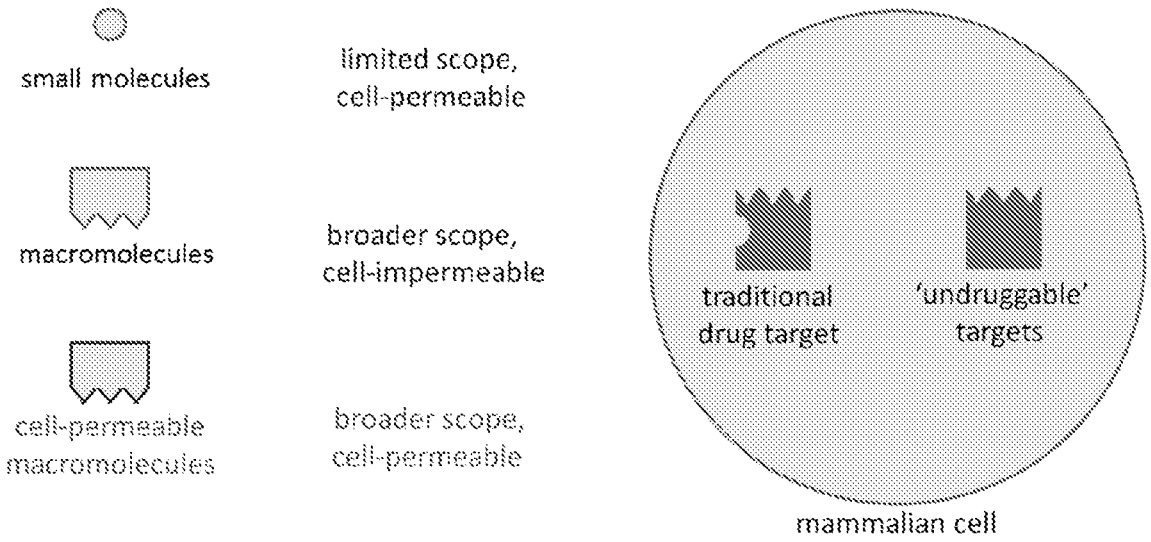


Figure 1

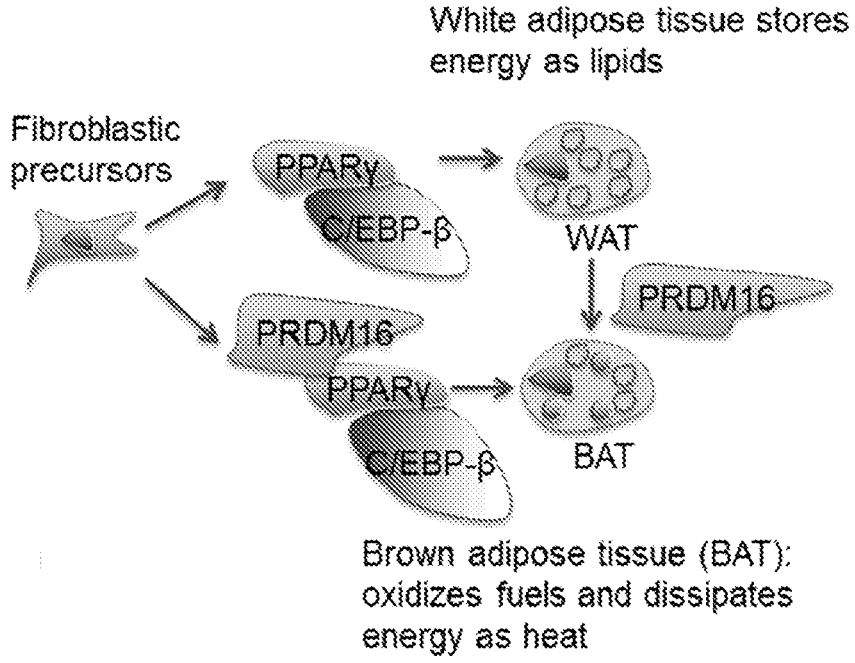


Figure 2

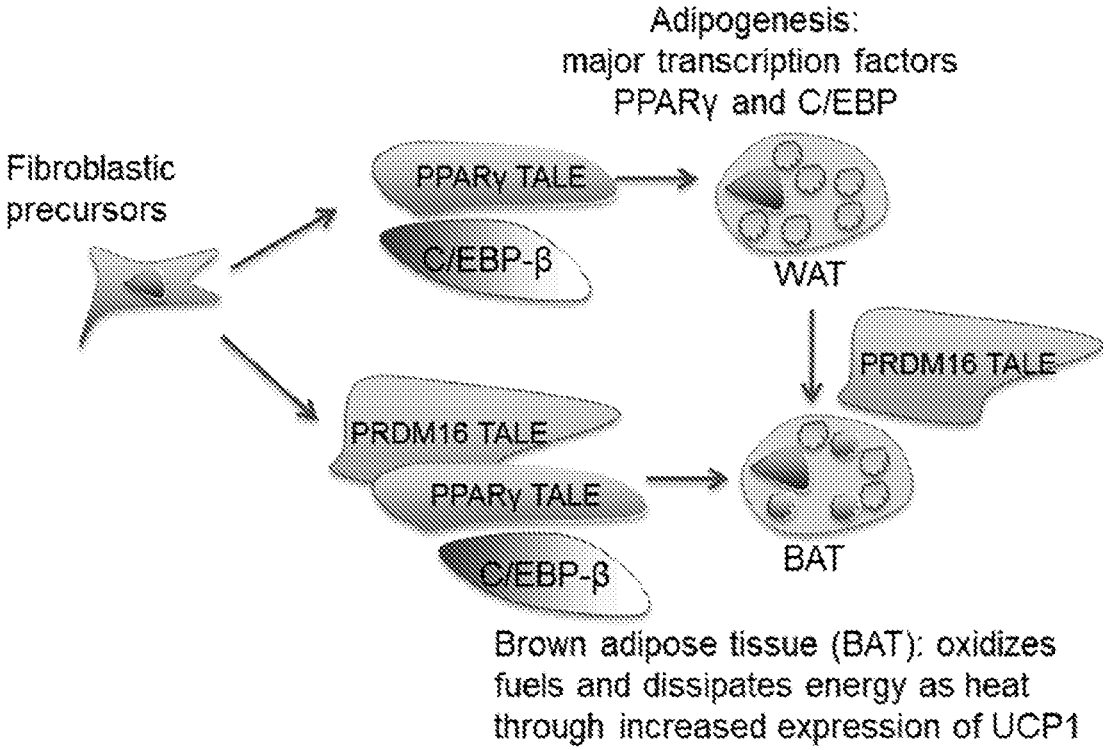


Figure 3

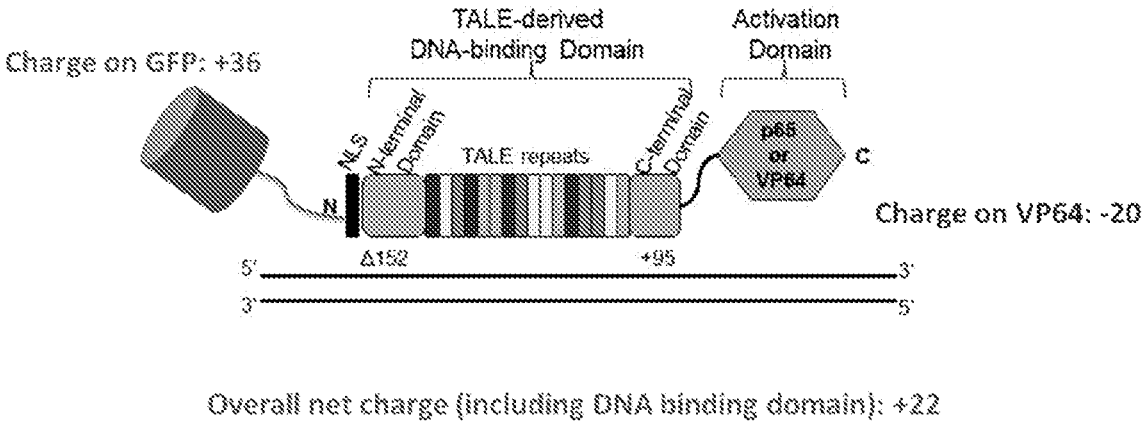


Figure 4

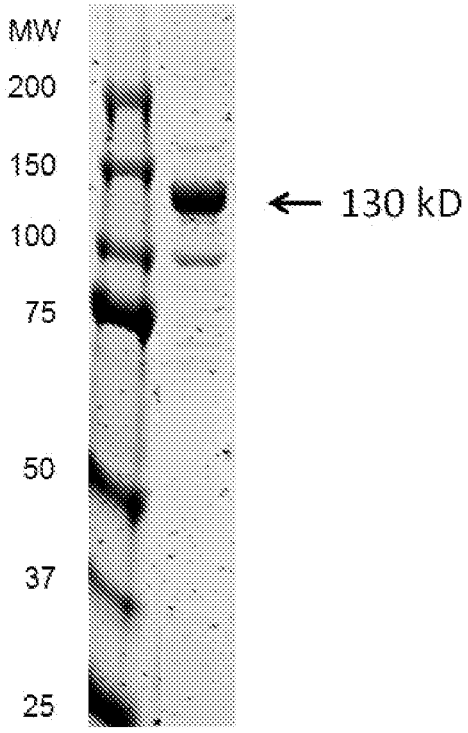


Figure 5

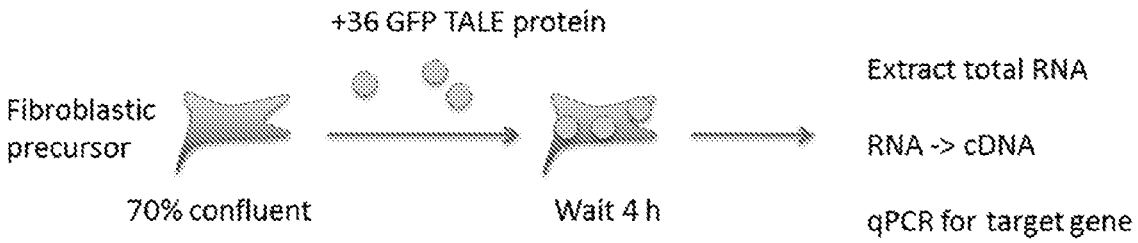


Figure 6

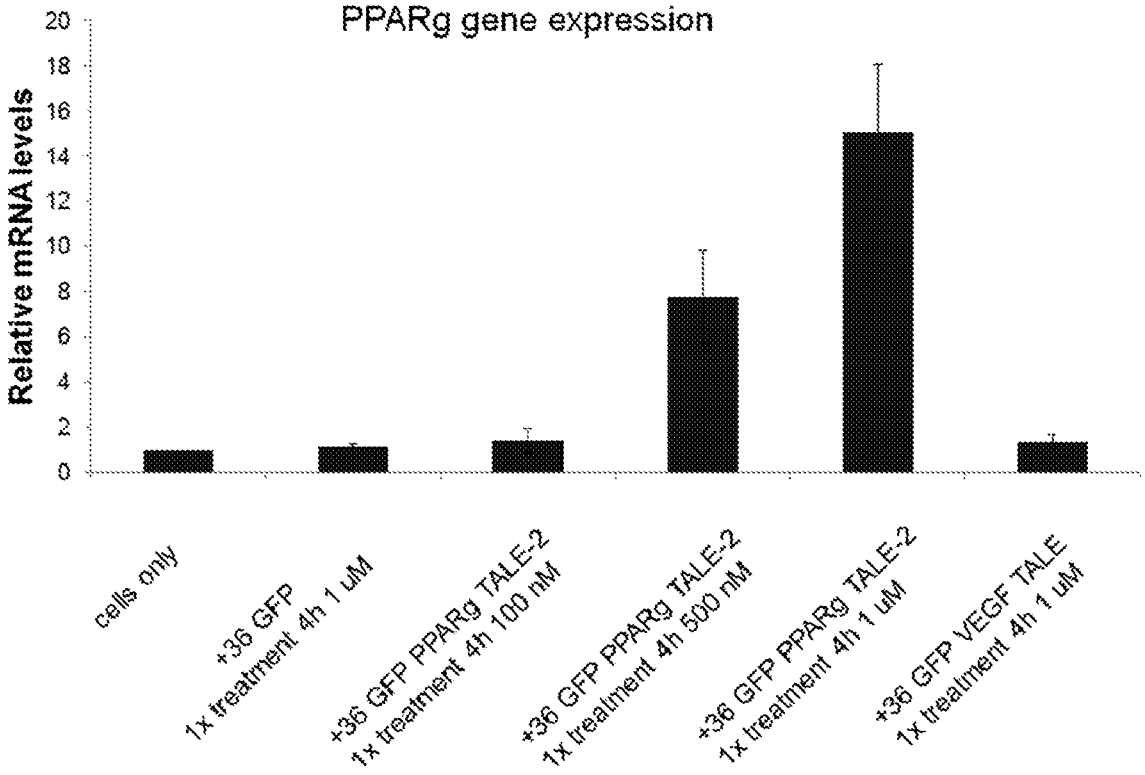

PPARg gene expression
Relative mRNA levels
20
18
16
14
12
10
8
6
4
2
0
cells only
+36 GFP 1x treatment 4h 1 uM
+36 GFP PPARg TALE-2 1x treatment 4h 100 nM
+36 GFP PPARg TALE-2 1x treatment 4h 500 nM
+36 GFP PPARg TALE-2 1x treatment 4h 1 uM
+36 GFP VEGF TALE 1x treatment 4h 1 uM

Figure 7

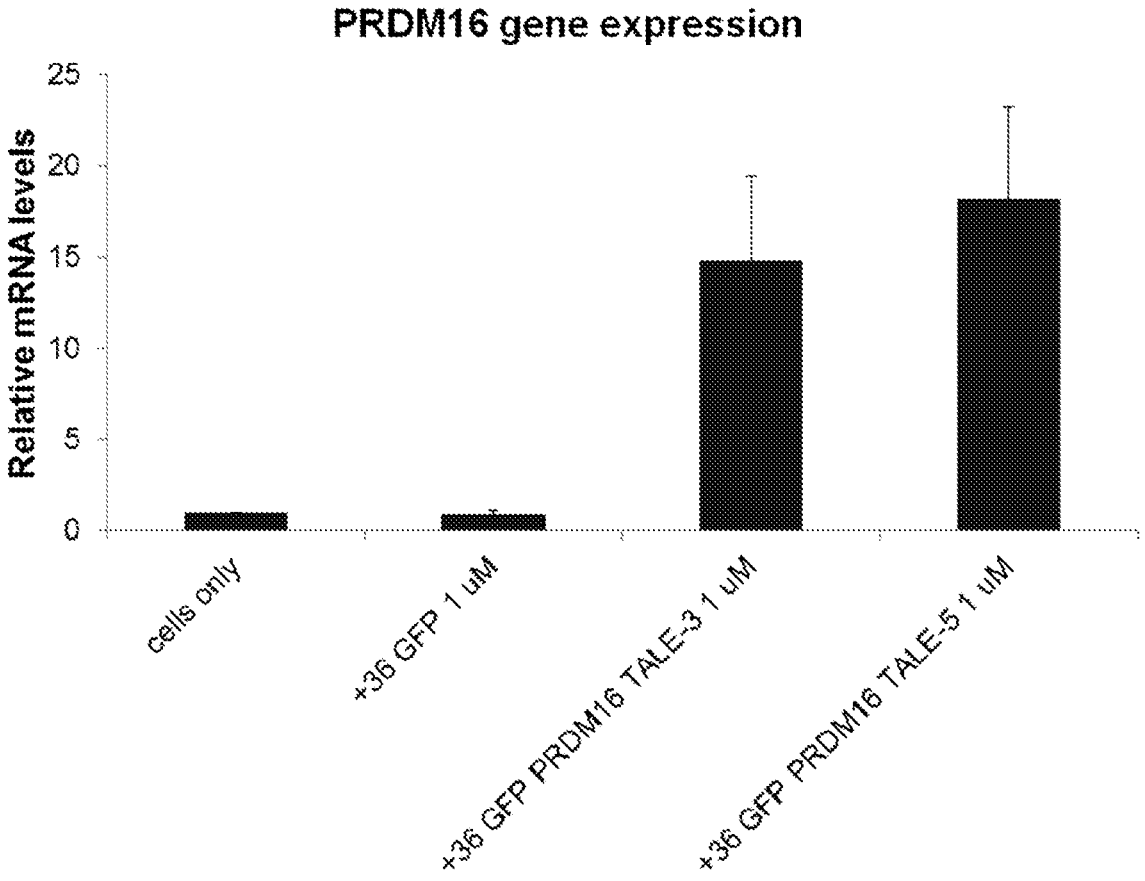

PRDM16 gene expression
Relative mRNA levels
25
20
15
10
5
0
cells only
+36 GFP 1 uM
+36 GFP PRDM16 TALE-3 1 uM
+36 GFP PRDM16 TALE-5 1 uM

Figure 8

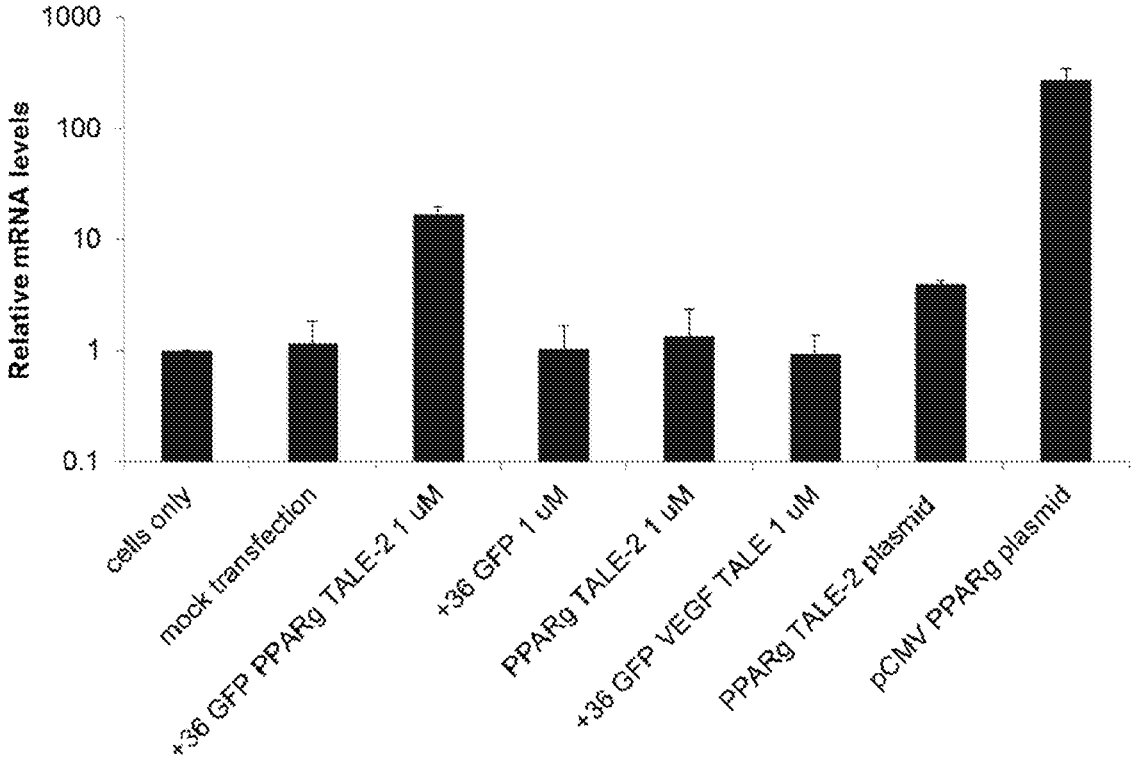

Relative mRNA levels
1000
100
10
1
0.1
cells only
mock transfection
+36 GFP PPARg TALE-2 1 uM
+36 GFP 1 uM
PPARg TALE-2 1 uM
+36 GFP VEGF TALE 1 uM
PPARg TALE-2 plasmid
pCMV PPARg plasmid

Figure 9

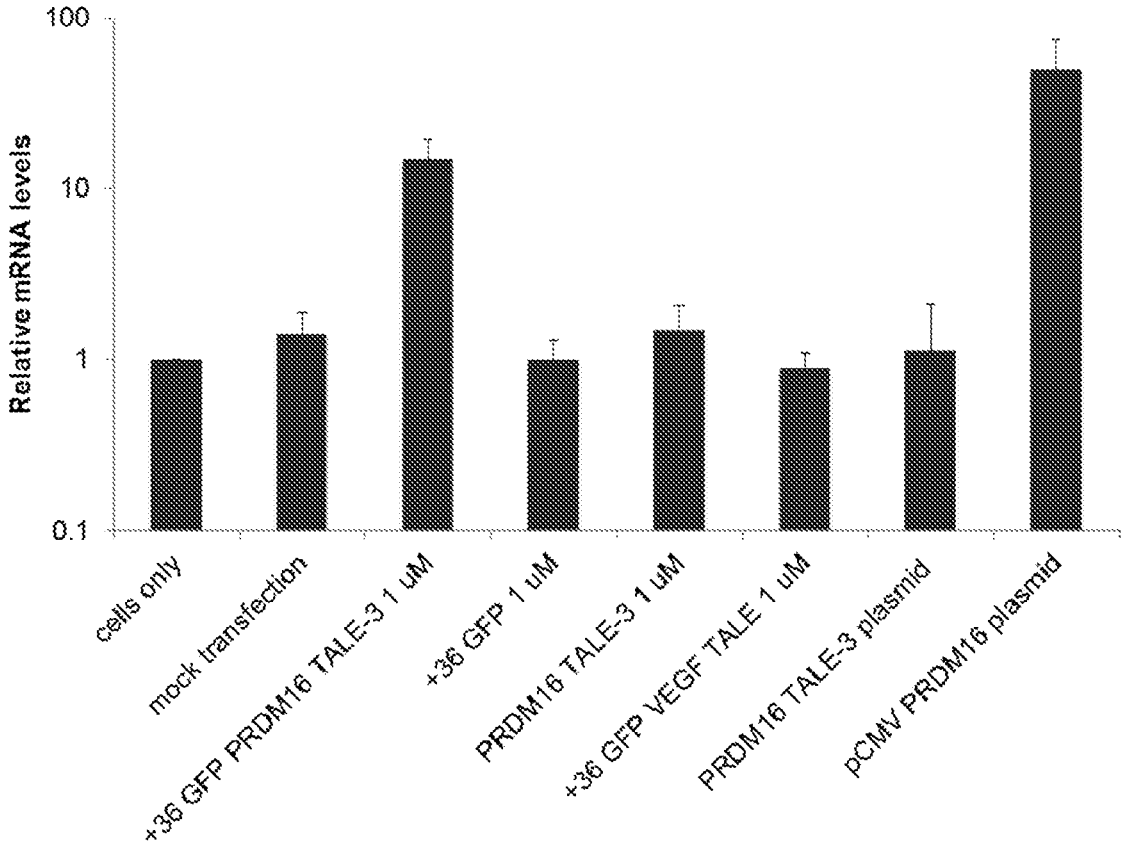

100
10
1
0.1
Relative mRNA levels
cells only
mock transfection
+36 GFP PRDM16 TALE-3 1 uM
+36 GFP 1 uM
PRDM16 TALE-3 1 uM
+36 GFP VEGF TALE 1 uM
PRDM16 TALE-3 plasmid
pCMV PRDM16 plasmid

Figure 10

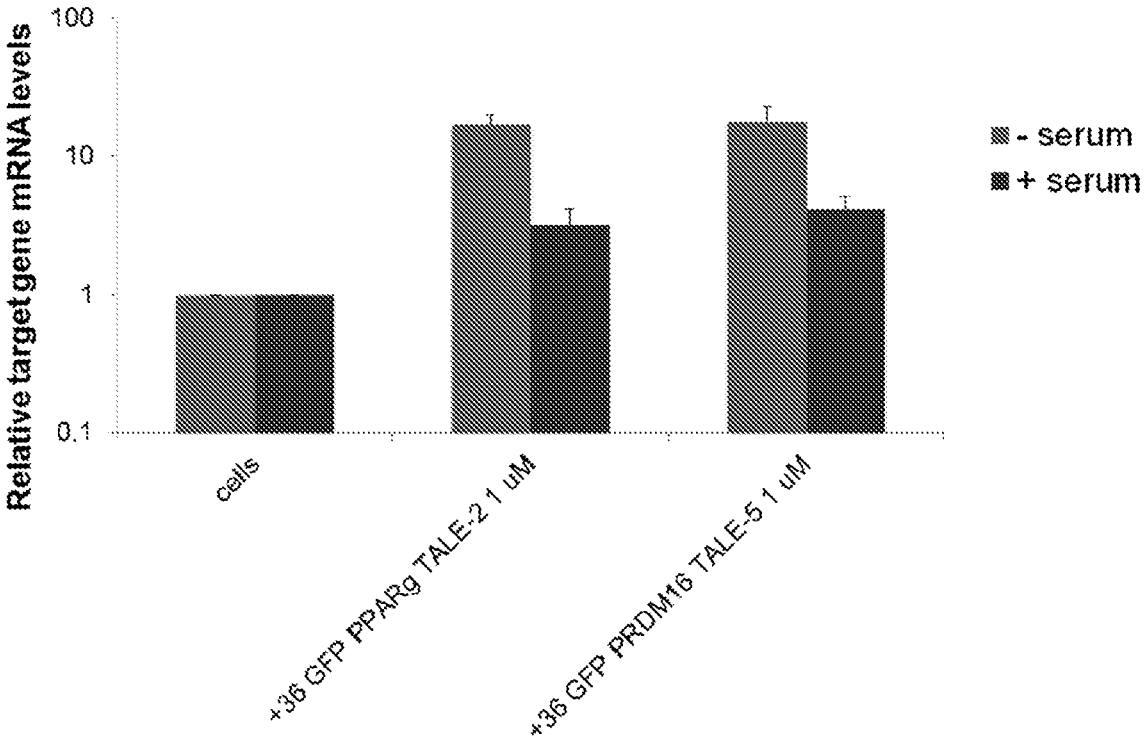

Relative target gene mRNA levels
100
10
1
0.1
- serum
+ serum
cells
+36 GFP PPARg TALE-2 1 uM
+36 GFP PRDM16 TALE-5 1 uM

Figure 11

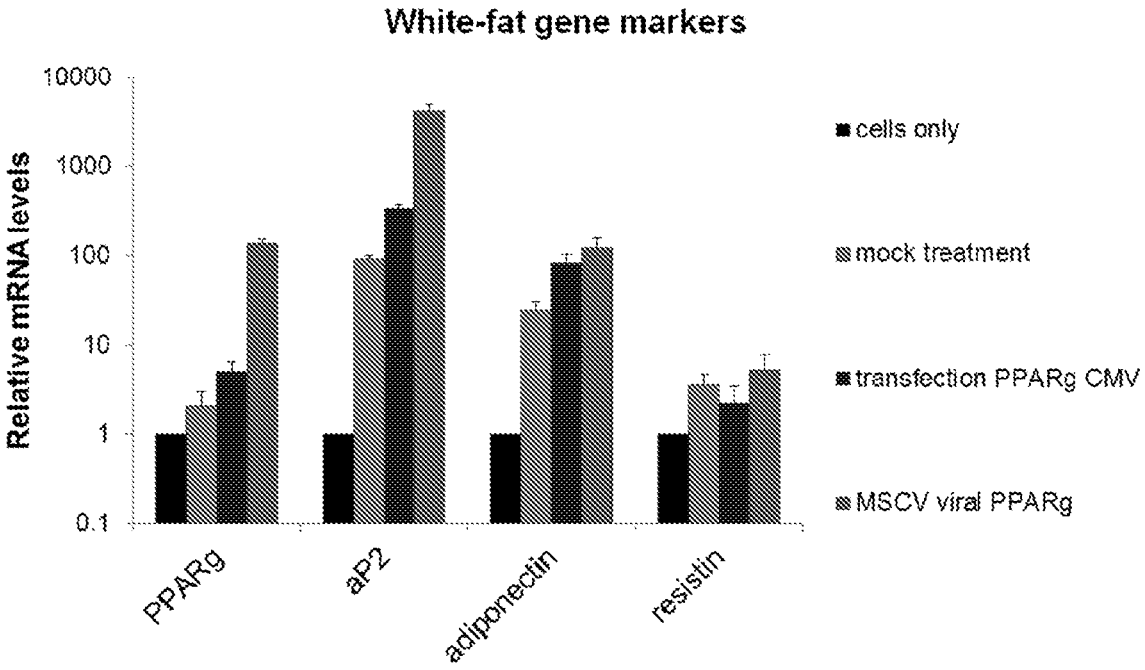


Figure 12

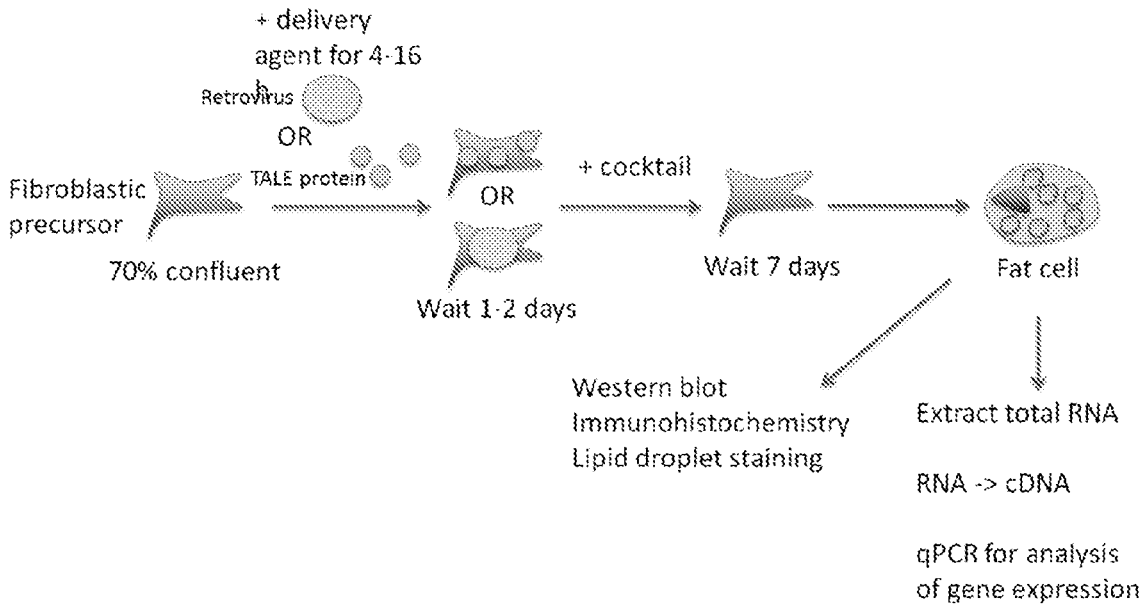


Figure 13

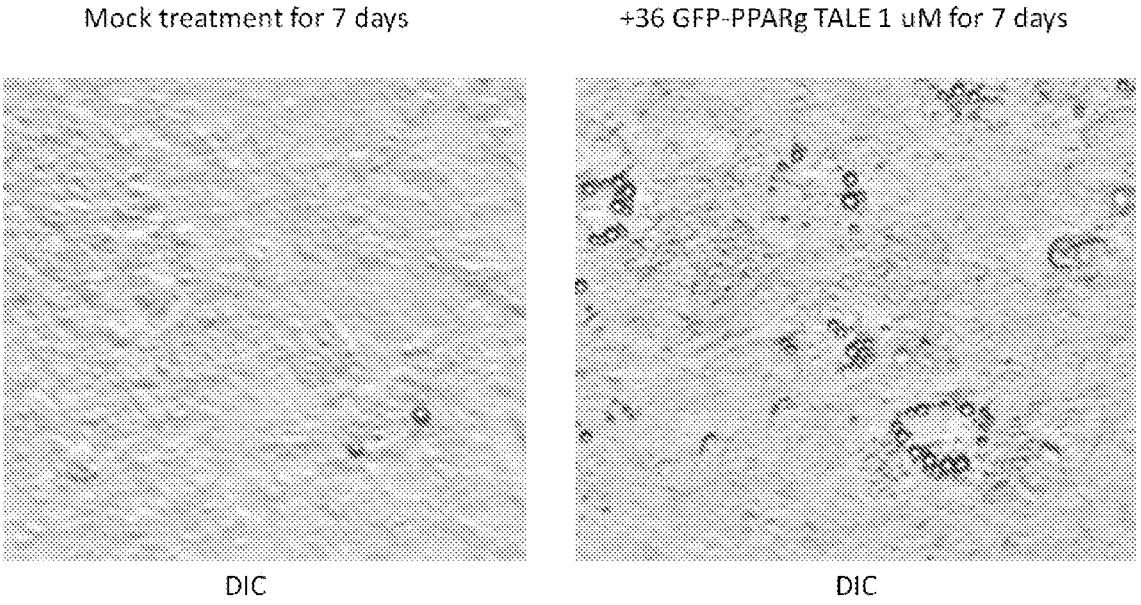


Figure 14

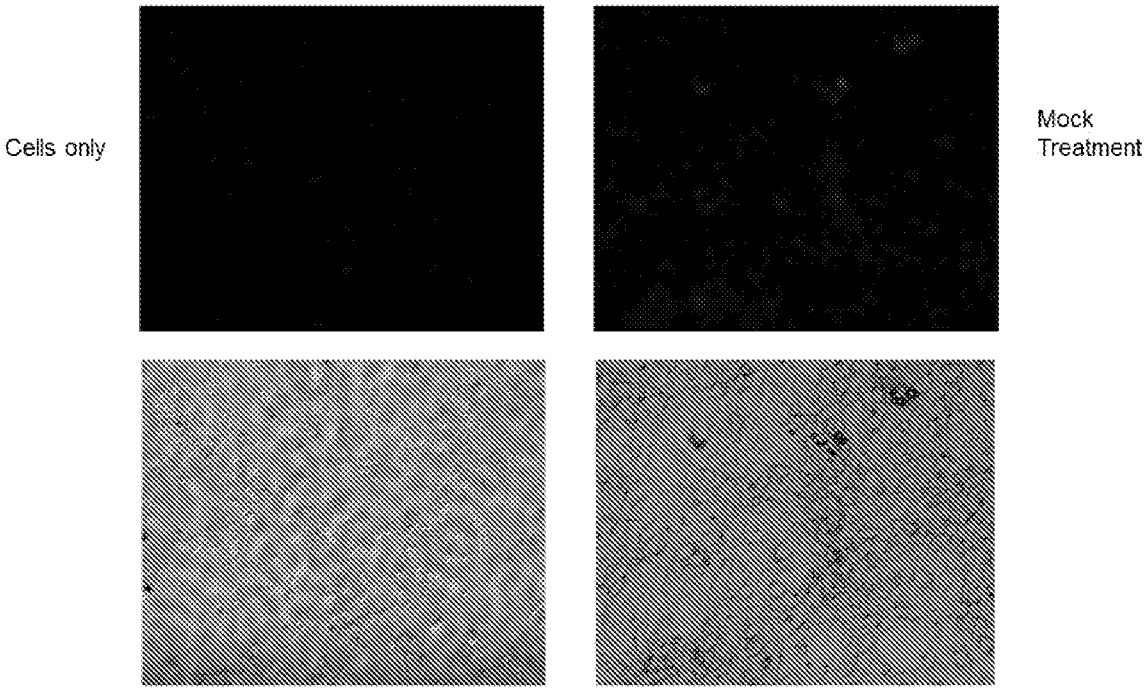


Figure 15

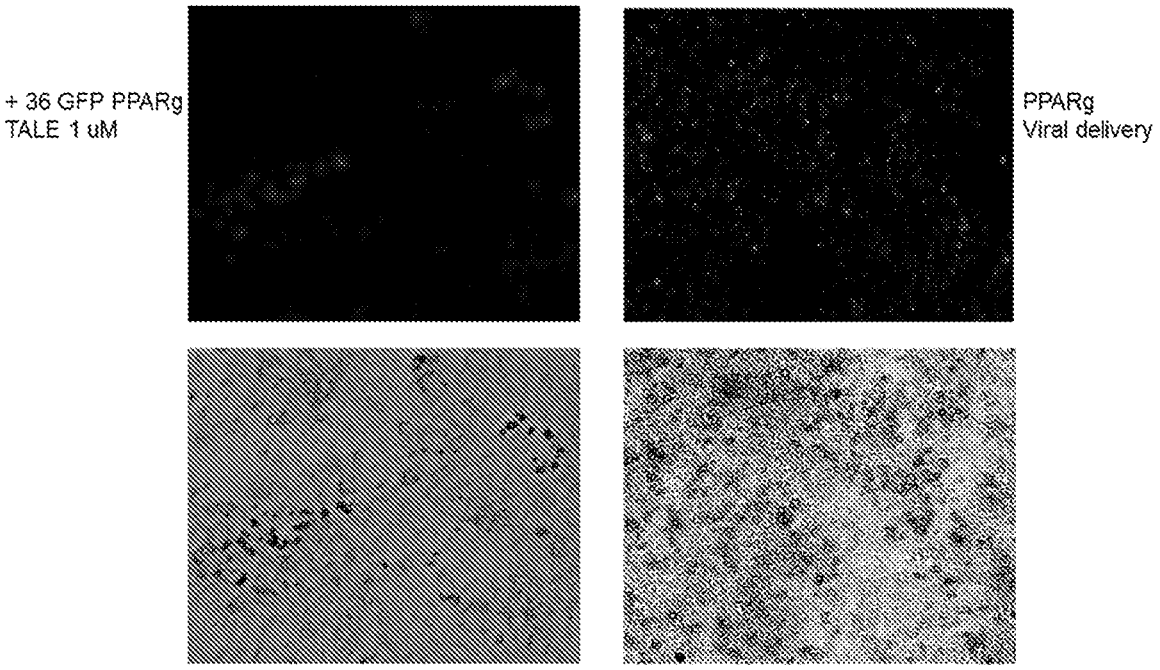


Figure 16

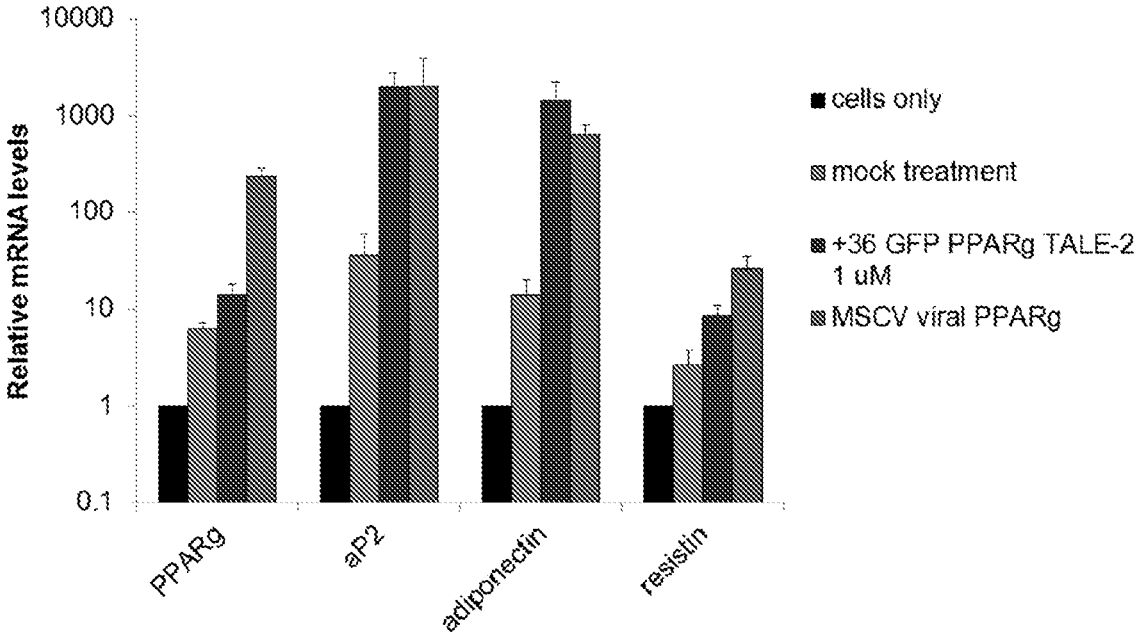


Figure 17

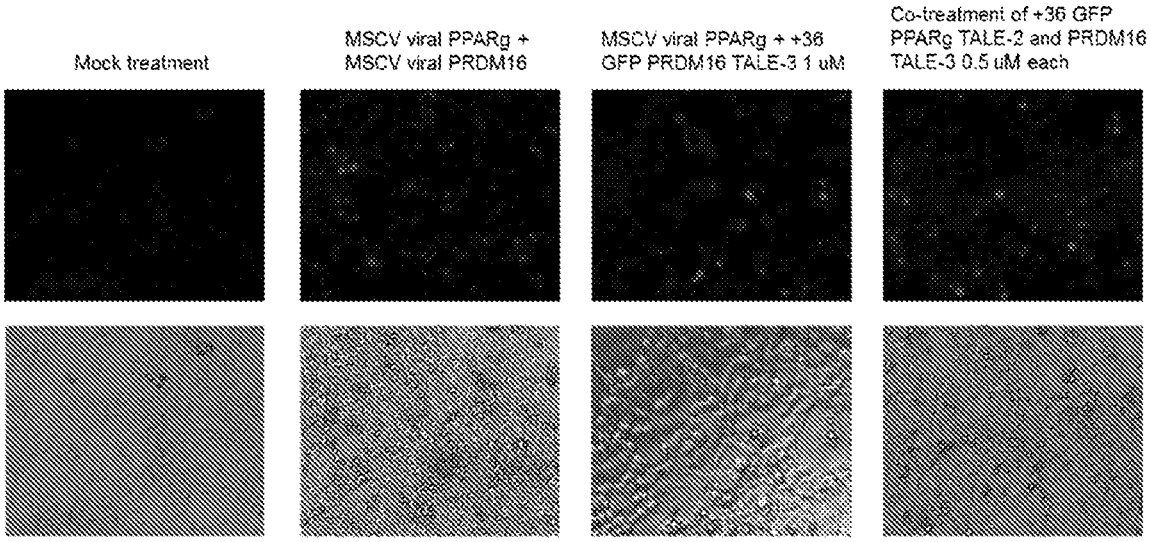


Figure 18

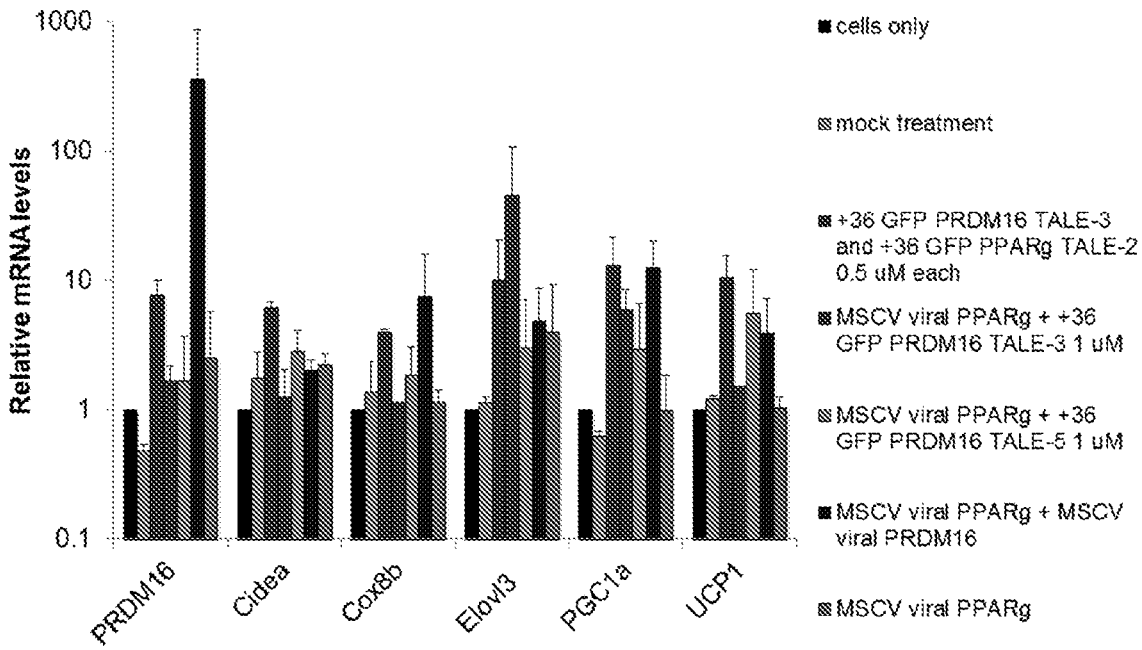


Figure 19

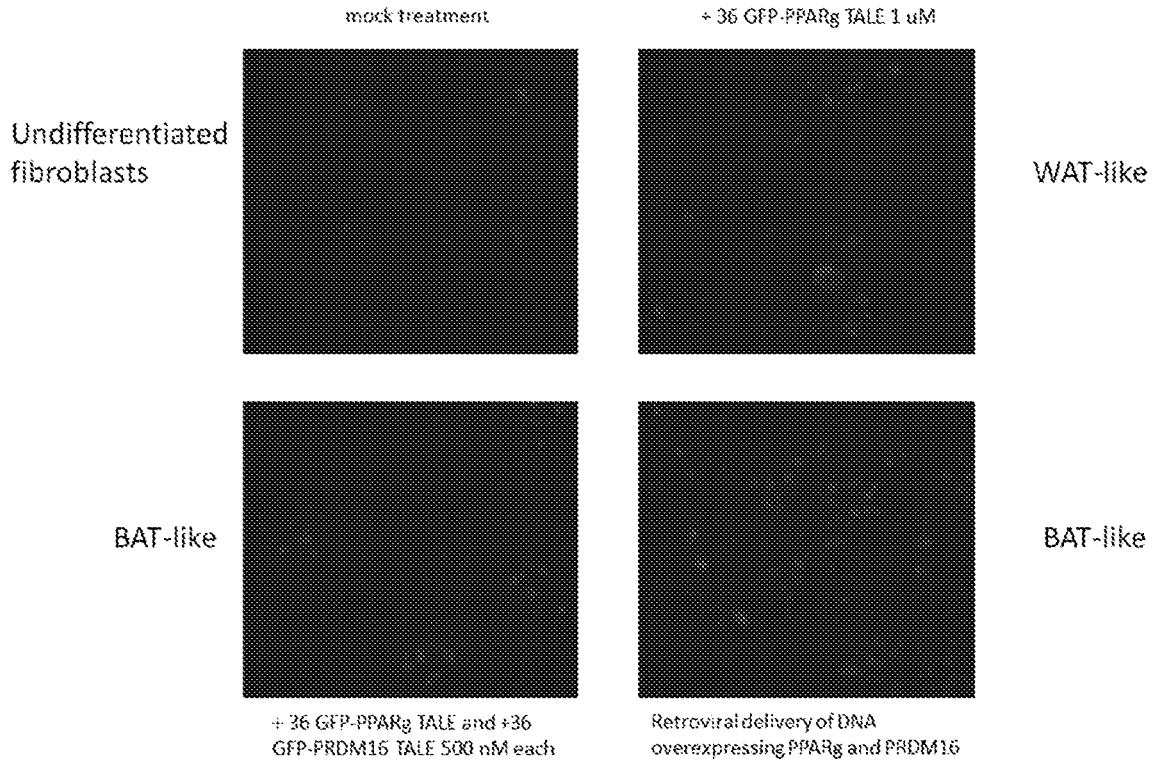

mock treatment
+ 36 GFP-PPARg TALE 1 uM
Undifferentiated fibroblasts
WAT-like
BAT-like
BAT-like
+ 36 GFP-PPARg TALE and +36 GFP-PRDM16 TALE 500 nM each
Retroviral delivery of DNA overexpressing PPARg and PRDM16

Figure 20

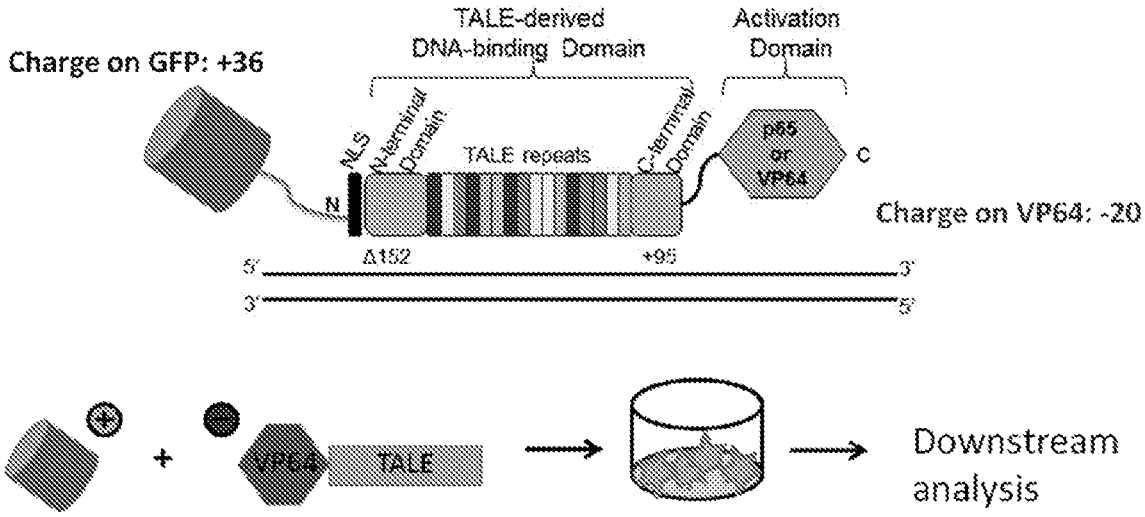

Charge on GFP: +36
TALE-derived
DNA-binding Domain
Activation
Domain
NLS
N-terminal
Domain
TALE repeats
C-terminal
Domain
p65
or
VP64
C
N
Charge on VP64: -20
Δ152
+95
5'
3'
3'
5'
+
VP64
TALE
Downstream
analysis

Figure 21

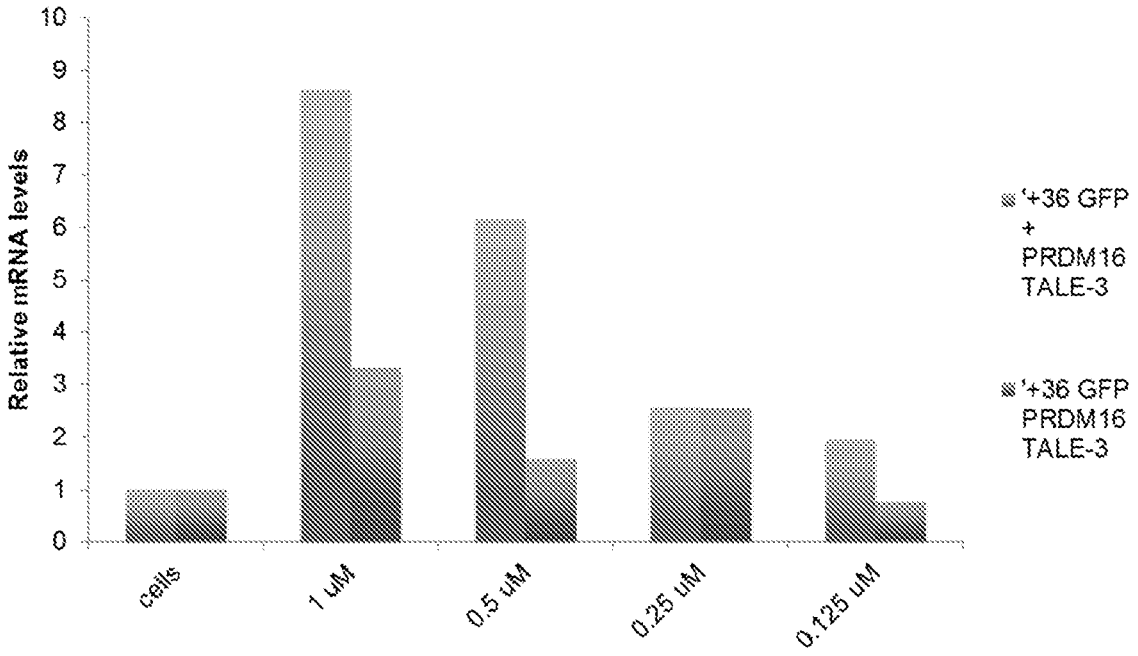

Relative mRNA levels
10
9
8
7
6
5
4
3
2
1
0
cells
1 uM
0.5 uM
0.25 uM
0.125 uM
'+36 GFP + PRDM16 TALE-3
'+36 GFP PRDM16 TALE-3

Figure 22

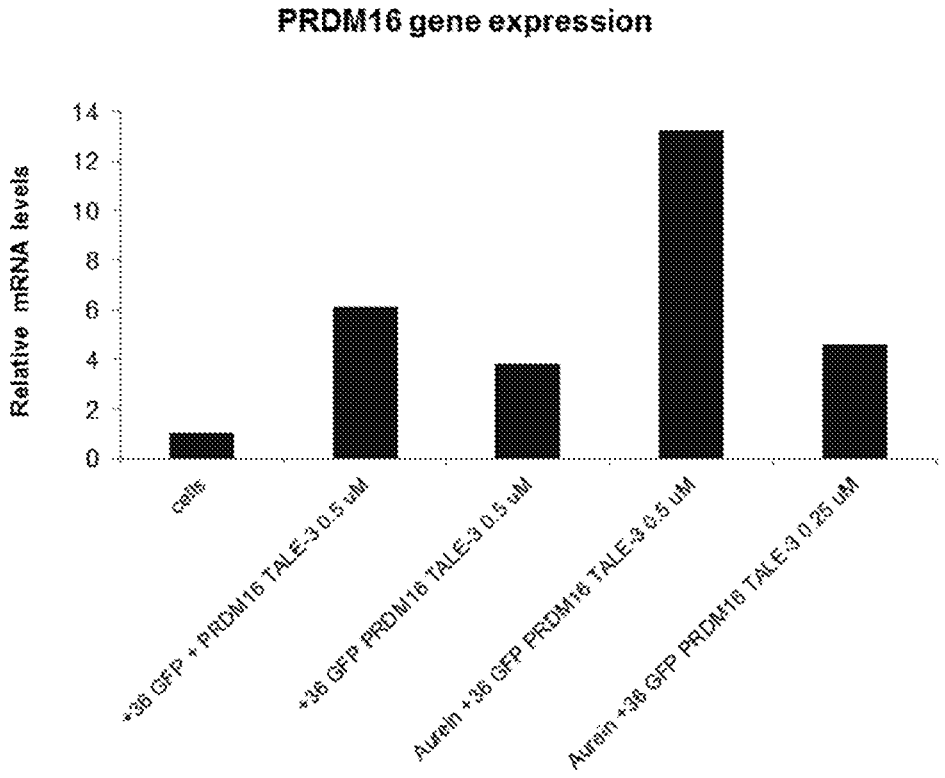

PRDM16 gene expression
Relative mRNA levels
14
12
10
8
6
4
2
0
cells
+36 GFP + PRDM16 TALE-3 0.5 uM
+36 GFP PRDM16 TALE-3 0.5 uM
Aurein +36 GFP PRDM16 TALE-3 0.5 uM
Aurein +36 GFP PRDM16 TALE-3 0.25 uM

Figure 23

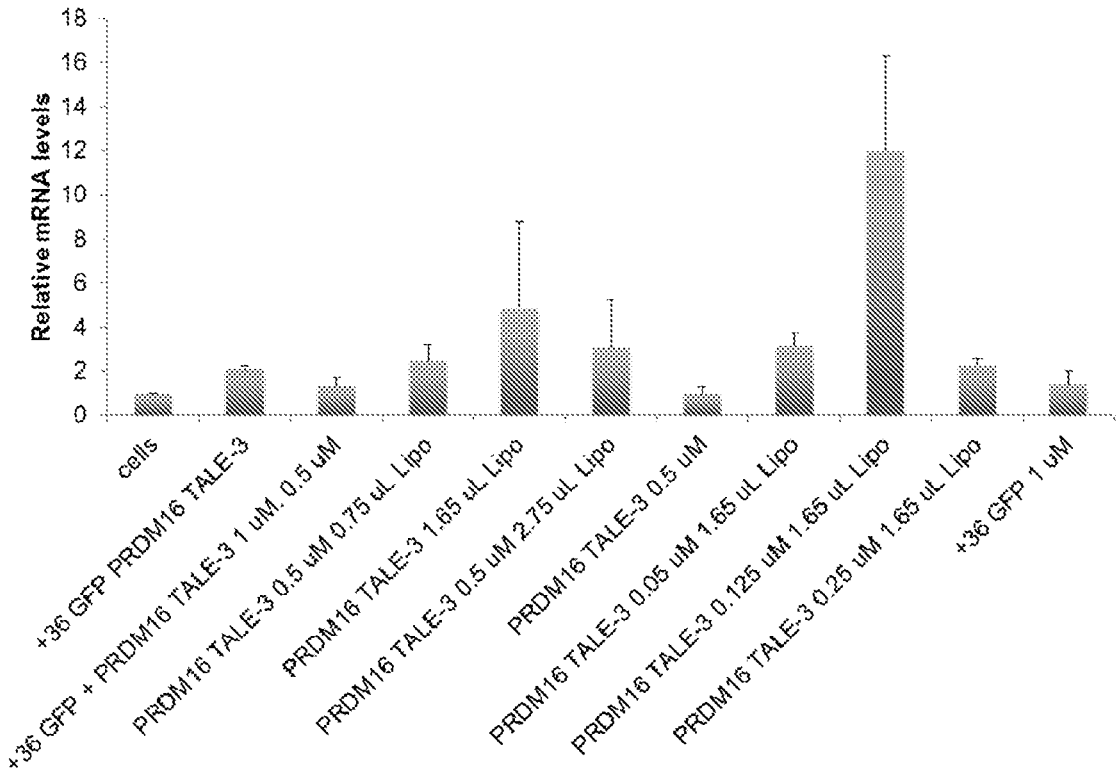

Relative mRNA levels
18
16
14
12
10
8
6
4
2
0
cells
+36 GFP PRDM16 TALE-3
+36 GFP + PRDM16 TALE-3 1 uM. 0.5 uM
PRDM16 TALE-3 0.5 uM 0.75 uL Lipo
PRDM16 TALE-3 1.65 uL Lipo
PRDM16 TALE-3 0.5 uM 2.75 uL Lipo
PRDM16 TALE-3 0.5 uM
PRDM16 TALE-3 0.05 uM 1.65 uL Lipo
PRDM16 TALE-3 0.125 uM 1.65 uL Lipo
PRDM16 TALE-3 0.25 uM 1.65 uL Lipo
+36 GFP 1 uM

Figure 24

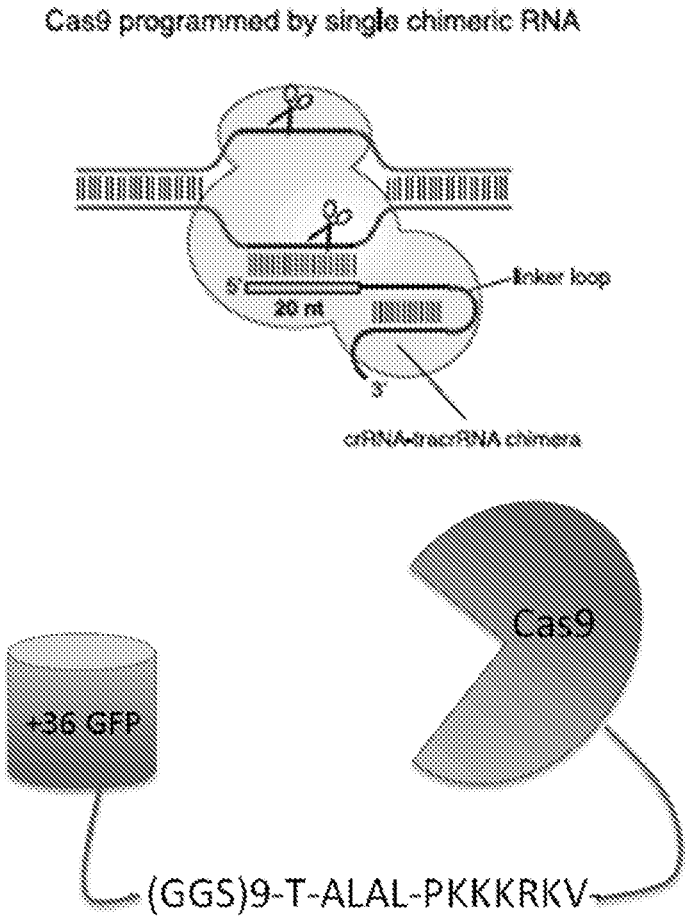

Cas9 programmed by single chimeric RNA
linker loop
20 nt
3'
crRNA-tracrRNA chimera
Cas9
+36 GFP
(GGS)9-T-ALAL-PKKKRKV

Figure 25

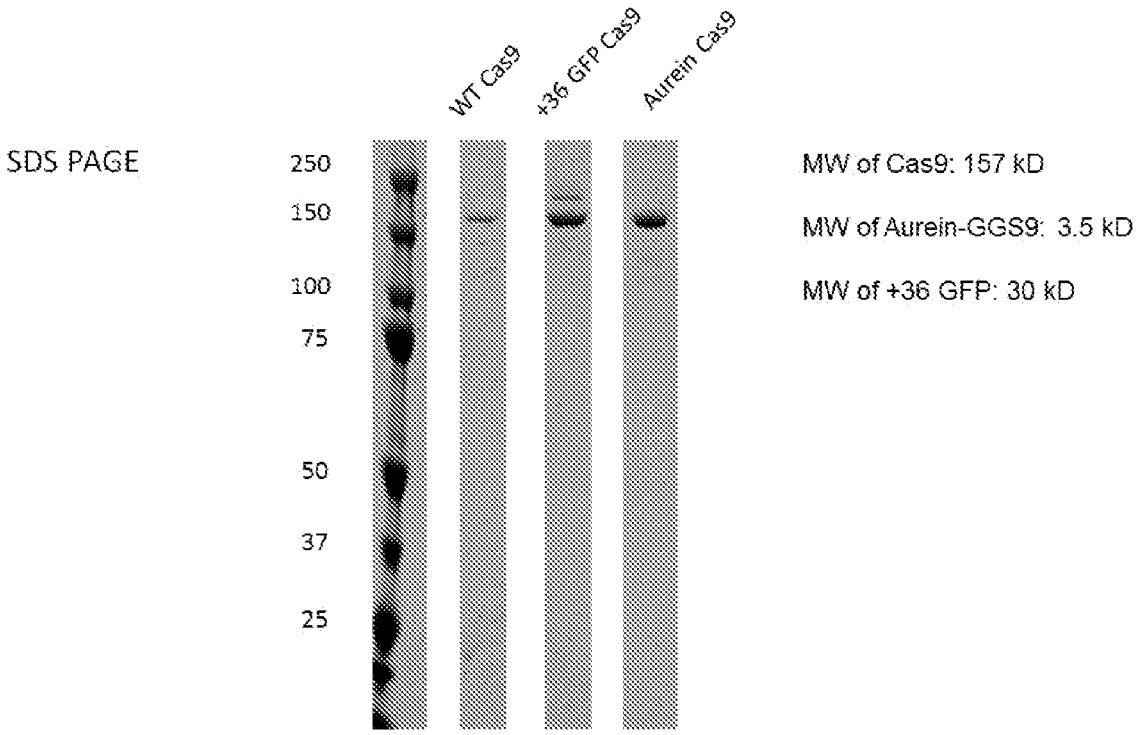

SDS PAGE
WT Cas9
+36 GFP Cas9
Aurein Cas9
250
150
100
75
50
37
25
MW of Cas9: 157 kD
MW of Aurein-GGS9: 3.5 kD
MW of +36 GFP: 30 kD

Figure 26

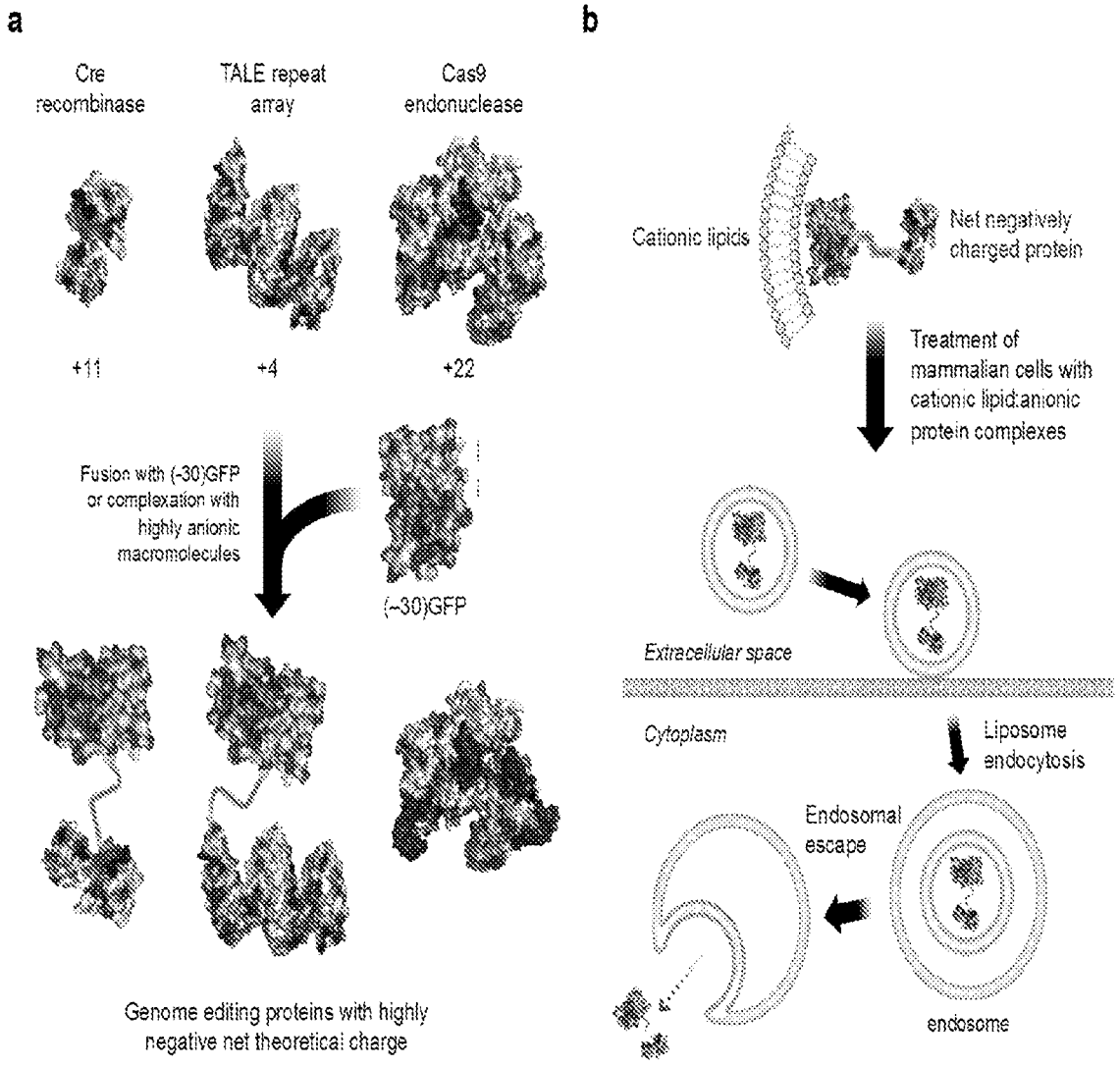

a
Cre recombinase
TALE repeat array
Cas9 endonuclease
+11
+4
+22
Fusion with (-30)GFP or complexation with highly anionic macromolecules
(-30)GFP
Genome editing proteins with highly negative net theoretical charge
b
Cationic lipids
Net negatively charged protein
Treatment of mammalian cells with cationic lipid:anionic protein complexes
Extracellular space
Cytoplasm
Liposome endocytosis
Endosomal escape
endosome

Figure 27

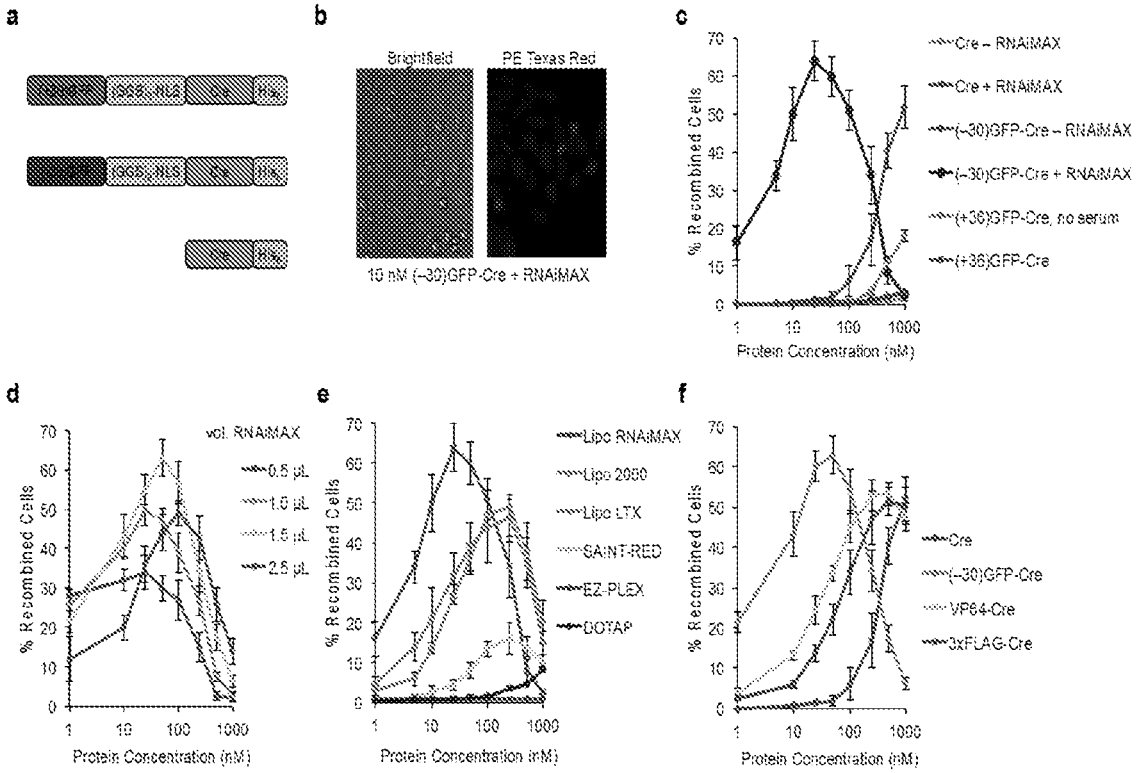
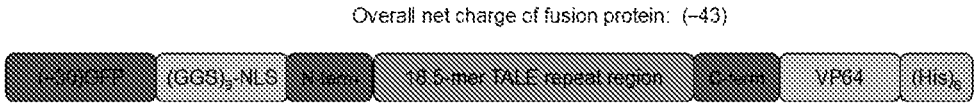

a
b
Brightfield
PE Texas Red
10 nM (-30)GFP-Cre + RNAiMAX
c
% Recombined Cells
Protein Concentration (nM)
Cre – RNAiMAX
Cre + RNAiMAX
(-30)GFP-Cre – RNAiMAX
(-30)GFP-Cre + RNAiMAX
(+36)GFP-Cre, no serum
(+36)GFP-Cre
d
vol. RNAiMAX
0.5 µL
1.0 µL
1.5 µL
2.5 µL
e
Lipo RNAiMAX
Lipo 2000
Lipo LTX
SAINT-RED
EZ-PLEX
DOTAP
f
Cre
(-30)GFP-Cre
VP64-Cre
3xFLAG-Cre

Figure 28

a


Overall net charge of fusion protein: (−43)
(GGS)3-NLS
VP64
(His)6

b

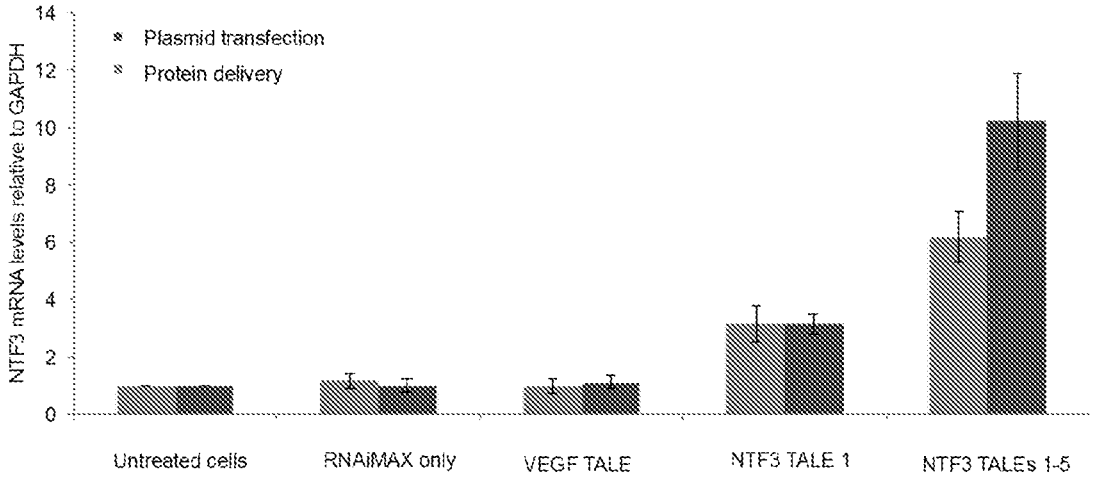

Plasmid transfection
Protein delivery
NTF3 mRNA levels relative to GAPDH
14
12
10
8
6
4
2
0
Untreated cells
RNAiMAX only
VEGF TALE
NTF3 TALE 1
NTF3 TALEs 1-5

Figure 29

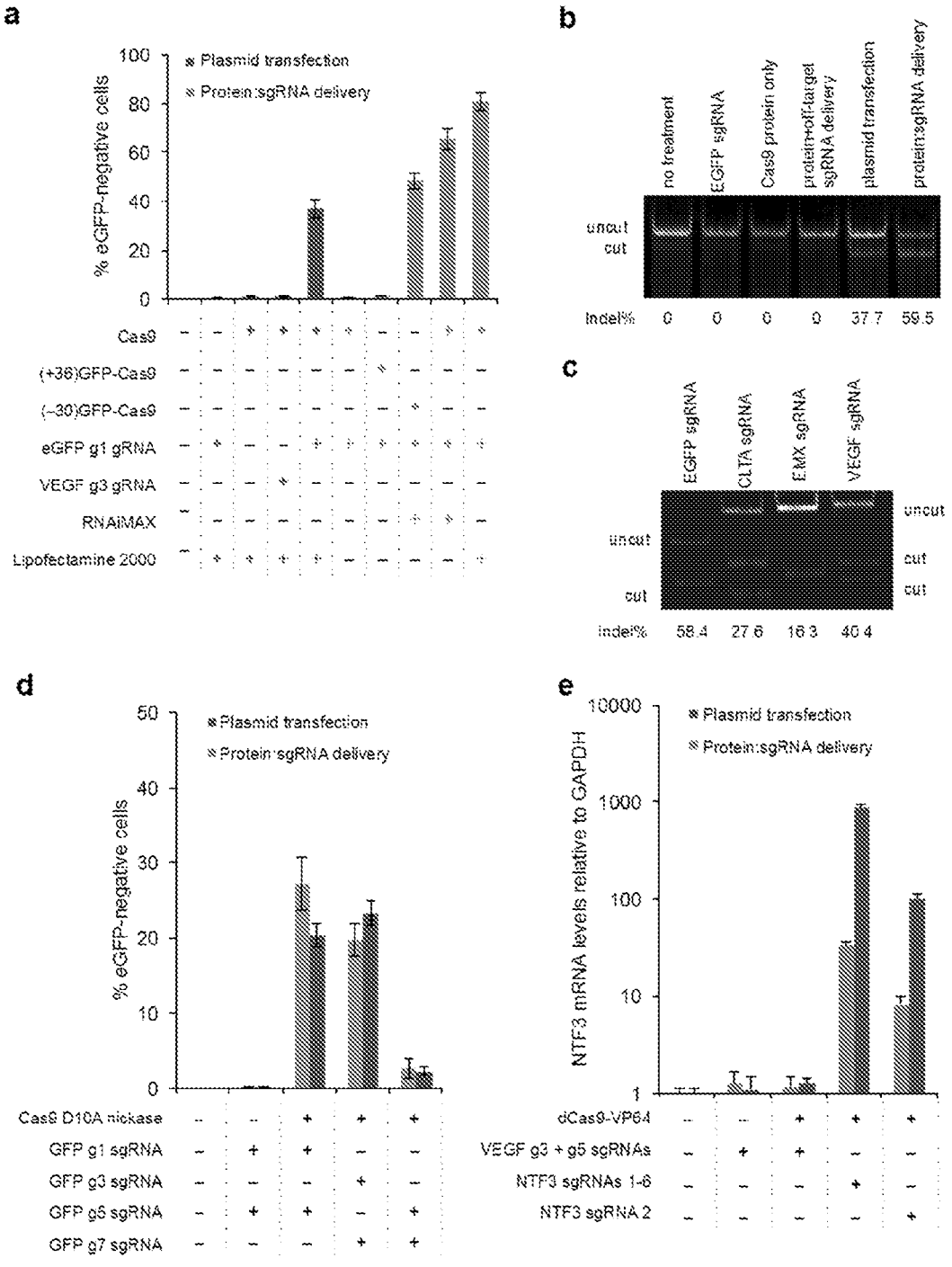

a
% eGFP-negative cells
Plasmid transfection
Protein:sgRNA delivery
Cas9
(+36)GFP-Cas9
(-30)GFP-Cas9
eGFP g1 gRNA
VEGF g3 gRNA
RNAiMAX
Lipofectamine 2000
b
no treatment
EGFP sgRNA
Cas9 protein only
protein+off-target sgRNA delivery
plasmid transfection
protein:sgRNA delivery
uncut
cut
Indel%
0 0 0 0 37.7 59.5
c
EGFP sgRNA
CLTA sgRNA
EMX sgRNA
VEGF sgRNA
uncut
cut
Indel%
58.4 27.6 16.3 40.4
d
% eGFP-negative cells
Plasmid transfection
Protein:sgRNA delivery
Cas9 D10A nickase
GFP g1 sgRNA
GFP g3 sgRNA
GFP g6 sgRNA
GFP g7 sgRNA
e
NTF3 mRNA levels relative to GAPDH
Plasmid transfection
Protein:sgRNA delivery
dCas9-VP64
VEGF g3 + g5 sgRNAs
NTF3 sgRNAs 1-6
NTF3 sgRNA 2

Figure 30

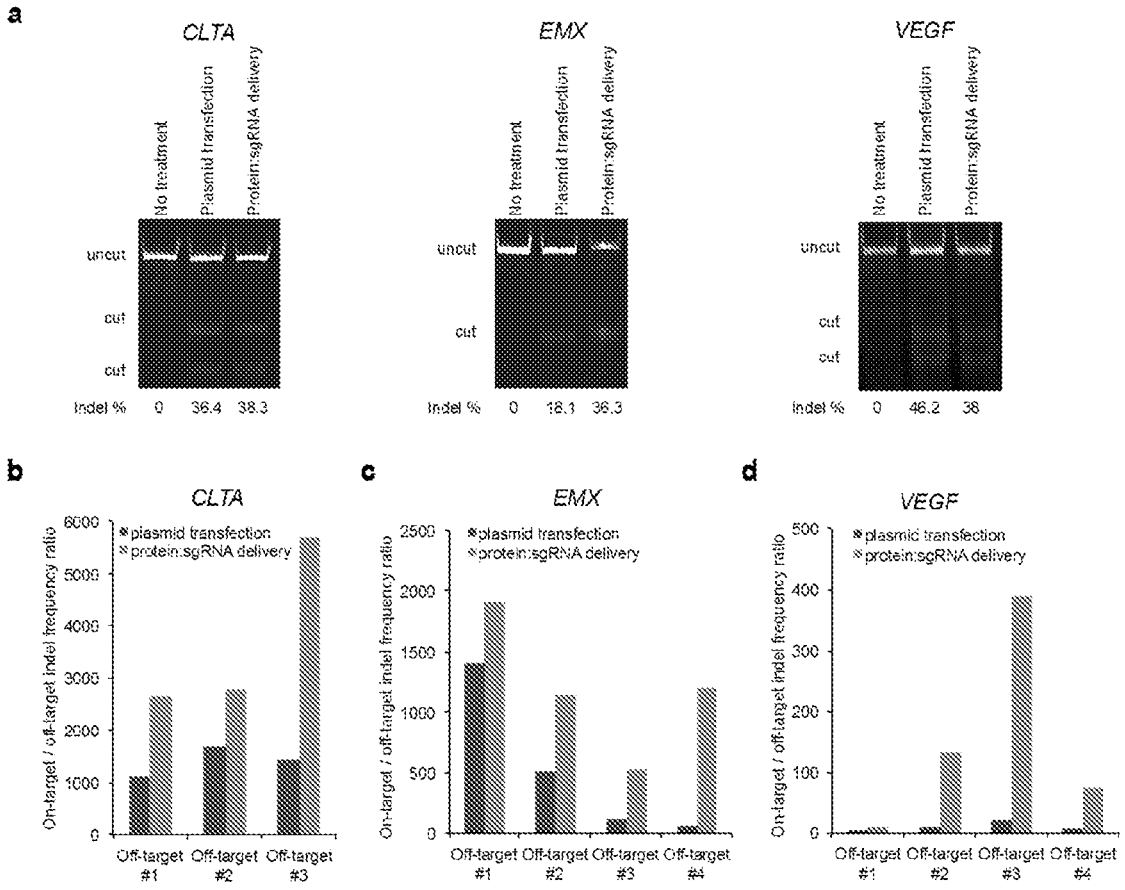

a
CLTA
EMX
VEGF
No treatment
Plasmid transfection
Protein:sgRNA delivery
uncut
cut
cut
Indel %
0
36.4
38.3
0
18.1
36.3
0
46.2
38
b
c
d
On-target / off-target indel frequency ratio
plasmid transfection
protein:sgRNA delivery
Off-target #1
Off-target #2
Off-target #3
Off-target #4

Figure 31

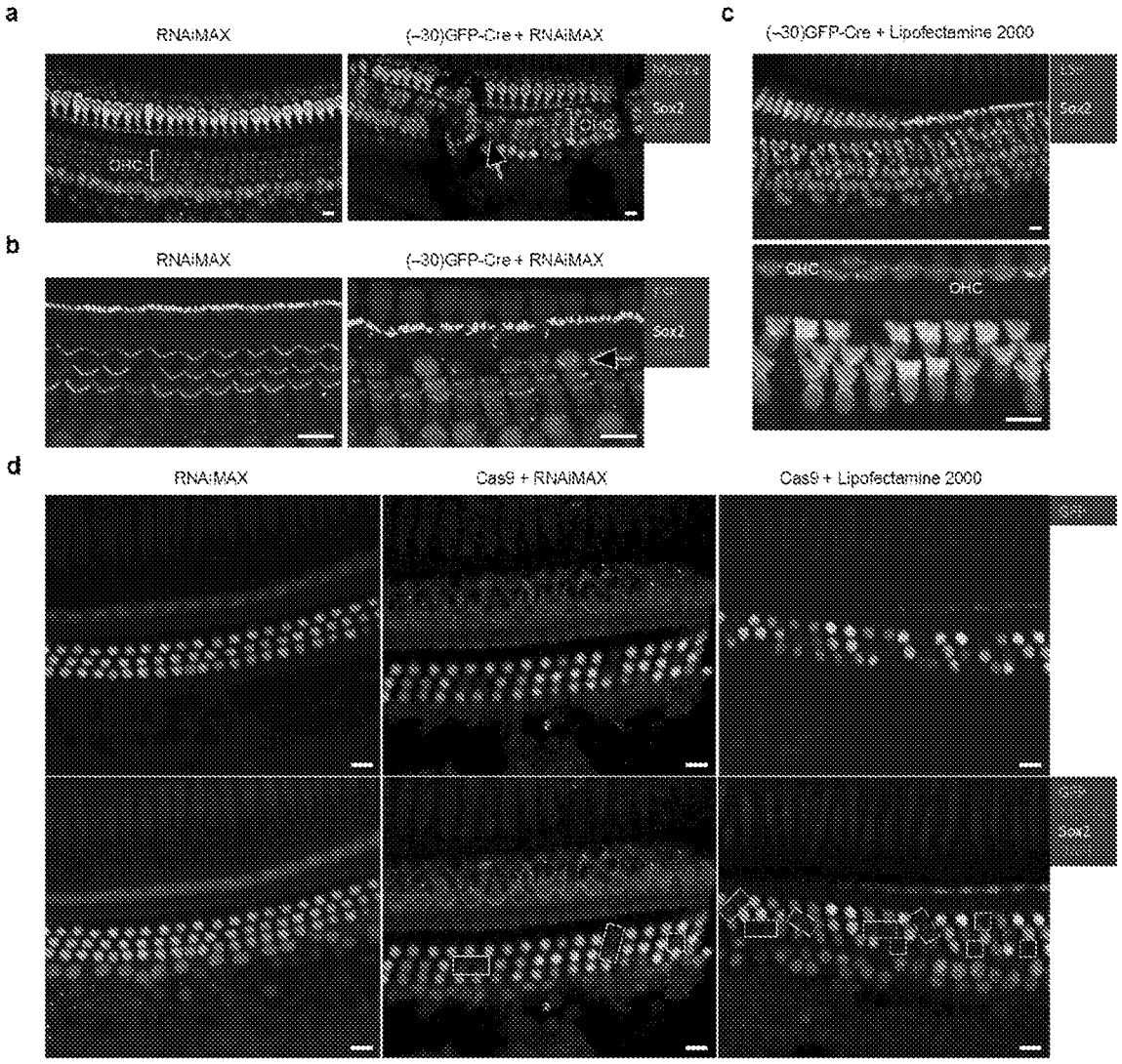

a
RNAiMAX
(-30)GFP-Cre + RNAiMAX
DHC
DHC
b
RNAiMAX
(-30)GFP-Cre + RNAiMAX
c
(-30)GFP-Cre + Lipofectamine 2000
DHC
DHC
d
RNAiMAX
Cas9 + RNAiMAX
Cas9 + Lipofectamine 2000

Figure 32

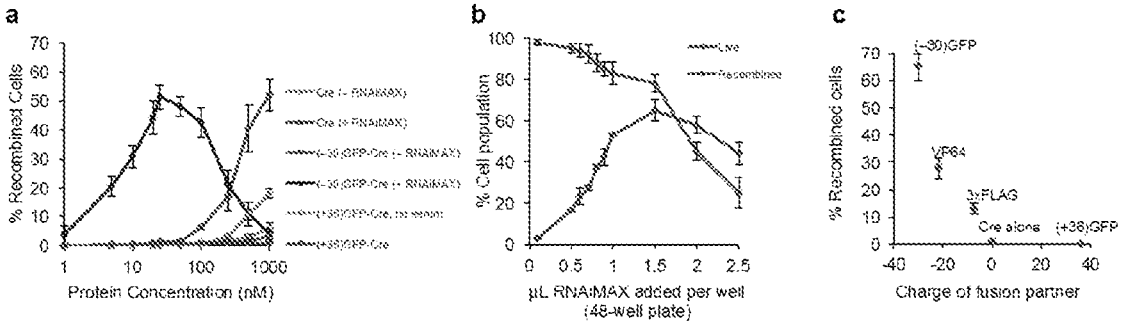


Figure 33

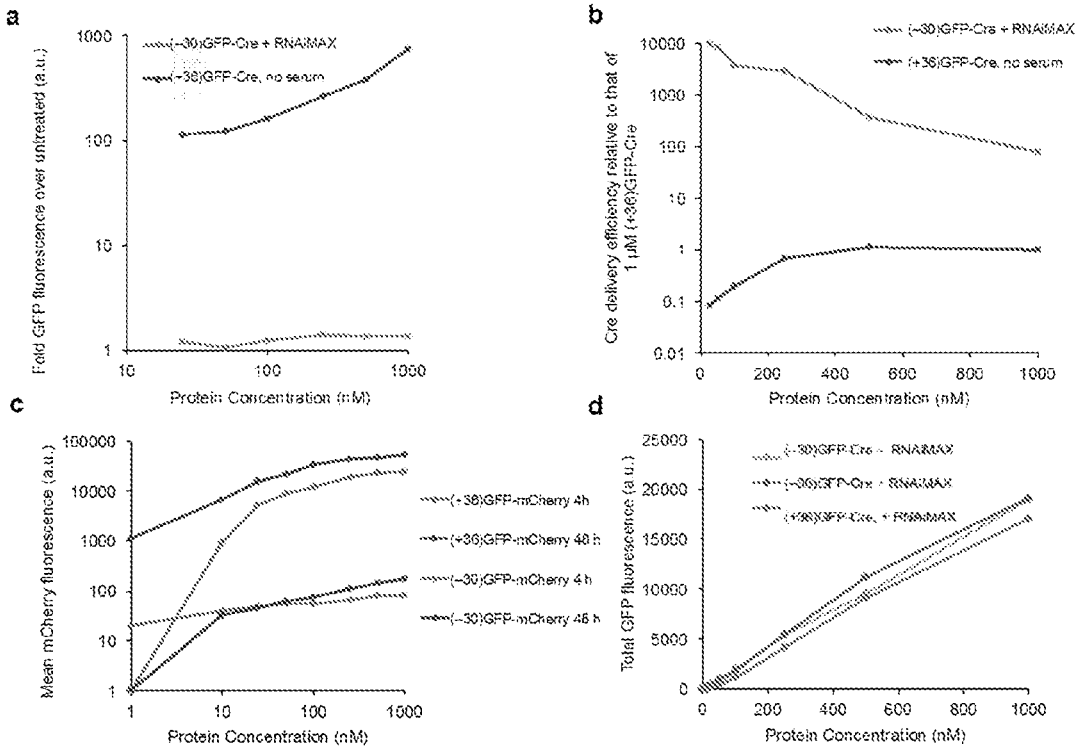


Figure 34

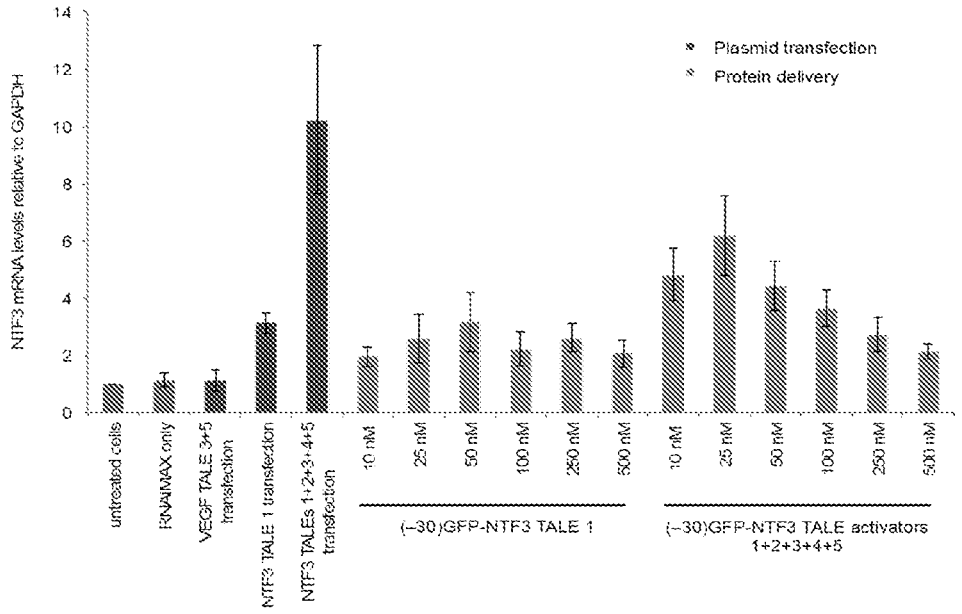


Figure 35

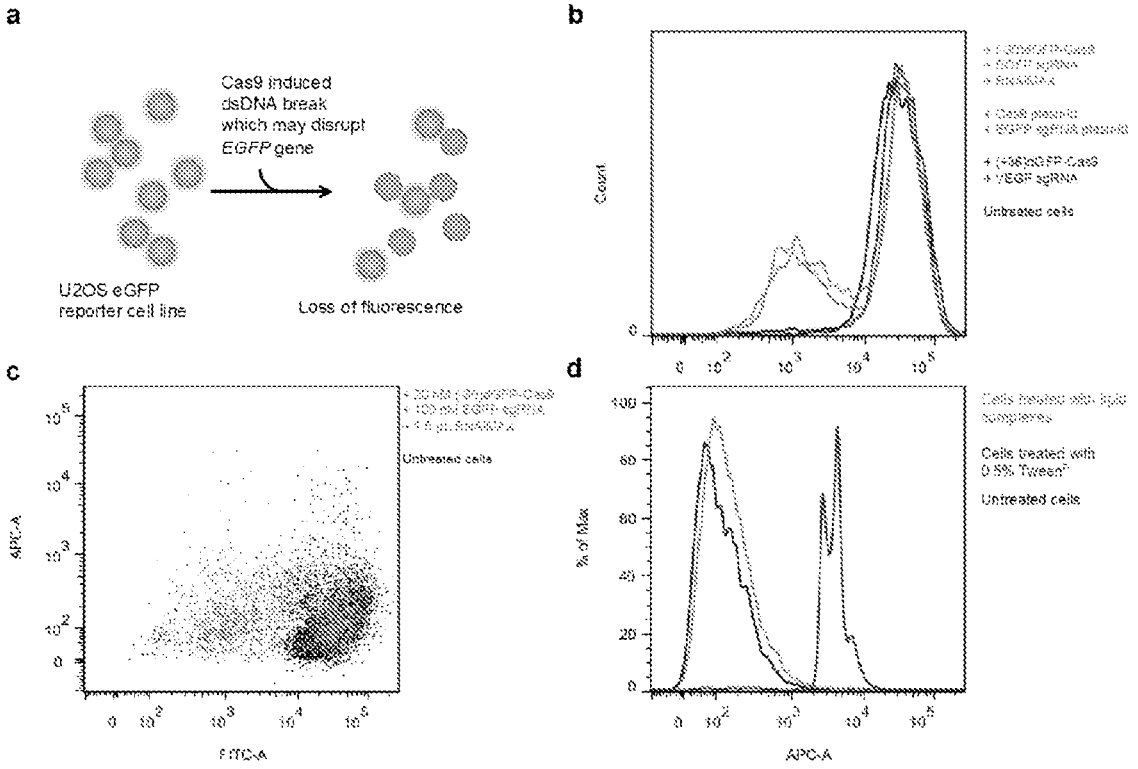


Figure 36

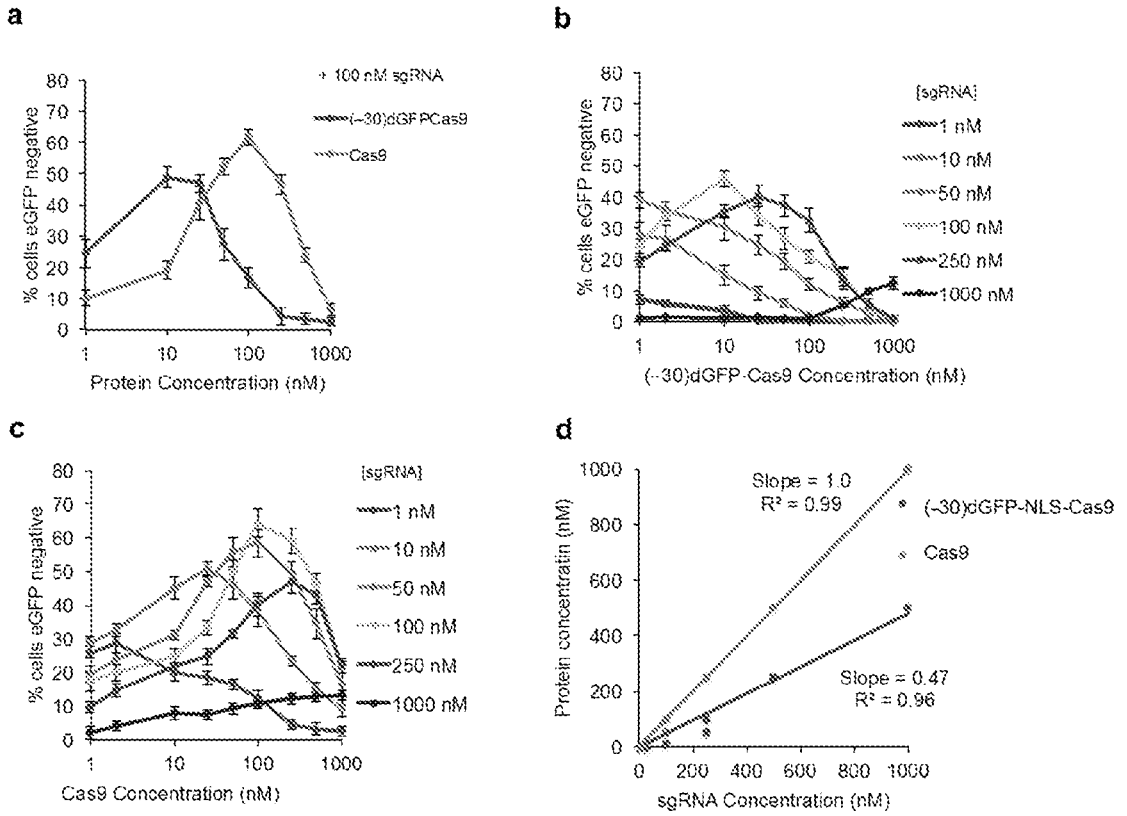


Figure 37

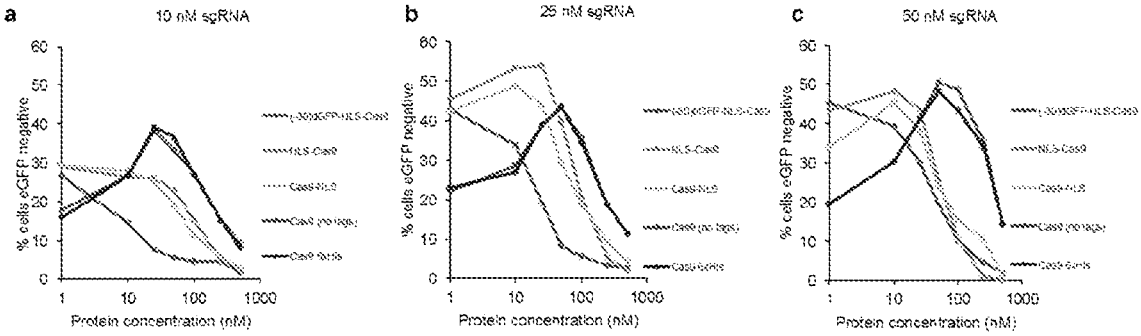


Figure 38

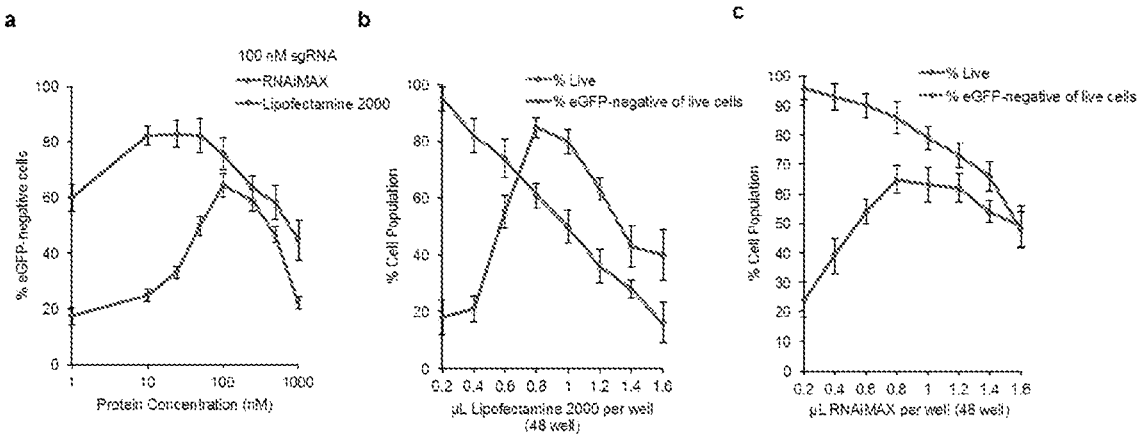


Figure 39

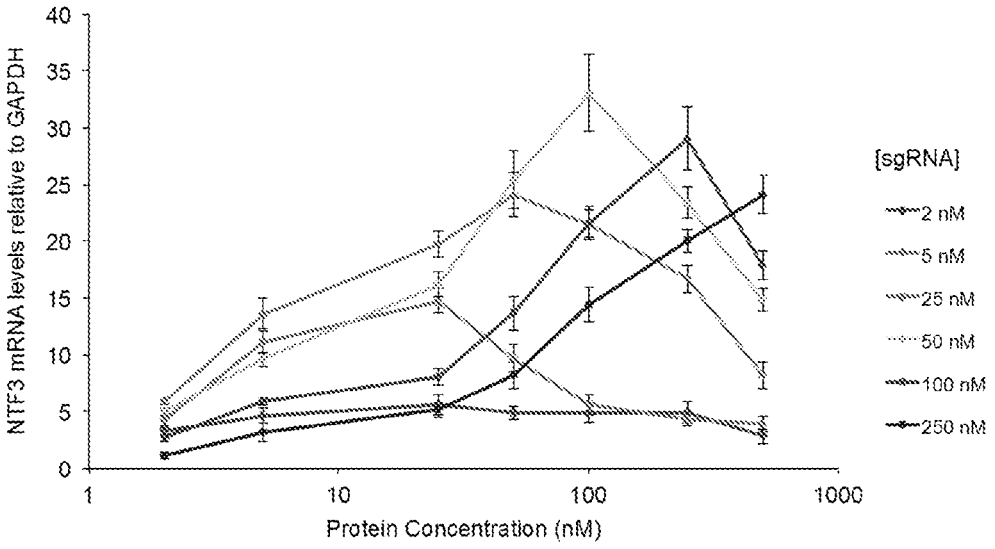


Figure 40

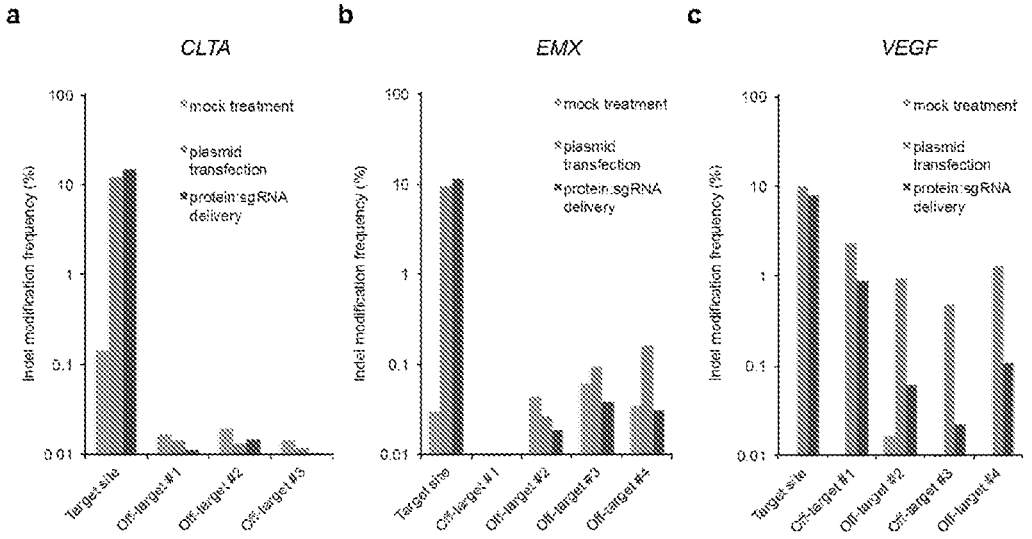


Figure 41

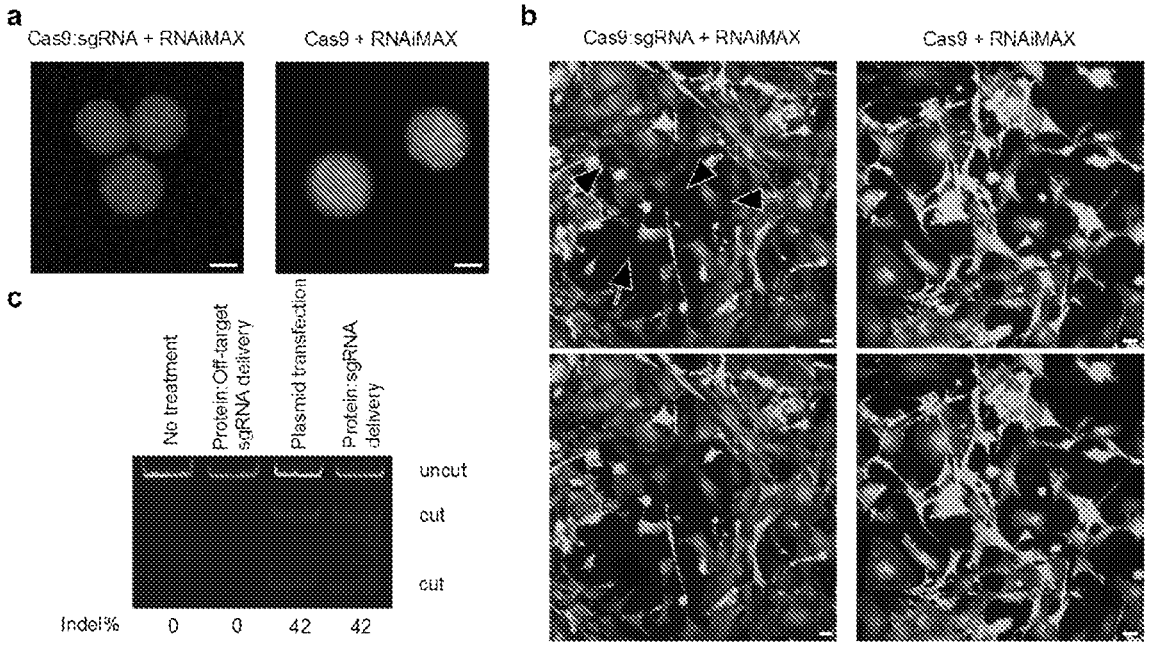


Figure 42

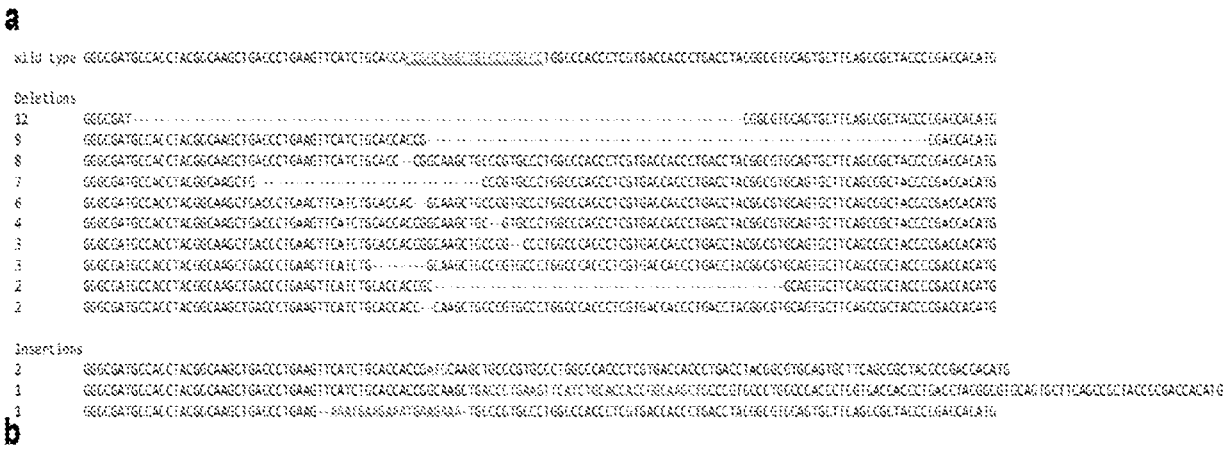

a
wild type
Deletions
12
9
8
7
6
4
3
3
2
2
Insertions
2
1
1
b
wild type
Deletions
58
8
7
6
6
5
4
3
3
3
Insertions
4
1
1

Figure 43