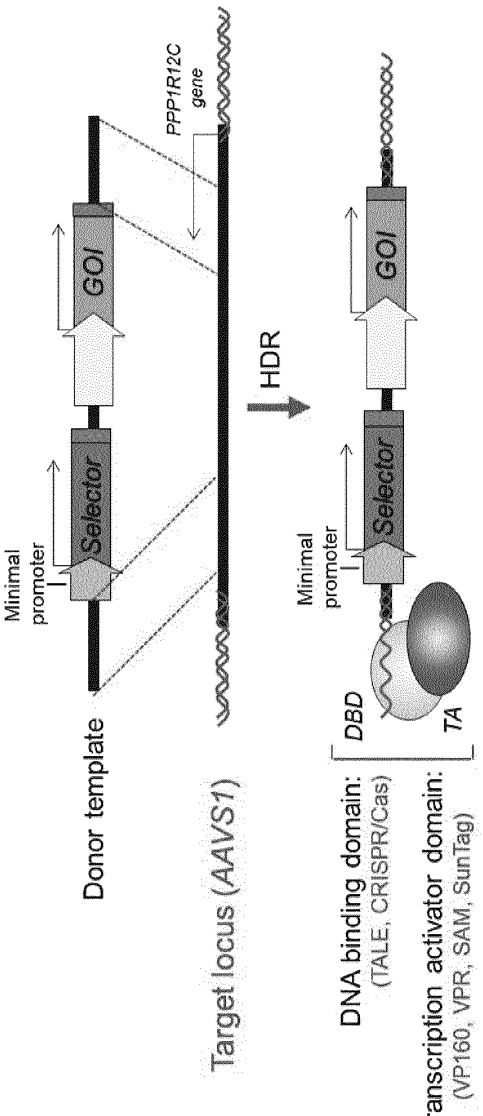


FIG. 1Ai

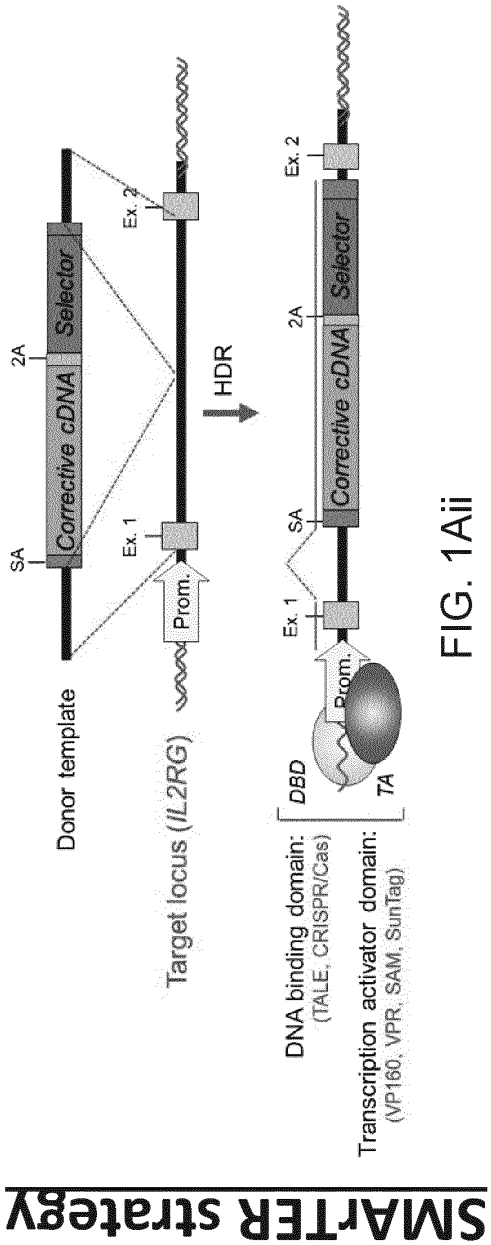


FIG. 1Aii

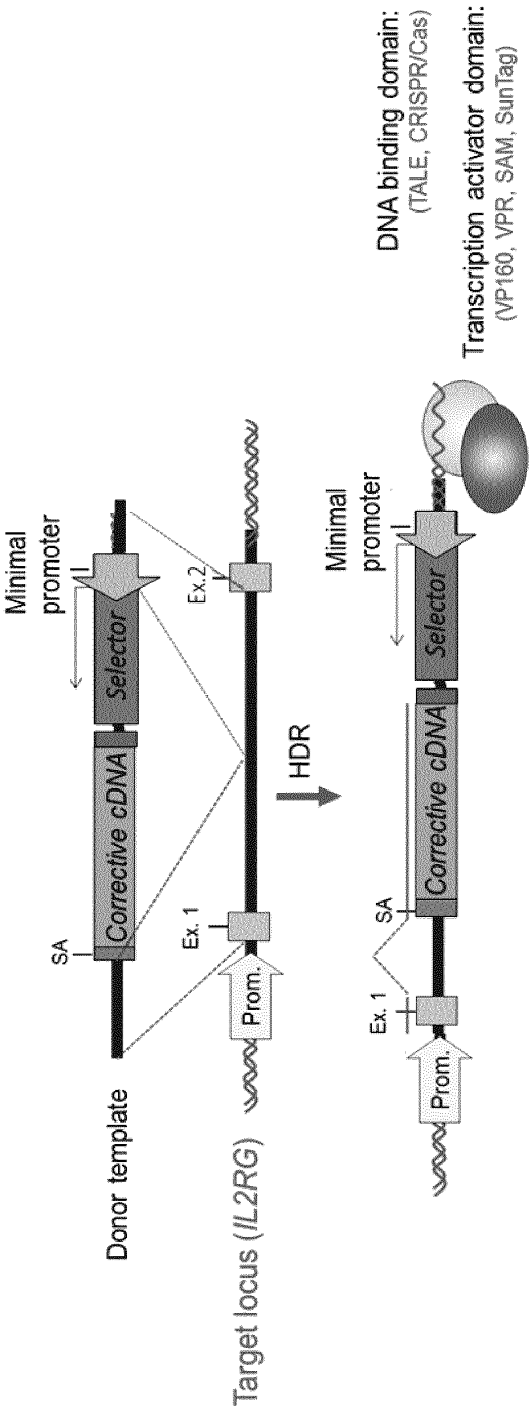


FIG. 1Aiii

SMART strategy for gene correction

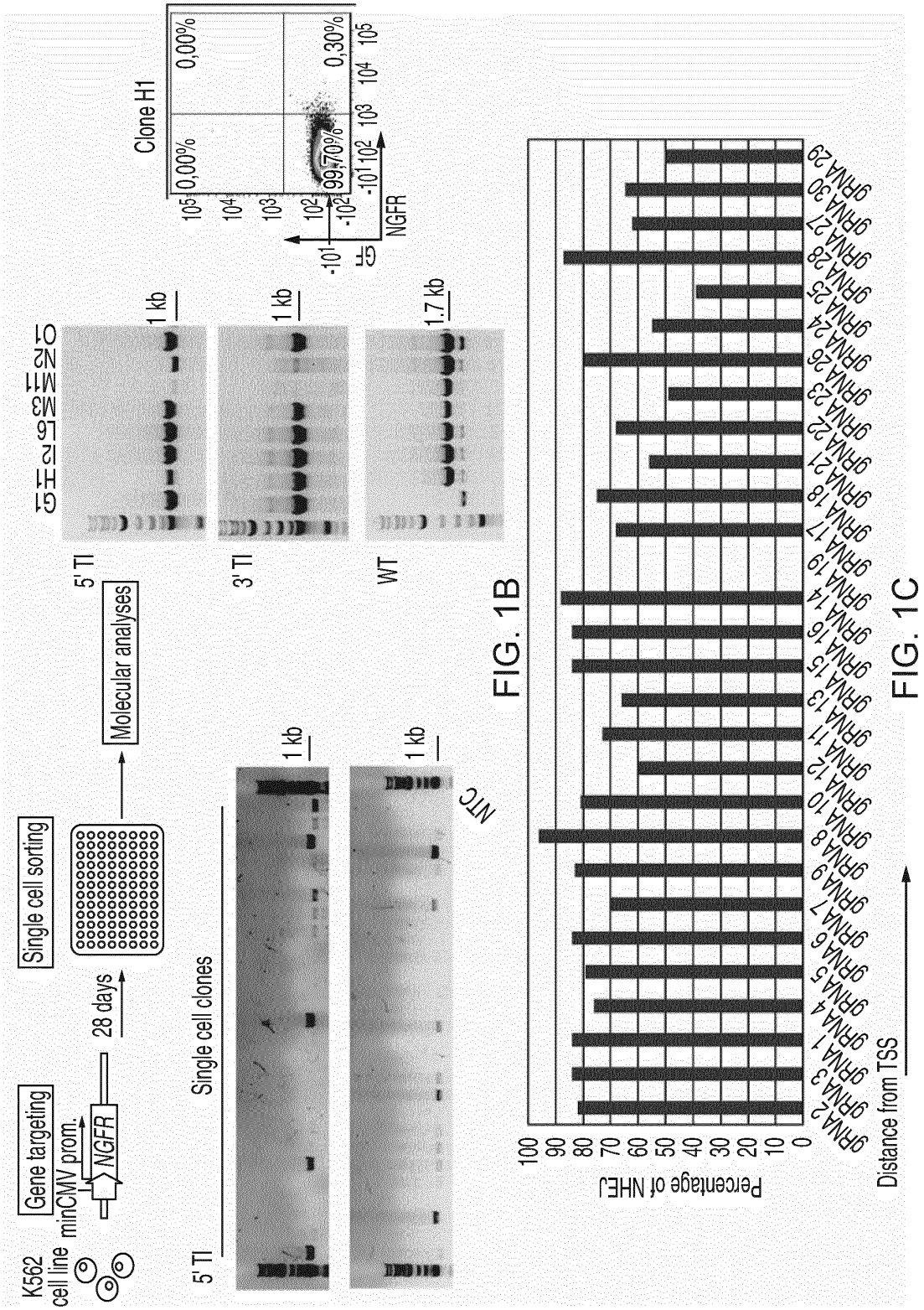

K562 cell line
Gene targeting
minCMV prom.
NGFR
28 days
Single cell sorting
Molecular analyses
Single cell clones
5' TI
1 kb
NTC
Clone H1
0,00%
0,00%
99,70%
0,30%
NGFR
G1
E1
F2
E6
M3
M11
N2
O1
5' TI
3' TI
WT
1 kb
1.7 kb
Percentage of NHEJ
Distance from TSS
gRNA 2
gRNA 3
gRNA 1
gRNA 4
gRNA 5
gRNA 6
gRNA 7
gRNA 9
gRNA 8
gRNA 10
gRNA 12
gRNA 11
gRNA 13
gRNA 15
gRNA 16
gRNA 14
gRNA 19
gRNA 17
gRNA 18
gRNA 21
gRNA 22
gRNA 23
gRNA 26
gRNA 24
gRNA 25
gRNA 28
gRNA 27
gRNA 30
gRNA 29

FIG. 1B

FIG. 1C

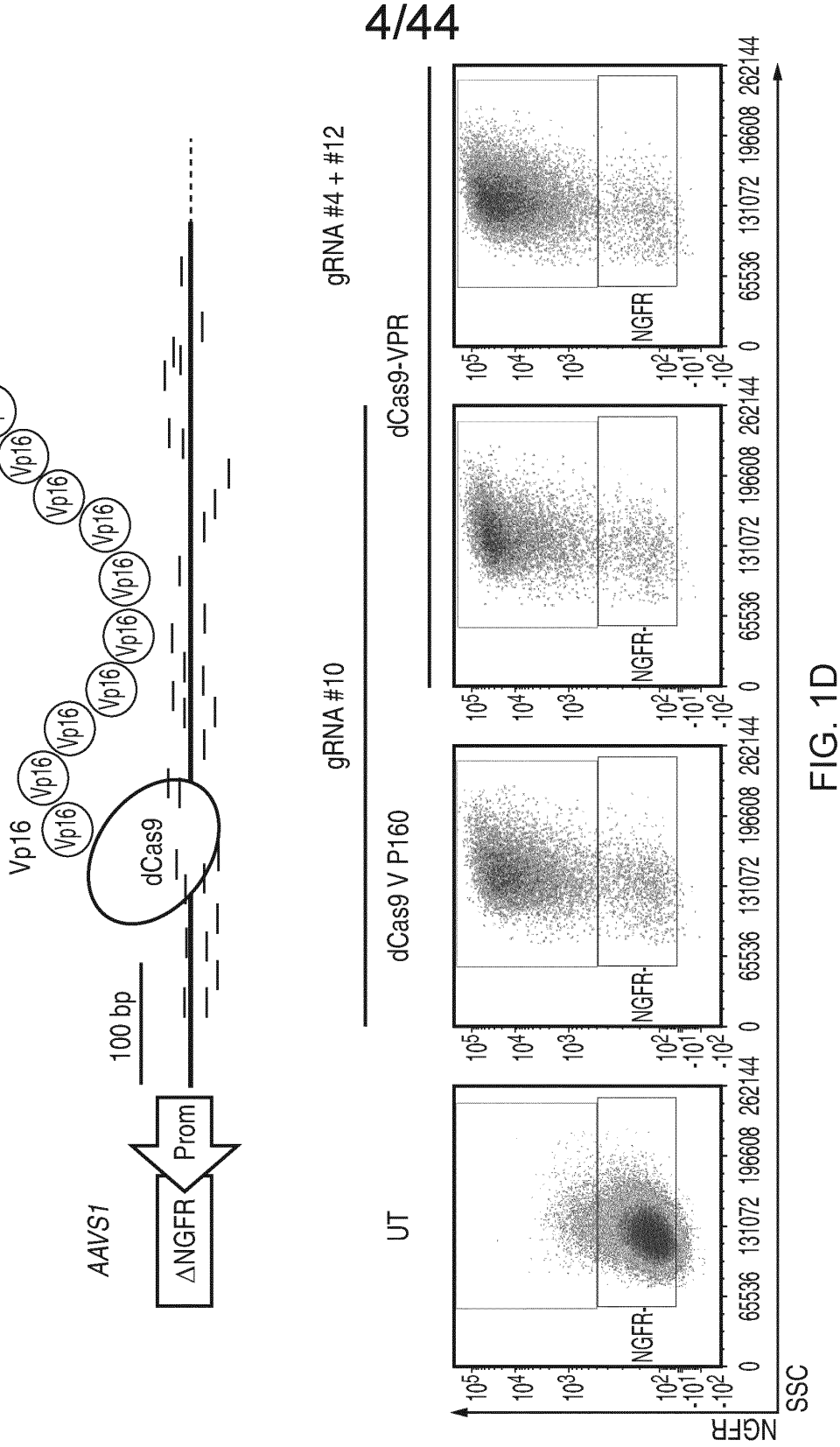

AAVS1
ΔNGFR
Prom
100 bp
dCas9
Vp16
gRNA #10
gRNA #4 + #12
dCas9 V P160
dCas9-VPR
UT
NGFR-
NGFR
SSC
0
65536
131072
196608
262144
10^5
10^4
10^3
10^2
10^1
-10^2

FIG. 1D

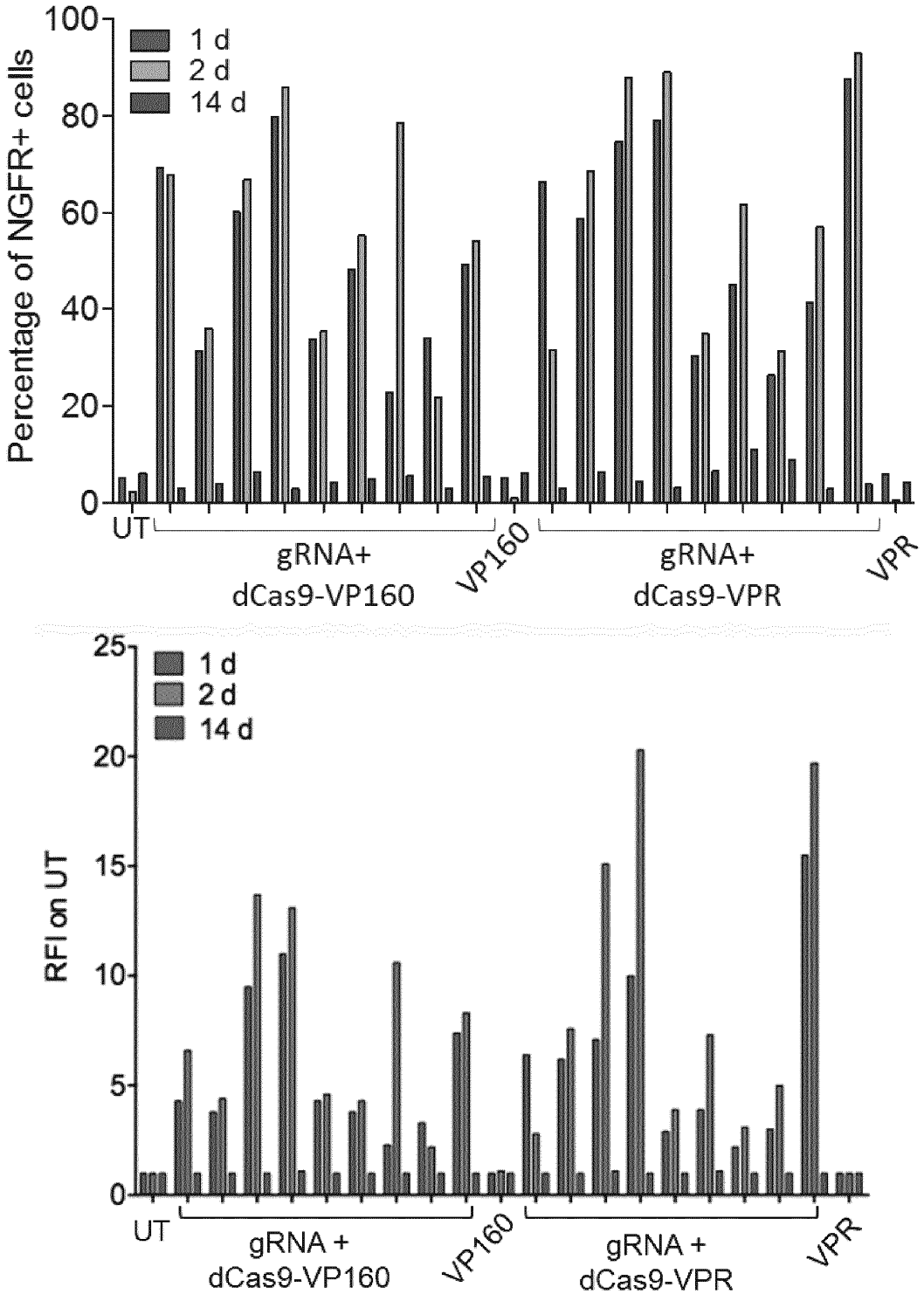

Percentage of NGFR+ cells
100
80
60
40
20
0
1 d
2 d
14 d
UT
gRNA+
dCas9-VP160
VP160
gRNA+
dCas9-VPR
VPR
RFI on UT
25
20
15
10
5
0
1 d
2 d
14 d
UT
gRNA +
dCas9-VP160
VP160
gRNA +
dCas9-VPR
VPR

FIG. 1E

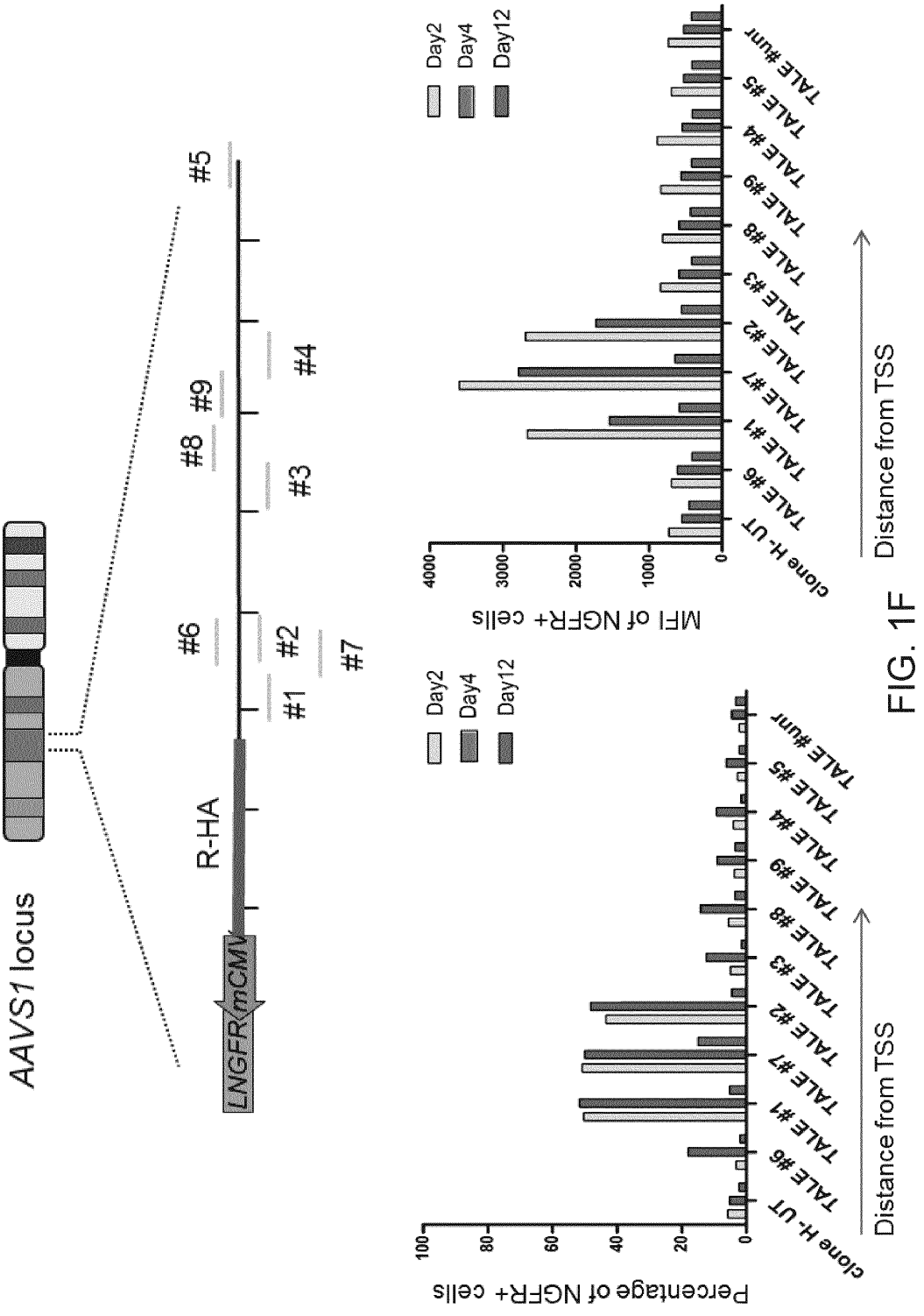

AAVS1 locus
R-HA
LNGFR
mCMV
#1
#2
#3
#4
#5
#6
#7
#8
#9
Day2
Day4
Day12
Percentage of NGFR+ cells
MFI of NGFR+ cells
clone H- UT
TALE #6
TALE #1
TALE #7
TALE #2
TALE #3
TALE #8
TALE #9
TALE #4
TALE #5
TALE #unr
Distance from TSS

FIG. 1F

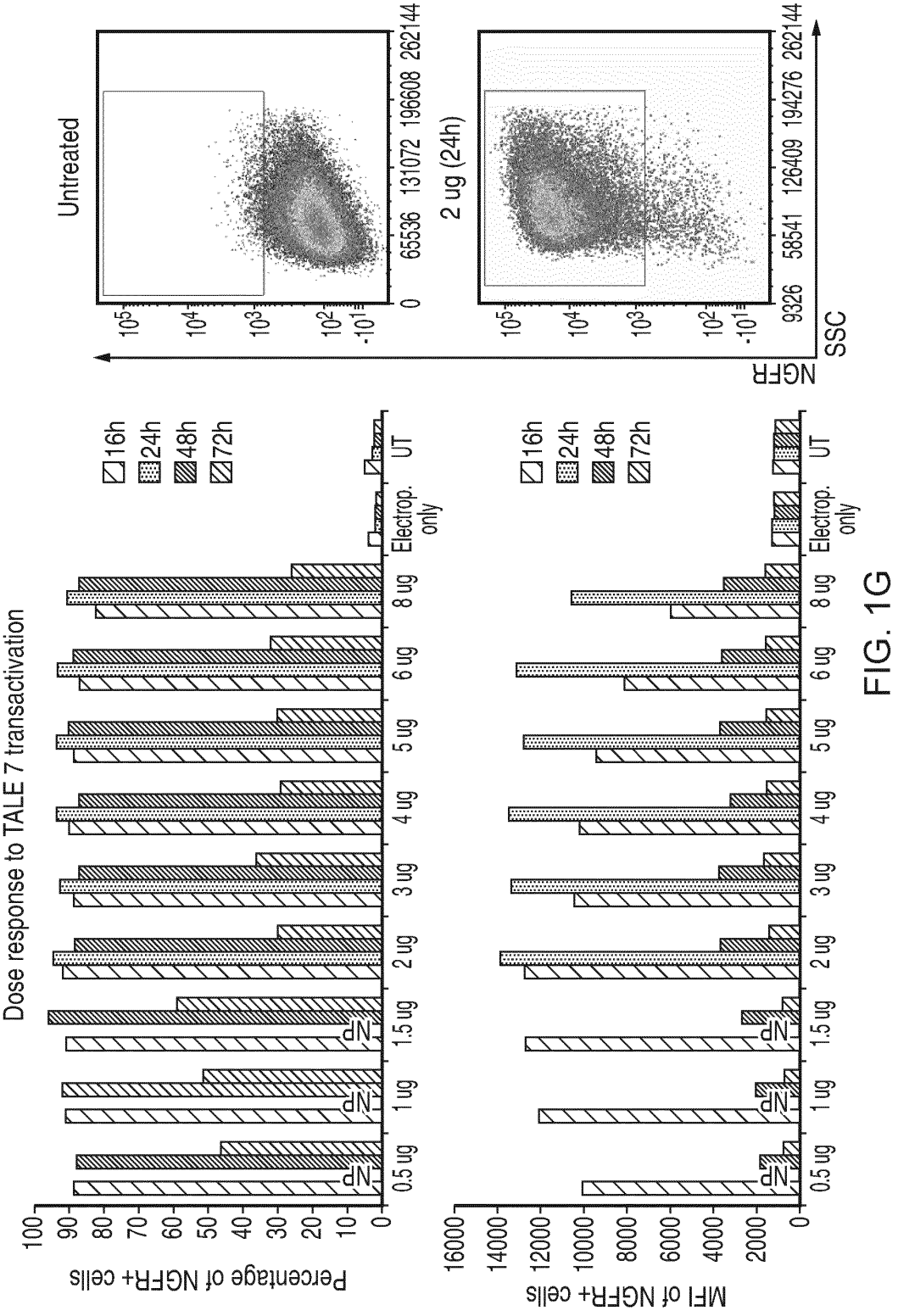
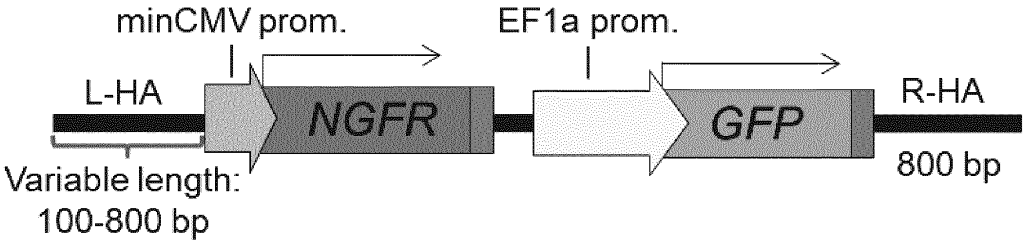

Untreated
2 ug (24h)
NGFR
SSC
0
65536
131072
196608
262144
9326
58541
126409
194276
262144
Dose response to TALE 7 transactivation
Percentage of NGFR+ cells
MFI of NGFR+ cells
16h
24h
48h
72h
NP
0.5 ug
1 ug
1.5 ug
2 ug
3 ug
4 ug
5 ug
6 ug
8 ug
Electrop. only
UT

FIG. 1G


minCMV prom.
EF1a prom.
L-HA
NGFR
GFP
R-HA
800 bp
Variable length:
100-800 bp

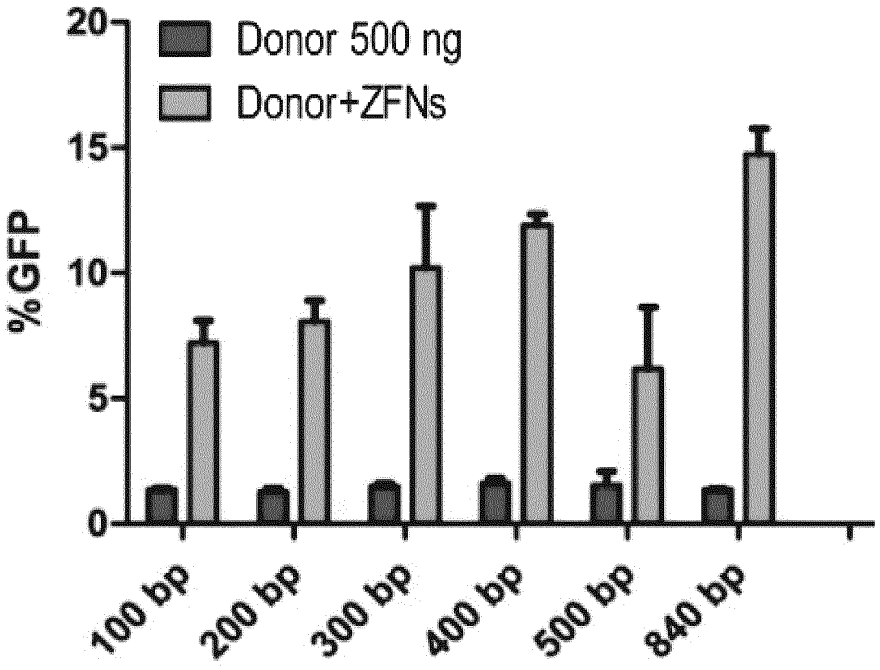

20
15
10
5
0
%GFP
Donor 500 ng
Donor+ZFNs
100 bp
200 bp
300 bp
400 bp
500 bp
840 bp

FIG. 2A

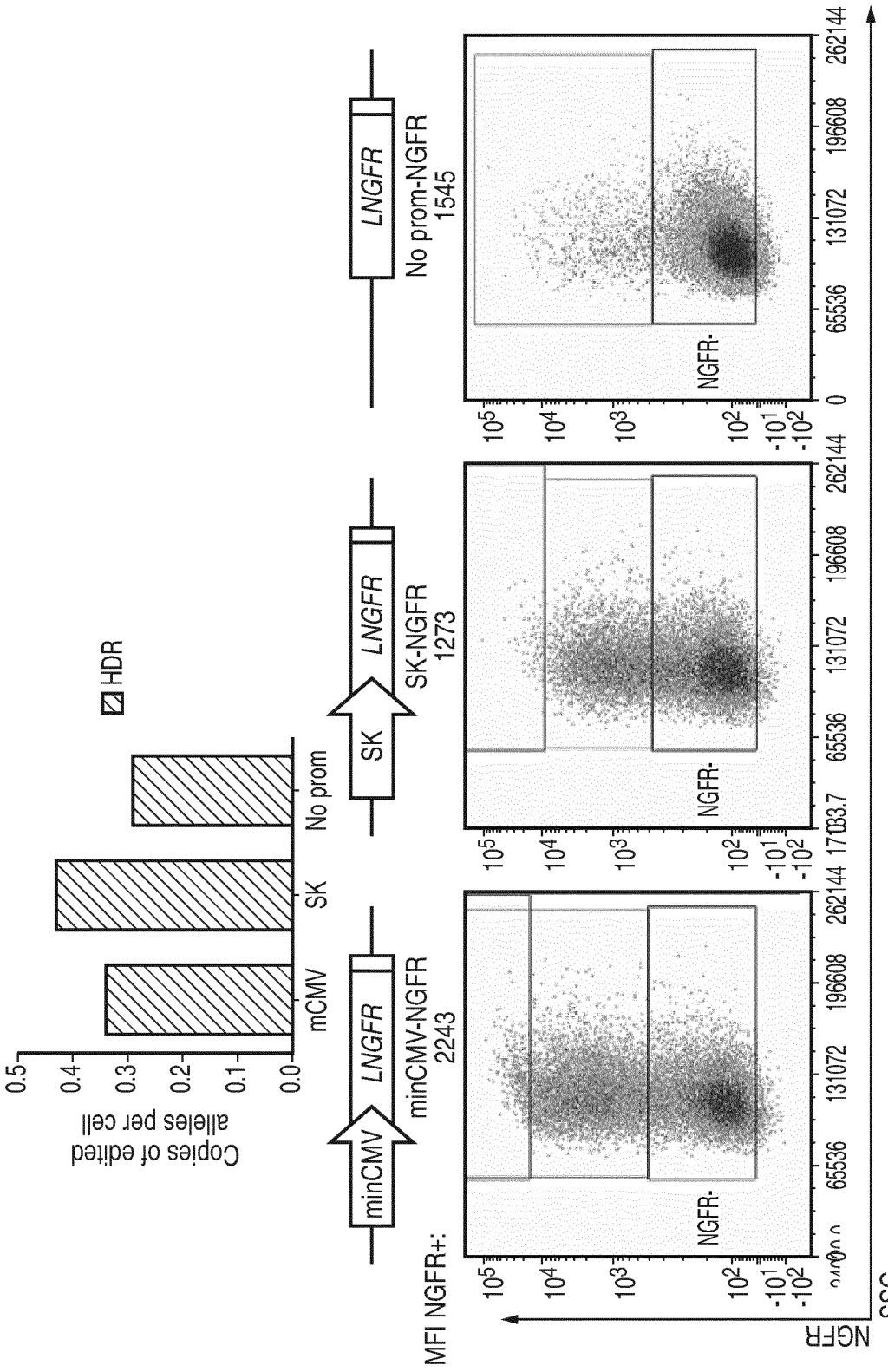

Copies of edited alleles per cell
0.5
0.4
0.3
0.2
0.1
0.0
HDR
mCMV
SK
No prom
minCMV
LNGFR
minCMV-NGFR
2243
SK
LNGFR
SK-NGFR
1273
LNGFR
No prom-NGFR
1545
MFI NGFR+:
NGFR-
NGFR
SSC
65536
131072
196608
262144
0

FIG. 2B

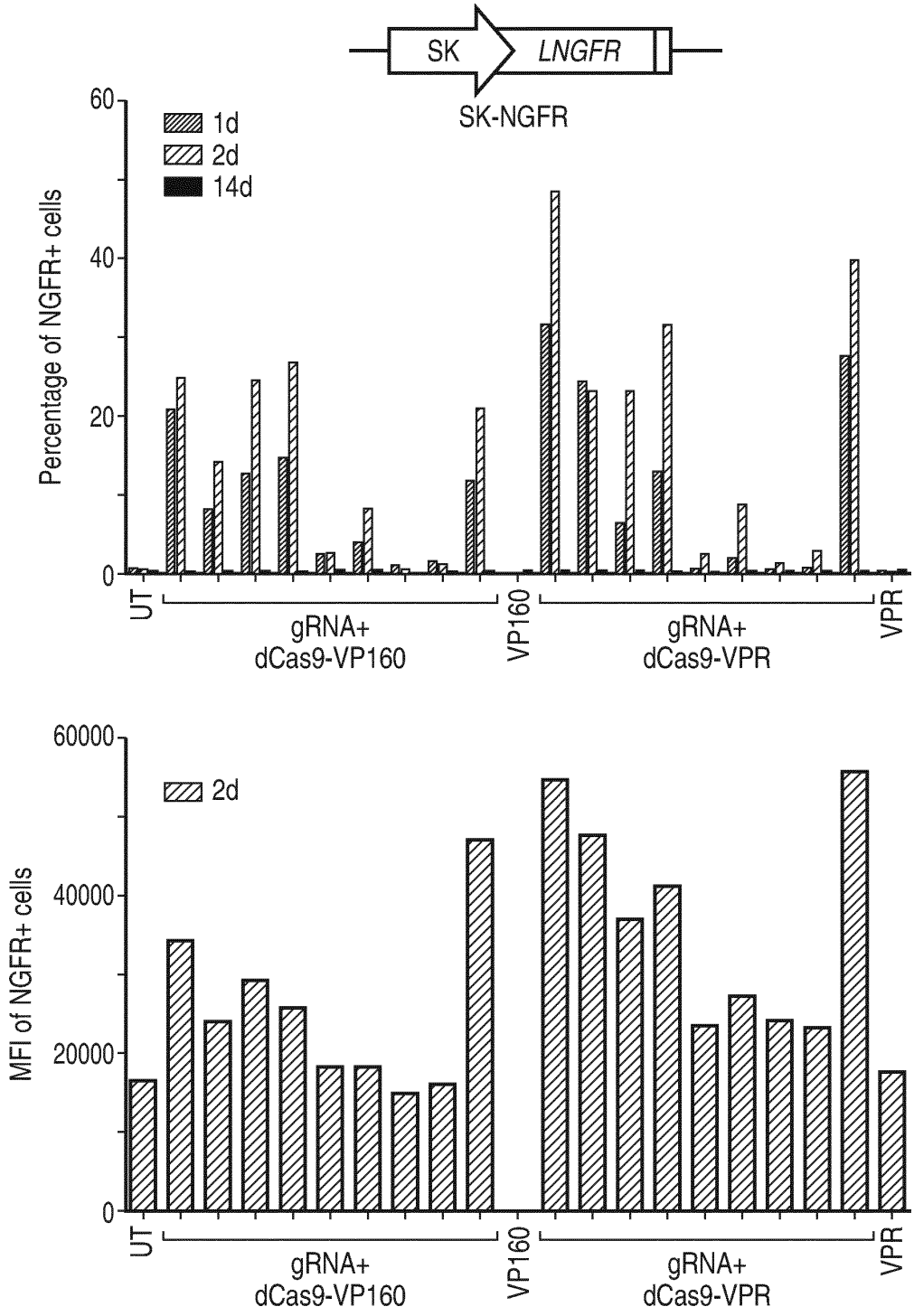

SK
LNGFR
SK-NGFR
1d
2d
14d
Percentage of NGFR+ cells
60
40
20
0
UT
gRNA+
dCas9-VP160
VP160
gRNA+
dCas9-VPR
VPR
MFI of NGFR+ cells
60000
40000
20000
0
2d
UT
gRNA+
dCas9-VP160
VP160
gRNA+
dCas9-VPR
VPR

FIG. 2C

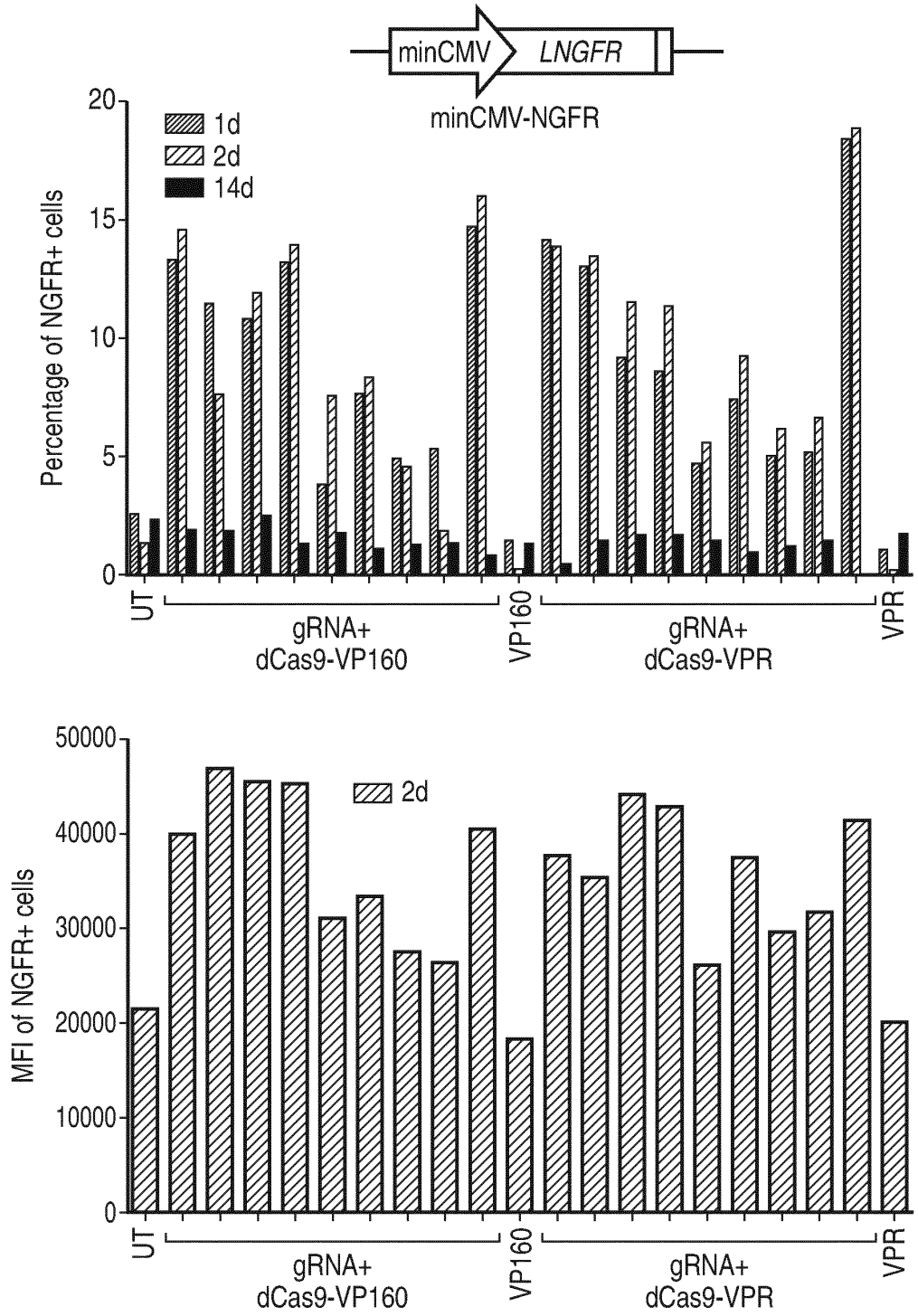


FIG. 2C (Continued)

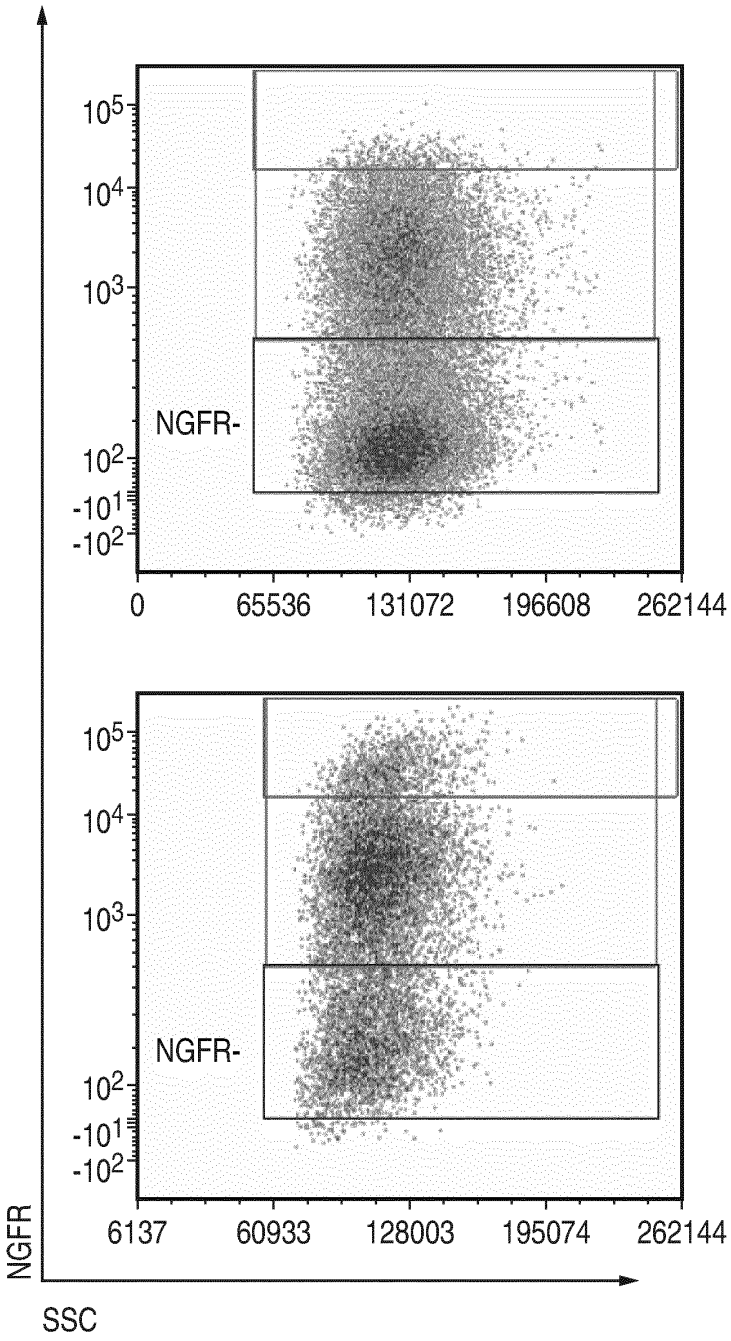


FIG. 2C (Continued)

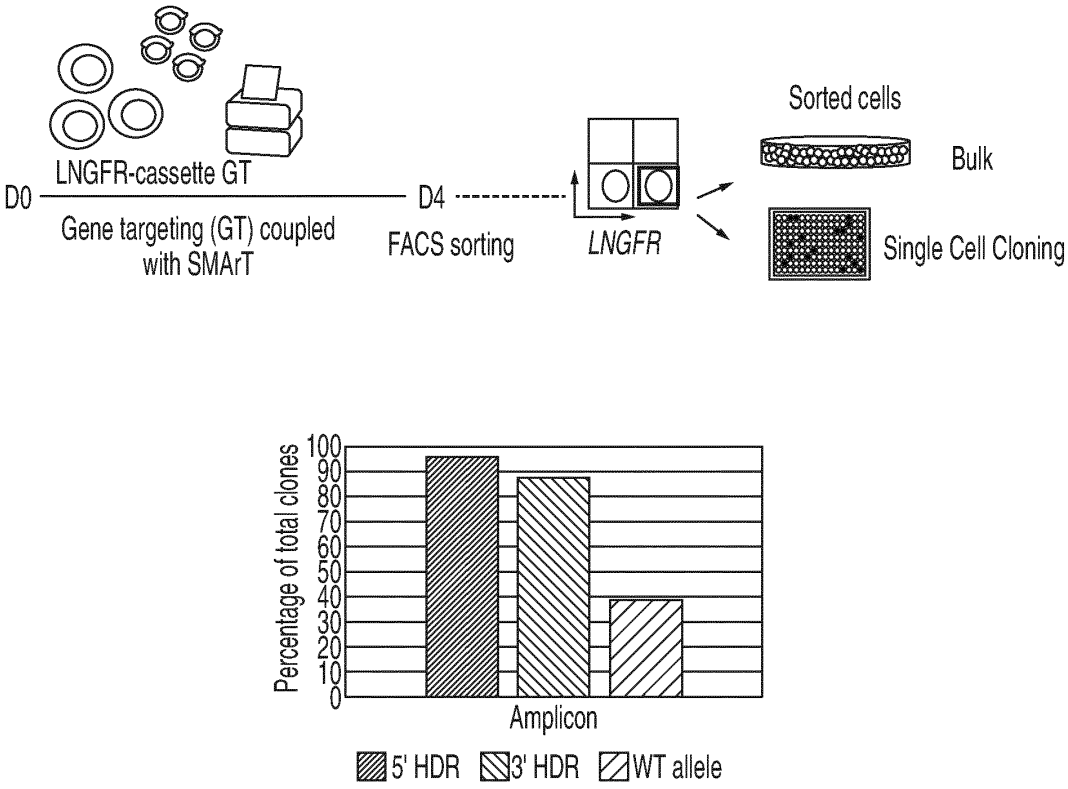

LNGFR-cassette GT
D0
Gene targeting (GT) coupled with SMArT
D4
FACS sorting
LNGFR
Sorted cells
Bulk
Single Cell Cloning
Percentage of total clones
100
90
80
70
60
50
40
30
20
10
0
Amplicon
5' HDR
3' HDR
WT allele

FIG. 2D

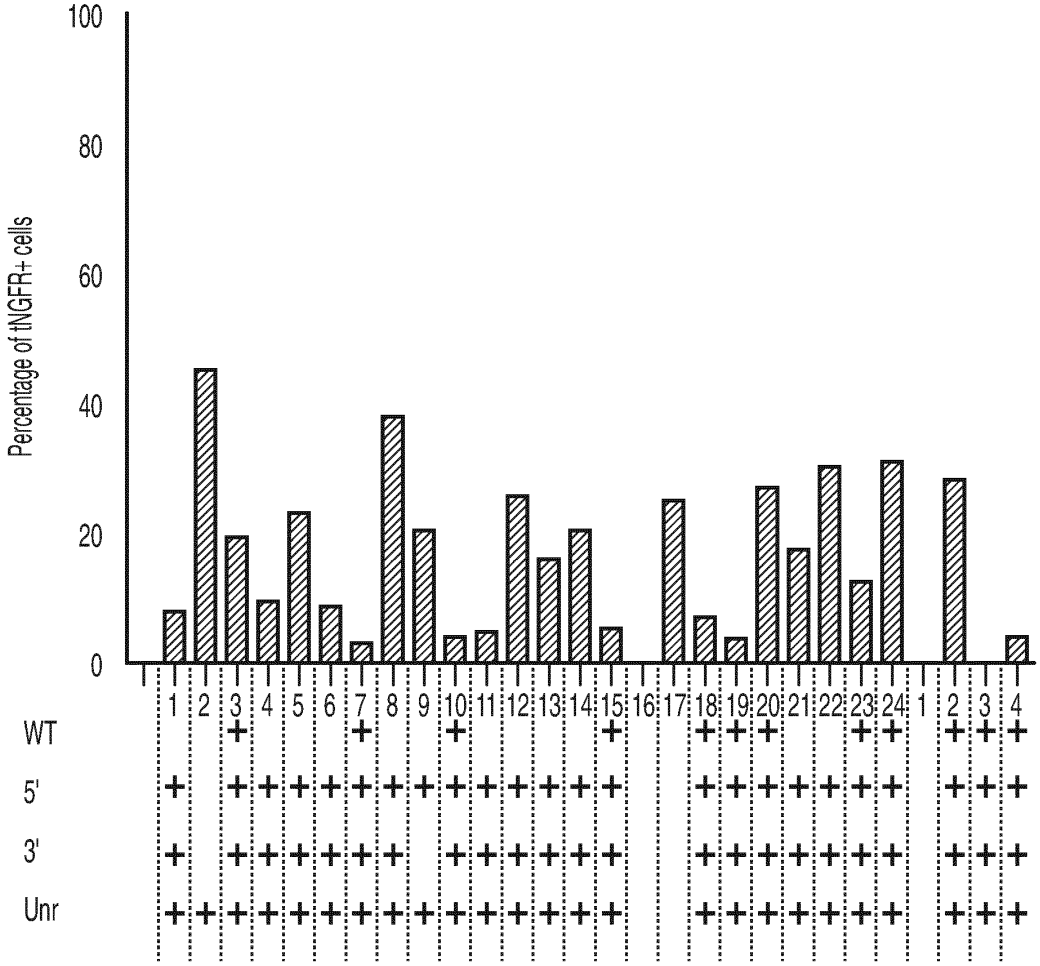


FIG. 2D (continued)

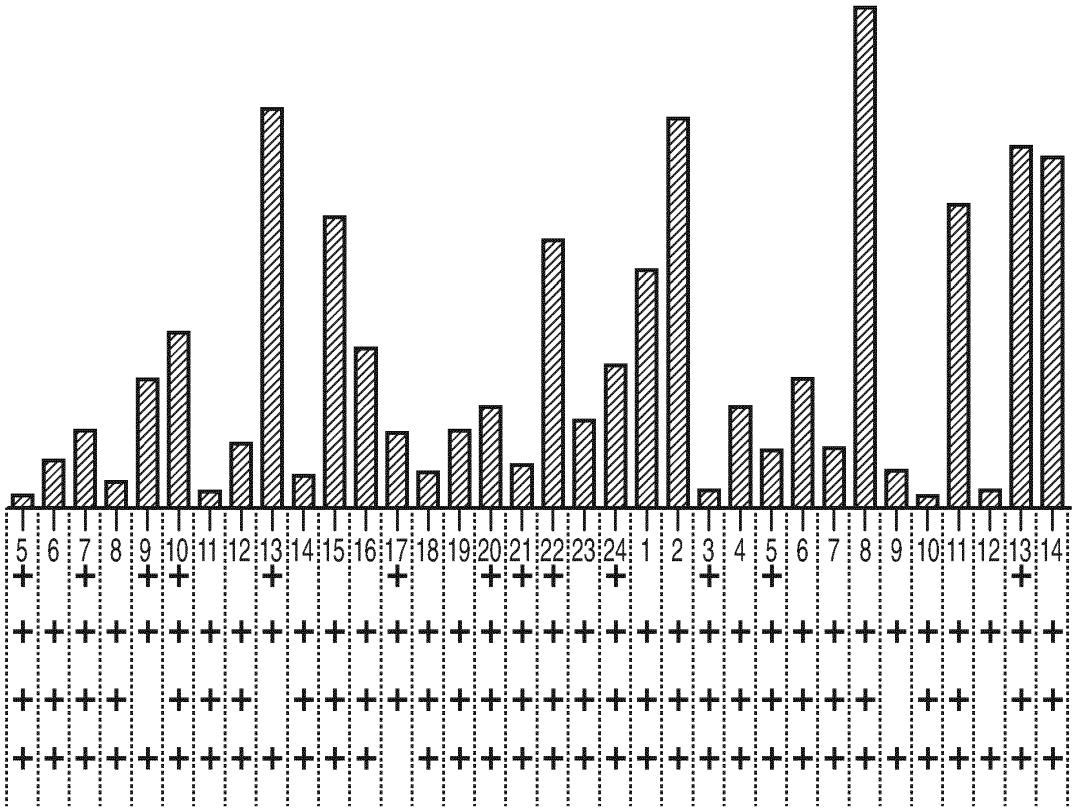


FIG. 2D (continued)

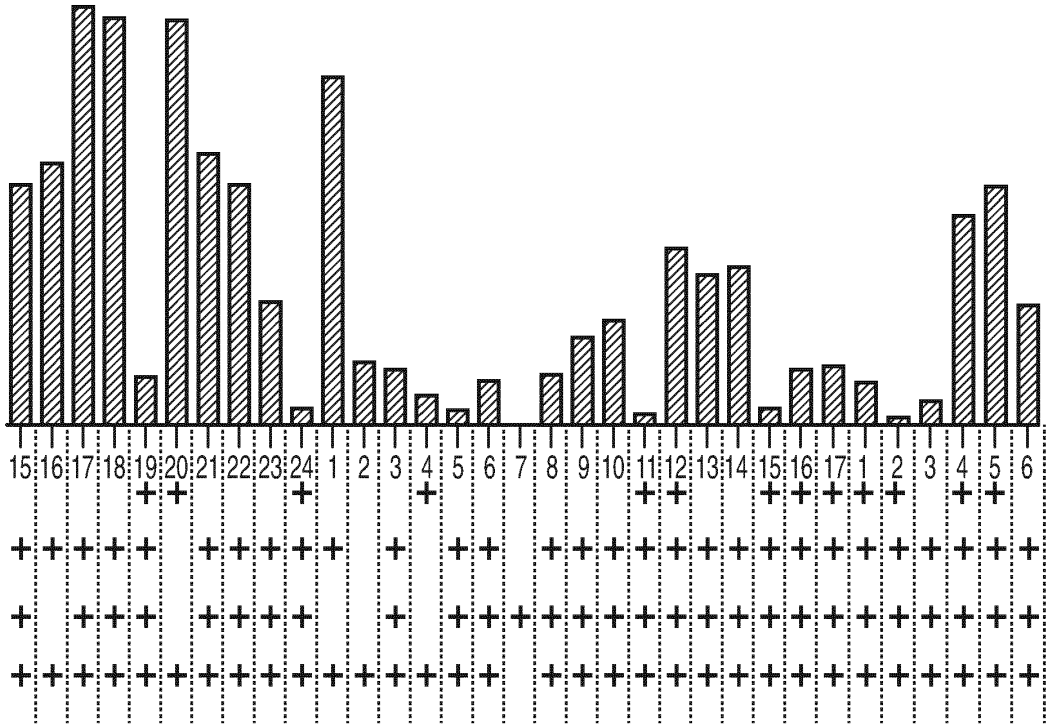


FIG. 2D (continued)

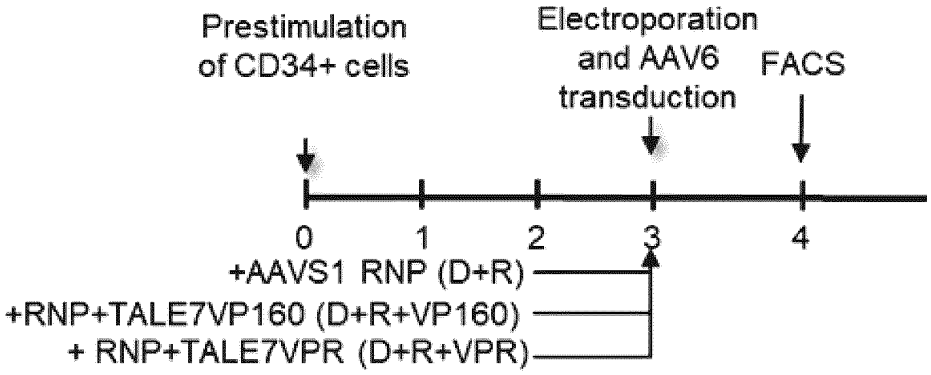

Prestimulation
of CD34+ cells
Electroporation
and AAV6
transduction
FACS
0
1
2
3
4
+AAVS1 RNP (D+R)
+RNP+TALE7VP160 (D+R+VP160)
+ RNP+TALE7VPR (D+R+VPR)

FIG. 3A

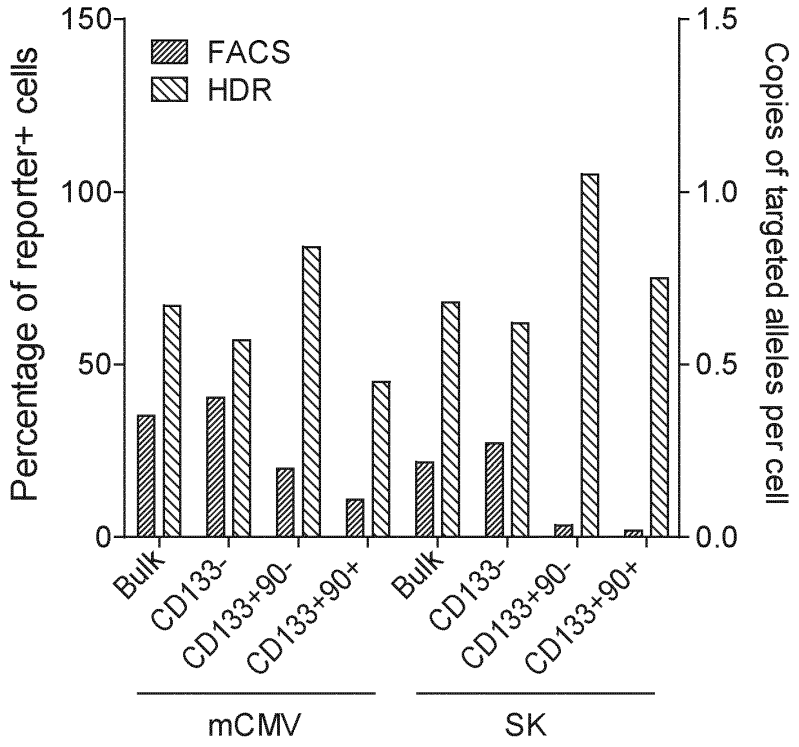

Percentage of reporter+ cells
Copies of targeted alleles per cell
FACS
HDR
150
100
50
0
1.5
1.0
0.5
0.0
Bulk
CD133-
CD133+90-
CD133+90+
Bulk
CD133-
CD133+90-
CD133+90+
mCMV
SK

FIG. 3C

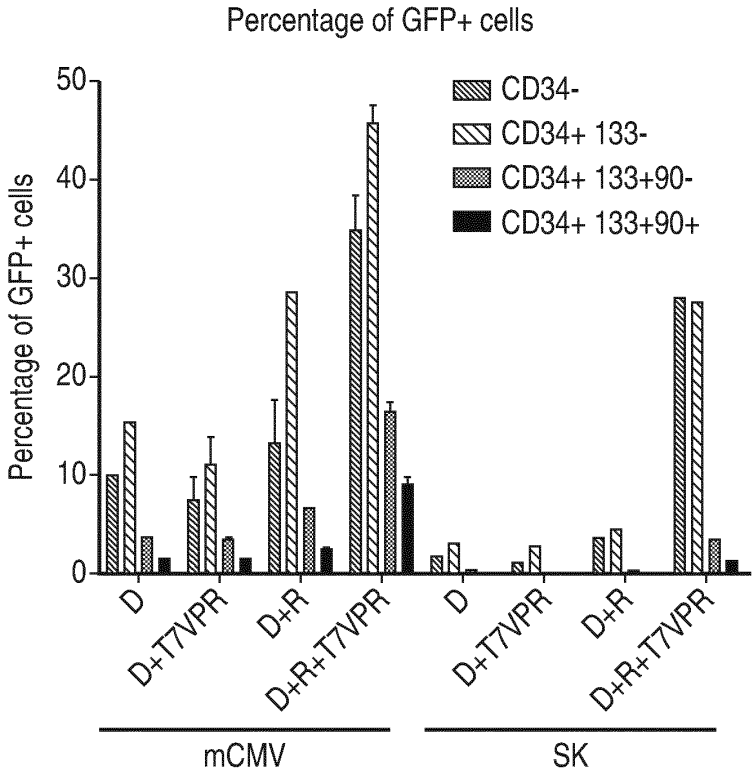


FIG. 3B

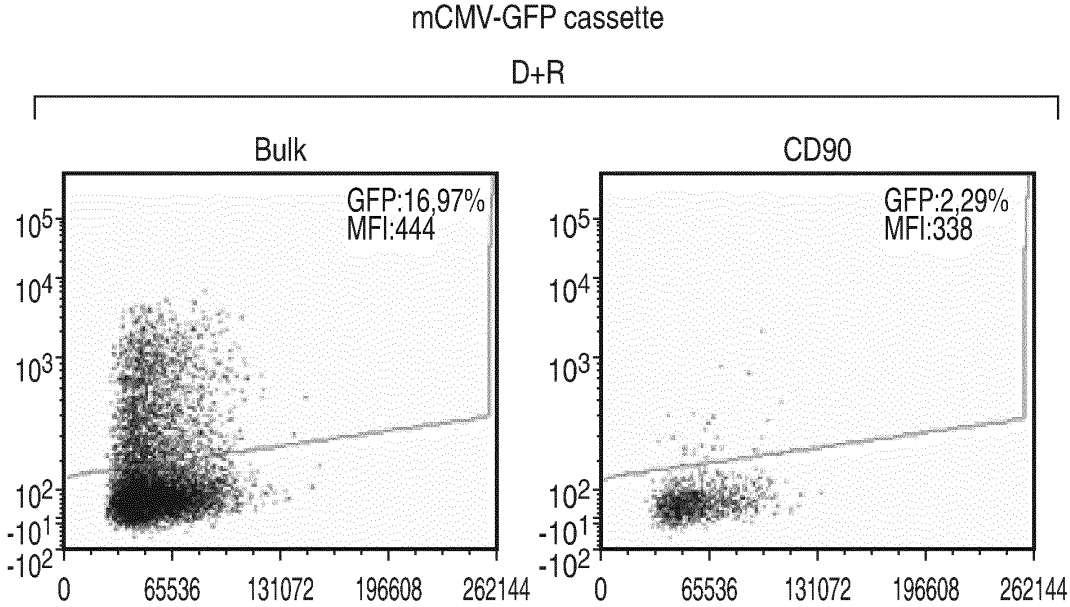


FIG. 3B (Continued)

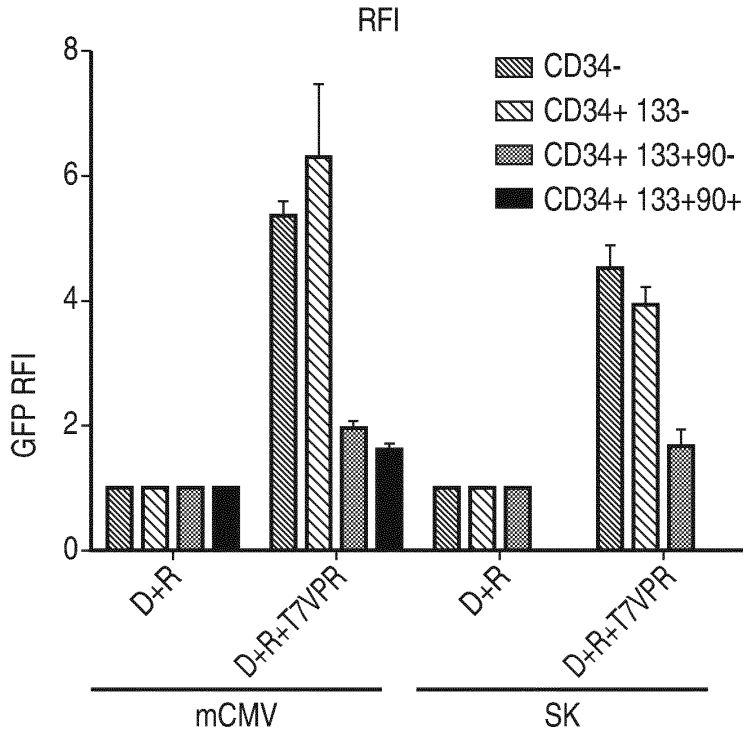


FIG. 3B (Continued)

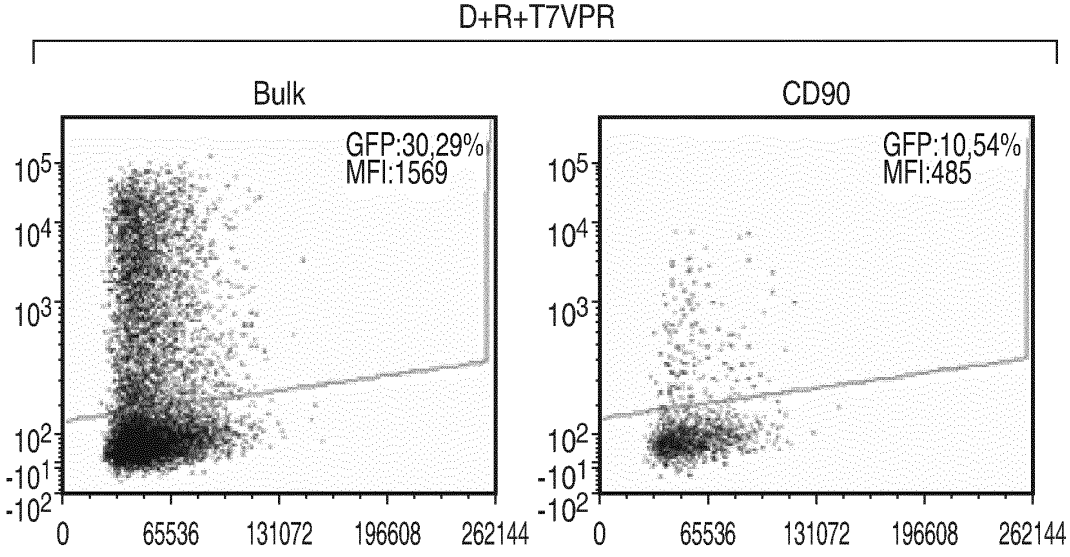


FIG. 3B (Continued)

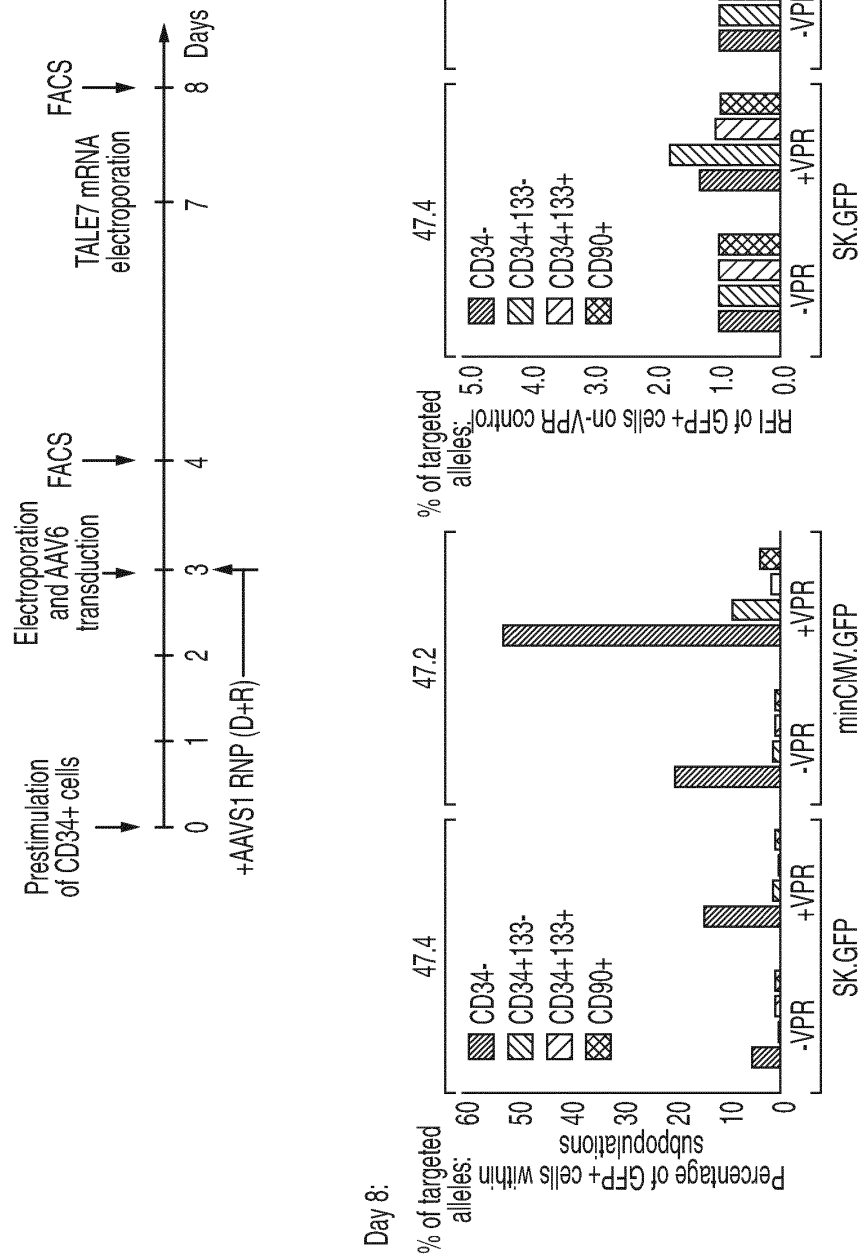

Prestimulation of CD34+ cells
Electroporation and AAV6 transduction
FACS
TALE7 mRNA electroporation
FACS
0 1 2 3 4 7 8 Days
+AAVS1 RNP (D+R)
Day 8:
% of targeted alleles:
Percentage of GFP+ cells within subpopulations
CD34-
CD34+133-
CD34+133+
CD90+
47.4
47.2
-VPR
+VPR
SK.GFP
minCMV.GFP
% of targeted alleles:
RFI of GFP+ cells on -VPR control
47.4
47.2
CD34-
CD34+133-
CD34+133+
CD90+
-VPR
+VPR
SK.GFP
minCMV.GFP

FIG. 3D

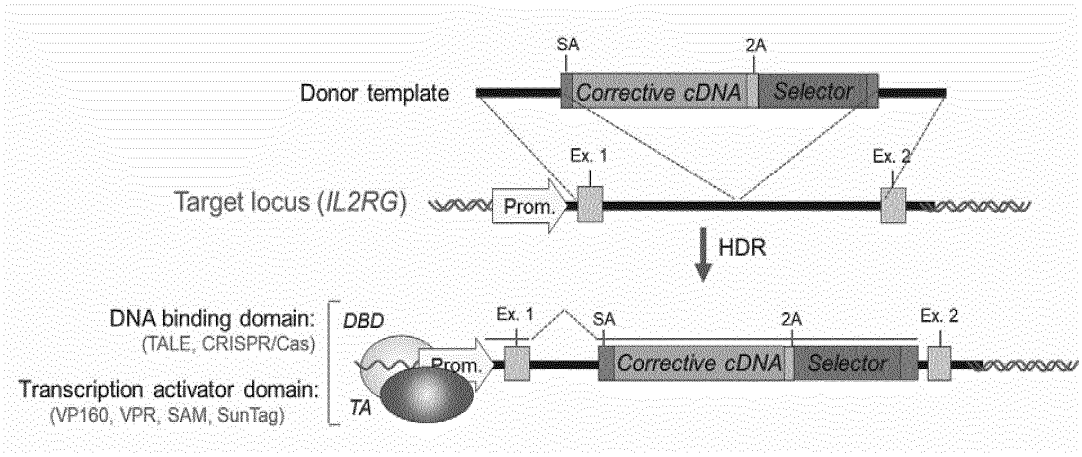

SA
2A
Donor template
Corrective cDNA
Selector
Ex. 1
Ex. 2
Target locus (IL2RG)
Prom.
HDR
DNA binding domain:
(TALE, CRISPR/Cas)
Transcription activator domain:
(VP160, VPR, SAM, SunTag)
DBD
TA
Ex. 1
SA
2A
Ex. 2
Prom.
Corrective cDNA
Selector

FIG. 4A

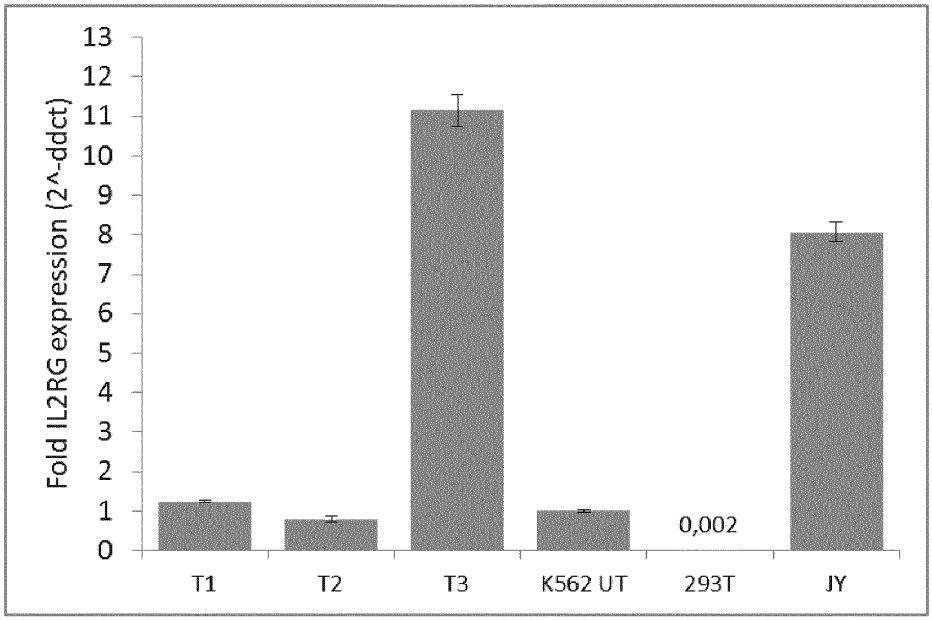

Fold IL2RG expression (2^-ddct)
13
12
11
10
9
8
7
6
5
4
3
2
1
0
0,002
T1
T2
T3
K562 UT
293T
JY

FIG. 4B

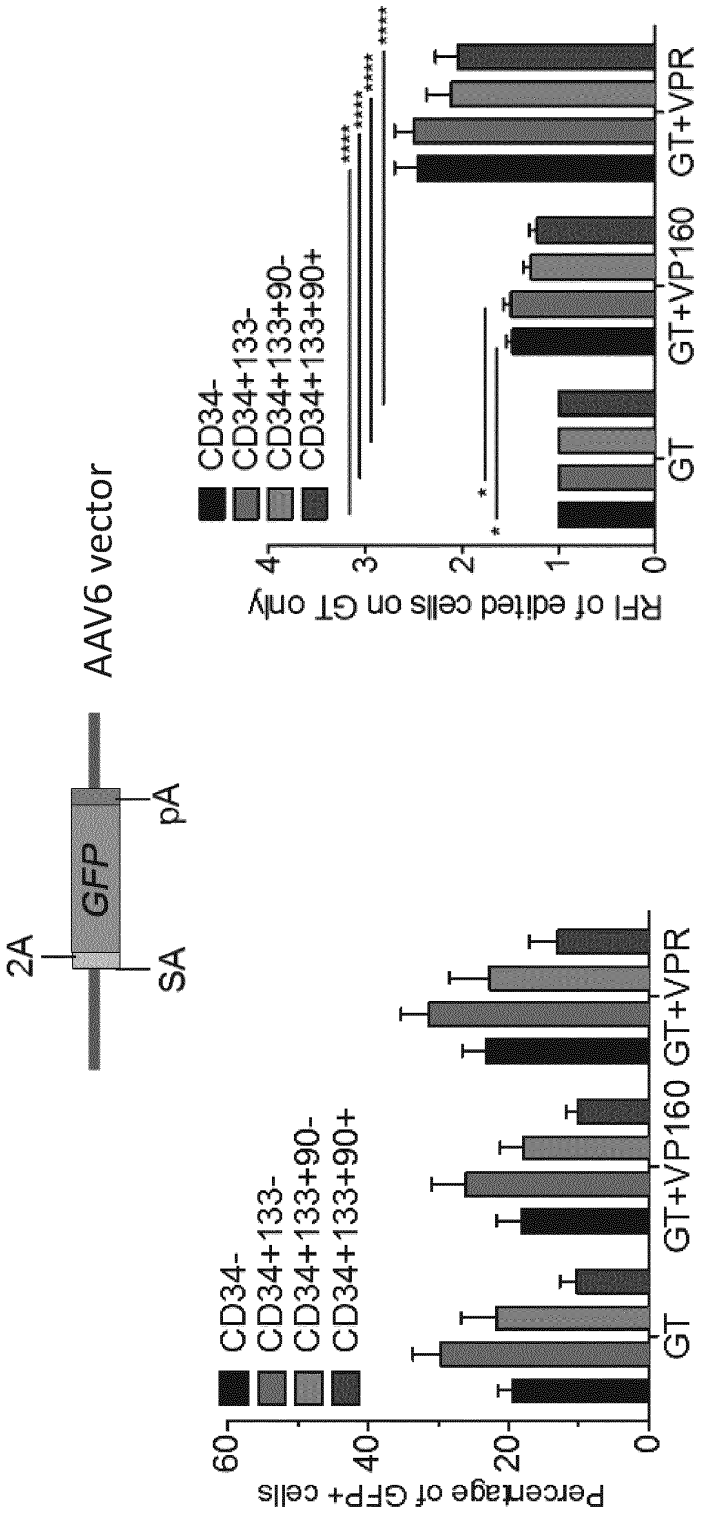

AAV6 vector
2A
SA
GFP
pA
CD34-
CD34+133-
CD34+133+90-
CD34+133+90+
Percentage of GFP+ cells
0
20
40
60
GT
GT+VP160
GT+VPR
RFI of edited cells on GT only
0
1
2
3
4
****
*

FIG. 4C

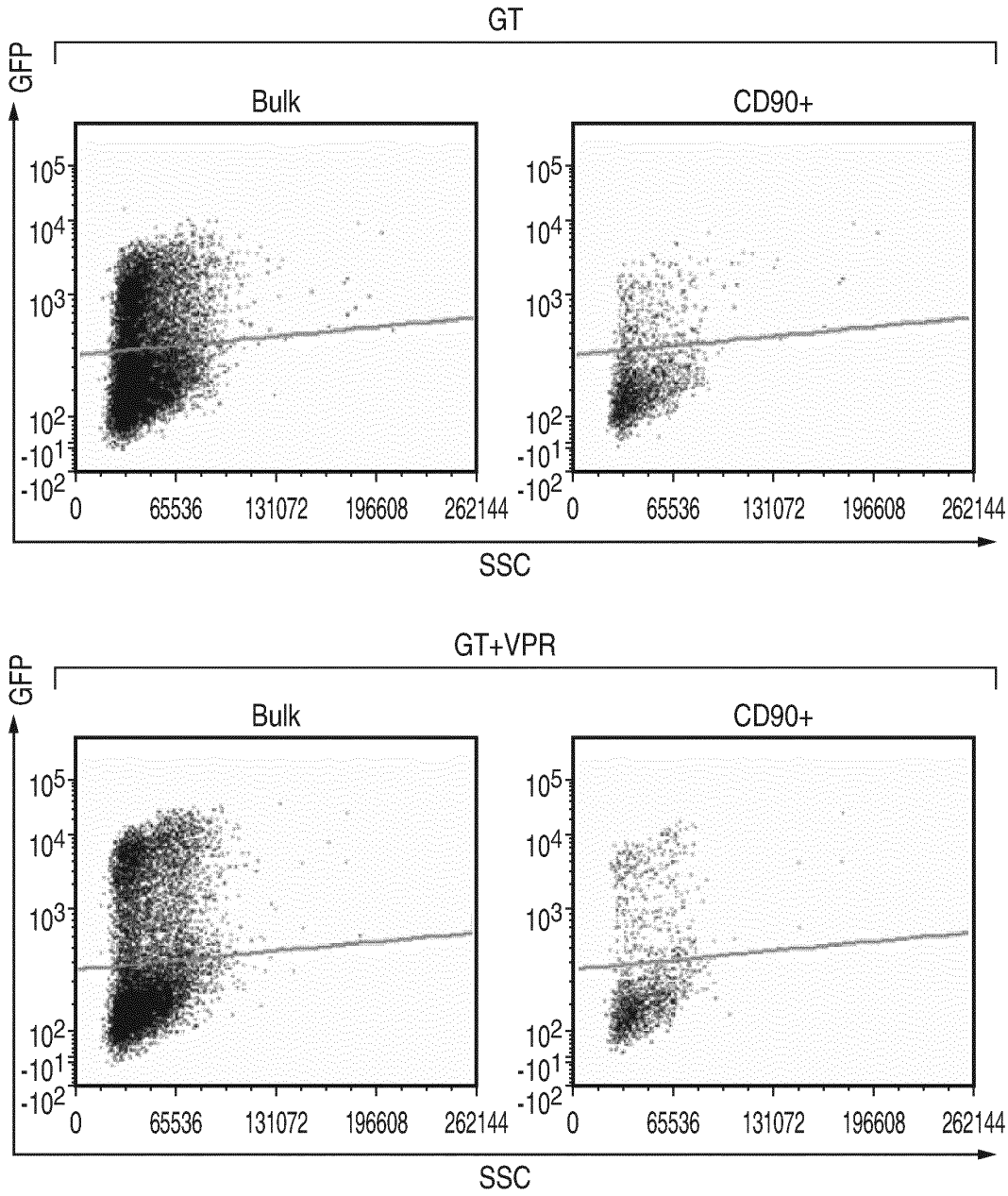

GT
GFP
Bulk
CD90+
10^5
10^4
10^3
10^2
-10^1
-10^2
0
65536
131072
196608
262144
SSC
GT+VPR
GFP
Bulk
CD90+
10^5
10^4
10^3
10^2
-10^1
-10^2
0
65536
131072
196608
262144
SSC

FIG. 4D

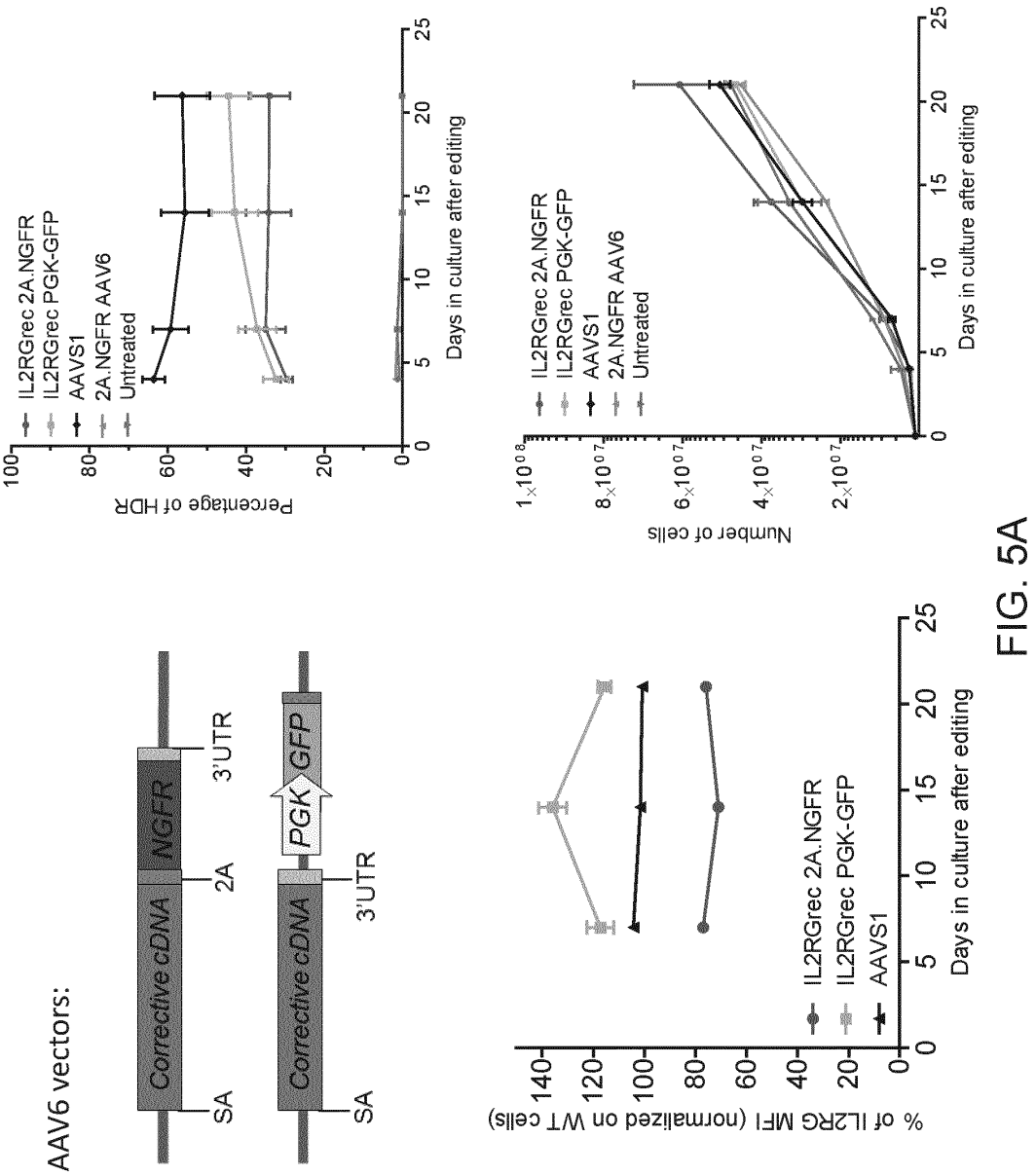

AAV6 vectors:
SA
Corrective cDNA
2A
NGFR
3'UTR
SA
Corrective cDNA
PGK
GFP
3'UTR
% of IL2RG MFI (normalized on WT cells)
Days in culture after editing
IL2RGrec 2A.NGFR
IL2RGrec PGK-GFP
AAVS1
Number of cells
Days in culture after editing
IL2RGrec 2A.NGFR
IL2RGrec PGK-GFP
AAVS1
2A.NGFR AAV6
Untreated
Percentage of HDR
Days in culture after editing
IL2RGrec 2A.NGFR
IL2RGrec PGK-GFP
AAVS1
2A.NGFR AAV6
Untreated

FIG. 5A

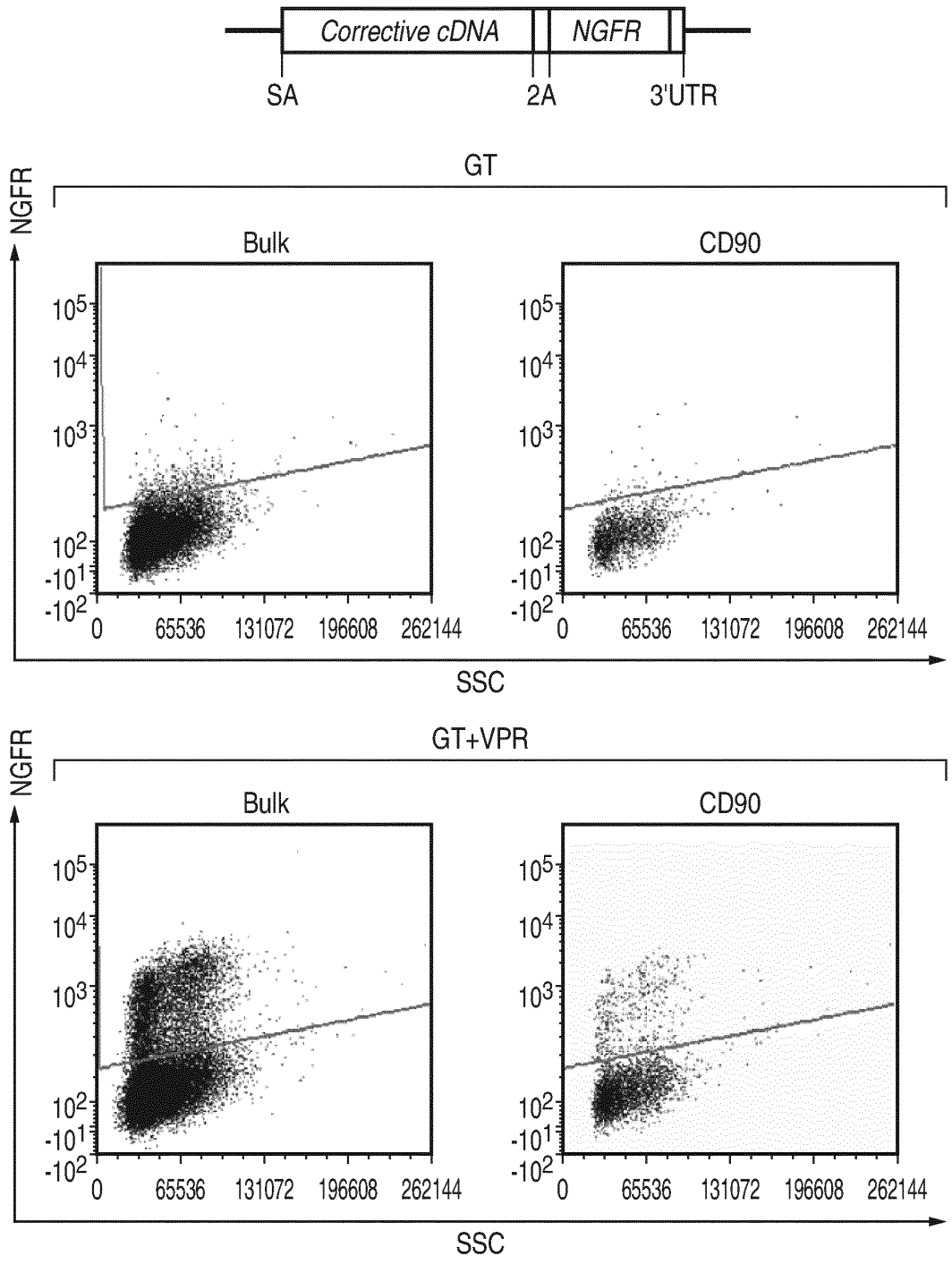
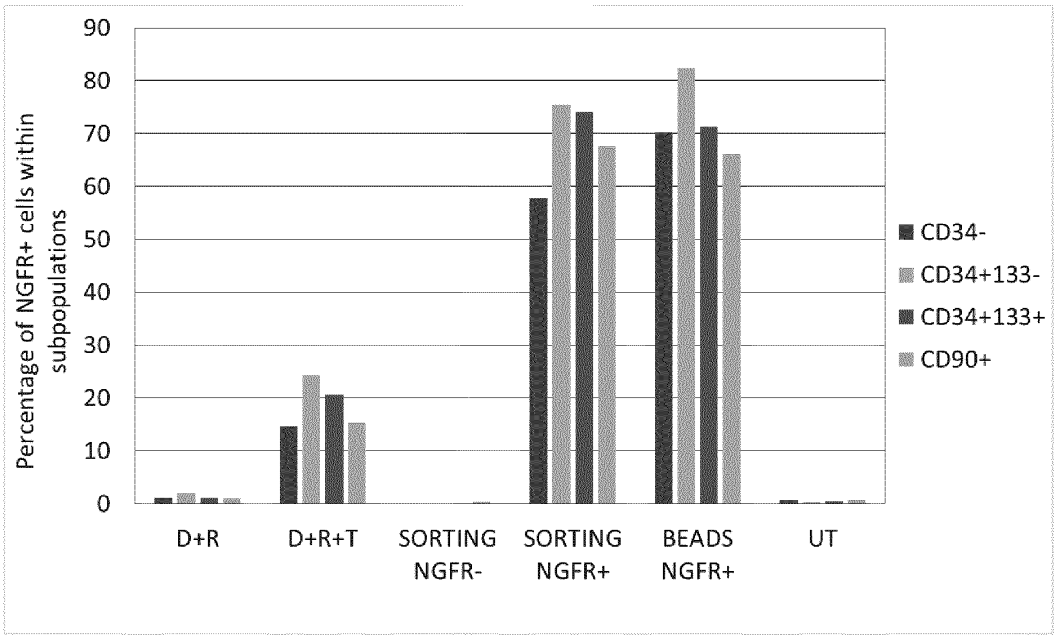

Corrective cDNA
NGFR
SA
2A
3'UTR
GT
NGFR
Bulk
CD90
10^5
10^4
10^3
10^2
-10^1
-10^2
0
65536
131072
196608
262144
SSC
GT+VPR
NGFR
Bulk
CD90
SSC

FIG. 5B


Percentage of NGFR+ cells within subpopulations
90
80
70
60
50
40
30
20
10
0
D+R
D+R+T
SORTING NGFR-
SORTING NGFR+
BEADS NGFR+
UT
CD34-
CD34+133-
CD34+133+
CD90+

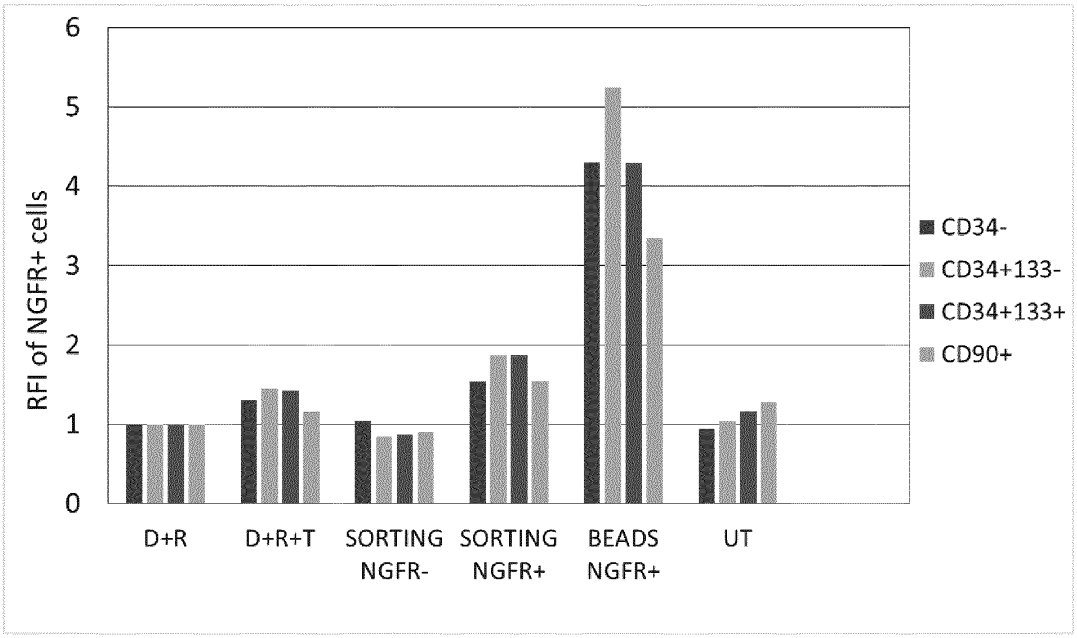
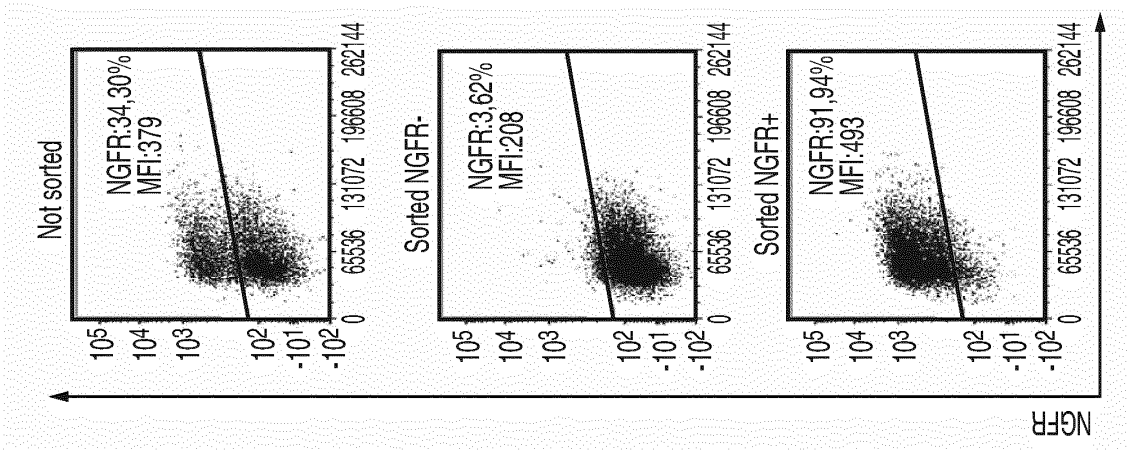

RFI of NGFR+ cells
6
5
4
3
2
1
0
D+R
D+R+T
SORTING NGFR-
SORTING NGFR+
BEADS NGFR+
UT
CD34-
CD34+133-
CD34+133+
CD90+

FIG. 5C


Not sorted
NGFR:34,30%
MFI:379
Sorted NGFR-
NGFR:3,62%
MFI:208
Sorted NGFR+
NGFR:91,94%
MFI:493
NGFR
105
104
103
102
-101
-102
0
65536
131072
196608
262144

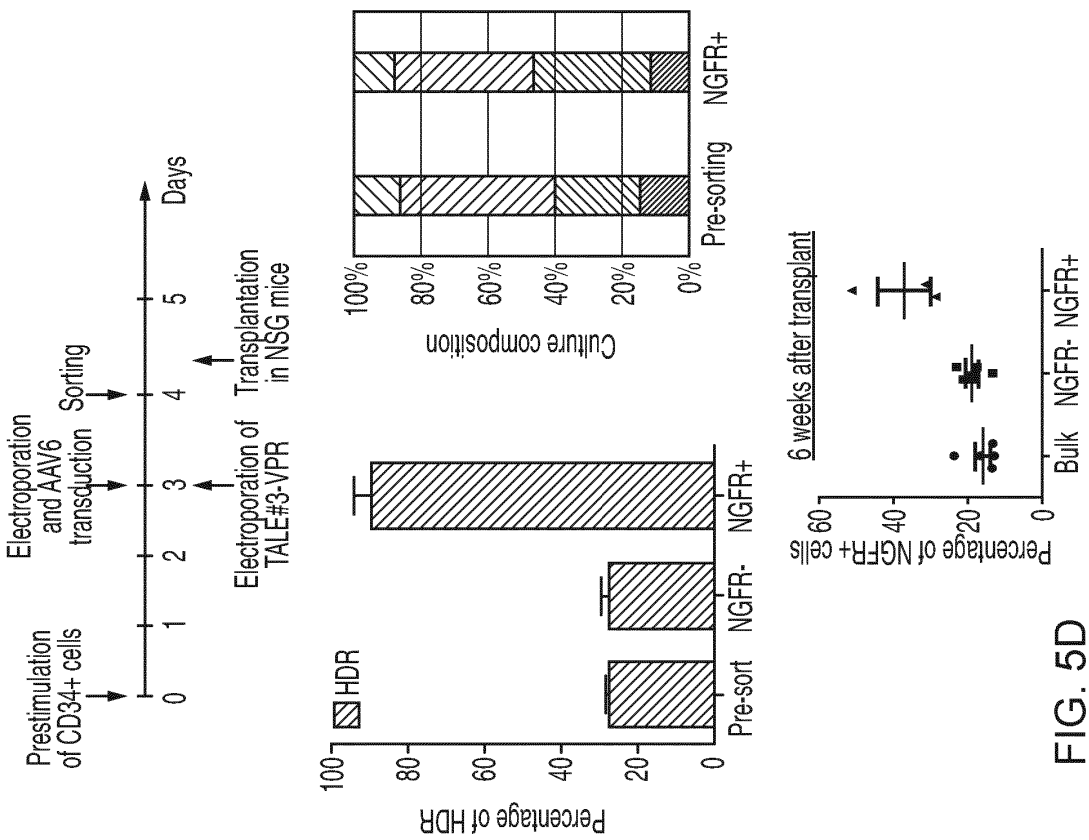

Prestimulation of CD34+ cells
Electroporation and AAV6 transduction
Electroporation of TALE#3-VPR
Sorting
Transplantation in NSG mice
0 1 2 3 4 5 Days
HDR
Percentage of HDR
100
80
60
40
20
0
Pre-sort
NGFR-
NGFR+
Culture composition
100%
80%
60%
40%
20%
0%
Pre-sorting
NGFR+
6 weeks after transplant
Percentage of NGFR+ cells
60
40
20
0
Bulk
NGFR-
NGFR+

FIG. 5D

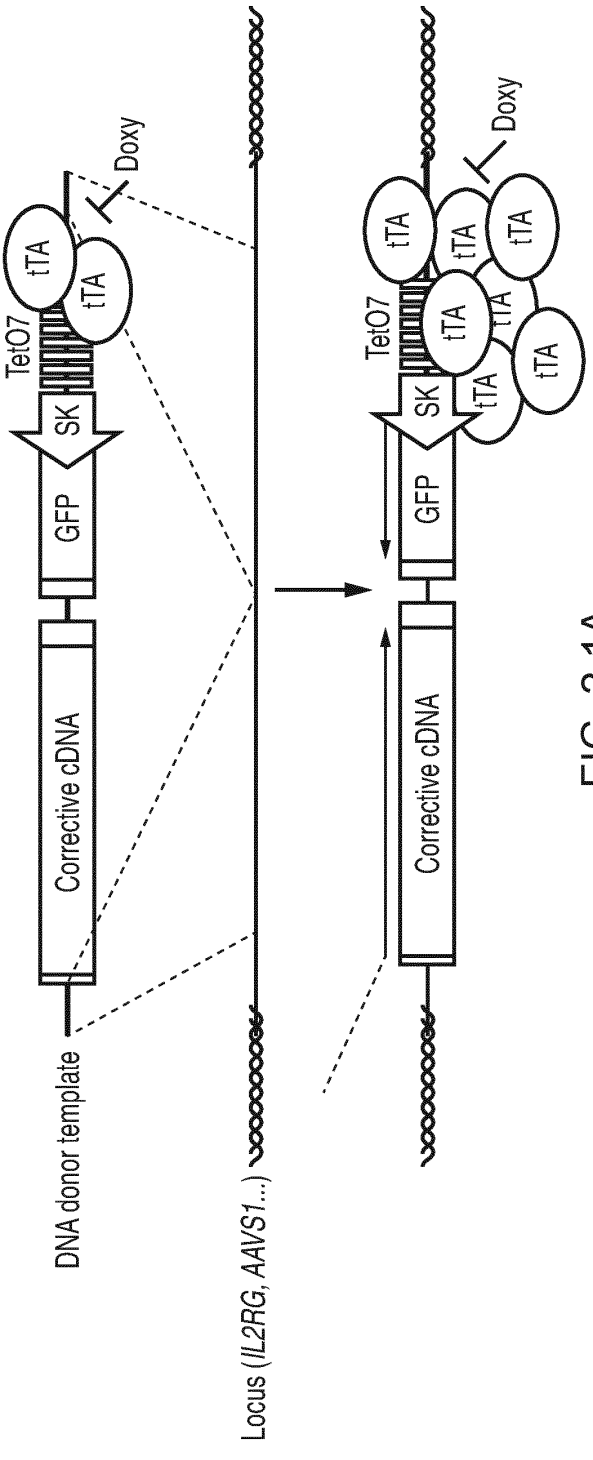


FIG. 2.1A

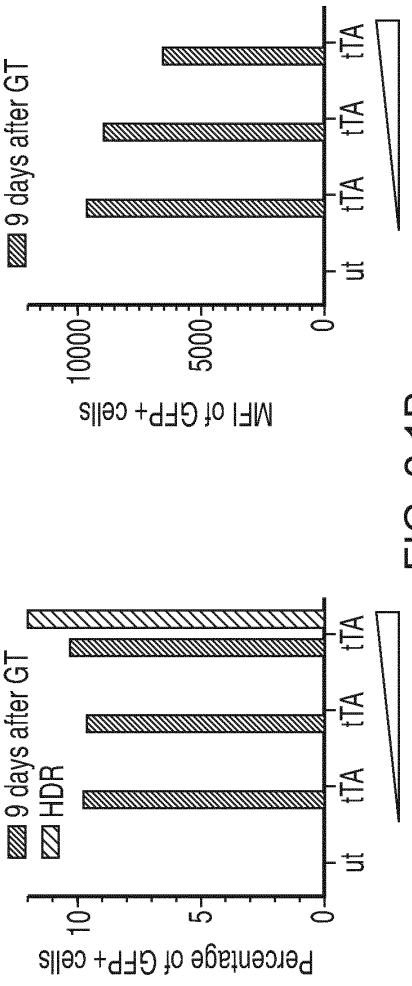


FIG. 2.1B

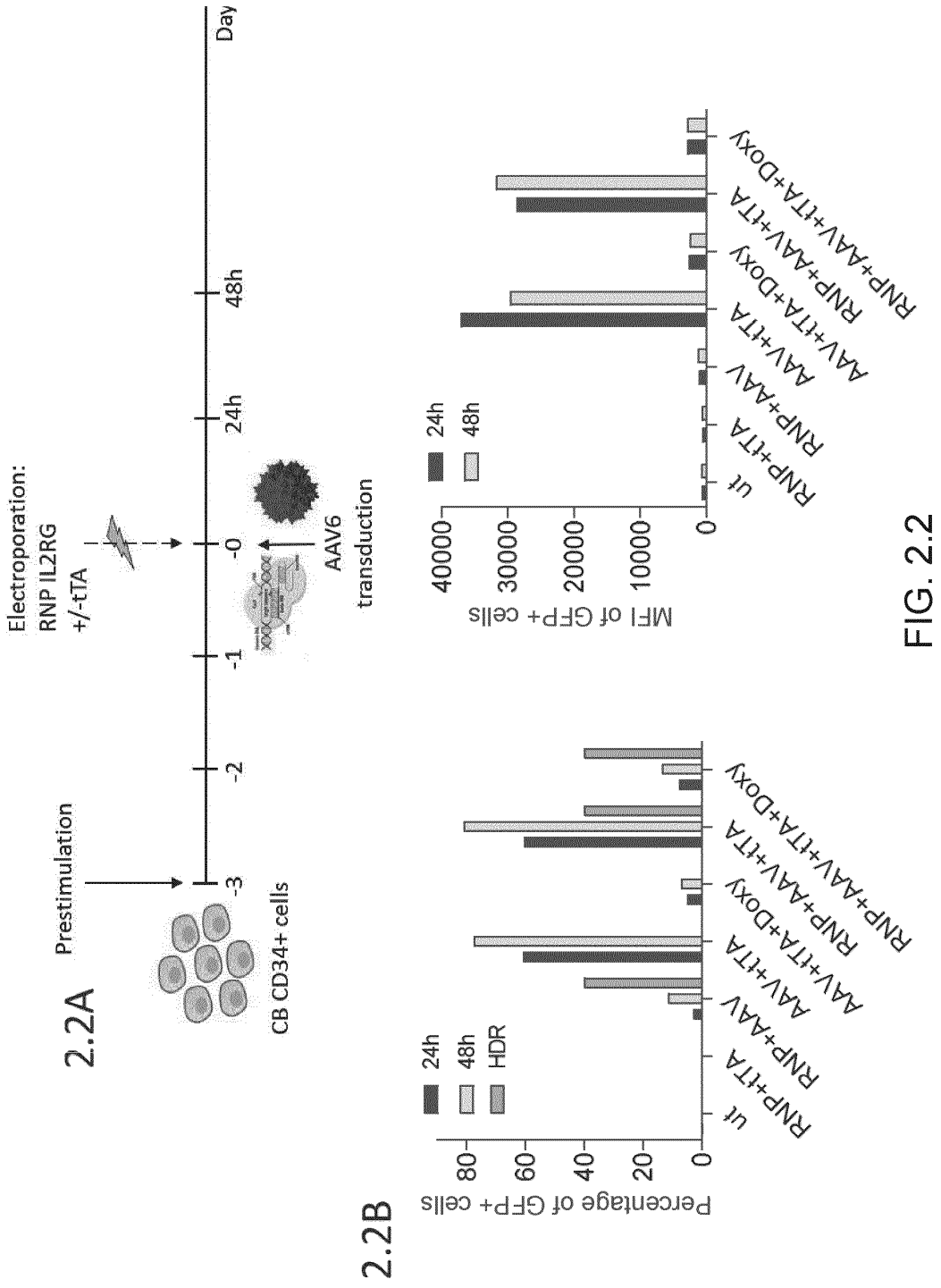

2.2A
Prestimulation
CB CD34+ cells
Electroporation:
RNP IL2RG
+/-tTA
AAV6
transduction
Day
-3
-2
-1
-0
24h
48h
2.2B
Percentage of GFP+ cells
MFI of GFP+ cells
24h
48h
HDR
ut
RNP+tTA
RNP+AAV
AAV+tTA
AAV+tTA+Doxy
RNP+AAV+tTA
RNP+AAV+tTA+Doxy

FIG. 2.2

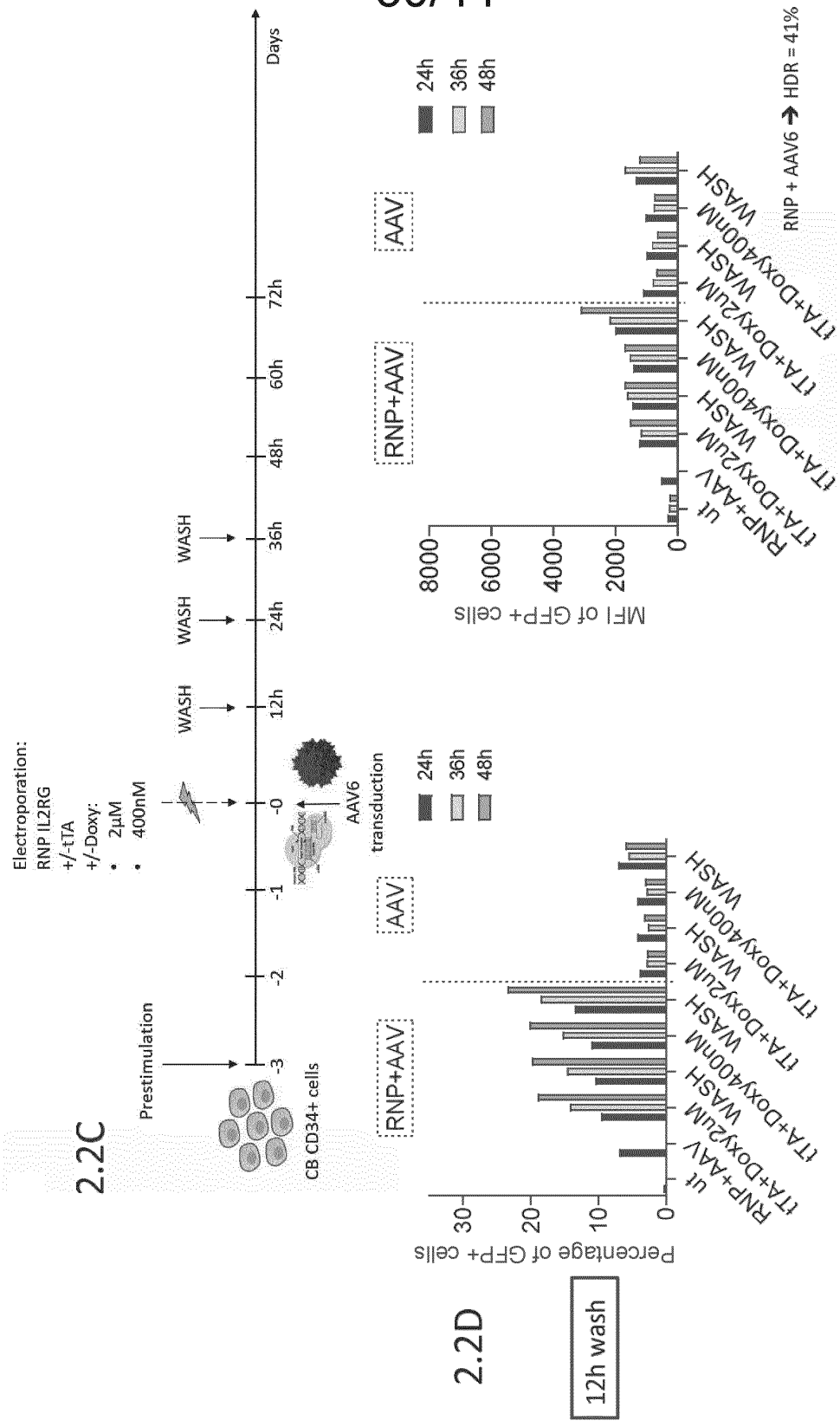


FIG. 2.2 (Continued)

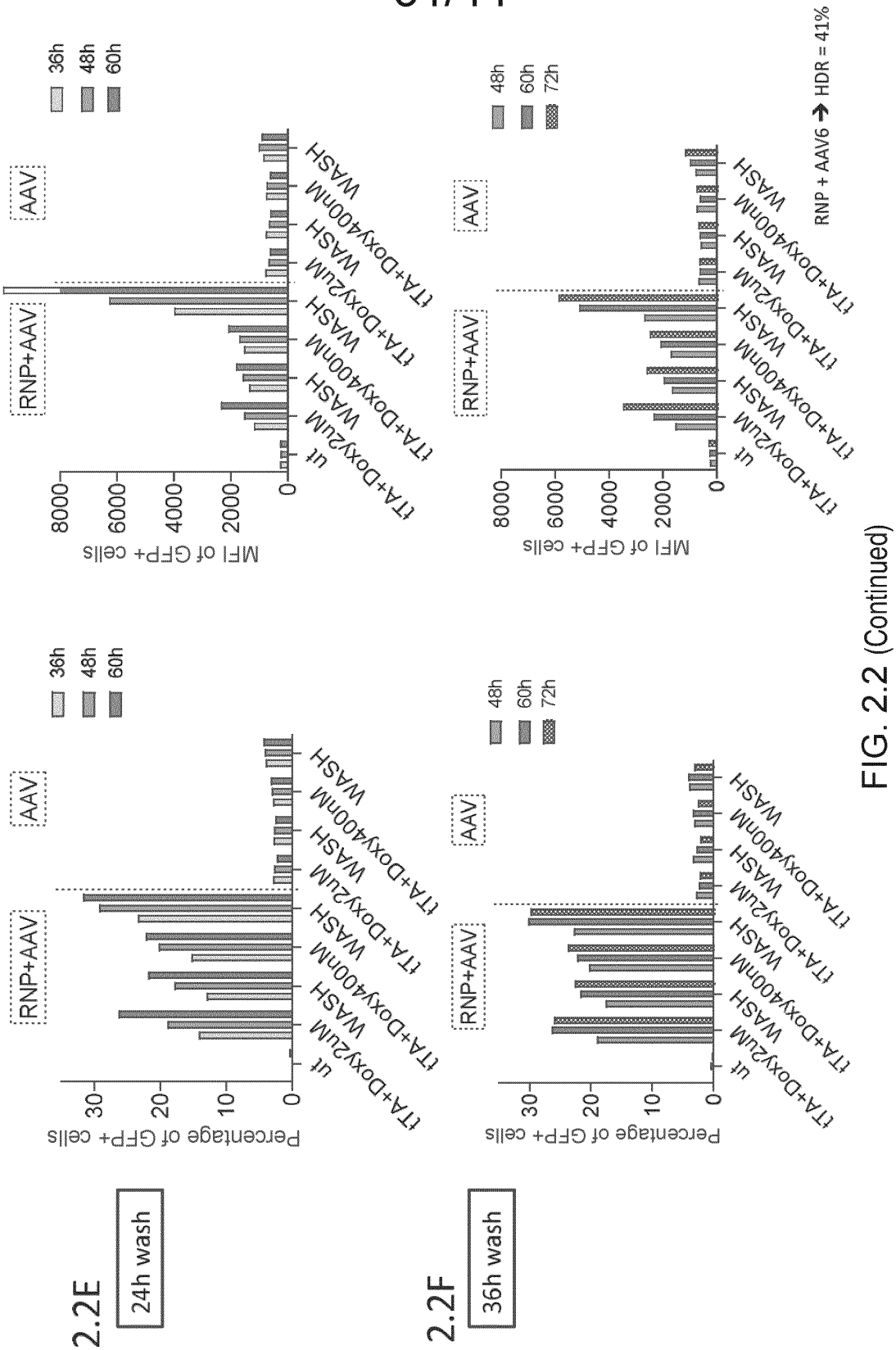


FIG. 2.2 (Continued)

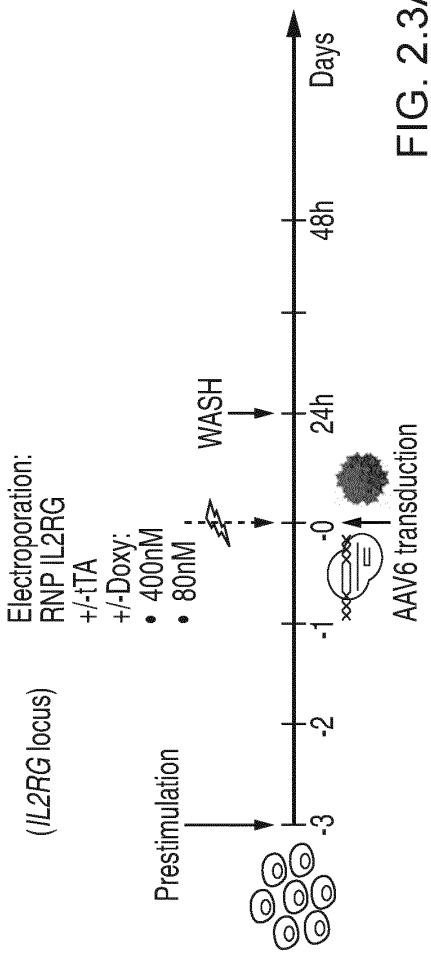


FIG. 2.3A

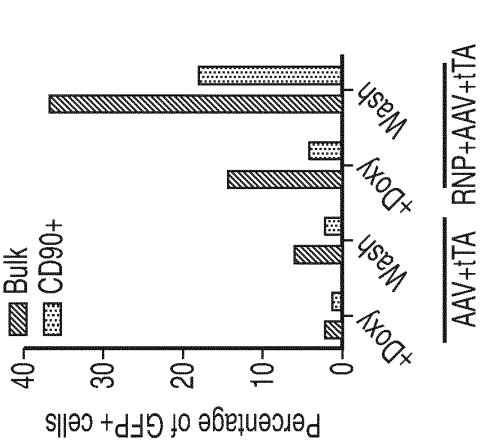


FIG. 2.3D

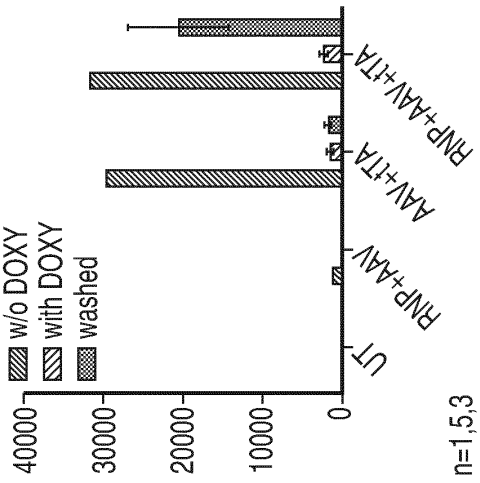


FIG. 2.3C

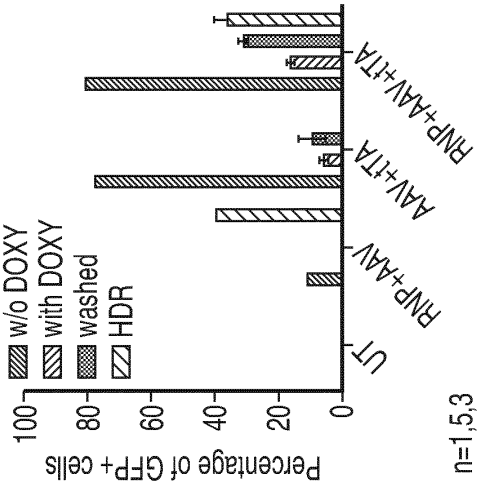


FIG. 2.3B

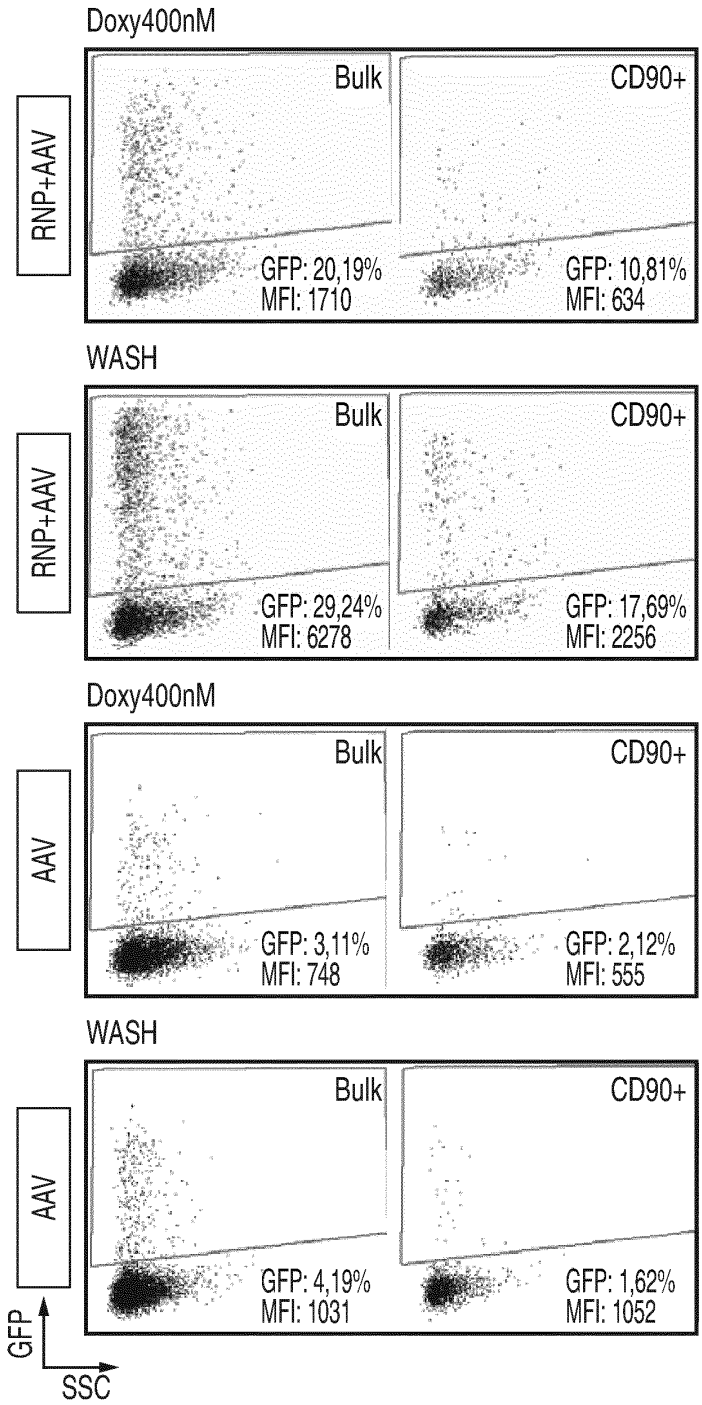

Doxy400nM
RNP+AAV
Bulk
CD90+
GFP: 20,19%
MFI: 1710
GFP: 10,81%
MFI: 634
WASH
RNP+AAV
Bulk
CD90+
GFP: 29,24%
MFI: 6278
GFP: 17,69%
MFI: 2256
Doxy400nM
AAV
Bulk
CD90+
GFP: 3,11%
MFI: 748
GFP: 2,12%
MFI: 555
WASH
AAV
Bulk
CD90+
GFP: 4,19%
MFI: 1031
GFP: 1,62%
MFI: 1052
GFP
SSC

FIG. 2.3E

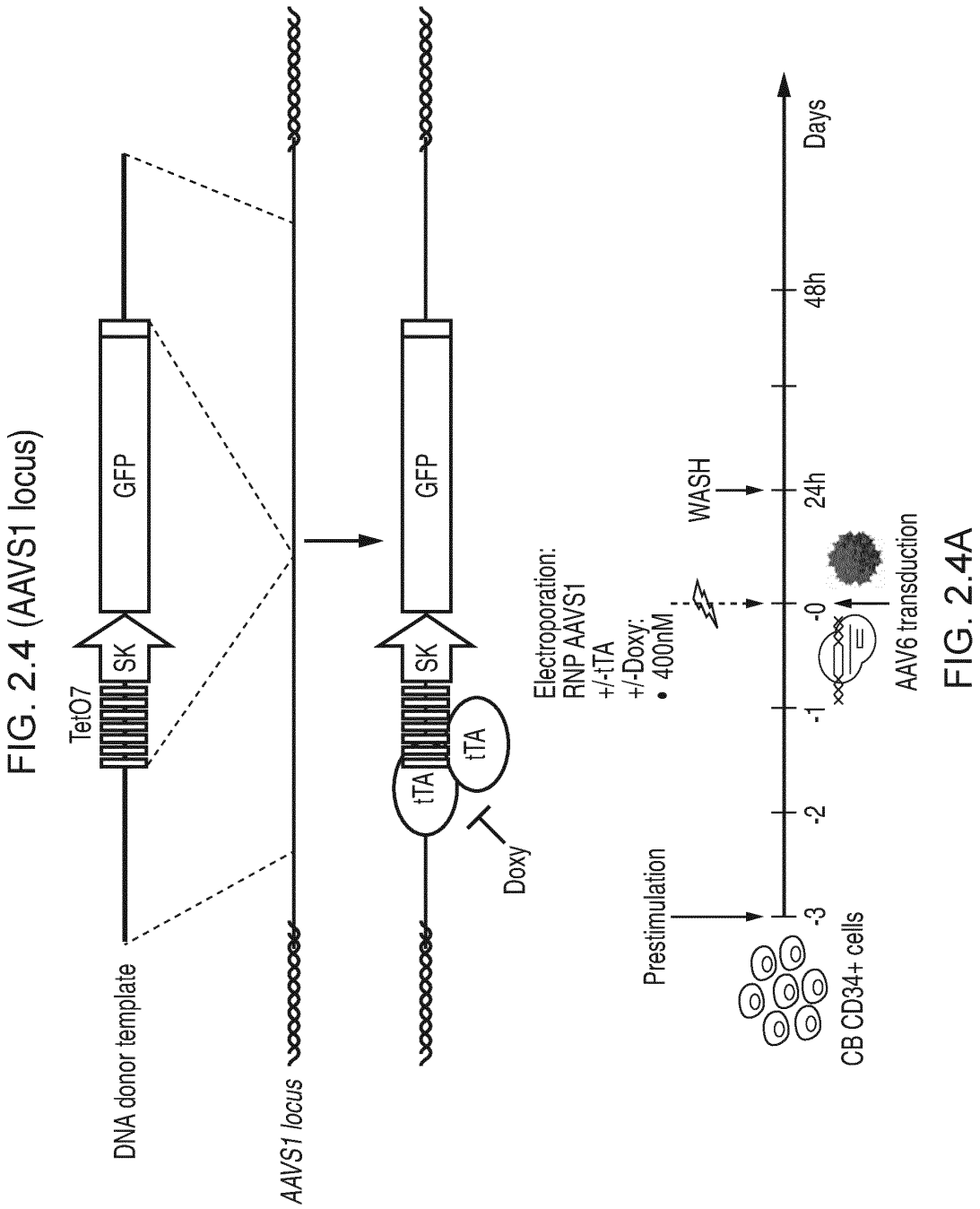


FIG. 2.4 (AAVS1 locus)

FIG. 2.4A

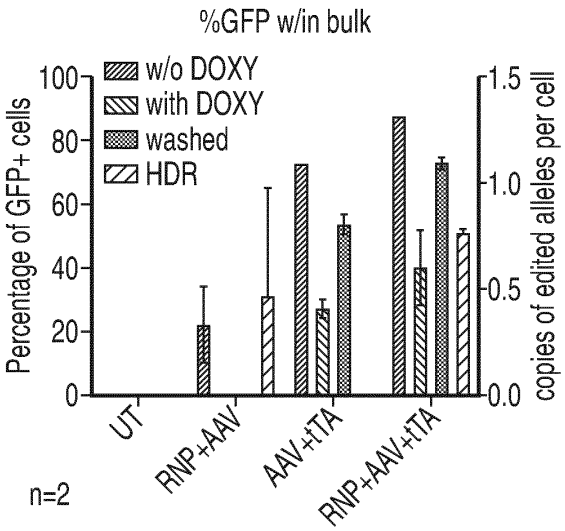


FIG. 2.4B

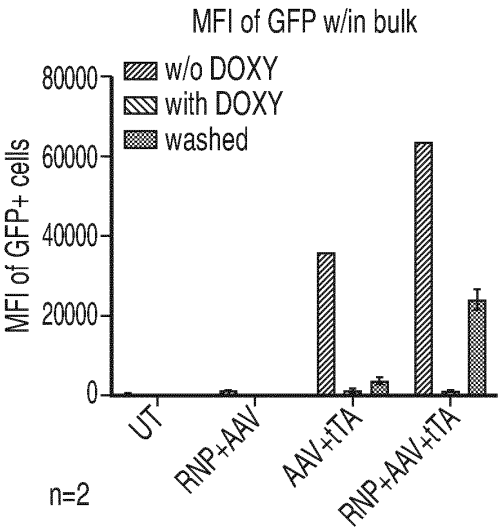


FIG. 2.4C

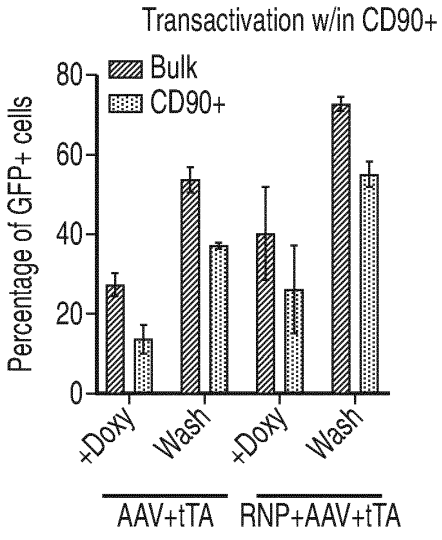


FIG. 2.4D

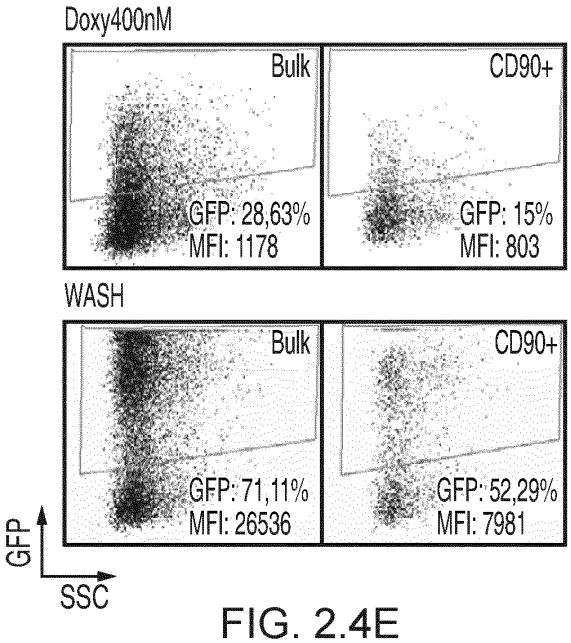


FIG. 2.4E

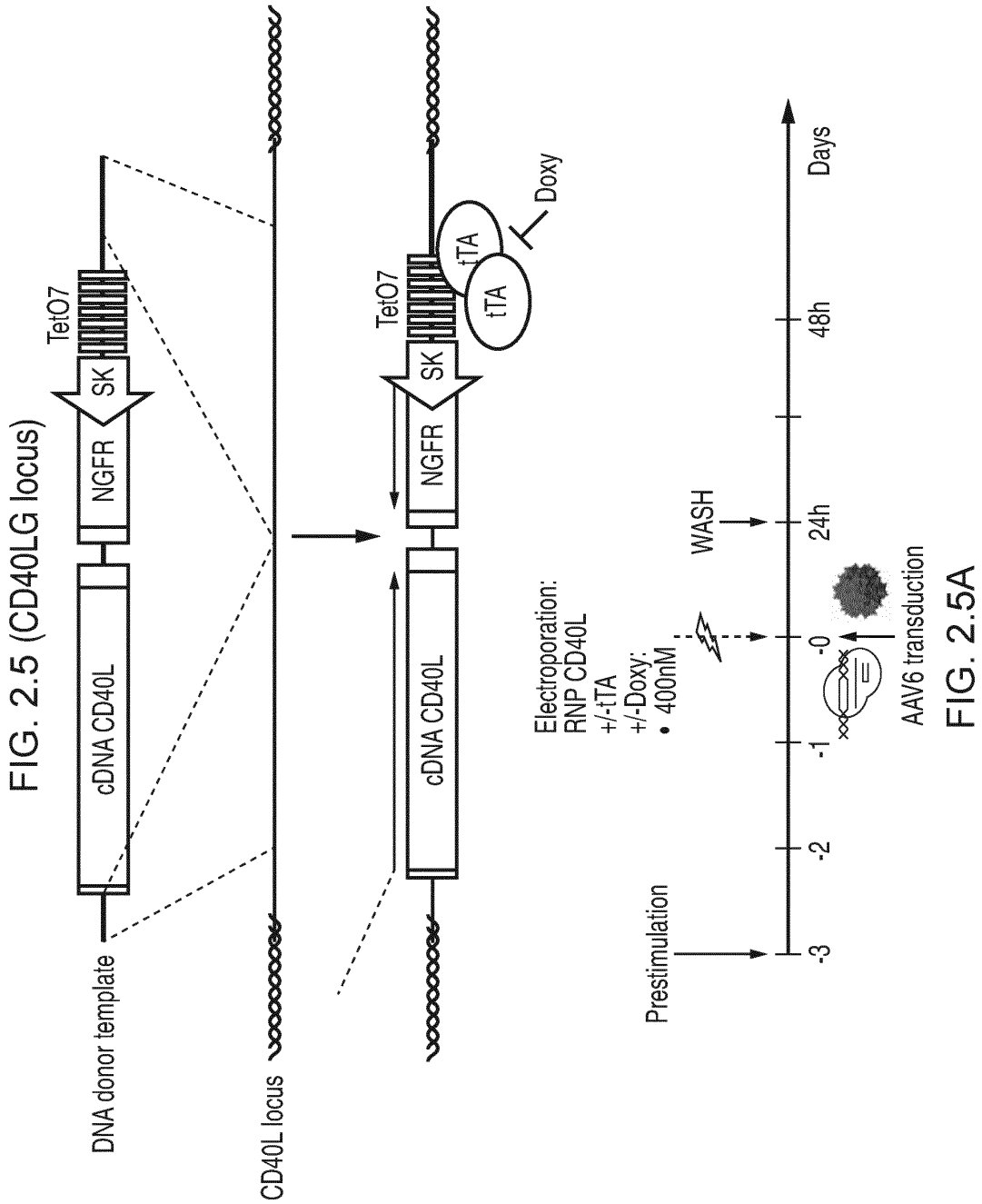

FIG. 2.5 (CD40LG locus)
DNA donor template
TetO7
SK
NGFR
cDNA CD40L
CD40L locus
TetO7
SK
NGFR
cDNA CD40L
tTA
tTA
Doxy
Electroporation:
RNP CD40L
+/-tTA
+/-Doxy:
• 400nM
WASH
Prestimulation
-3
-2
-1
-0
24h
48h
Days
AAV6 transduction

FIG. 2.5A

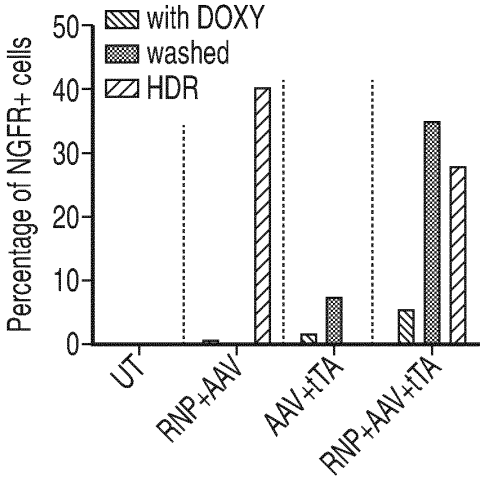


FIG. 2.5B

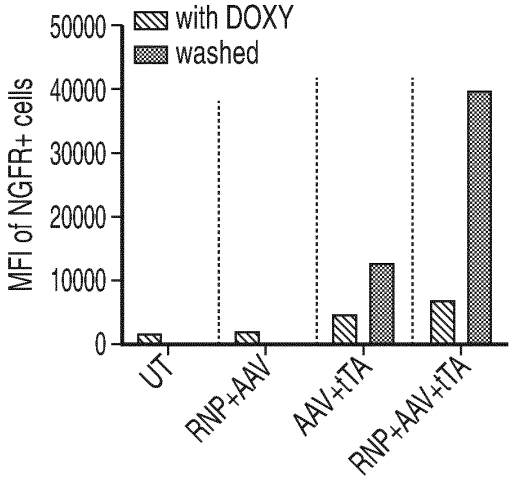


FIG. 2.5C

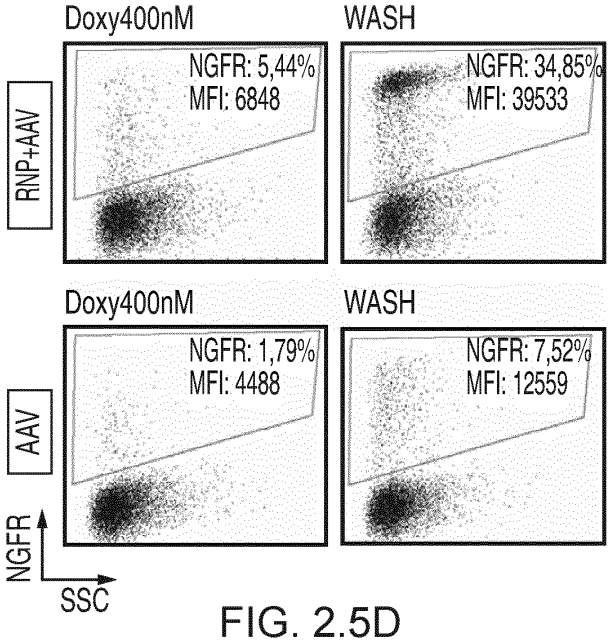


FIG. 2.5D

FIG. 2.6

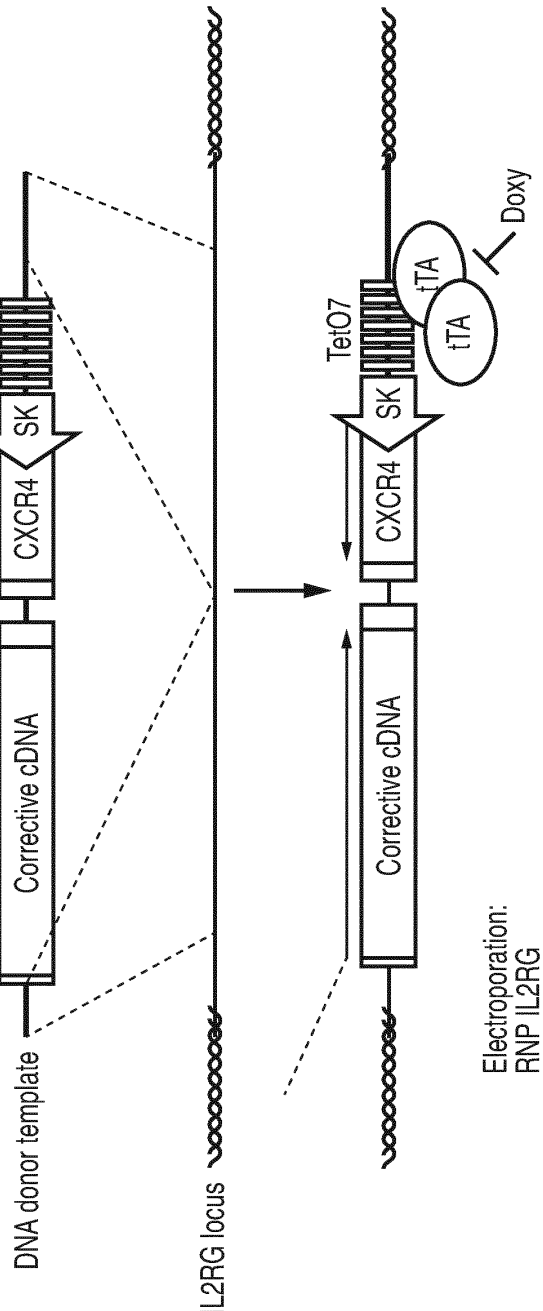
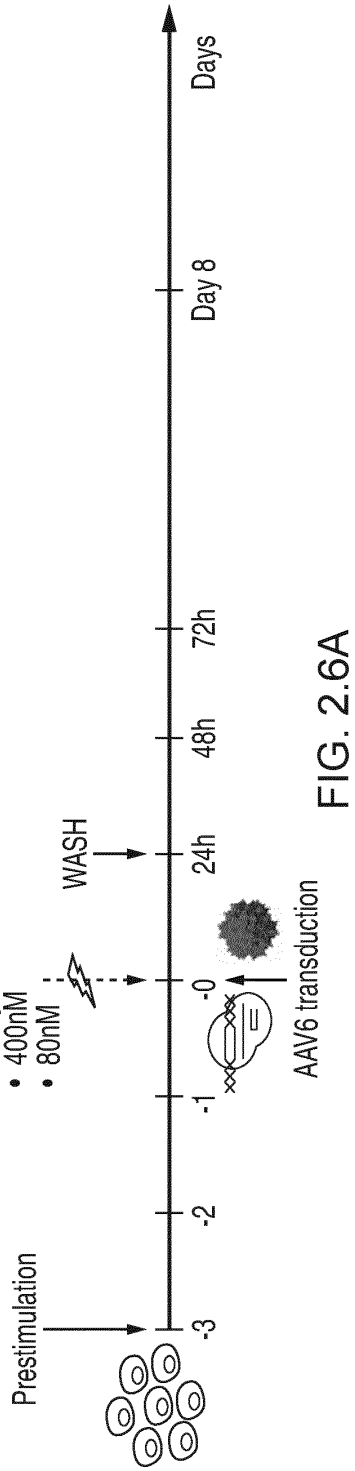


FIG. 2.6A



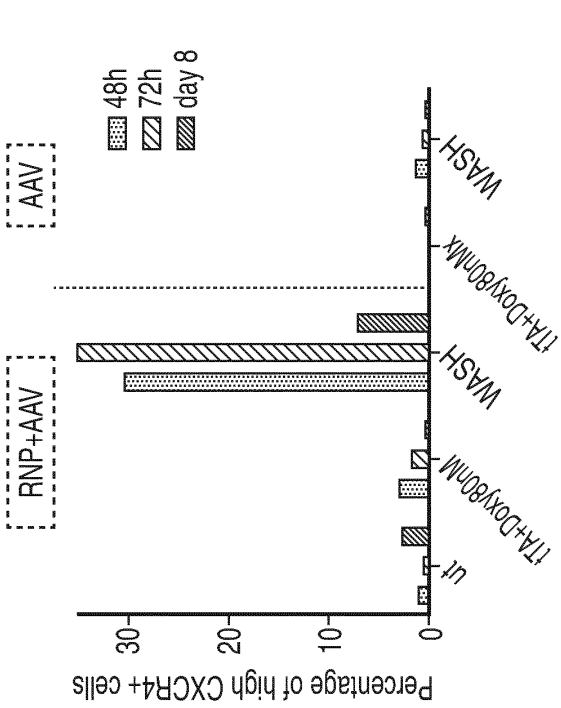


FIG. 2.6B

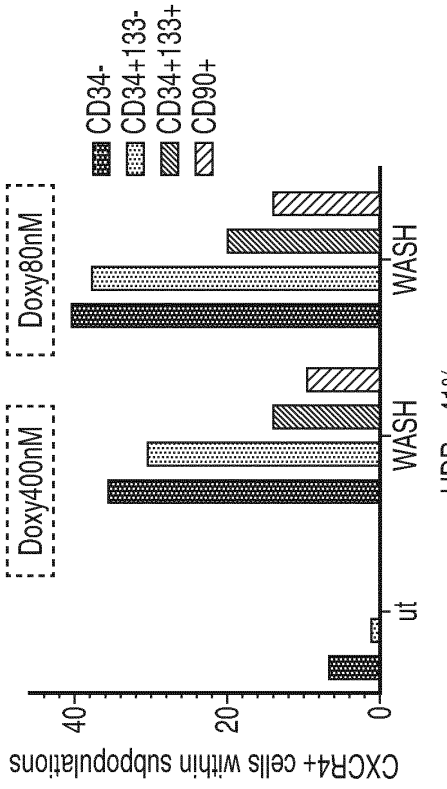


FIG. 2.6D

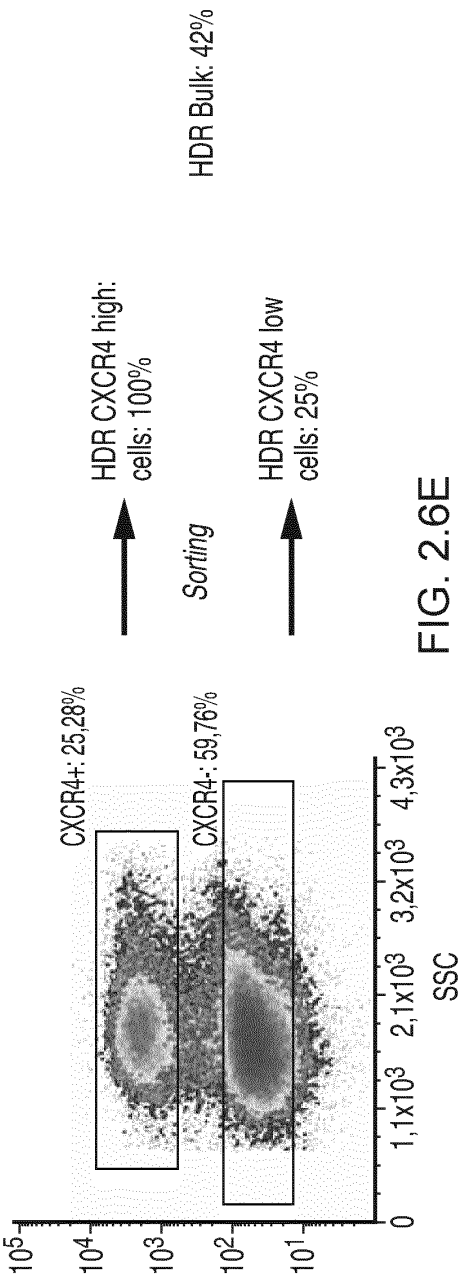


FIG. 2.6E

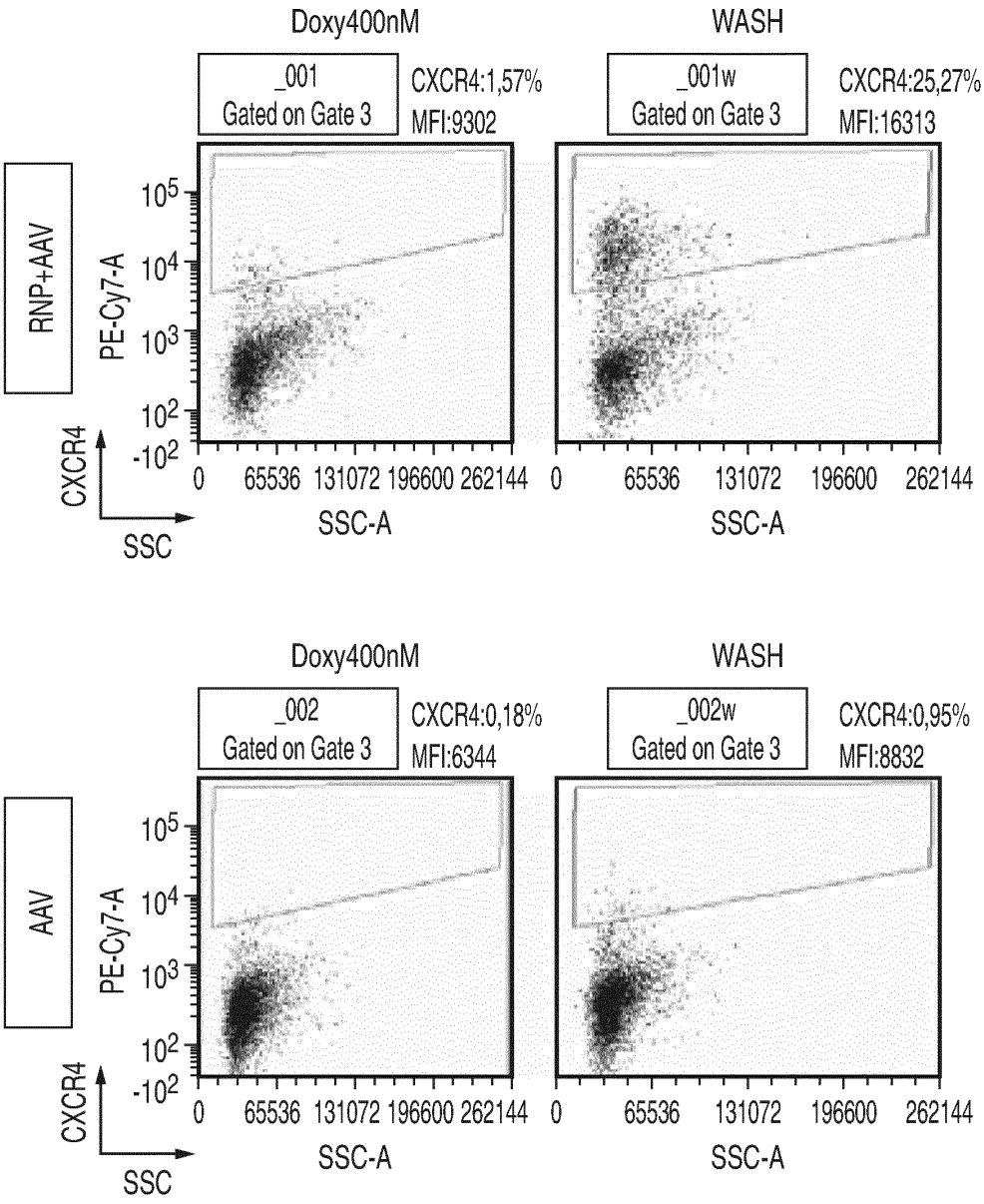

Doxy400nM
WASH
_001
Gated on Gate 3
CXCR4:1,57%
MFI:9302
_001w
Gated on Gate 3
CXCR4:25,27%
MFI:16313
RNP+AAV
PE-Cy7-A
CXCR4
SSC
SSC-A
0
65536
131072
196600
262144
Doxy400nM
WASH
_002
Gated on Gate 3
CXCR4:0,18%
MFI:6344
_002w
Gated on Gate 3
CXCR4:0,95%
MFI:8832
AAV

FIG. 2.6C

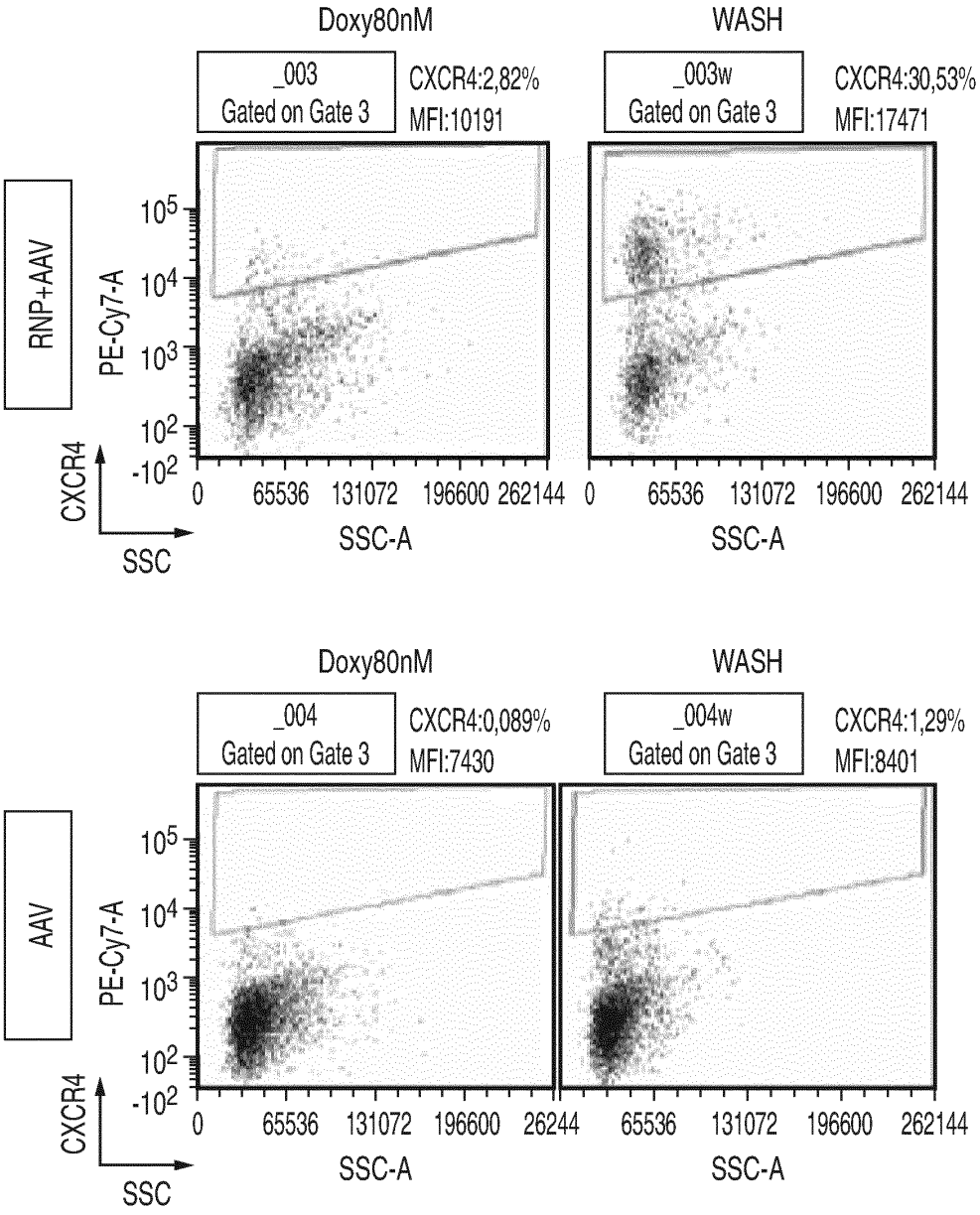


FIG. 2.6C (Continued)

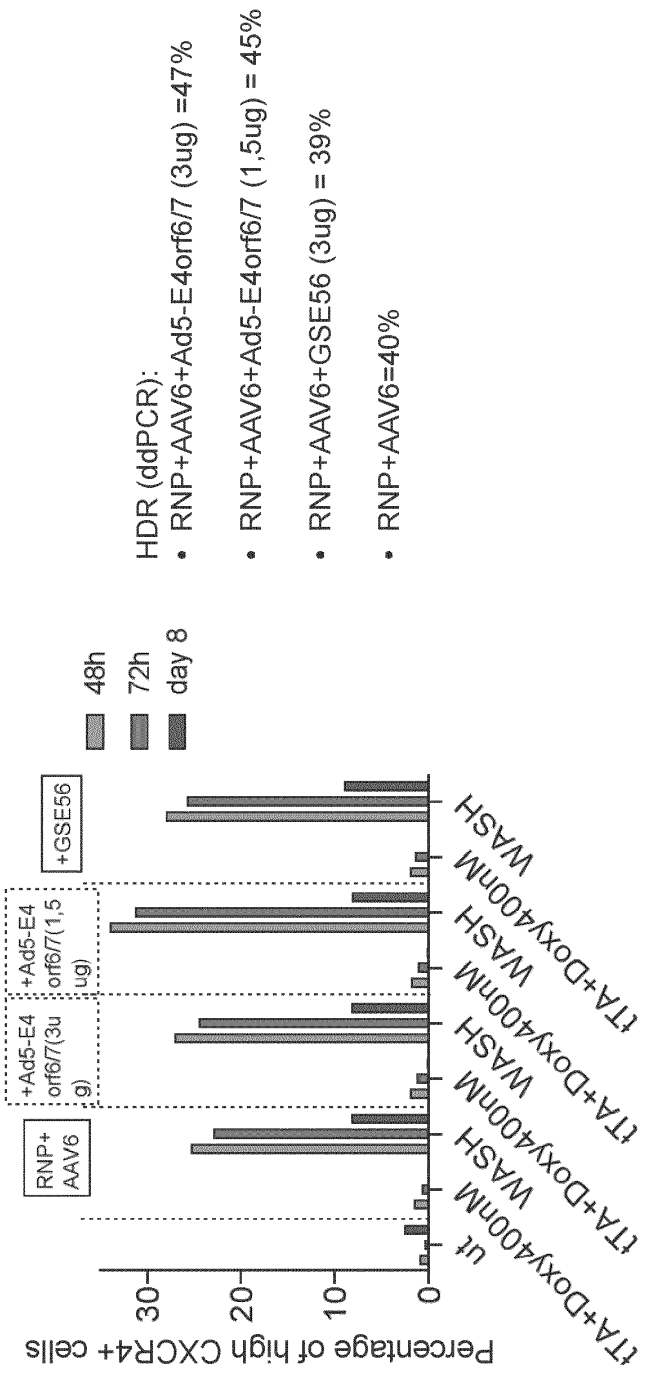

HDR (ddPCR):
• RNP+AAV6+Ad5-E4orf6/7 (3ug) =47%
• RNP+AAV6+Ad5-E4orf6/7 (1,5ug) = 45%
• RNP+AAV6+GSE56 (3ug) = 39%
• RNP+AAV6=40%
48h
72h
day 8
RNP+ AAV6
+Ad5-E4 orf6/7(3u g)
+Ad5-E4 orf6/7(1,5 ug)
+GSE56
Percentage of high CXCR4+ cells
0
10
20
30
ut
tTA+Doxy400nM WASH
tTA+Doxy400nM WASH
tTA+Doxy400nM WASH
tTA+Doxy400nM WASH
tTA+Doxy400nM WASH

FIG. 2.7A

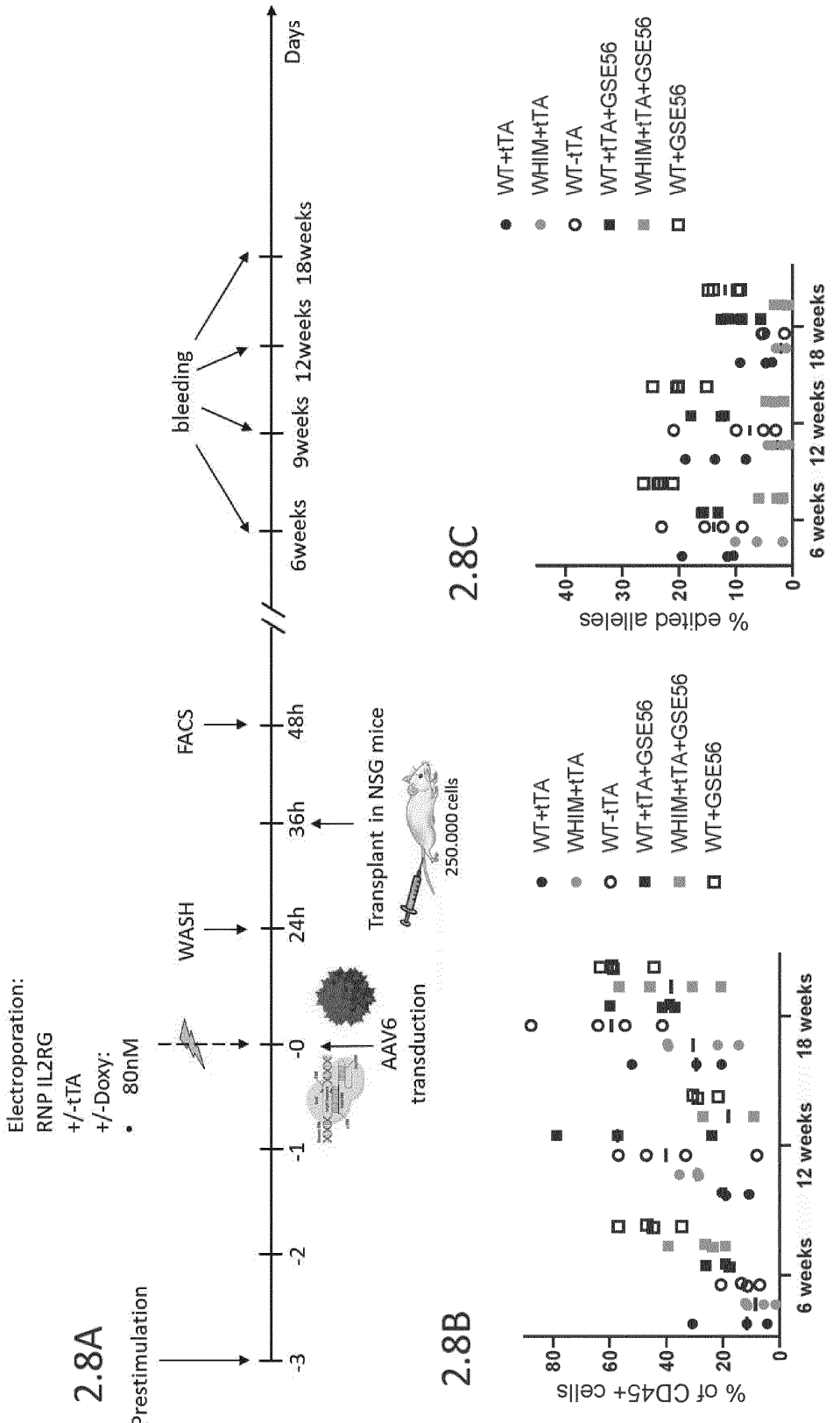

2.8A
Prestimulation
Electroporation:
RNP IL2RG
+/-tTA
+/-Doxy:
80nM
WASH
FACS
bleeding
Days
-3
-2
-1
-0
24h
36h
48h
6weeks
9weeks
12weeks
18weeks
AAV6
transduction
Transplant in NSG mice
250.000 cells
2.8B
% of CD45+ cells
6 weeks
12 weeks
18 weeks
WT+tTA
WHIM+tTA
WT-tTA
WT+tTA+GSE56
WHIM+tTA+GSE56
WT+GSE56
2.8C
% edited alleles
6 weeks
12 weeks
18 weeks
WT+tTA
WHIM+tTA
WT-tTA
WT+tTA+GSE56
WHIM+tTA+GSE56
WT+GSE56

FIG. 2.8

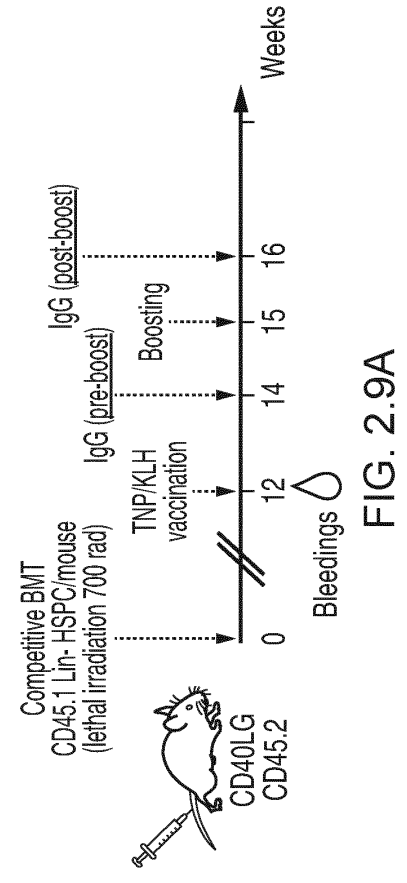


FIG. 2.9A

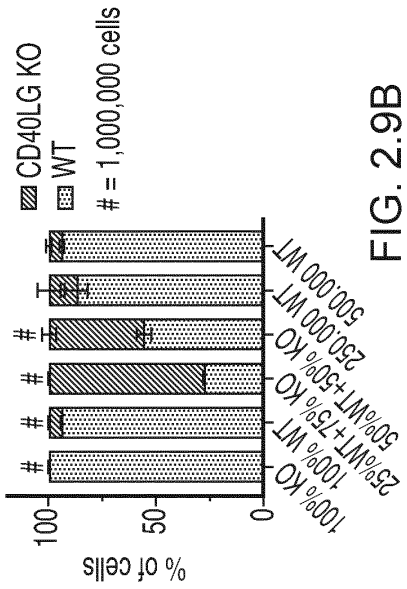


FIG. 2.9B

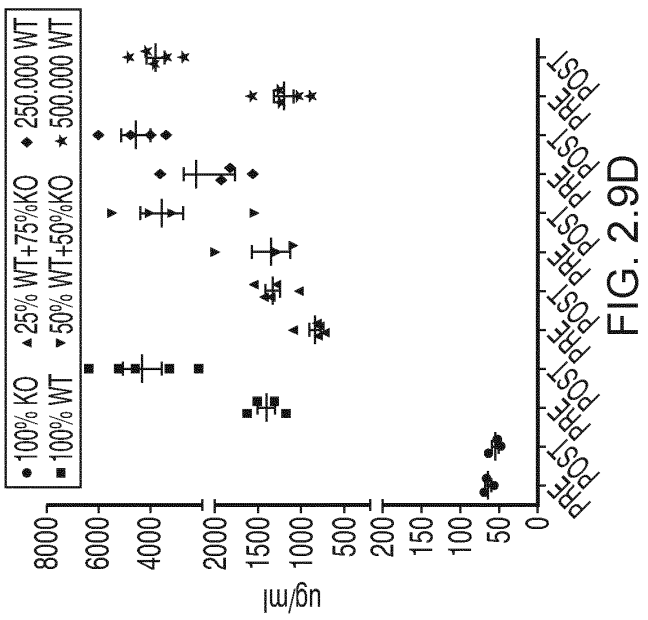


FIG. 2.9D

FIG. 2.9C