Fig. 1A

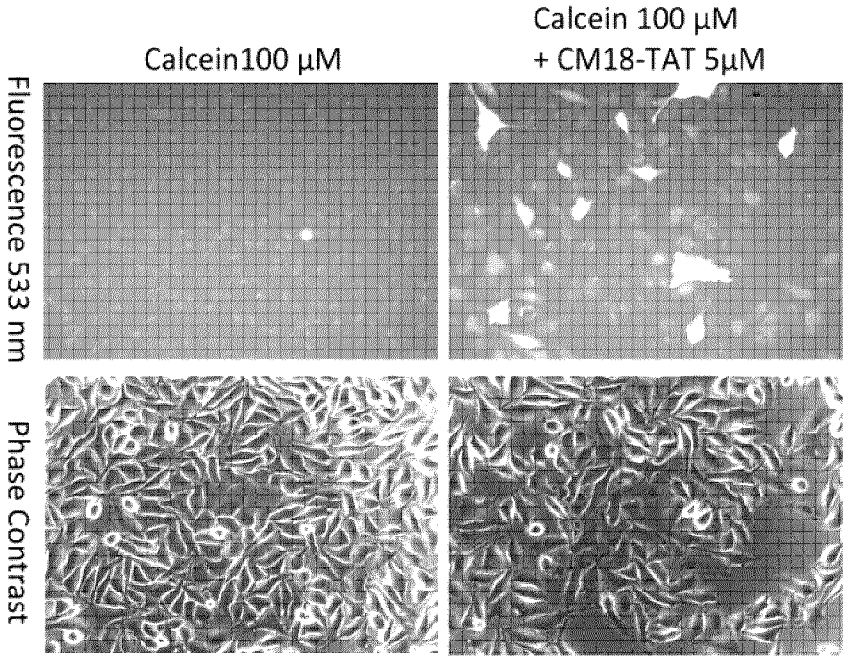

Calcein100 µM
Calcein 100 µM
+ CM18-TAT 5µM
Fluorescence 533 nm
Phase Contrast

Fig. 1B

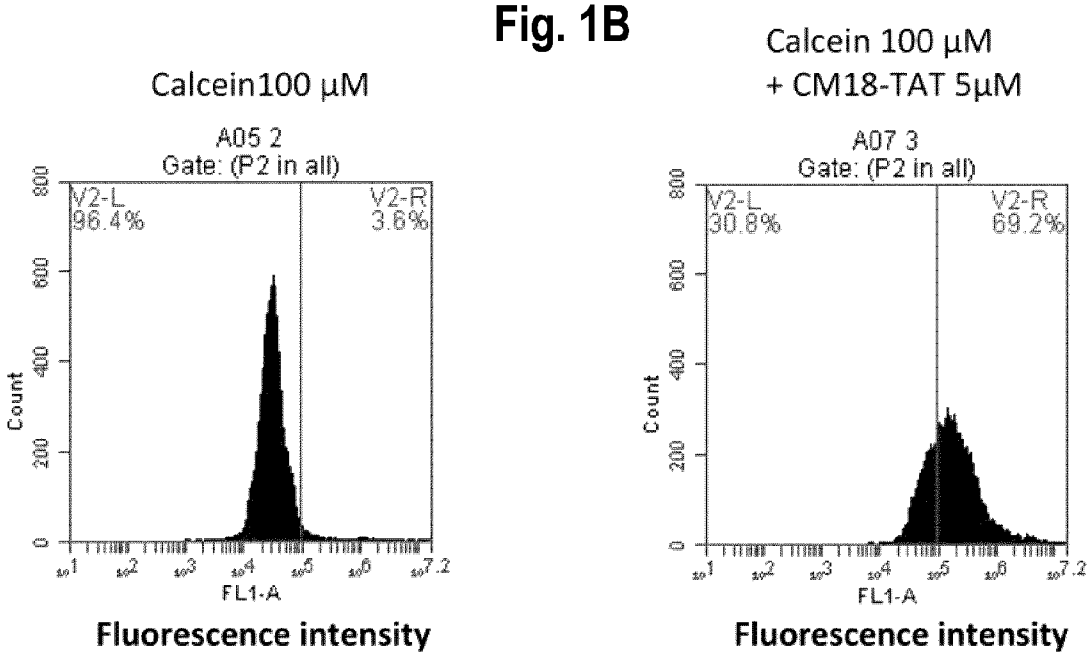

Calcein100 µM
A05 2
Gate: (P2 in all)
V2-L
96.4%
V2-R
3.6%
Count
FL1-A
Fluorescence intensity
Calcein 100 µM
+ CM18-TAT 5µM
A07 3
Gate: (P2 in all)
V2-L
30.8%
V2-R
69.2%
Count
FL1-A
Fluorescence intensity

Fig. 2

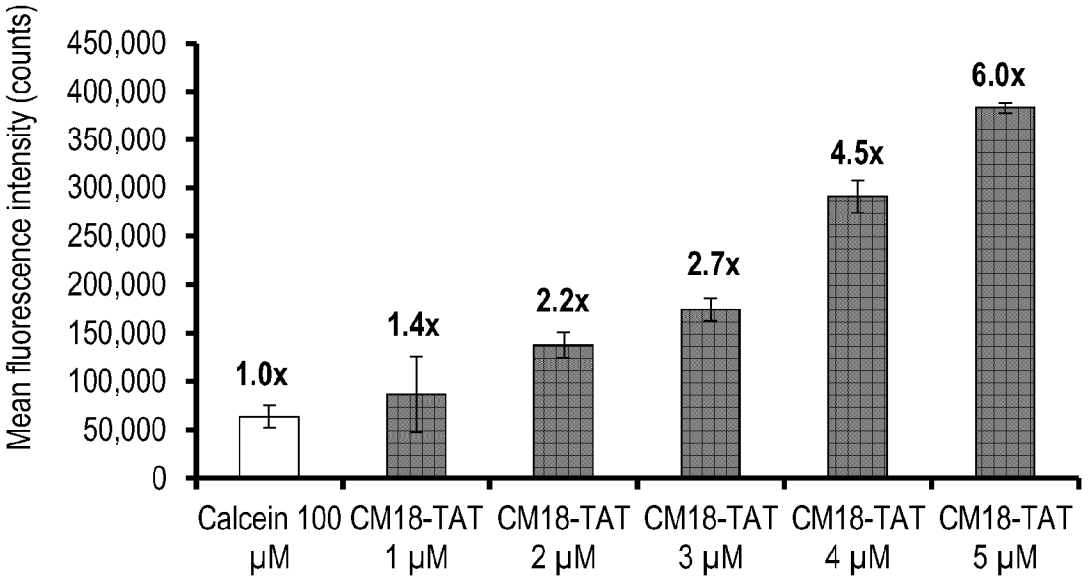

Mean fluorescence intensity (counts)
450,000
400,000
350,000
300,000
250,000
200,000
150,000
100,000
50,000
0
1.0x
1.4x
2.2x
2.7x
4.5x
6.0x
Calcein 100 µM
CM18-TAT 1 µM
CM18-TAT 2 µM
CM18-TAT 3 µM
CM18-TAT 4 µM
CM18-TAT 5 µM

Fig. 3

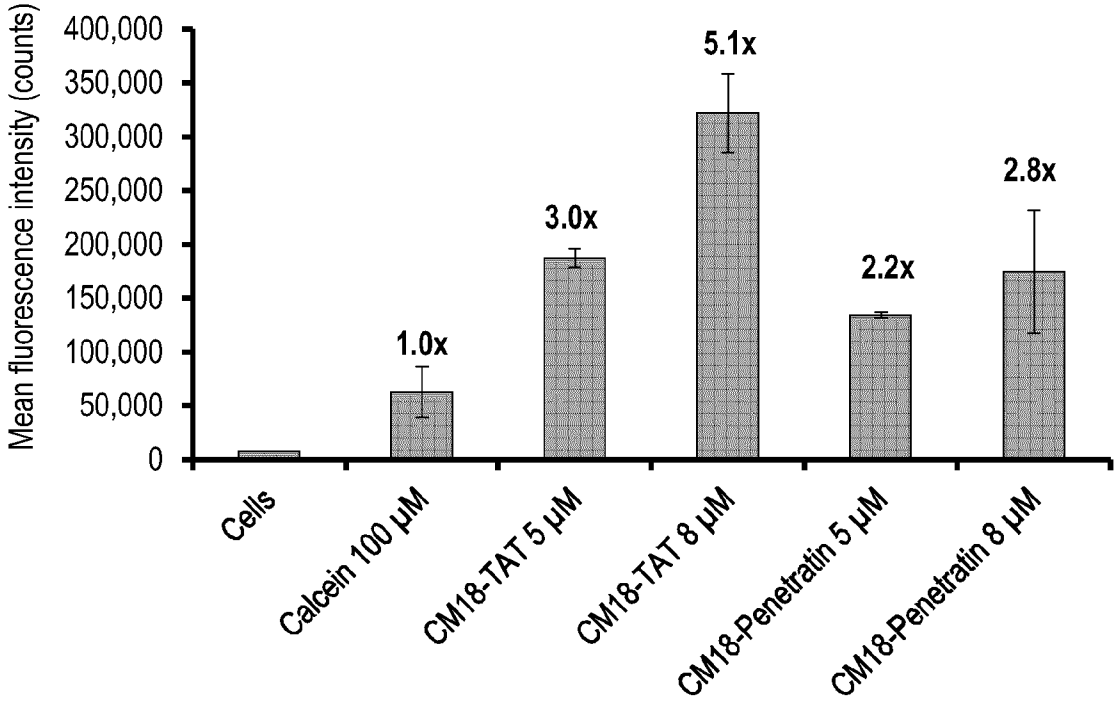

Mean fluorescence intensity (counts)
400,000
350,000
300,000
250,000
200,000
150,000
100,000
50,000
0
1.0x
3.0x
5.1x
2.2x
2.8x
Cells
Calcein 100 µM
CM18-TAT 5 µM
CM18-TAT 8 µM
CM18-Penetratin 5 µM
CM18-Penetratin 8 µM

Fig. 4

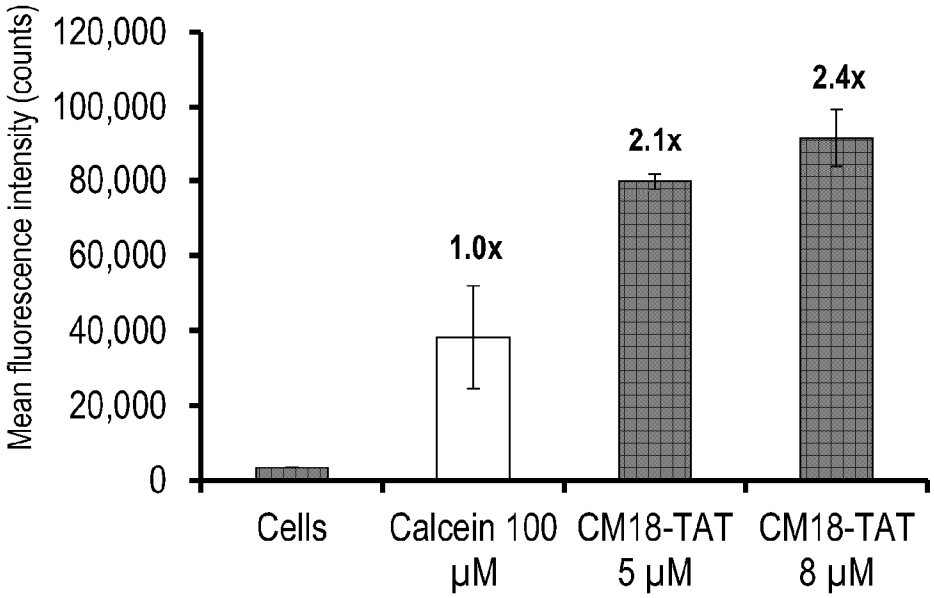

Mean fluorescence intensity (counts)
120,000
100,000
80,000
60,000
40,000
20,000
0
1.0x
2.1x
2.4x
Cells
Calcein 100 µM
CM18-TAT 5 µM
CM18-TAT 8 µM

Fig. 5

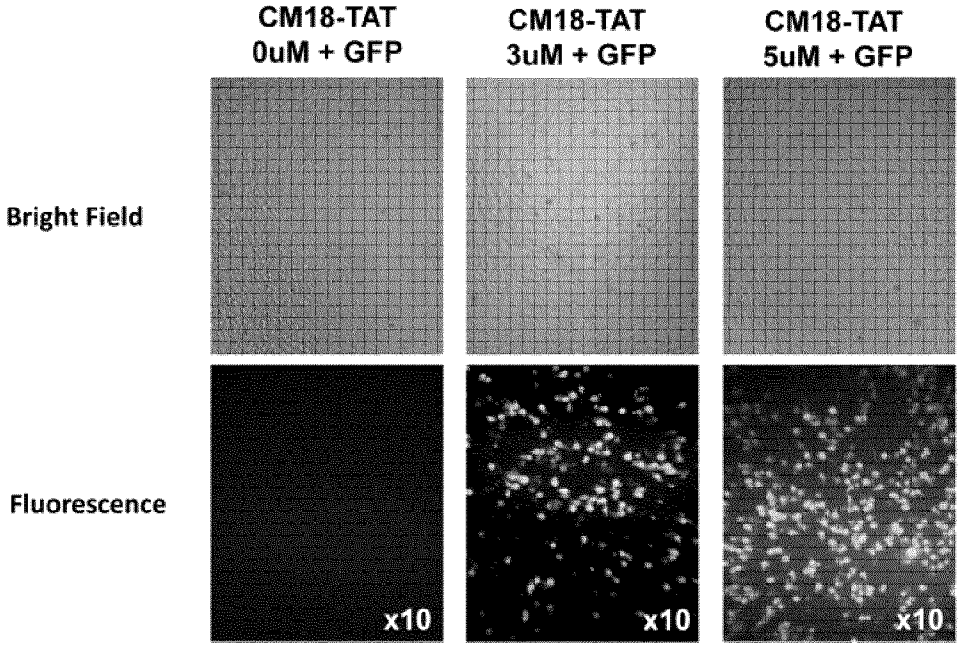

CM18-TAT 0uM + GFP
CM18-TAT 3uM + GFP
CM18-TAT 5uM + GFP
Bright Field
Fluorescence
x10
x10
x10

Fig. 6A

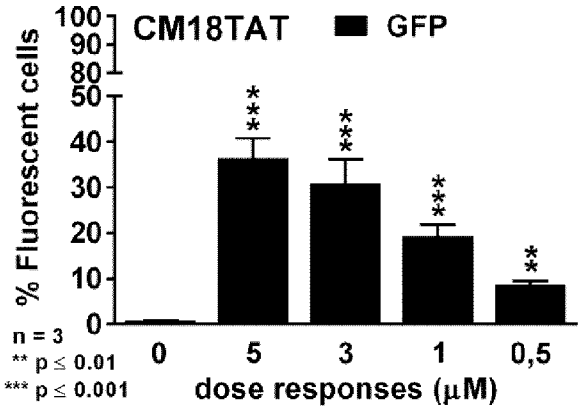


Fig. 6B

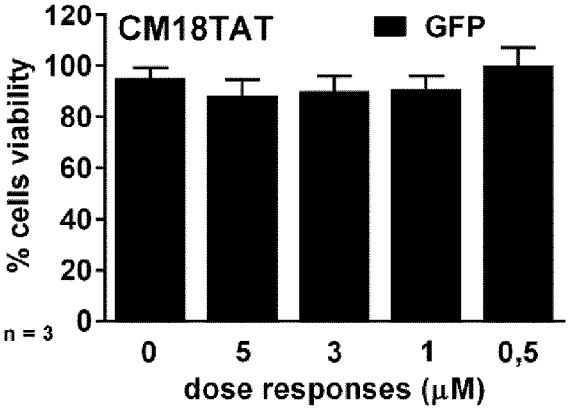


Fig. 7A

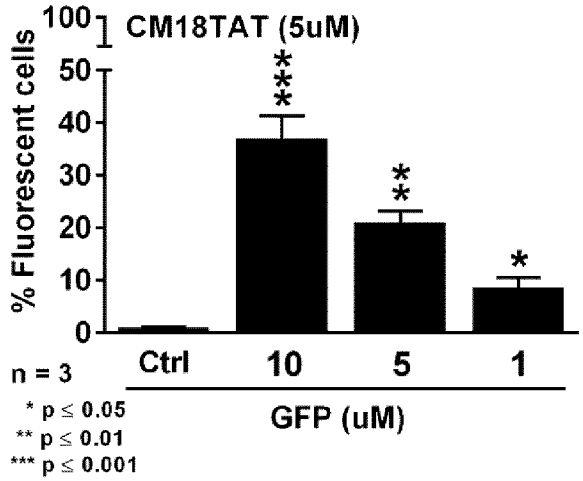


Fig. 7B

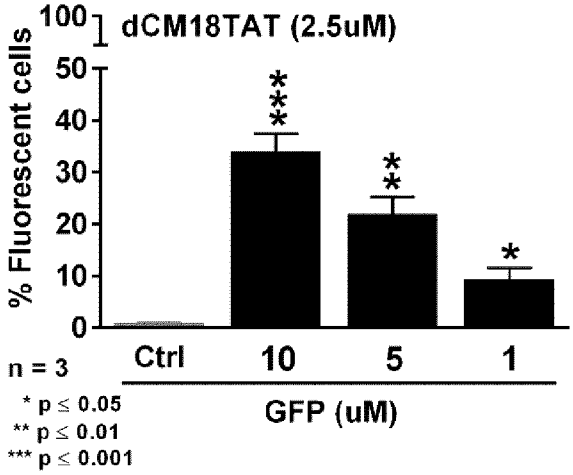


Fig. 8

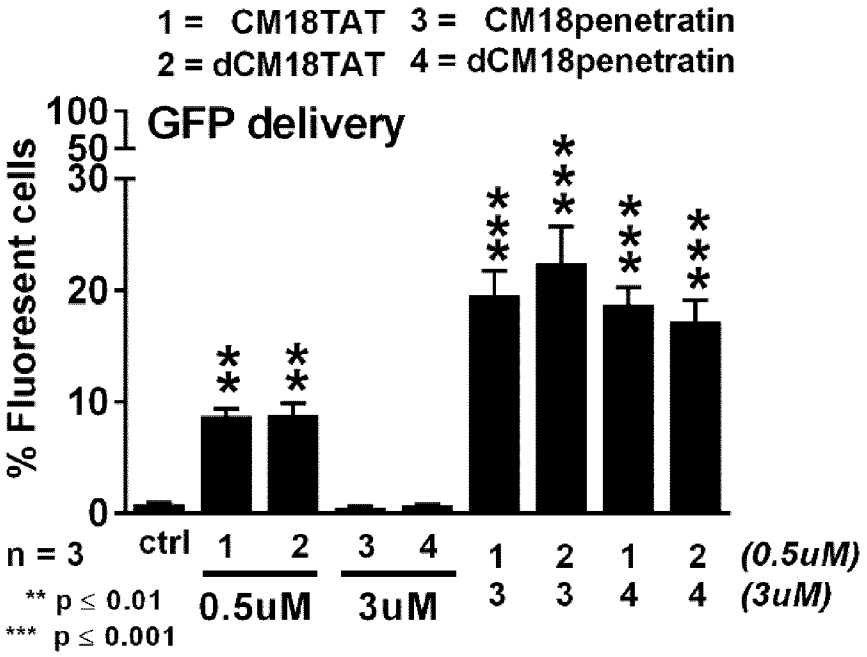

1 = CM18TAT 3 = CM18penetratin
2 = dCM18TAT 4 = dCM18penetratin
GFP delivery
% Fluoresent cells
100
50
30
20
10
0
n = 3
ctrl 1 2 3 4 1 2 1 2 (0.5uM)
1 3 3 4 4 (3uM)
0.5uM 3uM
** p ≤ 0.01
*** p ≤ 0.001

Fig. 9

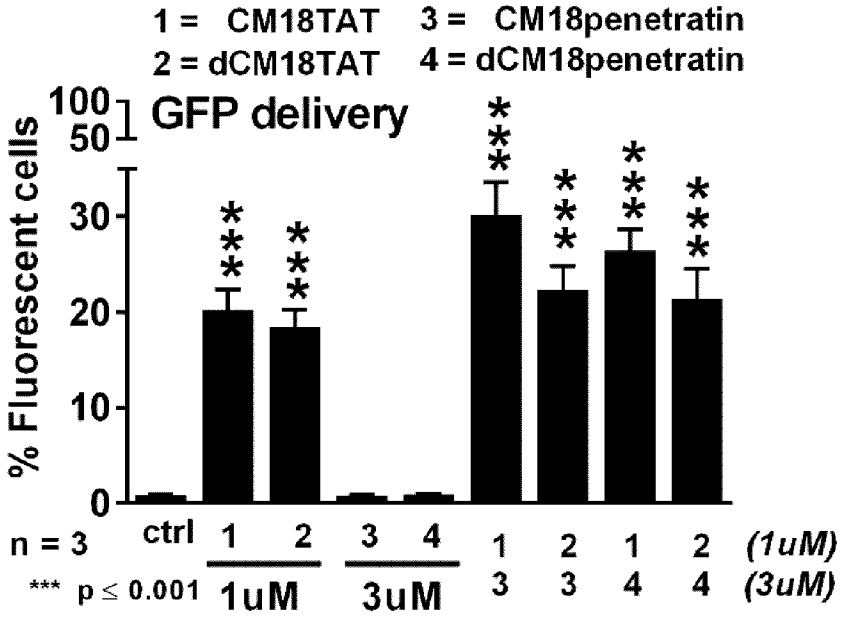

1 = CM18TAT 3 = CM18penetratin
2 = dCM18TAT 4 = dCM18penetratin
GFP delivery
% Fluorescent cells
100
50
30
20
10
0
n = 3
ctrl 1 2 3 4 1 2 1 2 (1uM)
1 3 3 4 4 (3uM)
1uM 3uM
*** p ≤ 0.001

Fig. 10

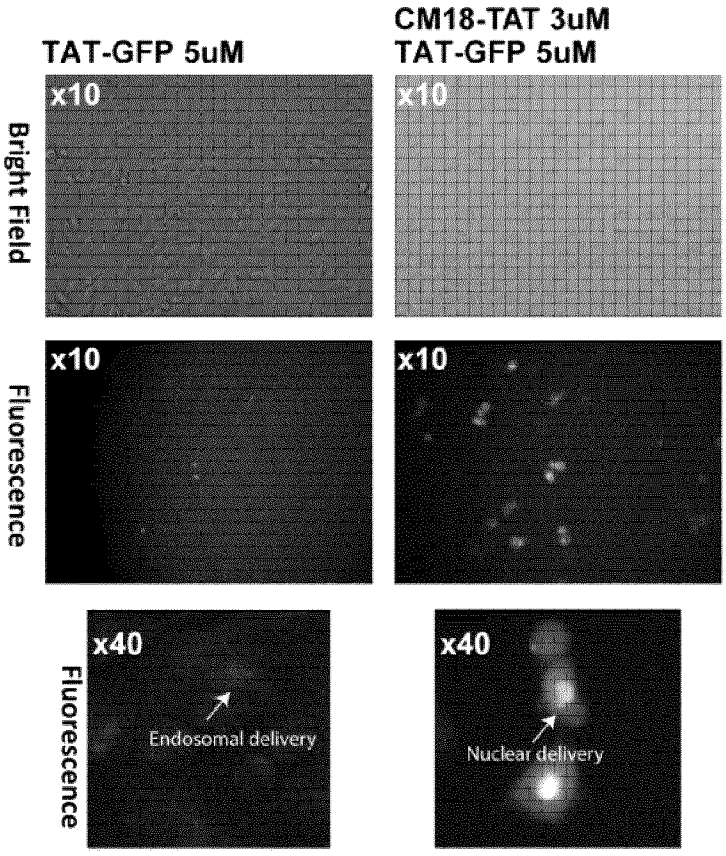


Fig. 11A

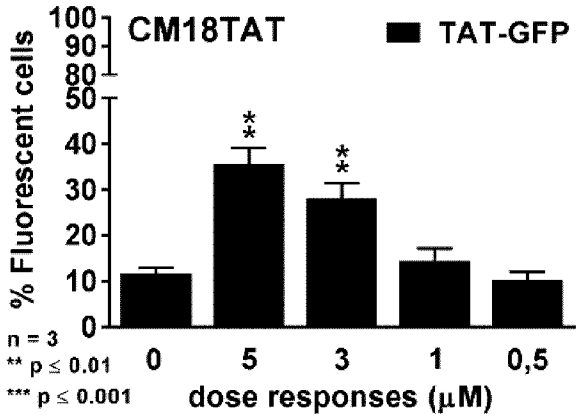


Fig. 11B

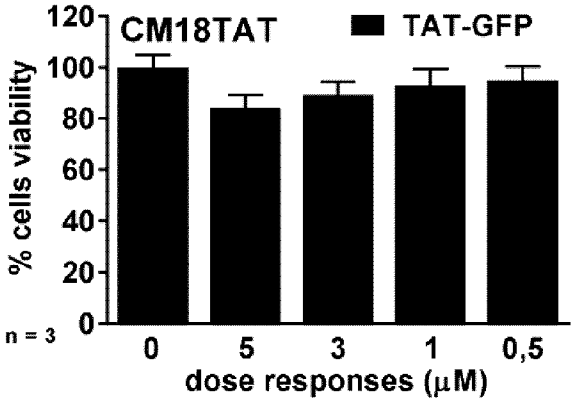


Fig. 12

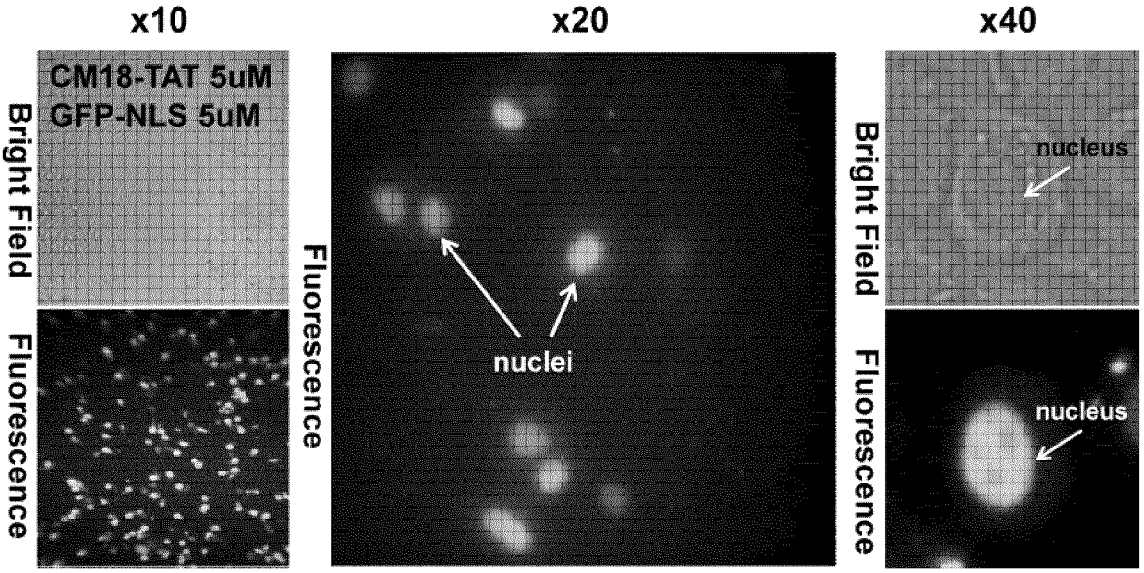


Fig. 13A

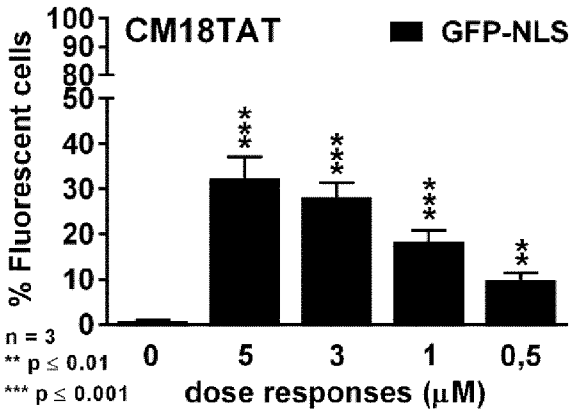


Fig. 13B

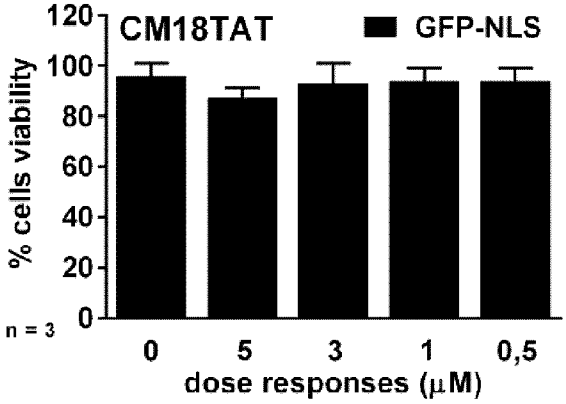


Fig. 14

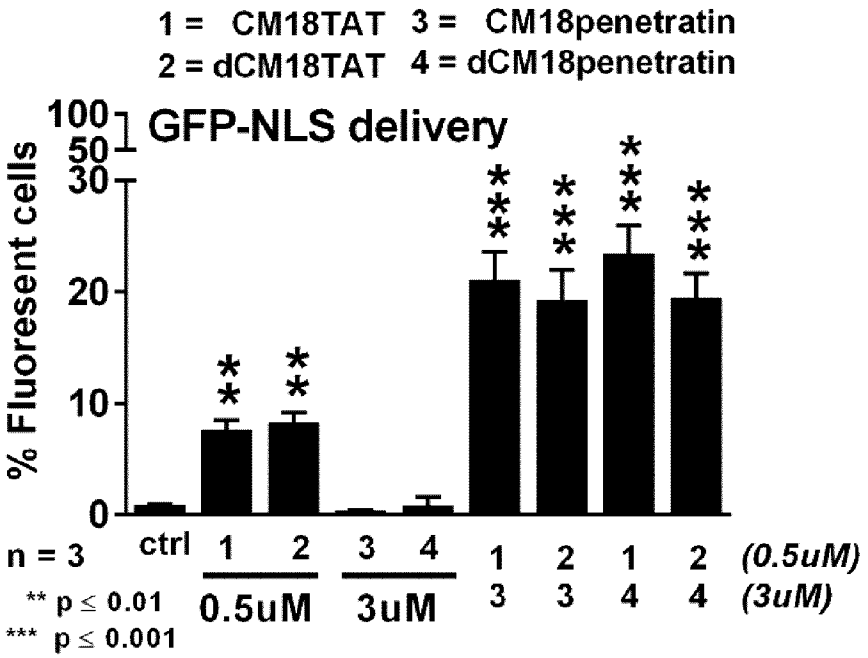

1 = CM18TAT 3 = CM18penetratin
2 = dCM18TAT 4 = dCM18penetratin
GFP-NLS delivery
% Fluoresent cells
100
50
30
20
10
0
n = 3
ctrl 1 2 3 4 1 2 1 2 (0.5uM)
0.5uM 3uM 3 3 4 4 (3uM)
** p ≤ 0.01
*** p ≤ 0.001

Fig. 15

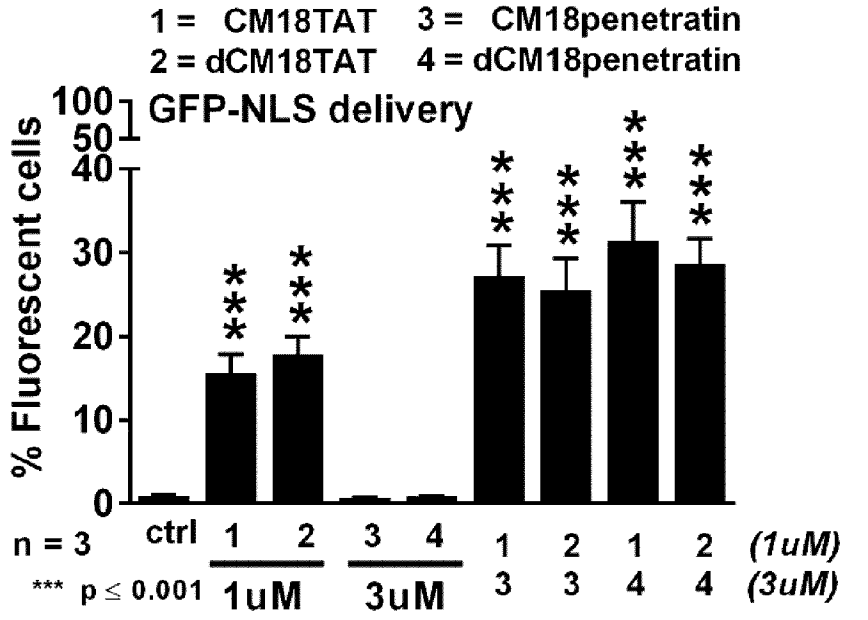

1 = CM18TAT 3 = CM18penetratin
2 = dCM18TAT 4 = dCM18penetratin
GFP-NLS delivery
% Fluorescent cells
100
50
40
30
20
10
0
n = 3
ctrl 1 2 3 4 1 2 1 2 (1uM)
1uM 3uM 3 3 4 4 (3uM)
*** p ≤ 0.001

Fig. 16

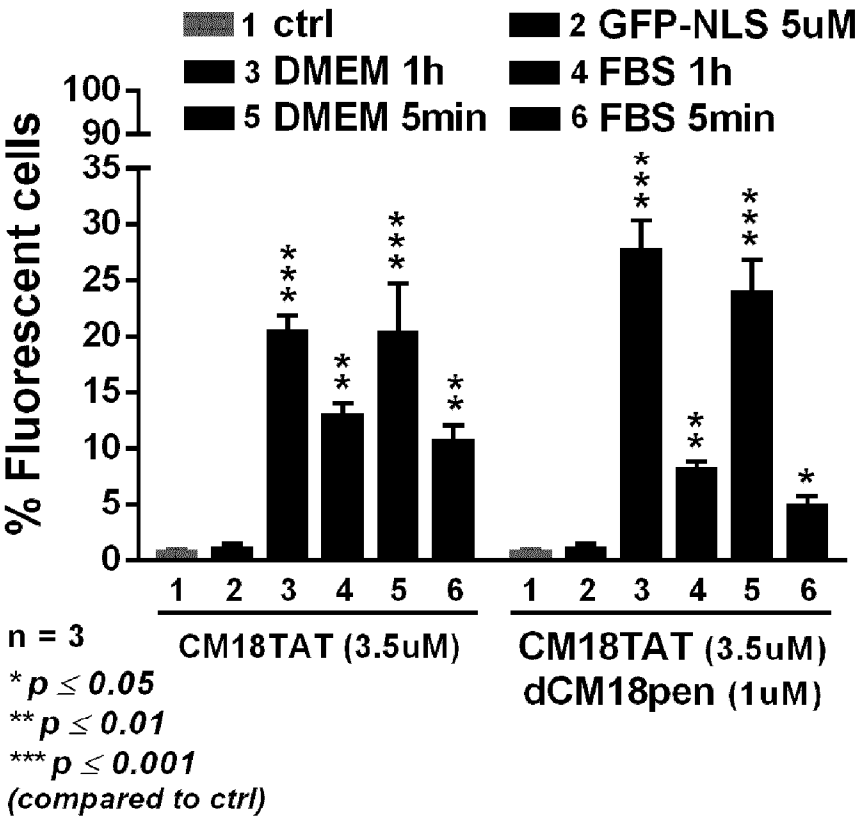


Fig. 17A

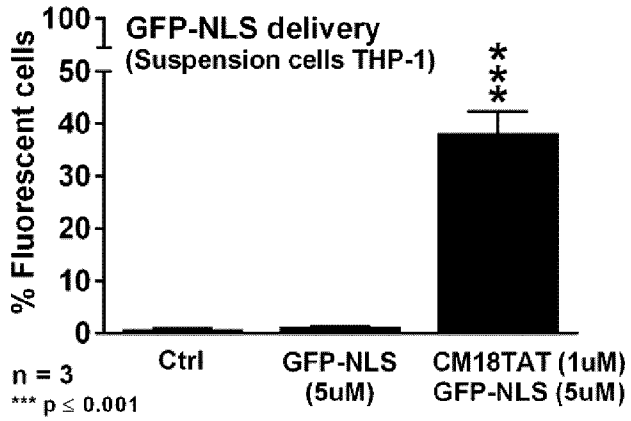


Fig. 17B

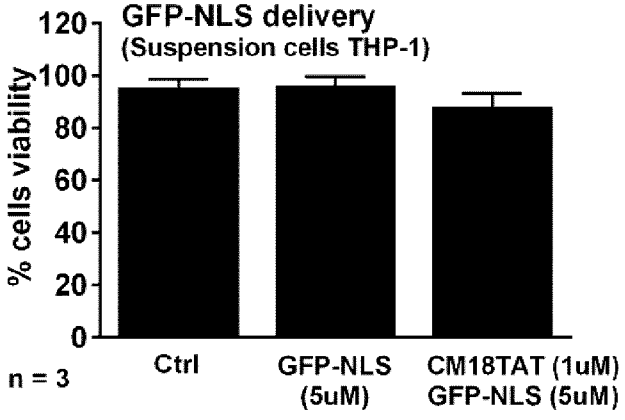


Fig. 18

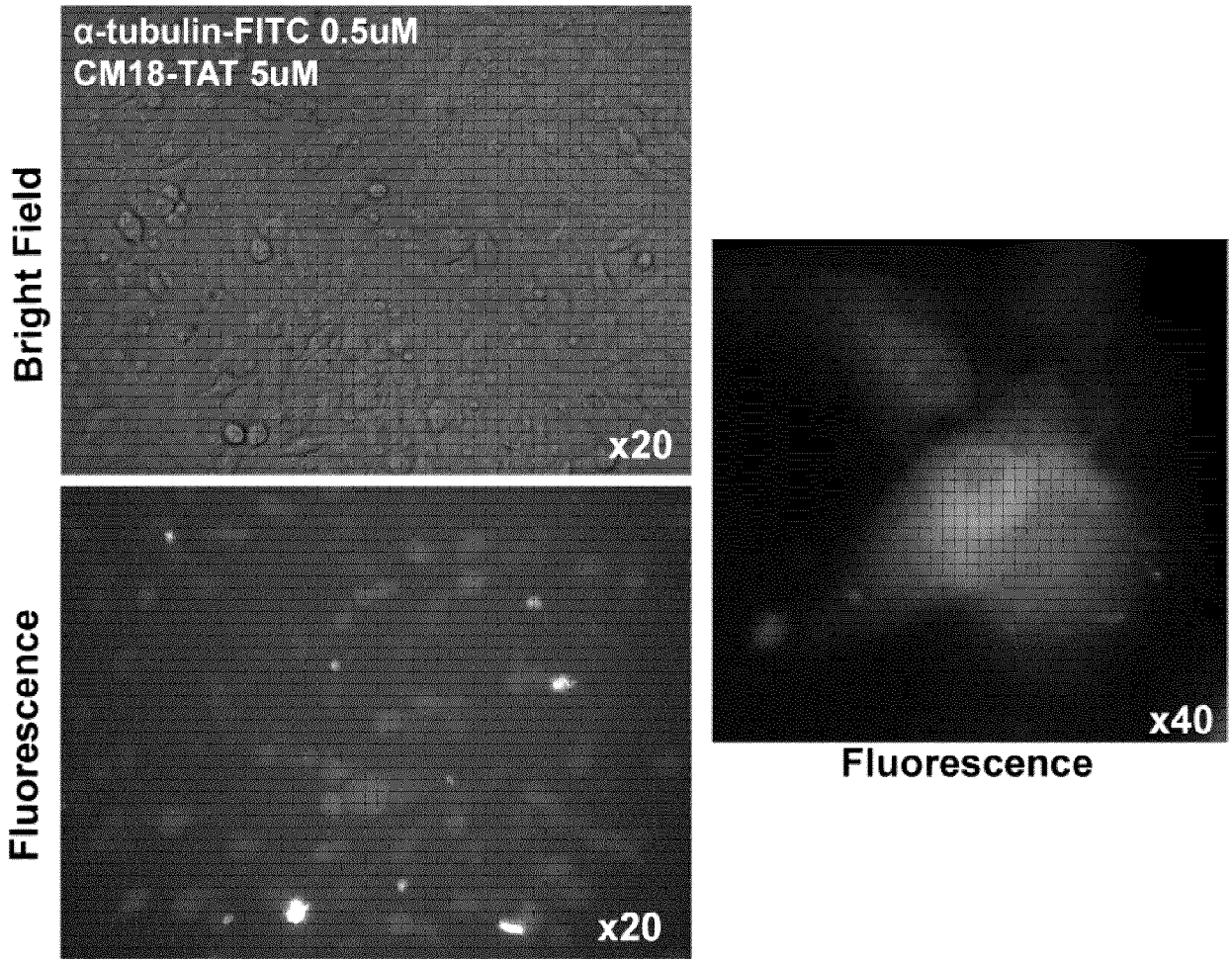

α-tubulin-FITC 0.5uM
CM18-TAT 5uM
Bright Field
x20
Fluorescence
x20
x40
Fluorescence

Fig. 19A

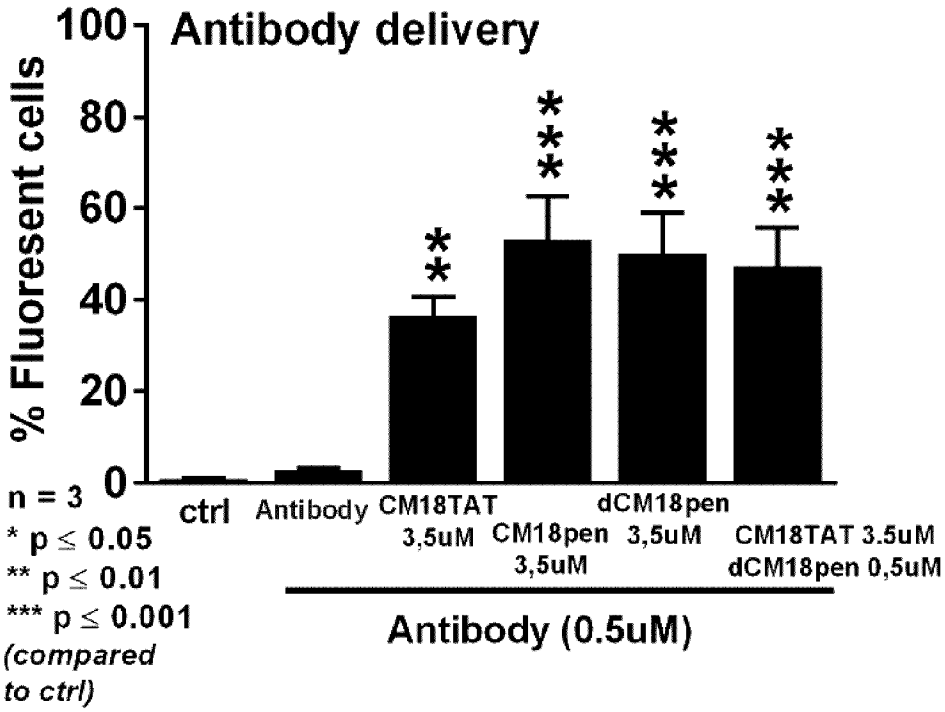


Fig. 19B

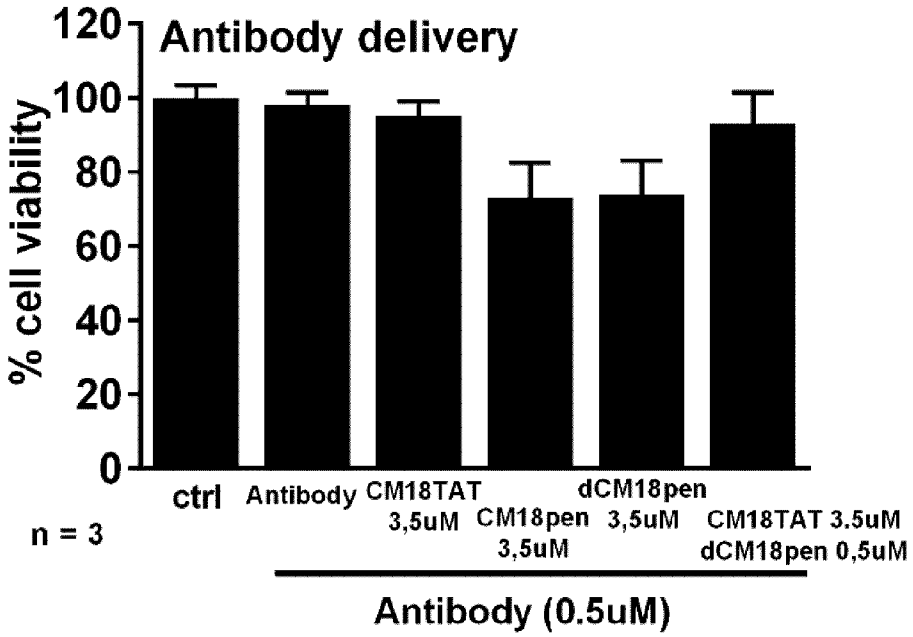


Fig. 20

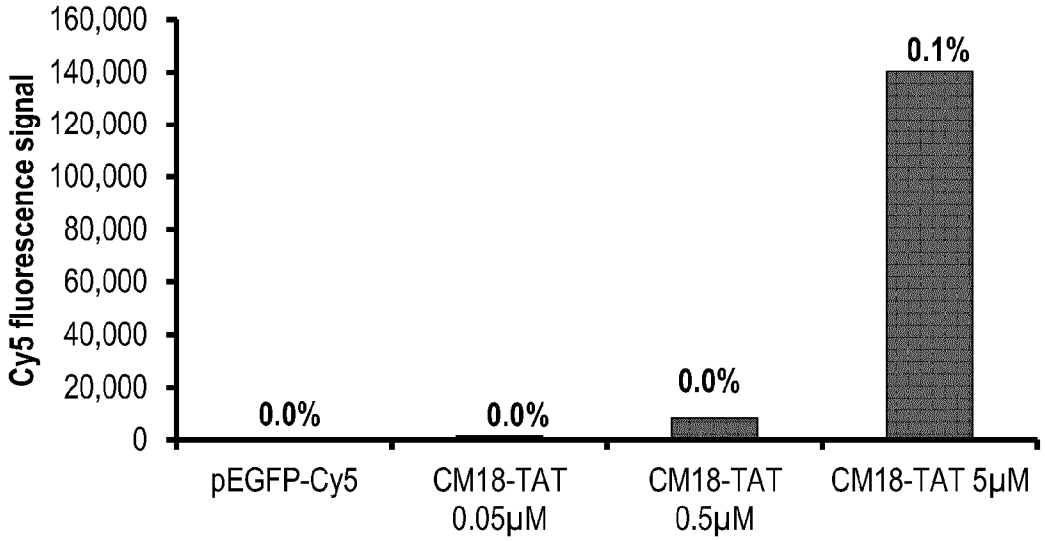


Fig. 21A

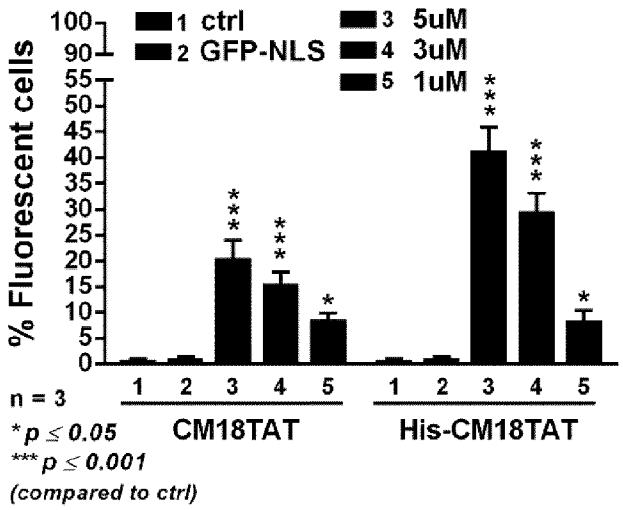


Fig. 21B

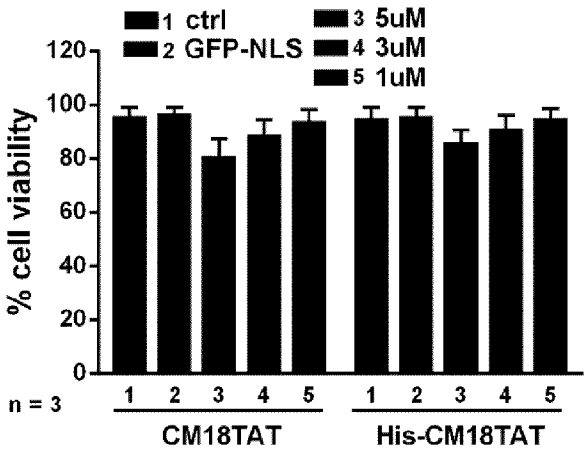


Fig. 22A

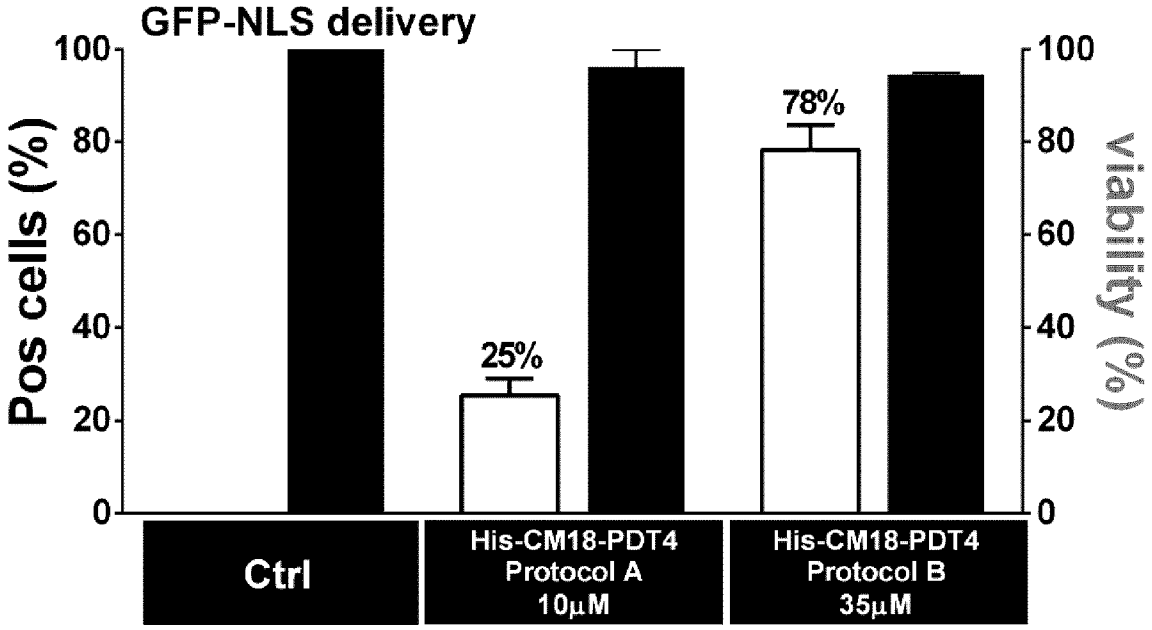


Fig. 22B

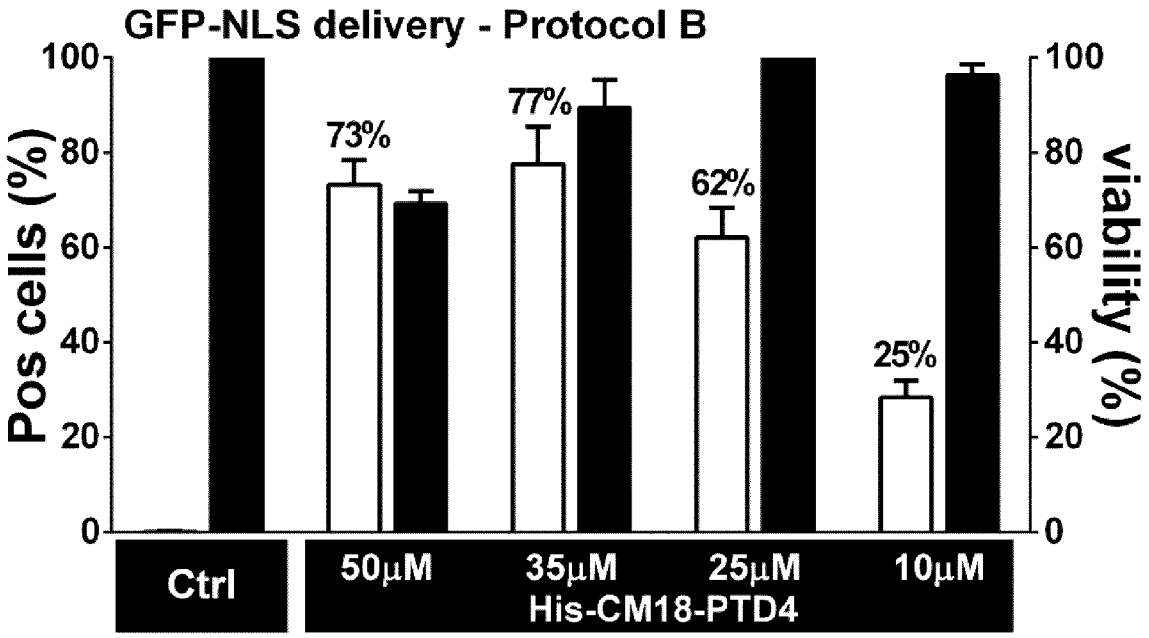
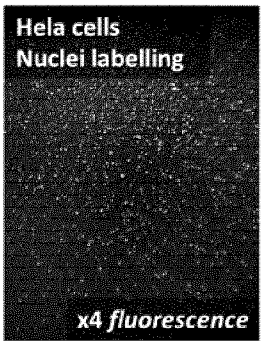


Fig. 23A



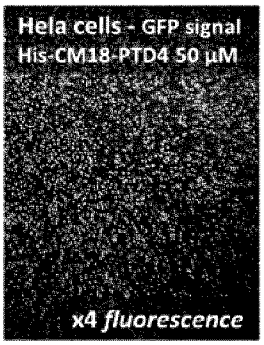
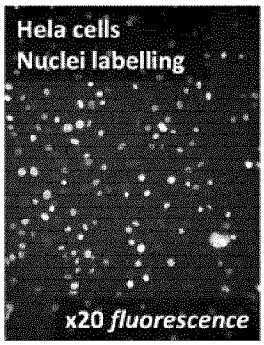


Fig. 23B



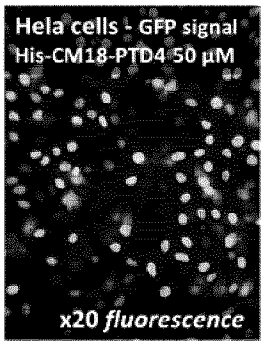
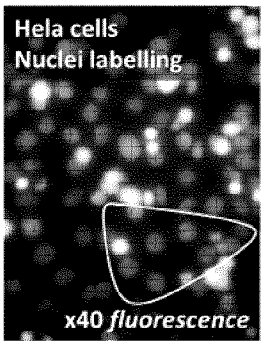


Fig. 23C



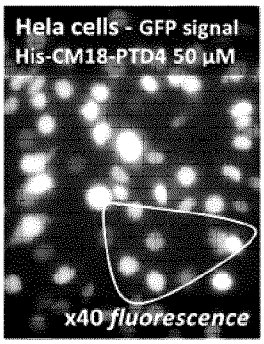
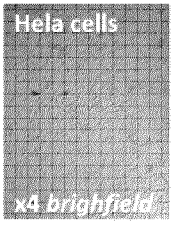
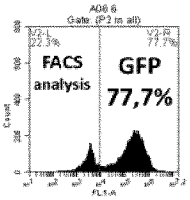


Fig. 23D





Viability = 95%

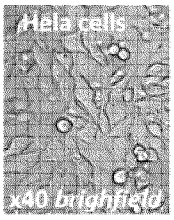


Fig. 24A

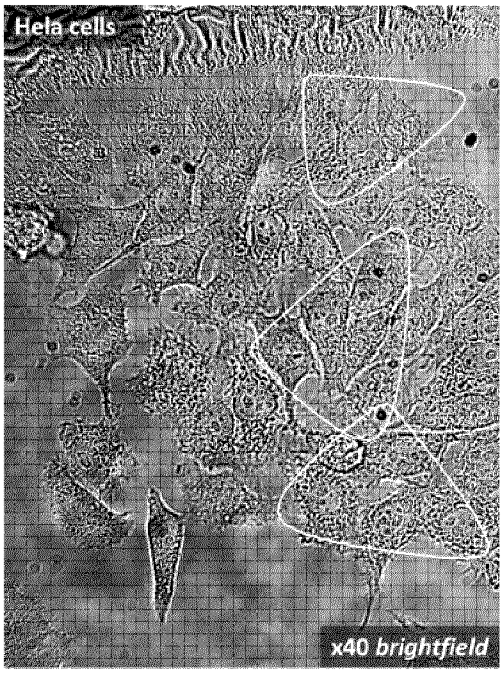


Fig. 24B

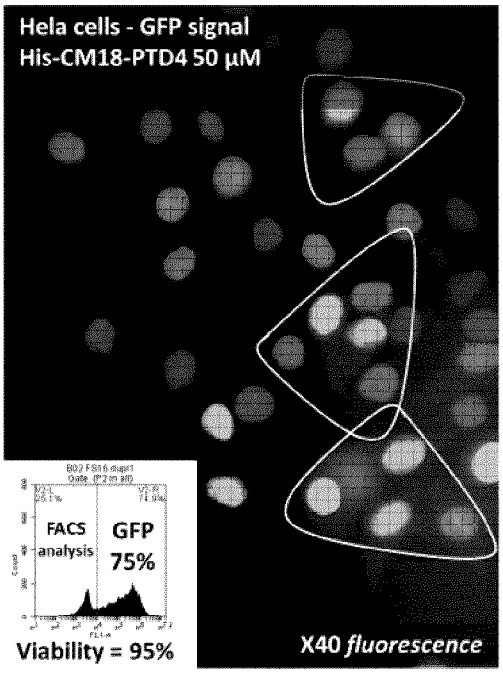


Fig. 24C

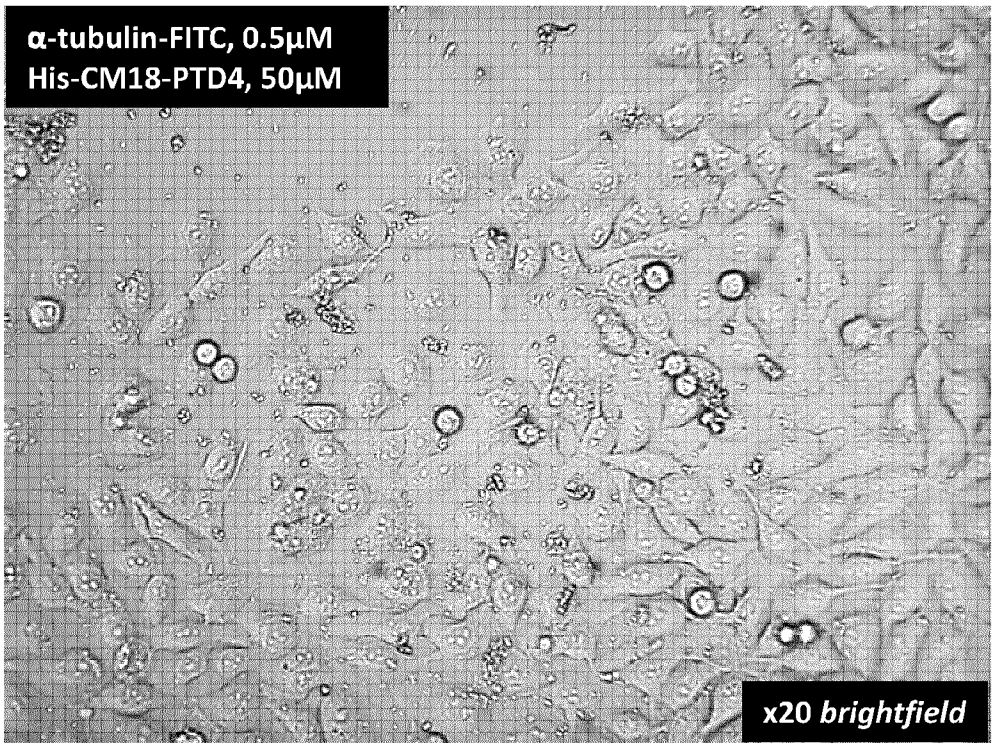


Fig. 24D

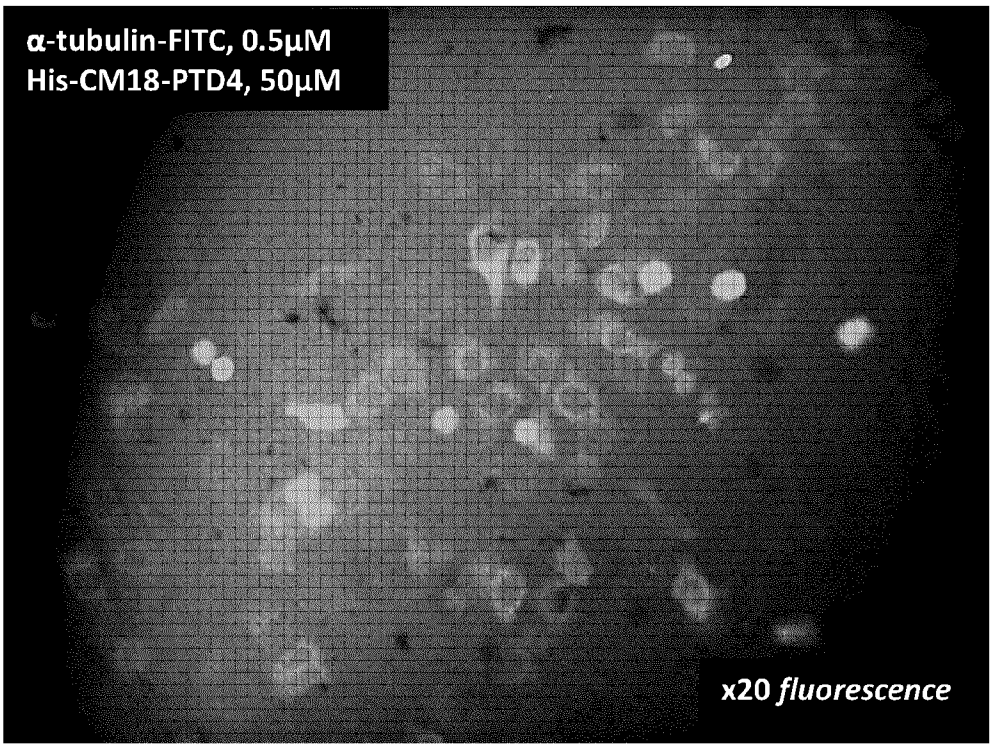


Fig. 25A

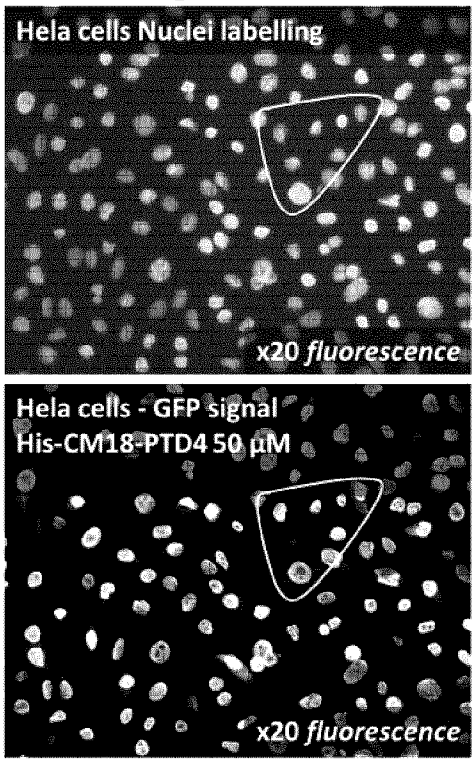


Fig. 25B

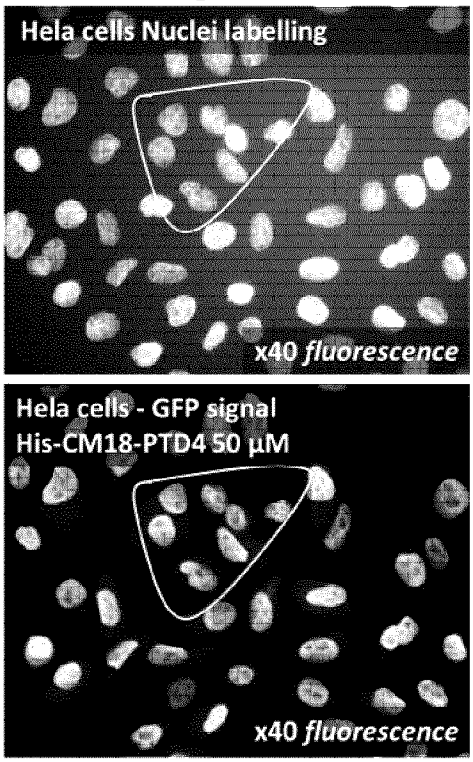


Fig. 26A

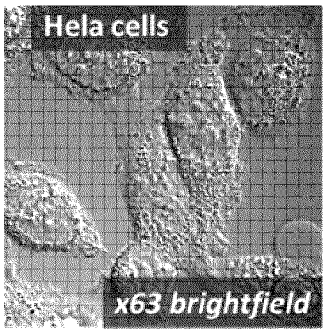


Fig. 26B

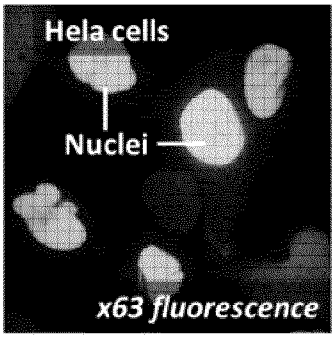


Fig. 26C

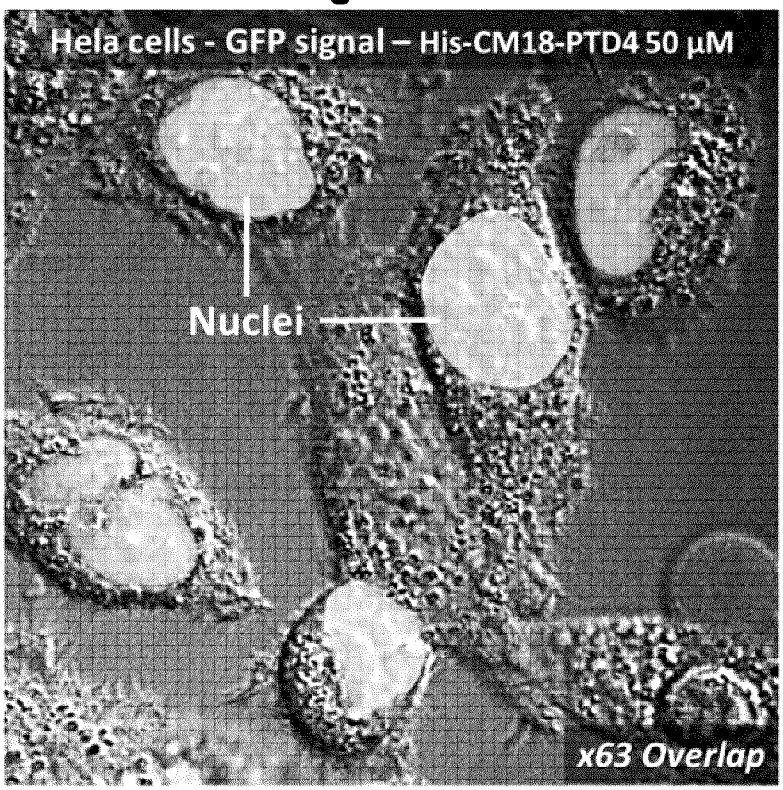


Fig. 27A

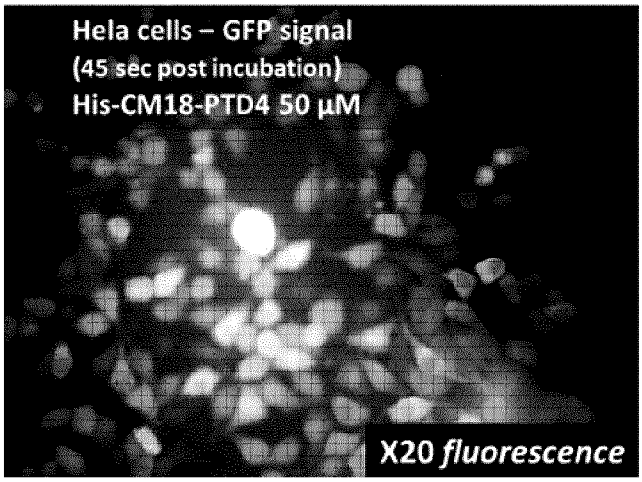


Fig. 27B

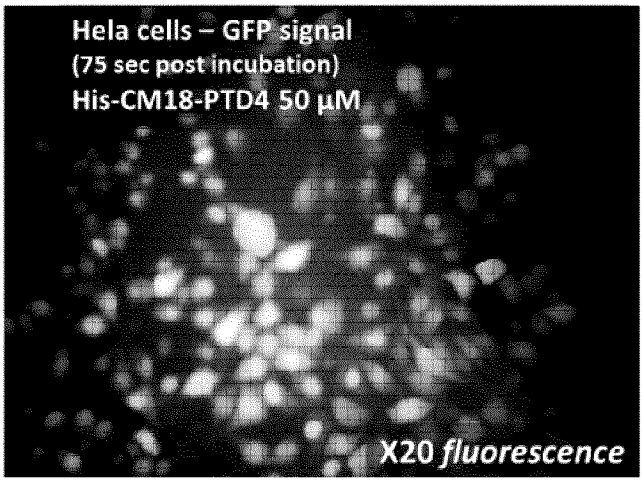


Fig. 27C

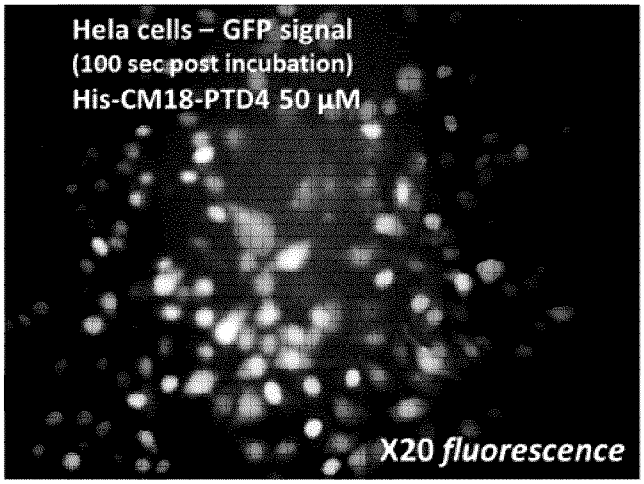


Fig. 27D

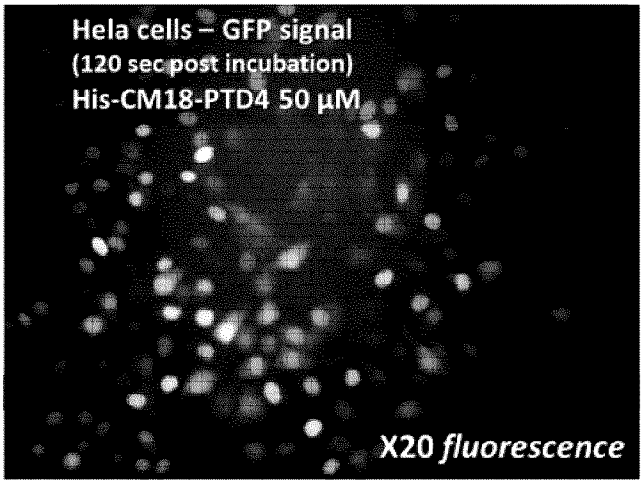


Fig. 28A

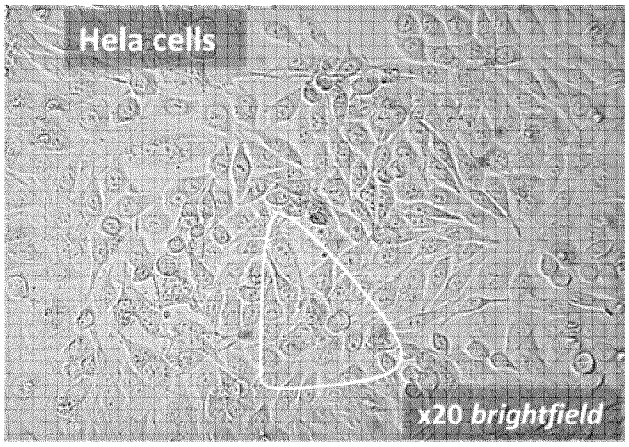


Fig. 28B

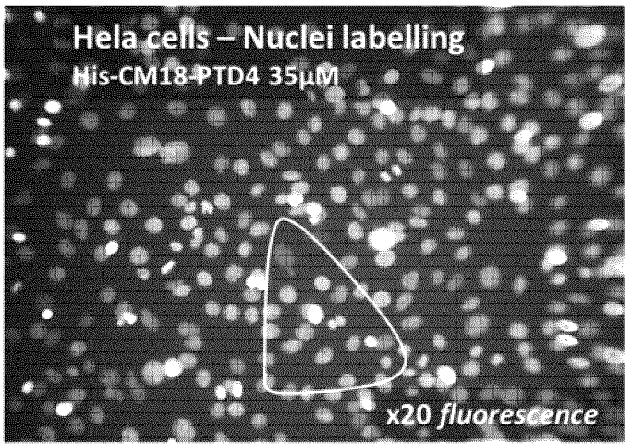


Fig. 28C

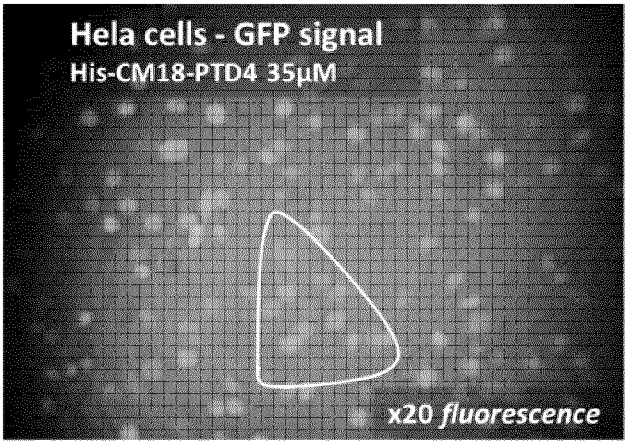


Fig. 28D

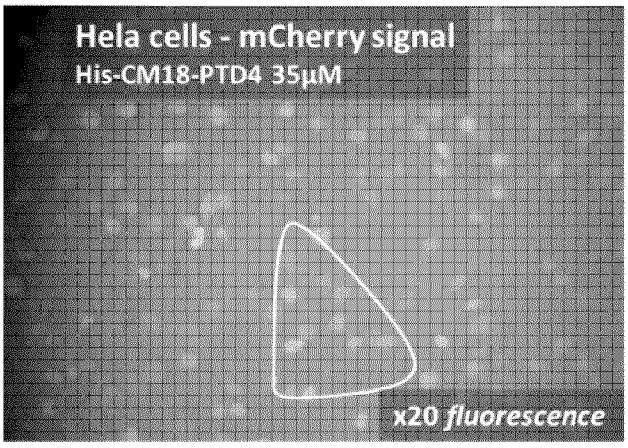


Fig. 29A

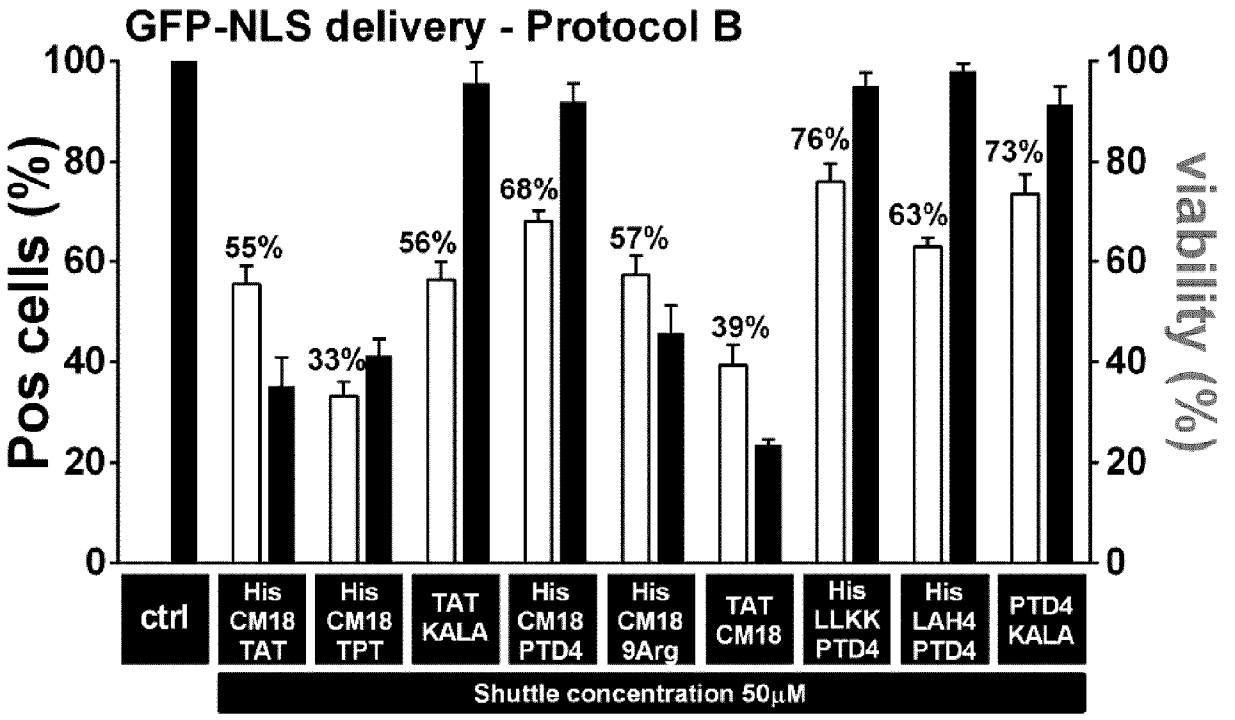


Fig. 29B

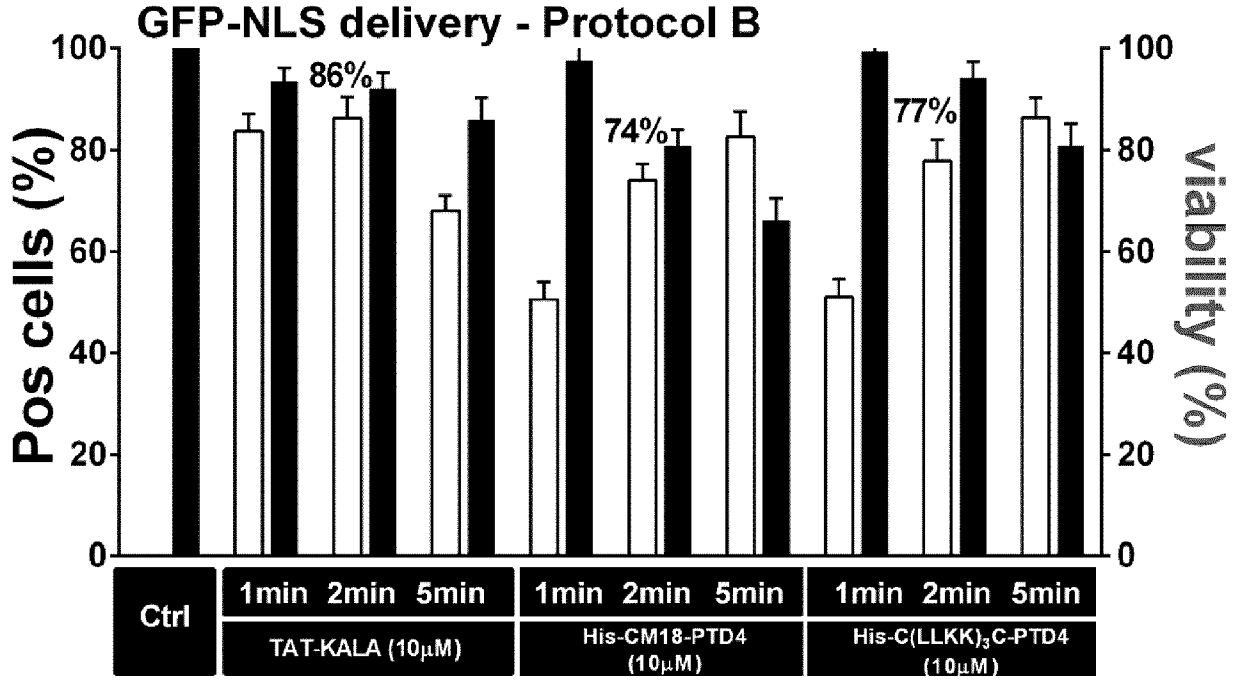


Fig. 29C

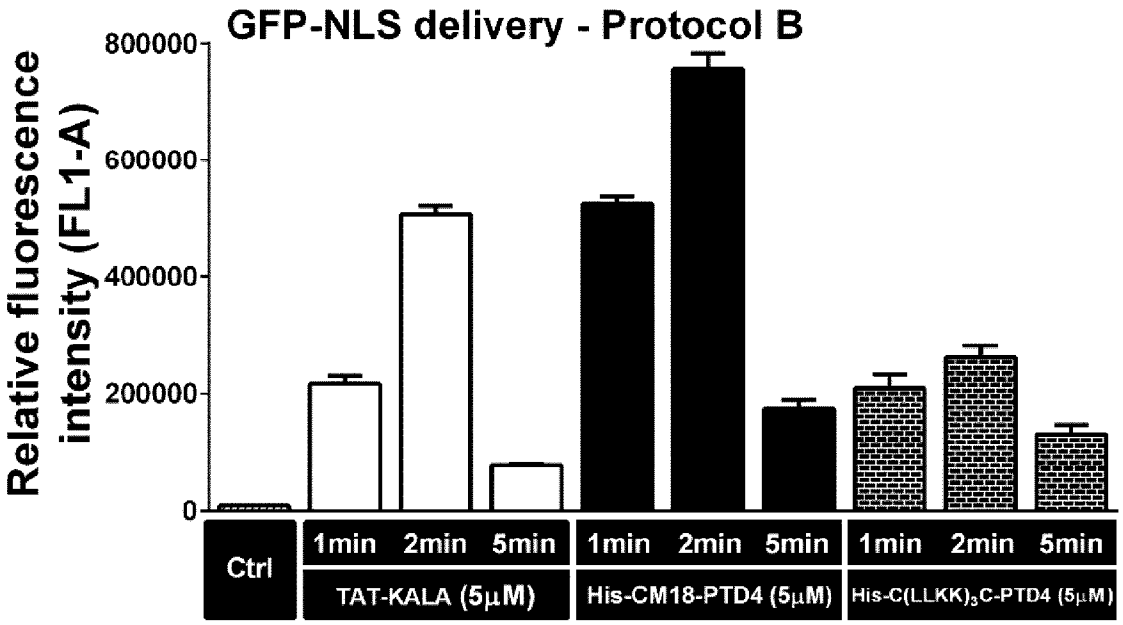


Fig. 29D

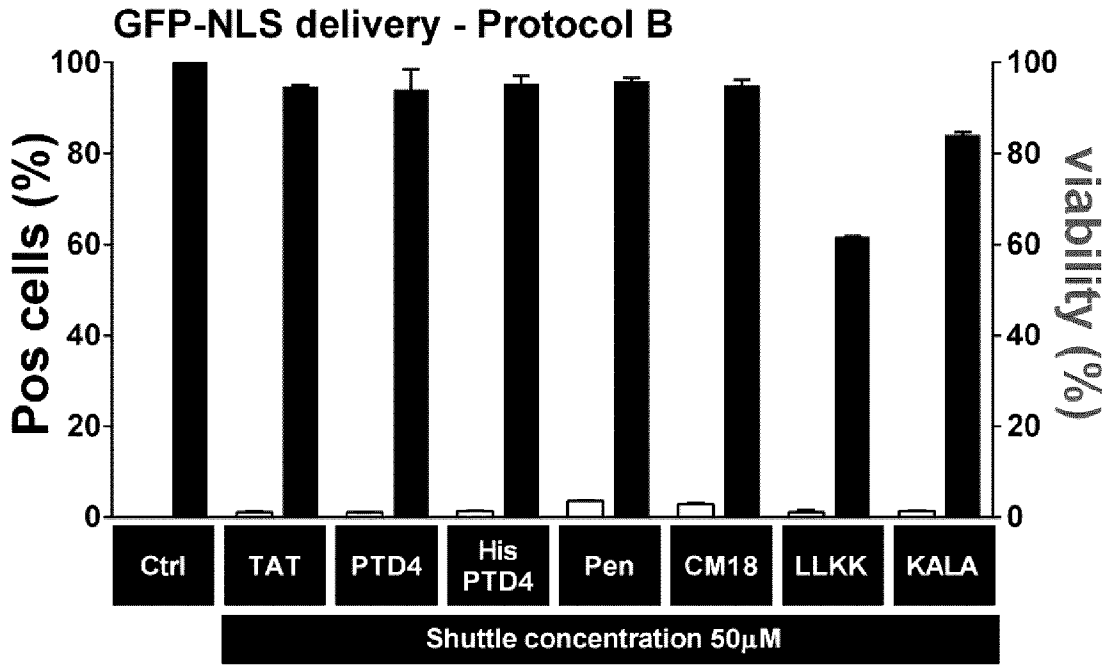


Fig. 29E

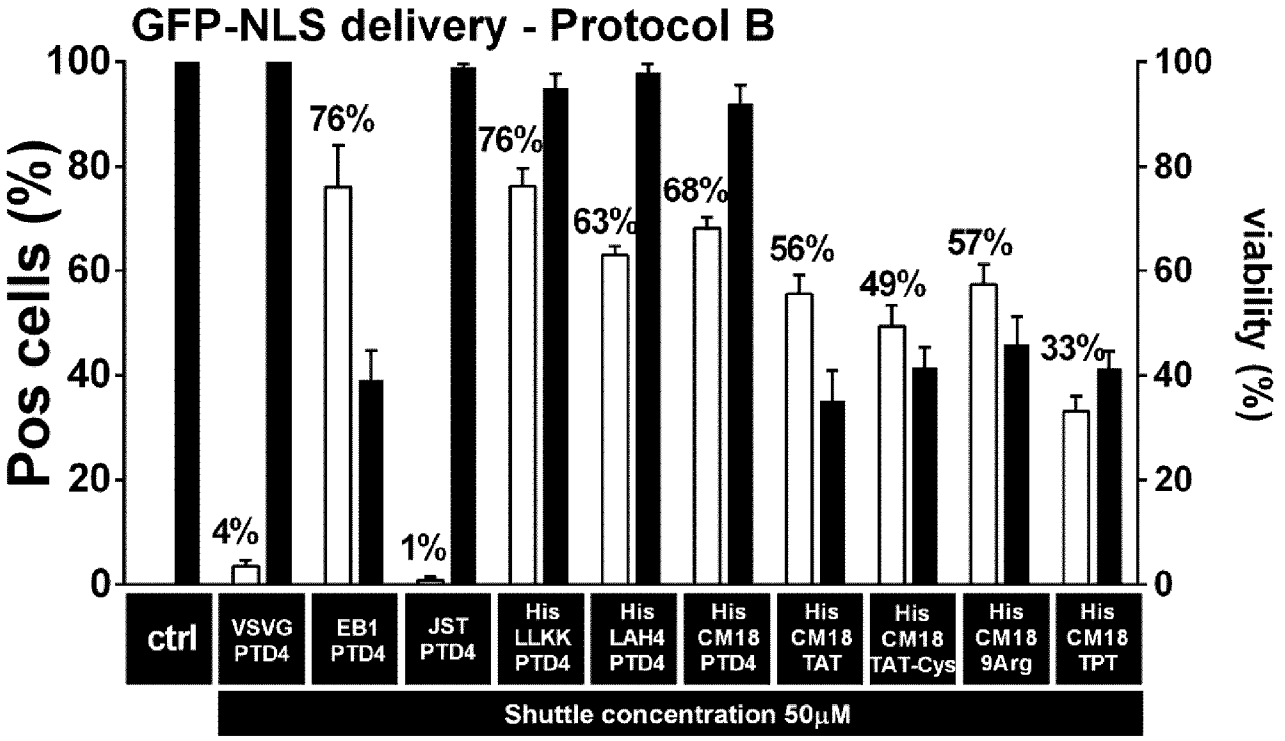


Fig. 29F

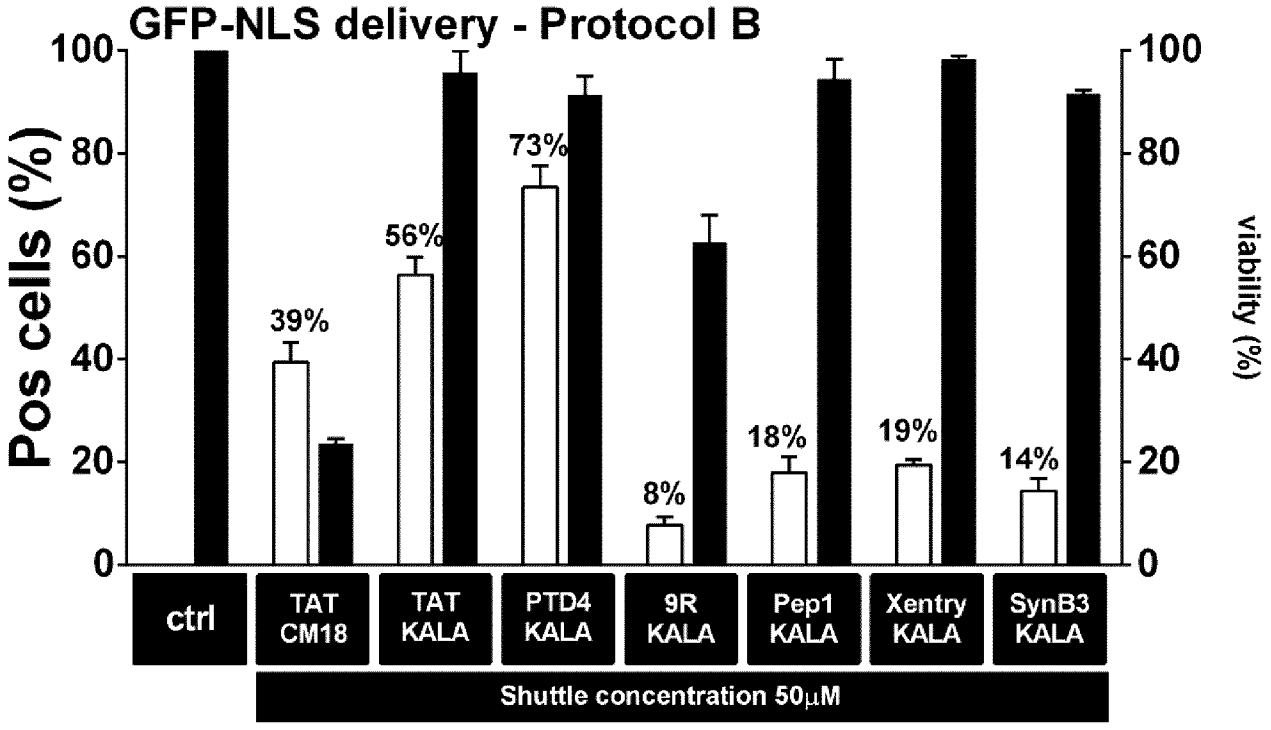


Fig. 29G

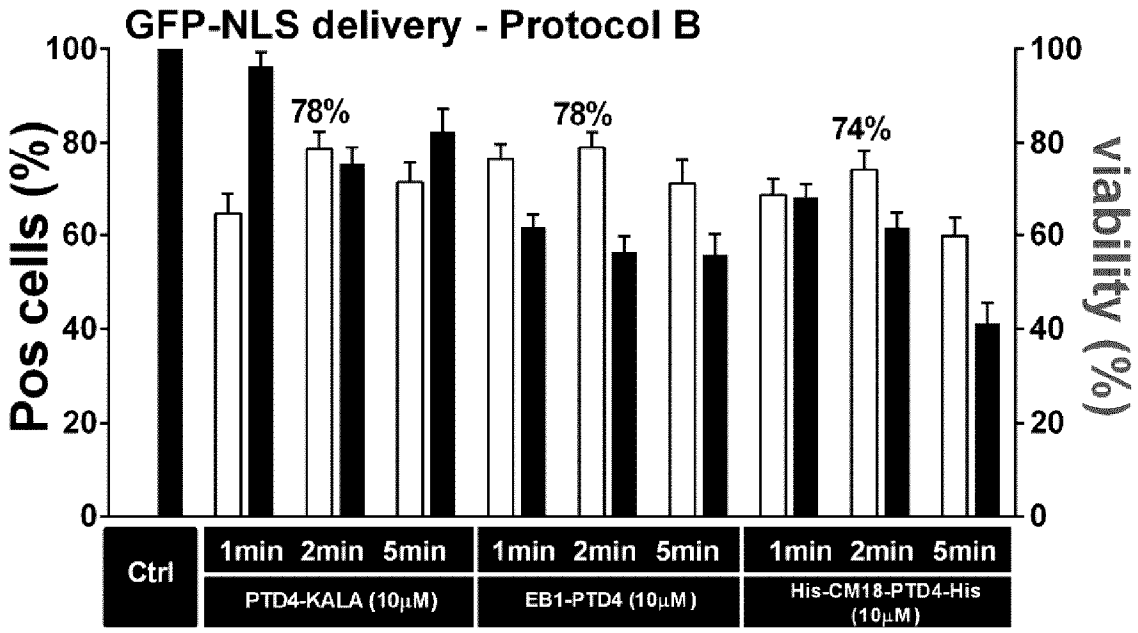


Fig. 29H

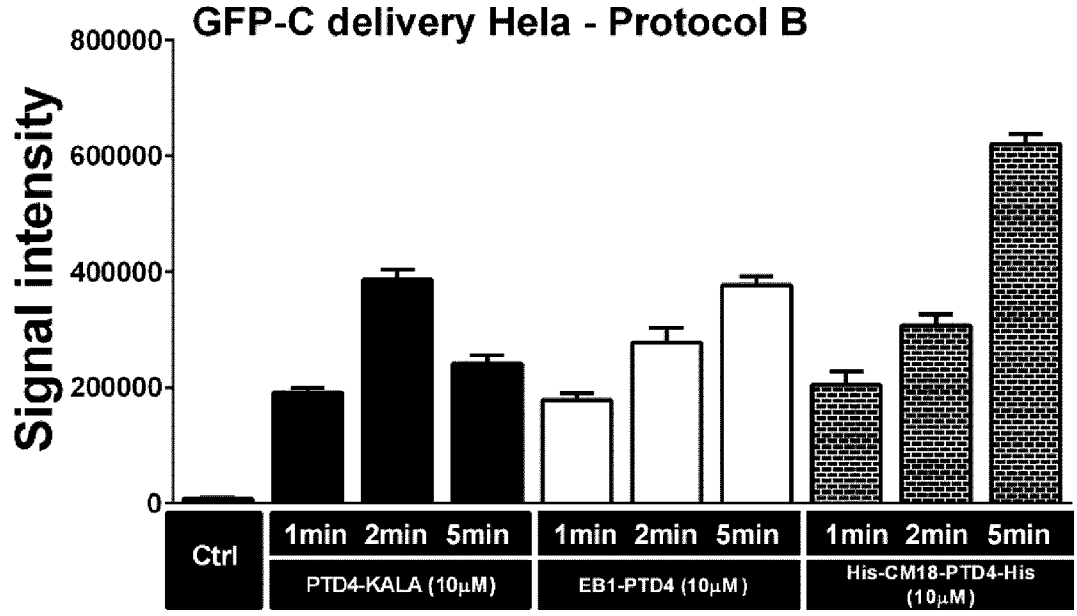


Fig. 29I

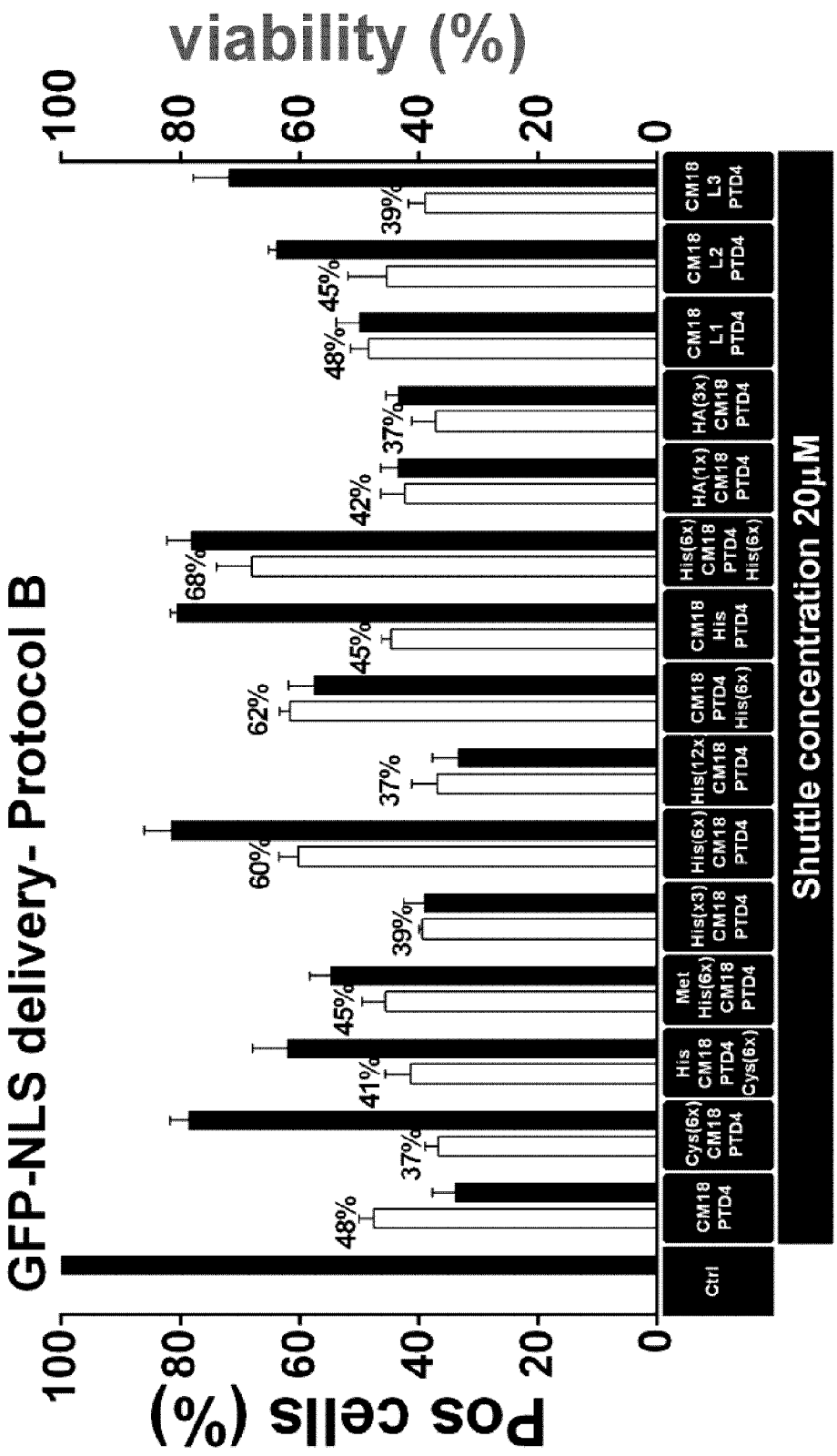

viability (%)
100
80
60
40
20
0
GFP-NLS delivery- Protocol B
Pos cells (%)
Shuttle concentration 20µM
Ctrl
CM18 PTD4
Cys(6x) CM18 PTD4
His CM18 PTD4 Cys(6x)
Met His(6x) CM18 PTD4
His(x3) CM18 PTD4
His(6x) CM18 PTD4
His(12x CM18 PTD4
CM18 PTD4 His(6x)
CM18 His PTD4
His(6x) CM18 PTD4 His(6x)
HA(1x) CM18 PTD4
HA(3x) CM18 PTD4
CM18 L1 PTD4
CM18 L2 PTD4
CM18 L3 PTD4
48%
37%
41%
45%
39%
60%
37%
62%
45%
68%
42%
37%
48%
45%
39%

Fig. 30A

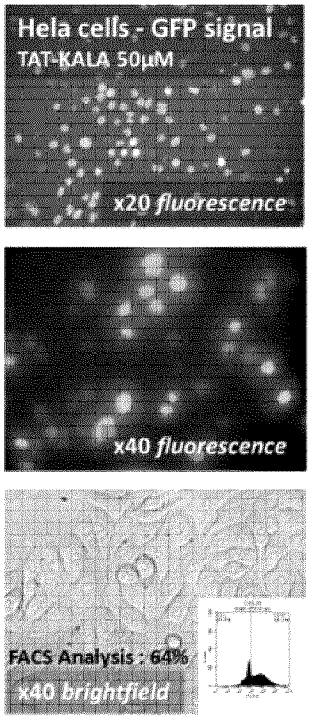


Fig. 30B

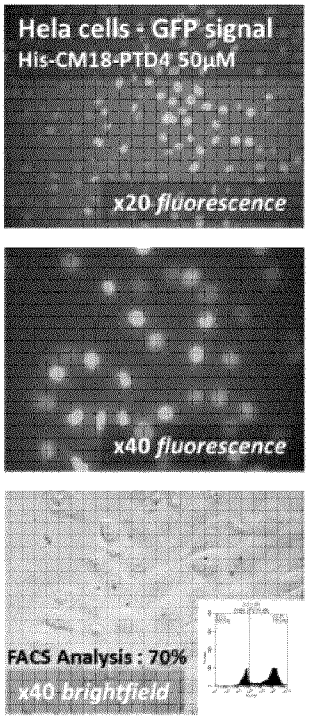


Fig. 30C

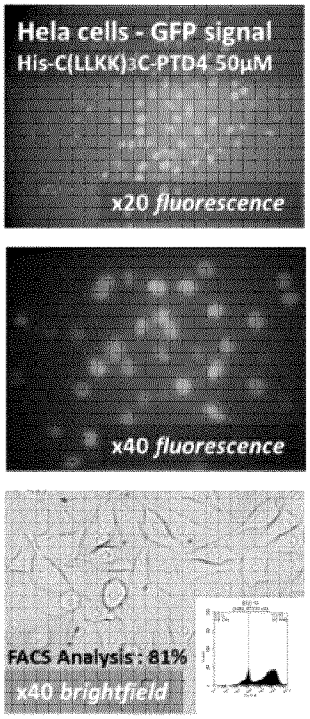


Fig. 30D

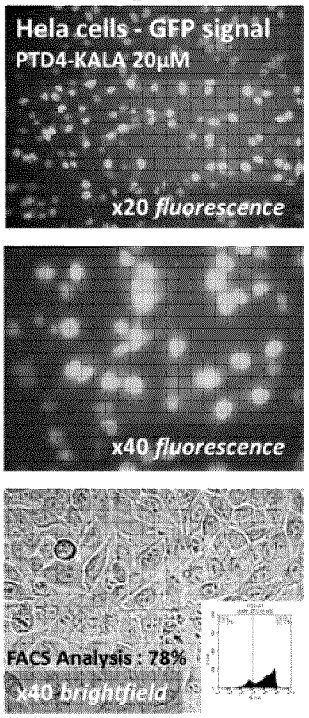


Fig. 30E

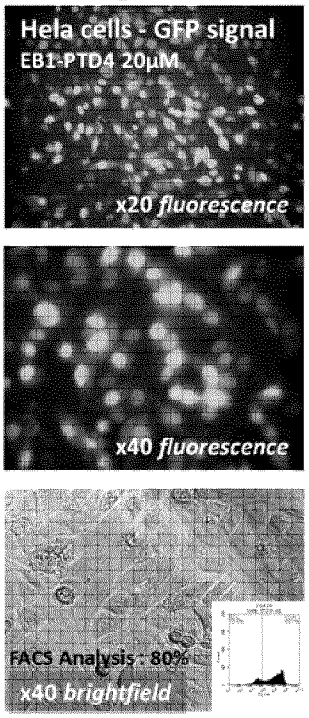


Fig. 30F

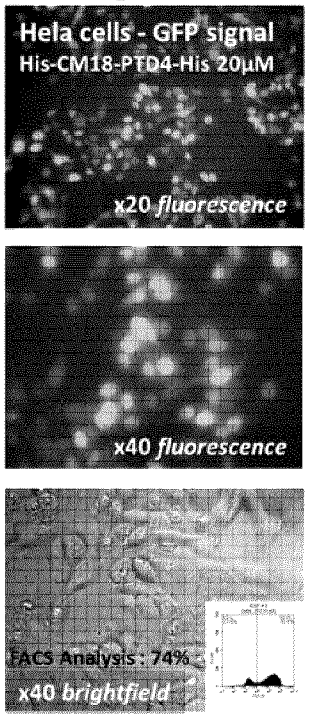


Fig. 31

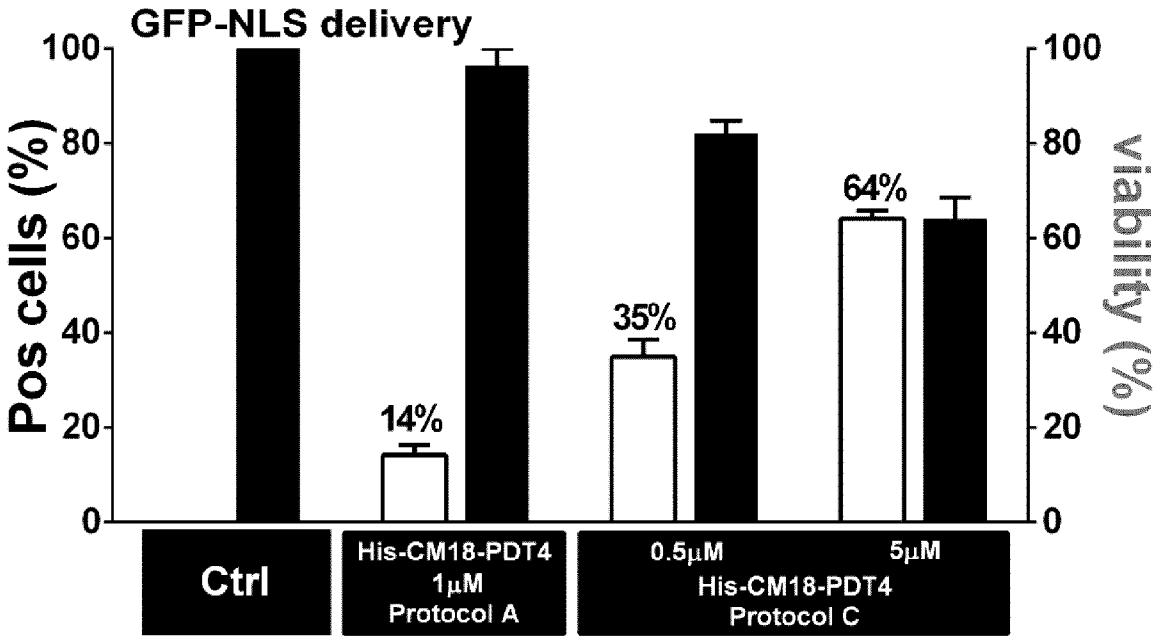

GFP-NLS delivery
Pos cells (%)
viability (%)
100
80
60
40
20
0
14%
35%
64%
Ctrl
His-CM18-PDT4
1μM
Protocol A
0.5μM
5μM
His-CM18-PDT4
Protocol C

Fig. 32A

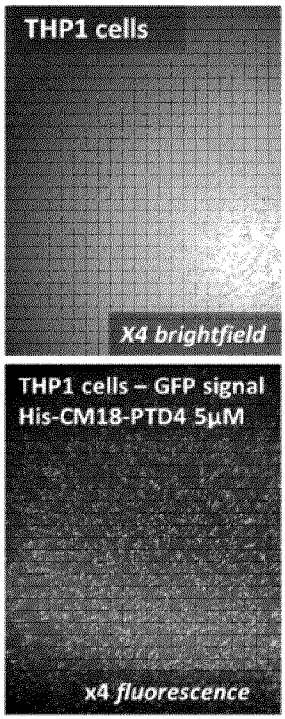


Fig. 32B

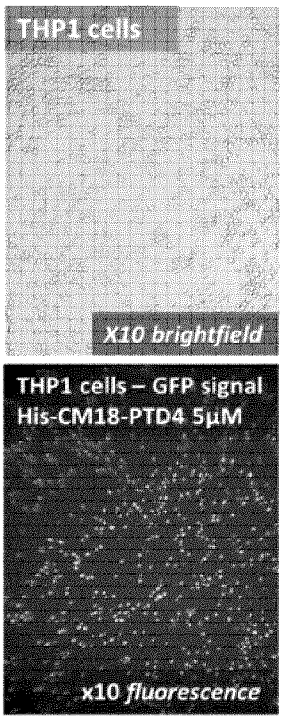


Fig. 32C

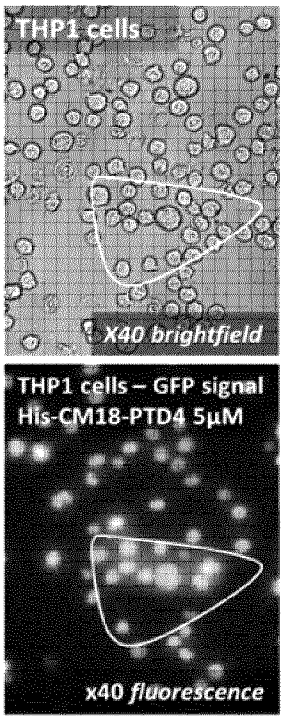
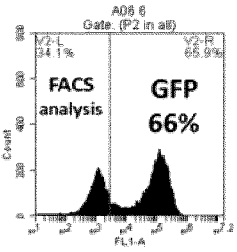


Fig. 32D



Viability = 95%

Fig. 33A

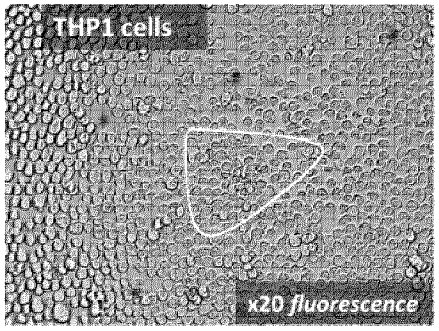


Fig. 33C

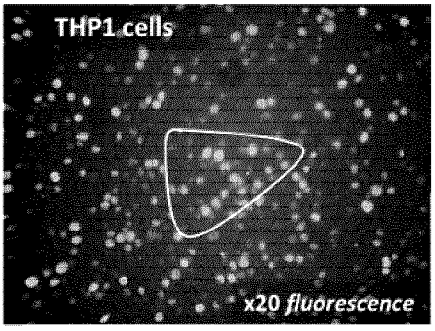


Fig. 33B

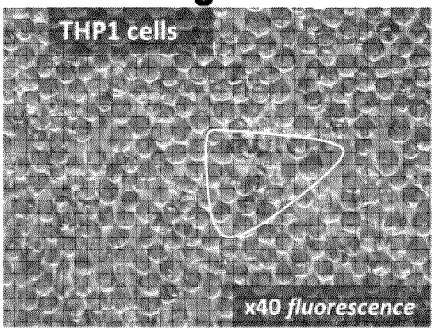
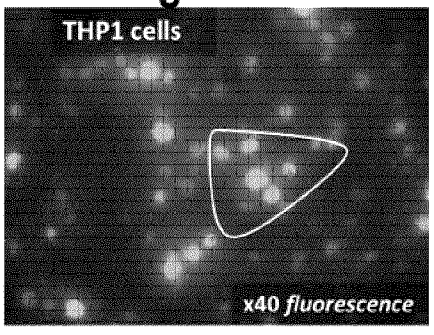
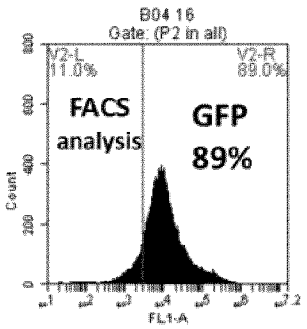


Fig. 33D





Viability = 75%

Fig. 34A

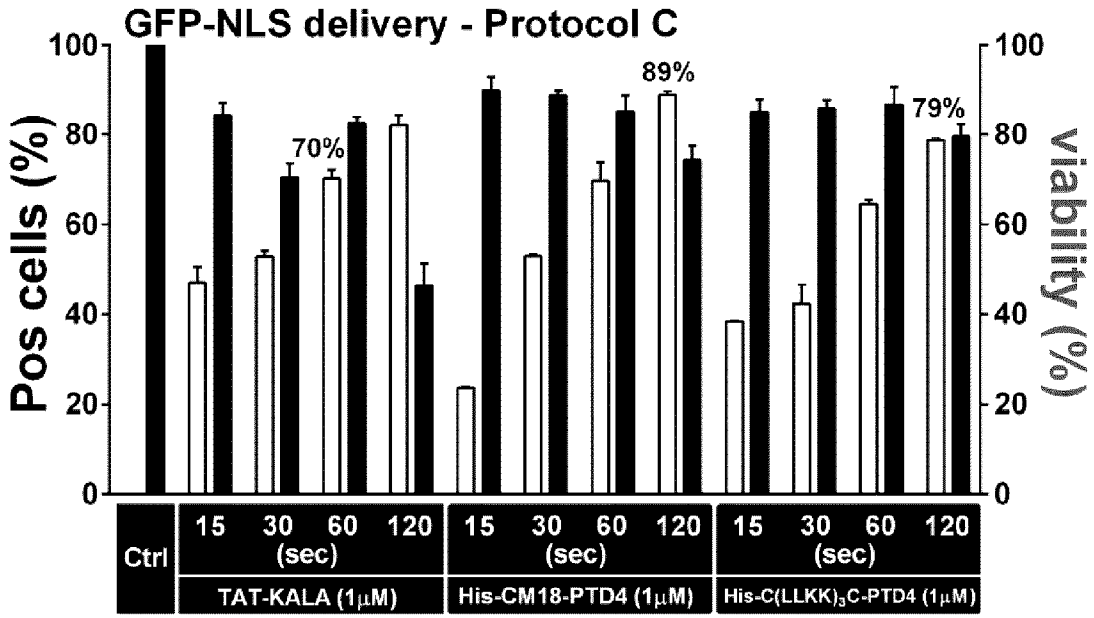


Fig. 34B

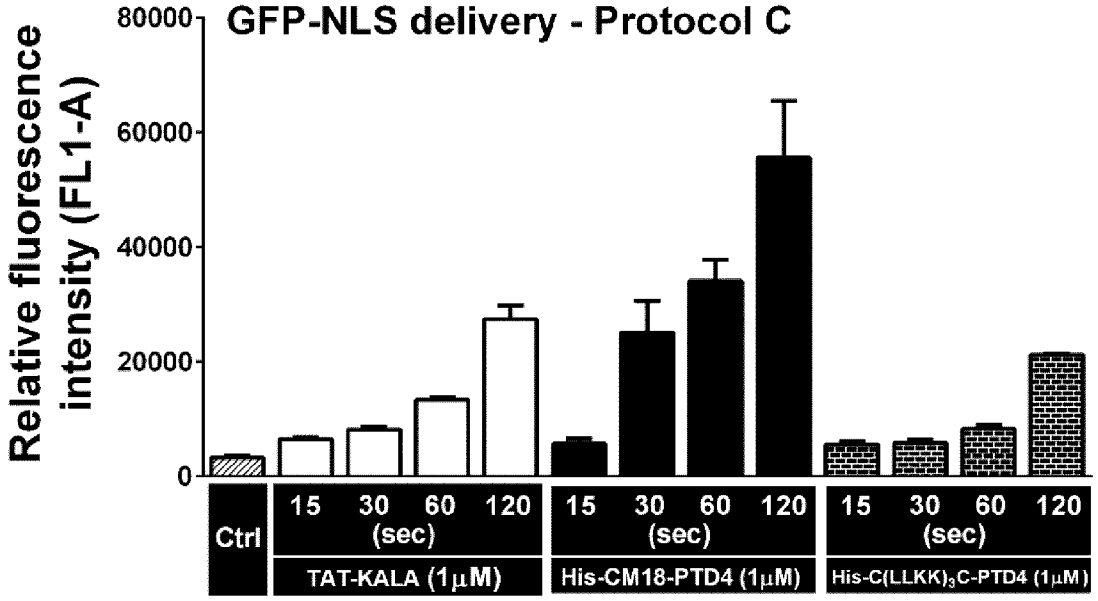


Fig. 35A

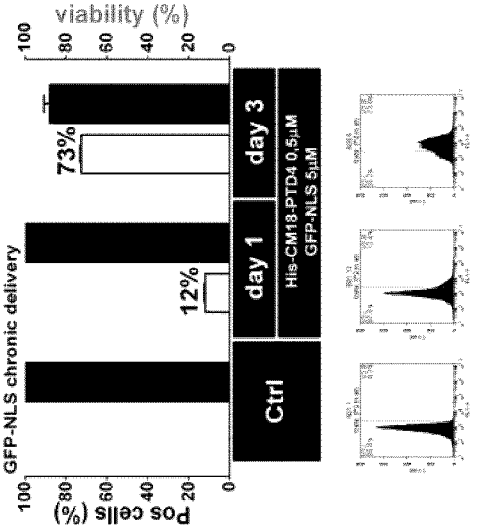


Fig. 35B

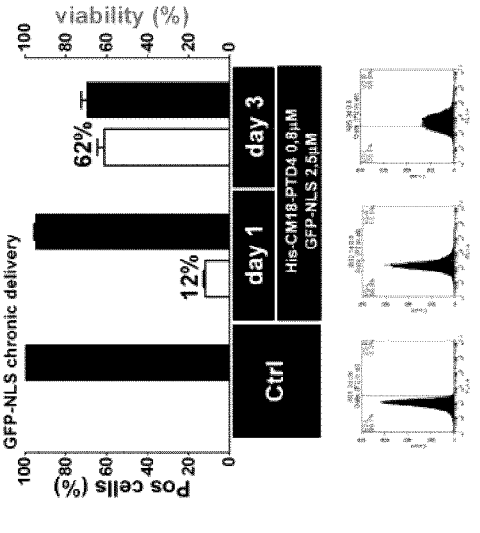


Fig. 35C

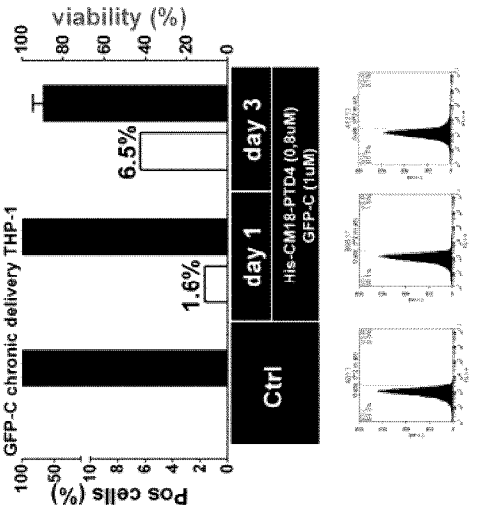


Fig. 35D

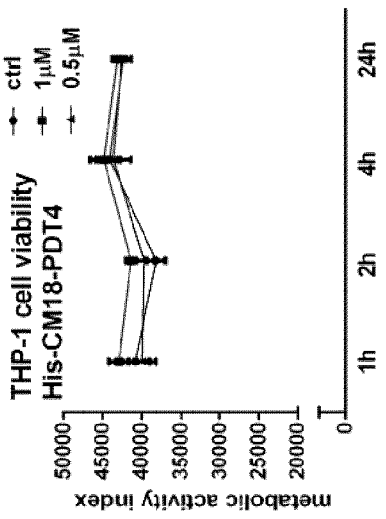


Fig. 35E

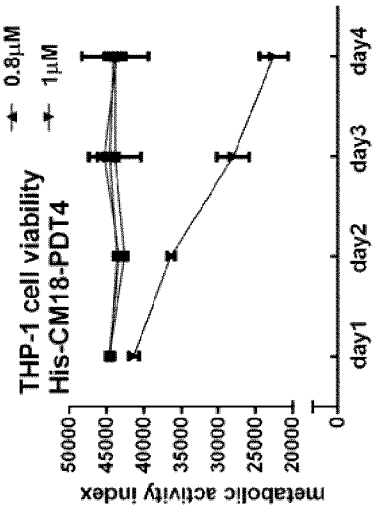


Fig. 35F

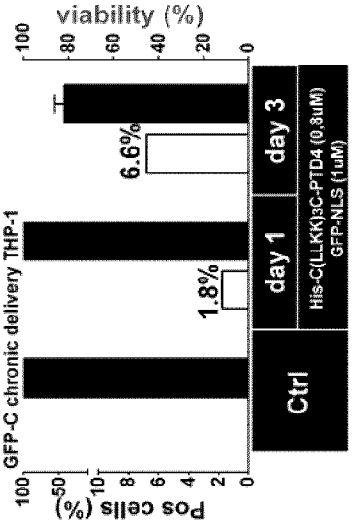


Fig. 36

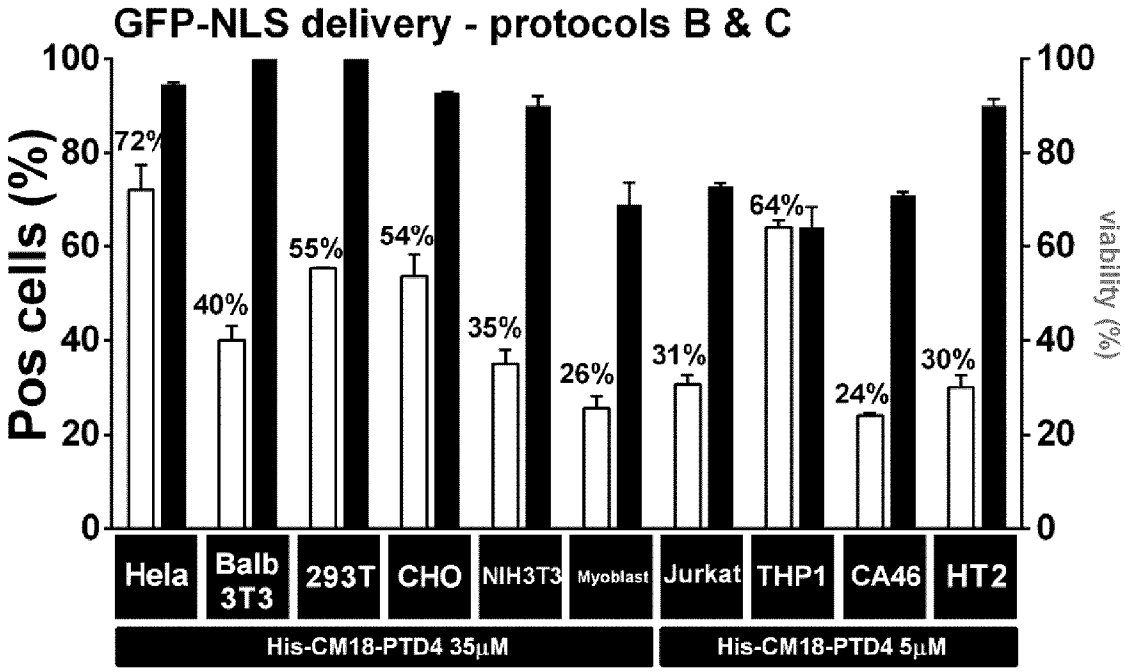


Fig. 37A

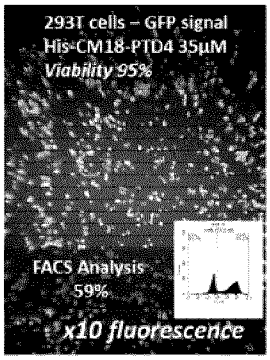


Fig. 37B

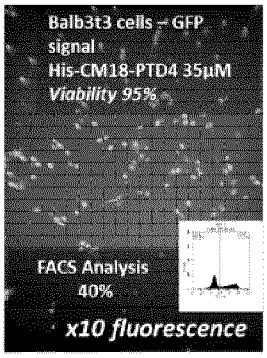


Fig. 37C

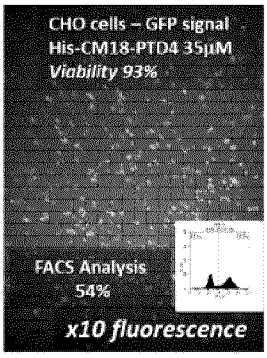


Fig. 37D

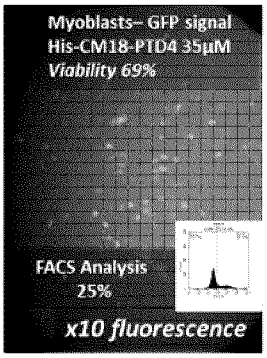


Fig. 37E

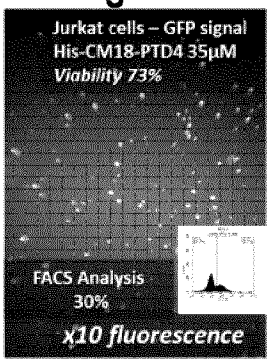


Fig. 37F

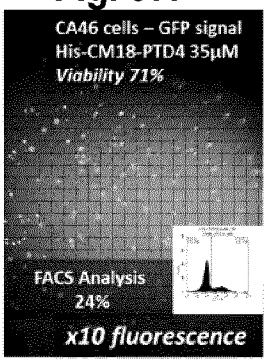


Fig. 37G

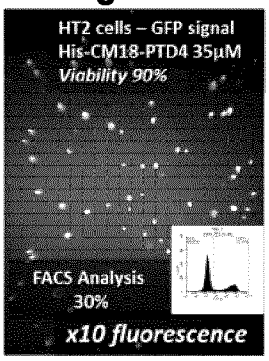


Fig. 37H

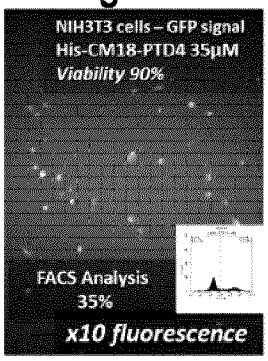


Fig. 38A

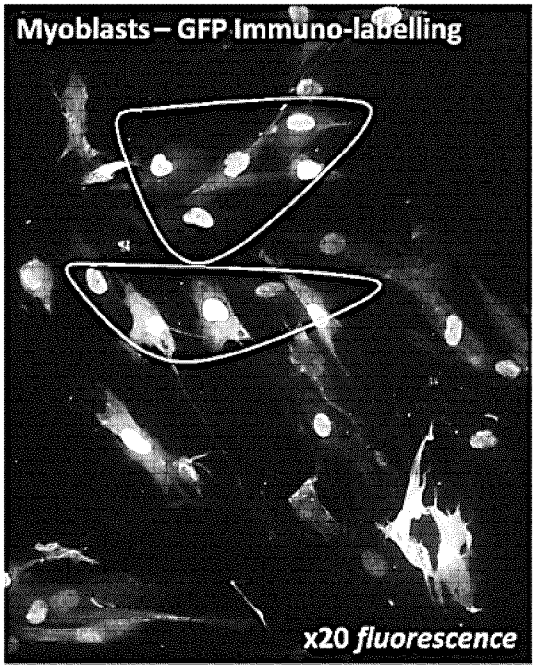


Fig. 38B

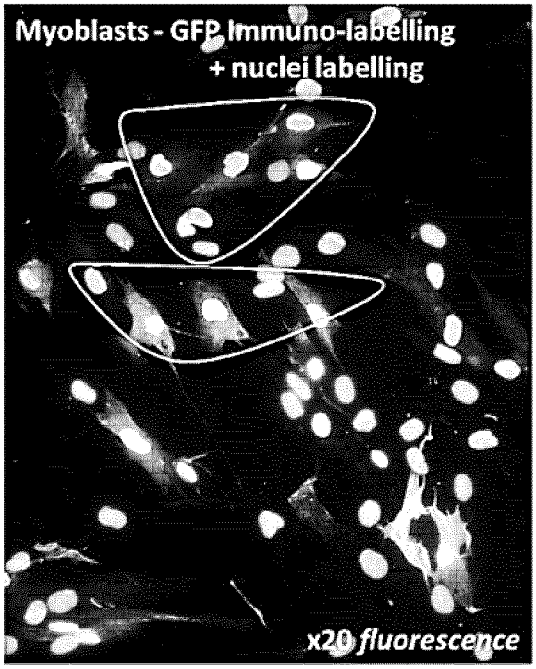


Fig. 39A

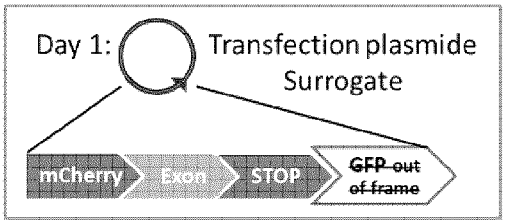


Fig. 39B

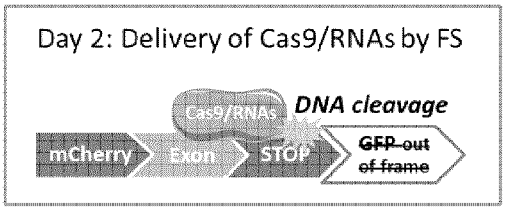


Fig. 39C

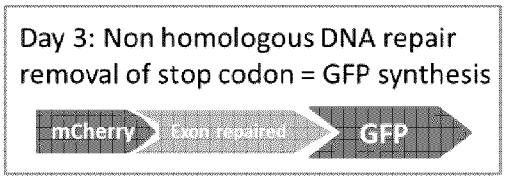


Fig. 39D

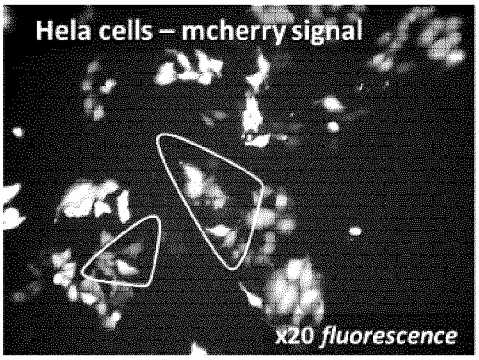


Fig. 39E

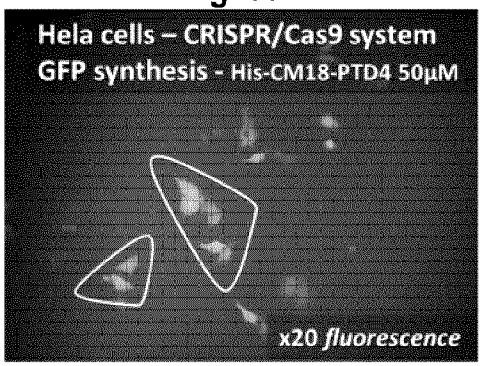


Fig. 40A

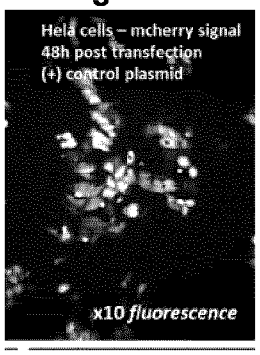


Fig. 40B

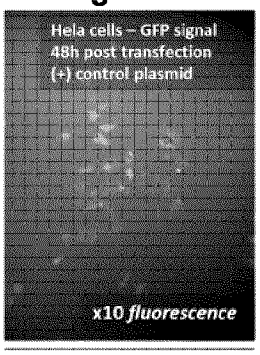


Fig. 40C

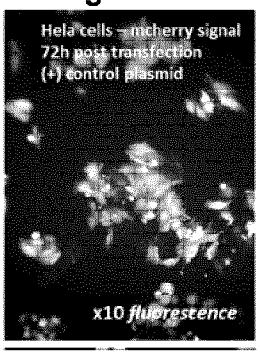


Fig. 40D

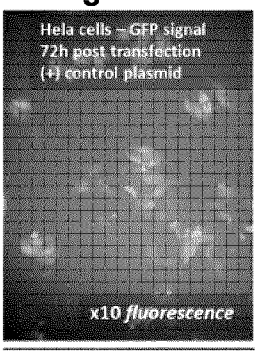


Fig. 40E

Fig. 40F

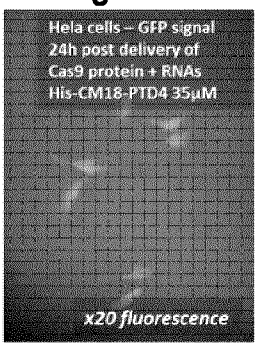


Fig. 40G

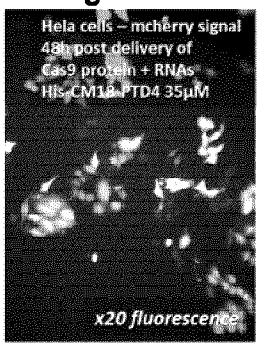


Fig. 40H

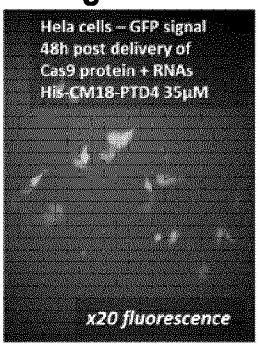


Fig. 41A

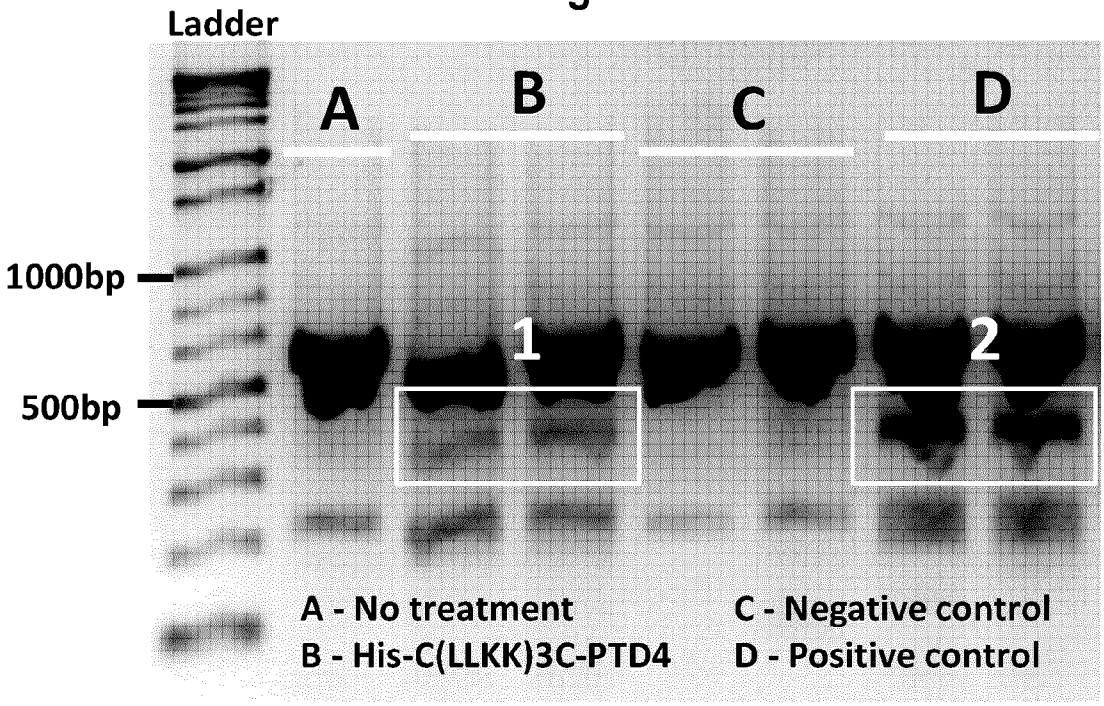


Fig. 41B

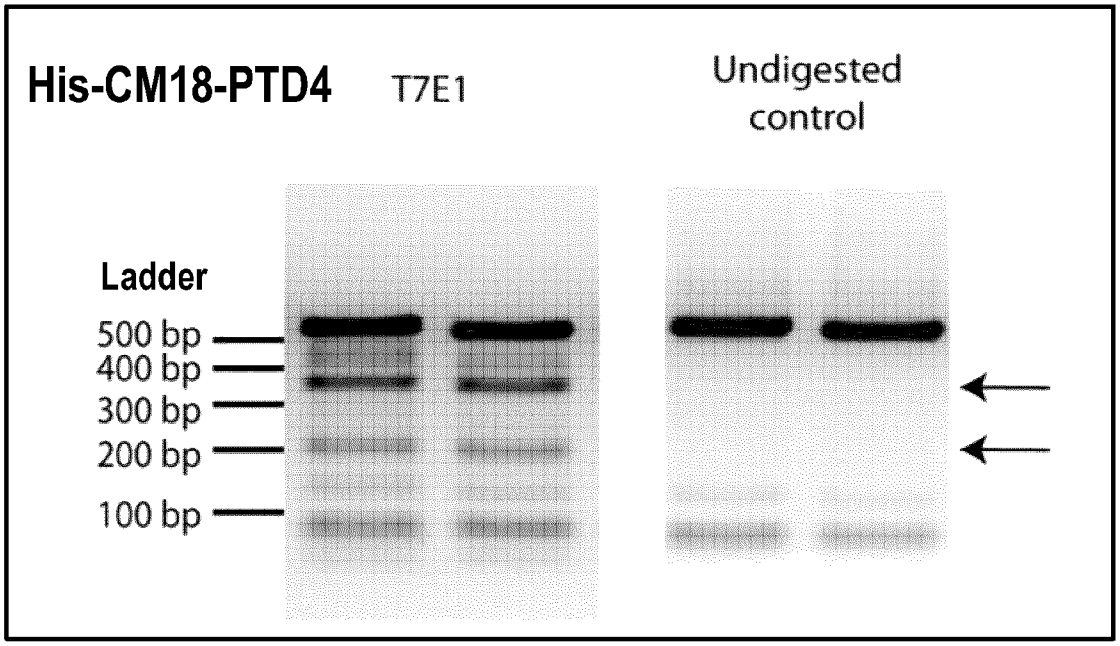


Fig. 41C

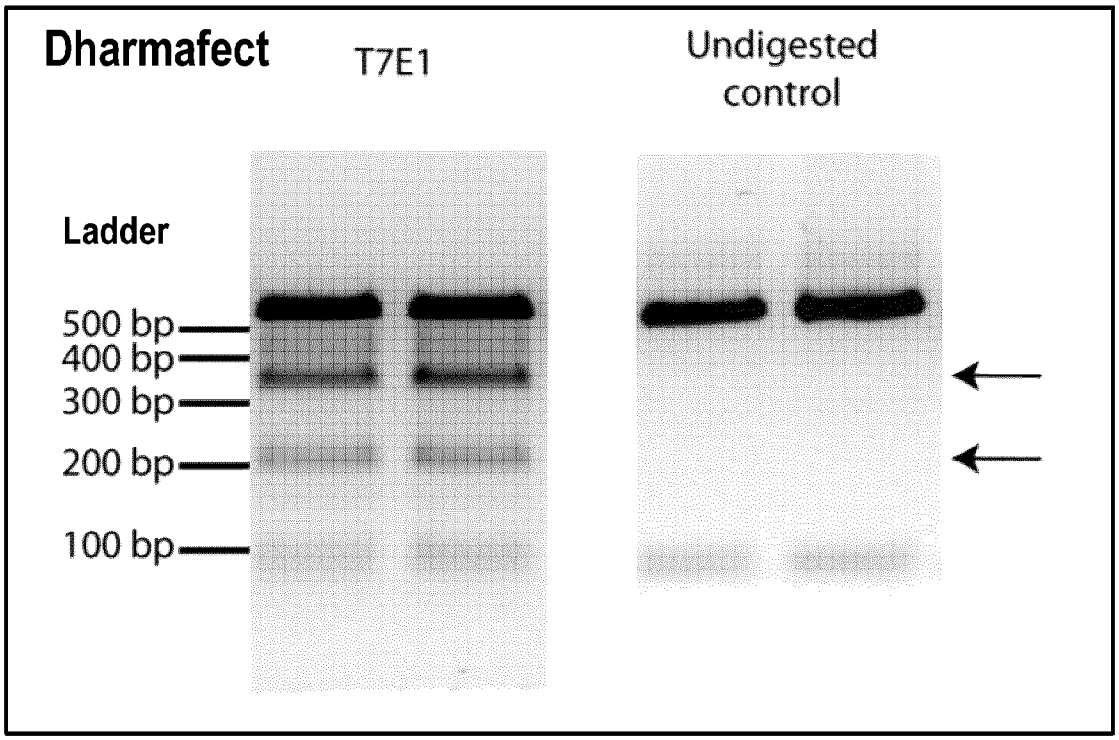


Fig. 42

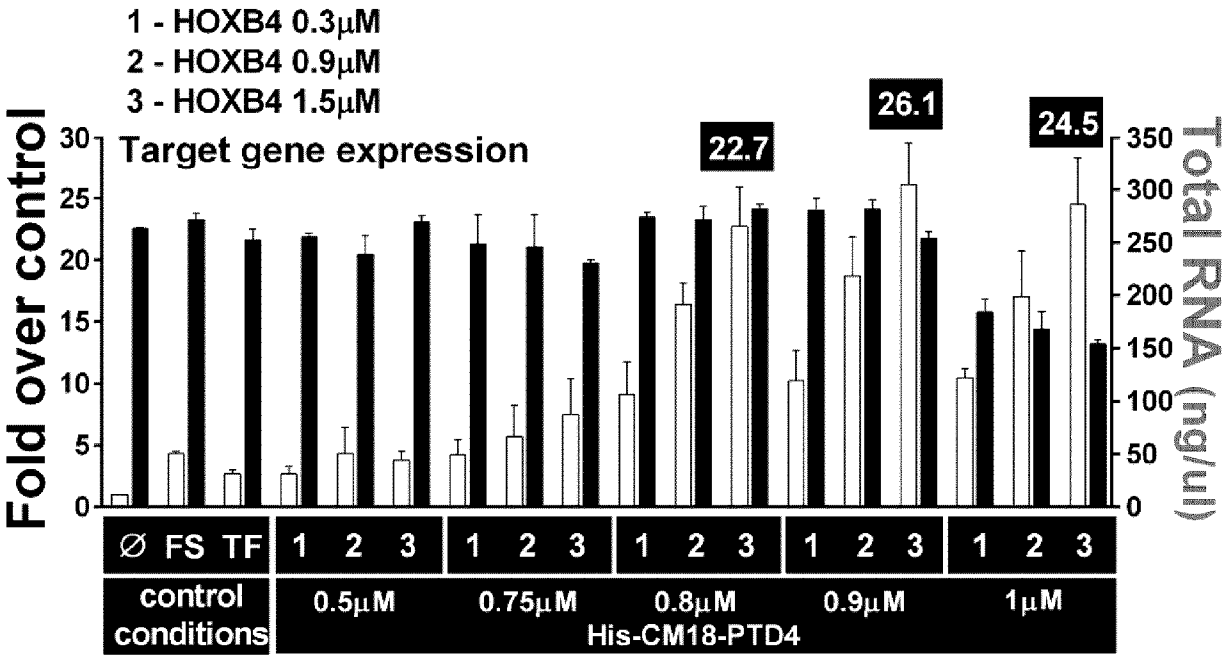

1 - HOXB4 0.3µM
2 - HOXB4 0.9µM
3 - HOXB4 1.5µM
Target gene expression
Fold over control
Total RNA (ng/ul)
22.7
26.1
24.5
Ø FS TF
control conditions
0.5µM
0.75µM
0.8µM
0.9µM
1µM
His-CM18-PTD4

Fig. 43

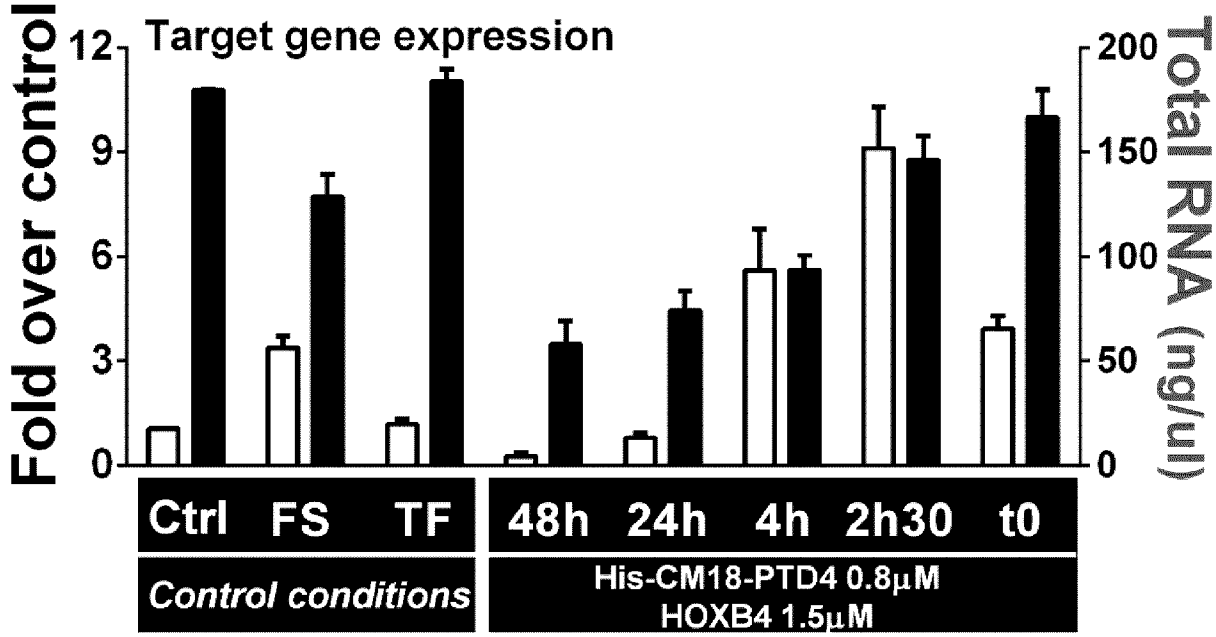

Target gene expression
Fold over control
Total RNA (ng/ul)
Ctrl FS TF
Control conditions
48h 24h 4h 2h30 t0
His-CM18-PTD4 0.8µM
HOXB4 1.5µM

Fig. 44

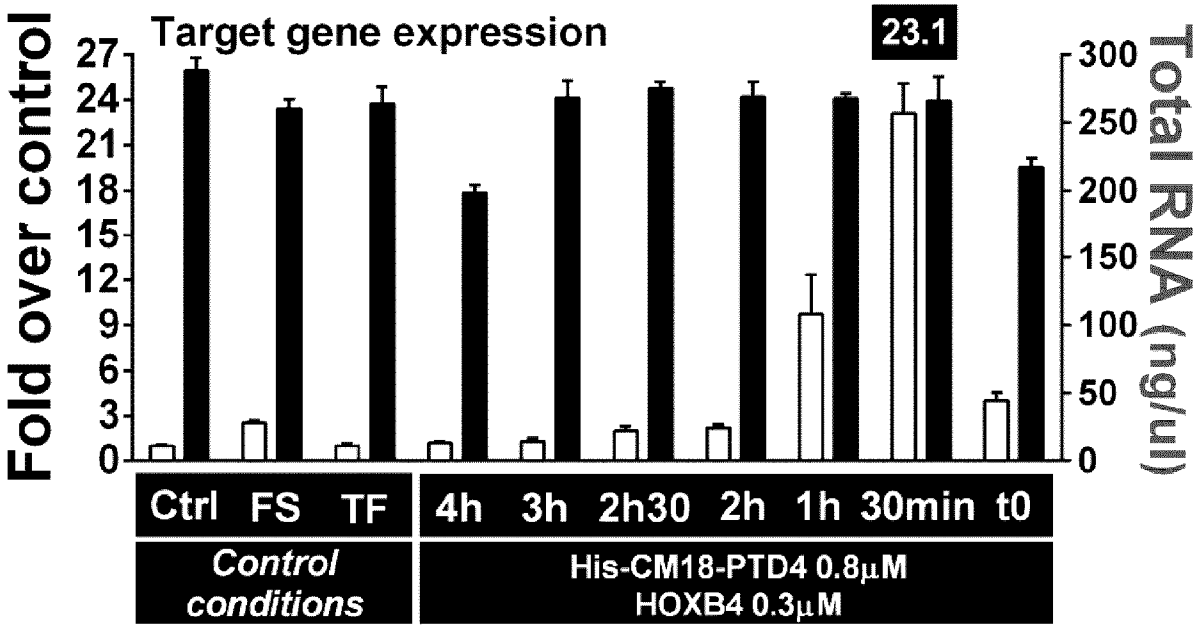


Fig. 45A

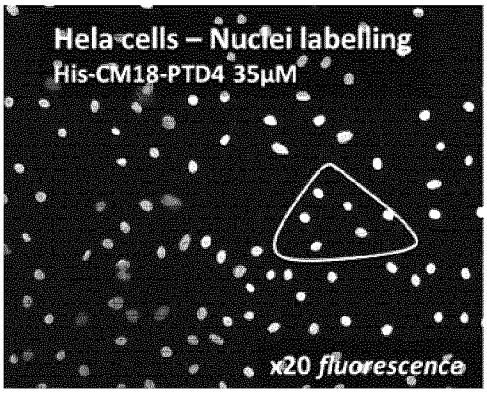


Fig. 45B

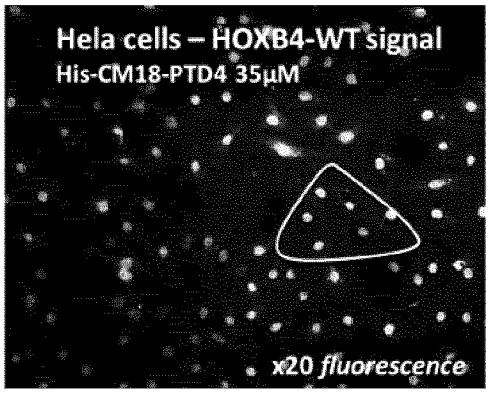


Fig. 45C

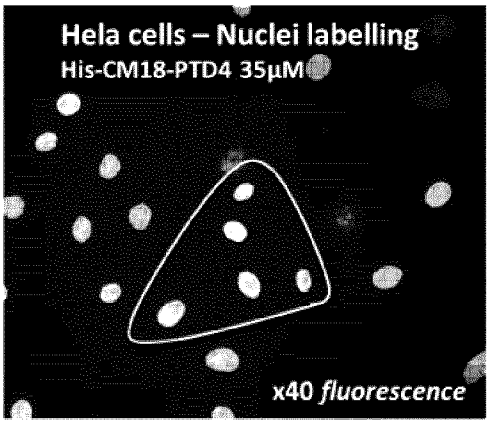


Fig. 45D

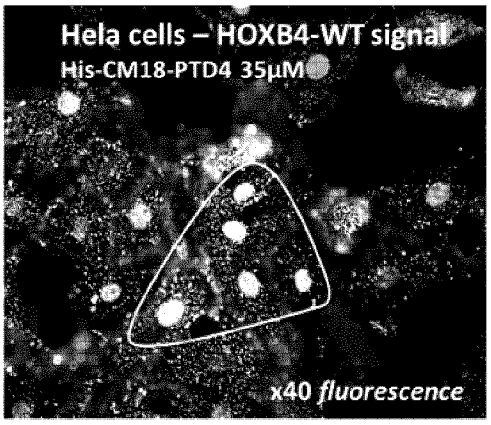


Fig. 46A

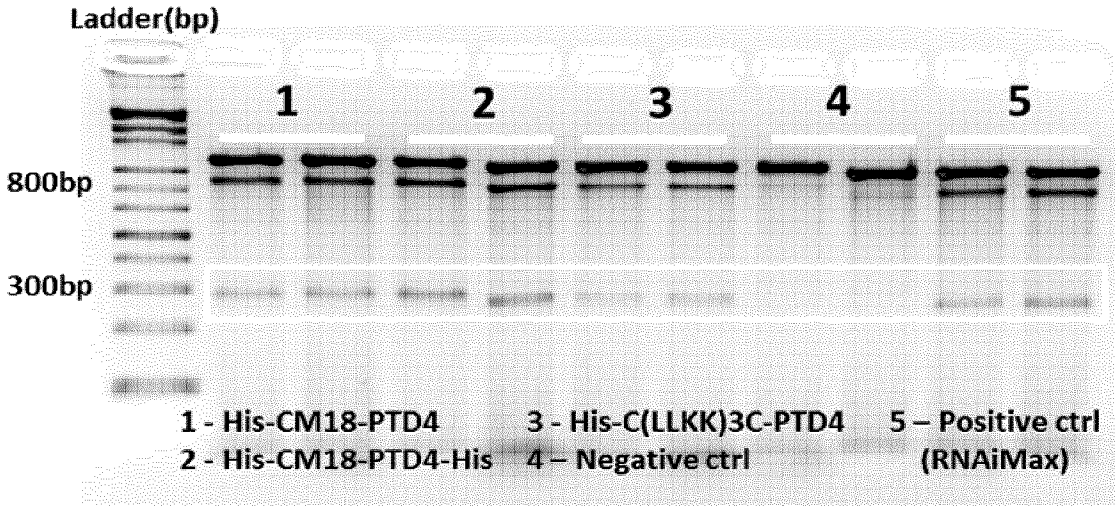


Fig. 46B

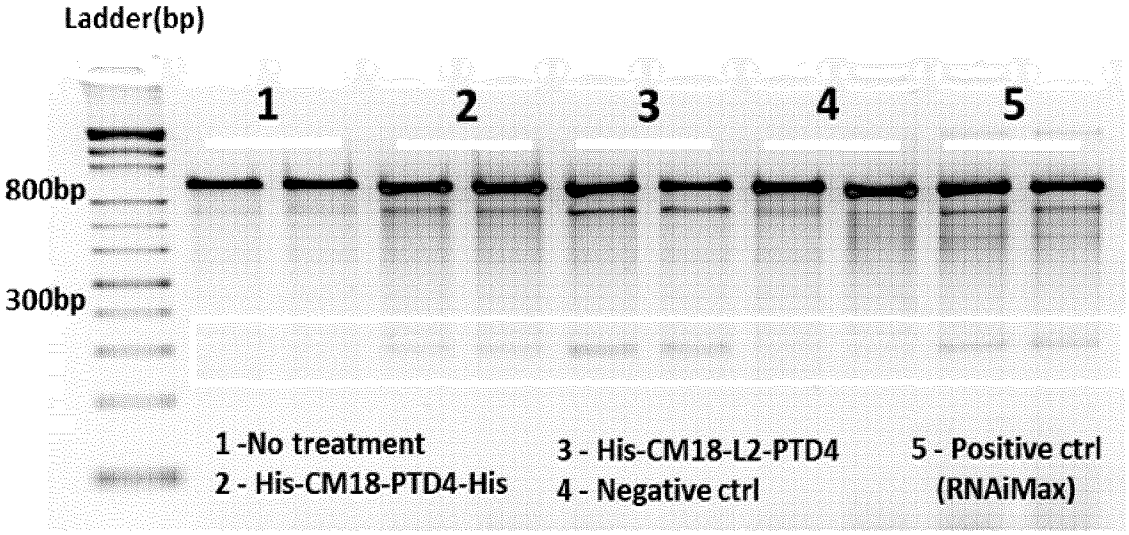


Fig. 47

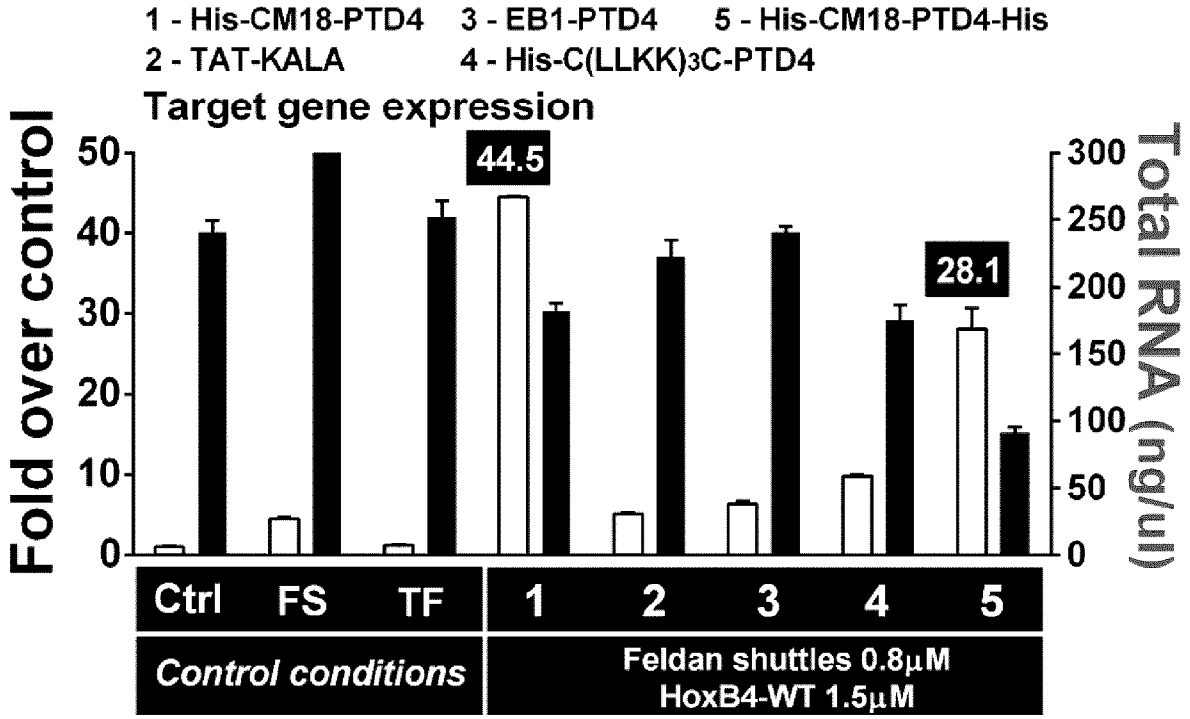

1 - His-CM18-PTD4
2 - TAT-KALA
3 - EB1-PTD4
4 - His-C(LLKK)3C-PTD4
5 - His-CM18-PTD4-His
Target gene expression
Fold over control
Total RNA (ng/µl)
44.5
28.1
Ctrl
FS
TF
1
2
3
4
5
Control conditions
Feldan shuttles 0.8µM
HoxB4-WT 1.5µM

Fig. 48

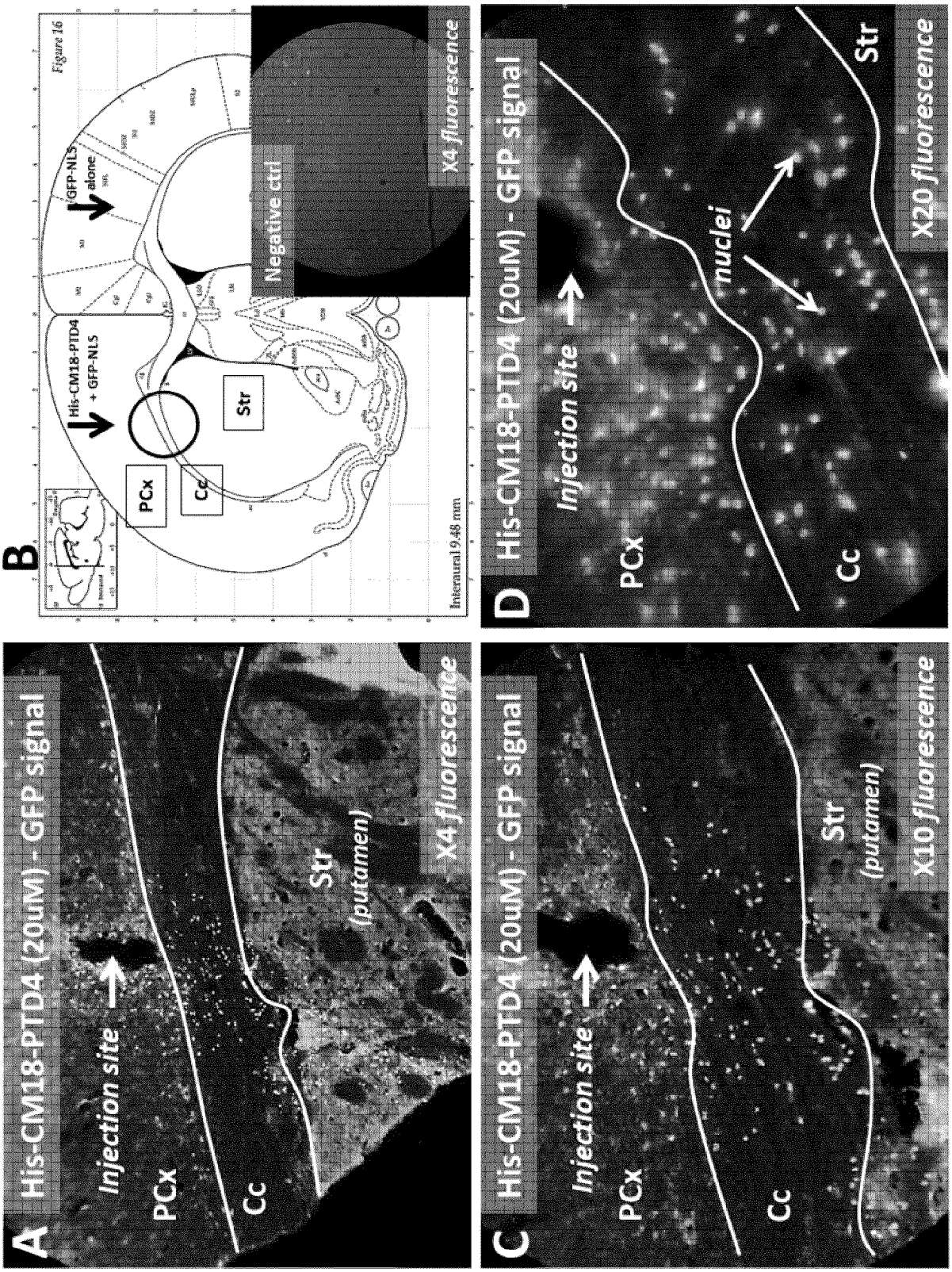

A
His-CM18-PTD4 (20uM) - GFP signal
Injection site
PCx
Cc
Str
(putamen)
X4 fluorescence
B
Figure 16
His-CM18-PTD4
+ GFP-NLS
GFP-NLS
alone
PCx
Cc
Str
Negative ctrl
X4 fluorescence
Interaural 9.48 mm
C
His-CM18-PTD4 (20uM) - GFP signal
Injection site
PCx
Cc
Str
(putamen)
X10 fluorescence
D
His-CM18-PTD4 (20uM) - GFP signal
Injection site
PCx
nuclei
Cc
Str
X20 fluorescence

Fig. 49A

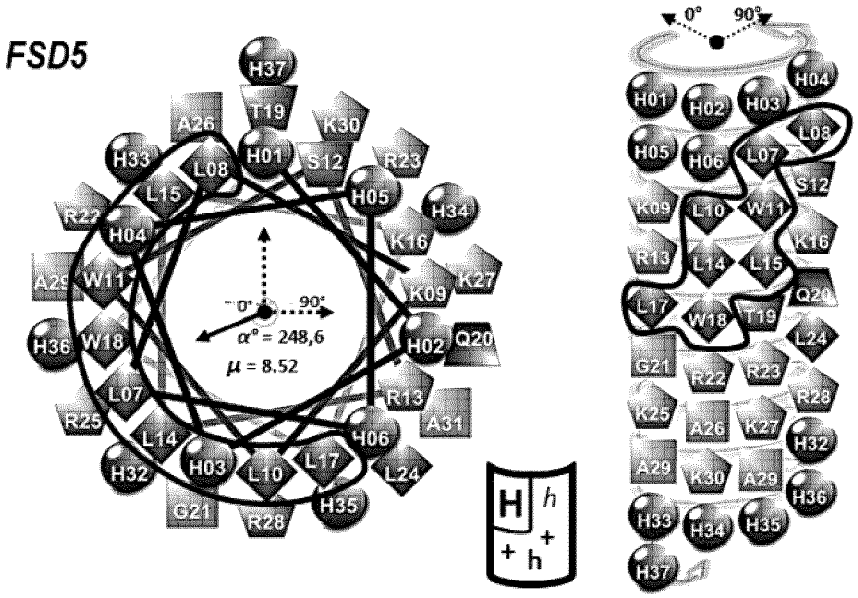
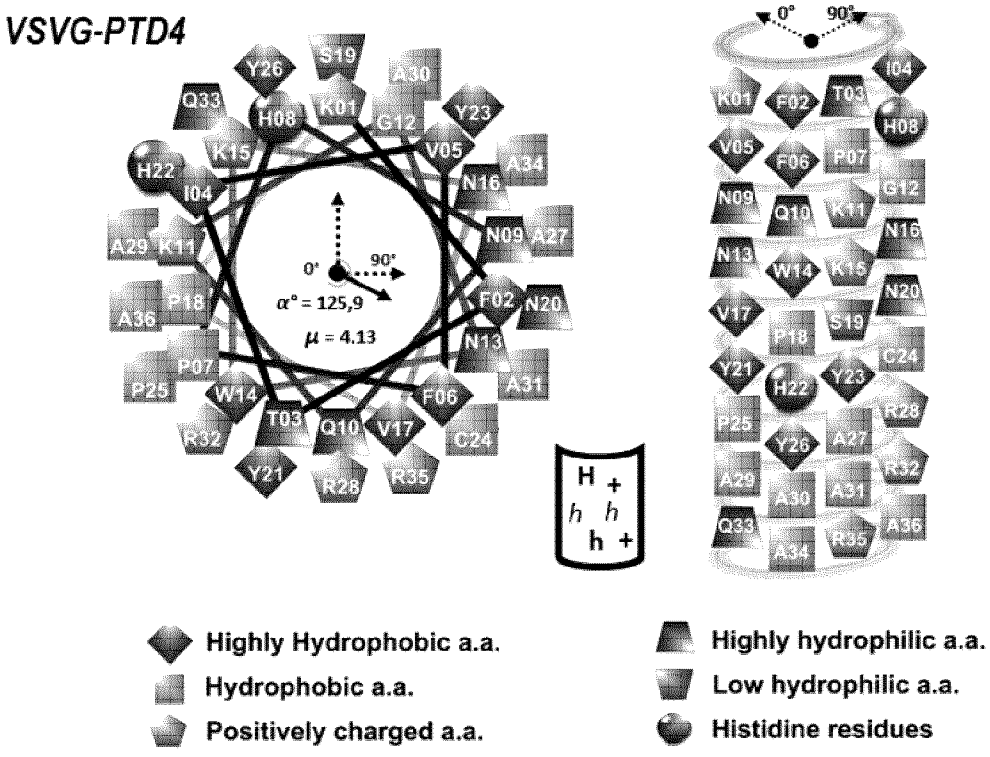


Fig. 49B



Highly Hydrophobic a.a.
Hydrophobic a.a.
Positively charged a.a.
Highly hydrophilic a.a.
Low hydrophilic a.a.
Histidine residues

Fig. 49C

Fig. 49D

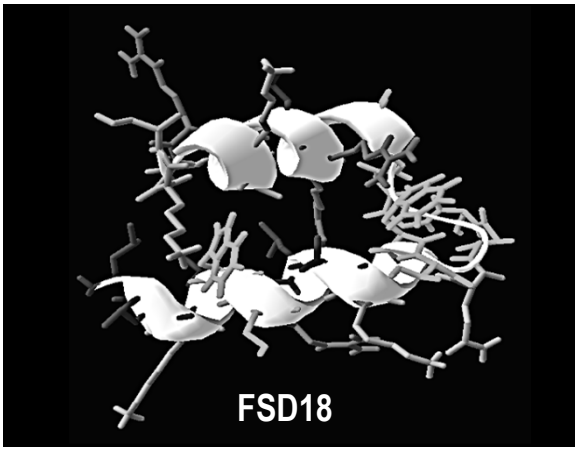


Fig. 49E

Fig. 49F

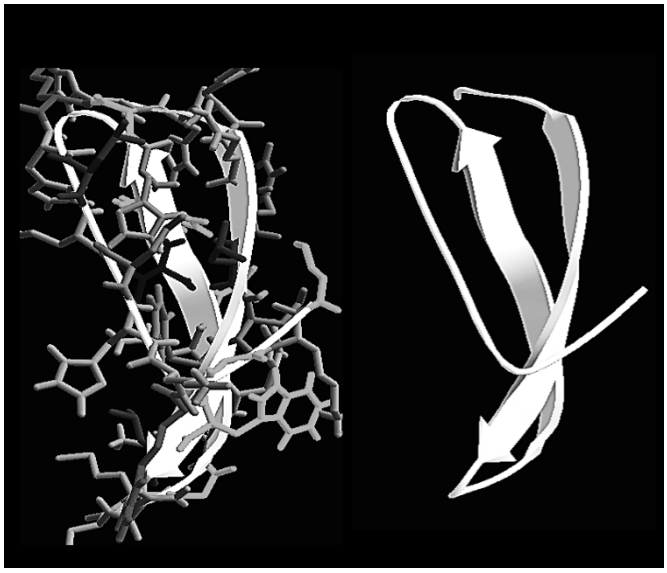


Fig. 49G

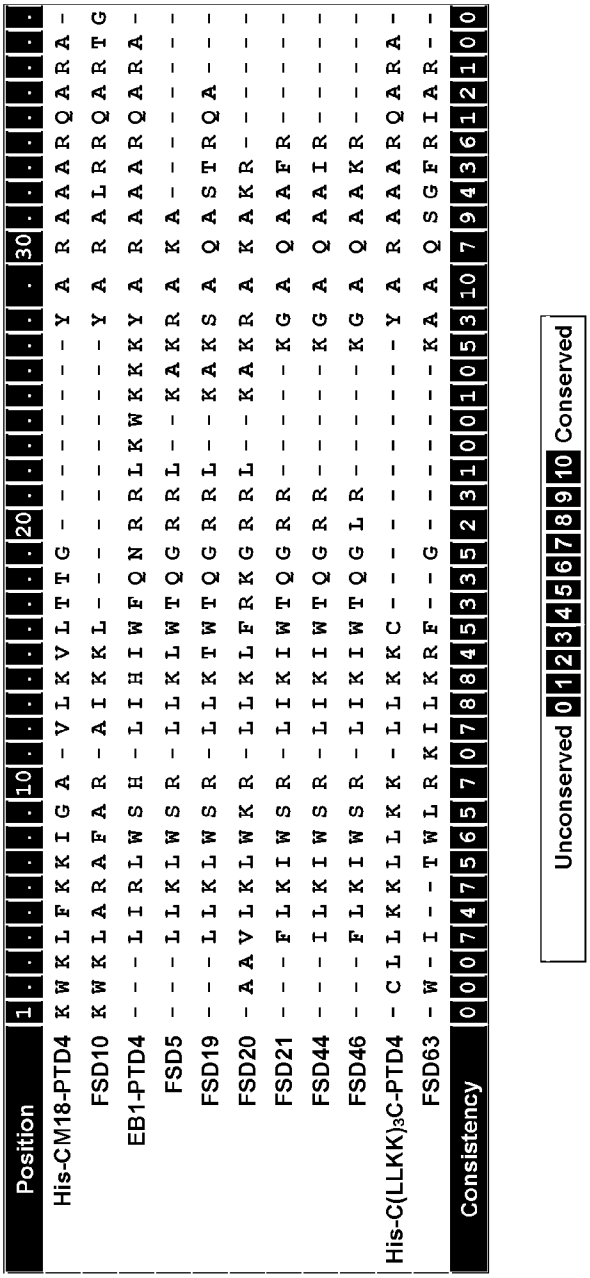

Position
1 . . . . . 10 . . . . . 20 . . . . . 30 . . . . . .
His-CM18-PTD4 K W K L F K K I G A - V L K V L T T G - - - - - - - - - - Y A R A A A R Q A R A -
FSD10 K W K L A R A F A R - A I K K L - - - - - - - - - - Y A R A L R R Q A R T G
EB1-PTD4 - - - L I R L W S H - L I H I W F Q N R R L K W K K K Y A R A A A R Q A R A -
FSD5 - - - L I K L W S R - L L K L W T Q G R R L - - K A K R A K A - - - - - - - -
FSD19 - - - L L K L W S R - L L K T W T Q G R R L - - K A K S A Q A S T R Q A - - -
FSD20 - A A V L K L W K R - L L K L F R K G R R L - - K A K R A K A K R - - - - - -
FSD21 - - - F L K I W S R - L I K I W T Q G R R - - - - K G A Q A A F R - - - - -
FSD44 - - - I L K I W S R - L I K I W T Q G R R - - - - K G A Q A A I R - - - - -
FSD46 - - - F L K I W S R - L I K I W T Q G L R - - - - K G A Q A A K R - - - - -
His-C(LLKK)3C-PTD4 - C L L K K L L K K K C - - - - - - - - - - Y A R A A A R Q A R A -
FSD63 - W - I - - T W L R K I L K R F - - G - - - - - - - K A A Q S G F R I A R - -
Consistency 0 0 7 4 7 5 6 5 7 0 7 8 4 5 3 3 5 2 3 1 0 0 1 0 5 3 10 7 9 4 3 6 1 2 1 0 0
Unconserved 0 1 2 3 4 5 6 7 8 9 10 Conserved

Fig. 50A

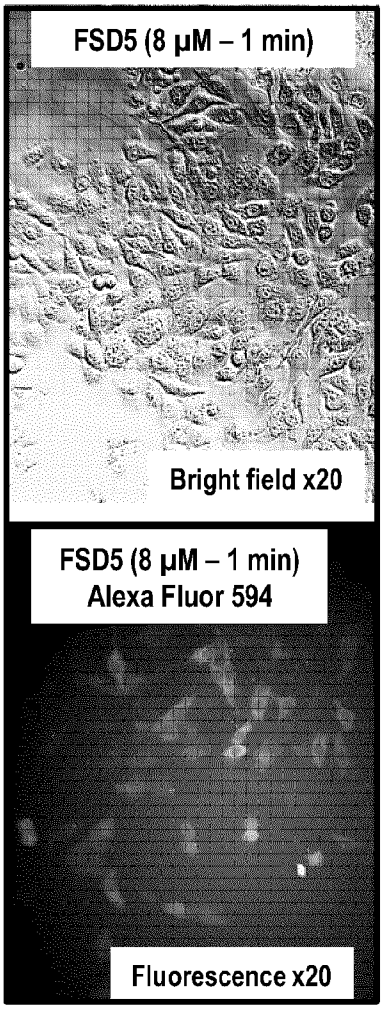


Fig. 50B

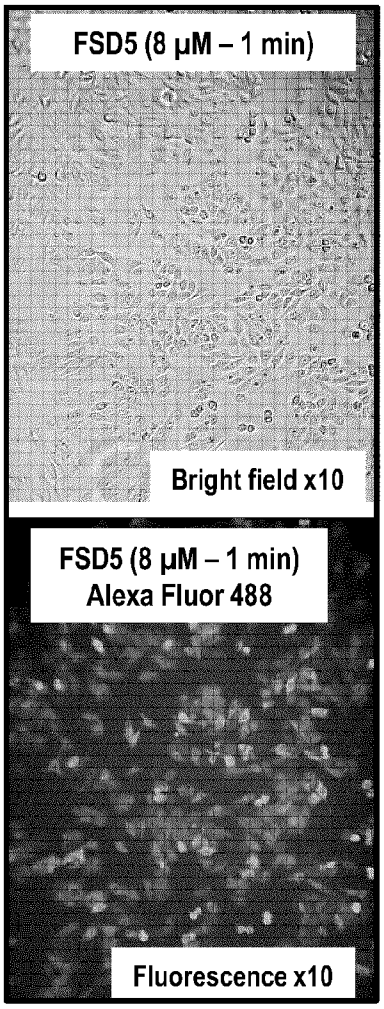


Fig. 50C

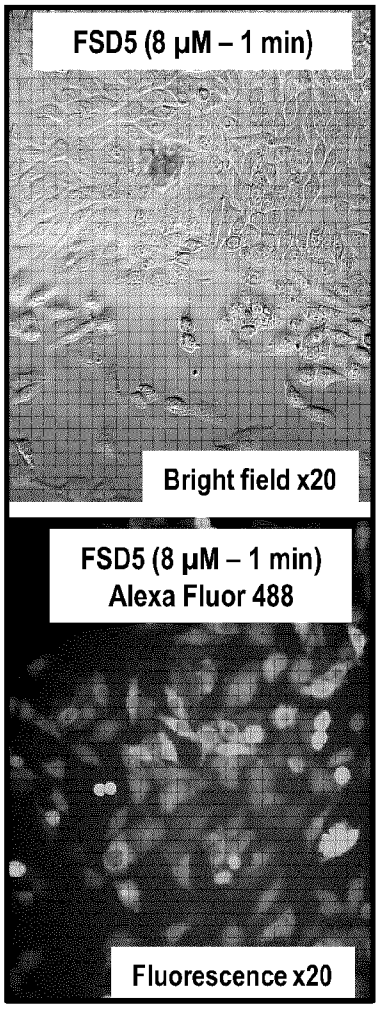
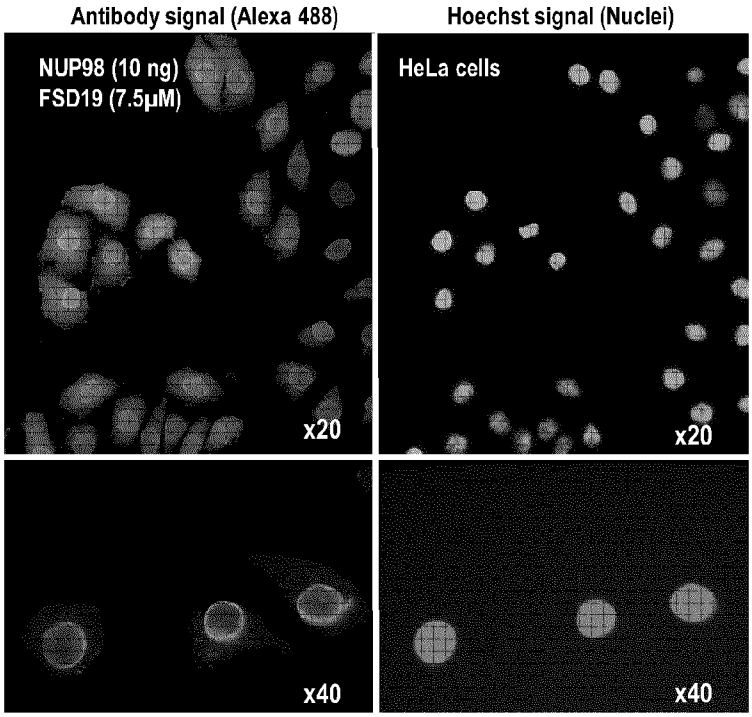


Fig. 50D


Antibody signal (Alexa 488)
Hoechst signal (Nuclei)
NUP98 (10 ng)
FSD19 (7.5µM)
HeLa cells
x20
x20
x40
x40

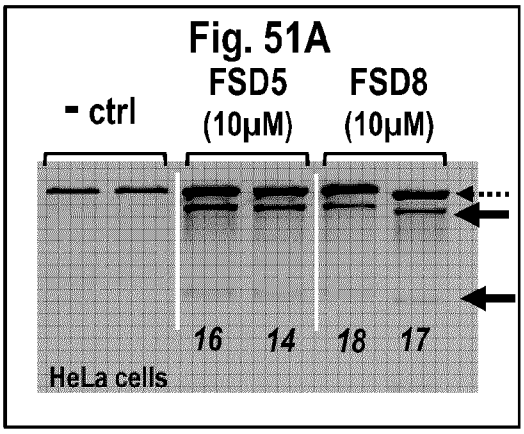


Fig. 51A
- ctrl
FSD5 (10μM)
FSD8 (10μM)
16
14
18
17
HeLa cells

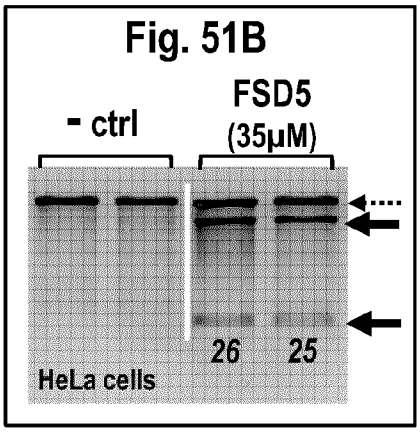


Fig. 51B
- ctrl
FSD5 (35μM)
26
25
HeLa cells

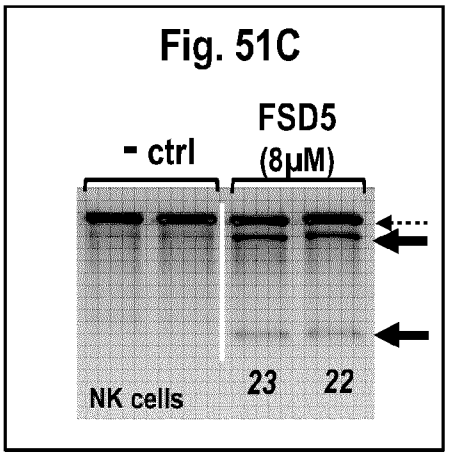


Fig. 51C
- ctrl
FSD5 (8μM)
23
22
NK cells

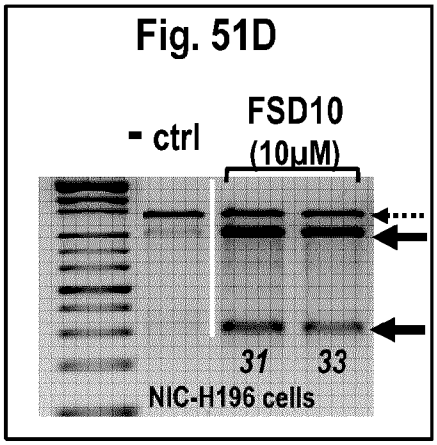


Fig. 51D
- ctrl
FSD10 (10μM)
31
33
NIC-H196 cells

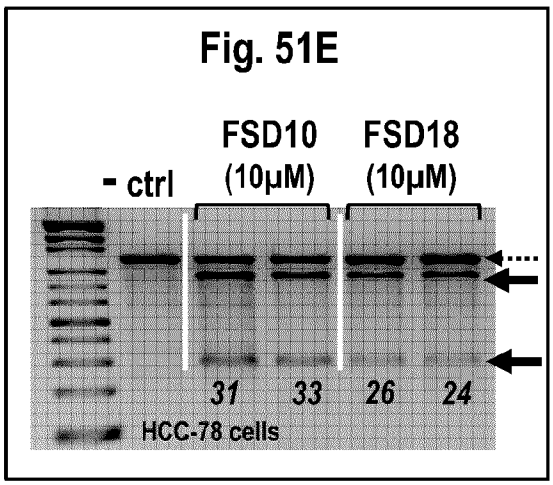


Fig. 51E
- ctrl
FSD10 (10μM)
FSD18 (10μM)
31
33
26
24
HCC-78 cells

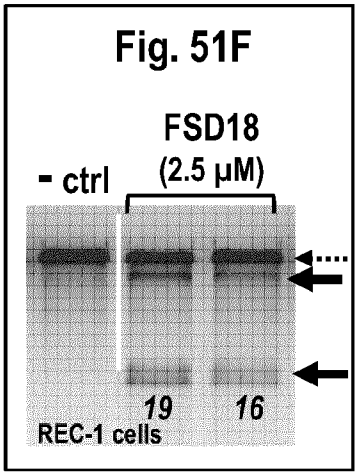


Fig. 51F
- ctrl
FSD18 (2.5 μM)
19
16
REC-1 cells

Fig. 51G

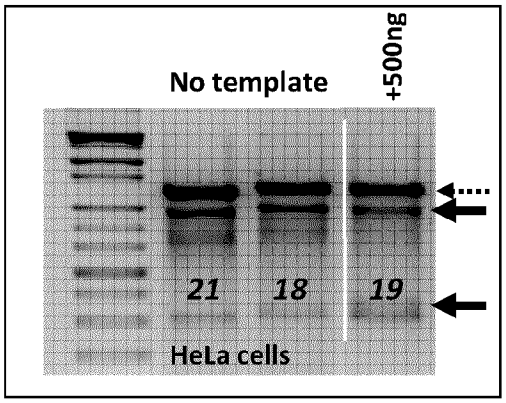


Fig. 51H

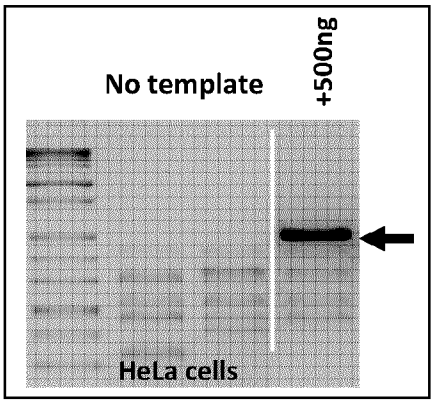


Fig. 51I

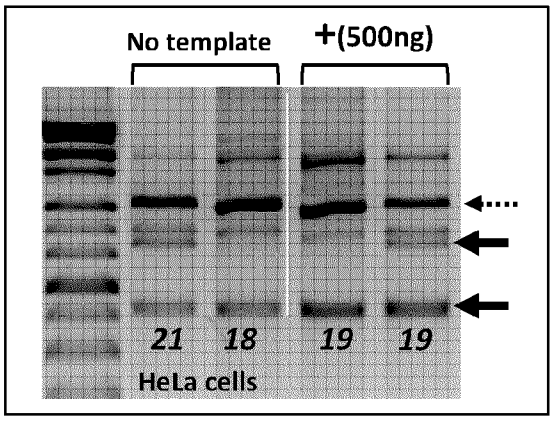


Fig. 51J

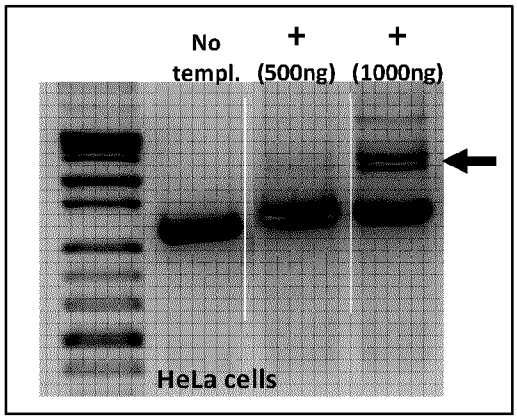


Fig. 51K

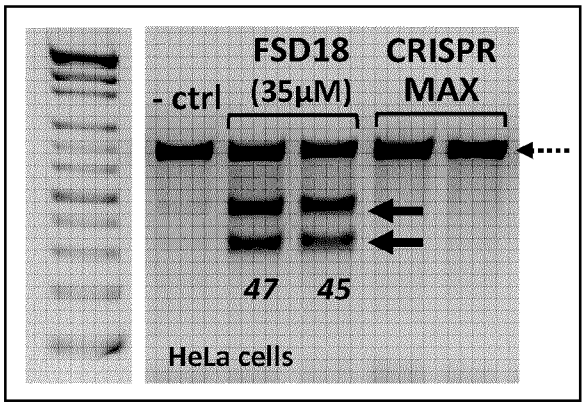


Fig. 51L

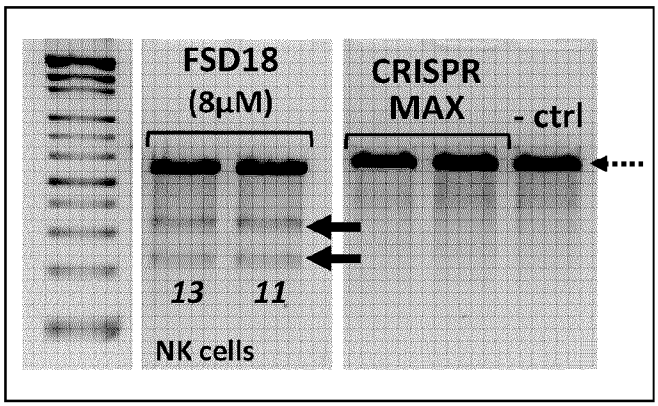
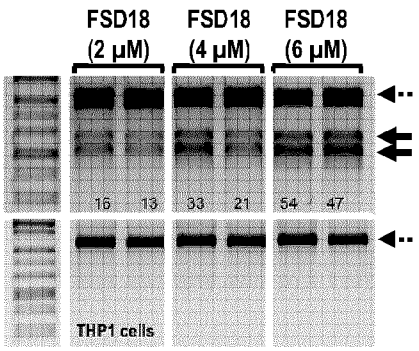
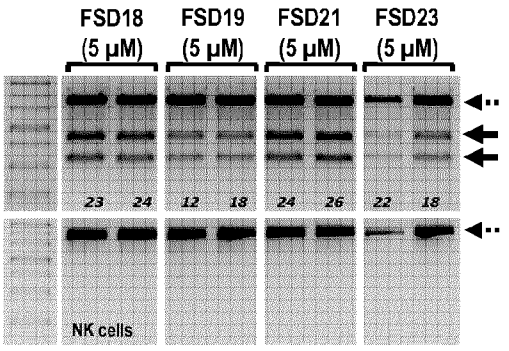


Fig. 52A



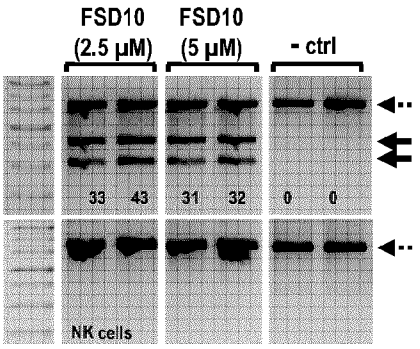
B2M - Incubation: 90 sec THP1 cells
1.33 µM Cpf1
2, 4, 6 µM FSD18

Fig. 52B



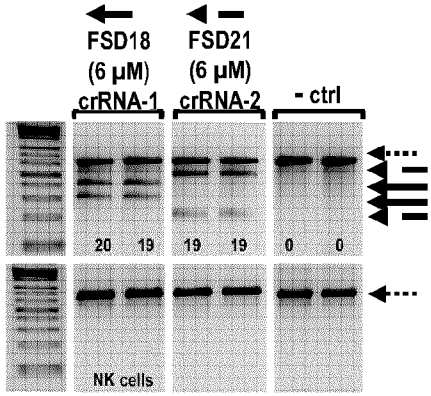
B2M – Incubation: 90sec - NK cells
1.33 µM Cpf1
5 µM FSD18, 19, 21, 23

Fig. 52C



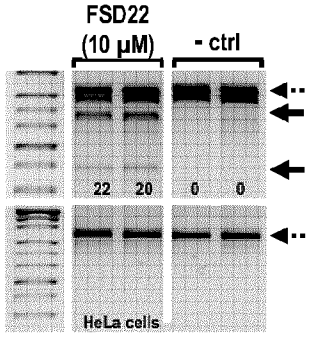
B2M – Incubation: 48h NK cells
1.33 µM Cpf1
2.5 & 5 µM FSD10

Fig. 52D



B2M - Incubation: 2 min NK cells
1.33 µM Cpf1
6 µM FSD18, 21

Fig. 52E



B2M - Incubation:
1h HeLa
2.5 µM Cas9
10 µM FSD43

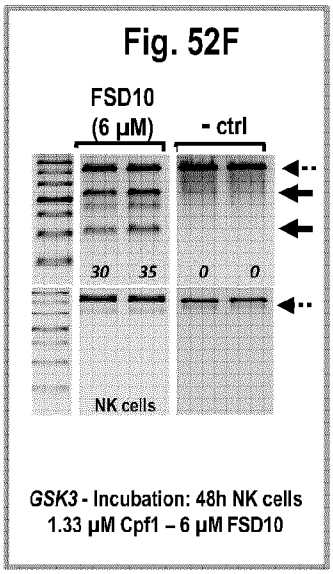


Fig. 52F
FSD10
(6 µM)
- ctrl
30
35
0
0
NK cells
GSK3 - Incubation: 48h NK cells
1.33 µM Cpf1 – 6 µM FSD10

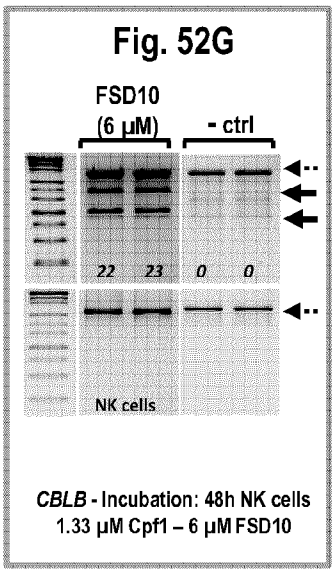


Fig. 52G
FSD10
(6 µM)
- ctrl
22
23
0
0
NK cells
CBLB - Incubation: 48h NK cells
1.33 µM Cpf1 – 6 µM FSD10

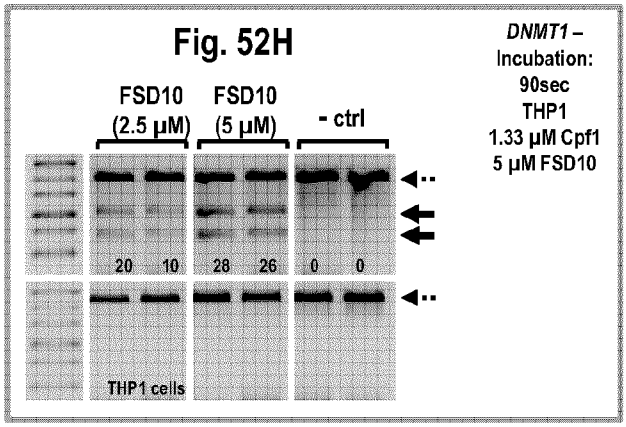


Fig. 52H
FSD10
(2.5 µM)
FSD10
(5 µM)
- ctrl
20
10
28
26
0
0
THP1 cells
DNMT1 –
Incubation:
90sec
THP1
1.33 µM Cpf1
5 µM FSD10

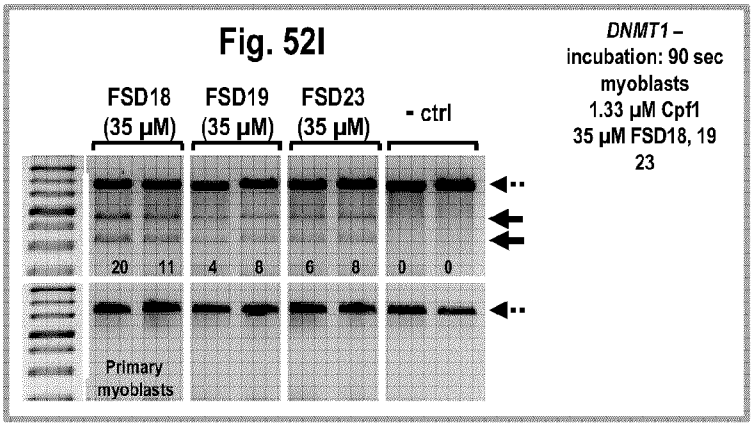


Fig. 52I
FSD18
(35 µM)
FSD19
(35 µM)
FSD23
(35 µM)
- ctrl
20
11
4
8
6
8
0
0
Primary
myoblasts
DNMT1 –
incubation: 90 sec
myoblasts
1.33 µM Cpf1
35 µM FSD18, 19
23

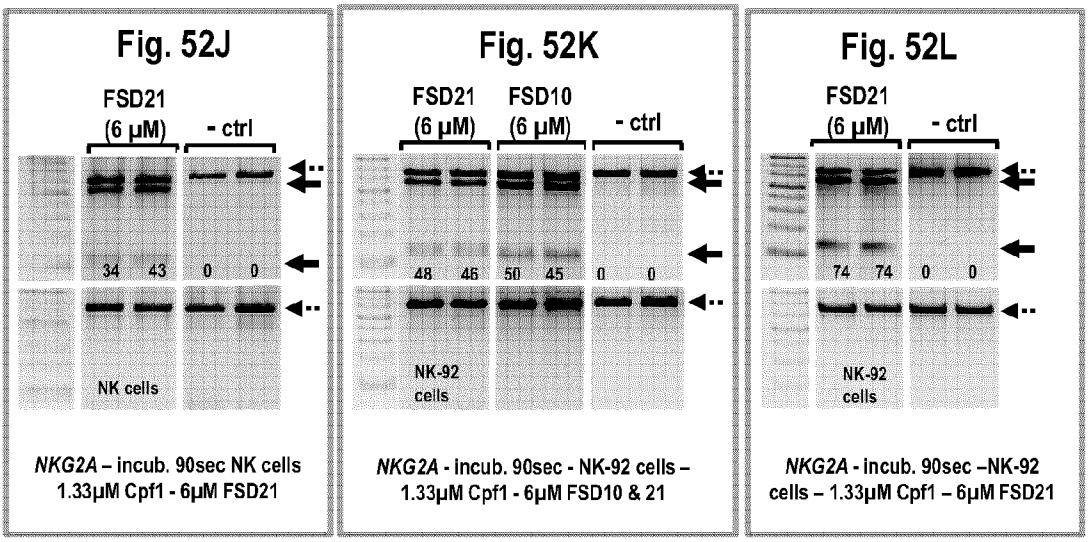


Fig. 52J
FSD21 (6 µM)
- ctrl
34 43 0 0
NK cells
NKG2A – incub. 90sec NK cells 1.33µM Cpf1 - 6µM FSD21
Fig. 52K
FSD21 (6 µM)
FSD10 (6 µM)
- ctrl
48 46 50 45 0 0
NK-92 cells
NKG2A - incub. 90sec - NK-92 cells – 1.33µM Cpf1 - 6µM FSD10 & 21
Fig. 52L
FSD21 (6 µM)
- ctrl
74 74 0 0
NK-92 cells
NKG2A - incub. 90sec –NK-92 cells – 1.33µM Cpf1 – 6µM FSD21

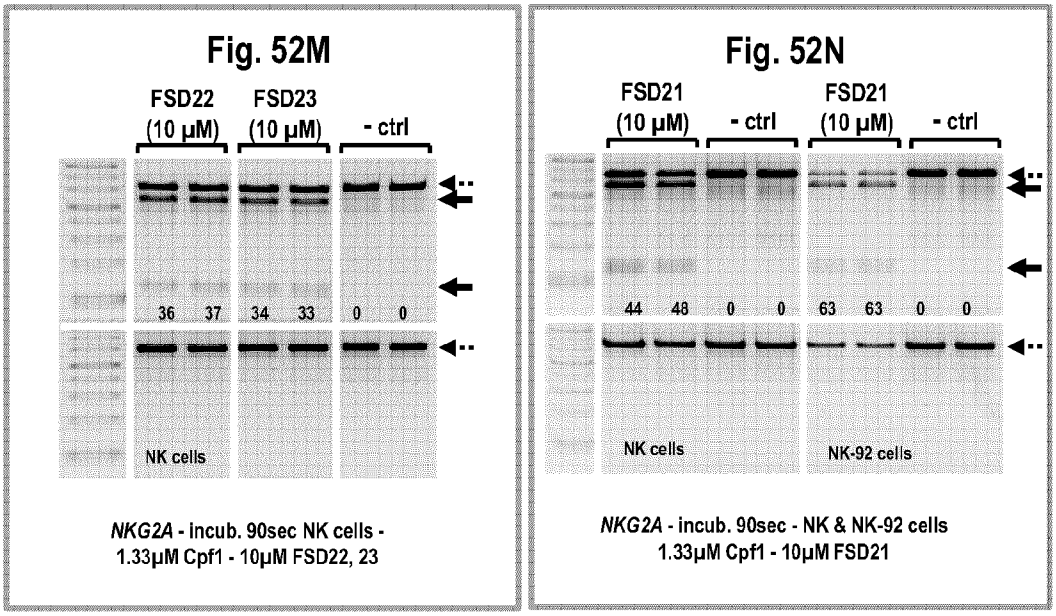


Fig. 52M
FSD22 (10 µM)
FSD23 (10 µM)
- ctrl
36 37 34 33 0 0
NK cells
NKG2A - incub. 90sec NK cells - 1.33µM Cpf1 - 10µM FSD22, 23
Fig. 52N
FSD21 (10 µM)
- ctrl
FSD21 (10 µM)
- ctrl
44 48 0 0 63 63 0 0
NK cells
NK-92 cells
NKG2A - incub. 90sec - NK & NK-92 cells 1.33µM Cpf1 - 10µM FSD21

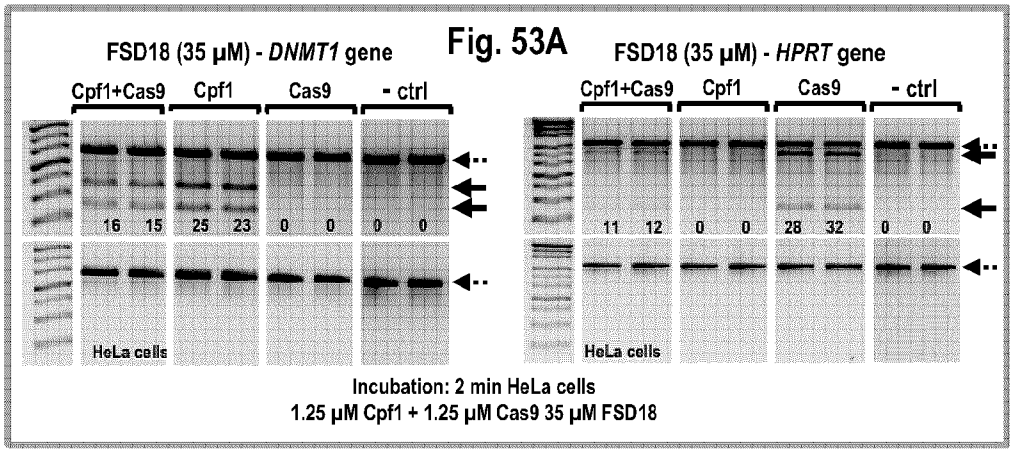


Fig. 53A
FSD18 (35 µM) - DNMT1 gene
Cpf1+Cas9
Cpf1
Cas9
- ctrl
16 15 25 23 0 0 0 0
HeLa cells
FSD18 (35 µM) - HPRT gene
Cpf1+Cas9
Cpf1
Cas9
- ctrl
11 12 0 0 28 32 0 0
HeLa cells
Incubation: 2 min HeLa cells
1.25 µM Cpf1 + 1.25 µM Cas9 35 µM FSD18

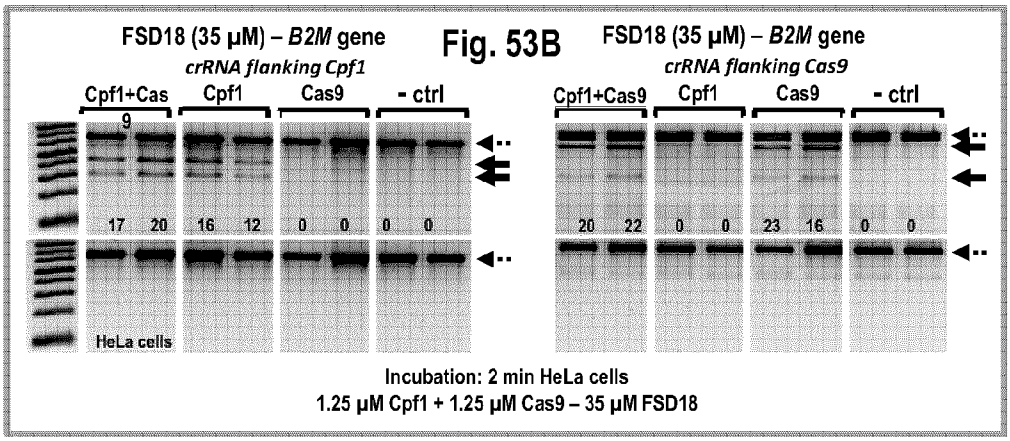


Fig. 53B
FSD18 (35 µM) – B2M gene
crRNA flanking Cpf1
Cpf1+Cas9
Cpf1
Cas9
- ctrl
17 20 16 12 0 0 0 0
HeLa cells
FSD18 (35 µM) – B2M gene
crRNA flanking Cas9
Cpf1+Cas9
Cpf1
Cas9
- ctrl
20 22 0 0 23 16 0 0
Incubation: 2 min HeLa cells
1.25 µM Cpf1 + 1.25 µM Cas9 – 35 µM FSD18

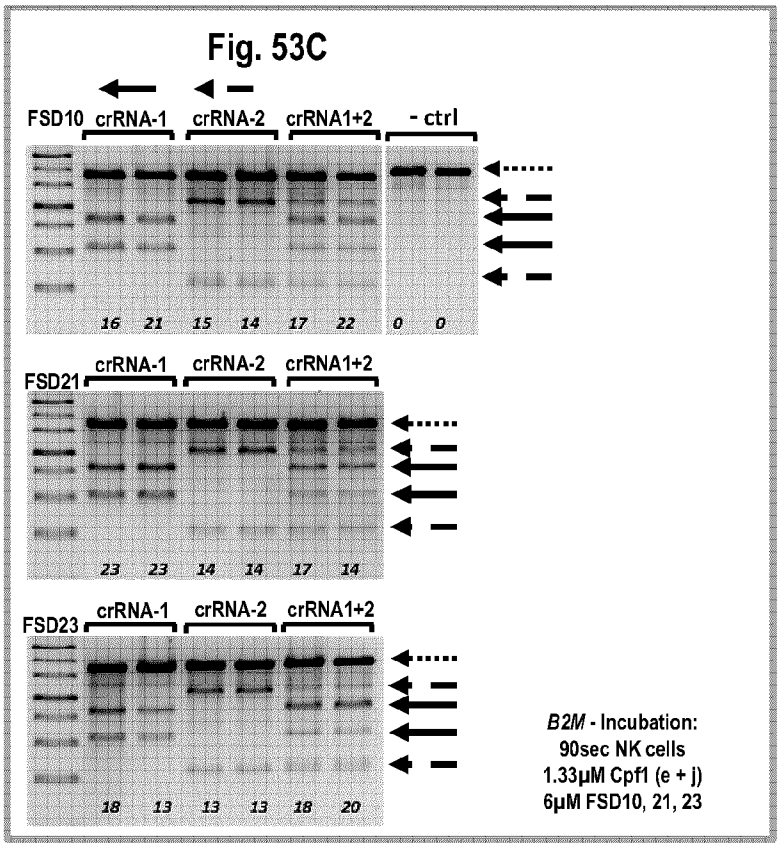


Fig. 53C
FSD10
crRNA-1
crRNA-2
crRNA1+2
- ctrl
16 21 15 14 17 22 0 0
FSD21
crRNA-1
crRNA-2
crRNA1+2
23 23 14 14 17 14
FSD23
crRNA-1
crRNA-2
crRNA1+2
18 13 13 13 18 20
B2M - Incubation:
90sec NK cells
1.33µM Cpf1 (e + j)
6µM FSD10, 21, 23

Fig. 54A

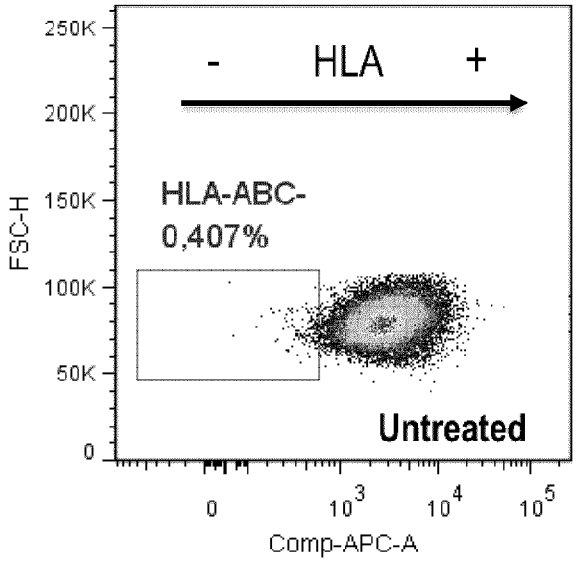


Fig. 54B

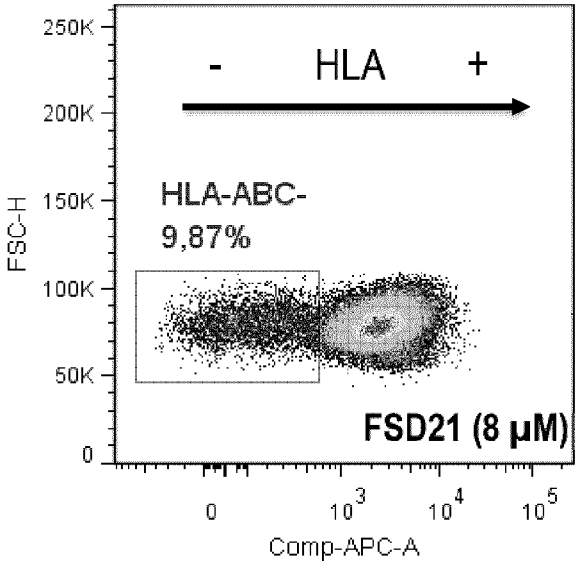


Fig. 54C

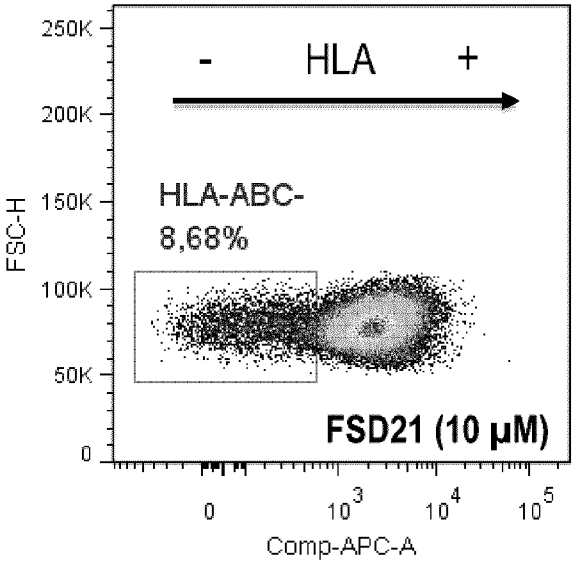


Fig. 54D

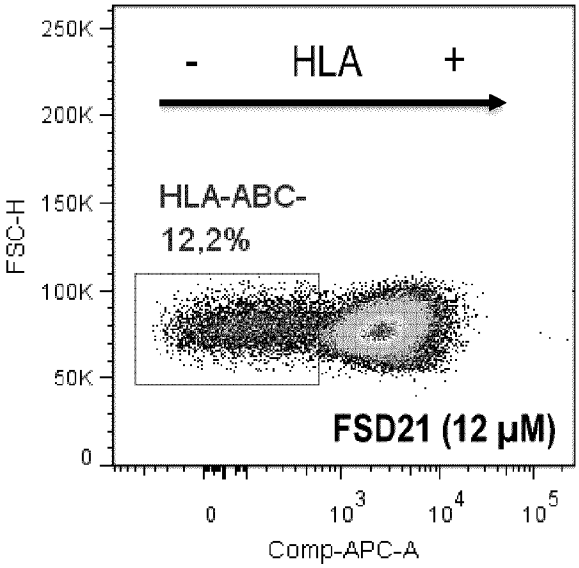


Fig. 55A

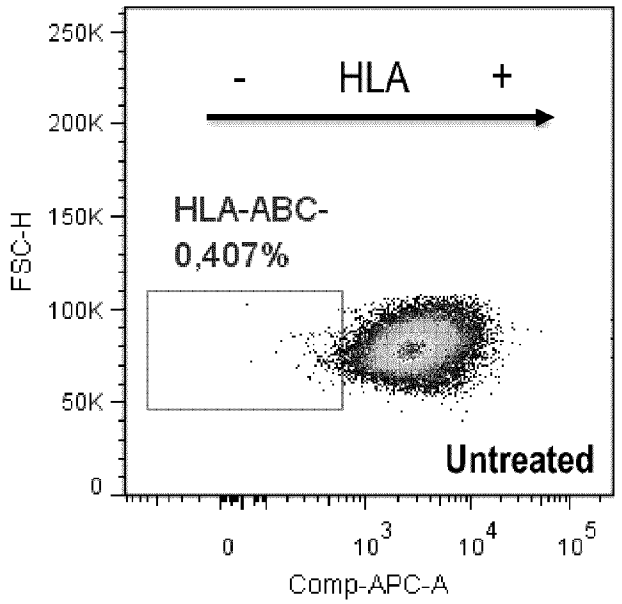


Fig. 55B

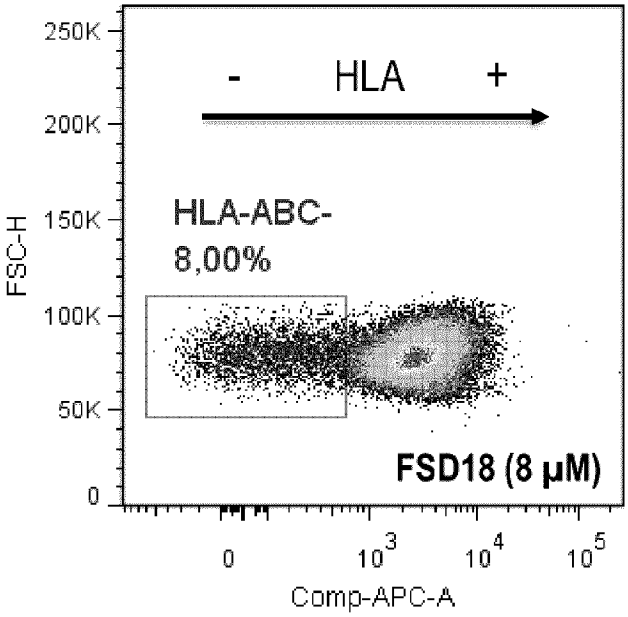


Fig. 55C

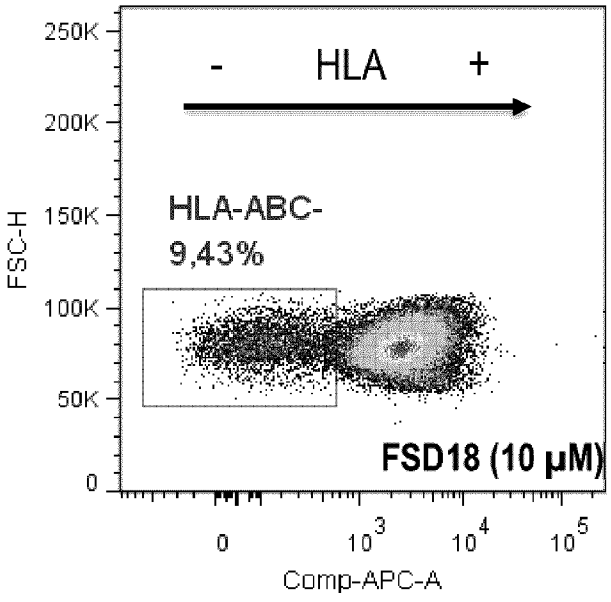


Fig. 55D

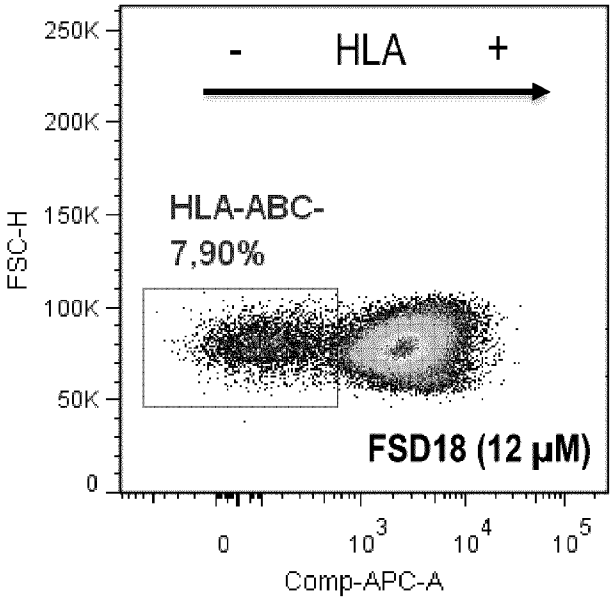


Fig. 56A

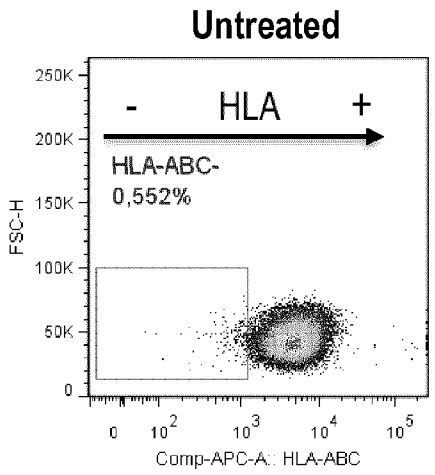


Fig. 56B

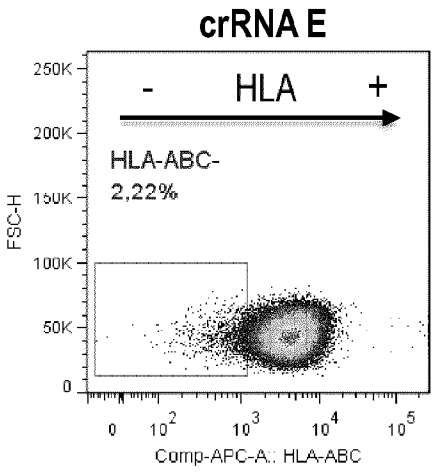


Fig. 56C

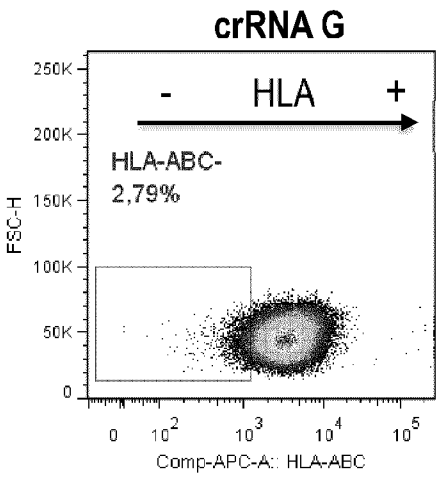


Fig. 56D

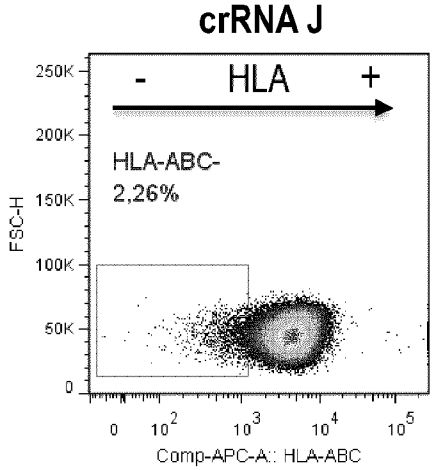


Fig. 56E

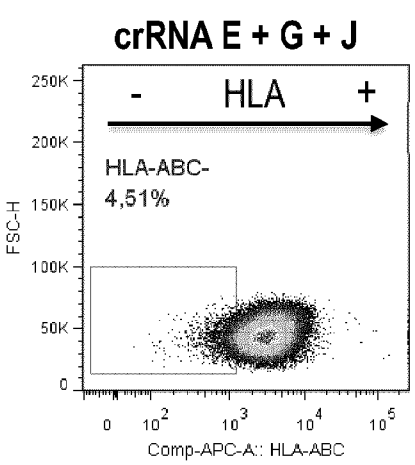


Fig. 57A

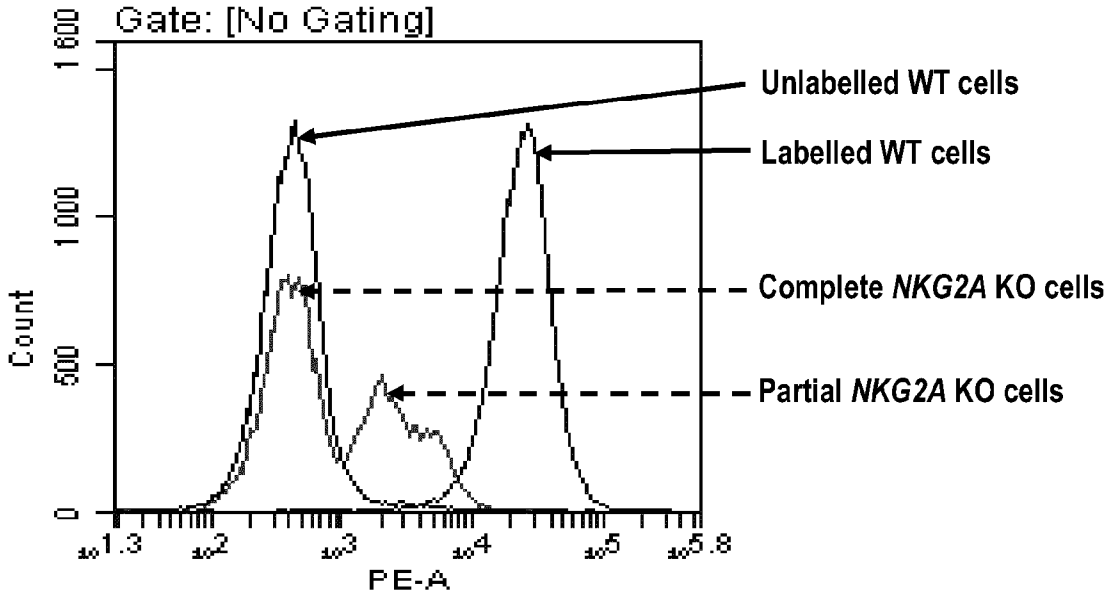


Fig. 57B

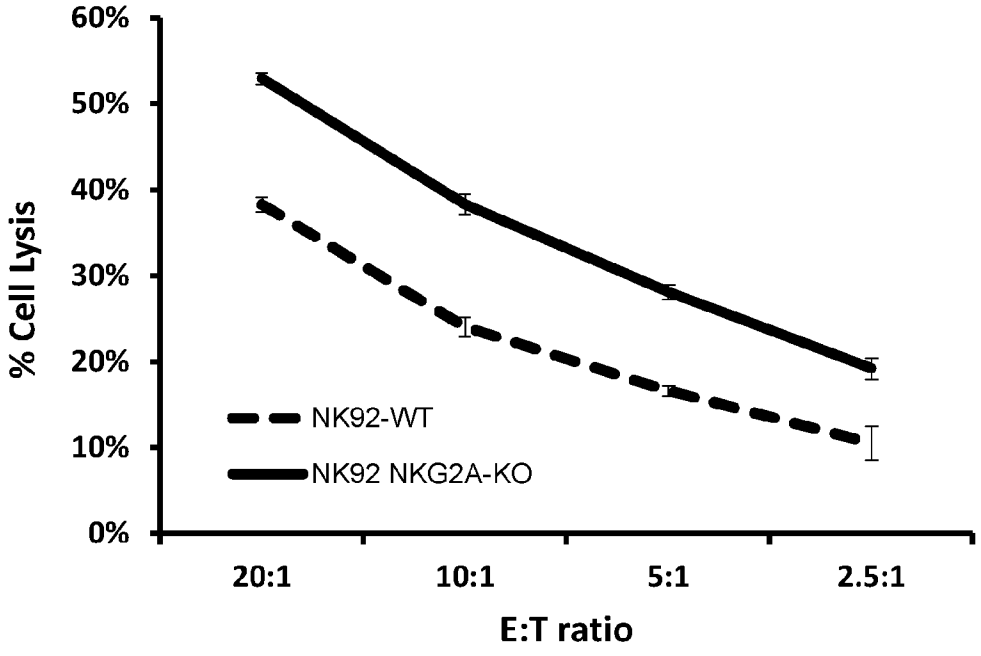


Fig. 58A

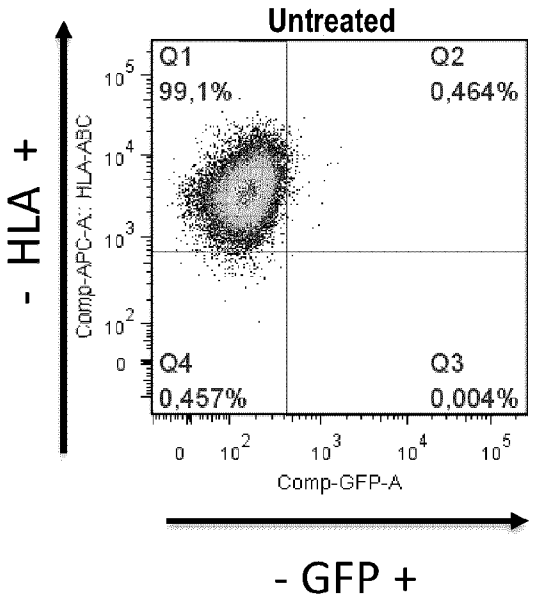


Fig. 58B

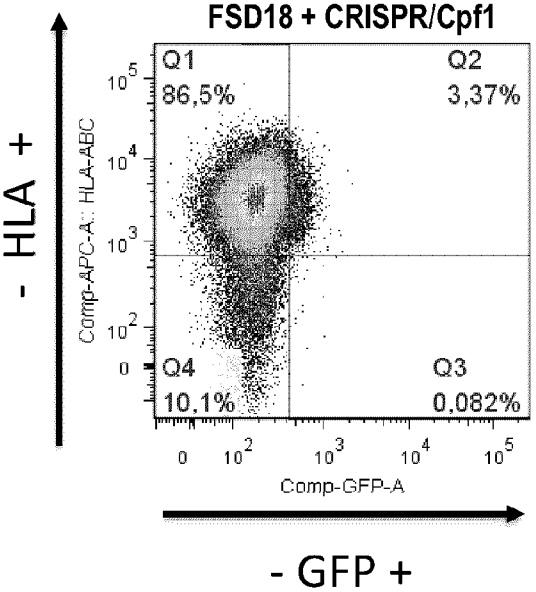


Fig. 58C

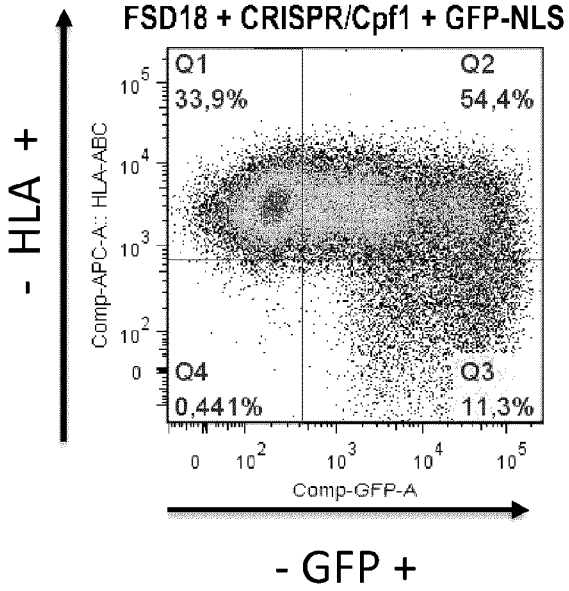
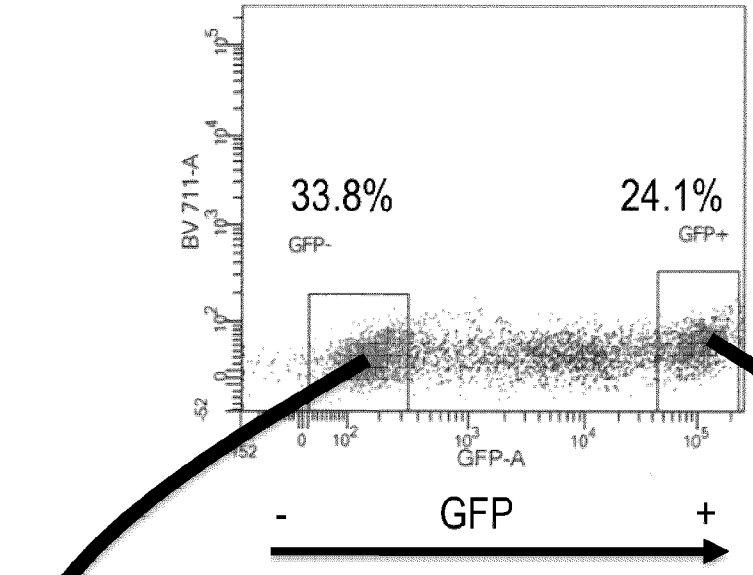


Fig. 58D



FACS sorting for GFP-negative cells

FACS sorting for GFP-positive cells

Fig. 58E

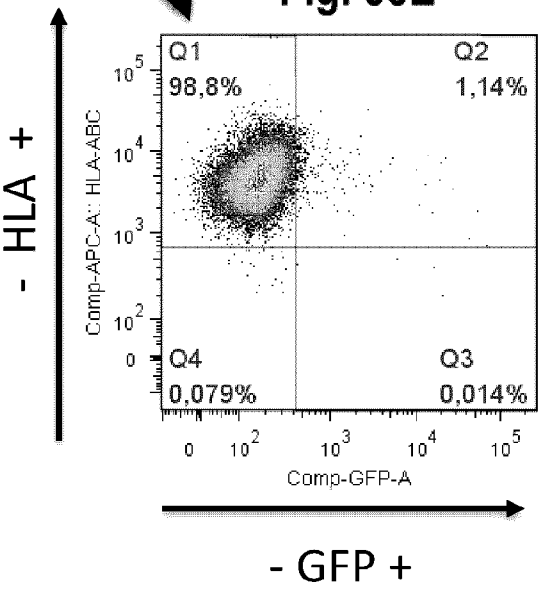


Fig. 58F

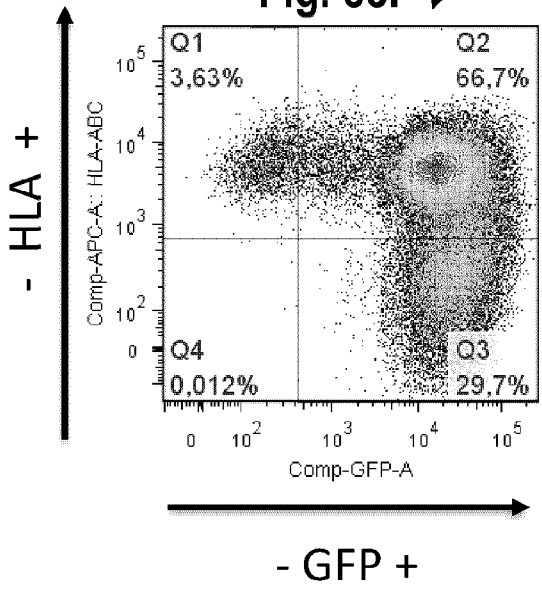


Fig.58G

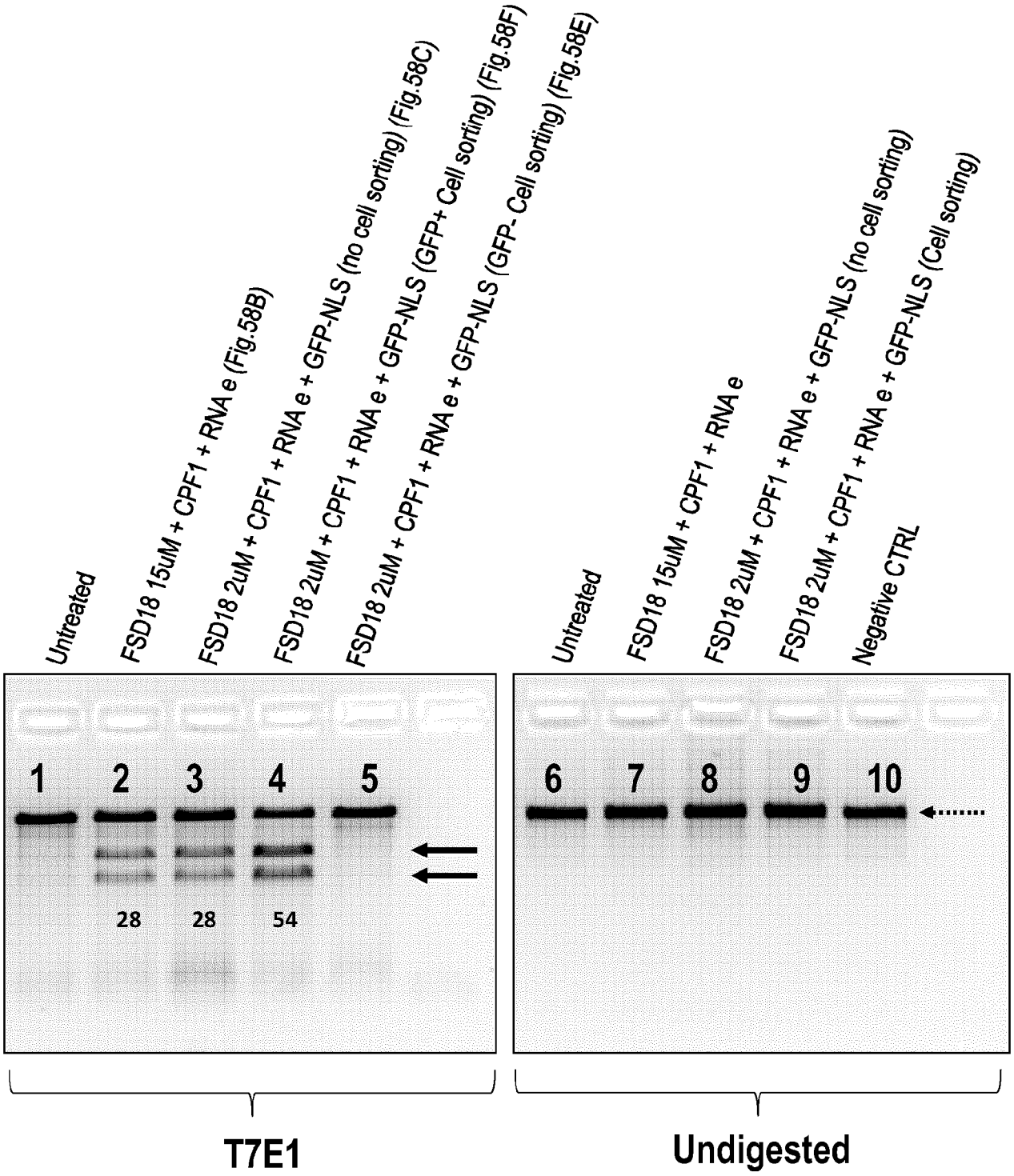

Untreated
FSD18 15uM + CPF1 + RNA e (Fig.58B)
FSD18 2uM + CPF1 + RNA e + GFP-NLS (no cell sorting) (Fig.58C)
FSD18 2uM + CPF1 + RNA e + GFP-NLS (GFP+ Cell sorting) (Fig.58F)
FSD18 2uM + CPF1 + RNA e + GFP-NLS (GFP- Cell sorting) (Fig.58E)
Untreated
FSD18 15uM + CPF1 + RNA e
FSD18 2uM + CPF1 + RNA e + GFP-NLS (no cell sorting)
FSD18 2uM + CPF1 + RNA e + GFP-NLS (Cell sorting)
Negative CTRL
1
2
3
4
5
6
7
8
9
10
28
28
54
T7E1
Undigested

Fig. 59A

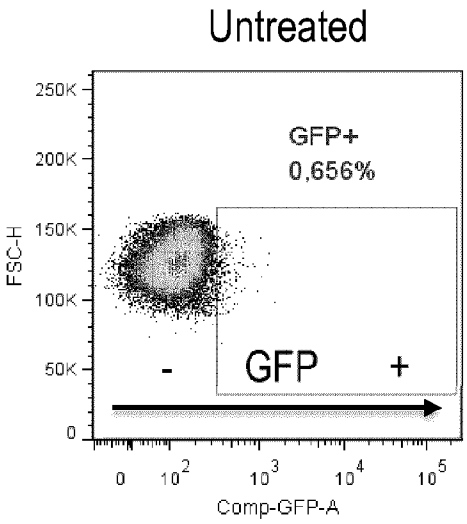


Fig. 59B

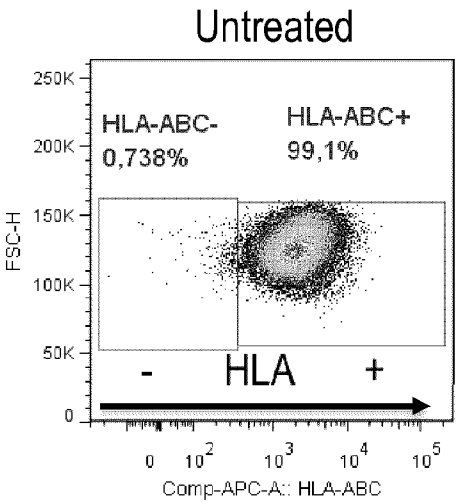


Fig. 59C

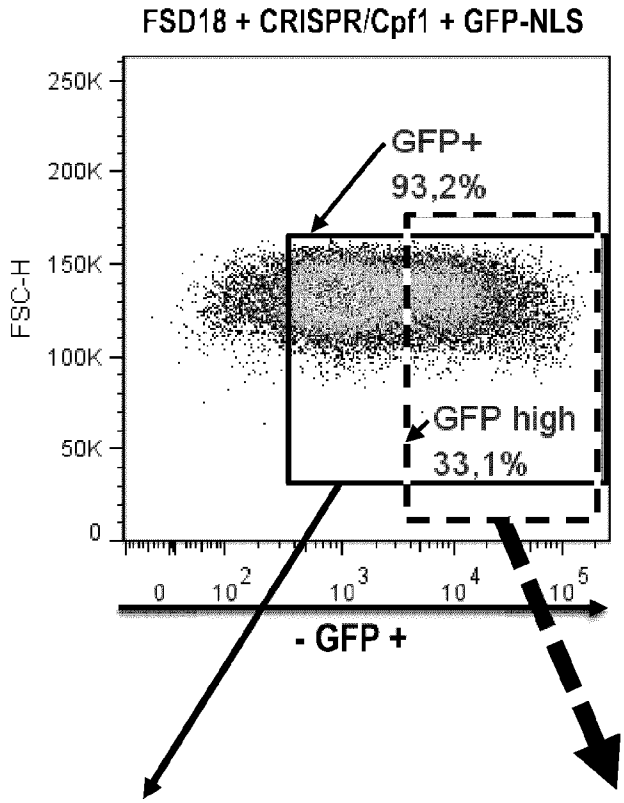


Fig. 59D

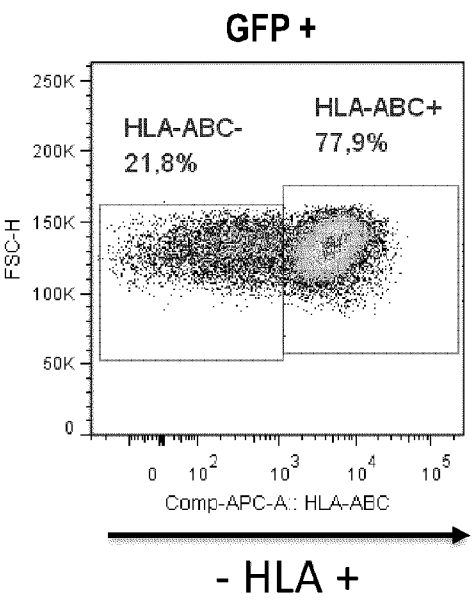


Fig. 59E

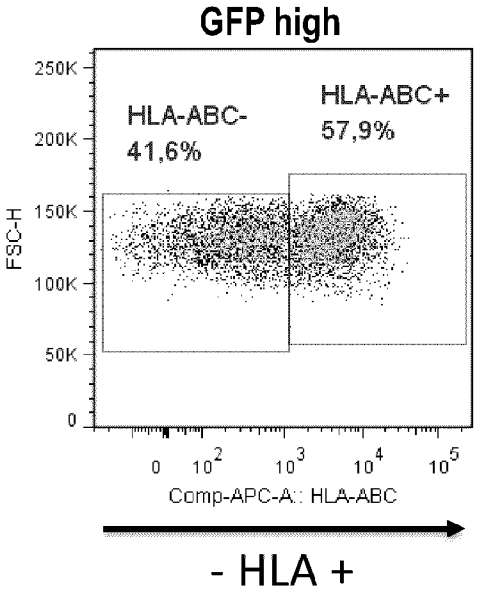


Fig. 60A

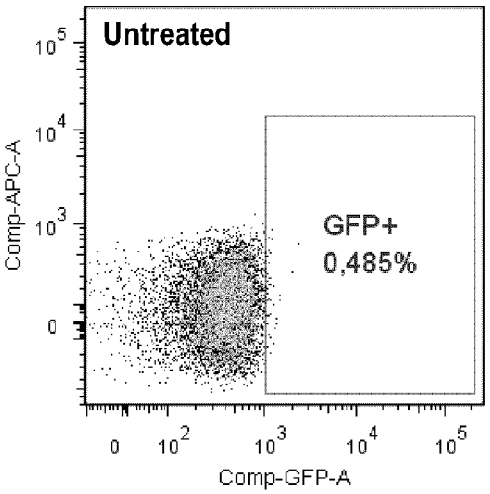
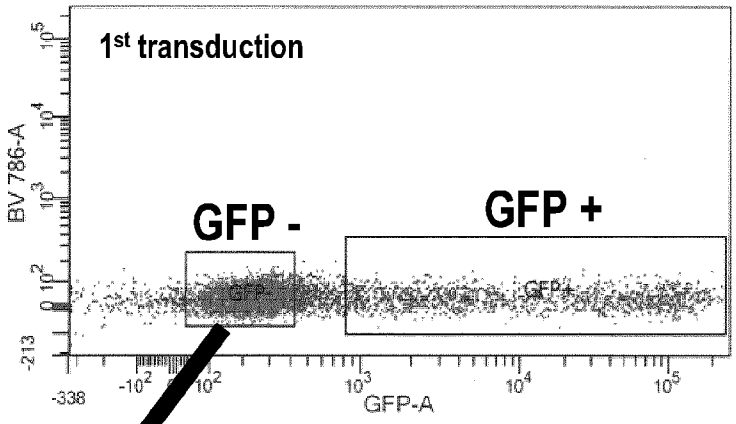


Fig. 60B



Sorting

Fig. 60C

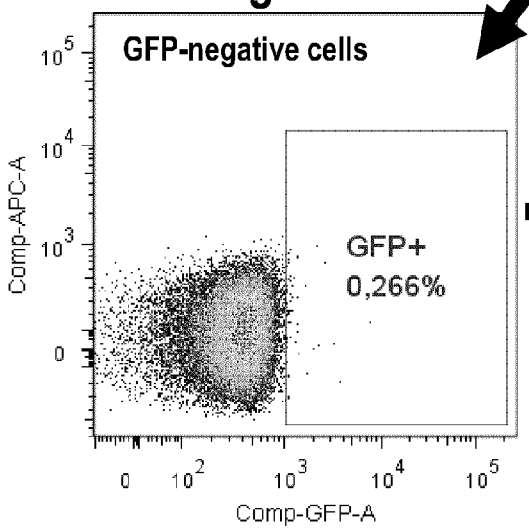
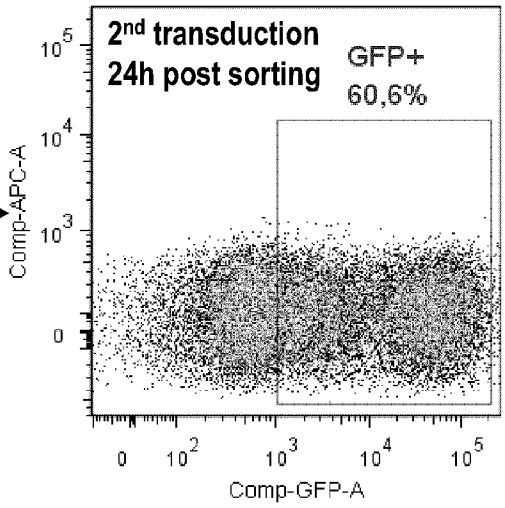



Fig. 60D