FIGURE 1

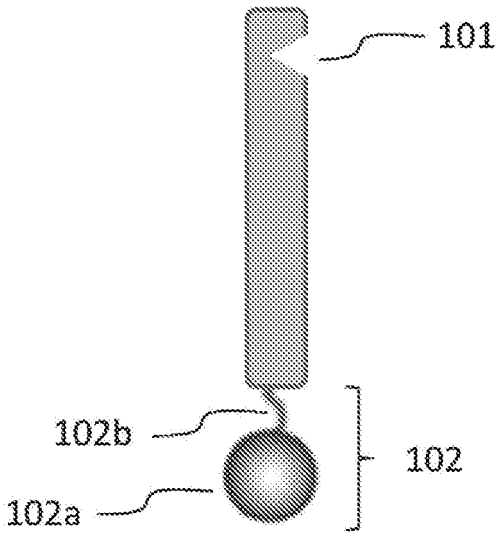


FIGURE 2

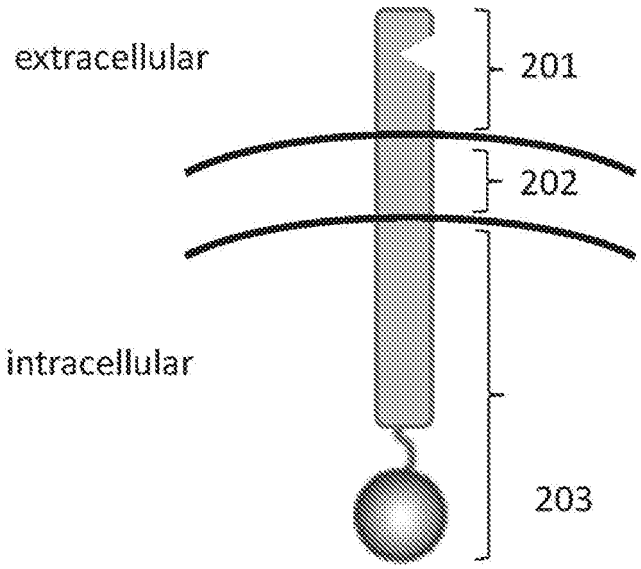


FIGURE 3A

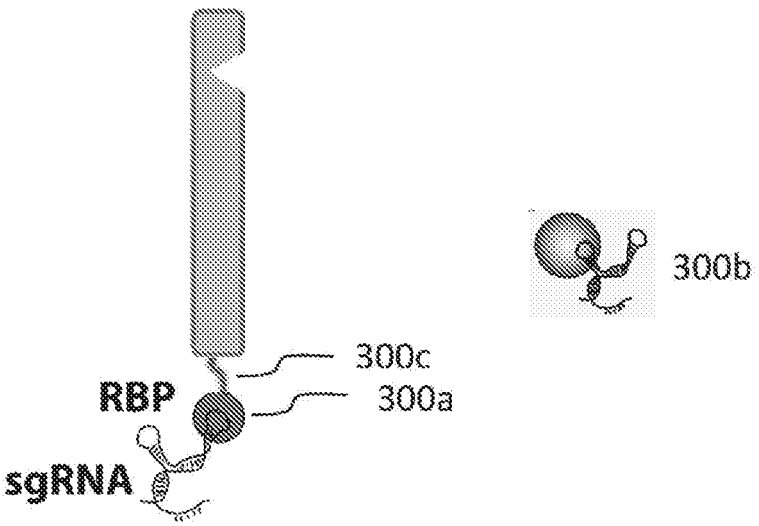


FIGURE 3B

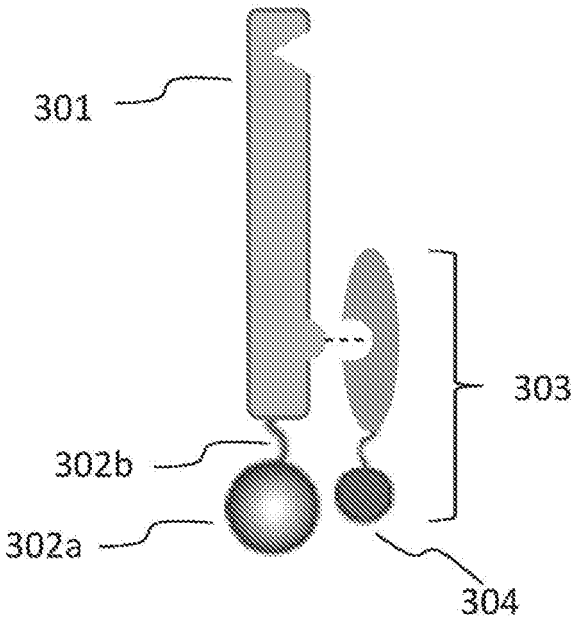


FIGURE 4A

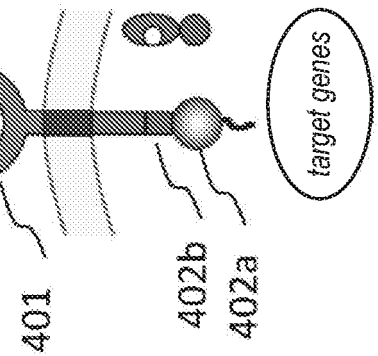


FIGURE 4B

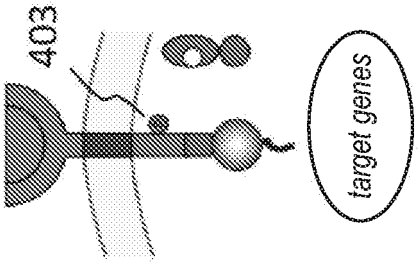


FIGURE 4C

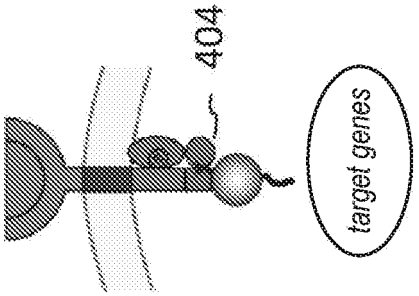


FIGURE 4D

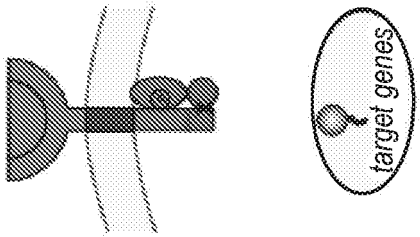


FIGURE 4E

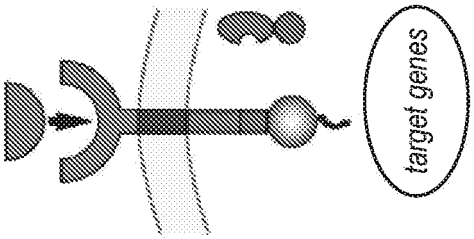


FIGURE 4F

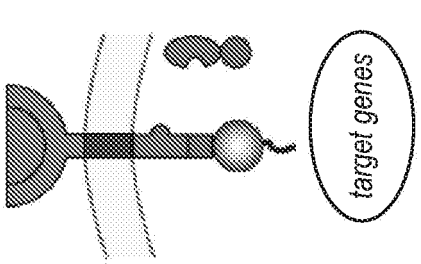


FIGURE 4G

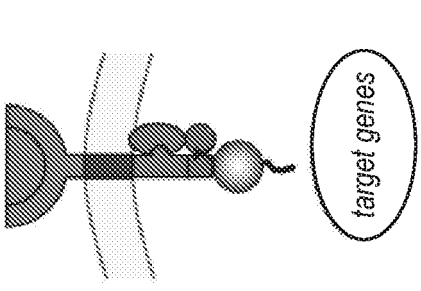


FIGURE 4H

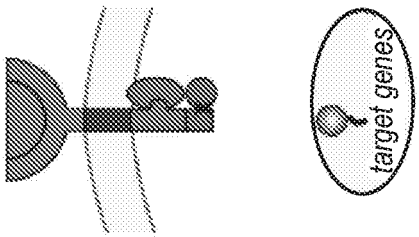


FIGURE 5

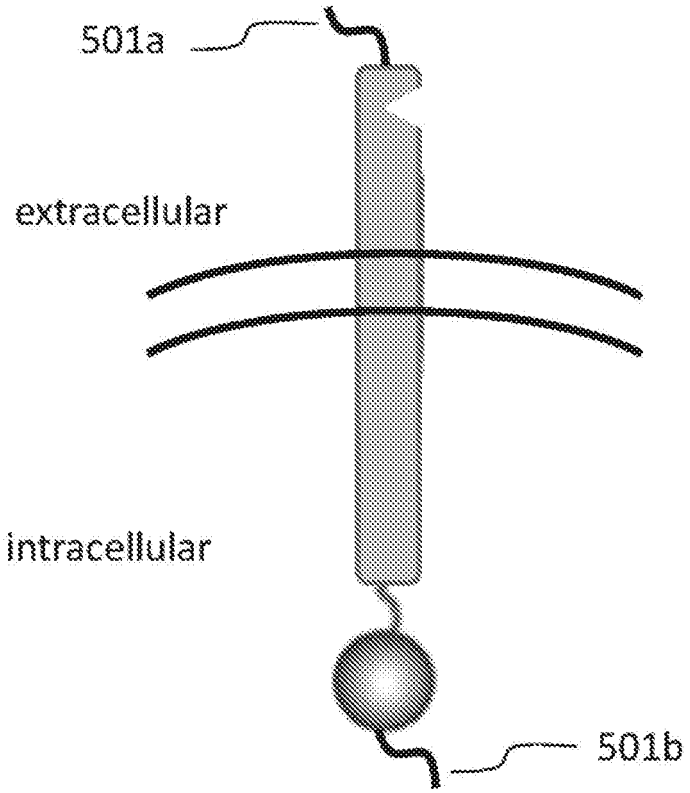


FIGURE 6A

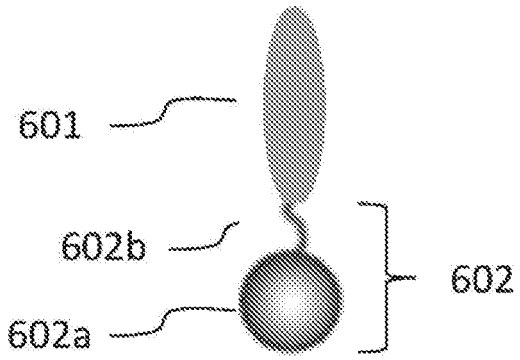


FIGURE 6B

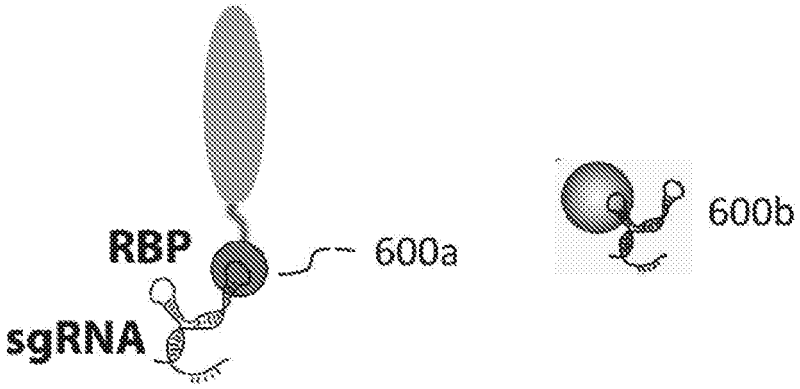
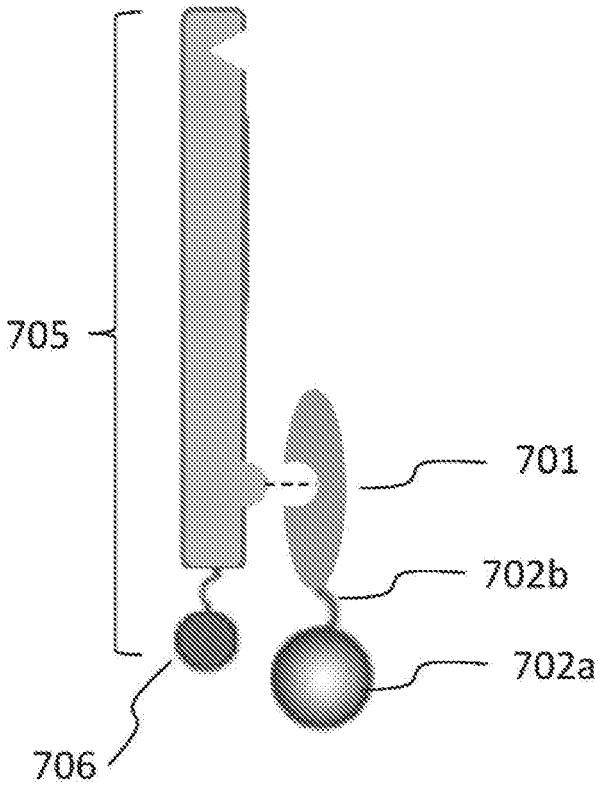


FIGURE 7



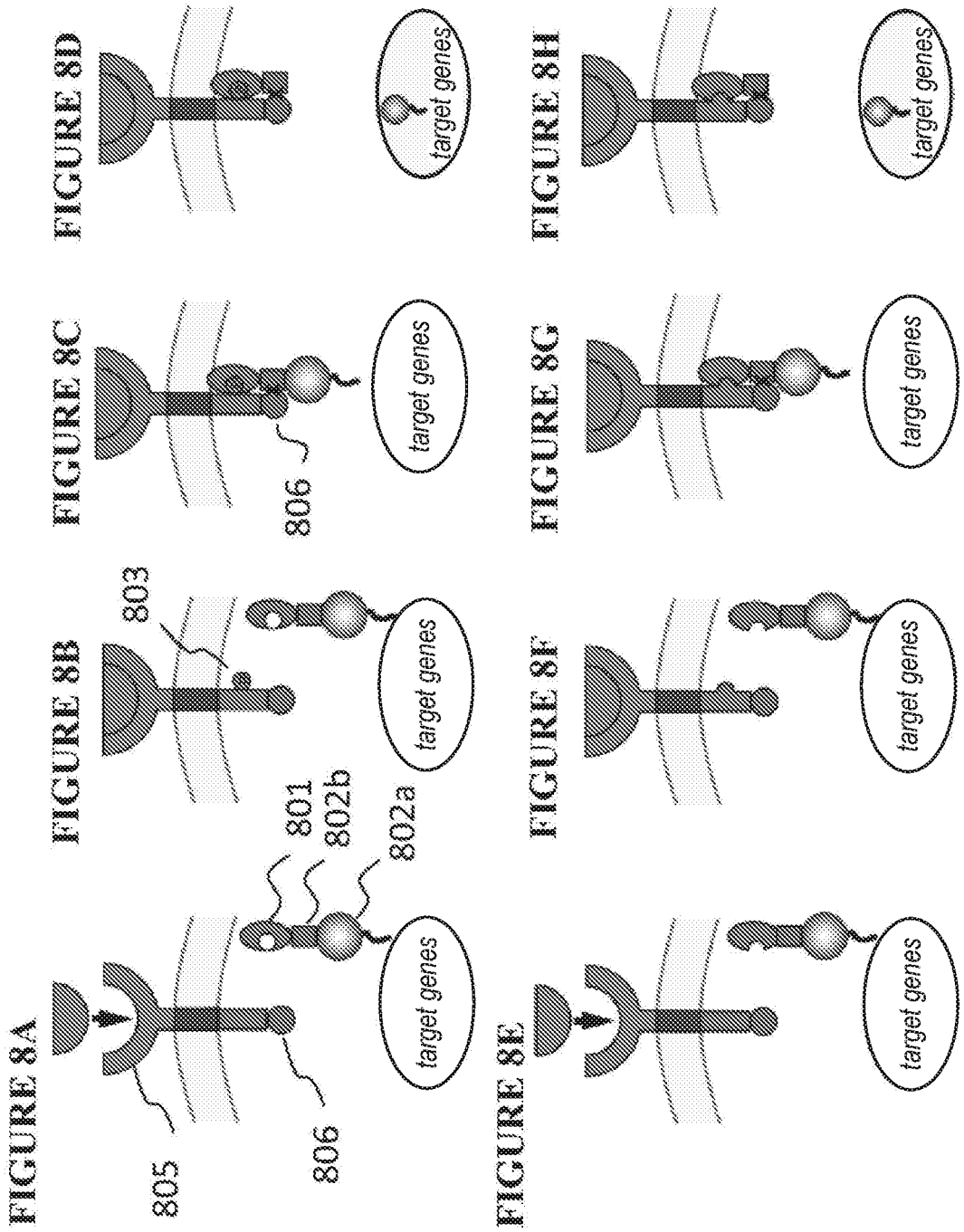
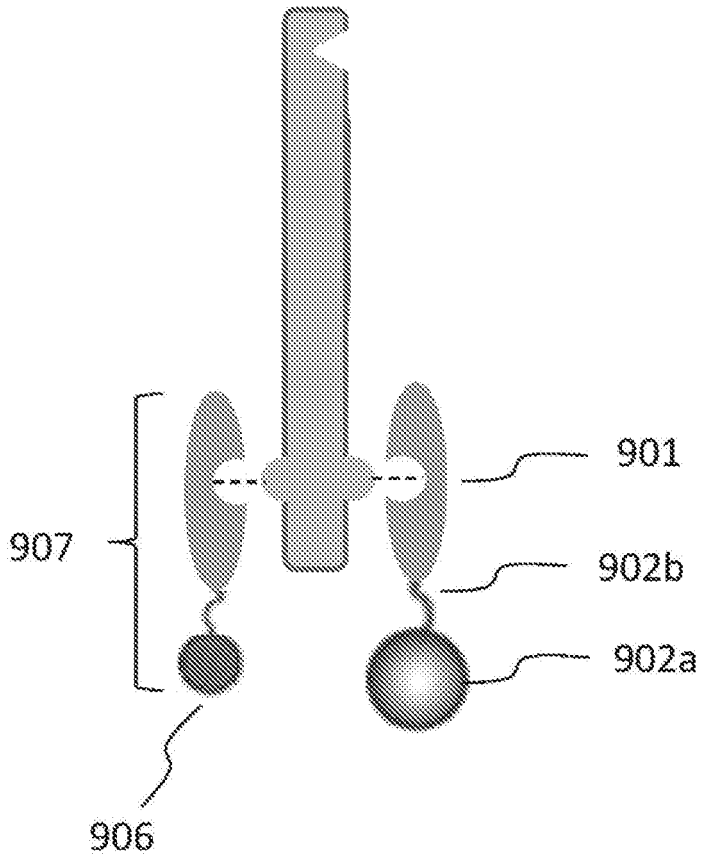

FIGURE 8A
805
806
801
802b
802a
target genes
FIGURE 8B
803
target genes
FIGURE 8C
806
target genes
FIGURE 8D
target genes
FIGURE 8E
target genes
FIGURE 8F
target genes
FIGURE 8G
target genes
FIGURE 8H
target genes

FIGURE 9



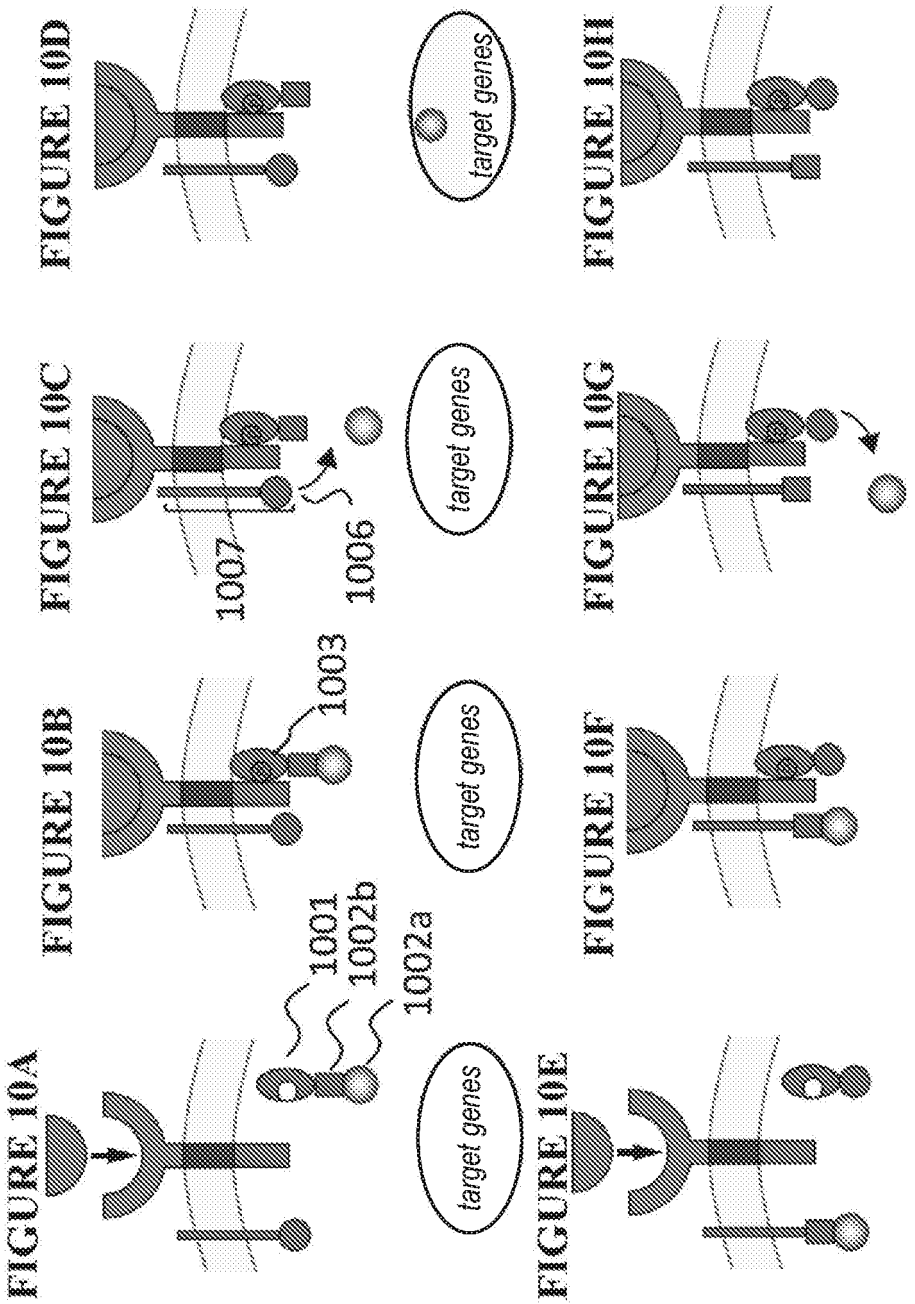

FIGURE 10A
1001
1002b
1002a
target genes
FIGURE 10B
1003
target genes
FIGURE 10C
1007
1006
target genes
FIGURE 10D
target genes
FIGURE 10E
FIGURE 10F
FIGURE 10G
FIGURE 10H

FIGURE 11

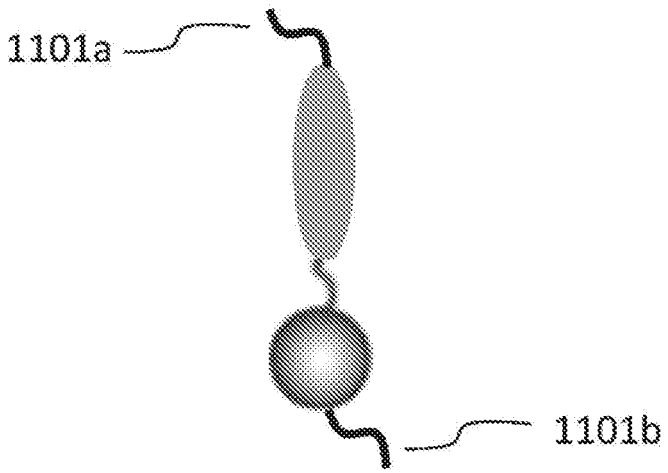


FIGURE 12A

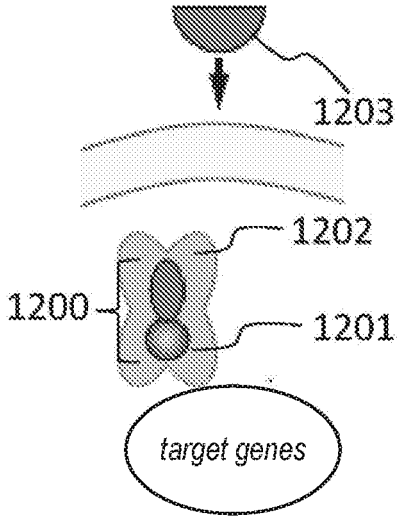


FIGURE 12B

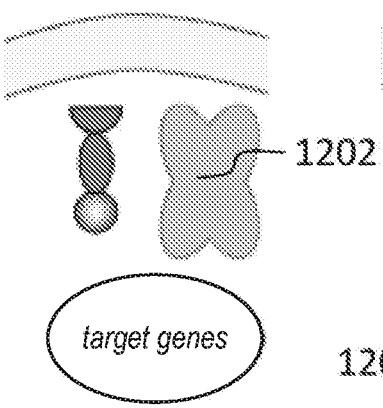
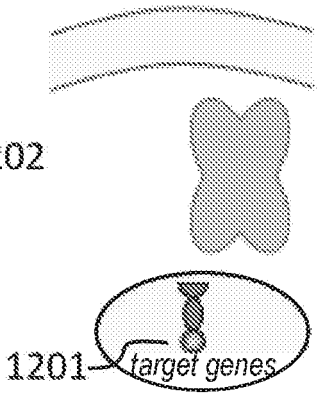
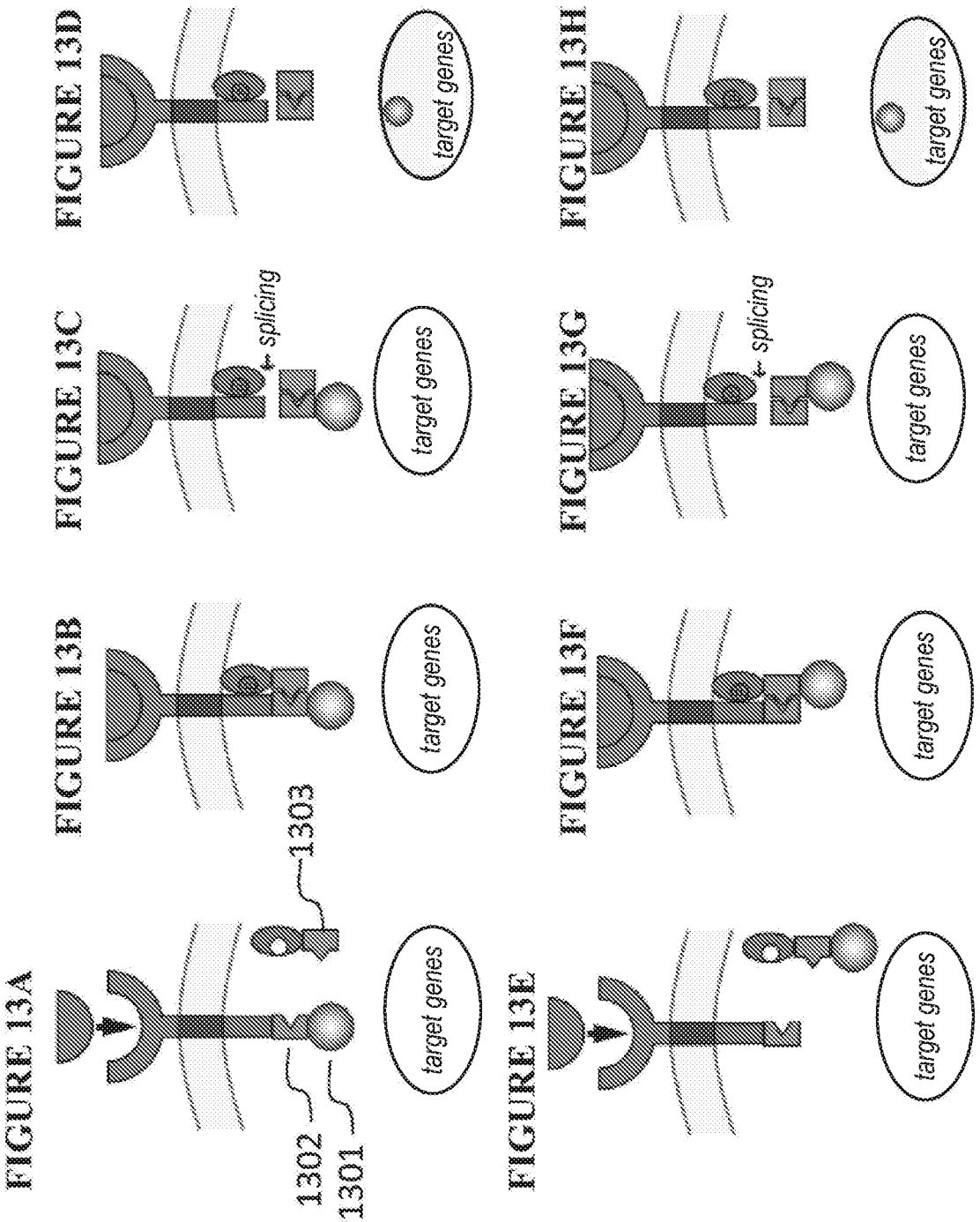


FIGURE 12C




FIGURE 13A
1302
1301
1303
target genes
FIGURE 13B
target genes
FIGURE 13C
splicing
target genes
FIGURE 13D
target genes
FIGURE 13E
target genes
FIGURE 13F
target genes
FIGURE 13G
splicing
target genes
FIGURE 13H
target genes

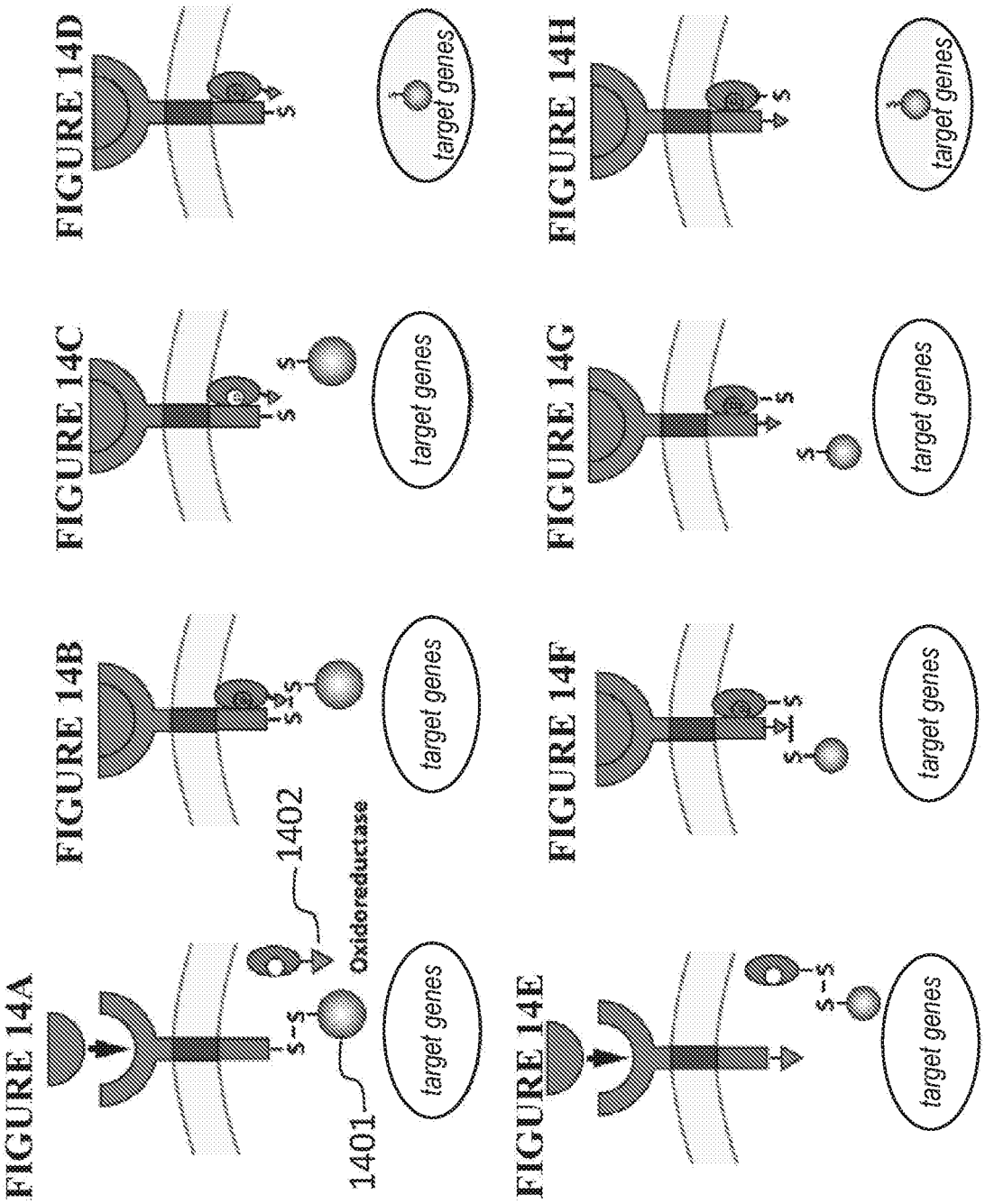
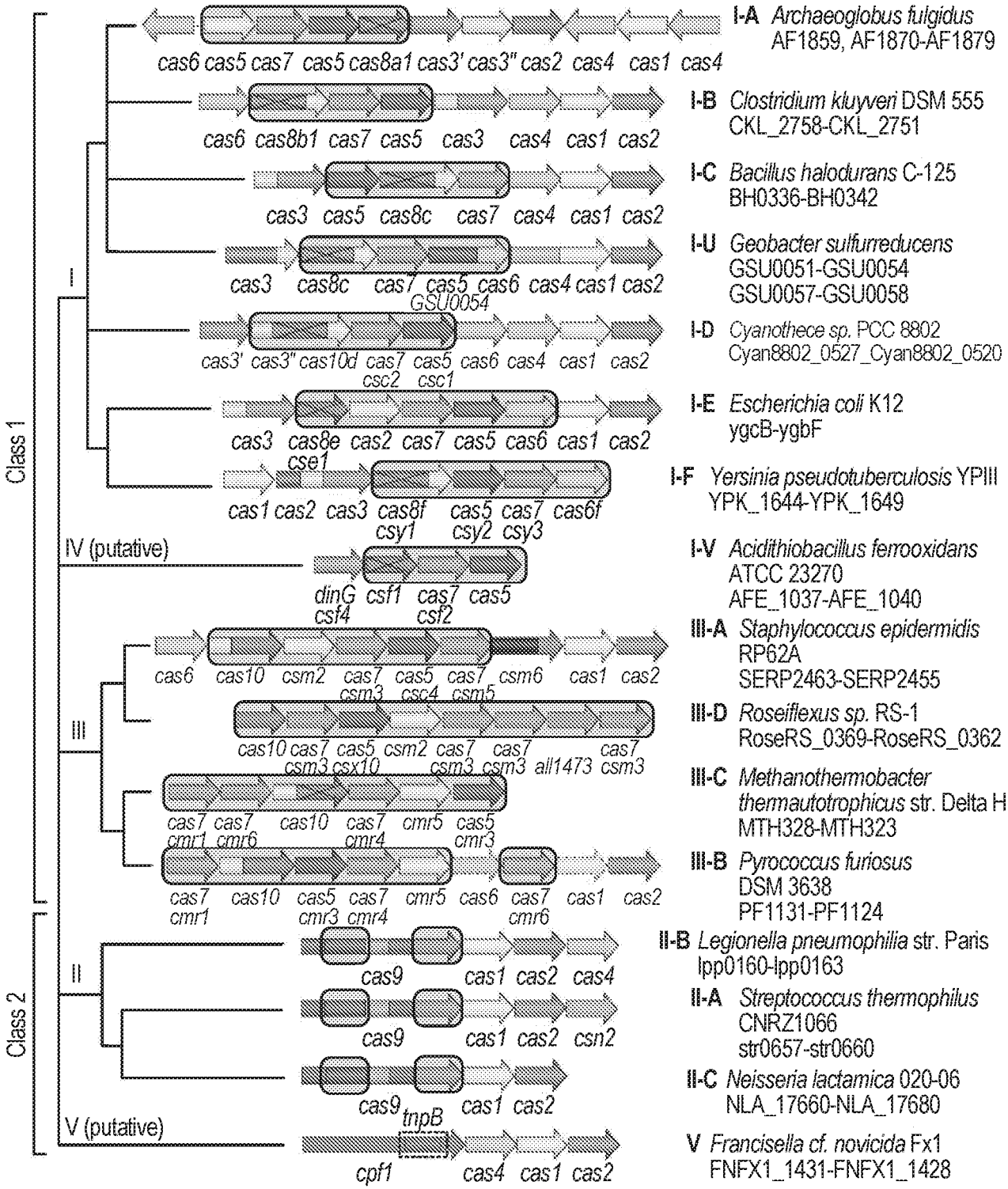
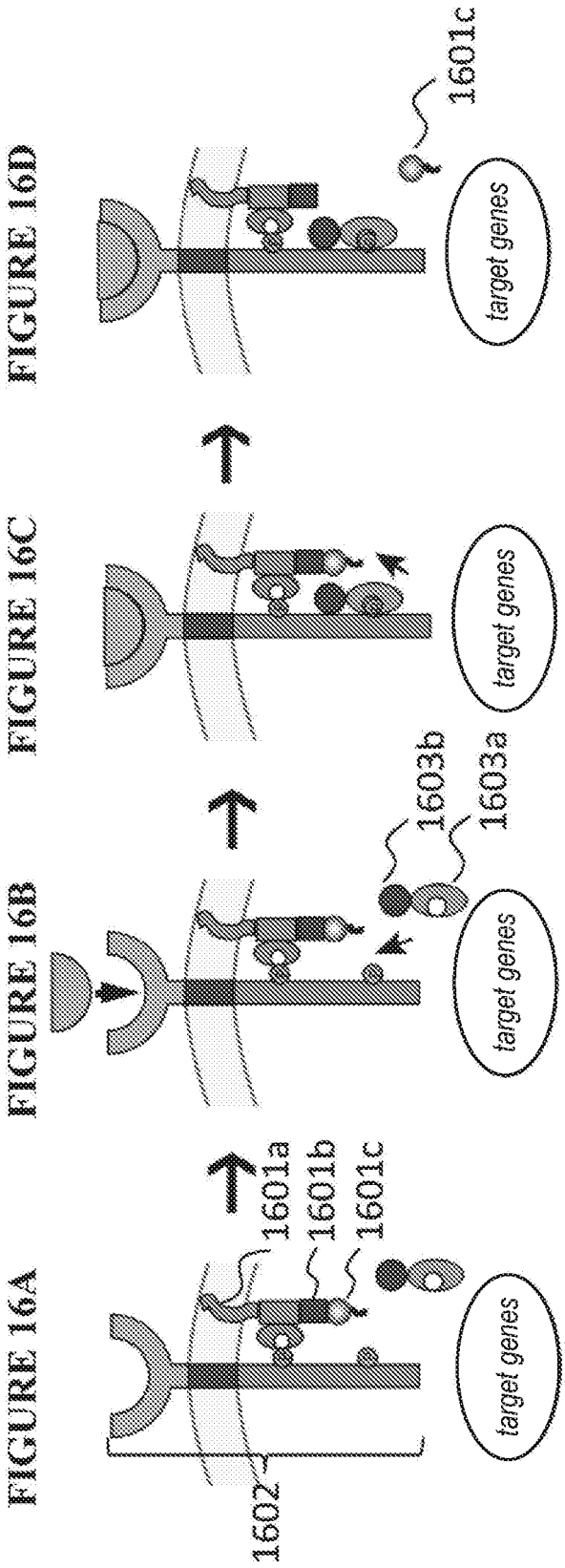

FIGURE 14A
1401
1402
Oxidoreductase
target genes
FIGURE 14B
target genes
FIGURE 14C
target genes
FIGURE 14D
target genes
FIGURE 14E
target genes
FIGURE 14F
target genes
FIGURE 14G
target genes
FIGURE 14H
target genes

FIGURE 15


Class 1
Class 2
I
III
II
IV (putative)
V (putative)
cas6 cas5 cas7 cas5 cas8a1 cas3' cas3" cas2 cas4 cas1 cas4
I-A Archaeoglobus fulgidus AF1859, AF1870-AF1879
cas6 cas8b1 cas7 cas5 cas3 cas4 cas1 cas2
I-B Clostridium kluyveri DSM 555 CKL_2758-CKL_2751
cas3 cas5 cas8c cas7 cas4 cas1 cas2
I-C Bacillus halodurans C-125 BH0336-BH0342
cas3 cas8c cas7 cas5 cas6 cas4 cas1 cas2
GSU0054
I-U Geobacter sulfurreducens GSU0051-GSU0054 GSU0057-GSU0058
cas3' cas3" cas10d cas7 cas5 cas6 cas4 cas1 cas2
csc2 csc1
I-D Cyanothece sp. PCC 8802 Cyan8802_0527_Cyan8802_0520
cas3 cas8e cas2 cas7 cas5 cas6 cas1 cas2
cse1
I-E Escherichia coli K12 ygcB-ygbF
cas1 cas2 cas3 cas8f cas5 cas7 cas6f
csy1 csy2 csy3
I-F Yersinia pseudotuberculosis YPIII YPK_1644-YPK_1649
dinG csf1 cas7 cas5
csf4 csf2
I-V Acidithiobacillus ferrooxidans ATCC 23270 AFE_1037-AFE_1040
cas6 cas10 csm2 cas7 cas5 cas7 csm6 cas1 cas2
csm3 csc4 csm5
III-A Staphylococcus epidermidis RP62A SERP2463-SERP2455
cas10 cas7 cas5 csm2 cas7 cas7 cas7
csm3 csx10 csm3 csm3 all1473 csm3
III-D Roseiflexus sp. RS-1 RoseRS_0369-RoseRS_0362
cas7 cas7 cas10 cas7 cmr5 cas5
cmr1 cmr6 cmr4 cmr3
III-C Methanothermobacter thermautotrophicus str. Delta H MTH328-MTH323
cas7 cas10 cas5 cas7 cmr5 cas6 cas7 cas1 cas2
cmr1 cmr3 cmr4 cmr6
III-B Pyrococcus furiosus DSM 3638 PF1131-PF1124
cas9 cas1 cas2 cas4
II-B Legionella pneumophilia str. Paris lpp0160-lpp0163
cas9 cas1 cas2 csn2
II-A Streptococcus thermophilus CNRZ1066 str0657-str0660
cas9 cas1 cas2
II-C Neisseria lactamica 020-06 NLA_17660-NLA_17680
cas9 tnpB cas1 cas2
cpf1 cas4 cas1 cas2
V Francisella cf. novicida Fx1 FNFX1_1431-FNFX1_1428


FIGURE 16A
FIGURE 16B
FIGURE 16C
FIGURE 16D
1601a
1601b
1601c
1602
1603b
1603a
target genes
target genes
target genes
target genes
1601c

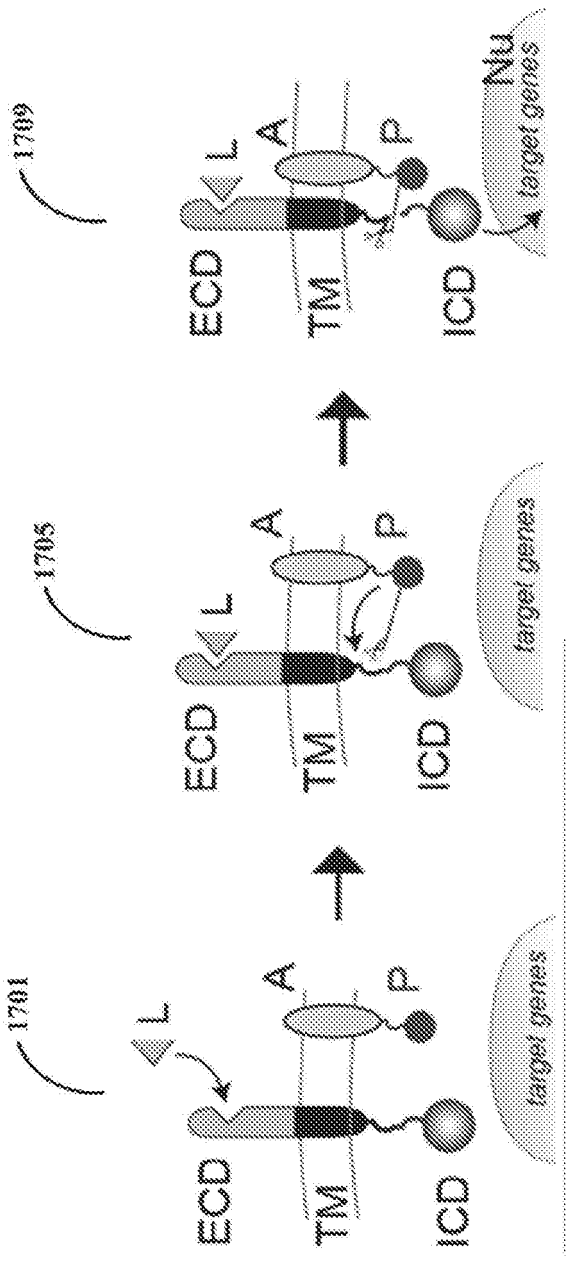

1701
L
A
P
ECD
TM
ICD
target genes
1705
L
A
P
ECD
TM
ICD
target genes
1709
L
A
P
ECD
TM
ICD
Nu
target genes

FIGURE 17

FIGURE 18

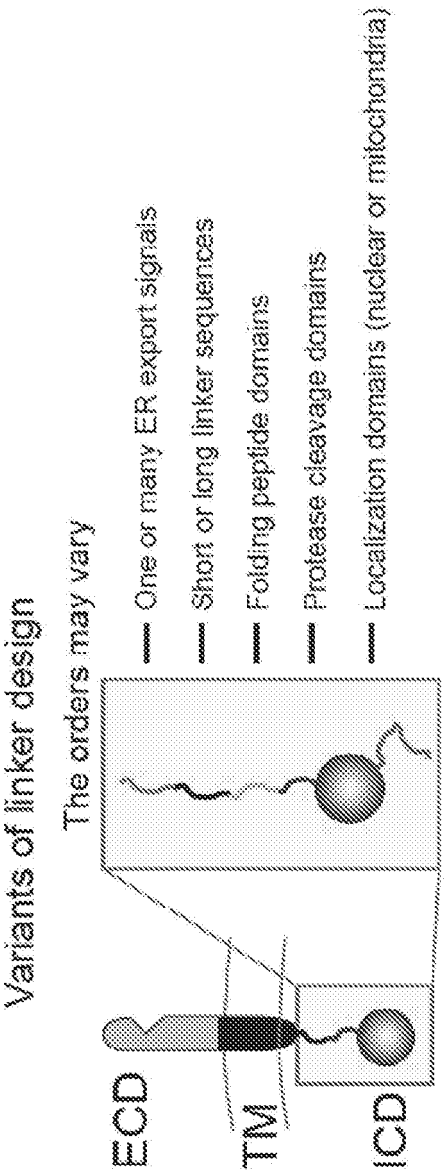

Variants of linker design
The orders may vary
One or many ER export signals
Short or long linker sequences
Folding peptide domains
Protease cleavage domains
Localization domains (nuclear or mitochondria)
ECD
TM
ICD

FIGURE 19A

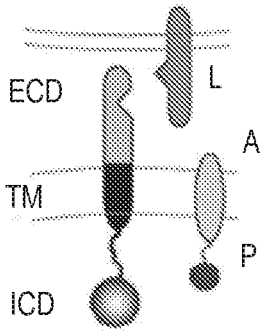


FIGURE 19B

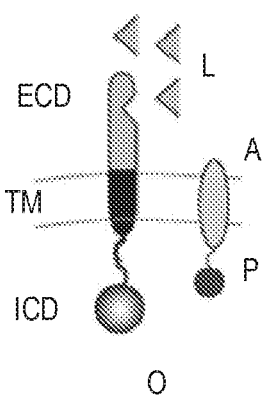


FIGURE 19C

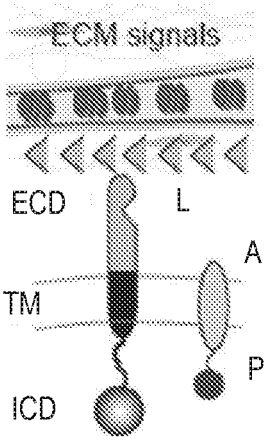


FIGURE 19D

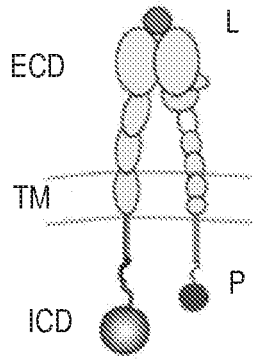


FIGURE19E

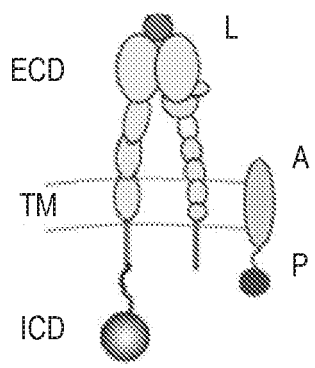
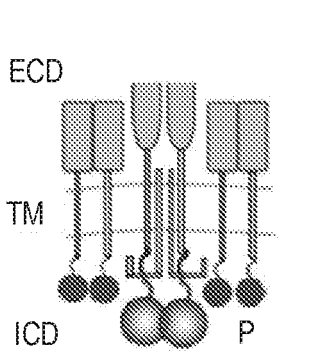


FIGURE 19F



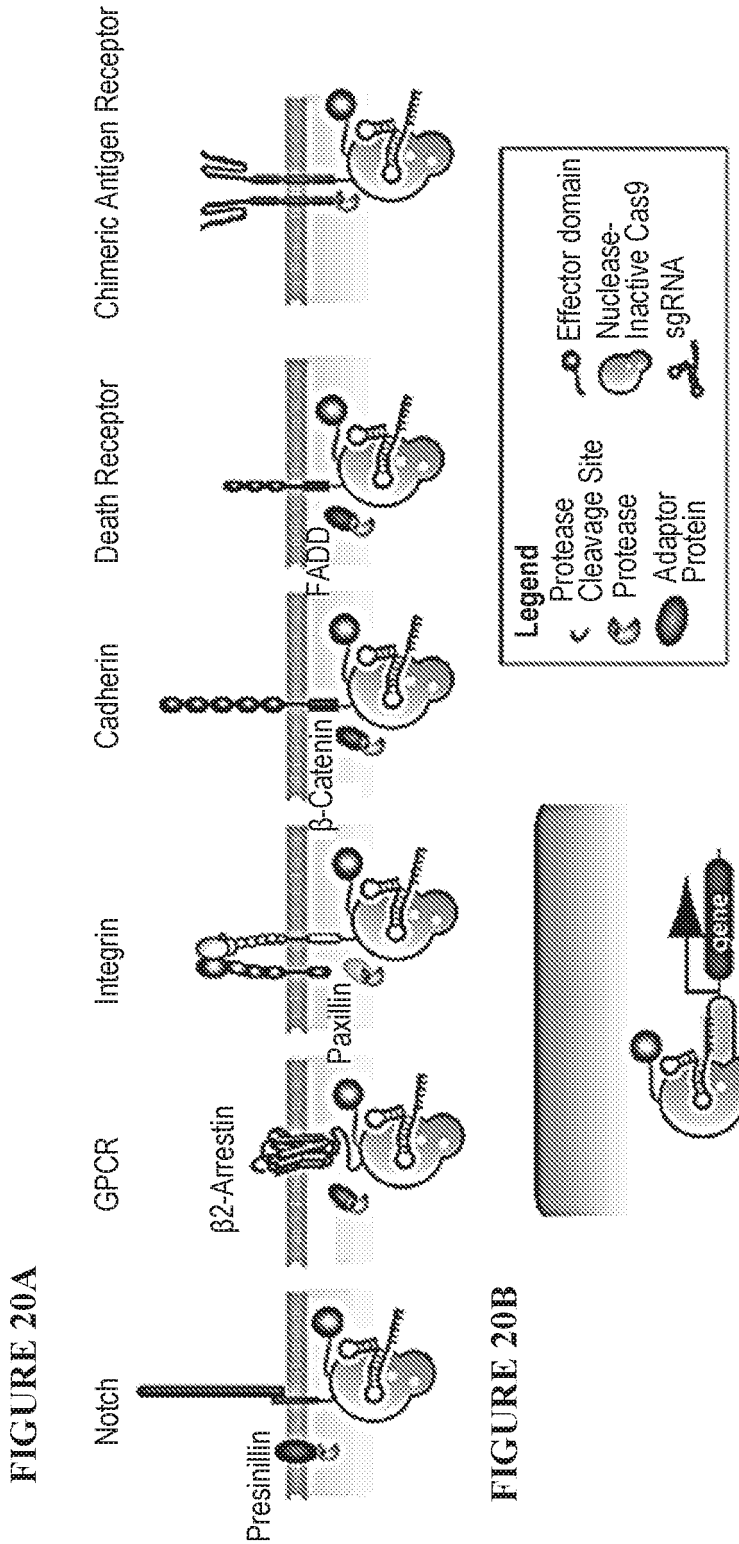


FIGURE 20A

FIGURE 20B

FIGURE 21A

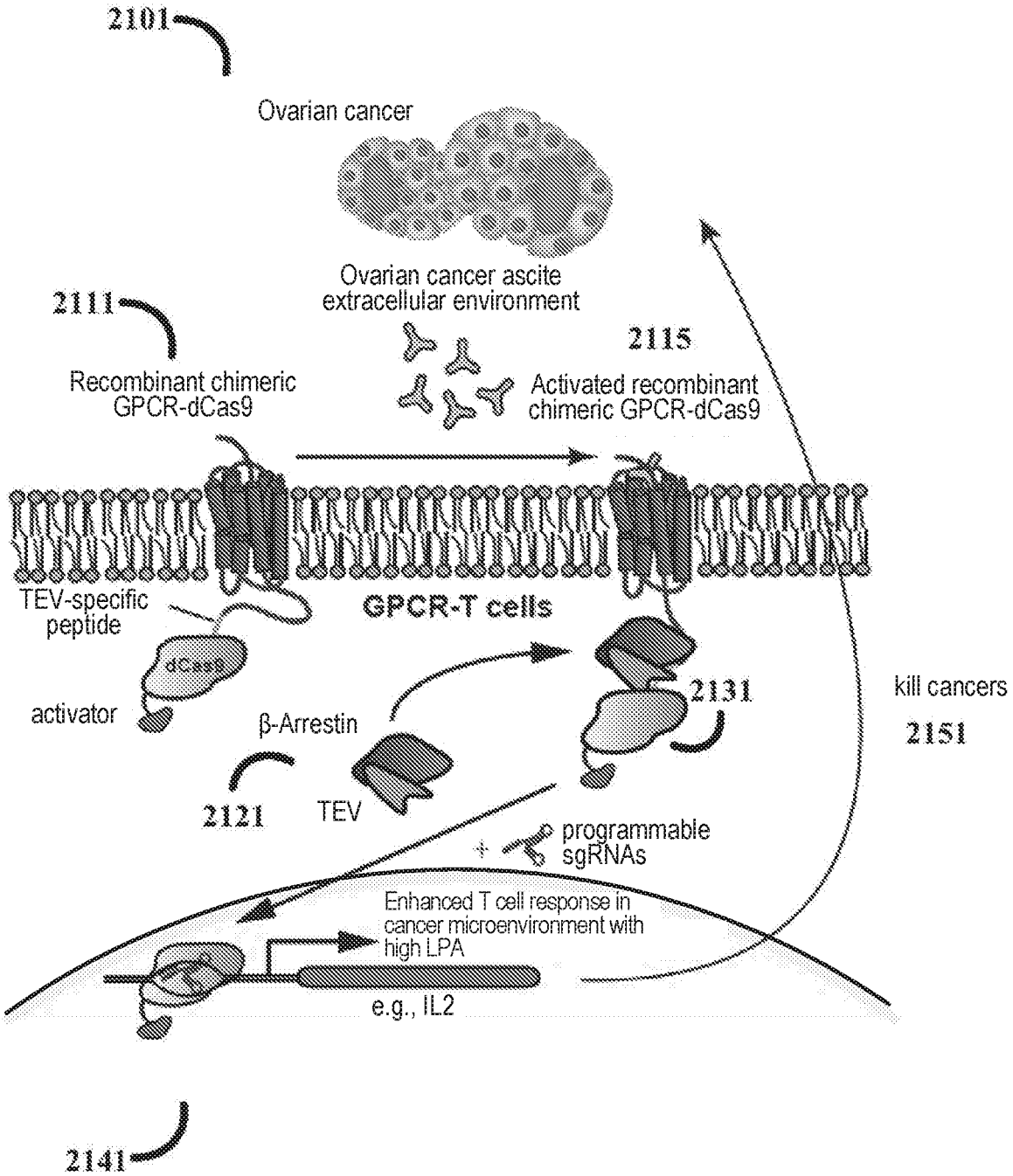

2101
Ovarian cancer
Ovarian cancer ascite extracellular environment
2111
Recombinant chimeric GPCR-dCas9
2115
Activated recombinant chimeric GPCR-dCas9
TEV-specific peptide
GPCR-T cells
dCas9
activator
β-Arrestin
2121
TEV
2131
kill cancers
2151
programmable sgRNAs
Enhanced T cell response in cancer microenvironment with high LPA
e.g., IL2
2141

FIGURE 21B

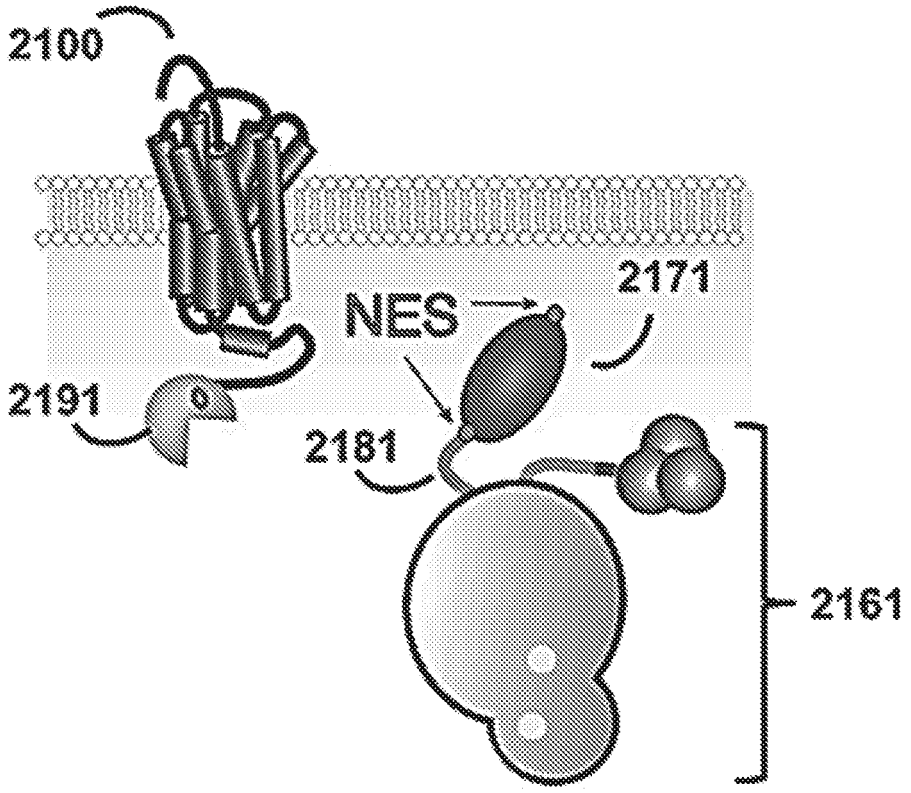


FIGURE 22A

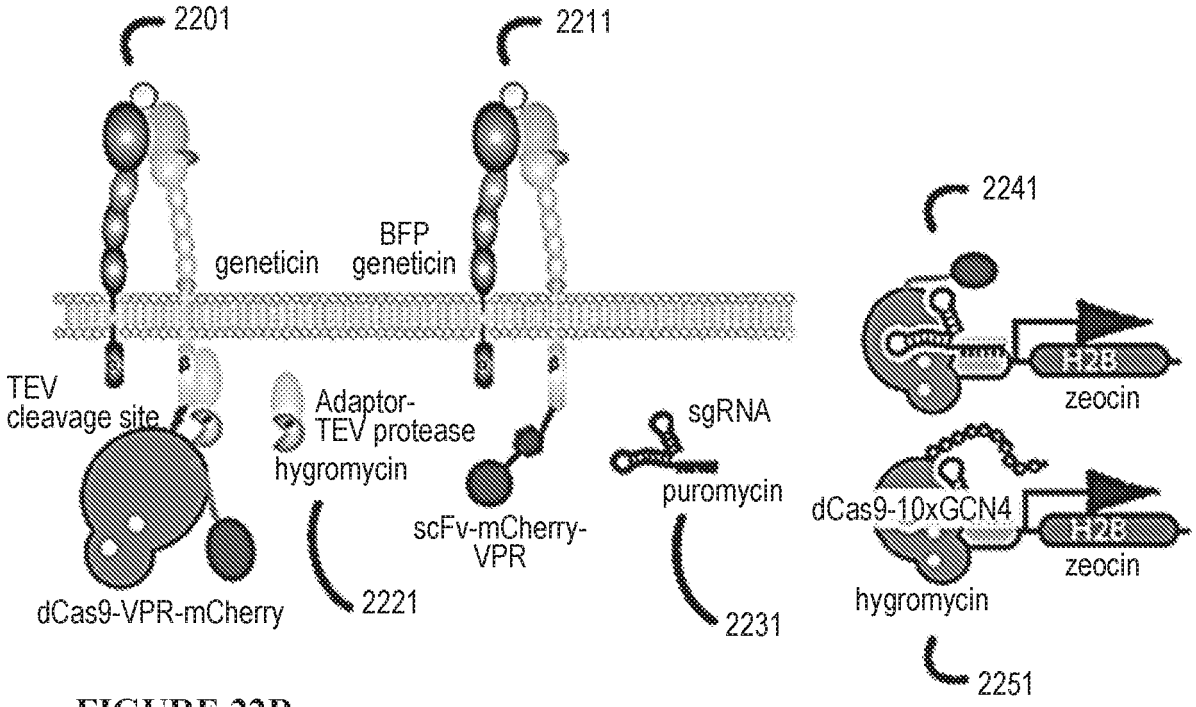


FIGURE 22B

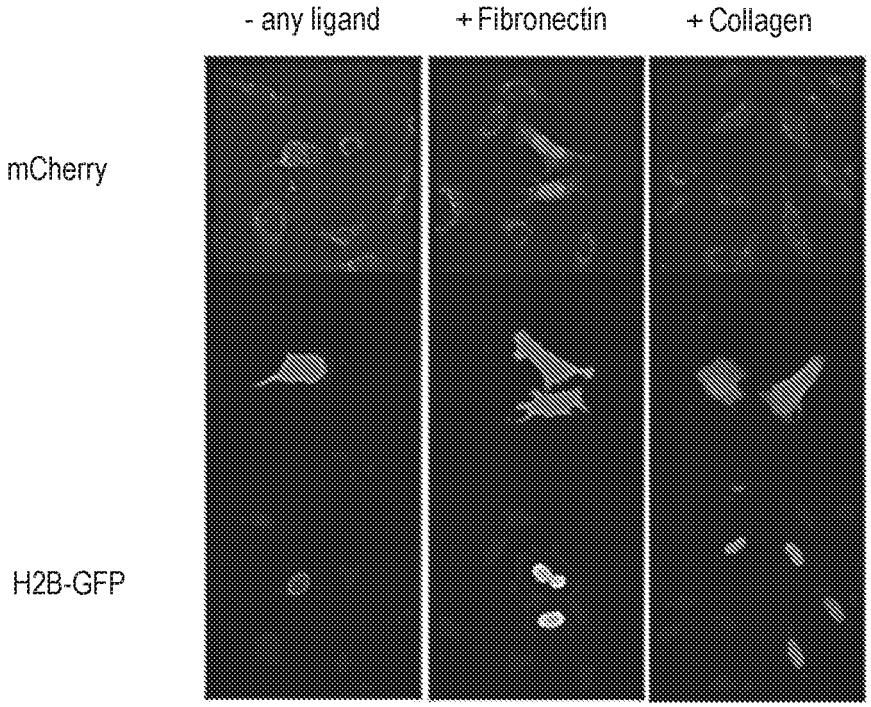


FIGURE 22C

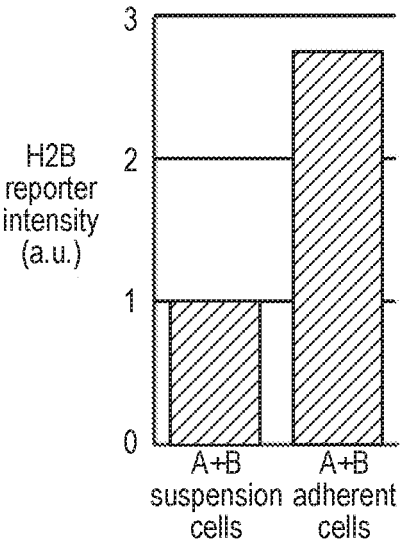


FIGURE 22D

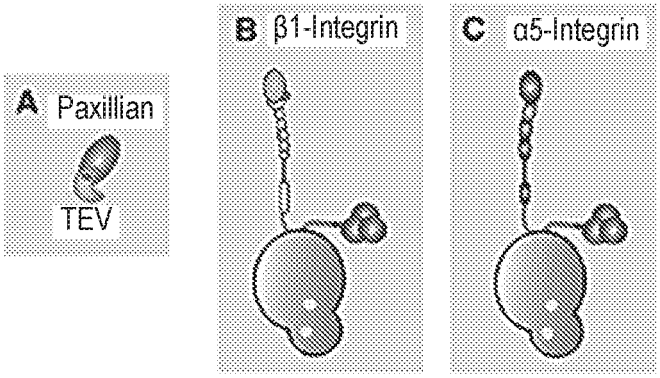


FIGURE 22E

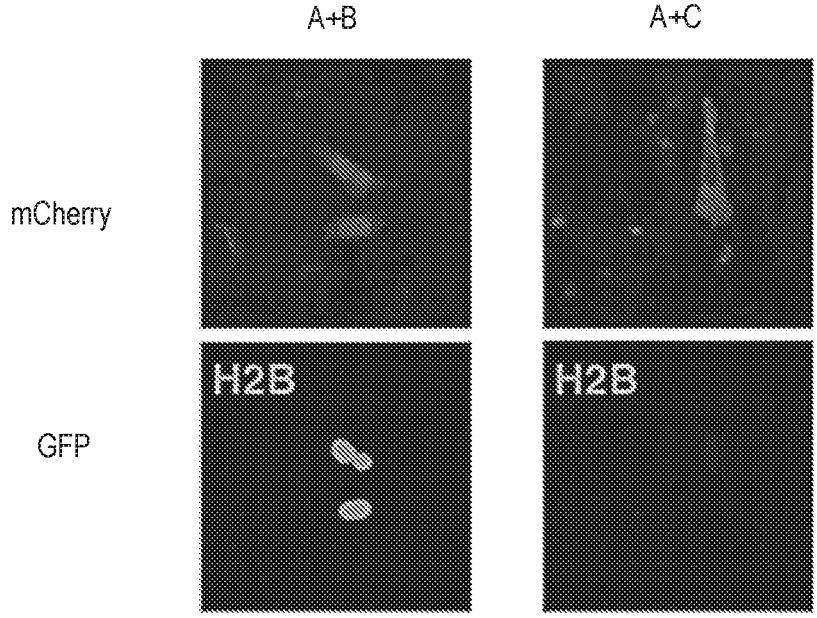


FIGURE 23A

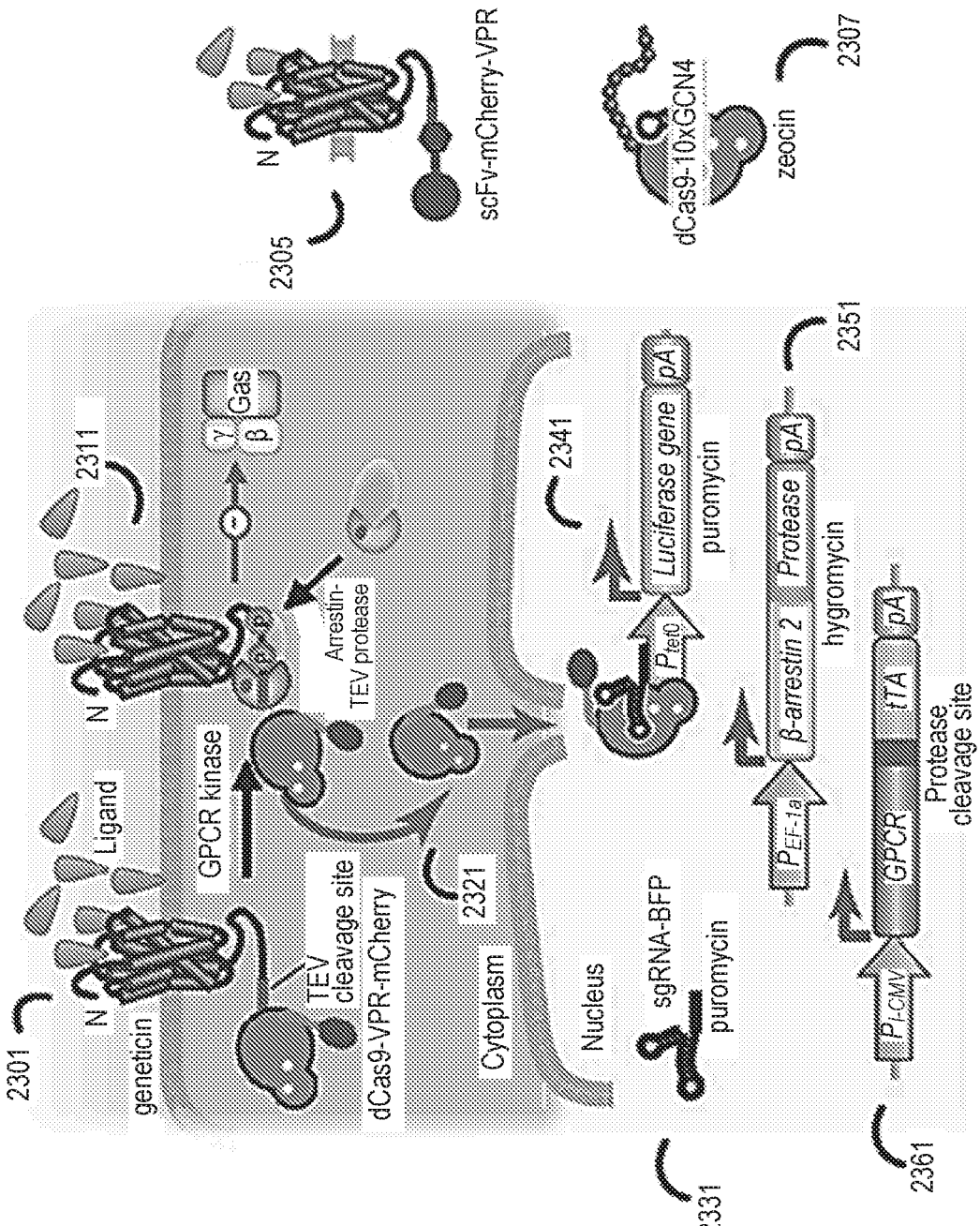

2301
N
Ligand
geneticin
GPCR kinase
TEV cleavage site
dCas9-VPR-mCherry
2311
N
Arrestin-TEV protease
γ
Gas
β
2321
Cytoplasm
Nucleus
2341
Luciferase gene
pA
puromycin
sgRNA-BFP
puromycin
2331
PTet0
PEF-1a
β-arrestin 2
Protease
pA
hygromycin
2351
PLCMV
GPCR
tTA
pA
Protease cleavage site
2361
2305
N
scFv-mCherry-VPR
dCas9-10xGCN4
zeocin
2307

FIGURE 23B

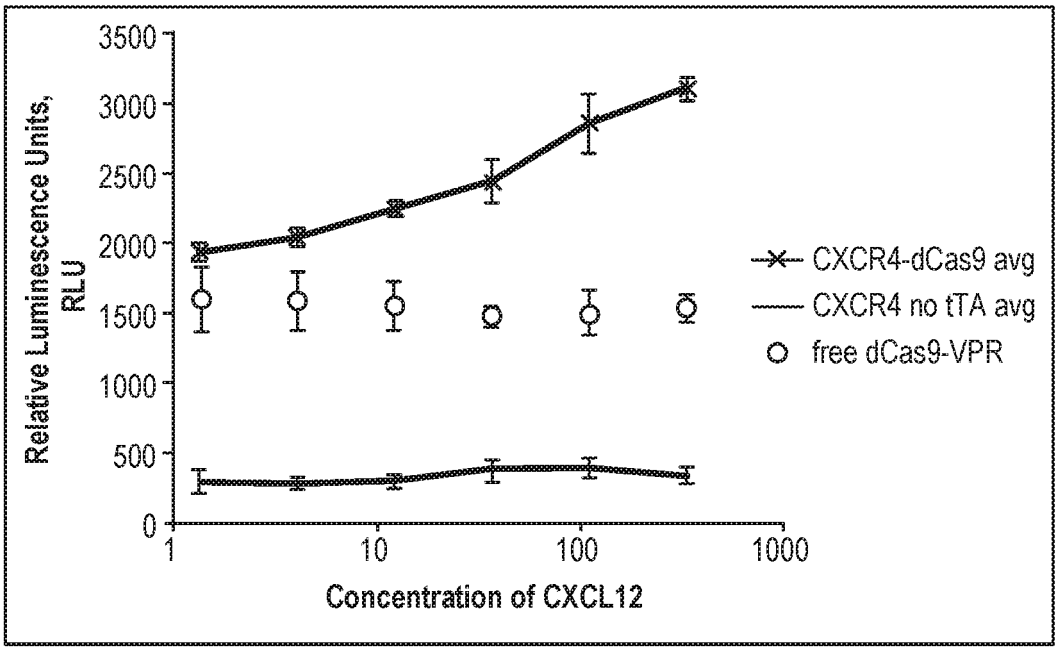

Relative Luminescence Units, RLU
3500
3000
2500
2000
1500
1000
500
0
1
10
100
1000
Concentration of CXCL12
CXCR4-dCas9 avg
CXCR4 no tTA avg
free dCas9-VPR

FIGURE 23C

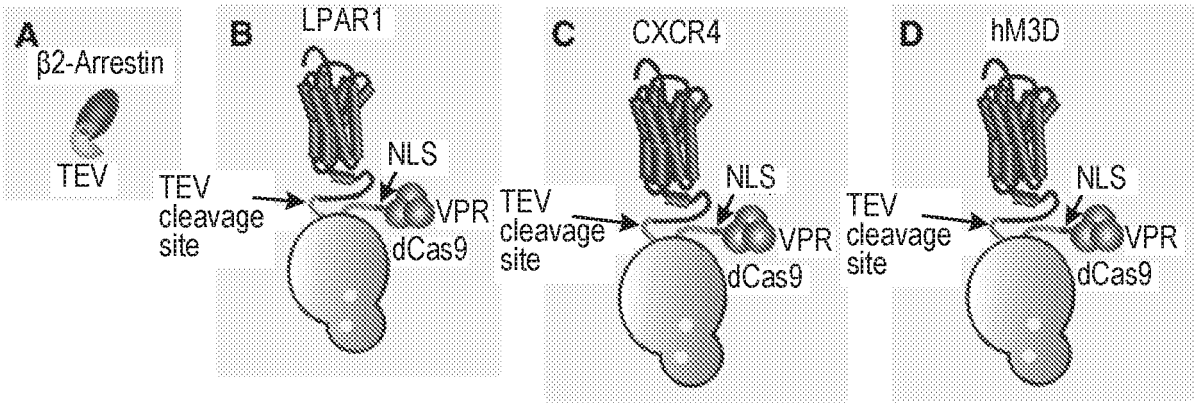


FIGURE 23D

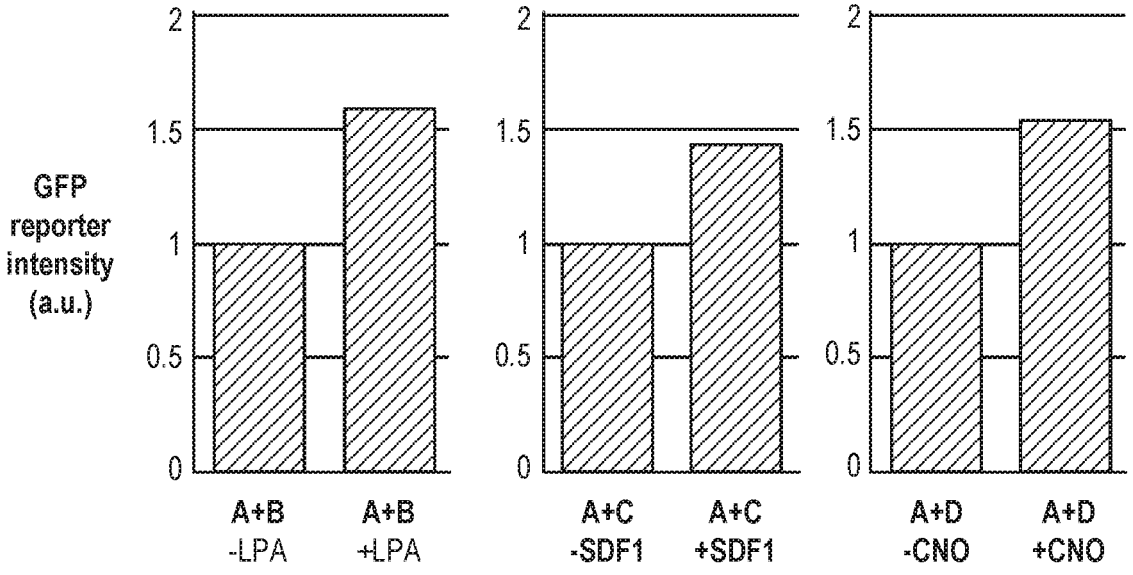


FIGURE 23E

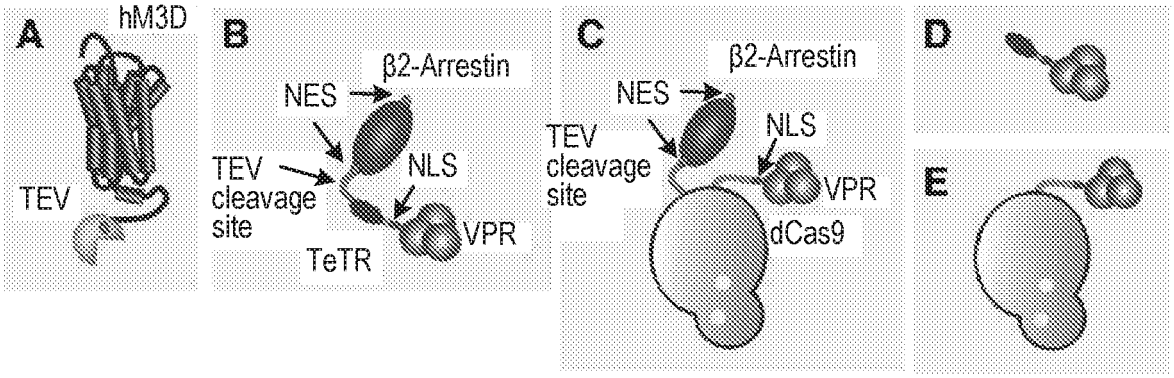
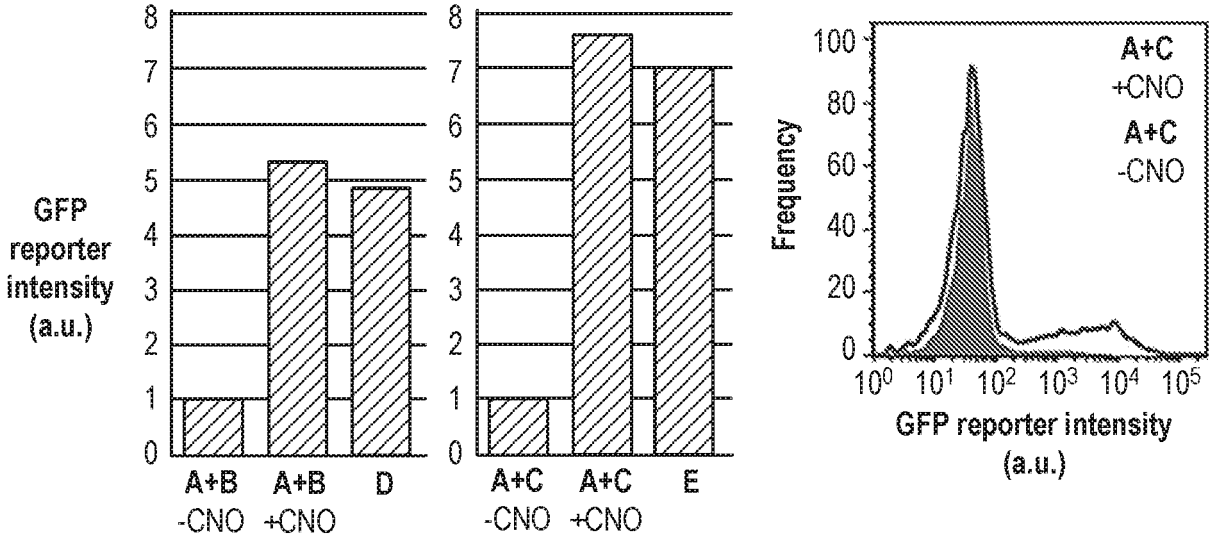


FIGURE 23F



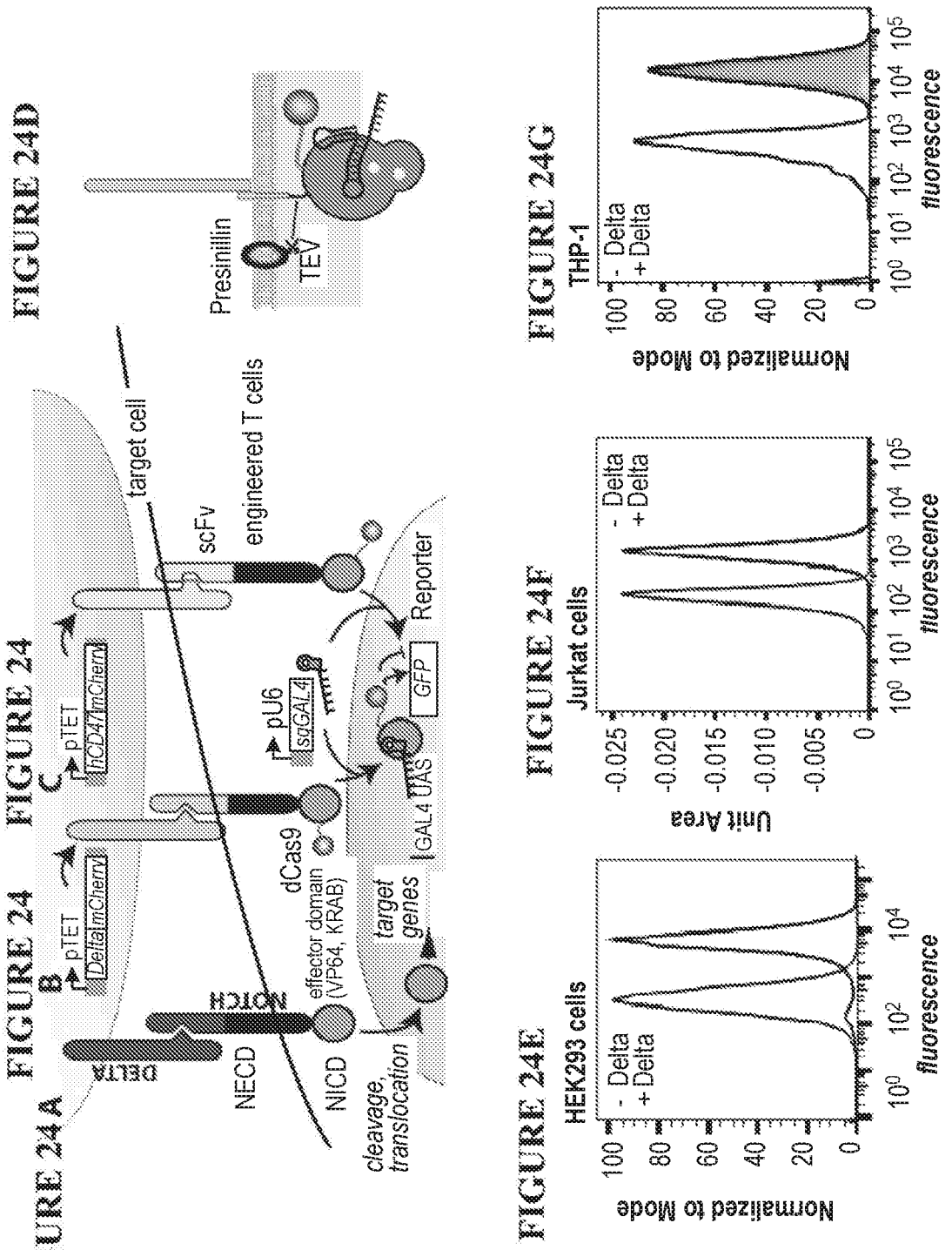
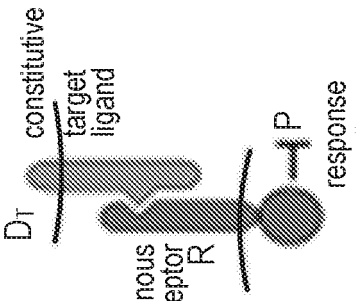

FIGURE 24A
FIGURE 24
B
C
pTET
DeltaImCherry
NOTCH
DELTA
NECD
NICD
effector domain
(VP64, KRAB)
dCas9
pU6
sgGAL4
GAL4 UAS
target
genes
cleavage,
translocation
pTET
tnCD247mCherry
scFv
target cell
engineered T cells
Presinillin
TEV
Reporter
GFP
FIGURE 24D
FIGURE 24E
HEK293 cells
- Delta
+ Delta
Normalized to Mode
fluorescence
FIGURE 24F
Jurkat cells
Unit Area
FIGURE 24G
THP-1

FIGURE 25A



$$\frac{dR}{dt} = \beta_R - Y_R R - \frac{RD_T}{k_{RD_T}}$$

$$\frac{dP}{dt} = \frac{\alpha_p}{1 + (RD_T)^{Y_R}} - Y_R P$$

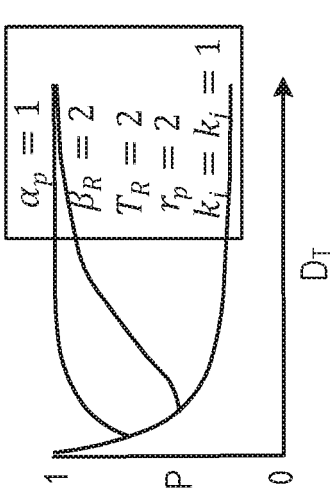
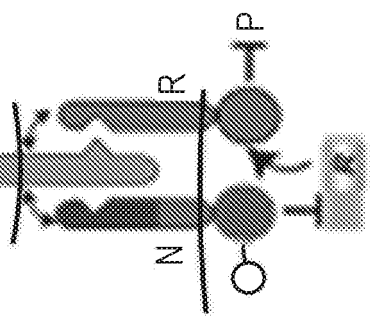


FIGURE 25B



$$\frac{dR}{dt} = \frac{\beta_R}{1 + (ND_T)^{T_R}} - Y_R R - \frac{RD_T}{k_{RD_T}}$$

$$\frac{dN}{dt} = \beta_N - Y_N N - \frac{ND_T}{k_{ND_T}}$$

$$\frac{dP}{dt} = \frac{\alpha_p}{1 + (RD_T)^{Y_R}} - Y_R P$$

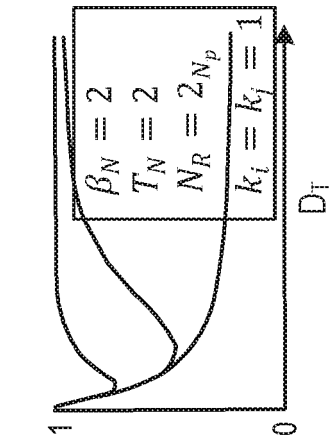
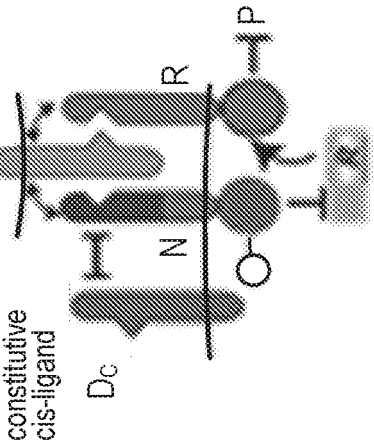


FIGURE 25C



$$\frac{dR}{dt} = \frac{\beta_R}{1 + (ND_T)^{T_R}} - Y_R R - \frac{RD_T}{k_{RD_T}}$$

$$\frac{dN}{dt} = \beta_N - Y_N N - \frac{ND_T}{k_{ND_T}} \cdot \frac{ND_C}{k_{ND_C}}$$

$$\frac{dP}{dt} = \frac{\alpha_p}{1 + (RD_T)^{Y_R}} - Y_R P$$

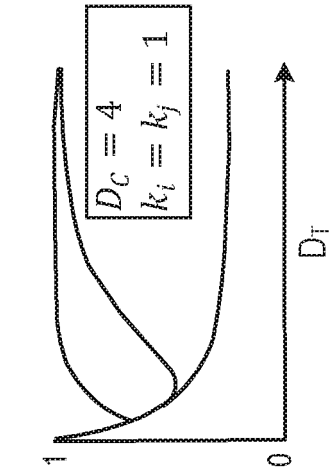


FIGURE 26A

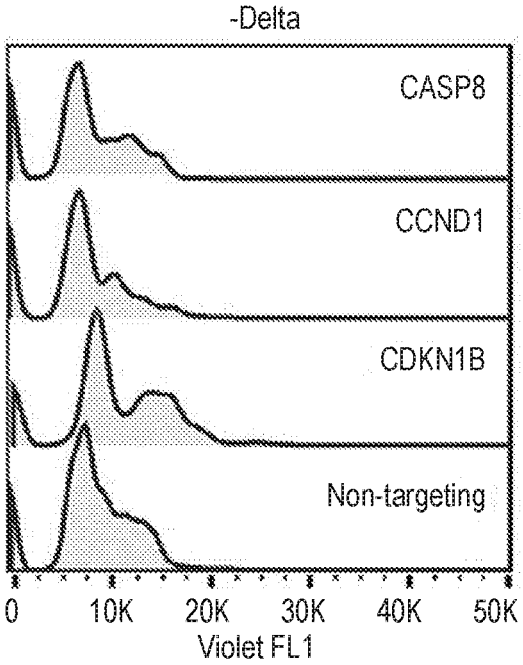


FIGURE 26B

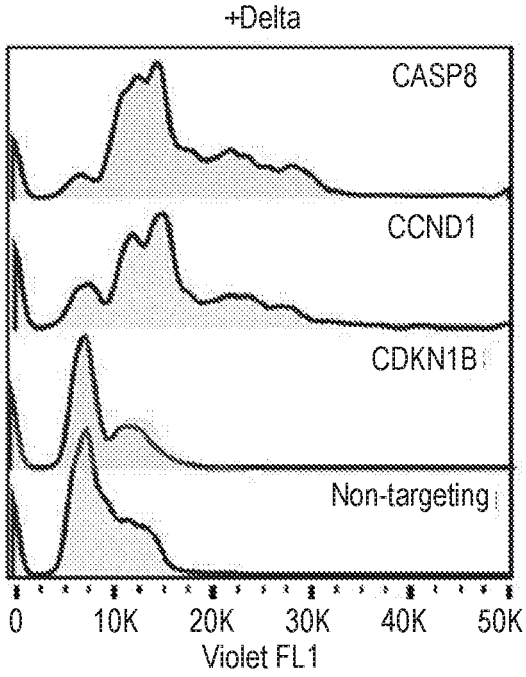


FIGURE 26C

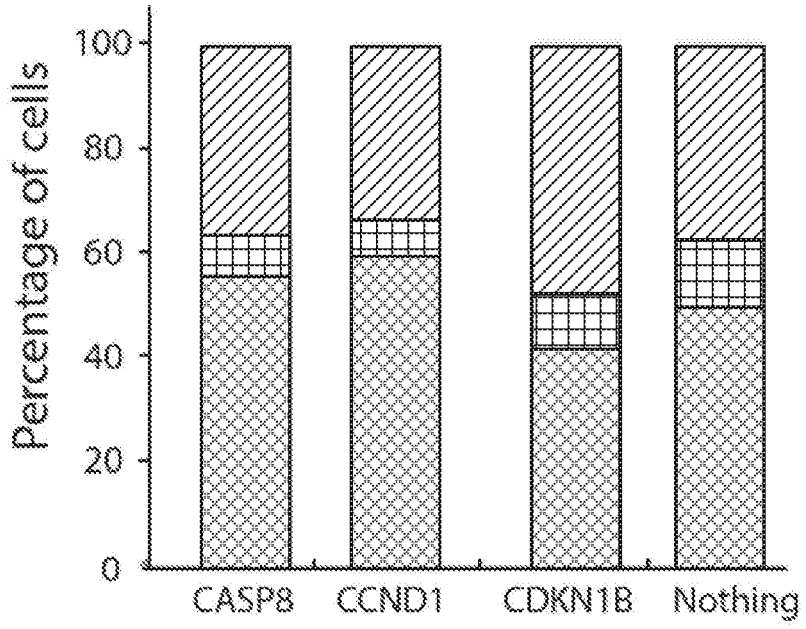


FIGURE 26D

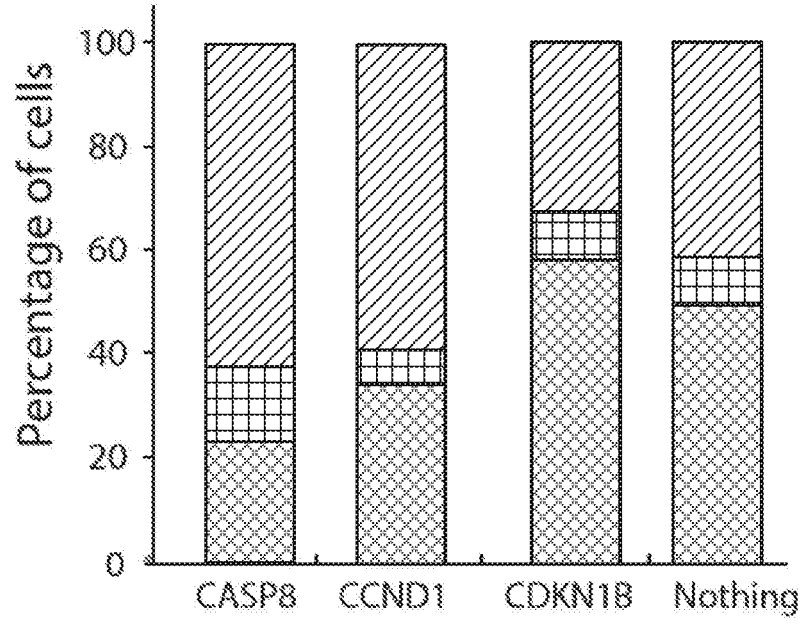


FIGURE 27A

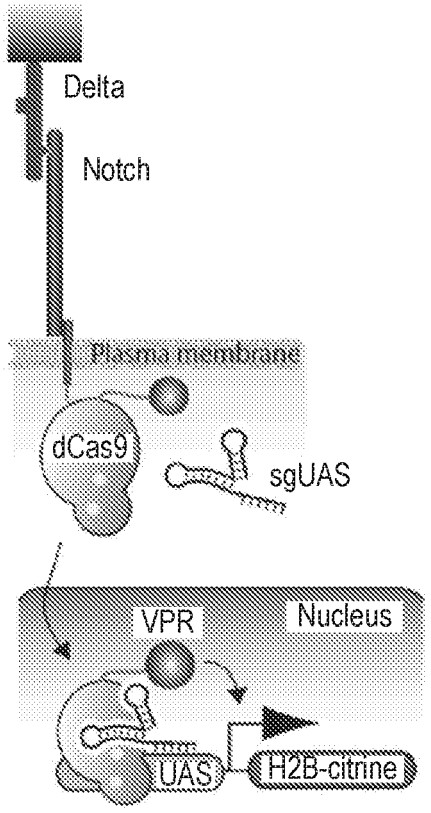


FIGURE 27B

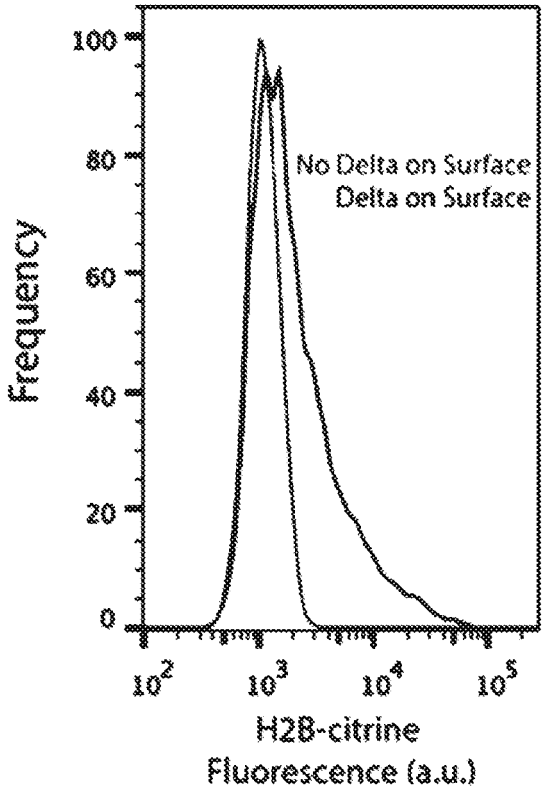


FIGURE 28A

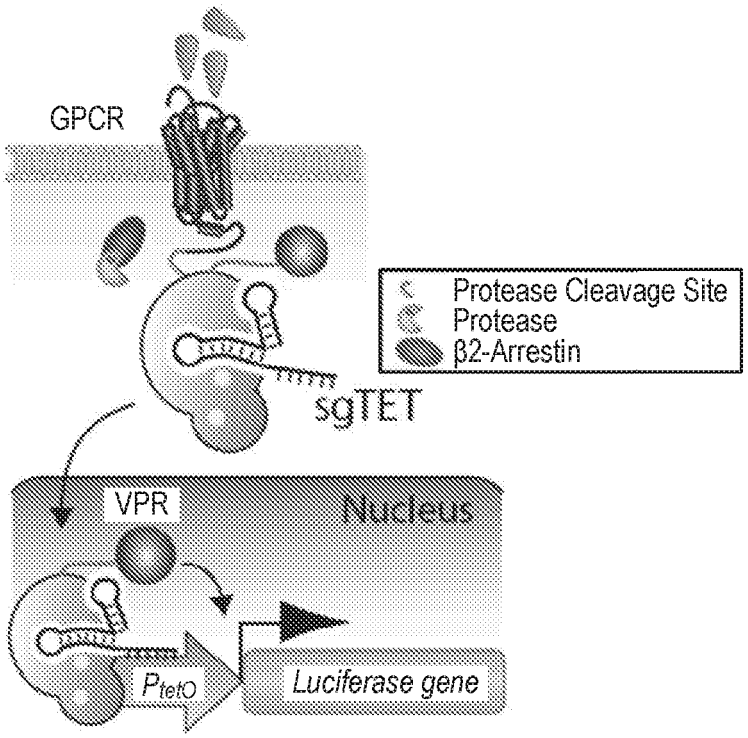


FIGURE 28B

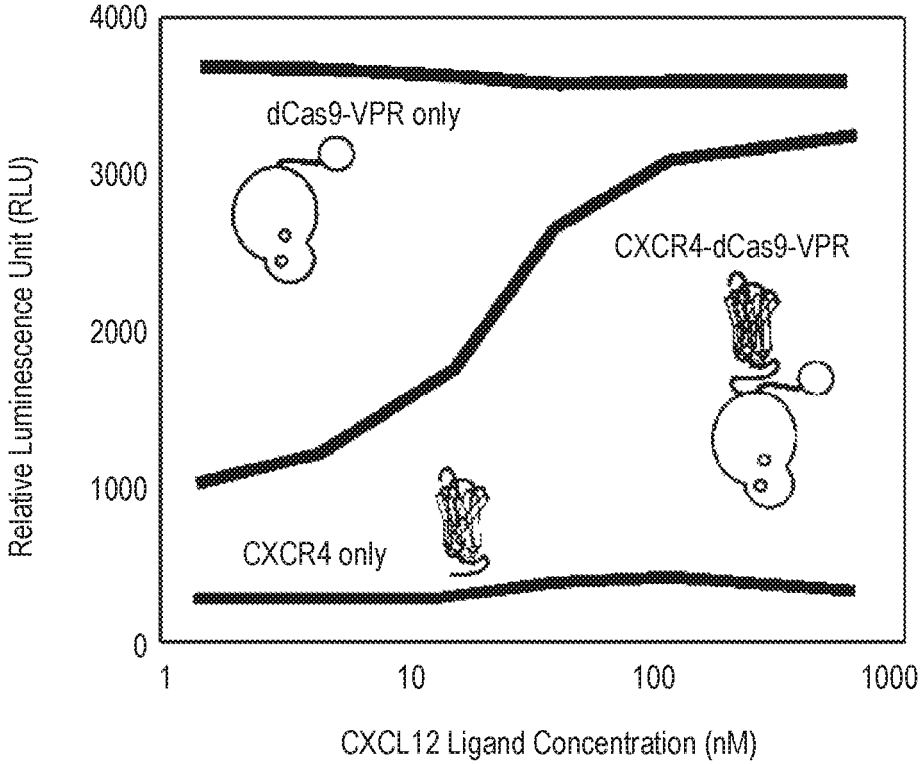


FIGURE 29A

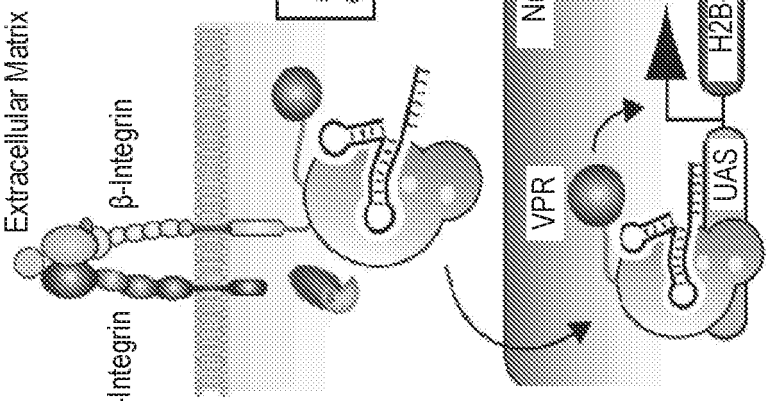


FIGURE 29B

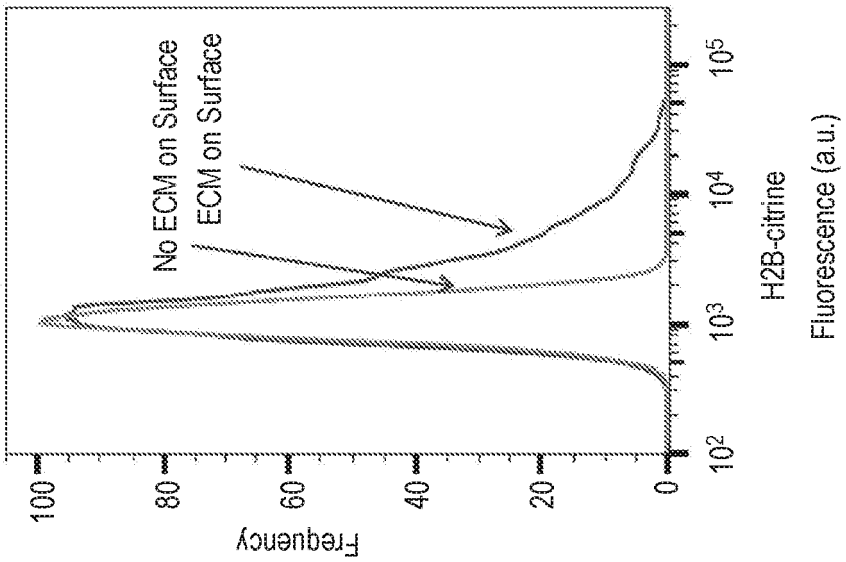


FIGURE 30A

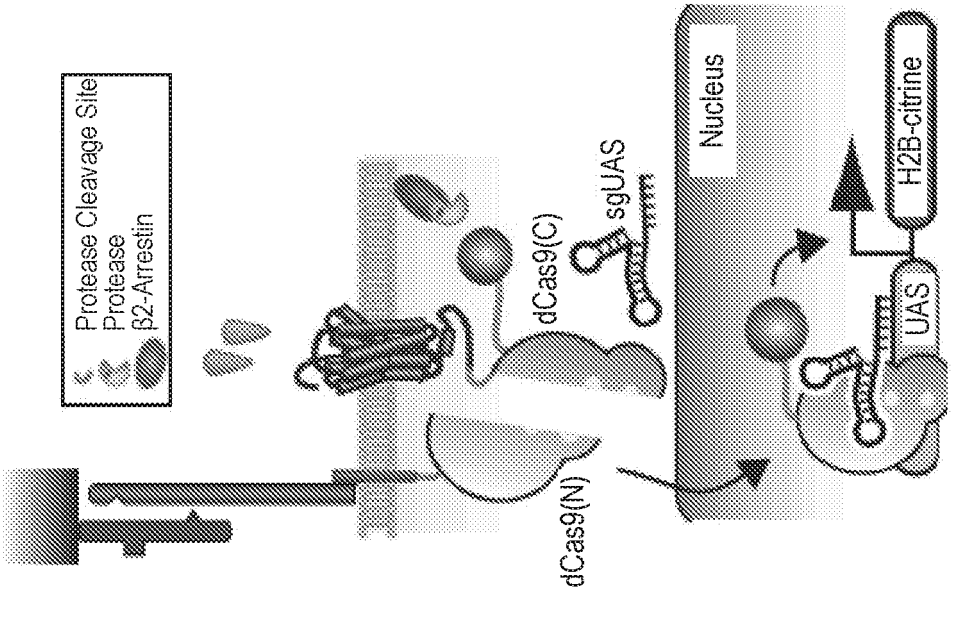


FIGURE 30B

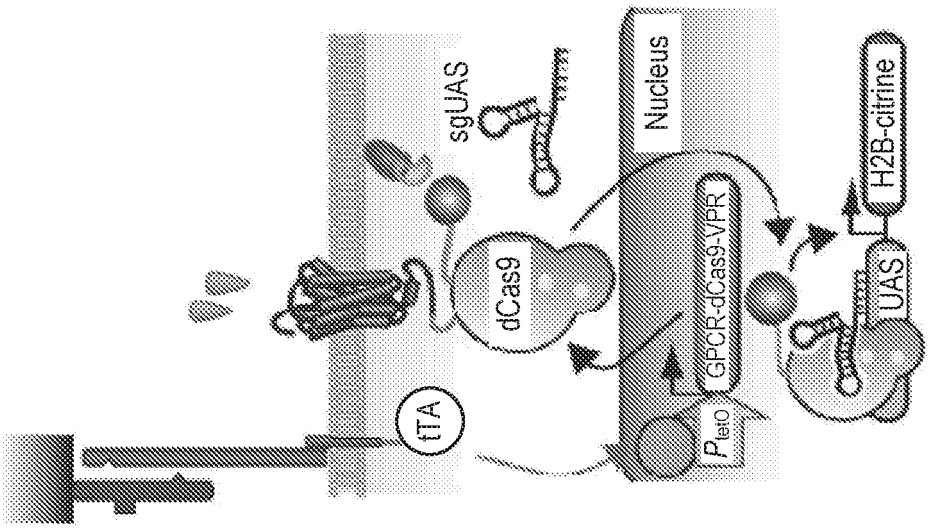


FIGURE 31A

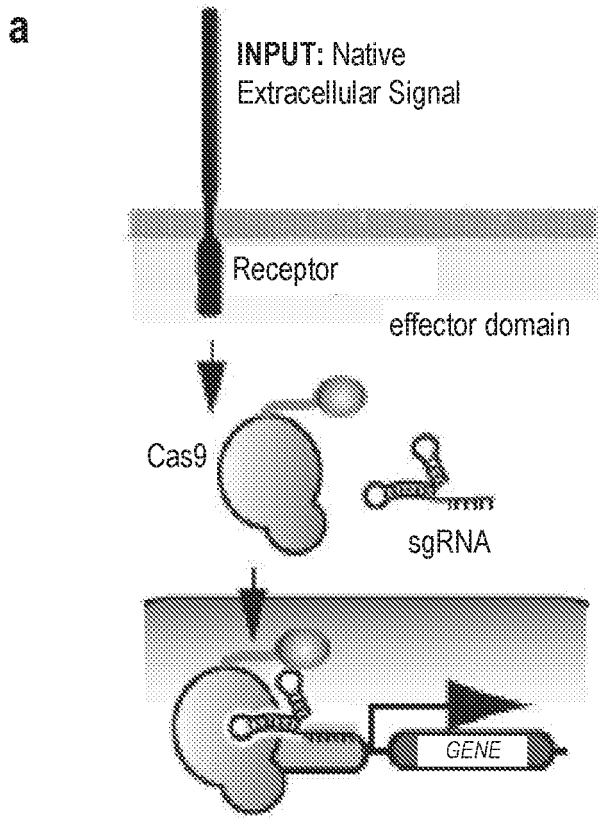

a
INPUT: Native
Extracellular Signal
Receptor
effector domain
Cas9
sgRNA
GENE
OUTPUT: Programmable Gene Regulation

FIGURE 31B

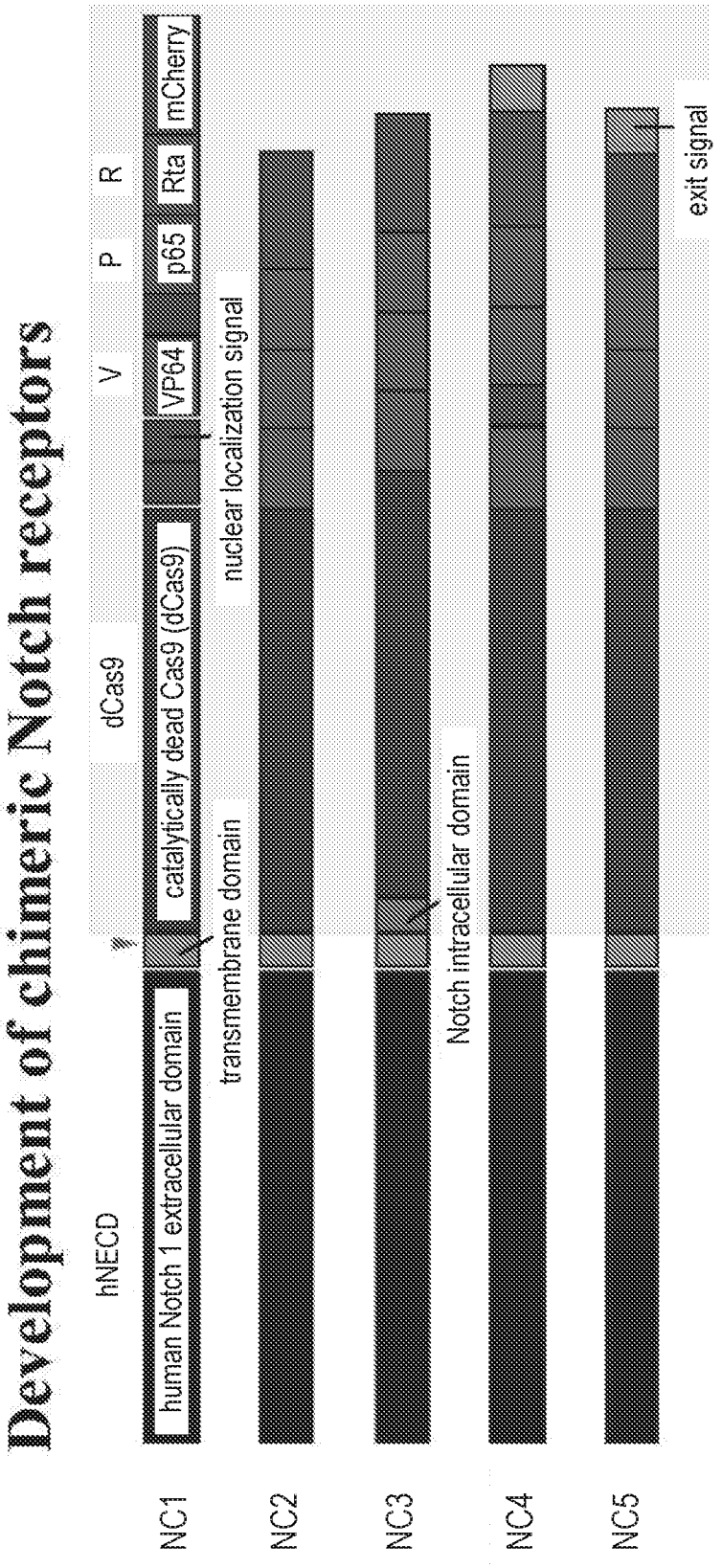

Development of chimeric Notch receptors
hNECD
dCas9
V
P
R
human Notch 1 extracellular domain
catalytically dead Cas9 (dCas9)
VP64
p65
Rta
mCherry
transmembrane domain
nuclear localization signal
Notch intracellular domain
exit signal
NC1
NC2
NC3
NC4
NC5

FIGURE 31C

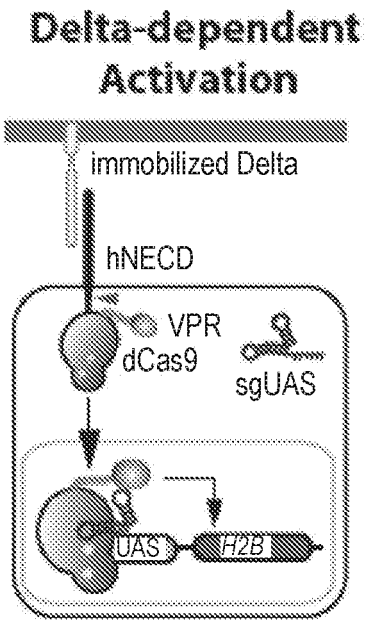


FIGURE 31D

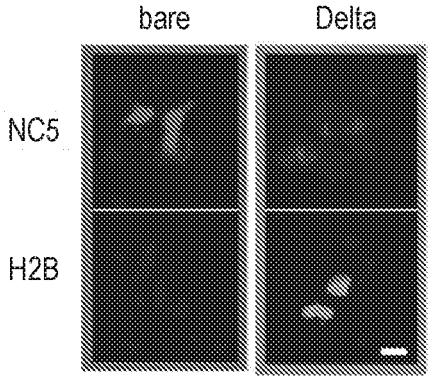


FIGURE 31E

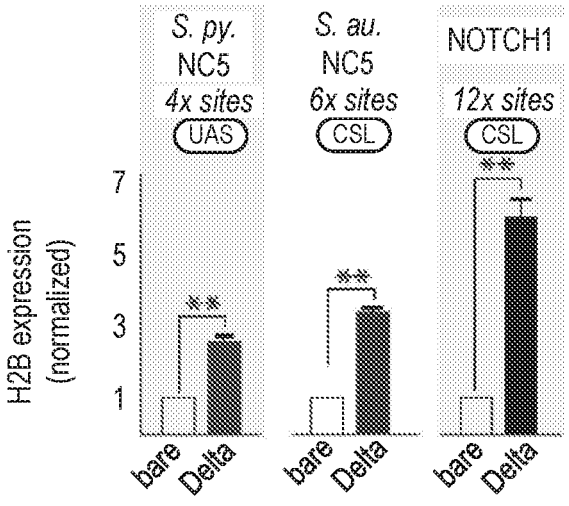


FIGURE 31F

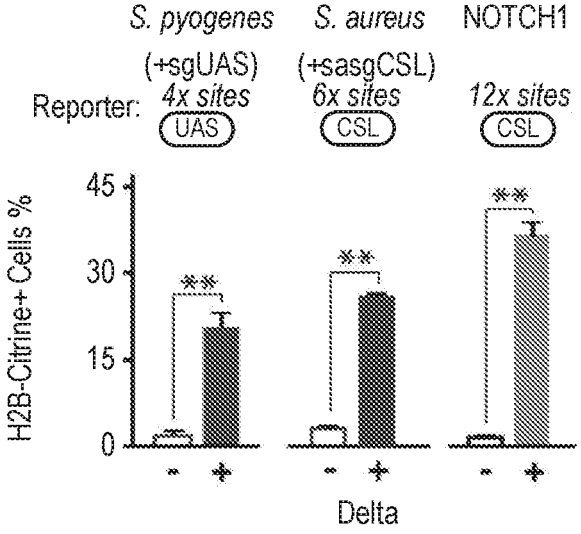


FIGURE 31G

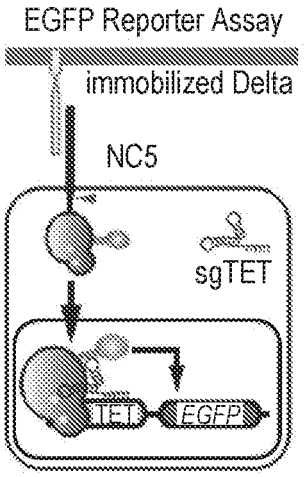


FIGURE 31H

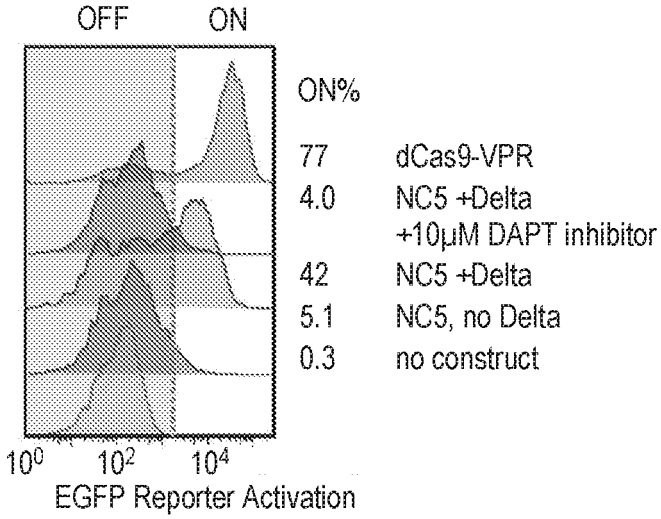


FIGURE 31I

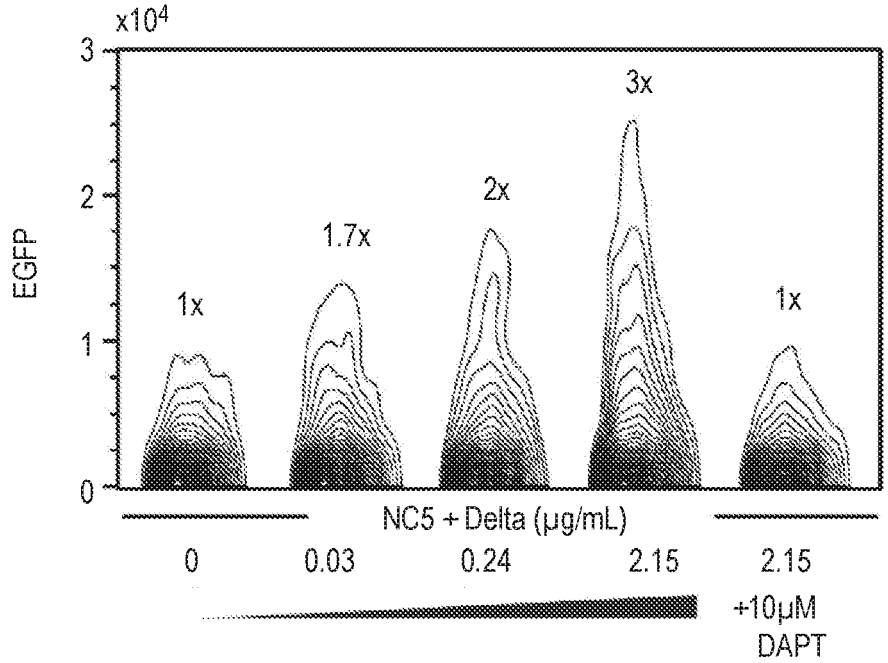


FIGURE 32A

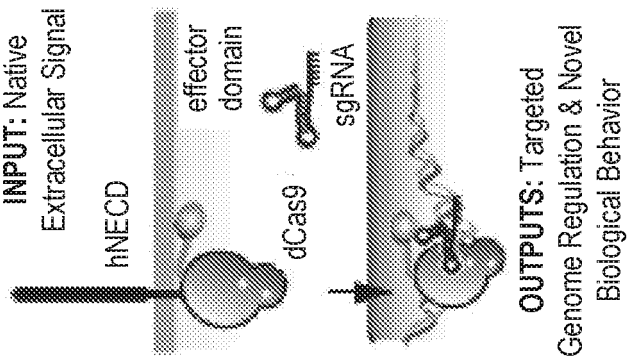


FIGURE 32B

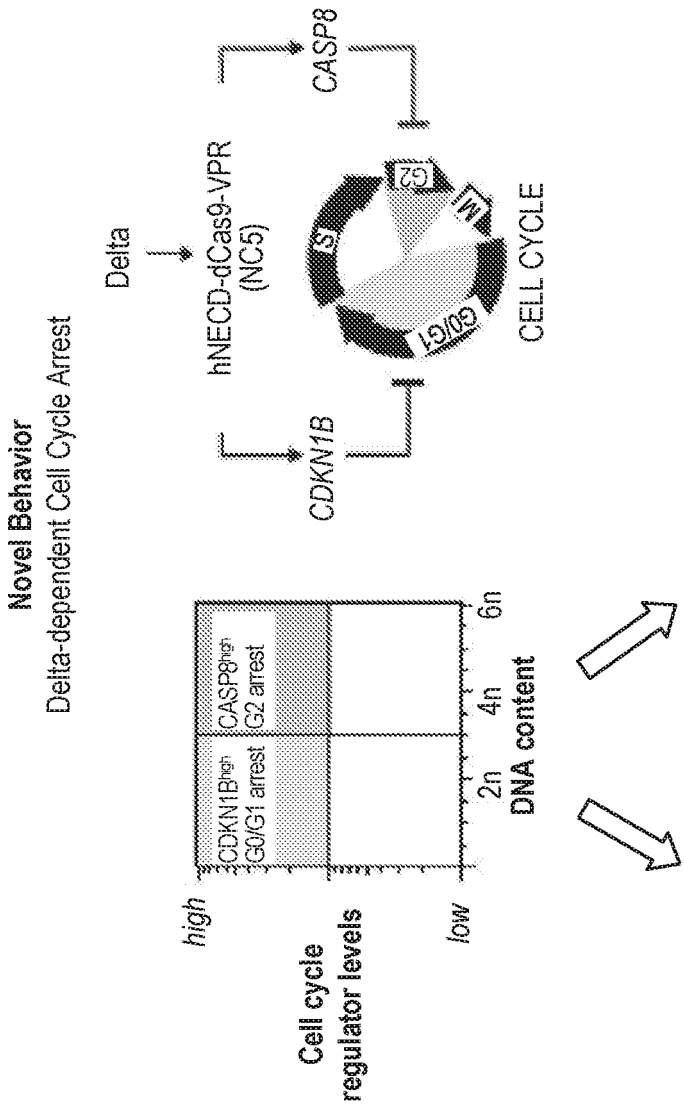


FIGURE 32C

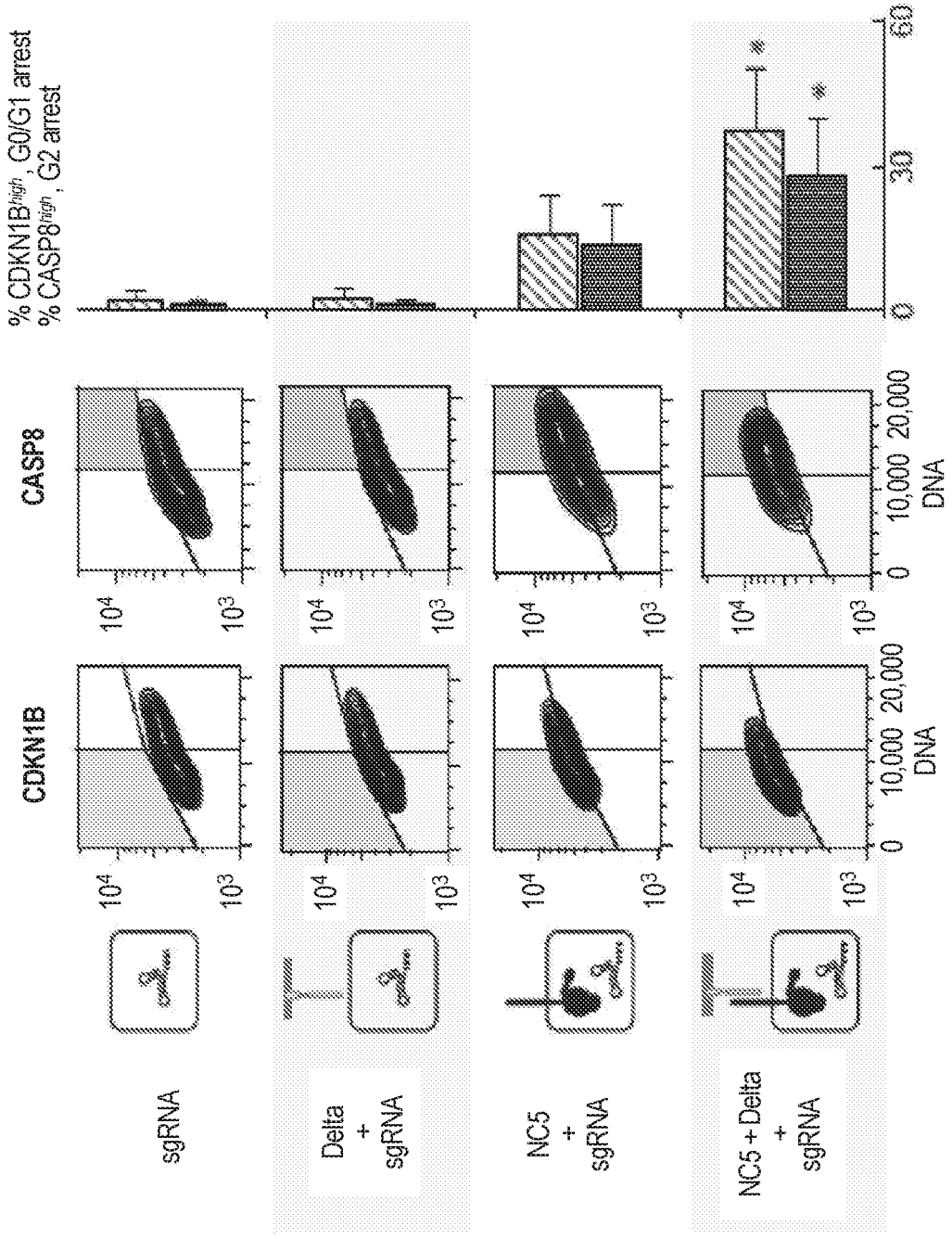

% CDKN1B^high, G0/G1 arrest
% CASP8^high, G2 arrest
CDKN1B
CASP8
sgRNA
Delta + sgRNA
NC5 + sgRNA
NC5 + Delta + sgRNA
DNA
0
30
60
10,000
20,000

FIGURE 33

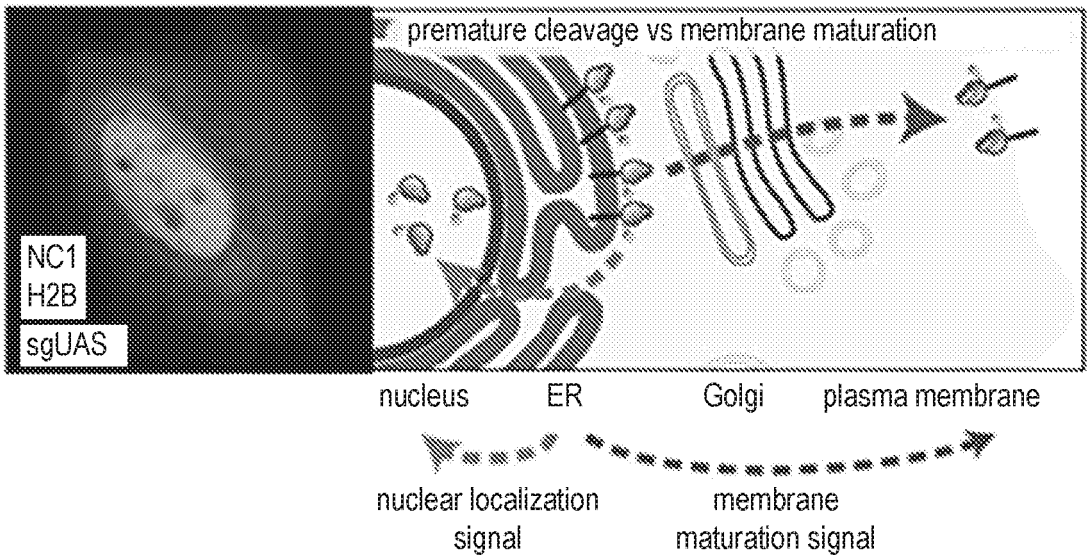

premature cleavage vs membrane maturation
NC1
H2B
sgUAS
nucleus
ER
Golgi
plasma membrane
nuclear localization signal
membrane maturation signal

FIGURE 34

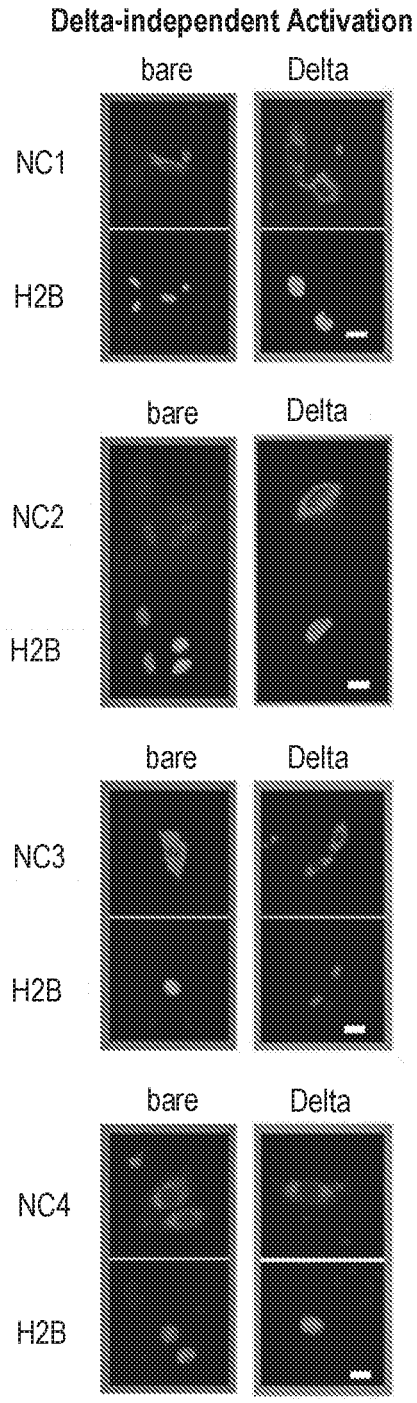

Delta-independent Activation
bare
Delta
NC1
H2B
bare
Delta
NC2
H2B
bare
Delta
NC3
H2B
bare
Delta
NC4
H2B

FIGURE 35A

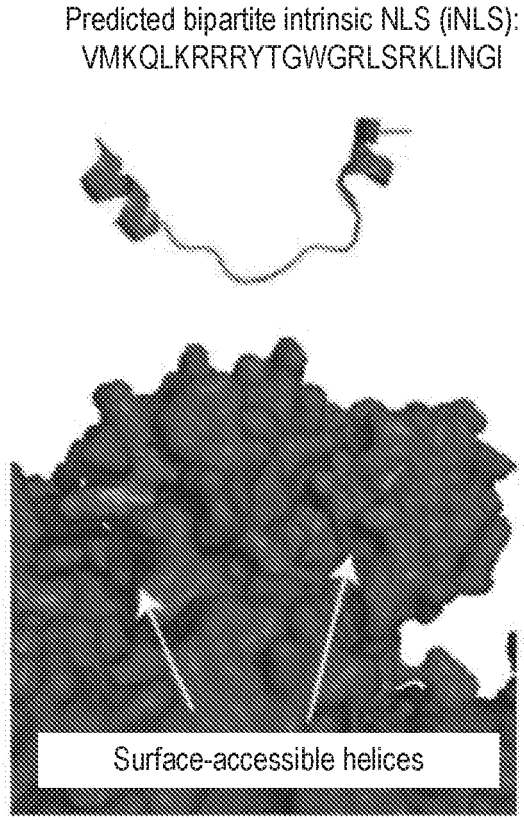


FIGURE 35B

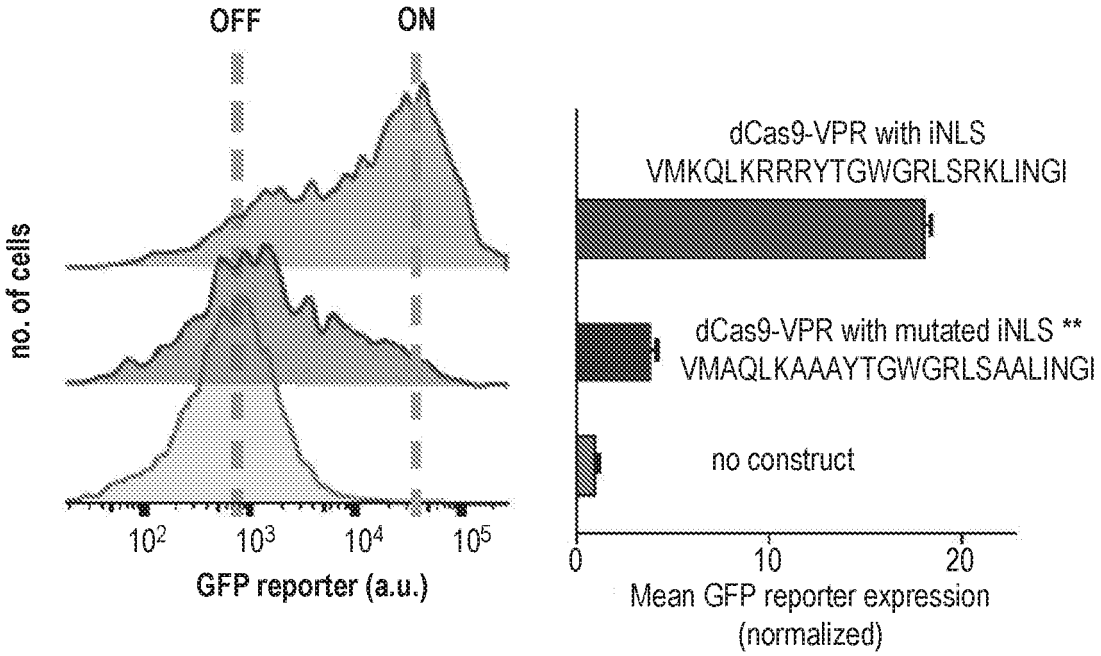


FIGURE 35C

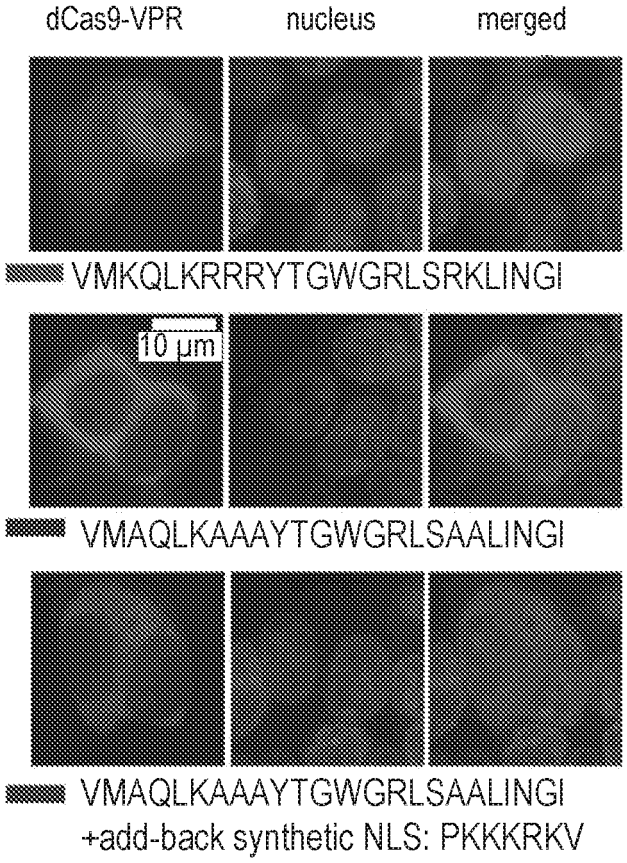


FIGURE 35D

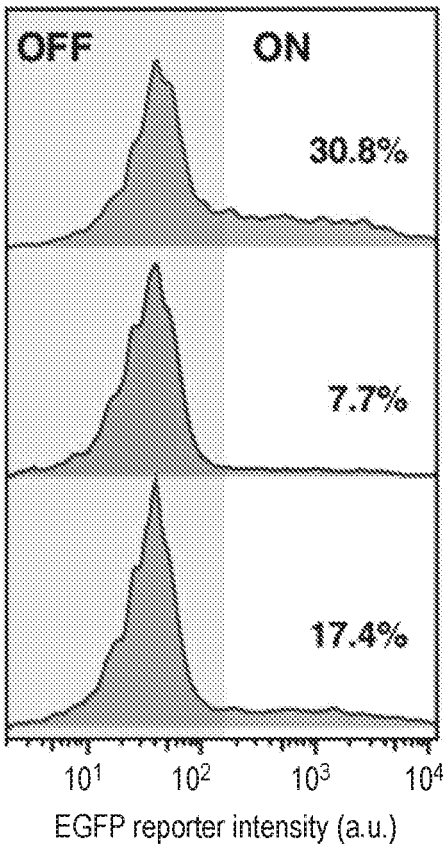


FIGURE 36A

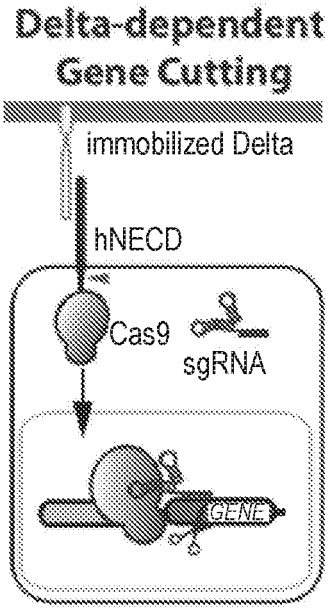


FIGURE 36B

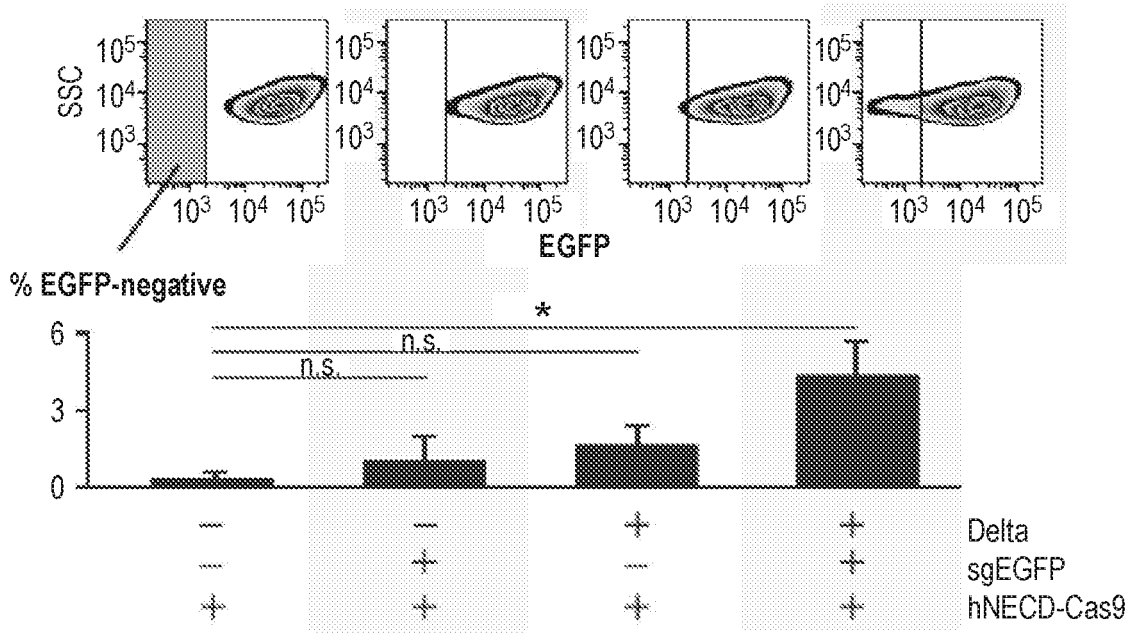


FIGURE 36C

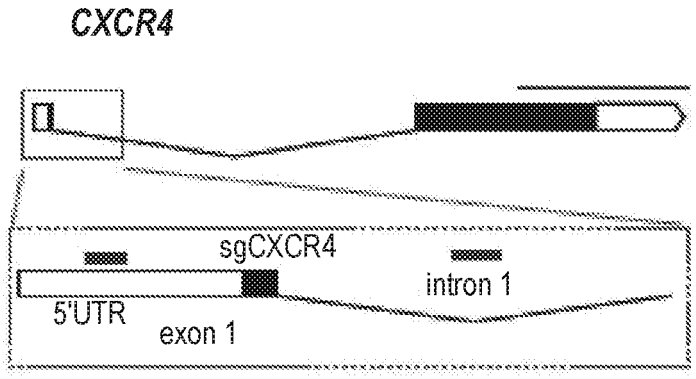


FIGURE 36D

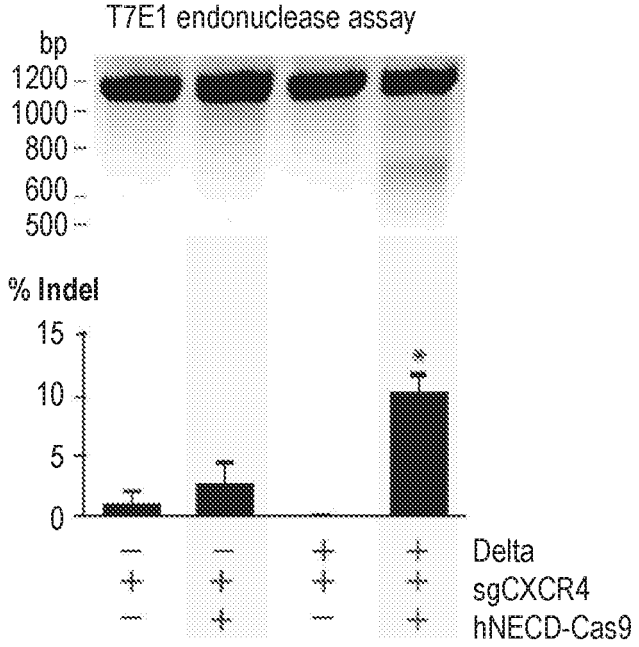


FIGURE 36E

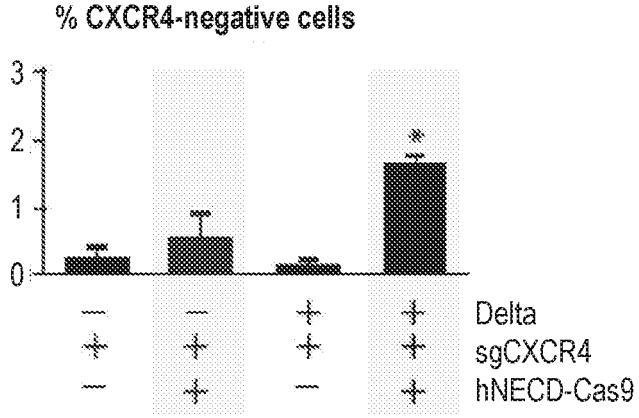


FIGURE 37A

Delta-binding unit of NECD: EGF repeats 11 & 12

```
Human      QDVDECSLGANPCEHAGKCINTLGSFECQCLQGYTGPRCEIDVNECVSNPCQNDATCLDQIGEFQCMCMPGYEGVHCE
Xenopus    NDVDECSLGANPCEHGGRCTNTLGSFQCNCPQGYAGPRCEIDVNECLSNPCQNDSTCLDQIGEFQCICMPGYEGLYCE
Zebrafish  QDIDECSLGANPCEHGGRCLNTKGSFQCRCLQGYEGPRCEMDVNECKSNPCQNDATCLDQIGGFRCICMPGYEGVFCQ
Drosophila EDIDECDQG SPCEHNGICVNTPGSYRCNCSQGFTGPRCETNINECESHPCQNEGSCLDDPGTFRCVCMPGFTGTQCE
           * * *   *   * *   * * *   *   * * * *   *     * *     * * *
```

FIGURE 37B

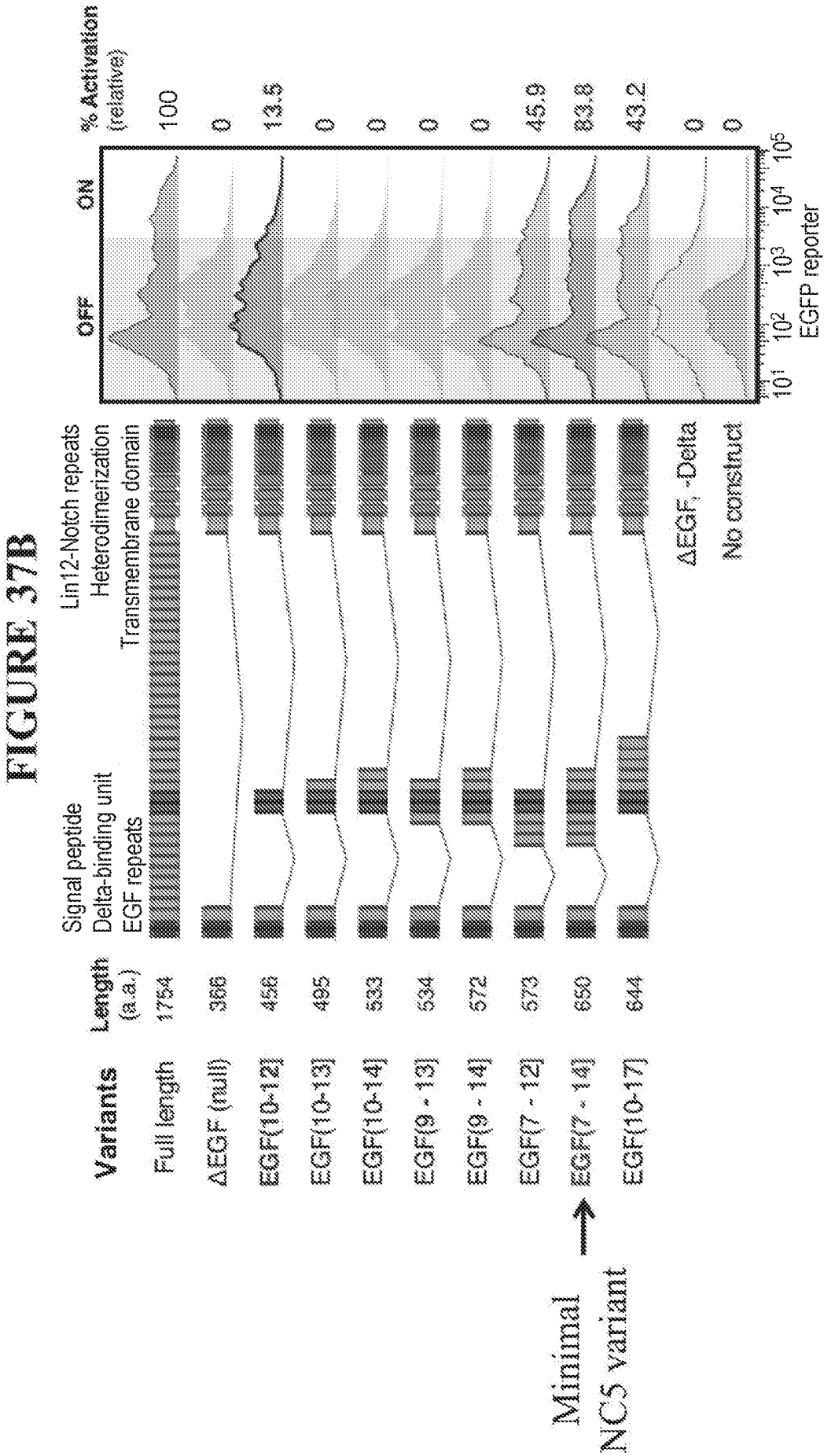

Signal peptide
Delta-binding unit
EGF repeats
Lin12-Notch repeats
Heterodimerization
Transmembrane domain
Variants
Length (a.a.)
% Activation (relative)
OFF
ON
EGFP reporter
Full length 1754 100
ΔEGF (null) 366 0
EGF(10-12] 456 13.5
EGF(10-13] 495 0
EGF(10-14] 533 0
EGF(9 - 13] 534 0
EGF(9 - 14] 572 0
EGF(7 - 12] 573 45.9
EGF(7 - 14] 650 83.8
EGF(10-17] 644 43.2
ΔEGF, -Delta 0
No construct 0
Minimal NC5 variant

FIGURE 38

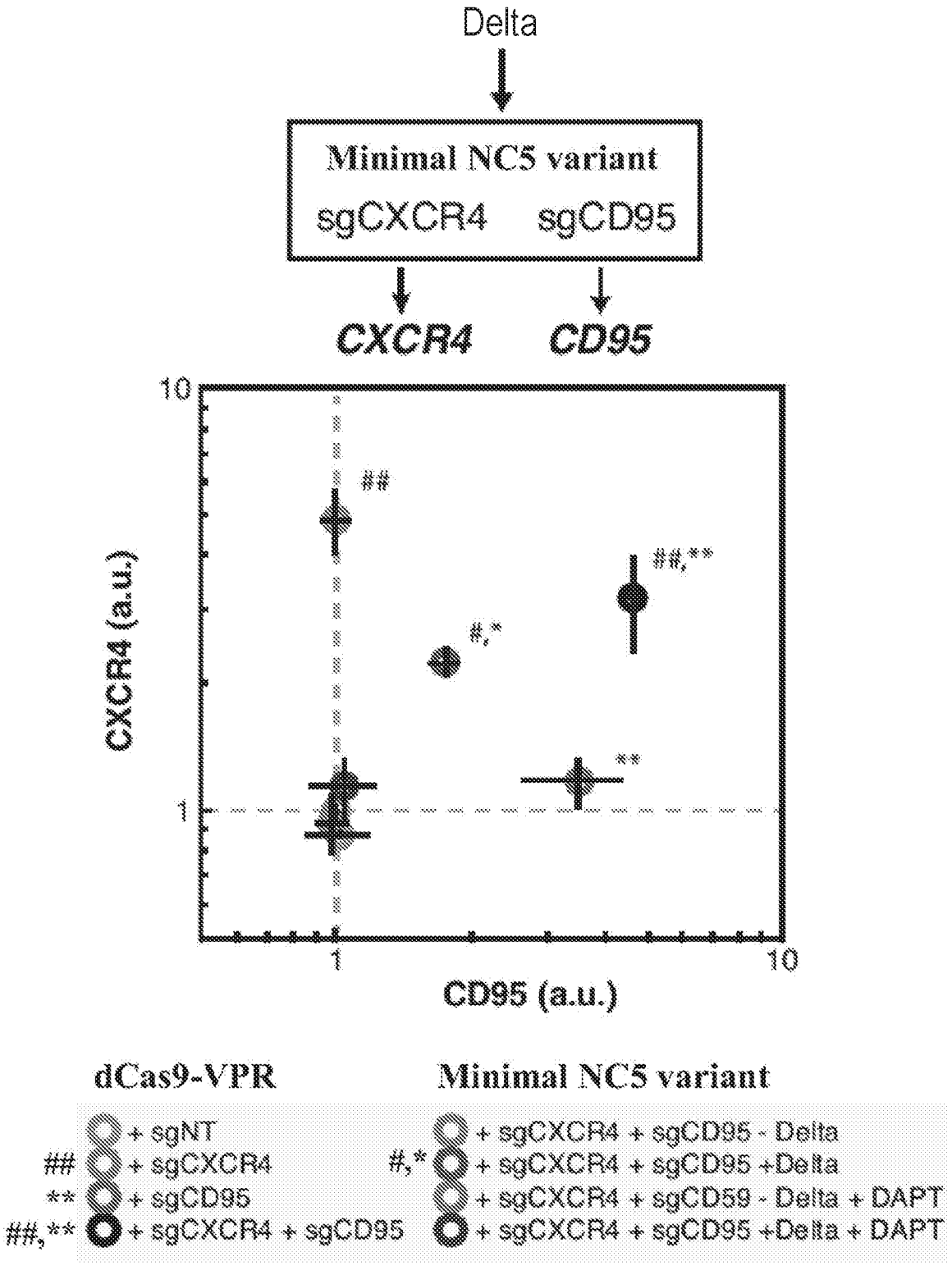

Delta
Minimal NC5 variant
sgCXCR4 sgCD95
CXCR4
CD95
10
1
1
10
CXCR4 (a.u.)
CD95 (a.u.)
##
#,*
##,**
**
dCas9-VPR
+ sgNT
+ sgCXCR4
** + sgCD95
##,** + sgCXCR4 + sgCD95
Minimal NC5 variant
+ sgCXCR4 + sgCD95 - Delta
#,* + sgCXCR4 + sgCD95 +Delta
+ sgCXCR4 + sgCD59 - Delta + DAPT
+ sgCXCR4 + sgCD95 +Delta + DAPT

FIGURE 39B

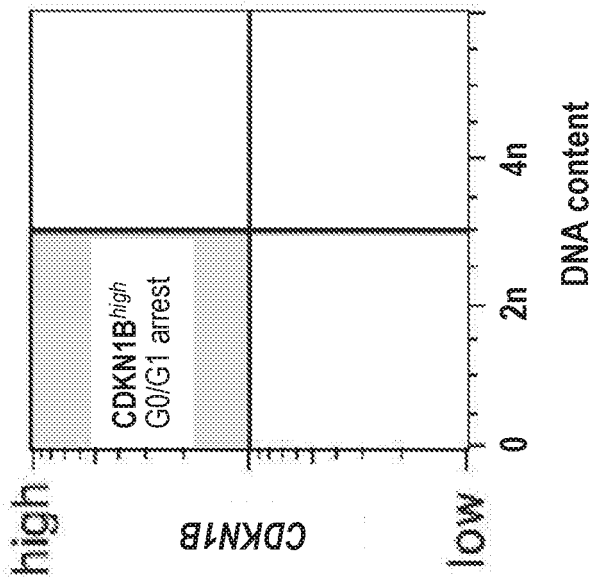


FIGURE 39A

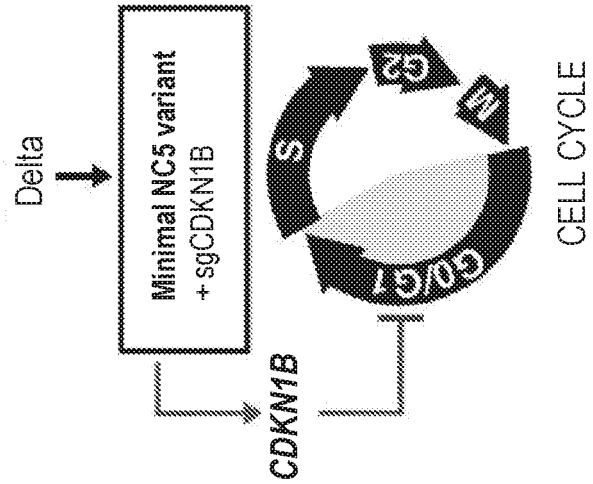


FIGURE 39C

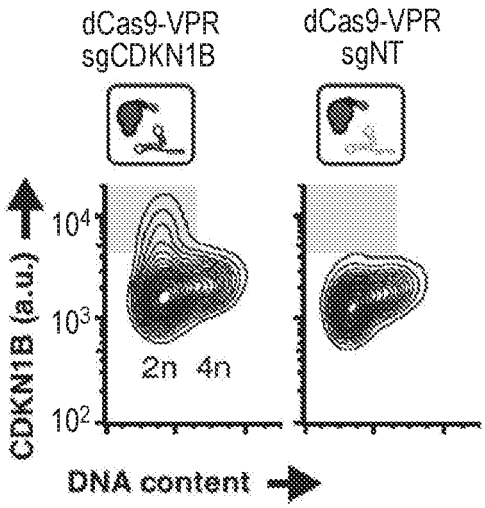


FIGURE 39D

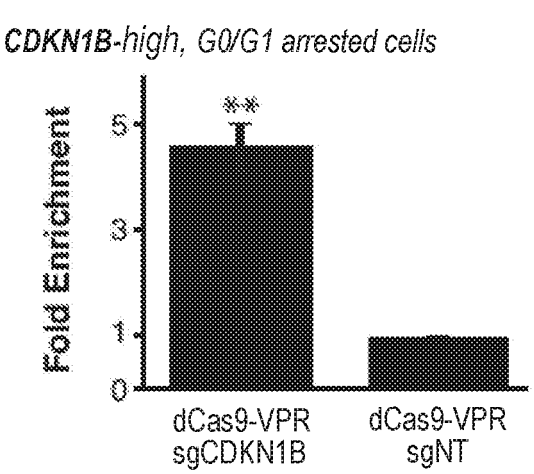


FIGURE 39E

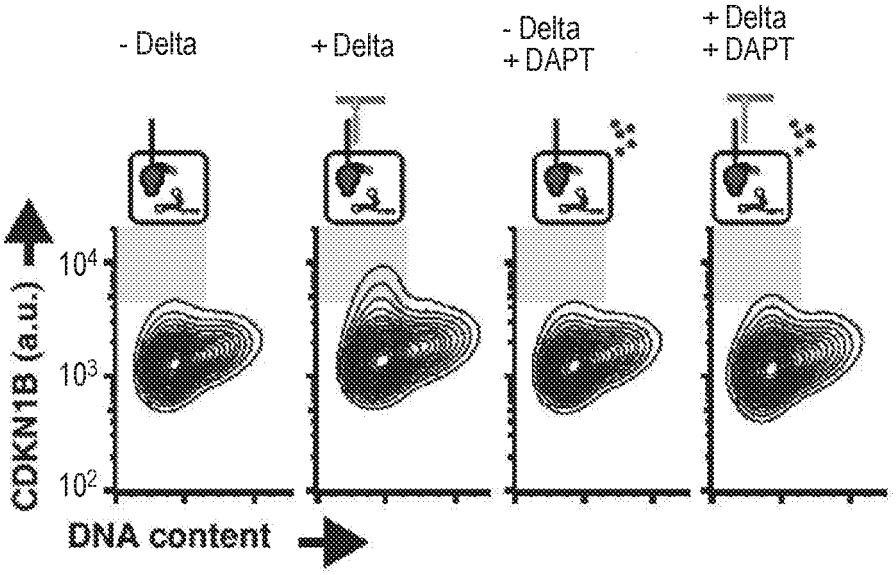


FIGURE 39F

