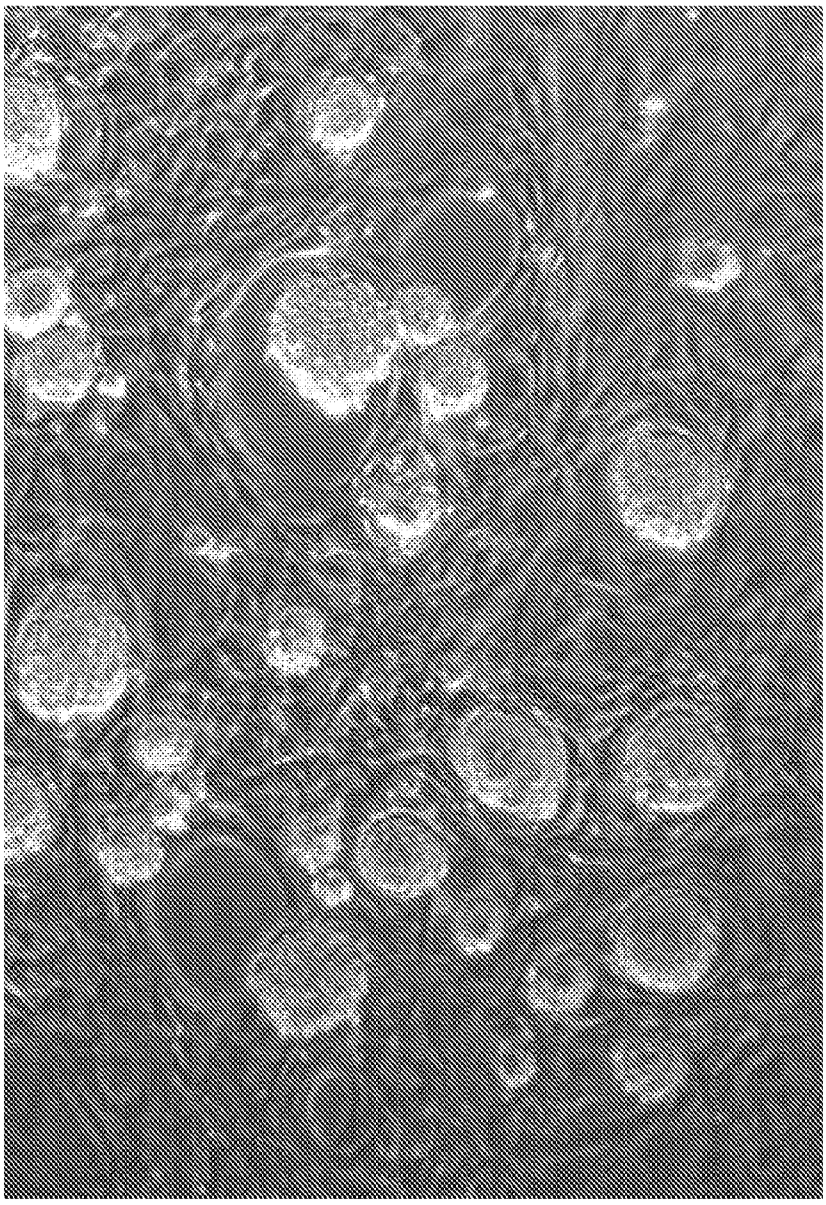


FIG. 1

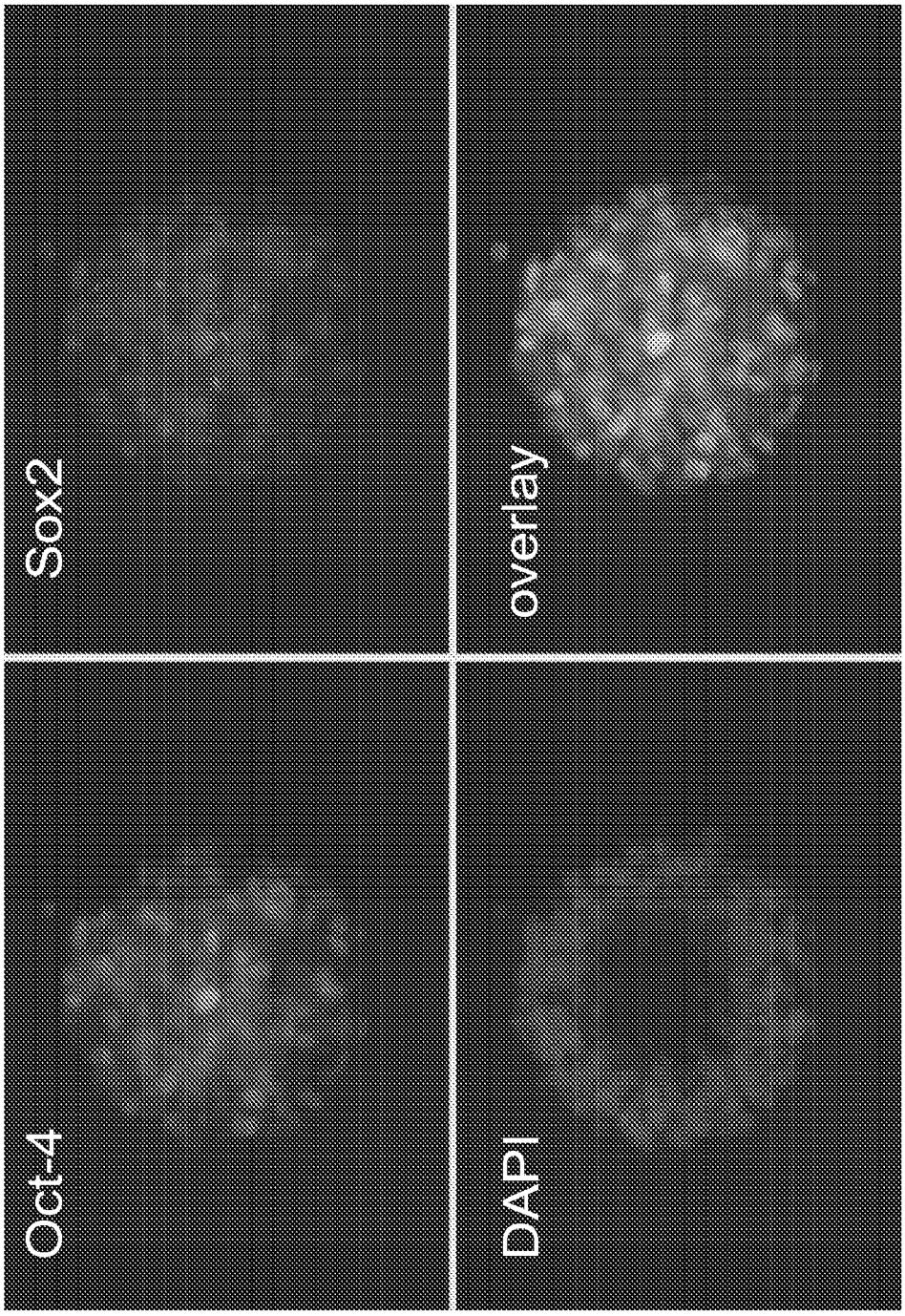


FIG. 2B FIG. 2D

FIG. 2A FIG. 2C

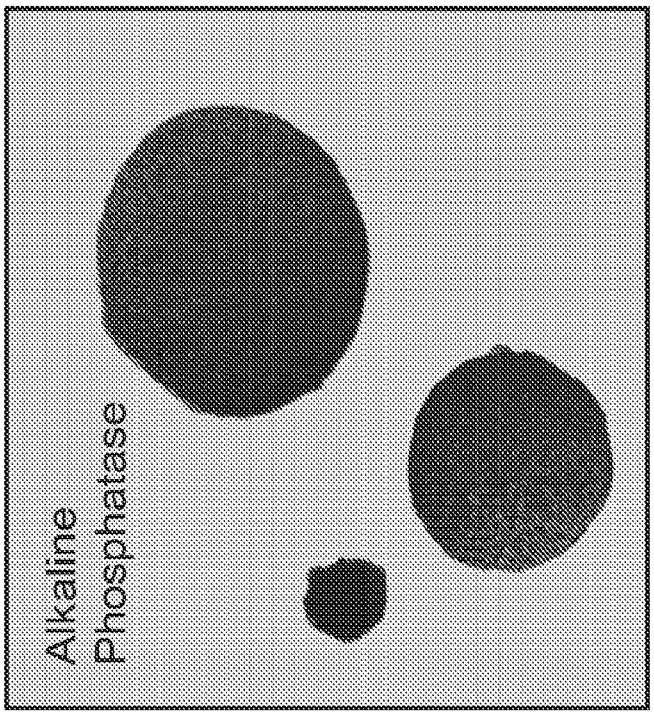


FIG. 3

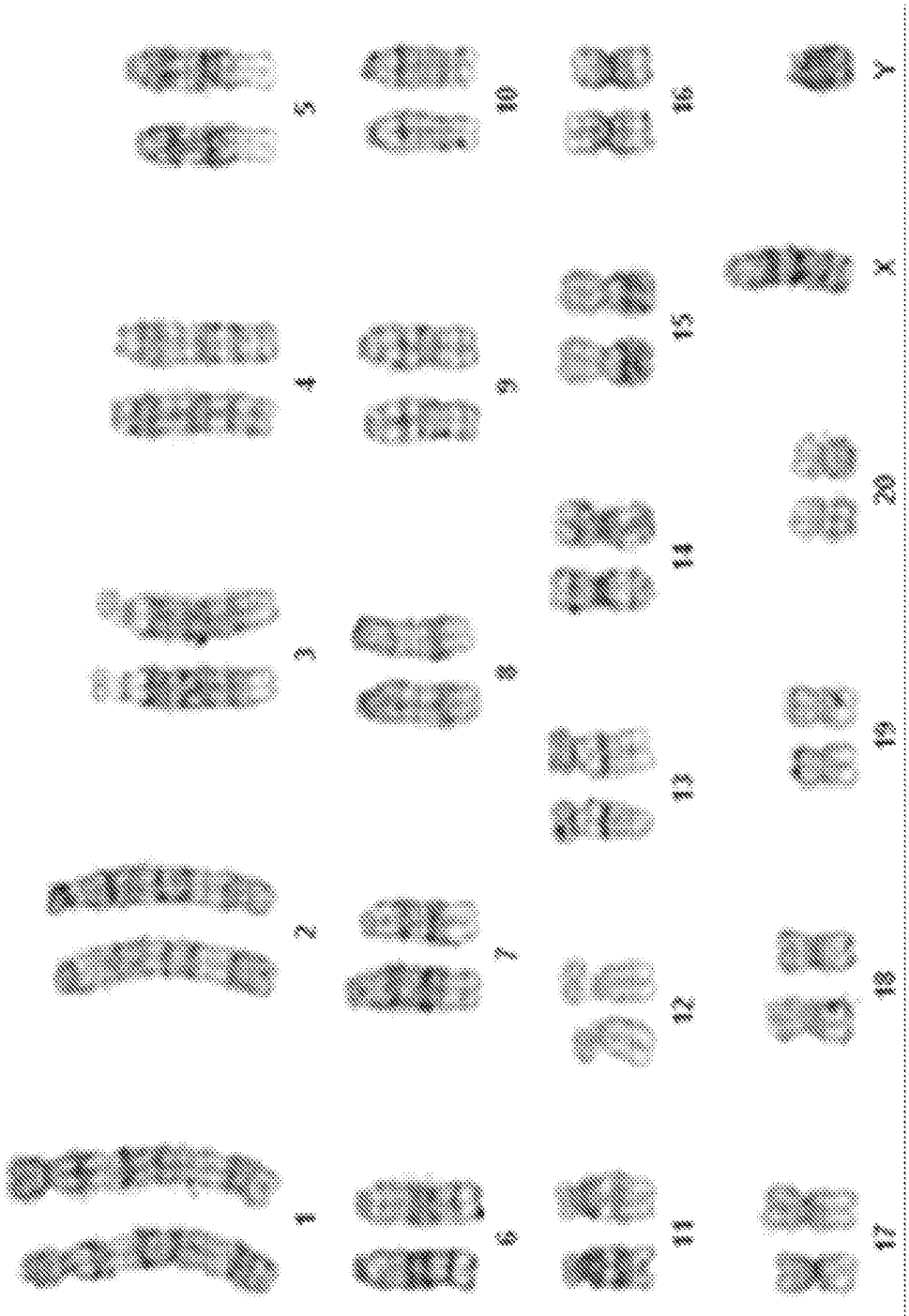


FIG. 4



Cell Results: 42, XY

FIG. 5A

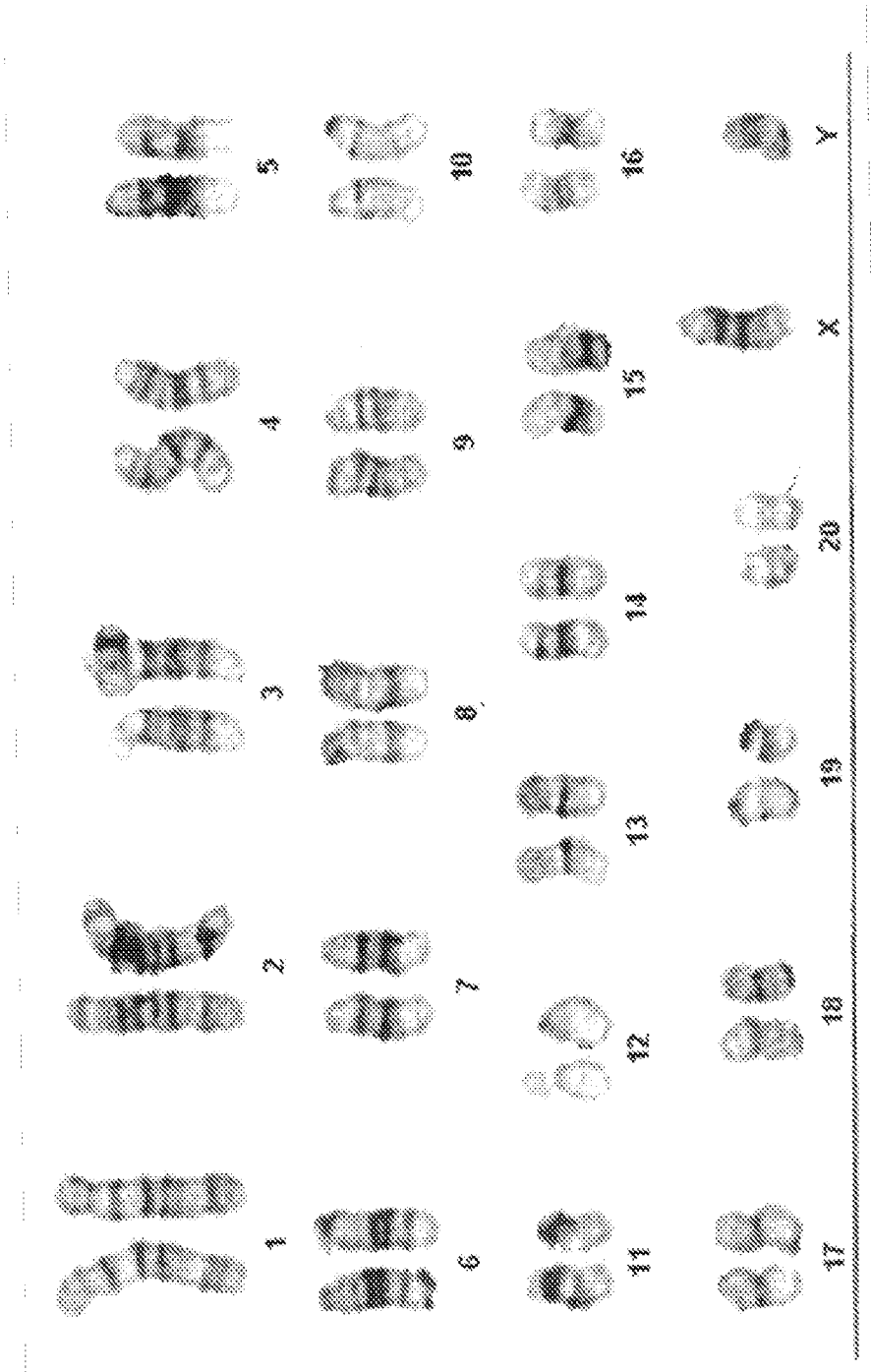


FIG. 5B



Cell Results: 42, XY

FIG. 6A

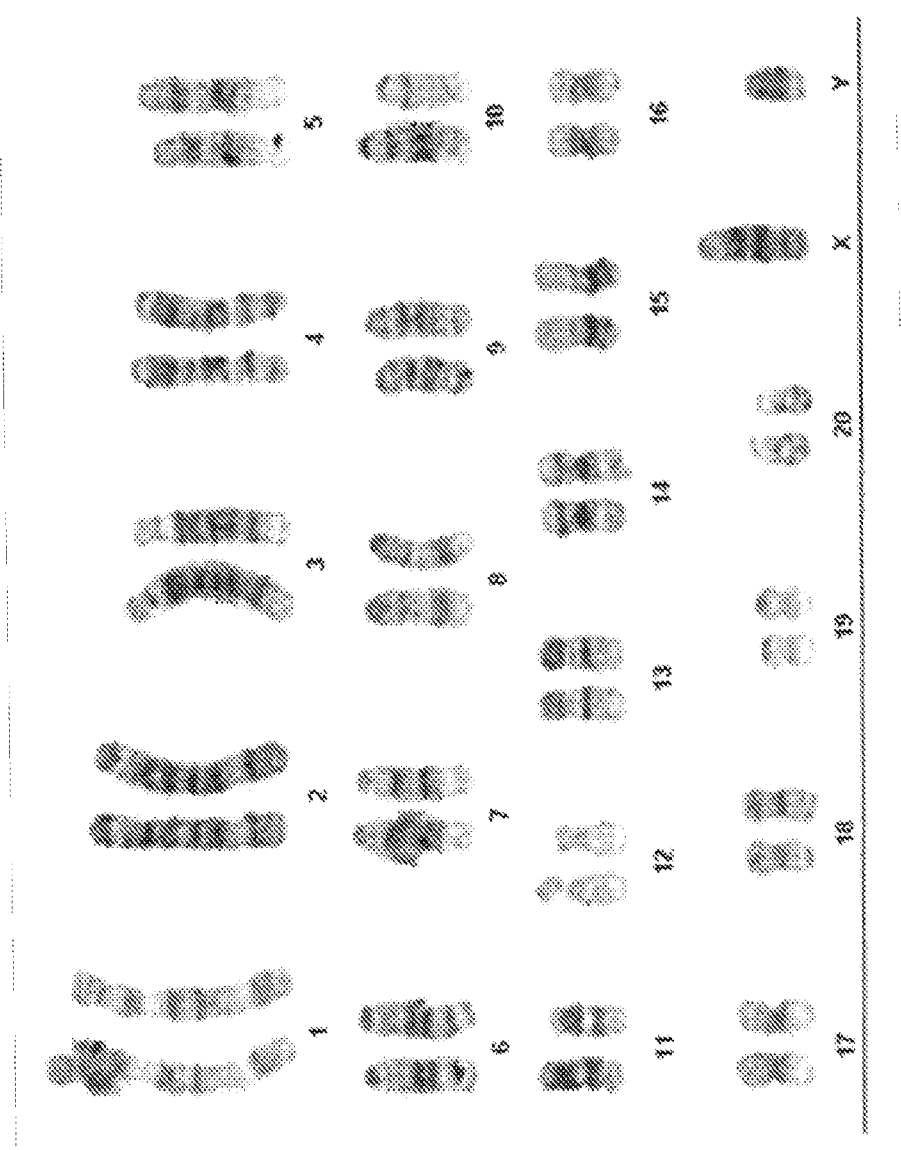


FIG. 6B



FIG. 7A



FIG. 7B

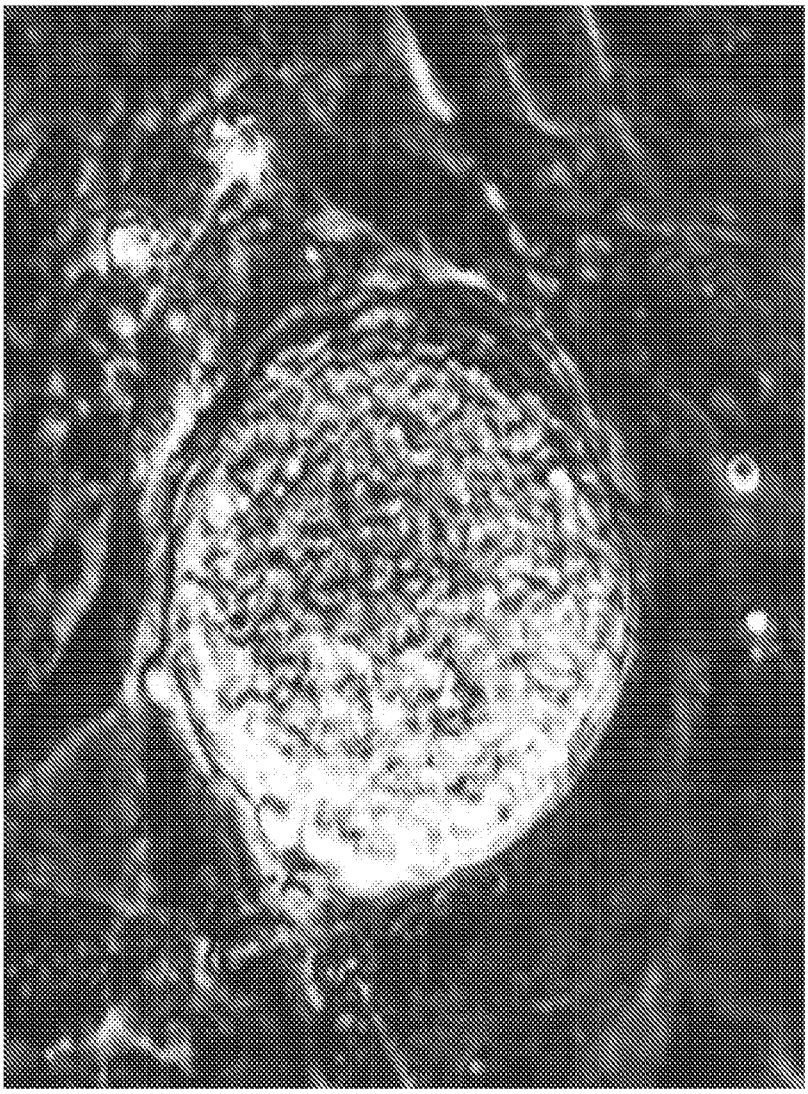


FIG. 8

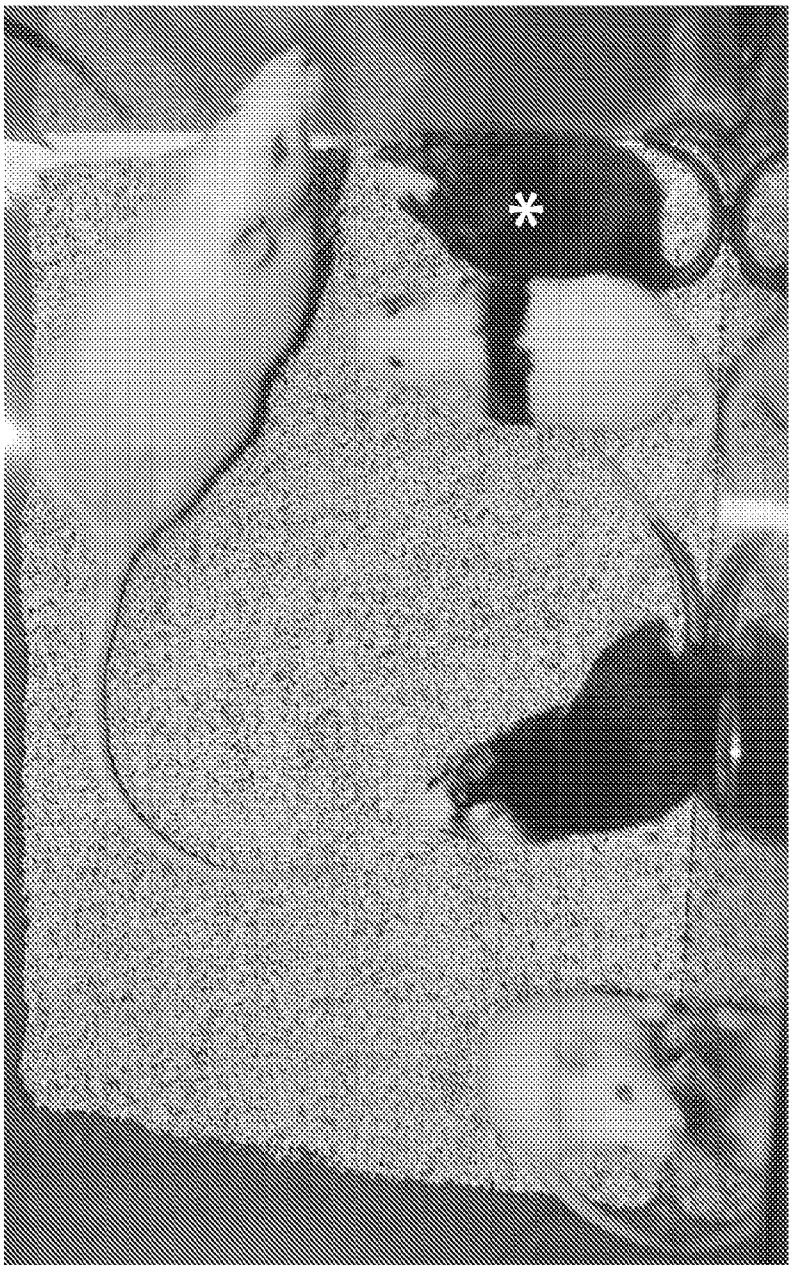


FIG. 9

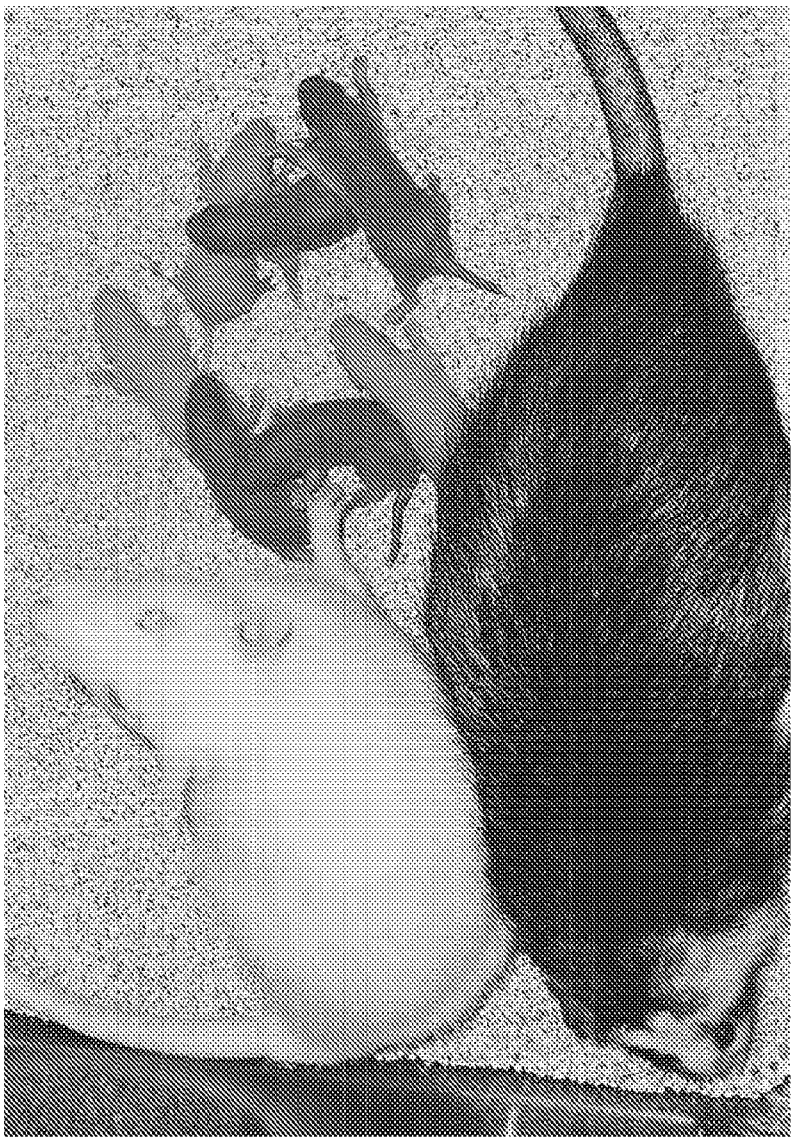


FIG. 10

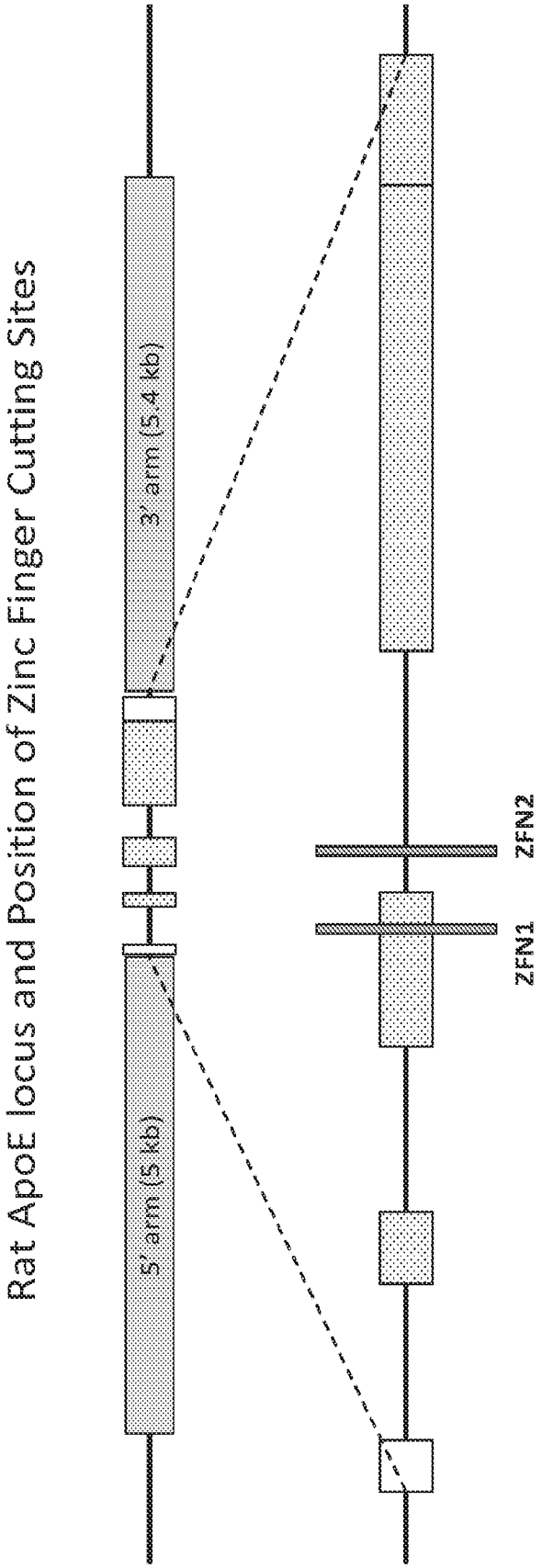
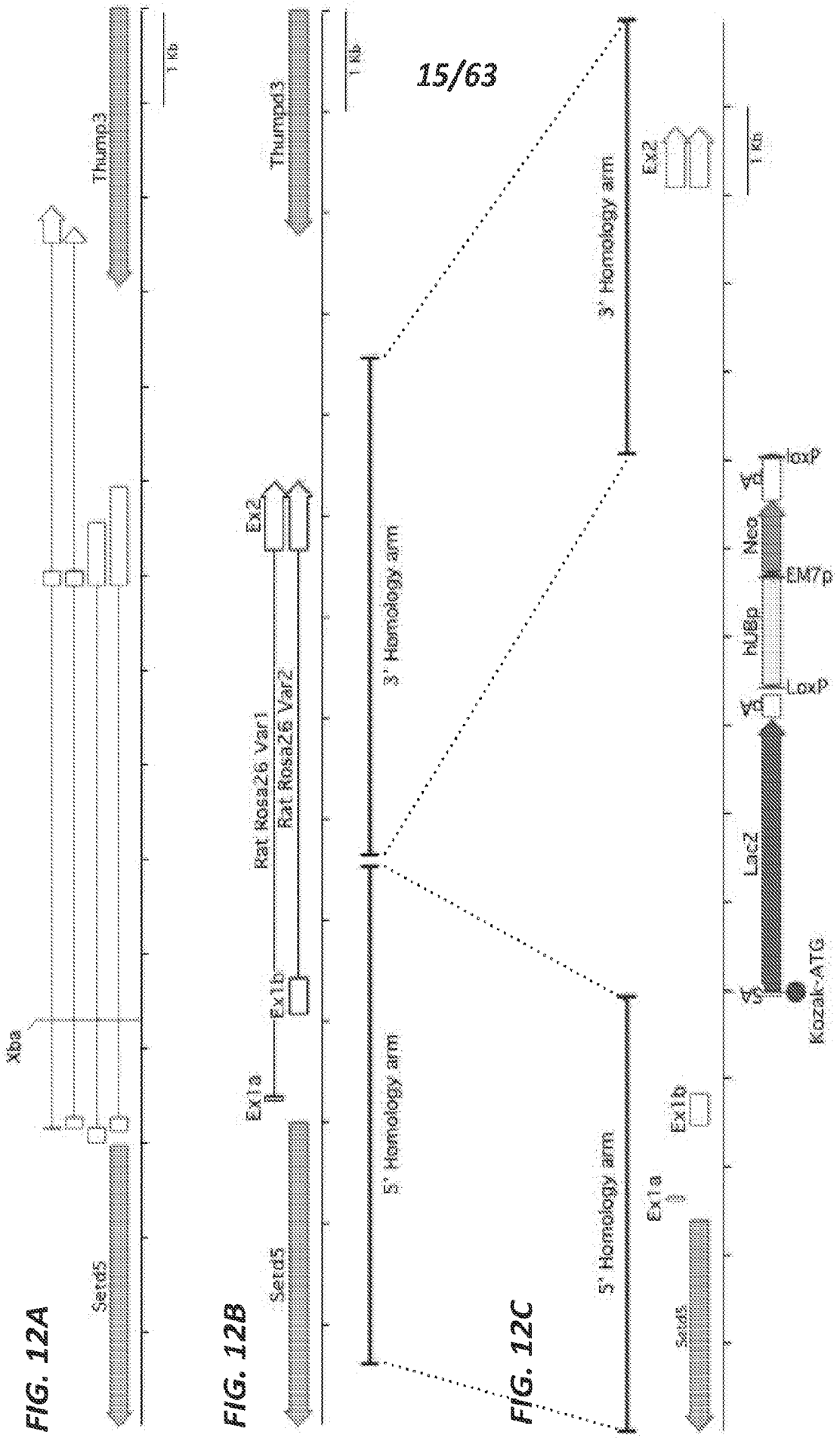

Rat ApoE locus and Position of Zinc Finger Cutting Sites
3' arm (5.4 kb)
5' arm (5 kb)
ZFN2
ZFN1

FIG. 11


15/63
FIG. 12A
Xba
Thump3
Sard5
1 Kb
FIG. 12B
Ex1a
Ex1b
Ex2
Rat Rosa26 Var1
Rat Rosa26 Var2
5' Homology arm
3' Homology arm
FIG. 12C
LoxP
Neo
EM7p
hUBp
LacZ
Kozak-ATG

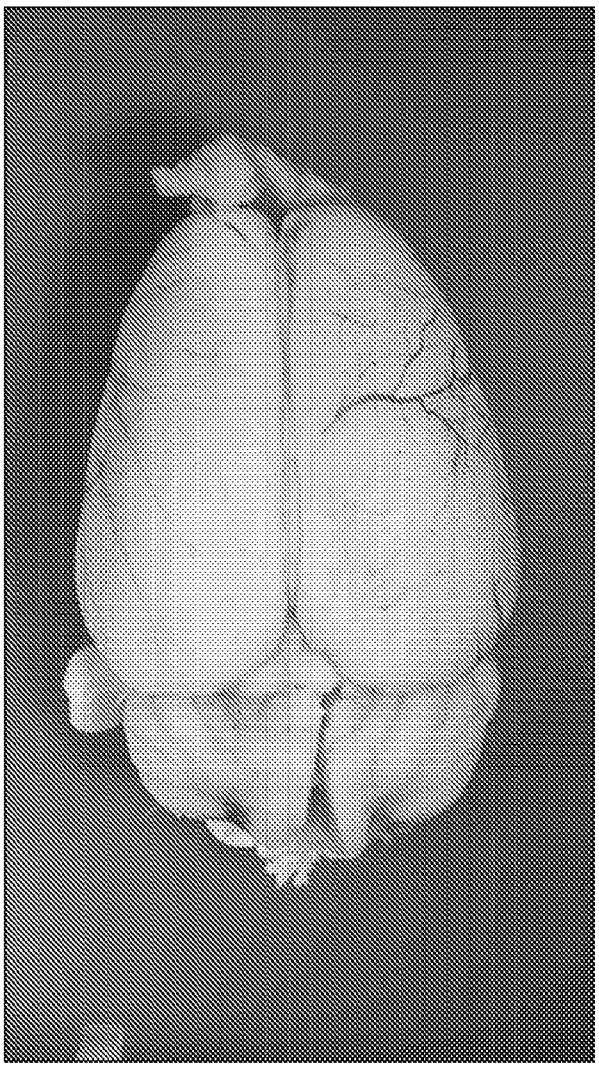


FIG. 13A

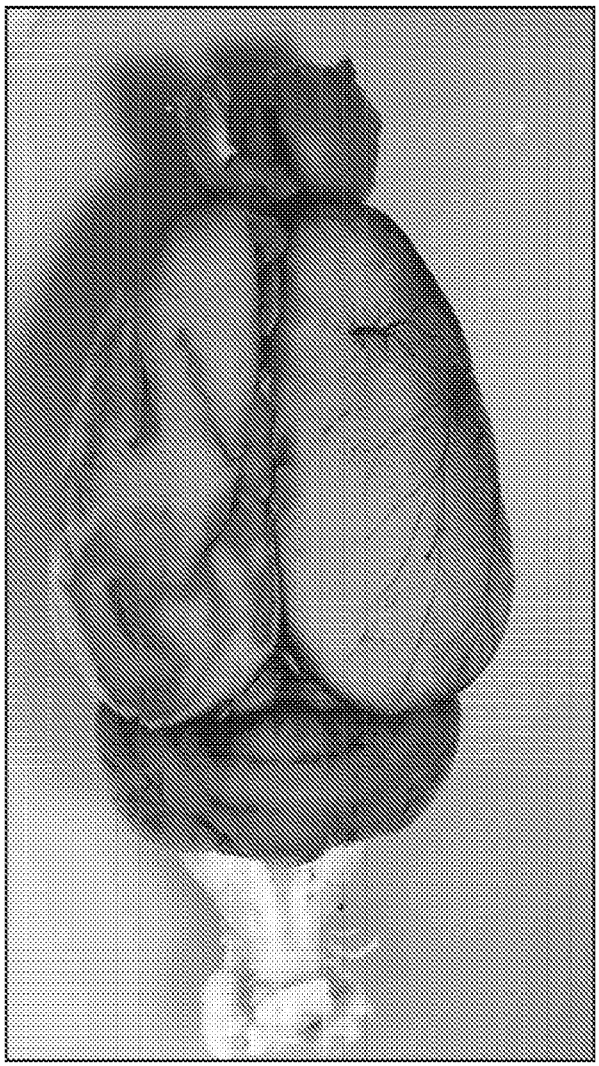


FIG. 13B

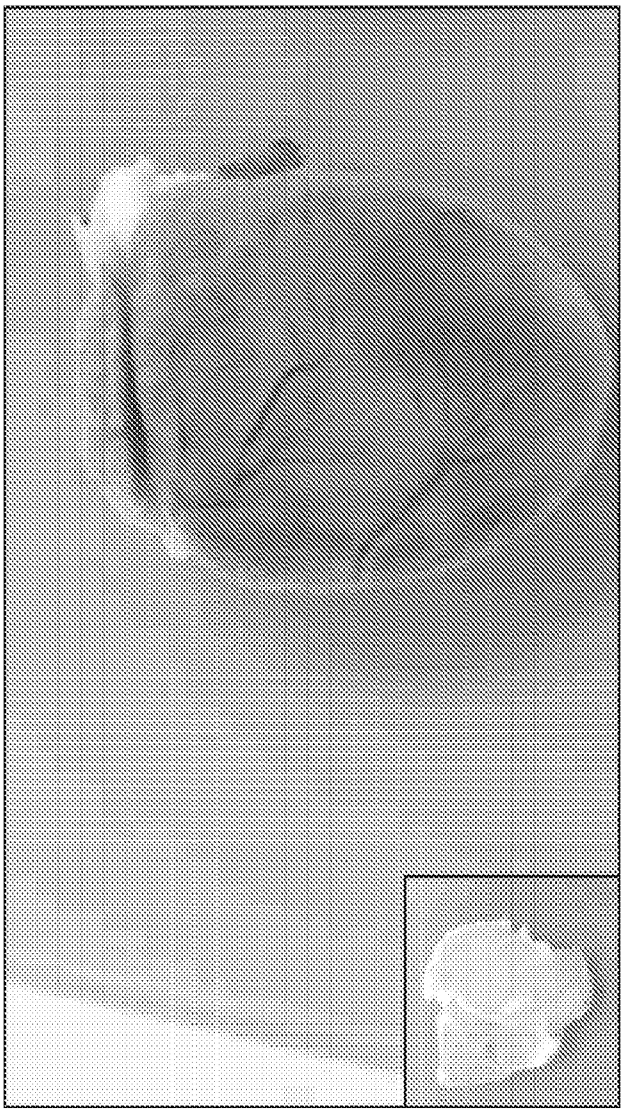


FIG. 13C

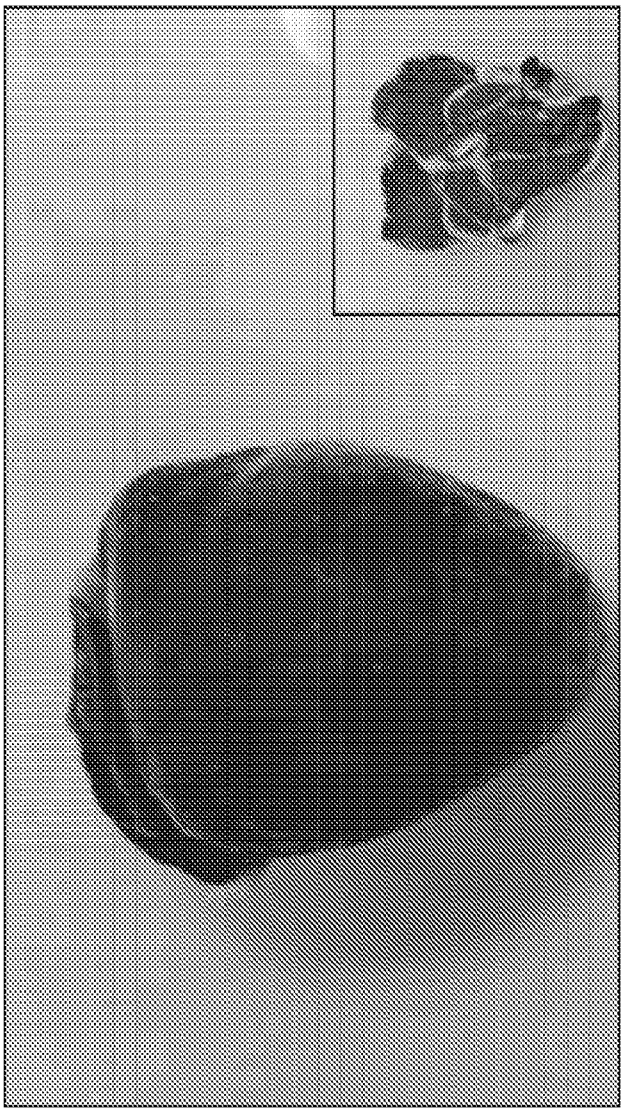


FIG. 13D



FIG. 13E

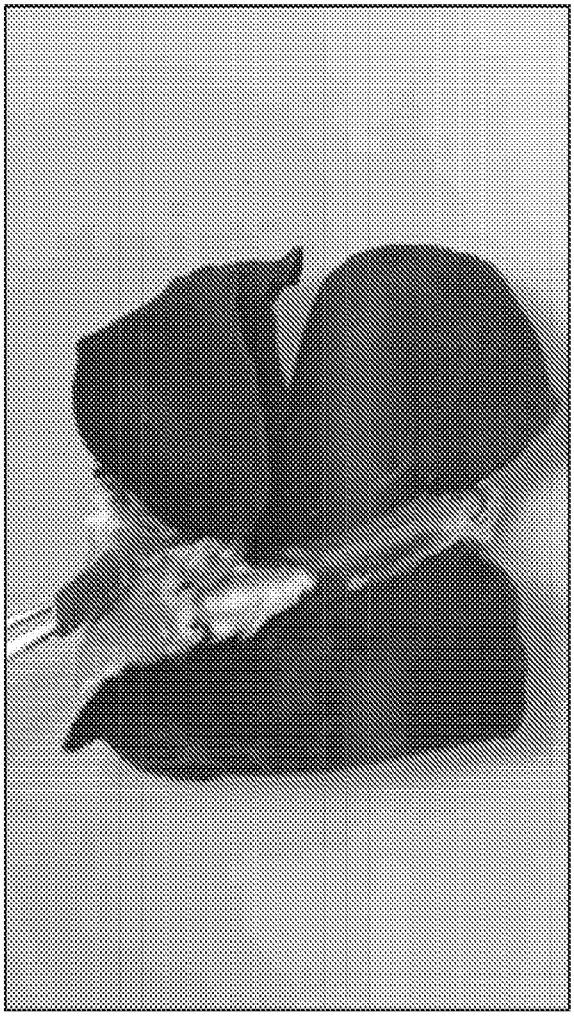


FIG. 13F

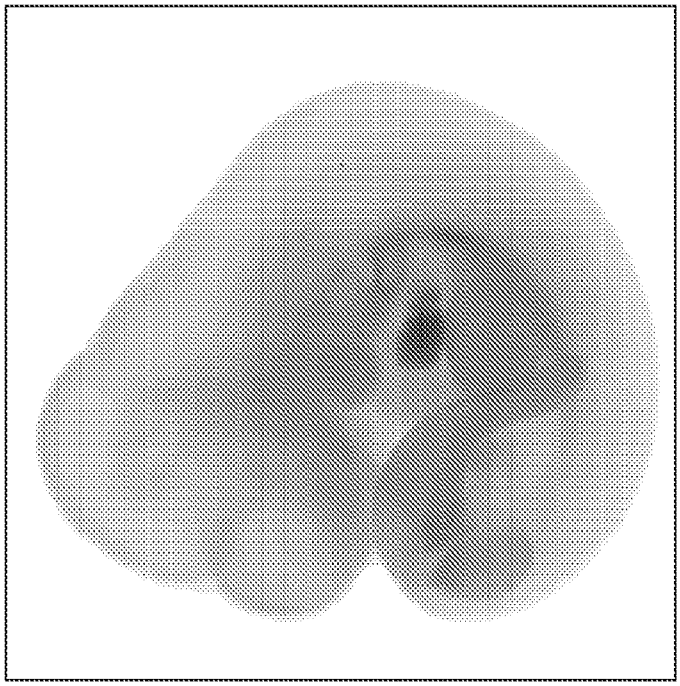


FIG. 13H

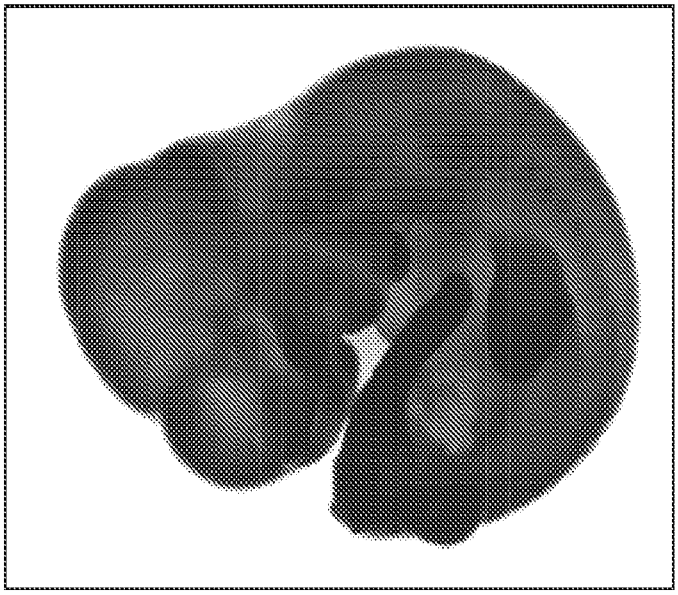


FIG. 13G

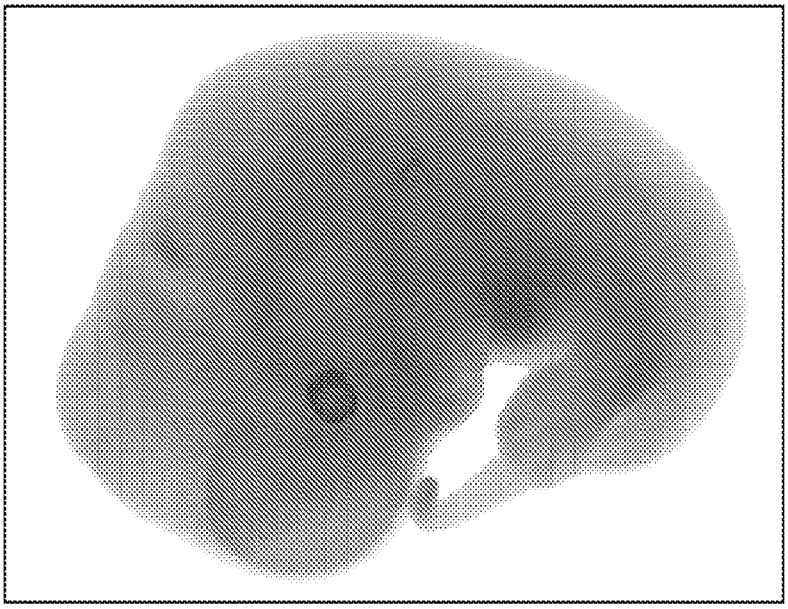


FIG. 13J

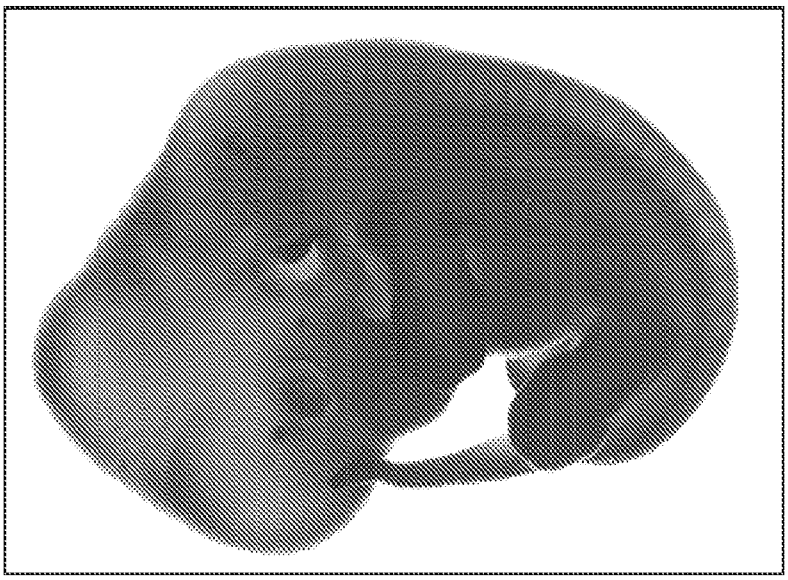


FIG. 13I

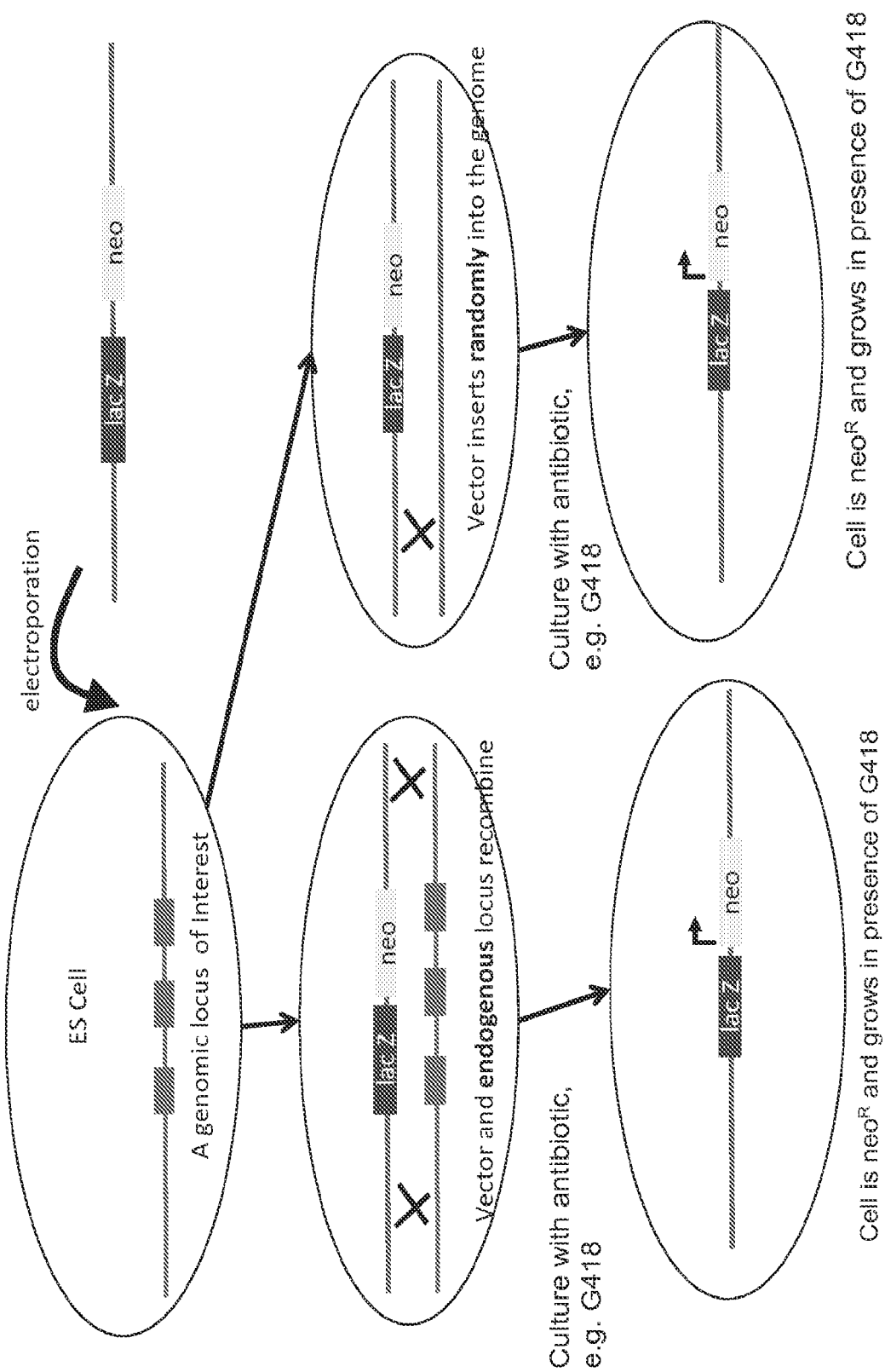

ES Cell
A genomic locus of Interest
electroporation
lacZ
neo
Vector and endogenous locus recombine
Vector inserts randomly into the genome
Culture with antibiotic, e.g. G418
Culture with antibiotic, e.g. G418
Cell is neoR and grows in presence of G418 typically 1-20% (at best) of insertions
Cell is neoR and grows in presence of G418

FIG. 14

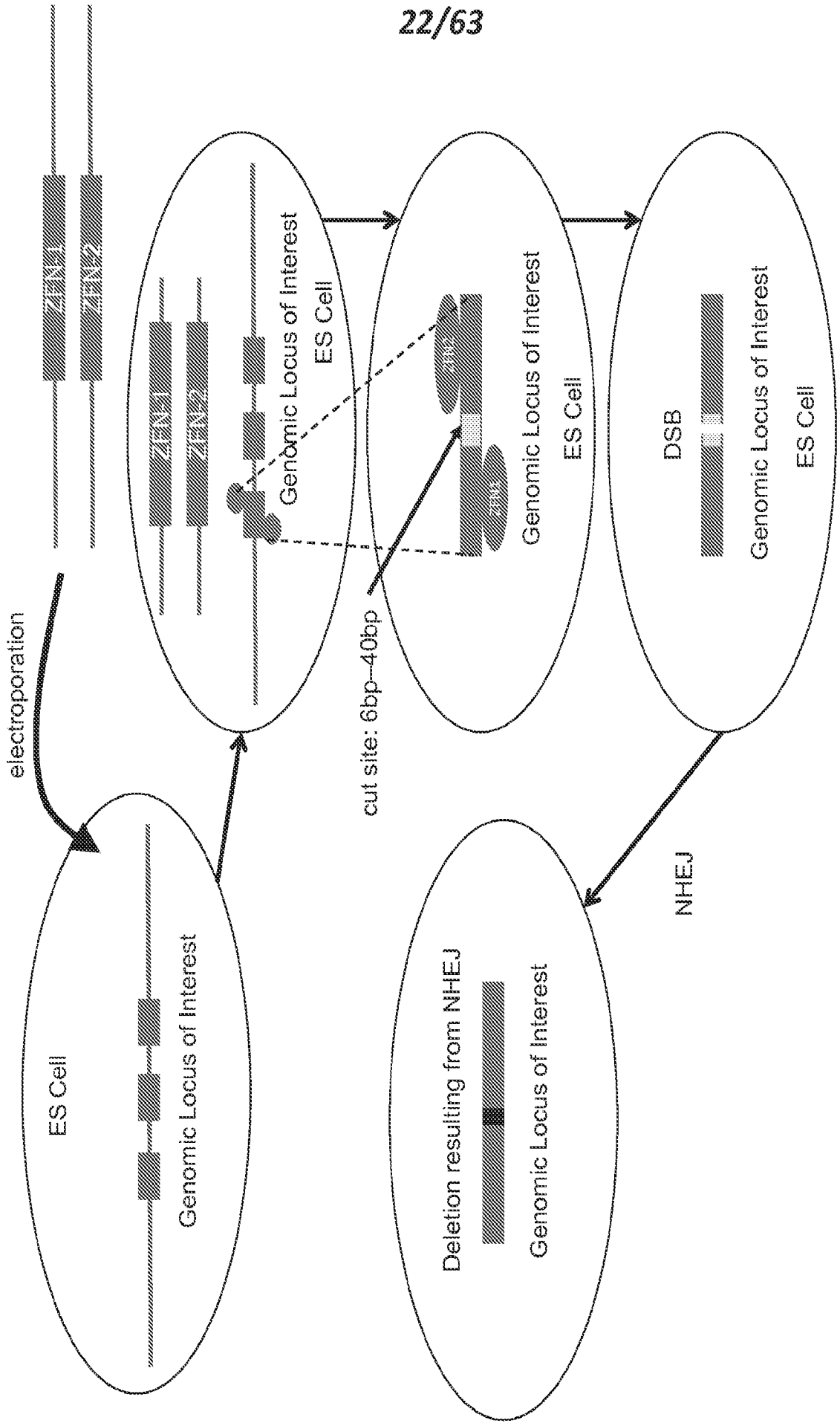

ZFN1
ZFN2
Genomic Locus of Interest
ES Cell
electroporation
cut site: 6bp–40bp
DSB
NHEJ
Deletion resulting from NHEJ

FIG. 15

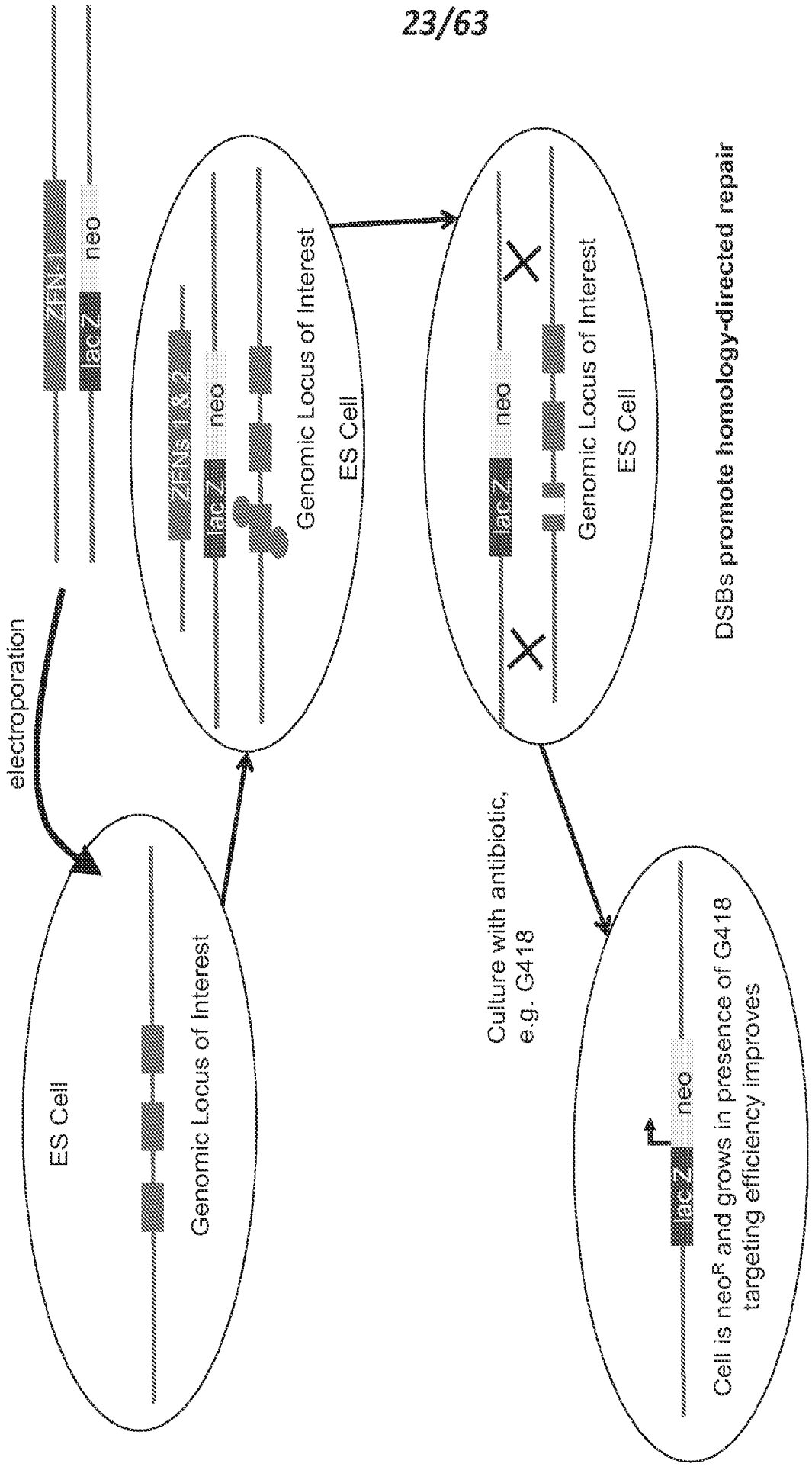

ZFN1
lacZ
neo
electroporation
ES Cell
Genomic Locus of Interest
ZFN1&2
lacZ
neo
ES Cell
Genomic Locus of Interest
DSBs promote homology-directed repair
lacZ
neo
ES Cell
Genomic Locus of Interest
Culture with antibiotic, e.g. G418
lacZ
neo
Cell is neo^R and grows in presence of G418
targeting efficiency improves

FIG. 16



FIG. 17

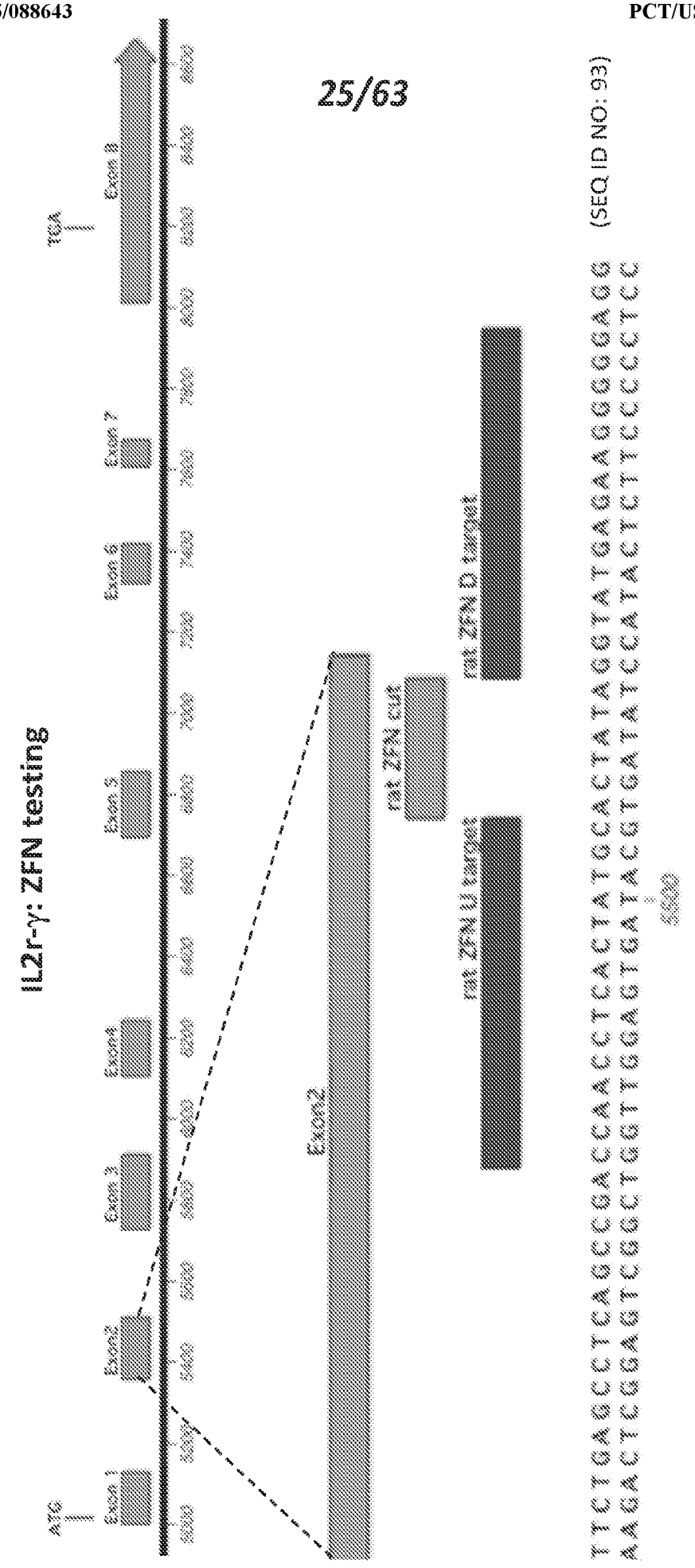
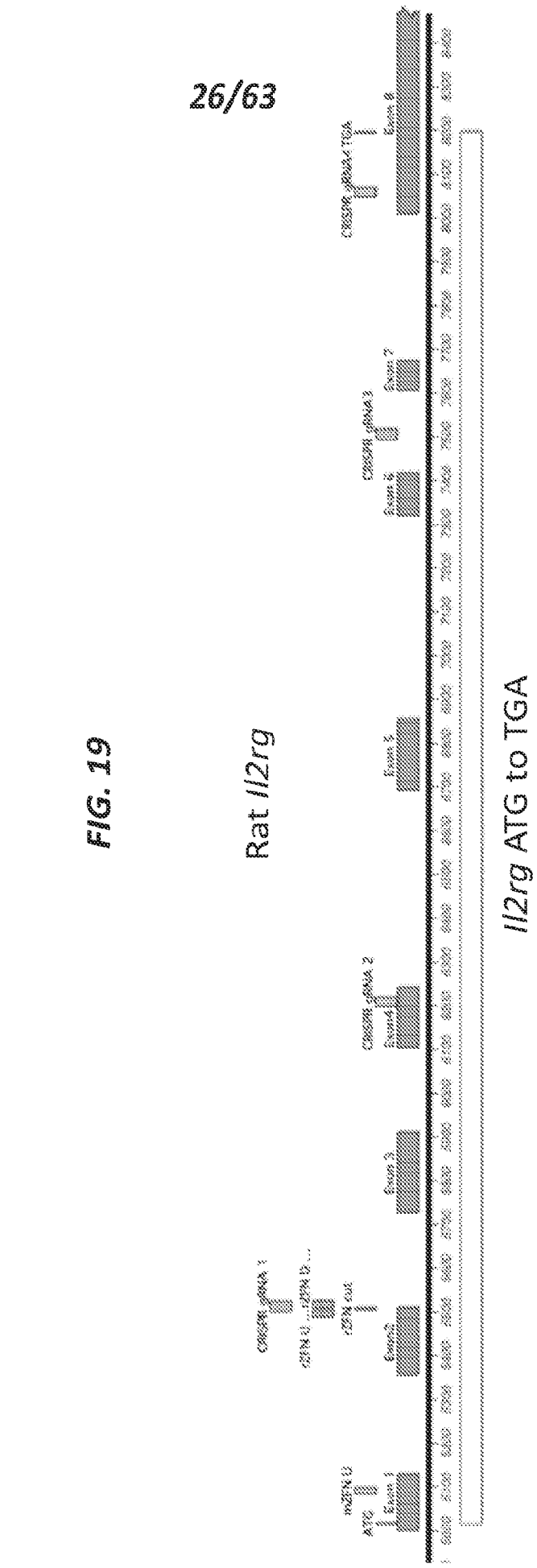

IL2r-γ: ZFN testing
ATG
TGA
Exon 1
Exon2
Exon 3
Exon4
Exon 5
Exon 6
Exon 7
Exon 8
Exon2
rat ZFN cut
rat ZFN D target
rat ZFN U target
5000
6000
6200
6400
6600
6800
7000
7200
7400
7600
7800
8000
8200
8400
8600
8800
TTCTGAGGCCTCAGCCCGACCAACCCTCACTATGCACTATAGGTATGAGAAGGGGGAGGG (SEQ ID NO: 93)
AAGACTCCGGAGTCGGCTTGGTTGGAGTGATACGTGATATCCATACTCTTCCCCCCTCC

FIG. 18

FIG. 19



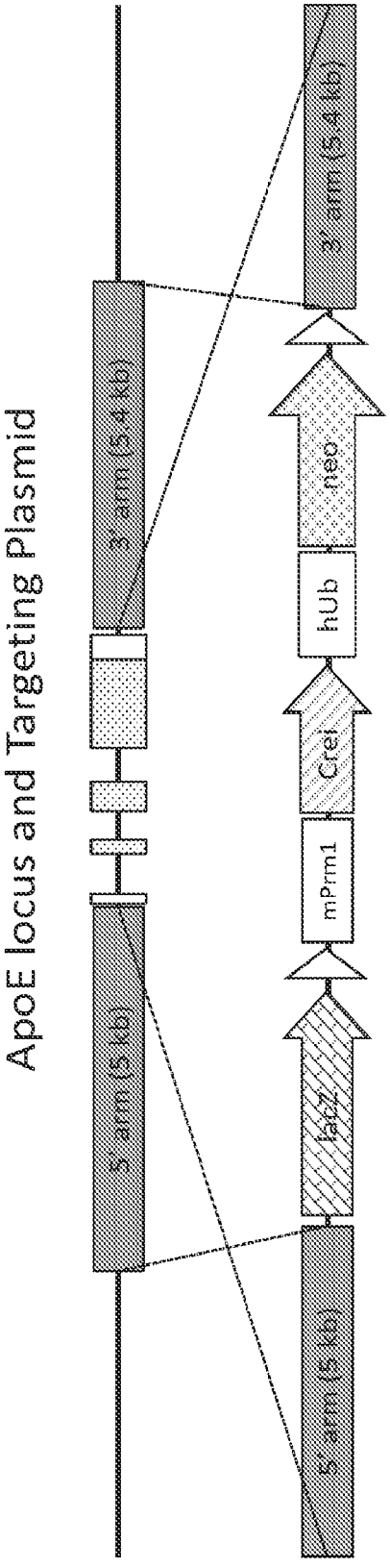

ApoE locus and Targeting Plasmid
5' arm (5 kb)
lacZ
mPrm1
Cre
hUb
neo
3' arm (5.4 kb)
5' arm (5 kb)
3' arm (5.4 kb)

FIG. 20

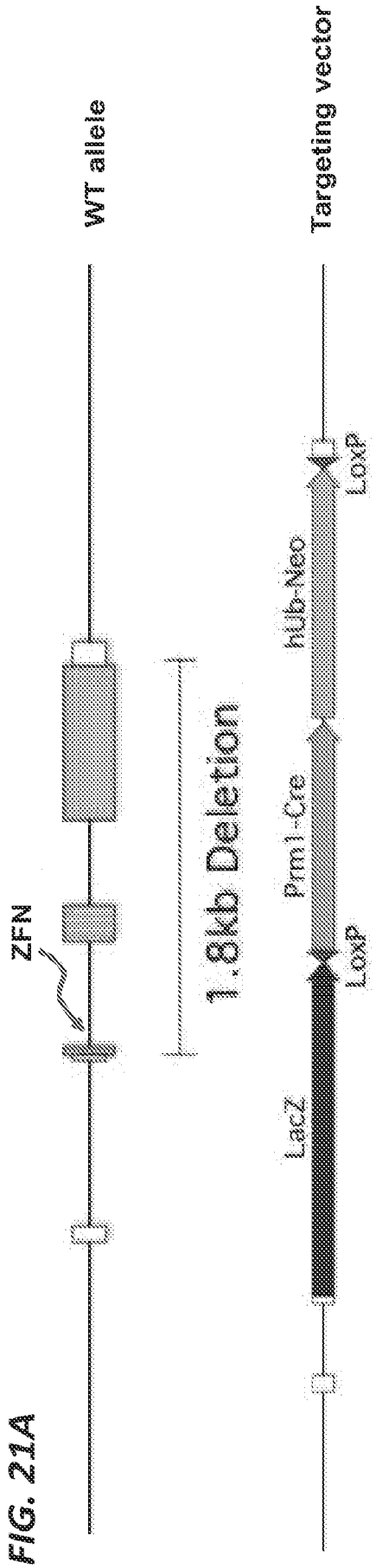


FIG. 21A

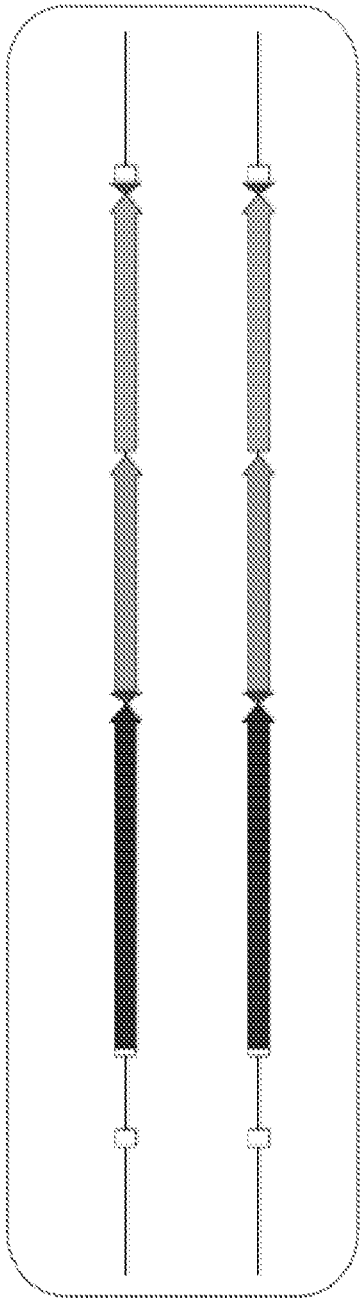


FIG. 21B

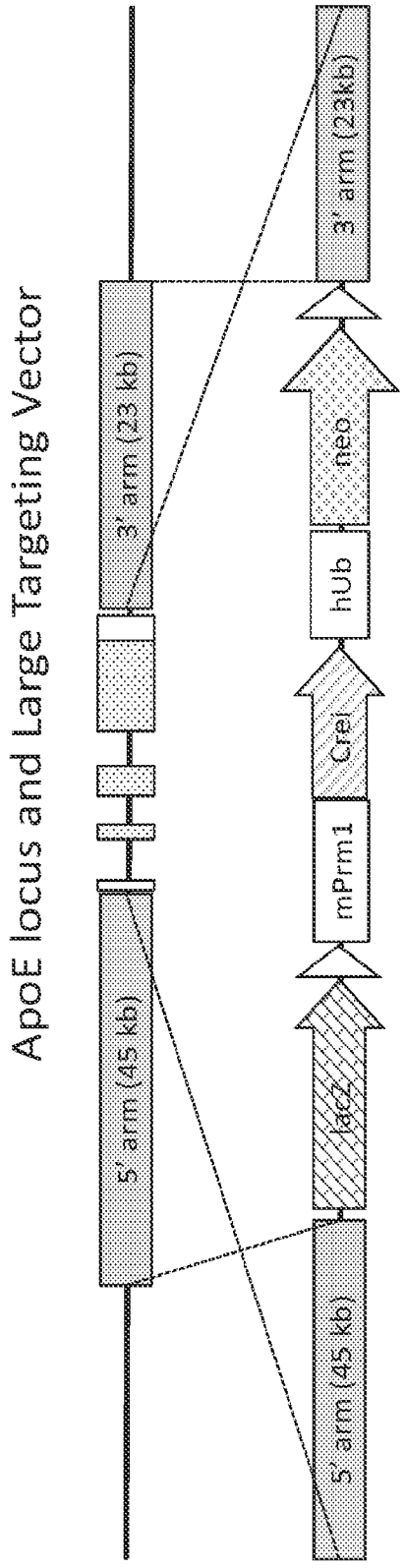

ApoE locus and Large Targeting Vector
5' arm (45 kb)
3' arm (23 kb)
lacZ
mPrm1
Cre
hUb
neo
3' arm (23kb)
5' arm (45 kb)

FIG. 22

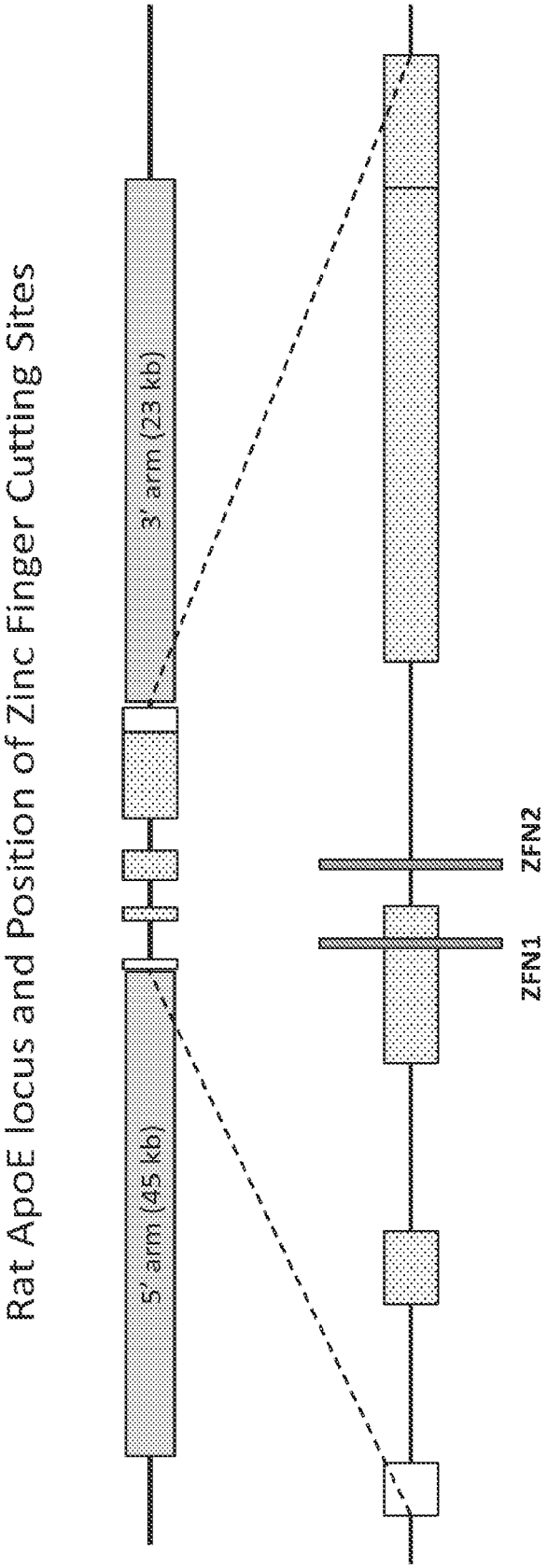

Rat ApoE locus and Position of Zinc Finger Cutting Sites
5' arm (45 kb)
3' arm (23 kb)
ZFN1
ZFN2

FIG. 23

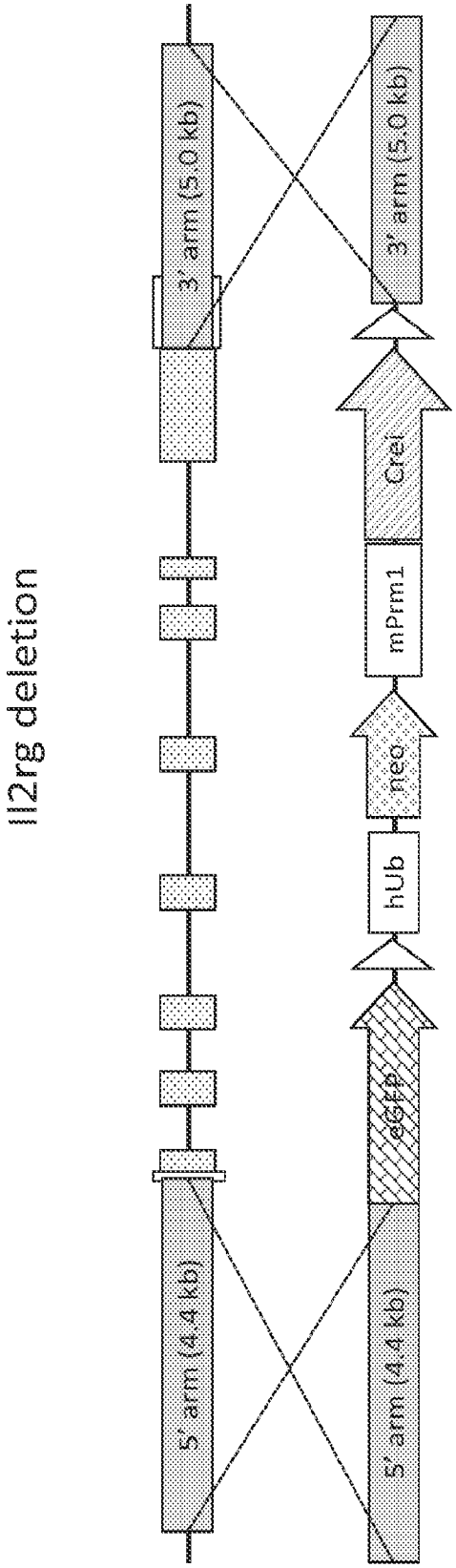

Il2rg deletion
5' arm (4.4 kb)
3' arm (5.0 kb)
eGFP
hUb
neo
mPrm1
Cre
5' arm (4.4 kb)
3' arm (5.0 kb)

FIG. 24

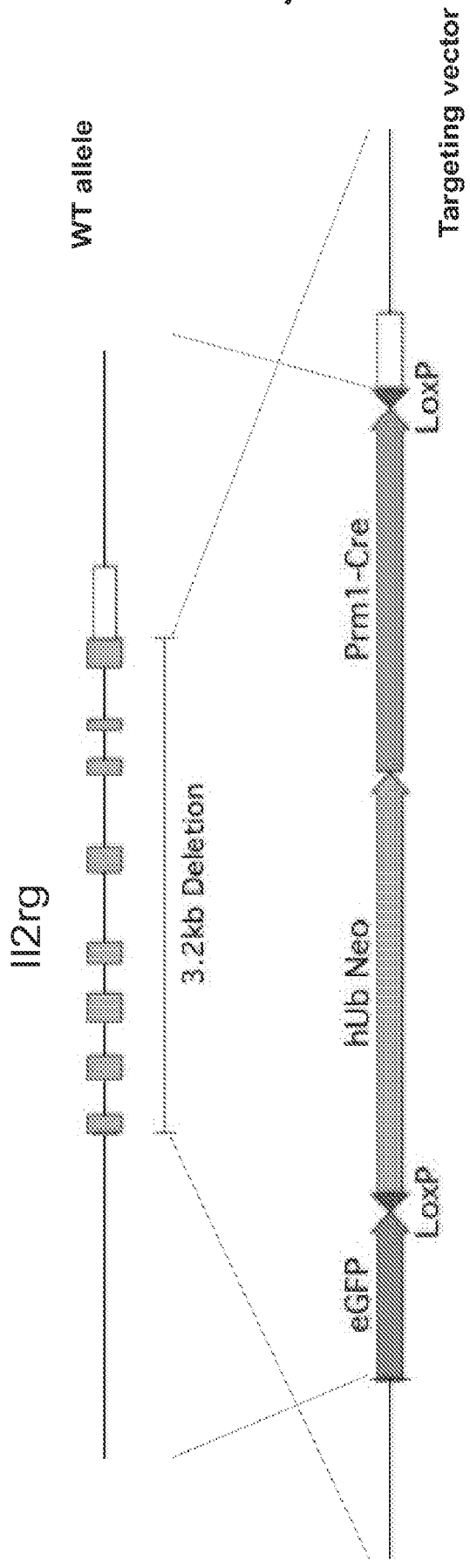

WT allele
Targeting vector
Il2rg
3.2kb Deletion
Prm1-Cre
hUb Neo
eGFP
LoxP
LoxP

FIG. 25

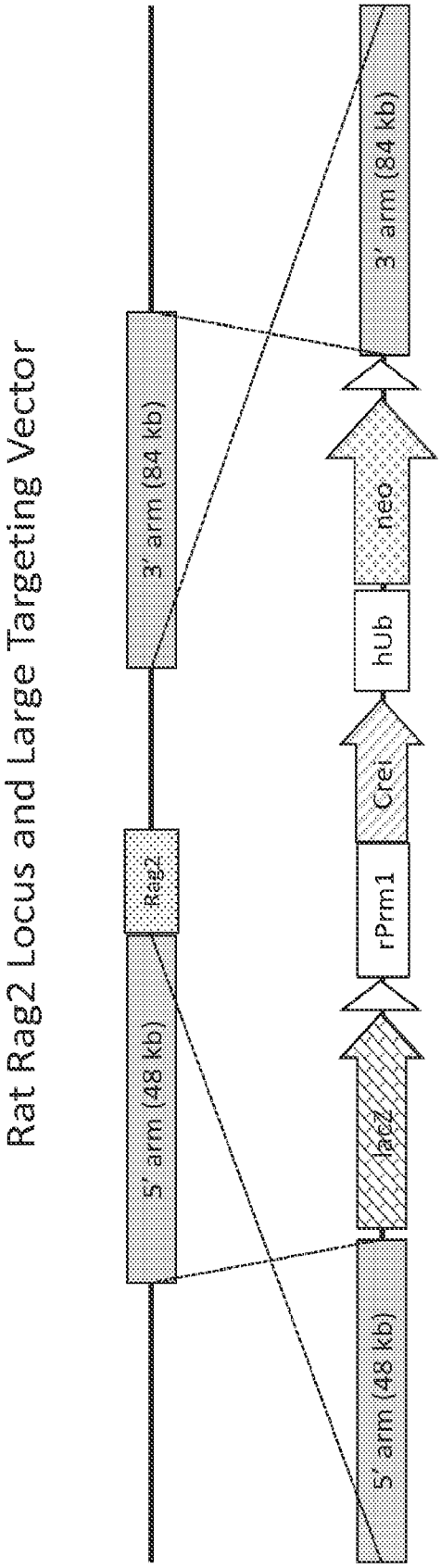

Rat Rag2 Locus and Large Targeting Vector
3' arm (84 kb)
Rag2
5' arm (48 kb)
3' arm (84 kb)
neo
hUb
Cre1
rPrm1
lacZ
5' arm (48 kb)

FIG. 26

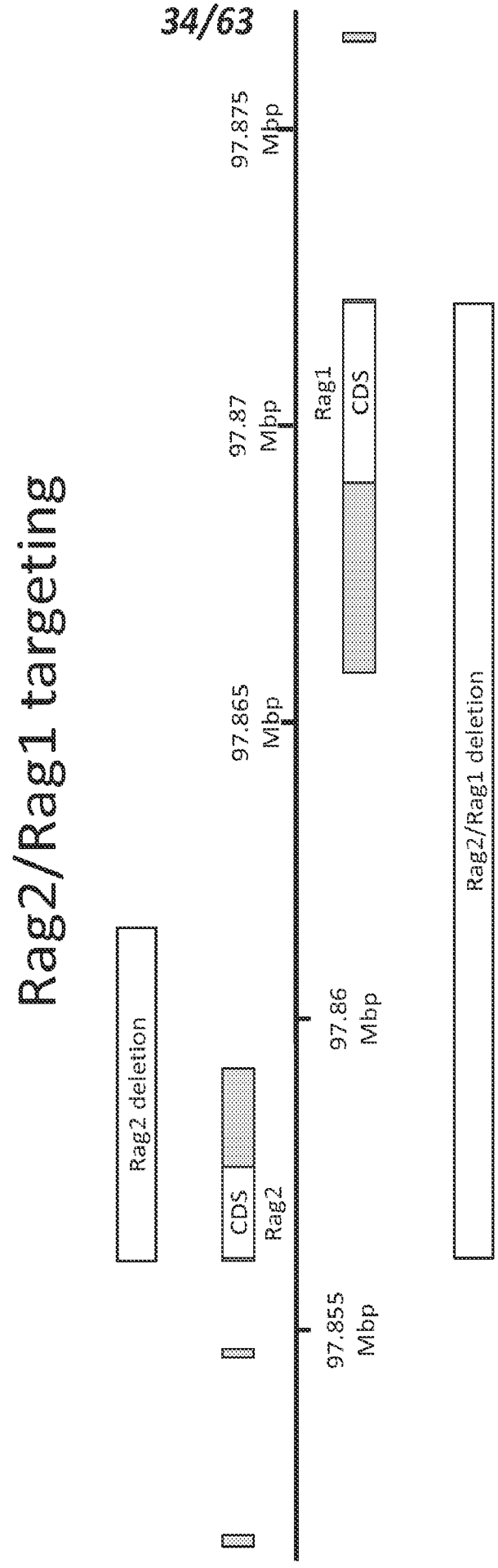

Rag2/Rag1 targeting
Rag2 deletion
CDS
Rag2
Rag1
CDS
Rag2/Rag1 deletion
97.855 Mbp
97.86 Mbp
97.865 Mbp
97.87 Mbp
97.875 Mbp

FIG. 27

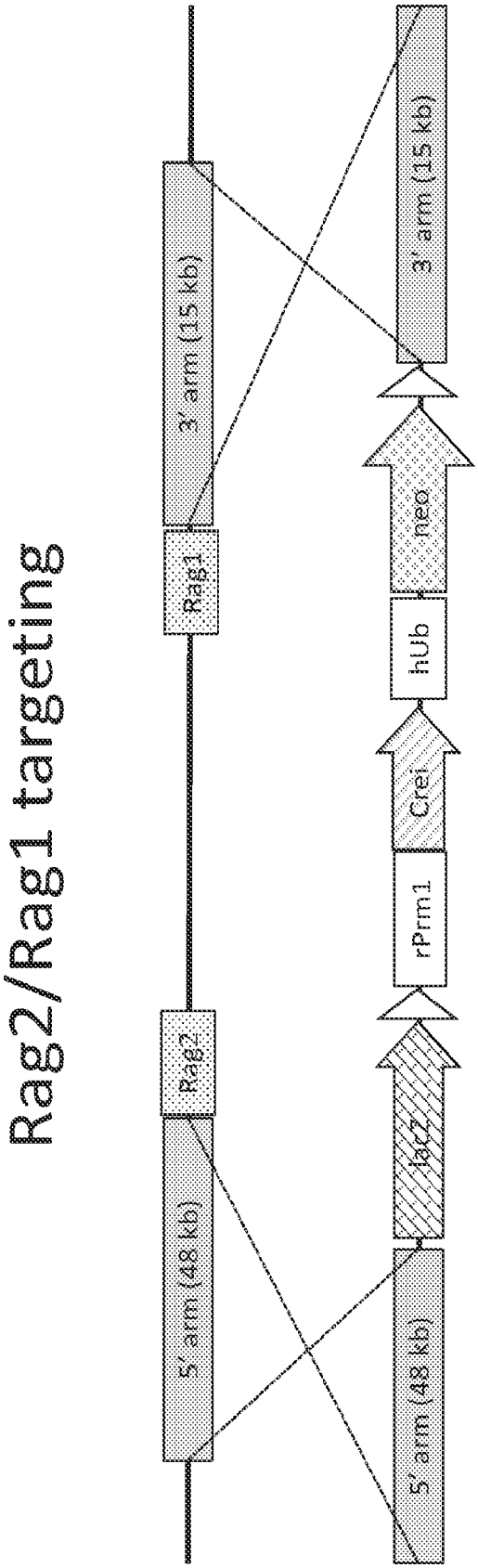
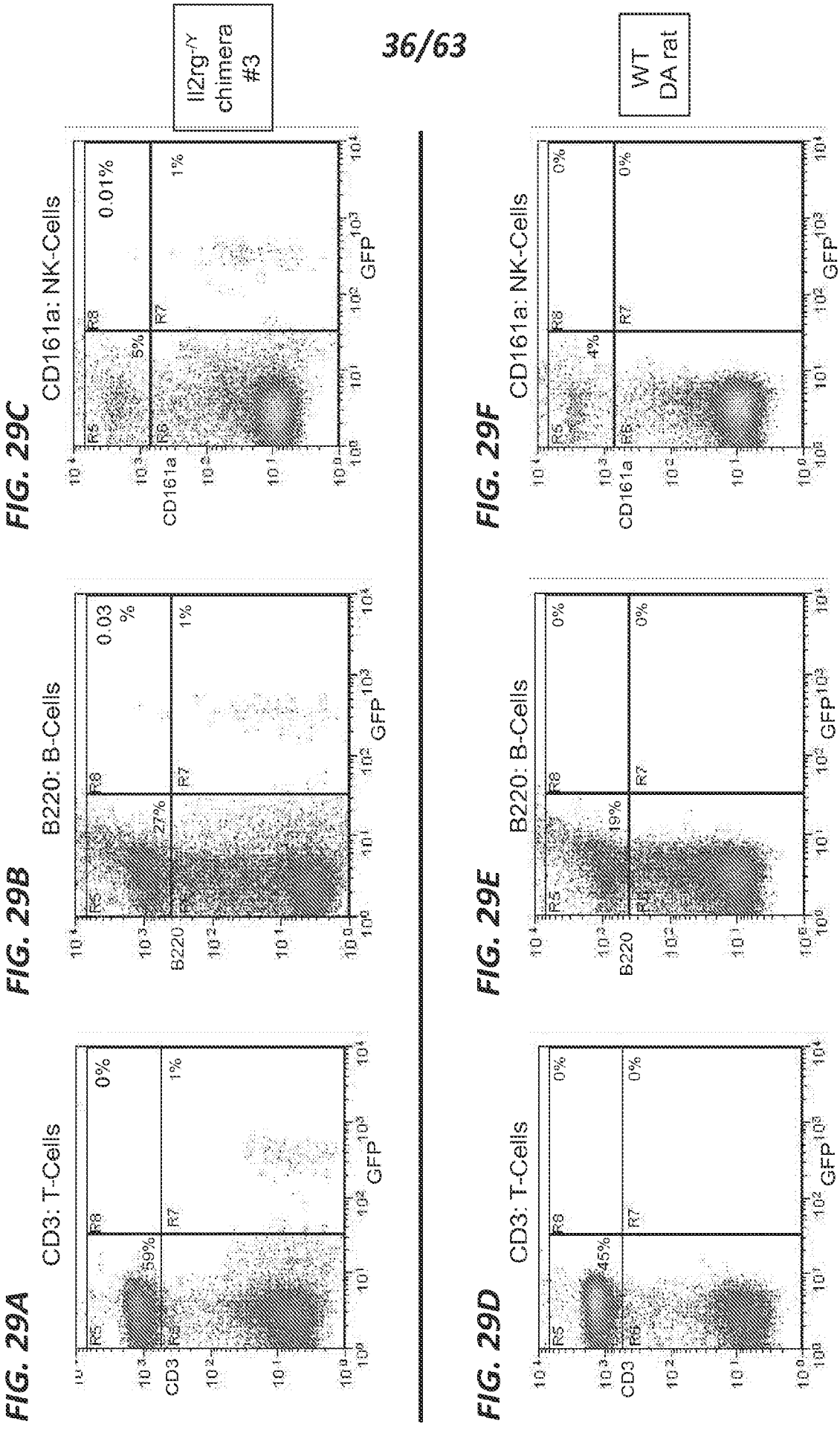

Rag2/Rag1 targeting
5' arm (48 kb)
Rag2
3' arm (15 kb)
Rag1
5' arm (48 kb)
iaC2
rPrm1
Crei
hUb
neo
3' arm (15 kb)

FIG. 28


FIG. 29A
CD3: T-Cells
CD3
GFP
R5
R6
R8
R7
59%
0%
1%
FIG. 29B
B220: B-Cells
B220
GFP
R5
R6
R8
R7
27%
0.03 %
1%
FIG. 29C
CD161a: NK-Cells
CD161a
GFP
R5
R6
R8
R7
5%
0.01%
1%
Il2rg-/Y chimera #3
FIG. 29D
CD3: T-Cells
CD3
GFP
R5
R6
R8
R7
45%
0%
0%
FIG. 29E
B220: B-Cells
B220
GFP
R5
R6
R8
R7
19%
0%
0%
FIG. 29F
CD161a: NK-Cells
CD161a
GFP
R5
R6
R8
R7
4%
0%
0%
WT DA rat

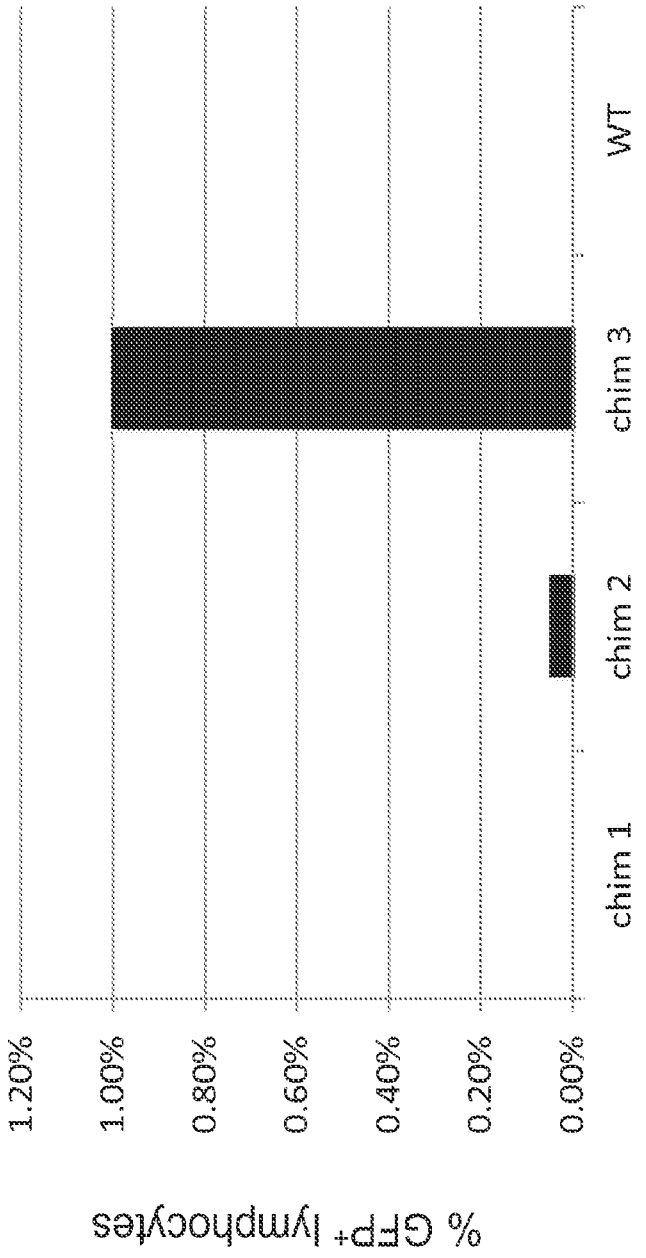

% GFP+ lymphocytes
1.20%
1.00%
0.80%
0.60%
0.40%
0.20%
0.00%
chim 1
chim 2
chim 3
WT

FIG. 30

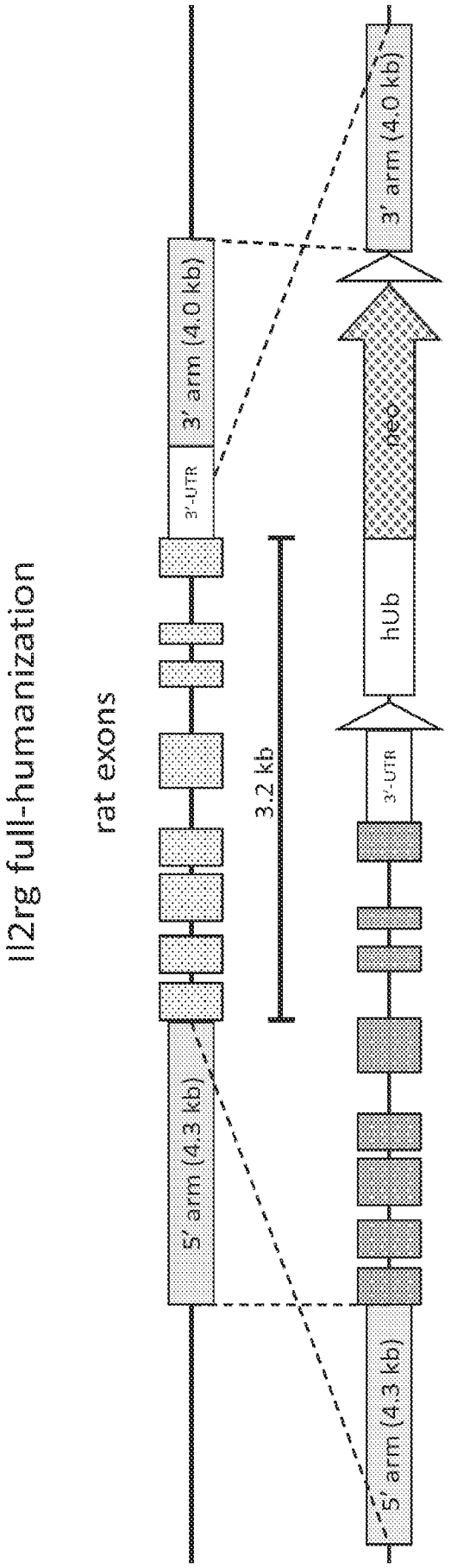

Il2rg full-humanization
rat exons
5' arm (4.3 kb)
3' arm (4.0 kb)
3'-UTR
3.2 kb
hUb
neo
3'-UTR
5' arm (4.3 kb)
3' arm (4.0 kb)
human exons

FIG. 31

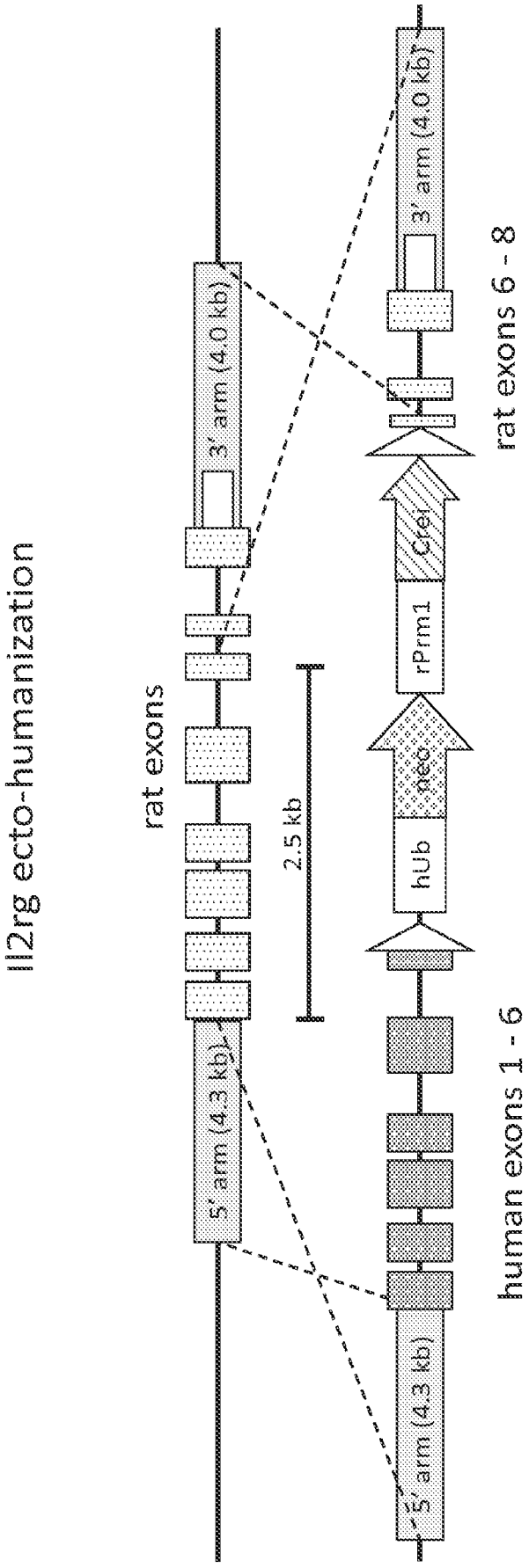

Il2rg ecto-humanization
rat exons
5' arm (4.3 kb)
2.5 kb
3' arm (4.0 kb)
human exons 1 - 6
hUb
neo
rPrm1
Cre
rat exons 6 - 8
3' arm (4.0 kb)
5' arm (4.3 kb)

FIG. 32

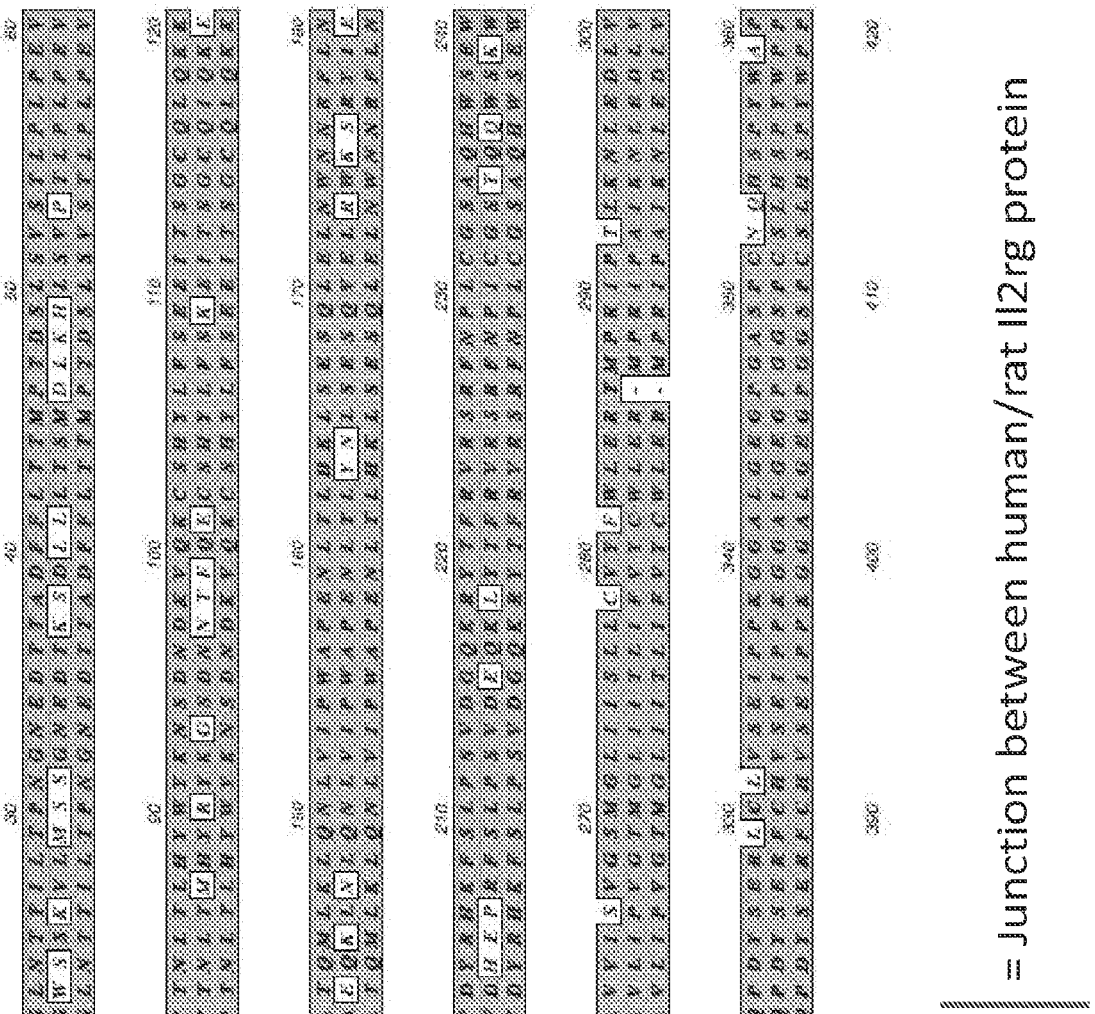
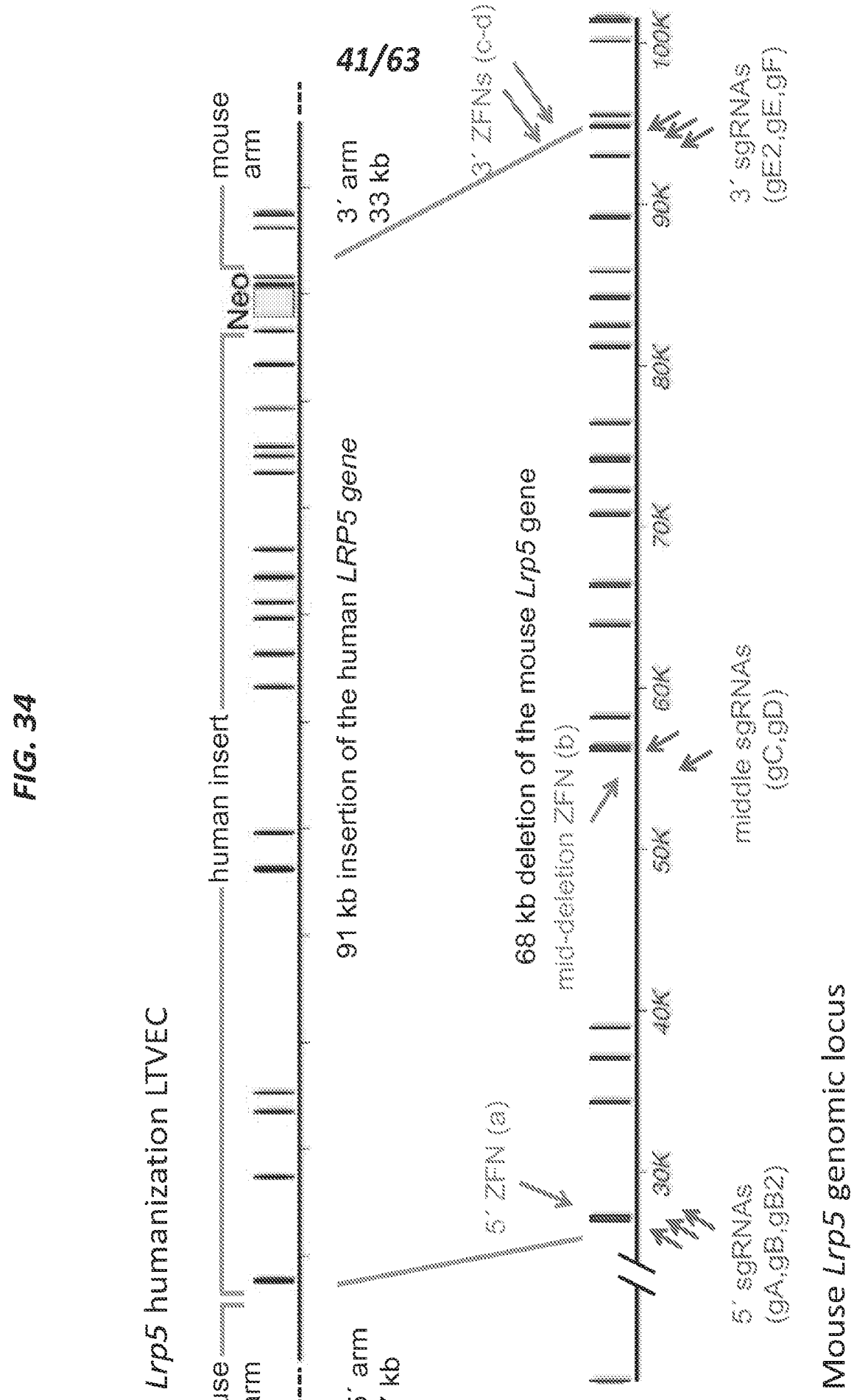

Hsa_Il2rg_NP_000197.1
Rno_Il2rg_NP_543165.1
Rsgn_Il2rg_human
= Junction between human/rat Il2rg protein
(SEQ ID NO: 20)
(SEQ ID NO: 21)
(SEQ ID NO: 22)

FIG. 33

FIG. 34


Lrp5 humanization LTVEC
mouse arm
human insert
mouse arm
Neo
5´ arm 7 kb
91 kb insertion of the human LRP5 gene
3´ arm 33 kb
3´ ZFNs (c-d)
68 kb deletion of the mouse Lrp5 gene
mid-deletion ZFN (b)
5´ ZFN (a)
30K
40K
50K
60K
70K
80K
90K
100K
5´ sgRNAs (gA,gB,gB2)
middle sgRNAs (gC,gD)
3´ sgRNAs (gE2,gE,gF)
Mouse Lrp5 genomic locus

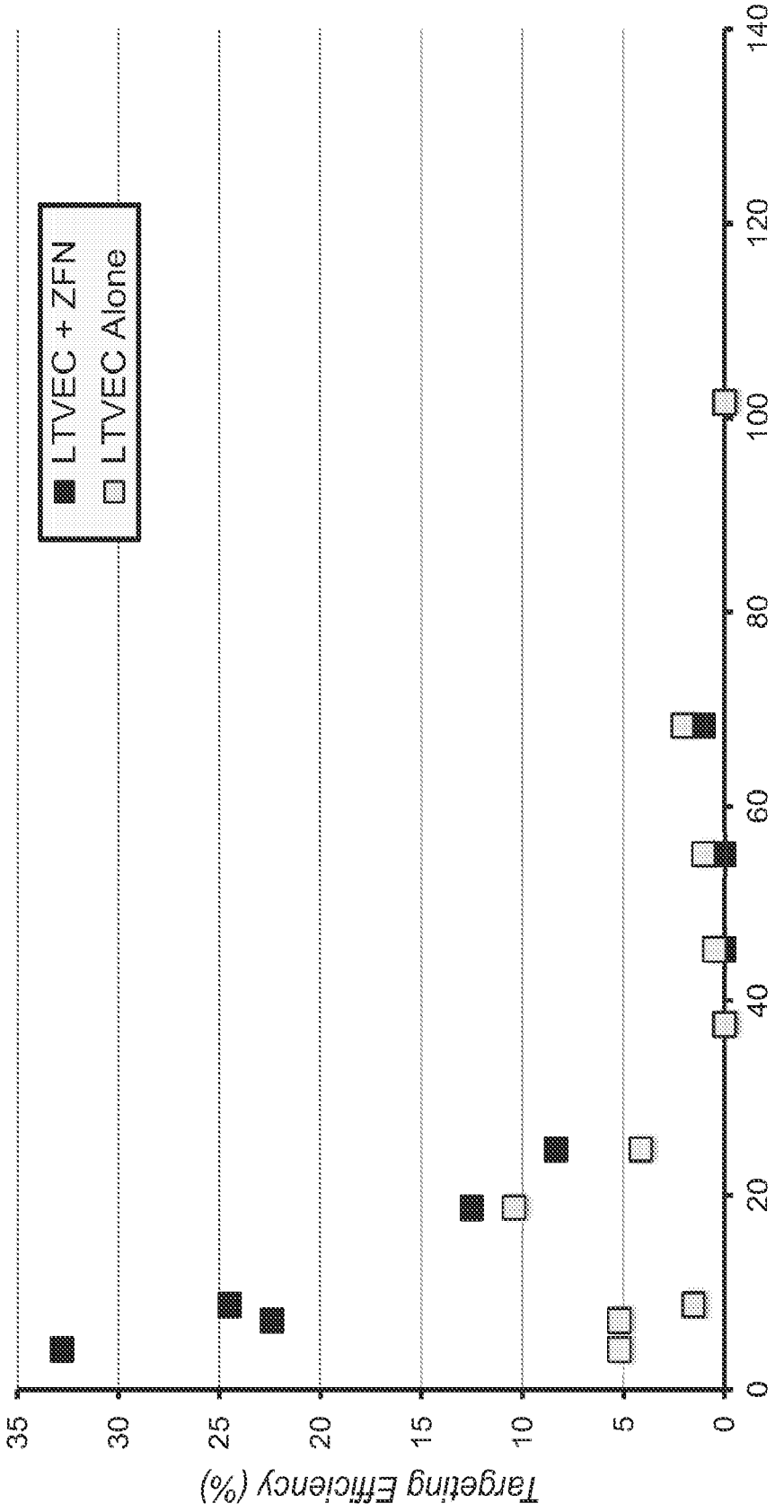

LTVEC + ZFN
LTVEC Alone
Targeting Efficiency (%)
35
30
25
20
15
10
5
0
0
20
40
60
80
100
120
140
Mouse Gene Deletion Size (kb)

FIG. 35A

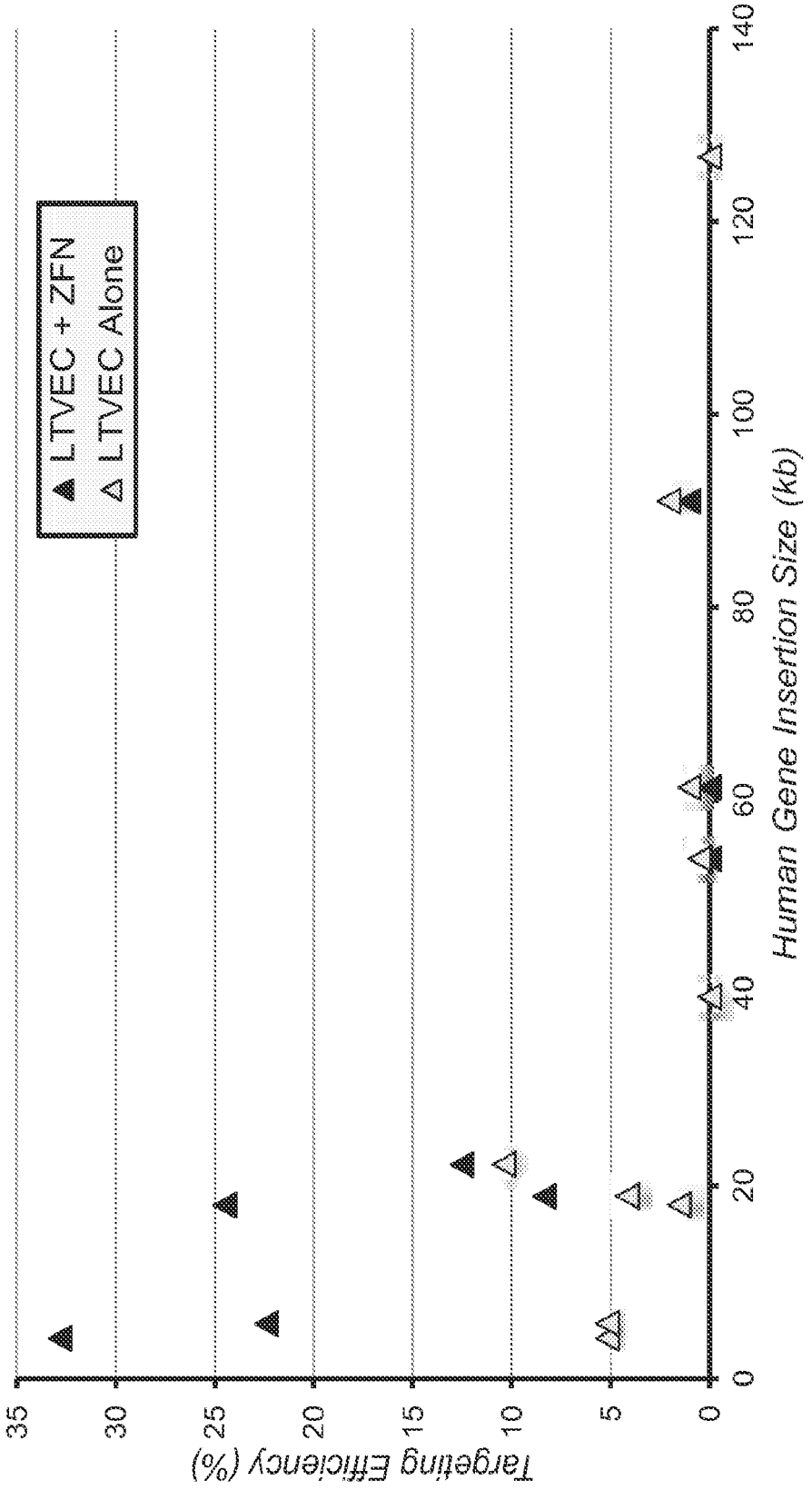

▲ LTVEC + ZFN
△ LTVEC Alone
Targeting Efficiency (%)
0
5
10
15
20
25
30
35
0
20
40
60
80
100
120
140
Human Gene Insertion Size (kb)

FIG. 35B

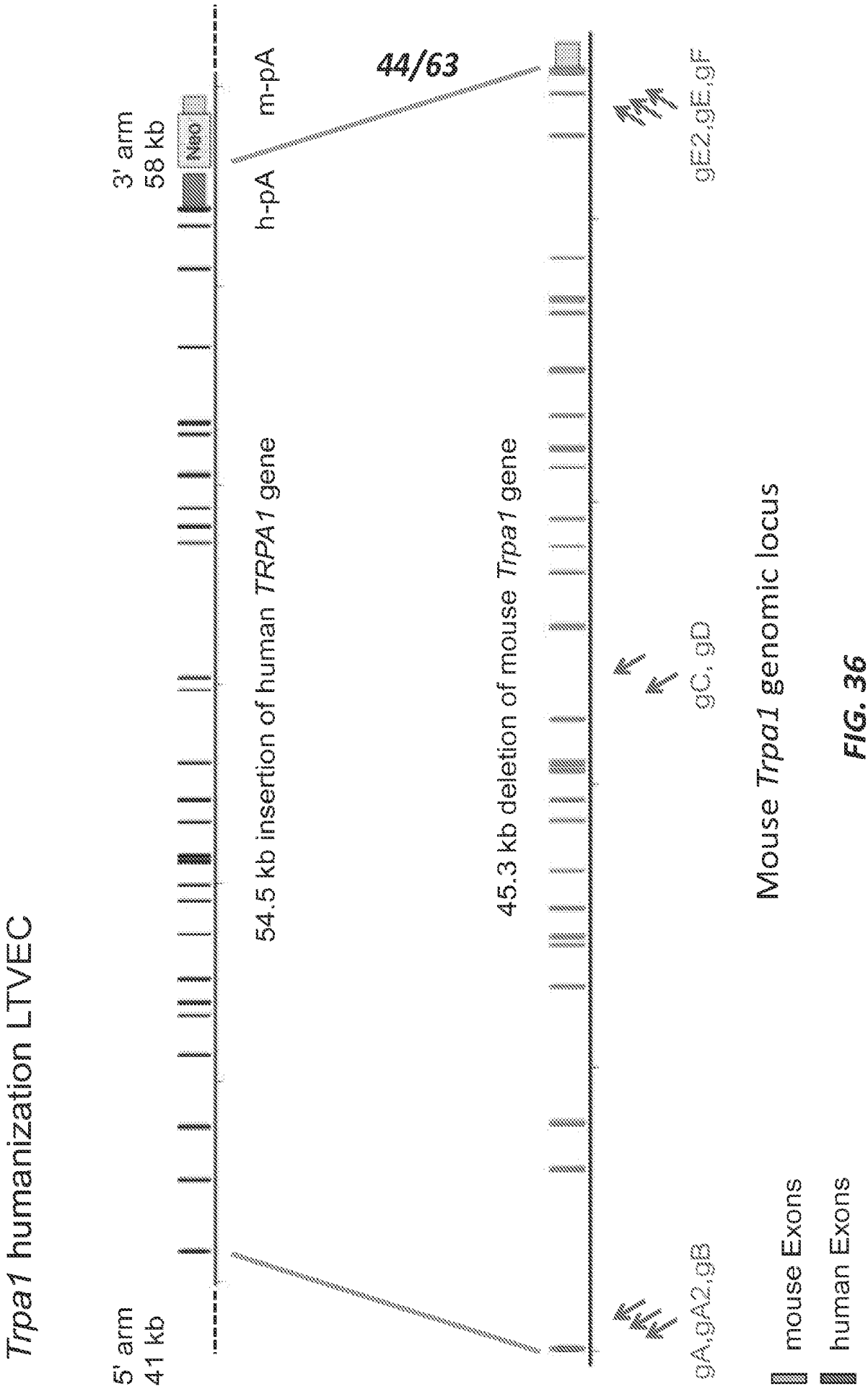

Trpa1 humanization LTVEC
5' arm
41 kb
3' arm
58 kb
Neo
h-pA
m-pA
44/63
54.5 kb insertion of human TRPA1 gene
45.3 kb deletion of mouse Trpa1 gene
gA,gA2,gB
gC, gD
gE2,gE,gF
Mouse Trpa1 genomic locus
mouse Exons
human Exons

FIG. 36

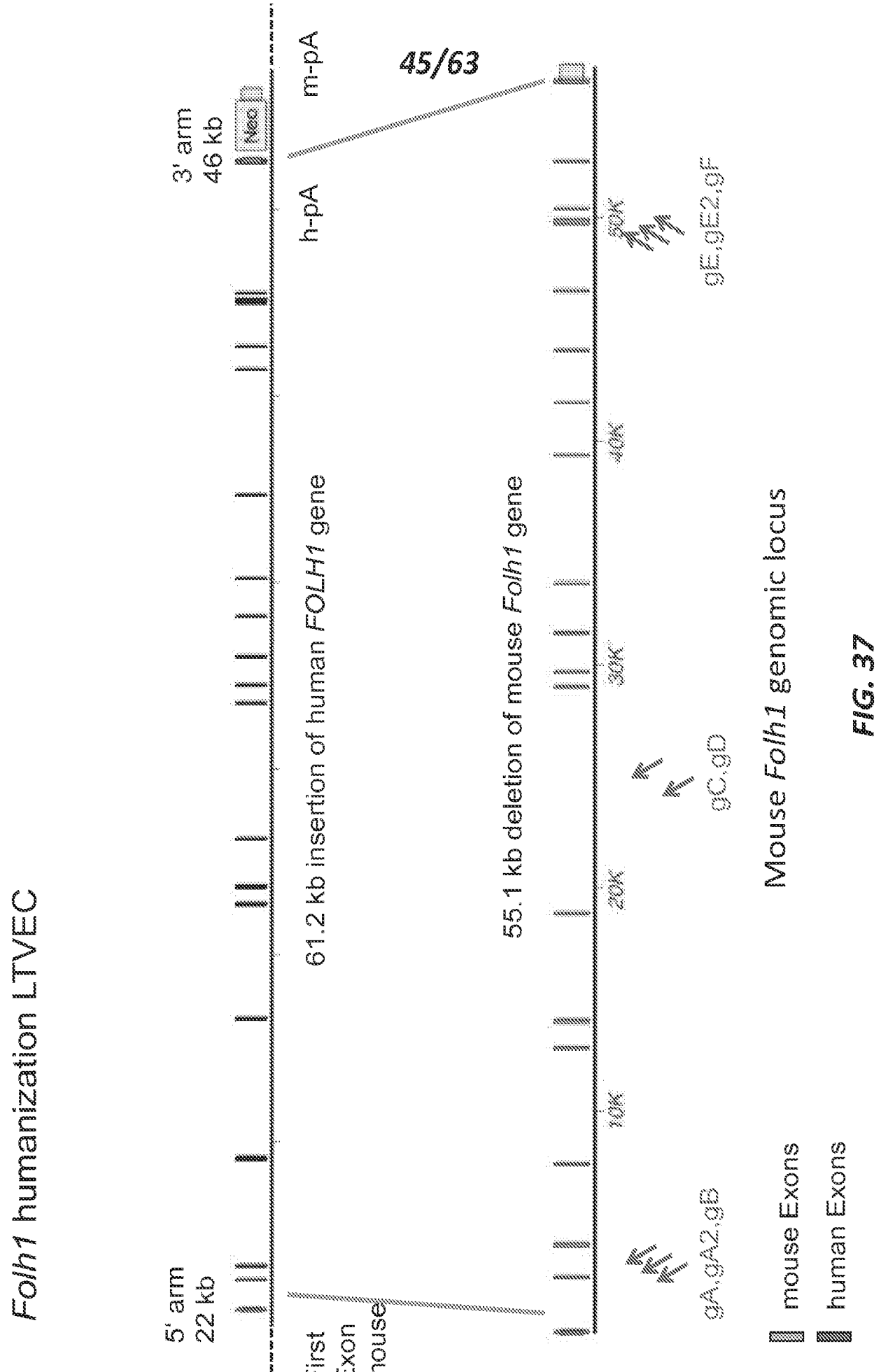

Folh1 humanization LTVEC
5' arm
22 kb
First
Exon
mouse
61.2 kb insertion of human FOLH1 gene
h-pA
3' arm
46 kb
Neo
m-pA
45/63
55.1 kb deletion of mouse Folh1 gene
10K
20K
30K
40K
50K
gA,gA2,gB
gC,gD
gE,gE2,gF
Mouse Folh1 genomic locus
mouse Exons
human Exons

FIG. 37

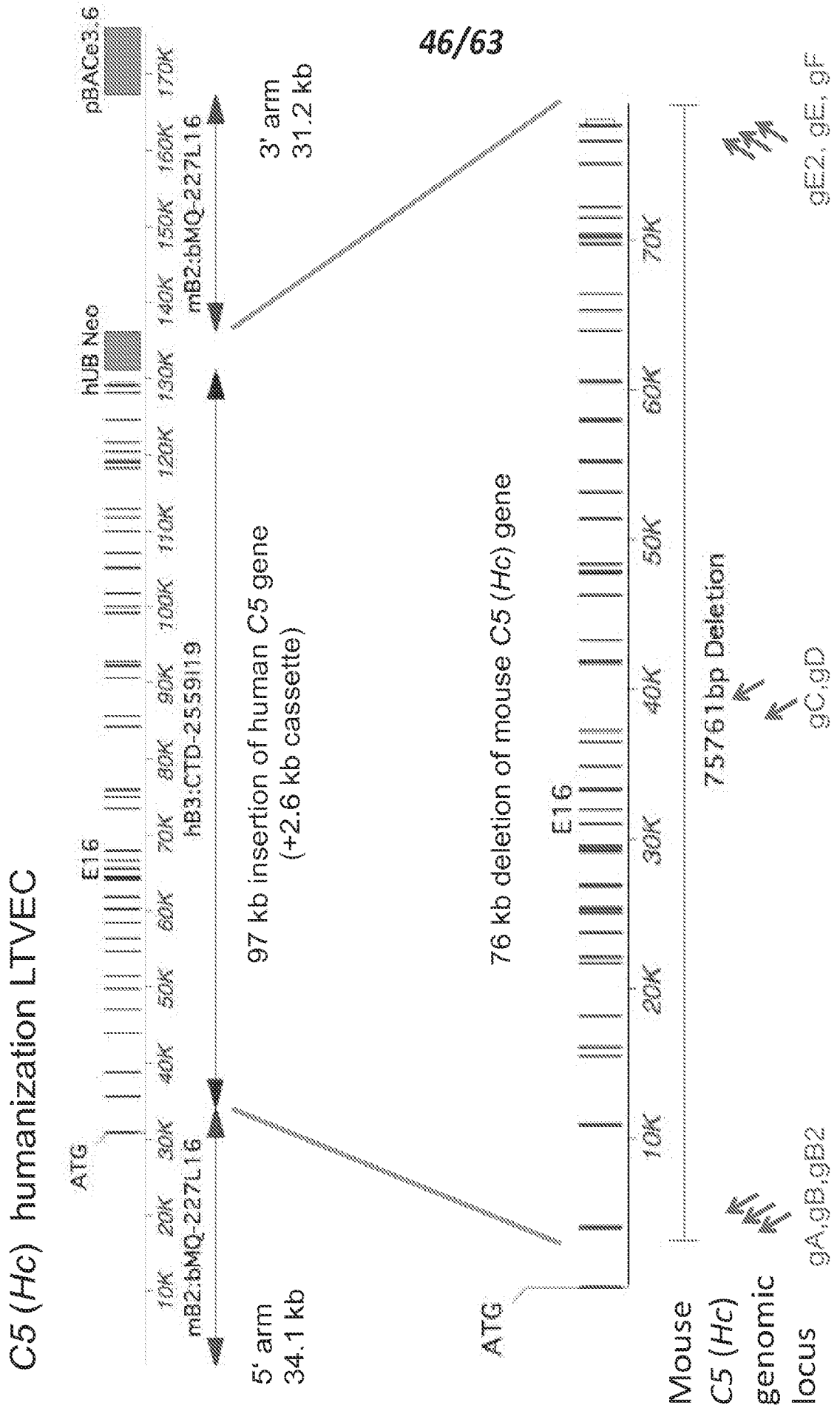

C5 (Hc) humanization LTVEC
pBACe3.6
hUB Neo
E16
ATG
10K 20K 30K 40K 50K 60K 70K 80K 90K 100K 110K 120K 130K 140K 150K 160K 170K
mB2:bMQ-227L16
hB3:CTD-2559I19
mB2:bMQ-227L16
3' arm
31.2 kb
97 kb insertion of human C5 gene
(+2.6 kb cassette)
5' arm
34.1 kb
76 kb deletion of mouse C5 (Hc) gene
E16
ATG
10K 20K 30K 40K 50K 60K 70K
757761bp Deletion
Mouse
C5 (Hc)
genomic
locus
gA,gB,gB2
gC,gD
gE2, gE, gF

FIG. 38

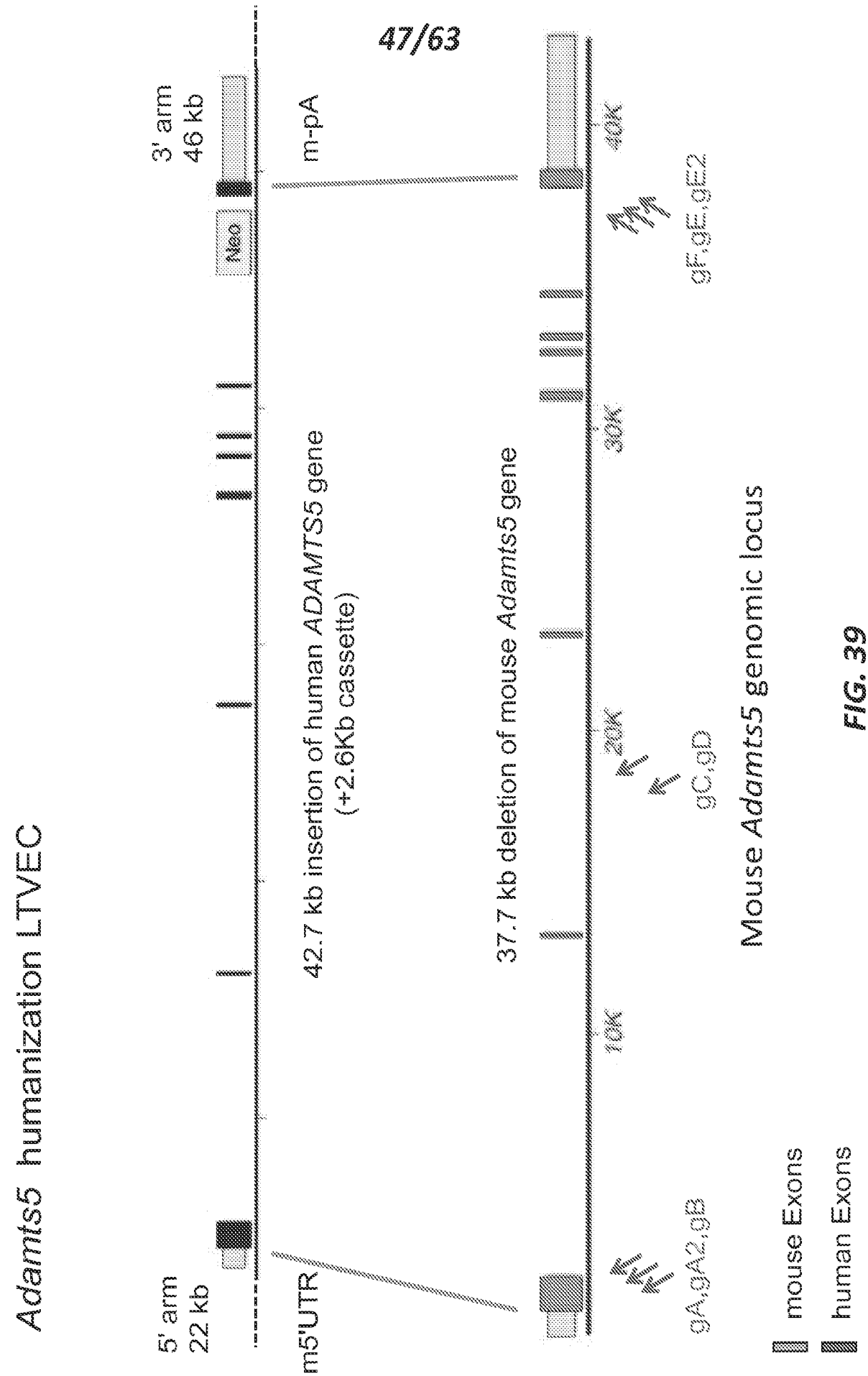

Adamts5 humanization LTVEC
5' arm
22 kb
m5'UTR
42.7 kb insertion of human ADAMTS5 gene
(+2.6Kb cassette)
37.7 kb deletion of mouse Adamts5 gene
3' arm
46 kb
Neo
m-pA
10K
20K
30K
40K
gA,gA2,gB
gC,gD
gF,gE,gE2
Mouse Adamts5 genomic locus
mouse Exons
human Exons

FIG. 39

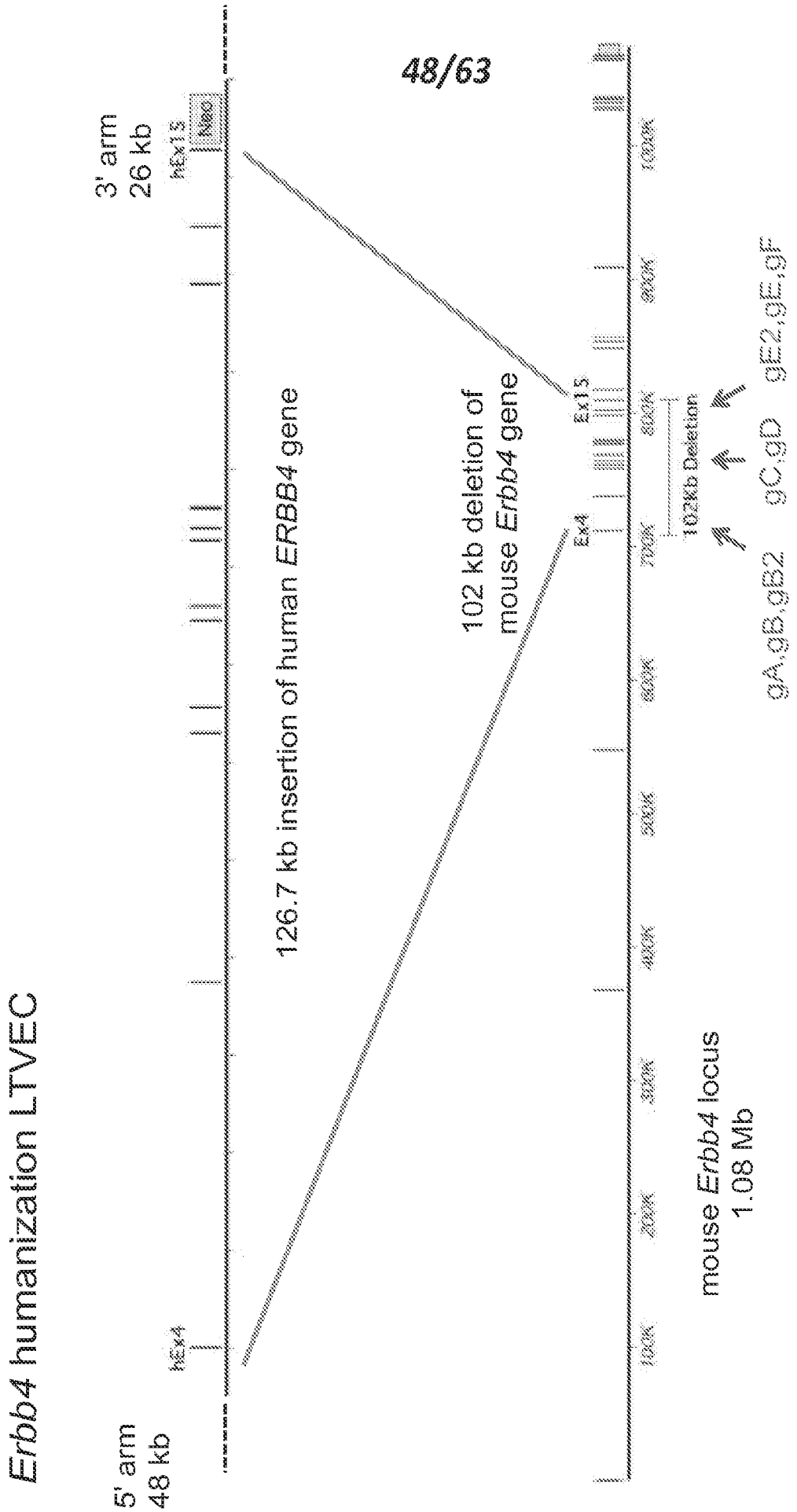

Erbb4 humanization LTVEC
5' arm
48 kb
hEx4
3' arm
26 kb
hEx15
Neo
126.7 kb insertion of human ERBB4 gene
102 kb deletion of
mouse Erbb4 gene
Ex4
Ex15
102Kb Deletion
gA,gB,gB2
gC,gD
gE2,gE,gF
mouse Erbb4 locus
1.08 Mb
Mouse Erbb4 genomic locus

FIG. 40

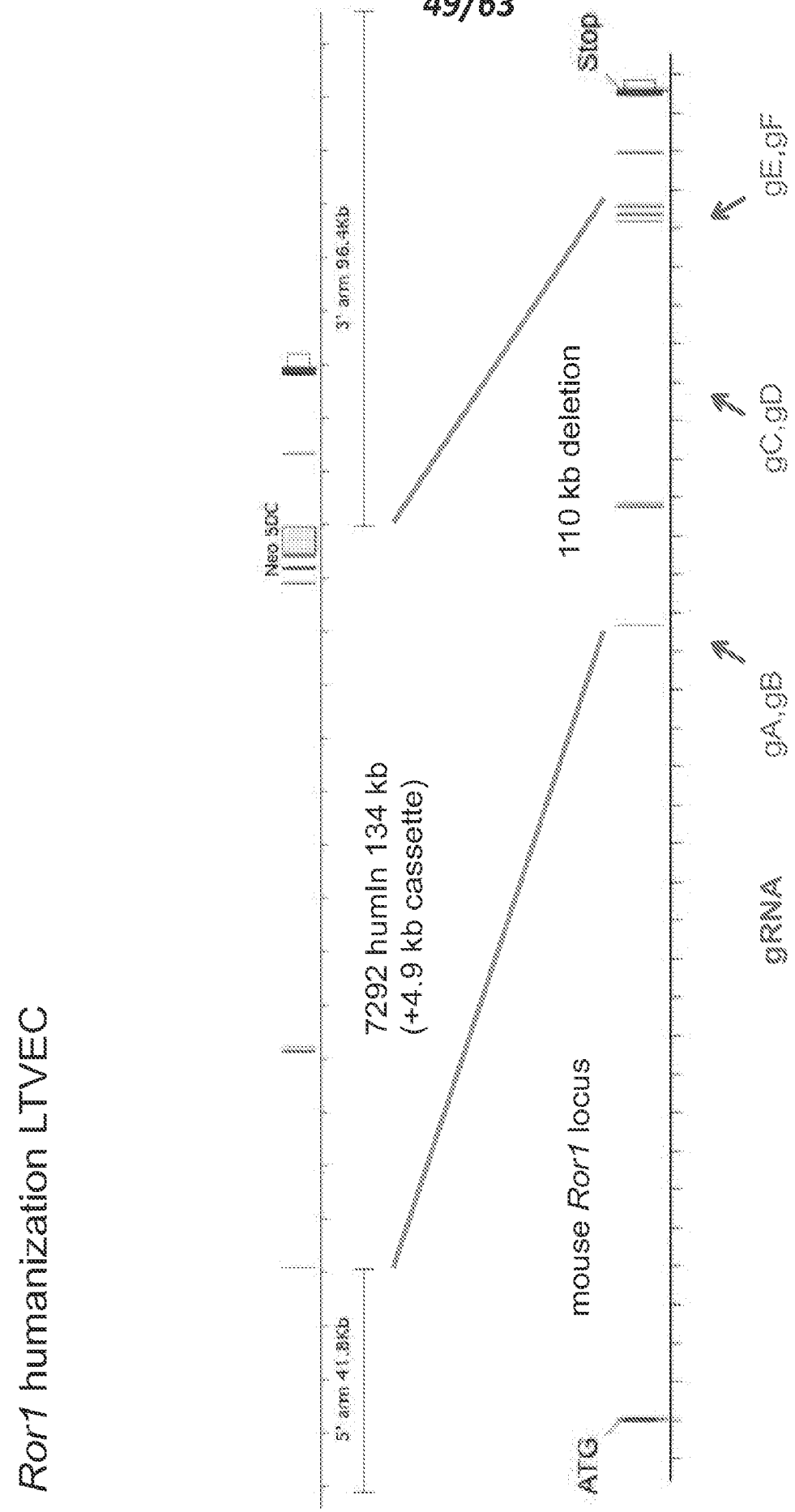

Ror1 humanization LTVEC
5' arm 41.8kb
Neo SDC
3' arm 96.4kb
7292 humIn 134 kb
(+4.9 kb cassette)
mouse Ror1 locus
110 kb deletion
ATG
Stop
gRNA
gA,gB
gC,gD
gE,gF

FIG. 41

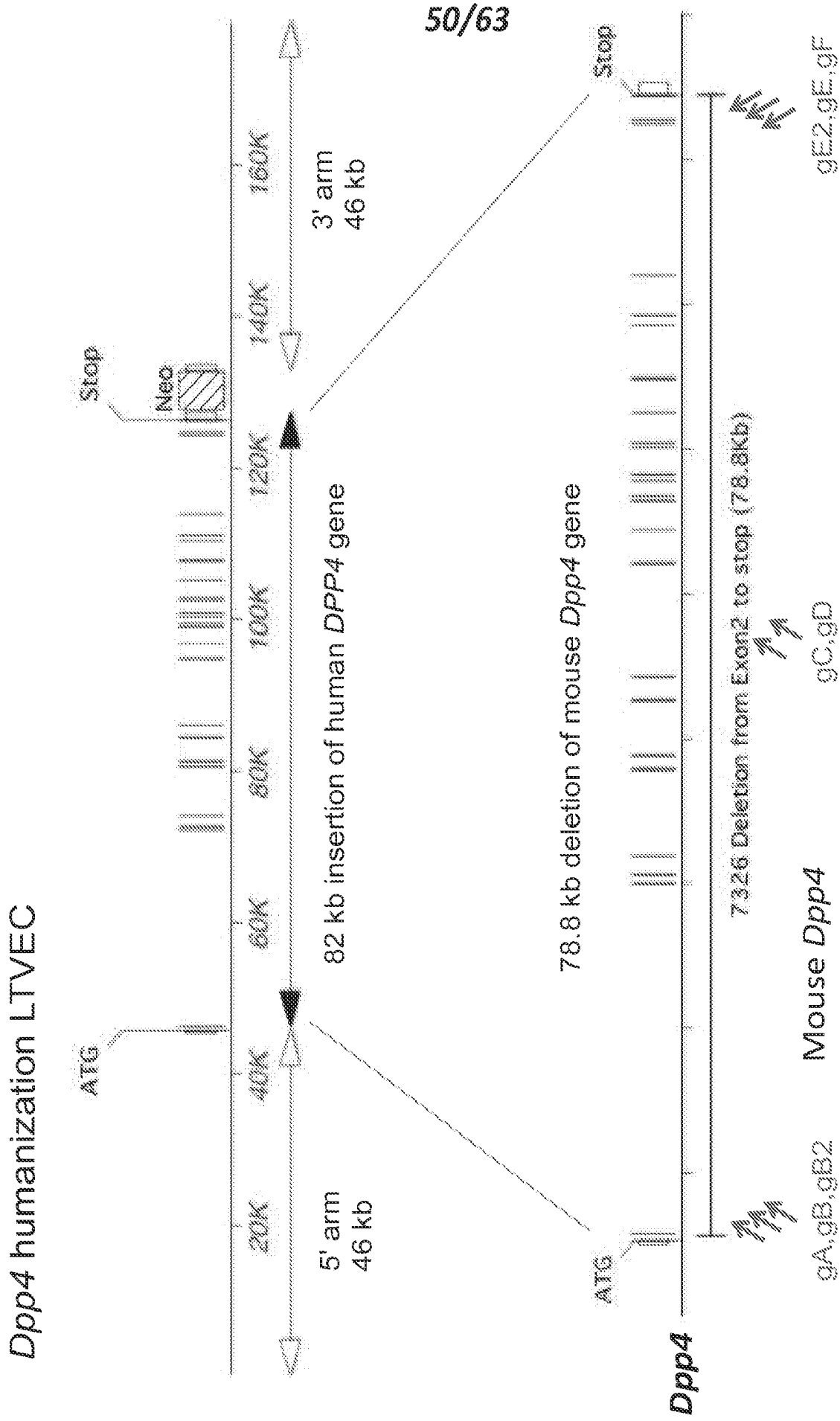

Dpp4 humanization LTVEC
ATG
Stop
Neo
20K
40K
60K
80K
100K
120K
140K
160K
5' arm
46 kb
3' arm
46 kb
82 kb insertion of human DPP4 gene
78.8 kb deletion of mouse Dpp4 gene
7326 Deletion from Exon2 to stop (78.8Kb)
gA,gB,gB2
gC,gD
gE2,gE,gF
Mouse Dpp4
genomic locus
ATG
Stop
Dpp4

FIG. 42

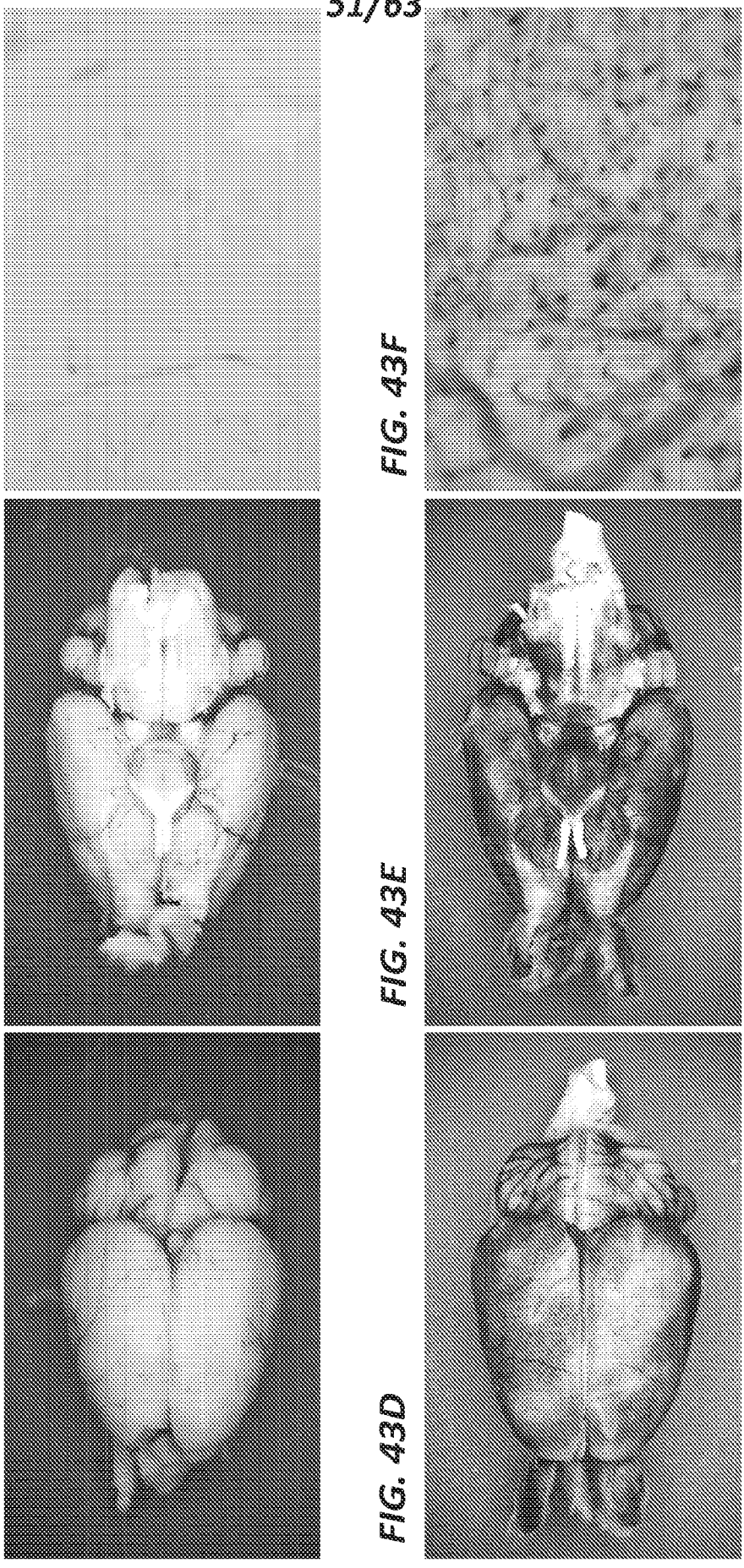
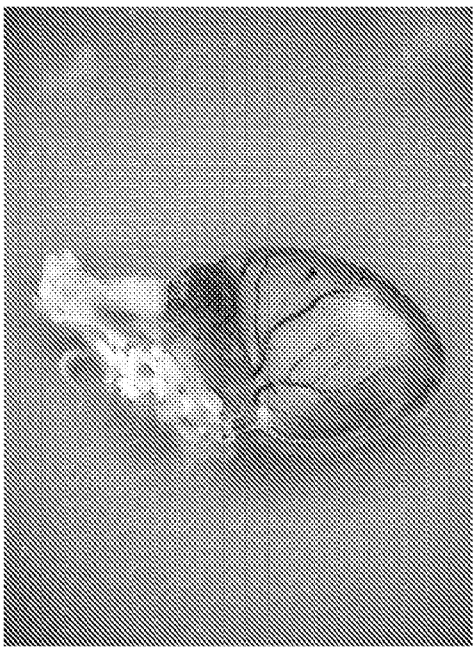

FIG. 43A
FIG. 43B
FIG. 43C
FIG. 43D
FIG. 43E
FIG. 43F
Wild type
ApoE+/-

FIG. 44A

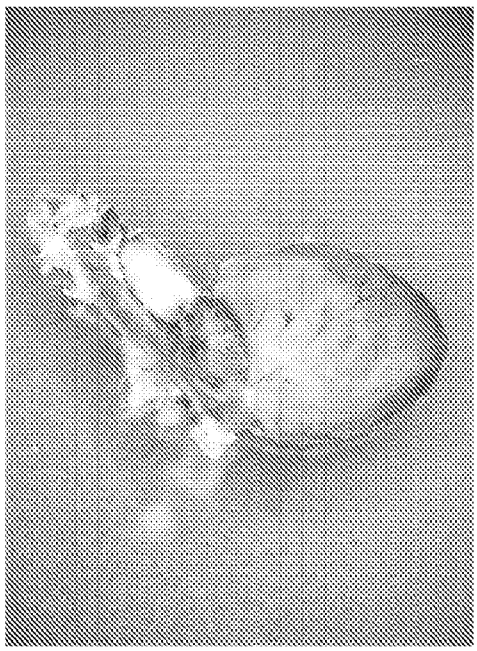


Wild type

FIG. 44B



FIG. 44C

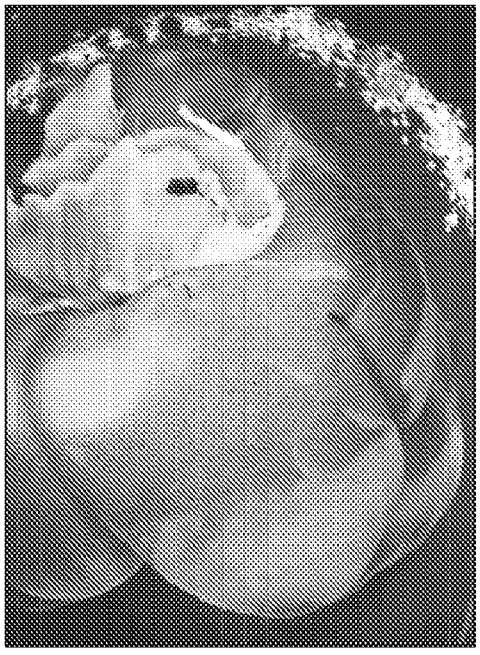


ApoE$^{+/-}$

FIG. 44D



FIG. 45A



Wild type

FIG. 45B

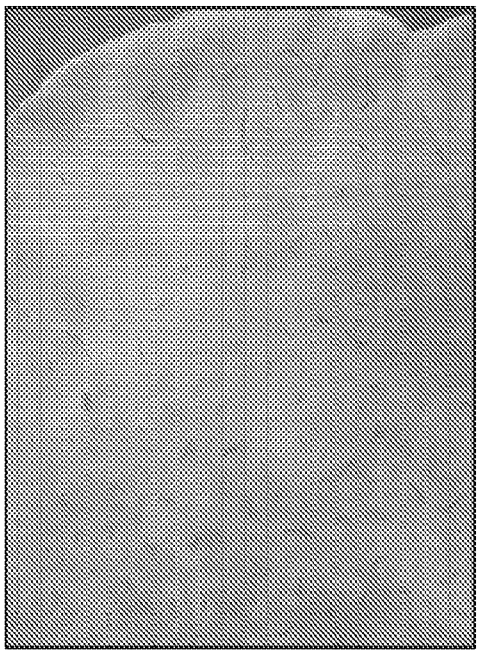
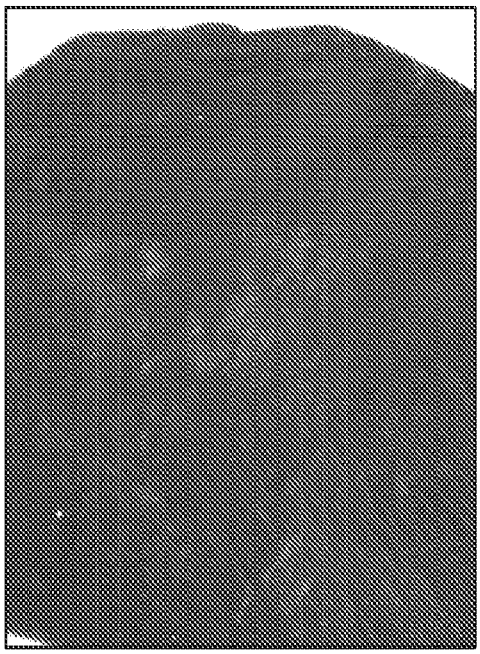


FIG. 45C



ApoE$^{+/-}$

FIG. 45D



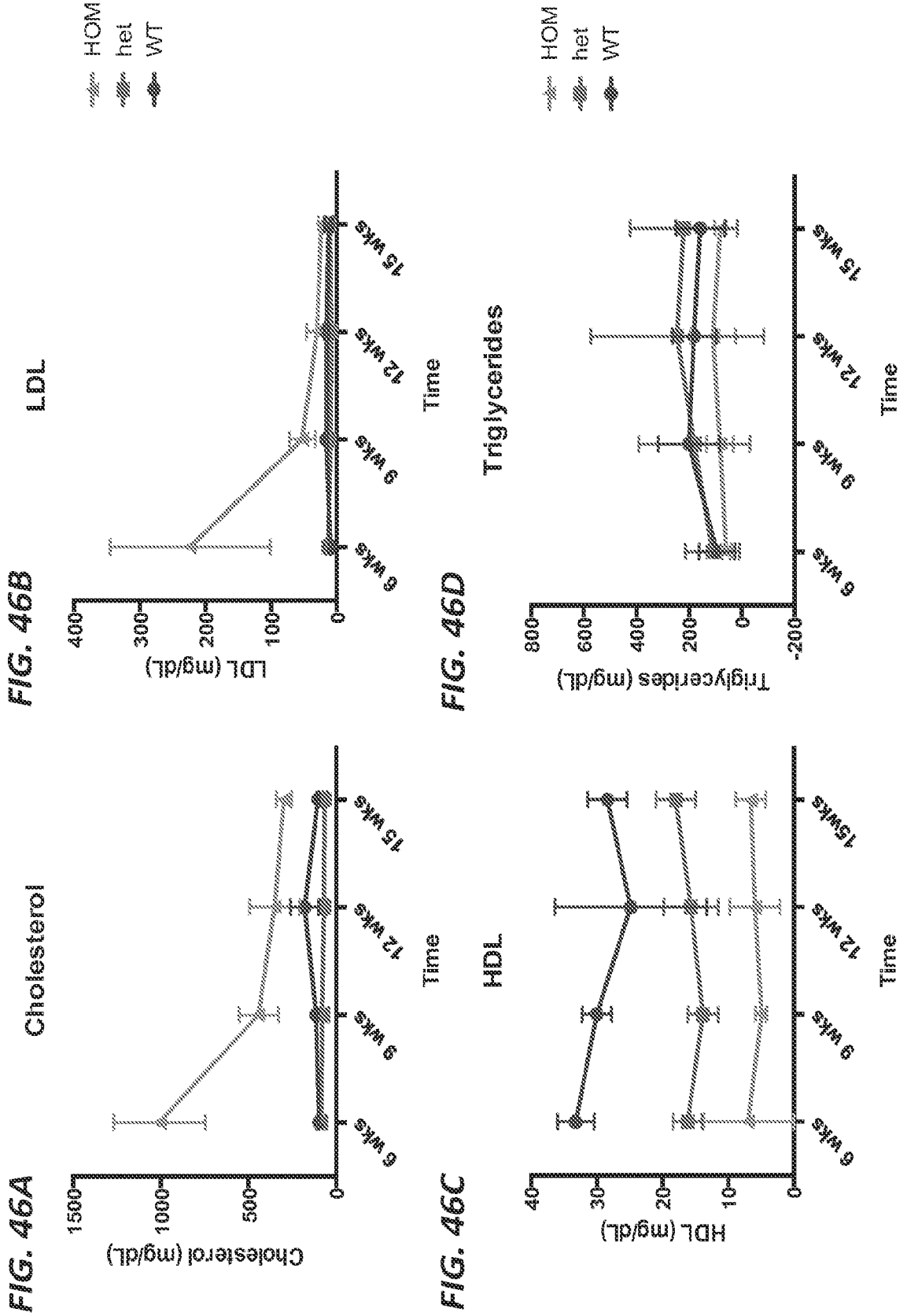

FIG. 46A
Cholesterol
Cholesterol (mg/dL)
1500
1000
500
0
6 wks
9 wks
12 wks
15 wks
Time
FIG. 46B
LDL
LDL (mg/dL)
400
300
200
100
0
HOM
het
WT
FIG. 46C
HDL
HDL (mg/dL)
40
30
20
10
0
FIG. 46D
Triglycerides
Triglycerides (mg/dL)
800
600
400
200
0
-200

FIG. 47

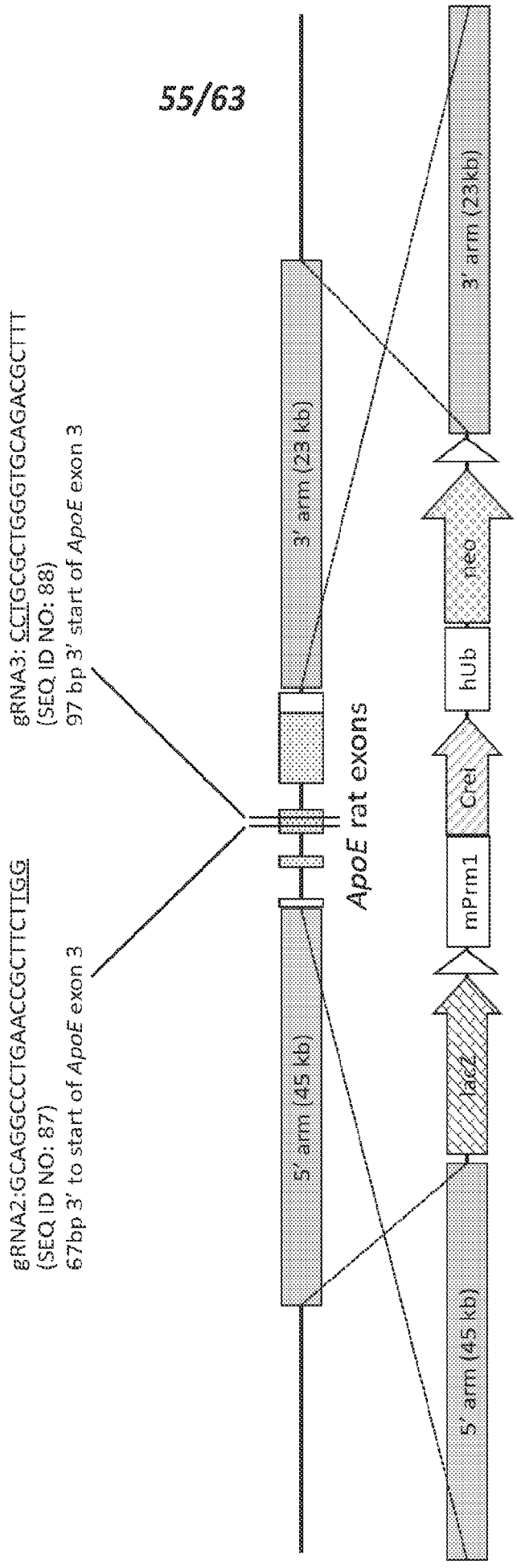

gRNA2:GCAGGCCCTGAACCGCTTCTTGG
(SEQ ID NO: 87)
67bp 3' to start of ApoE exon 3
gRNA3: CCTGCGCTGGGTGCAGACGCTTT
(SEQ ID NO: 88)
97 bp 3' start of ApoE exon 3
5' arm (45 kb)
3' arm (23 kb)
ApoE rat exons
lacZ
mPrm1
Crei
hUb
neo
3' arm (23kb)
5' arm (45 kb)

FIG. 48

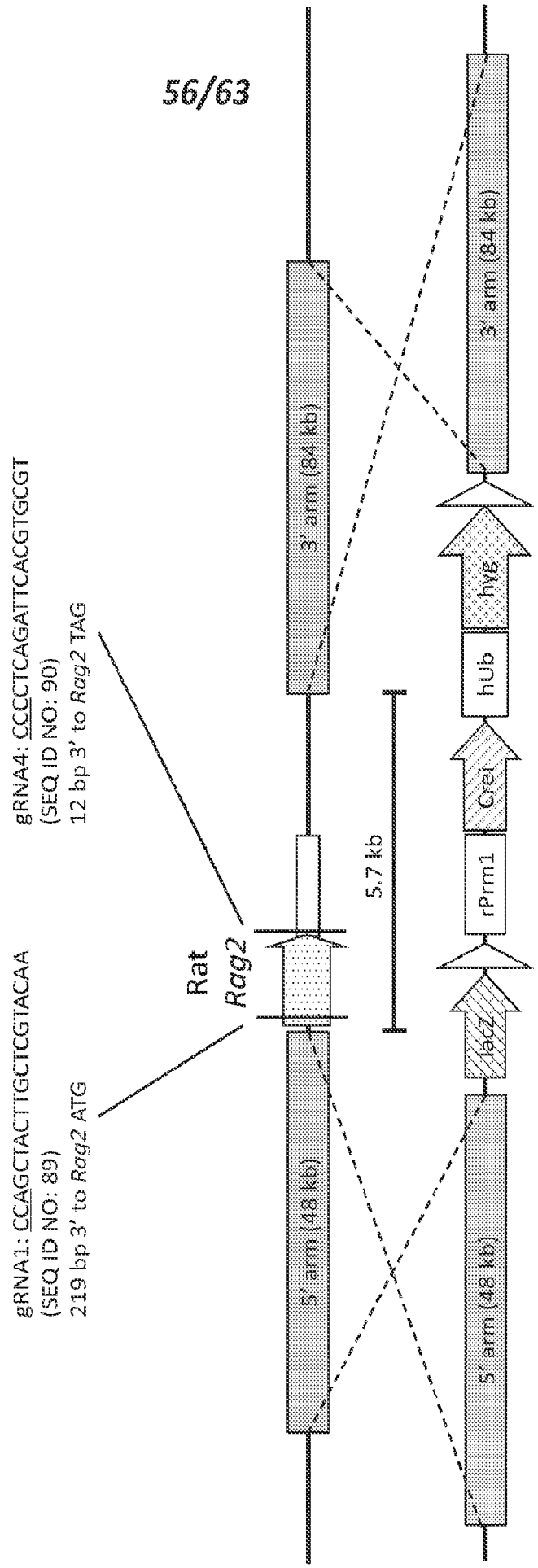
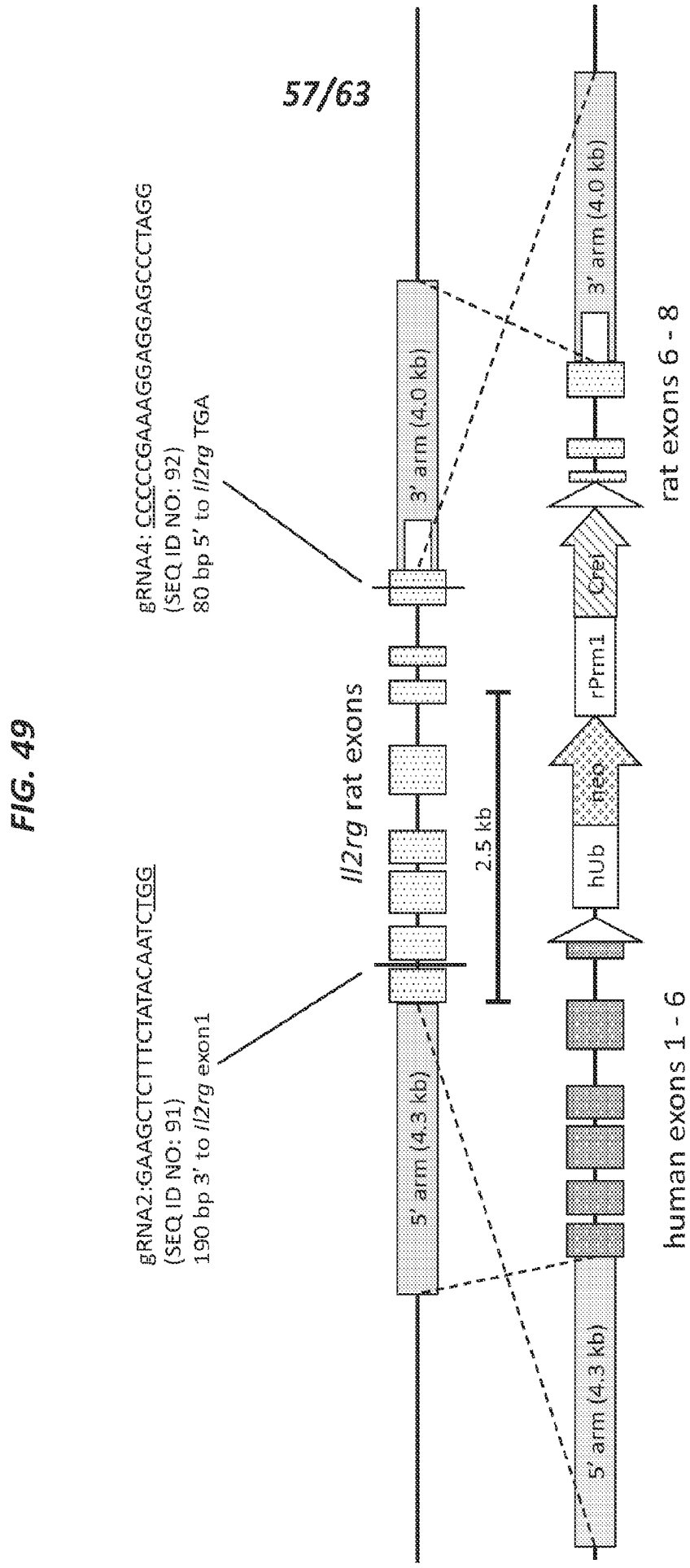

gRNA1: CCAGCTACTTGCTCGTACAA
(SEQ ID NO: 89)
219 bp 3' to Rag2 ATG
gRNA4: CCCCTCAGATTCACGTGCGT
(SEQ ID NO: 90)
12 bp 3' to Rag2 TAG
Rat
Rag2
5' arm (48 kb)
3' arm (84 kb)
5.7 kb
lacZ
rPrm1
Cre
hUb
hyg
5' arm (48 kb)
3' arm (84 kb)

FIG. 49


gRNA4: CCCCCGAAAGGAGGAGCCCTAGG
(SEQ ID NO: 92)
80 bp 5' to Il2rg TGA
gRNA2:GAAGCTCTTTCTATACAATCTGG
(SEQ ID NO: 91)
190 bp 3' to Il2rg exon1
Il2rg rat exons
2.5 kb
5' arm (4.3 kb)
hUb
neo
rPrm1
Cre
3' arm (4.0 kb)
human exons 1 - 6
rat exons 6 - 8

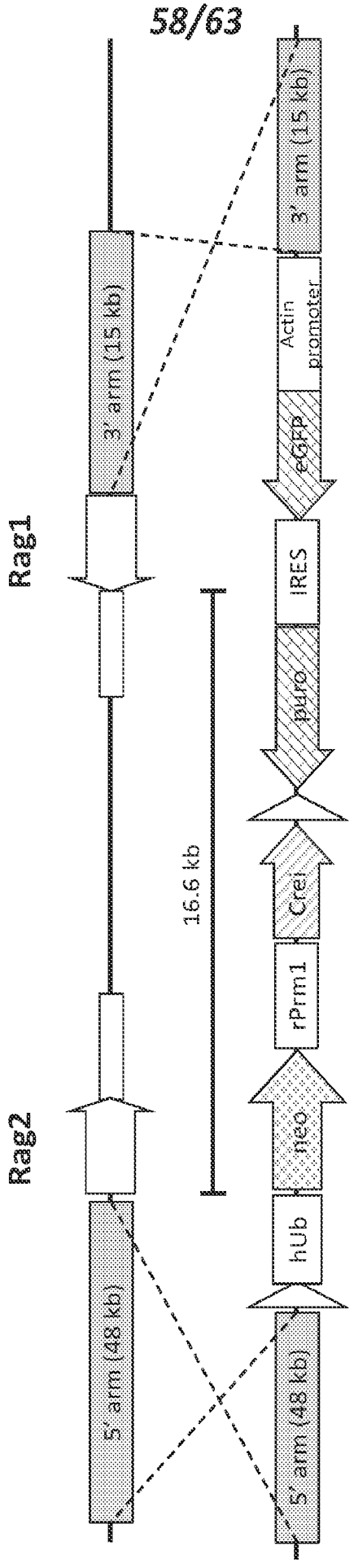

Rag1
Rag2
3' arm (15 kb)
5' arm (48 kb)
16.6 kb
hUb
neo
rPrm1
Cre1
IRES
puro
Actin promoter
eGFP
3' arm (15 kb)
5' arm (48 kb)

FIG. 50

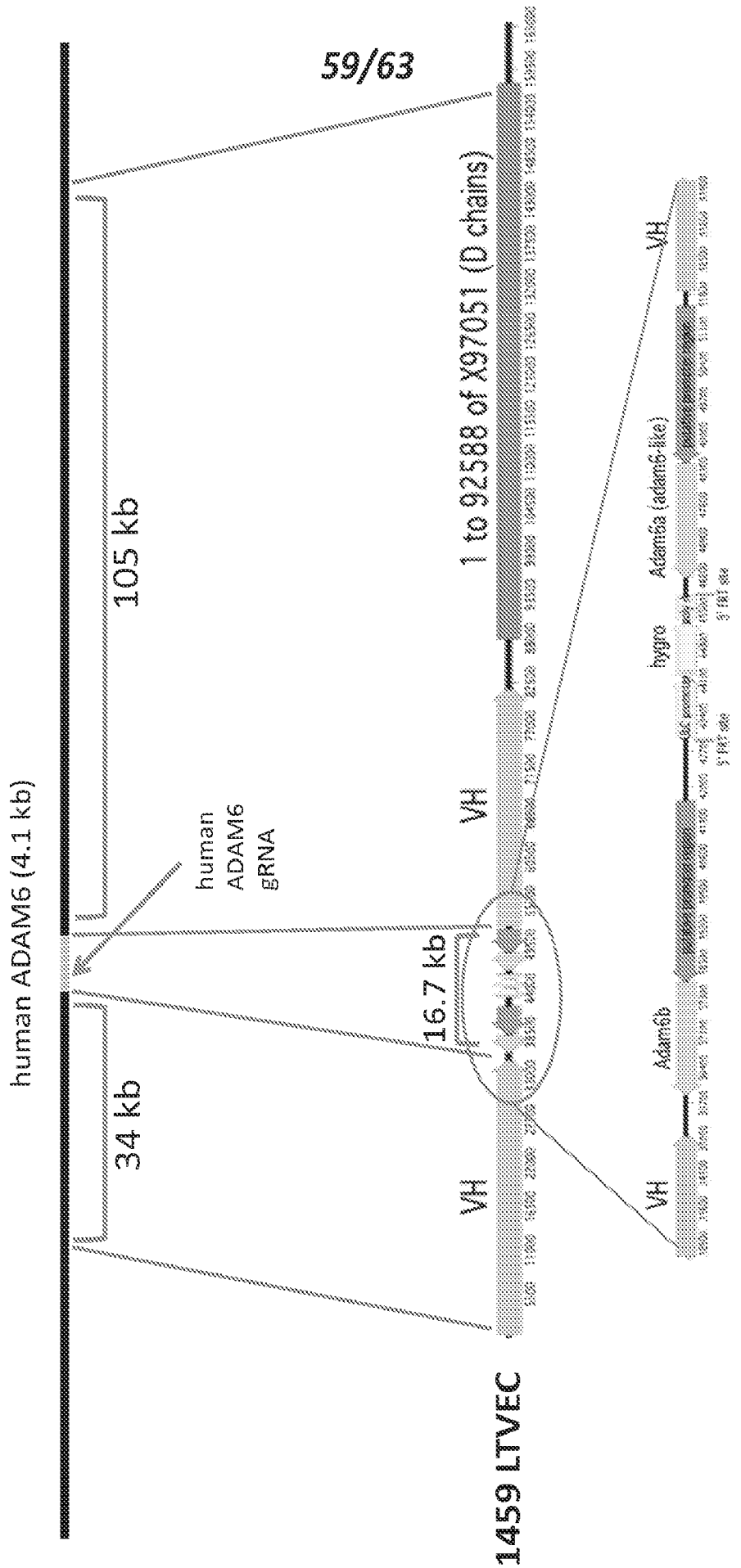

human ADAM6 (4.1 kb)
105 kb
34 kb
human ADAM6 gRNA
16.7 kb
VH
VH
1 to 92588 of X97051 (D chains)
1459 LTVEC
VH
Adam6b
hygro
Adam6a (adam6-like)
VH

FIG. 51

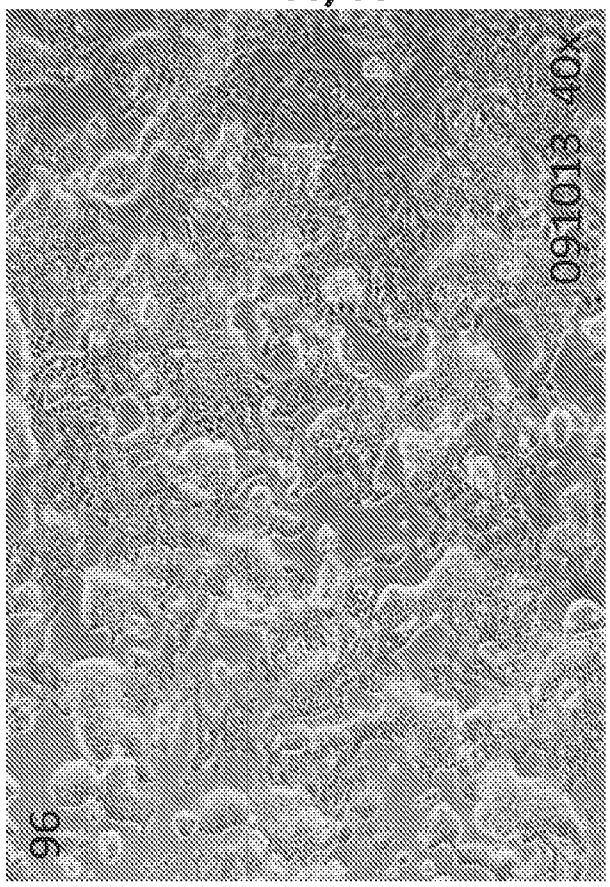


FIG. 52B

12 days in 2i

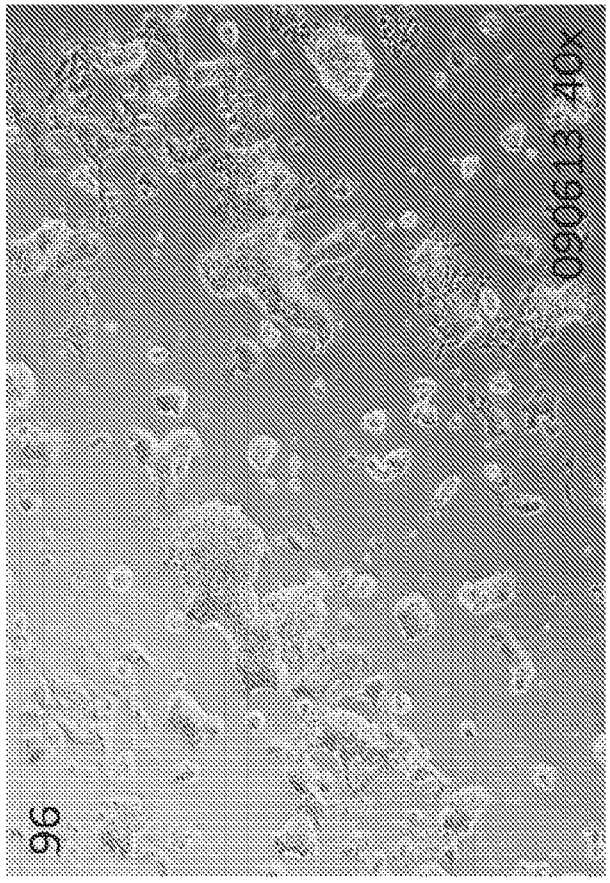


FIG. 52A

8 days in 2i

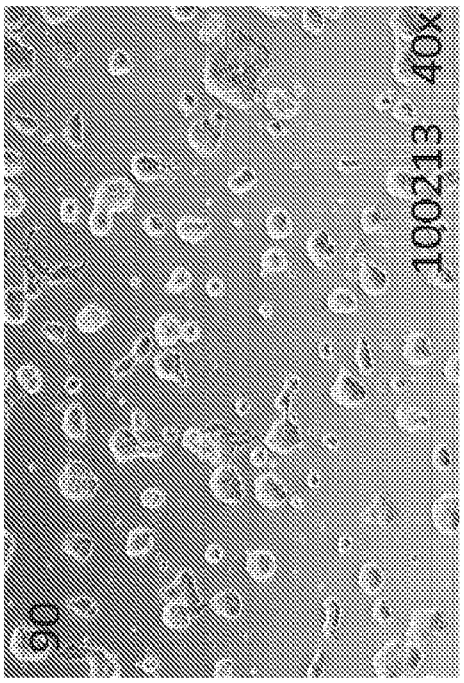


FIG. 53A

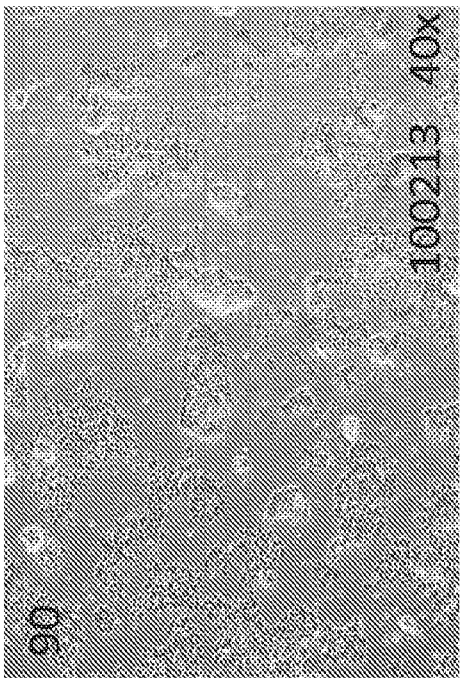


FIG. 53B

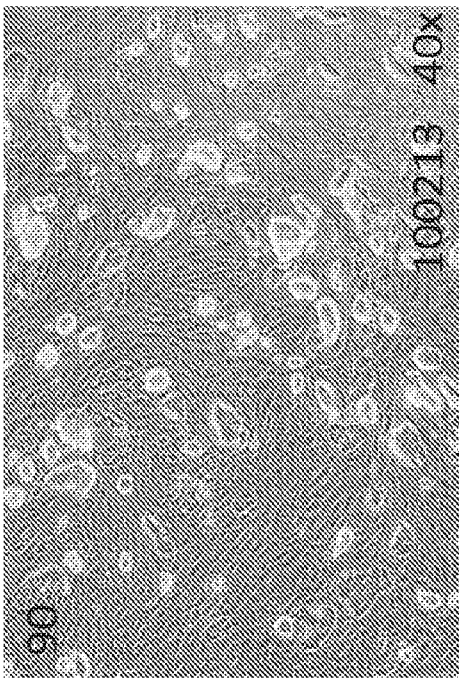


FIG. 53C

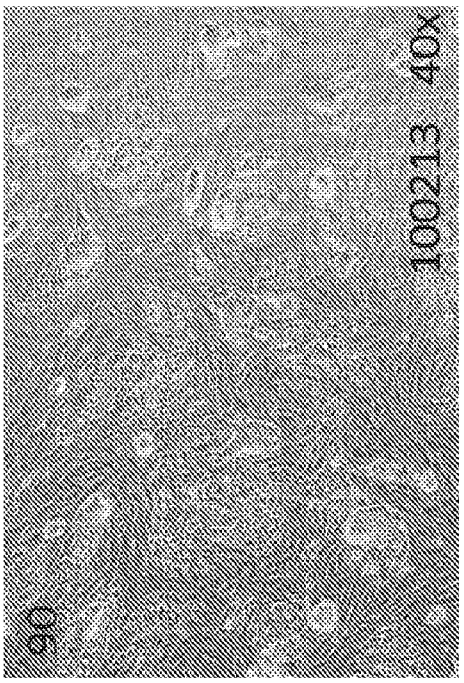


FIG. 53D

mTeSR-hLIF Matrigel hVG2i

mTeSR-hLIF NuFF feeder hVG2i

FIG. 54C

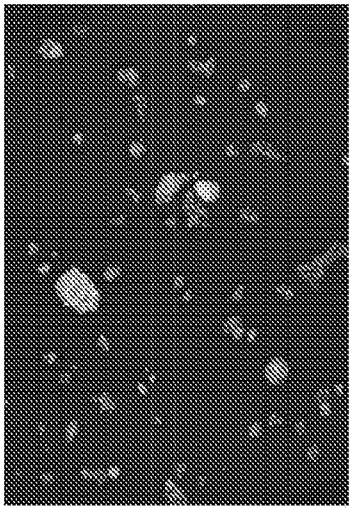


FIG. 54B

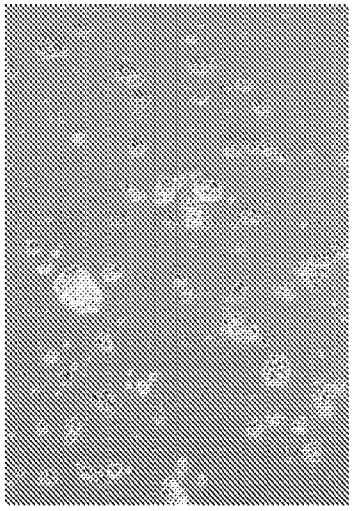
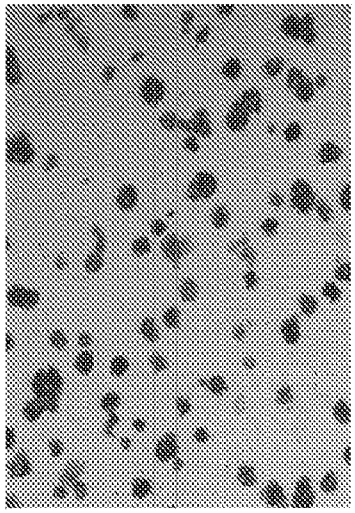
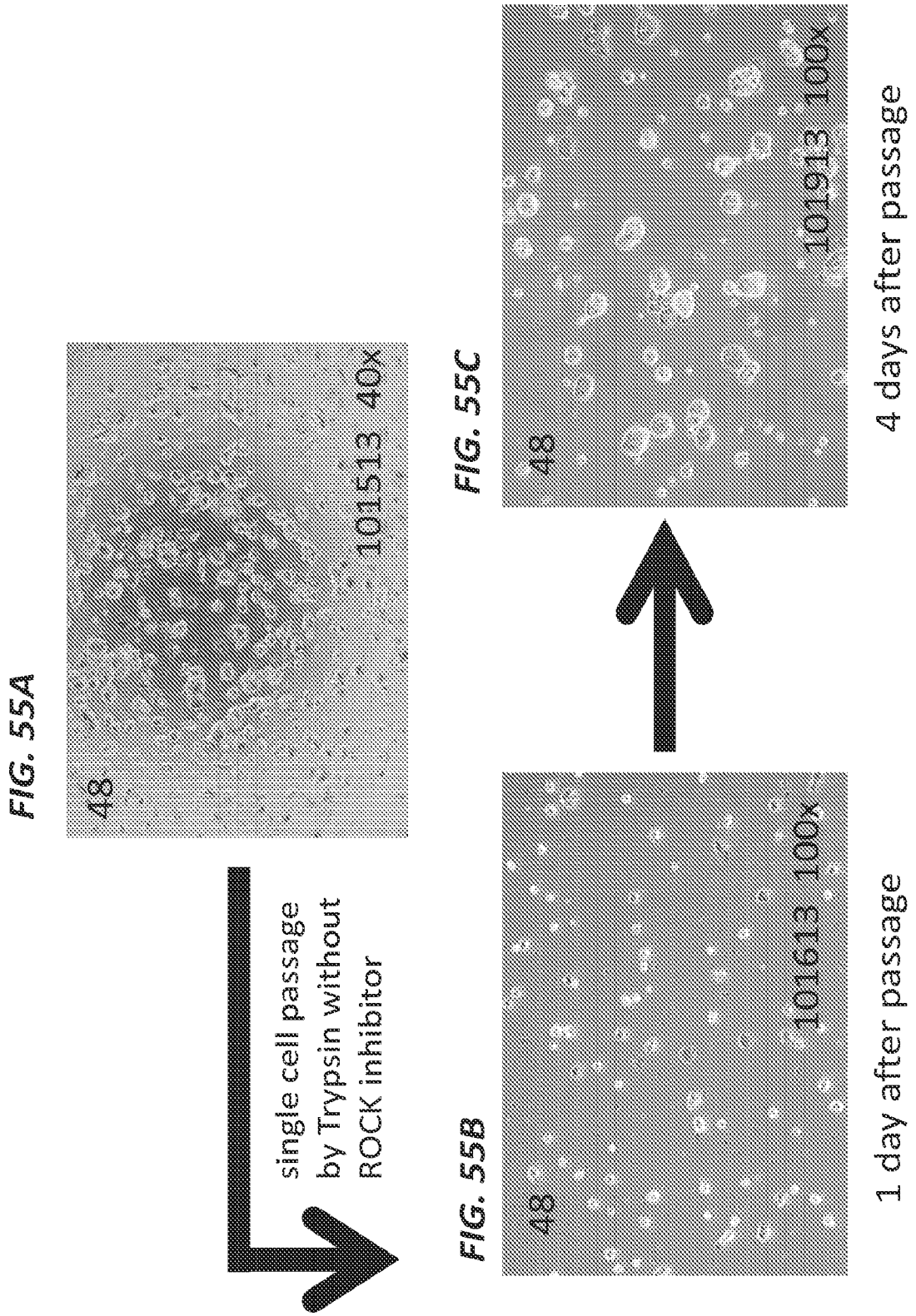


FIG. 54A




FIG. 55A
48
101513 40x
single cell passage
by Trypsin without
ROCK inhibitor
FIG. 55B
48
101613 100x
1 day after passage
FIG. 55C
48
101913 100x
4 days after passage