
Luminescence signal only
1 Hr
6 Hr
12 Hr
24 Hr
48 Hr

Fig. 34 A

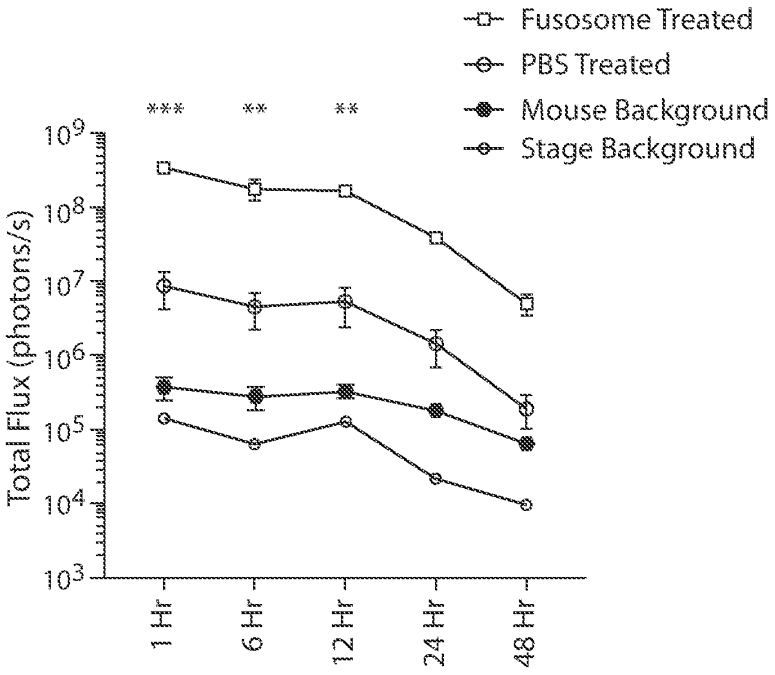
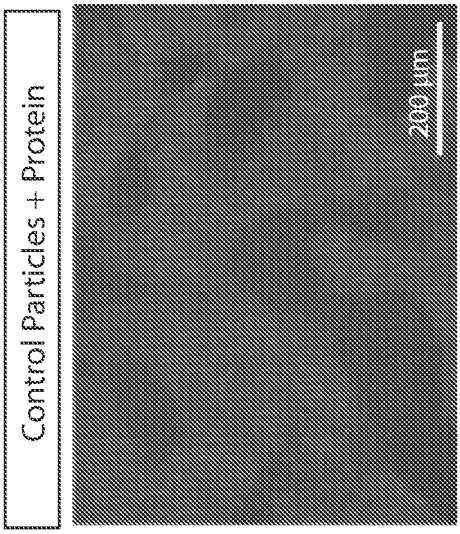
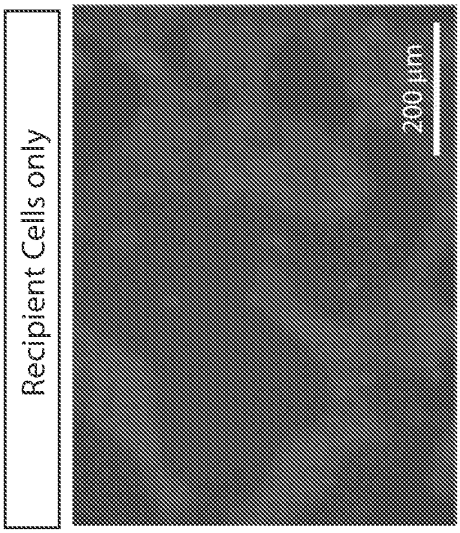

Fusosome Treated
PBS Treated
Mouse Background
Stage Background
***
**
**
Total Flux (photons/s)
10^9
10^8
10^7
10^6
10^5
10^4
10^3
1 Hr
6 Hr
12 Hr
24 Hr
48 Hr

Fig. 34B


Control Particles + Protein
200 μm


Recipient Cells only
200 μm

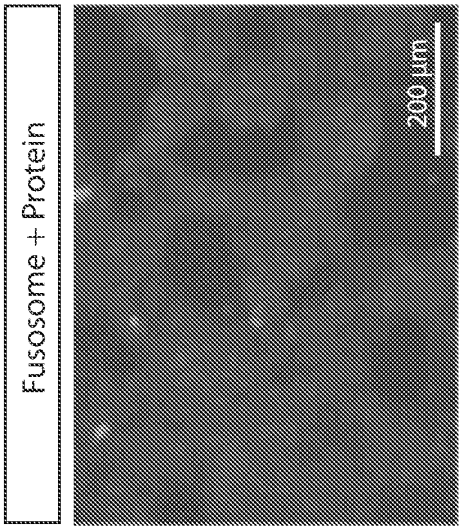

Fusosome + Protein
200 μm

Fig. 35

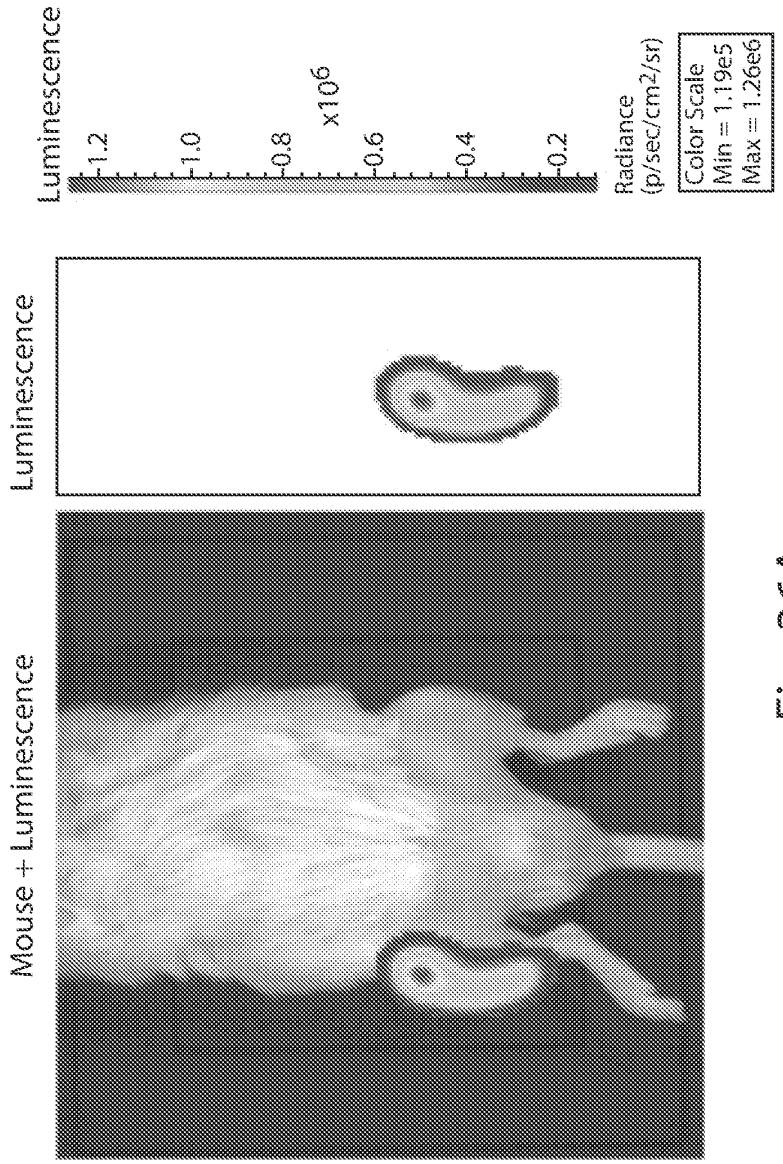

Mouse + Luminescence
Luminescence
Luminescence
1.2
1.0
0.8
x10^6
0.6
0.4
0.2
Radiance (p/sec/cm²/sr)
Color Scale
Min = 1.19e5
Max = 1.26e6

Fig. 36A

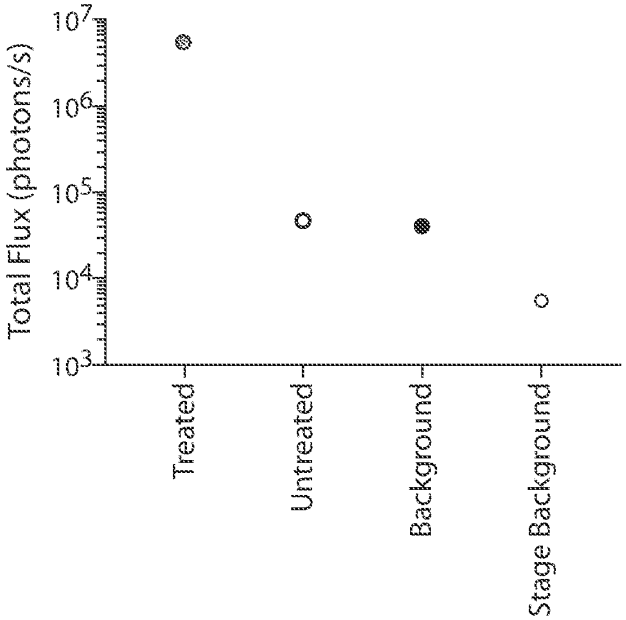

Total Flux (photons/s)
10^7
10^6
10^5
10^4
10^3
Treated
Untreated
Background
Stage Background

Fig. 36B

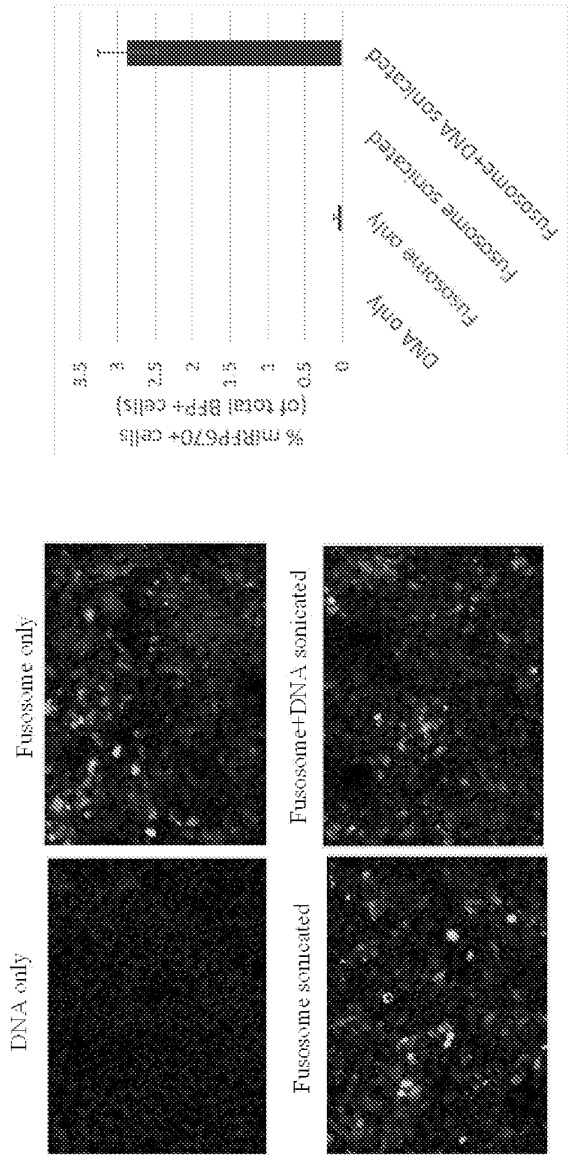

% mRFP670+ cells
(of total BFP+ cells)
3.5
3
2.5
2
1.5
1
0.5
0
DNA only
Fusosome only
Fusosome sonicated
Fusosome+DNA sonicated
DNA only
Fusosome only
Fusosome sonicated
Fusosome+DNA sonicated

Fig. 37

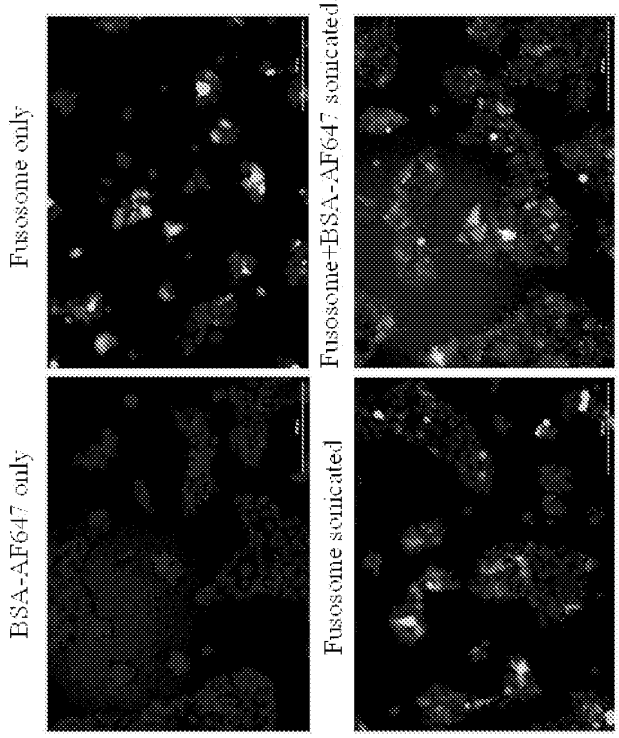

BSA-AF647 only
Fusosome only
Fusosome sonicated
Fusosome+BSA-AF647 sonicated
BSA-AF647 only
Fusosome only
Fusosome sonicated
Fusosome+BSA-AF647 sonicated
0
5
10
15
20
25
30
35
40
45
50
% BSA-AF647+ cells
(of total BFP+ cells)

Fig. 38

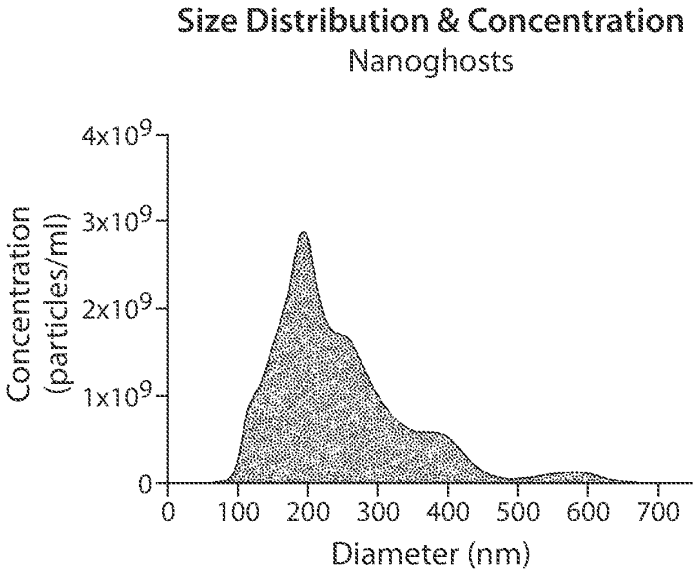

Size Distribution & Concentration
Nanoghosts
Concentration (particles/ml)
4x10^9
3x10^9
2x10^9
1x10^9
0
0
100
200
300
400
500
600
700
Diameter (nm)

Fig. 39

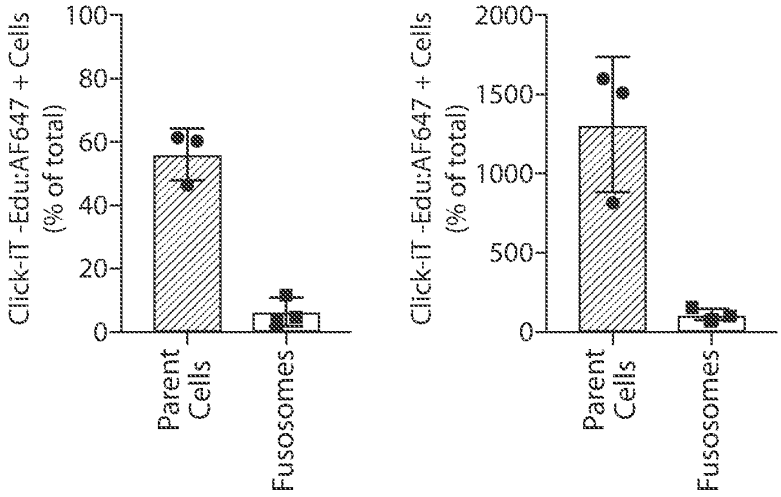

Click-iT -Edu:AF647 + Cells
(% of total)
100
80
60
40
20
0
Parent Cells
Fusosomes
Click-iT -Edu:AF647 + Cells
(% of total)
2000
1500
1000
500
0
Parent Cells
Fusosomes

Fig. 40

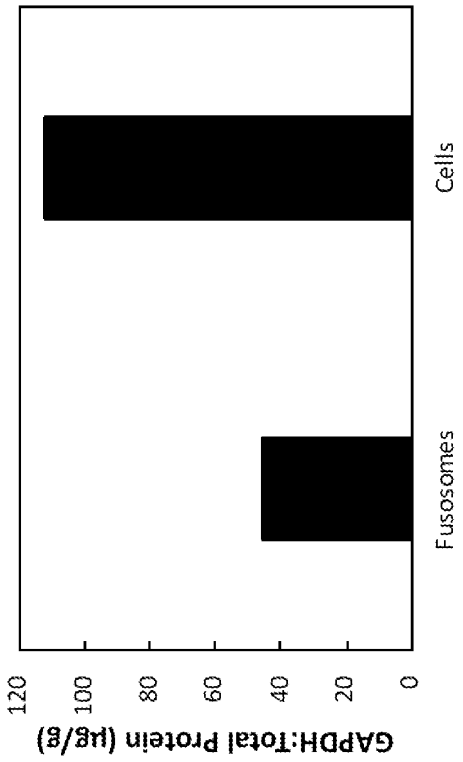


Fig. 41

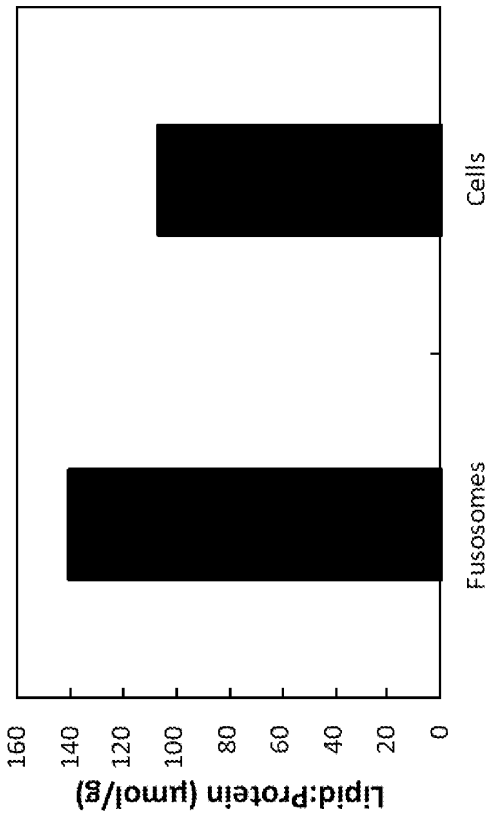

Lipid:Protein (μmol/g)
0
20
40
60
80
100
120
140
160
Fusosomes
Cells

Fig. 42

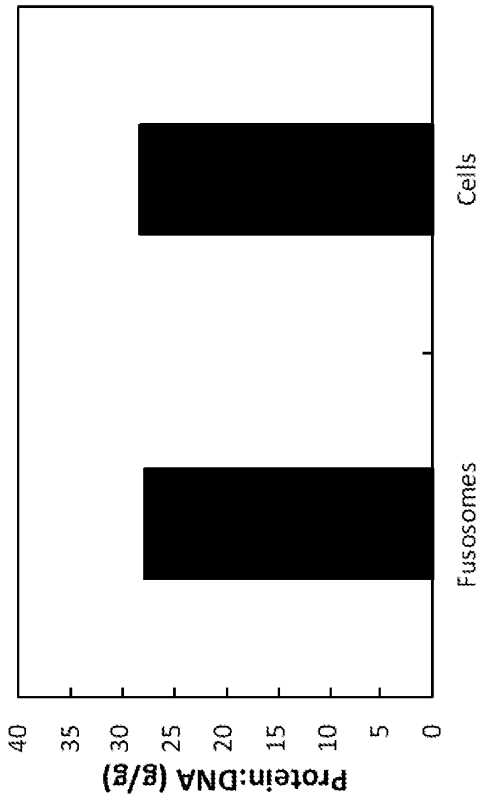


Fig. 43

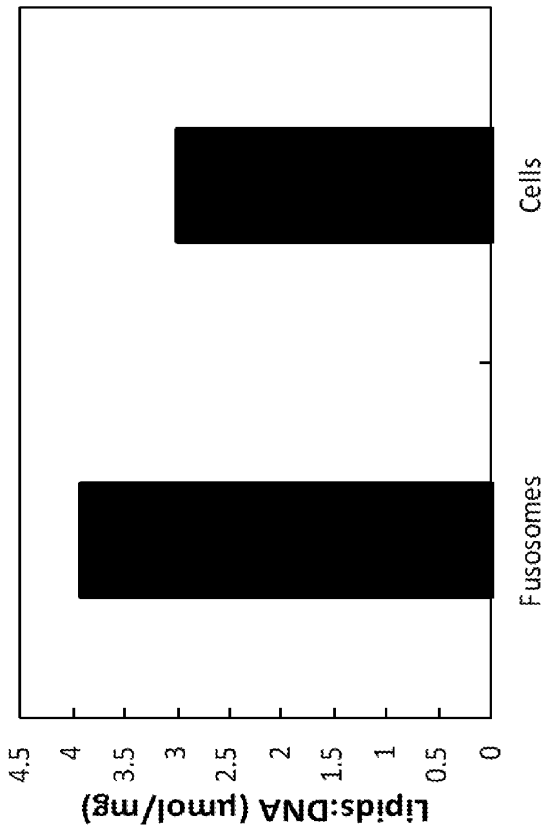


Fig. 44

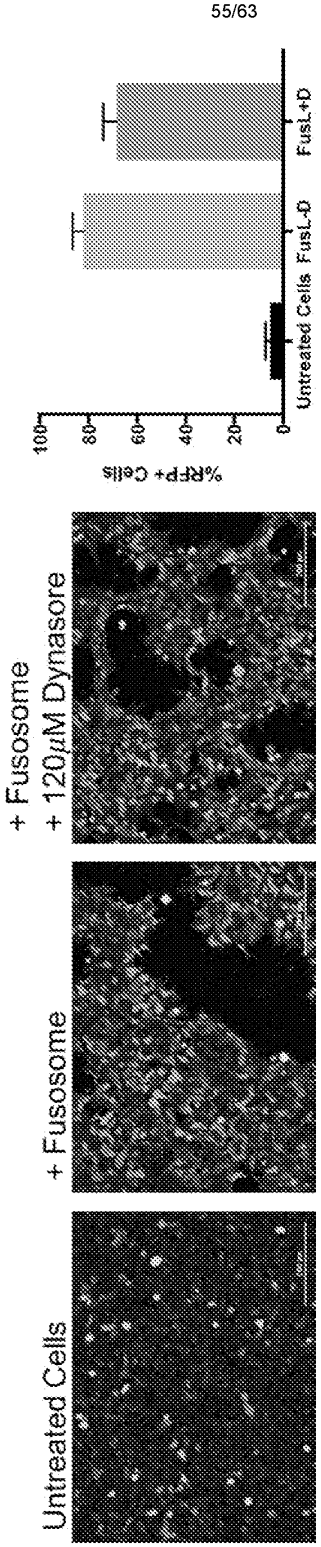

Untreated Cells
+ Fusosome
+ Fusosome
+ 120μM Dynasore
%RFP+ Cells
100
80
60
40
20
0
Untreated Cells
FusL-D
FusL+D

Fig. 45

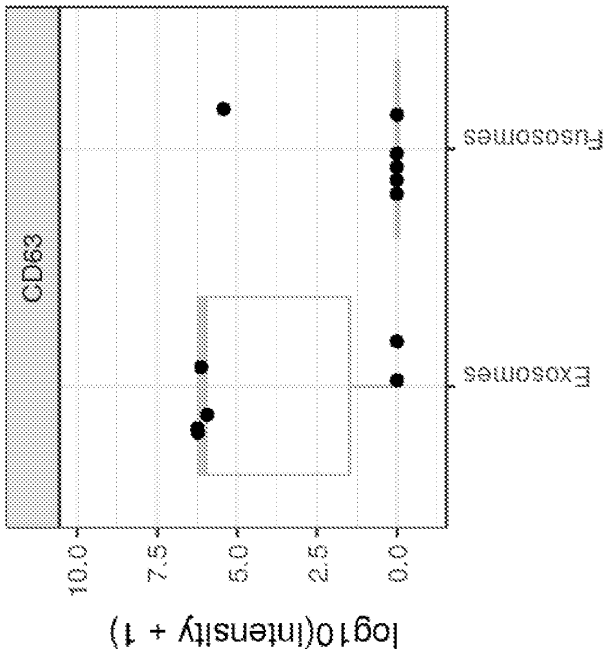

CD63
10.0
7.5
5.0
2.5
0.0
log10(intensity + 1)
Exosomes
Fusosomes
cohort

Fig. 46

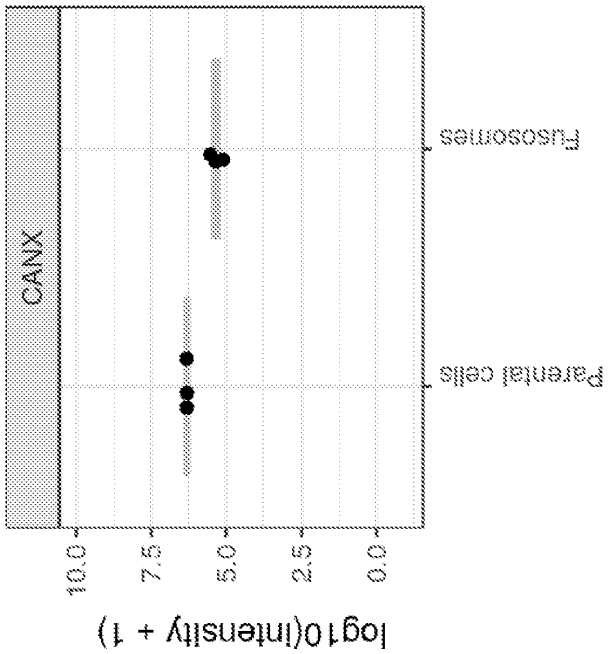

CANX
10.0
7.5
5.0
2.5
0.0
log10(Intensity + 1)
Parental cells
Fusosomes
cohort

Fig. 47

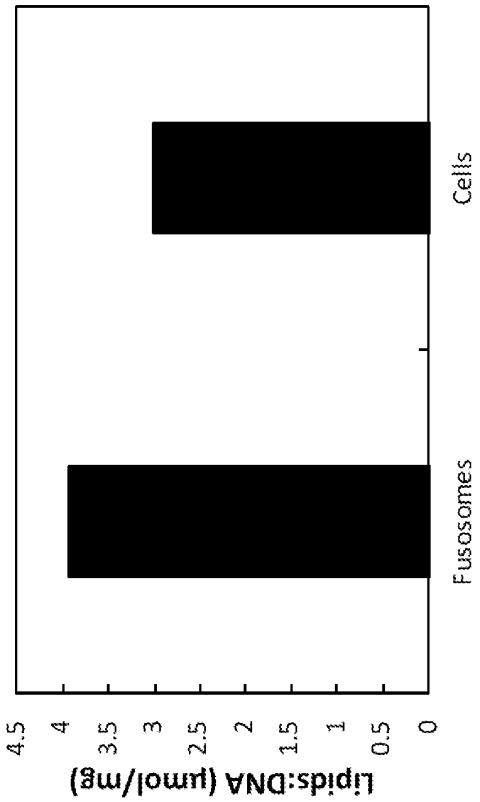


Fig. 48

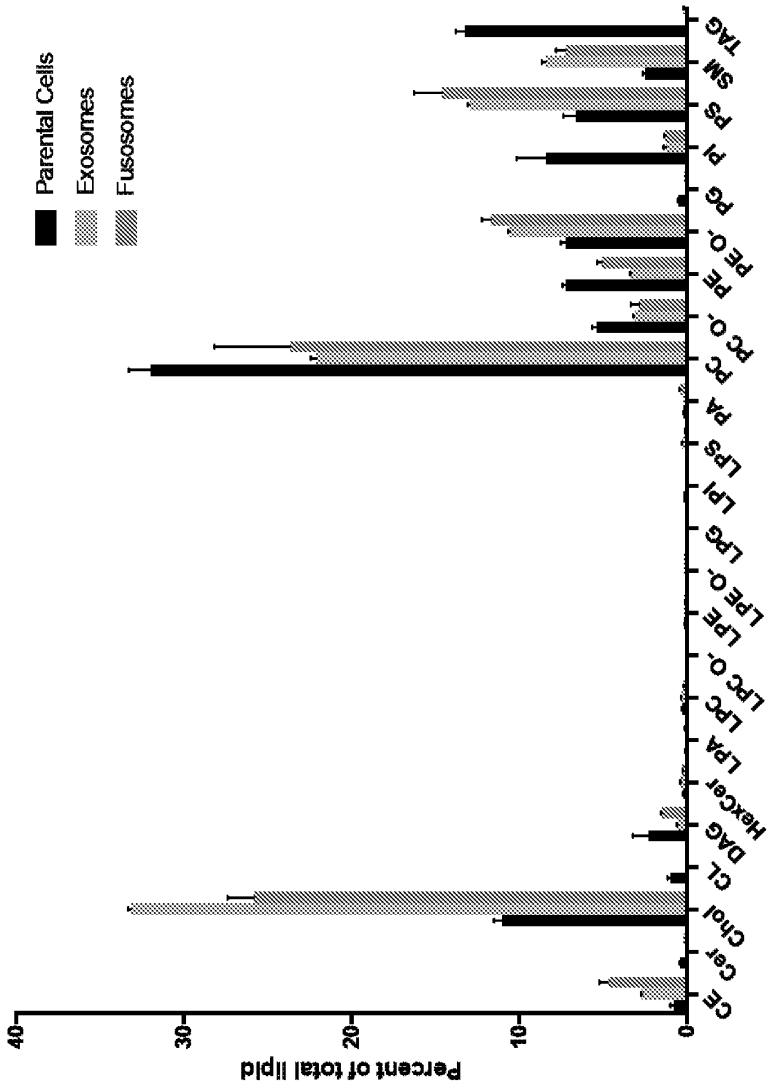

Parental Cells
Exosomes
Fusosomes
Percent of total lipid
0
10
20
30
40
CE
Cer
Chol
CL
DAG
Hexcer
LPA
LPC
LPC O-
LPE
LPE O-
LPG
LPI
LPS
PA
PC
PC O-
PE
PE O-
PG
PI
PS
SM
TAG

Fig. 49A

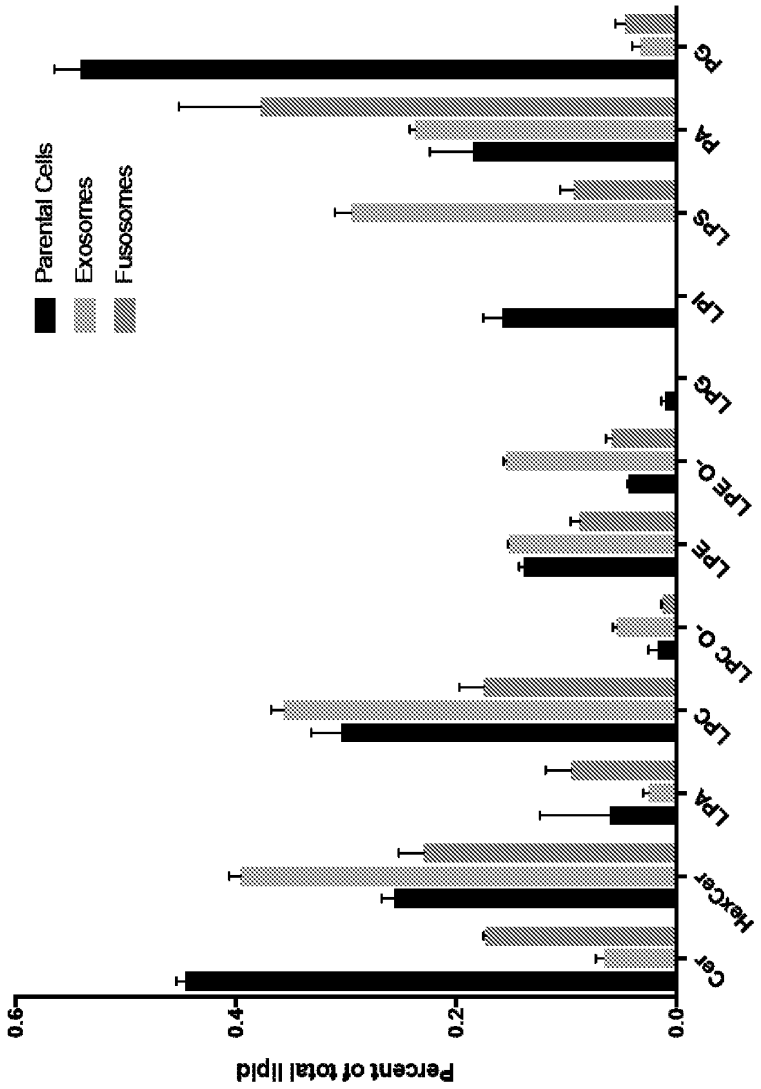

Parental Cells
Exosomes
Fusosomes
Percent of total lipid
0.0
0.2
0.4
0.6
Cer
hexCer
LPA
LPC
LPC O-
LPE
LPE O-
LPG
LPI
LPS
PA
PG

Fig. 49B

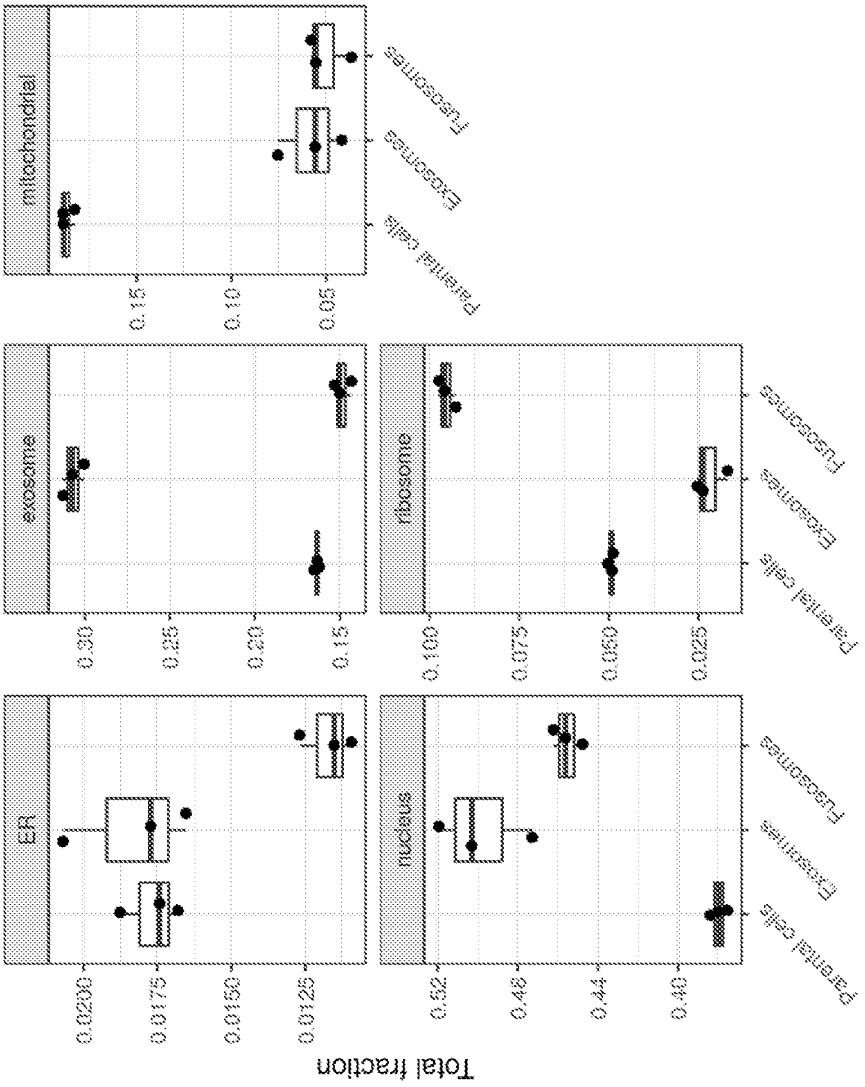

mitochondrial
exosome
ER
ribosome
nucleus
Total fraction
0.0200
0.0175
0.0150
0.0125
0.30
0.25
0.20
0.15
0.100
0.075
0.050
0.025
0.52
0.48
0.44
0.40
0.15
0.10
0.05
Parental cells
Exosomes
Fusosomes

Fig. 50

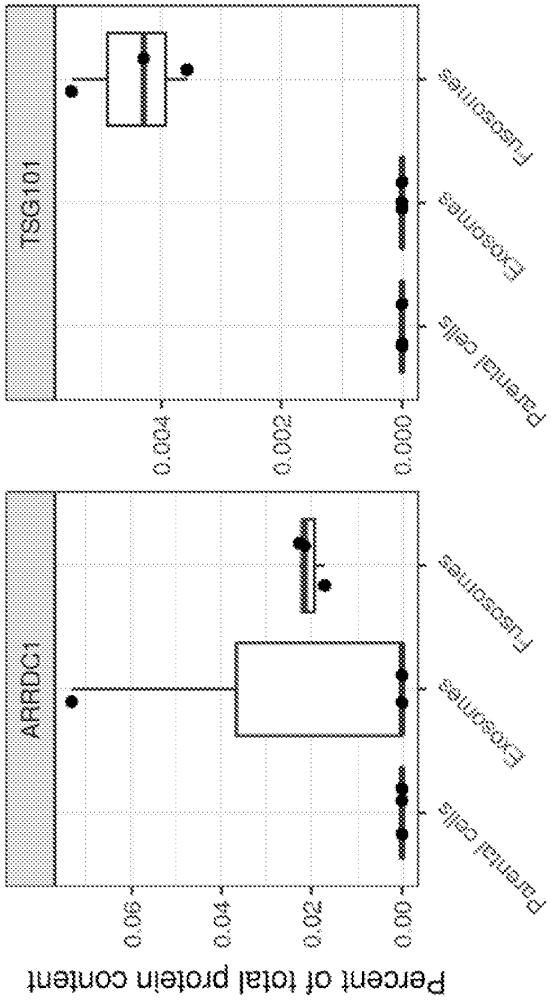
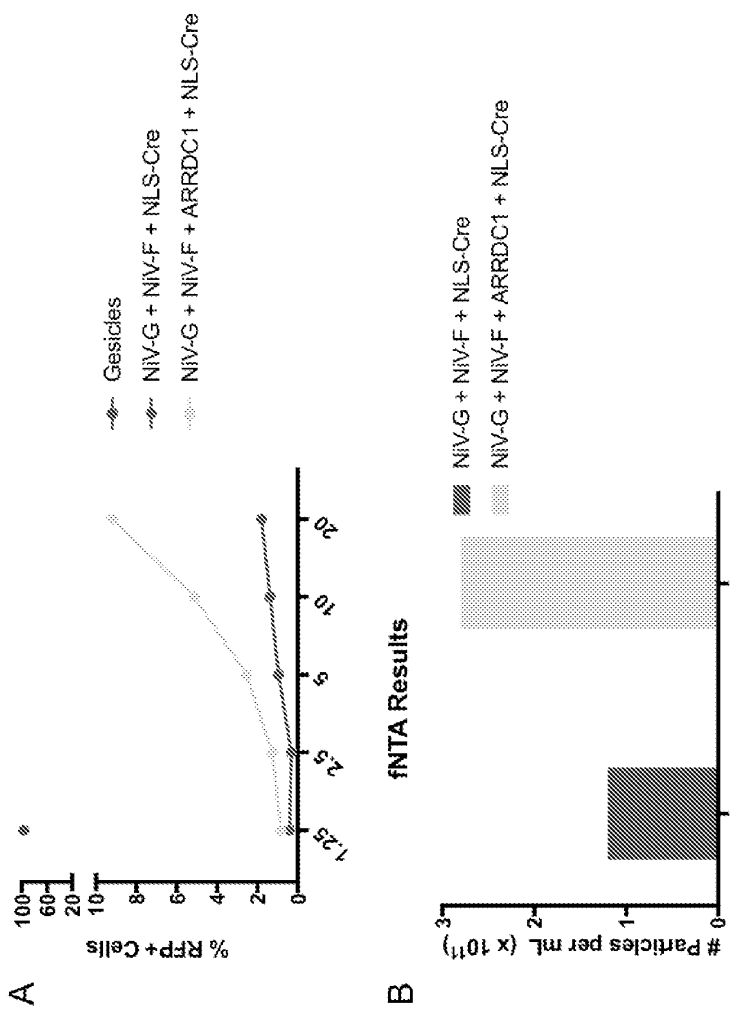

Percent of total protein content
ARRDC1
TSG101
0.00
0.02
0.04
0.06
0.000
0.002
0.004
Parental cells
Exosomes
Fusosomes

Fig. 51


A
% RFP+ Cells
100
60
20
10
8
6
4
2
0
1.25
2.5
5
10
20
Gesicles
NiV-G + NiV-F + NLS-Cre
NiV-G + NiV-F + ARRDC1 + NLS-Cre
B
fNTA Results
Particles per mL (x 10^11)
3
2
1
0
NiV-G + NiV-F + NLS-Cre
NiV-G + NiV-F + ARRDC1 + NLS-Cre

Figs. 52A-52B