FIG. 1

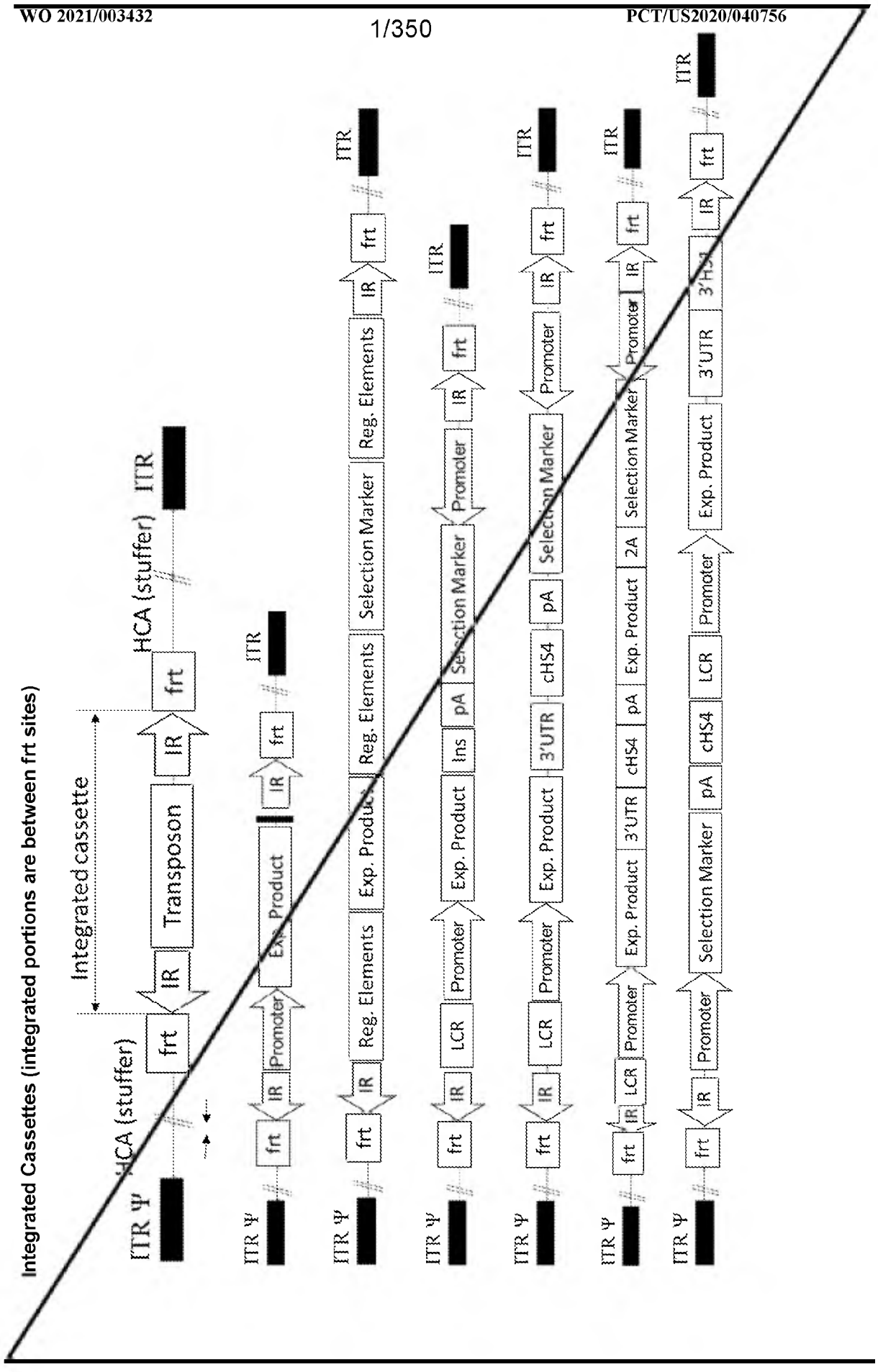

Integrated Cassettes (integrated portions are between frt sites)
Integrated cassette
HCA (stuffer)
ITR Ψ
frt
IR
Transposon
ITR
Promoter
Exp. Product
Reg. Elements
LCR
Selection Marker
pA
Ins
cHS4
3'UTR
2A
3'HS1
3'UTR

FIG. 1 cont'd

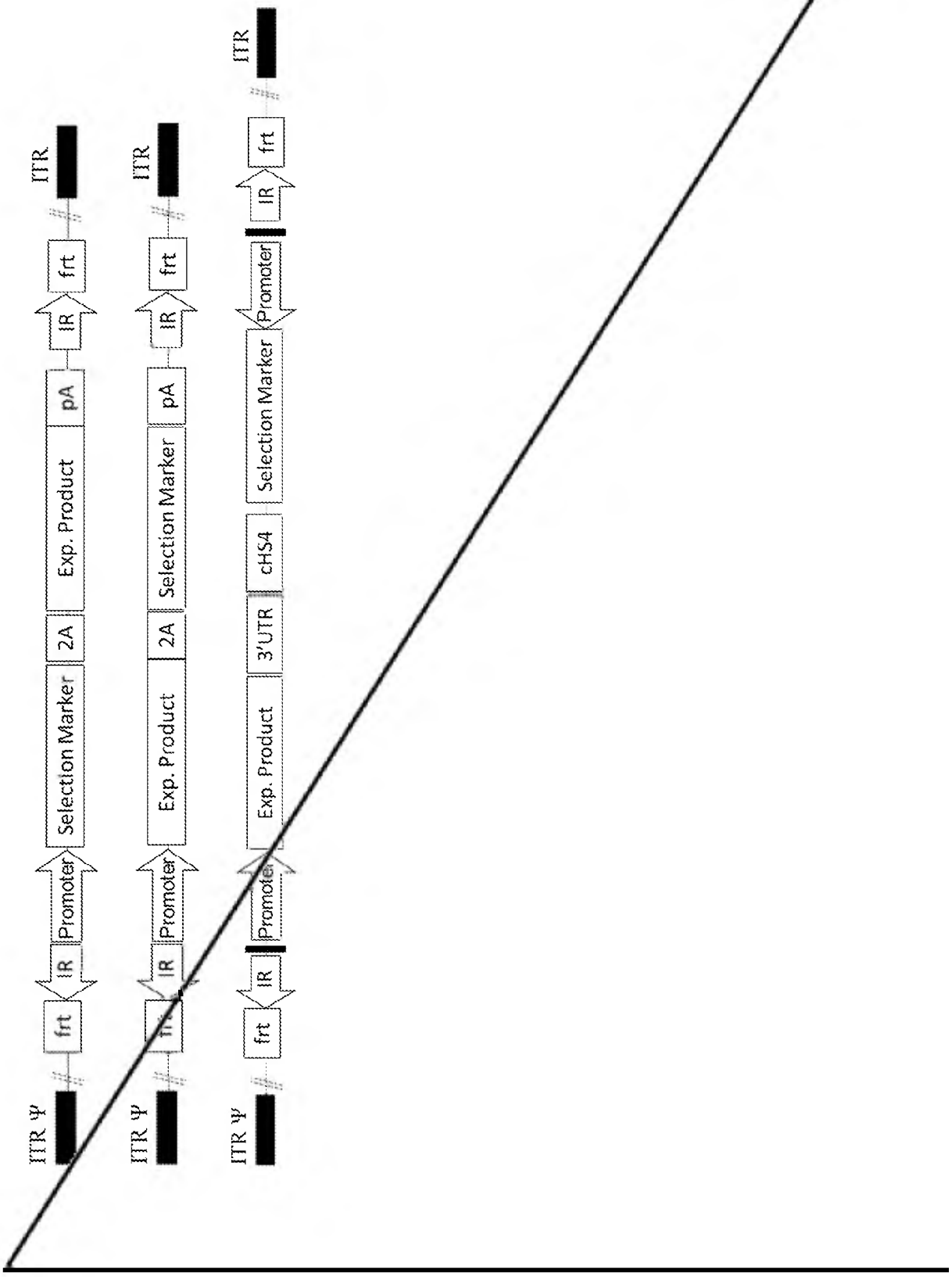

ITR Ψ
frt
IR
Promoter
Selection Marker
2A
Exp. Product
pA
IR
frt
ITR
ITR Ψ
frt
IR
Promoter
Exp. Product
2A
Selection Marker
pA
IR
frt
ITR
ITR Ψ
frt
IR
Promoter
Exp. Product
3'UTR
cHS4
Selection Marker
Promoter
IR
frt
ITR

FIG. 1 cont'd

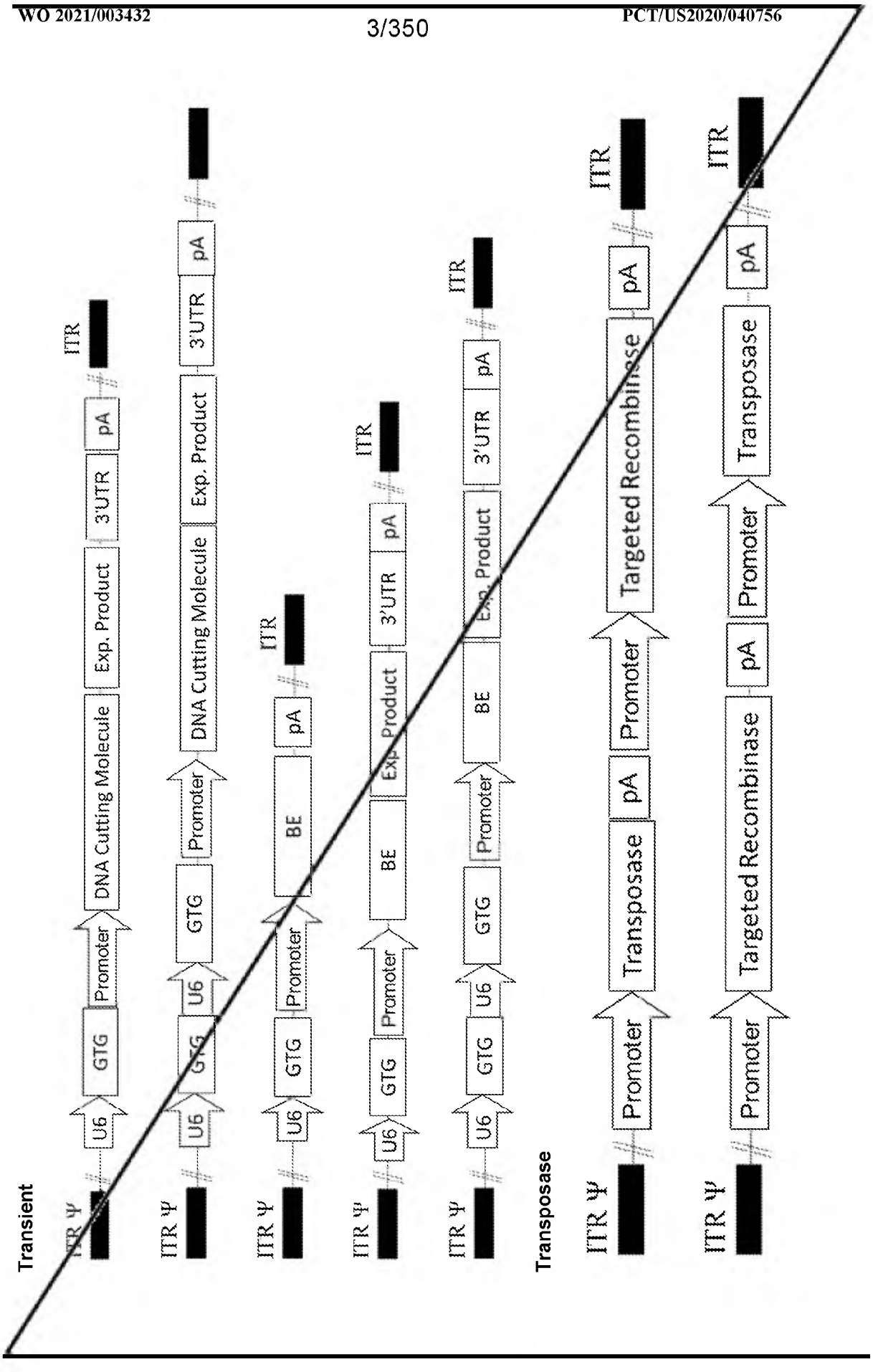

Transient
ITR Ψ
U6
GTG
Promoter
DNA Cutting Molecule
Exp. Product
3'UTR
pA
ITR
BE
Transposase
Targeted Recombinase

FIG. 2A

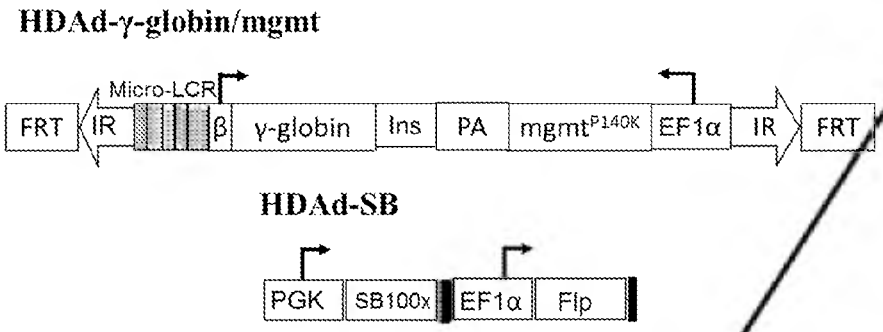


FIG. 2B

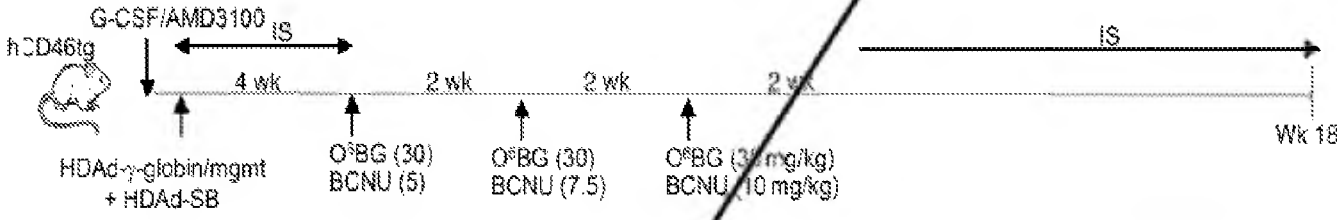


FIG. 2C

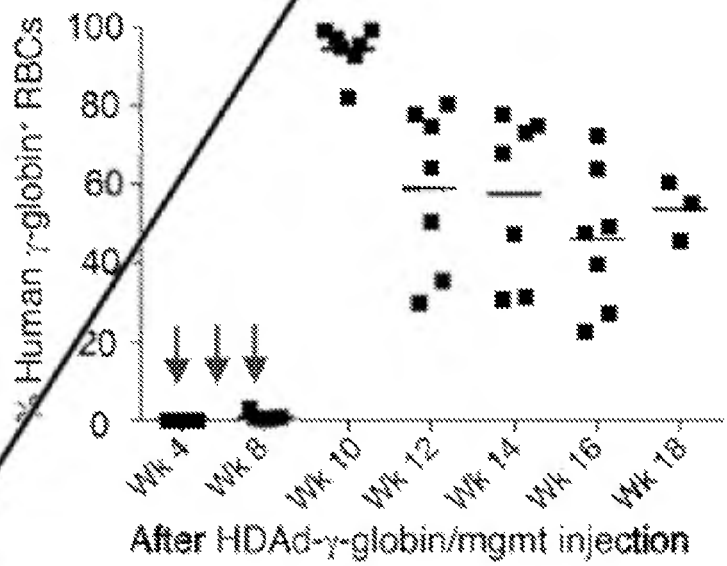


FIG. 2D

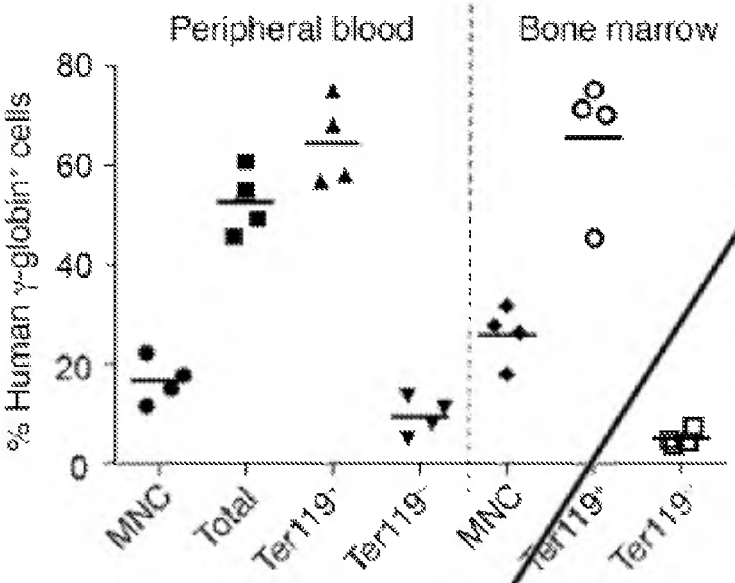

Peripheral blood
Bone marrow
% Human γ-globin+ cells
80
60
40
20
0
MNC
Total
Ter119+
Ter119-
MNC
Ter119+
Ter119-

FIG. 2E

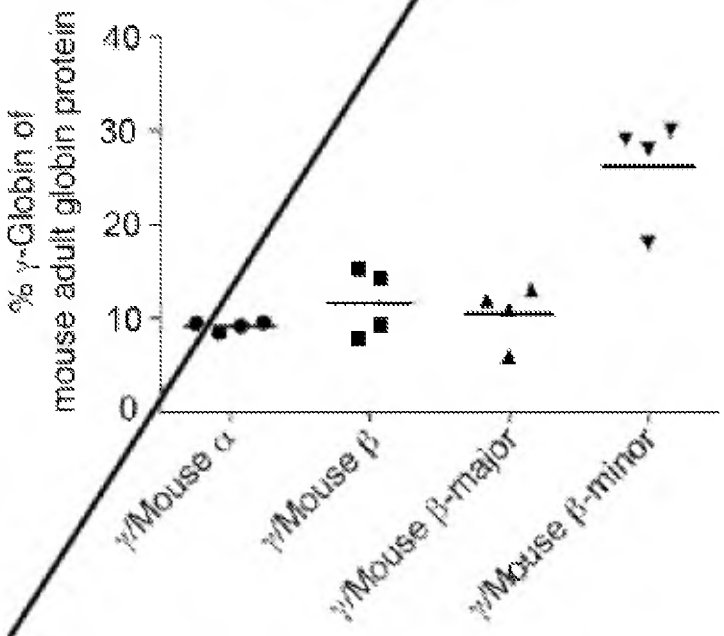

% γ-Globin of mouse adult globin protein
40
30
20
10
0
γ/Mouse α
γ/Mouse β
γ/Mouse β-major
γ/Mouse β-minor

FIG. 2F

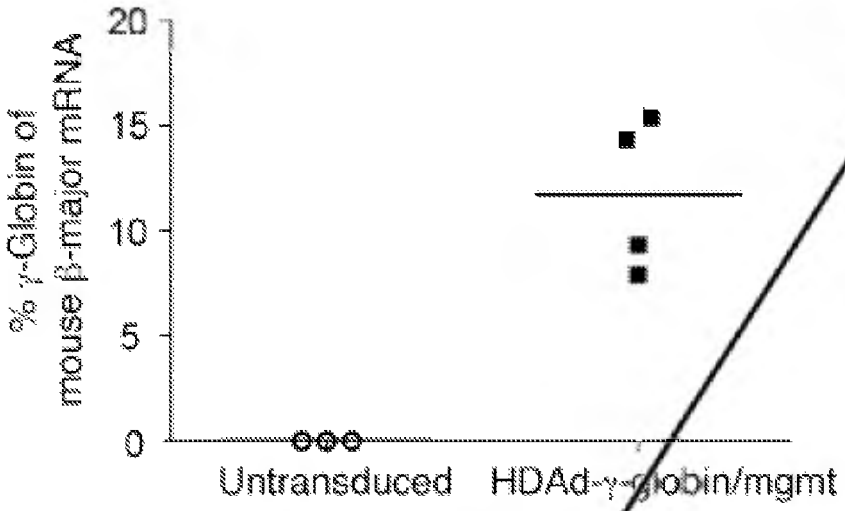

20
15
10
5
0
% γ-Globin of
mouse β-major mRNA
Untransduced
HDAd-γ-globin/mgmt

FIG. 3

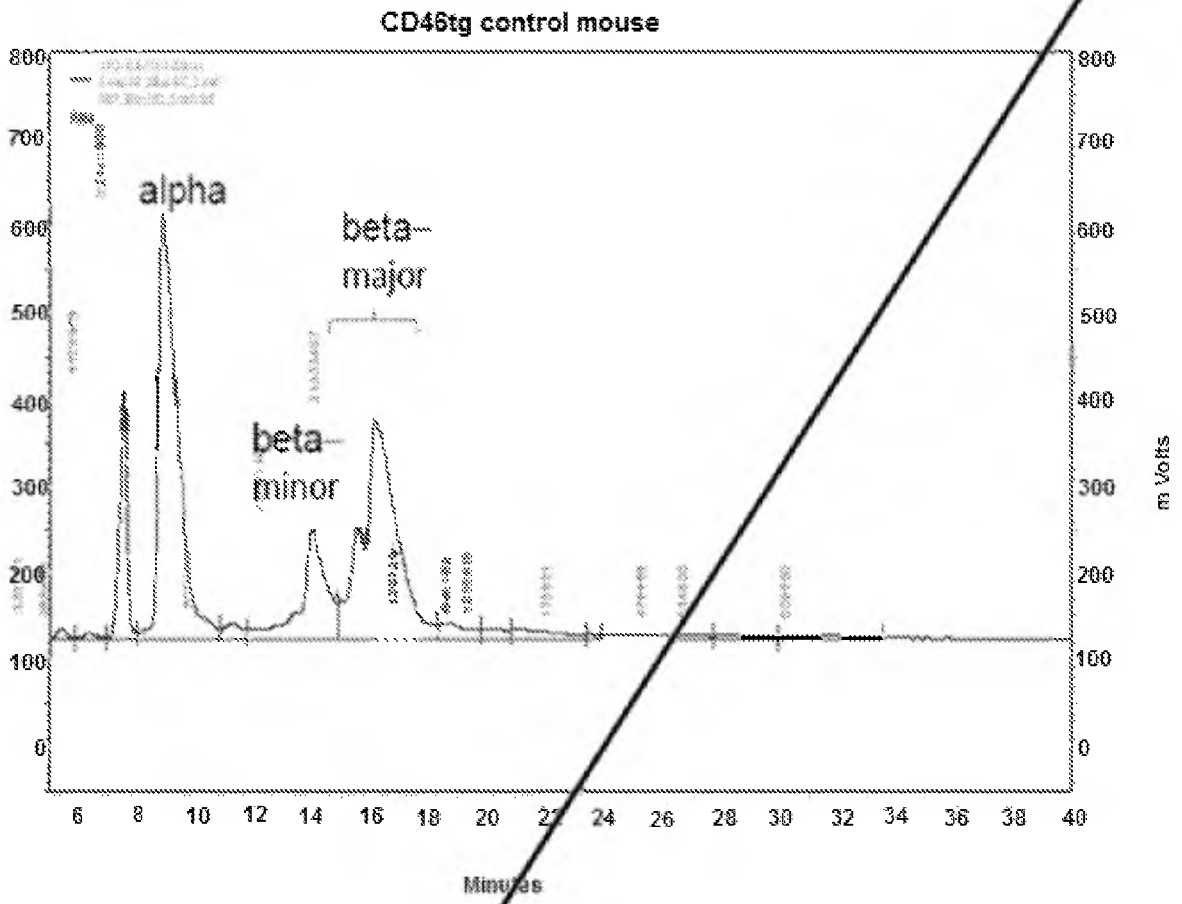

CD46tg control mouse
alpha
beta-major
beta-minor
800
700
600
500
400
300
200
100
0
m Volts
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
Minutes

FIG. 3 cont'd

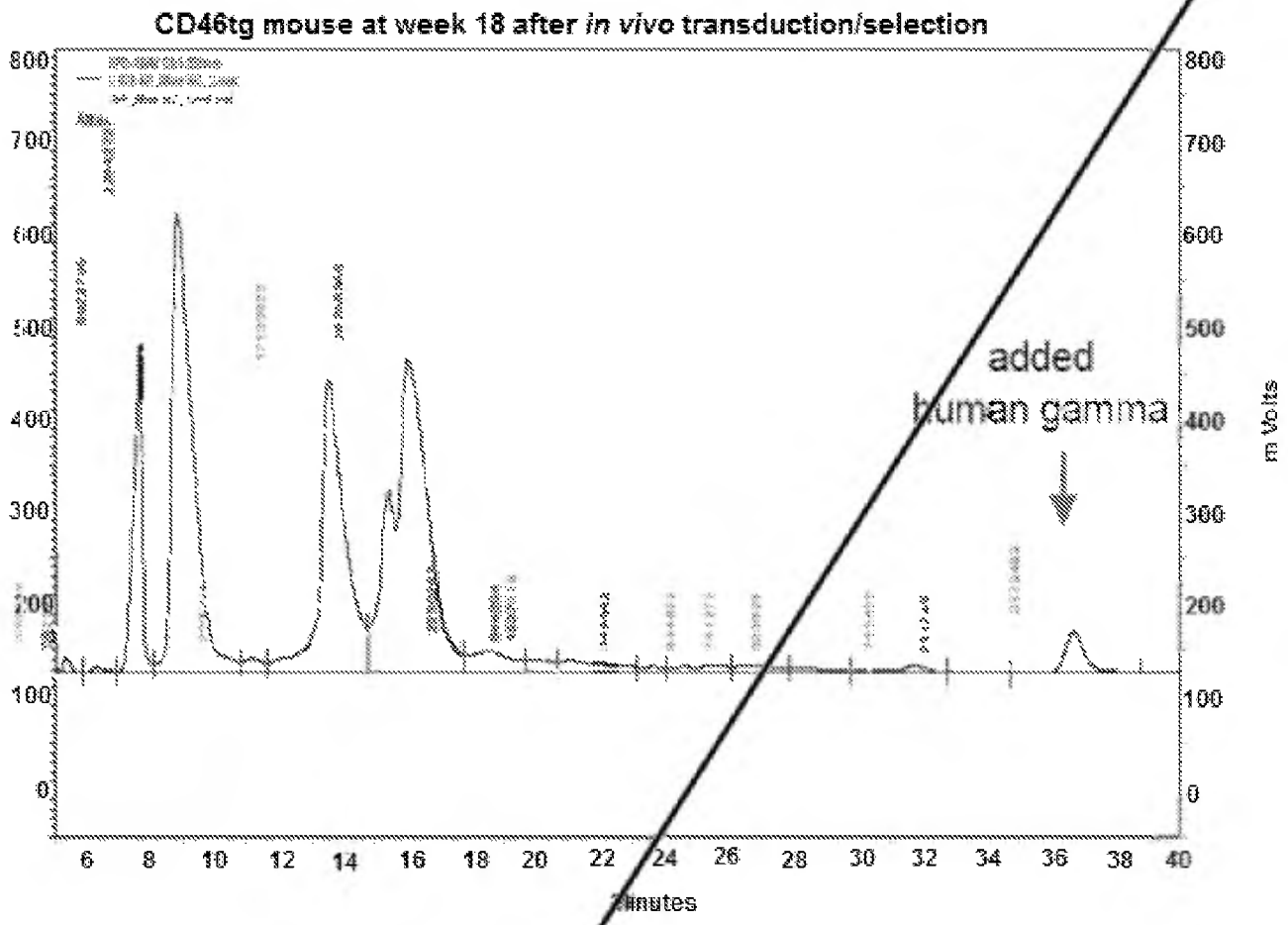

CD46tg mouse at week 18 after *in vivo* transduction/selection
added
human gamma
800
700
600
500
400
300
200
100
0
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
Minutes
m Volts

FIG. 4A

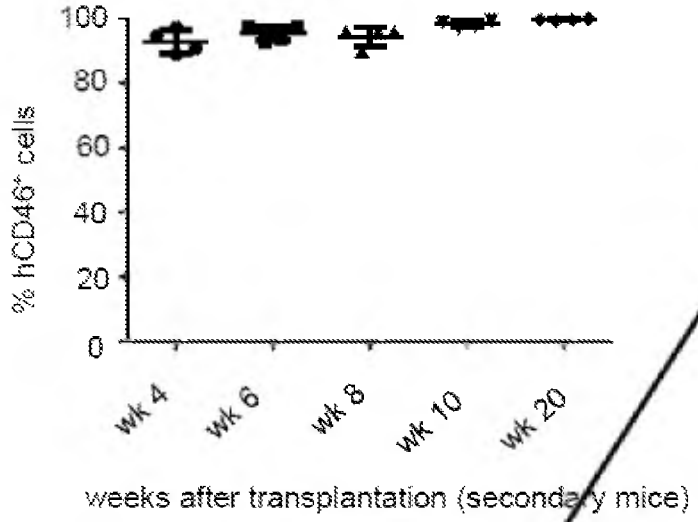


FIG. 4B

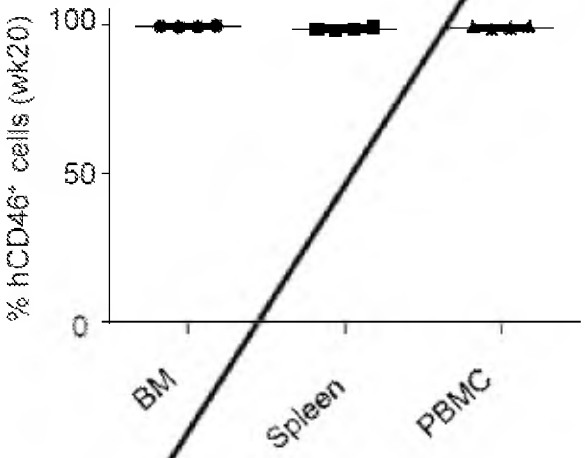


FIG. 4C

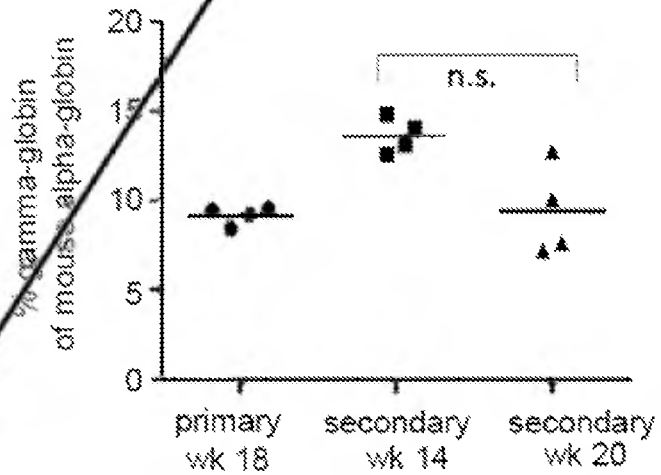


FIG. 5A

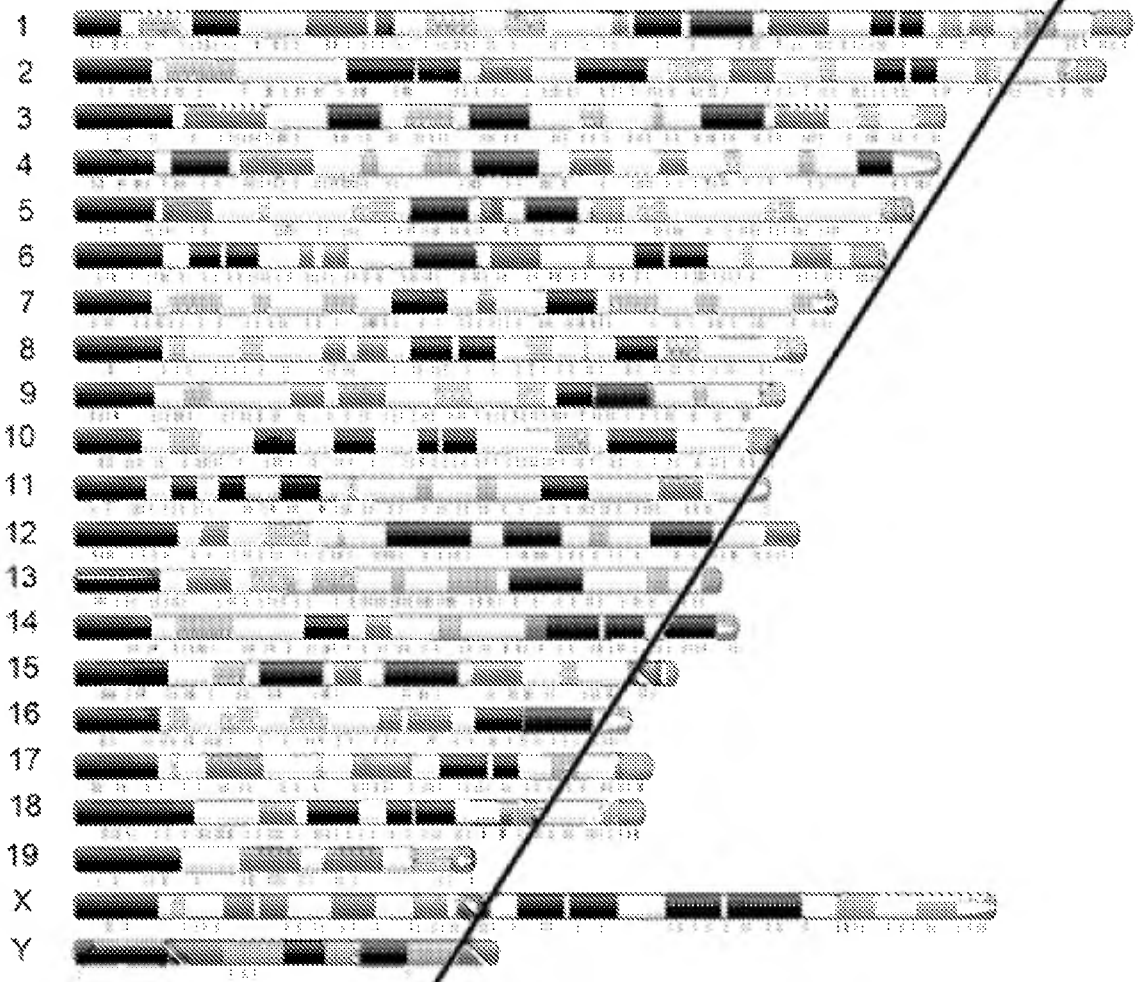


FIG. 5B

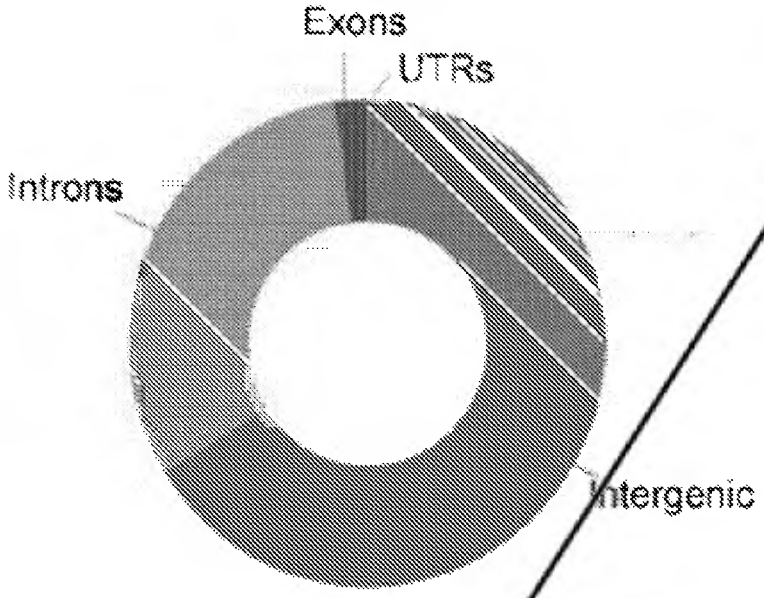

Exons
UTRs
Introns
Intergenic

FIG. 5C

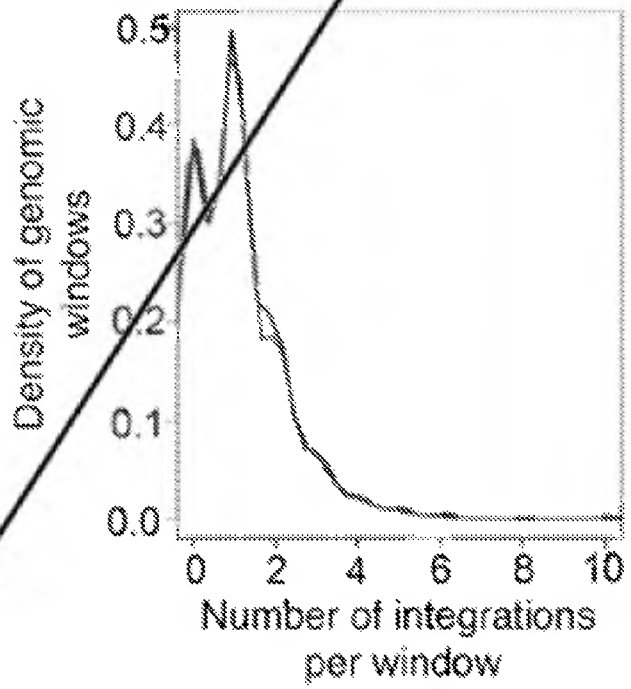

Density of genomic windows
0.5
0.4
0.3
0.2
0.1
0.0
0
2
4
6
8
10
Number of integrations per window

FIG. 5D

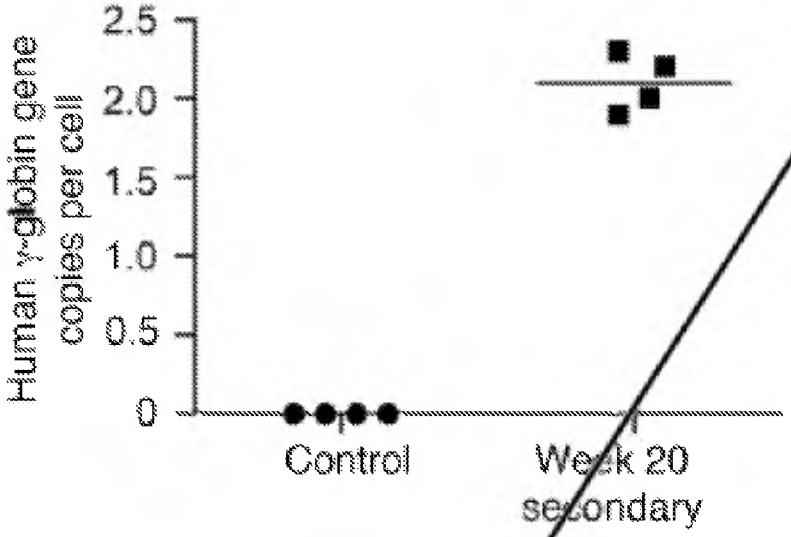

Human γ-globin gene copies per cell
2.5
2.0
1.5
1.0
0.5
0
Control
Week 20 secondary

FIG. 5E

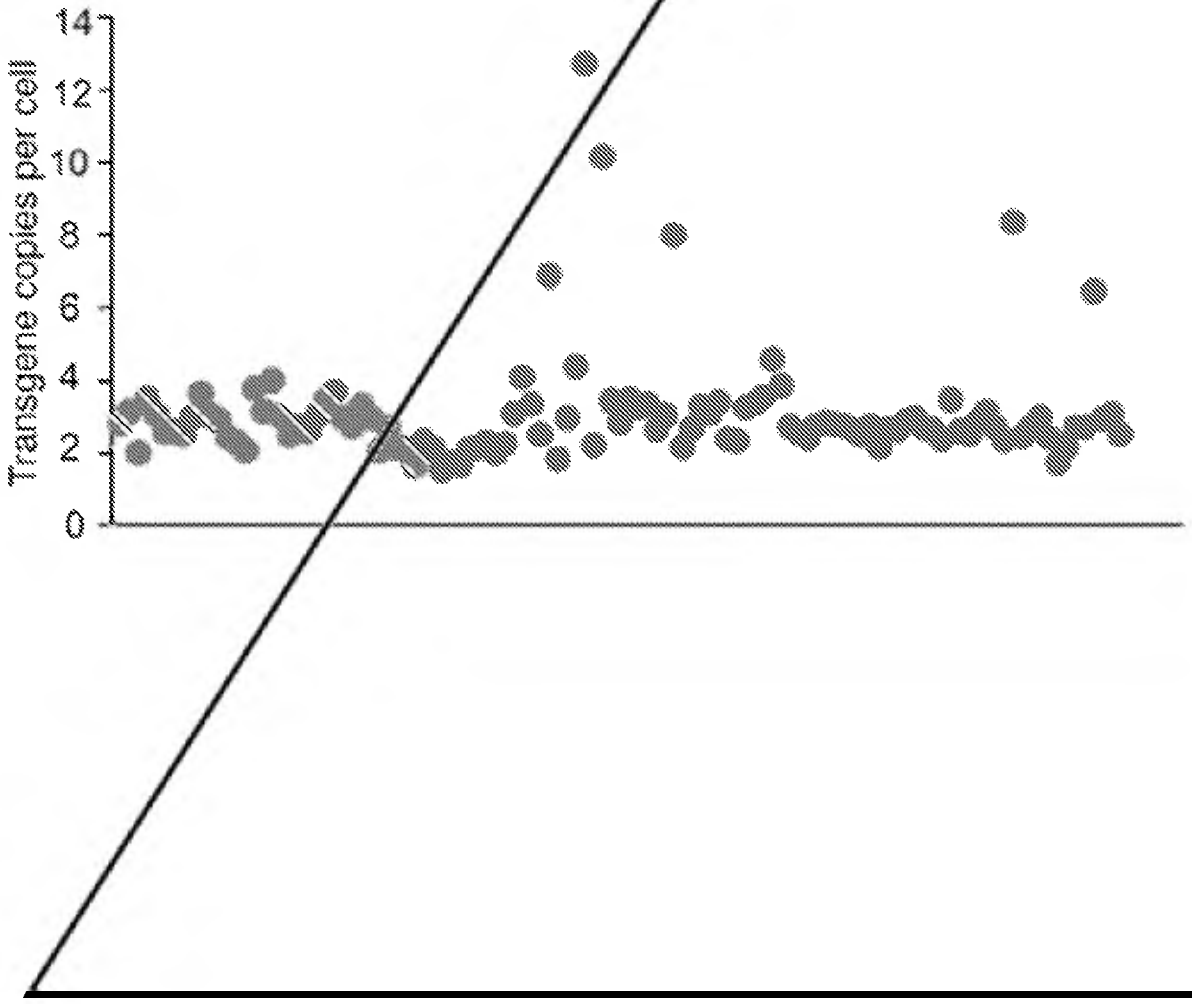

Transgene copies per cell
14
12
10
8
6
4
2
0

FIG. 6

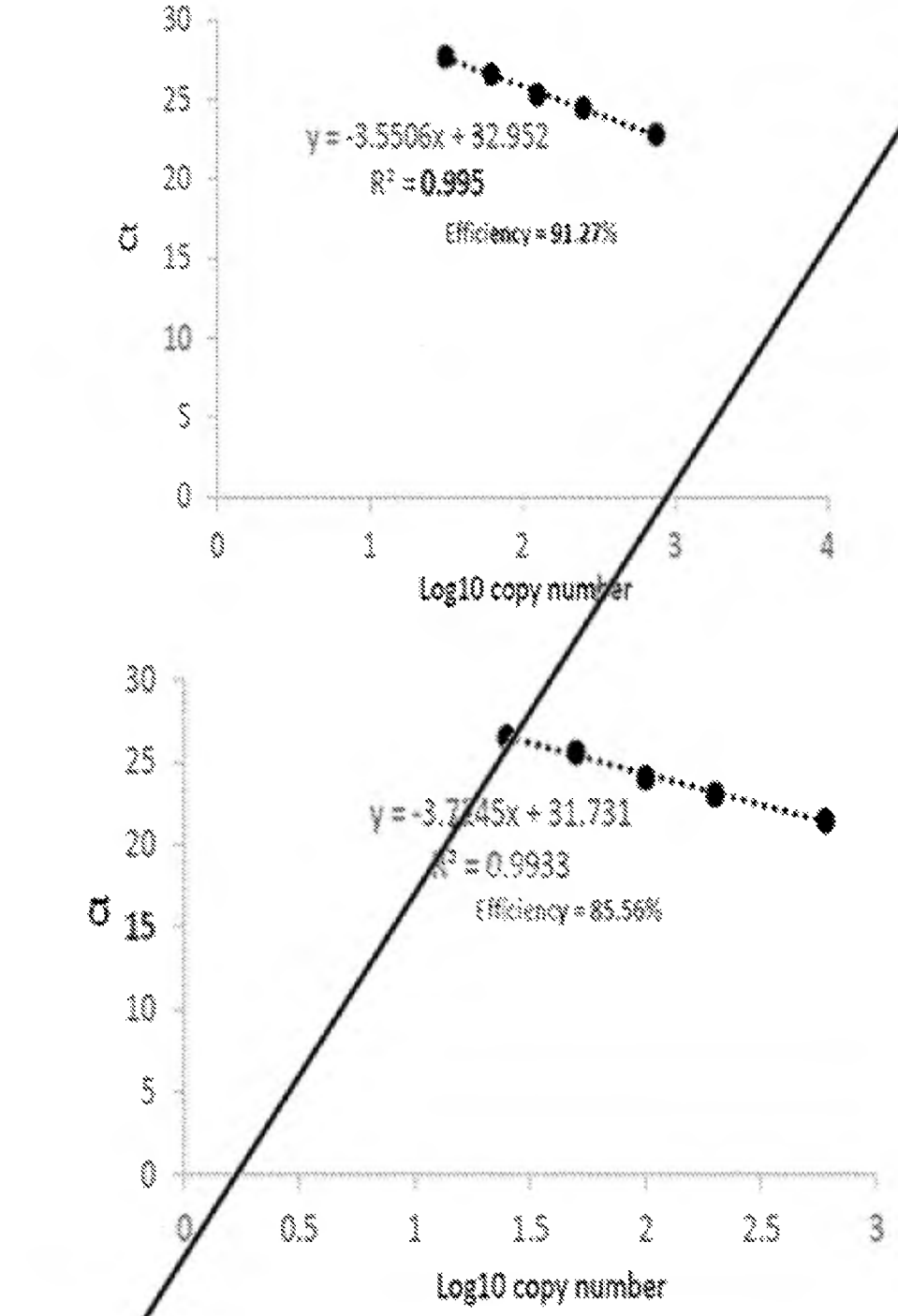

30
25
20
15
10
5
0
σ
y = -3.5506x + 32.952
R² = 0.995
Efficiency = 91.27%
0
1
2
3
4
Log10 copy number
30
25
20
15
10
5
0
σ
y = -3.7245x + 31.731
R² = 0.9933
Efficiency = 85.56%
0
0.5
1
1.5
2
2.5
3
Log10 copy number

FIG. 7A

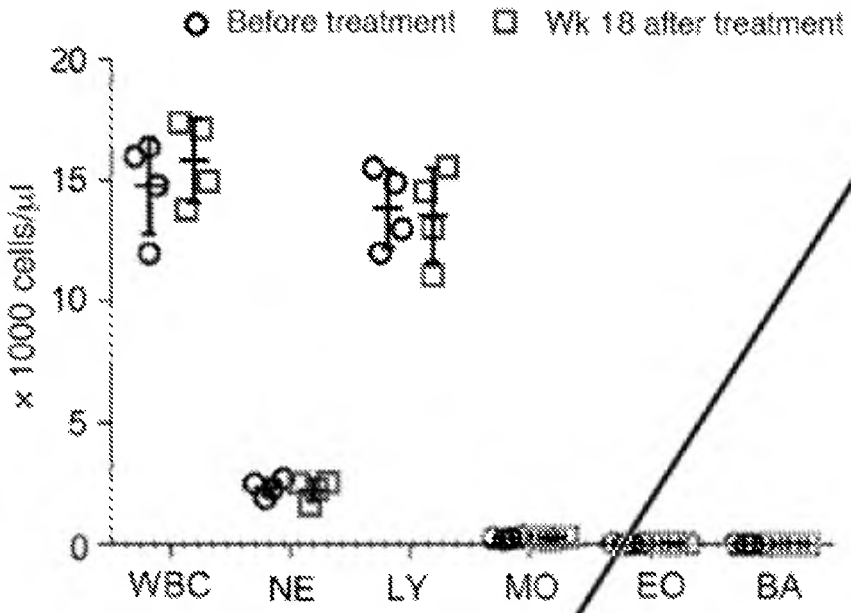

Before treatment
Wk 18 after treatment
× 1000 cells/μl
20
15
10
5
0
WBC
NE
LY
MO
EO
BA

FIG. 7B

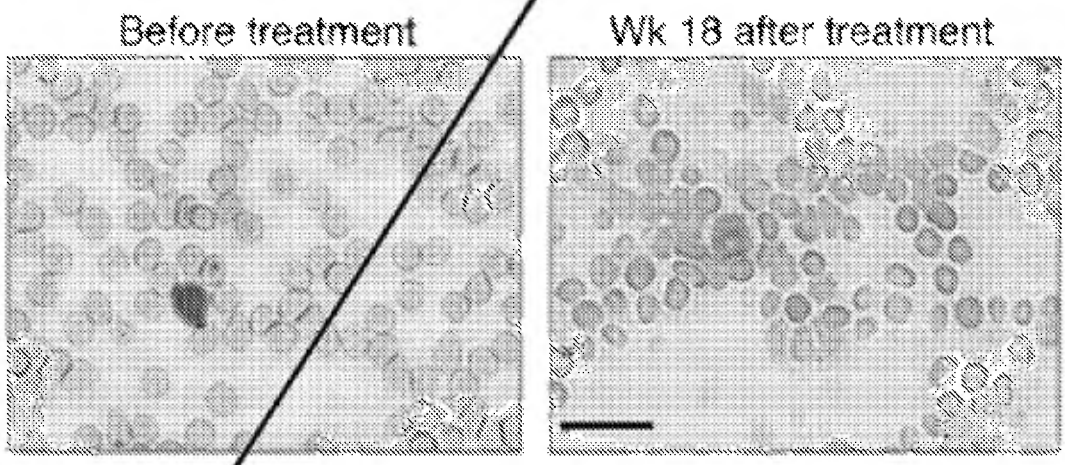

Before treatment
Wk 18 after treatment

FIG. 7C

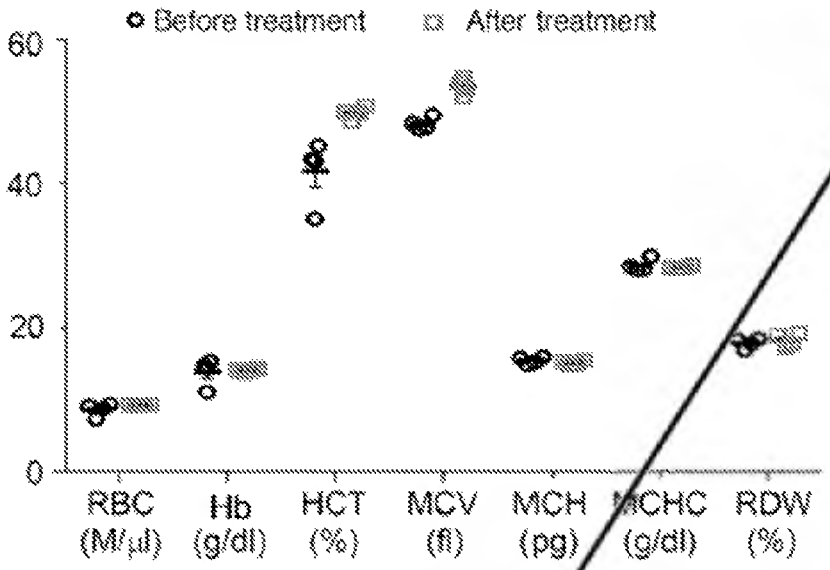

Before treatment
After treatment
60
40
20
0
RBC (M/μl)
Hb (g/dl)
HCT (%)
MCV (fl)
MCH (pg)
MCHC (g/dl)
RDW (%)

FIG. 7D

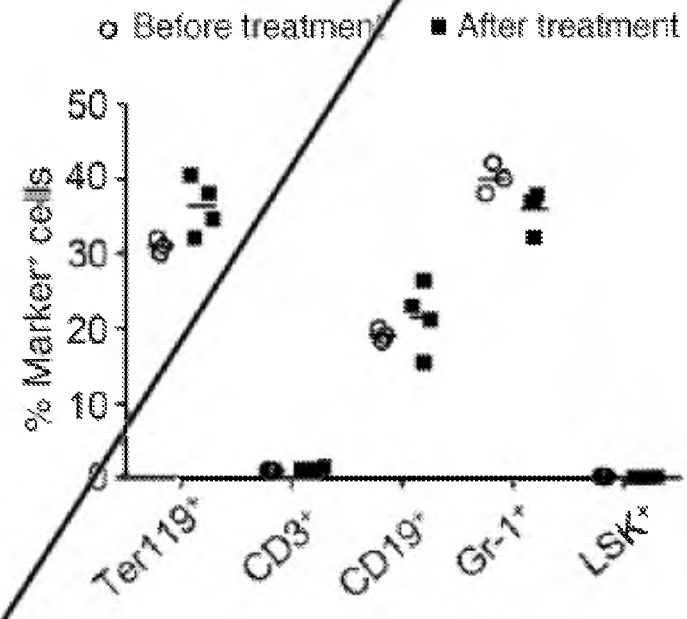

Before treatment
After treatment
% Marker+ cells
50
40
30
20
10
0
Ter119+
CD3+
CD19+
Gr-1+
LSK+

FIG. 7E

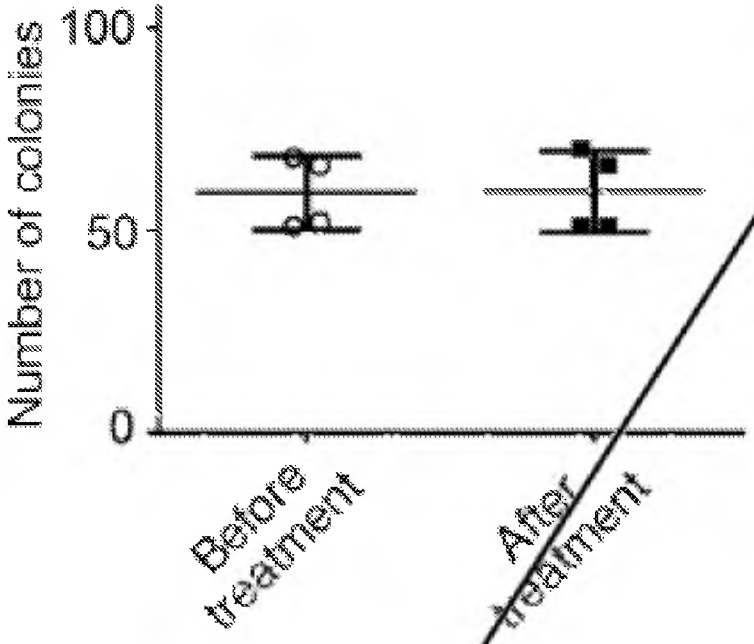

Number of colonies
100
50
0
Before treatment
After treatment

FIG. 8

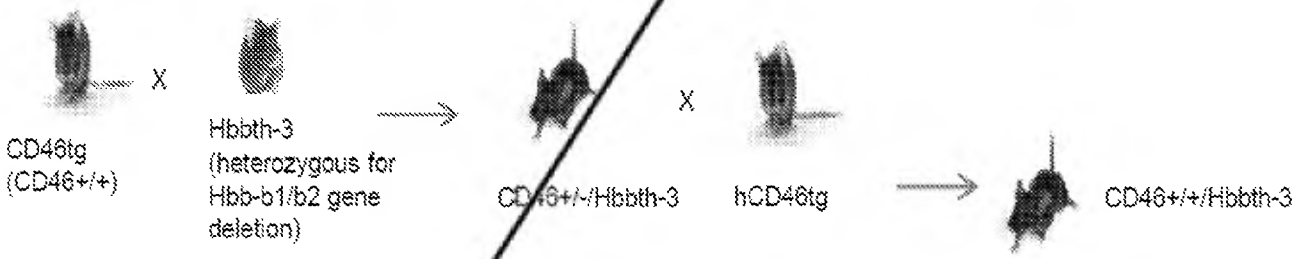

X
CD46tg
(CD46+/+)
Hbbth-3
(heterozygous for
Hbb-b1/b2 gene
deletion)
CD46+/-/Hbbth-3
X
hCD46tg
CD46+/+/Hbbth-3

FIG. 9A

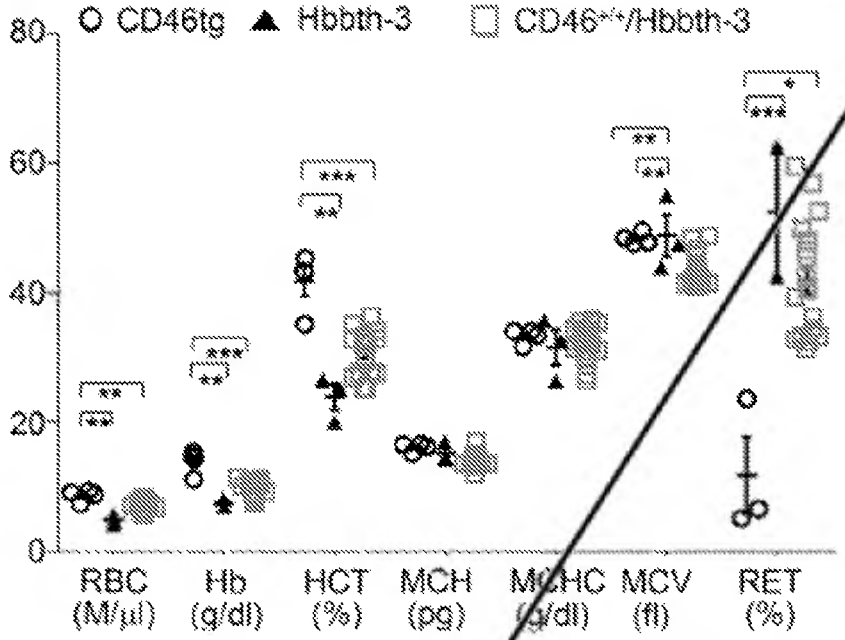

CD46tg
Hbbth-3
CD46-/-/Hbbth-3
80
60
40
20
0
RBC (M/μl)
Hb (g/dl)
HCT (%)
MCH (pg)
MCHC (g/dl)
MCV (fl)
RET (%)

FIG. 9B

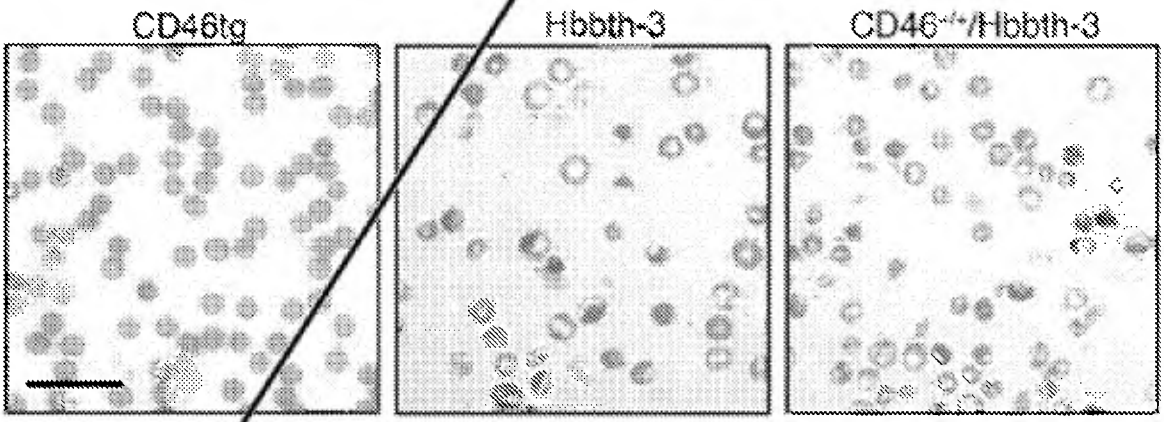

CD46tg
Hbbth-3
CD46-/-/Hbbth-3

FIG. 9C

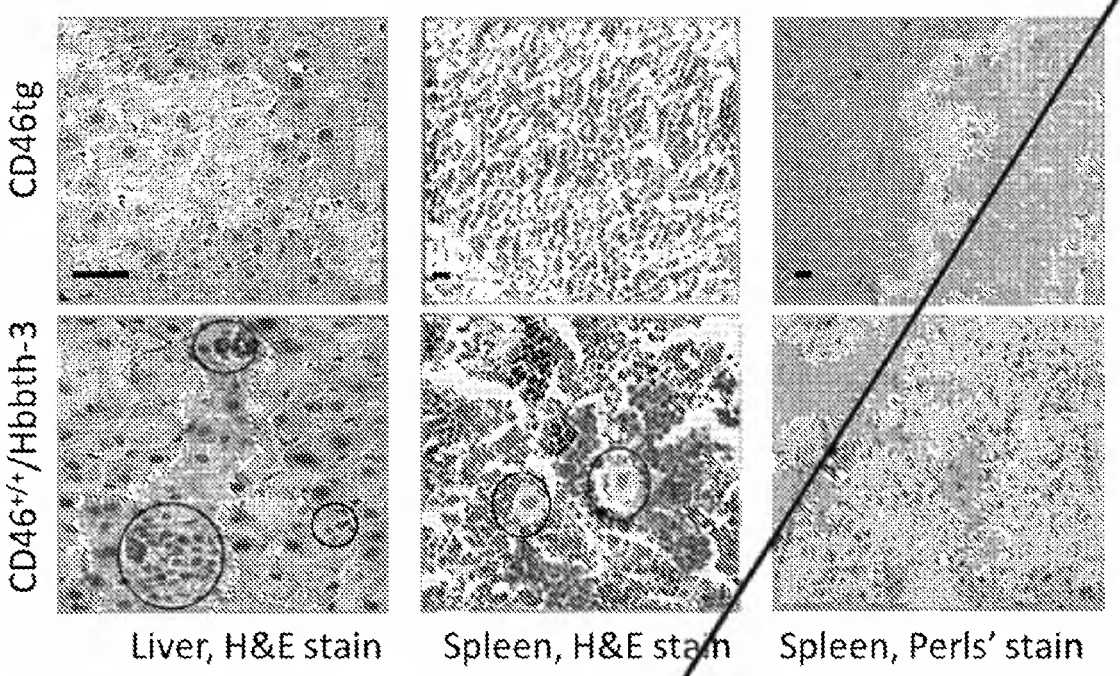

CD46tg
CD46+/+/Hbbth-3
Liver, H&E stain
Spleen, H&E stain
Spleen, Perls' stain

FIG. 10

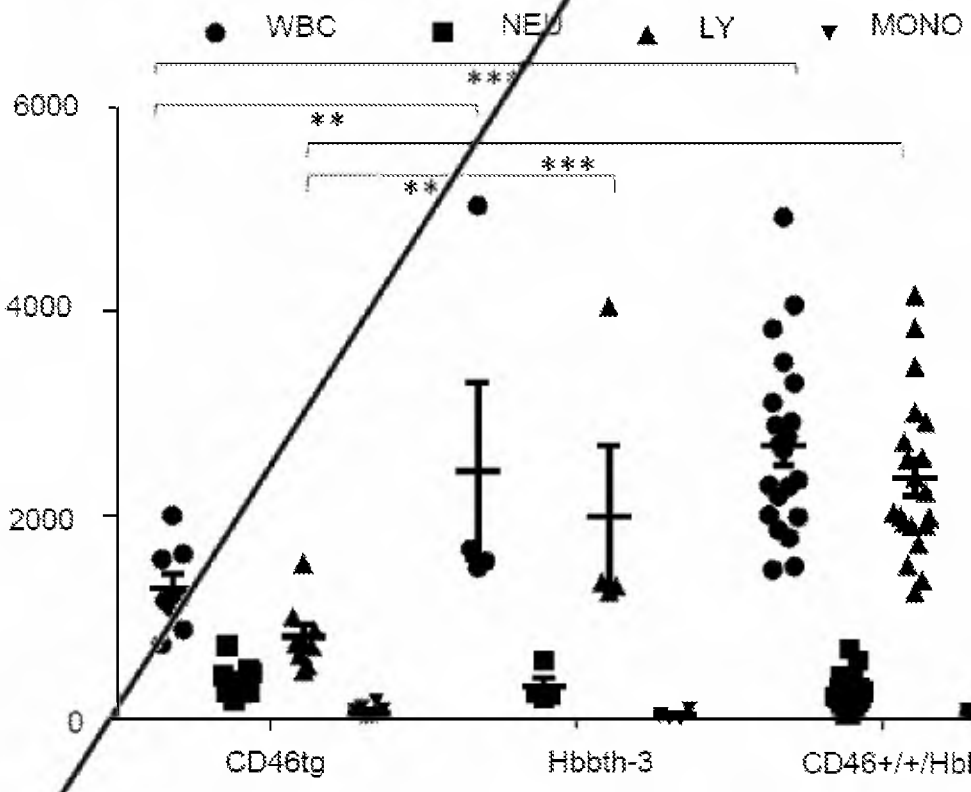

WBC
NEU
LY
MONO
6000
4000
2000
0
CD46tg
Hbbth-3
CD46+/+/Hbbth-3

FIG. 11

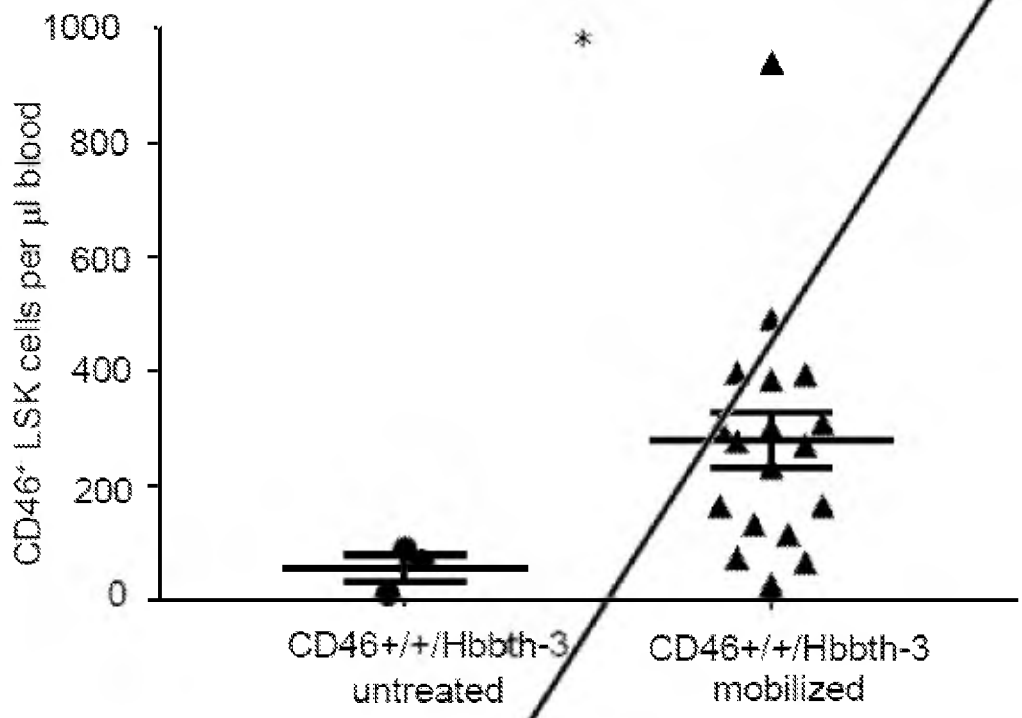

1000
800
600
400
200
0
CD46+ LSK cells per µl blood
*
CD46+/+/Hbbth-3
untreated
CD46+/+/Hbbth-3
mobilized

FIG. 12

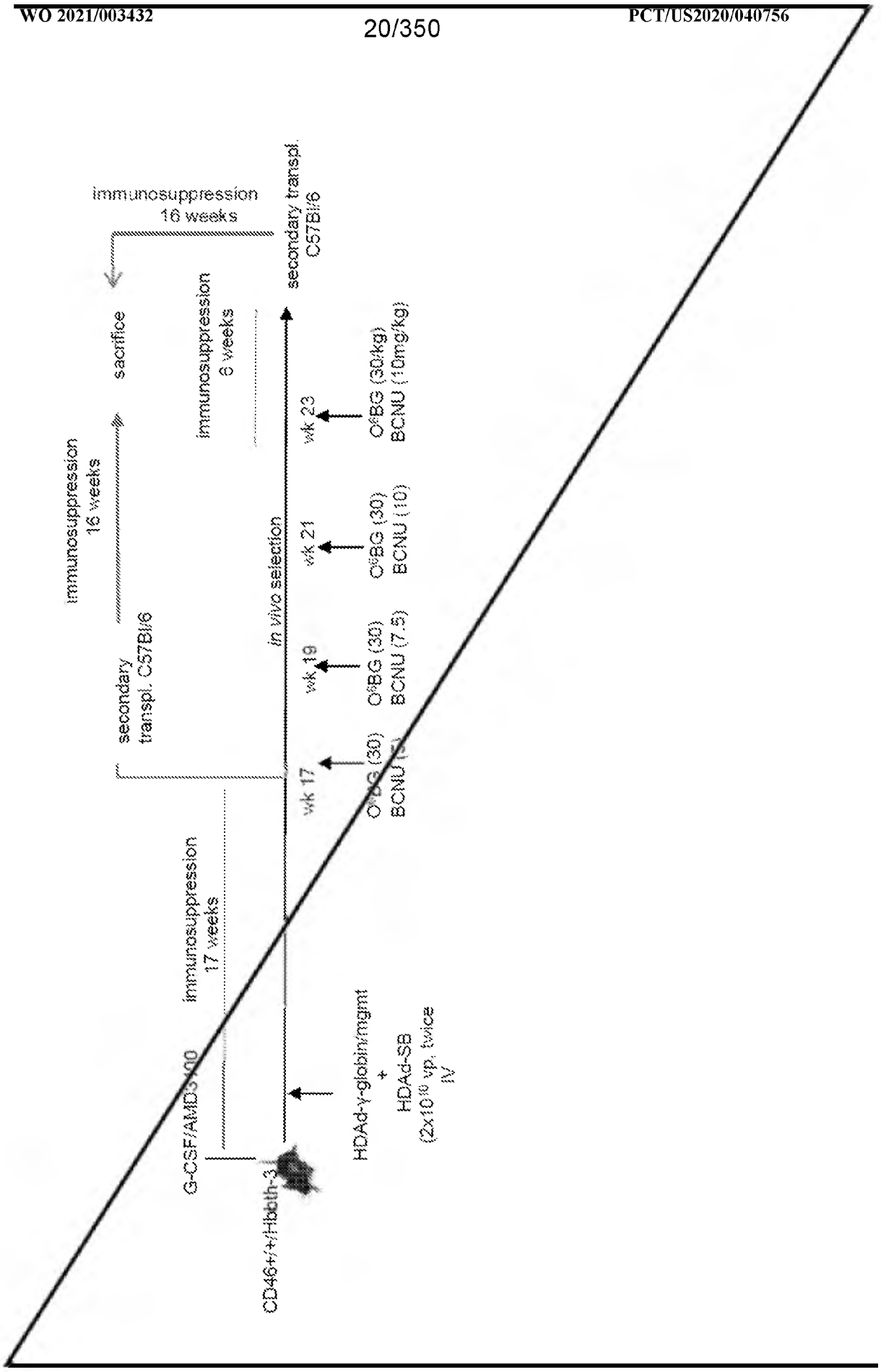

CD46+/+/Hbb^th-3
G-CSF/AMD3100
immunosuppression
17 weeks
HDAd-γ-globin/mgmt
+
HDAd-SB
(2x10^10 vp, twice
IV
wk 17
O^6BG (30)
BCNU (7.5)
wk 19
O^6BG (30)
BCNU (7.5)
in vivo selection
wk 21
O^6BG (30)
BCNU (10)
wk 23
O^6BG (30/kg)
BCNU (10mg/kg)
secondary transpl. C57Bl/6
immunosuppression
16 weeks
sacrifice
immunosuppression
6 weeks
secondary transpl.
C57Bl/6
immunosuppression
16 weeks

FIG. 13A

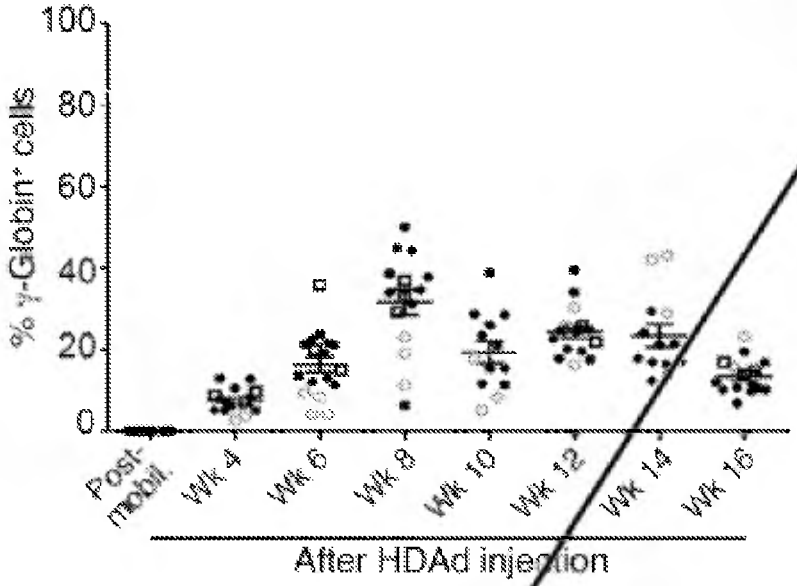


FIG. 13B

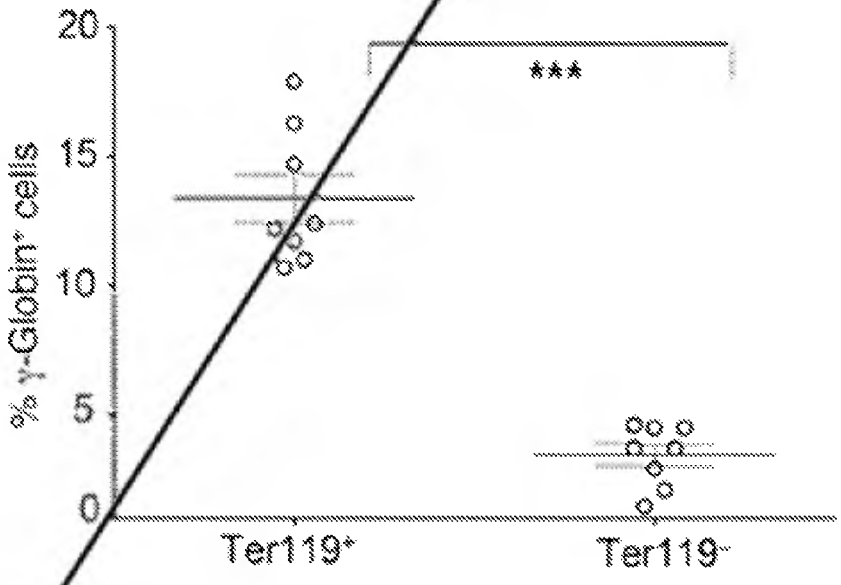


FIG. 13C

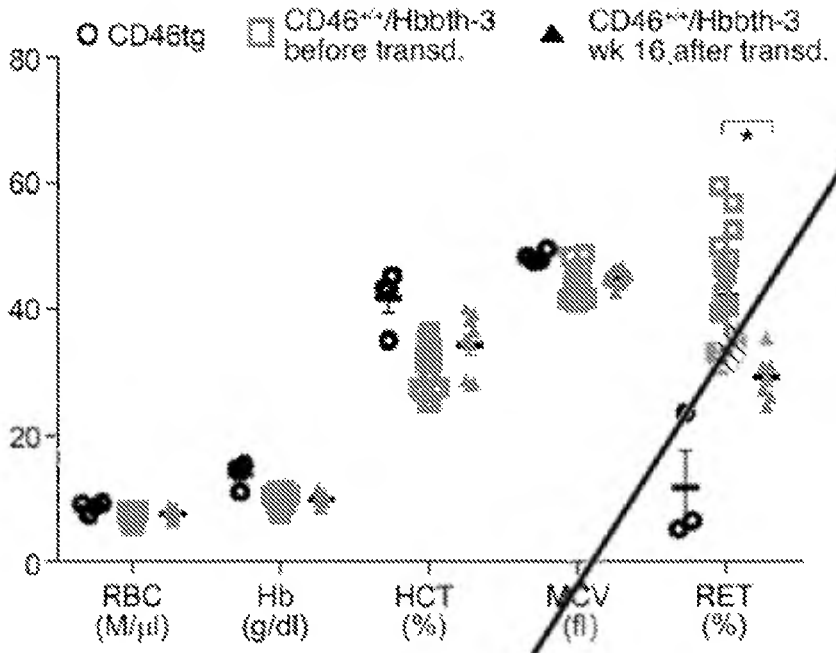


FIG. 13D

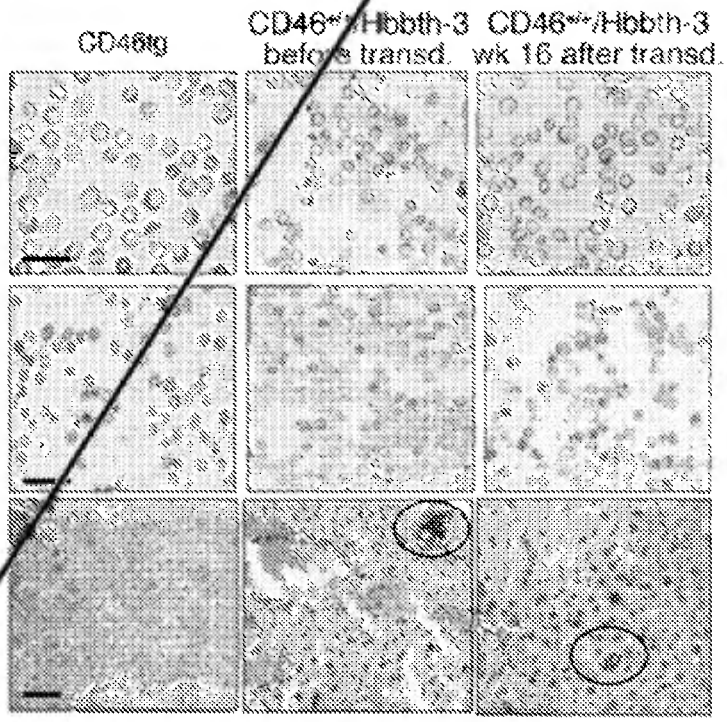


FIG. 13E

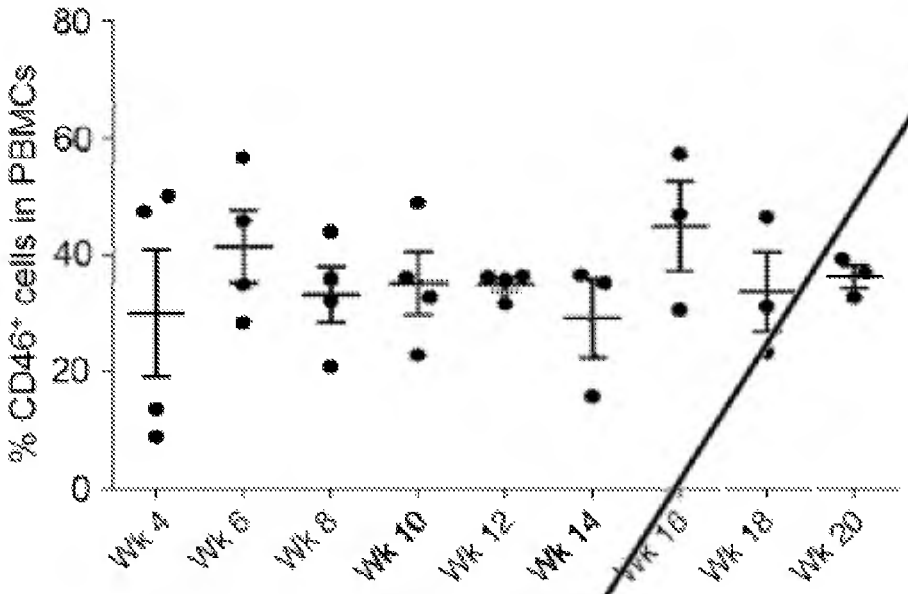


FIG. 13F

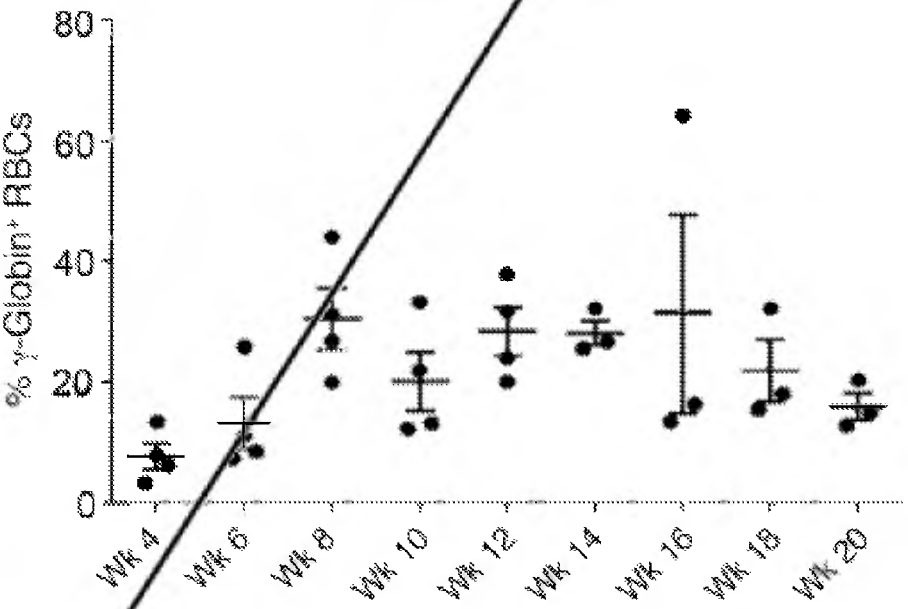


FIG. 14A

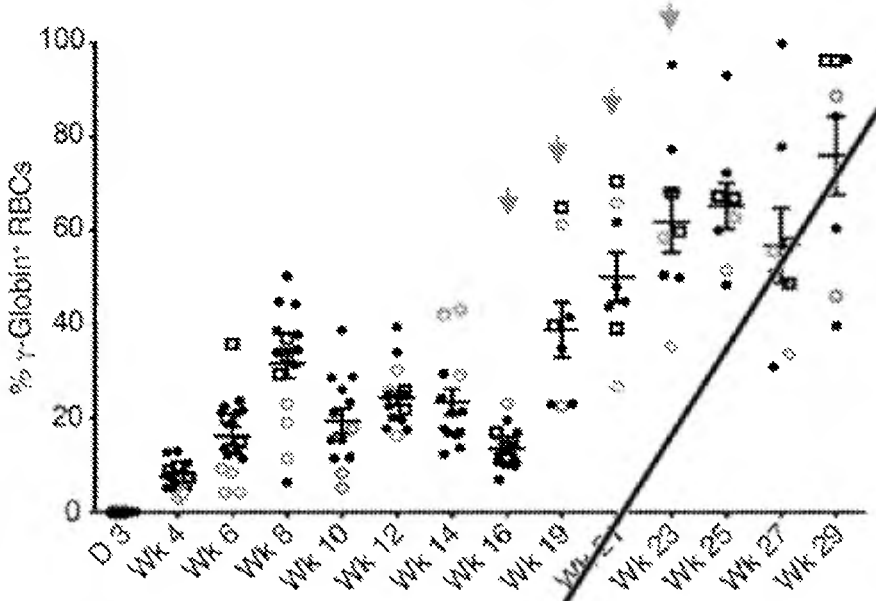


FIG. 14B

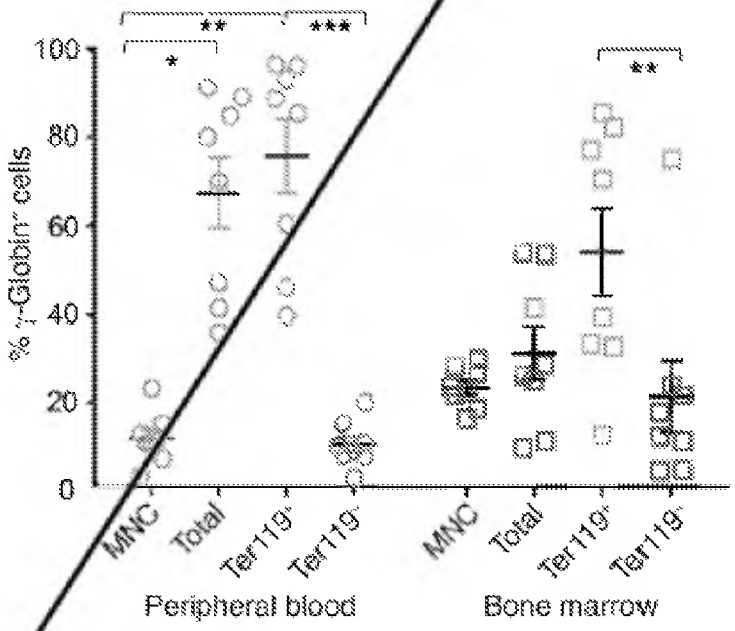


FIG. 14C

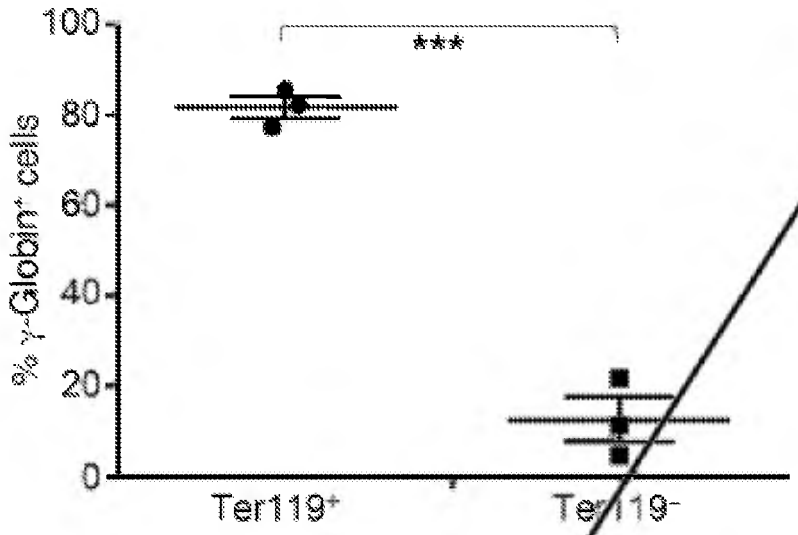


FIG. 14D

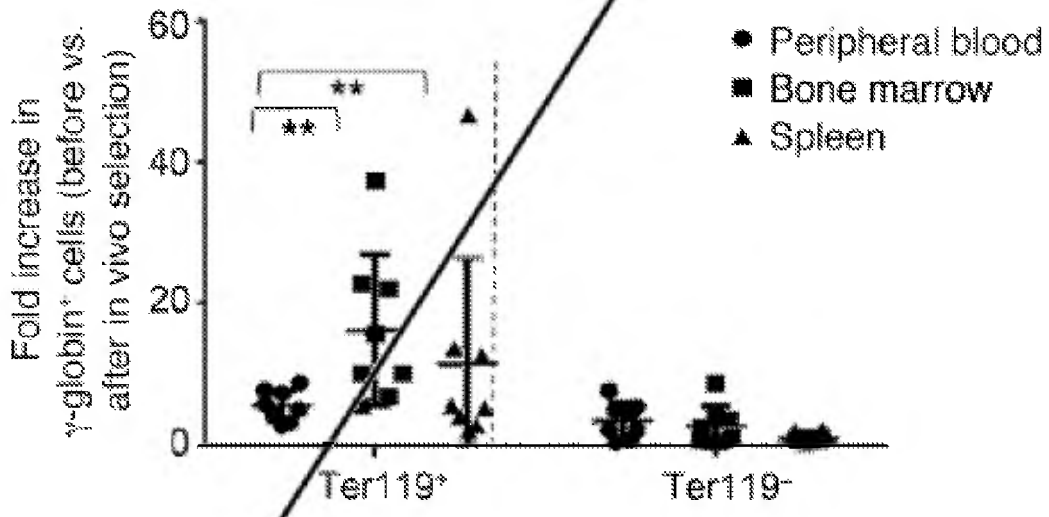


FIG. 14E

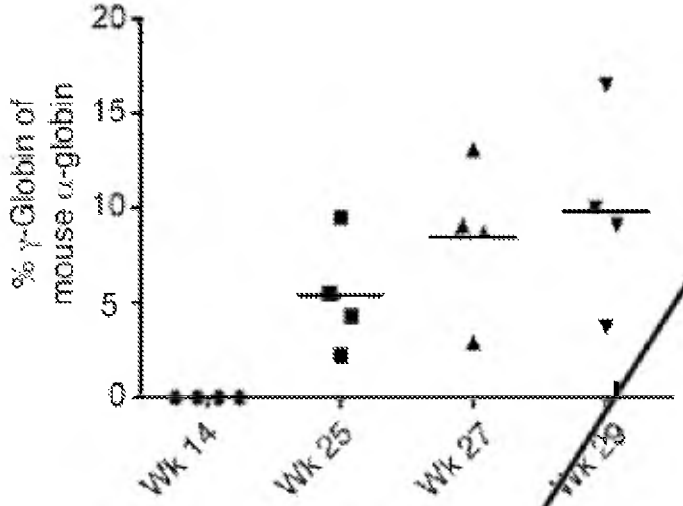


FIG. 14F

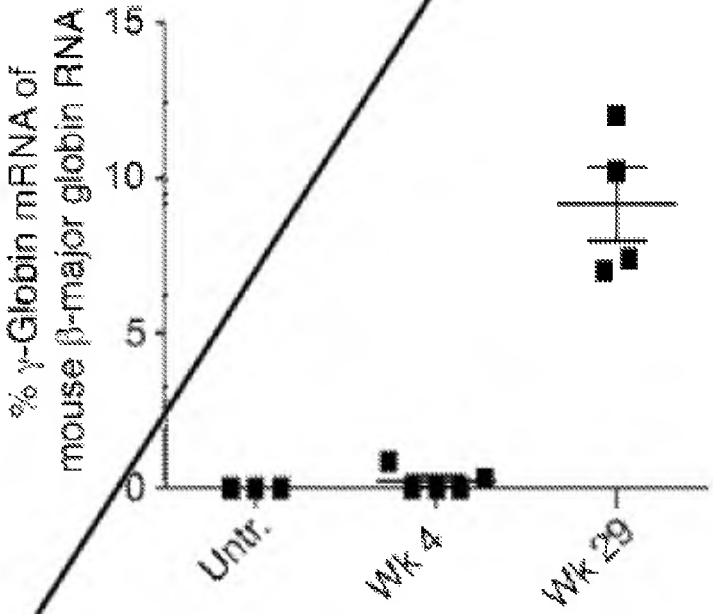


FIG. 15A

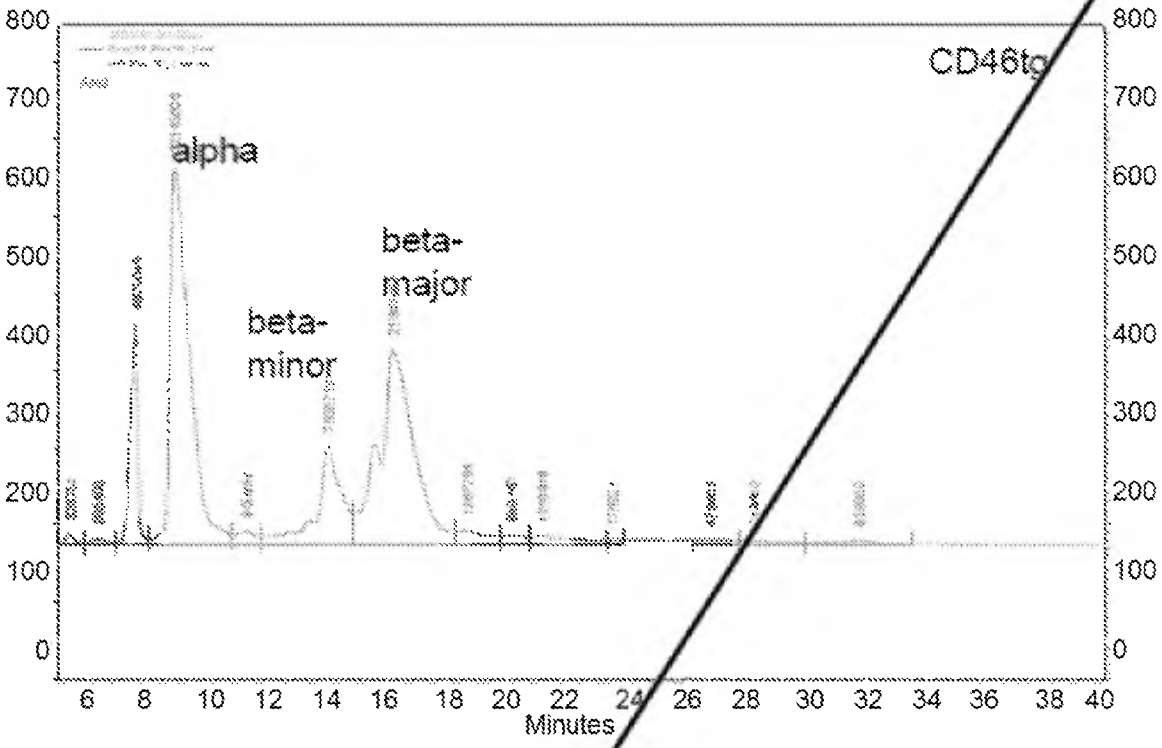


FIG. 15B

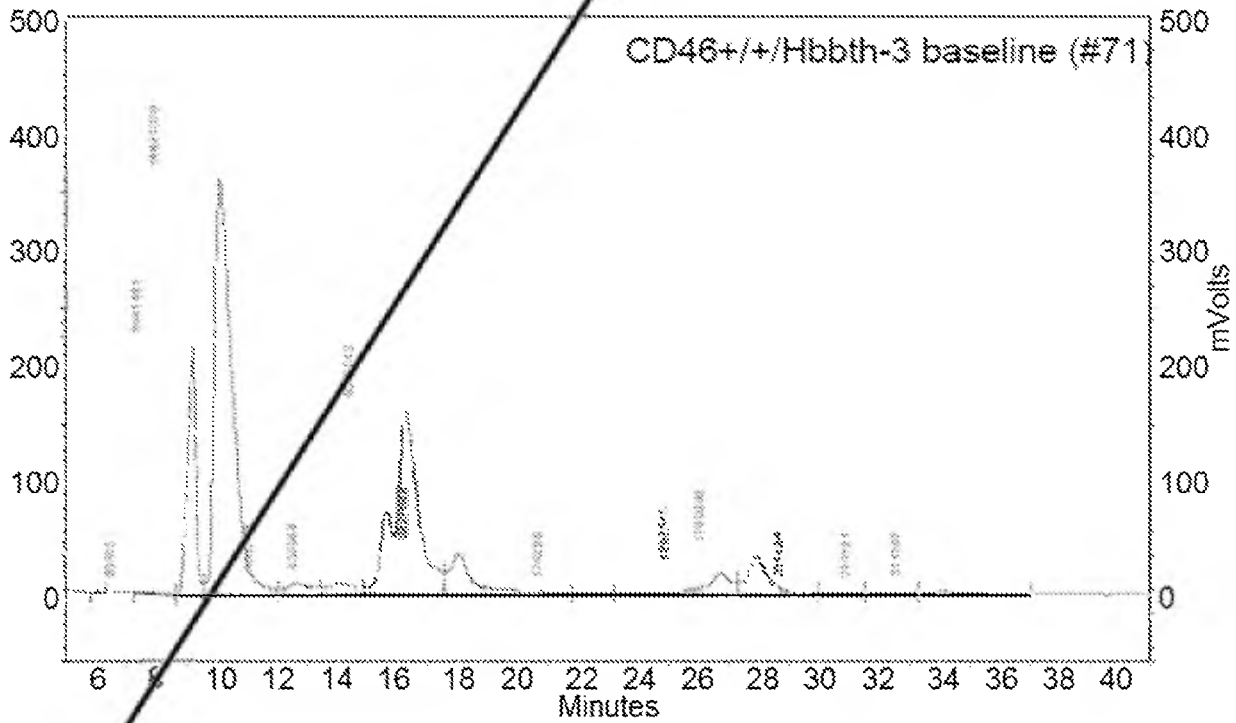


FIG. 15C

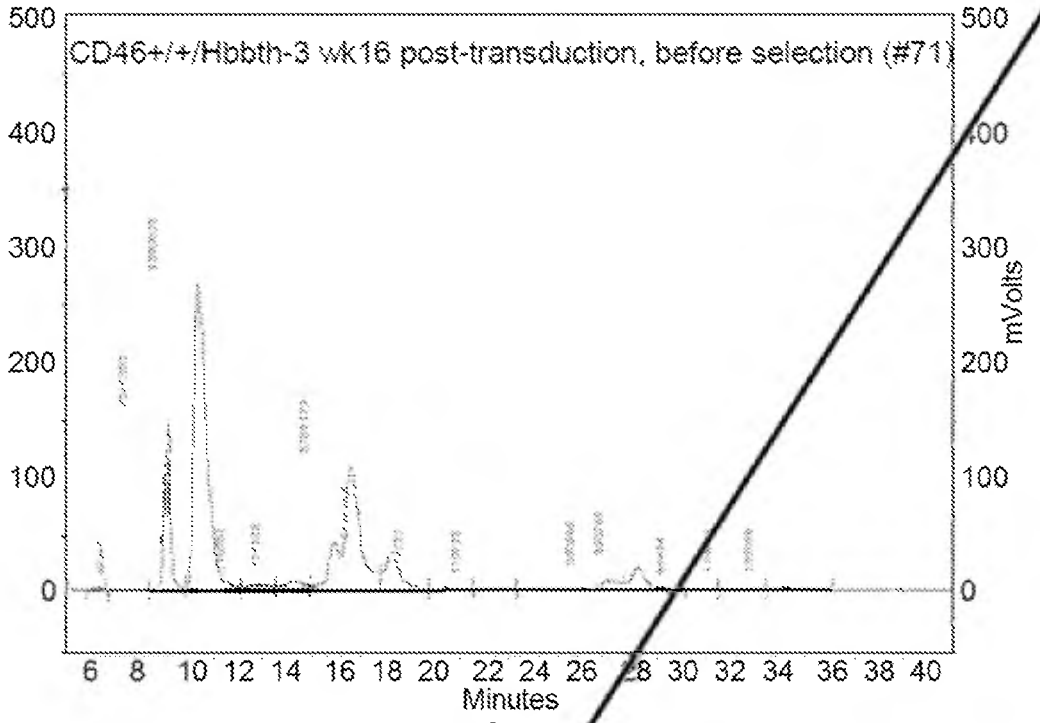


FIG. 15D

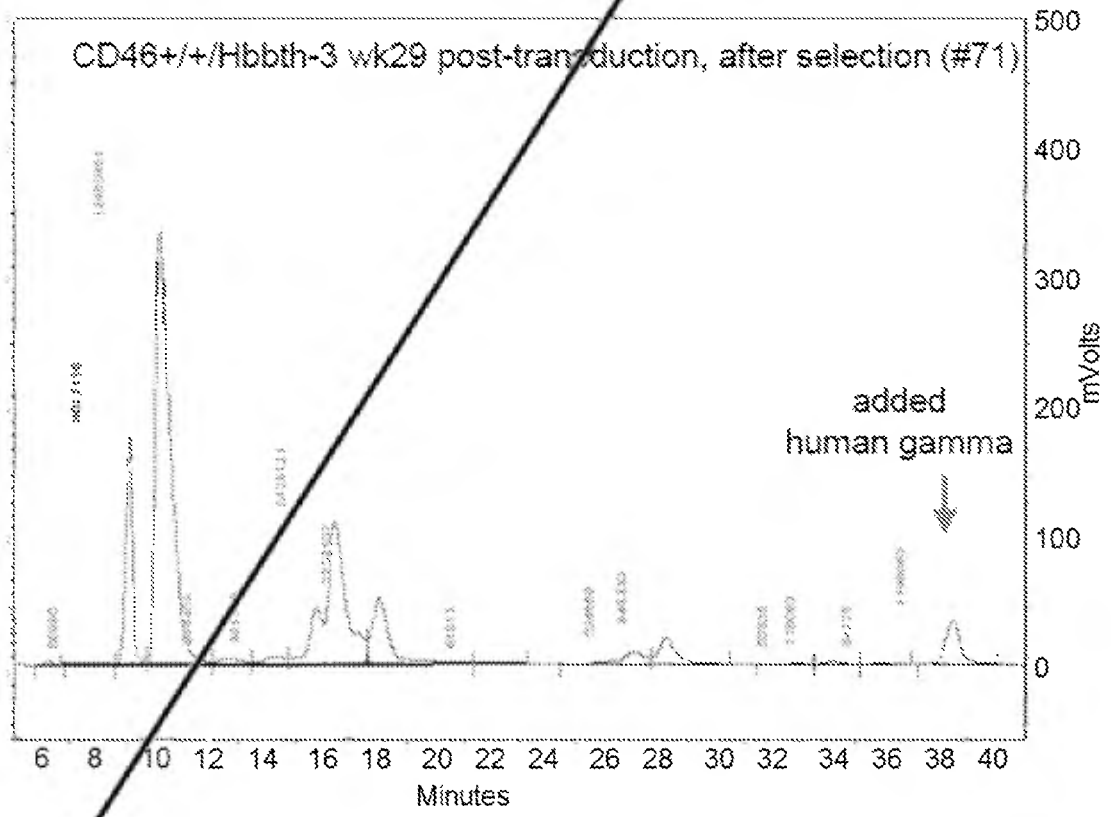


FIG. 16

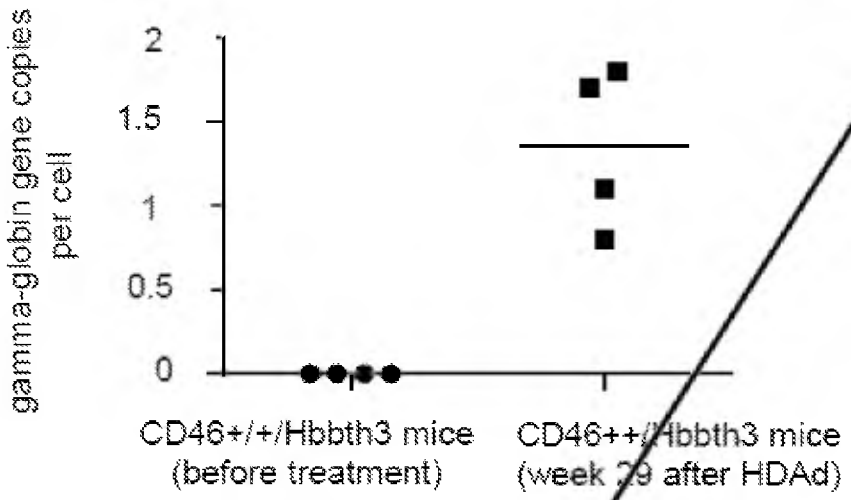


FIG. 17A

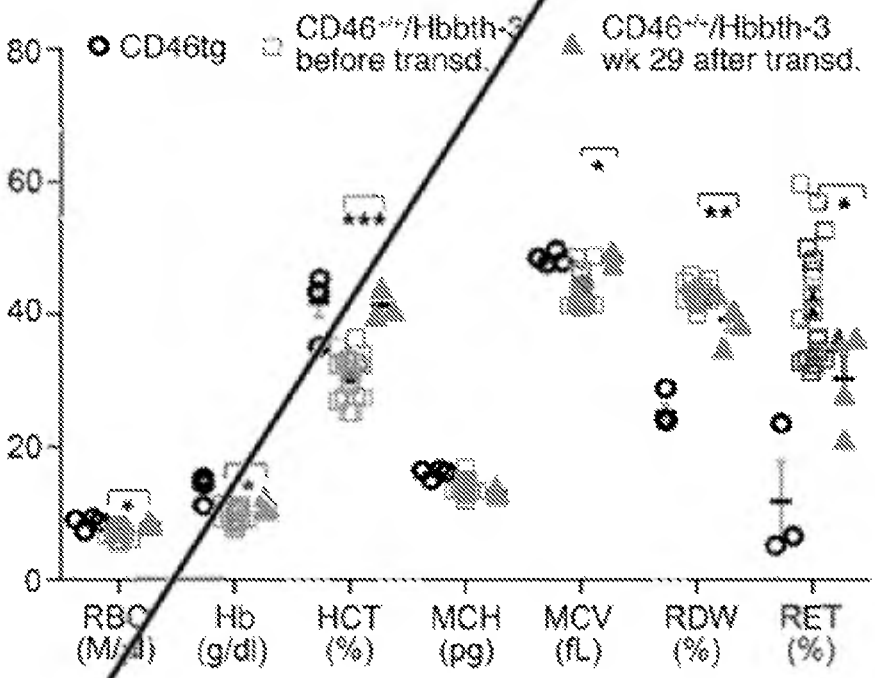


FIG. 17B

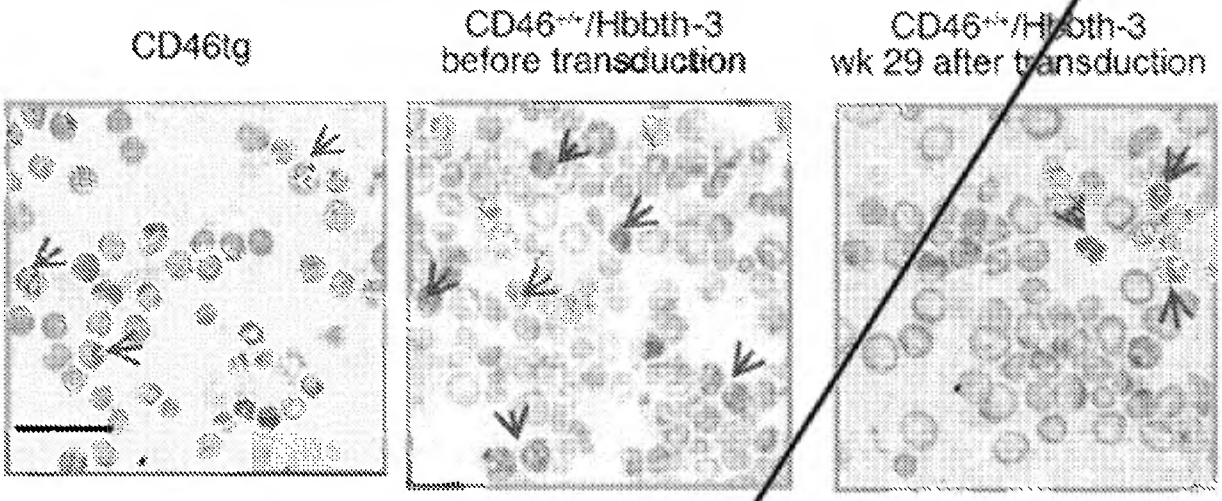


FIG. 17C

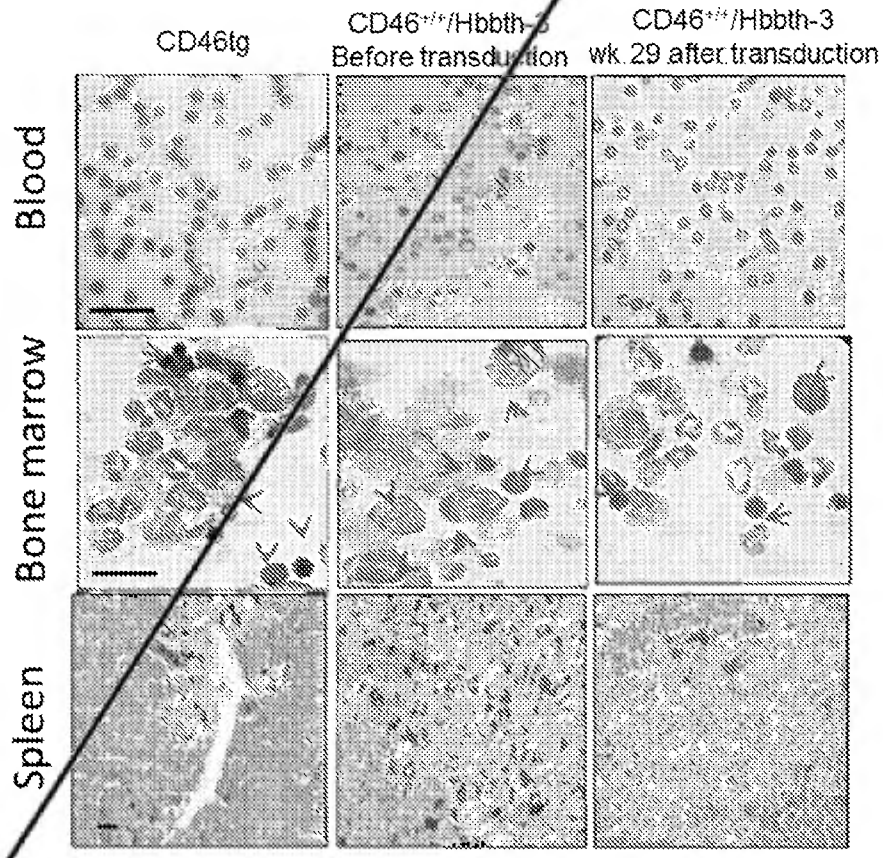


FIG. 17D

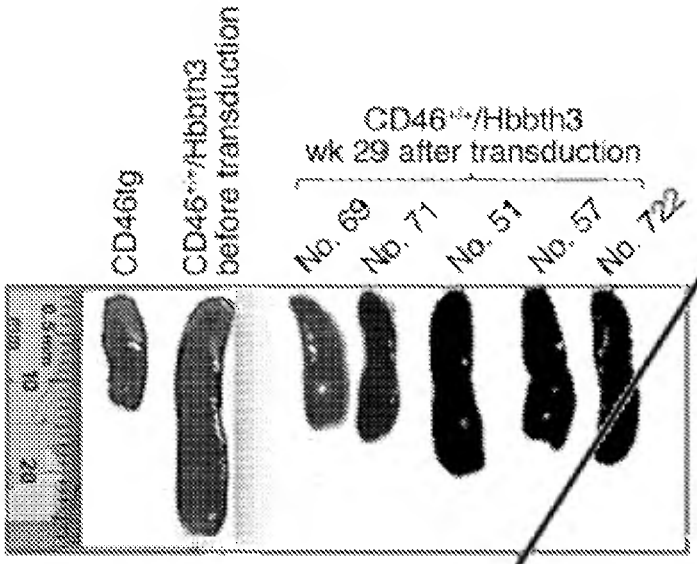


FIG. 17E

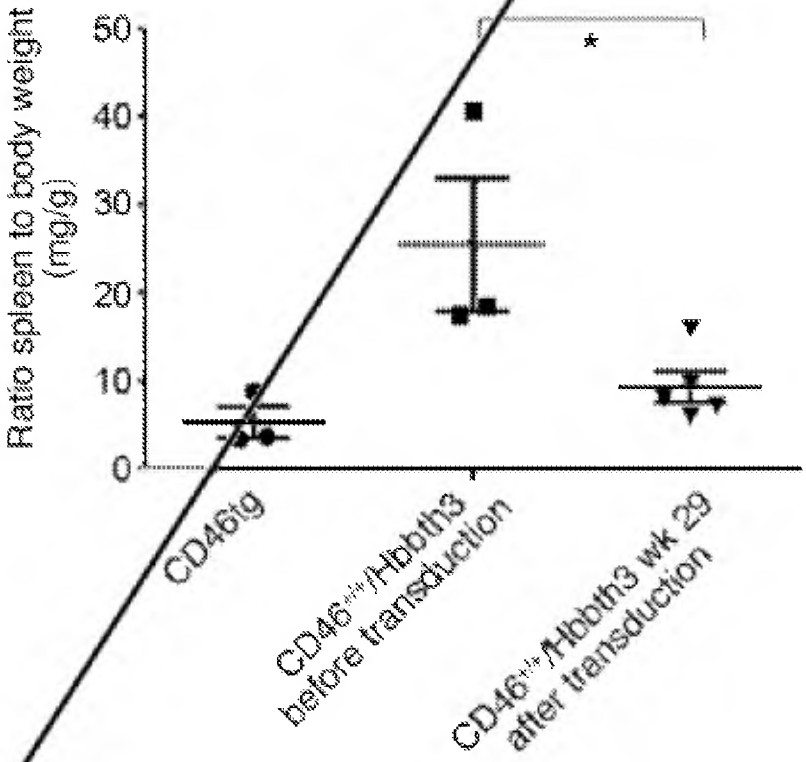


FIG. 18A

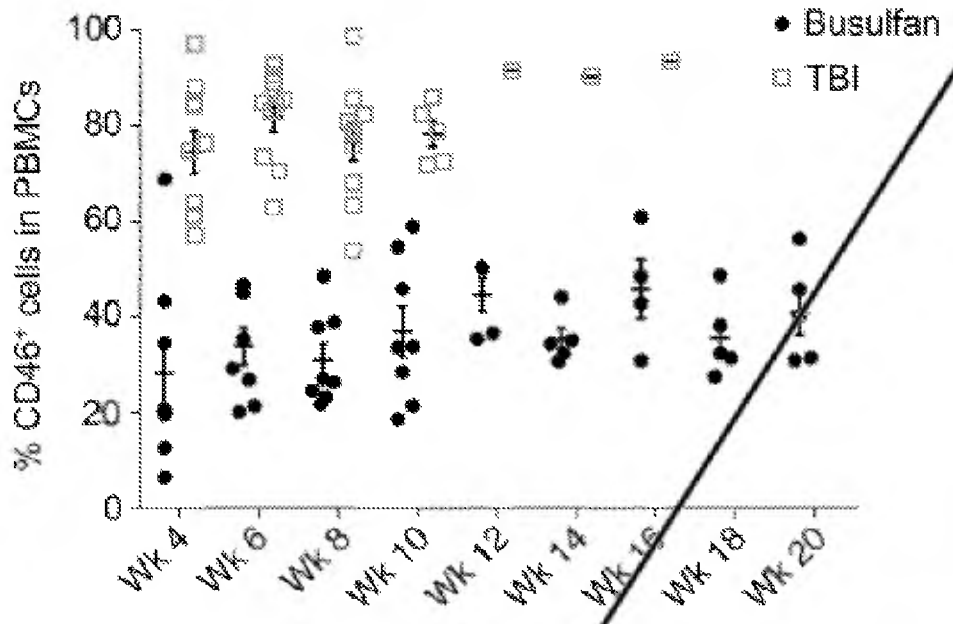


FIG. 18B

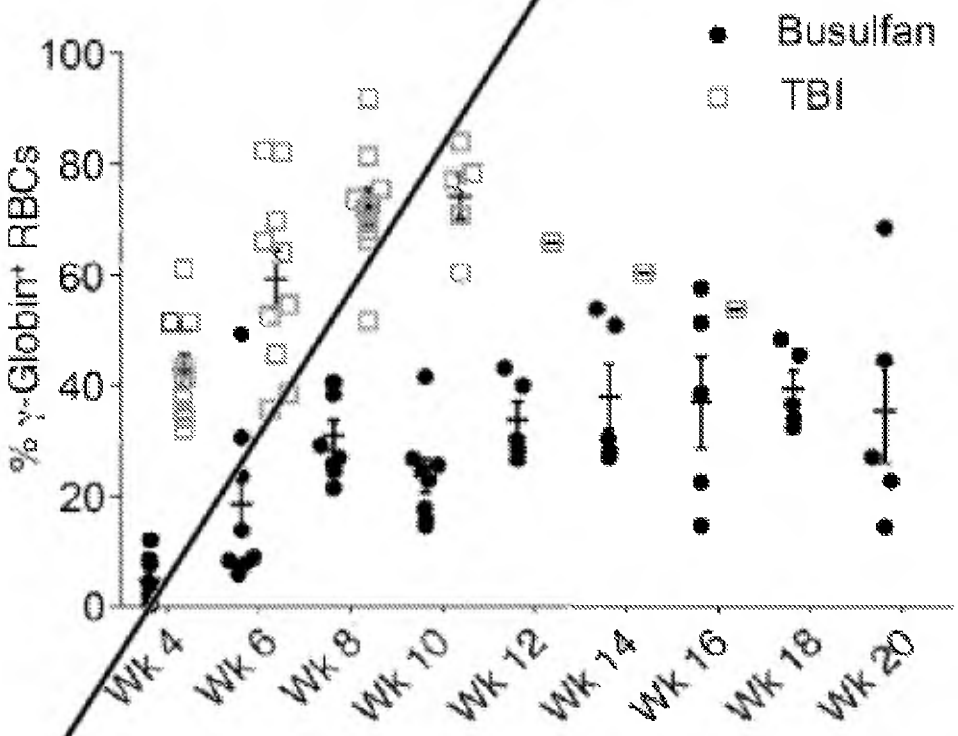


FIG. 18C

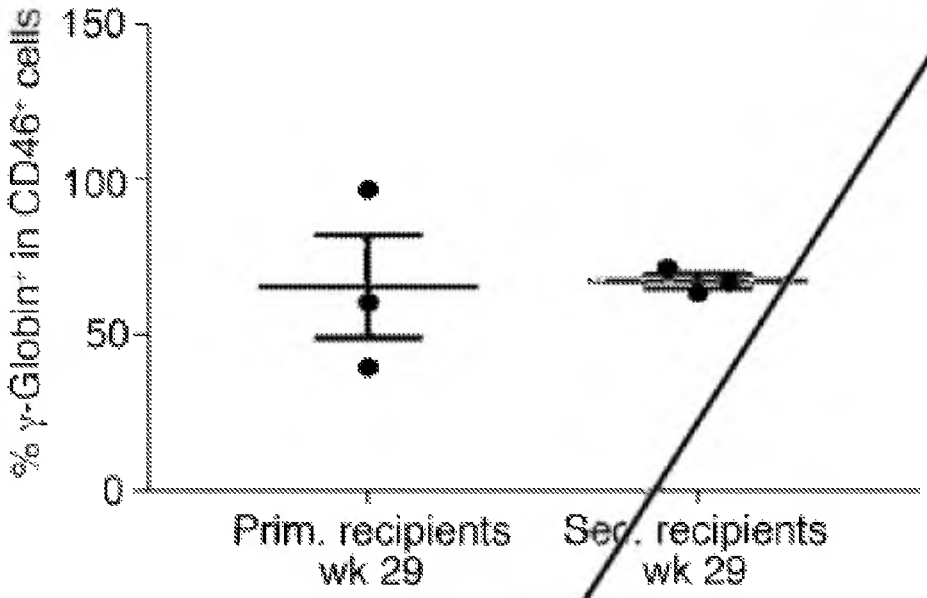


FIG. 18D

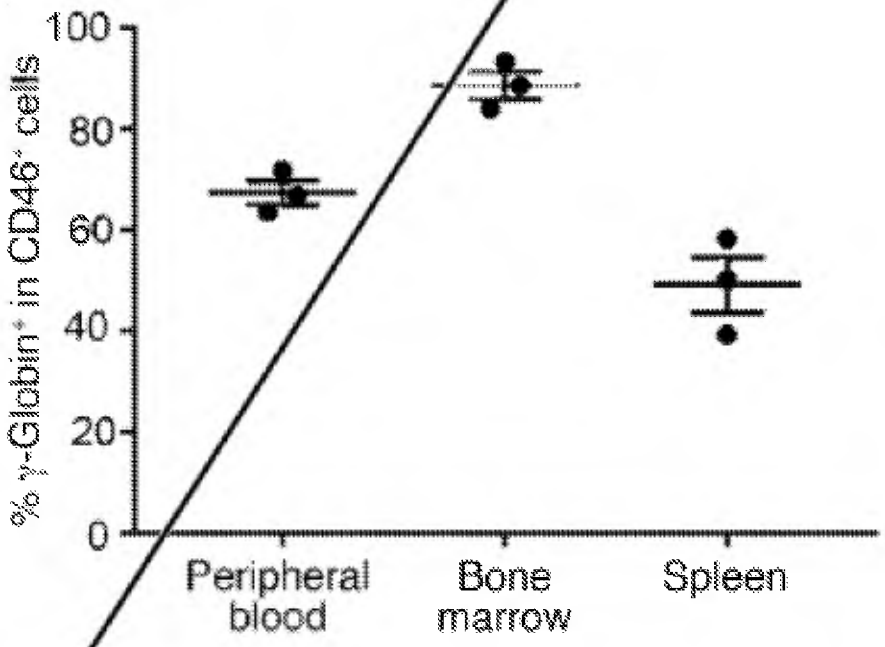


FIG. 18E

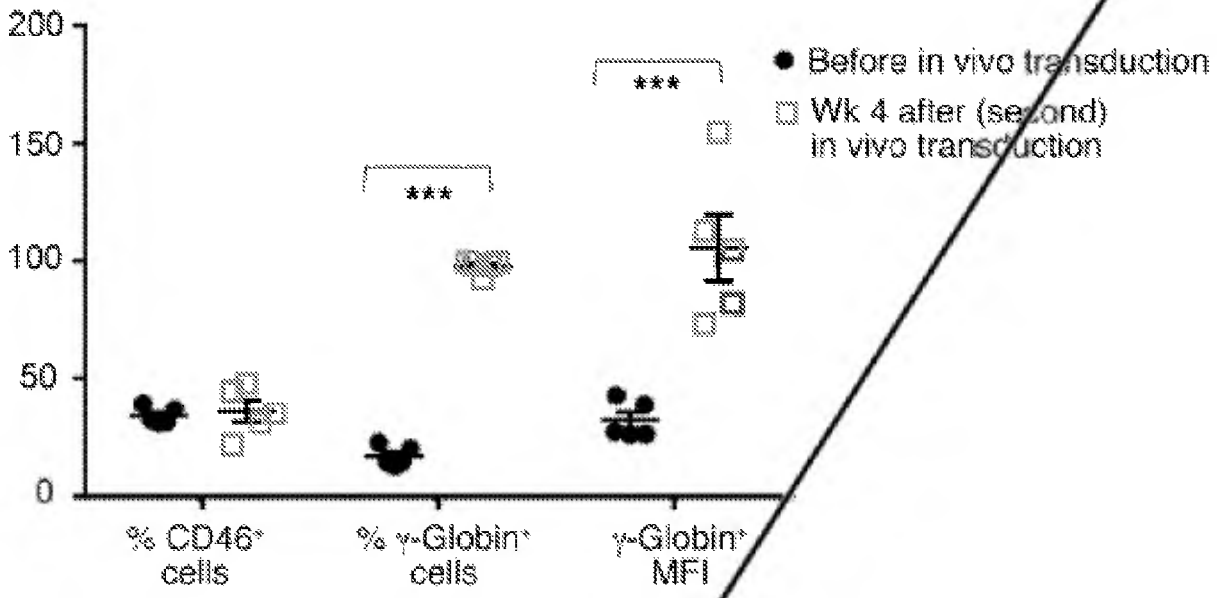


FIG. 19A

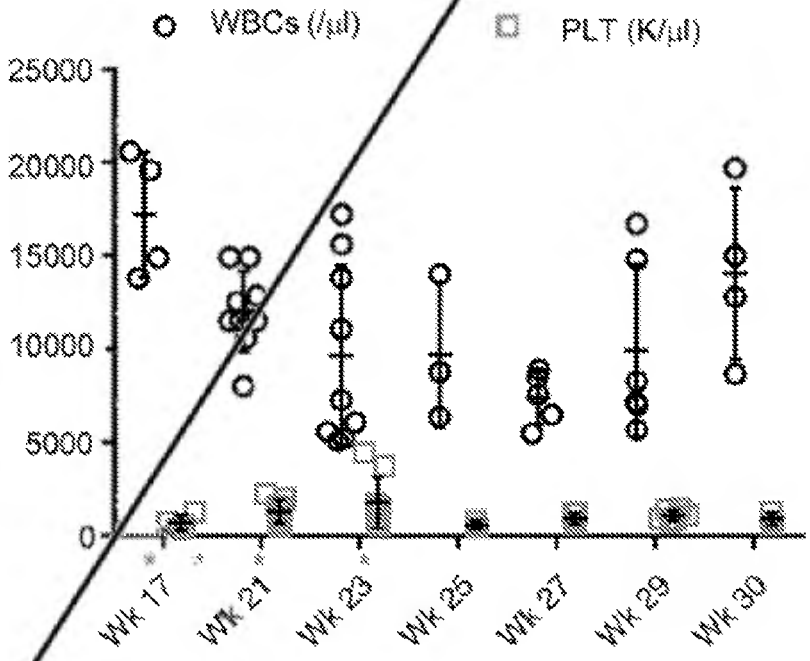


FIG. 19B

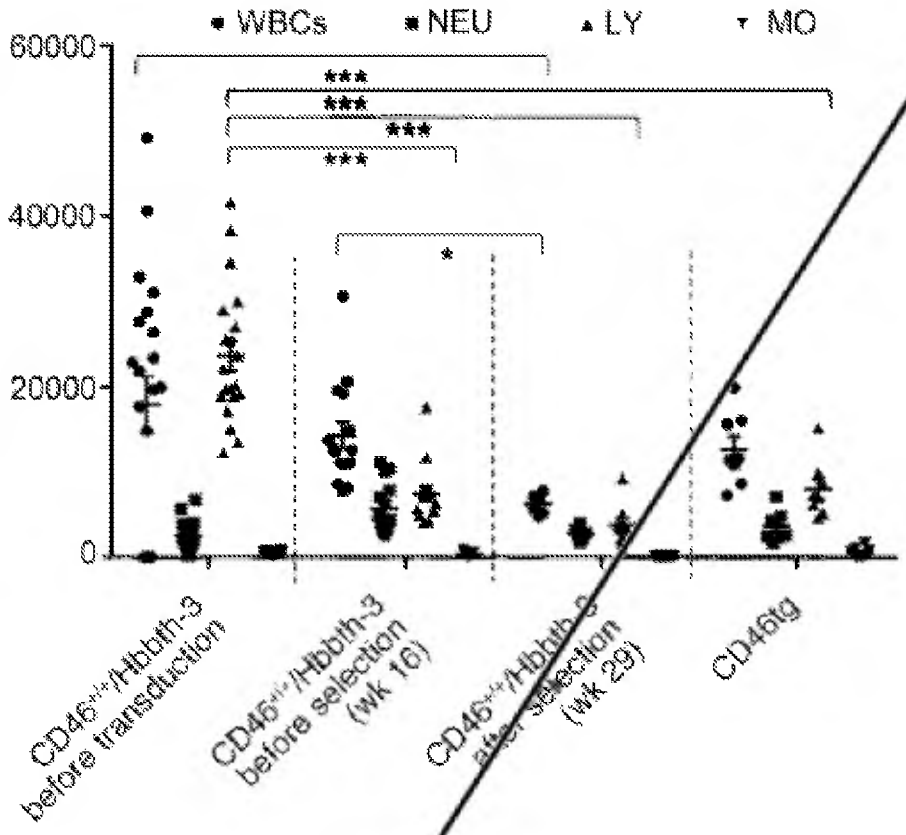


FIG. 19C

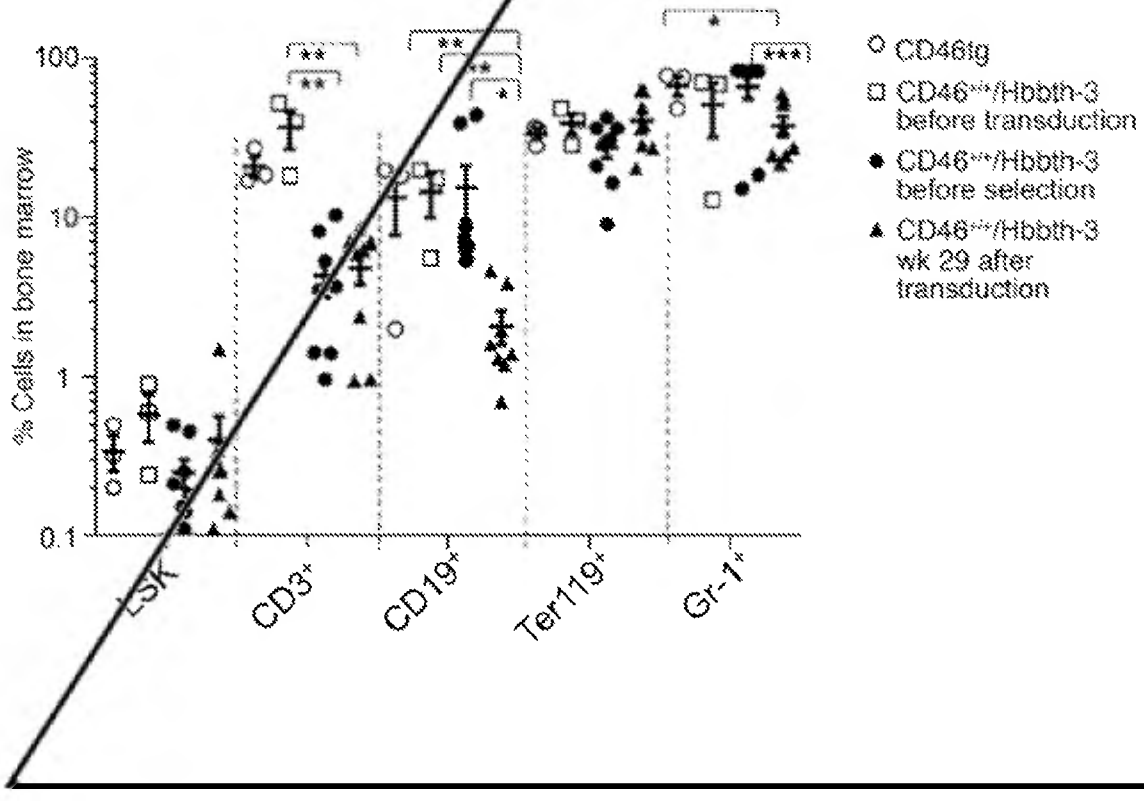


FIG. 19D

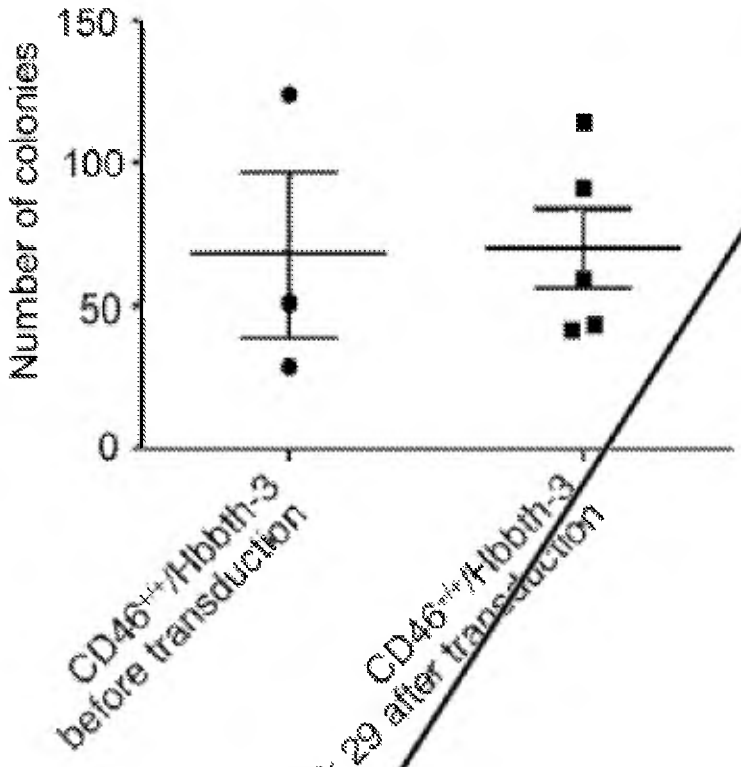

150
100
50
0
Number of colonies
CD46+/+/Hbbth-3 before transduction
CD46+/+/Hbbth-3 wk 29 after transduction

FIG. 20A

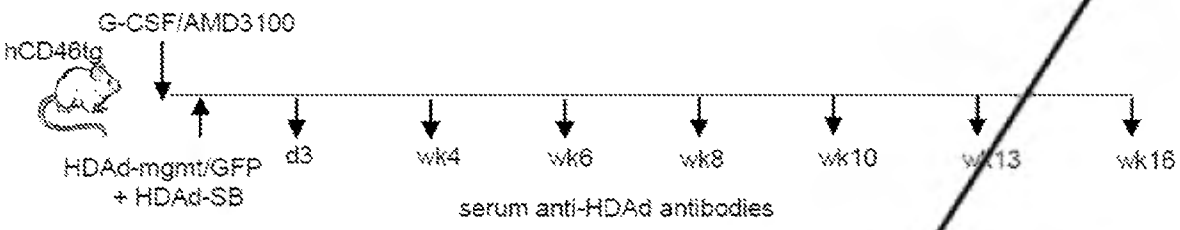


FIG. 20B

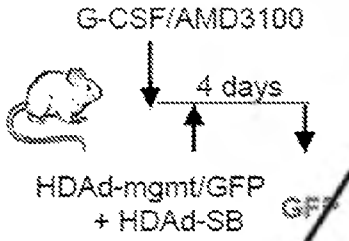


FIG. 20C

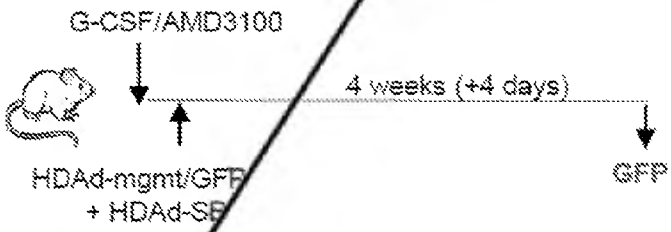


FIG. 20D

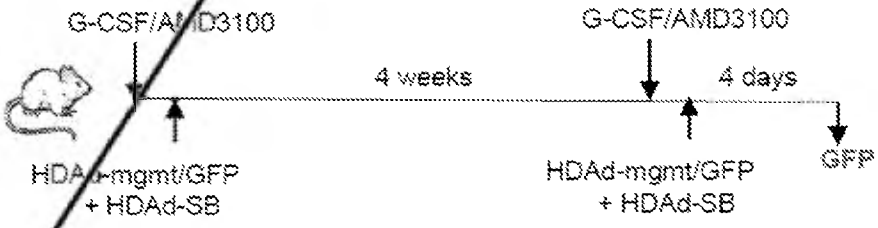


FIG. 20E

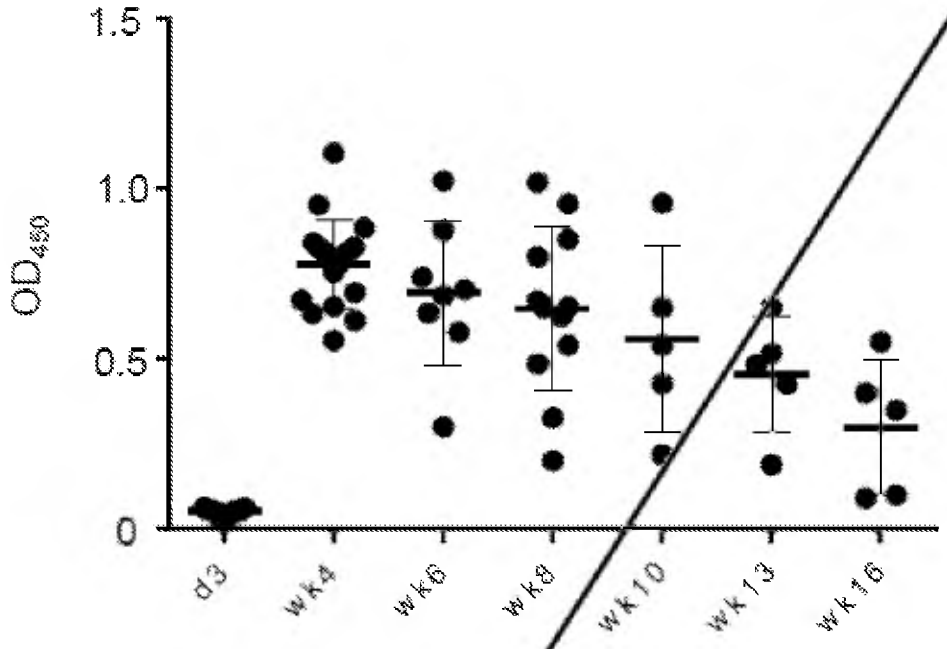


FIG. 20F

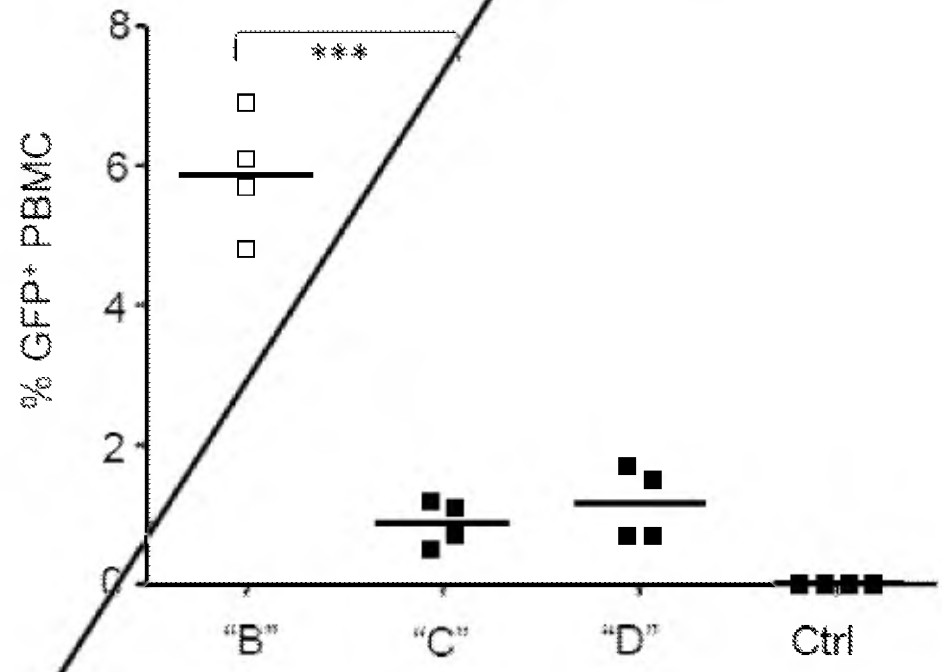


FIG. 21A

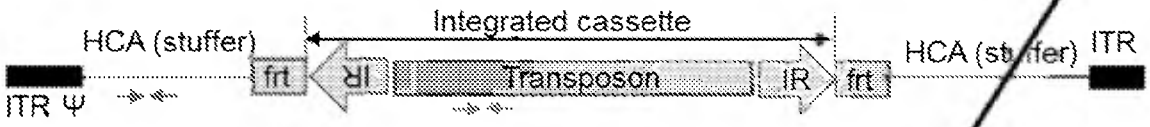


FIG. 21B

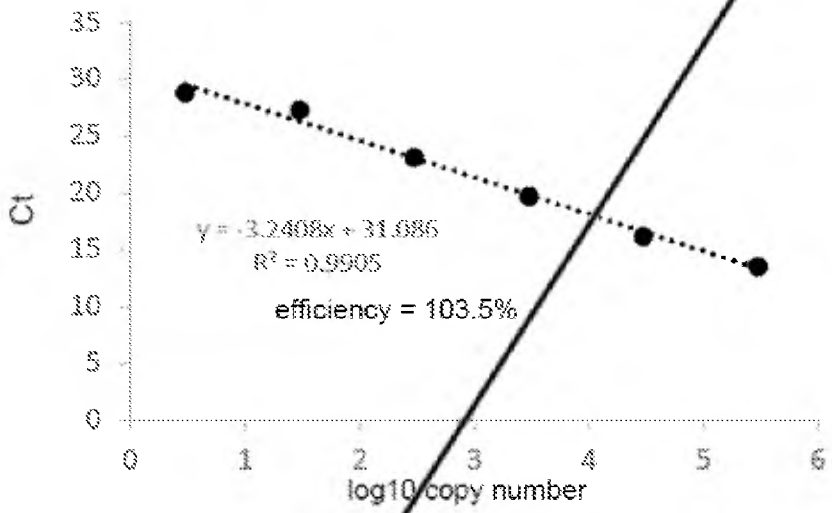


FIG. 21C

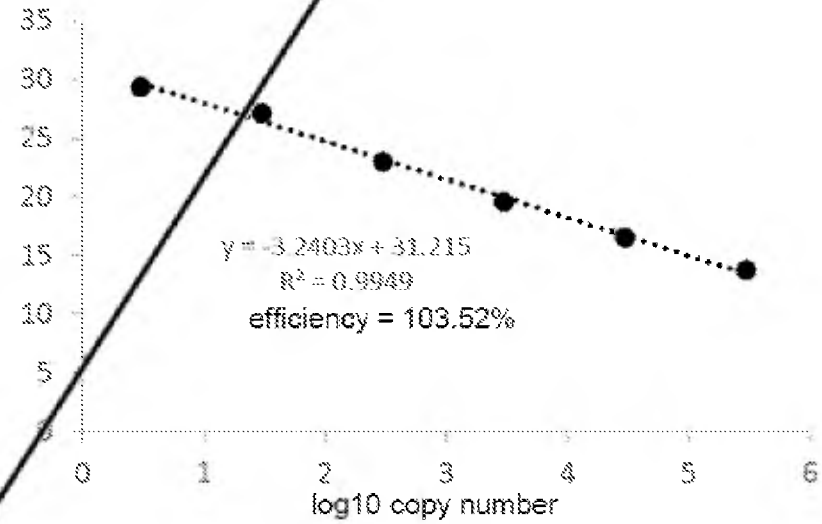


FIG. 21D

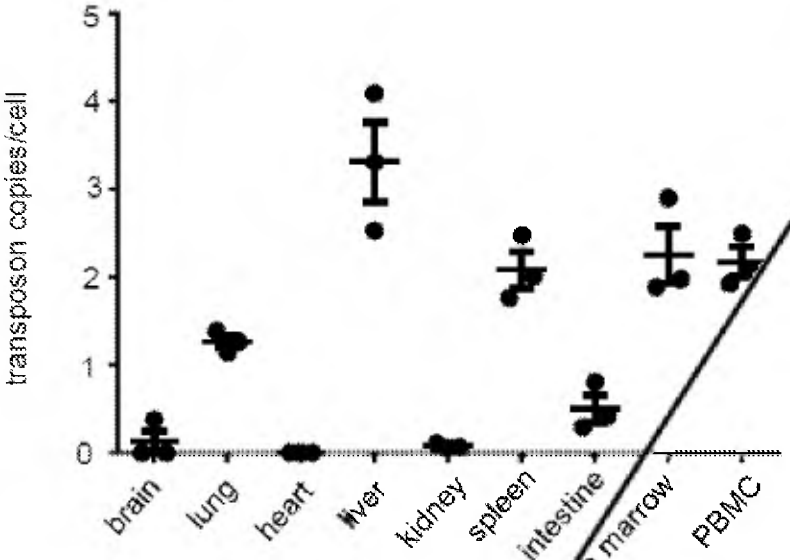

transposon copies/cell
5
4
3
2
1
0
brain
lung
heart
liver
kidney
spleen
intestine
bone marrow
PBMC

FIG. 22A

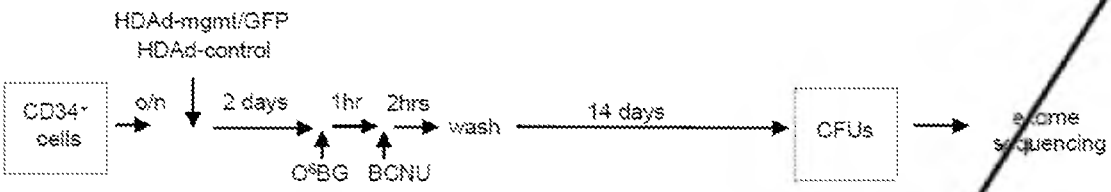


FIG. 22B

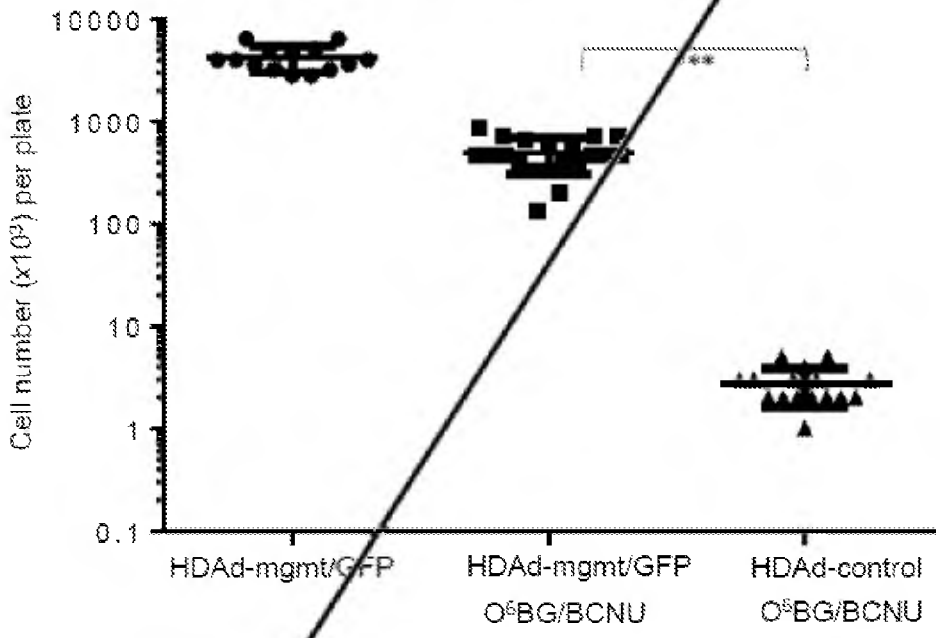
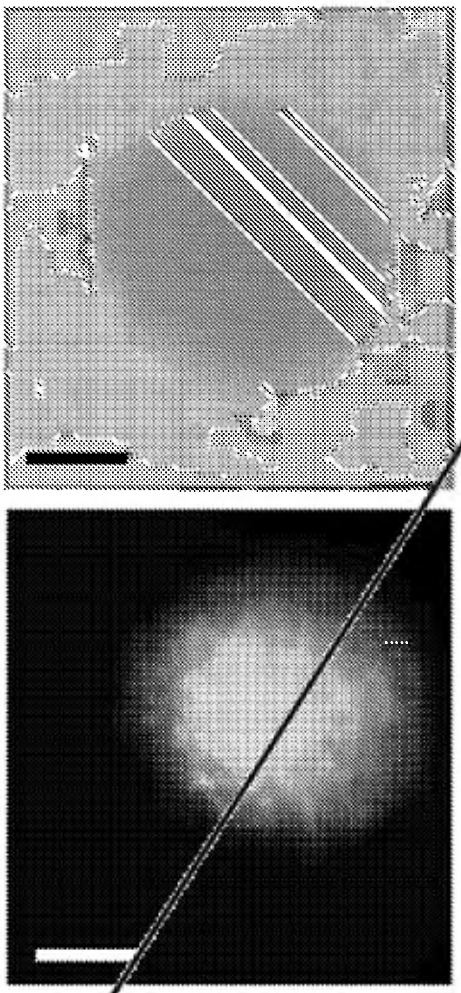


FIG. 22C



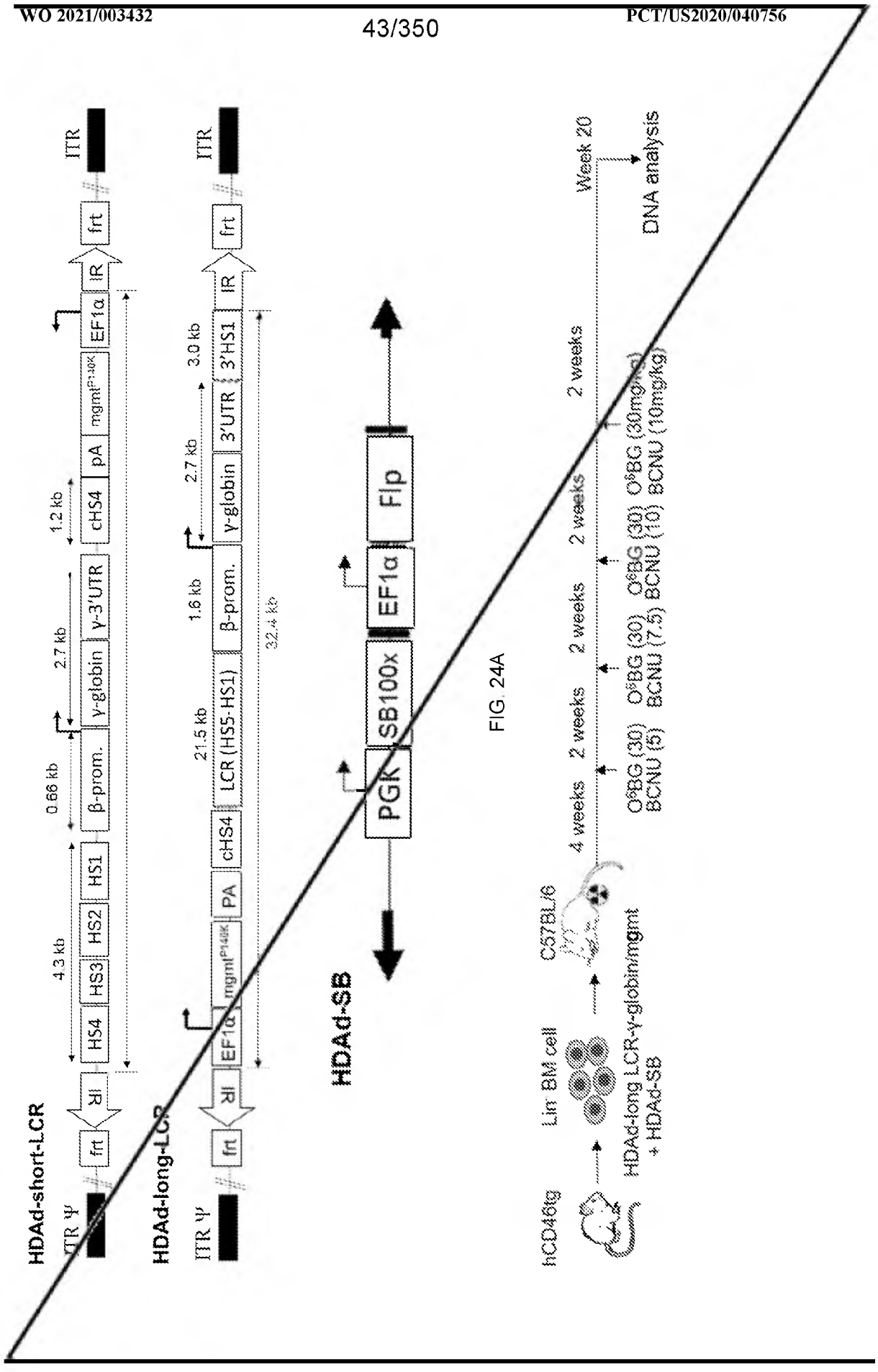


FIG. 23

FIG. 24A

FIG. 24B

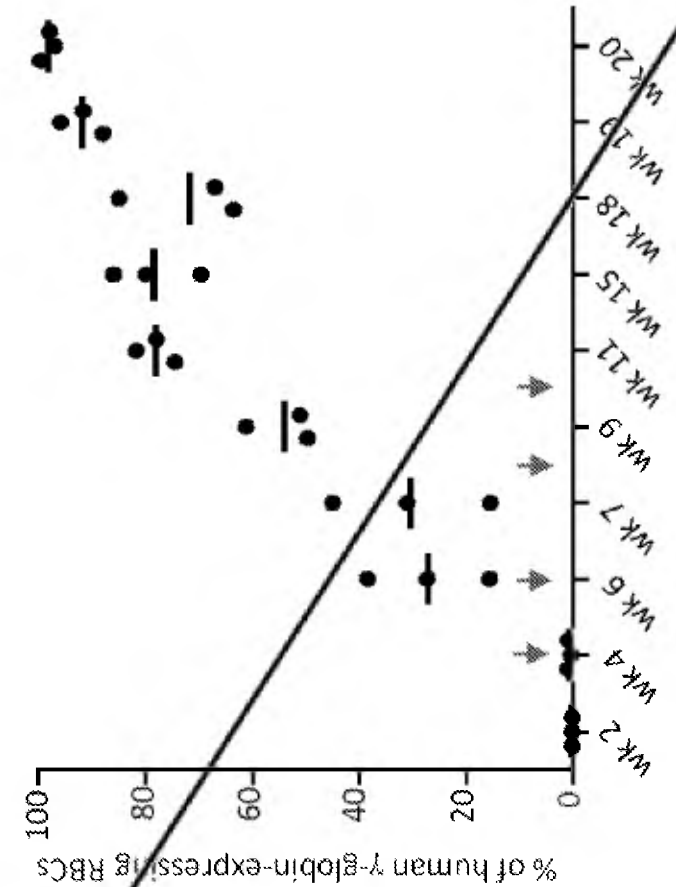


FIG. 24C

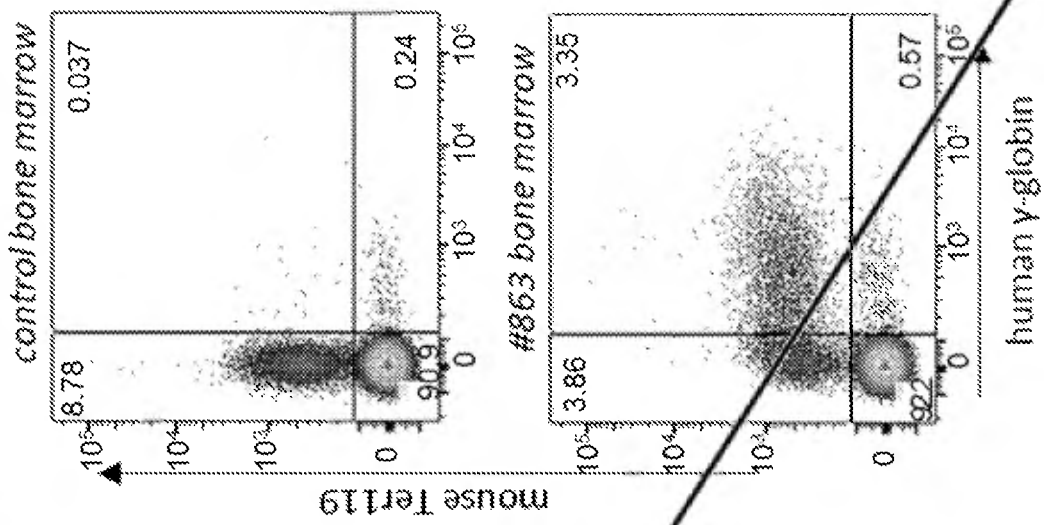


FIG. 24D

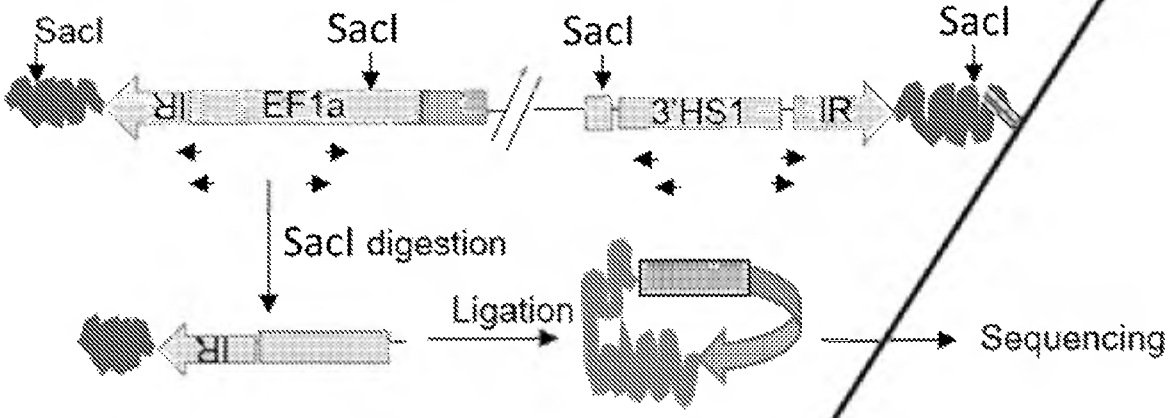


FIG. 24E

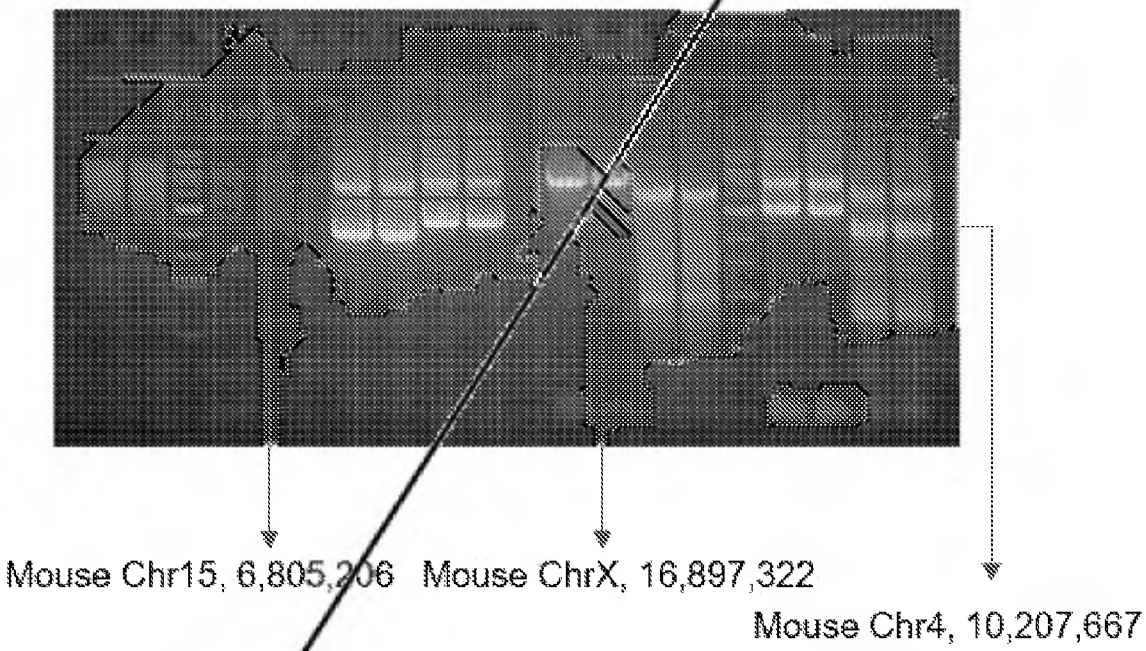
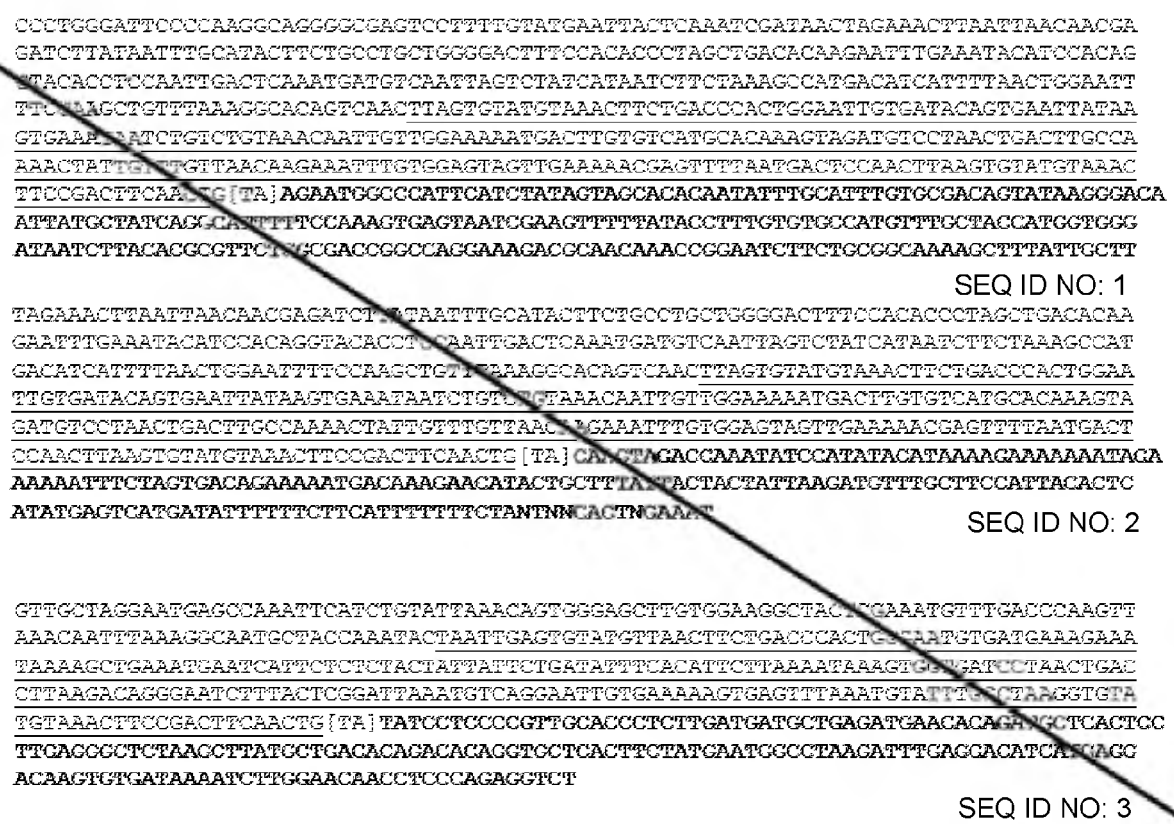


FIG. 24F


5'end vector sequence
Sleeping beauty IR/DR sequence
Integration junction (chr15, 68052067
SEQ ID NO: 1
5'end vector sequence
Sleeping beauty IR/DR sequence
Integration junction (chrX, 16897322)
SEQ ID NO: 2
3'end vector sequence
Sleeping beauty IR/DR sequence
Integration junction (chr4, 10207667)
SEQ ID NO: 3

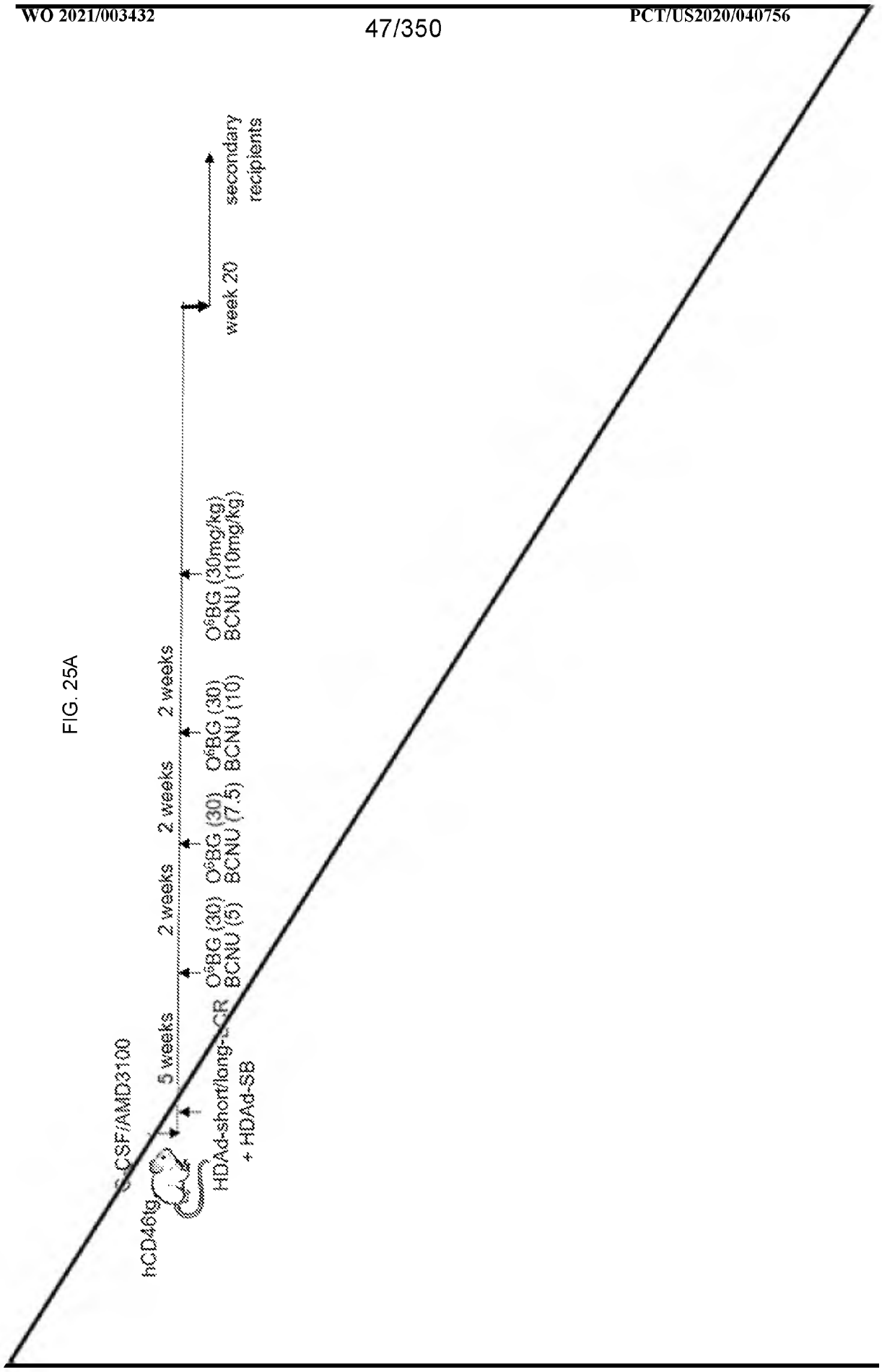

hCD46tg
G-CSF/AMD3100
5 weeks
HDAd-shortflang-CR
+ HDAd-SB
2 weeks
O6BG (30)
BCNU (5)
2 weeks
O6BG (30)
BCNU (7.5)
2 weeks
O6BG (30)
BCNU (10)
week 20
O6BG (30mg/kg)
BCNU (10mg/kg)
secondary recipients

FIG. 25A

FIG. 25B

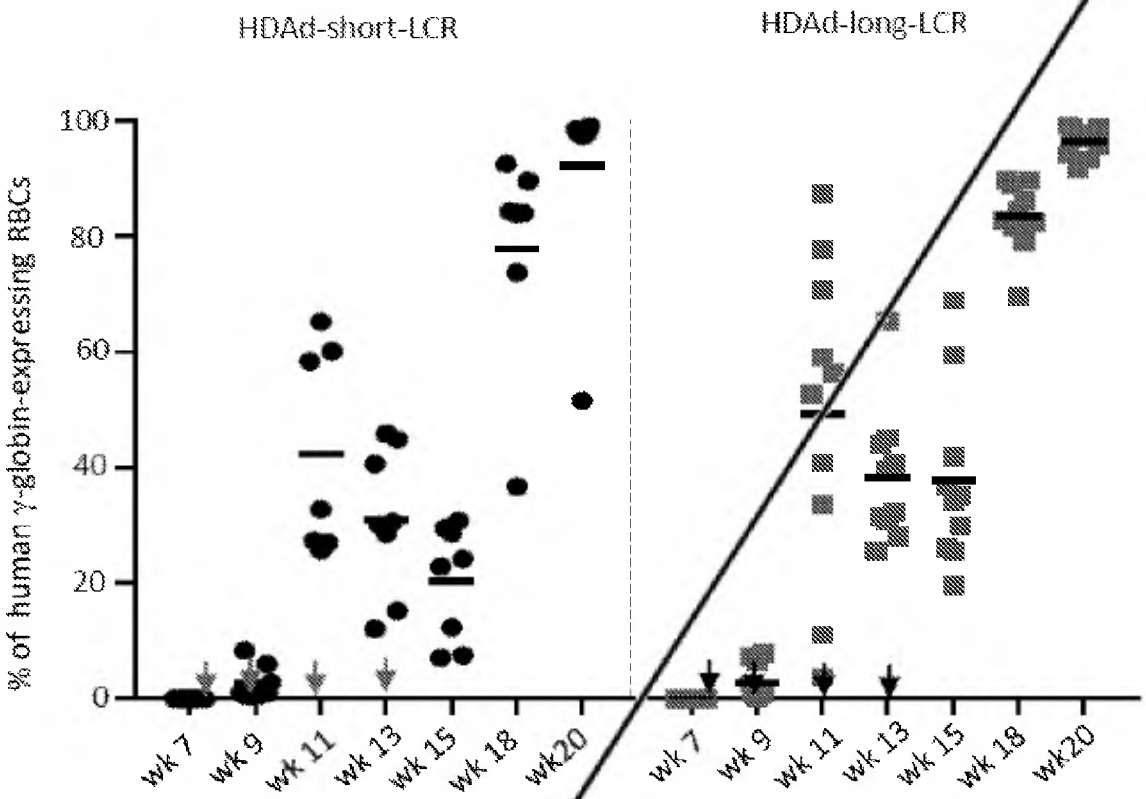


FIG. 25C

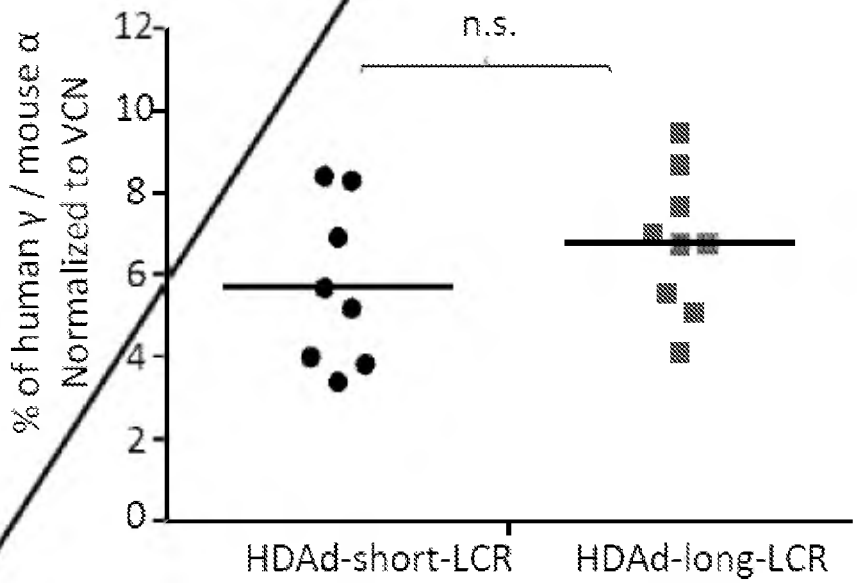


FIG. 25D

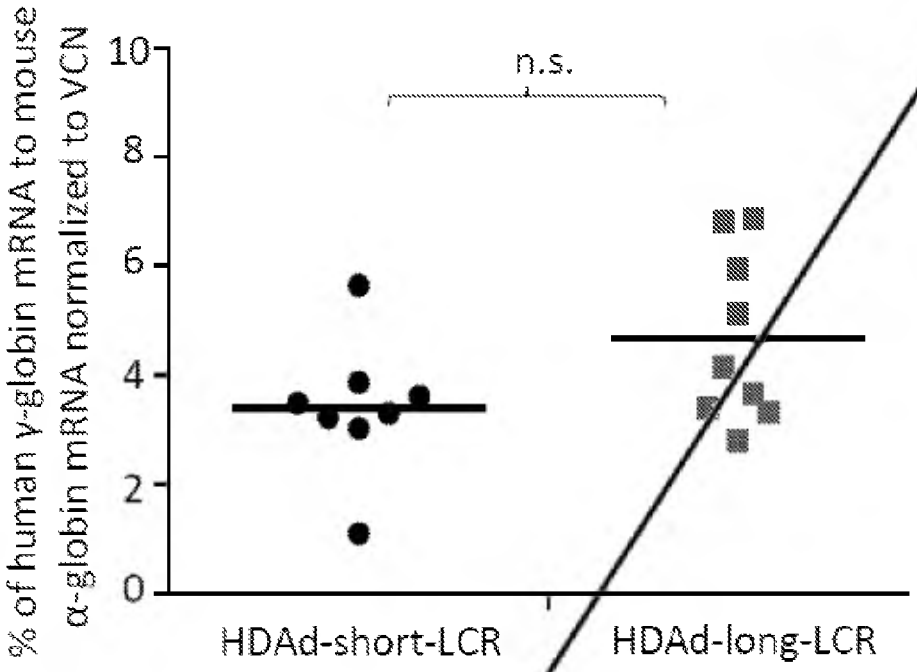


FIG. 25E

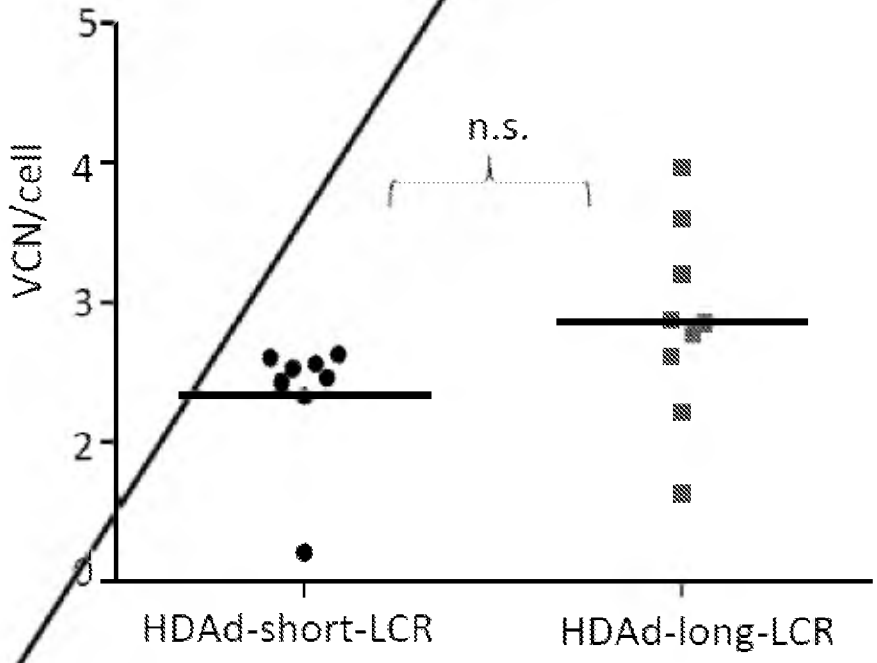


FIG. 26A

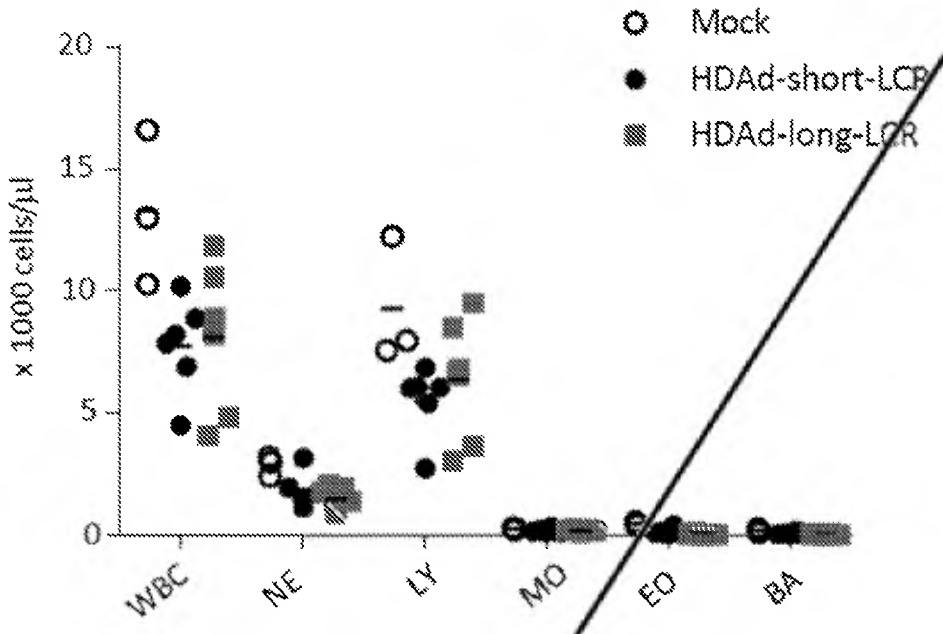


FIG. 26B

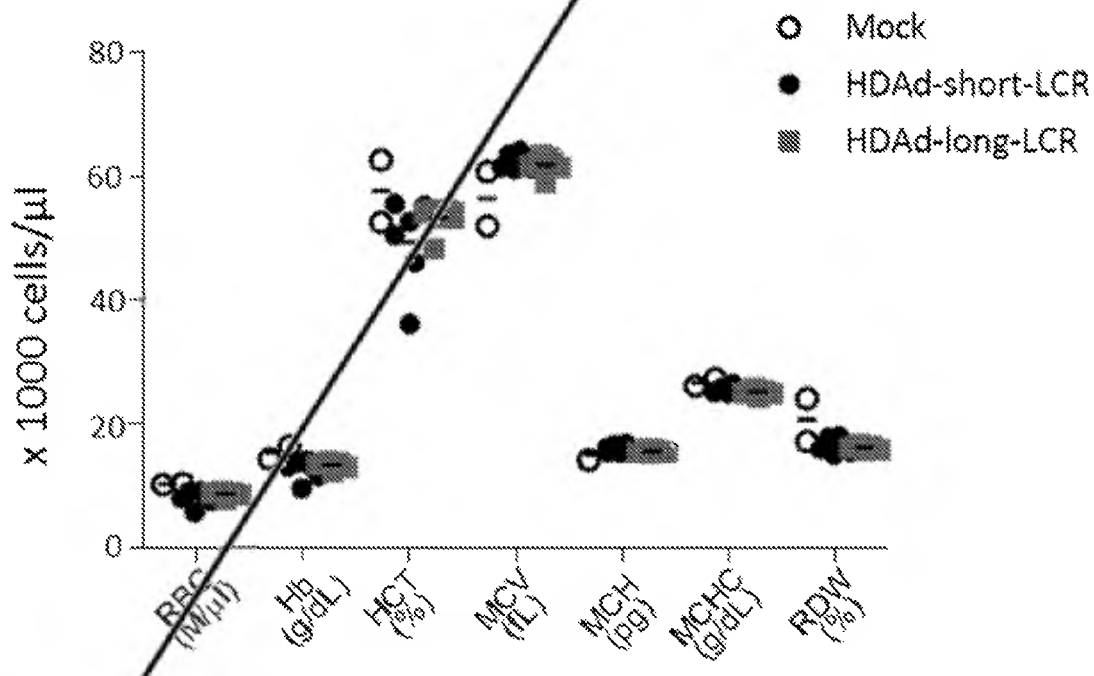


FIG. 26C

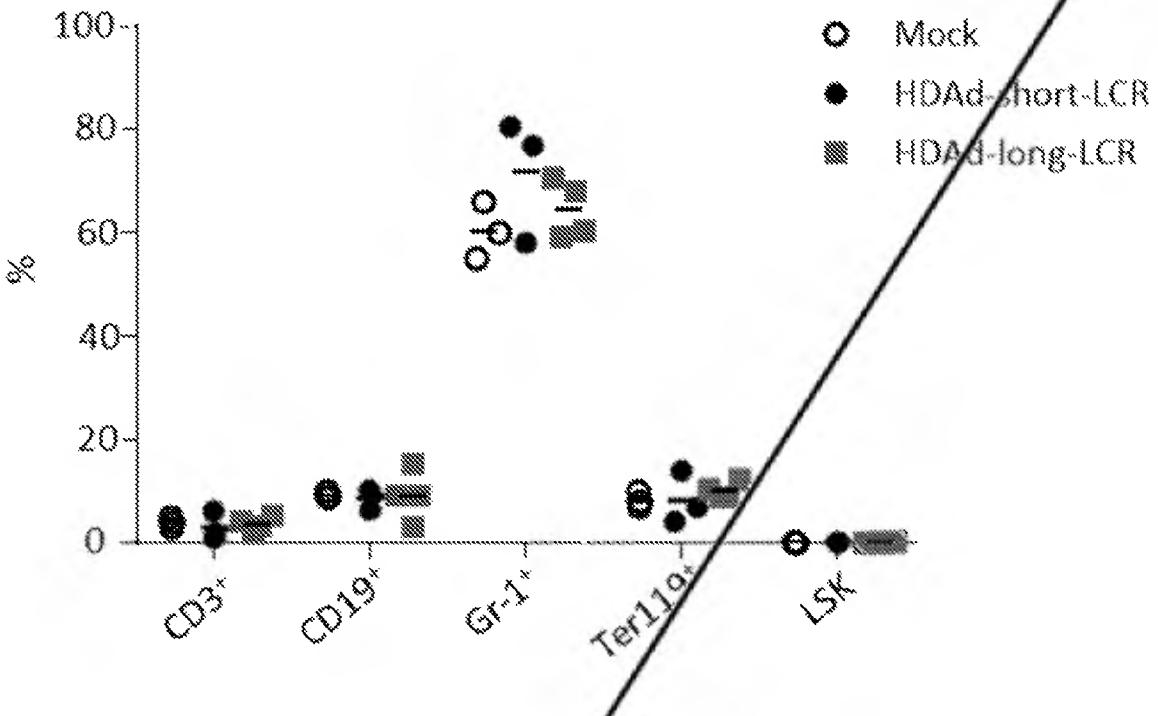


FIG. 26D

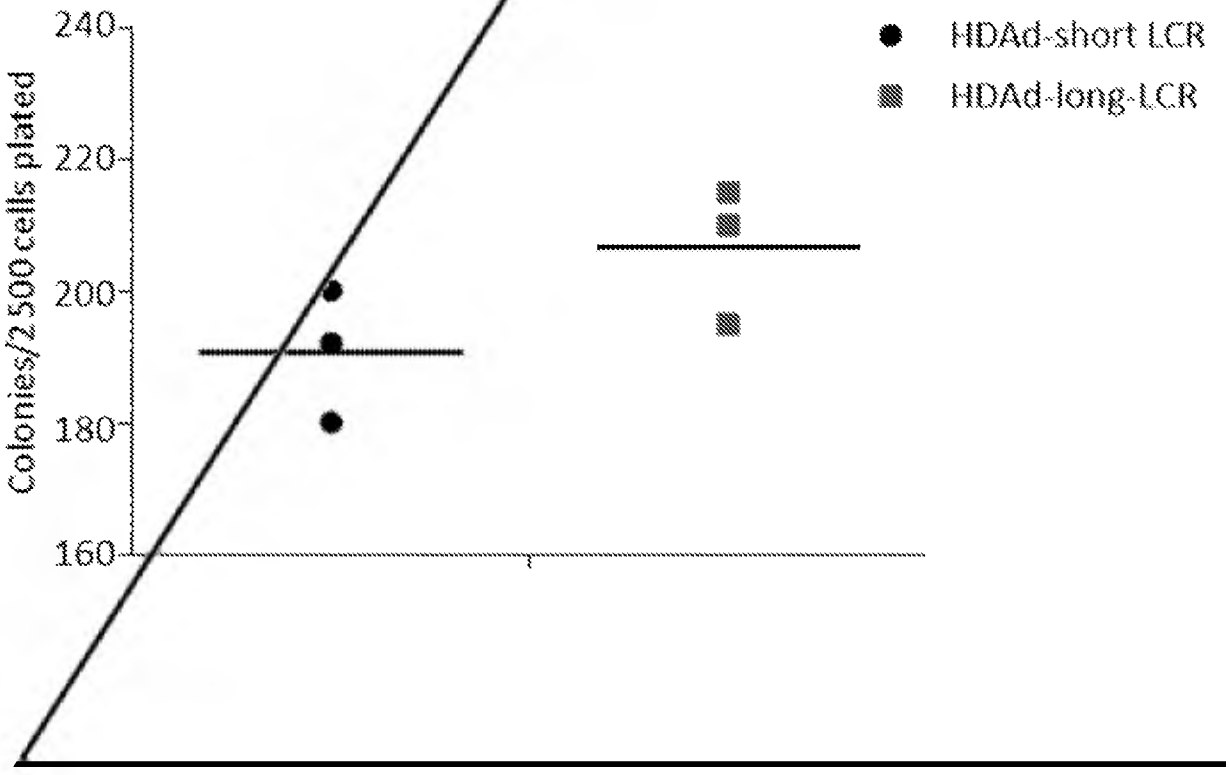


FIG. 27

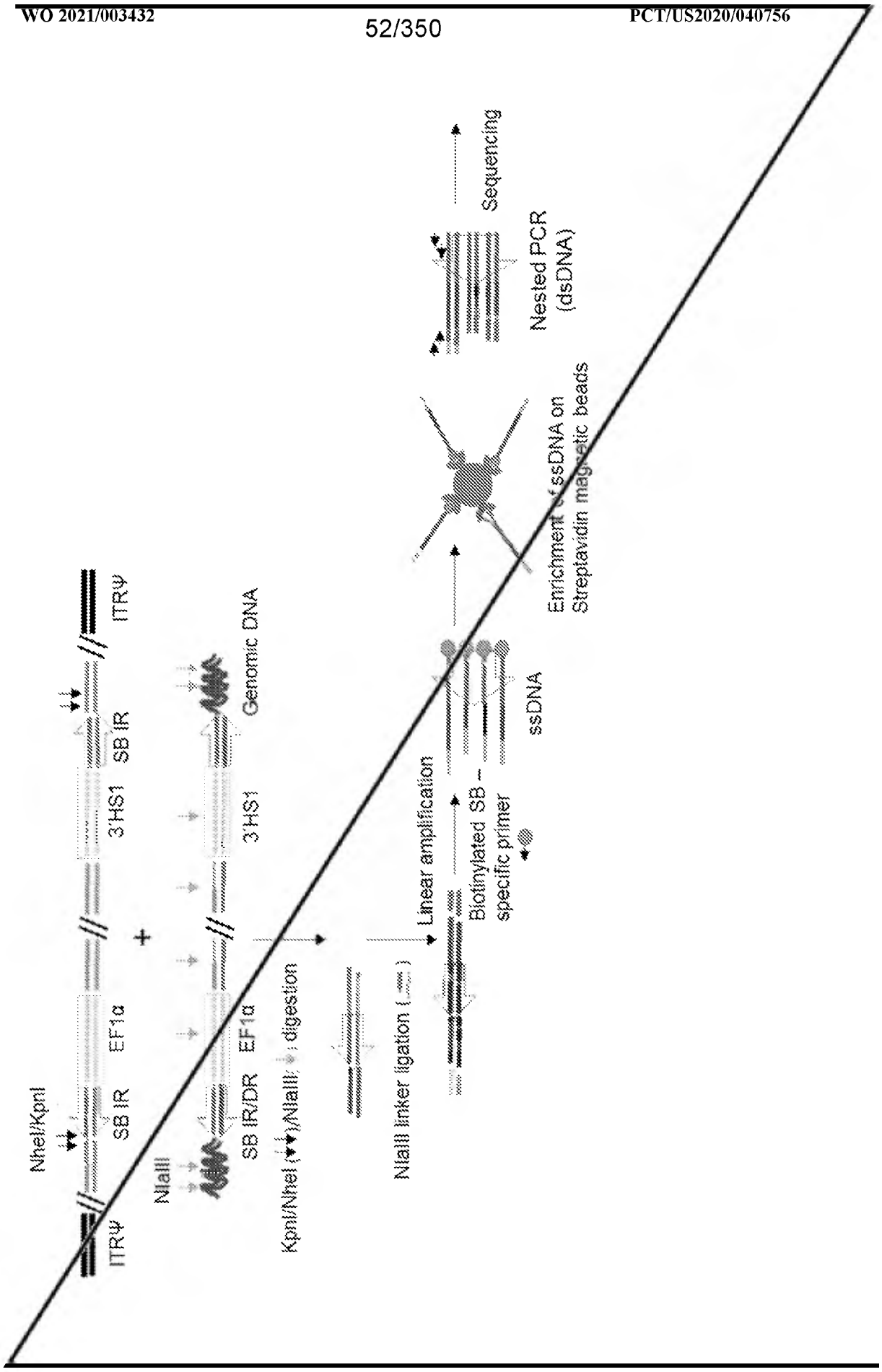

NheI/KpnI
ITRΨ
SB IR
EF1α
3'HS1
SB IR
ITRΨ
NlaIII
SB IR/DR
EF1α
3'HS1
Genomic DNA
KpnI/NheI (▼▼)/NlaIII (▲▲) digestion
NlaIII linker ligation (——)
Linear amplification
Biotinylated SB – specific primer
ssDNA
Enrichment of ssDNA on Streptavidin magnetic beads
Nested PCR (dsDNA)
Sequencing

FIG. 28A

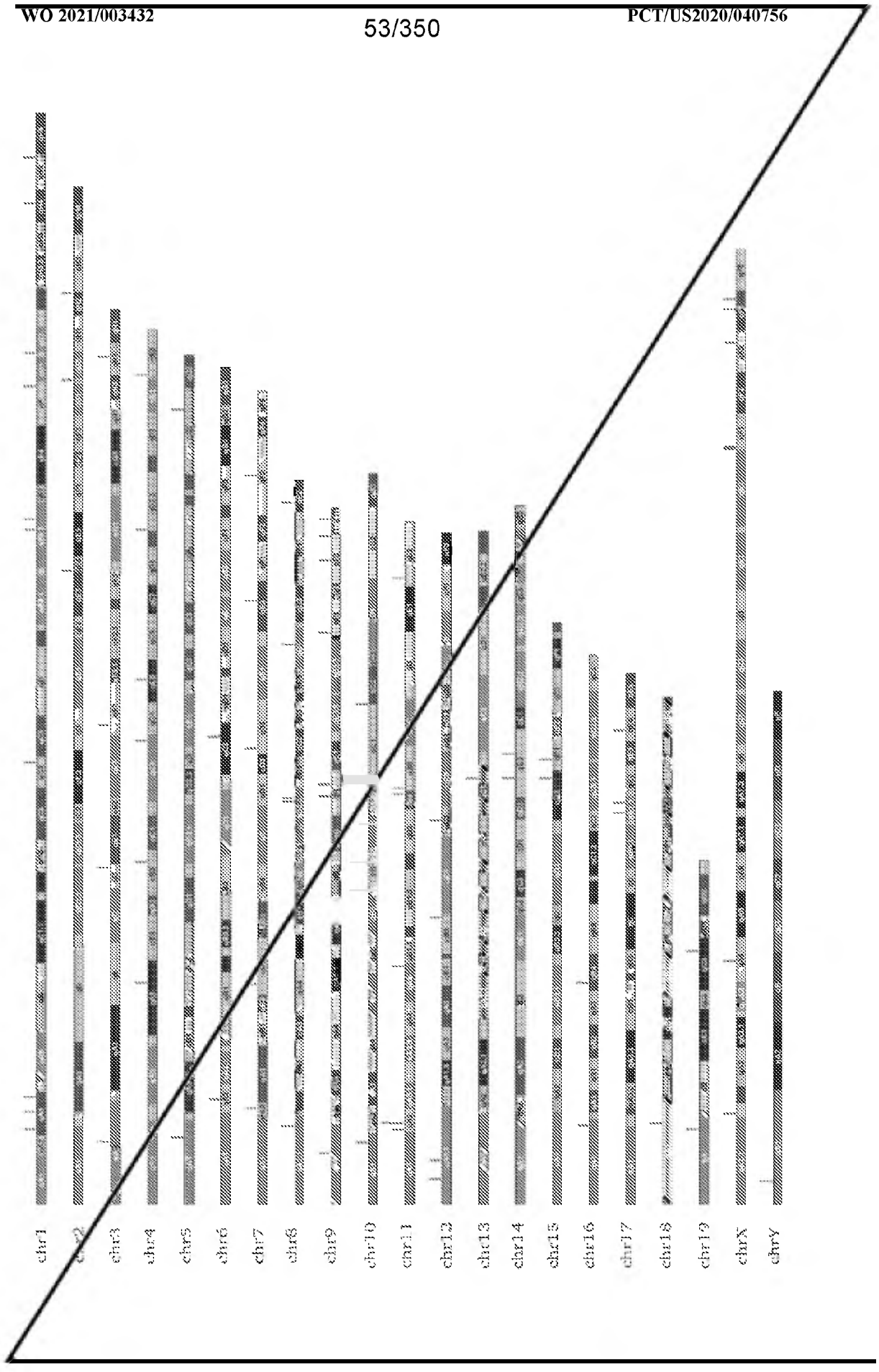

chr1
chr2
chr3
chr4
chr5
chr6
chr7
chr8
chr9
chr10
chr11
chr12
chr13
chr14
chr15
chr16
chr17
chr18
chr19
chrX
chrY

FIG 28B

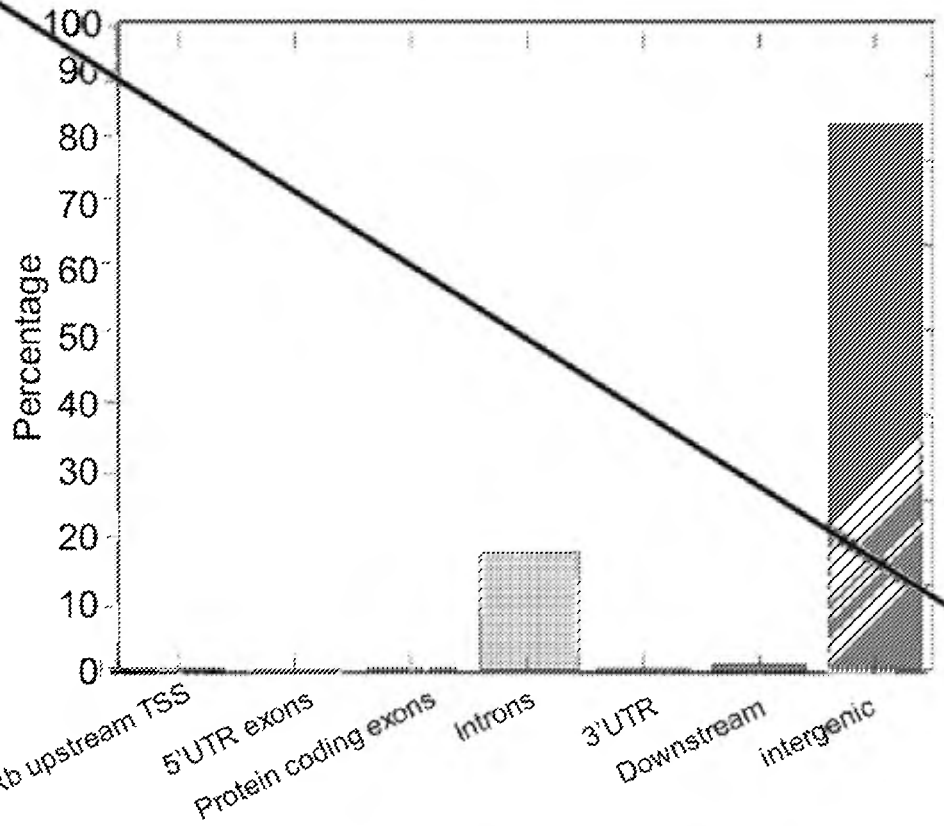
Sleeping beauty IR/DR sequence
integration junction (chr7, 79796094) ACTTAAGTGT ATGTAAACTT CCGACTTCAA CTGTAGCCTA CCTGATTCTC TGGCCATCTG TGCCCACTAC CATG

SEQ ID NO: 4

Sleeping beauty IR/DR sequence
integration junction (repeat region) ACTTAAGTGT ATGTAAACTT CCGACTTCAA CTGTAAATTT TCCACCTTTT TCAGTTTTCC TCGCCATATT TCATG

SEQ ID NO: 5

FIG. 28C



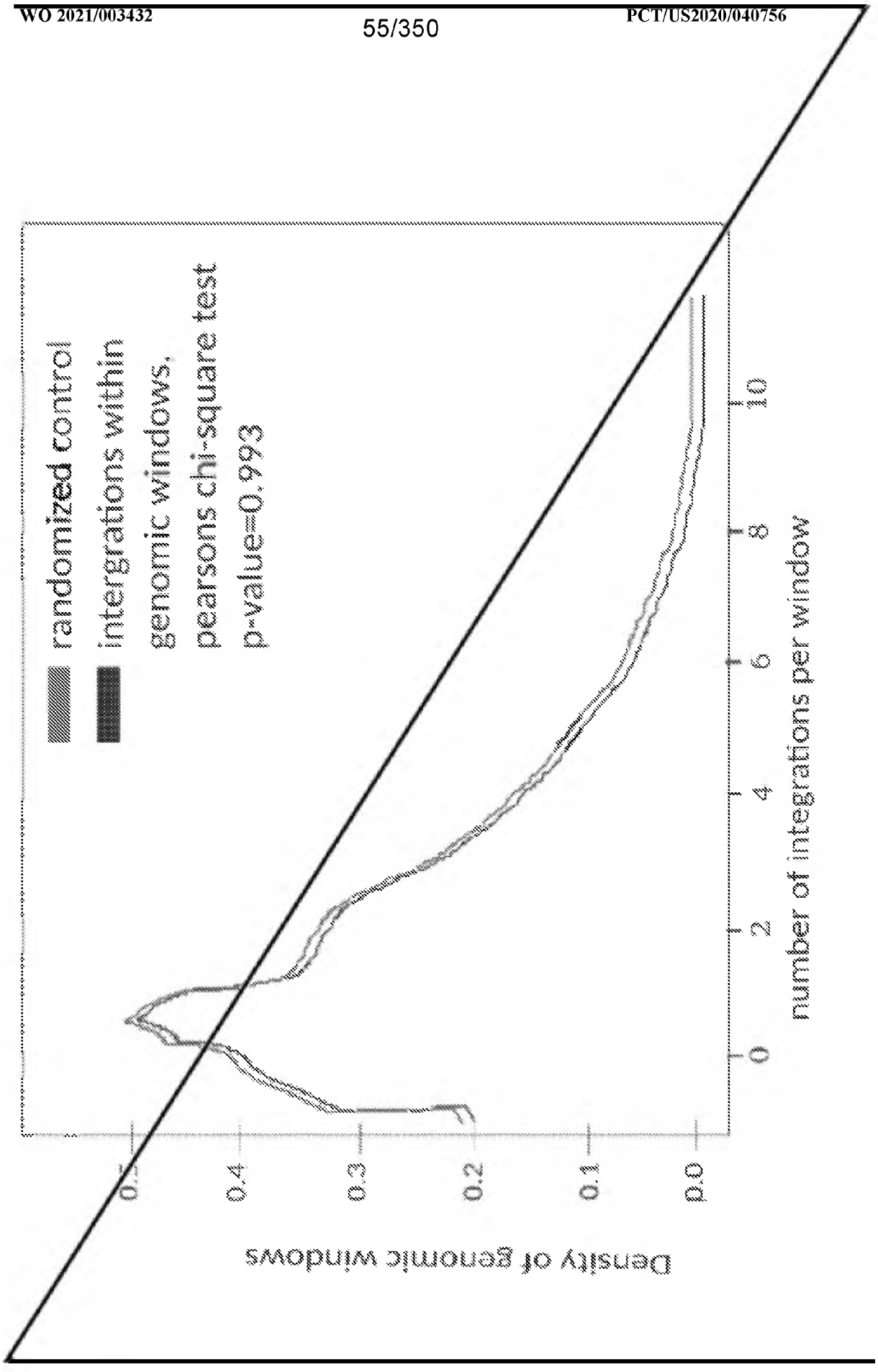
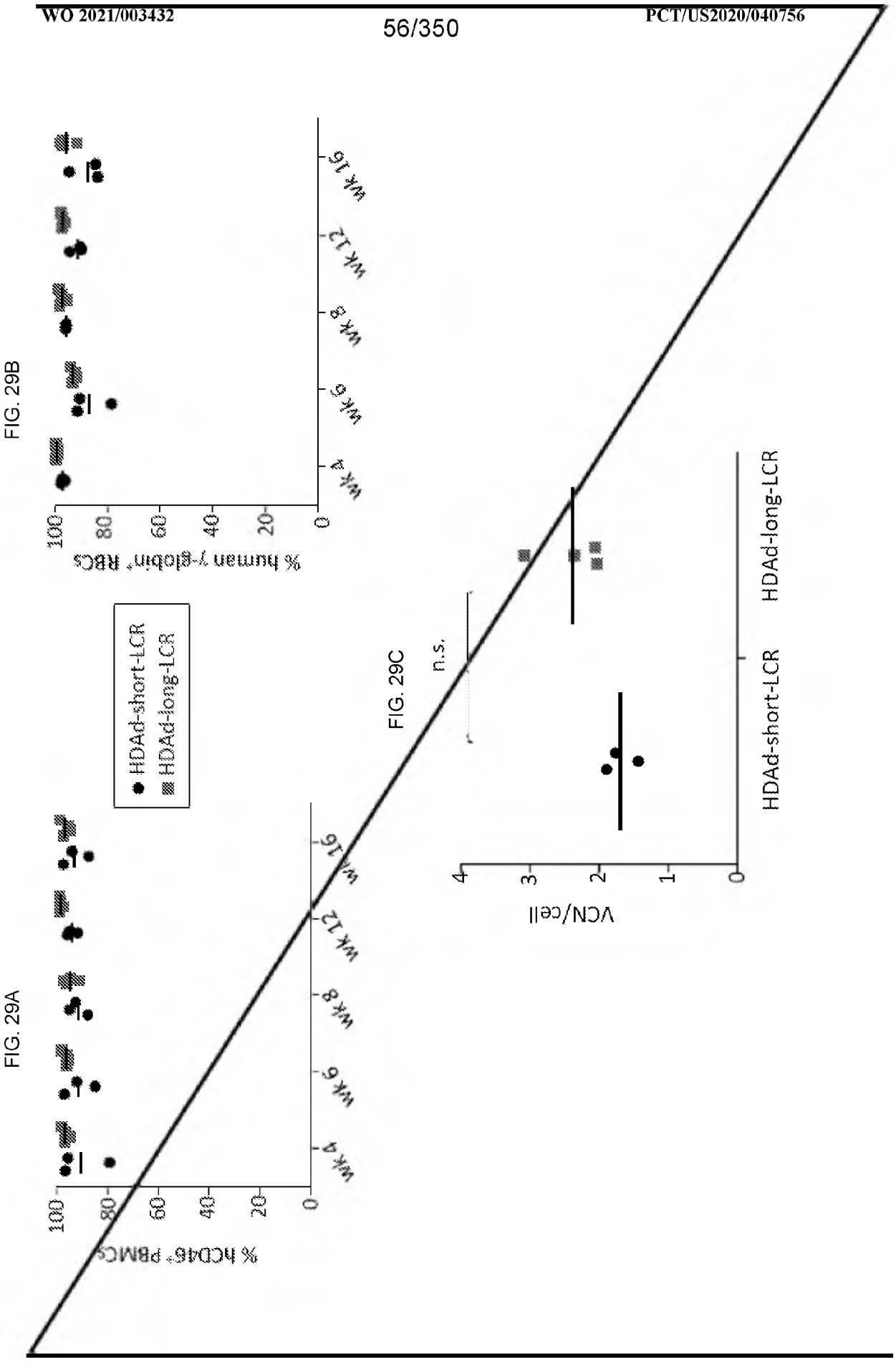

randomized control
integrations within genomic windows,
pearsons chi-square test
p-value=0.993
Density of genomic windows
0.0
0.1
0.2
0.3
0.4
0
2
4
6
8
10
number of integrations per window

FIG. 28D


FIG. 29A
% hCD46+ PBMCs
100
80
60
40
20
0
wk 4
wk 6
wk 8
wk 12
wk 16
HDAd-short-LCR
HDAd-long-LCR
FIG. 29B
% human γ-globin+ RBCs
100
80
60
40
20
0
wk 4
wk 6
wk 8
wk 12
wk 16
FIG. 29C
n.s.
VCN/cell
4
3
2
1
0
HDAd-short-LCR
HDAd-long-LCR

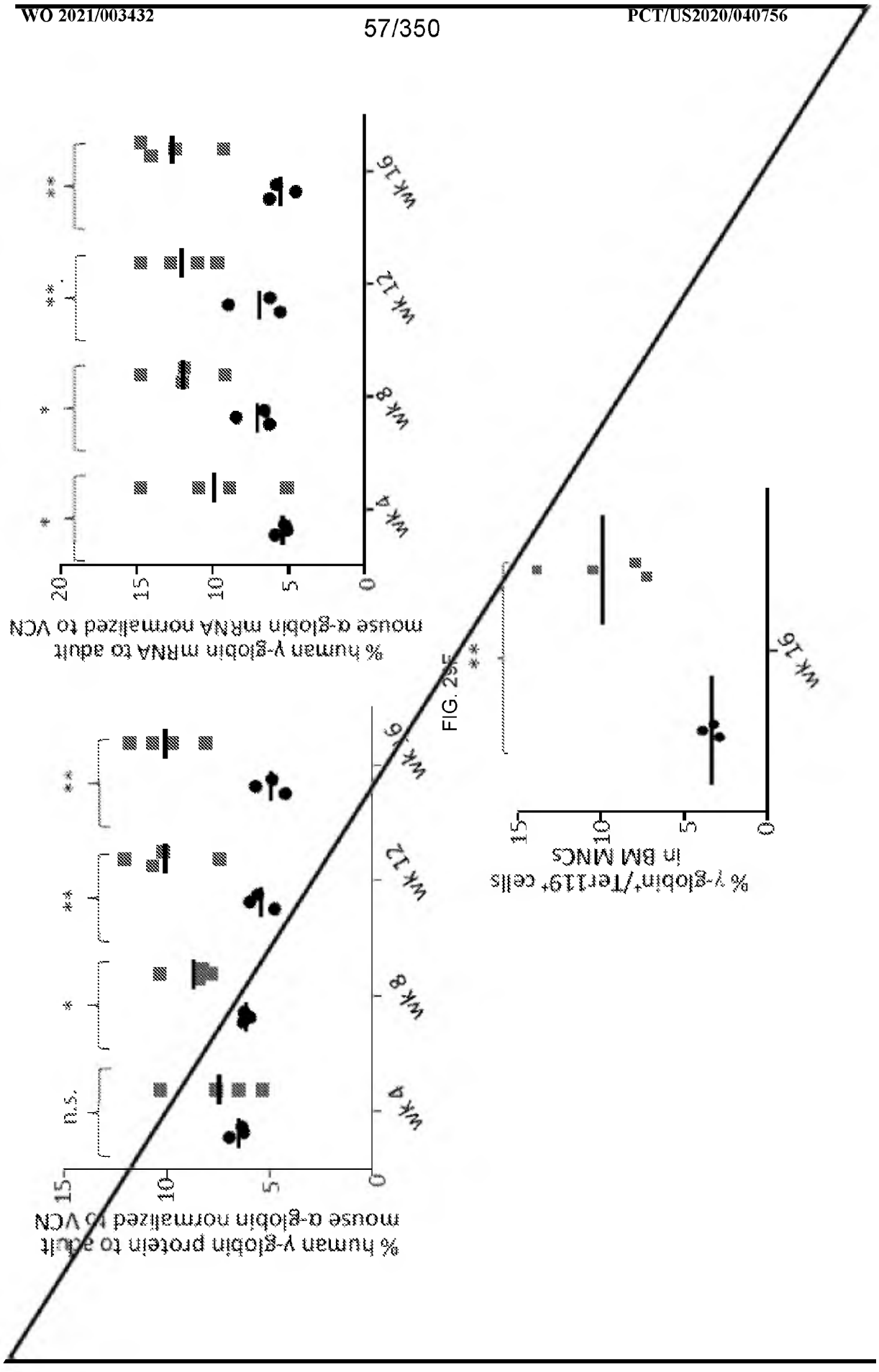

FIG.29D
% human γ-globin protein to adult mouse α-globin normalized to VCN
n.s.
*
**
**
Wk 4
Wk 8
Wk 12
Wk 16
FIG. 29E
% human γ-globin mRNA to adult mouse α-globin mRNA normalized to VCN
*
*
**
**
Wk 4
Wk 8
Wk 12
Wk 16
FIG. 29F
% γ-globin+/Ter119+ cells in BM MNCs
**
Wk 16

FIG. 29G

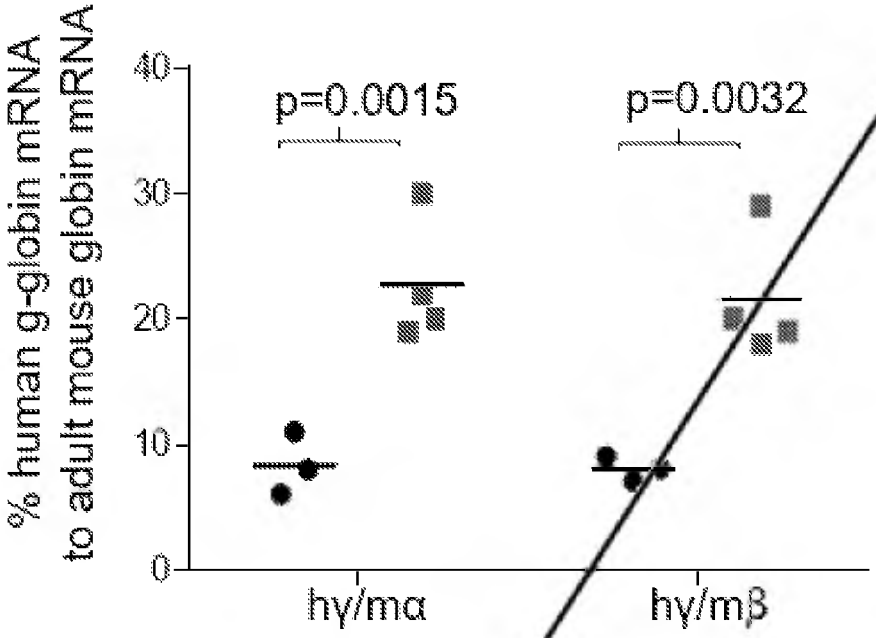

% human g-globin mRNA
to adult mouse globin mRNA
40
30
20
10
0
p=0.0015
p=0.0032
hγ/mα
hγ/mβ

FIG. 29H

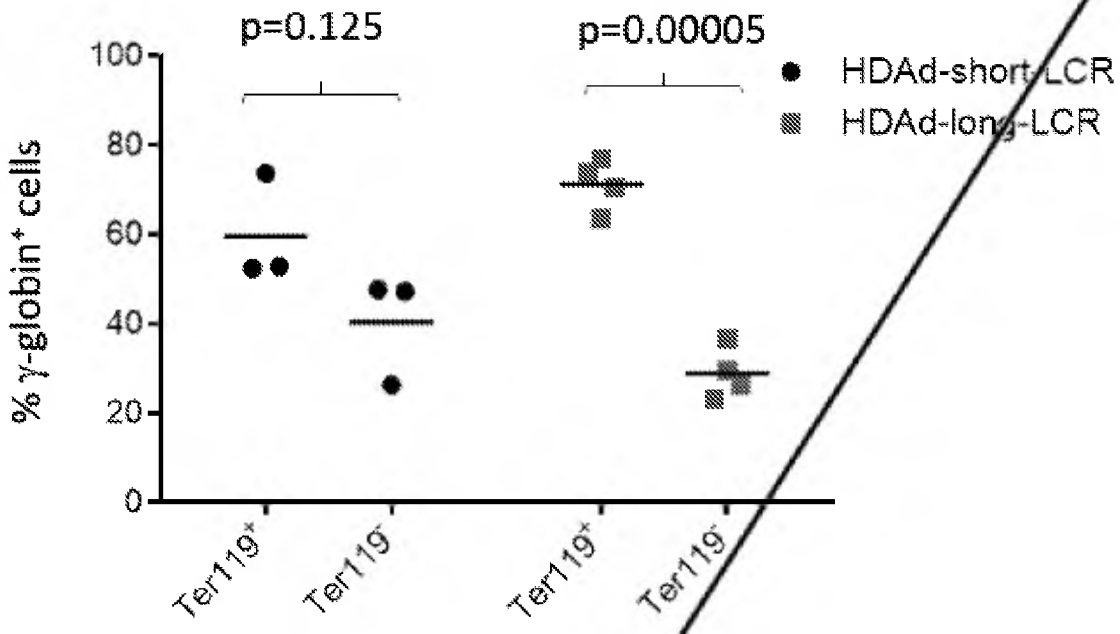
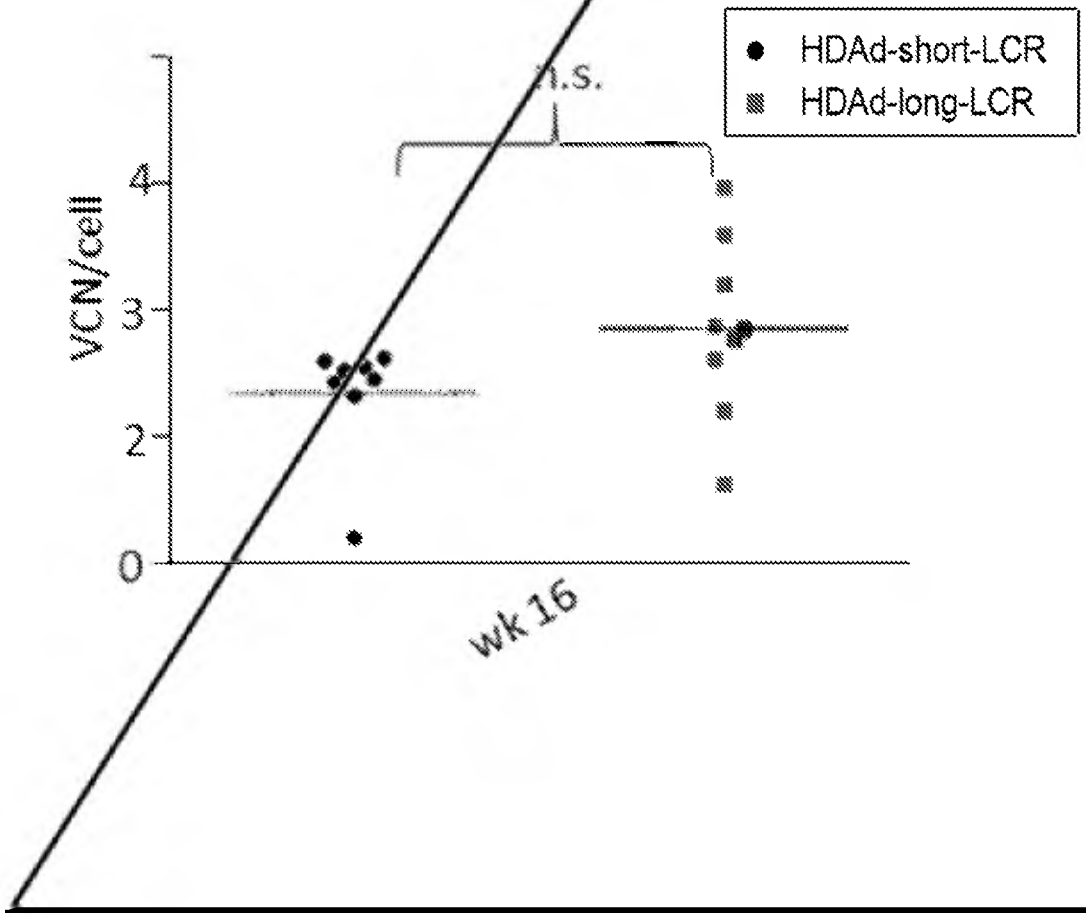


FIG. 29I



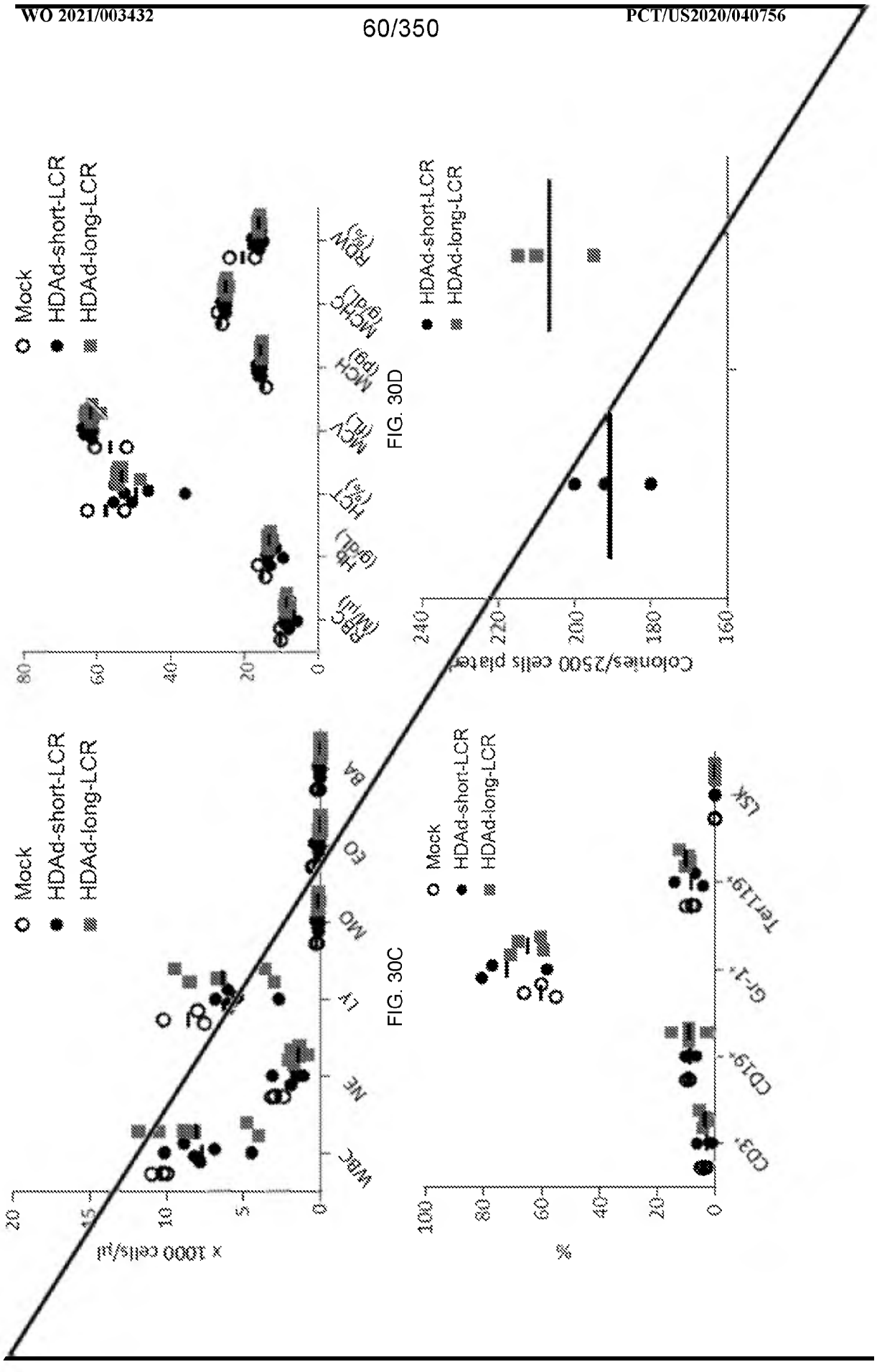

FIG. 30A
Mock
HDAd-short-LCR
HDAd-long-LCR
x 1000 cells/µl
WBC
NE
LY
MO
EO
BA
FIG. 30B
Mock
HDAd-short-LCR
HDAd-long-LCR
RBC (M/µl)
Hb (g/dL)
HCT (%)
MCV (fL)
MCH (pg)
MCHC (g/dL)
RDW (%)
FIG. 30C
Mock
HDAd-short-LCR
HDAd-long-LCR
%
CD3+
CD19+
Gr-1+
Ter119+
LSK
FIG. 30D
HDAd-short-LCR
HDAd-long-LCR
Colonies/2500 cells plated

FIG. 31A

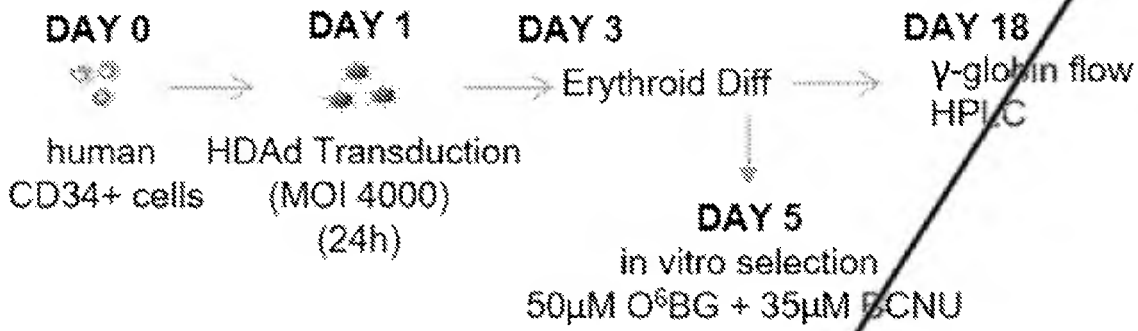


FIG. 31B

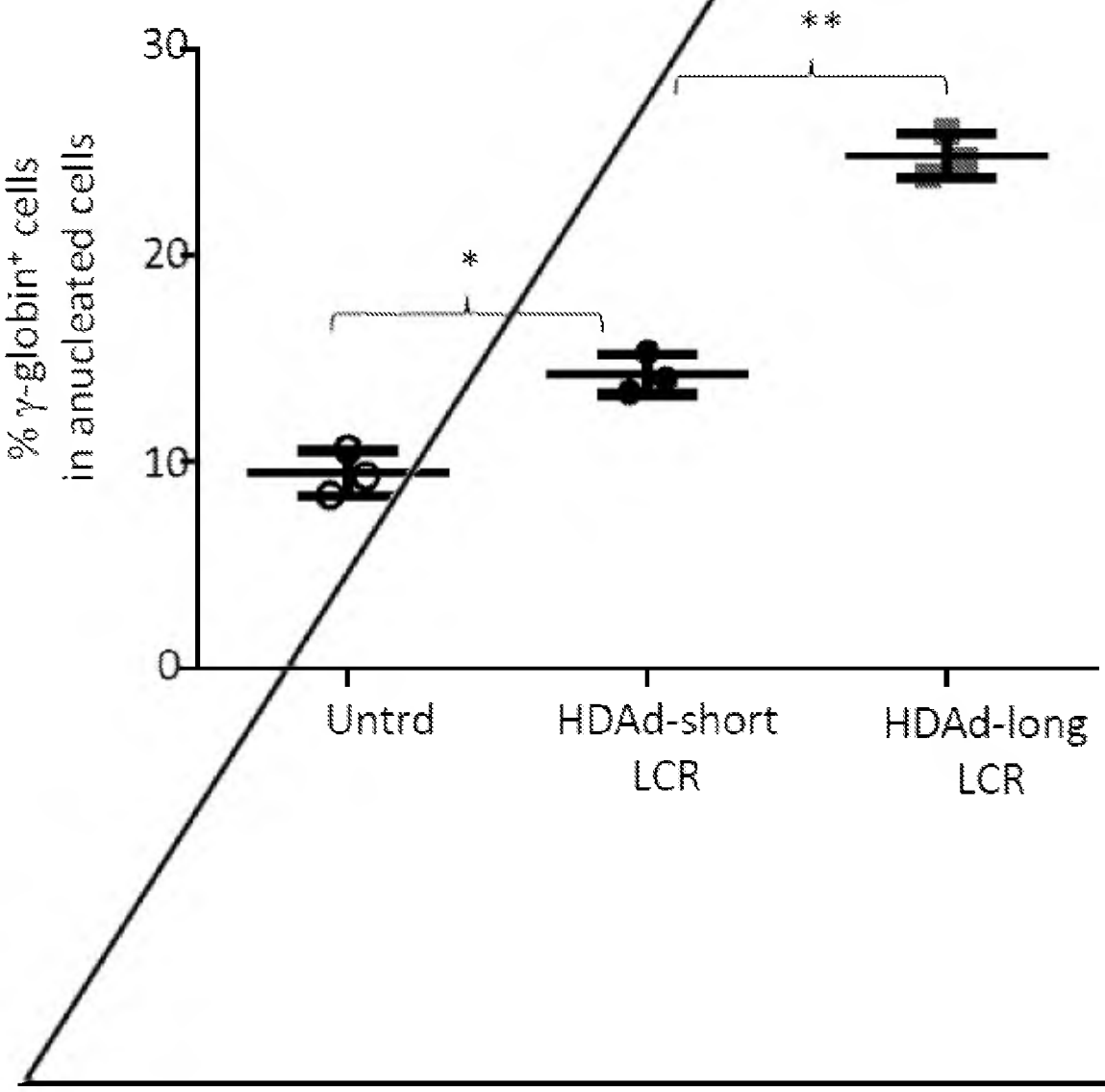


FIG. 31C

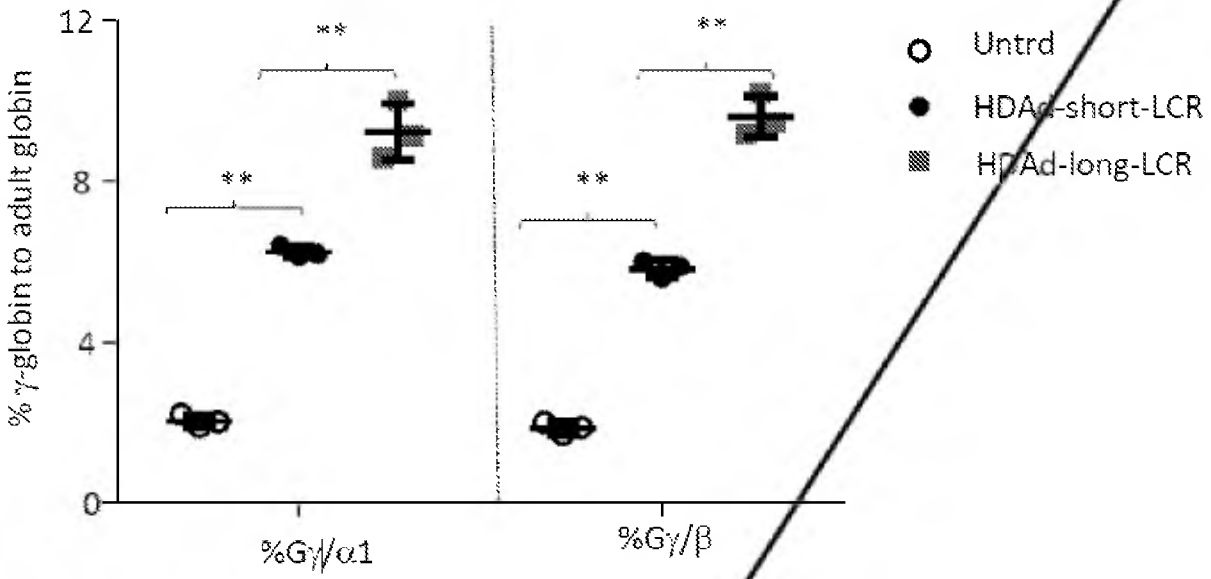


FIG. 31D

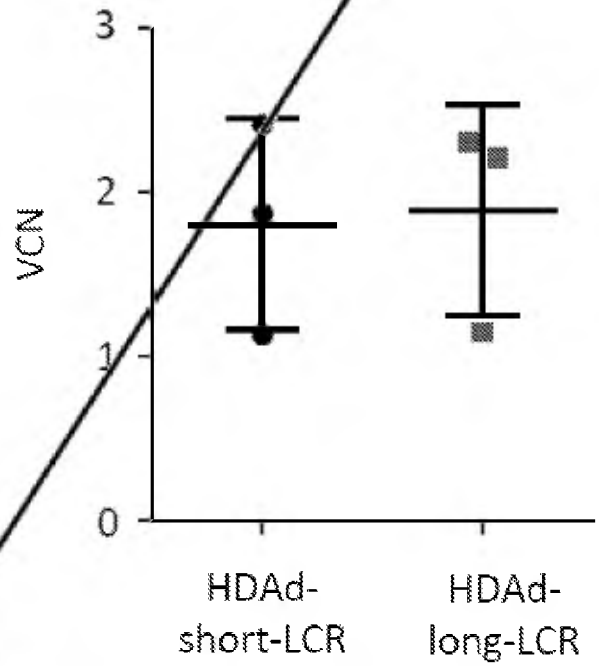


FIG. 32A

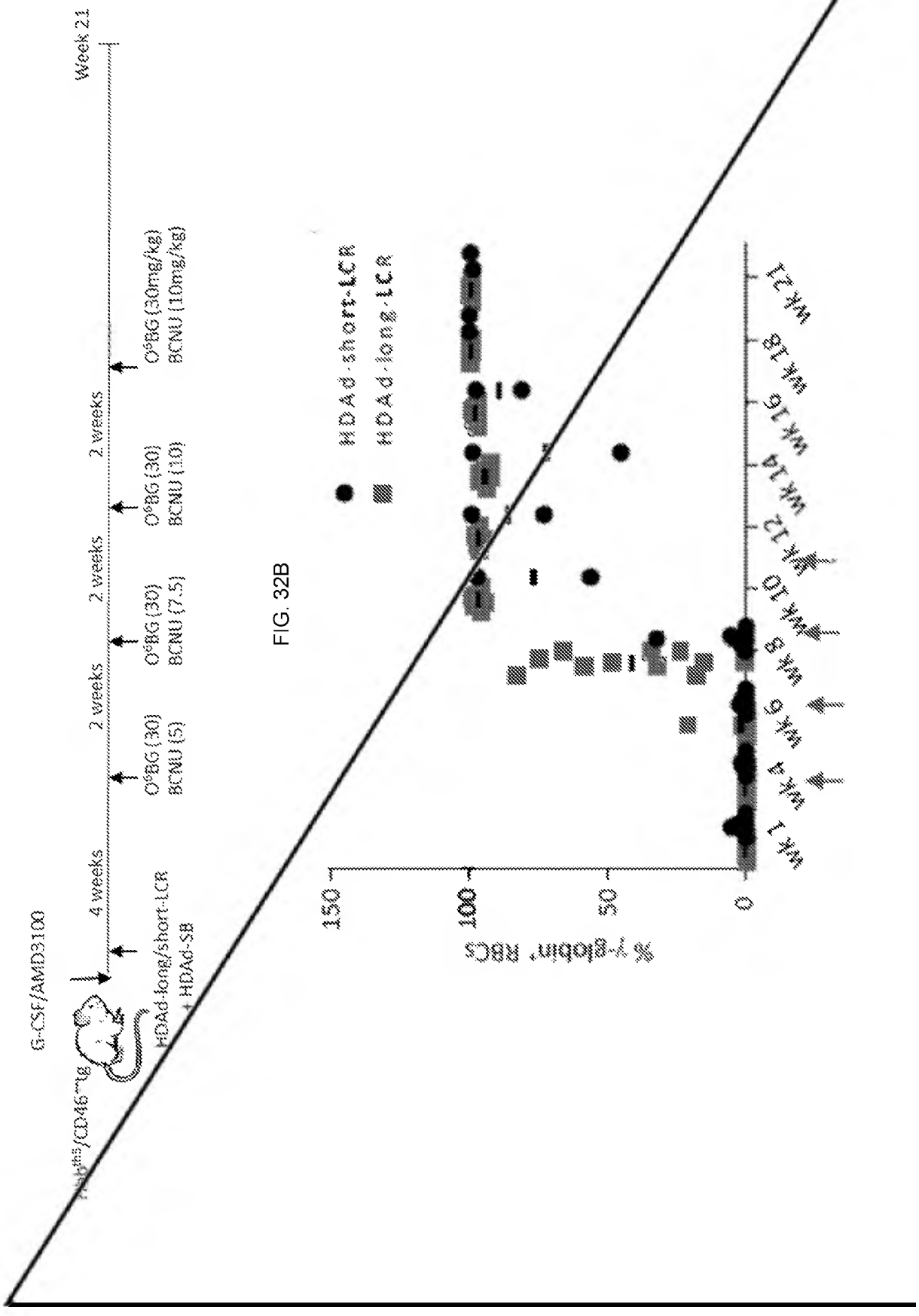


FIG. 32B

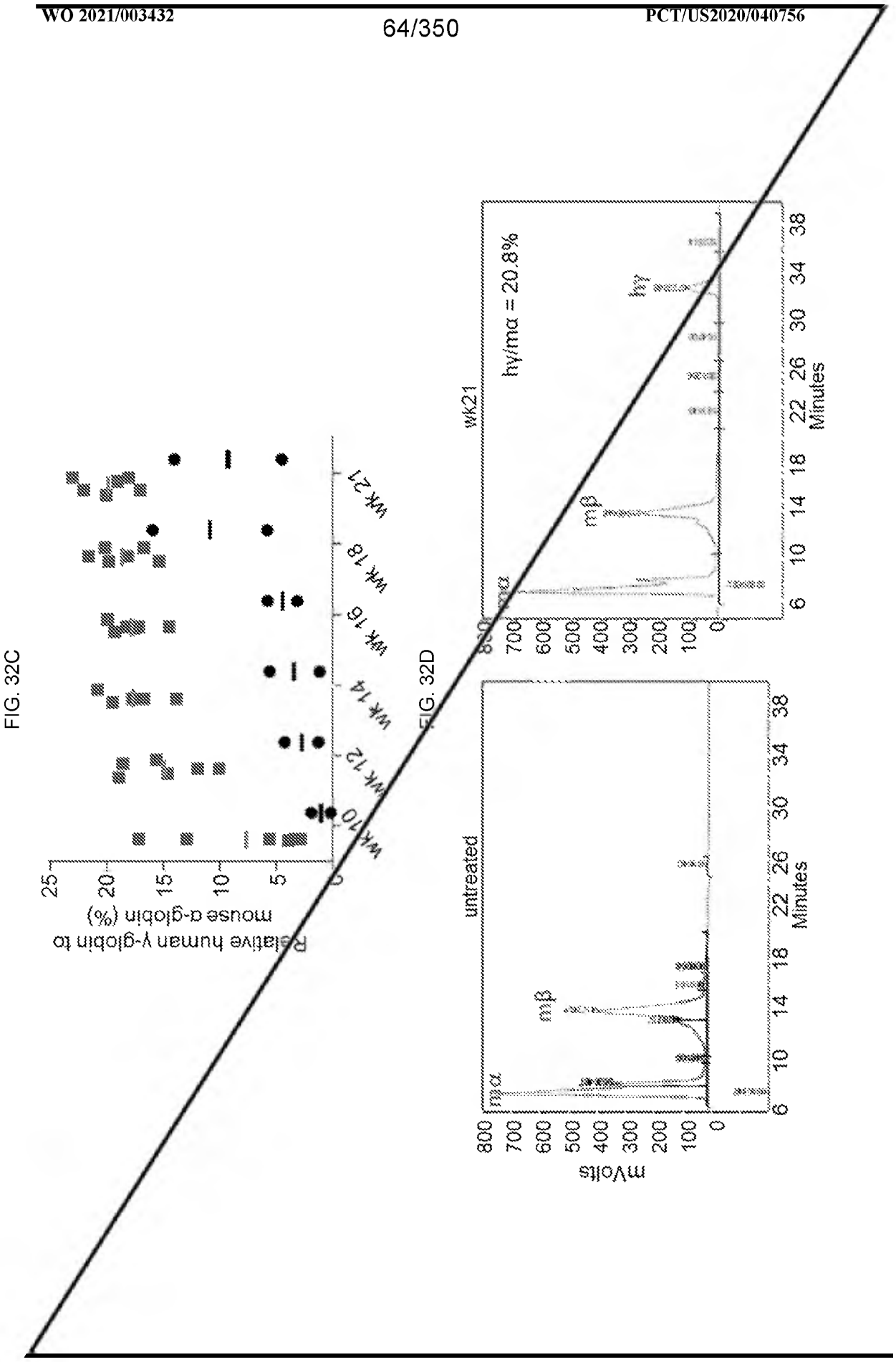

FIG. 32C
Relative human γ-globin to mouse α-globin (%)
25
20
15
10
5
0
wk 10
wk 12
wk 14
wk 16
wk 18
wk 21
FIG. 32D
untreated
mVolts
800
700
600
500
400
300
200
100
0
mα
mβ
6
10
14
18
22
26
30
34
38
Minutes
wk21
hγ/mα = 20.8%
hγ

FIG. 32E

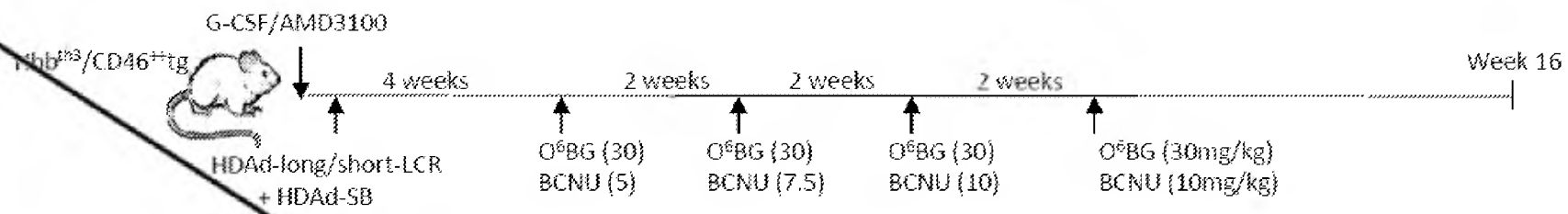
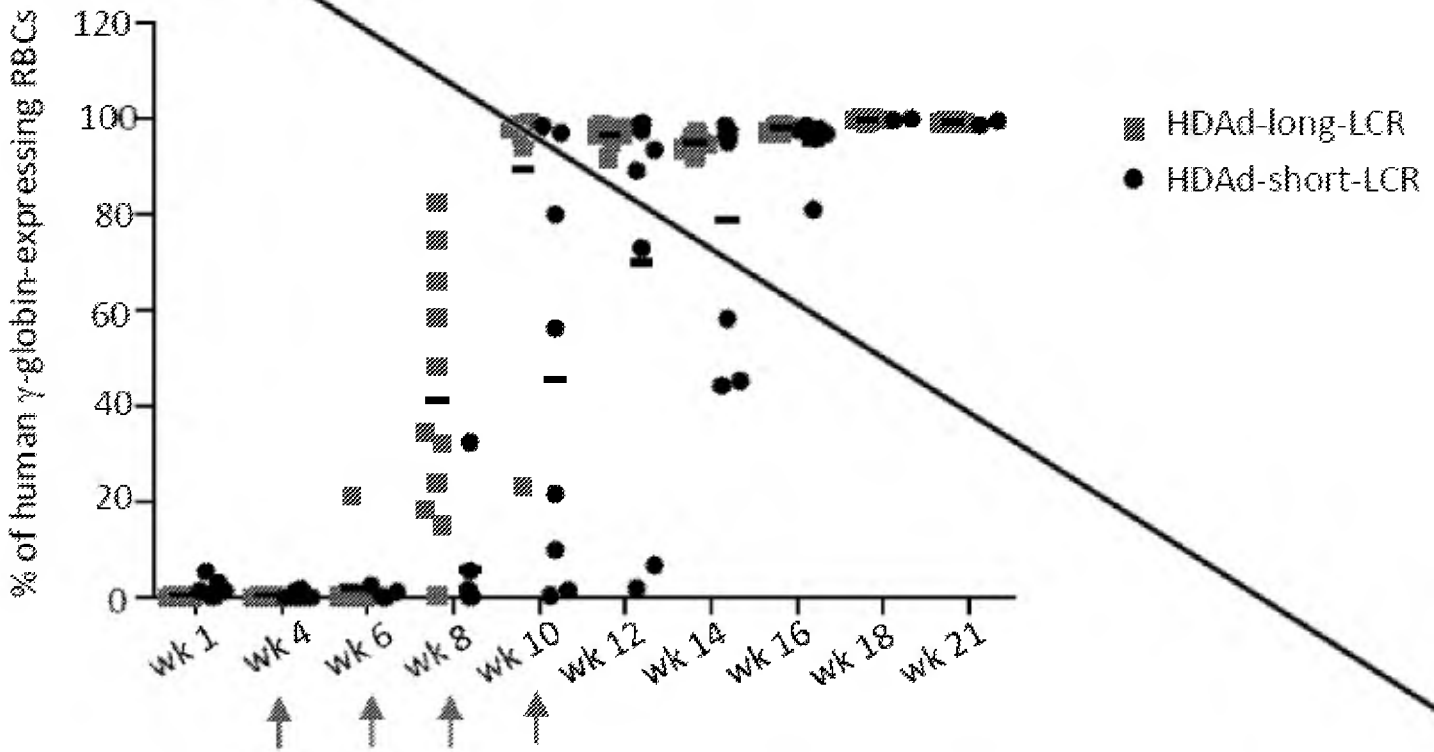


FIG. 32F



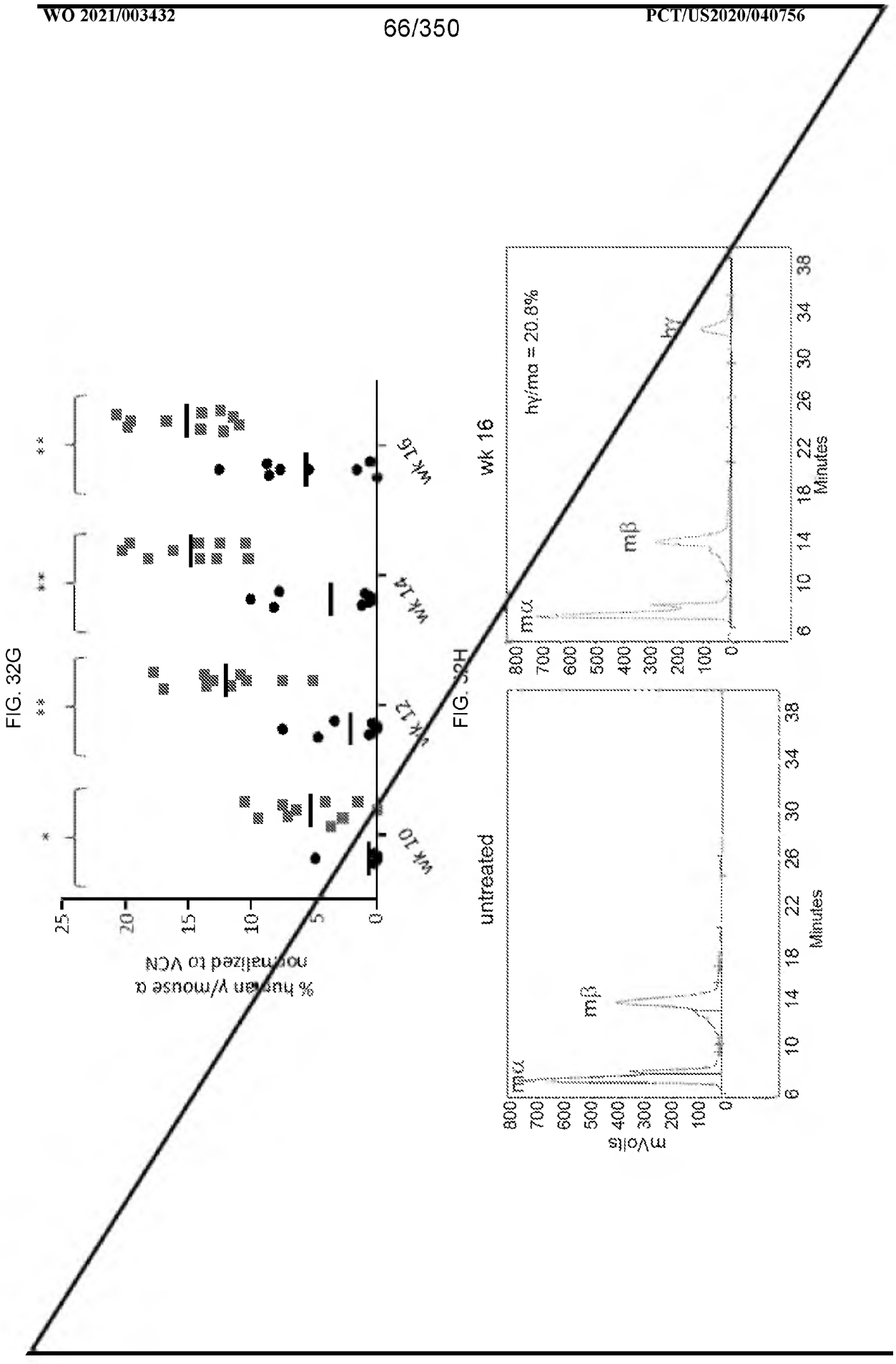

FIG. 32G
% human γ/mouse α normalized to VCN
25
20
15
10
5
0
*
**
**
**
wk 10
wk 12
wk 14
wk 16
FIG. 32H
untreated
wk 16
mVolts
mα
mβ
hγ
hγ/mα = 20.8%
Minutes
6
10
14
18
22
26
30
34
38
0
100
200
300
400
500
600
700
800

FIG. 33A

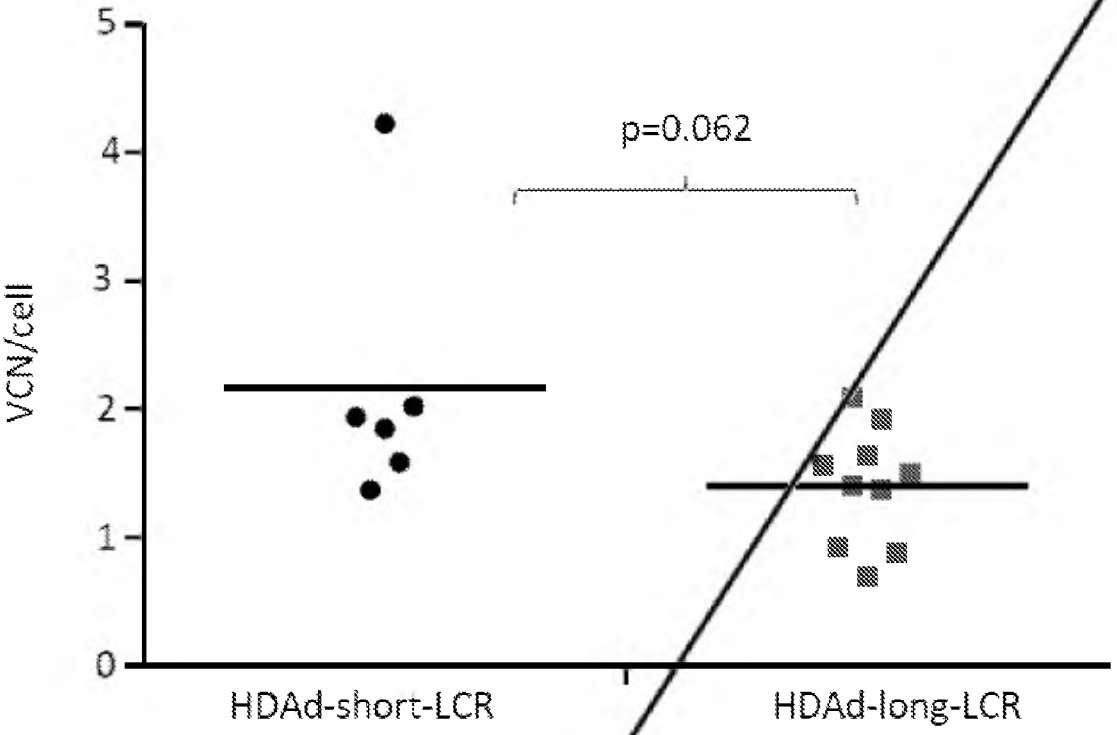


FIG. 33B

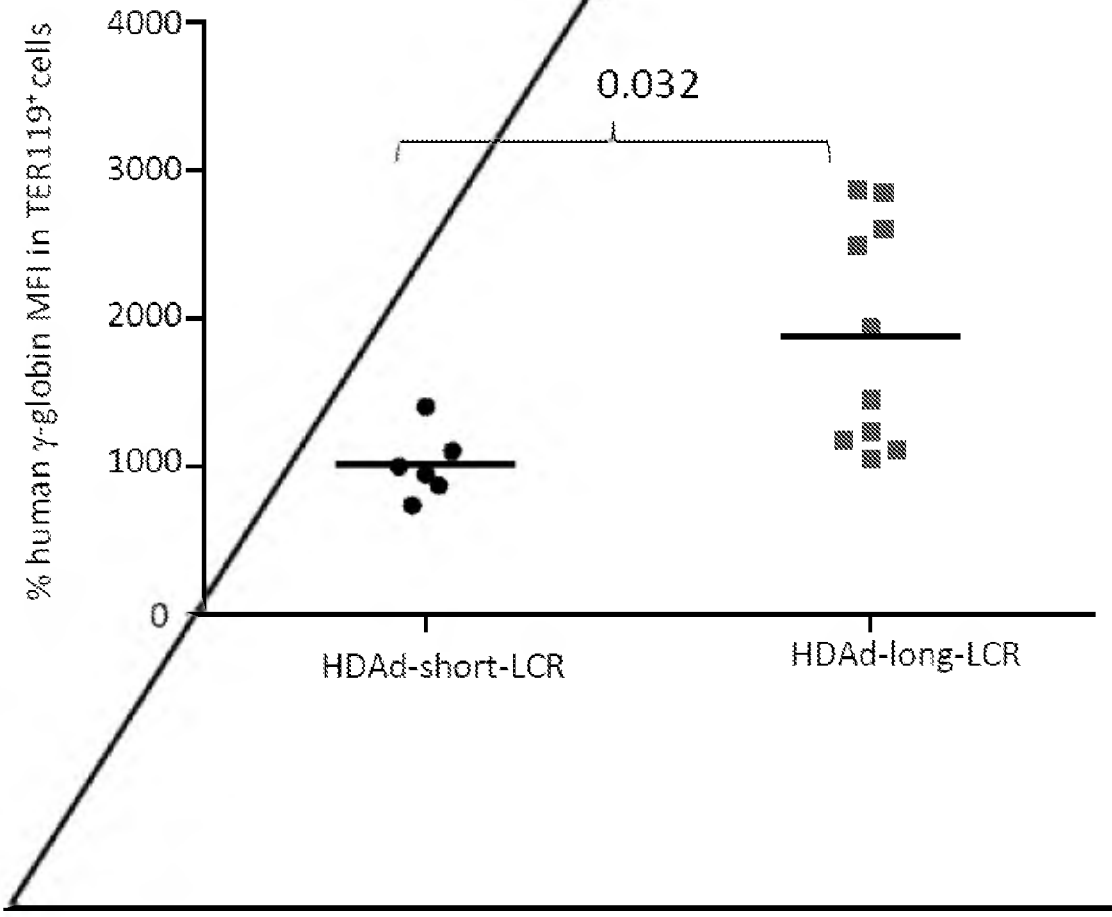


FIG. 34

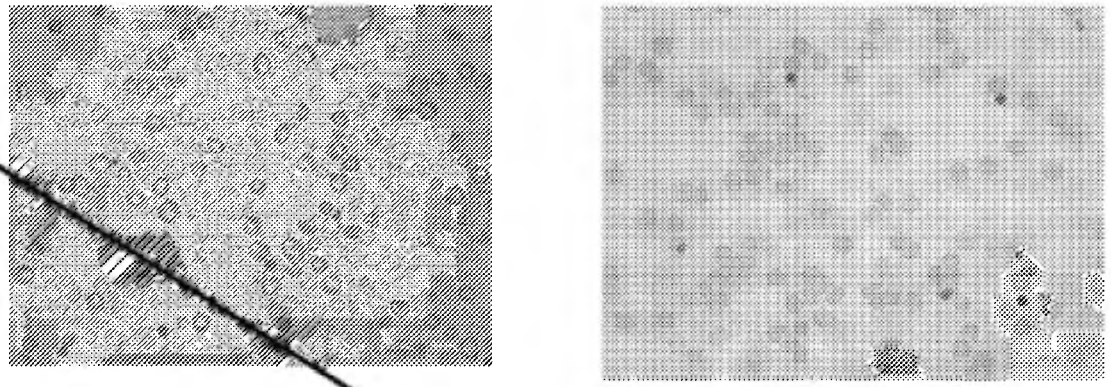

Before treatment
Week 10 after treatment

FIG. 35

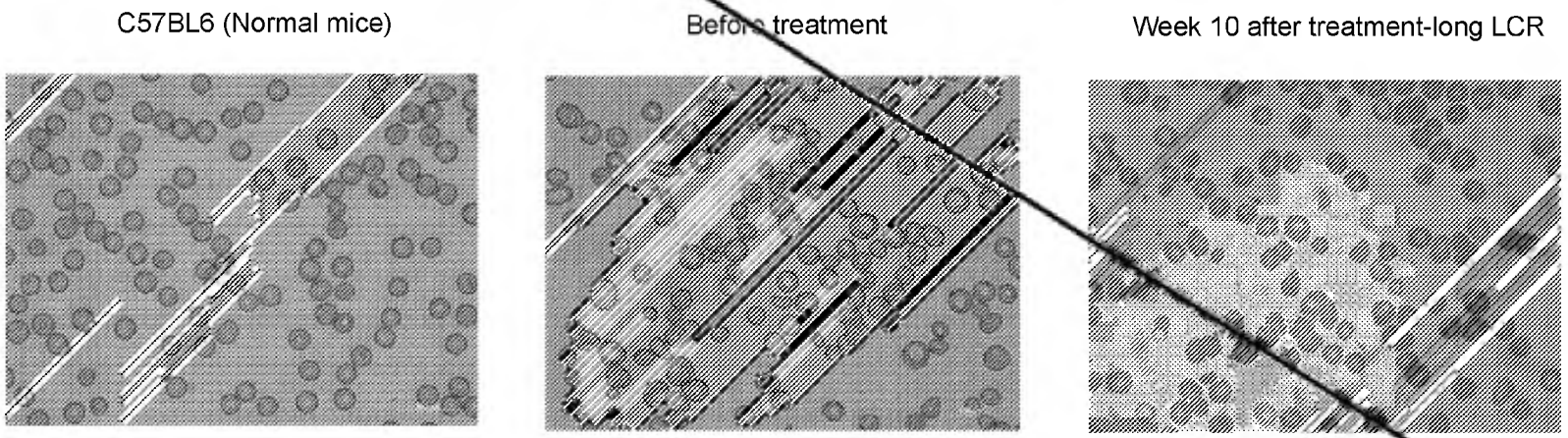

C57BL6 (Normal mice)
Before treatment
Week 10 after treatment-long LCR

FIG. 36A

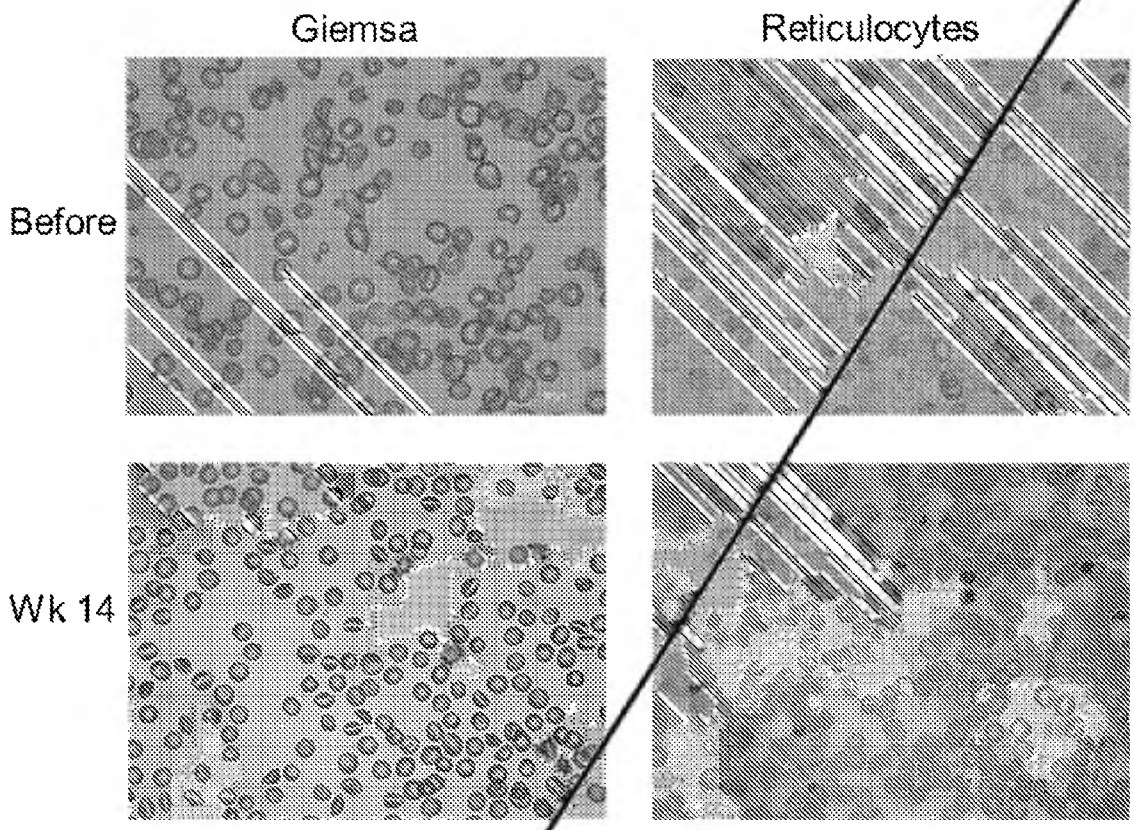


FIG. 36B

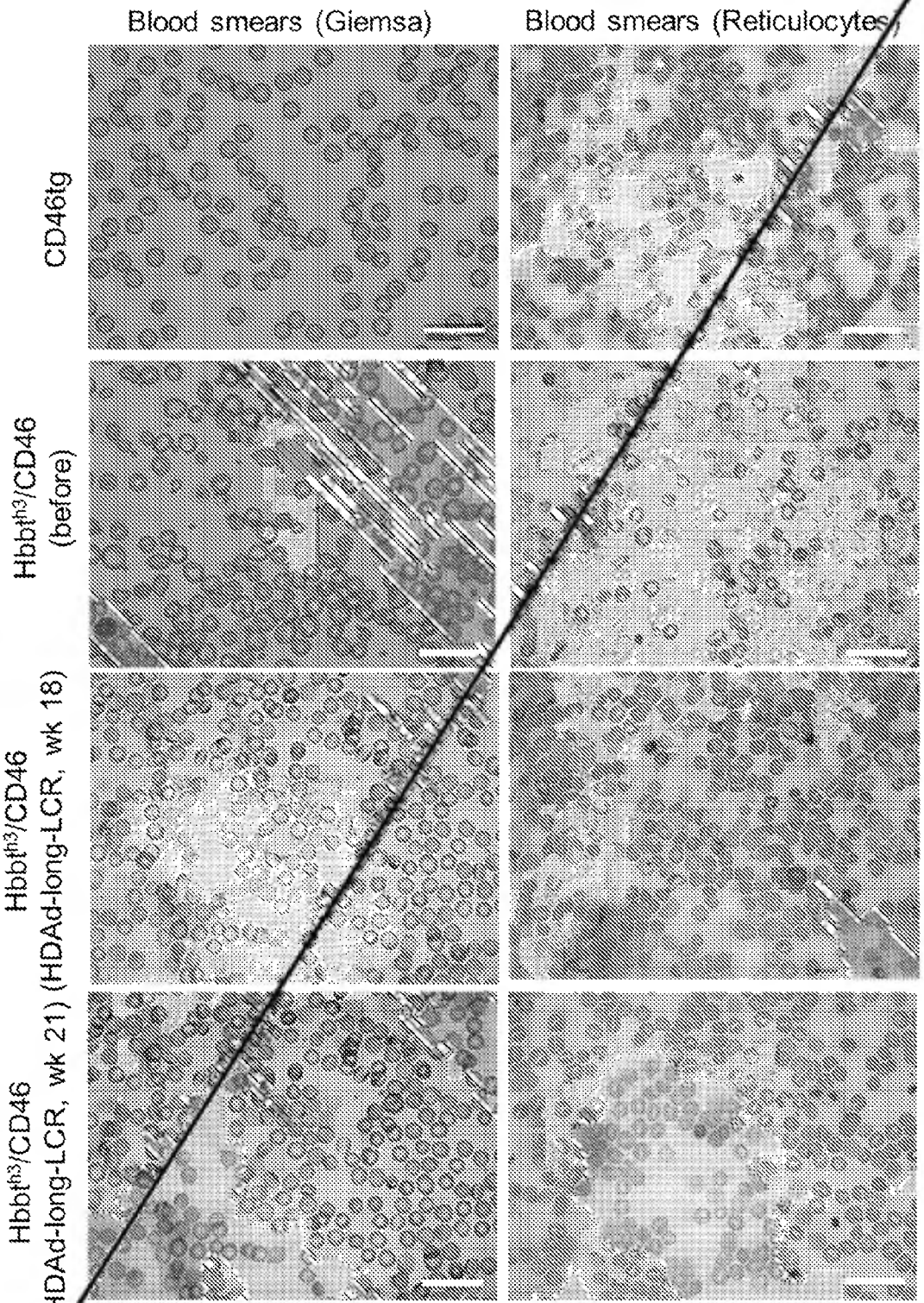

Blood smears (Giemsa)
Blood smears (Reticulocytes)
CD46tg
Hbbth3/CD46 (before)
Hbbth3/CD46 (HDAd-long-LCR, wk 18)
Hbbth3/CD46 (HDAd-long-LCR, wk 21)

FIG. 36C

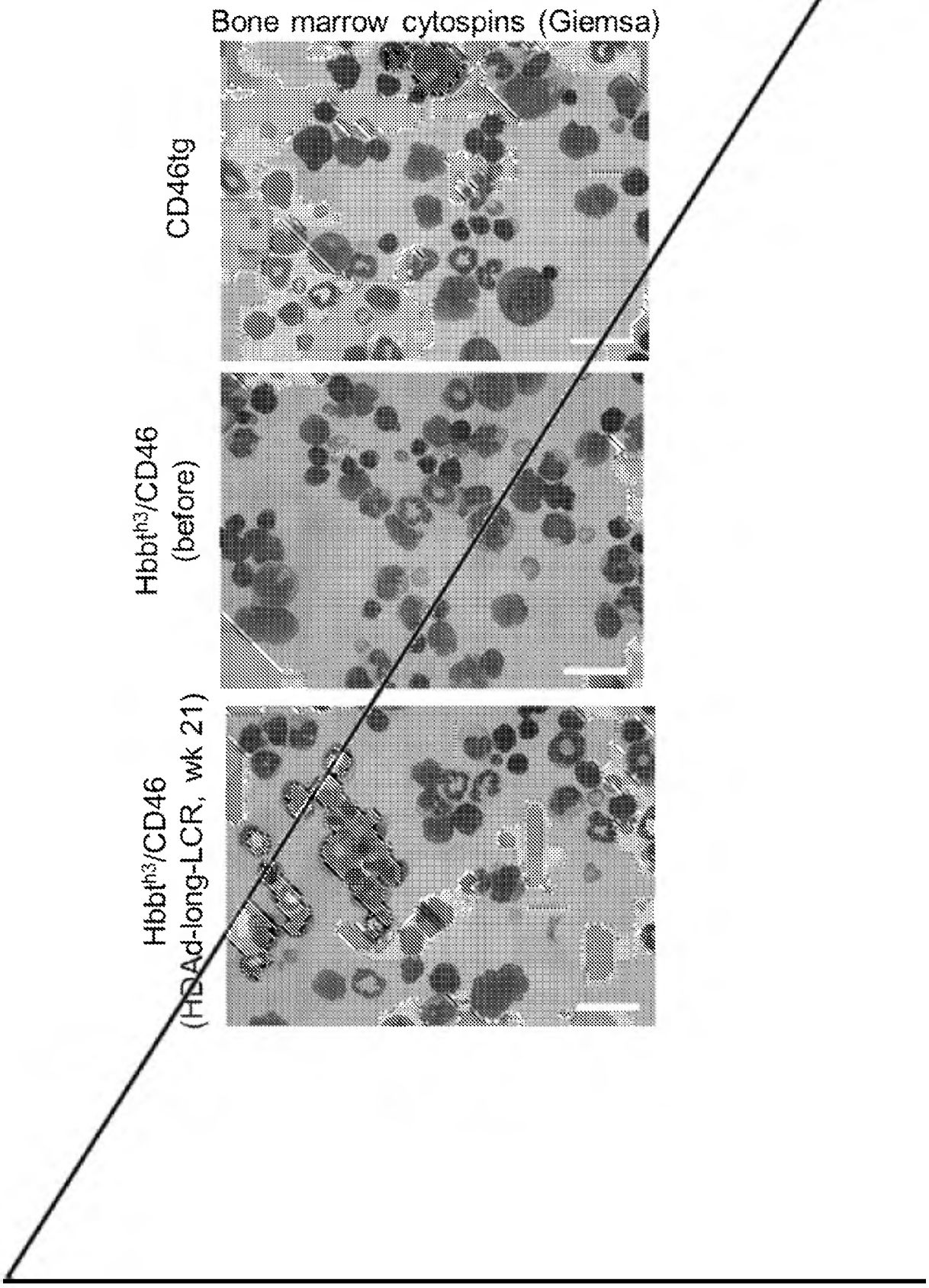

Bone marrow cytospins (Giemsa)
CD46tg
Hbbth3/CD46 (before)
Hbbth3/CD46 (HBAd-long-LCR, wk 21)

FIG. 37A

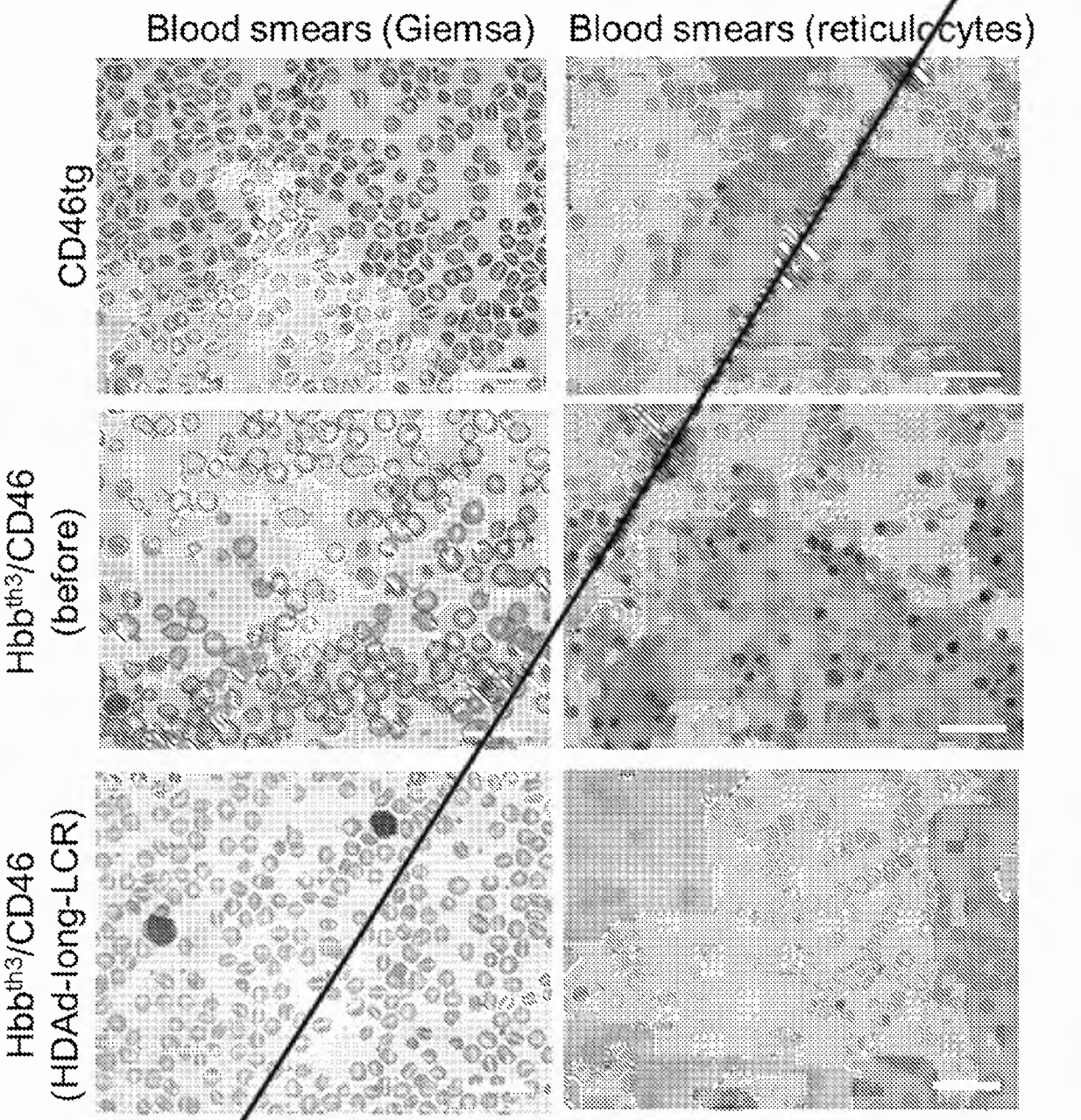

Blood smears (Giemsa)
Blood smears (reticulocytes)
CD46tg
Hbb^{th3}/CD46 (before)
Hbb^{th3}/CD46 (HDAd-long-LCR)

FIG. 37B

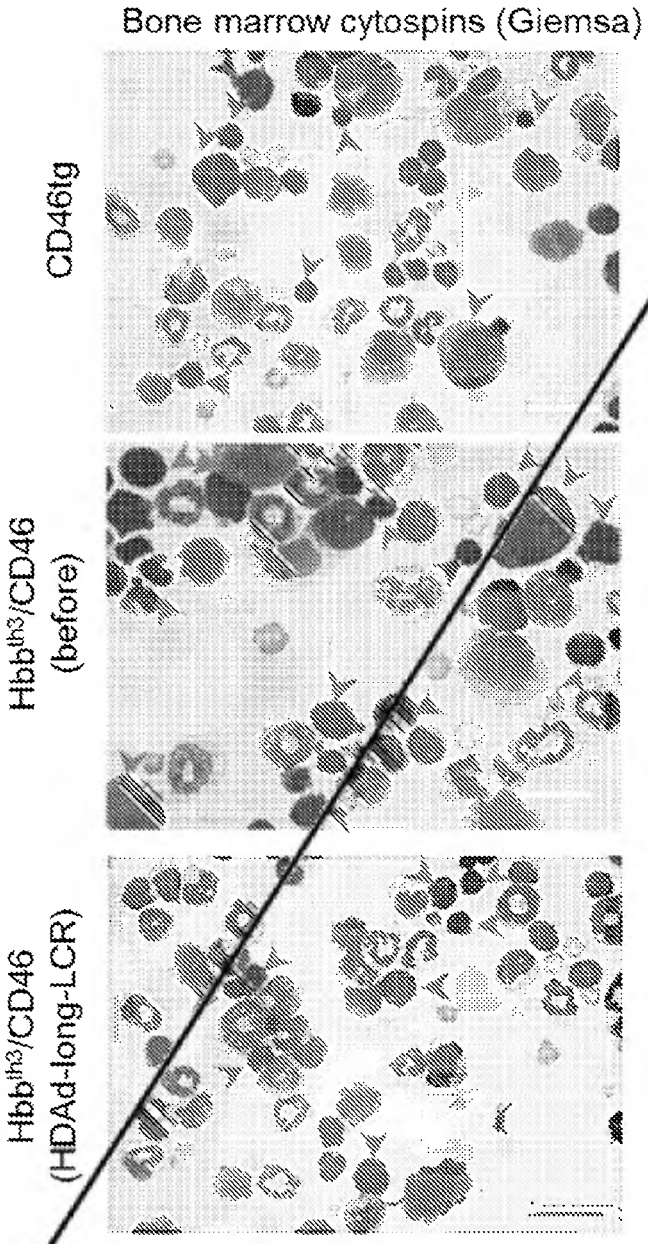

Bone marrow cytospins (Giemsa)
CD46tg
Hbb^th3/CD46 (before)
Hbb^th3/CD46 (HDAd-long-LCR)

FIG. 38

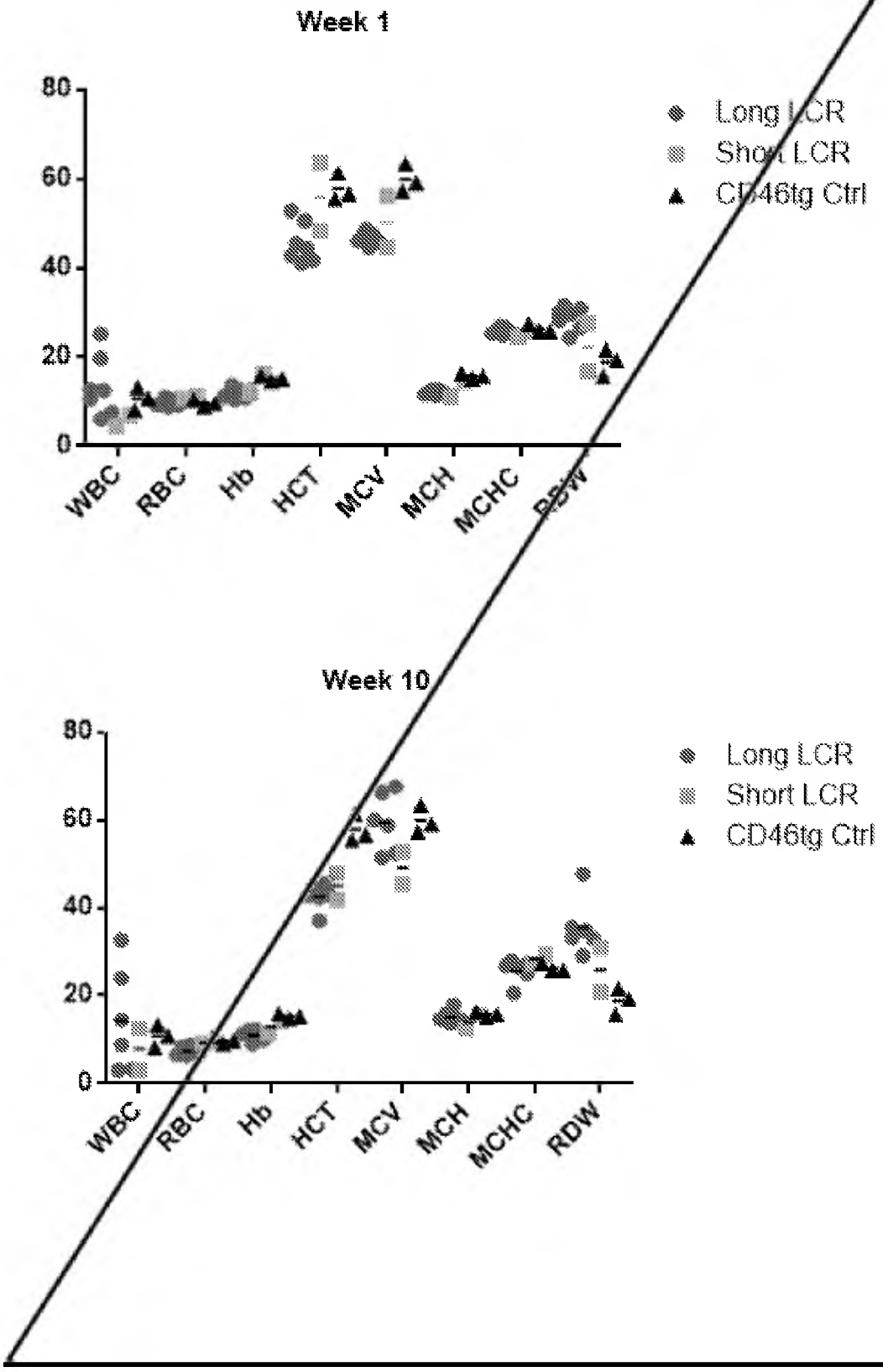

Week 1
80
60
40
20
0
WBC
RBC
Hb
HCT
MCV
MCH
MCHC
RDW
Long LCR
Short LCR
CD46tg Ctrl
Week 10
80
60
40
20
0
WBC
RBC
Hb
HCT
MCV
MCH
MCHC
RDW
Long LCR
Short LCR
CD46tg Ctrl

FIG. 39A

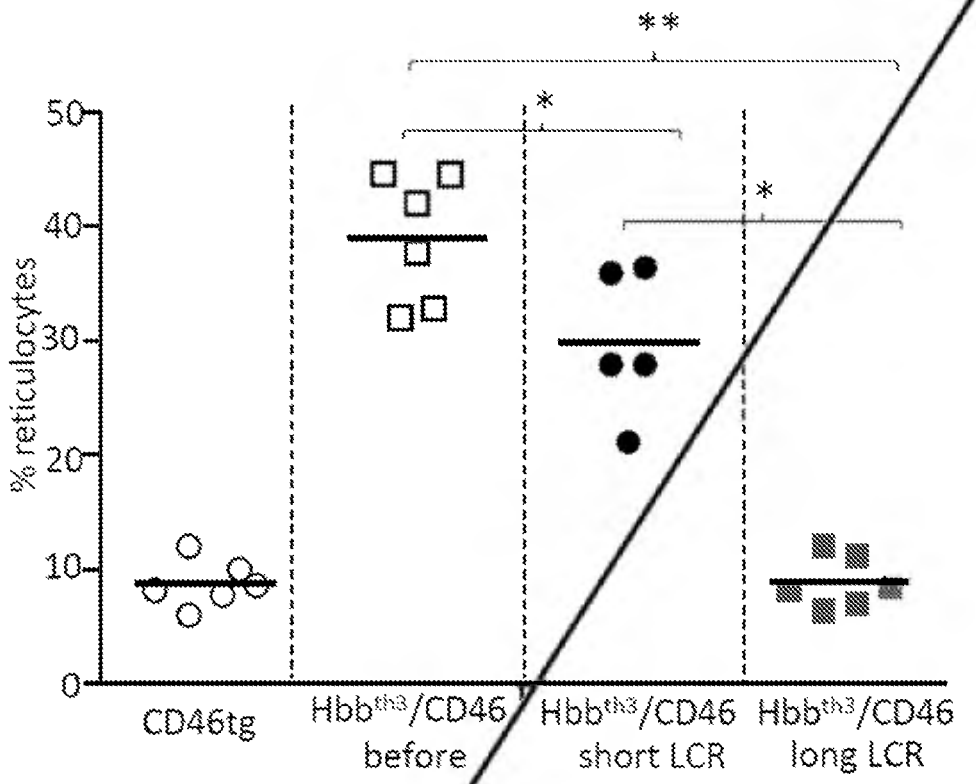

**
*
*
50
40
30
20
10
0
% reticulocytes
CD46tg
Hbb^th3/CD46 before
Hbb^th3/CD46 short LCR
Hbb^th3/CD46 long LCR

FIG. 39B

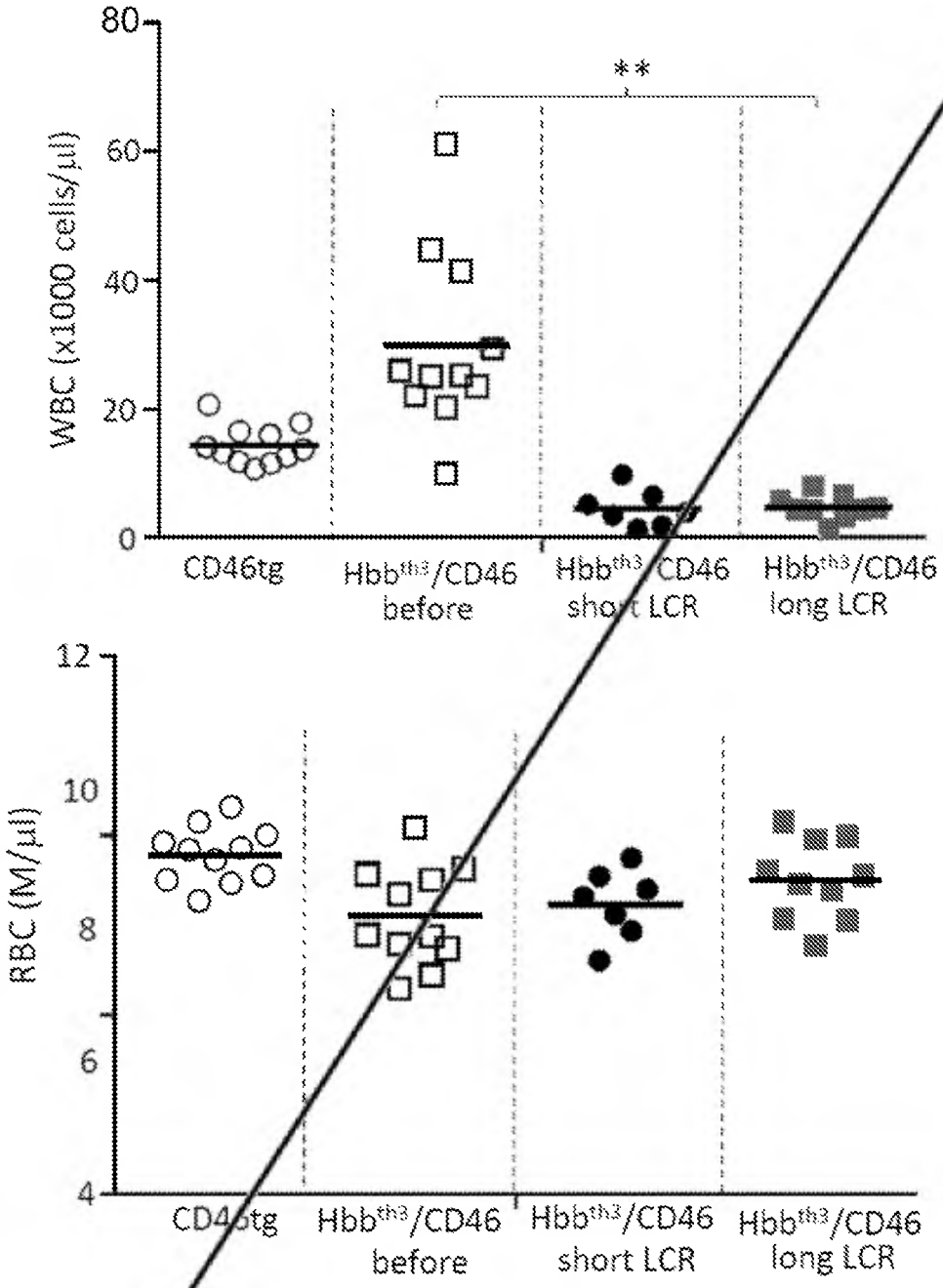

80
60
40
20
0
WBC (x1000 cells/μl)
**
CD46tg
Hbb^th3/CD46 before
Hbb^th3/CD46 short LCR
Hbb^th3/CD46 long LCR
12
10
8
6
4
RBC (M/μl)
CD46tg
Hbb^th3/CD46 before
Hbb^th3/CD46 short LCR
Hbb^th3/CD46 long LCR

FIG. 39B cont'd

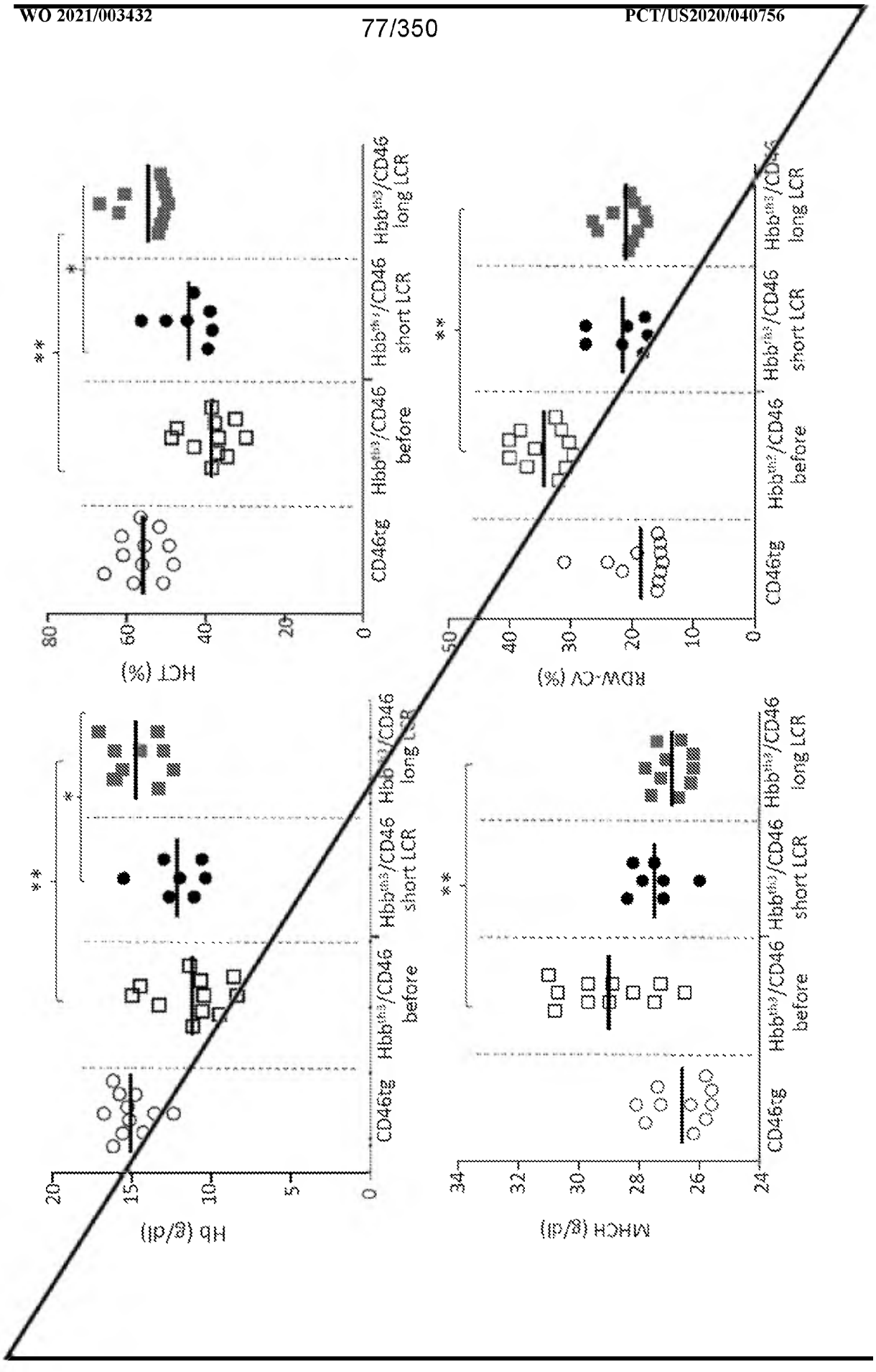
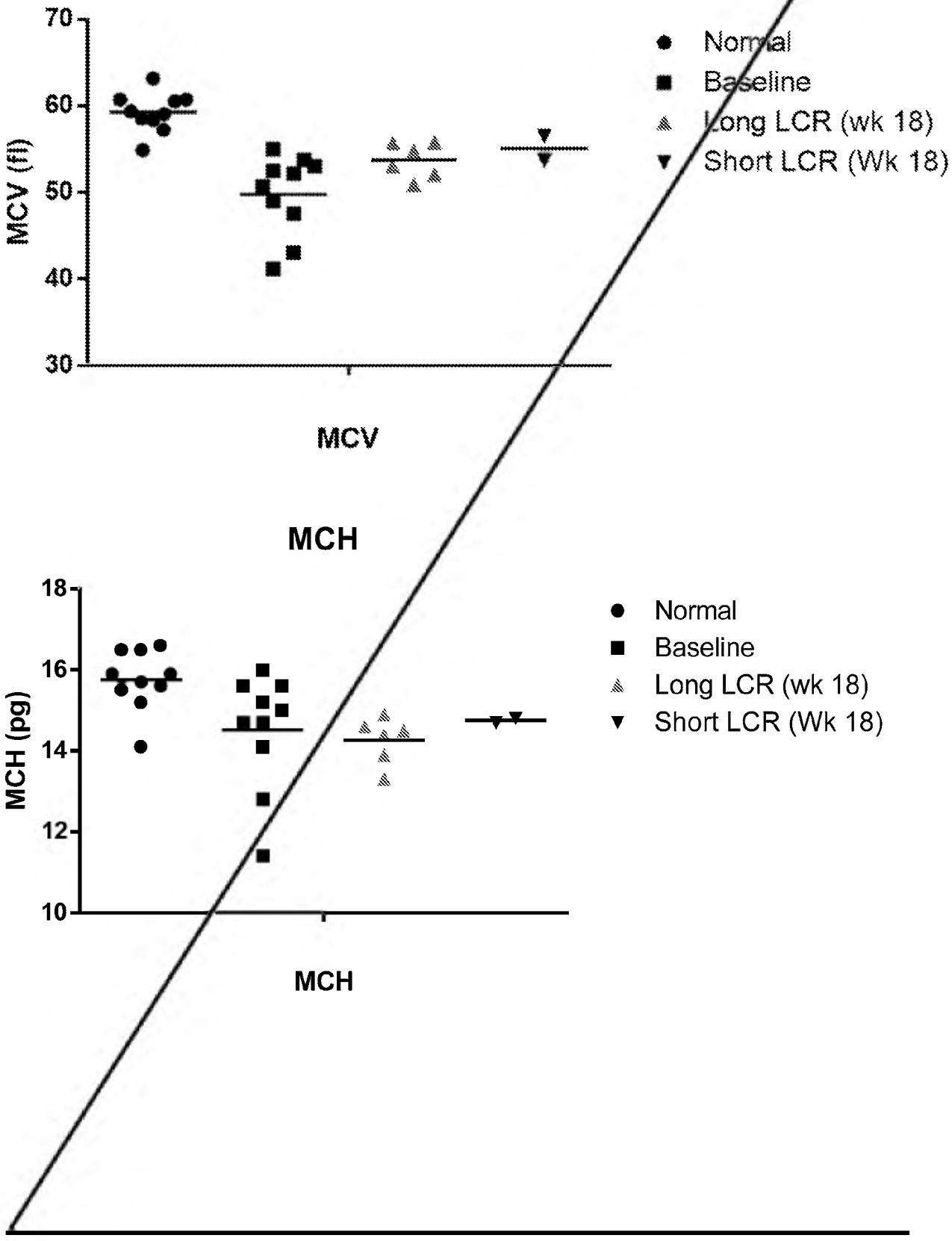


FIG. 39B cont'd



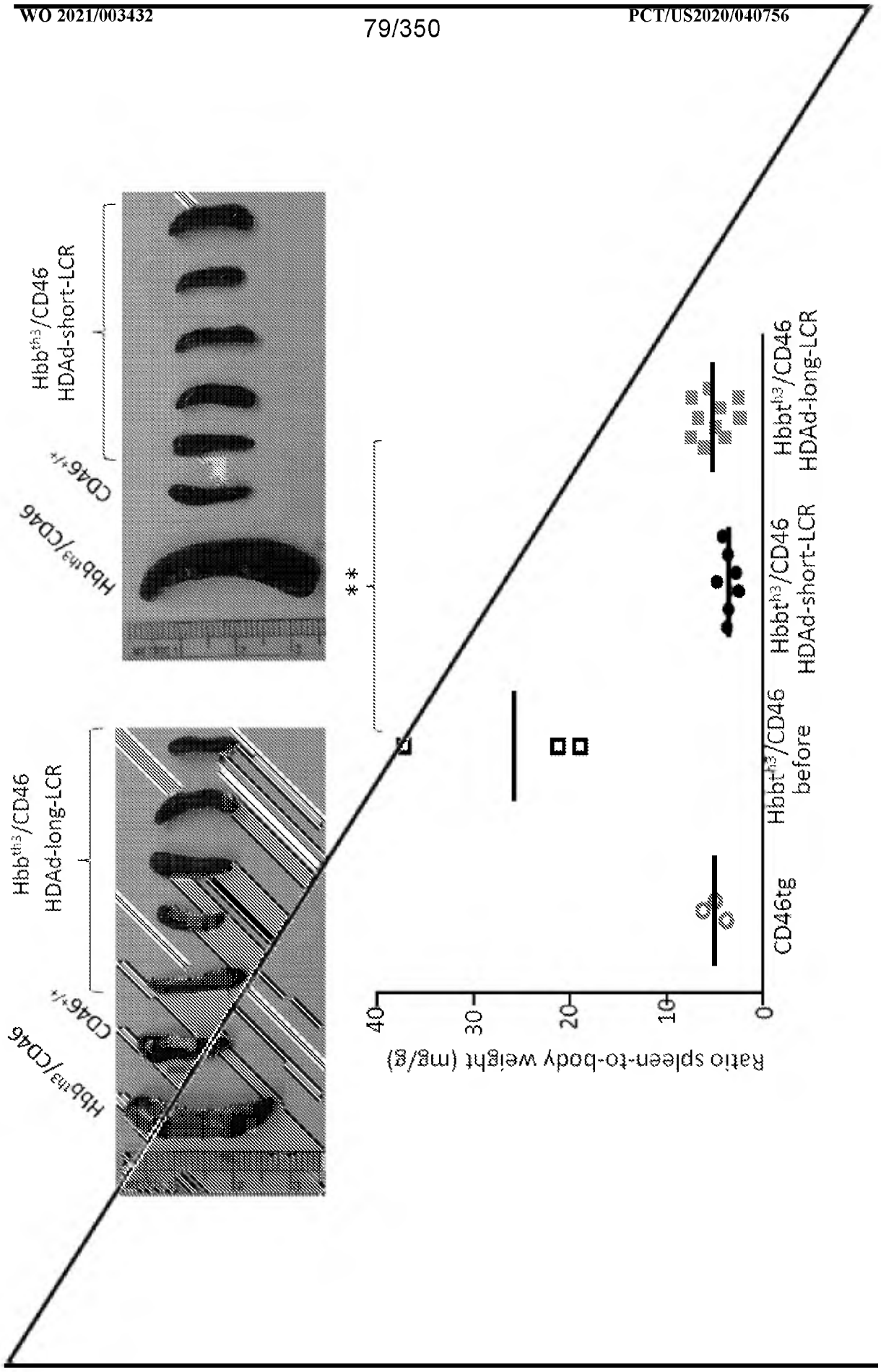

Hbb^th3/CD46
HDAd-long-LCR
CD46+/+
Hbb^th3/CD46
Hbb^th3/CD46
HDAd-short-LCR
CD46+/+
Hbb^th3/CD46
**
Ratio spleen-to-body weight (mg/g)
40
30
20
10
0
CD46tg
Hbbt^h3/CD46
before
Hbbt^h3/CD46
HDAd-short-LCR
Hbbt^h3/CD46
HDAd-long-LCR

FIG. 40A

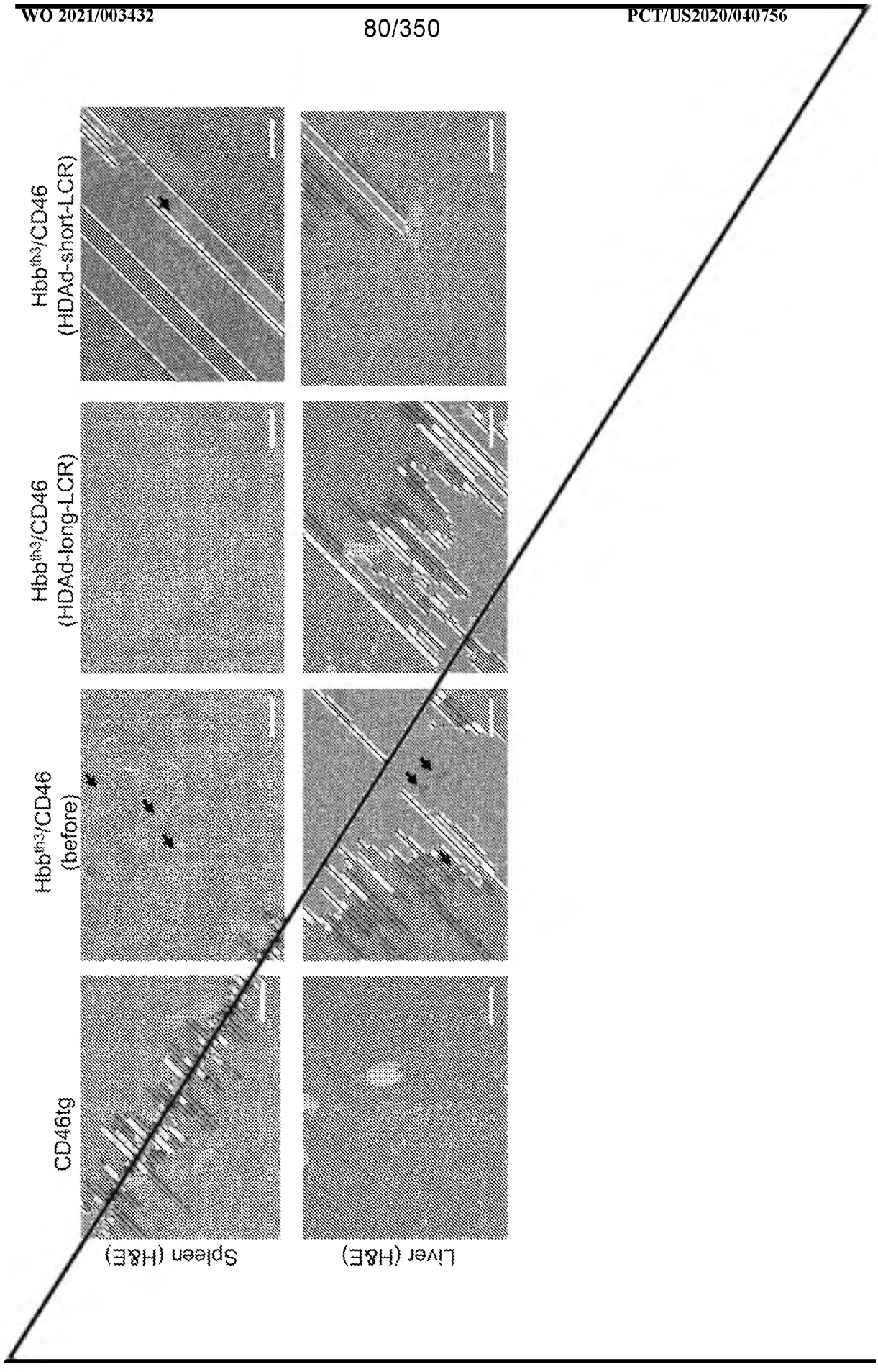

CD46tg
Hbb^{th3}/CD46 (before)
Hbb^{th3}/CD46 (HDAd-long-LCR)
Hbb^{th3}/CD46 (HDAd-short-LCR)
Spleen (H&E)
Liver (H&E)

FIG. 40B

FIG. 41

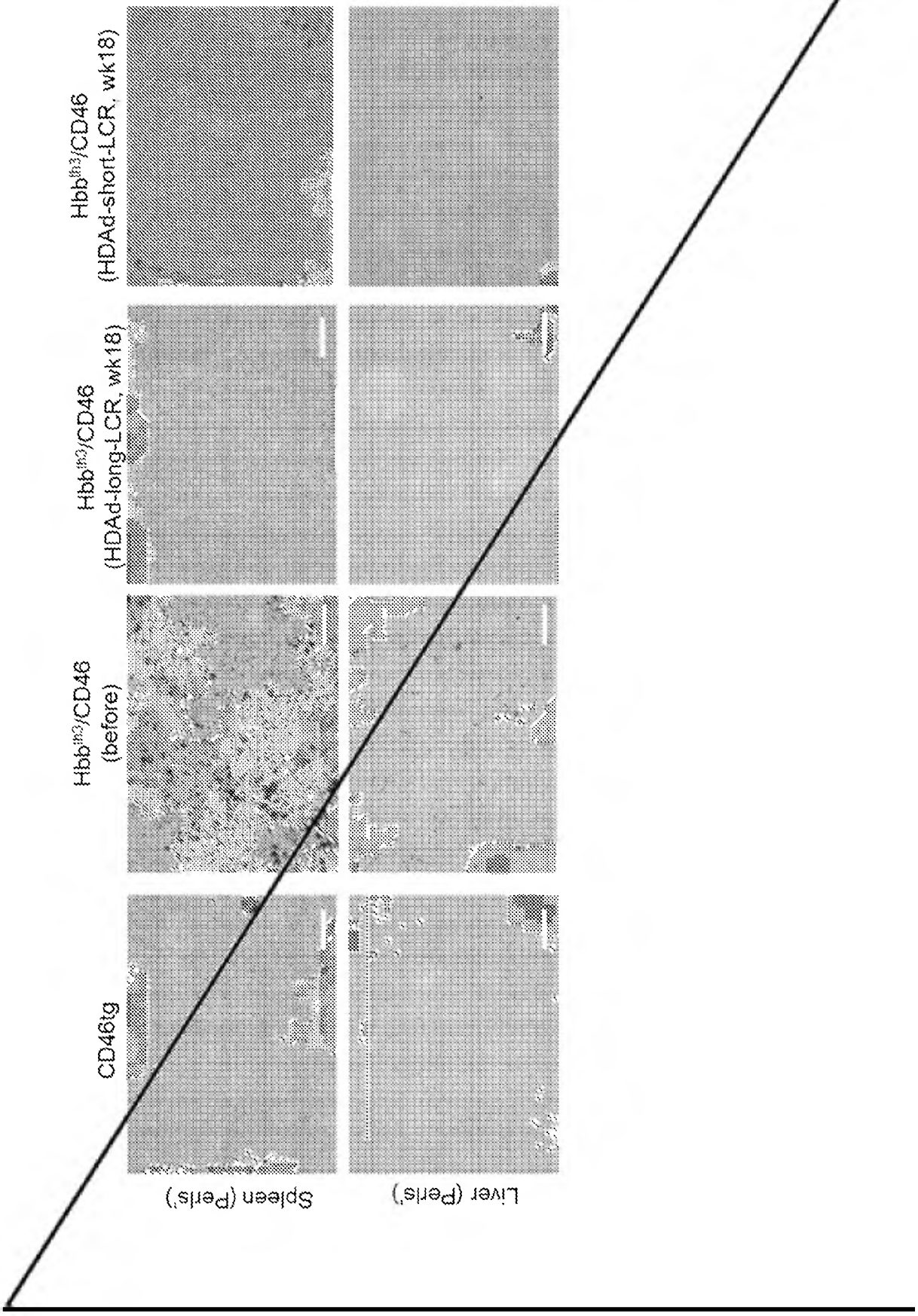

Liver (Perls')
Spleen (Perls')
CD46tg
Hbb^th3/CD46 (before)
Hbb^th3/CD46 (HDAd-long-LCR, wk18)
Hbb^th3/CD46 (HDAd-short-LCR, wk18)

FIG. 42A

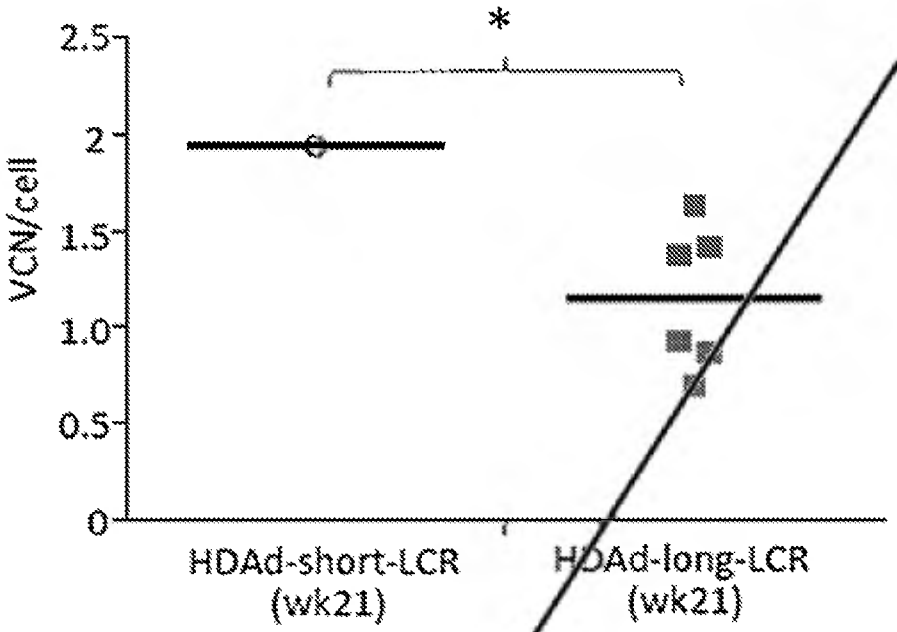


FIG. 42B

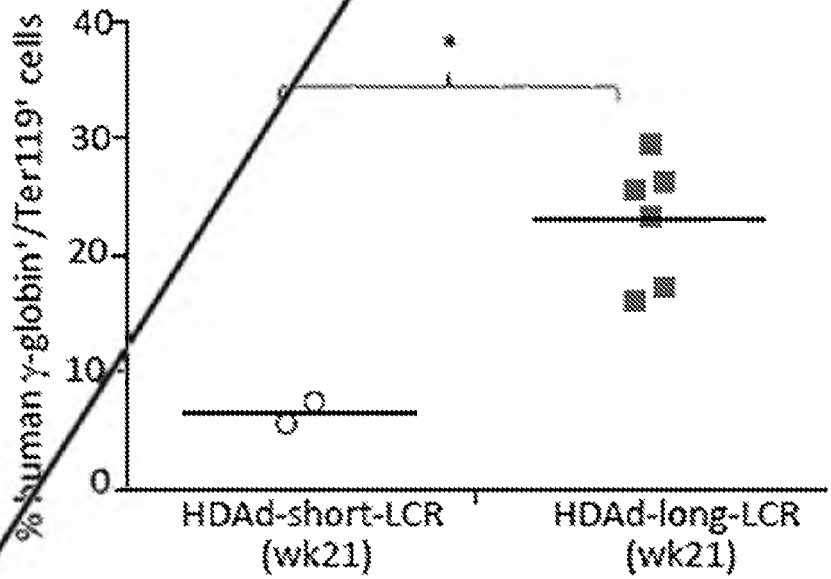
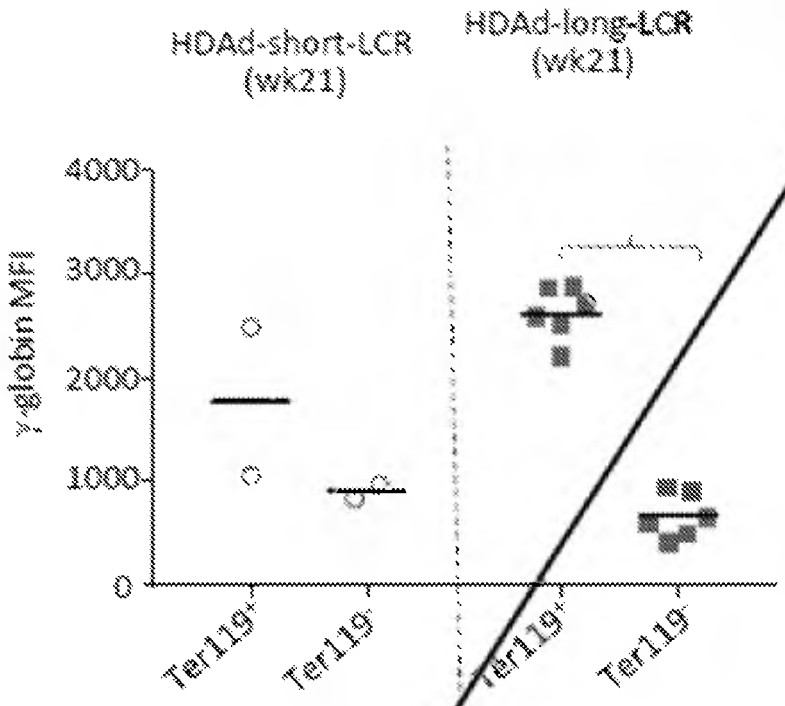


FIG. 42C


HDAd-short-LCR
(wk21)
HDAd-long-LCR
(wk21)
γ-globin MFI
4000
3000
2000
1000
0
Ter119+
Ter119-
Ter119+
Ter119-

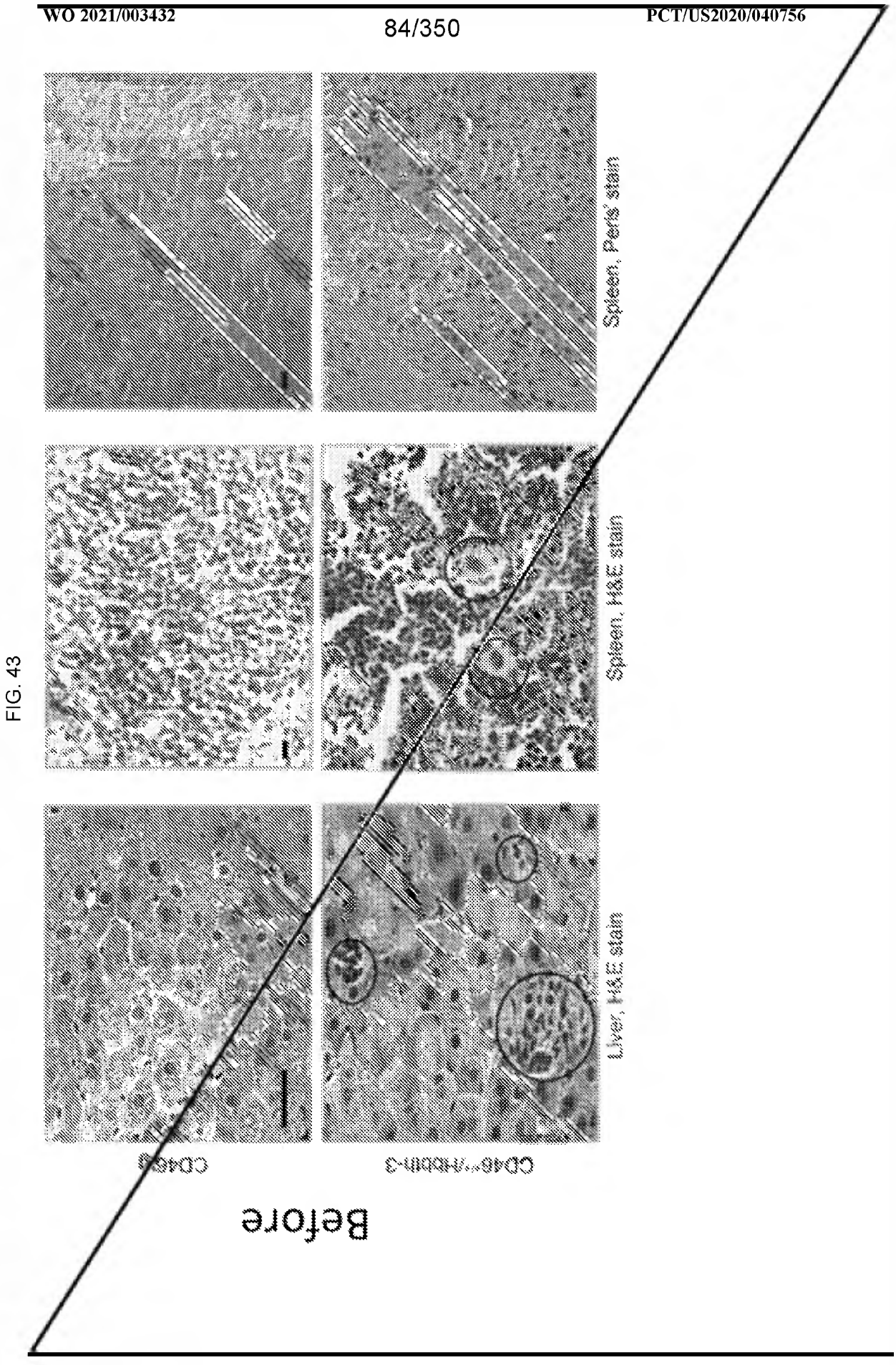

Before
CD45-/-Rabbit-3
CD45-/-
Liver, H&E stain
Spleen, H&E stain
Spleen, Perls' stain

FIG. 43

FIG. 44A

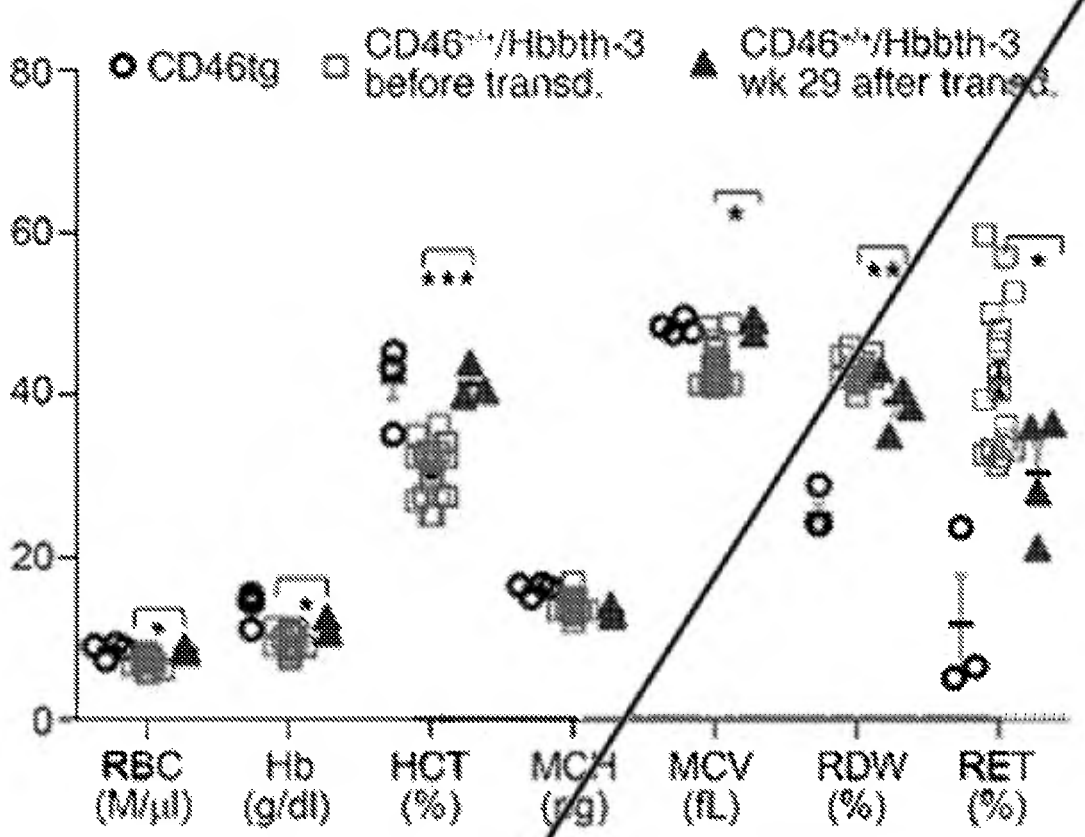


FIG. 44B

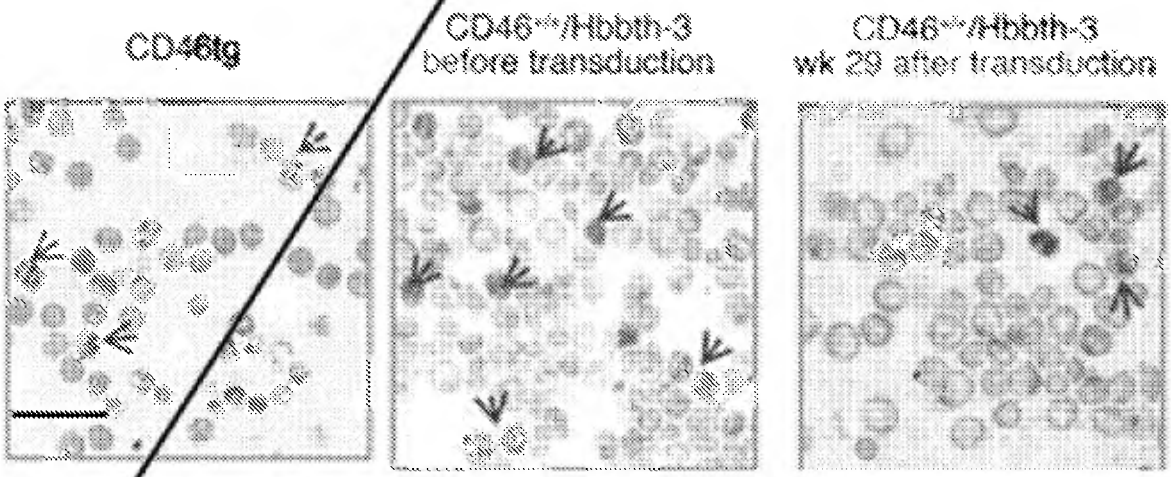


FIG. 44C

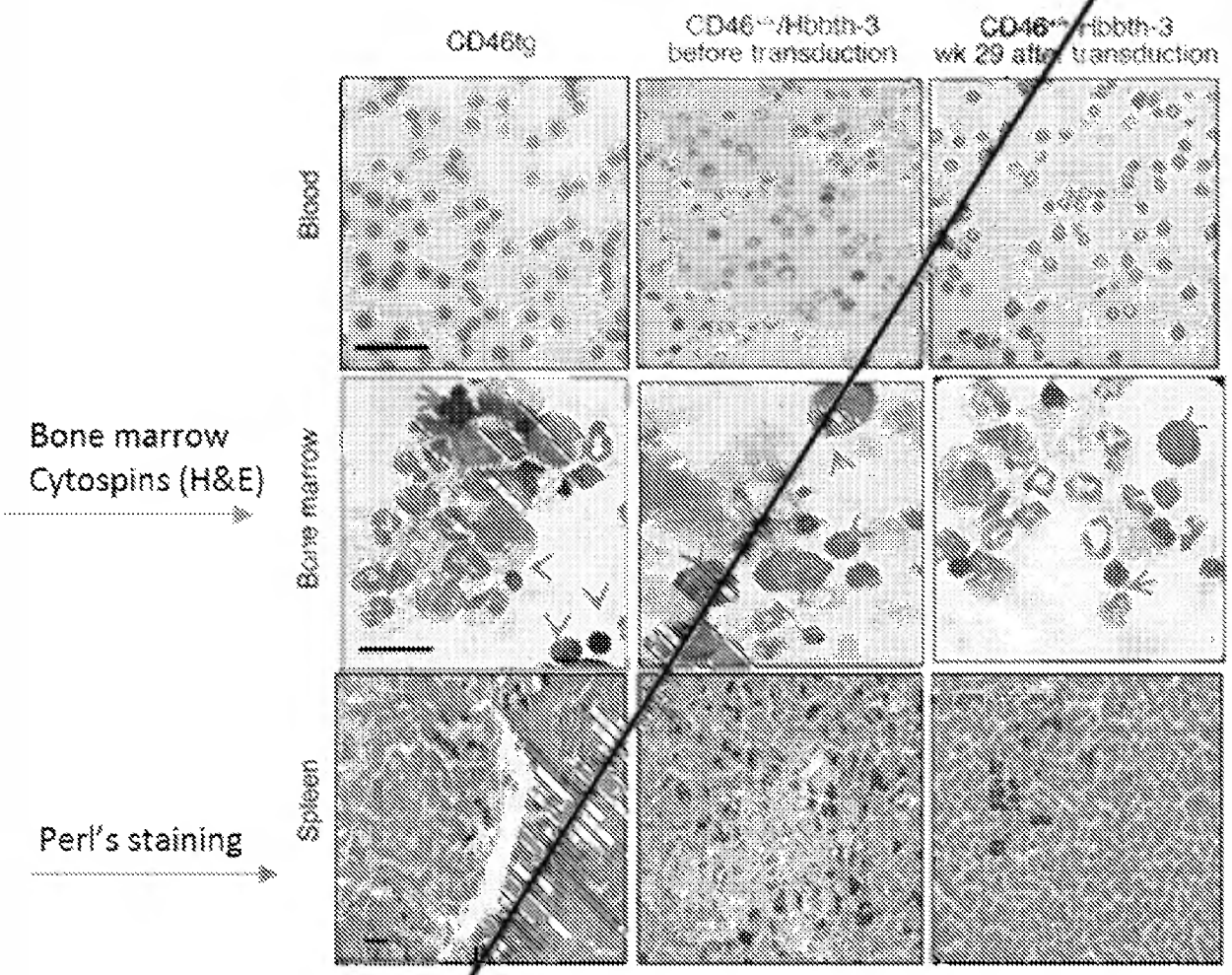

CD46tg
CD46-/-hbbth-3
before transduction
CD46-/-hbbth-3
wk 29 after transduction
Blood
Bone marrow
Spleen
Bone marrow
Cytospins (H&E)
Perl's staining

FIG. 44D

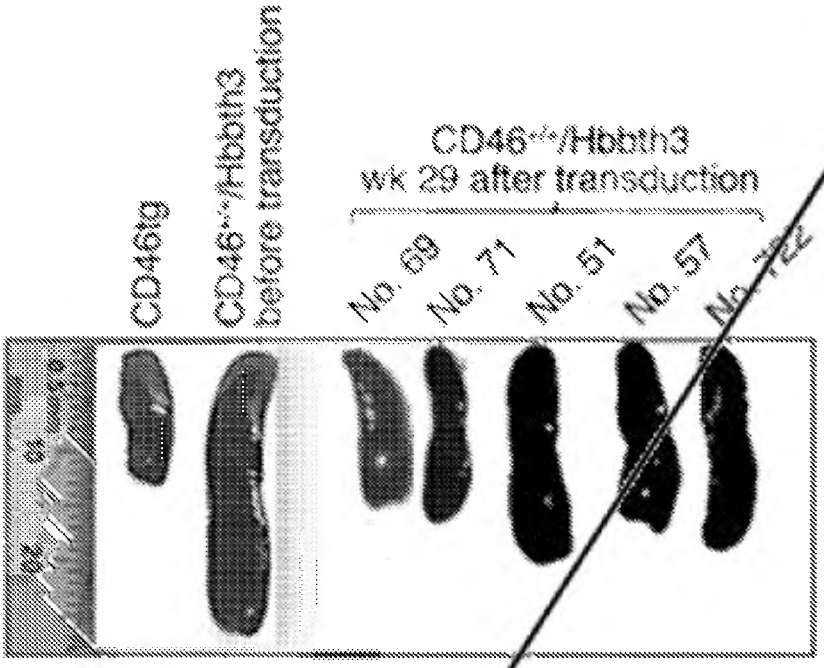
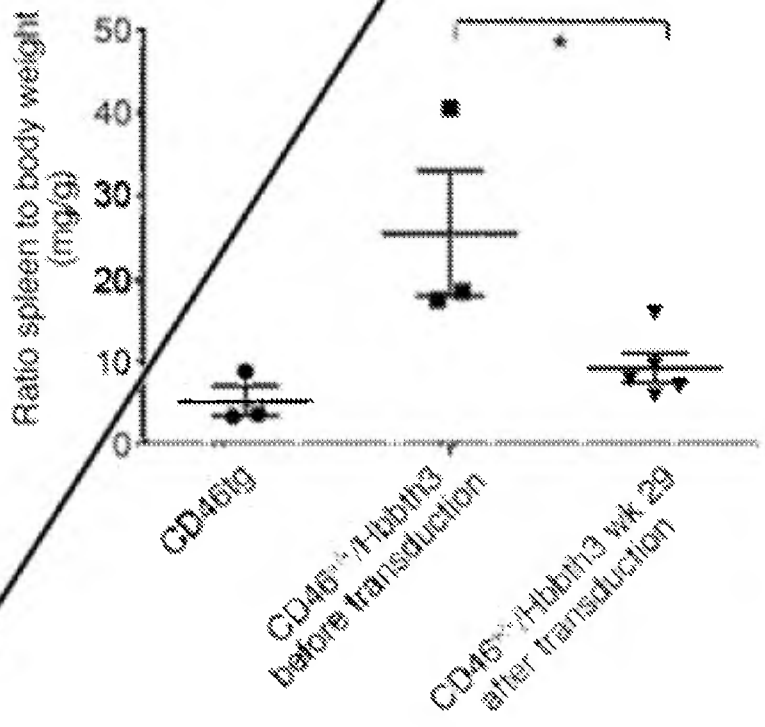


FIG. 44E



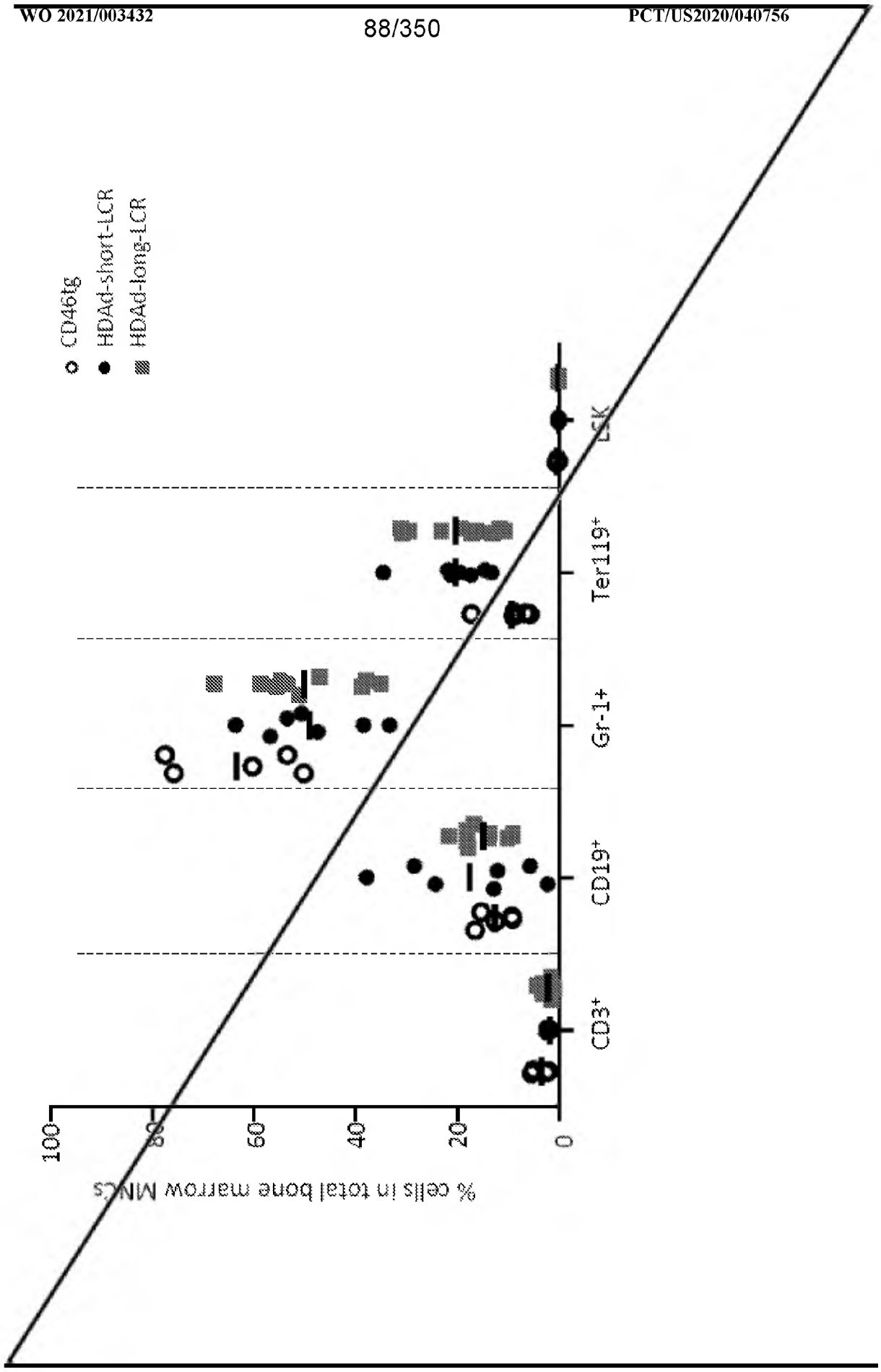

CD46tg
HDAd-short-LCR
HDAd-long-LCR
% cells in total bone marrow MNCs
100
80
60
40
20
0
CD3+
CD19+
Gr-1+
Ter119+
LSK

FIG. 45

FIG. 46

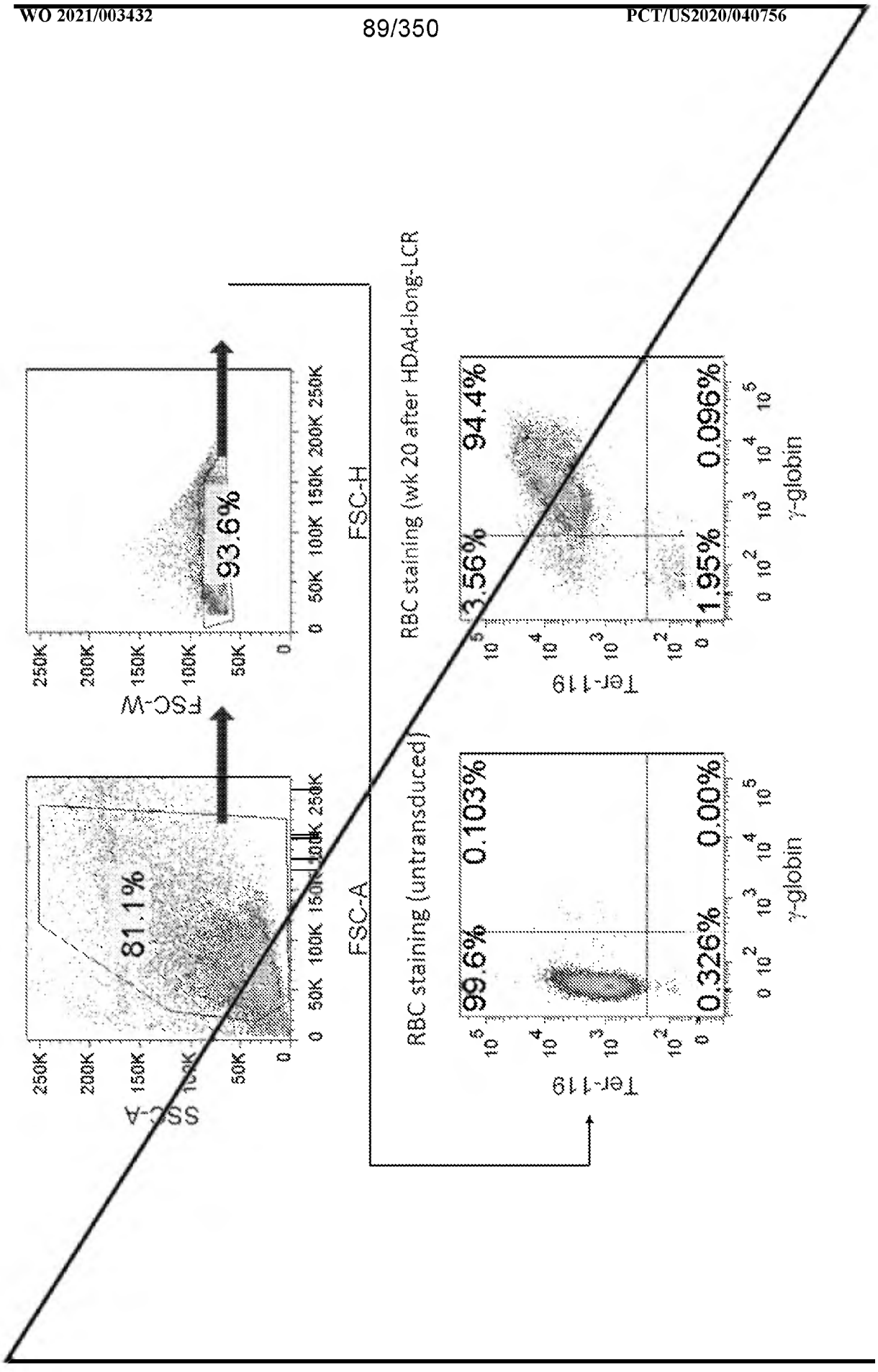
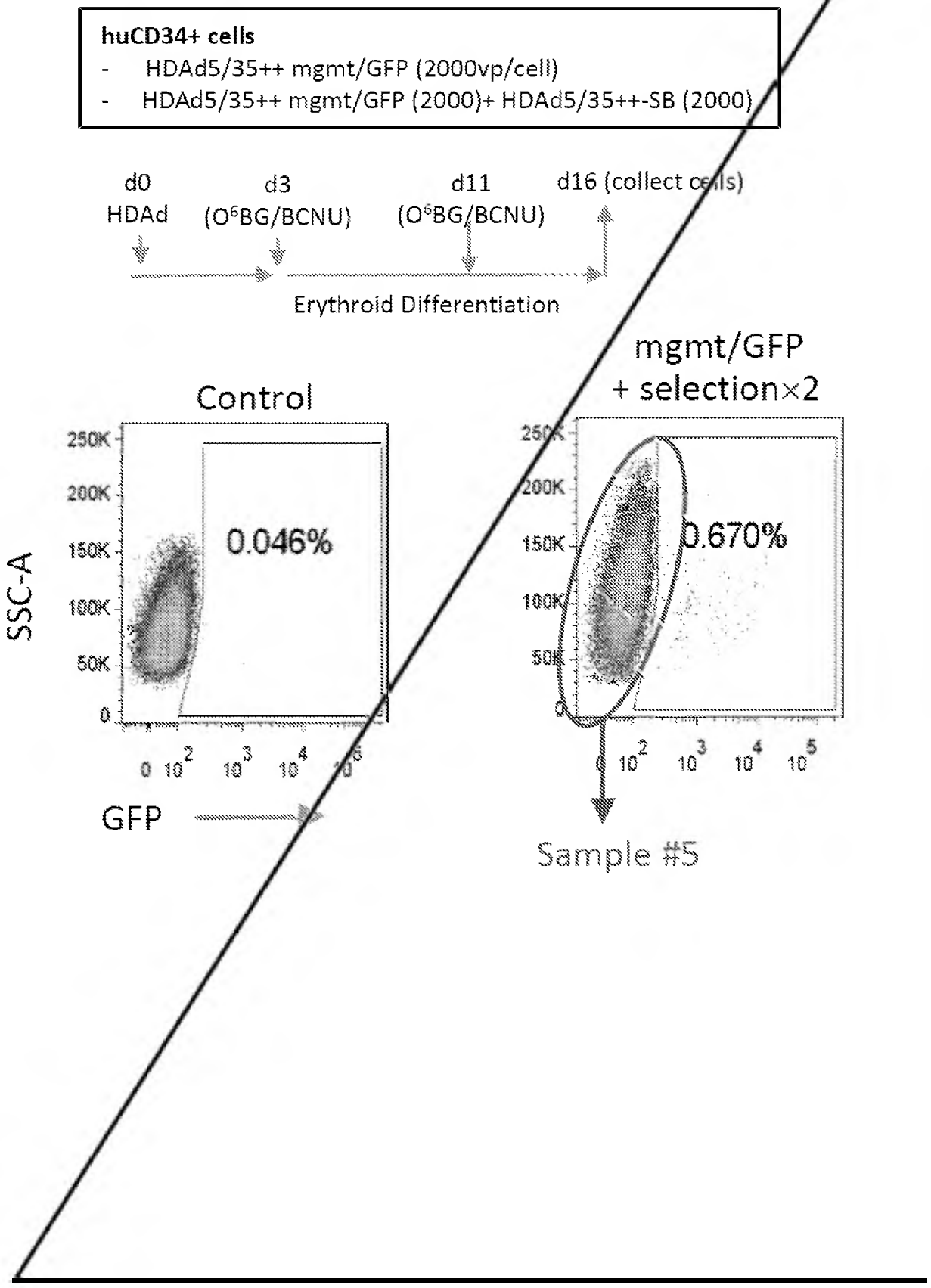

FSC-A
FSC-H
FSC-W
SSC-A
81.1%
93.6%
RBC staining (untransduced)
RBC staining (wk 20 after HDAd-long-LCR
Ter-119
γ-globin
99.6%
0.103%
0.326%
0.00%
3.56%
94.4%
1.95%
0.096%

FIG. 47A


huCD34+ cells
- HDAd5/35++ mgmt/GFP (2000vp/cell)
- HDAd5/35++ mgmt/GFP (2000)+ HDAd5/35++-SB (2000)
d0
HDAd
d3
(O6BG/BCNU)
d11
(O6BG/BCNU)
d16 (collect cells)
Erythroid Differentiation
Control
mgmt/GFP
+ selection×2
SSC-A
GFP
0.046%
0.670%
Sample #5

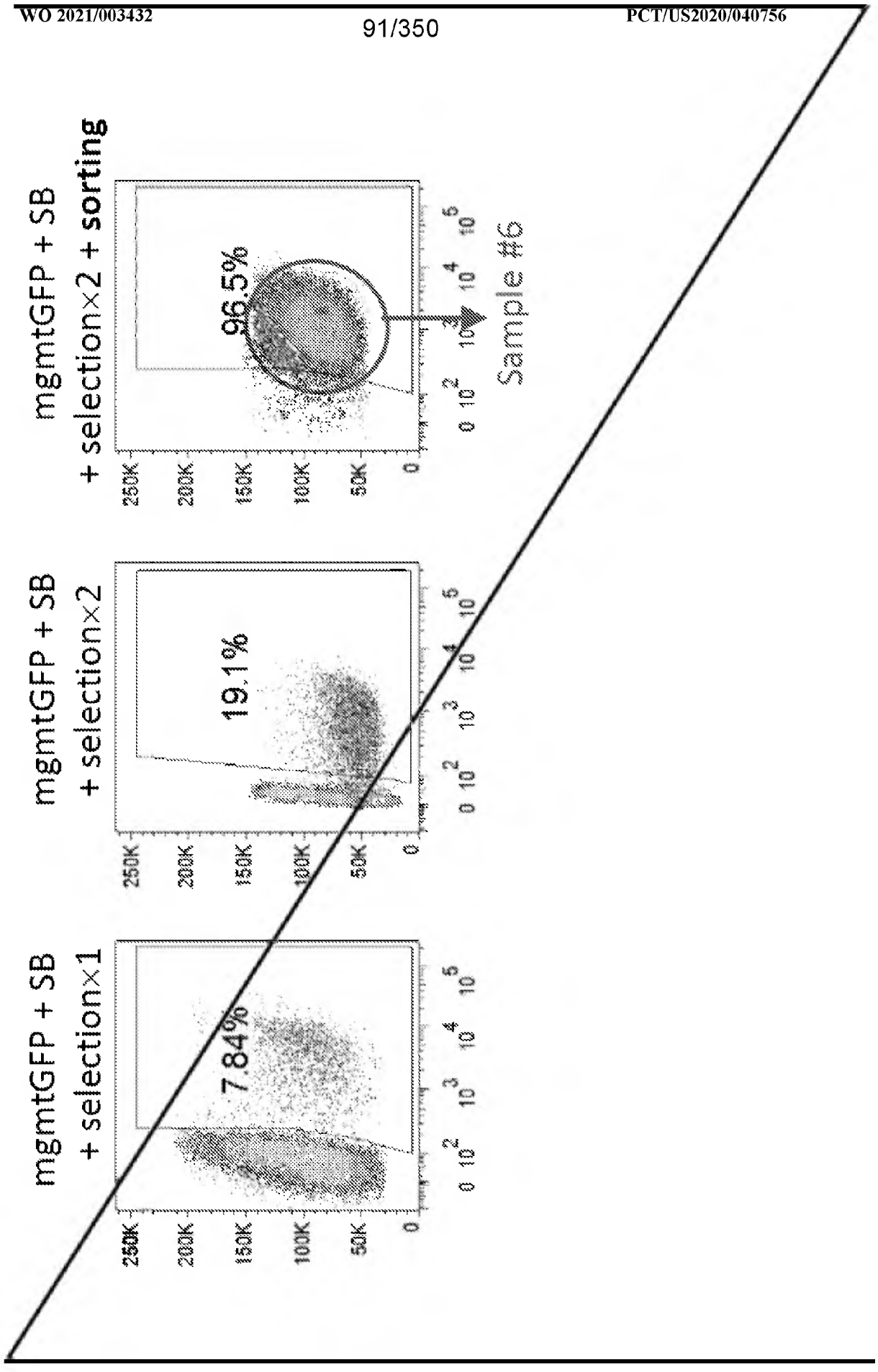


FIG. 47A cont'd

FIG. 47B

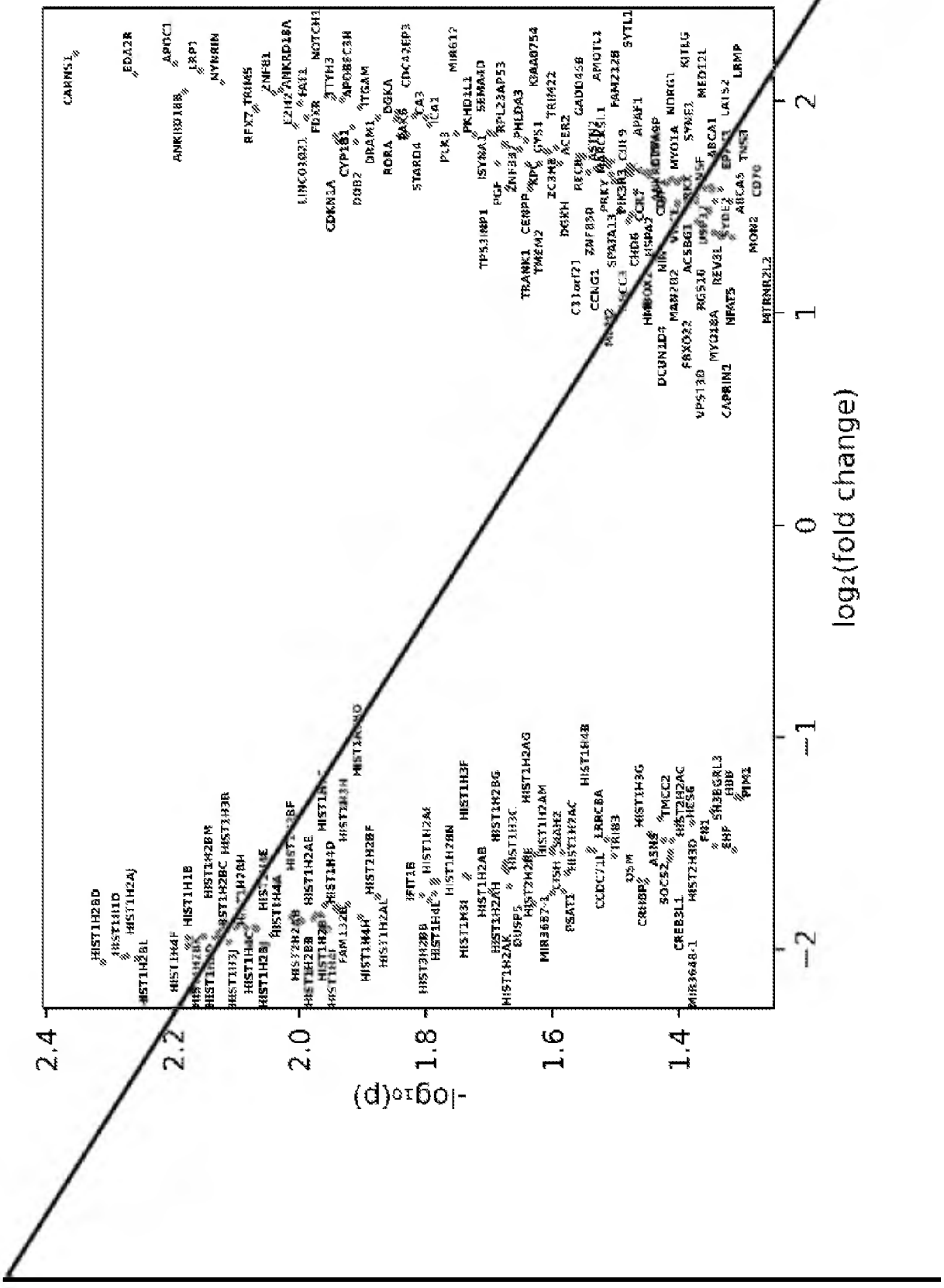

-log10(p)
log2(fold change)
2.4
2.2
2.0
1.8
1.6
1.4
−2
−1
0
1
2
CARNS1
EDA2R
APOC1
LRP3
NYNRIN
RFX7
TRIM5
ZNF81
KCNQ1DN
FBXW
NOTCH1
CDKN2A
CYP1B1
DGB2
DRAM1
ITGAM
RORA
DGKA
STARD4
CDC42EP3
PLK3
PKMYT1
MIR612
TP53INP1
SEMA4D
EGF
PHLDA3
TRANK1
TMEM2
ZC3H8
ACER2
TRIM22
DGKH
RECK
CCNG1
ZNF850
SPATA13
PRKY
PIK3R3
CUL9
APAF1
FAM212B
SYTL1
C11orf21
CADM4
AMOTL1
MYO1A
MORG1
KITLG
SYNE1
MED12L
ABCA1
LAIR2
LRMP
MOB2
CD70
MTRNR2L2
DCUN1D3
MAP2K2
ACSBG1
FBXO32
RGS16
VPS13D
MYO18A
NFAT5
CAPRIN2
REV3L
ABCA5
TNS1
HIST1H2BD
HIST1H1D
HIST1H2AJ
HIST1H2BL
HIST1H4F
HIST1H1B
HIST2H2BE
HIST2H2AA3
HIST1H2BC
HIST1H3B
HIST1H2BH
HIST1H2BJ
HIST1H2BF
HIST1H4A
HIST2H2BF
HIST1H2AE
HIST1H2BB
HIST1H1E
HIST1H4D
HIST1H3H
HIST2H2AC
FAM132B
HIST1H4H
HIST2H2BF
HIST1H2AL
HIST1H2AG
HIST1H2BB
HIST1H4E
HIST1H2BM
HIST1H3I
HIST1H2AB
HIST1H3F
HIST3H2A
HIST1H2BG
HIST1H2AK
HIST1H3C
BUSP5
HIST2H2BE
HIST1H2AM
MIR3687-3
SOAT1
HIST1H2AC
HIST1H4B
CCDC71L
LRRC8A
TRIB3
USP8
CREBBP
ASNS
HIST1H3G
SOCS2
TMEM2
CREB3L1
HIST2H3D
HESX
FN1
SH3BGRL3
EHF
HBB
PIM1

FIG. 48

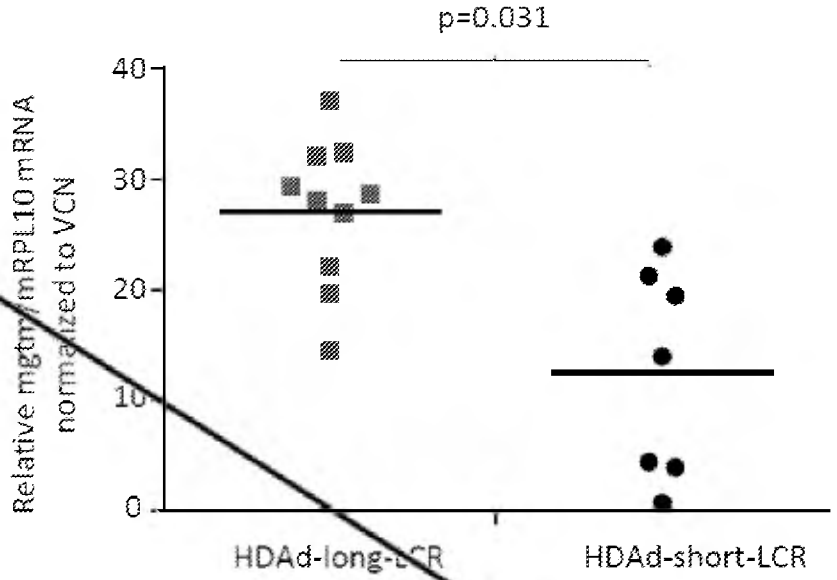
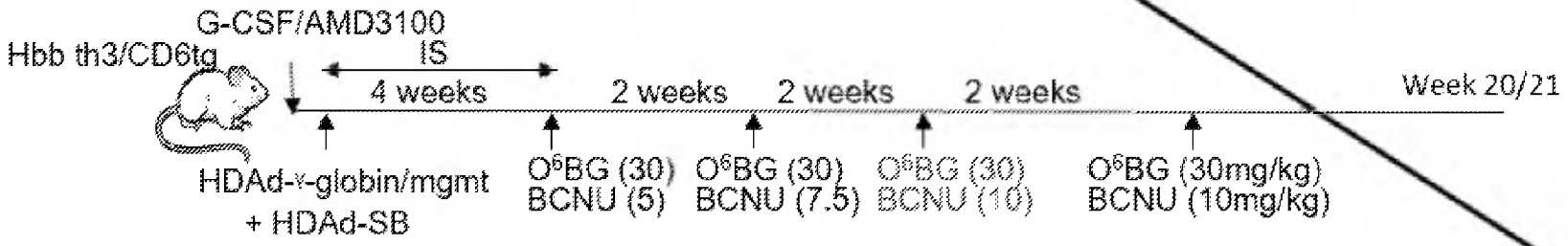

p=0.031
Relative mgtm/mRPL10 mRNA normalized to VCN
40
30
20
10
0
HDAd-long-LCR
HDAd-short-LCR

FIG. 49


G-CSF/AMD3100
IS
Hbb th3/CD6tg
4 weeks
2 weeks
2 weeks
2 weeks
Week 20/21
HDAd-γ-globin/mgmt
+ HDAd-SB
O6BG (30)
BCNU (5)
O6BG (30)
BCNU (7.5)
O6BG (30)
BCNU (10)
O6BG (30mg/kg)
BCNU (10mg/kg)

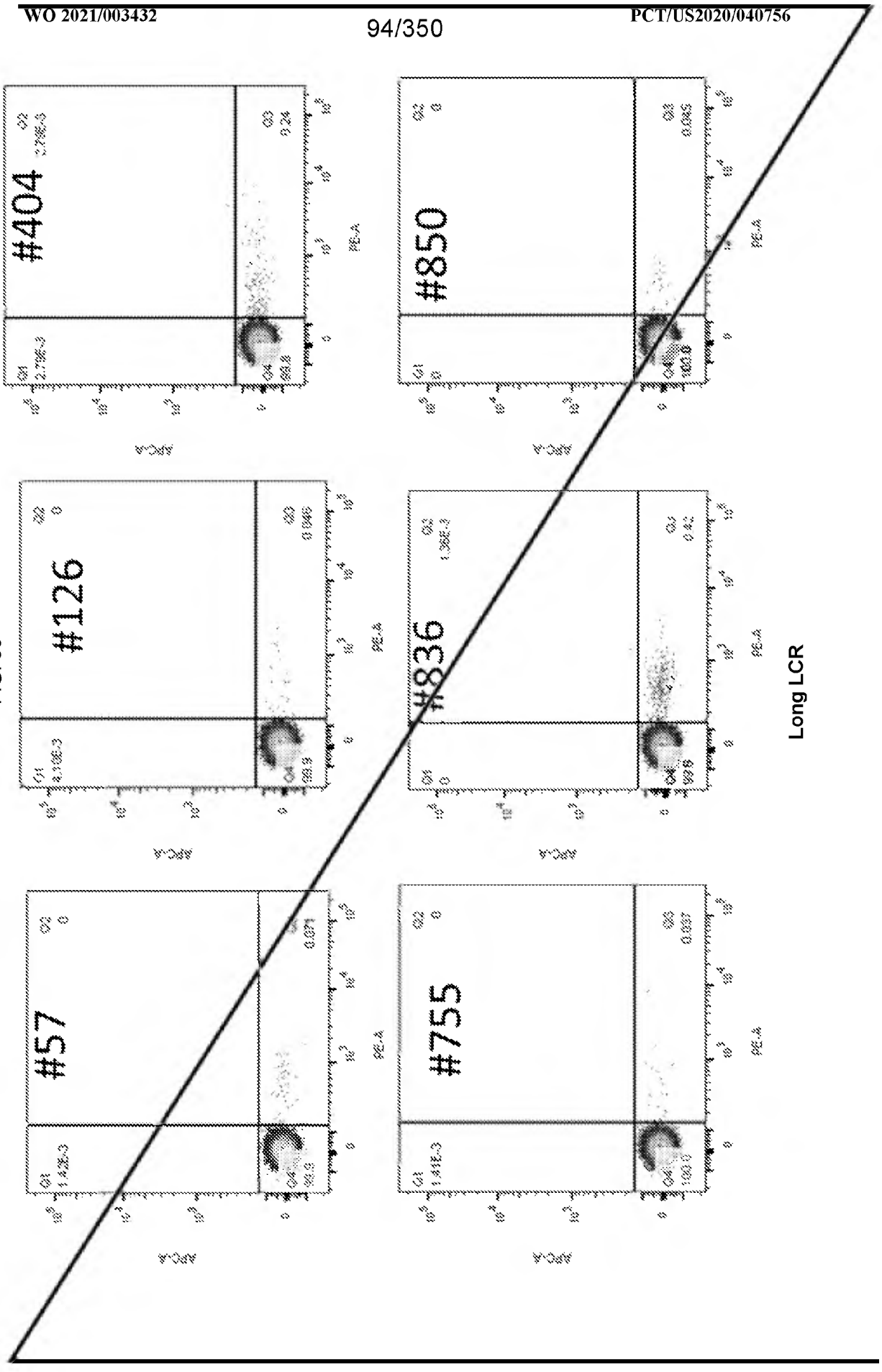
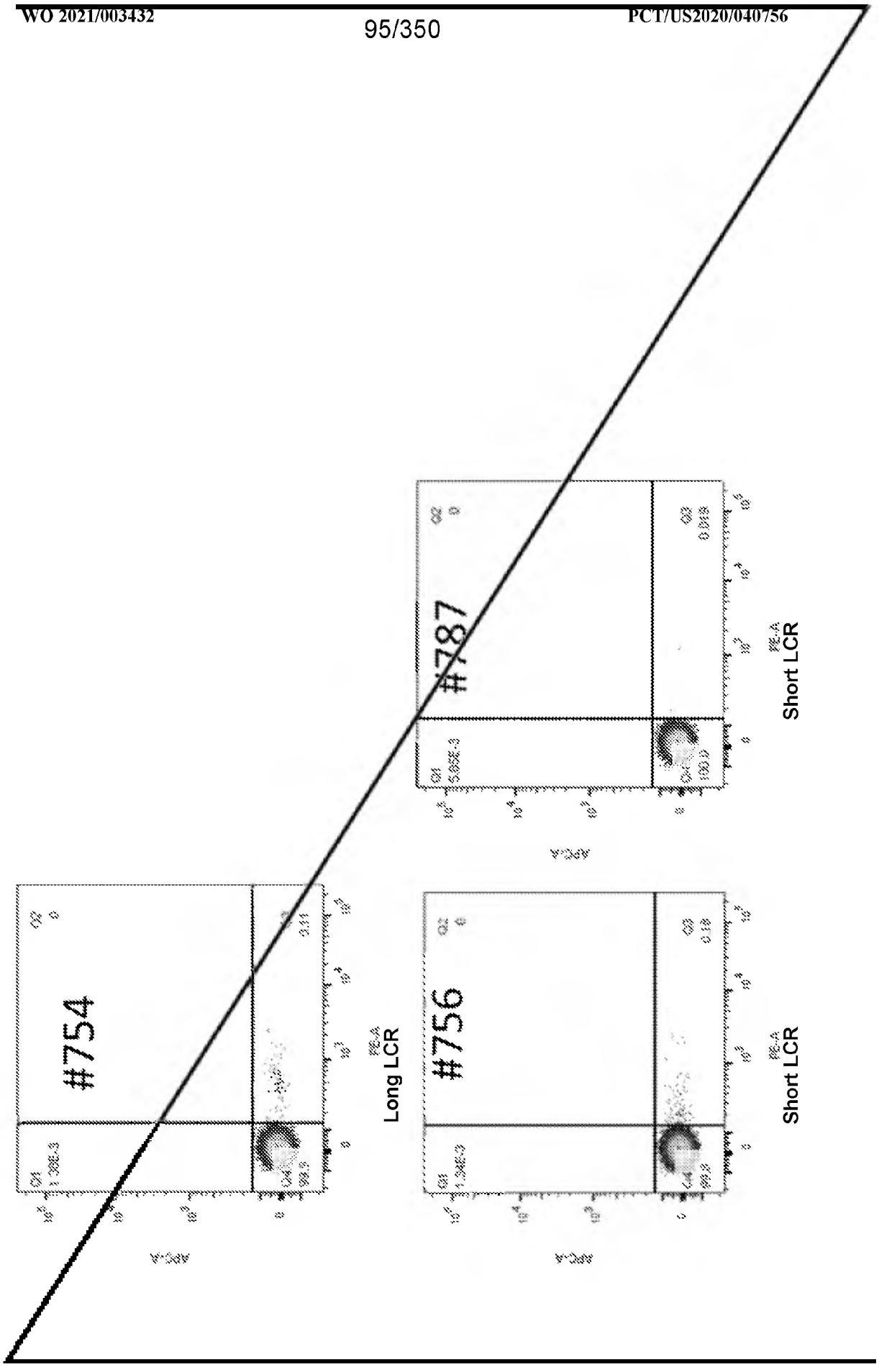


FIG. 50

FIG. 50 cont'd



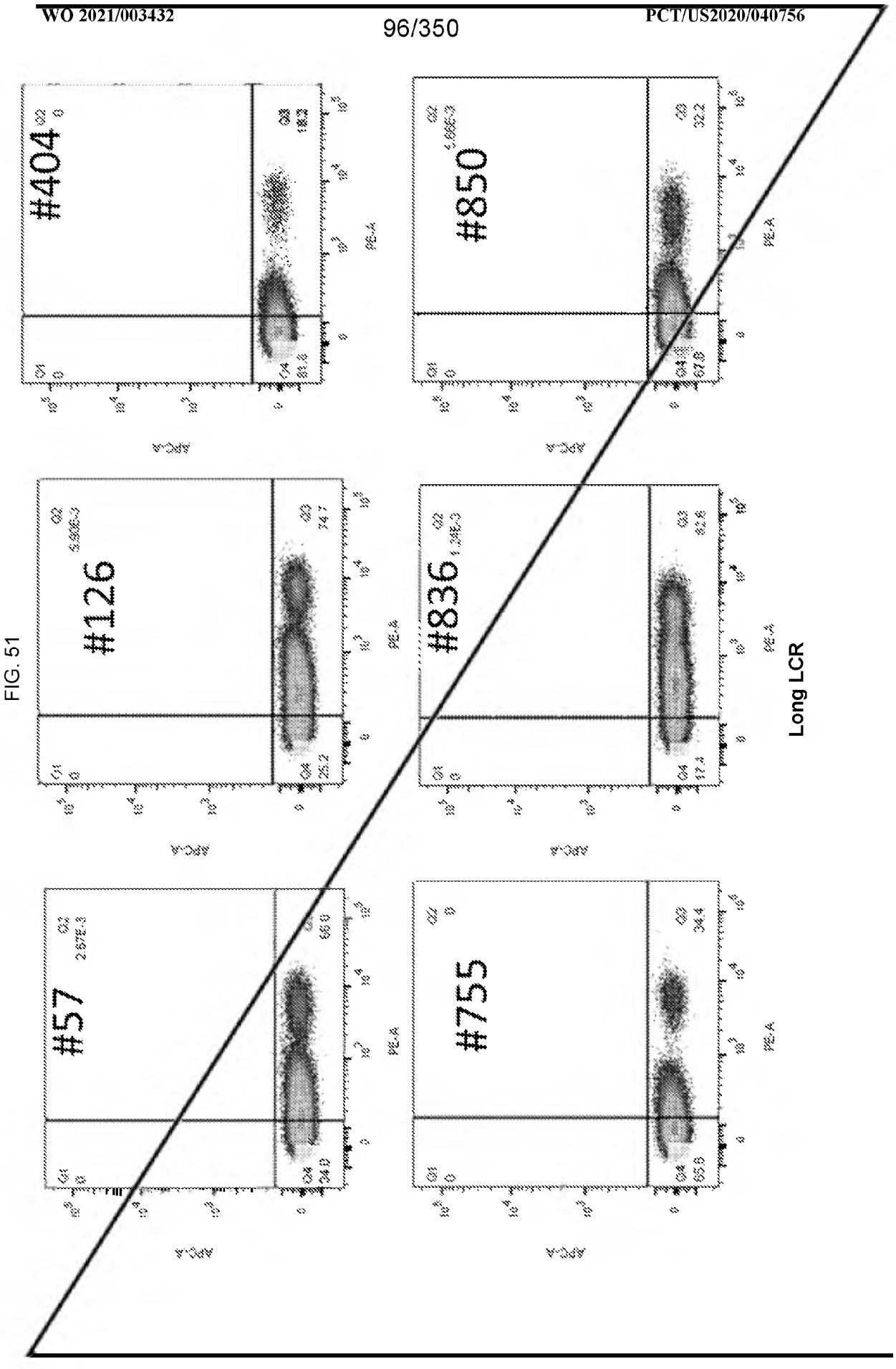


FIG. 51

FIG. 51 cont'd

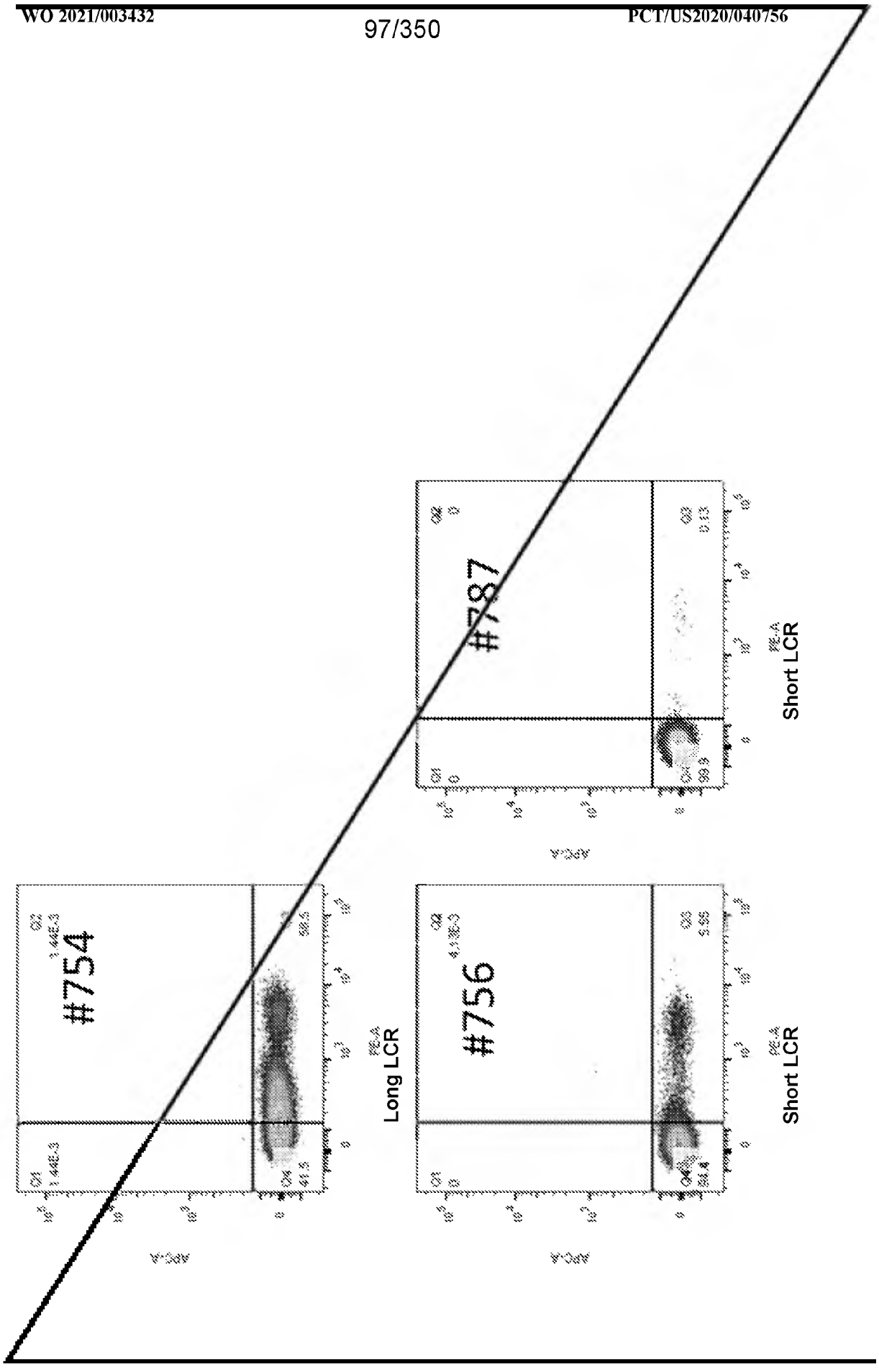


FIG. 52

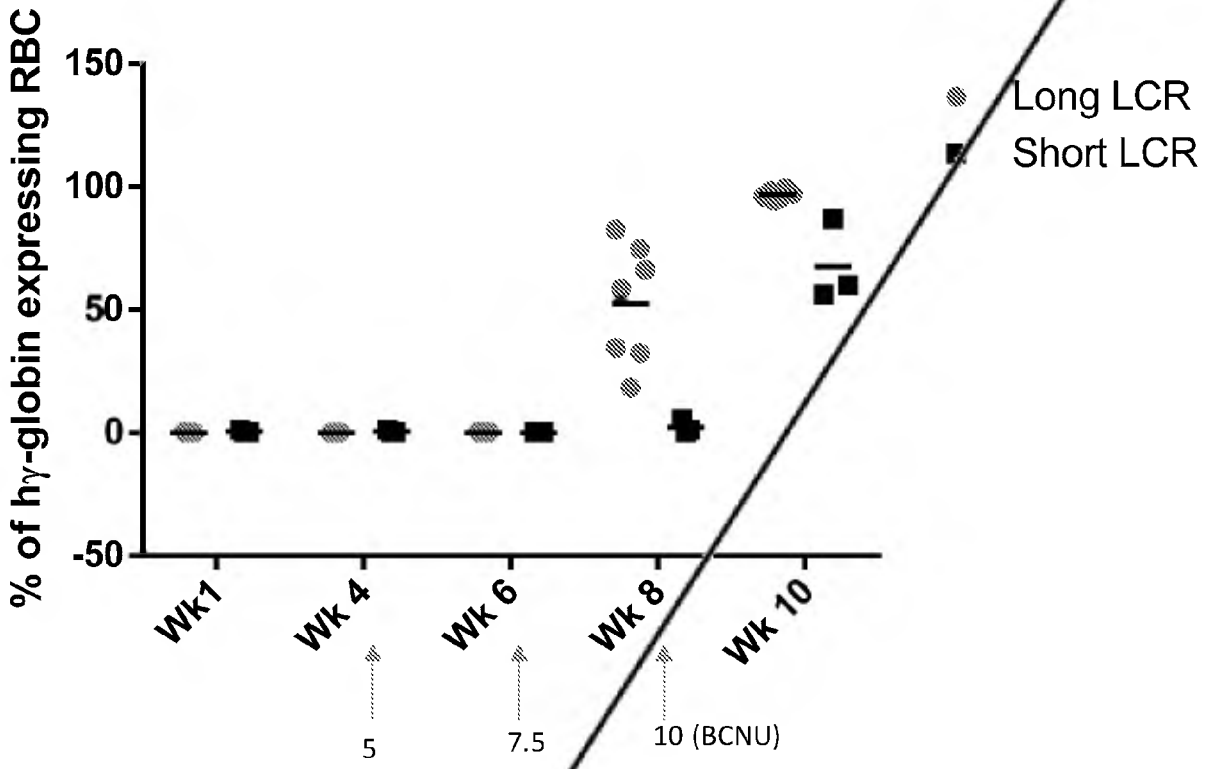

% of hγ-globin expressing RBC
150
100
50
0
-50
Wk1
Wk 4
Wk 6
Wk 8
Wk 10
5
7.5
10 (BCNU)
Long LCR
Short LCR

FIG. 53

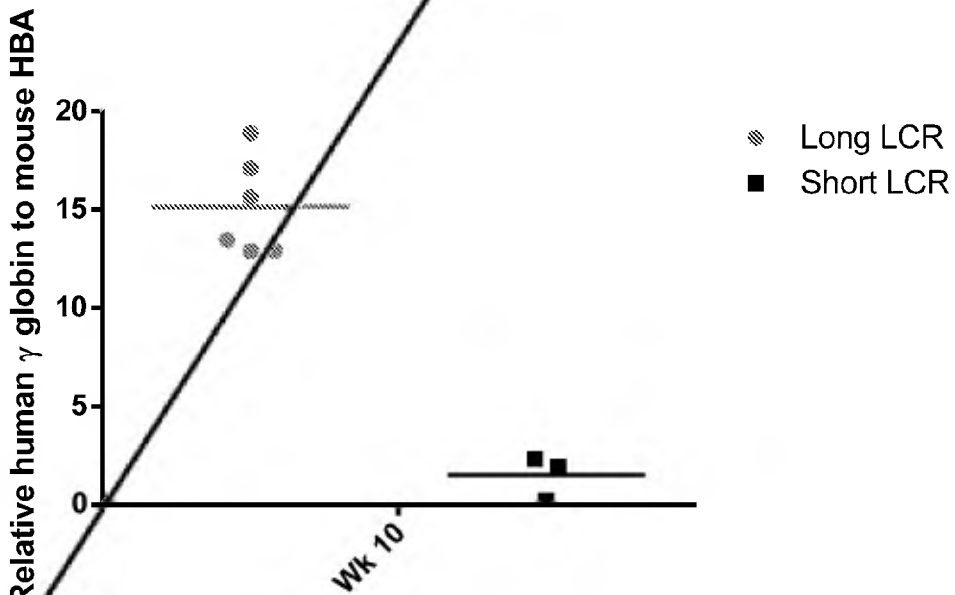

Relative human γ globin to mouse HBA
20
15
10
5
0
Wk 10
Long LCR
Short LCR

FIG. 54

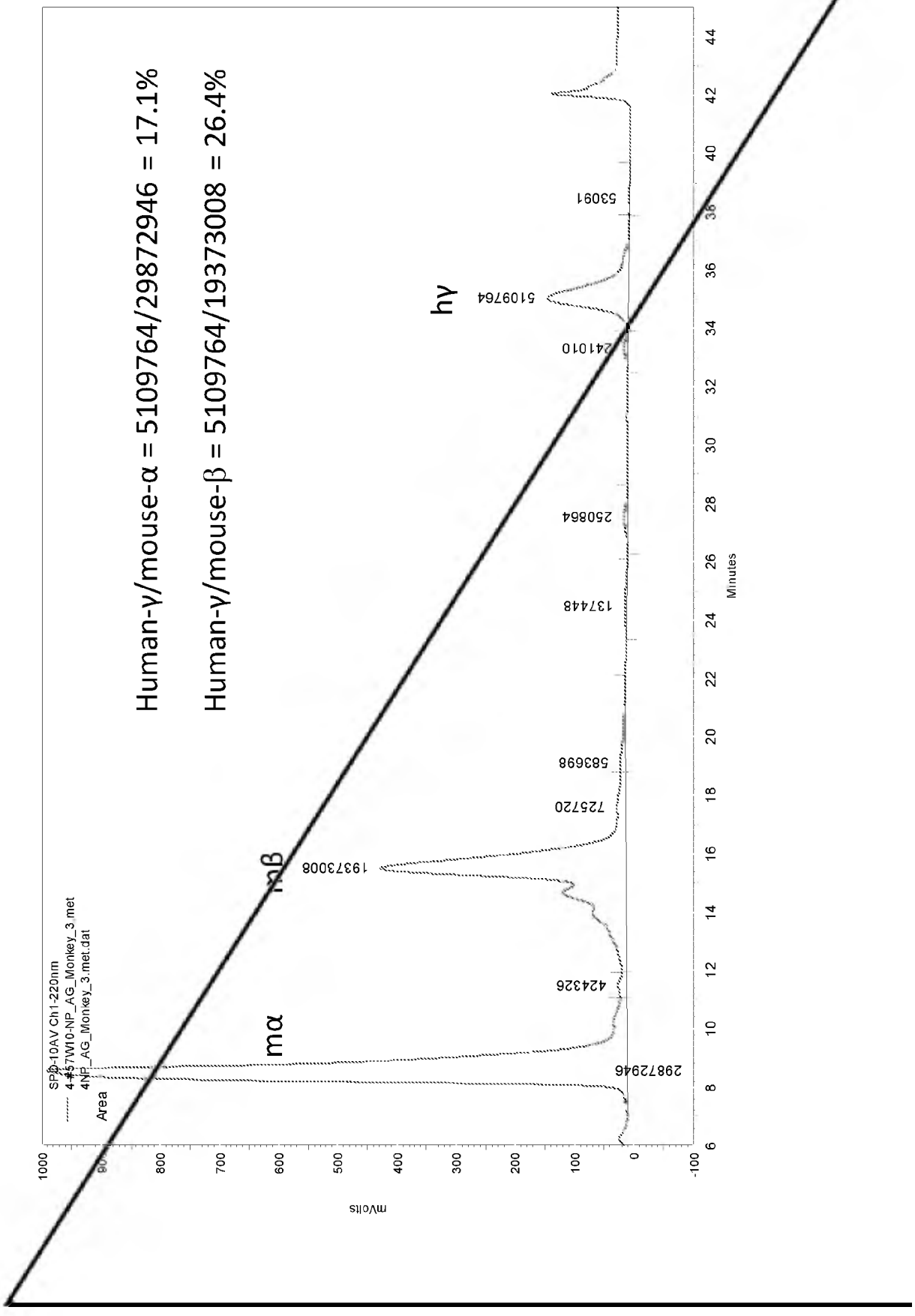

Human-γ/mouse-α = 5109764/29872946 = 17.1%
Human-γ/mouse-β = 5109764/19373008 = 26.4%
mα
mβ
hγ
SPD-10AV Ch1-220nm
4-457WI0-NP_AG_Monkey_3.met
4NP_AG_Monkey_3.met.dat
Area
mVolts
Minutes
29872946
424326
19373008
725720
583698
137448
250864
241010
5109764
53091

FIG. 55A

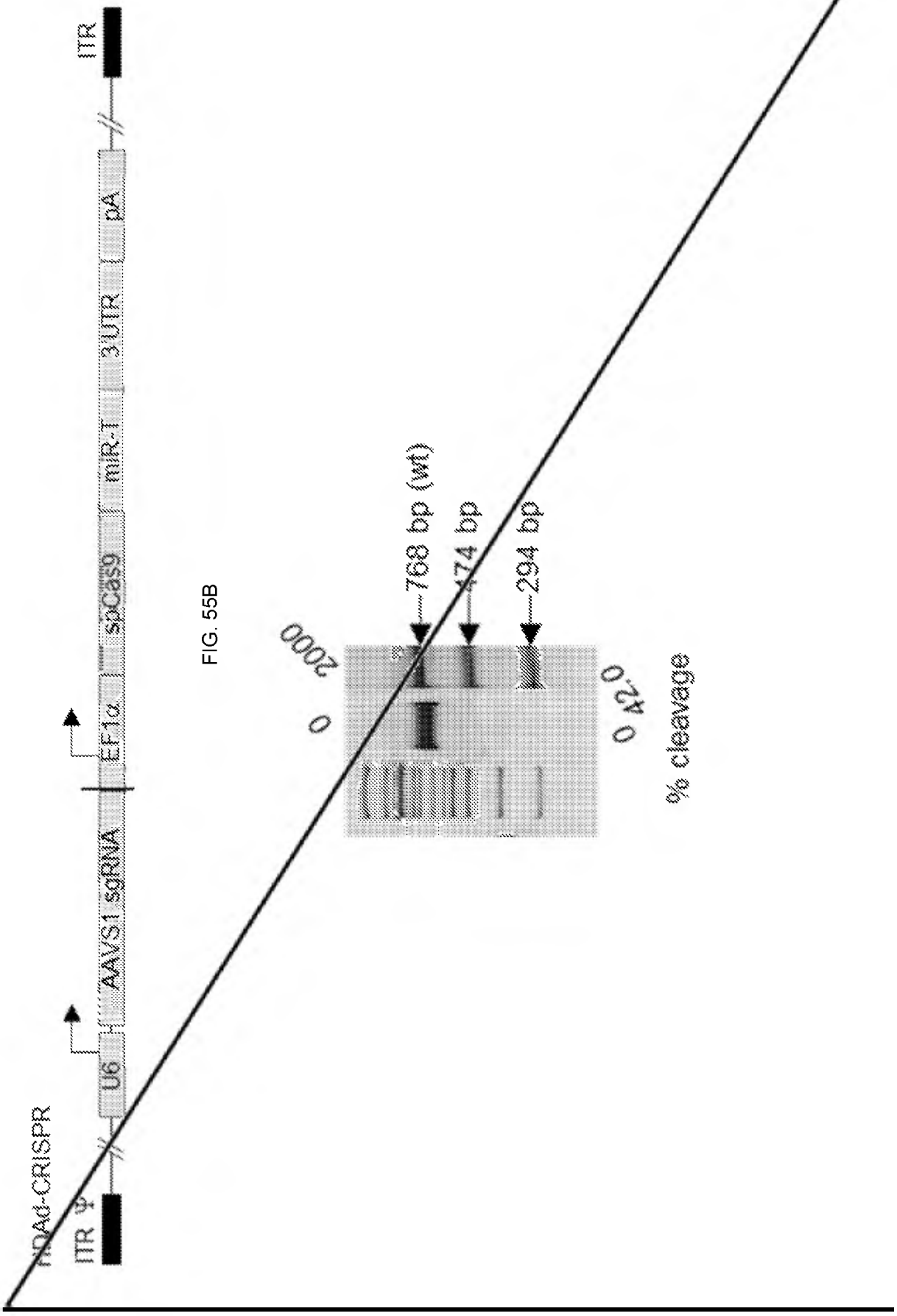
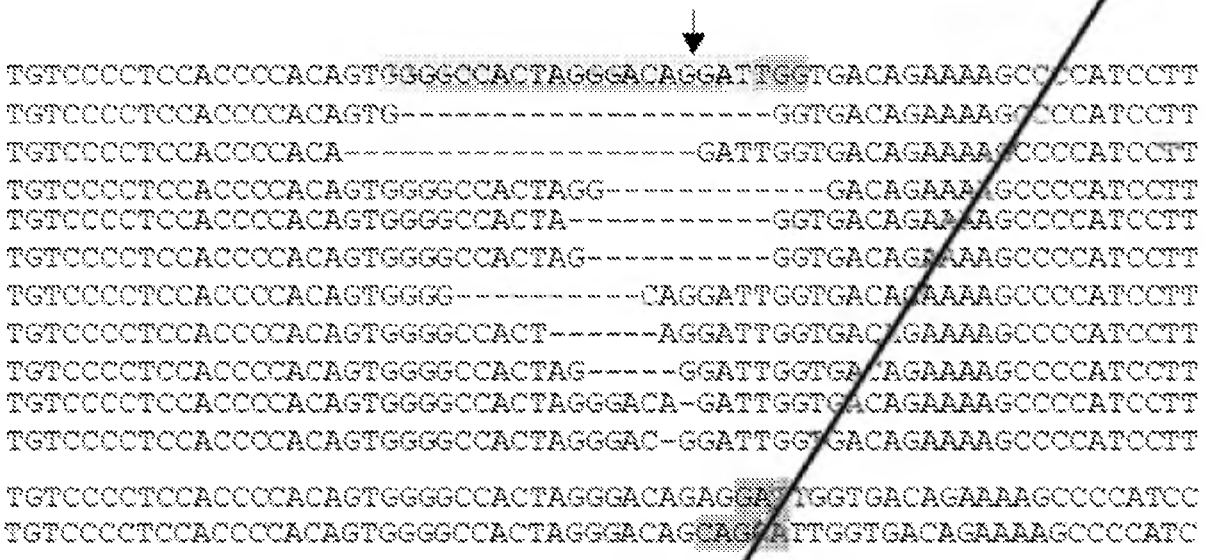


FIG. 55B

FIG. 55C



(SEQ ID NOs: 6-18)

FIG. 55D

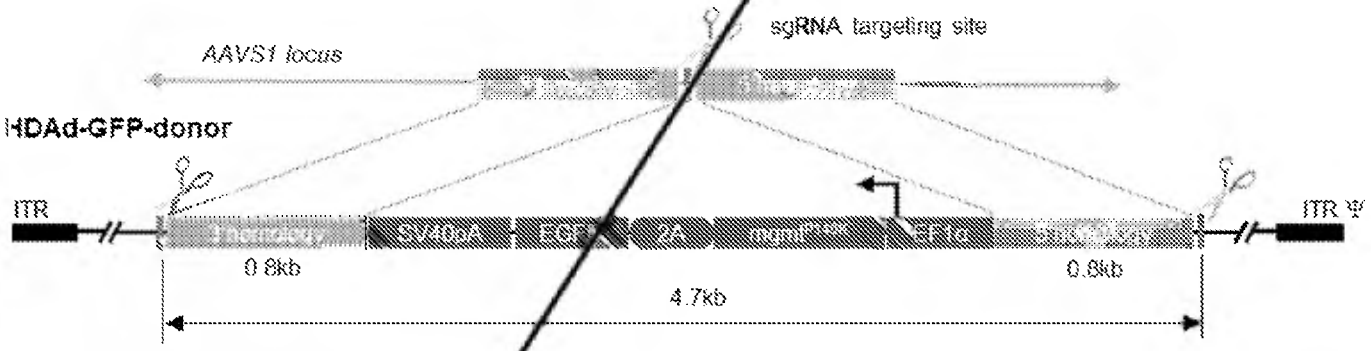


FIG. 55E

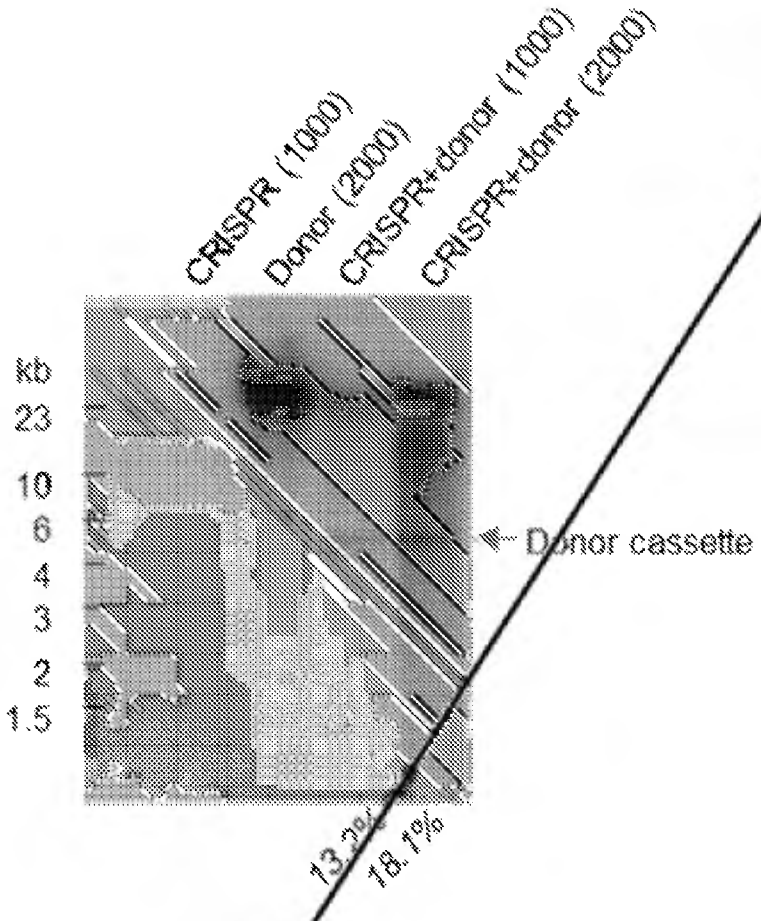
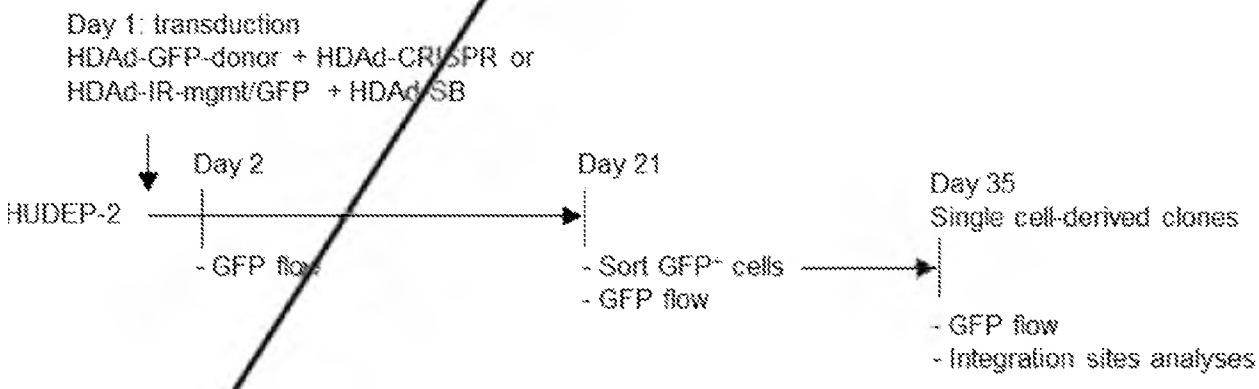


FIG. 56A



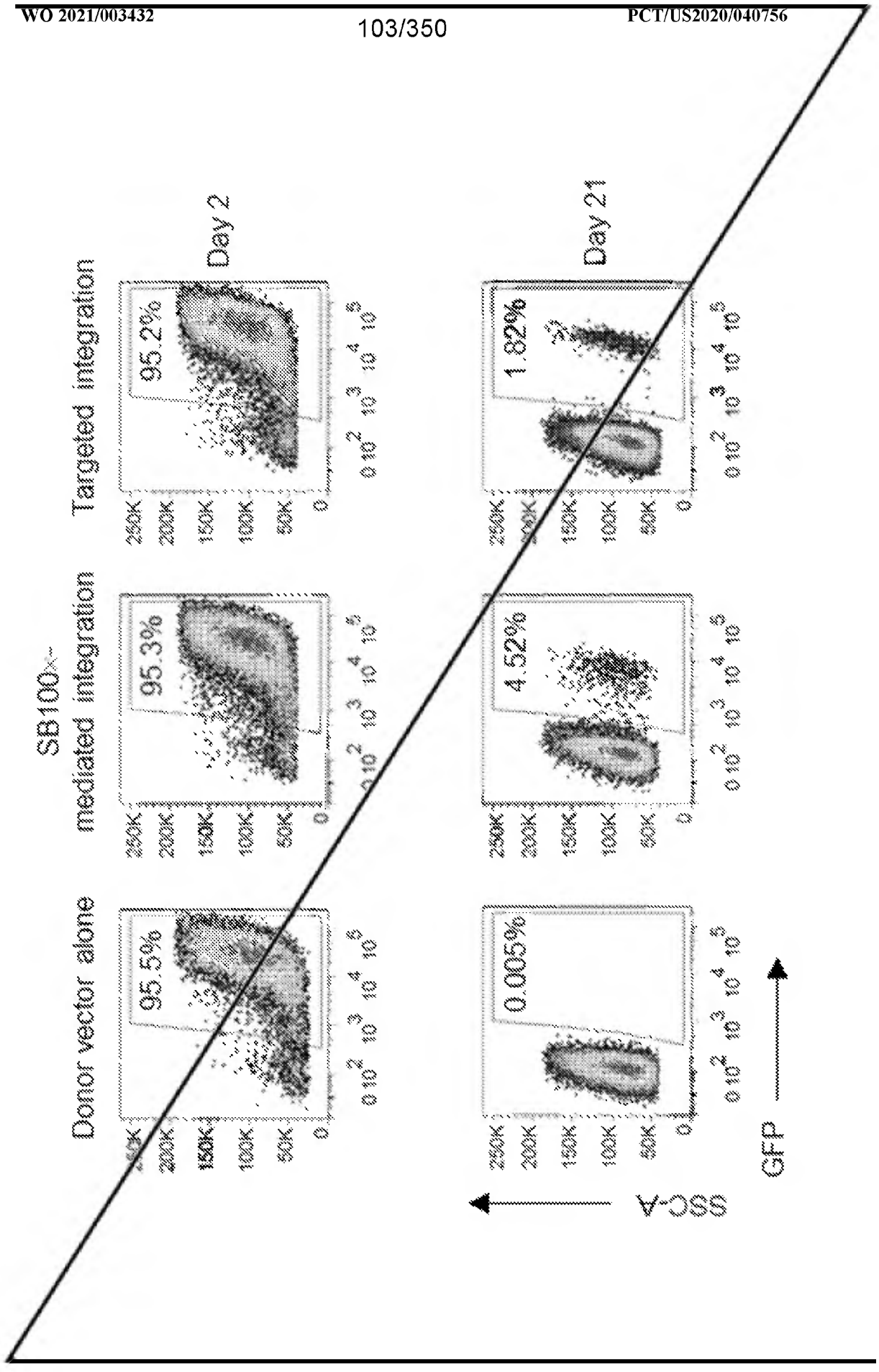

Donor vector alone
SB100X-mediated integration
Targeted integration
Day 2
Day 21
95.5%
95.3%
95.2%
0.005%
4.52%
1.82%
SSC-A
GFP

FIG. 56B

FIG. 56C

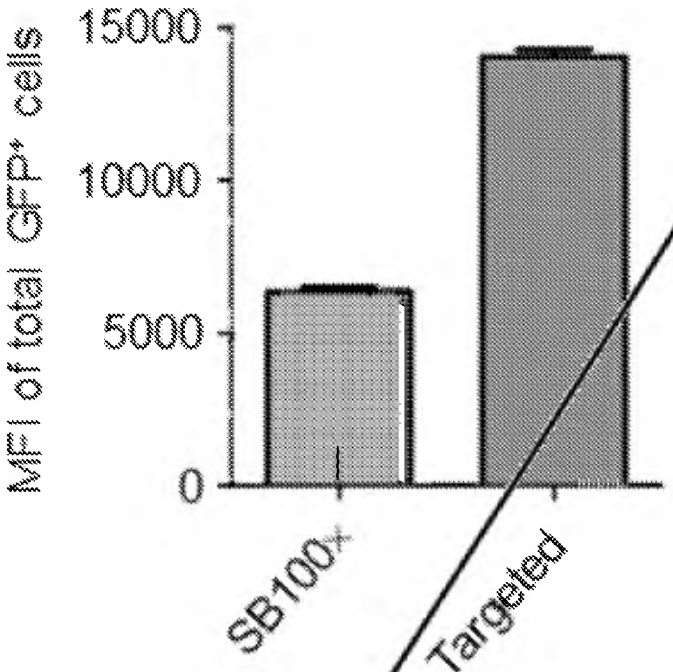
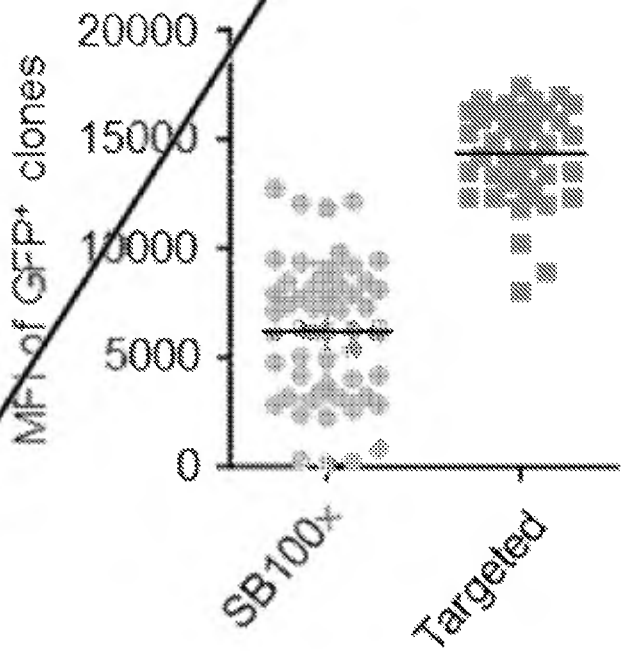


FIG. 56D



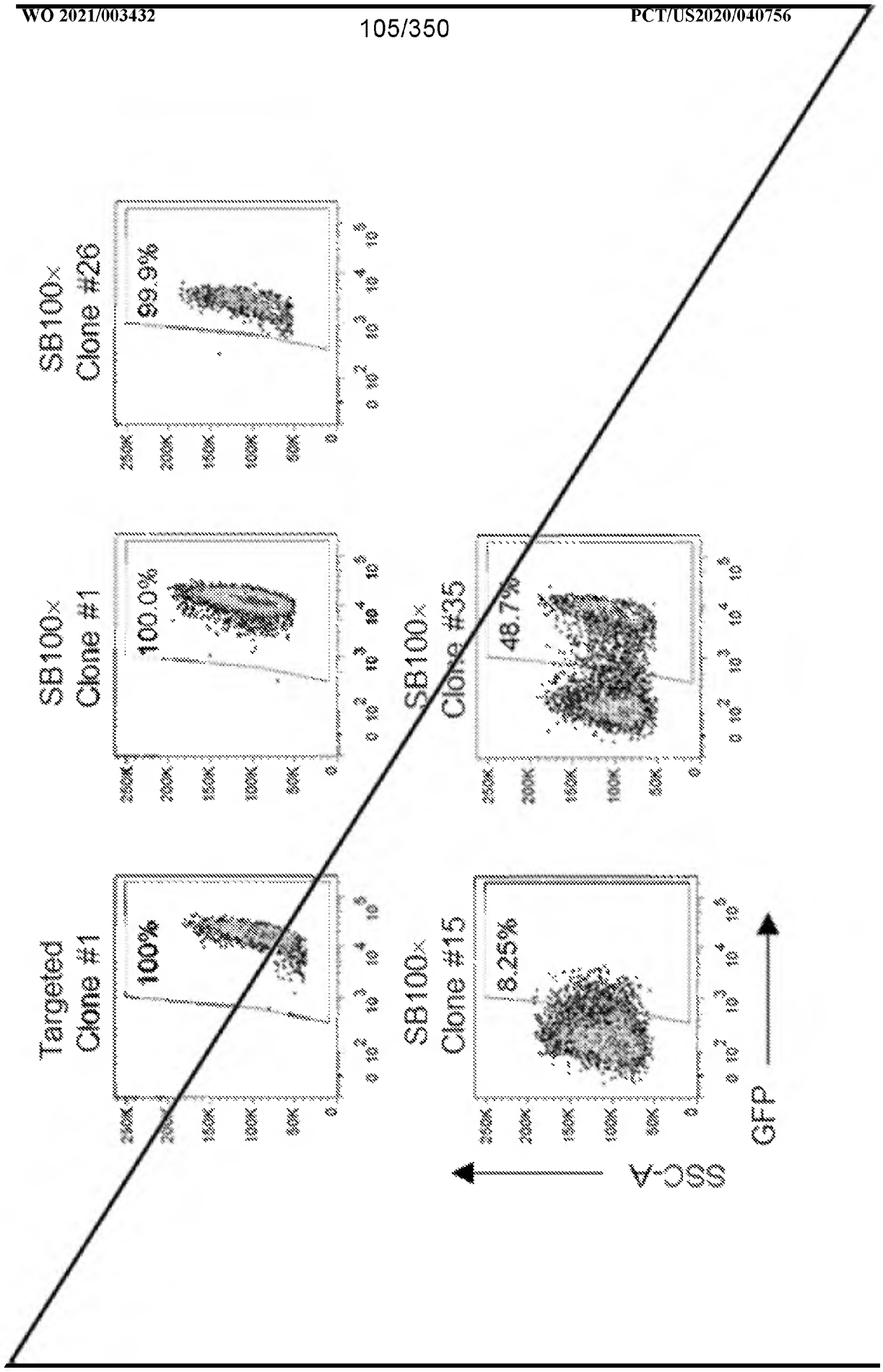

Targeted
Clone #1
100%
SB100x
Clone #1
100.0%
SB100x
Clone #26
99.9%
SB100x
Clone #15
8.25%
SB100x
Clone #35
48.7%
SSC-A
GFP

FIG. 56E

FIG. 56F

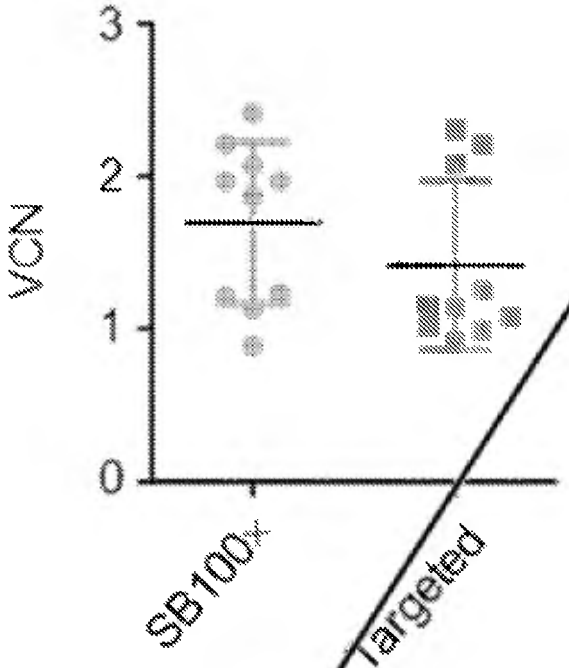
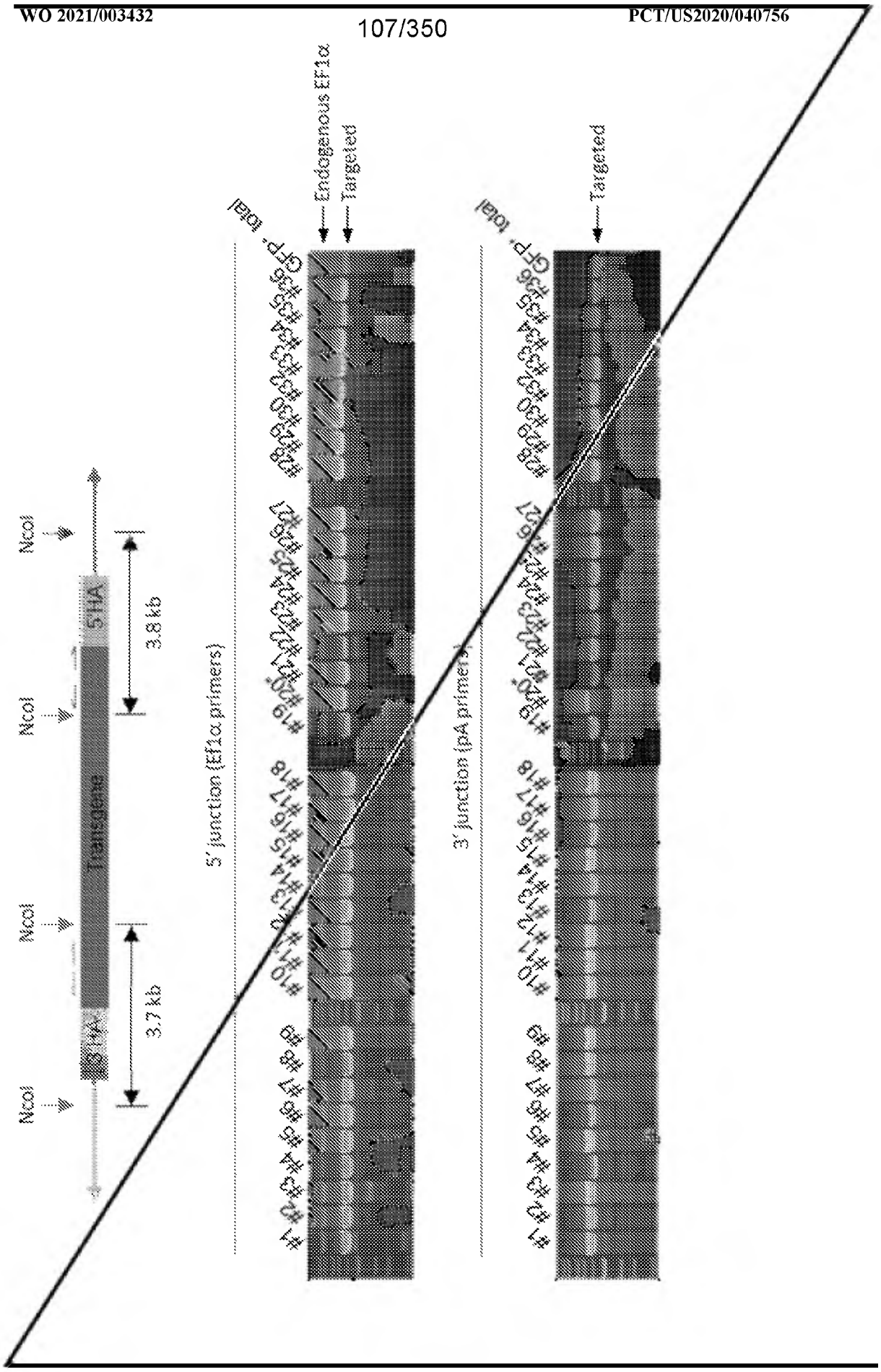


FIG. 57A


NcoI
NcoI
NcoI
NcoI
5'HA
Transgene
3'HA
3.8 kb
3.7 kb
5' junction (EF1α primers)
3' junction (pA primers)
Endogenous EF1α
Targeted
Targeted
total
GFP+
GFP-

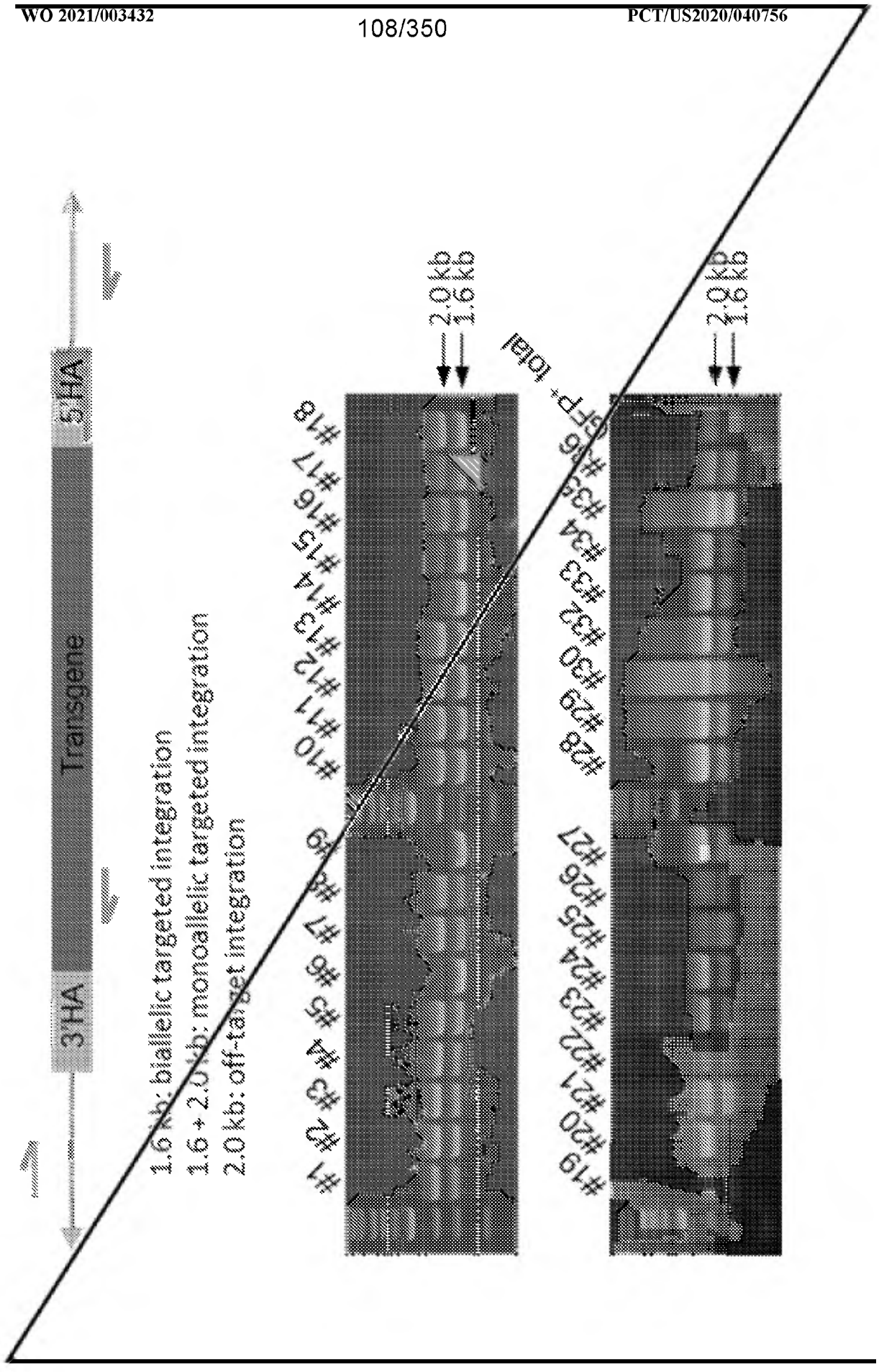

5'HA
Transgene
3'HA
1.6 kb: biallelic targeted integration
1.6 + 2.0 kb: monoallelic targeted integration
2.0 kb: off-target integration
2.0kb
1.6kb
#1 #2 #3 #4 #5 #6 #7 #8 #9 #10 #11 #12 #13 #14 #15 #16 #17 #18
#19 #20 #21 #22 #23 #24 #25 #26 #27 #28 #29 #30 #32 #33 #34 #35 #36 GFP+ total

FIG. 57B

FIG. 58A

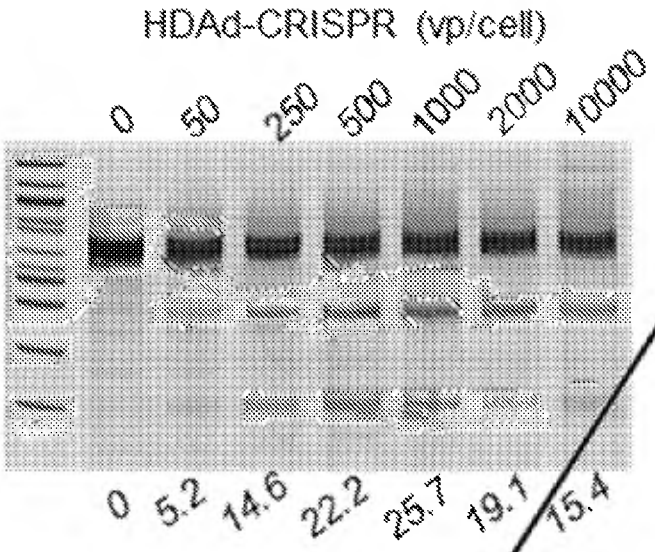


FIG. 58B

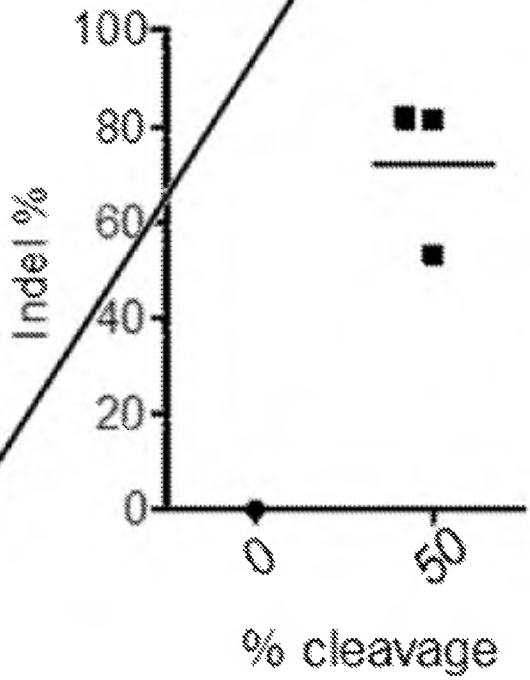


FIG. 58C

(SEQ ID NOs: 19-23, 21, 21, 26-30, 27, 32, 28, 34-47)

FIG. 59A

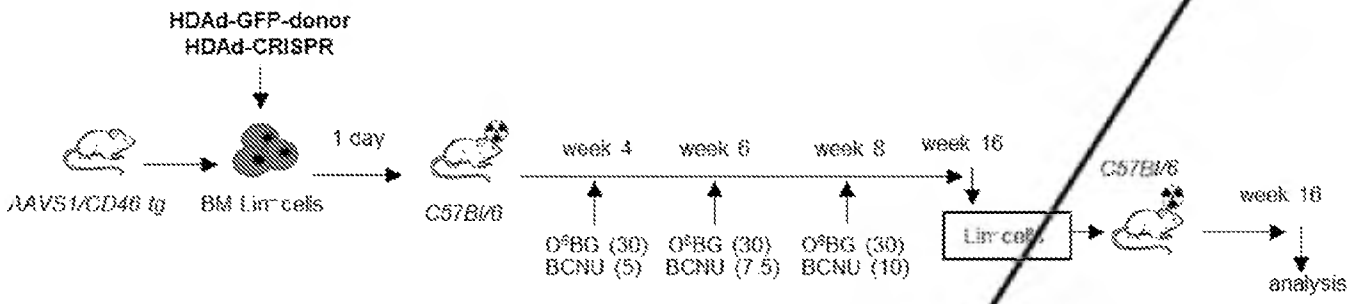


FIG. 59B

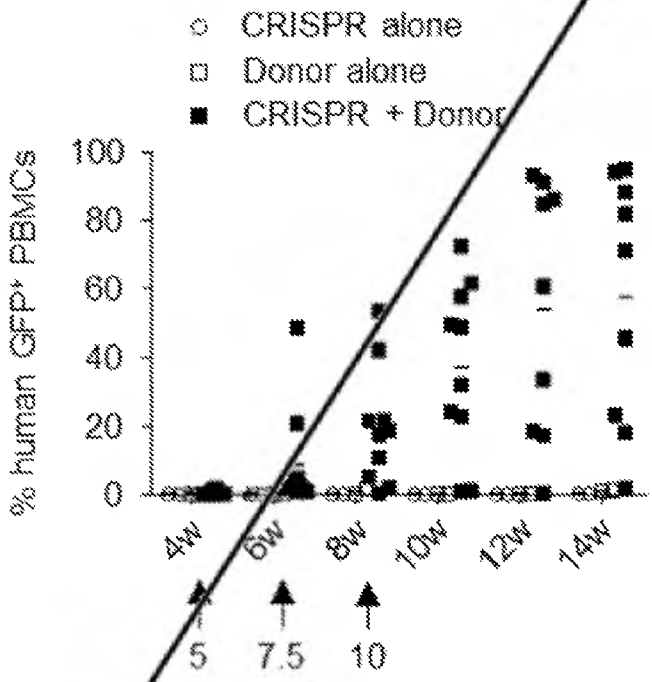


FIG. 59C

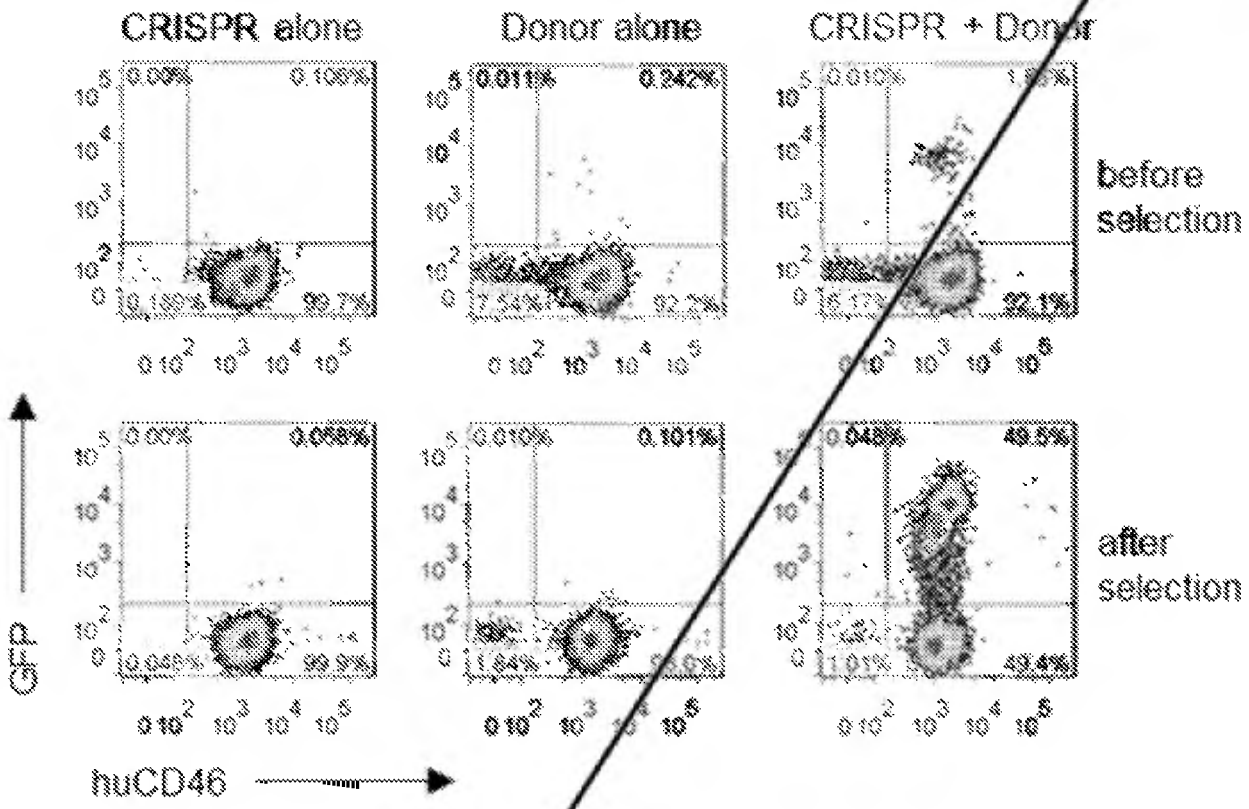


FIG. 59D

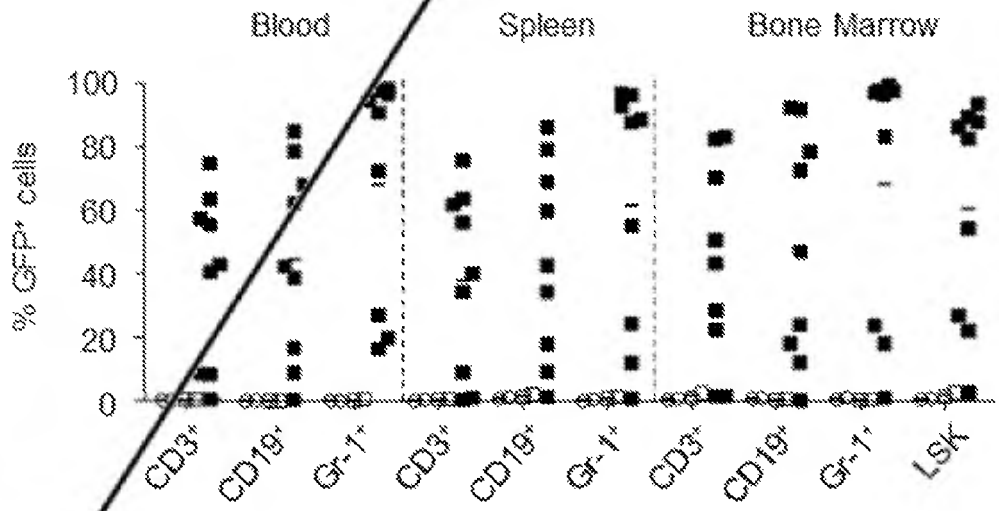


FIG. 60A

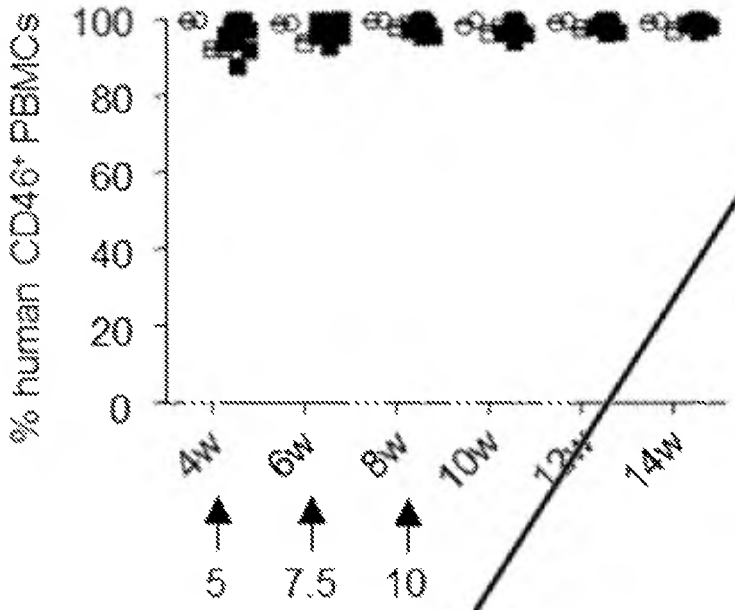


FIG. 60B

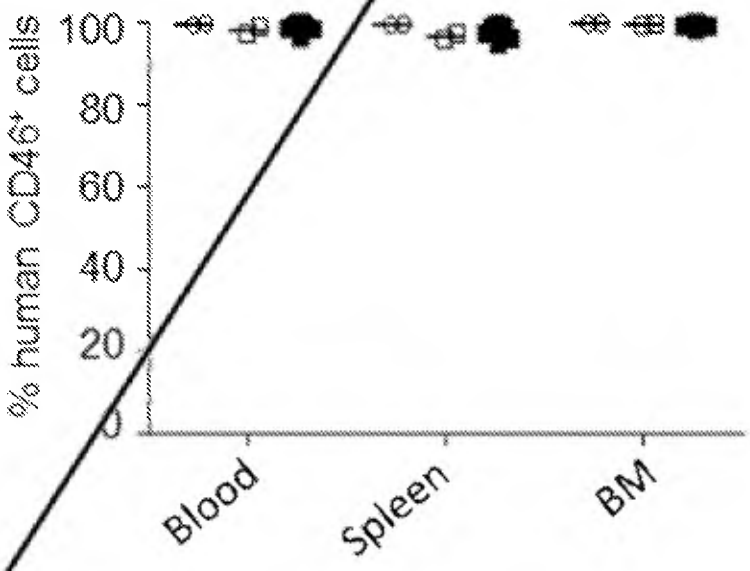


FIG. 60C

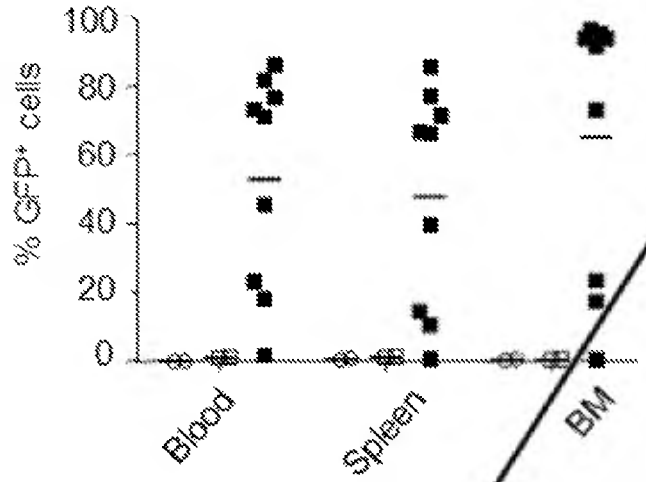


FIG. 60D

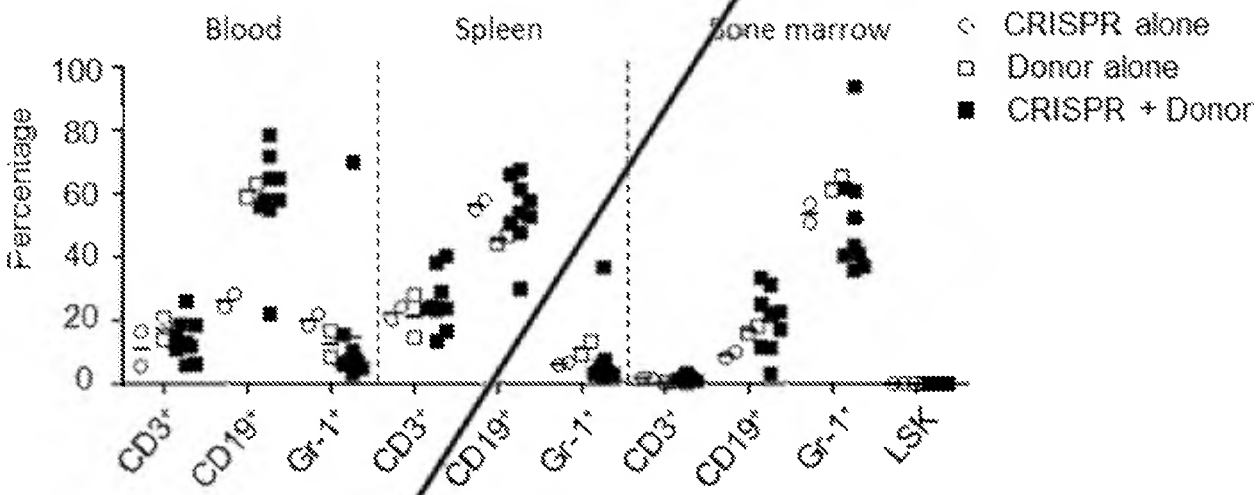


FIG. 60E

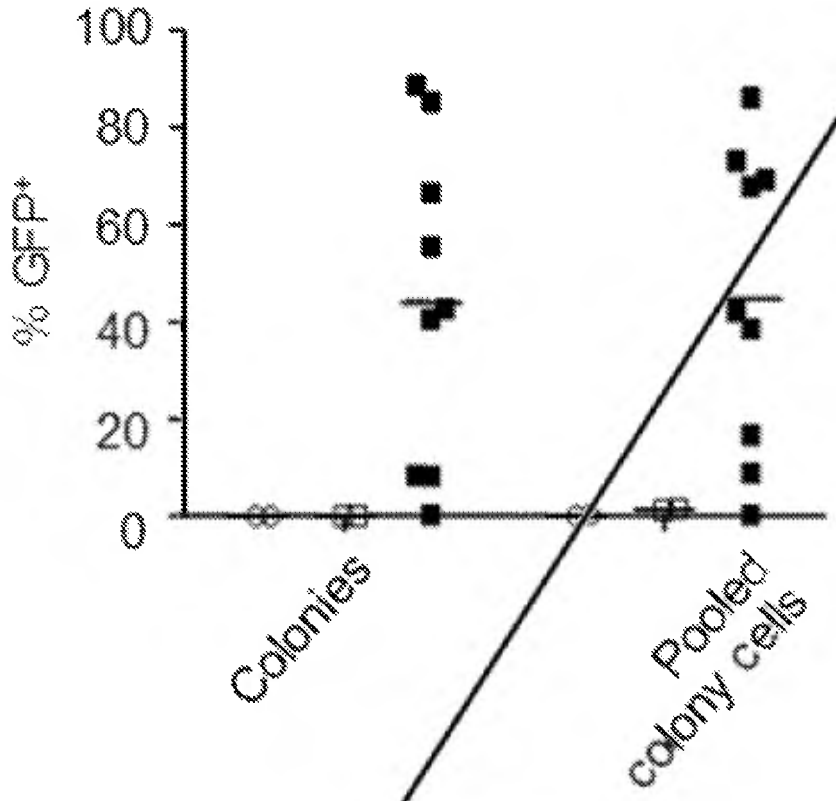


FIG. 61A

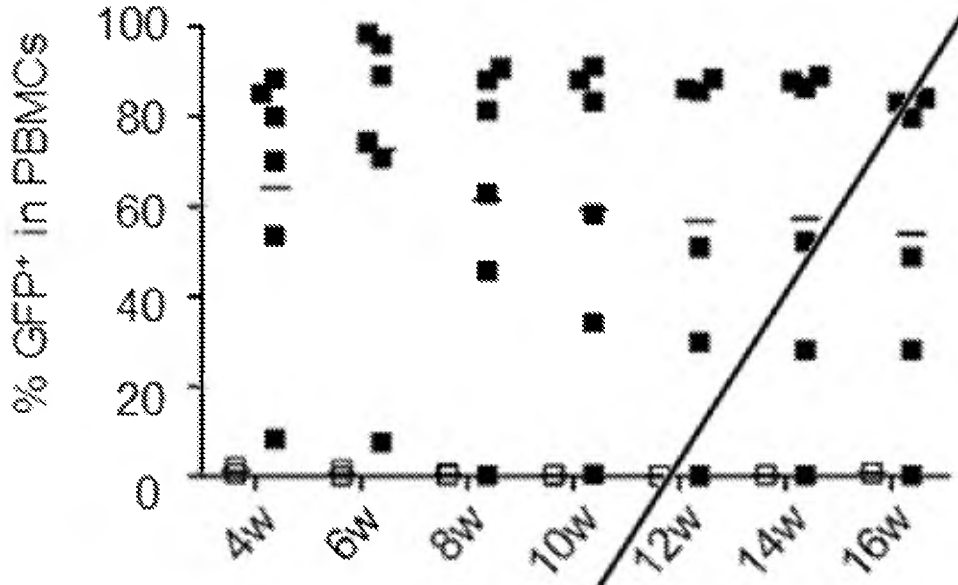


FIG. 61B

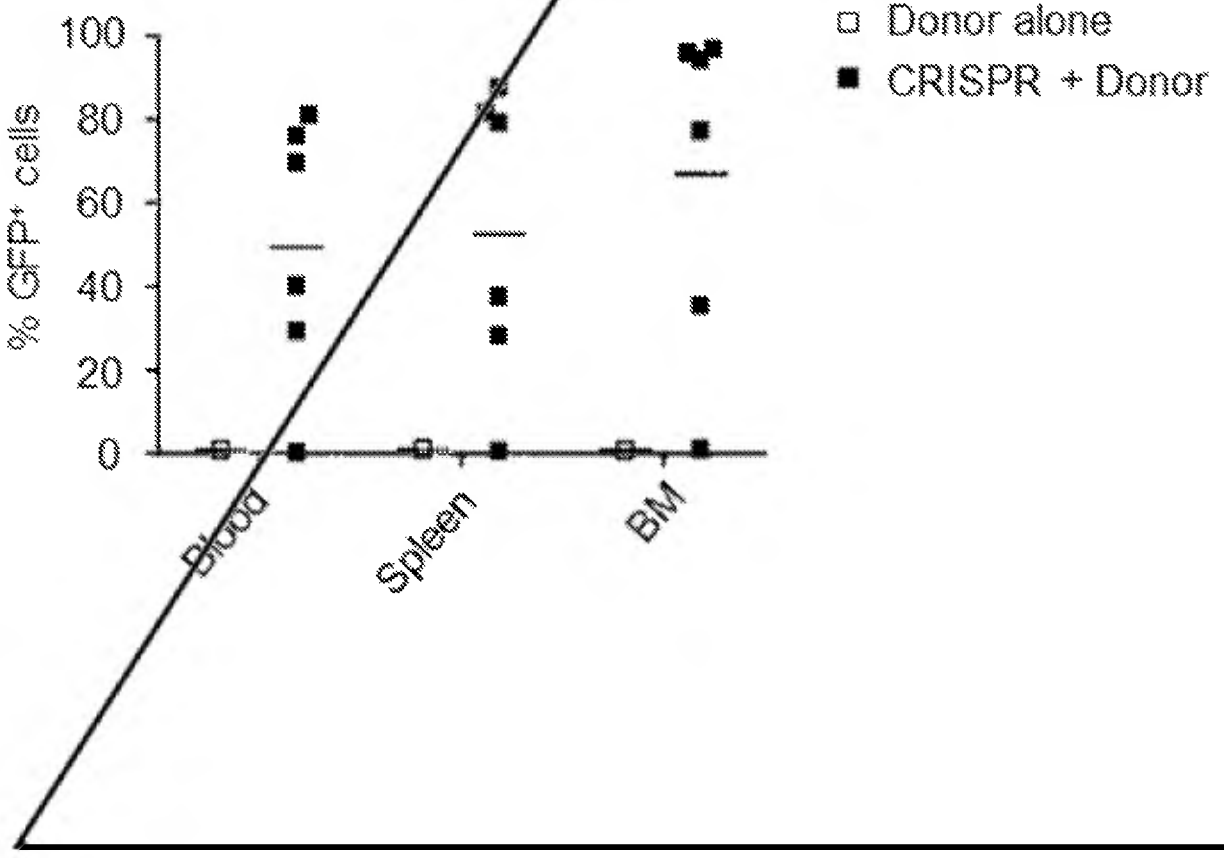


FIG. 61C

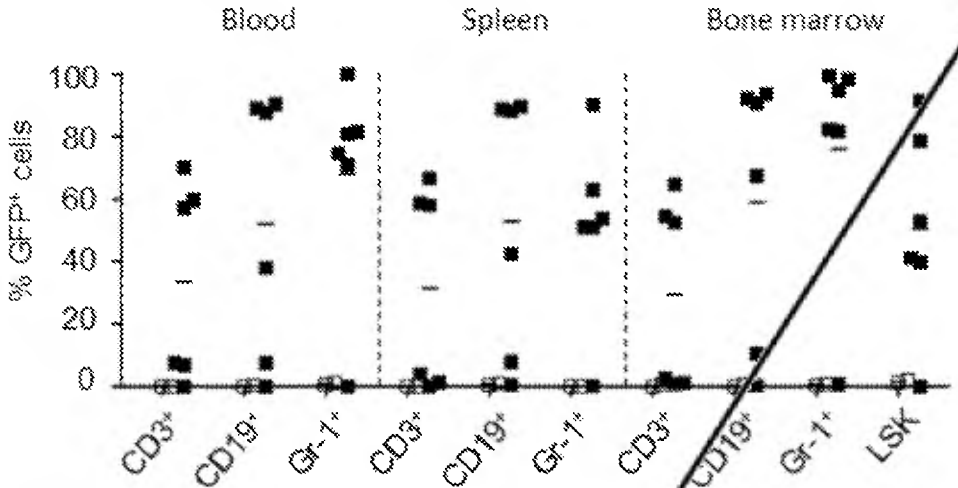


FIG. 61D

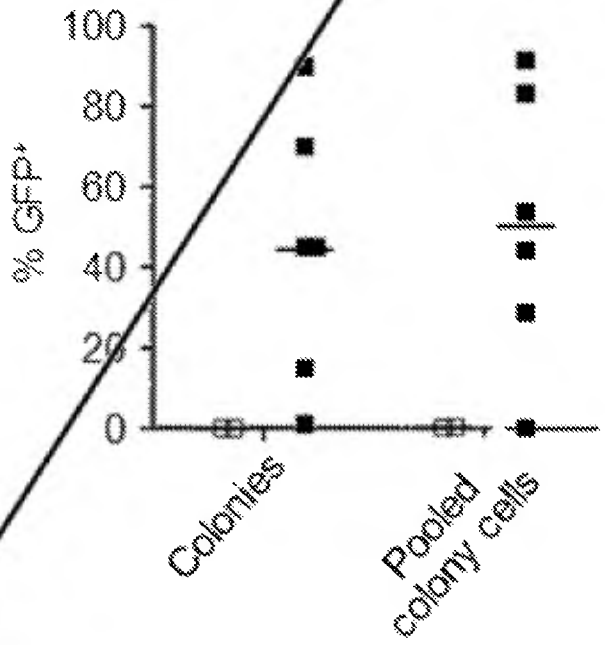


FIG. 61E

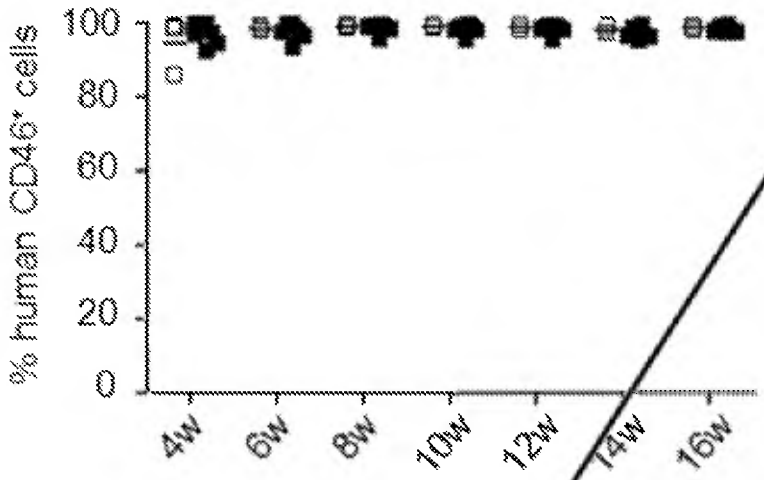


FIG. 61F

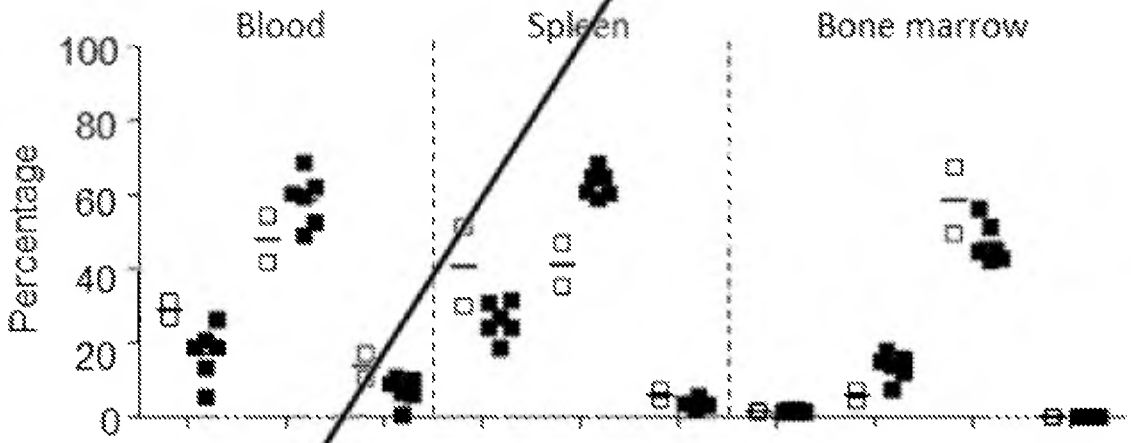


FIG. 62A

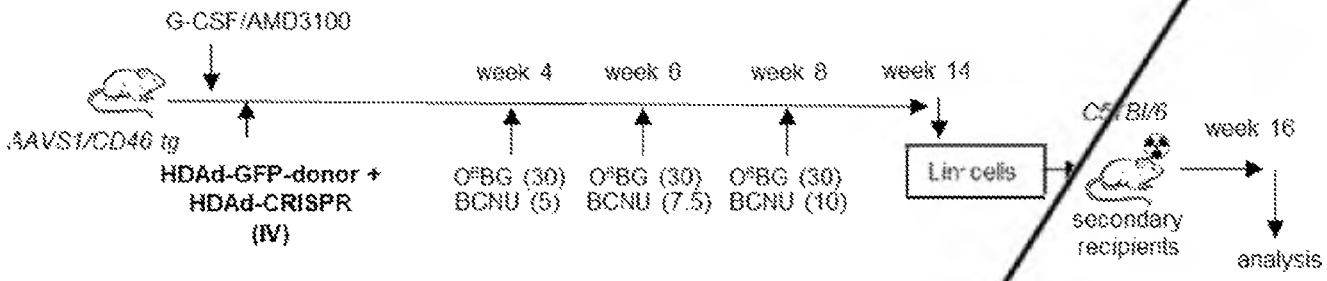


FIG. 62B

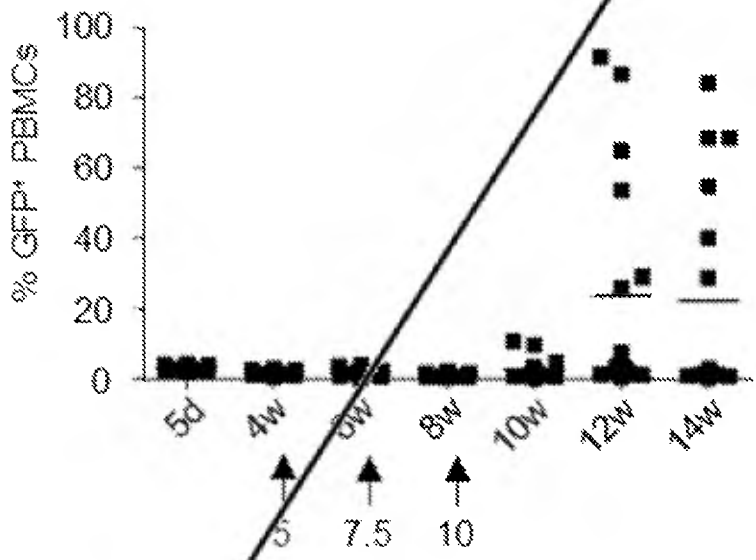


FIG. 62C

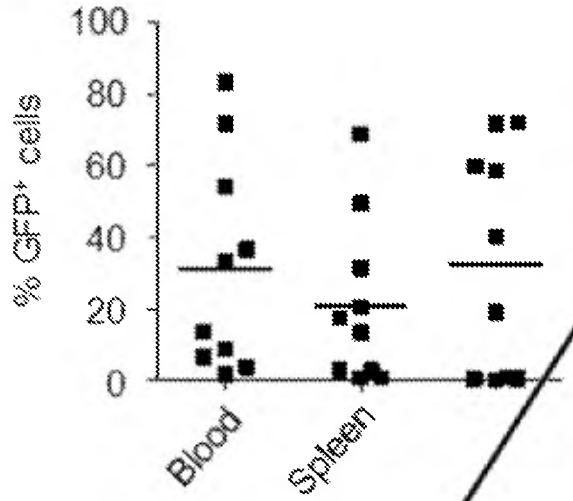


FIG. 62D

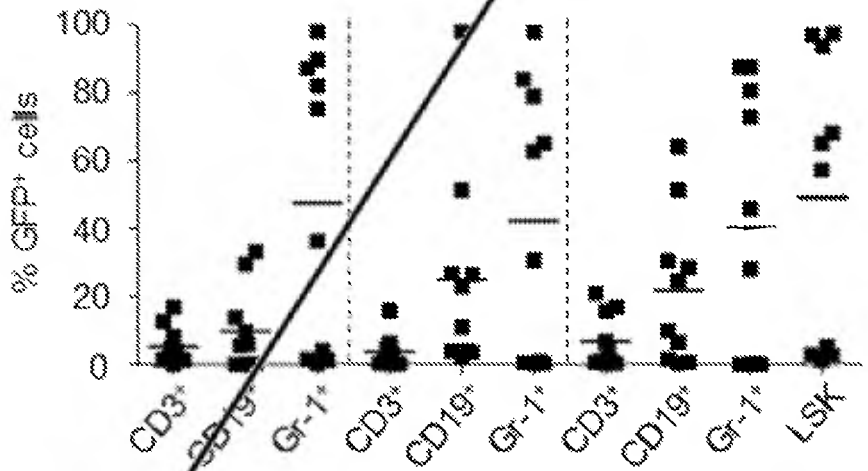


FIG. 62E

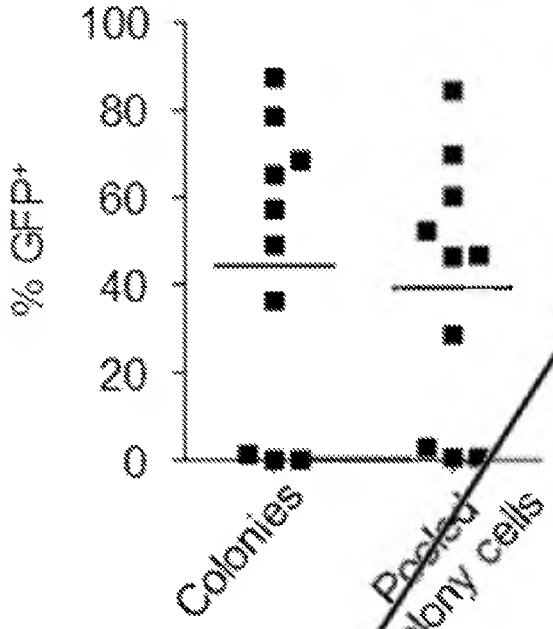


FIG. 62F

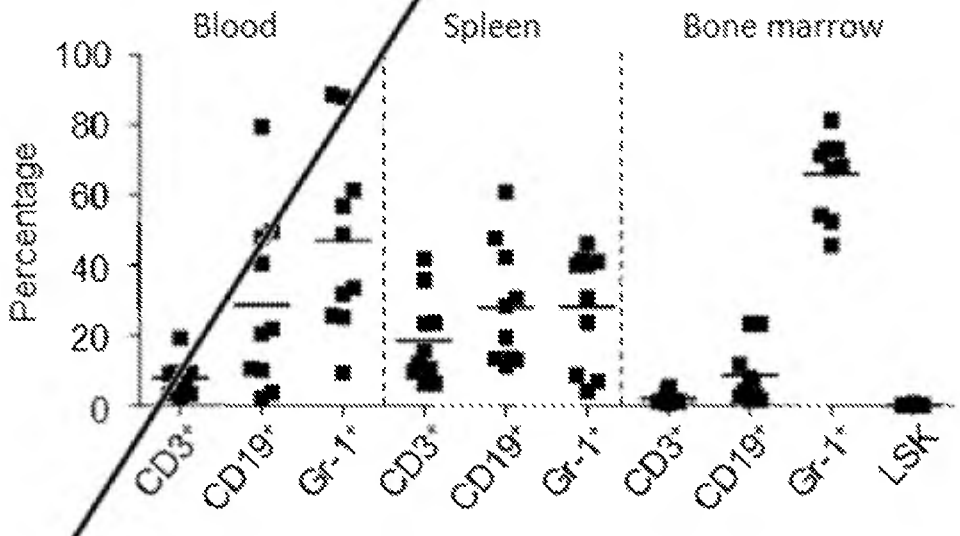


FIG. 63A

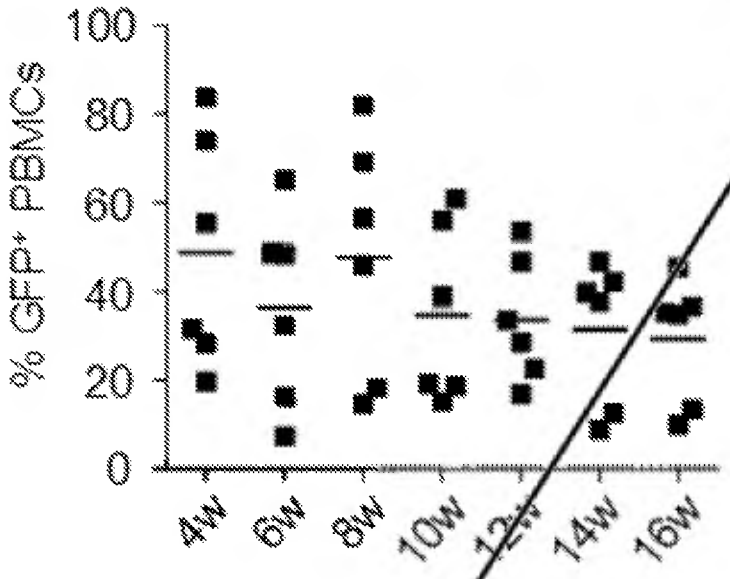


FIG. 63B

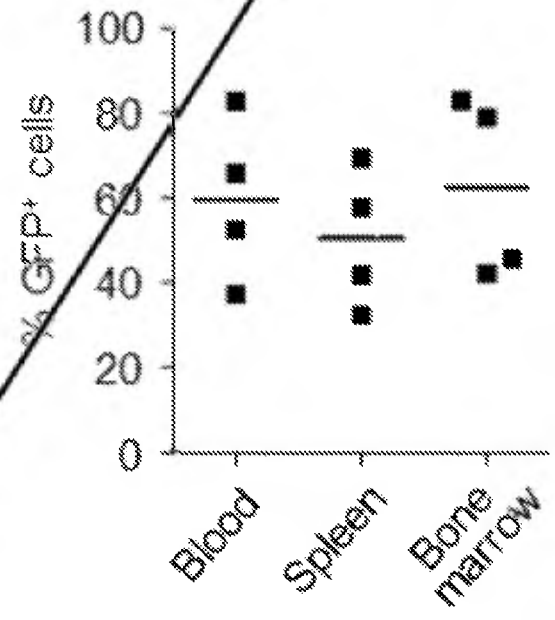


FIG. 63C

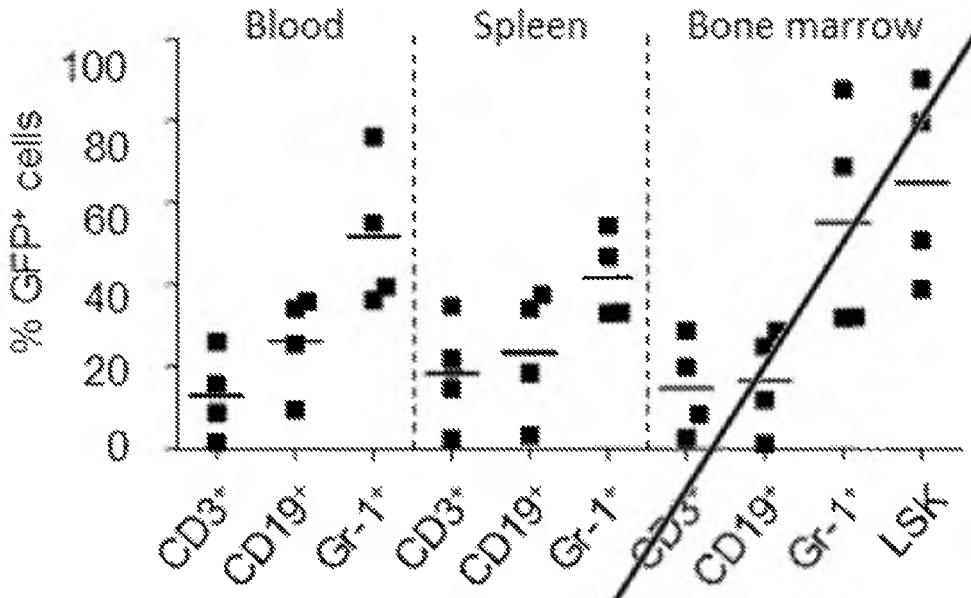


FIG. 63D

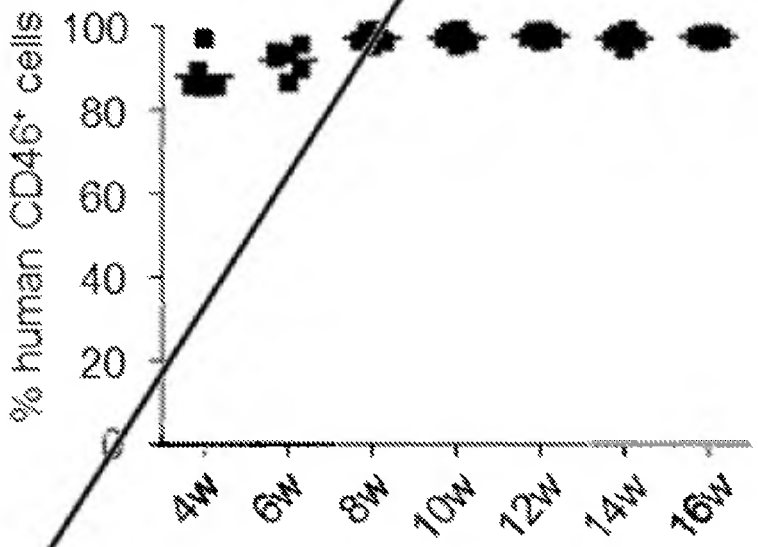


FIG. 63E

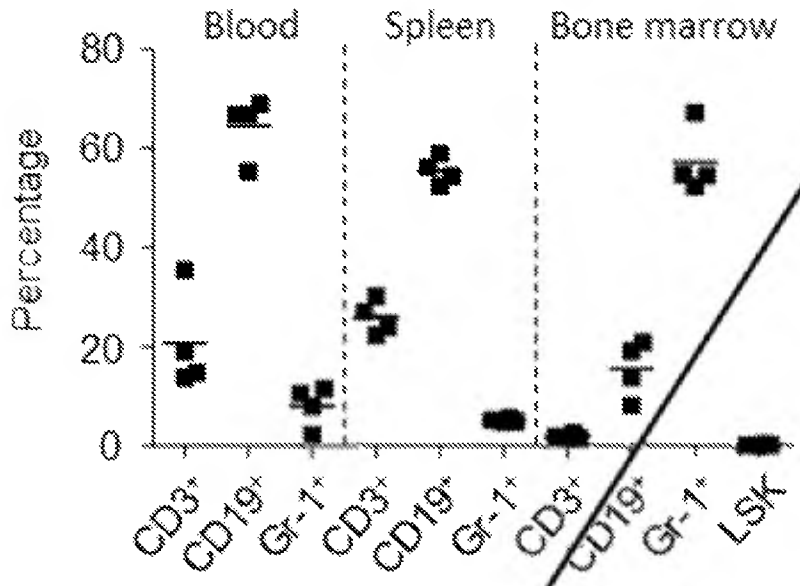


FIG. 64A

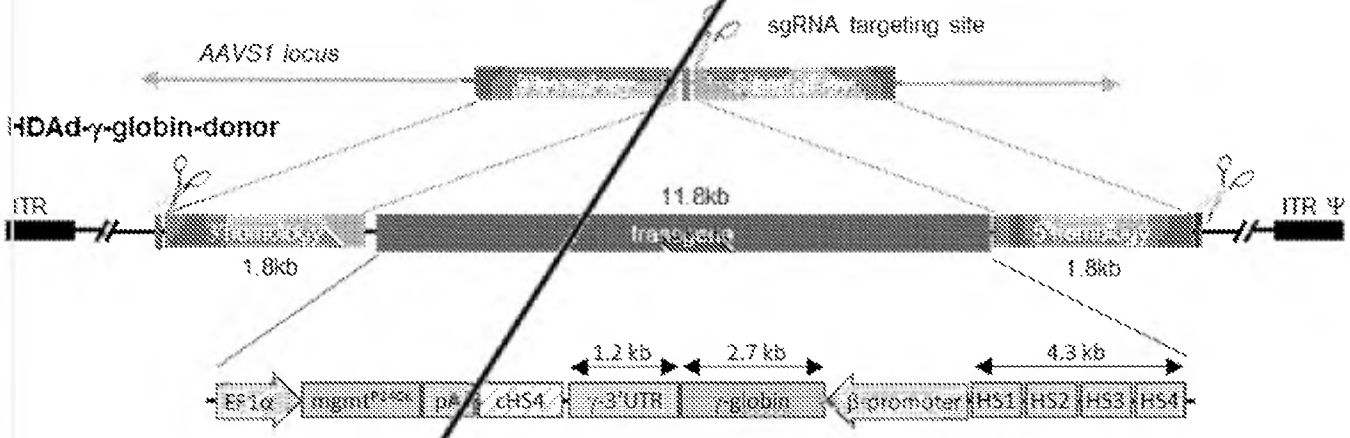


FIG. 64B

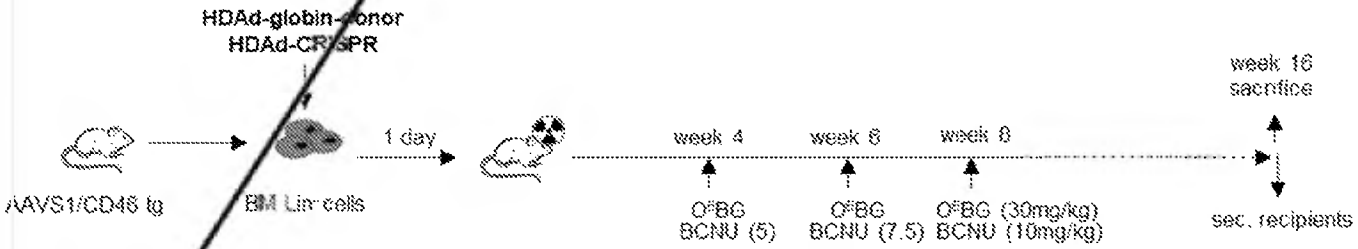


FIG. 64C

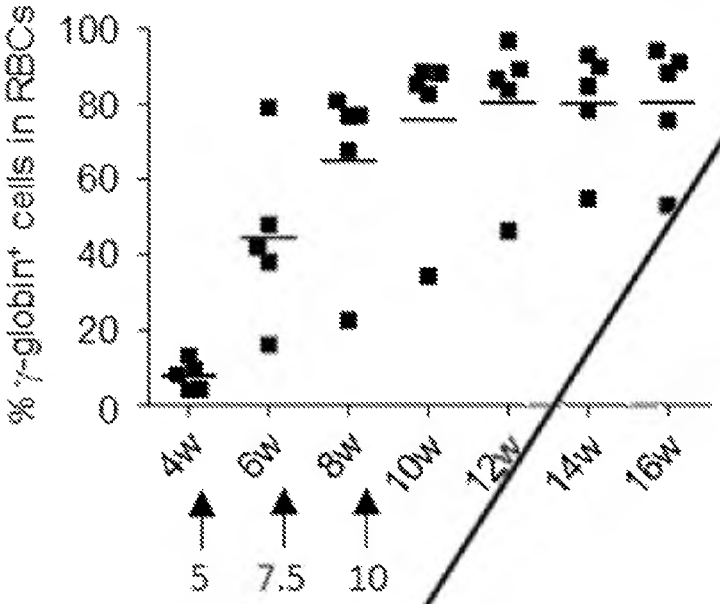


FIG. 64D

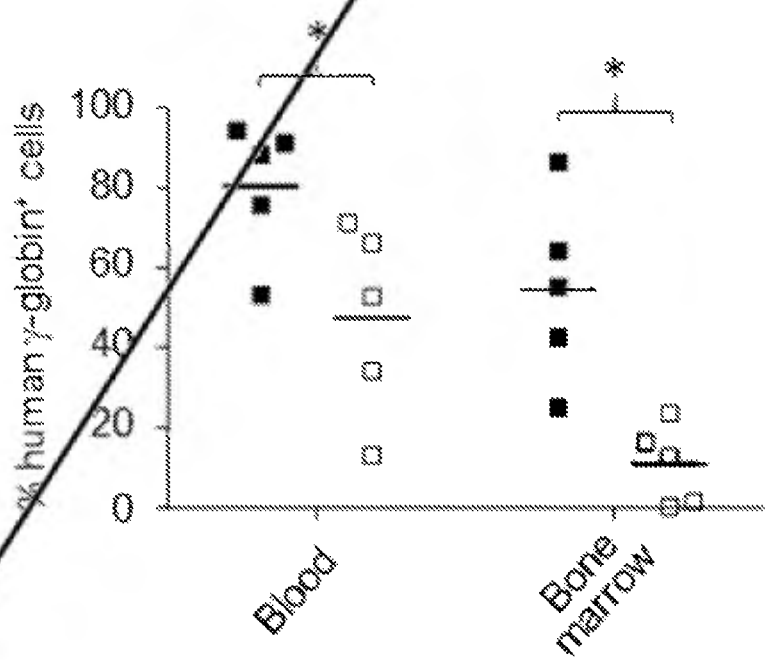


FIG. 64E

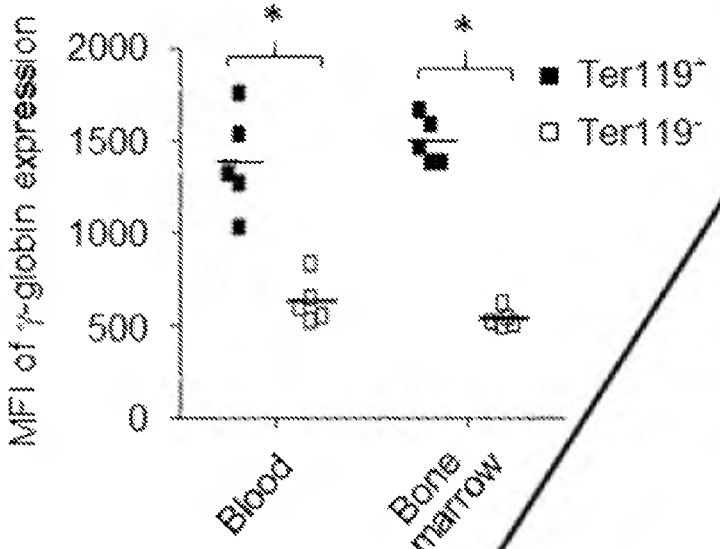


FIG. 64F

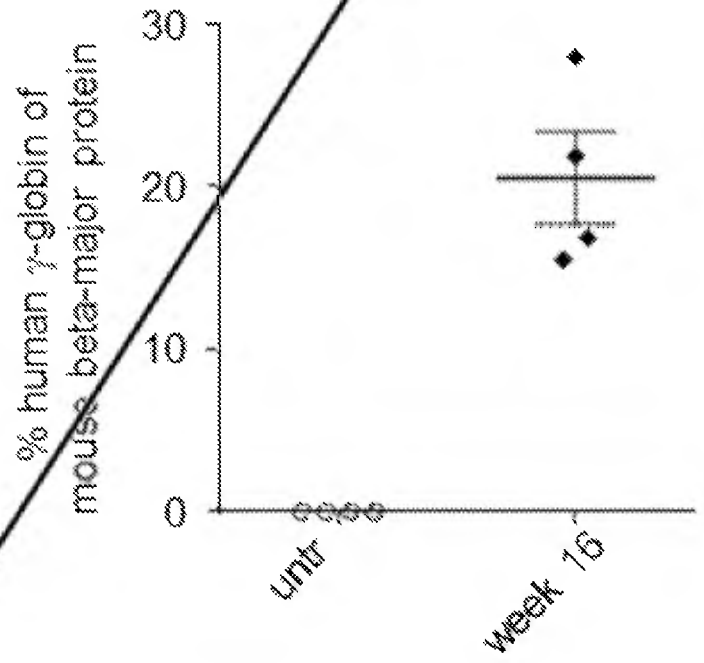


FIG. 64G

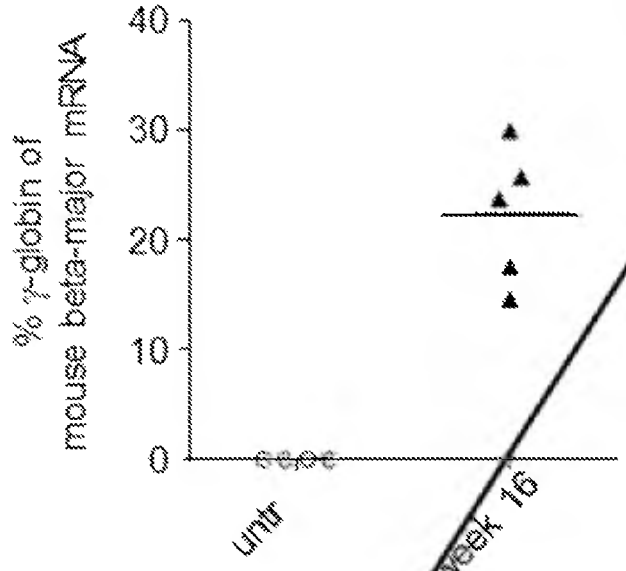


FIG. 64H

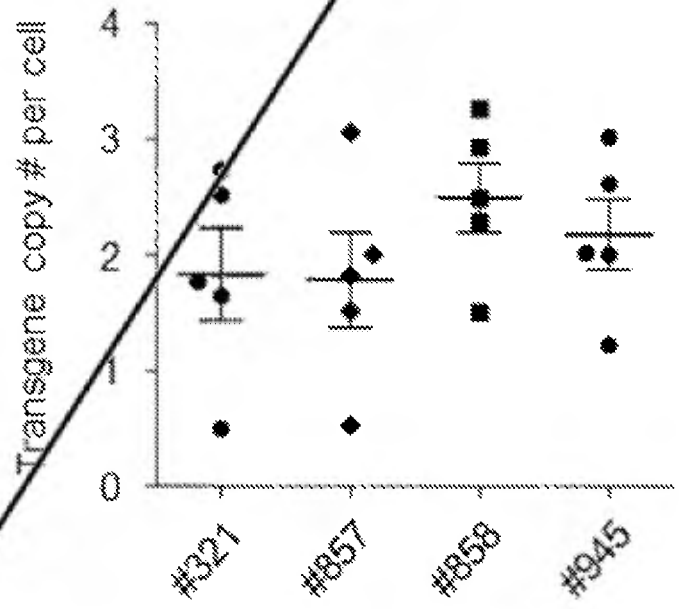


FIG. 65A

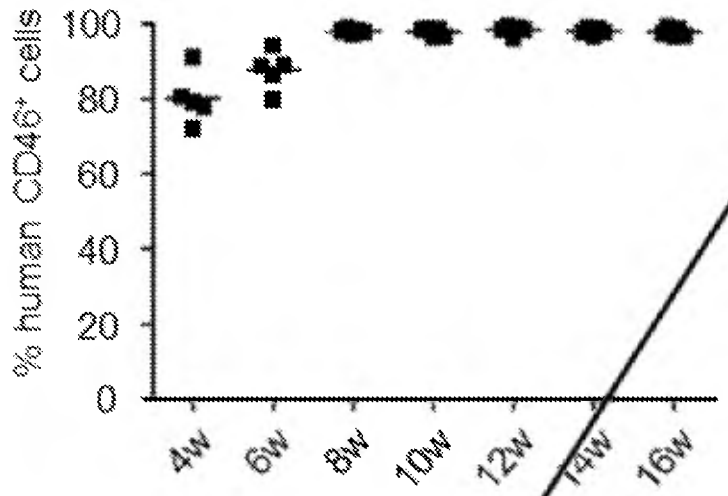


FIG. 65B

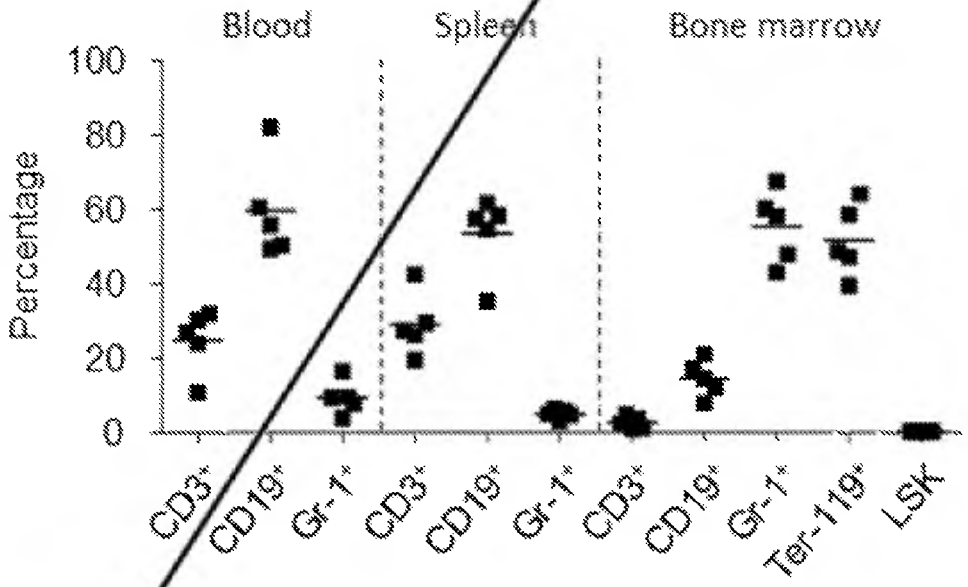


FIG. 66A

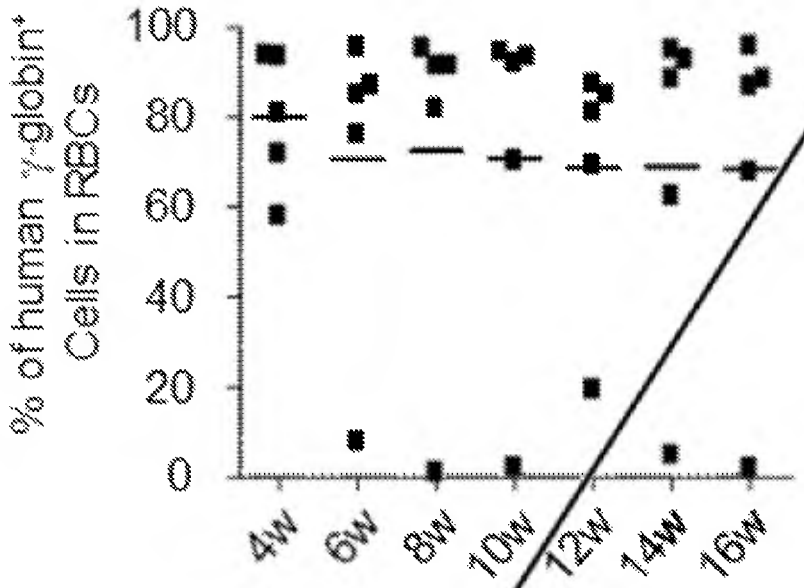


FIG. 66B

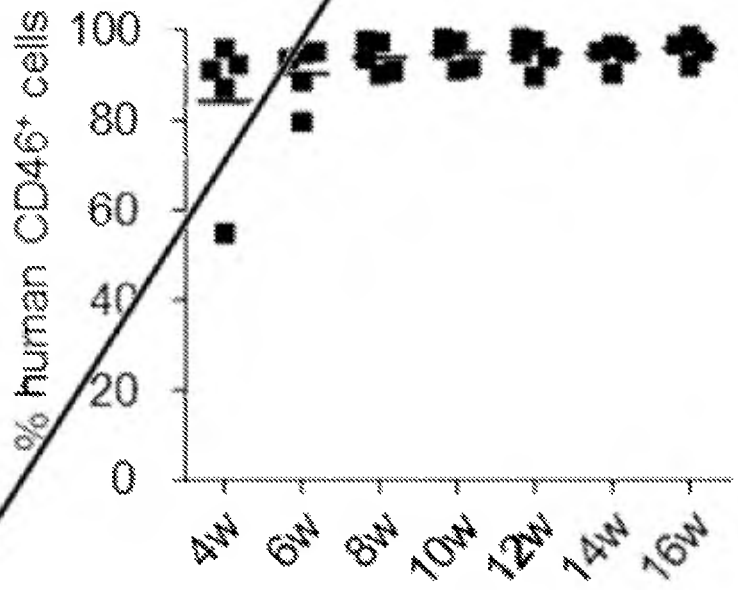


FIG. 66C

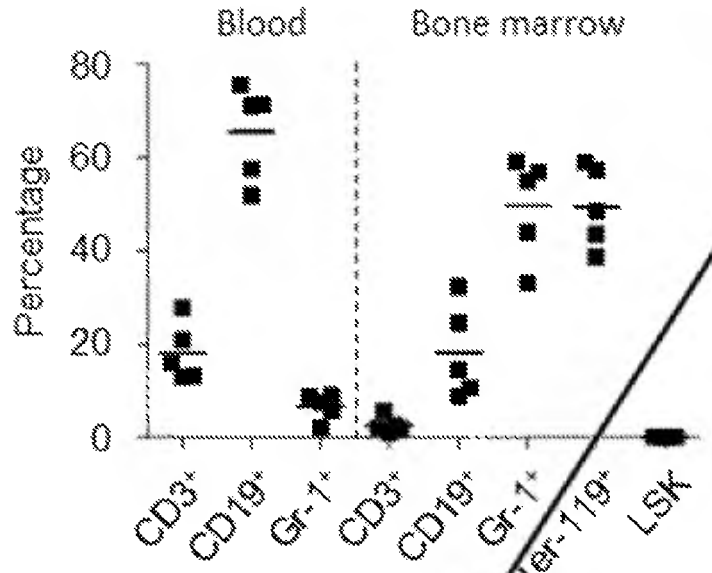

Blood
Bone marrow
Percentage
80
60
40
20
0
CD3+
CD19+
Gr-1+
CD3+
CD19+
Gr-1+
Ter-119+
LSK

FIG. 67A

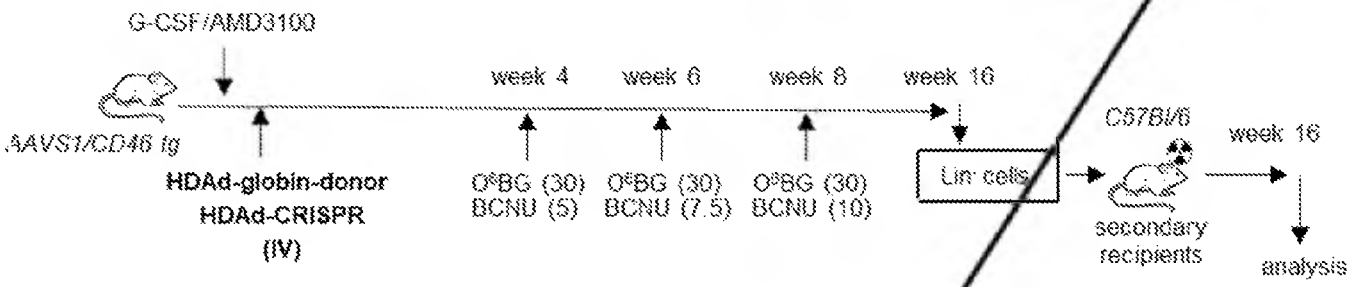


FIG. 67B

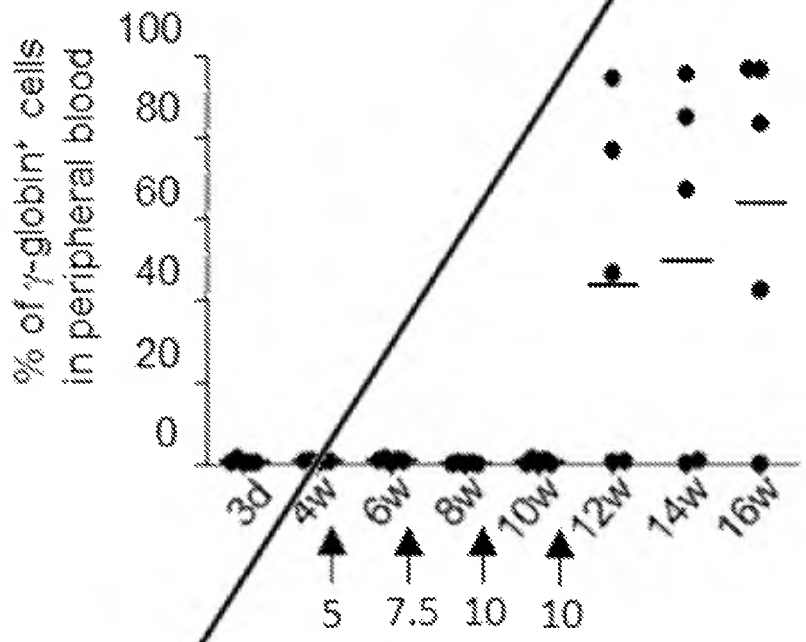


FIG. 67C

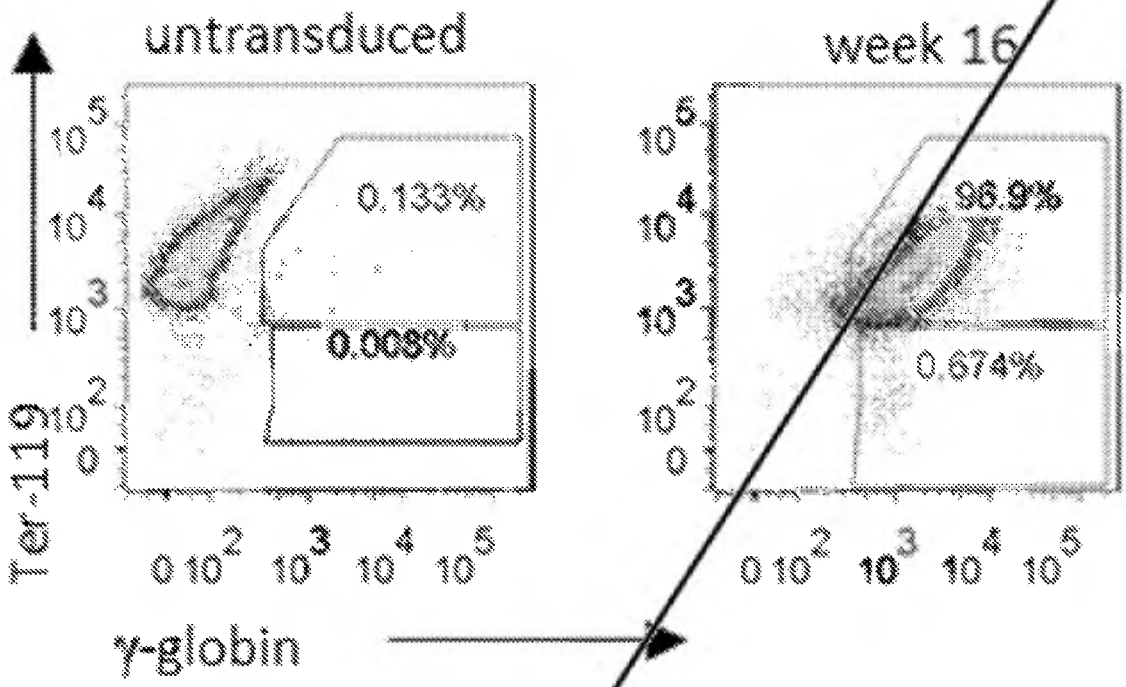


FIG. 67D

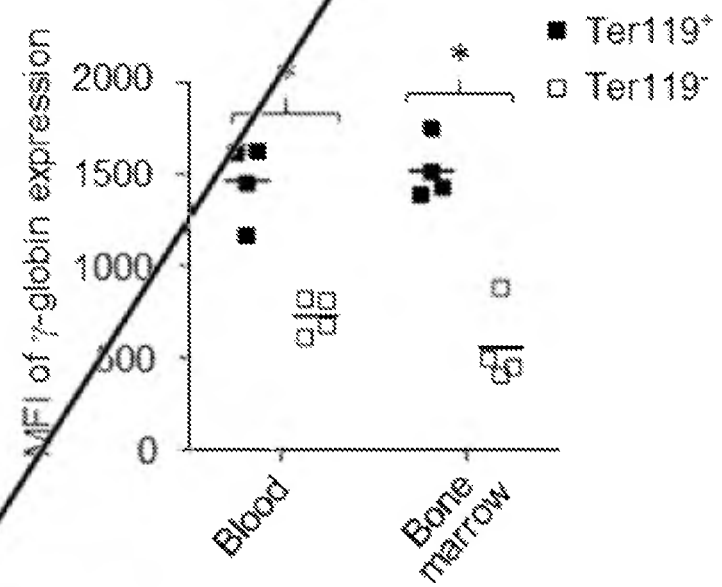


FIG. 67E

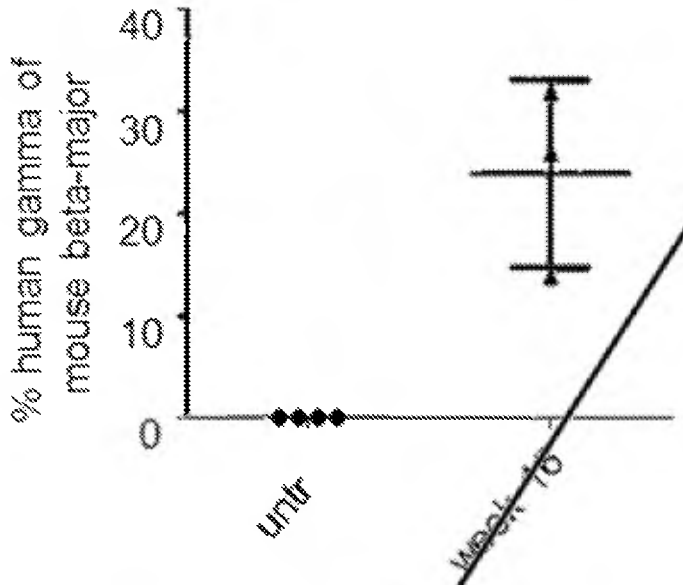


FIG. 67F

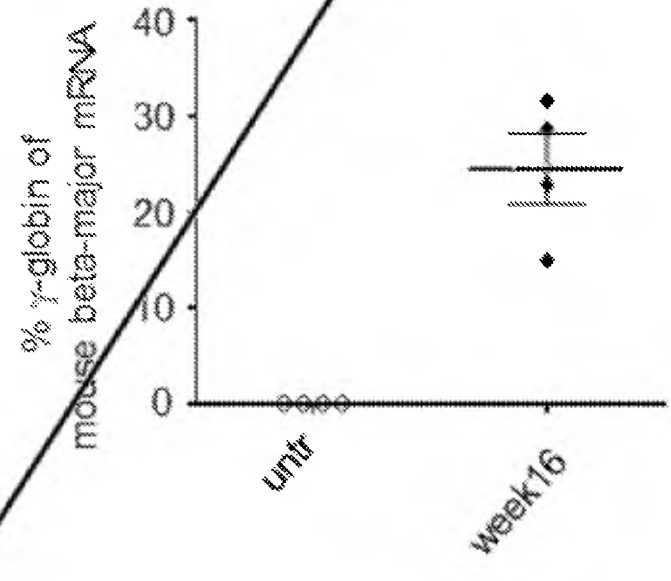


FIG. 67G

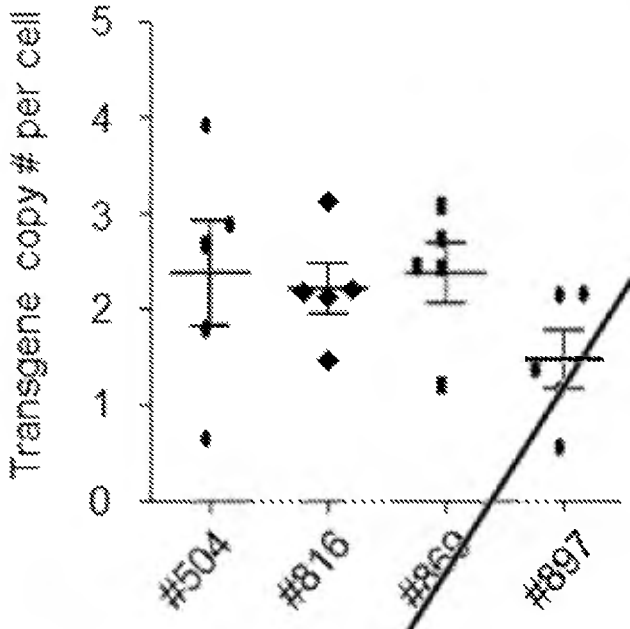


FIG. 67H

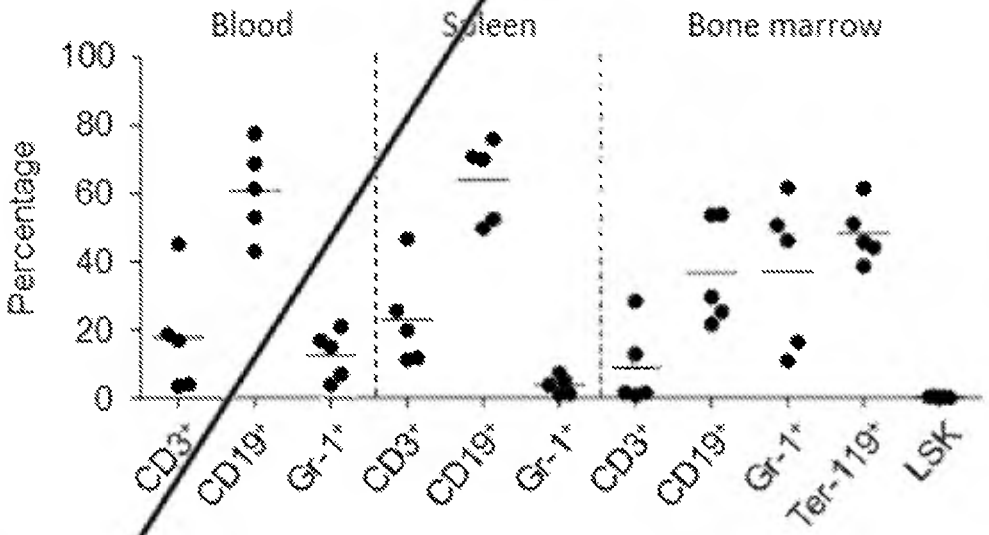


FIG. 68A

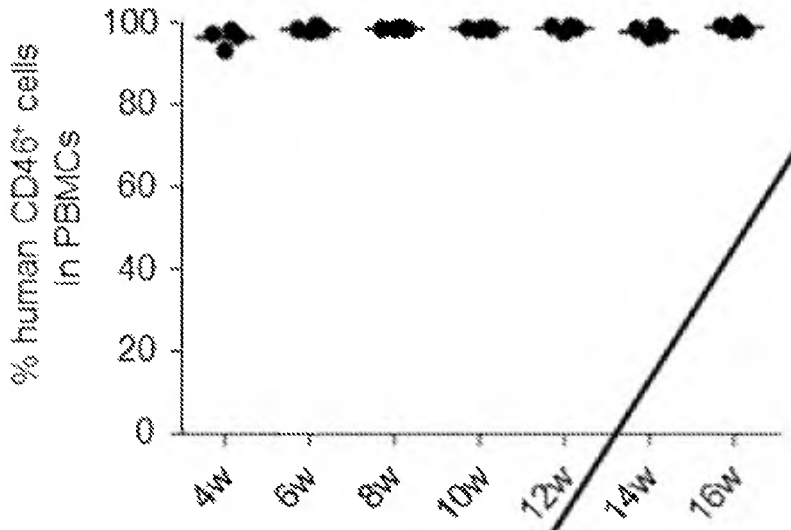


FIG. 68B

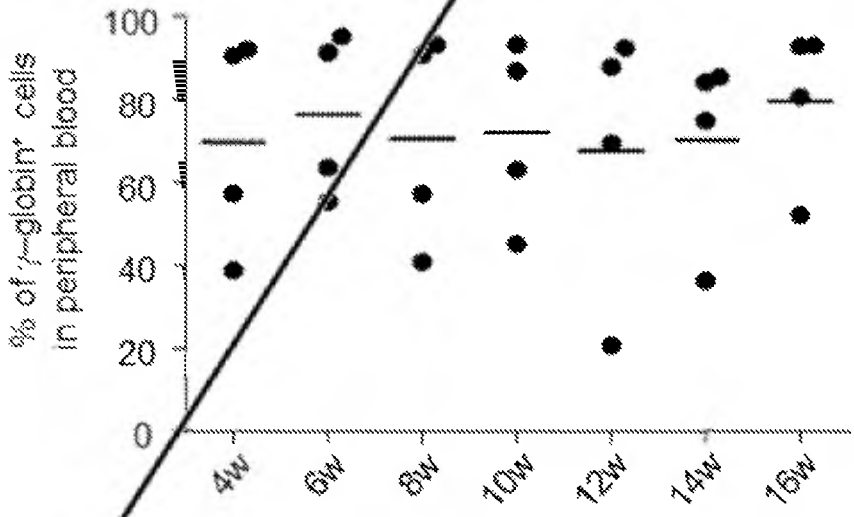


FIG. 68C

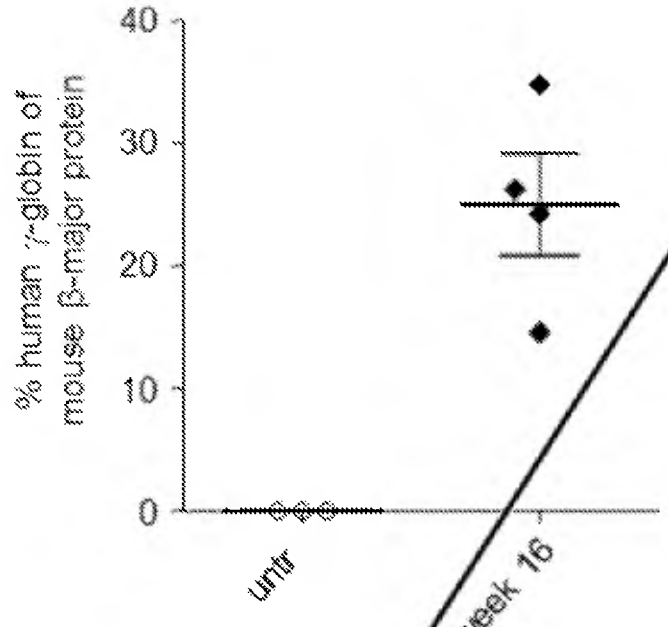


FIG. 68D

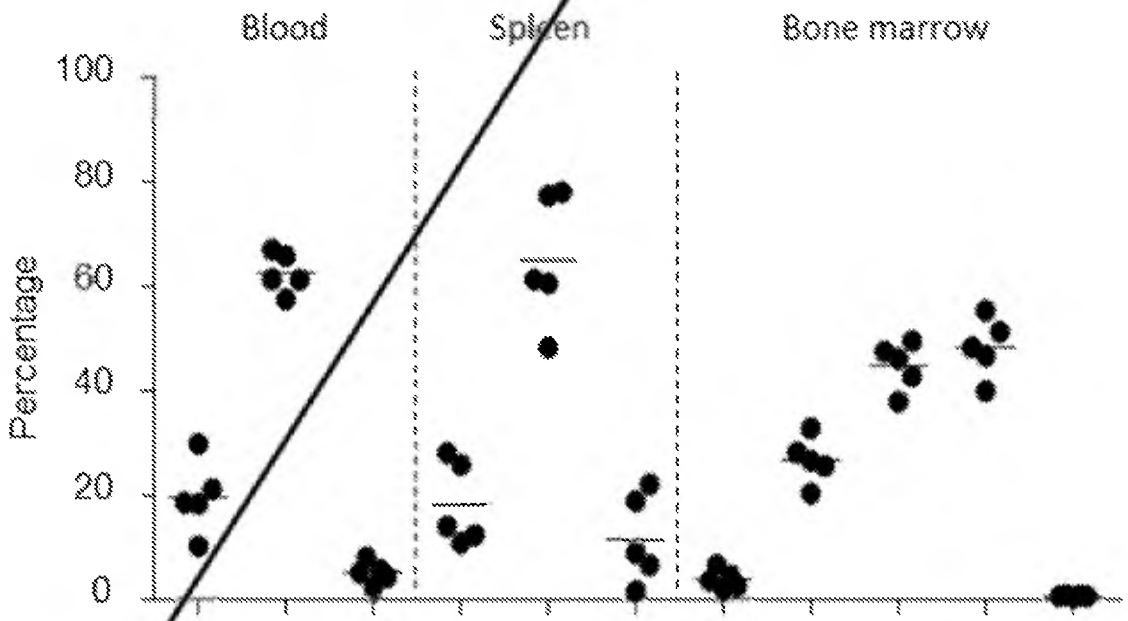


FIG. 69A

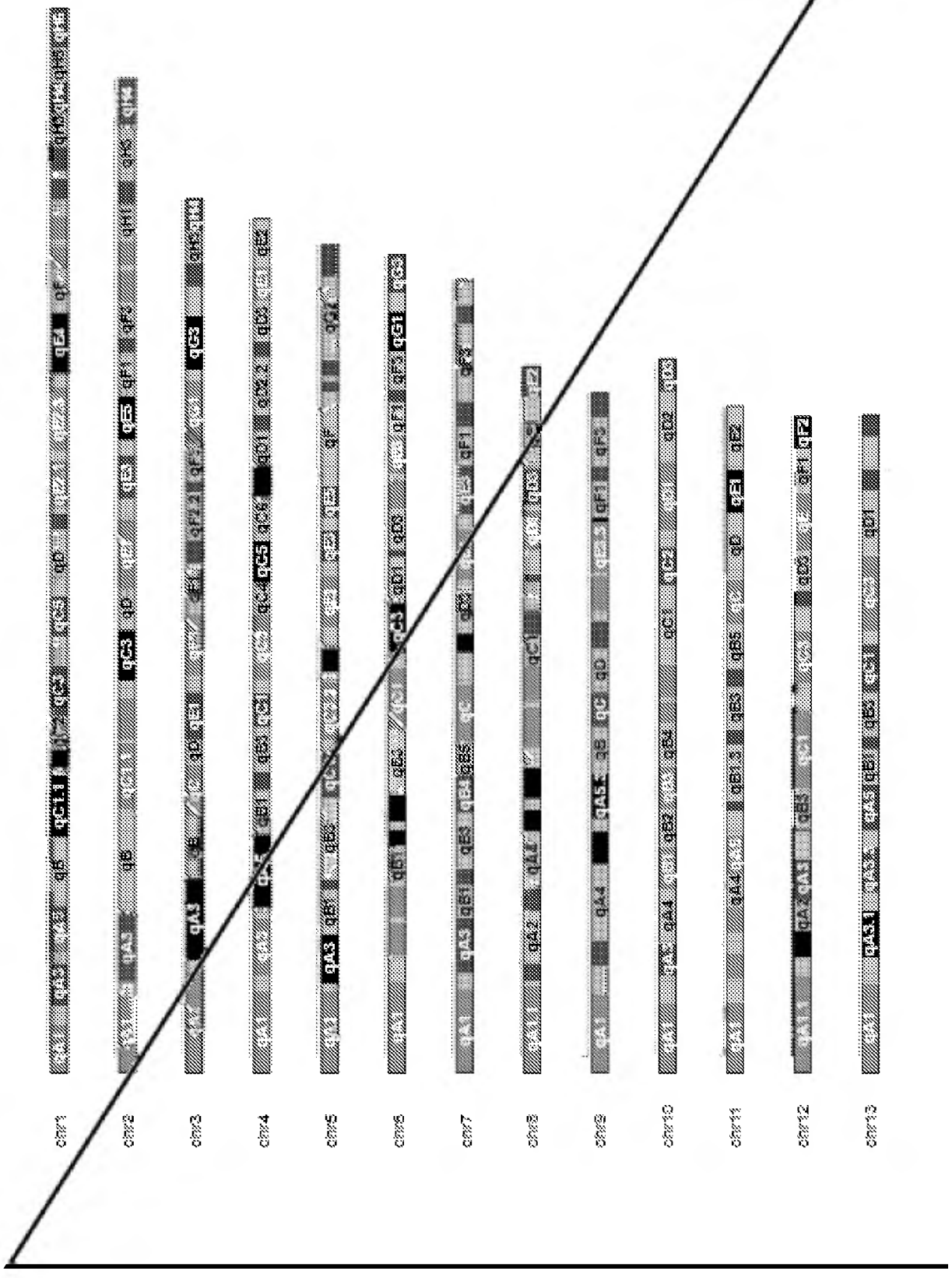

chr1
chr2
chr3
chr4
chr5
chr6
chr7
chr8
chr9
chr10
chr11
chr12
chr13

FIG. 69B

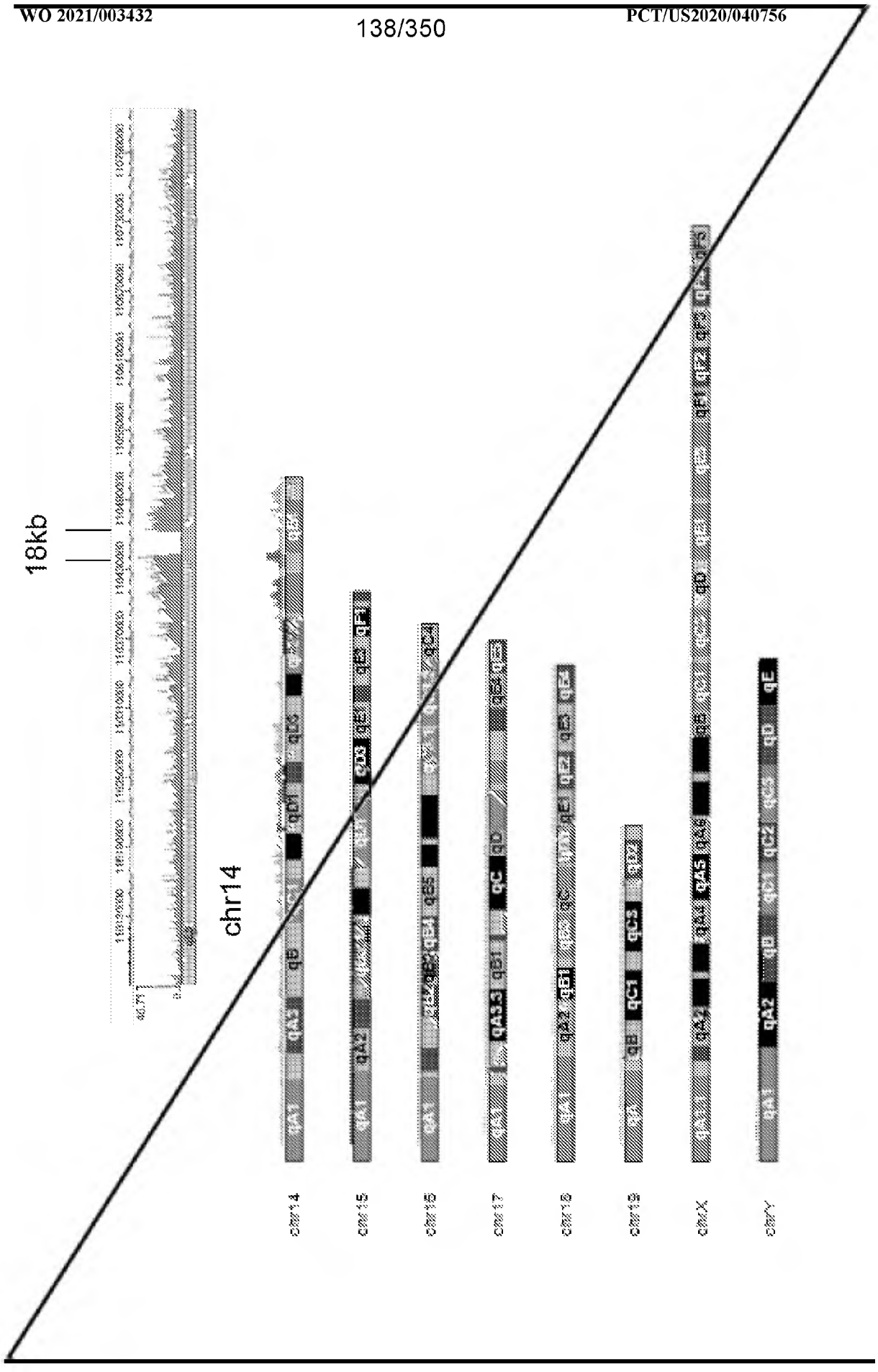


FIG. 70

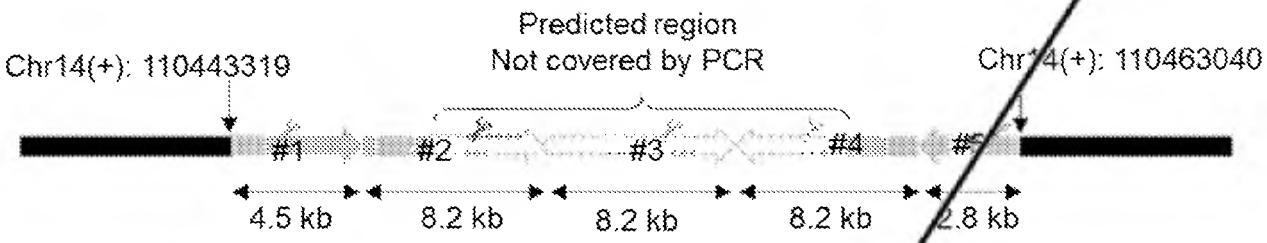


FIG. 71A

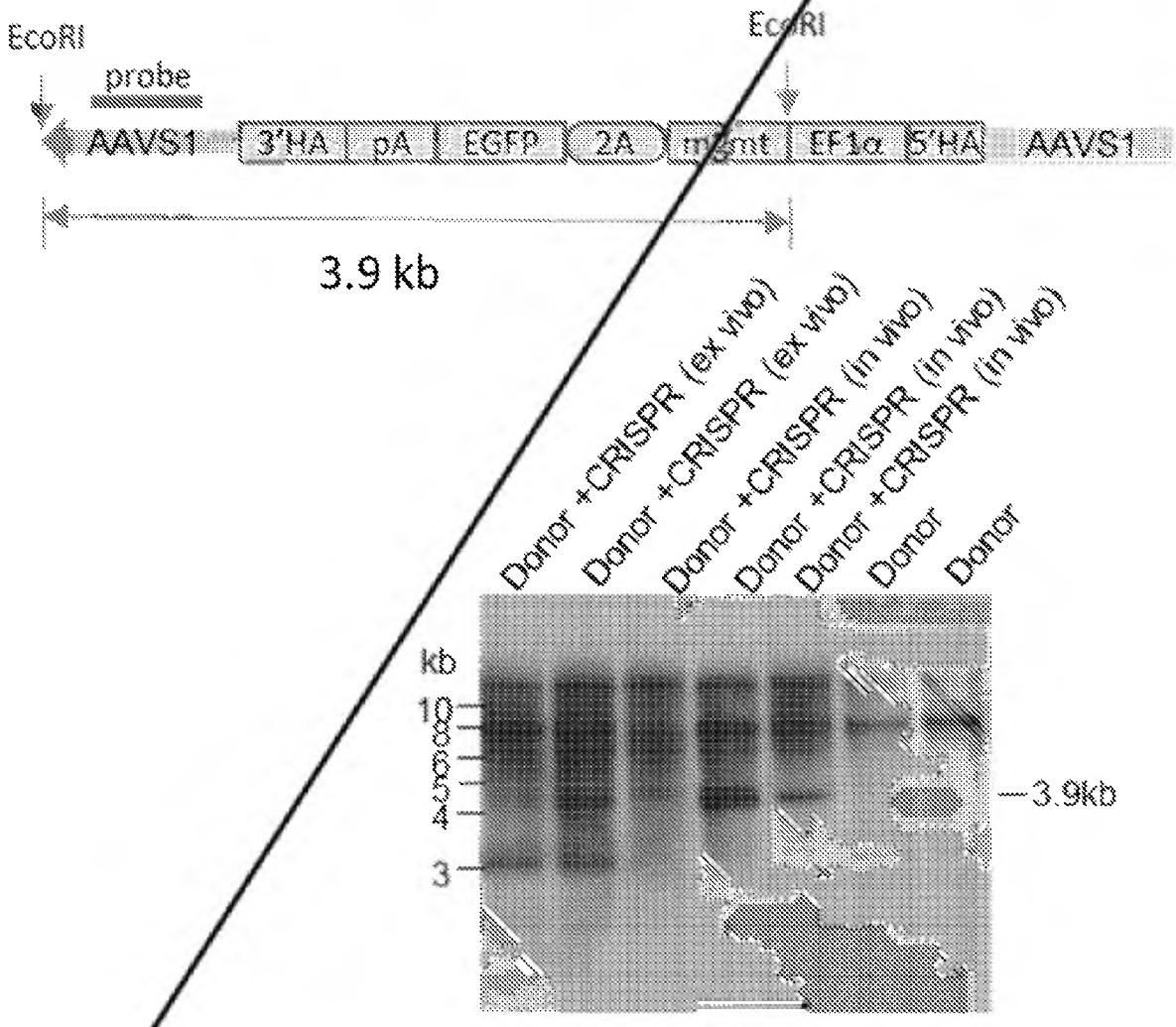


FIG. 71B

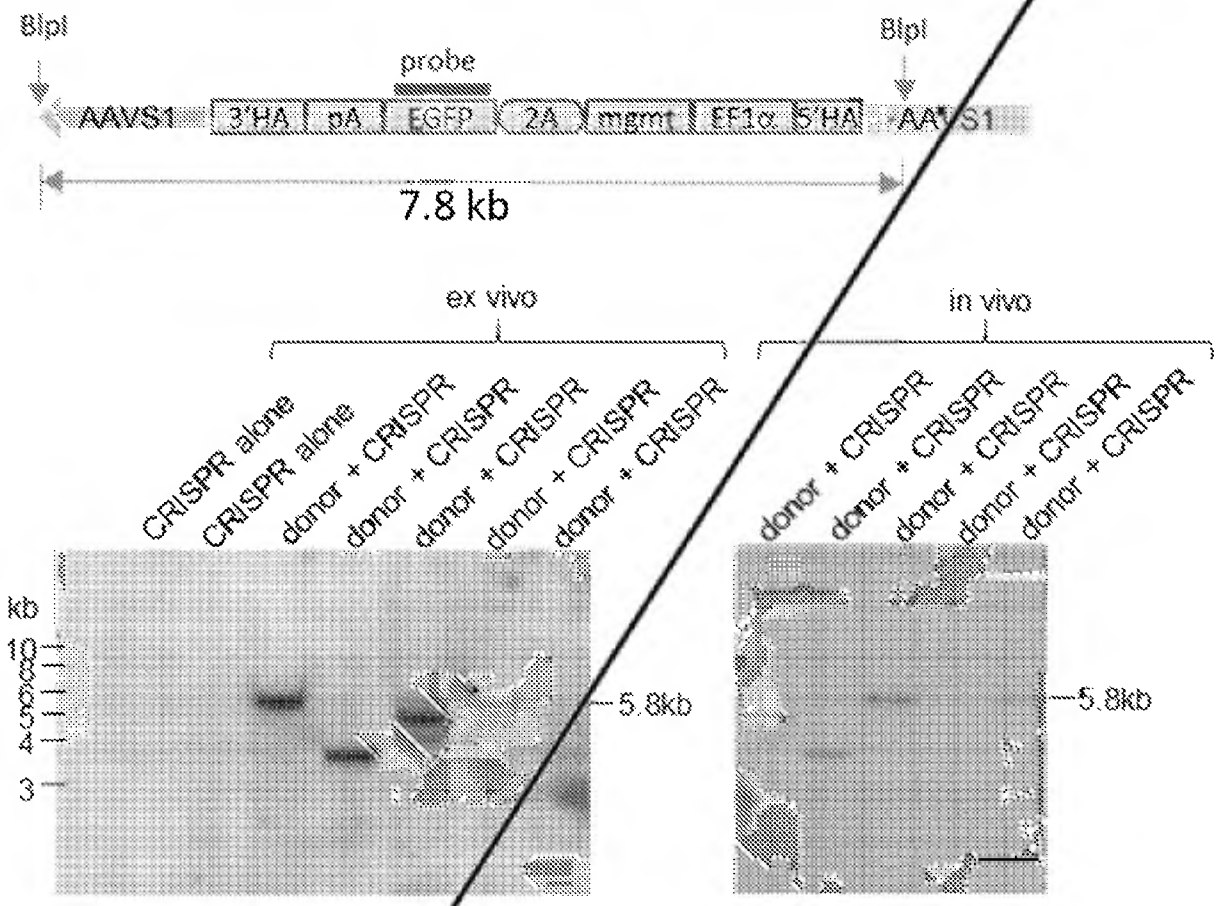

BlpI
probe
BlpI
AAVS1
3'HA
pA
EGFP
2A
mgmt
EF1α
5'HA
AAVS1
7.8 kb
ex vivo
in vivo
CRISPR alone
CRISPR alone
donor + CRISPR
donor + CRISPR
donor + CRISPR
donor + CRISPR
donor + CRISPR
donor + CRISPR
donor + CRISPR
donor + CRISPR
donor + CRISPR
donor + CRISPR
kb
10
4
3
5.8kb
5.8kb

FIG. 72A

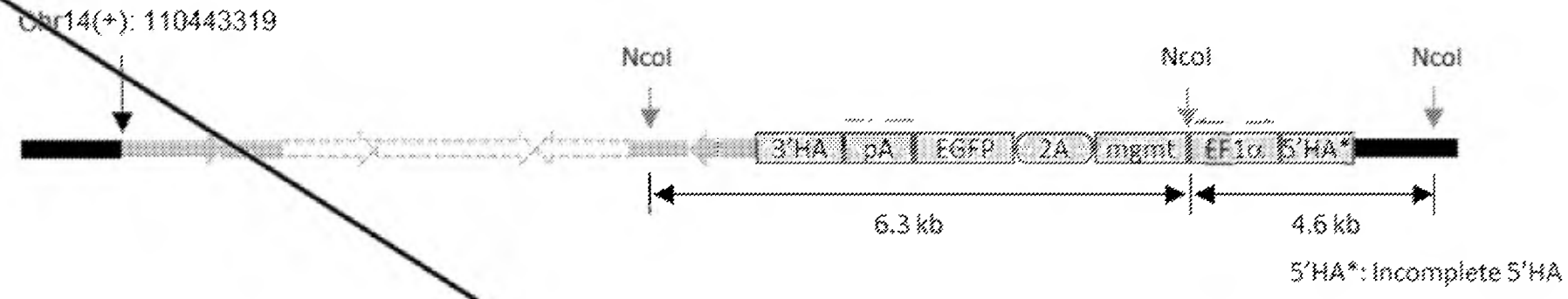


FIG. 72B

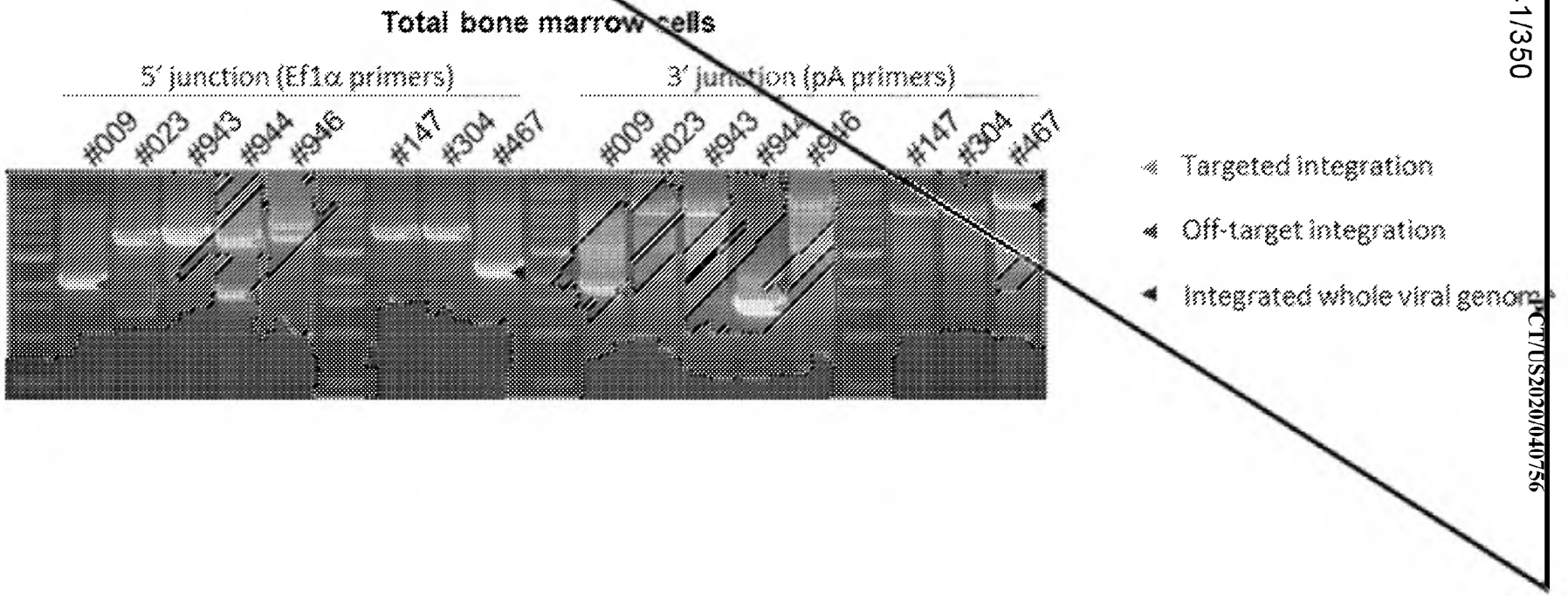


FIG. 72C

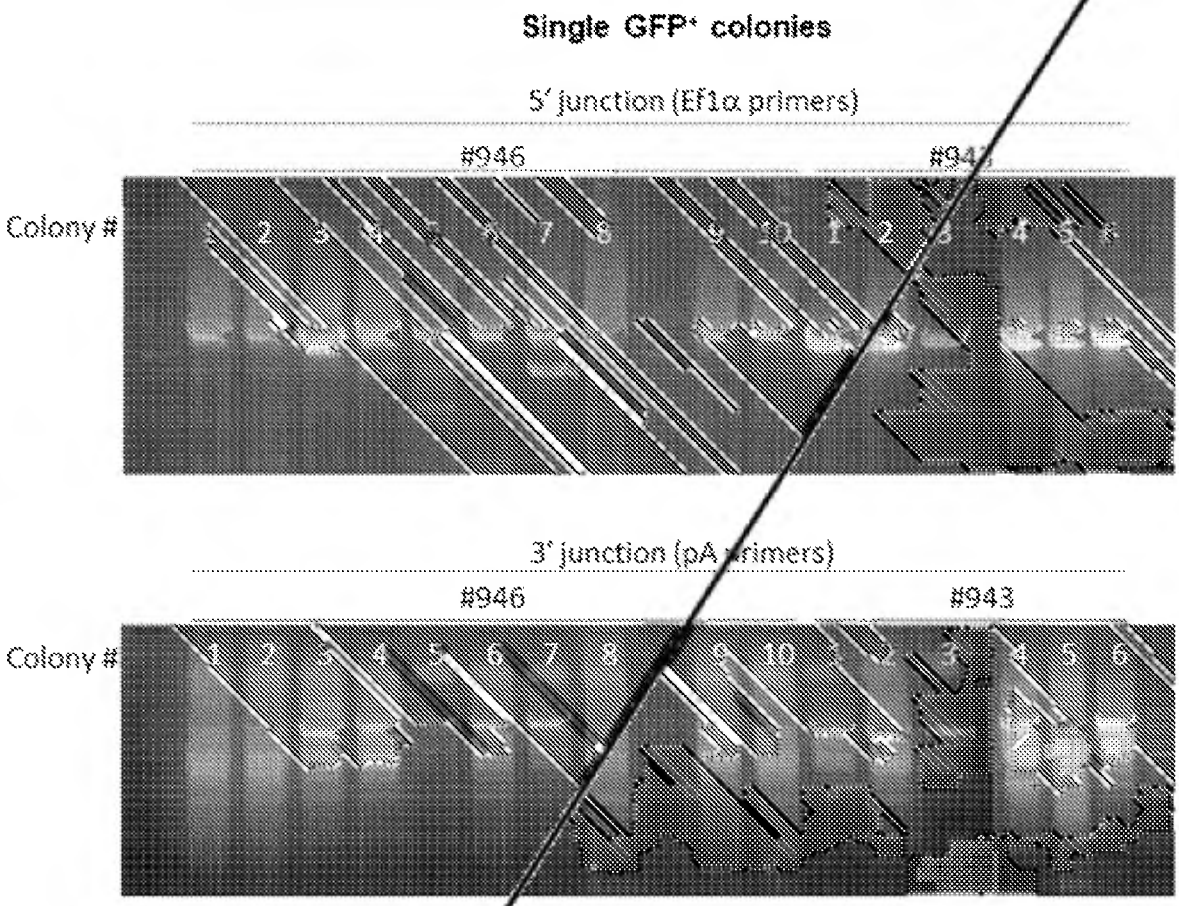

Single GFP+ colonies
5' junction (Ef1α primers)
#946
#943
Colony #
1 2 3 4 5 6 7 8 9 10 1 2 3 4 5 6
3' junction (pA primers)
#946
#943
Colony #
1 2 3 4 5 6 7 8 9 10 1 2 3 4 5 6

FIG. 73A

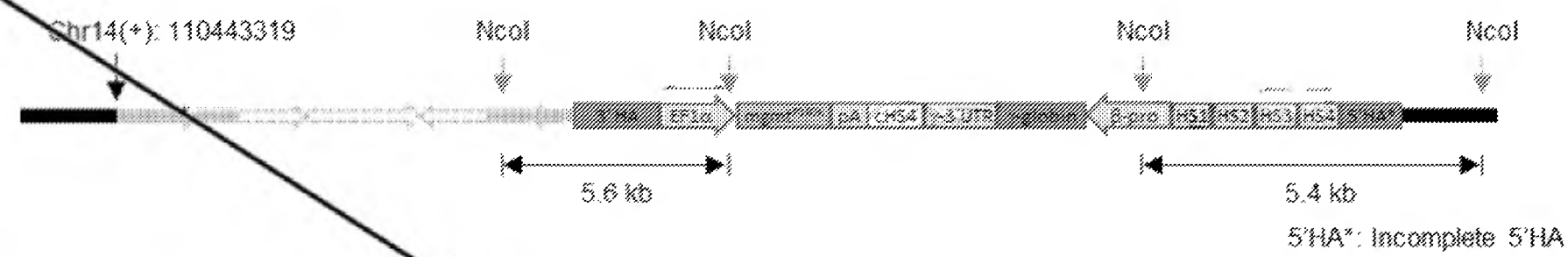


FIG. 73B

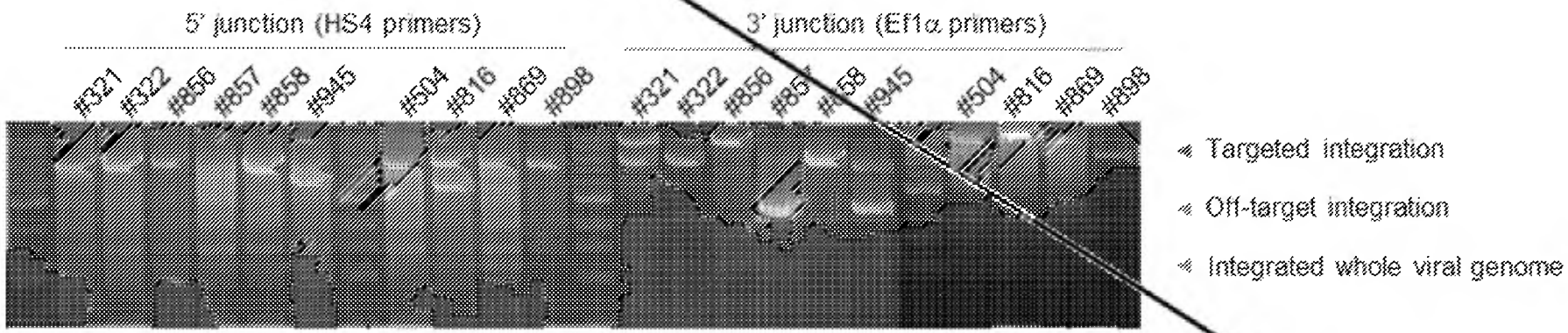


FIG. 74A

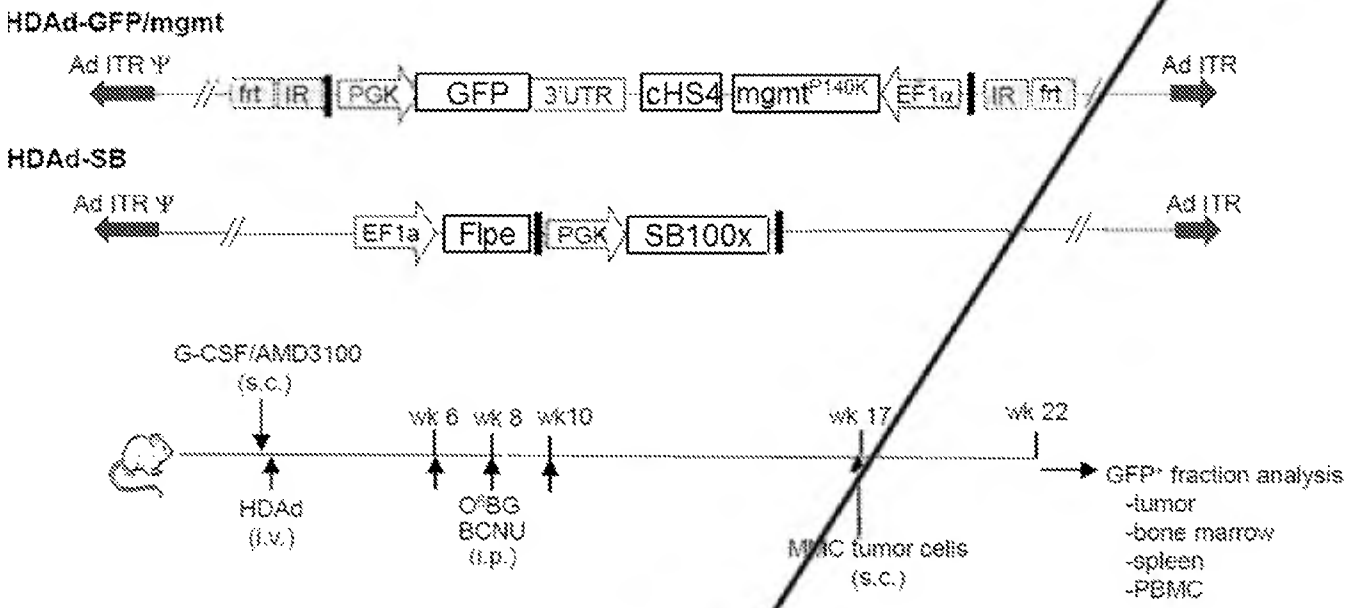


FIG. 74B

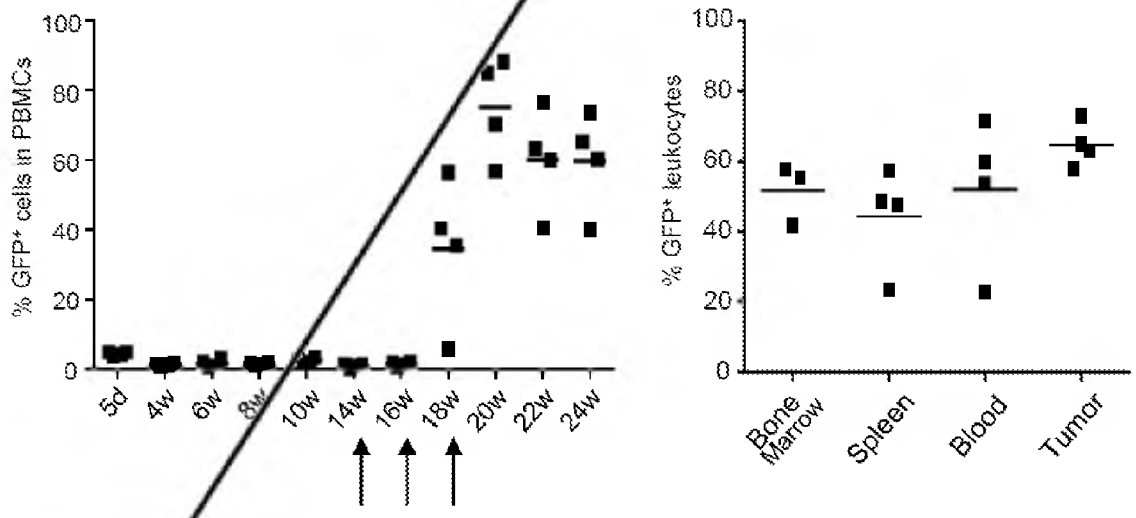


FIG. 74C

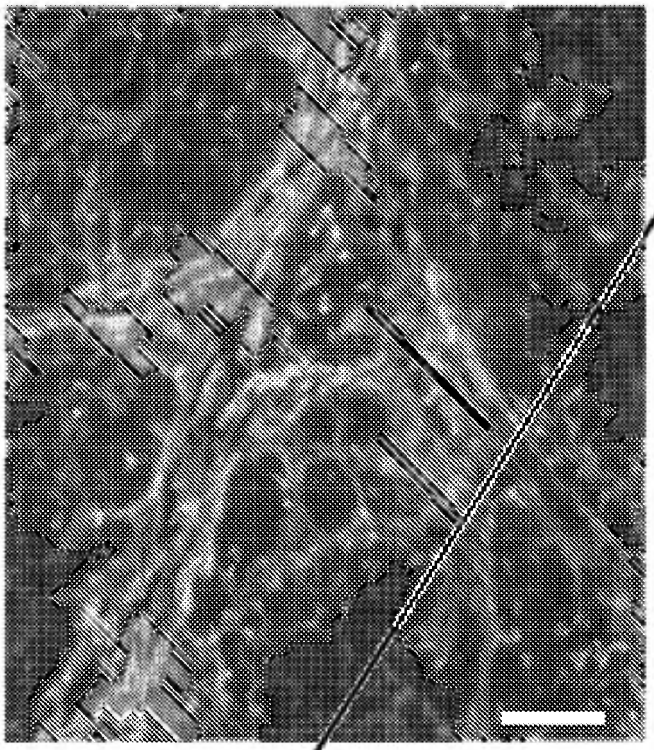


FIG. 74D

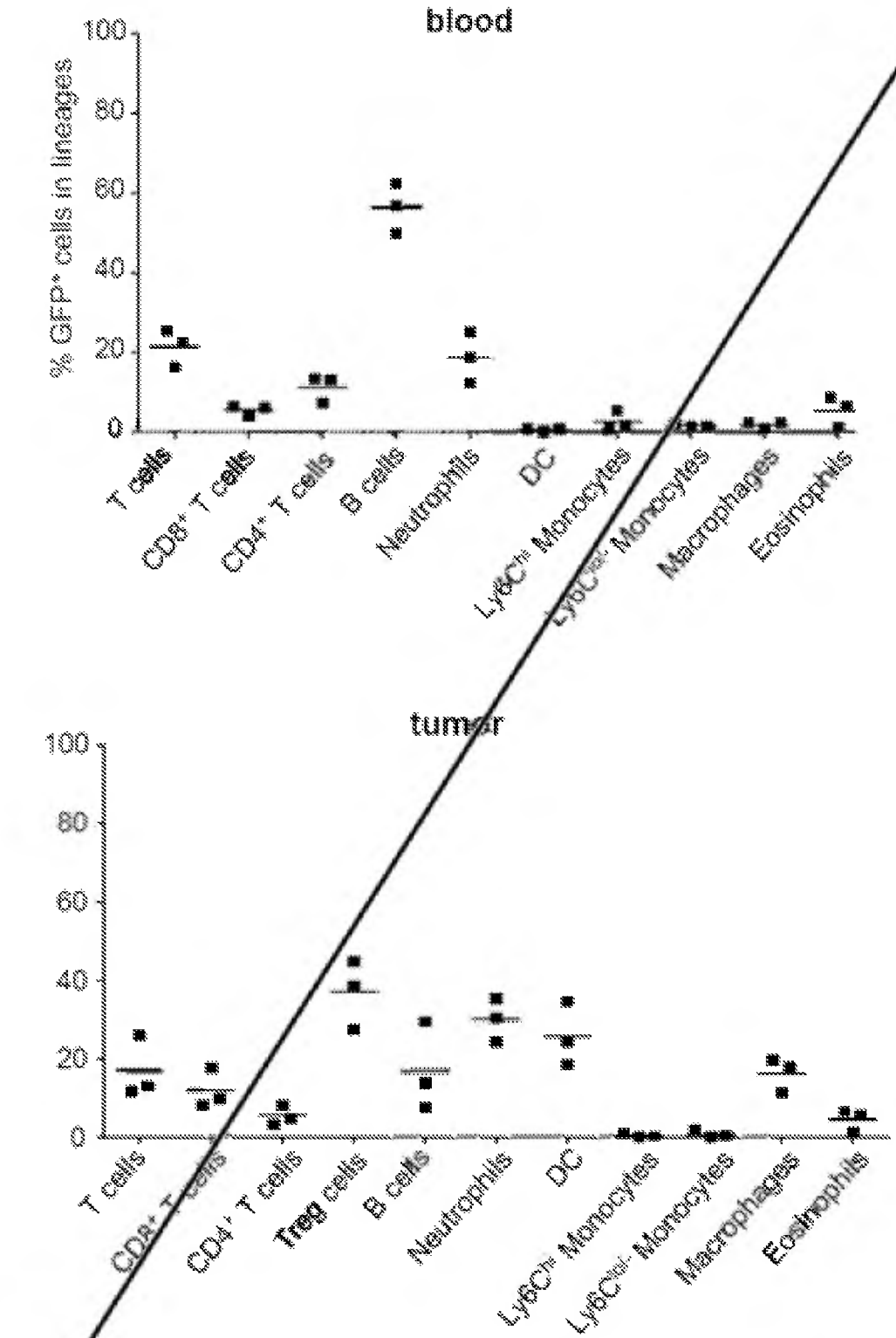
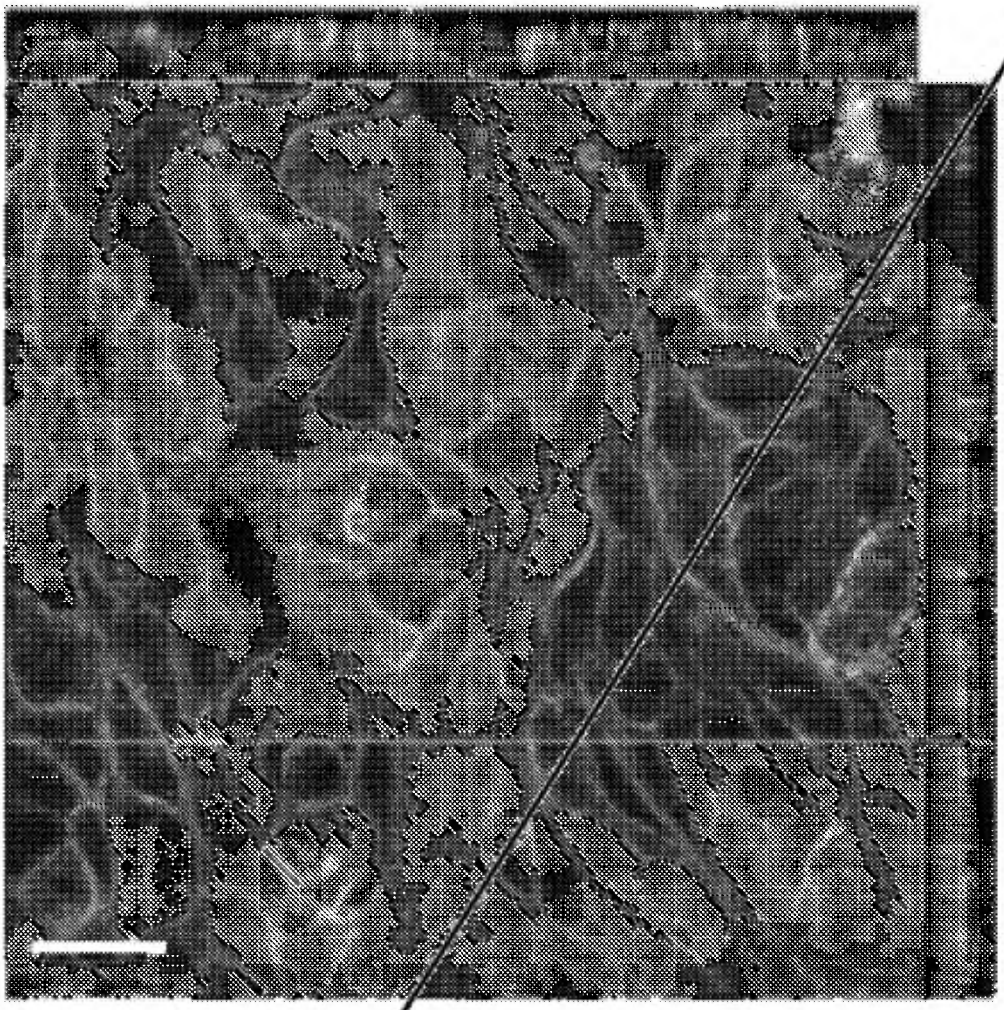

blood
% GFP+ cells in lineages
100
80
60
40
20
0
T cells
CD8+ T cells
CD4+ T cells
B cells
Neutrophils
DC
Ly6Chi Monocytes
Ly6Clo/- Monocytes
Macrophages
Eosinophils
tumor
100
80
60
40
20
0
T cells
CD8+ T cells
CD4+ T cells
Treg cells
B cells
Neutrophils
DC
Ly6Chi Monocytes
Ly6Clo/- Monocytes
Macrophages
Eosinophils

FIG. 75



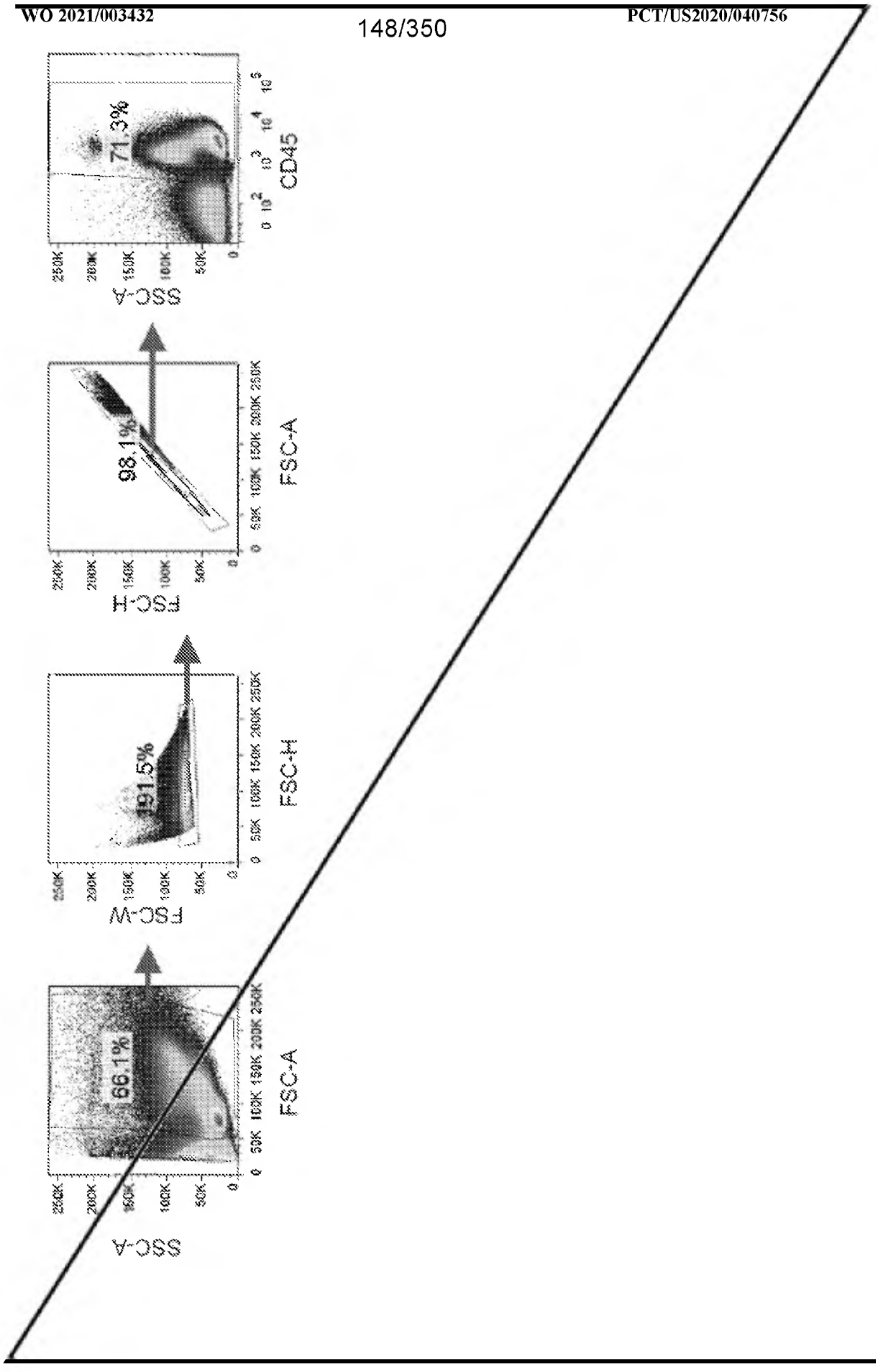


FIG. 76

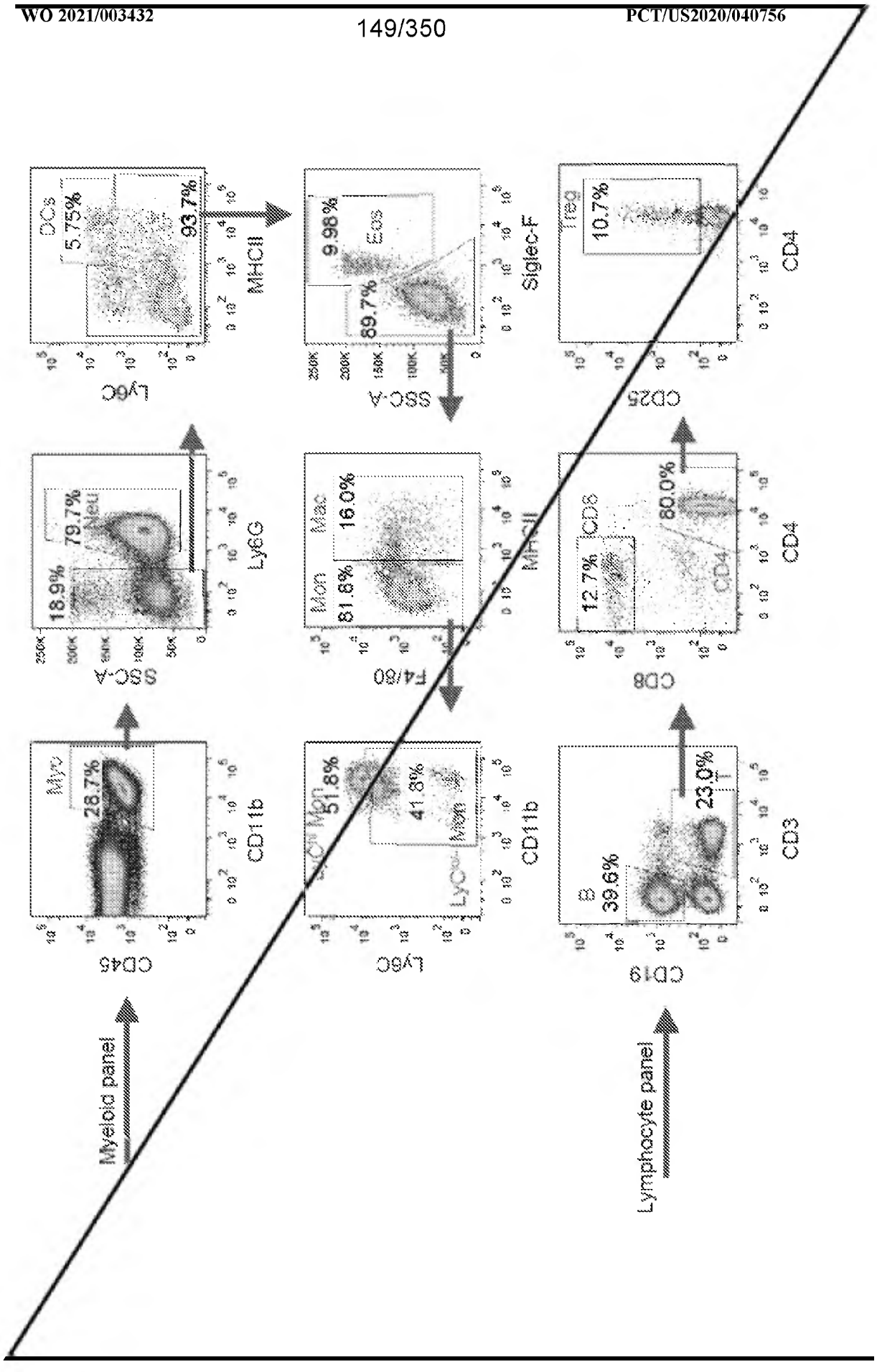

Myeloid panel
Lymphocyte panel
CD45
CD11b
Myc
28.7%
Ly6C
Ly6G
SSC-A
18.9%
79.7%
Neu
DCs
5.75%
93.7%
MHCII
Ly6C
CD11b
Mon
51.8%
41.8%
F4/80
MHCII
Mon
Mac
16.0%
81.8%
SSC-A
Siglec-F
9.98%
Eos
89.7%
CD19
CD3
B
39.6%
T
23.0%
CD8
CD4
12.7%
CD8
CD4
80.0%
CD25
CD4
Treg
10.7%

FIG. 76 cont'd

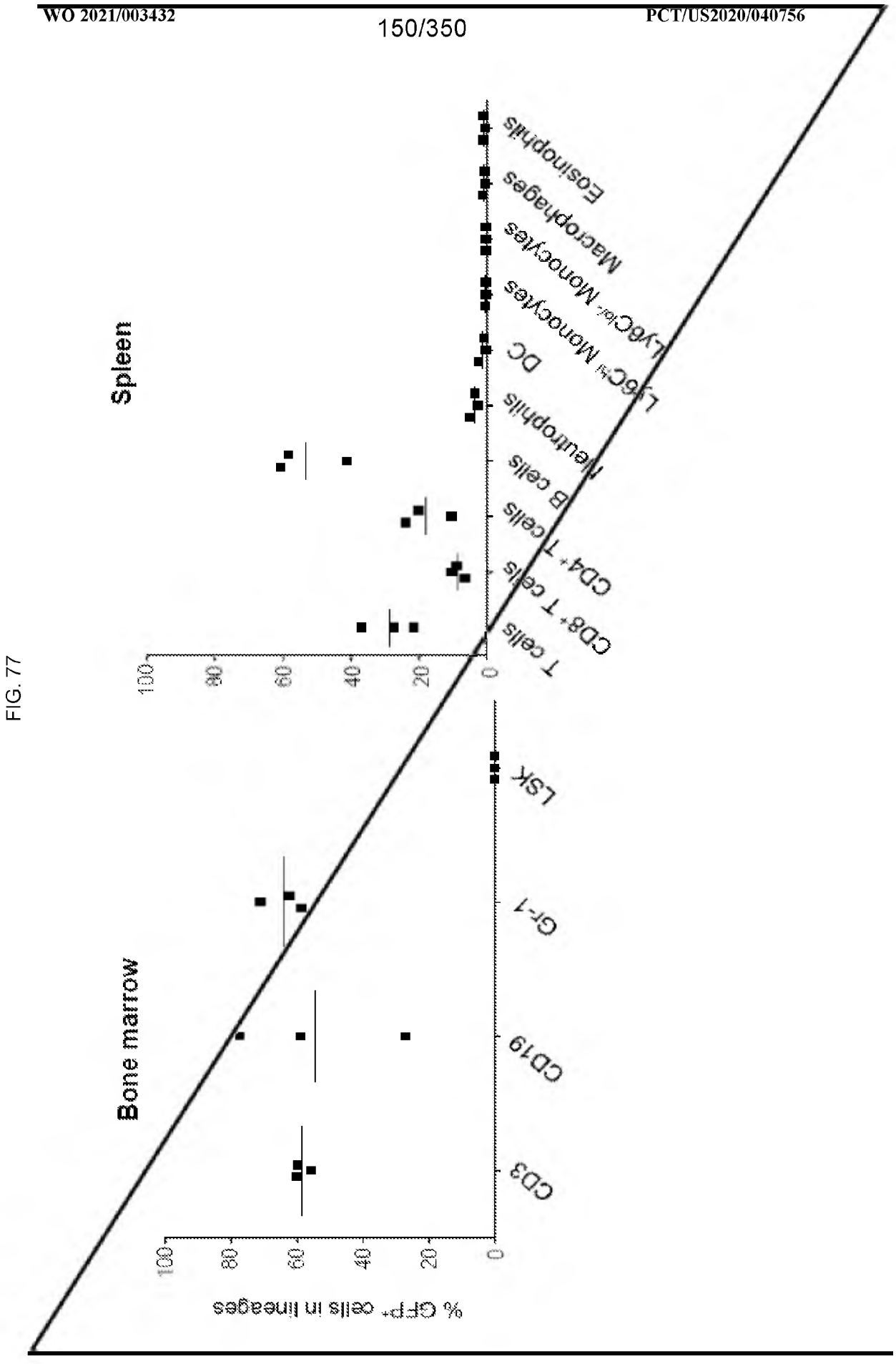

% GFP+ cells in lineages
Bone marrow
CD3
CD19
Gr-1
LSK
Spleen
T cells
CD8+ T cells
CD4+ T cells
B cells
Neutrophils
DC
Ly6Chi Monocytes
Ly6Clo- Monocytes
Macrophages
Eosinophils
0
20
40
60
80
100

FIG. 77

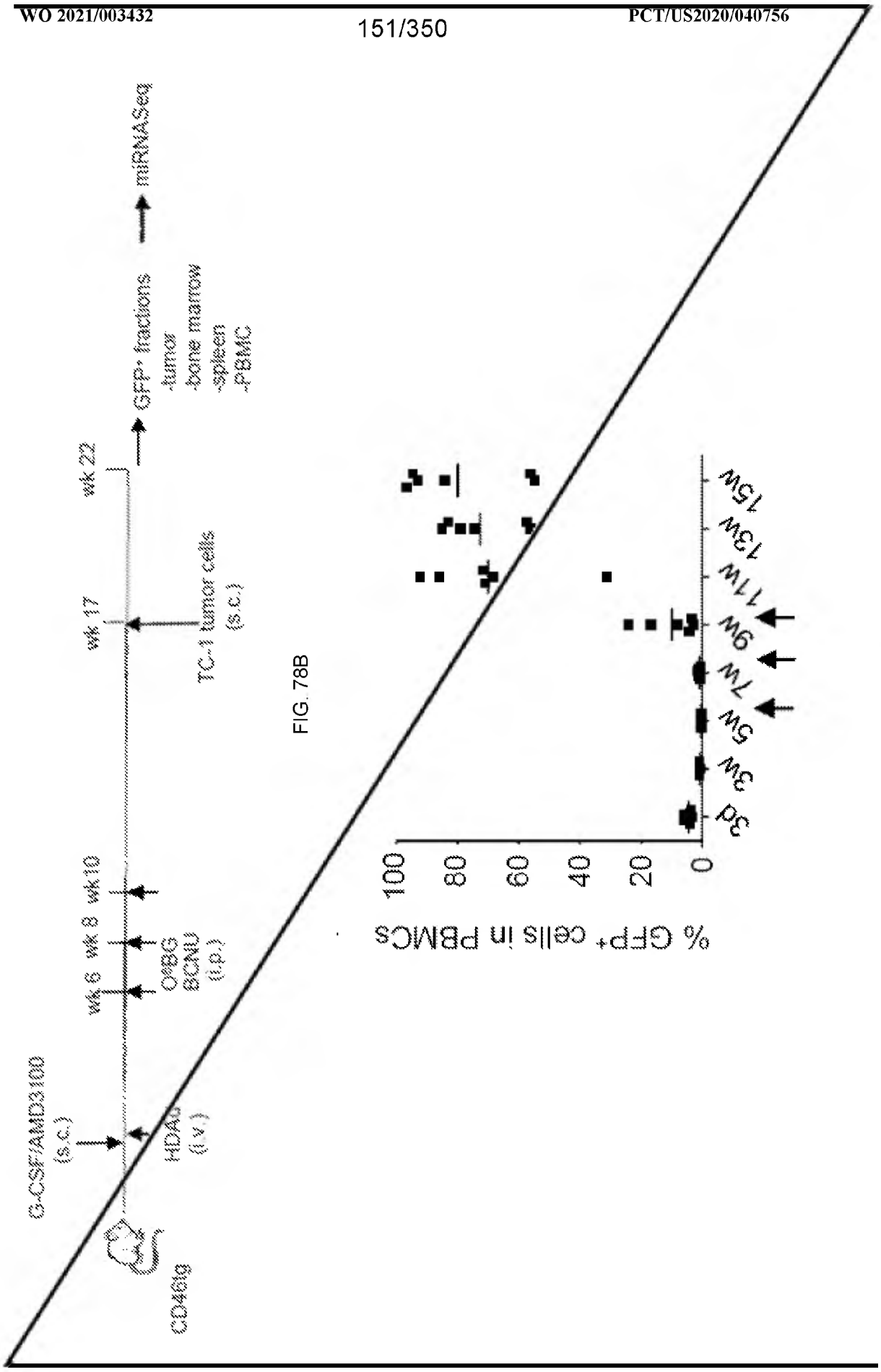


FIG. 78A

FIG. 78B

FIG. 78C

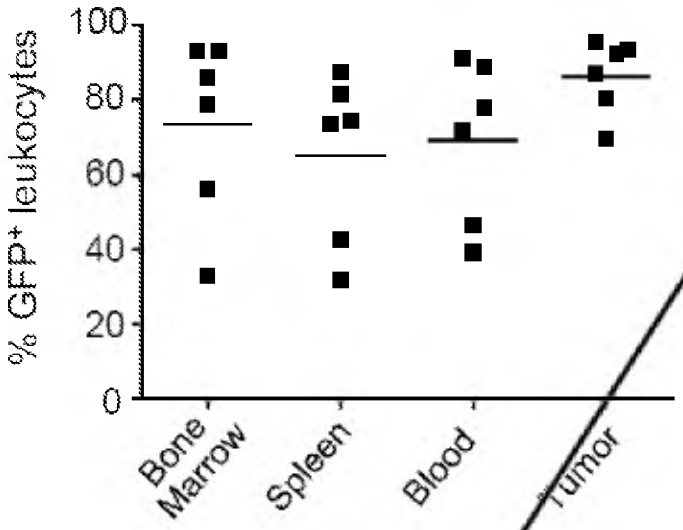


FIG. 78D

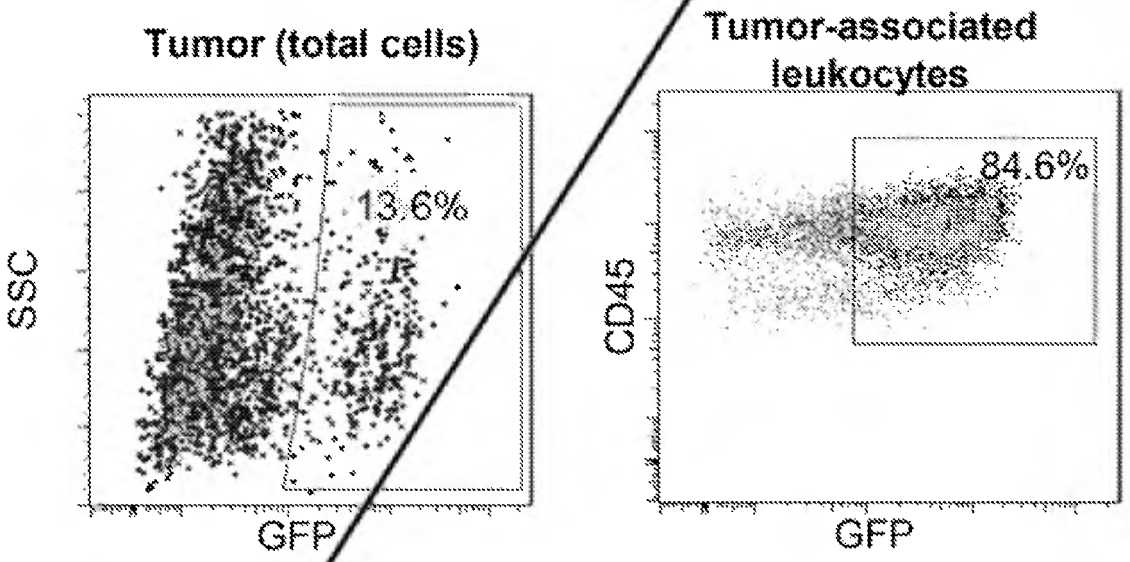


FIG. 78E

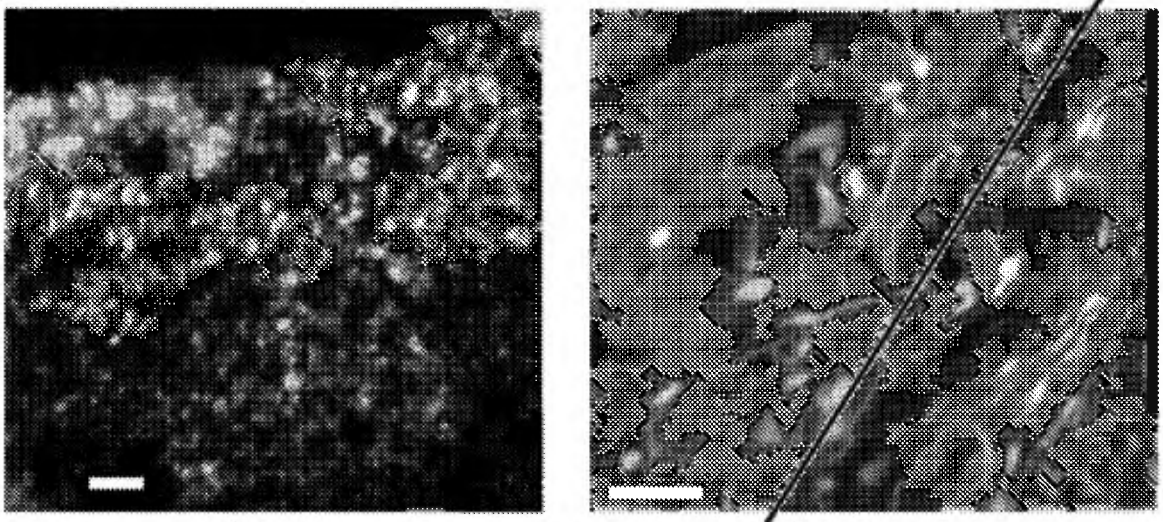


FIG. 78F

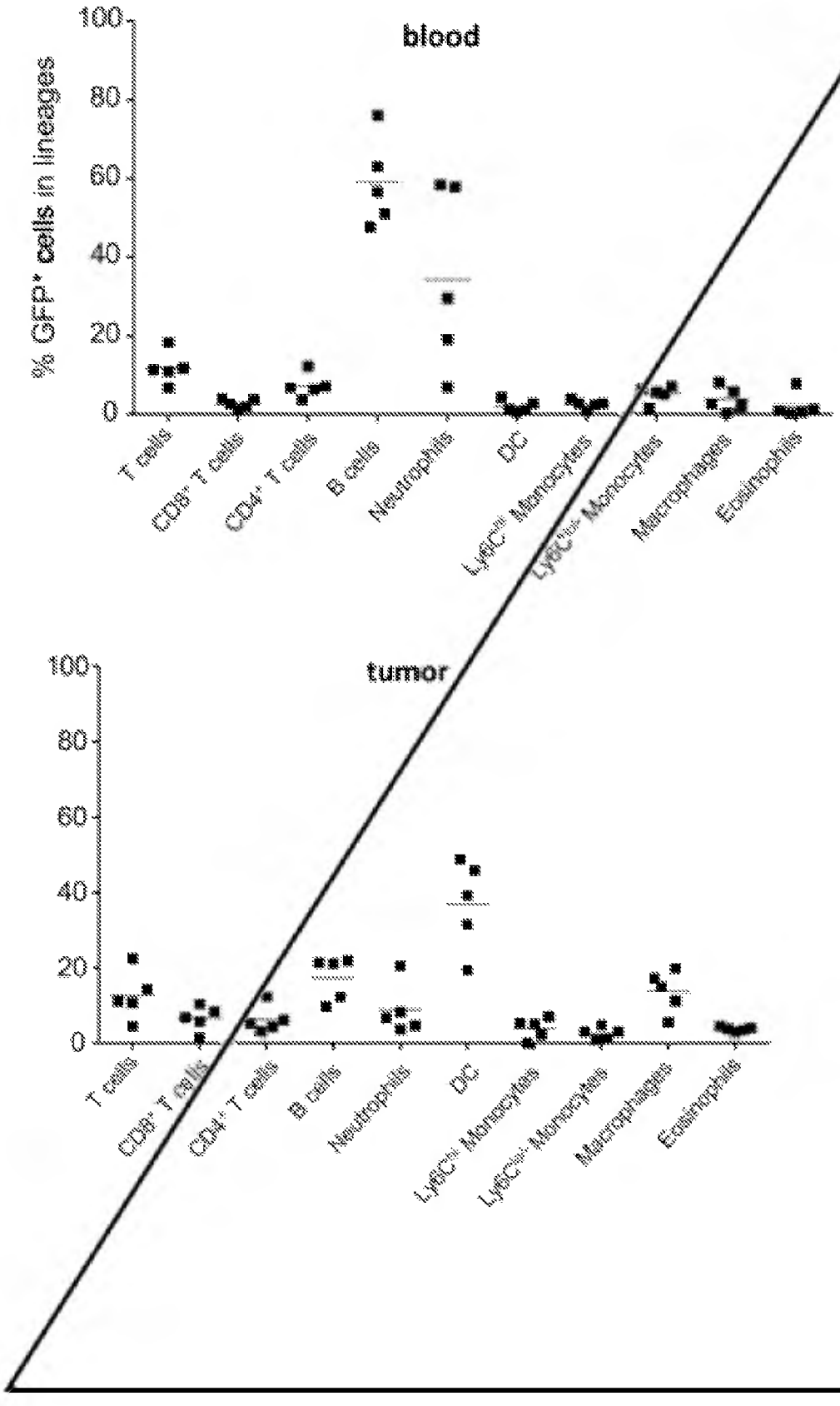

blood
% GFP+ cells in lineages
100
80
60
40
20
0
T cells
CD8+ T cells
CD4+ T cells
B cells
Neutrophils
DC
Ly6C^hi Monocytes
Ly6C^lo Monocytes
Macrophages
Eosinophils
tumor
100
80
60
40
20
0
T cells
CD8+ T cells
CD4+ T cells
B cells
Neutrophils
DC
Ly6C^hi Monocytes
Ly6C^lo Monocytes
Macrophages
Eosinophils

FIG. 79A

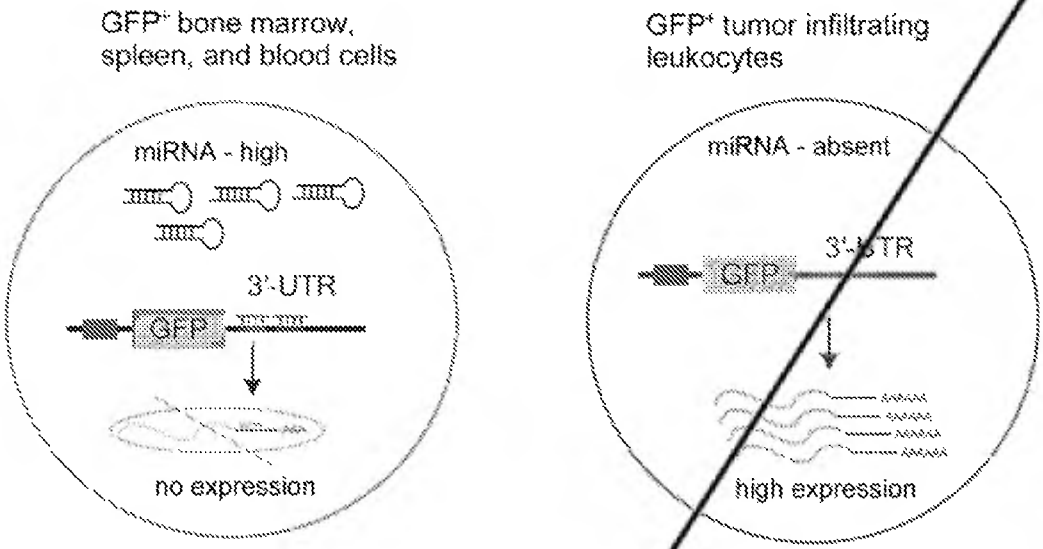


FIG. 79B

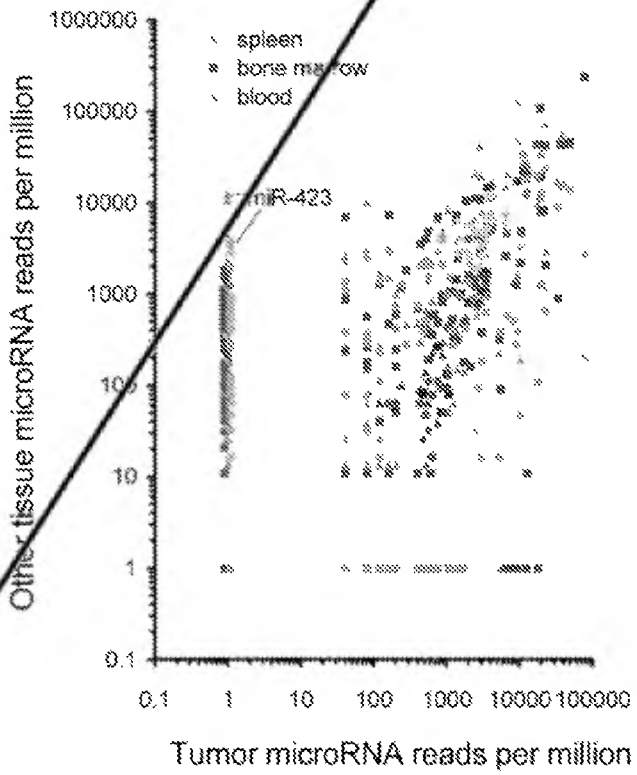


FIG. 79C

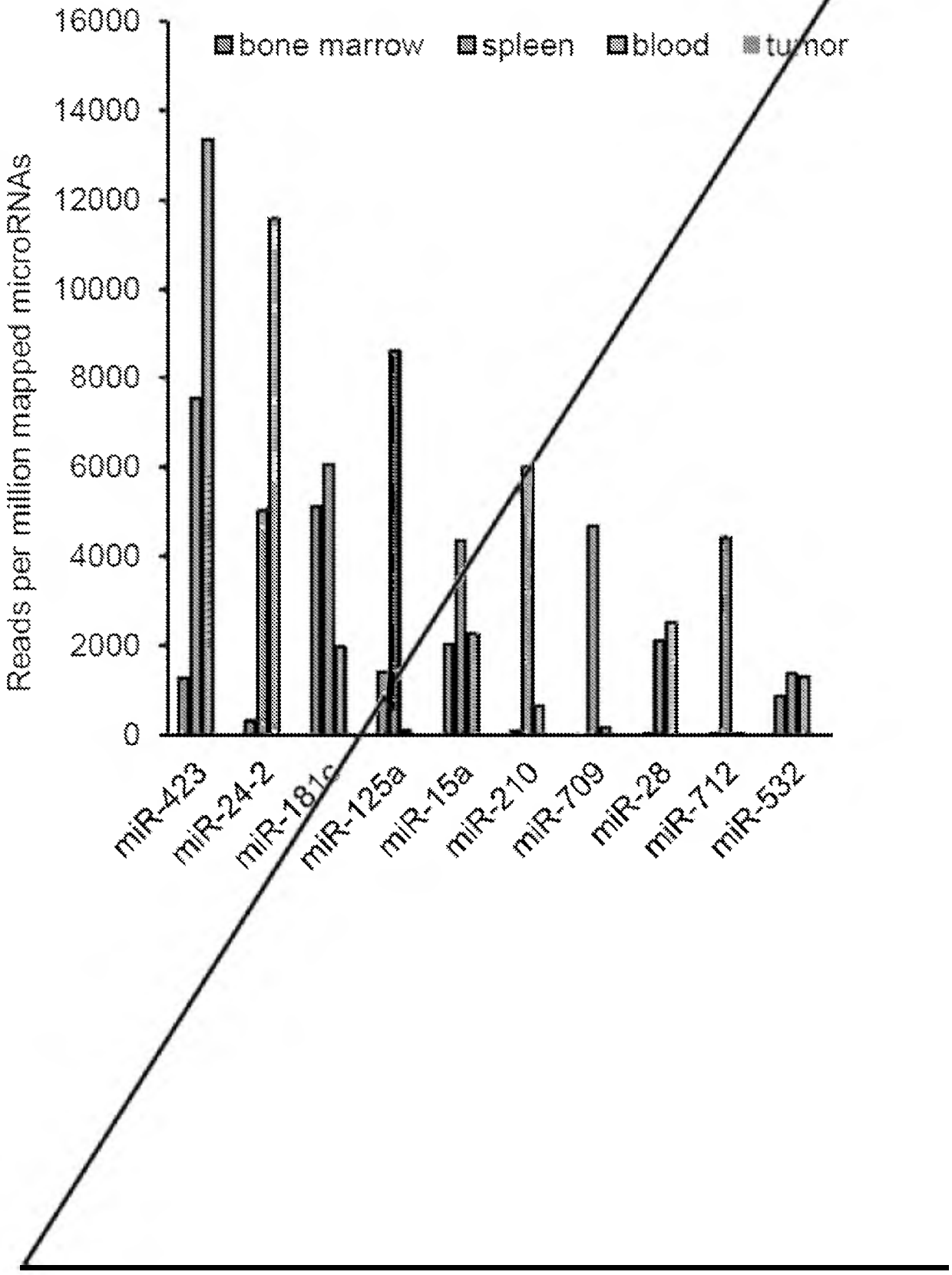

16000
14000
12000
10000
8000
6000
4000
2000
0
Reads per million mapped microRNAs
bone marrow
spleen
blood
tumor
miR-423
miR-24-2
miR-181c
miR-125a
miR-15a
miR-210
miR-709
miR-28
miR-712
miR-532

FIG. 80A

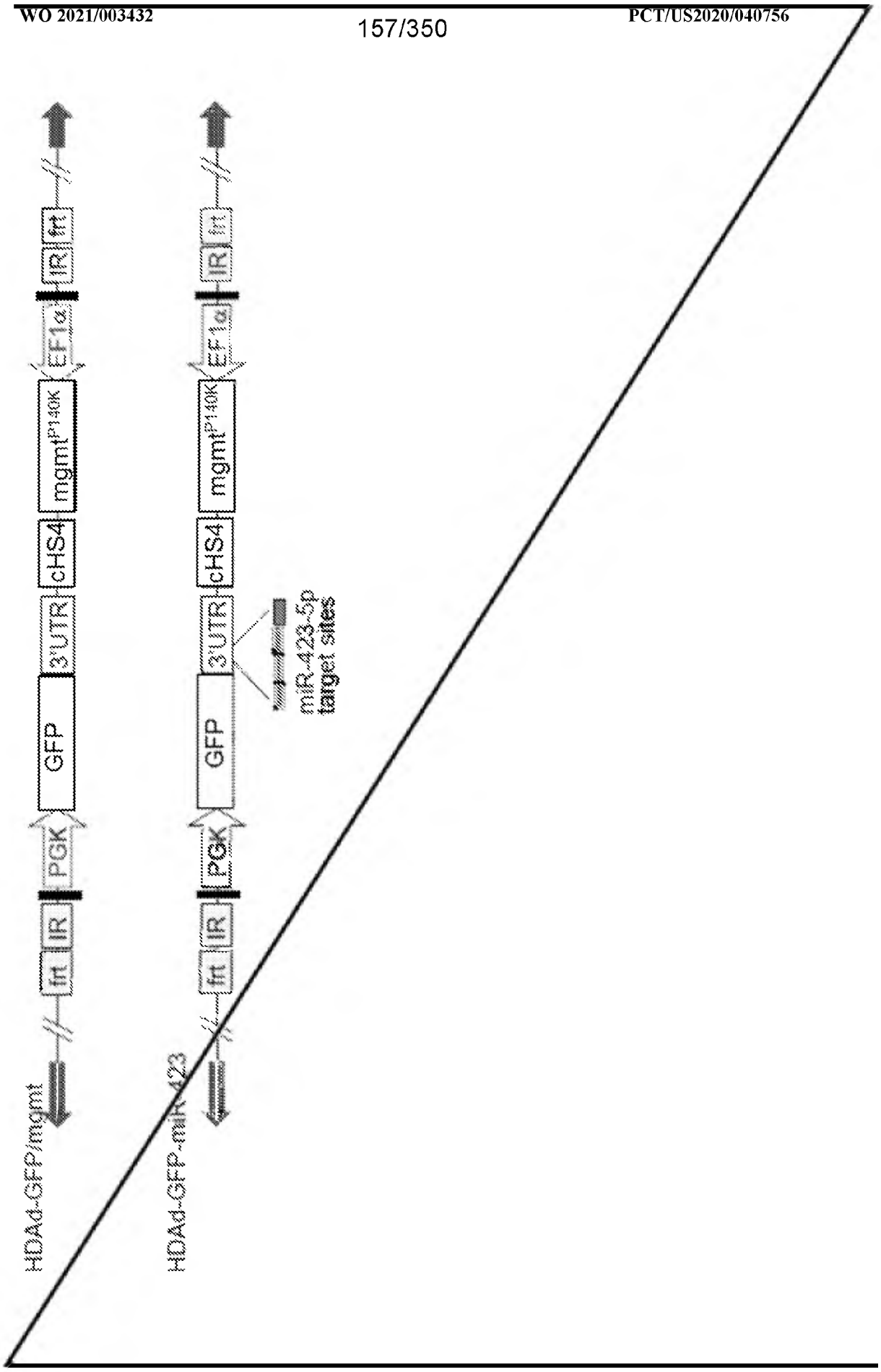

HDAd-GFP/mgmt
frt
IR
PGK
GFP
3'UTR
cHS4
mgmt^{P140K}
EF1α
IR
frt
HDAd-GFP-miR-423
frt
IR
PGK
GFP
3'UTR
cHS4
mgmt^{P140K}
EF1α
IR
frt
miR-423-5p
target sites

FIG. 80B

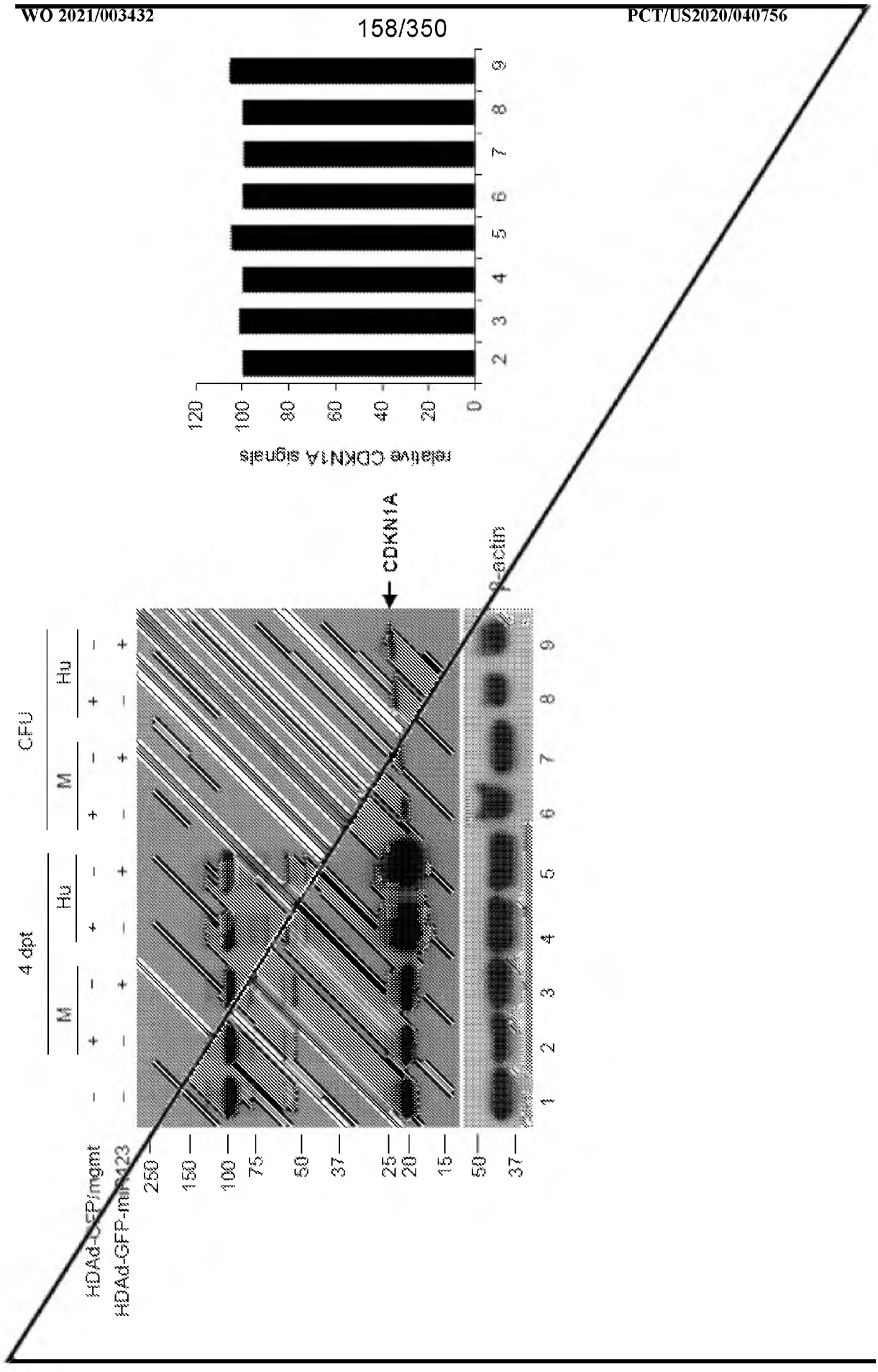

4 dpt
CFU
M
Hu
M
Hu
HDAd-GFP/mgmt
HDAd-GFP-miR-9-9*-122-123
250
150
100
75
50
37
25
20
15
50
37
CDKN1A
β-actin
1 2 3 4 5 6 7 8 9
relative CDKN1A signals
120
100
80
60
40
20
0
2 3 4 5 6 7 8 9

FIG. 80C

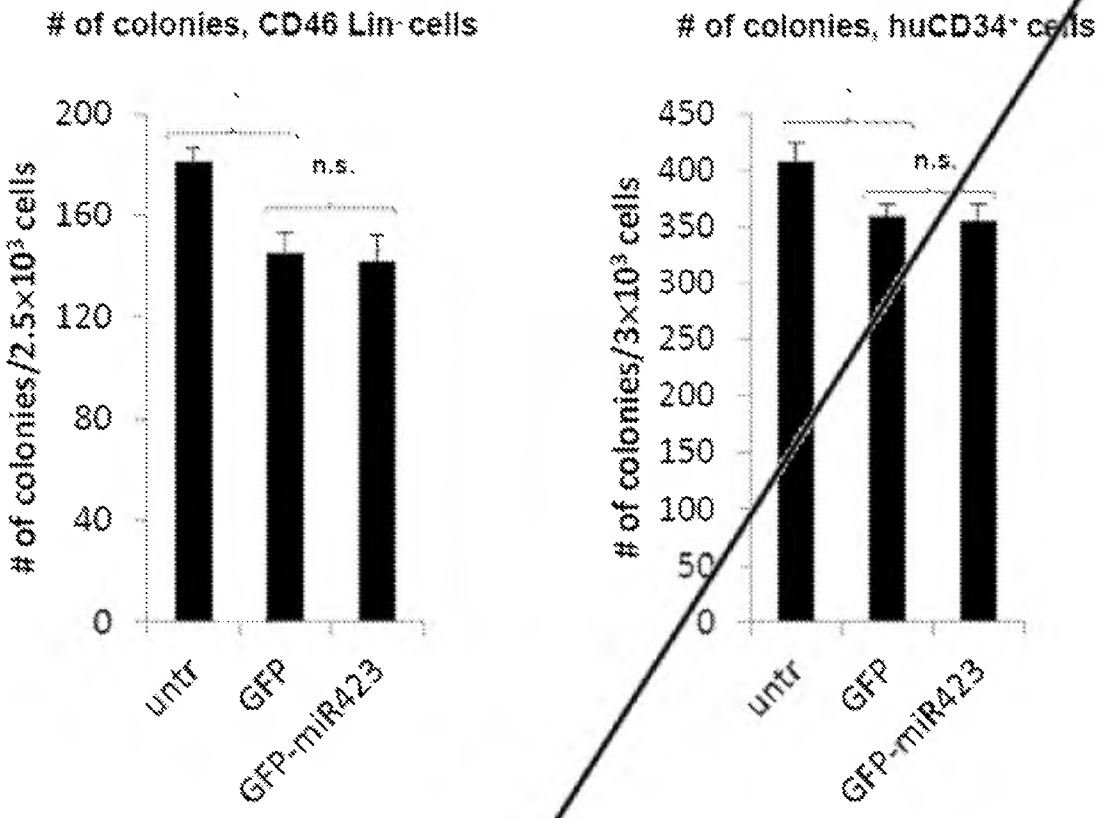
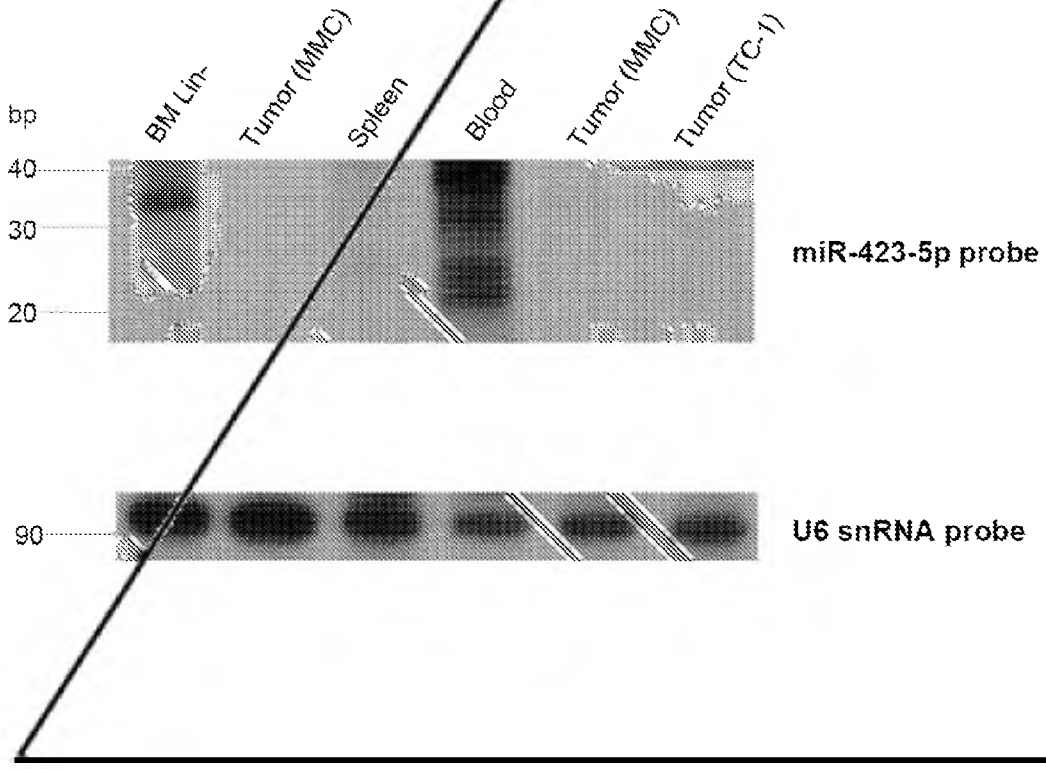


FIG. 81



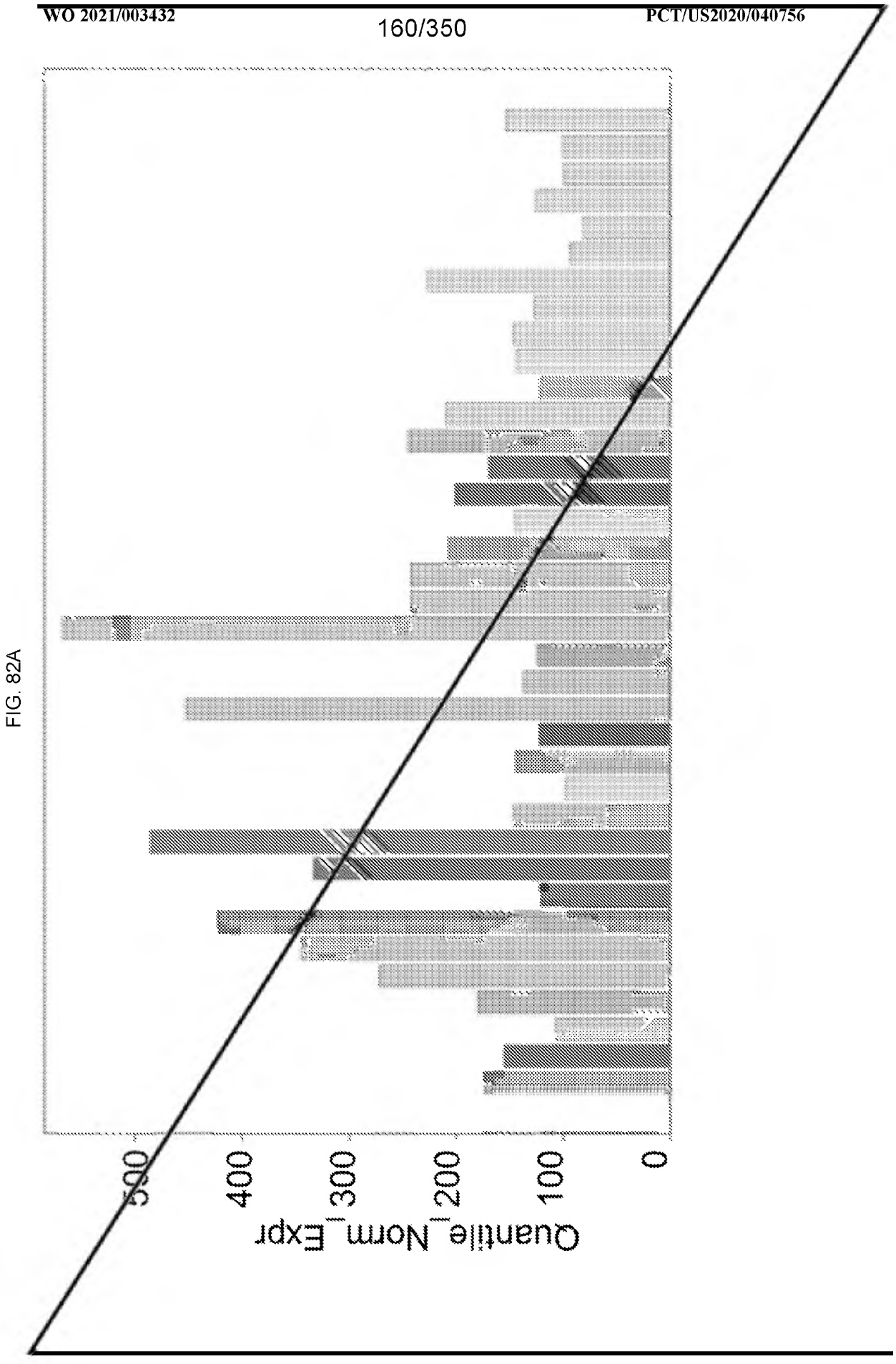


FIG. 82A

FIG. 82B

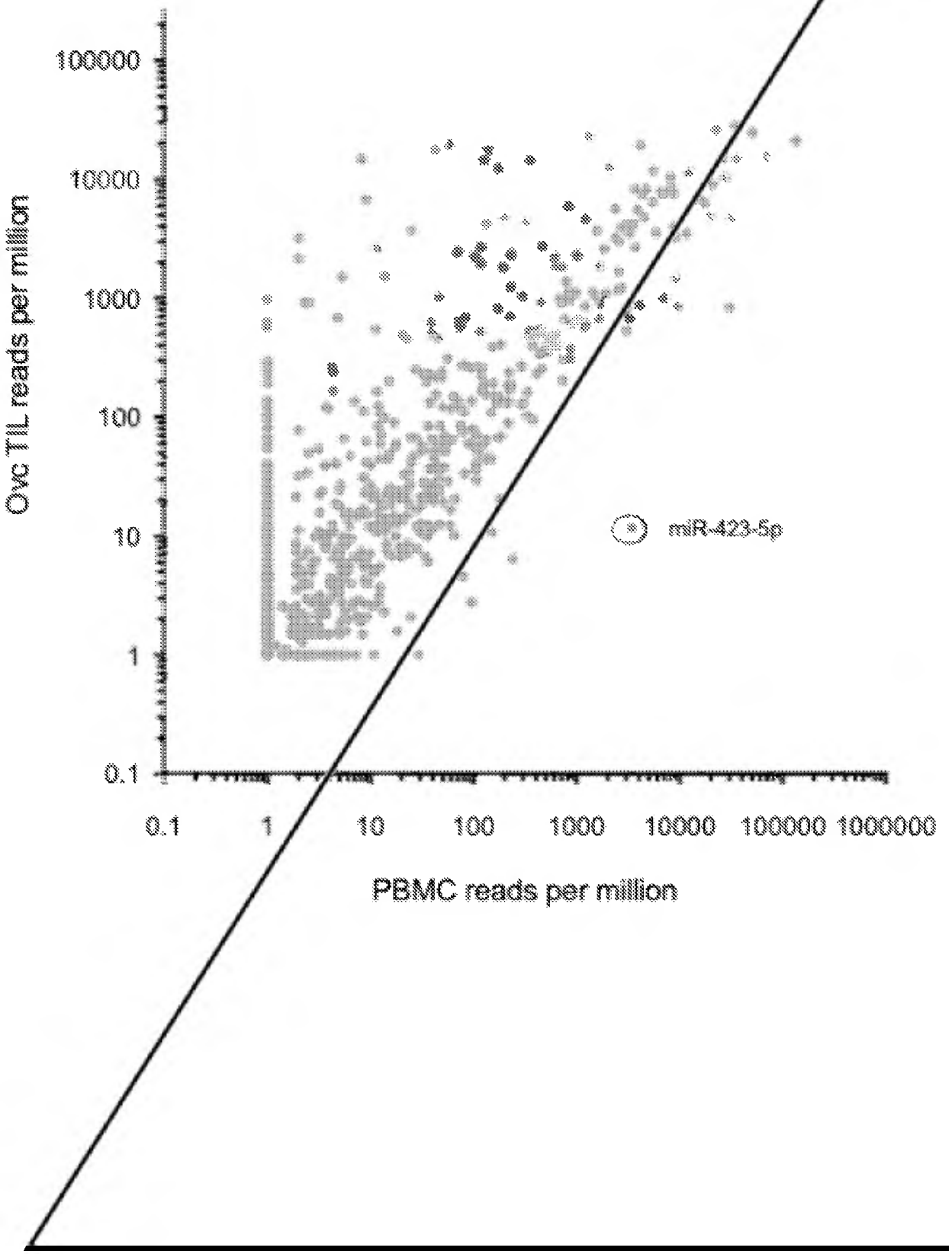

Ovc TIL reads per million
100000
10000
1000
100
10
1
0.1
0.1
1
10
100
1000
10000
100000
1000000
PBMC reads per million
miR-423-5p

FIG. 83A

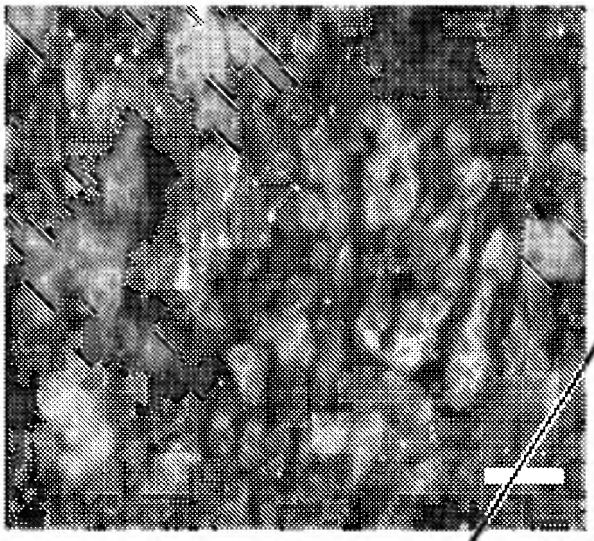


FIG. 83B

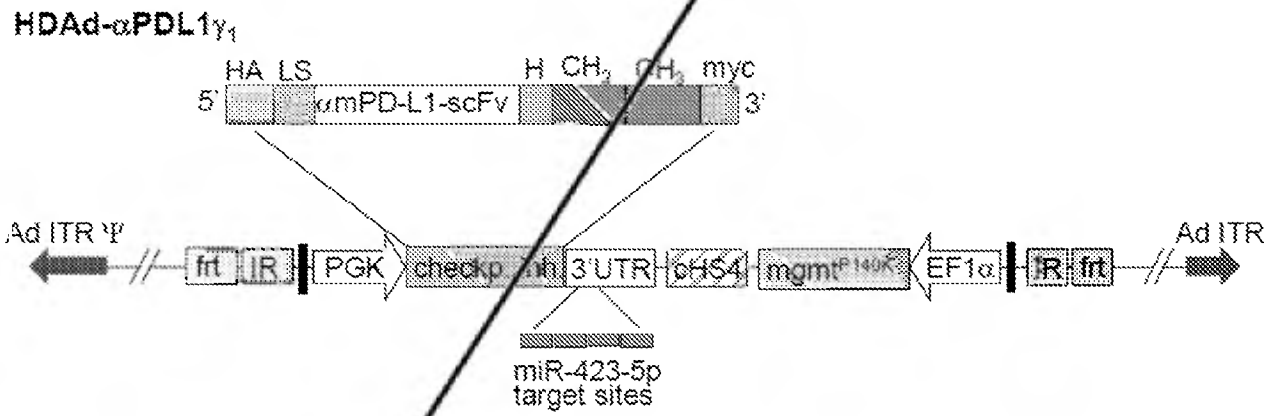


FIG. 83C

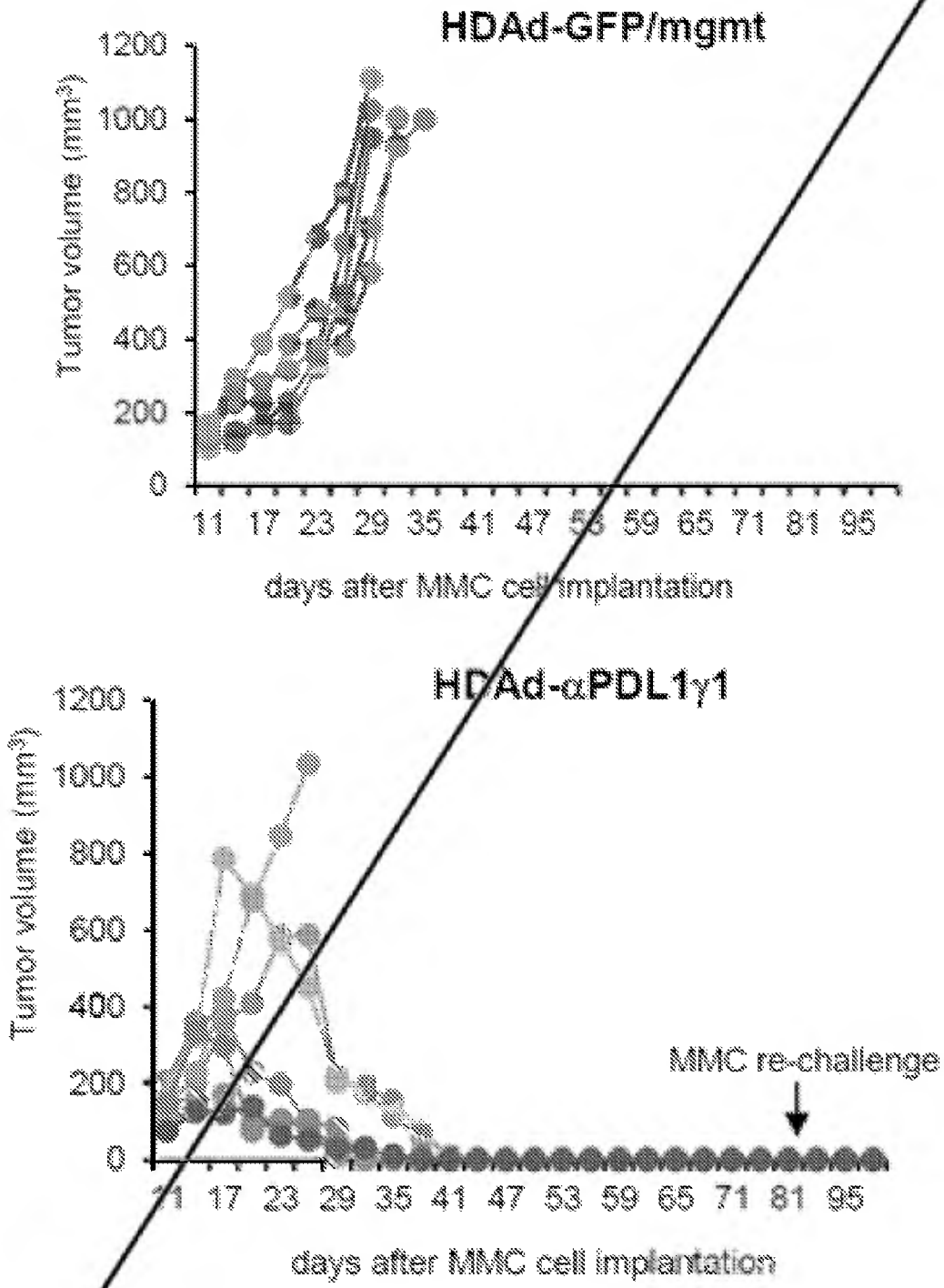

HDAd-GFP/mgmt
Tumor volume (mm3)
1200
1000
800
600
400
200
0
11 17 23 29 35 41 47 53 59 65 71 81 95
days after MMC cell implantation
HDAd-αPDL1γ1
Tumor volume (mm3)
1200
1000
800
600
400
200
0
MMC re-challenge
11 17 23 29 35 41 47 53 59 65 71 81 95
days after MMC cell implantation

FIG. 83D

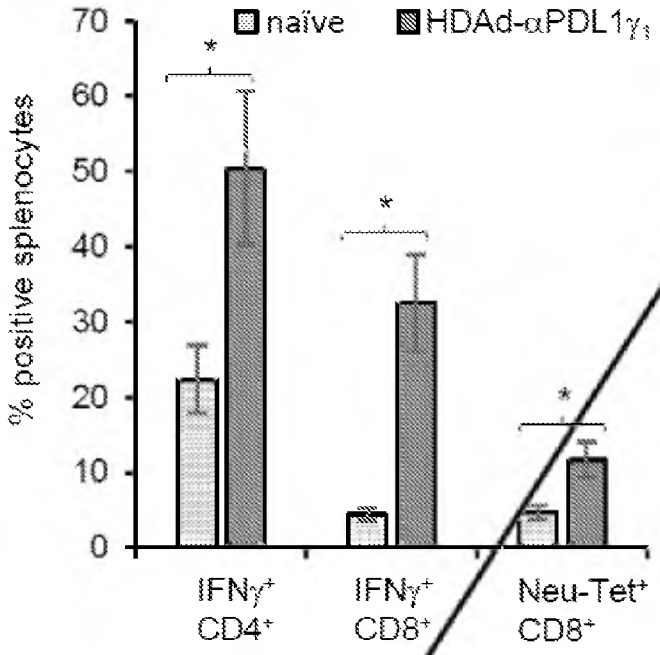


FIG. 83E

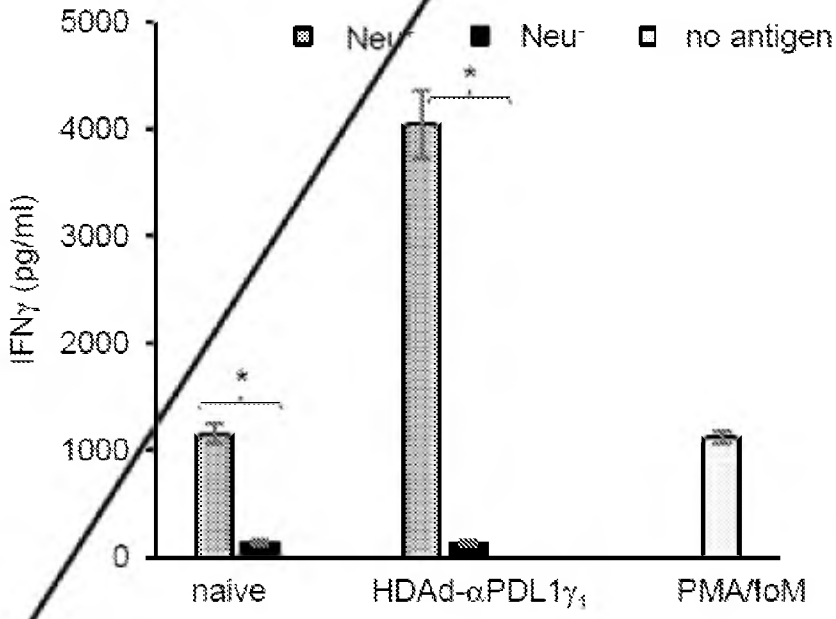
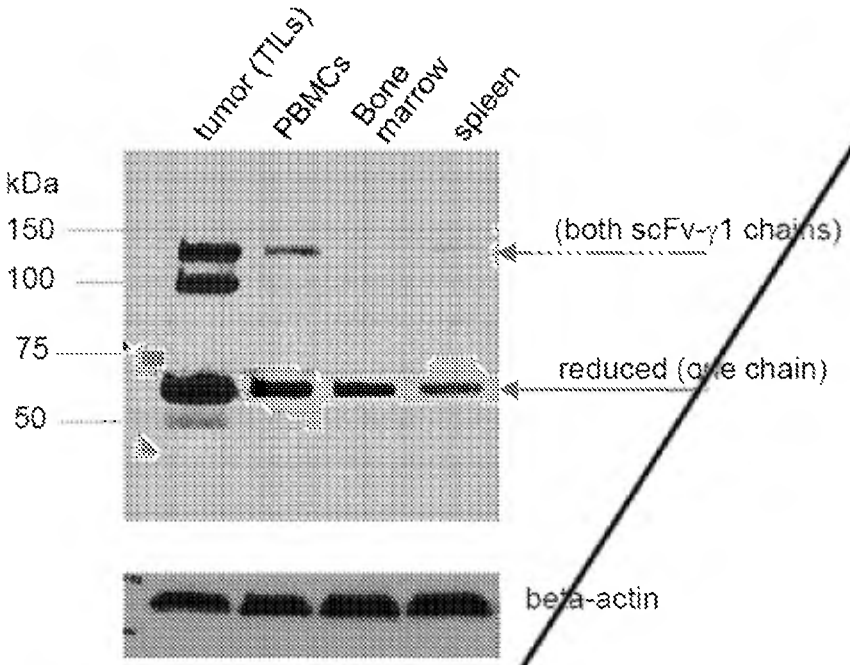


FIG. 84A


tumor (TILs)
PBMCs
Bone marrow
spleen
kDa
150
100
75
50
(both scFv-γ1 chains)
reduced (one chain)
beta-actin

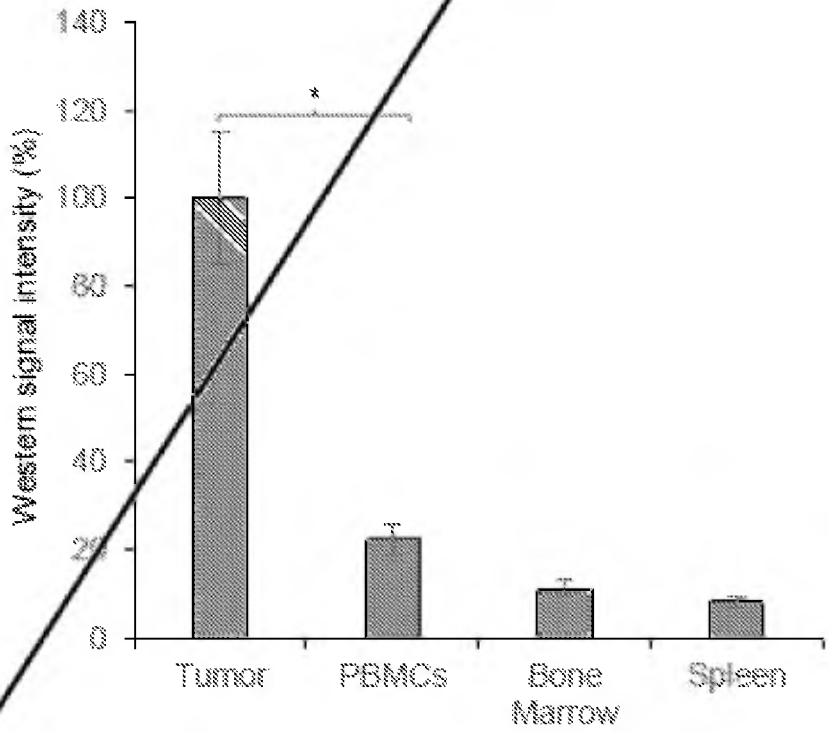

Western signal intensity (%)
140
120
100
80
60
40
20
0
*
Tumor
PBMCs
Bone Marrow
Spleen

FIG. 84B

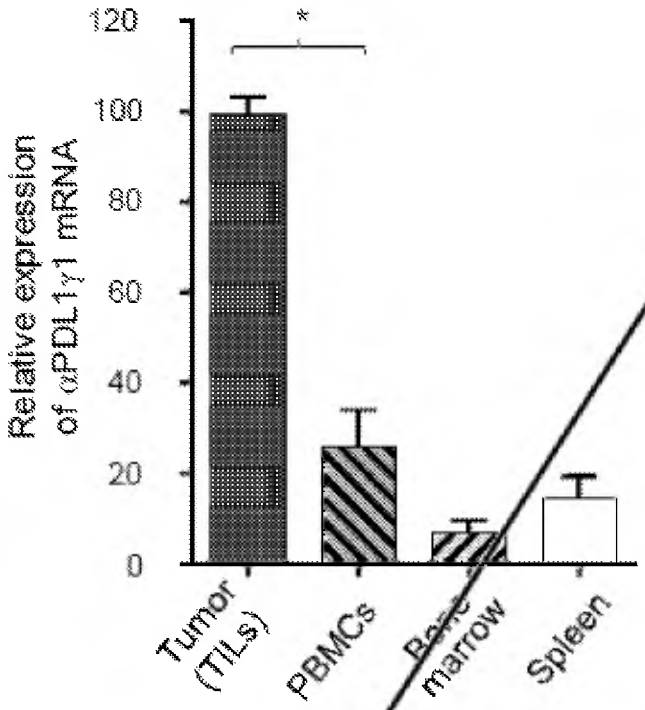
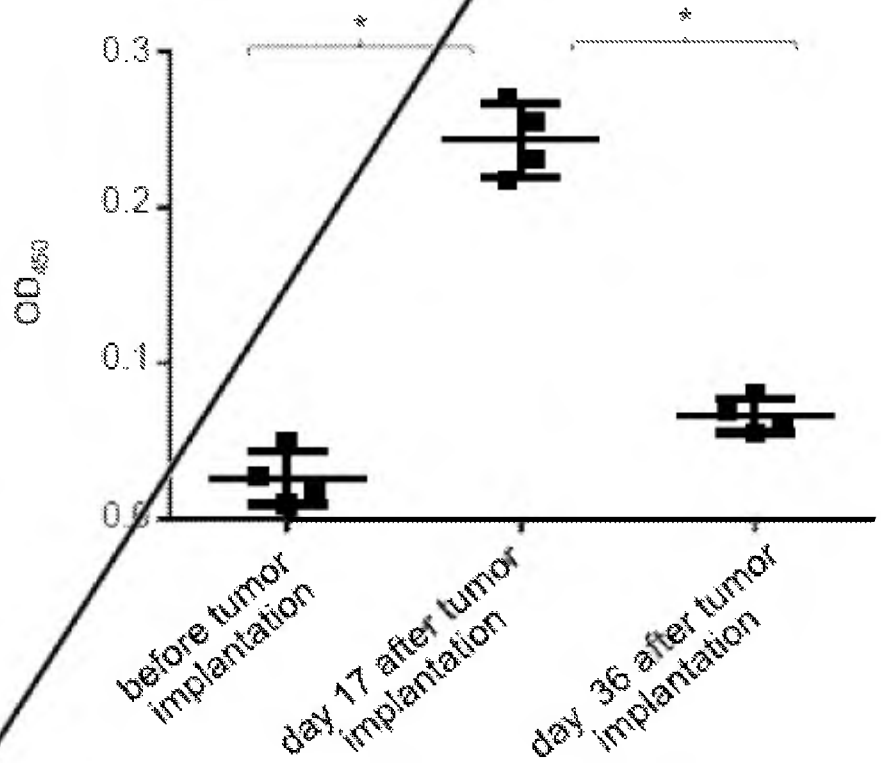


FIG. 84C



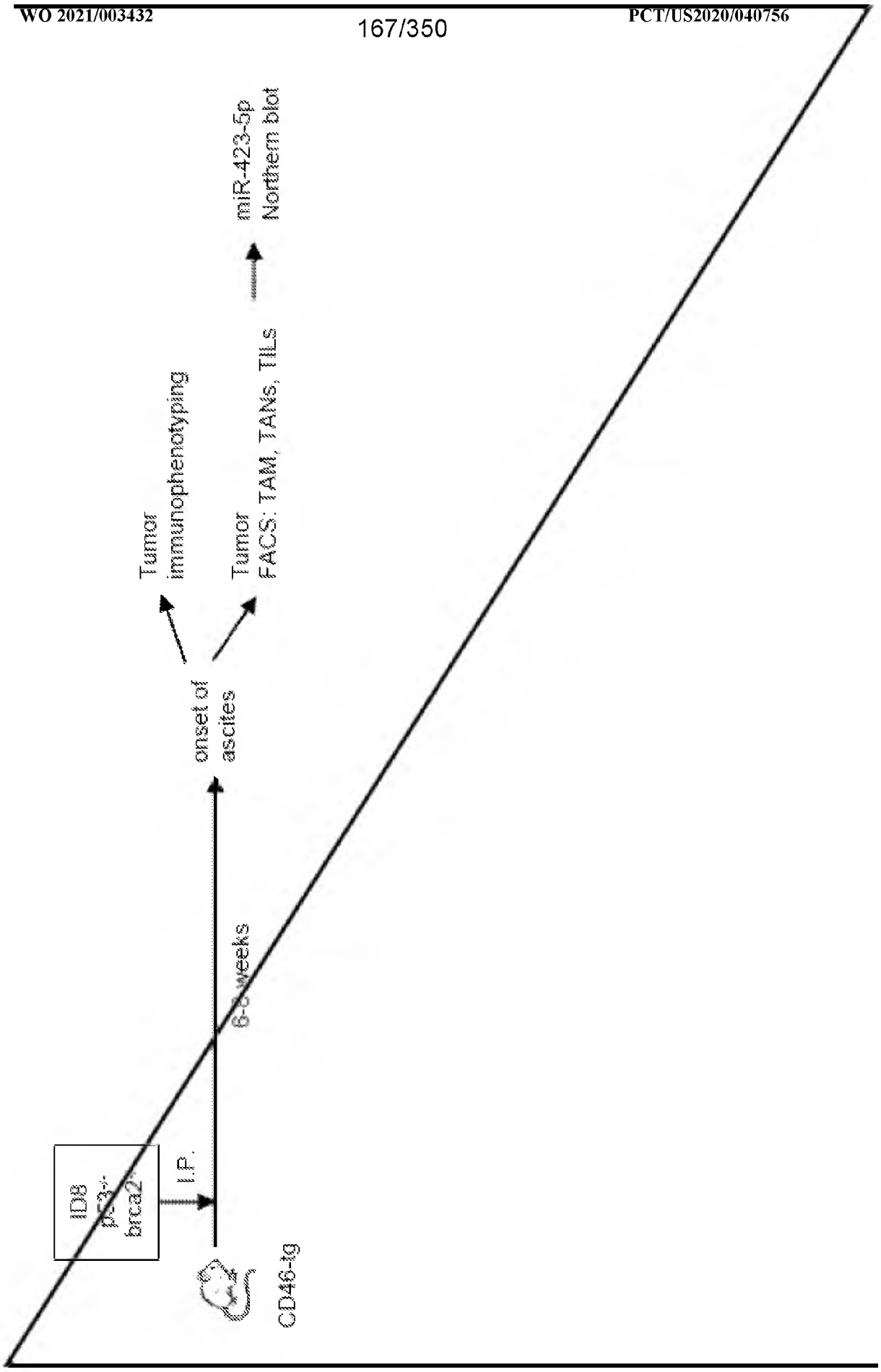

ID8
p53-/-
brca2-/-
I.P.
CD46-tg
6-8 weeks
onset of ascites
Tumor immunophenotyping
Tumor FACS: TAM, TANs, TILs
miR-423-5p Northern blot

FIG. 85A

FIG. 85B

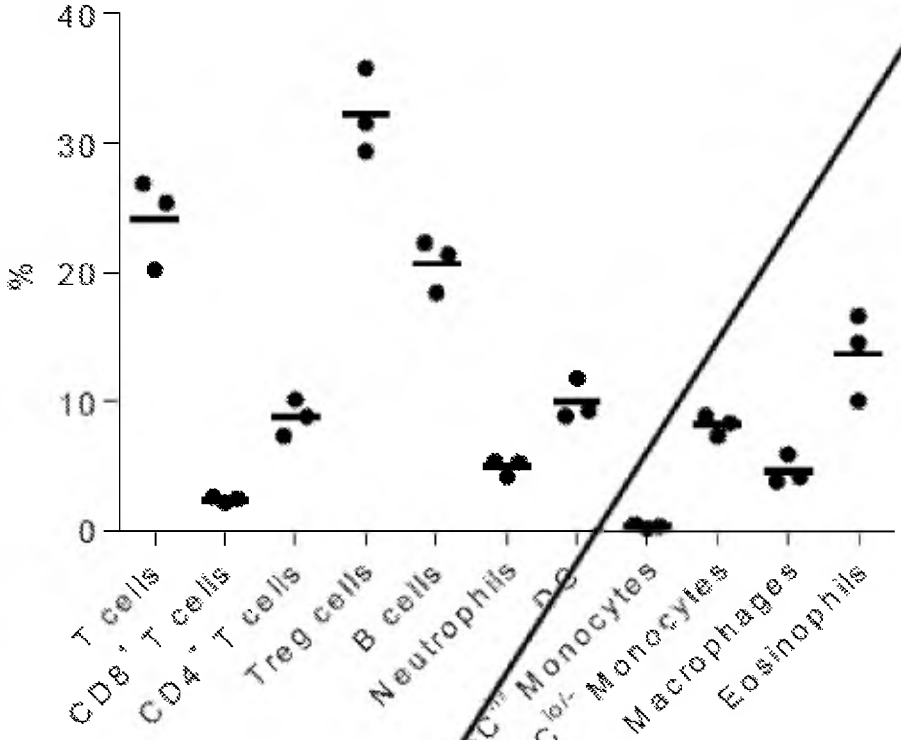
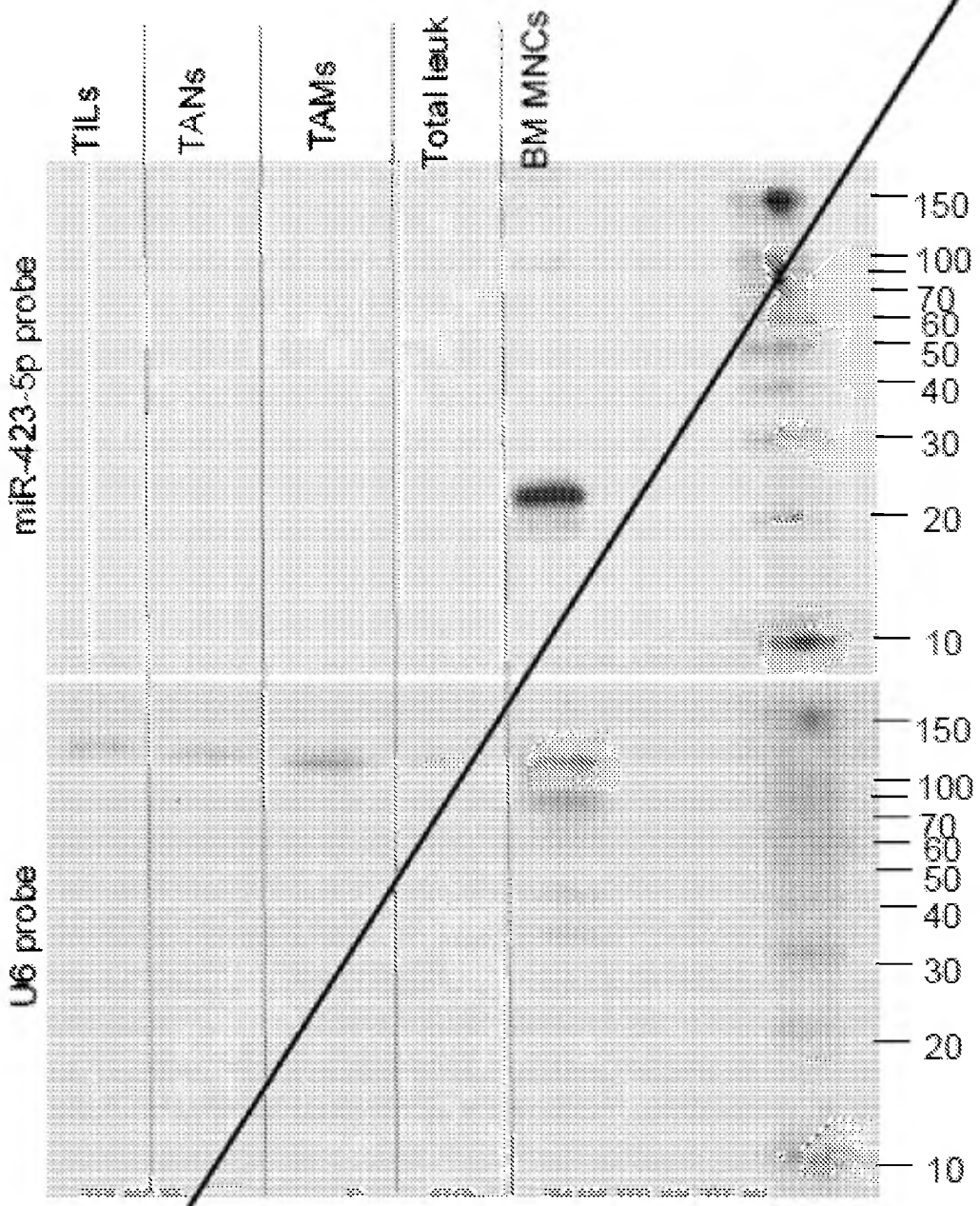

40
30
20
10
0
%
T cells
CD8+ T cells
CD4+ T cells
Treg cells
B cells
Neutrophils
DC
Ly6C hi Monocytes
Ly6C lo/- Monocytes
Macrophages
Eosinophils

FIG. 85C


TILs
TANs
TAMs
Total leuk
BM MNCs
miR-423-5p probe
U6 probe
150
100
70
60
50
40
30
20
10

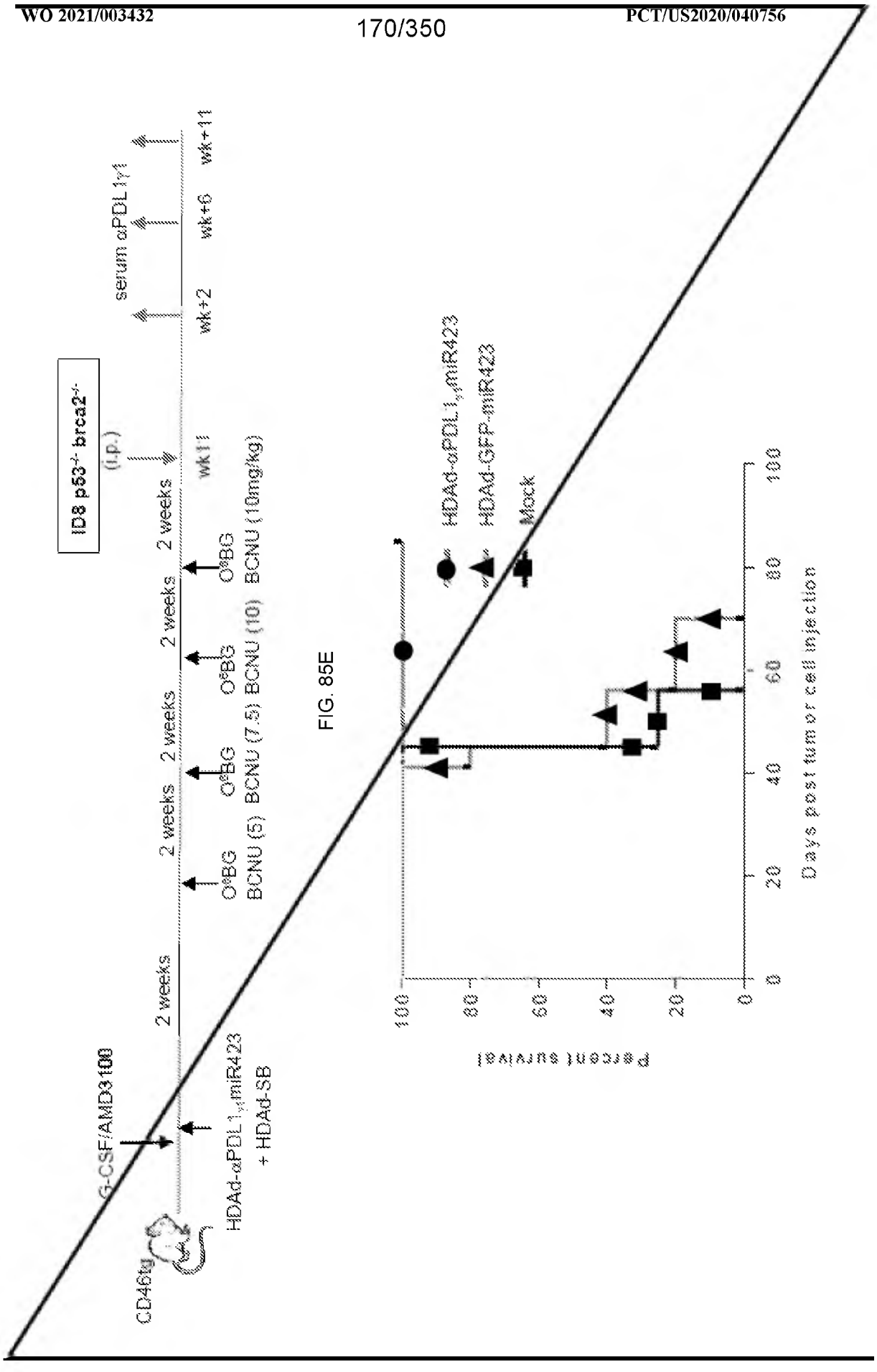
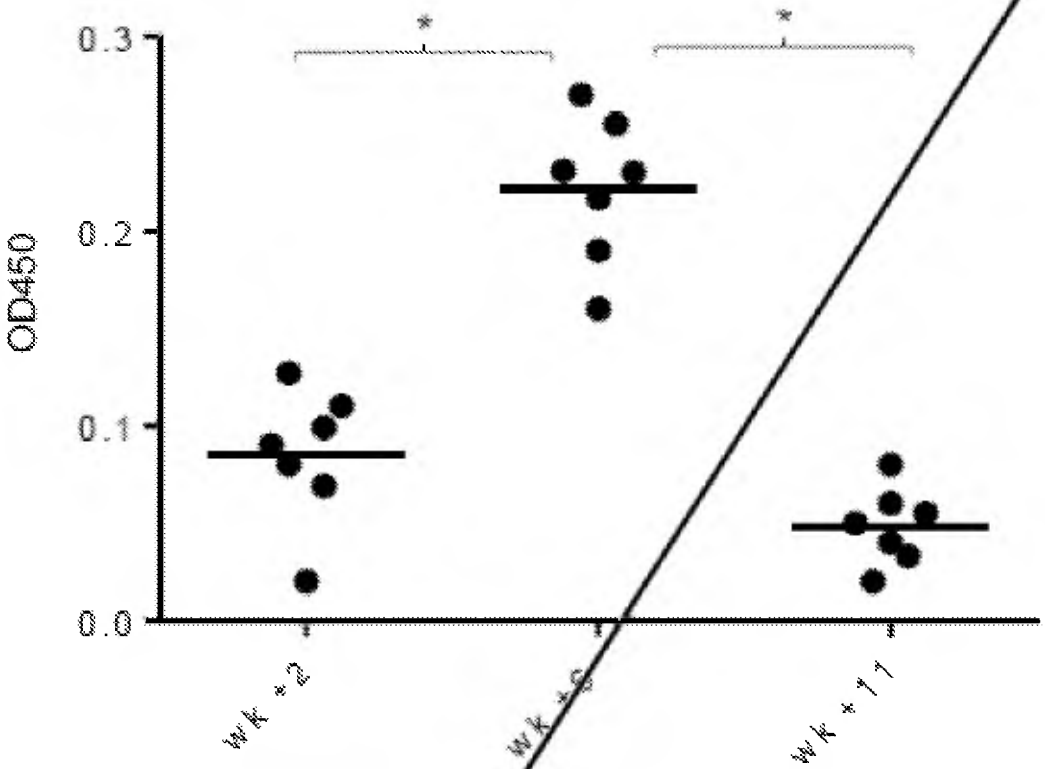


FIG. 85D

FIG. 85E

FIG. 85F



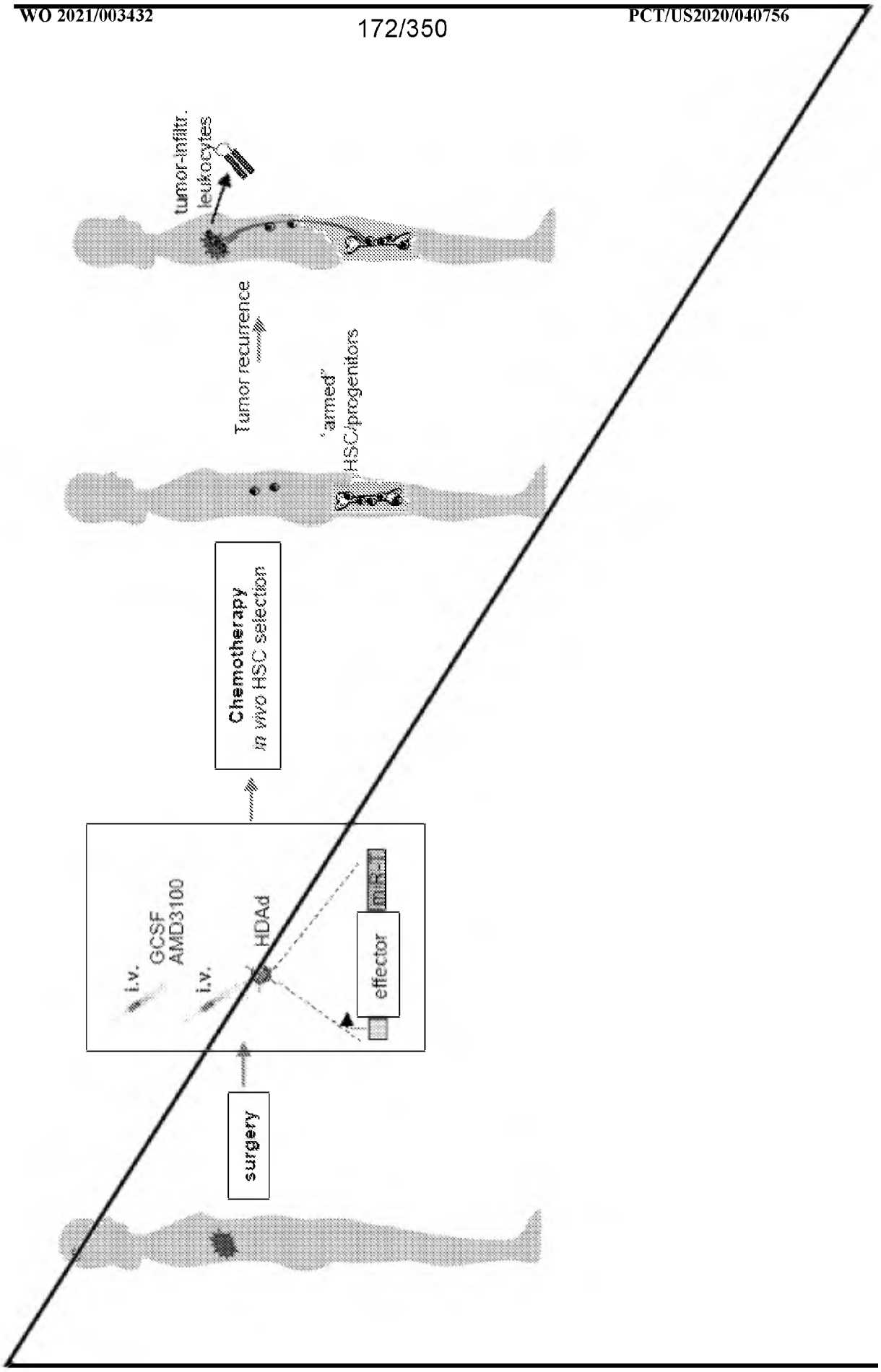

surgery
i.v.
GCSF
AMD3100
i.v.
HDAd
effector
miR-1
Chemotherapy
in vivo HSC selection
'armed'
HSC/progenitors
Tumor recurrence
tumor-infiltr.
leukocytes

FIG. 86A

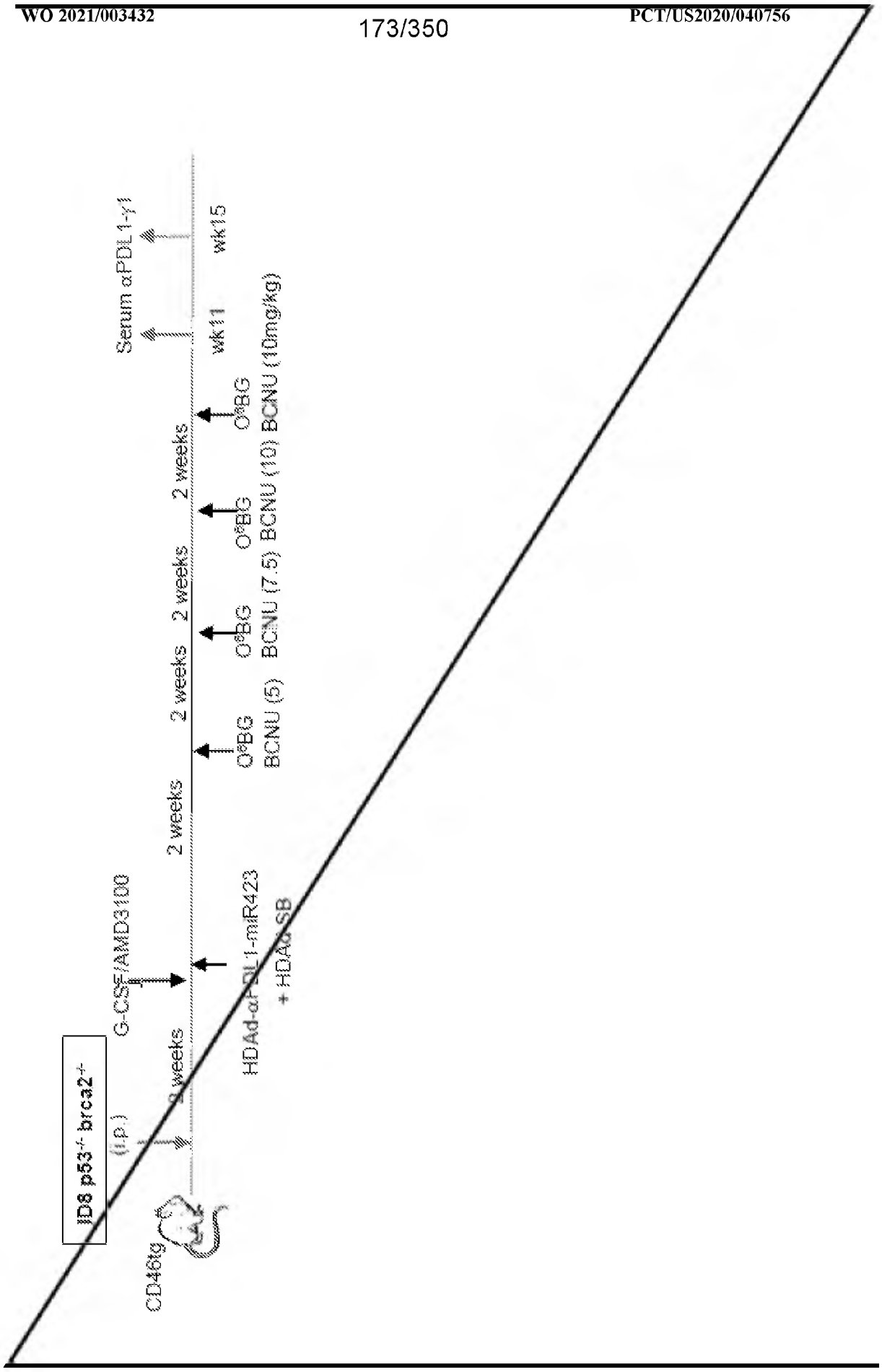

ID8 p53-/- brca2-/-
(i.p.)
CD46tg
3 weeks
G-CSF/AMD3100
HDAd-αPDL1-miR423
+ HDAd-SB
2 weeks
2 weeks
2 weeks
2 weeks
2 weeks
O6BG
BCNU (5)
O6BG
BCNU (7.5)
O6BG
BCNU (10)
O6BG
BCNU (10mg/kg)
wk11
Serum αPDL1-γ1
wk15

FIG. 86B

FIG. 86C

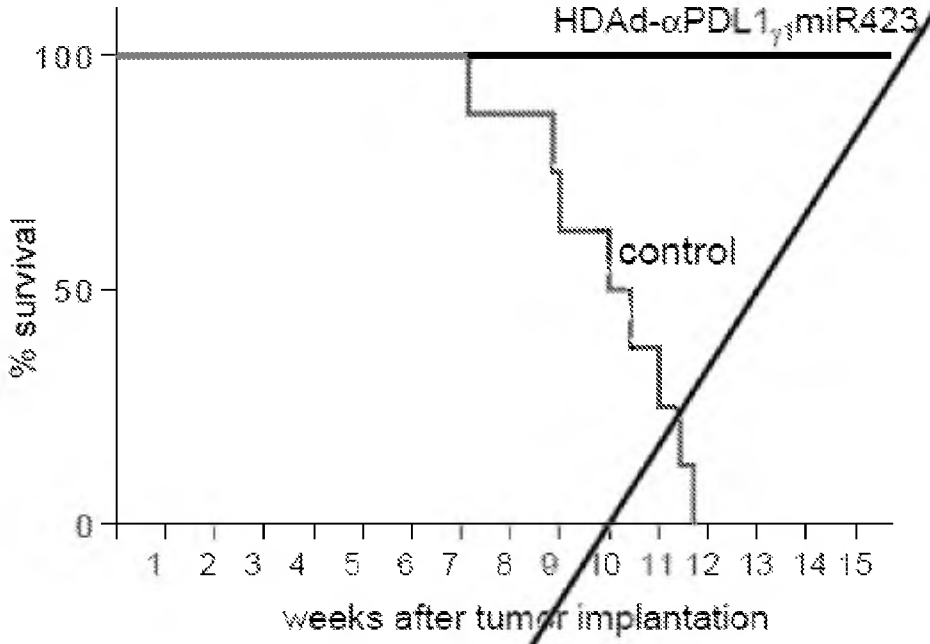


FIG. 86D

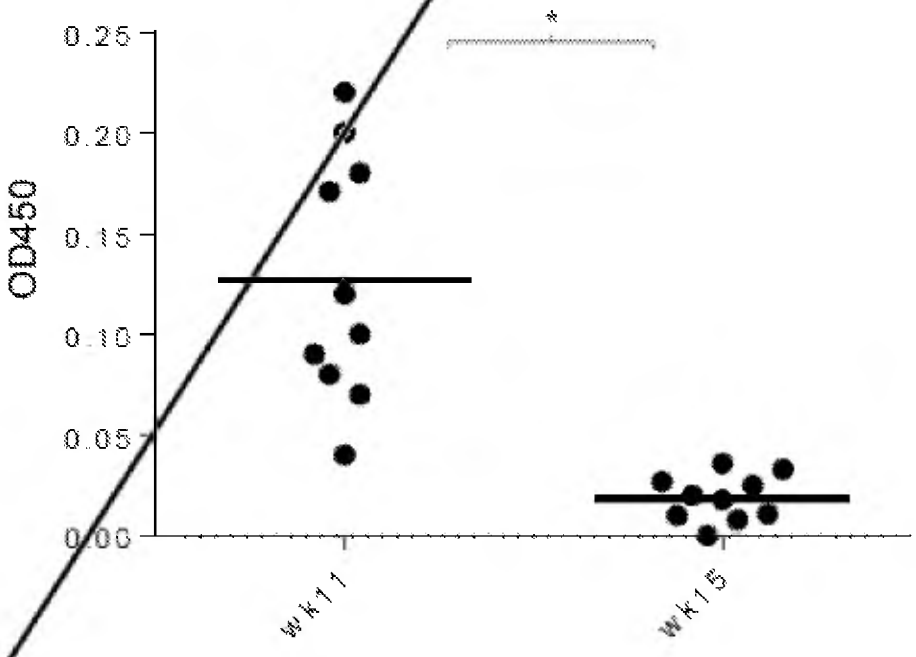


FIG. 87A

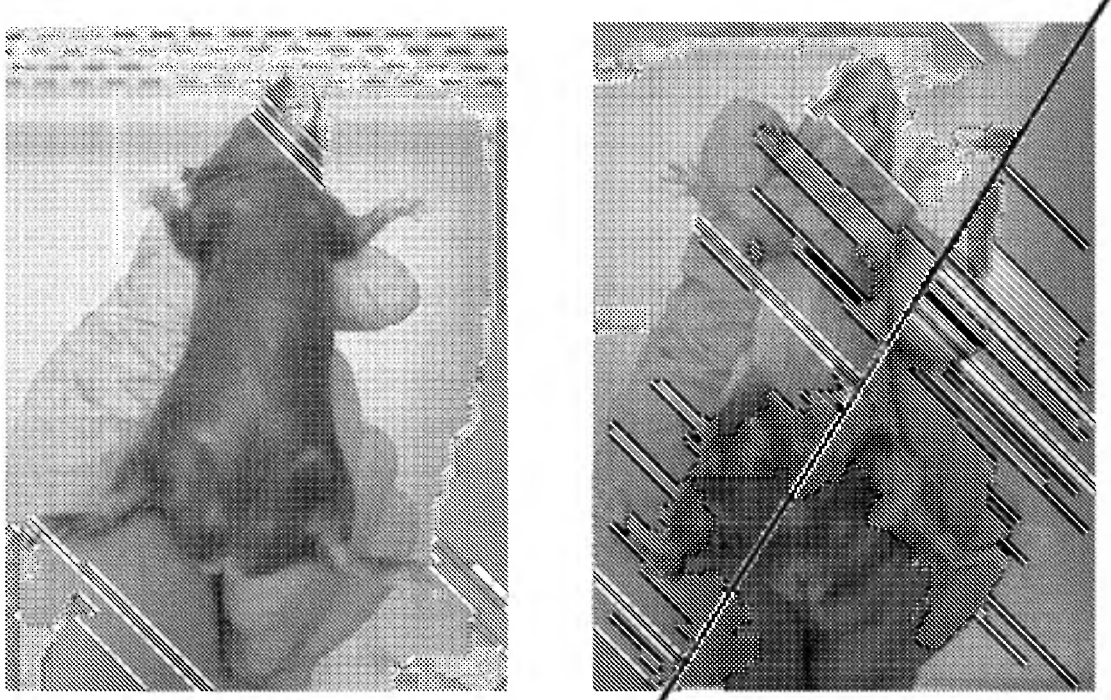


FIG. 87B

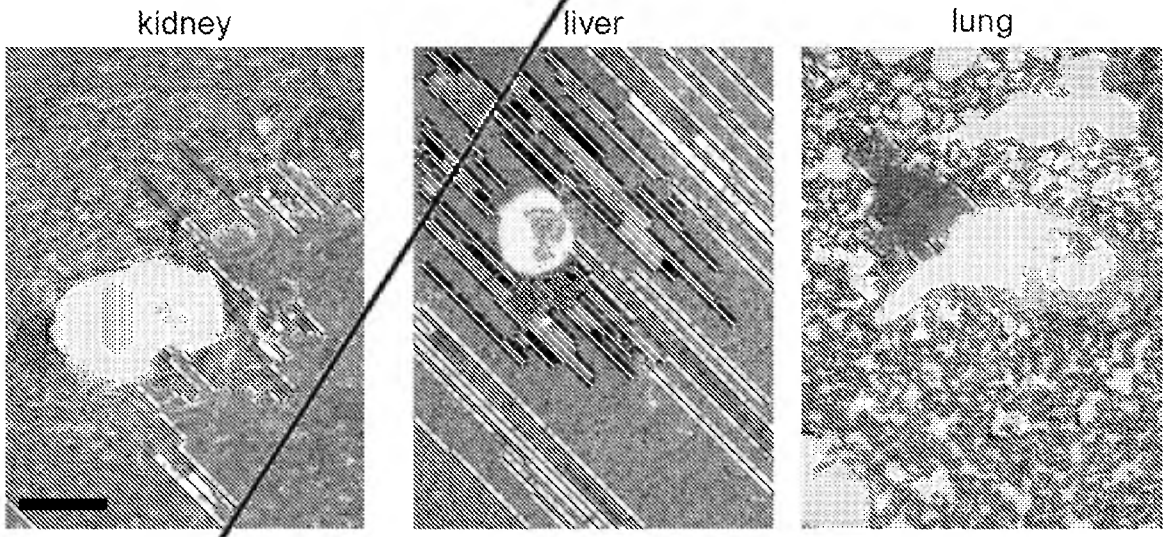
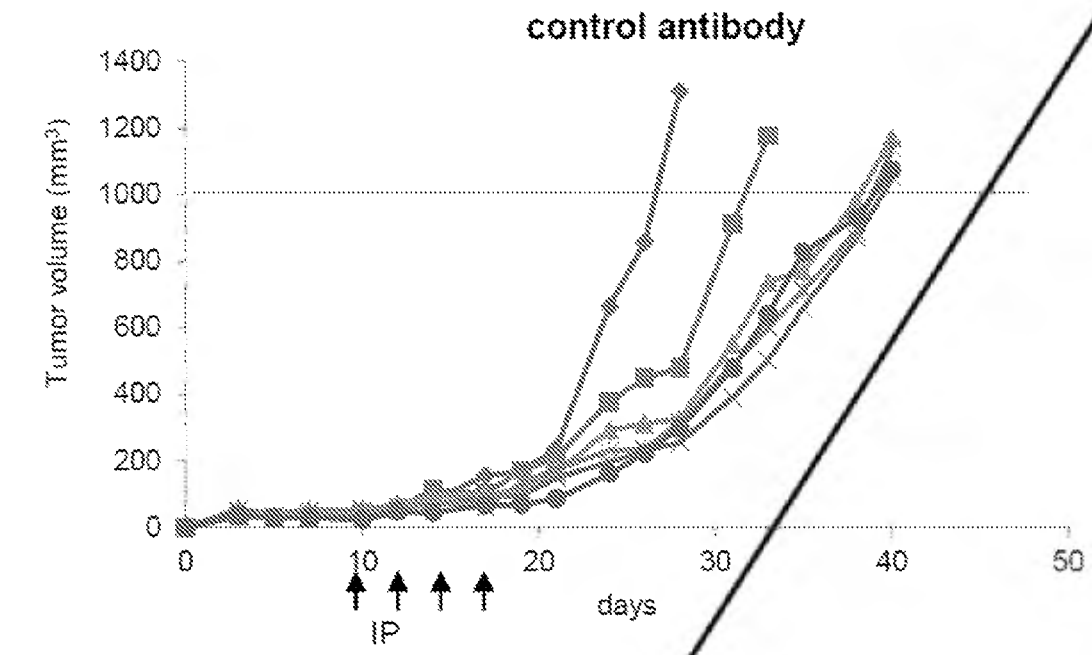


FIG. 88A


control antibody
Tumor volume (mm³)
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
days
IP

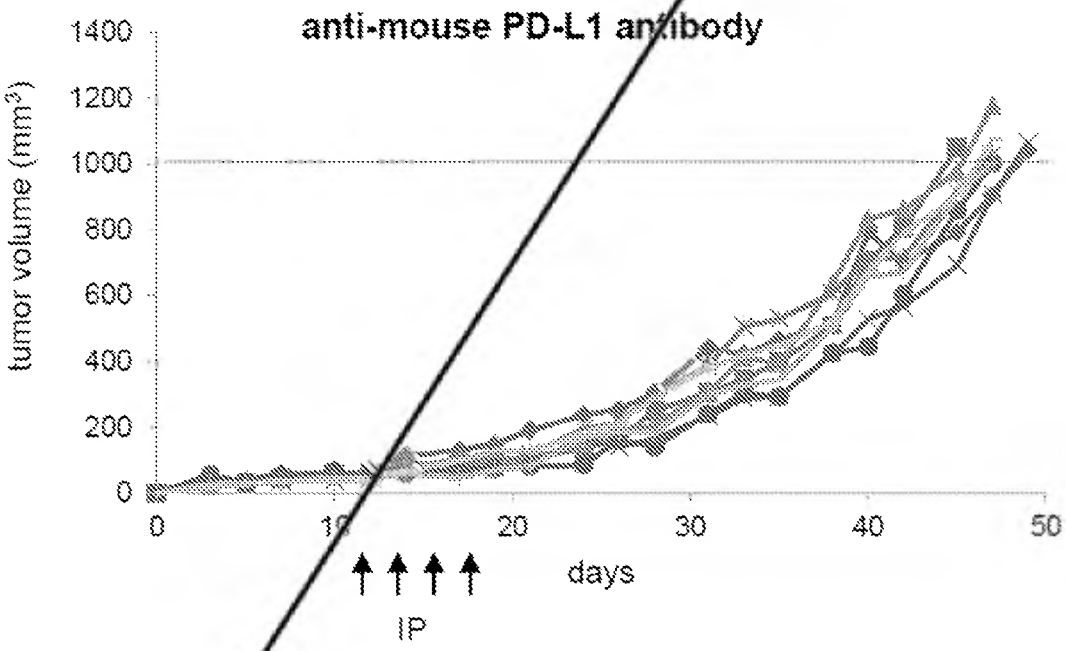

anti-mouse PD-L1 antibody
tumor volume (mm³)
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
days
IP

FIG. 88B

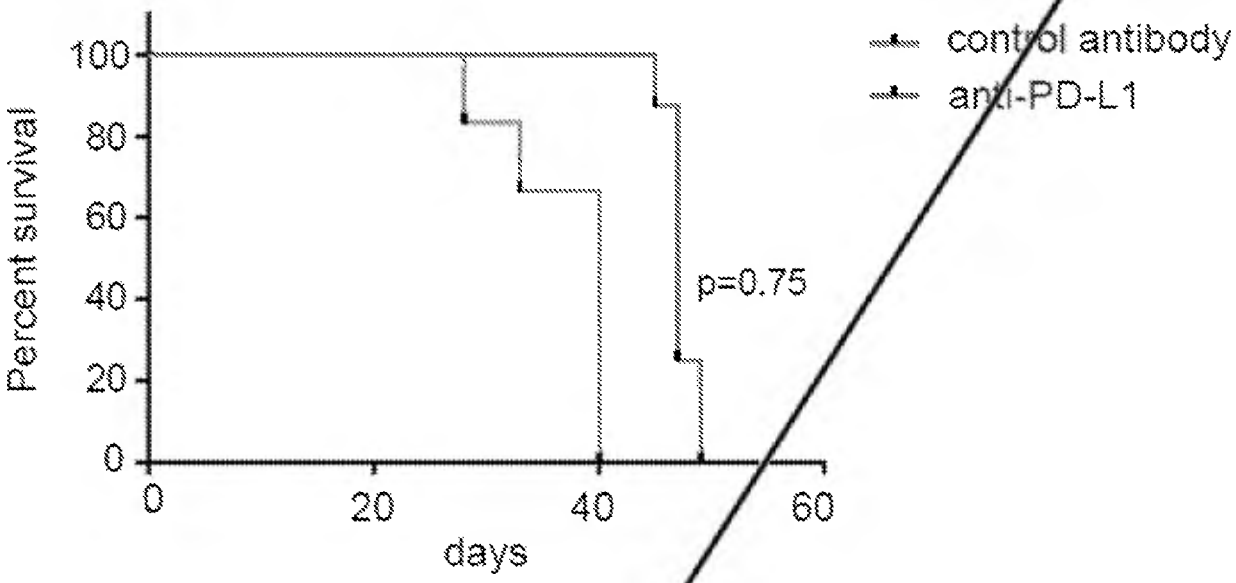

control antibody
anti-PD-L1
Percent survival
100
80
60
40
20
0
0
20
40
60
days
p=0.75

FIG. 88C

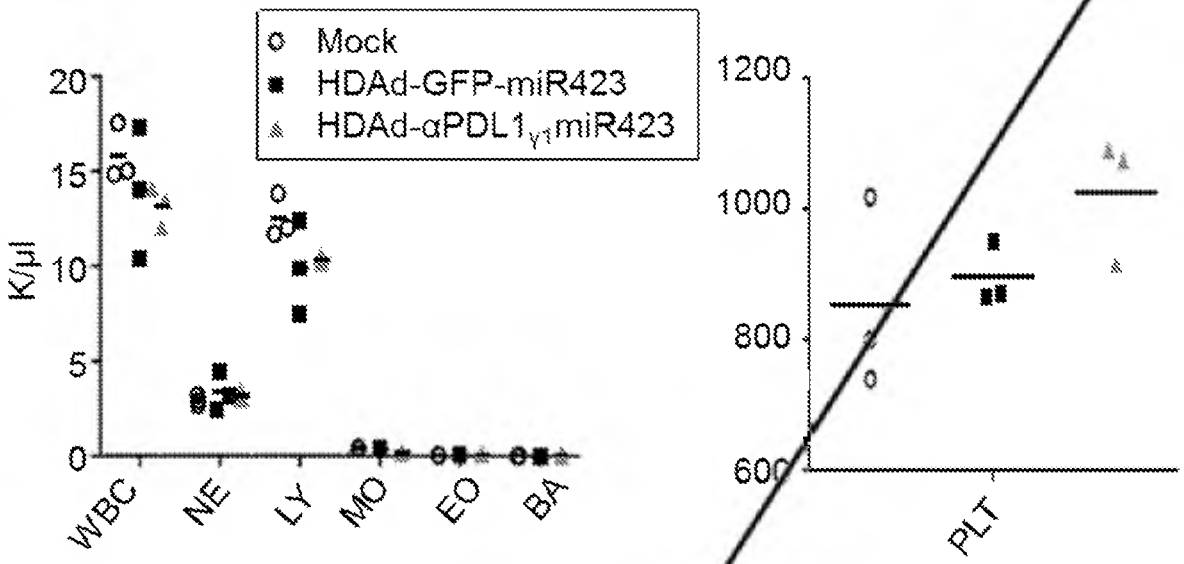


FIG. 88D

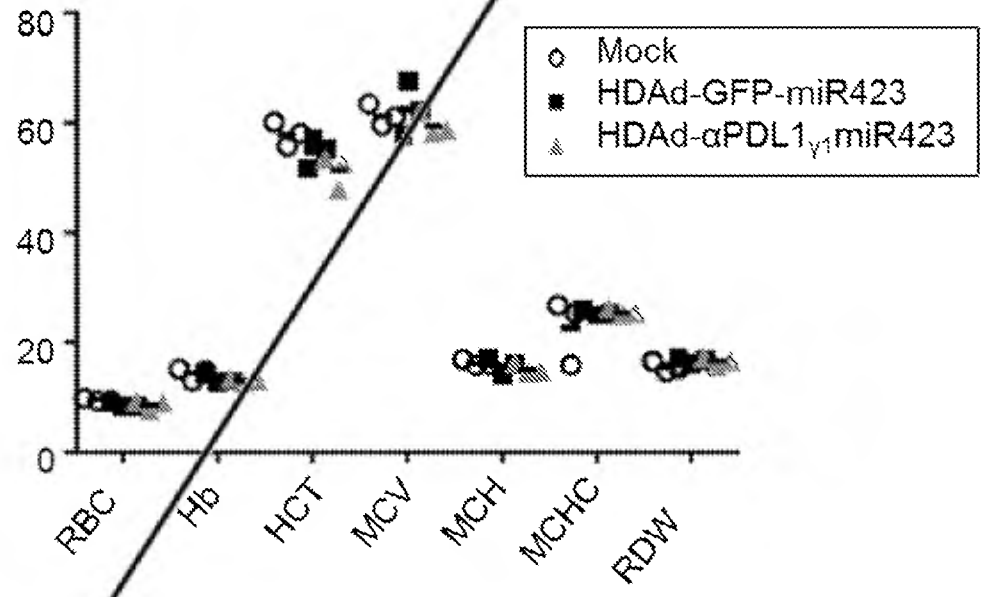
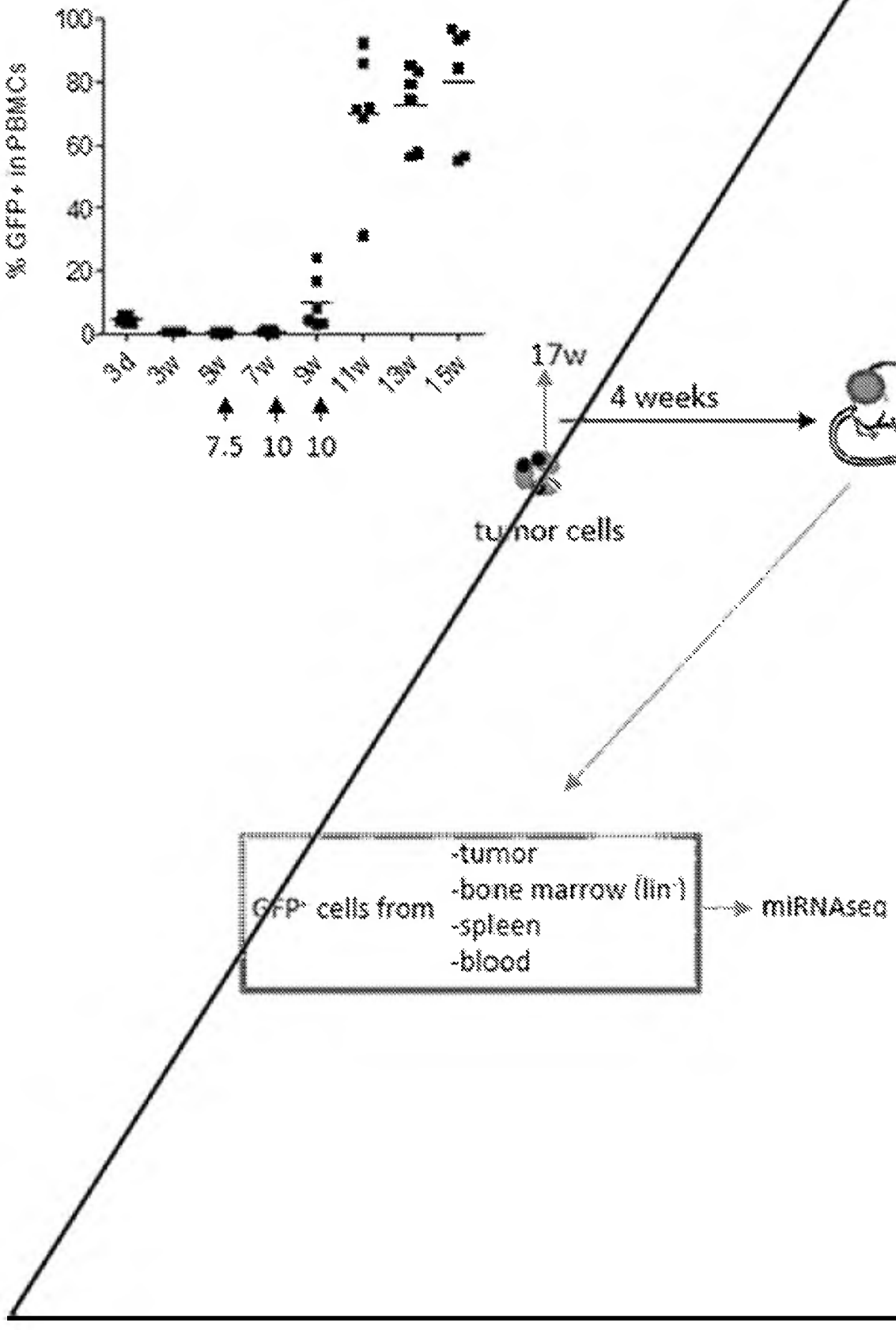
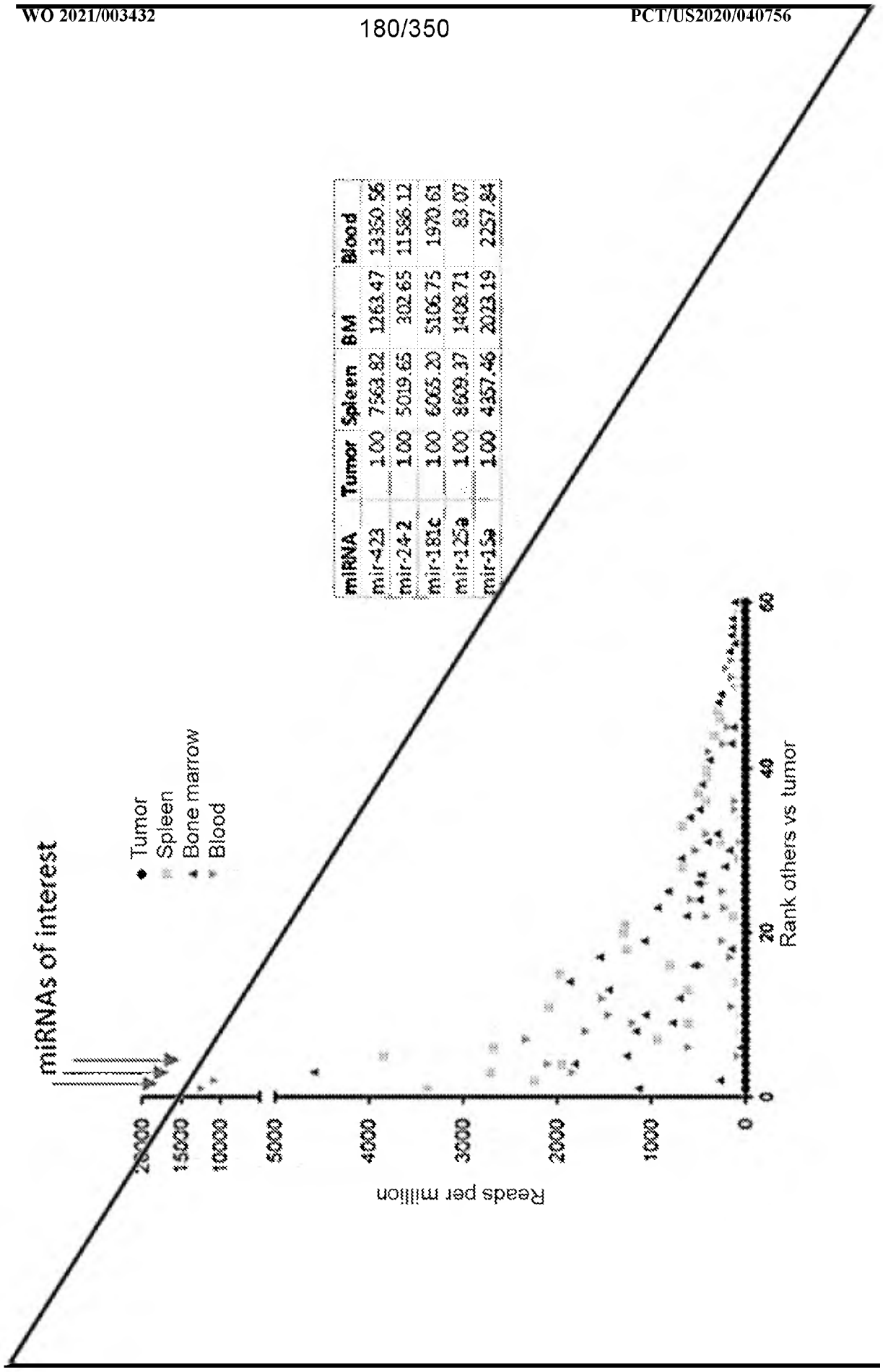


FIG. 88E


% GFP+ in PBMCs
100
80
60
40
20
0
3d
3w
5w
7w
9w
11w
13w
15w
7.5
10
10
17w
4 weeks
tumor cells
GFP+ cells from
-tumor
-bone marrow (lin-)
-spleen
-blood
miRNAseq



miRNA	Tumor	Spleen	BM	Blood
mir-423	1.00	7563.82	1263.47	13350.56
mir-24-2	1.00	5019.65	302.65	11566.12
mir-181c	1.00	6065.20	5106.75	1970.61
mir-125a	1.00	8609.37	1408.71	83.07
mir-15a	1.00	4357.46	2023.19	2257.84

FIG. 88E cont'd

FIG. 88F

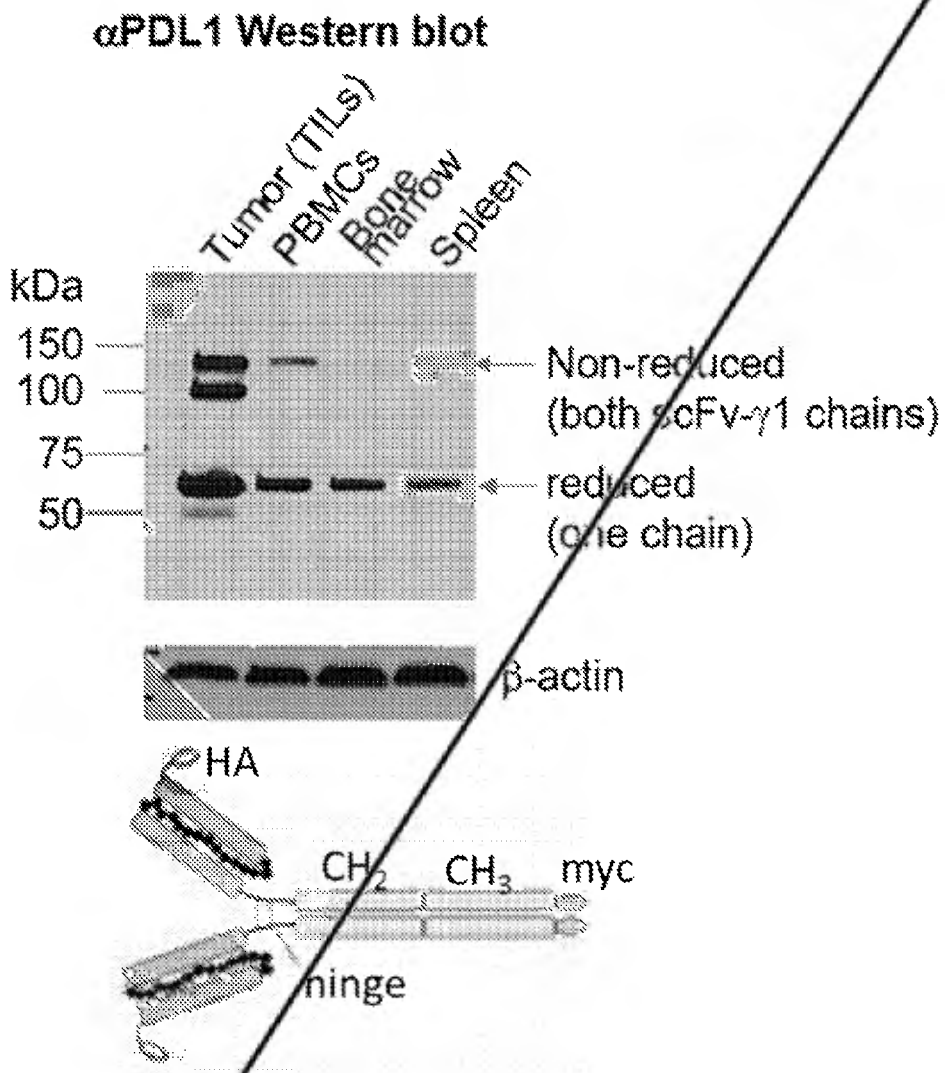

αPDL1 Western blot
Tumor (TILs)
PBMCs
Bone marrow
Spleen
kDa
150
100
75
50
Non-reduced
(both scFv-γ1 chains)
reduced
(one chain)
β-actin
HA
CH2
CH3
myc
ninge

FIG. 88F cont'd

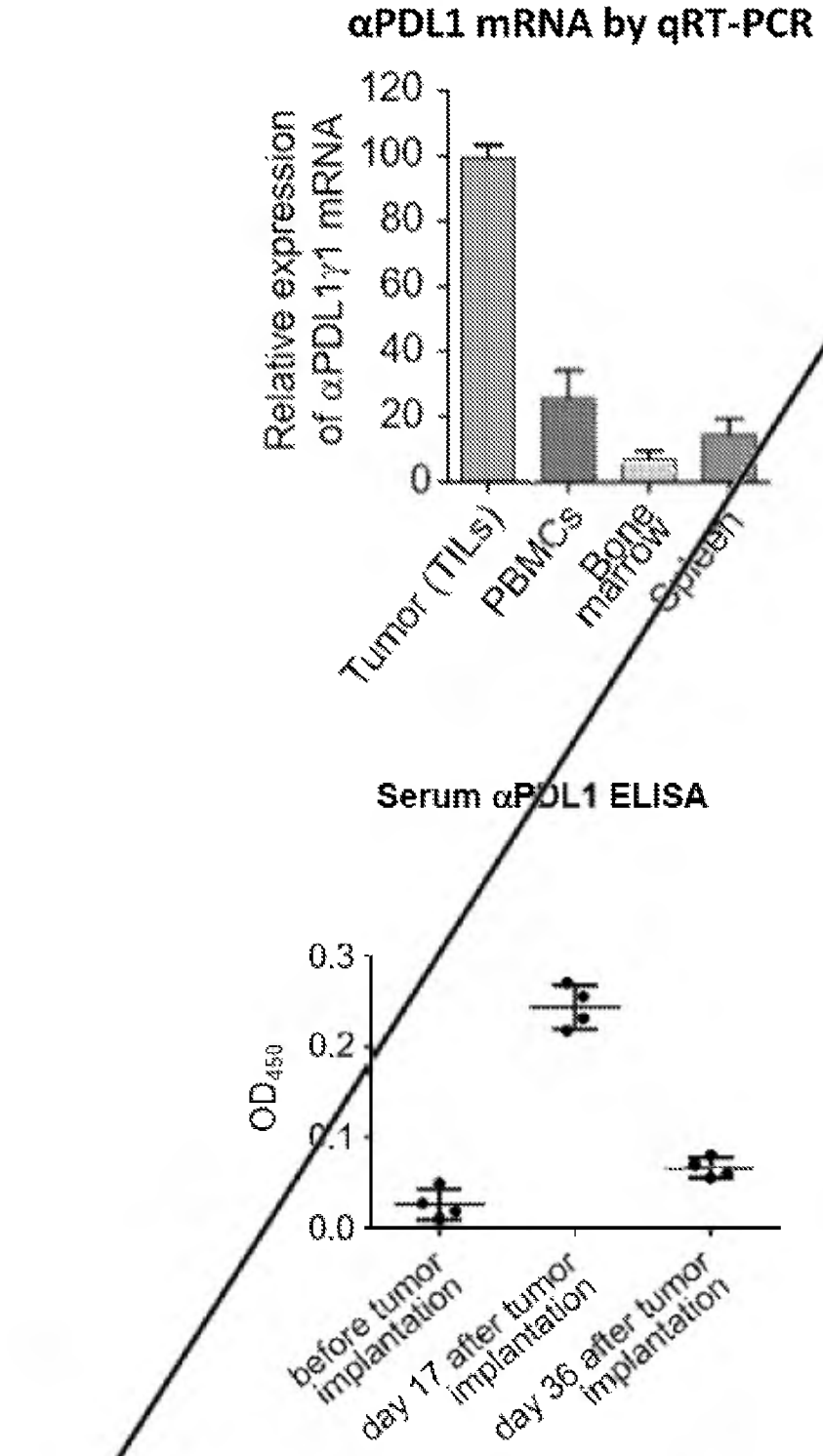


FIG. 88G

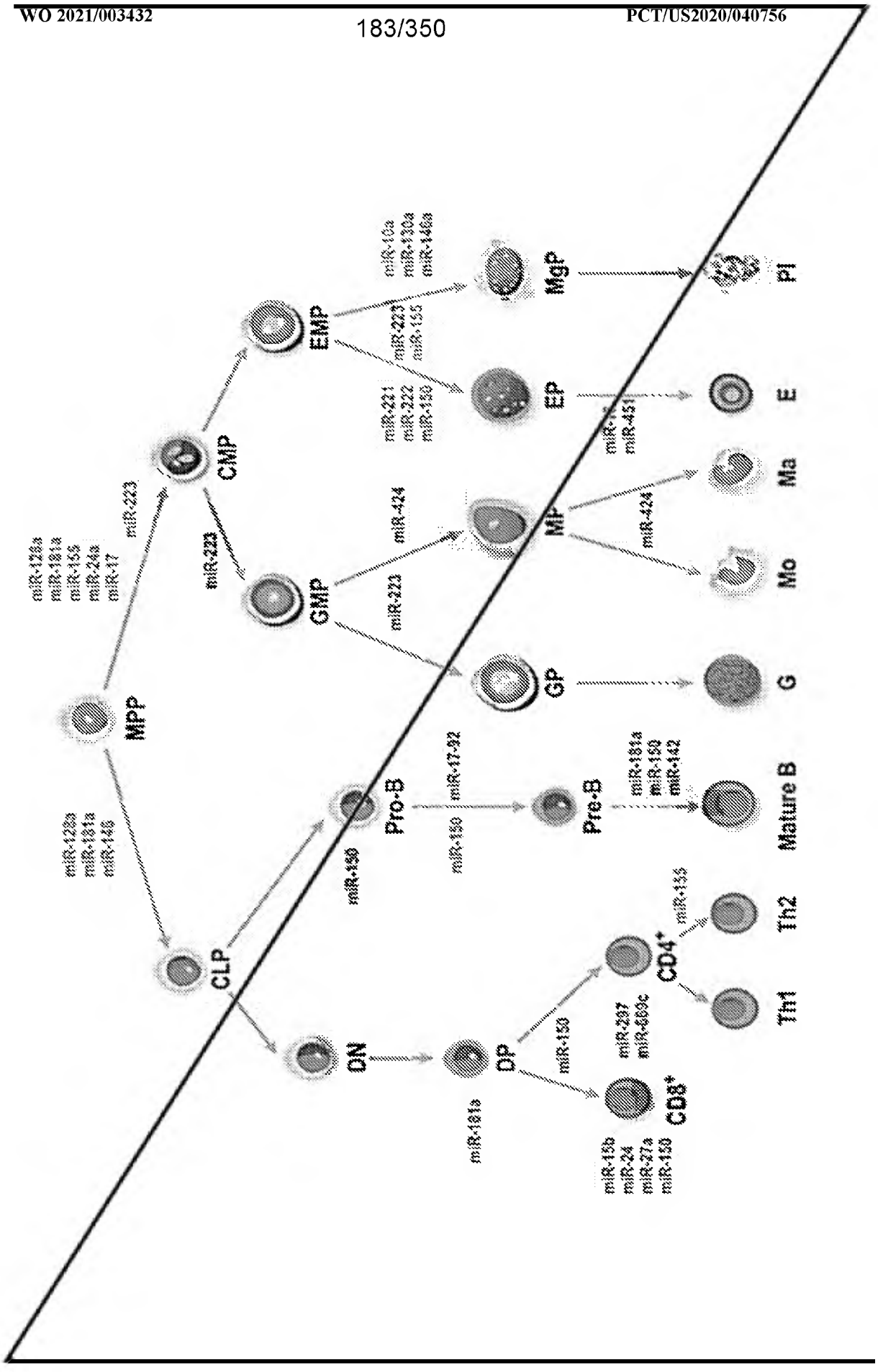
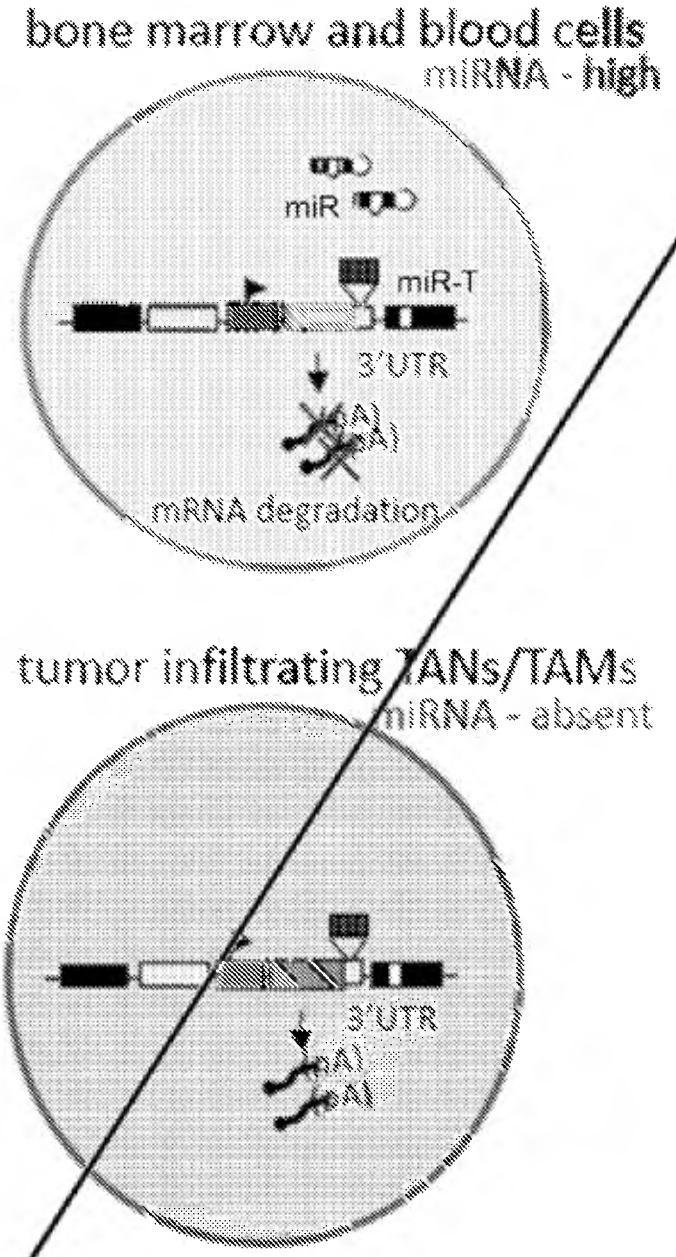

MPP
CLP
CMP
GMP
EMP
MEP
GP
MP
MgP
EP
ON
Pro-B
Pre-B
DP
Mature B
CD4+
CD8+
Th1
Th2
Mo
Ma
G
E
Pl
miR-223
miR-128a
miR-181a
miR-155
miR-24a
miR-17
miR-221
miR-424
miR-150
miR-17-92
miR-181a
miR-142
miR-155
miR-237
miR-486c
miR-15b
miR-24
miR-27a
miR-150
miR-16a
miR-130a
miR-146a
miR-222
miR-451

FIG. 88G cont'd



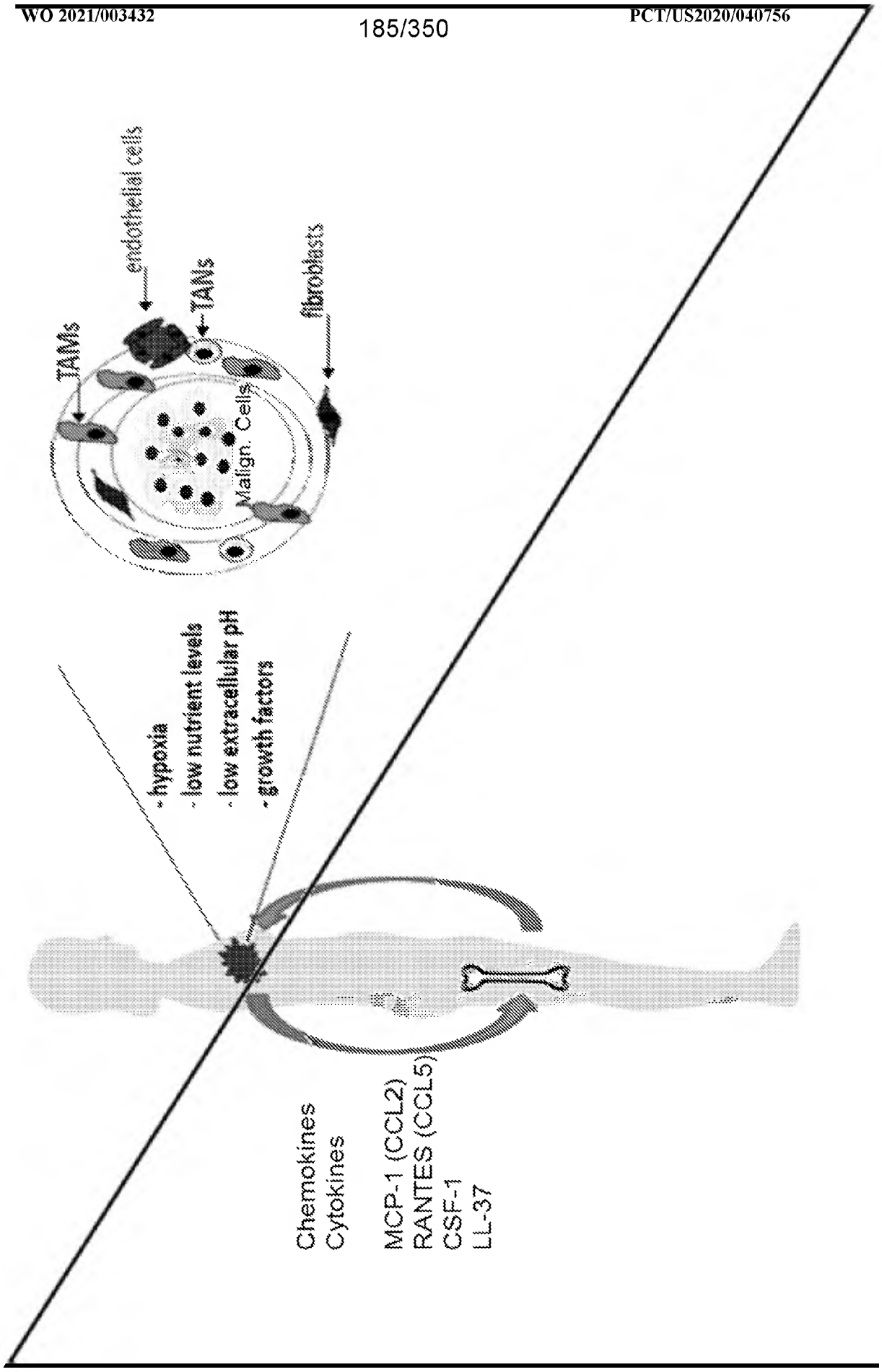

endothelial cells
TAMs
TANs
fibroblasts
Malign. Cells
- hypoxia
- low nutrient levels
- low extracellular pH
- growth factors
Chemokines
Cytokines
MCP-1 (CCL2)
RANTES (CCL5)
CSF-1
LL-37

FIG. 88H

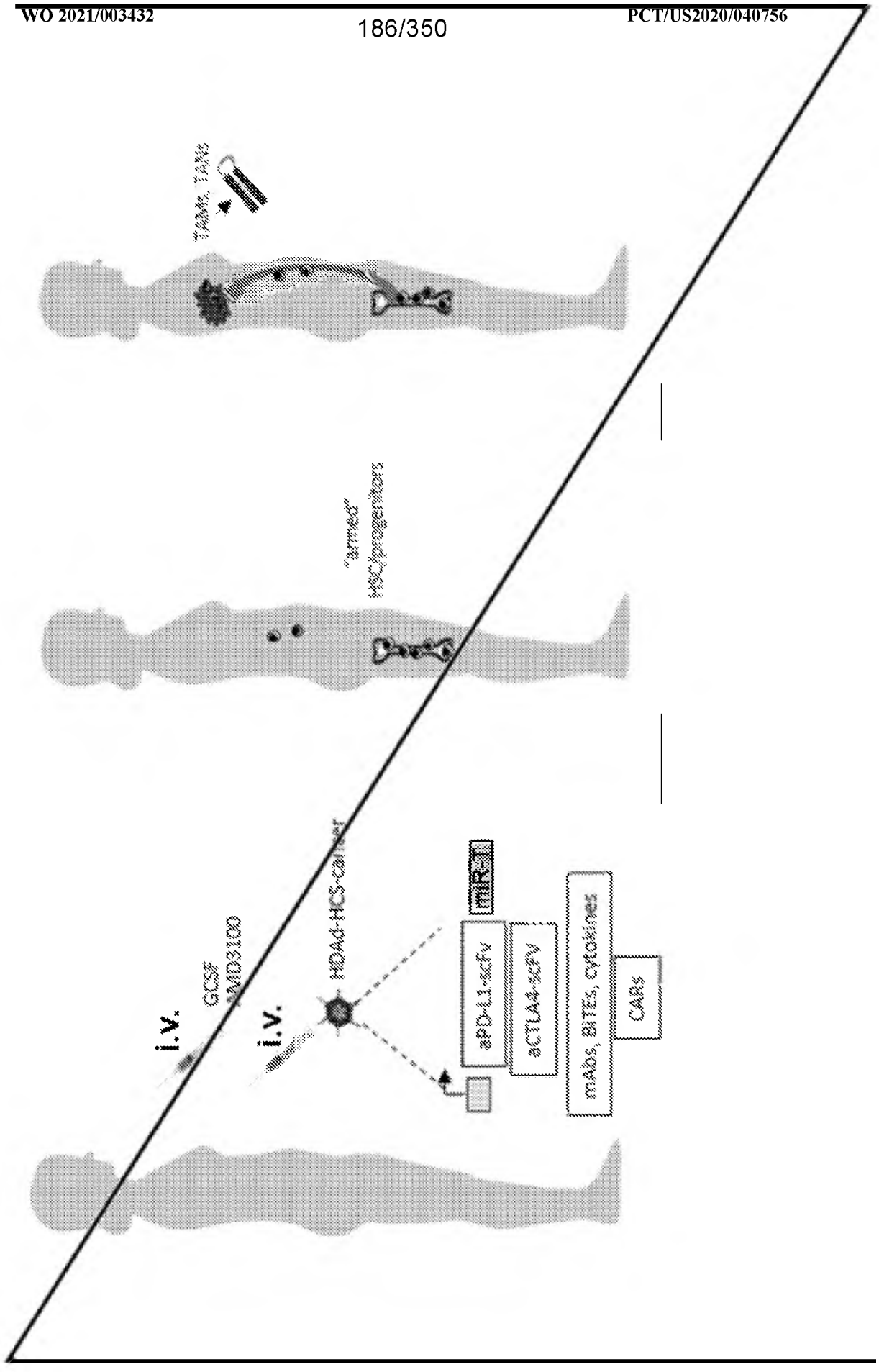


FIG. 88H cont'd

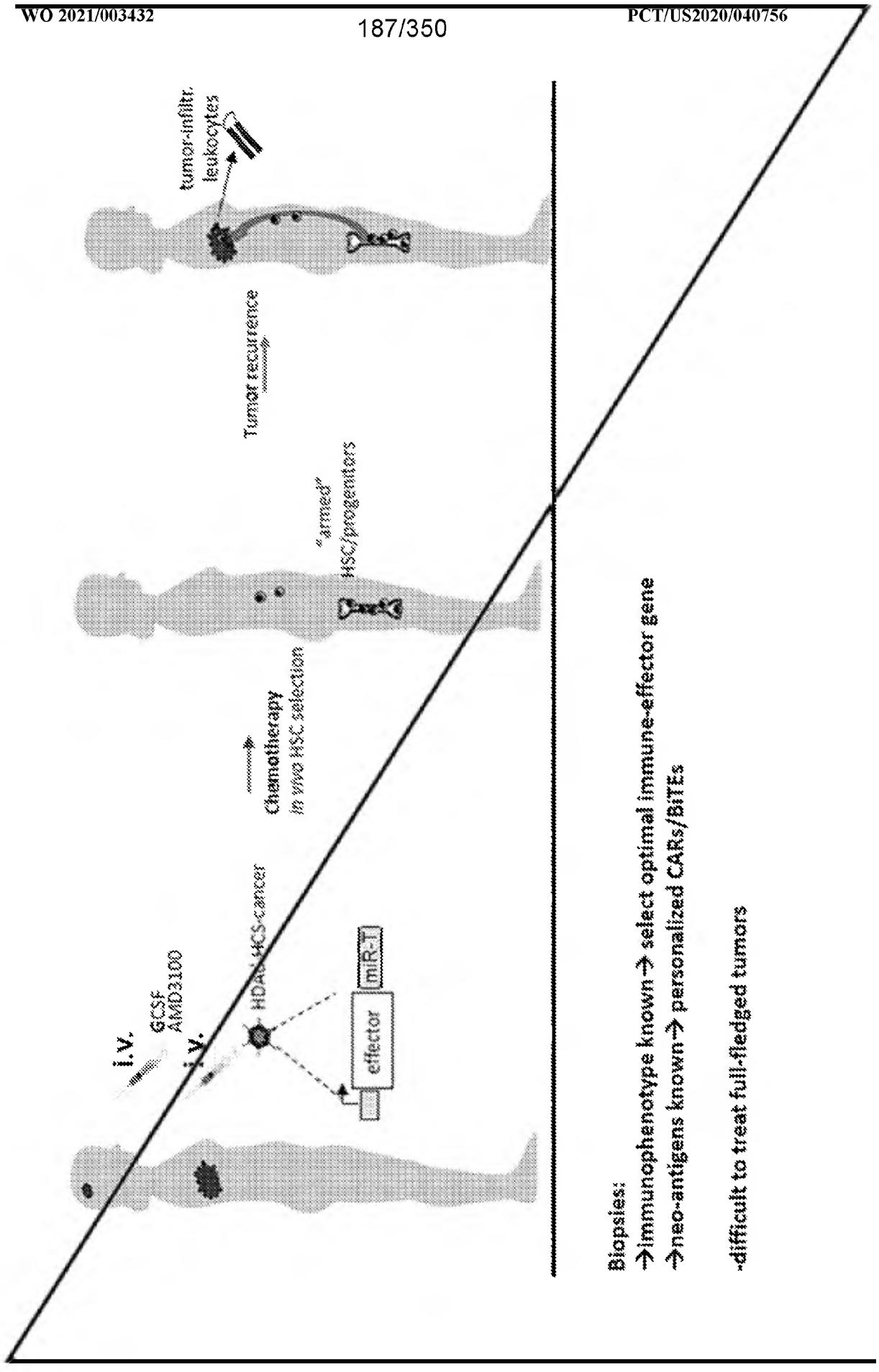


FIG. 88H cont'd

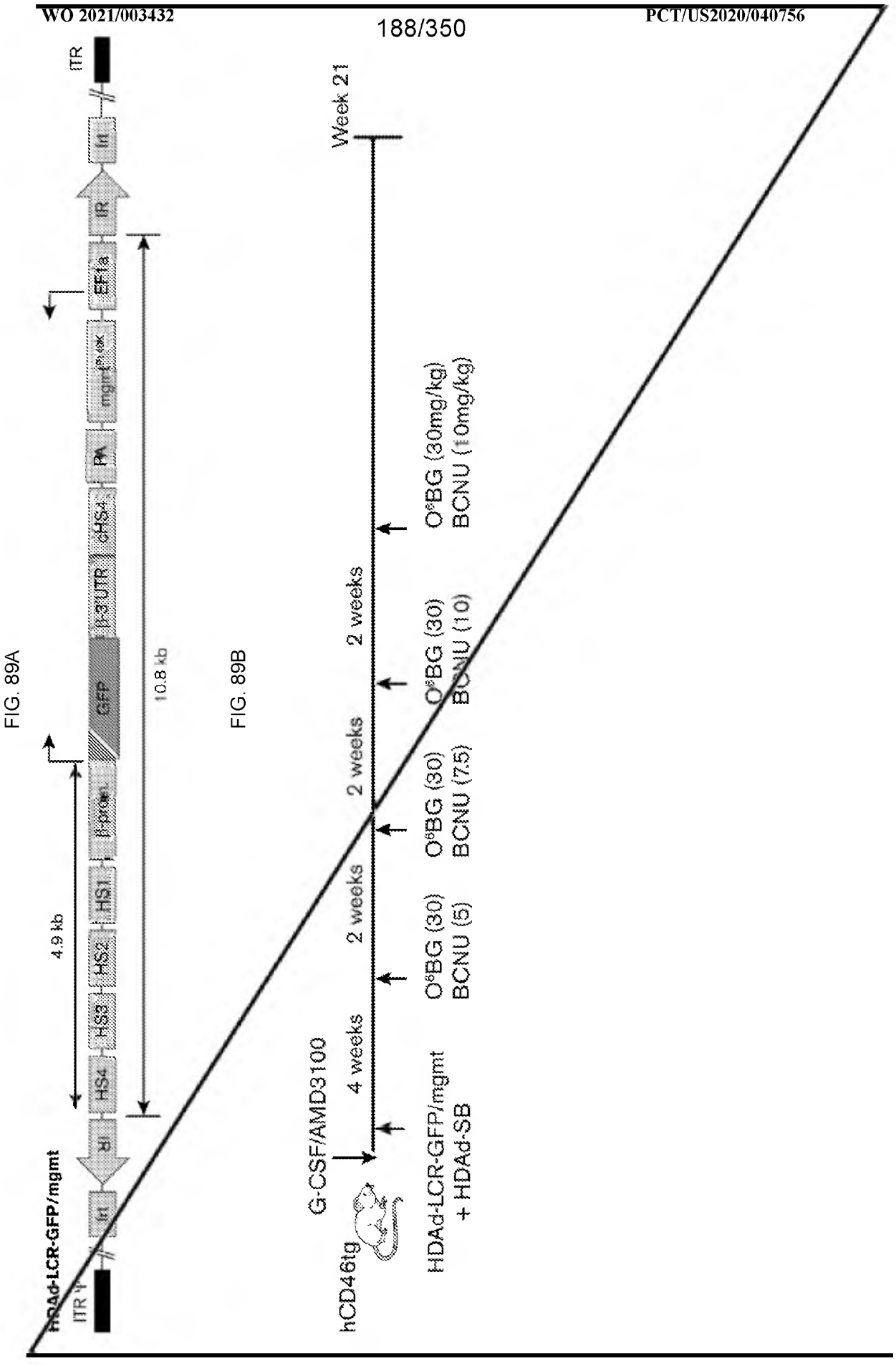


FIG. 89A

FIG. 89B

FIG. 89C

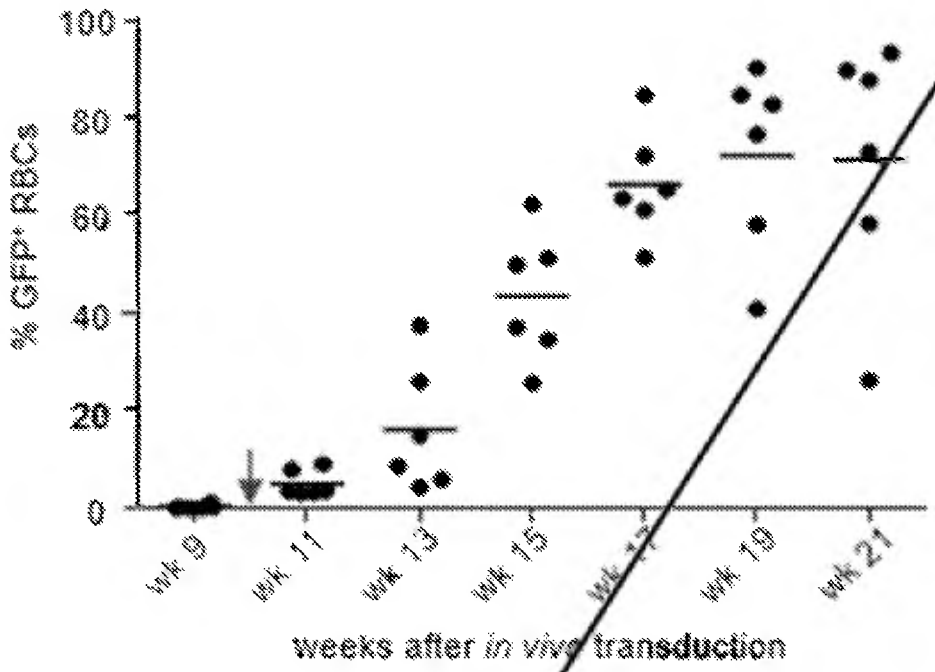


FIG. 89D

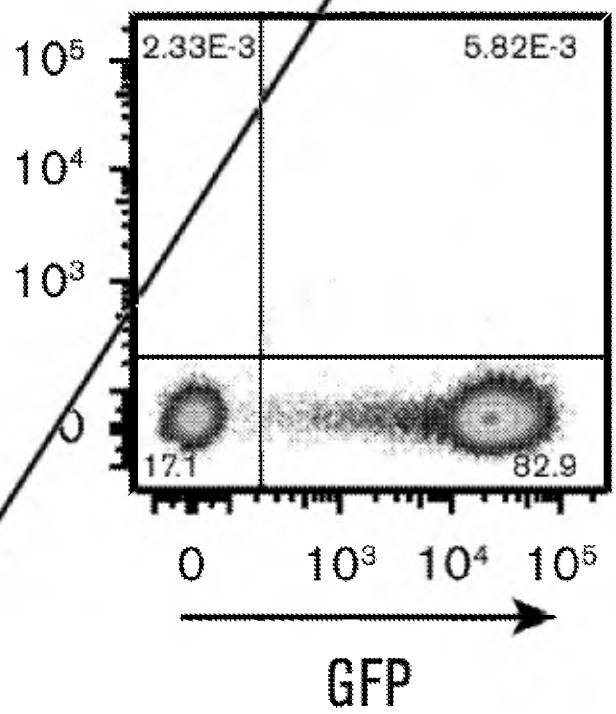


FIG. 89E

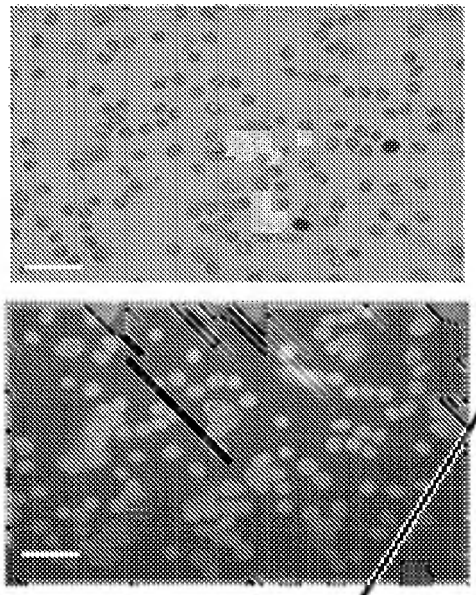


FIG. 89F

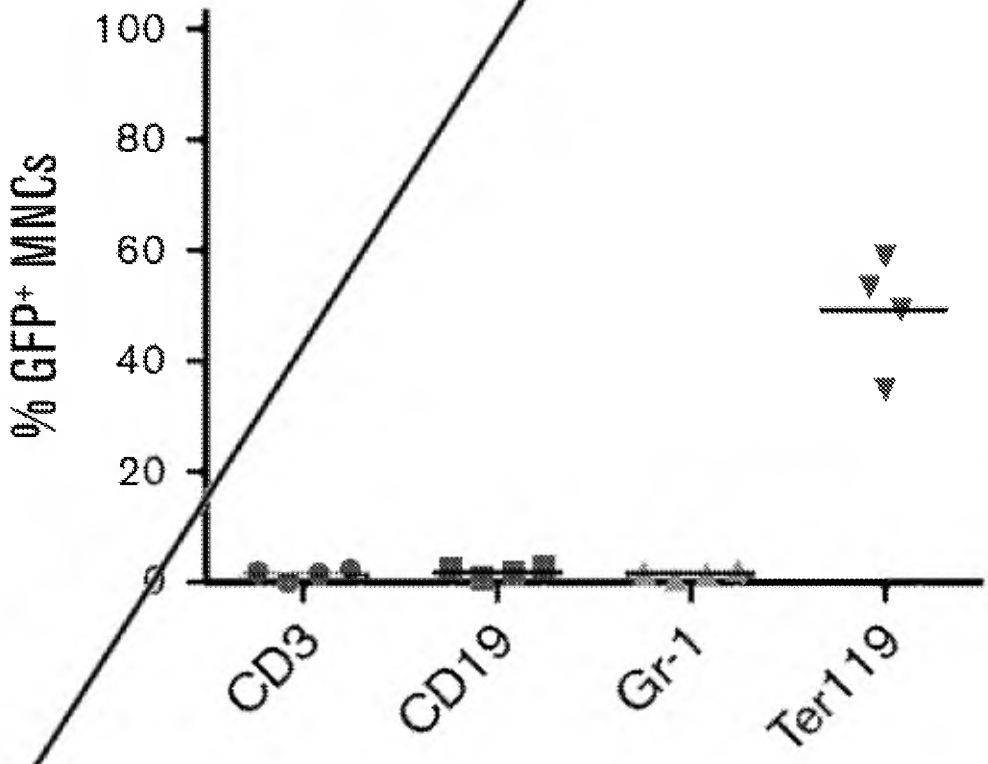


FIG. 89G

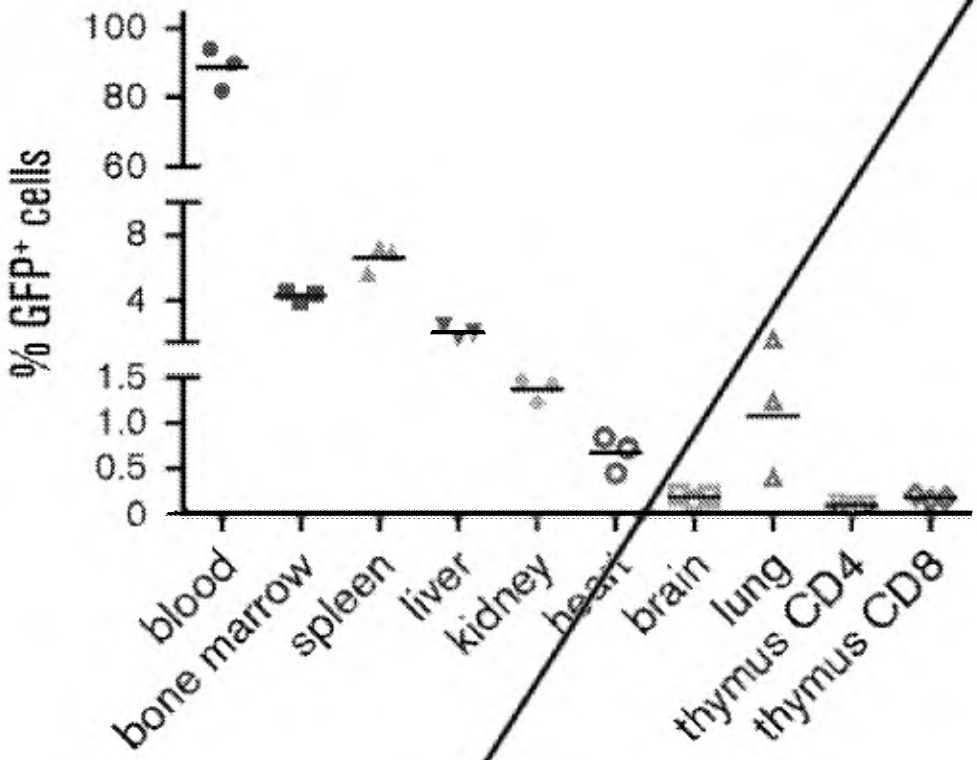


FIG. 89H

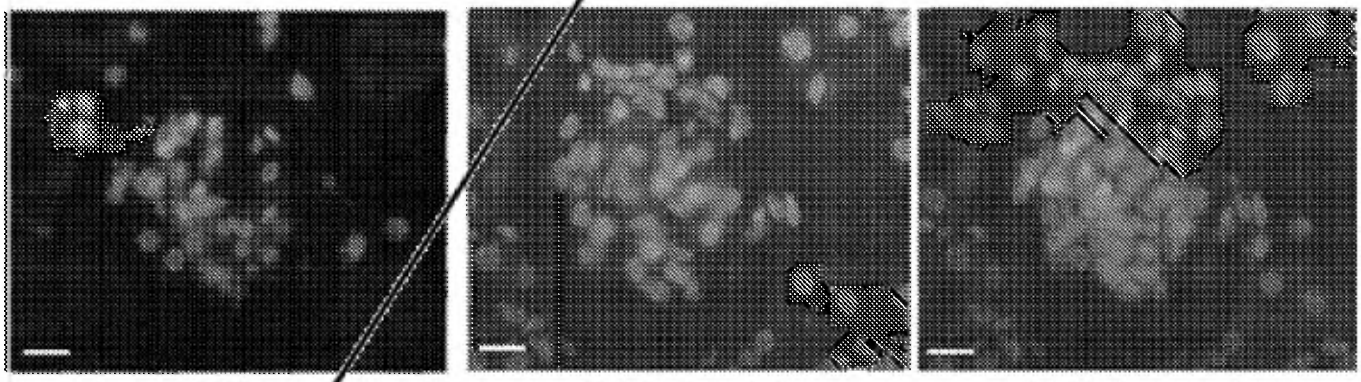


FIG. 90A

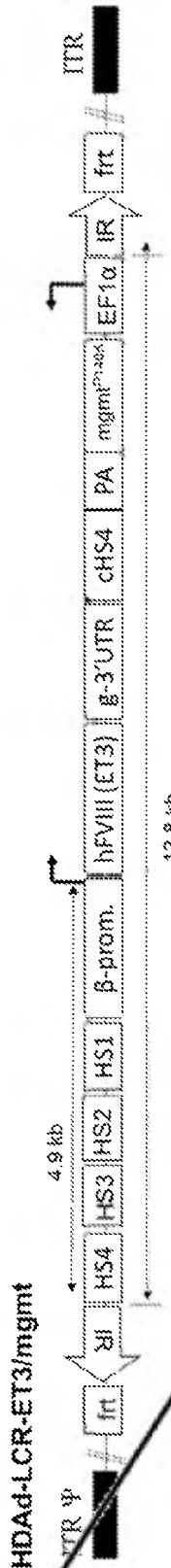


FIG. 90B

FIG. 90C

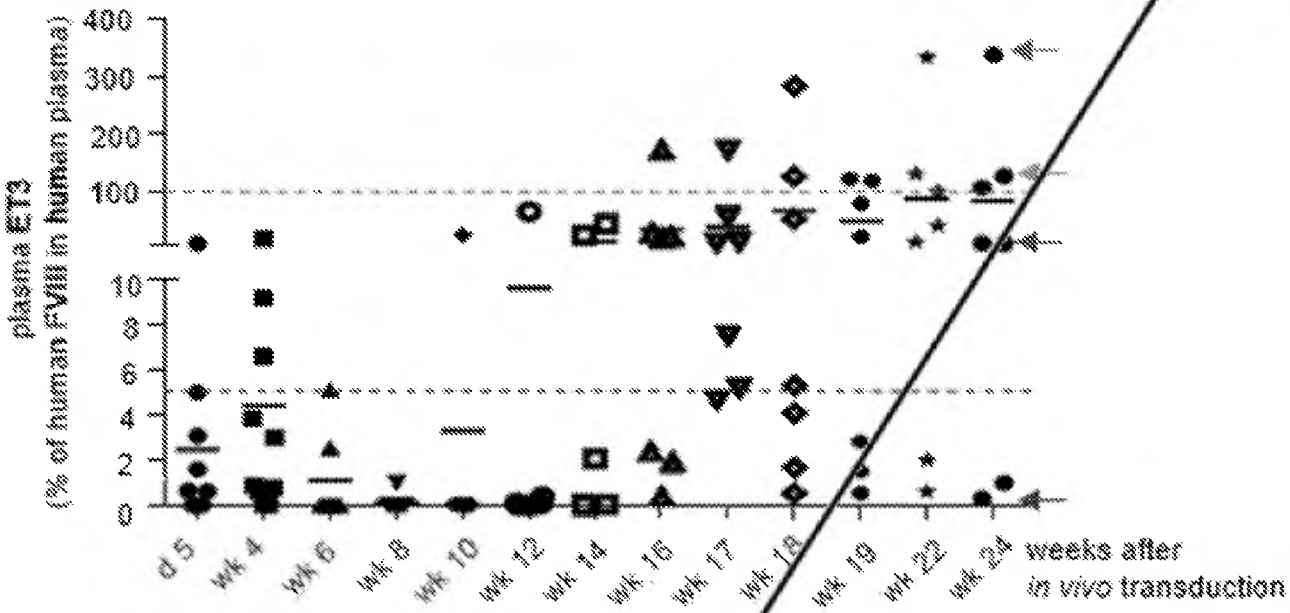


FIG. 90D

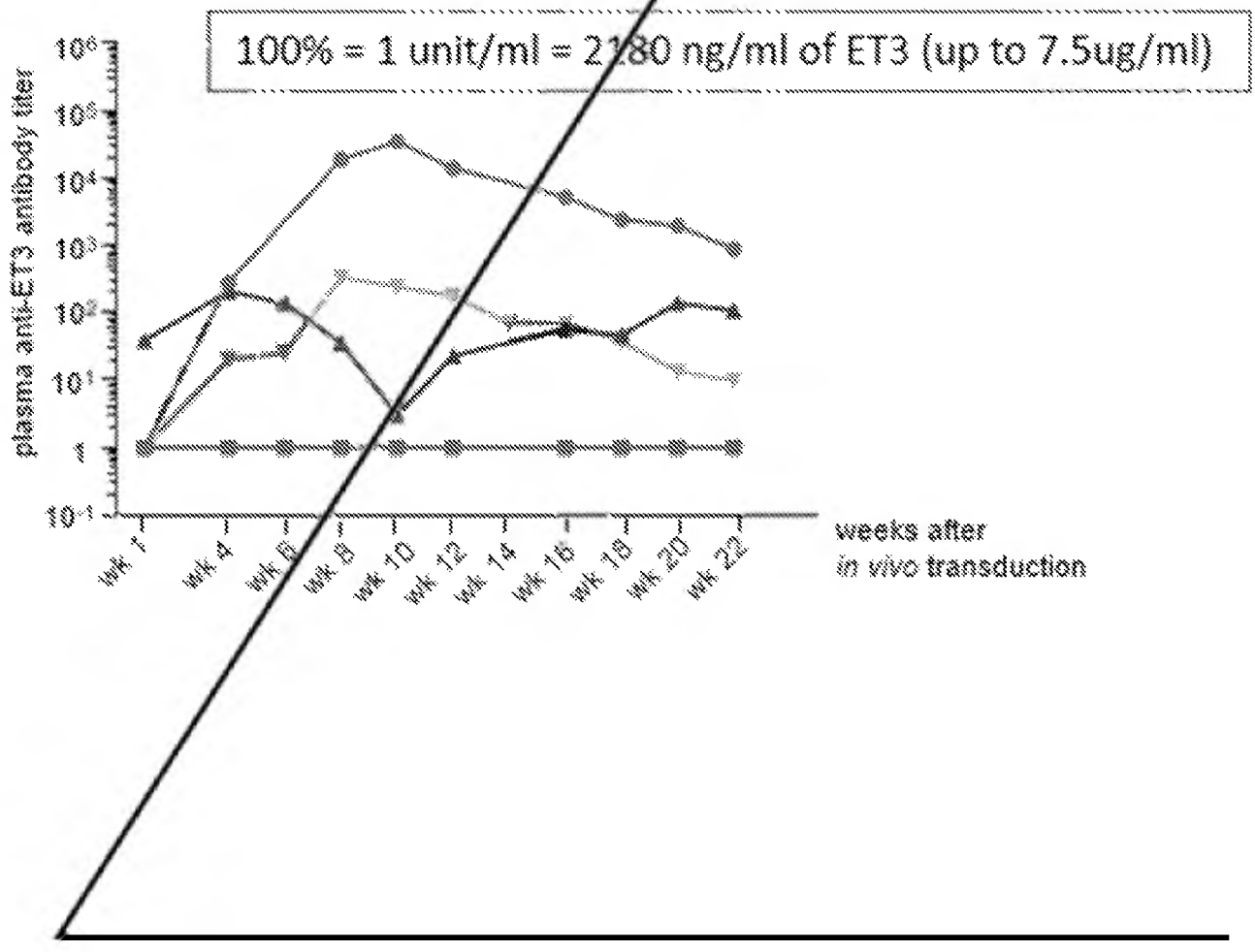


FIG. 90E

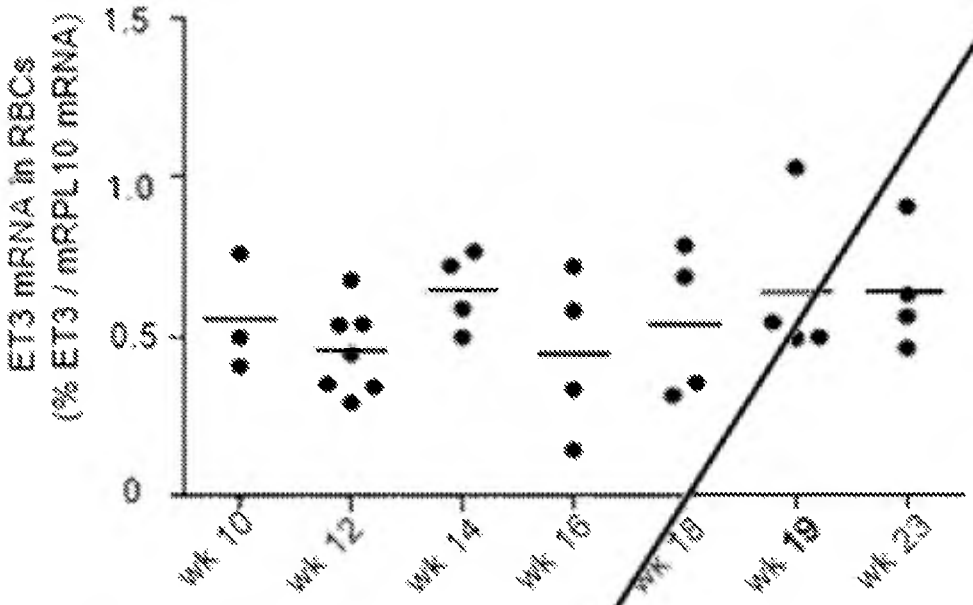


FIG. 90F

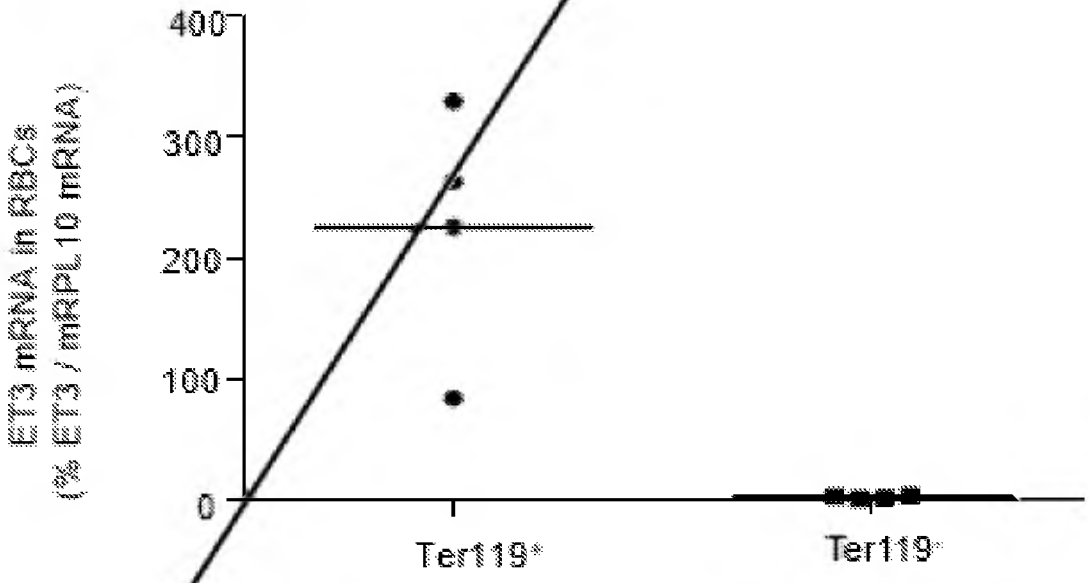


FIG. 90G

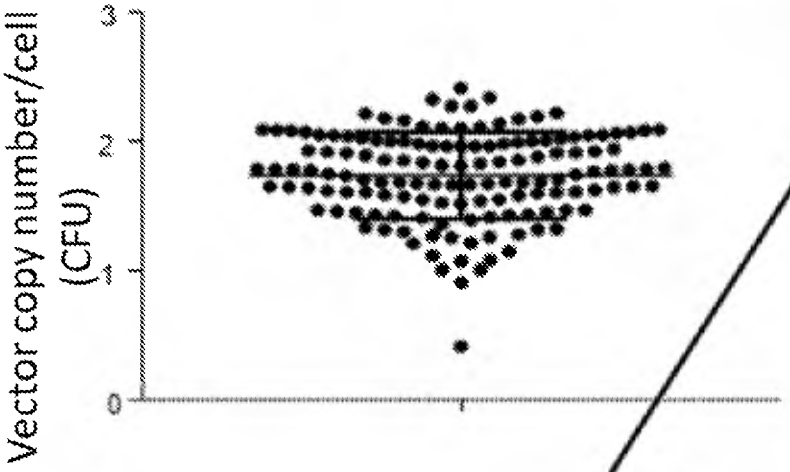


FIG. 90H

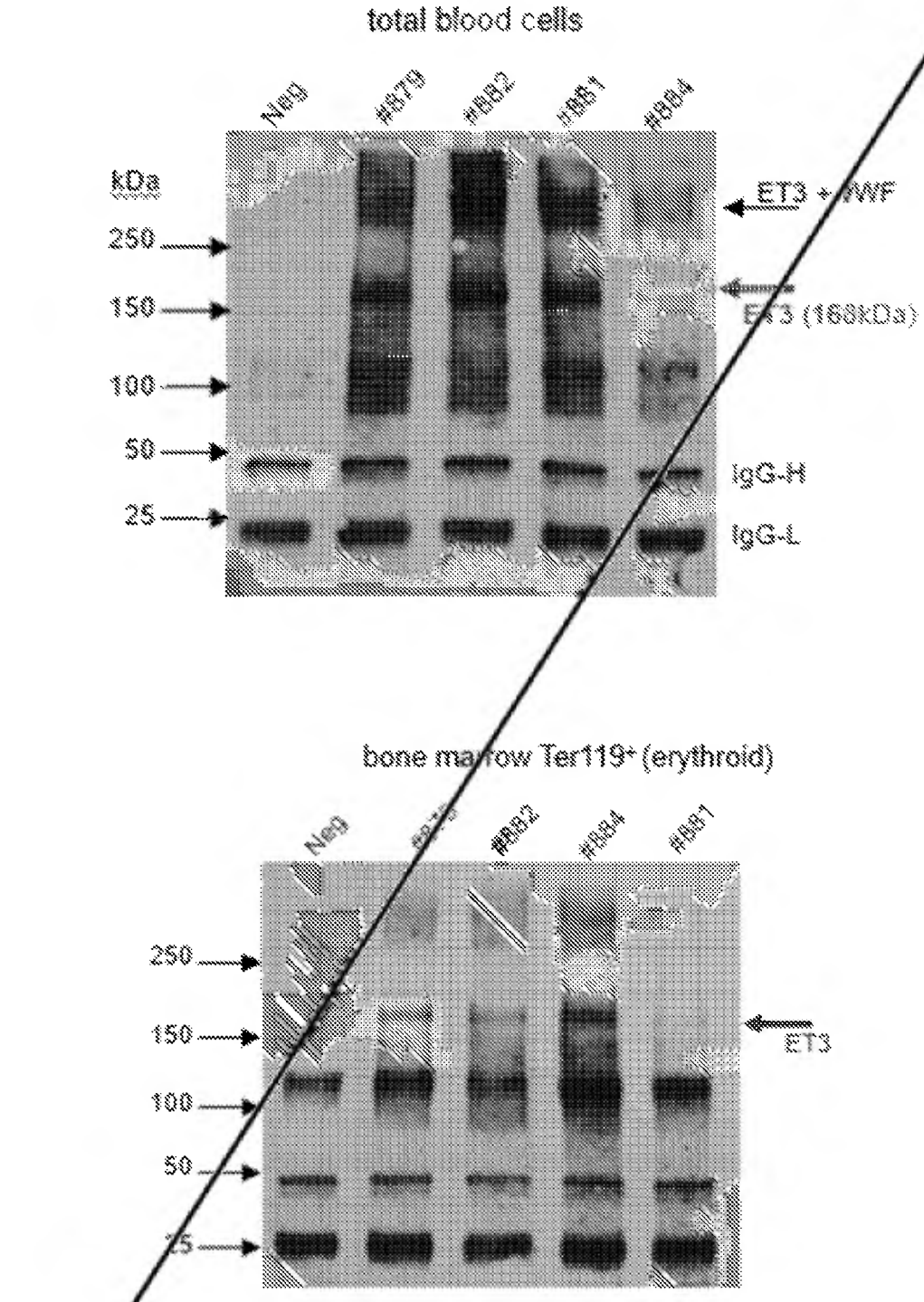

total blood cells
Neg
#879
#882
#881
#884
kDa
250
150
100
50
25
ET3 + VWF
ET3 (168kDa)
IgG-H
IgG-L
bone marrow Ter119+ (erythroid)
Neg
#882
#884
#881
250
150
100
50
25
ET3

FIG. 90H cont'd

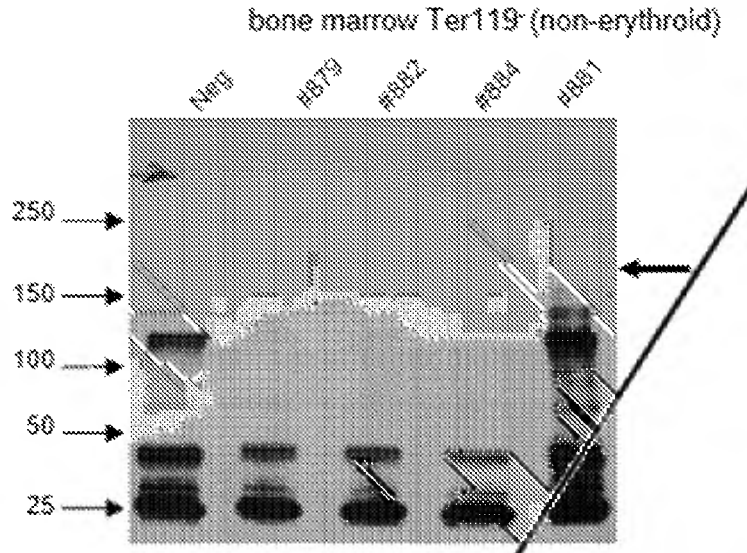


FIG. 90I

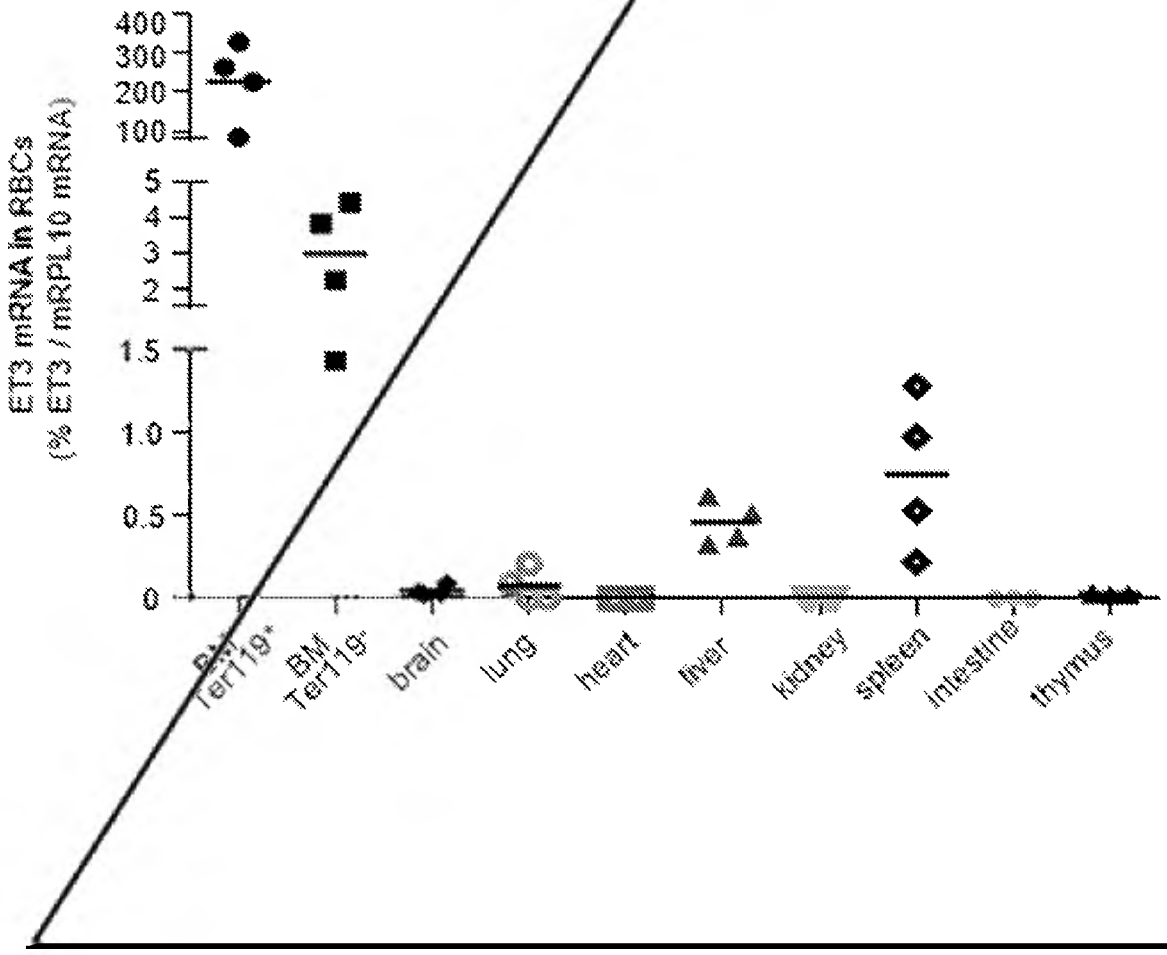


FIG. 91A

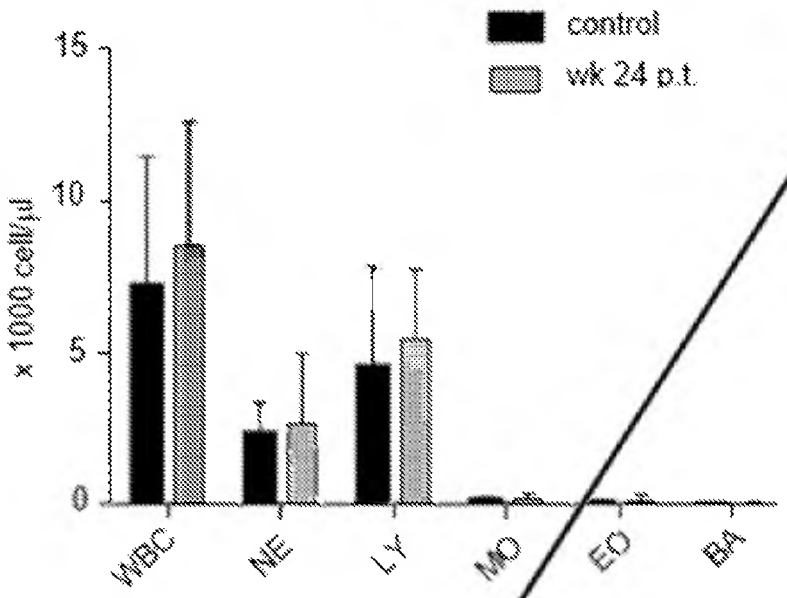


FIG. 91B

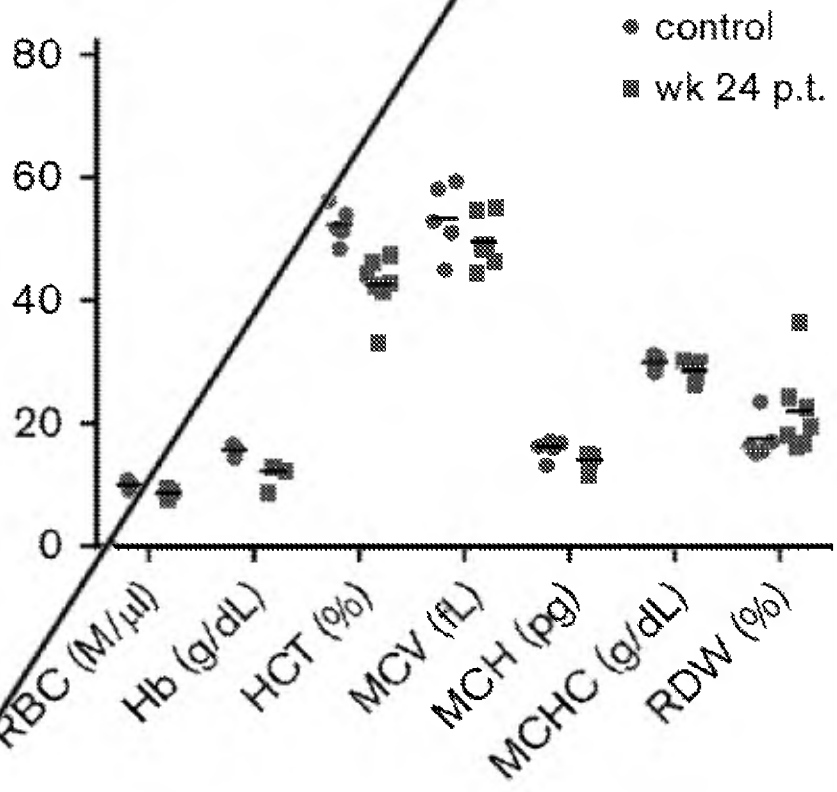


FIG. 91C

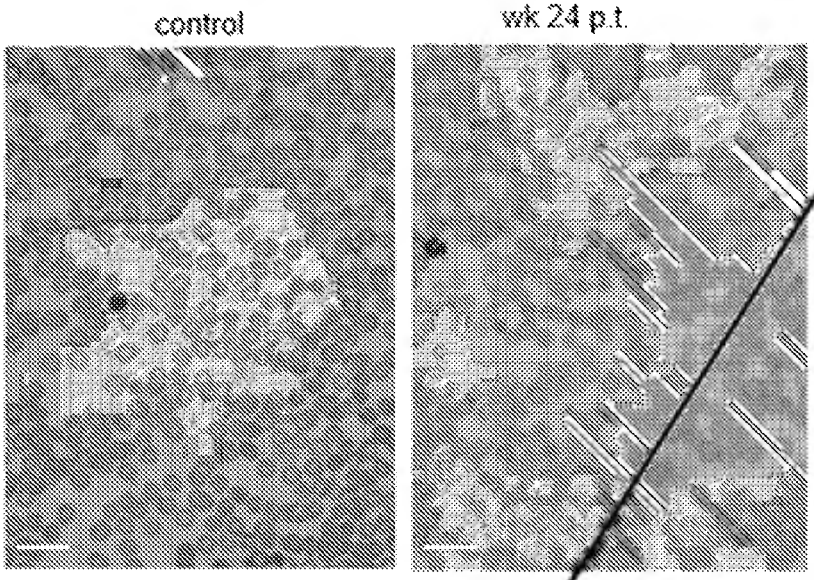
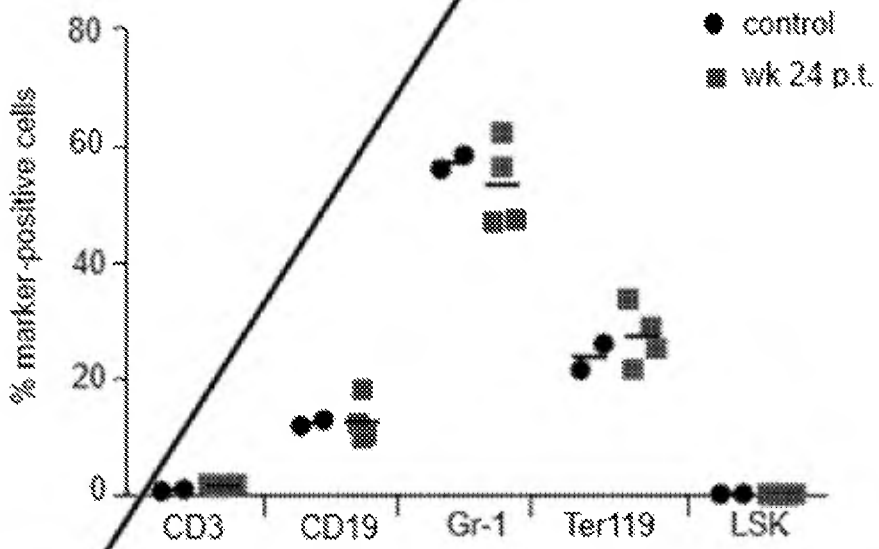


FIG. 91D



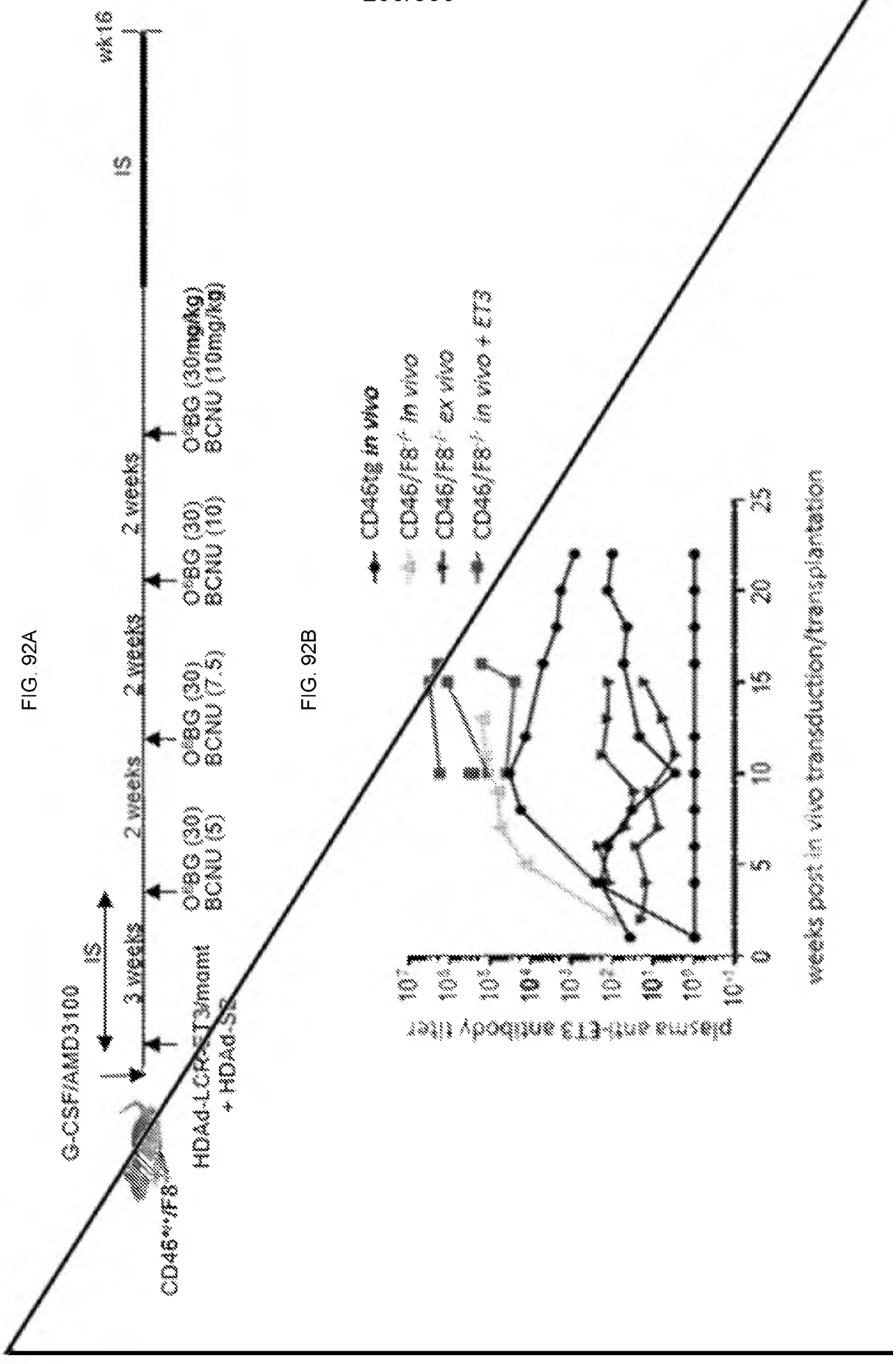


FIG. 92A

FIG. 92B

FIG. 92C

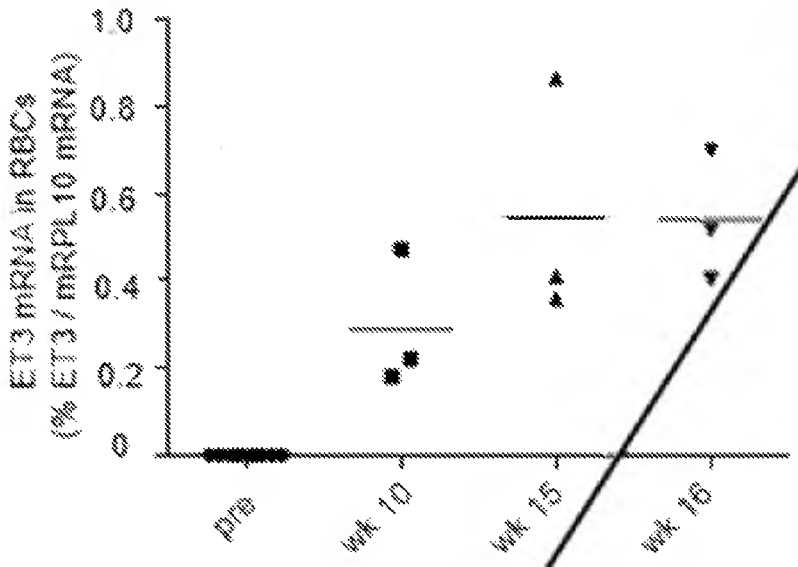


FIG. 92D

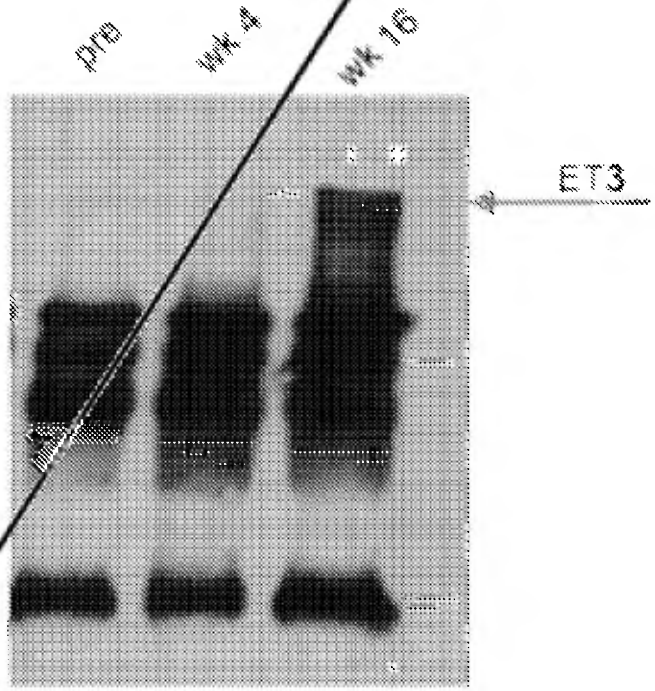


FIG. 92E

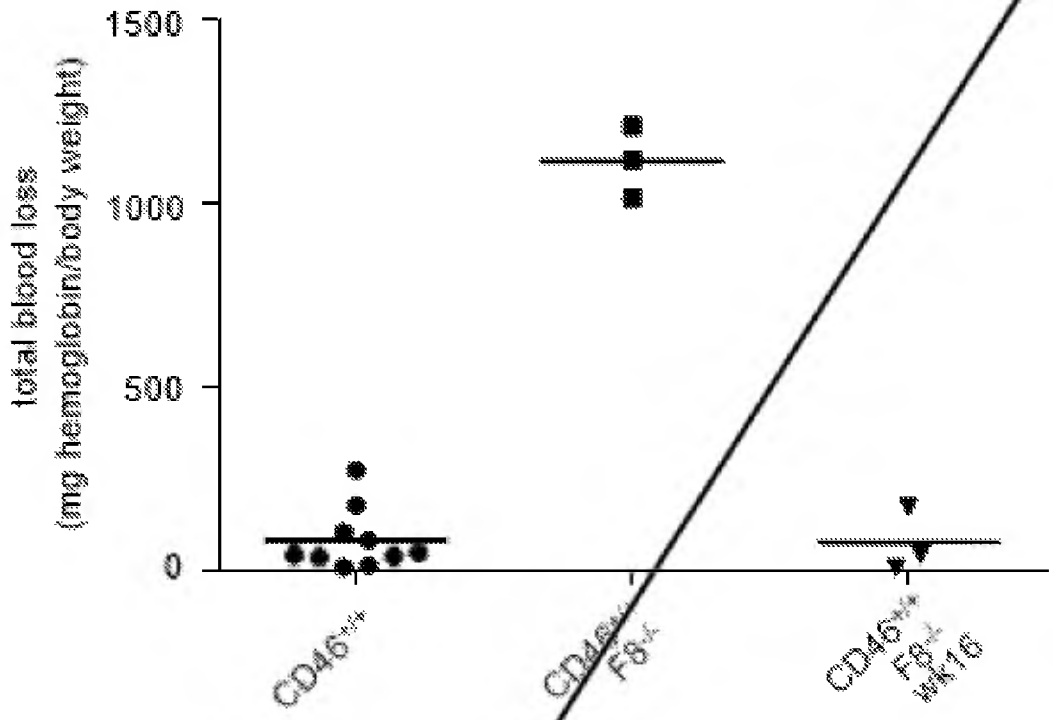


FIG. 92F

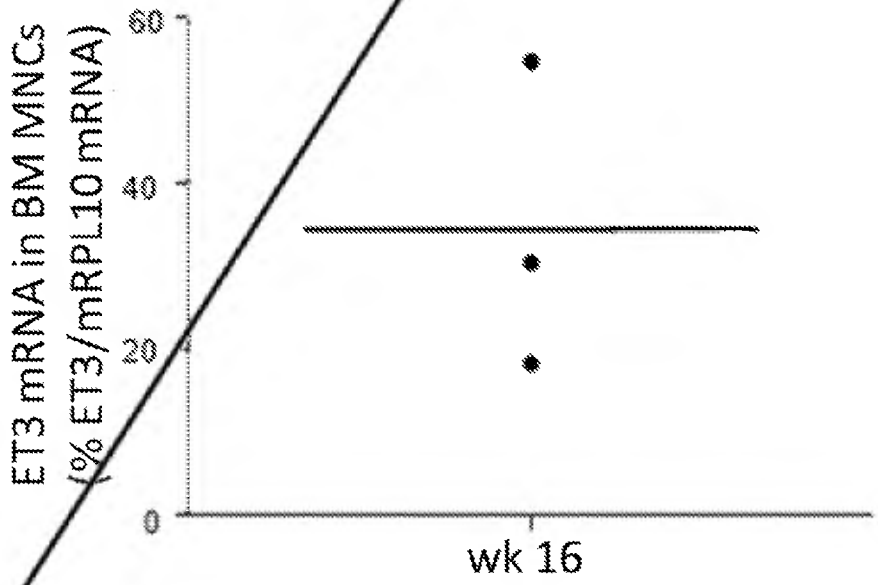


FIG. 92G

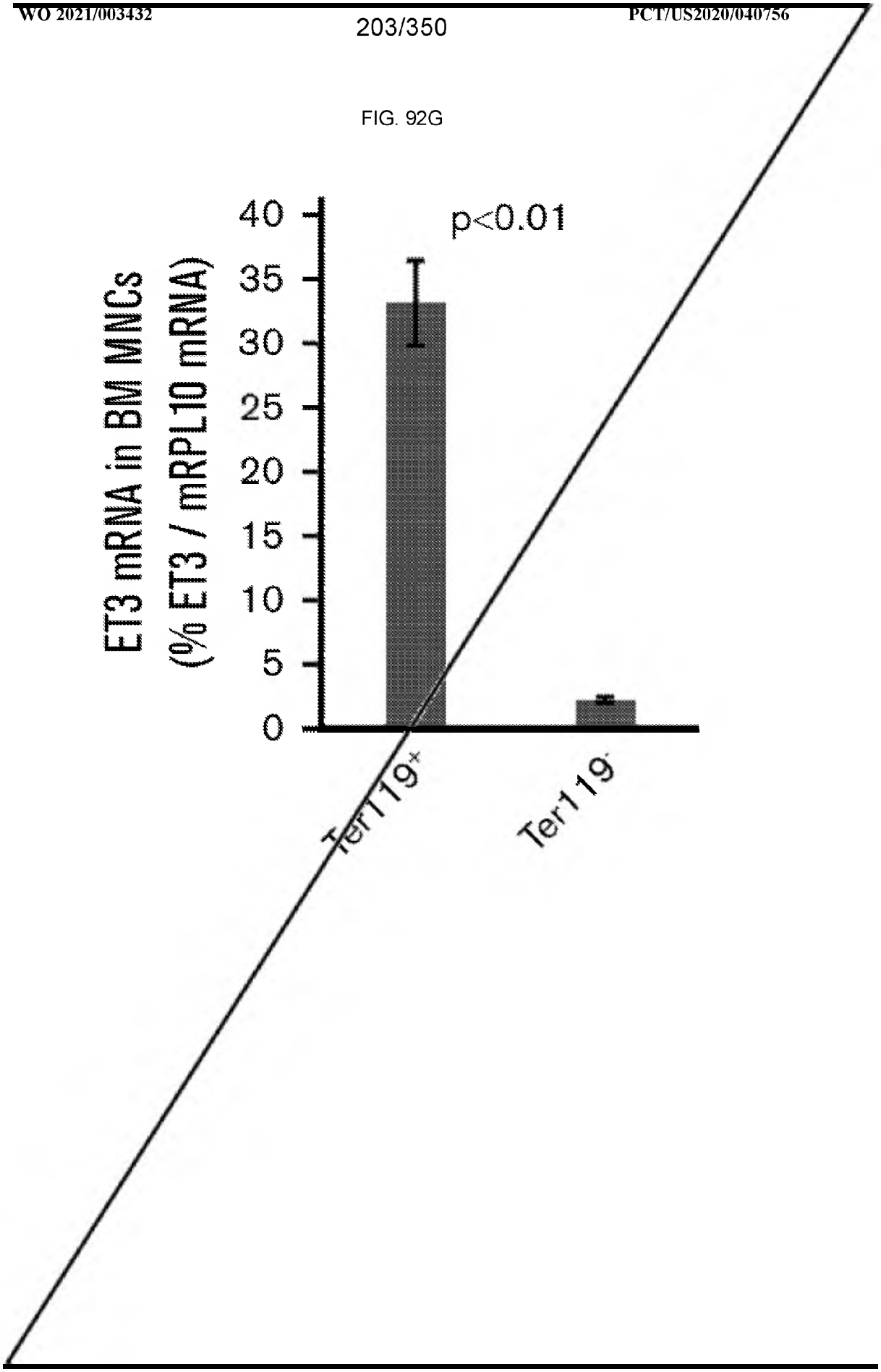

ET3 mRNA in BM MNCs
(% ET3 / mRPL10 mRNA)
40
35
30
25
20
15
10
5
0
p<0.01
Ter119+
Ter119-

FIG. 93A

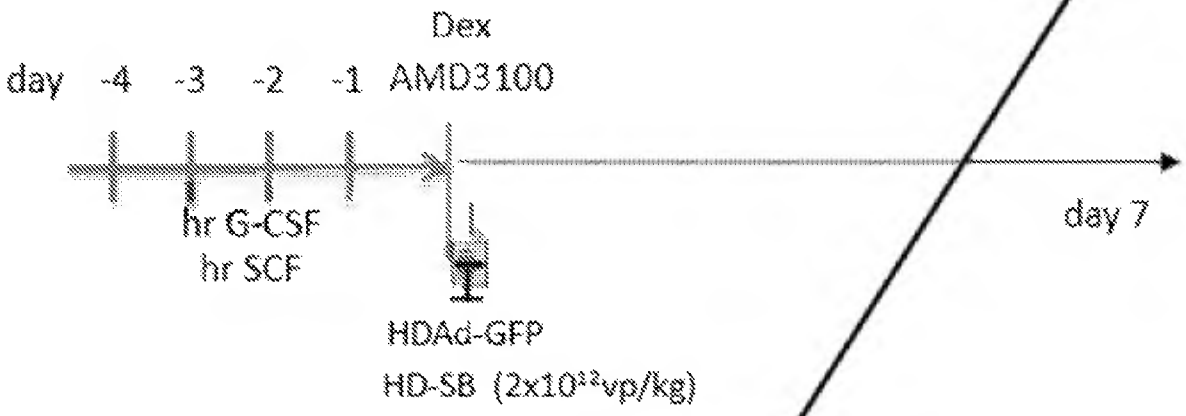


FIG. 93B

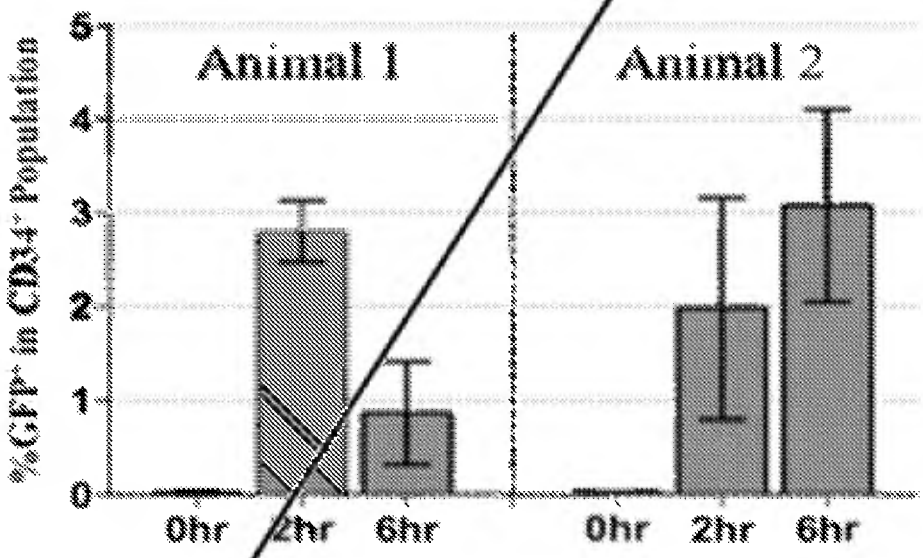


FIG. 93C

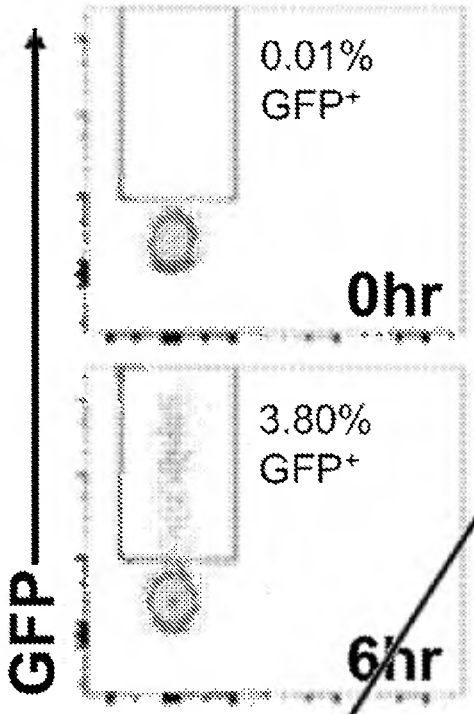


FIG. 93D

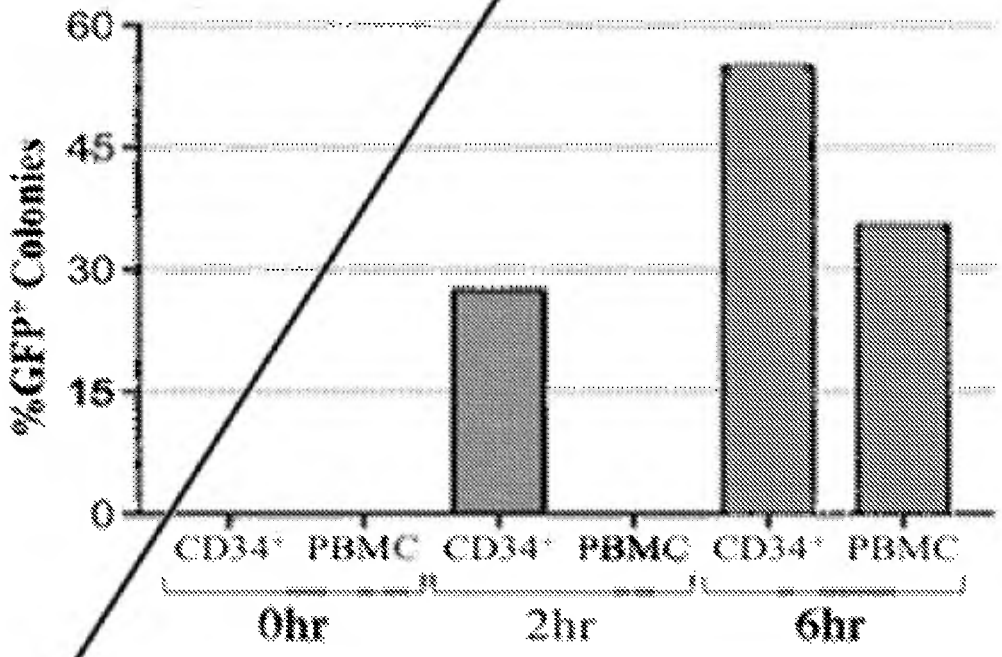


FIG. 93E

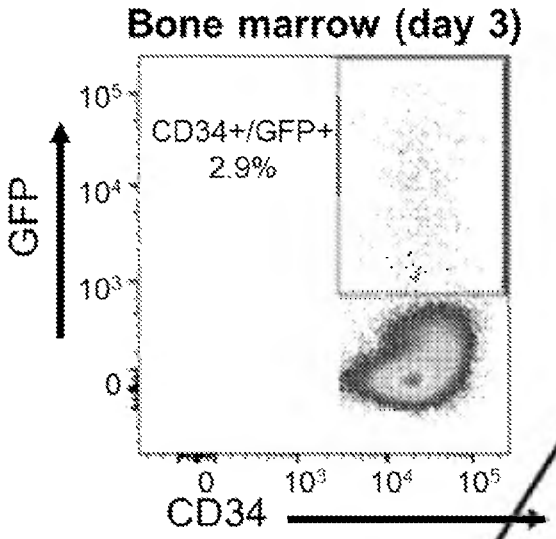

Bone marrow (day 3)
CD34+/GFP+
2.9%
GFP
10^5
10^4
10^3
0
0
10^3
10^4
10^5
CD34

FIG. 94A

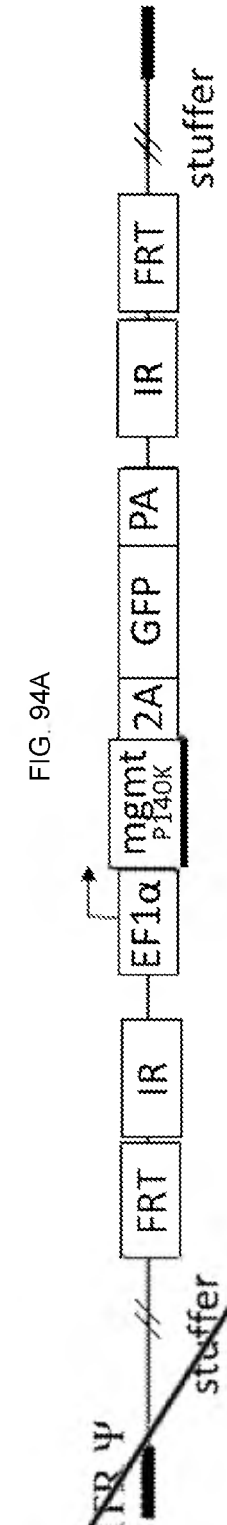


FIG. 94B

FIG. 94C

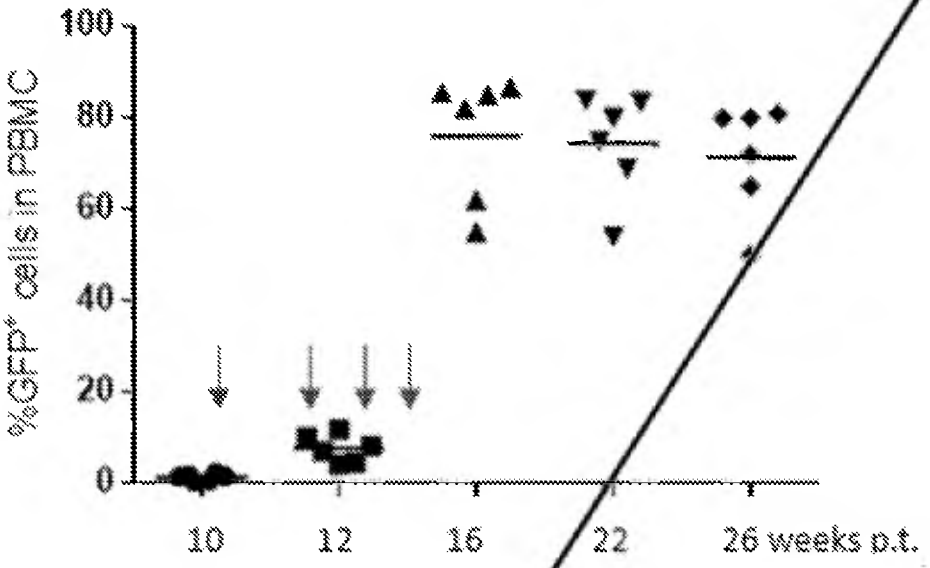


FIG. 94D

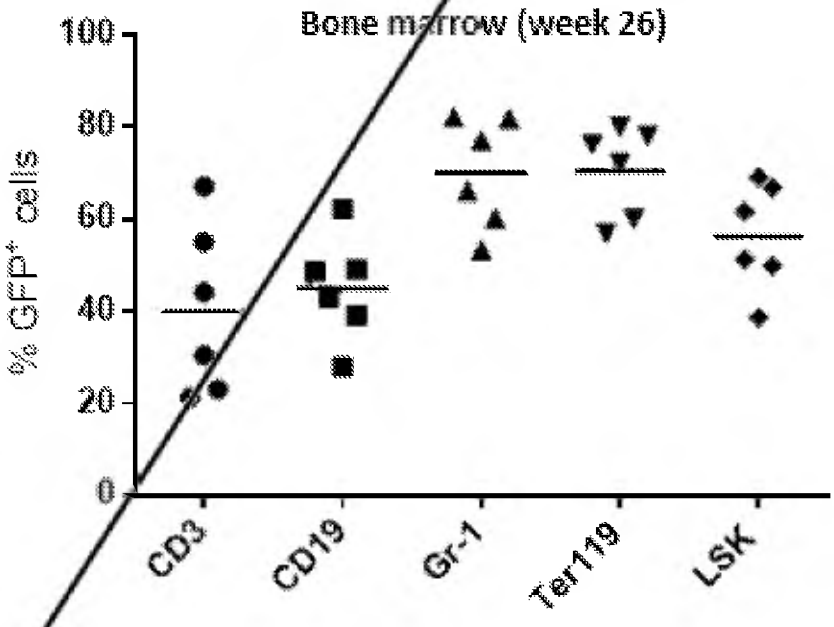


FIG. 94E

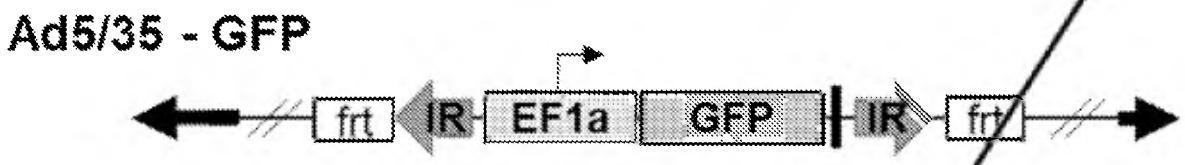


FIG. 94F

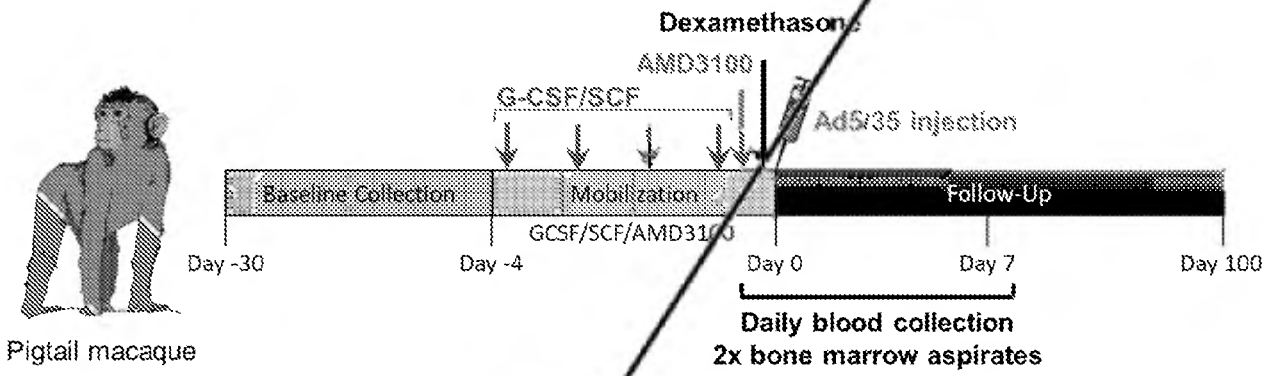


FIG. 94G

Animal ID	G-CSF	SCF	AMD3100	Dose
Z12406	50µg/kg	50µg/kg	-	$2x10^{12}$vp/kg
Z13126	50µg/kg	50µg/kg	4mg/kg	$4x10^{12}$vp/kg
Z15263	50µg/kg	50µg/kg	4mg/kg	$3x10^{12}$vp/kg

FIG. 94H

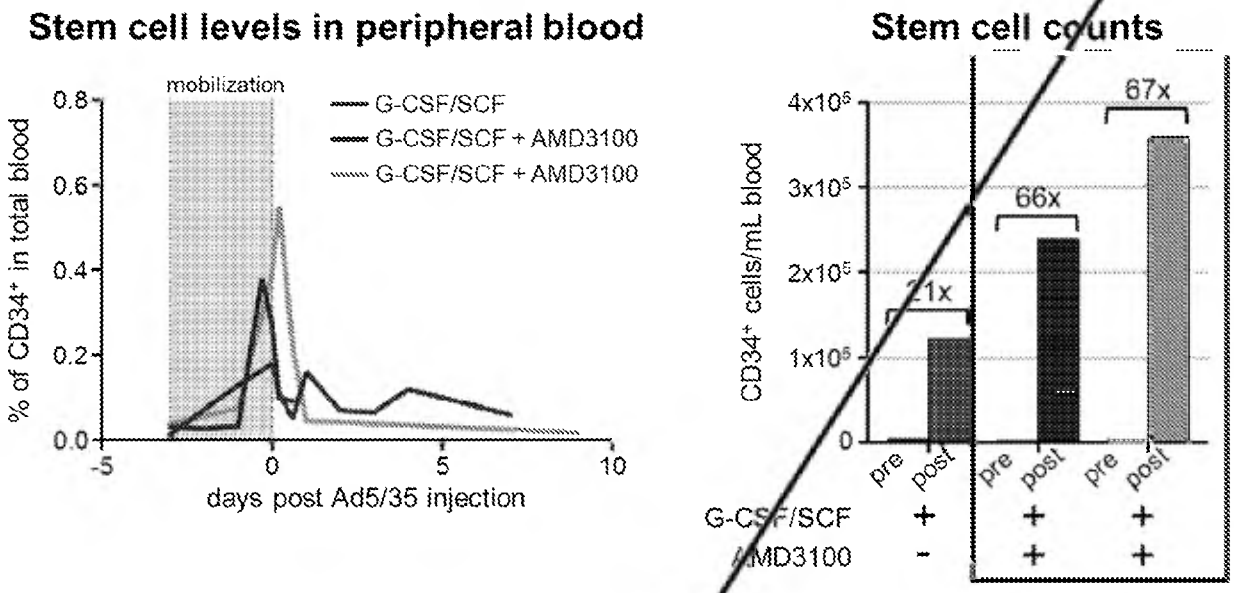
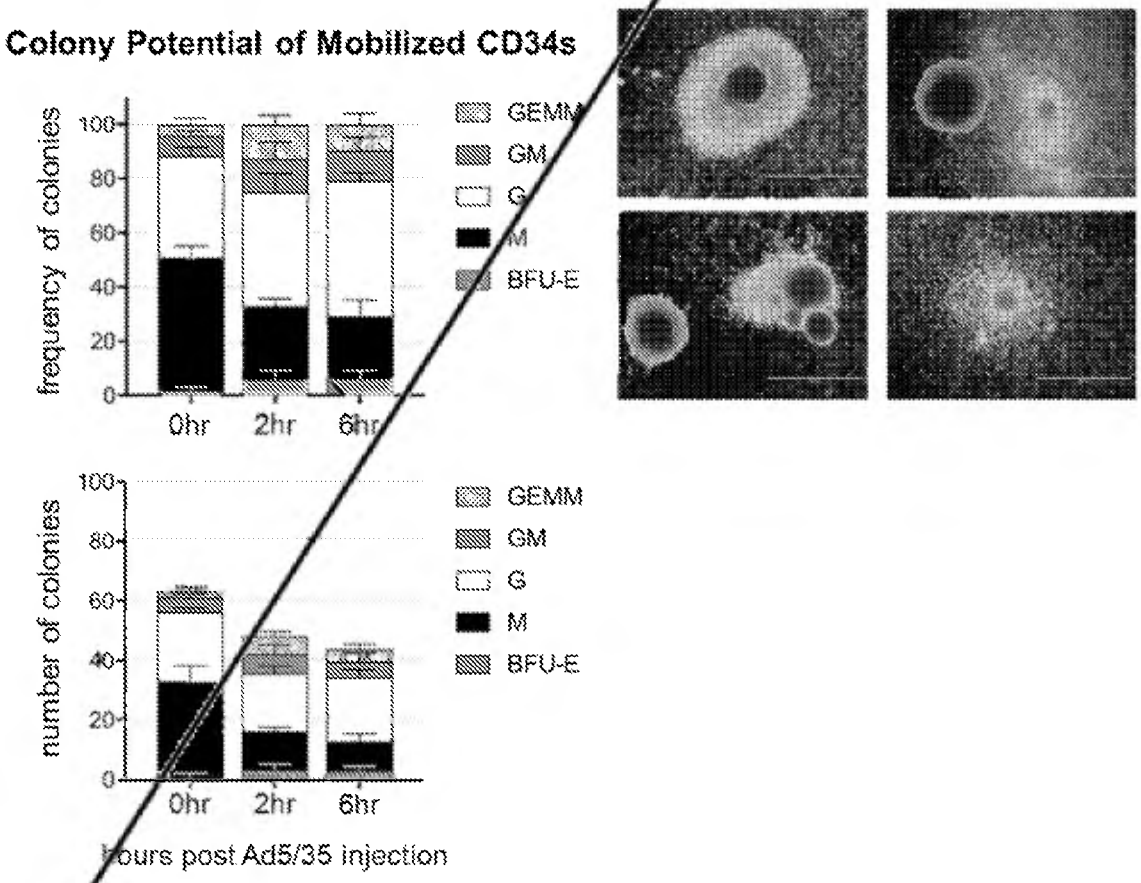


FIG. 94I



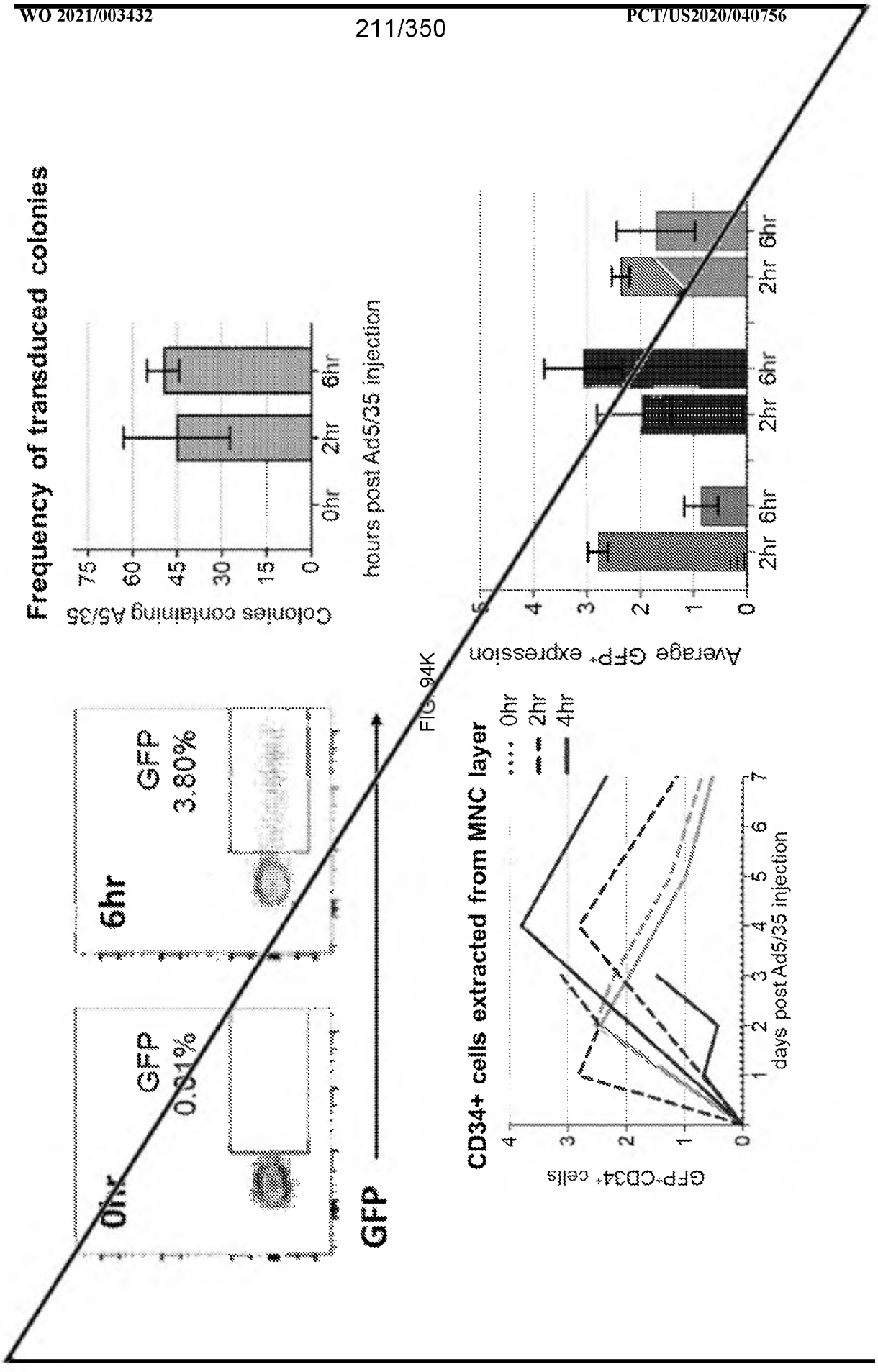


FIG. 94J

FIG. 94K

FIG. 94L

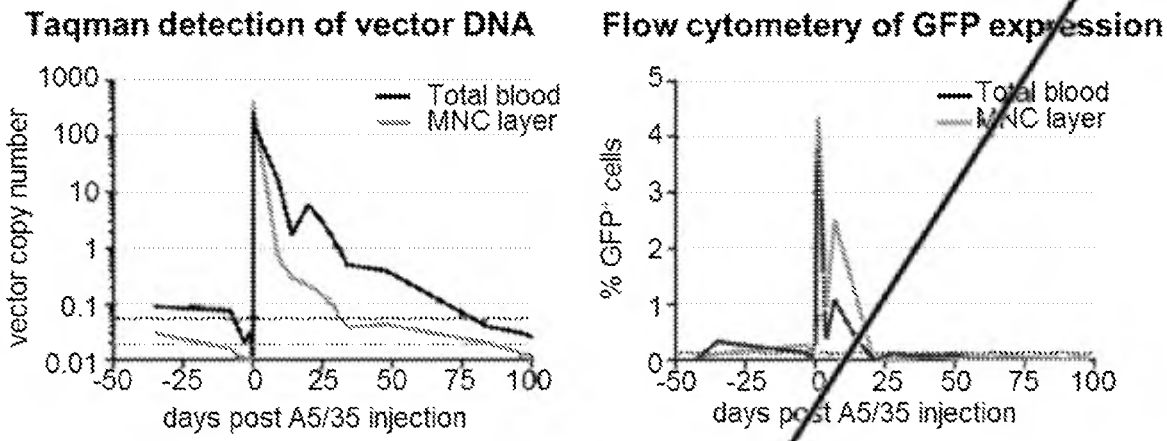
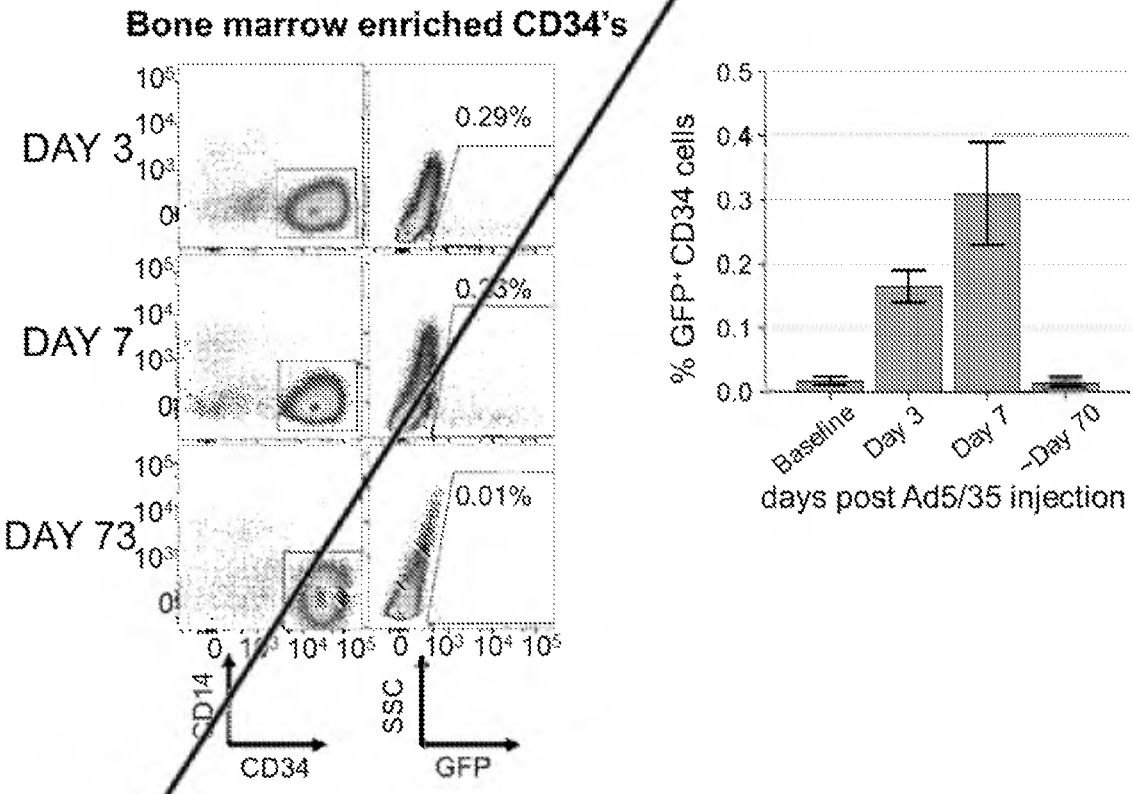


FIG. 94M



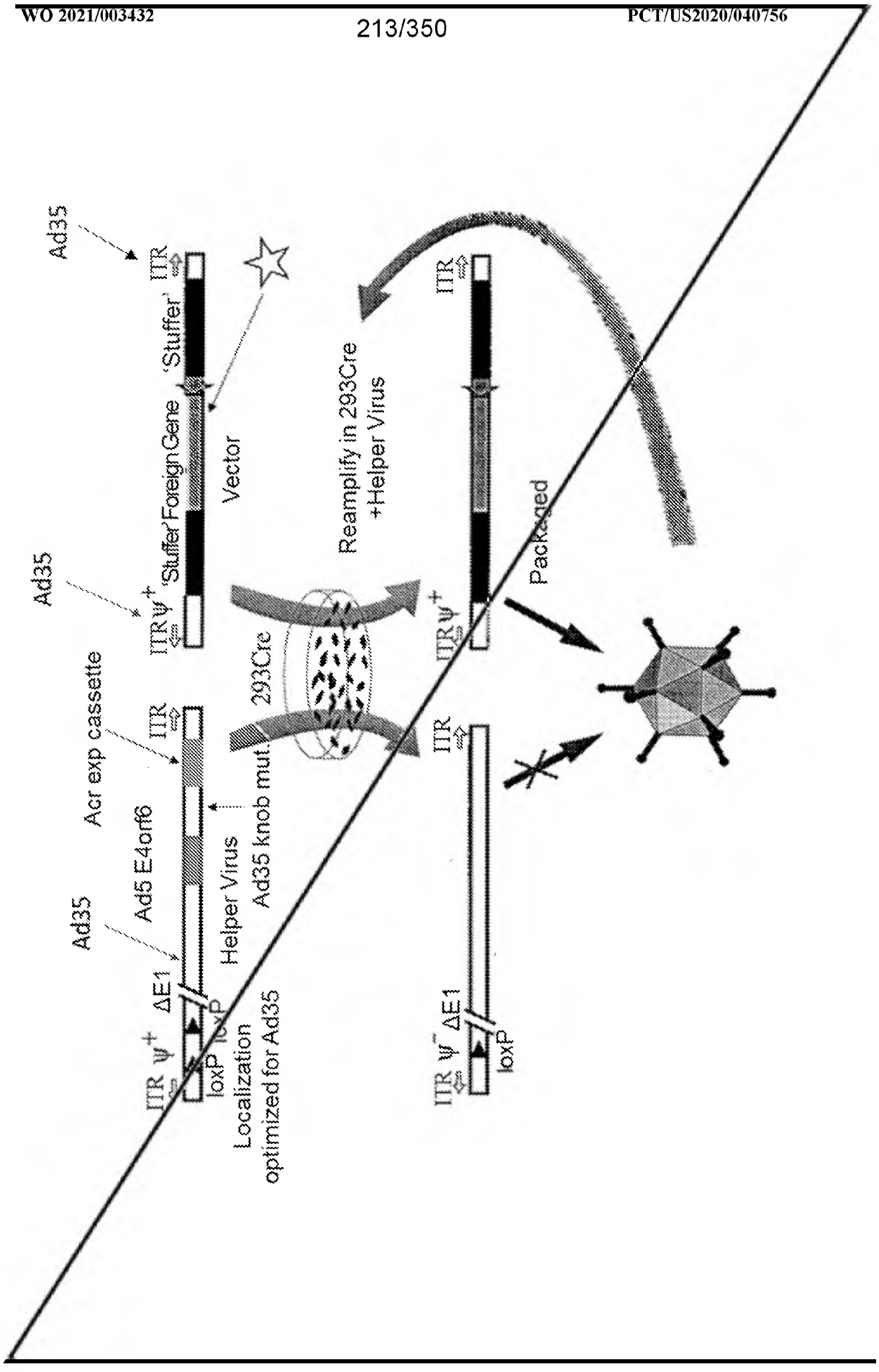

Ad35
ITR
'Stuffer'
Foreign Gene
Vector
Ad35
ITR ψ+
'Stuffer'
Reamplify in 293Cre
+Helper Virus
ITR
Packaged
293Cre
ITR ψ+
Acr exp cassette
ITR
Ad35
Ad5 E4orf6
Helper Virus
Ad35 knob mut
ITR
ITR ψ+ ΔE1
loxP
loxP
Localization
optimized for Ad35
ITR ψ- ΔE1
loxP

FIG. 95

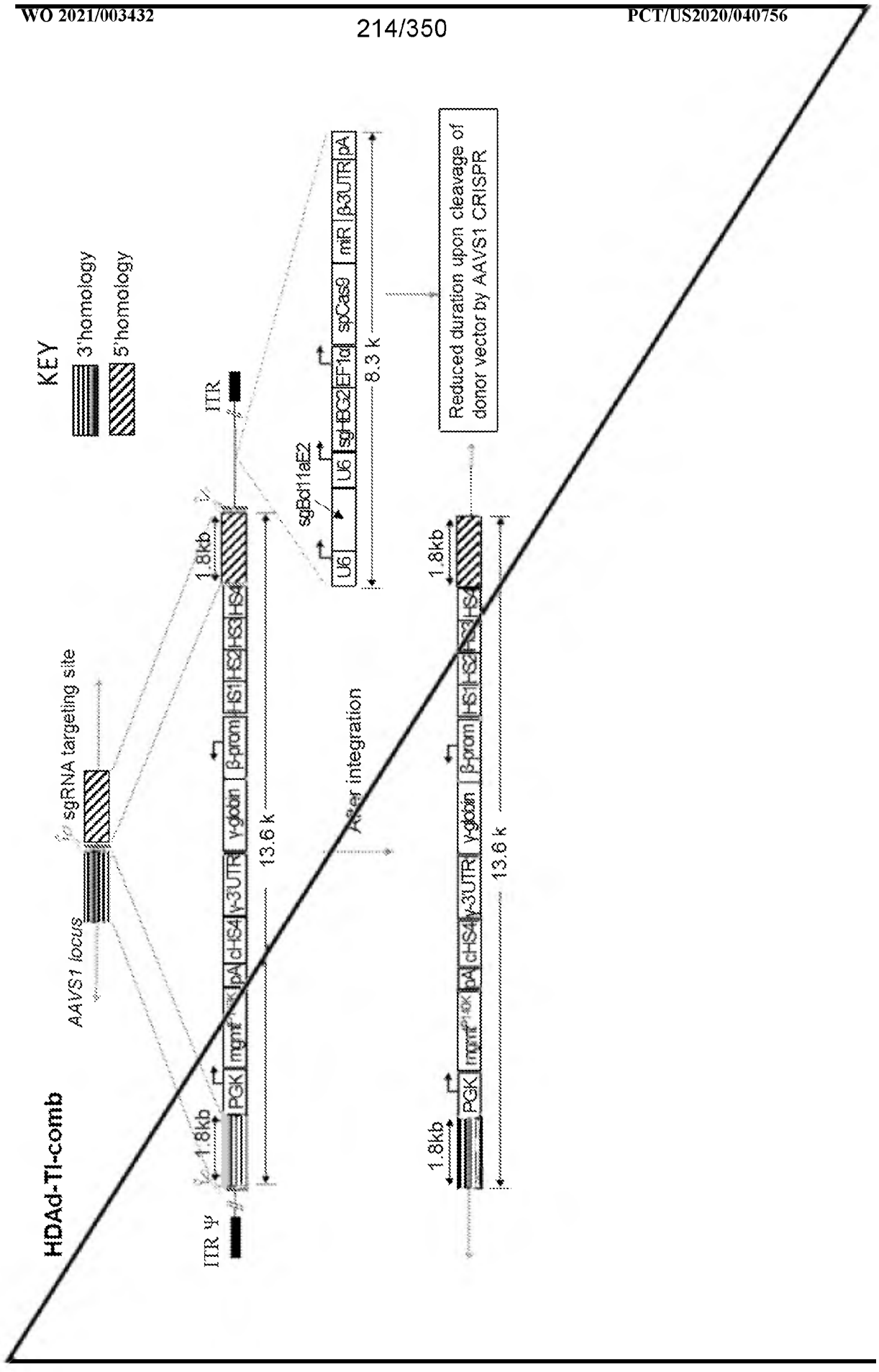

HDAd-TI-comb
KEY
3'homology
5'homology
sgRNA targeting site
AAVS1 locus
ITR Ψ
ITR
PGK
mgmt
pA
cHS4
γ-3UTR
γ-globin
β-prom
HS1
HS2
HS3
HS4
1.8kb
13.6 k
sgBcl11aE2
U6
sgRNA
EC2
EF1α
spCas9
miR
β-3UTR
pA
8.3 k
After integration
Reduced duration upon cleavage of donor vector by AAVS1 CRISPR

FIG. 96

FIG. 97A

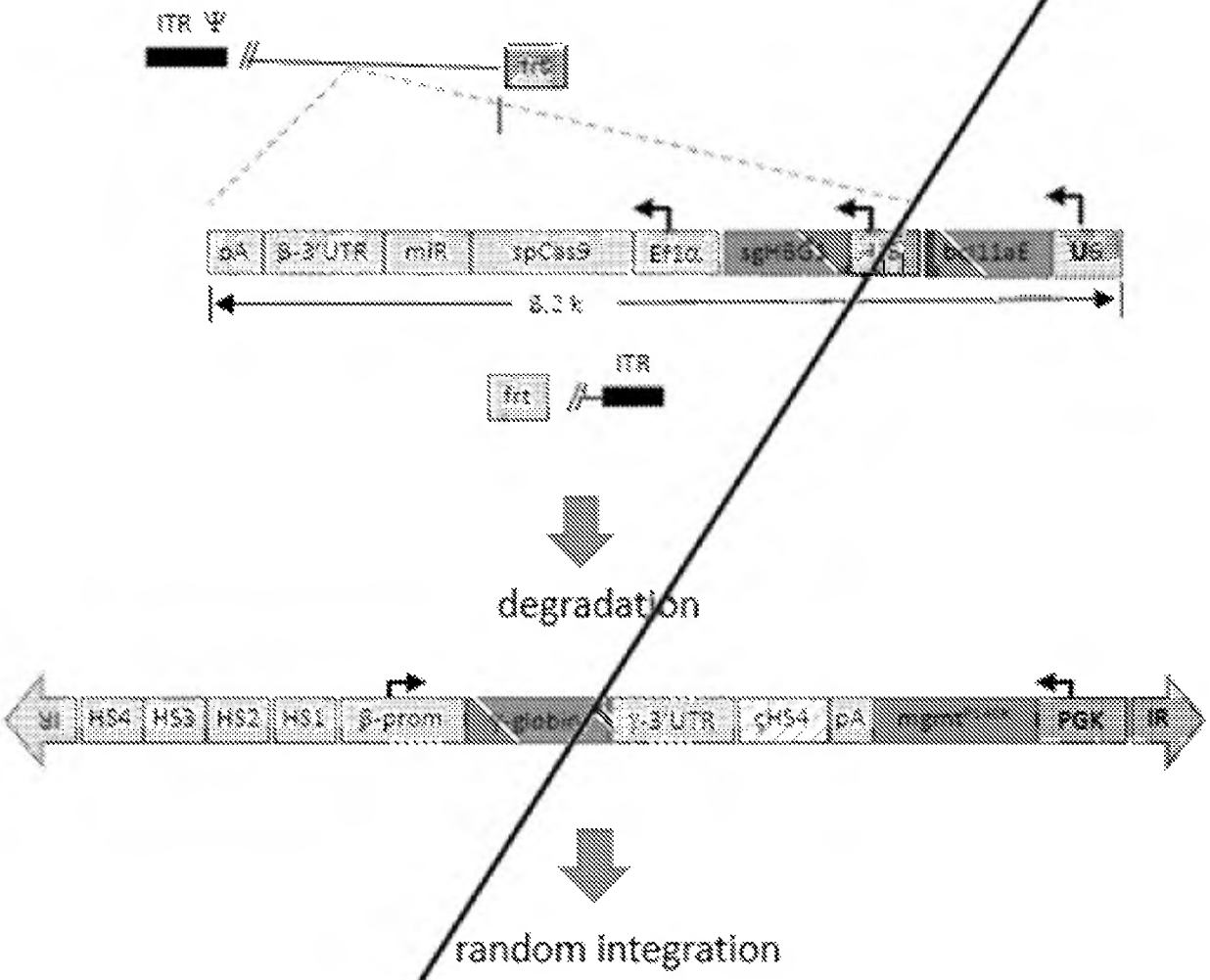

ITR
ITR
degradation
HS4
HS3
HS2
HS1
β-prom
γ-3'UTR
cHS4
pA
PGK
IR
random integration

FIG. 97B

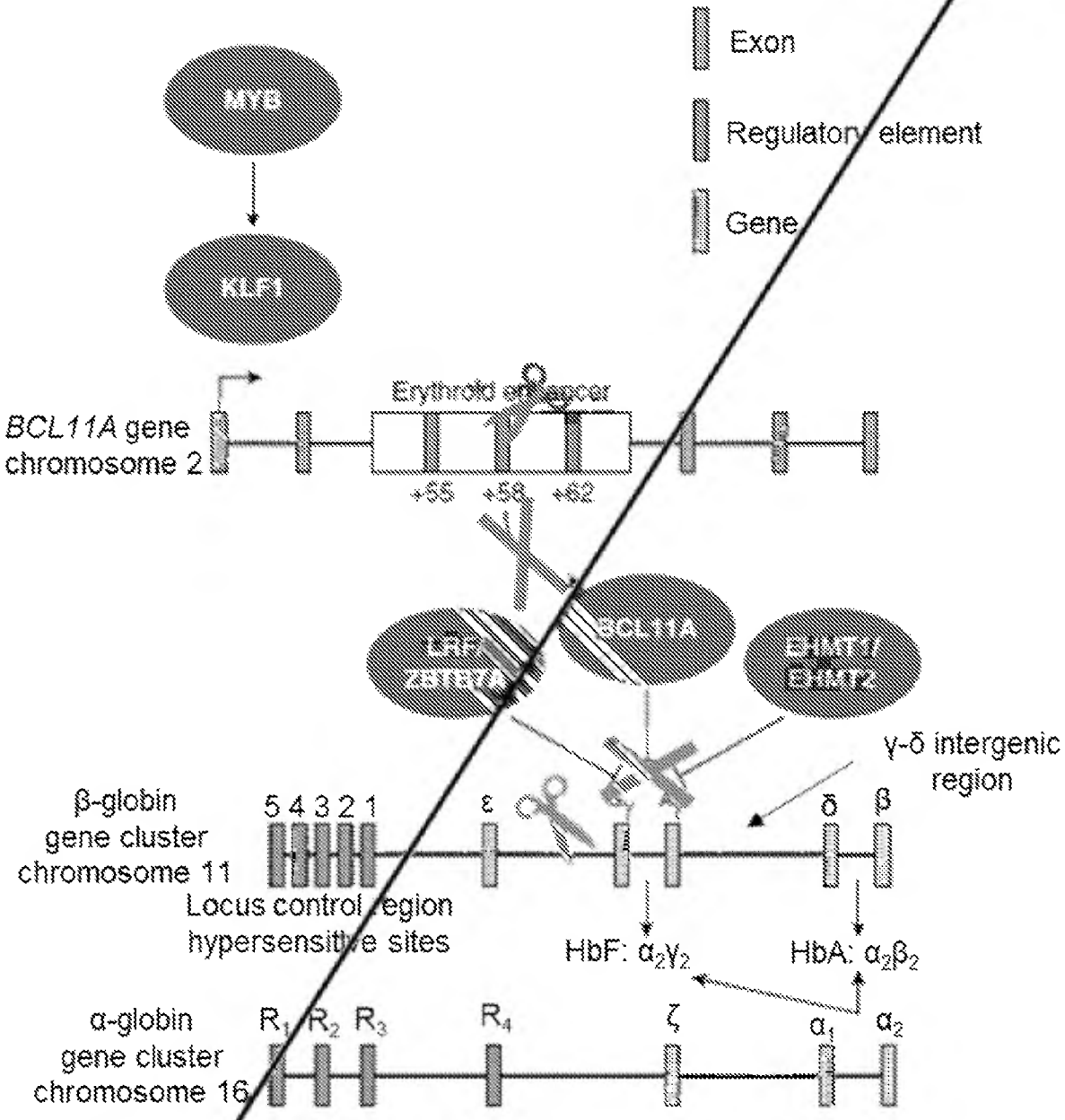

Exon
Regulatory element
Gene
MYB
KLF1
BCL11A gene
chromosome 2
Erythroid enhancer
+55
+58
+62
LRF
ZBTB7A
BCL11A
EHMT1/
EHMT2
γ-δ intergenic
region
β-globin
gene cluster
chromosome 11
5 4 3 2 1
ε
δ
β
Locus control region
hypersensitive sites
HbF: α2γ2
HbA: α2β2
α-globin
gene cluster
chromosome 16
R1 R2 R3
R4
ζ
α1
α2

FIG. 97C

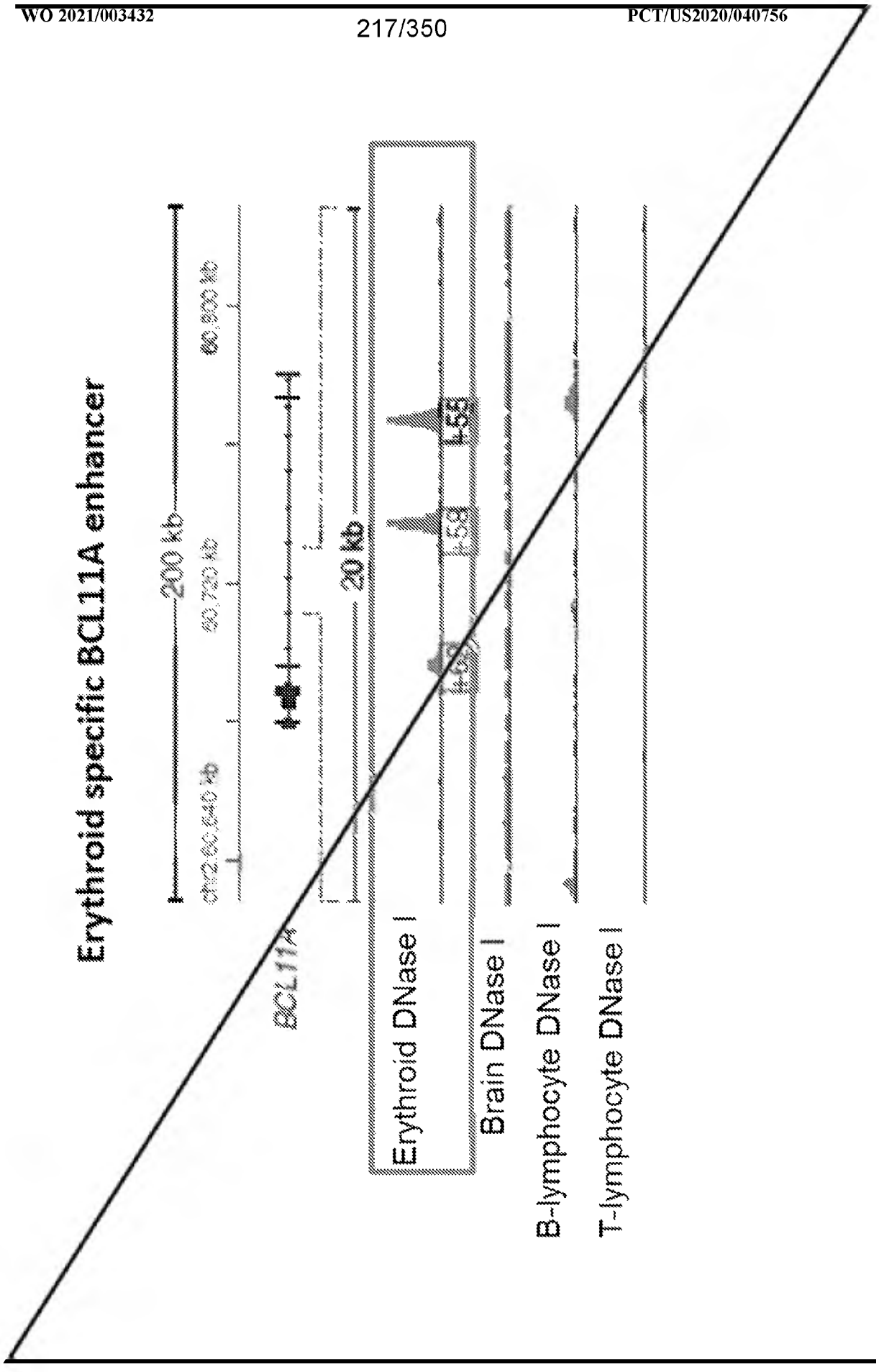

Erythroid specific BCL11A enhancer
200 kb
chr2:60,640 kb
60,720 kb
60,800 kb
BCL11A
20 kb
Erythroid DNase I
Brain DNase I
B-lymphocyte DNase I
T-lymphocyte DNase I
153
153
153

FIG. 97D

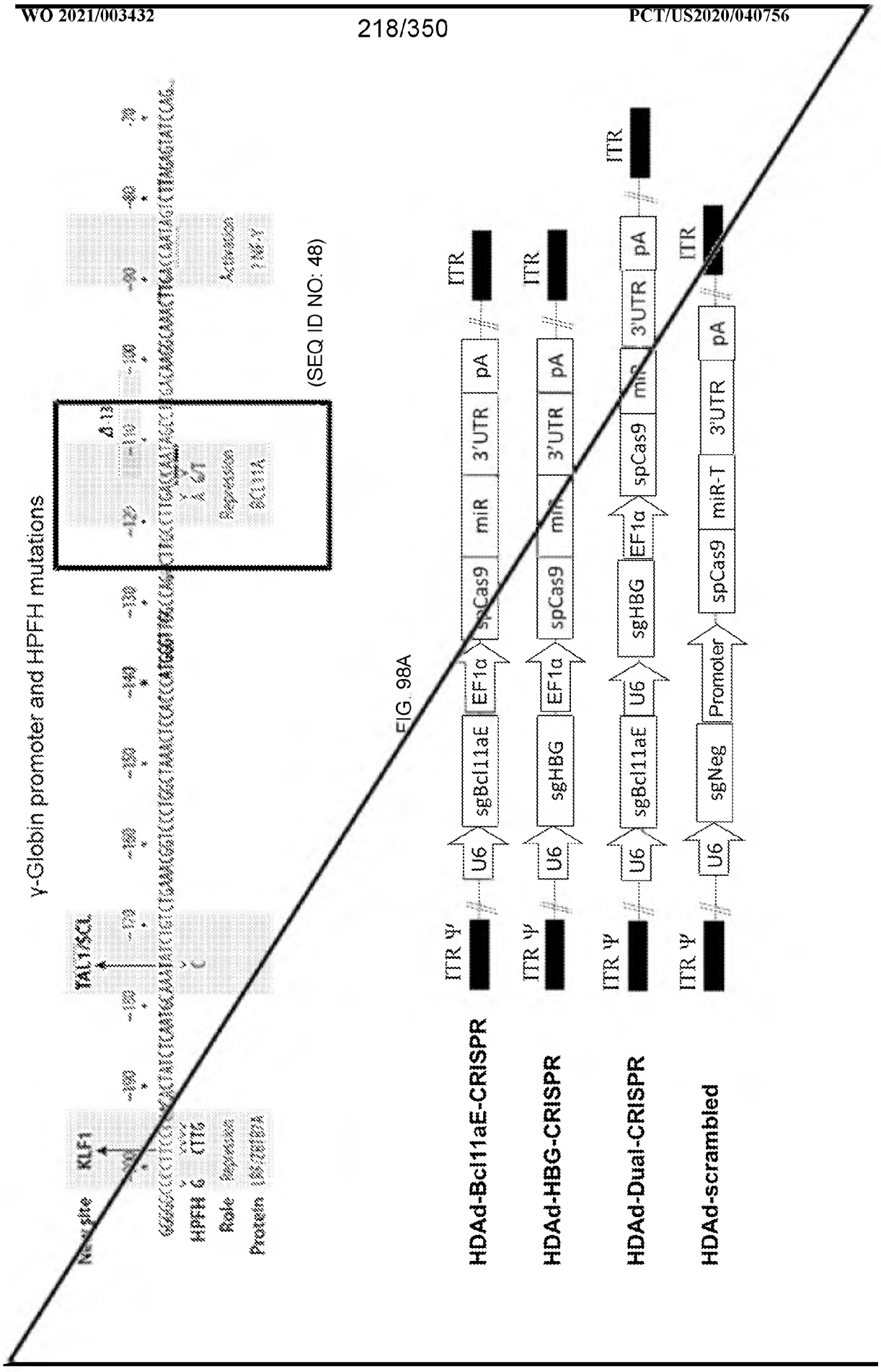


FIG. 98A

FIG. 98B

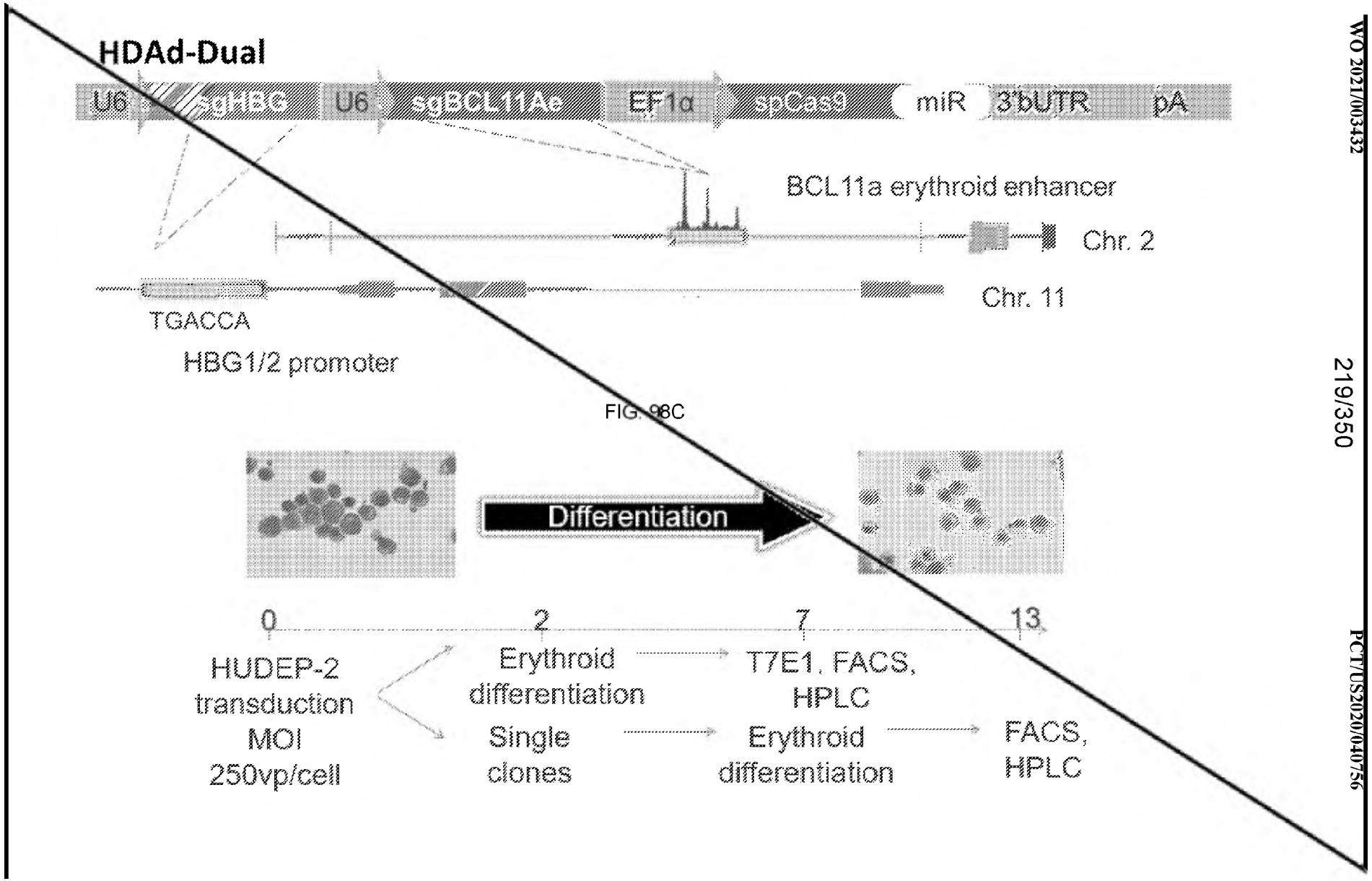


FIG. 98C

FIG. 98D

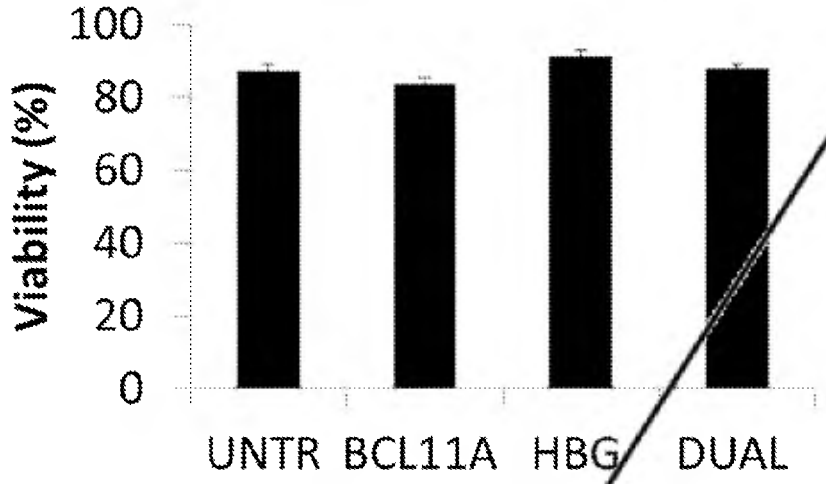


FIG. 98E

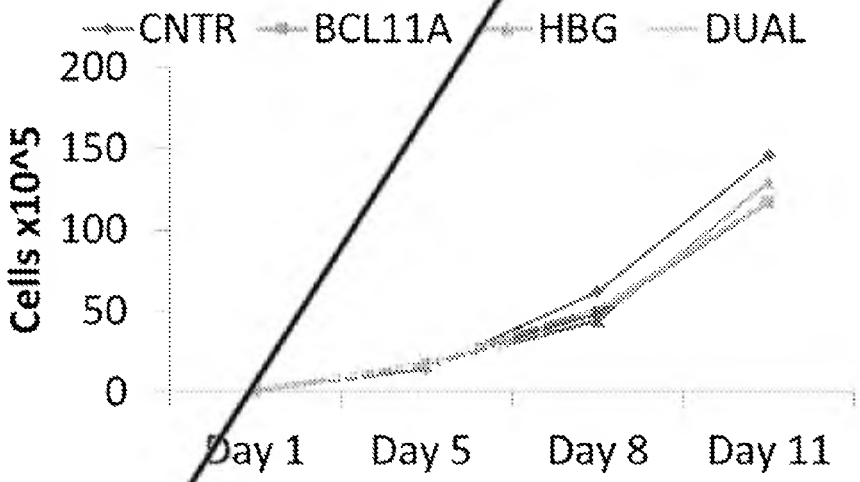


FIG. 98F

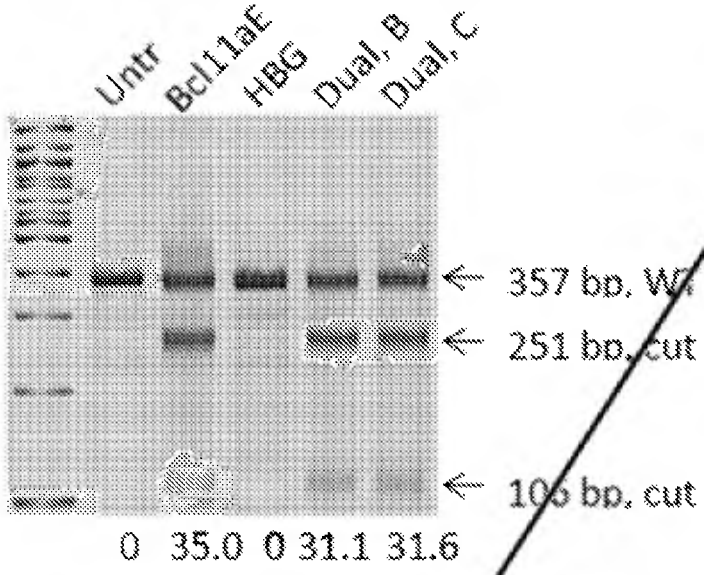


FIG. 98G

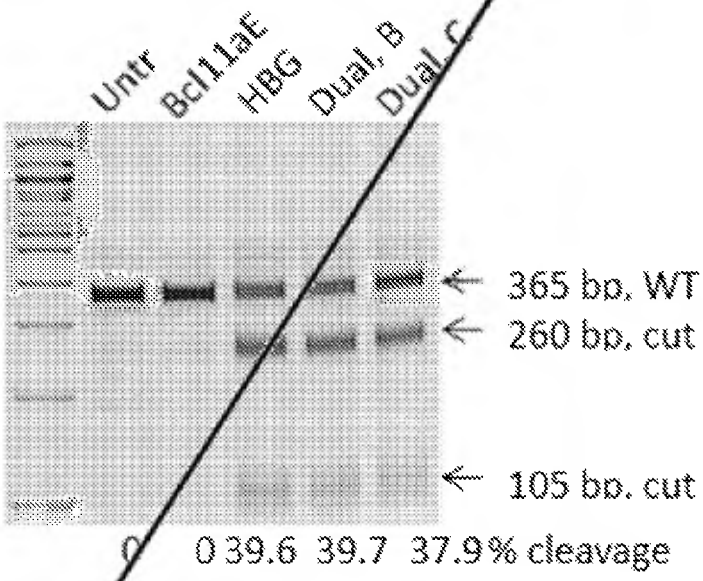


FIG. 98H

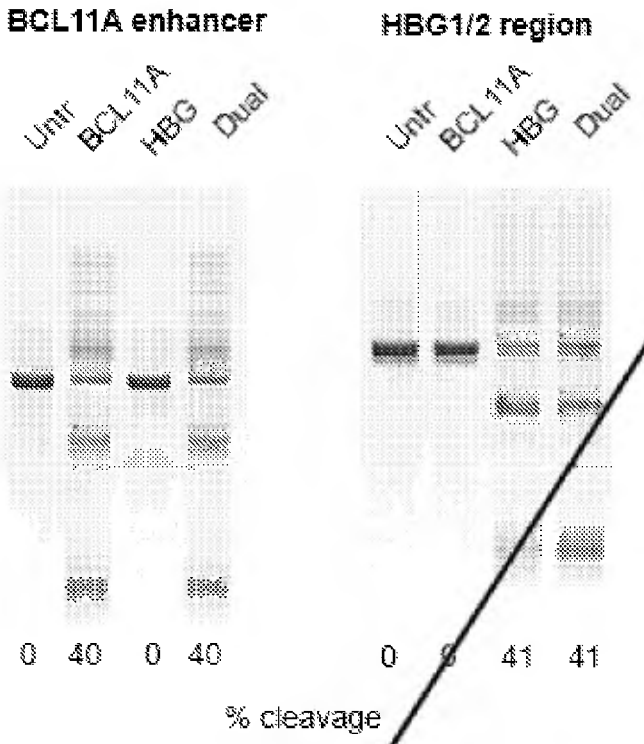

BCL11A enhancer
HBG1/2 region
Untr
BCL11A
HBG
Dual
Untr
BCL11A
HBG
Dual
0
40
0
40
0
0
41
41
% cleavage

FIG. 98I

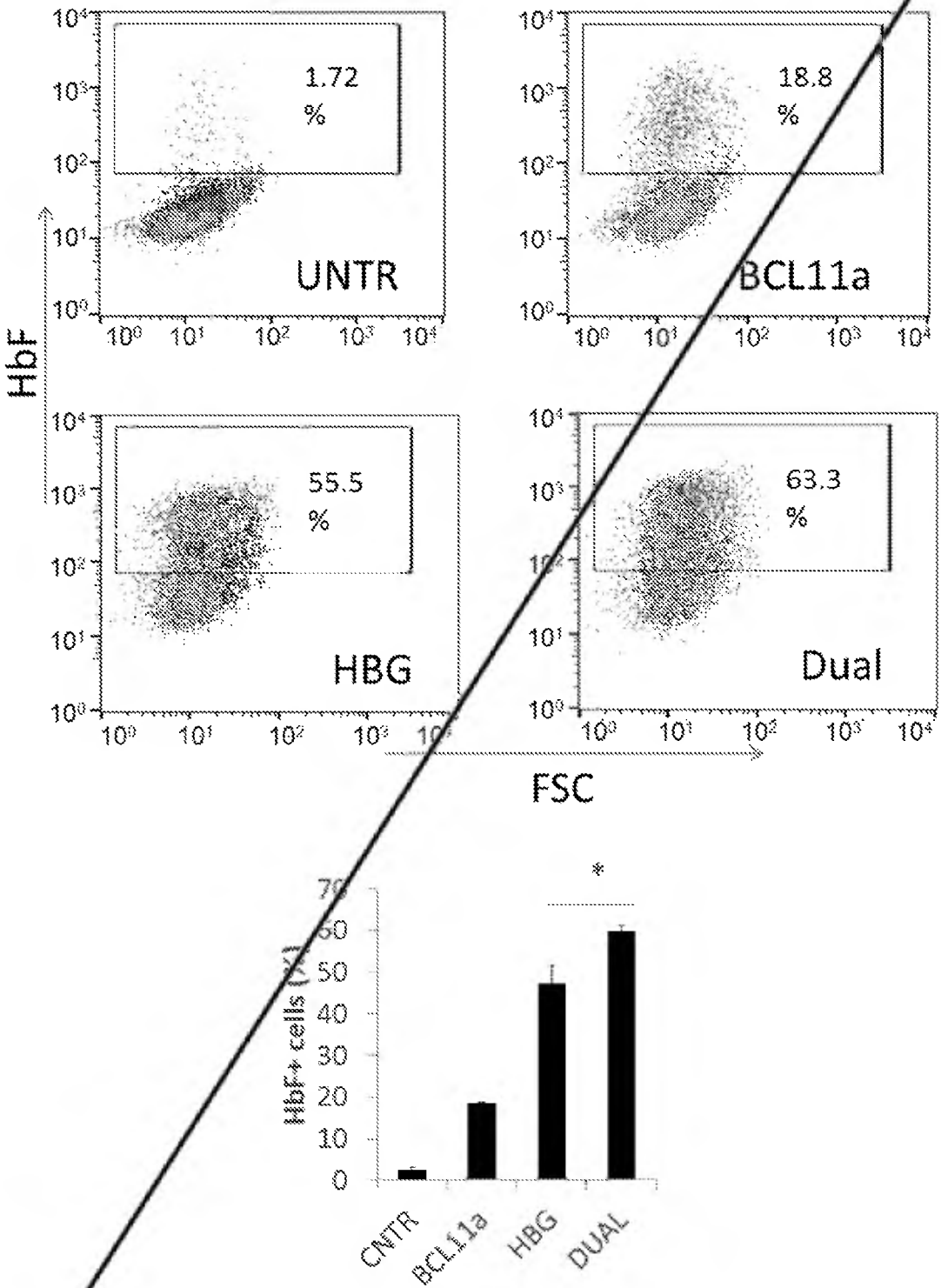
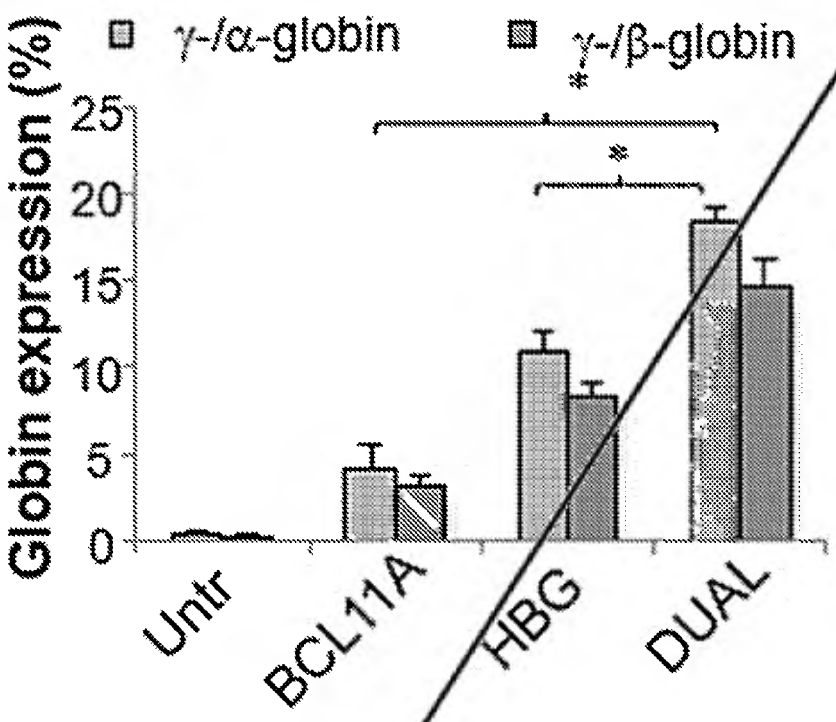

1.72 %
UNTR
18.8 %
BCL11a
55.5 %
HBG
63.3 %
Dual
HbF
FSC
HbF+ cells (%)
CNTR
BCL11a
HBG
DUAL
*

FIG. 98J


γ-/α-globin
γ-/β-globin
Globin expression (%)
25
20
15
10
5
0
*
*
Untr
BCL11A
HBG
DUAL

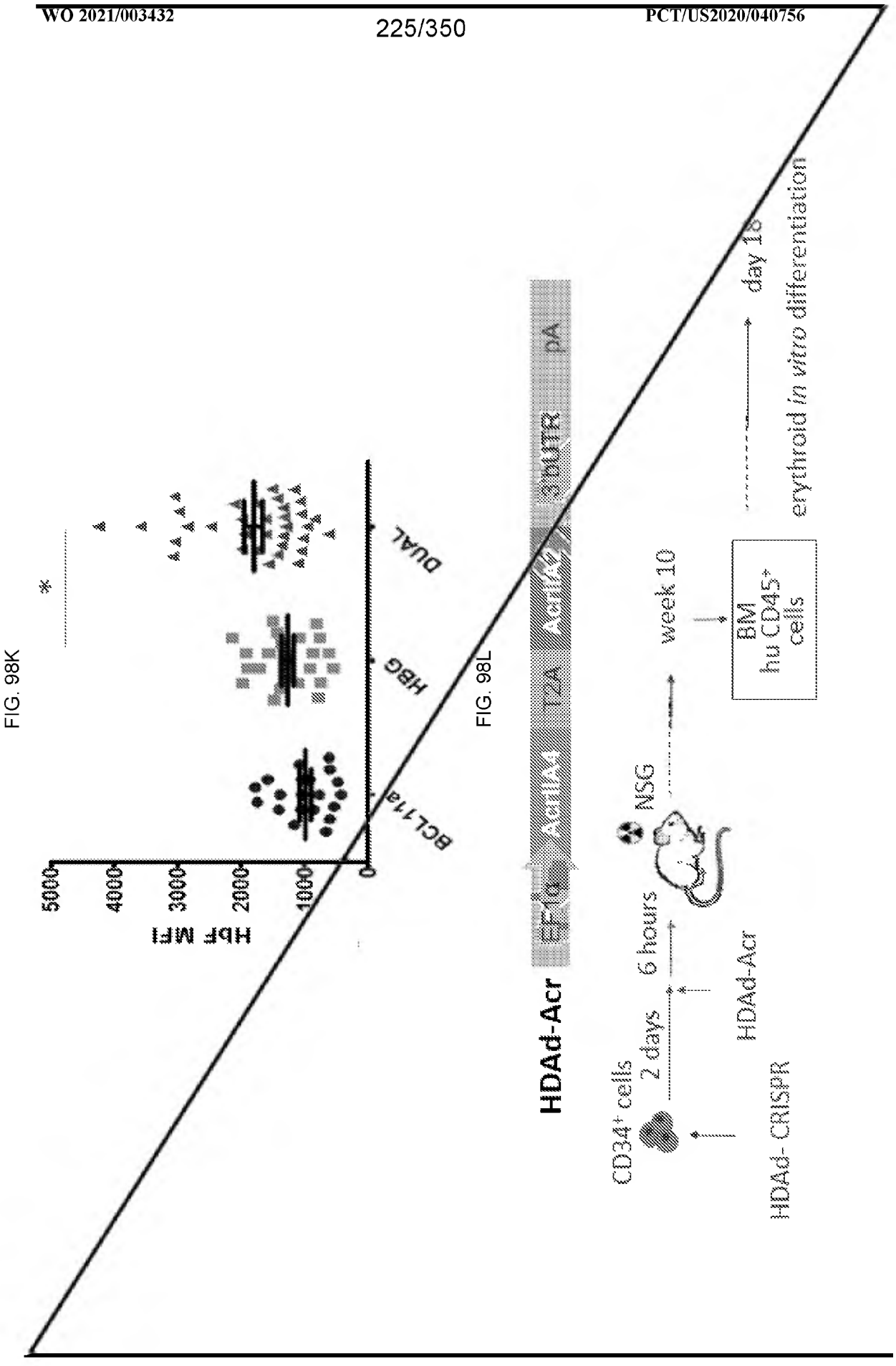


FIG. 98K

FIG. 98L

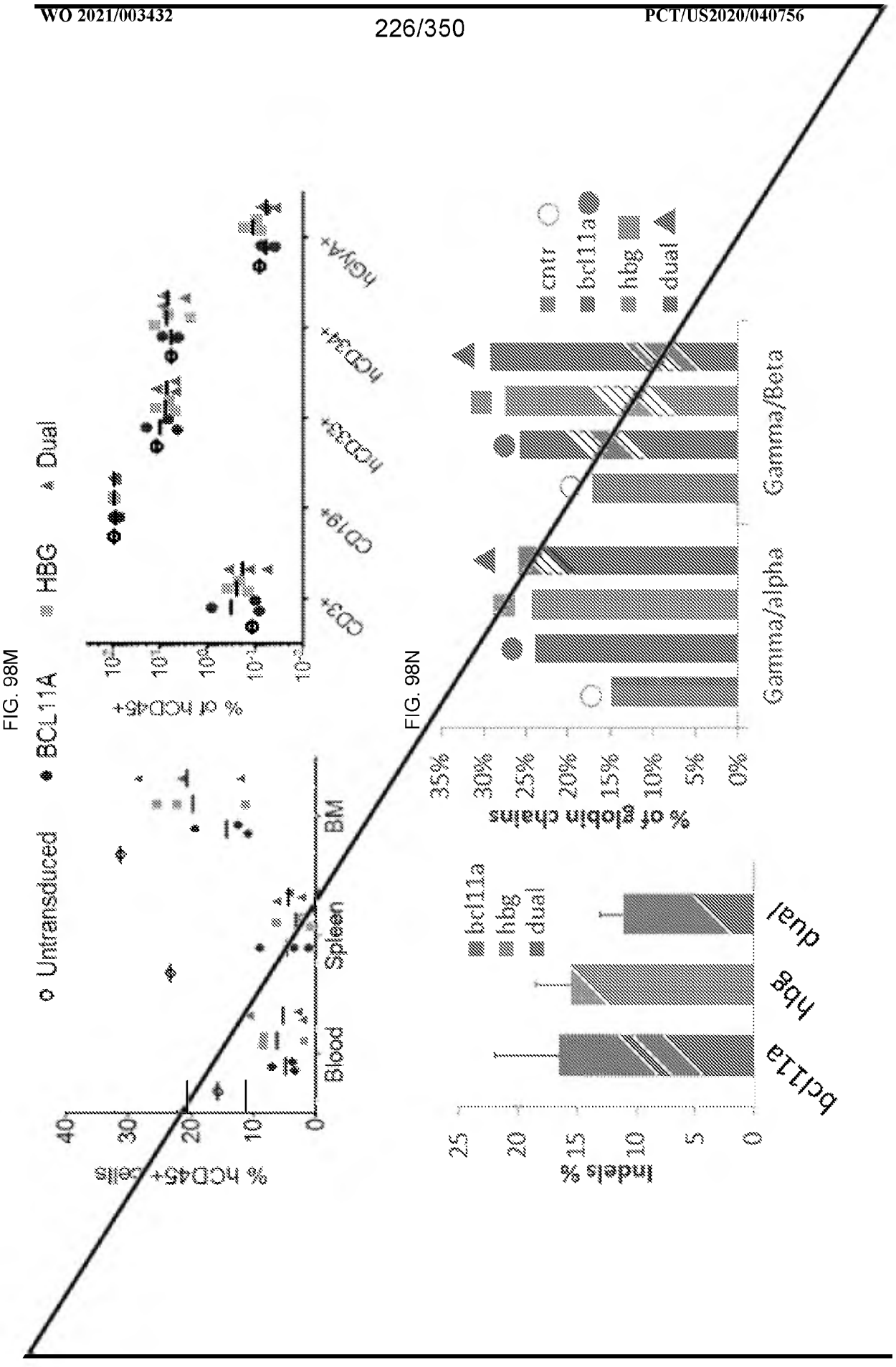

FIG. 98M
Untransduced
BCL11A
HBG
Dual
% hCD45+ cells
40
30
20
10
0
Blood
Spleen
BM
% of hCD45+
10^-1
10^-2
10^-3
10^-4
10^-5
CD3+
CD19+
hCD33+
hCD34+
hGlyA+
FIG. 98N
% of globin chains
35%
30%
25%
20%
15%
10%
5%
0%
Gamma/alpha
Gamma/Beta
cntr
bcl11a
hbg
dual
Indels %
25
20
15
10
5
0
bcl11a
hbg
dual

FIG. 99A

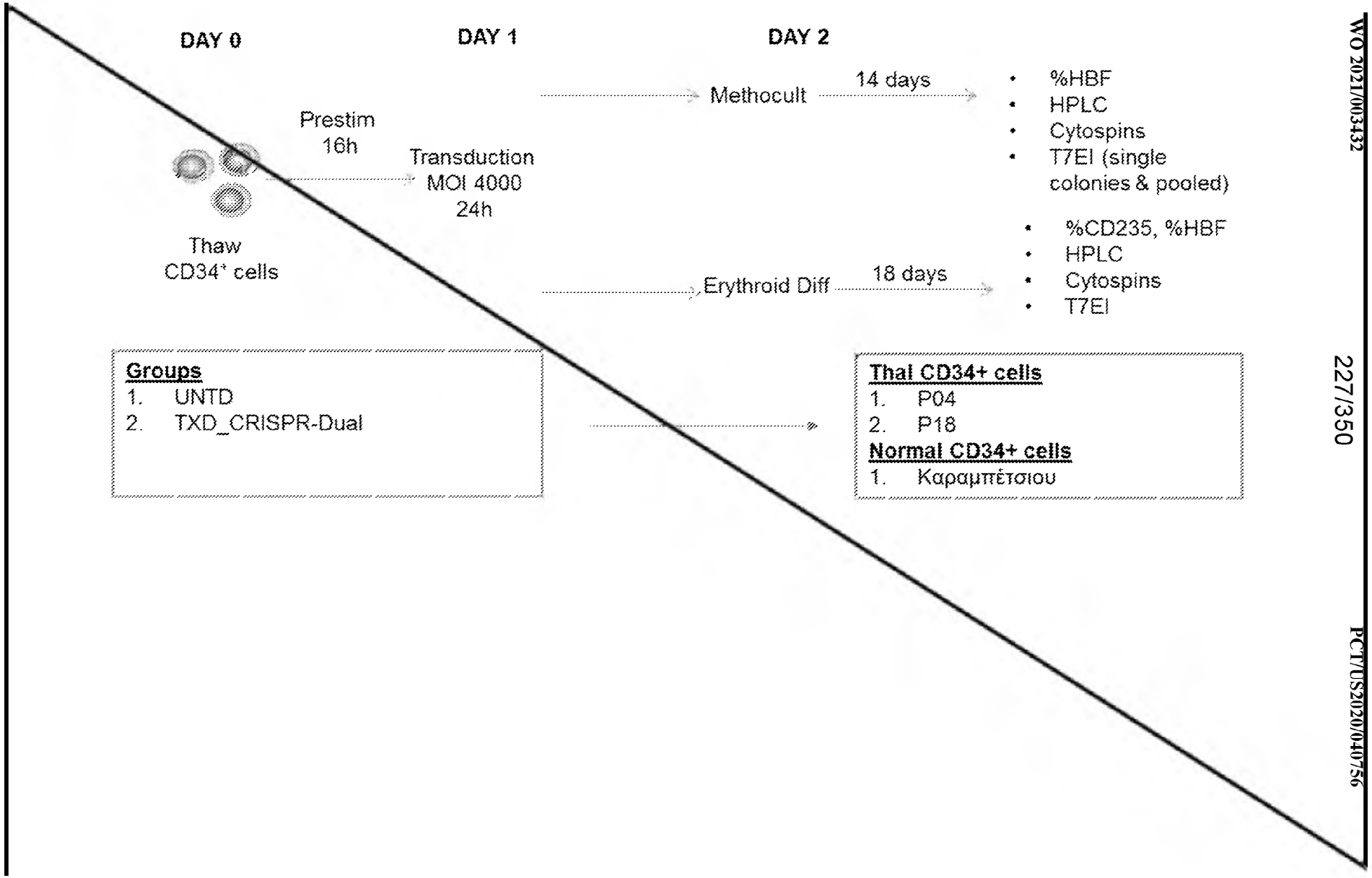

DAY 0
DAY 1
DAY 2
Prestim
16h
Transduction
MOI 4000
24h
Thaw
CD34+ cells
Methocult
14 days
%HBF
HPLC
Cytospins
T7EI (single colonies & pooled)
Erythroid Diff
18 days
%CD235, %HBF
HPLC
Cytospins
T7EI
Groups
1. UNTD
2. TXD_CRISPR-Dual
Thal CD34+ cells
1. P04
2. P18
Normal CD34+ cells
1. Καραμπέτσιου

FIG. 99B

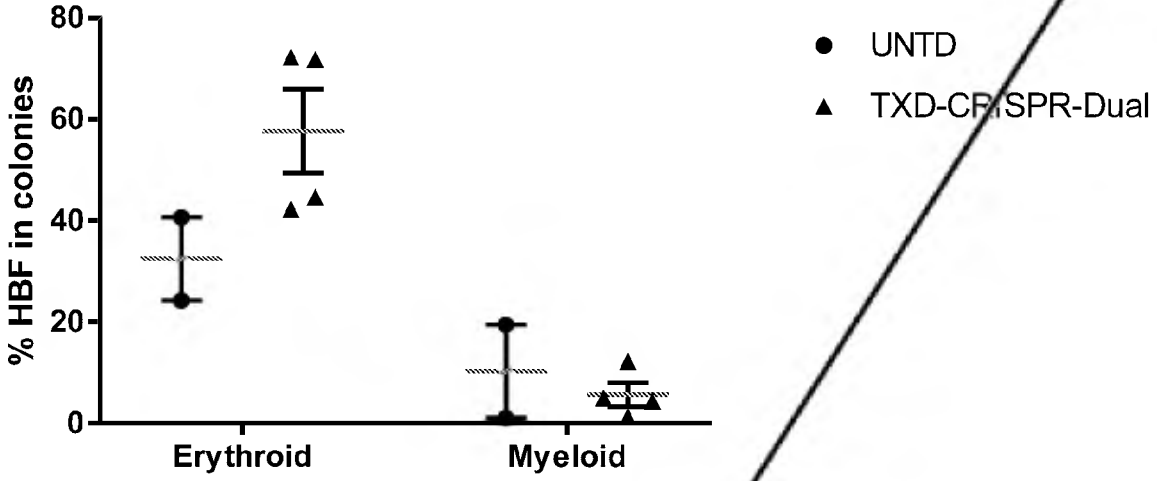


FIG. 99C

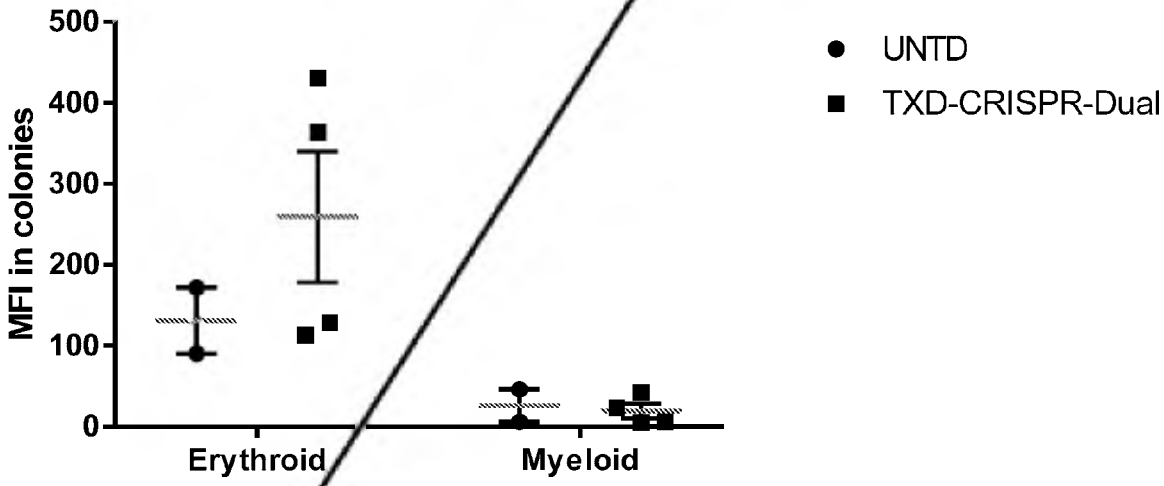


FIG. 99D

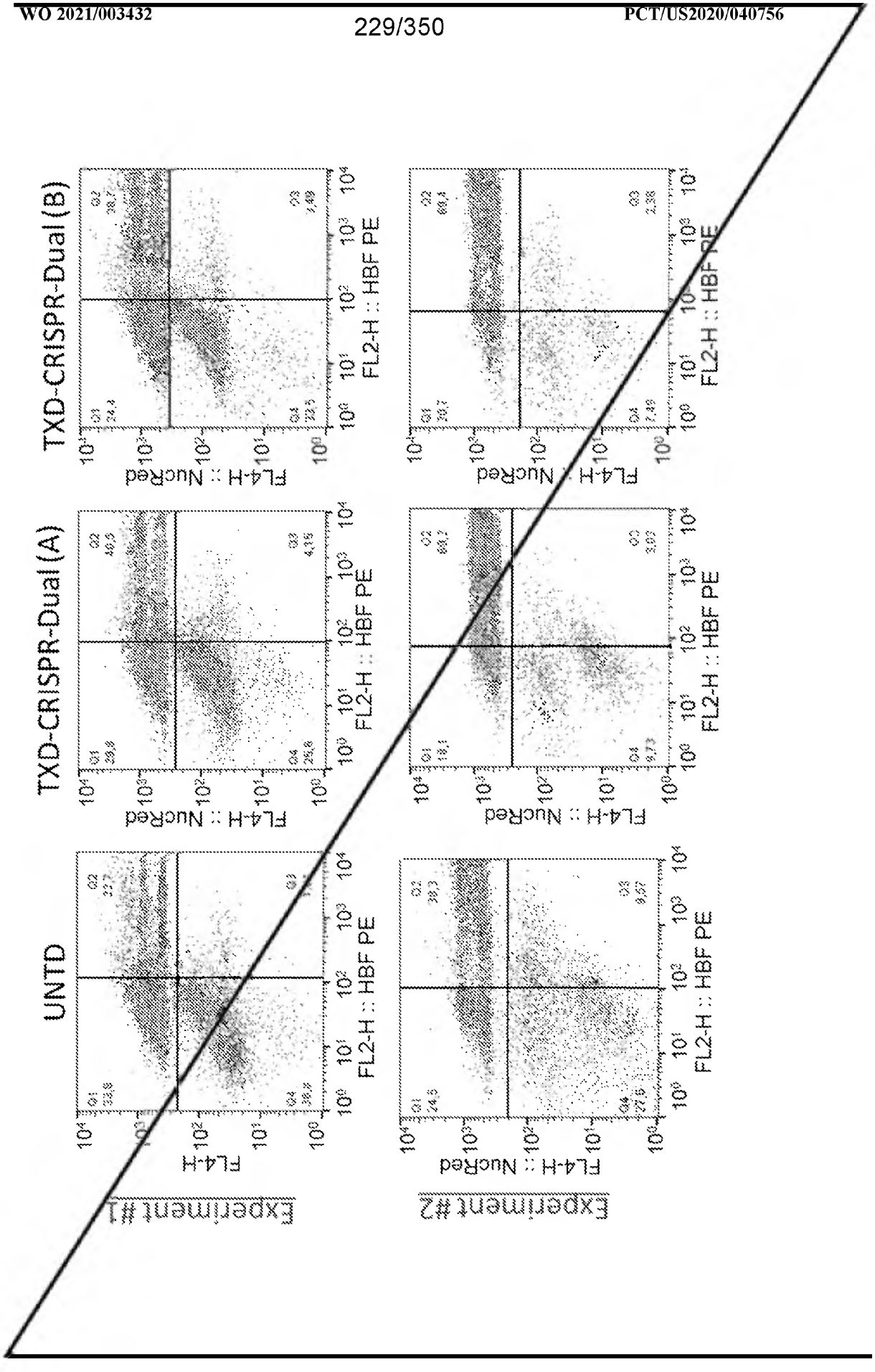

UNTD
TXD-CRISPR-Dual (A)
TXD-CRISPR-Dual (B)
Experiment #1
Experiment #2
FL4-H
FL4-H :: NucRed
FL2-H :: HBF PE
Q1
Q2
Q3
Q4

FIG. 99E

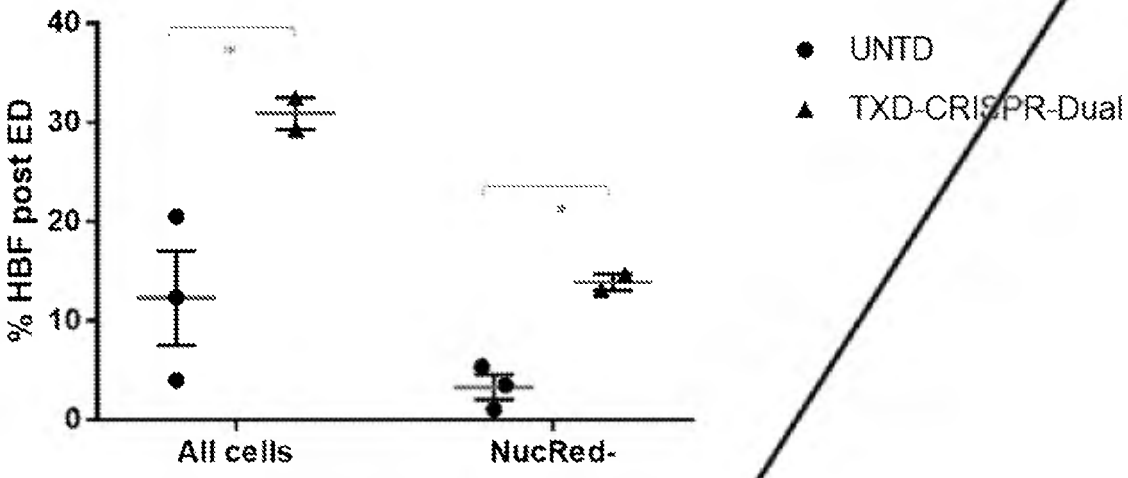


FIG. 99F

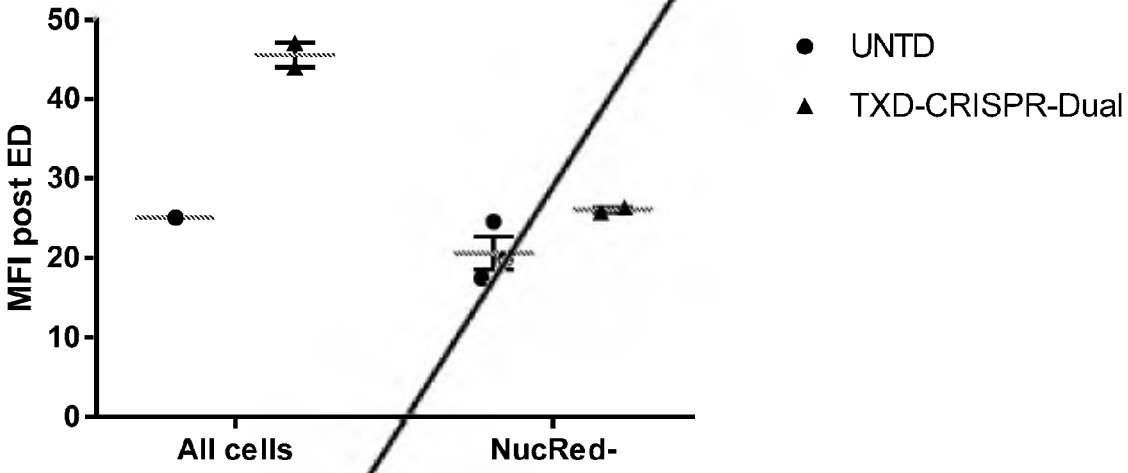


FIG. 99G

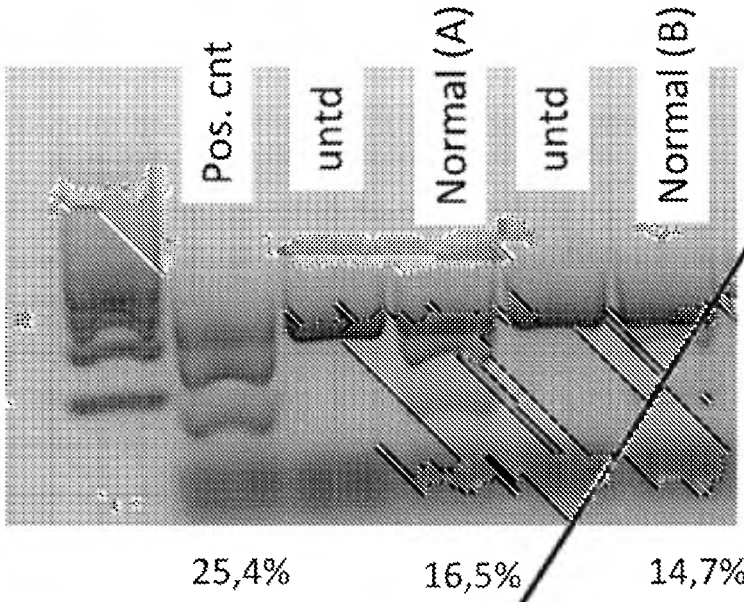
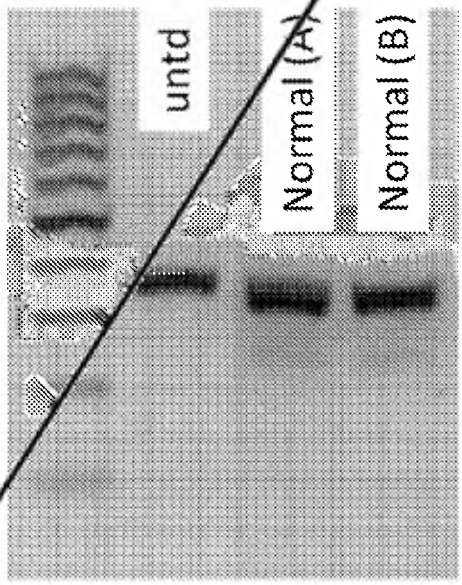


FIG. 99H



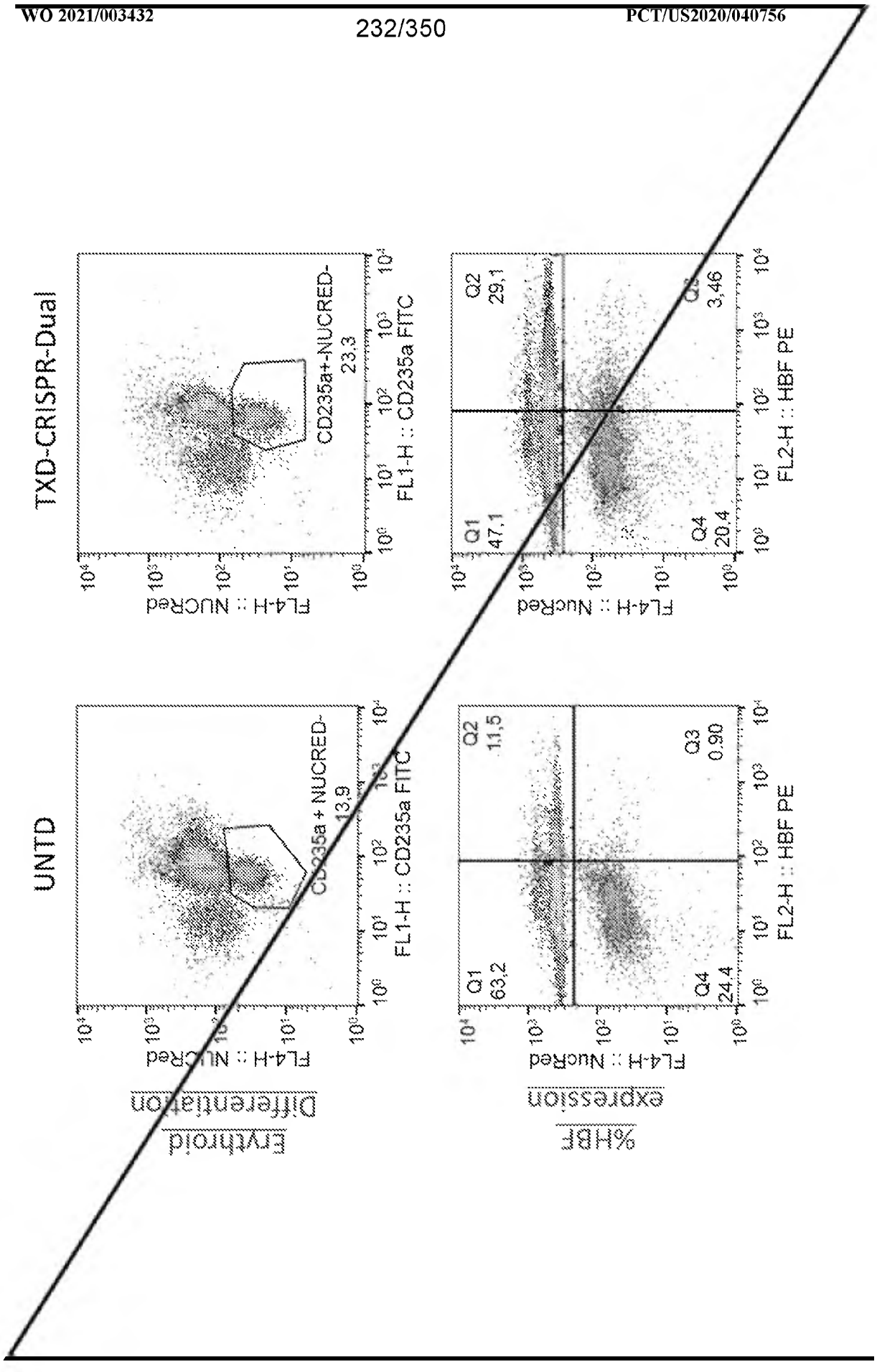

UNTD
TXD-CRISPR-Dual
Erythroid Differentiation
%HBF expression
FL4-H :: NUCRed
FL1-H :: CD235a FITC
CD235a + NUCRED- 13,9
CD235a+-NUCRED- 23,3
FL4-H :: NucRed
FL2-H :: HBF PE
Q1 63,2
Q2 11,5
Q3 0,90
Q4 24,4
Q1 47,1
Q2 29,1
Q3 3,46
Q4 20,4

FIG. 99I

FIG. 99J

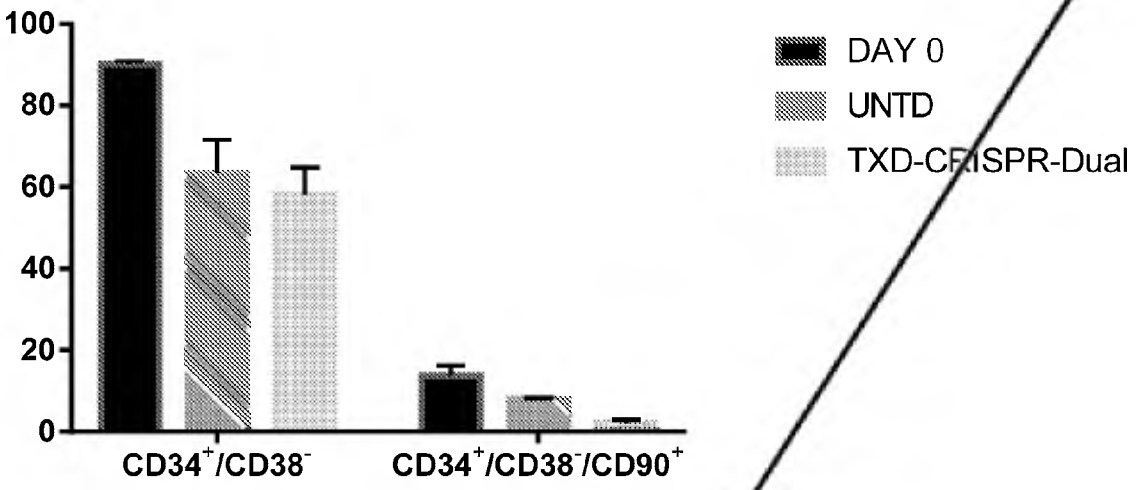


FIG. 99 K

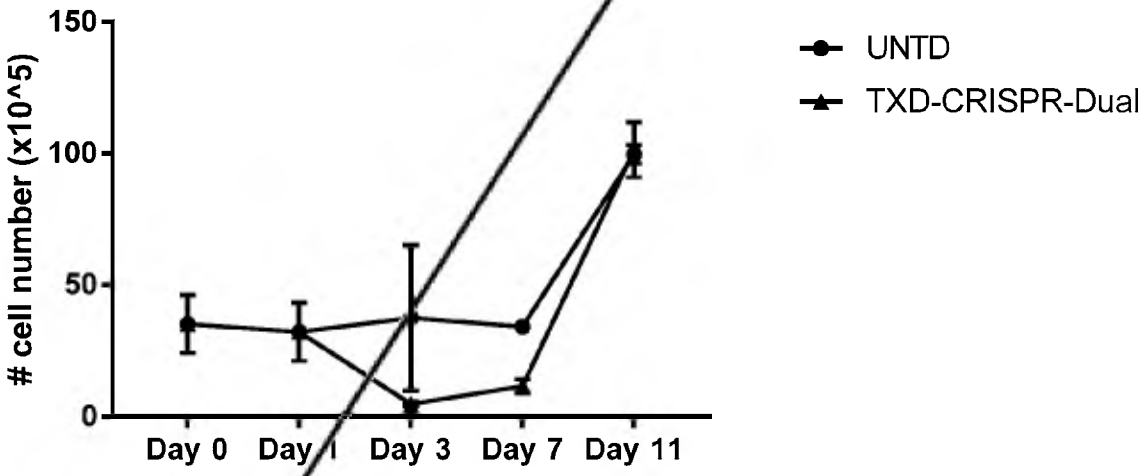


FIG. 99L

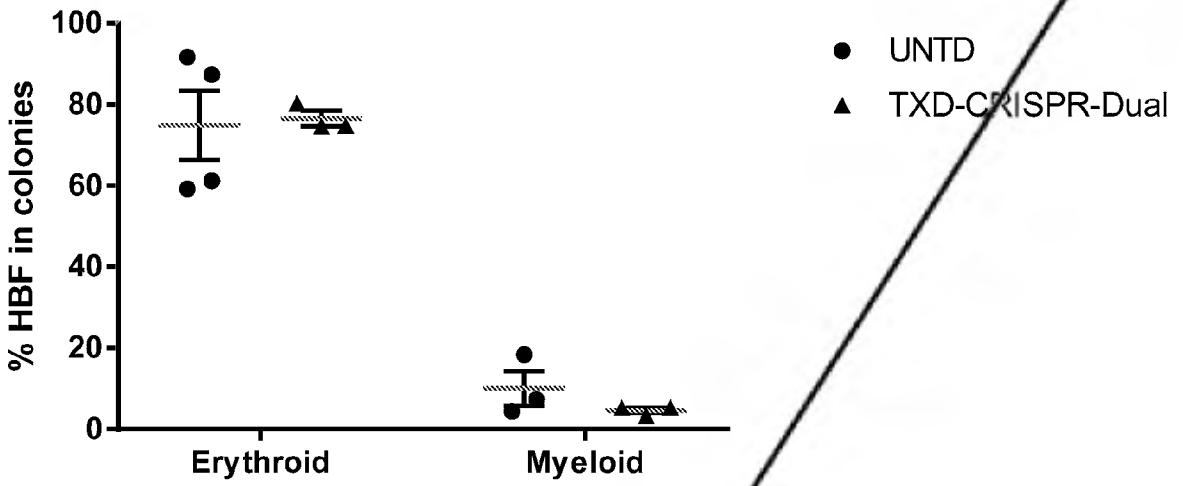


FIG. 99M

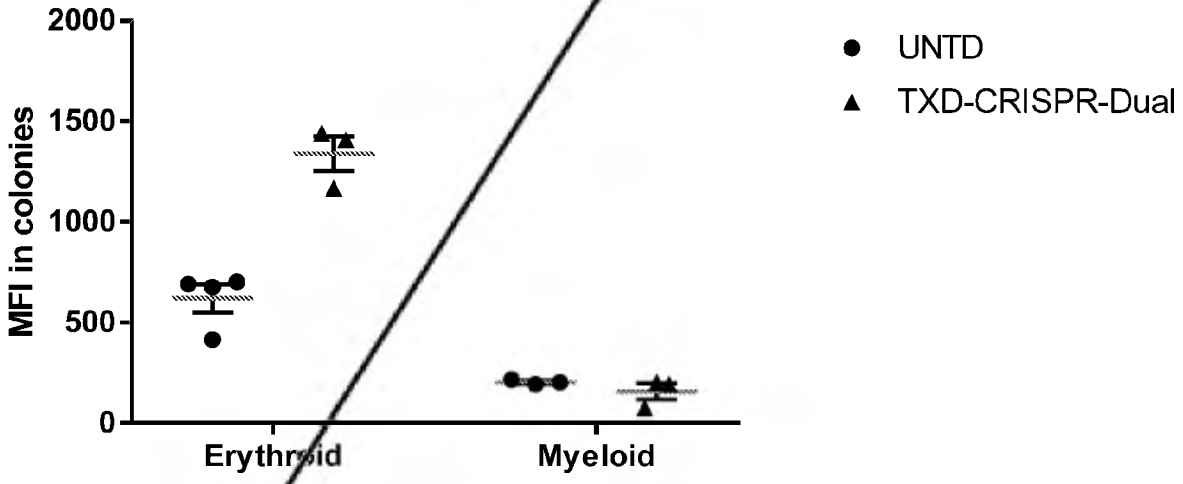
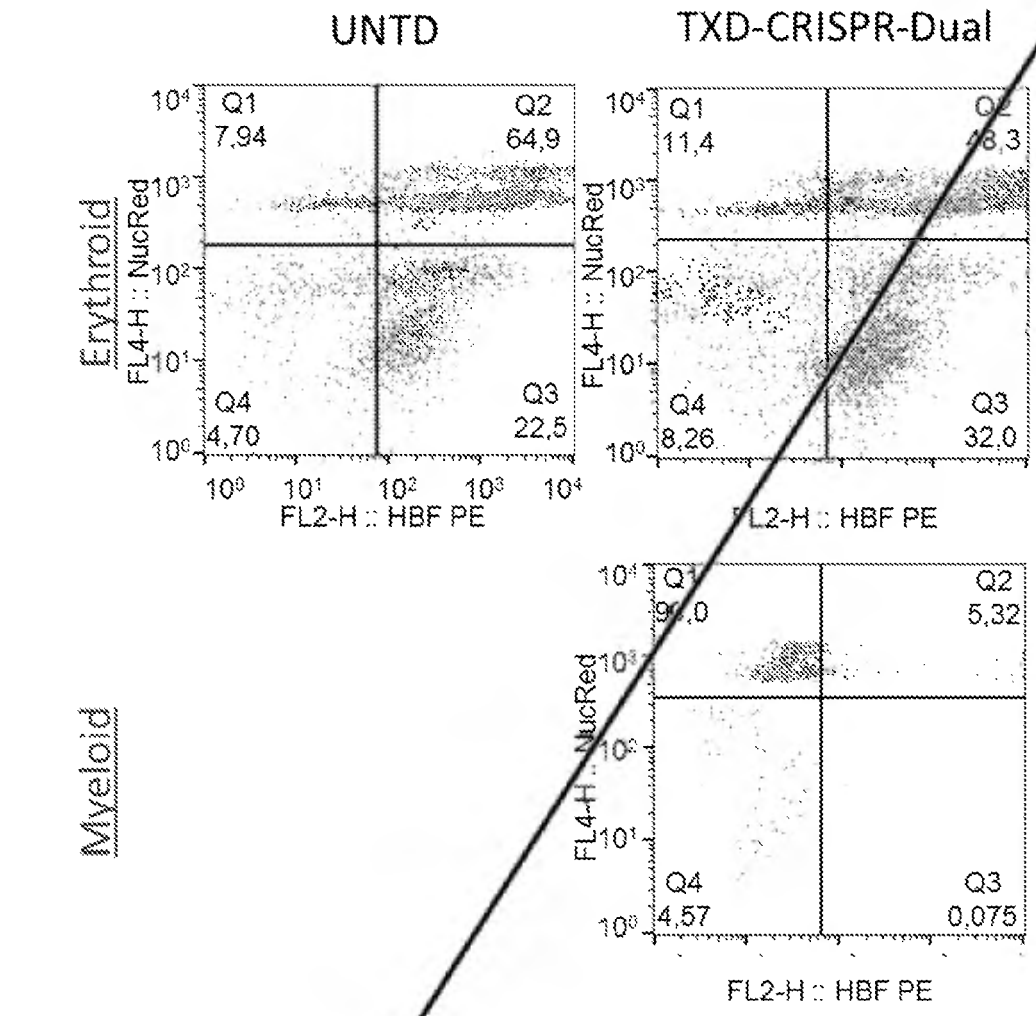


FIG. 99N


UNTD
TXD-CRISPR-Dual
Erythroid
Myeloid
FL4-H :: NucRed
FL2-H :: HBF PE
Q1
7,94
Q2
64,9
Q4
4,70
Q3
22,5
Q1
11,4
Q2
48,3
Q4
8,26
Q3
32,0
Q2
5,32
Q4
4,57
Q3
0,075

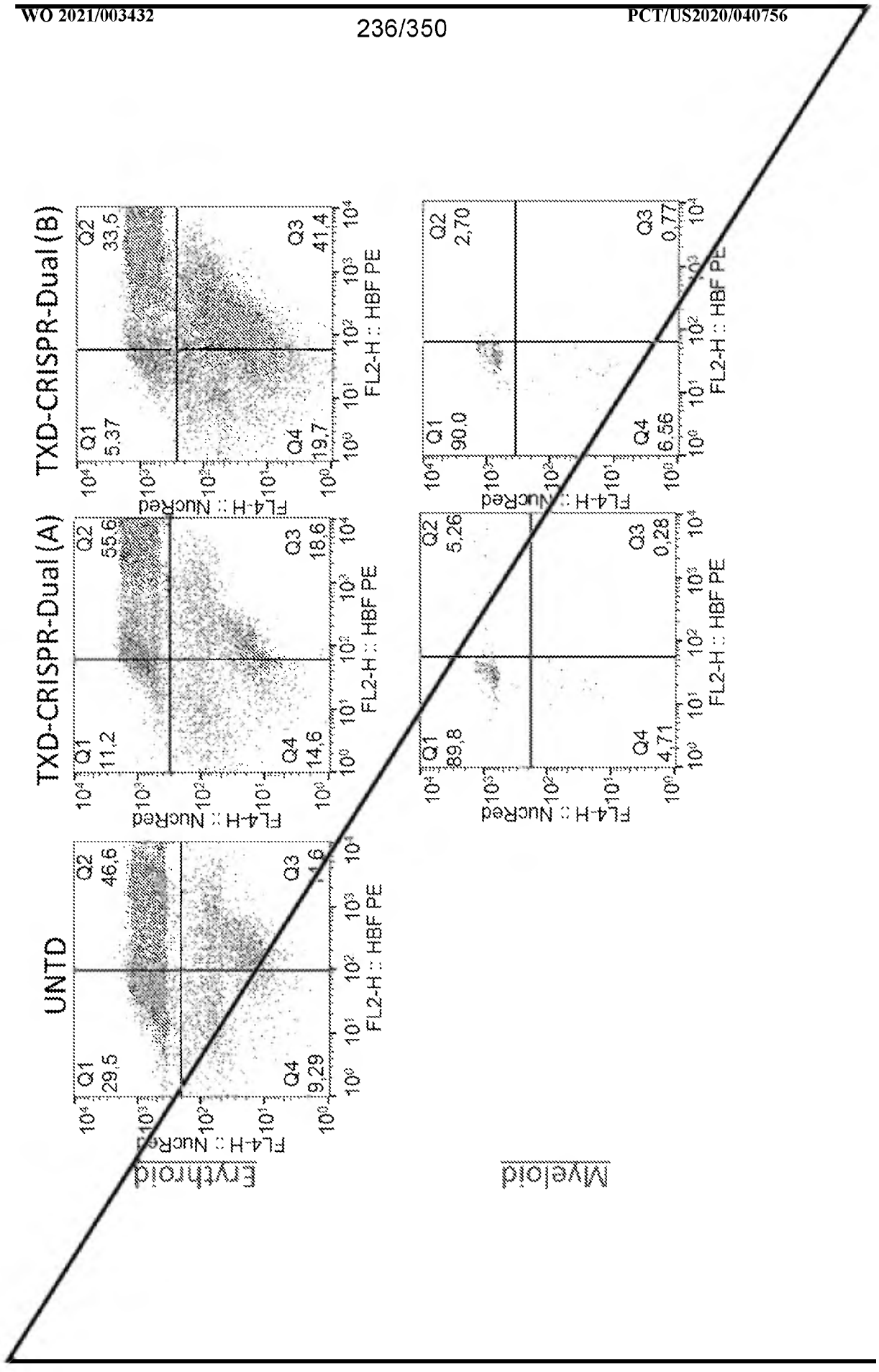

Erythroid
Myeloid
UNTD
TXD-CRISPR-Dual (A)
TXD-CRISPR-Dual (B)
Q1 29,5
Q2 46,6
Q3 1,6
Q4 9,29
Q1 11,2
Q2 55,6
Q3 18,6
Q4 14,6
Q1 5,37
Q2 33,5
Q3 41,4
Q4 19,7
Q1 89,8
Q2 5,26
Q3 0,28
Q4 4,71
Q1 90,0
Q2 2,70
Q3 0,77
Q4 6,56
FL4-H :: NucRed
FL2-H :: HBF PE

FIG. 99O

FIG. 99P

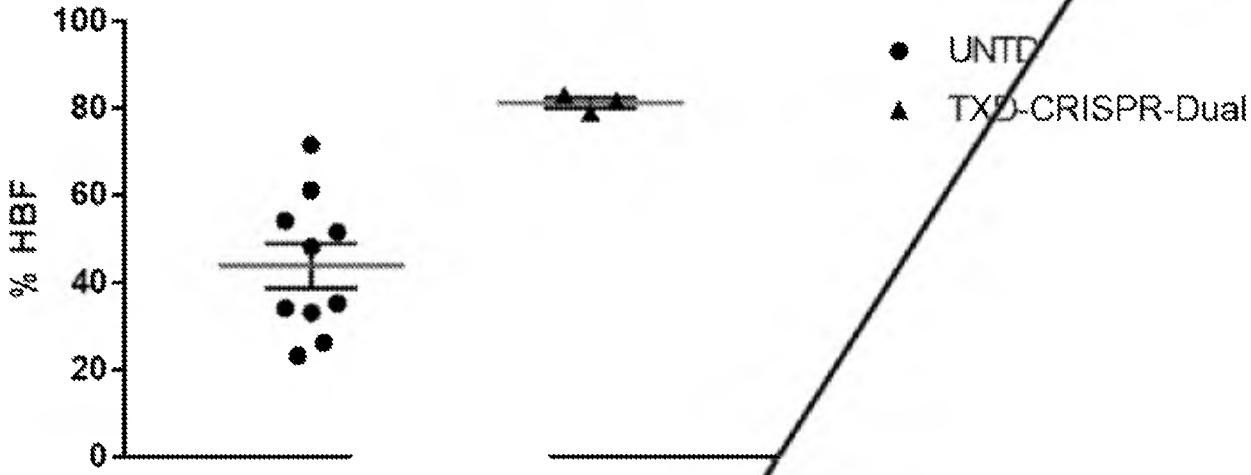


FIG. 99Q

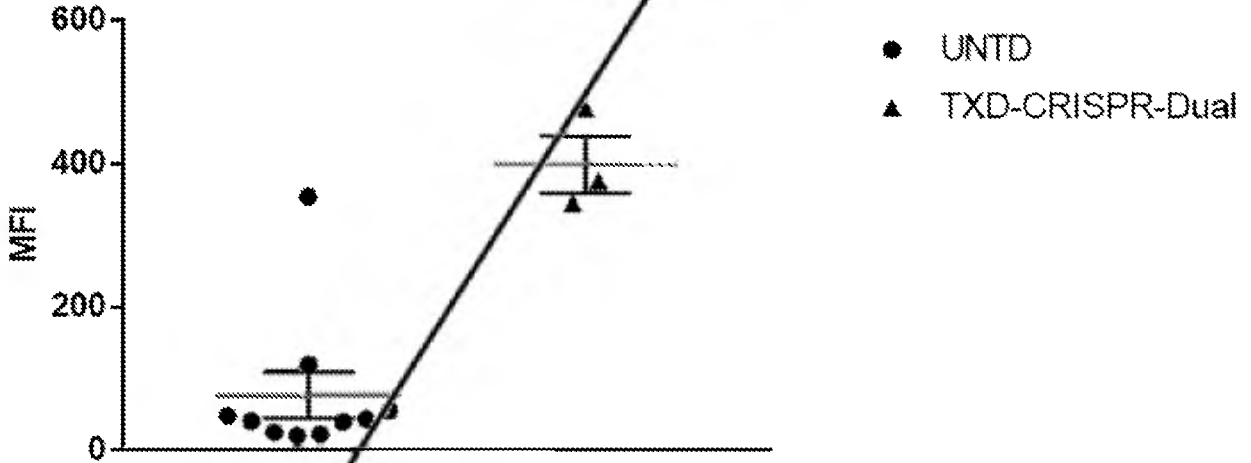


FIG. 99R

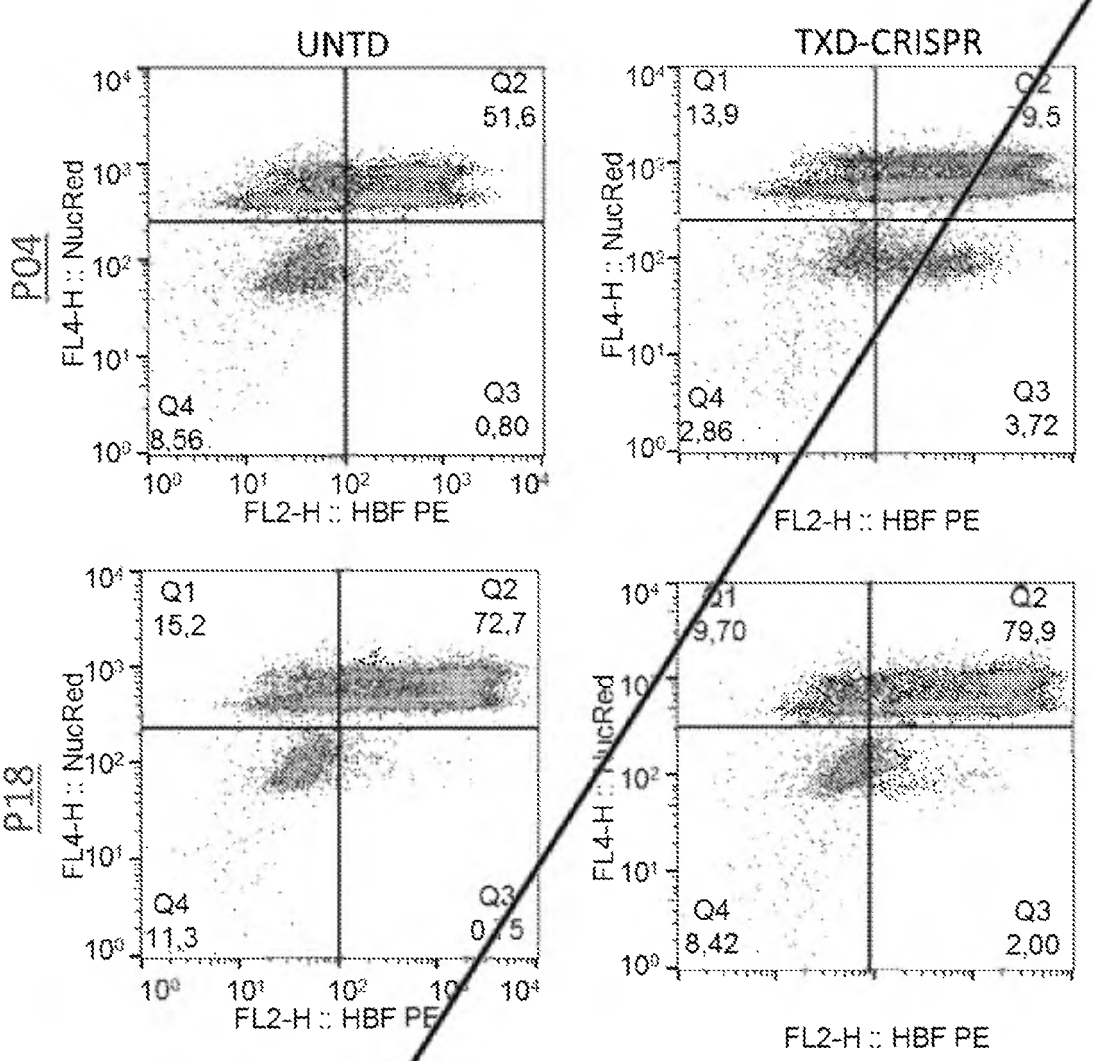

UNTD
TXD-CRISPR
P04
P18
FL4-H :: NucRed
FL2-H :: HBF PE
Q1
13,9
Q2
51,6
9,5
Q3
0,80
3,72
Q4
8,56
2,86
15,2
72,7
9,70
79,9
11,3
0,75
8,42
2,00

FIG. 99S

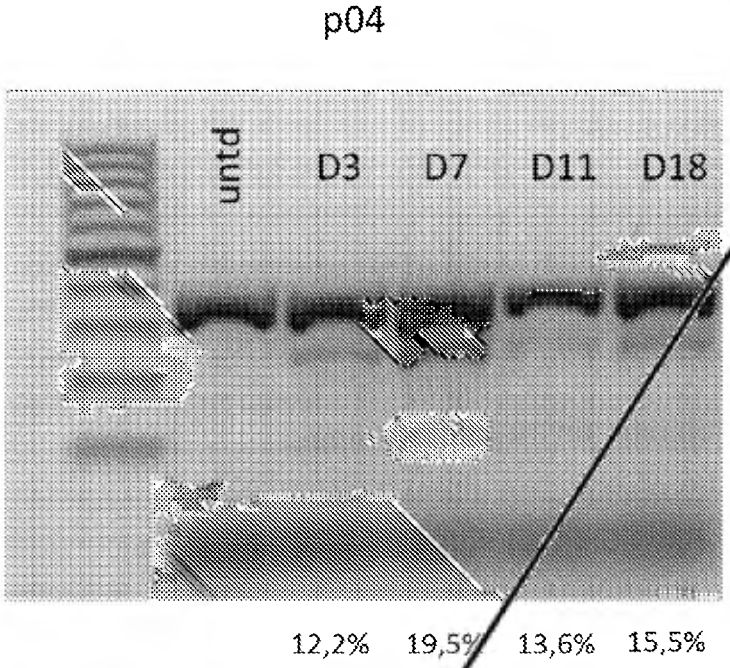


FIG. 99T

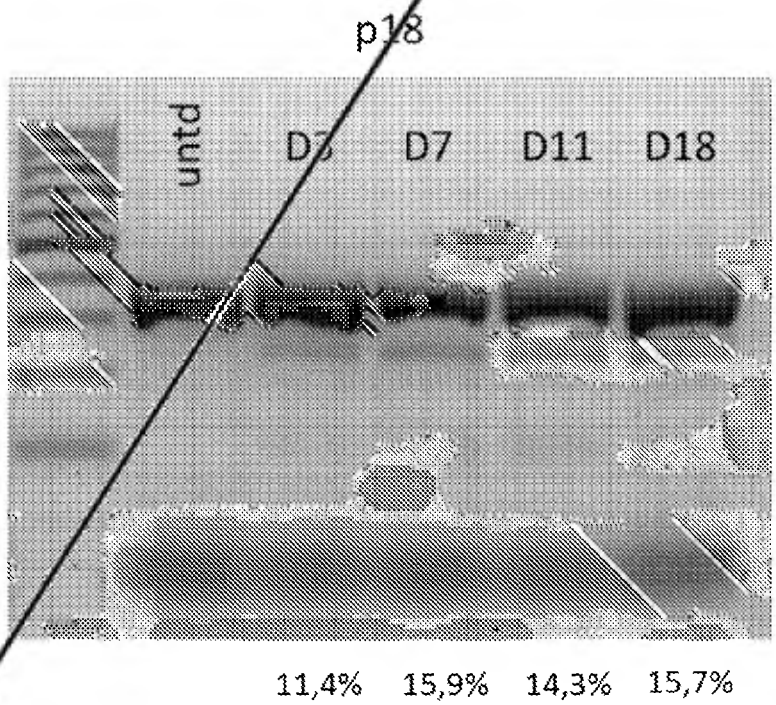


FIG. 99U

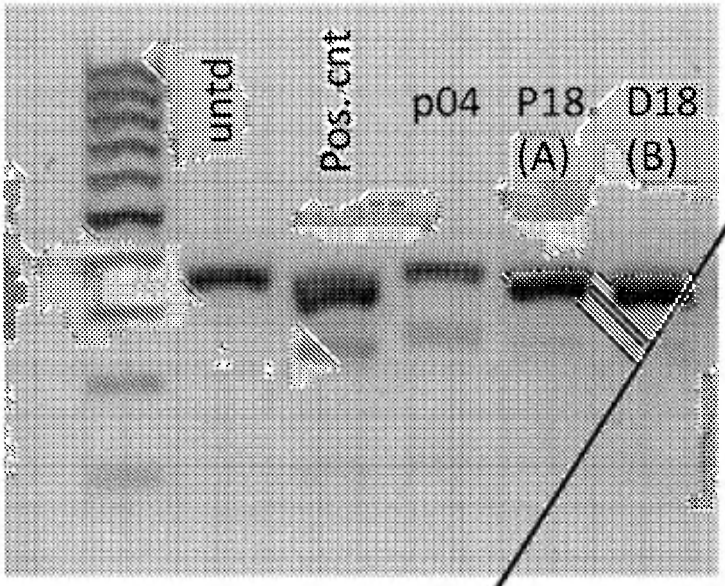


FIG. 100

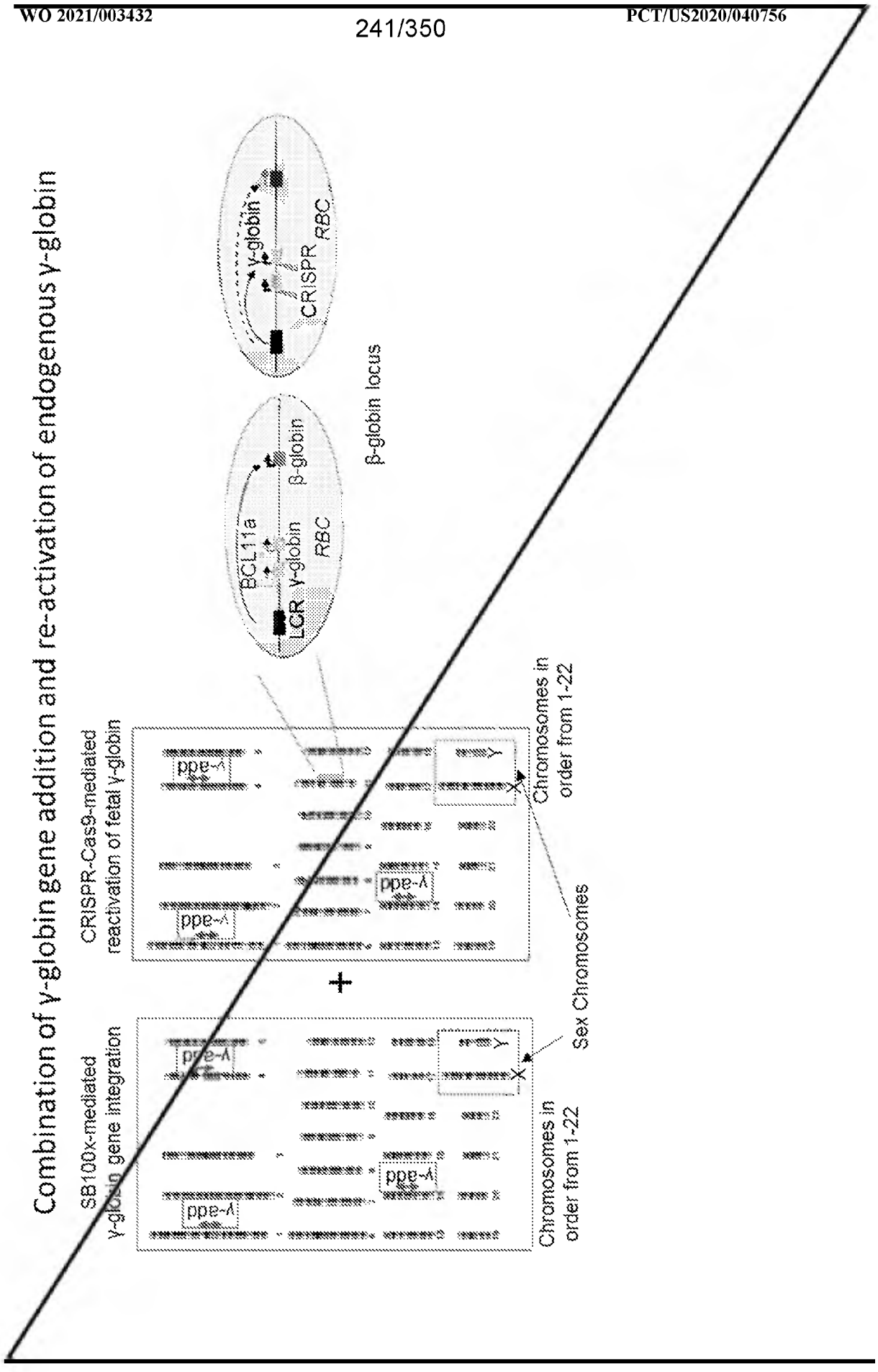

Combination of γ-globin gene addition and re-activation of endogenous γ-globin
SB100x-mediated γ-globin gene integration
γ-add
CRISPR-Cas9-mediated reactivation of fetal γ-globin
BCL11a
LCR
γ-globin
β-globin
RBC
β-globin locus
CRISPR
γ-globin
RBC
Chromosomes in order from 1-22
Sex Chromosomes
Chromosomes in order from 1-22

FIG. 101

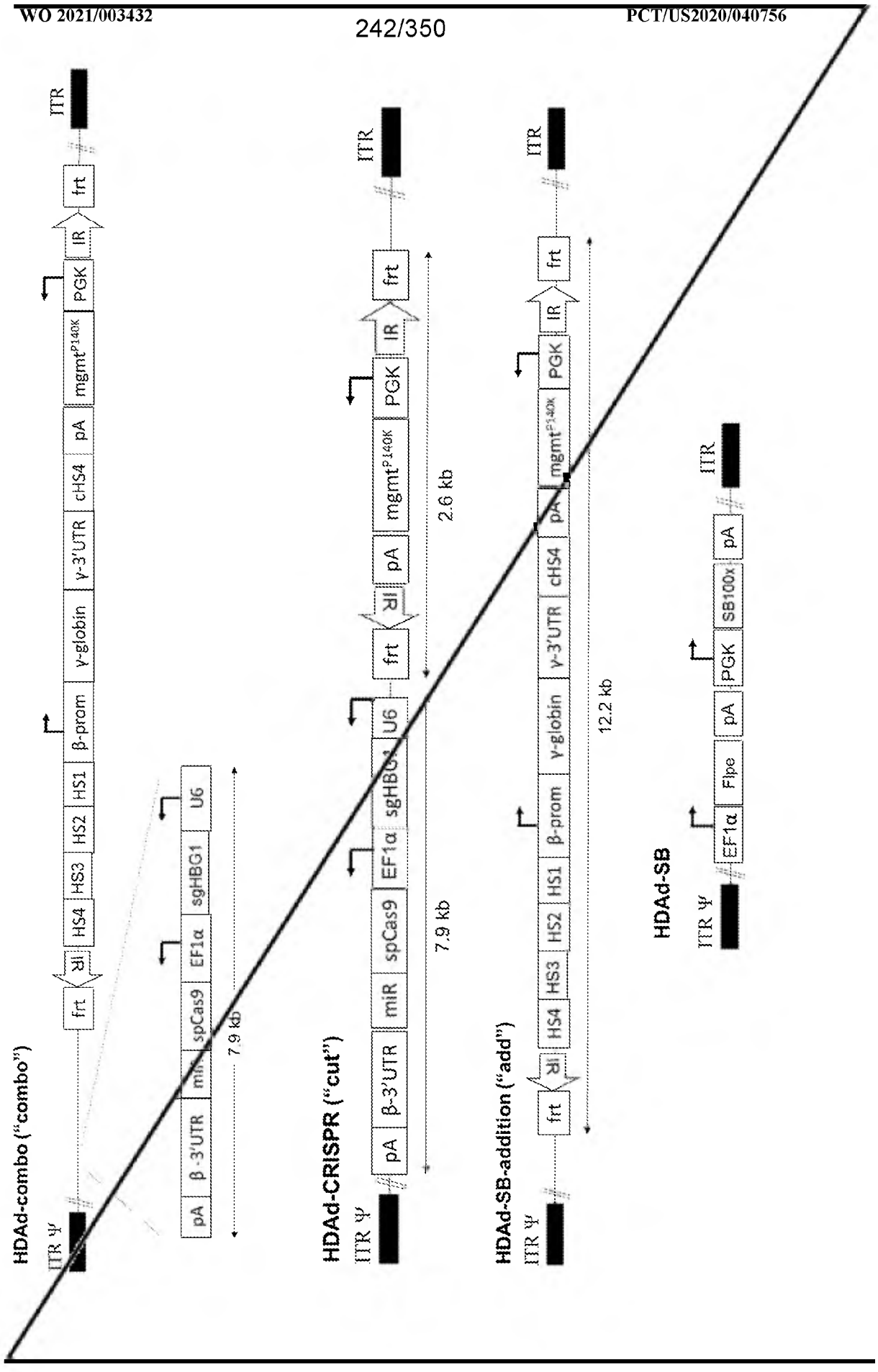

HDAd-combo ("combo")
ITR Ψ
frt
IR
HS4
HS3
HS2
HS1
β-prom
γ-globin
γ-3'UTR
cHS4
pA
mgmt^{P140K}
PGK
IR
frt
ITR
pA
β-3'UTR
miR
spCas9
EF1α
sgHBG1
U6
7.9 kb
HDAd-CRISPR ("cut")
ITR Ψ
pA
β-3'UTR
miR
spCas9
EF1α
sgHBG1
U6
frt
IR
pA
mgmt^{P140K}
PGK
IR
frt
ITR
7.9 kb
2.6 kb
HDAd-SB-addition ("add")
ITR Ψ
frt
IR
HS4
HS3
HS2
HS1
β-prom
γ-globin
γ-3'UTR
cHS4
pA
mgmt^{P140K}
PGK
IR
frt
ITR
12.2 kb
HDAd-SB
ITR Ψ
EF1α
Flpe
pA
PGK
SB100x
pA
ITR

FIG. 102

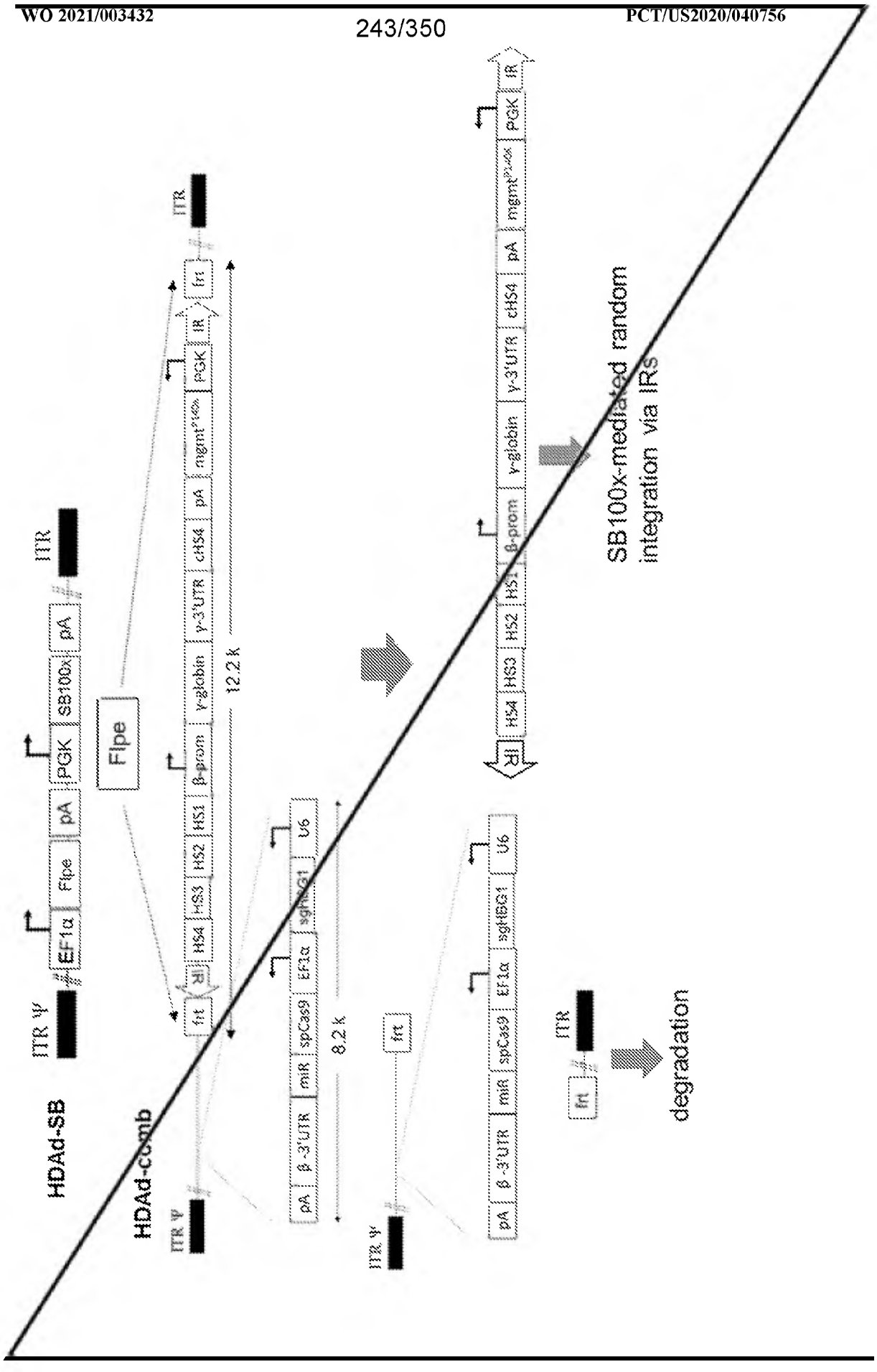

HDAd-SB
ITR Ψ
EF1α
Flpe
pA
PGK
SB100x
pA
ITR
HDAd-comb
ITR Ψ
frt
IR
HS4
HS3
HS2
HS1
β-prom
γ-globin
γ-3'UTR
cHS4
pA
mgmt
PGK
IR
frt
ITR
Flpe
12.2 k
pA
β-3'UTR
miR
spCas9
EF1α
sgRNG1
U6
8.2 k
ITR Ψ
frt
pA
β-3'UTR
miR
spCas9
EF1α
sgRNG1
U6
IR
HS4
HS3
HS2
HS1
β-prom
γ-globin
γ-3'UTR
cHS4
pA
mgmt
PGK
IR
SB100x-mediated random
integration via IRs
ITR
frt
degradation

FIG. 103A

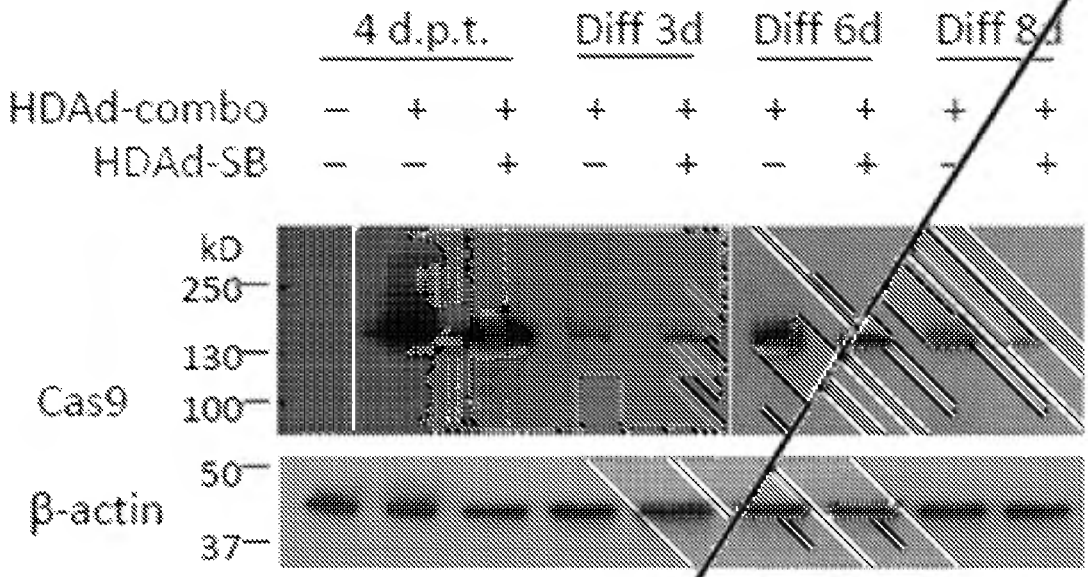


FIG. 103B

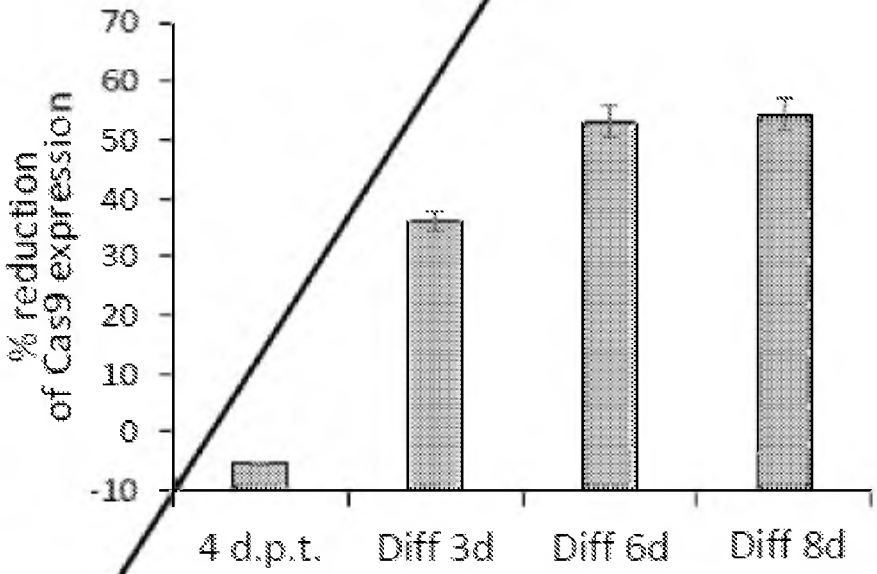


FIG. 103C

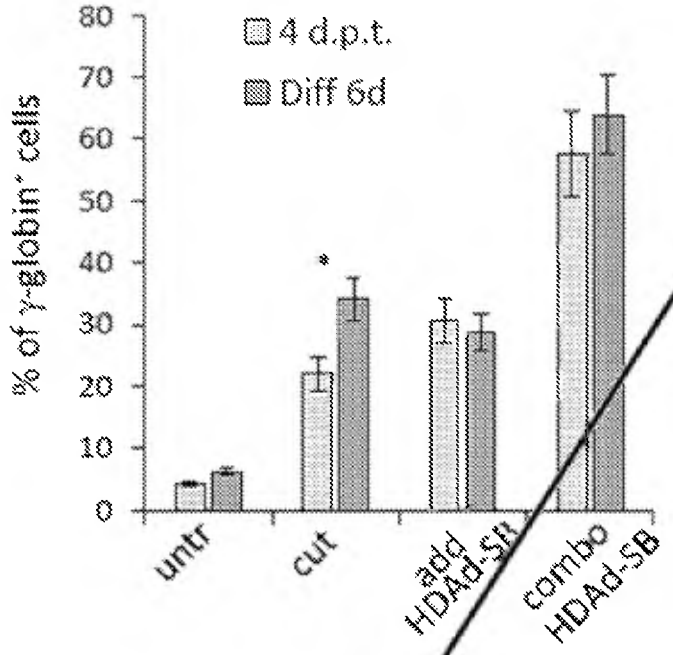


FIG. 103D

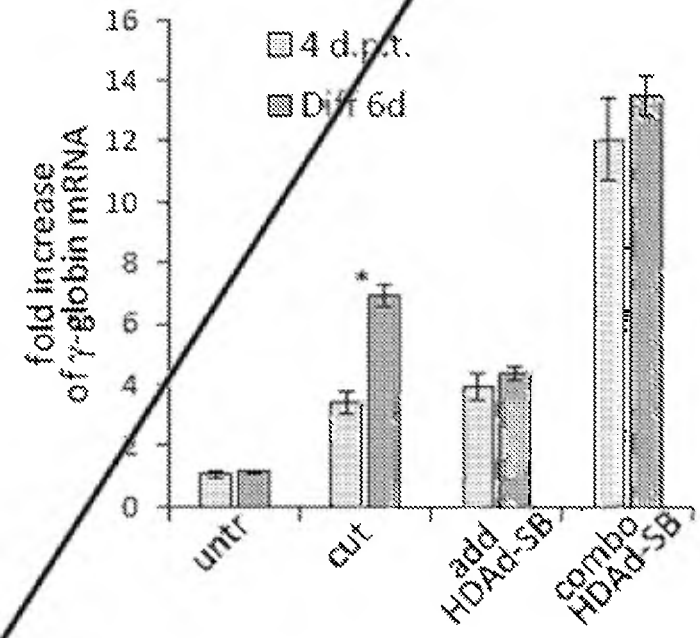


FIG. 104A

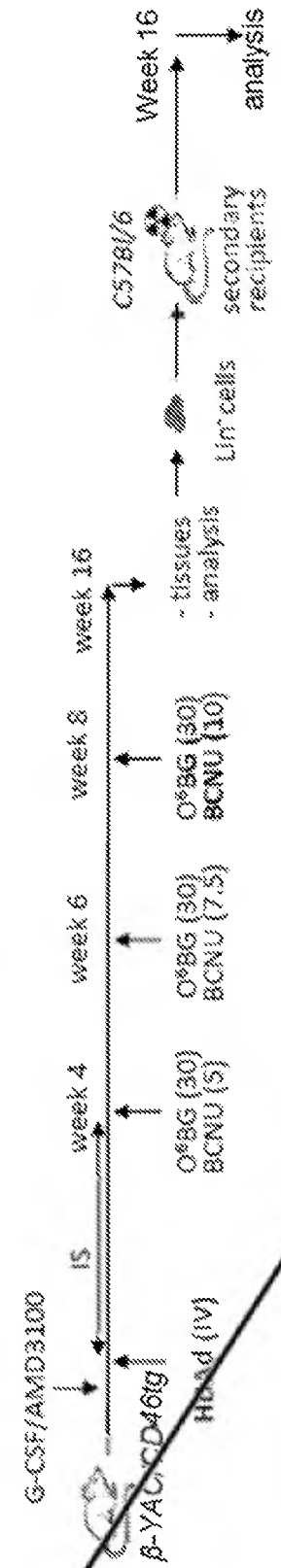
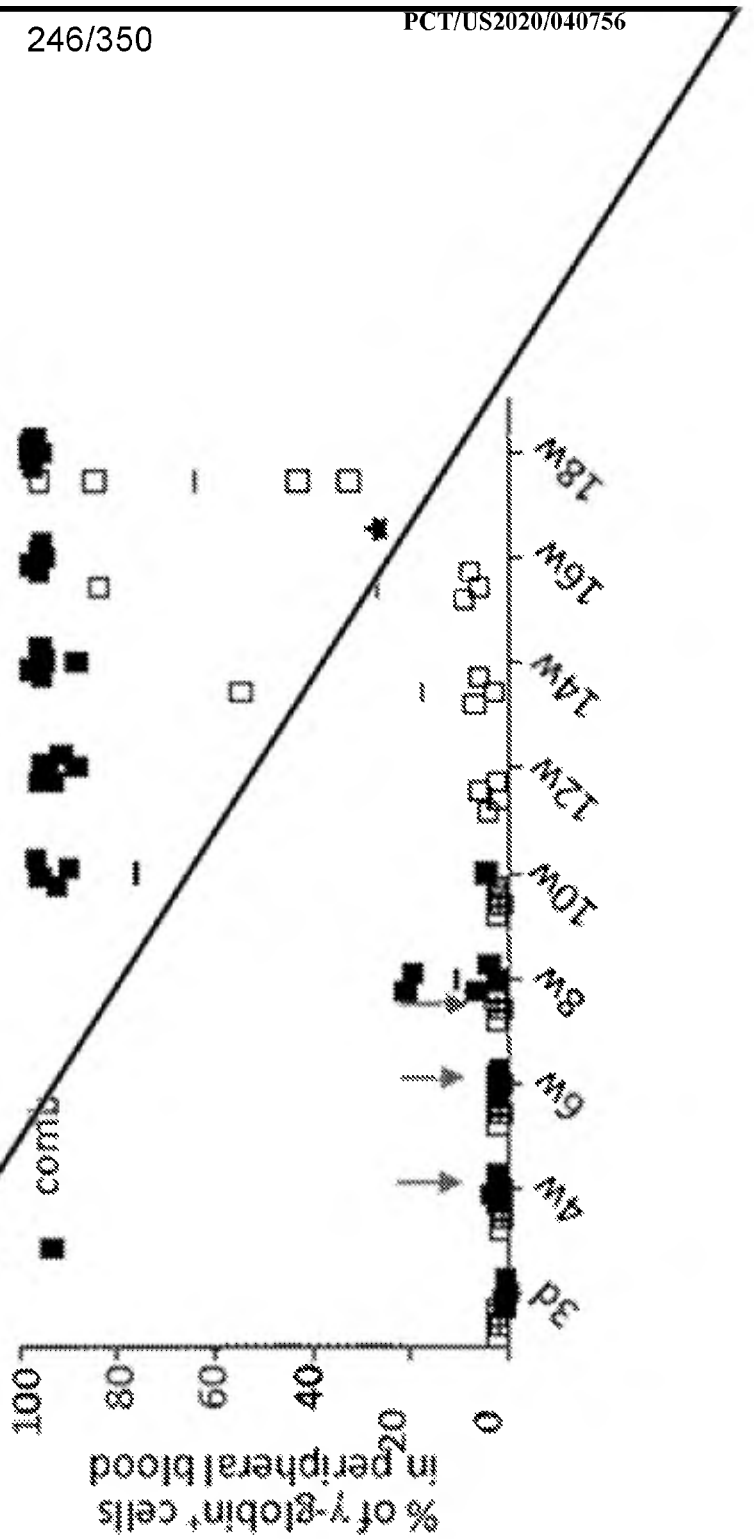


FIG. 104B



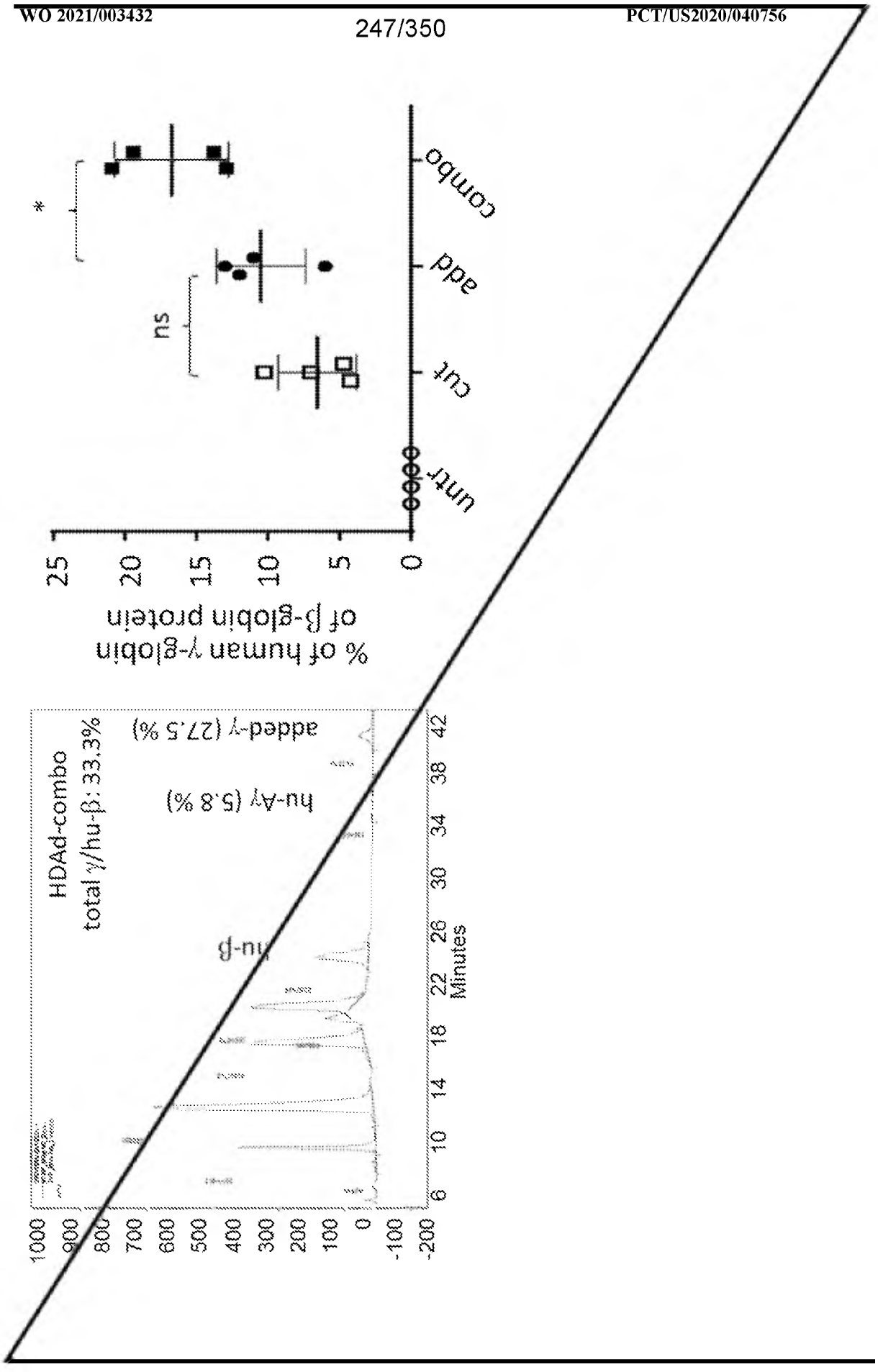

HDAd-combo
total γ/hu-β: 33.3%
hu-Aγ (5.8 %)
added-γ (27.5 %)
hu-β
1000
900
800
700
600
500
400
300
200
100
0
-100
-200
6
10
14
18
22
26
30
34
38
42
Minutes
% of human γ-globin
of β-globin protein
25
20
15
10
5
0
untr
cut
add
combo
ns
*

FIG. 104C

FIG. 104D

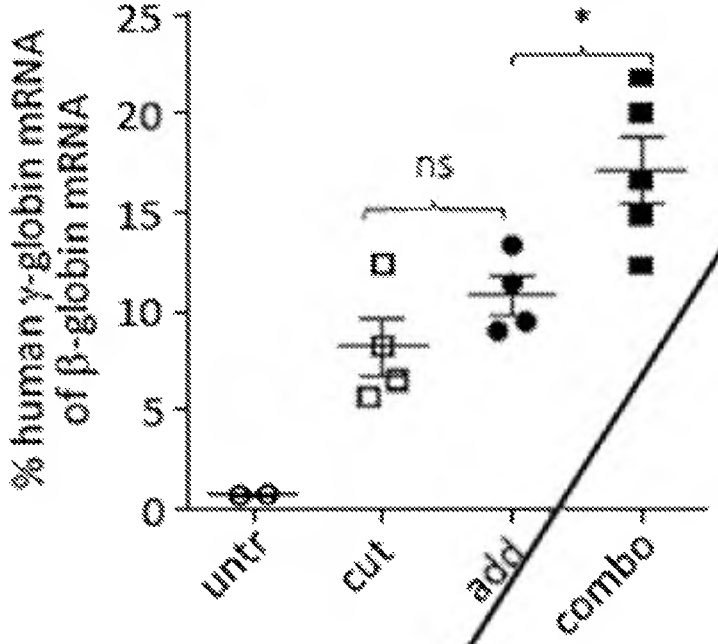


FIG. 104E

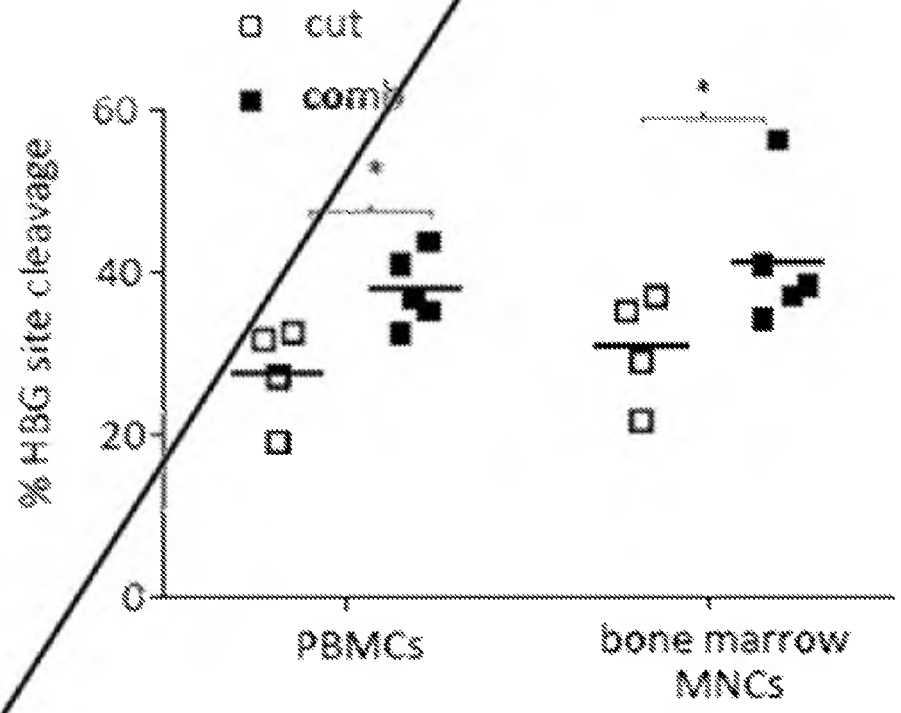


FIG. 104F

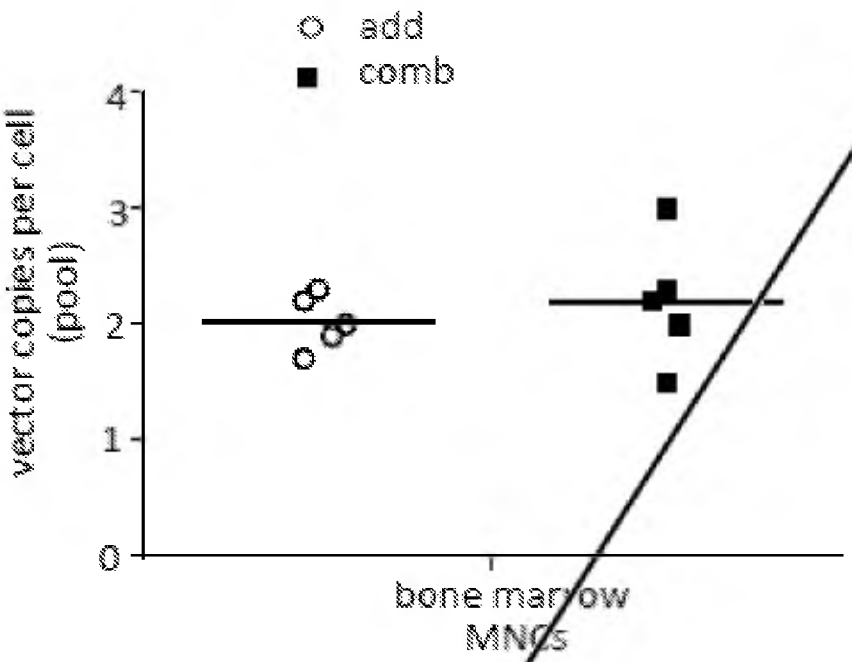


FIG. 104G

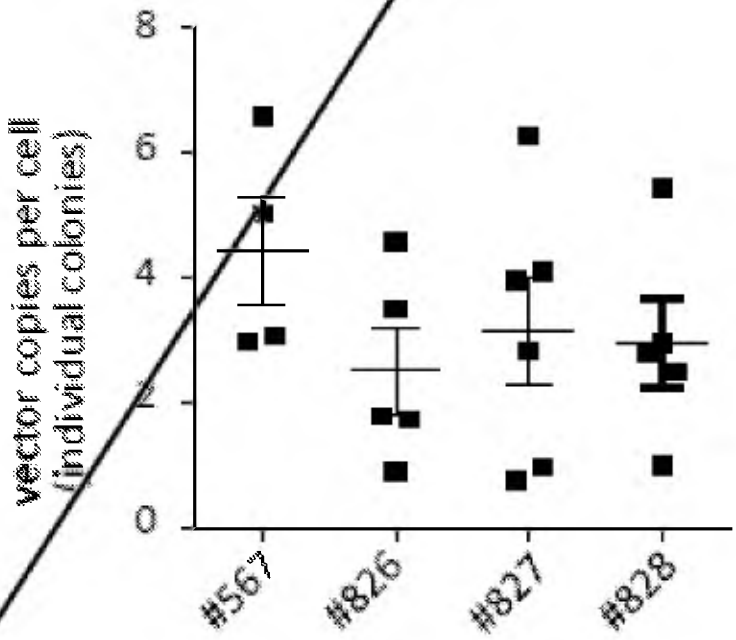


FIG. 104H

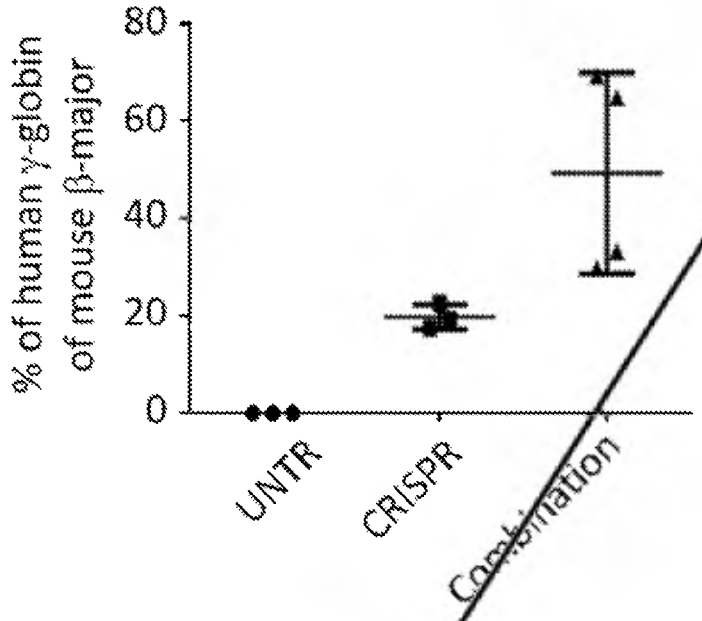


FIG. 105

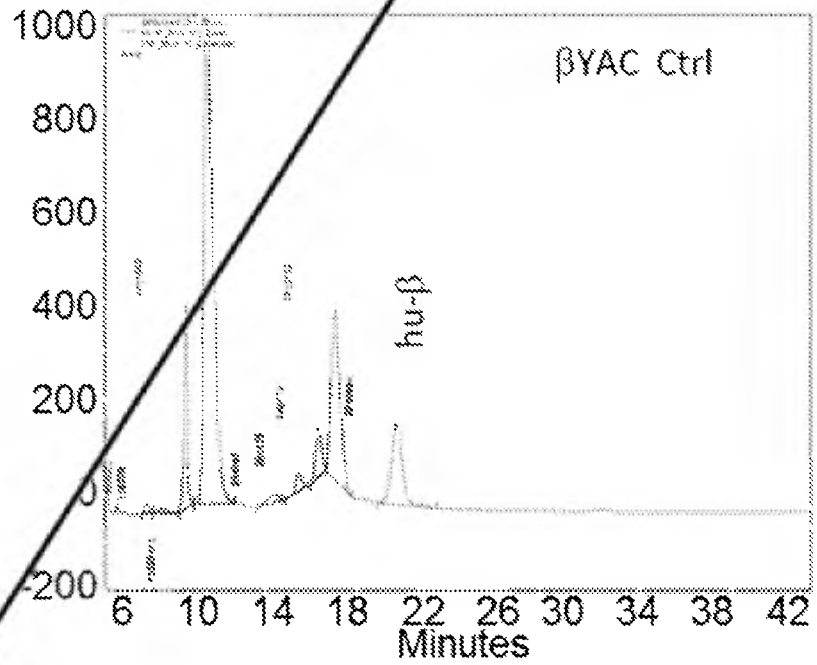
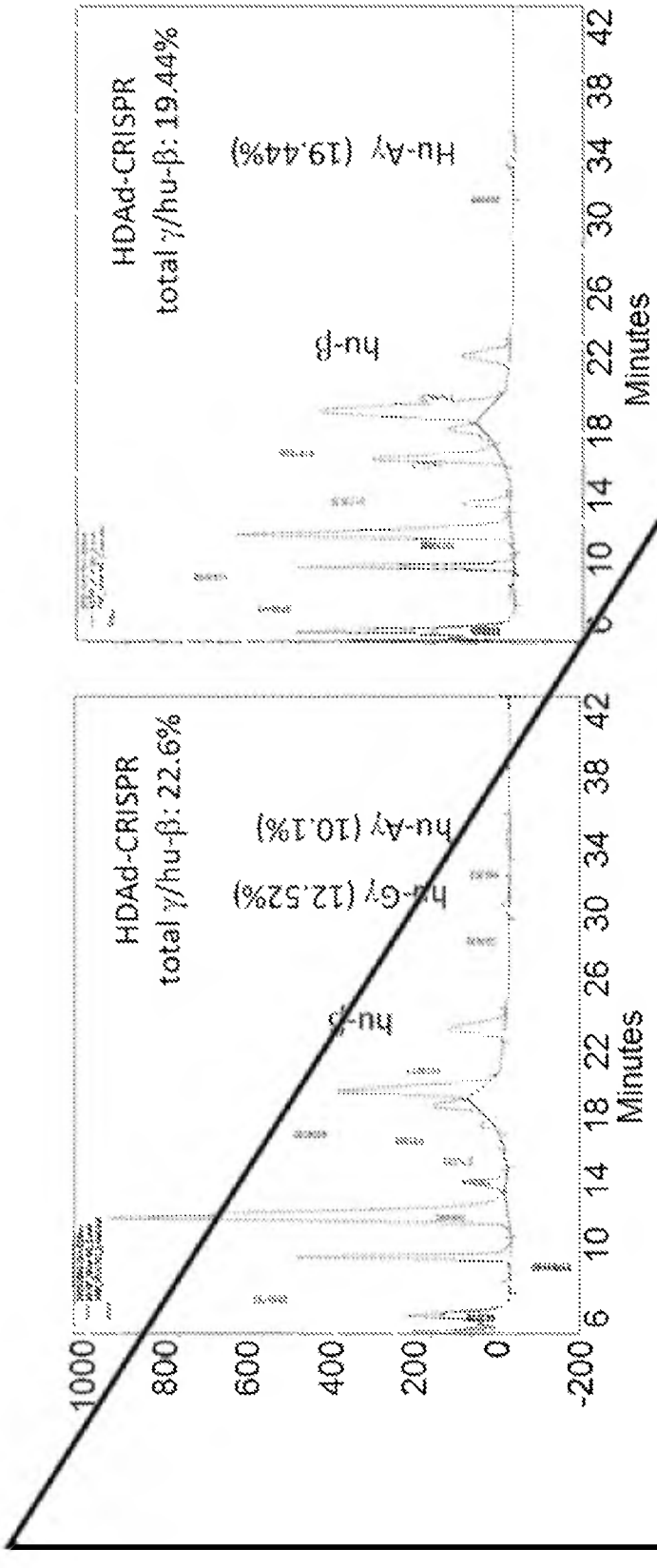


FIG. 105 cont'd



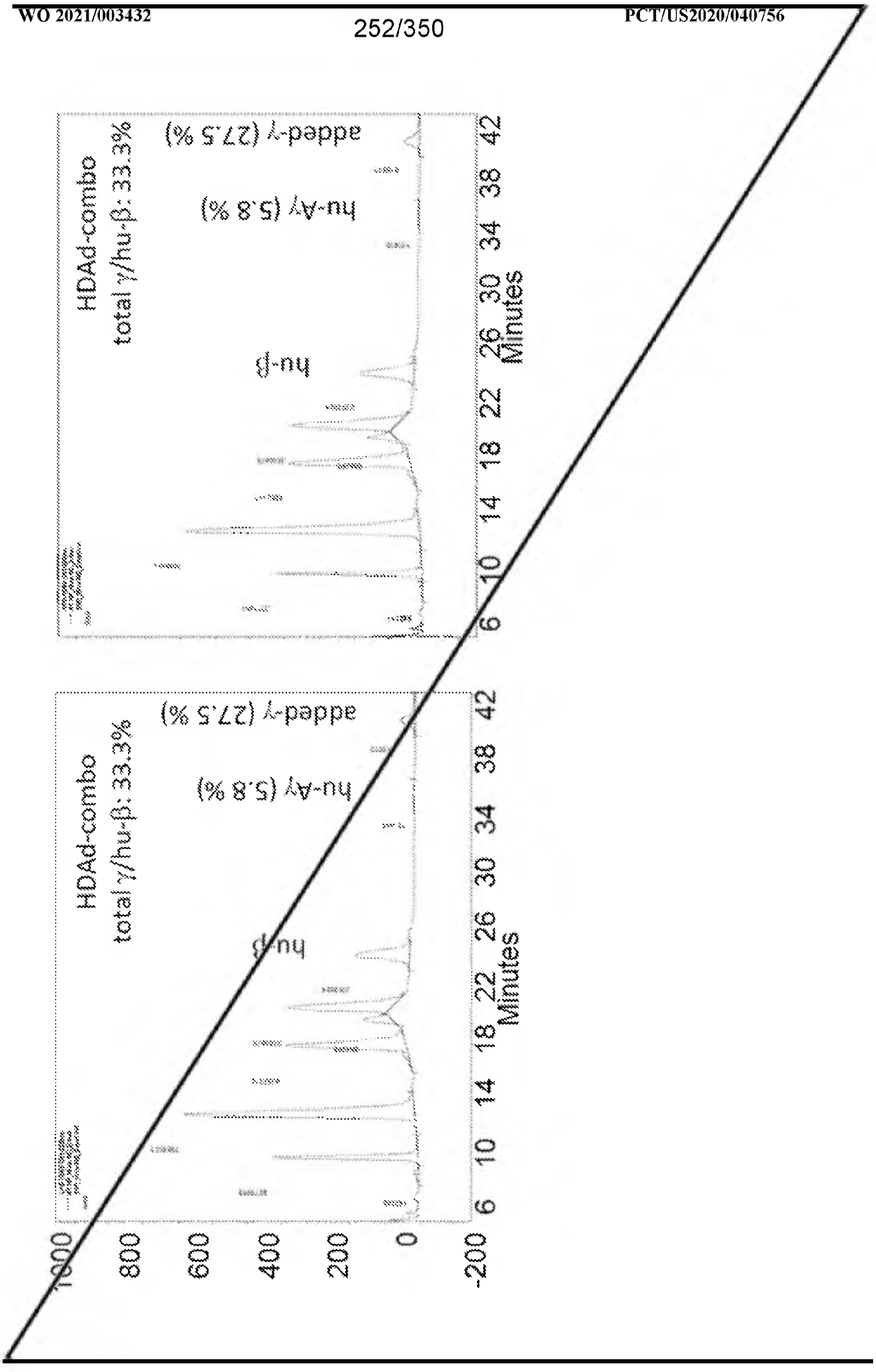
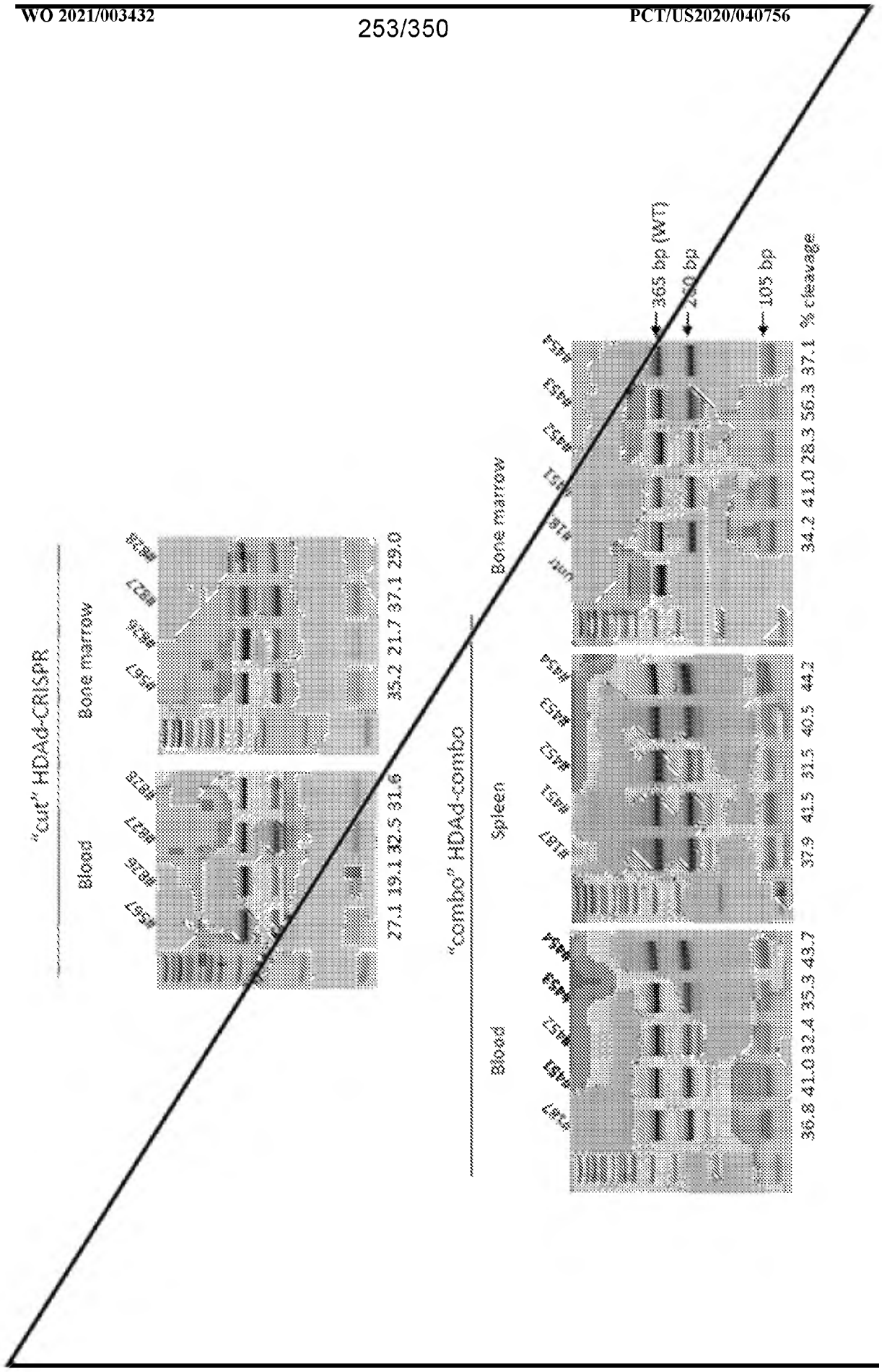


FIG. 105 cont'd

FIG. 106


"cut" HDAd-CRISPR
Blood
Bone marrow
#657
#828
#827
#826
27.1 19.1 32.5 31.6
35.2 21.7 37.1 29.0
"combo" HDAd-combo
Blood
Spleen
Bone marrow
#657
#828
#827
#826
#654
#653
#652
#651
#650
36.8 41.0 32.4 35.3 43.7
37.9 41.5 31.5 40.5 44.2
34.2 41.0 28.3 36.3 37.1
365 bp (WT)
105 bp
% cleavage

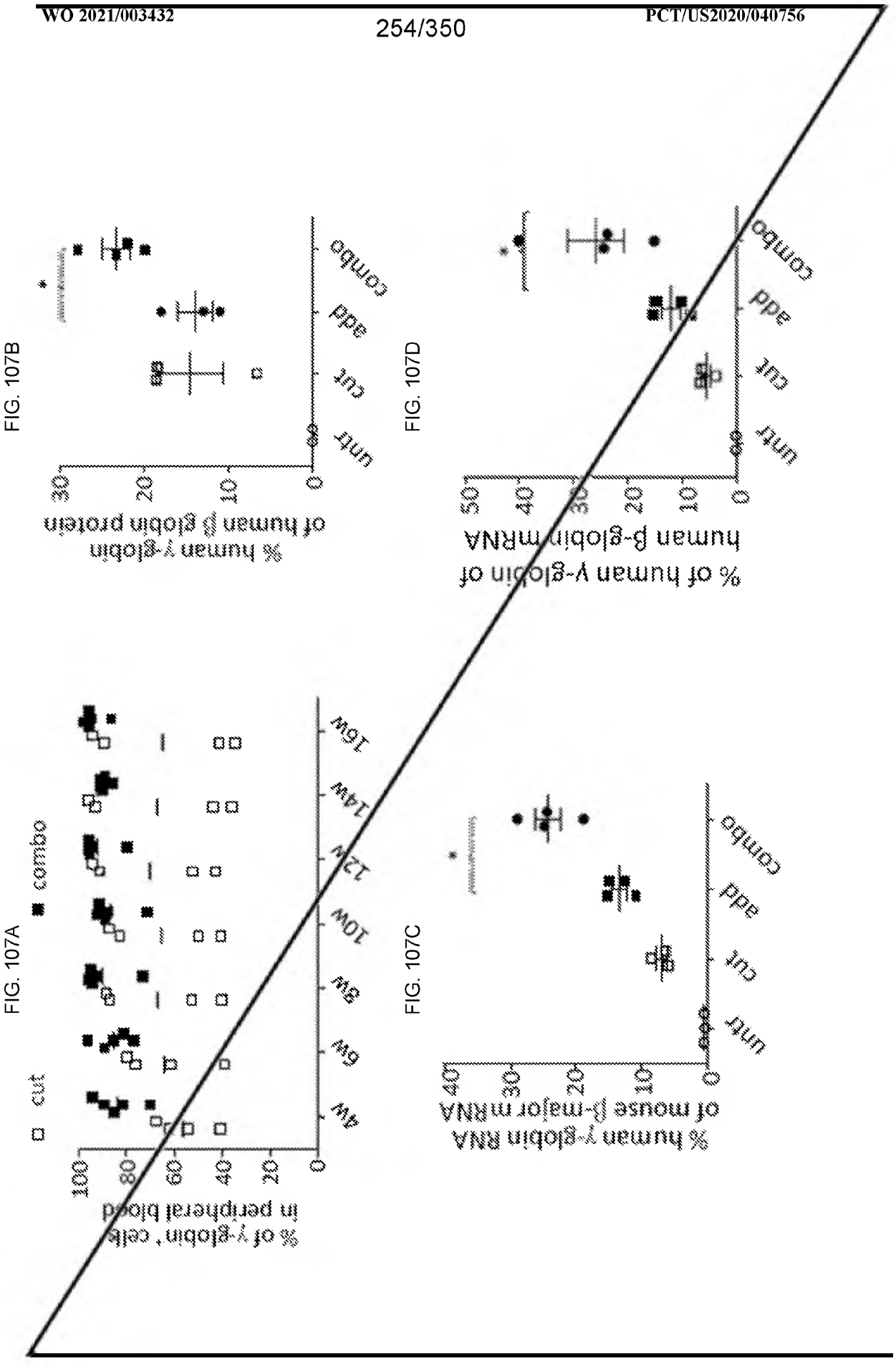

FIG. 107A
% of γ-globin+ cells in peripheral blood
combo
cut
4w
6w
8w
10w
12w
14w
16w
0
20
40
60
80
100
FIG. 107B
% human γ-globin of human β globin protein
untr
cut
add
combo
0
10
20
30
FIG. 107C
% human γ-globin RNA of mouse β-major mRNA
untr
cut
add
combo
0
10
20
30
40
FIG. 107D
% of human γ-globin of human β-globin mRNA
untr
cut
add
combo
0
10
20
30
40
50

FIG. 107E

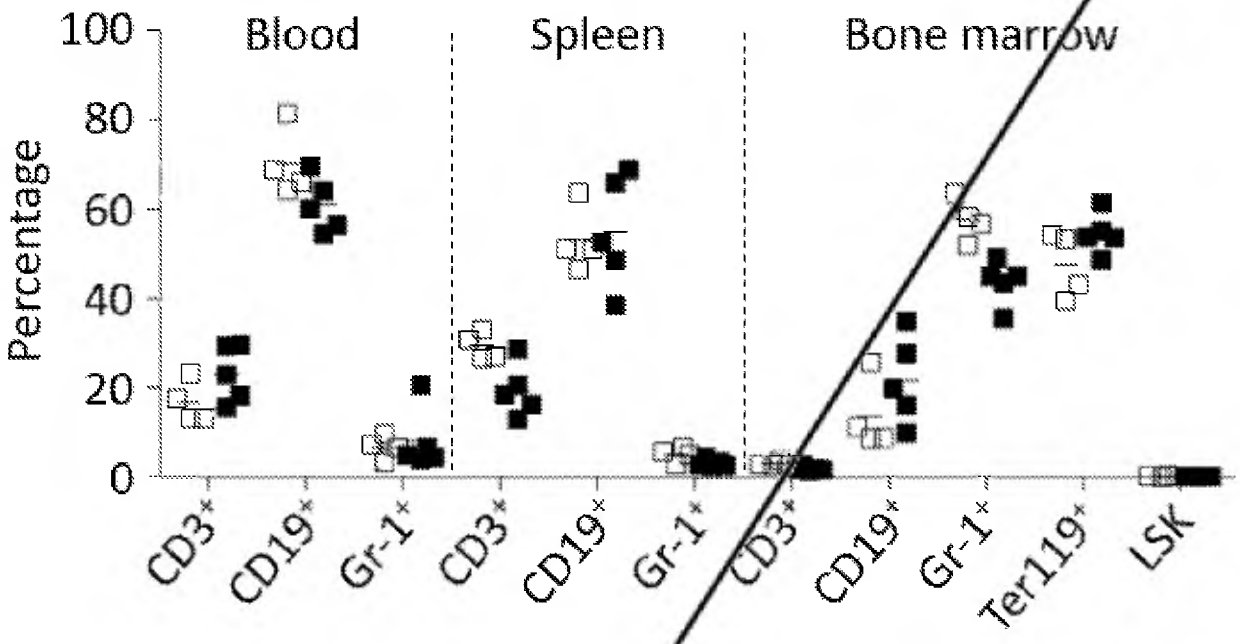


FIG. 107F

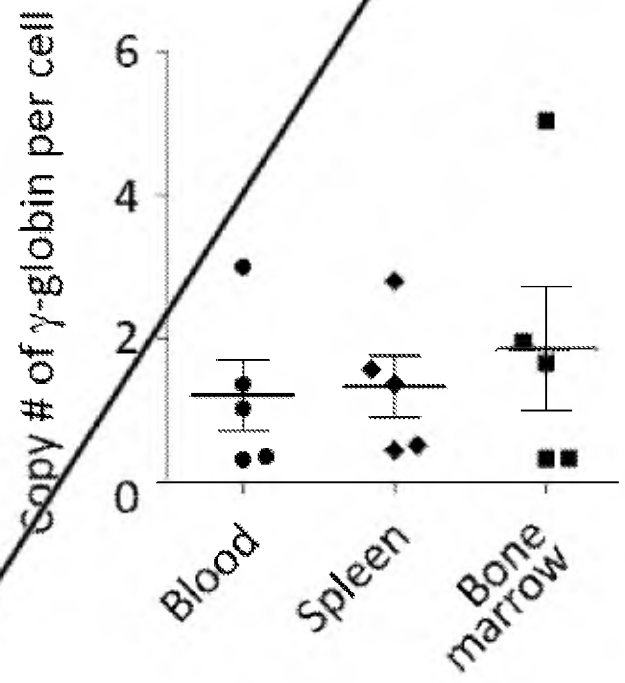


FIG. 108A

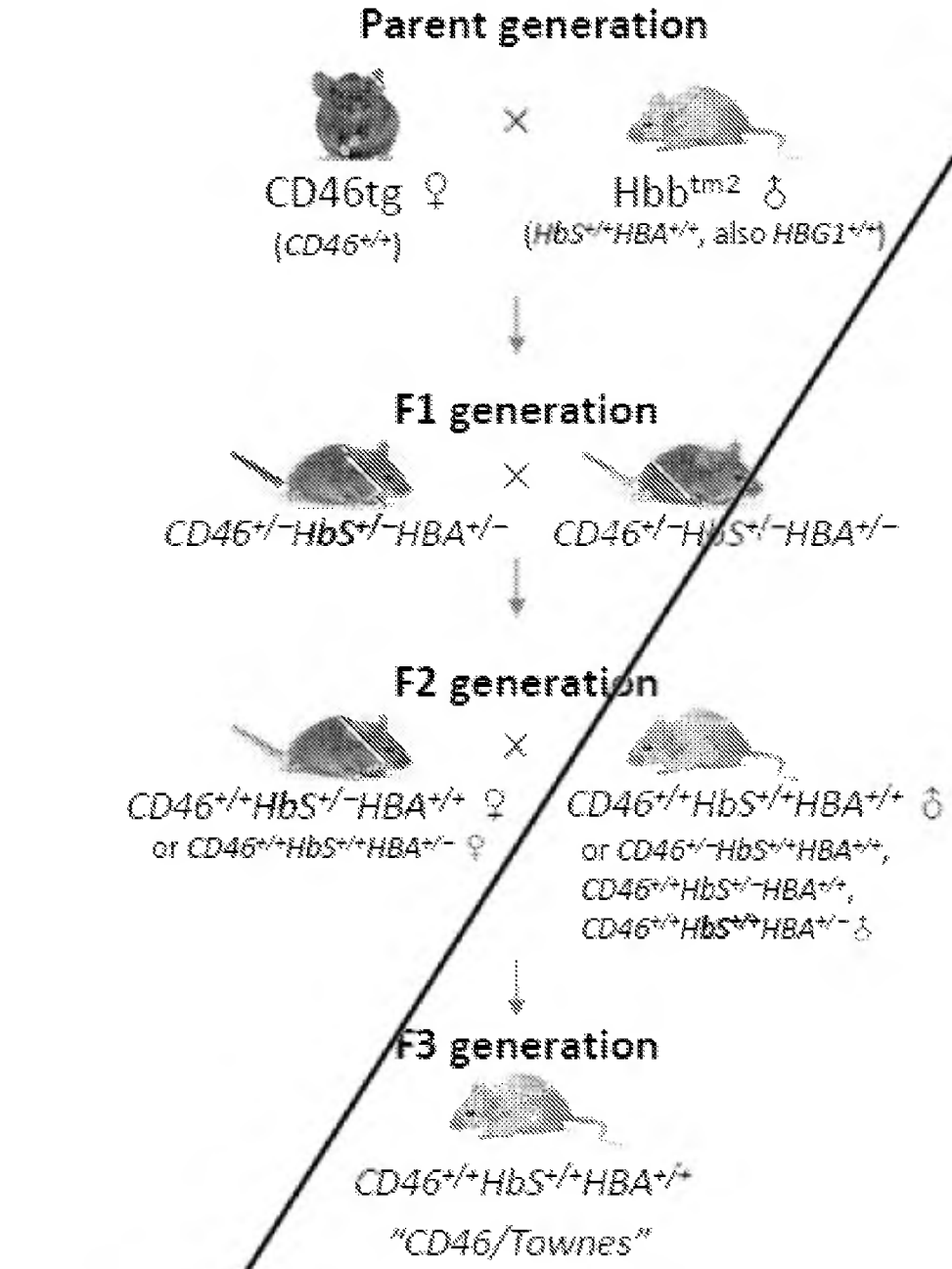

Parent generation
CD46tg ♀
(CD46+/+)
×
Hbbtm2 ♂
(HbS+/+HBA+/+, also HBG1+/+)
F1 generation
CD46+/−HbS+/−HBA+/−
×
CD46+/−HbS+/−HBA+/−
F2 generation
CD46+/+HbS+/−HBA+/+ ♀
or CD46+/+HbS+/+HBA+/− ♀
×
CD46+/+HbS+/+HBA+/+ ♂
or CD46+/−HbS+/+HBA+/+,
CD46+/+HbS+/−HBA+/+,
CD46+/+HbS+/+HBA+/− ♂
F3 generation
CD46+/+HbS+/+HBA+/+
"CD46/Townes"

FIG. 108B

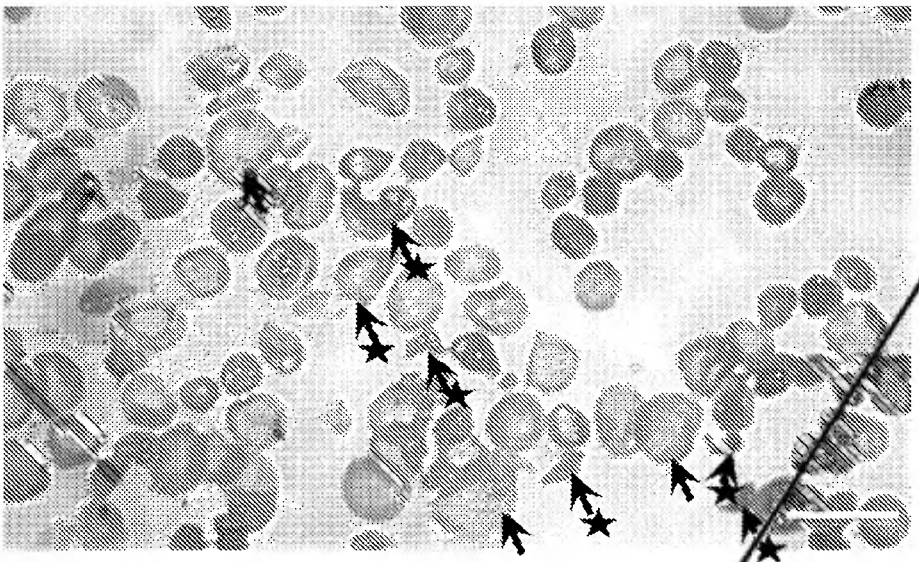


FIG. 108C

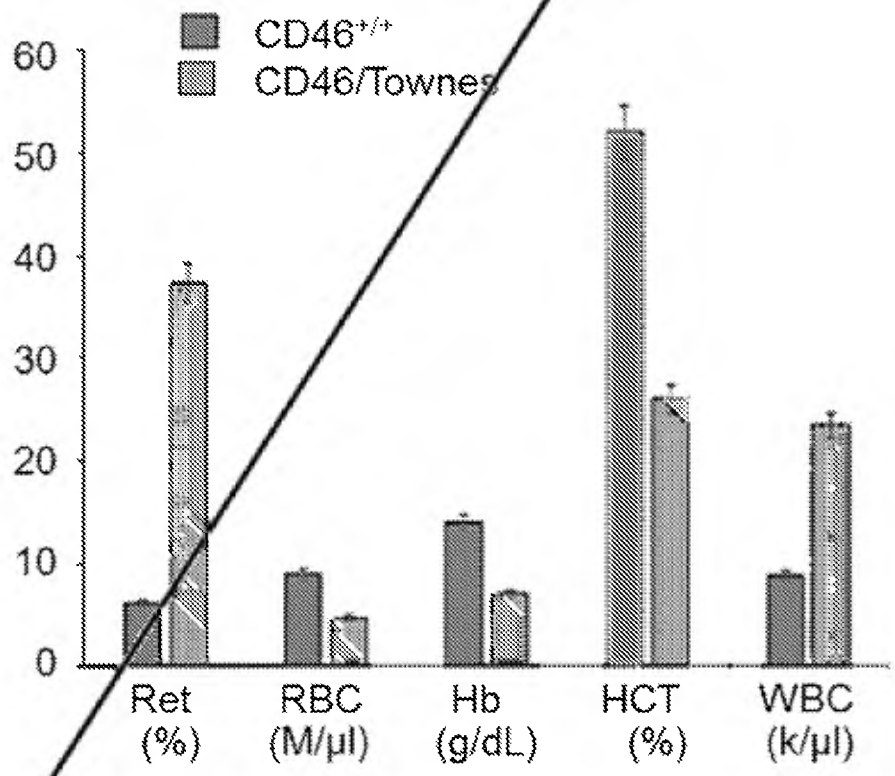


FIG. 108D

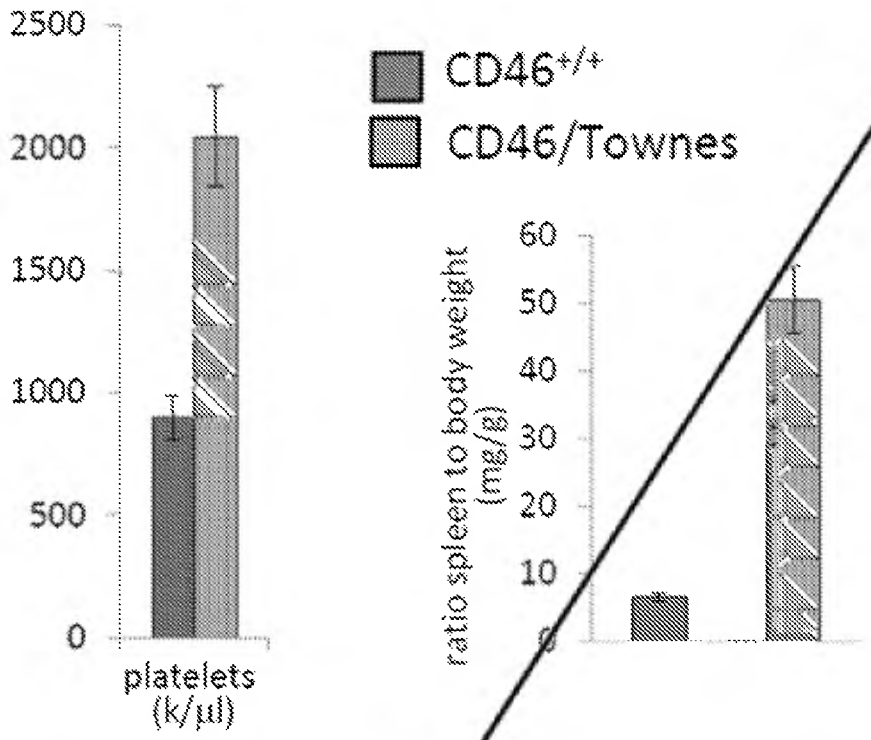
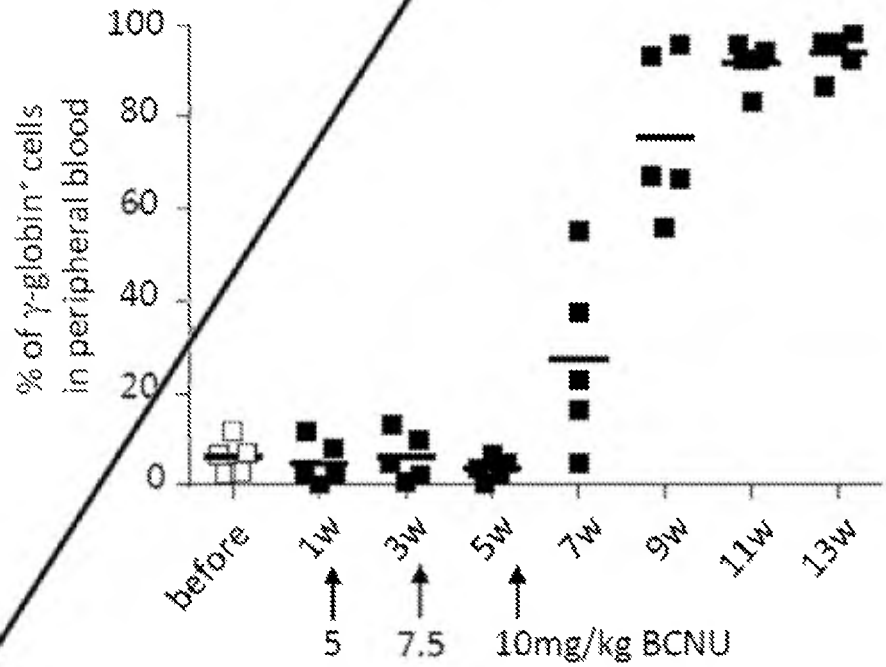


FIG. 109A



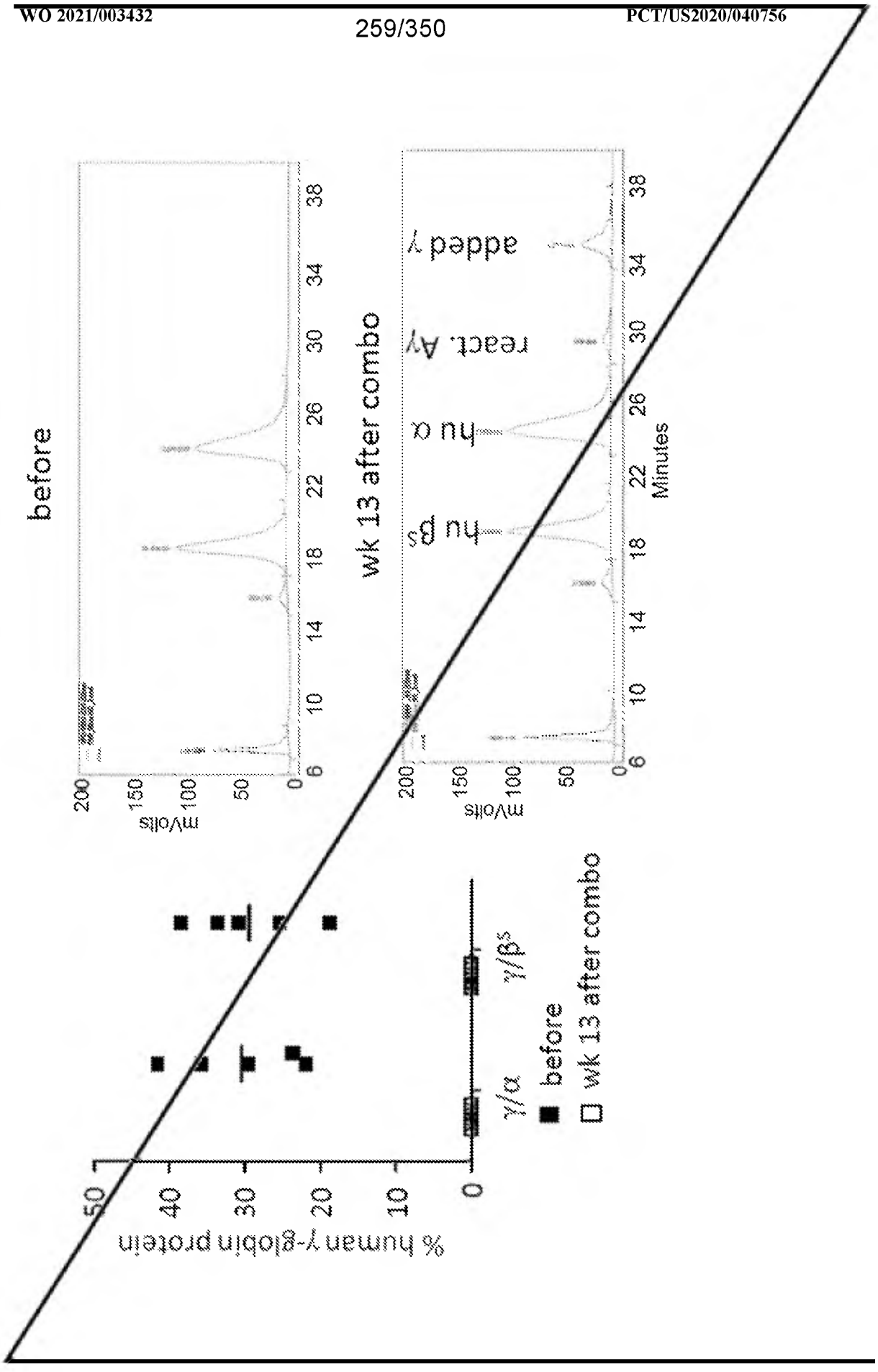

% human γ-globin protein
50
40
30
20
10
0
γ/α
γ/βS
before
wk 13 after combo
before
mVolts
200
150
100
50
0
6
10
14
18
22
26
30
34
38
wk 13 after combo
huβS
huα
react. Ay
added γ
Minutes

FIG. 109B

FIG. 109C

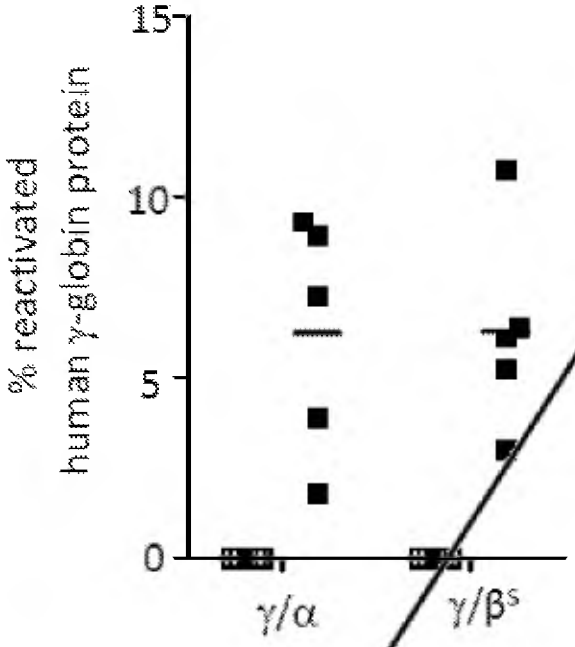


FIG. 109D

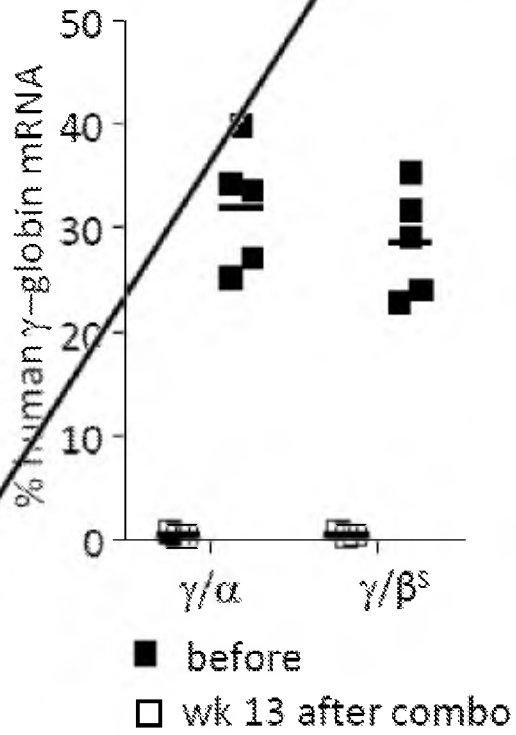


FIG. 109E

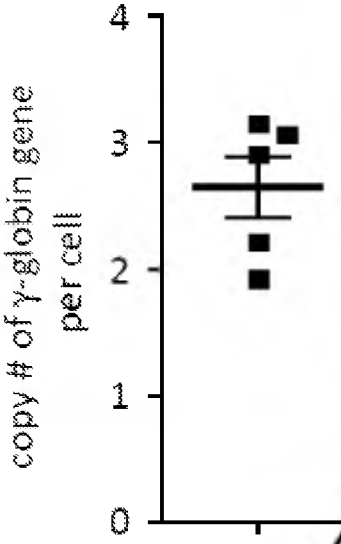


FIG. 109F

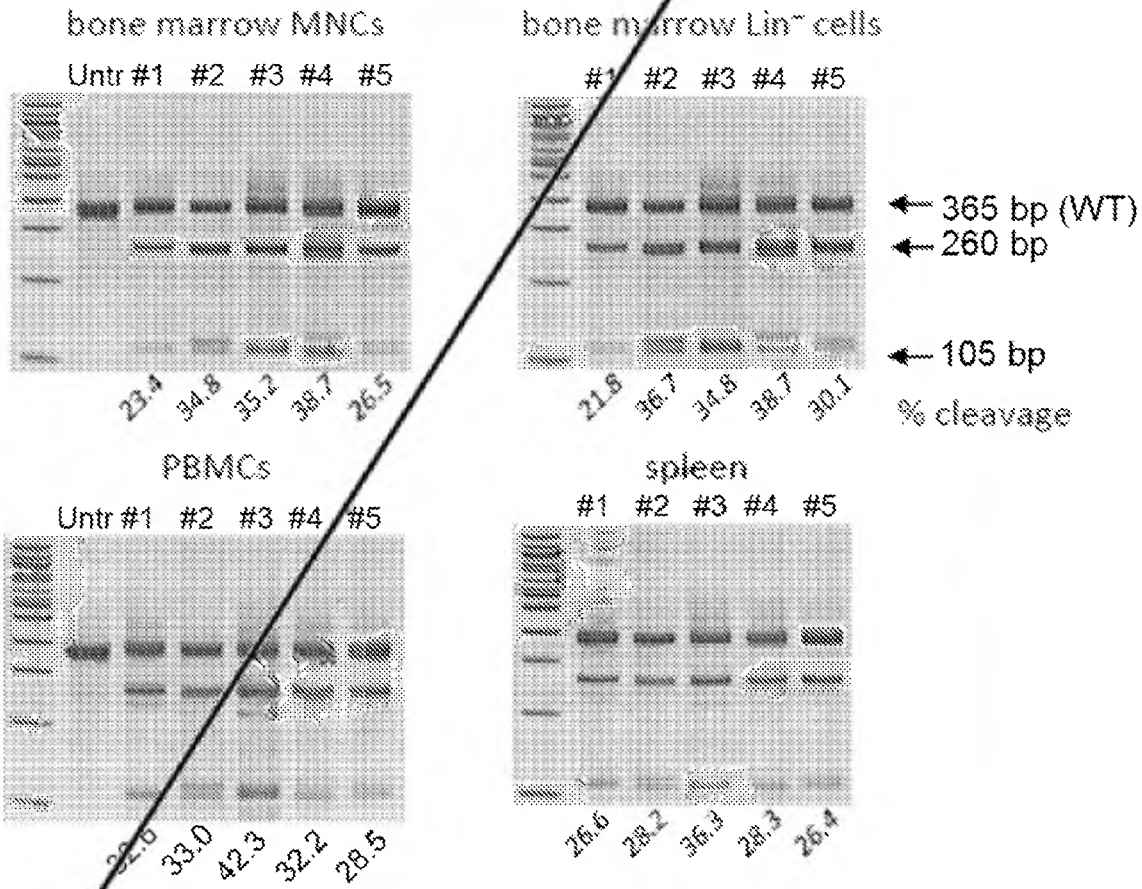


FIG. 110A

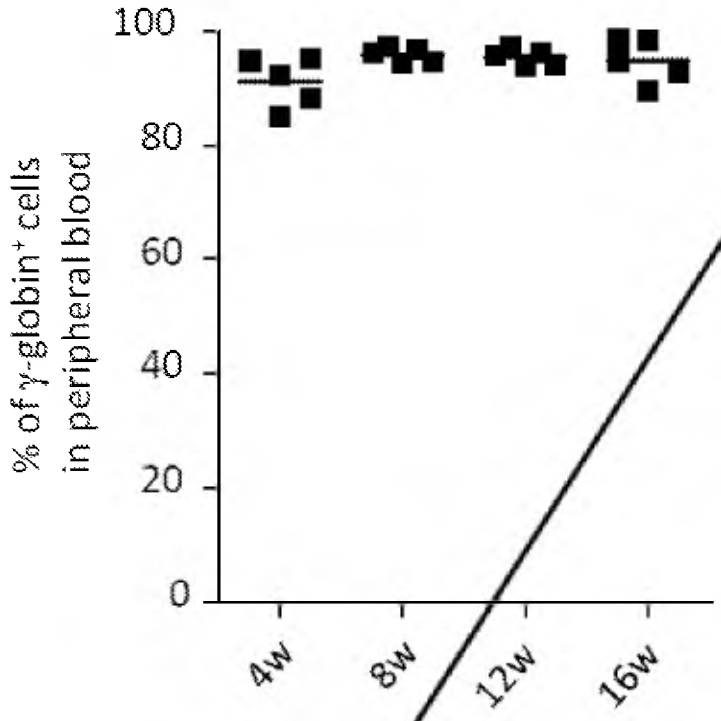
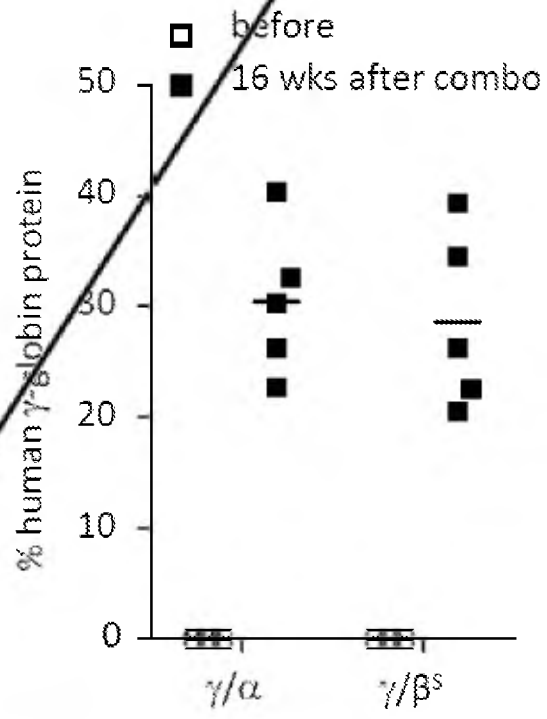


FIG. 110B



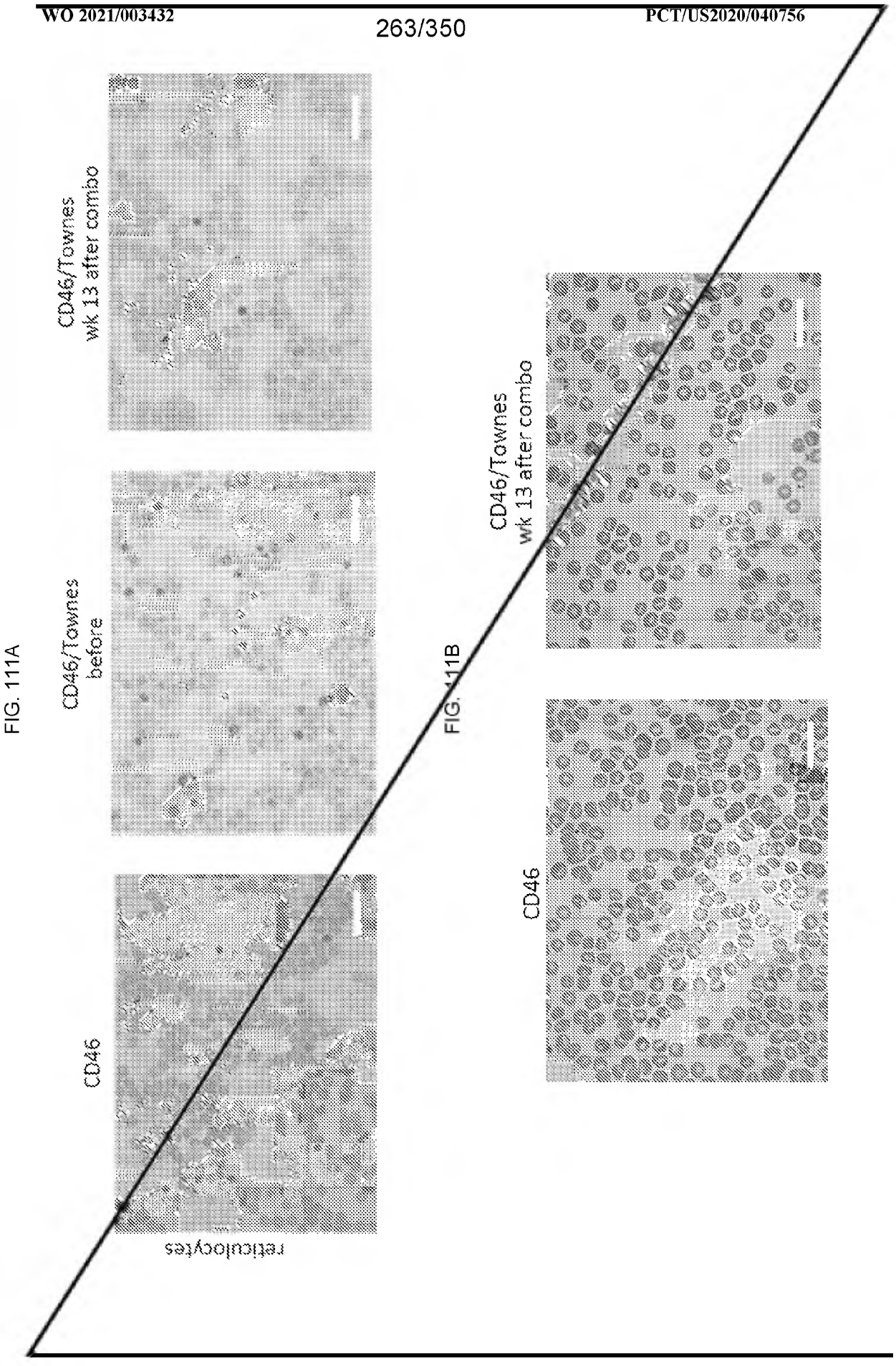


FIG. 111A

FIG. 111B

FIG. 111C

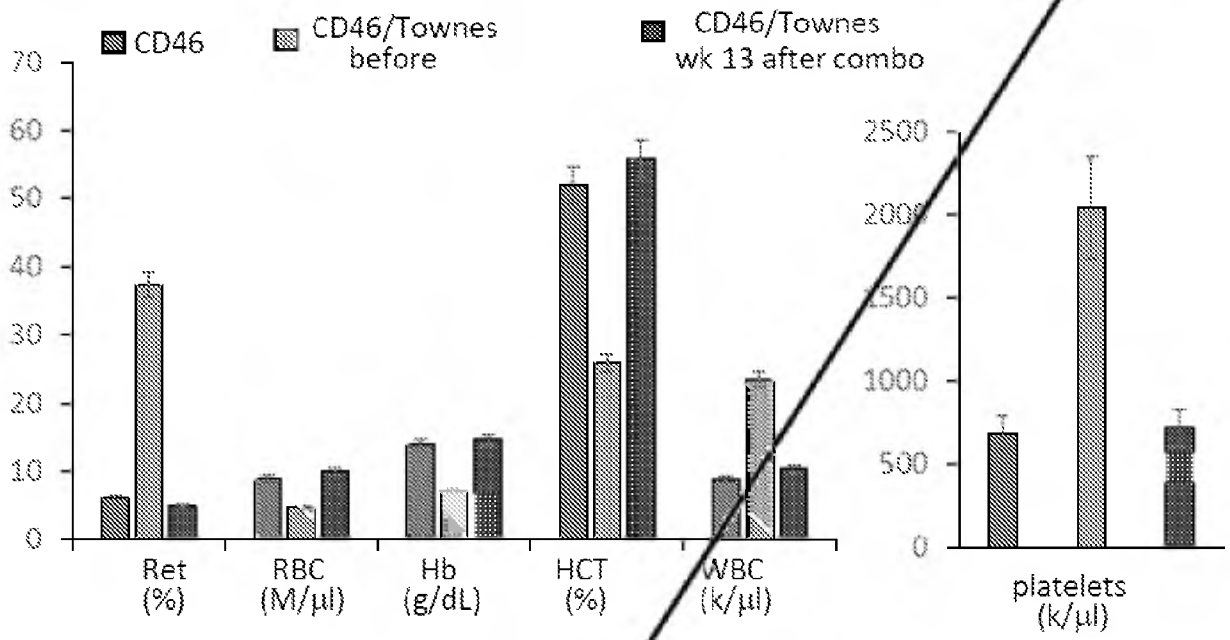

CD46
CD46/Townes before
CD46/Townes wk 13 after combo
70
60
50
40
30
20
10
0
Ret (%)
RBC (M/µl)
Hb (g/dL)
HCT (%)
WBC (k/µl)
2500
2000
1500
1000
500
0
platelets (k/µl)

FIG. 112A

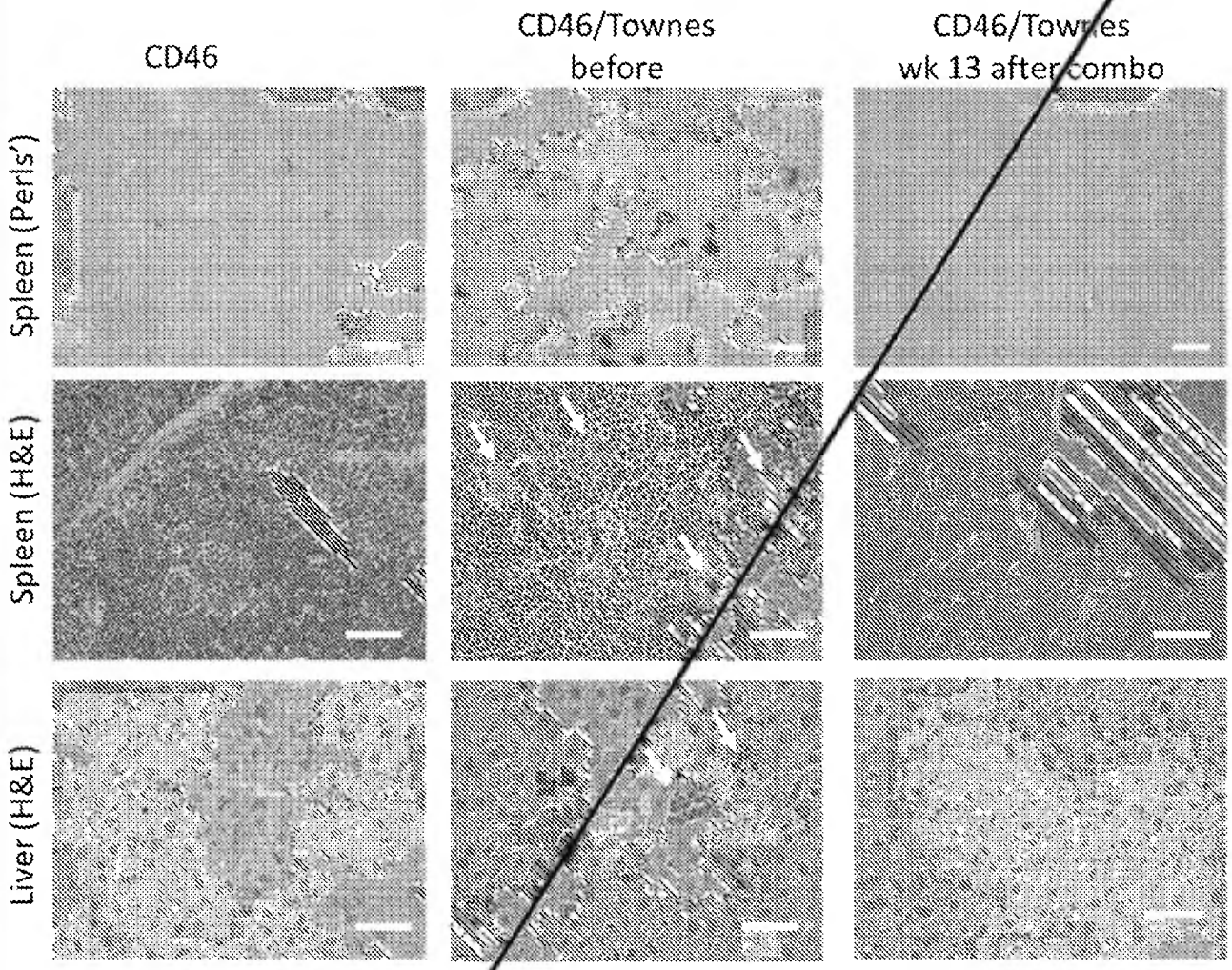
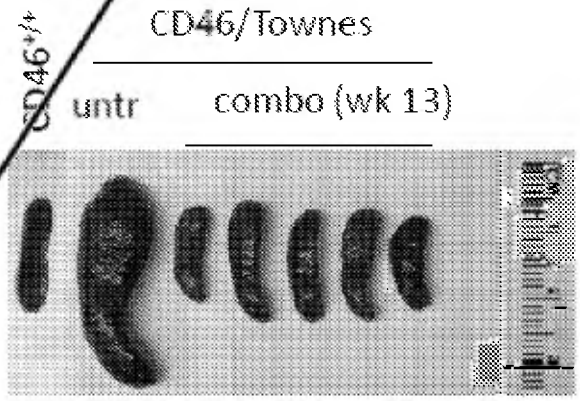


FIG. 112B



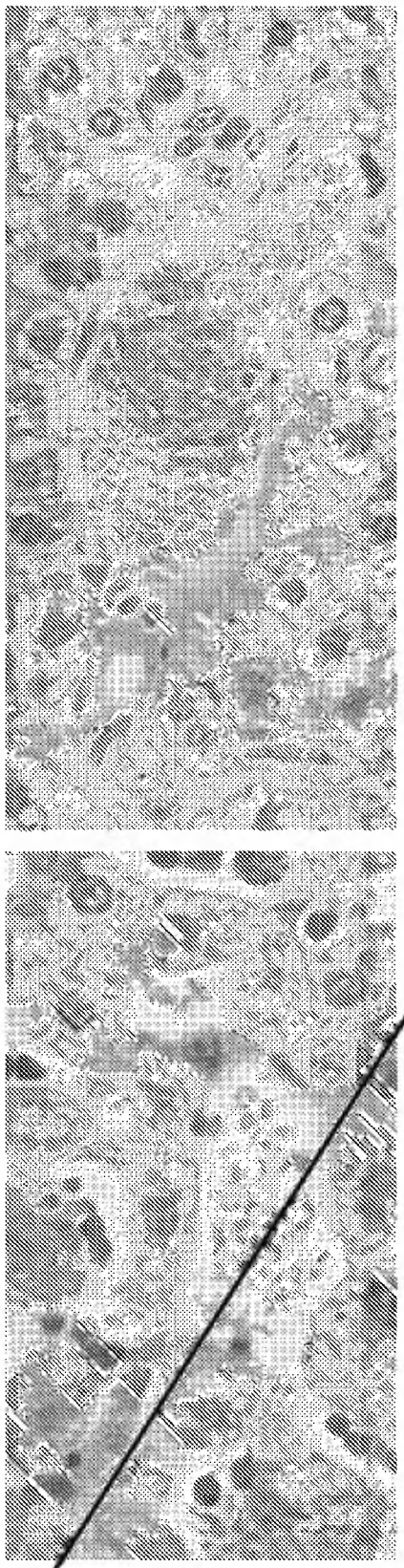
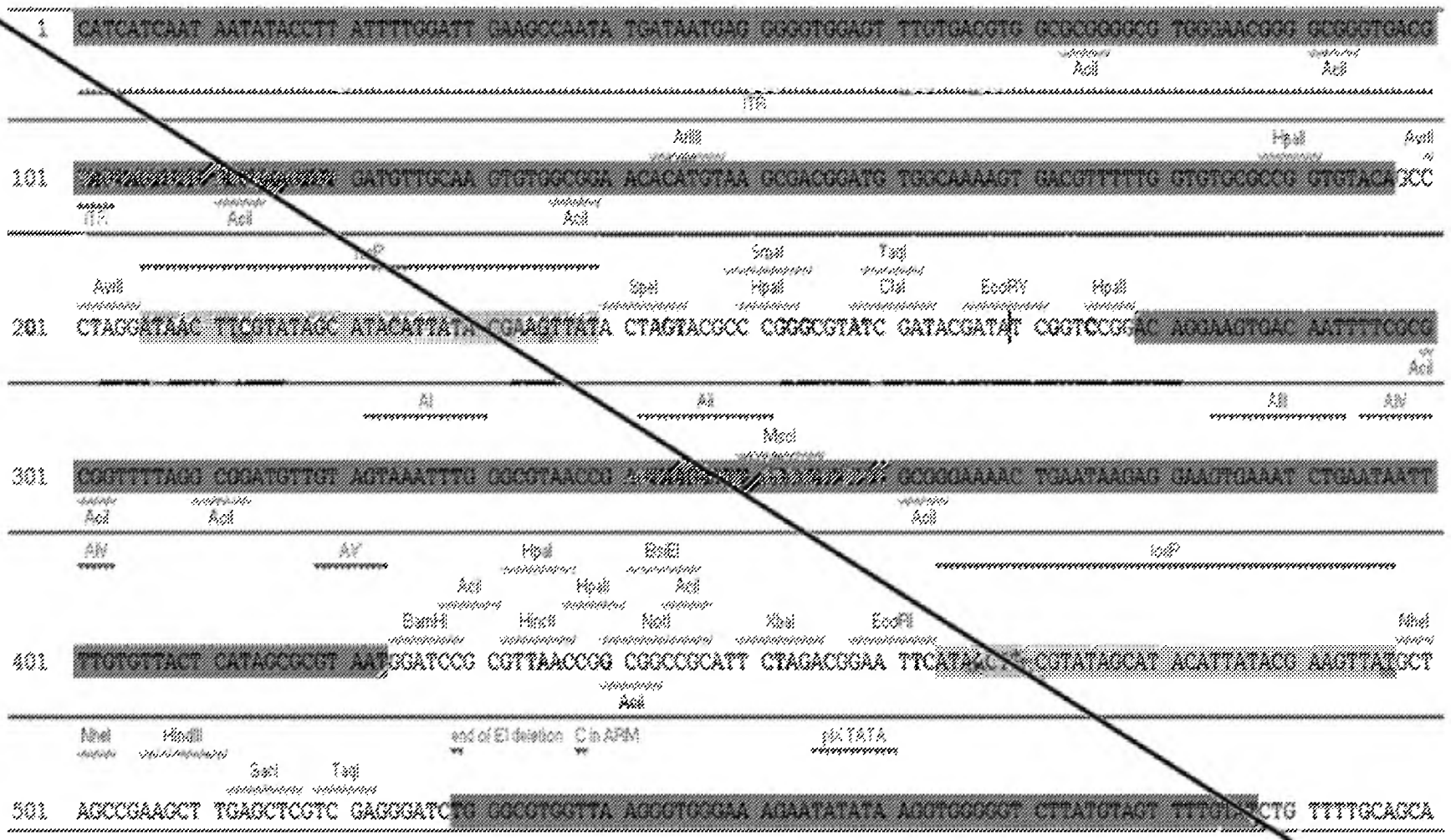


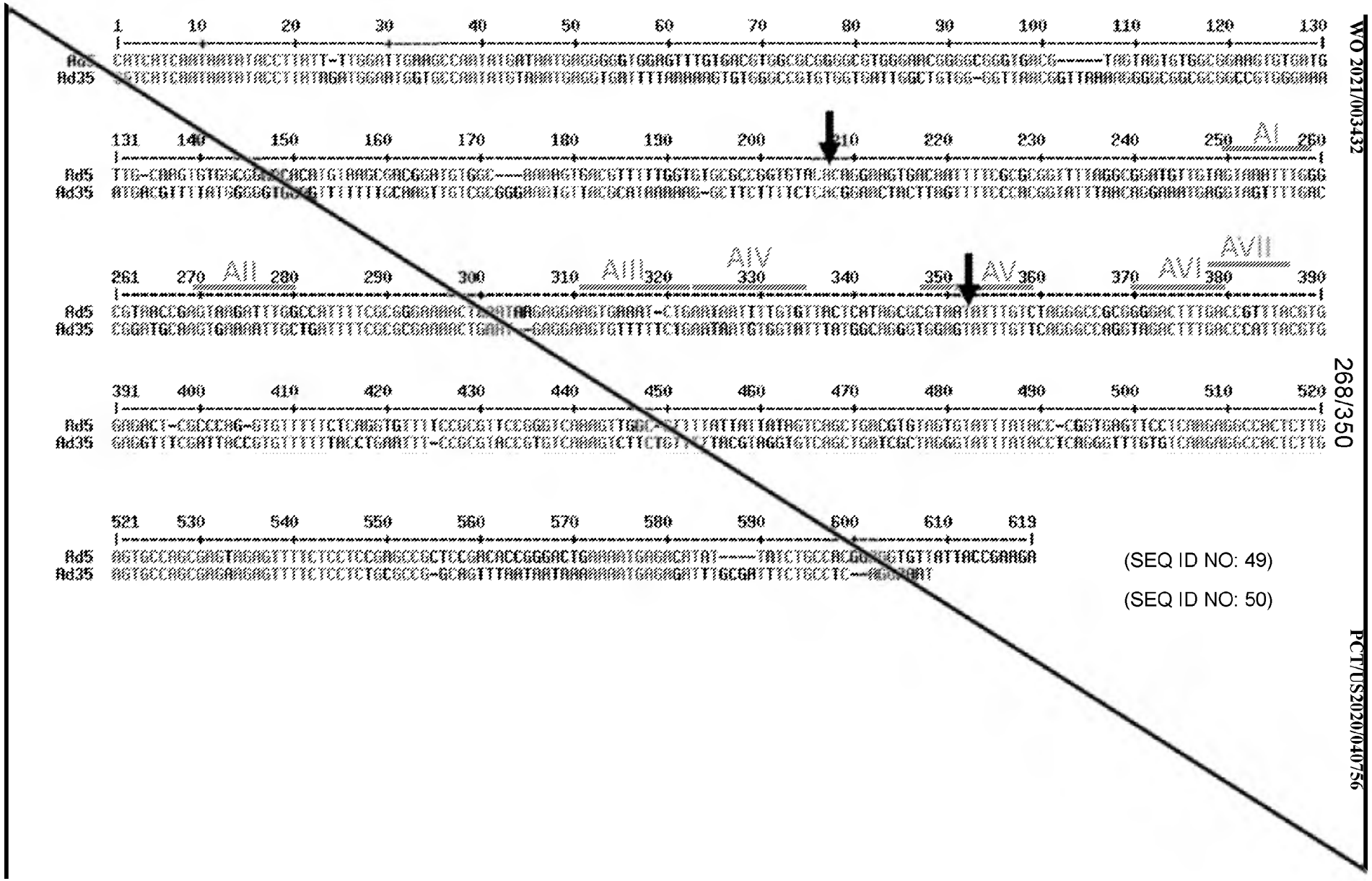
FIG. 112C

FIG. 113



SEQ ID NO: 262

FIG. 114



(SEQ ID NO: 49)

(SEQ ID NO: 50)

FIG. 115

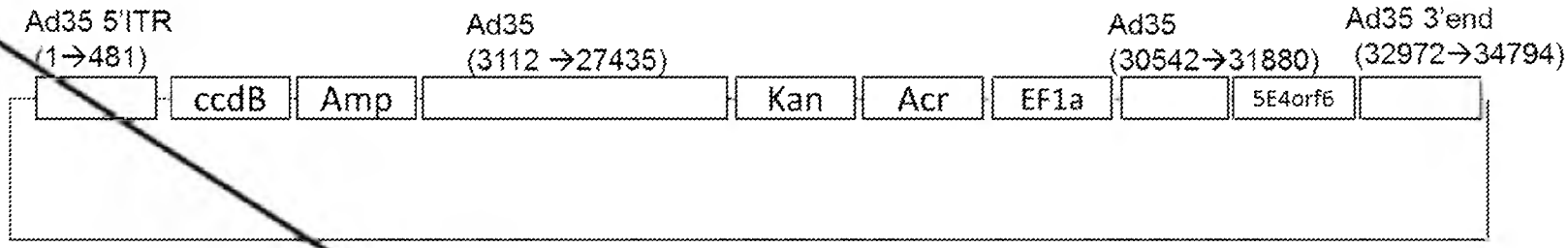


FIG. 116

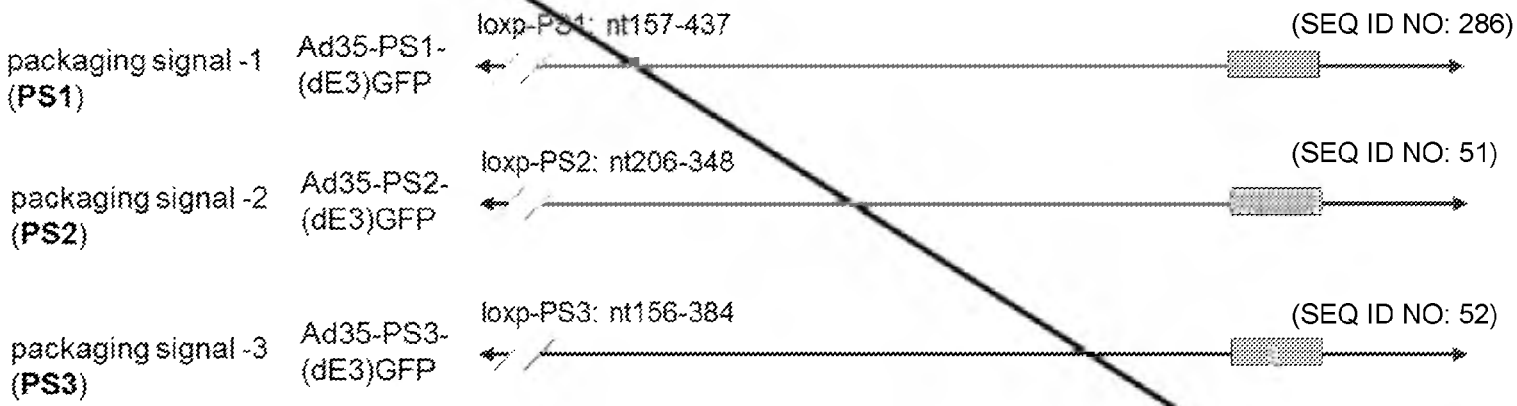


FIG. 117

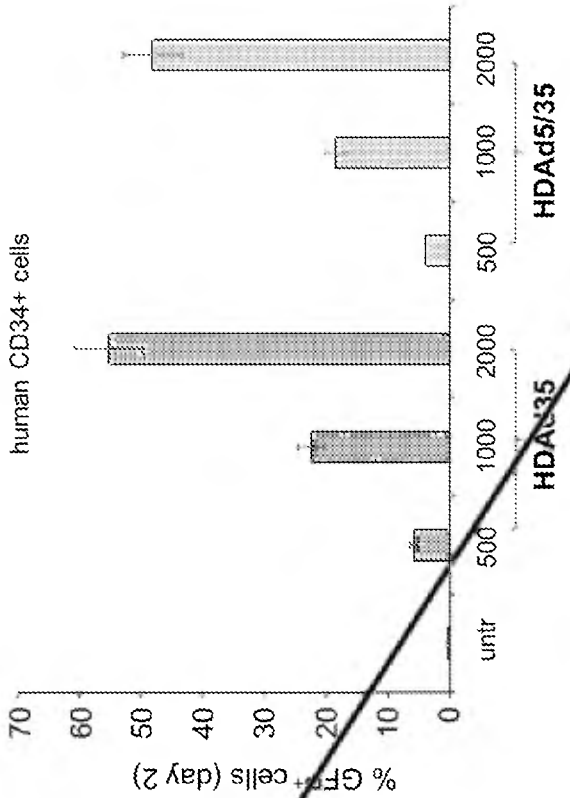


FIG. 118

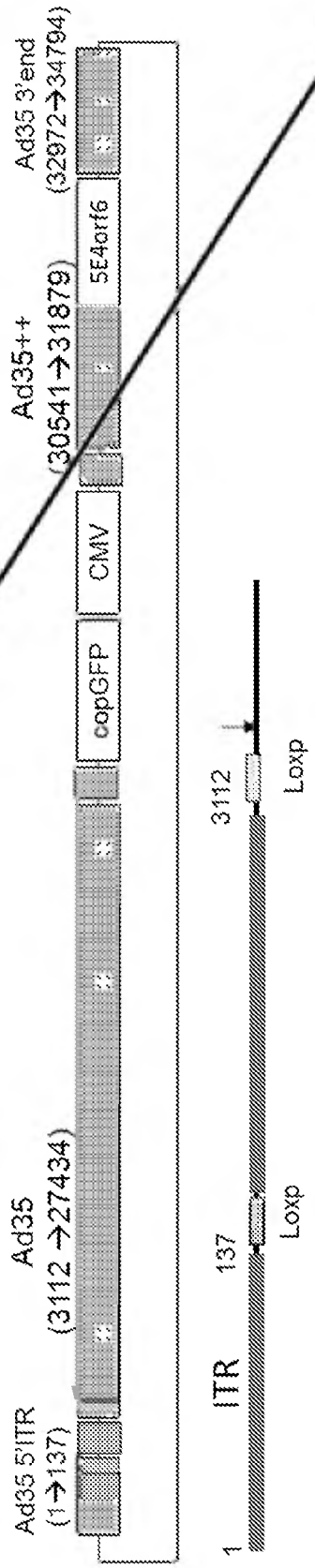


FIG. 119

CATCATCAATAATATACCTTATAGATGGAATGGTGCCAATATGTAAATGAGGTGATTTTAAAAAGTGTGGGCCGTGTGGTGATTGG
GTGTGGGGTTAACGGTTAAAAGGGGCGGCGCGGCCGTGGGAAAATGACGTT**ATTTAAAT**ATAACTTCGTATAGCATACATTATA
CGAAGTTAT<u>GAGGTAGTTTTGTTCAGGGGCAAGTGAAAATTGACCCATTACGCGCGAAAACTGAATGAGGAAGTGTTTTTCTGAA
TAATGTGGTATTTATGGCAGGGTGGAGTATTTGACCGGATCCAGGTAGACTTTGCTGATTTTCGTGGAGGTTT</u>*ATAACTTCGTATA
GCATACATTATACGAAGTTAT***ATTTAAAT*** (SEQ ID NO: 264)

Ad35 ITR 1→137bp: (positions 1-137 of SEQ ID NO: 264)

CATCATCAATAATATACCTTATAGATGGAATGGTGCCAATATGTAAATGAGGTGATTTTAAAAAGTGTGGGCCGTGT
GGTGATTGGCTGTGGGGTTAACGGTTAAAAGGGGCGGCGCGGCCGTGGGAAAATGACGTT

SwaI site: (positions 146-145 and 363-370 of SEQ ID NO: 264) ATTTAAAT

LoxP site: (positions 138-179 and 329-362 of SEQ ID NO: 264) ATAACTTCGTATAGCATACATTATACGAAGTTAT

Mutated packaging signal: (positions 138-145 of SEQ ID NO: 264)

GAGGTAGTTTTGTTCAGGGGCAAGTGAAAATTGACCCATTACGCGCGAAAACTGAATGAGGAAGTGTTTTTCTGAAT
AATGTGGTATTTATGGCAGGGTGGAGTATTTGACCGGATCCAGGTAGACTTTGCTGATTTTCGTGGAGGTTT

FIG. 120A

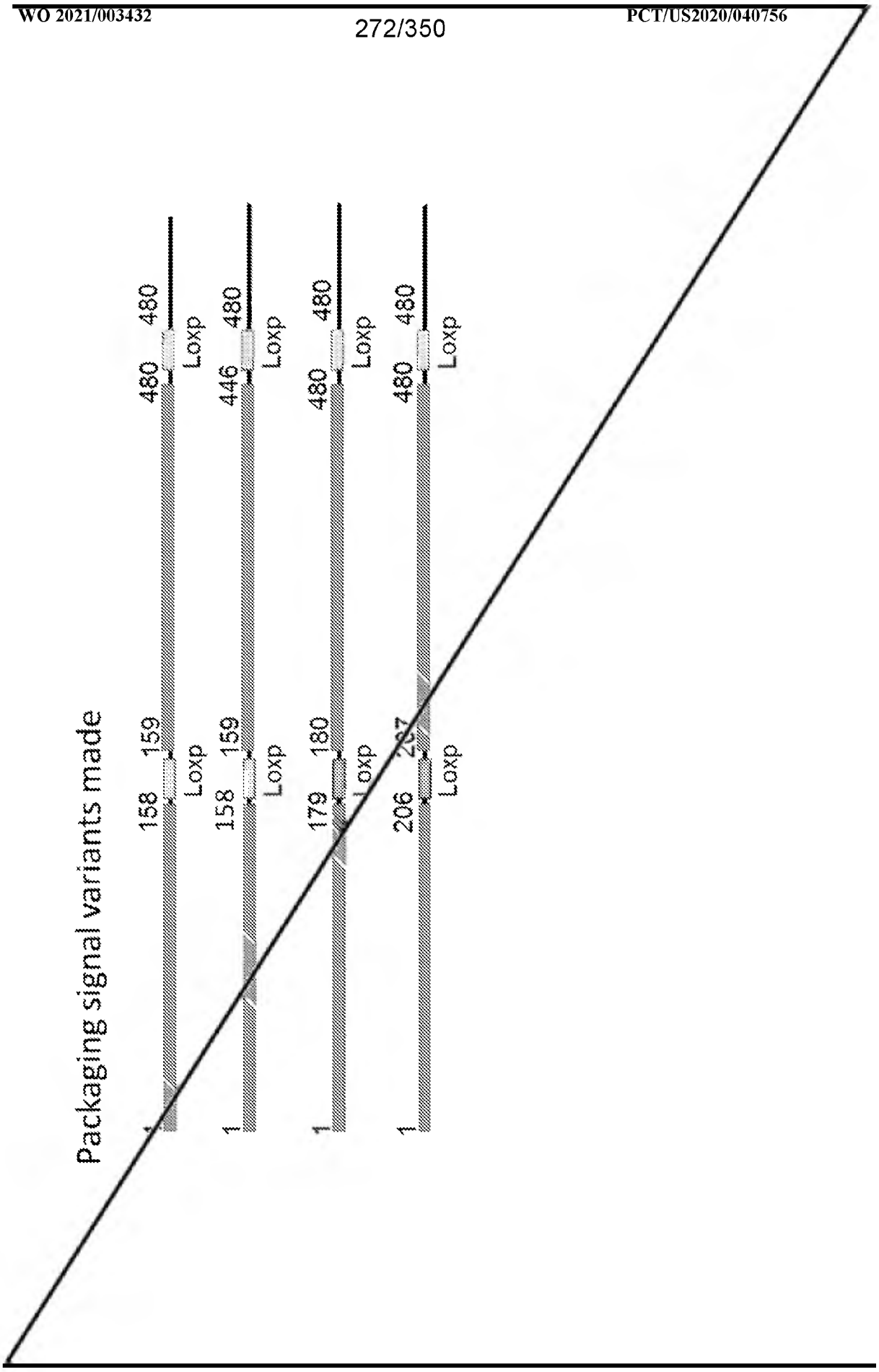
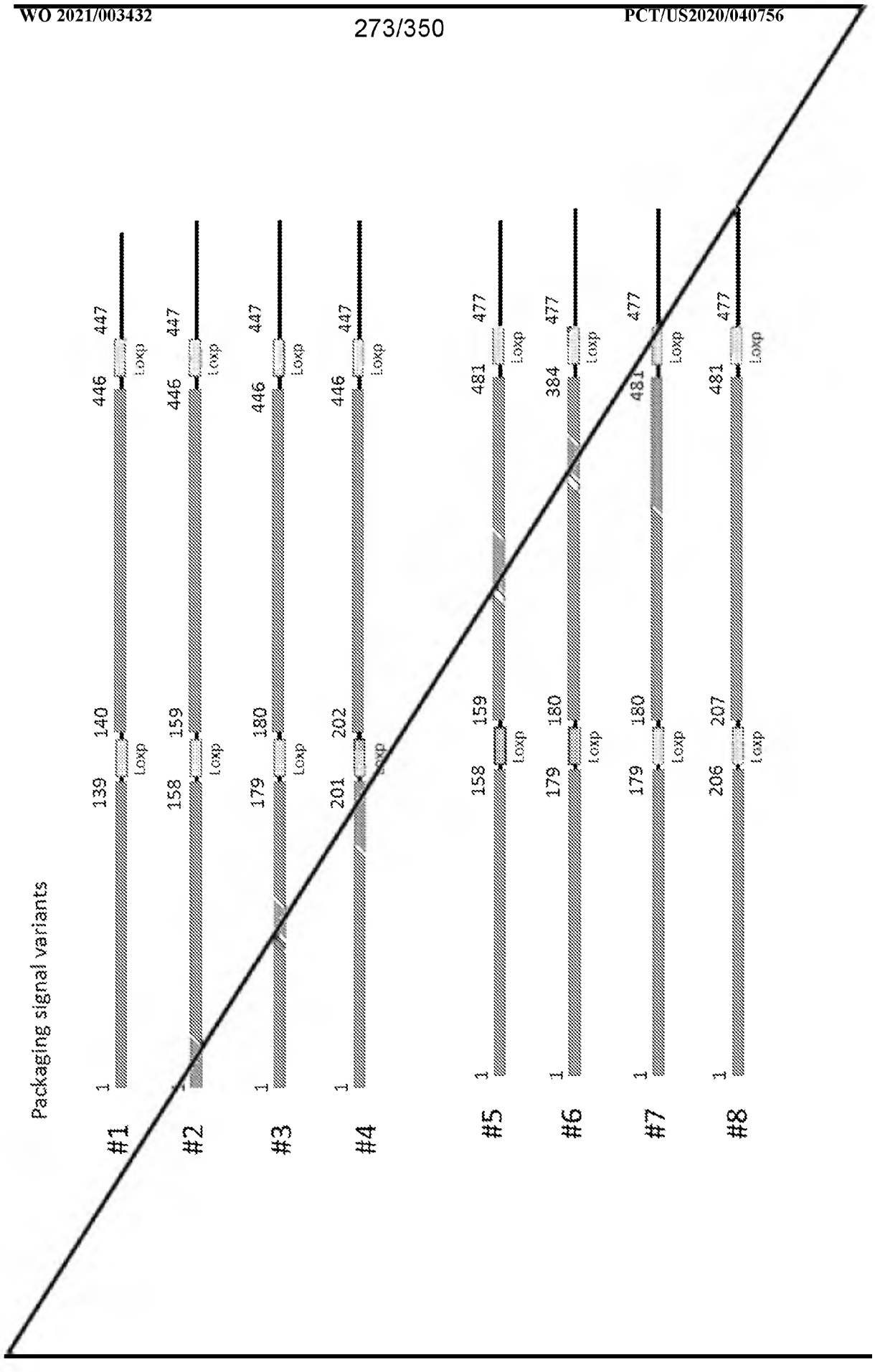

Packaging signal variants made
1
158
159
Loxp
480
480
1
158
159
Loxp
446
480
1
179
180
Loxp
480
480
1
206
227
Loxp
480
480

FIG. 120B


Packaging signal variants
#1
#2
#3
#4
#5
#6
#7
#8
1
139
140
446
447
loxp
158
159
179
180
201
202
481
477
384

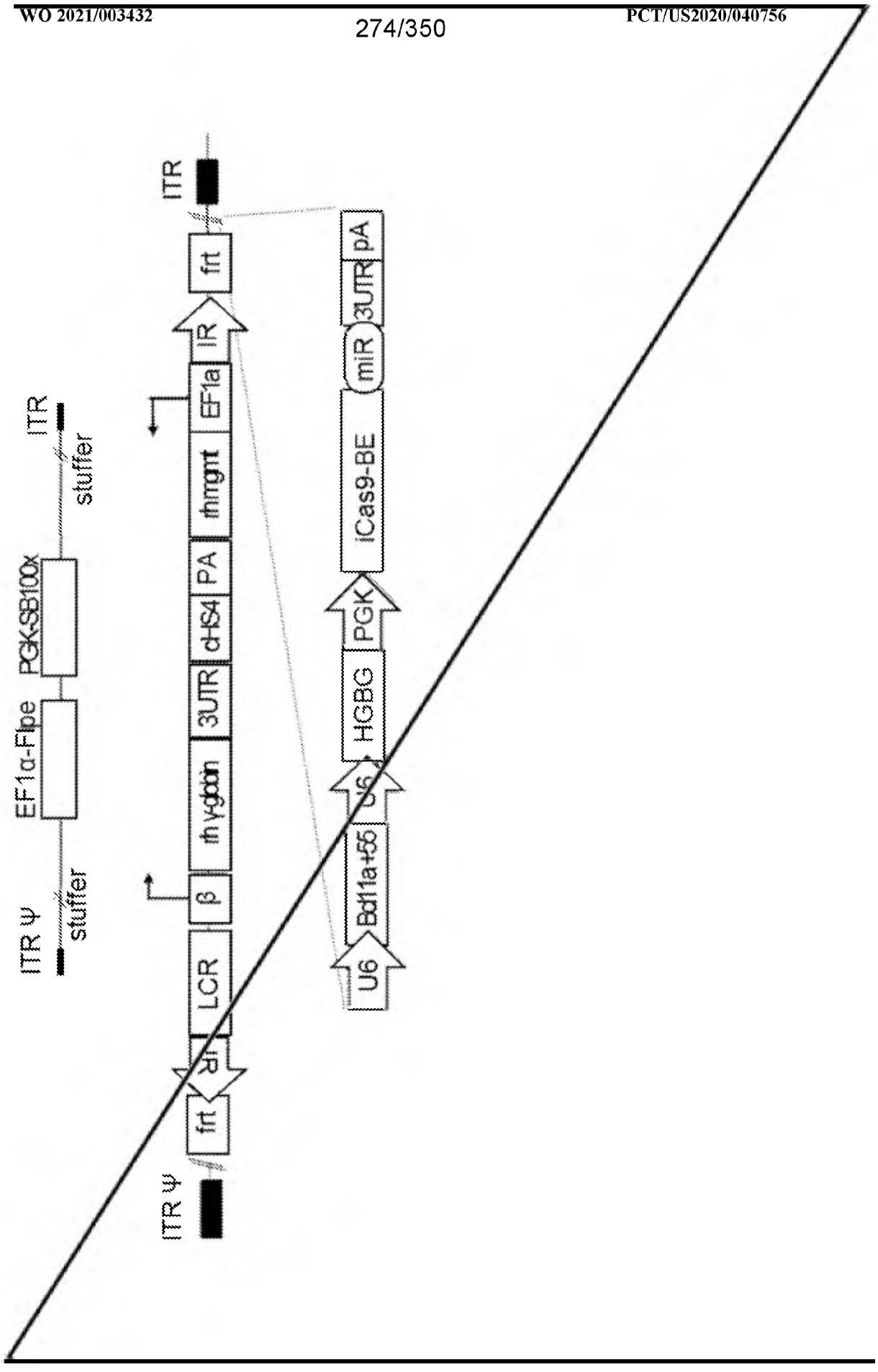

ITR Ψ
stuffer
EF1α-Flpe
PGK-SB100x
stuffer
ITR
ITR Ψ
frt
IR
LCR
β
hygro
3'UTR
cHS4
PA
mmgmt
EF1a
IR
frt
ITR
U6
Bcl11a+55
HGBG
PGK
iCas9-BE
miR
3'UTR
pA

FIG. 121

FIG. 122

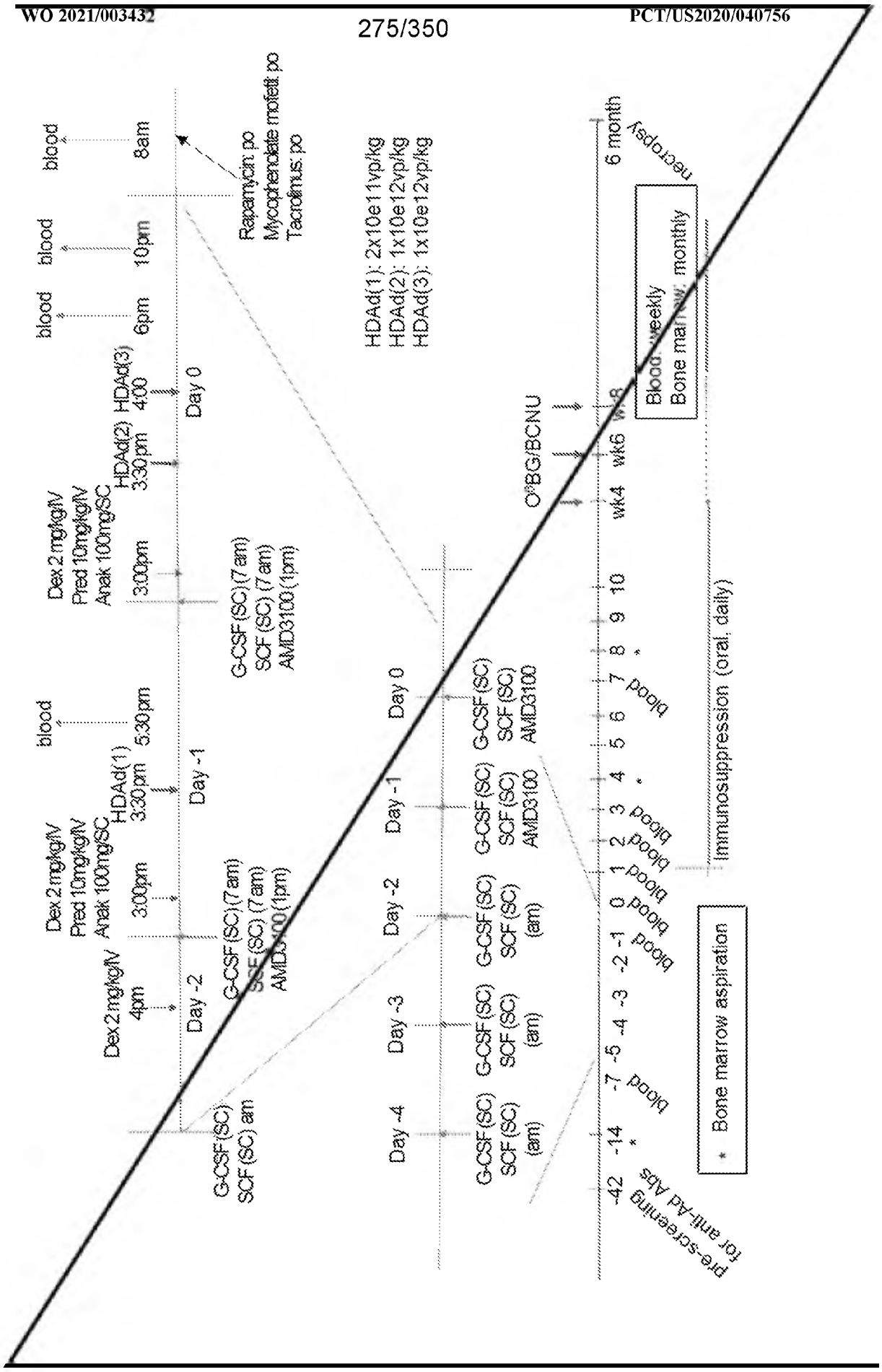

Dex 2 mg/kg/IV
4pm
Day -2
G-CSF (SC)
SCF (SC) am
Dex 2 mg/kg/IV
Pred 10mg/kg/IV
Anak 100mg/SC
3:00pm
HDAd(1)
3:30pm
5:30pm
blood
Day -1
G-CSF (SC)(7 am)
SCF (SC) (7 am)
AMD3100 (1pm)
Day -4
Day -3
Day -2
Day -1
Day 0
G-CSF (SC)
SCF (SC)
(am)
G-CSF (SC)
SCF (SC)
AMD3100
Dex 2 mg/kg/IV
Pred 10mg/kg/IV
Anak 100mg/SC
3:00pm
HDAd(2)
3:30pm
HDAd(3)
4:00
6pm
10pm
8am
blood
Day 0
G-CSF (SC)(7 am)
SCF (SC) (7 am)
AMD3100 (1pm)
Rapamycin po
Mycophenolate mofetil po
Tacrolimus po
HDAd(1): 2x10e11vp/kg
HDAd(2): 1x10e12vp/kg
HDAd(3): 1x10e12vp/kg
O6BG/BCNU
-42
-14
-7
-5
-4
-3
-2
-1
0
1
2
3
4
5
6
7
8
9
10
wk4
wk6
wk8
6 month
necropsy
Pre-screening for anti-Ad Abs
blood
Blood: weekly
Bone marrow: monthly
Immunosuppression (oral, daily)
* Bone marrow aspiration

FIG. 123

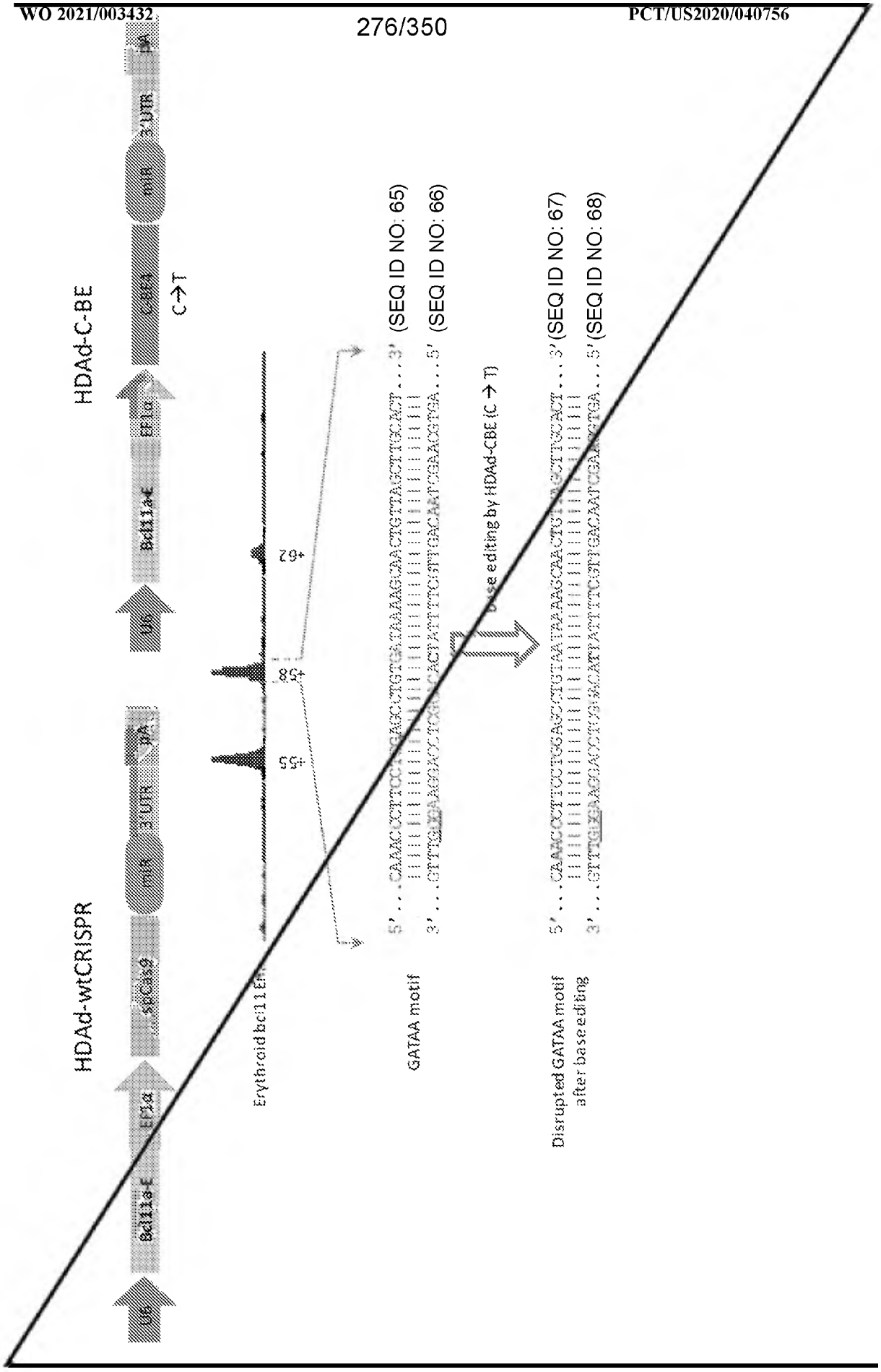

HDAd-wtCRISPR
HDAd-C-BE
UE
BclI1a-E
EF1a
SpCas9
miR
3'UTR
pA
BclI1a-E
EF1a
C-BEA
miR
3'UTR
pA
C→T
Erythroid bcl11 En
+55
+58
+62
GATAA motif
5'...CAAACCTTCCTGAAGCTTGTGATAAAAGCAACTGTTAAGCTTGCACT...3' (SEQ ID NO: 65)
3'...GTTTGGAAGGACTTCGAACACTATTTTCGTTGACAATTCGAACGTGA...5' (SEQ ID NO: 66)
Base editing by HDAd-CBE (C → T)
Disrupted GATAA motif after base editing
5'...CAAACCTTCCTGAAGCTTGTAATAAAAGCAACTGTTAAGCTTGCACT...3' (SEQ ID NO: 67)
3'...GTTTGGAAGGACTTCGAACATTATTTTCGTTGACAATTCGAACGTGA...5' (SEQ ID NO: 68)

FIG. 124A

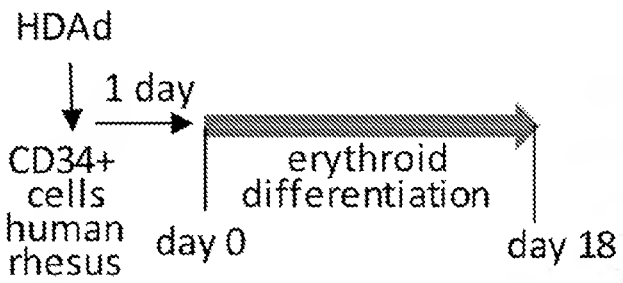


FIG. 124B

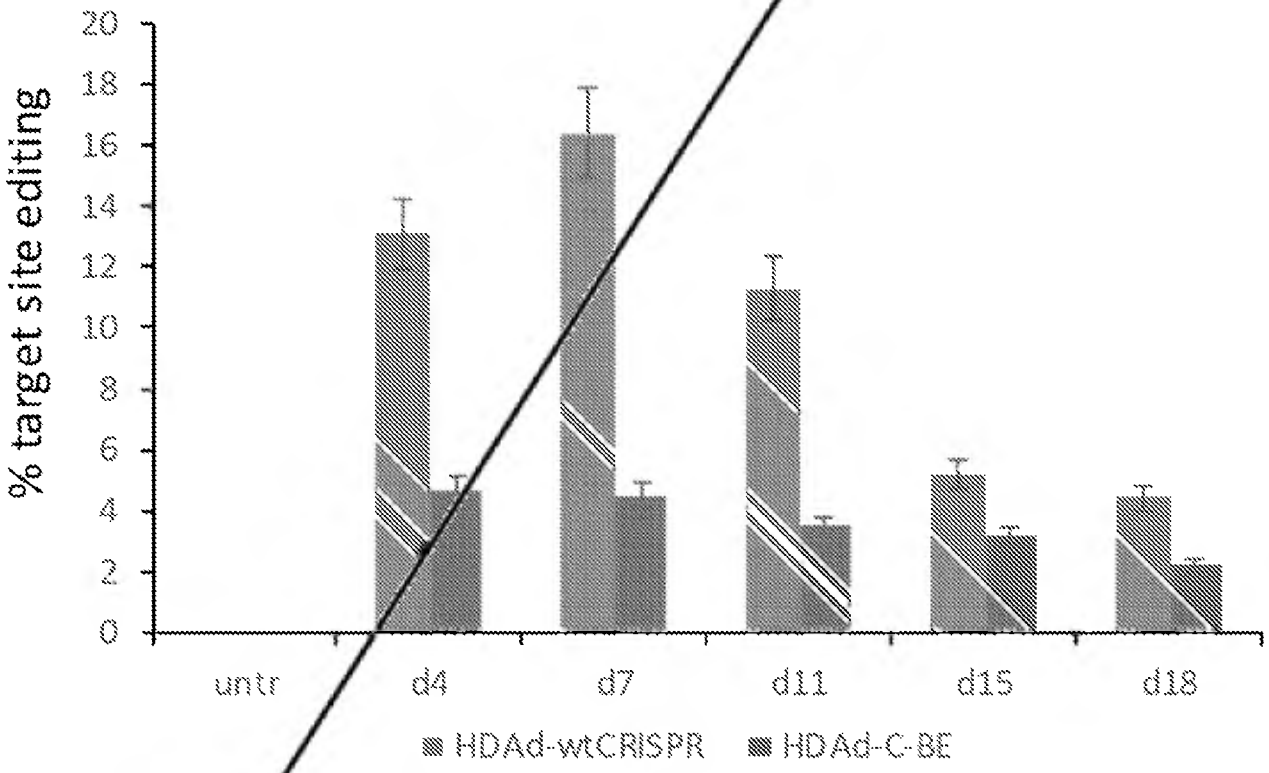


FIG. 124C

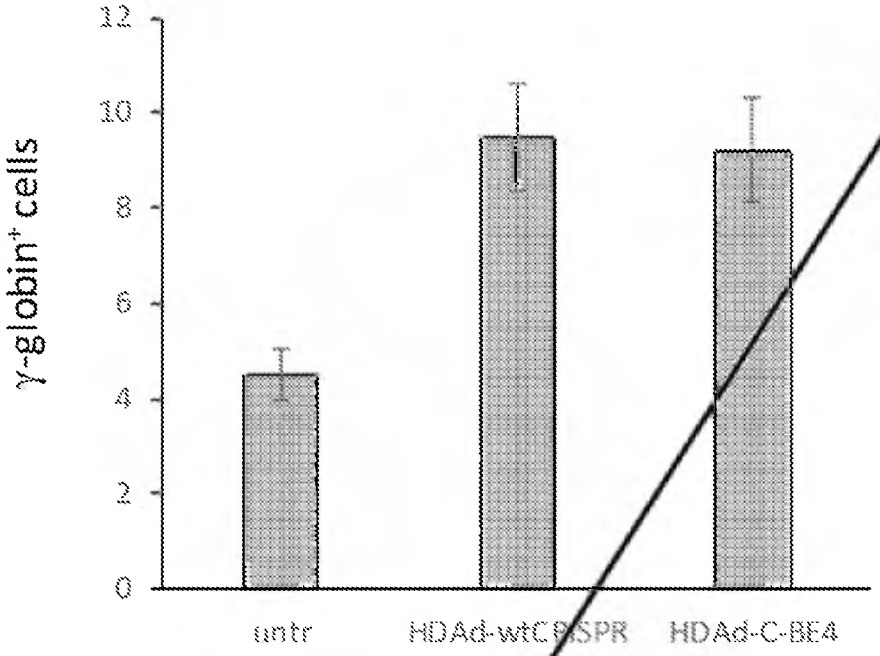


FIG. 125

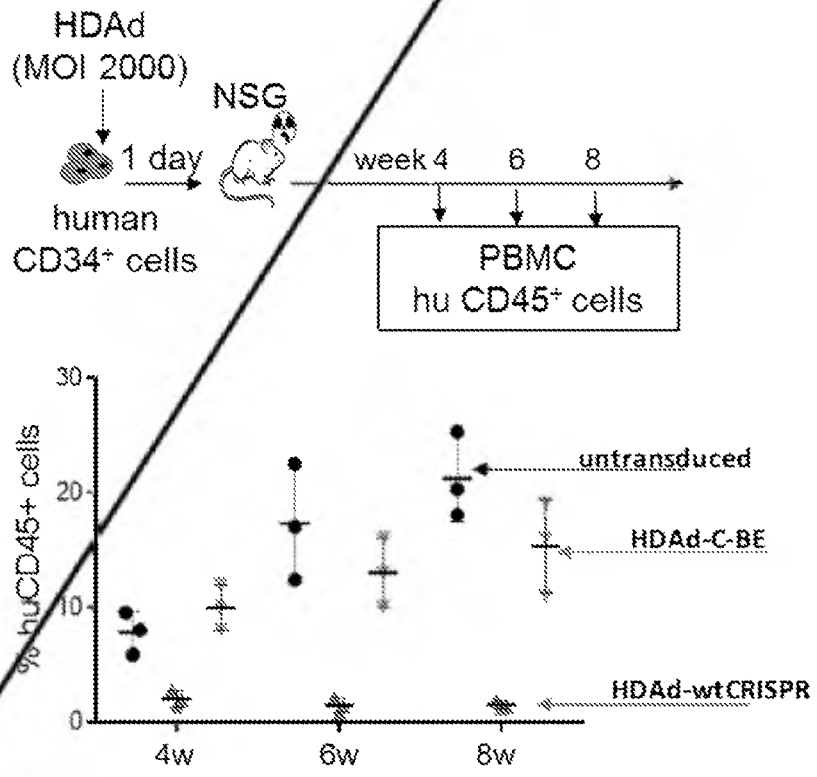


FIG. 126

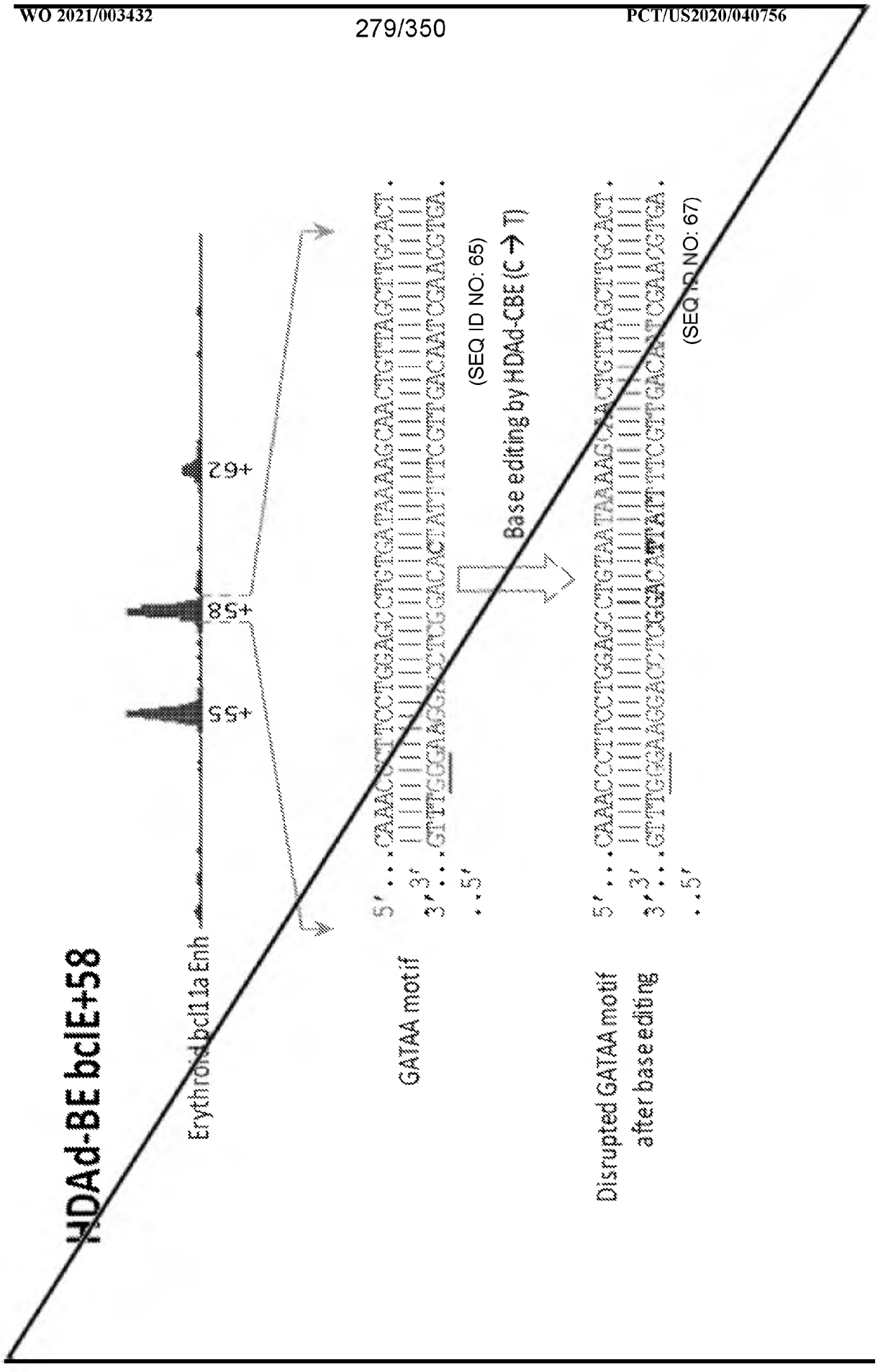

HDAd-BE bclE+58
Erythroid bcl11a Enh
+55
+58
+62
GATAA motif
5' . . . CAAACCTTCCTGGAGCCTGTGATAAAGCAACTGTTAGCTTGCACT .
3' . . . GTTTGGAAGGACCTCGACACTATTTCGTTGACAATCGAACGTGA .
. . 5'
(SEQ ID NO: 65)
Base editing by HDAd-CBE (C → T)
Disrupted GATAA motif
after base editing
5' . . . CAAACCTTCCTGGAGCCTGTAATAAAGCAACTGTTAGCTTGCACT .
3' . . . GTTTGGAAGGACCTCGACATTATTTCGTTGACAATCGAACGTGA .
. . 5'
(SEQ ID NO: 67)

FIG. 126 cont'd

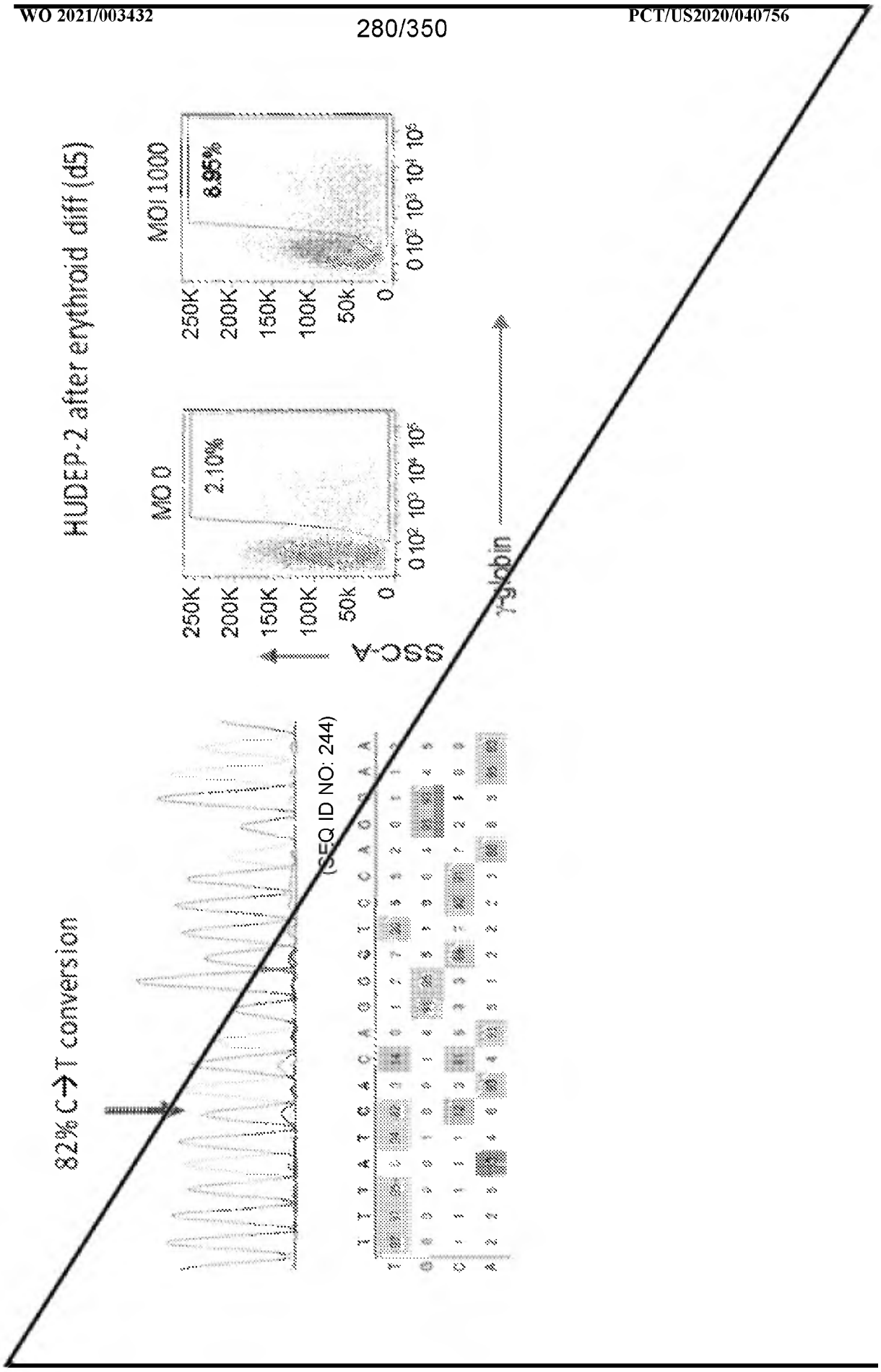
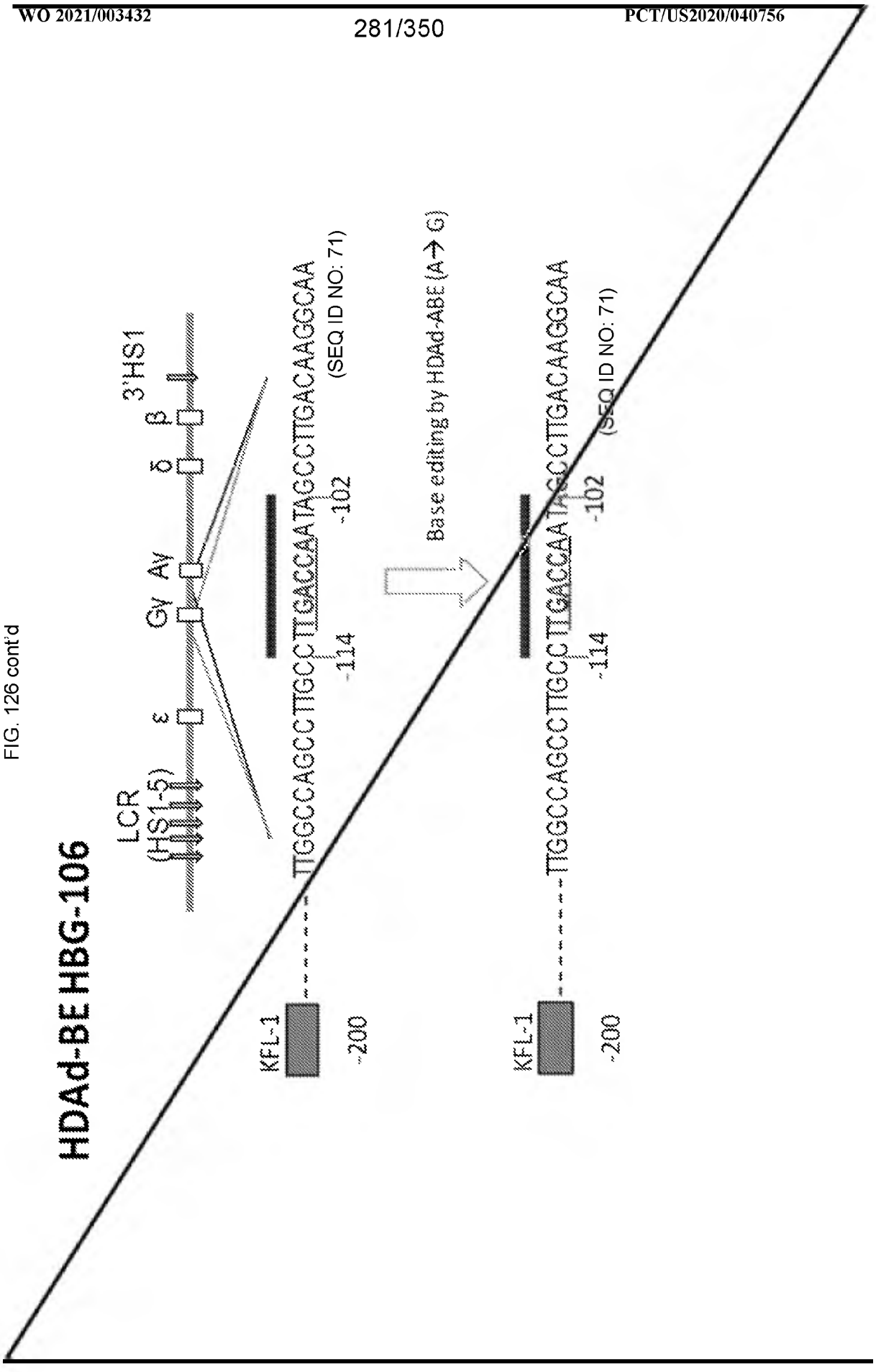


FIG. 126 cont'd



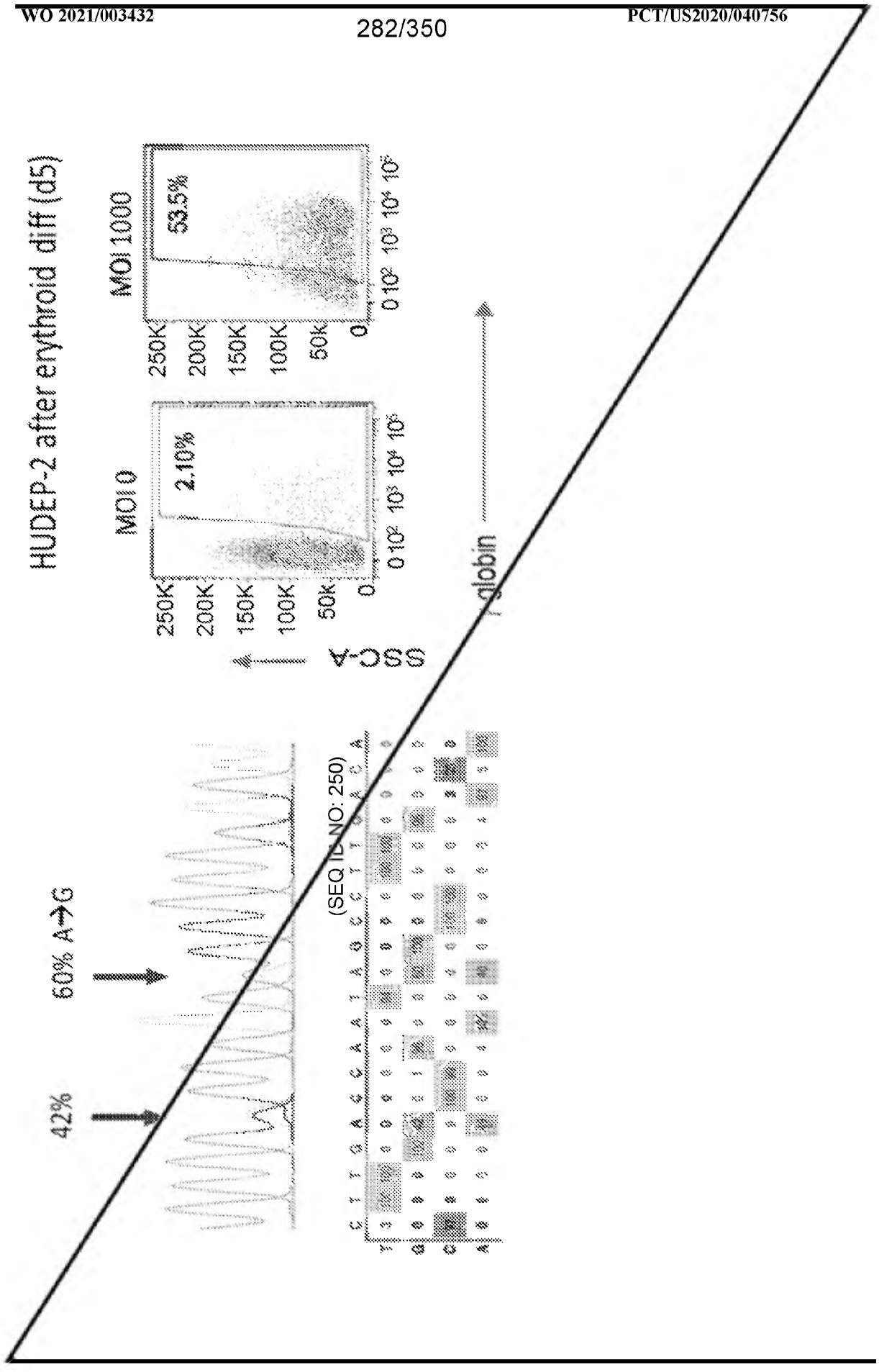


FIG. 126 cont'd

FIG.127

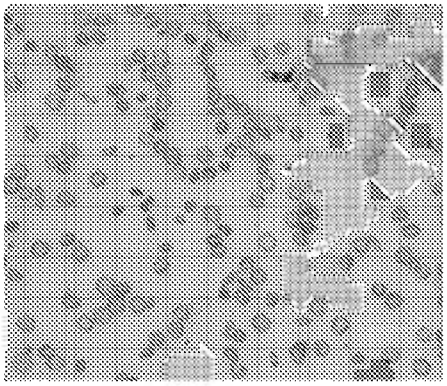


FIG. 127B

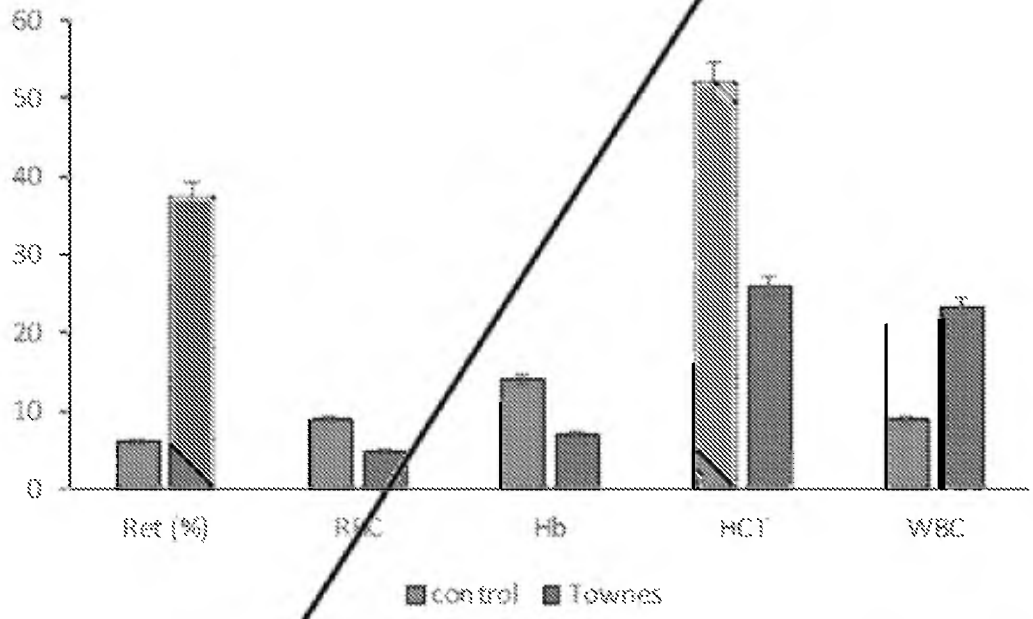


FIG. 128A

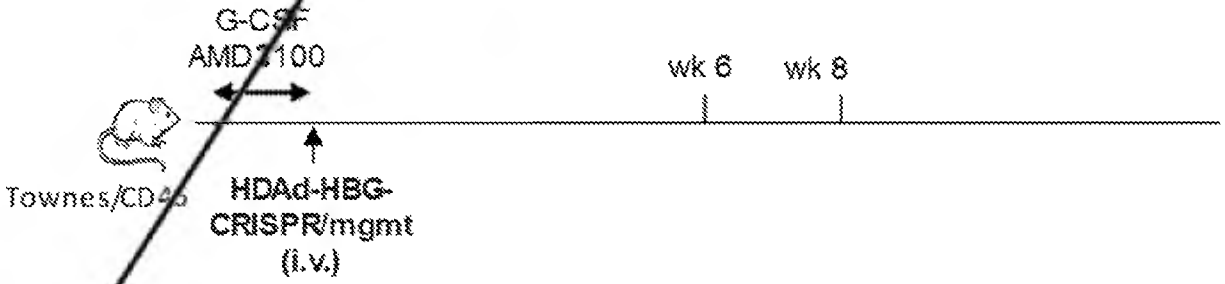


FIG. 128B

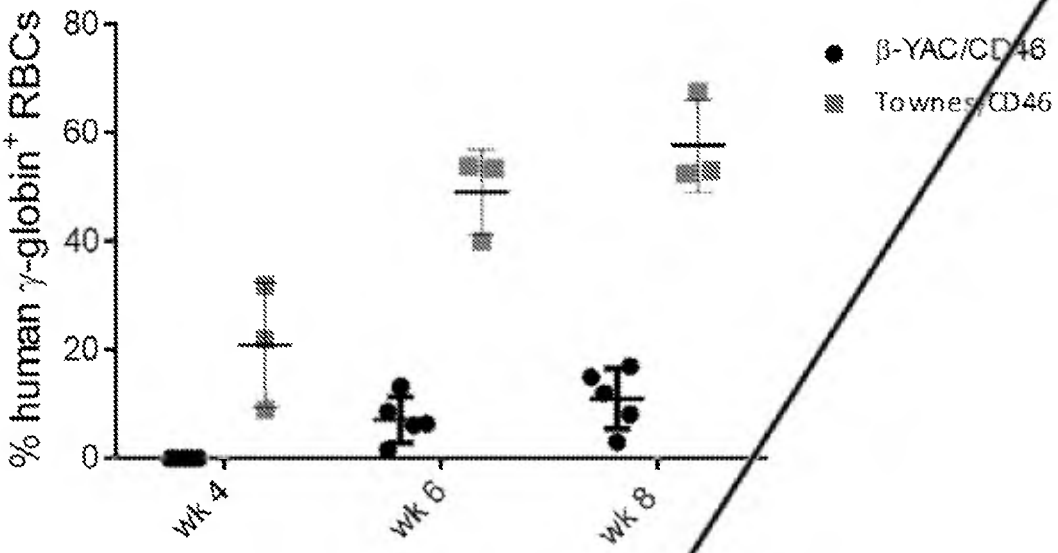


FIG. 128C

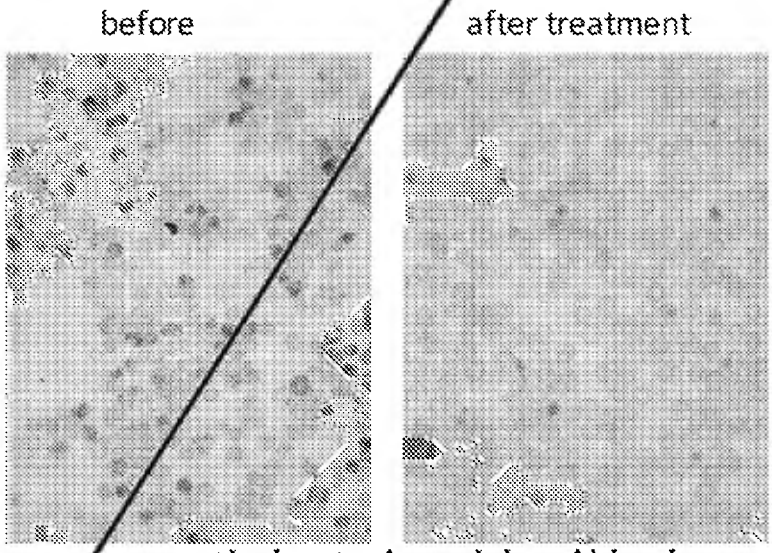


FIG. 129A

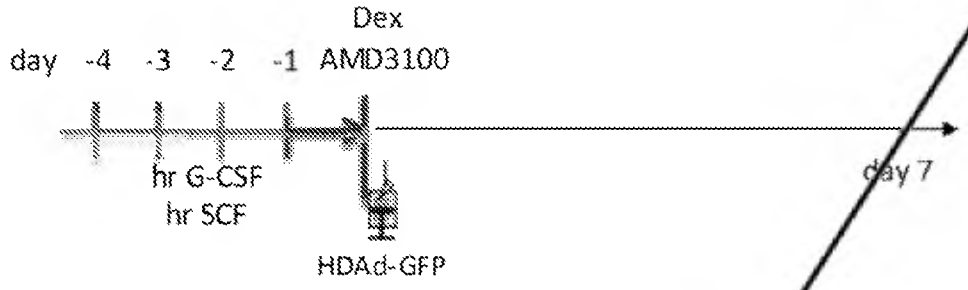


FIG.129B

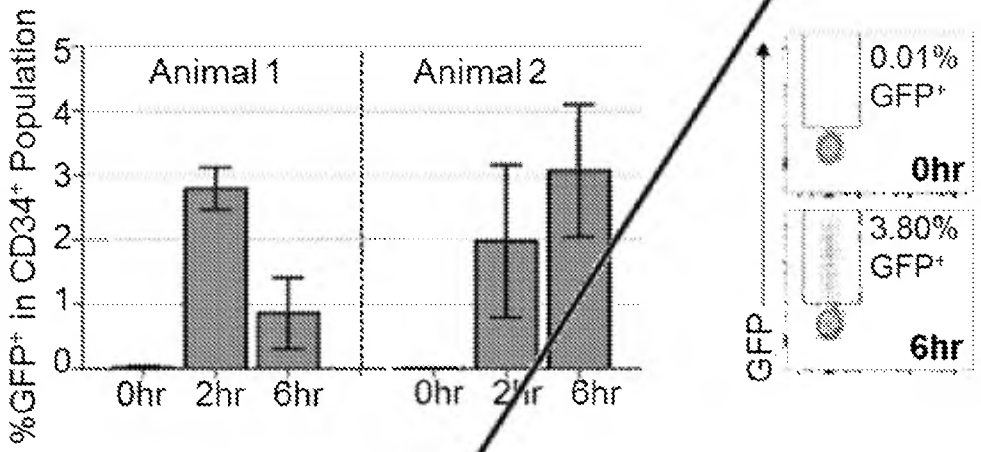


FIG. 129C

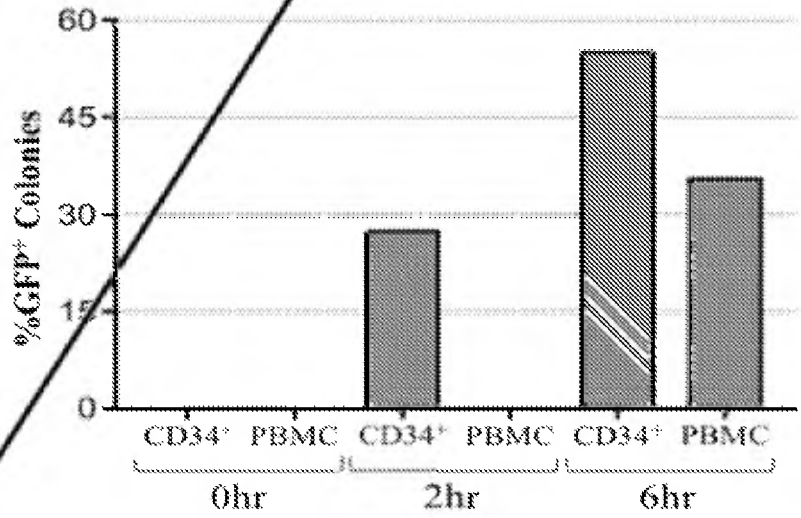


FIG. 129D

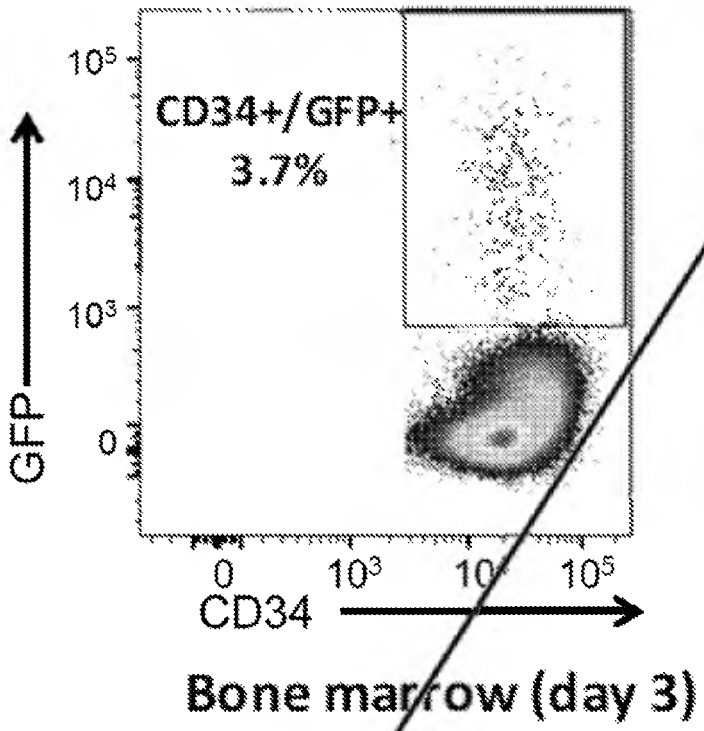


FIG. 130

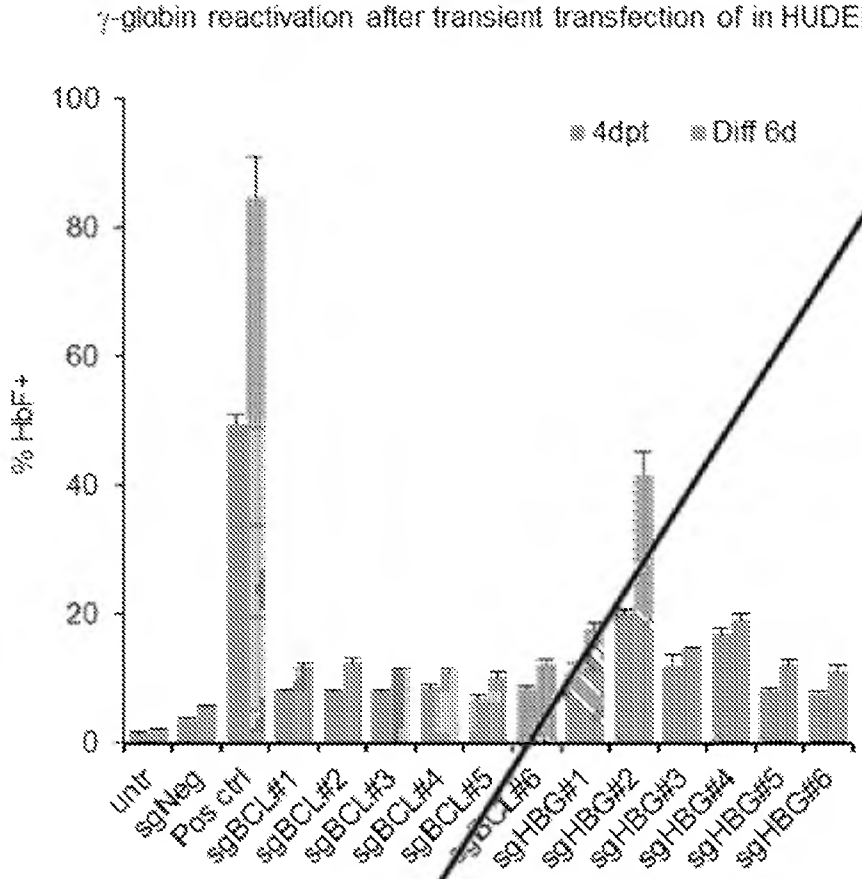


FIG. 131A

FIG. 131B

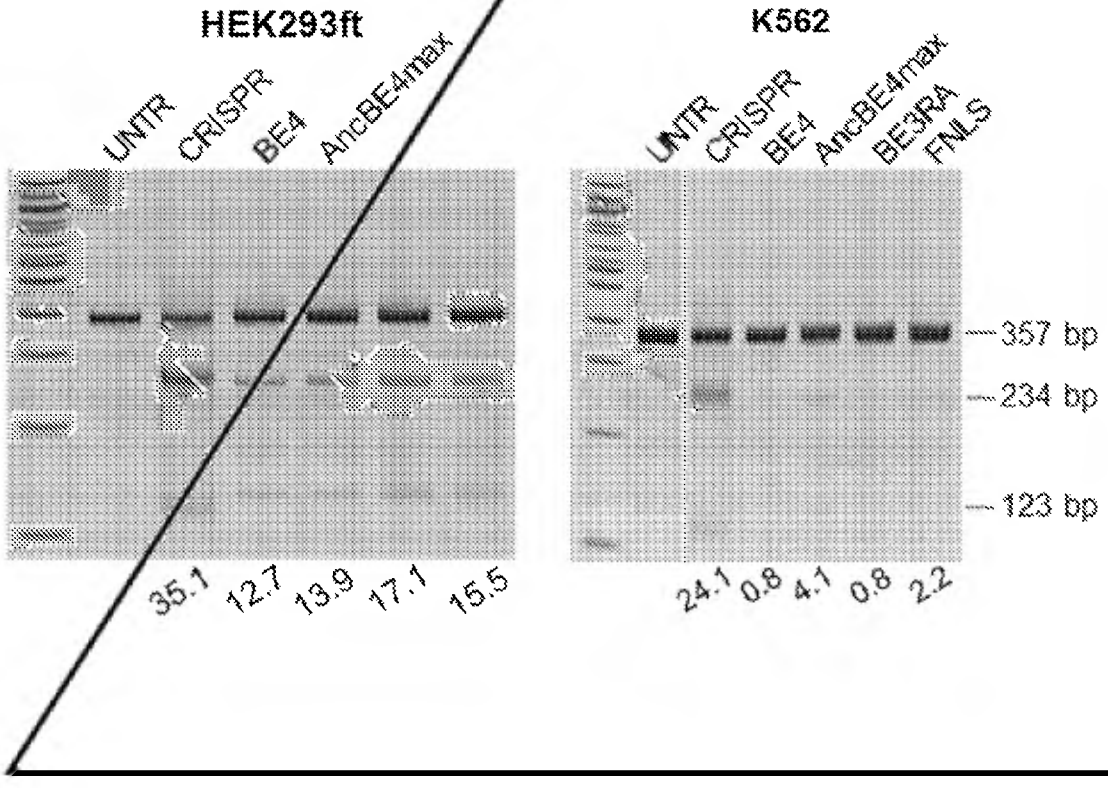


FIG. 132A

FIG. 132B

FIG. 132C

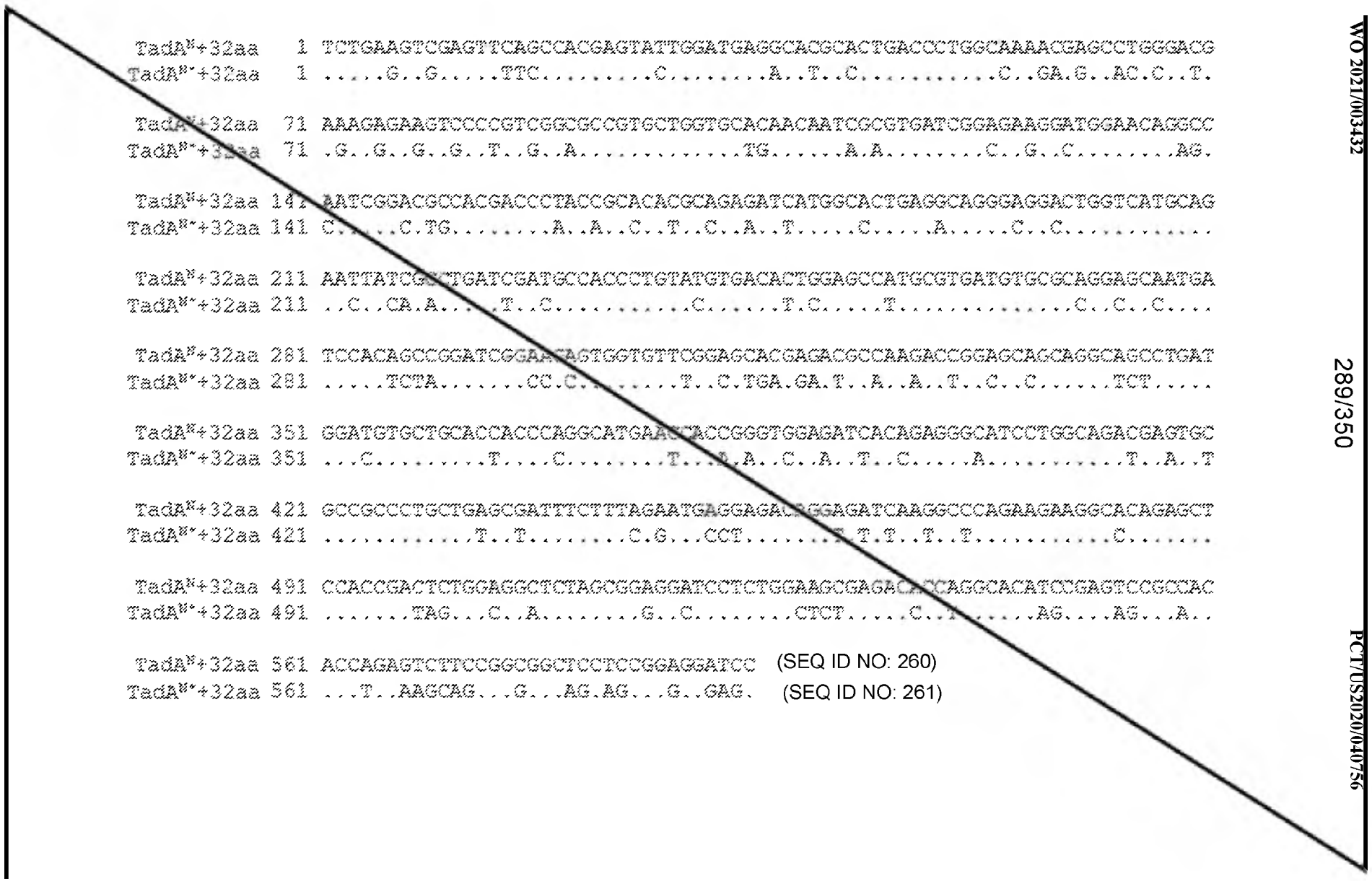

TadA^N+32aa 1 TCTGAAGTCGAGTTCAGCCACGAGTATTGGATGAGGCACGCACTGACCCTGGCAAAACGAGCCTGGGACG
TadA^N*+32aa 1 .....G..G.....TTC.....C.....A..T..C.....C..GA.G..AC.C..T.
TadA^N+32aa 71 AAAGAGAAGTCCCCGTCGGCGCCGTGCTGGTGCACAACAATCGCGTGATCGGAGAAGGATGGAACAGGCC
TadA^N*+32aa 71 .G..G..G..G..T..G..A.....TG.....A.A.....C..G..C.....AG.
TadA^N+32aa 141 AATCGGACGCCACGACCCTACCGCACACGCAGAGATCATGGCACTGAGGCAGGGAGGACTGGTCATGCAG
TadA^N*+32aa 141 C.....C.TG.....A..A..C..T..C..A..T.....C.....A.....C..C.....
TadA^N+32aa 211 AATTATCGACTGATCGATGCCACCCTGTATGTGACACTGGAGCCATGCGTGATGTGCGCAGGAGCAATGA
TadA^N*+32aa 211 ..C..CA.A.....T..C.....C.....T.C.....T.....C..C..C....
TadA^N+32aa 281 TCCACAGCCCGGATCGGAATAGTGGTGTTCGGAGCACGAGACGCCAAGACCGGAGCAGCAGGCAGCCTGAT
TadA^N*+32aa 281 .....TCTA.....CC.C.....T..C.TGA.GA.T..A..A..T..C..C.....TCT.....
TadA^N+32aa 351 GGATGTGCTGCACCACCCAGGCATGAACCACCGGGTGGAGATCACAGAGGGCATCCTGGCAGACGAGTGC
TadA^N*+32aa 351 ...C.....T....C.....T...A..C..A..T..C.....A.....T..A..T
TadA^N+32aa 421 GCCGCCCTGCTGAGCGATTTCTTTAGAATGAGGAGACGGAGATCAAGGCCCAGAAGAAGGCACAGAGCT
TadA^N*+32aa 421 .....T..T.....C.G...CCT.....T.T..T..T.....C.....
TadA^N+32aa 491 CCACCGACTCTGGAGGCTCTAGCGGAGGATCCTCTGGAAGCGAGACCCAGGCACATCCGAGTCCGCCAC
TadA^N*+32aa 491 .....TAG...C..A.....G..C.....CTCT.....C.T.....AG....AG...A..
TadA^N+32aa 561 ACCAGAGTCTTCCGGCGGCTCCTCCGGAGGATCC (SEQ ID NO: 260)
TadA^N*+32aa 561 ...T..AAGCAG...G...AG.AG...G..GAG. (SEQ ID NO: 261)

FIG. 133A

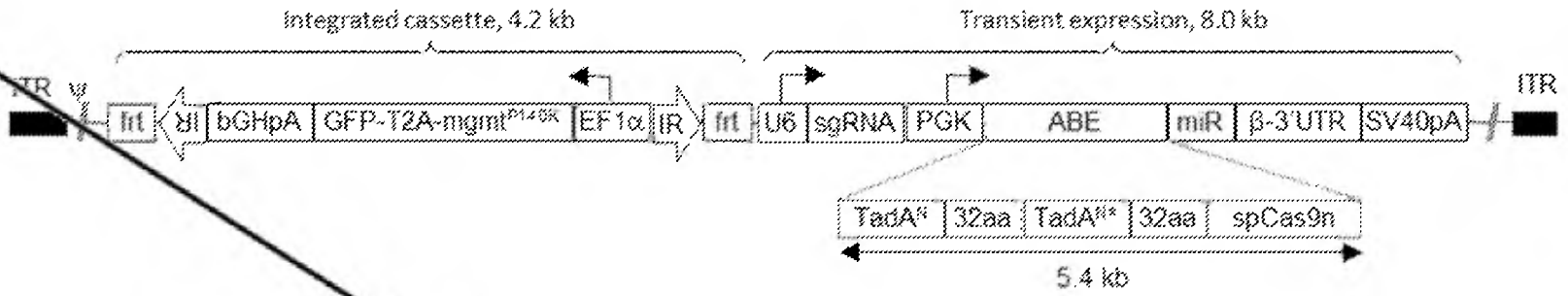


FIG. 133B

Full name	Editor	Guide RNA	Yield (× 10^{12} vp)
HDAd5/35++_BE4max-sgBCL#1-FI-mgmt/GFP	CBE	sgBCL#1	1.2
HDAd5/35++_BE4max-sgHBG#1-FI-mgmt/GFP	CBE	sgHBG#1	0.8
HDAd5/35++_ABEopti-sgHBG#2-FI-mgmt/GFP	ABE	sgHBG#2	3.8
HDAd5/35++_ABEopti-sgHBG#4-FI-mgmt/GFP	ABE	sgHBG#4	2.8
HDAd5/35++_BE4max-Dual#1-FI-mgmt/GFP	CBE	sgBCL#1 + sgHBG#1	0.5
HDAd5/35++_BE4max-sgNeg-FI-mgmt/GFP	CBE	sgNeg	1.5

FIG. 133C

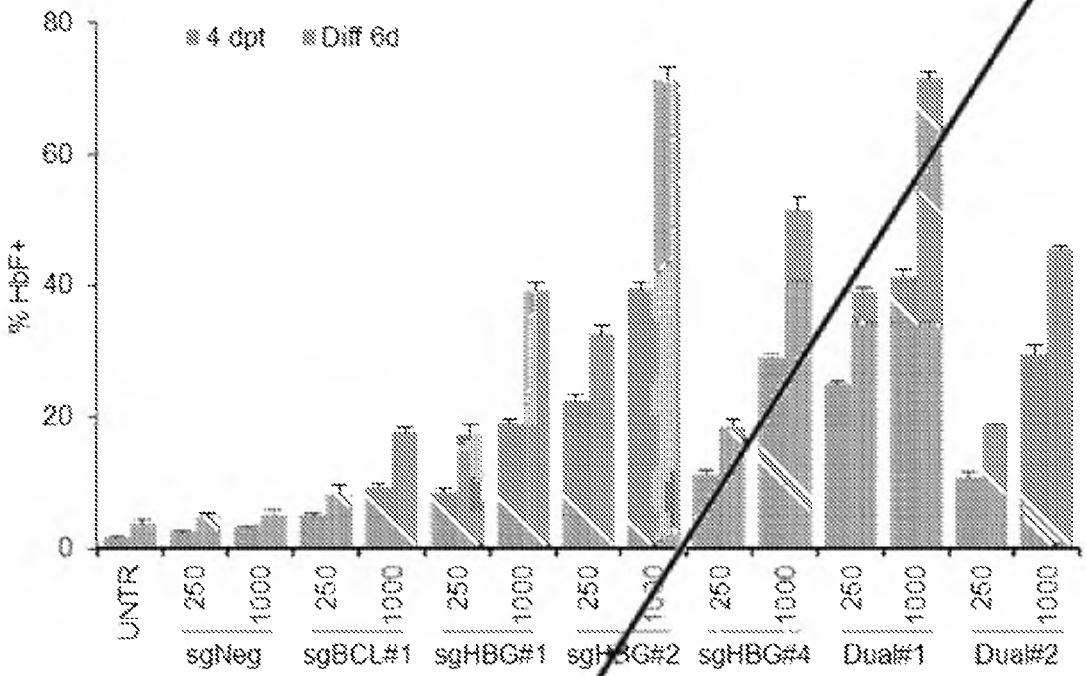
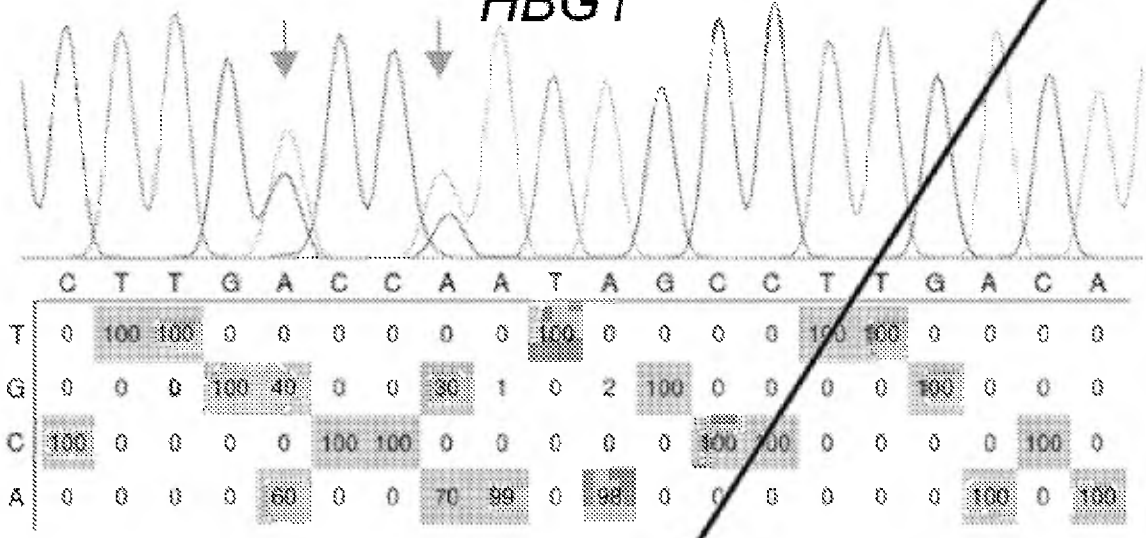

4 dpt
Diff 6d
% HbF+
80
60
40
20
0
UNTR
250
1000
sgNeg
sgBCL#1
sgHBG#1
sgHBG#2
sgHBG#4
Dual#1
Dual#2

FIG. 133D

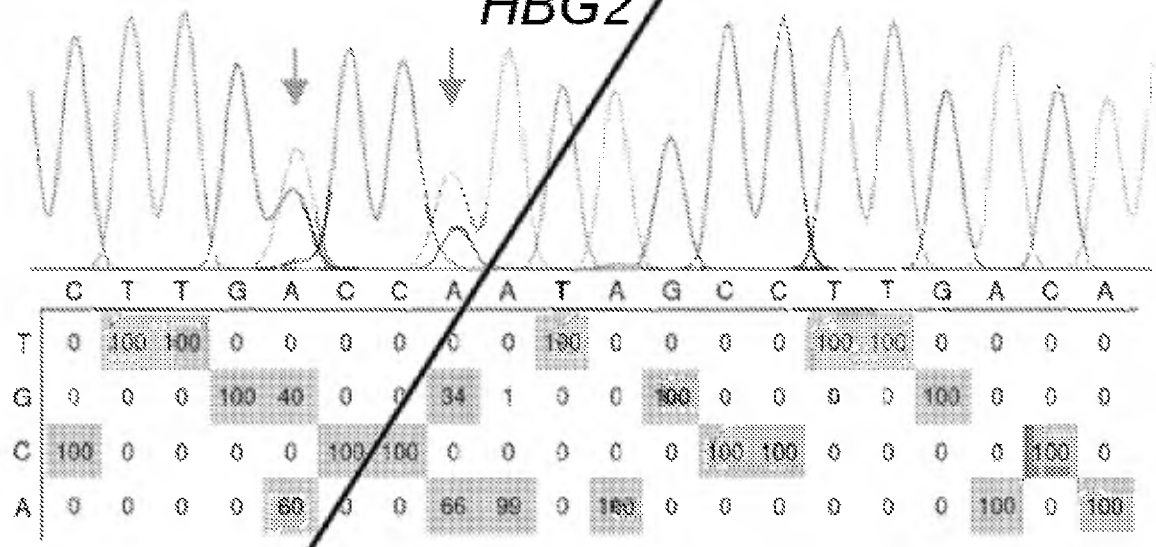
sgHBG#2

HBG1



(SEQ ID NO: 250)

HBG2



(SEQ ID NO: 250)

FIG. 133E

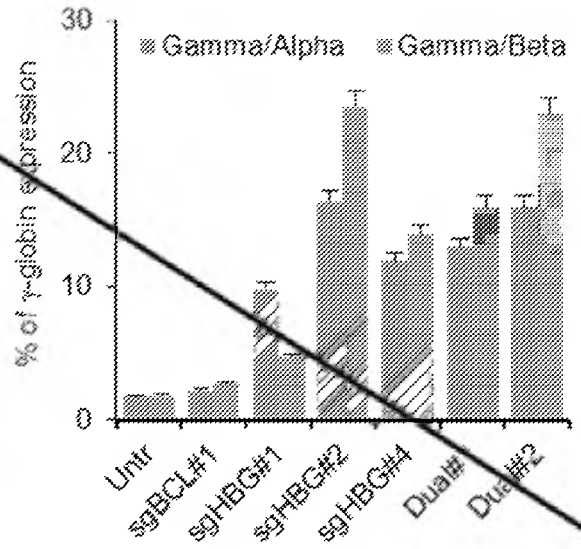


FIG. 133F

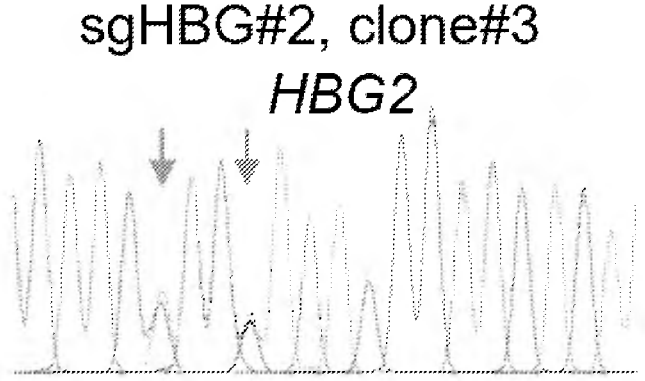


FIG. 133G

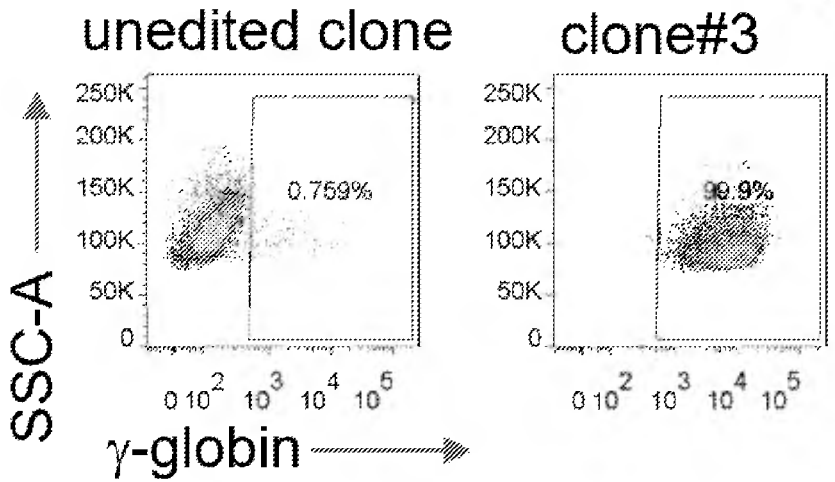


FIG. 133H

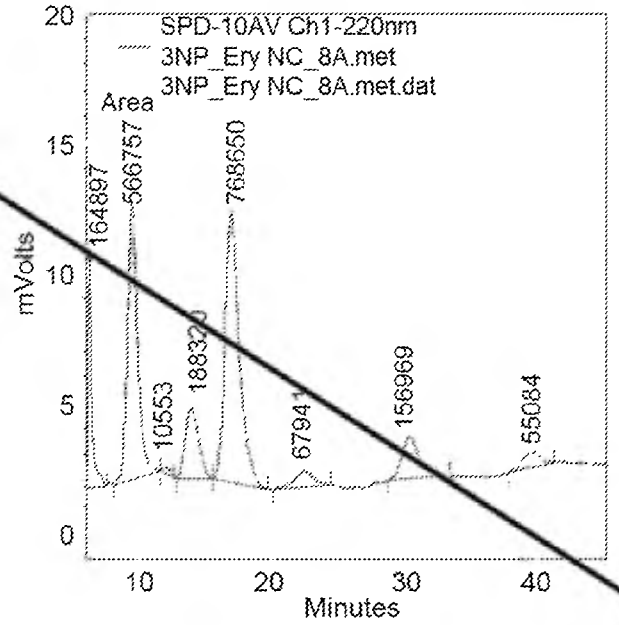


FIG. 134A

sgBCL#1
Bcl11A

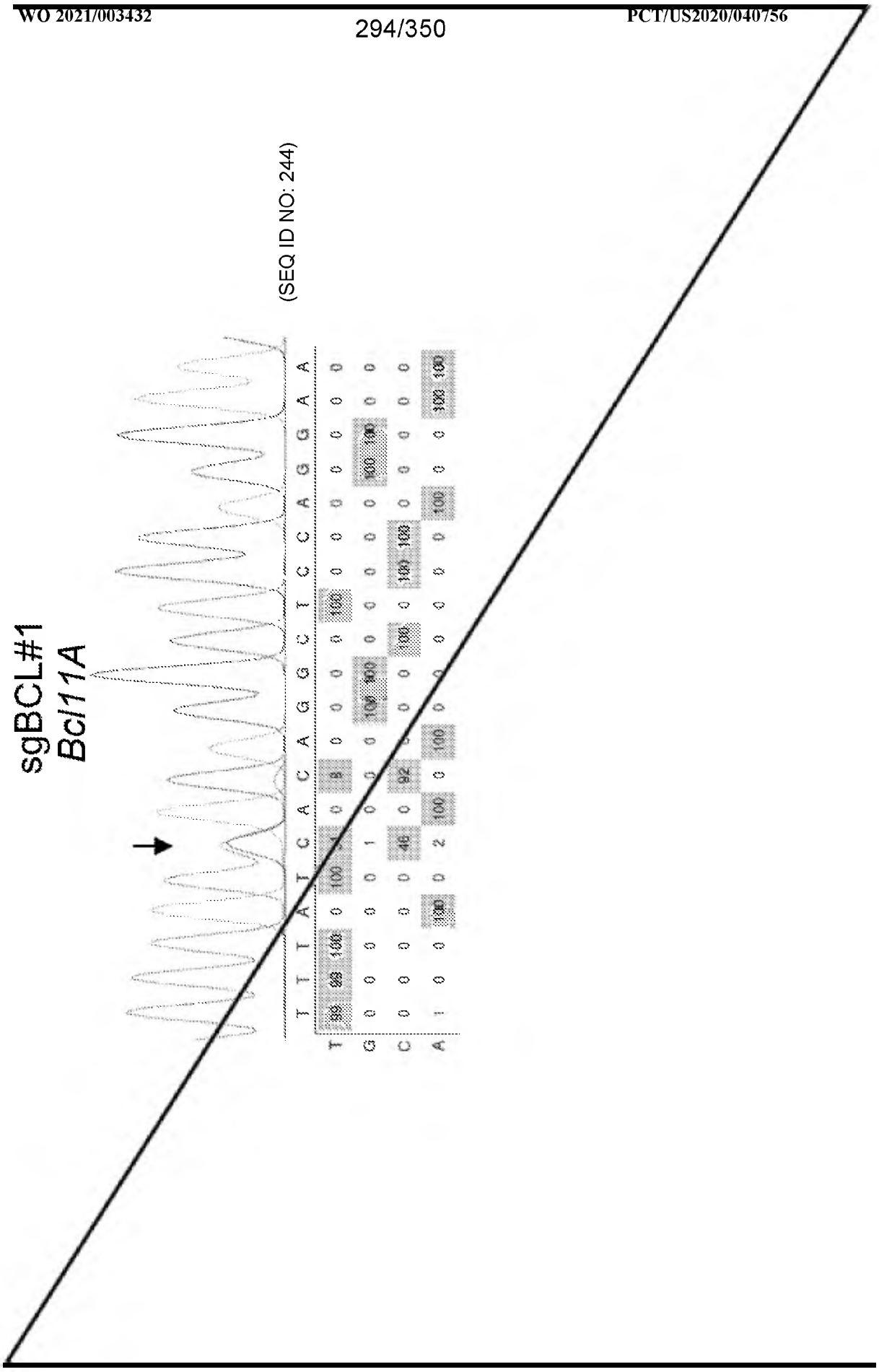


FIG. 134A cont'd

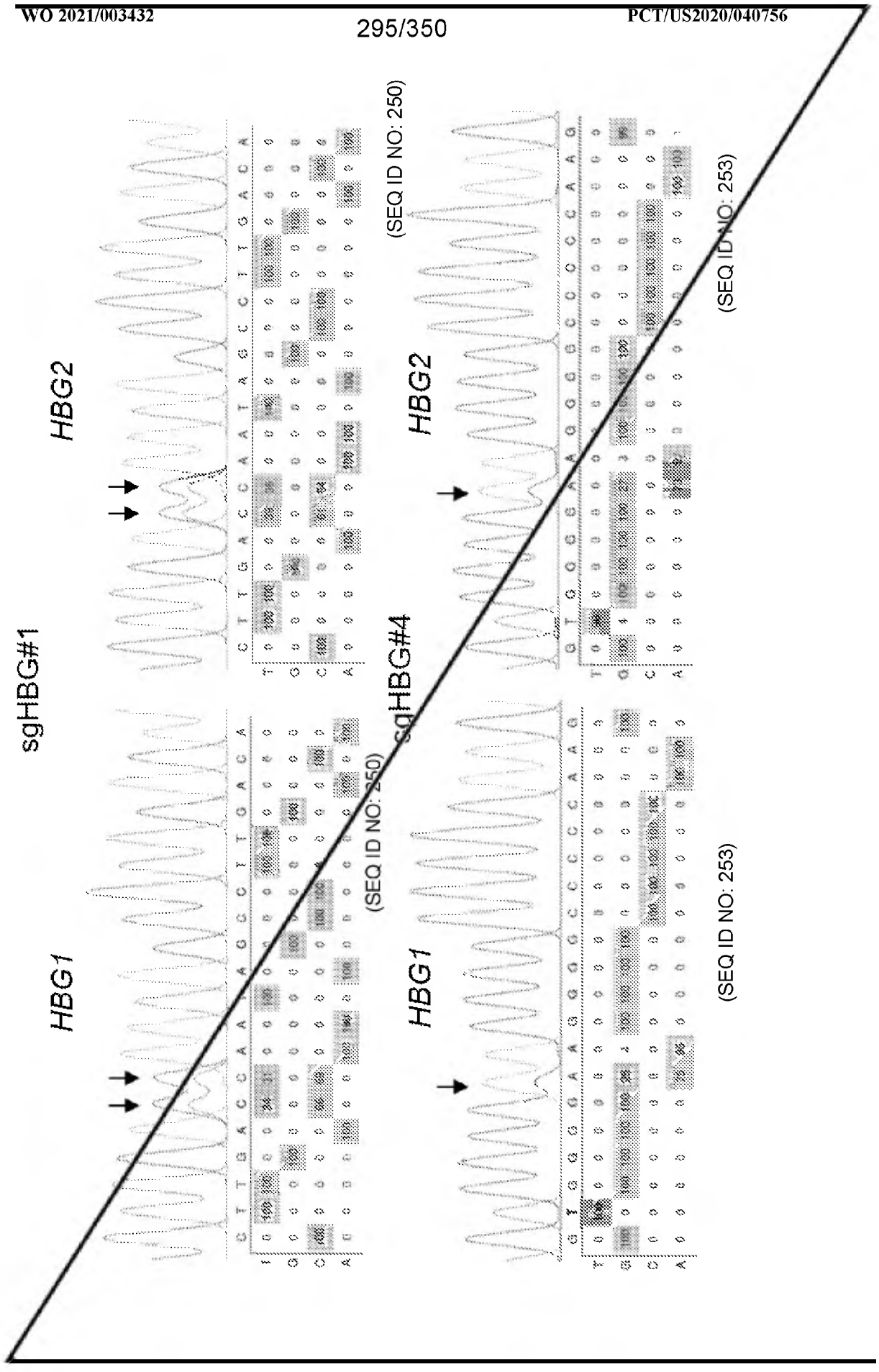


FIG. 134B

sgBCL#1, Clone#5
Bcl11A

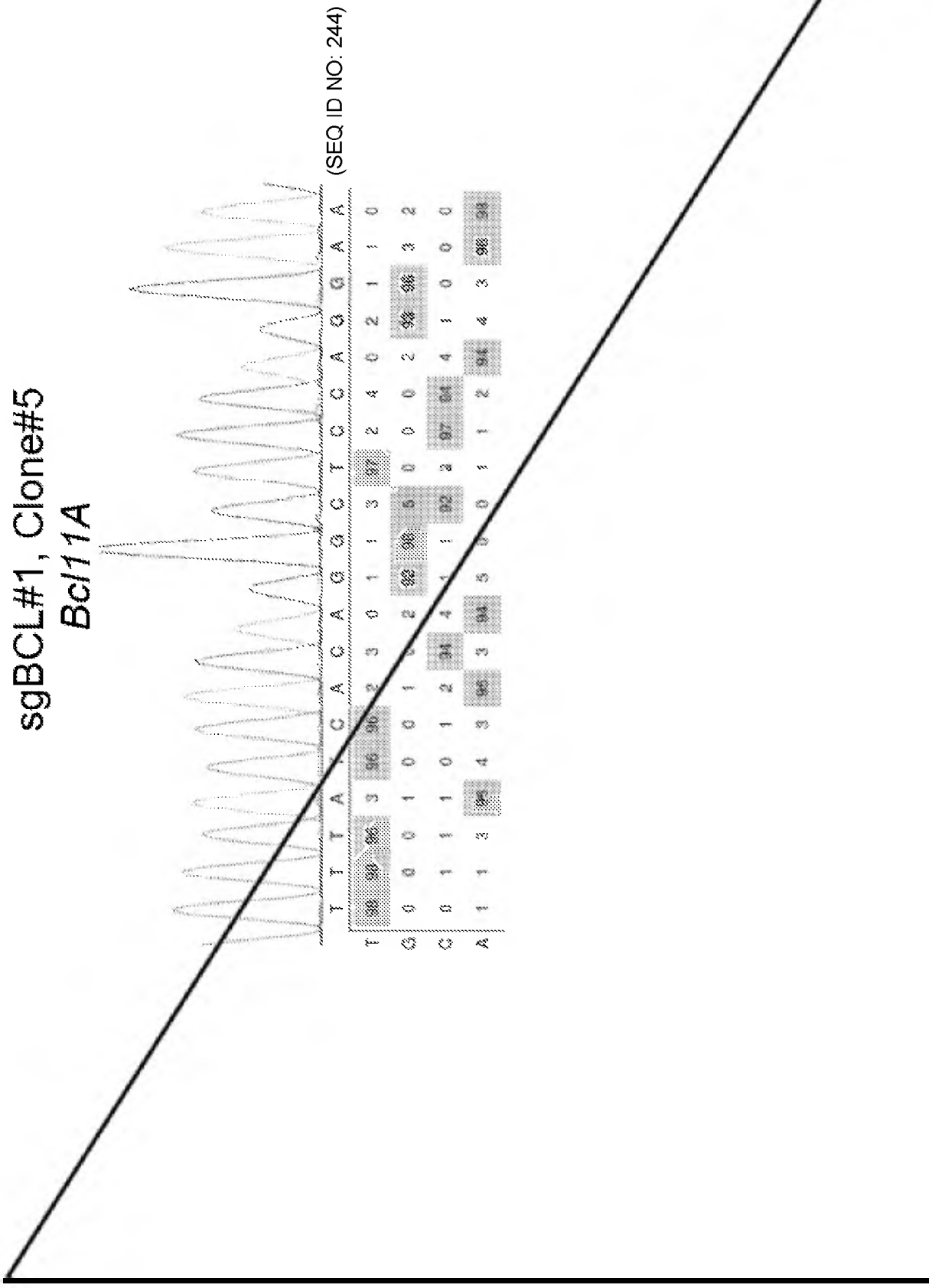


FIG. 134B cont'd

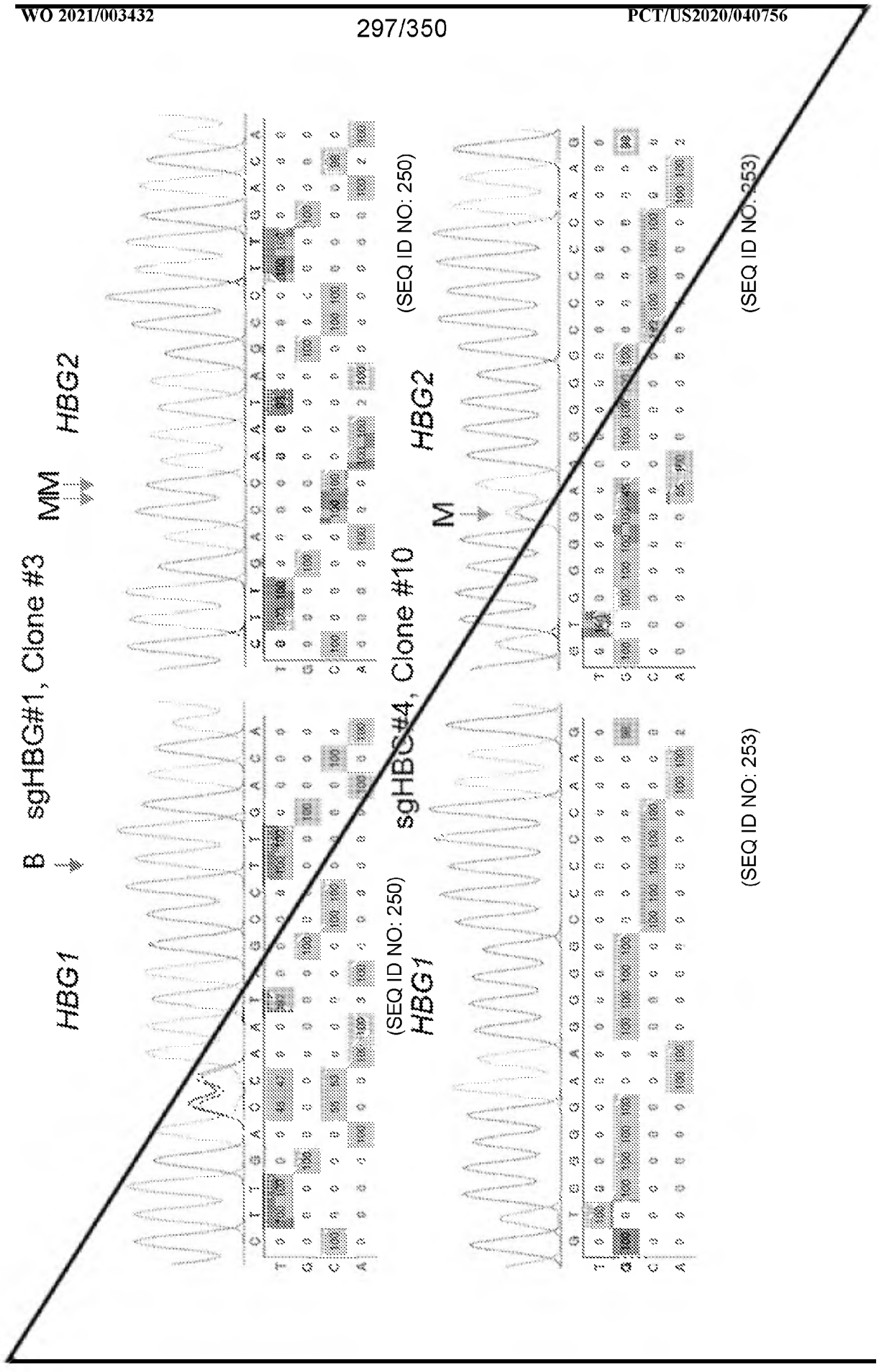


FIG. 134C

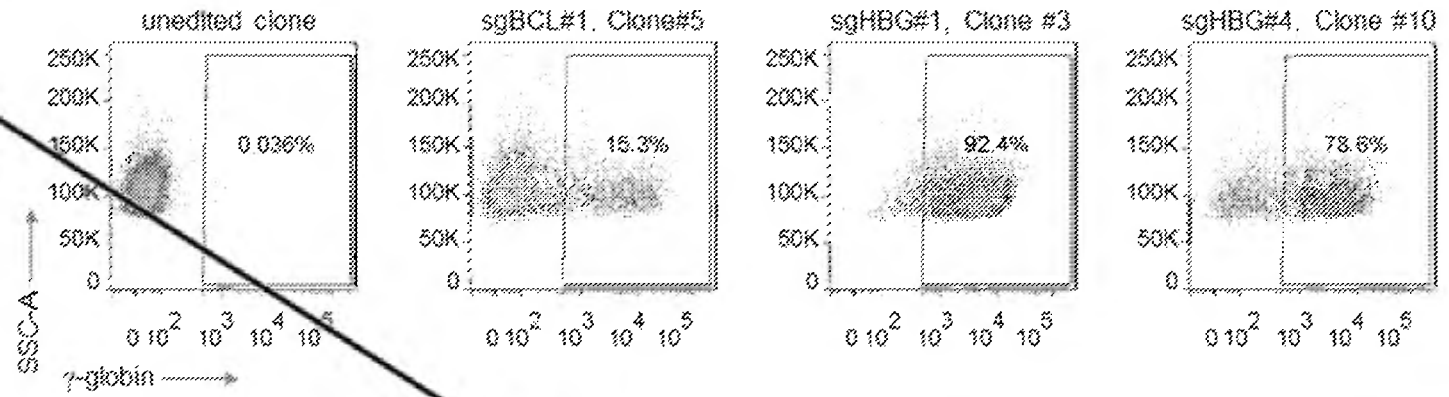


FIG. 135A

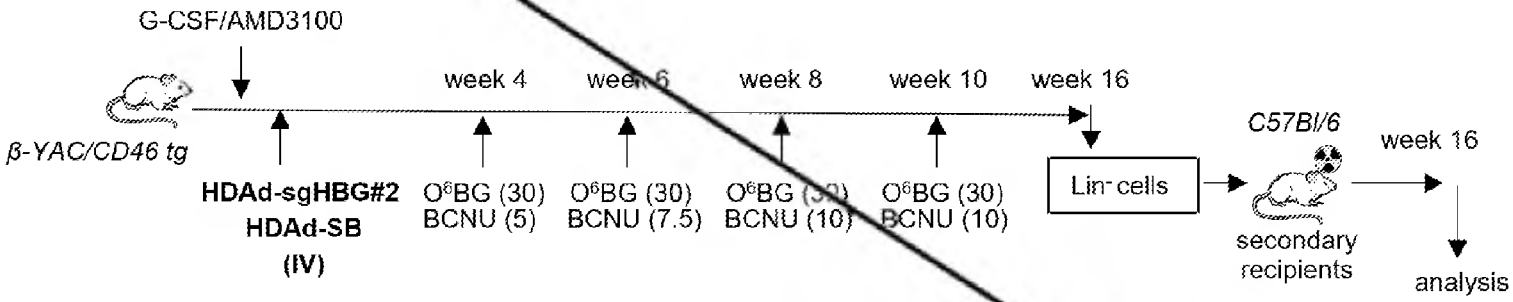


FIG. 135B

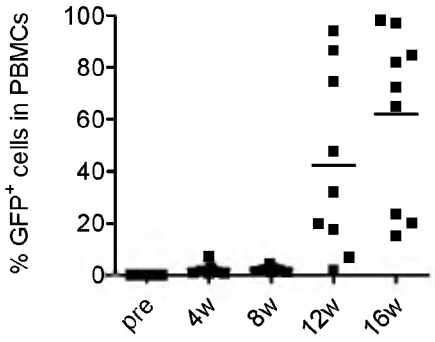


FIG. 135C

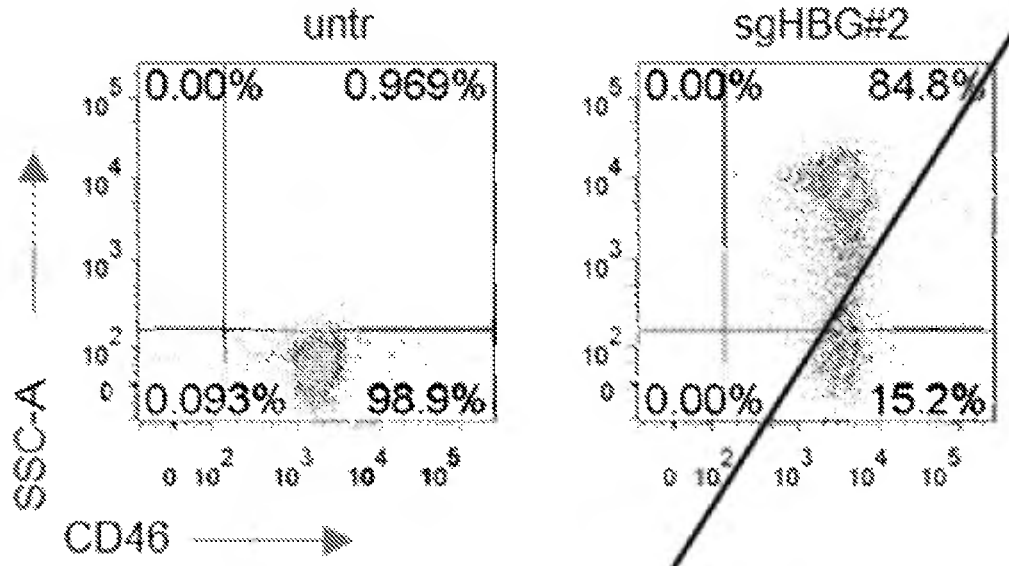


FIG. 135D

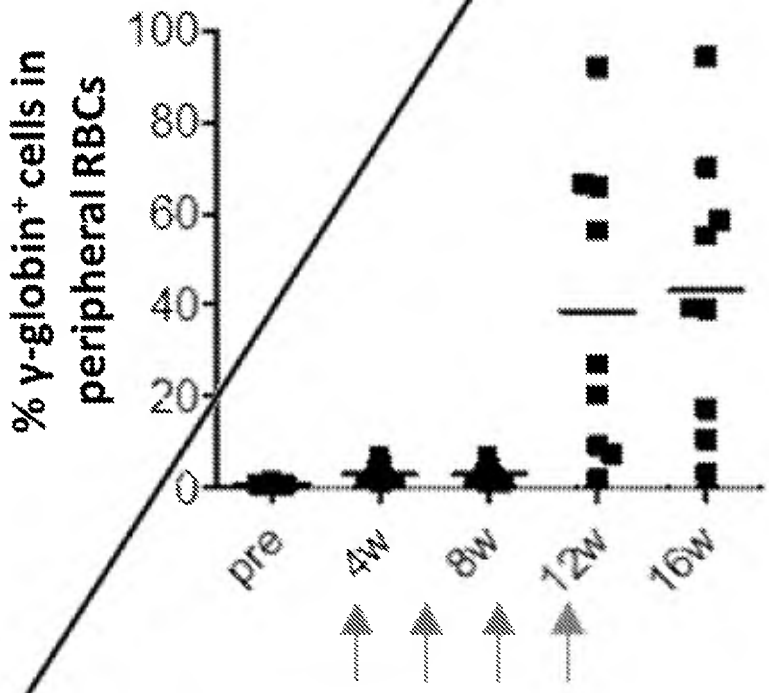


FIG. 135E

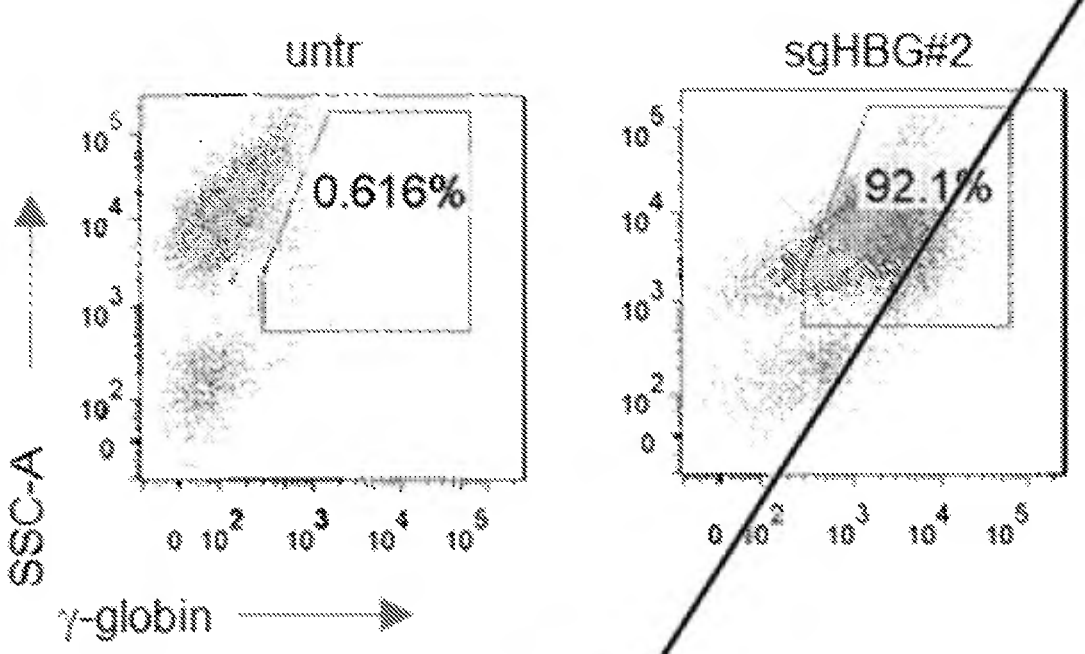


FIG. 135F

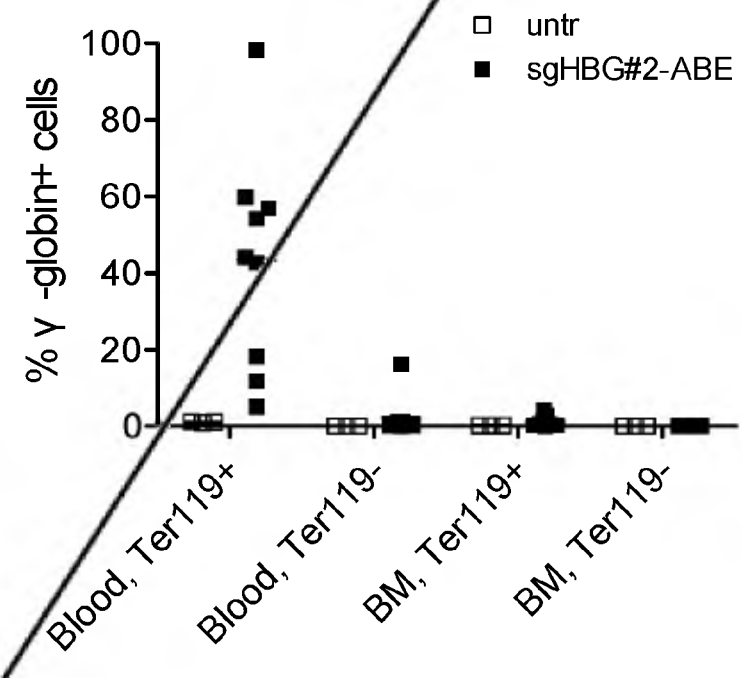


FIG. 135G

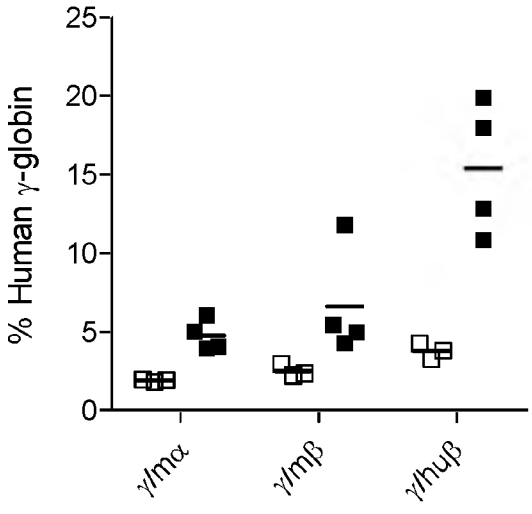


FIG. 135H

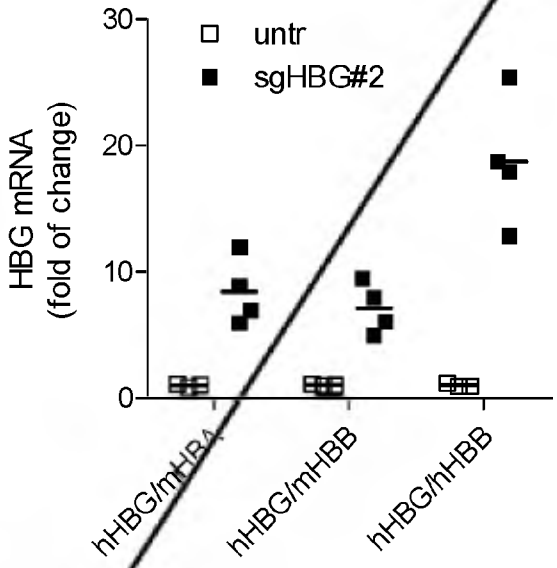


FIG. 135I

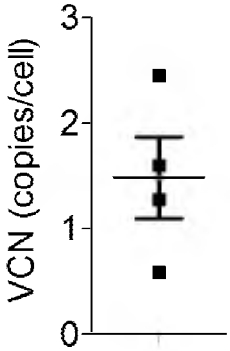


FIG. 136

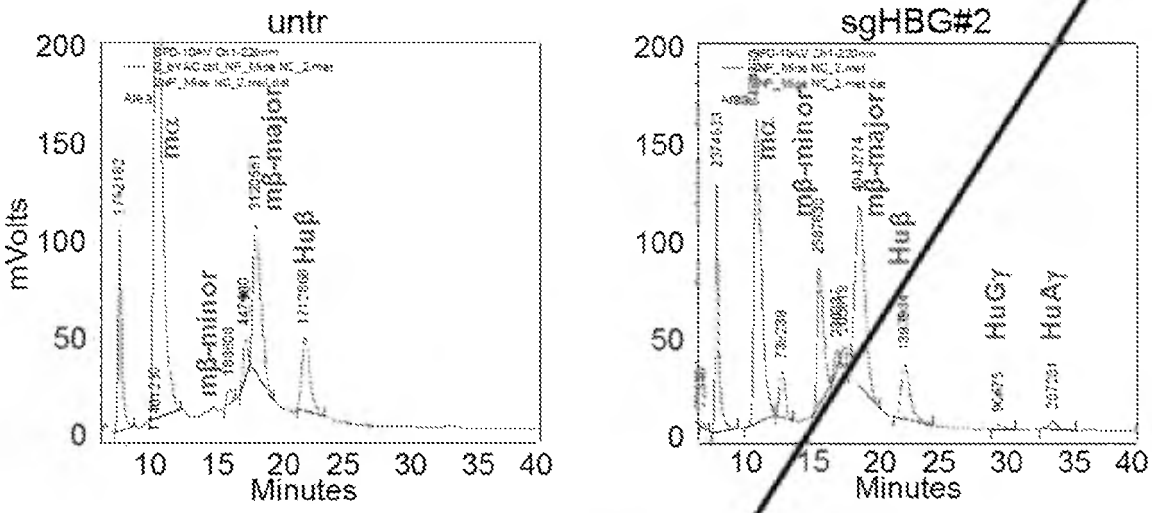
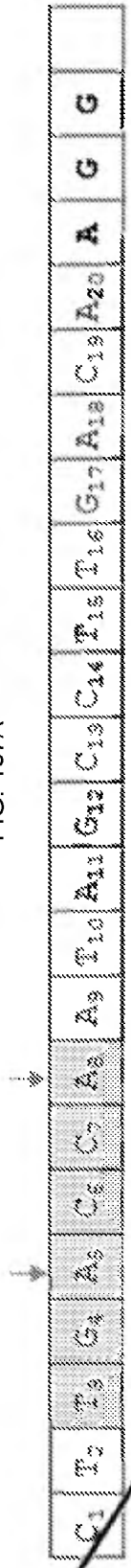

untr
sgHBG#2
mVolts
Minutes
mα
mβ-minor
mβ-major
Huβ
HuGγ
HuAγ
0
50
100
150
200
10
15
20
25
30
35
40

FIG. 137A



(SEQ ID NO: 276)

FIG. 137B

FIG. 137C

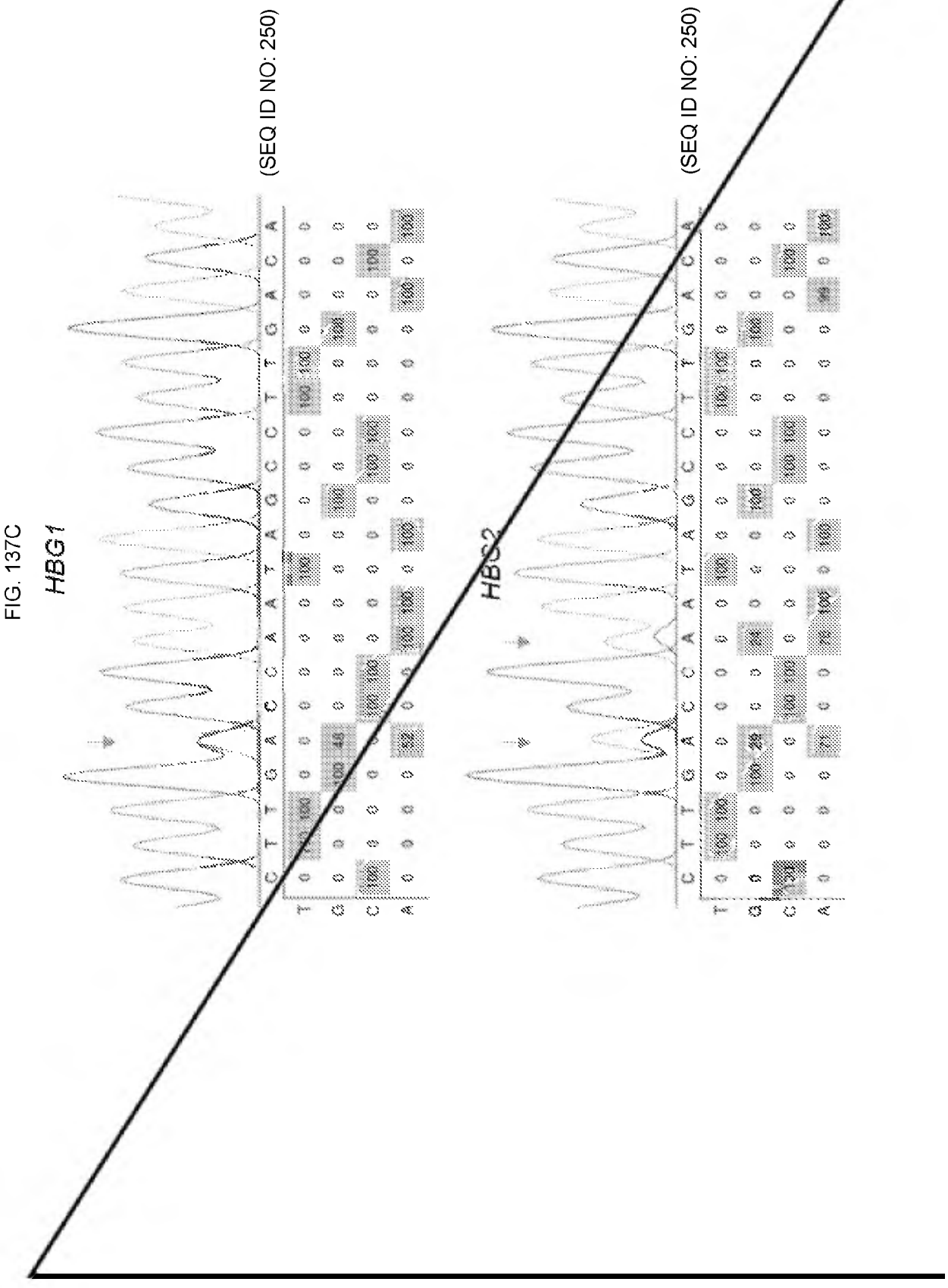


FIG. 137D

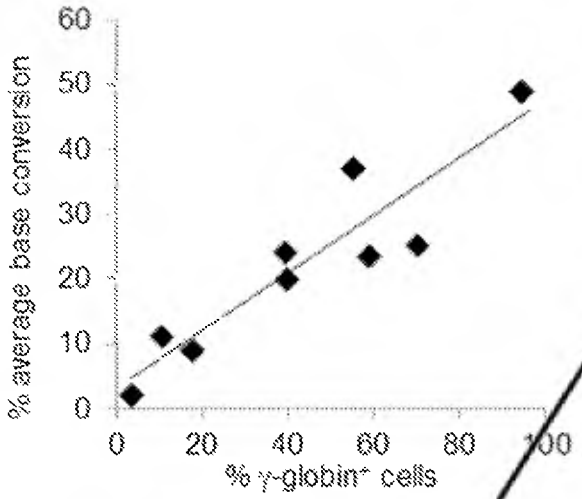


FIG. 137E

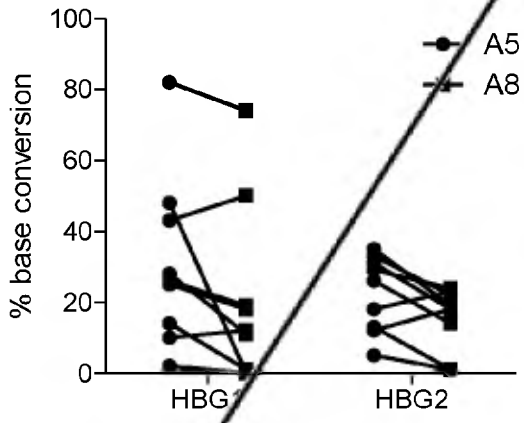
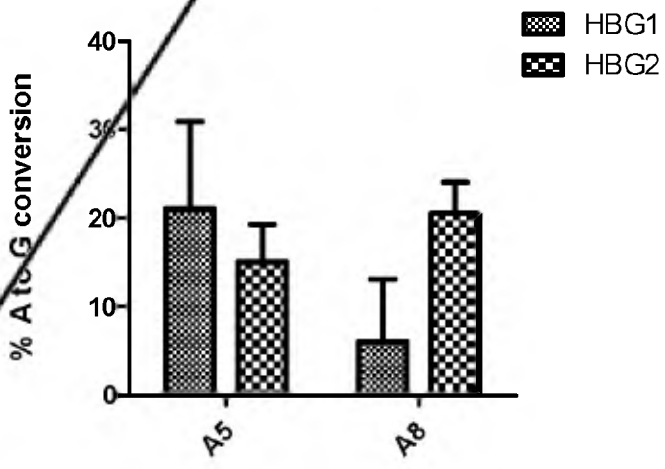


FIG. 137F



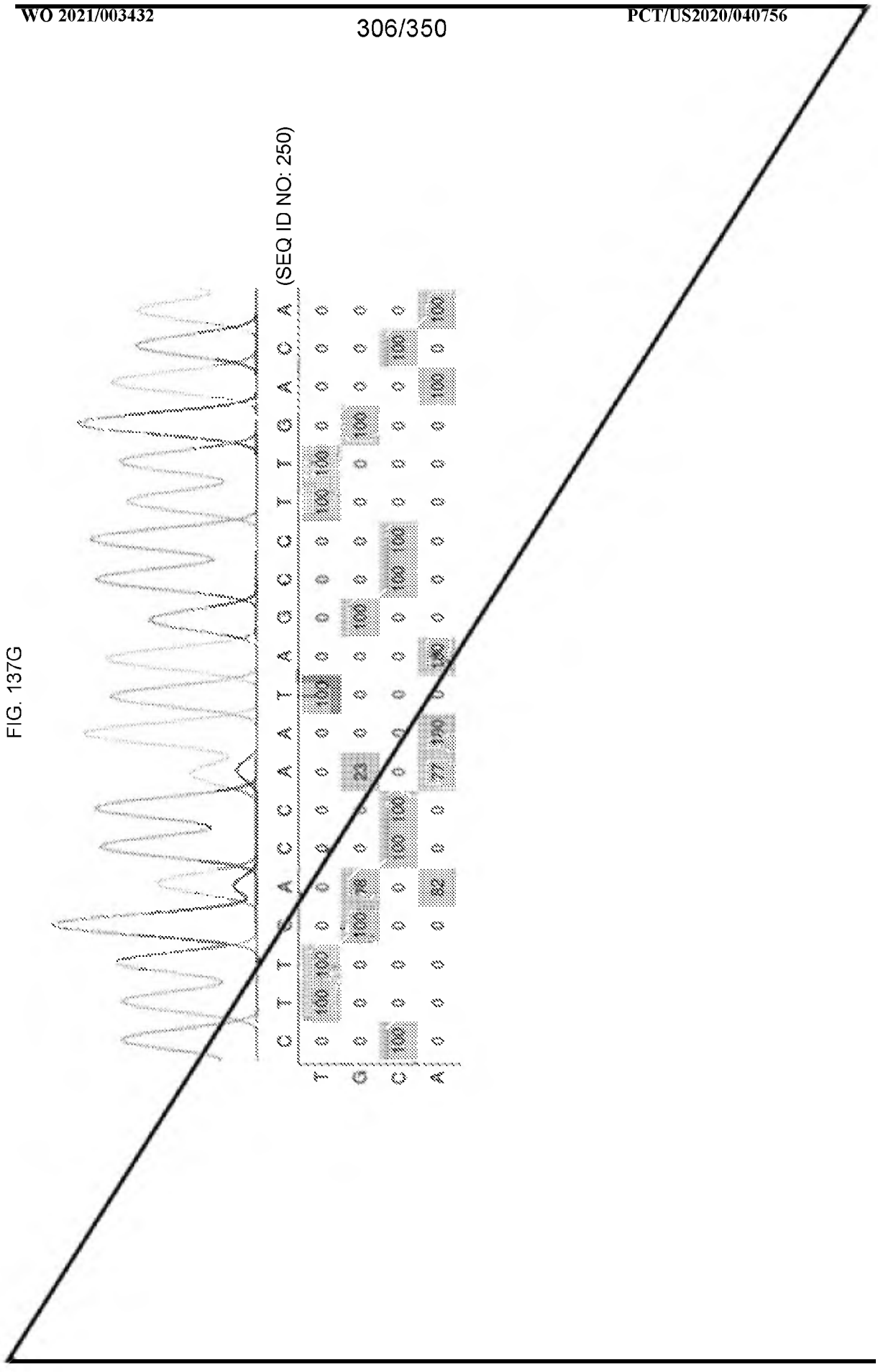


FIG. 137G

FIG. 138A

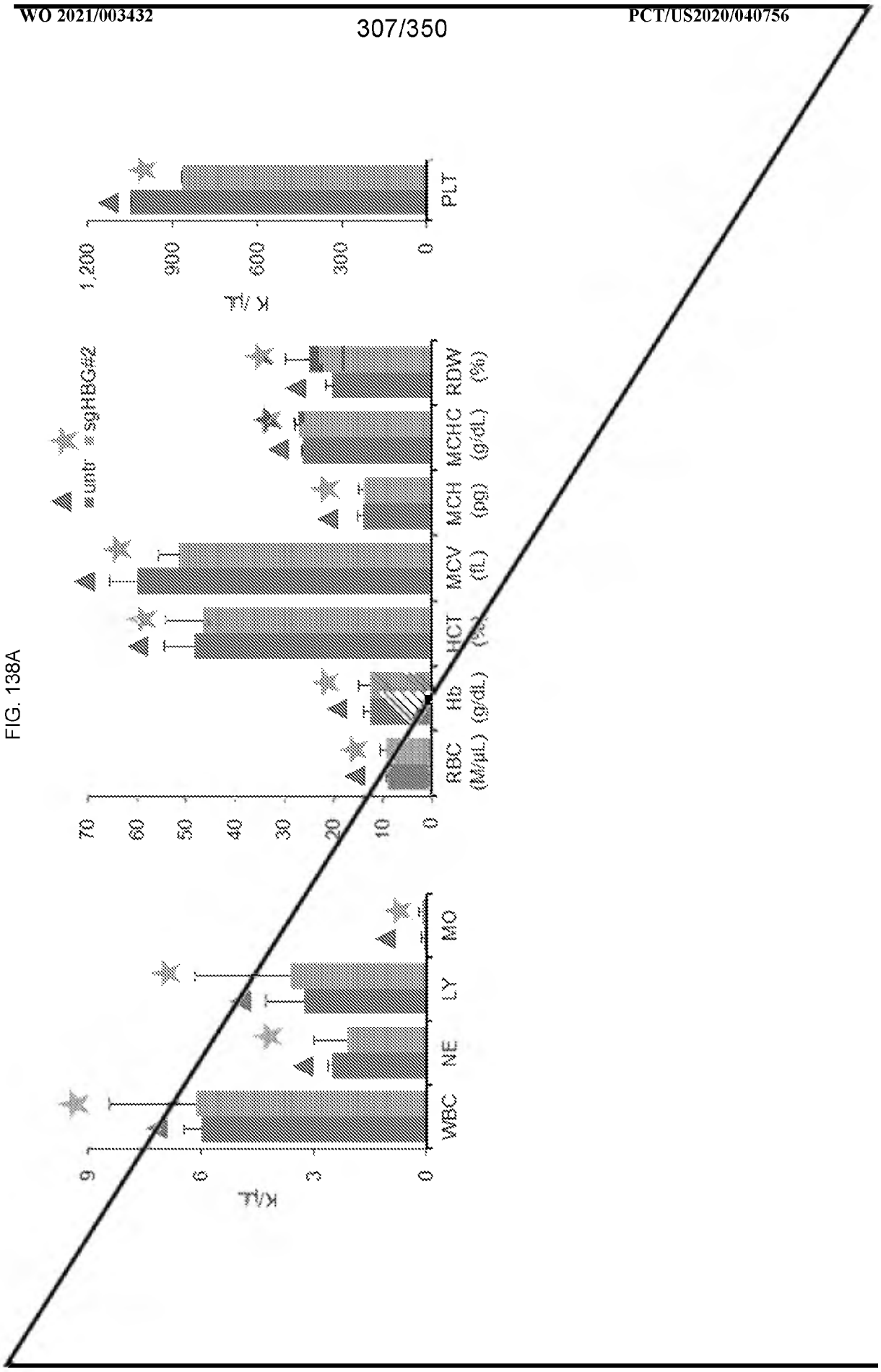

untr
sgHBG#2
K/μL
WBC
NE
LY
MO
RBC (M/μL)
Hb (g/dL)
HCT (%)
MCV (fL)
MCH (pg)
MCHC (g/dL)
RDW (%)
PLT

FIG. 138B

FIG. 138C

FIG. 138D

FIG. 139B

FIG. 139A

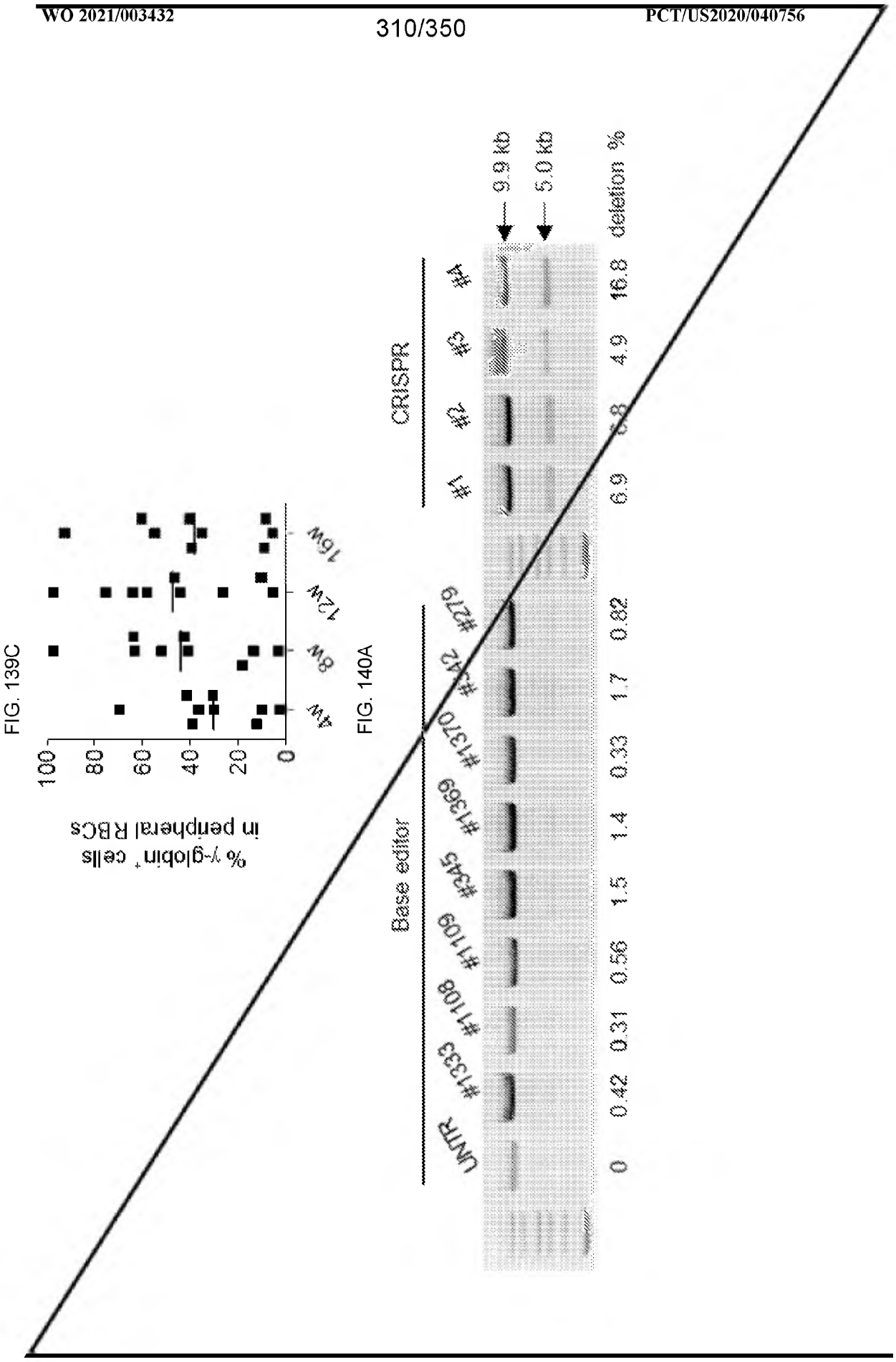

UNTR
#133
#1108
#1109
#345
#1369
#1370
#42
#279
Base editor
#1
#2
#3
#4
CRISPR
9.9 kb
5.0 kb
0
0.42
0.31
0.56
1.5
1.4
0.33
1.7
0.82
6.9
3.8
4.9
16.8
deletion %
FIG. 140A
% γ-globin+ cells
in peripheral RBCs
100
80
60
40
20
0
4w
8w
12w
16w
FIG. 139C

FIG. 140B

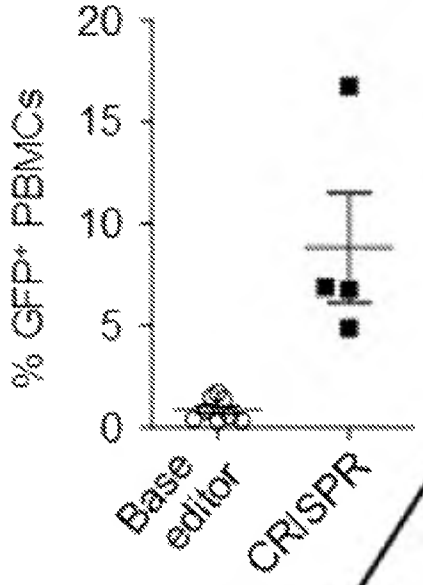


FIG. 141

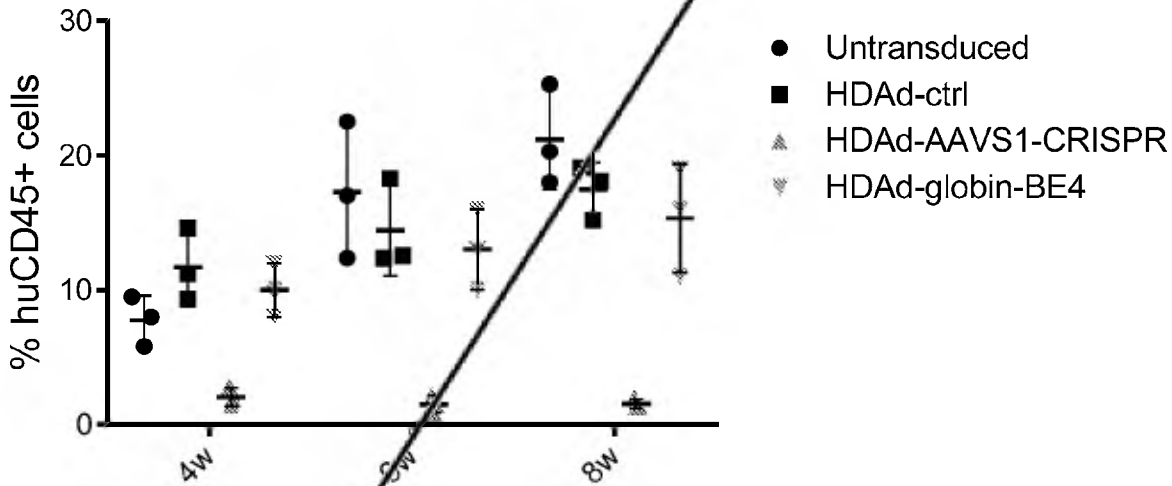


FIG. 142

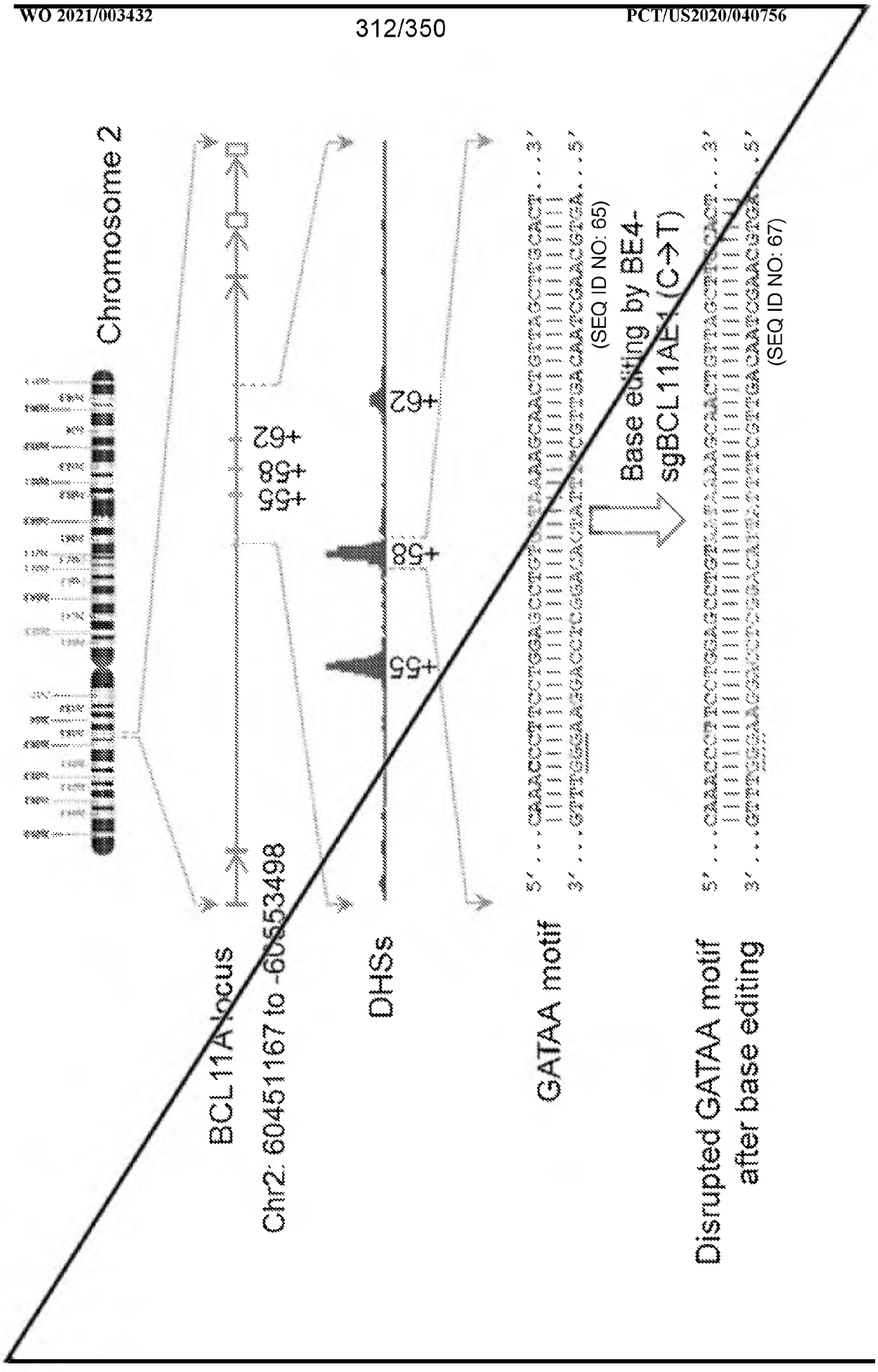

Chromosome 2
BCL11A locus
Chr2: 60451167 to -60553498
+62
+58
+55
DHSs
GATAA motif
5' . . . CAAACCTTCCTGGAGCCTGTTAAAGCAACTGTTAGCTTGCACT . . . 3'
3' . . . GTTTGGAAGGACCTCGGACACAATTTCGTTGACAATCGAACGTGA . . . 5'
(SEQ ID NO: 65)
Base editing by BE4-sgBCL11AE1 (C→T)
Disrupted GATAA motif after base editing
5' . . . CAAACCTTCCTGGAGCCTGTTAAAGCAACTGTTAGCTTGCACT . . . 3'
3' . . . GTTTGGAAGGACCTCGGACATTTTCGTTGACAATCGAACGTGA . . . 5'
(SEQ ID NO: 67)

FIG. 143

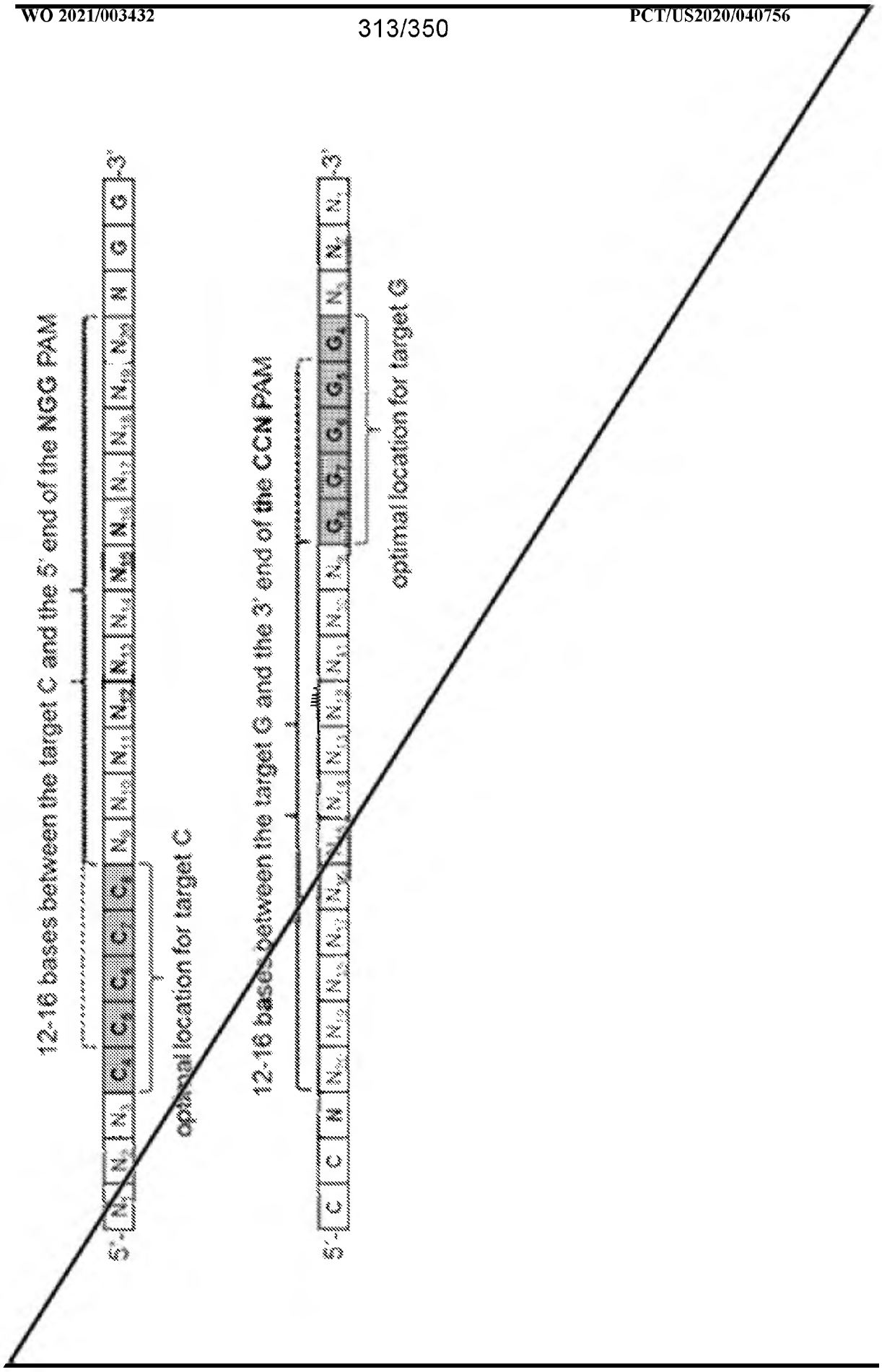
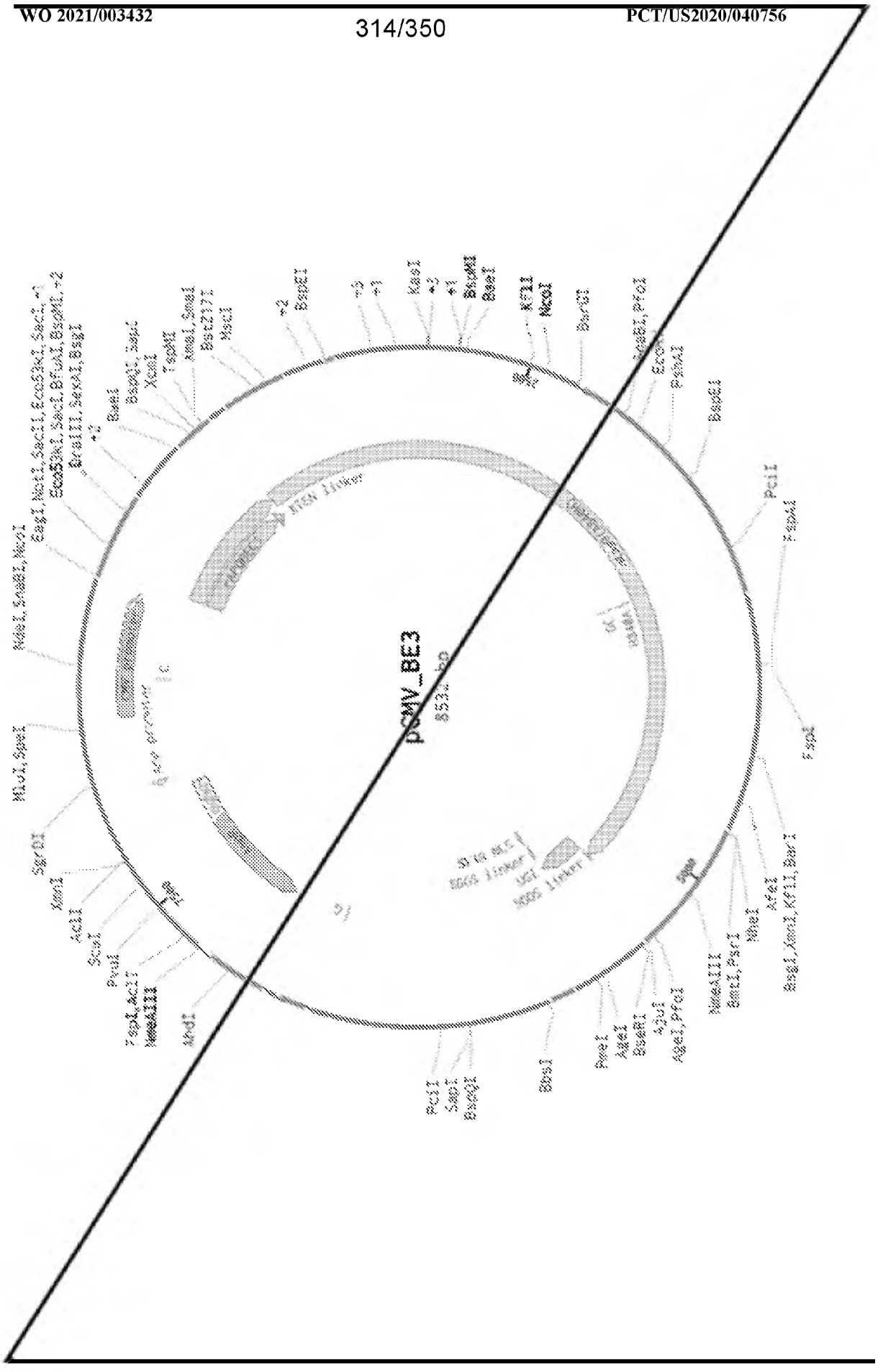

12-16 bases between the target C and the 5' end of the NGG PAM
optimal location for target C
12-16 bases between the target G and the 3' end of the CCN PAM
optimal location for target G

FIG. 144


pCMV_BE3
8531 bp
CMV promoter
NLS
rAPOBEC1
XTEN Linker
UGI
NLS
2A
NLS
UGI
NLS
pA
ori
AmpR promoter
AmpR
NotI, SnaBI, NcoI
EagI, NruI, SacII, EcoO109I, SacI, +1
Eco53kI, SacI, BfuAI, BspMI, +2
BraI III, SacaI, BsgI
BseRI
BspQI, SapI
XcmI
TspMI
XmaI, SmaI
Bst2Z7I
NsiI
+2
BspEI
+2
+1
KasI
+2
+1
BspMI
BsaI
KflI
NaeI
BsrGI
AgeI, PfoI
EcoRI
PasaI
BspEI
PciI
FseI
SgrDI
XmnI
AclI
ScaI
PvuI
TspMI, AclI
NheXIII
XhoI
PciI
SanI
BsaXI
BbvI
PmeI
AflII
BseRI
AjuI
AgeI, PfoI
NheXIII
BmtI, PsrI
NheI
AfeI
BsgI, XmnI, KflI, BsrI
FseI

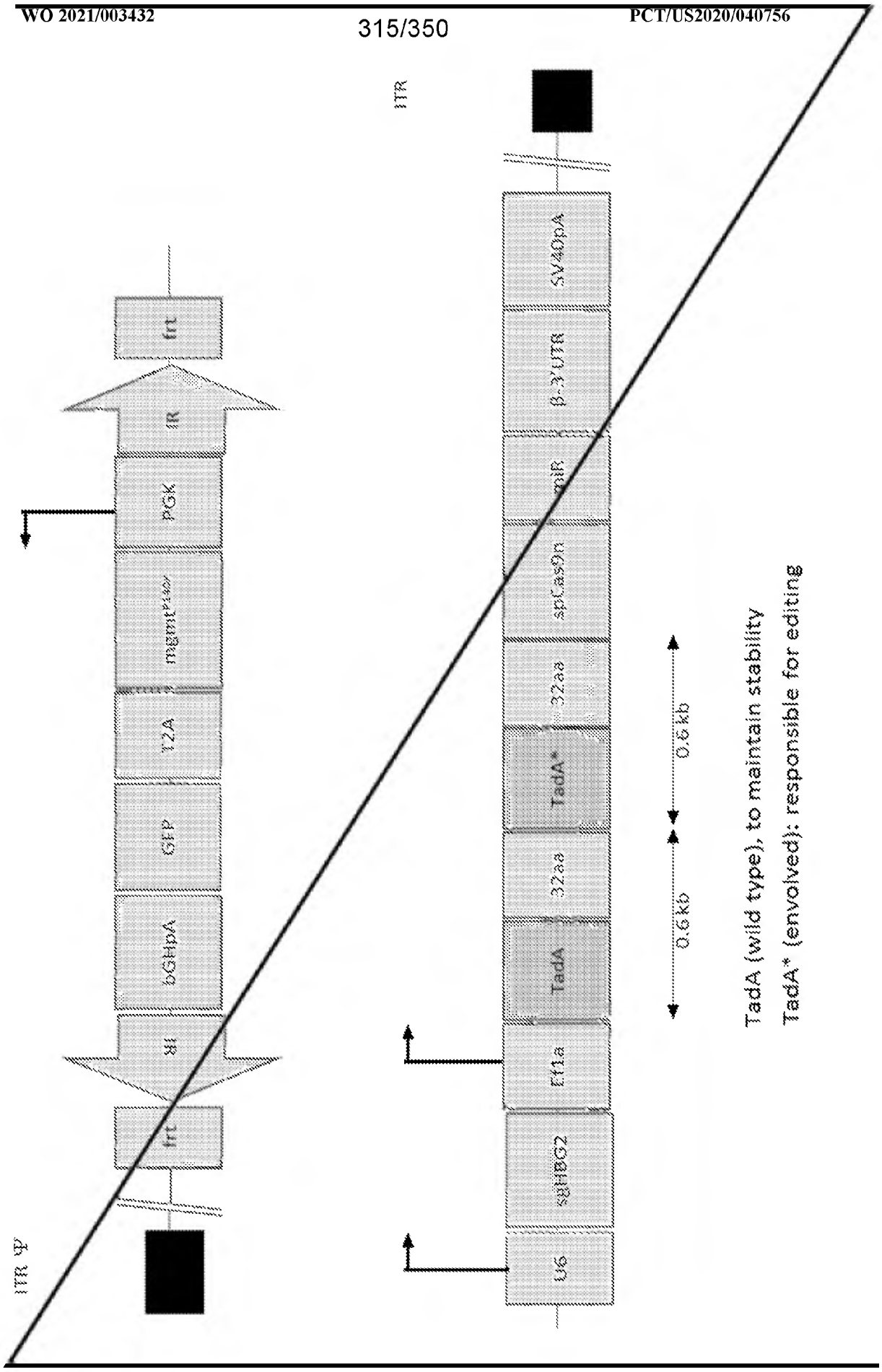

ITR Ψ
frt
SI
bGHpA
GFP
T2A
mgmtP140K
PGK
IR
frt
ITR
U6
sgHBG2
EF1a
TadA
32aa
TadA*
32aa
spCas9n
NLS
β-3'UTR
SV40pA
0.6 kb
0.6 kb
TadA (wild type), to maintain stability
TadA* (envolved): responsible for editing

FIG. 145

FIG. 146



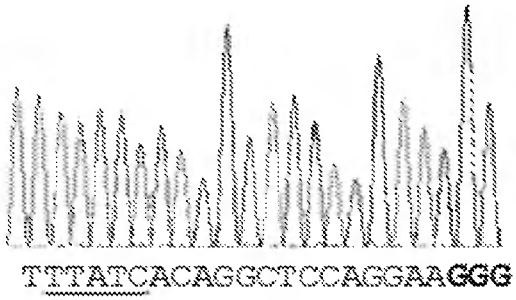
(SEQ ID NO: 265)
(SEQ ID NO: 266)

DNA sequence of two 'TadA + 32aa'

(SEQ ID NO: 267)
(SEQ ID NO: 268)

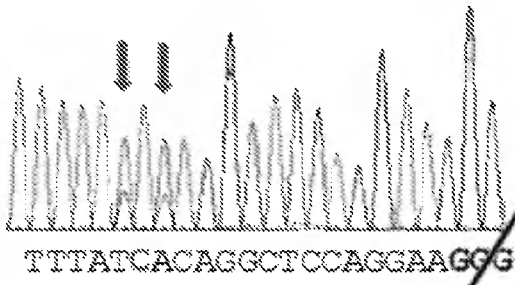
FIG. 147





Wild type sequence
Underlined: GATAA motif
Bold: PAM motif

(SEQ ID NO: 269)



Base edited sequence
Arrows: edited bases
Purple: disrupted base of GATAA motif

(SEQ ID NO: 269)

FIG. 148

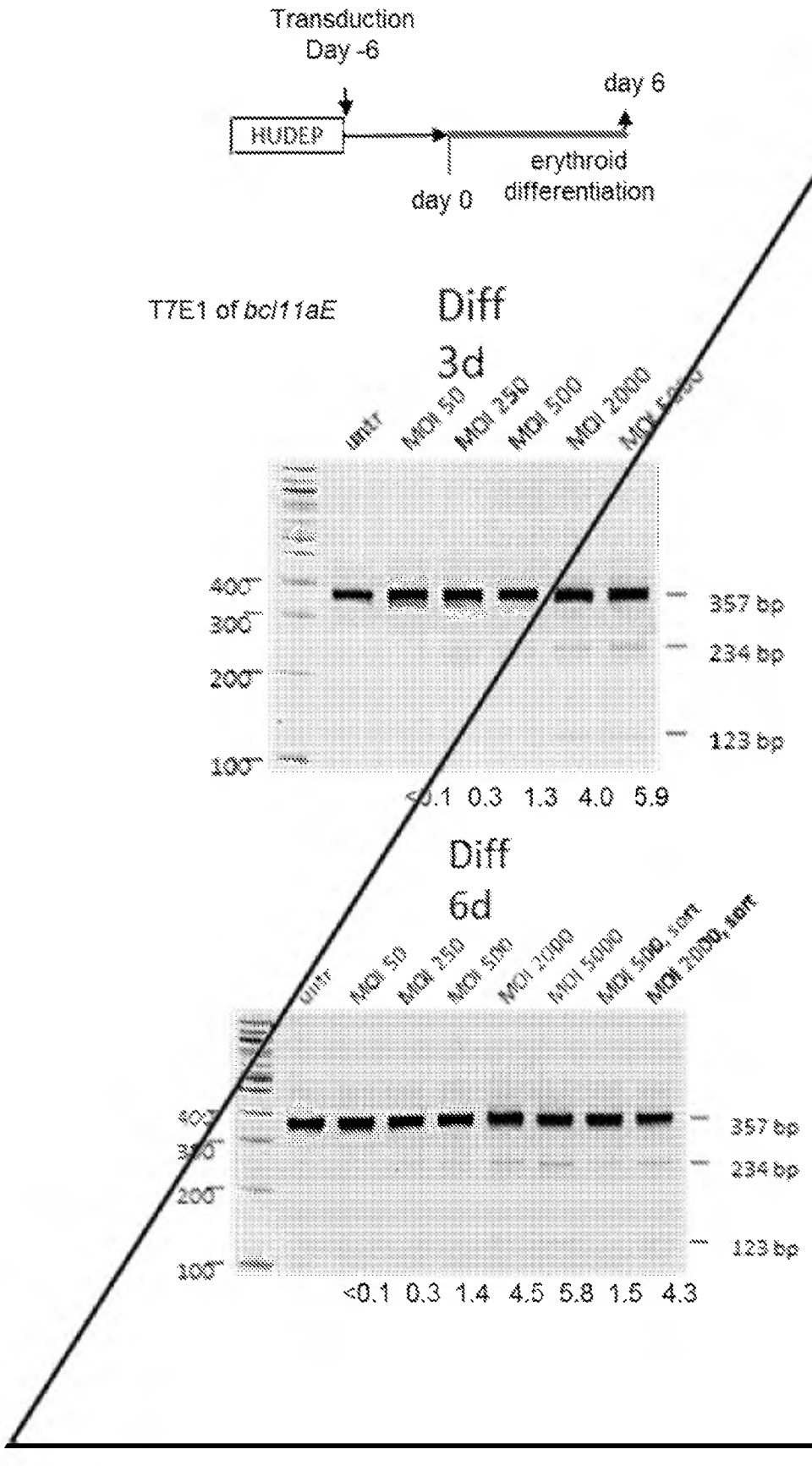

Transduction
Day -6
day 6
HUDEP
erythroid
differentiation
day 0
T7E1 of bcl11aE
Diff
3d
untr
MOI 50
MOI 150
MOI 500
MOI 2000
MOI 5000
400
300
200
100
357 bp
234 bp
123 bp
<0.1 0.3 1.3 4.0 5.9
Diff
6d
untr
MOI 50
MOI 150
MOI 500
MOI 2000
MOI 5000
MOI 500, stim
MOI 2000, stim
400
300
200
100
357 bp
234 bp
123 bp
<0.1 0.3 1.4 4.5 5.8 1.5 4.3

FIG. 149

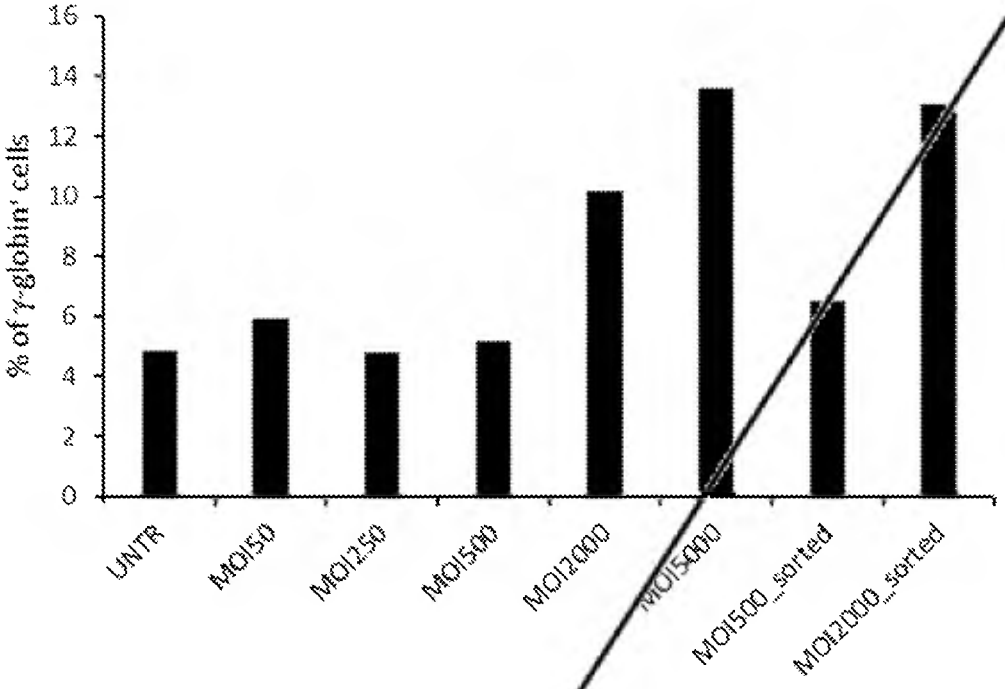


FIG. 150

Reactivation of HbF by Base editing

Cytidine base editor: C → T
Adenine base editor: A → G

Constructed vectors:

pBS-AncBE4max-sgBCL11AE1	+58 GATA motif, G --> A
pBS-AncBE4max-sgBCL11AE2	+58 GATA motif, G --> A
pBS-ABEmax-sgBCL11AE3	+58 GATA motif, T --> C
pBS-ABEmax-sgBCL11AE4	+58 GATA motif, T --> C
pBS-AncBE4max-sgHBG1	13bp deletion, CCAAT box, CC --> TT
pBS-ABEmax-sgHBG2	13bp deletion, CCAAT box, A --> G
pBS-ABEmax-sgHBG3	13bp deletion, CCAAT box, T --> C
pBS-ABEmax-sgHBG4	-198 T --> C
pBS-AncBE4max-sgRS-1	control

FIG. 151

	Date	Full name	Titer	Note
	101918-3	HDAd5/35++_BE4max-sgBCL11Ae1-FI-mgmtGFP	0.5 x 10e12	
→	041619-2	HDAd5/35++_BE4max-sgHBG1-FI-mgmtGFP	very low (use as 0.05 x 10e12)	
→	032819-1	HDAd5/35++_ABEopti-sgHBG2-FI-mgmtGFP	2.8 x 10e12	
→	041819-1	HDAd5/35++_ABEopti-sgHBG4-FI-mgmtGFP	1.52 x 10e12	
→	041619-3	HDAd5/35++_BE4max-Dual1-FI-mgmtGFP	very low (use as 0.05 x 10e12)	sgBcl1 + sgHBG1 sgHBG2 + sgHBB-Stop
	041619-1	HDAd5/35++_ABEopti-Dual-skip-FI-mgmtGFP	2.5 x 10e12	
	041819-2	HDAd5/35++_BE4max-sgR5-1-FI-mgmtGFP	0.9 x 10e12	Non-targeting ctrl

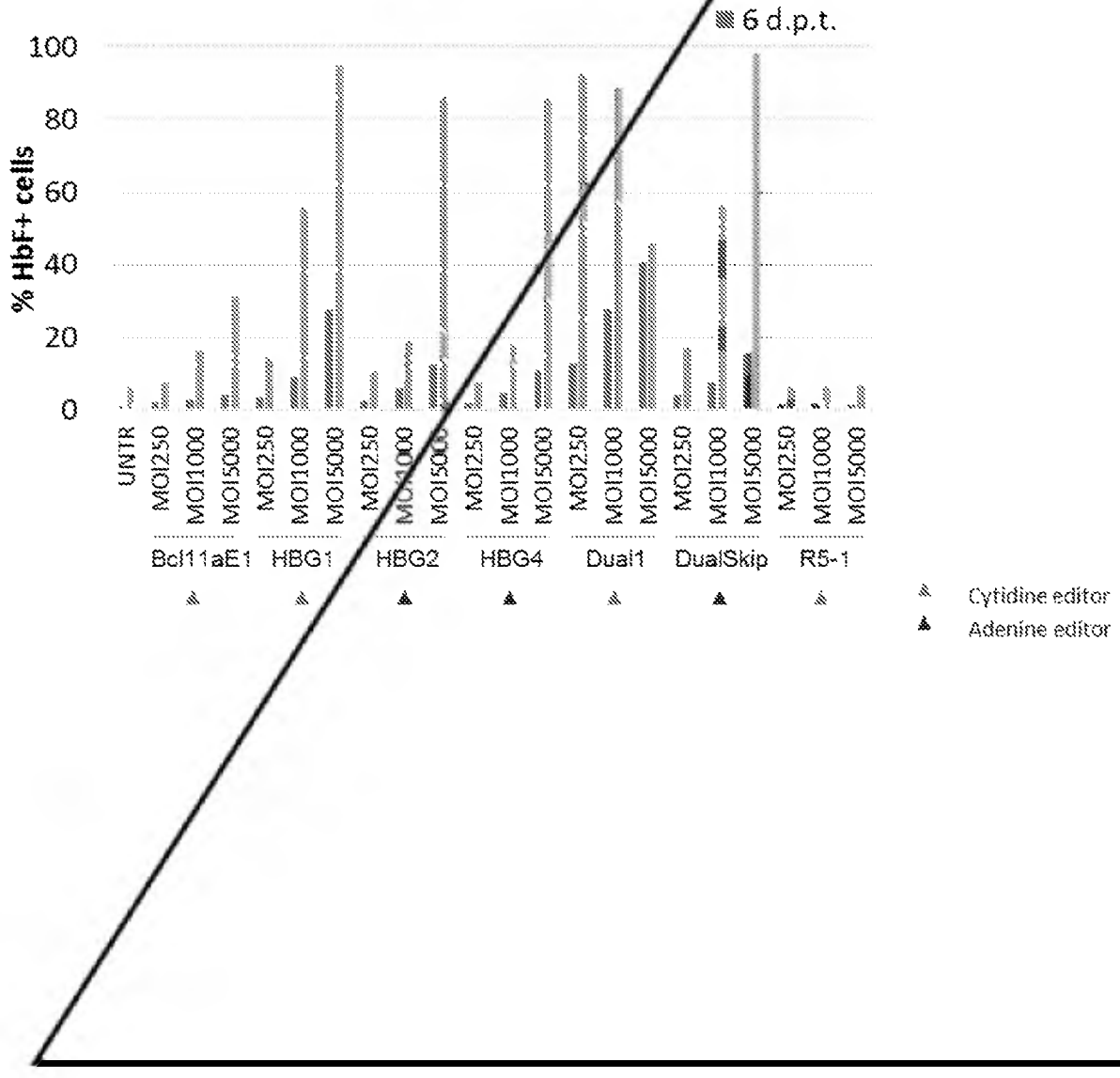


FIG. 152

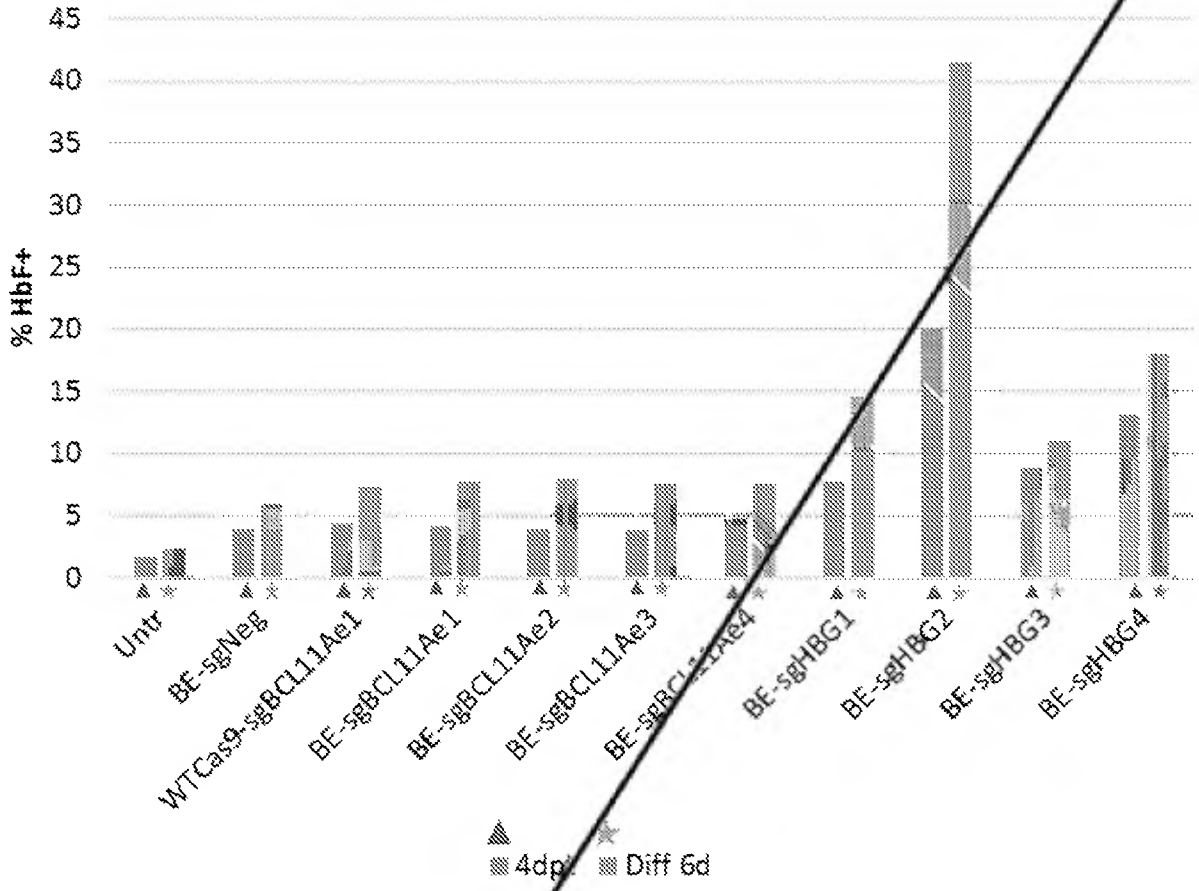
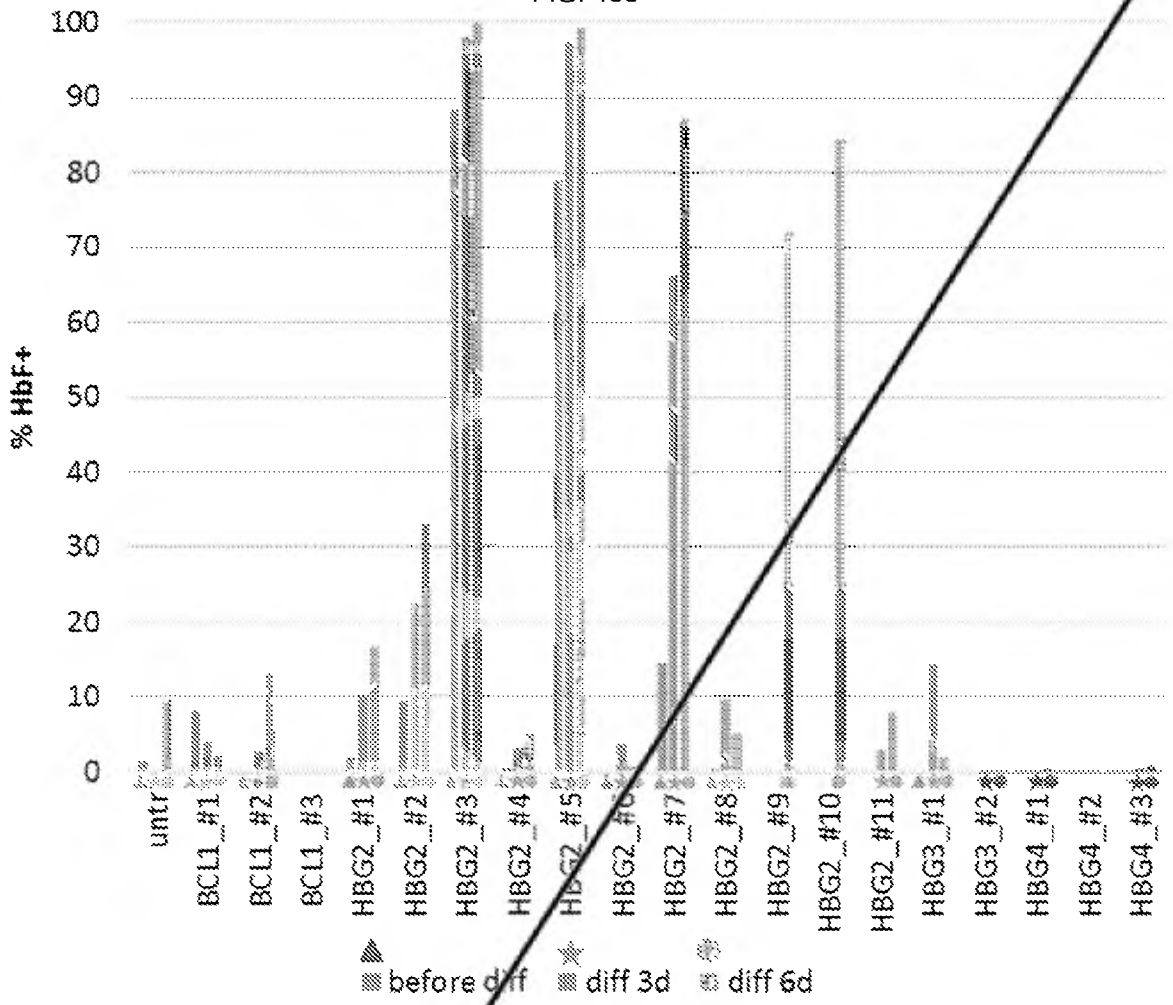

% HbF+
45
40
35
30
25
20
15
10
5
0
Untr
BE-sgNeg
WTCas9-sgBCL11Ae1
BE-sgBCL11Ae1
BE-sgBCL11Ae2
BE-sgBCL11Ae3
BE-sgBCL11Ae4
BE-sgHBG1
BE-sgHBG2
BE-sgHBG3
BE-sgHBG4
4dpt
Diff 6d

FIG. 153


% HbF+
100
90
80
70
60
50
40
30
20
10
0
untr
BCL1_#1
BCL1_#2
BCL1_#3
HBG2_#1
HBG2_#2
HBG2_#3
HBG2_#4
HBG2_#5
HBG2_#6
HBG2_#7
HBG2_#8
HBG2_#9
HBG2_#10
HBG2_#11
HBG3_#1
HBG3_#2
HBG4_#1
HBG4_#2
HBG4_#3
before diff
diff 3d
diff 6d

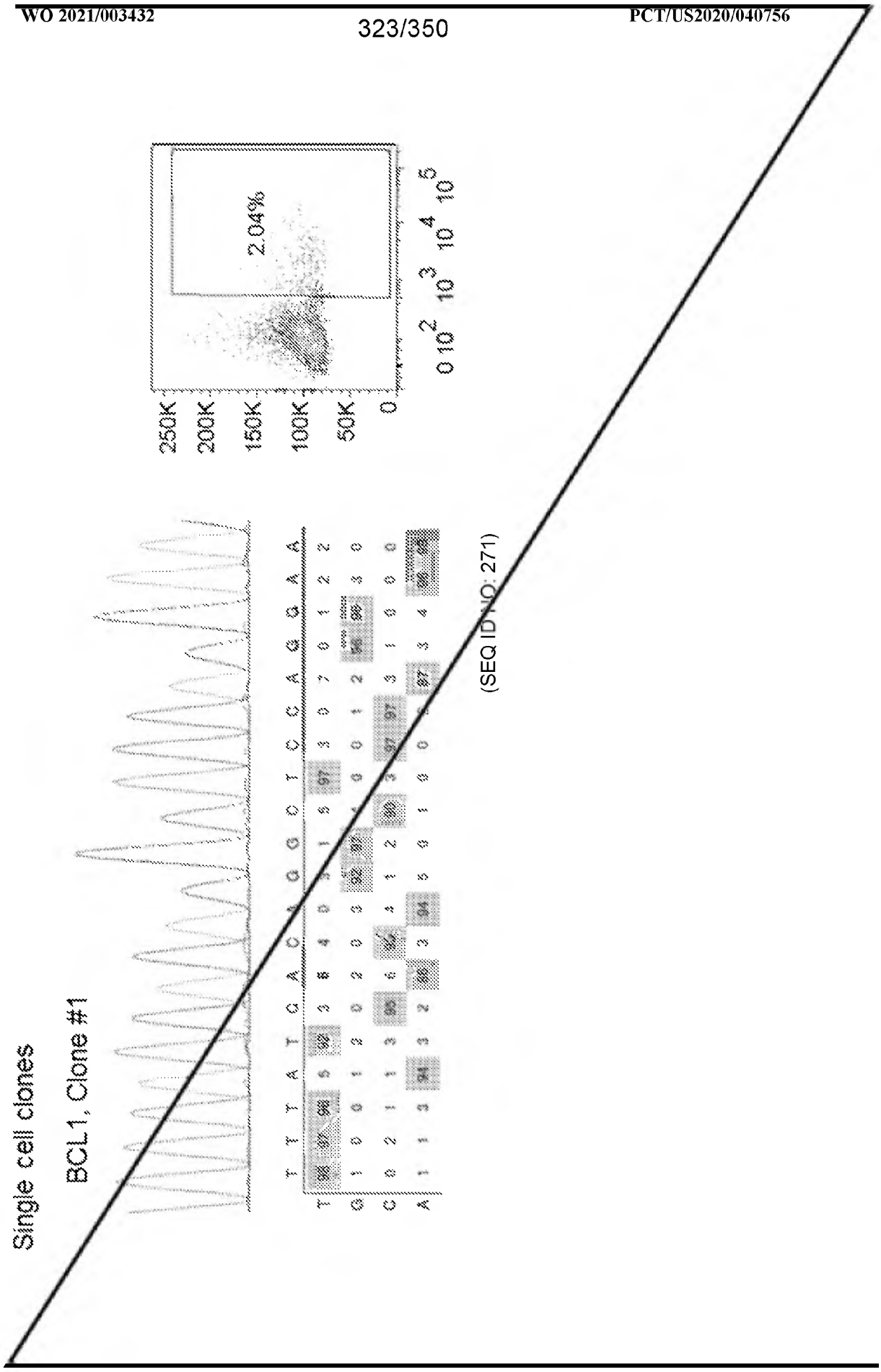

Single cell clones
BCL1, Clone #1
2.04%
250K
200K
150K
100K
50K
0
0 10^2 10^3 10^4 10^5
(SEQ ID NO: 271)

FIG. 154A

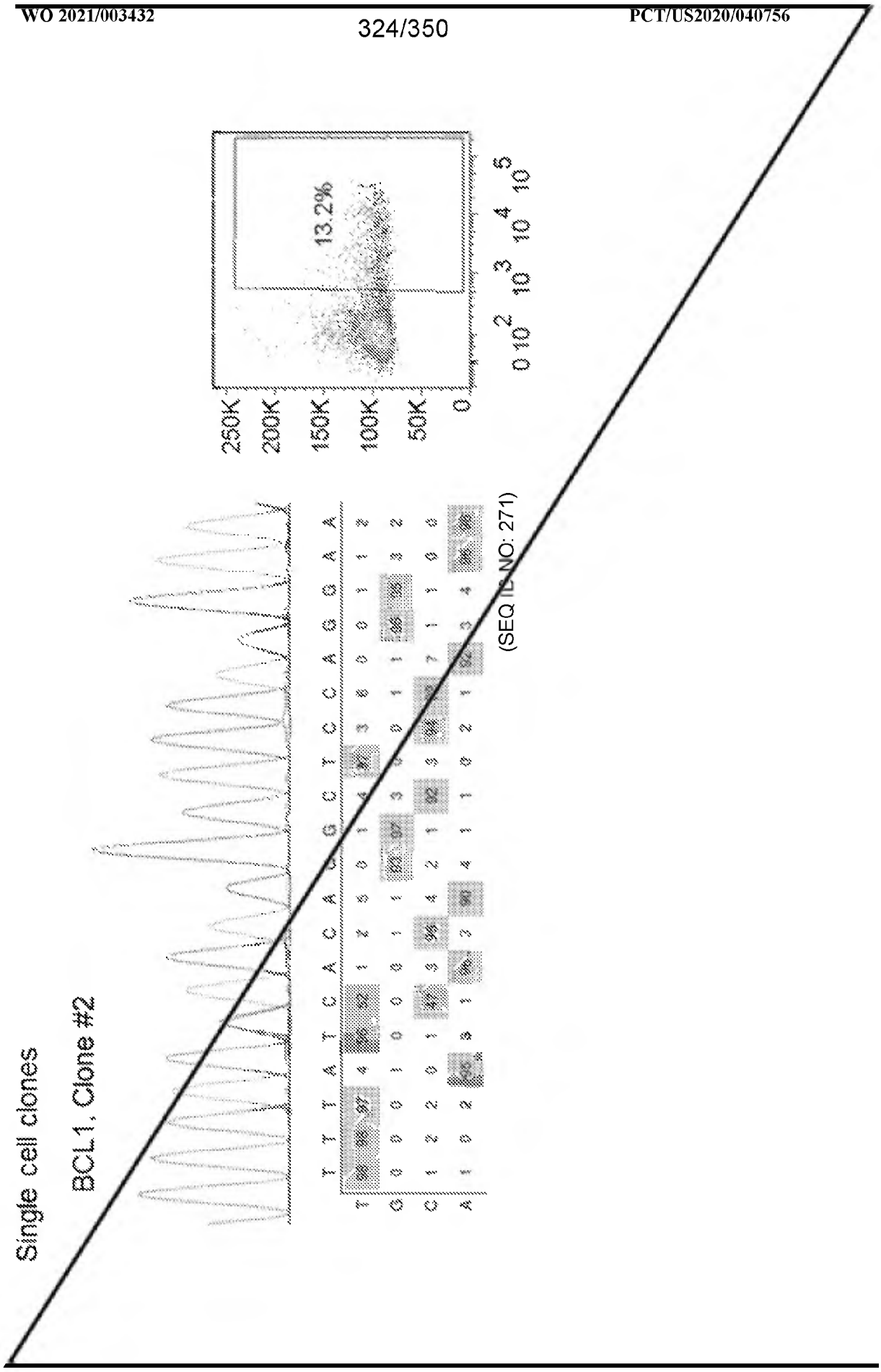

Single cell clones
BCL1, Clone #2
T T T A T C A C A G G C T C C A G G A A
(SEQ ID NO: 271)
250K
200K
150K
100K
50K
0
13.2%
0 10^2 10^3 10^4 10^5

FIG. 154B

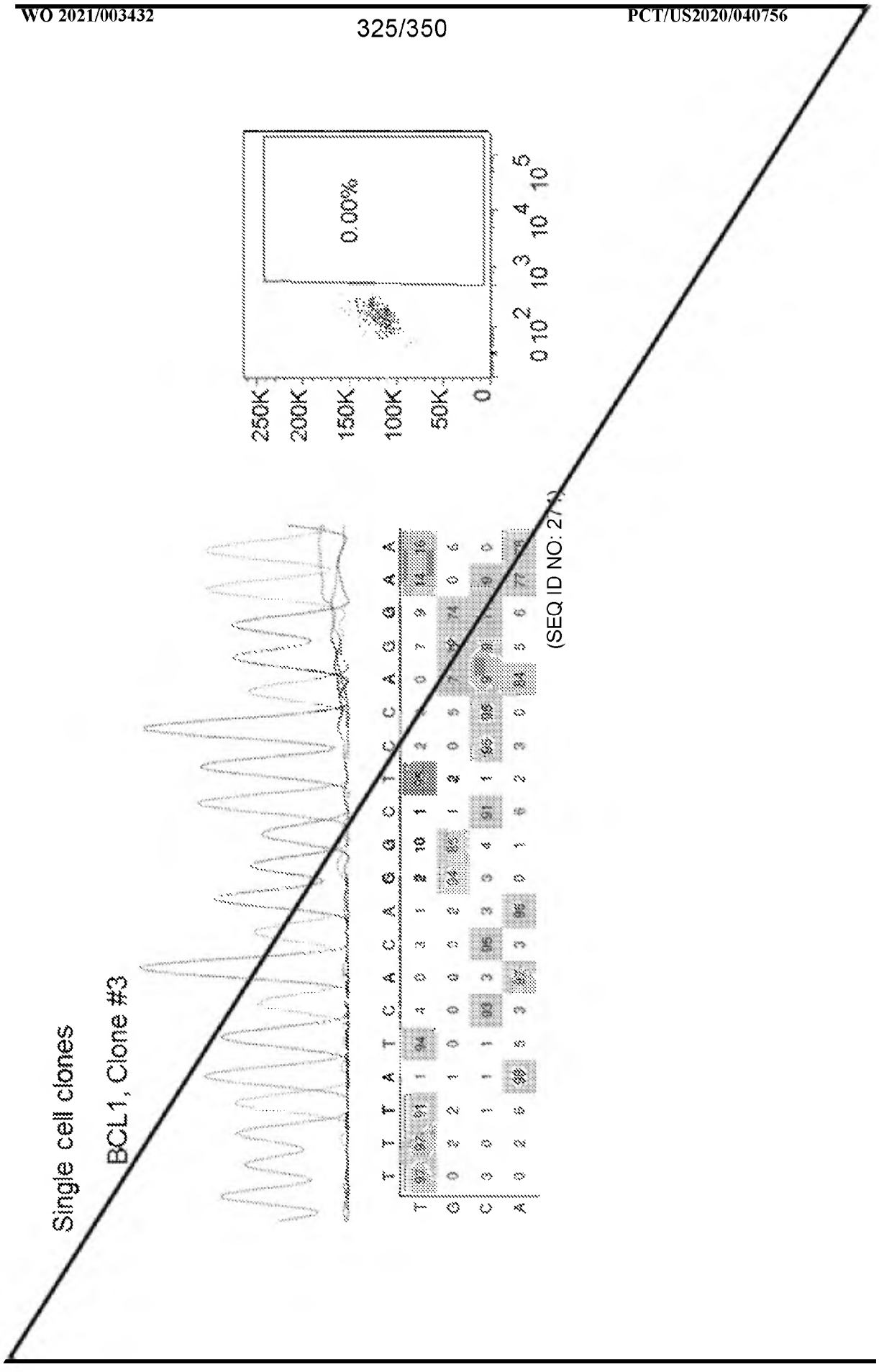

Single cell clones
BCL1, Clone #3
0.00%
250K
200K
150K
100K
50K
0
0 10^2 10^3 10^4 10^5
T T T A T C A C A G G C T C C A G G A A
T
G
C
A
(SEQ ID NO: 272)

FIG. 154C

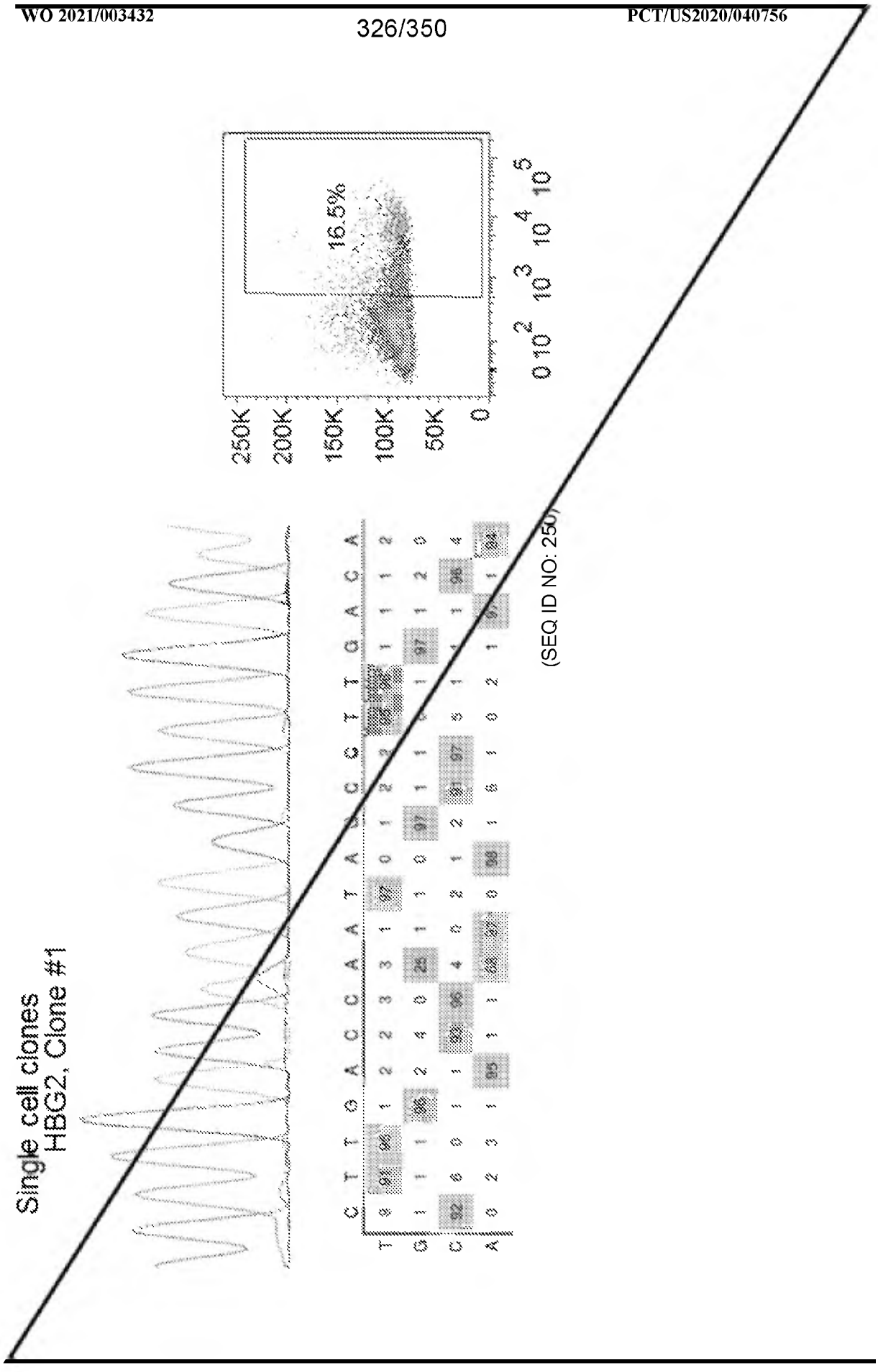

Single cell clones
HBG2, Clone #1
16.5%
250K
200K
150K
100K
50K
0
0 10^2 10^3 10^4 10^5
C T T G A C C A A T A G C C T T G A C A
T
G
C
A
(SEQ ID NO: 250)

FIG. 154D

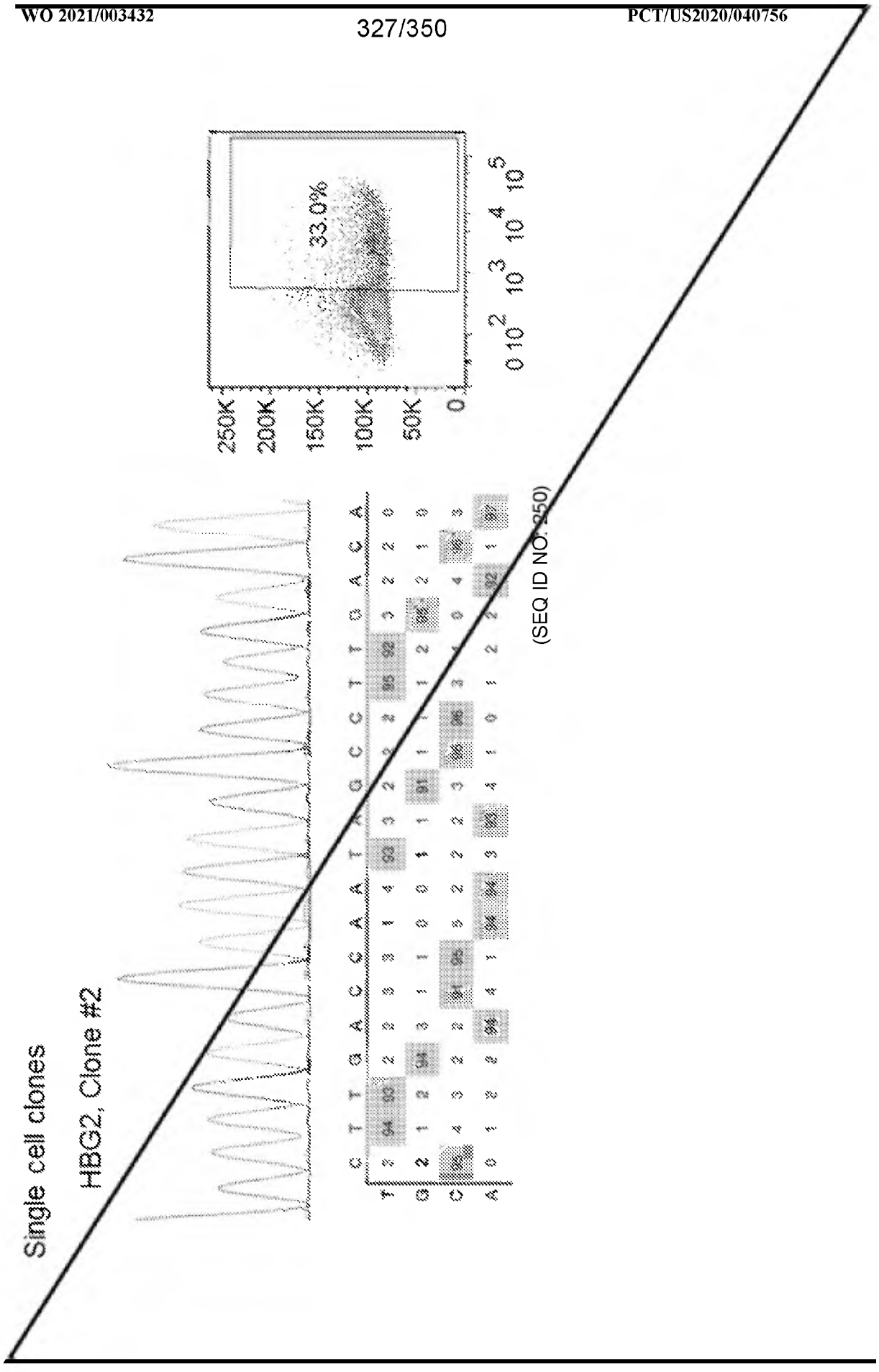

Single cell clones
HBG2, Clone #2
33.0%
250K
200K
150K
100K
50K
0
0 10^2 10^3 10^4 10^5
(SEQ ID NO: 250)

FIG. 154E

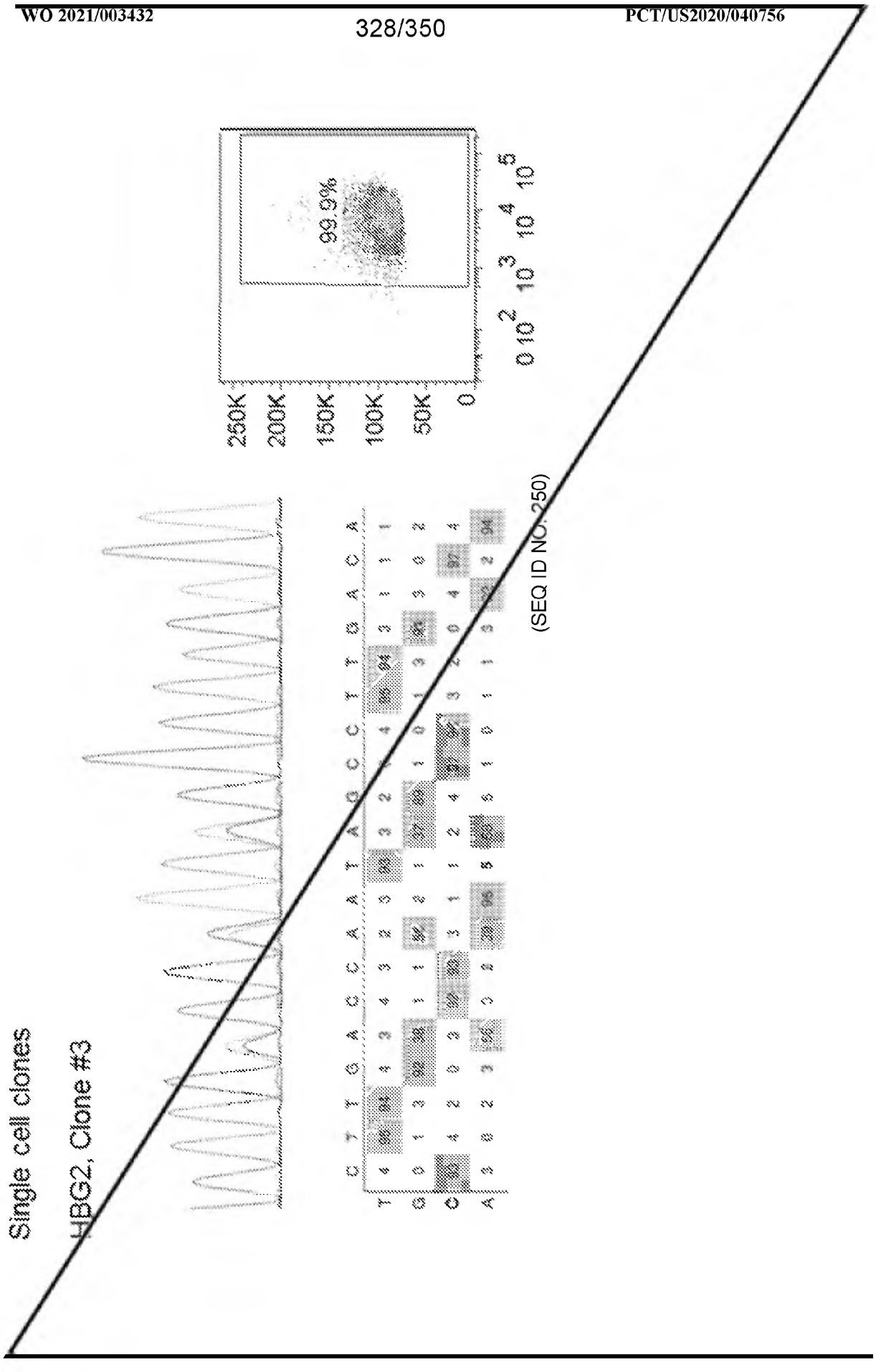

Single cell clones
HBG2, Clone #3
99.9%
250K
200K
150K
100K
50K
0
0 10^2 10^3 10^4 10^5
(SEQ ID NO: 250)

FIG. 154F

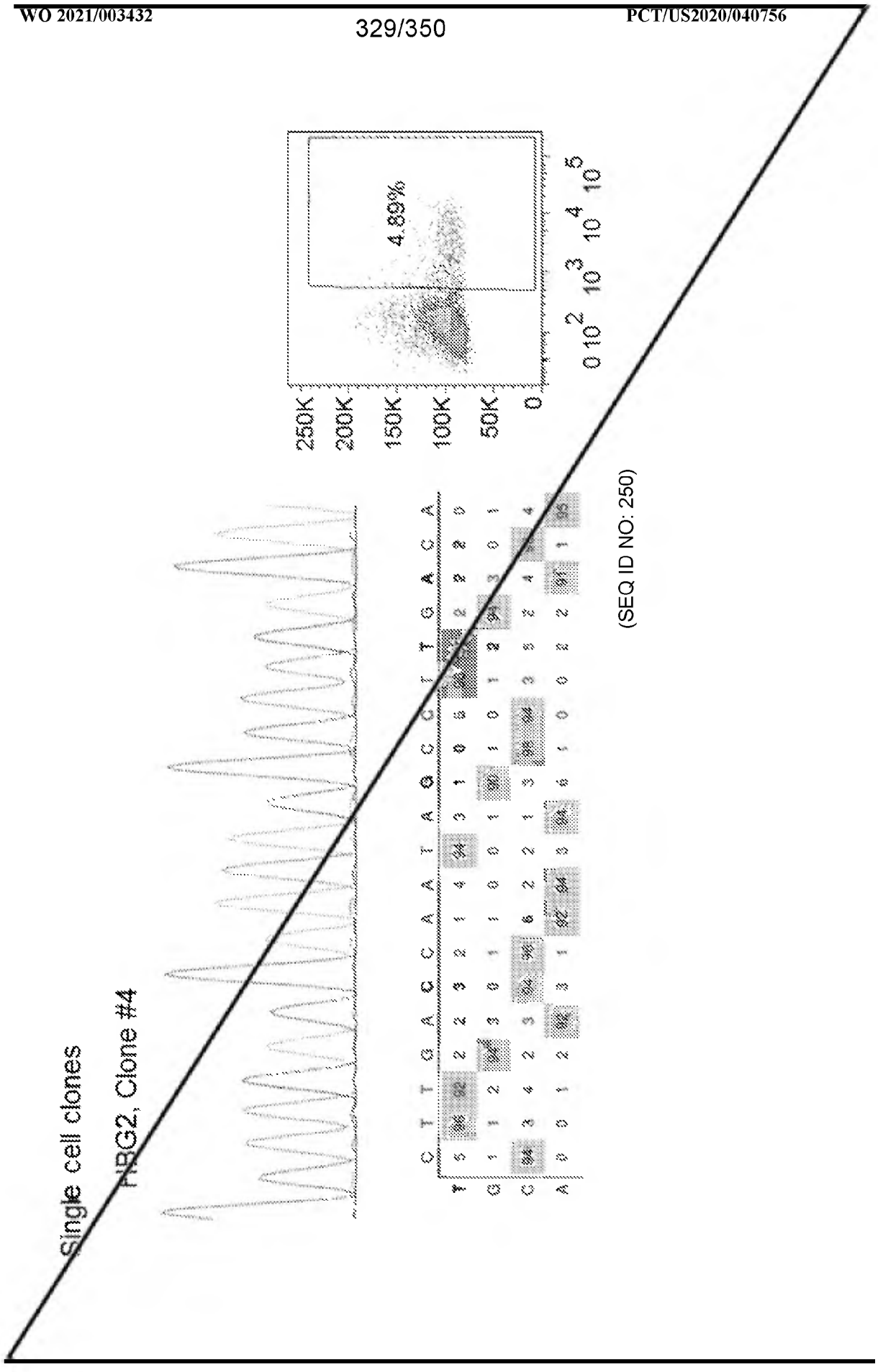
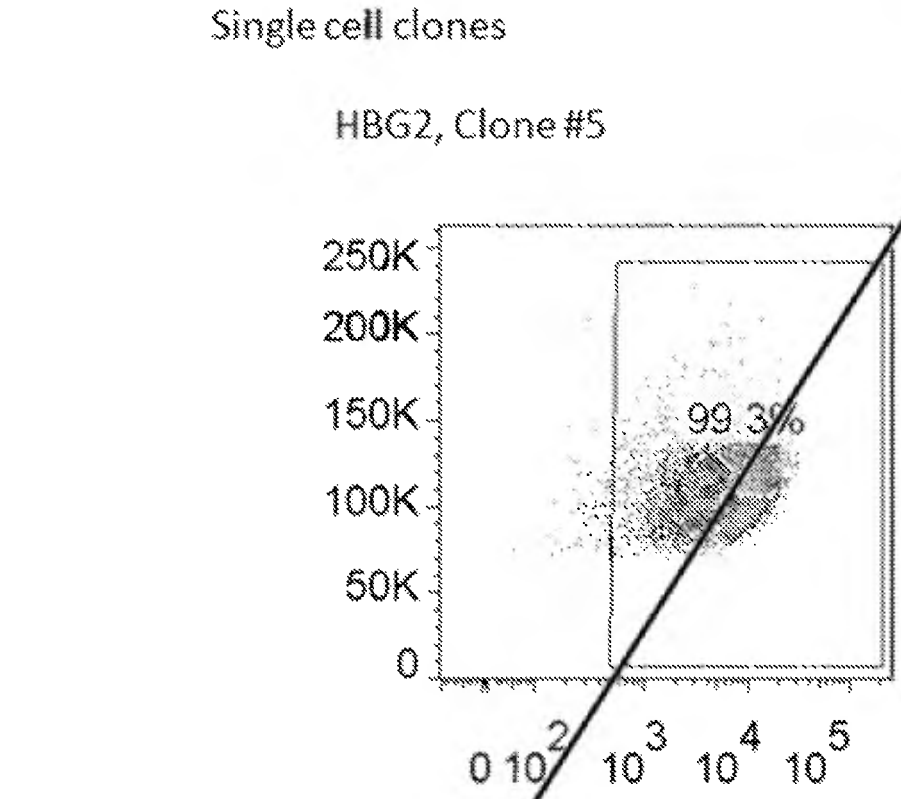

Single cell clones
HBG2, Clone #4
4.89%
250K
200K
150K
100K
50K
0
0 10^2 10^3 10^4 10^5
(SEQ ID NO: 250)

FIG. 154G

FIG. 154H


Single cell clones
HBG2, Clone #5
250K
200K
150K
100K
50K
0
99.3%
0
10^2
10^3
10^4
10^5

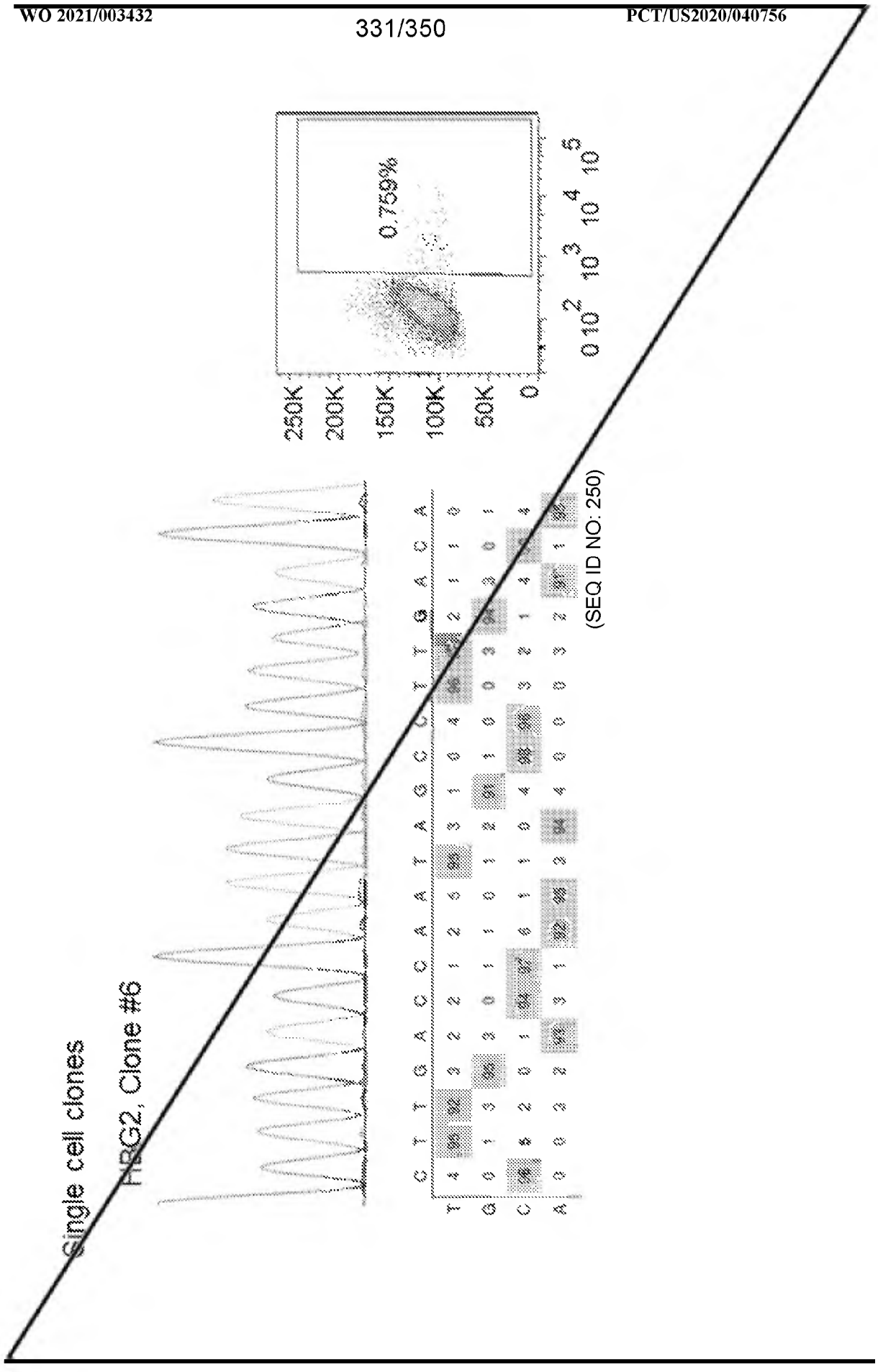

Single cell clones
HBG2, Clone #6
C T T G A C C C A A T A G C C C T T G A C A
T
G
C
A
0.759%
250K
200K
150K
100K
50K
0
0 10^2 10^3 10^4 10^5
(SEQ ID NO: 250)

FIG. 154I

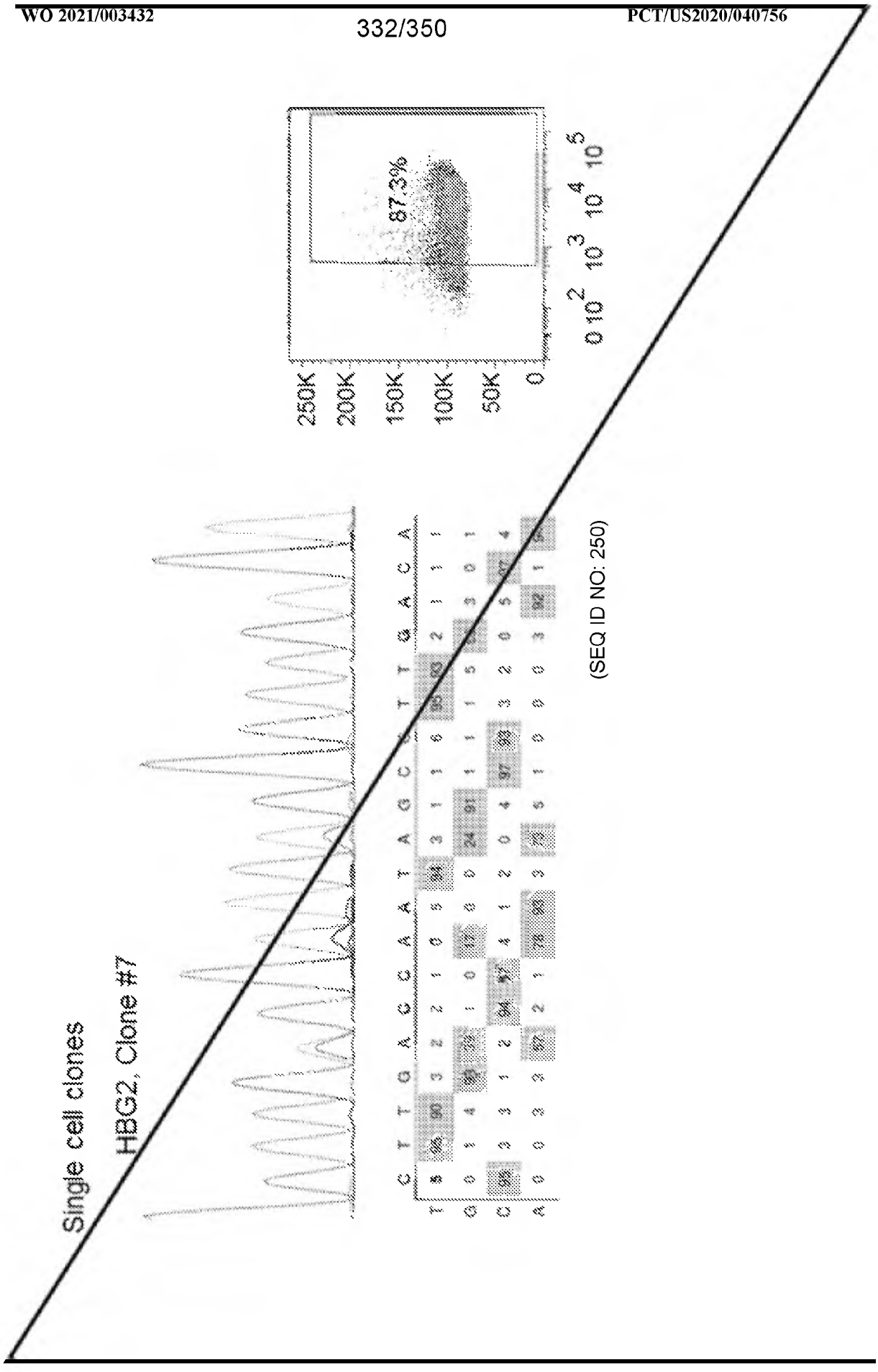

Single cell clones
HBG2, Clone #7
87.3%
250K
200K
150K
100K
50K
0
0 10^2 10^3 10^4 10^5
(SEQ ID NO: 250)

FIG. 154J

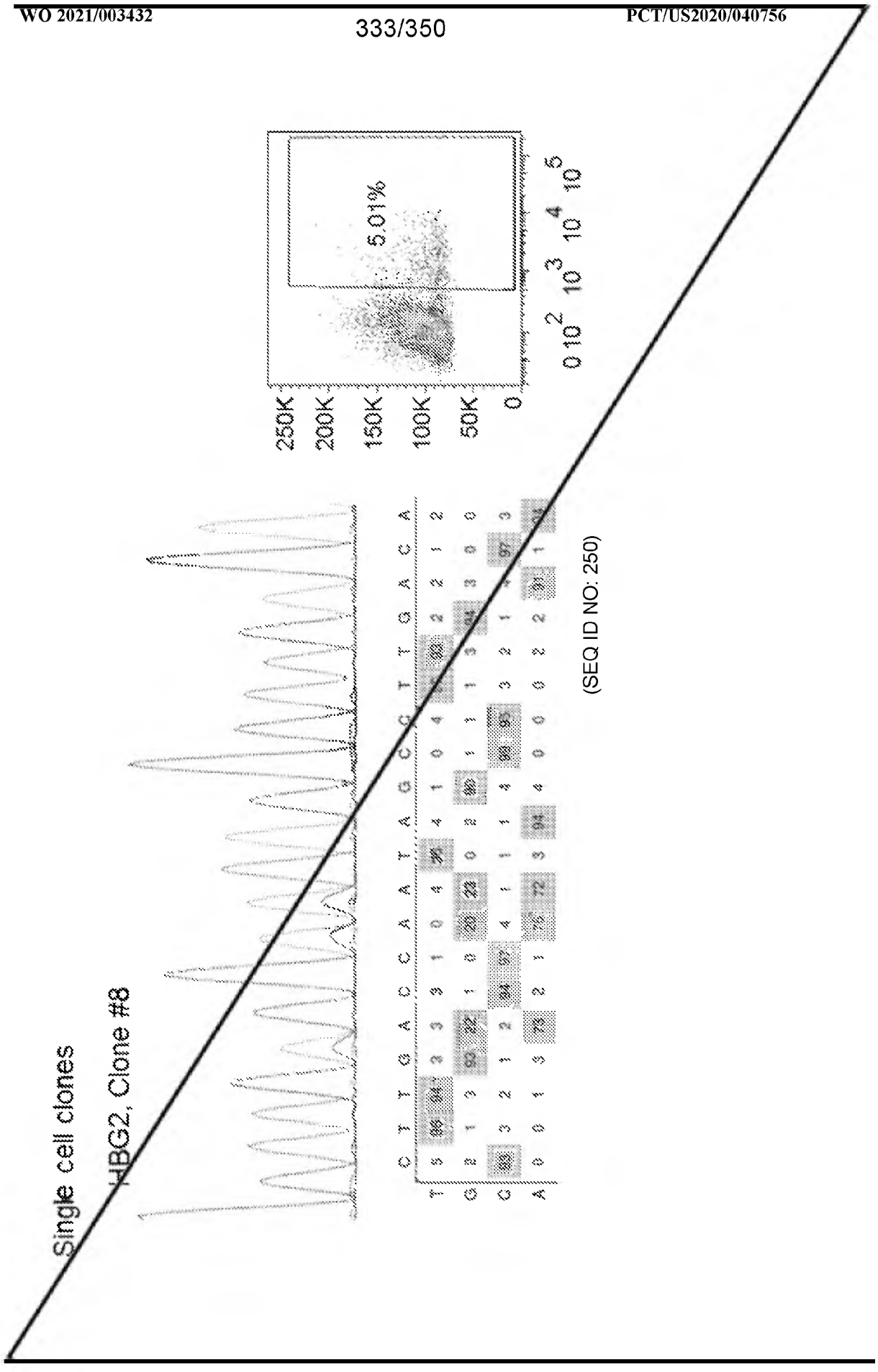

Single cell clones
HBG2, Clone #8
5.01%
250K
200K
150K
100K
50K
0
0 10^2 10^3 10^4 10^5
C T T G A C C C A A T A G G C C T T G A C A
T
G
C
A
(SEQ ID NO: 250)

FIG. 154K

FIG. 154L

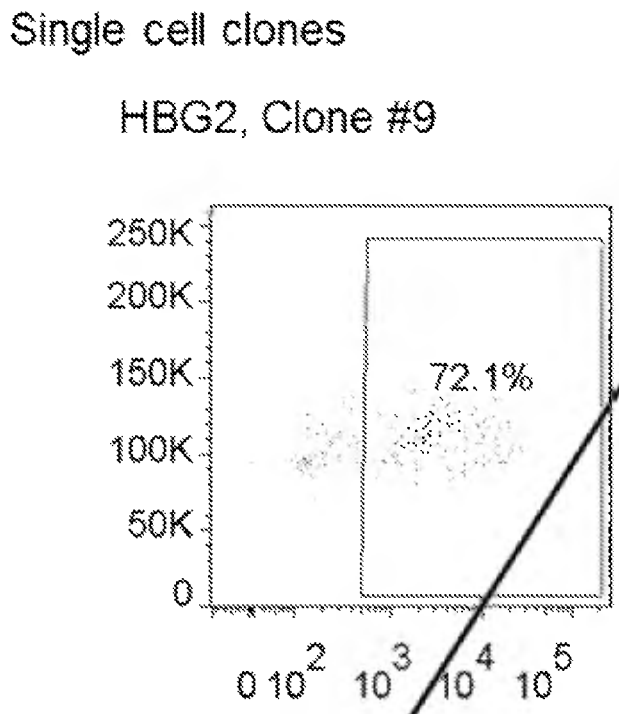


FIG. 154M

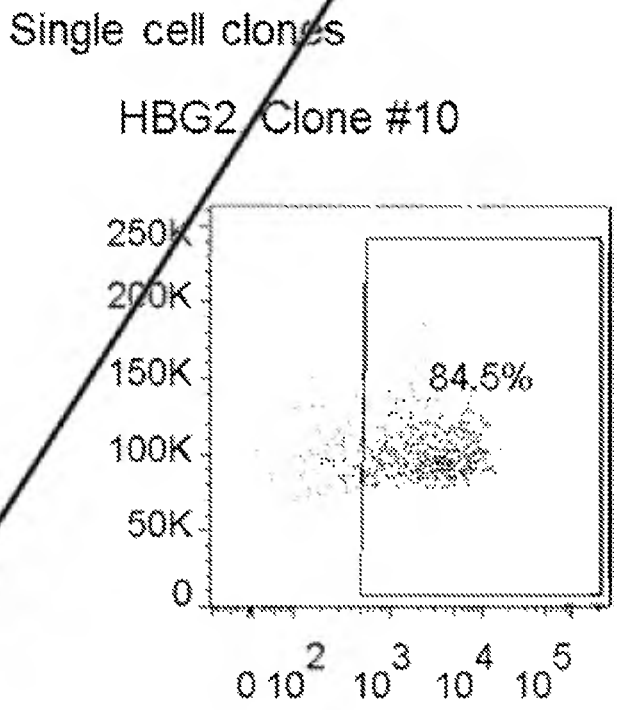
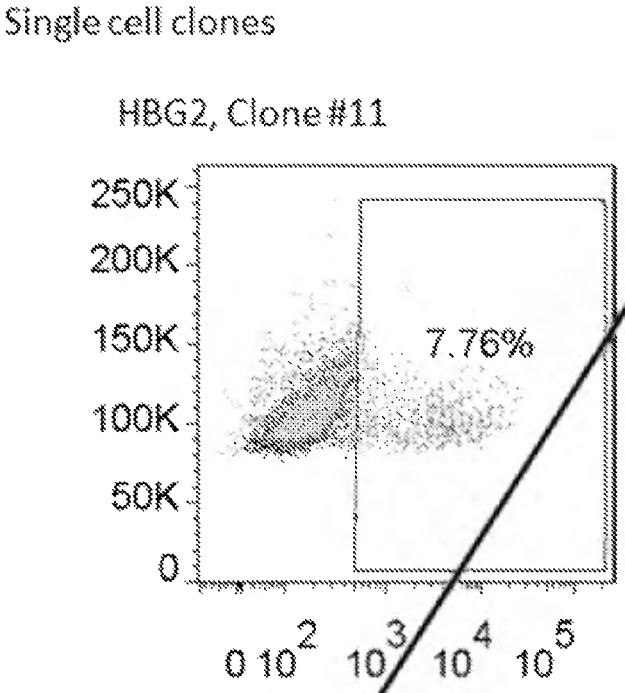


FIG. 154N


Single cell clones
HBG2, Clone #11
250K
200K
150K
100K
50K
0
7.76%
0 10^2 10^3 10^4 10^5

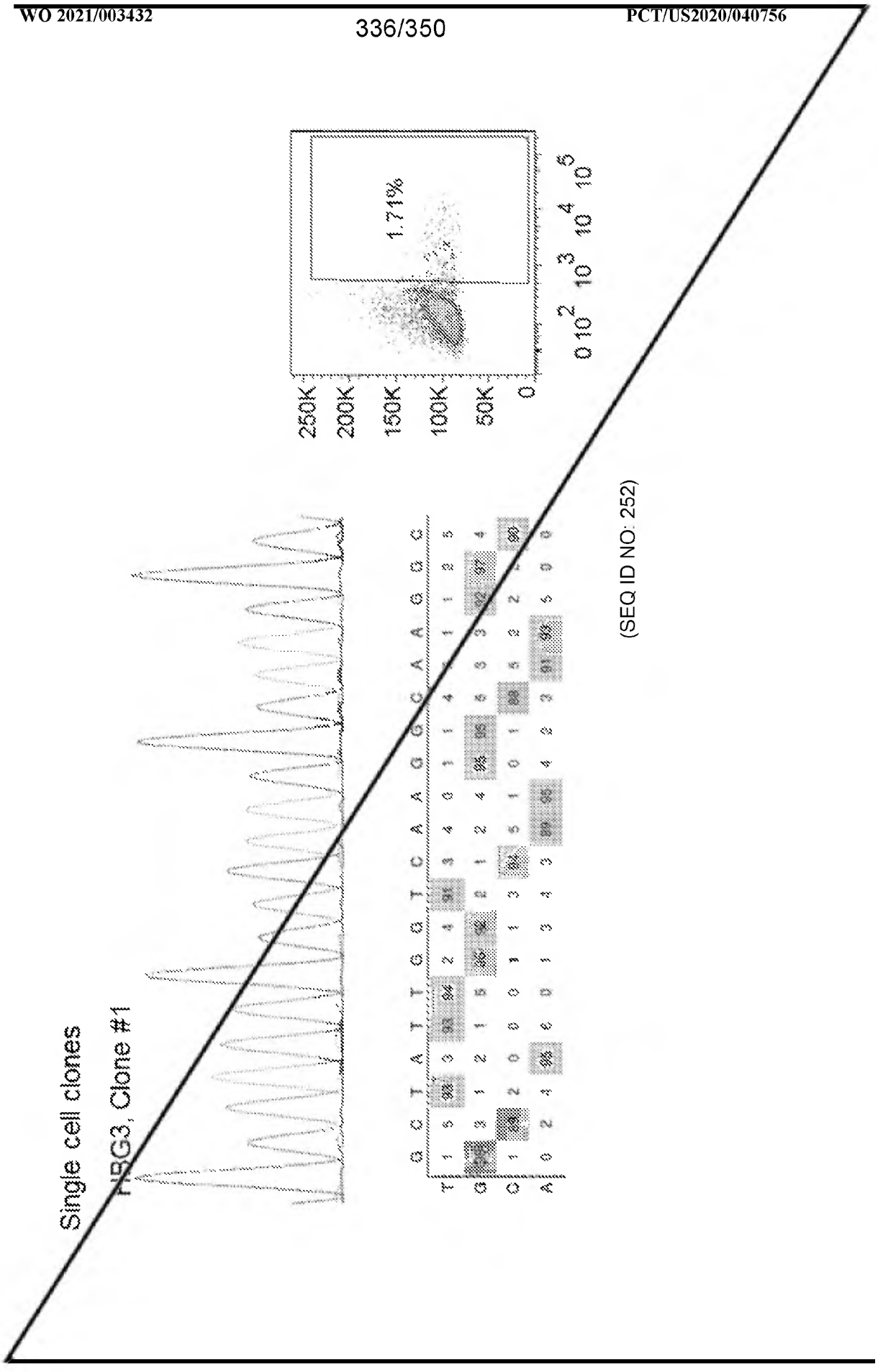

Single cell clones
RBG3, Clone #1
1.71%
250K
200K
150K
100K
50K
0
0 10^2 10^3 10^4 10^5
T
G
C
A
(SEQ ID NO: 252)

FIG. 154O

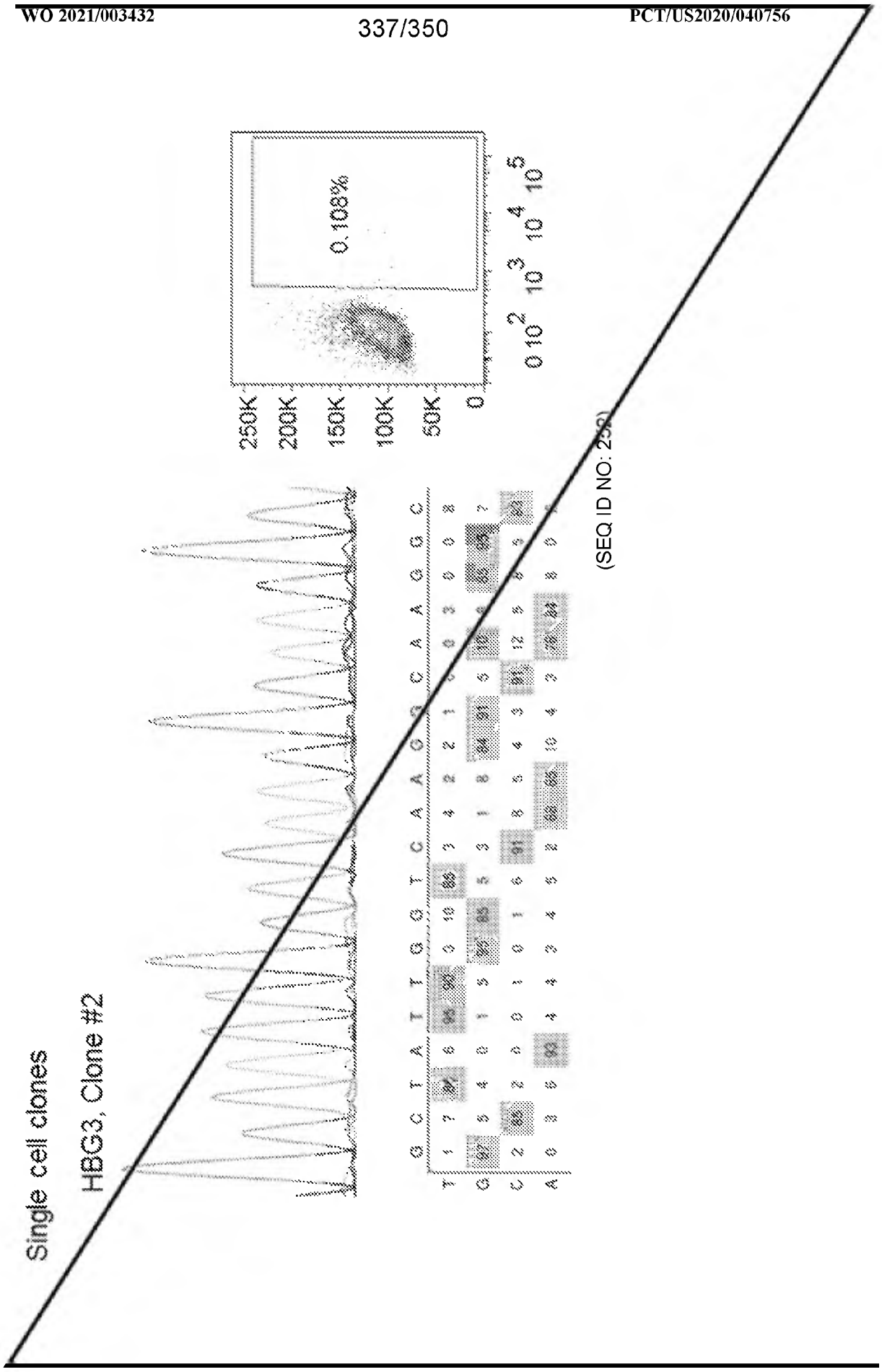

Single cell clones
HBG3, Clone #2
0.108%
250K
200K
150K
100K
50K
0
0 10^2 10^3 10^4 10^5
(SEQ ID NO: 258)

FIG. 154P

FIG. 154Q

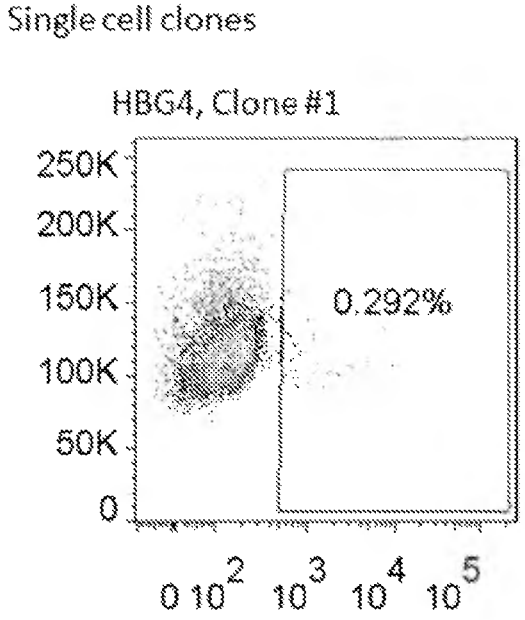


FIG. 154R

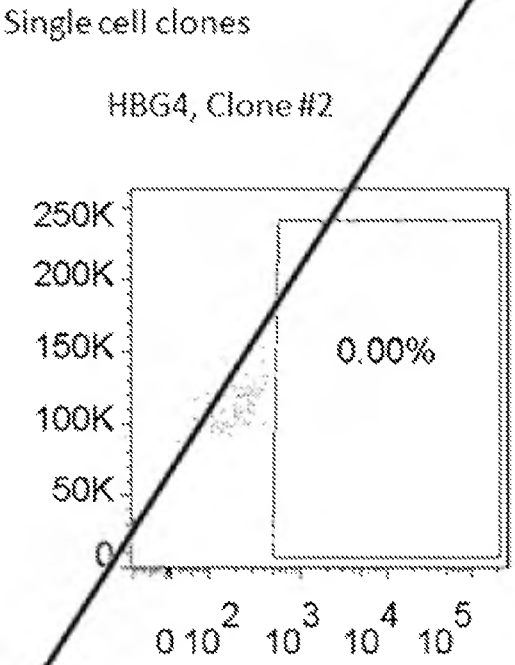
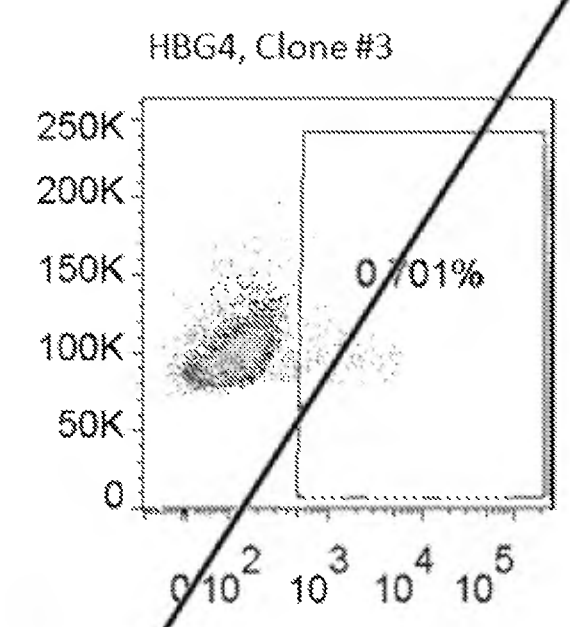


FIG. 154S



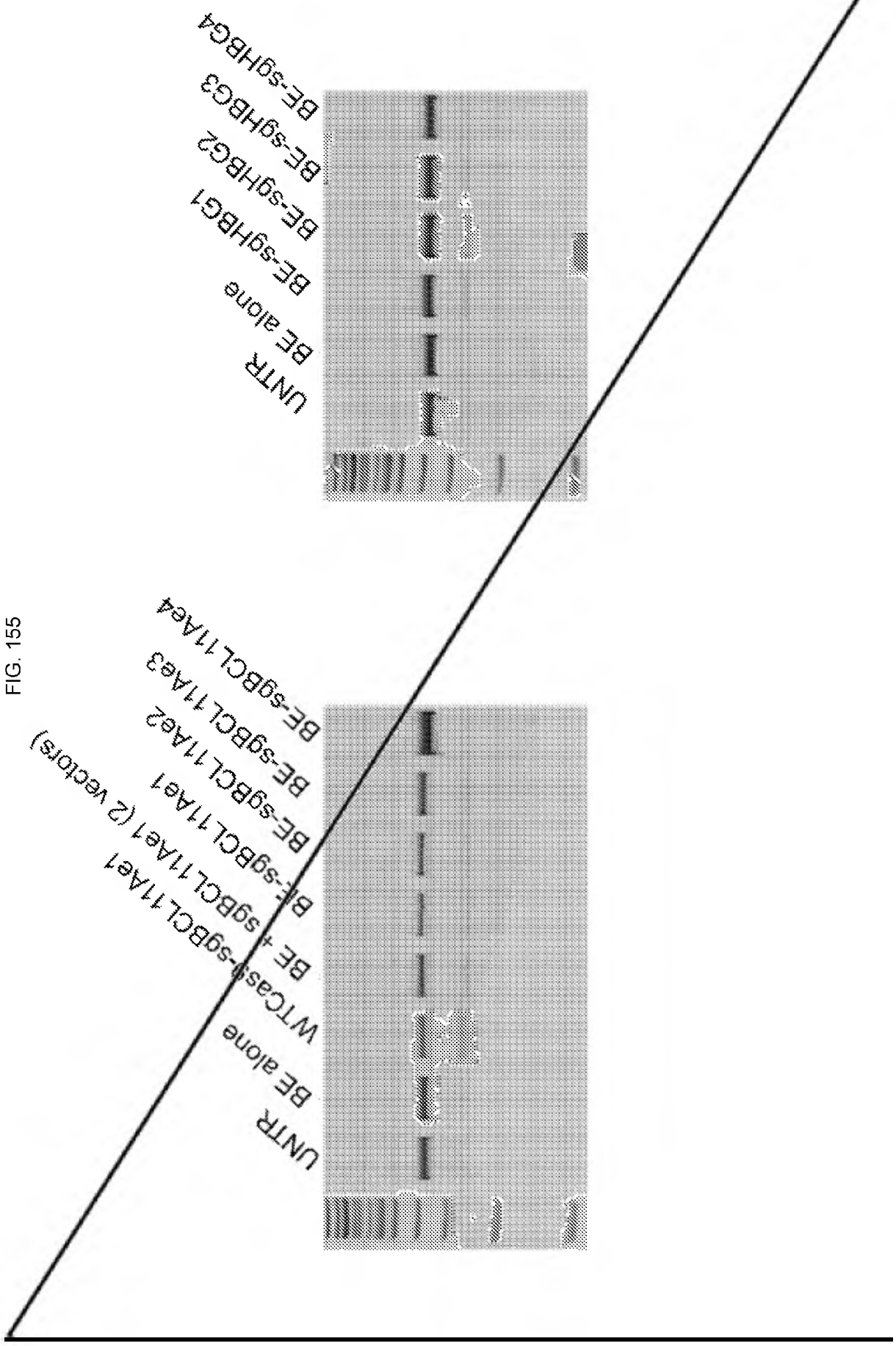

UNTR
BE alone
BE-sgHBG1
BE-sgHBG2
BE-sgHBG3
BE-sgHBG4
UNTR
BE alone
WTCas9-sgBCL11Ae1
BE + sgBCL11Ae1 (2 vectors)
BE-sgBCL11Ae1
BE-sgBCL11Ae2
BE-sgBCL11Ae3
BE-sgBCL11Ae4

FIG. 155

FIG. 156A

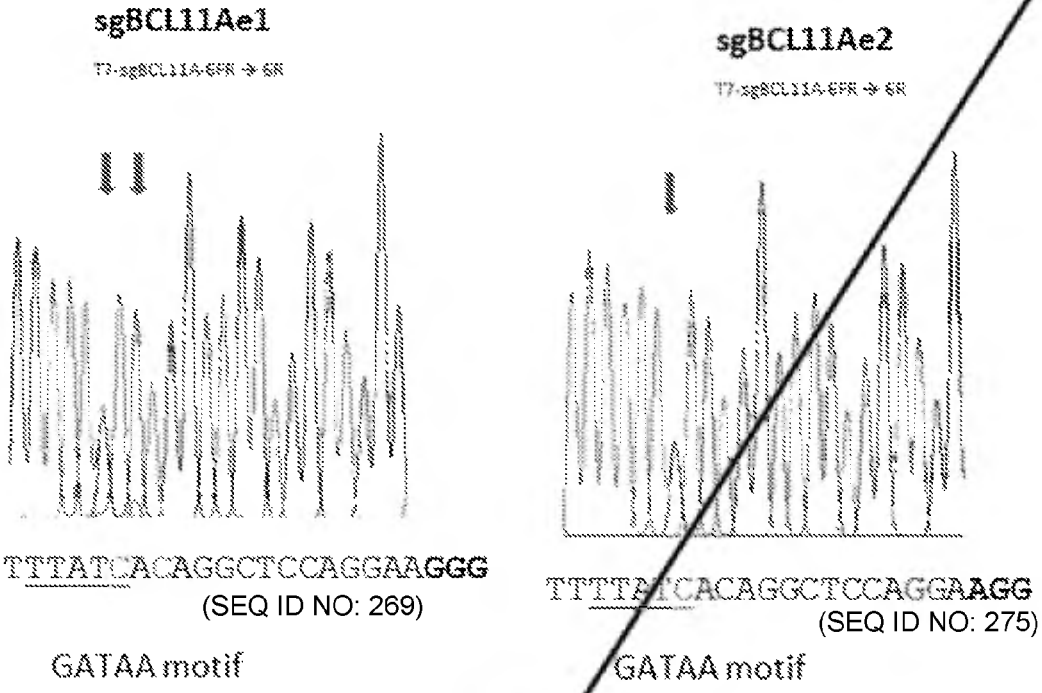


FIG. 156B

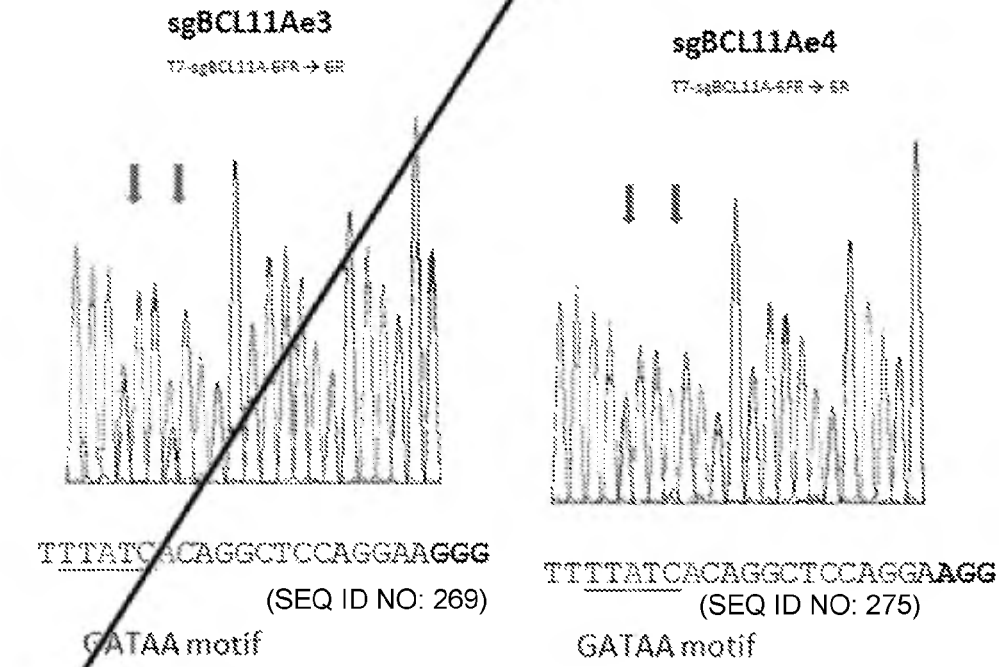


FIG. 156C

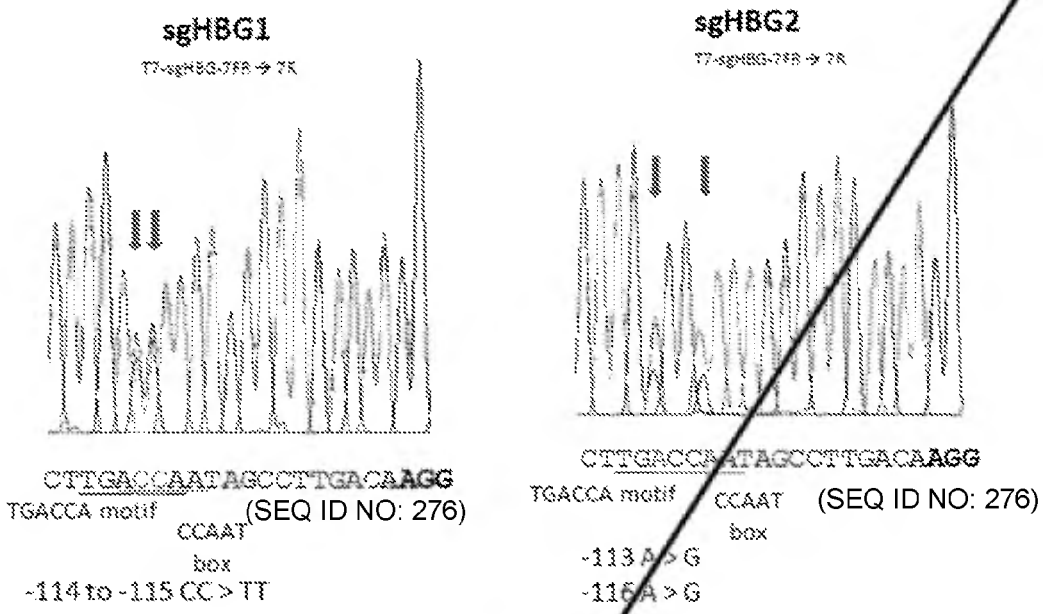


FIG. 156D

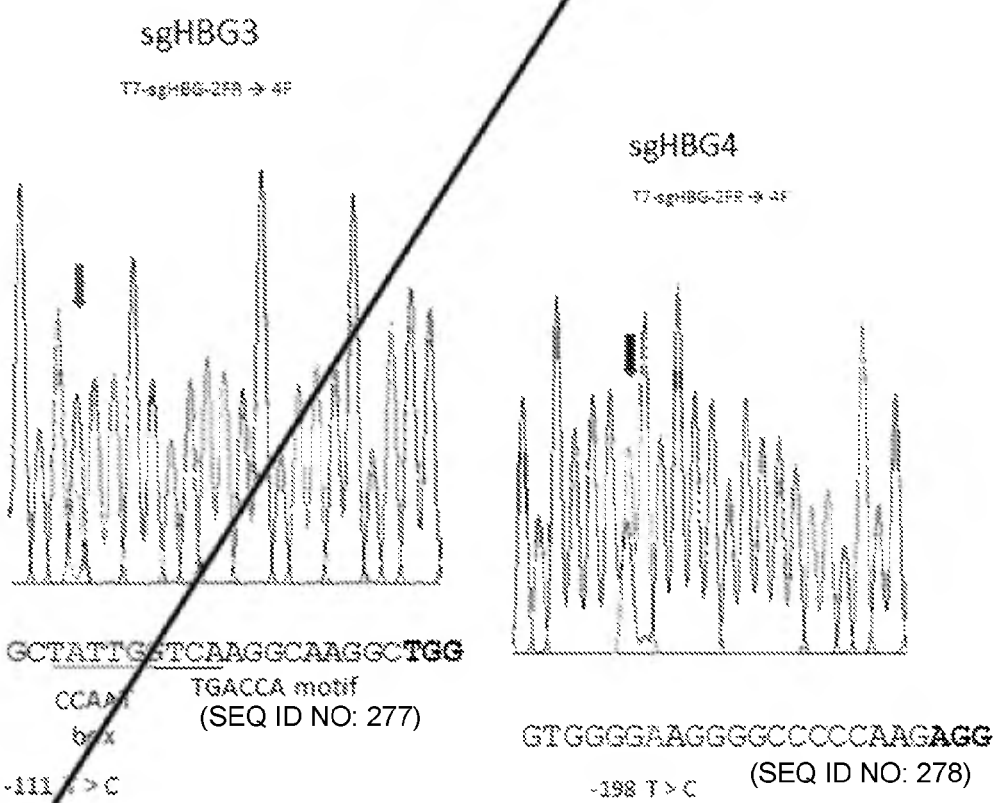


FIG. 157

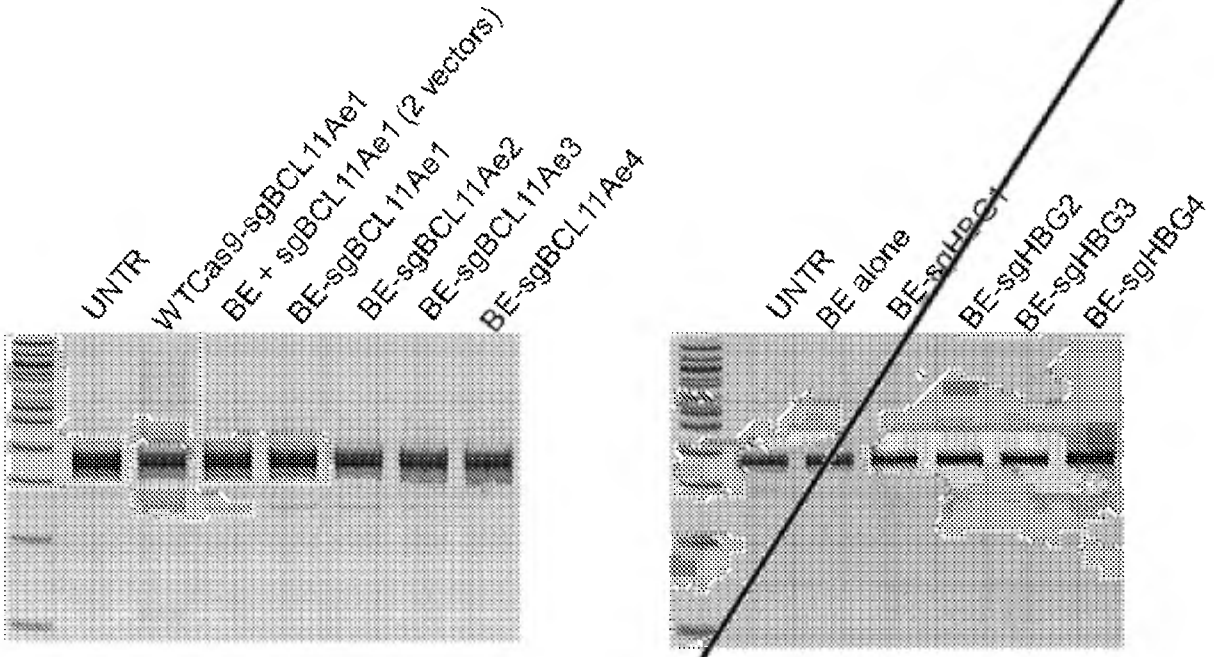


FIG. 158

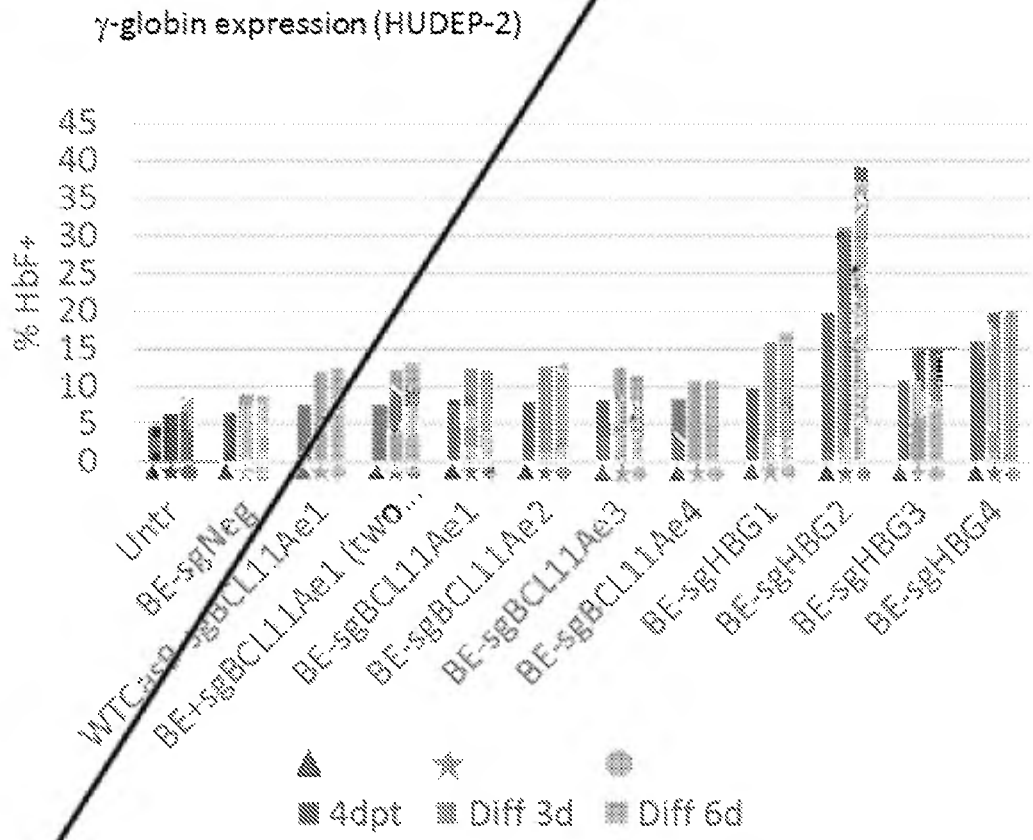


FIG. 159A

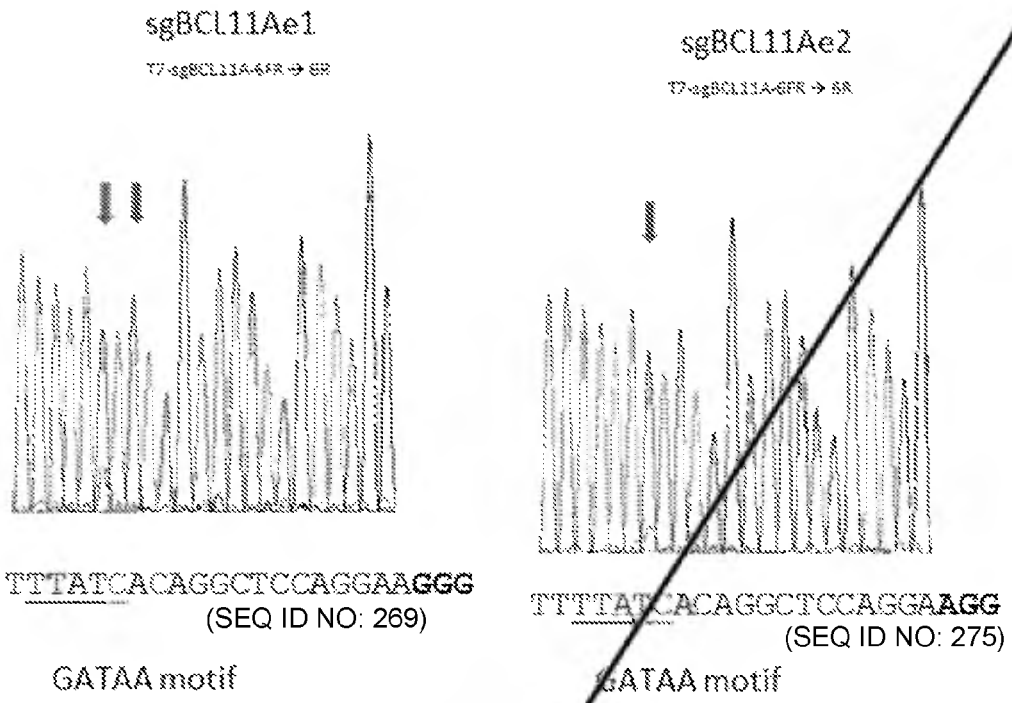


FIG. 159B

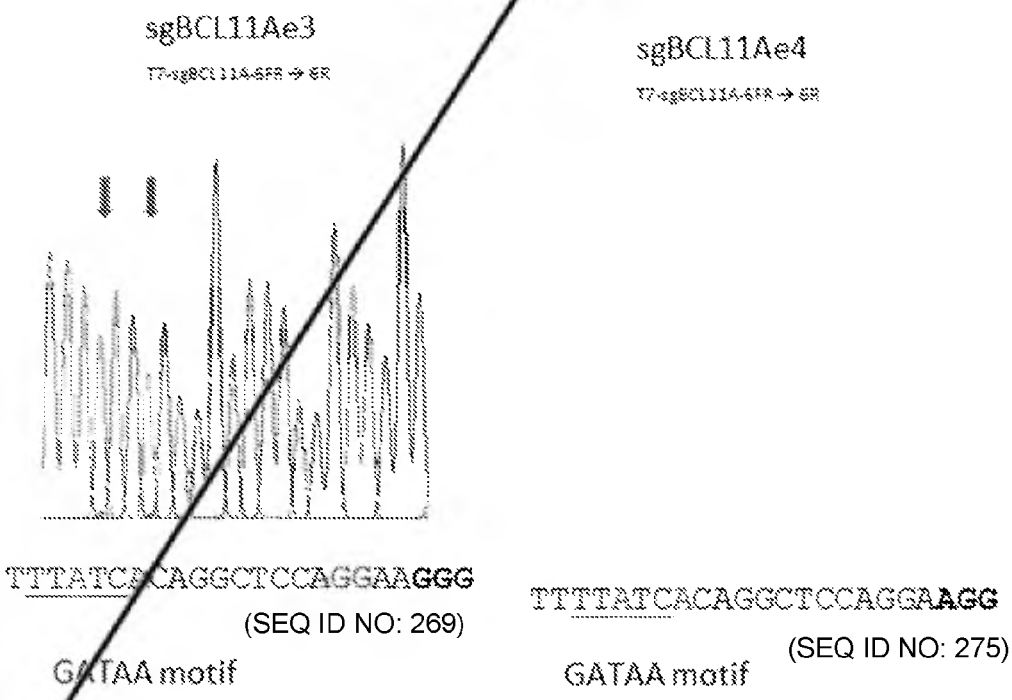


FIG. 159C

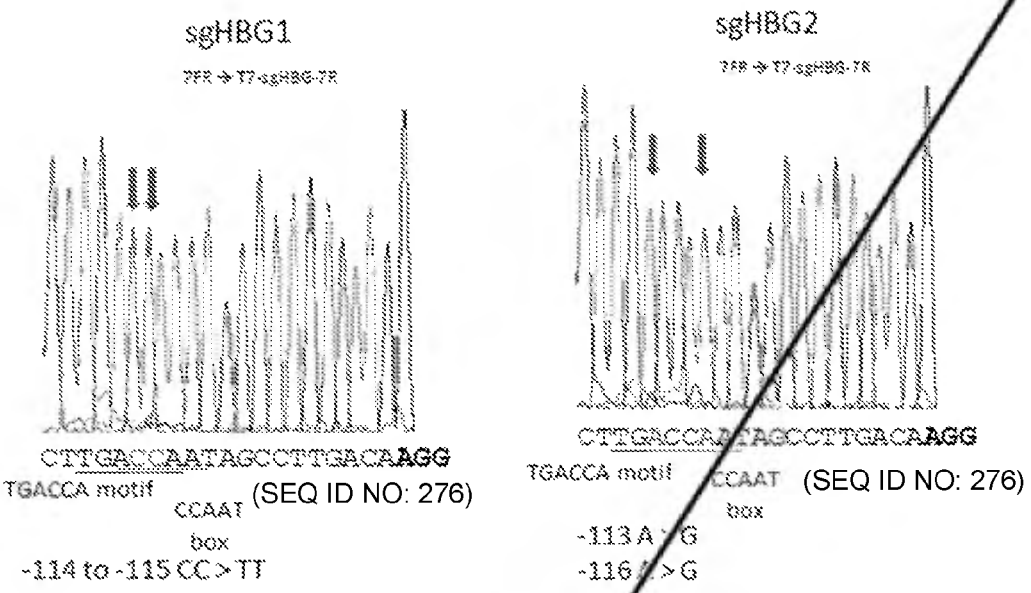


FIG. 159D

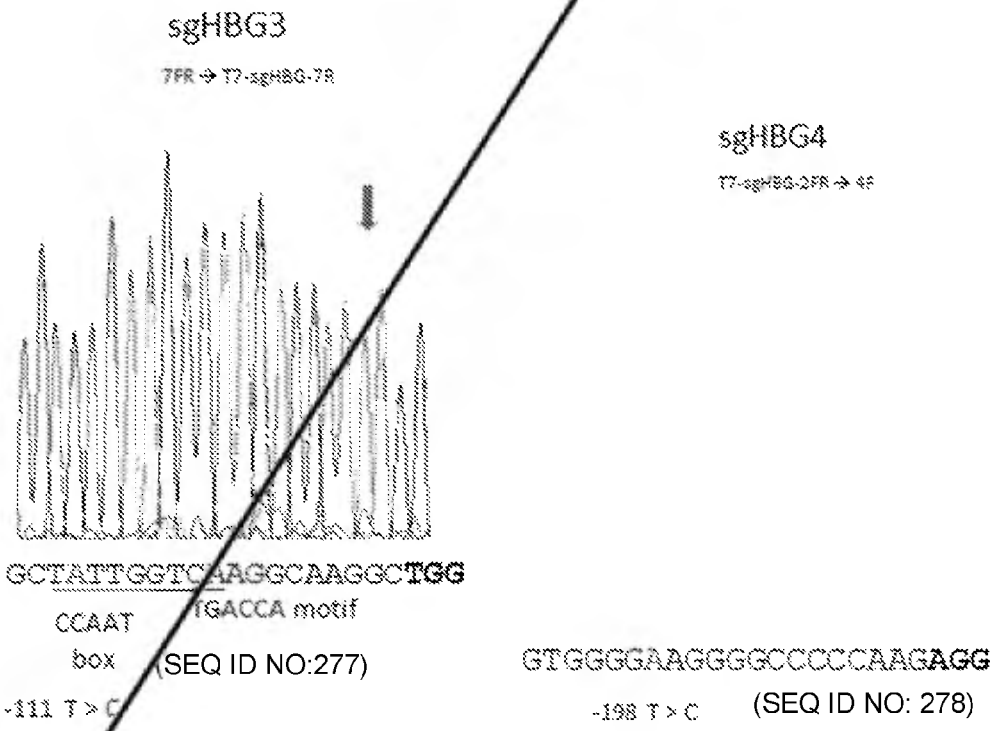


FIG. 160

Selected constructs for HDAd virus production (under Maxi preparation)

Constructed vectors:		Corresponding Ad constructs:
pBS-AncBE4max-sgBCL11AE1	+58 GATA motif, G --> A	pHCA-BE4max-sgBCL11Ae1-FI-mgmt/GFP
pBS-AncBE4max-sgBCL11AE2	+58 GATA motif, G --> A	
pBS-ABEmax-sgBCL11AE3	+58 GATA motif, T --> C	
pBS-ABEmax-sgBCL11AE4	+58 GATA motif, T --> C	
pBS-AncBE4max-sgHBG1-1	13bp deletion, CCAAT box, CC --> TT	
pBS-ABEmax-sgHBG1-2	13bp deletion, CCAAT box, A --> G	pHCA-ABEmax-sgHBG2-FI-mgmt/GFP
pBS-ABEmax-sgHBG1-3	13bp deletion, CCAAT box, T --> C	
pBS-ABEmax-sgHBG1-4	-198 T --> C	pHCA-ABEmax-sgHBG4-FI-mgmt/GFP
pBS-AncBE4max-sgR5-1	control	pHCA-BE4max-sgR5-1-FI-mgmt/GFP (Ctrl)

FIG. 161

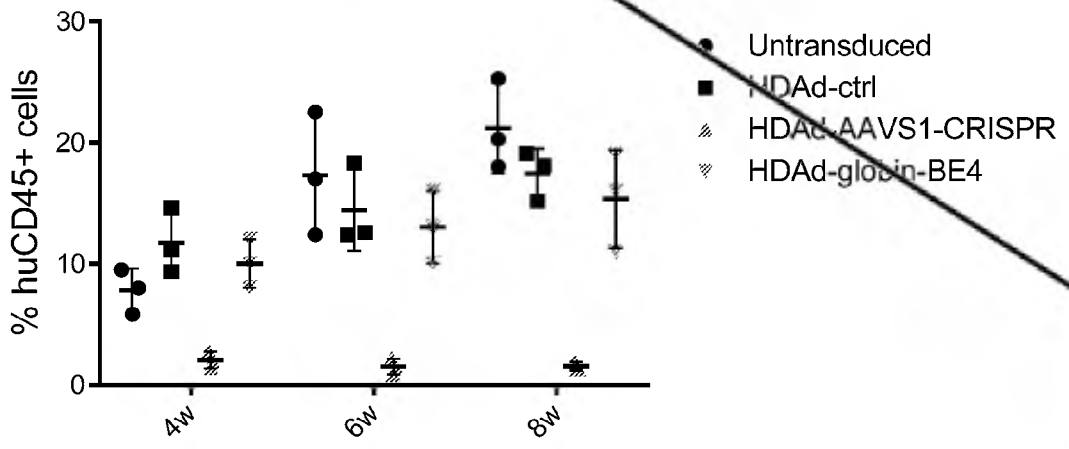


FIG. 162

Transient transfection of HUDEP-2 cells:
(cleavage by T7EI)

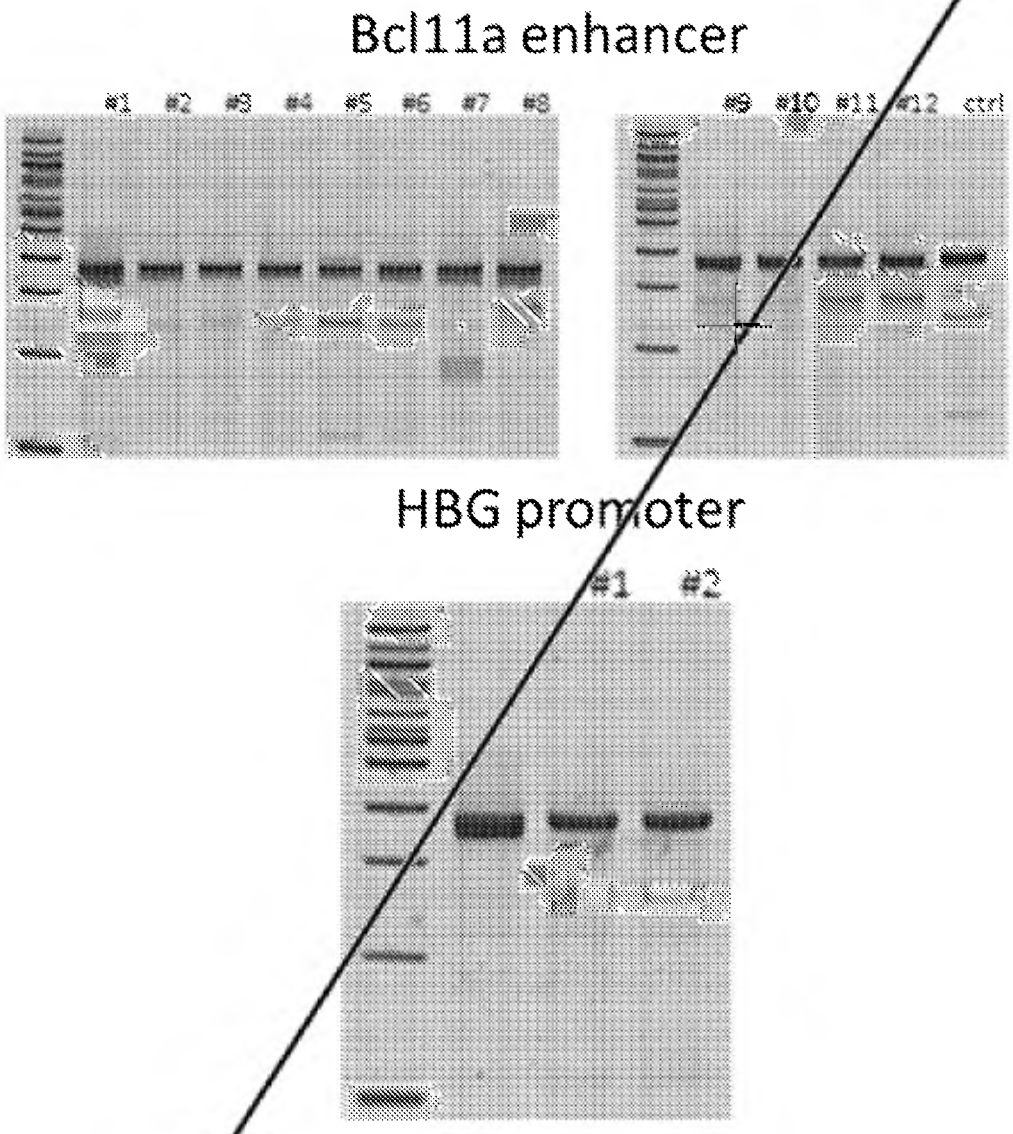


FIG. 163

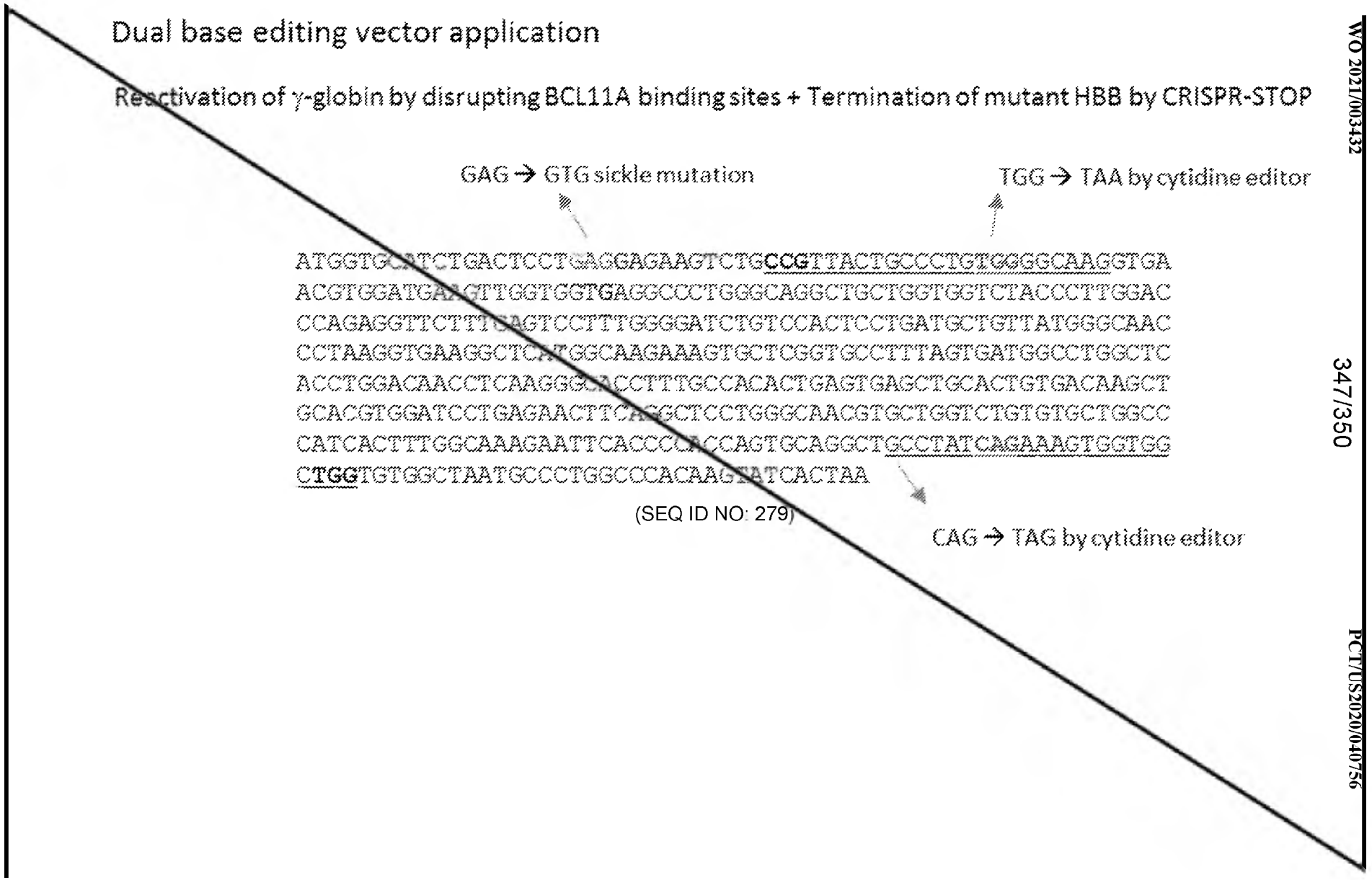

Dual base editing vector application
Reactivation of γ-globin by disrupting BCL11A binding sites + Termination of mutant HBB by CRISPR-STOP
GAG → GTG sickle mutation
TGG → TAA by cytidine editor
ATGGTGCATCTGACTCCTGAGGAGAAGTCTGCCGTTACTGCCCTGTGGGGCAAGGTGA
ACGTGGATGAAGTTGGTGGTGAGGCCCTGGGCAGGCTGCTGGTGGTCTACCCTTGGAC
CCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTCCACTCCTGATGCTGTTATGGGCAAC
CCTAAGGTGAAGGCTCATGGCAAGAAAGTGCTCGGTGCCTTTAGTGATGGCCTGGCTC
ACCTGGACAACCTCAAGGGCACCTTTGCCACACTGAGTGAGCTGCACTGTGACAAGCT
GCACGTGGATCCTGAGAACTTCAGGCTCCTGGGCAACGTGCTGGTCTGTGTGCTGGCC
CATCACTTTGGCAAAGAATTCACCCCACCAGTGCAGGCTGCCTATCAGAAAGTGGTGG
CTGGTGTGGCTAATGCCCTGGCCCACAAGTATCACTAA
(SEQ ID NO: 279)
CAG → TAG by cytidine editor

FIG. 164

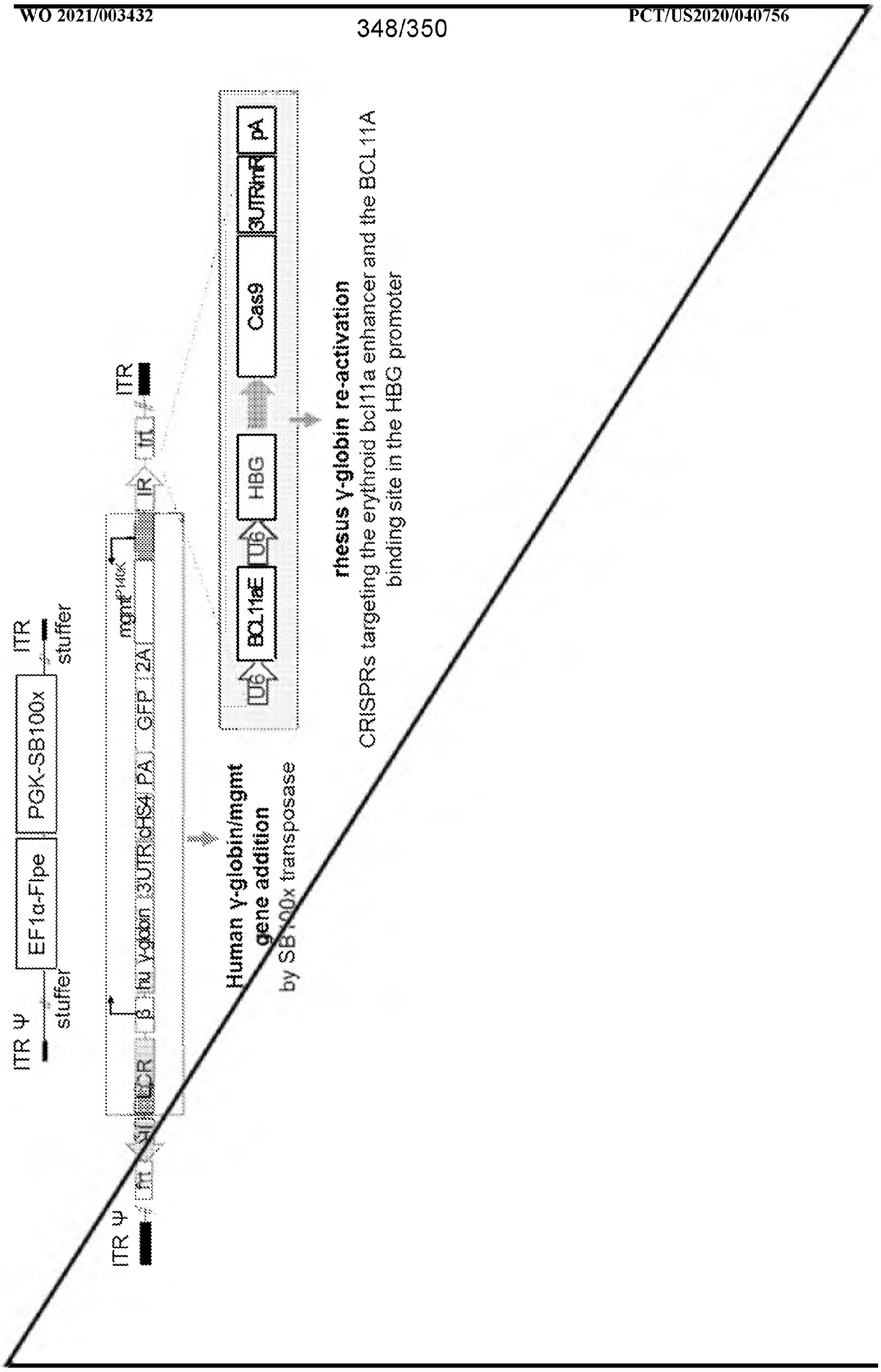

ITR Ψ
stuffer
EF1α-Flpe
PGK-SB100x
ITR
stuffer
ITR Ψ
ITR
LCR
β
hu γ-globin
3'UTR
cHS4
PA
GFP
2A
mgmt140K
IR
ITR
ITR
U6
BCL11aE
U6
HBG
Cas9
3'UTR
pA
Human γ-globin/mgmt gene addition
by SB100x transposase
rhesus γ-globin re-activation
CRISPRs targeting the erythroid bcl11a enhancer and the BCL11A binding site in the HBG promoter

FIG. 165

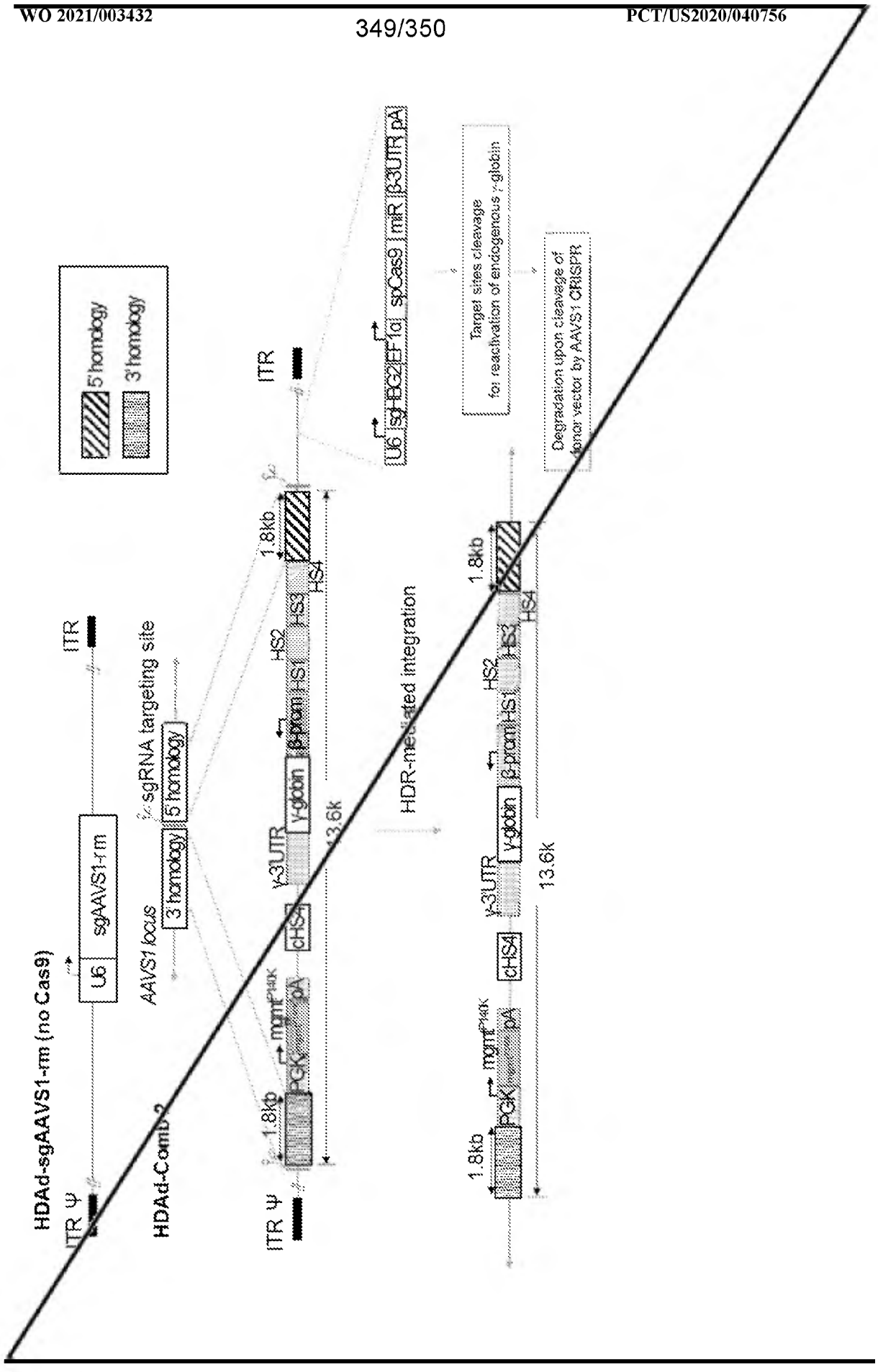

HDAd-sgAAVS1-rm (no Cas9)
ITR Ψ
U6
sgAAVS1-rm
ITR
HDAd-Comb2
AAVS1 locus
3' homology
5' homology
sgRNA targeting site
ITR Ψ
1.8kb
PGK
mgmt P140K
pA
cHS4
γ-3'UTR
γ-globin
β-prom
HS1
HS2
HS3
HS4
13.6k
HDR-mediated integration
ITR
1.8kb
U6
sgHBG2/EF1α
spCas9
miR
β-3'UTR
pA
5' homology
3' homology
Target sites cleavage
for reactivation of endogenous γ-globin
Degradation upon cleavage of
donor vector by AAVS1 CRISPR

FIG. 166

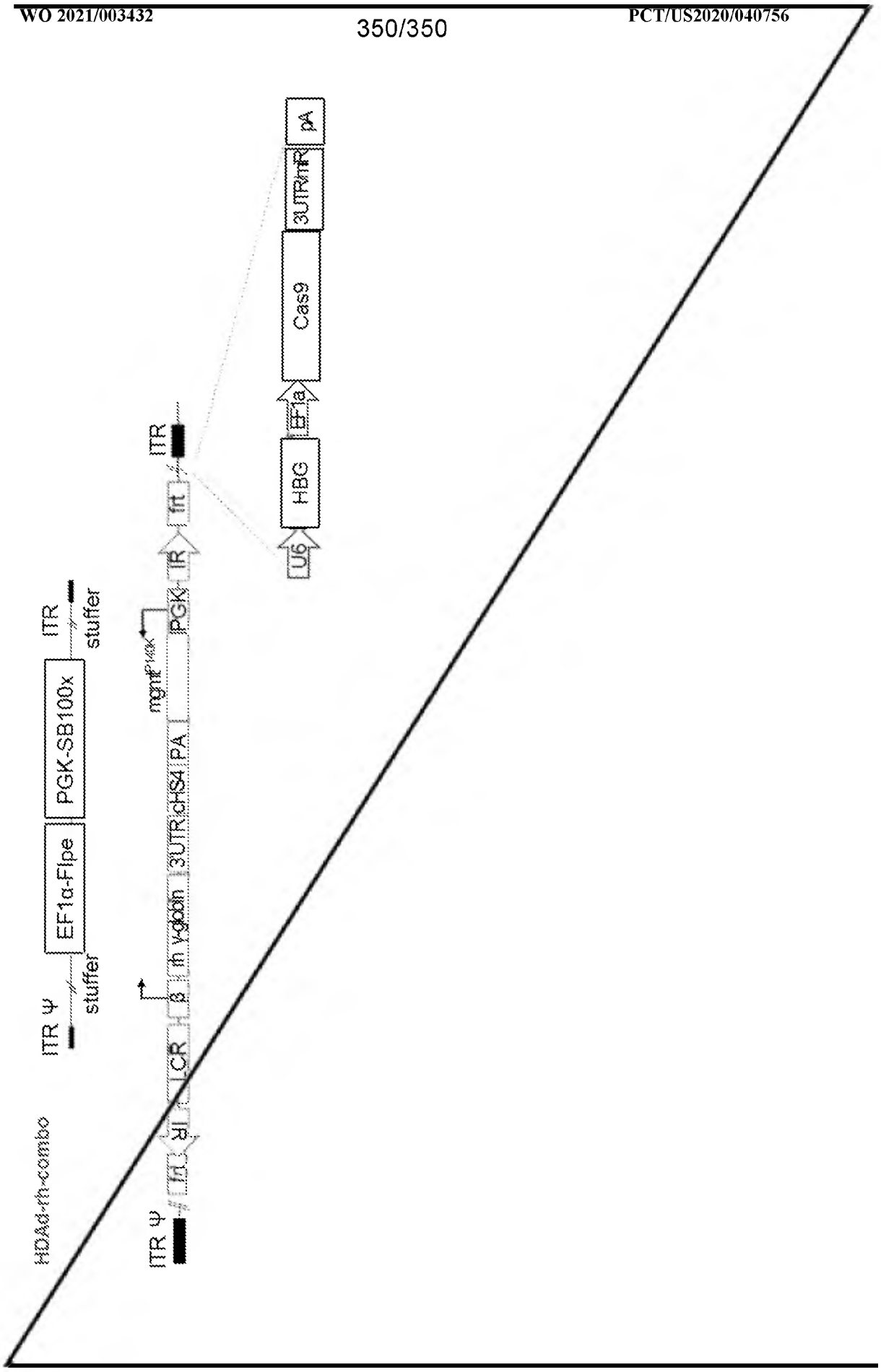

HDAd-rh-combo
ITR Ψ
stuffer
EF1α-Flpe
PGK-SB100x
ITR
stuffer
ITR Ψ
rh
CR
β
rh γ-globin
3'UTR-cHS4
PA
mgmt^P140K
PGK
IR
ITR
U6
HBG
EF1a
Cas9
3'UTR-mR
pA

FIG. 1

Integrated Cassettes (integrated portions are between frt sites)

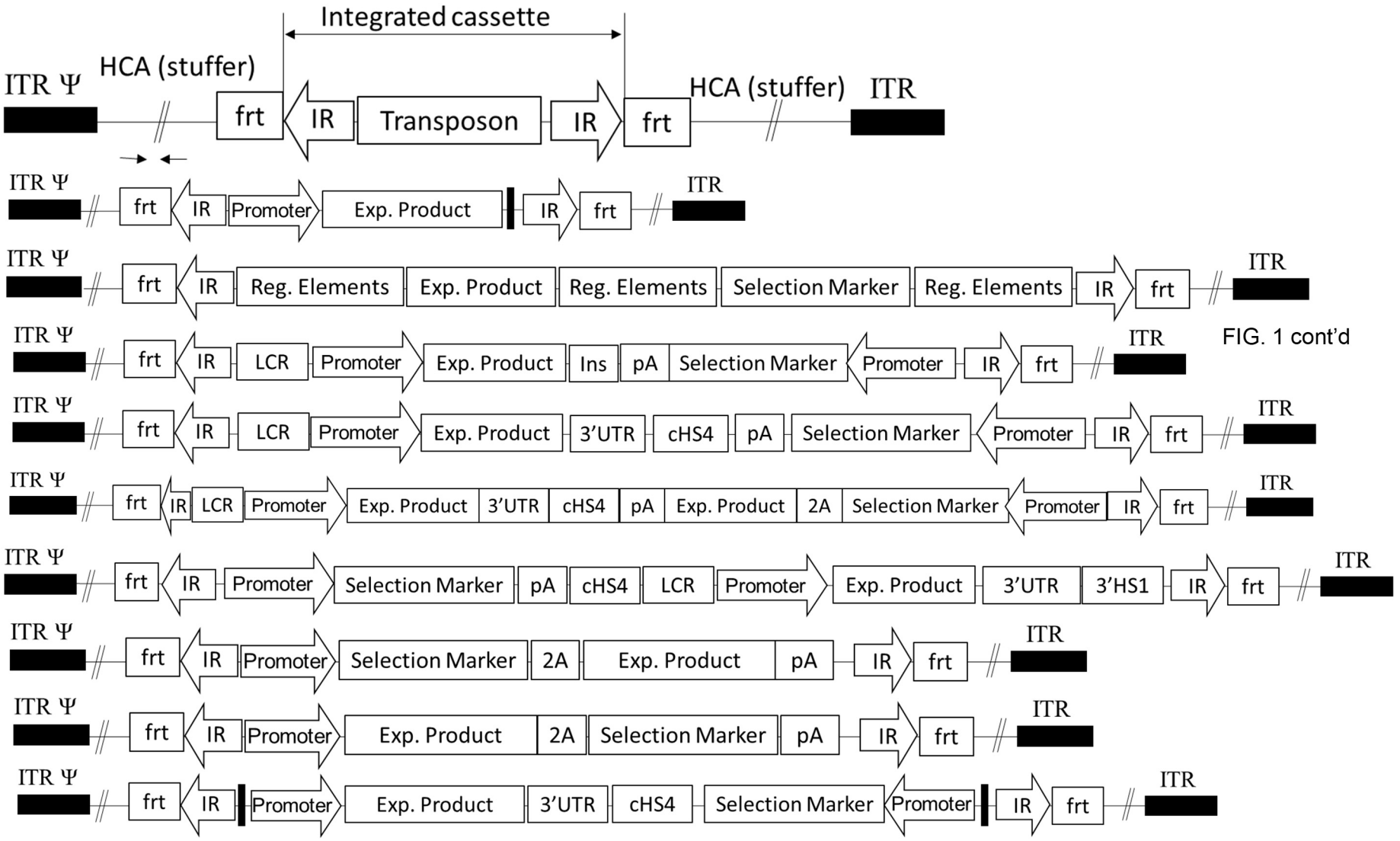
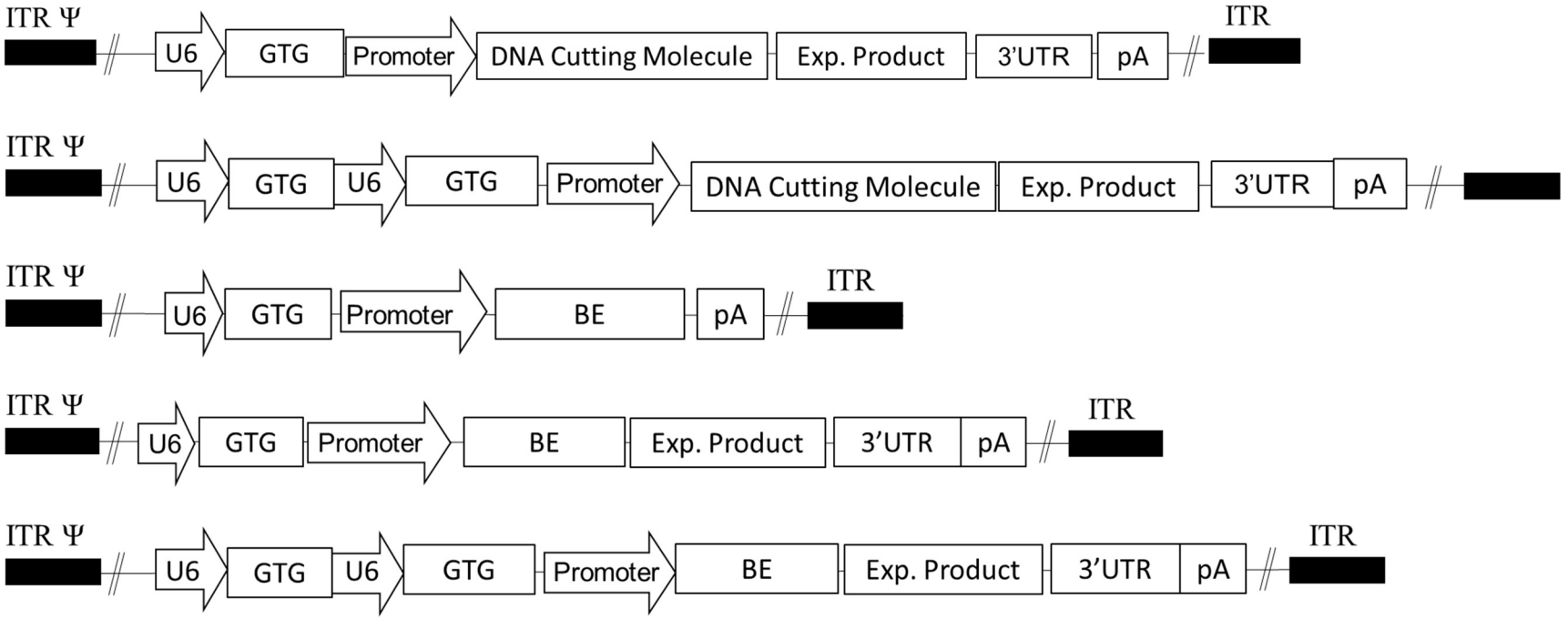


FIG. 1 cont'd

FIG. 1 cont'd

Transient



Transposase

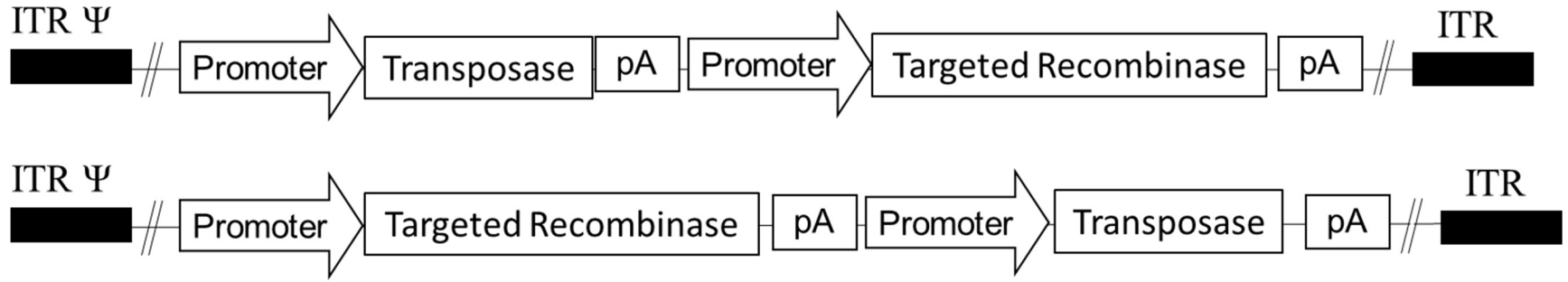


FIG. 2A

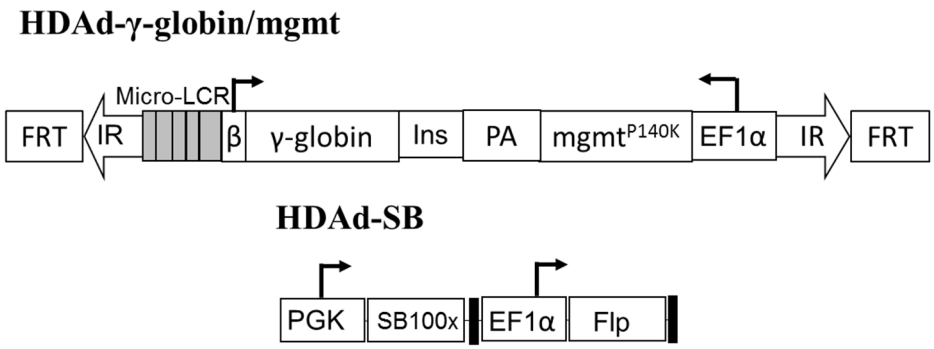


FIG. 2B

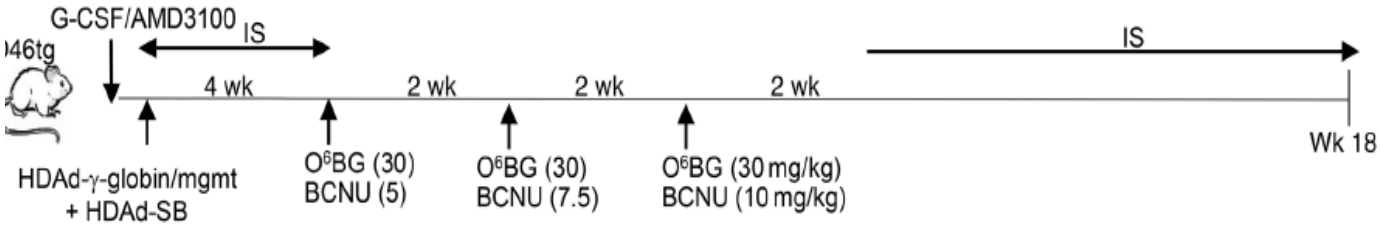


FIG. 2C

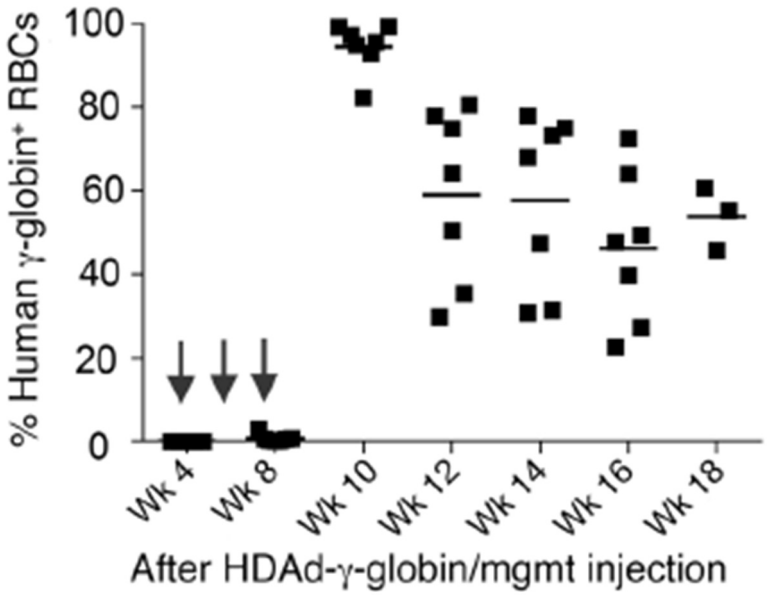


FIG. 2D

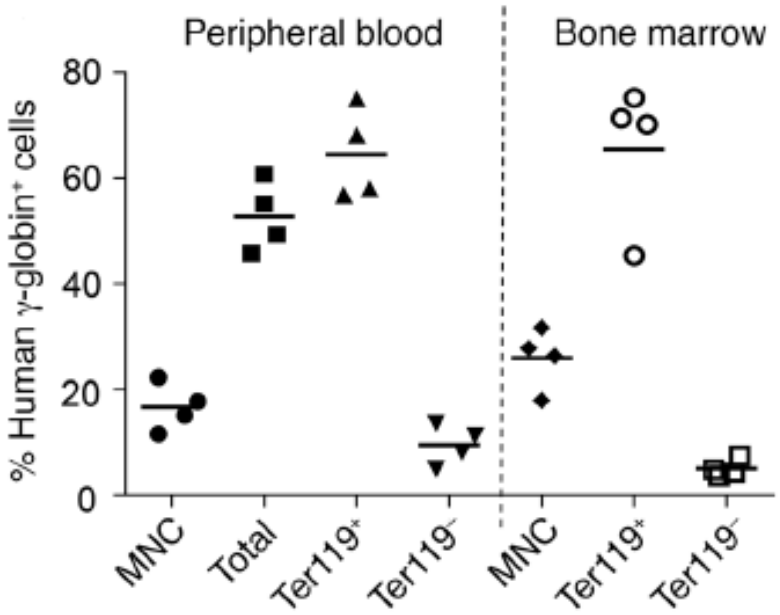

Peripheral blood
Bone marrow
% Human γ-globin+ cells
80
60
40
20
0
MNC
Total
Ter119+
Ter119-
MNC
Ter119+
Ter119-

FIG. 2E

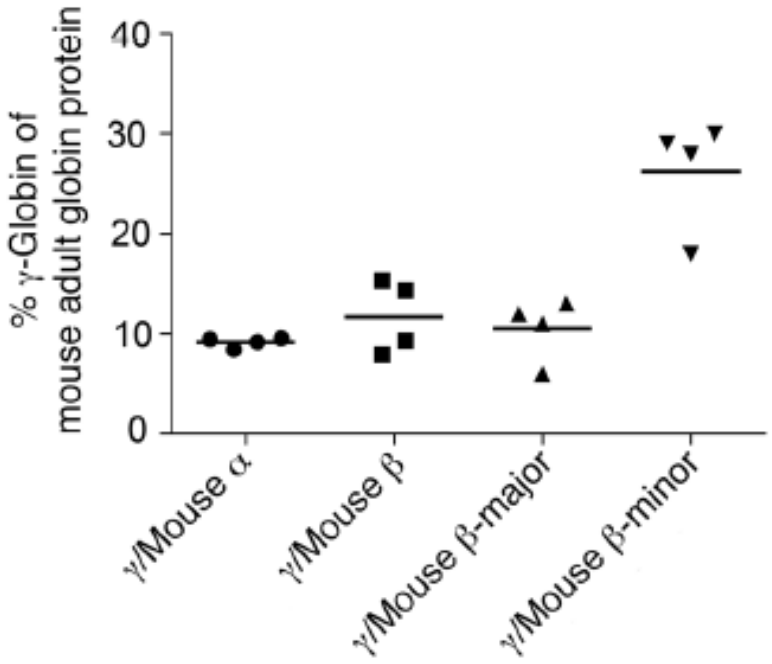

% γ-Globin of mouse adult globin protein
40
30
20
10
0
γ/Mouse α
γ/Mouse β
γ/Mouse β-major
γ/Mouse β-minor

FIG. 2F

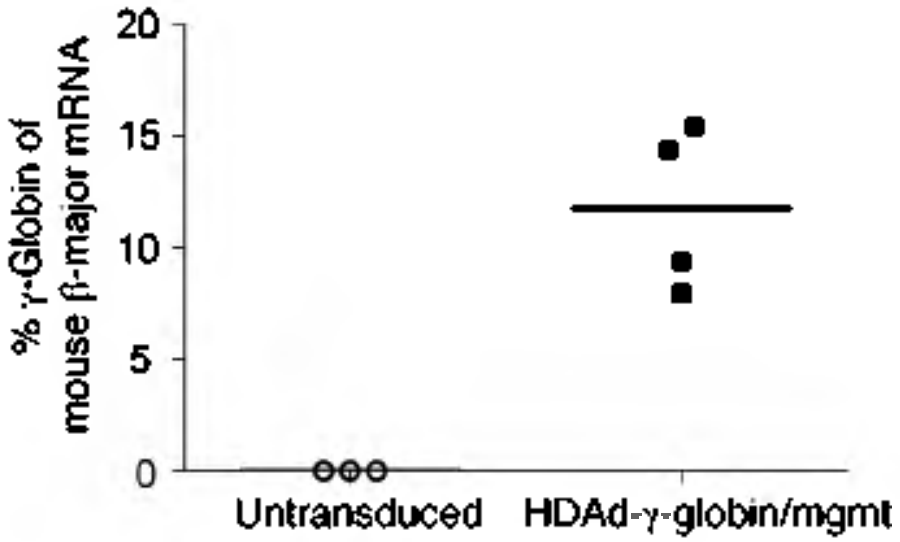

% γ-Globin of mouse β-major mRNA
20
15
10
5
0
Untransduced
HDAd-γ-globin/mgmt

FIG. 3

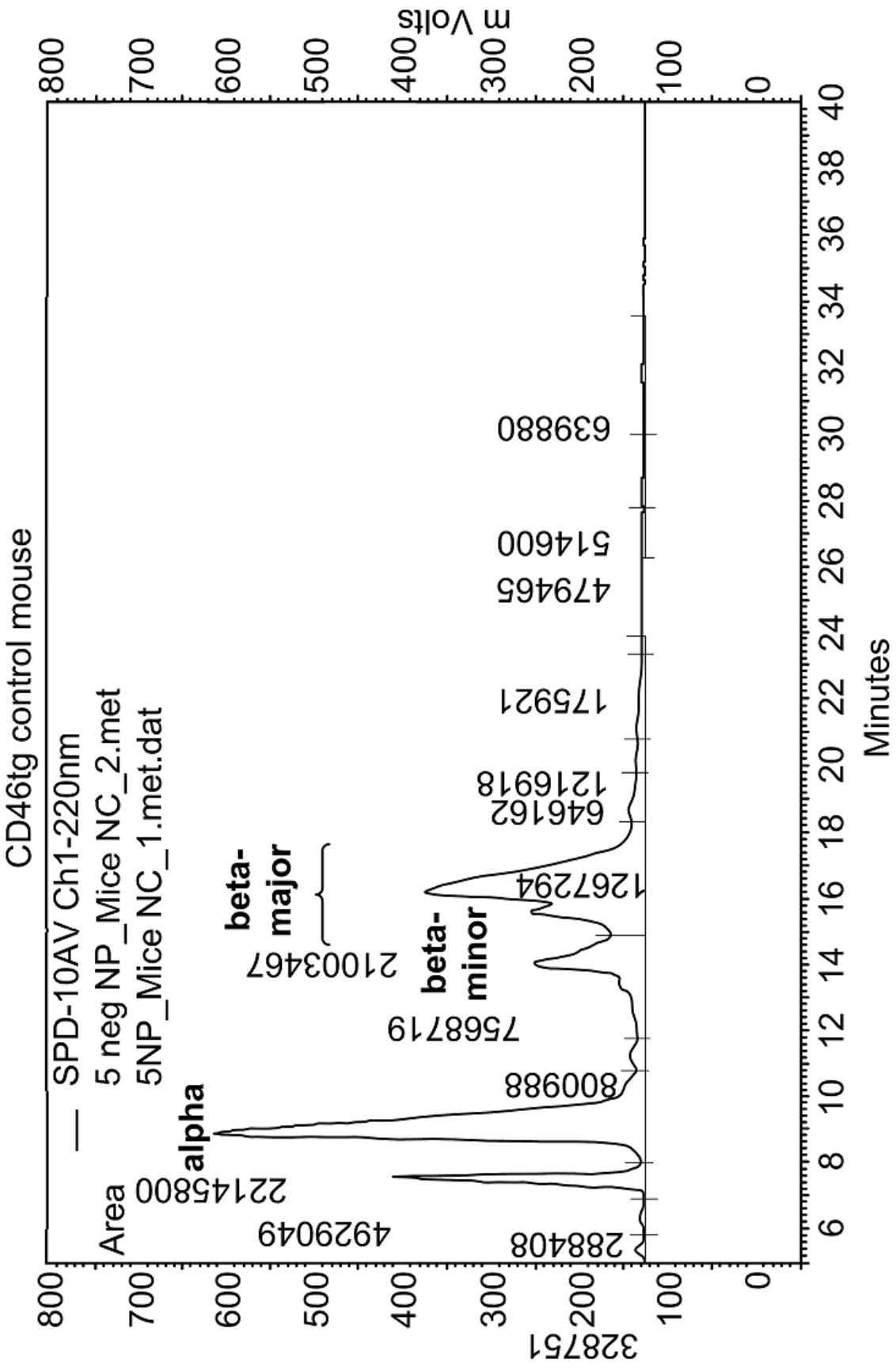

CD46tg control mouse
SPD-10AV Ch1-220nm
5 neg NP_Mice NC_2.met
5NP_Mice NC_1.met.dat
Area
alpha
2214580
beta-major
2100346
beta-minor
756871
4929049
288408
800988
1267294
646162
1216918
175921
479465
514600
639880
3287511
m Volts
Minutes

FIG. 3 cont'd

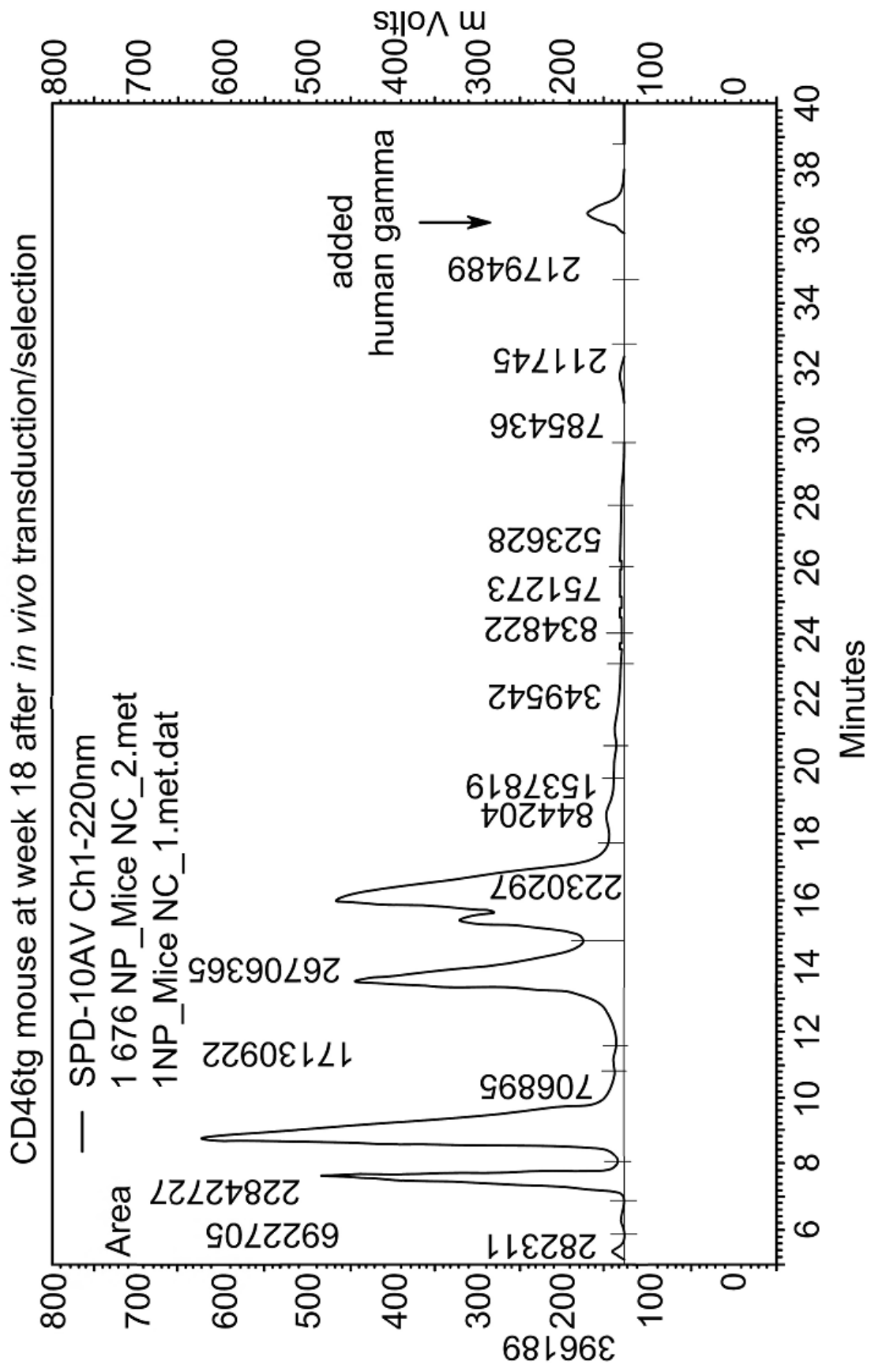

CD46tg mouse at week 18 after *in vivo* transduction/selection
— SPD-10AV Ch1-220nm
1 676 NP_Mice NC_2.met
1NP_Mice NC_1.met.dat
Area
2284272 7
6922705
1713092 2
2670636 5
706895
2230297
8420 4
15378 19
349542
834822
751273
523628
785436
211745
2179489
added
human gamma
396189
282311
m Volts
Minutes

FIG. 4A

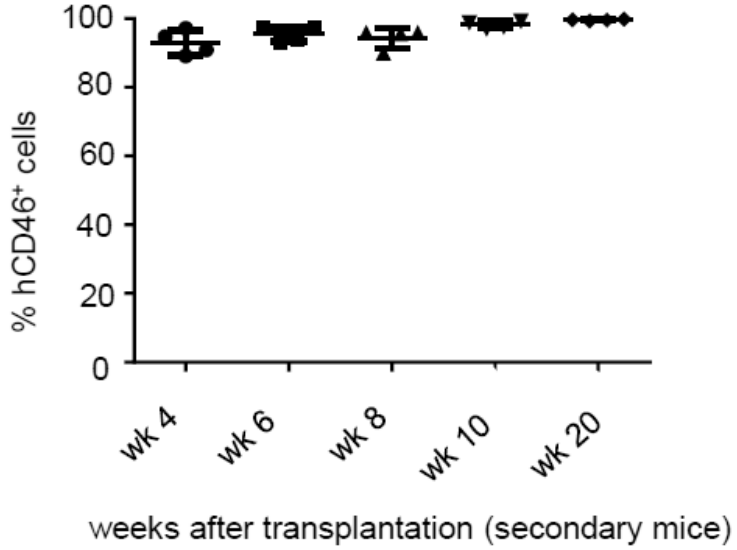


FIG. 4B

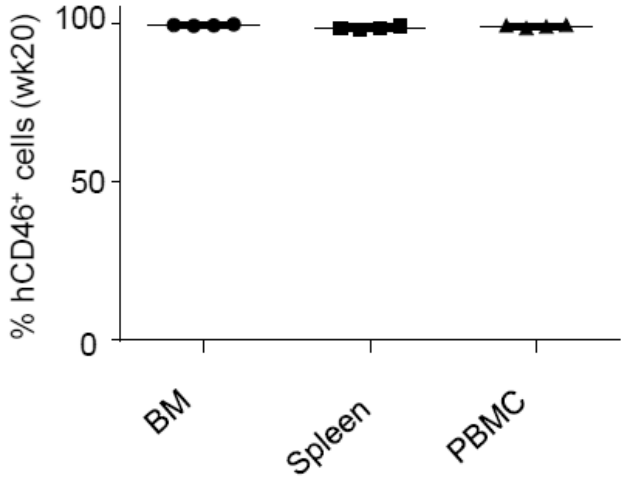


FIG. 4C

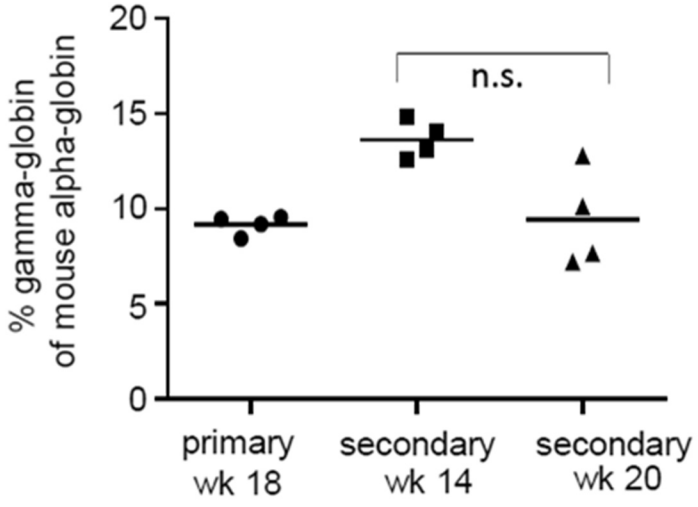


FIG. 5A

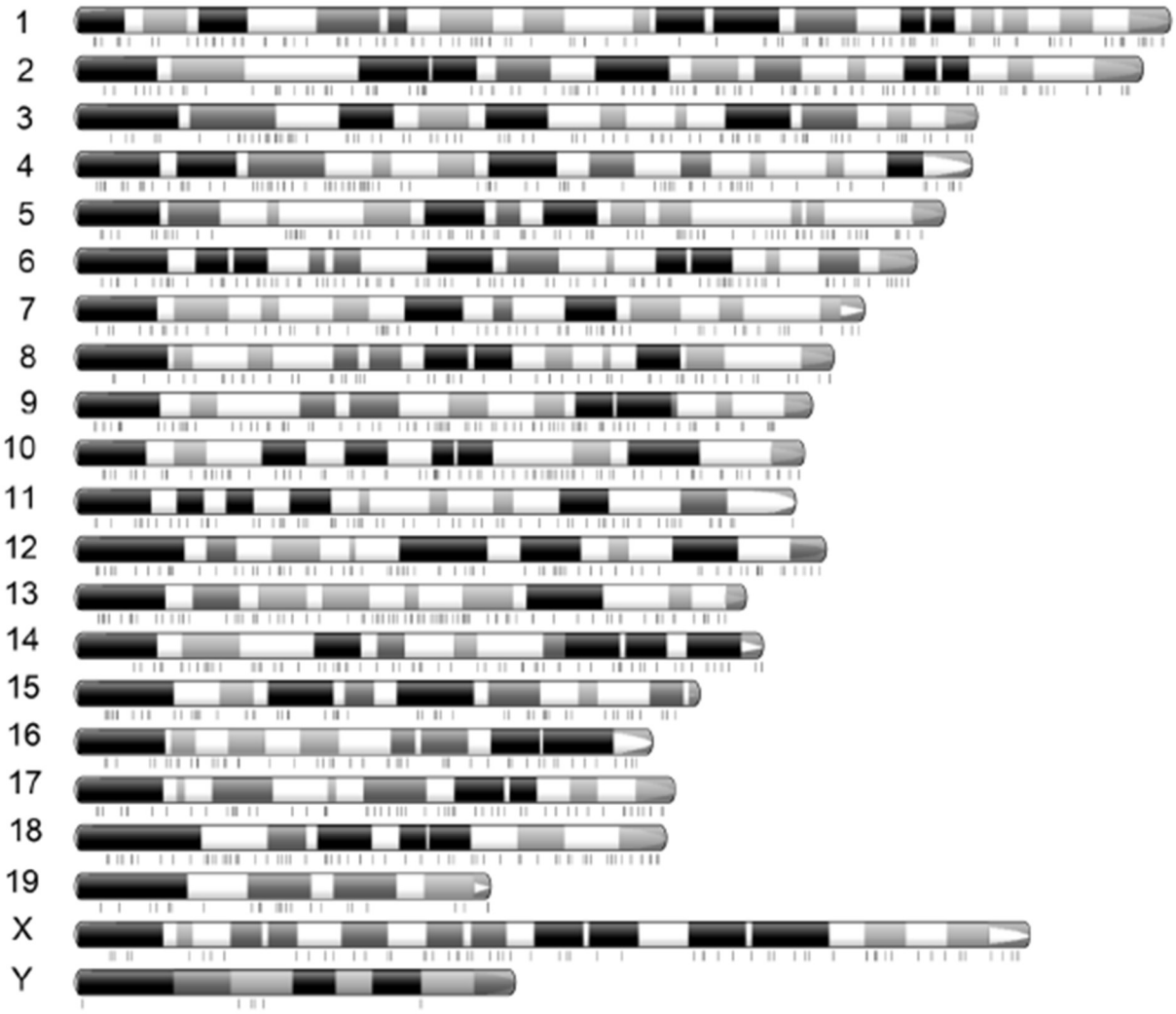


FIG. 5B

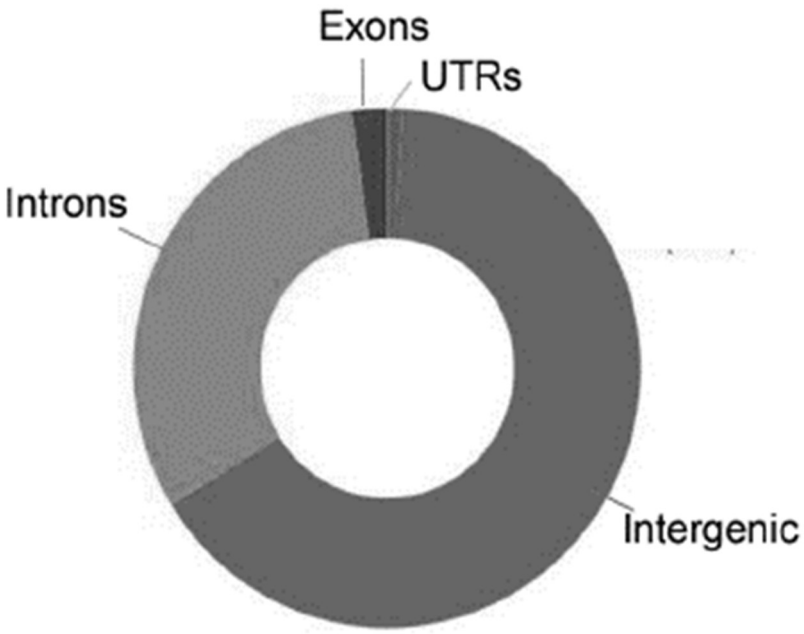

Exons
UTRs
Introns
Intergenic

FIG. 5C

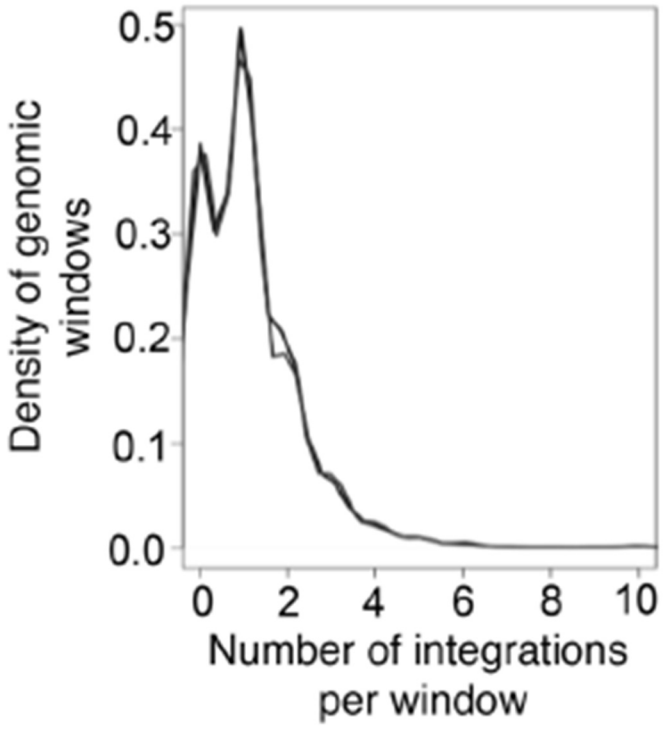

0.5
0.4
0.3
0.2
0.1
0.0
Density of genomic windows
0
2
4
6
8
10
Number of integrations per window

FIG. 5D

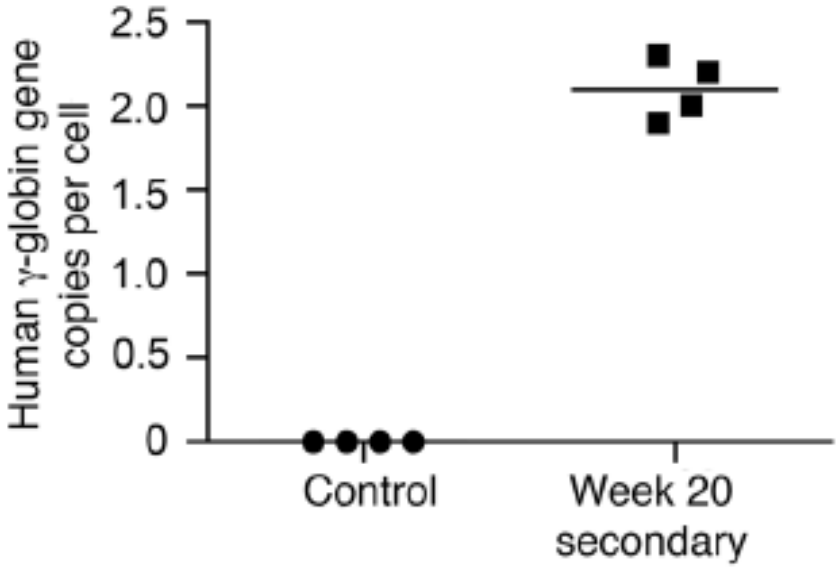

Human γ-globin gene copies per cell
2.5
2.0
1.5
1.0
0.5
0
Control
Week 20 secondary

FIG. 5E

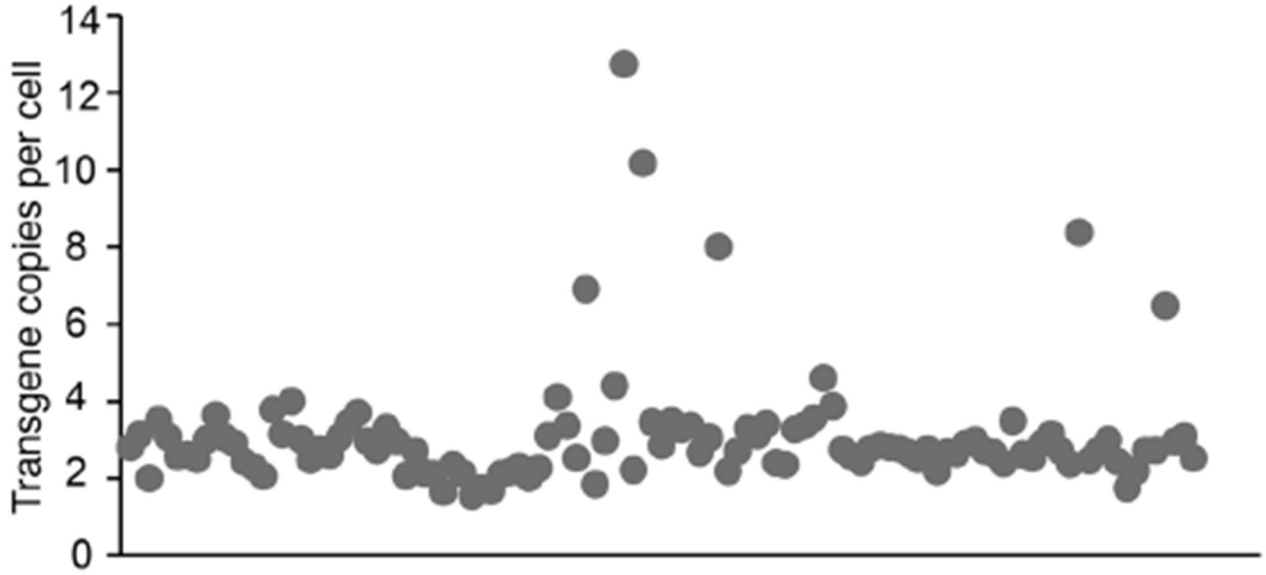
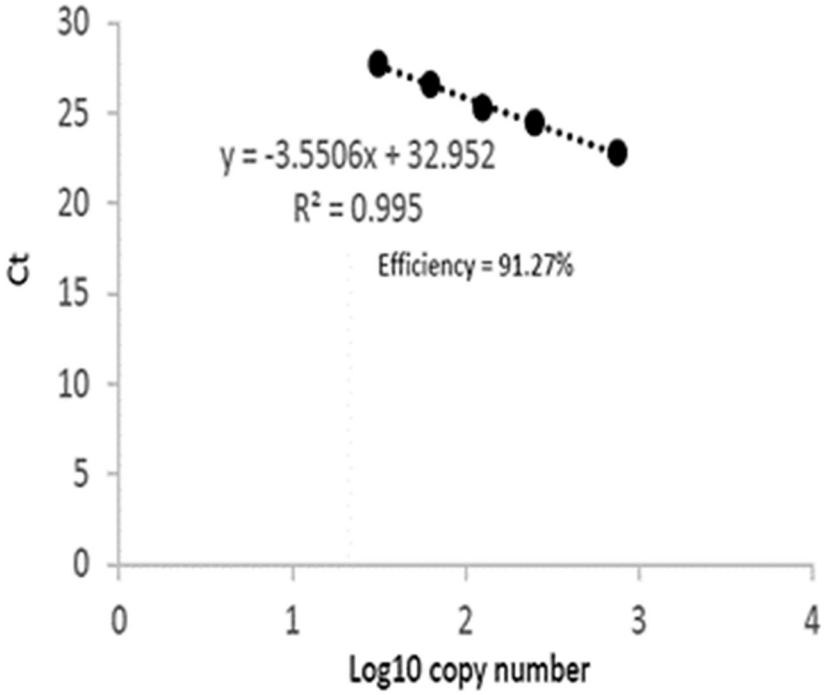

Transgene copies per cell
14
12
10
8
6
4
2
0

FIG. 6


30
25
20
15
10
5
0
Ct
y = -3.5506x + 32.952
R² = 0.995
Efficiency = 91.27%
0
1
2
3
4
Log10 copy number

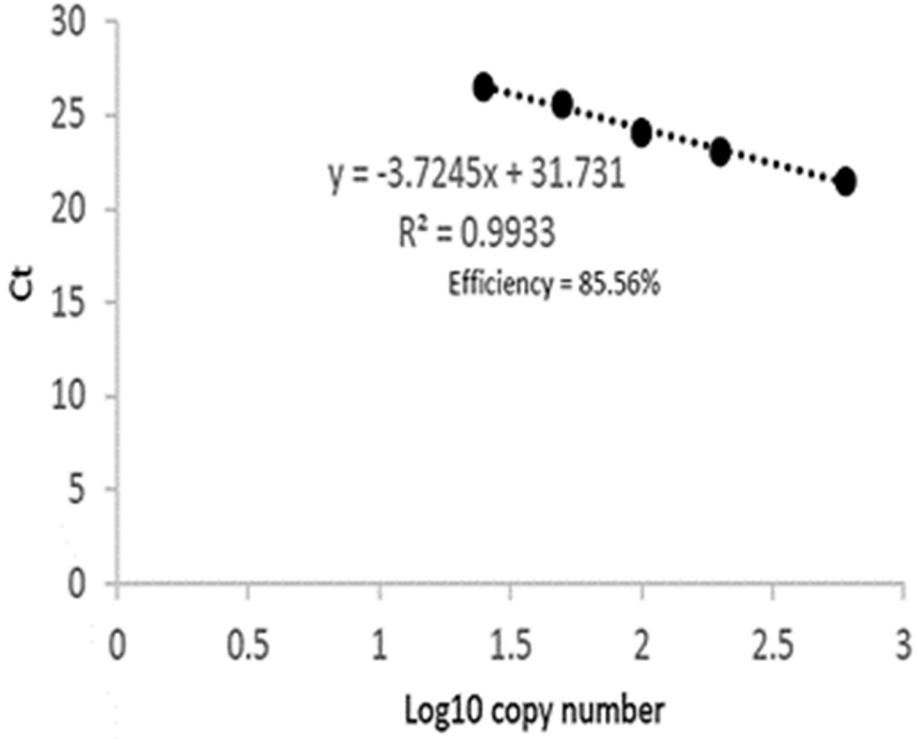

30
25
20
15
10
5
0
Ct
y = -3.7245x + 31.731
R² = 0.9933
Efficiency = 85.56%
0
0.5
1
1.5
2
2.5
3
Log10 copy number

FIG. 7A

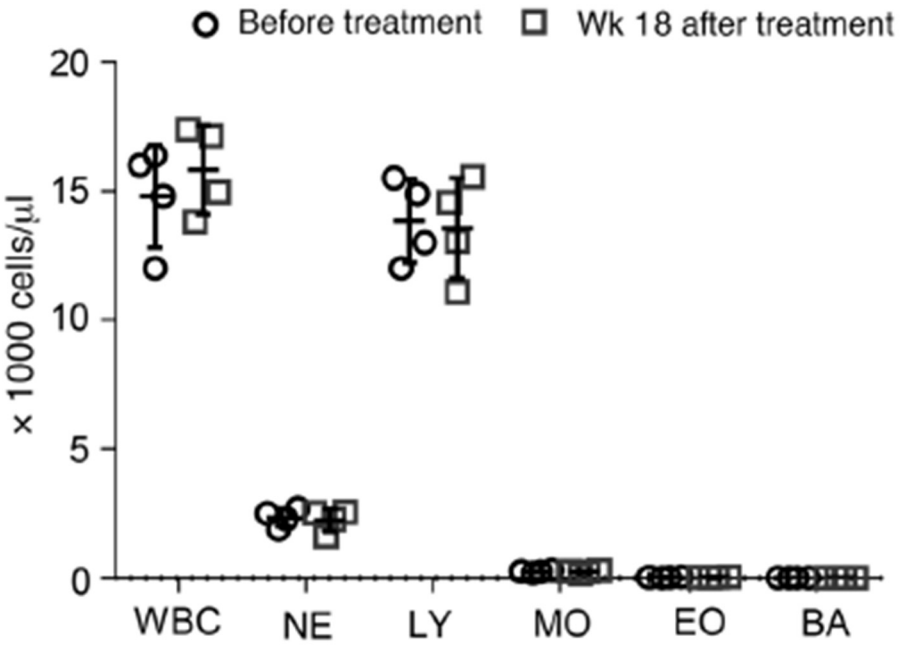

Before treatment
Wk 18 after treatment
20
15
10
5
0
× 1000 cells/μl
WBC
NE
LY
MO
EO
BA

FIG. 7B

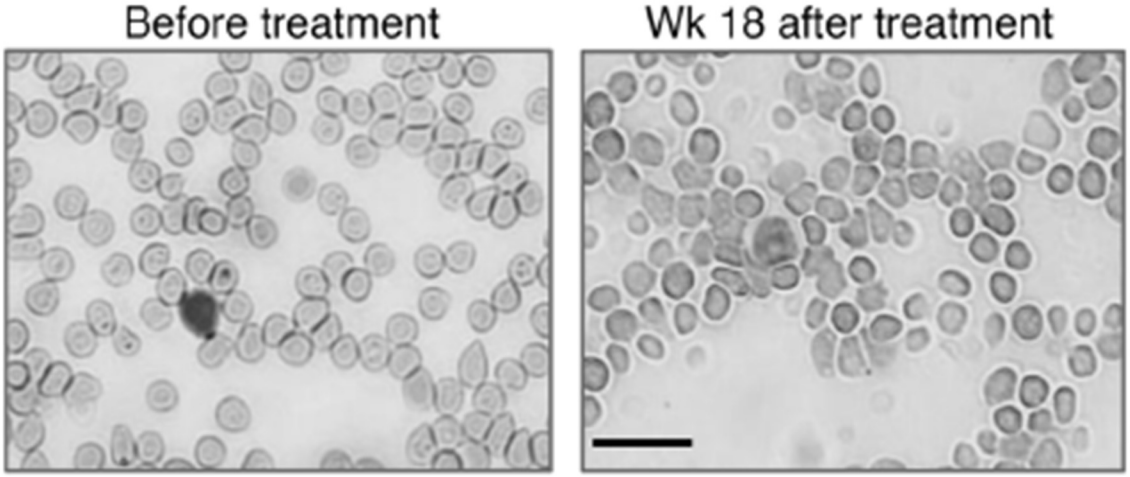

Before treatment
Wk 18 after treatment

FIG. 7C

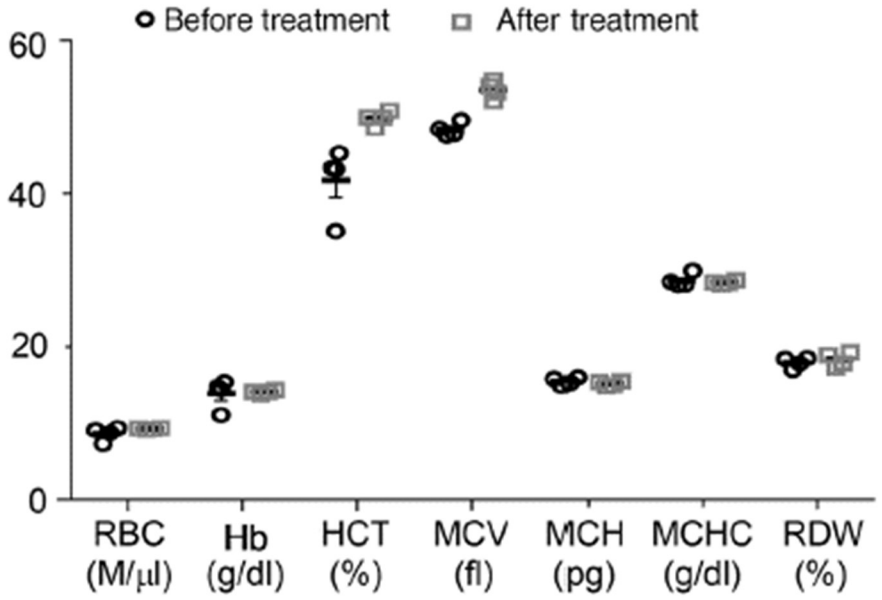

Before treatment
After treatment
60
40
20
0
RBC (M/μl)
Hb (g/dl)
HCT (%)
MCV (fl)
MCH (pg)
MCHC (g/dl)
RDW (%)

FIG. 7D

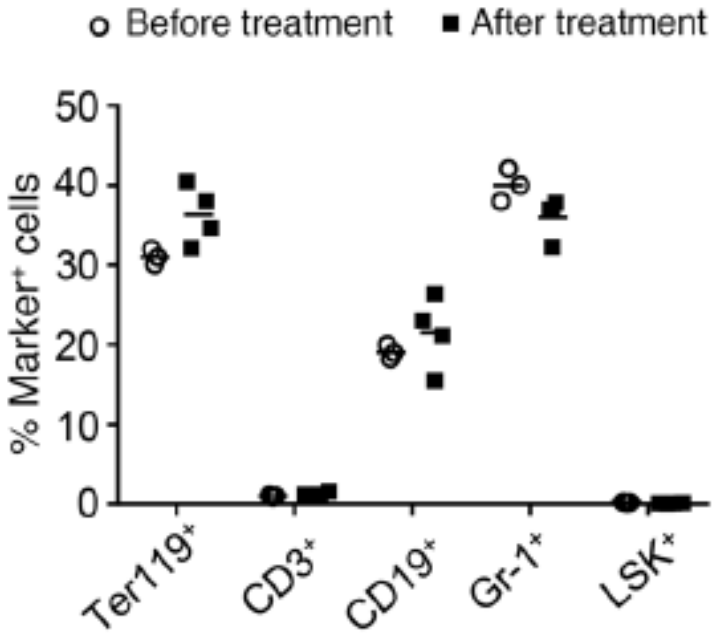

Before treatment
After treatment
% Marker+ cells
50
40
30
20
10
0
Ter119+
CD3+
CD19+
Gr-1+
LSK+

FIG. 7E

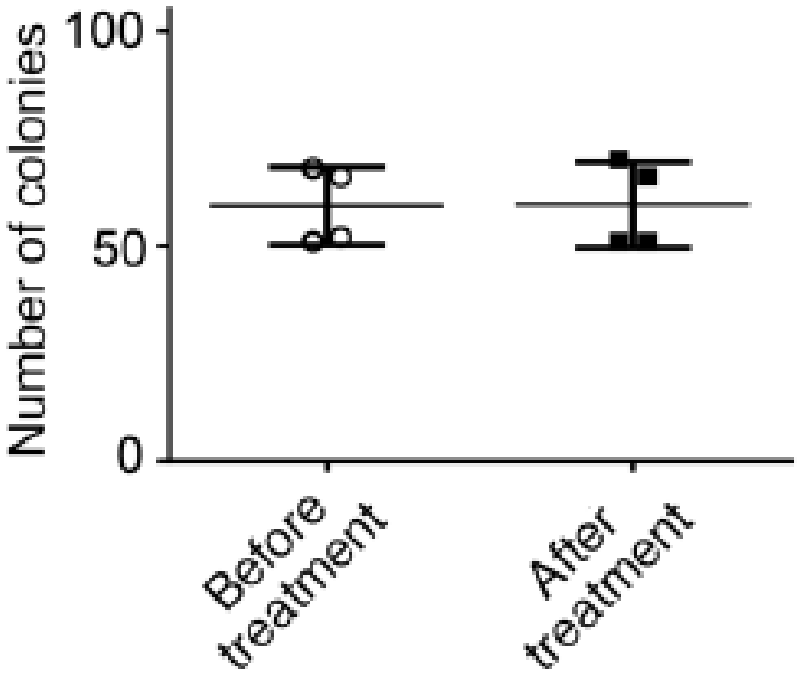

Number of colonies
100
50
0
Before treatment
After treatment

FIG. 8

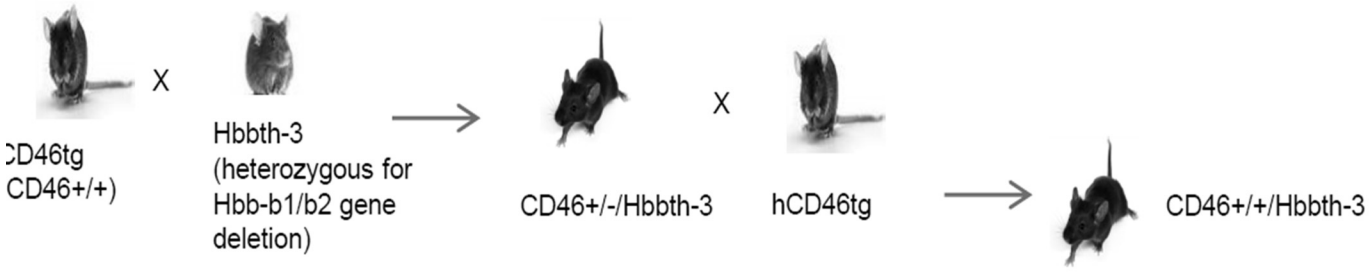

X
CD46tg
CD46+/+)
Hbbth-3
(heterozygous for
Hbb-b1/b2 gene
deletion)
CD46+/-/Hbbth-3
X
hCD46tg
CD46+/+/Hbbth-3

FIG. 9A

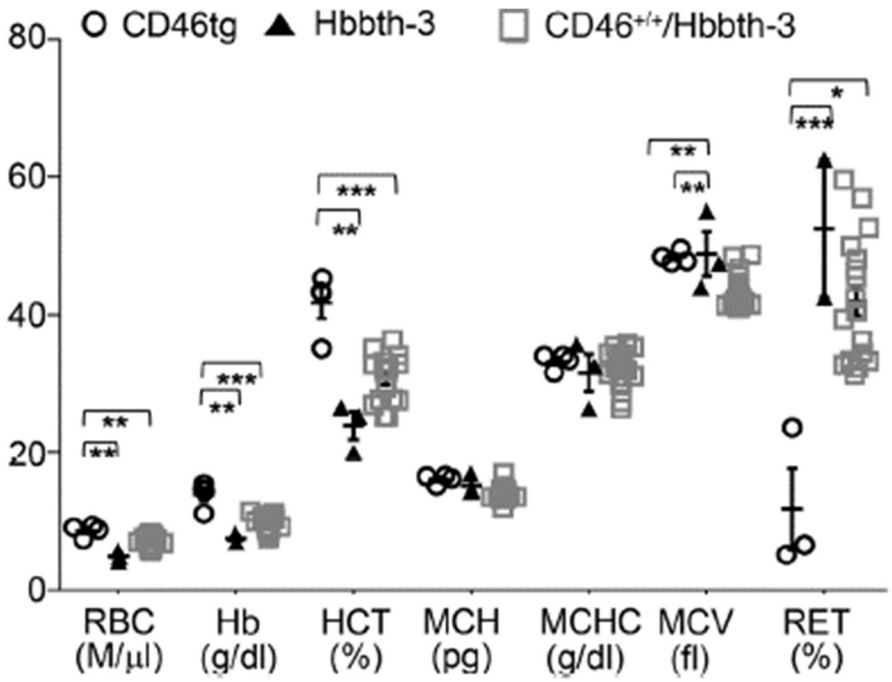

CD46tg
Hbbth-3
CD46+/+/Hbbth-3
80
60
40
20
0
RBC (M/μl)
Hb (g/dl)
HCT (%)
MCH (pg)
MCHC (g/dl)
MCV (fl)
RET (%)

FIG. 9B

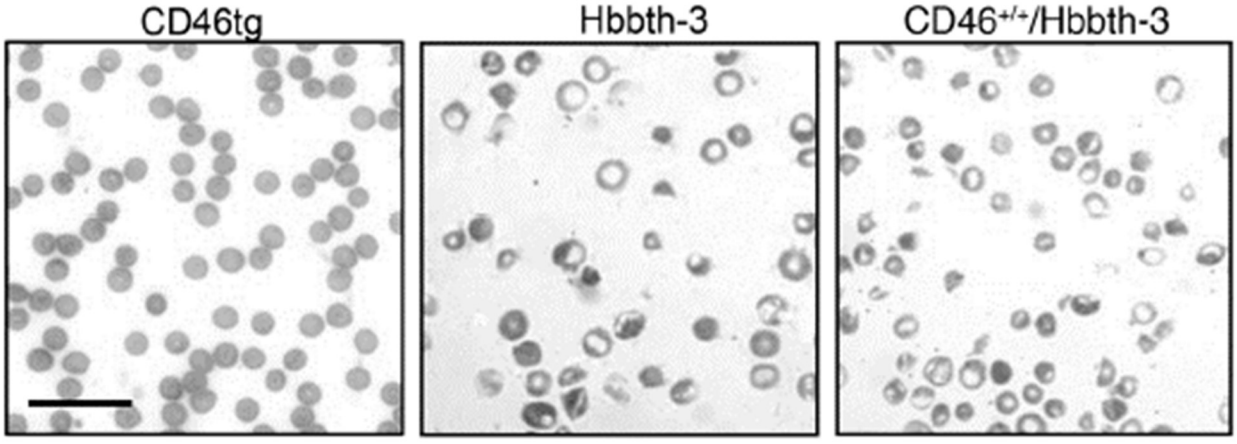

CD46tg
Hbbth-3
CD46+/+/Hbbth-3

FIG. 9C

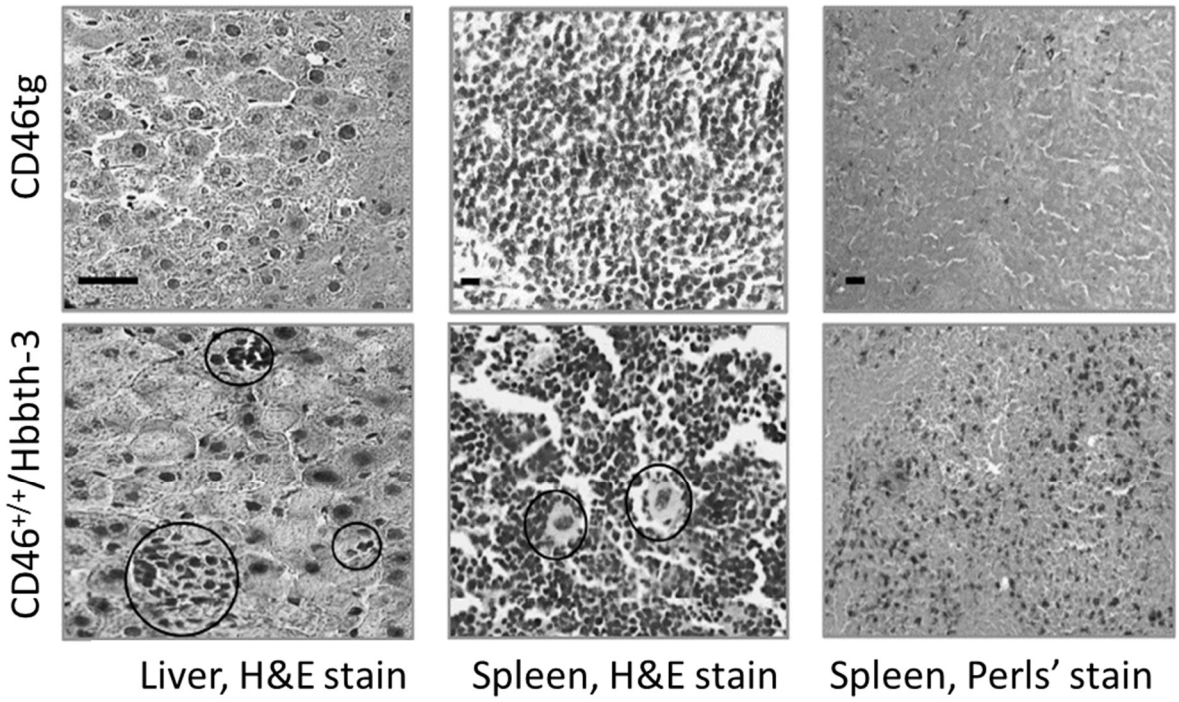

CD46tg
CD46+/+/Hbbth-3
Liver, H&E stain
Spleen, H&E stain
Spleen, Perls' stain

FIG. 10

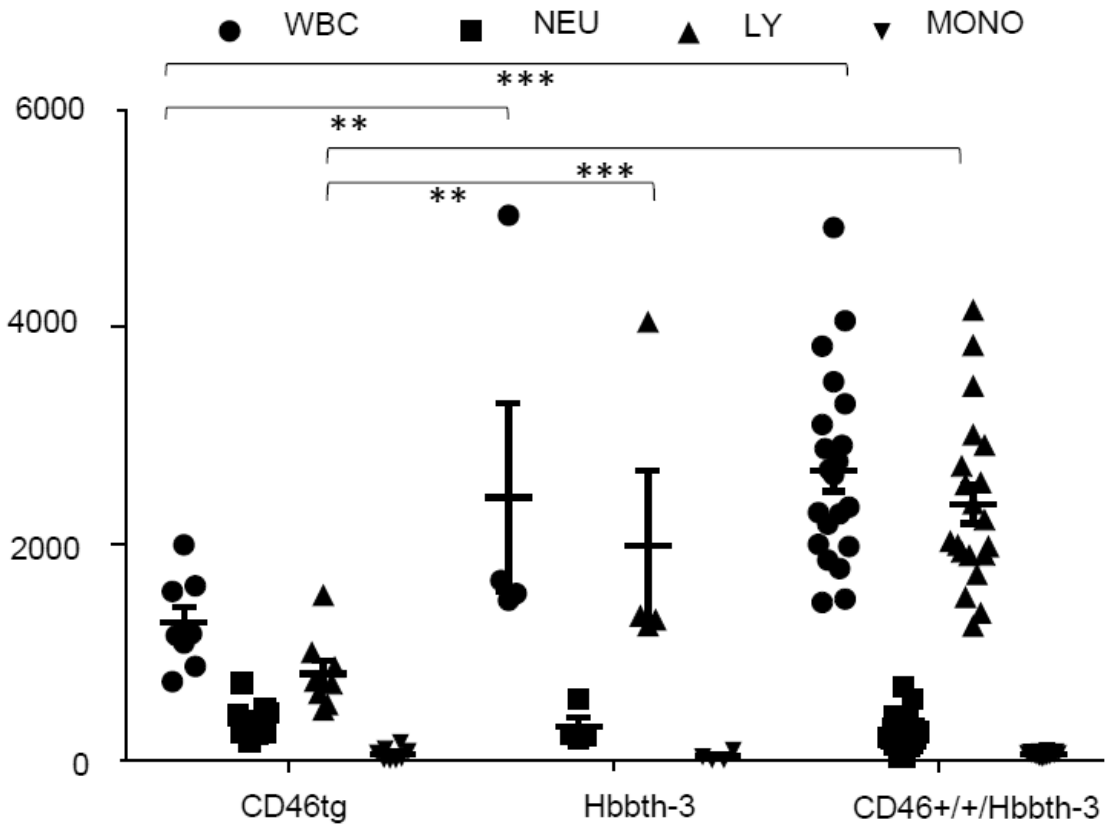

WBC
NEU
LY
MONO
***
**
***
**
6000
4000
2000
0
CD46tg
Hbbth-3
CD46+/+/Hbbth-3

FIG. 11

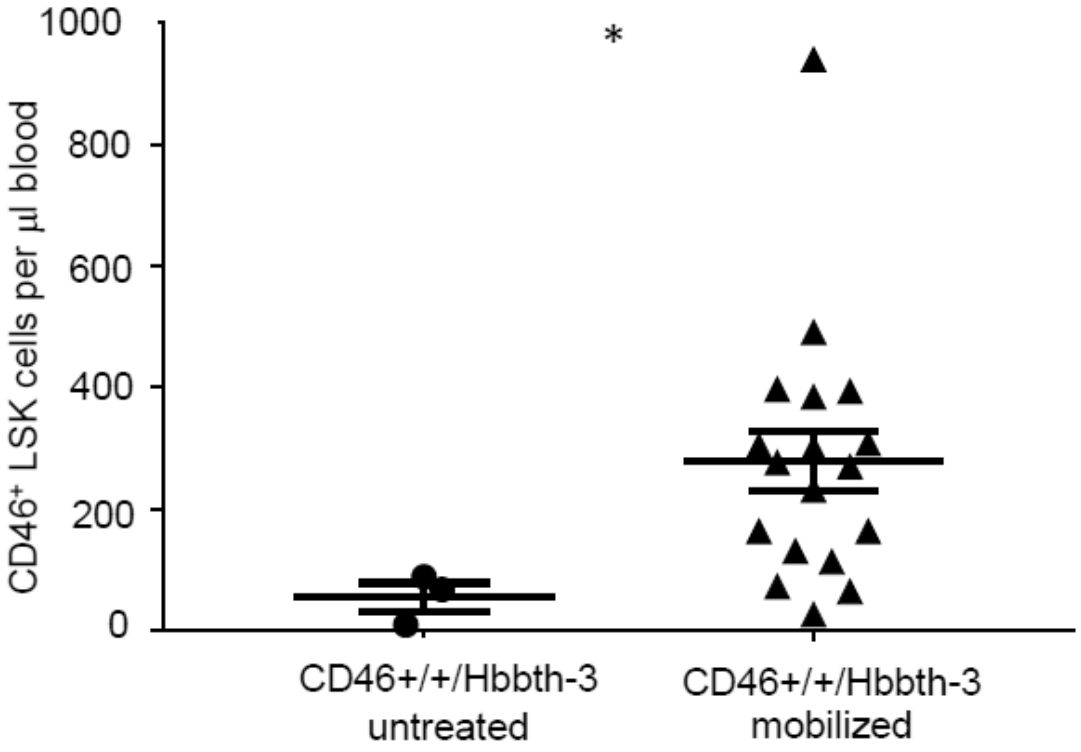

1000
800
600
400
200
0
CD46+ LSK cells per µl blood
*
CD46+/+/Hbbth-3
untreated
CD46+/+/Hbbth-3
mobilized

FIG. 12

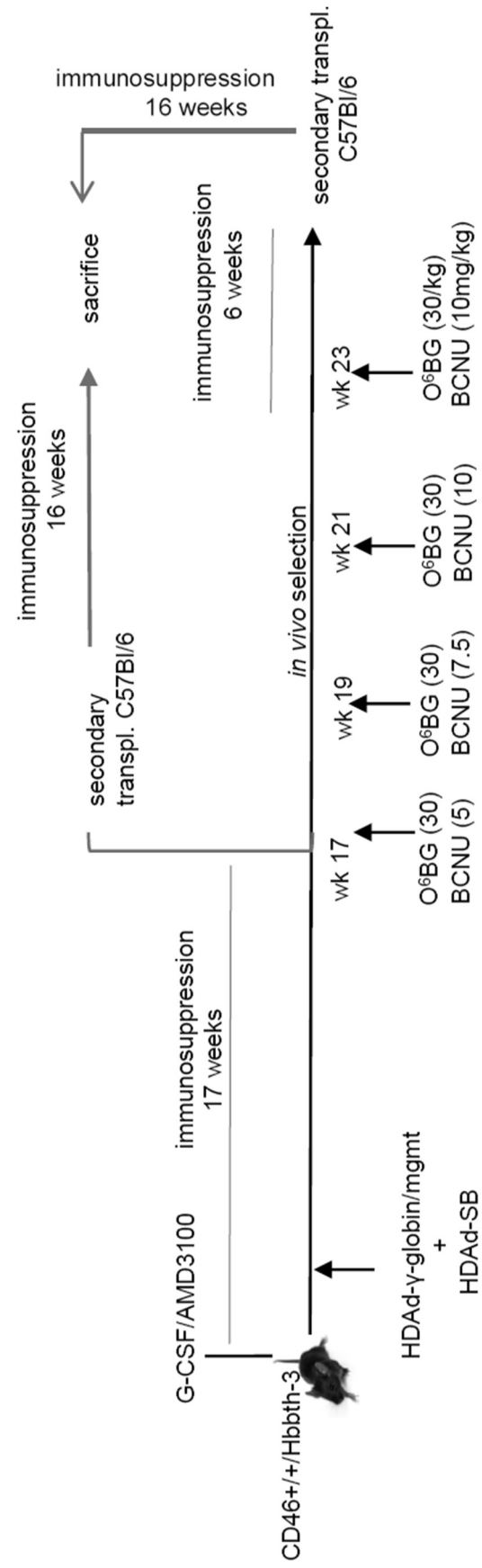

CD46+/+/Hbbth-3
G-CSF/AMD3100
immunosuppression
17 weeks
HDAd-γ-globin/mgmt
+
HDAd-SB
(2x10^10 vp, twice
IV
wk 17
O6BG (30)
BCNU (5)
wk 19
O6BG (30)
BCNU (7.5)
wk 21
O6BG (30)
BCNU (10)
wk 23
O6BG (30/kg)
BCNU (10mg/kg)
in vivo selection
secondary
transpl. C57Bl/6
immunosuppression
16 weeks
sacrifice
immunosuppression
6 weeks
secondary transpl.
C57Bl/6
immunosuppression
16 weeks

FIG. 13A

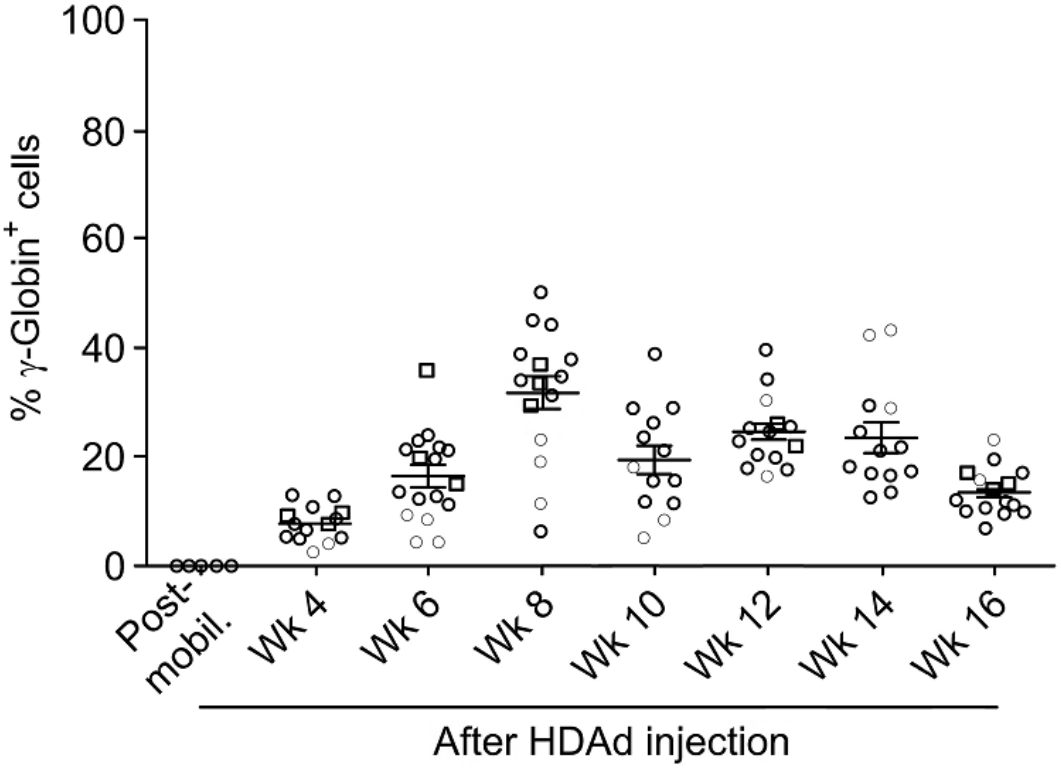


FIG. 13B

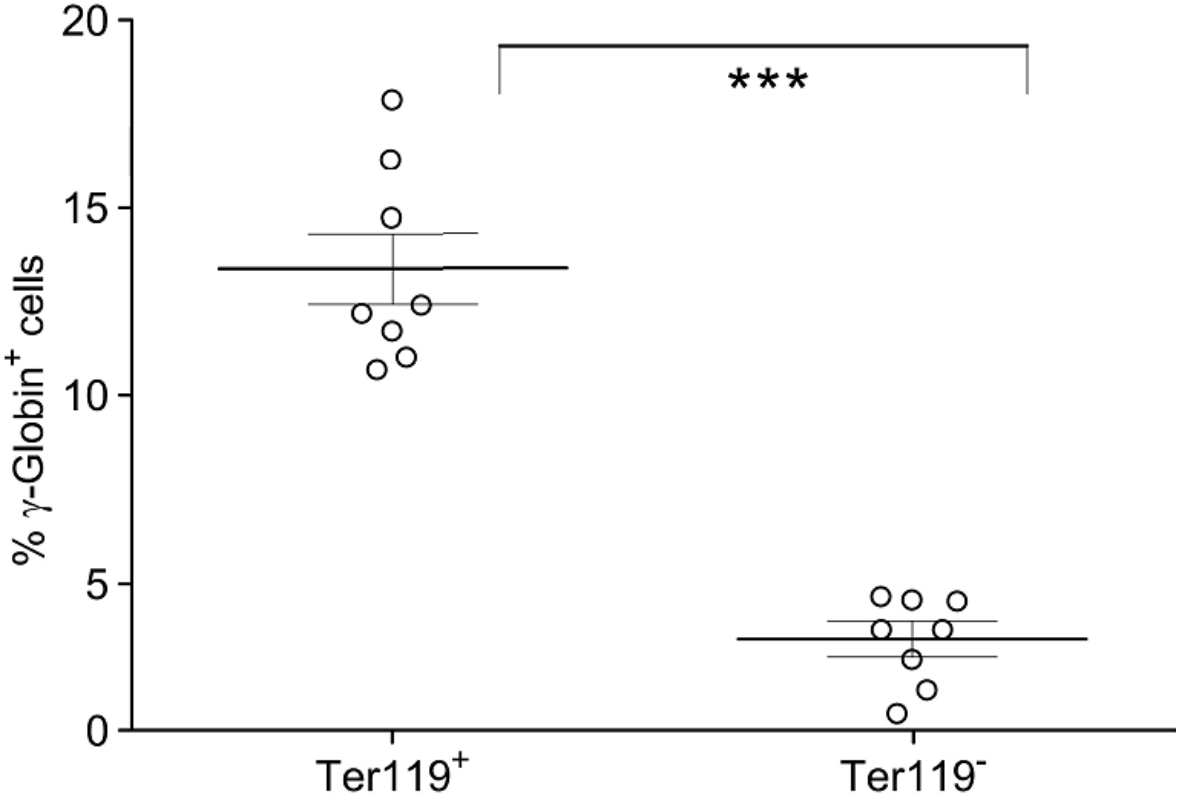


FIG. 13C

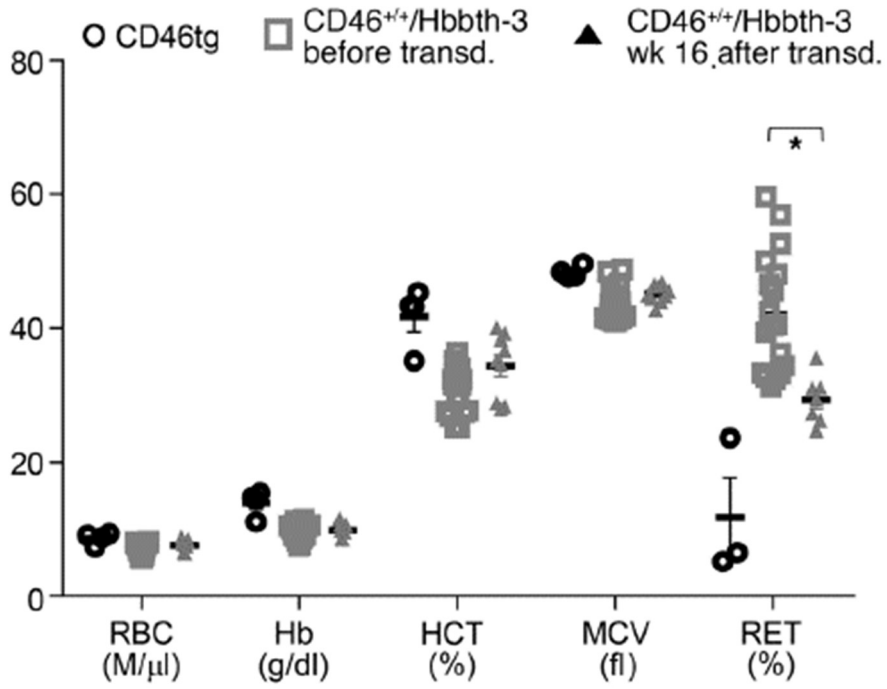


FIG. 13D

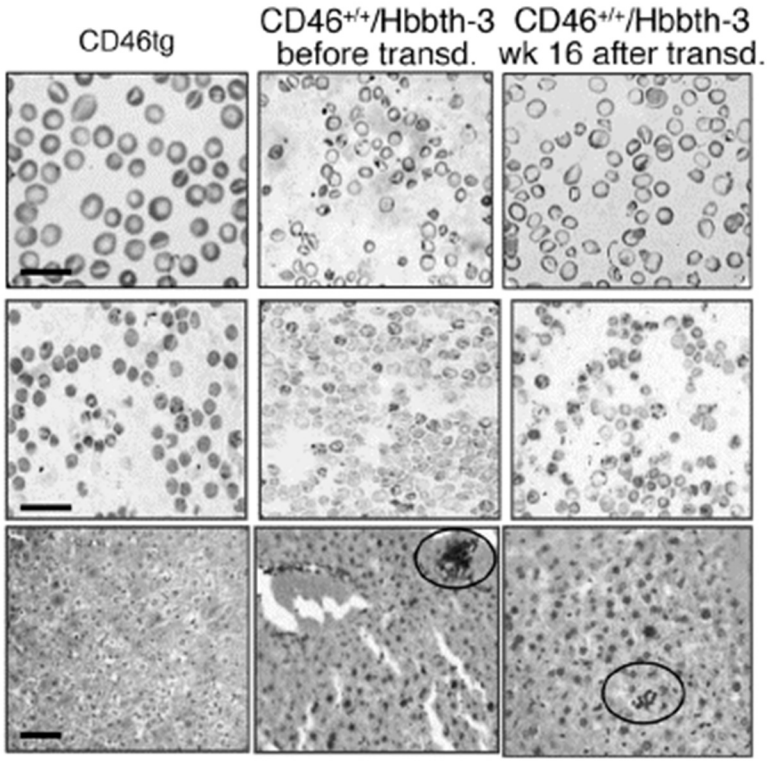


FIG. 13E

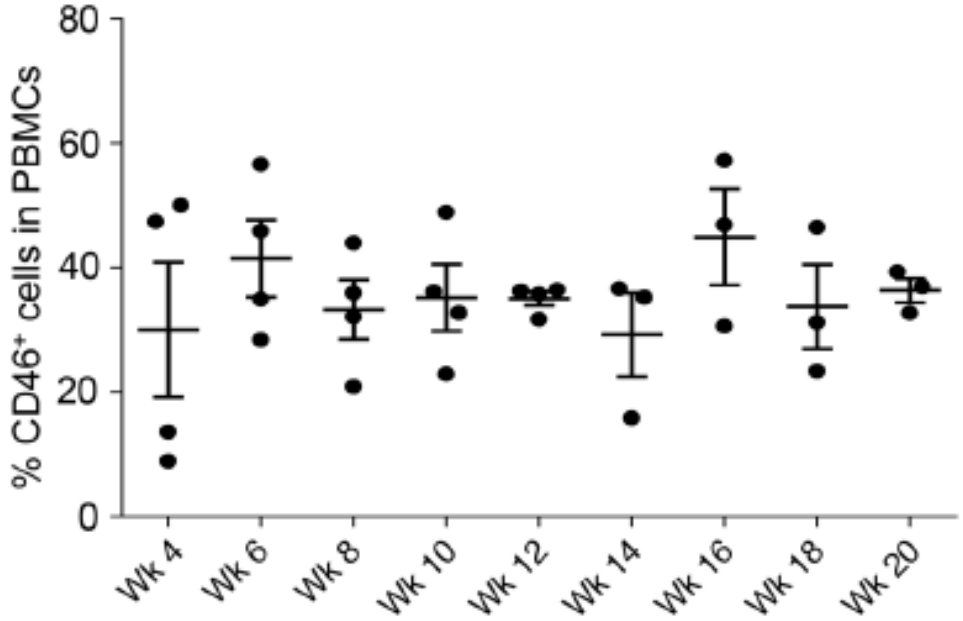


FIG. 13F

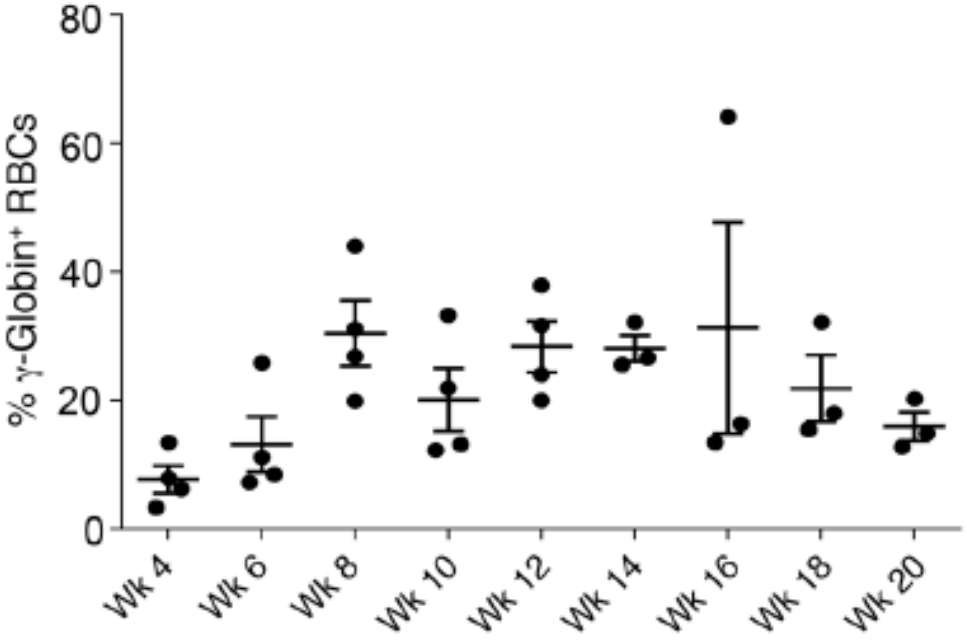


FIG. 14A

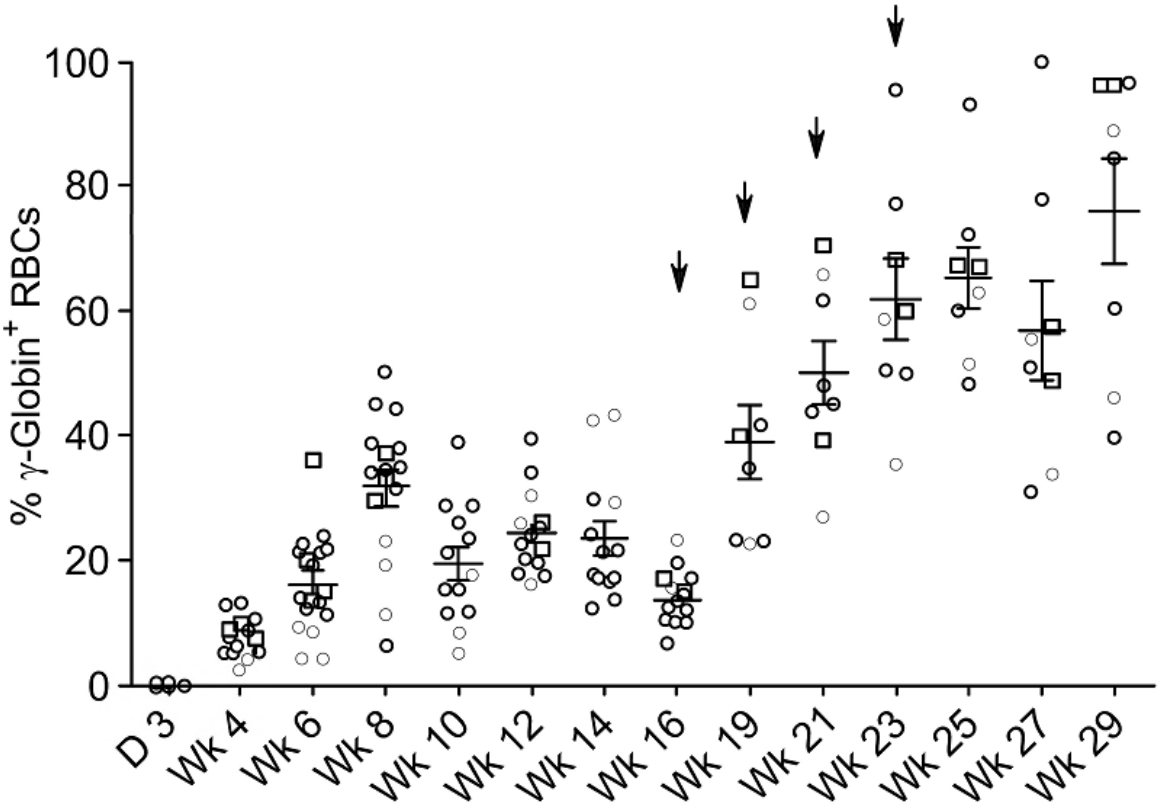


FIG. 14B

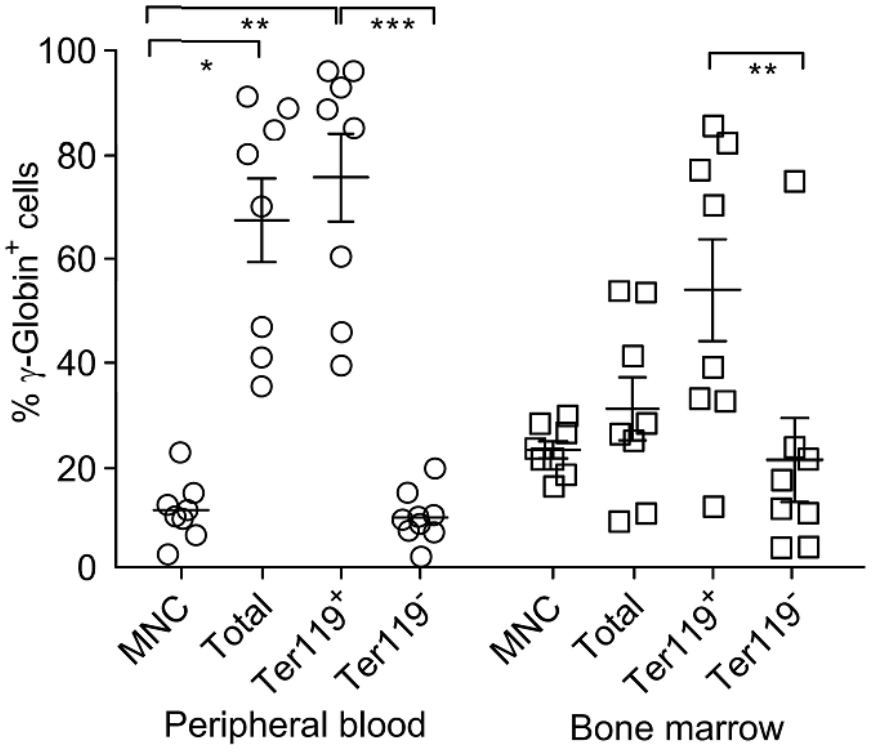


FIG. 14C

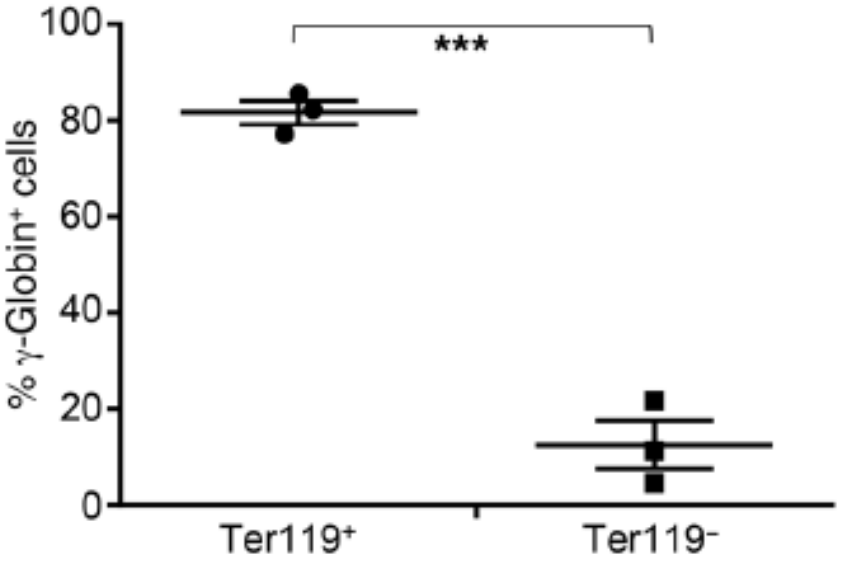


FIG. 14D

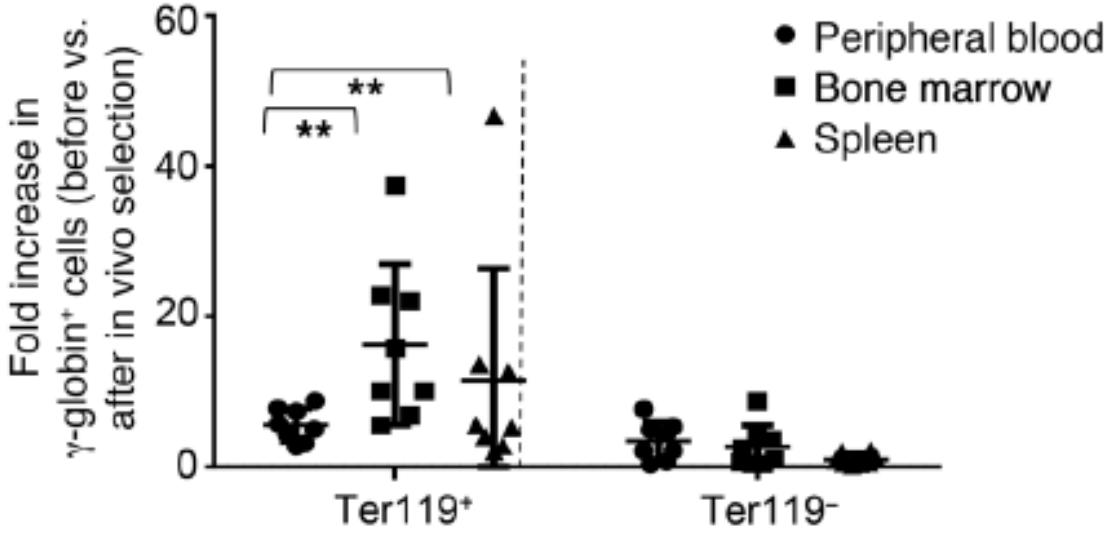


FIG. 14E

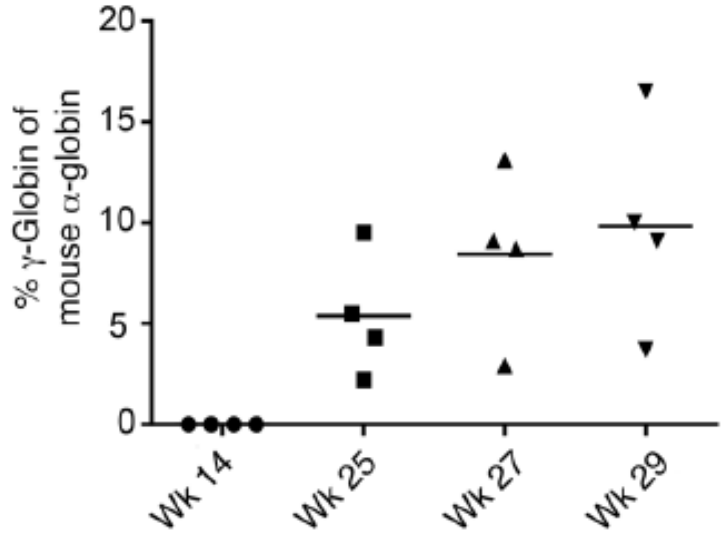


FIG. 14F

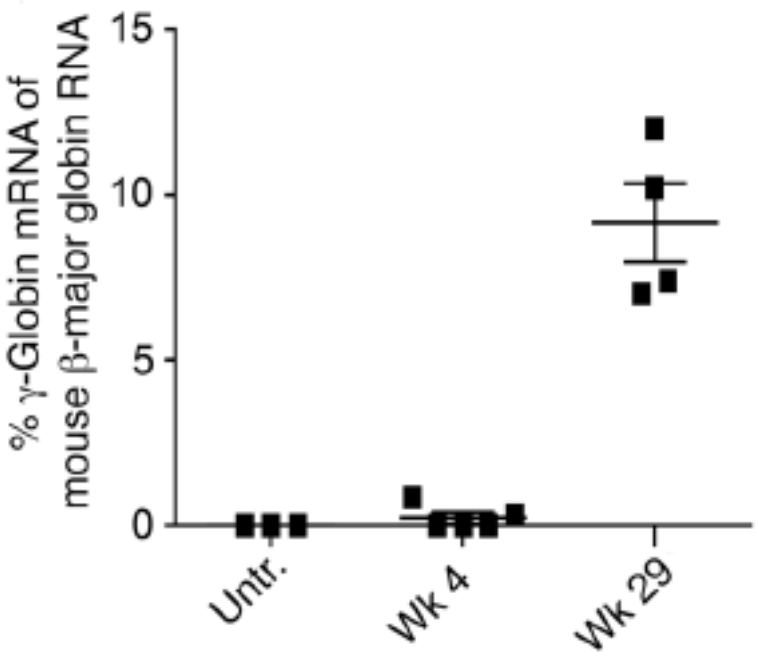


FIG. 15A

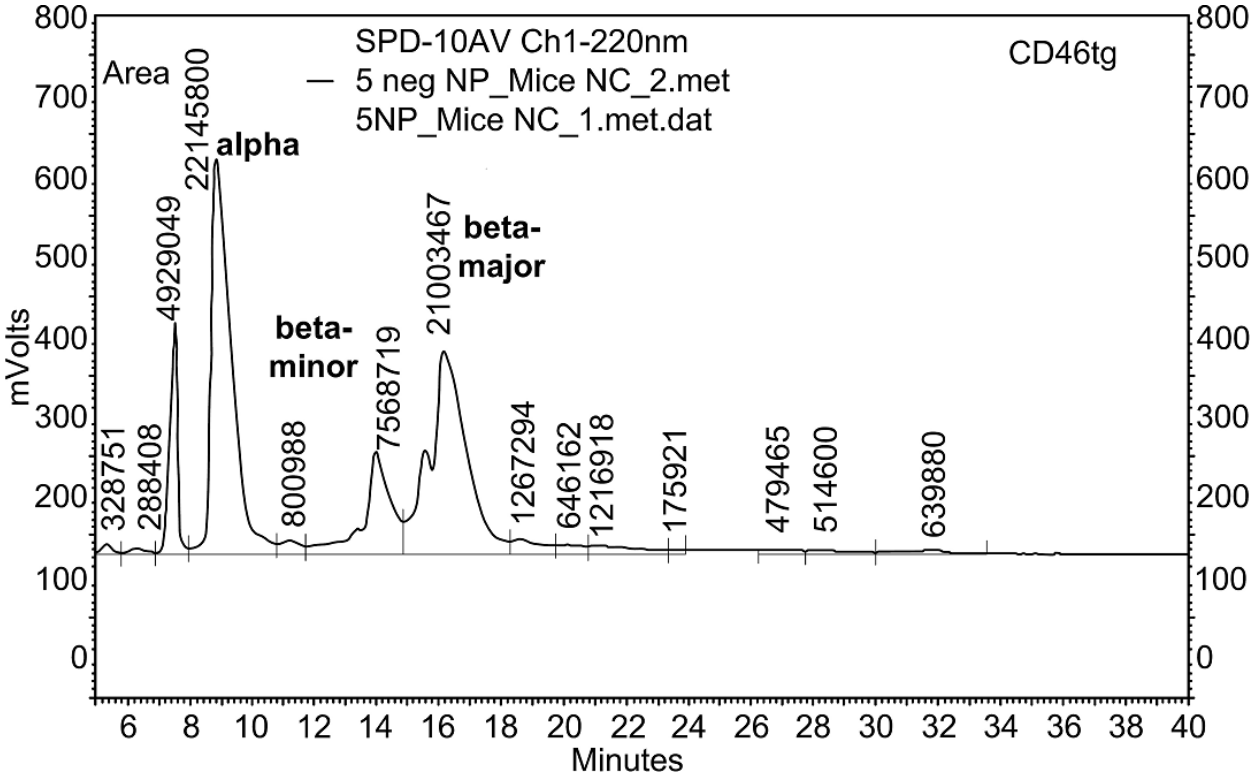


FIG. 15B

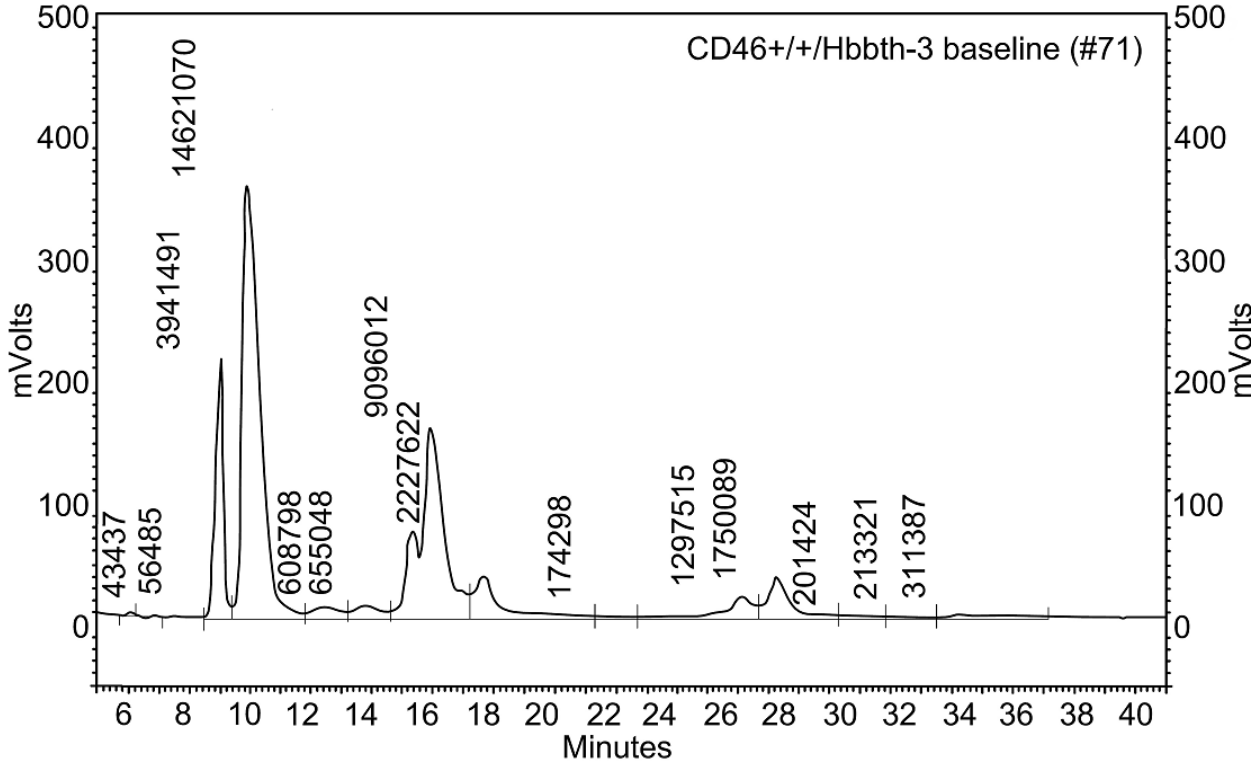


FIG. 15C

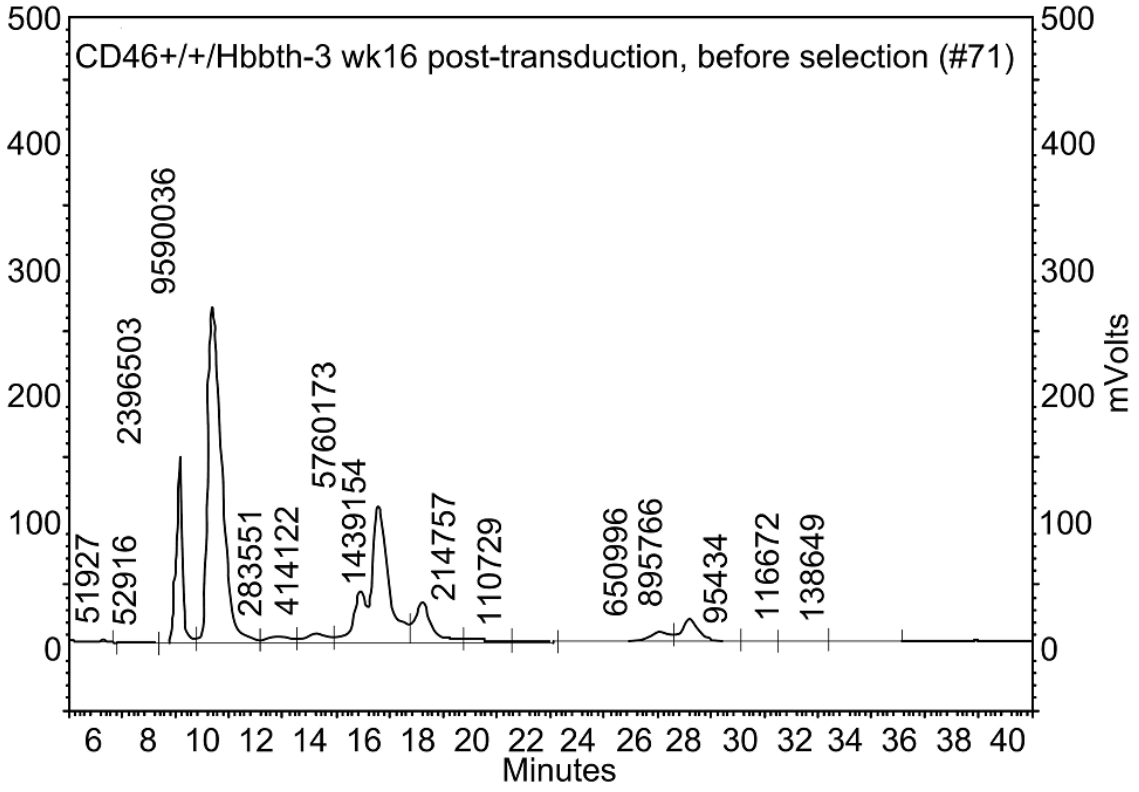


FIG. 15D

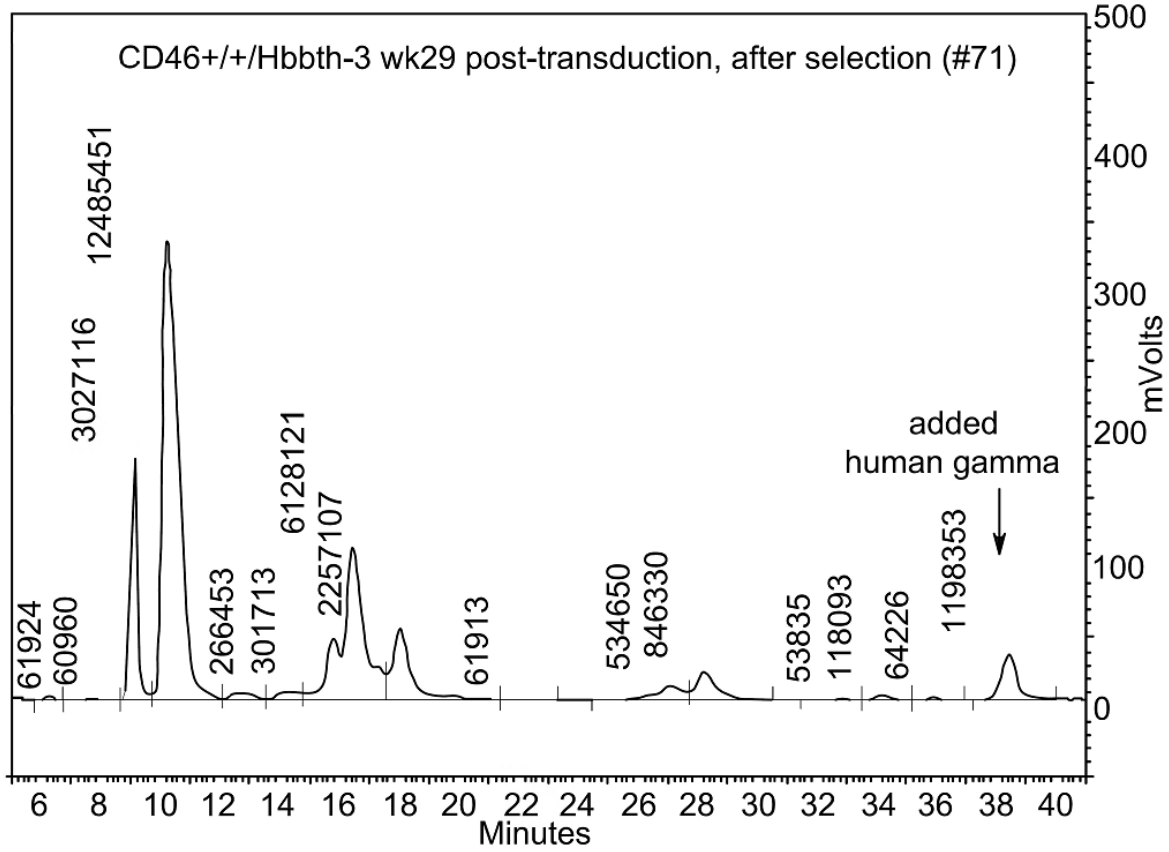


FIG. 16

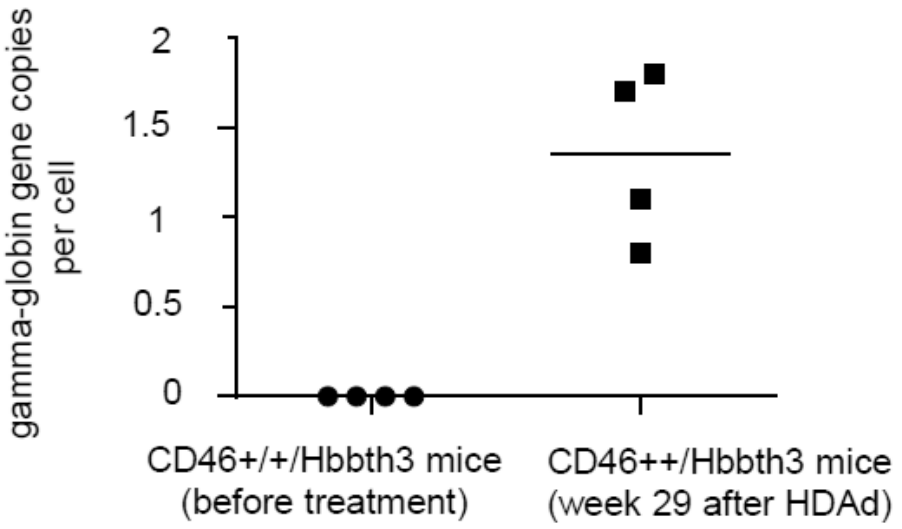


FIG. 17A

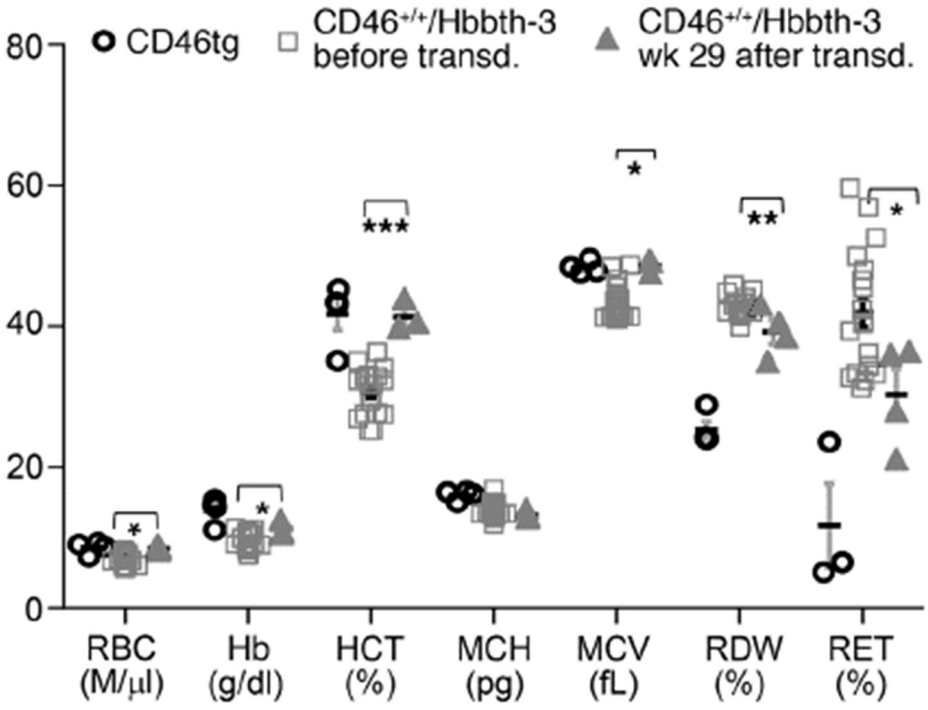


FIG. 17B

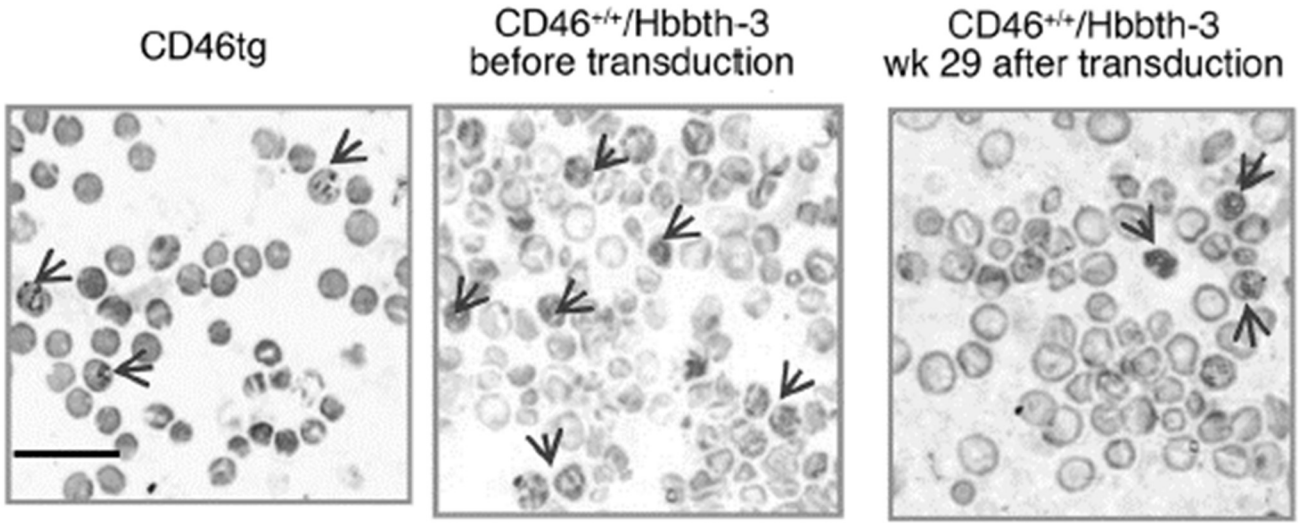


FIG. 17C

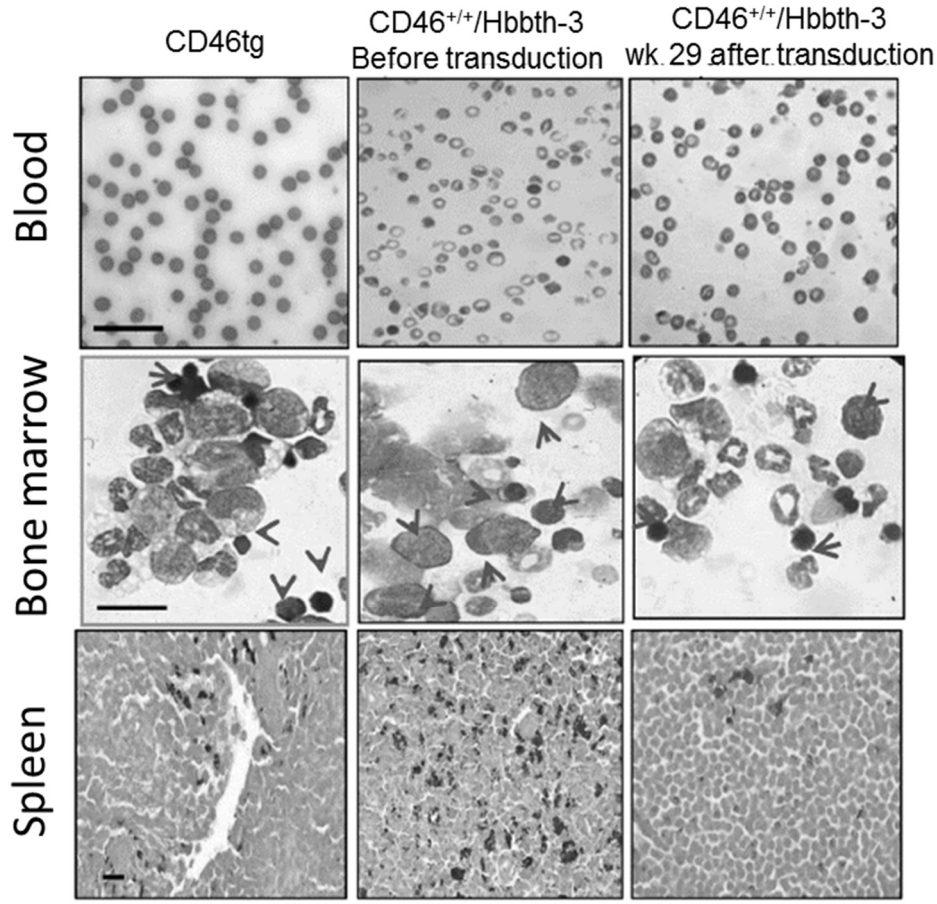


FIG. 17D

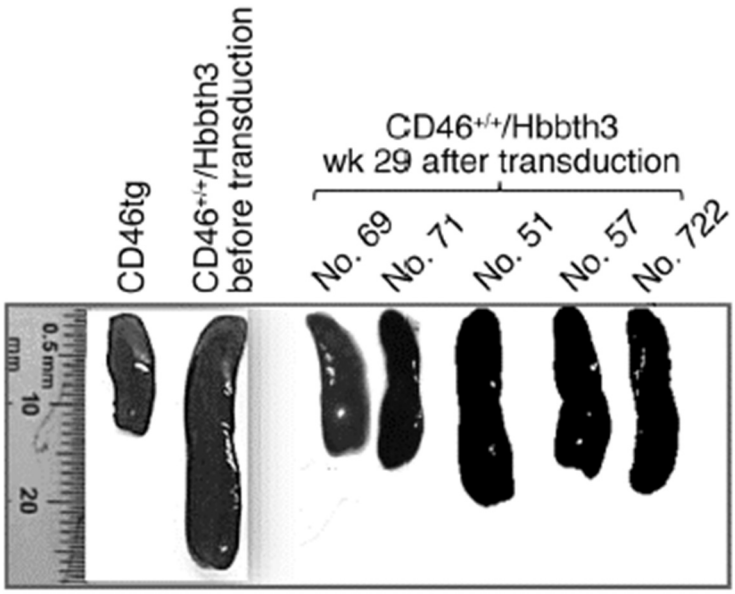


FIG. 17E

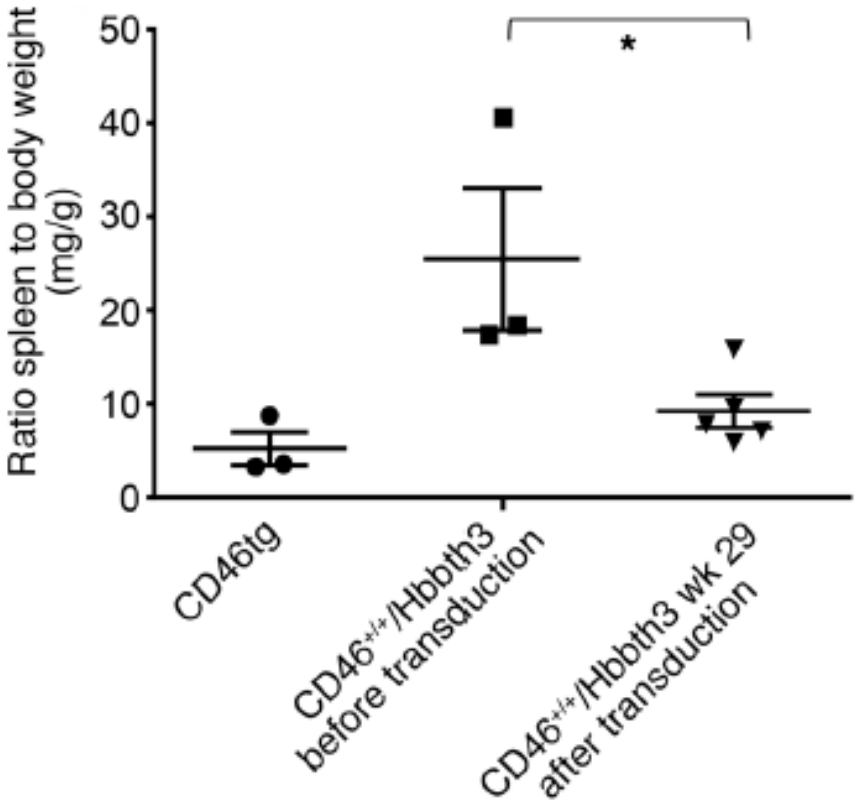


FIG. 18A

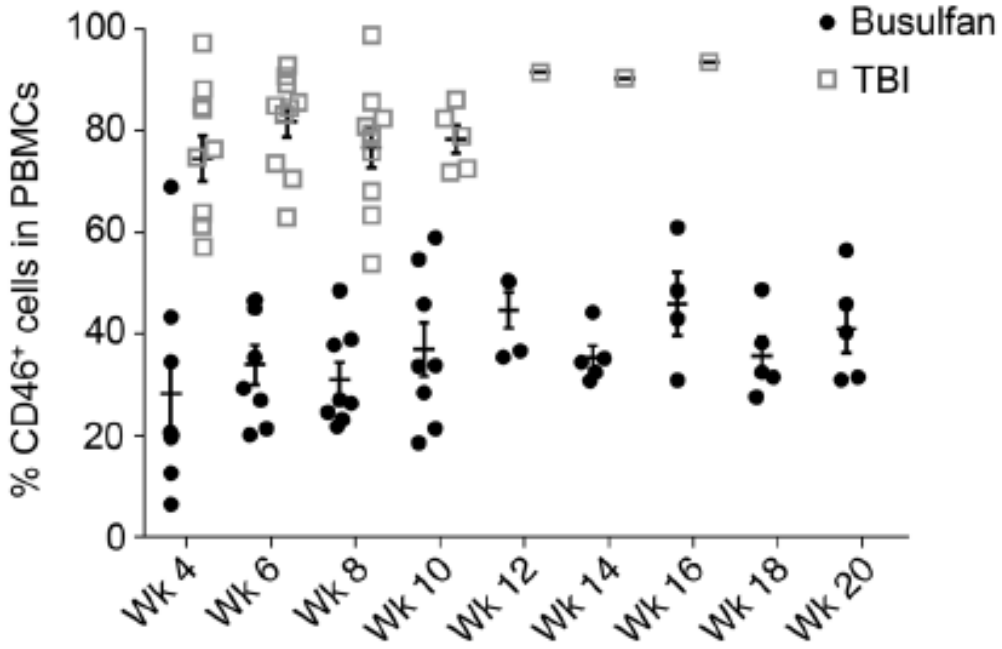


FIG. 18B

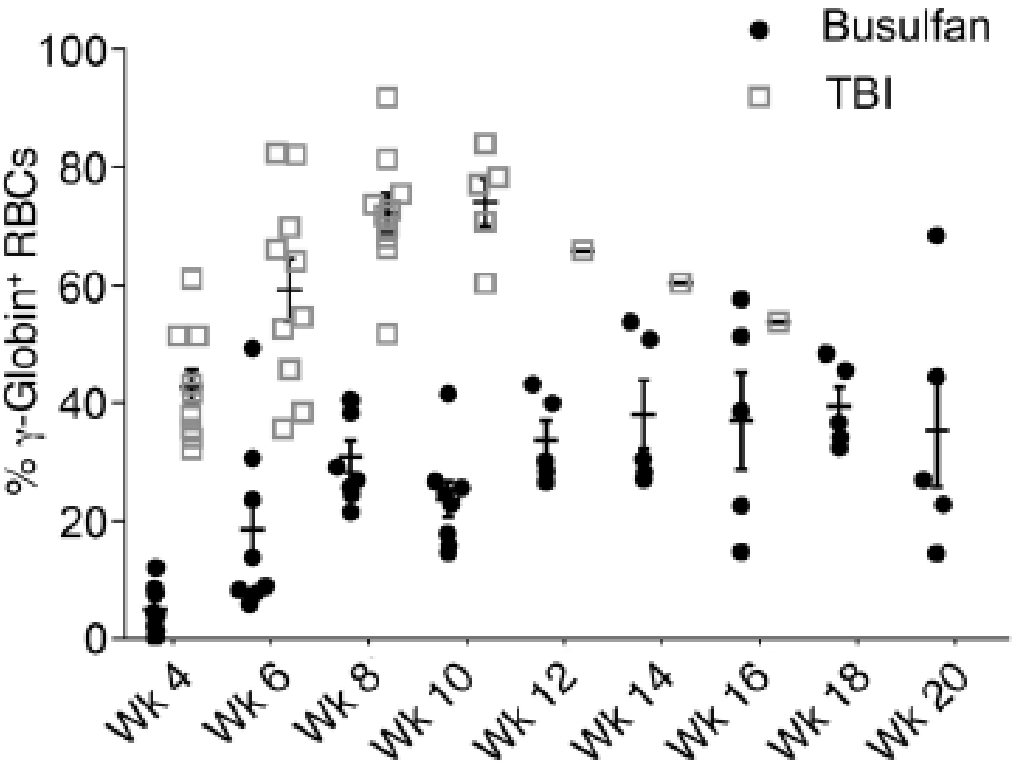


FIG. 18C

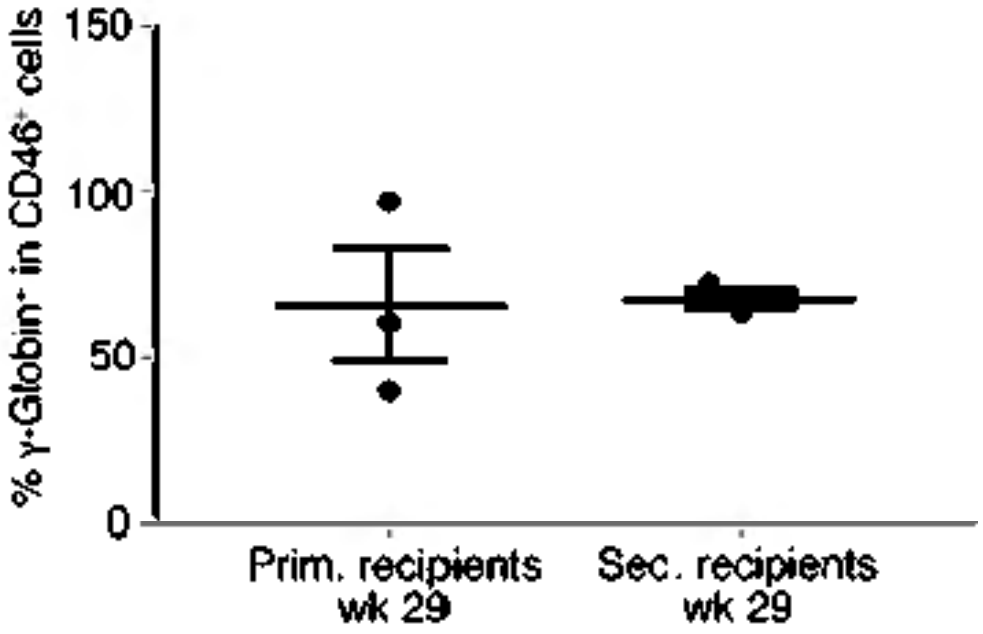


FIG. 18D

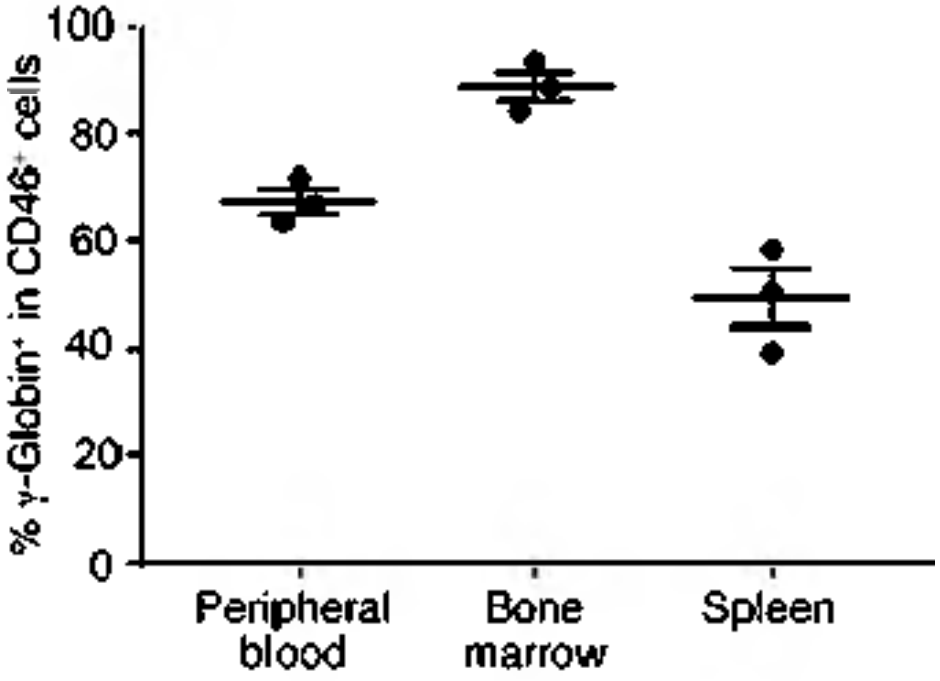


FIG. 18E

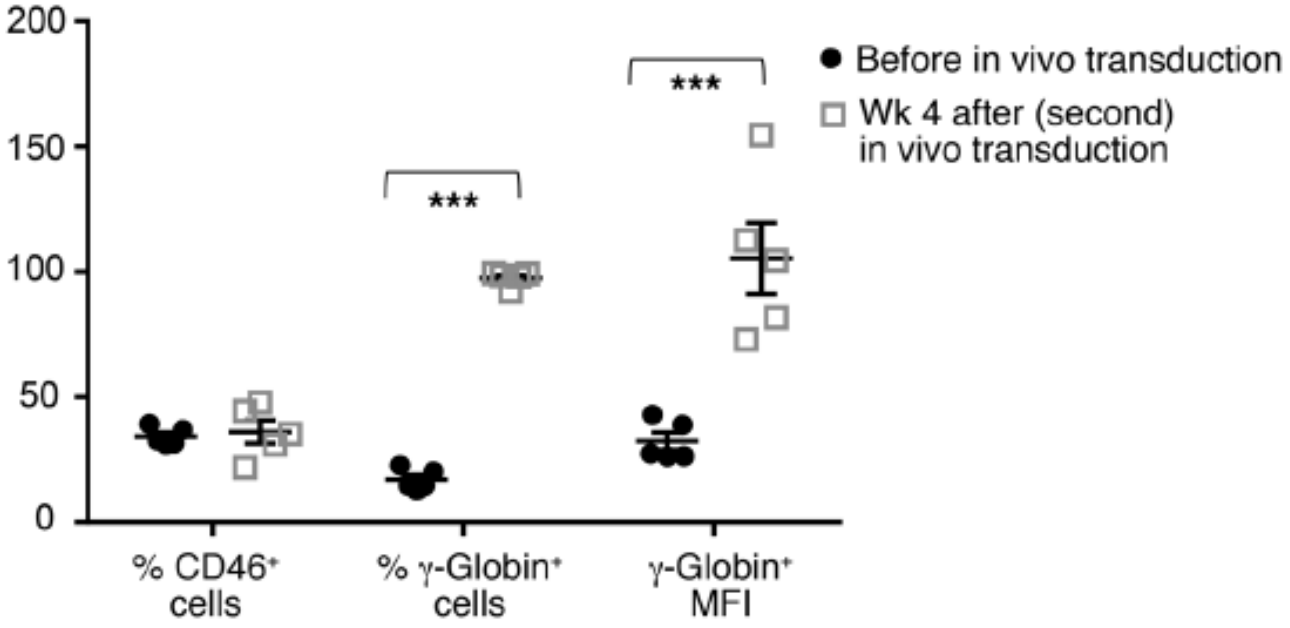


FIG. 19A

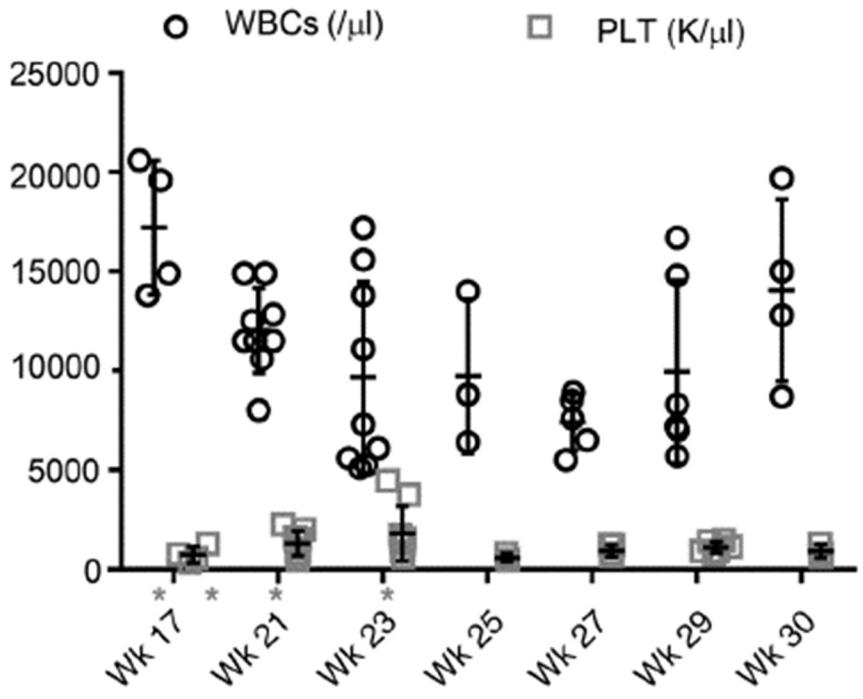


FIG. 19B

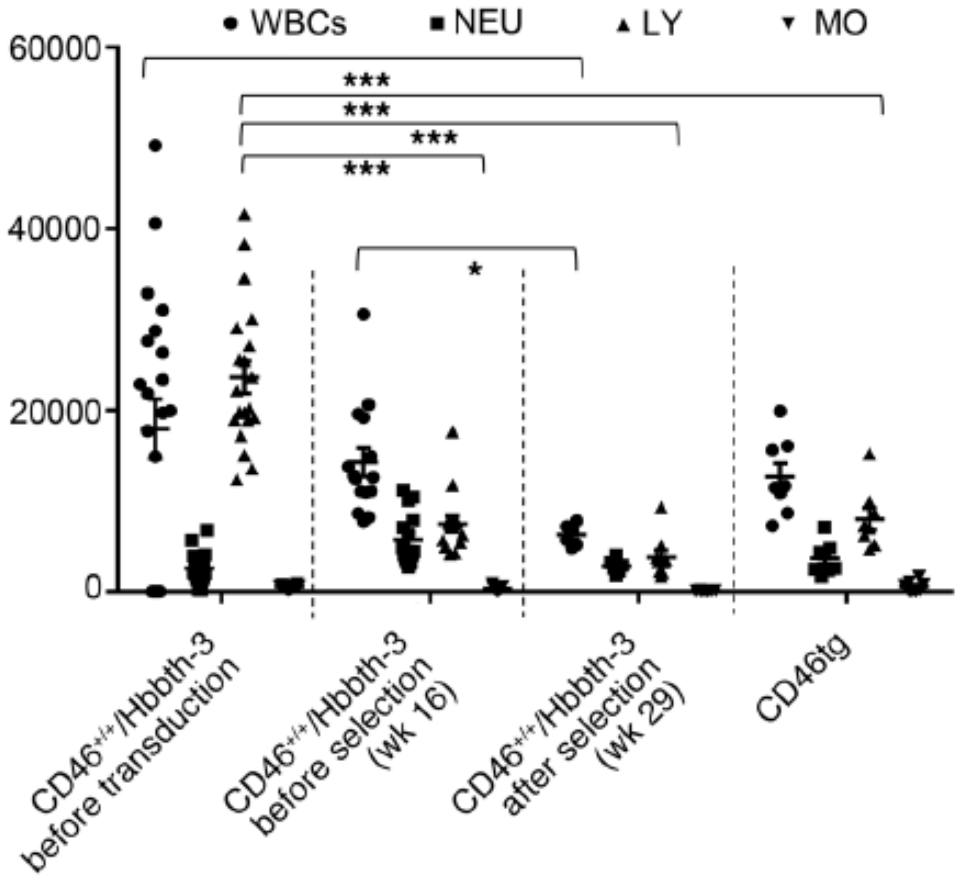


FIG. 19C

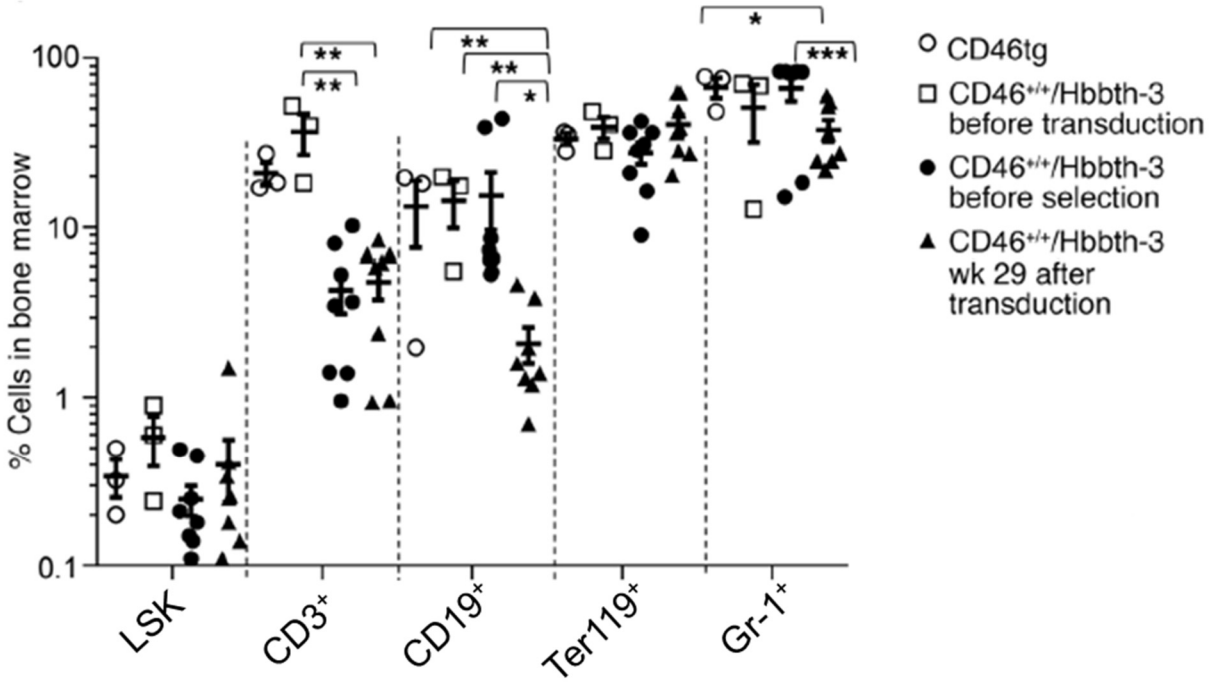


FIG. 19D

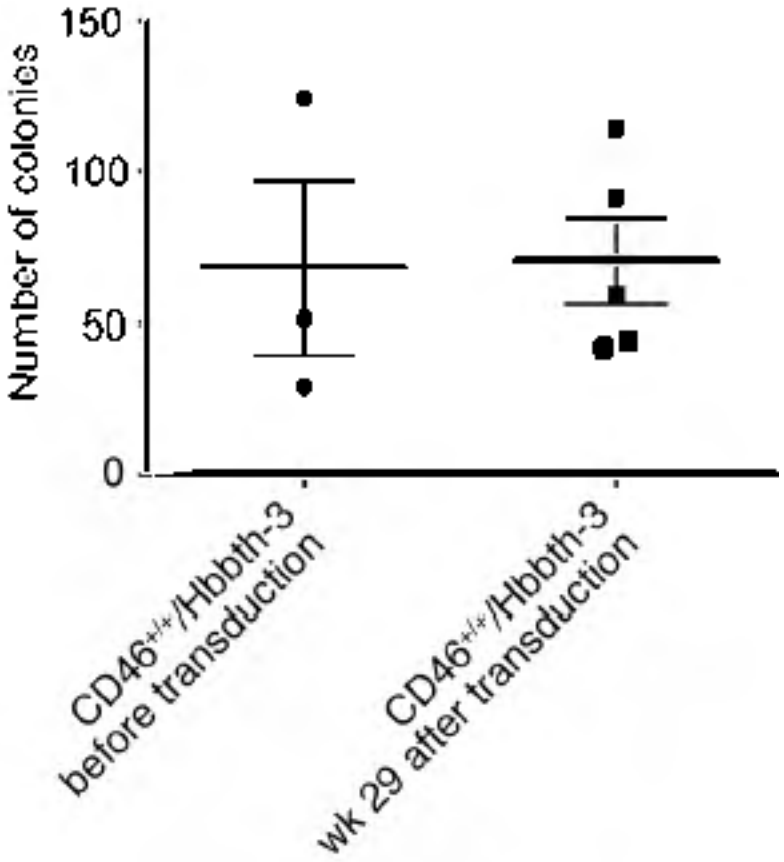

150
100
50
0
Number of colonies
CD46+/+/Hbbth-3 before transduction
CD46+/+/Hbbth-3 wk 29 after transduction

FIG. 20A

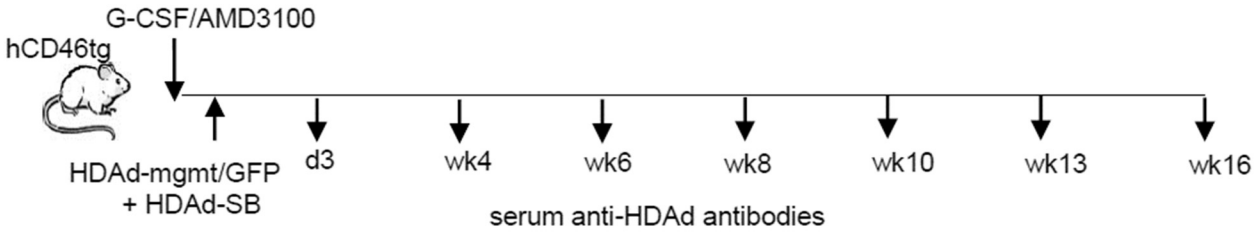


FIG. 20B

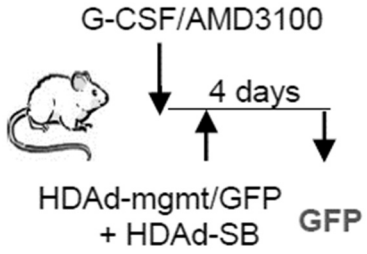


FIG. 20C

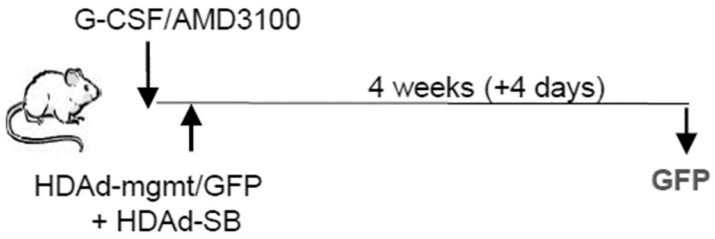


FIG. 20D

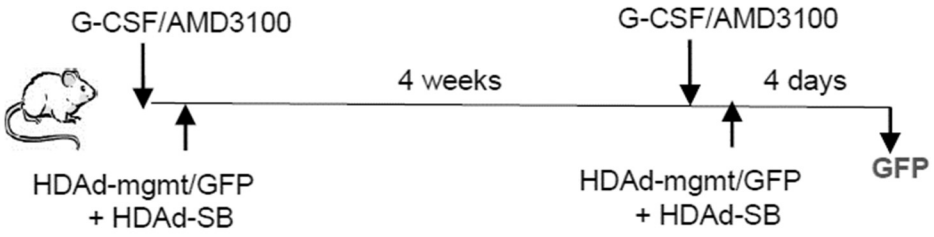


FIG. 20E

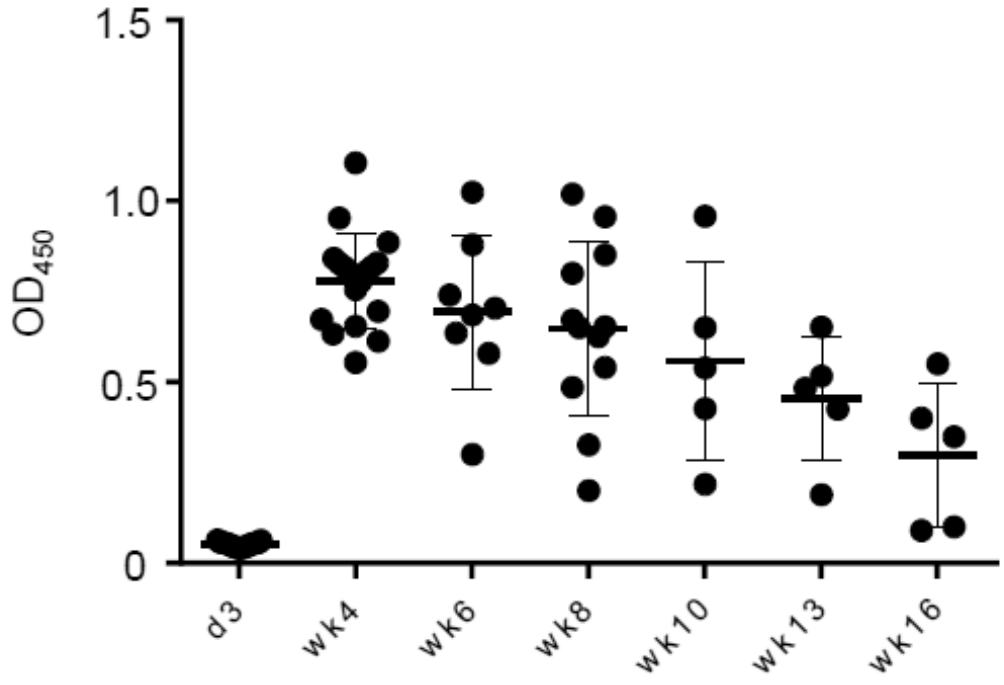


FIG. 20F

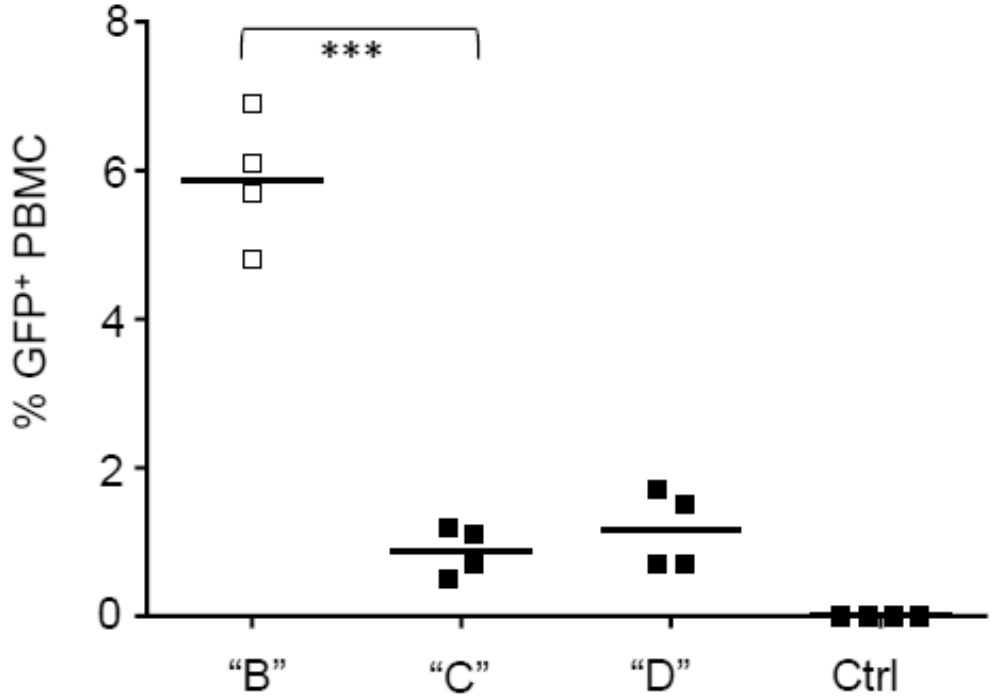


FIG. 21A

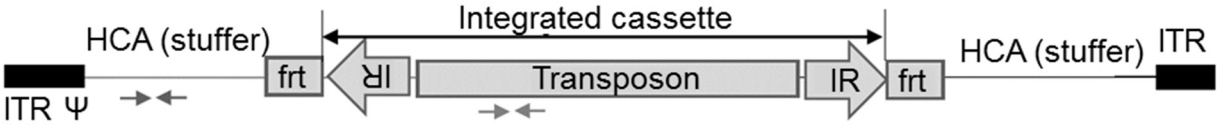


FIG. 21B

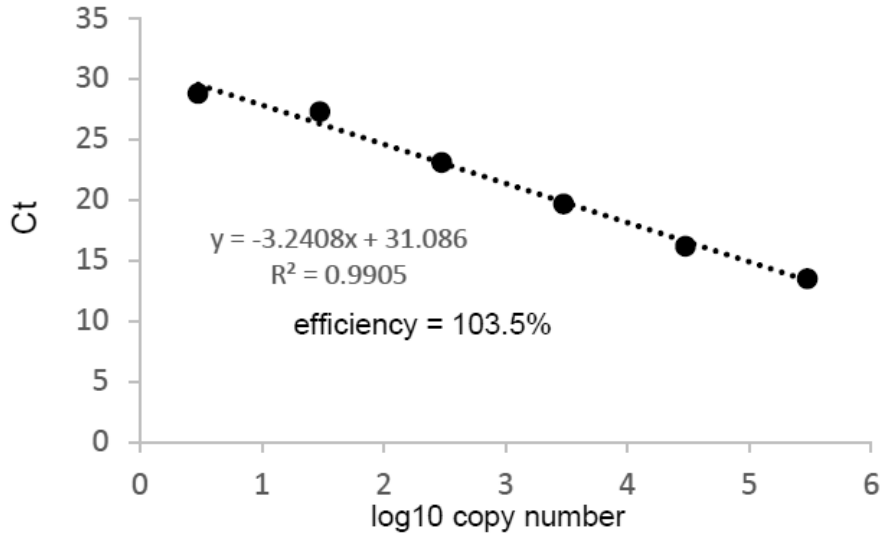


FIG. 21C

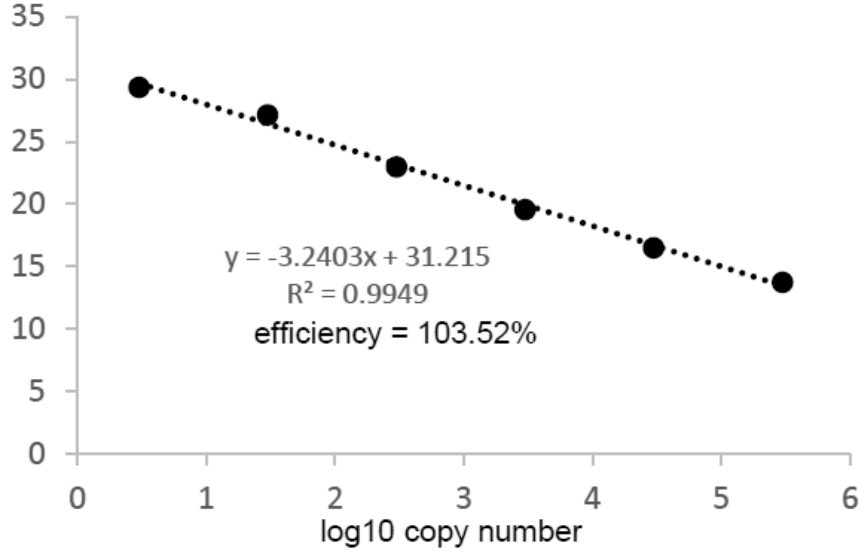


FIG. 21D

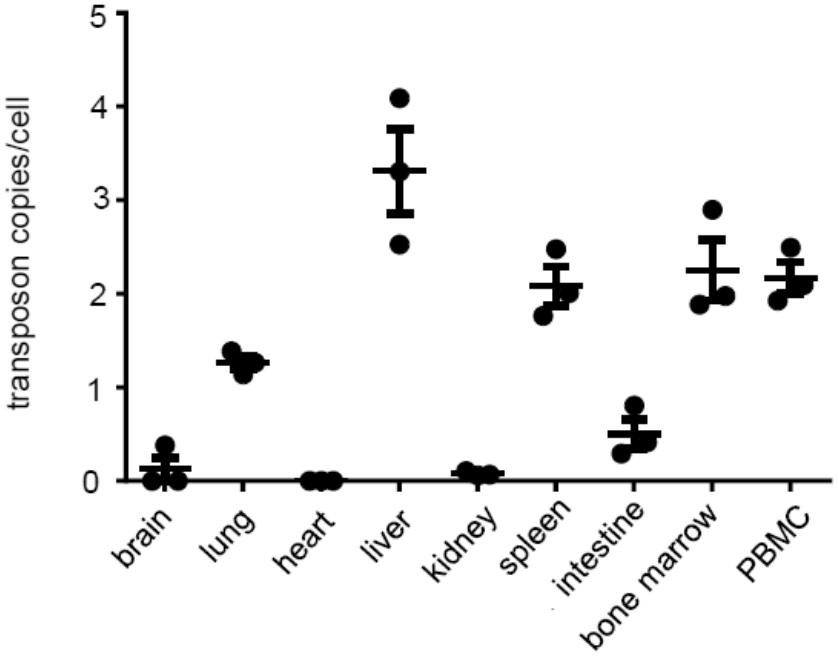

transposon copies/cell
5
4
3
2
1
0
brain
lung
heart
liver
kidney
spleen
intestine
bone marrow
PBMC

FIG. 22A

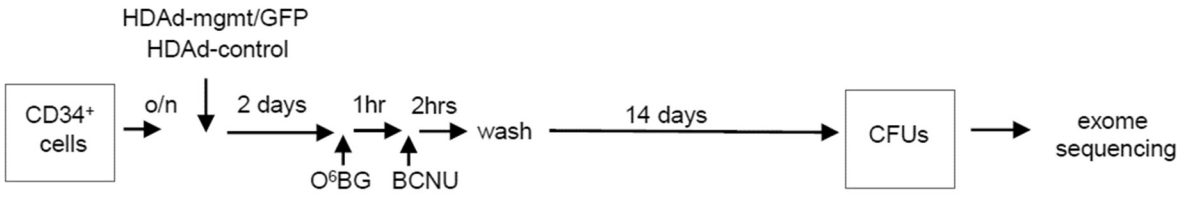


FIG. 22B

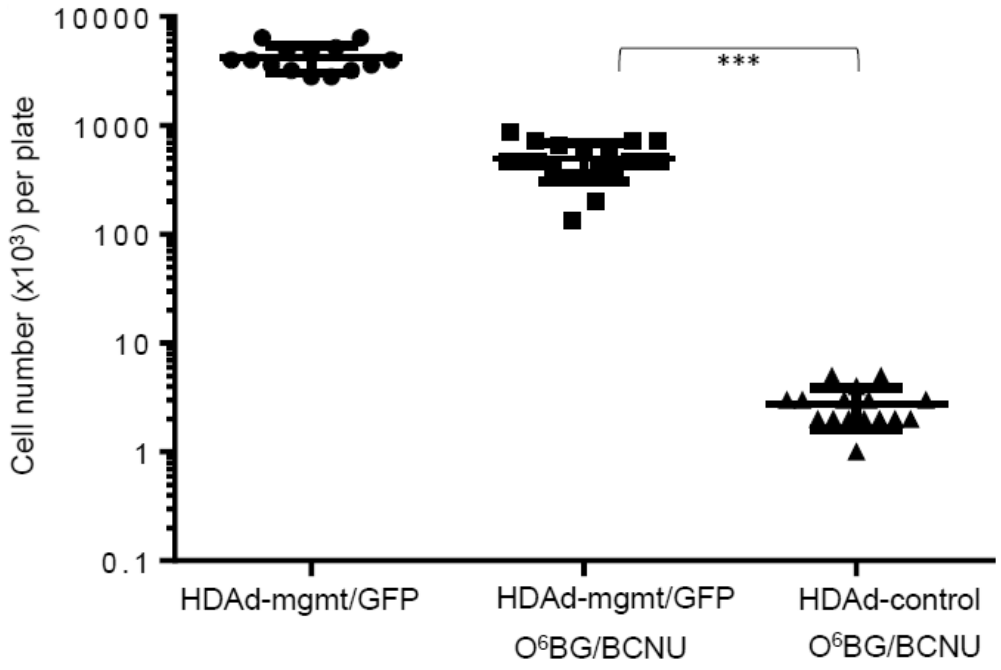


FIG. 22C

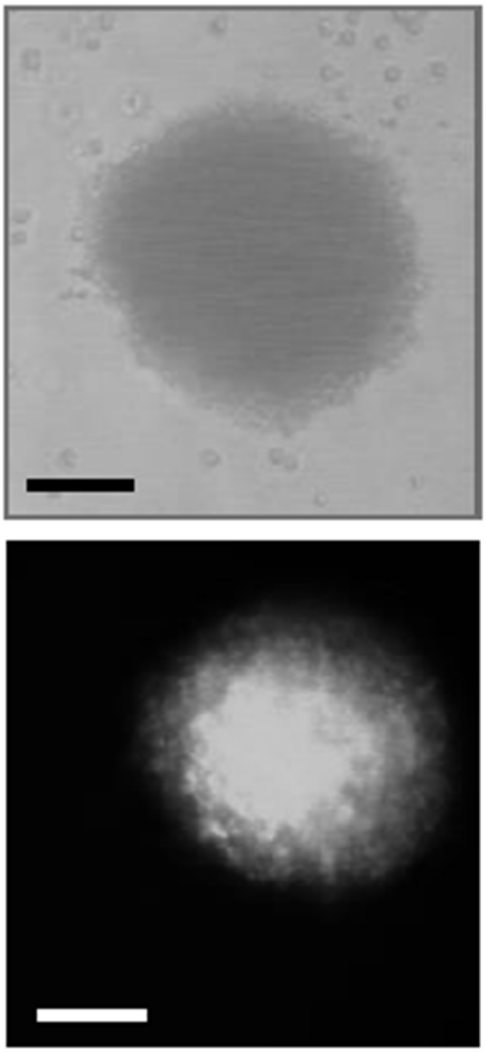


FIG. 23

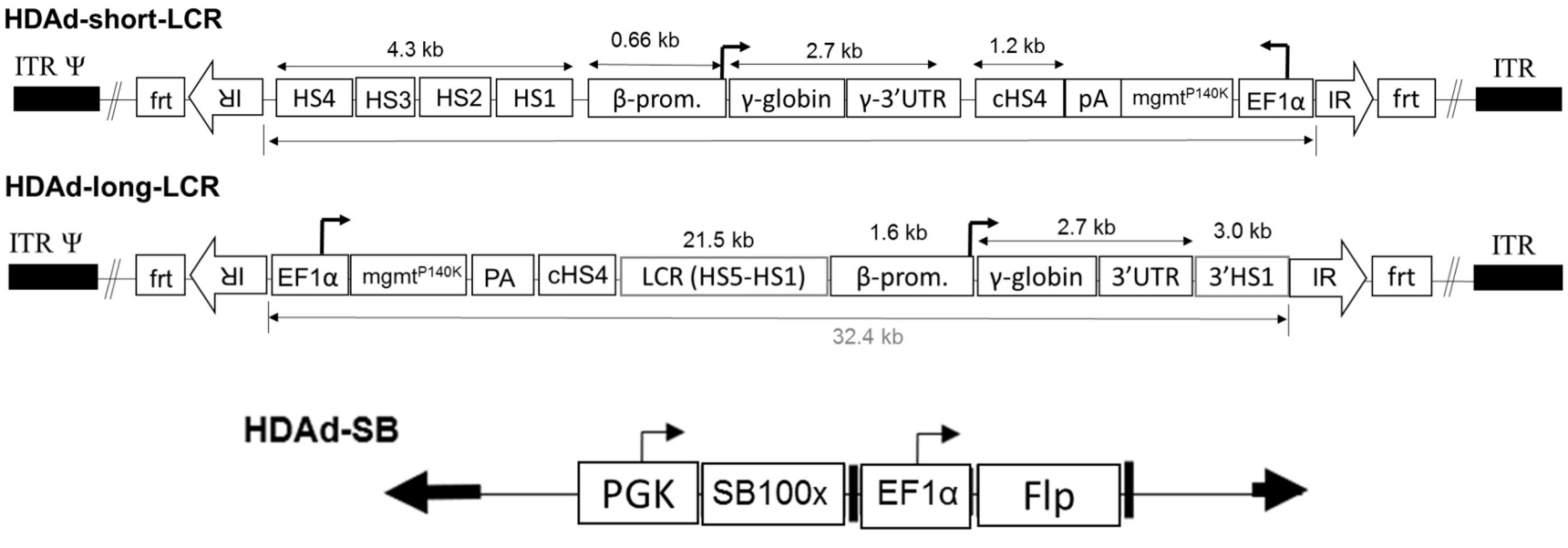


FIG. 24A

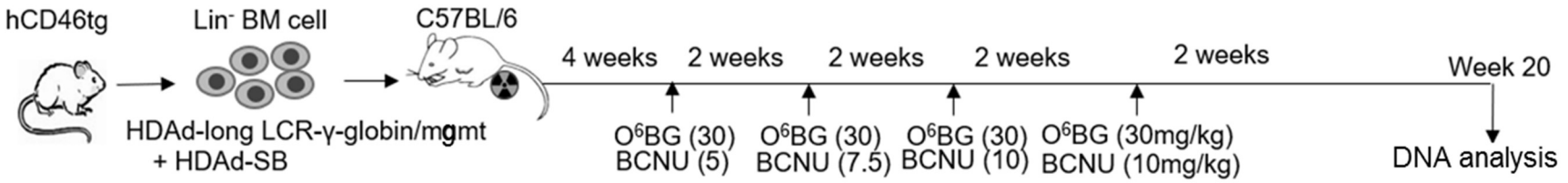


FIG. 24C

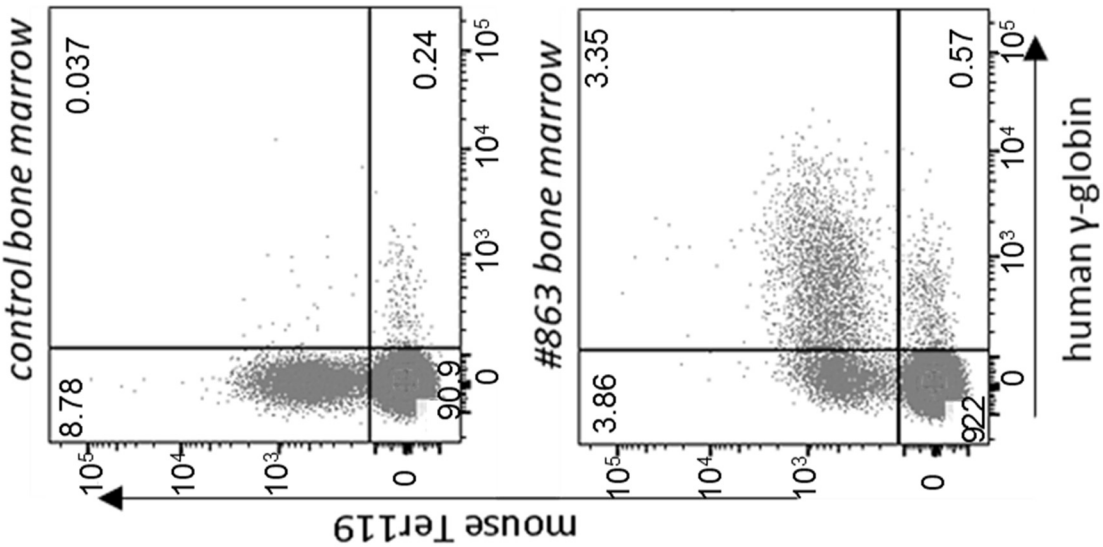


FIG. 24B

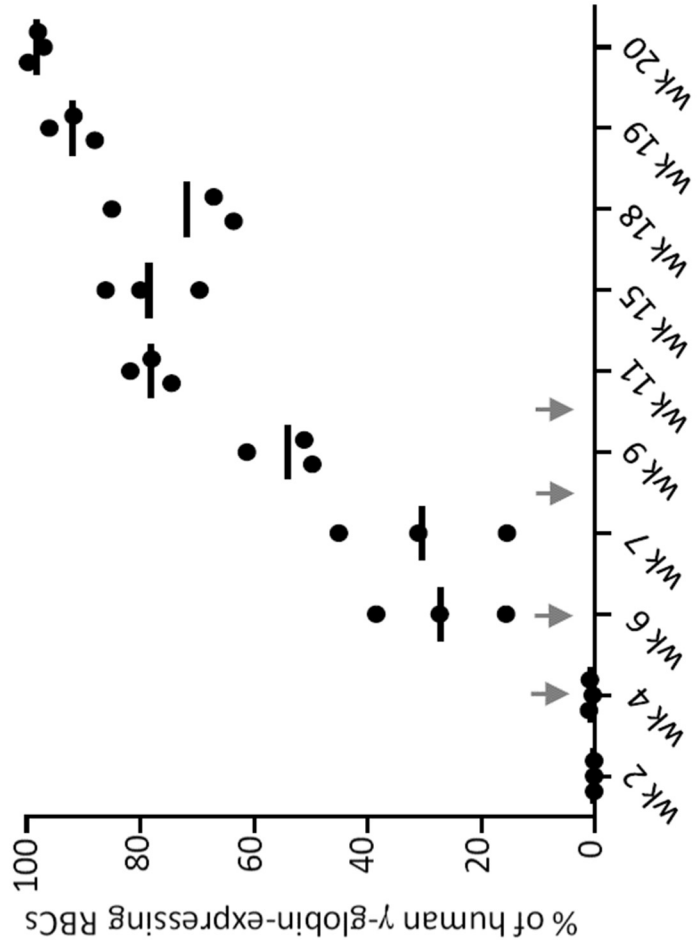


FIG. 24D

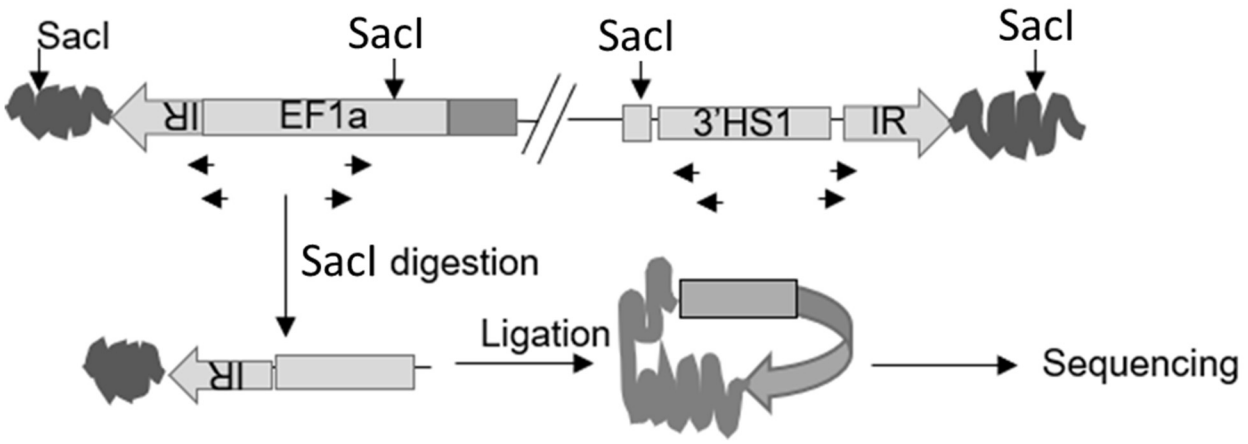


FIG. 24E

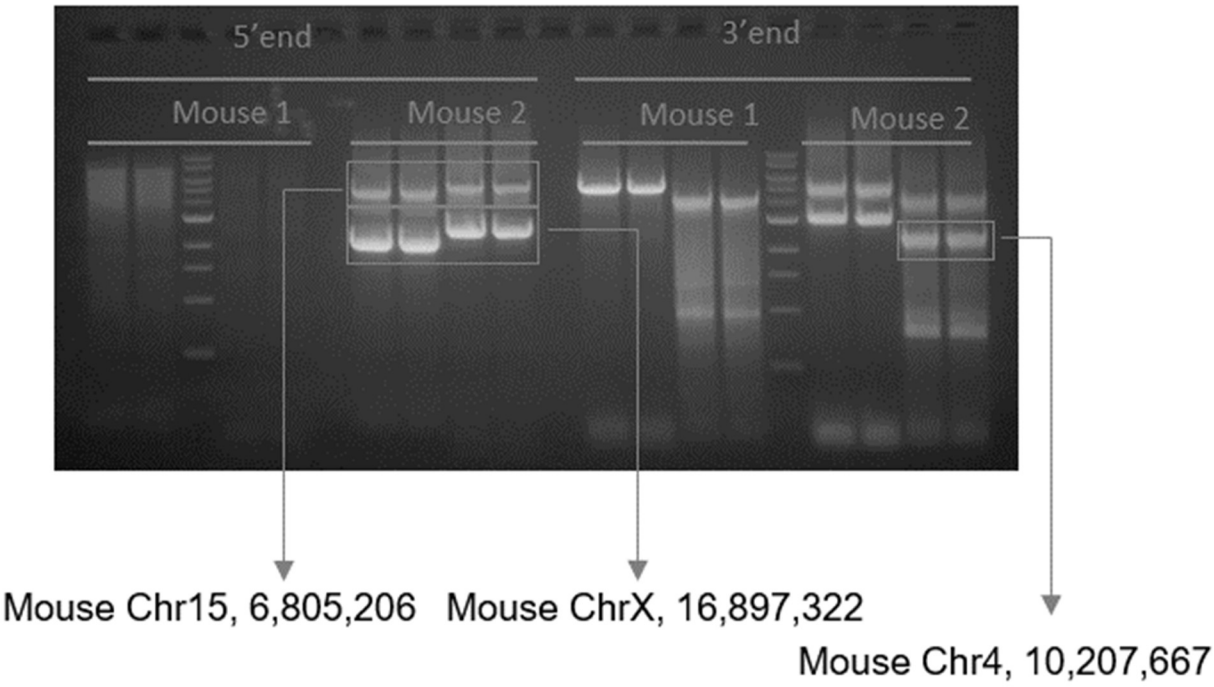


FIG. 24F

CCCTGGGATTCCCCAAGGCAGGGGCGAGTCCTTTTGTATGAATTACTCAAATCGATAACTAGAAACTTAATTAACAACGA
GATCTTATAATTTGCATACTTCTGCCTGCTGGGGACTTTCCACACCCTAGCTGACACAAGAATTTGAAATACATCCACAG
GTACACCTCCAATTGACTCAAATGATGTCAATTAGTCTATCATAATCTTCTAAAGCCATGACATCATTTTAACTGGAATT
TTCCAAGCTGTTTAAAGGCACAGTCAACTTAGTGTATGTAAACTTCTGACCCACTGGAATTGTGATACAGTGAATTATAA
GTGAAATAATCTGTCTGTAAACAATTGTTGGAAAAATGACTTGTGTCATGCACAAAGTAGATGTCCTAACTGACTTGCCA
AAACTATTGTTTGTTAACAAGAAATTTGTGGAGTAGTTGAAAAACGAGTTTTAATGACTCCAACTTAAGTGTATGTAAAC
TTCCGACTTCAACTG[TA]**AGAATGGCCCATTCATCTATAGTAGCACACAATATTTGCATTTGTGCGACAGTATAAGGGACA**
ATTATGCTATCAGGCATTTTTCCAAAGTGAGTAATCGAAGTTTTTATACCTTTGTGTGCCATGTTTGCTACCATGGTGGG
ATAATCTTACACGCGTTCTCGCGACCGGCCAGGAAAGACGCAACAAACCGGAATCTTCTGCGGCAAAAGCTTTATTGCTT

SEQ ID NO: 1

5’ end vector sequence Sleeping beauty IR/DR sequence **Integration junction (chr15, 6805206)**

TAGAAACTTAATTAACAACGAGATCTTATAATTTGCATACTTCTGCCTGCTGGGGACTTTCCACACCCTAGCTGACACAA
GAATTTGAAATACATCCACAGGTACACCTCCAATTGACTCAAATGATGTCAATTAGTCTATCATAATCTTCTAAAGCCAT
GACATCATTTTAACTGGAATTTTCCAAGCTGTTTAAAGGCACAGTCAACTTAGTGTATGTAAACTTCTGACCCACTGGAA
TTGTGATACAGTGAATTATAAGTGAAATAATCTGTCTGTAAACAATTGTTGGAAAAATGACTTGTGTCATGCACAAAGTA
GATGTCCTAACTGACTTGCCAAAACTATTGTTTGTTAACAAGAAATTTGTGGAGTAGTTGAAAAACGAGTTTTAATGACT
CCAACTTAAGTGTATGTAAACTTCCGACTTCAACTG[TA]**CAAGTAGACCAAATATCCATATACATAAAAGAAAAAAATAGA**
AAAAATTTCTAGTGACAGAAAAATGACAAAGAACATACTGCTTTATTACTACTATTAAGATGTTTGCTTCCATTACACTC
ATATGAGTCATGATATTTTTTCTTCATTTTTTTCTANTNNCACTNGAAAT

SEQ ID NO: 2

5’ end vector sequence Sleeping beauty IR/DR sequence **Integration junction (chrX, 16897322)**

GTTGCTAGGAATGAGCCAAATTCATCTGTATTAAACAGTGGGAGCTTGTGGAAGGCTACTCGAAATGTTTGACCCAAGTT
AAACAATTTAAAGGCAATGCTACCAAATACTAATTGAGTGTATGTTAACTTCTGACCCACTGGGAATGTGATGAAAGAAA
TAAAAGCTGAAATGAATCATTCTCTCTACTATTATTCTGATATTTCACATTCTTAAAATAAAGTGGTGATCCTAACTGAC
CTTAAGACAGGGAATCTTTACTCGGATTAAATGTCAGGAATTGTGAAAAAGTGAGTTTAAATGTATTTGGCTAAGGTGTA
TGTAAACTTCCGACTTCAACTG[TA]**TATCCTCCCCGTTGCACCCTCTTGATGATGCTGAGATGAACACAGATGCTCACTCC**
TTGAGGGCTCTAAGCTTATGCTGACACAGACACAGGTGCTCACTTCTATGAATGGCCTAAGATTTGAGGACATCATGAGG
ACAAGTGTGATAAAATCTTGGAACAACCTCCCAGAGGTCT

SEQ ID NO: 3

3’ end vector sequence Sleeping beauty IR/DR sequence **Integration junction (chr4, 10207667)**

FIG. 25A

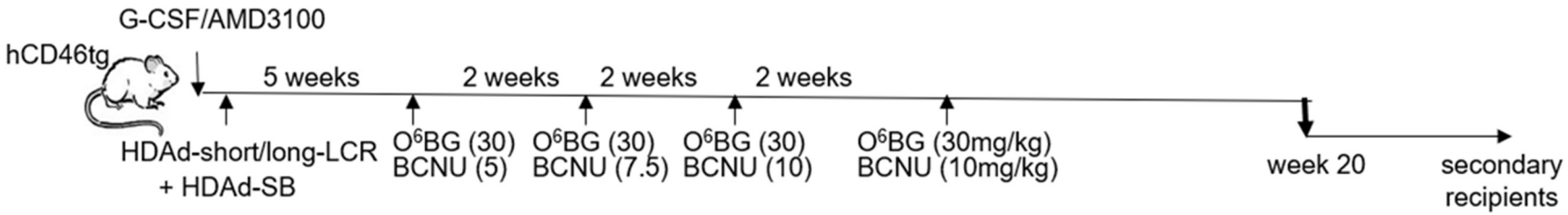

G-CSF/AMD3100
hCD46tg
5 weeks
2 weeks
2 weeks
2 weeks
HDAd-short/long-LCR
+ HDAd-SB
O6BG (30)
BCNU (5)
O6BG (30)
BCNU (7.5)
O6BG (30)
BCNU (10)
O6BG (30mg/kg)
BCNU (10mg/kg)
week 20
secondary
recipients

FIG. 25B

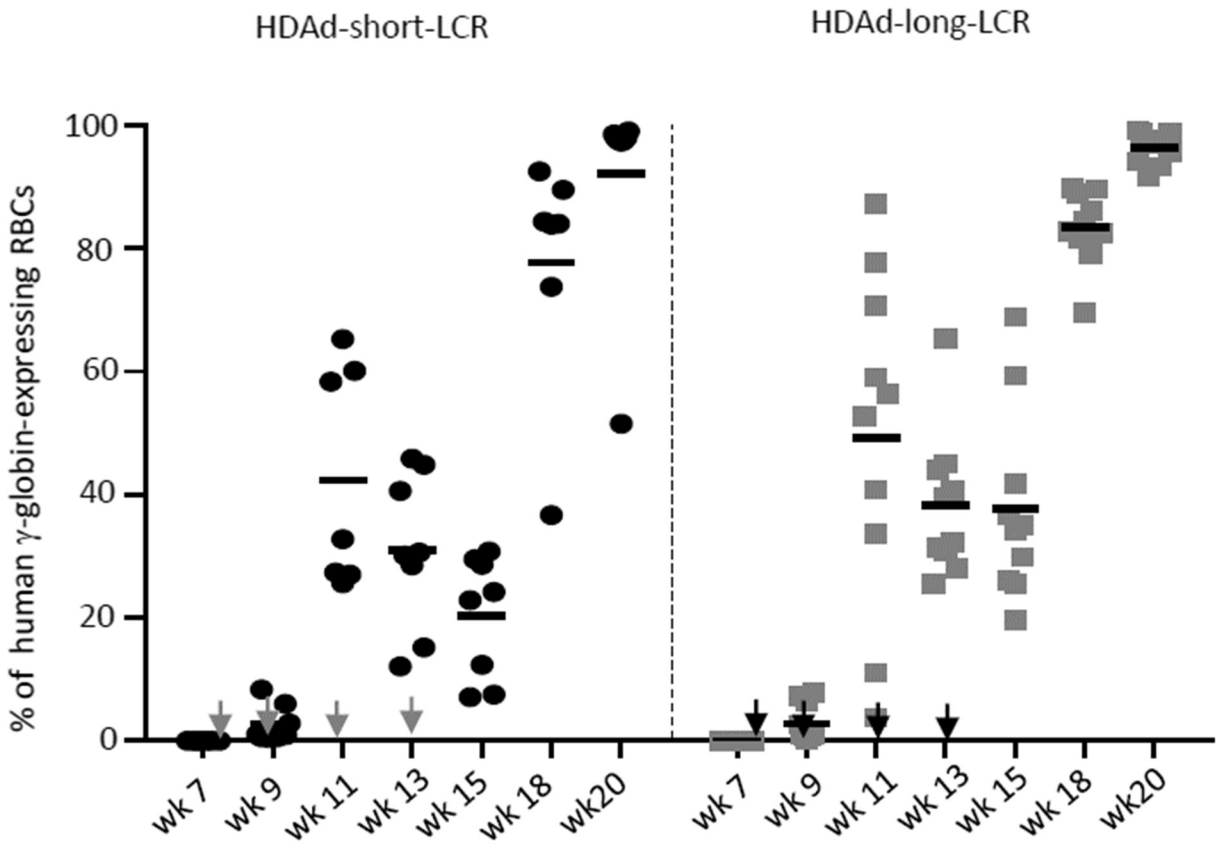


FIG. 25C

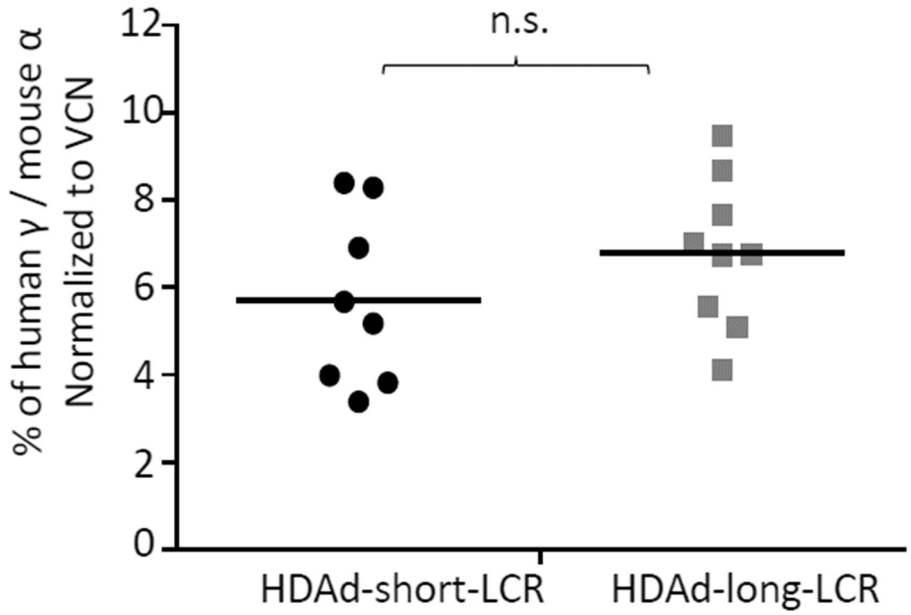


FIG. 25D

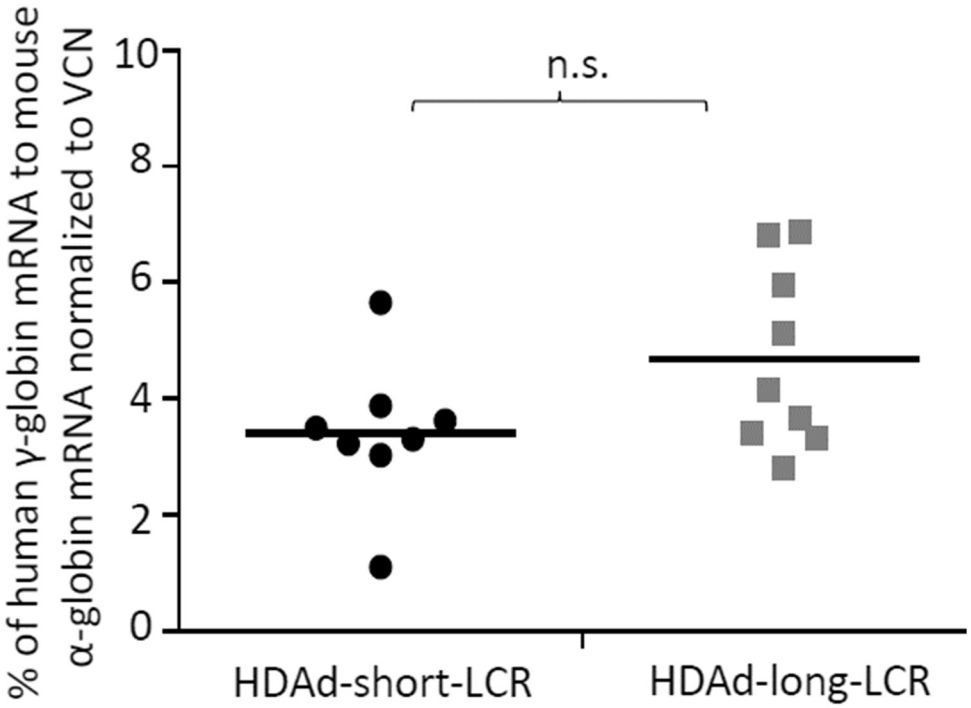


FIG. 25E

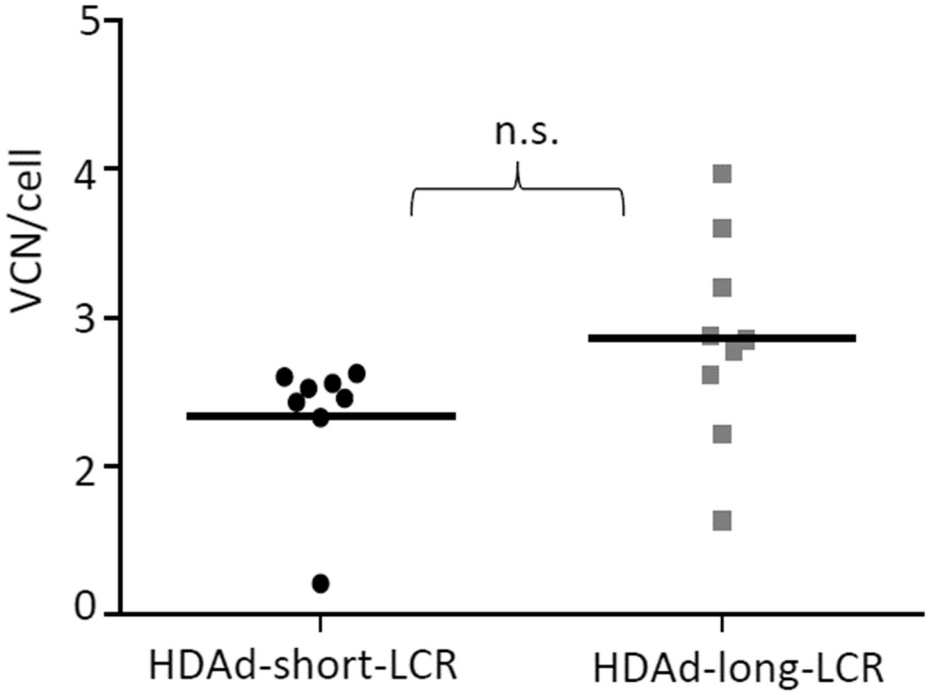


FIG. 26A

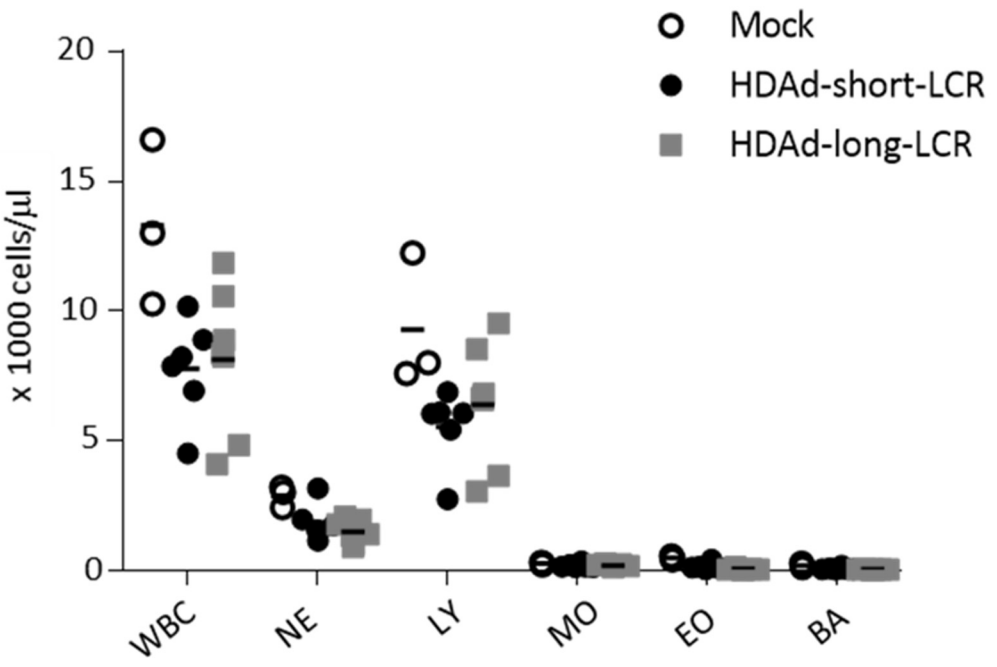


FIG. 26B

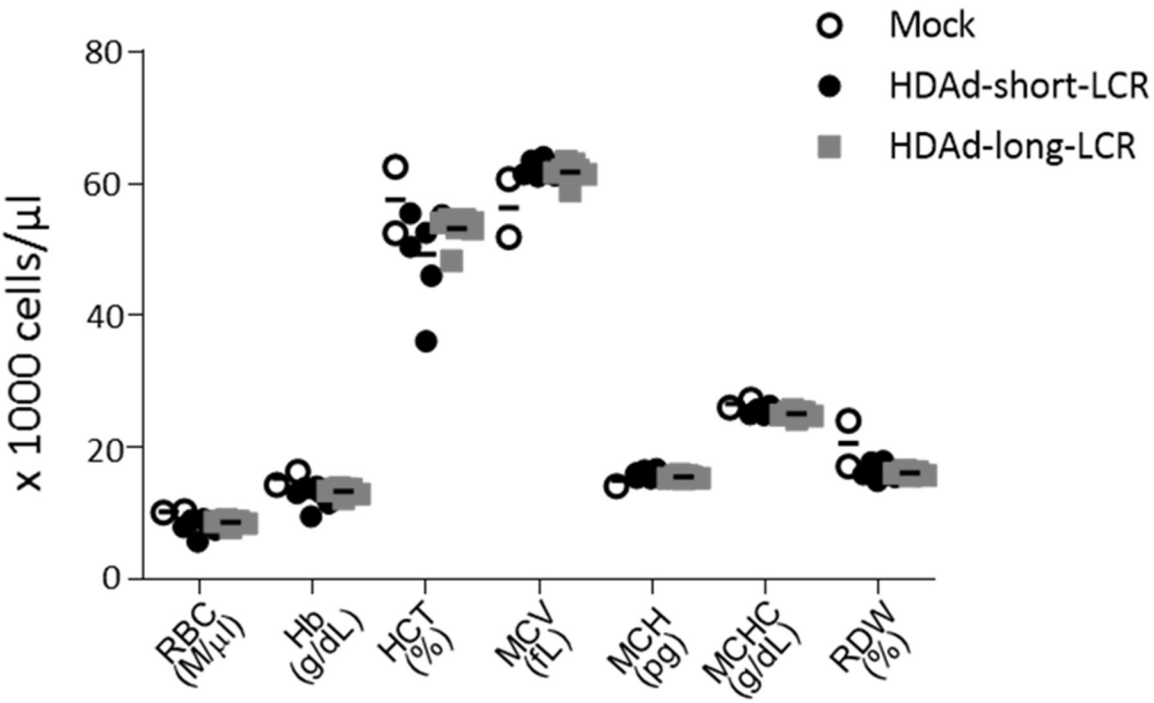


FIG. 26C

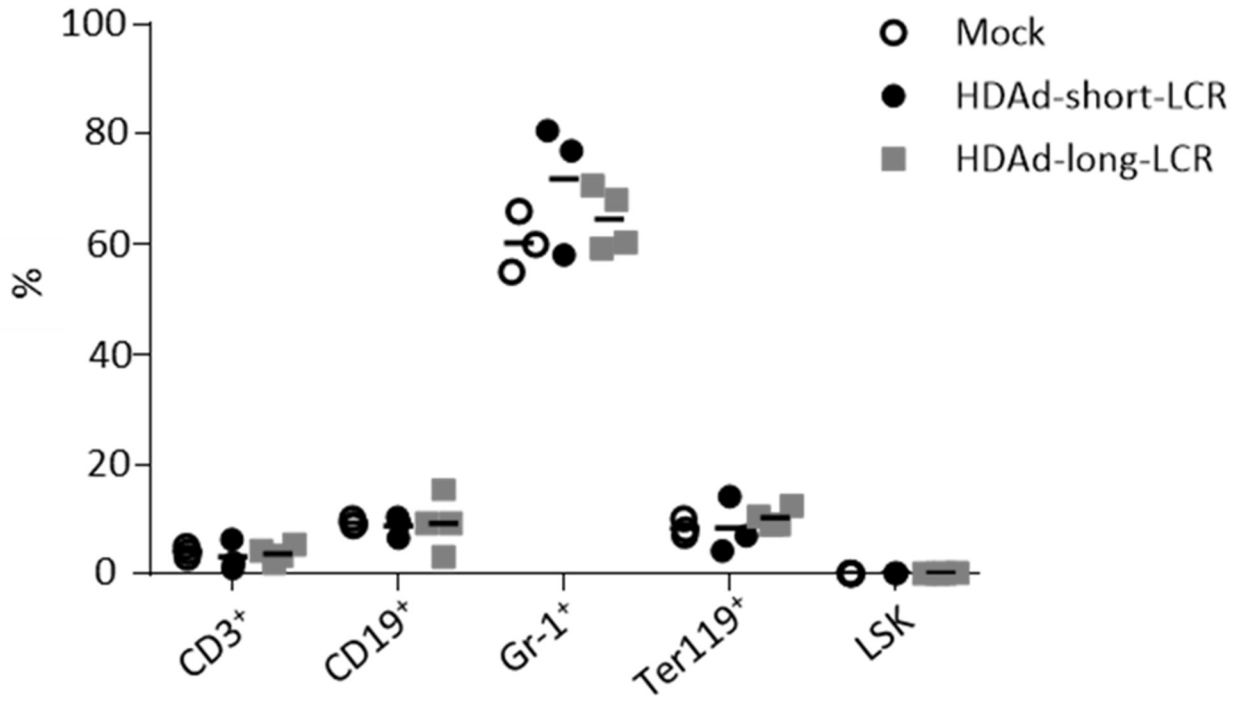


FIG. 26D

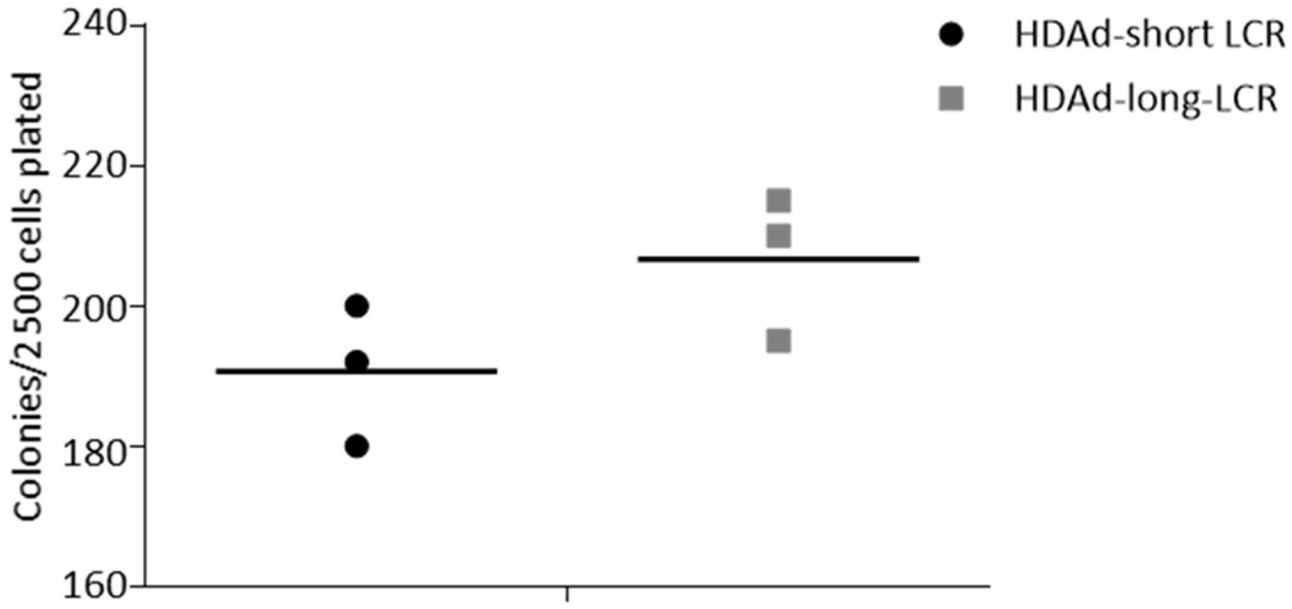


FIG. 27

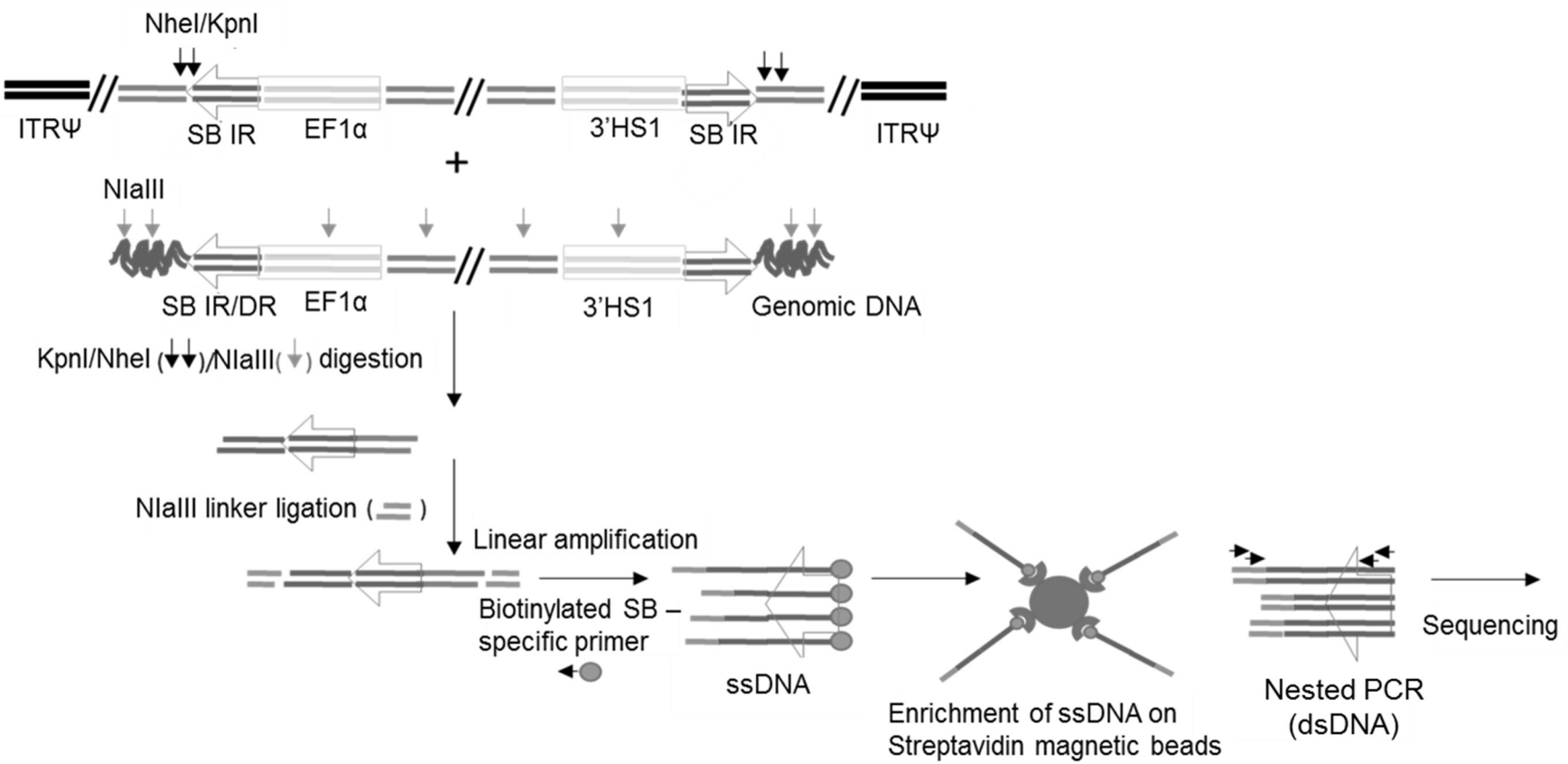

NheI/KpnI
ITRΨ
SB IR
EF1α
3'HS1
SB IR
ITRΨ
+
NlaIII
SB IR/DR
EF1α
3'HS1
Genomic DNA
KpnI/NheI ( )/NlaIII( ) digestion
NlaIII linker ligation ( )
Linear amplification
Biotinylated SB – specific primer
ssDNA
Enrichment of ssDNA on Streptavidin magnetic beads
Nested PCR (dsDNA)
Sequencing

FIG. 28A

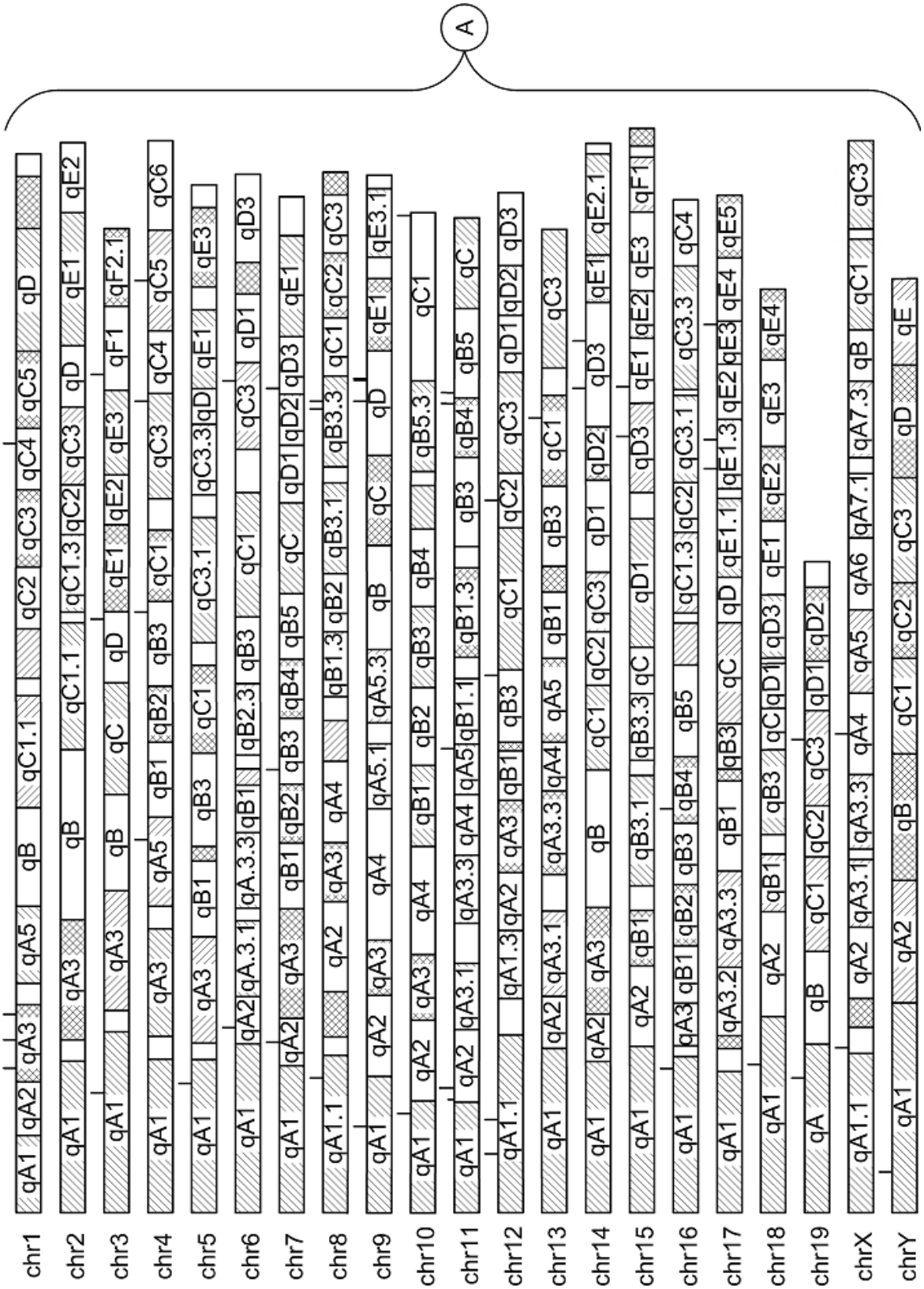

A
chr1
chr2
chr3
chr4
chr5
chr6
chr7
chr8
chr9
chr10
chr11
chr12
chr13
chr14
chr15
chr16
chr17
chr18
chr19
chrX
chrY

FIG. 28A cont'd.

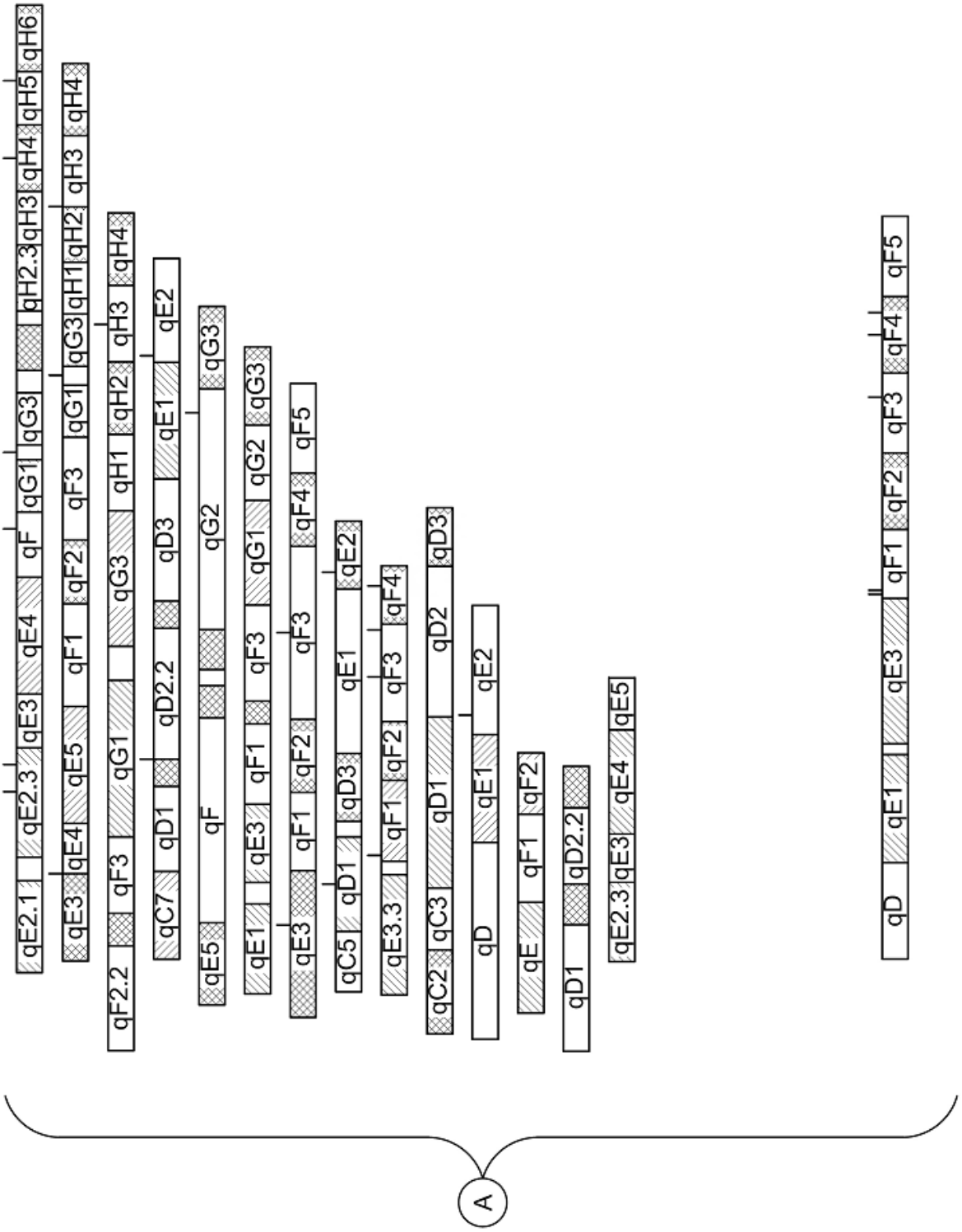


FIG 28B

Sleeping beauty IR/DR sequence
Integration junction (chr7, 79796094) ACTTAAGTGT ATGTAAACTT CCGACTTCAA CTGTAGGGTA CCTGATTCTC TGGGCATCTC TGCCCACTAC CATG

SEQ ID NO: 4

Sleeping beauty IR/DR sequence
Integration junction (repeat region) ACTTAAGTGT ATGTAAACTT CCGACTTCAA CTGTAAATTT TCCACCTTTT TCAGTTTTCC TCGCCATATT TCATG

SEQ ID NO: 5

FIG. 28C

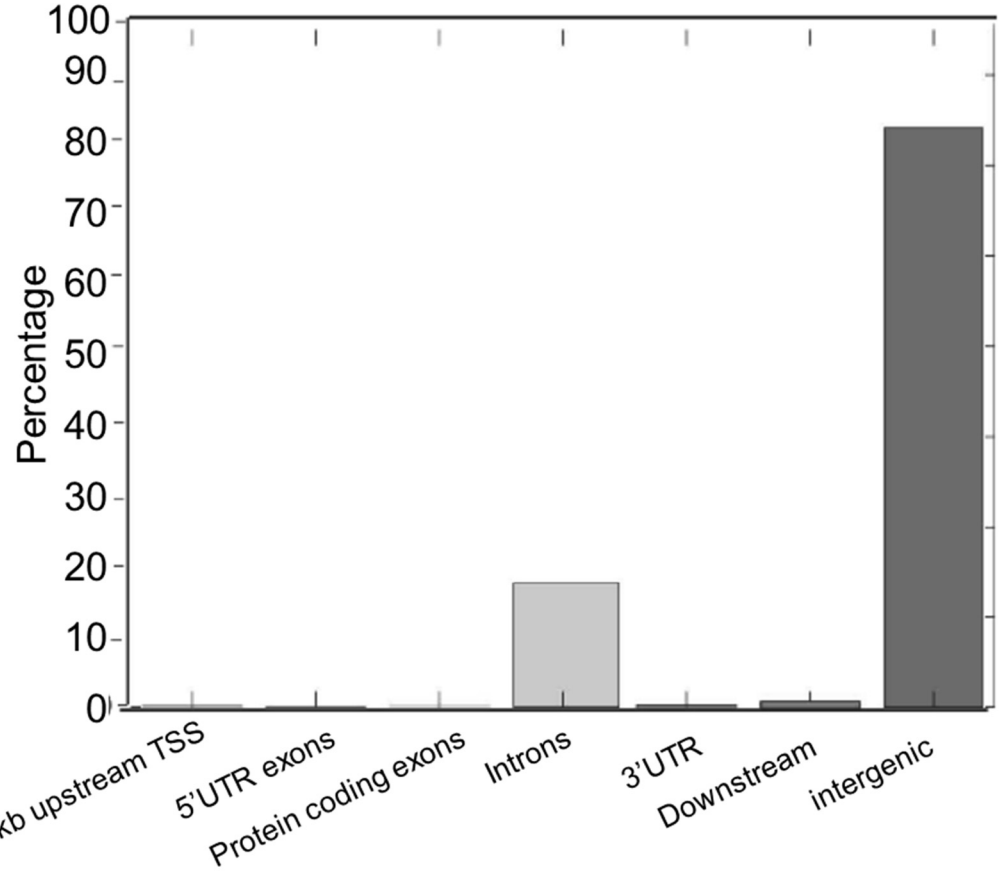


FIG. 28D

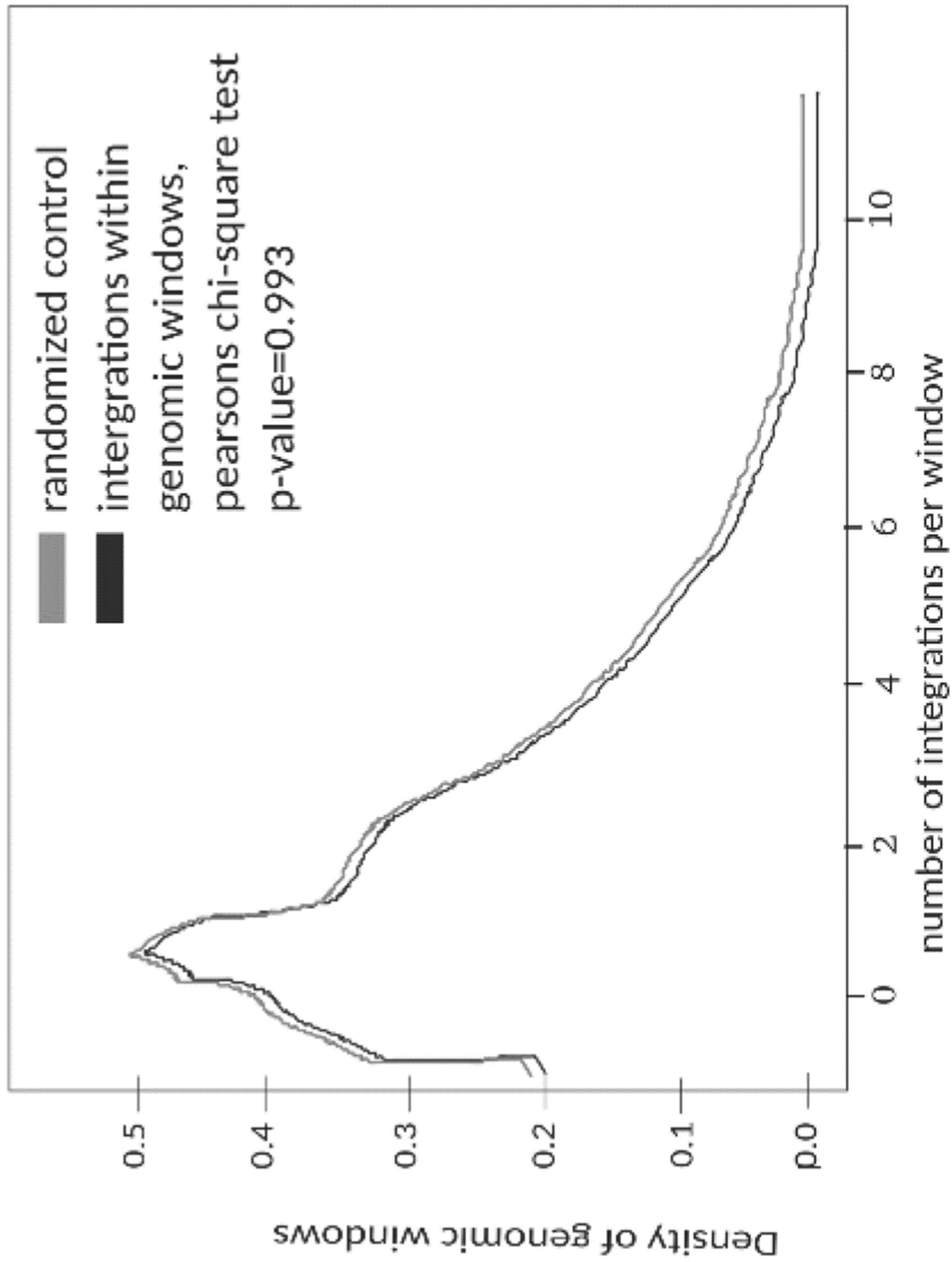

randomized control
intergrations within genomic windows,
pearsons chi-square test
p-value=0.993
Density of genomic windows
0.0
0.1
0.2
0.3
0.4
0.5
0
2
4
6
8
10
number of integrations per window

FIG. 29A

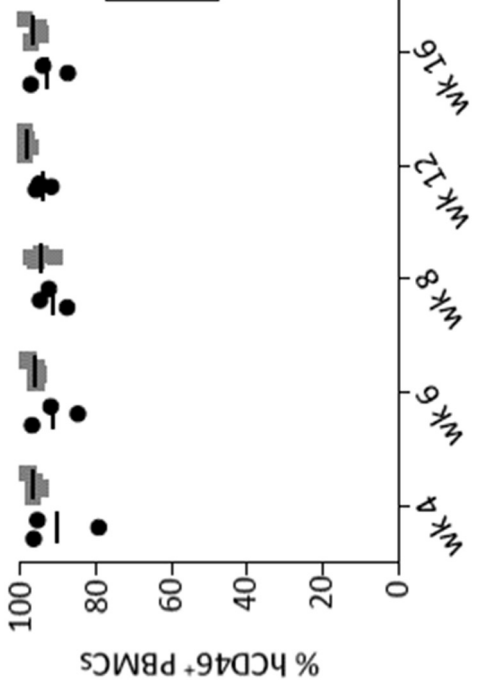


FIG. 29B

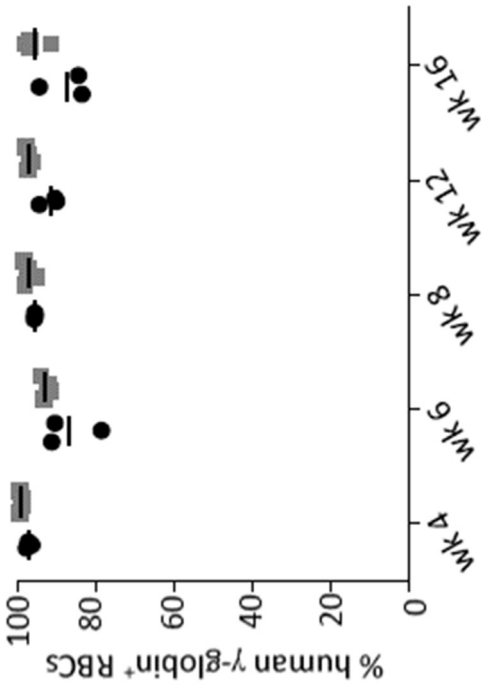
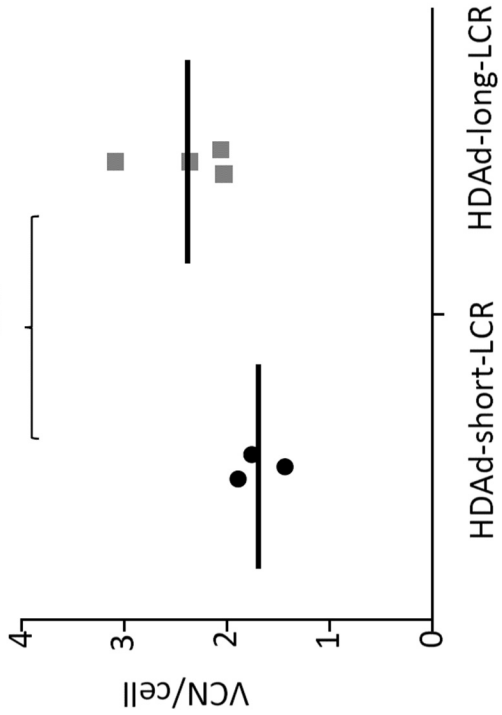


FIG. 29C



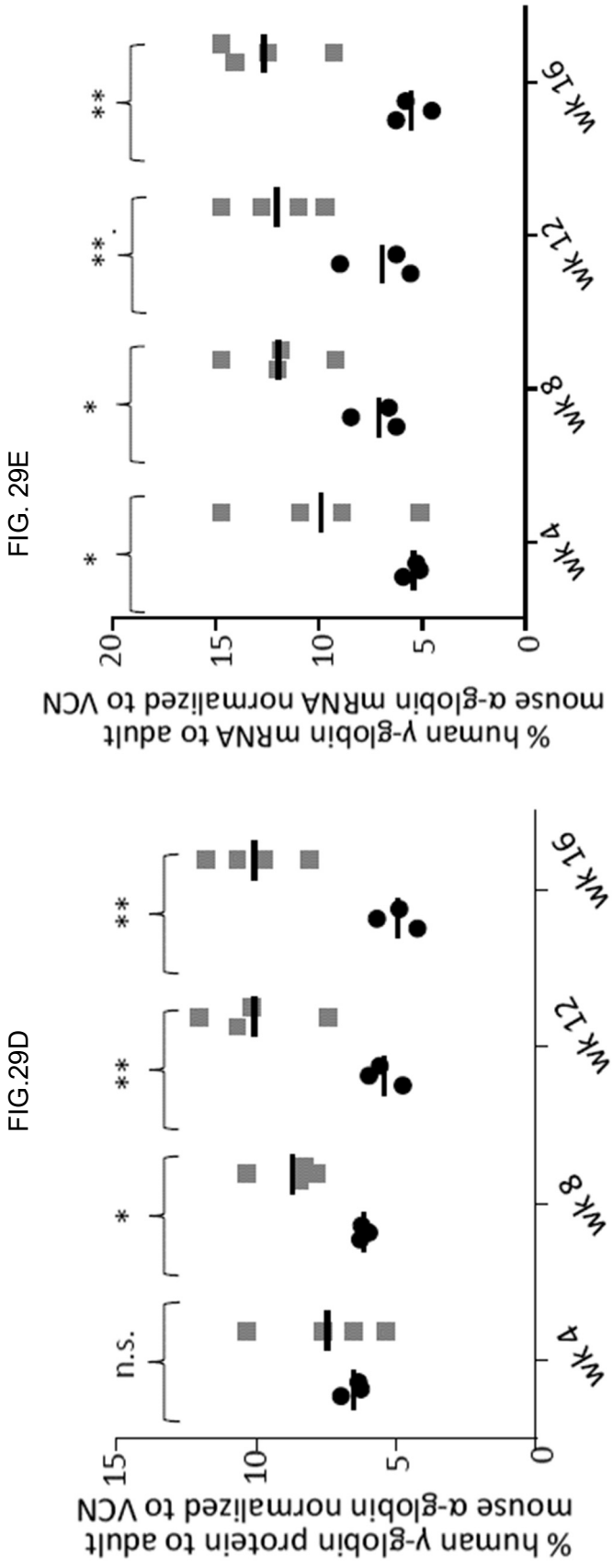

FIG.29D
% human γ-globin protein to adult mouse α-globin normalized to VCN
15
10
5
0
n.s.
*
**
**
wk 4
wk 8
wk 12
wk 16

FIG. 29E
% human γ-globin mRNA to adult mouse α-globin mRNA normalized to VCN
20
15
10
5
0
*
*
**
**
wk 4
wk 8
wk 12
wk 16

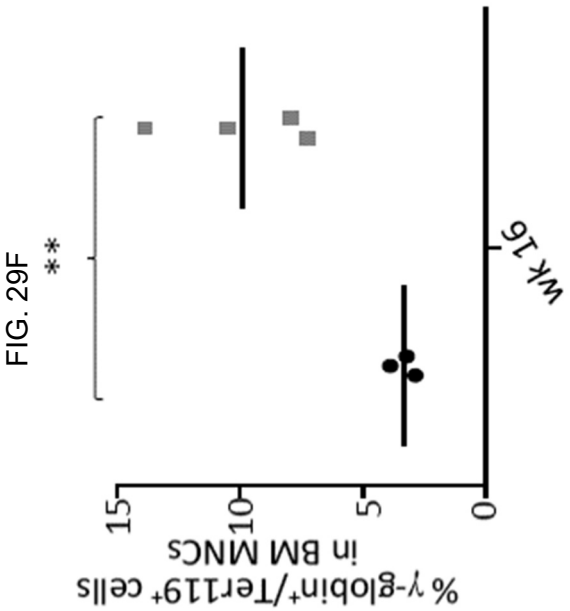

FIG. 29F
% γ-globin+/Ter119+ cells in BM MNCs
15
10
5
0
**
wk 16

FIG. 29G

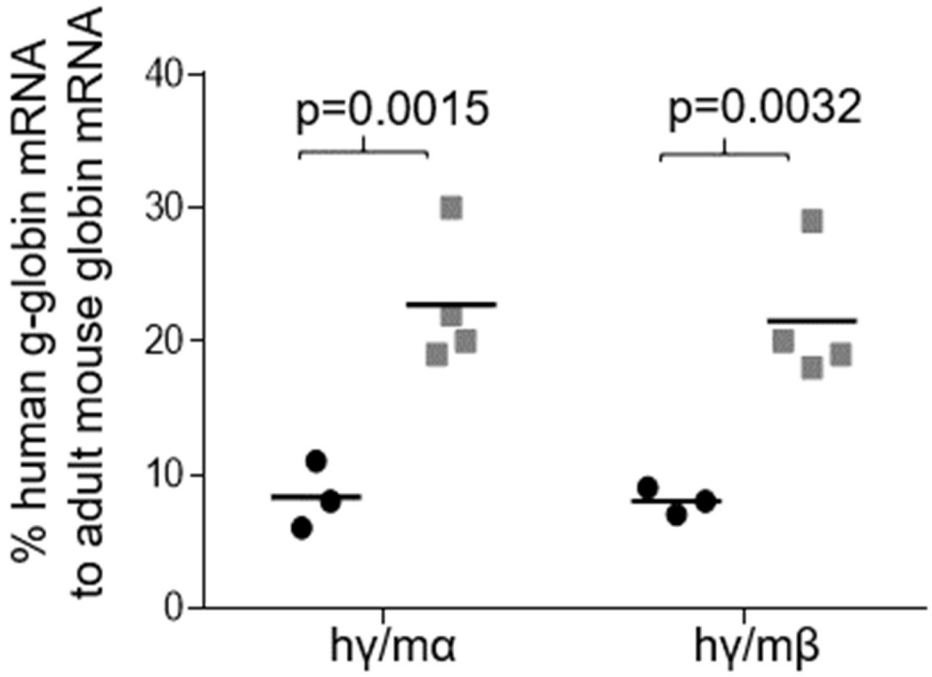

% human g-globin mRNA
to adult mouse globin mRNA
40
30
20
10
0
p=0.0015
p=0.0032
hγ/mα
hγ/mβ

FIG. 29H

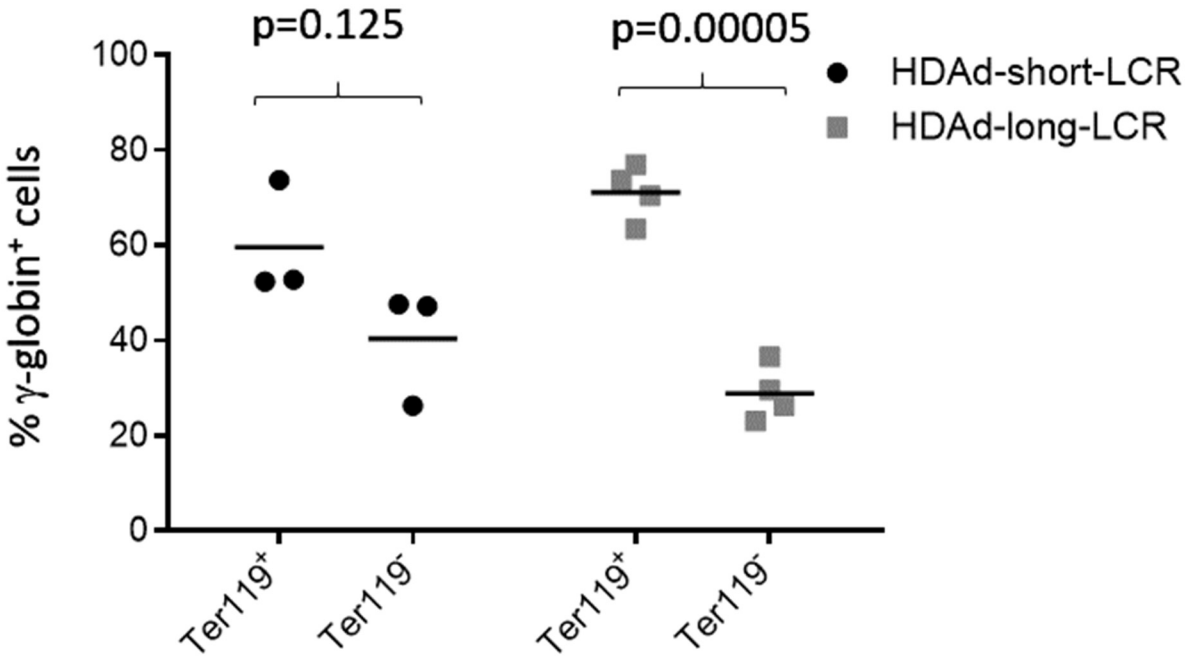
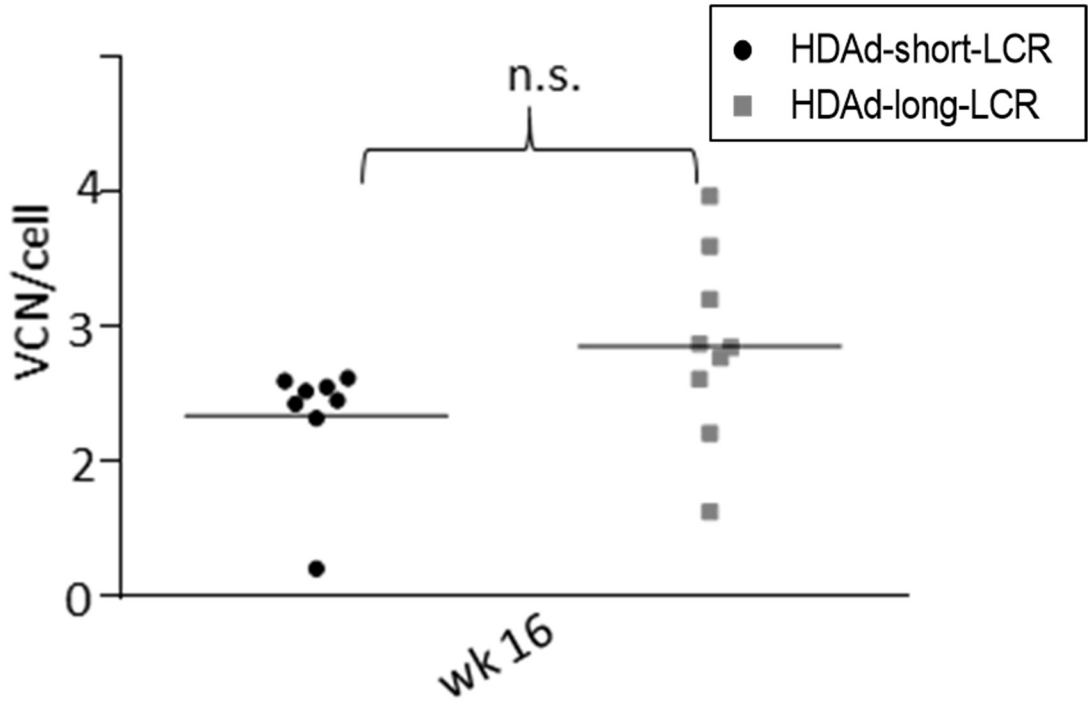


FIG. 29I



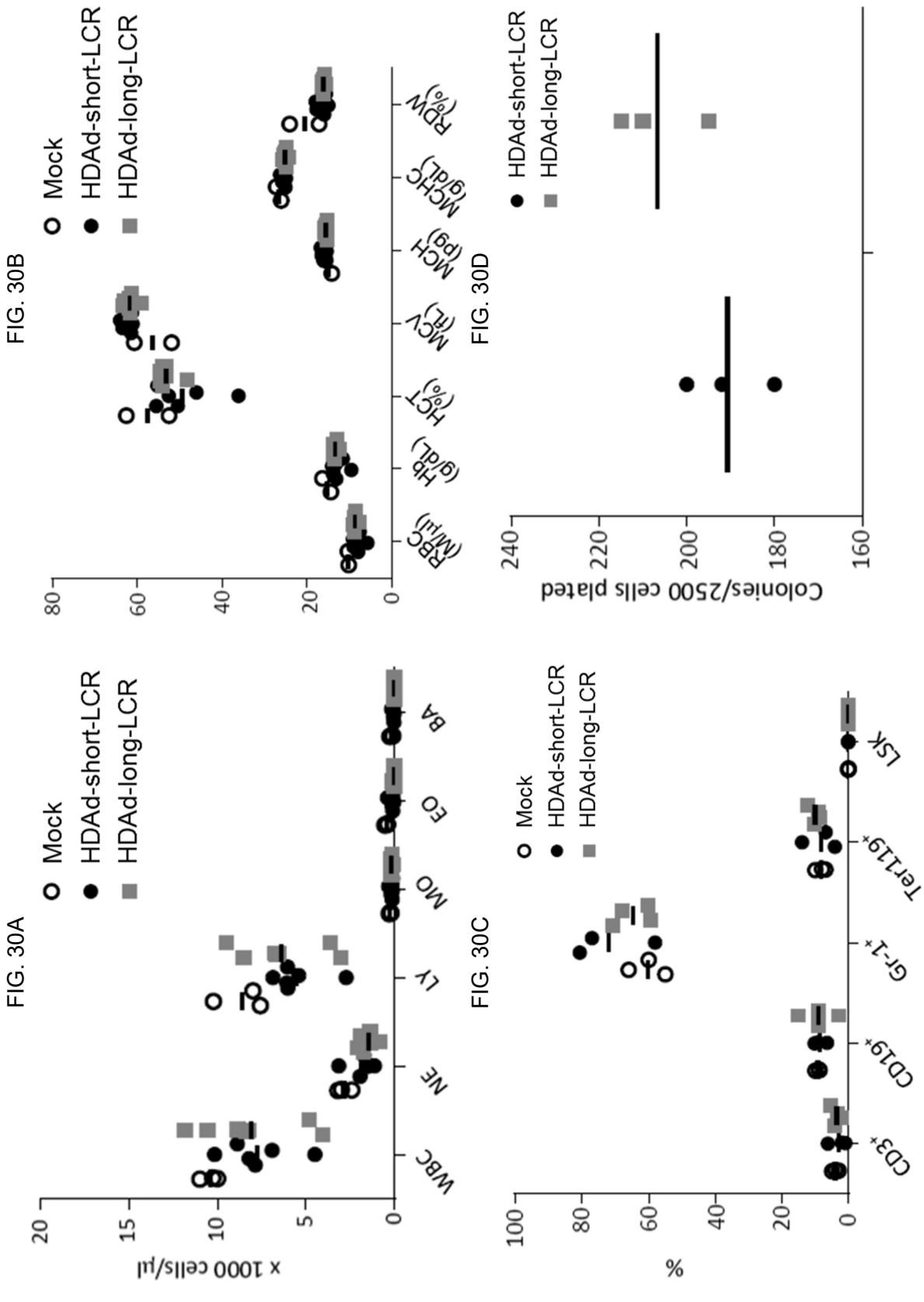

FIG. 30A
Mock
HDAd-short-LCR
HDAd-long-LCR
x 1000 cells/μl
WBC
NE
LY
MO
EO
BA
FIG. 30B
Mock
HDAd-short-LCR
HDAd-long-LCR
RBC (M/μl)
Hb (g/dL)
HCT (%)
MCV (fL)
MCH (pg)
MCHC (g/dL)
RDW (%)
FIG. 30C
Mock
HDAd-short-LCR
HDAd-long-LCR
%
CD3+
CD19+
Gr-1+
Ter119+
LSK
FIG. 30D
HDAd-short-LCR
HDAd-long-LCR
Colonies/2500 cells plated

FIG. 31A

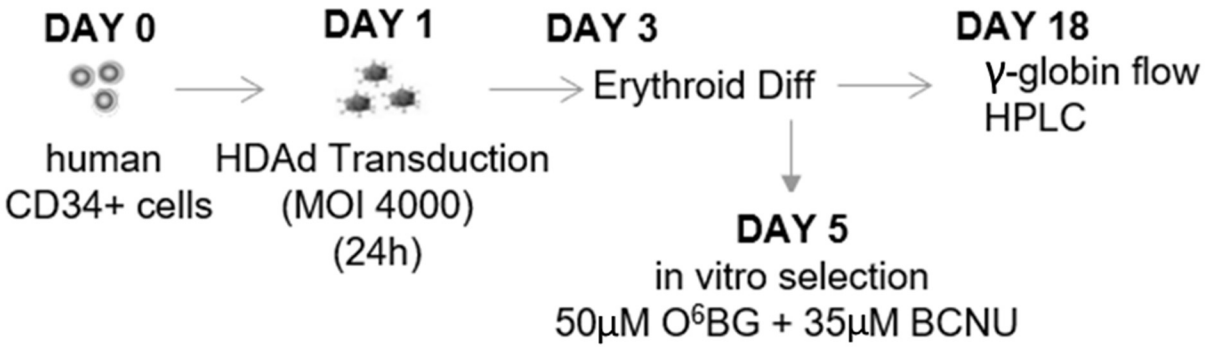


FIG. 31B

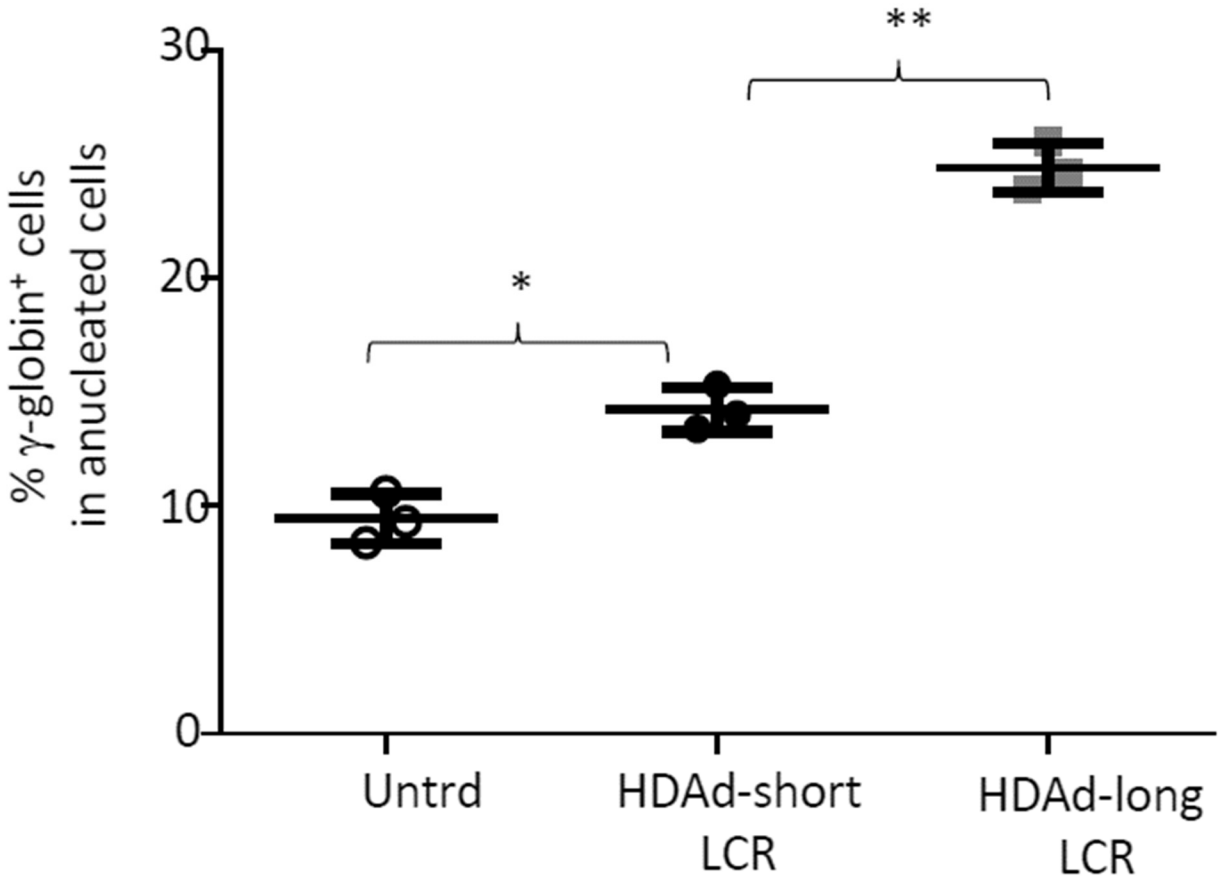


FIG. 31C

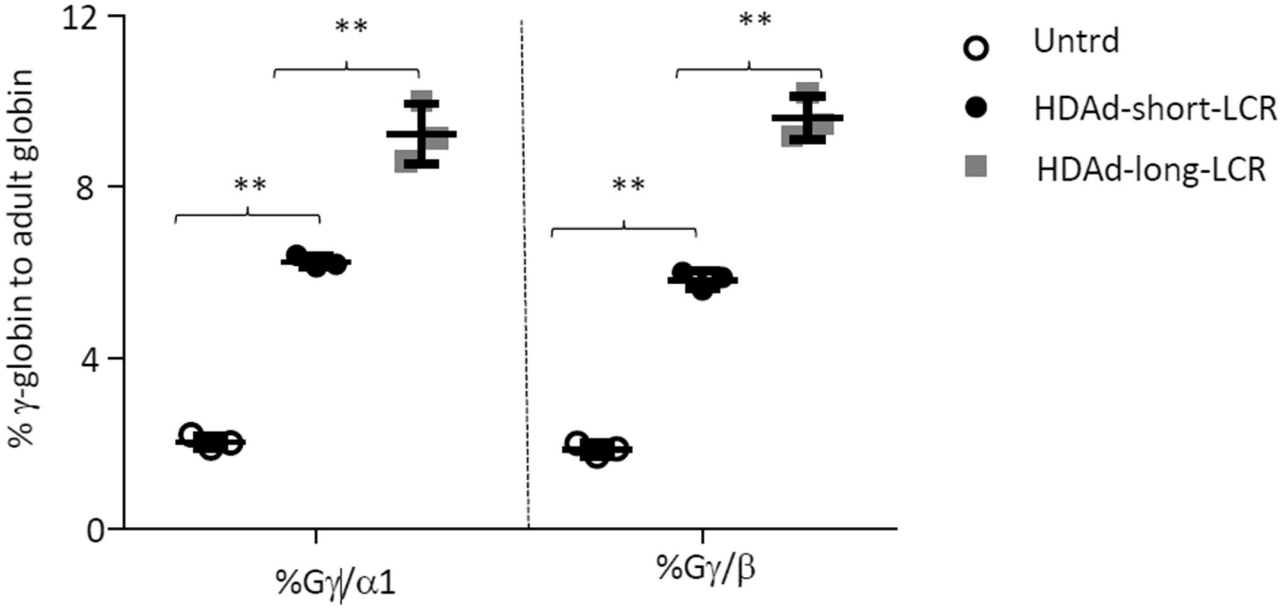


FIG. 31D

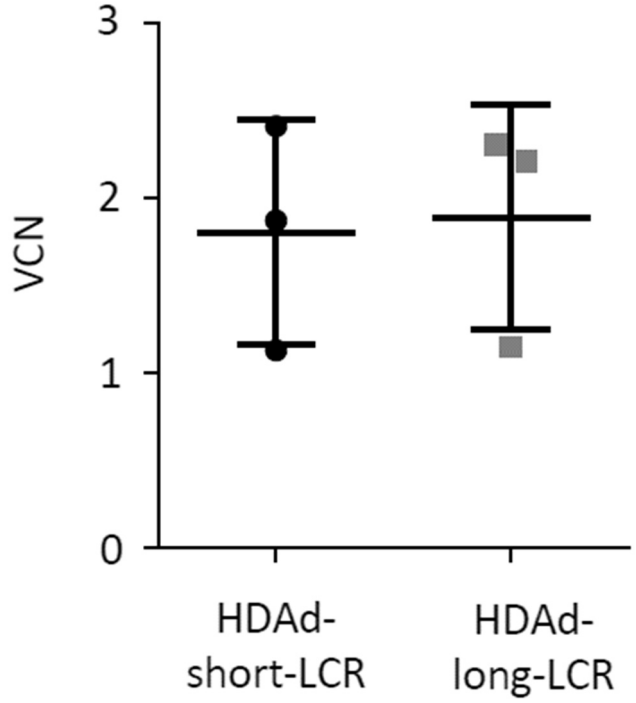


FIG. 32A

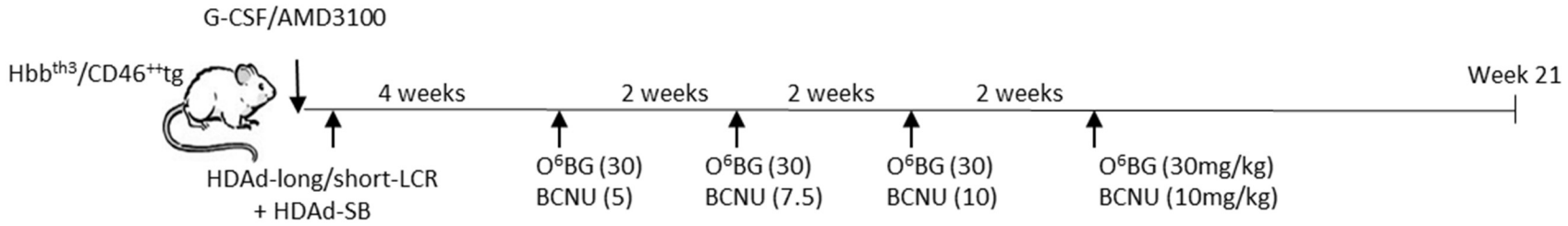


FIG. 32B

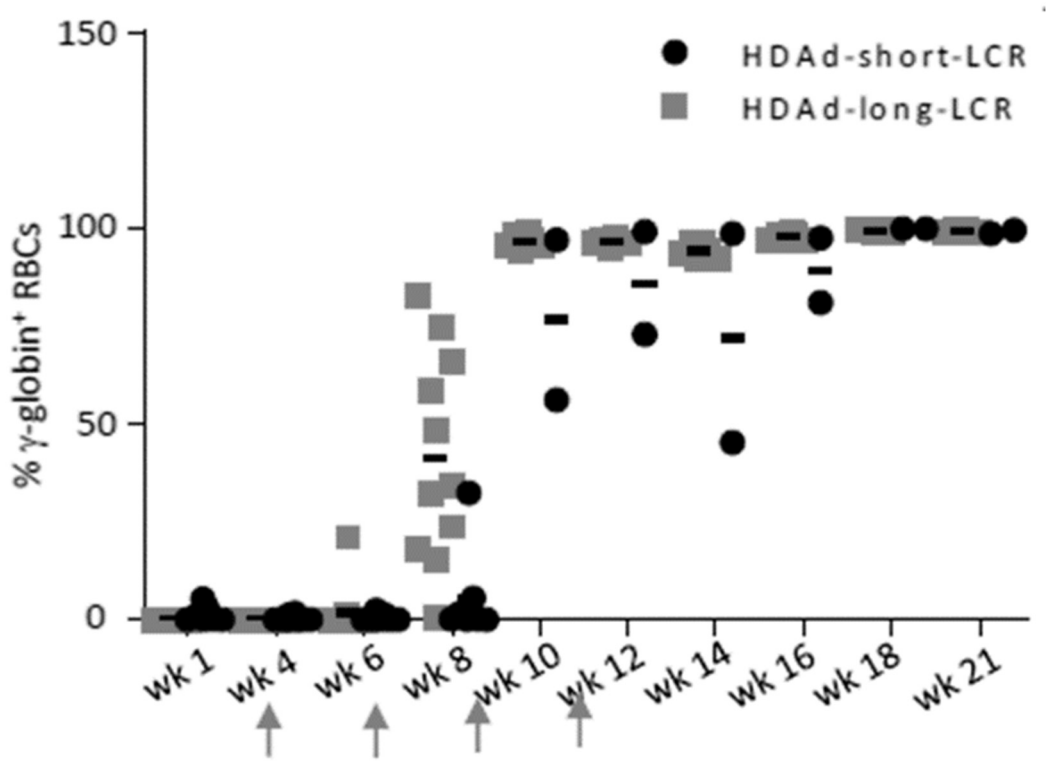


FIG. 32C

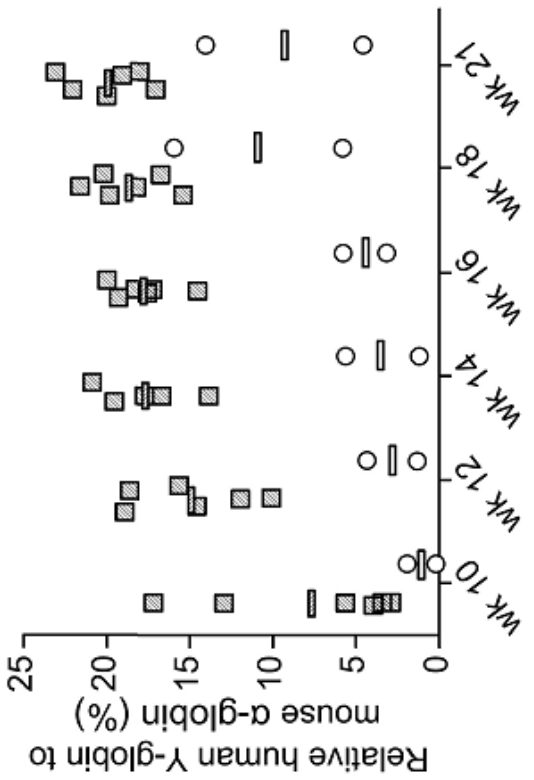


FIG. 32D

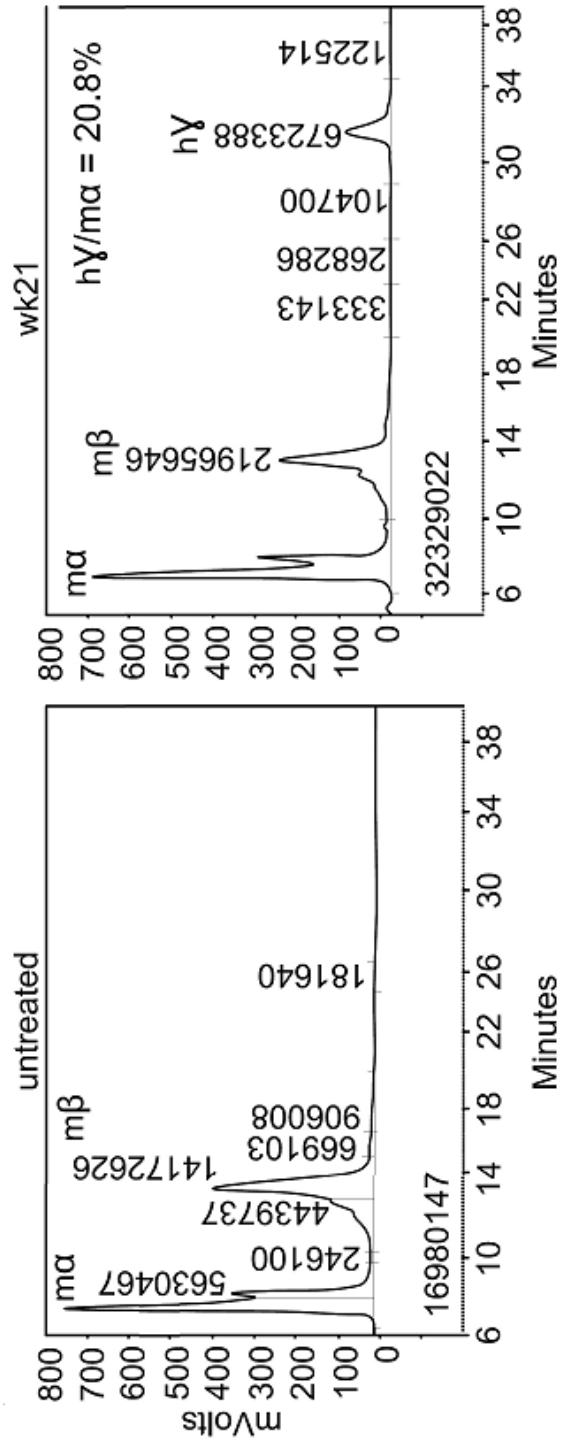


FIG. 32E

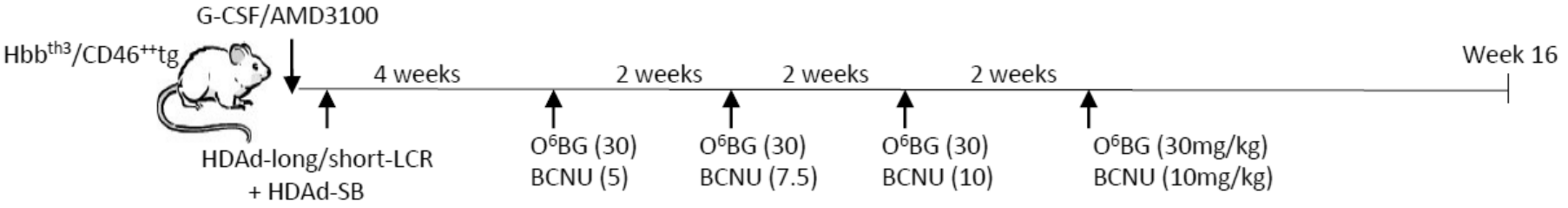


FIG. 32F

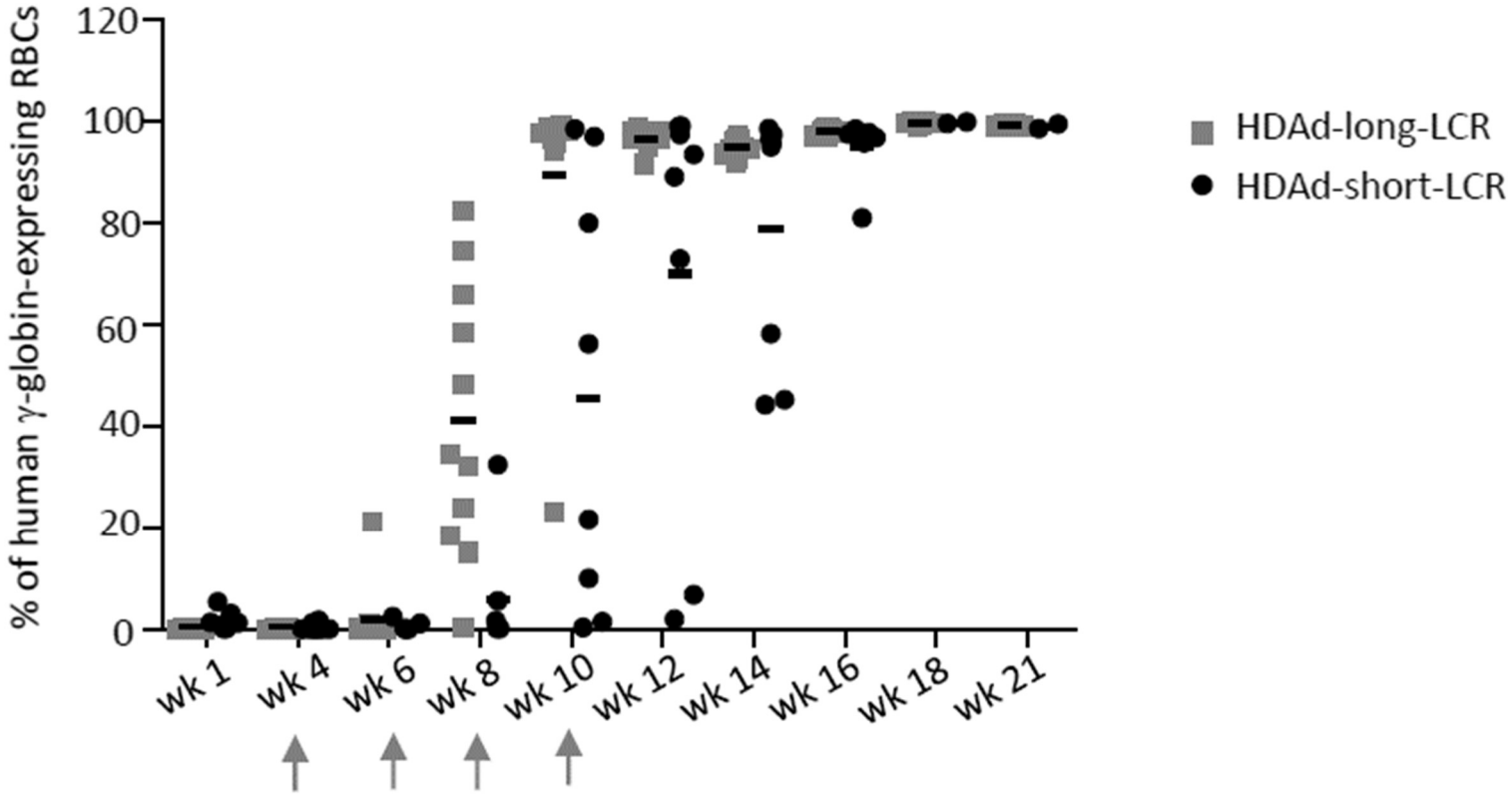


FIG. 32G

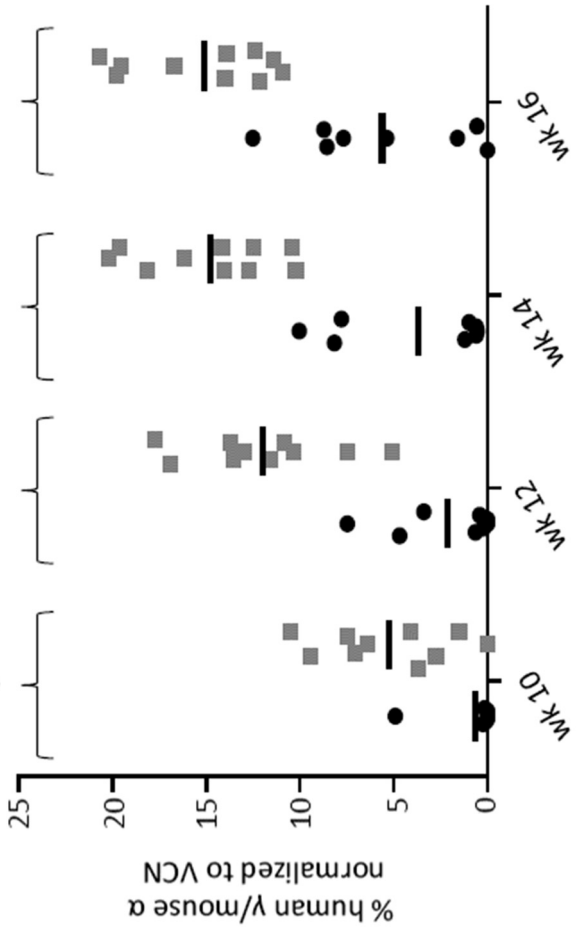


FIG. 32H

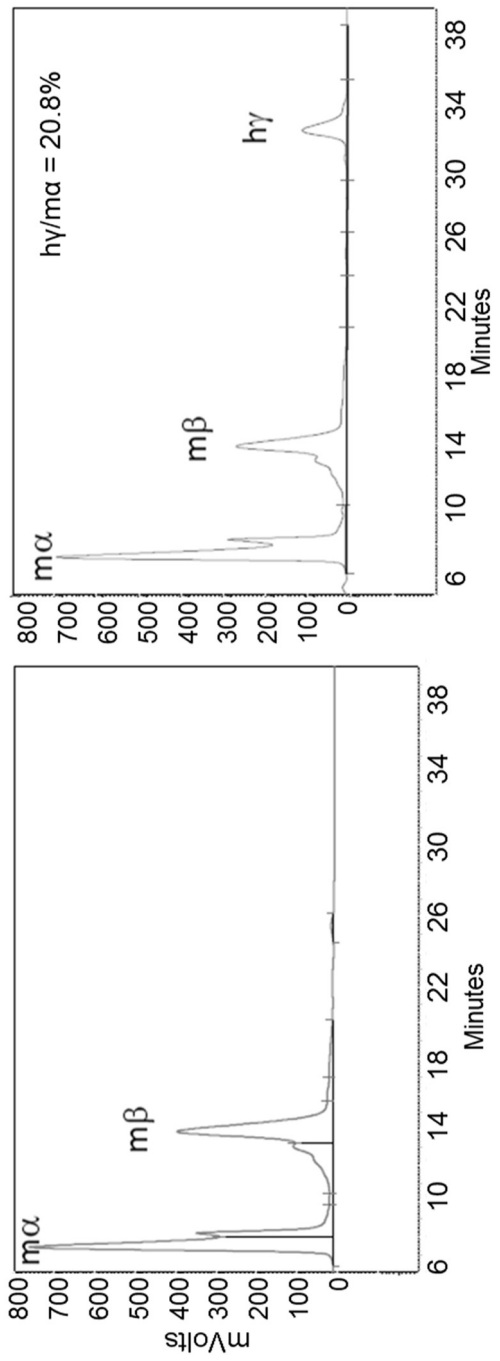


FIG. 33A

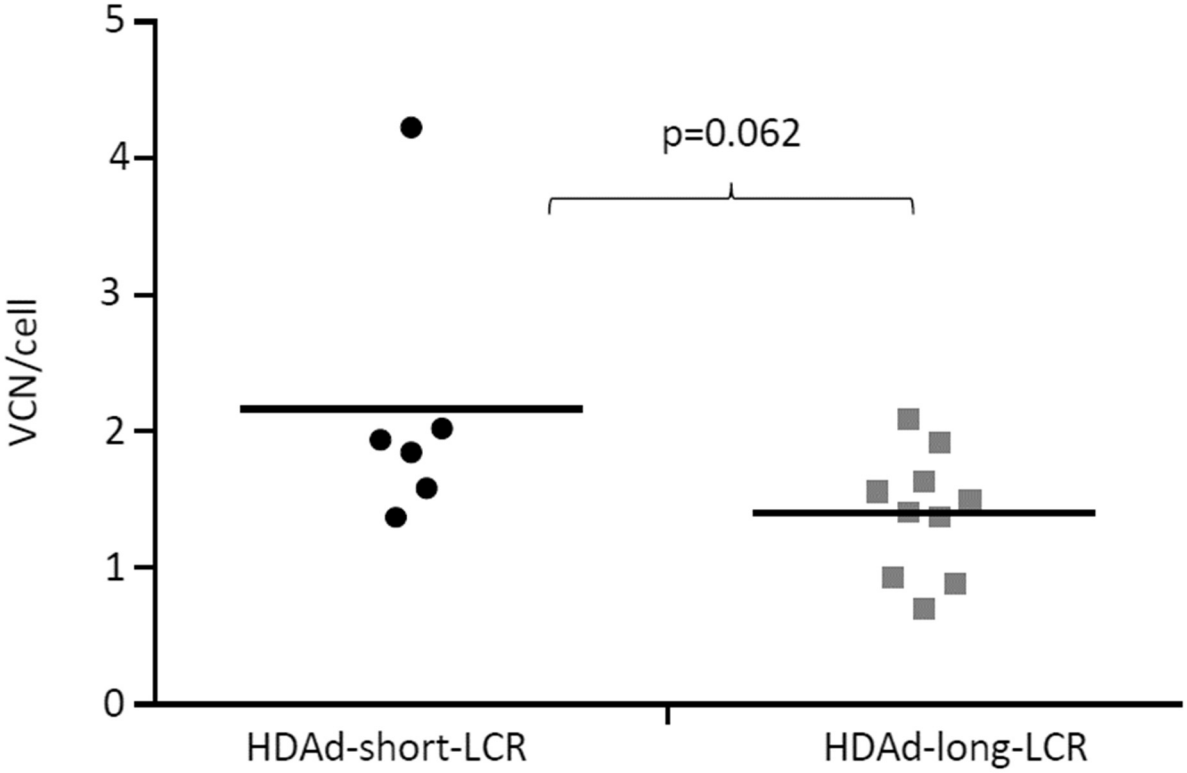


FIG. 33B

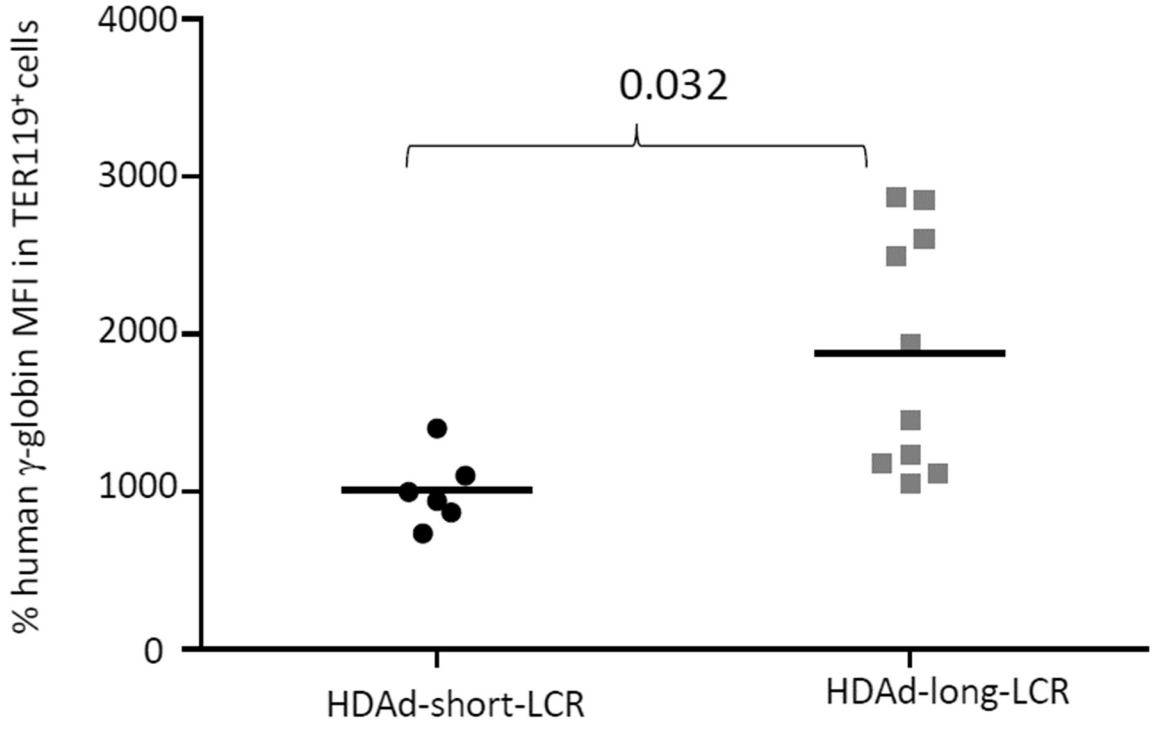


FIG. 34

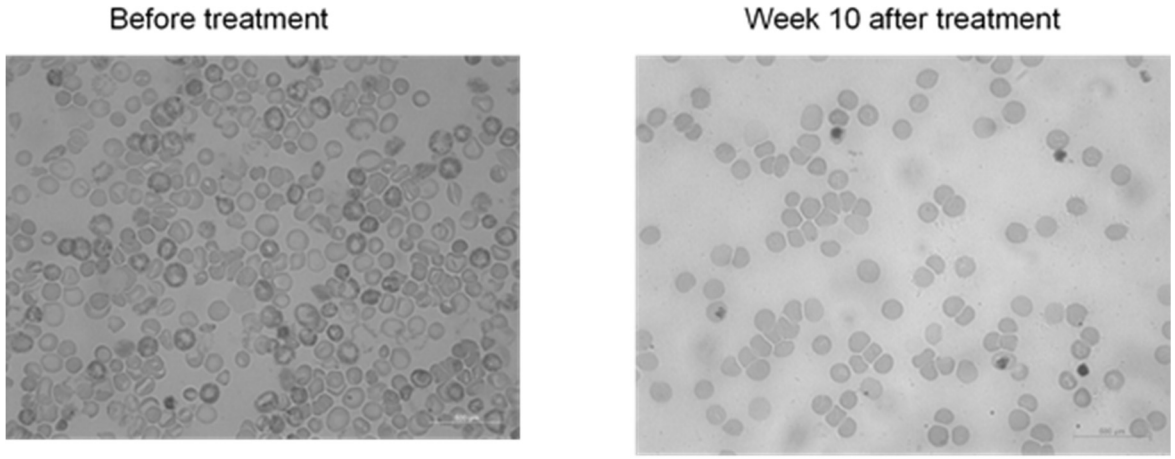

Before treatment
Week 10 after treatment

FIG. 35

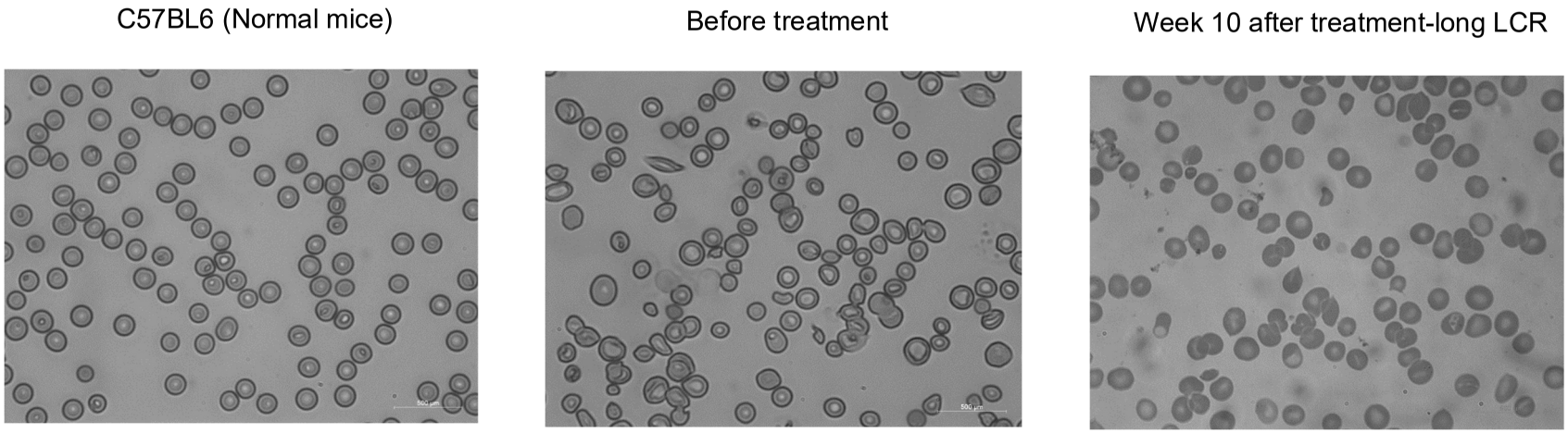

C57BL6 (Normal mice)
Before treatment
Week 10 after treatment-long LCR

FIG. 36A

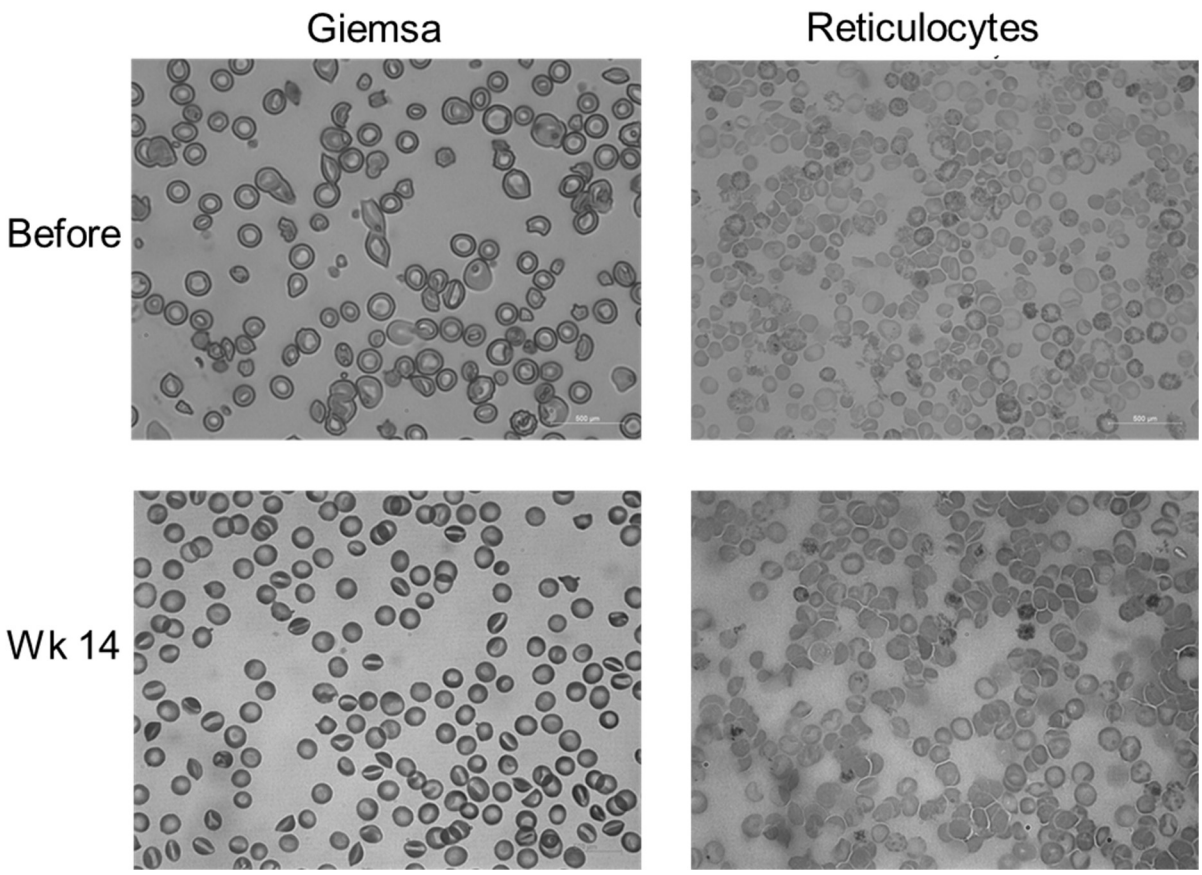


FIG. 36B

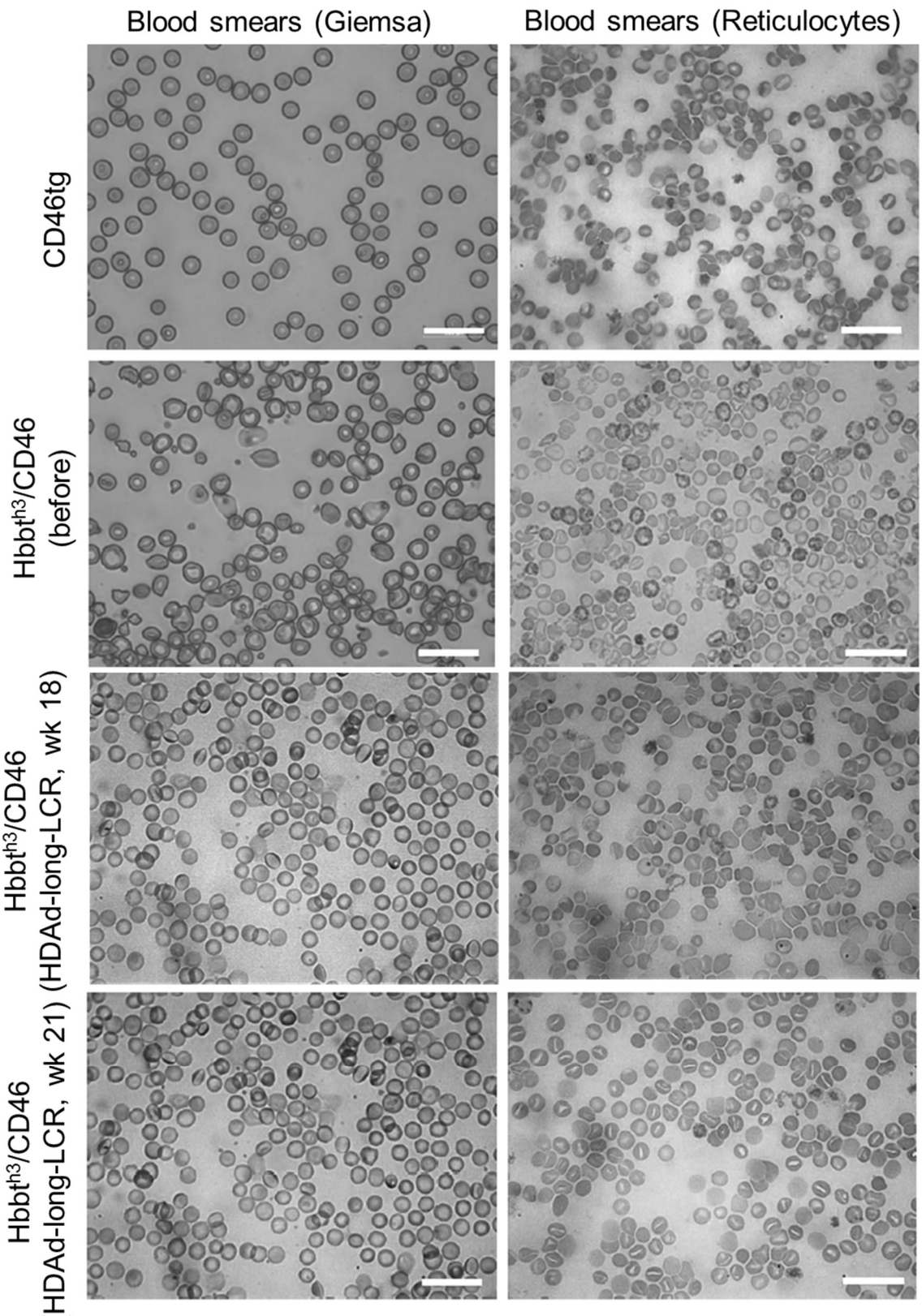

Blood smears (Giemsa)
Blood smears (Reticulocytes)
CD46tg
Hbbth3/CD46 (before)
Hbbth3/CD46 (HDAd-long-LCR, wk 18)
Hbbth3/CD46 (HDAd-long-LCR, wk 21)

FIG. 36C

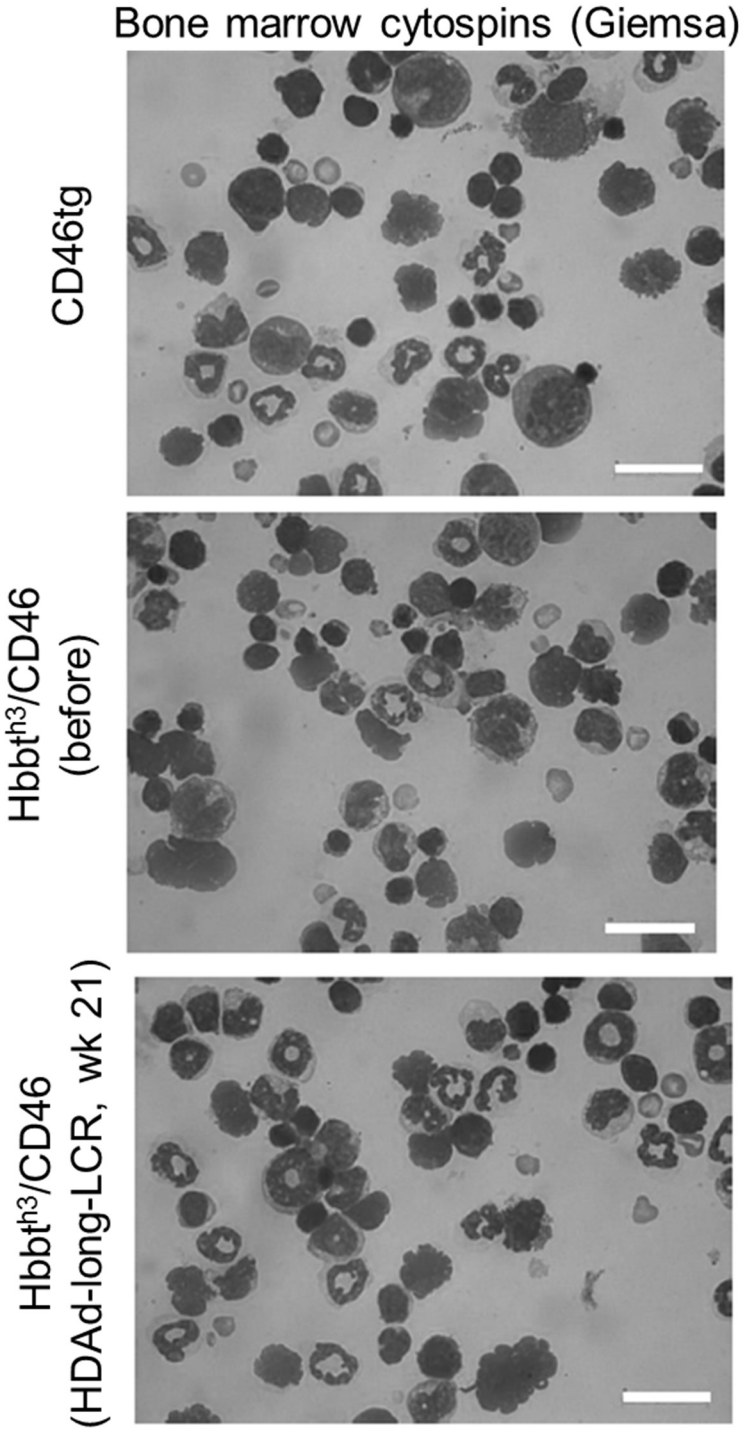

Bone marrow cytospins (Giemsa)
CD46tg
Hbbth3/CD46 (before)
Hbbth3/CD46 (HDAd-long-LCR, wk 21)

FIG. 37A

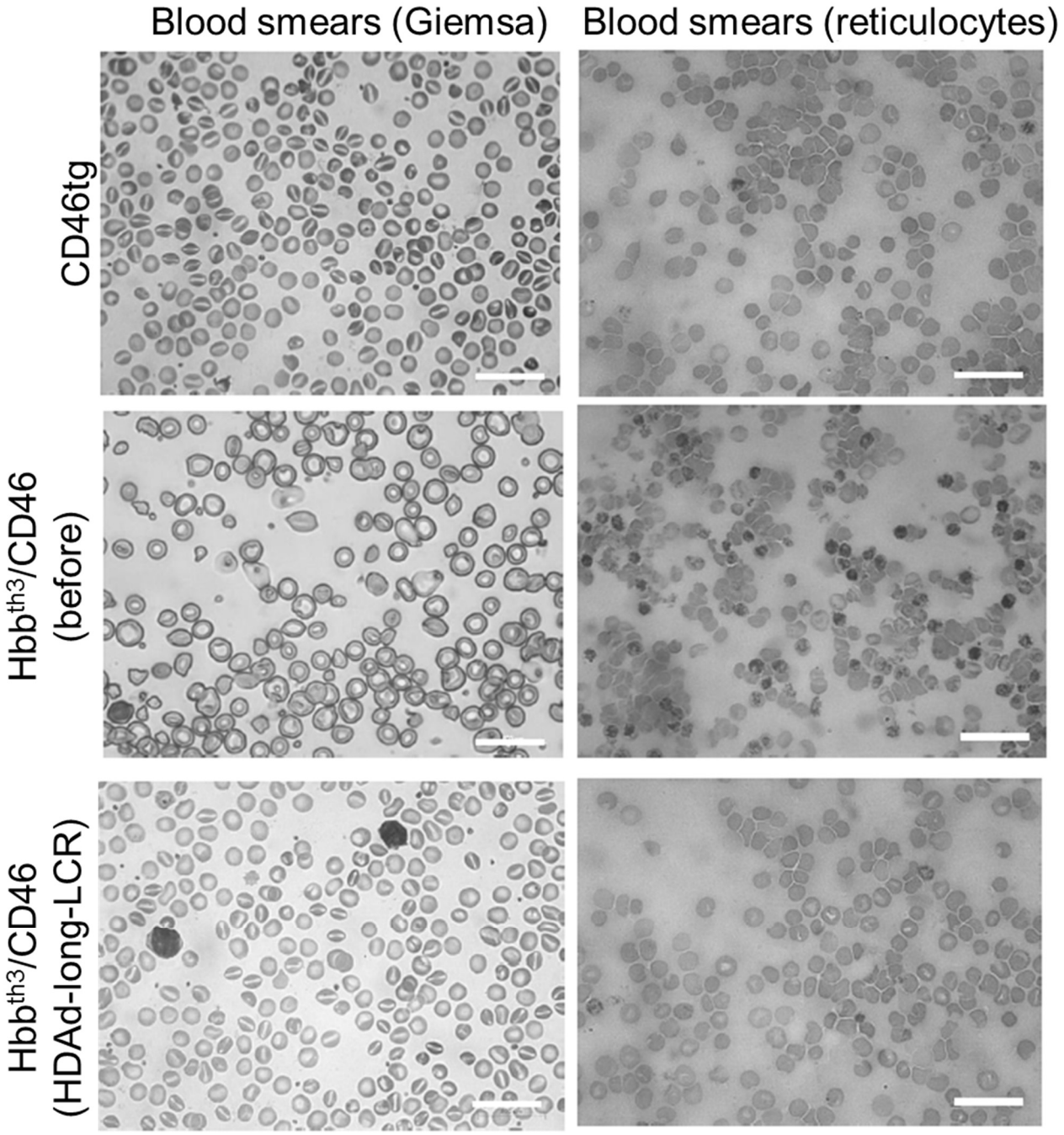

Blood smears (Giemsa)
Blood smears (reticulocytes)
CD46tg
Hbb^{th3}/CD46 (before)
Hbb^{th3}/CD46 (HDAd-long-LCR)

FIG. 37B

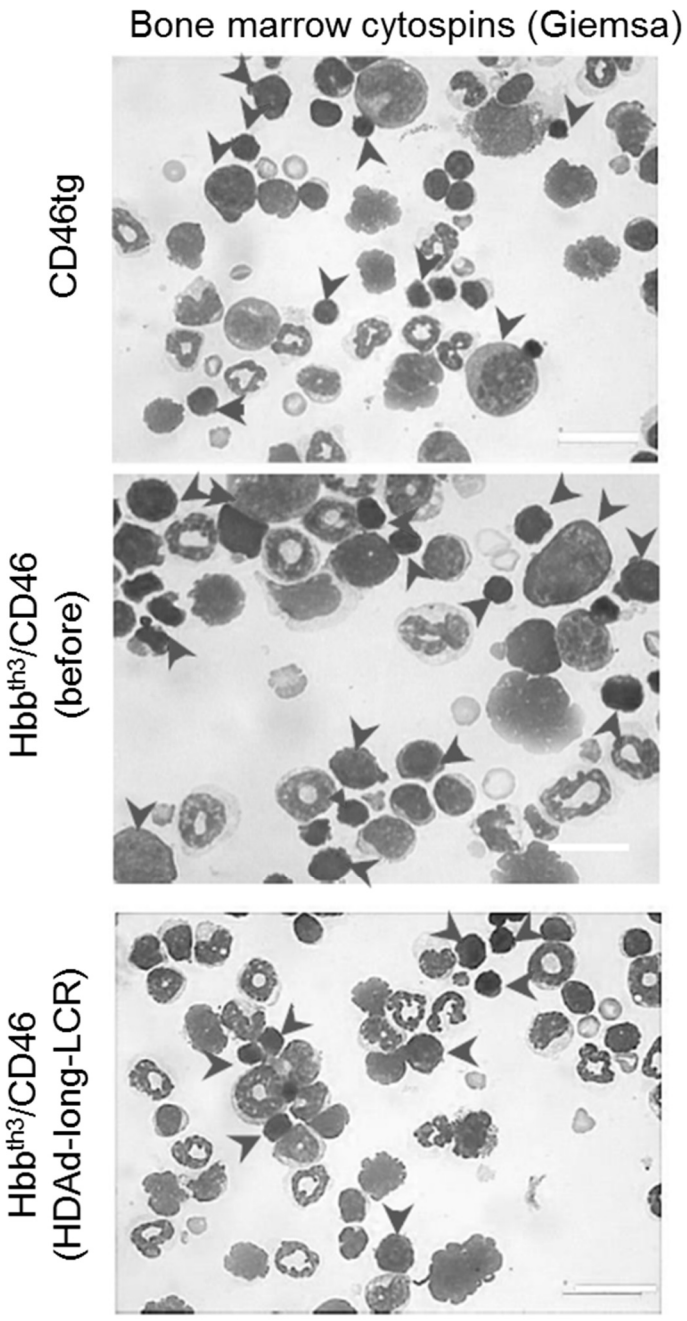

Bone marrow cytospins (Giemsa)
CD46tg
Hbb^{th3}/CD46 (before)
Hbb^{th3}/CD46 (HDAd-long-LCR)

FIG. 38

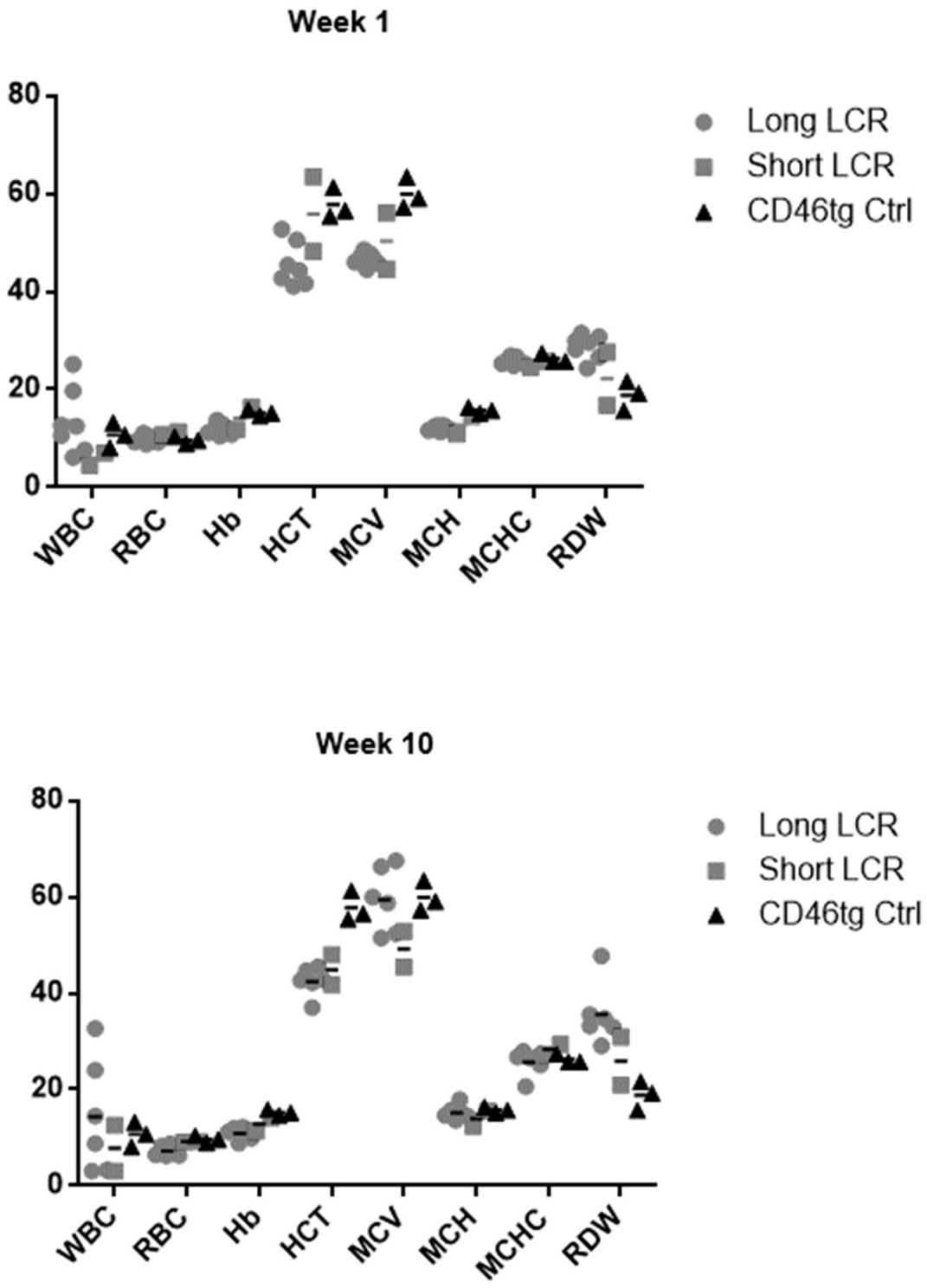

Week 1
80
60
40
20
0
WBC
RBC
Hb
HCT
MCV
MCH
MCHC
RDW
Long LCR
Short LCR
CD46tg Ctrl
Week 10
80
60
40
20
0
WBC
RBC
Hb
HCT
MCV
MCH
MCHC
RDW
Long LCR
Short LCR
CD46tg Ctrl

FIG. 39A

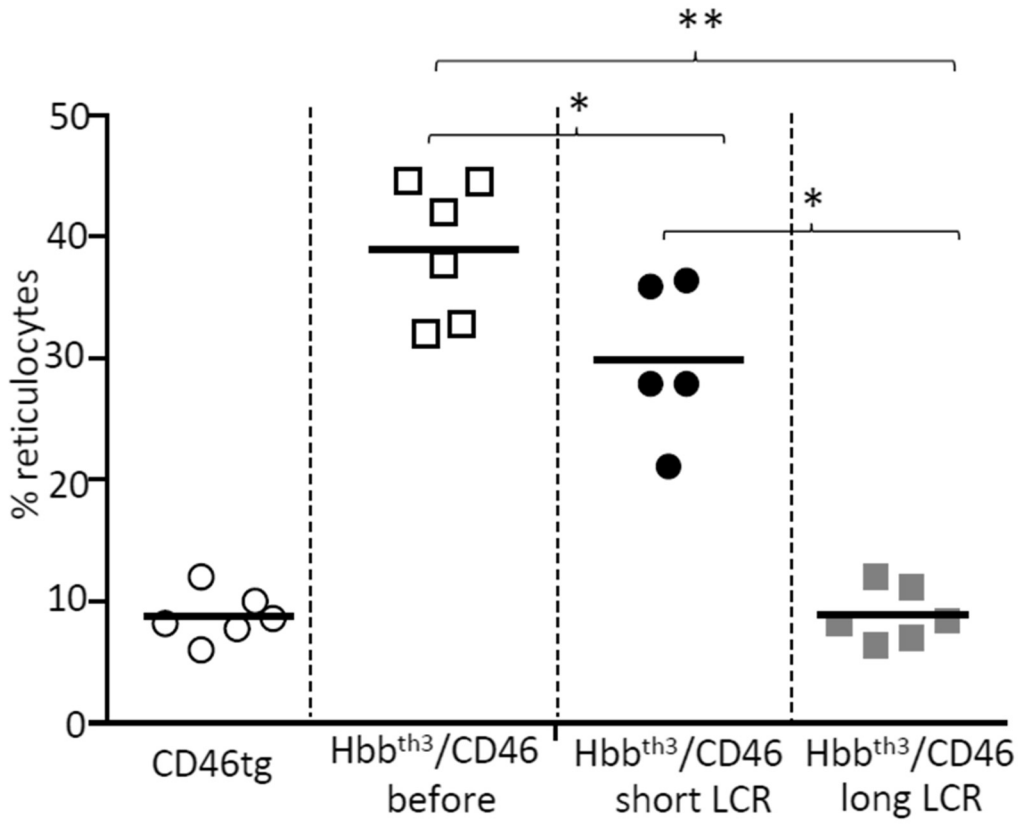

**
*
*
50
40
30
20
10
0
% reticulocytes
CD46tg
Hbb^th3/CD46 before
Hbb^th3/CD46 short LCR
Hbb^th3/CD46 long LCR

FIG. 39B

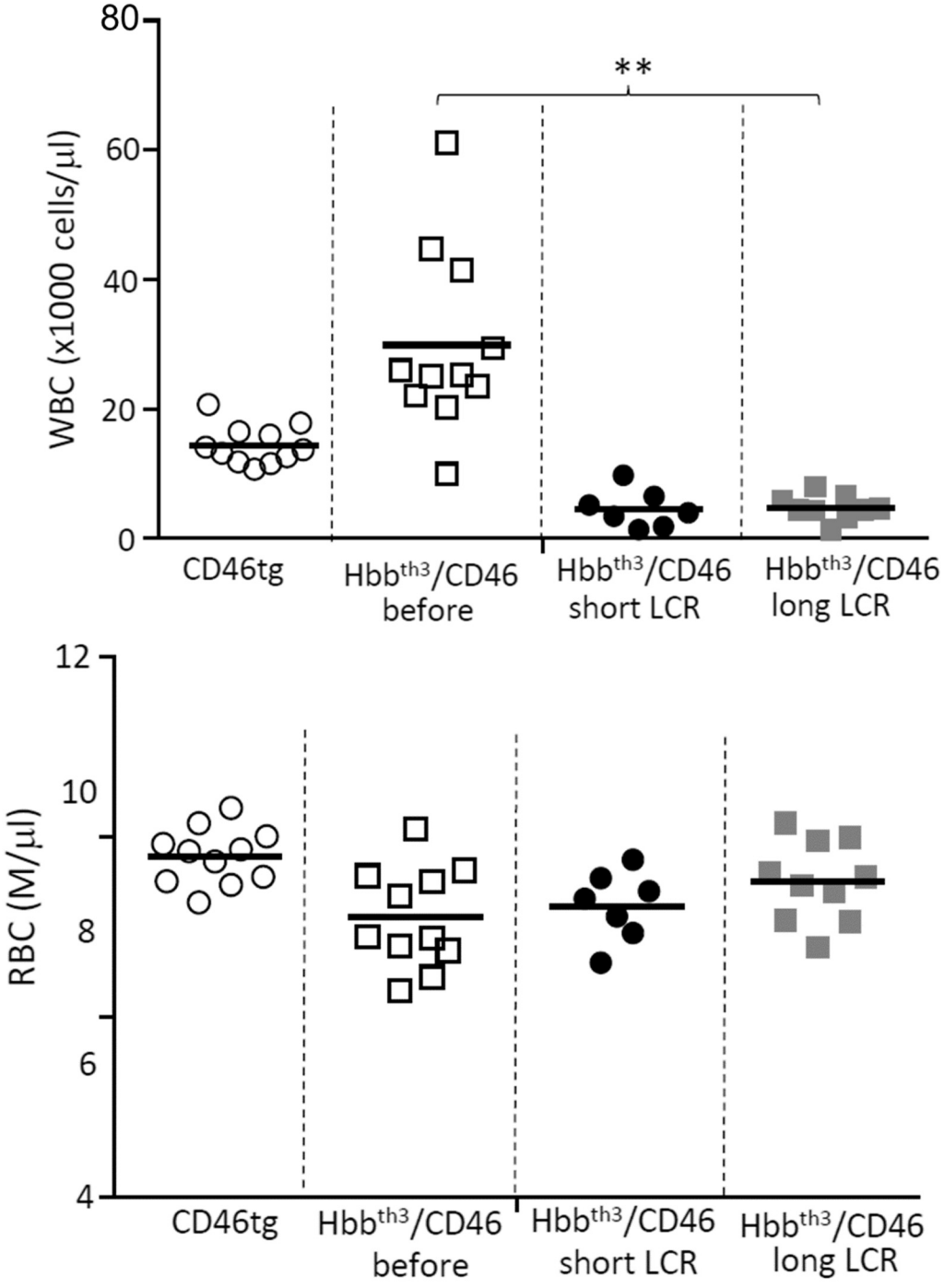

80
60
40
20
0
WBC (x1000 cells/µl)
**
CD46tg
Hbb^th3/CD46 before
Hbb^th3/CD46 short LCR
Hbb^th3/CD46 long LCR
12
10
8
6
4
RBC (M/µl)
CD46tg
Hbb^th3/CD46 before
Hbb^th3/CD46 short LCR
Hbb^th3/CD46 long LCR

FIG. 39B cont'd

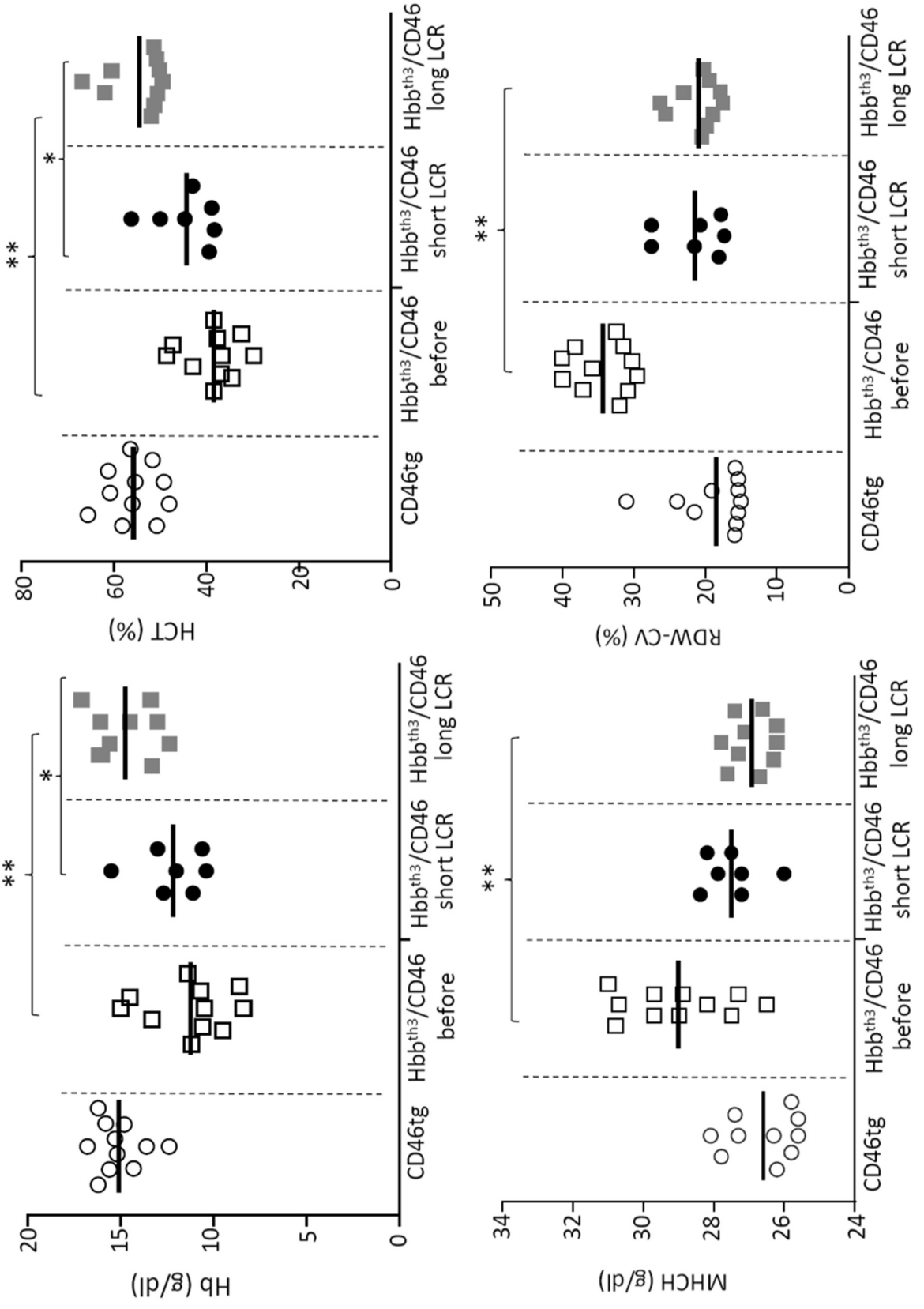
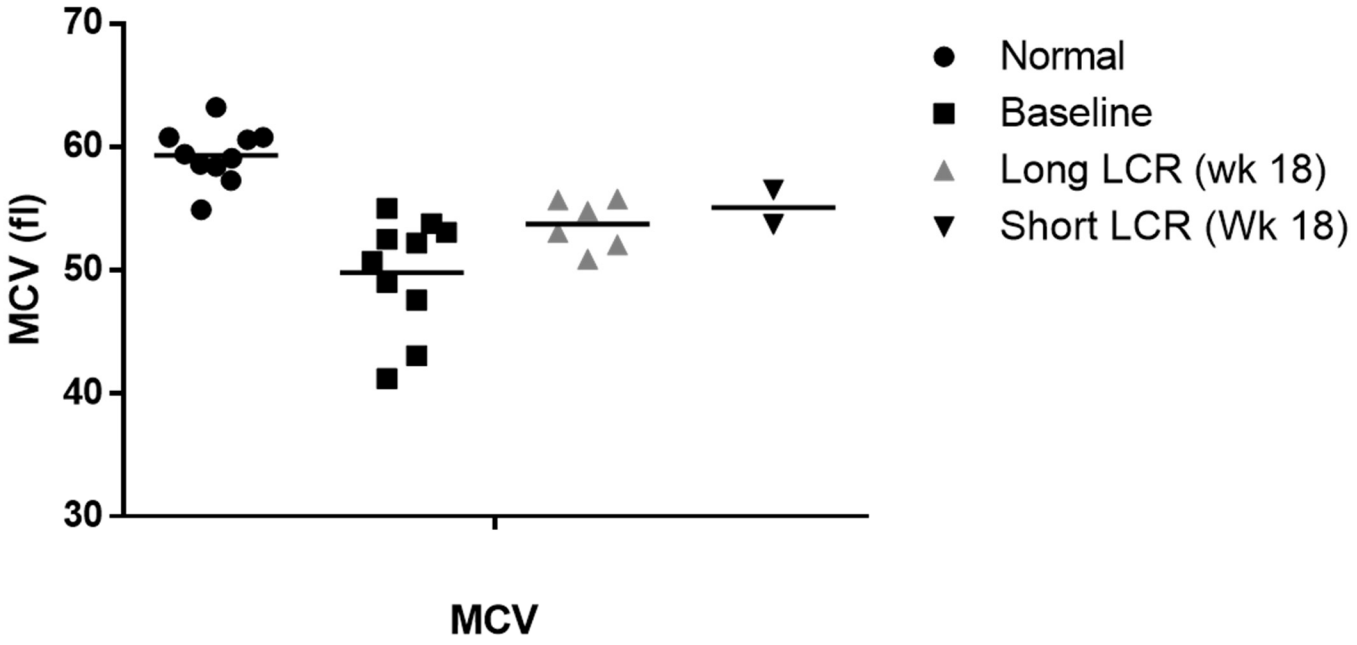


FIG. 39B cont'd



MCH

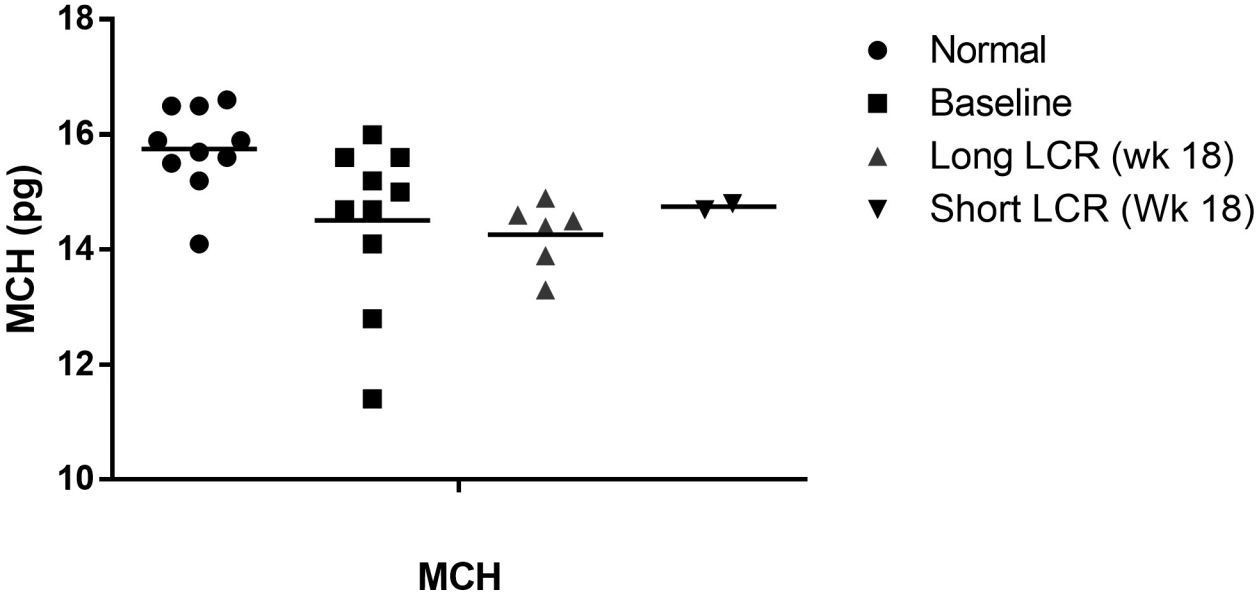
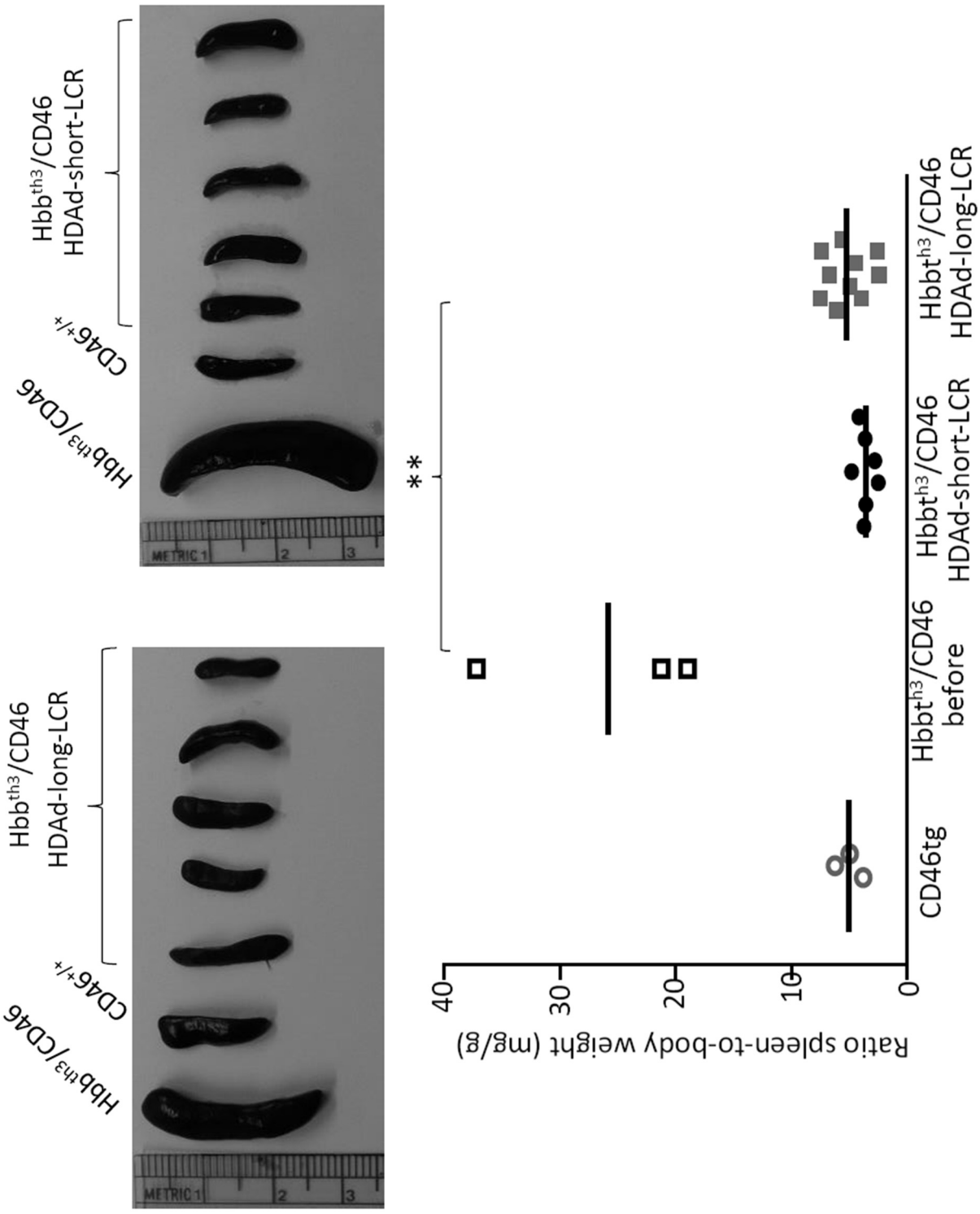


FIG. 40A


Hbb^th3/CD46
HDAd-long-LCR
CD46+/+
Hbb^th3/CD46
Hbb^th3/CD46
HDAd-short-LCR
CD46+/+
Hbb^th3/CD46
METRIC 1
2
3
**
Ratio spleen-to-body weight (mg/g)
40
30
20
10
0
CD46tg
Hbbt^h3/CD46
before
Hbbt^h3/CD46
HDAd-short-LCR
Hbbt^h3/CD46
HDAd-long-LCR

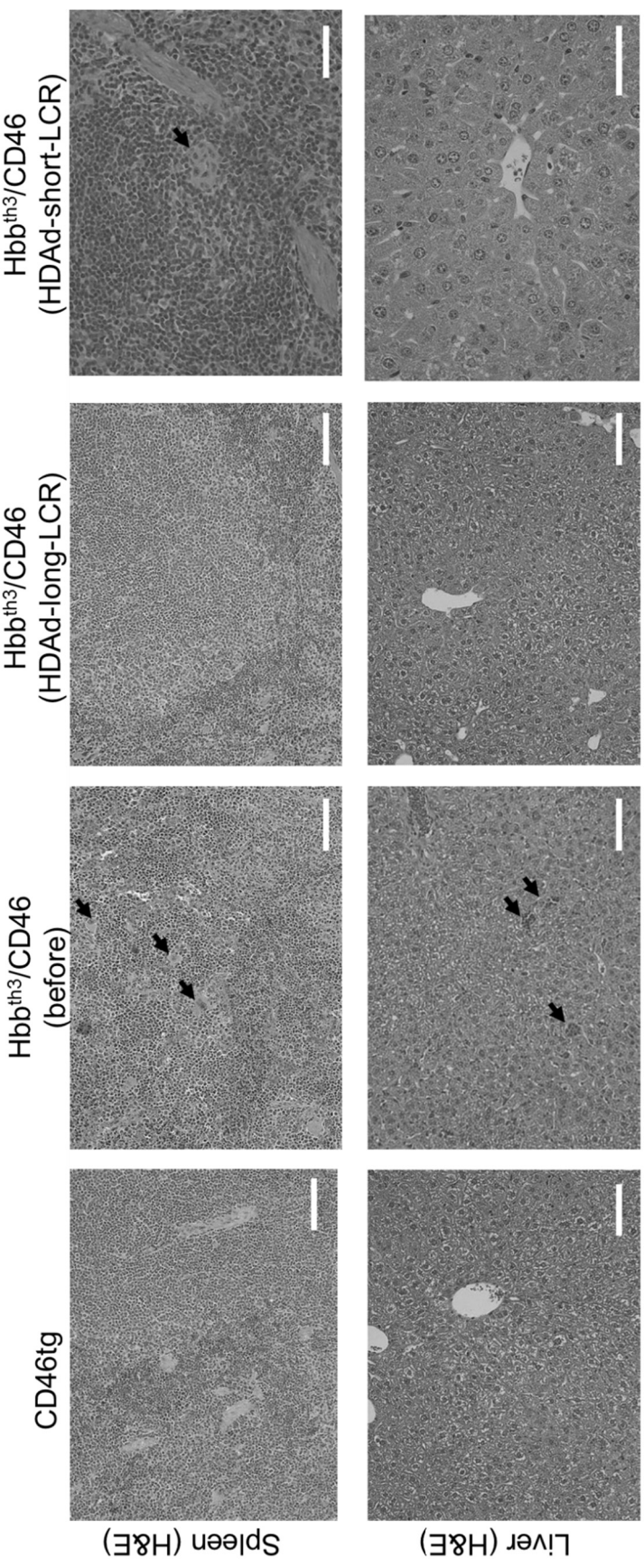

CD46tg
Hbb^{th3}/CD46 (before)
Hbb^{th3}/CD46 (HDAd-long-LCR)
Hbb^{th3}/CD46 (HDAd-short-LCR)
Spleen (H&E)
Liver (H&E)

FIG. 40B

FIG. 41

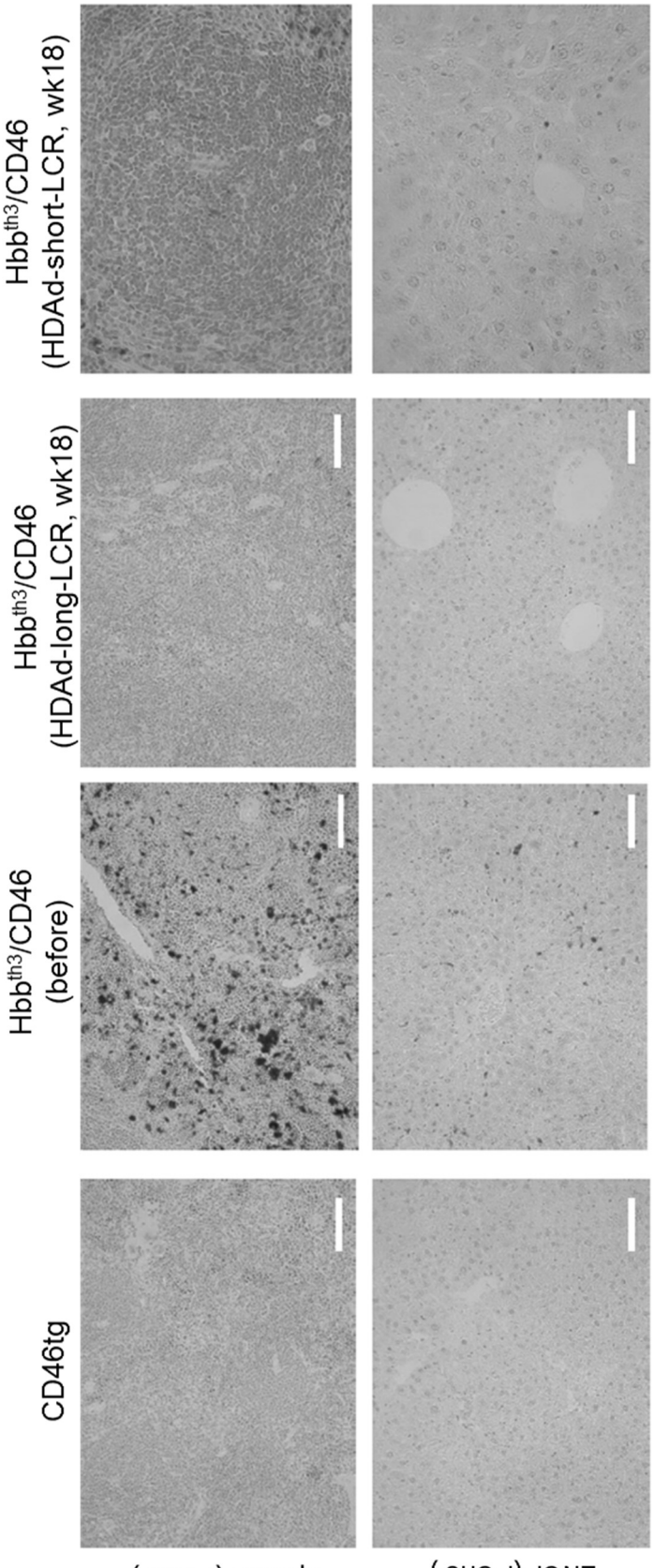

CD46tg
Hbb^{th3}/CD46 (before)
Hbb^{th3}/CD46 (HDAd-long-LCR, wk18)
Hbb^{th3}/CD46 (HDAd-short-LCR, wk18)
Spleen (Perls')
Liver (Perls')

FIG. 42A

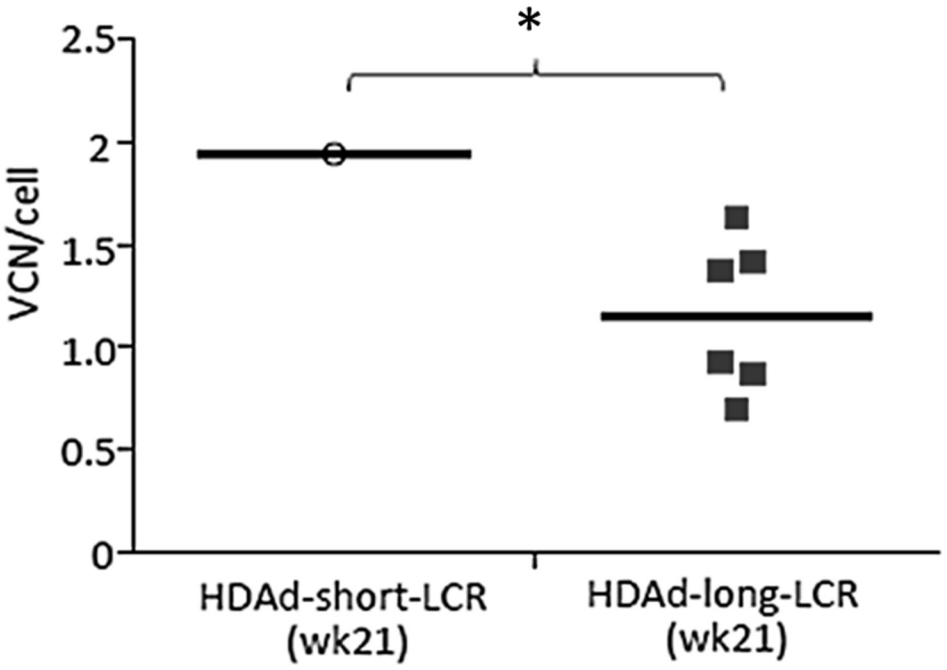


FIG. 42B

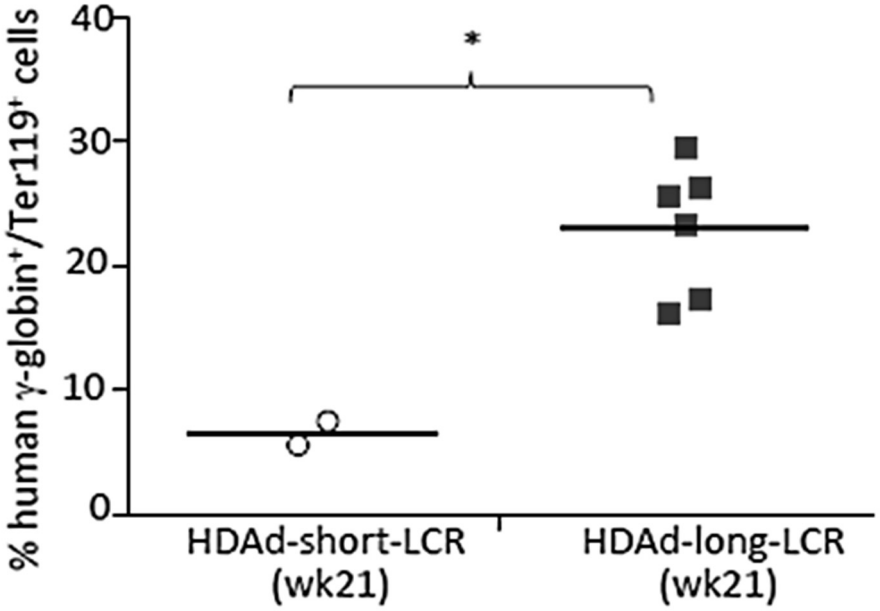


FIG. 42C

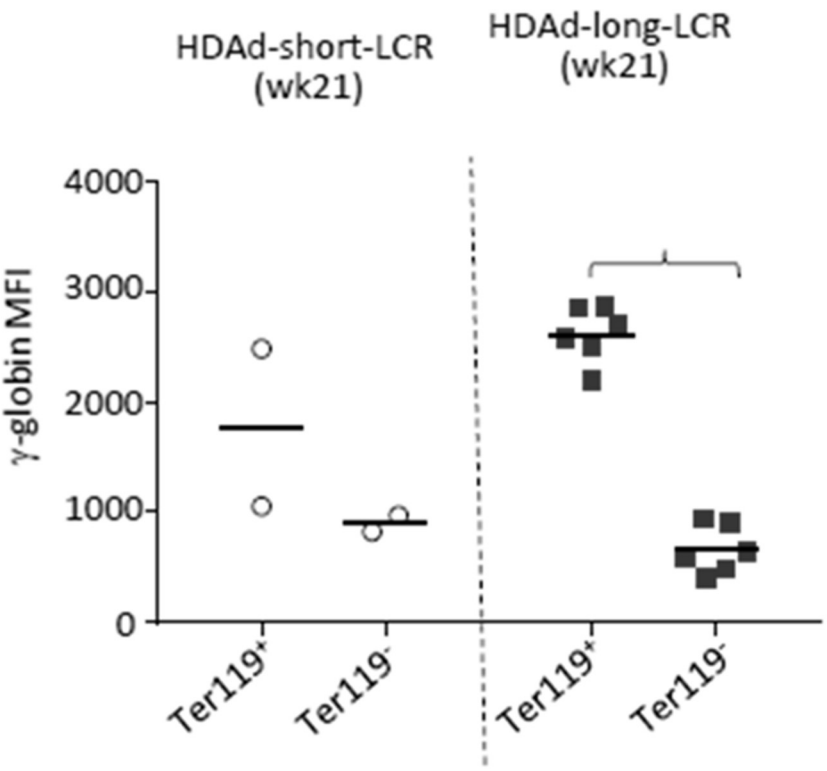

HDAd-short-LCR
(wk21)
HDAd-long-LCR
(wk21)
γ-globin MFI
4000
3000
2000
1000
0
Ter119+
Ter119-
Ter119+
Ter119-

FIG. 43

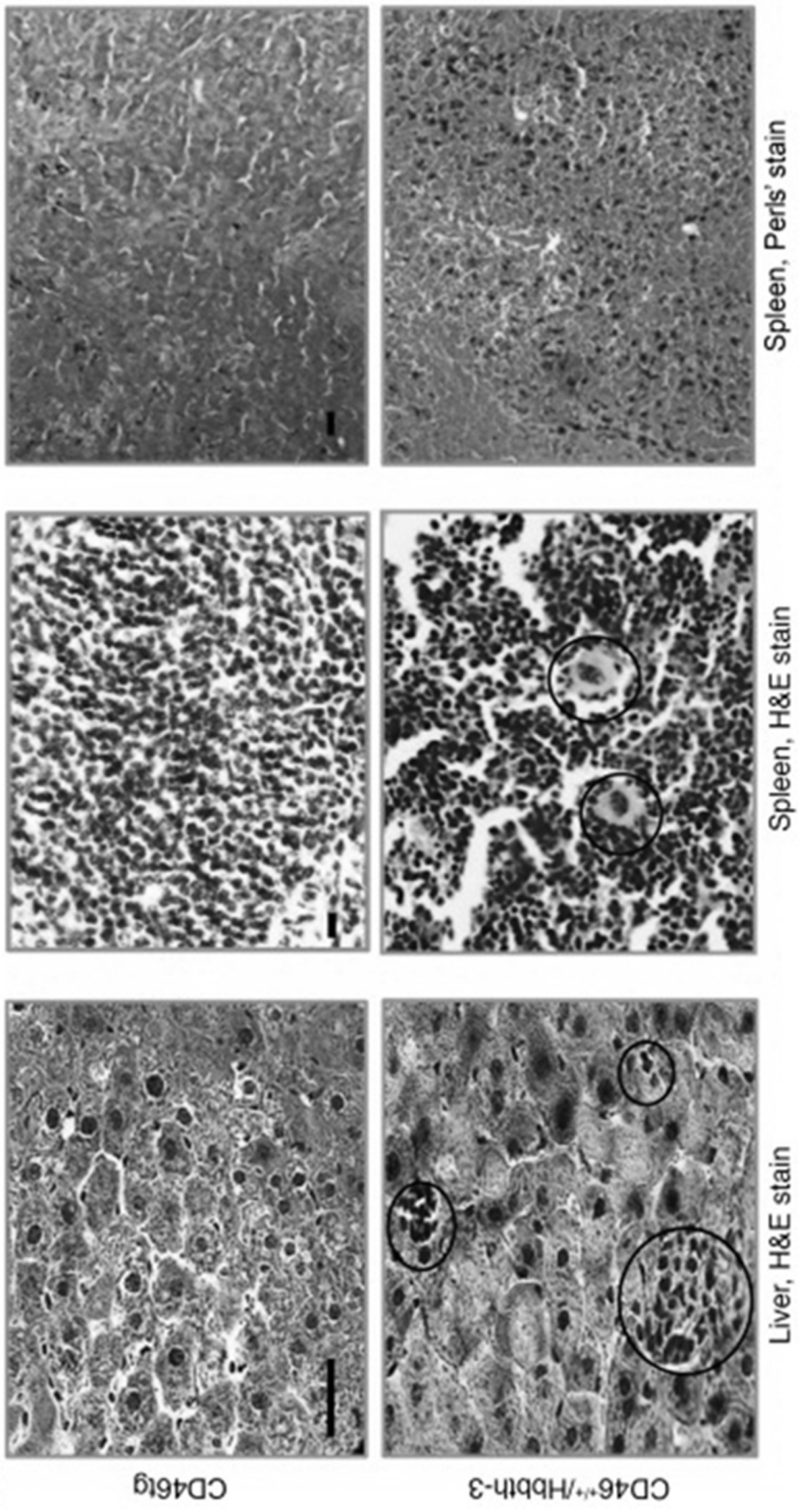

Before
CD46tg
CD46-/-/Hbb1h-3
Liver, H&E stain
Spleen, H&E stain
Spleen, Perls' stain

FIG. 44A

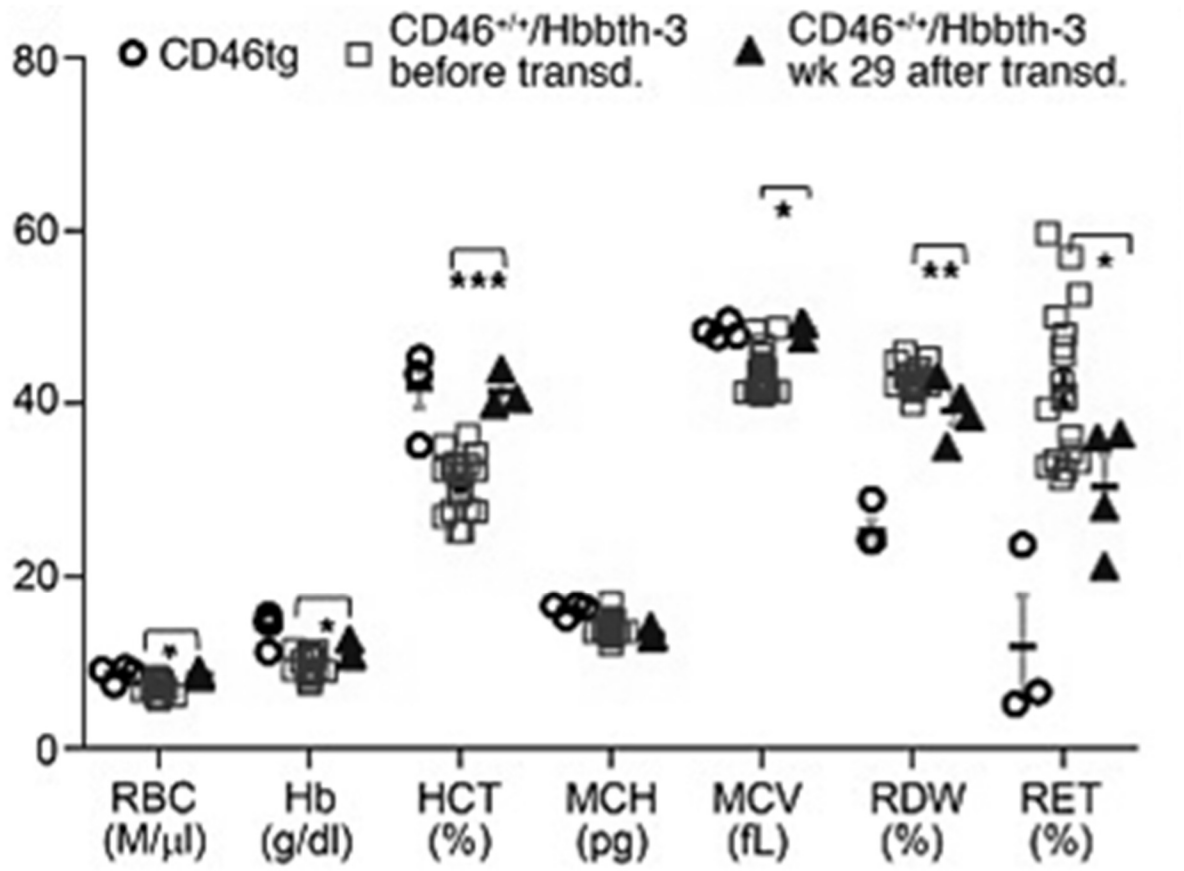


FIG. 44B

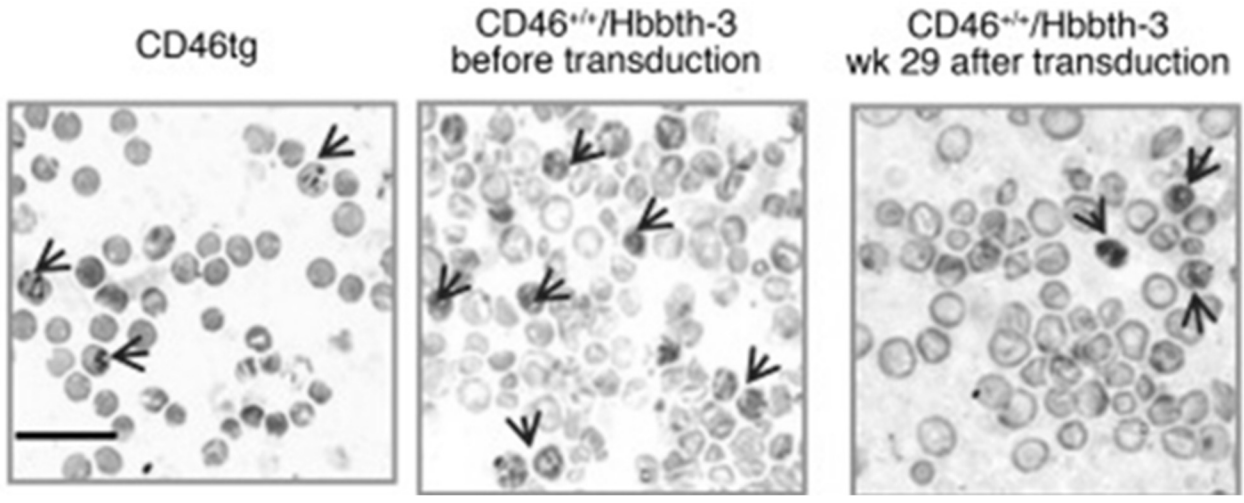


FIG. 44C

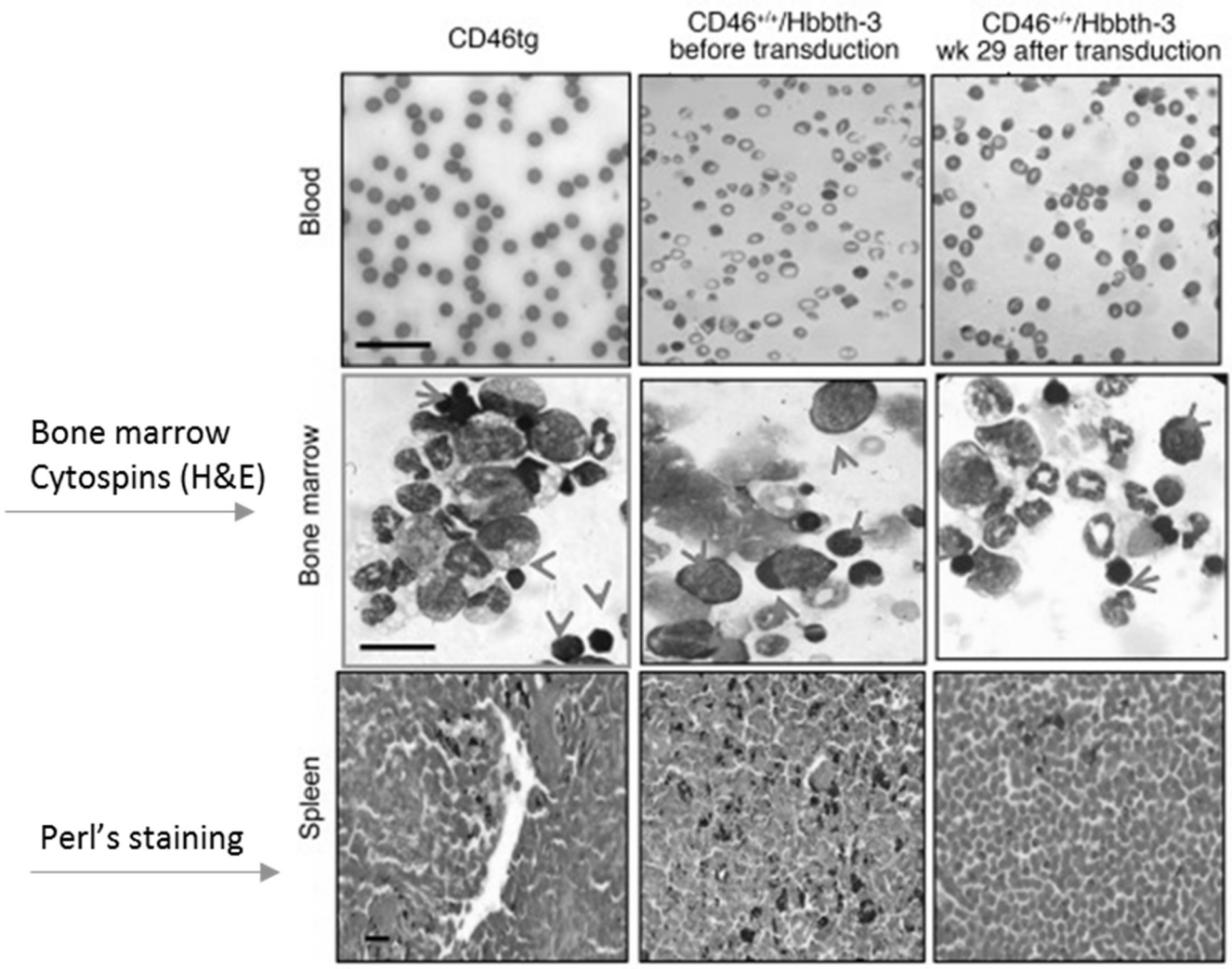

CD46tg
CD46+/+/Hbbth-3 before transduction
CD46+/+/Hbbth-3 wk 29 after transduction
Blood
Bone marrow
Spleen
Bone marrow Cytospins (H&E)
Perl's staining

FIG. 44D

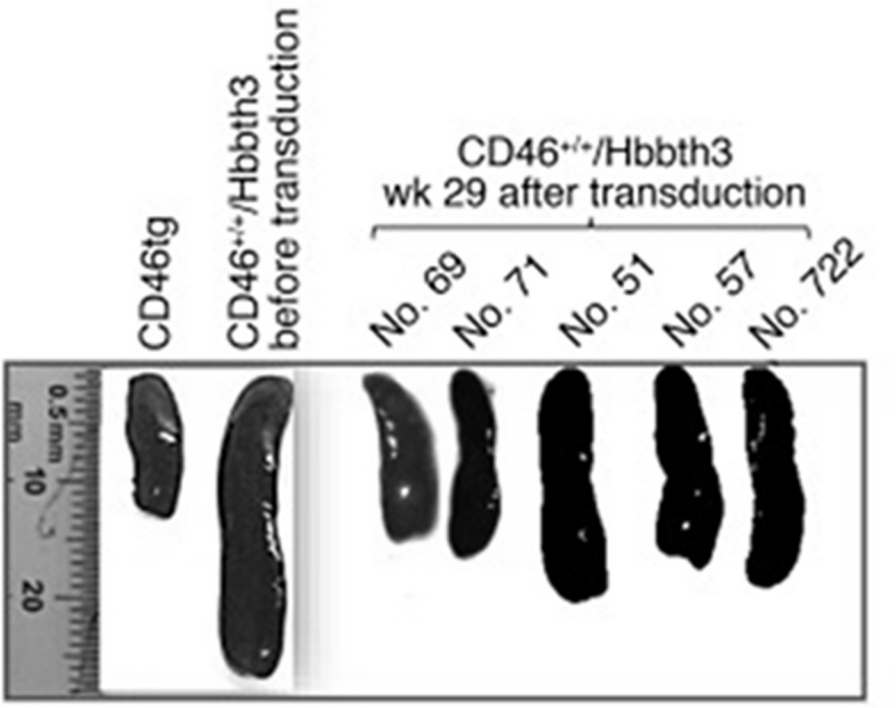
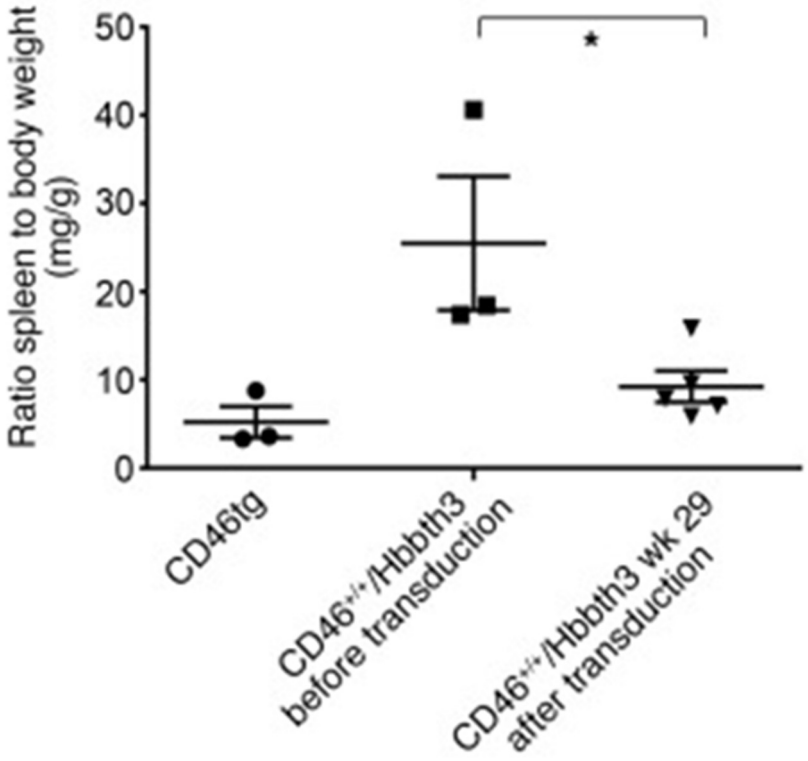


FIG. 44E



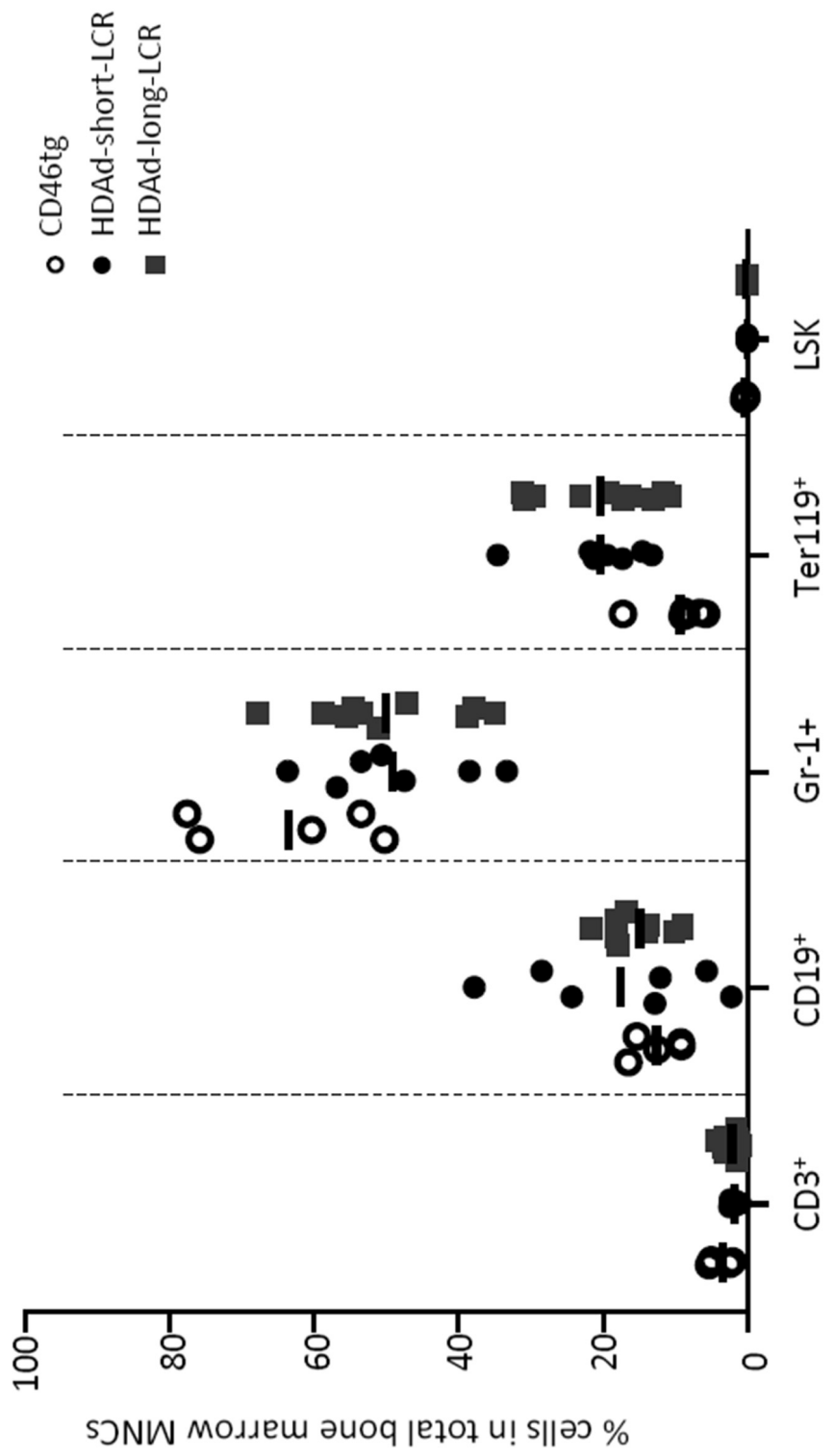

CD46tg
HDAd-short-LCR
HDAd-long-LCR
% cells in total bone marrow MNCs
100
80
60
40
20
0
CD3+
CD19+
Gr-1+
Ter119+
LSK

FIG. 45

FIG. 46

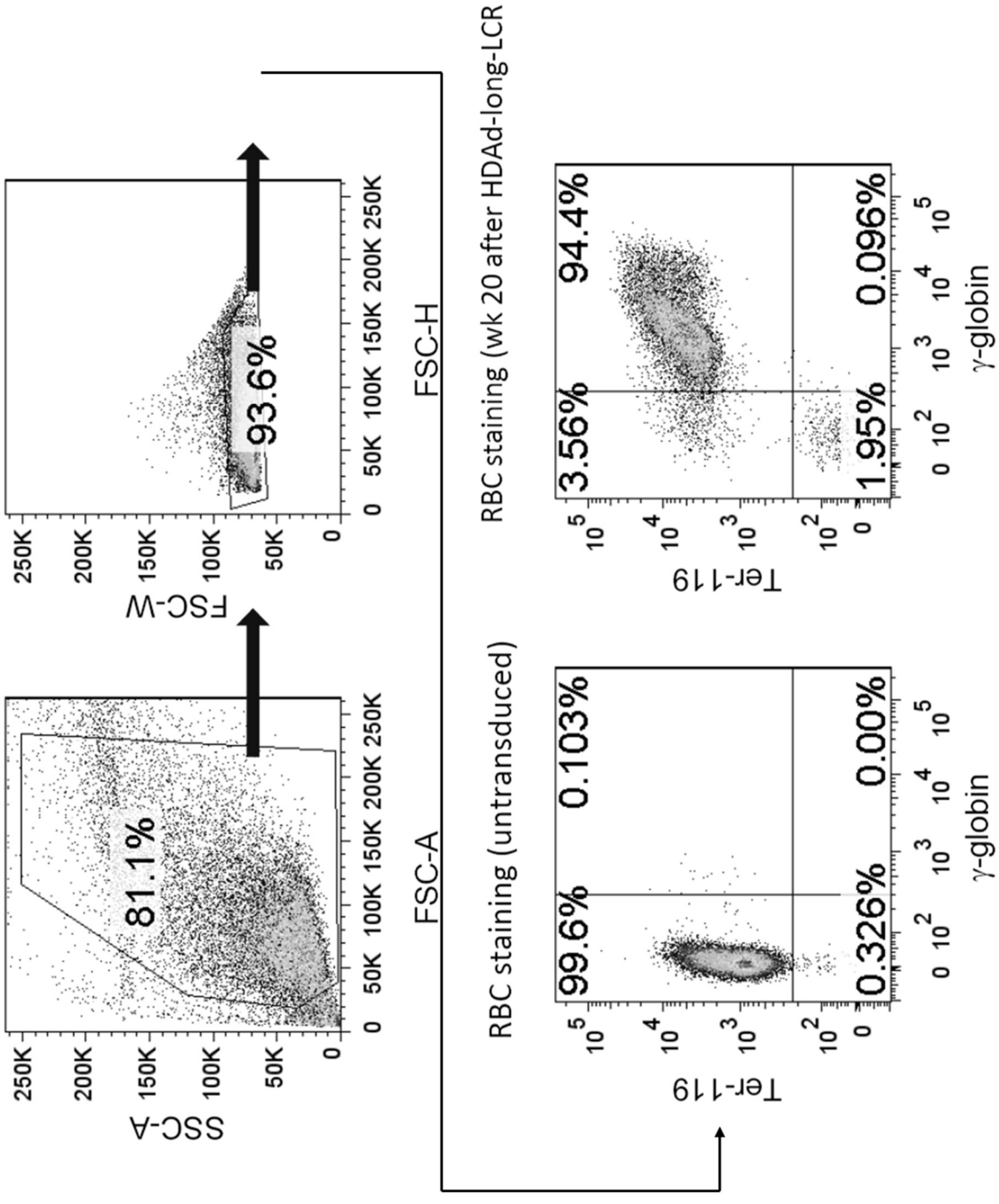

SSC-A
FSC-A
81.1%
FSC-W
FSC-H
93.6%
RBC staining (untransduced)
Ter-119
γ-globin
99.6%
0.103%
0.326%
0.00%
RBC staining (wk 20 after HDAd-long-LCR
3.56%
94.4%
1.95%
0.096%

FIG. 47A

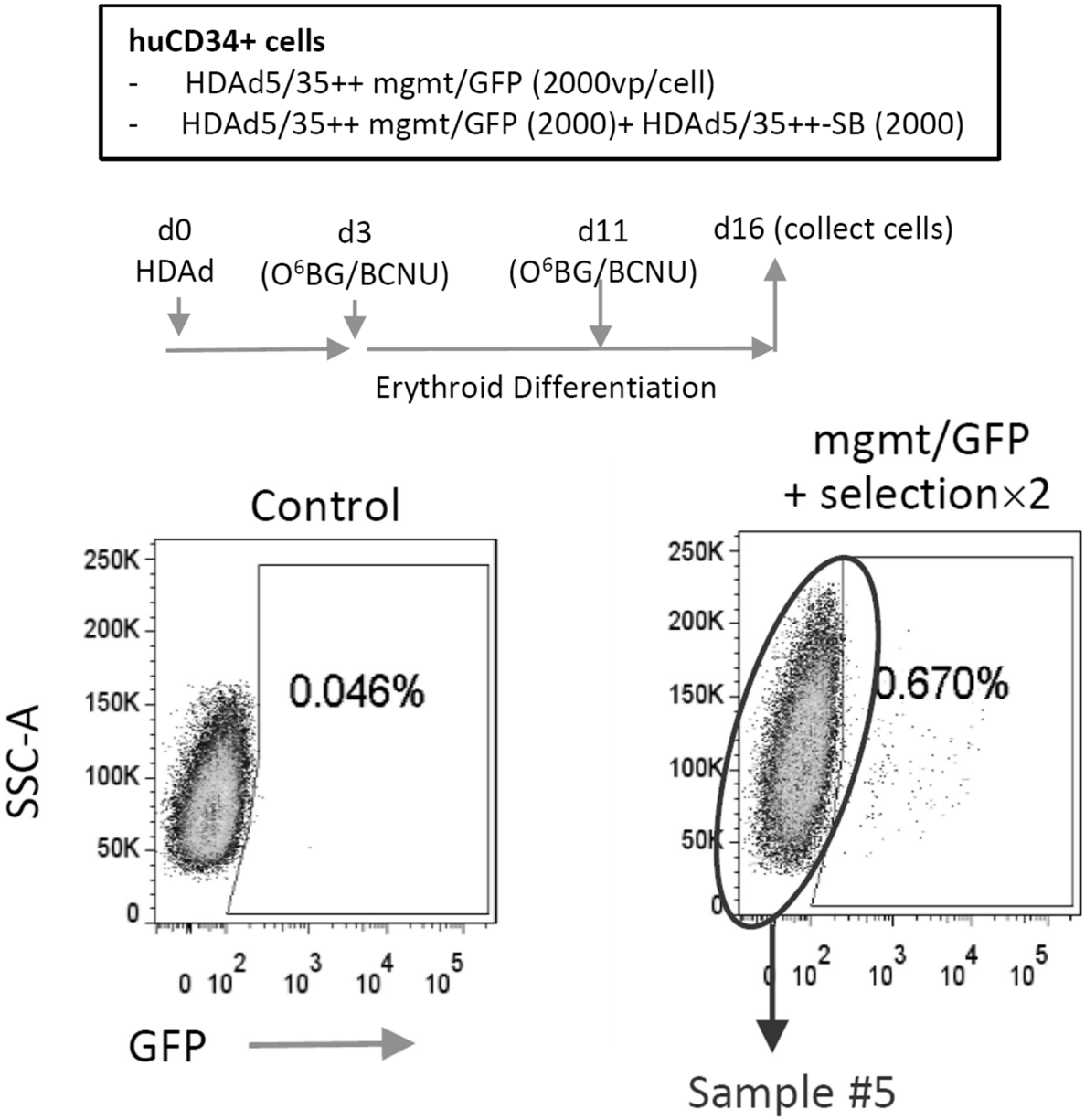

huCD34+ cells
- HDAd5/35++ mgmt/GFP (2000vp/cell)
- HDAd5/35++ mgmt/GFP (2000)+ HDAd5/35++-SB (2000)
d0
HDAd
d3
(O6BG/BCNU)
d11
(O6BG/BCNU)
d16 (collect cells)
Erythroid Differentiation
Control
mgmt/GFP
+ selection×2
SSC-A
250K
200K
150K
100K
50K
0
0.046%
0.670%
0 10^2 10^3 10^4 10^5
GFP
Sample #5

FIG. 47A cont'd

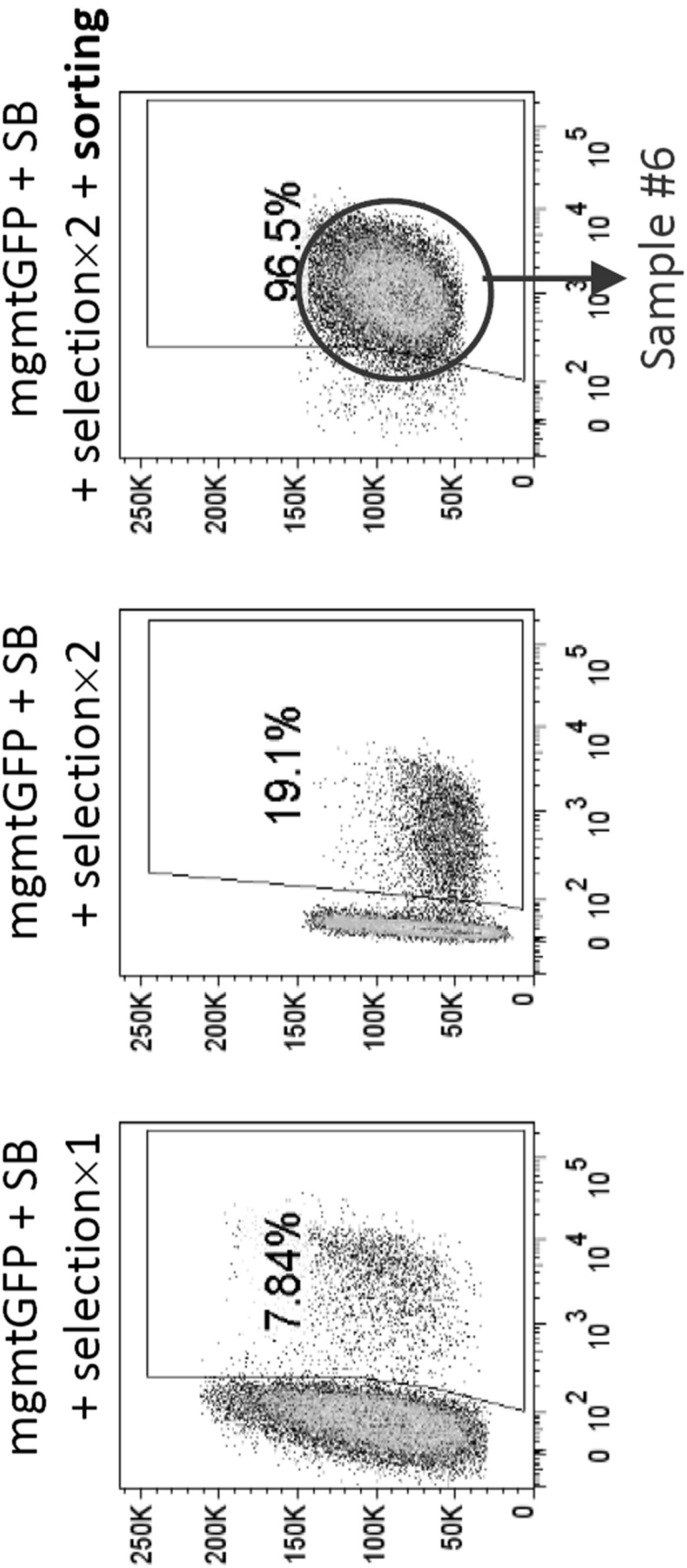


FIG. 47B

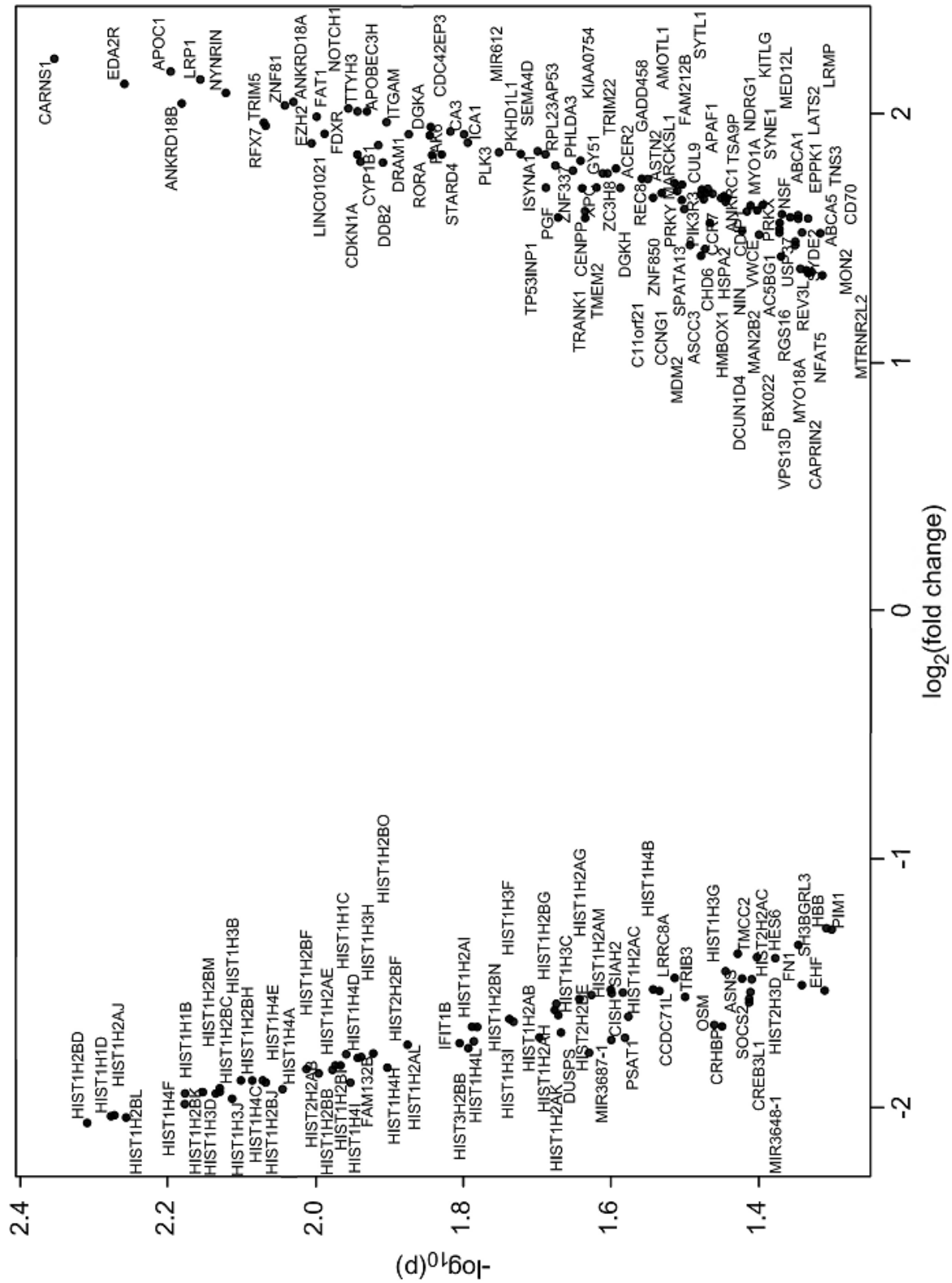

-log10(p)
log2(fold change)
2.4
2.2
2.0
1.8
1.6
1.4
-2
-1
0
1
2
CARNS1
EDA2R
APOC1
LRP1
NYNRIN
ANKRD18B
RFX7
TRIM5
ZNF81
EZH2
ANKRD18A
FAT1
NOTCH1
FDXR
TTYH3
APOBEC3H
ITGAM
LINC01021
CDKN1A
CYP1B1
DDB2
DRAM1
DGKA
RORA
CDC42EP3
STARD4
CA3
ICA1
PLK3
PKHD1L1
MIR612
SEMA4D
TP53INP1
ISYNA1
RPL23AP53
PHLDA3
PGF
ZNF33A
KIAA0754
TRIM22
GY51
ZC3H8
ACER2
DGKH
GADD458
REC8
ASTN2
AMOTL1
TRANK1
TMEM2
CENPP
FAM212B
SYTL1
C11orf21
ZNF850
CCNG1
SPATA13
MRCKSL1
CUL9
APAF1
MDM2
ASCC3
CHD6
HMBOX1
HSPA2
TSA9P
DCUN1D4
MAN2B2
NDRG1
KITLG
FBX022
AC5BG1
SYNE1
MED12L
VPS13D
RGS16
ABCA1
CAPRIN2
MYO18A
REV3L
EPPK1
LATS2
NFAT5
ABCA5
TNS3
LRMP
MON2
CD70
MTRNR2L2
HIST1H2BD
HIST1H1D
HIST1H2AJ
HIST1H2BL
HIST1H4F
HIST1H1B
HIST1H2BM
HIST1H2BC
HIST1H3B
HIST1H3J
HIST1H2BH
HIST1H4C
HIST1H4E
HIST1H2BJ
HIST1H4A
HIST2H2AB
HIST1H2BF
HIST1H2BB
HIST1H2AE
HIST1H1C
HIST1H4I
HIST1H4D
HIST1H3H
FAM132B
HIST1H2BO
HIST1H4H
HIST2H2BF
HIST1H2AL
IFIT1B
HIST3H2BB
HIST1H4L
HIST1H2AI
HIST1H2BN
HIST1H3F
HIST1H3I
HIST1H2AB
HIST1H2BG
HIST1H2AH
HIST1H3C
HIST1H2AG
HIST1H2AK
DUSP5
HIST2H2BE
HIST1H2AM
MIR3687-1
CISH
SIAH2
PSAT1
HIST1H2AC
HIST1H4B
CCDC71L
LRRC8A
TRIB3
OSM
HIST1H3G
CRHBP
ASNS
TMCC2
SOCS2
HIST2H2AC
CREB3L1
HES6
HIST2H3D
MIR3648-1
FN1
SH3BGRL3
HBB
EHF
PIM1

FIG. 48

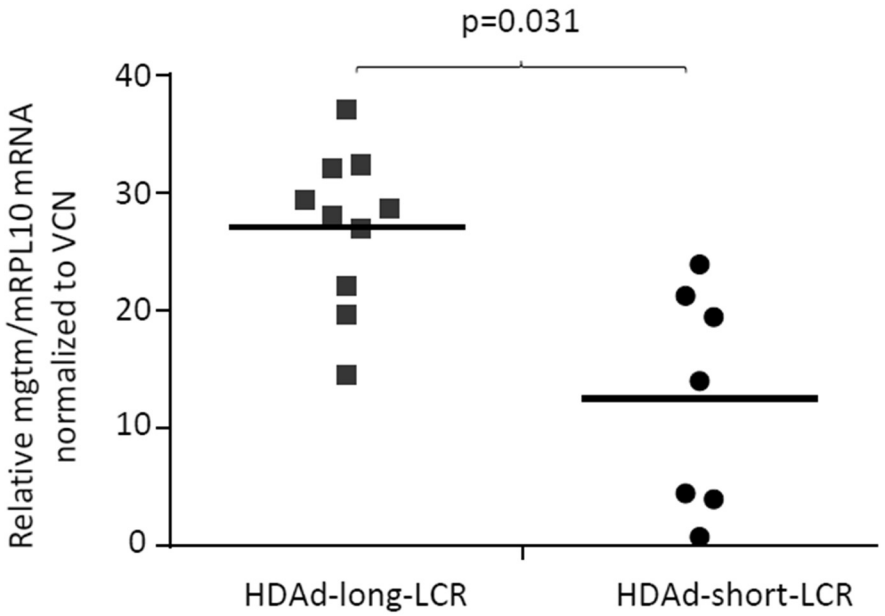

p=0.031
Relative mgtm/mRPL10 mRNA normalized to VCN
40
30
20
10
0
HDAd-long-LCR
HDAd-short-LCR

FIG. 49

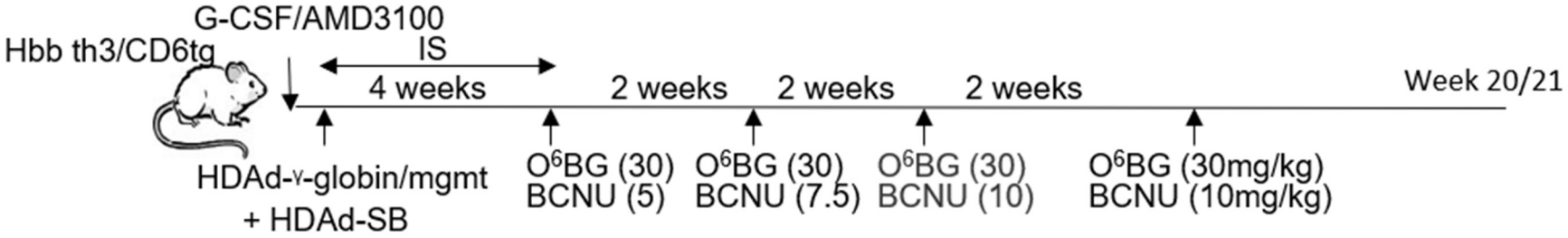

G-CSF/AMD3100
Hbb th3/CD6tg
IS
4 weeks
2 weeks
2 weeks
2 weeks
Week 20/21
HDAd-γ-globin/mgmt
+ HDAd-SB
O6BG (30)
BCNU (5)
O6BG (30)
BCNU (7.5)
O6BG (30)
BCNU (10)
O6BG (30mg/kg)
BCNU (10mg/kg)

FIG. 50

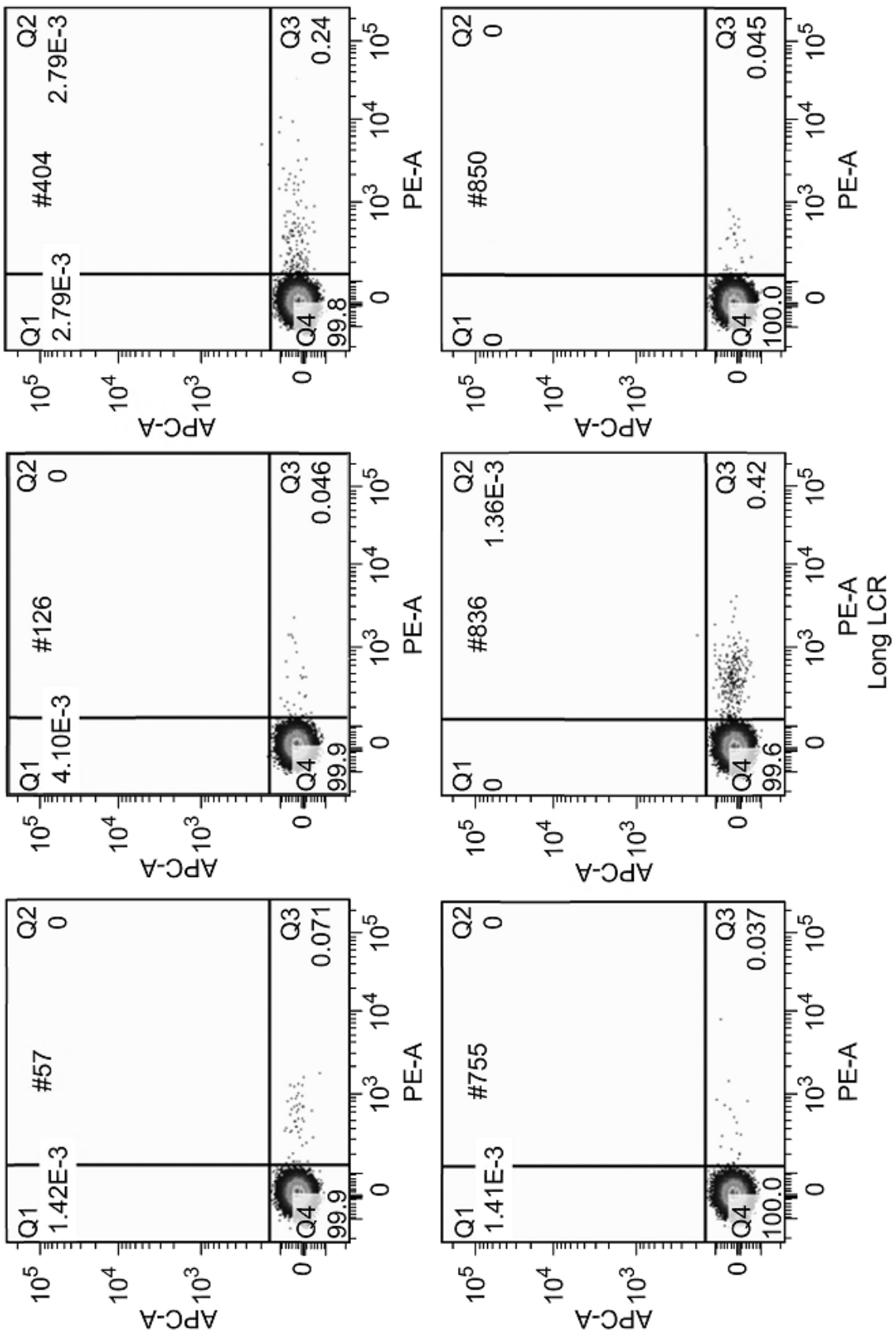
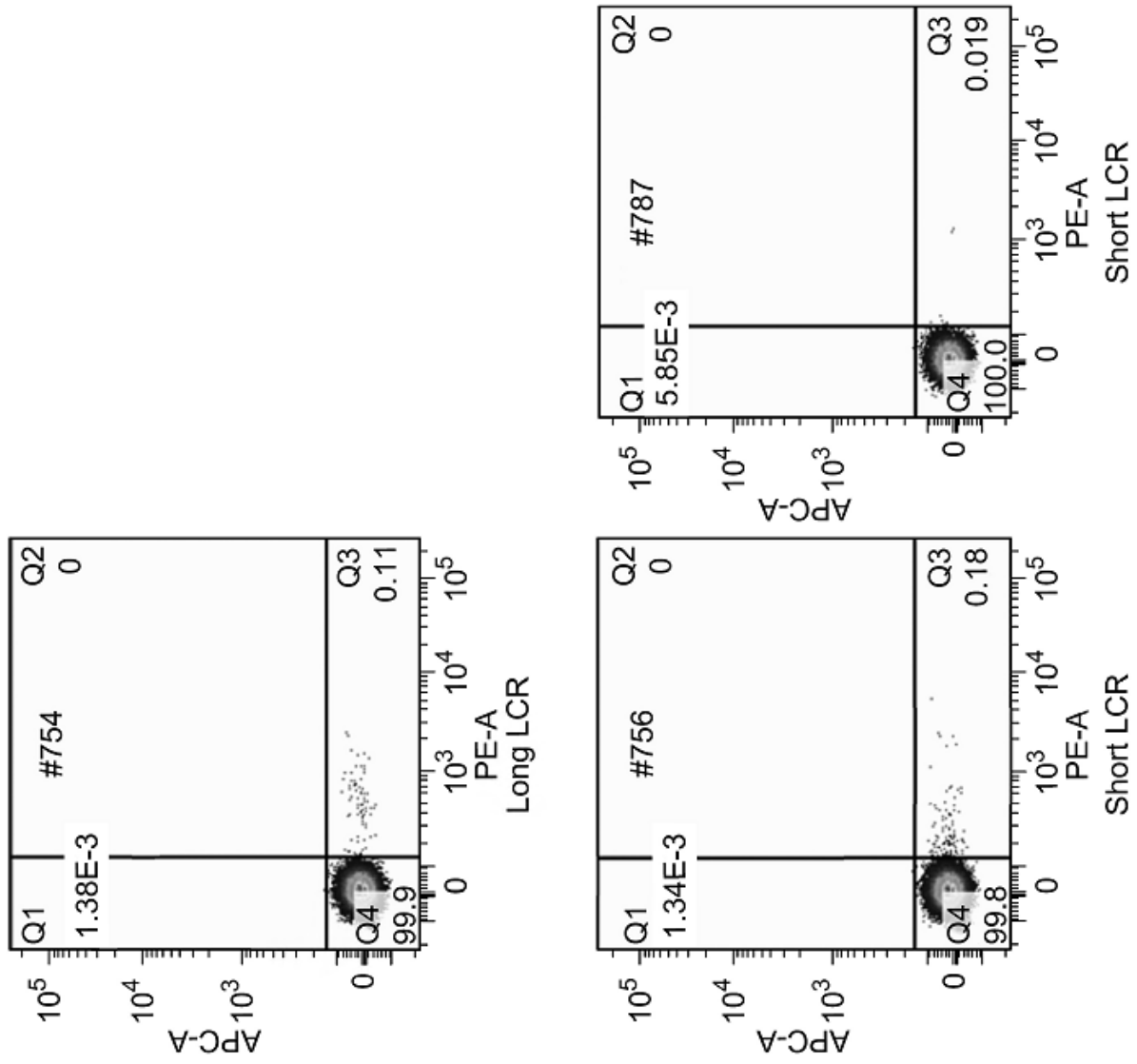

#57
Q1 1.42E-3
Q2 0
Q4 99.9
Q3 0.071
#126
Q1 4.10E-3
Q2 0
Q4 99.9
Q3 0.046
#404
Q1 2.79E-3
Q2 2.79E-3
Q4 99.8
Q3 0.24
#755
Q1 1.41E-3
Q2 0
Q4 100.0
Q3 0.037
#836
Q1 0
Q2 1.36E-3
Q4 99.6
Q3 0.42
#850
Q1 0
Q2 0
Q4 100.0
Q3 0.045
APC-A
PE-A
Long LCR

FIG. 50 cont'd.


#754
Q1
1.38E-3
Q2
0
Q4
99.9
Q3
0.11
APC-A
PE-A
Long LCR
#787
Q1
5.85E-3
Q2
0
Q4
100.0
Q3
0.019
APC-A
PE-A
Short LCR
#756
Q1
1.34E-3
Q2
0
Q4
99.8
Q3
0.18
APC-A
PE-A
Short LCR

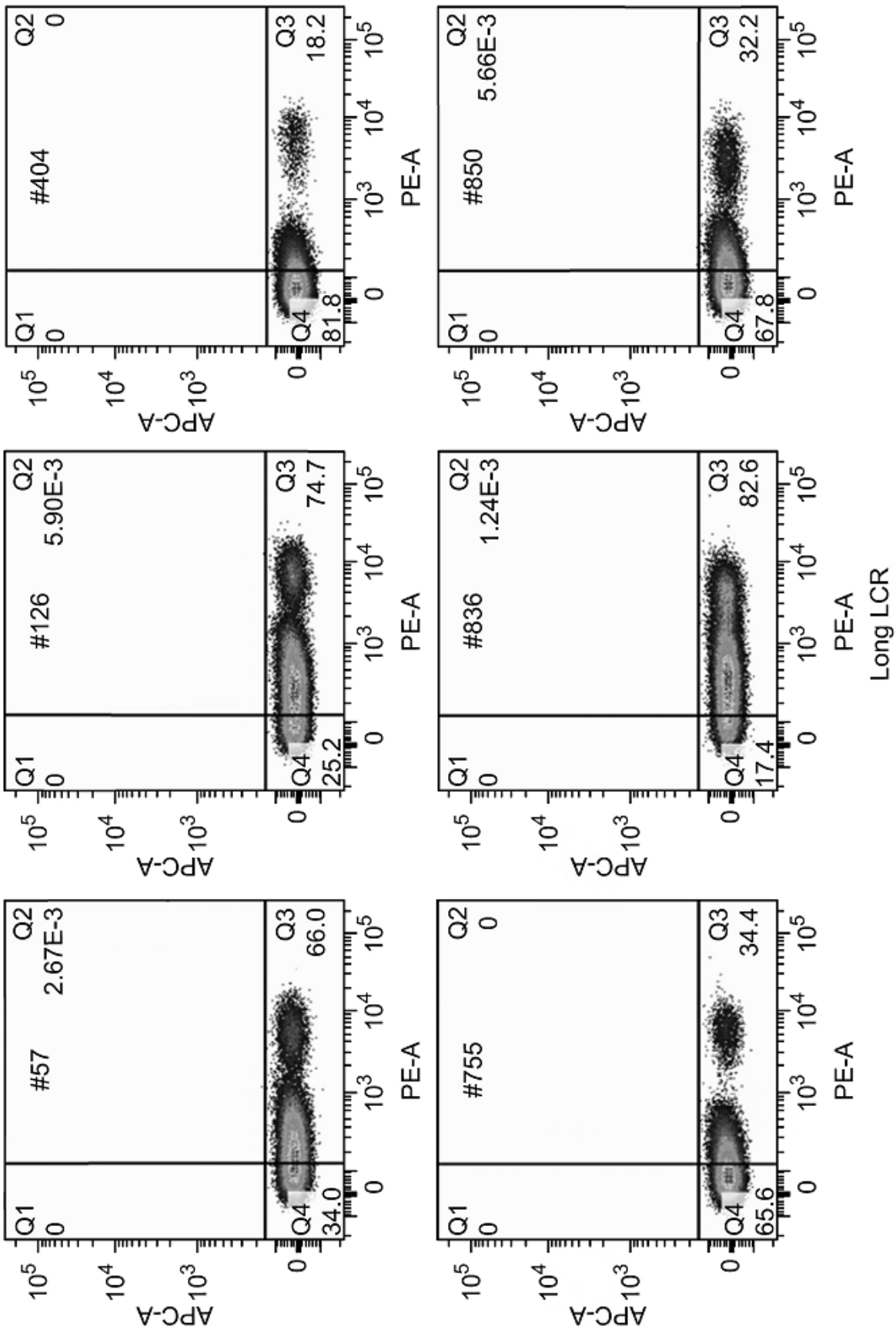

#57
Q1 0
Q2 2.67E-3
Q4 34.0
Q3 66.0
#126
Q1 0
Q2 5.90E-3
Q4 25.2
Q3 74.7
#404
Q1 0
Q2 0
Q4 81.8
Q3 18.2
#755
Q1 0
Q2 0
Q4 65.6
Q3 34.4
#836
Q1 0
Q2 1.24E-3
Q4 17.4
Q3 82.6
#850
Q1 0
Q2 5.66E-3
Q4 67.8
Q3 32.2
APC-A
PE-A
Long LCR

FIG. 51

FIG. 51 cont'd.

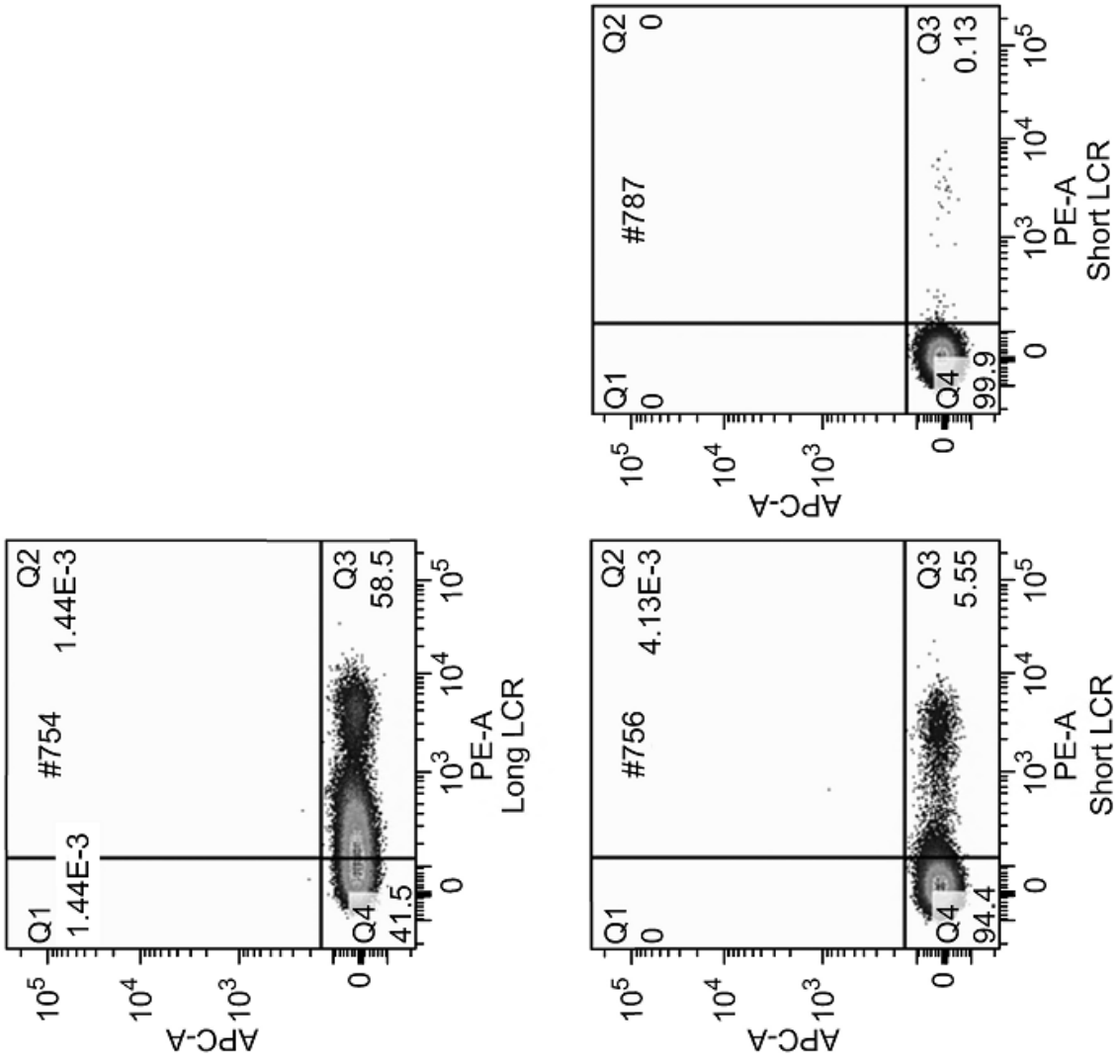

#754
Q1
1.44E-3
Q2
1.44E-3
Q4
41.5
Q3
58.5
APC-A
PE-A
Long LCR
#787
Q1
0
Q2
0
Q4
99.9
Q3
0.13
Short LCR
#756
Q1
0
Q2
4.13E-3
Q4
94.4
Q3
5.55
Short LCR

FIG. 52

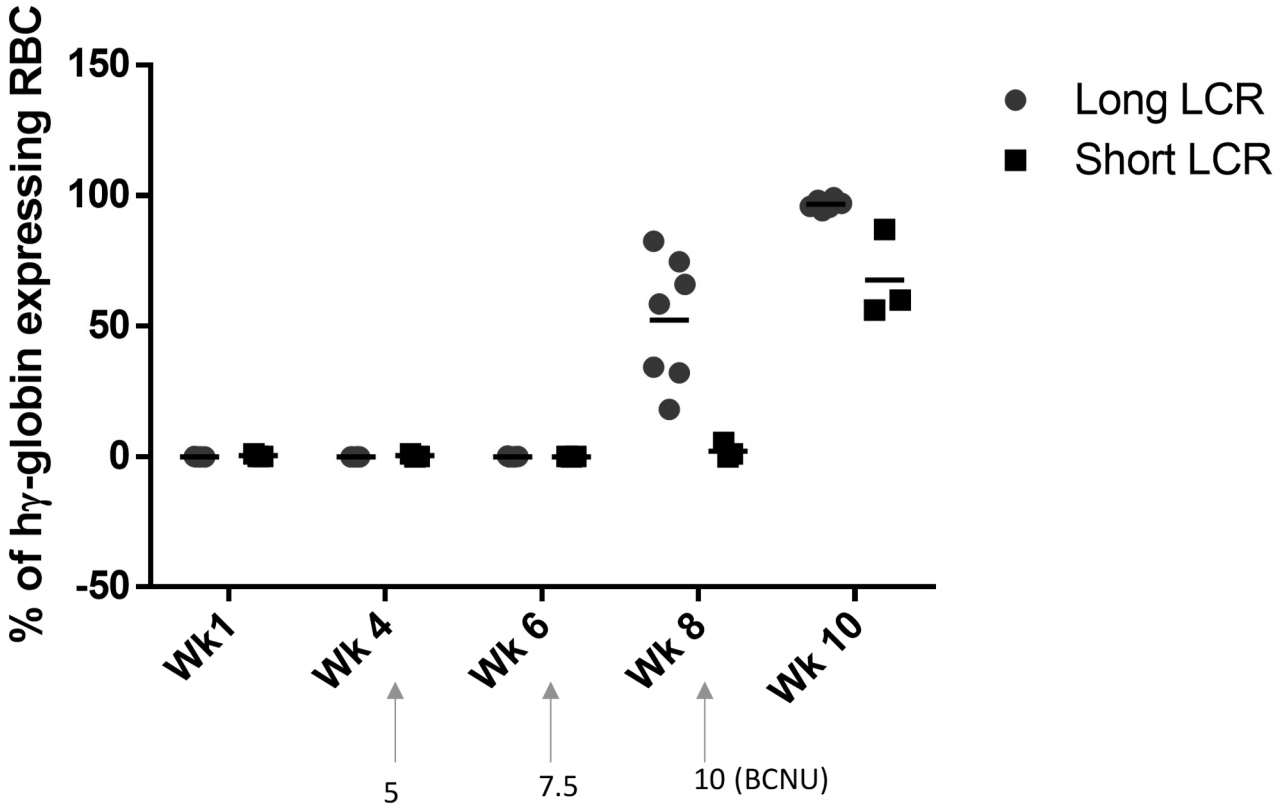

% of hγ-globin expressing RBC
150
100
50
0
-50
Wk1
Wk 4
Wk 6
Wk 8
Wk 10
5
7.5
10 (BCNU)
Long LCR
Short LCR

FIG. 53

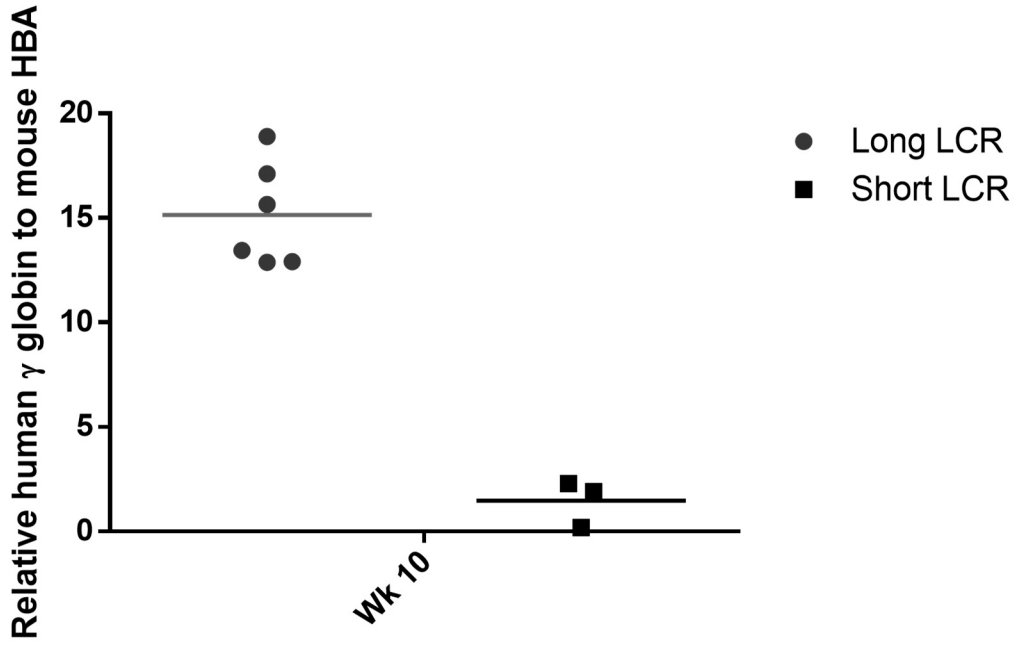

Relative human γ globin to mouse HBA
20
15
10
5
0
Wk 10
Long LCR
Short LCR

FIG. 54

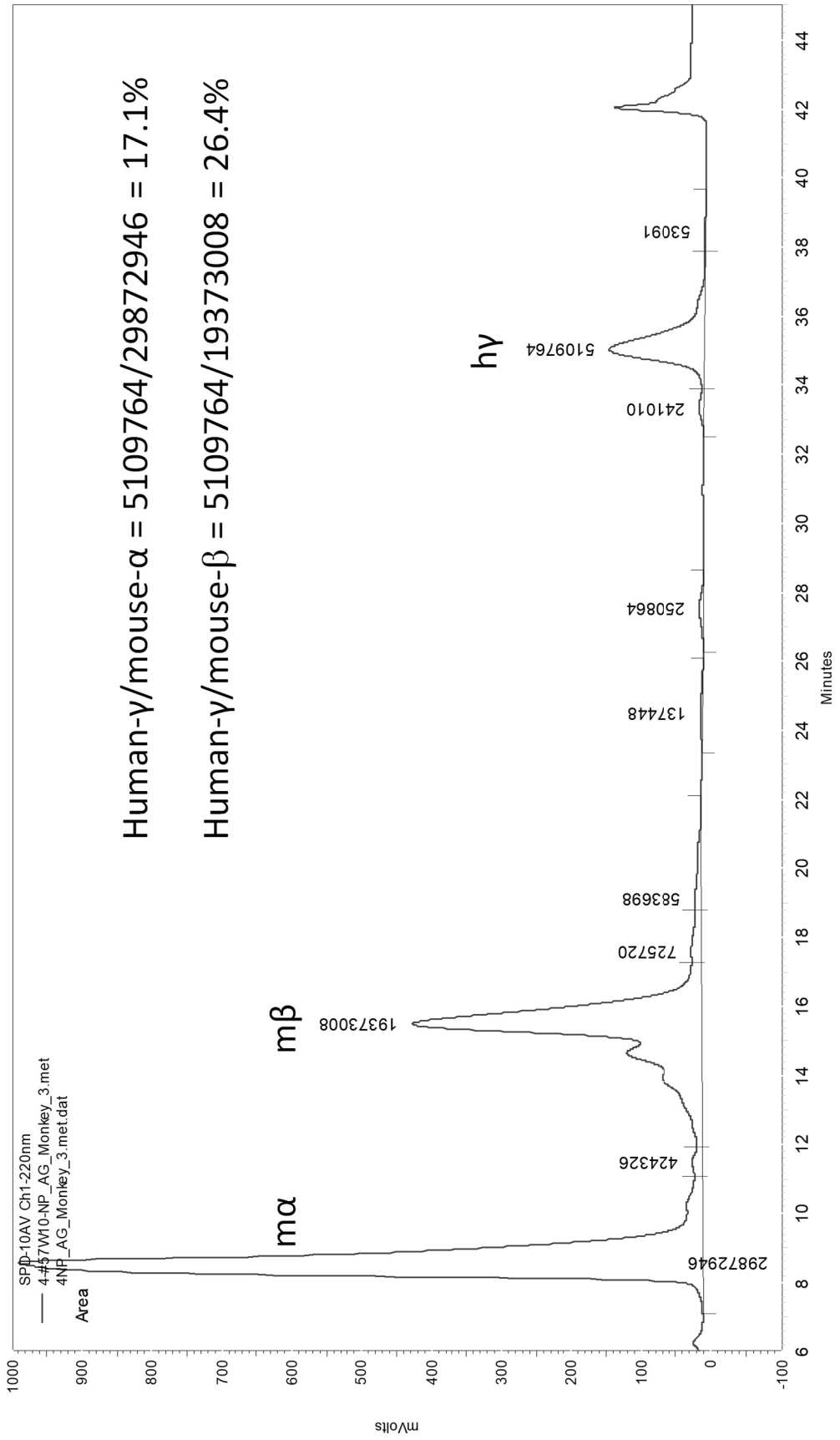

Human-γ/mouse-α = 5109764/29872946 = 17.1%
Human-γ/mouse-β = 5109764/19373008 = 26.4%
mα
mβ
hγ
SPD-10AV Ch1-220nm
4457W10-NP_AG_Monkey_3.met
4NP_AG_Monkey_3.met.dat
Area
29872946
424326
19373008
725720
583698
137448
250864
5109764
241010
53091
mVolts
Minutes

FIG. 55A



FIG. 55B

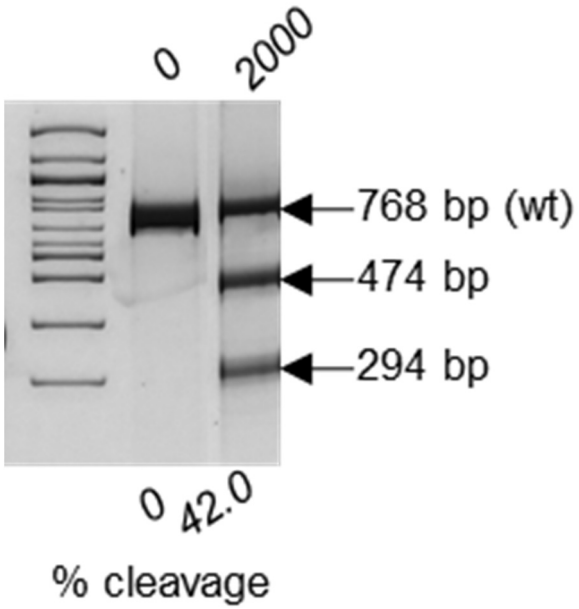


FIG. 55C



(SEQ ID NOs: 6-18)

FIG. 55D

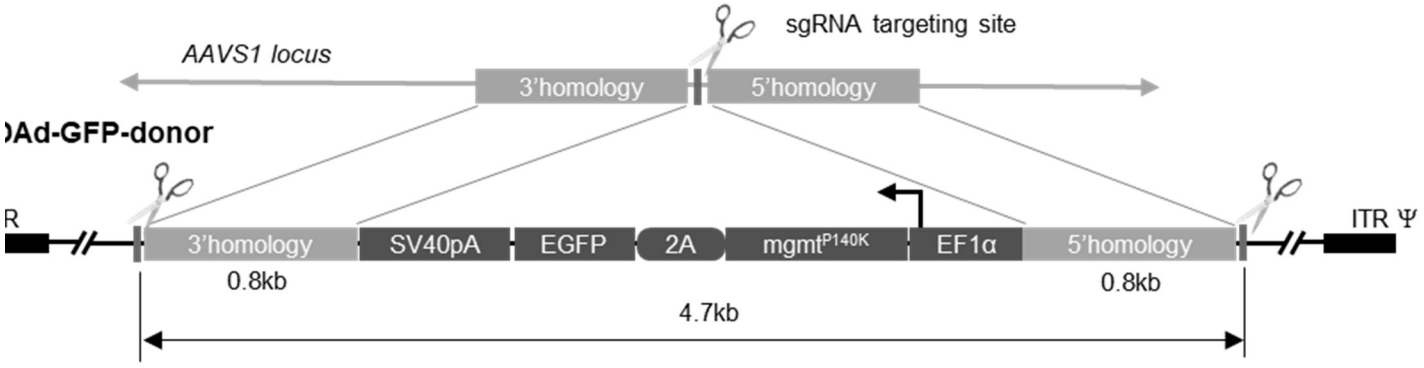


FIG. 55E

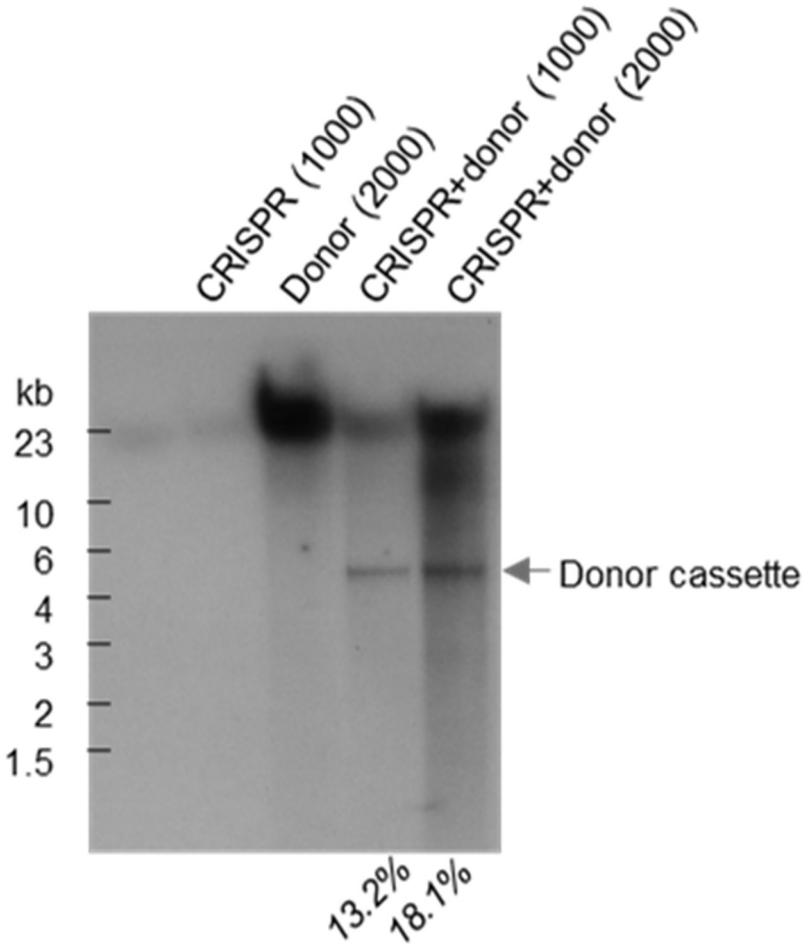


FIG. 56A

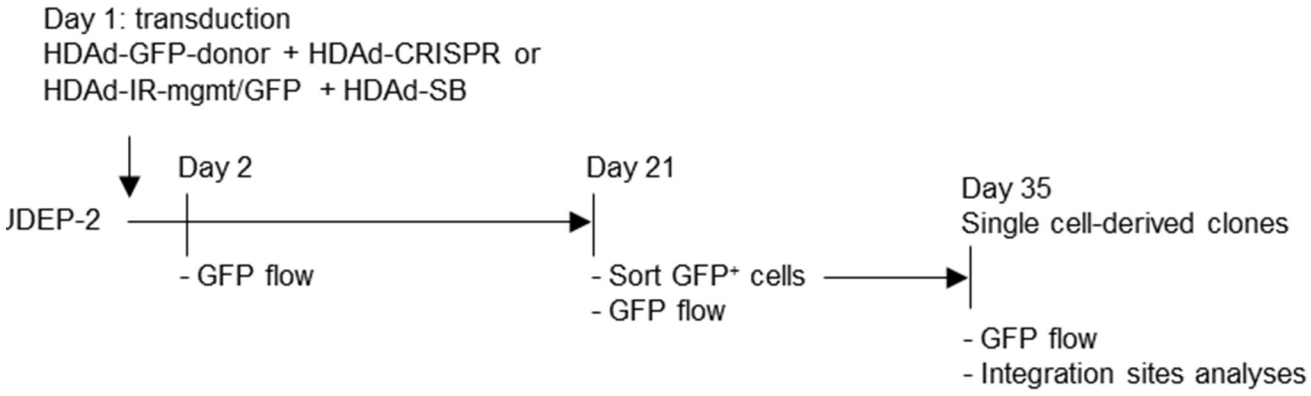


FIG. 56B

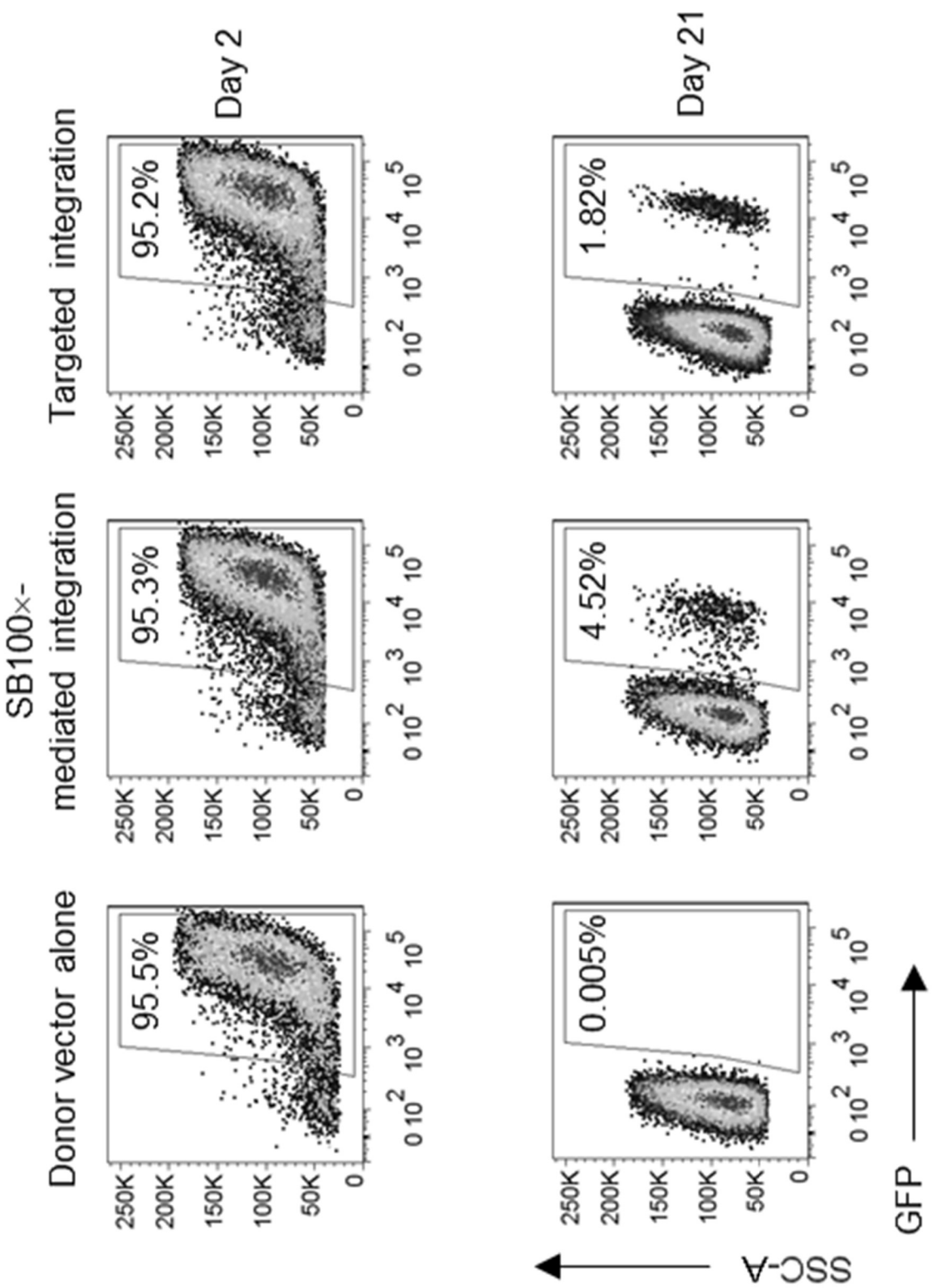

Donor vector alone
SB100×-mediated integration
Targeted integration
Day 2
Day 21
95.5%
95.3%
95.2%
0.005%
4.52%
1.82%
250K
200K
150K
100K
50K
0
0 10^2 10^3 10^4 10^5
SSC-A
GFP

FIG. 56C

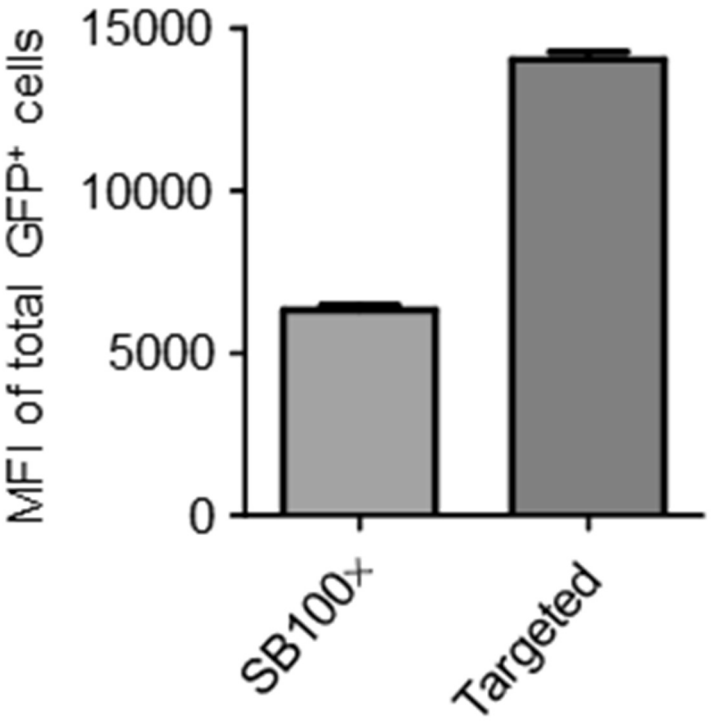


FIG. 56D

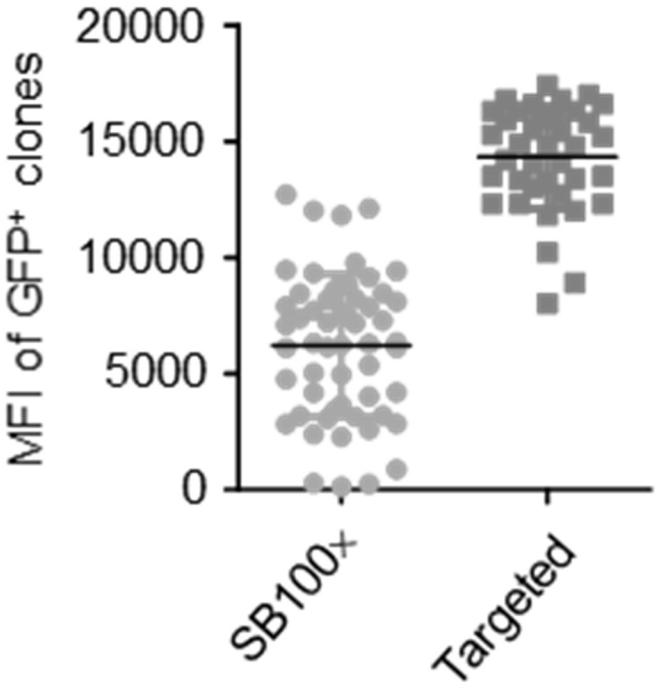


FIG. 56E

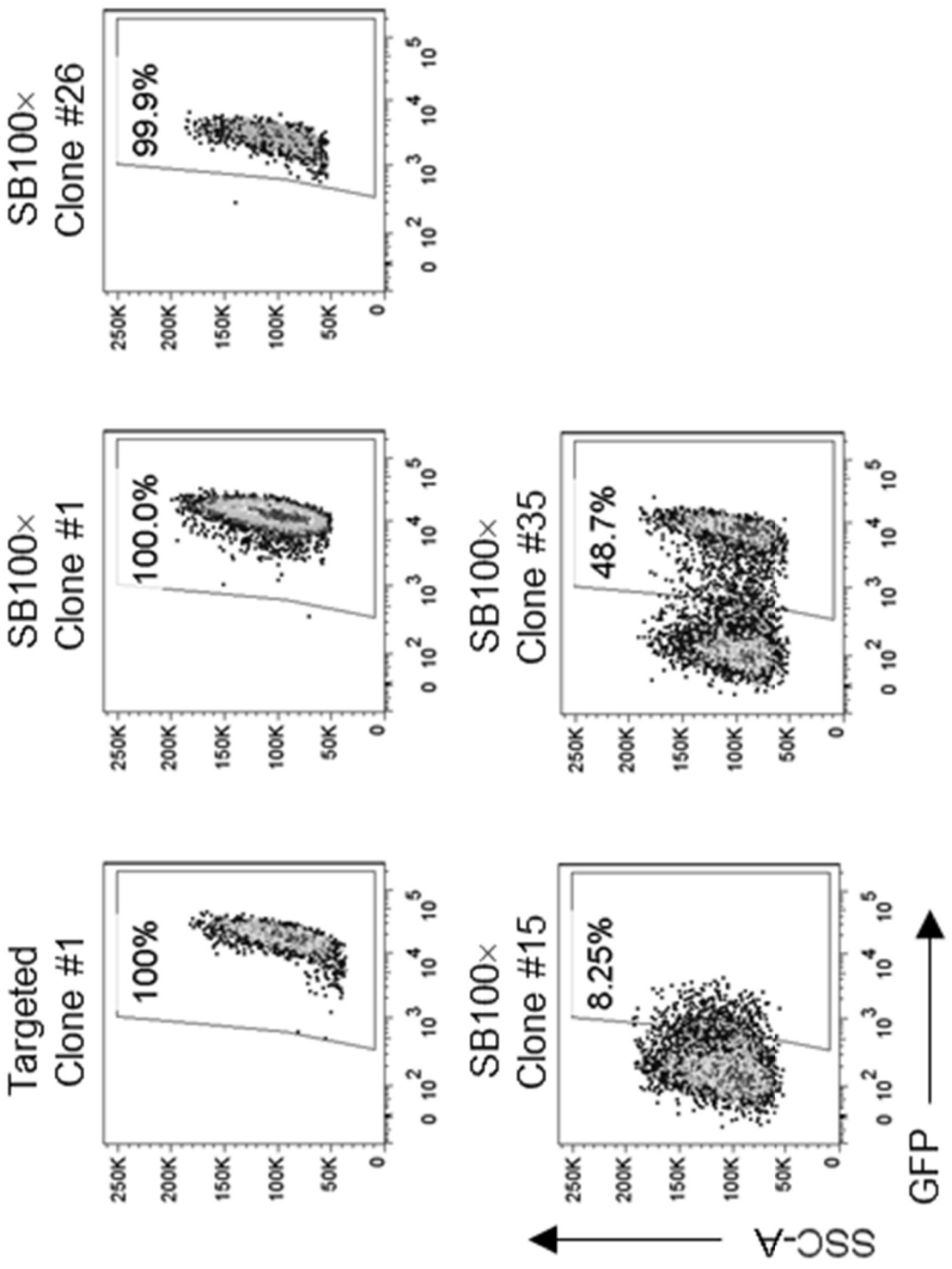

Targeted
Clone #1
100%
SB100×
Clone #1
100.0%
SB100×
Clone #26
99.9%
SB100×
Clone #15
8.25%
SB100×
Clone #35
48.7%
SSC-A
GFP
250K
200K
150K
100K
50K
0
0 10^2 10^3 10^4 10^5

FIG. 56F

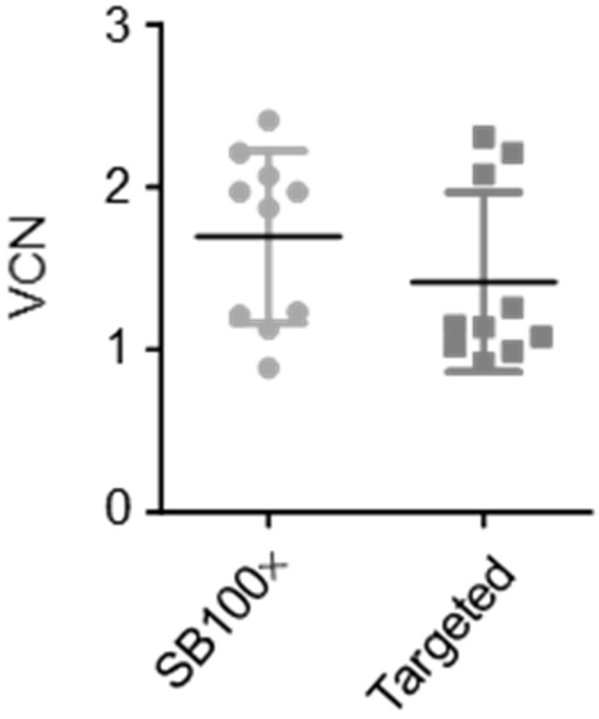


FIG. 57A

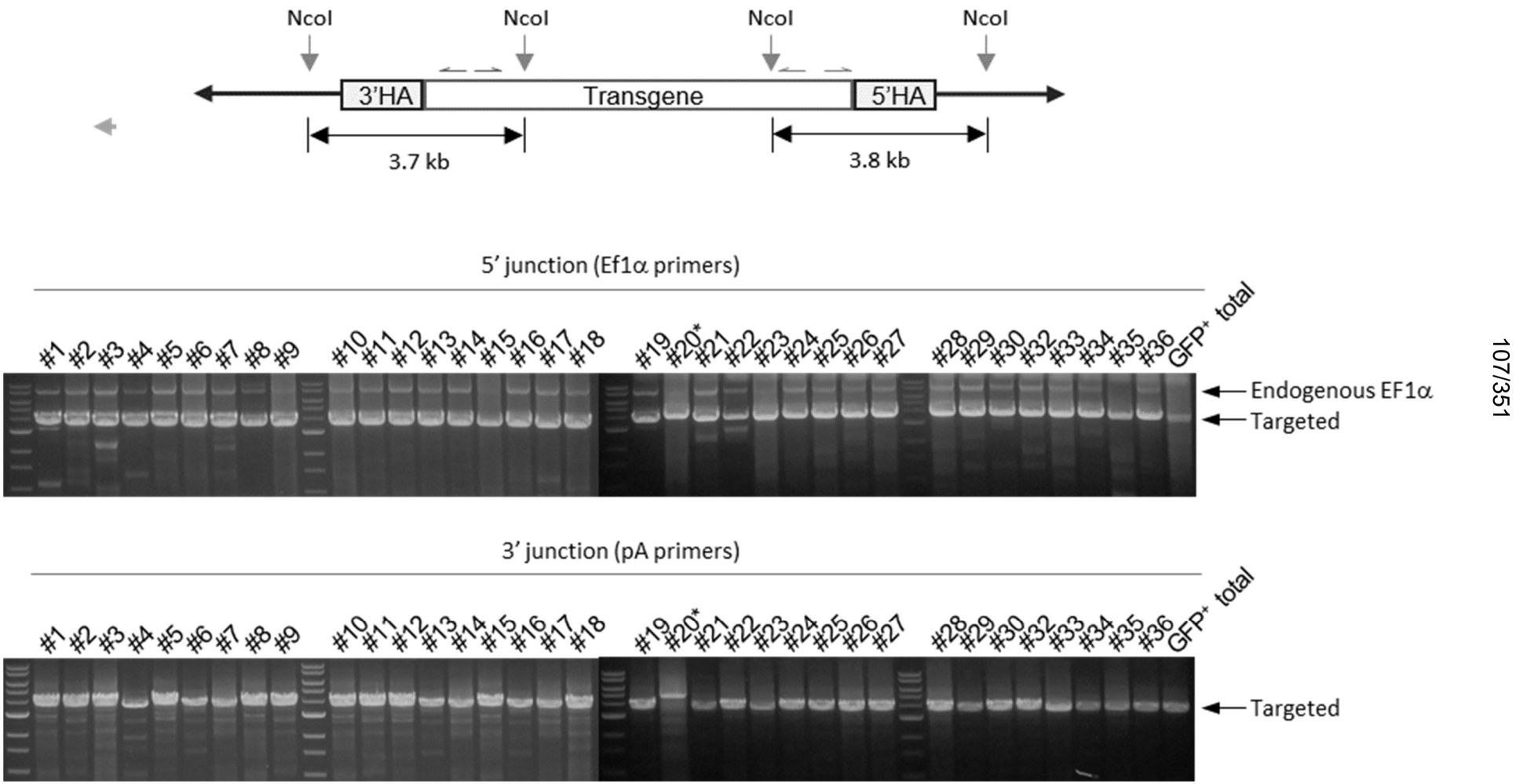

NcoI
NcoI
NcoI
NcoI
3'HA
Transgene
5'HA
3.7 kb
3.8 kb
5' junction (Ef1α primers)
#1 #2 #3 #4 #5 #6 #7 #8 #9 #10 #11 #12 #13 #14 #15 #16 #17 #18 #19 #20* #21 #22 #23 #24 #25 #26 #27 #28 #29 #30 #32 #33 #34 #35 #36 GFP+ total
Endogenous EF1α
Targeted
3' junction (pA primers)
#1 #2 #3 #4 #5 #6 #7 #8 #9 #10 #11 #12 #13 #14 #15 #16 #17 #18 #19 #20* #21 #22 #23 #24 #25 #26 #27 #28 #29 #30 #32 #33 #34 #35 #36 GFP+ total
Targeted

FIG. 57B

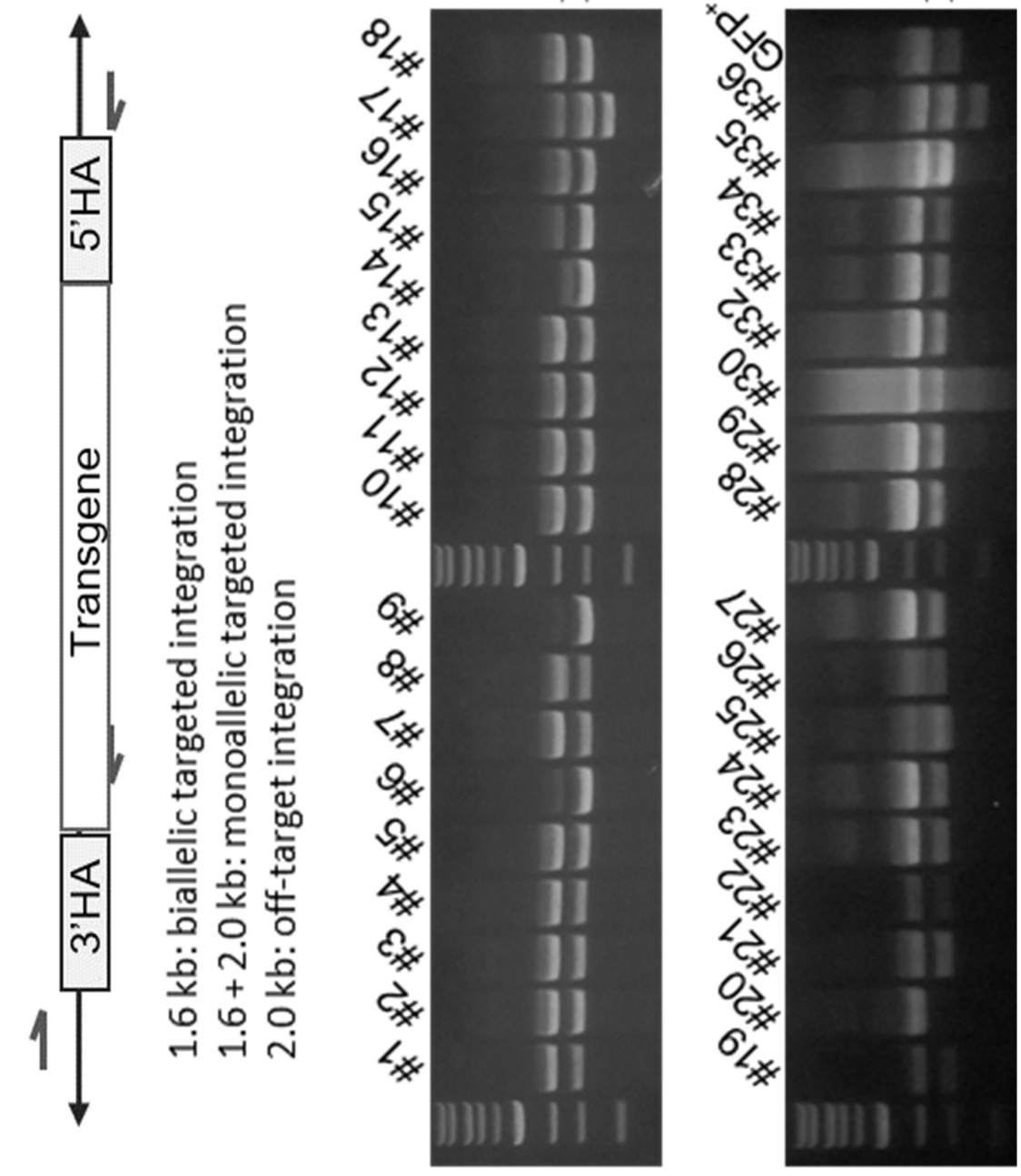

3'HA
Transgene
5'HA
1.6 kb: biallelic targeted integration
1.6 + 2.0 kb: monoallelic targeted integration
2.0 kb: off-target integration
#1 #2 #3 #4 #5 #6 #7 #8 #9 #10 #11 #12 #13 #14 #15 #16 #17 #18
#19 #20 #21 #22 #23 #24 #25 #26 #27 #28 #29 #30 #32 #33 #34 #35 #36 GFP+ total
2.0 kb
1.6 kb
2.0 kb
1.6 kb

FIG. 58A

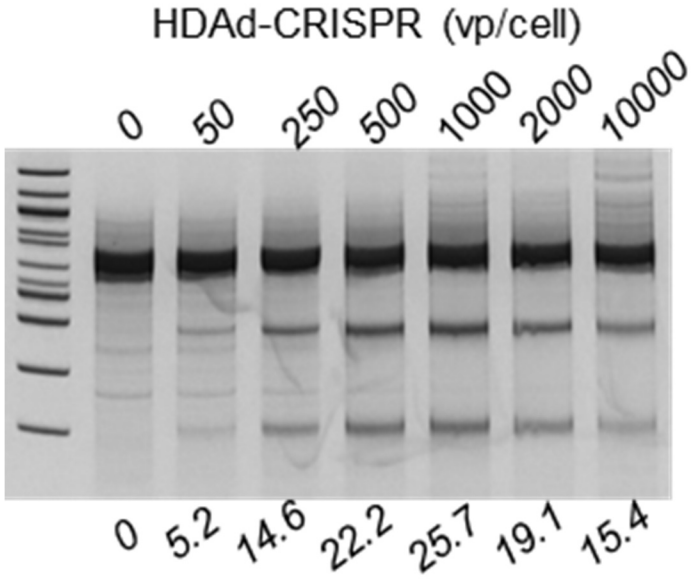


FIG. 58B

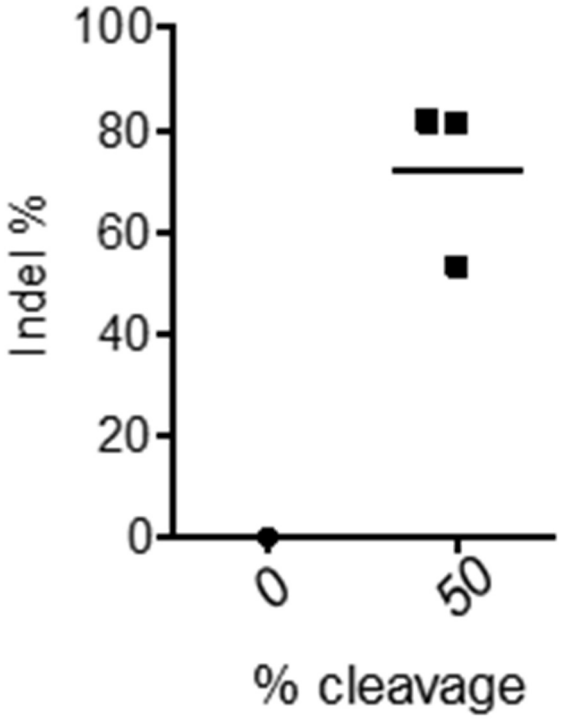
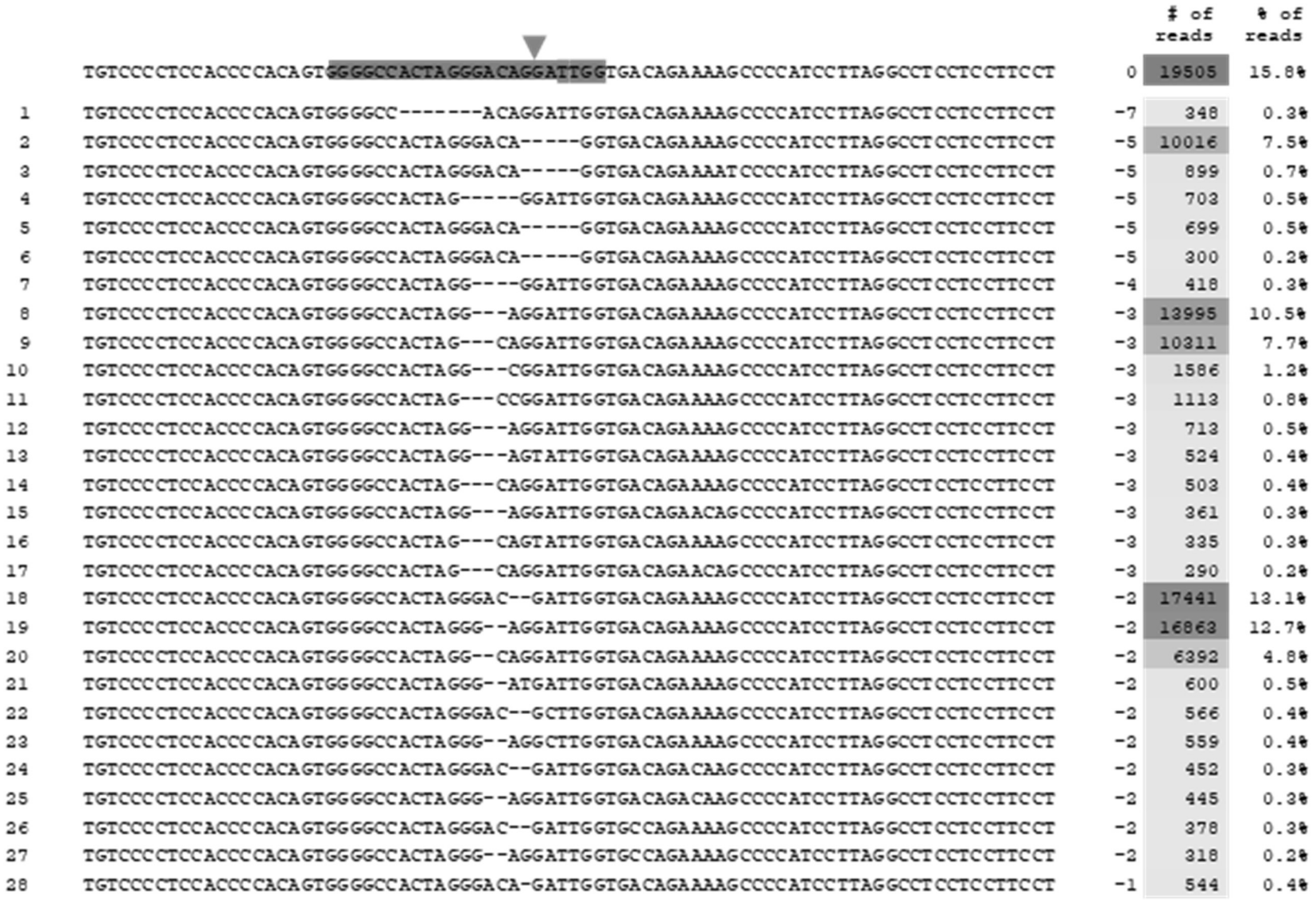


FIG. 58C



			# of reads	% of reads
	TGTCCCCTCCACCCCACAGTGGGGCCACTAGGGACAGGATTGGTGACAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCCT	0	19505	15.8%
1	TGTCCCCTCCACCCCACAGTGGGGCC-------ACAGGATTGGTGACAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCCT	-7	348	0.3%
2	TGTCCCCTCCACCCCACAGTGGGGCCACTAGGGACA-----GGTGACAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCCT	-5	10016	7.5%
3	TGTCCCCTCCACCCCACAGTGGGGCCACTAGGGACA-----GGTGACAGAAAATCCCCATCCTTAGGCCTCCTCCTTCCT	-5	899	0.7%
4	TGTCCCCTCCACCCCACAGTGGGGCCACTAG-----GGATTGGTGACAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCCT	-5	703	0.5%
5	TGTCCCCTCCACCCCACAGTGGGGCCACTAGGGACA-----GGTGACAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCCT	-5	699	0.5%
6	TGTCCCCTCCACCCCACAGTGGGGCCACTAGGGACA-----GGTGACAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCCT	-5	300	0.2%
7	TGTCCCCTCCACCCCACAGTGGGGCCACTAGG----GGATTGGTGACAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCCT	-4	418	0.3%
8	TGTCCCCTCCACCCCACAGTGGGGCCACTAGG---AGGATTGGTGACAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCCT	-3	13995	10.5%
9	TGTCCCCTCCACCCCACAGTGGGGCCACTAG---CAGGATTGGTGACAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCCT	-3	10311	7.7%
10	TGTCCCCTCCACCCCACAGTGGGGCCACTAGG---CGGATTGGTGACAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCCT	-3	1586	1.2%
11	TGTCCCCTCCACCCCACAGTGGGGCCACTAG---CCGGATTGGTGACAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCCT	-3	1113	0.8%
12	TGTCCCCTCCACCCCACAGTGGGGCCACTAGG---AGGATTGGTGACAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCCT	-3	713	0.5%
13	TGTCCCCTCCACCCCACAGTGGGGCCACTAGG---AGTATTGGTGACAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCCT	-3	524	0.4%
14	TGTCCCCTCCACCCCACAGTGGGGCCACTAG---CAGGATTGGTGACAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCCT	-3	503	0.4%
15	TGTCCCCTCCACCCCACAGTGGGGCCACTAGG---AGGATTGGTGACAGAACAGCCCCATCCTTAGGCCTCCTCCTTCCT	-3	361	0.3%
16	TGTCCCCTCCACCCCACAGTGGGGCCACTAG---CAGTATTGGTGACAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCCT	-3	335	0.3%
17	TGTCCCCTCCACCCCACAGTGGGGCCACTAG---CAGGATTGGTGACAGAACAGCCCCATCCTTAGGCCTCCTCCTTCCT	-3	290	0.2%
18	TGTCCCCTCCACCCCACAGTGGGGCCACTAGGGAC--GATTGGTGACAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCCT	-2	17441	13.1%
19	TGTCCCCTCCACCCCACAGTGGGGCCACTAGGG--AGGATTGGTGACAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCCT	-2	16863	12.7%
20	TGTCCCCTCCACCCCACAGTGGGGCCACTAGG--CAGGATTGGTGACAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCCT	-2	6392	4.8%
21	TGTCCCCTCCACCCCACAGTGGGGCCACTAGGG--ATGATTGGTGACAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCCT	-2	600	0.5%
22	TGTCCCCTCCACCCCACAGTGGGGCCACTAGGGAC--GCTTGGTGACAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCCT	-2	566	0.4%
23	TGTCCCCTCCACCCCACAGTGGGGCCACTAGGG--AGGCTTGGTGACAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCCT	-2	559	0.4%
24	TGTCCCCTCCACCCCACAGTGGGGCCACTAGGGAC--GATTGGTGACAGACAAGCCCCATCCTTAGGCCTCCTCCTTCCT	-2	452	0.3%
25	TGTCCCCTCCACCCCACAGTGGGGCCACTAGGG--AGGATTGGTGACAGACAAGCCCCATCCTTAGGCCTCCTCCTTCCT	-2	445	0.3%
26	TGTCCCCTCCACCCCACAGTGGGGCCACTAGGGAC--GATTGGTGCCAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCCT	-2	378	0.3%
27	TGTCCCCTCCACCCCACAGTGGGGCCACTAGGG--AGGATTGGTGCCAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCCT	-2	318	0.2%
28	TGTCCCCTCCACCCCACAGTGGGGCCACTAGGGACA-GATTGGTGACAGAAAAGCCCCATCCTTAGGCCTCCTCCTTCCT	-1	544	0.4%

(SEQ ID NOs: 19-23, 21, 21, 26-30, 27, 32, 28, 34-47)

FIG. 59A

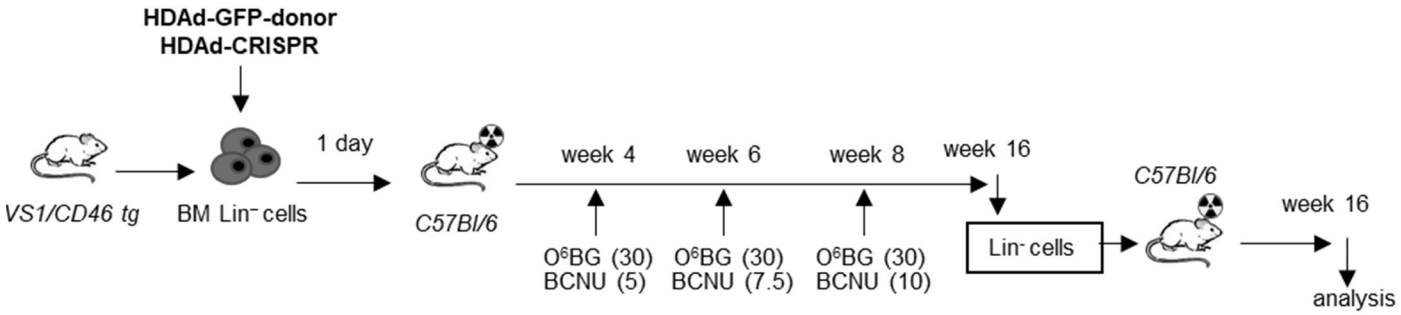


FIG. 59B

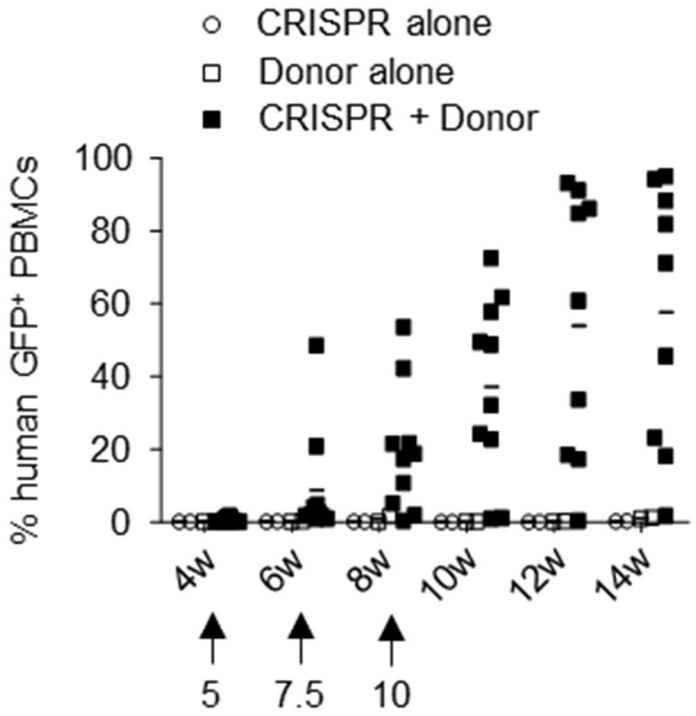


FIG. 59C

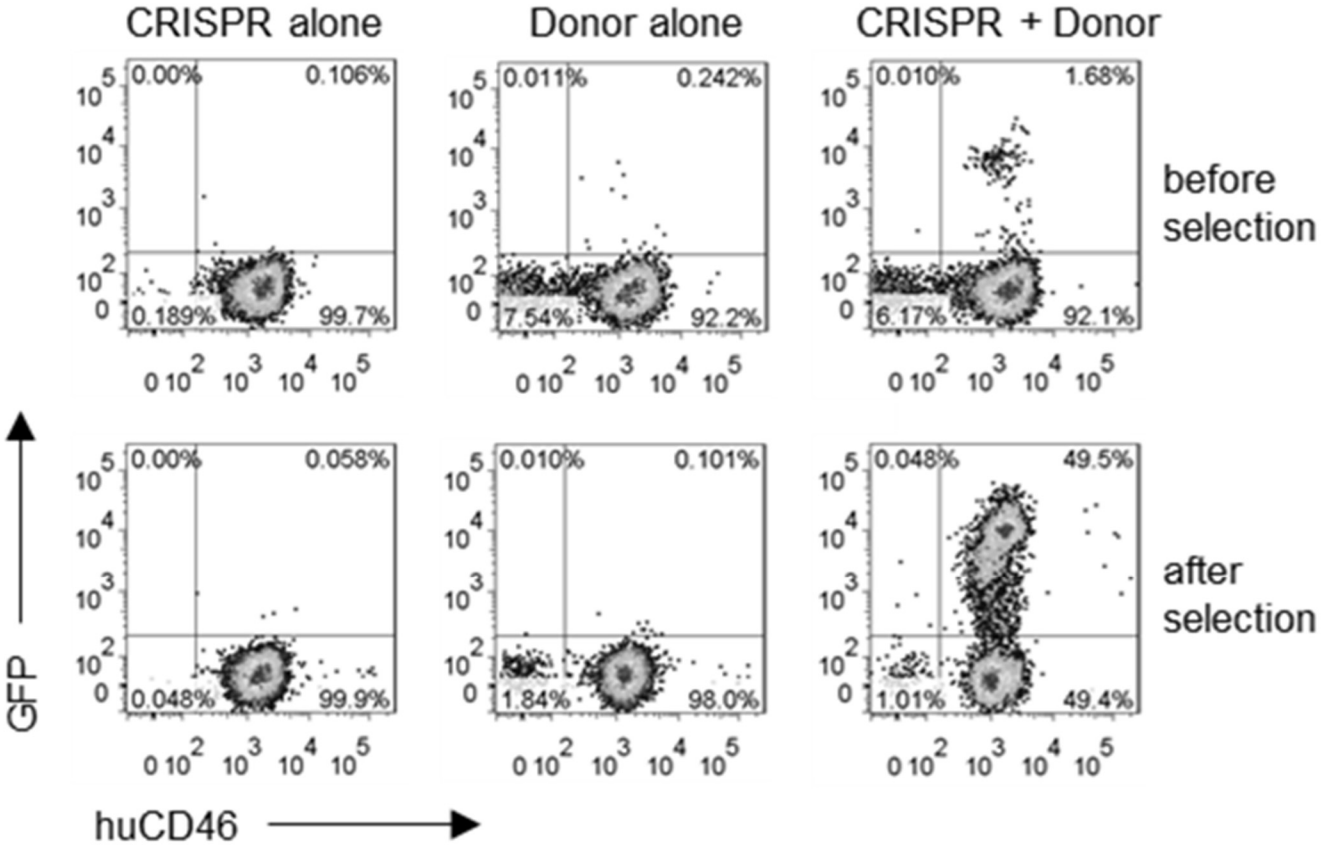


FIG. 59D

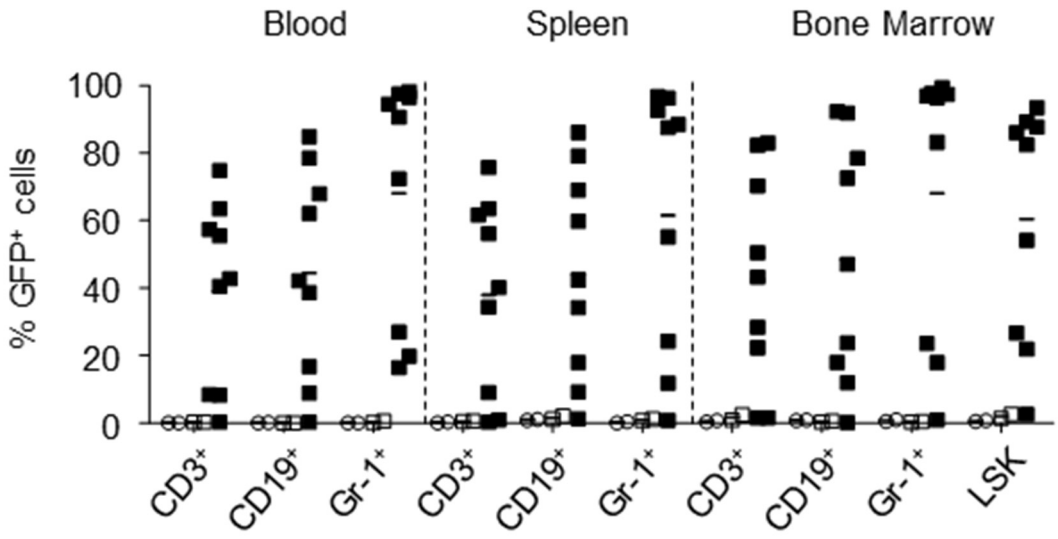


FIG. 60A

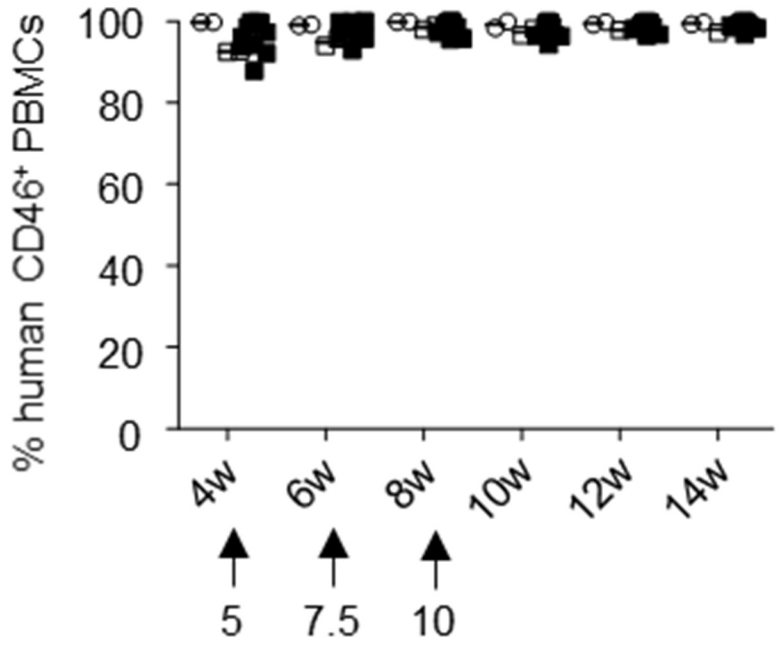


FIG. 60B

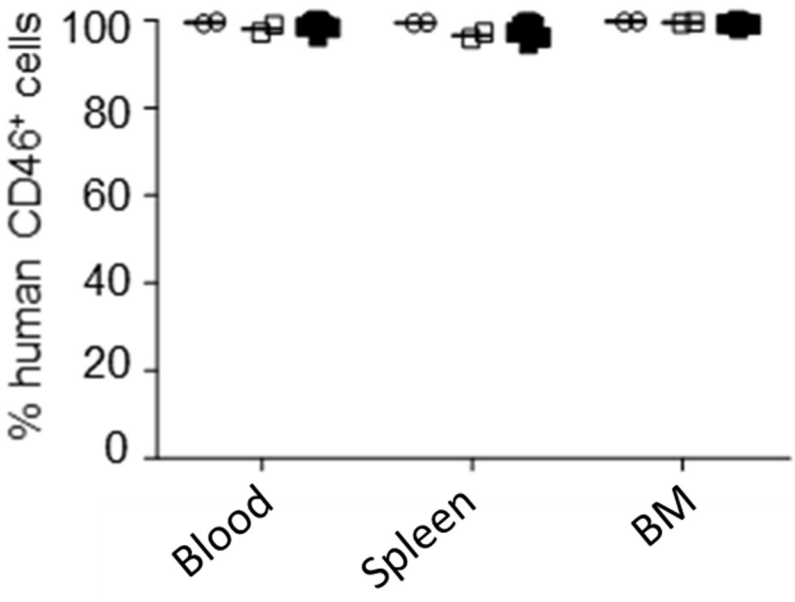


FIG. 60C

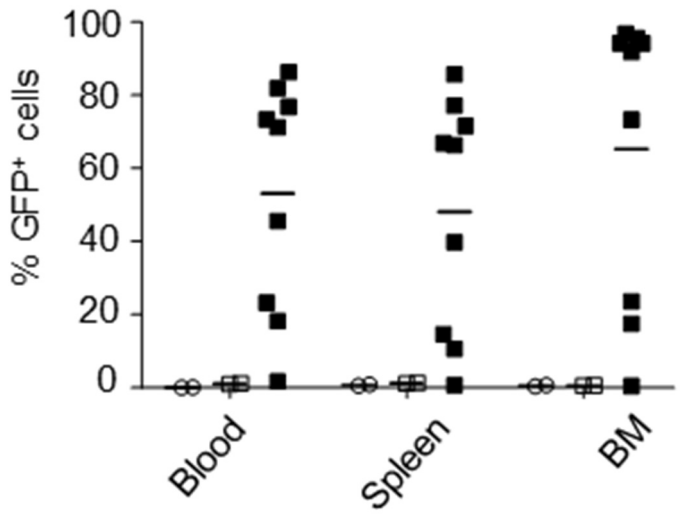


FIG. 60D

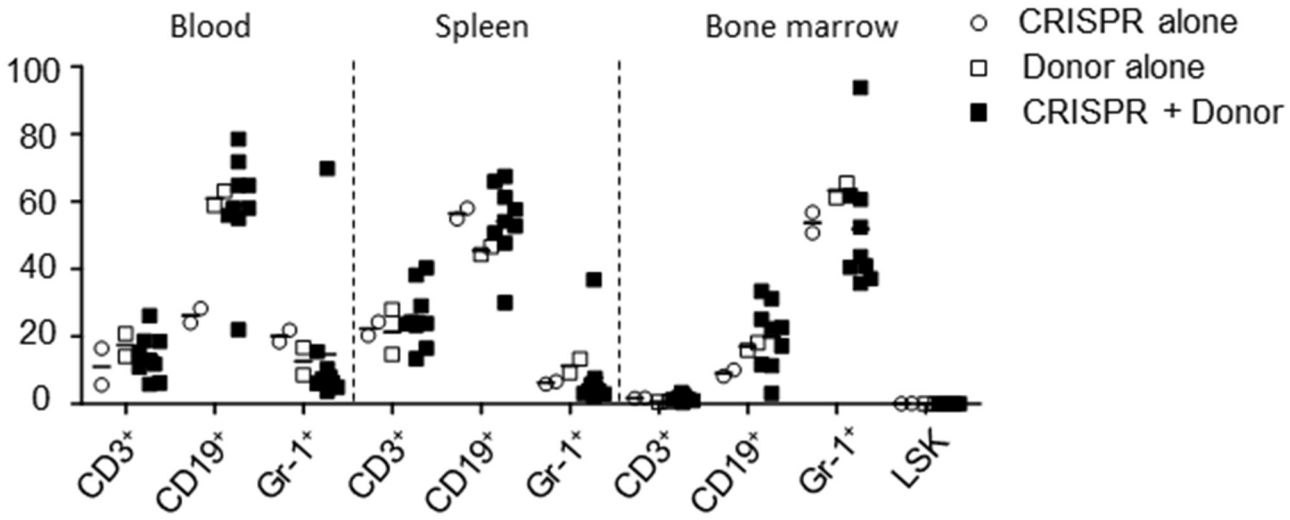


FIG. 60E

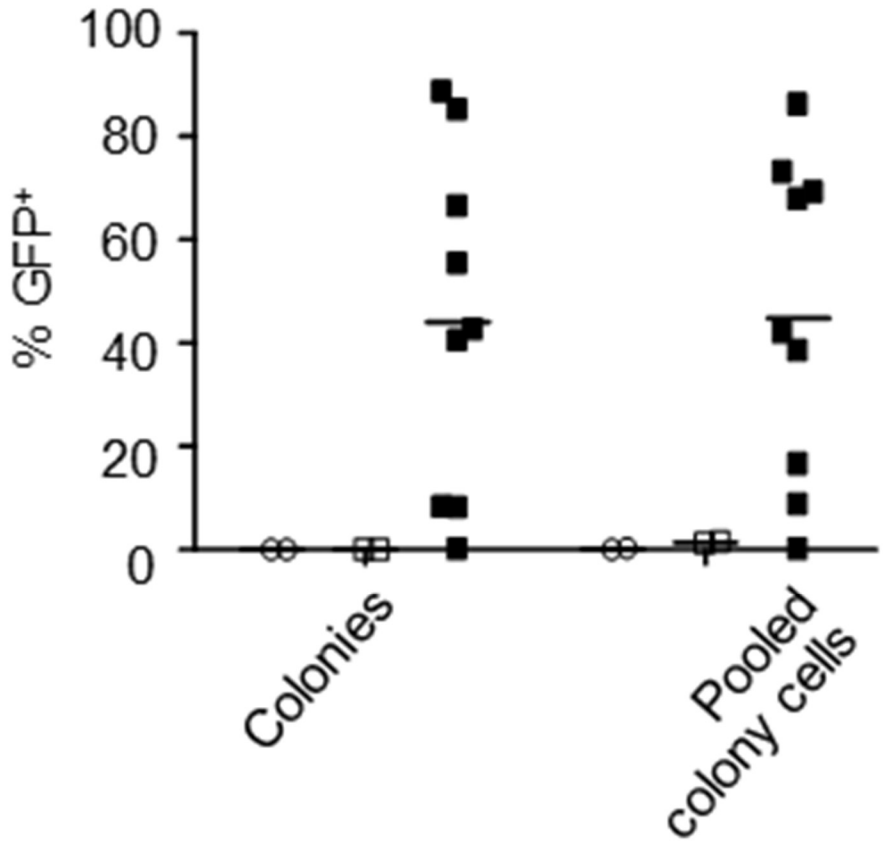


FIG. 61A

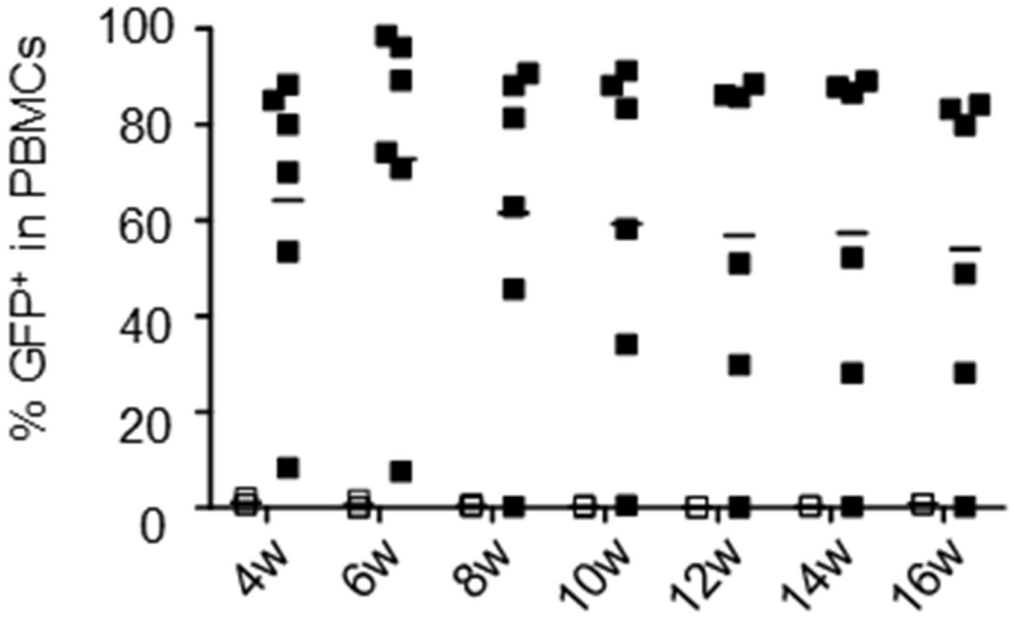


FIG. 61B

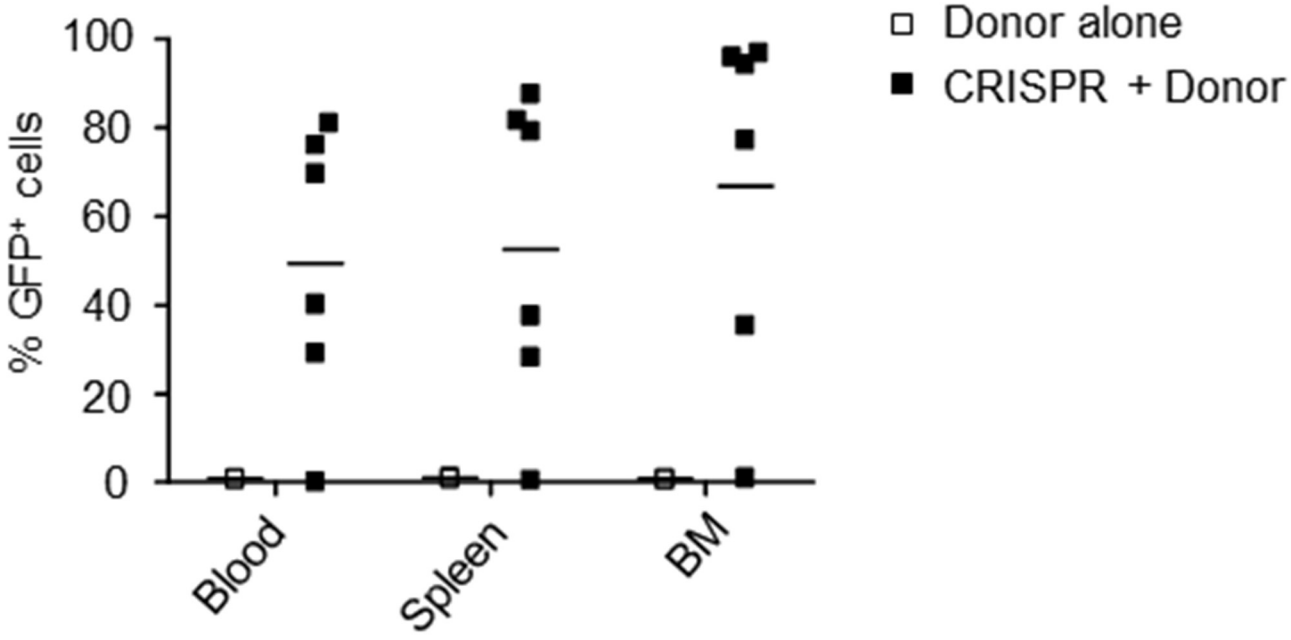


FIG. 61C

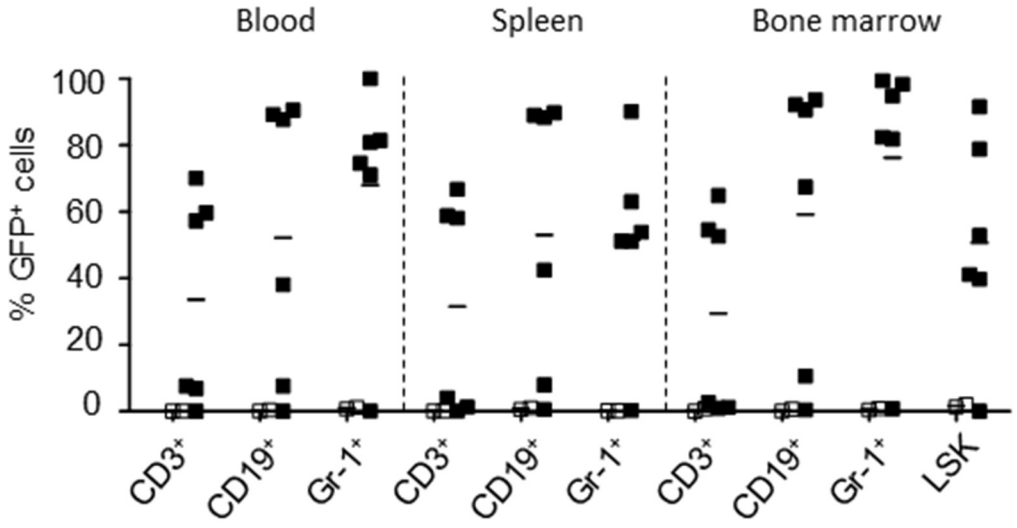


FIG. 61D

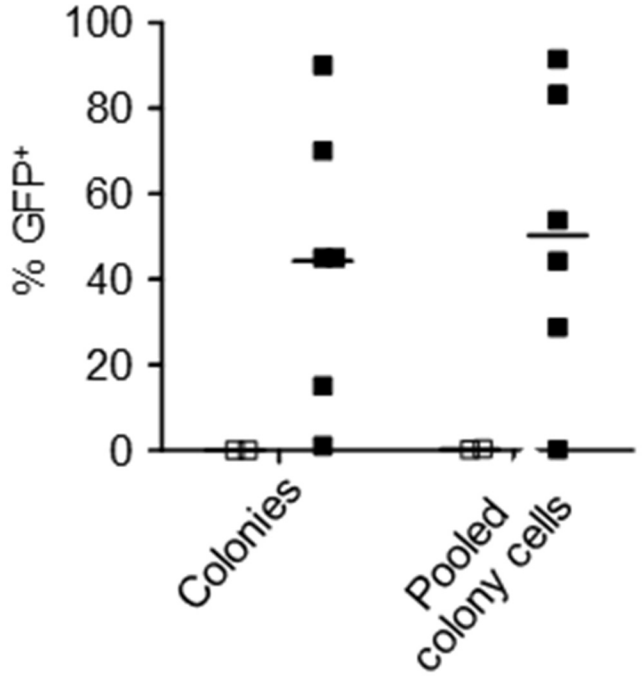


FIG. 61E

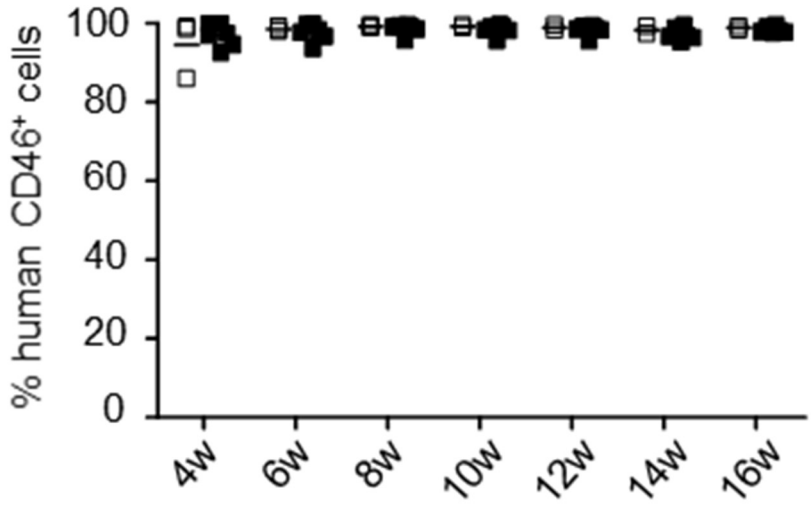


FIG. 61F

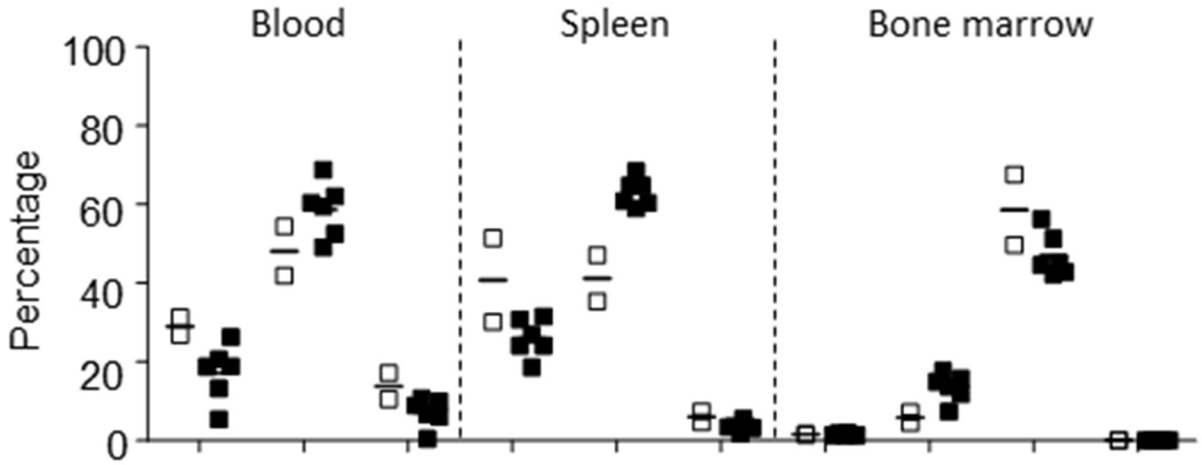


FIG. 62A

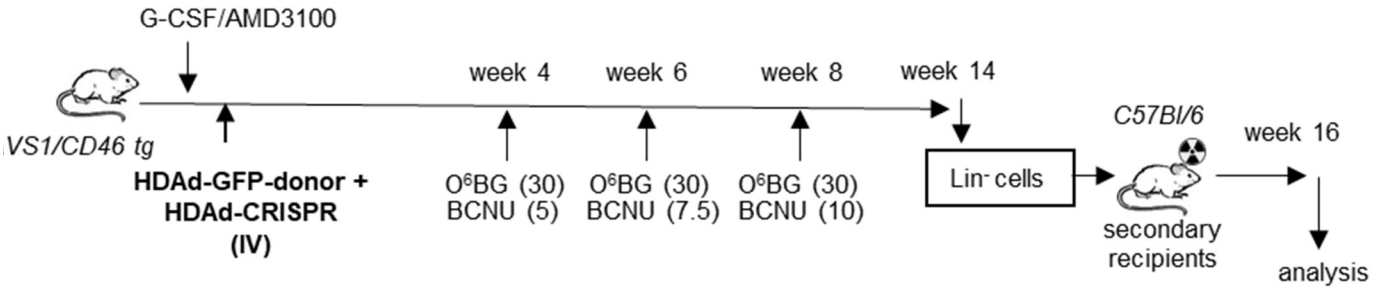


FIG. 62B

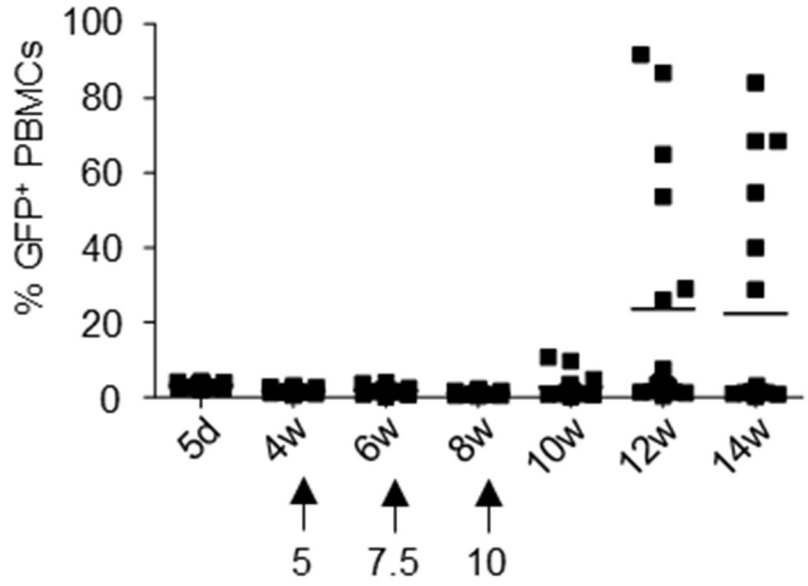


FIG. 62C

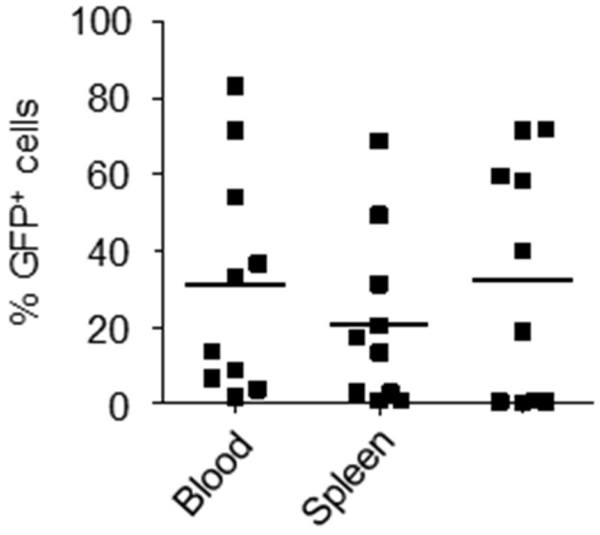


FIG. 62D

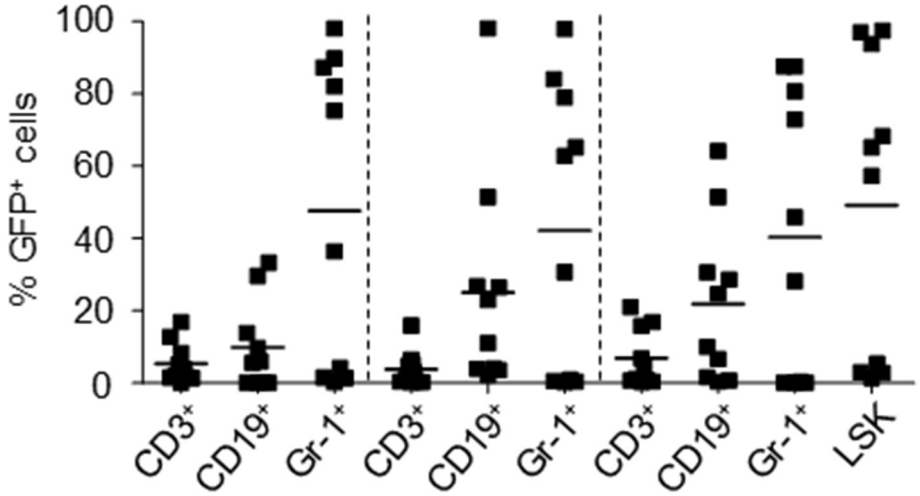


FIG. 62E

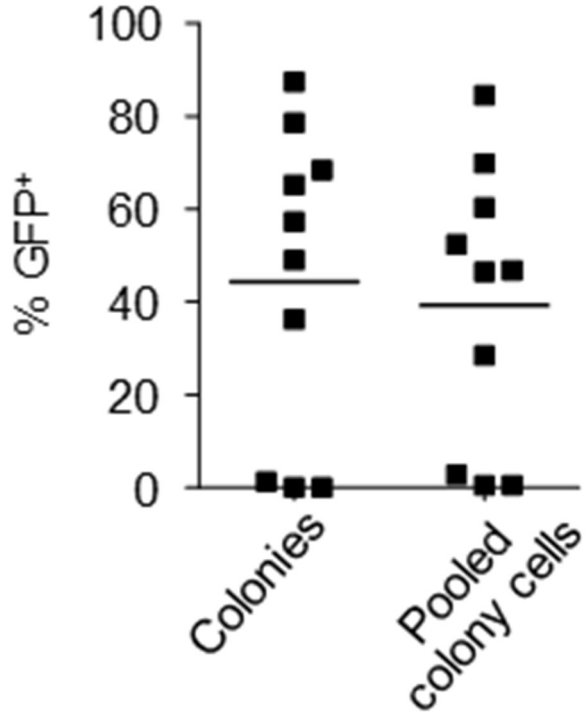


FIG. 62F

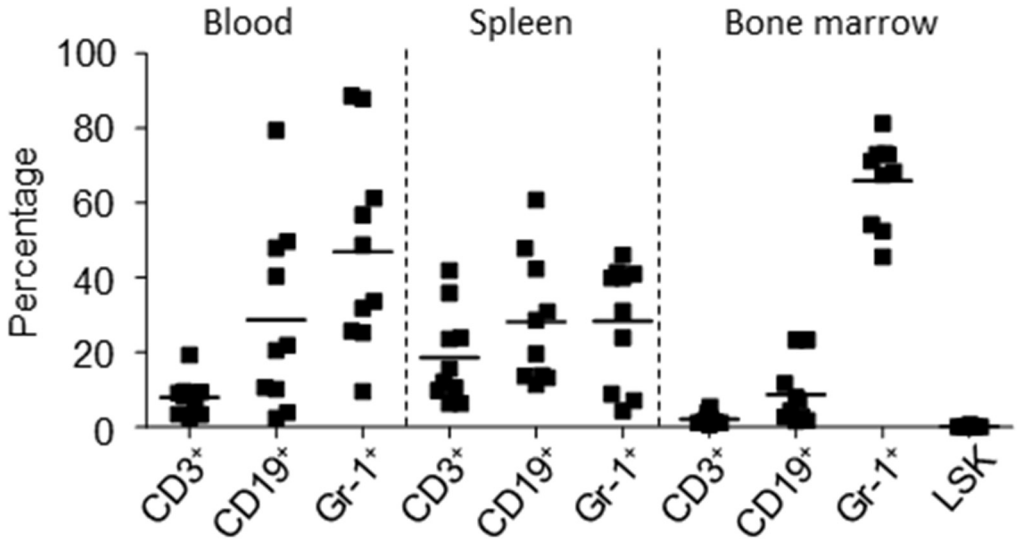


FIG. 63A

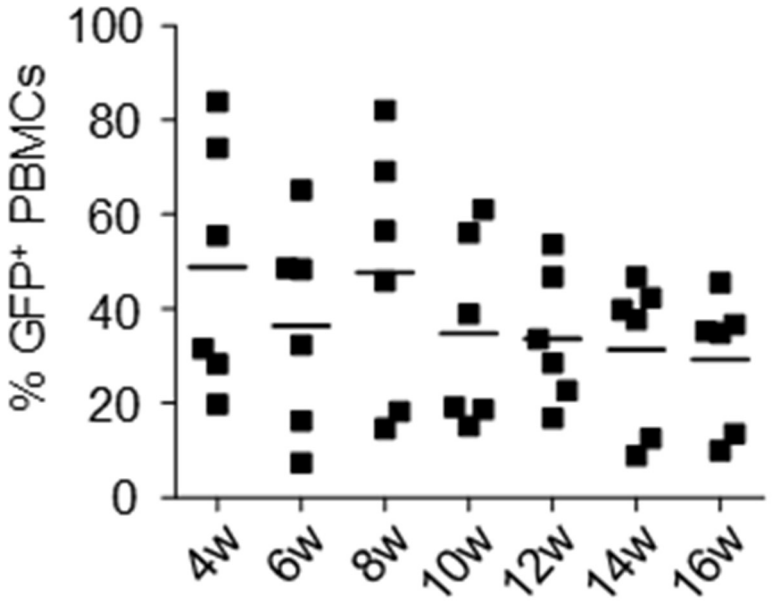


FIG. 63B

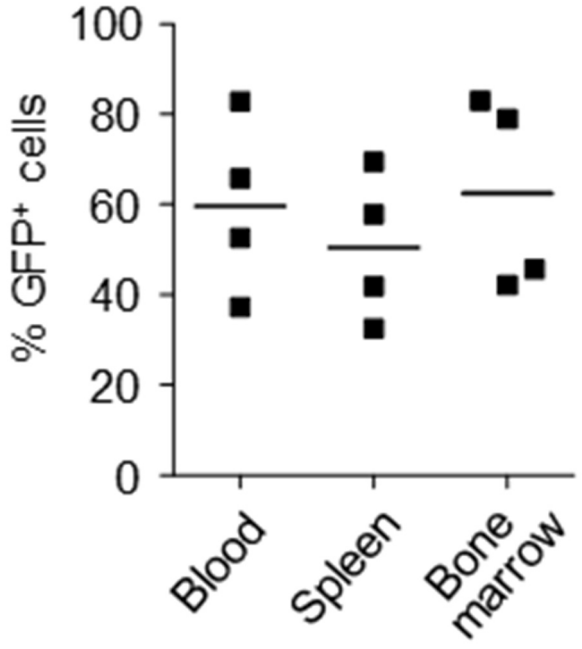


FIG. 63C

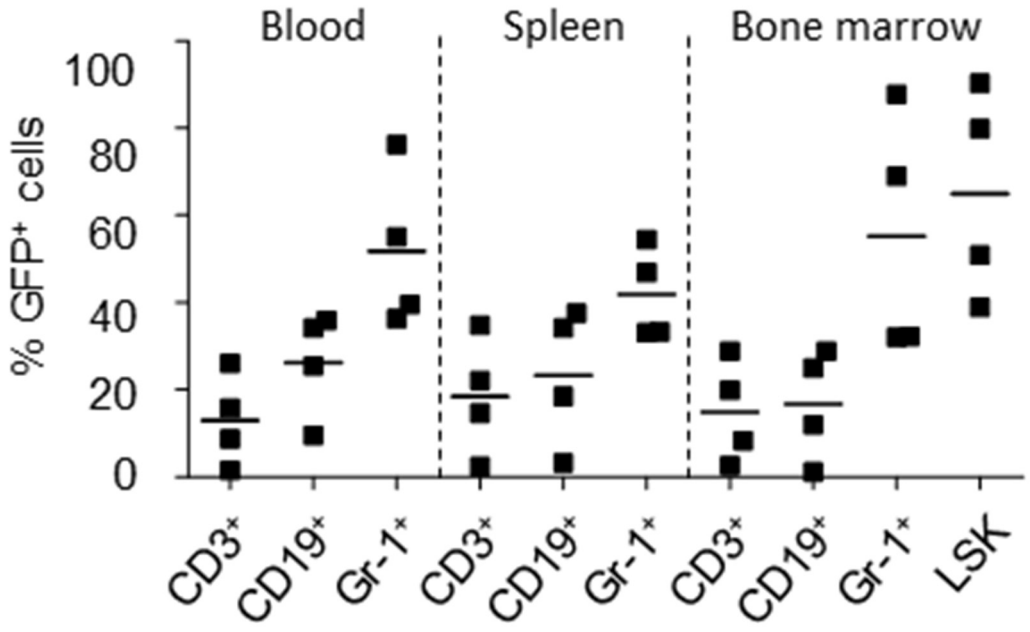


FIG. 63D

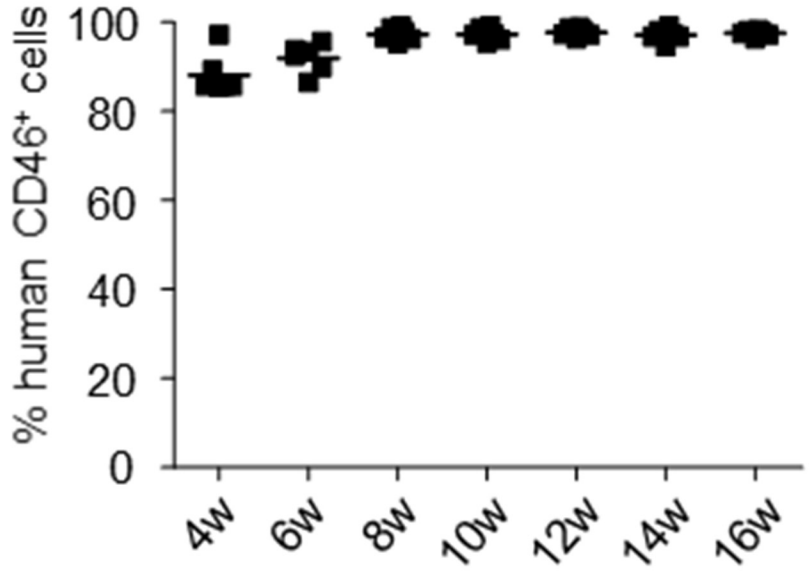


FIG. 63E

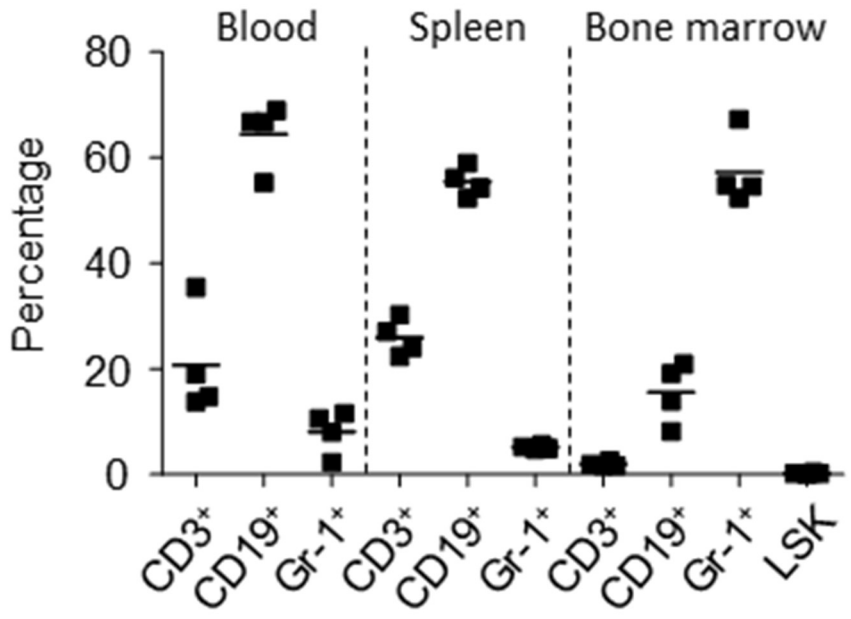


FIG. 64A

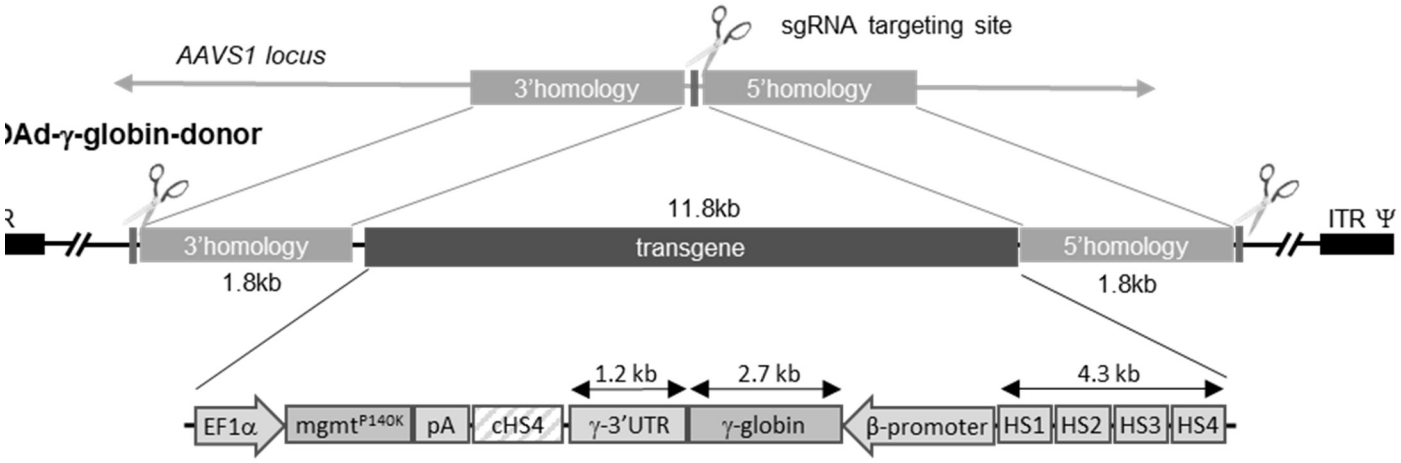


FIG. 64B

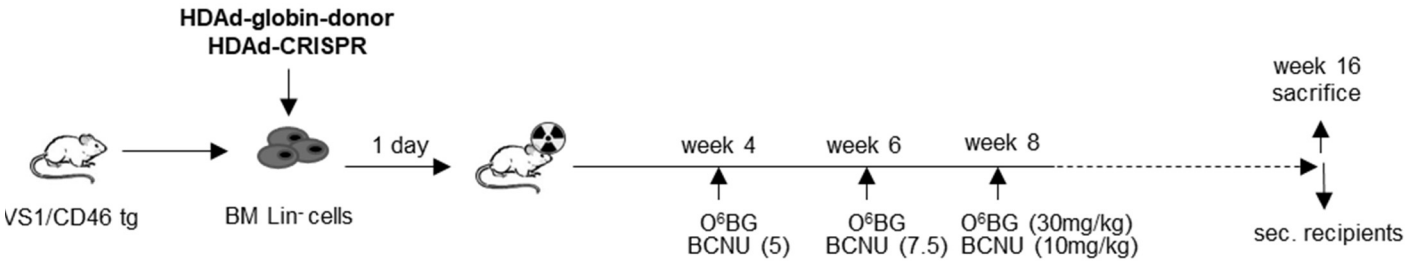


FIG. 64C

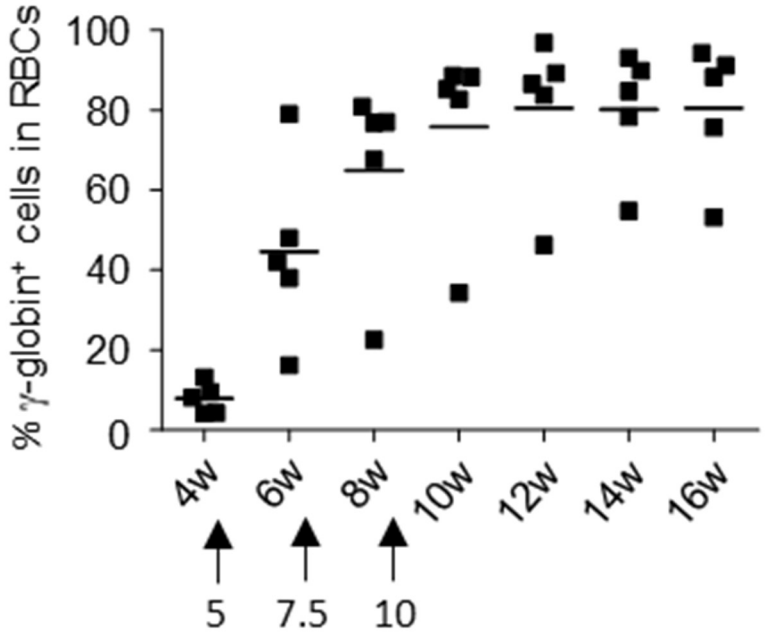


FIG. 64D

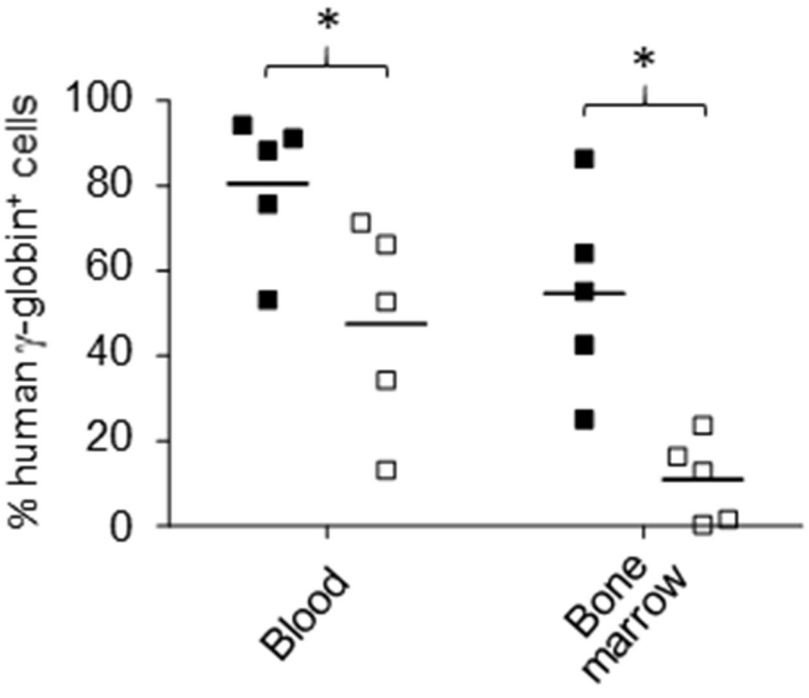


FIG. 64E

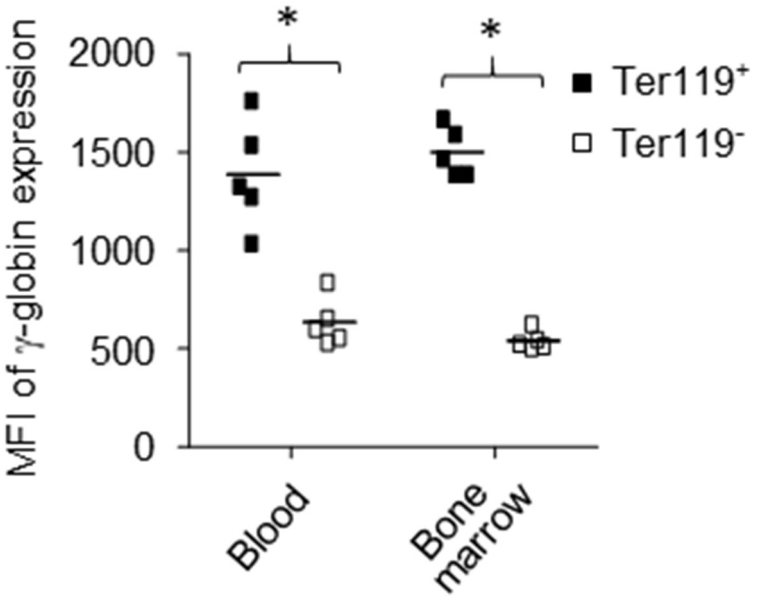


FIG. 64F

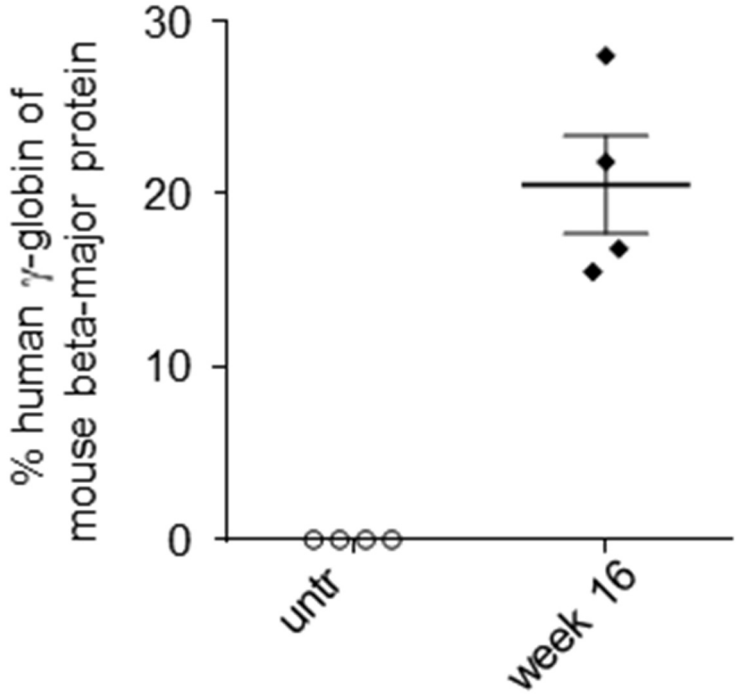


FIG. 64G

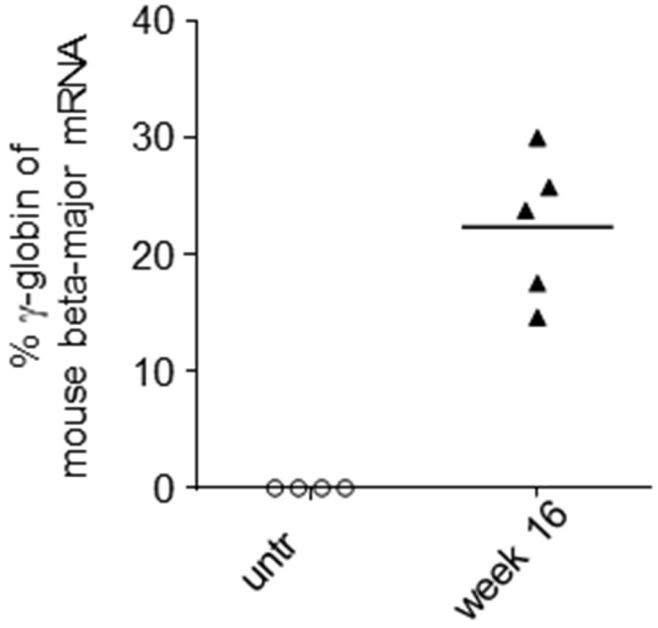


FIG. 64H

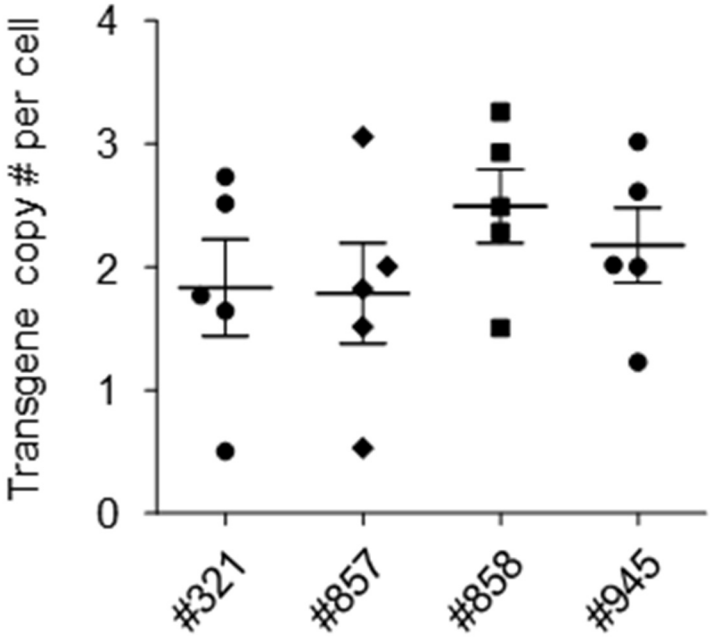


FIG. 65A

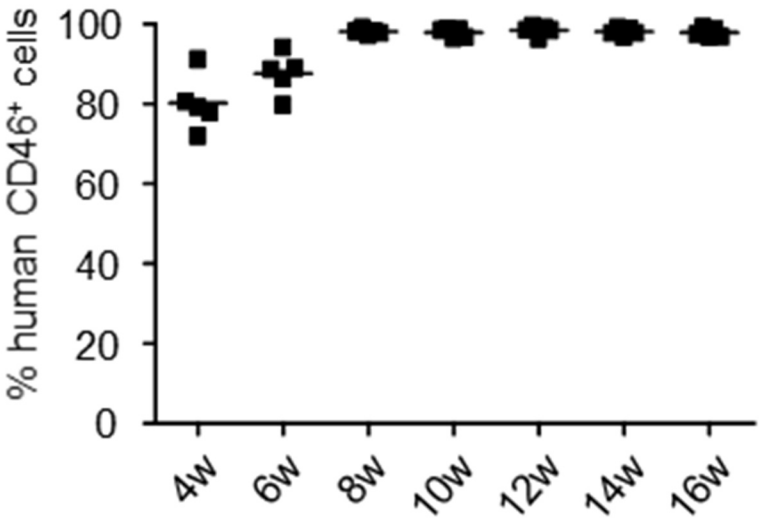


FIG. 65B

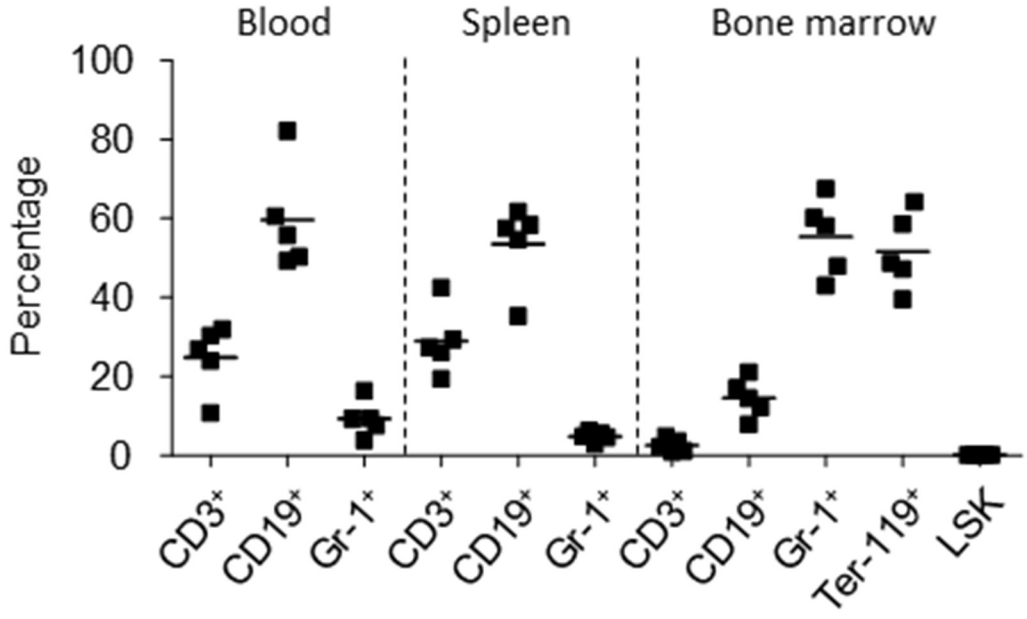


FIG. 66A

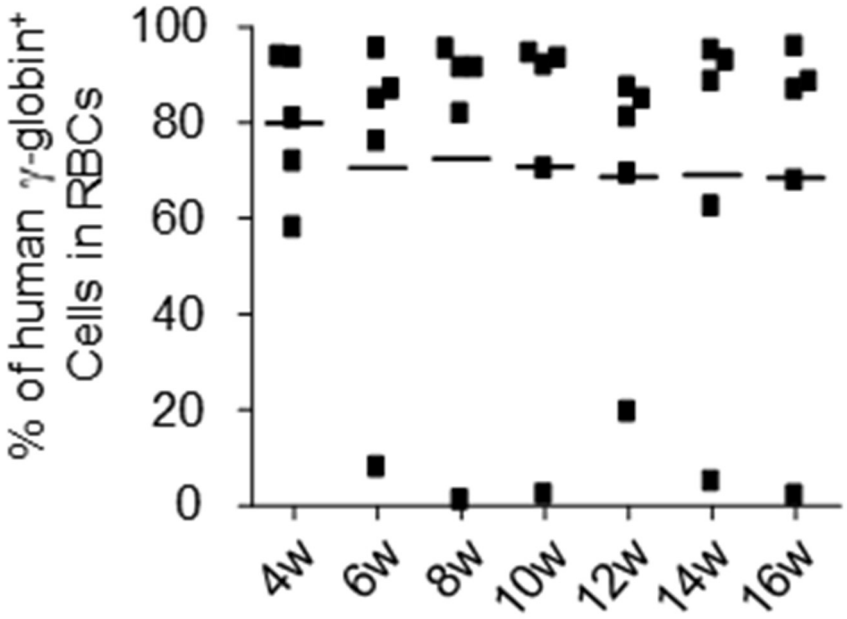


FIG. 66B

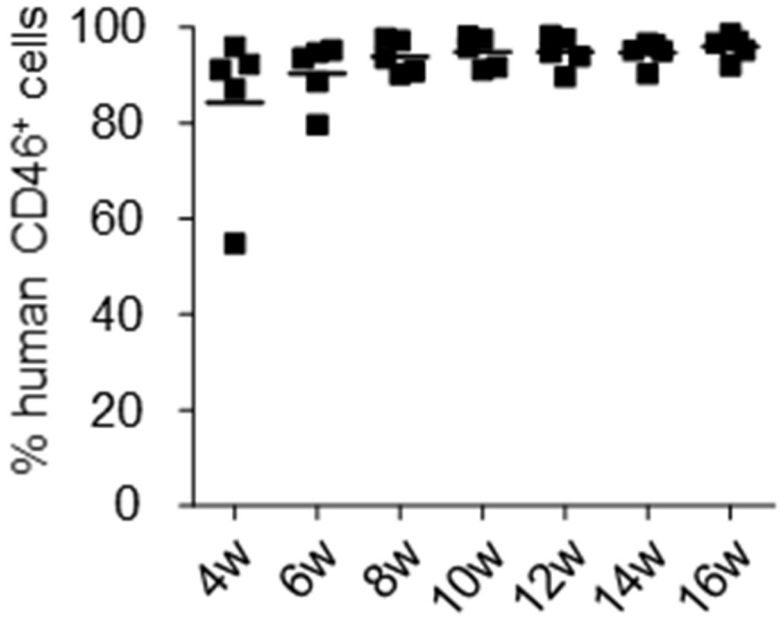


FIG. 66C

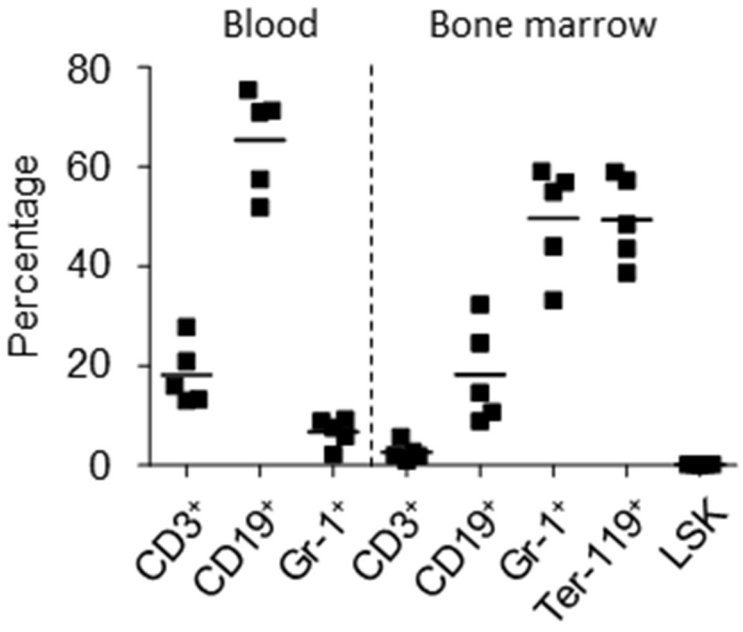

Blood
Bone marrow
Percentage
80
60
40
20
0
CD3+
CD19+
Gr-1+
CD3+
CD19+
Gr-1+
Ter-119+
LSK

FIG. 67A

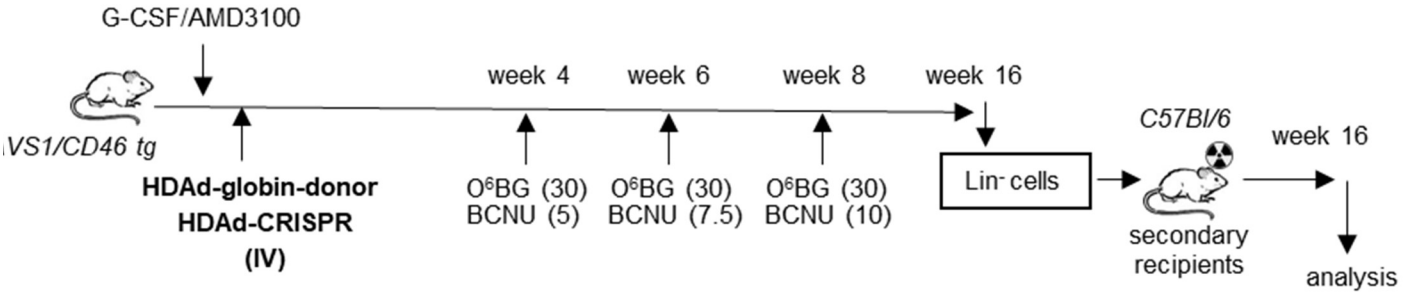


FIG. 67B

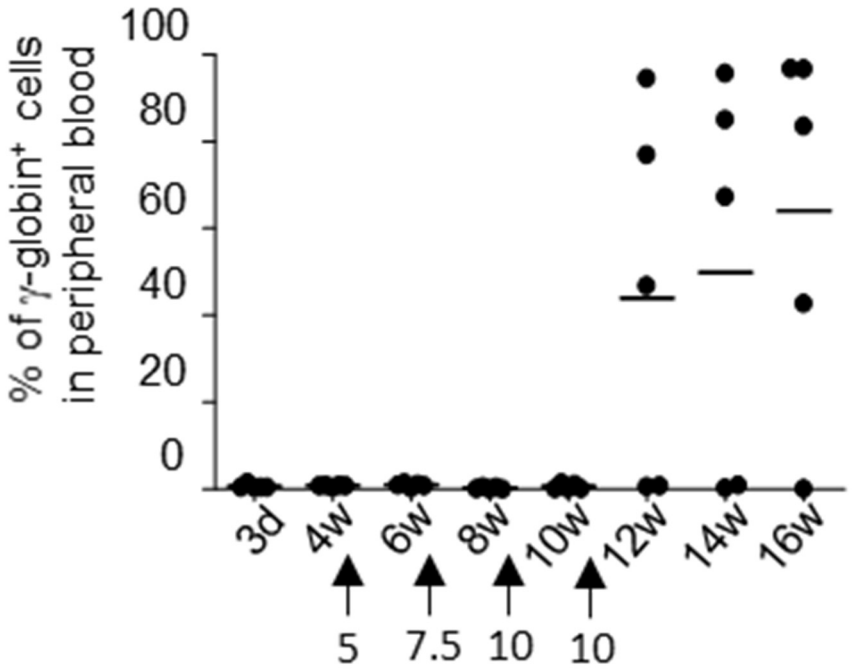


FIG. 67C

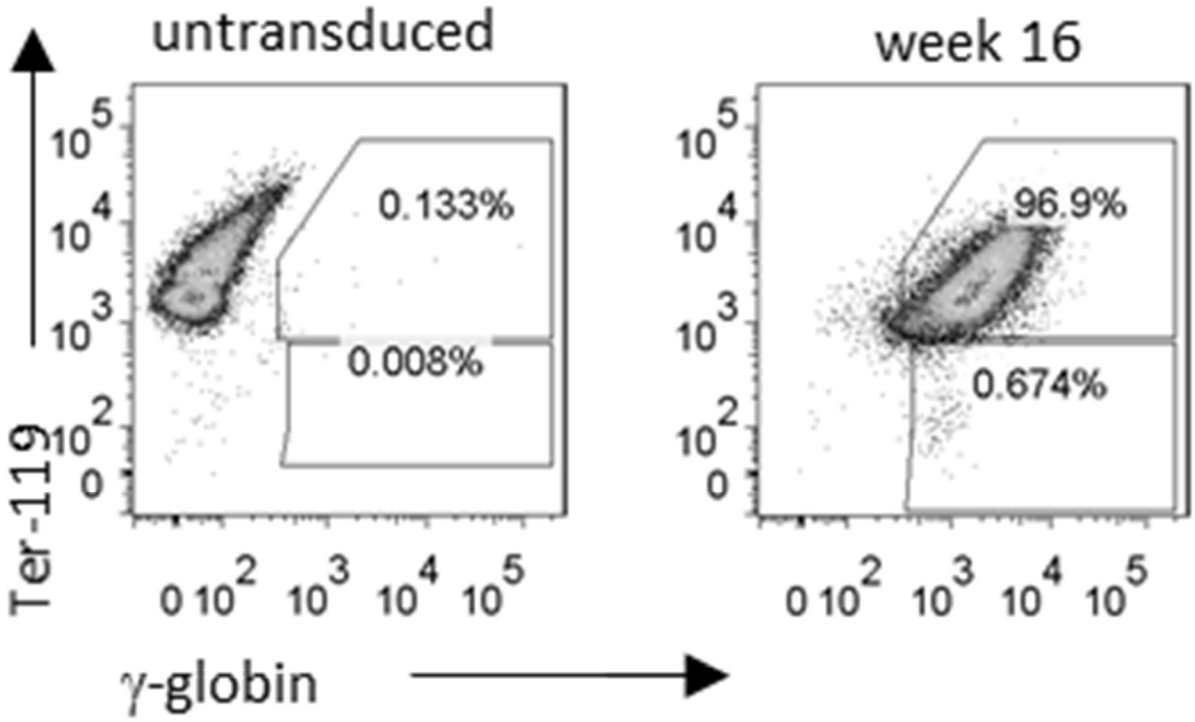


FIG. 67D

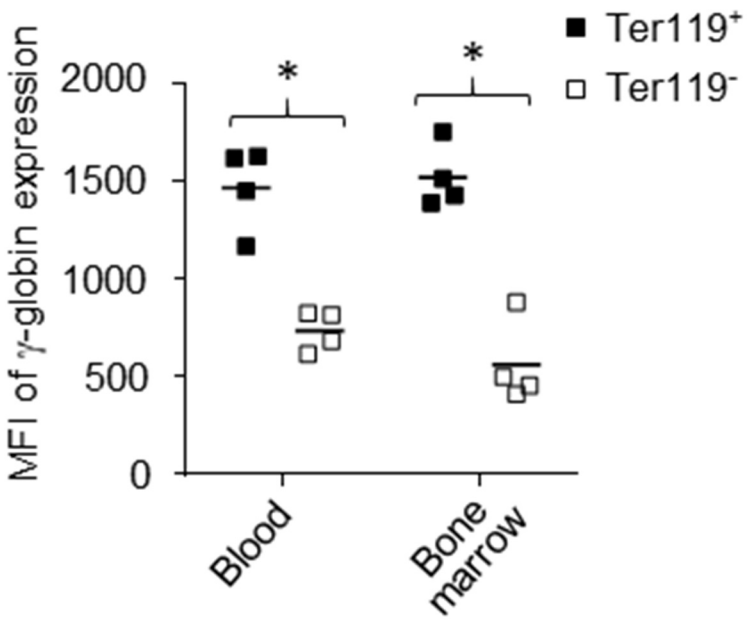


FIG. 67E

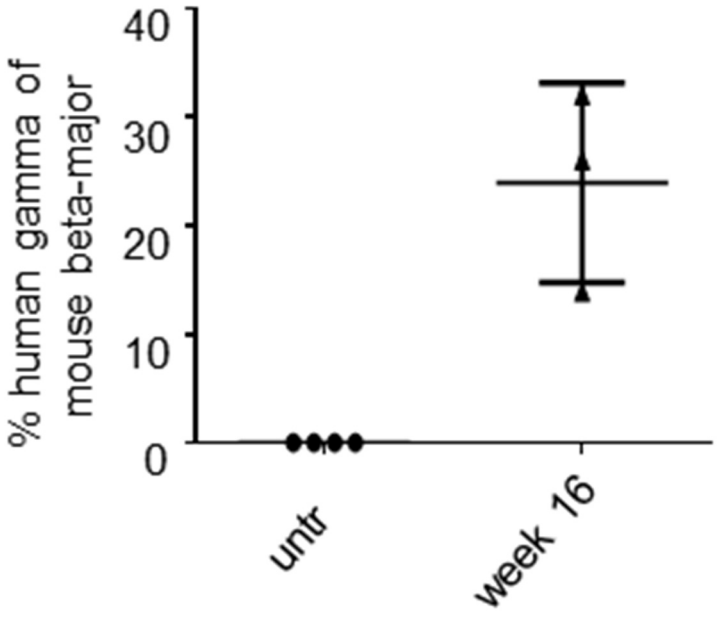


FIG. 67F

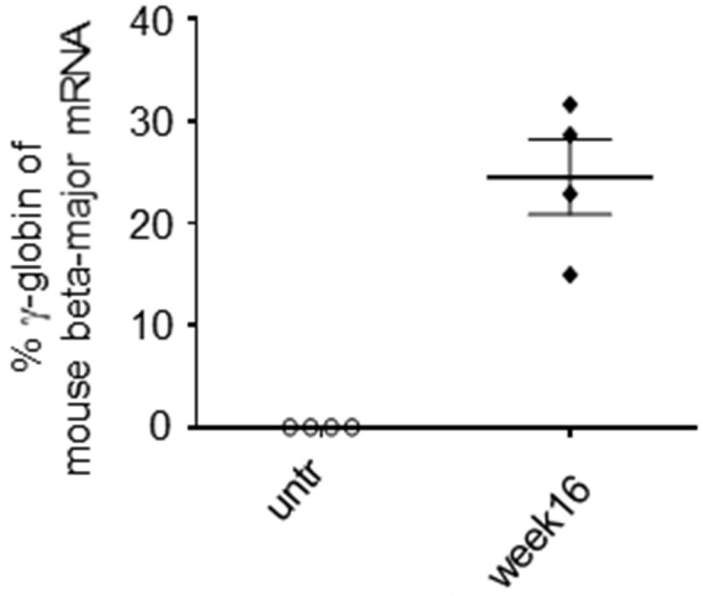


FIG. 67G

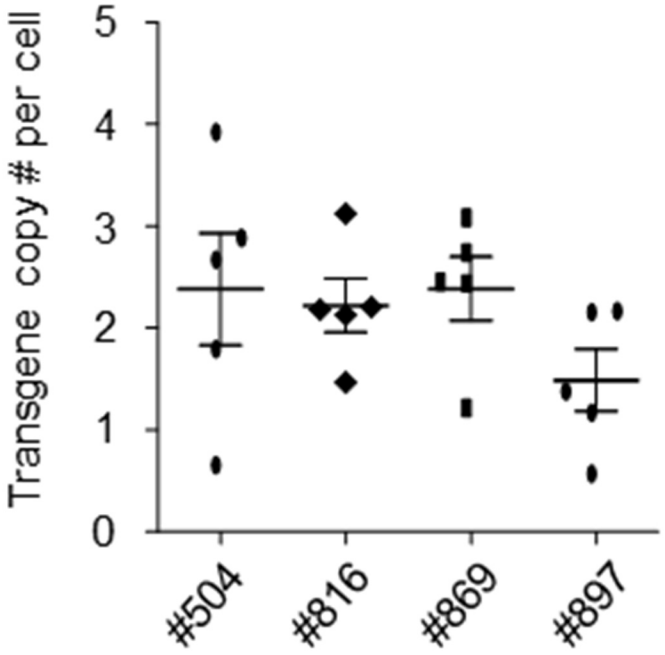


FIG. 67H

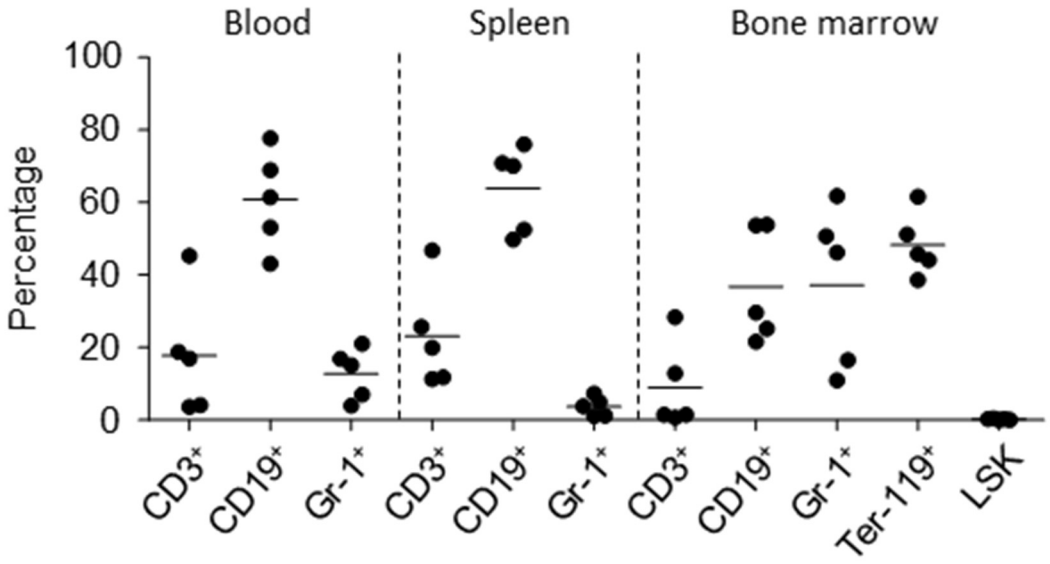


FIG. 68A

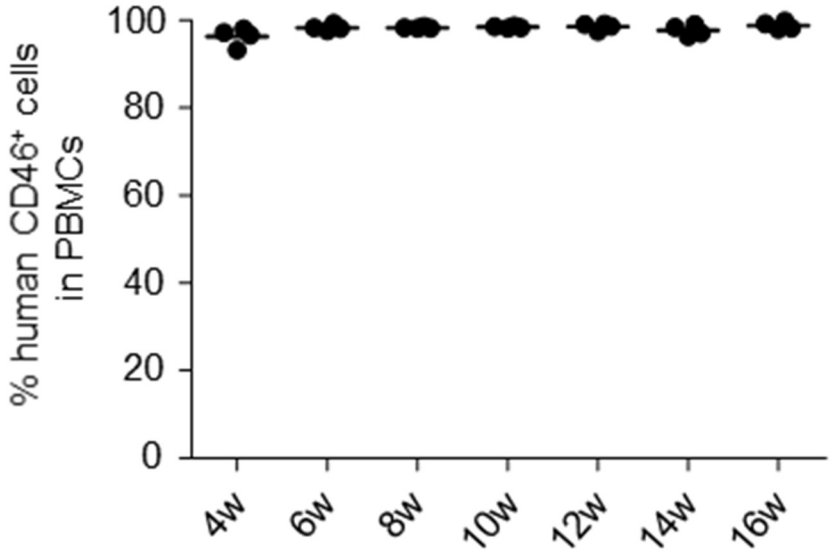


FIG. 68B

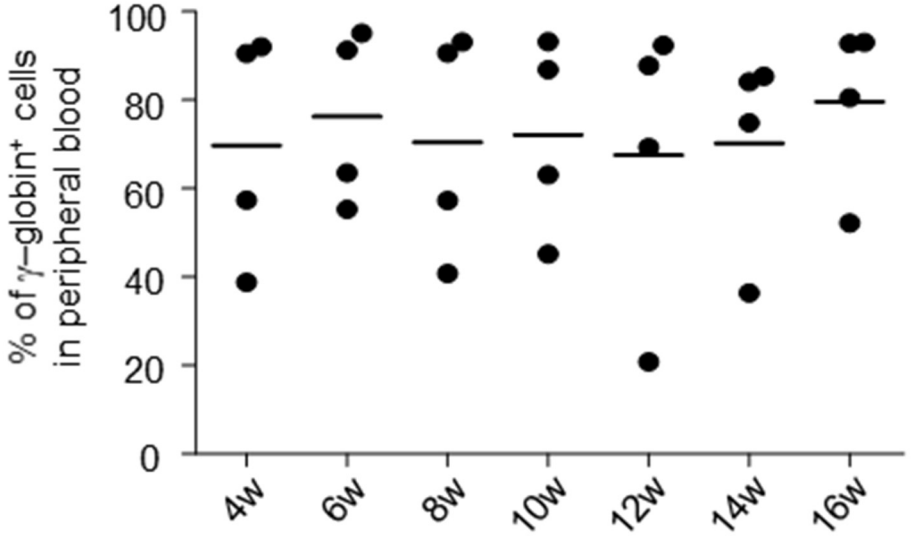


FIG. 68C

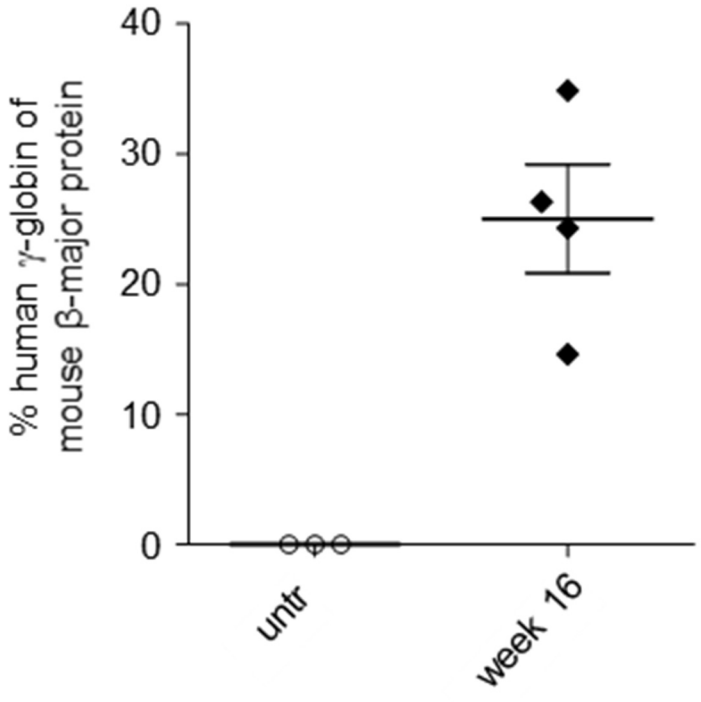


FIG. 68D

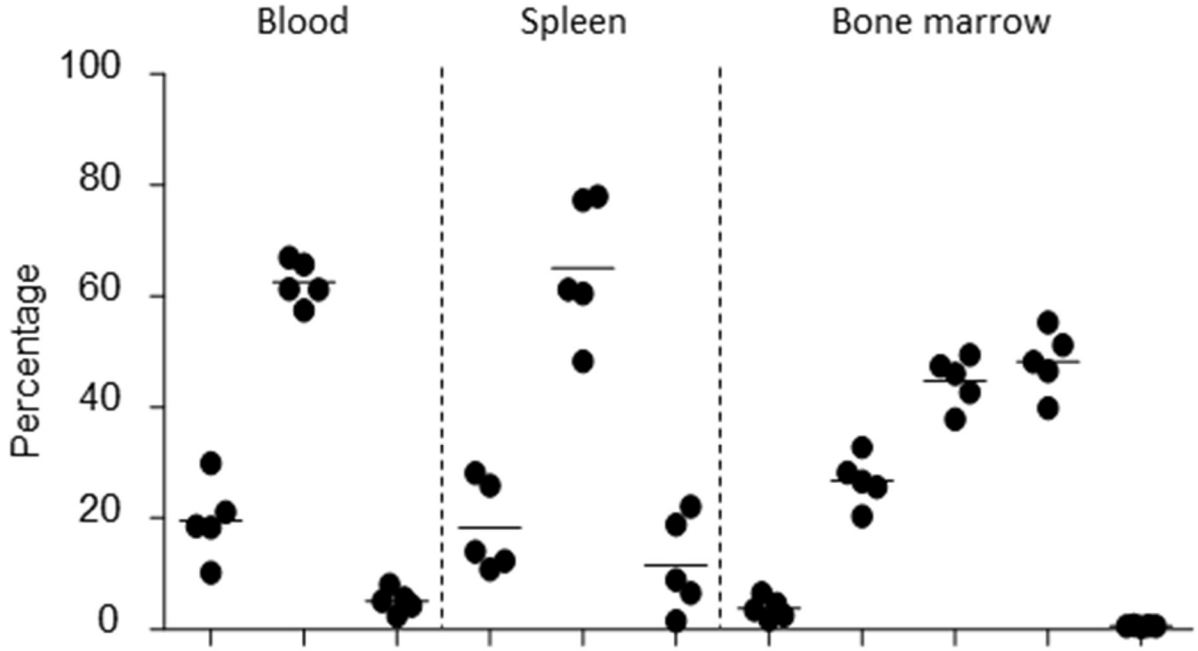


FIG. 69A


chr1 qA1 qA3 qA5 qB qC1.1 qC2 qC3 qC5 qD qE2.1 qE2.3 qE4 qF qH3 qH4 qH5 qH6
chr2 qA1 qA3 qB qC1.1 qC3 qD qE1 qE3 qE5 qF1 qF3 qH1 qH3 qH4
chr3 qA1 qA3 qB qC qD qE1 qE3 qF1 qF2.2 qF3 qG1 qG3 qH3 qH4
chr4 qA1 qA3 qA5 qB1 qB3 qC1 qC3 qC4 qC5 qC6 qD1 qD2.2 qD3 qE1 qE2
chr5 qA1 qA3 qB1 qB3 qC1 qC3.1 qE1 qE3 qE5 qF qG2
chr6 qA1 qB1 qB3 qC1 qC3 qD1 qD3 qE3 qF1 qF3 qG1 qG3
chr7 qA1 qA3 qB1 qB3 qB4 qB5 qC qD3 qE1 qE3 qF1 qF3
chr8 qA1.1 qA2 qA4 qC1 qD1 qD3 qE1 qE2
chr9 qA1 qA4 qA5.3 qB qC qD qE3.3 qF1 qF3
chr10 qA1 qA3 qA4 qB1 qB2 qB3 qB4 qC1 qC2 qD1 qD2 qD3
chr11 qA1 qA4 qA5 qB1.3 qB3 qB5 qC qD qE1 qE2
chr12 qA1.1 qA2 qA3 qB3 qC1 qC3 qD3 qE qF1 qF2
chr13 qA1 qA3.1 qA3.3 qA5 qB1 qB3 qC1 qC3 qD1

FIG. 69B

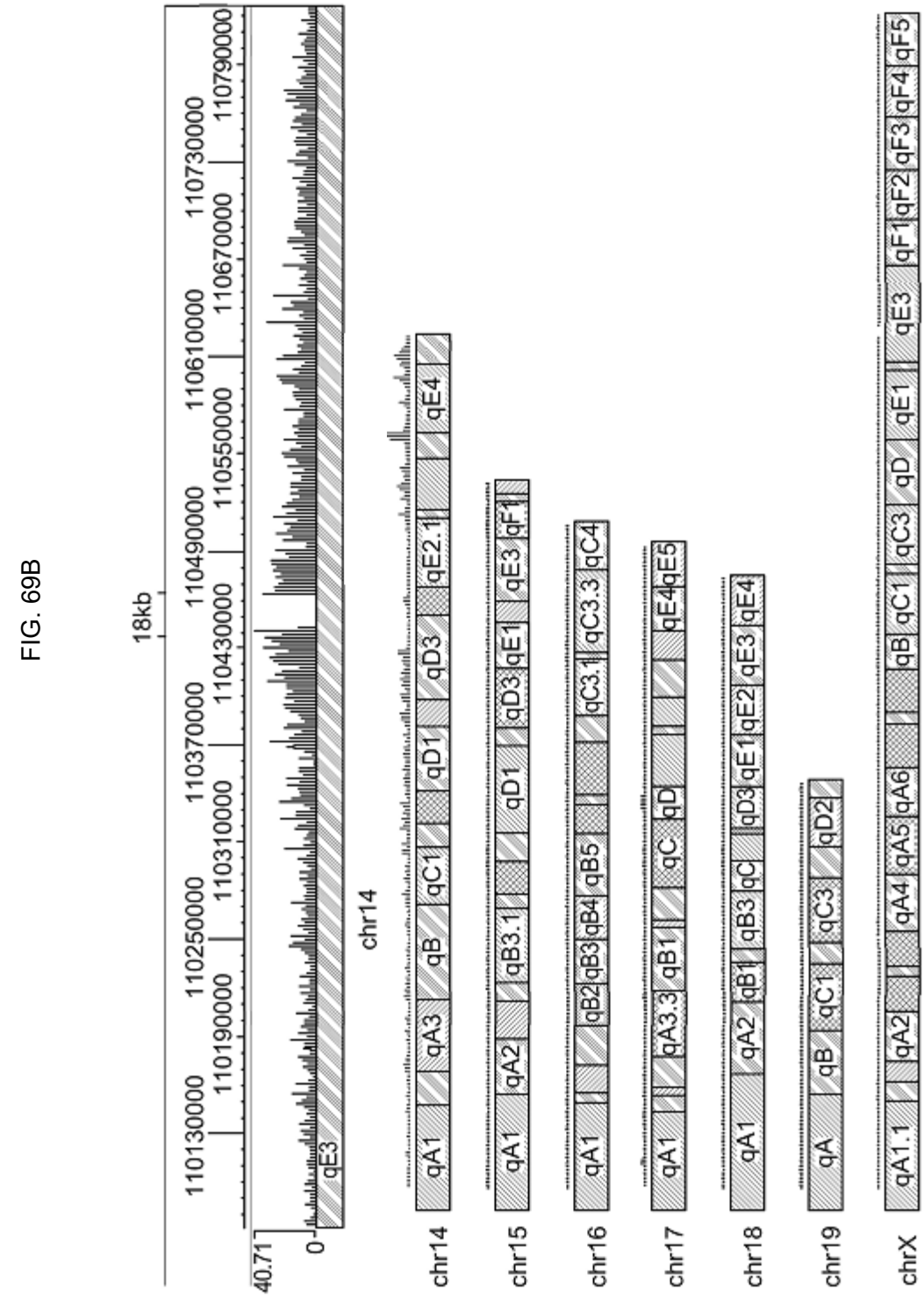

18kb
110130000
110190000
110250000
110310000
110370000
110430000
110490000
110550000
110610000
110670000
110730000
110790000
40.71
0
qE3
chr14
chr14 qA1 qA3 qB qC1 qD1 qD3 qE2.1 qE4
chr15 qA1 qA2 qB3.1 qD1 qD3 qE1 qE3 qF1
chr16 qA1 qB2 qB3 qB4 qB5 qC3.1 qC3.3 qC4
chr17 qA1 qA3.3 qB1 qC qD qE4 qE5
chr18 qA1 qA2 qB1 qB3 qC qD3 qE1 qE2 qE3 qE4
chr19 qA qB qC1 qC3 qD2
chrX qA1.1 qA2 qA4 qA5 qA6 qB qC1 qC3 qD qE1 qE3 qF1 qF2 qF3 qF4 qF5
chrY qA1 qA2 qB qC1 qC2 qC3 qD qE

FIG. 70

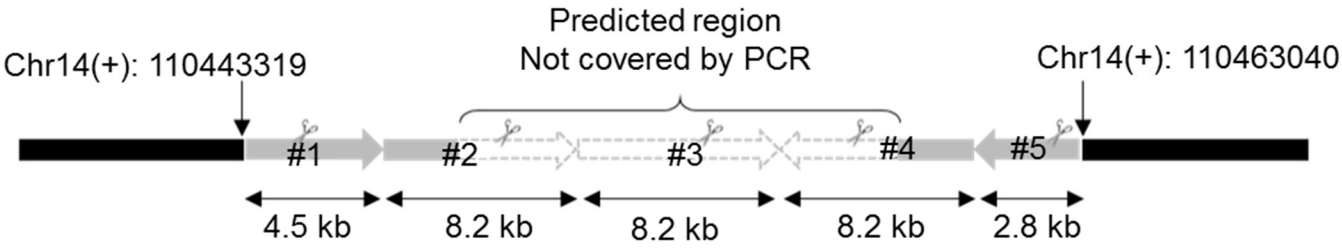


FIG. 71A

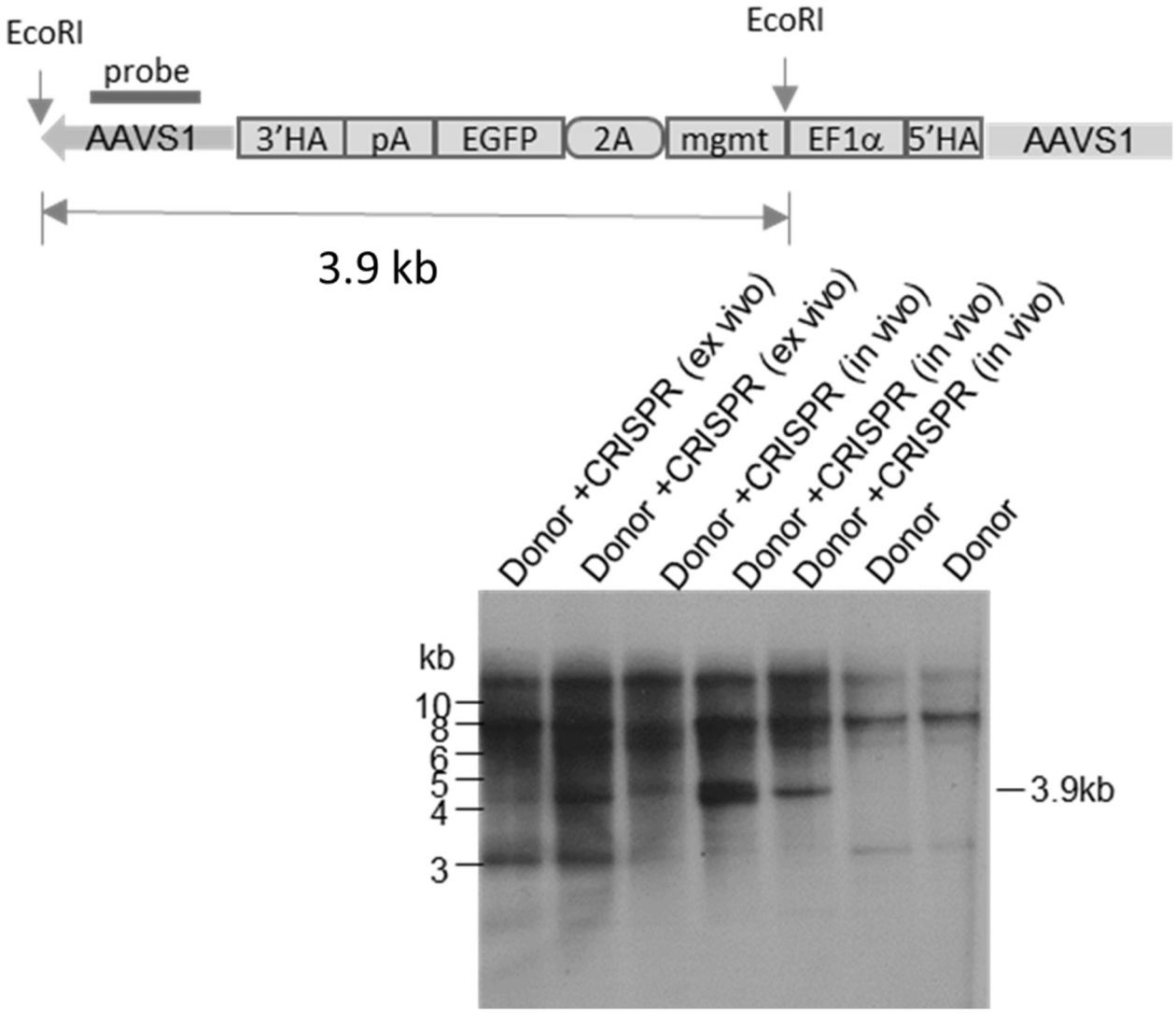


FIG. 71B

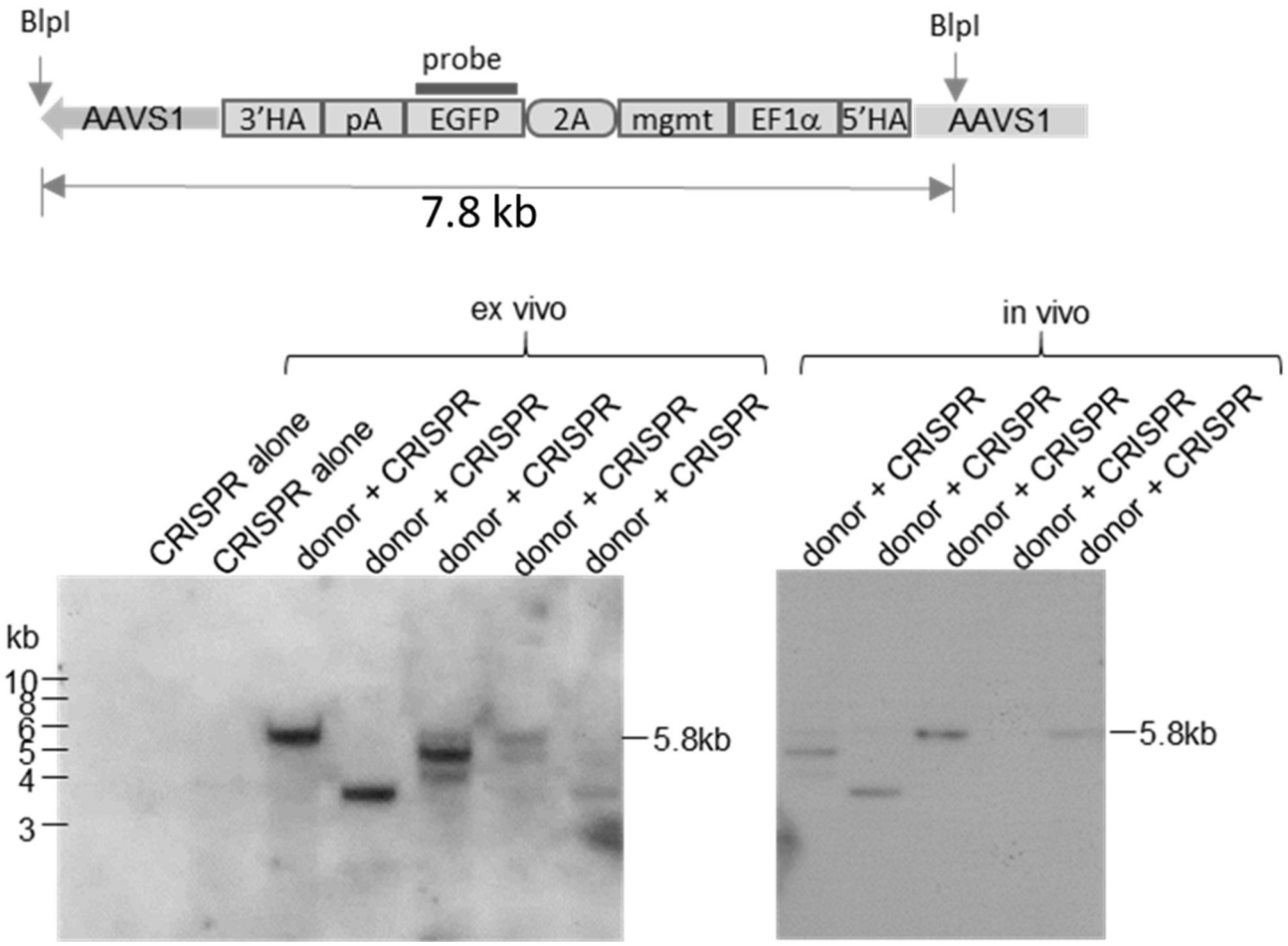

BlpI
probe
BlpI
AAVS1
3'HA
pA
EGFP
2A
mgmt
EF1α
5'HA
AAVS1
7.8 kb
ex vivo
in vivo
CRISPR alone
CRISPR alone
donor + CRISPR
donor + CRISPR
donor + CRISPR
donor + CRISPR
donor + CRISPR
donor + CRISPR
donor + CRISPR
donor + CRISPR
donor + CRISPR
kb
10
8
6
5
4
3
5.8kb
5.8kb

FIG. 72A

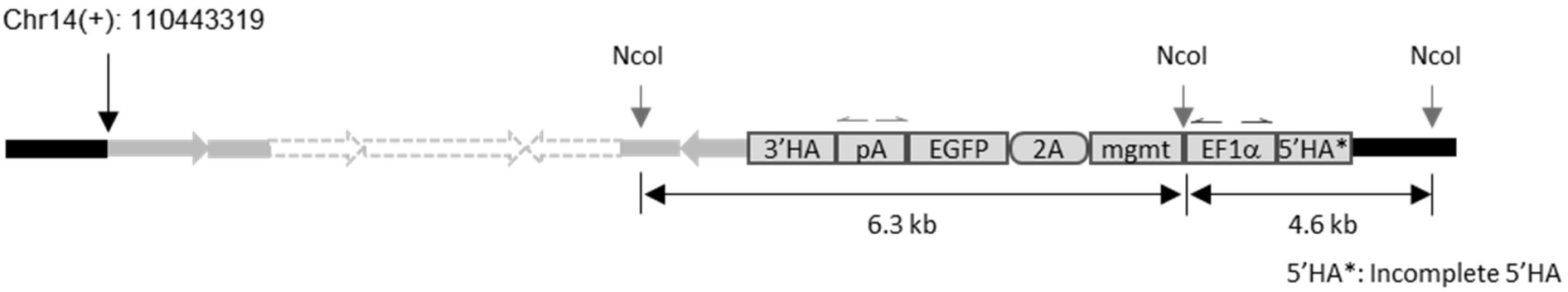


FIG. 72B

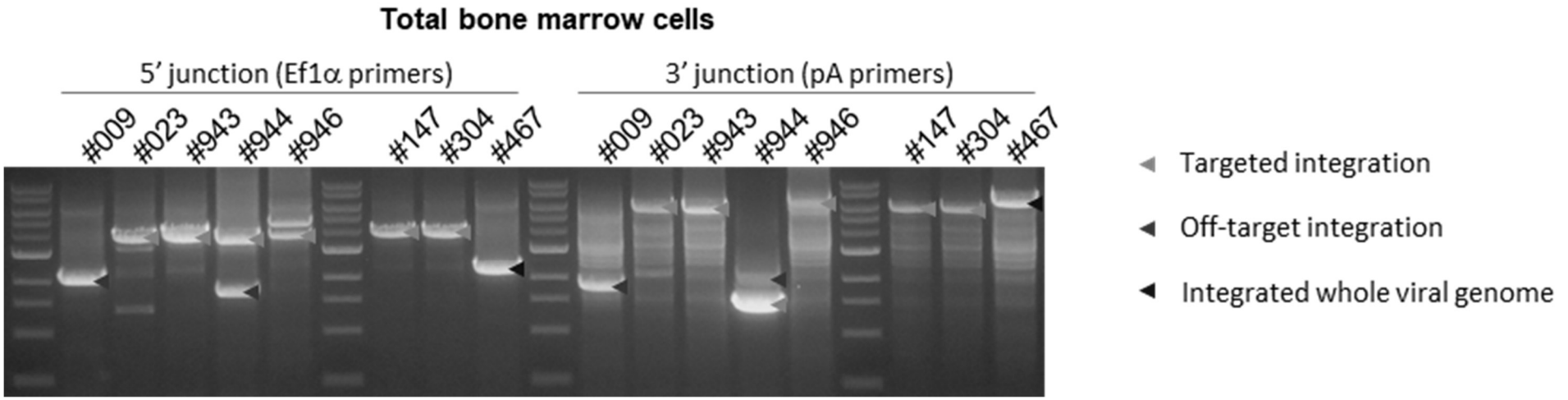


FIG. 72C

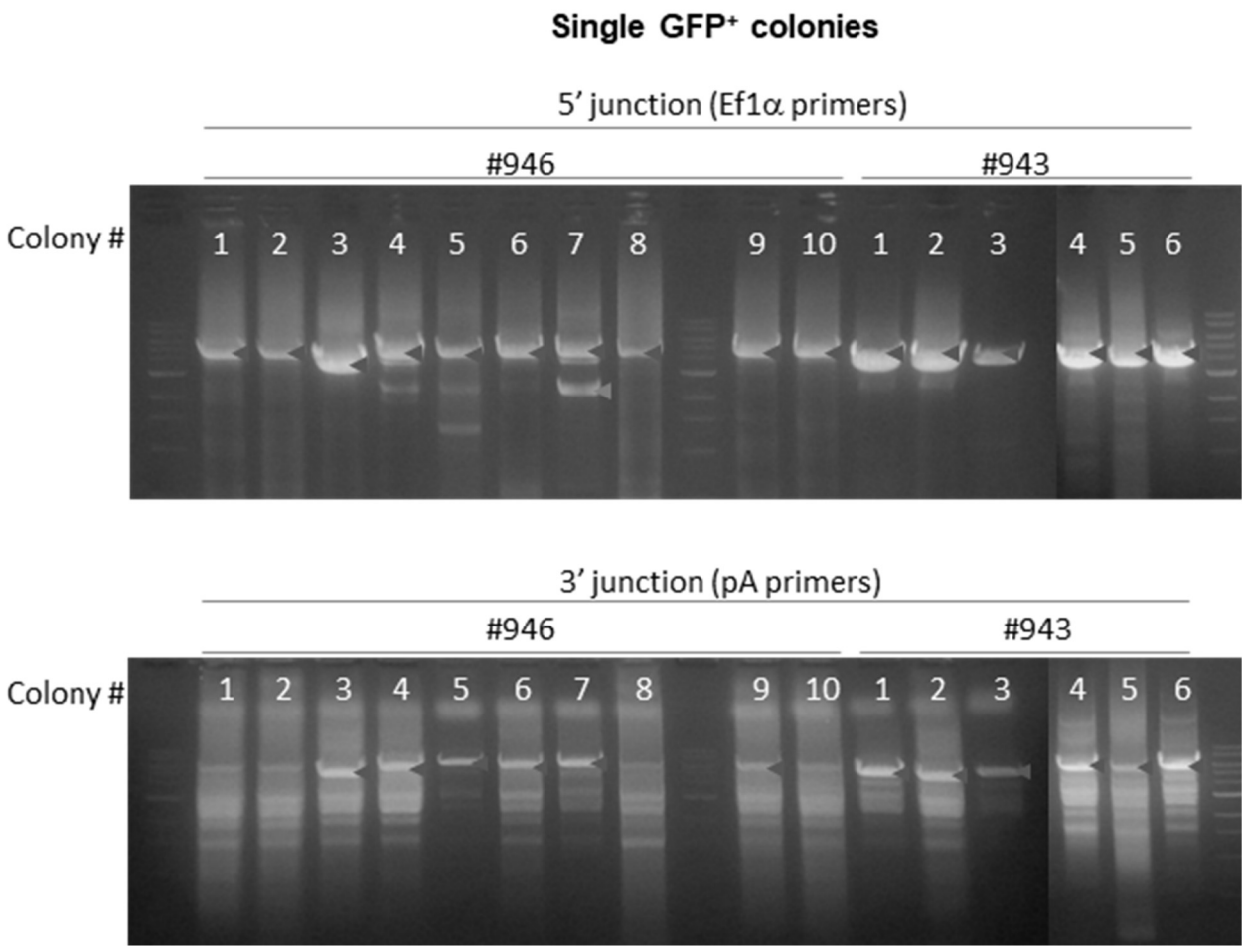
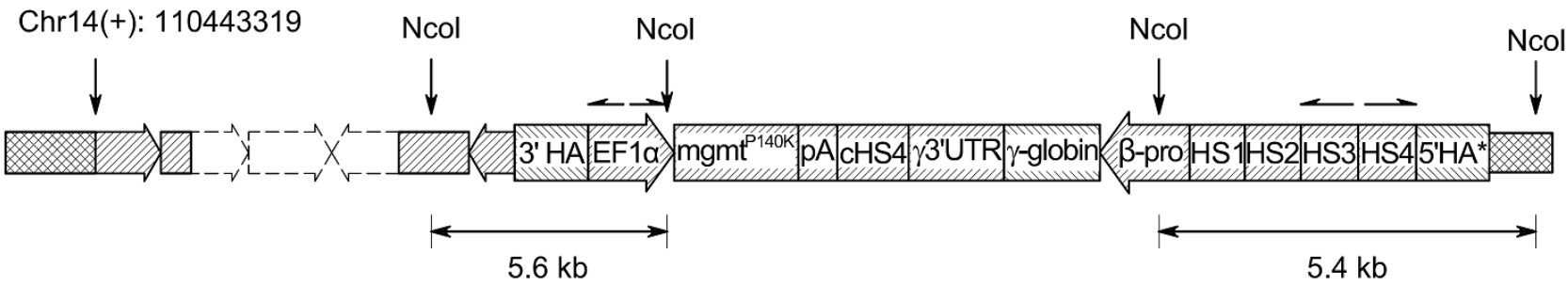

Single GFP+ colonies
5' junction (Ef1α primers)
#946
#943
Colony #
1 2 3 4 5 6 7 8 9 10 1 2 3 4 5 6
3' junction (pA primers)
#946
#943
Colony #
1 2 3 4 5 6 7 8 9 10 1 2 3 4 5 6

FIG. 73A



5'HA*: Incomplete 5'HA

FIG. 73B

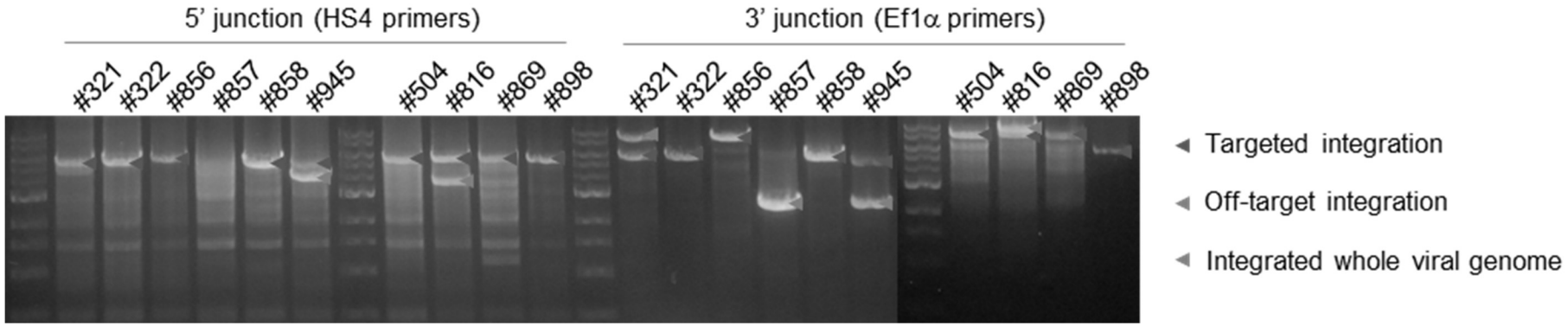


FIG. 74A

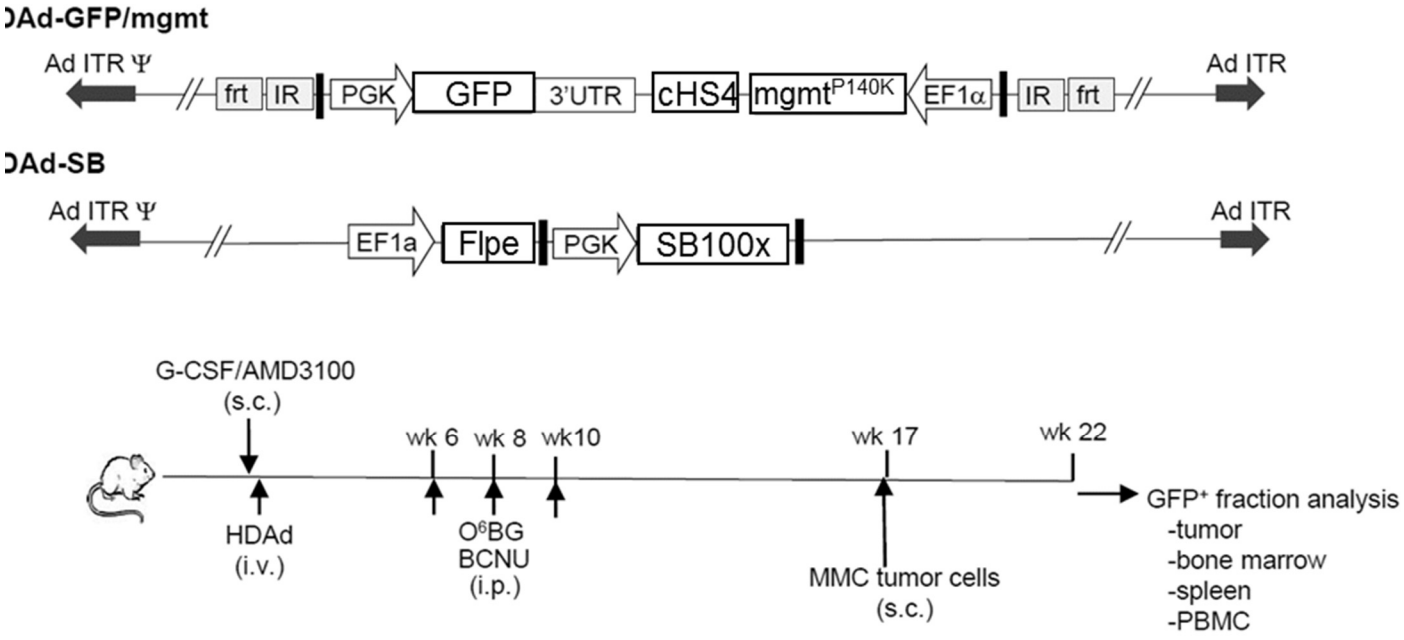


FIG. 74B

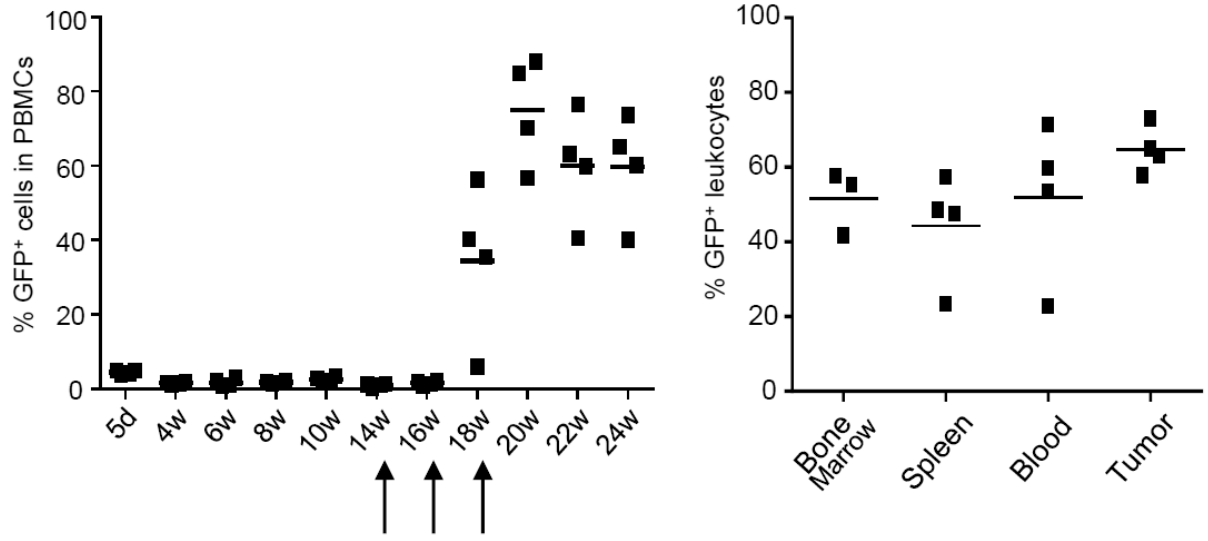
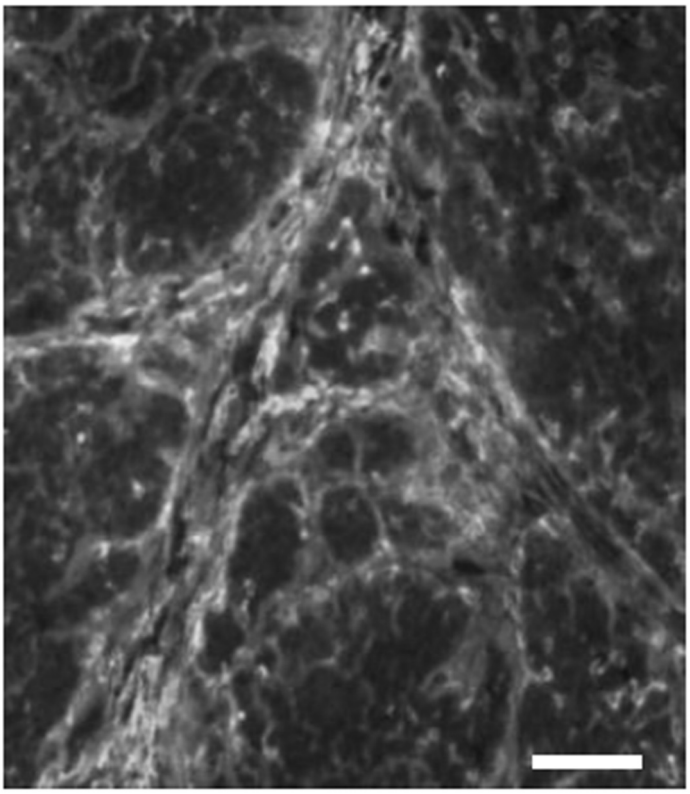


FIG. 74C



GFP/laminin

FIG. 74D

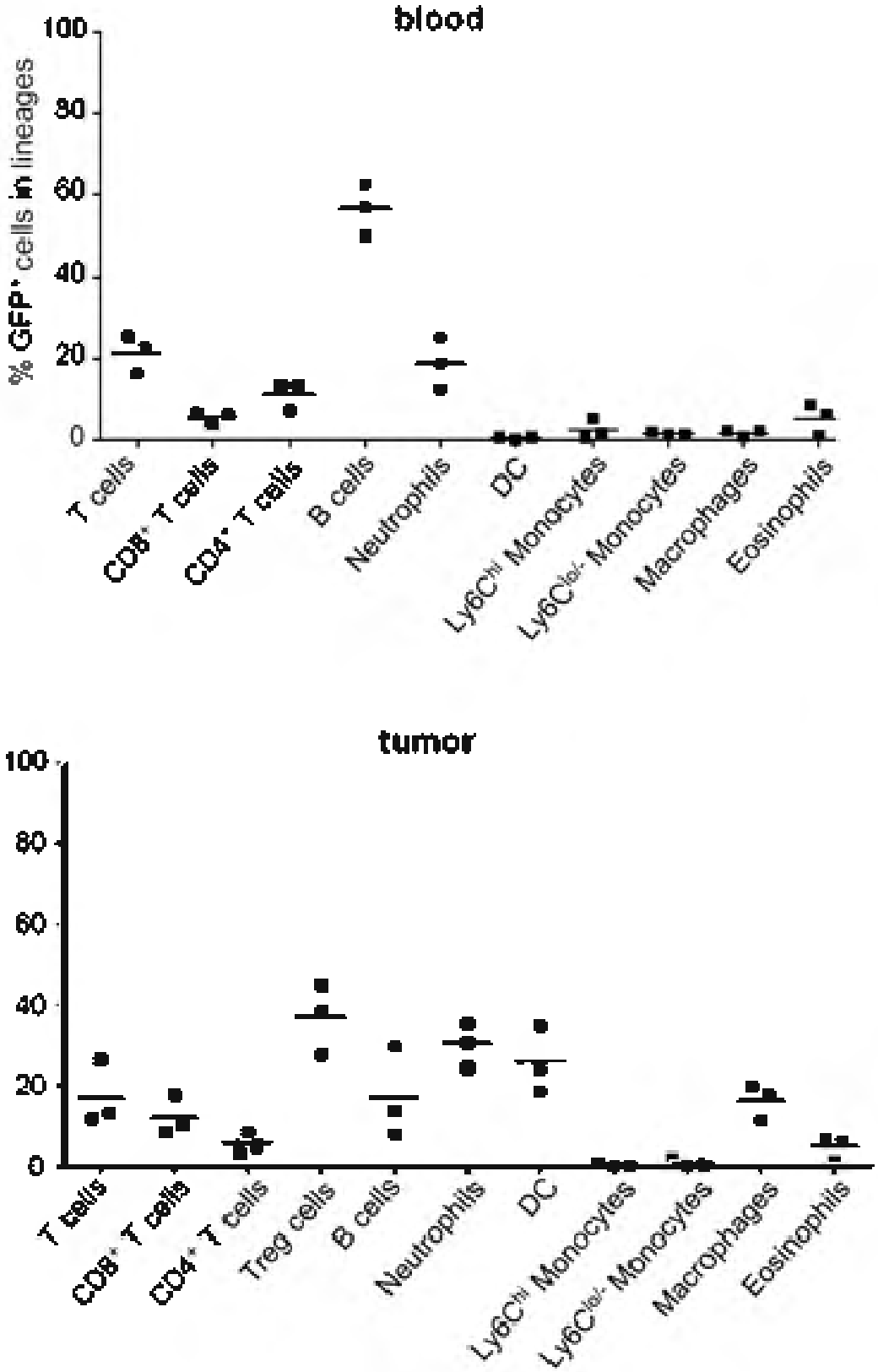
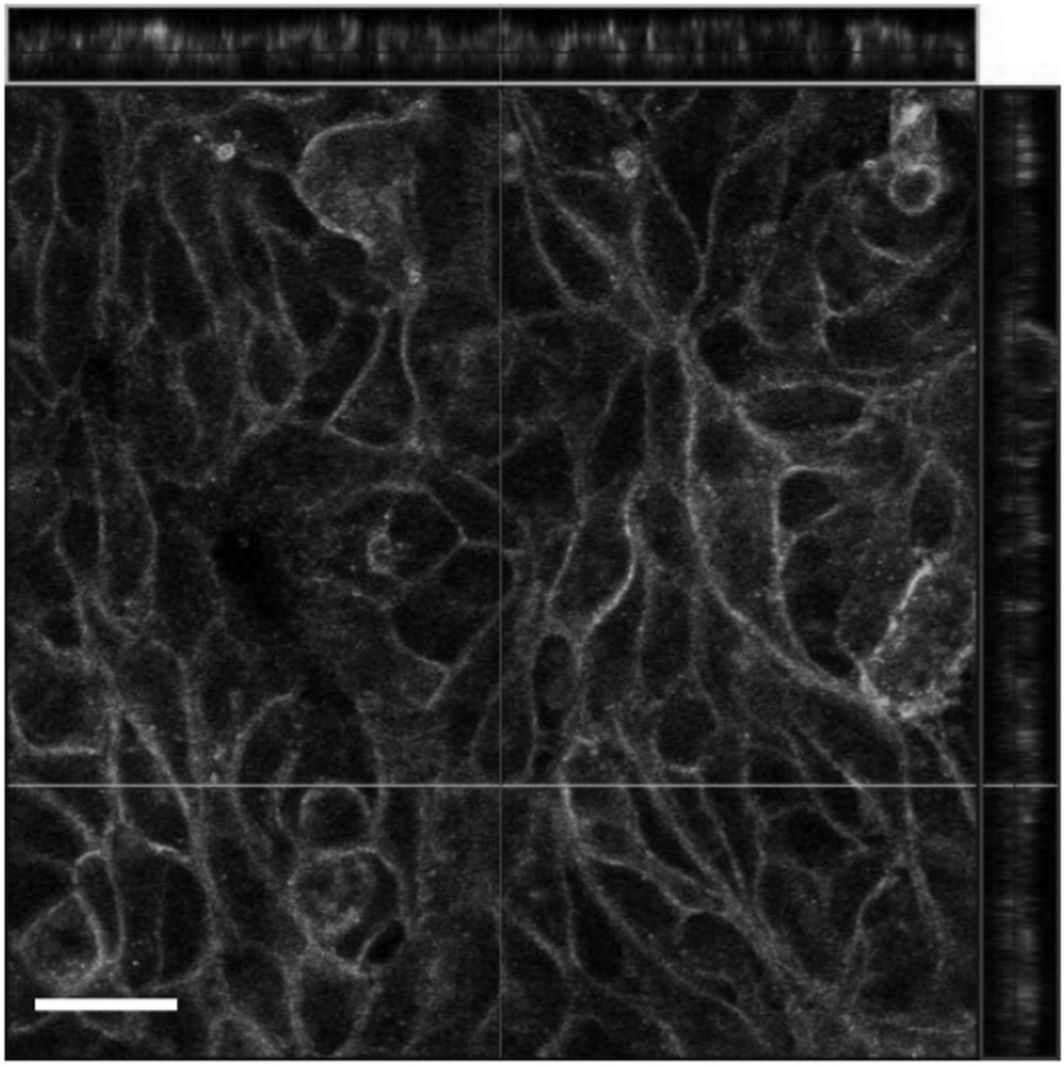

blood
% GFP+ cells in lineages
100
80
60
40
20
0
T cells
CD8+ T cells
CD4+ T cells
B cells
Neutrophils
DC
Ly6Chi Monocytes
Ly6Clo/- Monocytes
Macrophages
Eosinophils
tumor
100
80
60
40
20
0
T cells
CD8+ T cells
CD4+ T cells
Treg cells
B cells
Neutrophils
DC
Ly6Chi Monocytes
Ly6Clo/- Monocytes
Macrophages
Eosinophils

FIG. 75



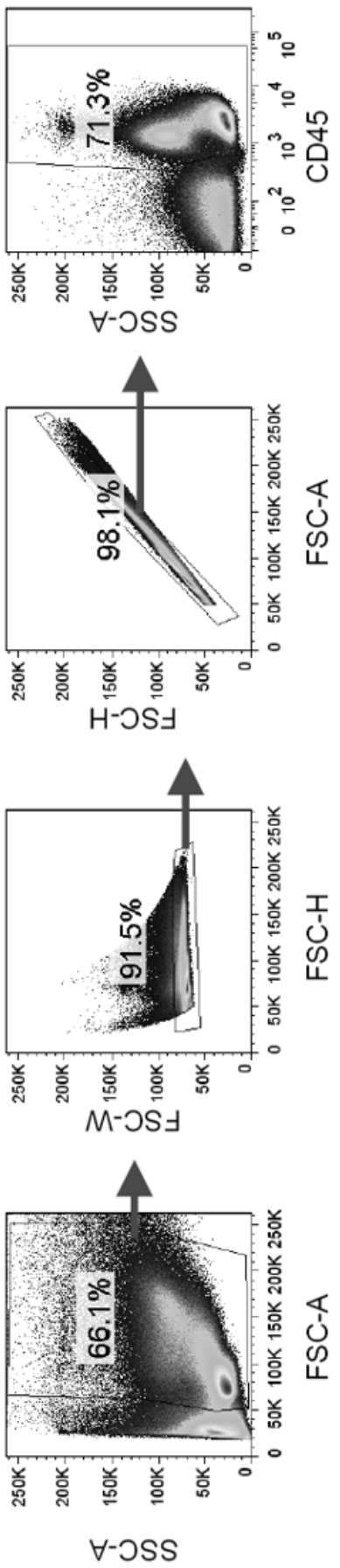


FIG. 76

FIG. 76 cont'd

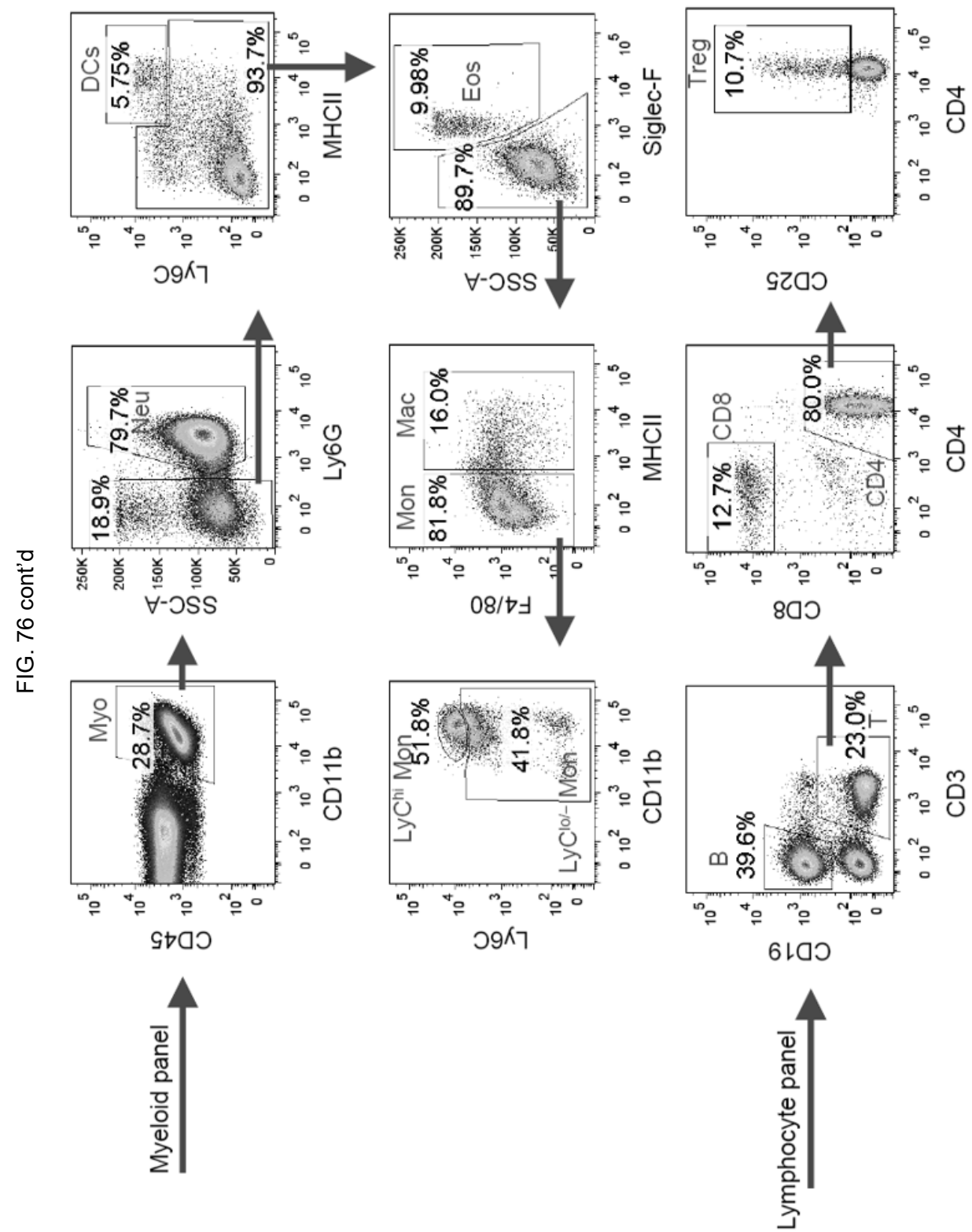

Myeloid panel
Lymphocyte panel
CD45
CD11b
Myo
28.7%
SSC-A
Ly6G
18.9%
79.7%
Neu
Ly6C
CD11b
LyChi Mon
51.8%
41.8%
LyClo/- Mon
F4/80
MHCII
Mon
Mac
81.8%
16.0%
SSC-A
Siglec-F
89.7%
9.98%
Eos
Ly6C
MHCII
DCs
5.75%
93.7%
CD19
CD3
B
39.6%
T
23.0%
CD8
CD4
12.7%
CD8
80.0%
CD4
CD25
CD4
Treg
10.7%

FIG. 77

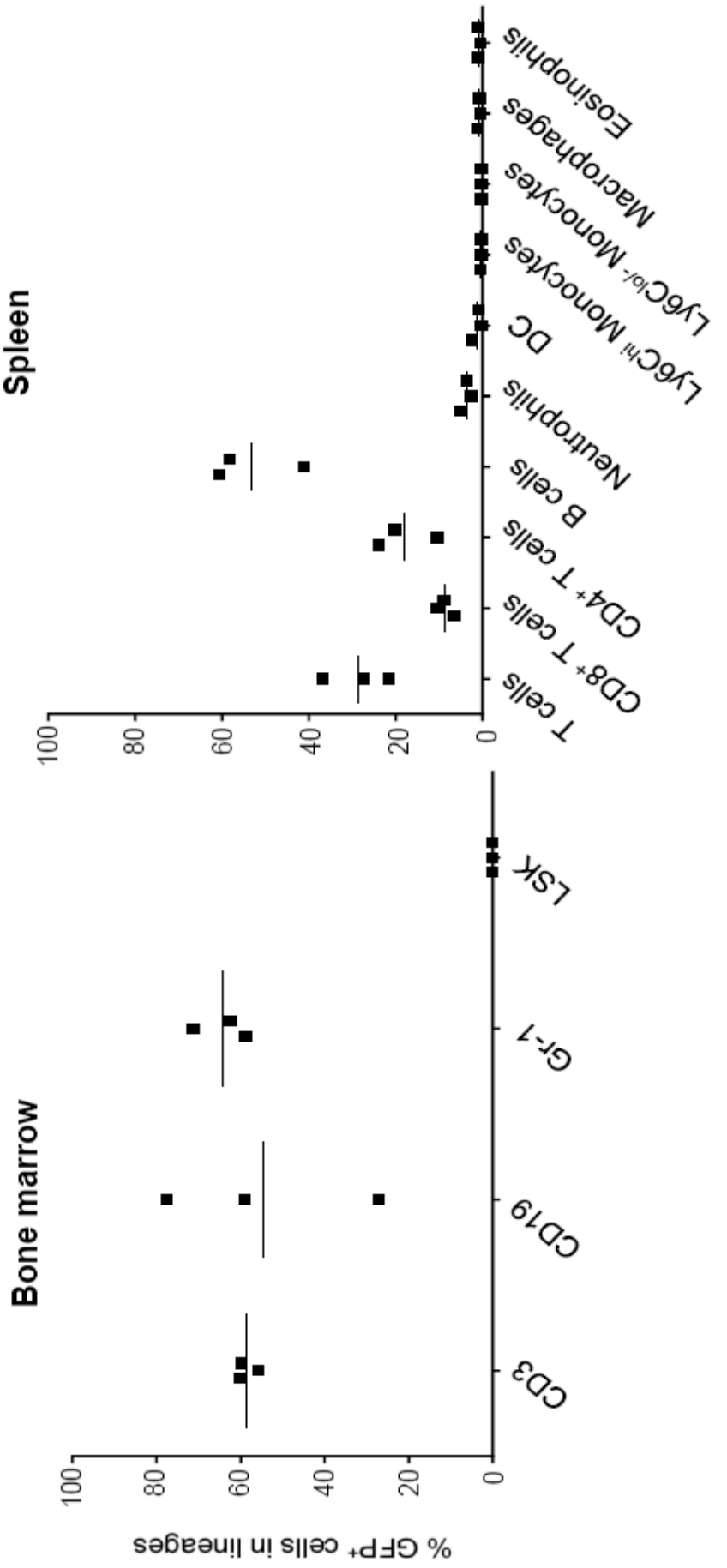

Bone marrow
Spleen
% GFP+ cells in lineages
100
80
60
40
20
0
CD3
CD19
Gr-1
LSK
T cells
CD8+ T cells
CD4+ T cells
B cells
Neutrophils
DC
Ly6Chi Monocytes
Ly6Clo- Monocytes
Macrophages
Eosinophils

FIG. 78A

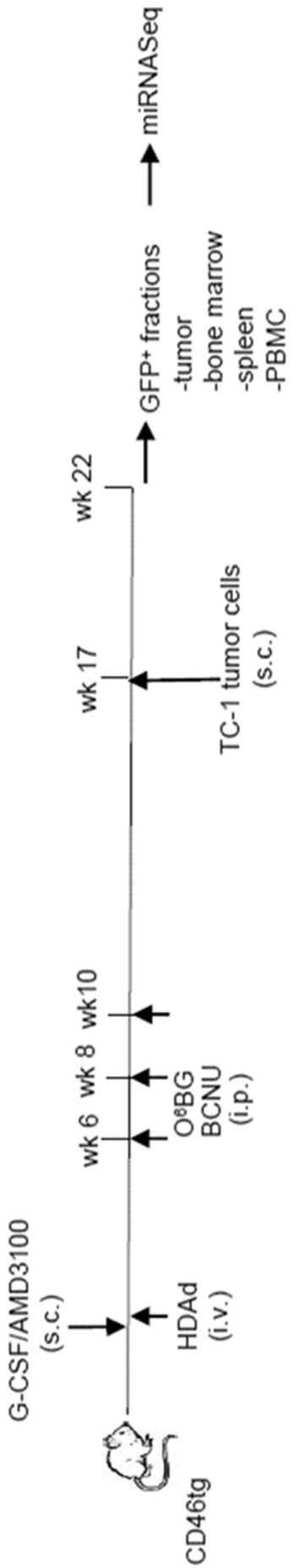


FIG. 78B

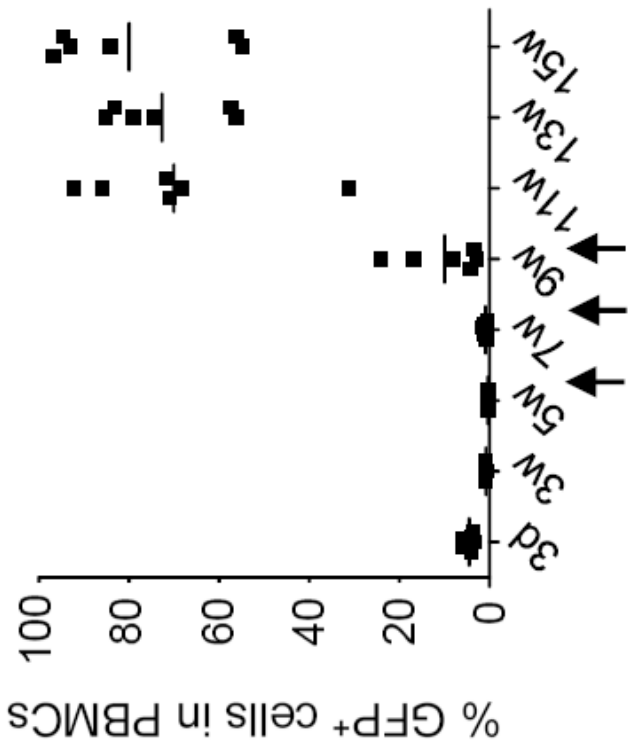


FIG. 78C

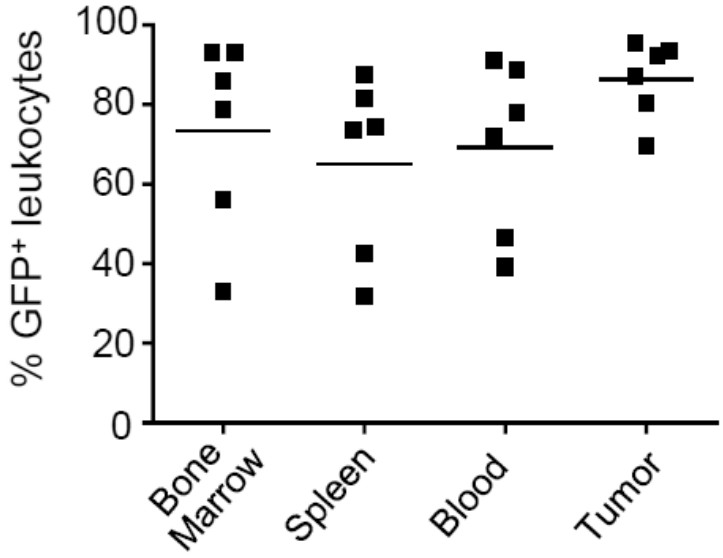


FIG. 78D

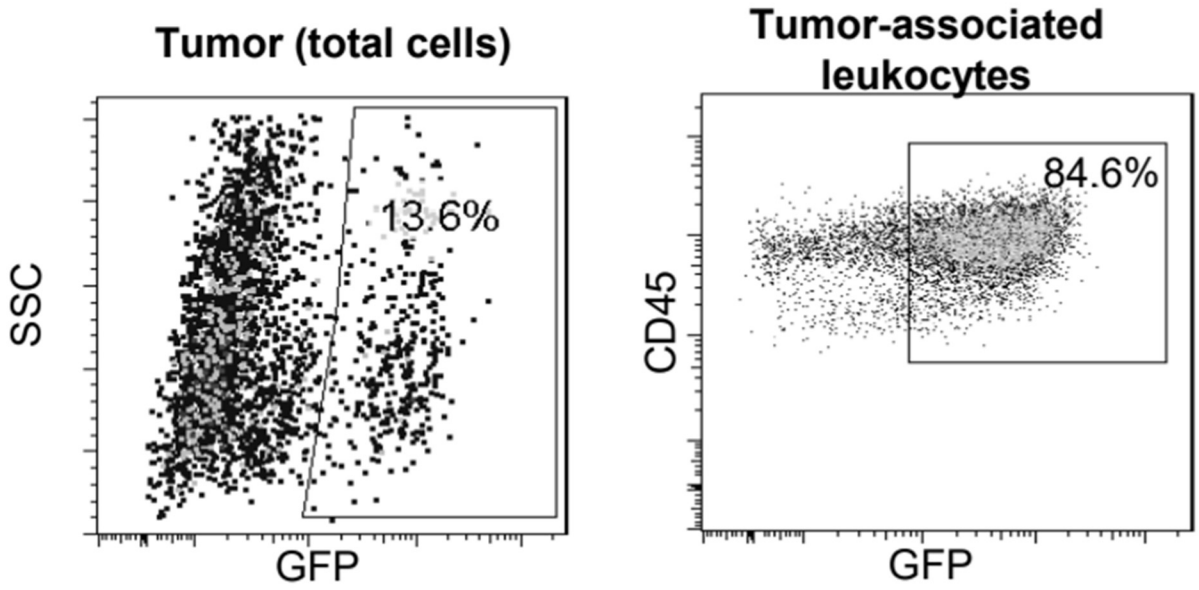


FIG. 78E

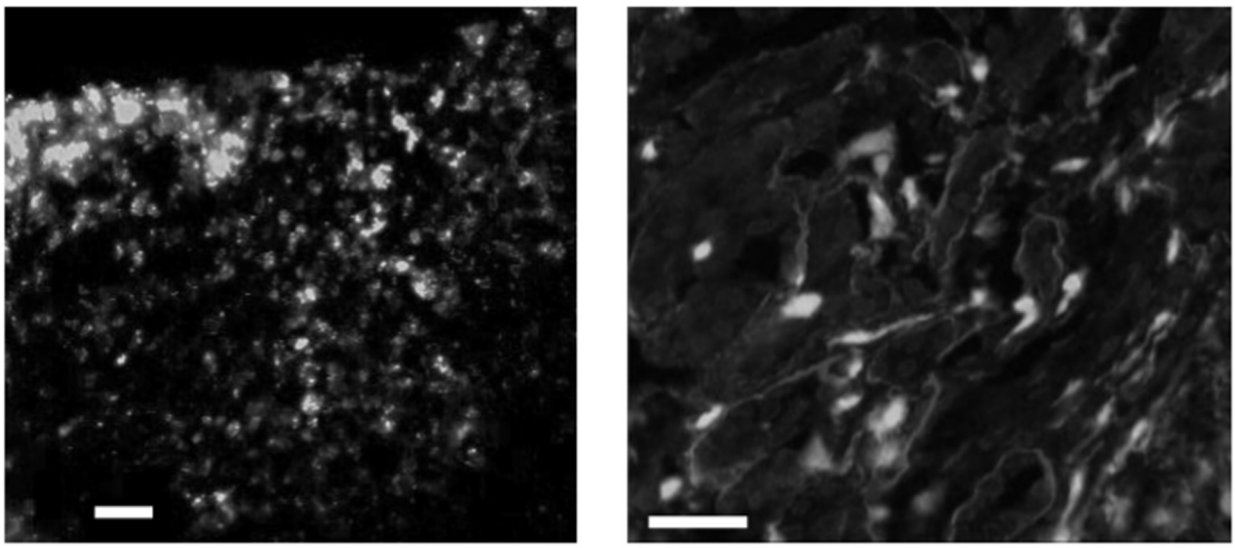


FIG. 7F

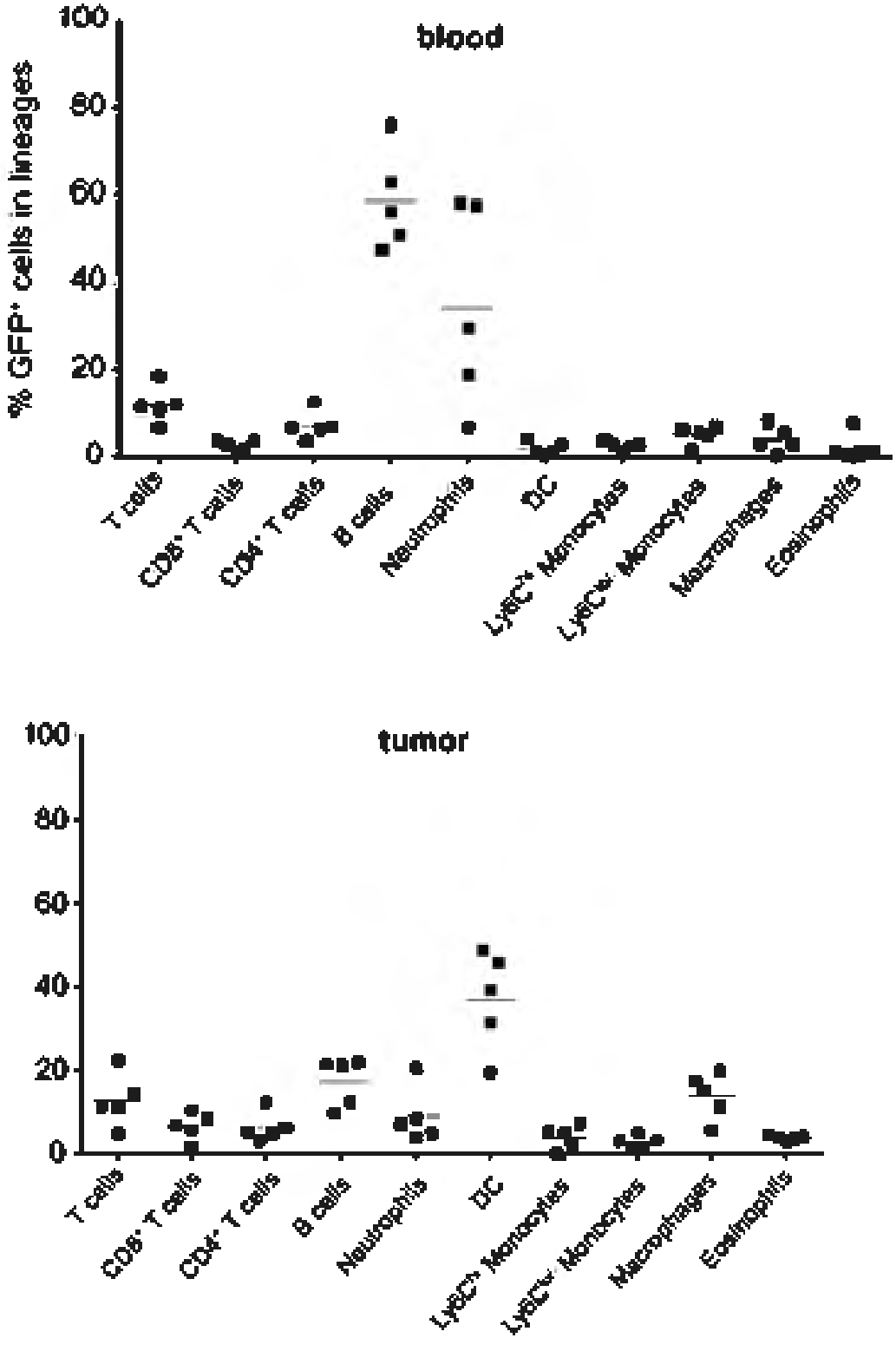

blood
% GFP+ cells in lineages
100
80
60
40
20
0
T cells
CD8+ T cells
CD4+ T cells
B cells
Neutrophils
DC
Ly6C+ Monocytes
Ly6C- Monocytes
Macrophages
Eosinophils
tumor
100
80
60
40
20
0
T cells
CD8+ T cells
CD4+ T cells
B cells
Neutrophils
DC
Ly6C+ Monocytes
Ly6C- Monocytes
Macrophages
Eosinophils

FIG. 79A

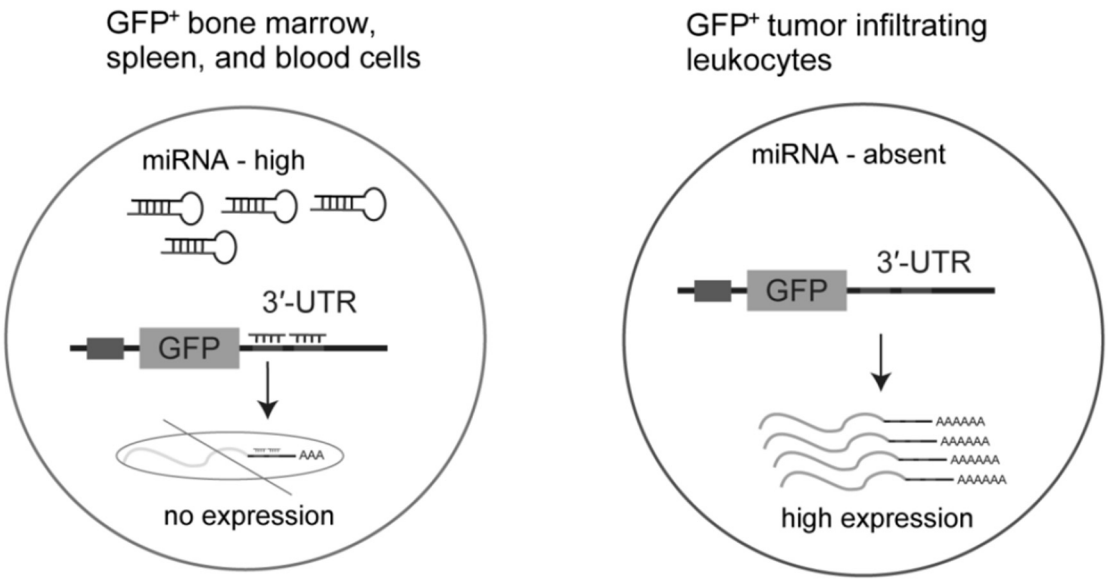


FIG. 79B

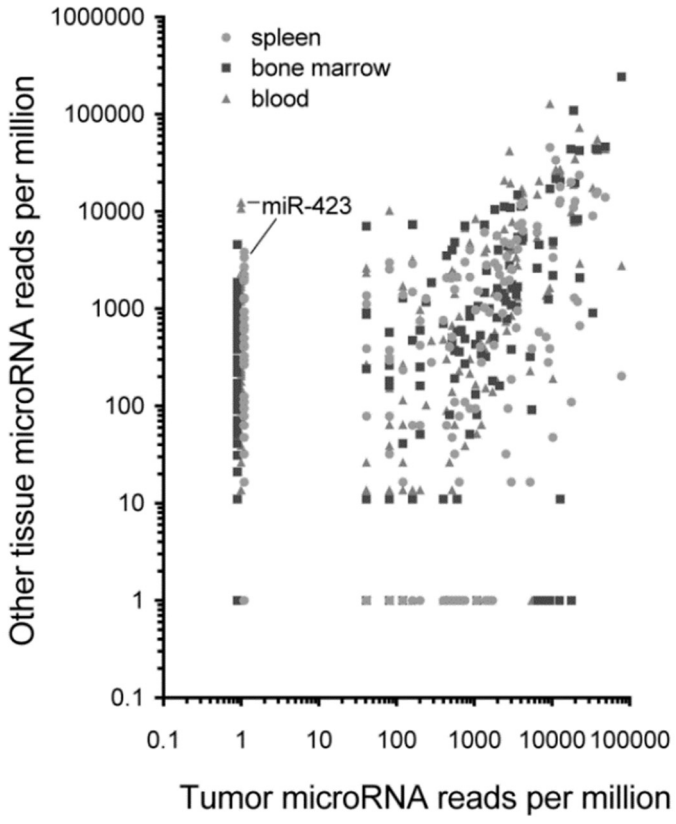


FIG. 79C

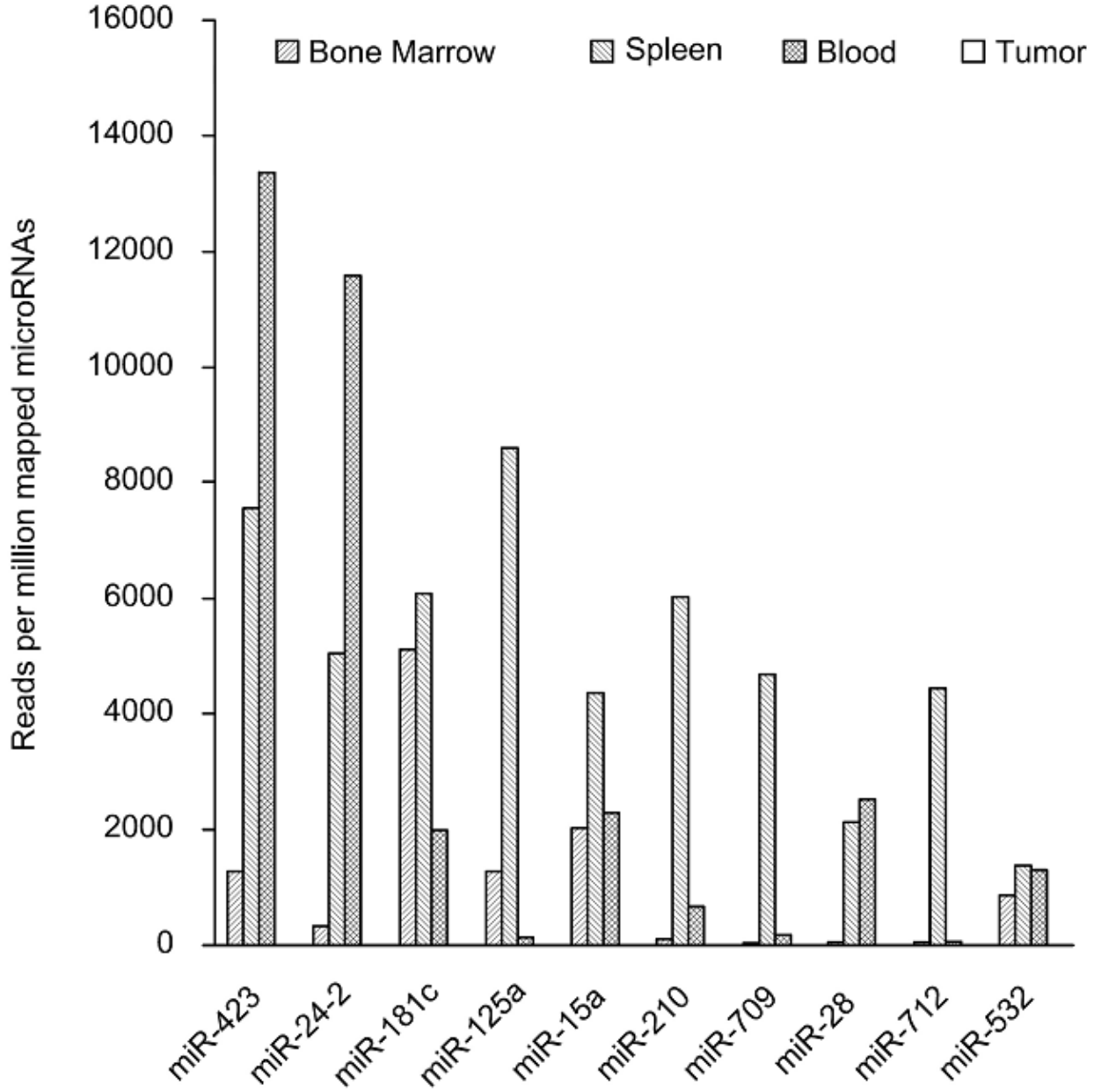

16000
14000
12000
10000
8000
6000
4000
2000
0
Reads per million mapped microRNAs
Bone Marrow
Spleen
Blood
Tumor
miR-423
miR-24-2
miR-181c
miR-125a
miR-15a
miR-210
miR-709
miR-28
miR-712
miR-532

FIG. 80A

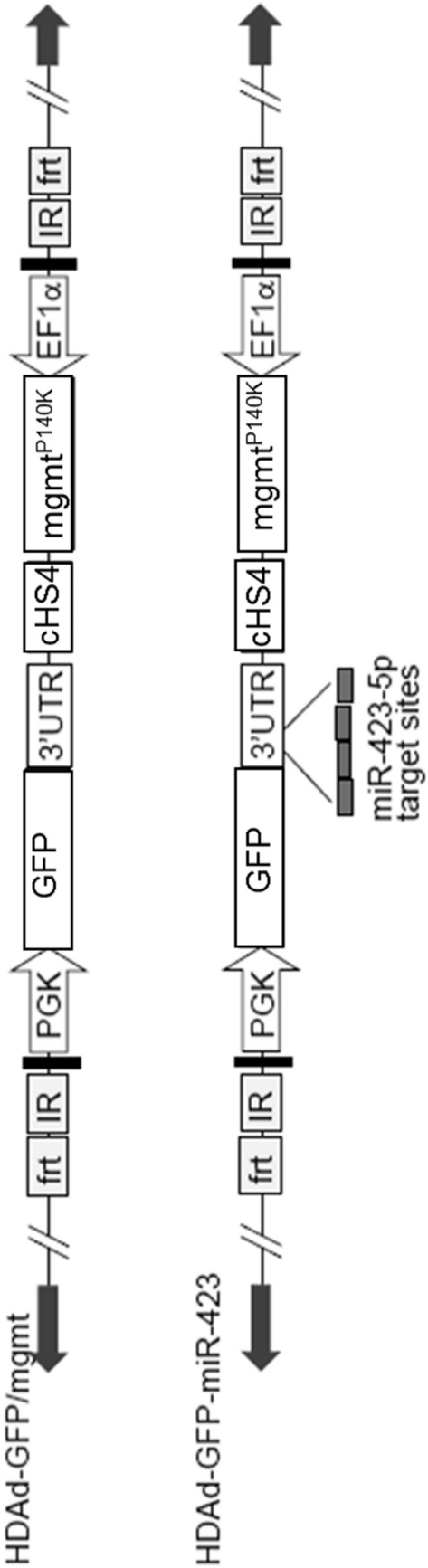

HDAd-GFP/mgmt
frt
IR
PGK
GFP
3'UTR
cHS4
mgmt^{P140K}
EF1α
IR
frt
HDAd-GFP-miR-423
frt
IR
PGK
GFP
3'UTR
cHS4
mgmt^{P140K}
EF1α
IR
frt
miR-423-5p
target sites

FIG. 80B

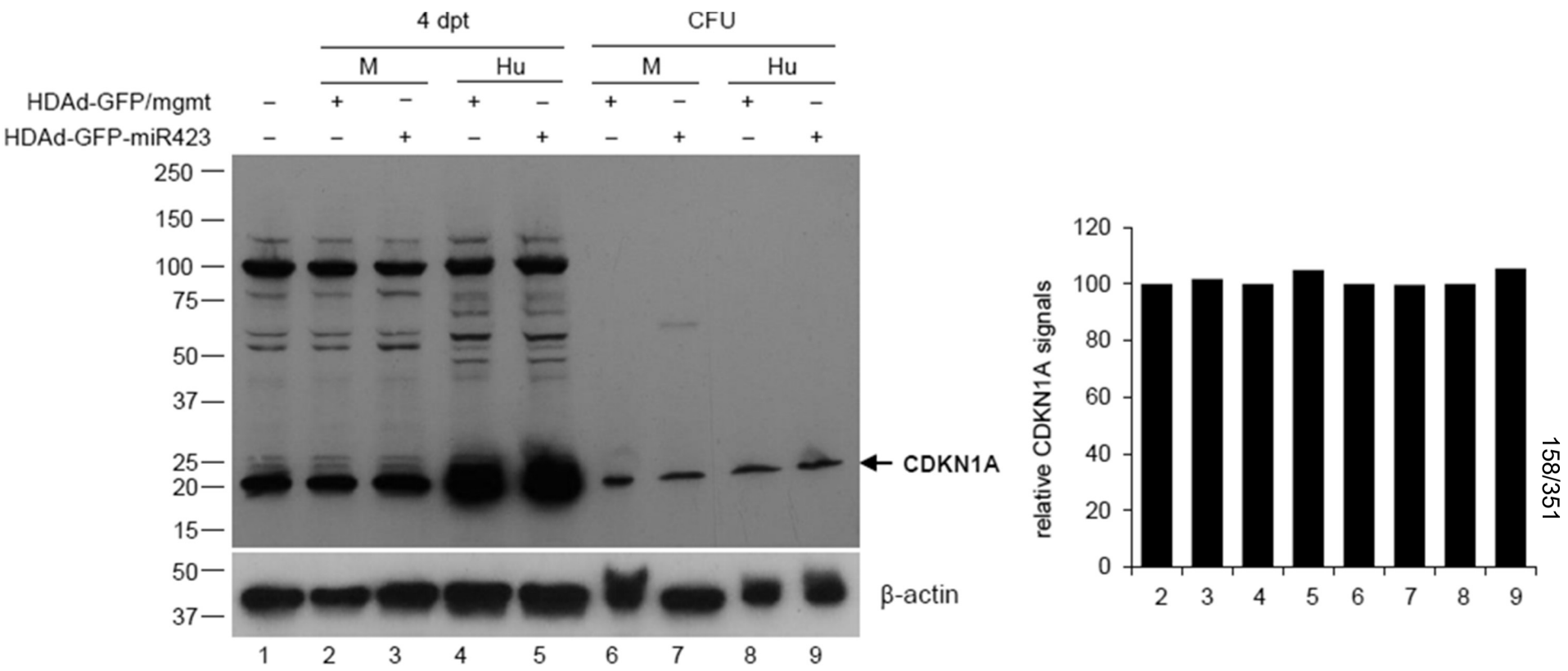

4 dpt
CFU
M
Hu
M
Hu
HDAd-GFP/mgmt
HDAd-GFP-miR423
250
150
100
75
50
37
25
20
15
50
37
CDKN1A
β-actin
1 2 3 4 5 6 7 8 9
relative CDKN1A signals
120
100
80
60
40
20
0
2 3 4 5 6 7 8 9

FIG. 80C

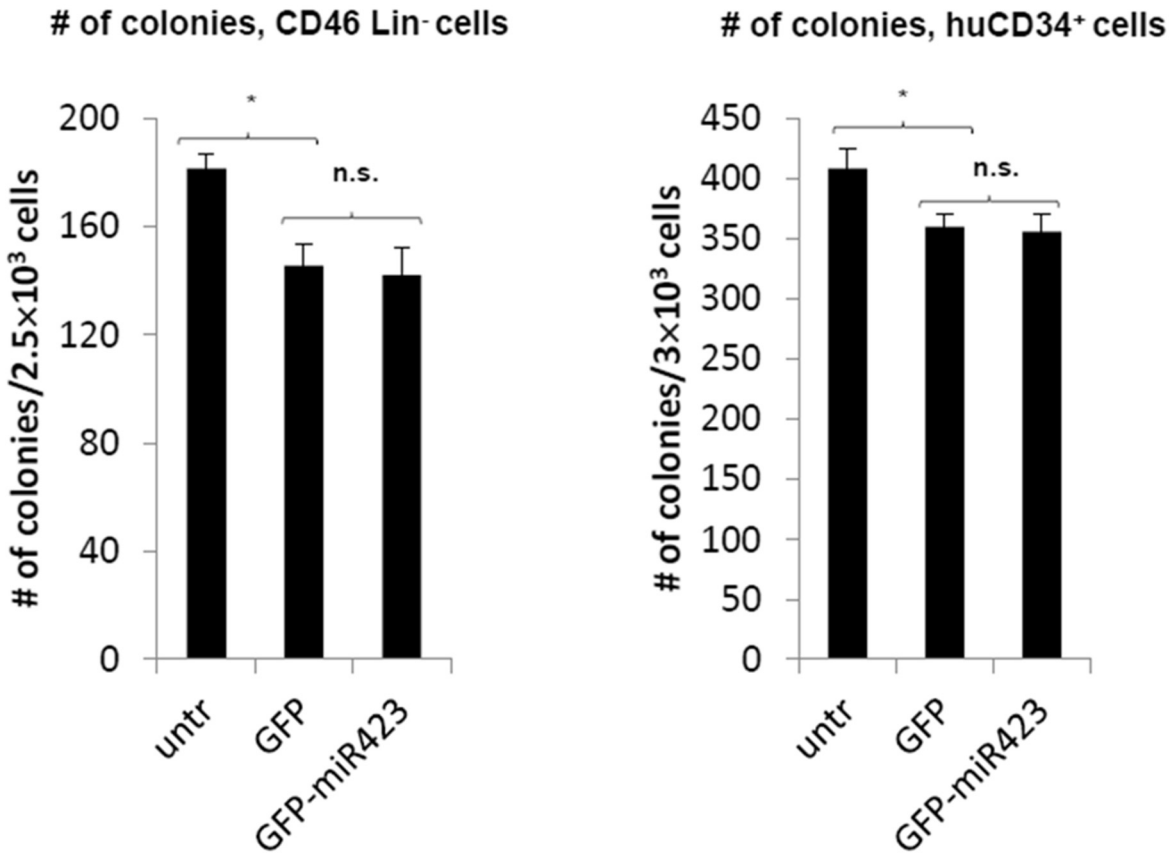
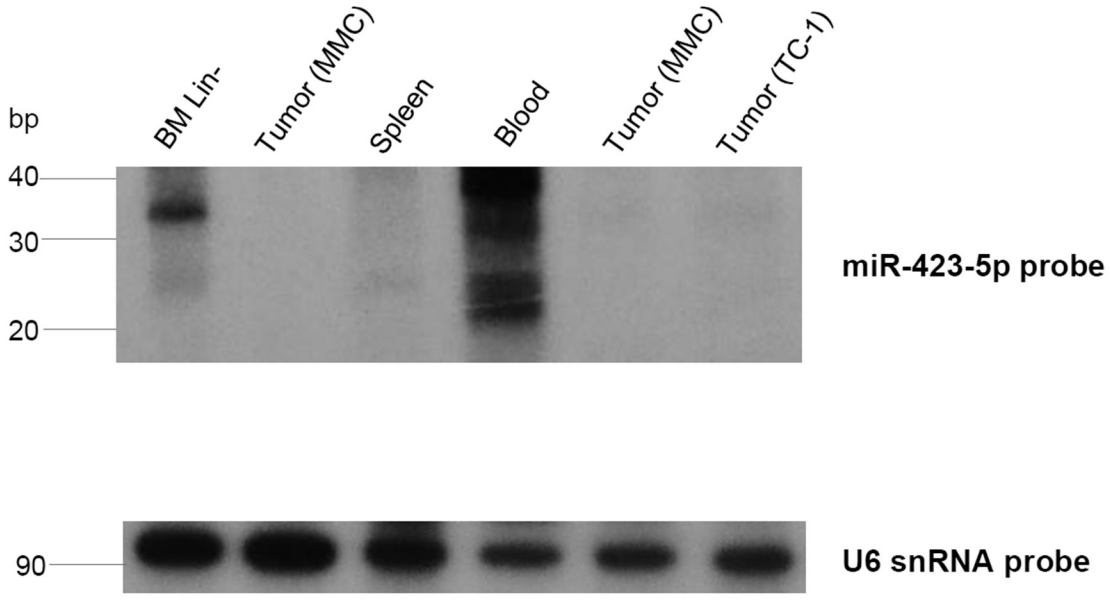


FIG. 81



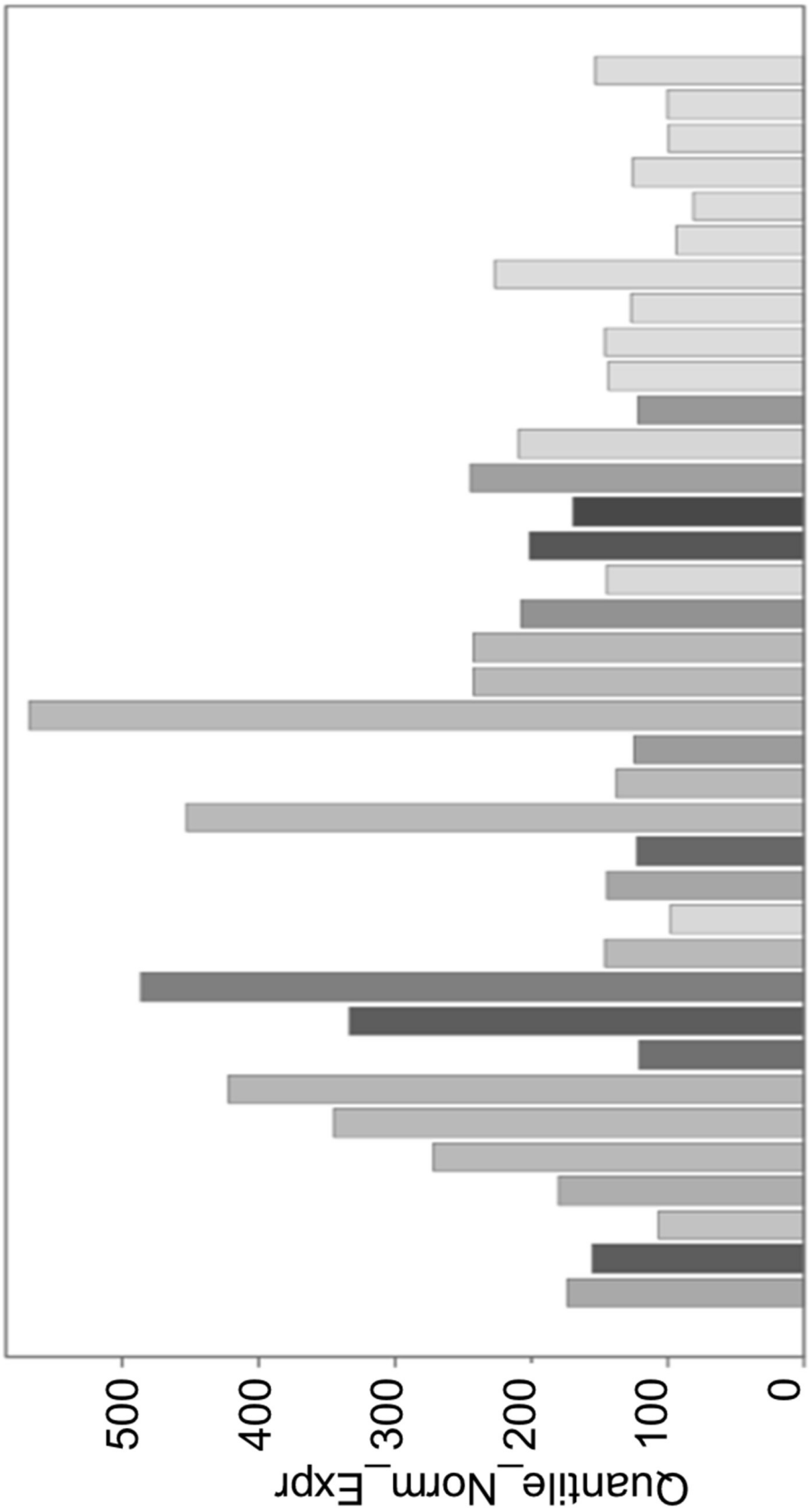


FIG. 82A

FIG. 82B

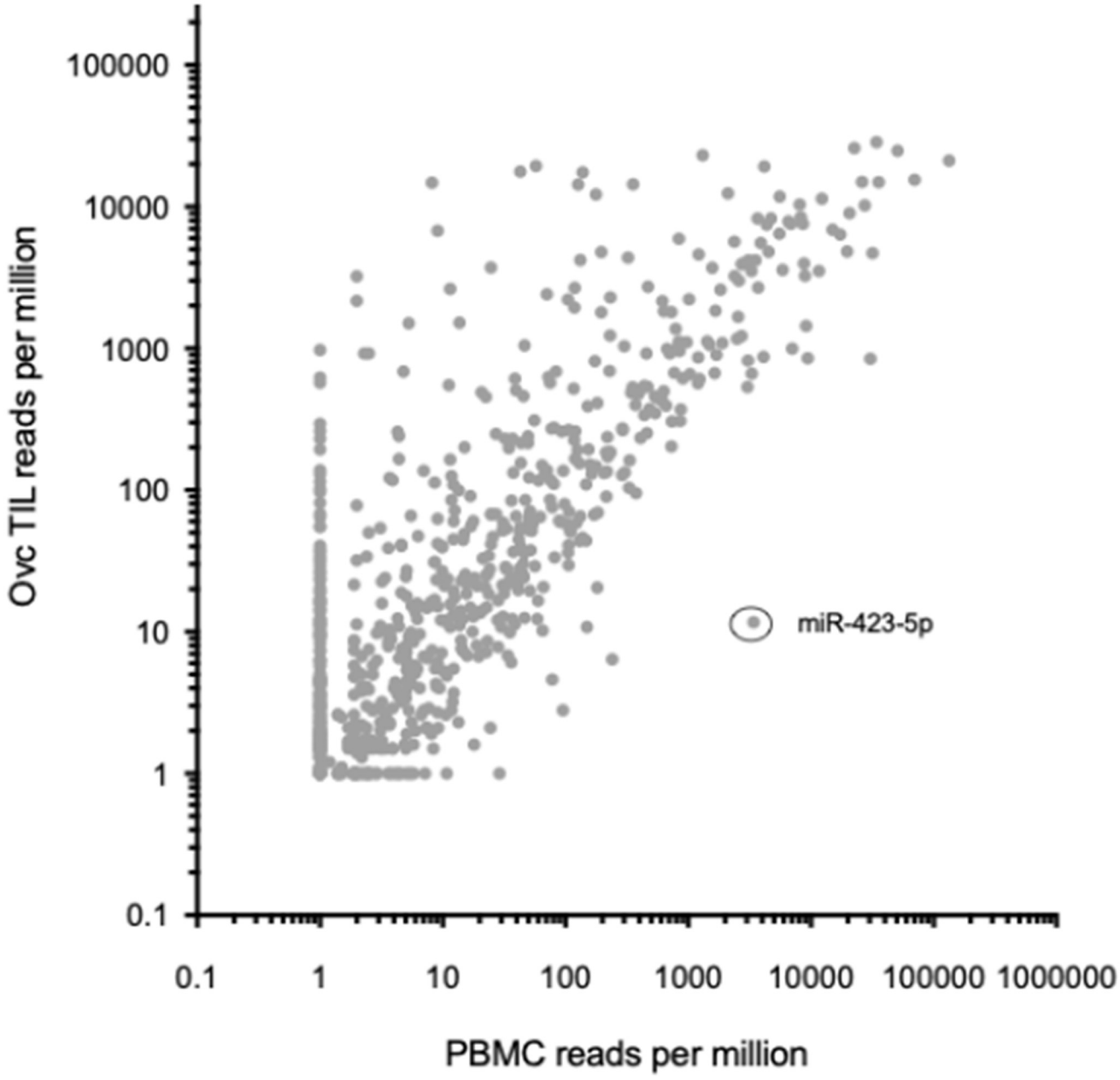

100000
10000
1000
100
10
1
0.1
Ovc TIL reads per million
miR-423-5p
0.1
1
10
100
1000
10000
100000
1000000
PBMC reads per million

FIG. 83A

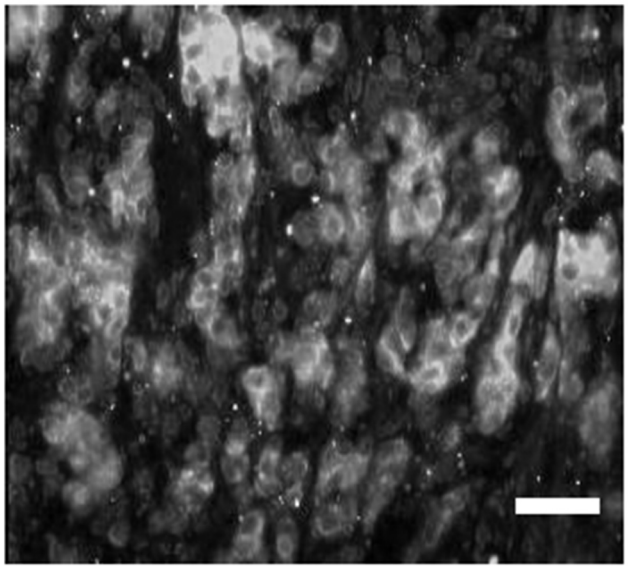


FIG. 83B

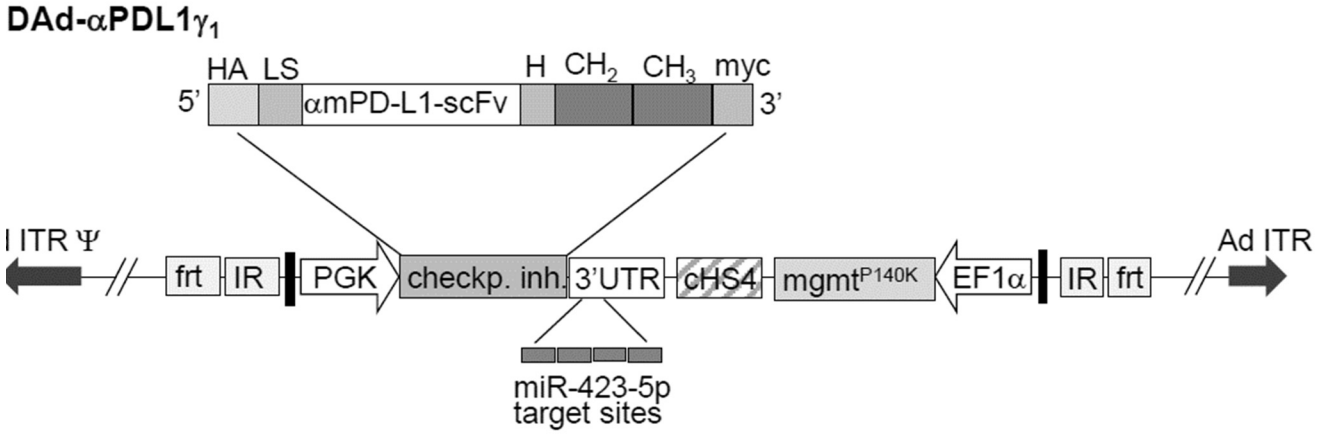


FIG 6C

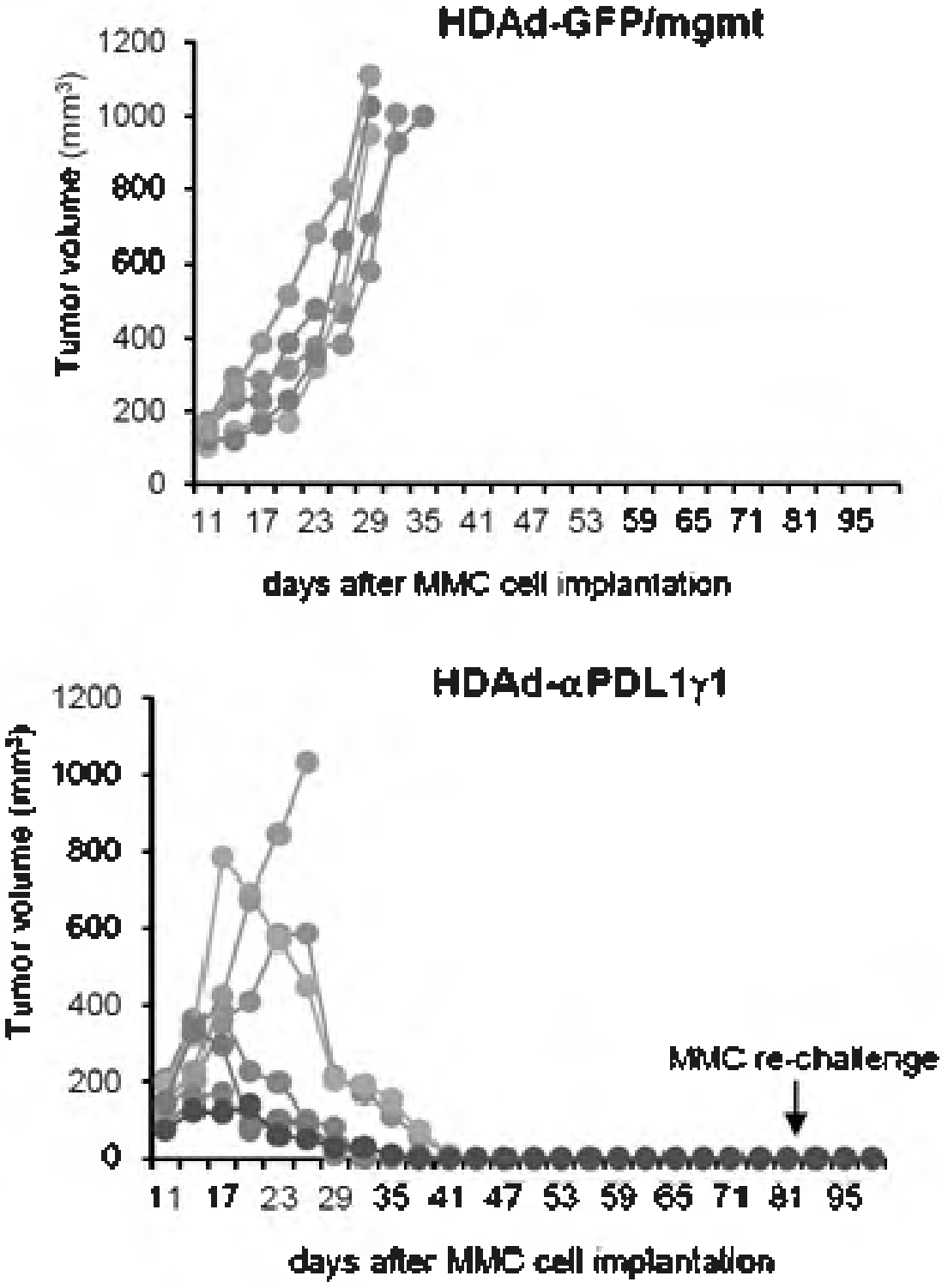

HDAd-GFP/mgmt
Tumor volume (mm³)
1200
1000
800
600
400
200
0
11 17 23 29 35 41 47 53 59 65 71 81 95
days after MMC cell implantation
HDAd-αPDL1γ1
Tumor volume (mm³)
1200
1000
800
600
400
200
0
MMC re-challenge
11 17 23 29 35 41 47 53 59 65 71 81 95
days after MMC cell implantation

FIG. 83D

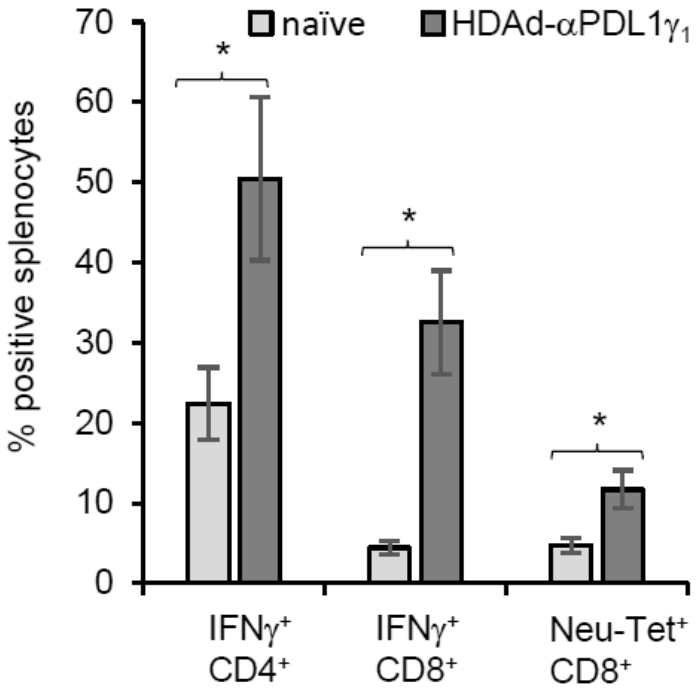


FIG. 83E

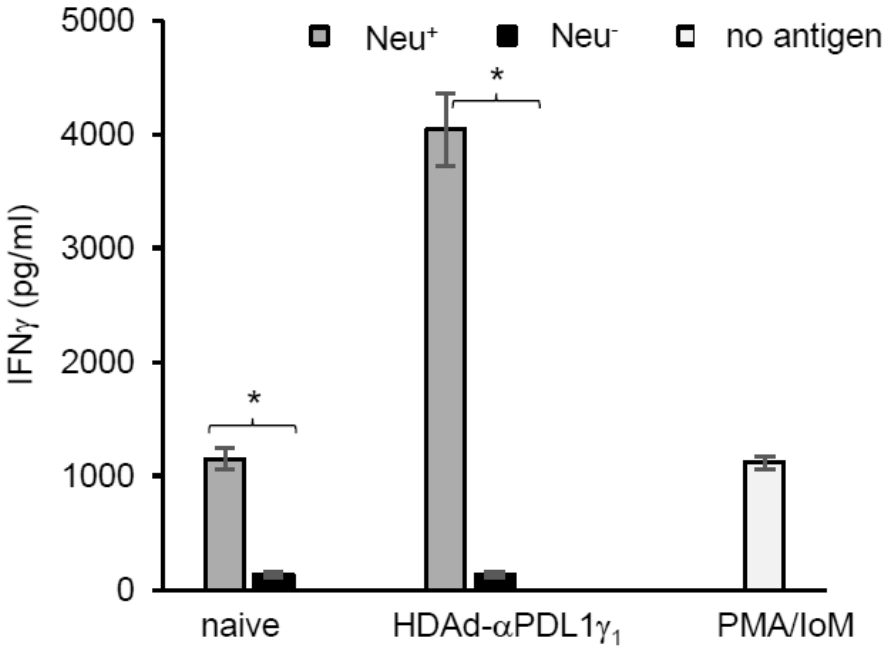
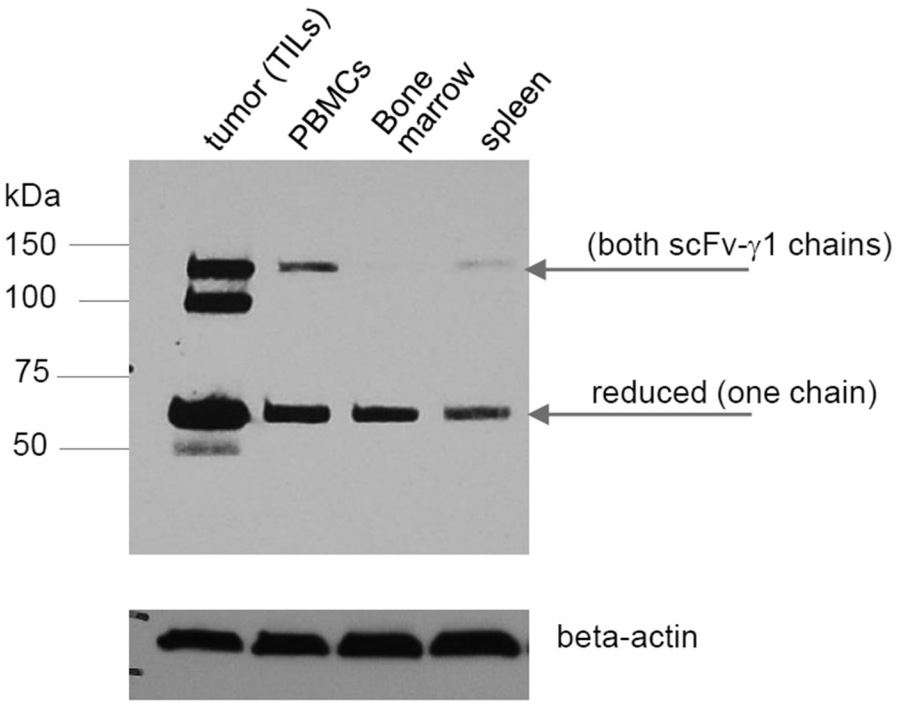


FIG. 84A


tumor (TILs)
PBMCs
Bone marrow
spleen
kDa
150
100
75
50
(both scFv-γ1 chains)
reduced (one chain)
beta-actin

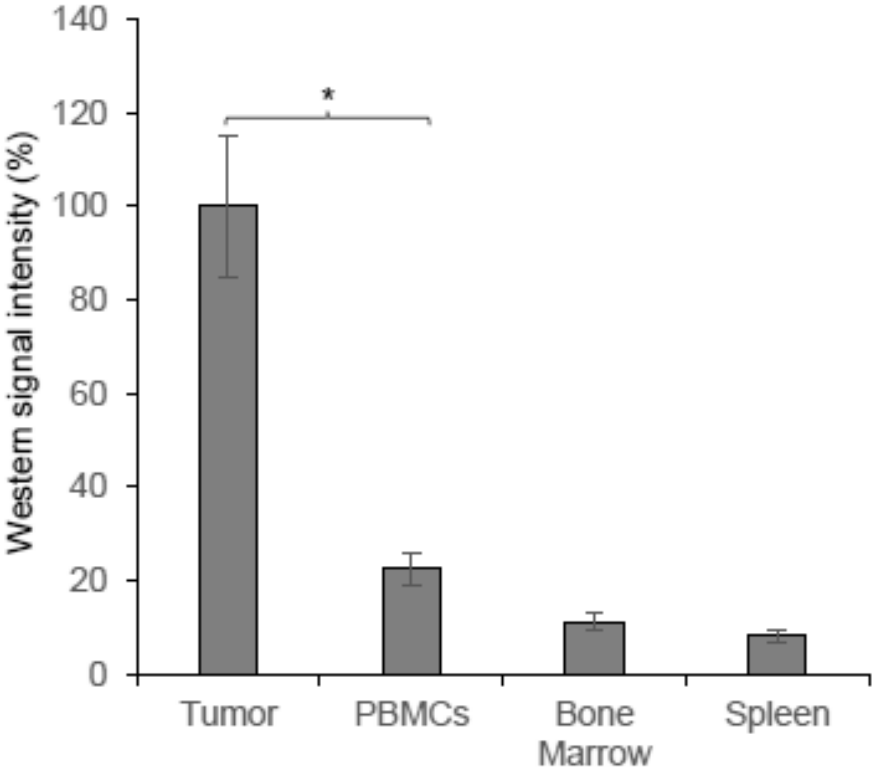

Western signal intensity (%)
140
120
100
80
60
40
20
0
*
Tumor
PBMCs
Bone Marrow
Spleen

FIG. 84B

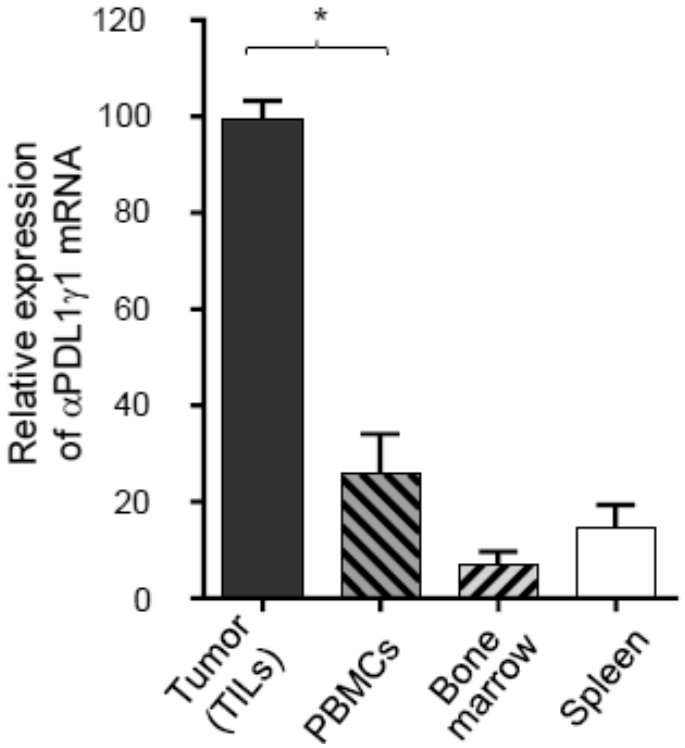


FIG. 84C

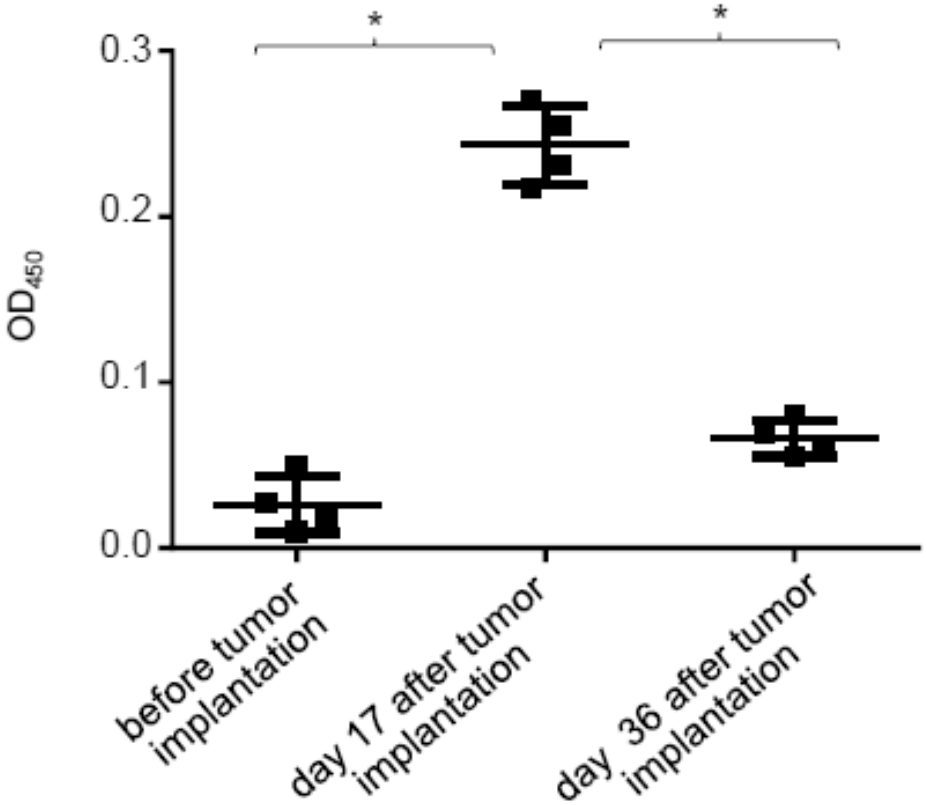


FIG. 85A

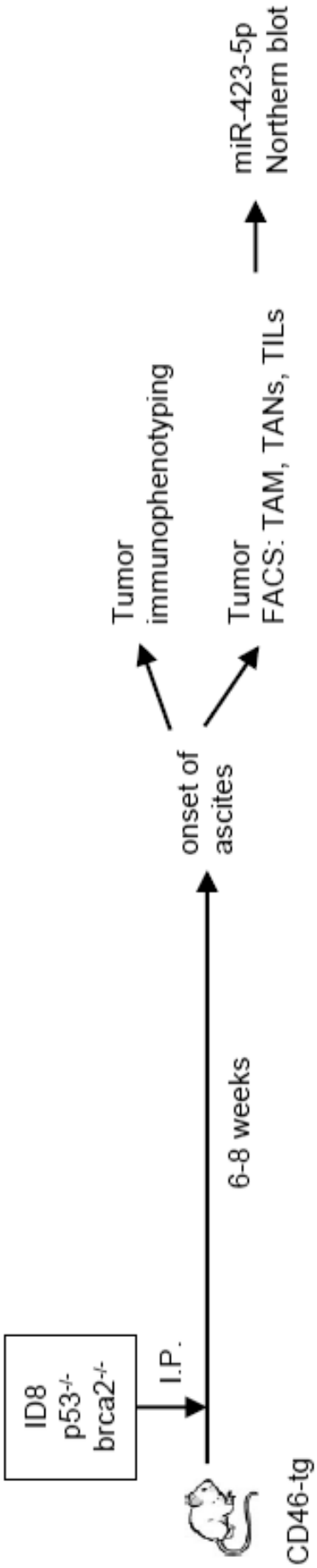

ID8
p53-/-
brca2-/-
I.P.
CD46-tg
6-8 weeks
onset of
ascites
Tumor
immunophenotyping
Tumor
FACS: TAM, TANs, TILs
miR-423-5p
Northern blot

FIG. 85B

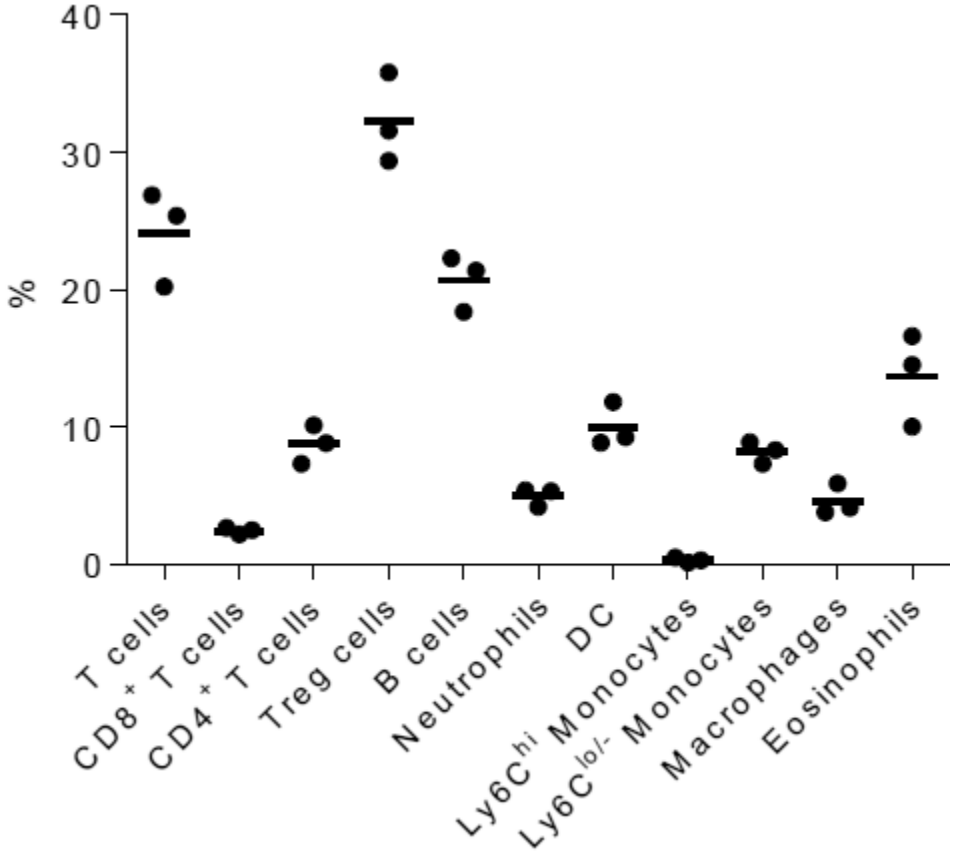

40
30
20
10
0
%
T cells
CD8+ T cells
CD4+ T cells
Treg cells
B cells
Neutrophils
DC
Ly6Chi Monocytes
Ly6Clo/- Monocytes
Macrophages
Eosinophils

FIG. 85C

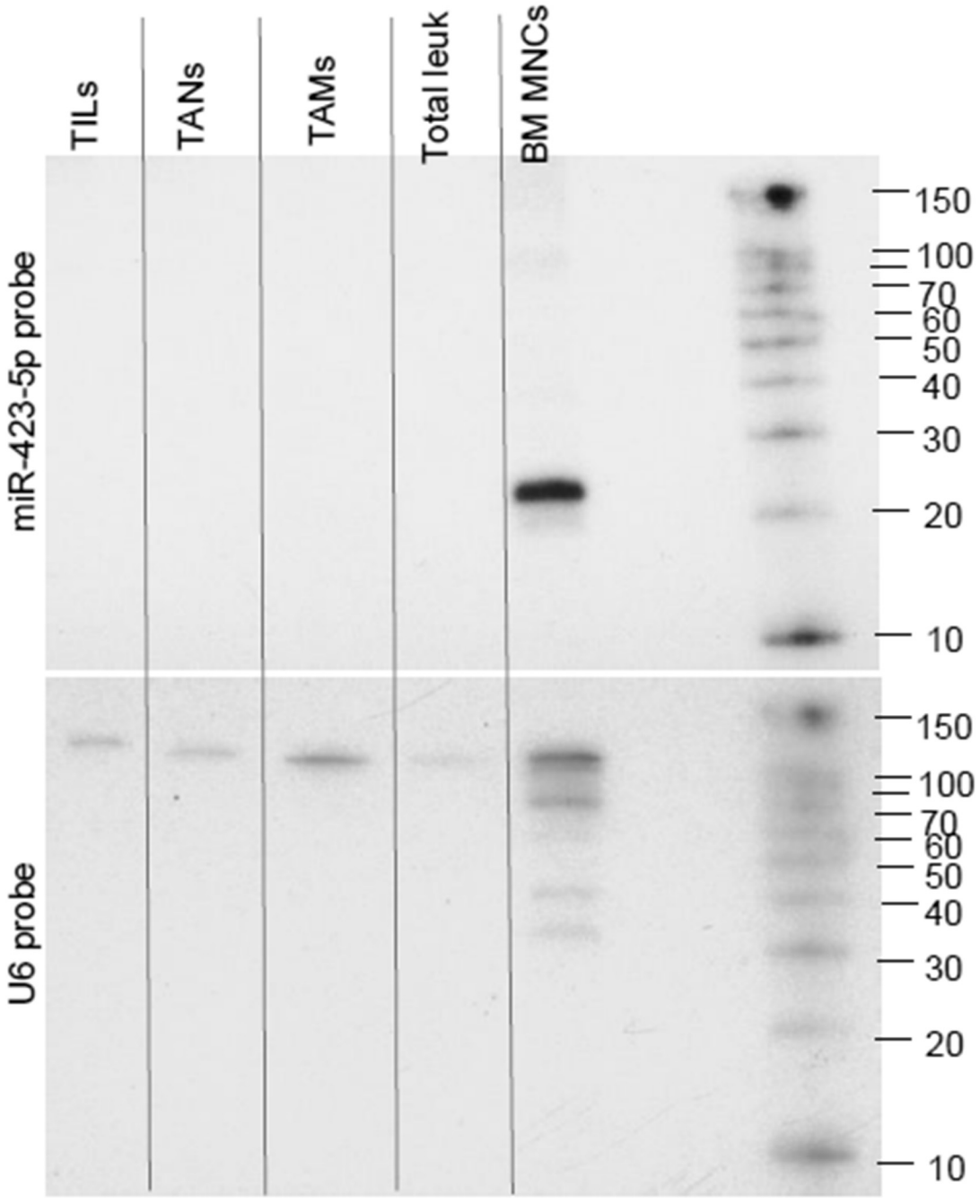

TILs
TANs
TAMs
Total leuk
BM MNCs
miR-423-5p probe
U6 probe
150
100
70
60
50
40
30
20
10

FIG. 85D

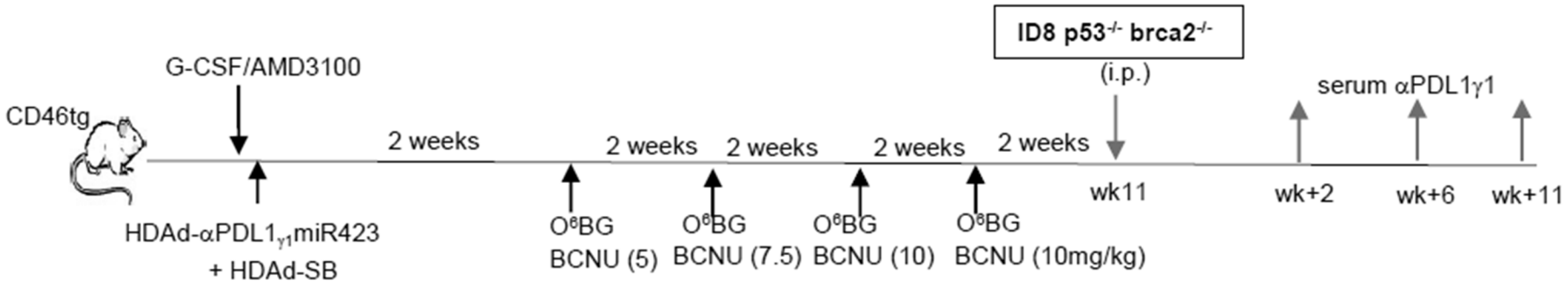


FIG. 85E

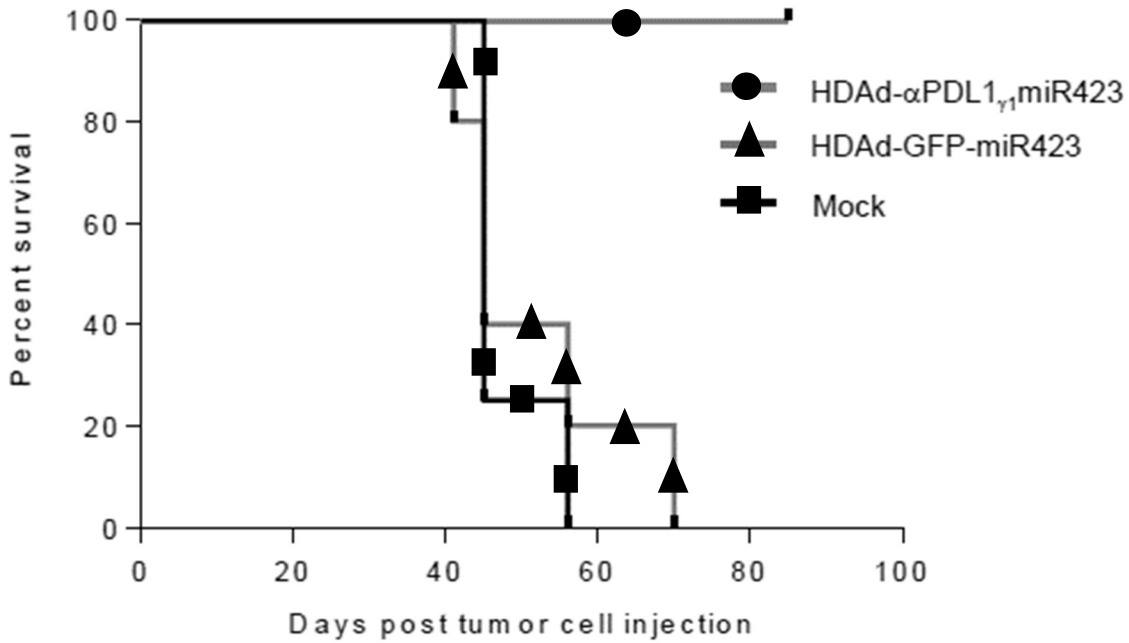


FIG. 6F

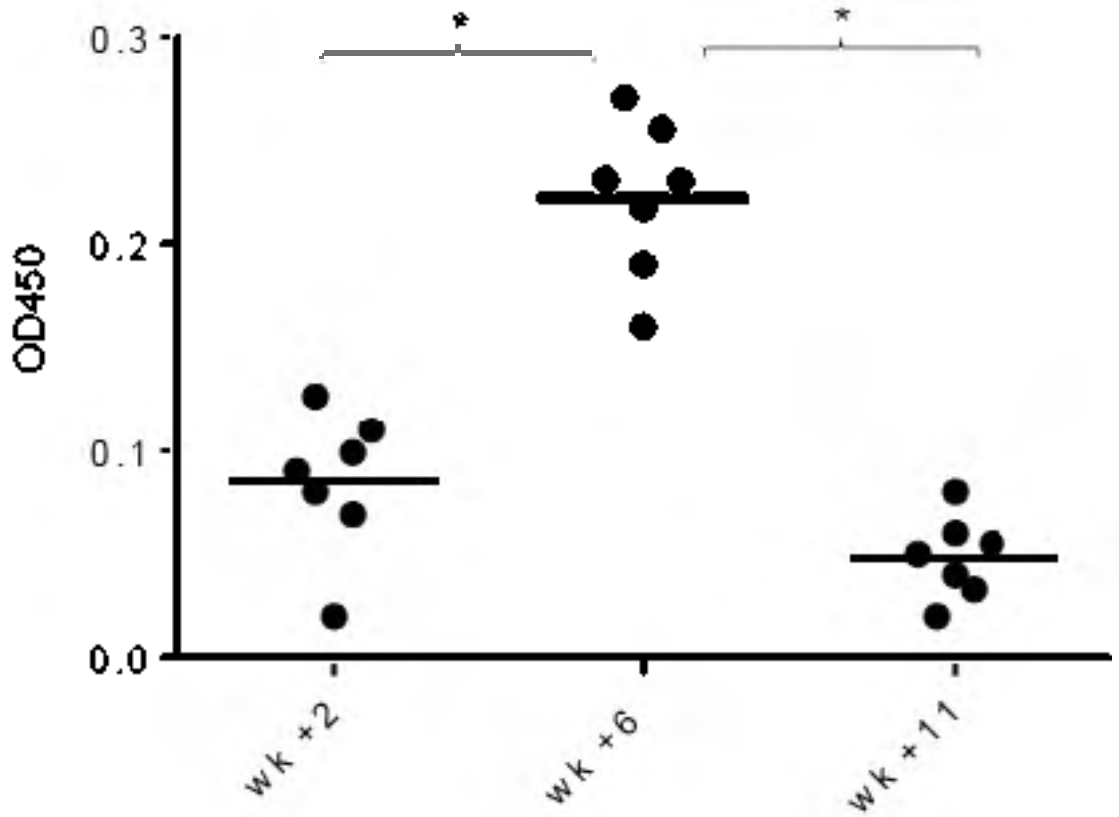


FIG. 86A

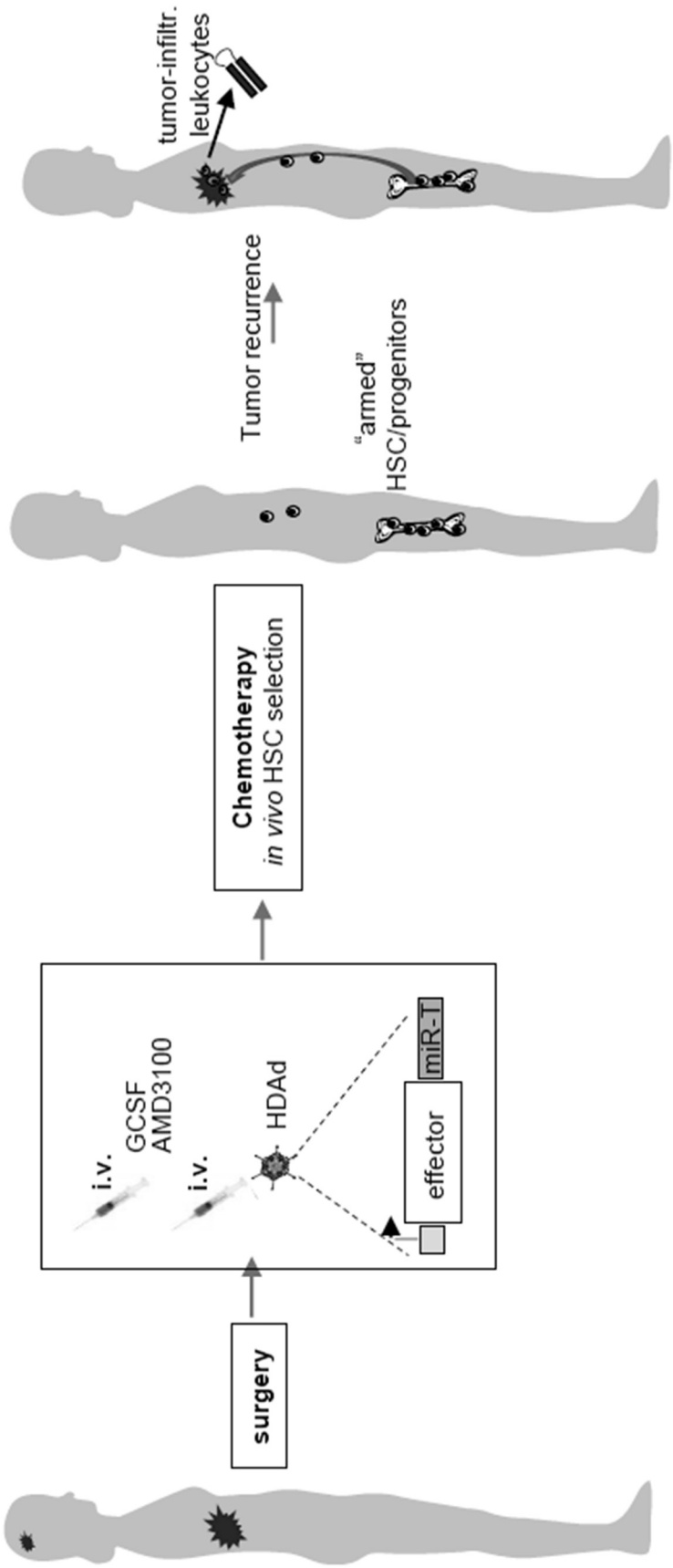

surgery
i.v.
GCSF
AMD3100
i.v.
HDAd
effector
miR-T
Chemotherapy
in vivo HSC selection
"armed"
HSC/progenitors
Tumor recurrence
tumor-infiltr.
leukocytes

FIG. 86B

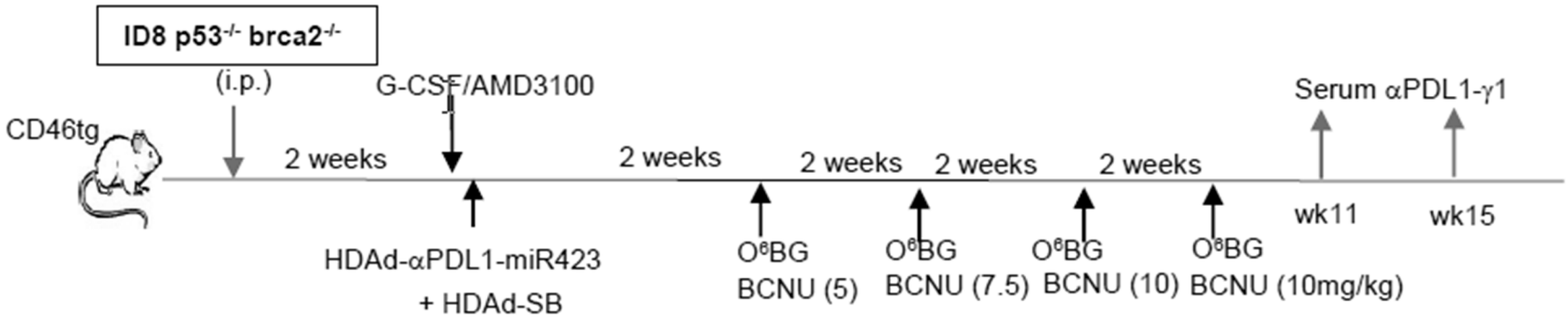

ID8 p53-/- brca2-/-
(i.p.)
G-CSF/AMD3100
CD46tg
2 weeks
2 weeks
2 weeks
2 weeks
2 weeks
Serum αPDL1-γ1
wk11
wk15
HDAd-αPDL1-miR423
+ HDAd-SB
O6BG
BCNU (5)
O6BG
BCNU (7.5)
O6BG
BCNU (10)
O6BG
BCNU (10mg/kg)

FIG. 86C

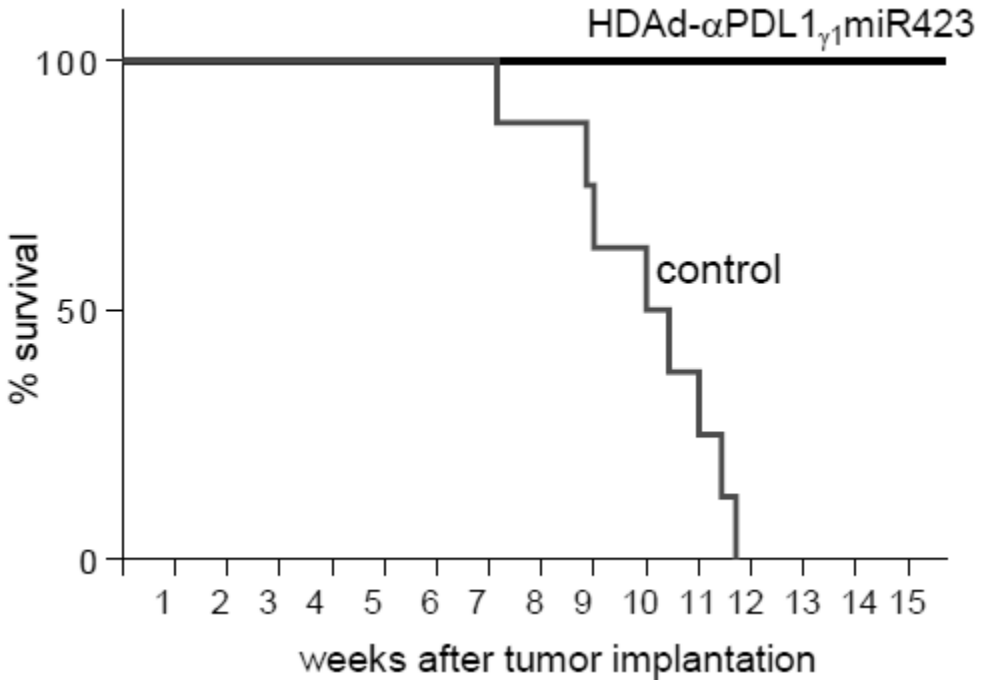


FIG. 86D

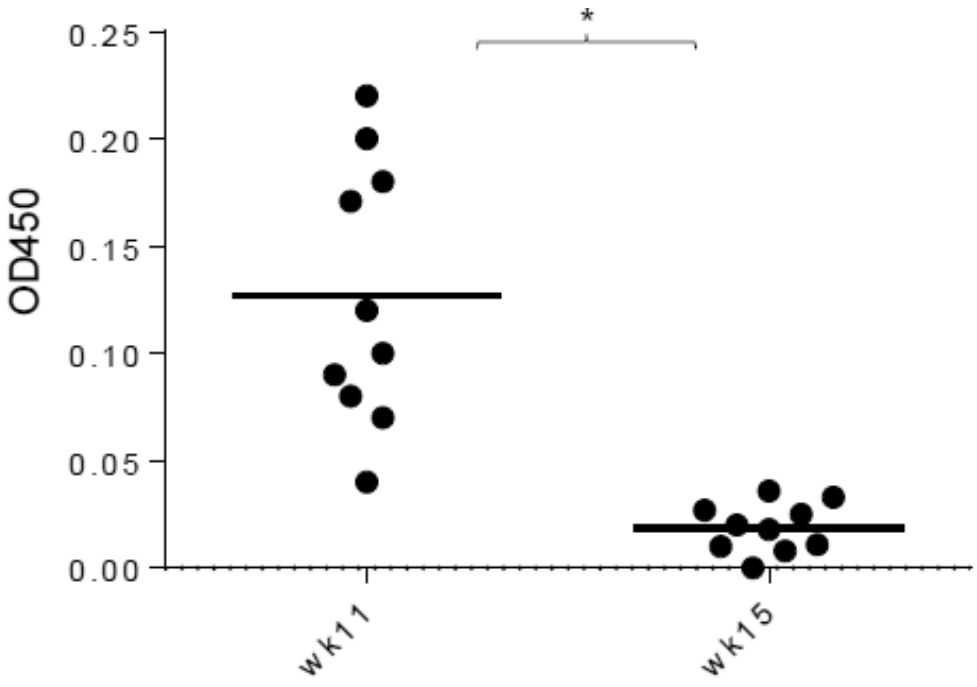


FIG. 87A

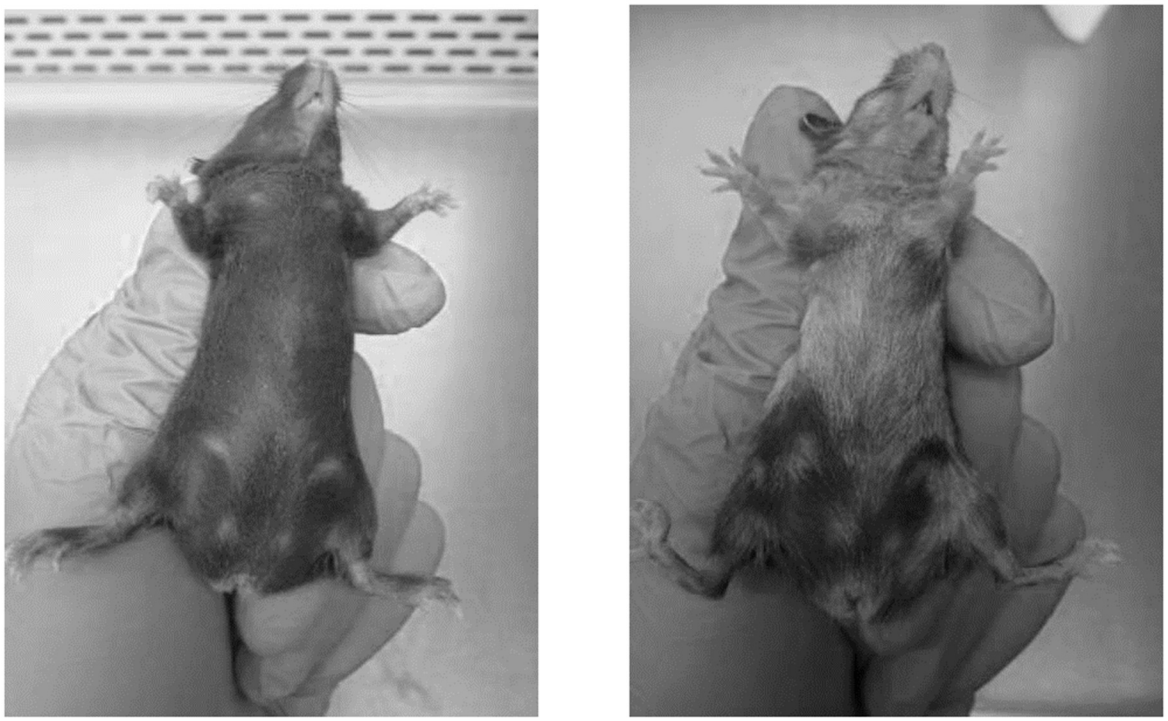


FIG. 87B

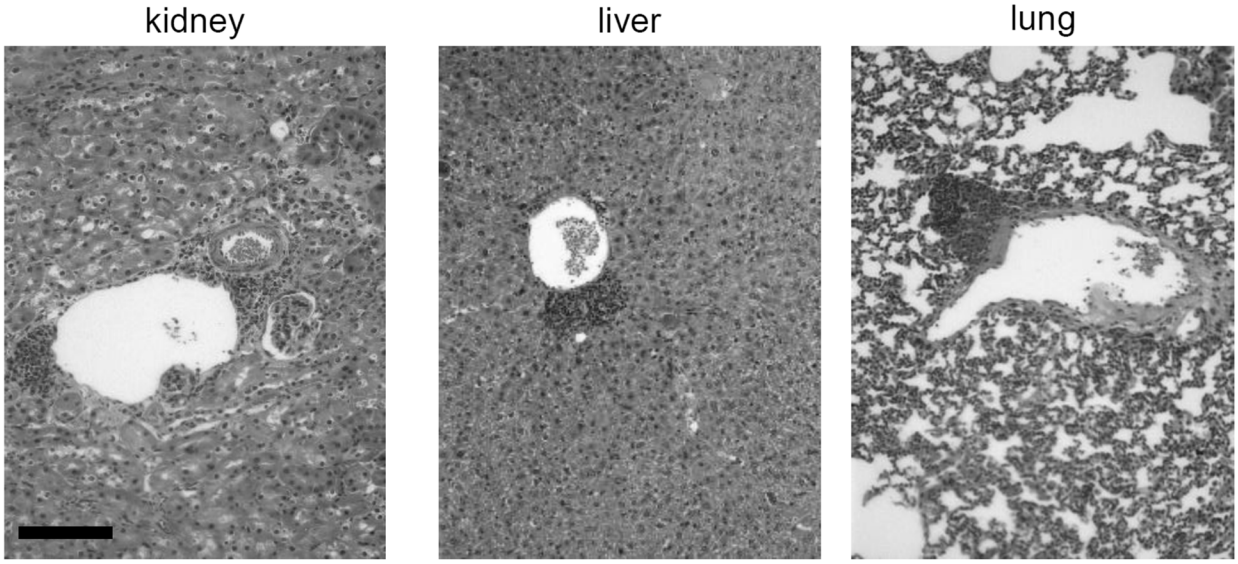


FIG. 88A

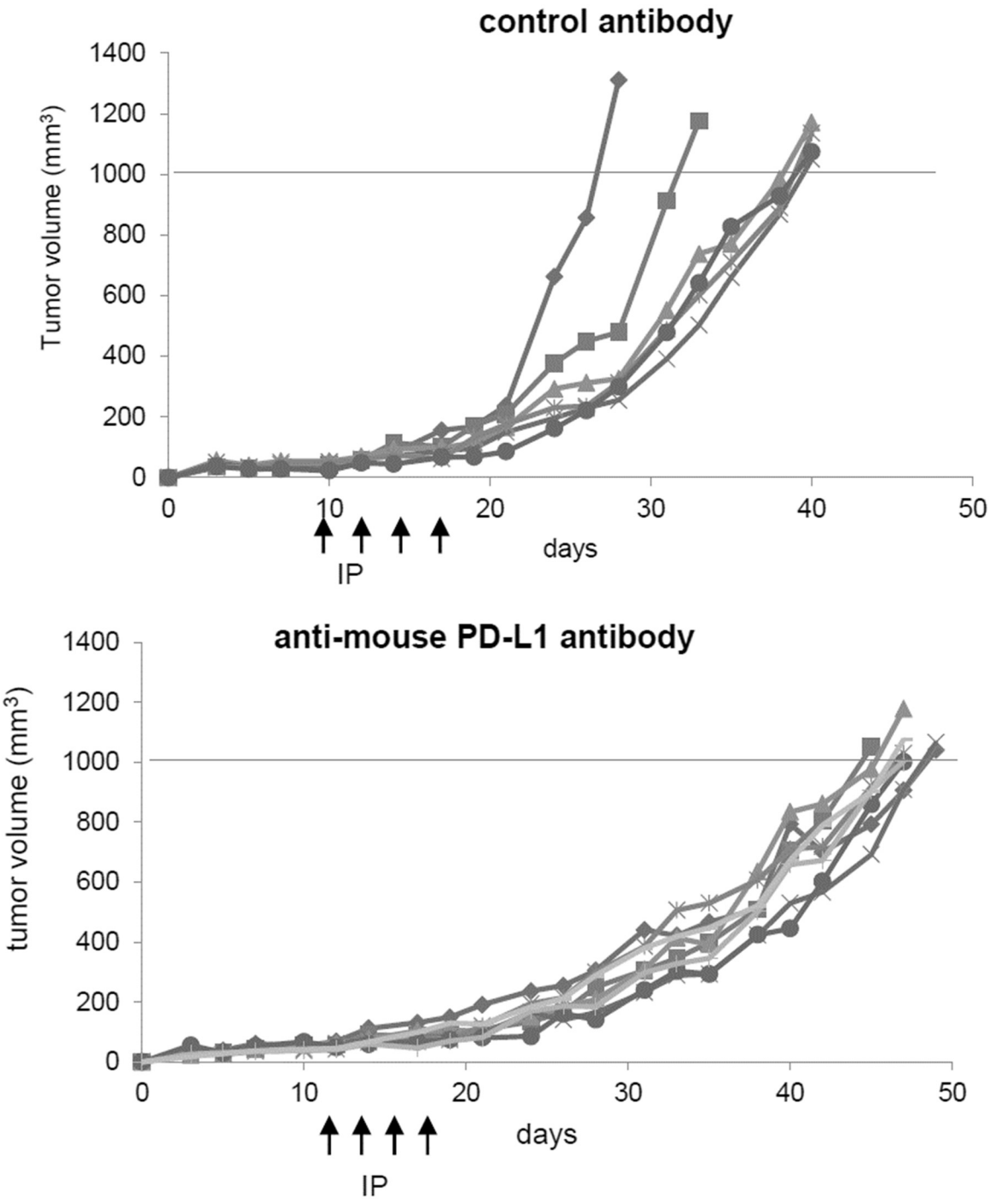

control antibody
Tumor volume (mm³)
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
days
IP
anti-mouse PD-L1 antibody
tumor volume (mm³)
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
days
IP

FIG. 88B

FIG. 88B

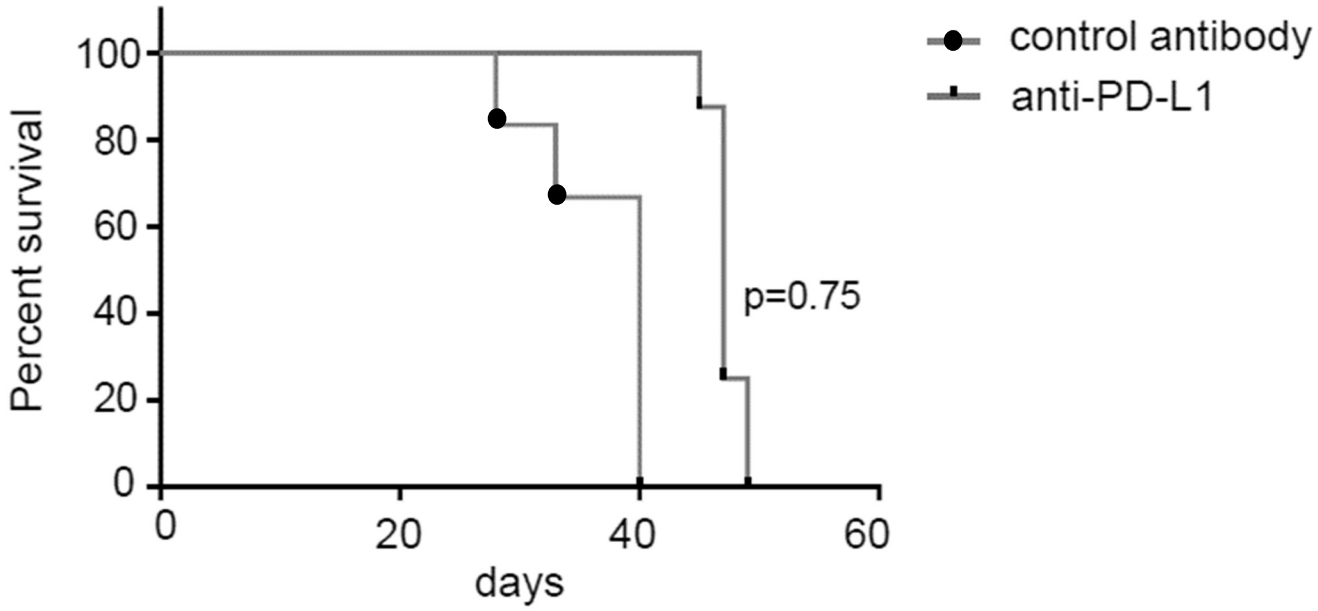


FIG. 88C

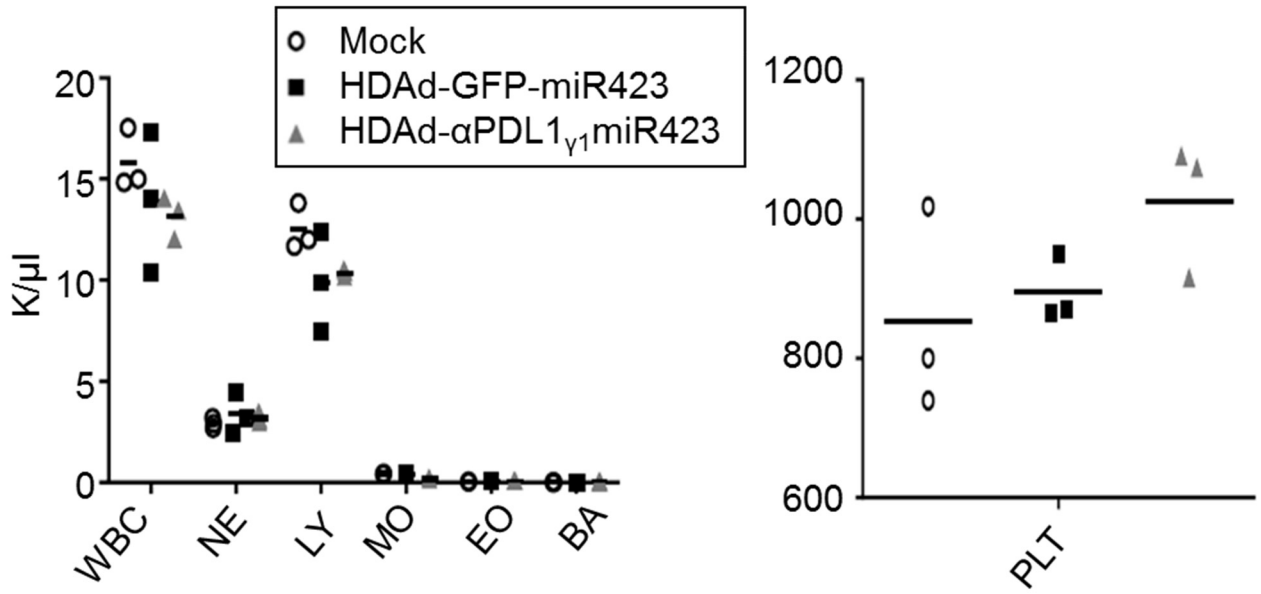


FIG. 88D

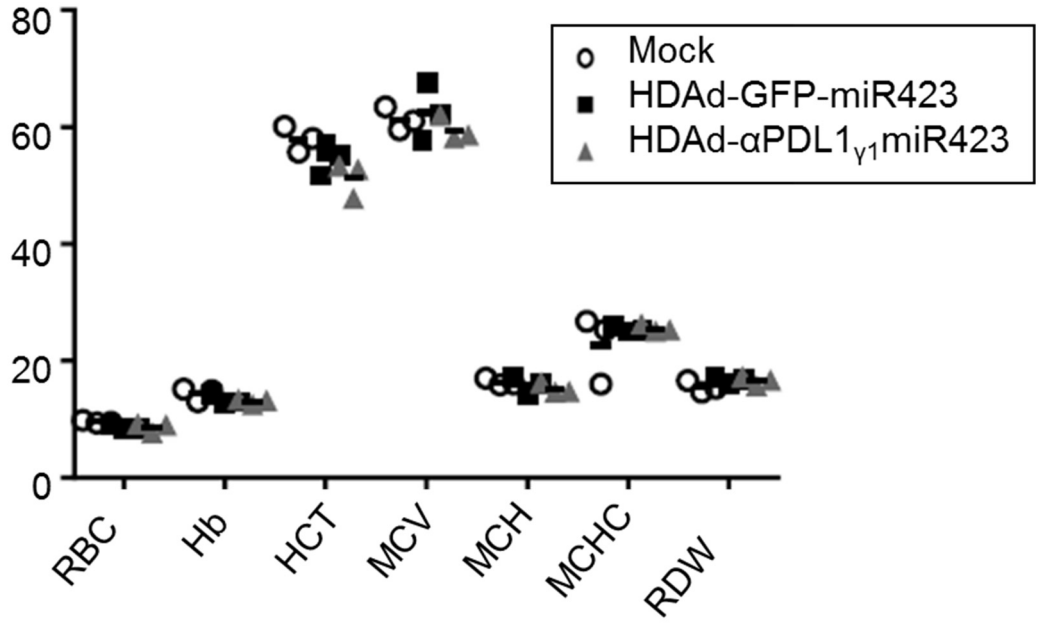


FIG. 88E

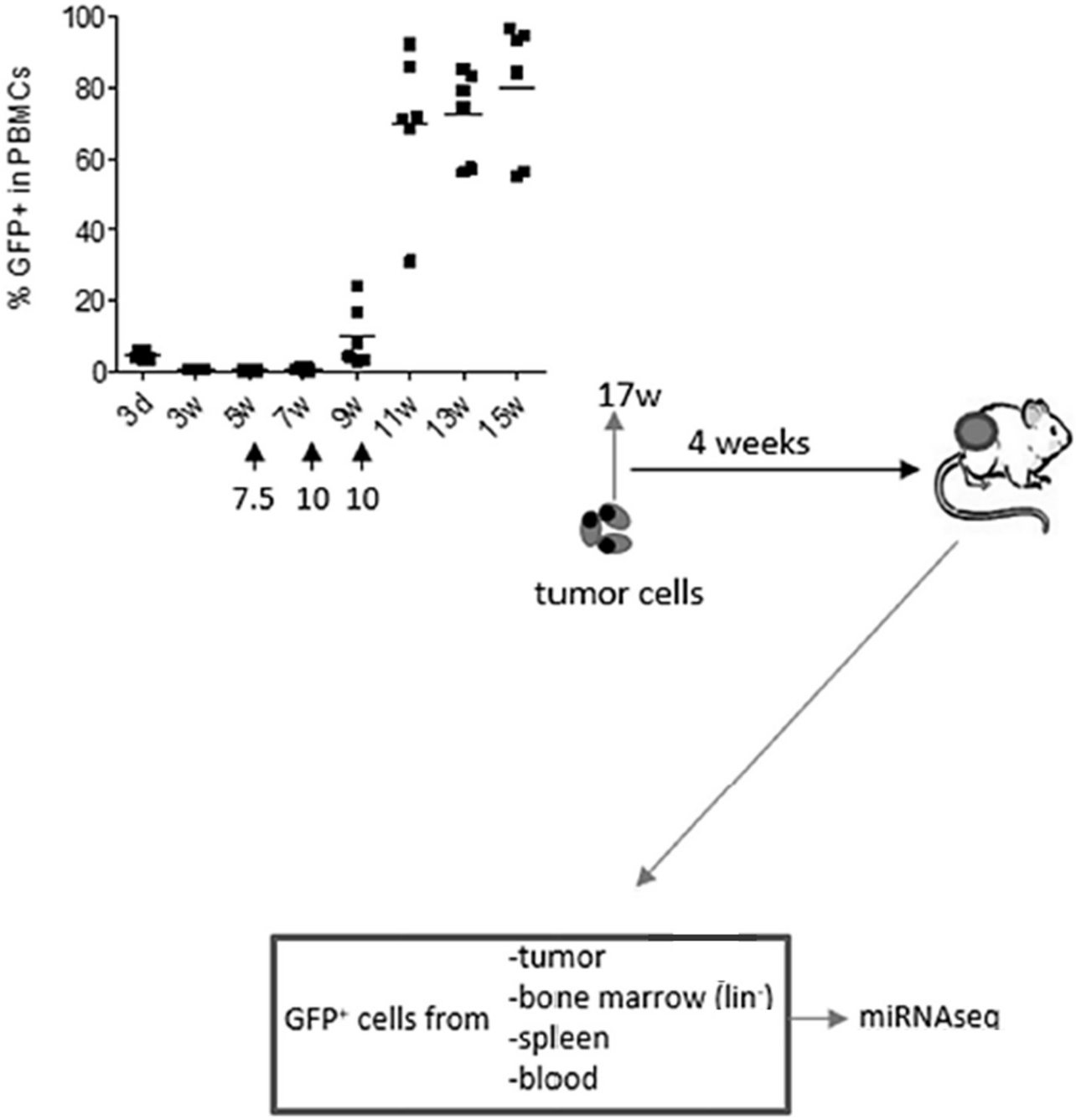
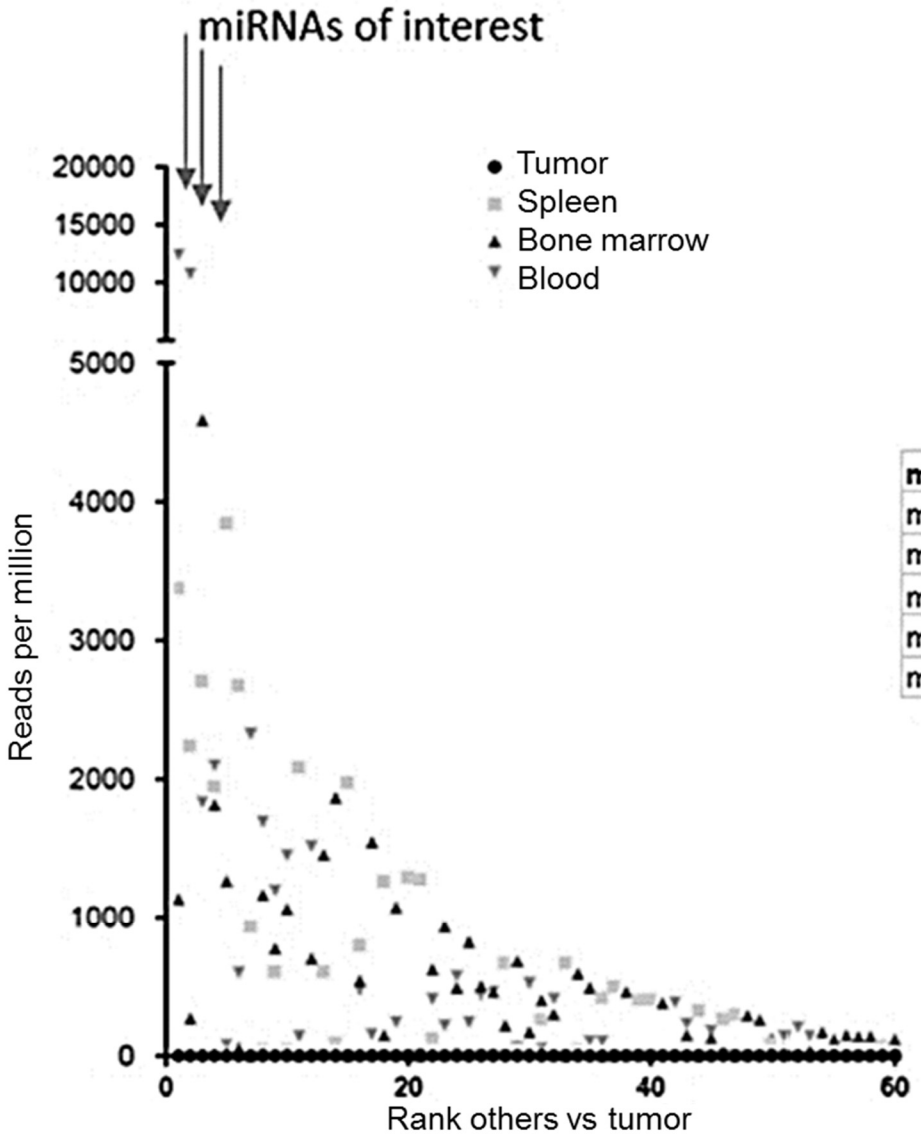

% GFP+ in PBMCs
100
80
60
40
20
0
3d
3w
5w
7w
9w
11w
13w
15w
7.5
10
10
17w
4 weeks
tumor cells
GFP+ cells from
-tumor
-bone marrow (lin-)
-spleen
-blood
miRNAseq

FIG. 88E cont'd



mIRNA	Tumor	Spleen	BM	Blood
mir-423	1.00	7563.82	1263.47	13350.56
mir-24-2	1.00	5019.65	302.65	11586.12
mir-181c	1.00	6065.20	5106.75	1970.61
mir-125a	1.00	8609.37	1408.71	83.07
mir-15a	1.00	4357.46	2023.19	2257.84

FIG. 88F

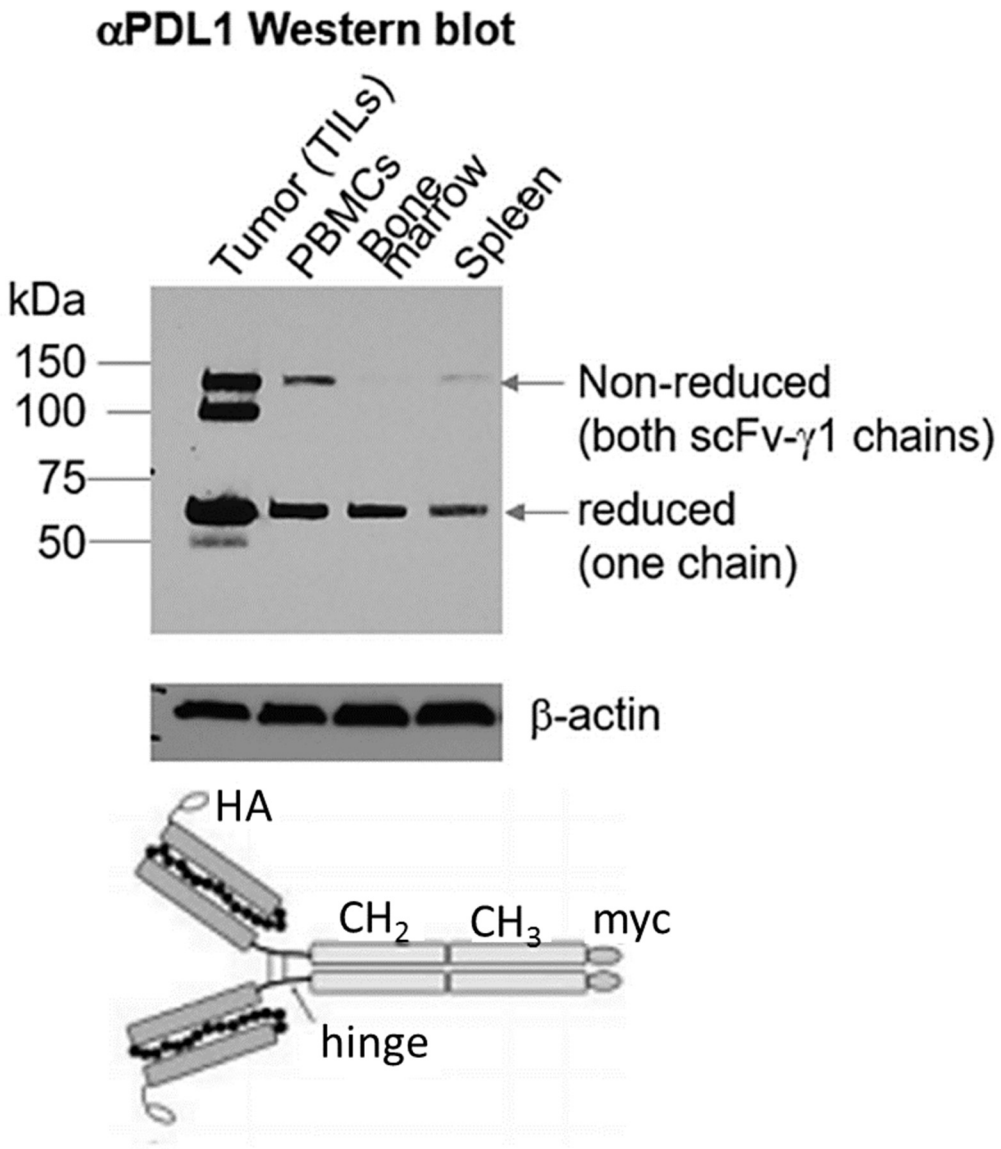
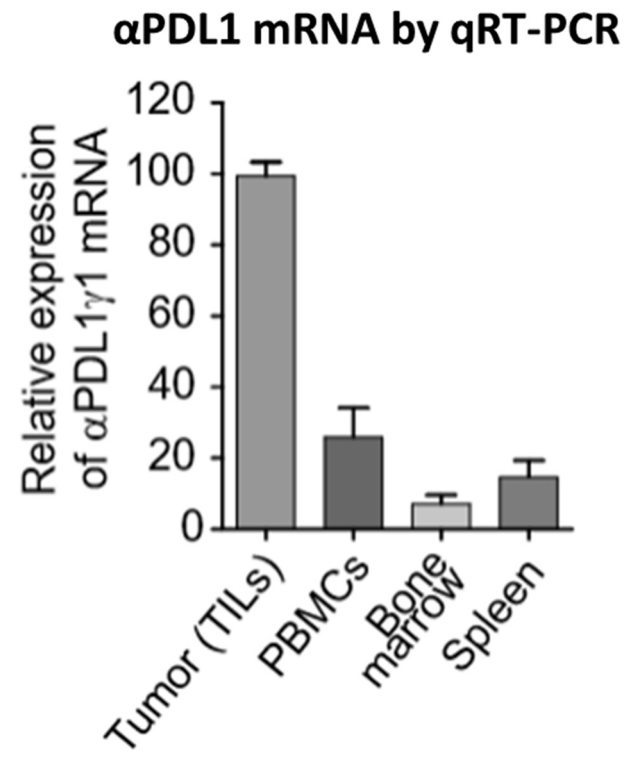

αPDL1 Western blot
Tumor (TILs)
PBMCs
Bone marrow
Spleen
kDa
150
100
75
50
Non-reduced
(both scFv-γ1 chains)
reduced
(one chain)
β-actin
HA
CH2
CH3
myc
hinge

FIG. 88F cont'd



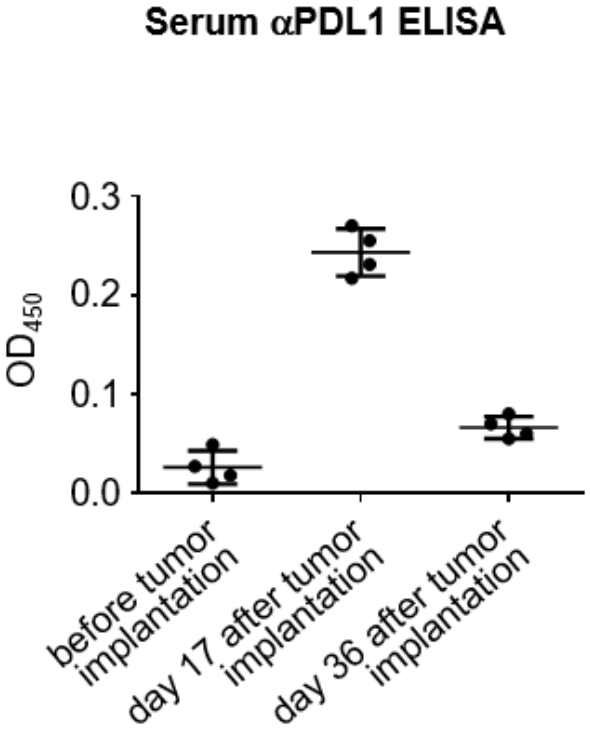


FIG. 88G

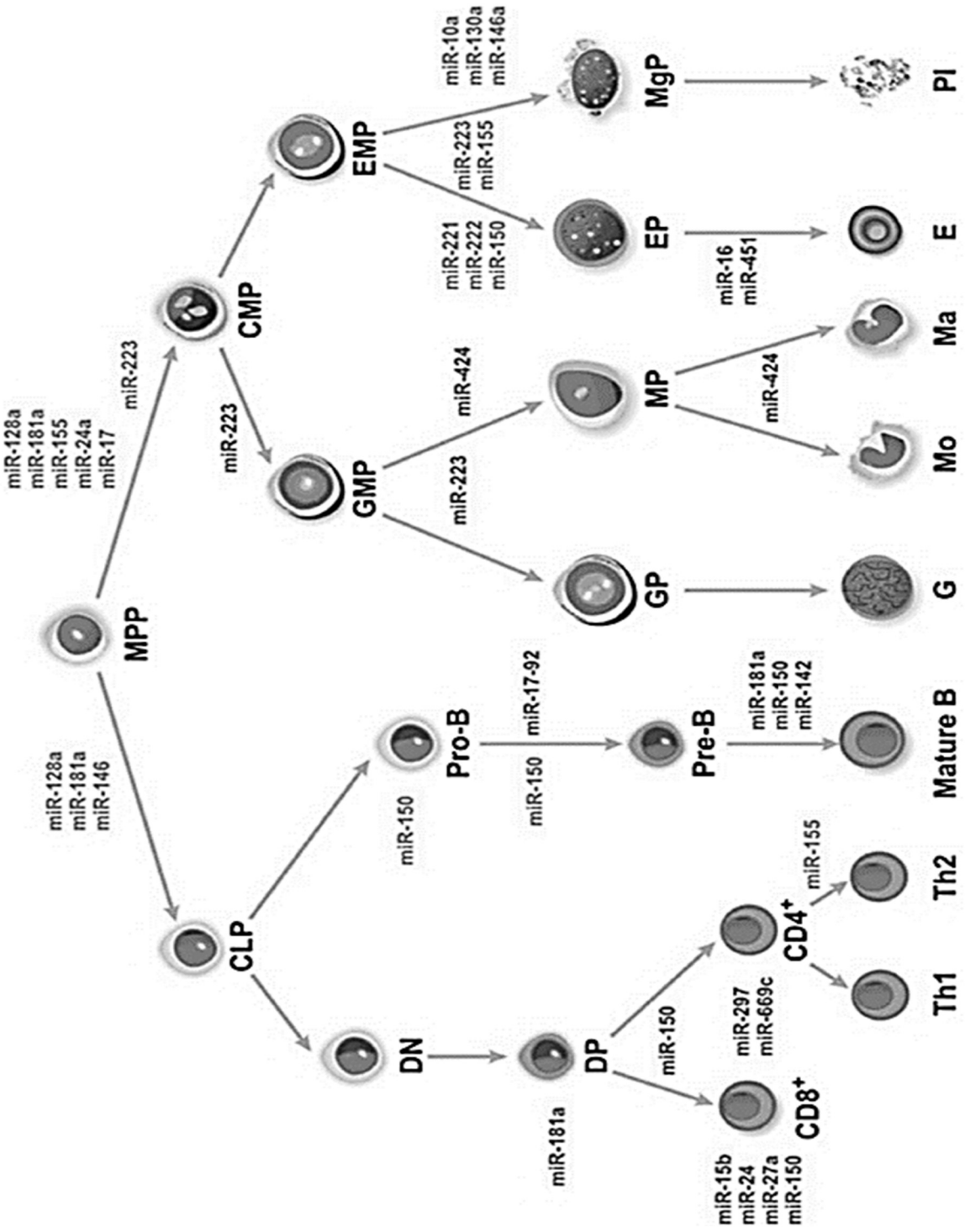

MPP
CLP
CMP
GMP
MP
GP
Mo
Ma
G
EMP
EP
MgP
E
Pl
DN
DP
CD4+
CD8+
Th1
Th2
Pro-B
Pre-B
Mature B
miR-128a
miR-181a
miR-155
miR-24a
miR-17
miR-223
miR-128a
miR-181a
miR-146
miR-223
miR-424
miR-223
miR-424
miR-10a
miR-130a
miR-146a
miR-223
miR-155
miR-221
miR-222
miR-150
miR-16
miR-451
miR-150
miR-17-92
miR-150
miR-181a
miR-150
miR-142
miR-155
miR-150
miR-297
miR-669c
miR-181a
miR-15b
miR-24
miR-27a
miR-150

FIG. 88G cont'd

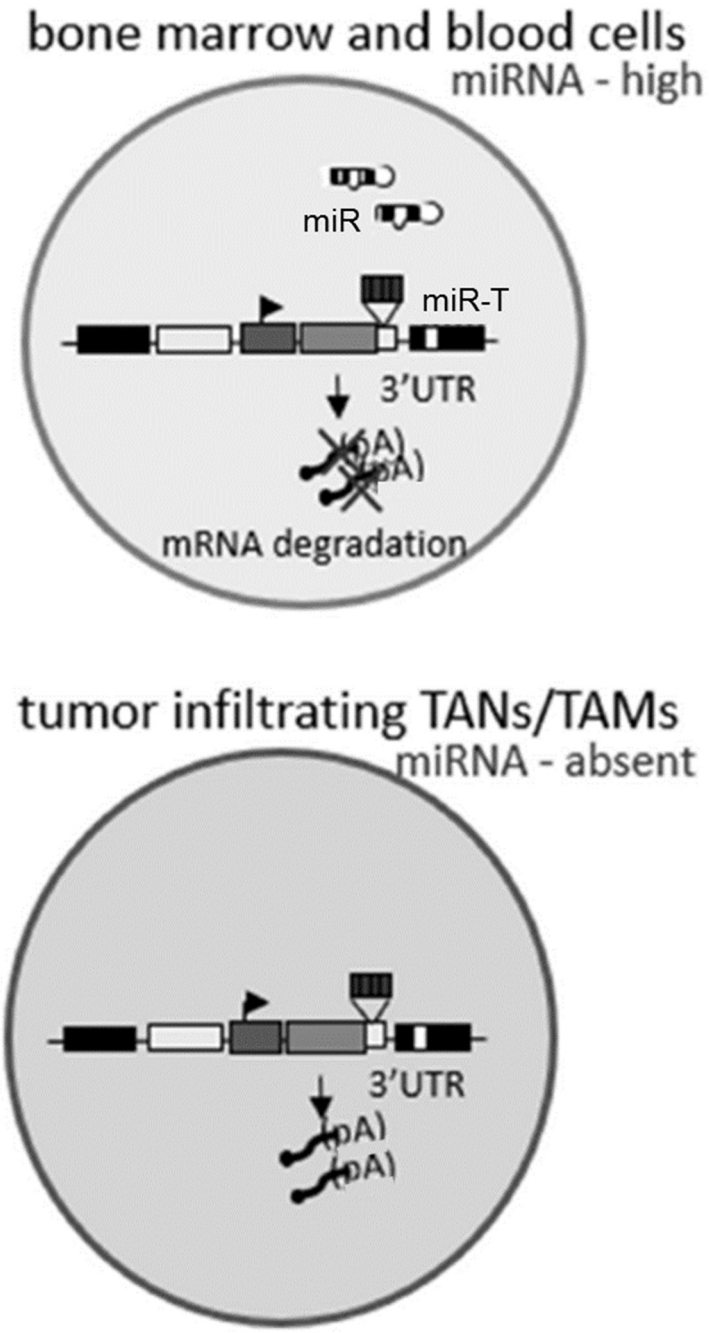


FIG. 88H

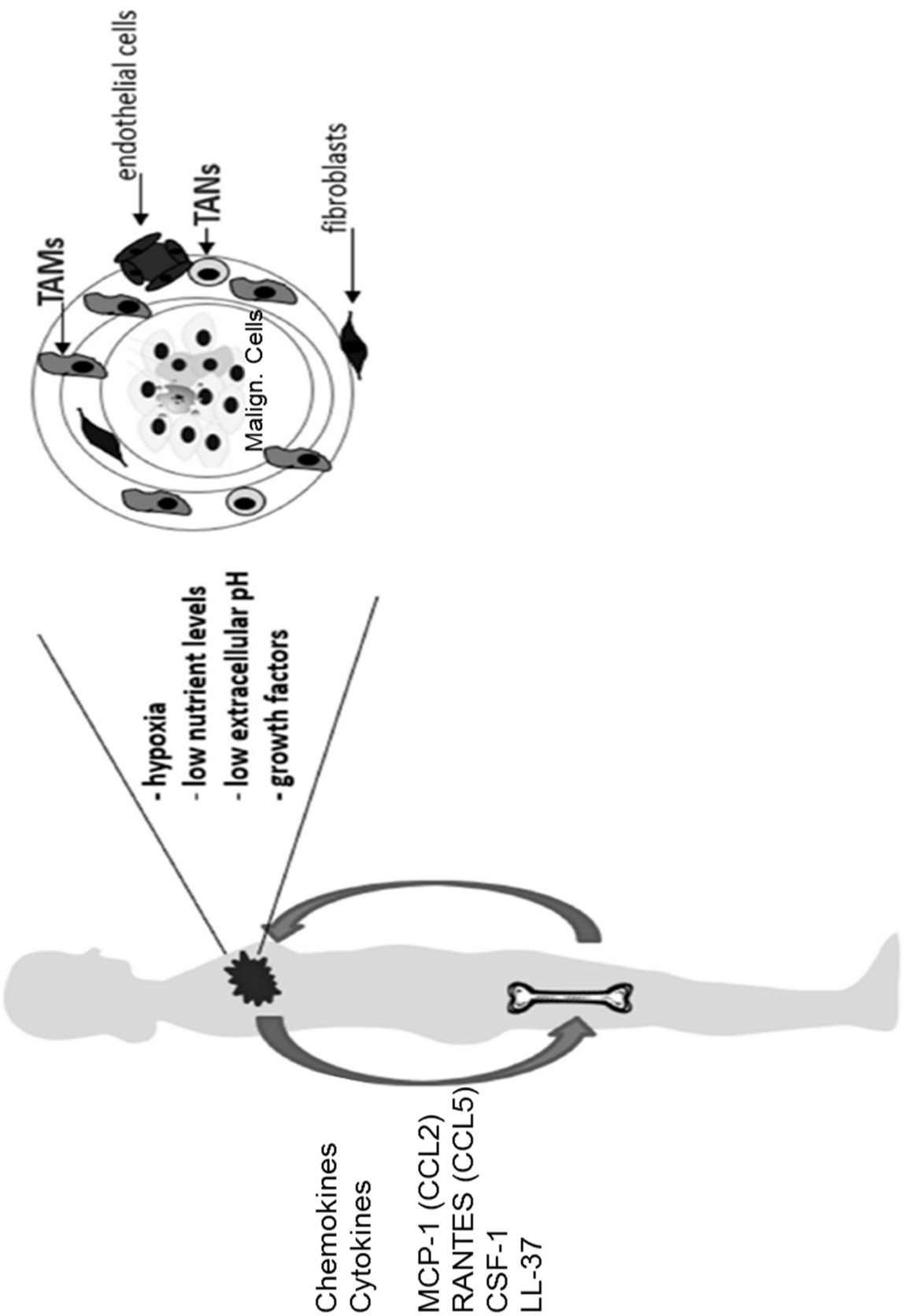

endothelial cells
TANs
TAMs
fibroblasts
Malign. Cells
- hypoxia
- low nutrient levels
- low extracellular pH
- growth factors
Chemokines
Cytokines
MCP-1 (CCL2)
RANTES (CCL5)
CSF-1
LL-37

FIG. 88H cont'd

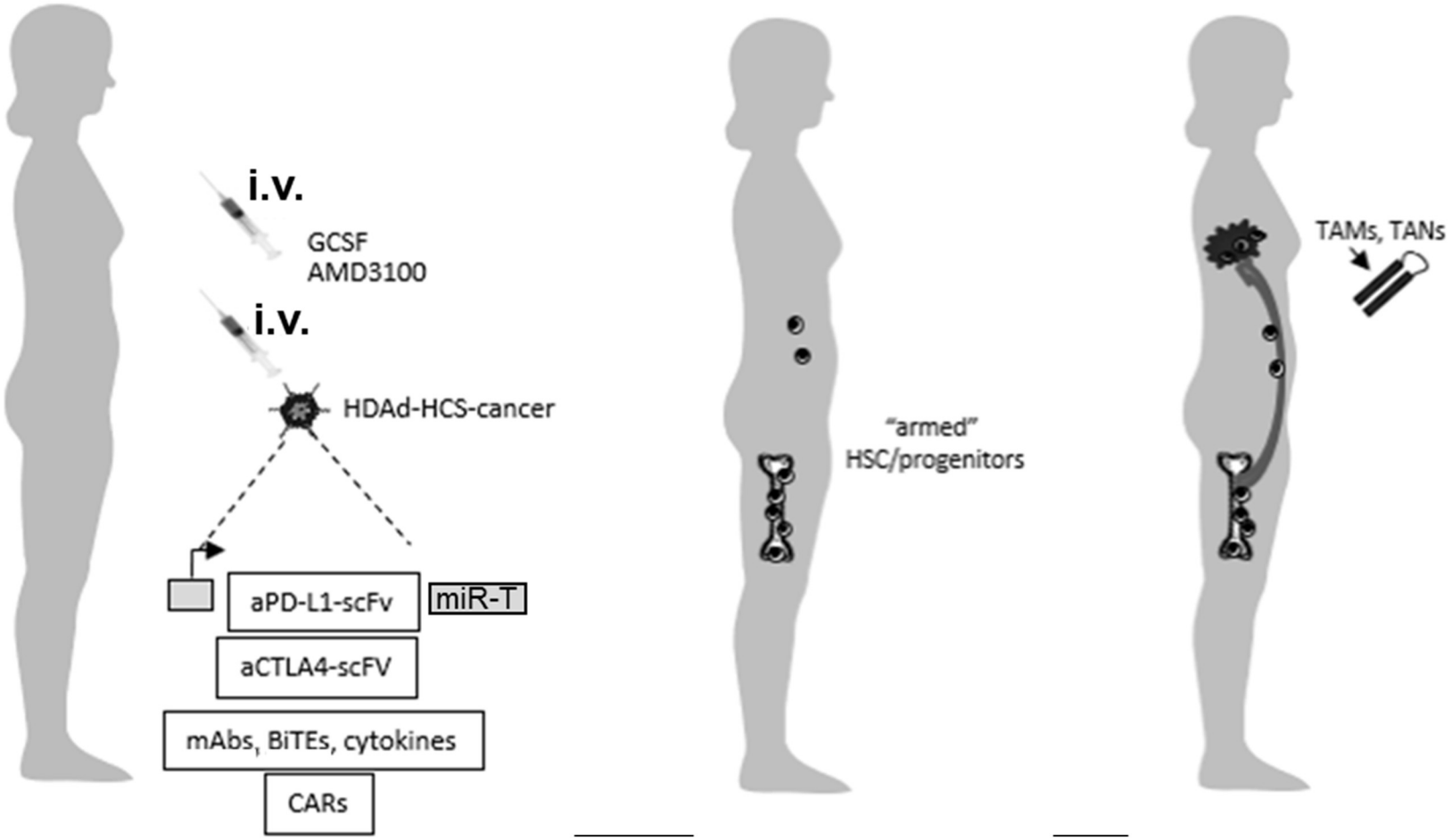
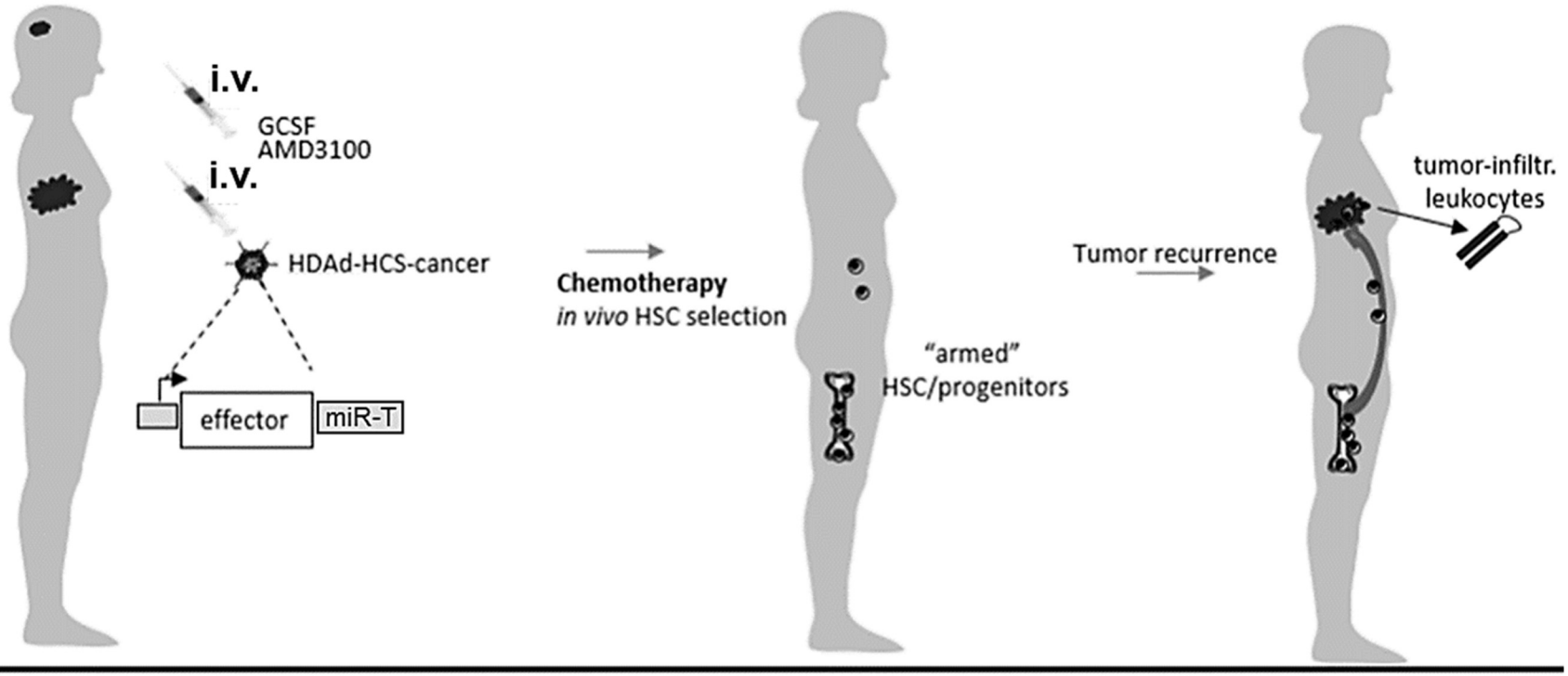


FIG. 88H cont'd



Biopsies:
→immunophenotype known→ select optimal immune-effector gene
→neo-antigens known→ personalized CARs/BiTEs

-difficult to treat full-fledged tumors

FIG. 89A

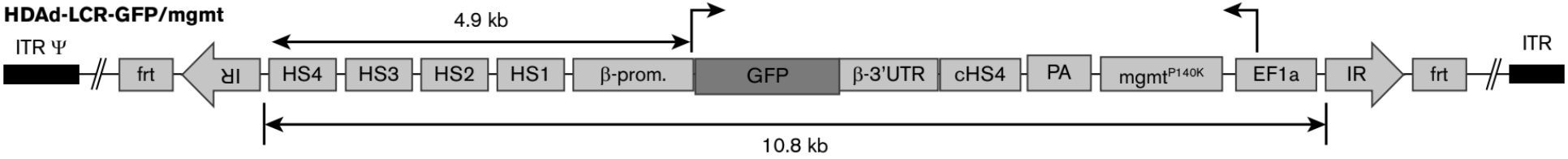


FIG. 89B

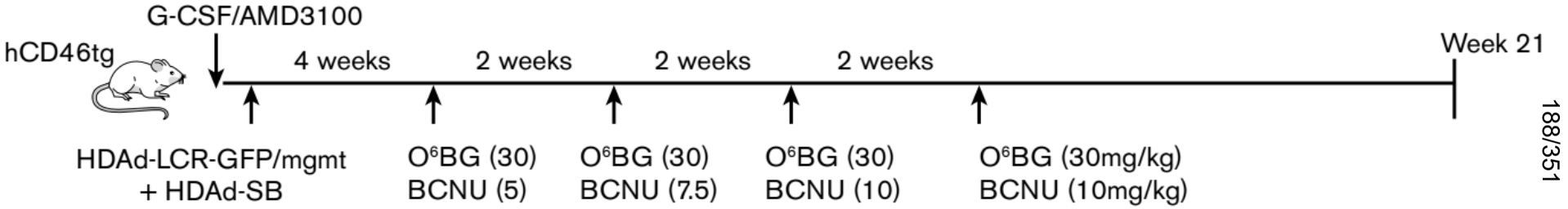


FIG. 89C

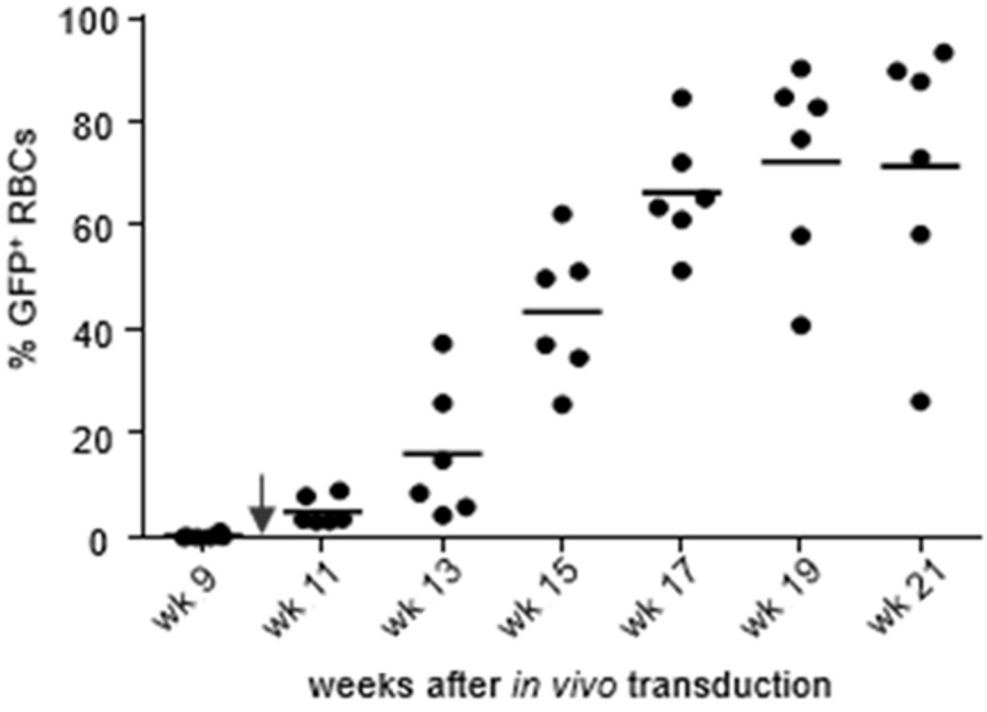


FIG. 89D

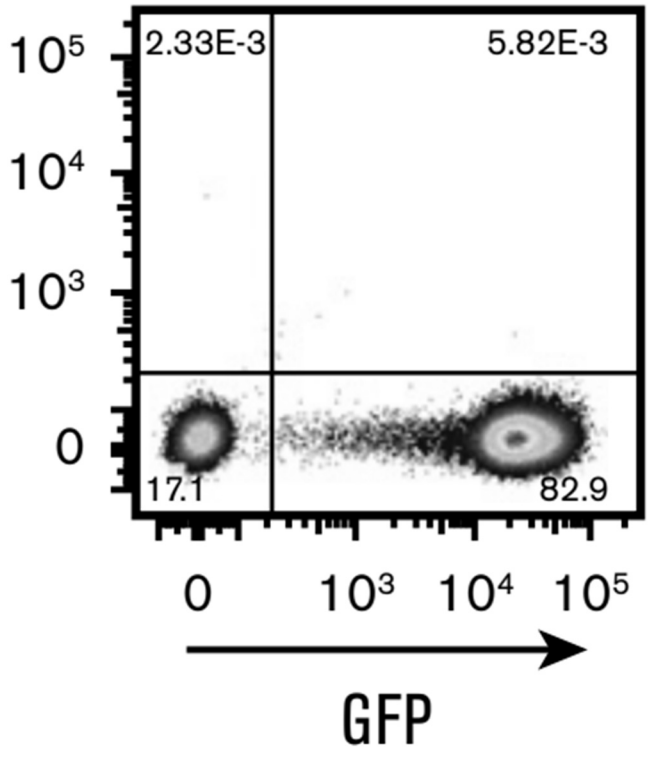


FIG. 89E

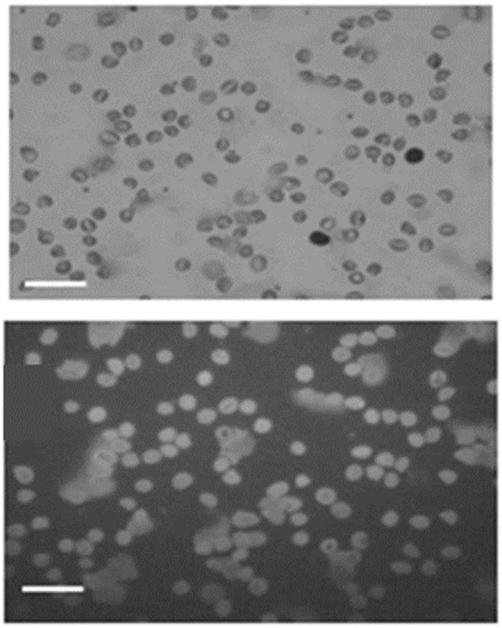


FIG. 89F

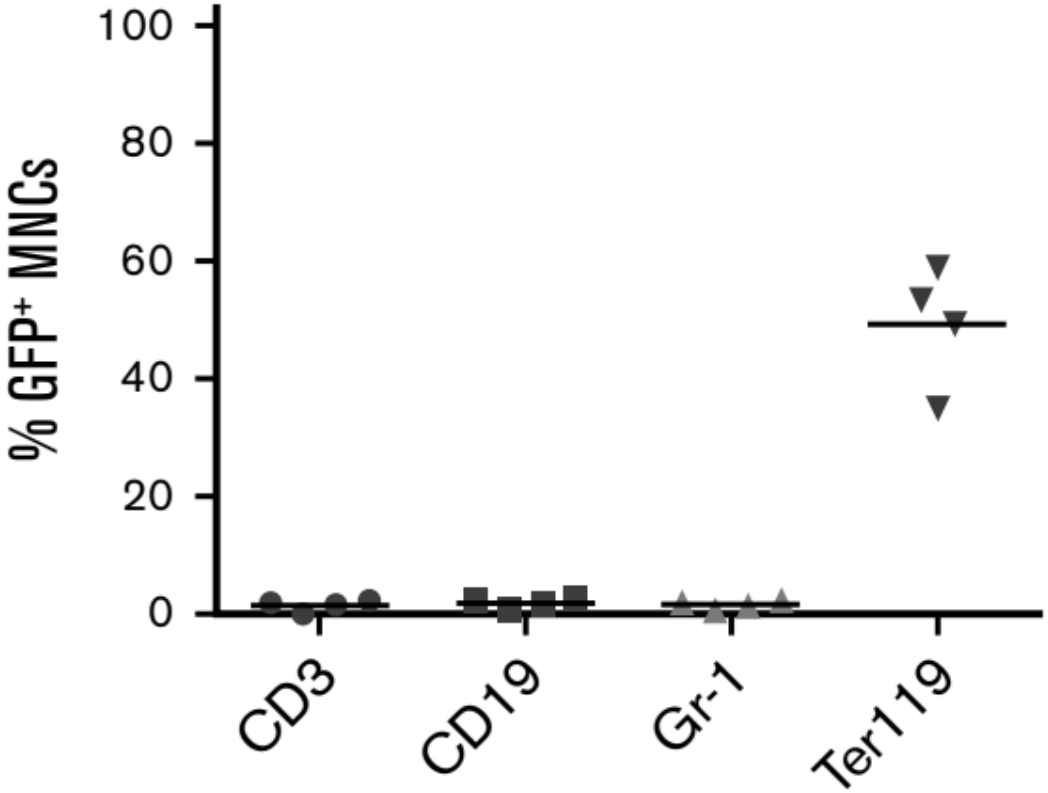


FIG. 89G

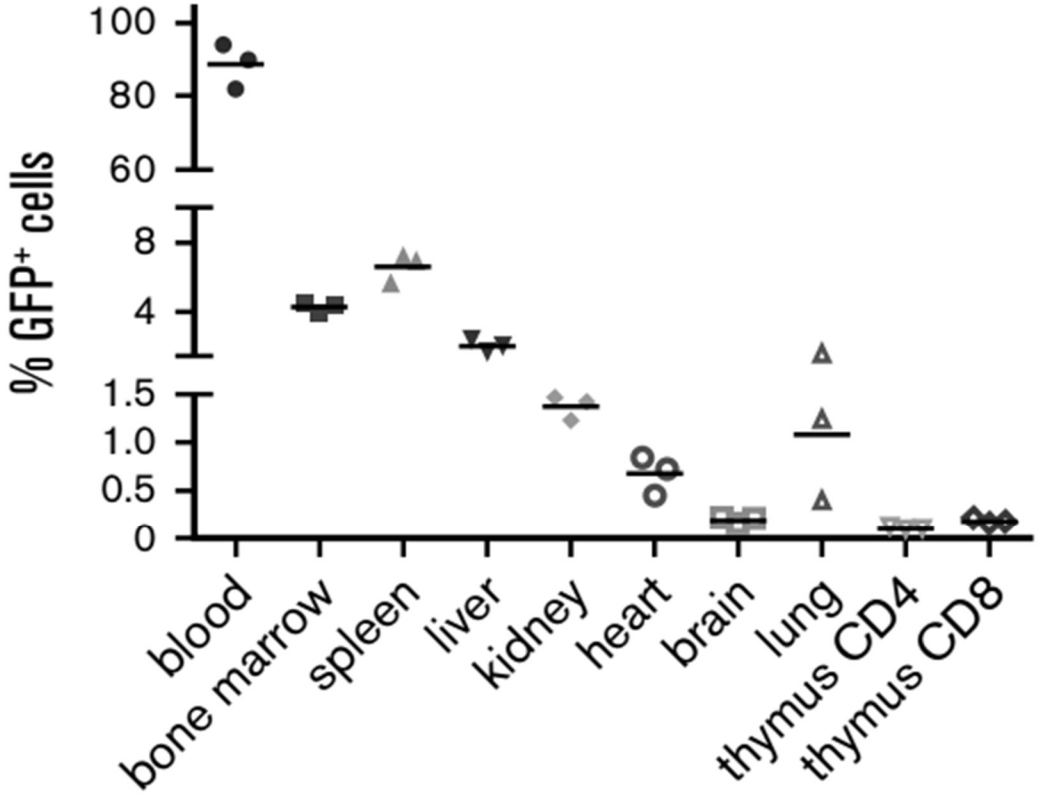


FIG. 89H

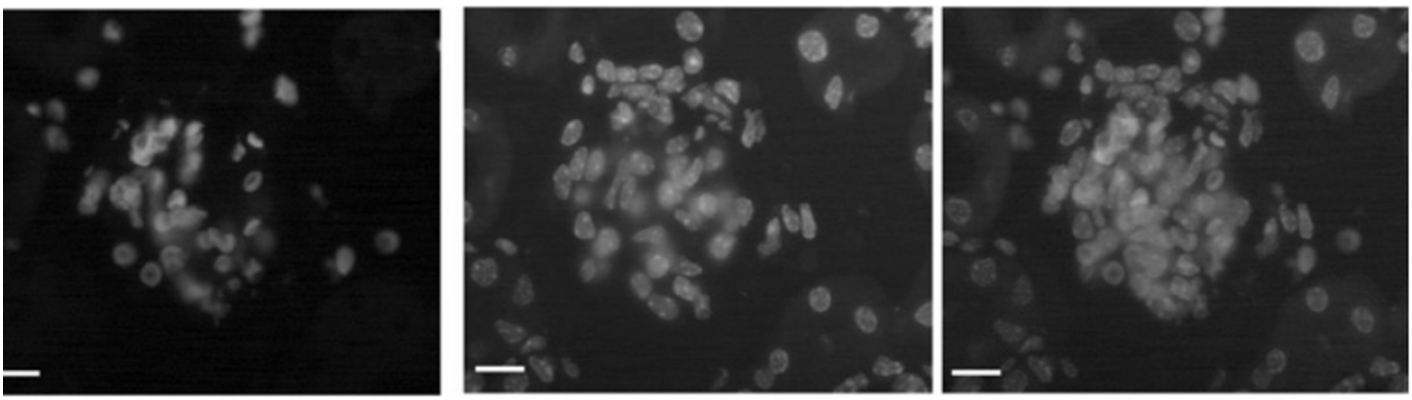


FIG. 90A

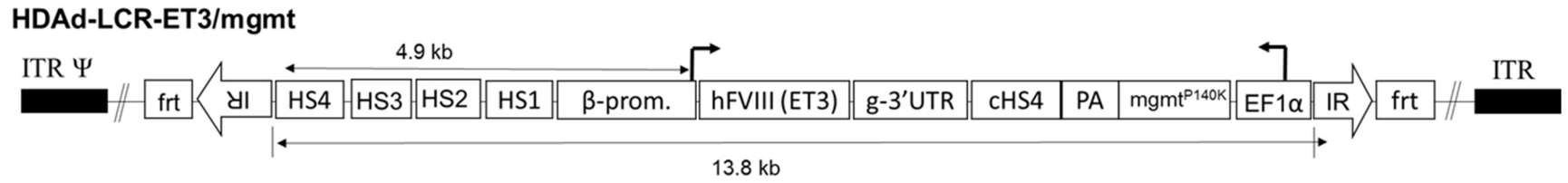


FIG. 90B

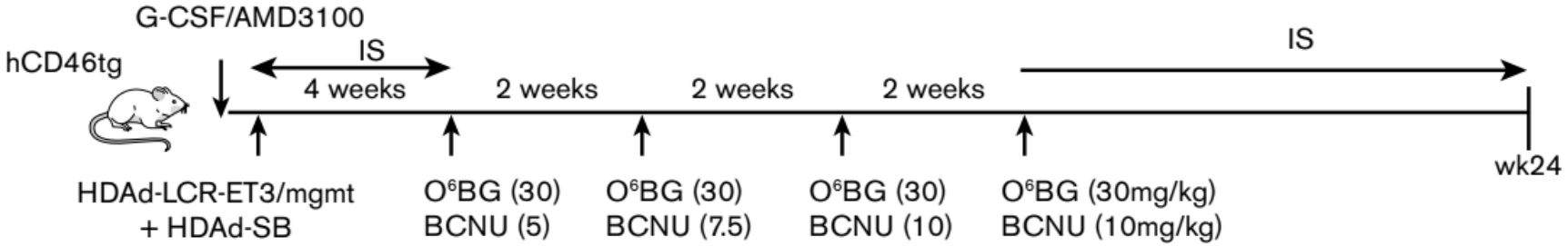


FIG. 90C

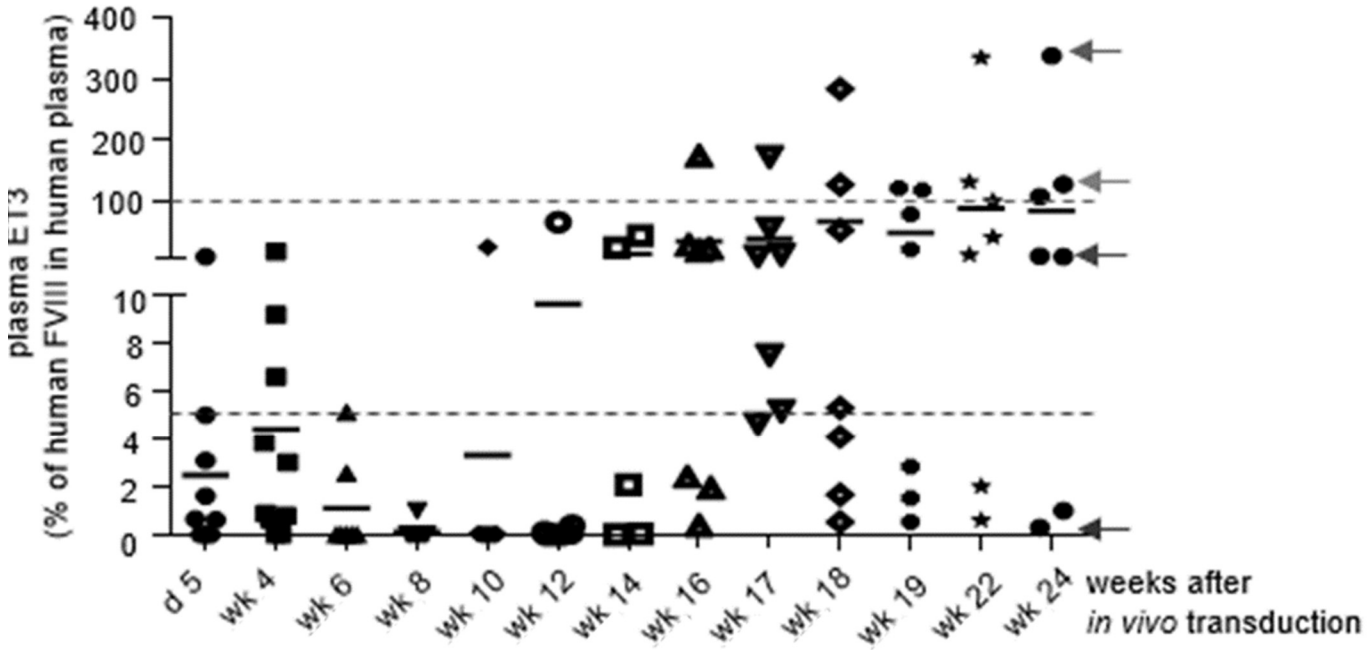


FIG. 90D

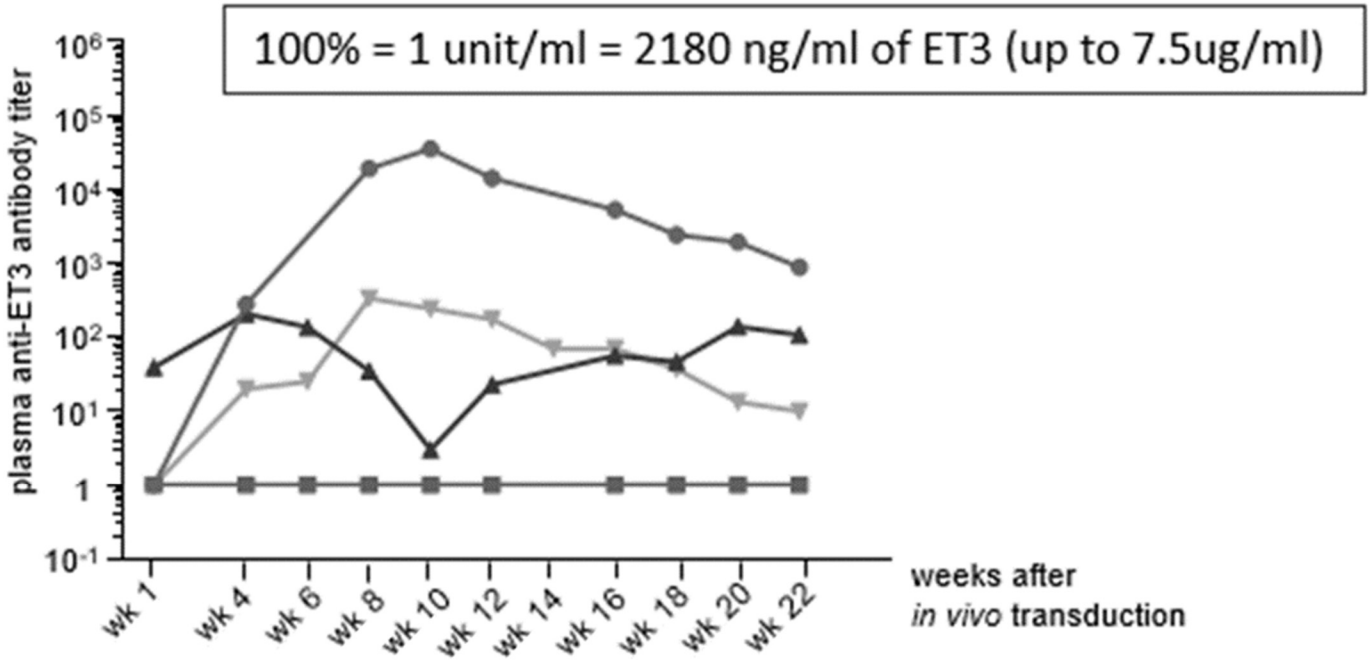


FIG. 90E

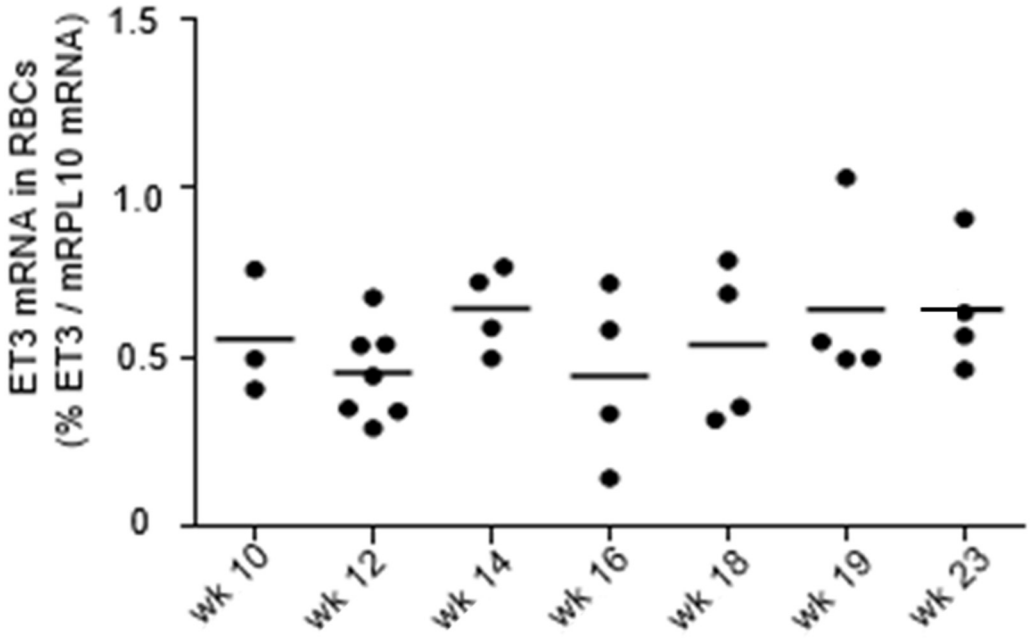


FIG. 90F

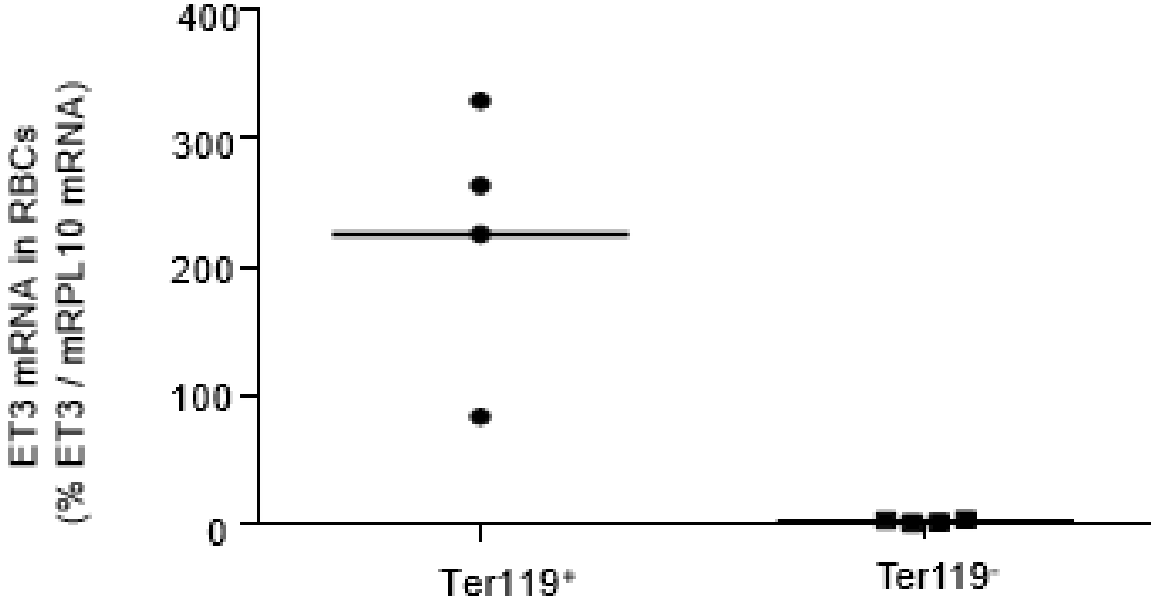


FIG. 90G

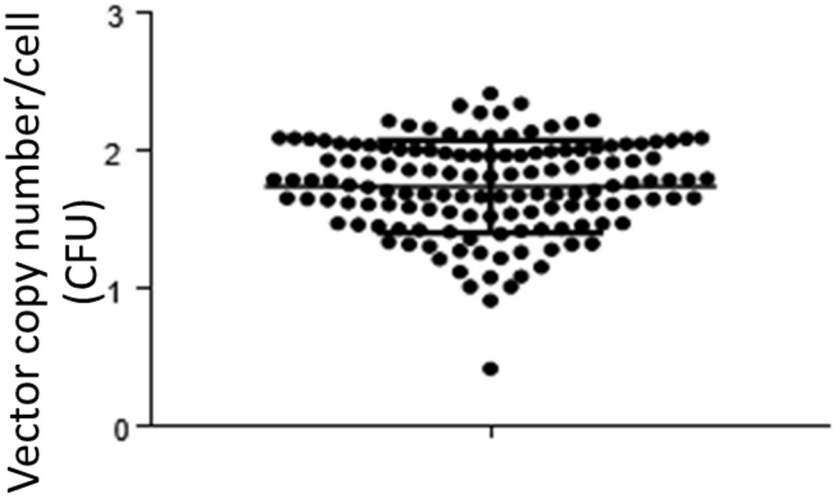


FIG. 90H

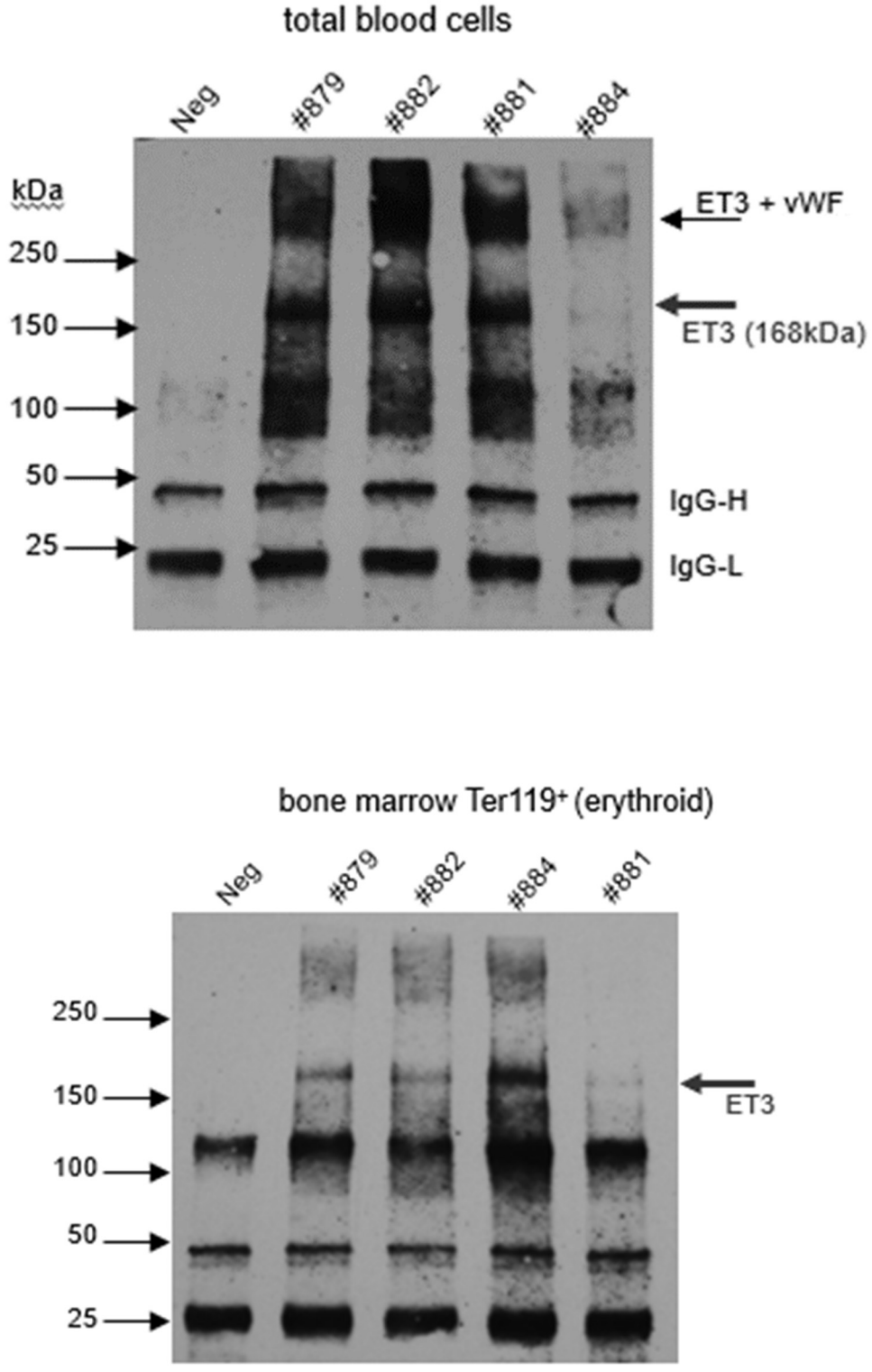

total blood cells
Neg
#879
#882
#881
#884
kDa
250
150
100
50
25
ET3 + vWF
ET3 (168kDa)
IgG-H
IgG-L
bone marrow Ter119+ (erythroid)
Neg
#879
#882
#884
#881
250
150
100
50
25
ET3

FIG. 90H cont'd

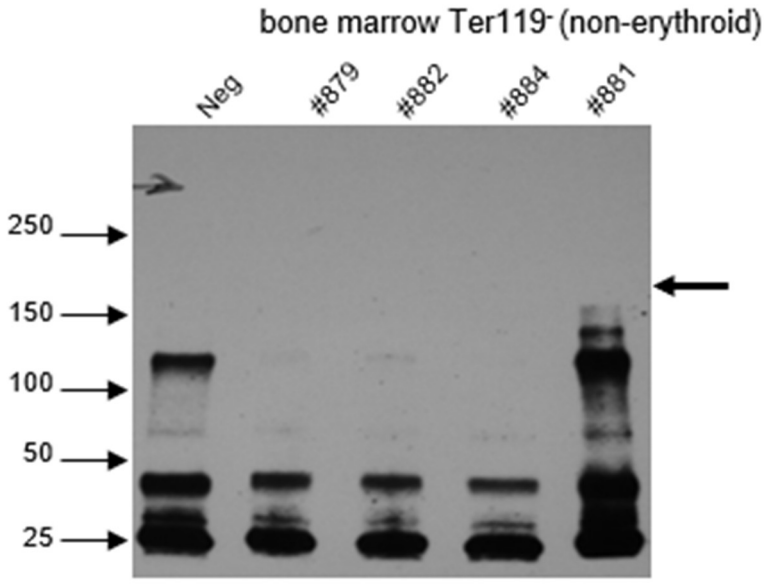


FIG. 90I

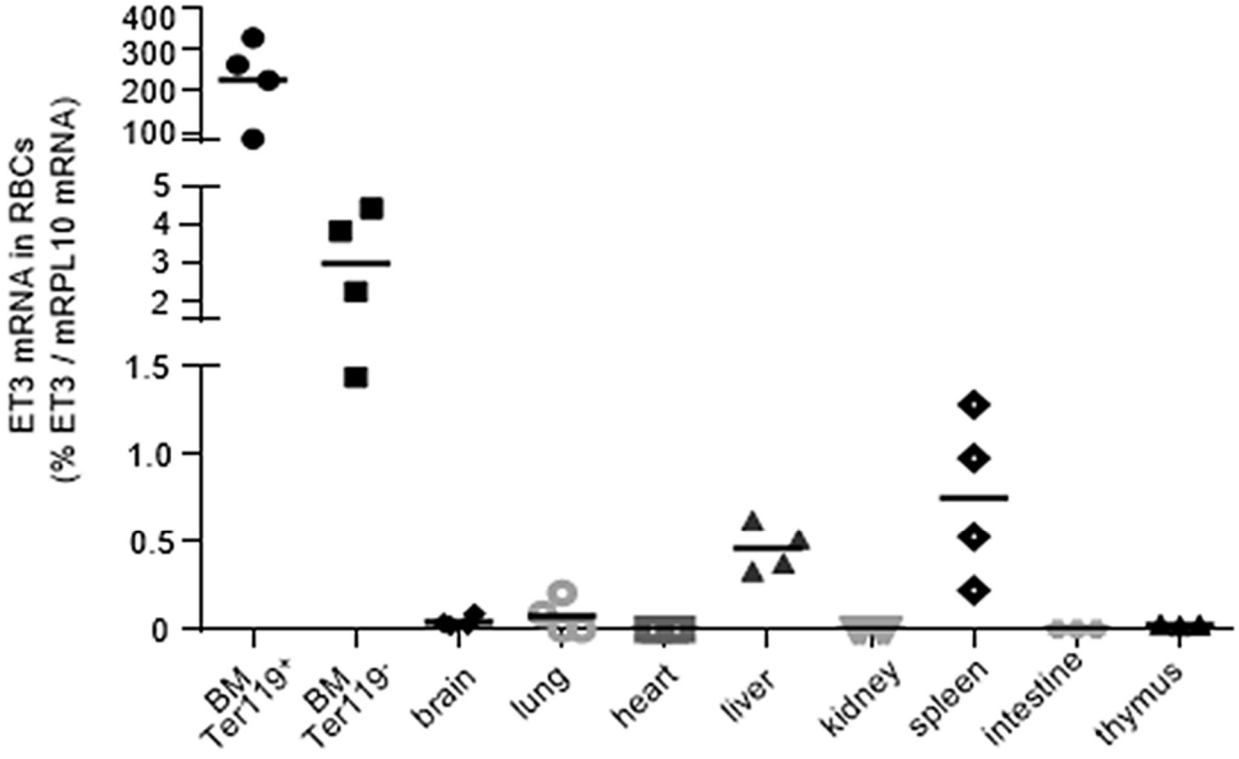


FIG. 91A

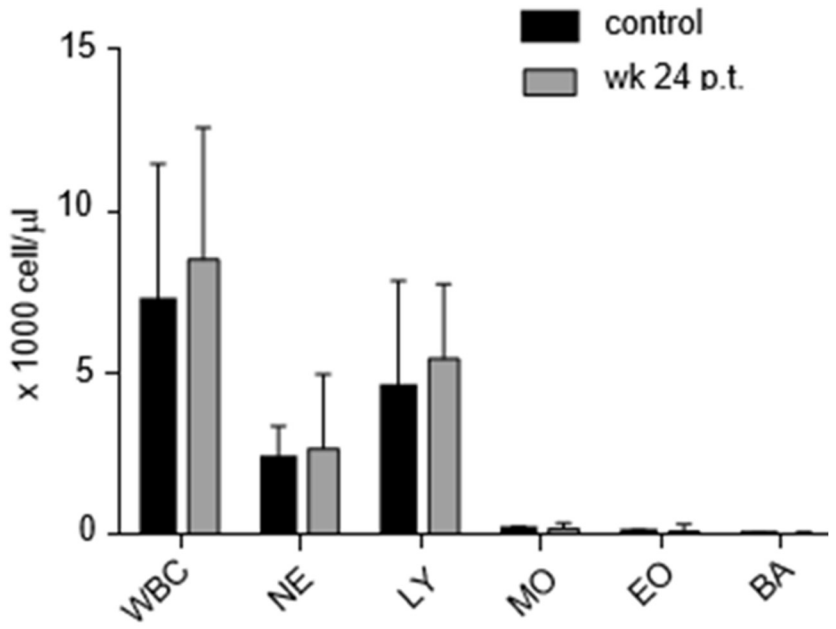


FIG. 91B

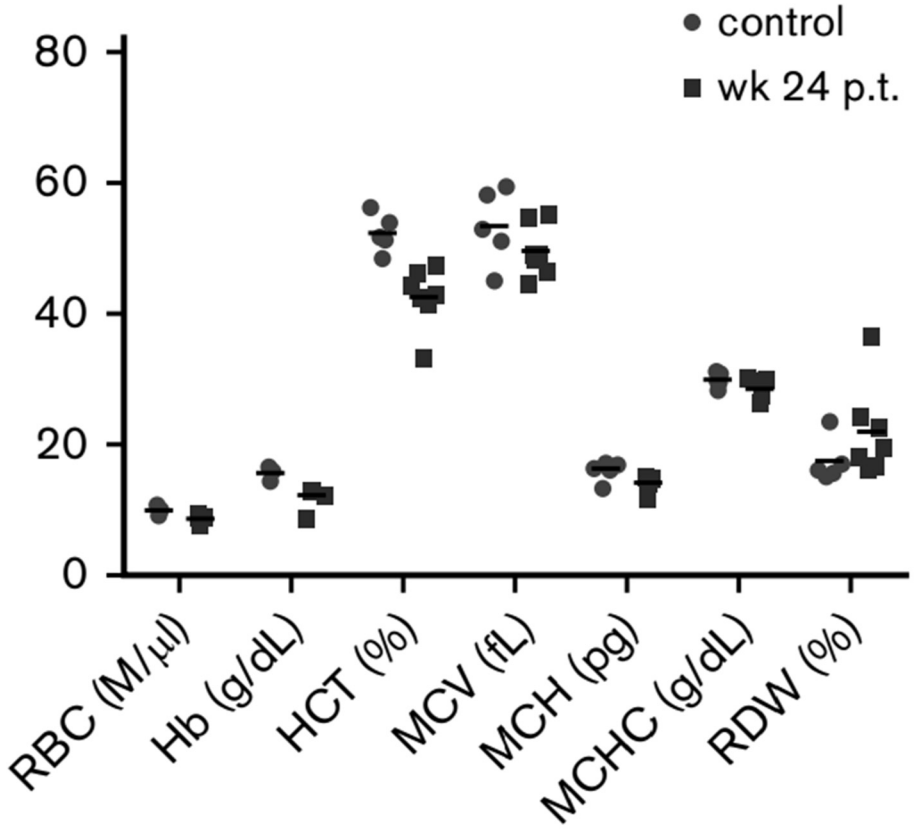


FIG. 91C

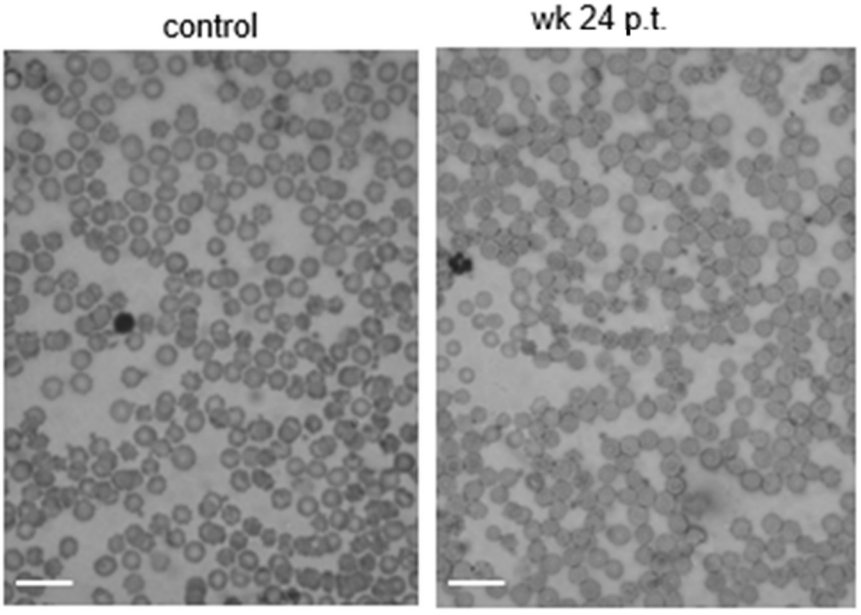


FIG. 91D

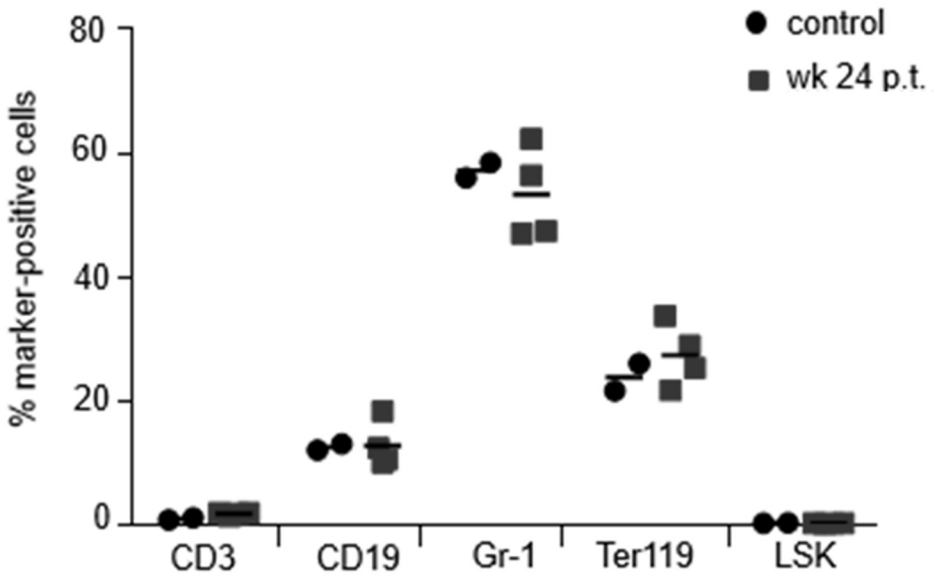


FIG. 92A

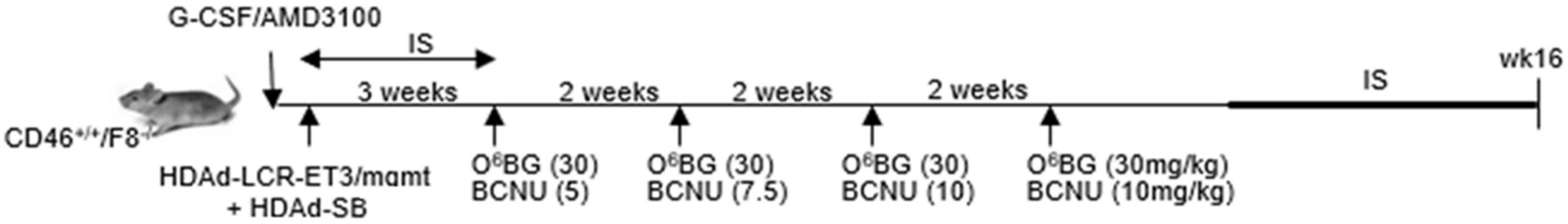


FIG. 92B

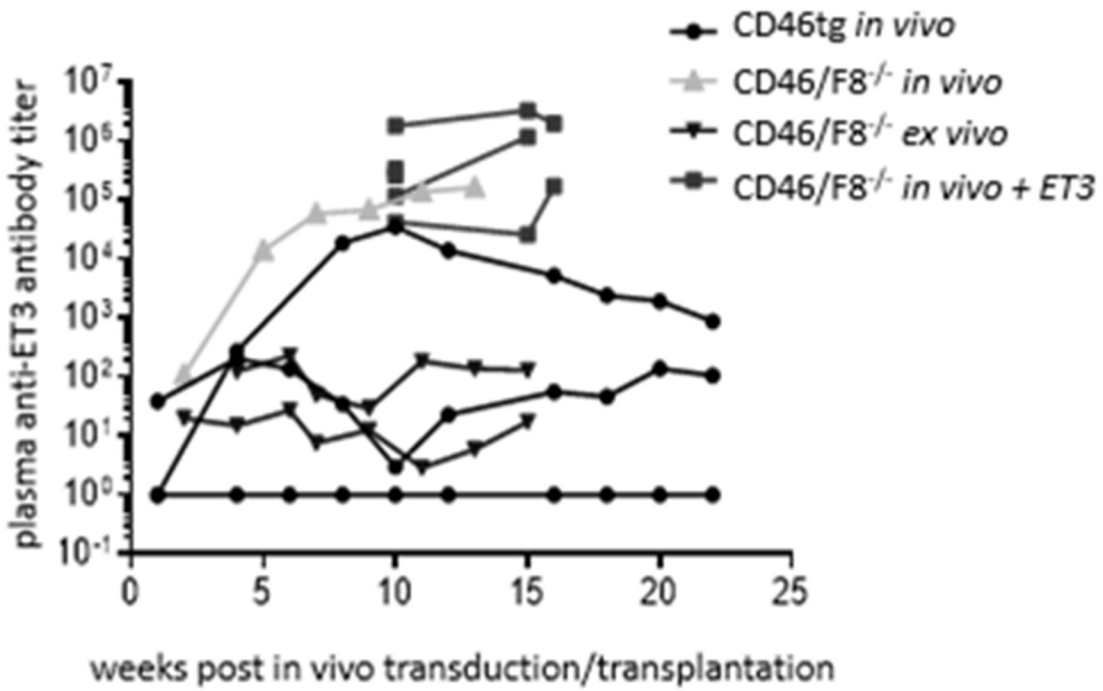


FIG. 92C

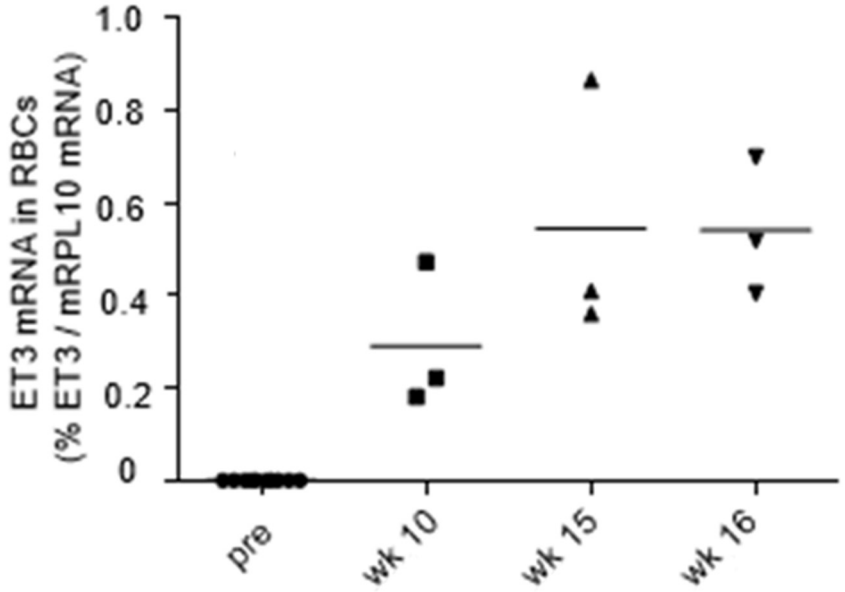


FIG. 92D

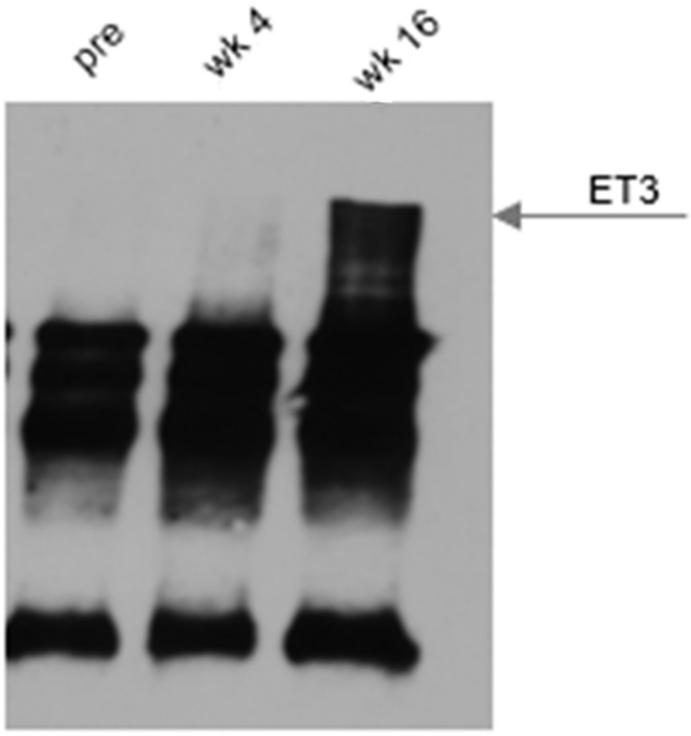


FIG. 92E

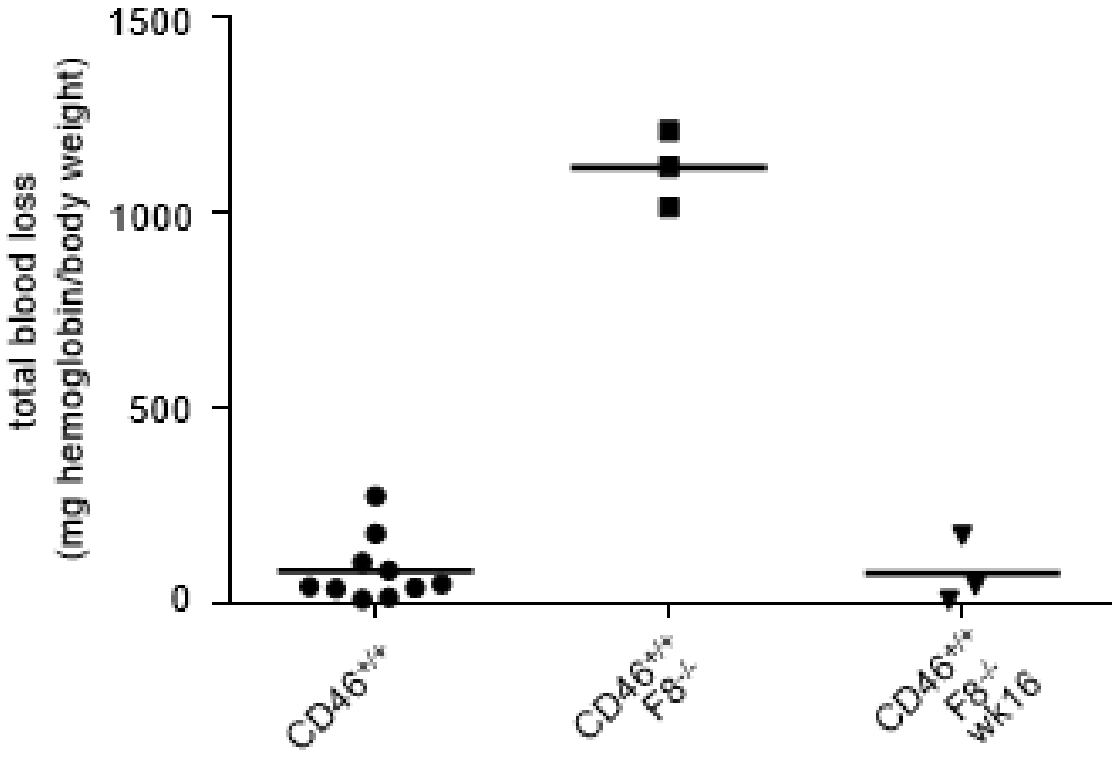


FIG. 92F

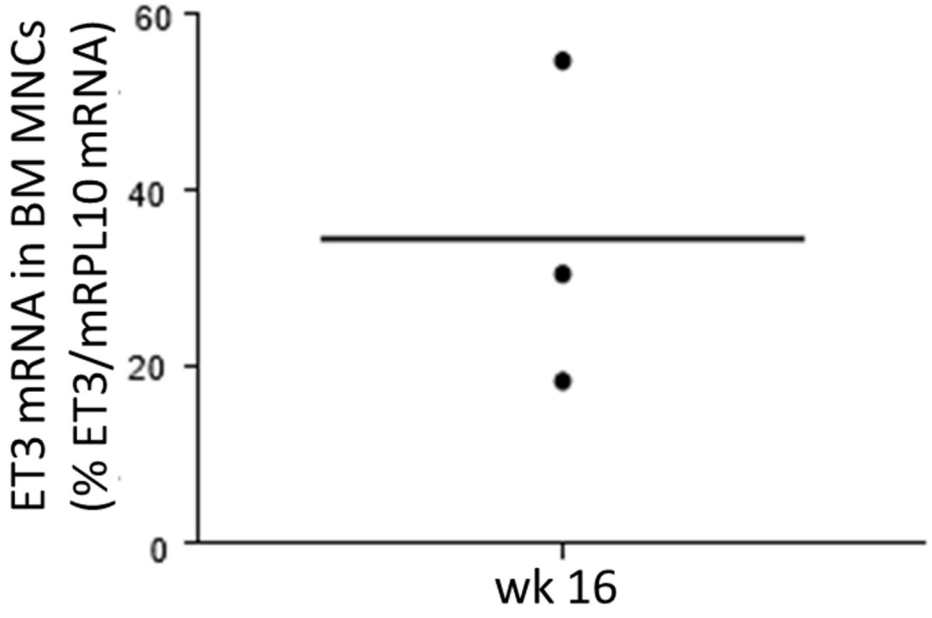


FIG. 92G

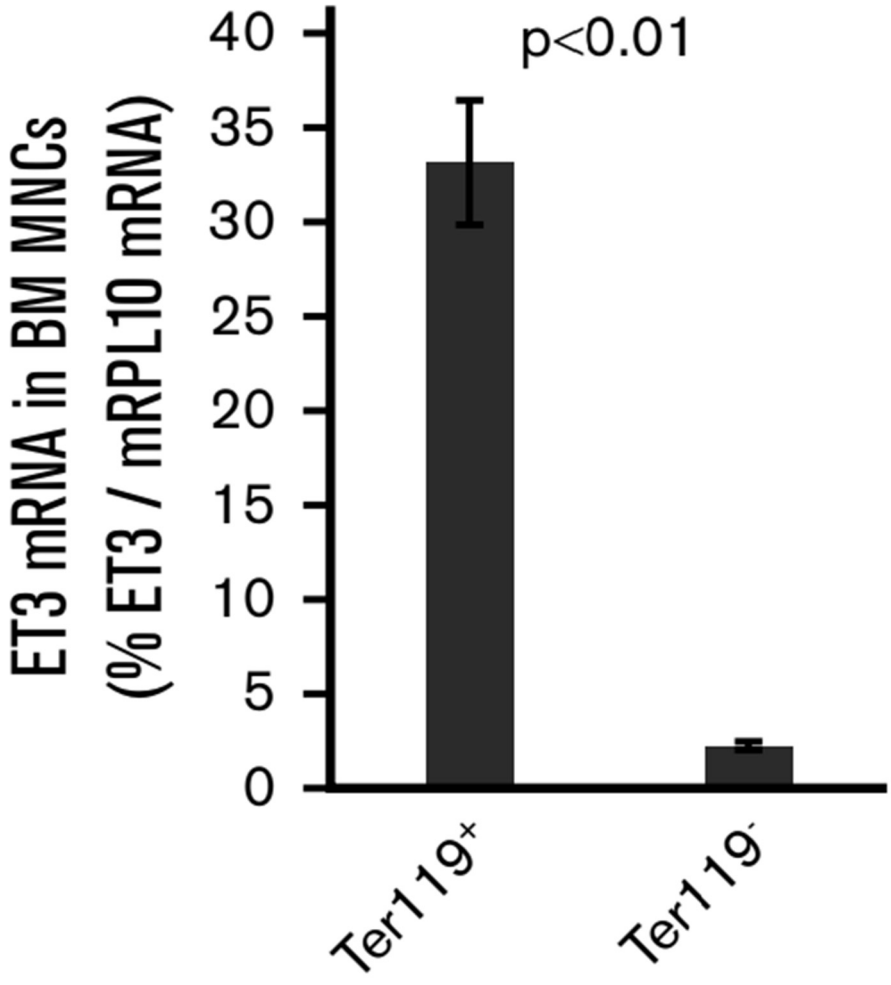

ET3 mRNA in BM MNCs
(% ET3 / mRPL10 mRNA)
40
35
30
25
20
15
10
5
0
p<0.01
Ter119+
Ter119-

FIG. 93A

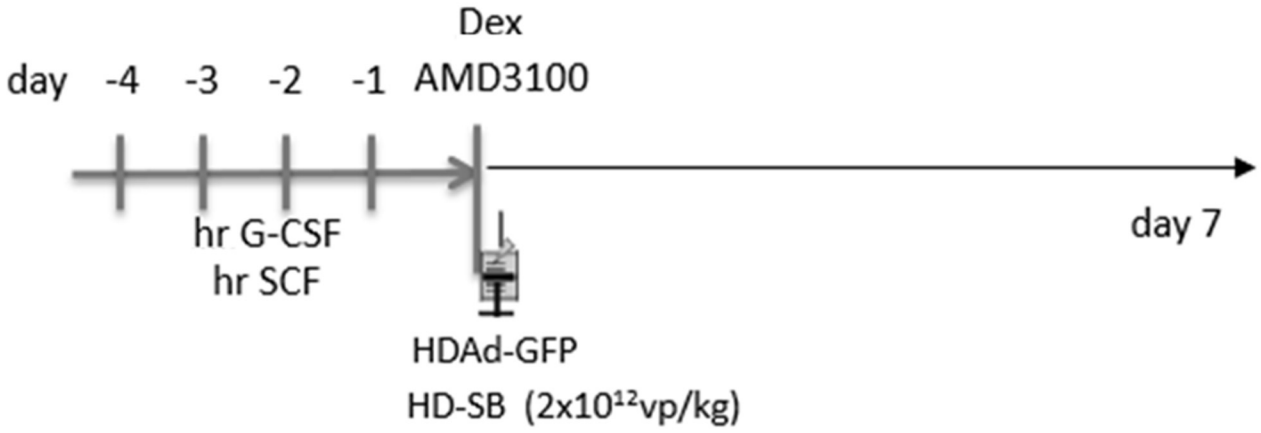


FIG. 93B

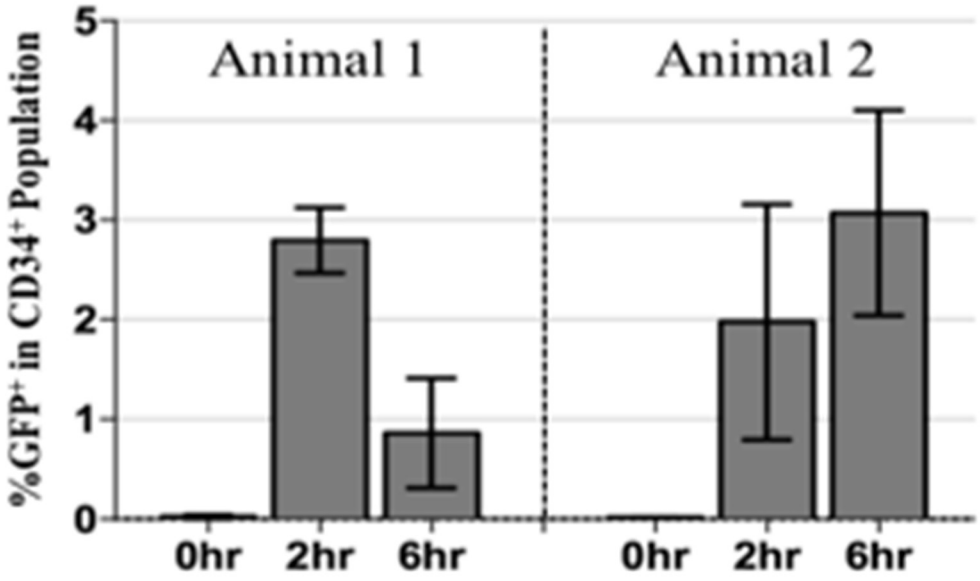


FIG. 93C

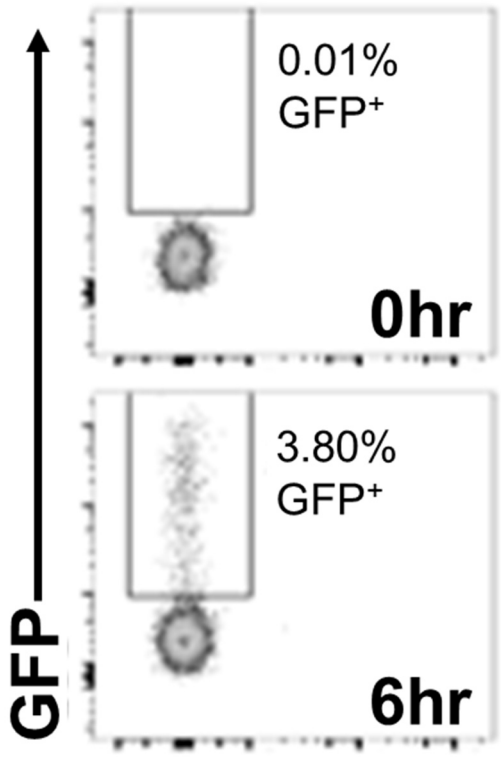


FIG. 93D

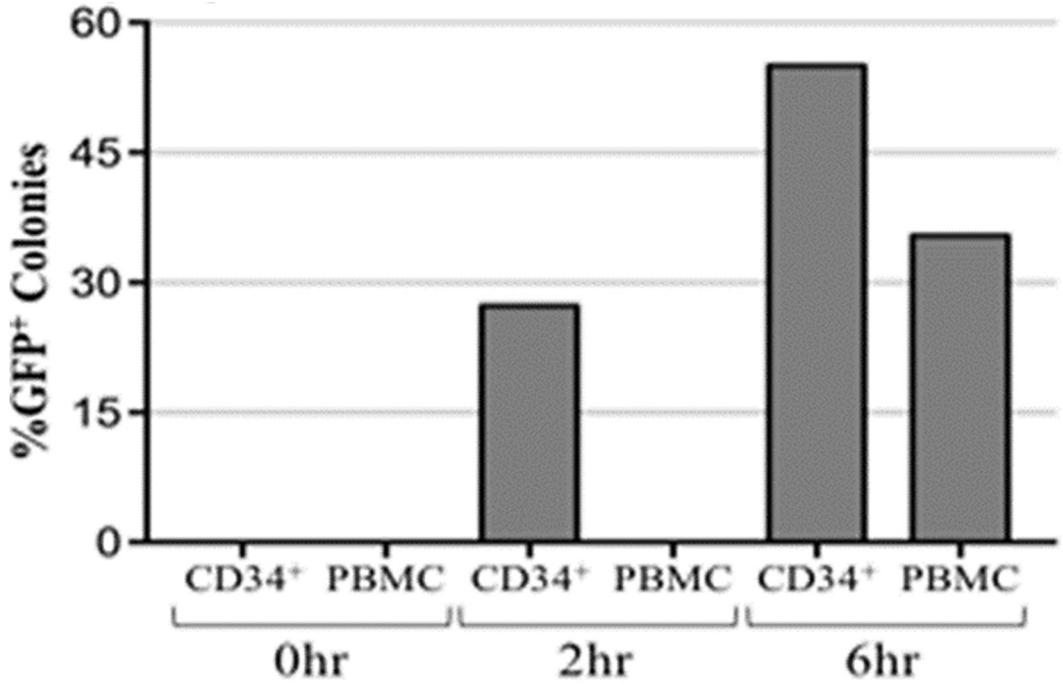


FIG. 93E

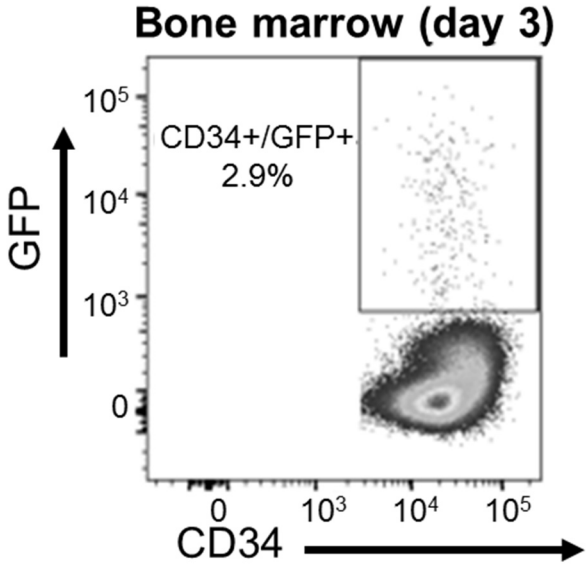

Bone marrow (day 3)
CD34+/GFP+
2.9%
GFP
10^5
10^4
10^3
0
0
10^3
10^4
10^5
CD34

FIG. 94A



FIG. 94B

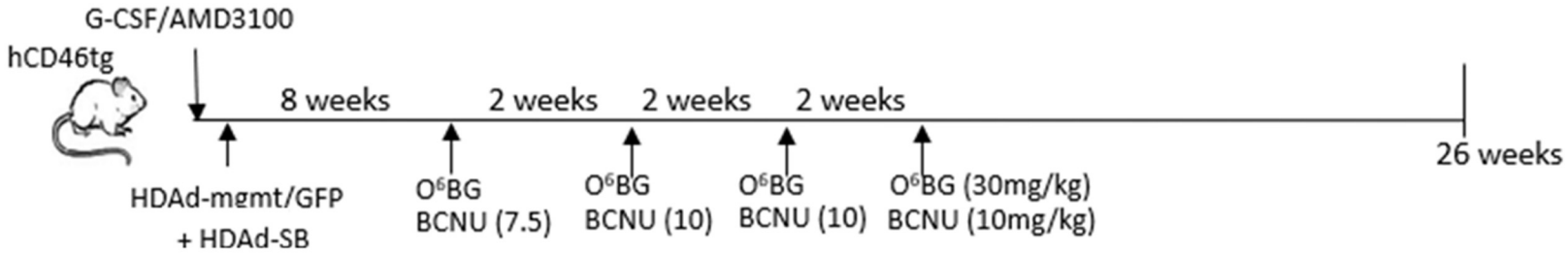


FIG. 94C

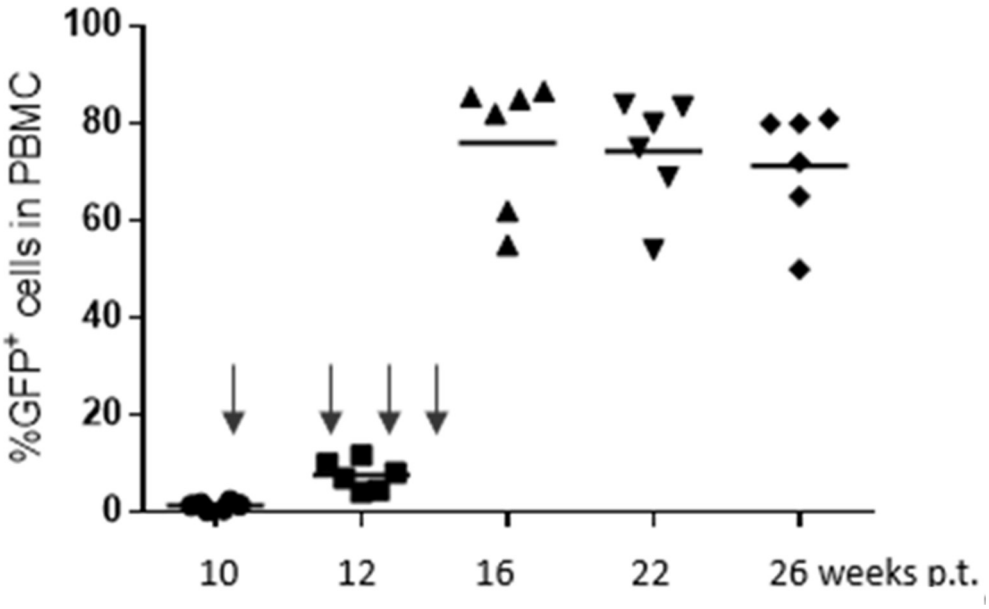


FIG. 94D

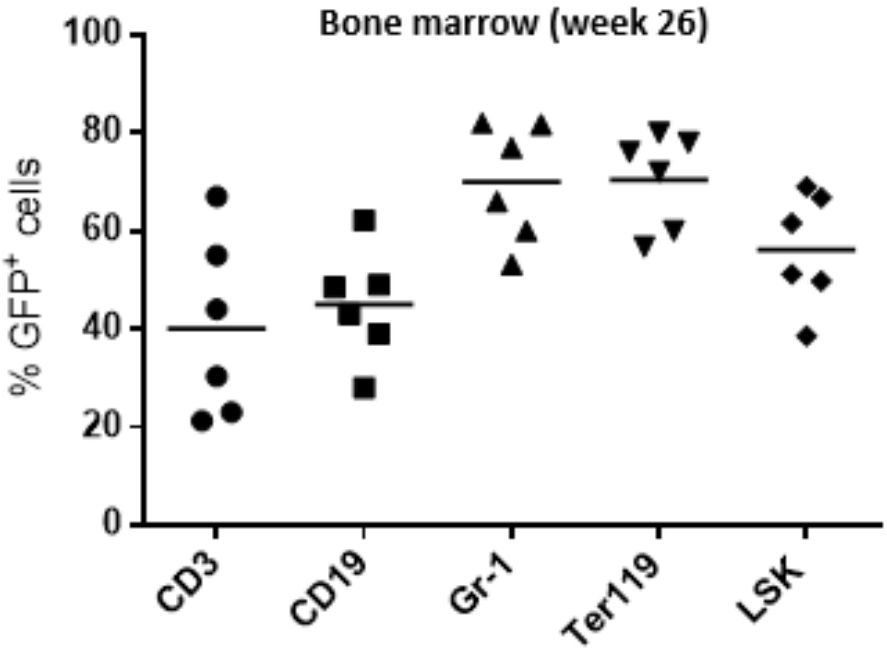


FIG. 94E

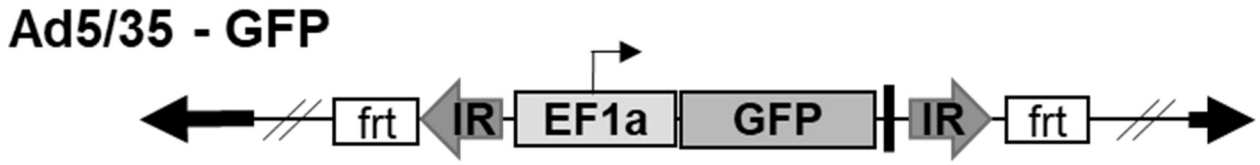


FIG. 94F

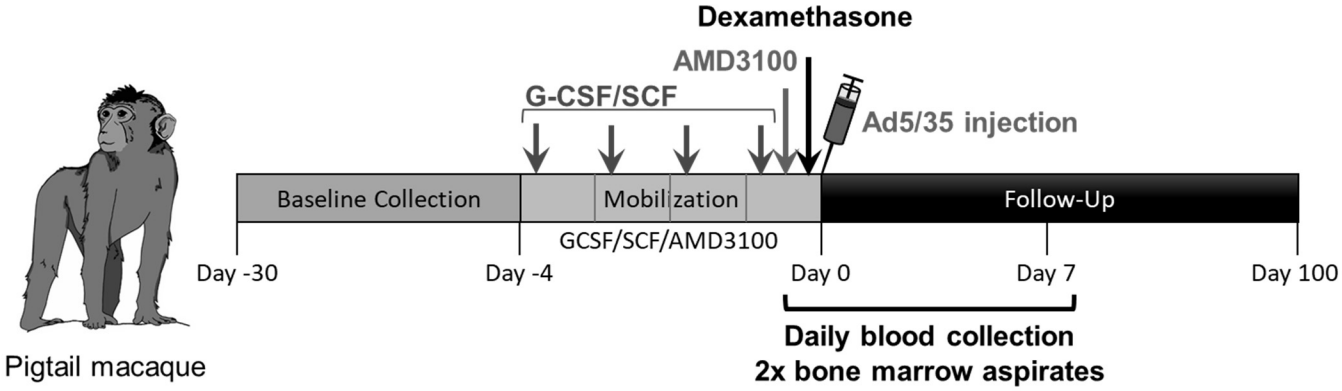


FIG. 94G

Animal ID	G-CSF	SCF	AMD3100	Dose
Z12406	50µg/kg	50µg/kg	-	$2x10^{12}$vp/kg
Z13126	50µg/kg	50µg/kg	4mg/kg	$4x10^{12}$vp/kg
Z15263	50µg/kg	50µg/kg	4mg/kg	$3x10^{12}$vp/kg

FIG. 94H

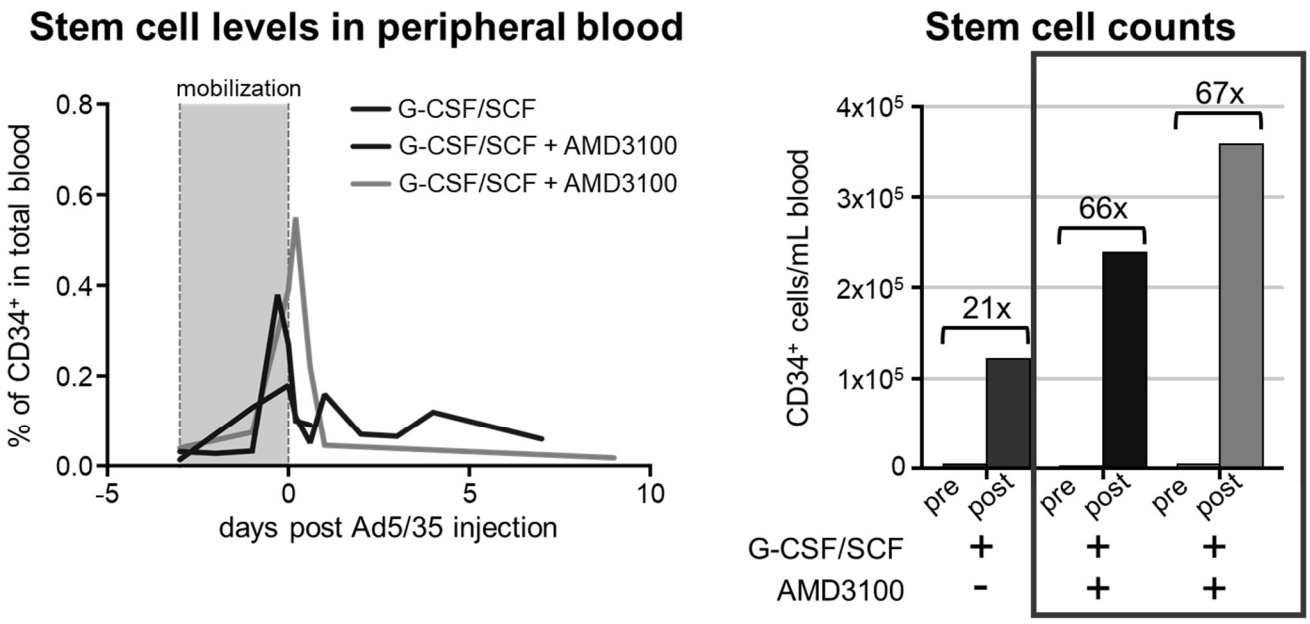


FIG. 94I

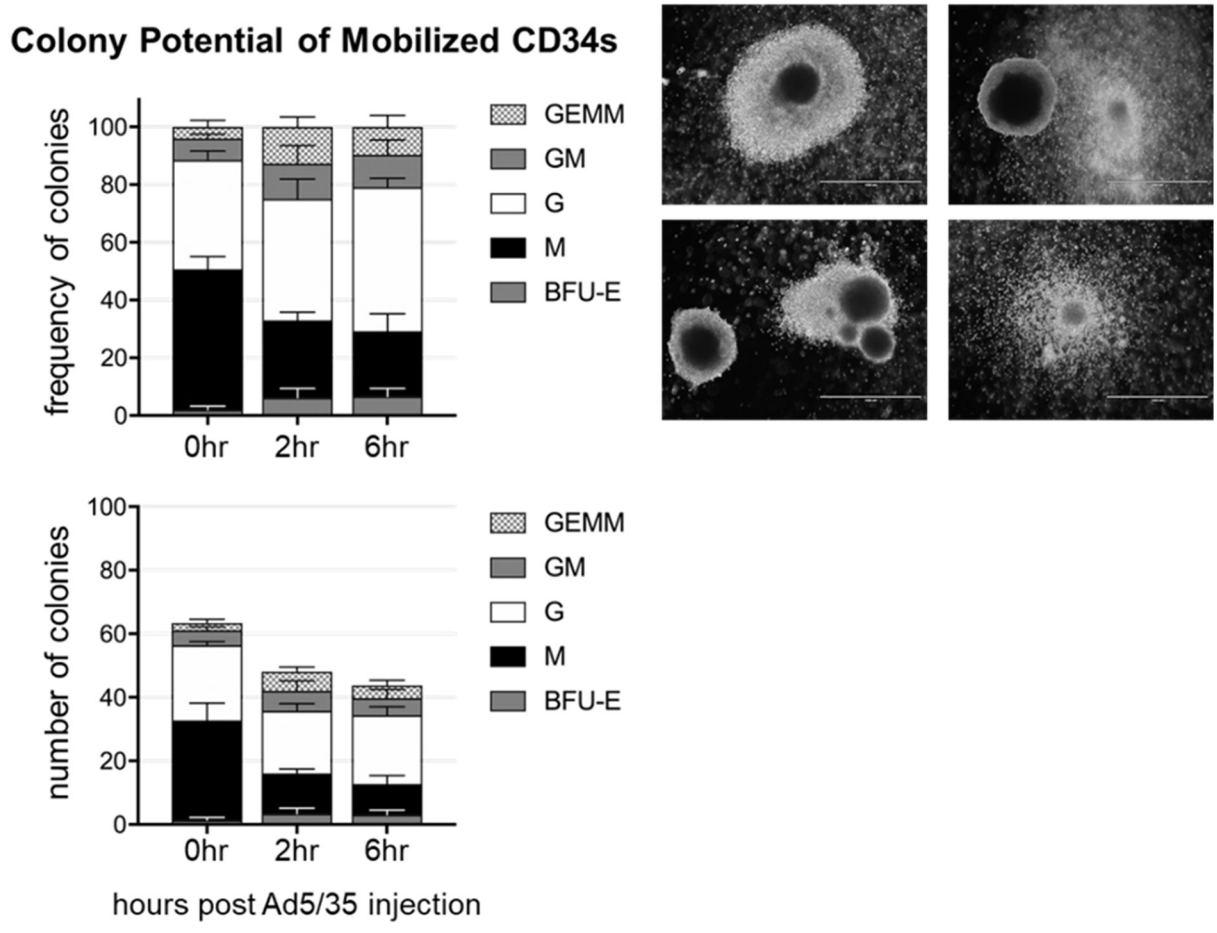


FIG. 94J

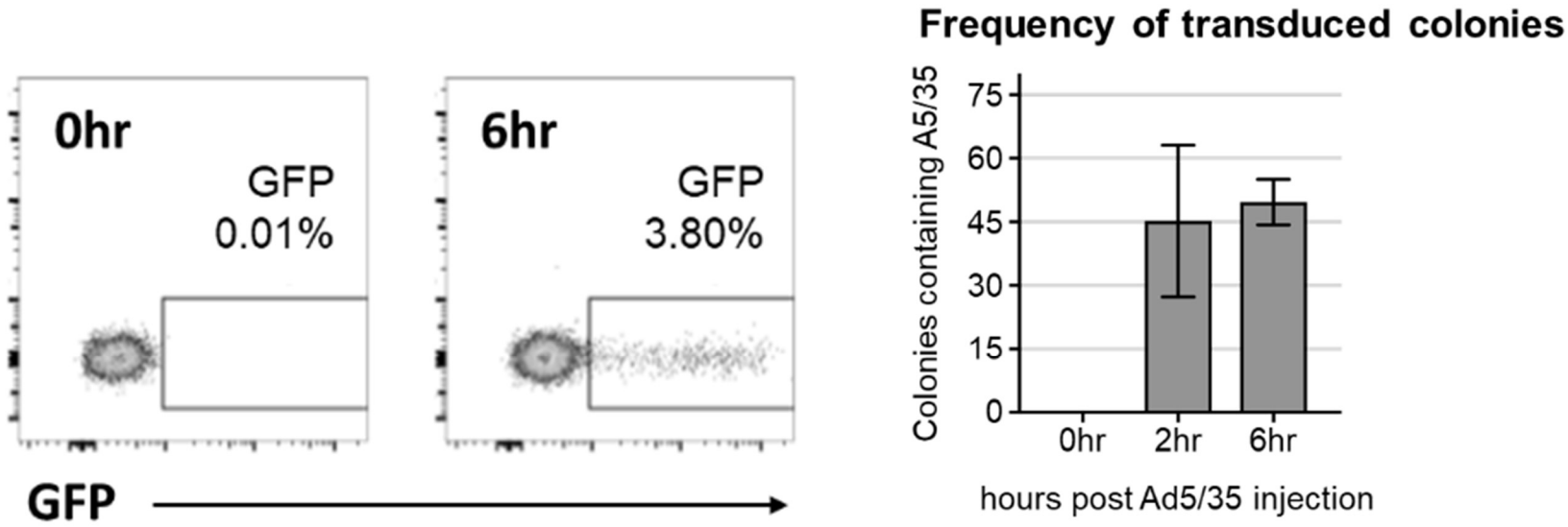


FIG. 94K

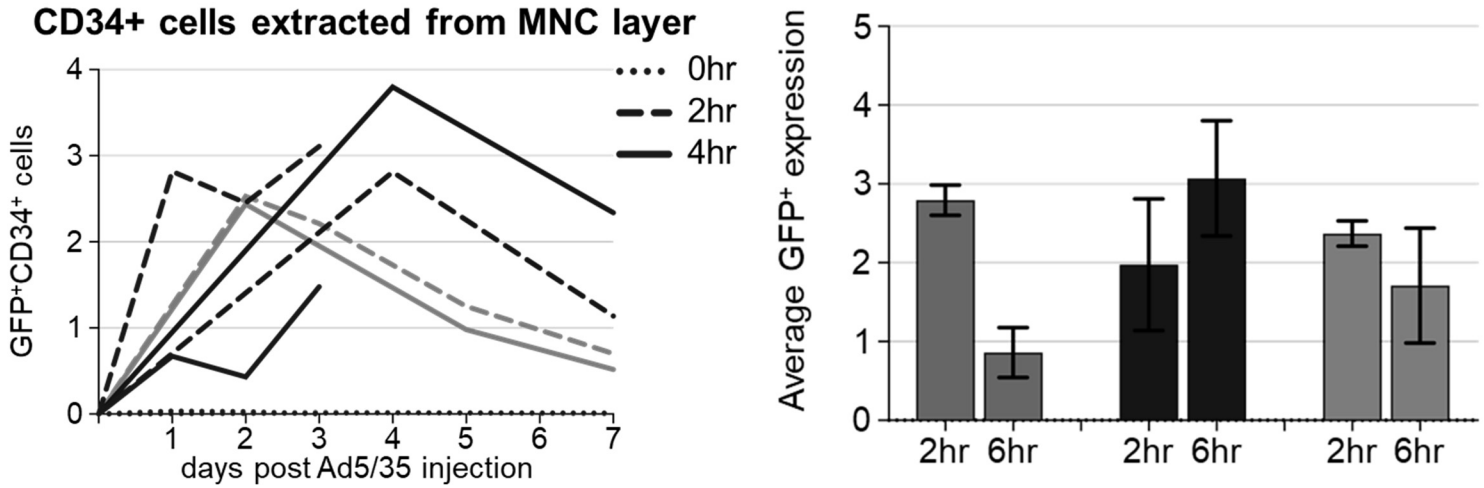


FIG. 94L

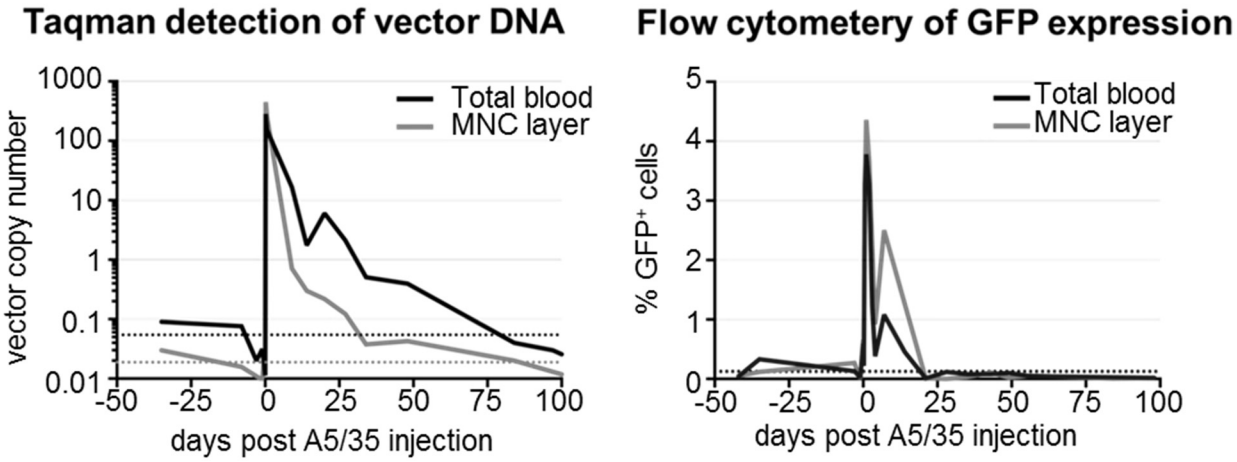


FIG. 94M

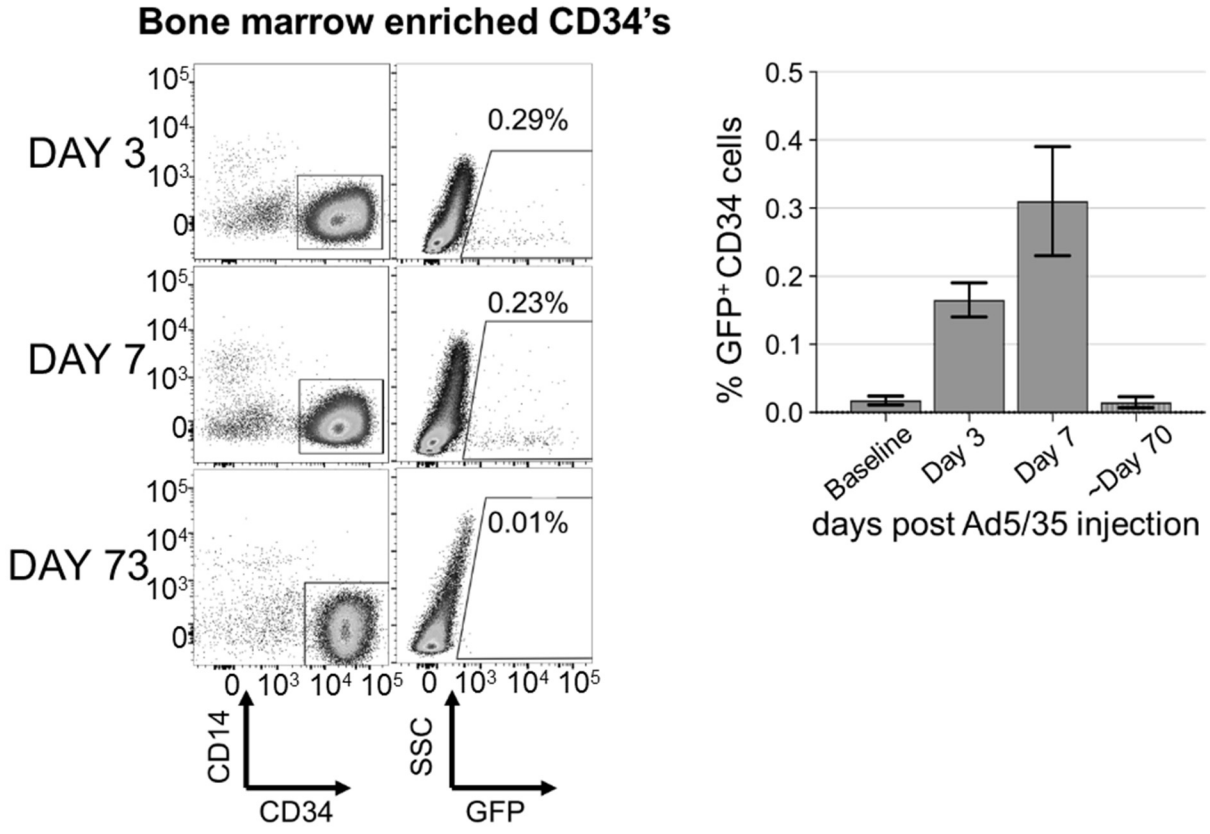


FIG. 95

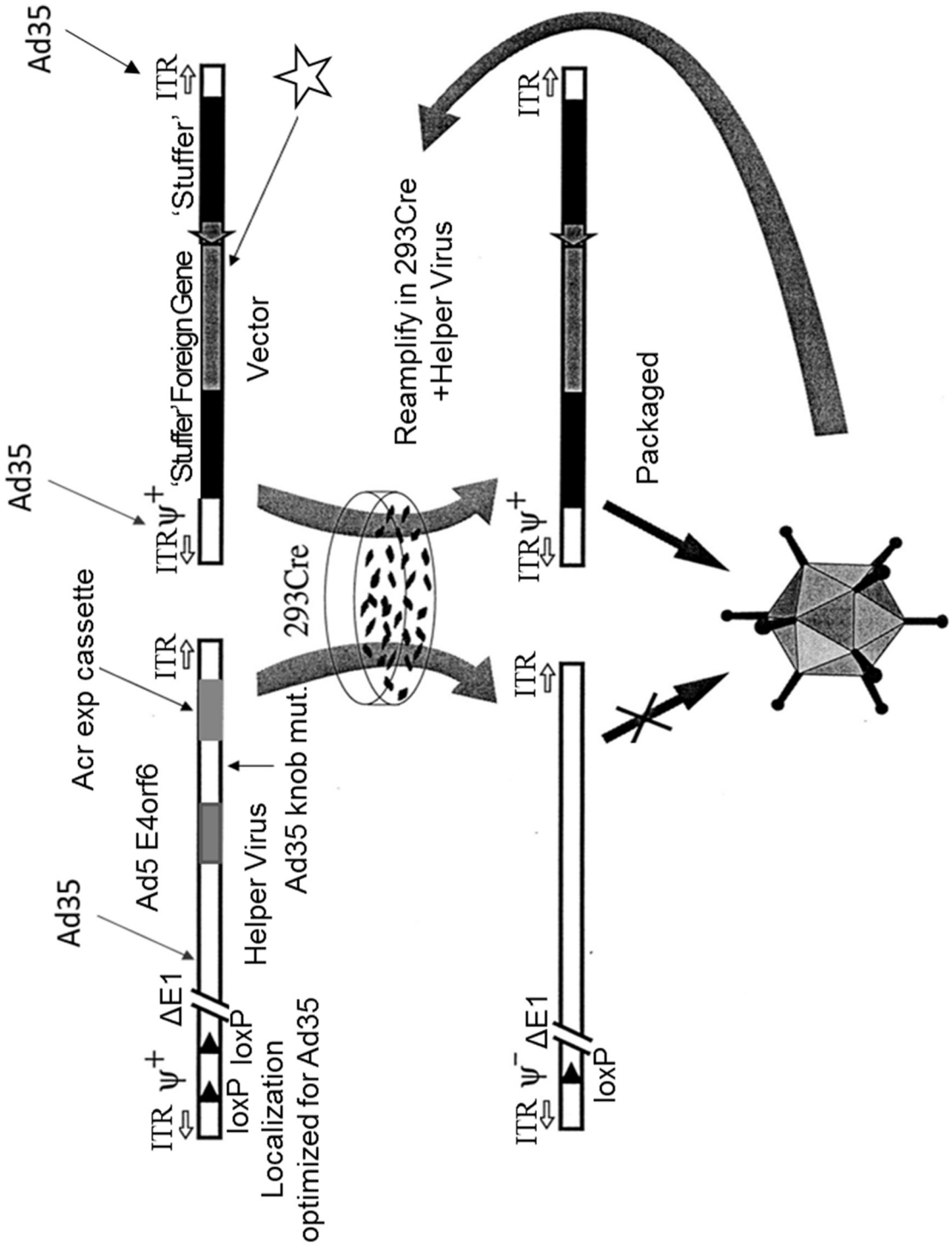

Ad35
ITR
'Stuffer'
Ad35
ITR ψ+
'Stuffer' Foreign Gene
Vector
Reamplify in 293Cre
+Helper Virus
ITR
Packaged
Ad35
Acr exp cassette
ITR
293Cre
ITR ψ+
Ad5 E4orf6
Helper Virus
Ad35 knob mut.
ITR
Ad35
ITR ψ+ ΔE1
loxP loxP
Localization
optimized for Ad35
ITR ψ- ΔE1
loxP

FIG. 96

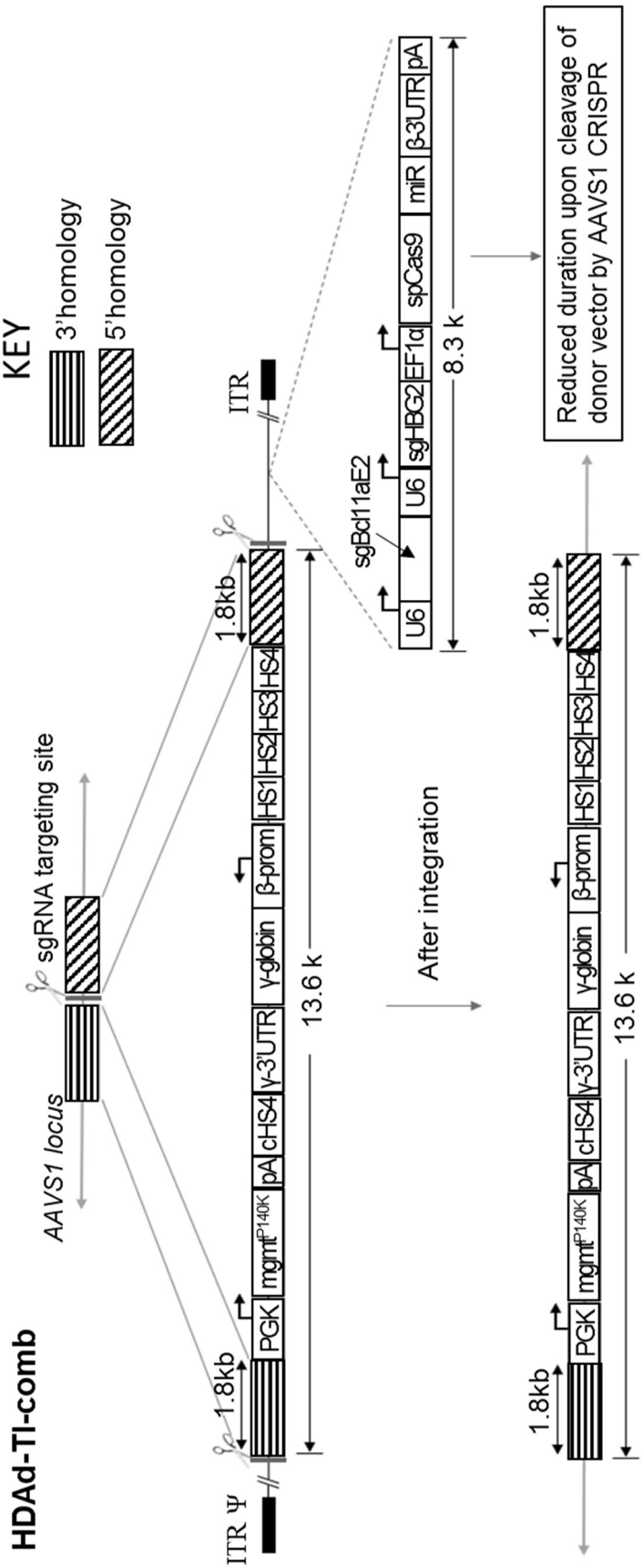

KEY
3'homology
5'homology
HDAd-TI-comb
AAVS1 locus
sgRNA targeting site
ITR Ψ
1.8kb
PGK
mgmt^P140K
pA
cHS4
γ-3'UTR
γ-globin
β-prom
HS1
HS2
HS3
HS4
13.6 k
ITR
sgBcl11aE2
U6
U6
sgHBG2
EF1d
spCas9
miR
β-3'UTR
pA
8.3 k
After integration
Reduced duration upon cleavage of donor vector by AAVS1 CRISPR

FIG. 97A

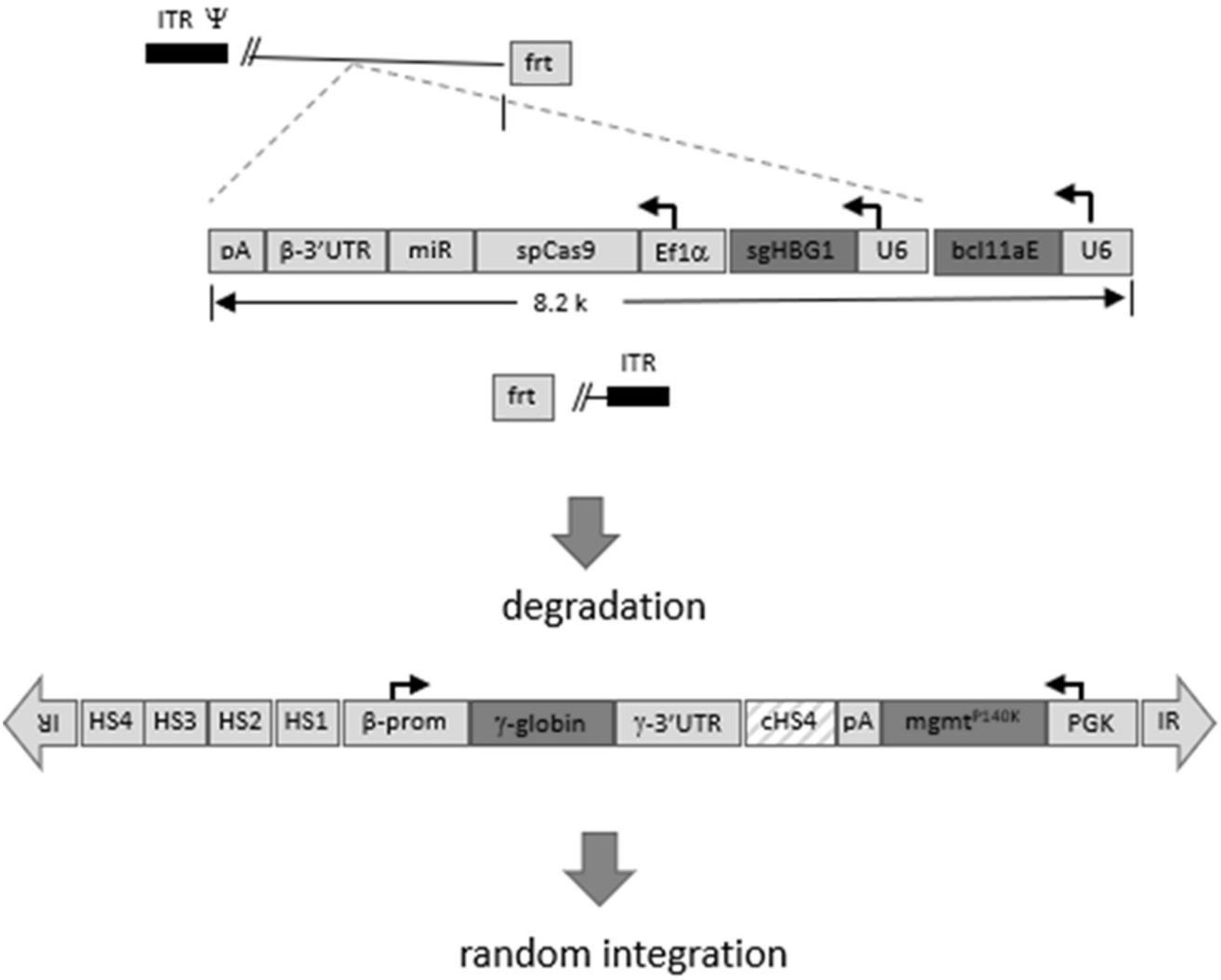

ITR Ψ
frt
pA
β-3'UTR
miR
spCas9
Ef1α
sgHBG1
U6
bcl11aE
U6
8.2 k
frt
ITR
degradation
IR
HS4
HS3
HS2
HS1
β-prom
γ-globin
γ-3'UTR
cHS4
pA
mgmt^{P140K}
PGK
IR
random integration

FIG. 97B

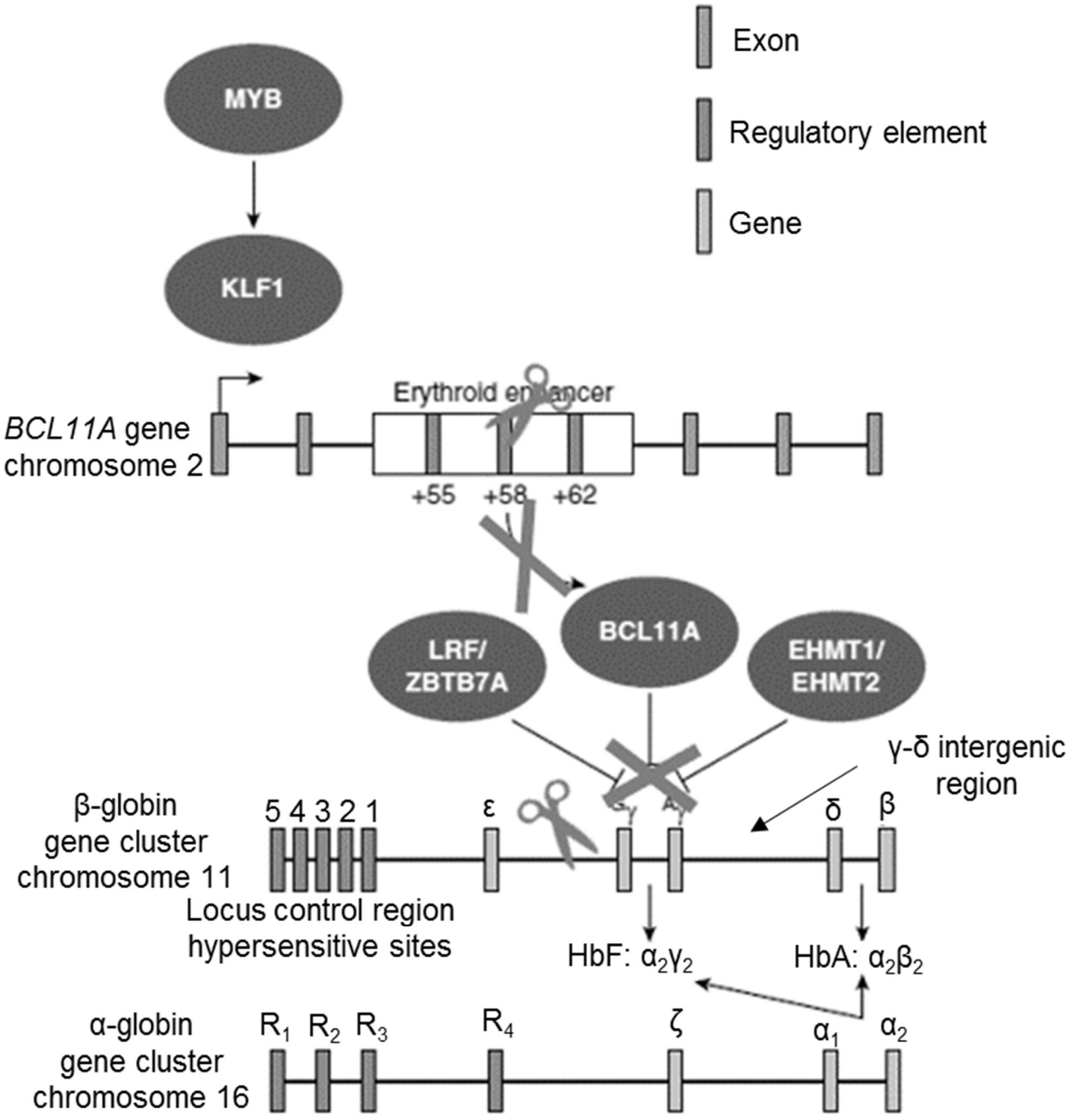

Exon
Regulatory element
Gene
MYB
KLF1
BCL11A gene
chromosome 2
Erythroid enhancer
+55
+58
+62
LRF/
ZBTB7A
BCL11A
EHMT1/
EHMT2
γ-δ intergenic
region
β-globin
gene cluster
chromosome 11
5 4 3 2 1
ε
Gγ
Aγ
δ
β
Locus control region
hypersensitive sites
HbF: α2γ2
HbA: α2β2
α-globin
gene cluster
chromosome 16
R1 R2 R3
R4
ζ
α1
α2

FIG. 97C

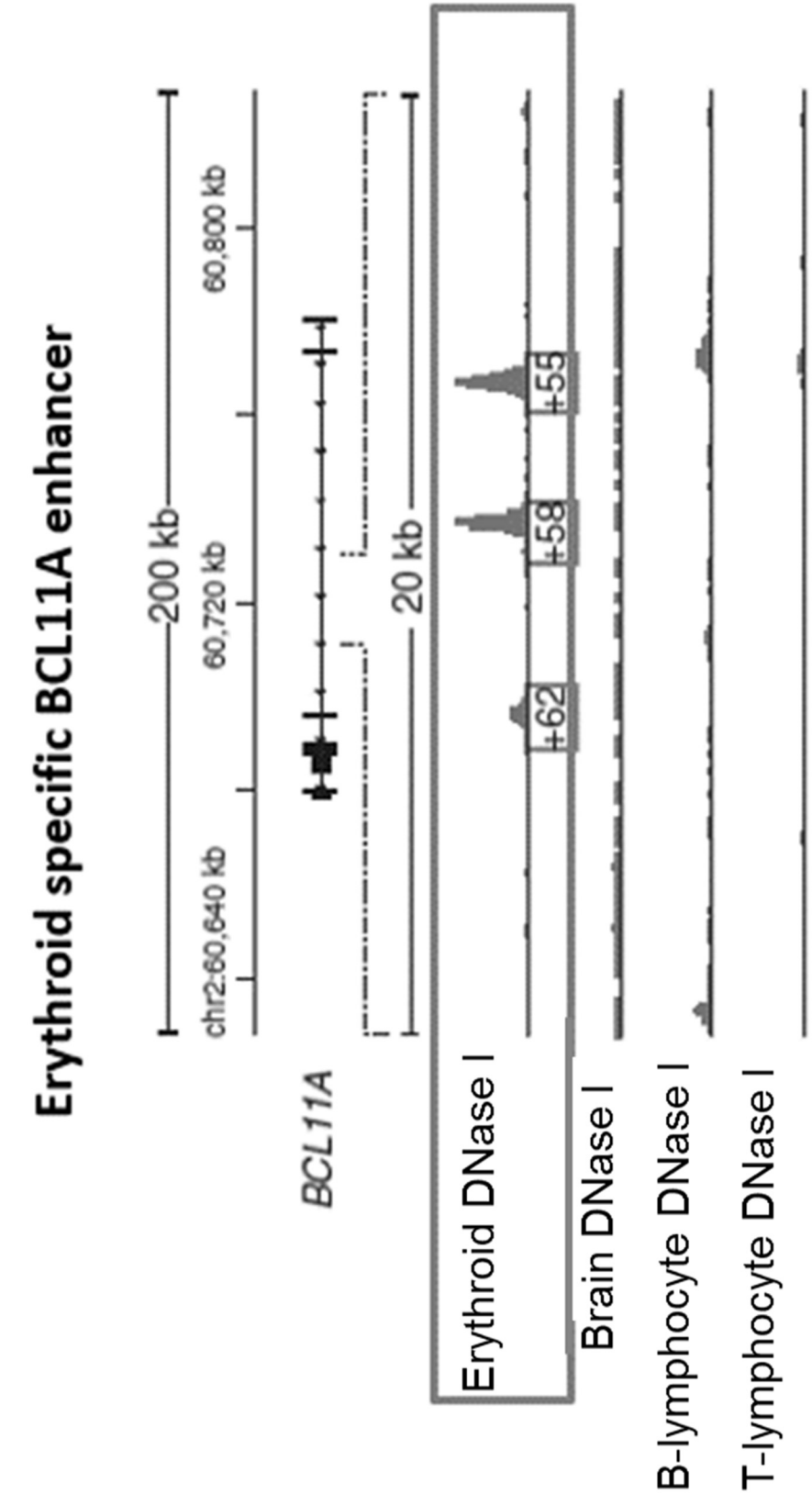

Erythroid specific BCL11A enhancer
200 kb
chr2:60,640 kb
60,720 kb
60,800 kb
BCL11A
20 kb
+62
+58
+55
Erythroid DNase I
Brain DNase I
B-lymphocyte DNase I
T-lymphocyte DNase I

FIG. 97D

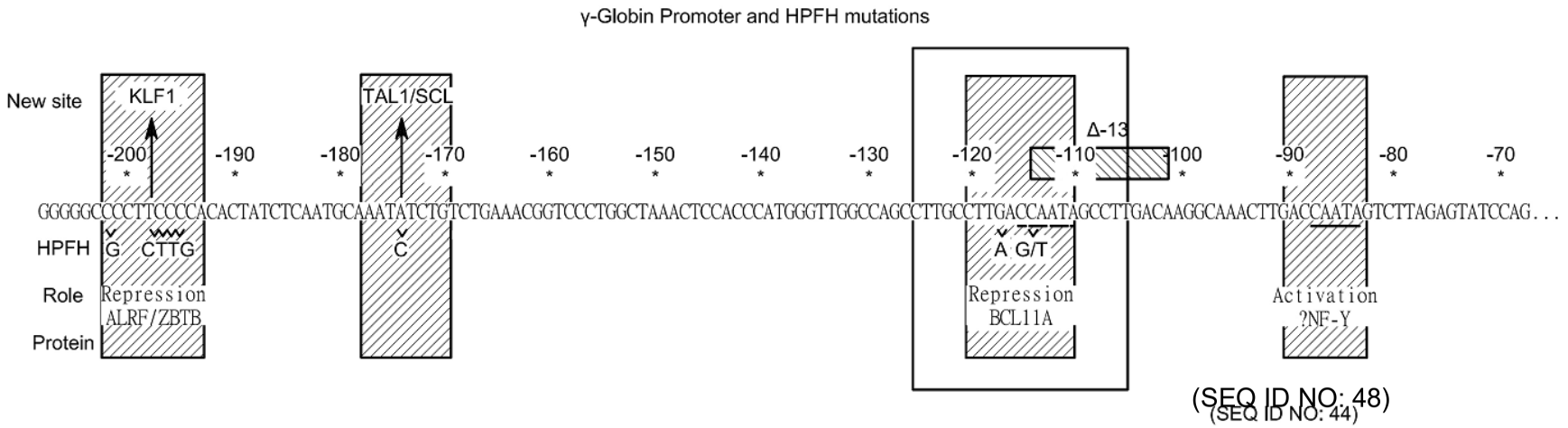


FIG. 98A

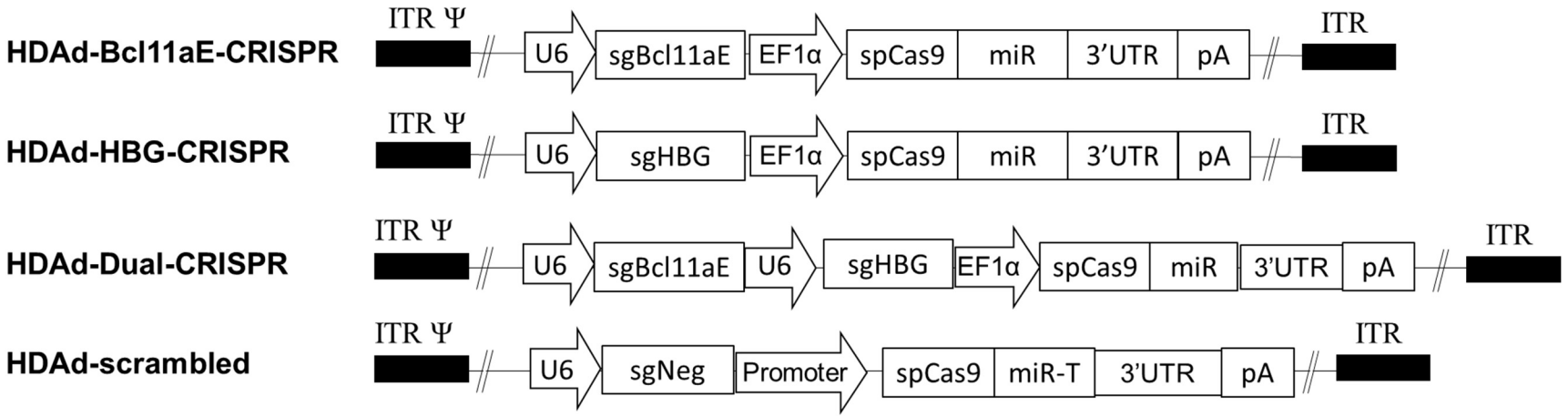


FIG. 98B

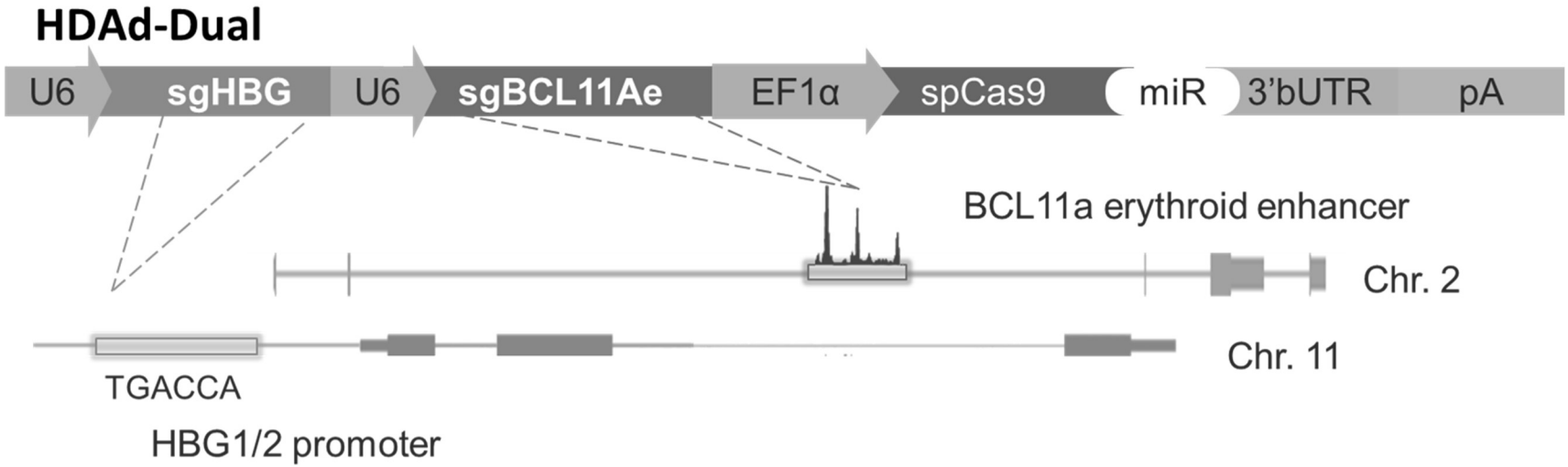


FIG. 98C

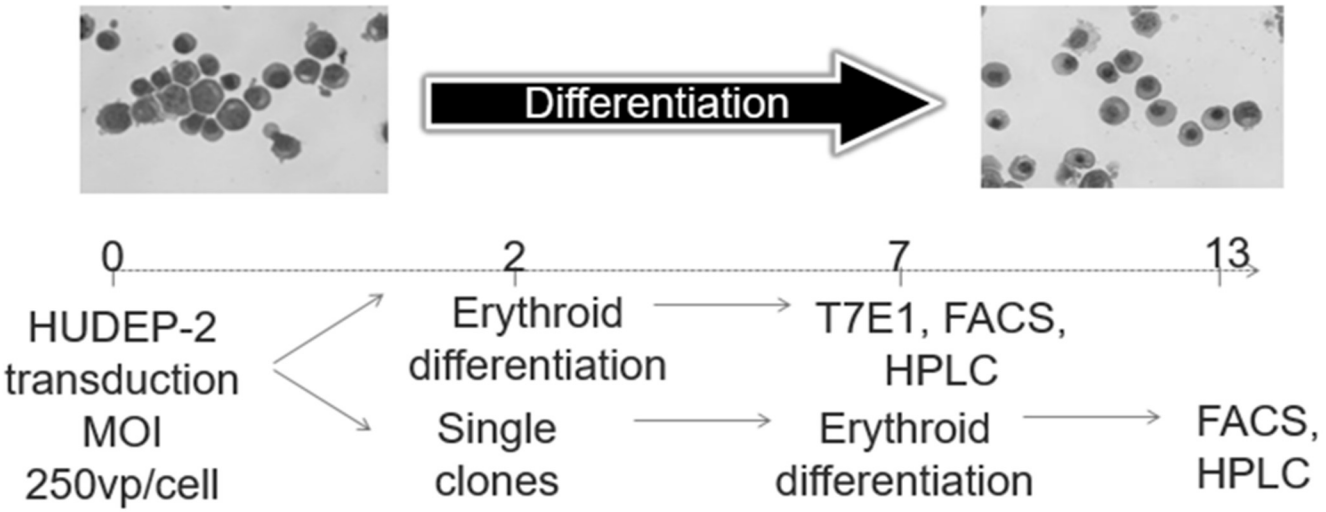


FIG. 98D

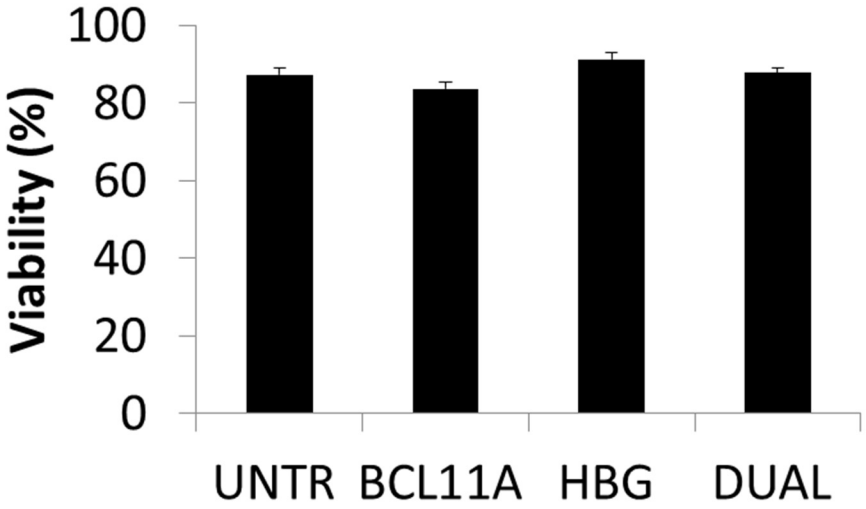


FIG. 98E

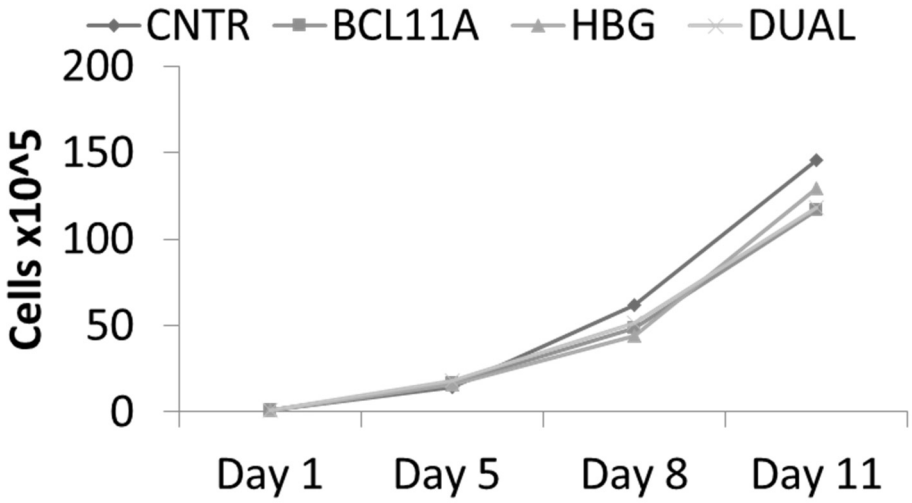


FIG. 98F

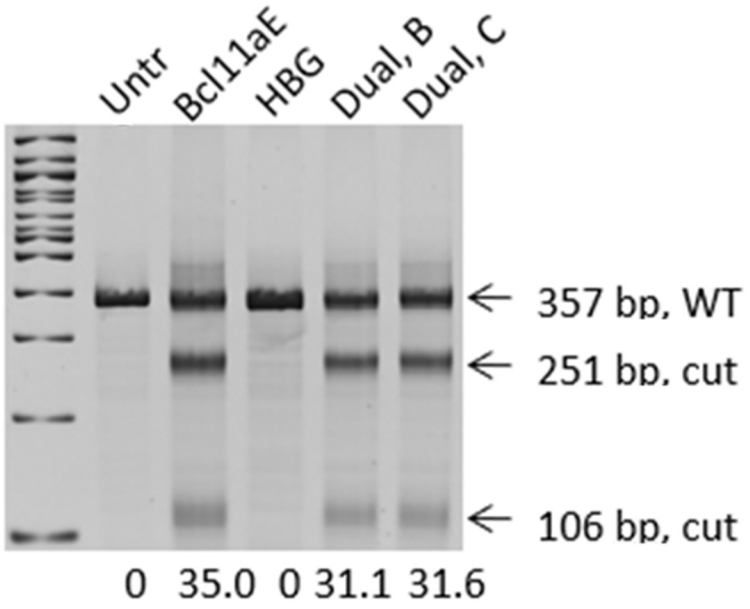


FIG. 98G

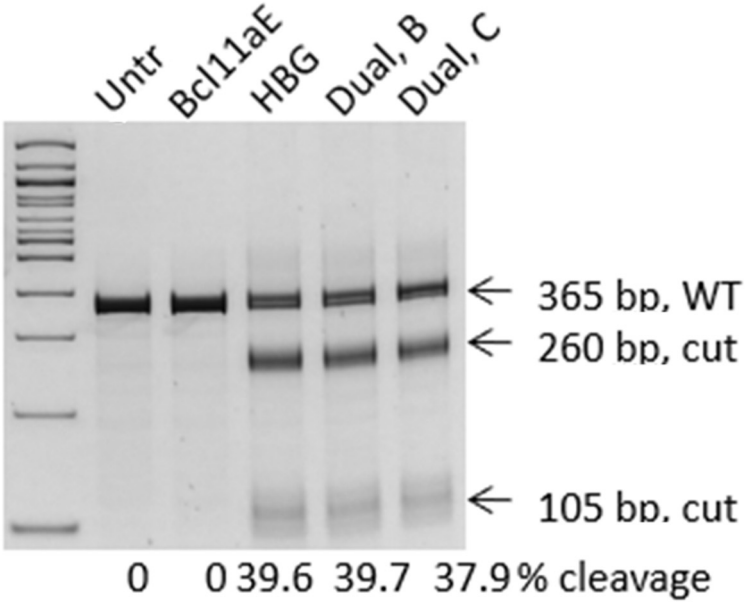


FIG. 98H

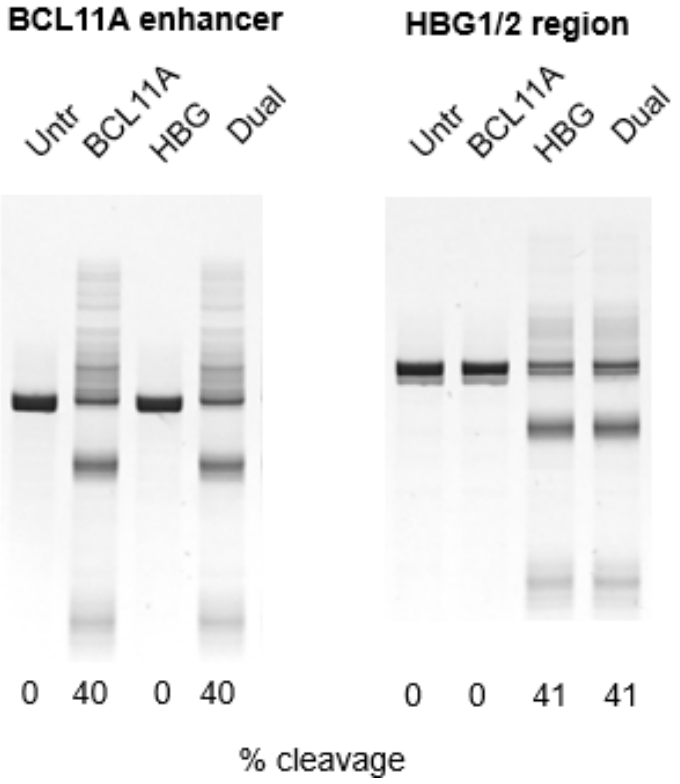
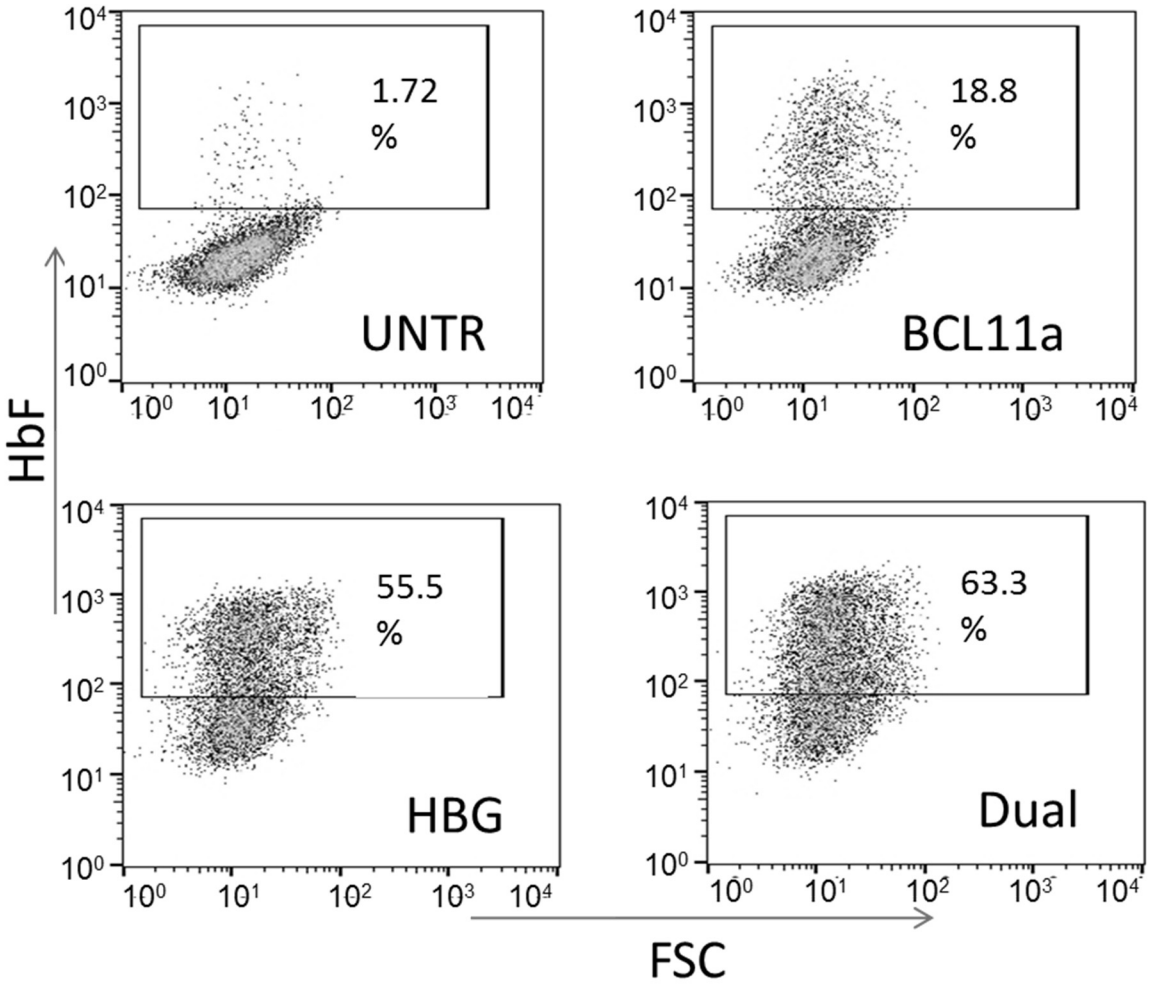

BCL11A enhancer
HBG1/2 region
Untr
BCL11A
HBG
Dual
Untr
BCL11A
HBG
Dual
0 40 0 40
0 0 41 41
% cleavage

FIG. 98I


1.72
%
UNTR
18.8
%
BCL11a
55.5
%
HBG
63.3
%
Dual
HbF
FSC

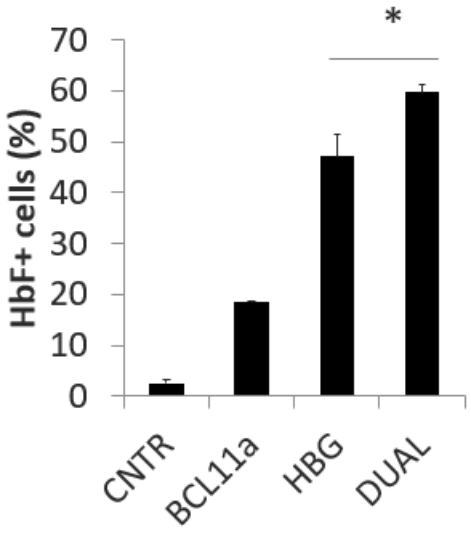

*
HbF+ cells (%)
70
60
50
40
30
20
10
0
CNTR
BCL11a
HBG
DUAL

FIG. 98J

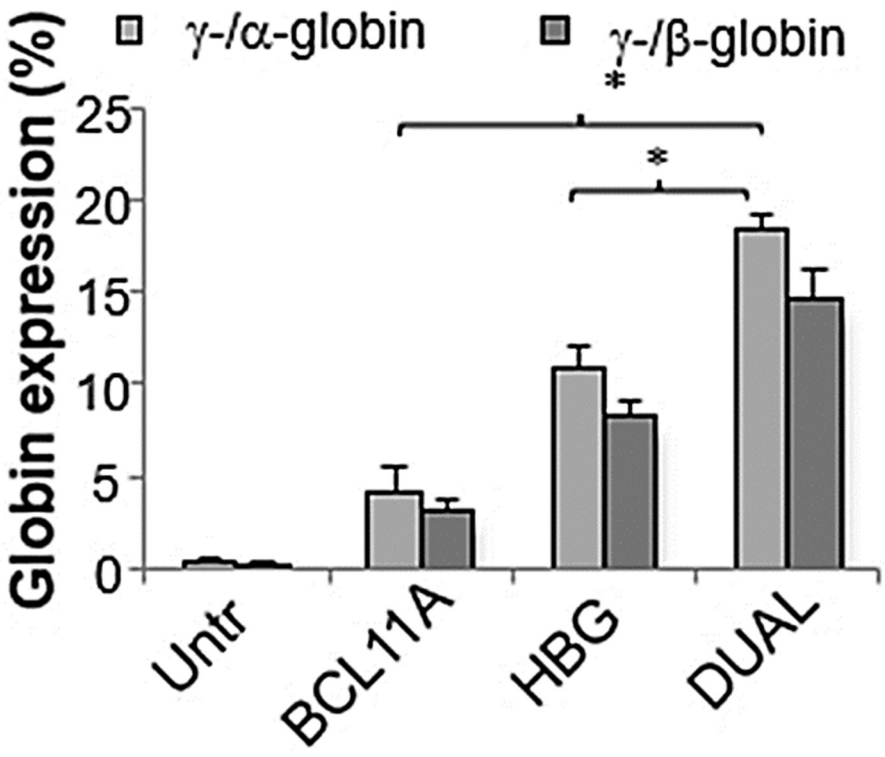

γ-/α-globin
γ-/β-globin
Globin expression (%)
25
20
15
10
5
0
*
*
Untr
BCL11A
HBG
DUAL

FIG. 98K

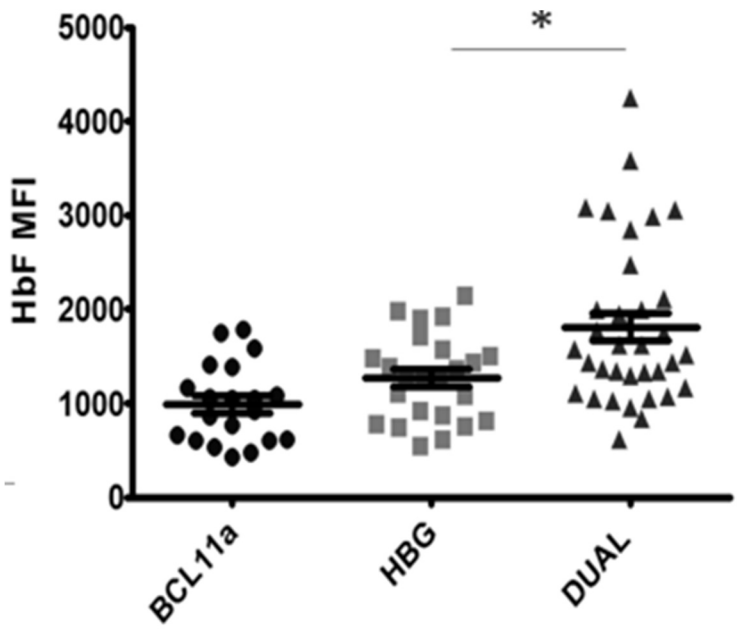


FIG. 98L

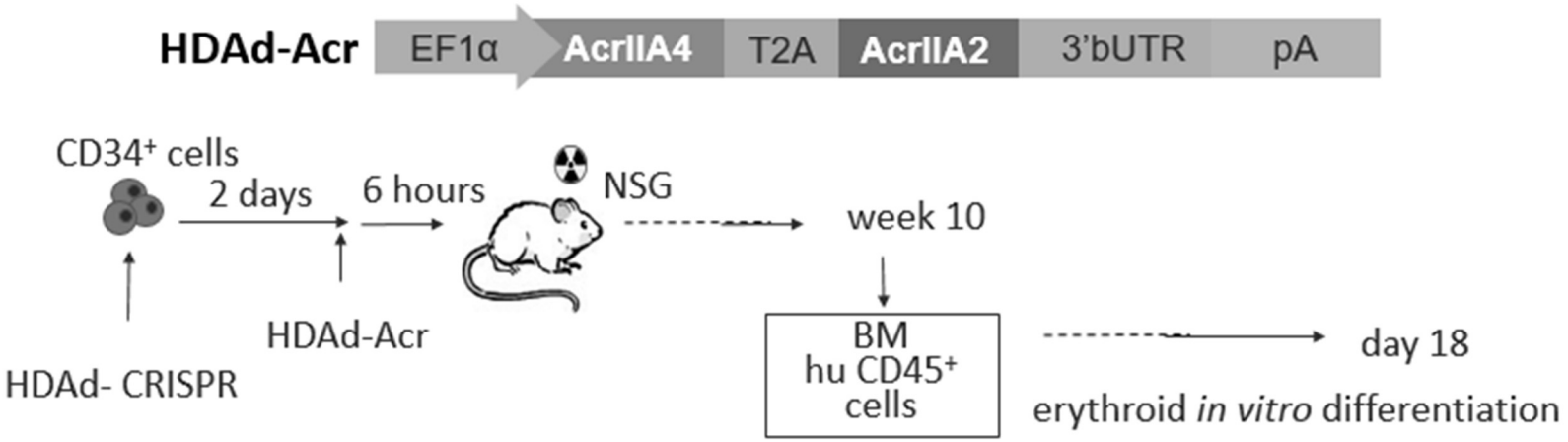


FIG. 98M

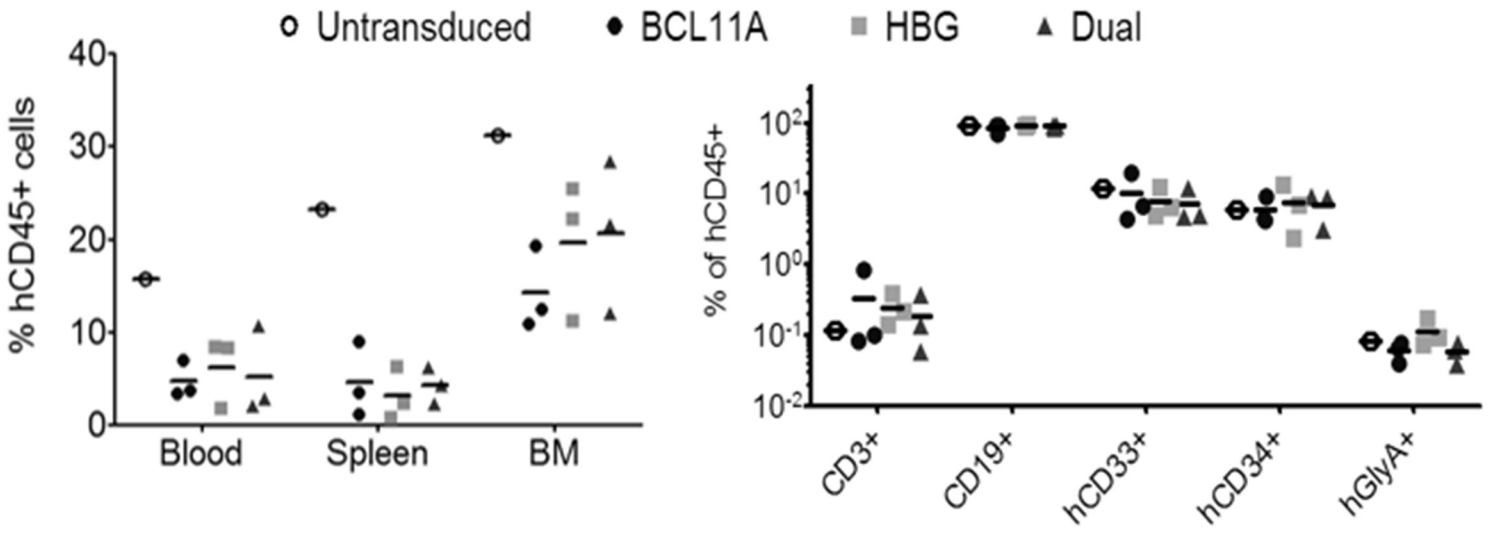


FIG. 98N

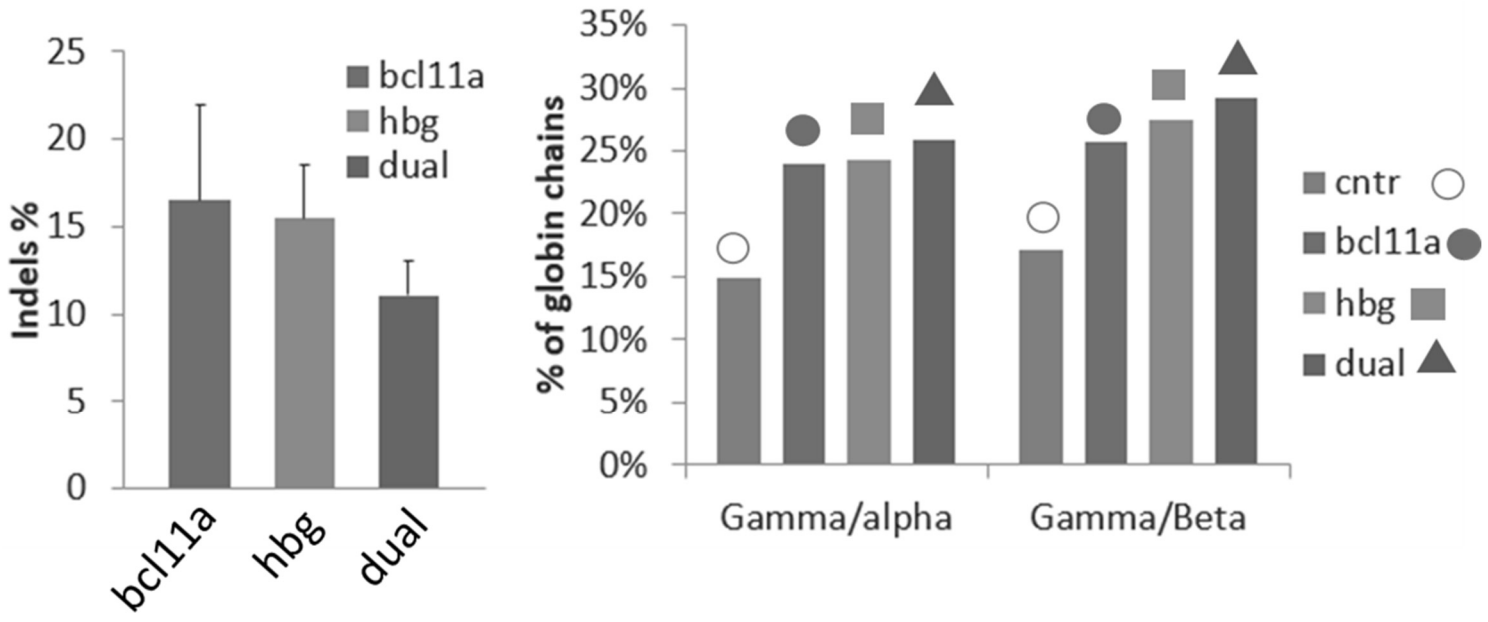


FIG. 99A

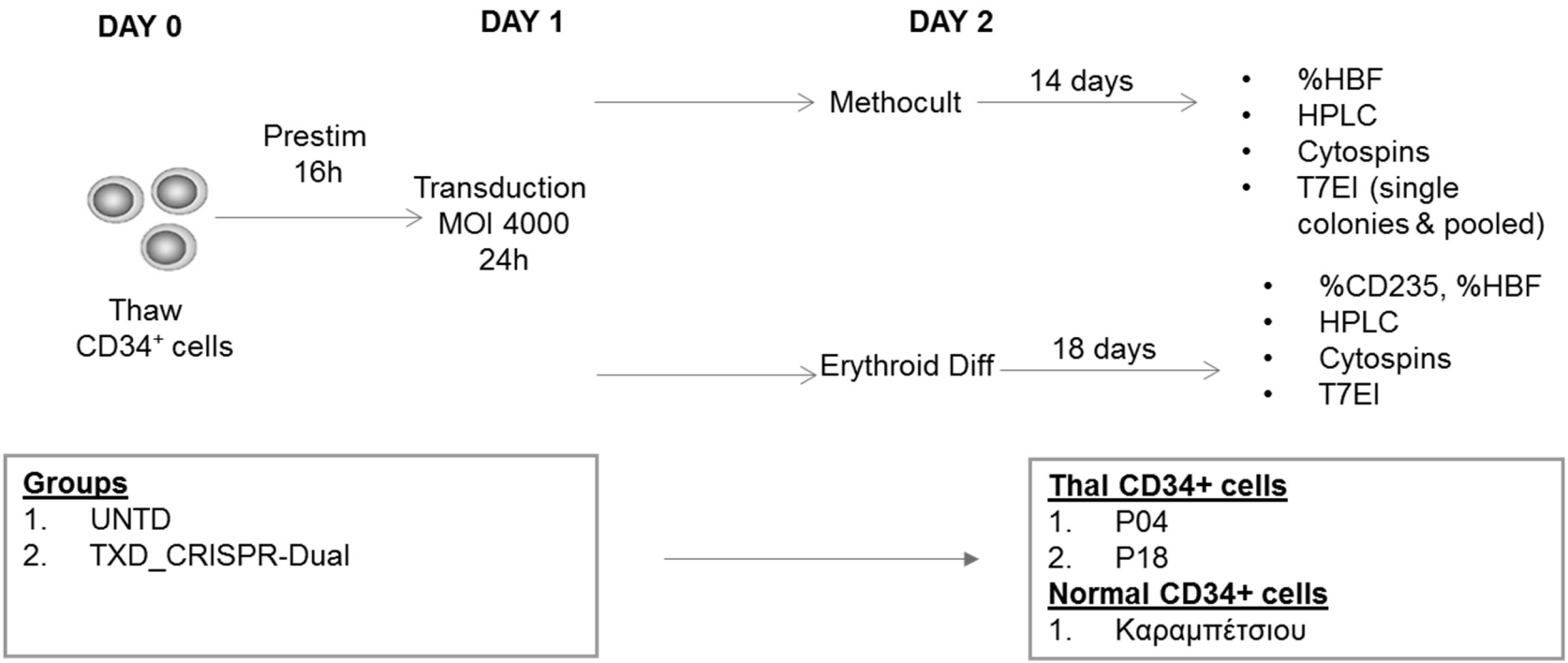

DAY 0
DAY 1
DAY 2
Prestim
16h
Transduction
MOI 4000
24h
Thaw
CD34+ cells
Methocult
14 days
%HBF
HPLC
Cytospins
T7EI (single colonies & pooled)
Erythroid Diff
18 days
%CD235, %HBF
HPLC
Cytospins
T7EI
Groups
1. UNTD
2. TXD_CRISPR-Dual
Thal CD34+ cells
1. P04
2. P18
Normal CD34+ cells
1. Καραμπέτσιου

FIG. 99B

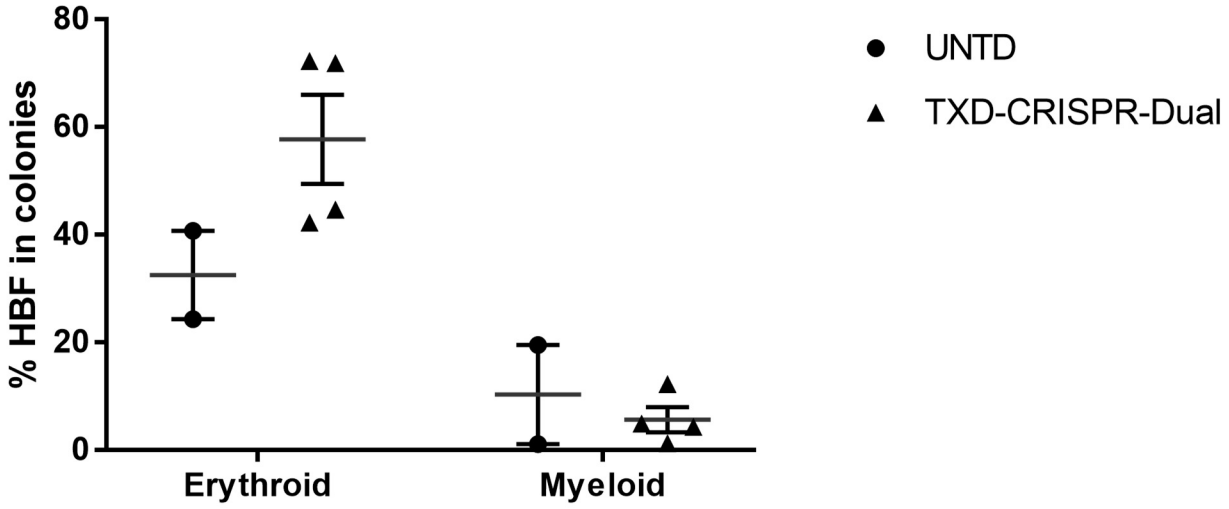


FIG. 99C

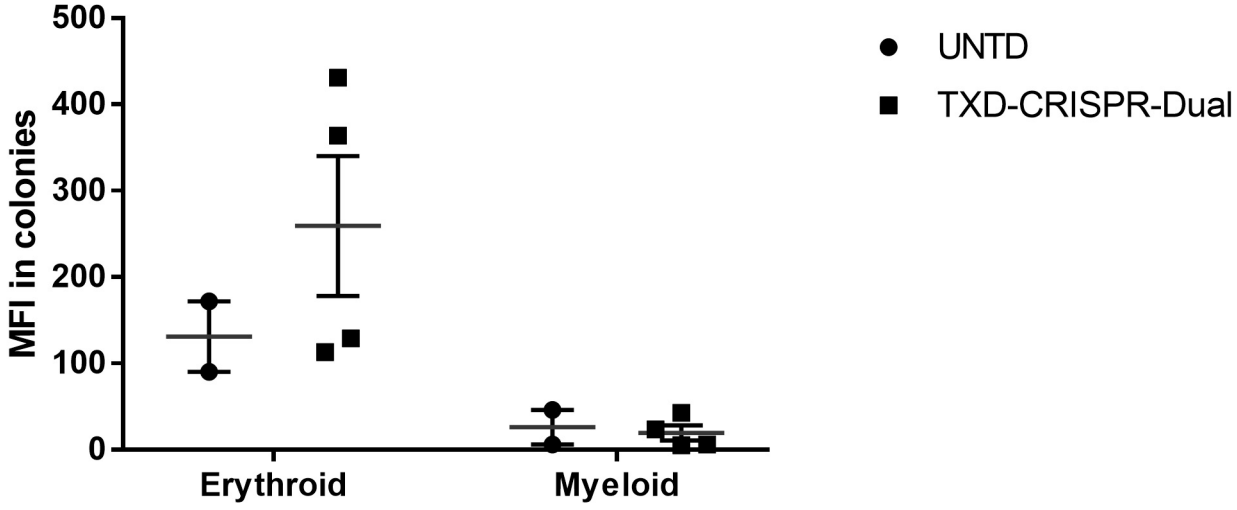


FIG. 99D

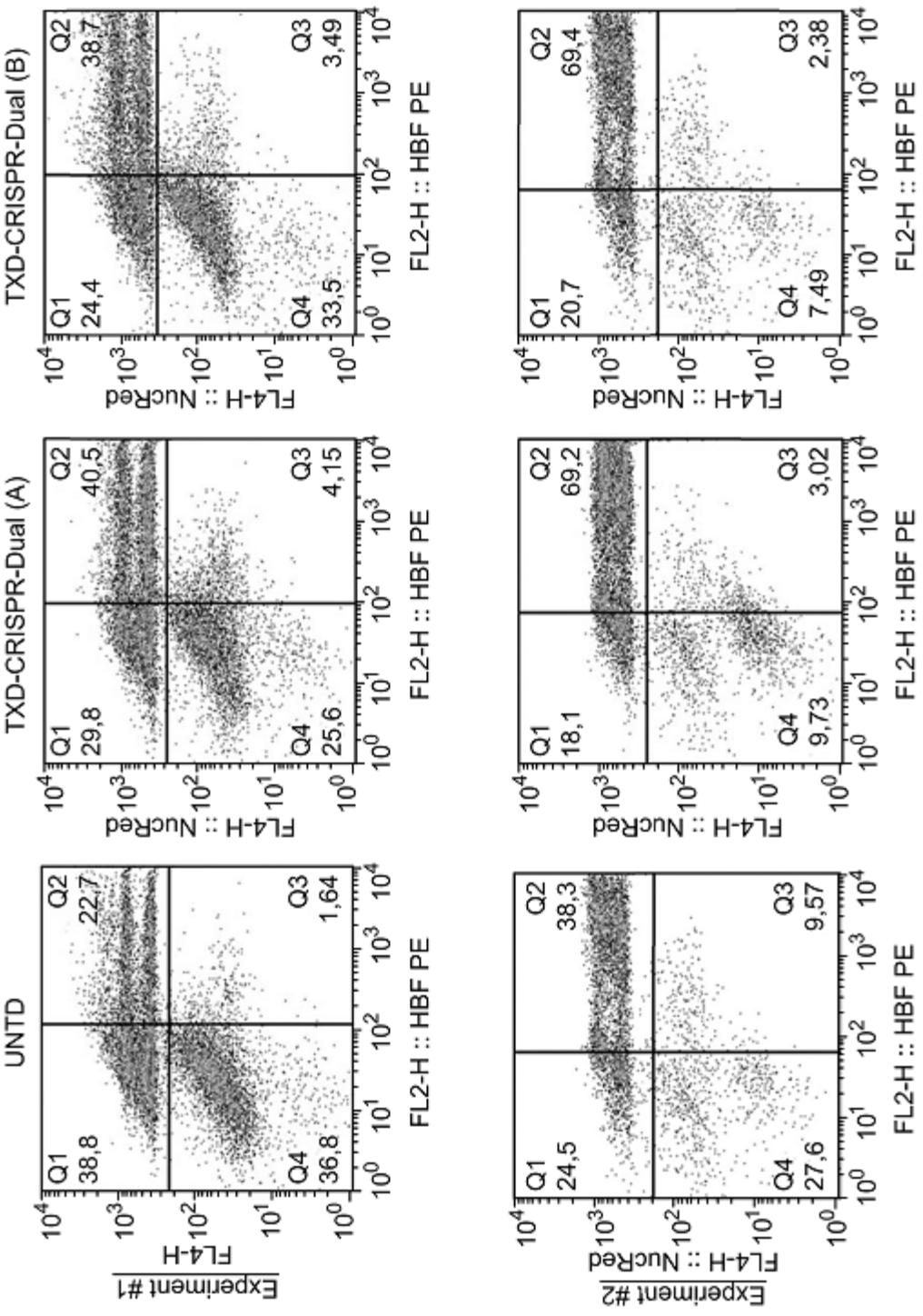

UNTD
TXD-CRISPR-Dual (A)
TXD-CRISPR-Dual (B)
Experiment #1
Experiment #2
FL4-H
FL4-H :: NucRed
FL2-H :: HBF PE
Q1 38,8
Q2 22,7
Q3 1,64
Q4 36,8
Q1 29,8
Q2 40,5
Q3 4,15
Q4 25,6
Q1 24,4
Q2 38,7
Q3 3,49
Q4 33,5
Q1 24,5
Q2 38,3
Q3 9,57
Q4 27,6
Q1 18,1
Q2 69,2
Q3 3,02
Q4 9,73
Q1 20,7
Q2 69,4
Q3 2,38
Q4 7,49

FIG. 99E

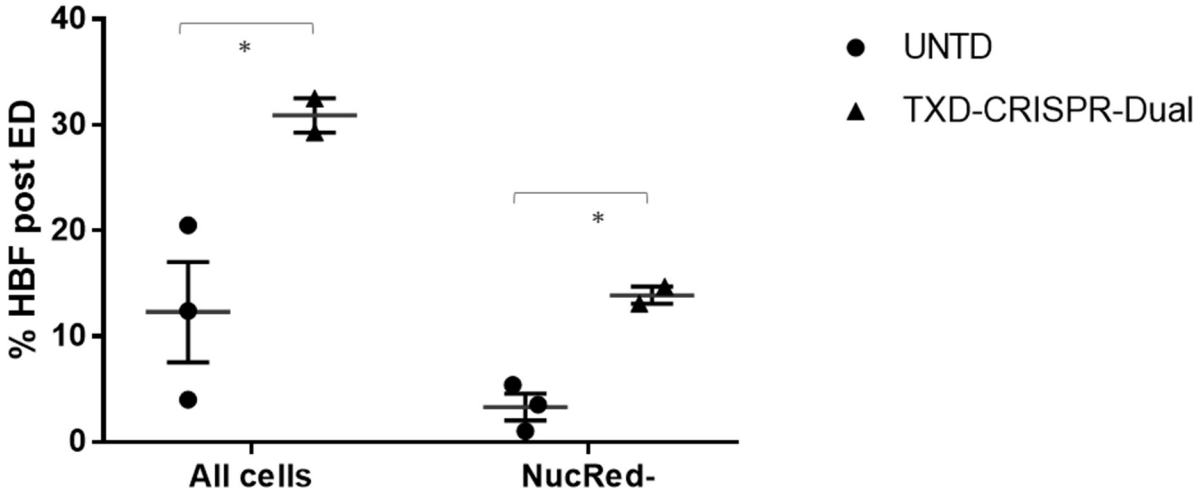


FIG. 99F

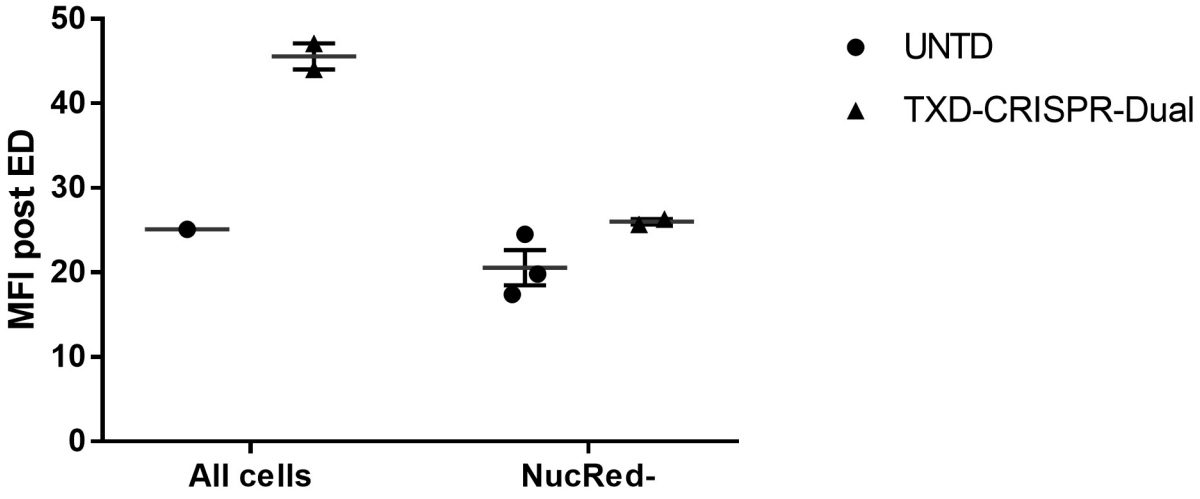


FIG. 99G

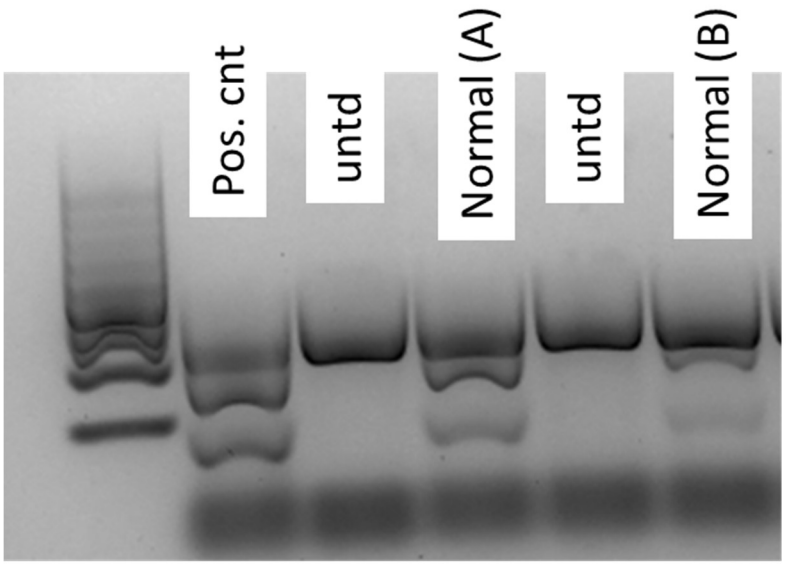
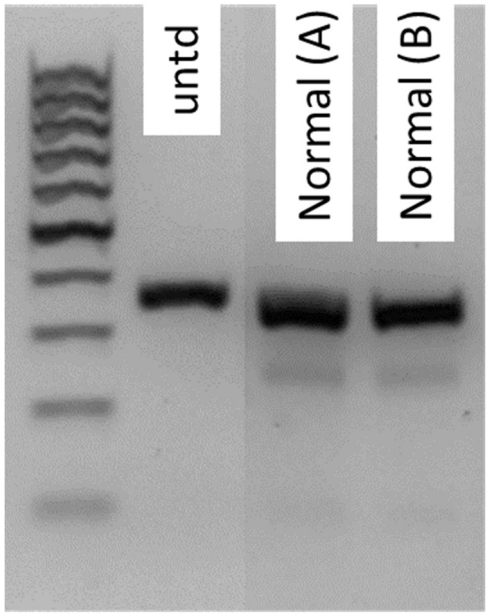


FIG. 99H



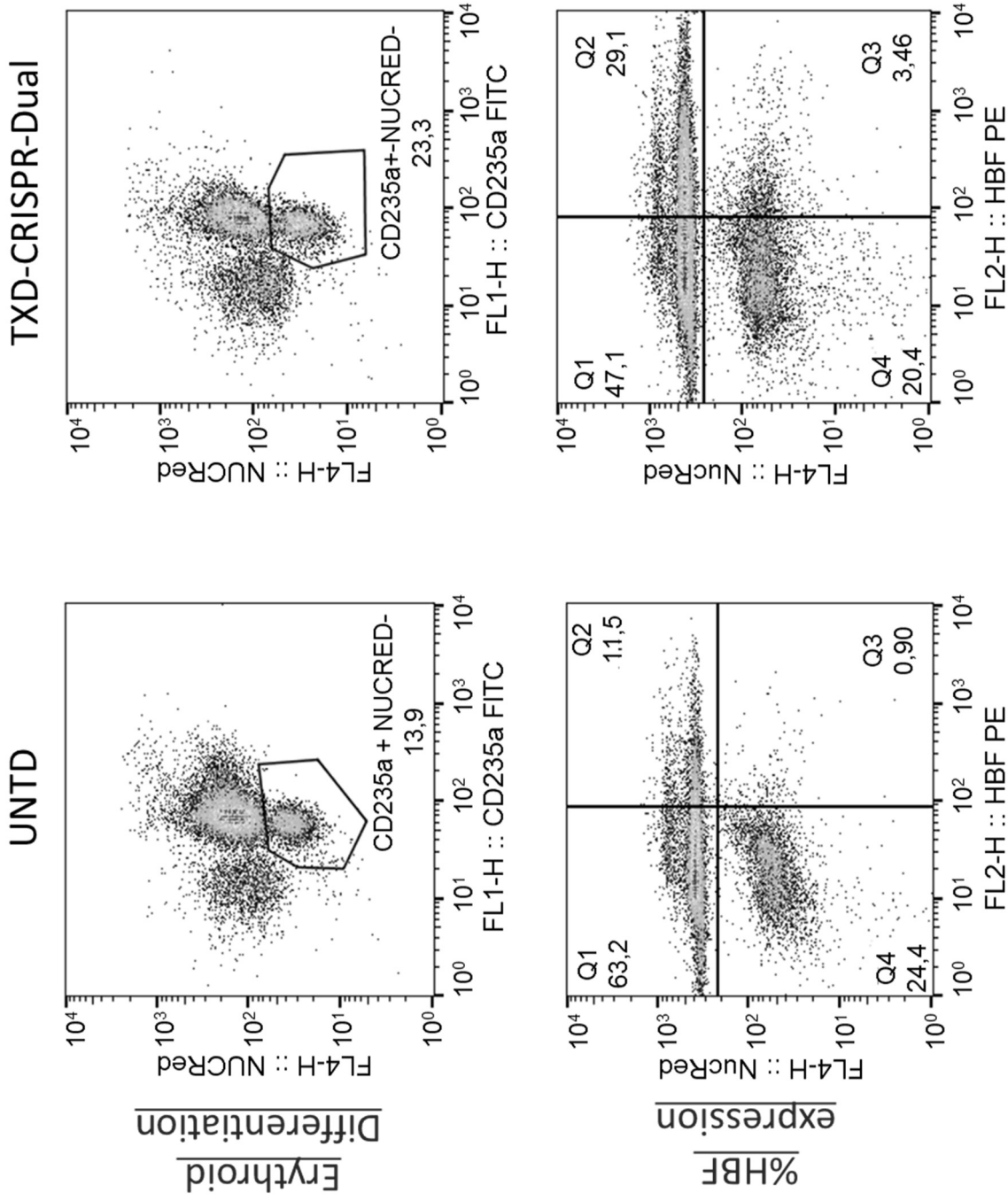

UNTD
TXD-CRISPR-Dual
Erythroid Differentiation
%HBF expression
FL4-H :: NUCRed
FL1-H :: CD235a FITC
FL2-H :: HBF PE
CD235a + NUCRED- 13,9
CD235a+-NUCRED- 23,3
Q1 63,2
Q2 11,5
Q3 0,90
Q4 24,4
Q1 47,1
Q2 29,1
Q3 3,46
Q4 20,4

FIG. 99I

FIG. 99J

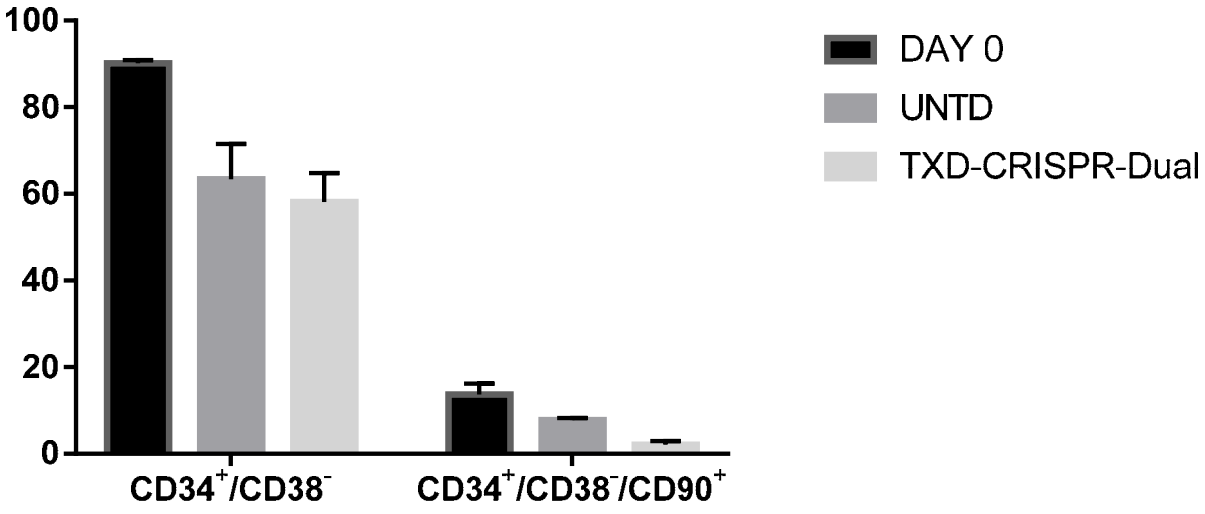


FIG. 99 K

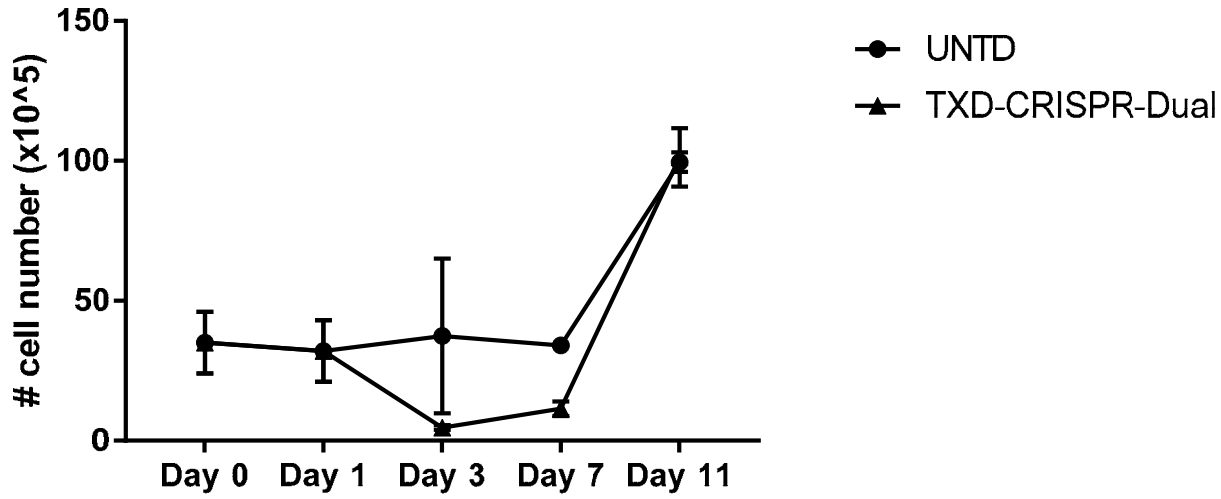


FIG. 99L

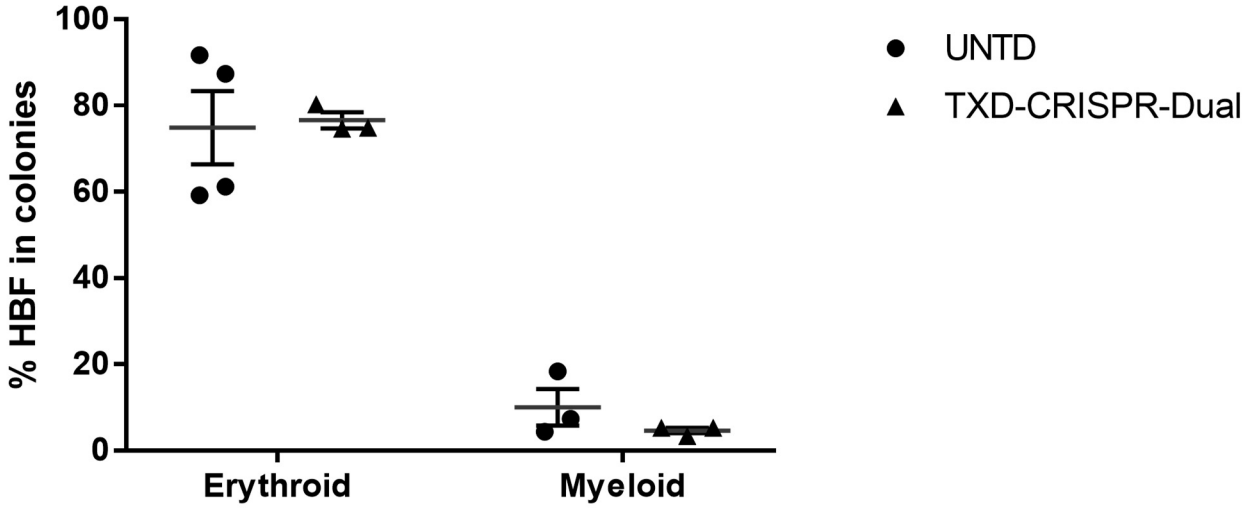


FIG. 99M

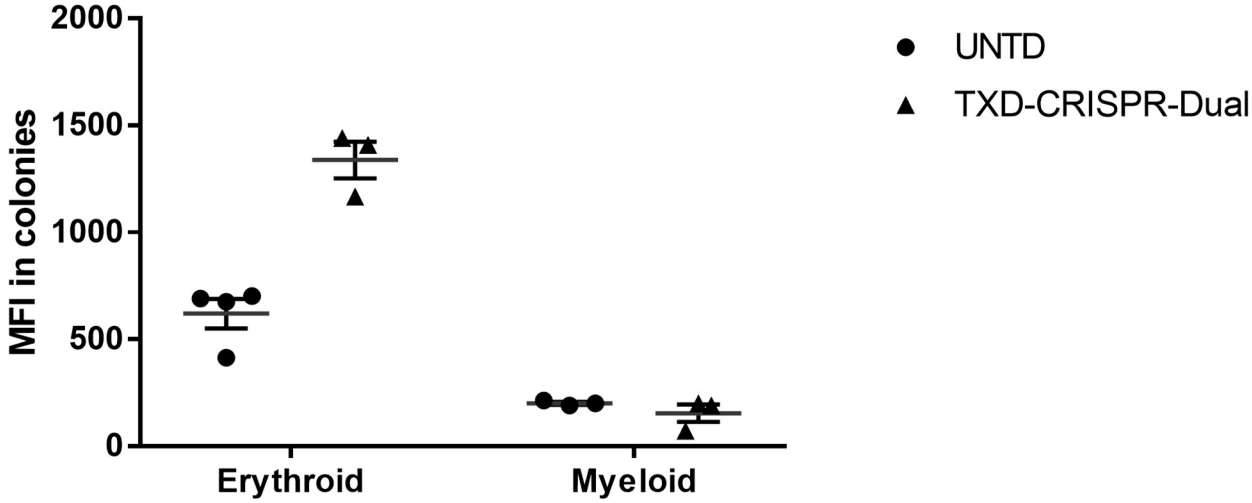


FIG. 99N

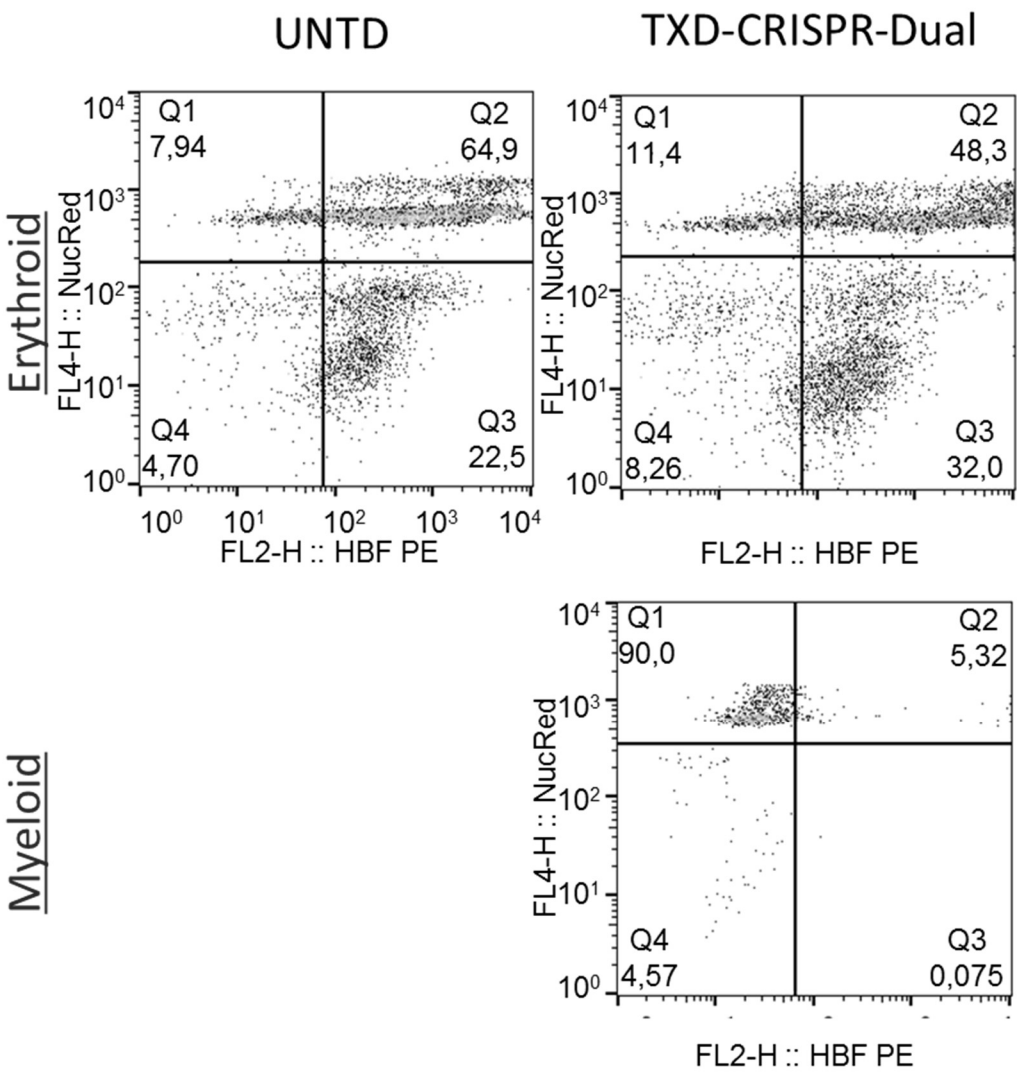

UNTD
TXD-CRISPR-Dual
Erythroid
Myeloid
FL4-H :: NucRed
FL2-H :: HBF PE
Q1
7,94
Q2
64,9
Q4
4,70
Q3
22,5
Q1
11,4
Q2
48,3
Q4
8,26
Q3
32,0
Q1
90,0
Q2
5,32
Q4
4,57
Q3
0,075

FIG. 99O

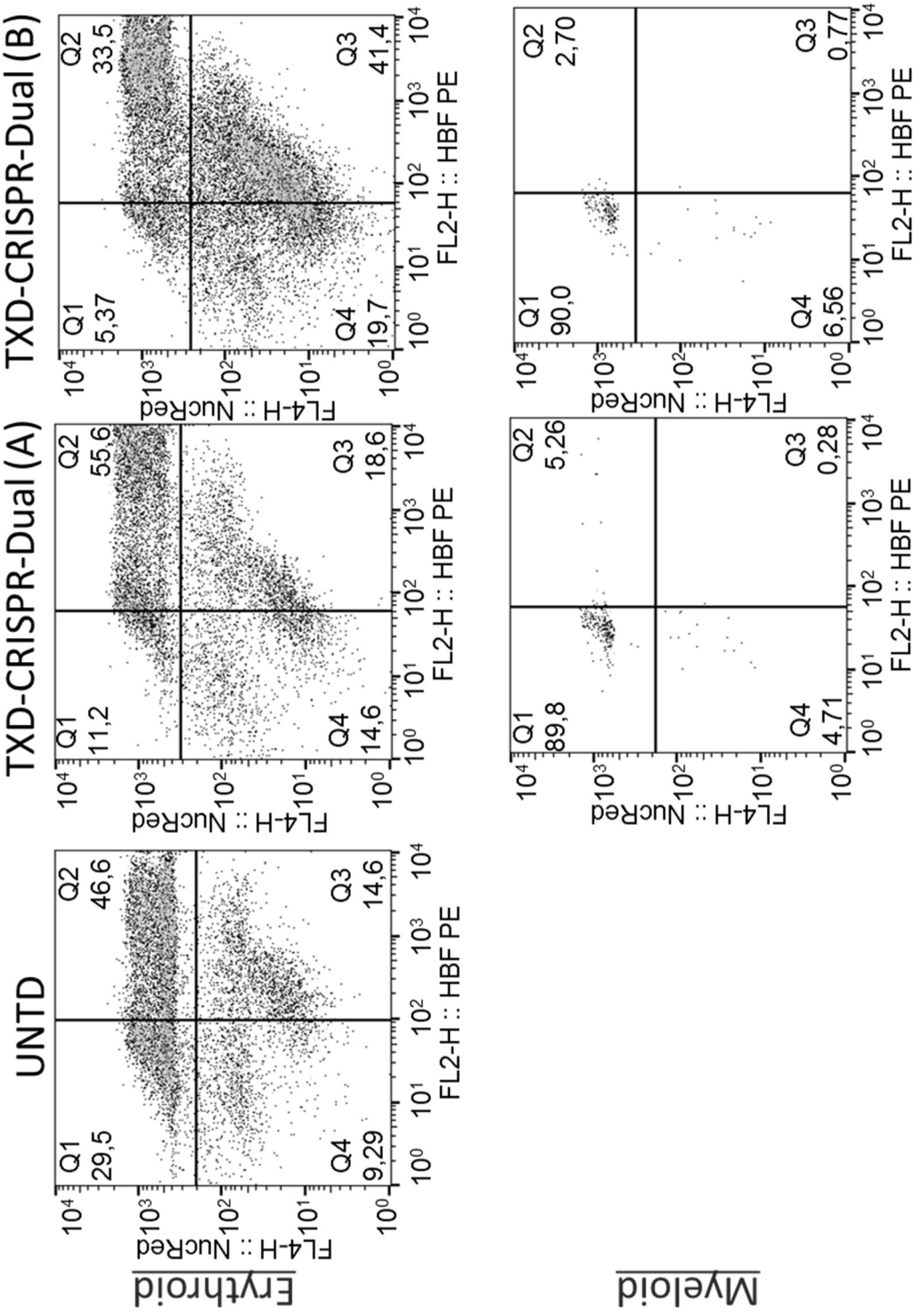

UNTD
TXD-CRISPR-Dual (A)
TXD-CRISPR-Dual (B)
Erythroid
Myeloid
FL4-H :: NucRed
FL2-H :: HBF PE
Q1 29,5
Q2 46,6
Q4 9,29
Q3 14,6
Q1 11,2
Q2 55,6
Q4 14,6
Q3 18,6
Q1 5,37
Q2 33,5
Q4 19,7
Q3 41,4
Q1 89,8
Q2 5,26
Q4 4,71
Q3 0,28
Q1 90,0
Q2 2,70
Q4 6,56
Q3 0,77

FIG. 99P

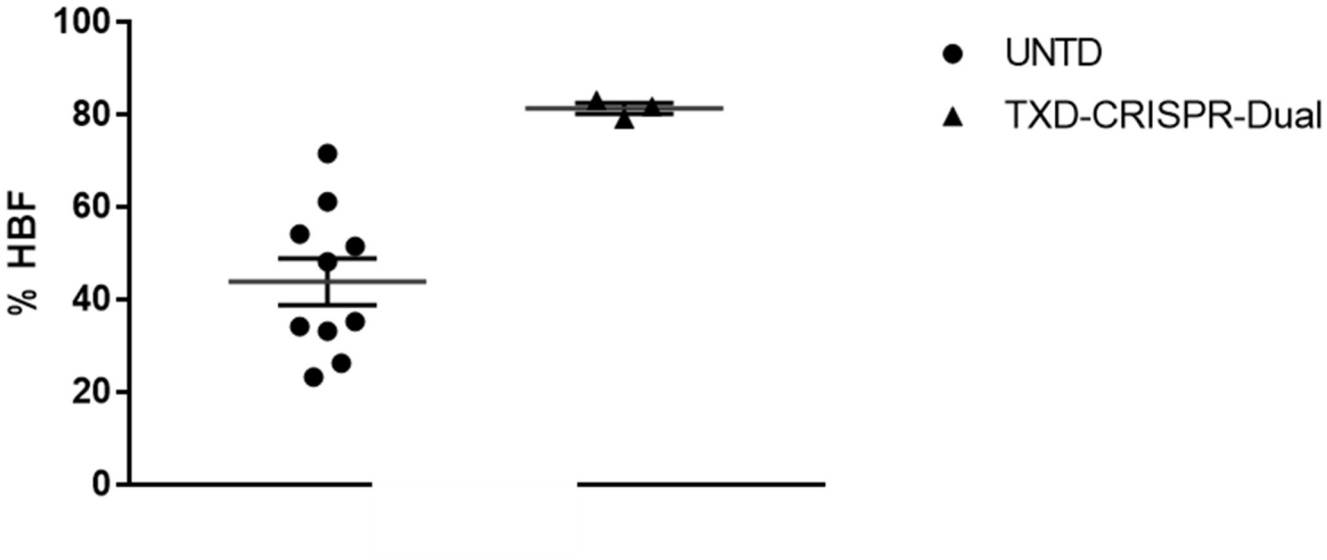


FIG. 99Q

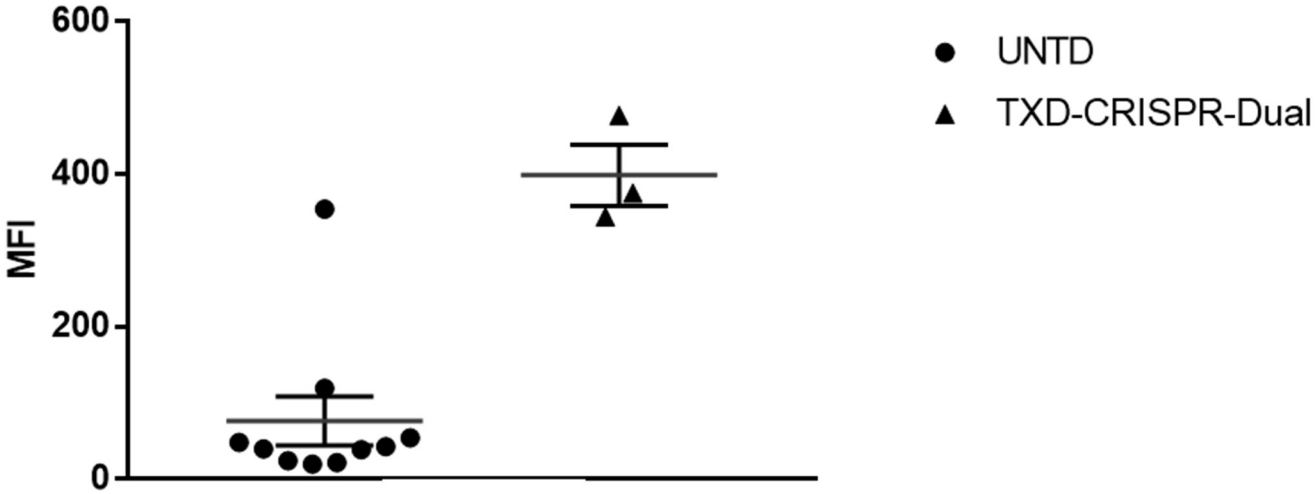


FIG. 99R

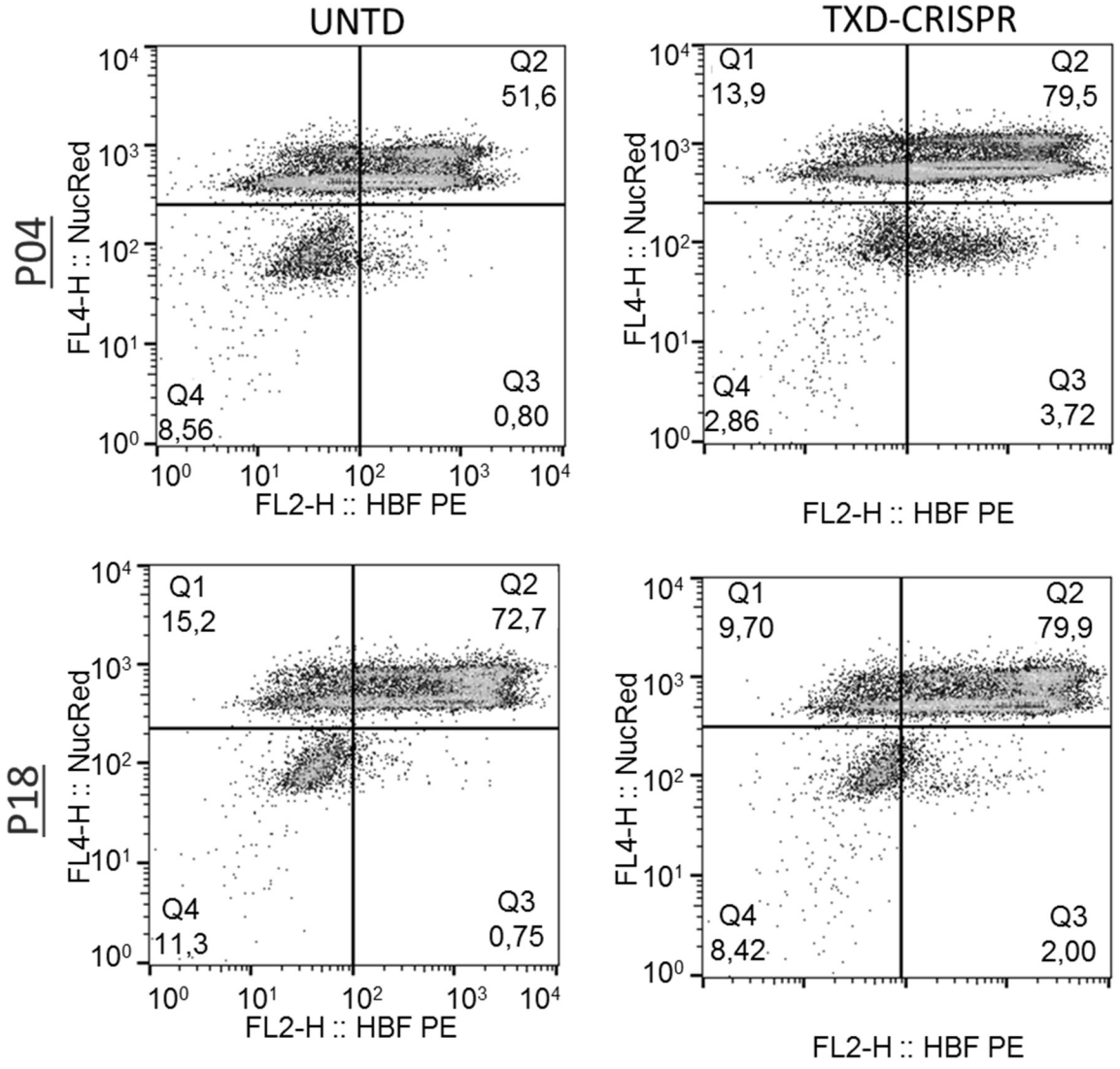

UNTD
TXD-CRISPR
P04
P18
FL4-H :: NucRed
FL2-H :: HBF PE
Q1
Q2
Q3
Q4
51,6
0,80
8,56
13,9
79,5
3,72
2,86
15,2
72,7
0,75
11,3
9,70
79,9
2,00
8,42

FIG. 99S

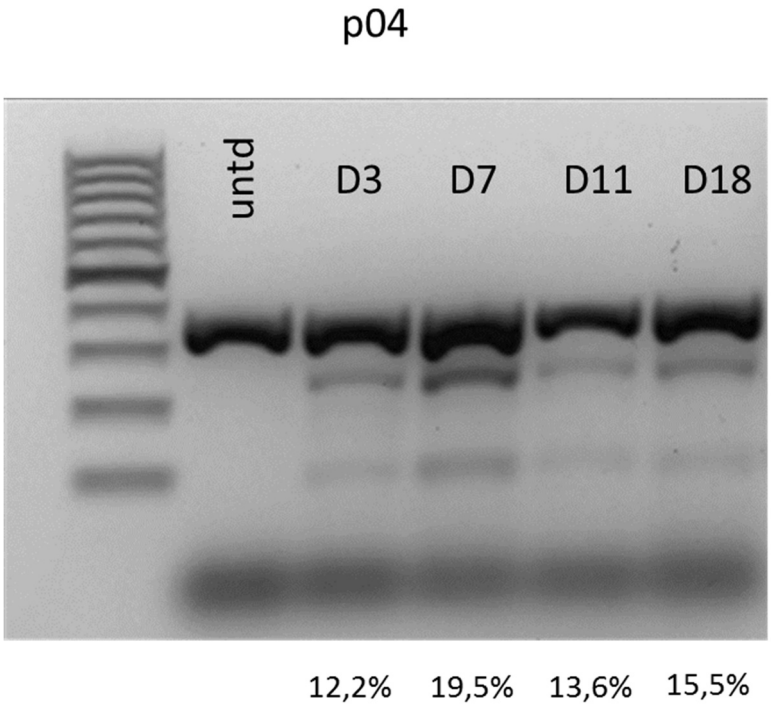


FIG. 99T

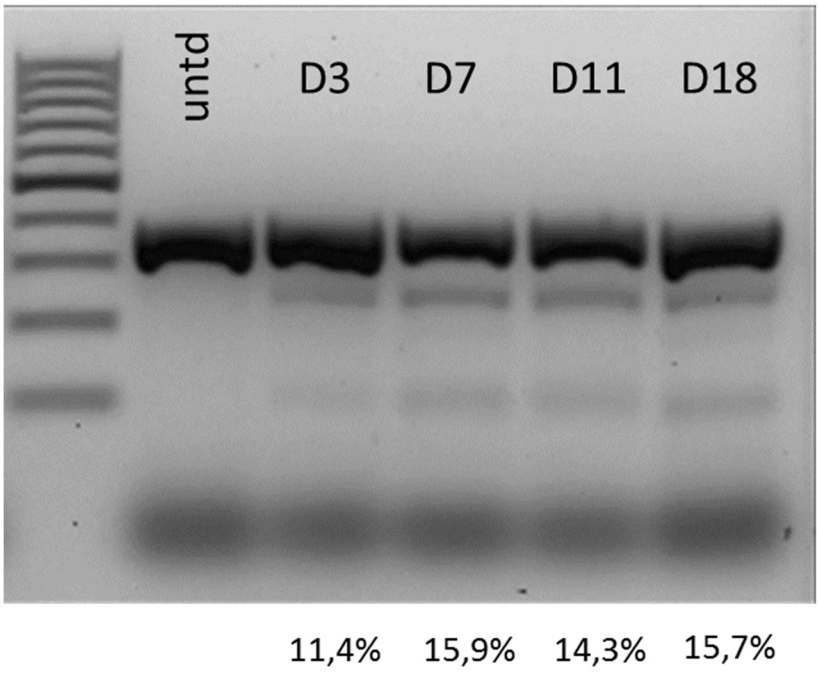


FIG. 99U

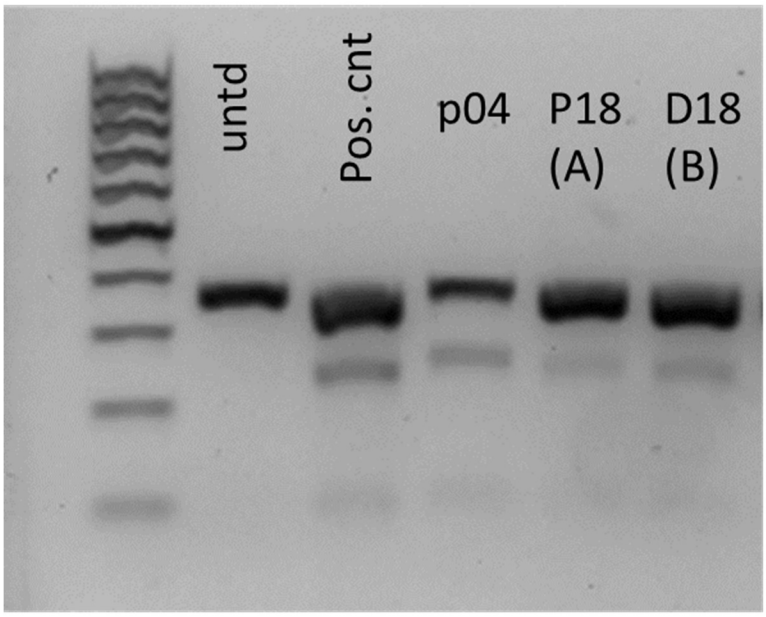


FIG. 100

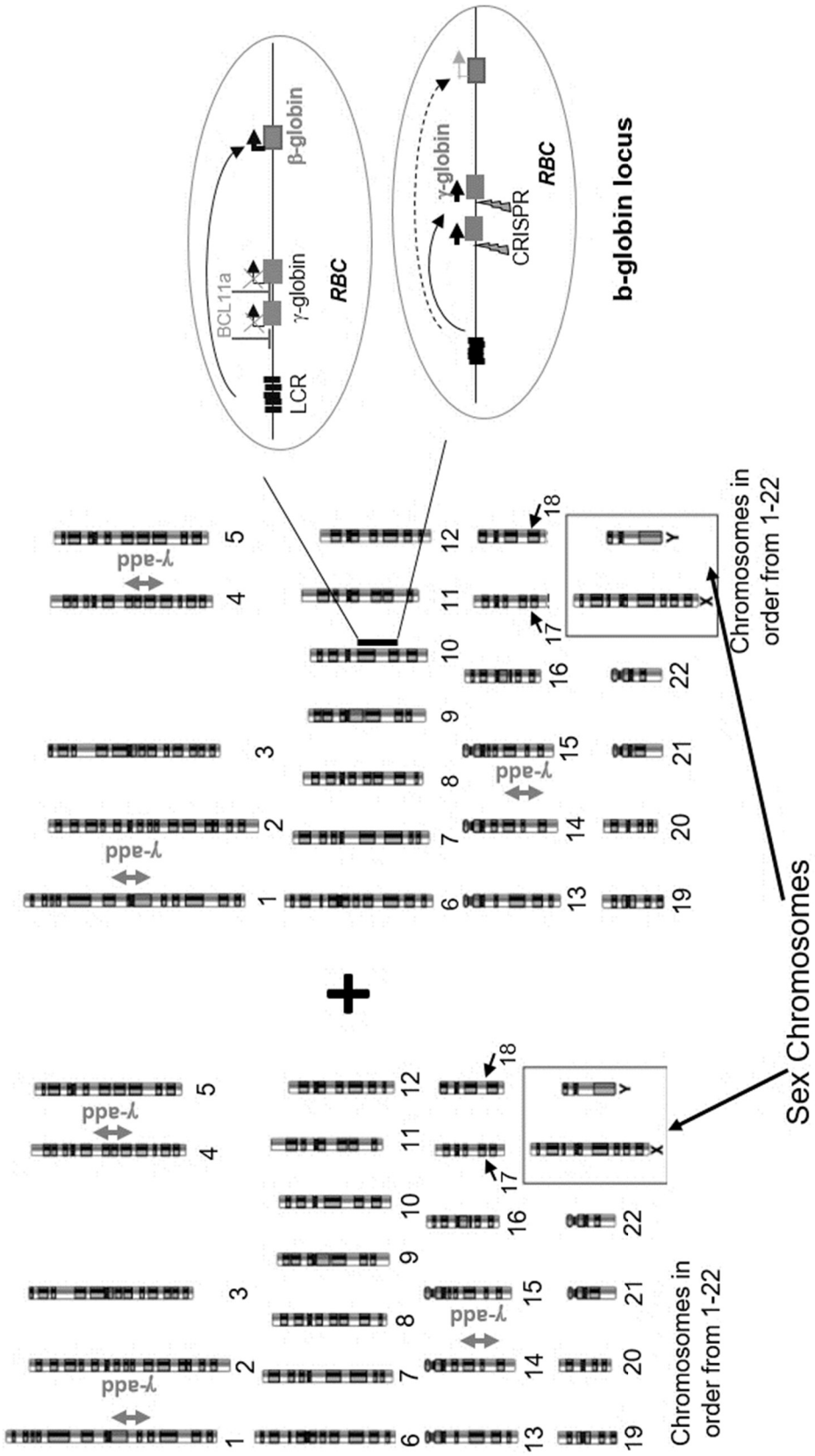

Combination of g-globin gene addition and re-activation of endogenous g-globin
SB100x-mediated
g-globin gene integration
γ-add
CRISPR-Cas9-mediated
reactivation of fetal g-globin
BCL11a
LCR
γ-globin
RBC
β-globin
γ-globin
CRISPR
RBC
b-globin locus
Chromosomes in
order from 1-22
Sex Chromosomes
Chromosomes in
order from 1-22

FIG. 101

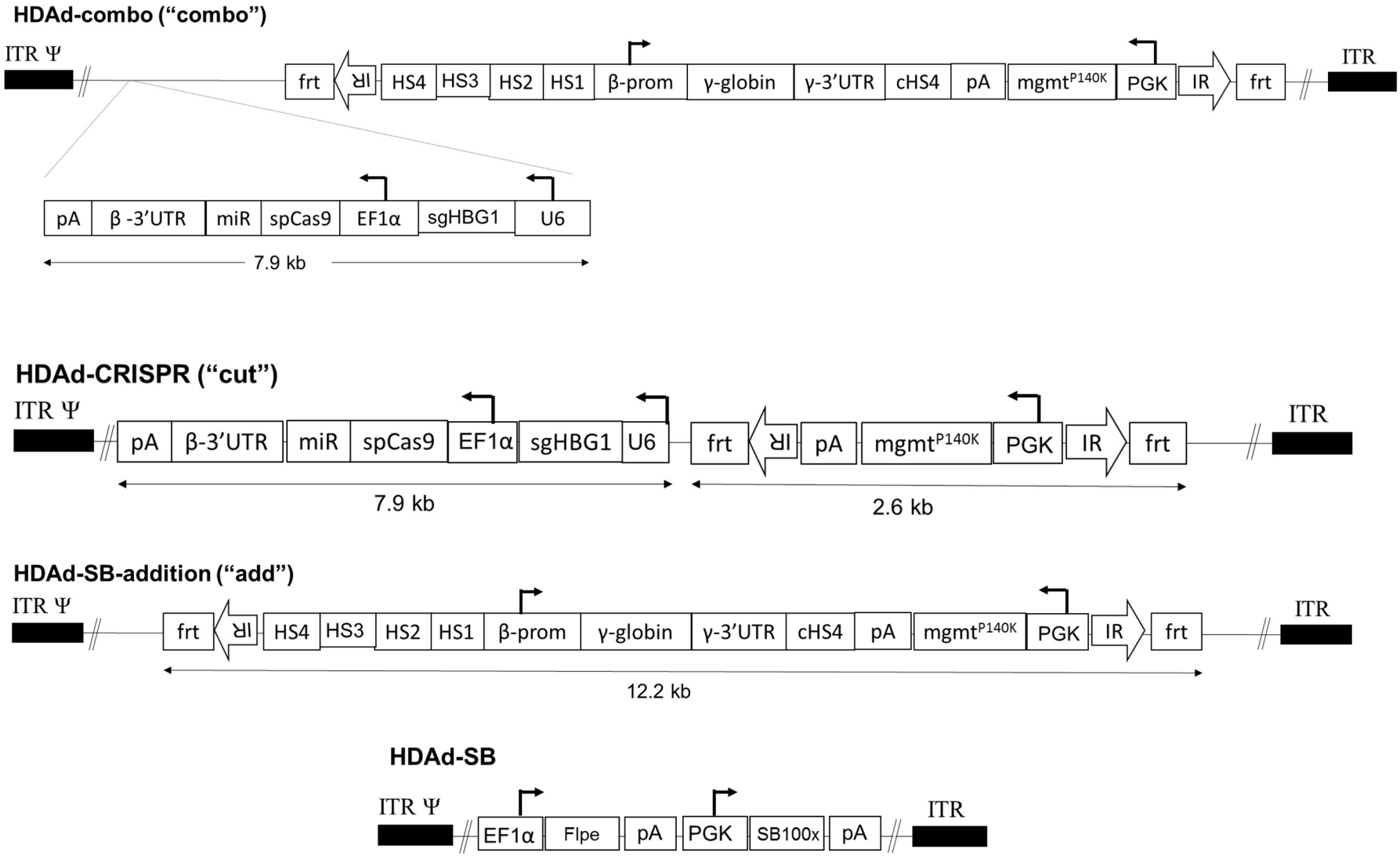

HDAd-combo ("combo")
ITR Ψ
frt
IR
HS4
HS3
HS2
HS1
β-prom
γ-globin
γ-3'UTR
cHS4
pA
mgmt^{P140K}
PGK
IR
frt
ITR
pA
β -3'UTR
miR
spCas9
EF1α
sgHBG1
U6
7.9 kb
HDAd-CRISPR ("cut")
ITR Ψ
pA
β-3'UTR
miR
spCas9
EF1α
sgHBG1
U6
7.9 kb
frt
IR
pA
mgmt^{P140K}
PGK
IR
frt
2.6 kb
ITR
HDAd-SB-addition ("add")
ITR Ψ
frt
IR
HS4
HS3
HS2
HS1
β-prom
γ-globin
γ-3'UTR
cHS4
pA
mgmt^{P140K}
PGK
IR
frt
12.2 kb
ITR
HDAd-SB
ITR Ψ
EF1α
Flpe
pA
PGK
SB100x
pA
ITR

FIG. 102

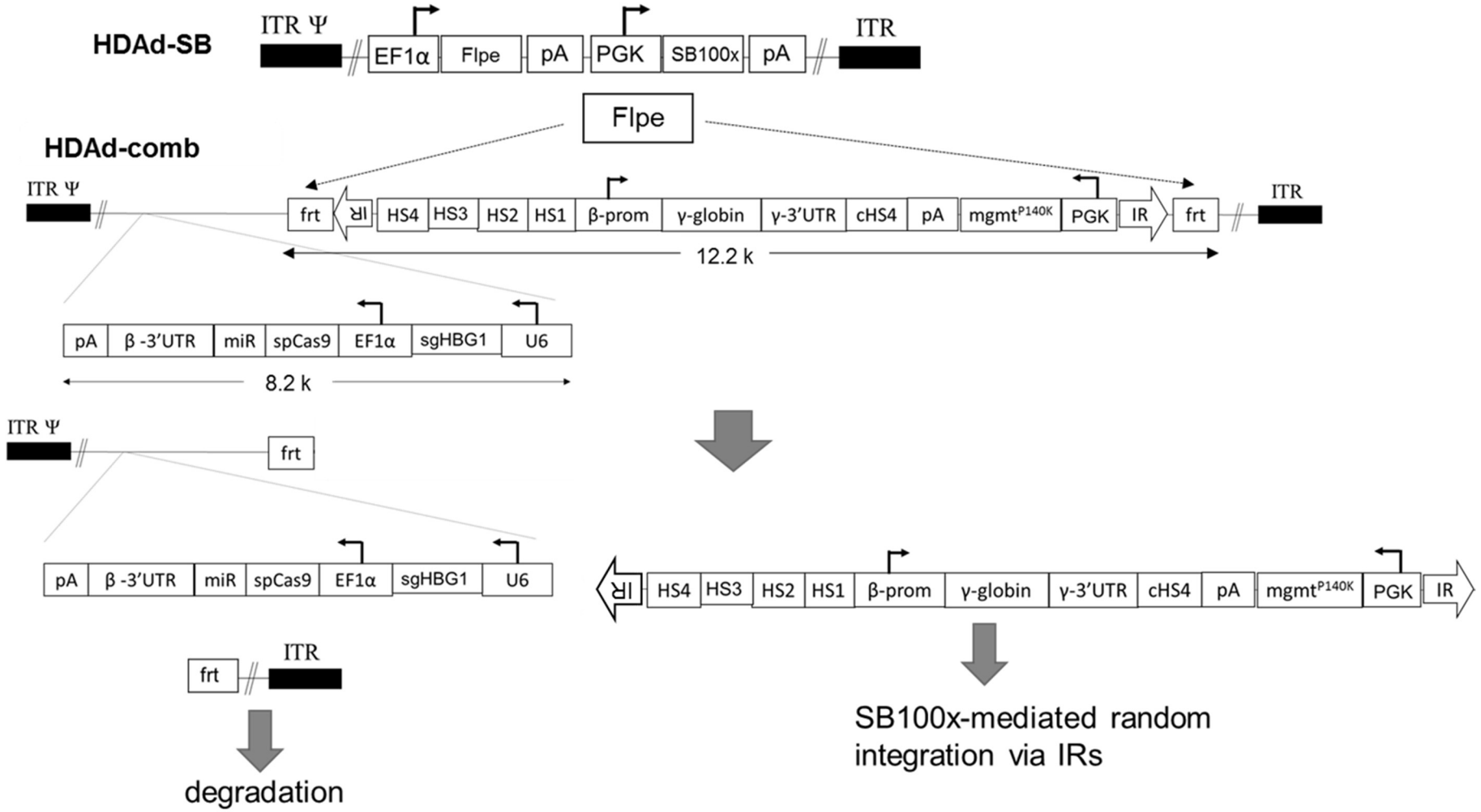

HDAd-SB
ITR Ψ
EF1α
Flpe
pA
PGK
SB100x
pA
ITR
HDAd-comb
Flpe
ITR Ψ
frt
IR
HS4
HS3
HS2
HS1
β-prom
γ-globin
γ-3'UTR
cHS4
pA
mgmtP140K
PGK
IR
frt
ITR
12.2 k
pA
β -3'UTR
miR
spCas9
EF1α
sgHBG1
U6
8.2 k
ITR Ψ
frt
pA
β -3'UTR
miR
spCas9
EF1α
sgHBG1
U6
frt
ITR
degradation
IR
HS4
HS3
HS2
HS1
β-prom
γ-globin
γ-3'UTR
cHS4
pA
mgmtP140K
PGK
IR
SB100x-mediated random integration via IRs

FIG. 103A

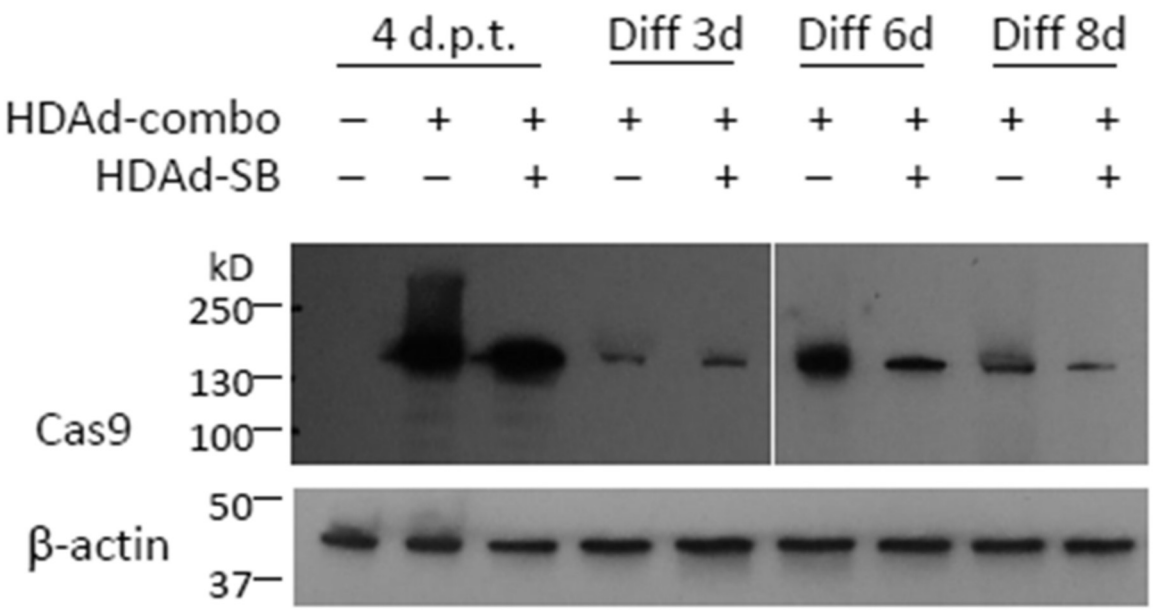


FIG. 103B

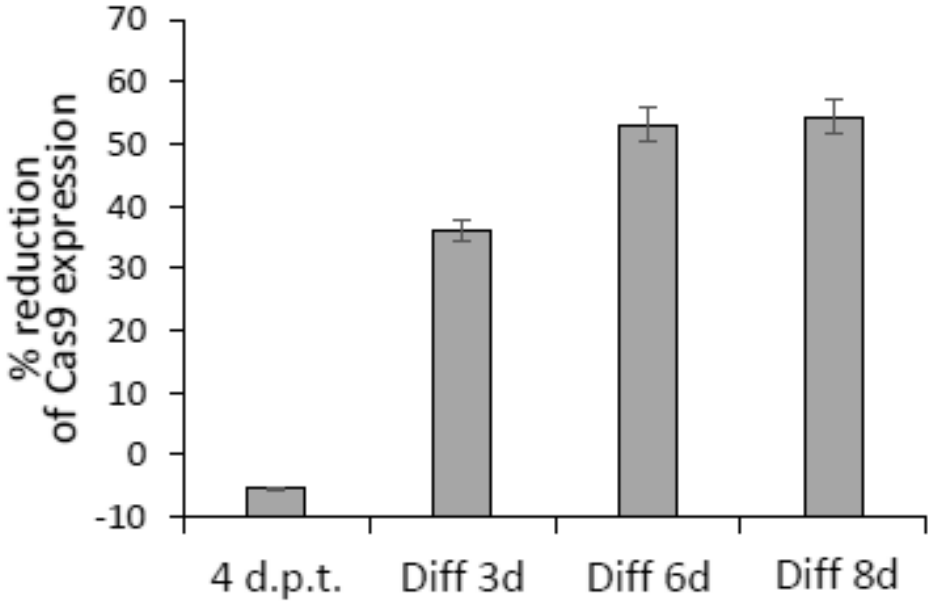


FIG. 103C

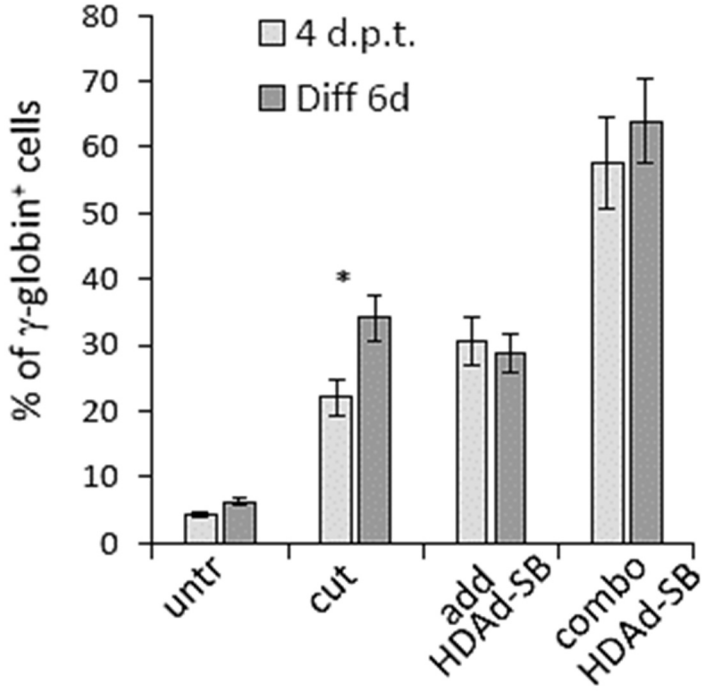


FIG. 103D

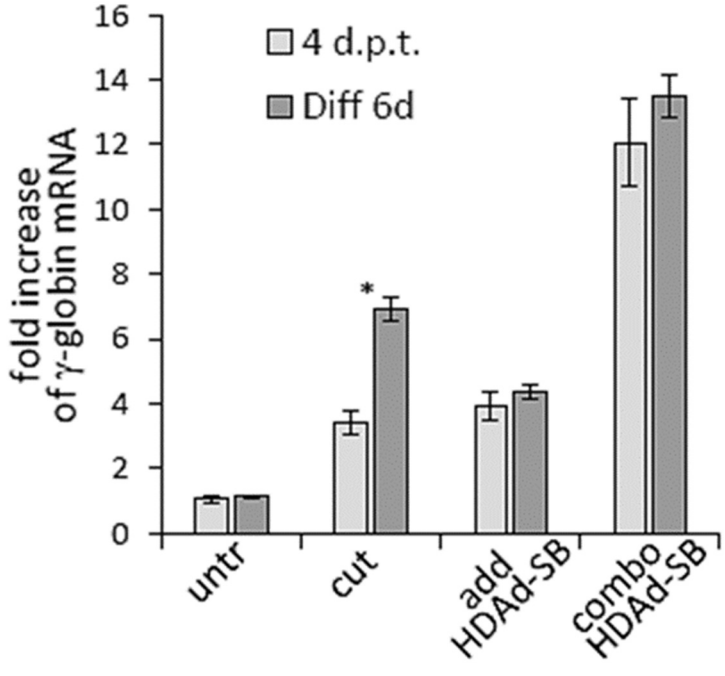


FIG. 104A

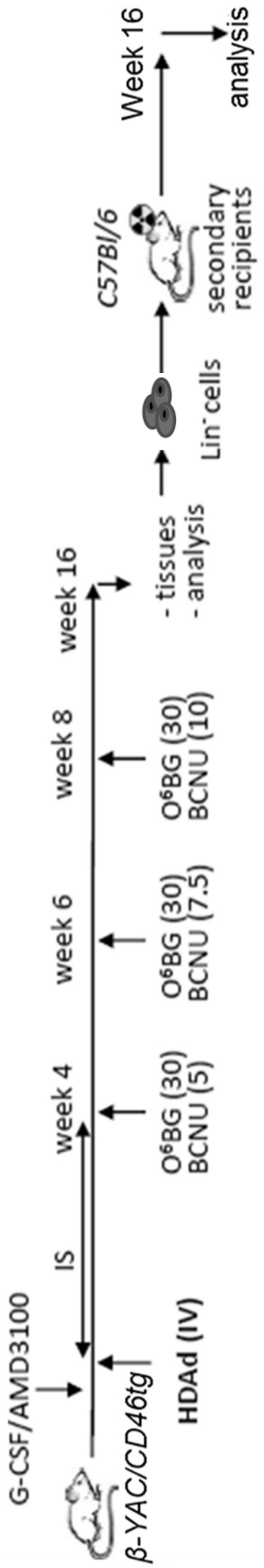


FIG. 104B

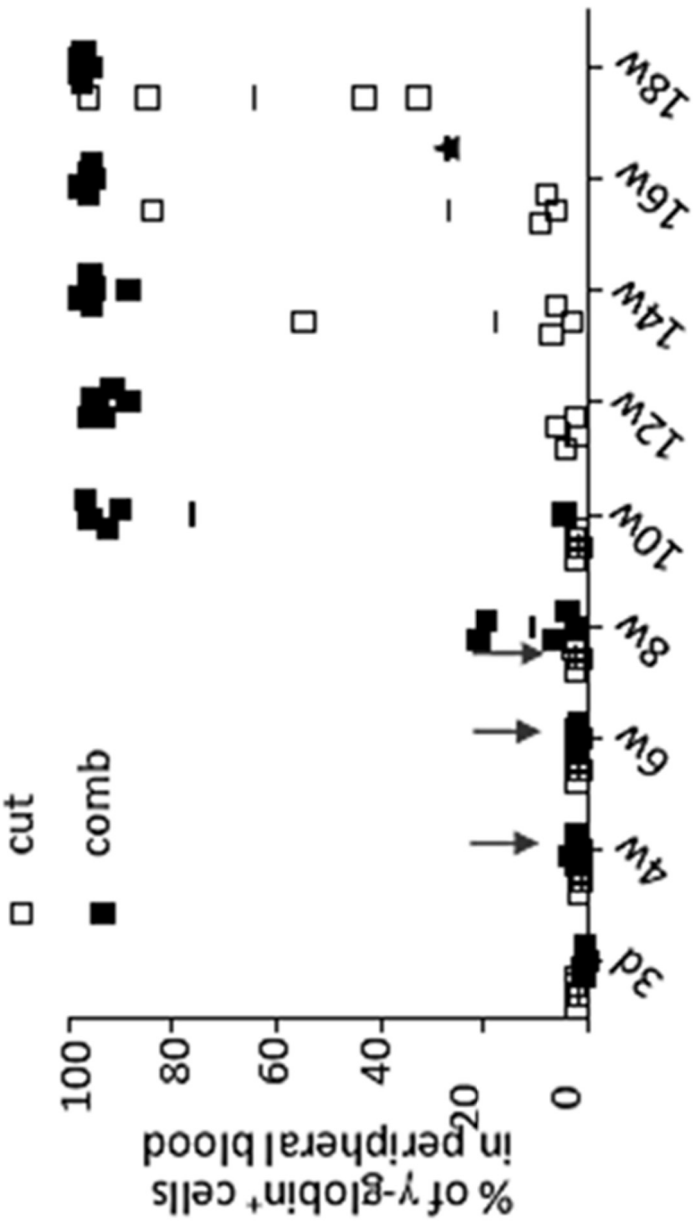
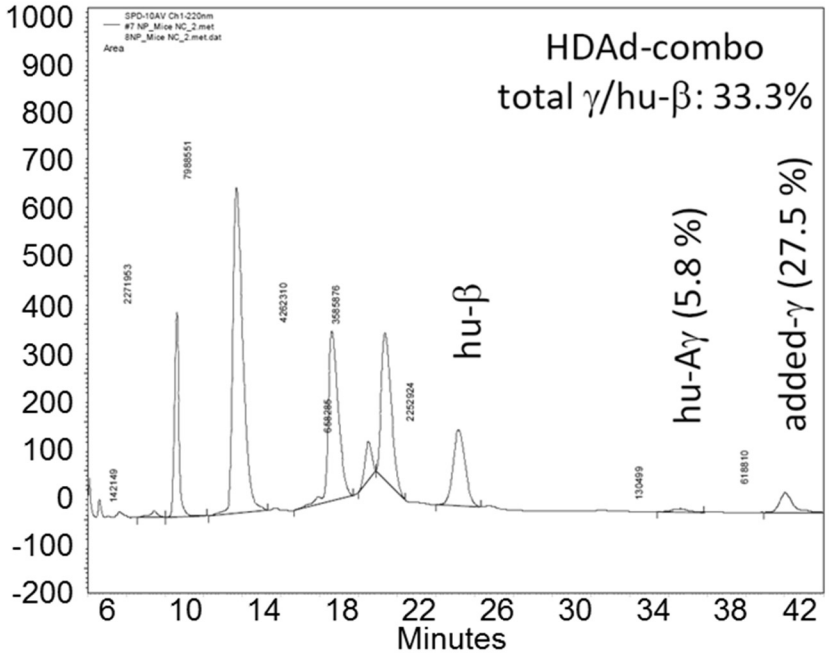


FIG. 104C


HDAd-combo
total γ/hu-β: 33.3%
hu-β
hu-Aγ (5.8 %)
added-γ (27.5 %)
Minutes

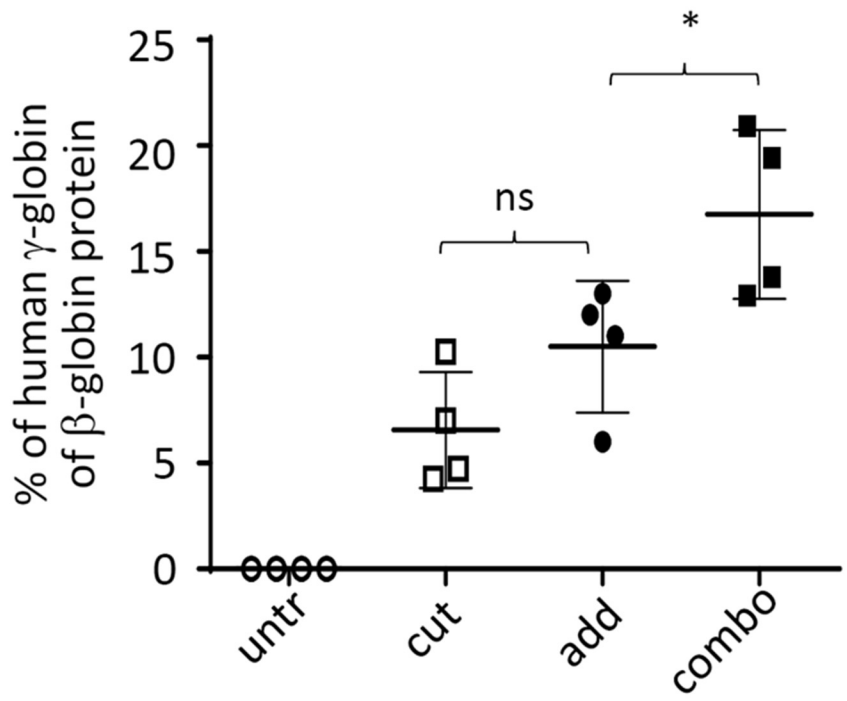

% of human γ-globin
of β-globin protein
ns
*
untr
cut
add
combo

FIG. 104D

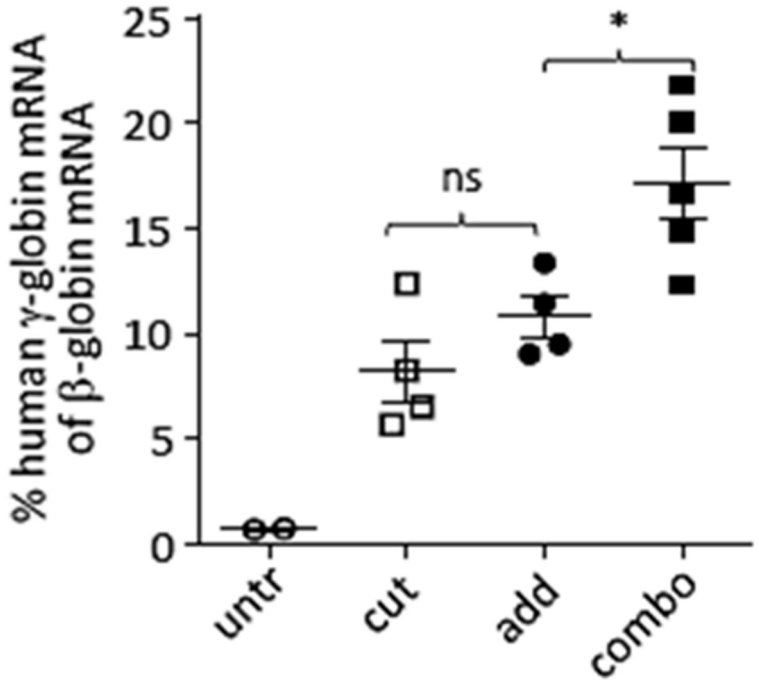


FIG. 104E

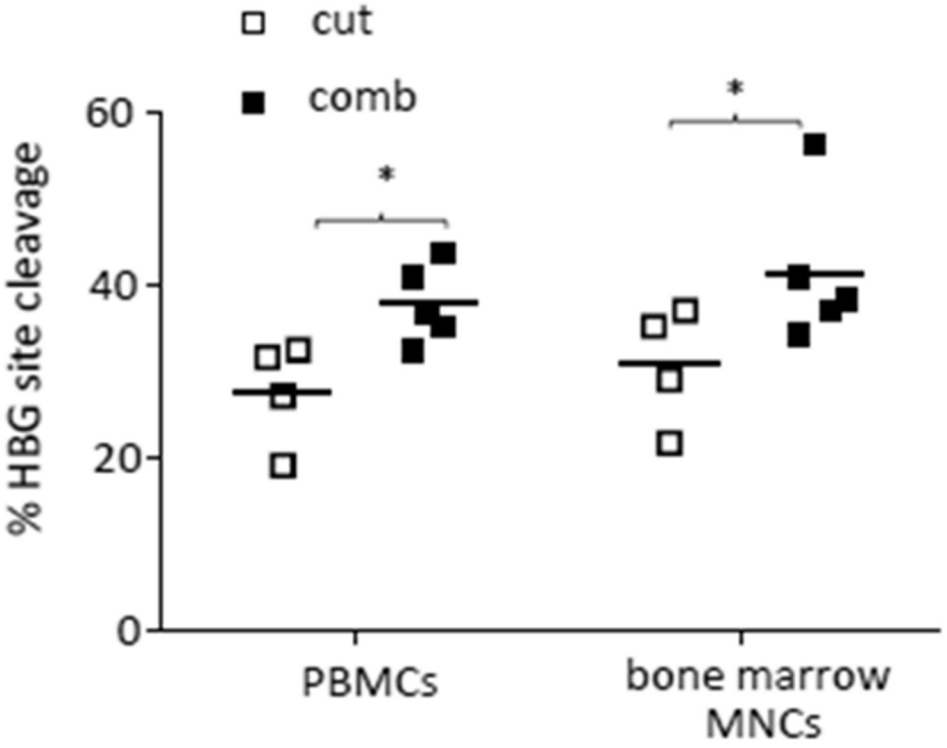


FIG. 104F

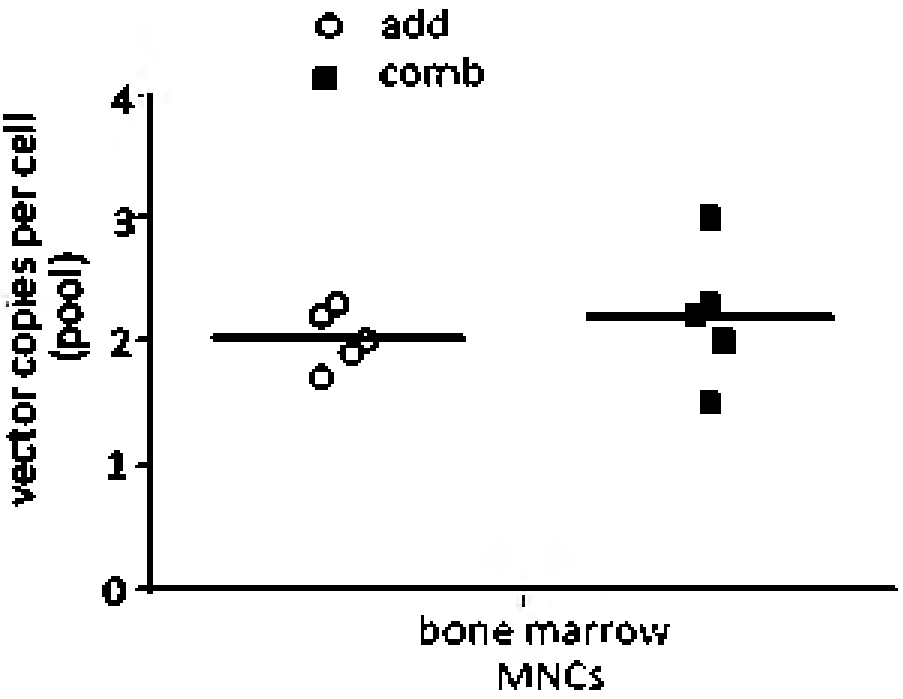


FIG. 104G

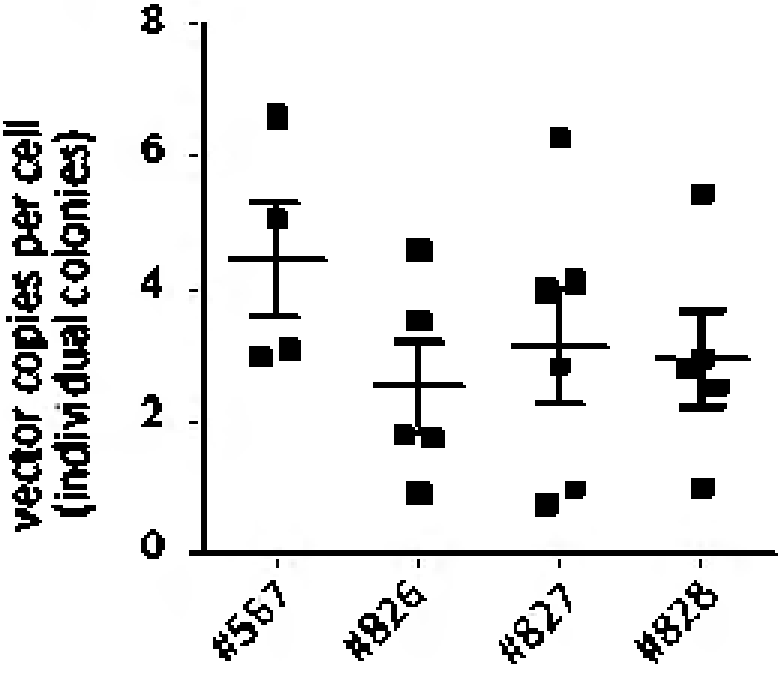


FIG. 104H

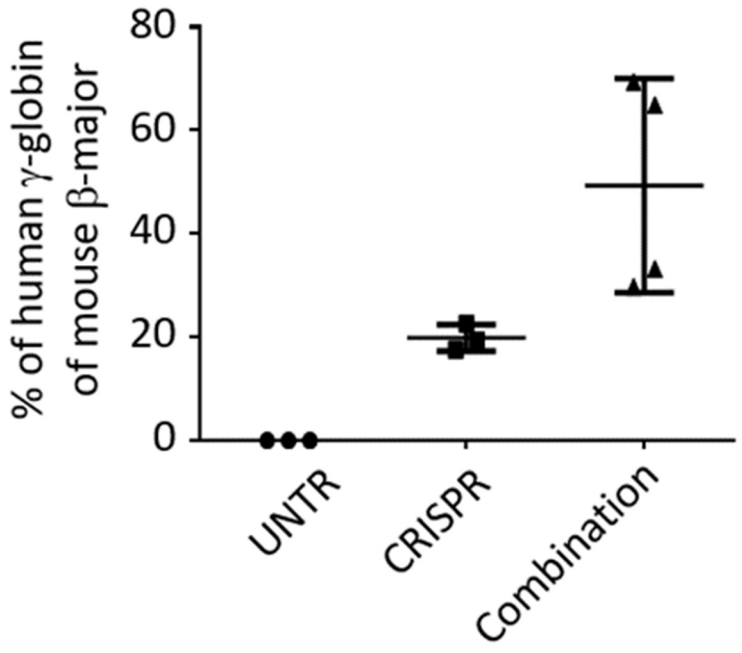

% of human γ-globin
of mouse β-major
80
60
40
20
0
UNTR
CRISPR
Combination

FIG. 105

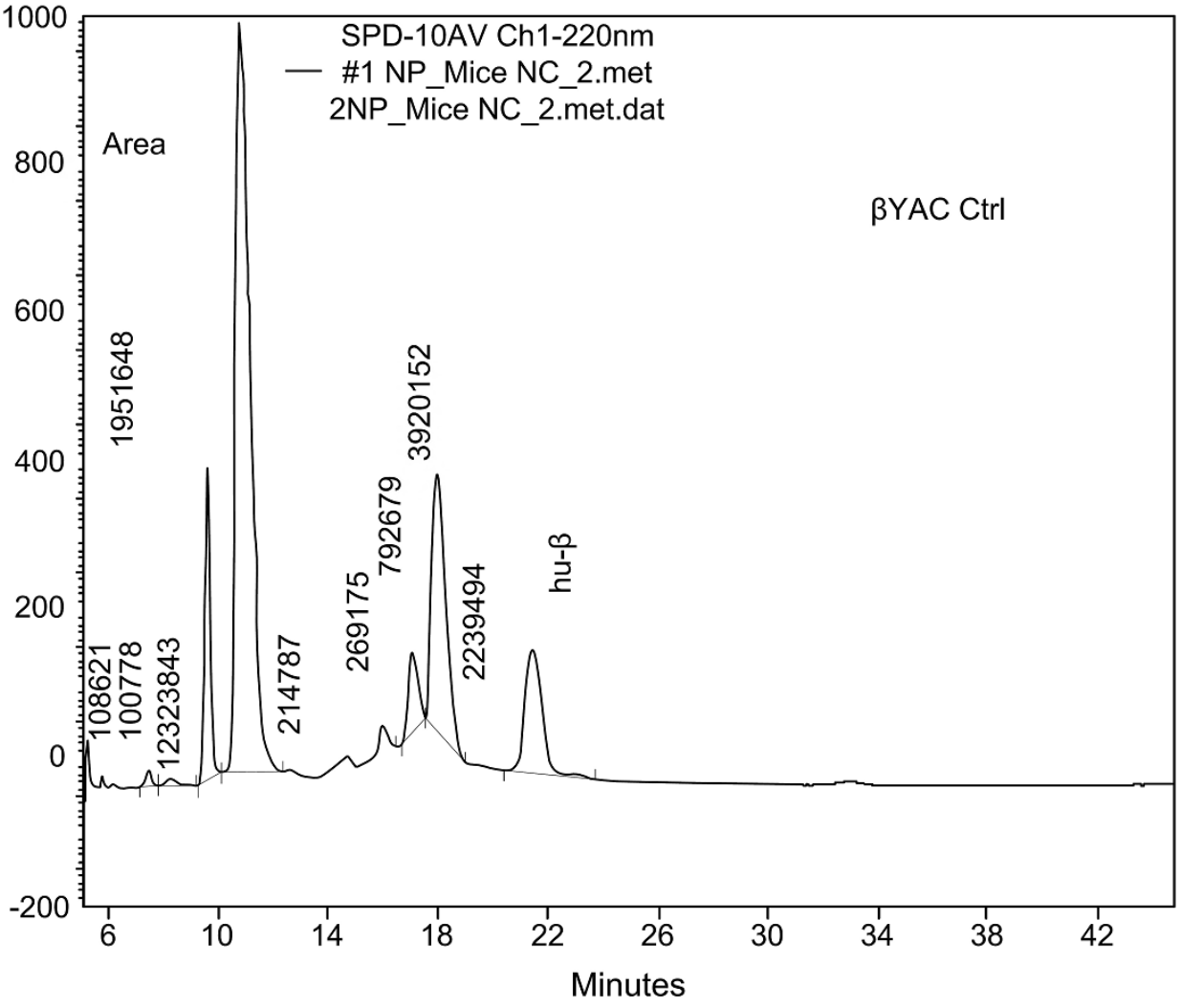

SPD-10AV Ch1-220nm
#1 NP_Mice NC_2.met
2NP_Mice NC_2.met.dat
Area
βYAC Ctrl
1951648
108621
100778
12323843
214787
269175
792679
3920152
2239494
hu-β
1000
800
600
400
200
0
-200
6
10
14
18
22
26
30
34
38
42
Minutes

FIG. 105 cont'd

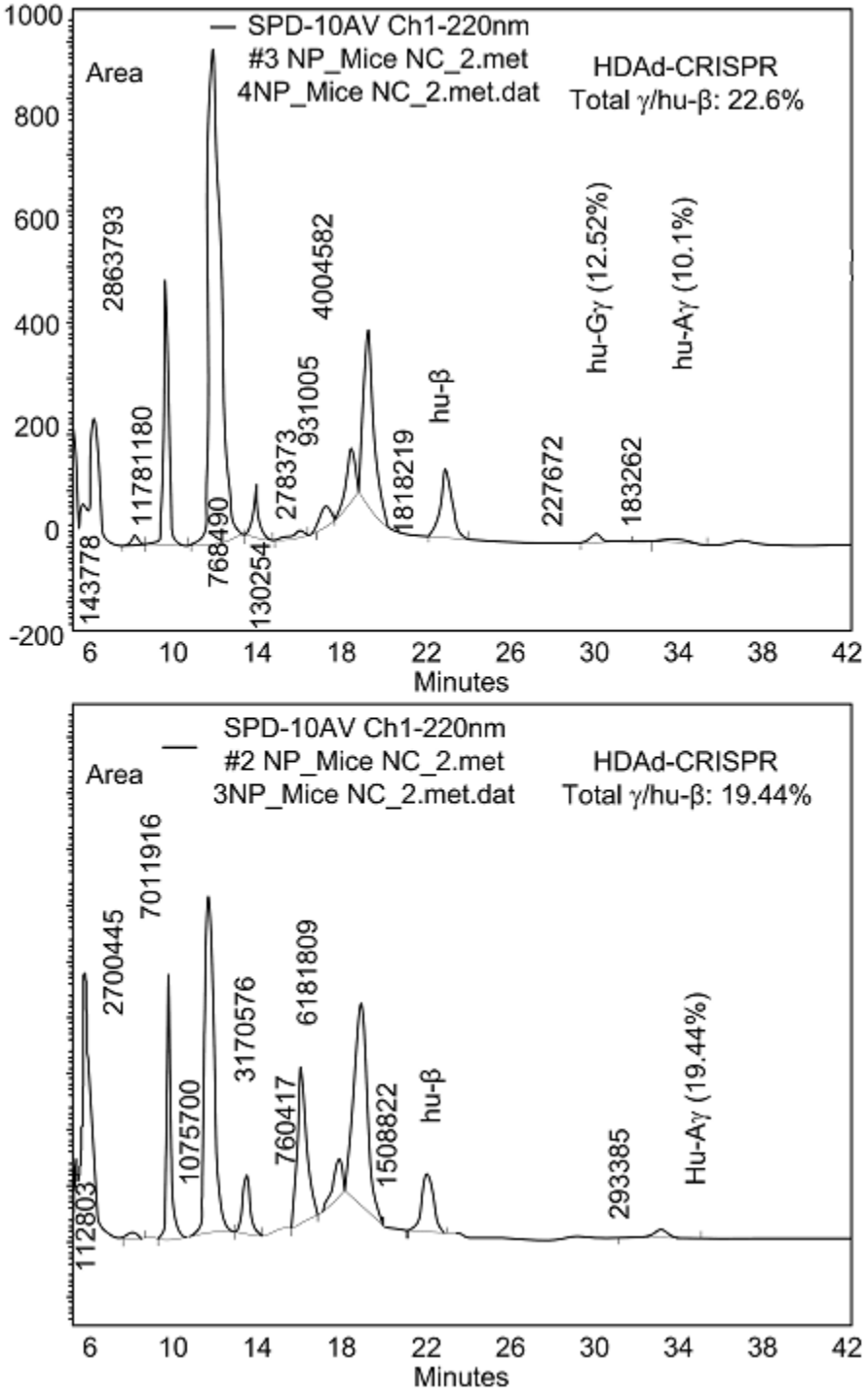


FIG. 105 cont'd

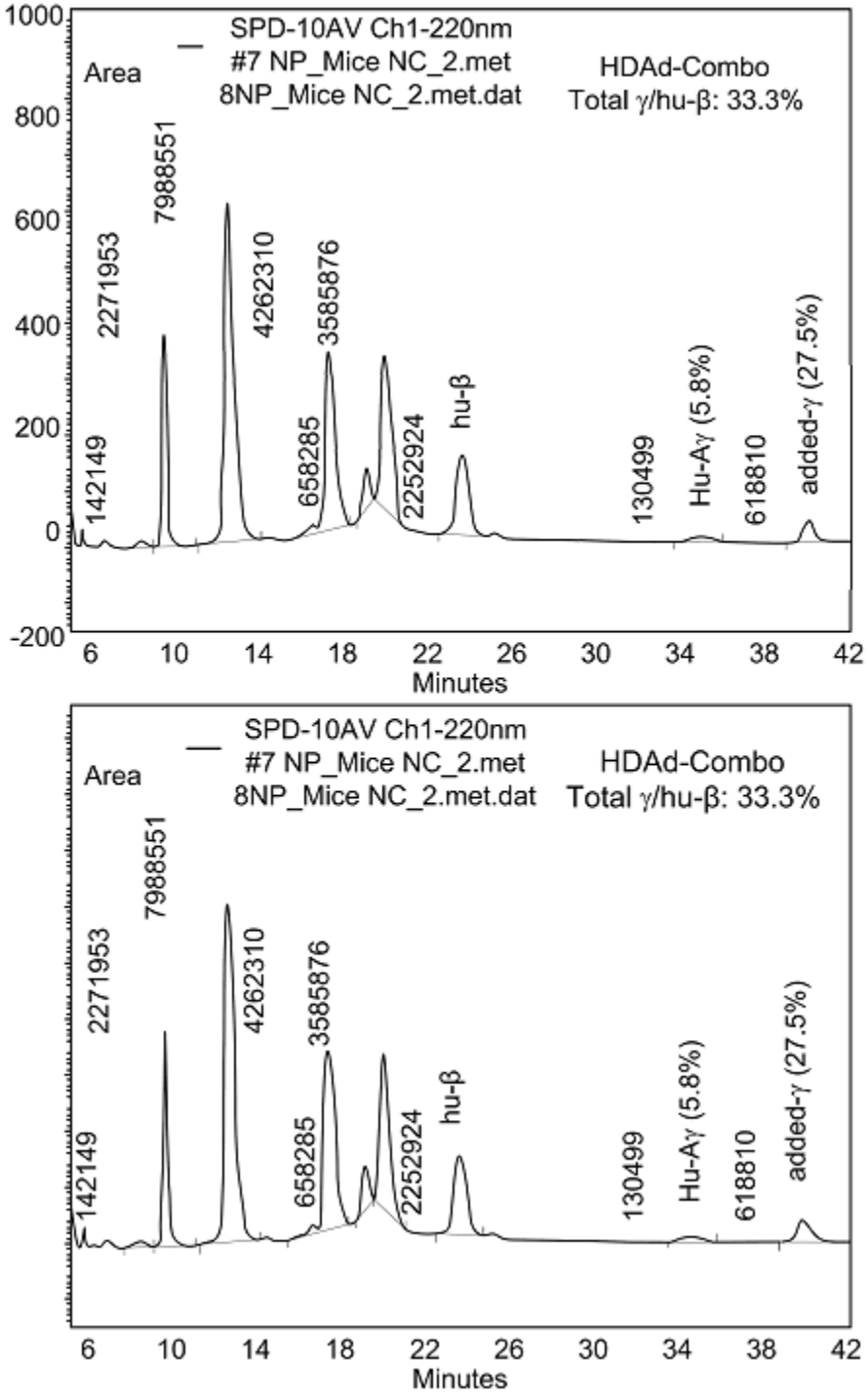


FIG. 106

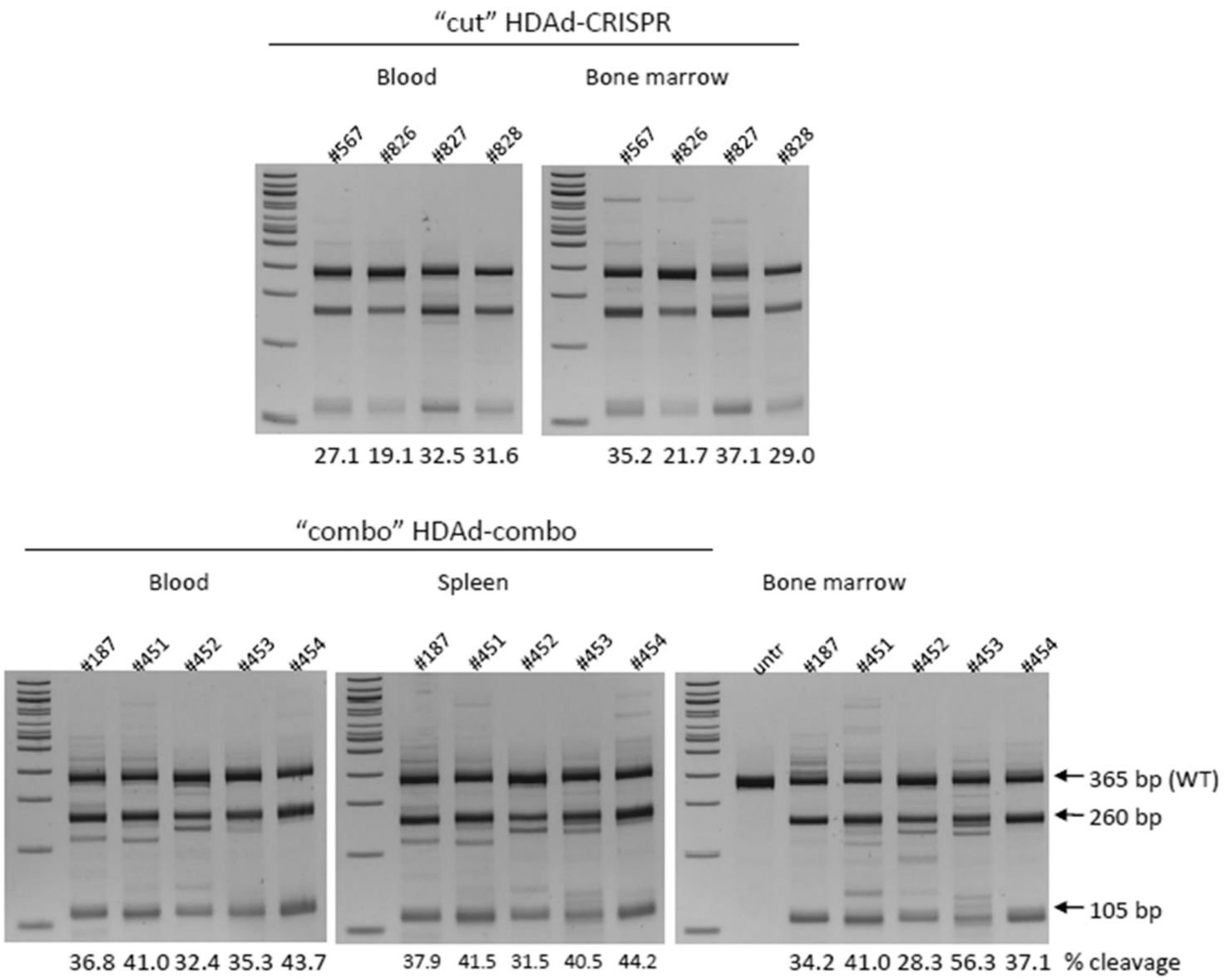

"cut" HDAd-CRISPR
Blood
Bone marrow
#567
#826
#827
#828
27.1 19.1 32.5 31.6
35.2 21.7 37.1 29.0
"combo" HDAd-combo
Blood
Spleen
Bone marrow
#187
#451
#452
#453
#454
untr
36.8 41.0 32.4 35.3 43.7
37.9 41.5 31.5 40.5 44.2
34.2 41.0 28.3 56.3 37.1 % cleavage
365 bp (WT)
260 bp
105 bp

FIG. 107A

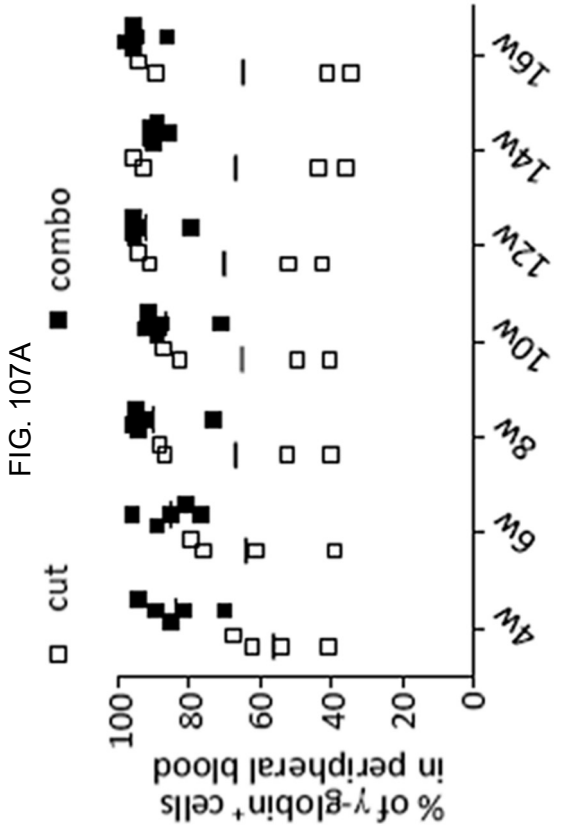


FIG. 107B

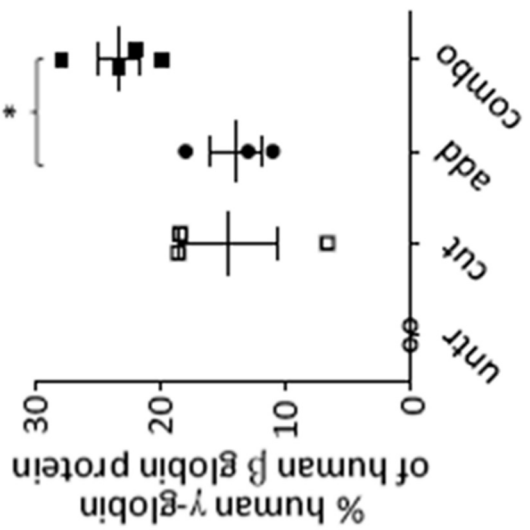


FIG. 107C

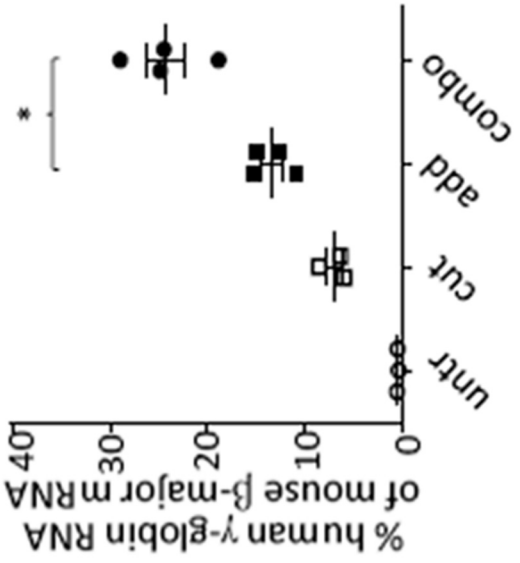


FIG. 107D

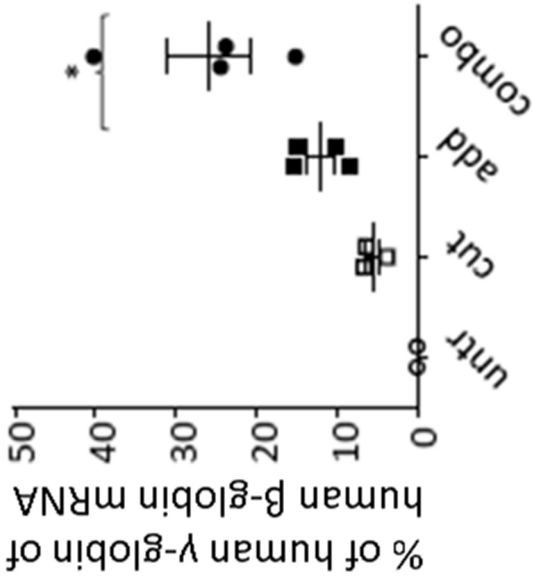


FIG. 107E

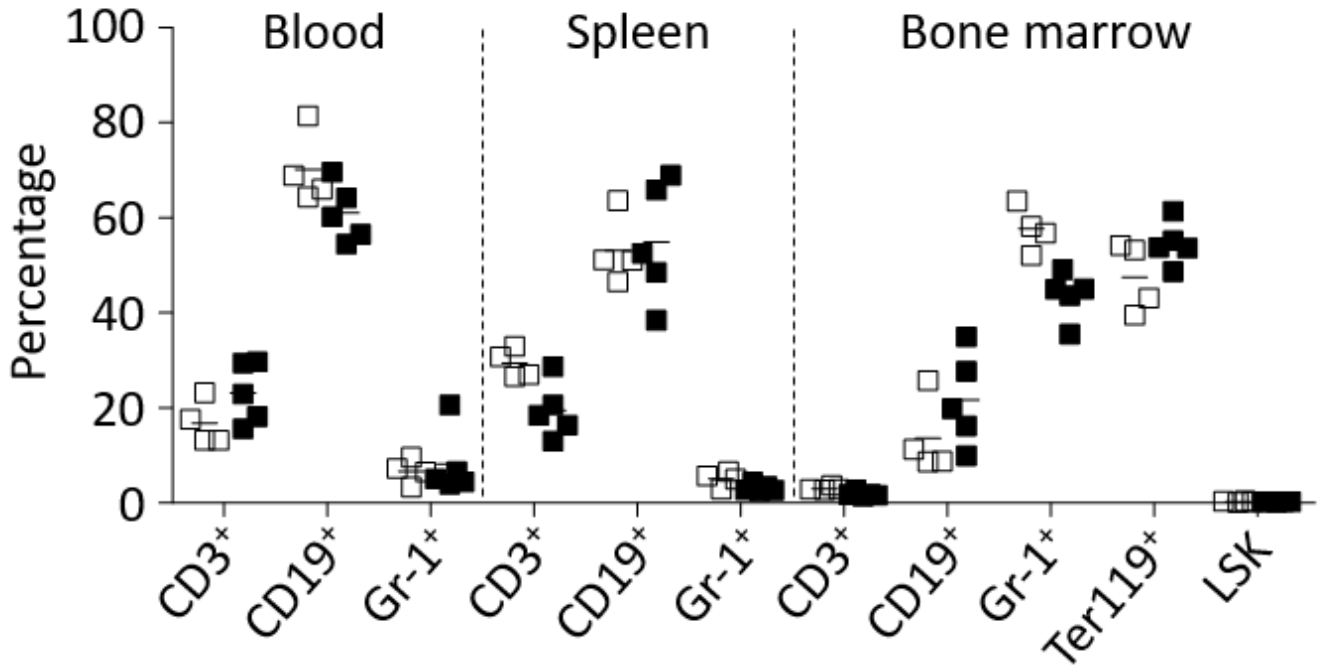


FIG. 107F

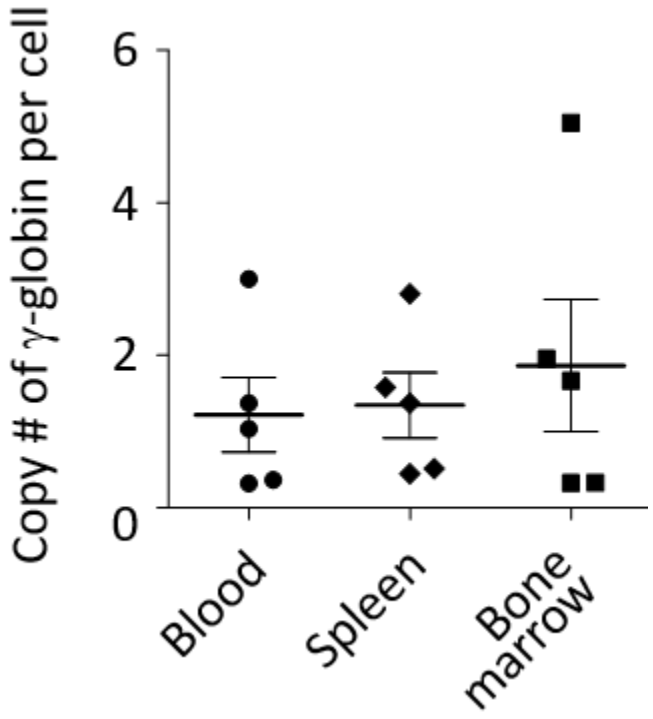


FIG. 108A

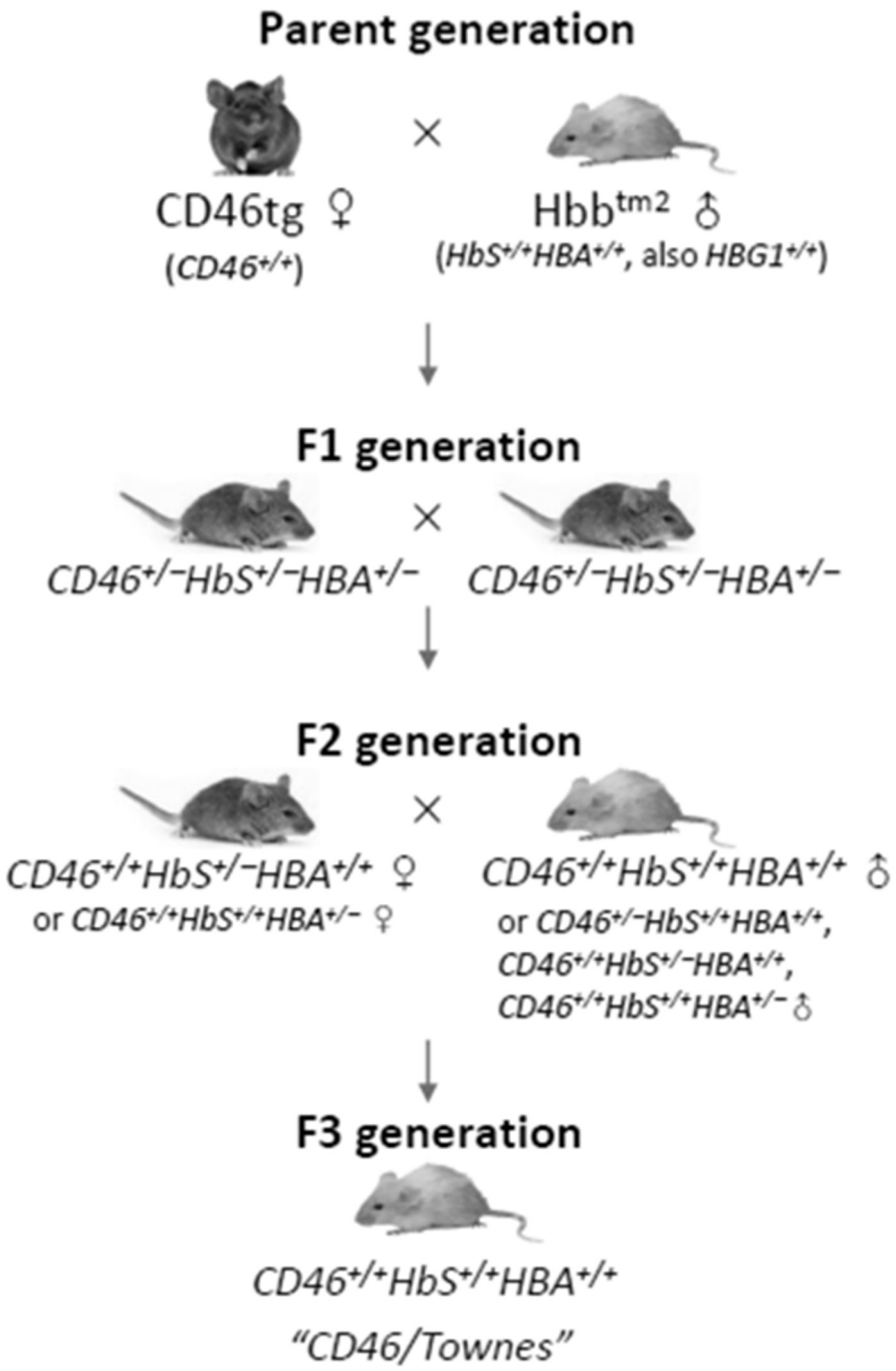

Parent generation
CD46tg ♀
(CD46+/+)
×
Hbbtm2 ♂
(HbS+/+HBA+/+, also HBG1+/+)
F1 generation
CD46+/−HbS+/−HBA+/−
×
CD46+/−HbS+/−HBA+/−
F2 generation
CD46+/+HbS+/−HBA+/+ ♀
or CD46+/+HbS+/+HBA+/− ♀
×
CD46+/+HbS+/+HBA+/+ ♂
or CD46+/−HbS+/+HBA+/+,
CD46+/+HbS+/−HBA+/+,
CD46+/+HbS+/+HBA+/− ♂
F3 generation
CD46+/+HbS+/+HBA+/+
"CD46/Townes"

FIG. 108B

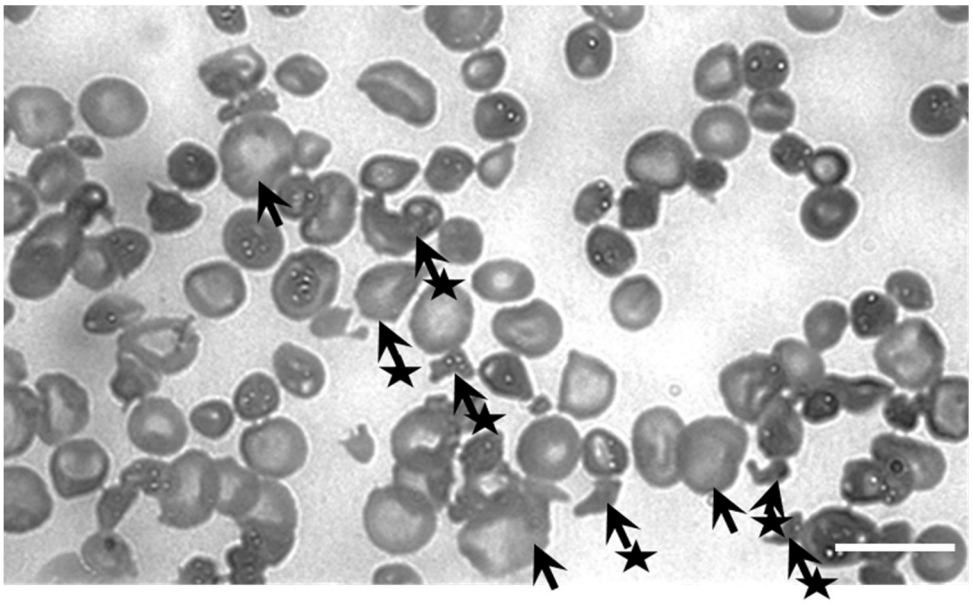


FIG. 108C

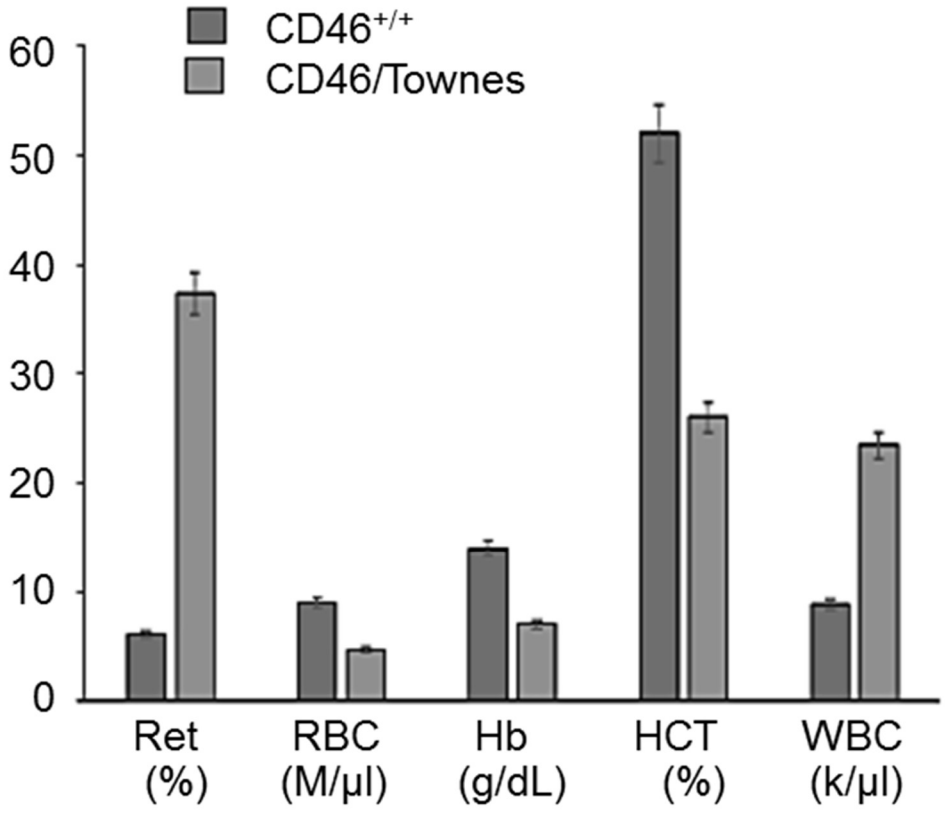


FIG. 108D

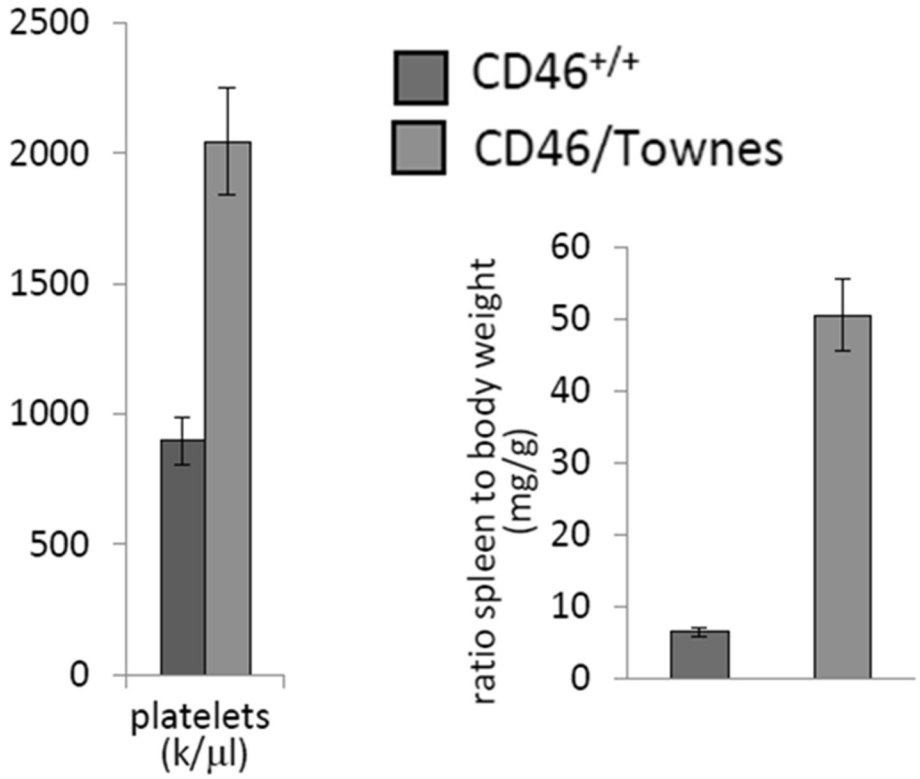


FIG. 109A

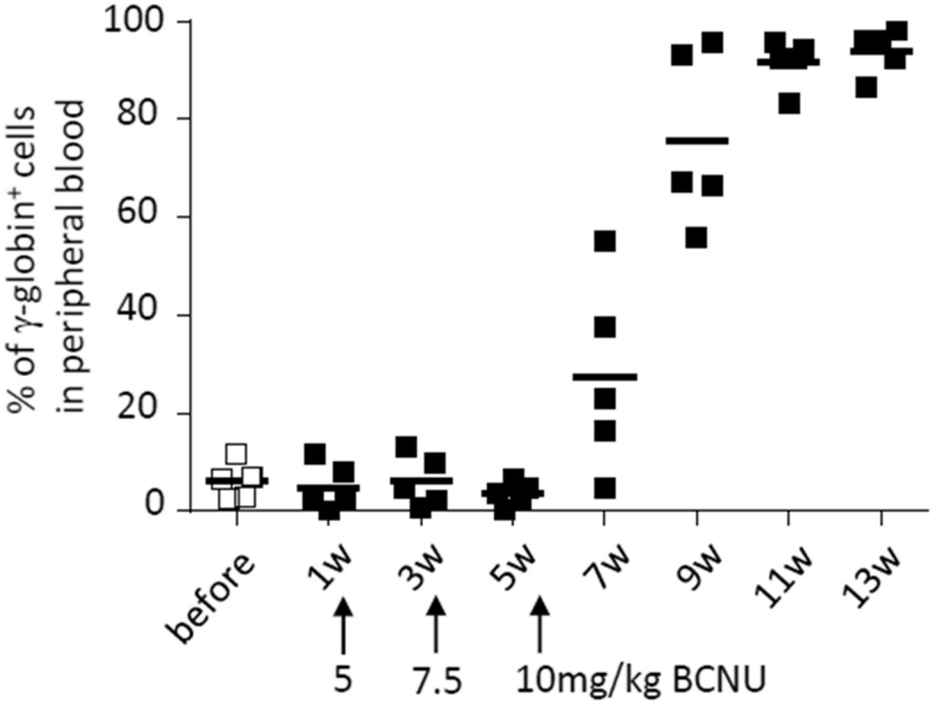


FIG. 109B

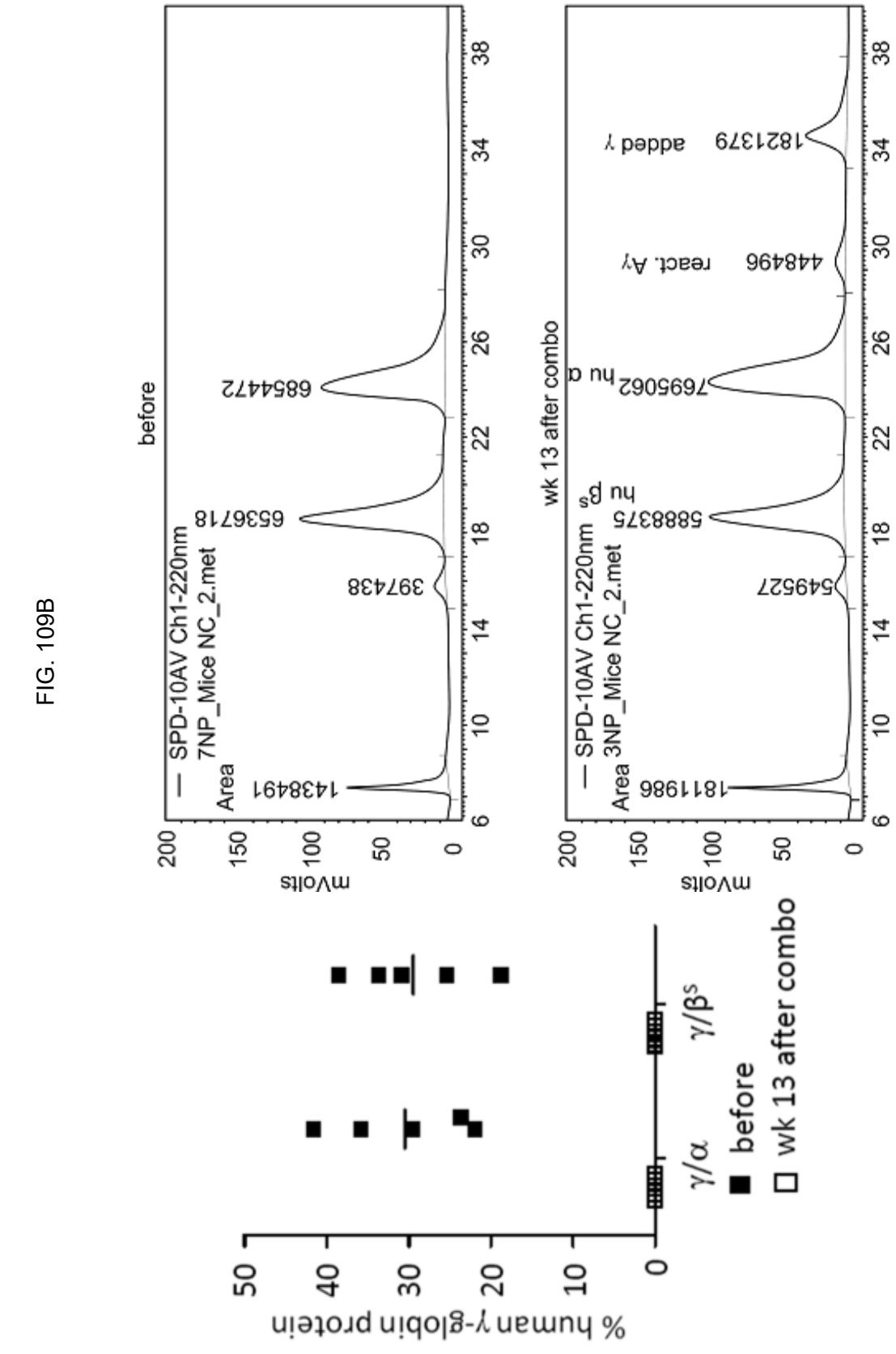

before
SPD-10AV Ch1-220nm
7NP_Mice NC_2.met
Area
1438491
653718
397438
685472
mVolts
wk 13 after combo
SPD-10AV Ch1-220nm
3NP_Mice NC_2.met
Area
1811986
588375
549527
769506
448496
1821379
added γ
react. Aγ
% human γ-globin protein
γ/α
γ/β^S
before
wk 13 after combo

FIG. 109C

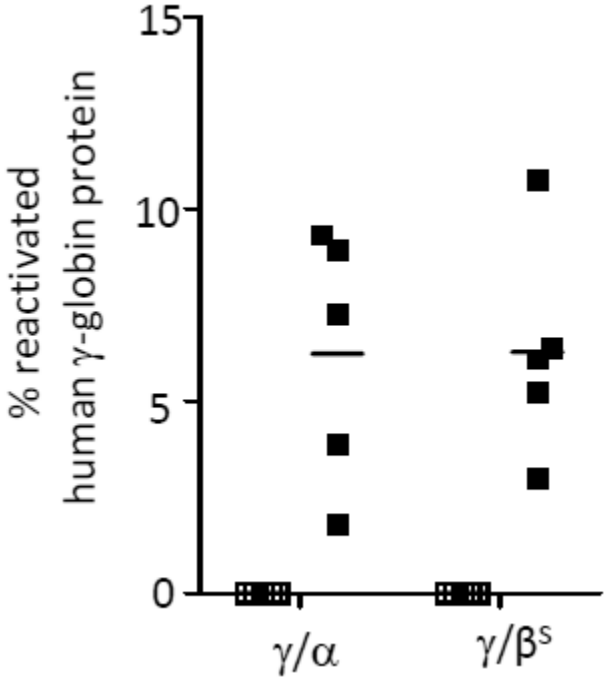


FIG. 109D

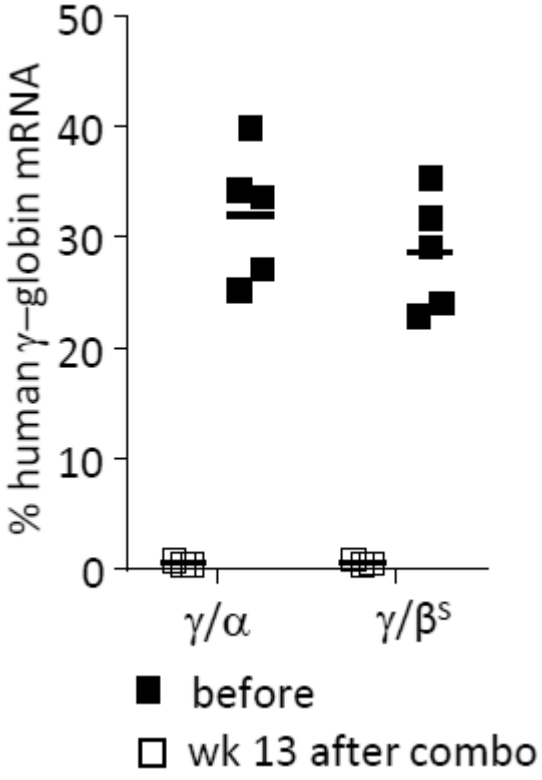


FIG. 109E

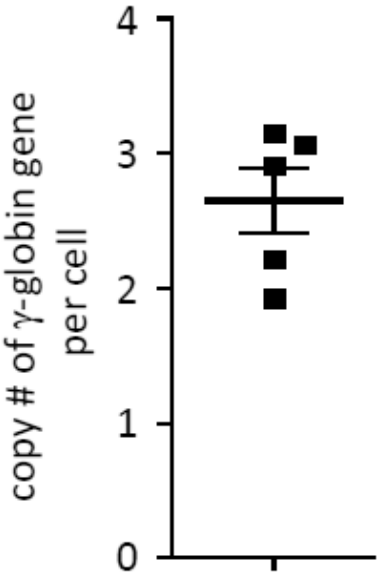


FIG. 109F

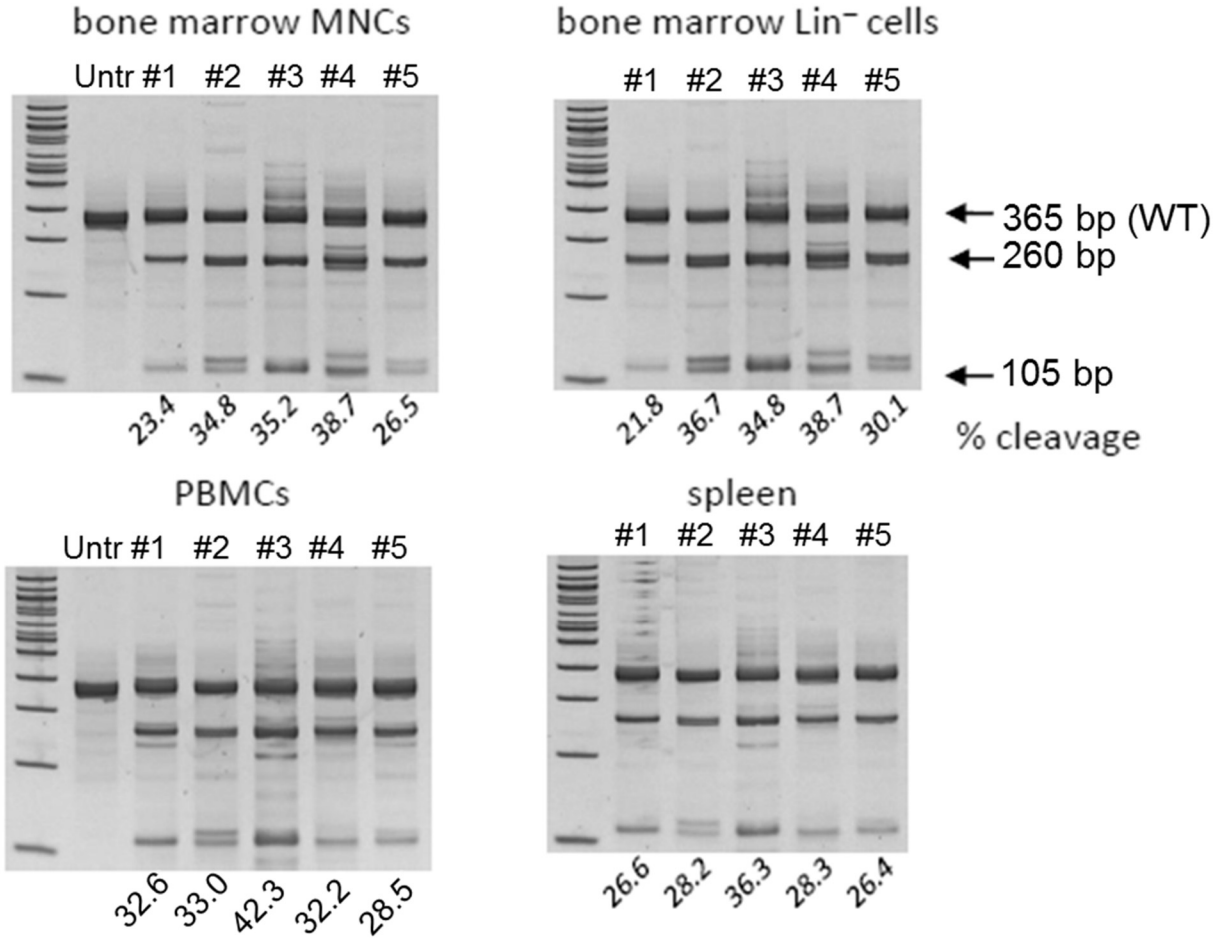


FIG. 110A

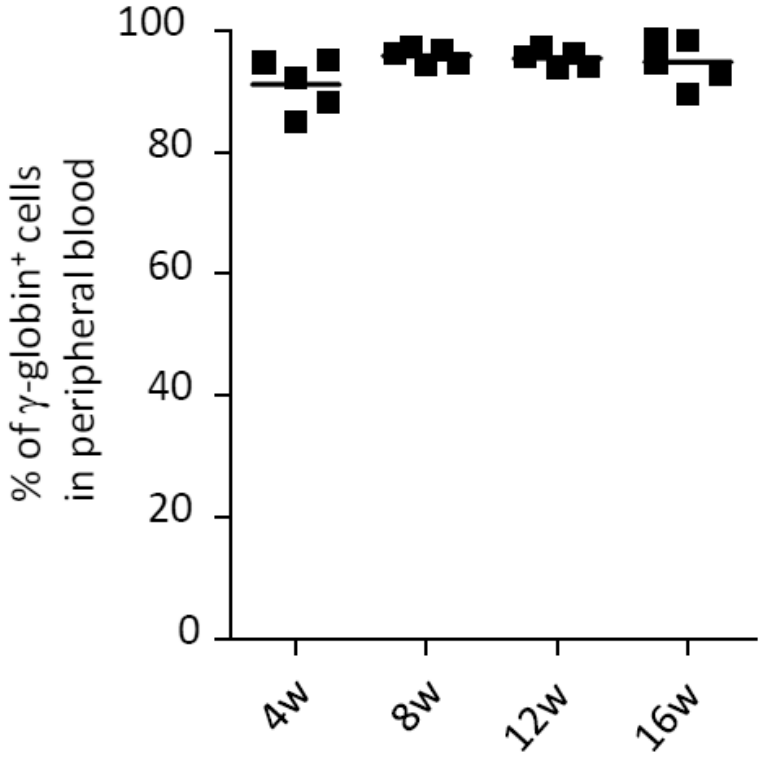


FIG. 110B

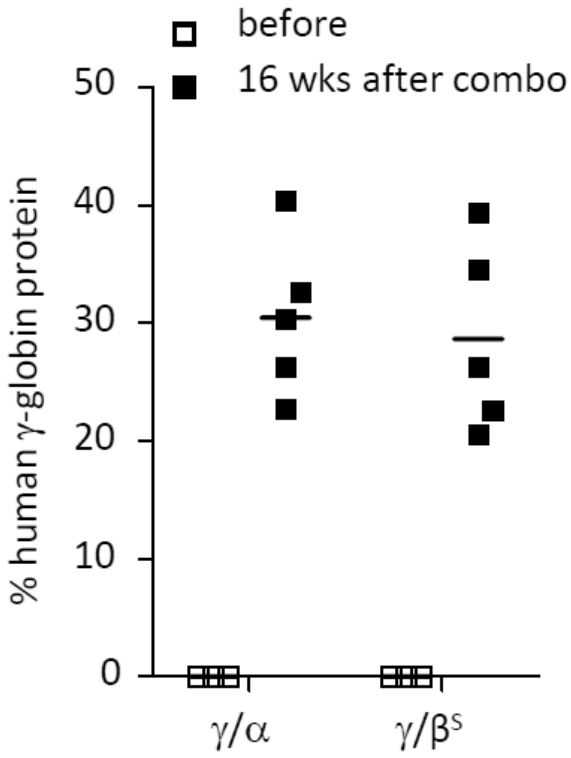


FIG. 111A

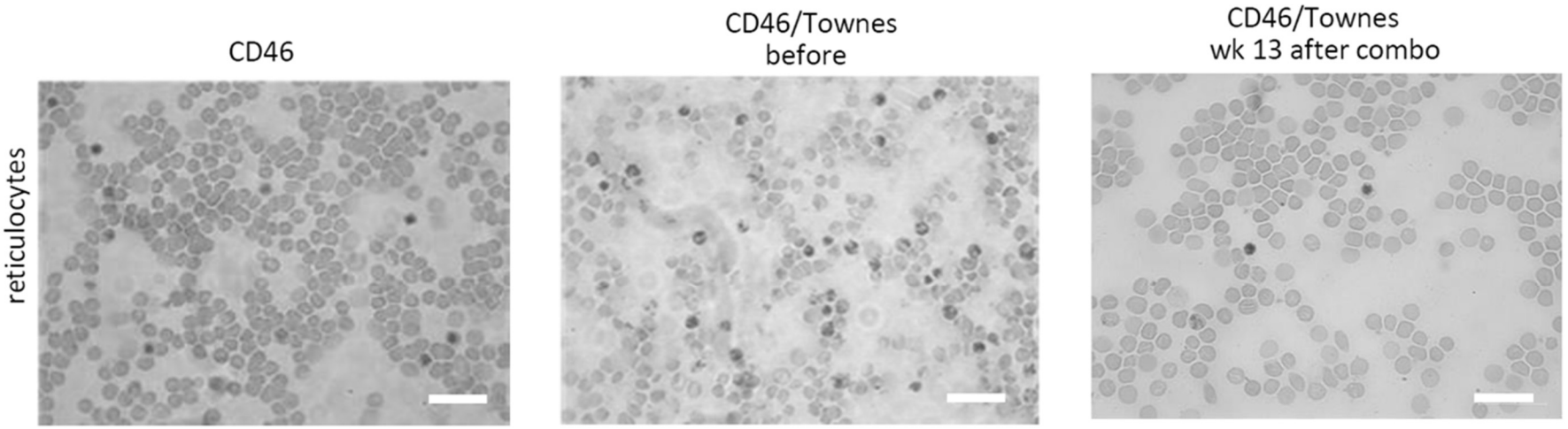


FIG. 111B

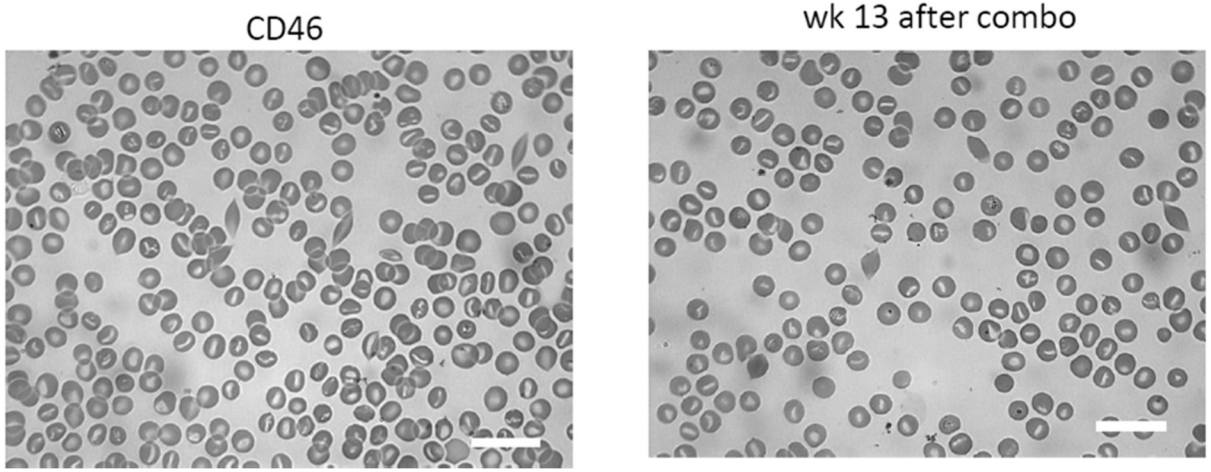


FIG. 111C

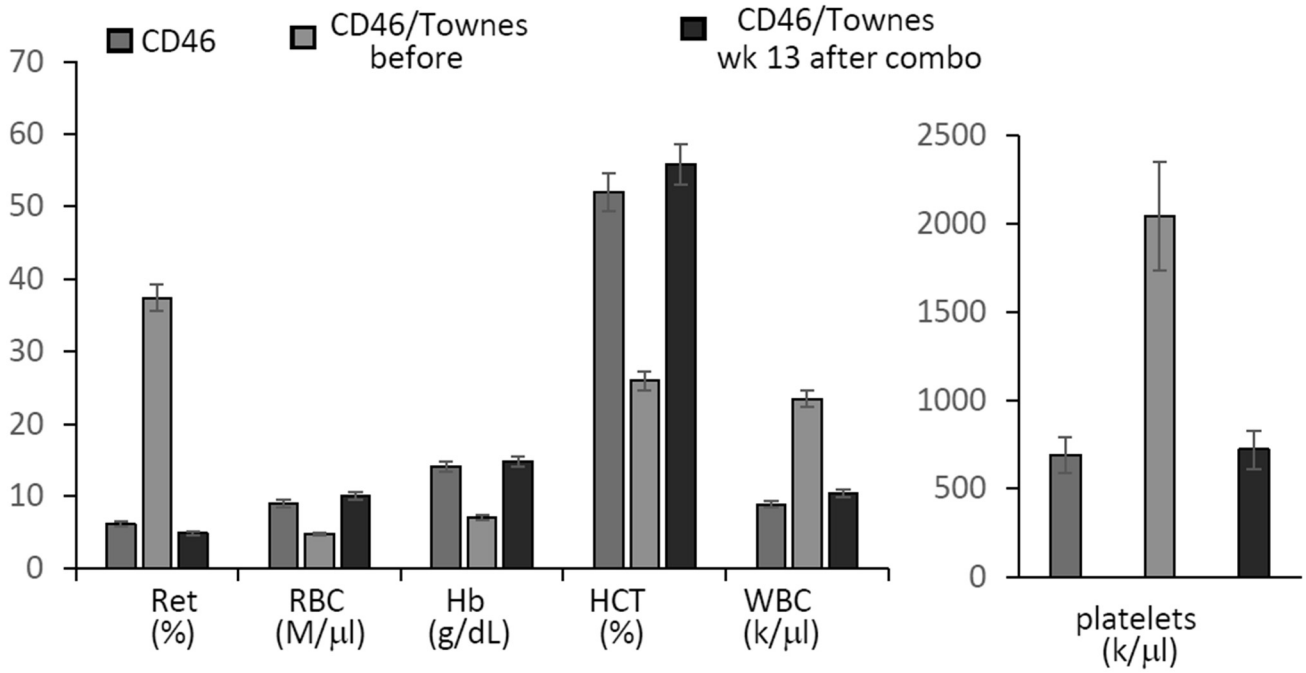

CD46
CD46/Townes before
CD46/Townes wk 13 after combo
70
60
50
40
30
20
10
0
2500
2000
1500
1000
500
0
Ret (%)
RBC (M/µl)
Hb (g/dL)
HCT (%)
WBC (k/µl)
platelets (k/µl)

FIG. 112A

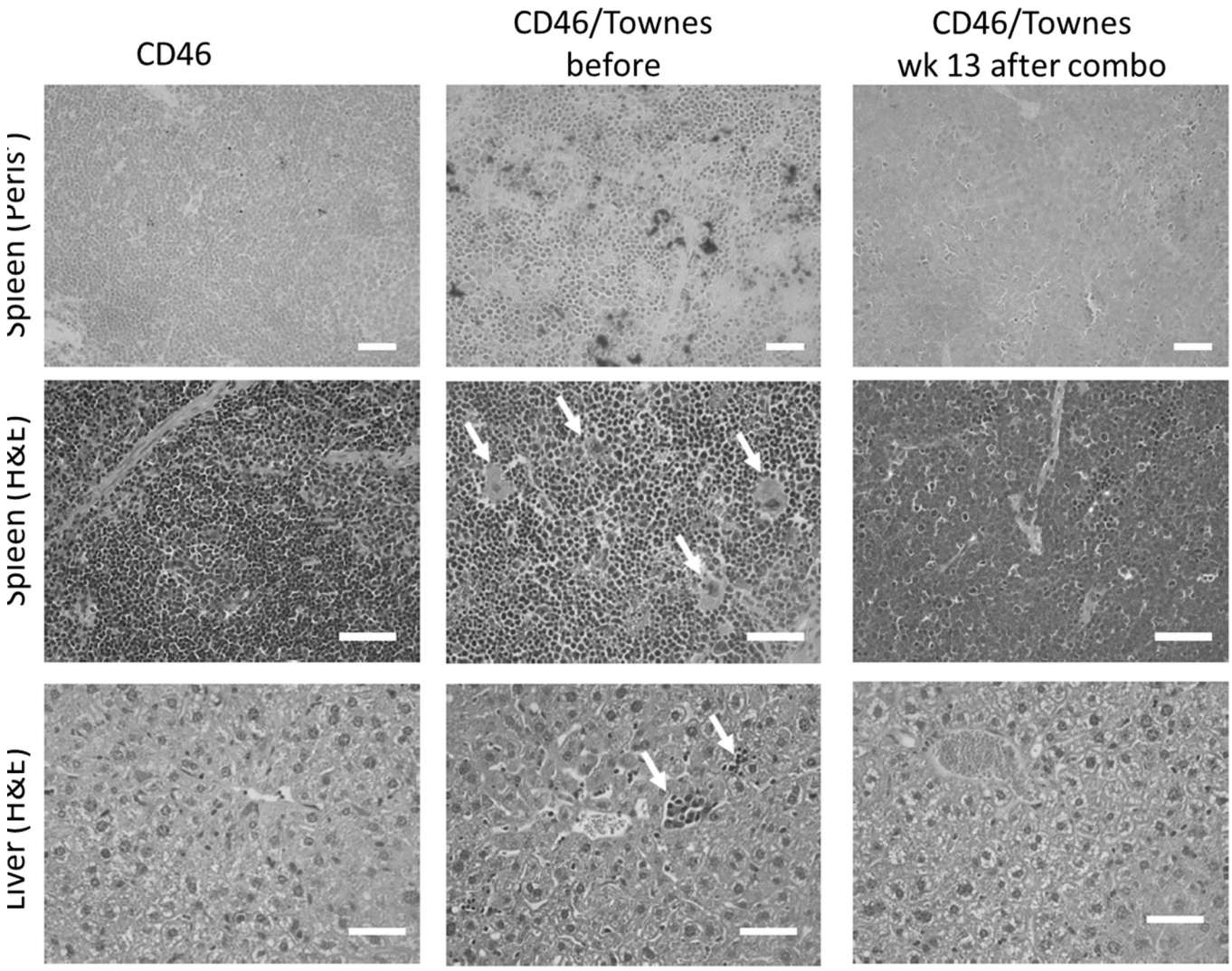
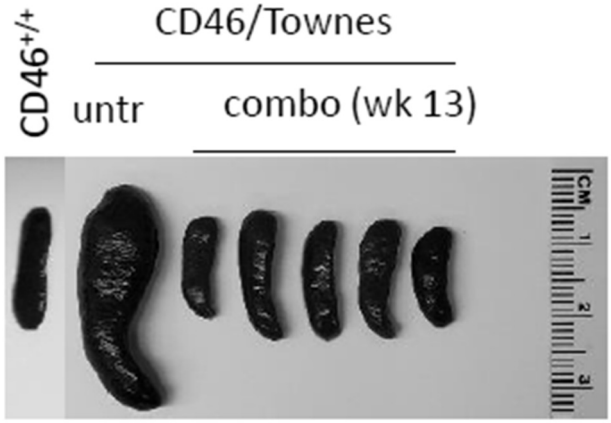


FIG. 112B



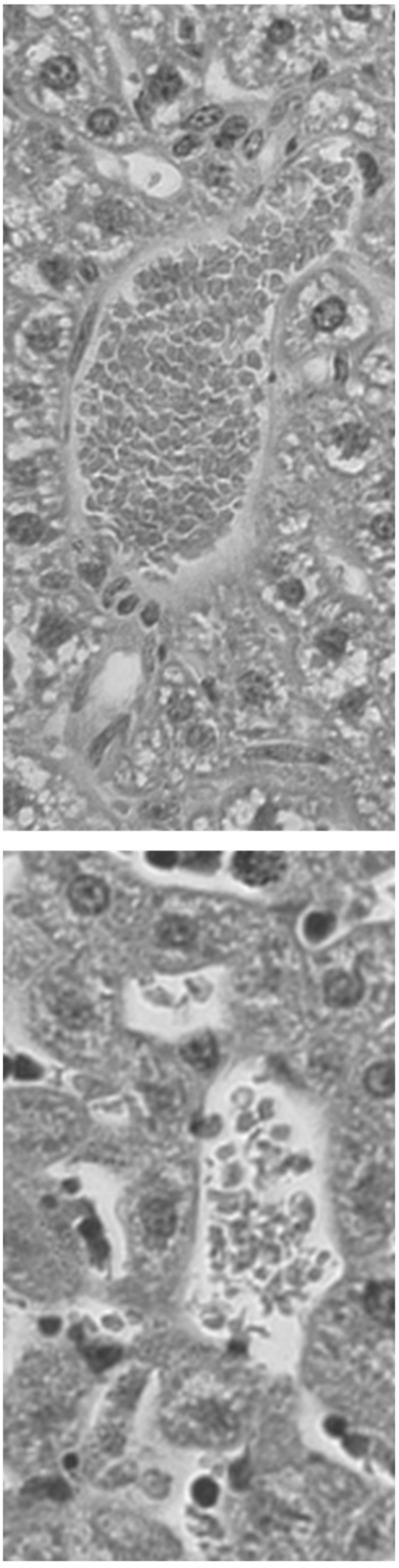
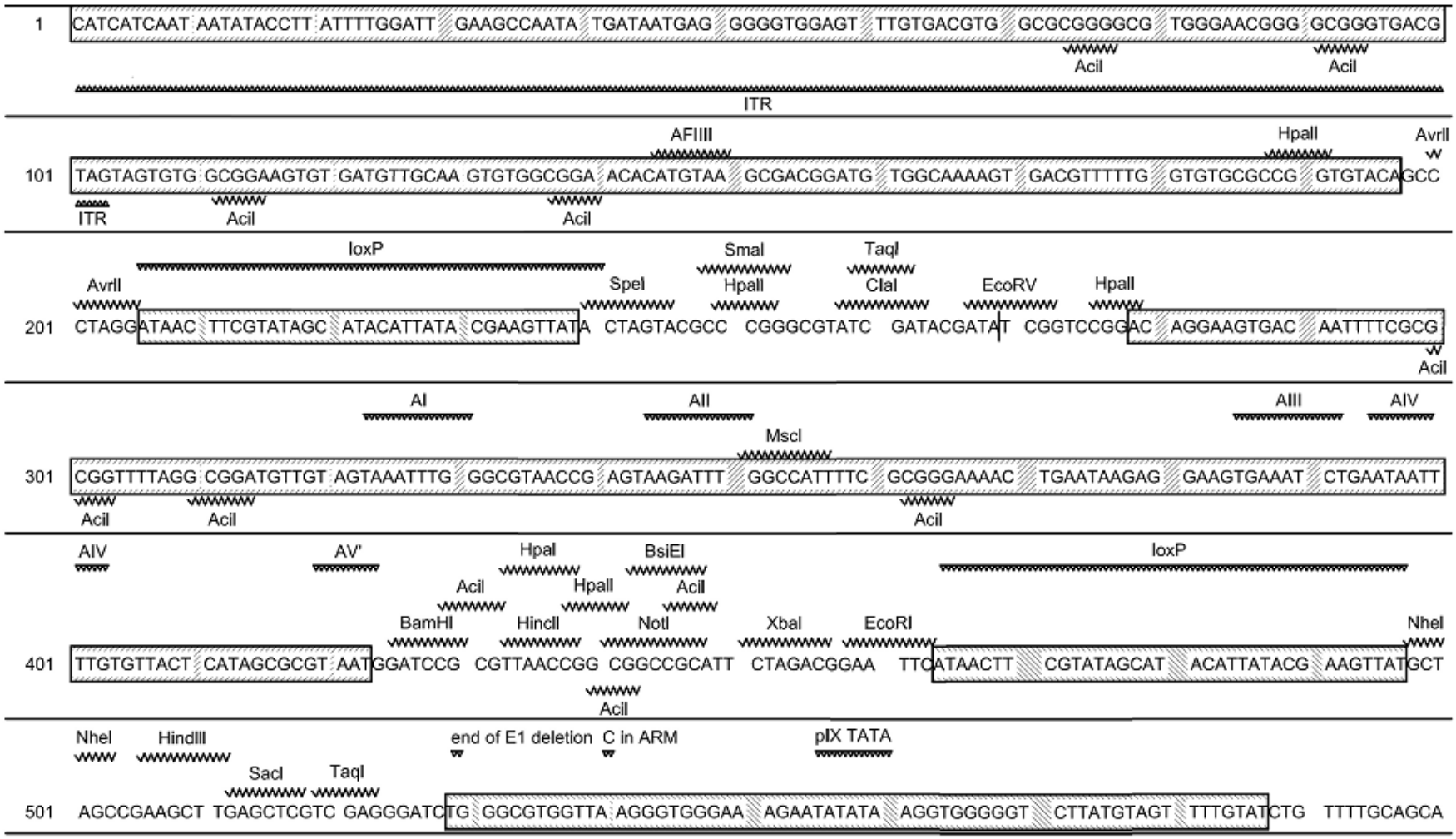


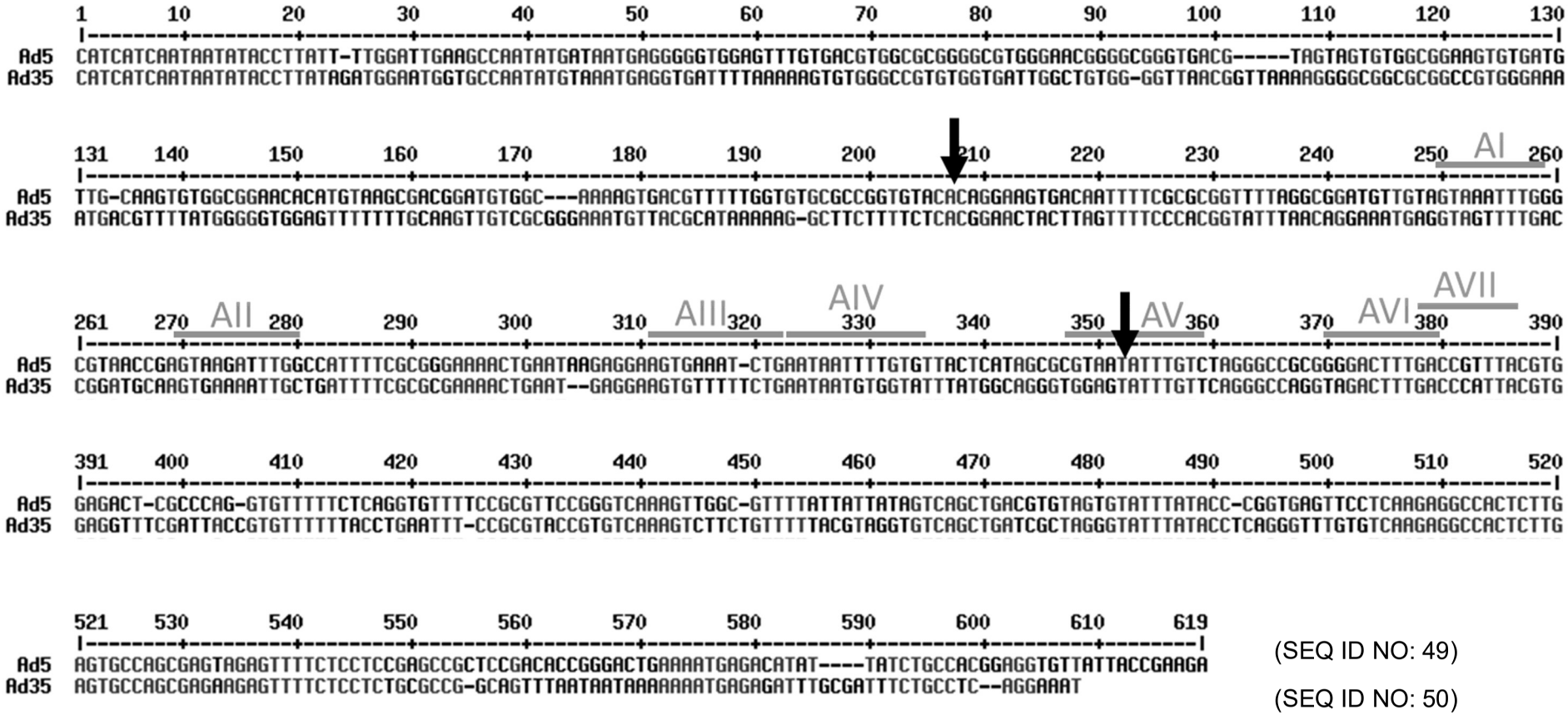
FIG. 112C

FIG. 113



SEQ ID NO: 262

FIG. 114



(SEQ ID NO: 49)

(SEQ ID NO: 50)

FIG. 115

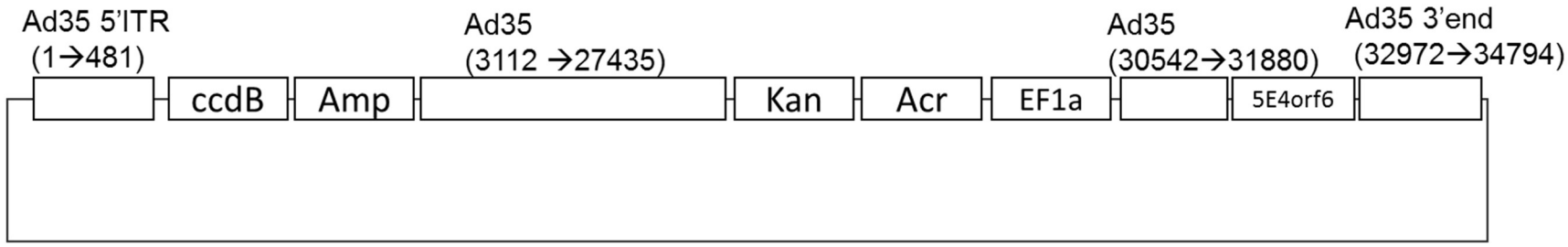


FIG. 116

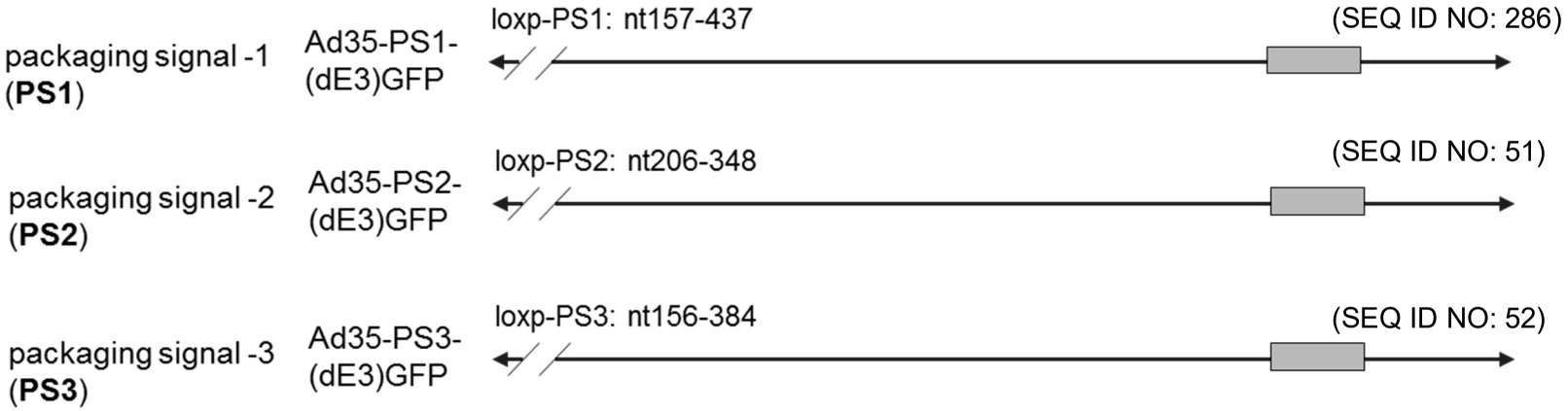


FIG. 117

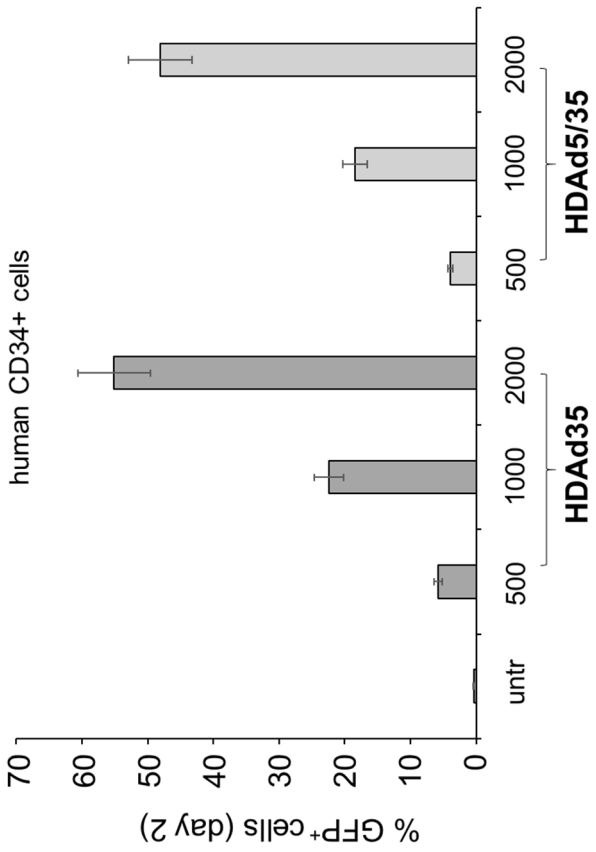


FIG. 118

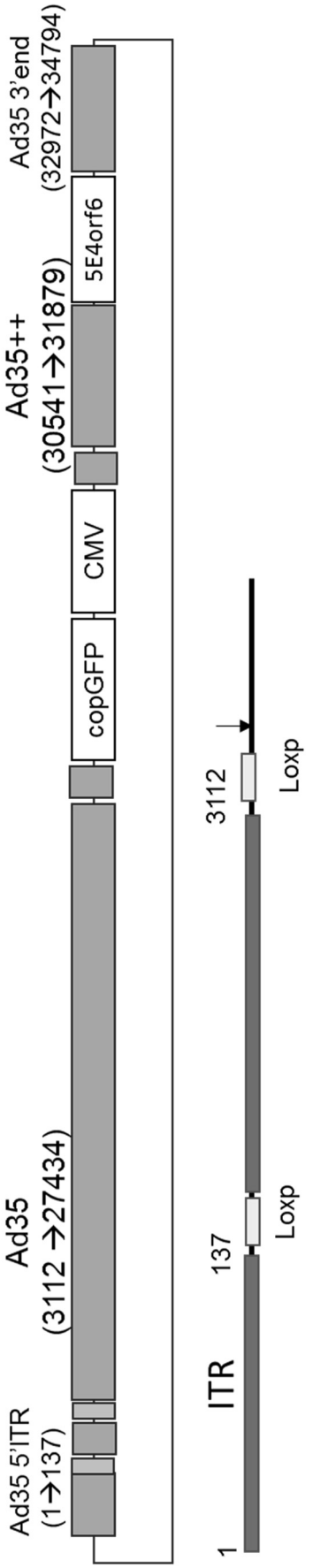


FIG. 119

CATCATCAATAATATACCTTATAGATGGAATGGTGCCAATATGTAAATGAGGTGATTTTAAAAAGTGTGGGCCGTGTGGTGATTGG
CTGTGGGGTTAACGGTTAAAAGGGGCGGCGCGGCCGTGGGAAAATGACGTT**ATTTAAAT**ATAACTTCGTATAGCATACATTATA
CGAAGTTATGAGGTAGTTTTGTTCAGGGGCAAGTGAAAATTGACCCATTACGCGCGAAAACTGAATGAGGAAGTGTTTTTCTGAA
TAATGTGGTATTTATGGCAGGGTGGAGTATTTGACCGGATCCAGGTAGACTTTGCTGATTTTCGTGGAGGTTT*ATAACTTCGTATA*
*GCATACATTATACGAAGTTAT***ATTTAAAT** (SEQ ID NO: 264)

Ad35 ITR 1→137bp: (positions 1-137 of SEQ ID NO: 264)

CATCATCAATAATATACCTTATAGATGGAATGGTGCCAATATGTAAATGAGGTGATTTTAAAAAGTGTGGGCCGTGT
GGTGATTGGCTGTGGGGTTAACGGTTAAAAGGGGCGGCGCGGCCGTGGGAAAATGACGTT

SwaI site: (positions 146-145 and 363-370 of SEQ ID NO: 264) ATTTAAAT

Loxp site: (positions 138-179 and 329-362 of SEQ ID NO: 264) ATAACTTCGTATAGCATACATTATACGAAGTTAT

Mutated packaging signal: (positions 138-145 of SEQ ID NO: 264)

GAGGTAGTTTTGTTCAGGGGCAAGTGAAAATTGACCCATTACGCGCGAAAACTGAATGAGGAAGTGTTTTTCTGAAT
AATGTGGTATTTATGGCAGGGTGGAGTATTTGACCGGATCCAGGTAGACTTTGCTGATTTTCGTGGAGGTTT

FIG. 120A

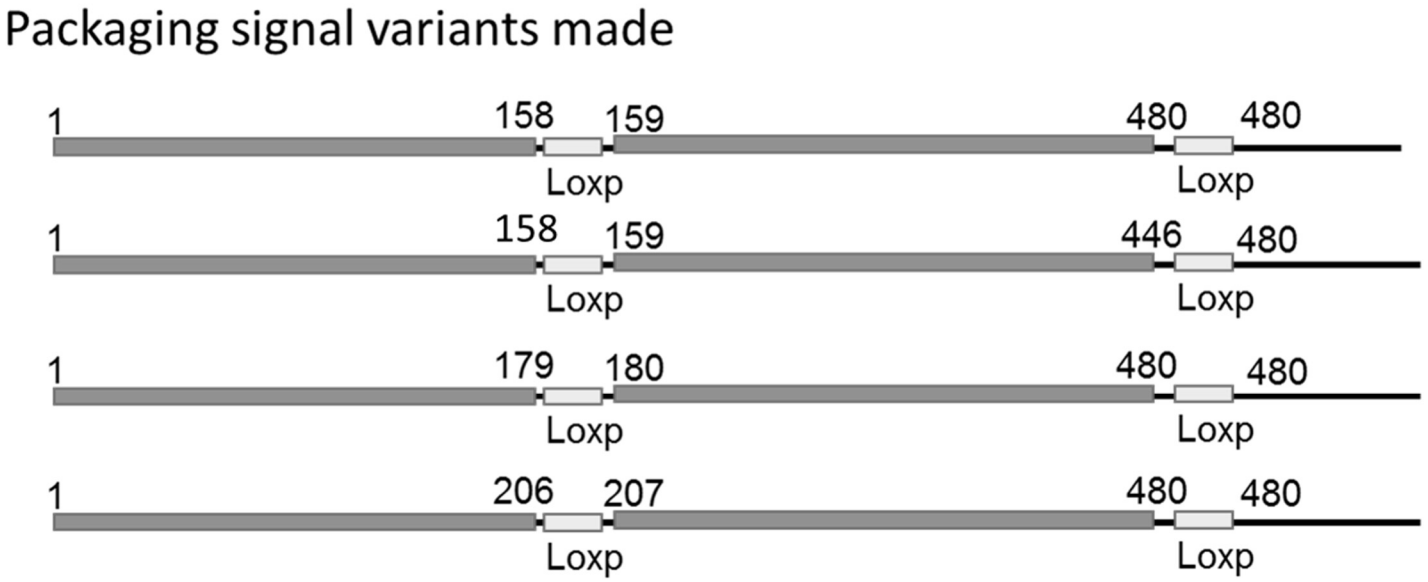

Packaging signal variants made
1
158
159
480
480
Loxp
Loxp
1
158
159
446
480
Loxp
Loxp
1
179
180
480
480
Loxp
Loxp
1
206
207
480
480
Loxp
Loxp

FIG. 120B

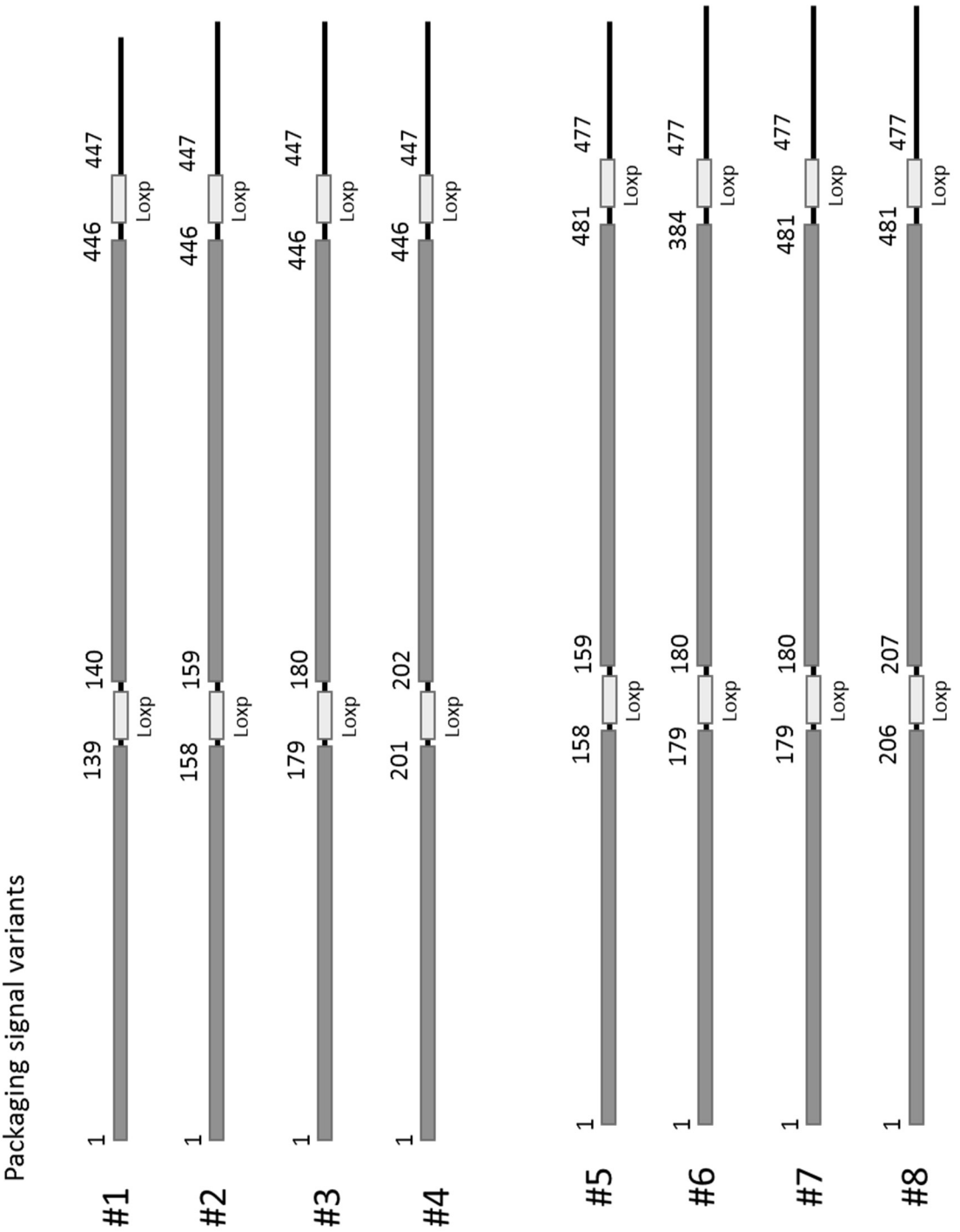

Packaging signal variants
#1
1
139
140
Loxp
446
447
Loxp
#2
1
158
159
Loxp
446
447
Loxp
#3
1
179
180
Loxp
446
447
Loxp
#4
1
201
202
Loxp
446
447
Loxp
#5
1
158
159
Loxp
481
477
Loxp
#6
1
179
180
Loxp
384
477
Loxp
#7
1
179
180
Loxp
481
477
Loxp
#8
1
206
207
Loxp
481
477
Loxp

FIG. 121

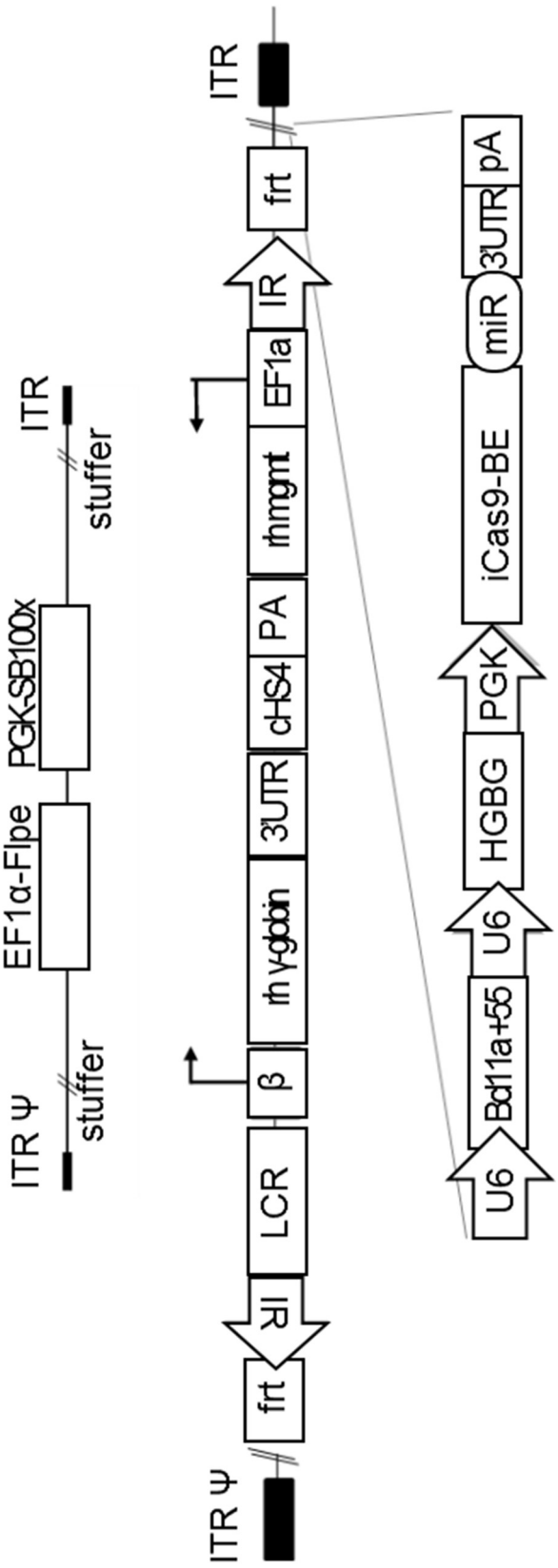

ITR Ψ
stuffer
EF1α-Flpe
PGK-SB100x
ITR
stuffer
ITR Ψ
frt
IR
LCR
β
rh γ-globin
3'UTR
cHS4
PA
rhmgmt
EF1a
IR
frt
ITR
U6
Bcl11a+55
U6
HGBG
PGK
iCas9-BE
miR
3'UTR
pA

FIG. 122

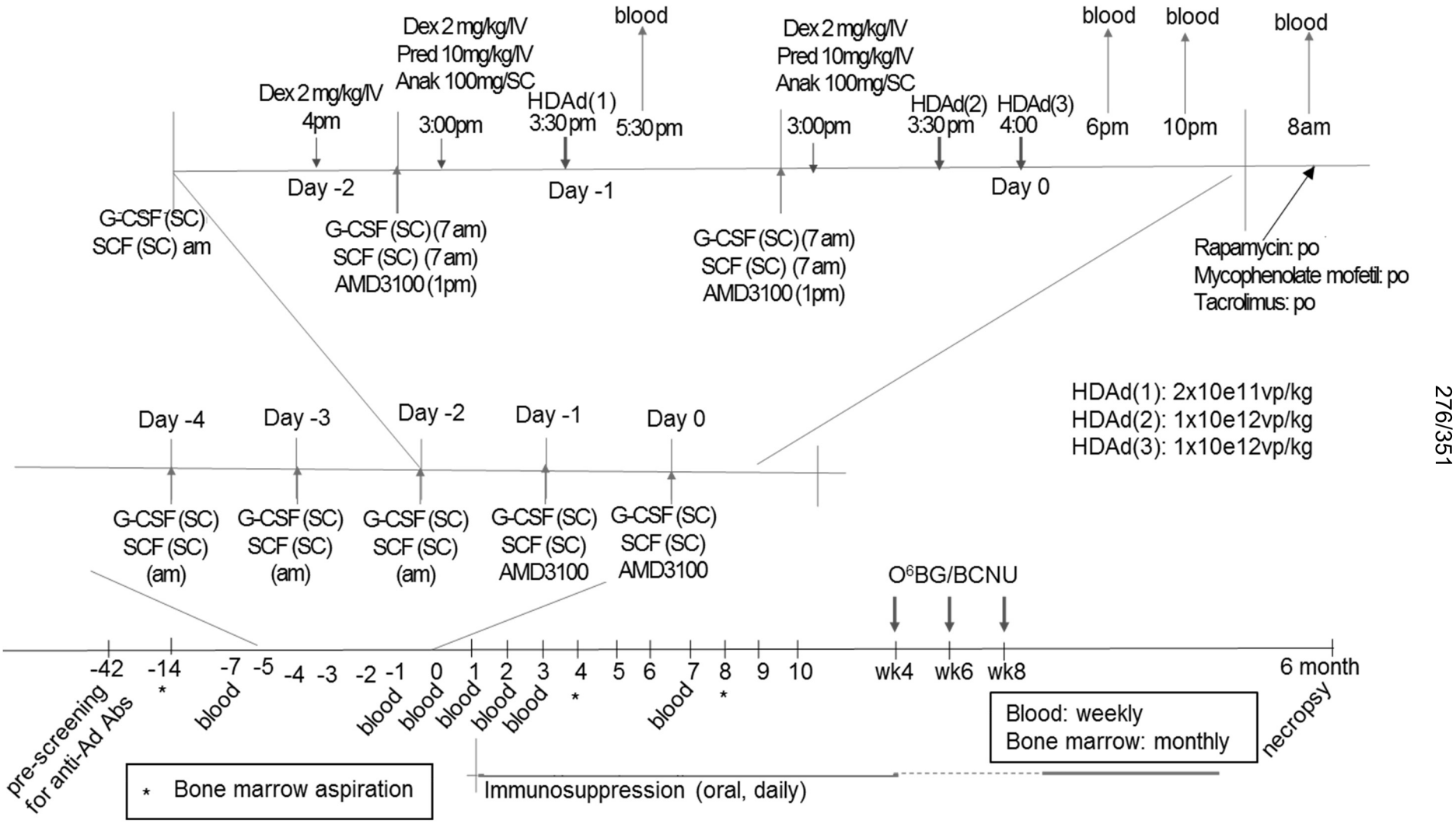

Dex 2 mg/kg/IV
Pred 10mg/kg/IV
Anak 100mg/SC
Dex 2 mg/kg/IV
4pm
3:00pm
HDAd(1)
3:30 pm
blood
5:30 pm
Dex 2 mg/kg/IV
Pred 10mg/kg/IV
Anak 100mg/SC
3:00pm
HDAd(2)
3:30 pm
HDAd(3)
4:00
blood
6pm
blood
10pm
blood
8am
Day -2
Day -1
Day 0
G-CSF (SC)
SCF (SC) am
G-CSF (SC) (7 am)
SCF (SC) (7 am)
AMD3100 (1pm)
G-CSF (SC) (7 am)
SCF (SC) (7 am)
AMD3100 (1pm)
Rapamycin: po
Mycophenolate mofetil: po
Tacrolimus: po
HDAd(1): 2x10e11vp/kg
HDAd(2): 1x10e12vp/kg
HDAd(3): 1x10e12vp/kg
Day -4
Day -3
Day -2
Day -1
Day 0
G-CSF (SC)
SCF (SC)
(am)
G-CSF (SC)
SCF (SC)
(am)
G-CSF (SC)
SCF (SC)
(am)
G-CSF (SC)
SCF (SC)
AMD3100
G-CSF (SC)
SCF (SC)
AMD3100
O6BG/BCNU
-42
-14
-7
-5
-4
-3
-2
-1
0
1
2
3
4
5
6
7
8
9
10
wk4
wk6
wk8
6 month
pre-screening
for anti-Ad Abs
blood
blood
blood
blood
blood
blood
blood
Blood: weekly
Bone marrow: monthly
necropsy
* Bone marrow aspiration
Immunosuppression (oral, daily)

FIG. 123

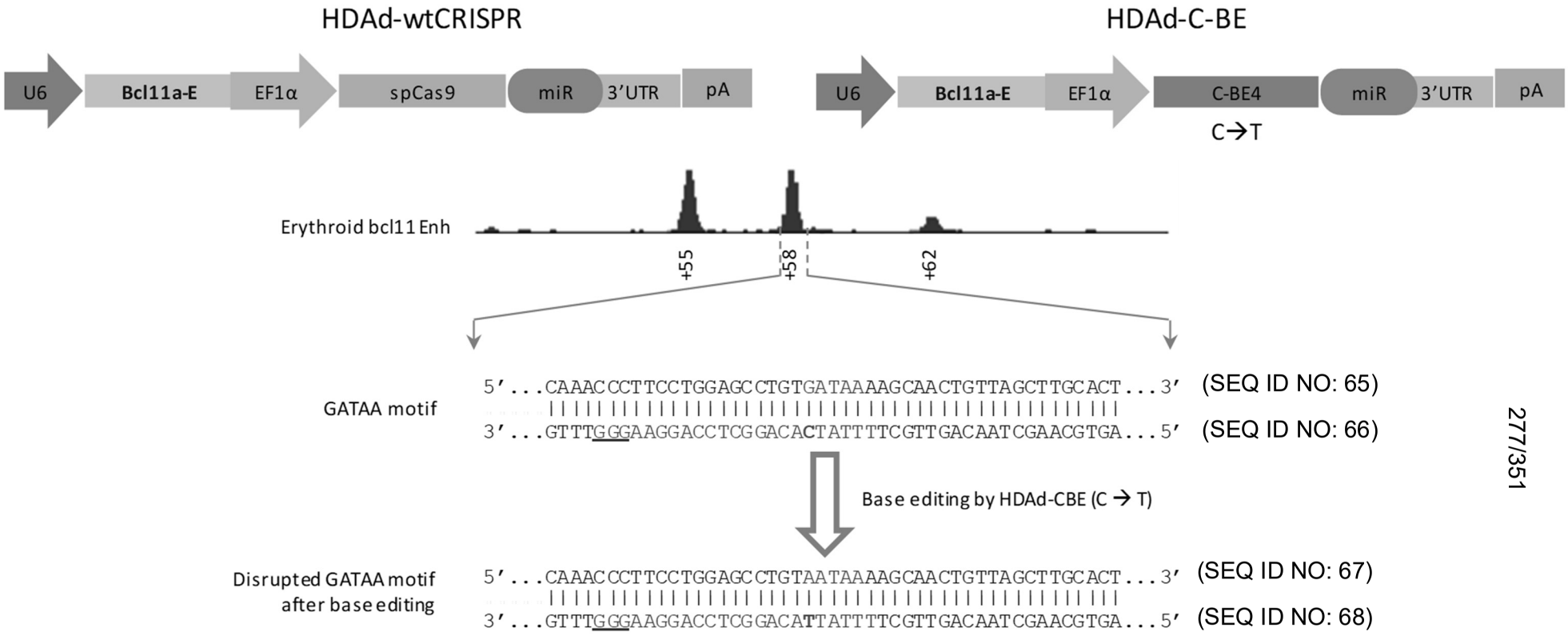

HDAd-wtCRISPR
U6
Bcl11a-E
EF1α
spCas9
miR
3'UTR
pA
HDAd-C-BE
U6
Bcl11a-E
EF1α
C-BE4
miR
3'UTR
pA
C→T
Erythroid bcl11 Enh
+55
+58
+92
GATAA motif
5'...CAAACCCTTCCTGGAGCCTGTGATAAAAGCAACTGTTAGCTTGCACT...3' (SEQ ID NO: 65)
3'...GTTTGGGAAGGACCTCGGACACTATTTTCGTTGACAATCGAACGTGA...5' (SEQ ID NO: 66)
Base editing by HDAd-CBE (C → T)
Disrupted GATAA motif after base editing
5'...CAAACCCTTCCTGGAGCCTGTAATAAAAGCAACTGTTAGCTTGCACT...3' (SEQ ID NO: 67)
3'...GTTTGGGAAGGACCTCGGACATTATTTTCGTTGACAATCGAACGTGA...5' (SEQ ID NO: 68)

FIG. 124A

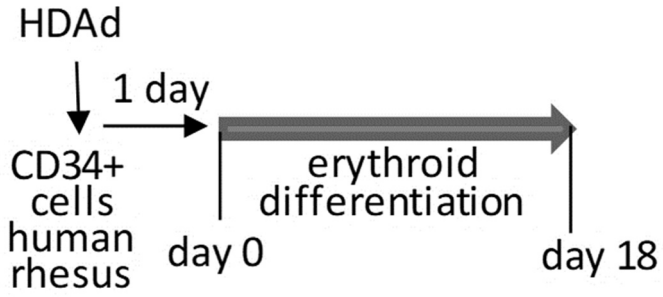


FIG. 124B

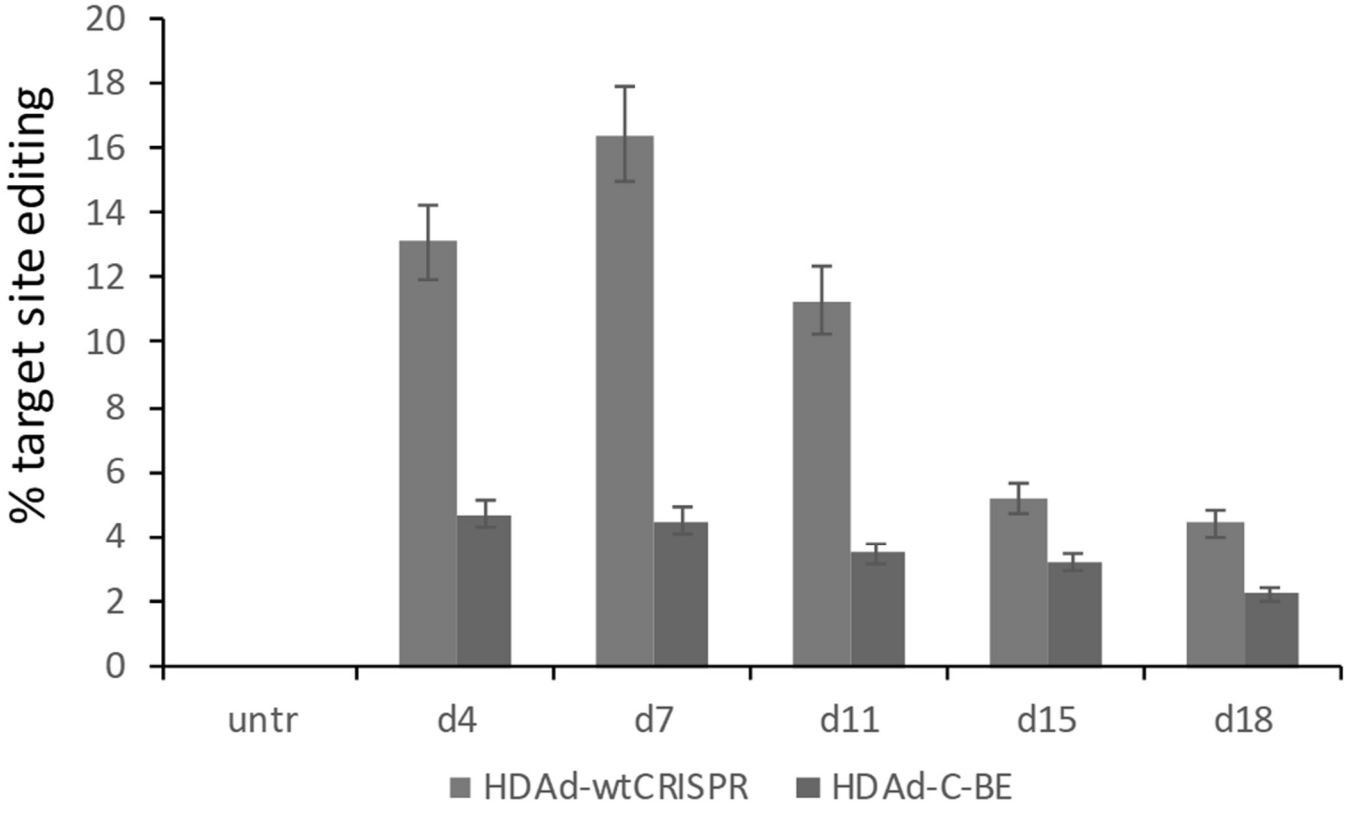


FIG. 124C

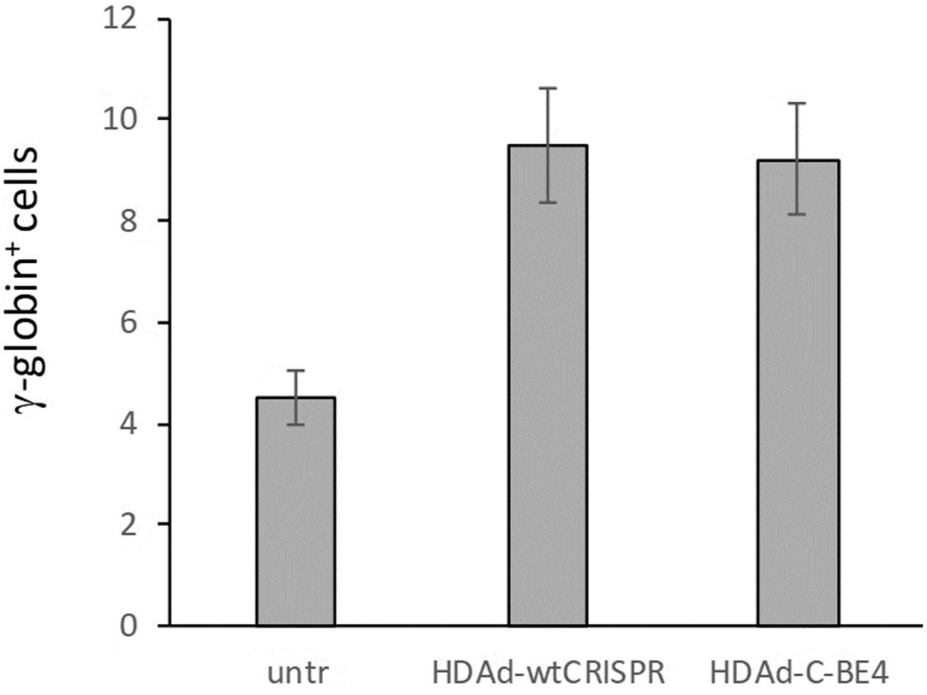


FIG. 125

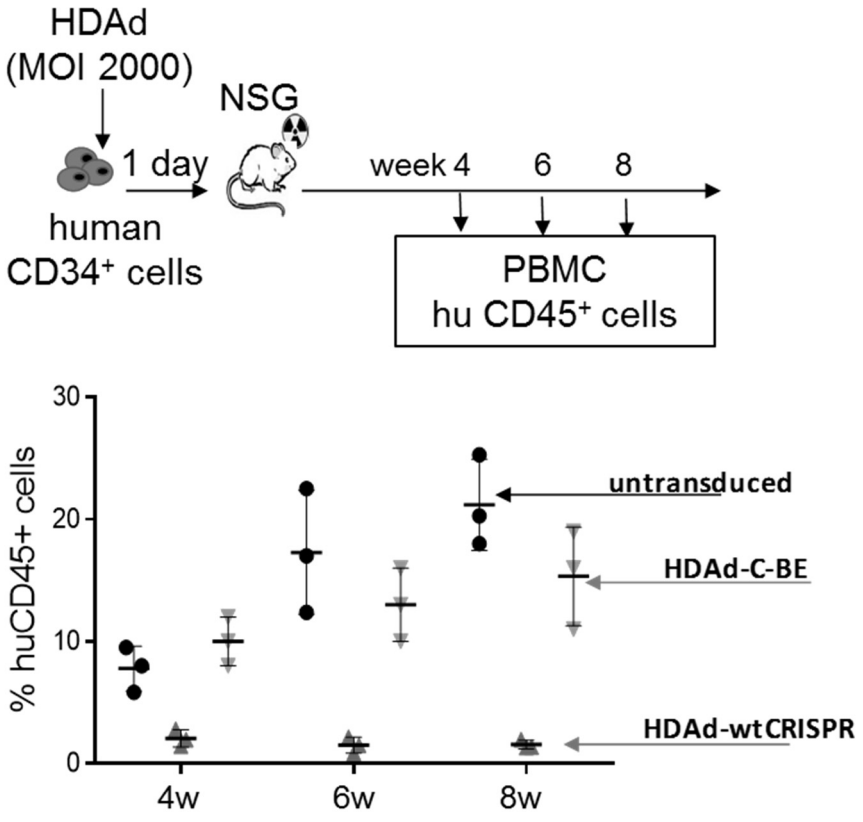


FIG. 126

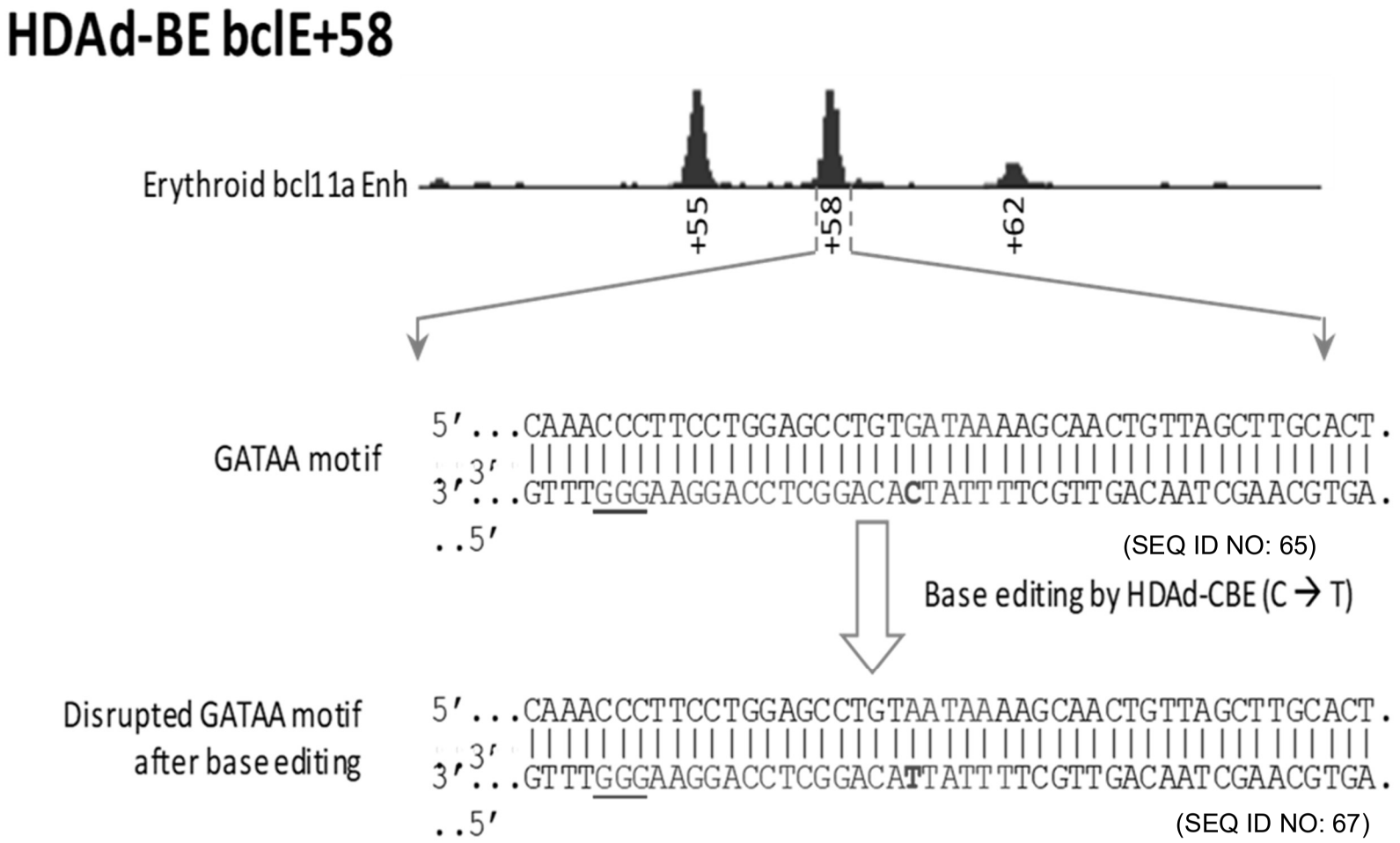
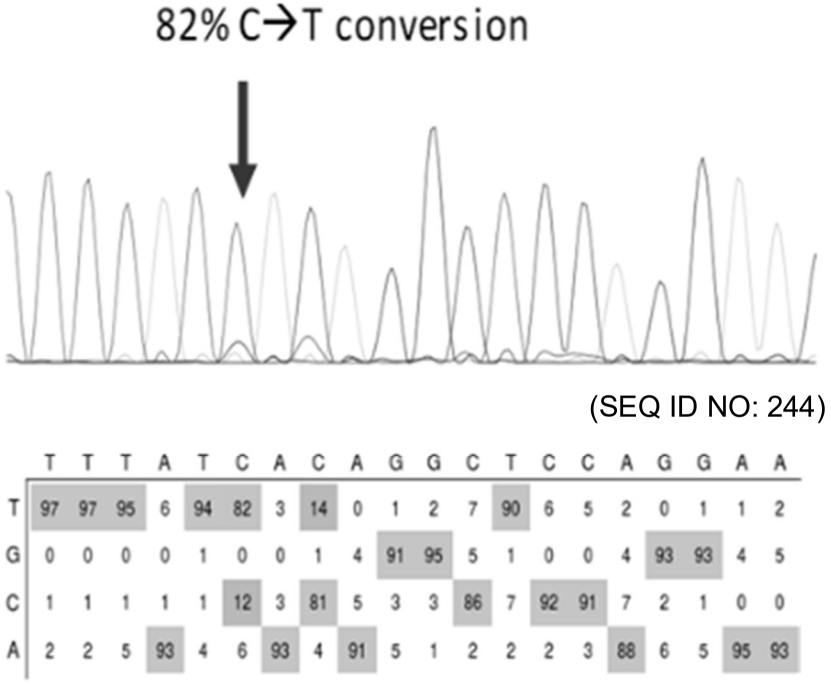

HDAd-BE bclE+58
Erythroid bcl11a Enh
+55
+58
+62
GATAA motif
5′...CAAACCCTTCCTGGAGCCTGTGATAAAAGCAACTGTTAGCTTGCACT.
3′...GTTTGGGAAGGACCTCGGACACTATTTTCGTTGACAATCGAACGTGA.
(SEQ ID NO: 65)
Base editing by HDAd-CBE (C → T)
Disrupted GATAA motif after base editing
5′...CAAACCCTTCCTGGAGCCTGTAATAAAAGCAACTGTTAGCTTGCACT.
3′...GTTTGGGAAGGACCTCGGACATTATTTTCGTTGACAATCGAACGTGA.
(SEQ ID NO: 67)

FIG. 126 cont'd



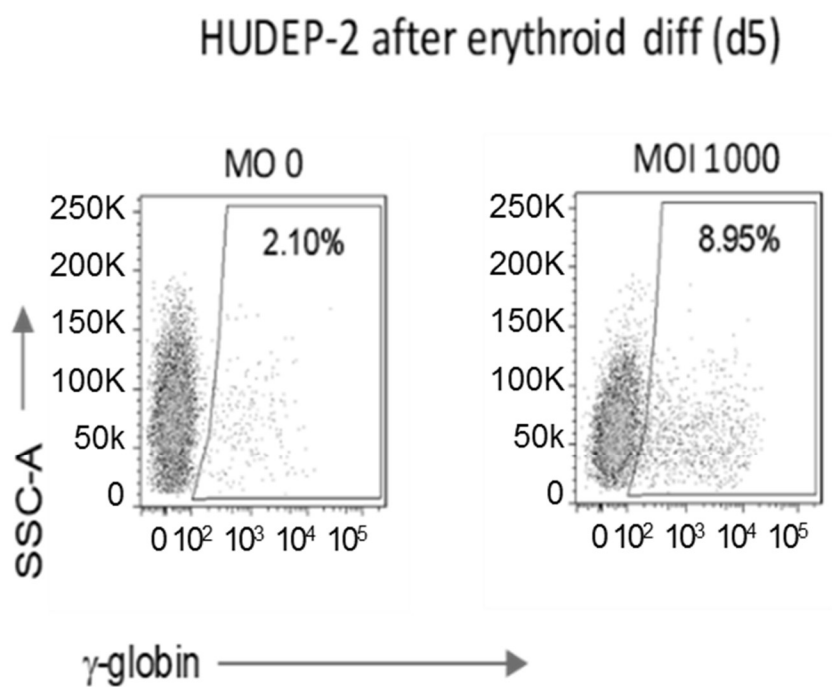


FIG. 126 cont'd

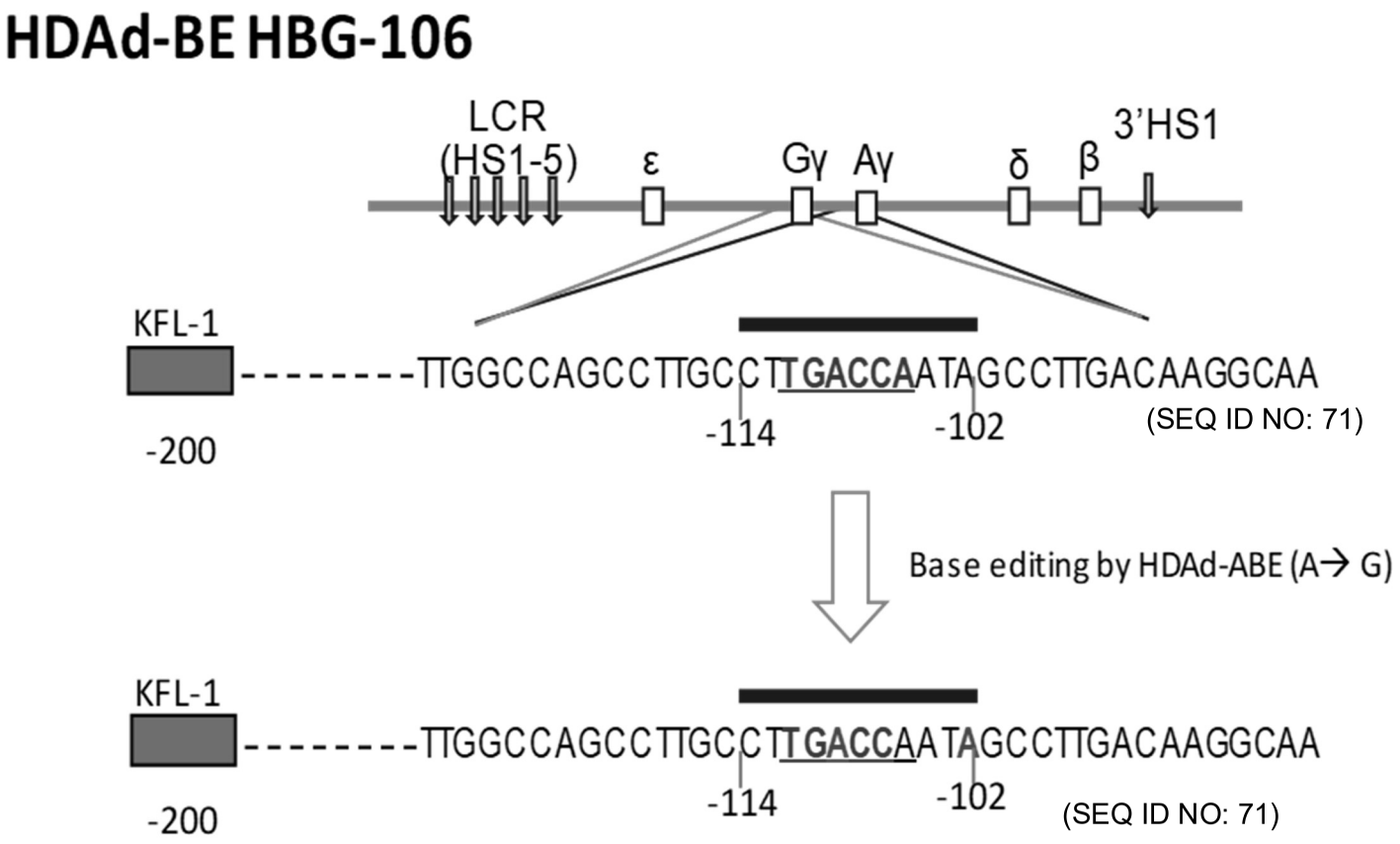
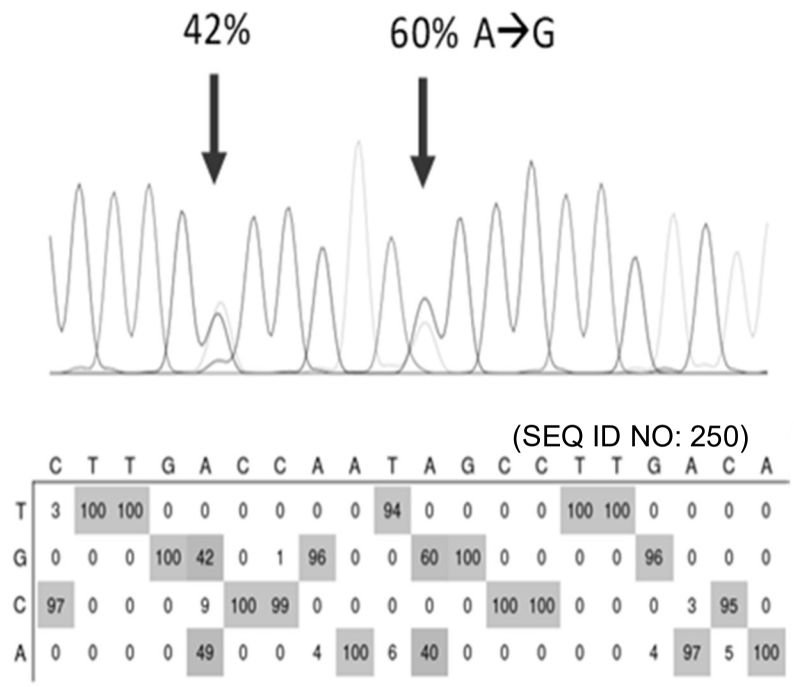


FIG. 126 cont'd



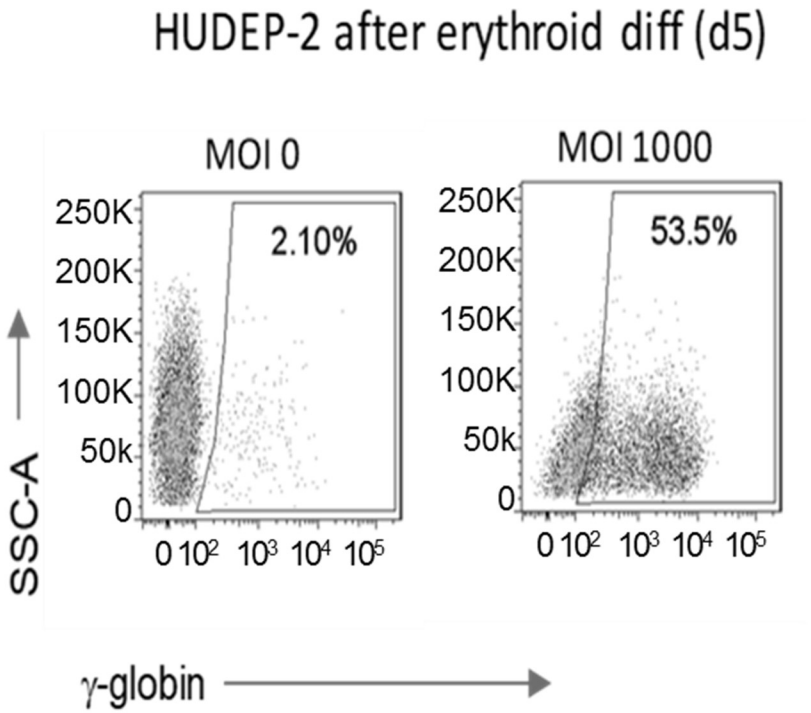


FIG.127

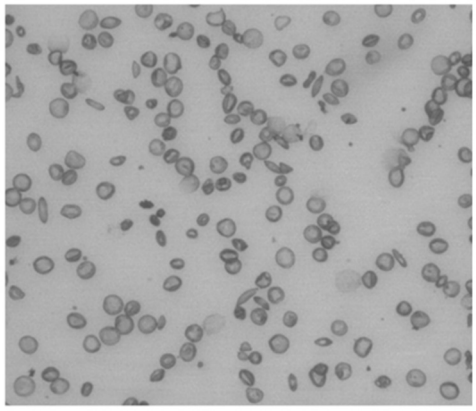


FIG. 127B

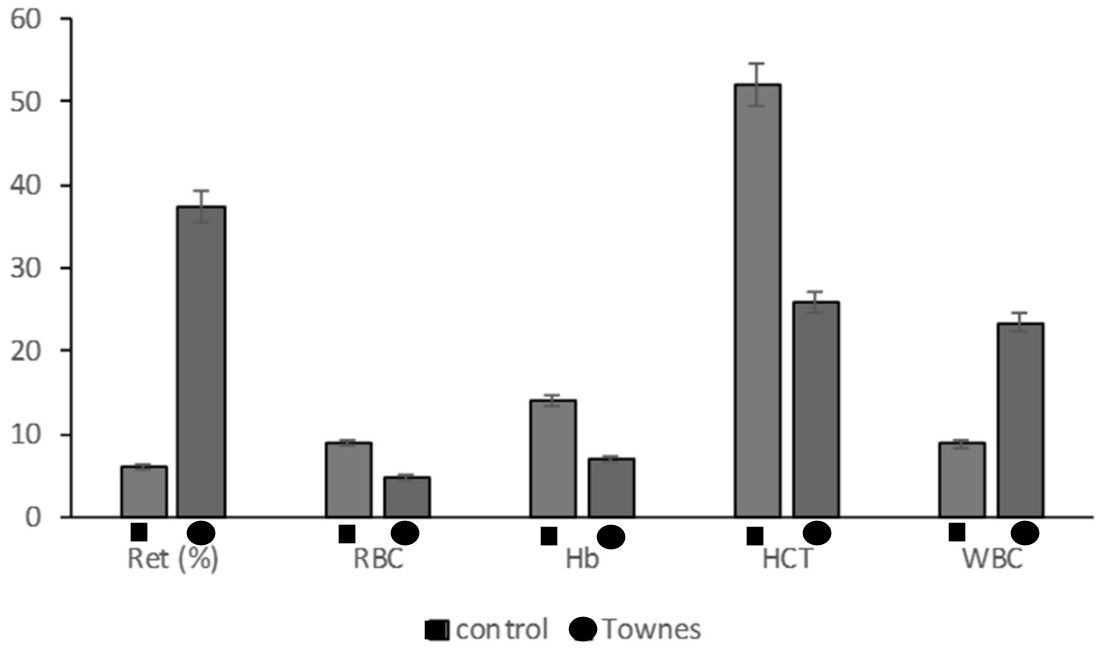


FIG. 128A

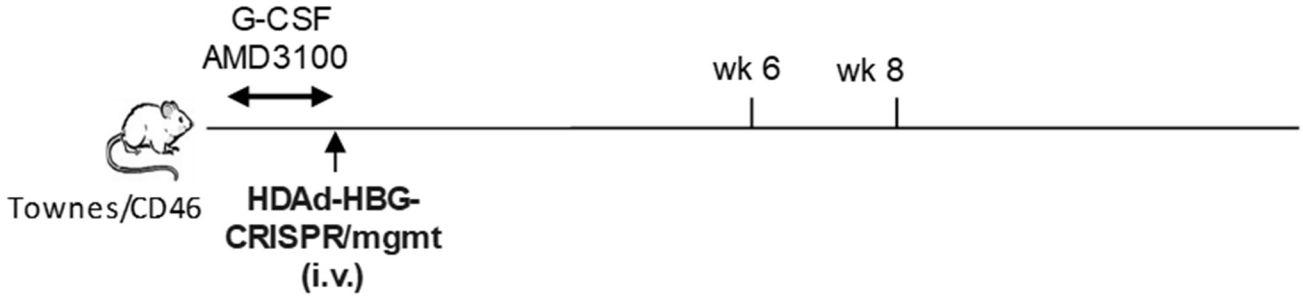


FIG. 128B

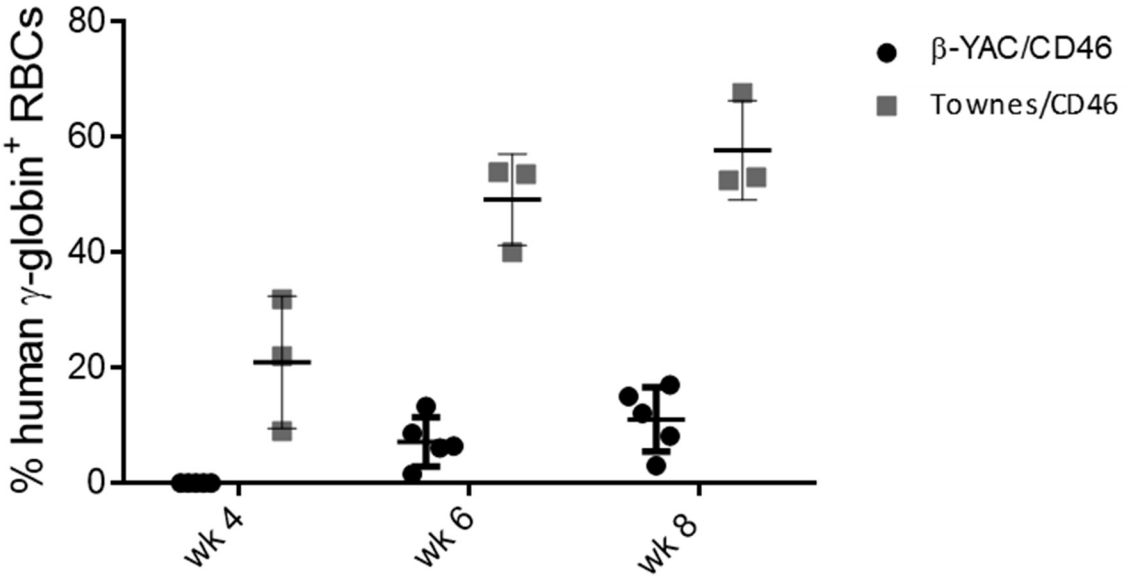


FIG. 128C

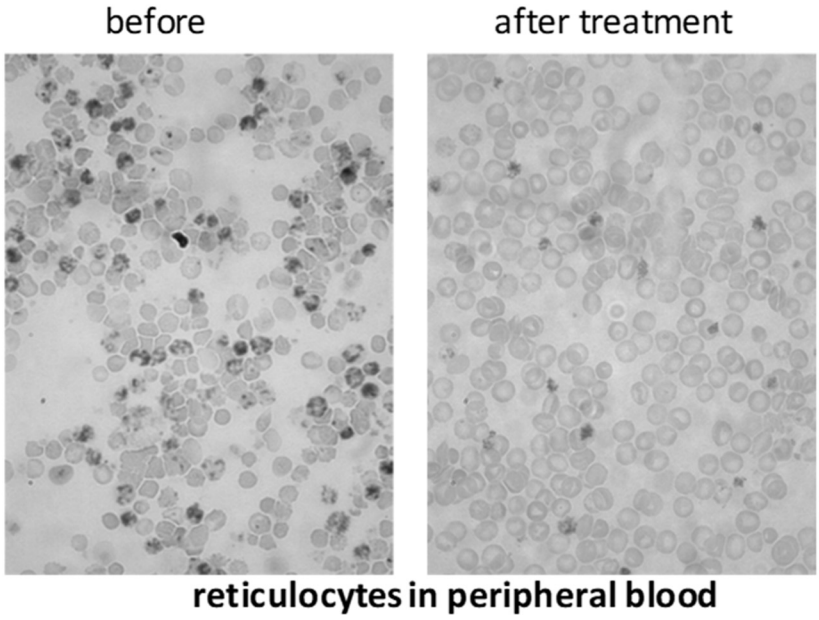


FIG. 129A

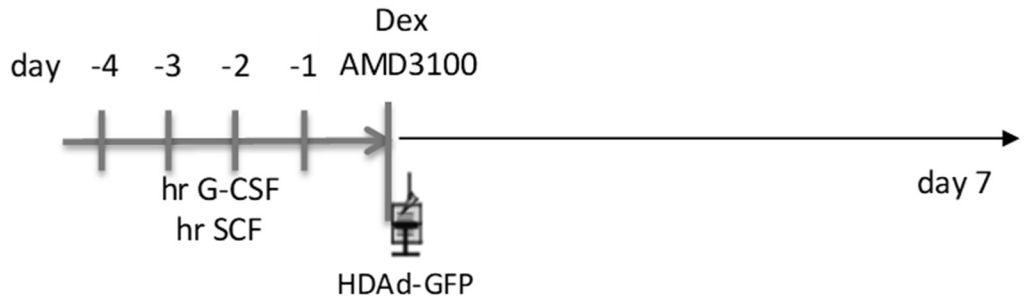


FIG.129B

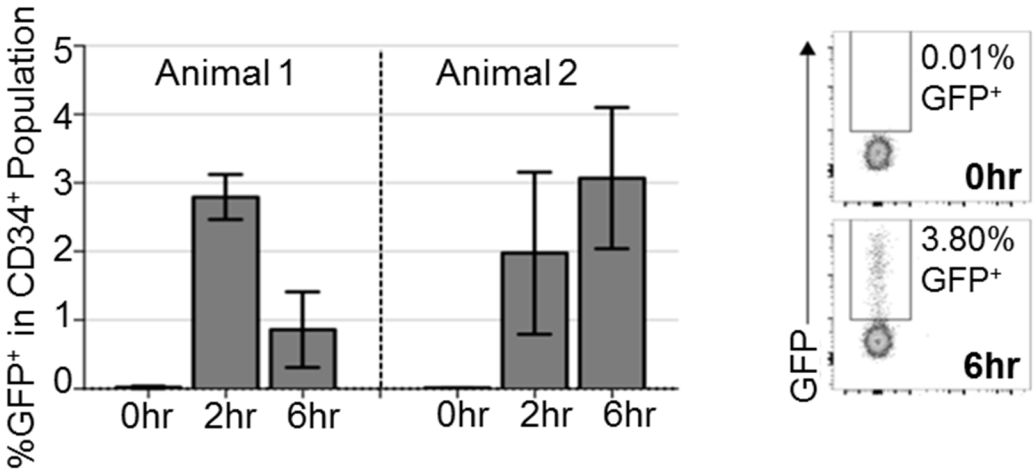


FIG. 129C

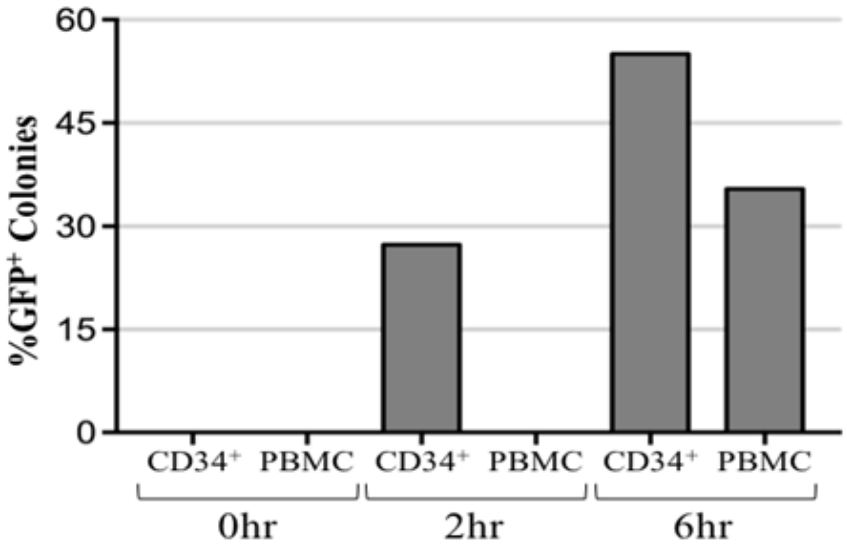


FIG. 129D

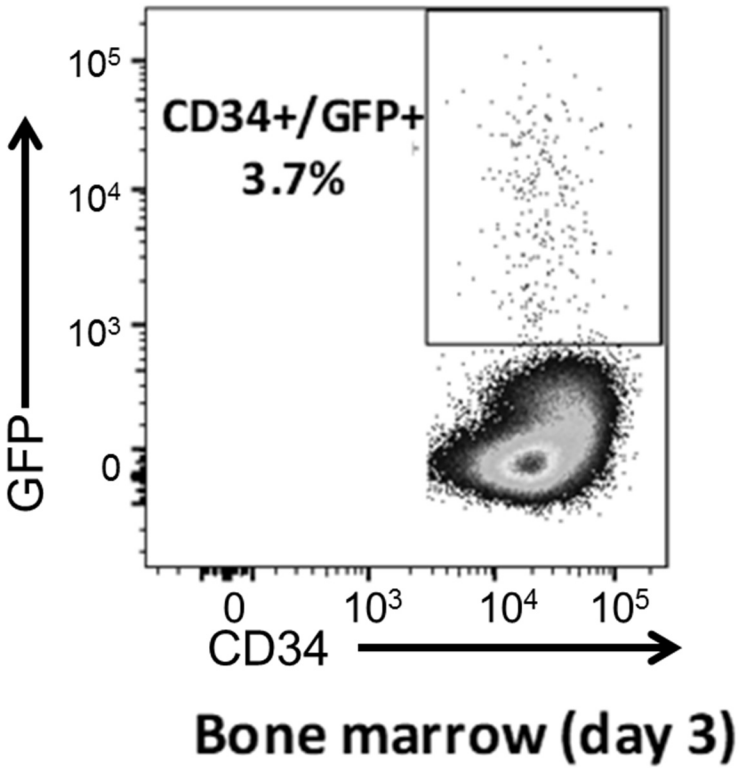

CD34+/GFP+
3.7%
10^5
10^4
10^3
0
GFP
0
10^3
10^4
10^5
CD34
Bone marrow (day 3)

FIG. 130

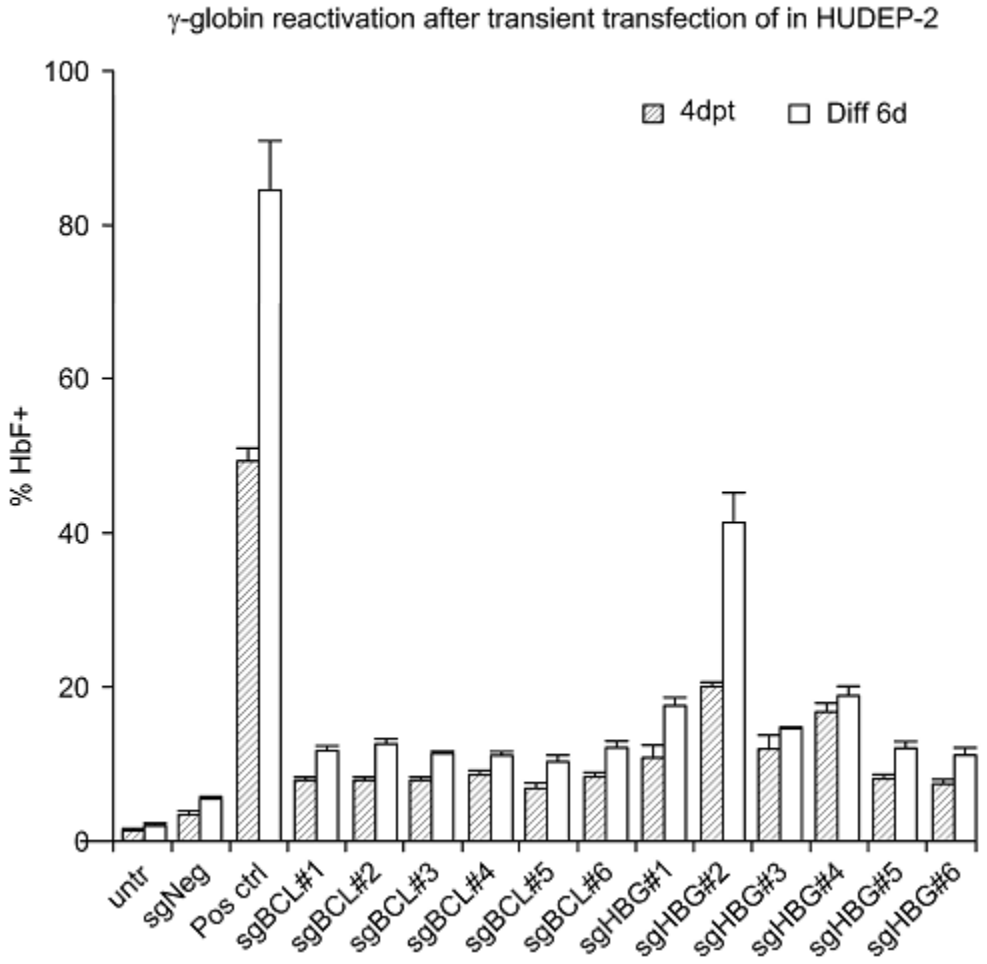


FIG. 131A

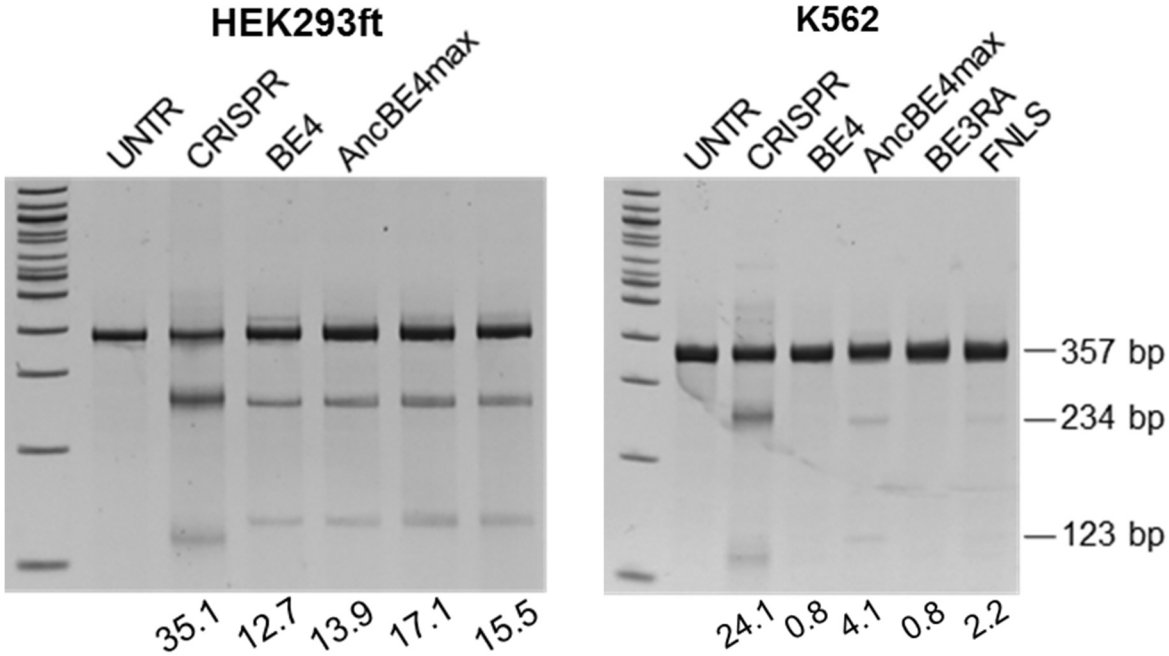


FIG. 131B

FIG. 132A

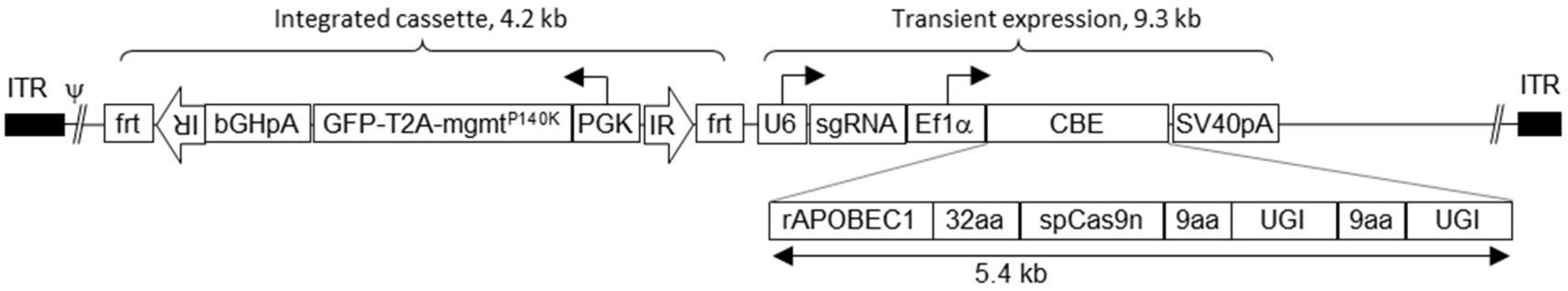


FIG. 132B

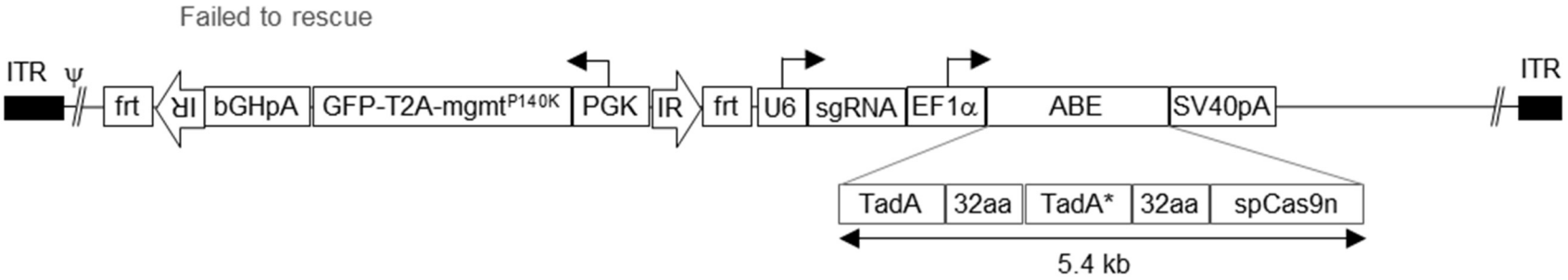


FIG. 132C

```
 TadA^N+32aa   1 TCTGAAGTCGAGTTCAGCCACGAGTATTGGATGAGGCACGCACTGACCCTGGCAAAACGAGCCTGGGACG
TadA^N*+32aa   1 .....G..G.....TTC.........C........A..T..C...........C..GA.G..AC.C..T.

 TadA^N+32aa  71 AAAGAGAAGTCCCCGTCGGCGCCGTGCTGGTGCACAACAATCGCGTGATCGGAGAAGGATGGAACAGGCC
TadA^N*+32aa  71 .G..G..G..G..T..G..A.............TG......A.A........C..G..C........AG.

 TadA^N+32aa 141 AATCGGACGCCACGACCCTACCGCACACGCAGAGATCATGGCACTGAGGCAGGGAGGACTGGTCATGCAG
TadA^N*+32aa 141 C.....C.TG........A..A..C..T..C..A..T.....C.....A.....C..C...........

 TadA^N+32aa 211 AATTATCGCCTGATCGATGCCACCCTGTATGTGACACTGGAGCCATGCGTGATGTGCGCAGGAGCAATGA
TadA^N*+32aa 211 ..C..CA.A.....T..C...........C......T.C.....T.............C..C..C....

 TadA^N+32aa 281 TCCACAGCCGGATCGGAAGAGTGGTGTTCGGAGCACGAGACGCCAAGACCGGAGCAGCAGGCAGCCTGAT
TadA^N*+32aa 281 .....TCTA.......CC.C.........T..C.TGA.GA.T..A..A..T..C..C......TCT.....

 TadA^N+32aa 351 GGATGTGCTGCACCACCCAGGCATGAACCACCGGGTGGAGATCACAGAGGGCATCCTGGCAGACGAGTGC
TadA^N*+32aa 351 ...C.........T....C.........T...A.A..C..A..T..C.....A...........T..A..T

 TadA^N+32aa 421 GCCGCCCTGCTGAGCGATTTCTTTAGAATGAGGAGACAGGAGATCAAGGCCCAGAAGAAGGCACAGAGCT
TadA^N*+32aa 421 ............T..T.........C.G...CCT.......T.T.T..T..T...........C.......

 TadA^N+32aa 491 CCACCGACTCTGGAGGCTCTAGCGGAGGATCCTCTGGAAGCGAGACACCAGGCACATCCGAGTCCGCCAC
TadA^N*+32aa 491 .......TAG...C..A.........G..C.........CTCT.....C..T......AG....AG...A..

 TadA^N+32aa 561 ACCAGAGTCTTCCGGCGGCTCCTCCGGAGGATCC  (SEQ ID NO: 260)
TadA^N*+32aa 561 ...T..AAGCAG...G...AG.AG...G..GAG.   (SEQ ID NO: 261)
```

FIG. 133A

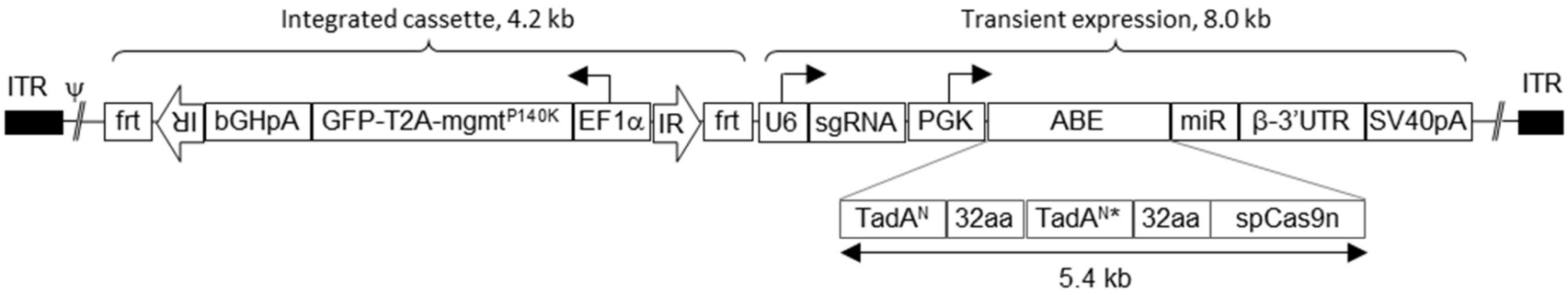


FIG. 133B

Full name	Editor	Guide RNA	Yield (× 10^{12} vp)
HDAd5/35++_BE4max-sgBCL#1-FI-mgmt/GFP	CBE	sgBCL#1	1.2
HDAd5/35++_BE4max-sgHBG#1-FI-mgmt/GFP	CBE	sgHBG#1	0.8
HDAd5/35++_ABEopti-sgHBG#2-FI-mgmt/GFP	ABE	sgHBG#2	3.8
HDAd5/35++_ABEopti-sgHBG#4-FI-mgmt/GFP	ABE	sgHBG#4	2.8
HDAd5/35++_BE4max-Dual#1-FI-mgmt/GFP	CBE	sgBCL#1 + sgHBG#1	0.5
HDAd5/35++_BE4max-sgNeg-FI-mgmt/GFP	CBE	sgNeg	1.5

FIG. 133C

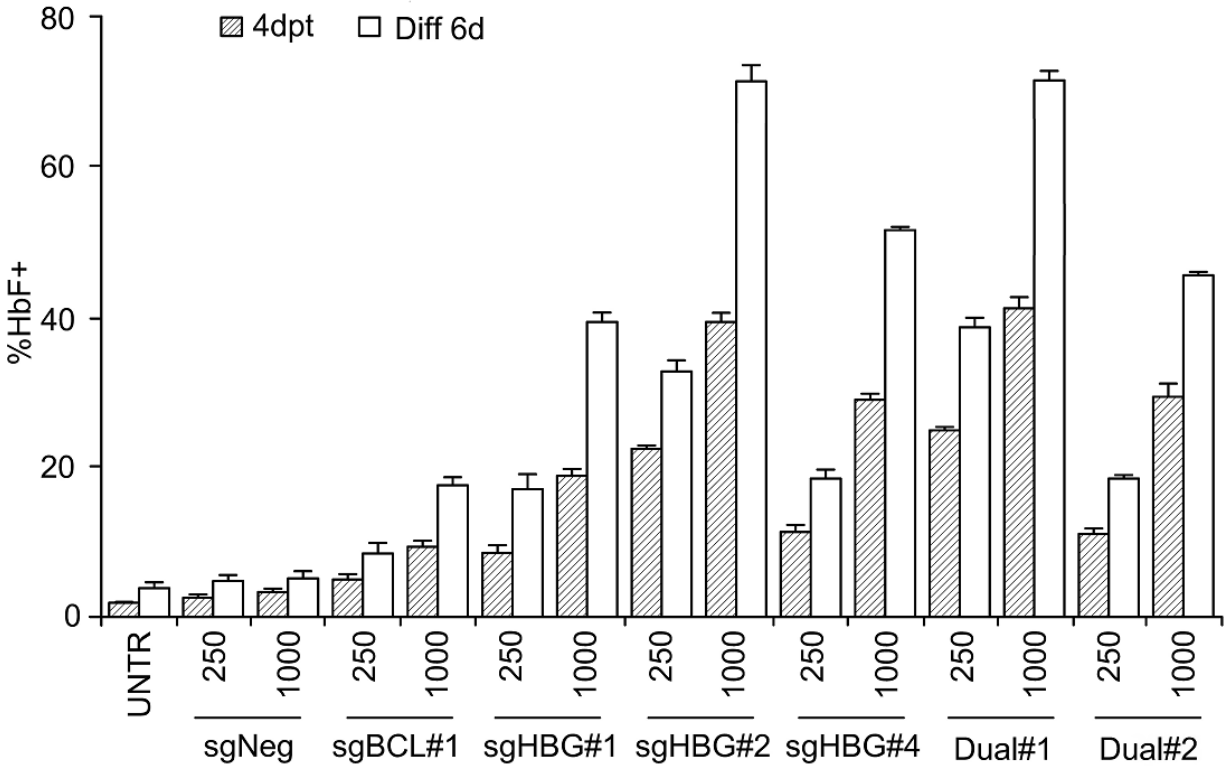
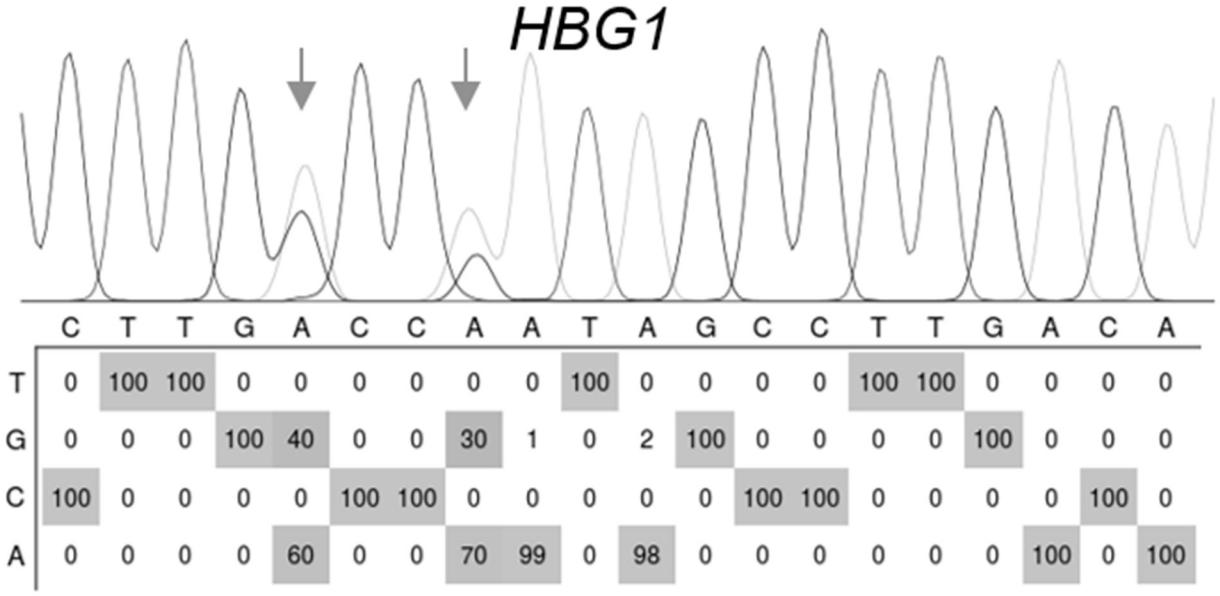

4dpt
Diff 6d
%HbF+
80
60
40
20
0
UNTR
250
1000
sgNeg
sgBCL#1
sgHBG#1
sgHBG#2
sgHBG#4
Dual#1
Dual#2

FIG. 133D

sgHBG#2

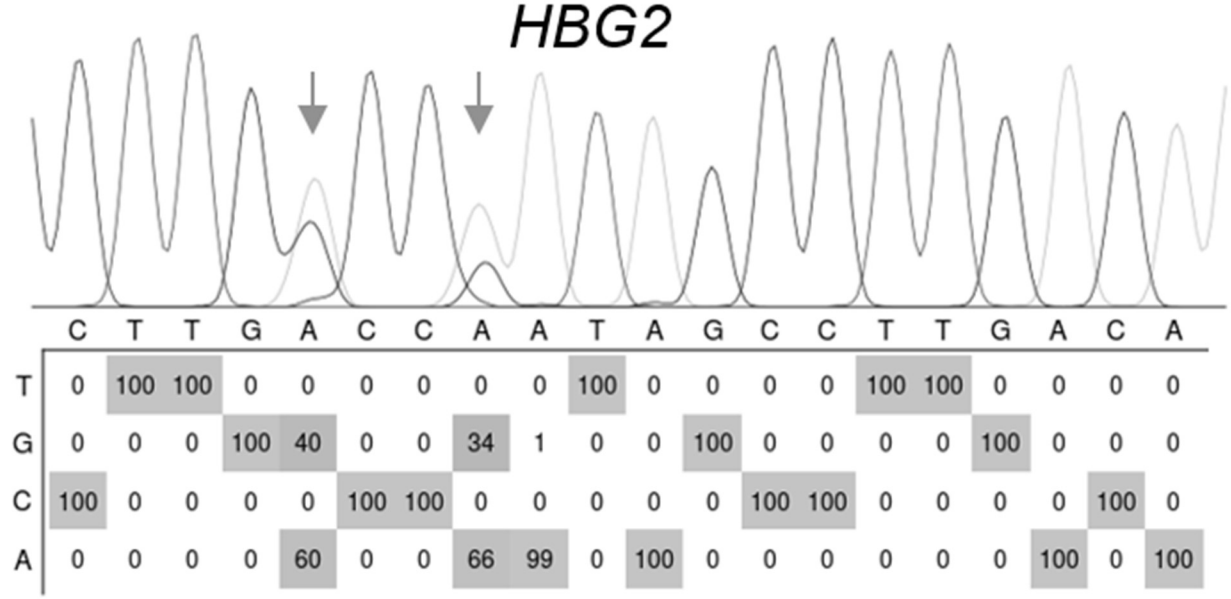
HBG1



	C	T	T	G	A	C	C	A	A	T	A	G	C	C	T	T	G	A	C	A
T	0	100	100	0	0	0	0	0	0	100	0	0	0	0	100	100	0	0	0	0
G	0	0	0	100	40	0	0	30	1	0	2	100	0	0	0	0	100	0	0	0
C	100	0	0	0	0	100	100	0	0	0	0	0	100	100	0	0	0	0	100	0
A	0	0	0	0	60	0	0	70	99	0	98	0	0	0	0	0	0	100	0	100

(SEQ ID NO: 250)

HBG2



	C	T	T	G	A	C	C	A	A	T	A	G	C	C	T	T	G	A	C	A
T	0	100	100	0	0	0	0	0	0	100	0	0	0	0	100	100	0	0	0	0
G	0	0	0	100	40	0	0	34	1	0	0	100	0	0	0	0	100	0	0	0
C	100	0	0	0	0	100	100	0	0	0	0	0	100	100	0	0	0	0	100	0
A	0	0	0	0	60	0	0	66	99	0	100	0	0	0	0	0	0	100	0	100

(SEQ ID NO: 250)

FIG. 133E

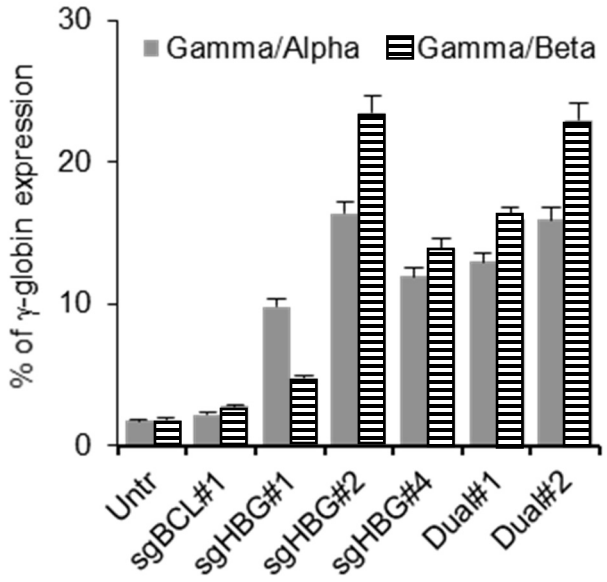


FIG. 133F

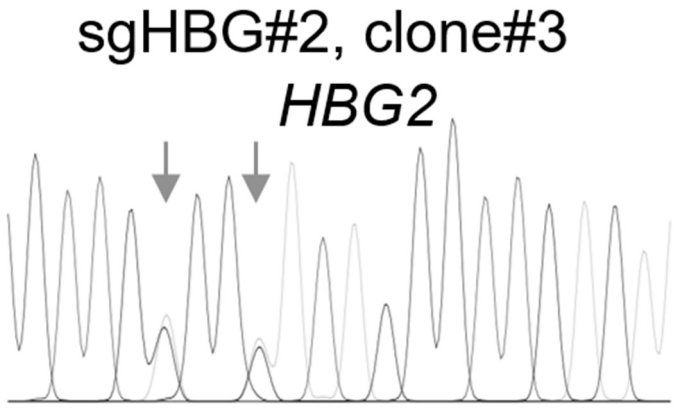


FIG. 133G

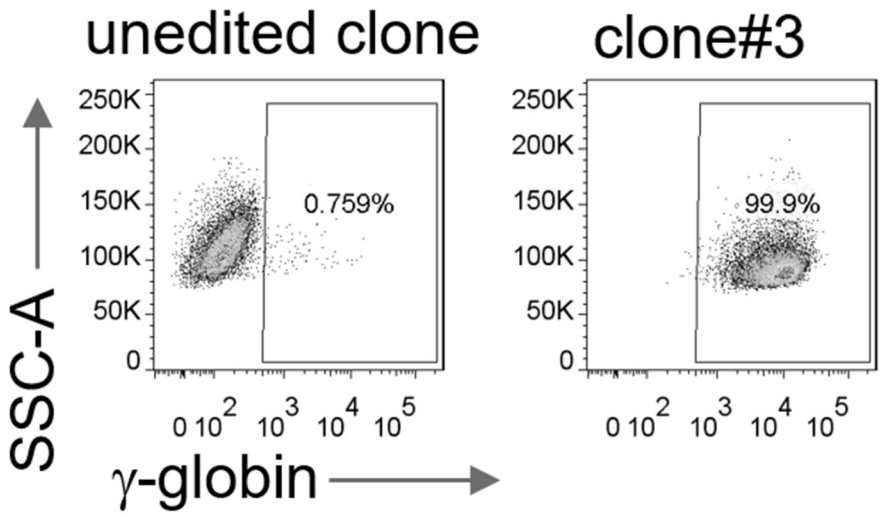


FIG. 133H

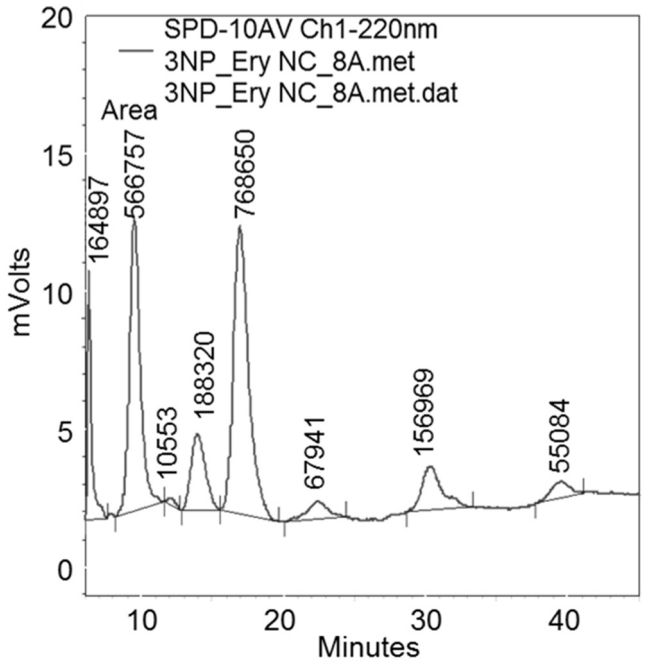
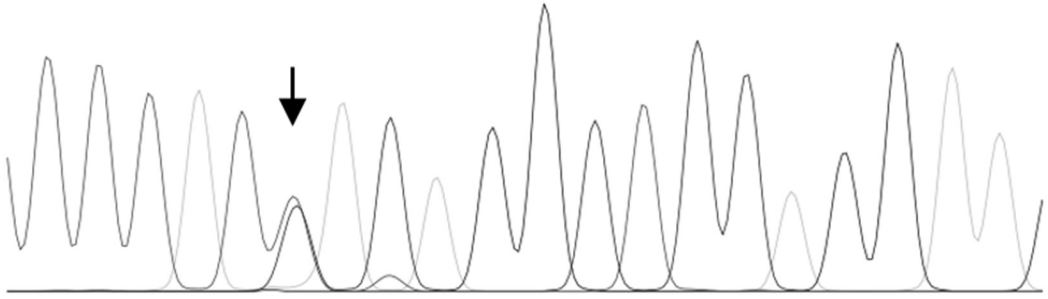


FIG. 134A

sgBCL#1

Bcl11A

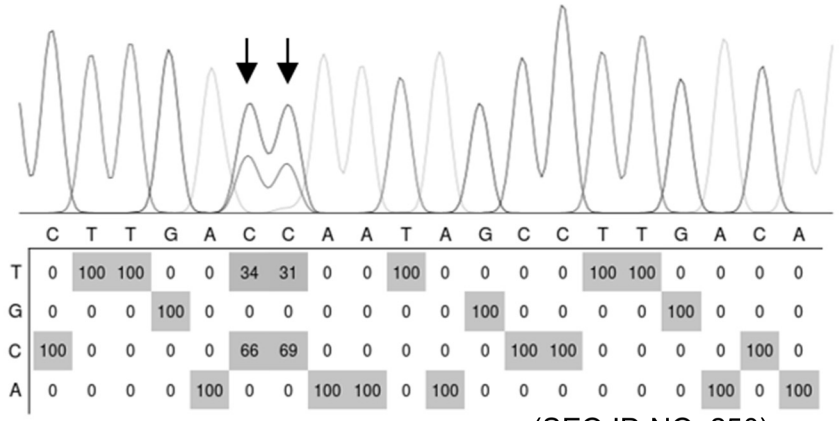


	T	T	T	A	T	C	A	C	A	G	G	C	T	C	C	A	G	G	A	A
T	99	99	100	0	100	51	0	8	0	0	0	0	100	0	0	0	0	0	0	0
G	0	0	0	0	0	1	0	0	0	100	100	0	0	0	0	0	100	100	0	0
C	0	0	0	0	0	46	0	92	0	0	0	100	0	100	100	0	0	0	0	0
A	1	0	0	100	0	2	100	0	100	0	0	0	0	0	0	100	0	0	100	100

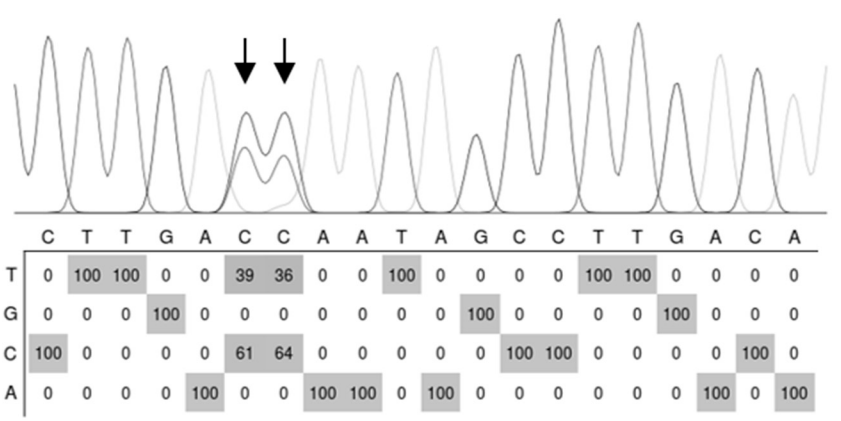
(SEQ ID NO: 244)

FIG. 134A cont'd

sgHBG#1

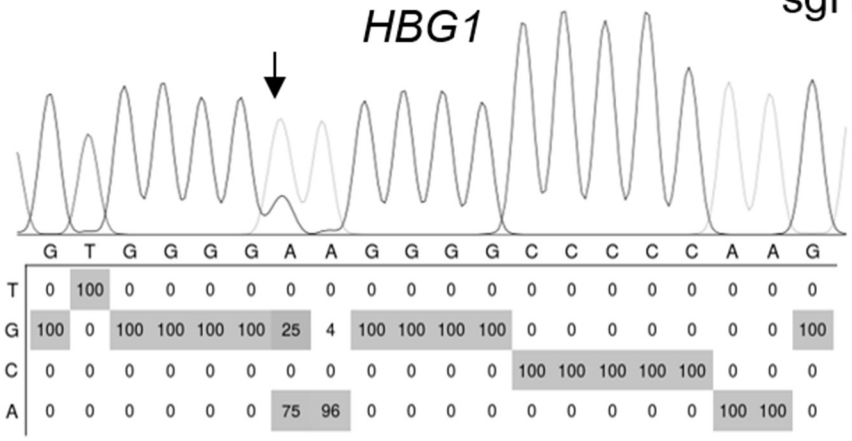


(SEQ ID NO: 250)

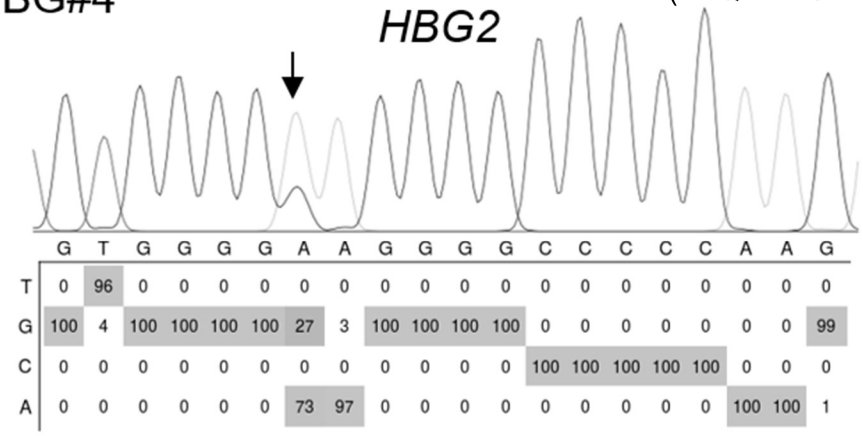


(SEQ ID NO: 250)

sgHBG#4



(SEQ ID NO: 253)

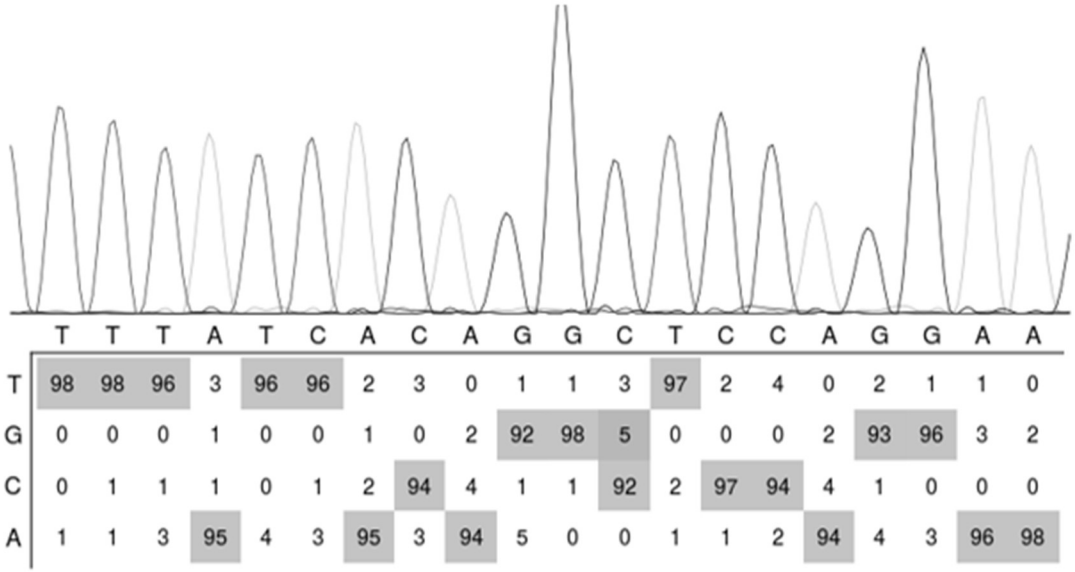


(SEQ ID NO: 253)

FIG. 134B

sgBCL#1, Clone#5

Bcl11A



	T	T	T	A	T	C	A	C	A	G	G	C	T	C	C	A	G	G	A	A
T	98	98	96	3	96	96	2	3	0	1	1	3	97	2	4	0	2	1	1	0
G	0	0	0	1	0	0	1	0	2	92	98	5	0	0	0	2	93	96	3	2
C	0	1	1	1	0	1	2	94	4	1	1	92	2	97	94	4	1	0	0	0
A	1	1	3	95	4	3	95	3	94	5	0	0	1	1	2	94	4	3	96	98

(SEQ ID NO: 244)

FIG. 134B cont'd

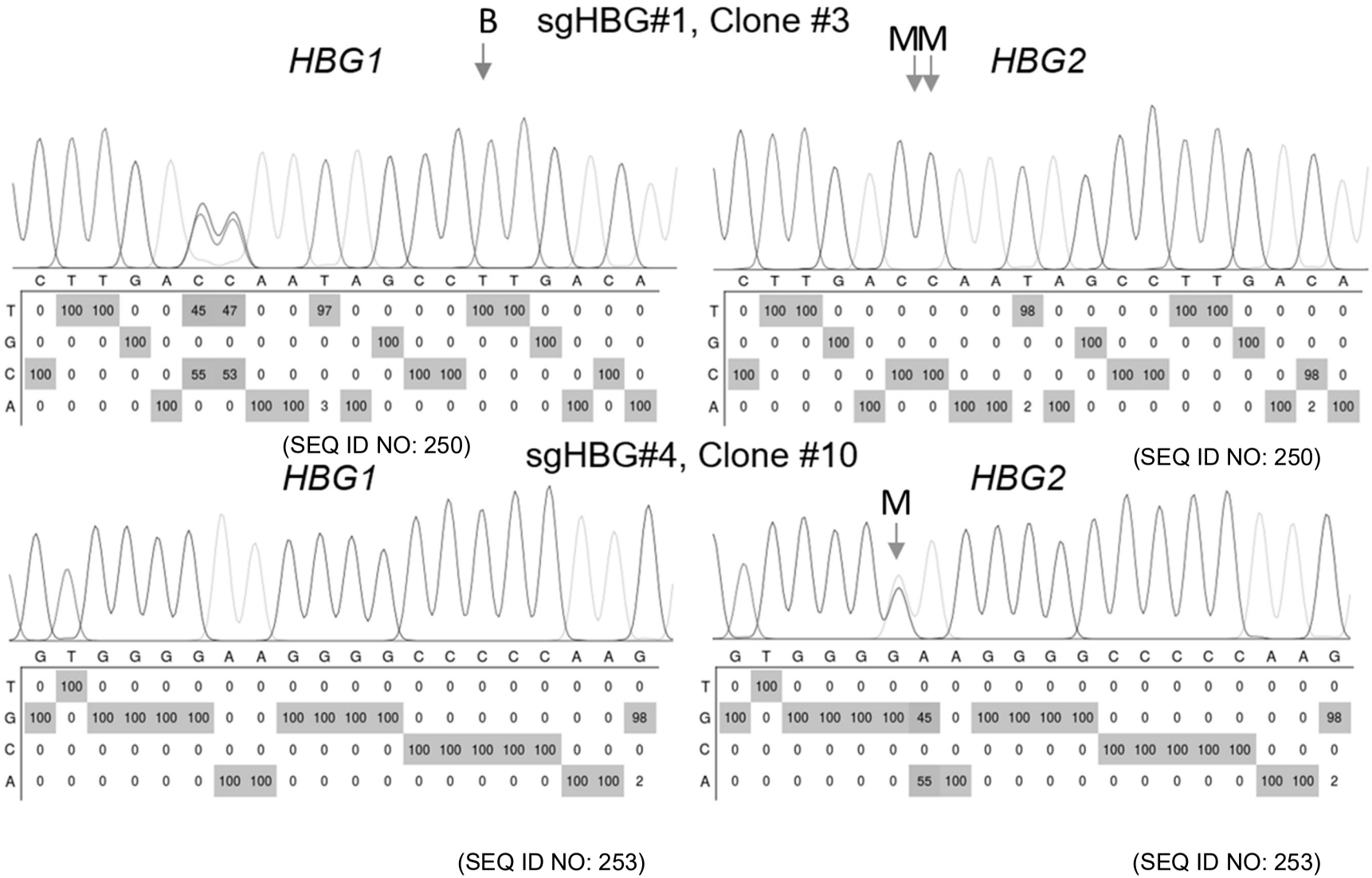


FIG. 134C

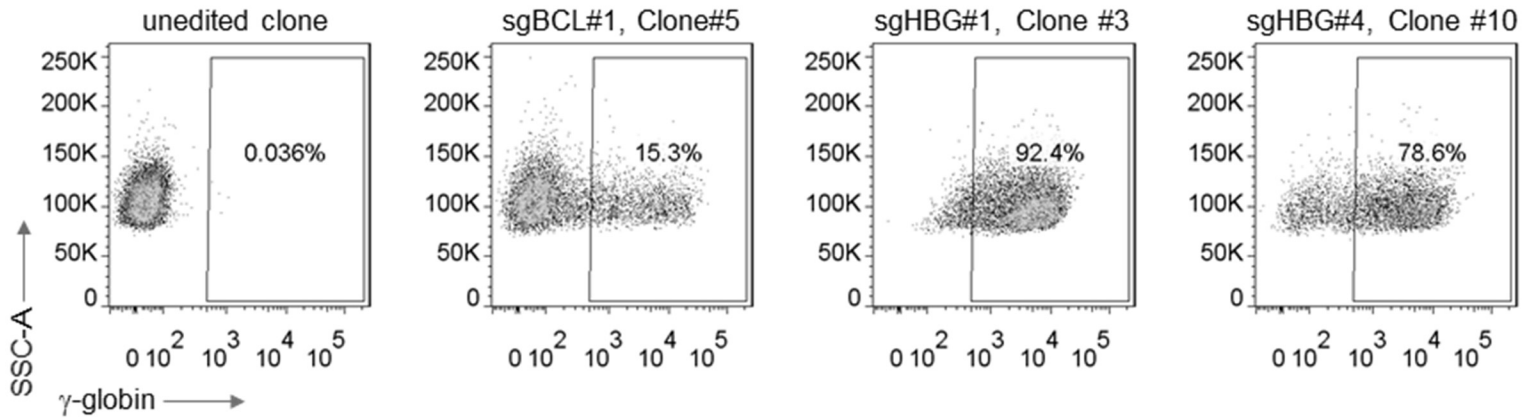


FIG. 135A

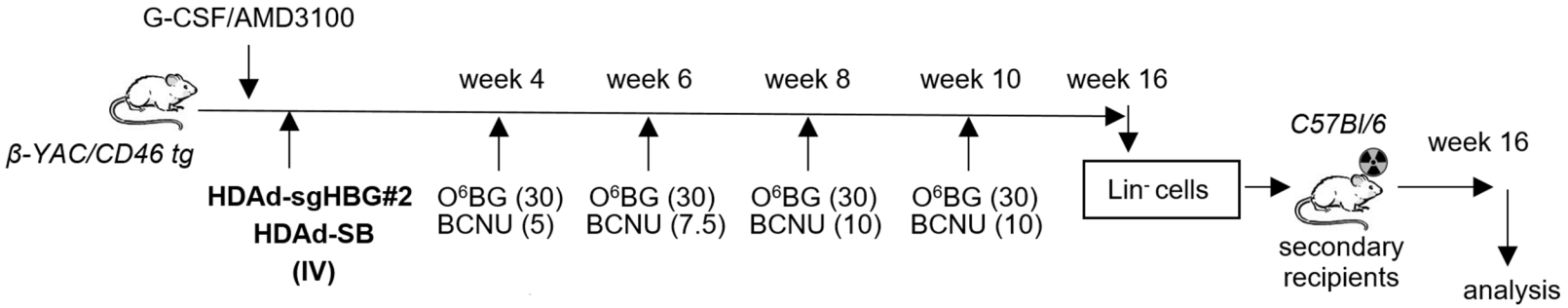


FIG. 135B

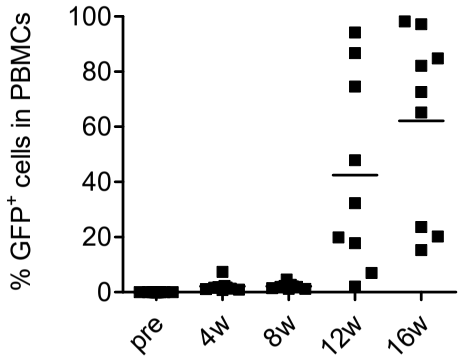


FIG. 135C

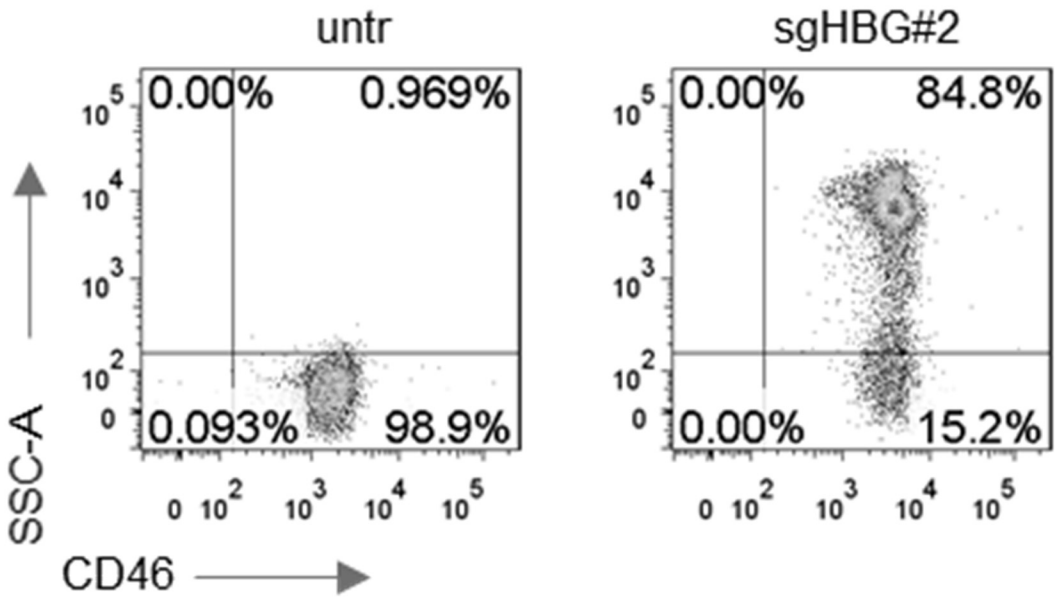


FIG. 135D

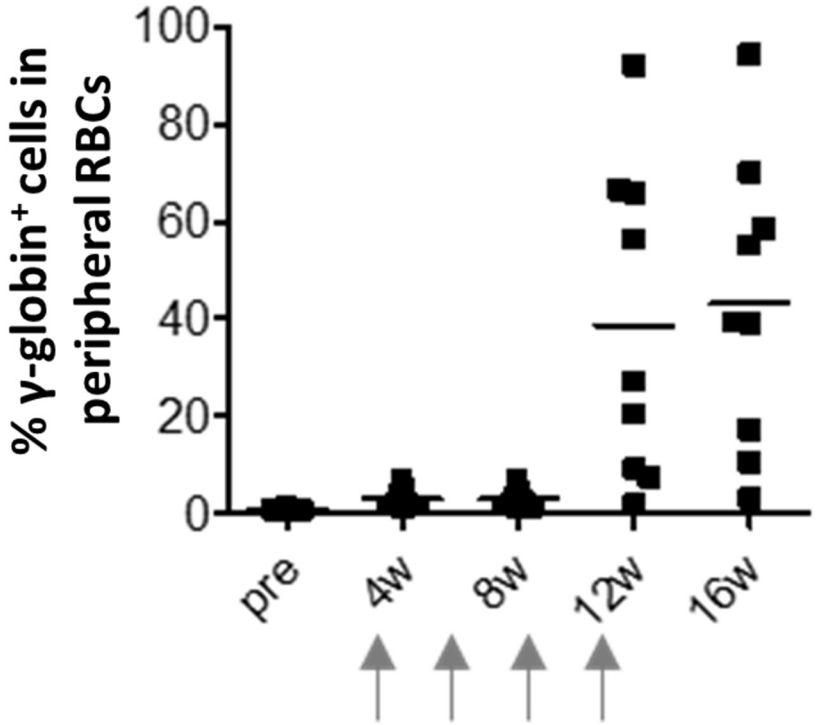


FIG. 135E

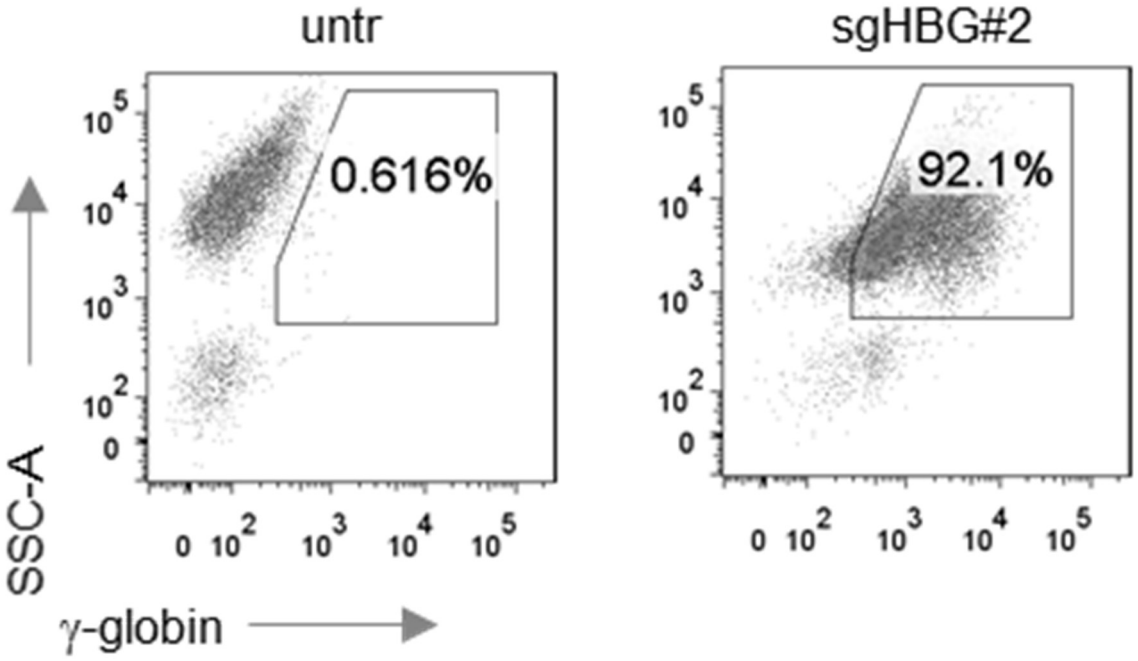


FIG. 135F

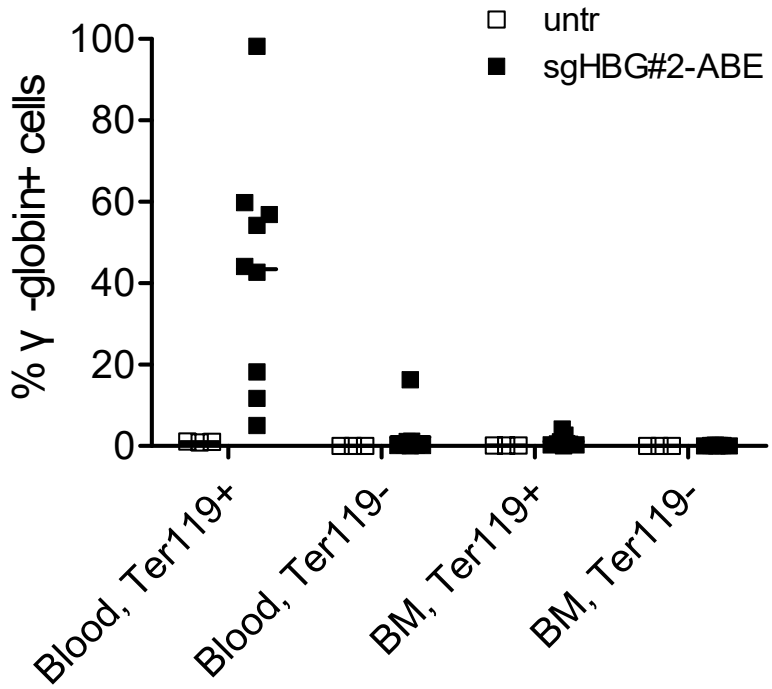


FIG. 135G

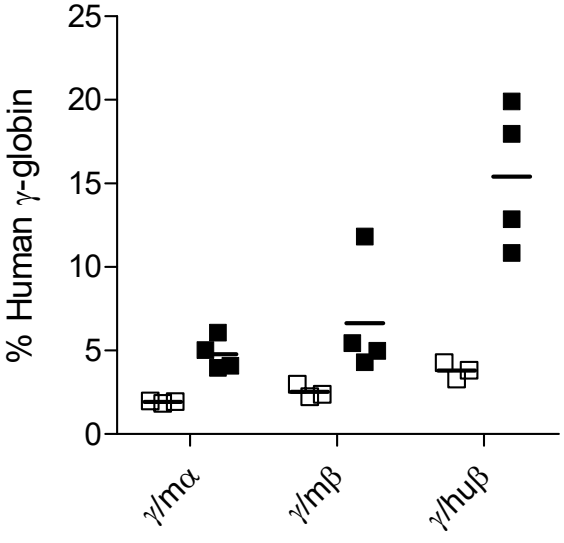


FIG. 135H

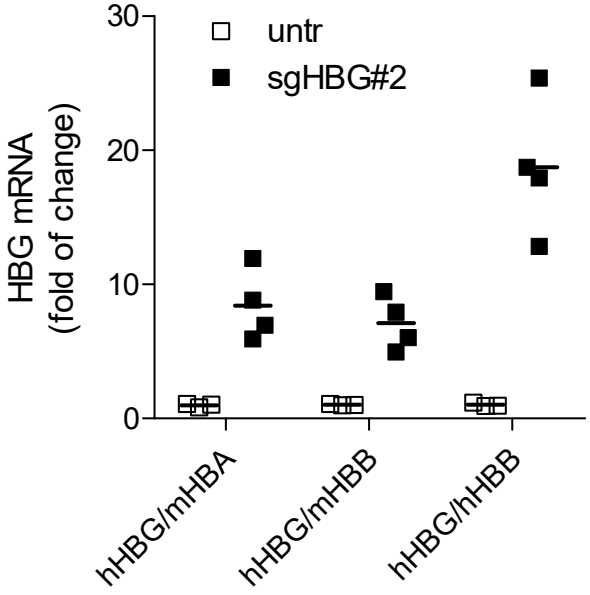


FIG. 135I

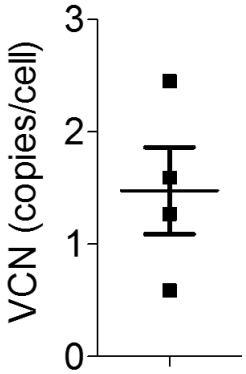


FIG. 136

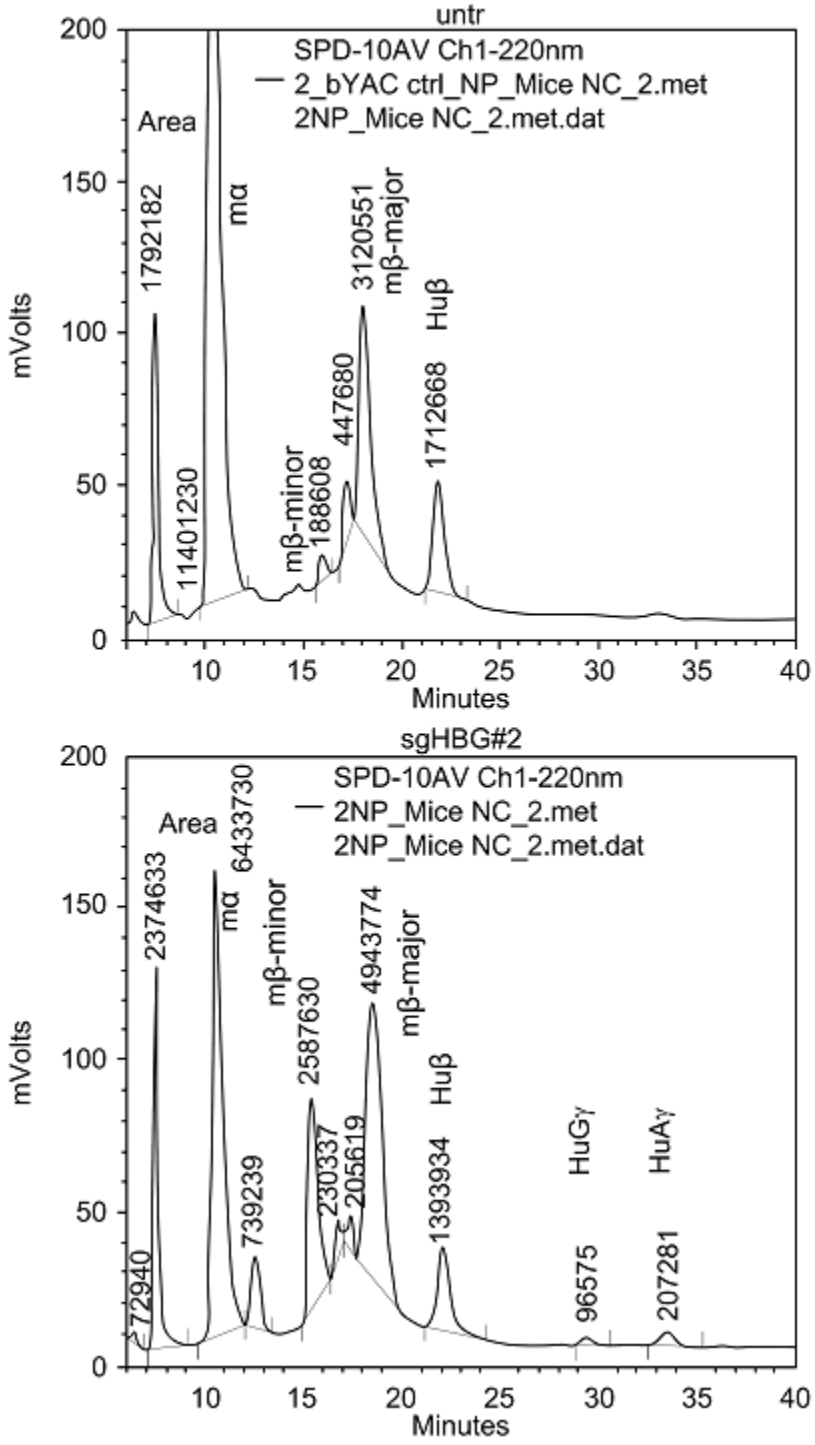
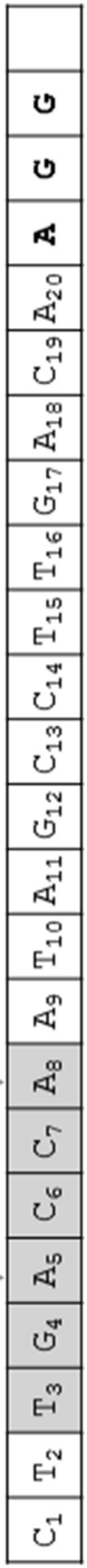

untr
SPD-10AV Ch1-220nm
2_bYAC ctrl_NP_Mice NC_2.met
2NP_Mice NC_2.met.dat
Area
1792182
11401230
mα
mβ-minor
188608
447680
3120551
mβ-major
Huβ
1712668
mVolts
Minutes
sgHBG#2
SPD-10AV Ch1-220nm
2NP_Mice NC_2.met
2NP_Mice NC_2.met.dat
Area
2374633
6433730
mα
mβ-minor
2587630
4943774
mβ-major
Huβ
72940
739239
230337
205619
1393934
HuGγ
HuAγ
96575
207281
mVolts
Minutes

FIG. 137A



(SEQ ID NO: 276)

FIG. 137B

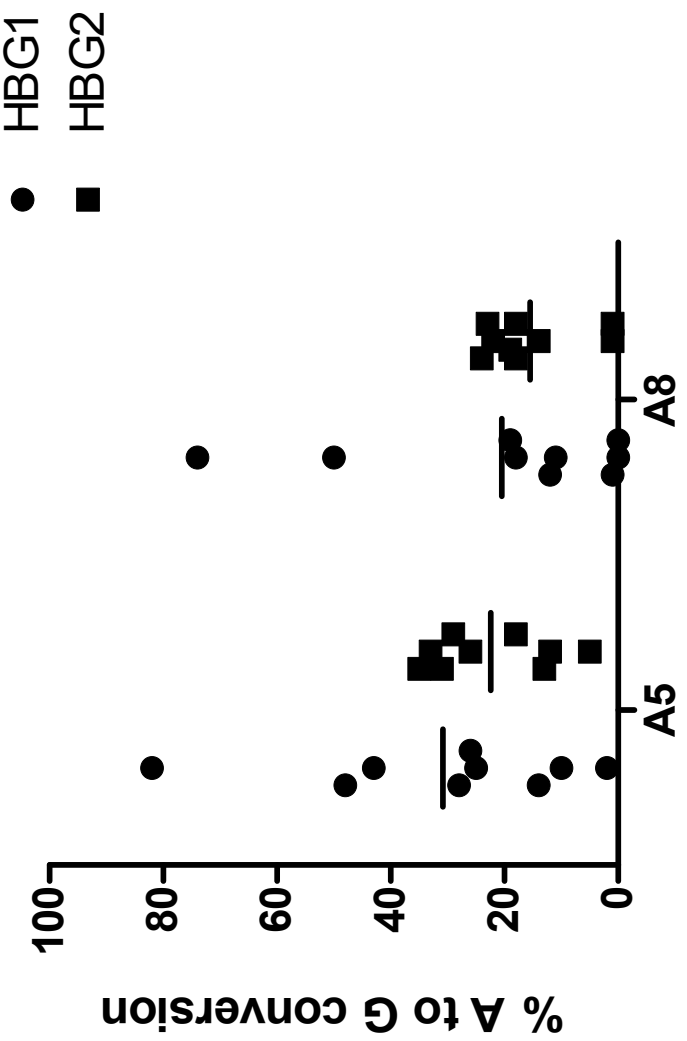
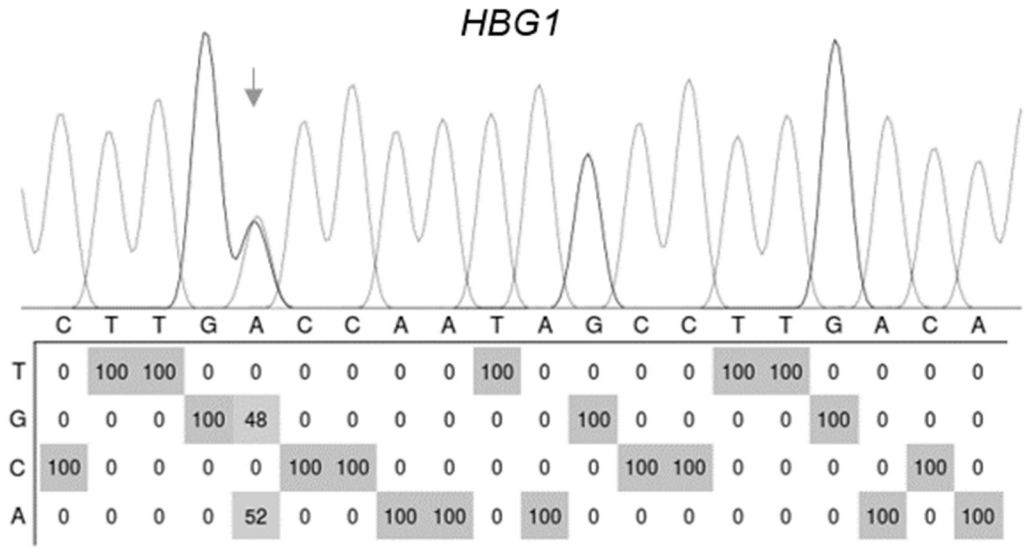
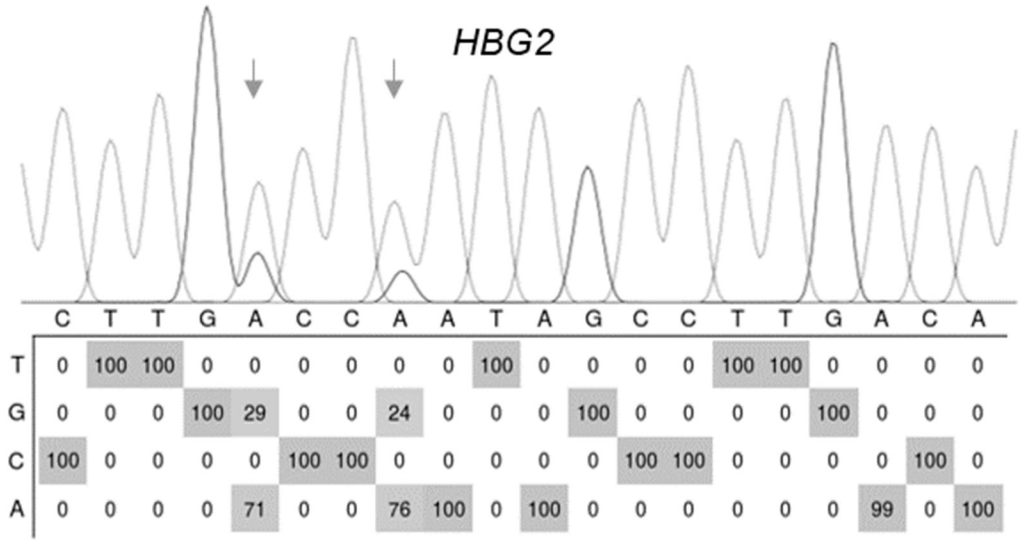


FIG. 137C



(SEQ ID NO: 250)



(SEQ ID NO: 250)

FIG. 137D

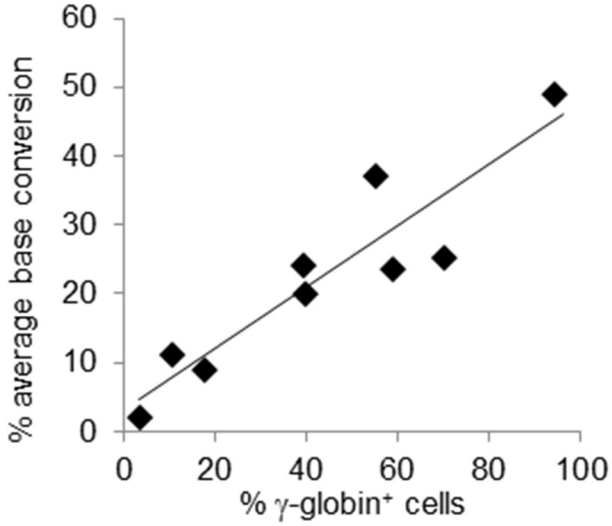


FIG. 137E

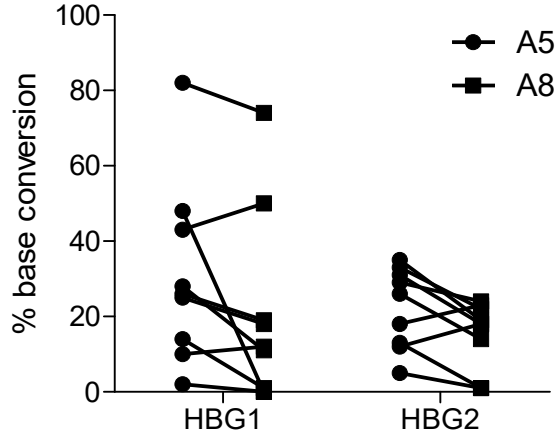


FIG. 137F

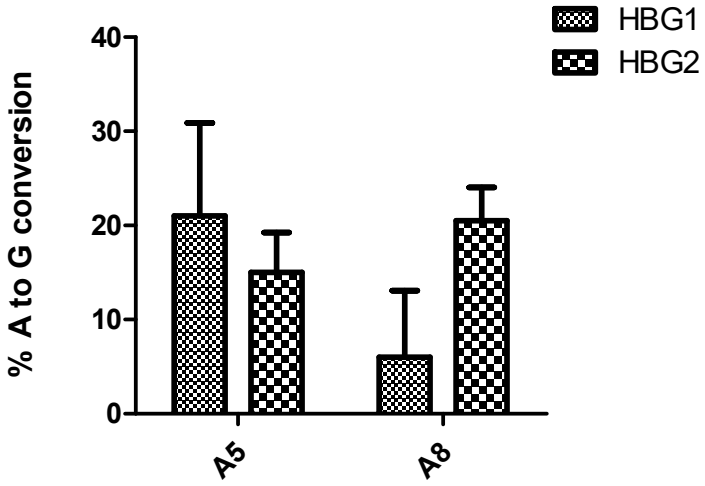


FIG. 137G

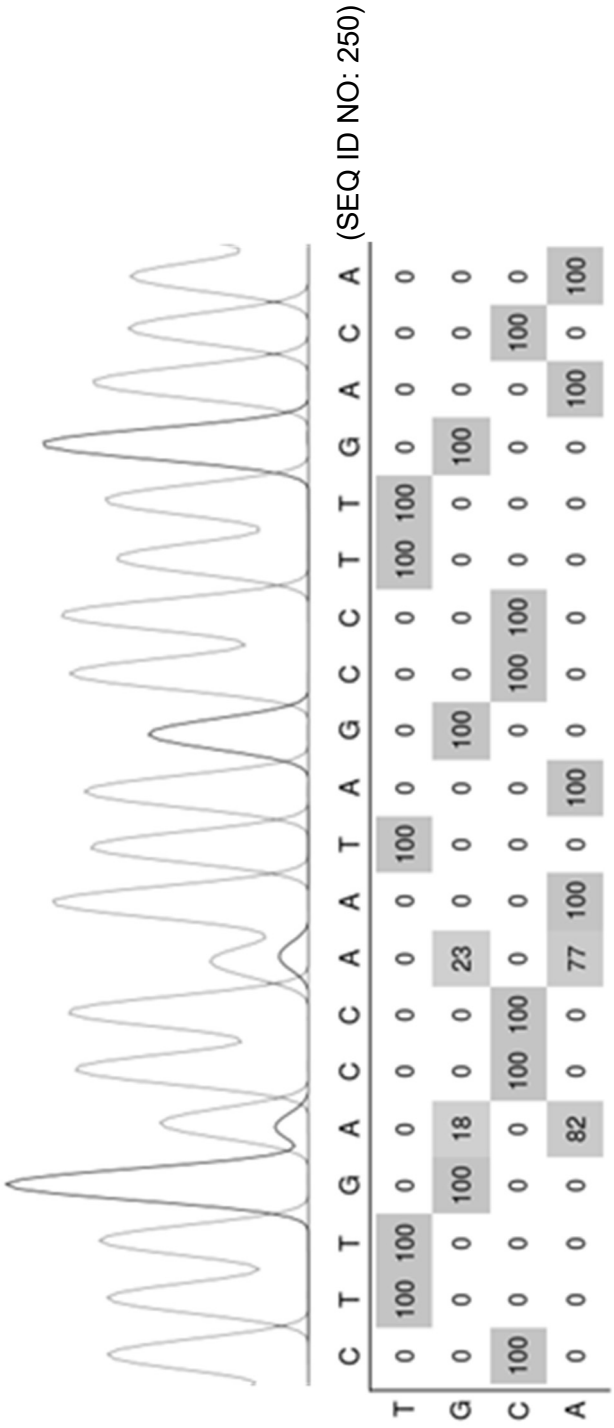

(SEQ ID NO: 250)
C T T G A C C A A T A G C C T T G A C A
T 0 100 100 0 0 0 0 100 0 0 0 100 100 0 0 0 0
G 0 0 0 100 18 0 0 23 0 0 0 100 0 0 0 0 0
C 100 0 0 0 0 100 100 0 0 0 0 0 100 100 0 0 0 100 0
A 0 0 0 0 82 0 0 77 100 0 100 0 0 0 0 0 100 0 100

FIG. 138A

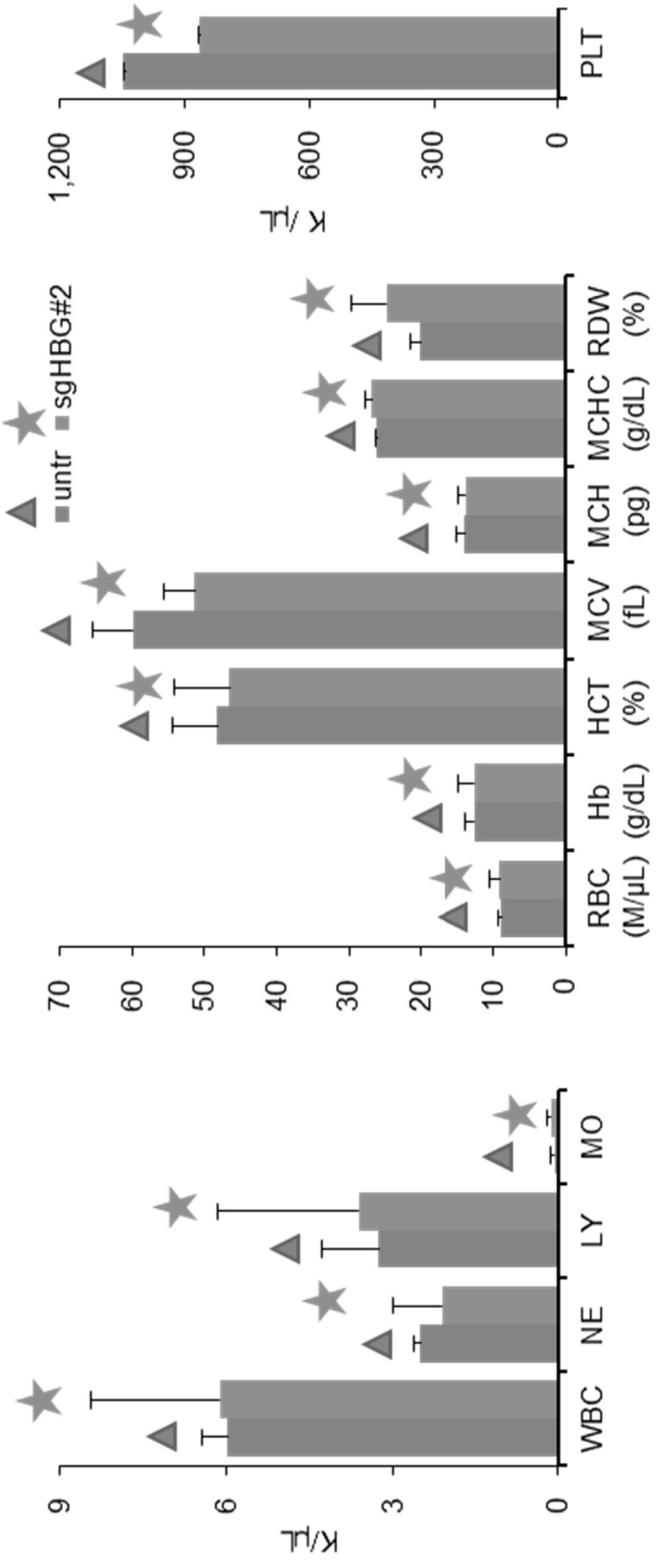

untr
sgHBG#2
K/μL
9
6
3
0
WBC
NE
LY
MO
70
60
50
40
30
20
10
0
RBC (M/μL)
Hb (g/dL)
HCT (%)
MCV (fL)
MCH (pg)
MCHC (g/dL)
RDW (%)
K /μL
1,200
900
600
300
0
PLT

FIG. 138B

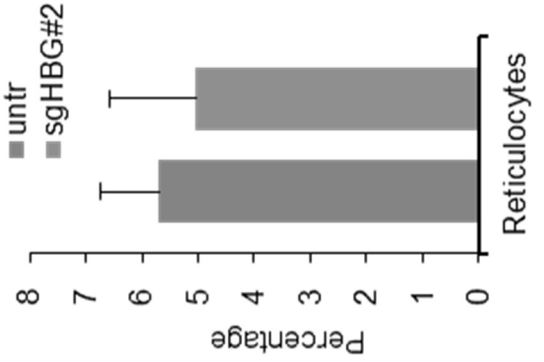


FIG. 138C

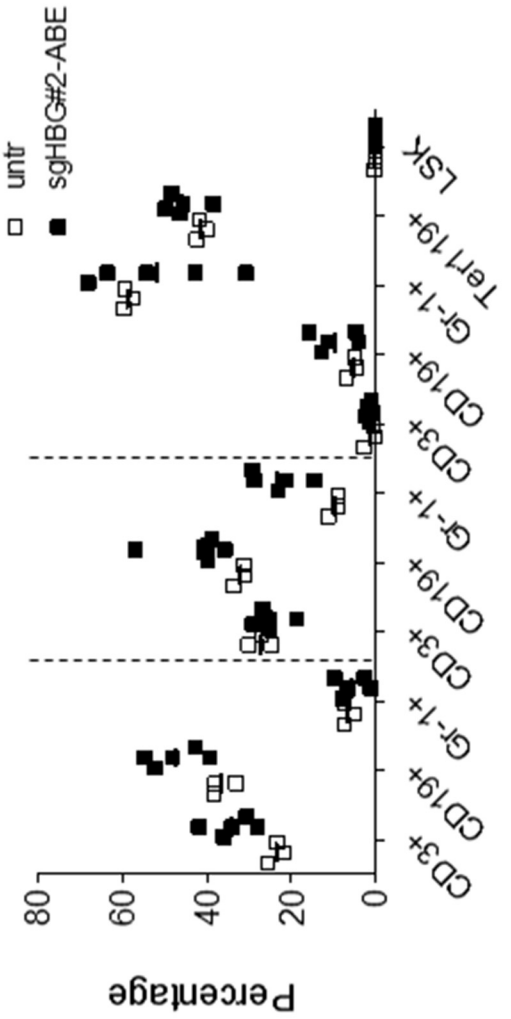


FIG. 138D

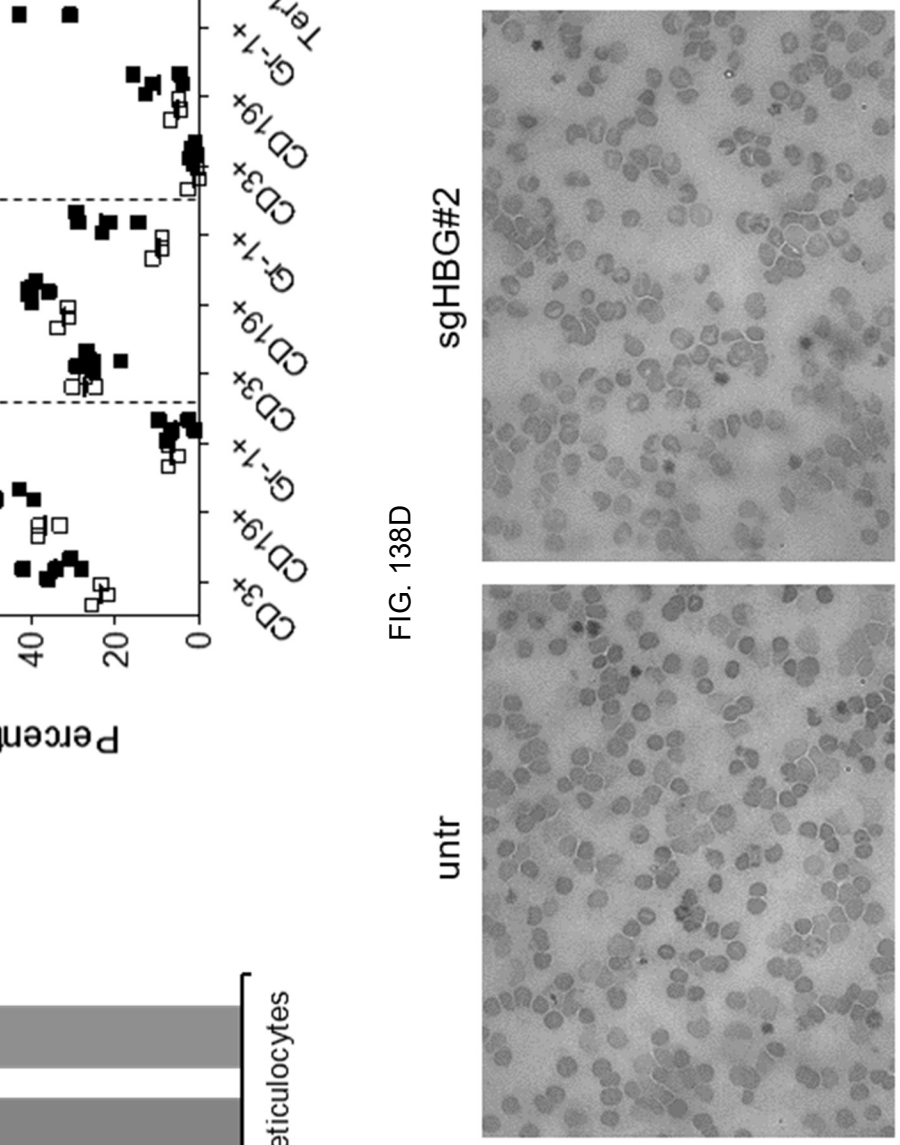


FIG. 139B

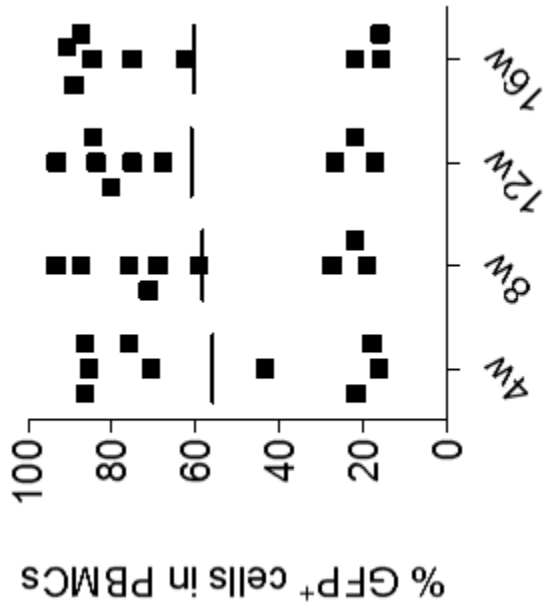


FIG. 139A

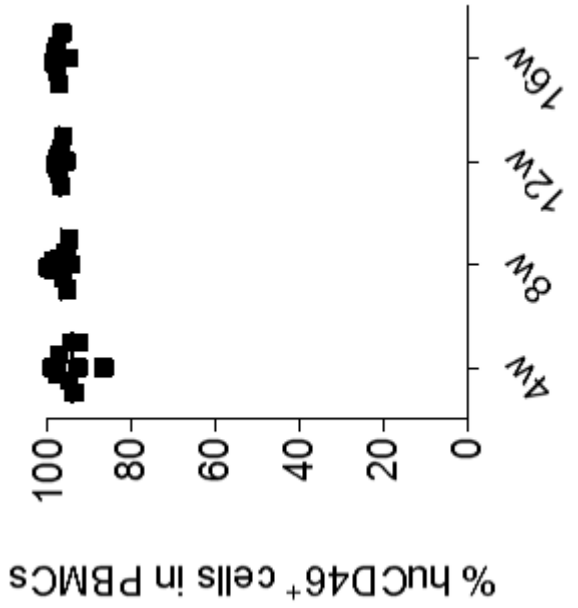


FIG. 139C

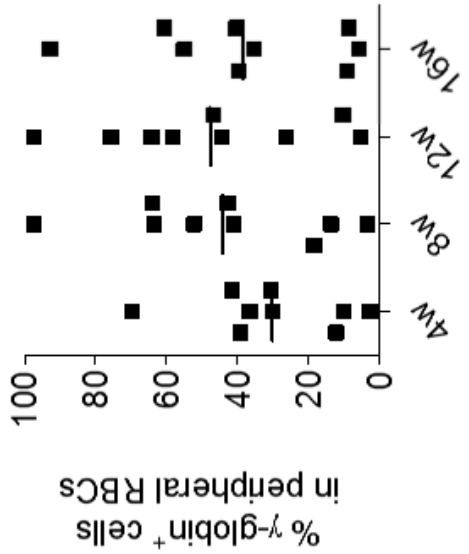


FIG. 140A

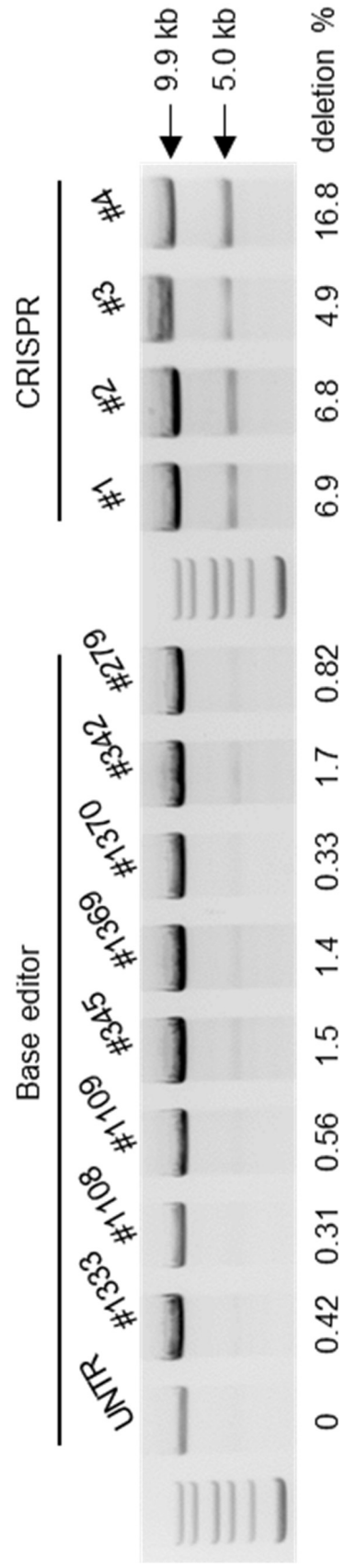


FIG. 140B

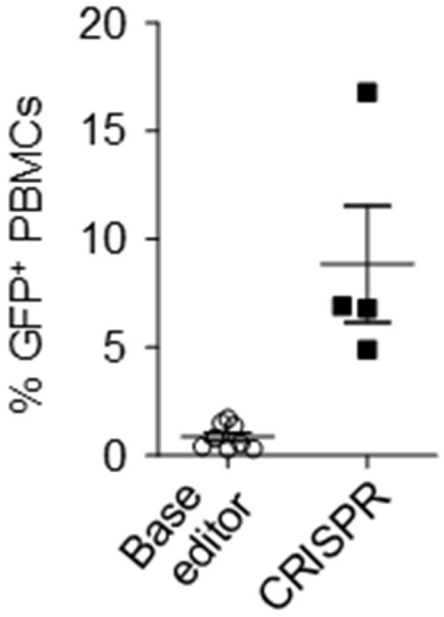


FIG. 141

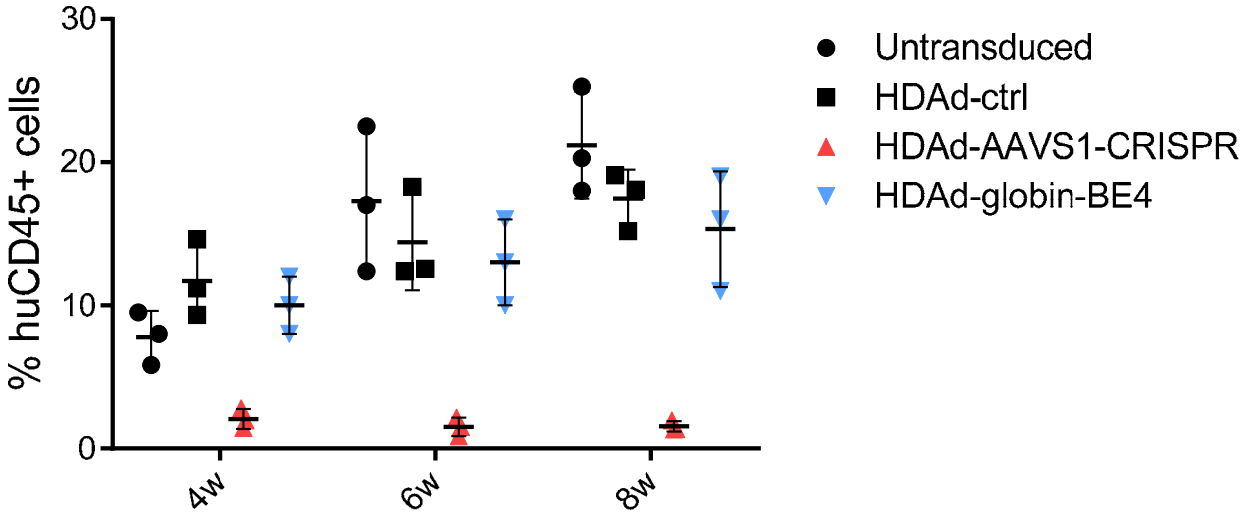


FIG. 142

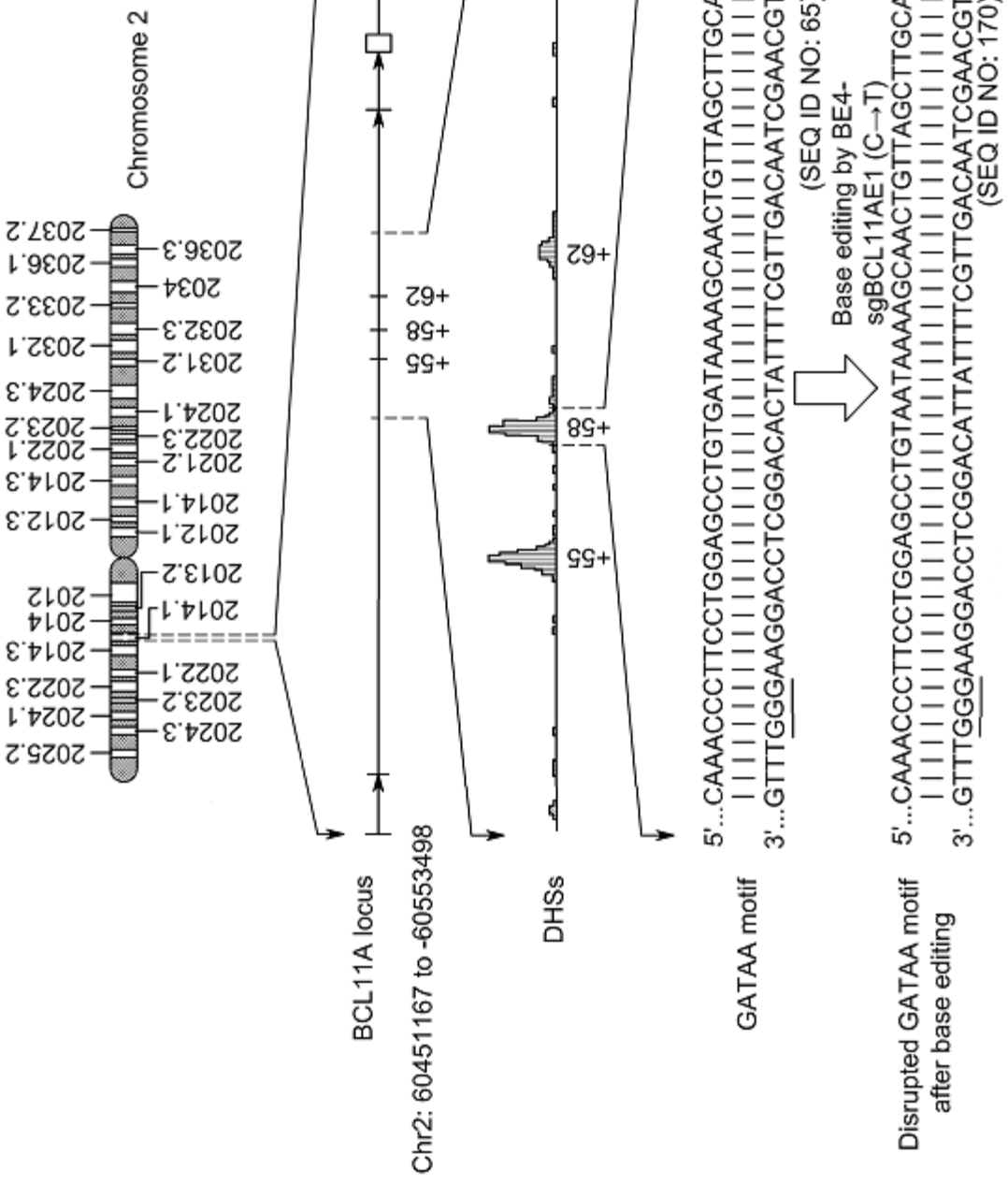

Chromosome 2
2037.2
2036.1
2036.3
2034
2033.2
2032.3
2032.1
2031.2
2024.3
2024.1
2023.2
2022.3
2022.1
2021.2
2014.3
2014.1
2012.3
2012.1
2013.2
2014.1
2012
2014
2014.3
2022.1
2022.3
2023.2
2024.1
2024.3
2025.2
BCL11A locus
Chr2: 60451167 to -60553498
+55
+58
+62
DHSs
+55
+58
+62
GATAA motif
5'...CAAACCCTTCCTGGAGCCTGTGATAAAAGCAACTGTTAGCTTGCACT...3'
3'...GTTTGGGAAGGACCTCGGACACTATTTTCGTTGACAATCGAACGTGA...5'
(SEQ ID NO: 65)
Base editing by BE4-
sgBCL11AE1 (C→T)
Disrupted GATAA motif
after base editing
5'...CAAACCCTTCCTGGAGCCTGTAATAAAAGCAACTGTTAGCTTGCACT...3'
3'...GTTTGGGAAGGACCTCGGACATTATTTTCGTTGACAATCGAACGTGA...5'
(SEQ ID NO: 170)

FIG. 143

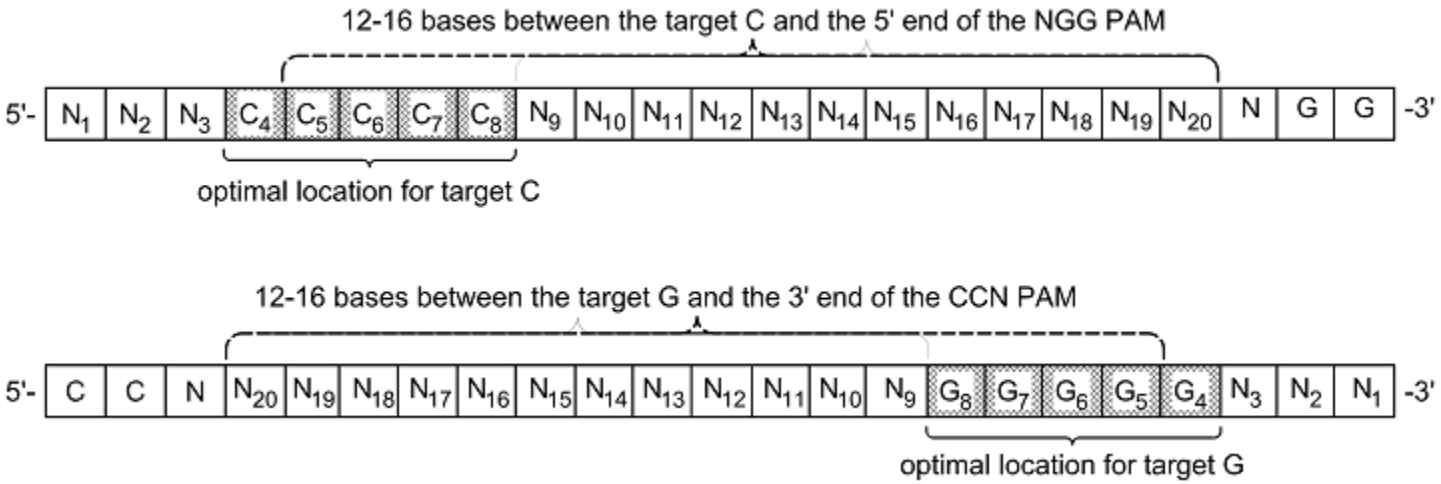

12-16 bases between the target C and the 5' end of the NGG PAM
5'-
N1 N2 N3 C4 C5 C6 C7 C8 N9 N10 N11 N12 N13 N14 N15 N16 N17 N18 N19 N20 N G G
-3'
optimal location for target C
12-16 bases between the target G and the 3' end of the CCN PAM
5'-
C C N N20 N19 N18 N17 N16 N15 N14 N13 N12 N11 N10 N9 G8 G7 G6 G5 G4 N3 N2 N1
-3'
optimal location for target G

FIG. 144

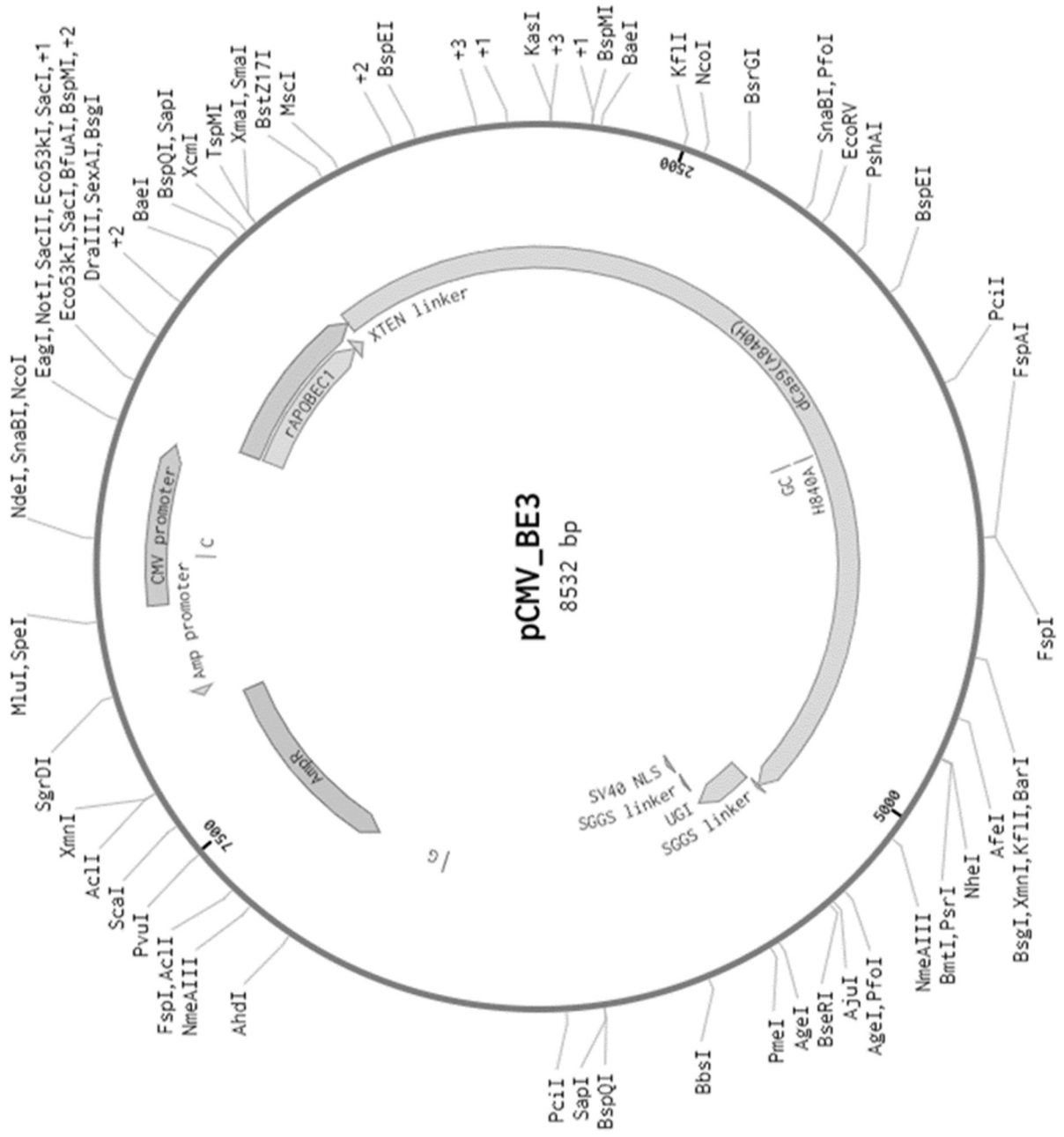
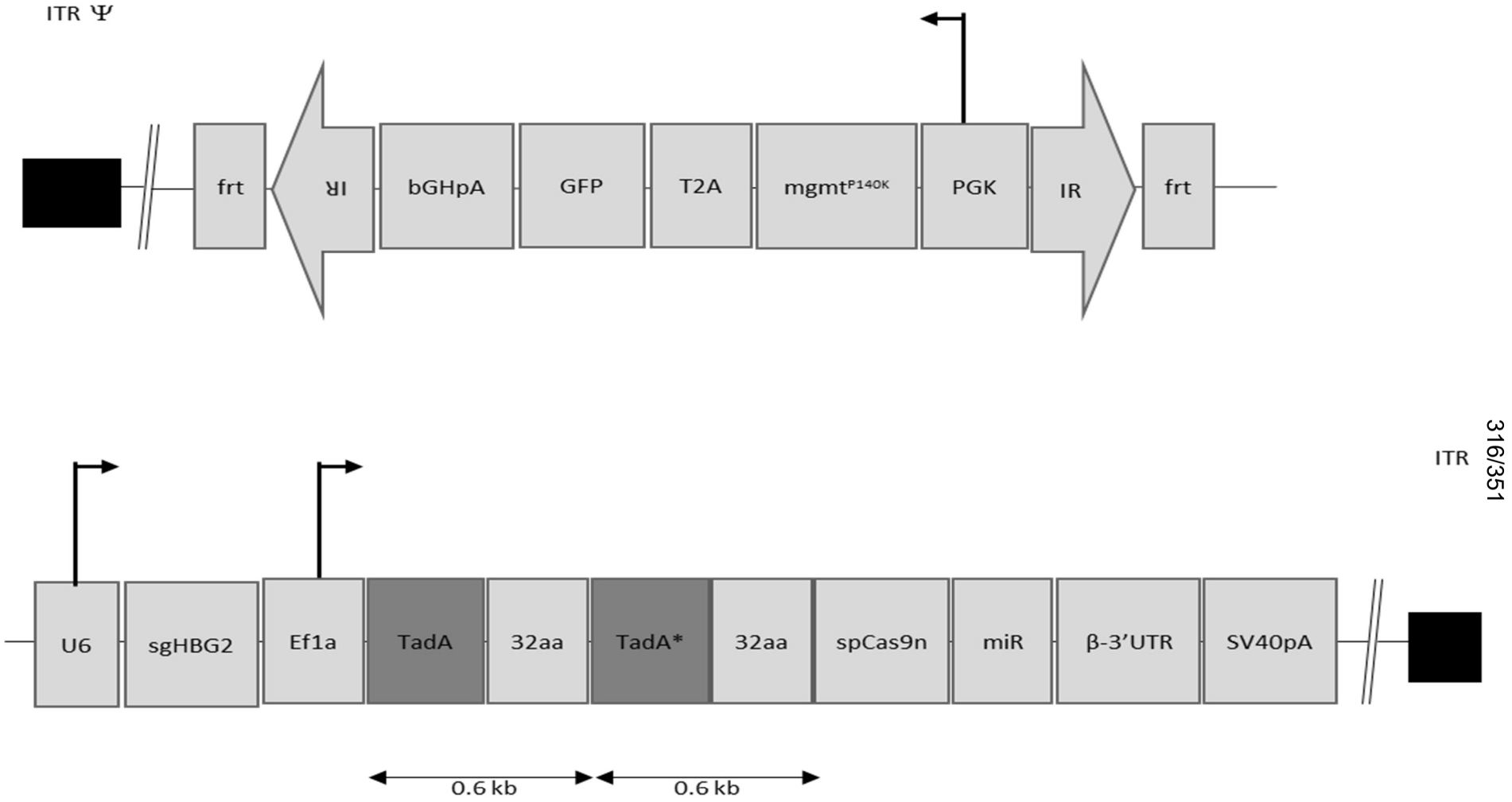

pCMV_BE3
8532 bp
CMV promoter
T7 promoter
rAPOBEC1
XTEN linker
dCas9 (A840H)
nCas9 (A840H)
GC
H840A
SV40 NLS
SGGS linker
UGI
SGGS linker
AmpR
2500
5000
7500
NdeI, SnaBI, NcoI
EagI, NotI, SacII, Eco53kI, SacI, +1
Eco53kI, SacI, BfuAI, BspMI, +2
DraIII, SexAI, BsgI
+2
BaeI
BspQI, SapI
XcmI
TspMI
XmaI, SmaI
BstZ17I
MscI
+2
BspEI
+3
+1
KasI
+3
+1
BspMI
BaeI
KflI
NcoI
BsrGI
SnaBI, PfoI
EcoRV
PshAI
BspEI
PciI
FspAI
FspI
BsgI, XmnI, KflI, BarI
AfeI
NheI
BmtI, PsrI
NmeAIII
AjuI
AgeI, PfoI
BseRI
AgeI
PmeI
BbsI
PciI
SapI
BspQI
AhdI
NmeAIII
FspI, AclI
PvuI
ScaI
AclI
XmnI
SgrDI
MluI, SpeI

FIG. 145



TadA (wild type), to maintain stability

TadA* (envolved): responsible for editing

FIG. 146

```
     1 SEVEFSHEYWMRHALTLAKRAWDEREVPVGAVLVHNNRVIGEGWNRPIGRHDPTAHAEIMALRQGGLVMQNYRLIDATLYVTLEPCVMCAGAMIHSRIGRVVFGARDAKTGAAGSLMDVL
TadA*1 .....R.....L.....A.L.....F.....V.N.....

   361 HHPGMNHRVEITEGILADECAALLSDFFRMRRQEIKAQKKAQSSTDSGGSSGGSSGSETPGTSESATPESSGGSSGGS
   361 .Y.....CY...P..VFN.....
```

(SEQ ID NO: 265)

(SEQ ID NO: 266)

DNA sequence of Two 'TadA + 32aa'

```
TadA  1 tctgaagtcgagtttagccacgagtattggatgaggcacgcactgaccctggcaaagcgagcatgggatgaaagagaagtccccgtgggcgccgtgctggtgcacaacaatagagtgatc
TadA*1 .....g..g.....tc.....c.....a..t..c.....c...a.g...c.c.....g..g..g..g..t.....a.....tg.....

   121 ggagagggatggaacaggccaatcggccgccacgaccctaccgcacacgcagagatcatggcactgaggcagggaggcctggtcatgcagaattaccgcctgatcgatgccaccctgtat
   121 ..c.....c.....ag.c.....tg.....a..a..c..t..c..a..t.....c.....a.....c.....c...a.a.....t..c.....c

   241 gtgacactggagccatgcgtgatgtgcgcaggagcaatgatccacagcaggatcggaagagtggtgttcggagcacgggacgccaagaccggcgcagcaggctccctgatggatgtgctg
   241 ......t.c.....t.....c..c..c.....tct.....cc.c.....t..c.tga..a...a..a.....c.....c.....

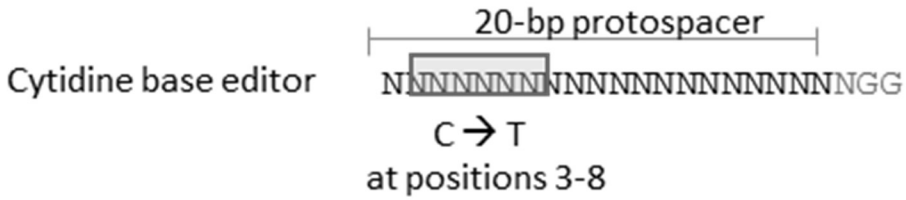
   361 caccaccccggcatgaaccaccgggtggagatcacagagggaatcctggcagacgagtgcgccgccctgctgagcgatttctttagaatgcggagacaggagatcaaggcccagaagaag
   361 ...t.....t.....c..c..a..t..c.....t..a..t.....t..t.....c.g....ct.....t.t....t..t.....

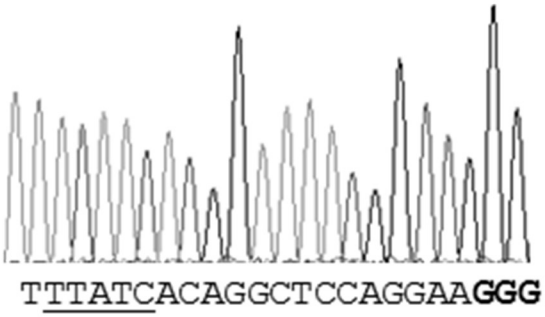
   481 gcacagagctccaccgactctggaggatctagcggaggatcctctggaagcgagacaccaggcacaagcgagtccgccacaccagagagctccggcggctcctccggaggatcc
   481 ..c.....c.....c.....ctct.....t.....ag...a.....t..a...ag...g...ag.ag...g..g..a
```

(SEQ ID NO: 267)

(SEQ ID NO: 268)

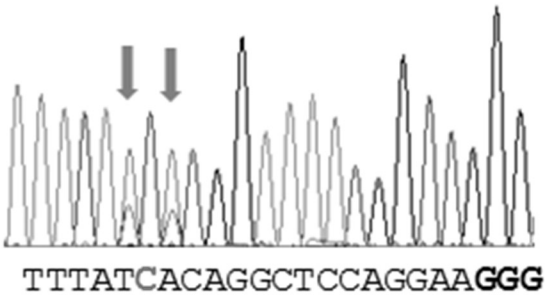
FIG. 147





Wild type sequence
Underlined: GATAA motif
Bold: PAM motif

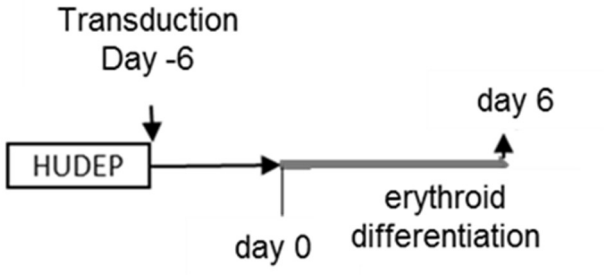
(SEQ ID NO: 269)

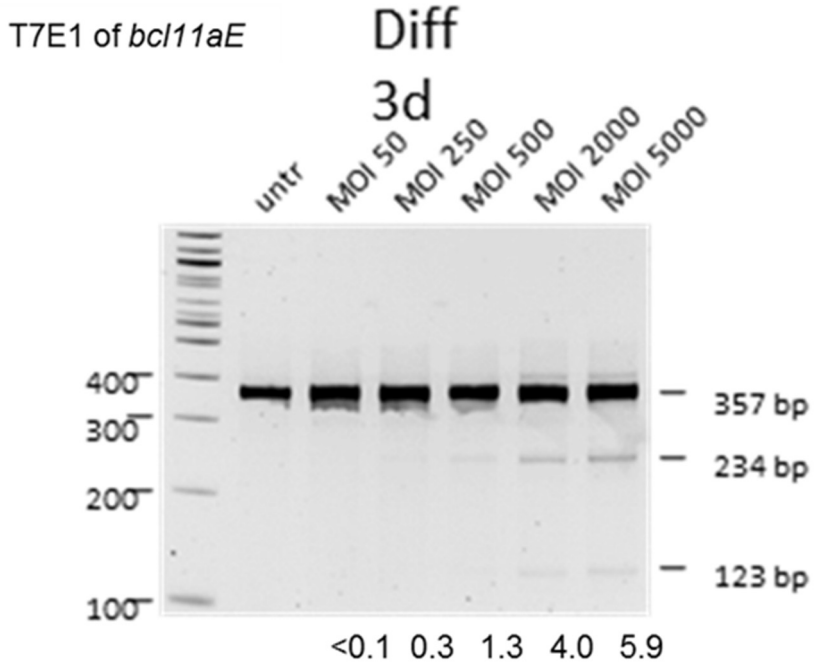


Base edited sequence
Arrows: edited bases
Purple: disrupted base of GATAA motif

(SEQ ID NO: 269)

FIG. 148


Transduction
Day -6
HUDEP
day 6
erythroid
differentiation
day 0


T7E1 of bcl11aE
Diff
3d
untr
MOI 50
MOI 250
MOI 500
MOI 2000
MOI 5000
400
300
200
100
357 bp
234 bp
123 bp
<0.1 0.3 1.3 4.0 5.9

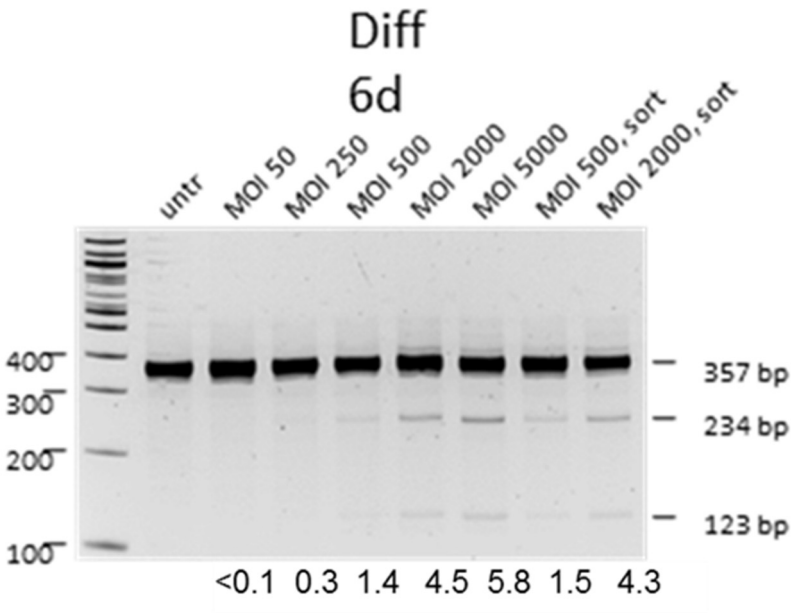

Diff
6d
untr
MOI 50
MOI 250
MOI 500
MOI 2000
MOI 5000
MOI 500, sort
MOI 2000, sort
400
300
200
100
357 bp
234 bp
123 bp
<0.1 0.3 1.4 4.5 5.8 1.5 4.3

FIG. 149

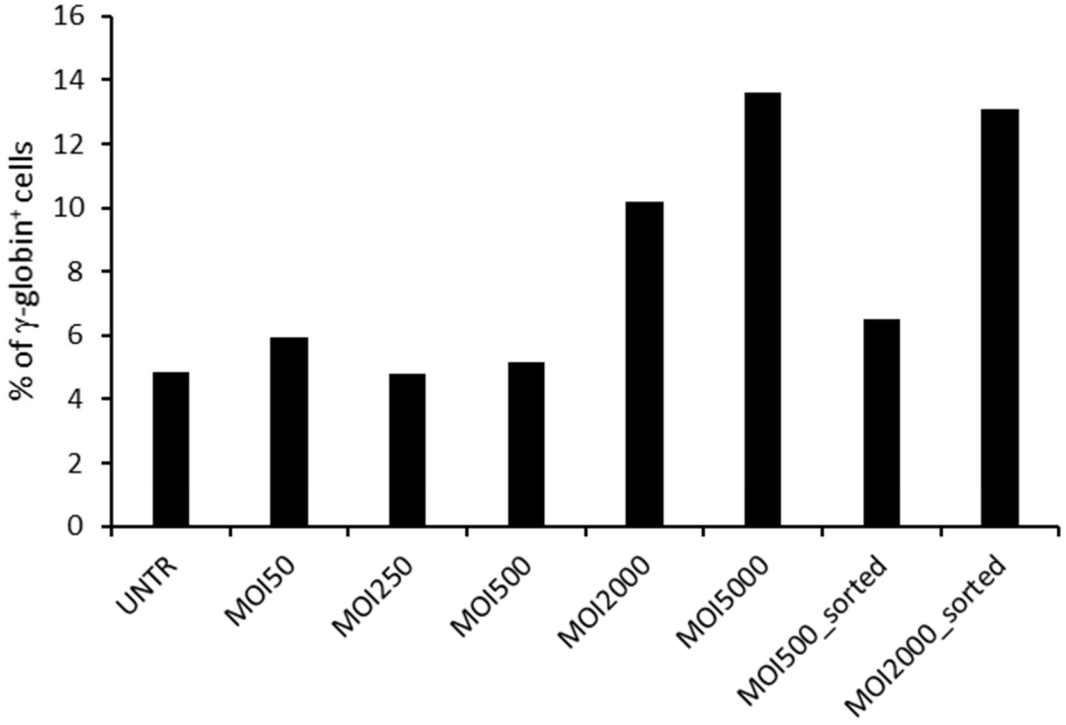


FIG. 150

Reactivation of HbF by Base editing

Cytidine base editor: C → T
Adenine base editor: A → G

Constructed vectors:

pBS-AncBE4max-sgBCL11AE1	+58 GATA motif, G --> A
pBS-AncBE4max-sgBCL11AE2	+58 GATA motif, G --> A
pBS-ABEmax-sgBCL11AE3	+58 GATA motif, T --> C
pBS-ABEmax-sgBCL11AE4	+58 GATA motif, T --> C
pBS-AncBE4max-sgHBG1	13bp deletion, CCAAT box, CC --> TT
pBS-ABEmax-sgHBG2	13bp deletion, CCAAT box, A --> G
pBS-ABEmax-sgHBG3	13bp deletion, CCAAT box, T --> C
pBS-ABEmax-sgHBG4	-198 T --> C
pBS-AncBE4max-sgR5-1	control

FIG. 151

	Date	Full name	Titer	Note
	101918-3	HDAd5/35++_BE4max-sgBCL11Ae1-FI-mgmtGFP	0.5 x 10e12	
→	041619-2	HDAd5/35++_BE4max-sgHBG1-FI-mgmtGFP	very low (use as 0.05 x 10e12)	
→	032819-1	HDAd5/35++_ABEopti-sgHBG2-FI-mgmtGFP	2.8 x 10e12	
→	041819-1	HDAd5/35++_ABEopti-sgHBG4-FI-mgmtGFP	1.52 x 10e12	
→	041619-3	HDAd5/35++_BE4max-Dual1-FI-mgmtGFP	very low (use as 0.05 x 10e12)	sgBcl1 + sgHBG1
	041619-1	HDAd5/35++_ABEopti-Dual-skip-FI-mgmtGFP	2.5 x 10e12	sgHBG2 + sgHBB-Stop
	041819-2	HDAd5/35++_BE4max-sgR5-1-FI-mgmtGFP	0.9 x 10e12	Non-targeting ctrl

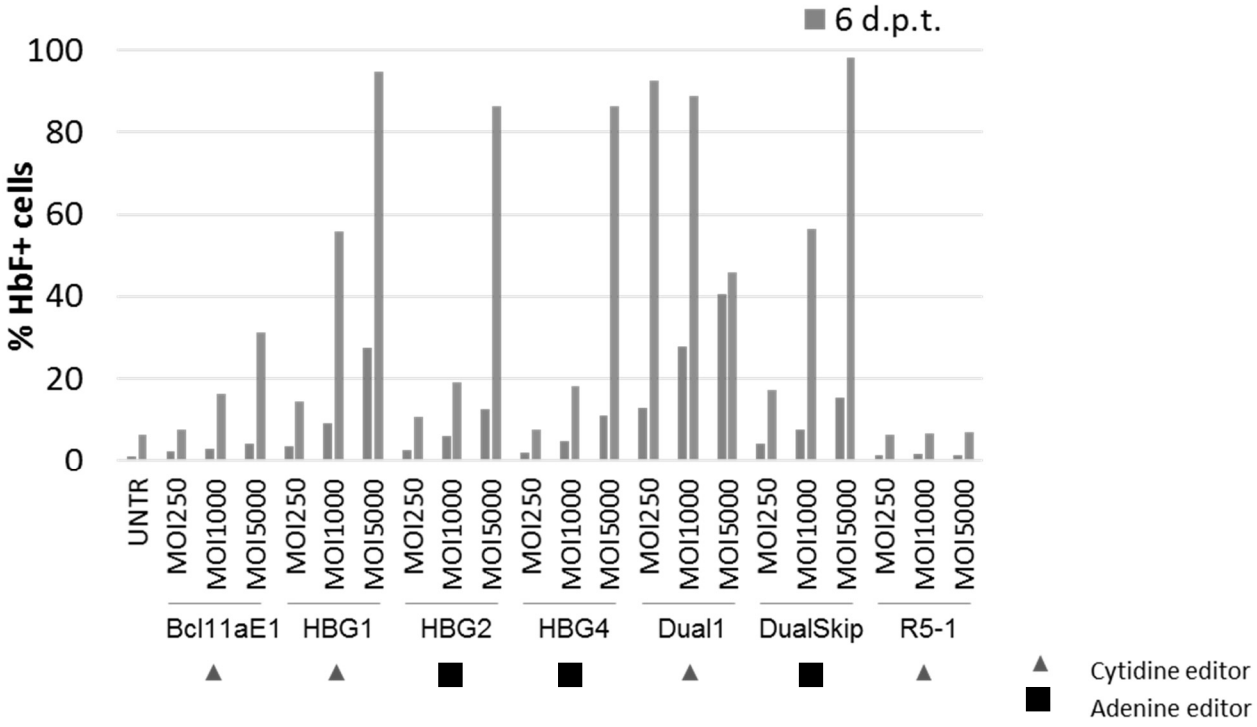


FIG. 152

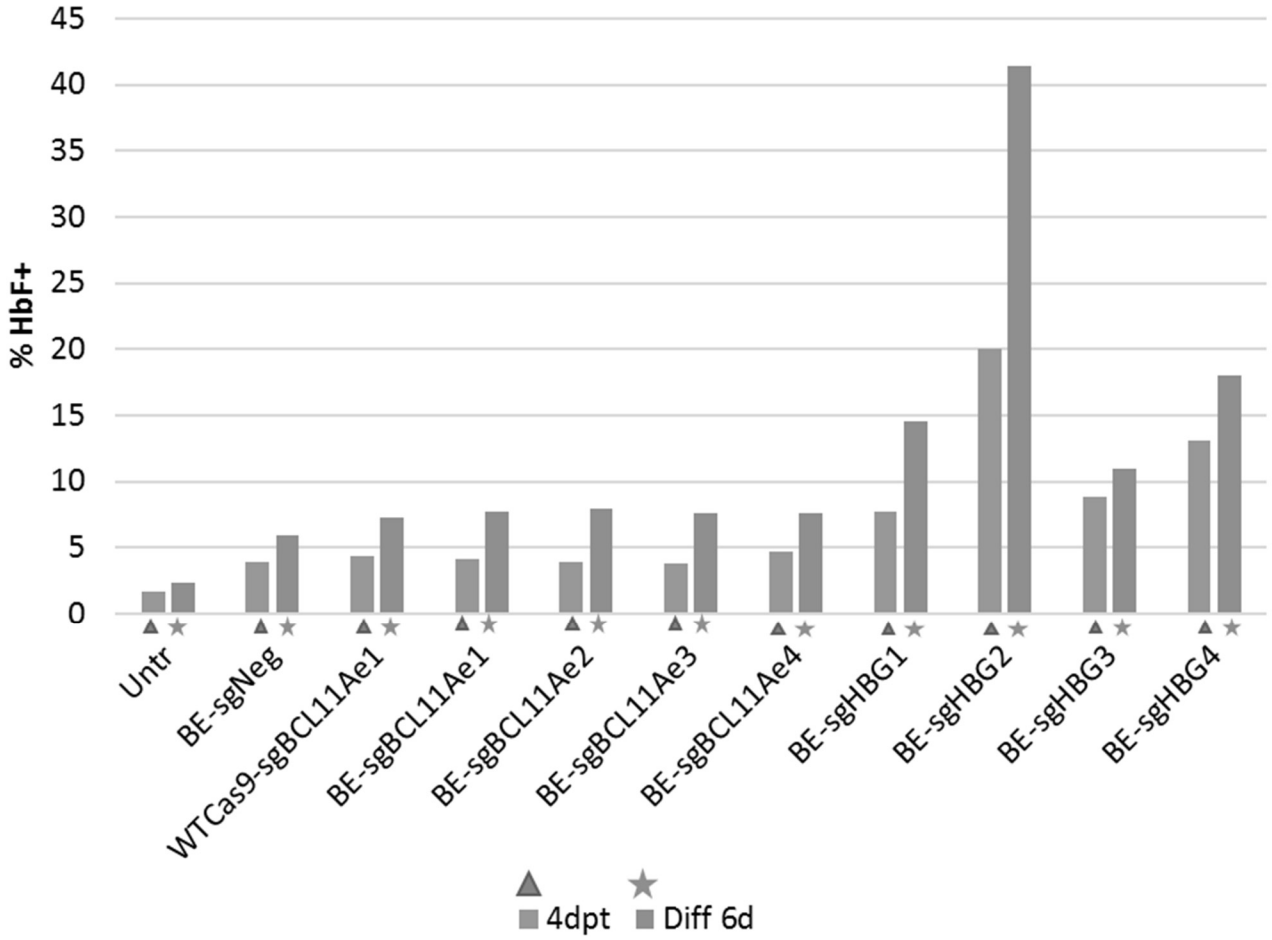

45
40
35
30
25
20
15
10
5
0
% HbF+
Untr
BE-sgNeg
WTCas9-sgBCL11Ae1
BE-sgBCl11Ae1
BE-sgBCL11Ae2
BE-sgBCL11Ae3
BE-sgBCL11Ae4
BE-sgHBG1
BE-sgHBG2
BE-sgHBG3
BE-sgHBG4
4dpt
Diff 6d

FIG. 153

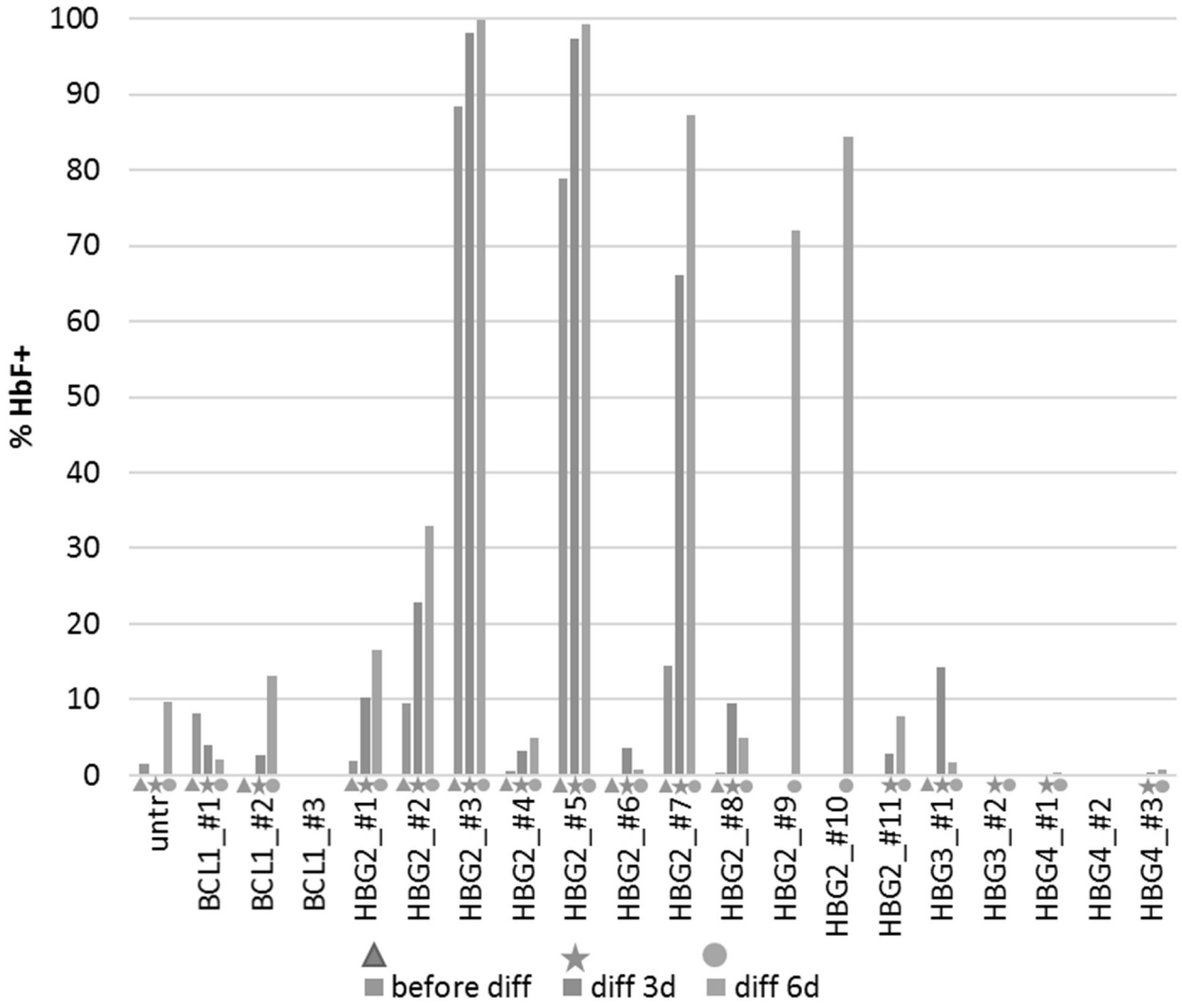
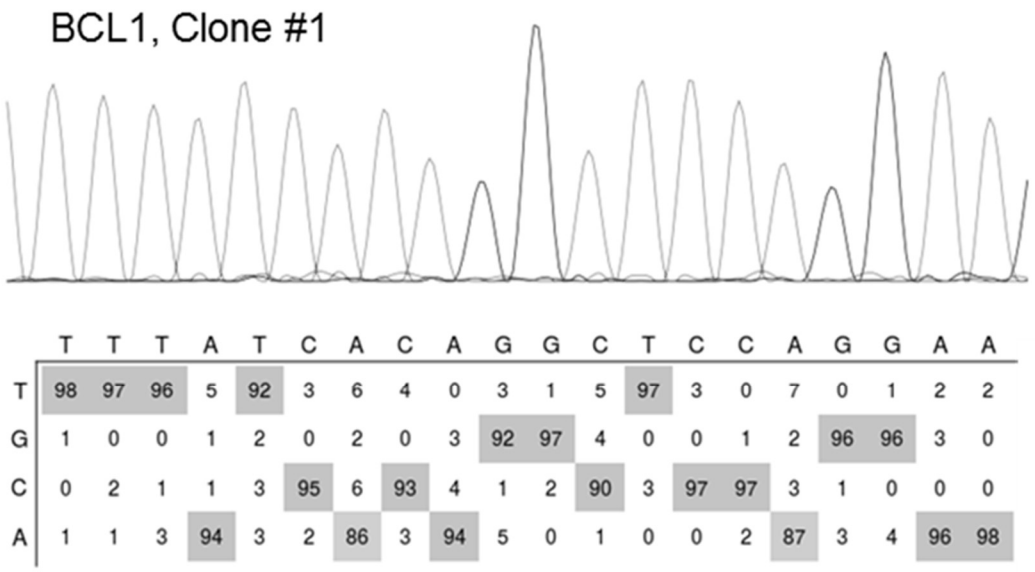
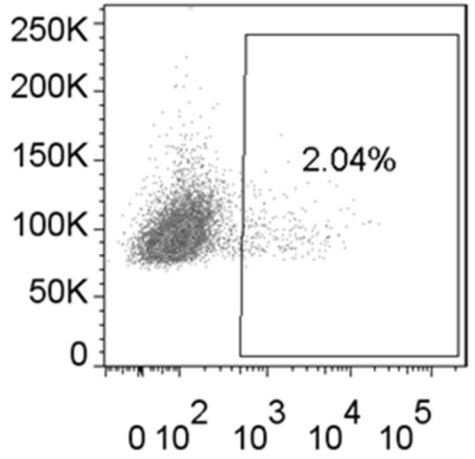

100
90
80
70
60
50
40
30
20
10
0
% HbF+
untr
BCL1_#1
BCL1_#2
BCL1_#3
HBG2_#1
HBG2_#2
HBG2_#3
HBG2_#4
HBG2_#5
HBG2_#6
HBG2_#7
HBG2_#8
HBG2_#9
HBG2_#10
HBG2_#11
HBG3_#1
HBG3_#2
HBG4_#1
HBG4_#2
HBG4_#3
before diff
diff 3d
diff 6d

FIG. 154A

Single cell clones



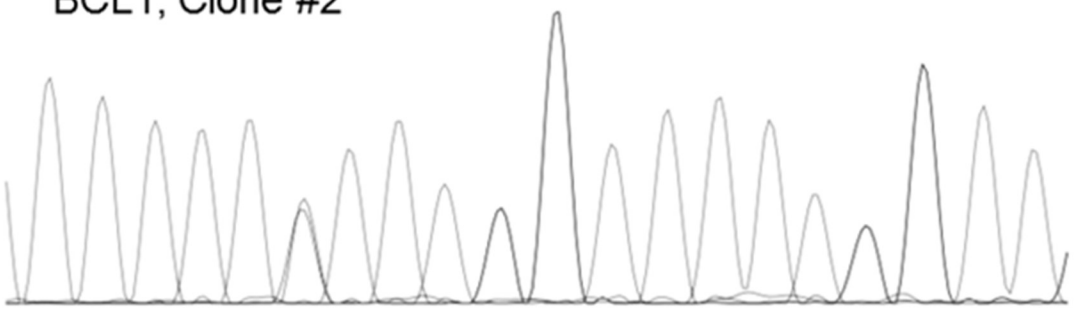


(SEQ ID NO: 271)

FIG. 154B

Single cell clones

BCL1, Clone #2



	T	T	T	A	T	C	A	C	A	G	G	C	T	C	C	A	G	G	A	A
T	98	98	97	4	96	52	1	2	5	0	1	4	97	3	6	0	0	1	1	2
G	0	0	0	1	0	0	0	1	1	93	97	3	0	0	1	1	96	95	3	2
C	1	2	2	0	1	47	3	95	4	2	1	92	3	94	92	7	1	1	0	0
A	1	0	2	95	3	1	96	3	90	4	1	1	0	2	1	92	3	4	96	96

(SEQ ID NO: 271)

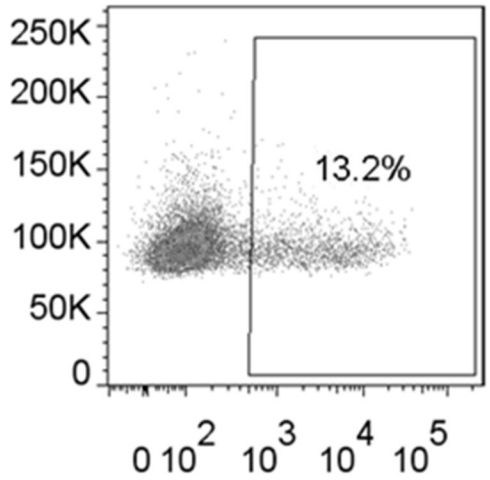
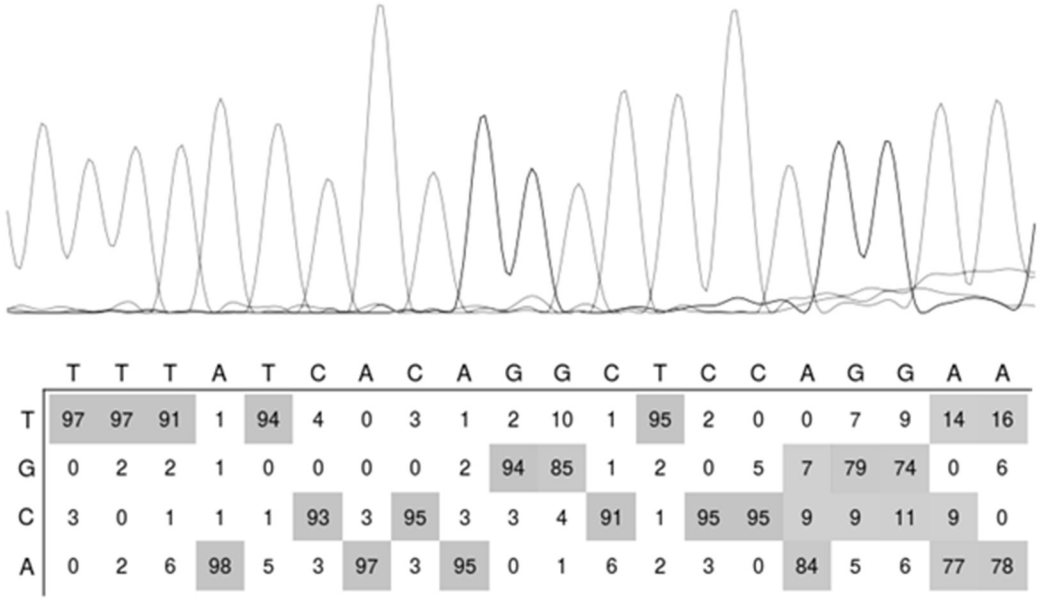


FIG. 154C

Single cell clones

BCL1, Clone #3



	T	T	T	A	T	C	A	C	A	G	G	C	T	C	C	A	G	G	A	A
T	97	97	91	1	94	4	0	3	1	2	10	1	95	2	0	0	7	9	14	16
G	0	2	2	1	0	0	0	0	2	94	85	1	2	0	5	7	79	74	0	6
C	3	0	1	1	1	93	3	95	3	3	4	91	1	95	95	9	9	11	9	0
A	0	2	6	98	5	3	97	3	95	0	1	6	2	3	0	84	5	6	77	78

(SEQ ID NO: 271)

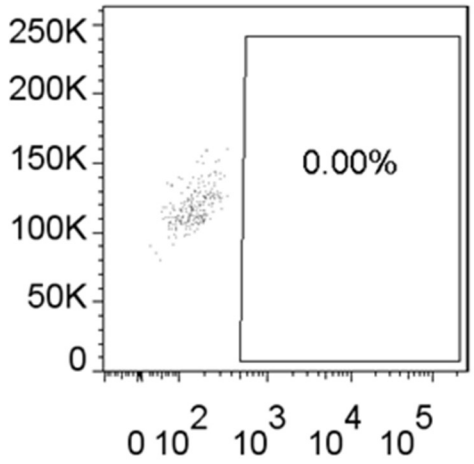
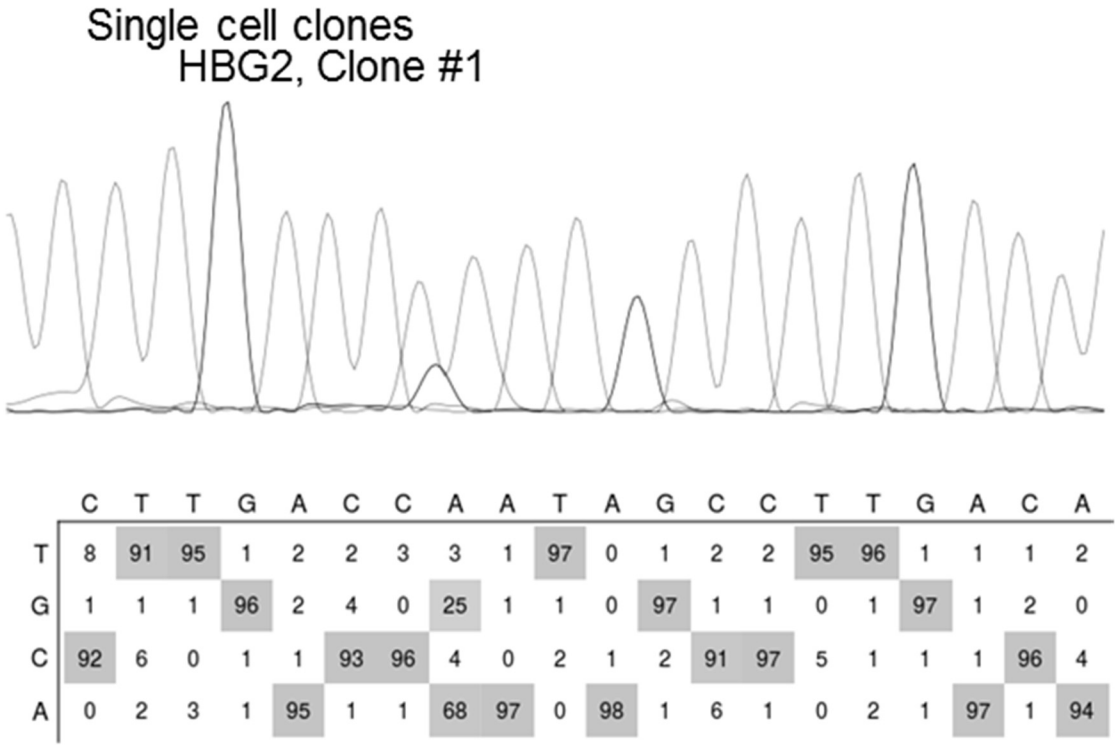


FIG. 154D



(SEQ ID NO: 250)

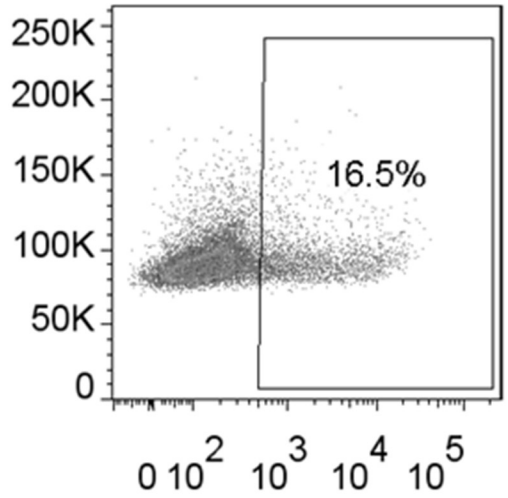
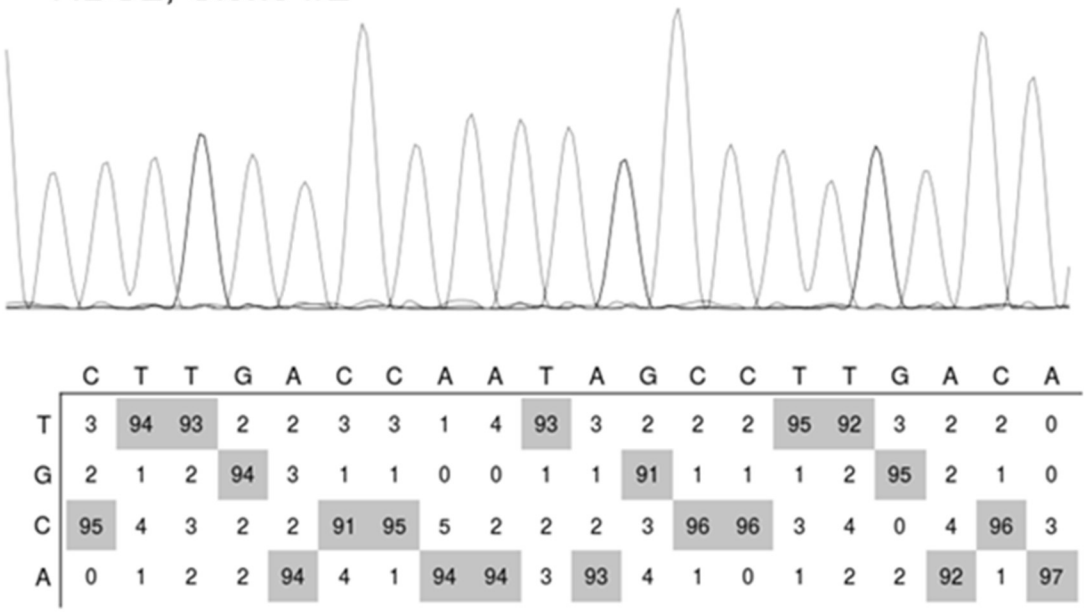


FIG. 154E

Single cell clones

HBG2, Clone #2



	C	T	T	G	A	C	C	A	A	T	A	G	C	C	T	T	G	A	C	A
T	3	94	93	2	2	3	3	1	4	93	3	2	2	2	95	92	3	2	2	0
G	2	1	2	94	3	1	1	0	0	1	1	91	1	1	1	2	95	2	1	0
C	95	4	3	2	2	91	95	5	2	2	2	3	96	96	3	4	0	4	96	3
A	0	1	2	2	94	4	1	94	94	3	93	4	1	0	1	2	2	92	1	97

(SEQ ID NO: 250)

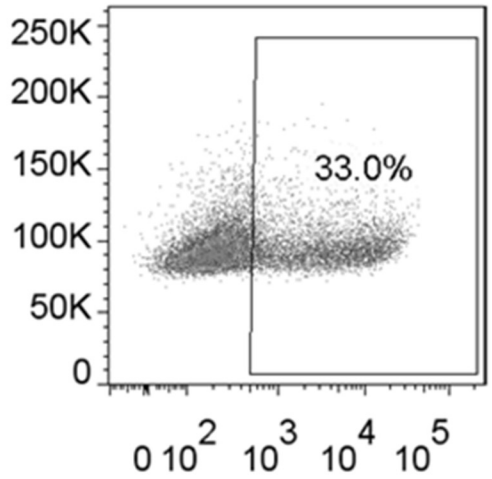
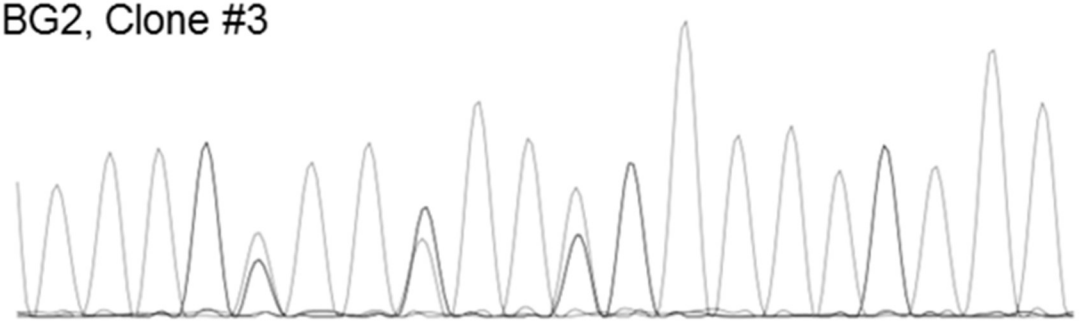


FIG. 154F

Single cell clones

HBG2, Clone #3



	C	T	T	G	A	C	C	A	A	T	A	G	C	C	T	T	G	A	C	A
T	4	95	94	4	3	4	3	2	3	93	3	2	0	4	95	94	3	1	1	1
G	0	1	3	92	38	1	1	55	2	1	37	89	1	0	1	3	94	3	0	2
C	93	4	2	0	3	92	93	3	1	1	2	4	97	96	3	2	0	4	97	4
A	3	0	2	3	56	3	2	39	95	5	58	5	1	0	1	1	3	92	2	94

(SEQ ID NO: 250)

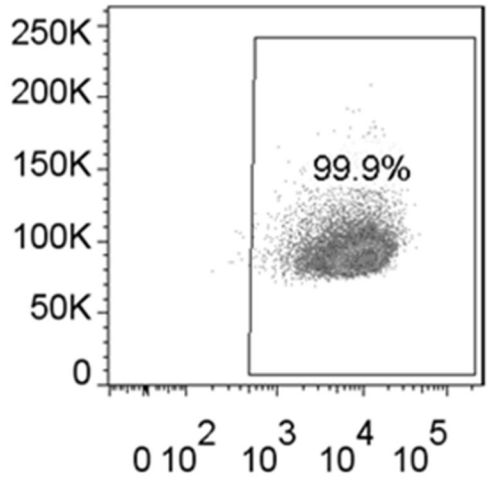
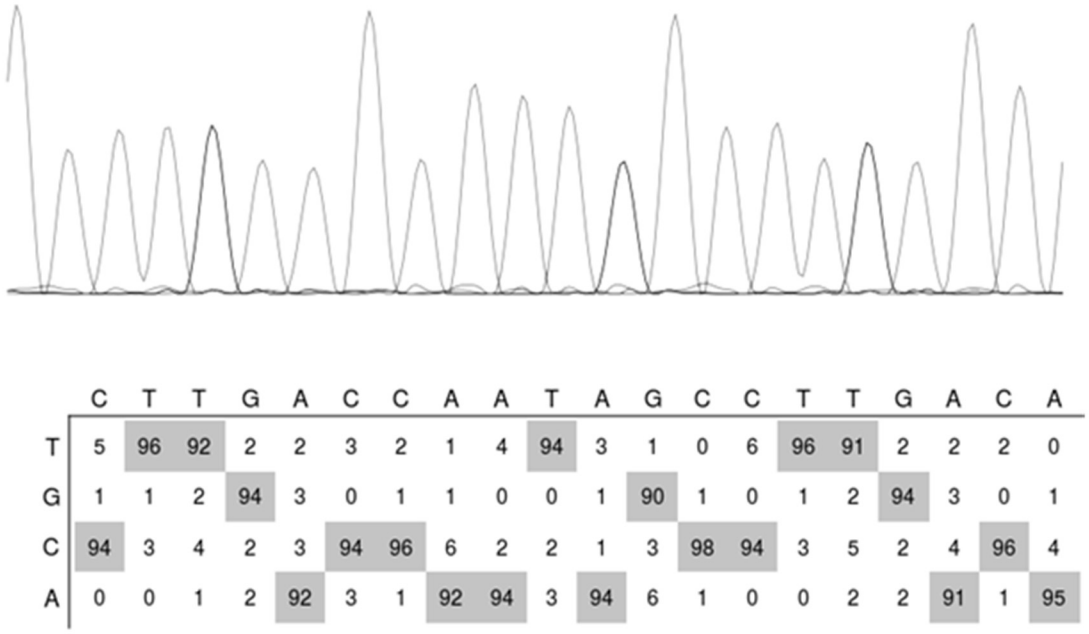


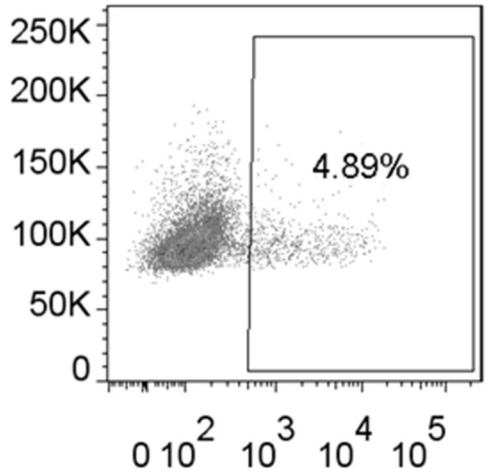
FIG. 154G

Single cell clones

HBG2, Clone #4



	C	T	T	G	A	C	C	A	A	T	A	G	C	C	T	T	G	A	C	A
T	5	96	92	2	2	3	2	1	4	94	3	1	0	6	96	91	2	2	2	0
G	1	1	2	94	3	0	1	1	0	0	1	90	1	0	1	2	94	3	0	1
C	94	3	4	2	3	94	96	6	2	2	1	3	98	94	3	5	2	4	96	4
A	0	0	1	2	92	3	1	92	94	3	94	6	1	0	0	2	2	91	1	95



(SEQ ID NO: 250)

FIG. 154H

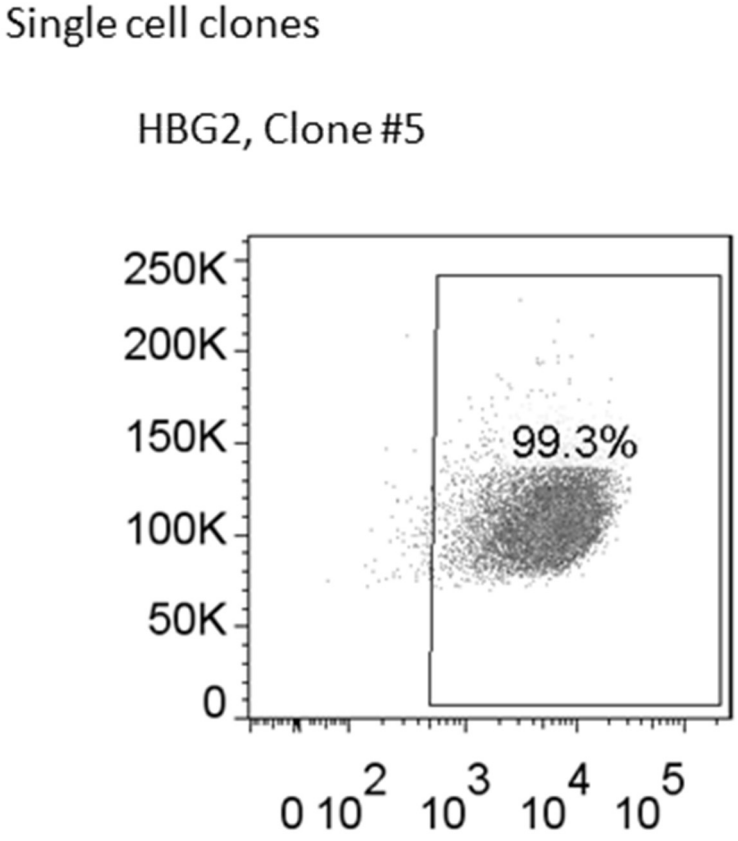
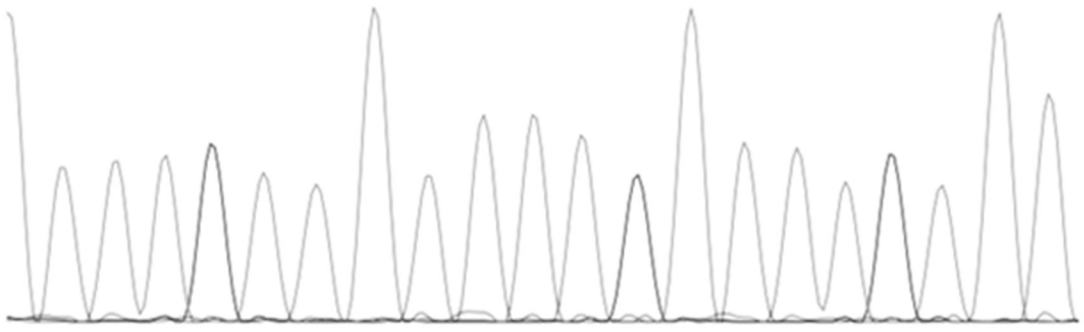

Single cell clones
HBG2, Clone #5
250K
200K
150K
100K
50K
0
99.3%
0 10^2 10^3 10^4 10^5

FIG. 154I

Single cell clones

HBG2, Clone #6



	C	T	T	G	A	C	C	A	A	T	A	G	C	C	T	T	G	A	C	A
T	4	95	92	3	2	2	1	2	5	95	3	1	0	4	96	92	2	1	1	0
G	0	1	3	95	3	0	1	1	0	1	2	91	1	0	0	3	94	3	0	1
C	96	5	2	0	1	94	97	6	1	1	0	4	98	96	3	2	1	4	98	4
A	0	0	3	2	93	3	1	92	95	3	94	4	0	0	0	3	2	91	1	95

(SEQ ID NO: 250)

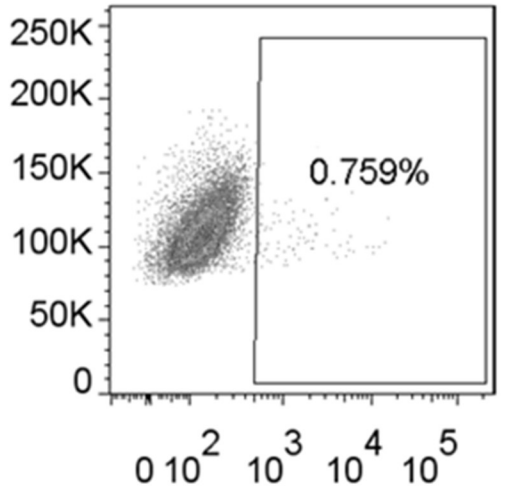


FIG. 154J

Single cell clones

HBG2, Clone #7



	C	T	T	G	A	C	C	A	A	T	A	G	C	C	T	T	G	A	C	A
T	5	95	90	3	2	2	1	0	5	94	3	1	1	6	95	93	2	1	1	1
G	0	1	4	93	39	1	0	17	0	0	24	91	1	1	1	5	95	3	0	1
C	95	3	3	1	2	94	97	4	1	2	0	4	97	93	3	2	0	5	97	4
A	0	0	3	3	57	2	1	78	93	3	73	5	1	0	0	0	3	92	1	94

(SEQ ID NO: 250)

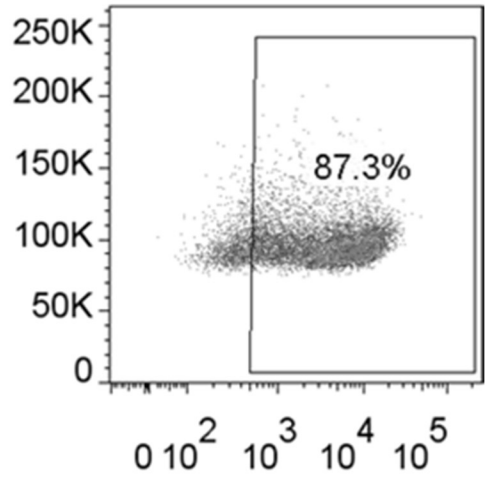
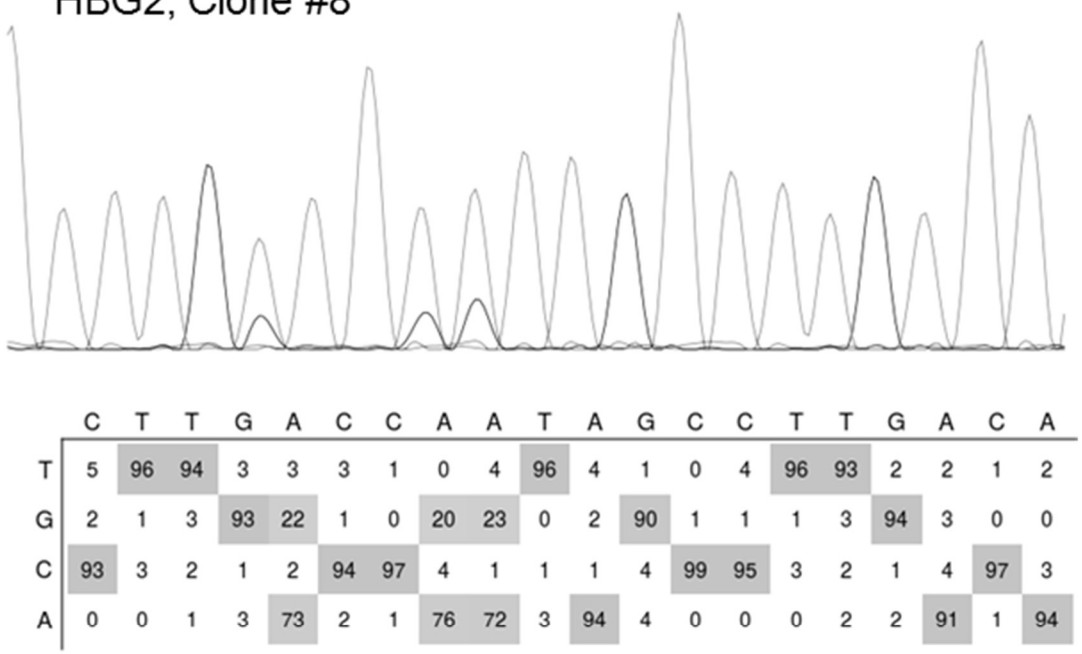


FIG. 154K

Single cell clones

HBG2, Clone #8



	C	T	T	G	A	C	C	A	A	T	A	G	C	C	T	T	G	A	C	A
T	5	96	94	3	3	3	1	0	4	96	4	1	0	4	96	93	2	2	1	2
G	2	1	3	93	22	1	0	20	23	0	2	90	1	1	1	3	94	3	0	0
C	93	3	2	1	2	94	97	4	1	1	1	4	99	95	3	2	1	4	97	3
A	0	0	1	3	73	2	1	76	72	3	94	4	0	0	0	2	2	91	1	94

(SEQ ID NO: 250)

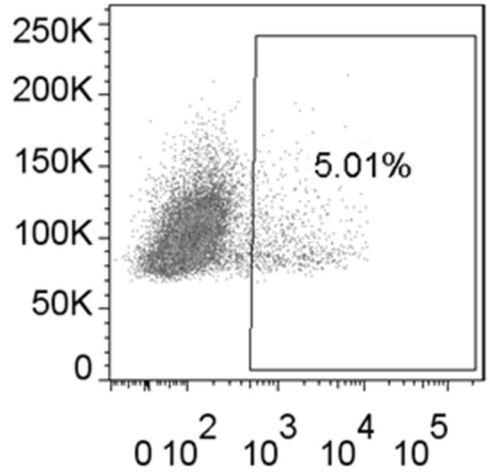


FIG. 154L

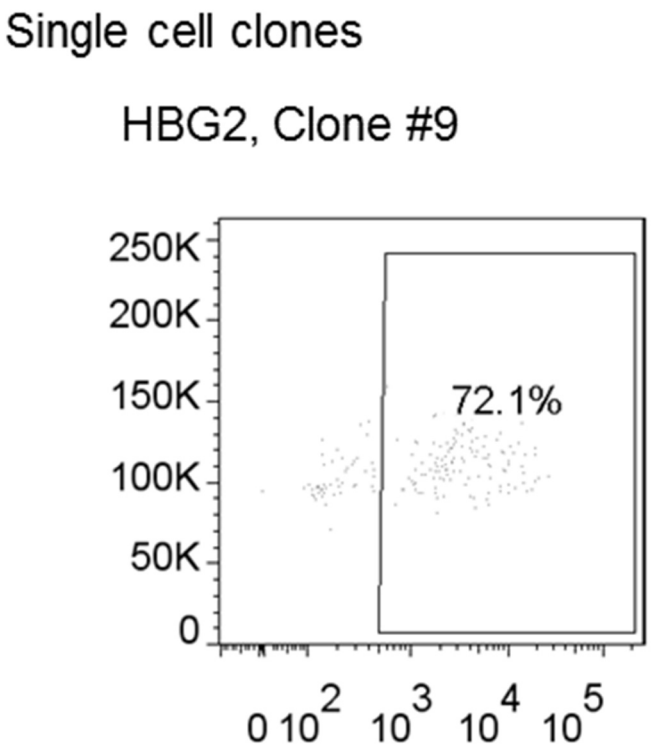


FIG. 154M

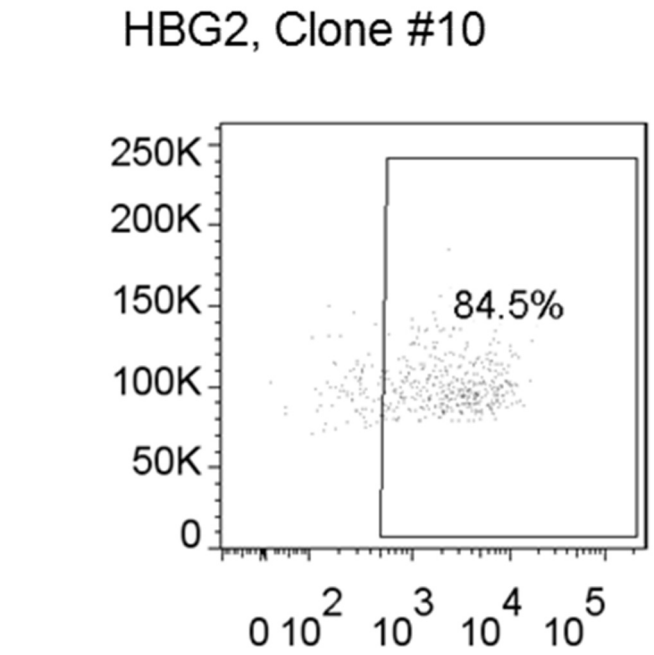


FIG. 154N

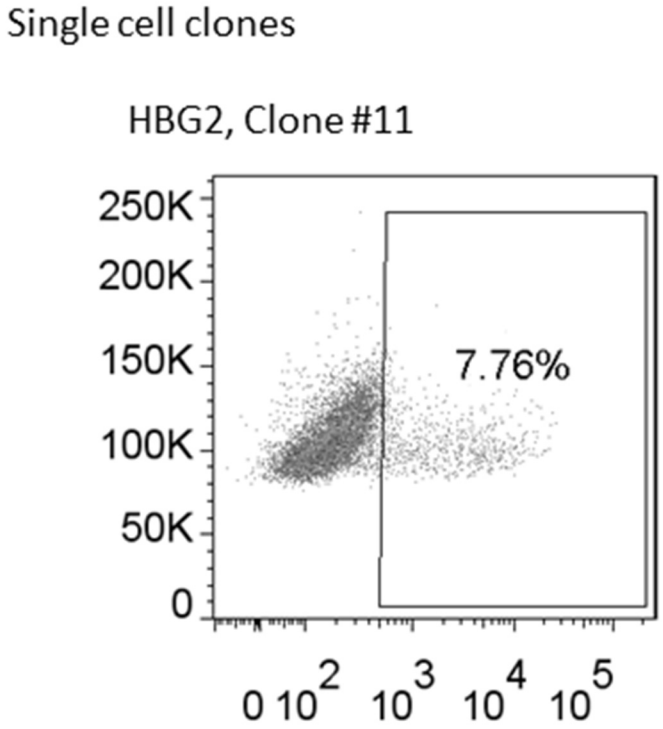
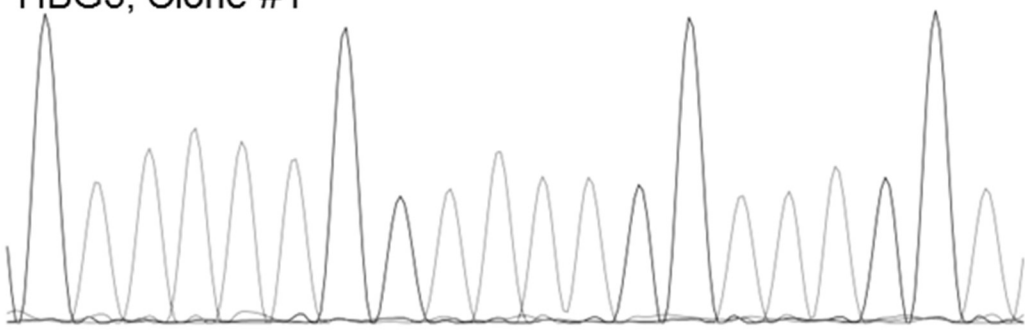

Single cell clones
HBG2, Clone #11
250K
200K
150K
100K
50K
0
7.76%
0 10^2 10^3 10^4 10^5

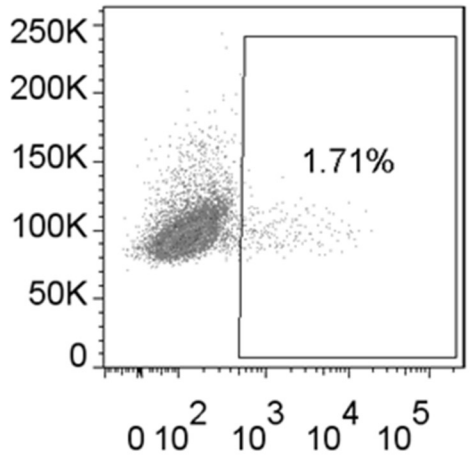
FIG. 154O

Single cell clones

HBG3, Clone #1



	G	C	T	A	T	T	G	G	T	C	A	A	G	G	C	A	A	G	G	C
T	1	5	93	3	93	94	2	4	91	3	4	0	1	1	4	0	1	1	2	5
G	98	3	1	2	1	5	96	92	2	1	2	4	95	95	5	3	3	92	97	4
C	1	89	2	0	0	0	1	1	3	94	5	1	0	1	88	5	2	2	2	90
A	0	2	4	95	6	0	1	3	4	3	89	95	4	2	3	91	93	5	0	0

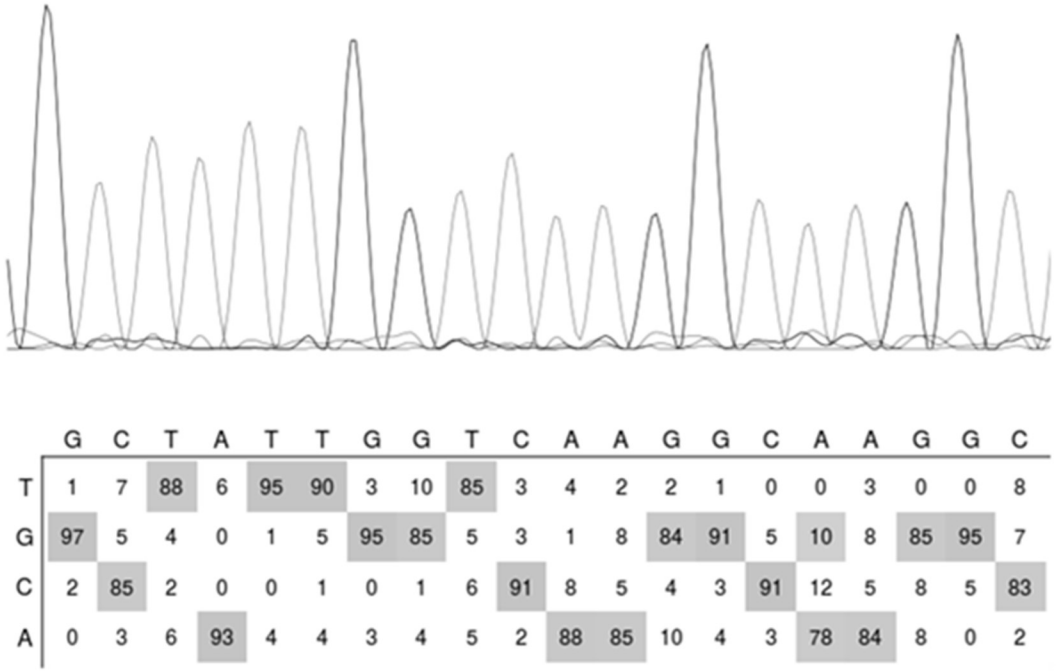


(SEQ ID NO: 252)

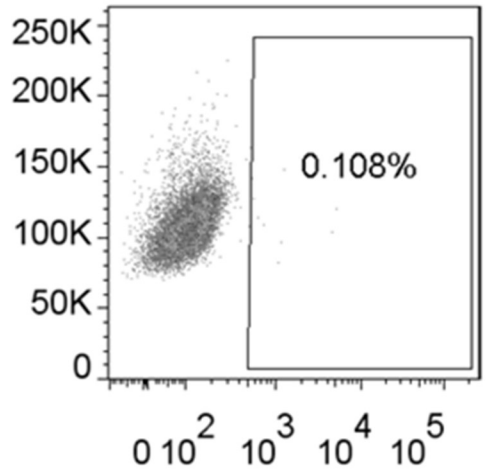
FIG. 154P

Single cell clones

HBG3, Clone #2



	G	C	T	A	T	T	G	G	T	C	A	A	G	G	C	A	A	G	G	C
T	1	7	88	6	95	90	3	10	85	3	4	2	2	1	0	0	3	0	0	8
G	97	5	4	0	1	5	95	85	5	3	1	8	84	91	5	10	8	85	95	7
C	2	85	2	0	0	1	0	1	6	91	8	5	4	3	91	12	5	8	5	83
A	0	3	6	93	4	4	3	4	5	2	88	85	10	4	3	78	84	8	0	2



(SEQ ID NO: 252)

FIG. 154Q

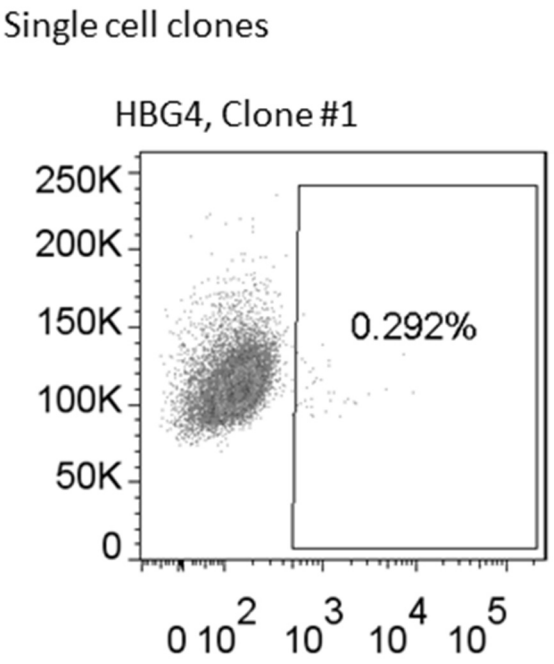


FIG. 154R

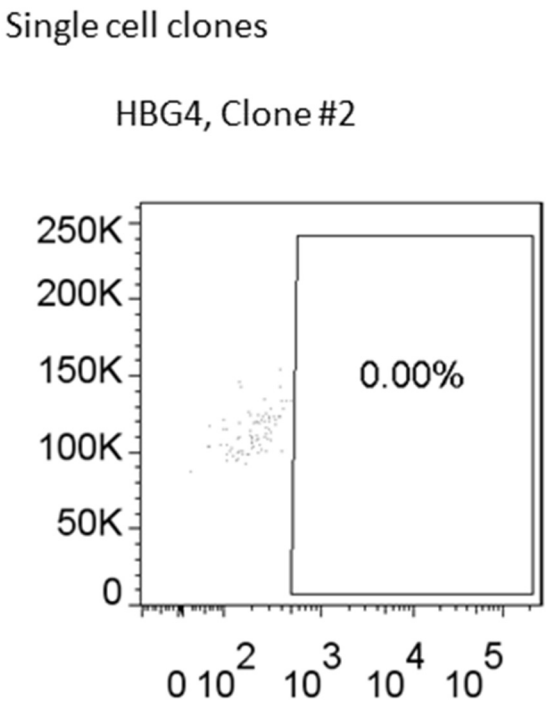
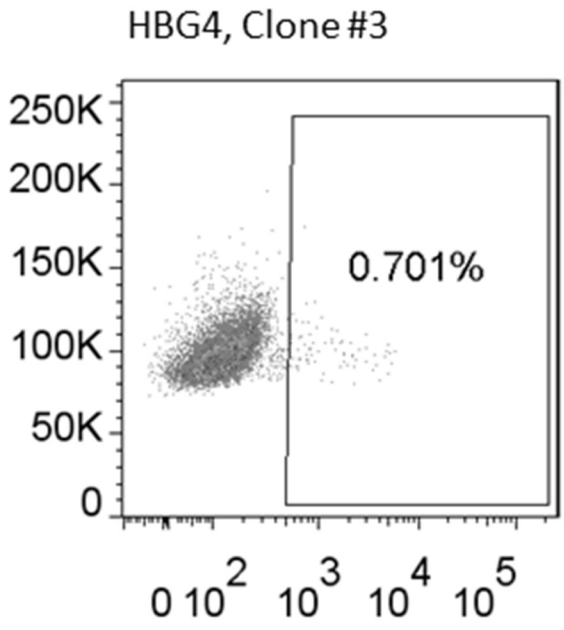
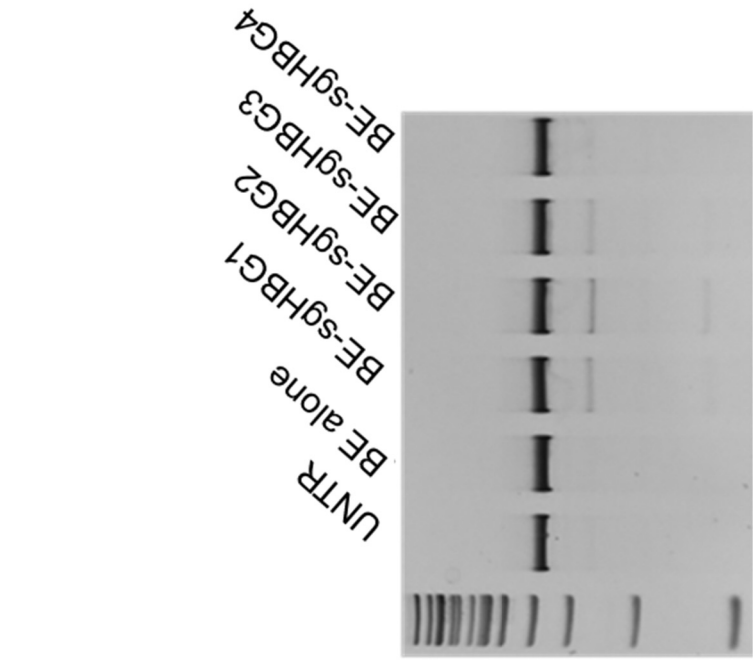


FIG. 154S




UNTR
BE alone
BE-sgHBG1
BE-sgHBG2
BE-sgHBG3
BE-sgHBG4

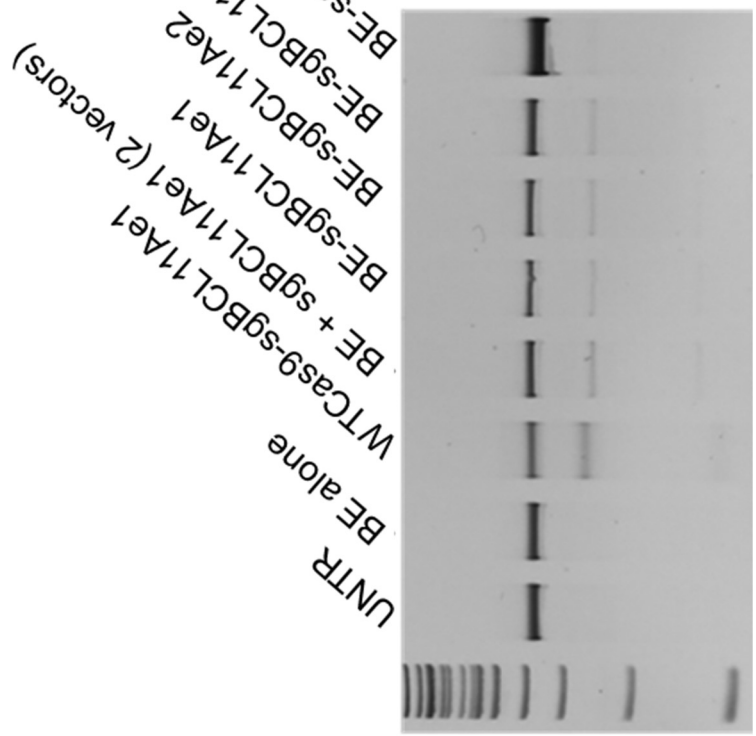

UNTR
BE alone
WTCas9-sgBCL11Ae1
BE + sgBCL11Ae1 (2 vectors)
BE-sgBCL11Ae1
BE-sgBCL11Ae2
BE-sgBCL11Ae3
BE-sgBCL11Ae4

FIG. 155

FIG. 156A

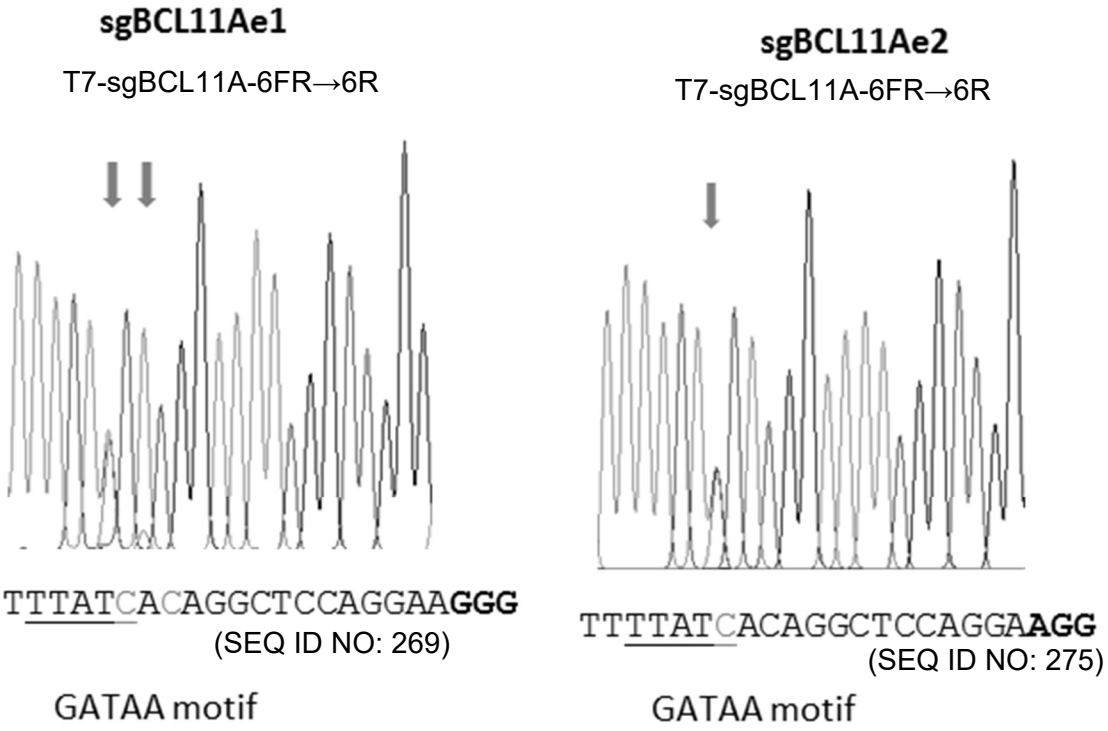


FIG. 156B

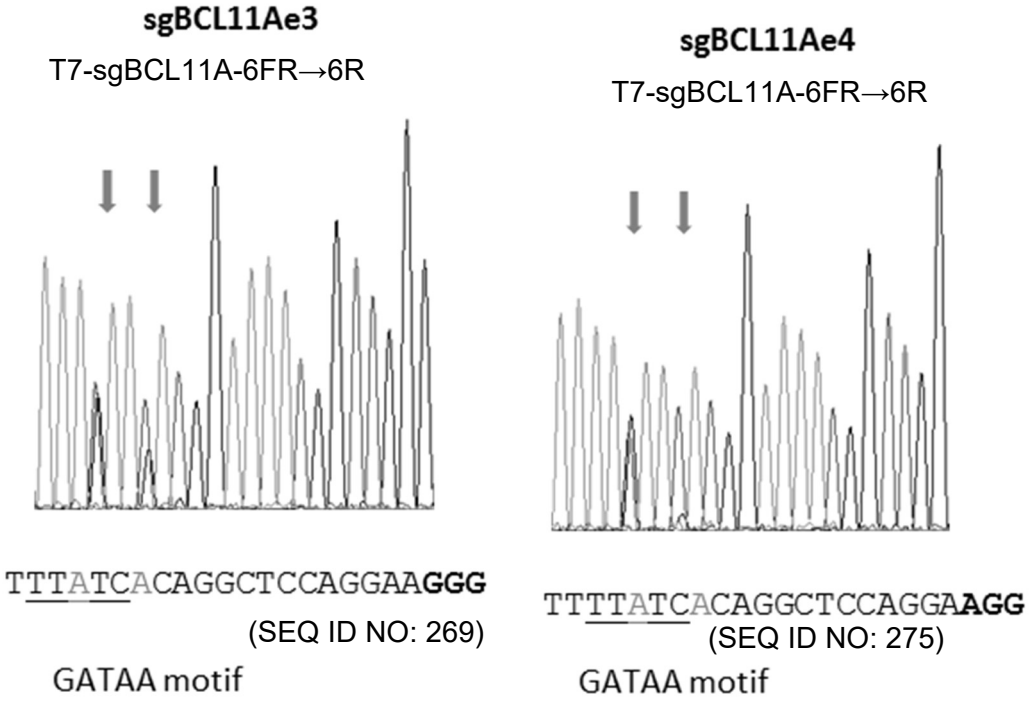


FIG. 156C

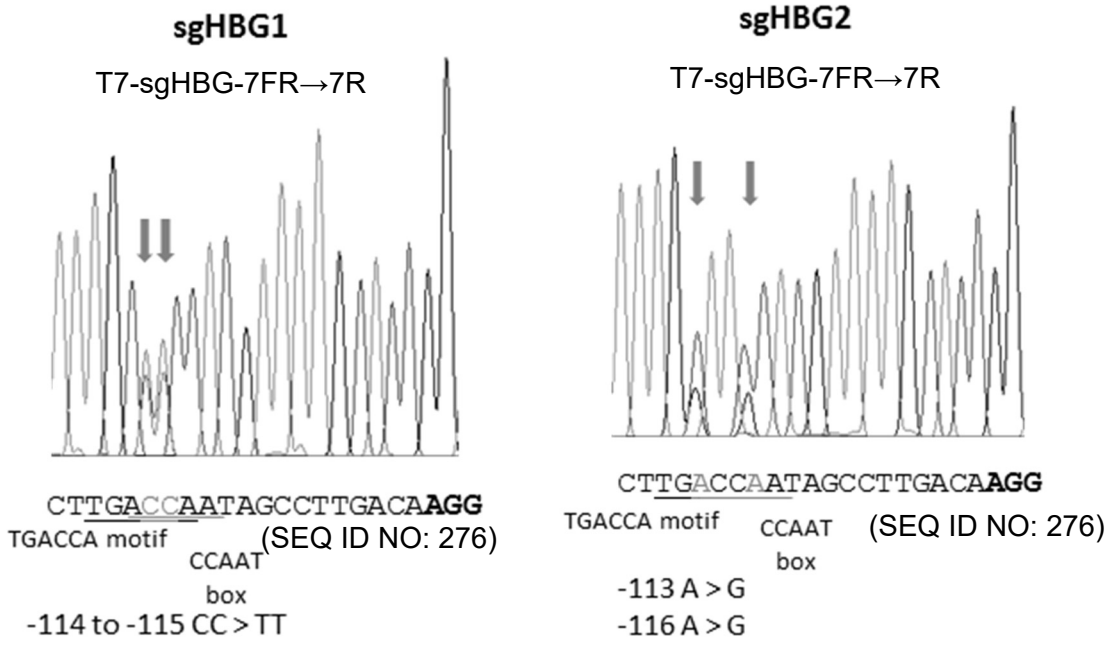


FIG. 156D

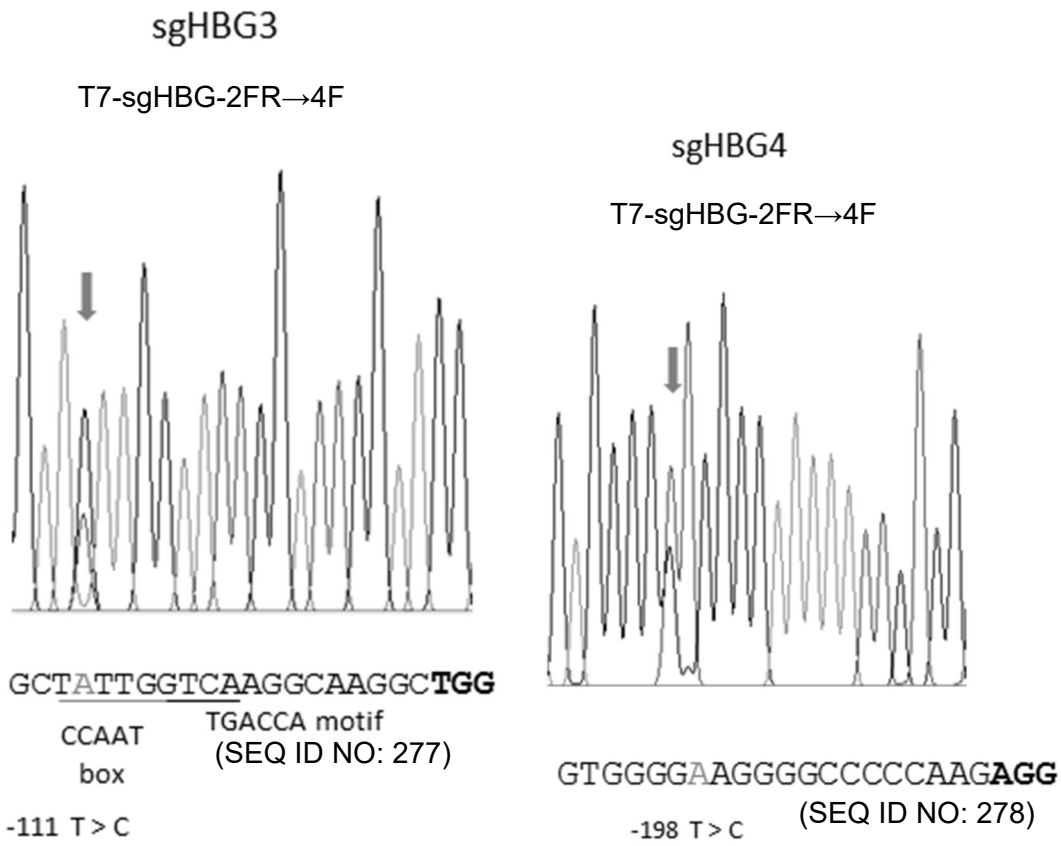


FIG. 157

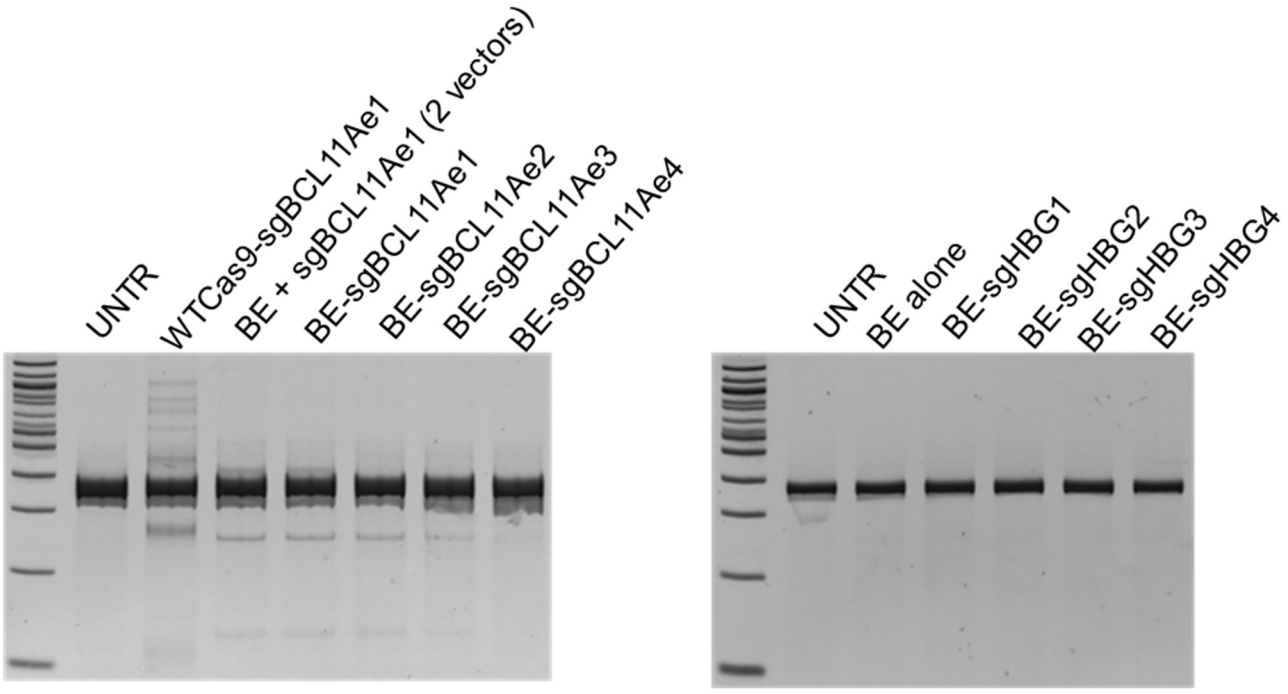


FIG. 158

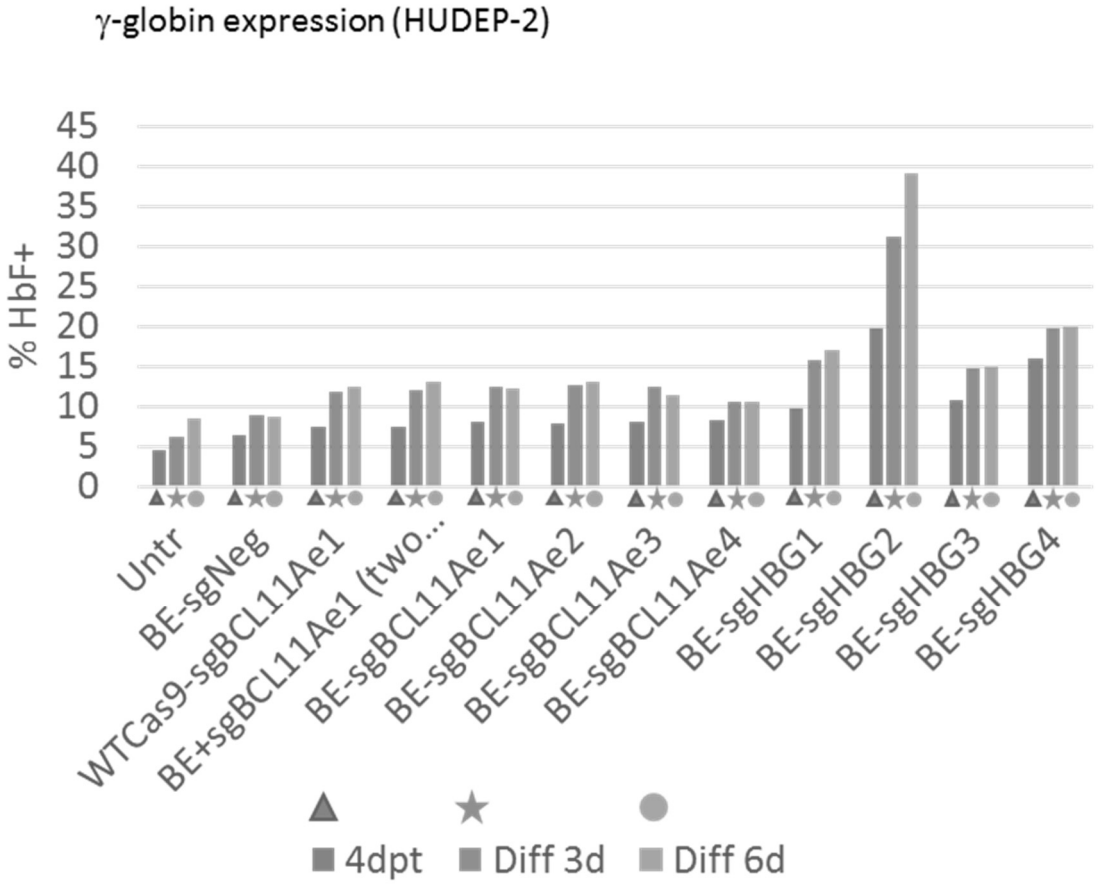


FIG. 159A

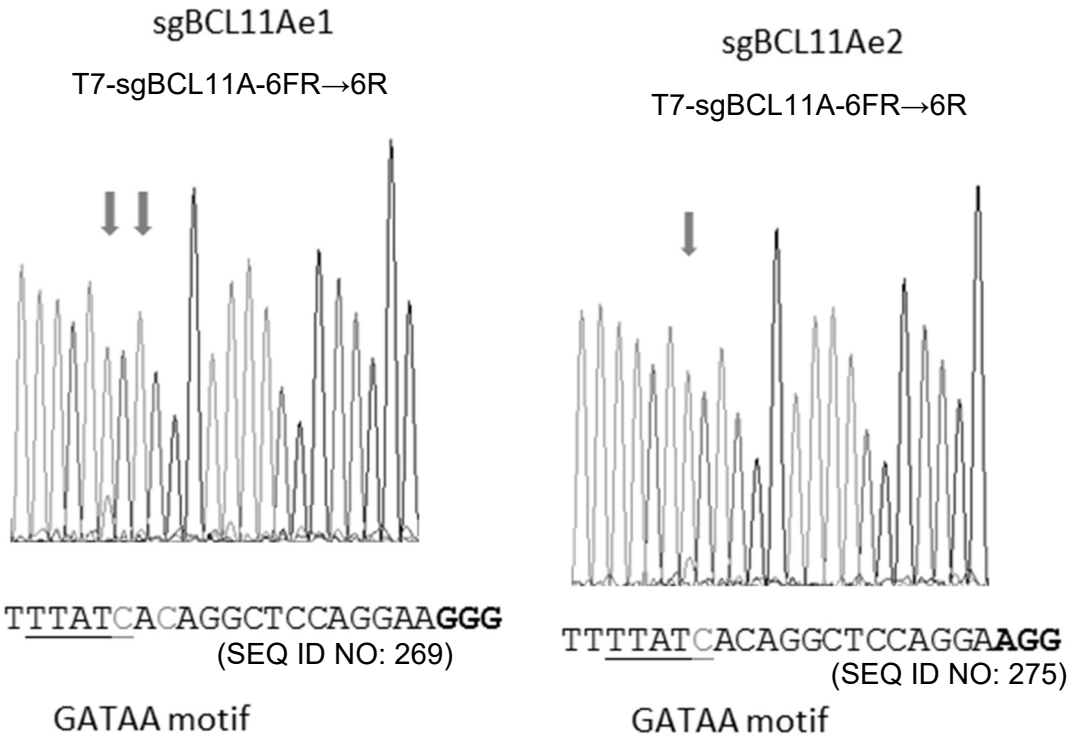


FIG. 159B

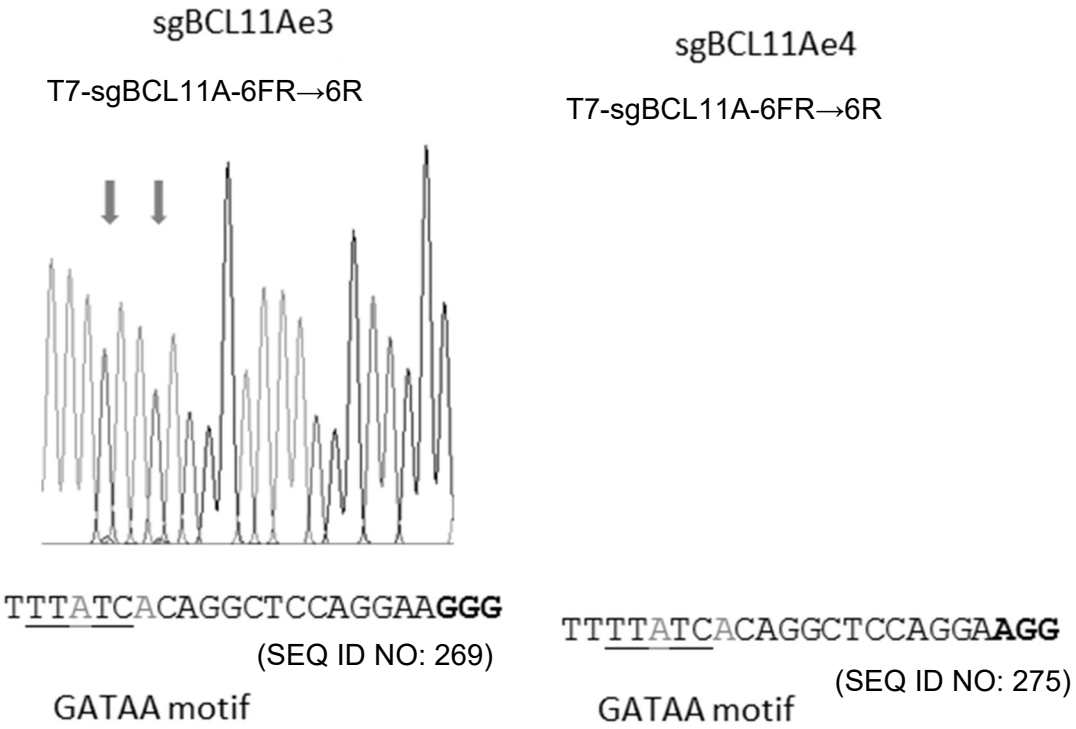


FIG. 159C

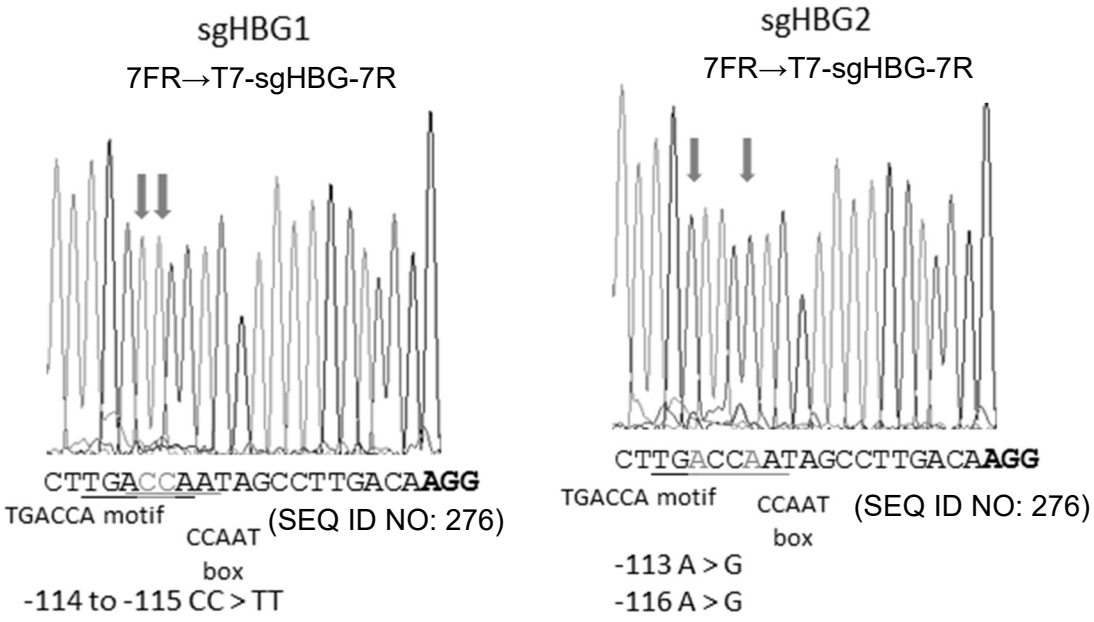


FIG. 159D

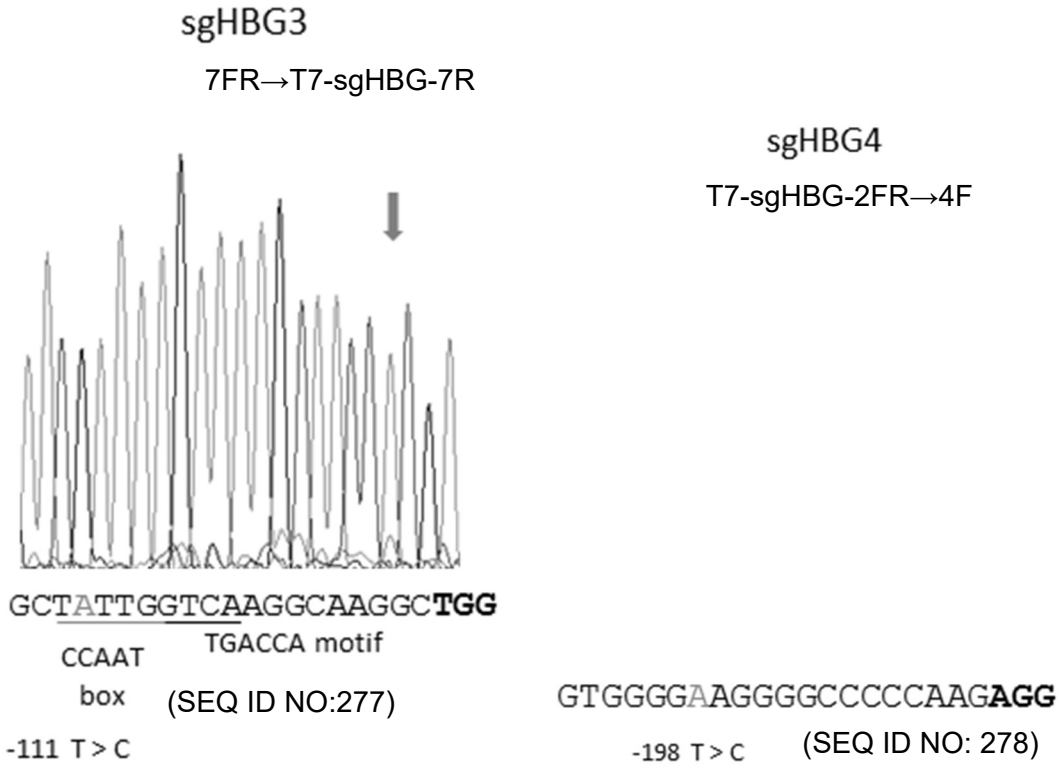


FIG. 160

Selected constructs for HDAd virus production (under Maxi preparation)

Constructed vectors:		Corresponding Ad constructs:
pBS-AncBE4max-sgBCL11AE1	+58 GATA motif, G --> A	**pHCA-BE4max-sgBCL11Ae1-FI-mgmt/GFP**
pBS-AncBE4max-sgBCL11AE2	+58 GATA motif, G --> A	
pBS-ABEmax-sgBCL11AE3	+58 GATA motif, T --> C	
pBS-ABEmax-sgBCL11AE4	+58 GATA motif, T --> C	
pBS-AncBE4max-sgHBG1-1	13bp deletion, CCAAT box, CC --> TT	
pBS-ABEmax-sgHBG1-2	13bp deletion, CCAAT box, A --> G	**pHCA-ABEmax-sgHBG2-FI-mgmt/GFP**
pBS-ABEmax-sgHBG1-3	13bp deletion, CCAAT box, T --> C	
pBS-ABEmax-sgHBG1-4	-198 T --> C	**pHCA-ABEmax-sgHBG4-FI-mgmt/GFP**
pBS-AncBE4max-sgR5-1	control	**pHCA-BE4max-sgR5-1-FI-mgmt/GFP (Ctrl)**

FIG. 161

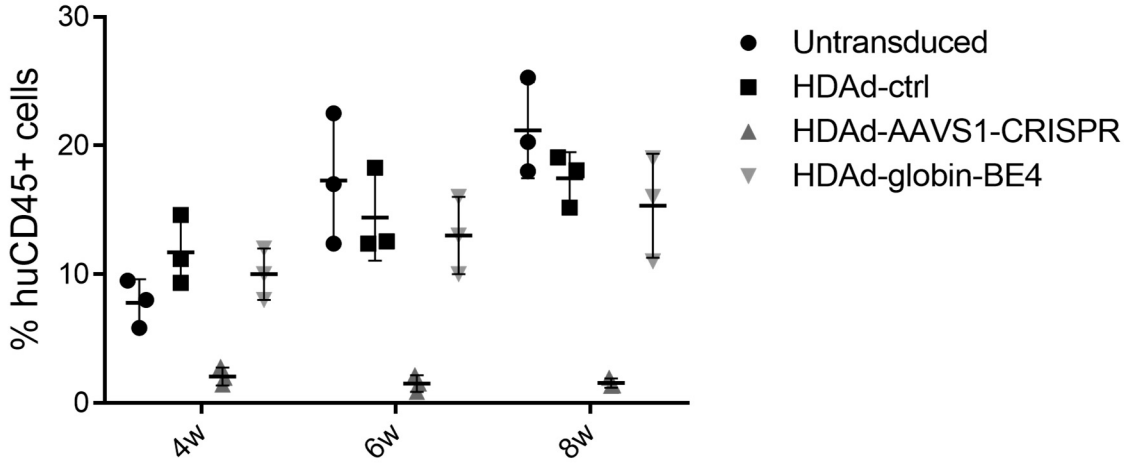
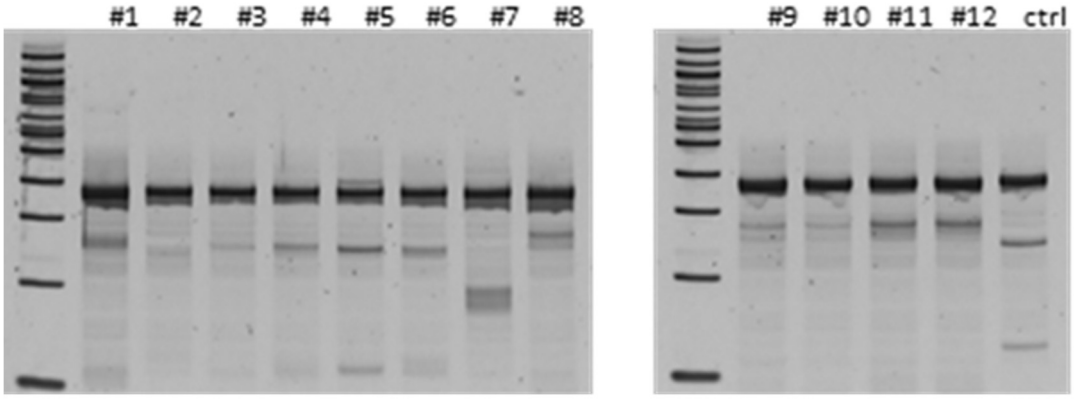


FIG. 162

Transient transfection of HUDEP-2 cells:
(cleavage by T7EI)

Bcl11a enhancer



HBG promoter

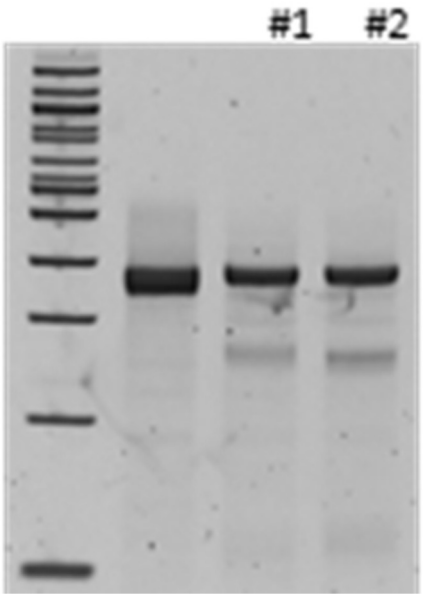


FIG. 163

Dual base editing vector application

Reactivation of γ-globin by disrupting BCL11A binding sites + Termination of mutant HBB by CRISPR-STOP

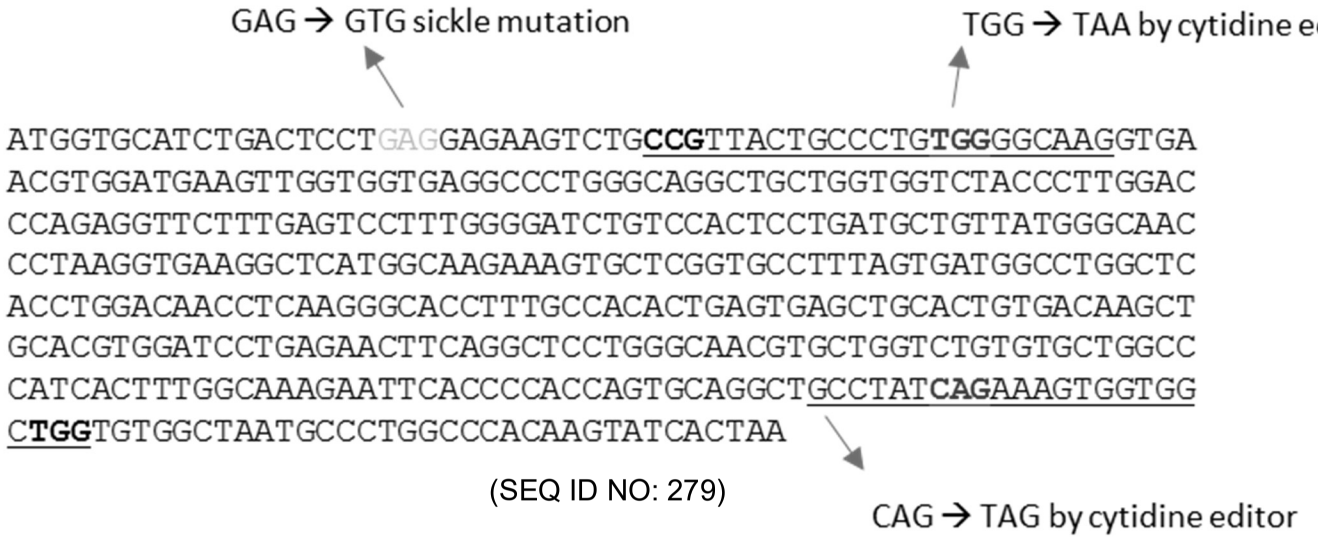


FIG. 164

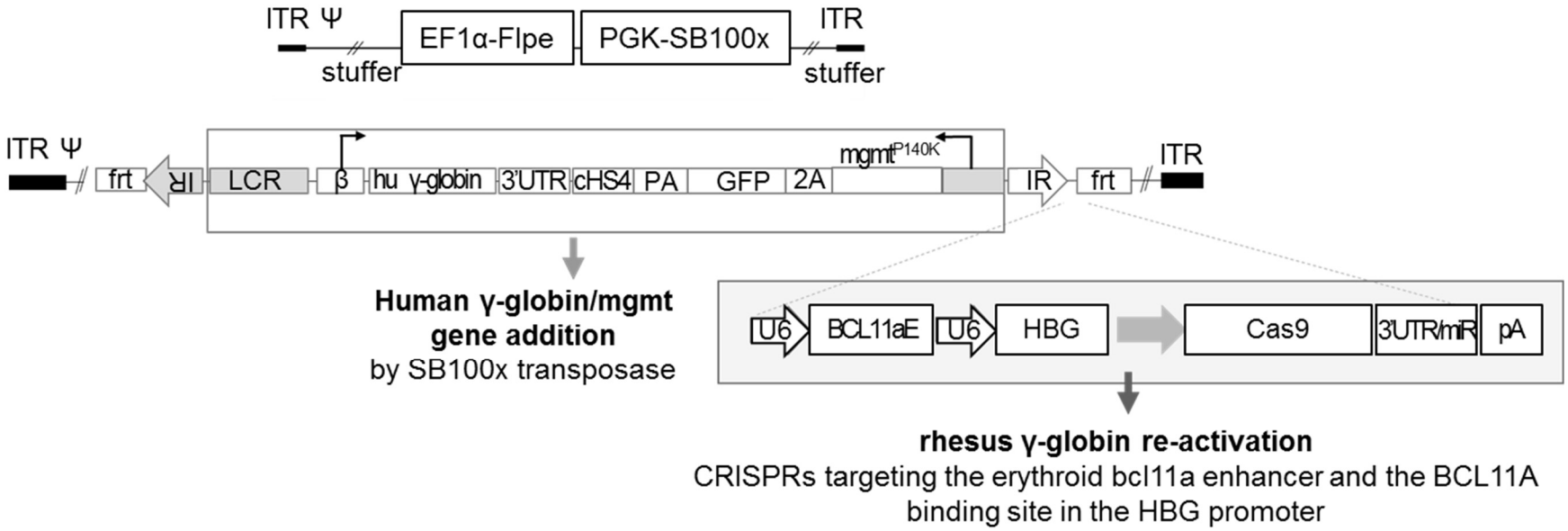

ITR Ψ
stuffer
EF1α-Flpe
PGK-SB100x
ITR
stuffer
ITR Ψ
frt
IR
LCR
β
hu γ-globin
3'UTR
cHS4
PA
GFP
2A
mgmtP140K
IR
frt
ITR
Human γ-globin/mgmt
gene addition
by SB100x transposase
U6
BCL11aE
U6
HBG
Cas9
3'UTR/miR
pA
rhesus γ-globin re-activation
CRISPRs targeting the erythroid bcl11a enhancer and the BCL11A
binding site in the HBG promoter

FIG. 165

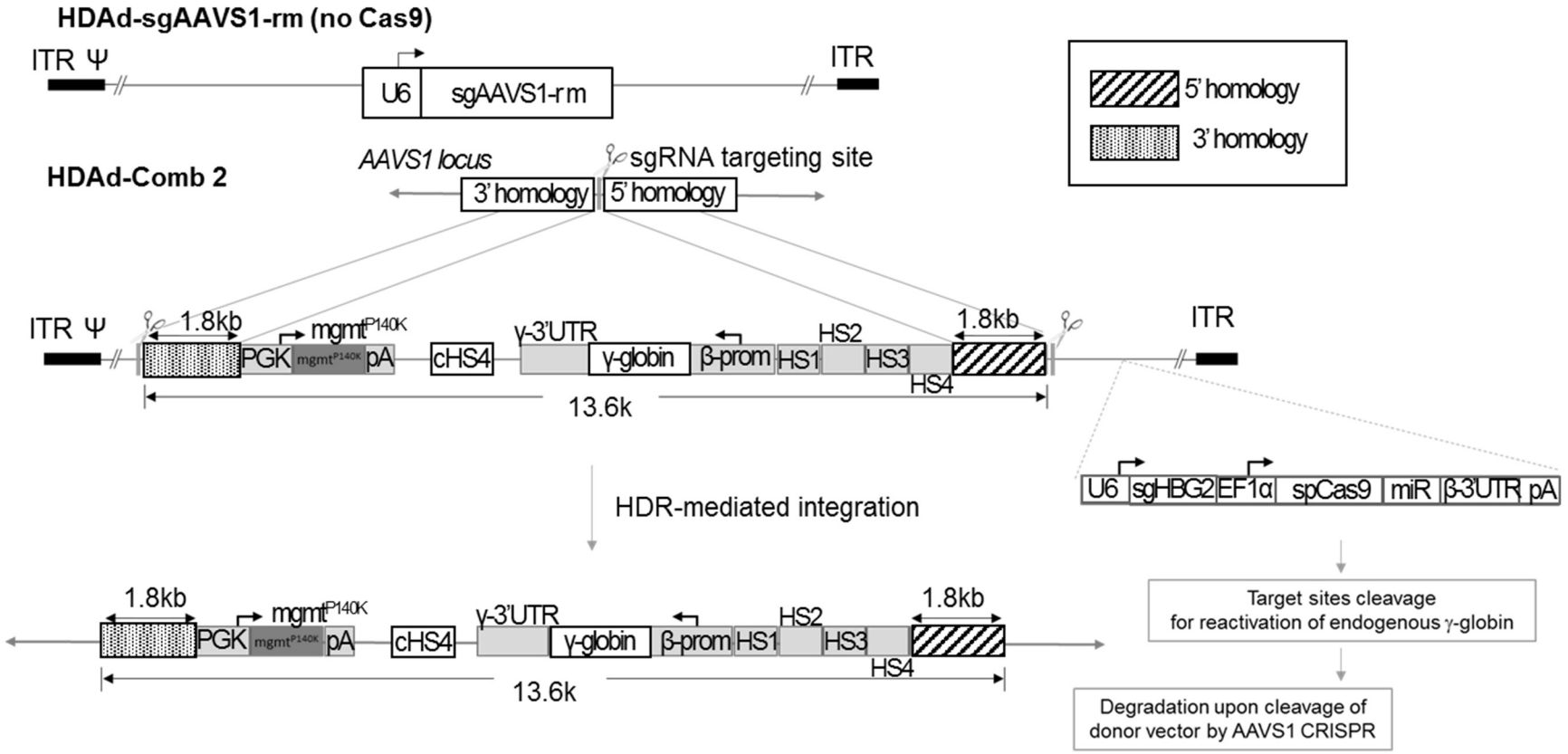
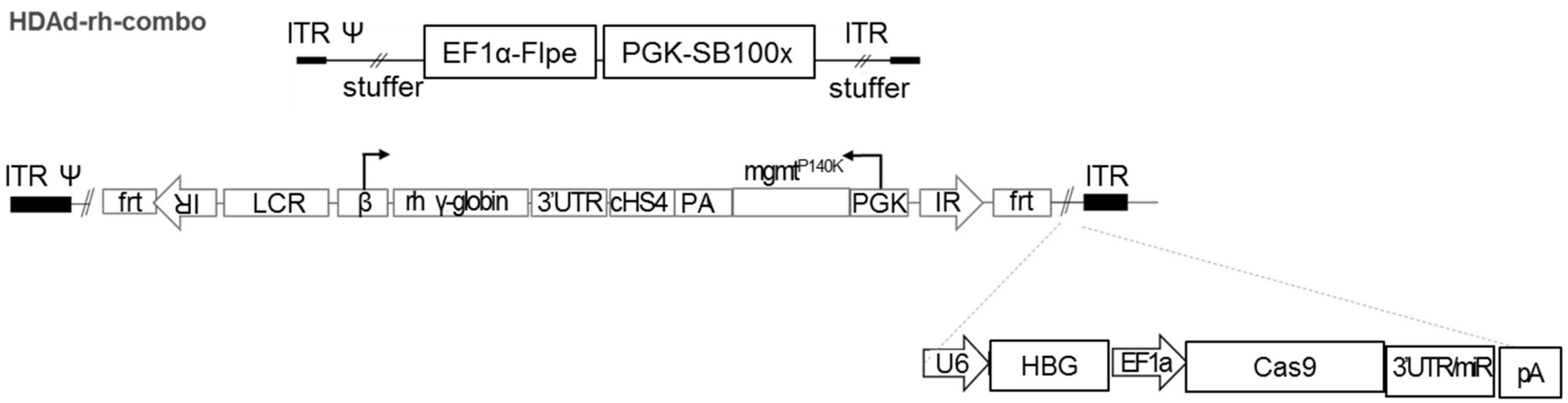

HDAd-sgAAVS1-rm (no Cas9)
ITR Ψ
U6
sgAAVS1-rm
ITR
5' homology
3' homology
HDAd-Comb 2
AAVS1 locus
sgRNA targeting site
3' homology
5' homology
ITR Ψ
1.8kb
mgmtP140K
PGK
mgmtP140K
pA
cHS4
γ-3'UTR
γ-globin
β-prom
HS1
HS2
HS3
HS4
1.8kb
ITR
13.6k
HDR-mediated integration
U6
sgHBG2
EF1α
spCas9
miR
β-3'UTR
pA
1.8kb
mgmtP140K
PGK
mgmtP140K
pA
cHS4
γ-3'UTR
γ-globin
β-prom
HS1
HS2
HS3
HS4
1.8kb
13.6k
Target sites cleavage
for reactivation of endogenous γ-globin
Degradation upon cleavage of
donor vector by AAVS1 CRISPR

FIG. 166


HDAd-rh-combo
ITR Ψ
stuffer
EF1α-Flpe
PGK-SB100x
ITR
stuffer
ITR Ψ
frt
IR
LCR
β
rh γ-globin
3'UTR
cHS4
PA
mgmtP140K
PGK
IR
frt
ITR
U6
HBG
EF1a
Cas9
3'UTRmiR
pA