FIG. 1

Integrated Cassettes (integrated portions are between frt sites)

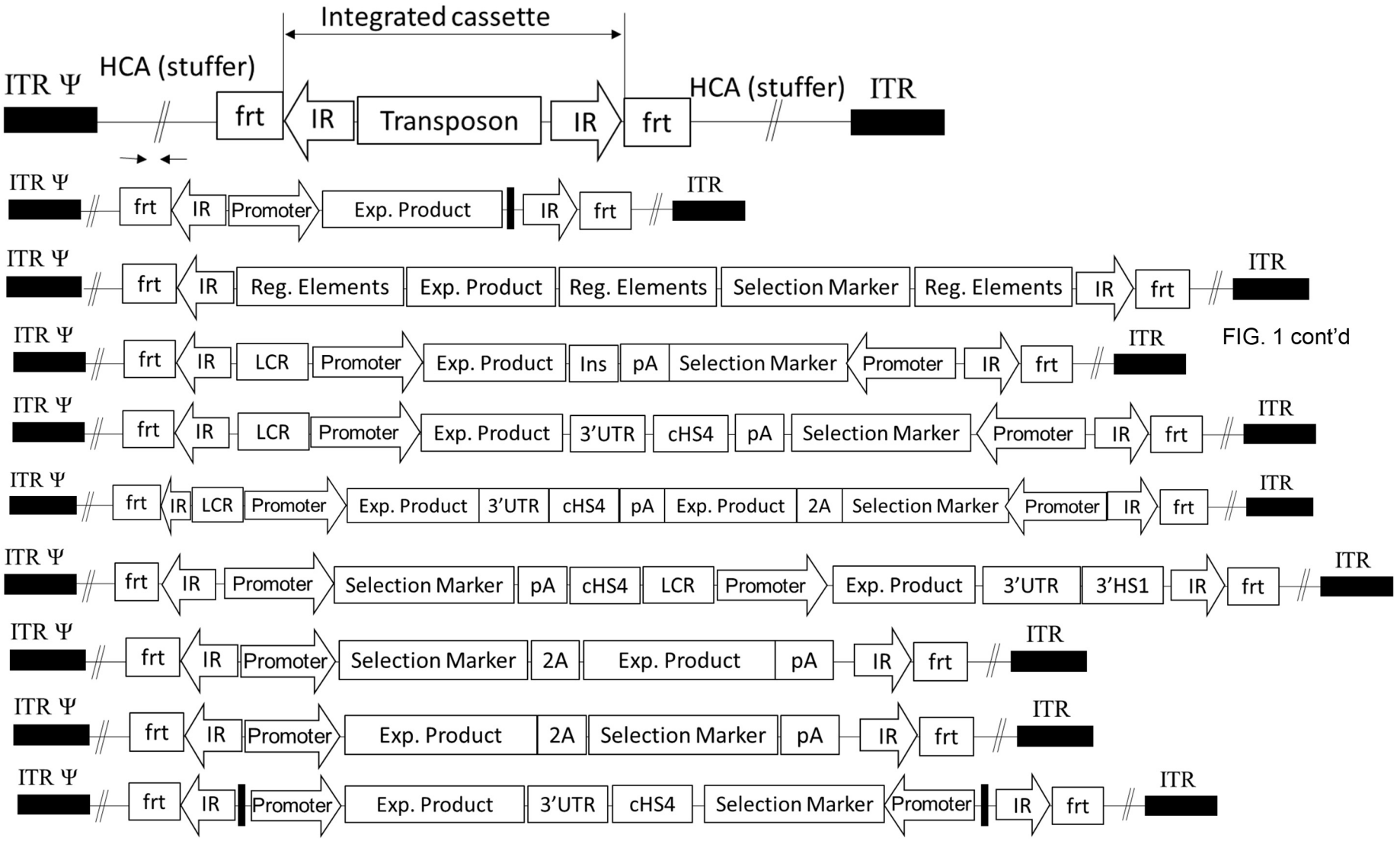


FIG. 1 cont'd

FIG. 1 cont'd

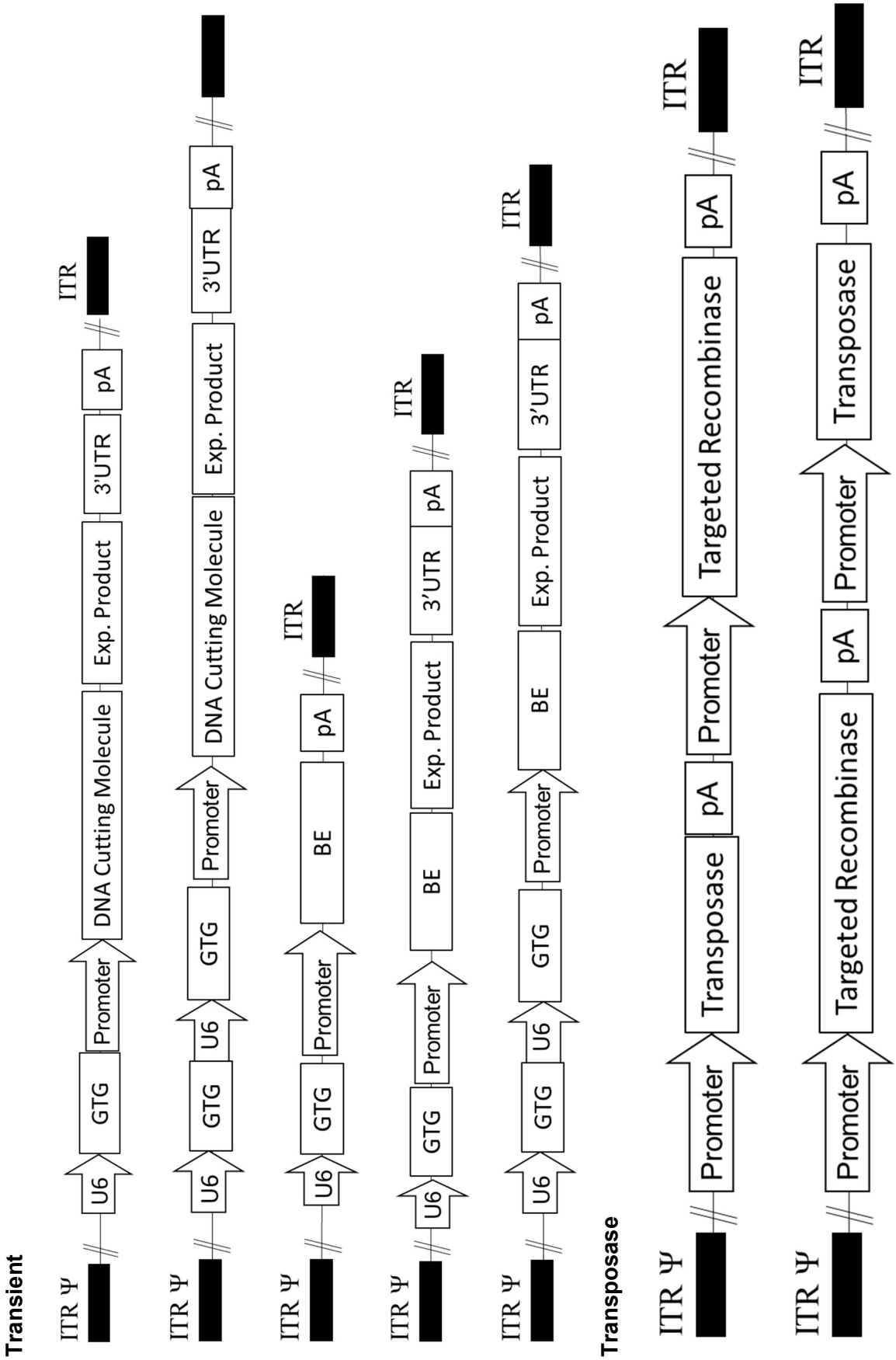

Transient
ITR Ψ
U6
GTG
Promoter
DNA Cutting Molecule
Exp. Product
3'UTR
pA
ITR
U6
GTG
U6
GTG
Promoter
DNA Cutting Molecule
Exp. Product
3'UTR
pA
ITR
U6
GTG
Promoter
BE
pA
ITR
U6
GTG
Promoter
BE
Exp. Product
3'UTR
pA
ITR
U6
GTG
U6
GTG
Promoter
BE
Exp. Product
3'UTR
pA
ITR
Transposase
ITR Ψ
Promoter
Transposase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

FIG. 2A

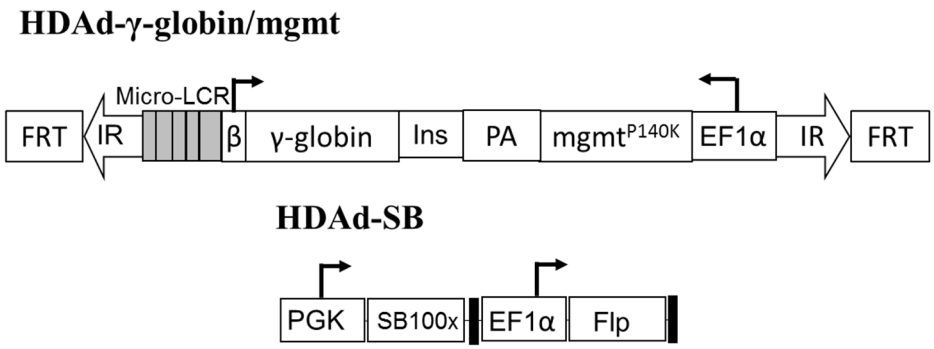


FIG. 2B

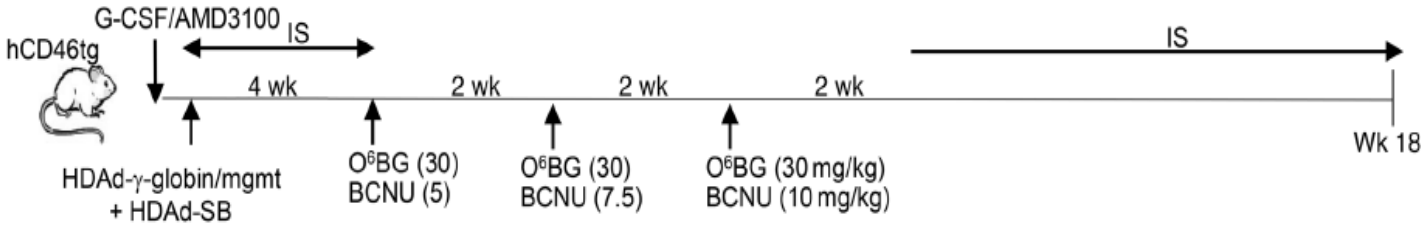


FIG. 2C

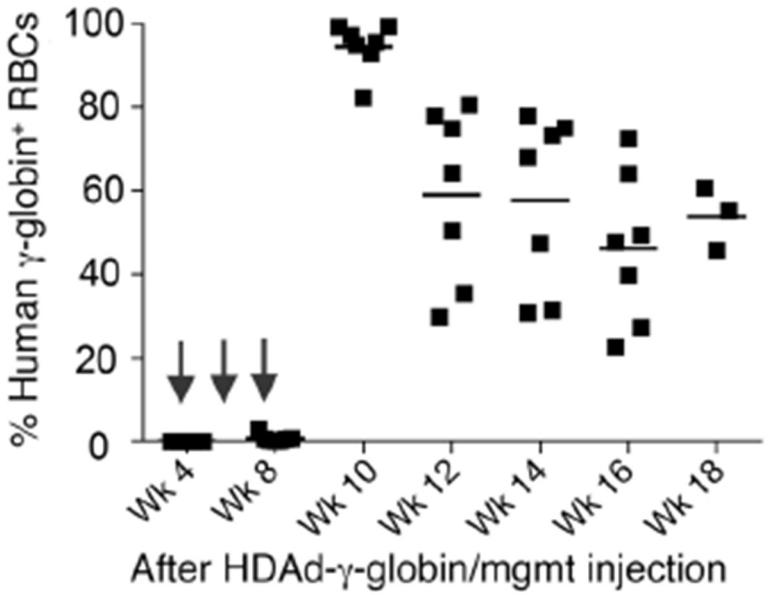


FIG. 2D

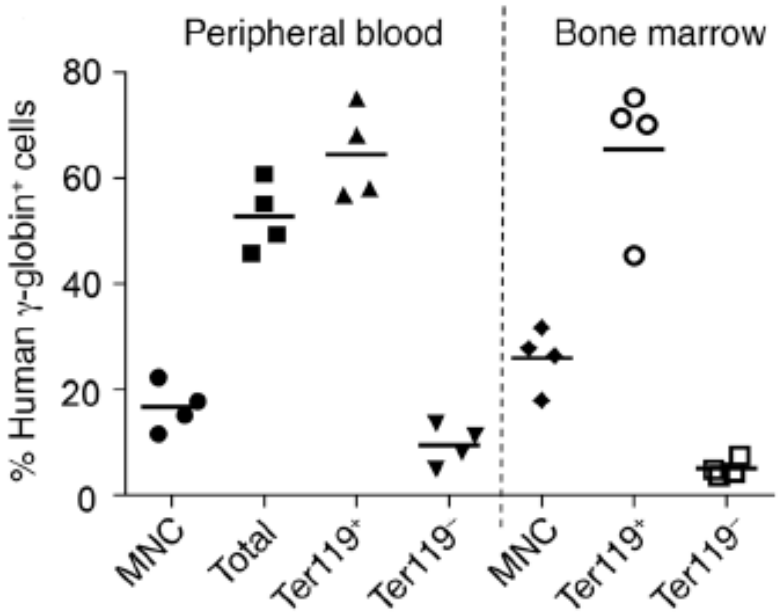

Peripheral blood
Bone marrow
% Human γ-globin+ cells
80
60
40
20
0
MNC
Total
Ter119+
Ter119-
MNC
Ter119+
Ter119-

FIG. 2E

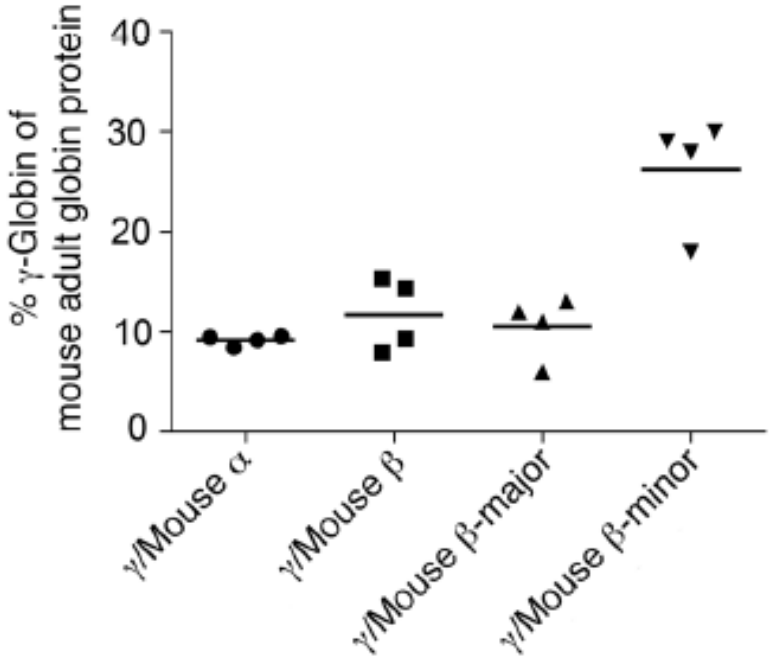

% γ-Globin of mouse adult globin protein
40
30
20
10
0
γ/Mouse α
γ/Mouse β
γ/Mouse β-major
γ/Mouse β-minor

FIG. 2F

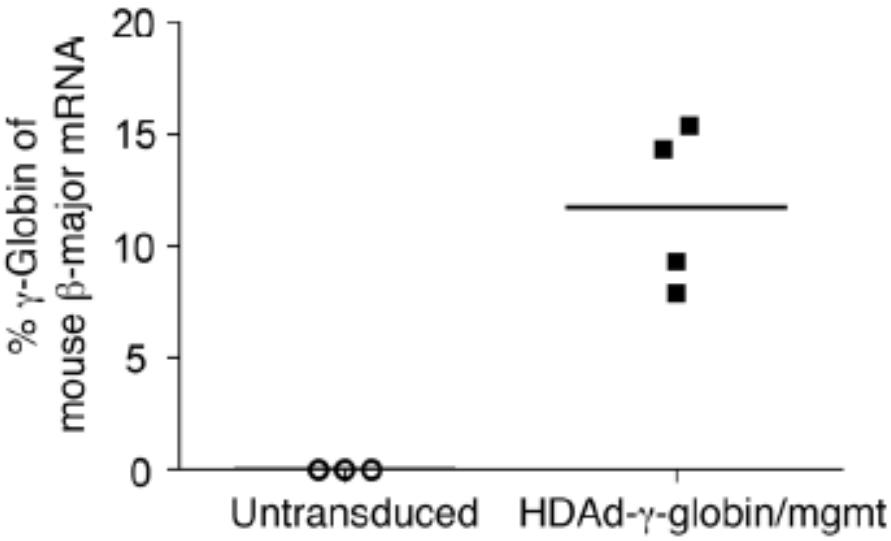

% γ-Globin of mouse β-major mRNA
20
15
10
5
0
Untransduced
HDAd-γ-globin/mgmt

FIG. 3

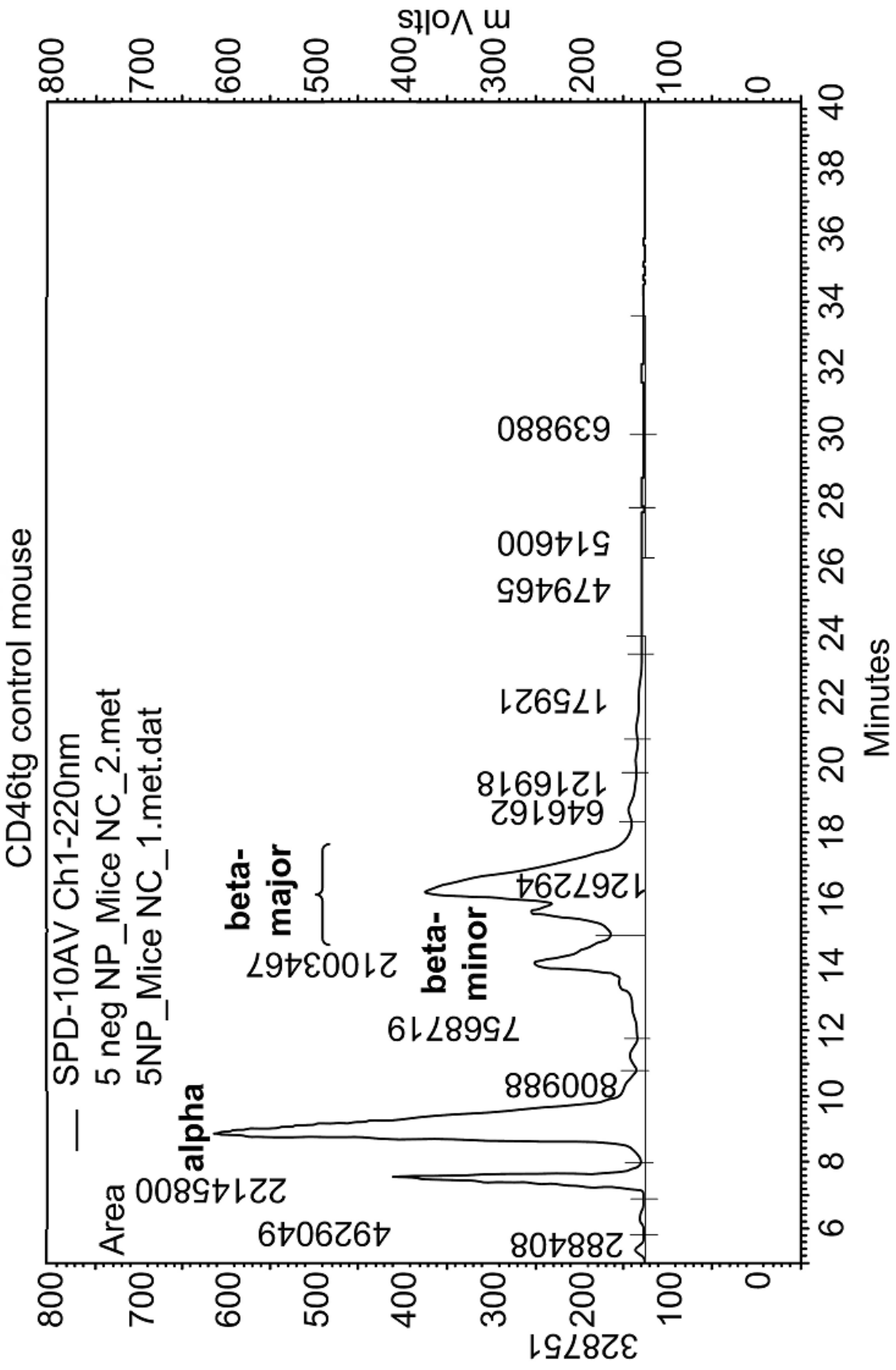

CD46tg control mouse
SPD-10AV Ch1-220nm
5 neg NP_Mice NC_2.met
5NP_Mice NC_1.met.dat
Area
alpha
2214580
beta-
major
2100346
beta-
minor
756871
4929049
328751
m Volts
Minutes
288408
800988
1267294
646162
1216918
175921
479465
514600
639880

FIG. 3 cont'd

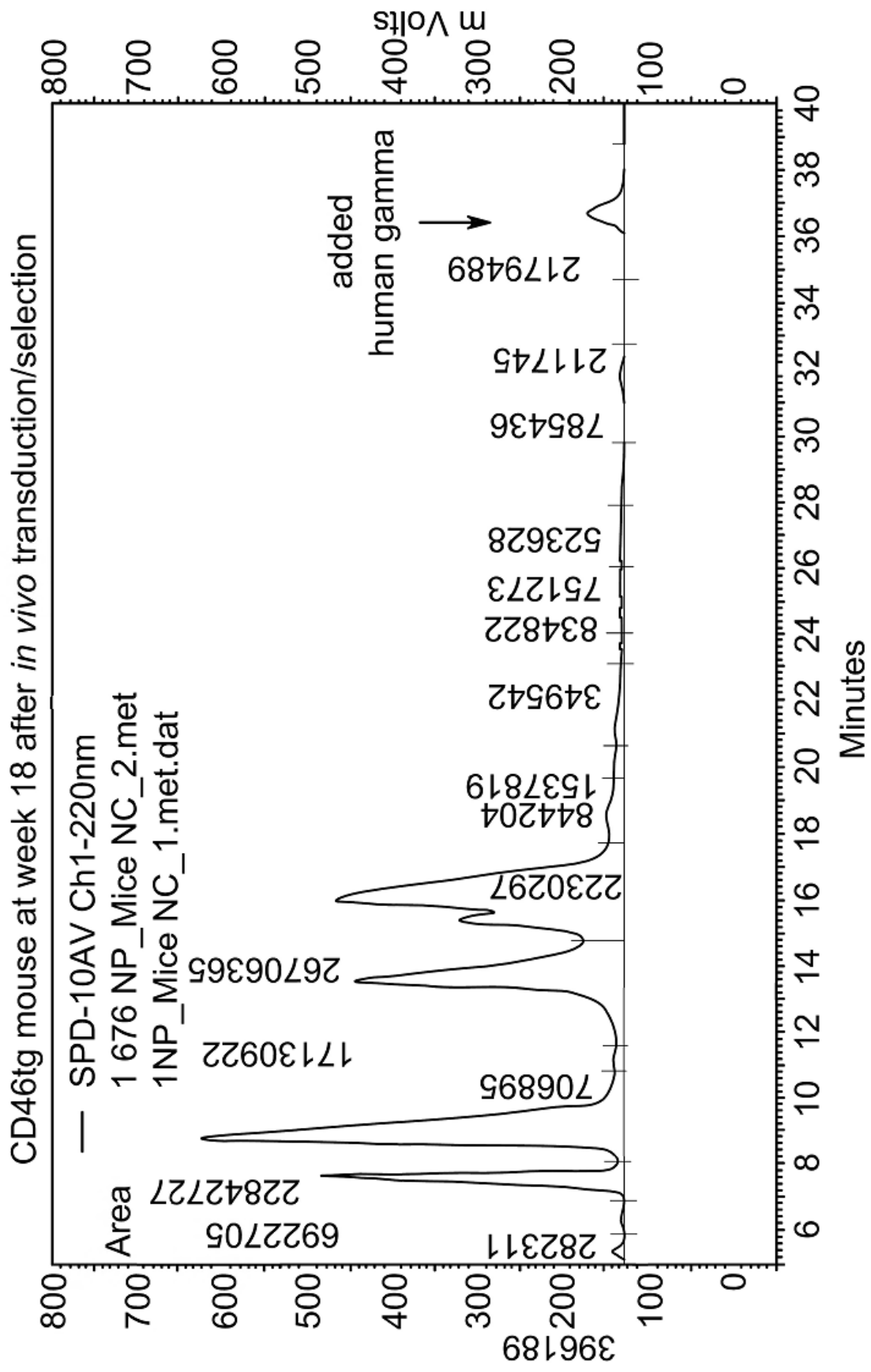

CD46tg mouse at week 18 after in vivo transduction/selection
SPD-10AV Ch1-220nm
1 676 NP_Mice NC_2.met
1NP_Mice NC_1.met.dat
Area
2284272 7
6922705
1713092 2
2670636 5
706895
2230297
8420 4
1537819
349542
834822
751273
523628
785436
211745
2179489
396189
282311
added
human gamma
m Volts
Minutes

FIG. 4A

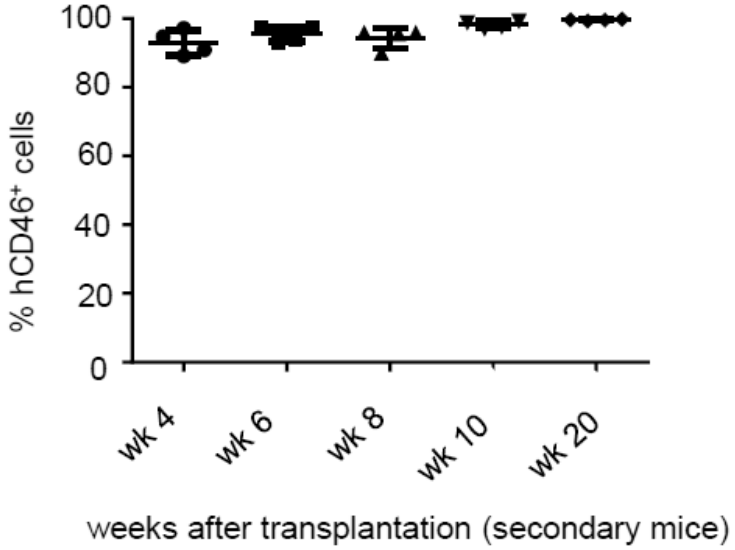


FIG. 4B

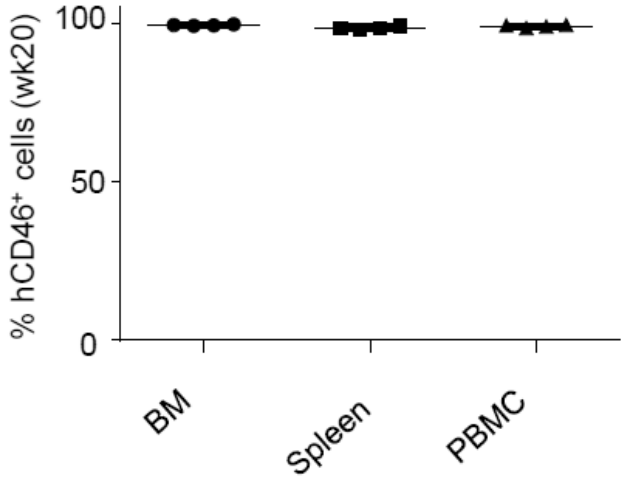


FIG. 4C

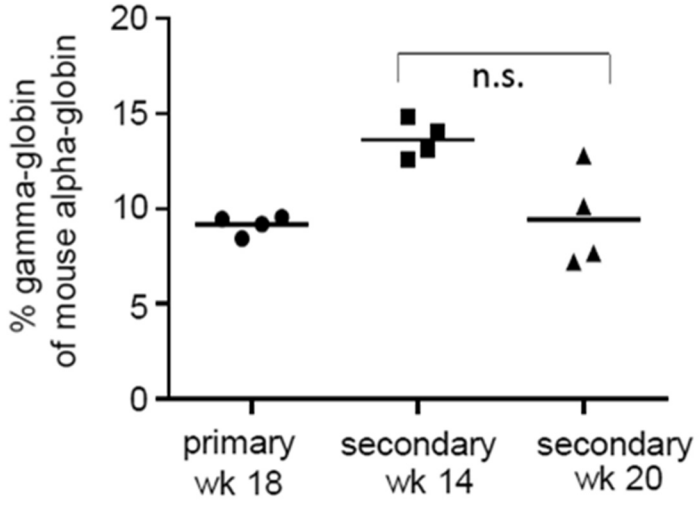


FIG. 5A

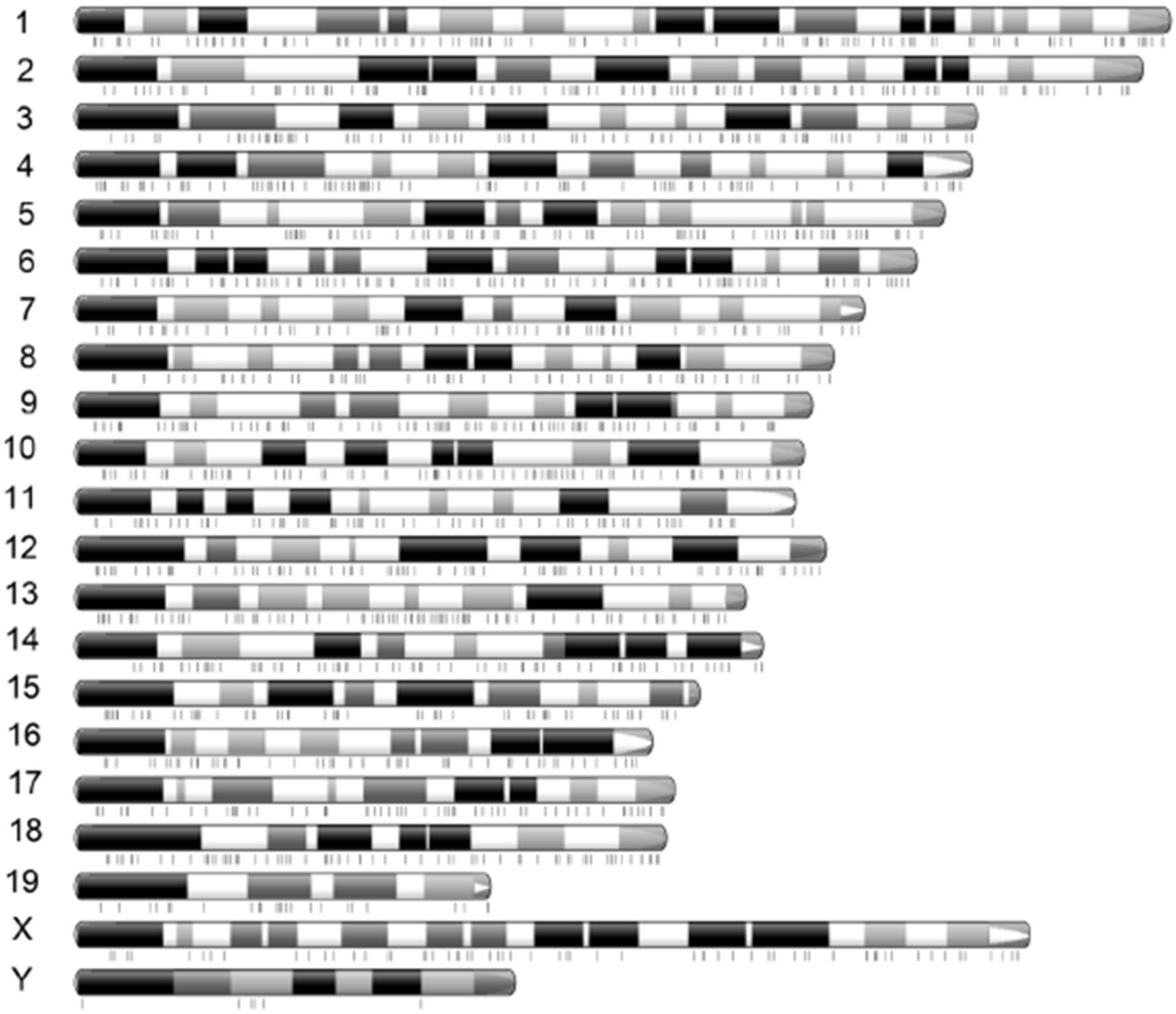


FIG. 5B

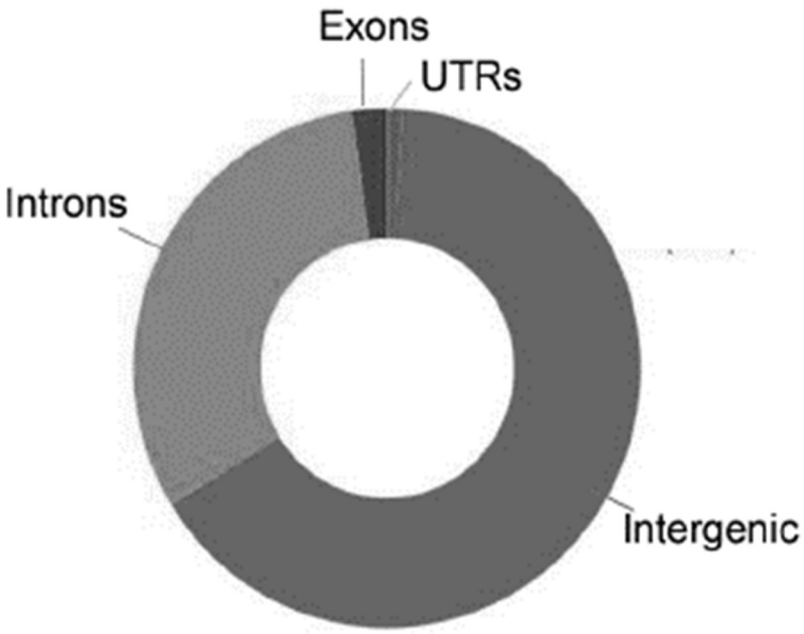

Exons
UTRs
Introns
Intergenic

FIG. 5C

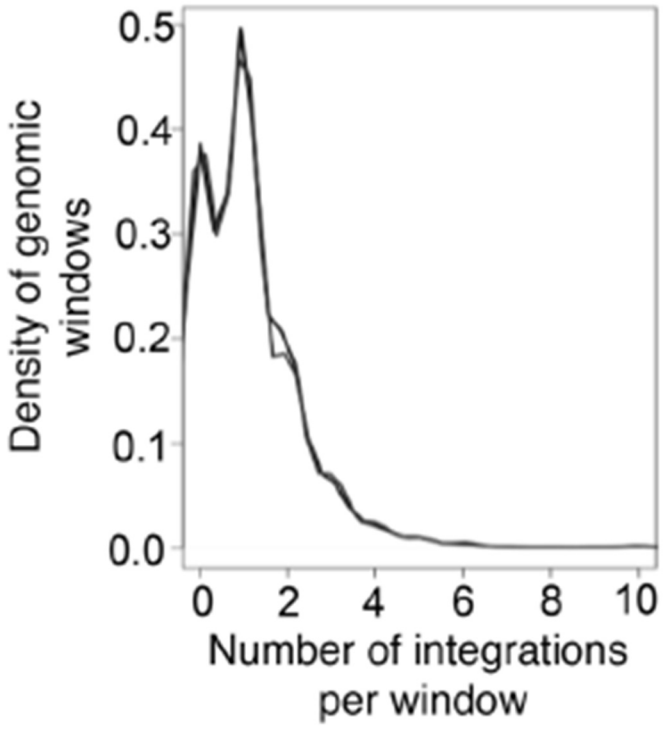

0.5
0.4
0.3
0.2
0.1
0.0
Density of genomic windows
0
2
4
6
8
10
Number of integrations per window

FIG. 5D

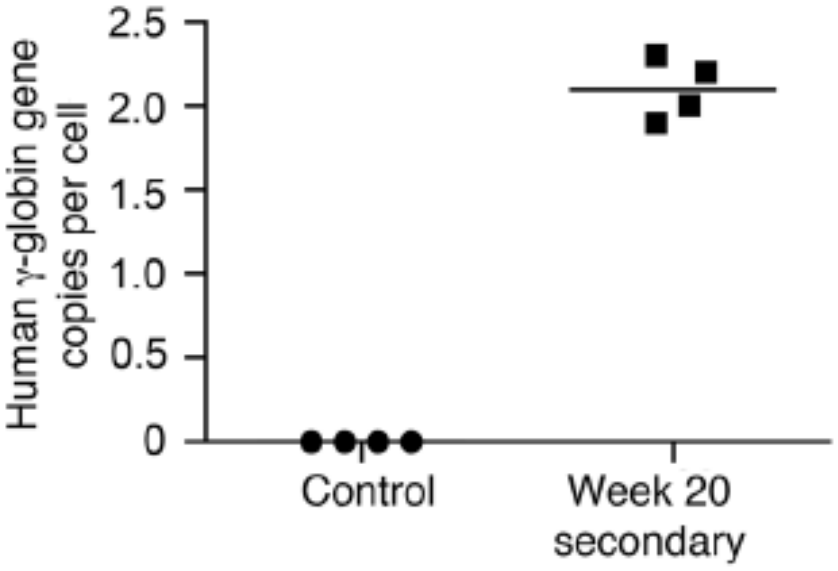

Human γ-globin gene copies per cell
2.5
2.0
1.5
1.0
0.5
0
Control
Week 20 secondary

FIG. 5E

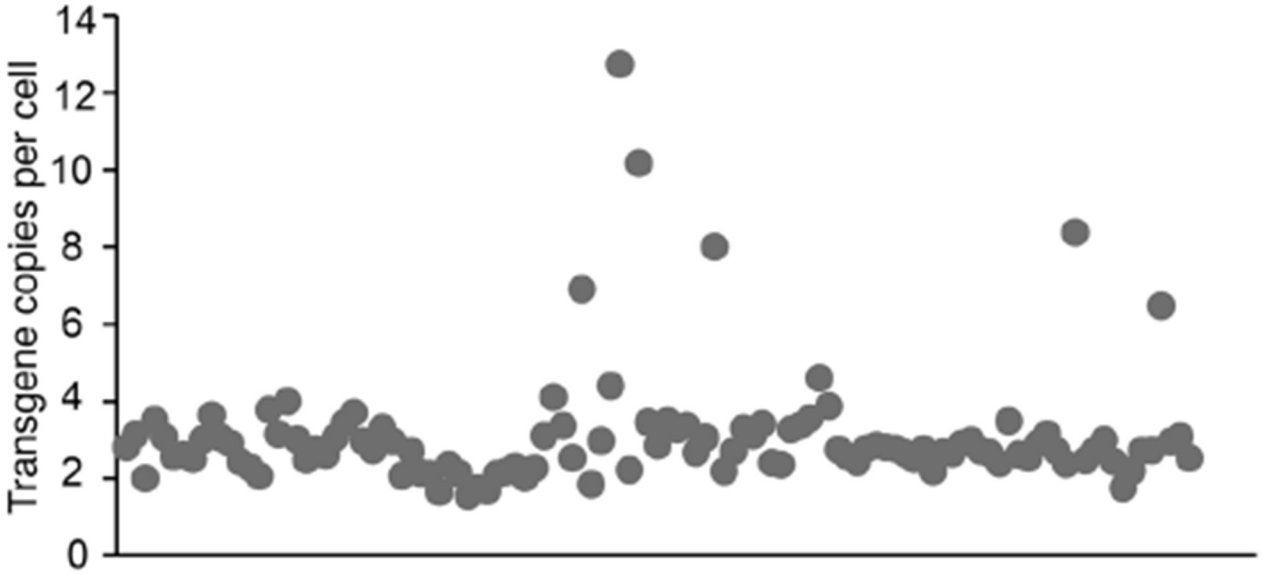
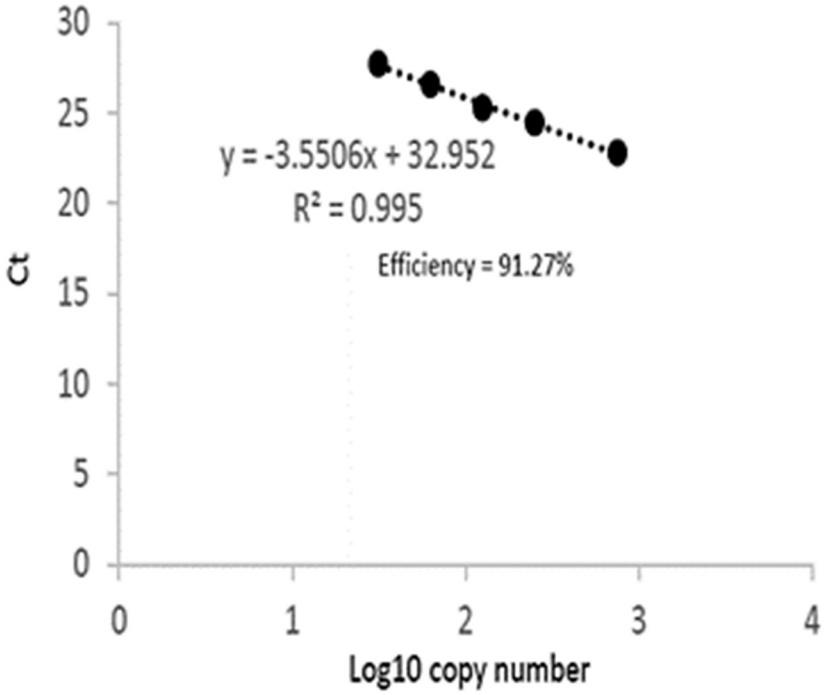

Transgene copies per cell
14
12
10
8
6
4
2
0

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

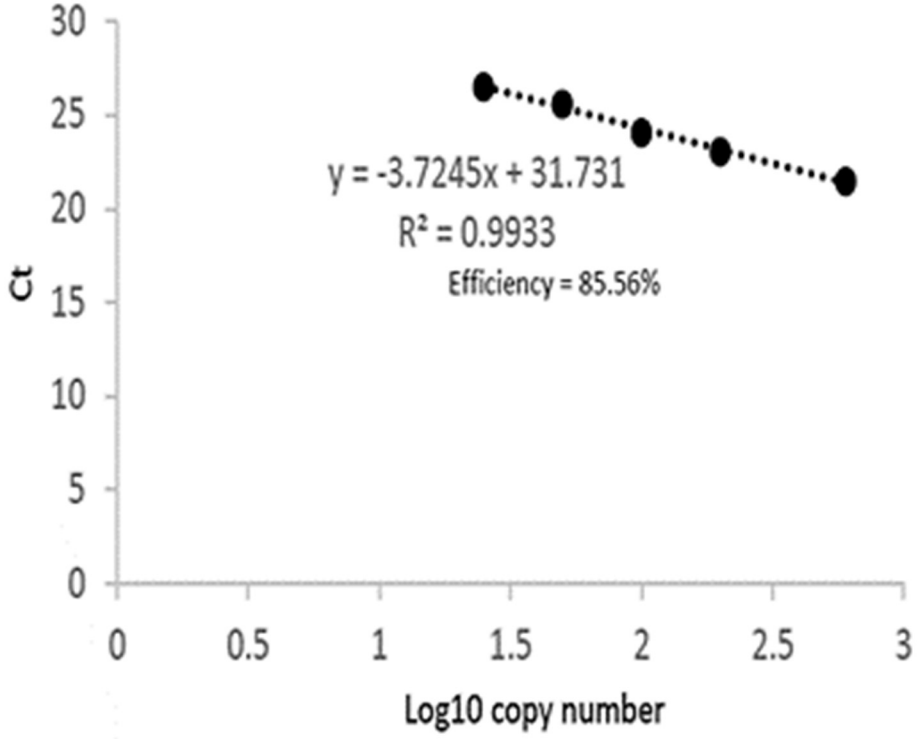

30
25
20
15
10
5
0
Ct
y = -3.7245x + 31.731
R² = 0.9933
Efficiency = 85.56%
0
0.5
1
1.5
2
2.5
3
Log10 copy number

FIG. 7A

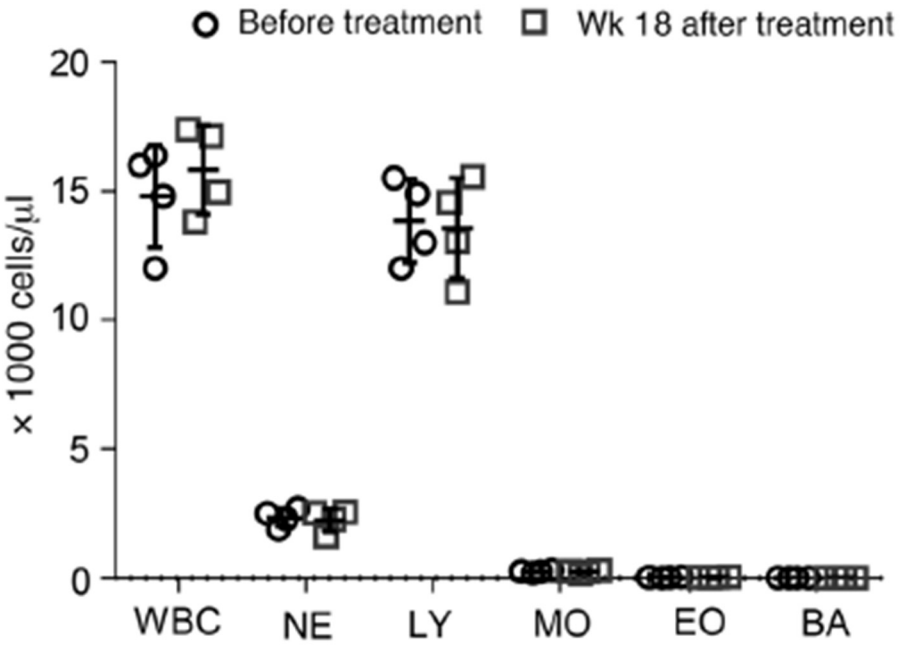

Before treatment
Wk 18 after treatment
20
15
10
5
0
× 1000 cells/μl
WBC
NE
LY
MO
EO
BA

FIG. 7B

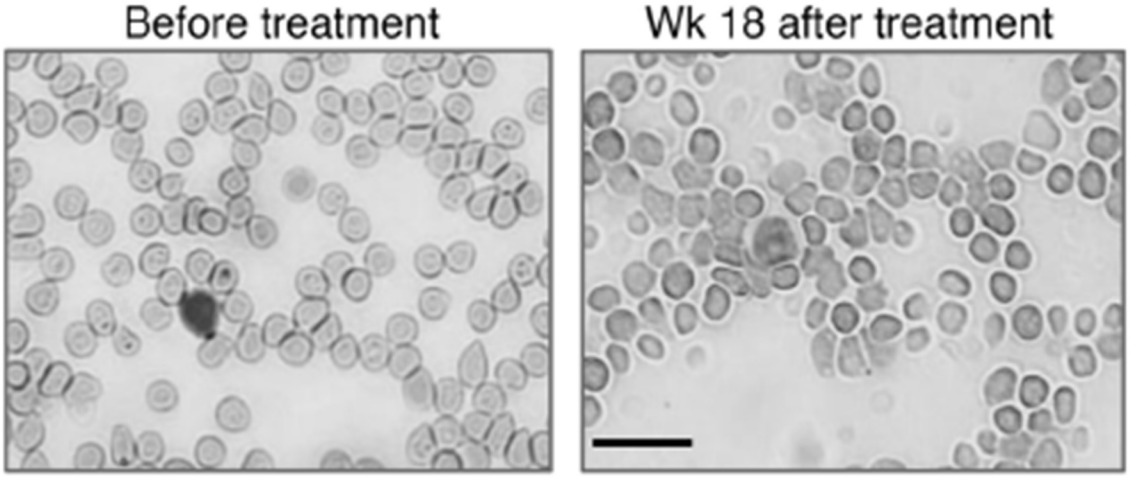

Before treatment
Wk 18 after treatment

FIG. 7C

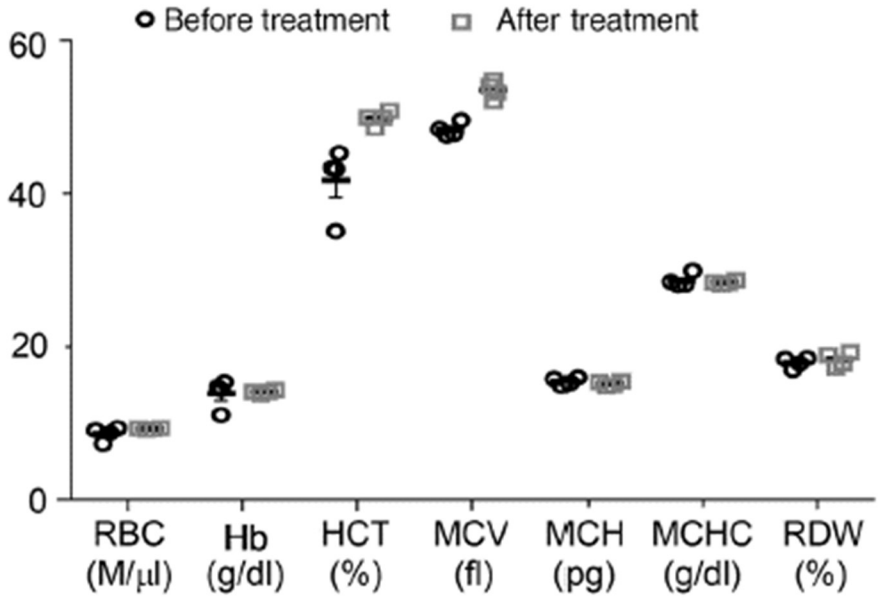

Before treatment
After treatment
60
40
20
0
RBC (M/μl)
Hb (g/dl)
HCT (%)
MCV (fl)
MCH (pg)
MCHC (g/dl)
RDW (%)

FIG. 7D

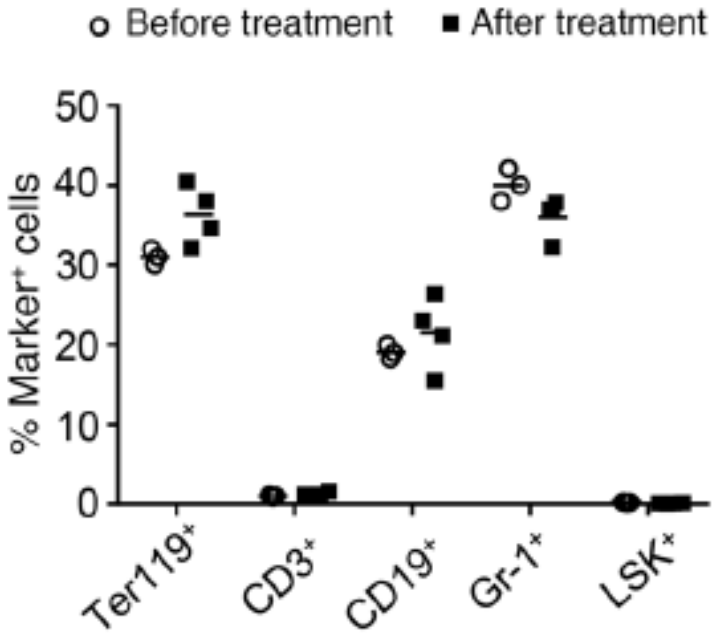

Before treatment
After treatment
% Marker+ cells
50
40
30
20
10
0
Ter119+
CD3+
CD19+
Gr-1+
LSK+

FIG. 7E

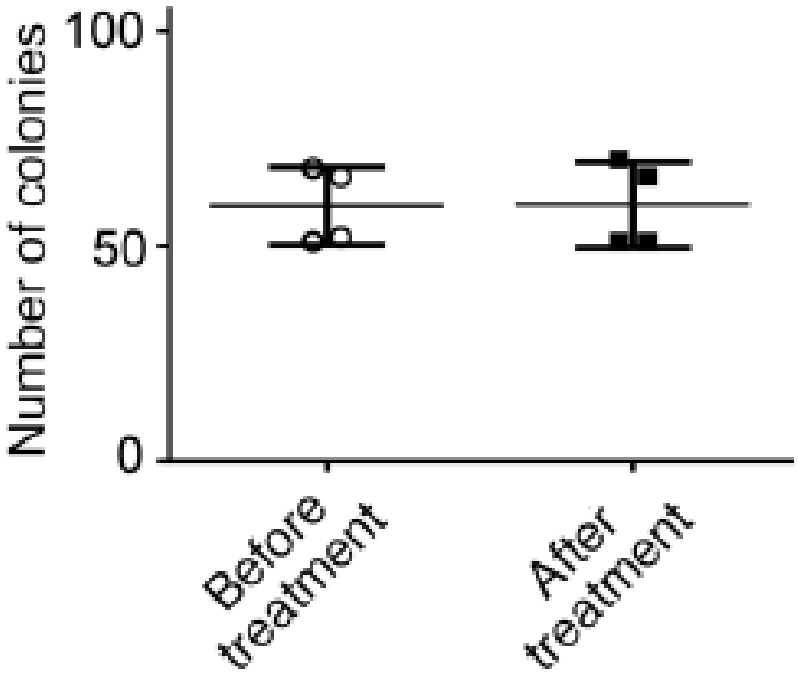

Number of colonies
100
50
0
Before treatment
After treatment

FIG. 8

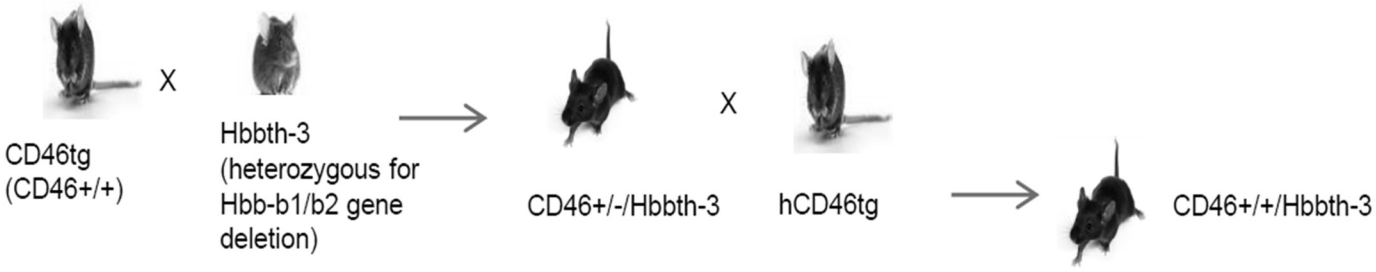

X
CD46tg
(CD46+/+)
Hbbth-3
(heterozygous for
Hbb-b1/b2 gene
deletion)
CD46+/-/Hbbth-3
X
hCD46tg
CD46+/+/Hbbth-3

FIG. 9A

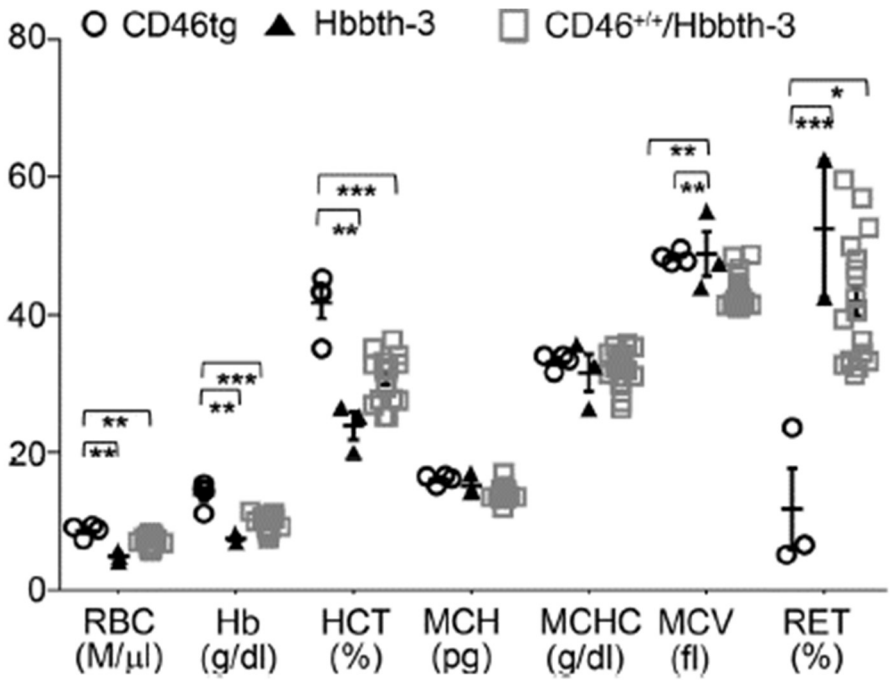

CD46tg
Hbbth-3
CD46+/+/Hbbth-3
80
60
40
20
0
RBC (M/μl)
Hb (g/dl)
HCT (%)
MCH (pg)
MCHC (g/dl)
MCV (fl)
RET (%)

FIG. 9B

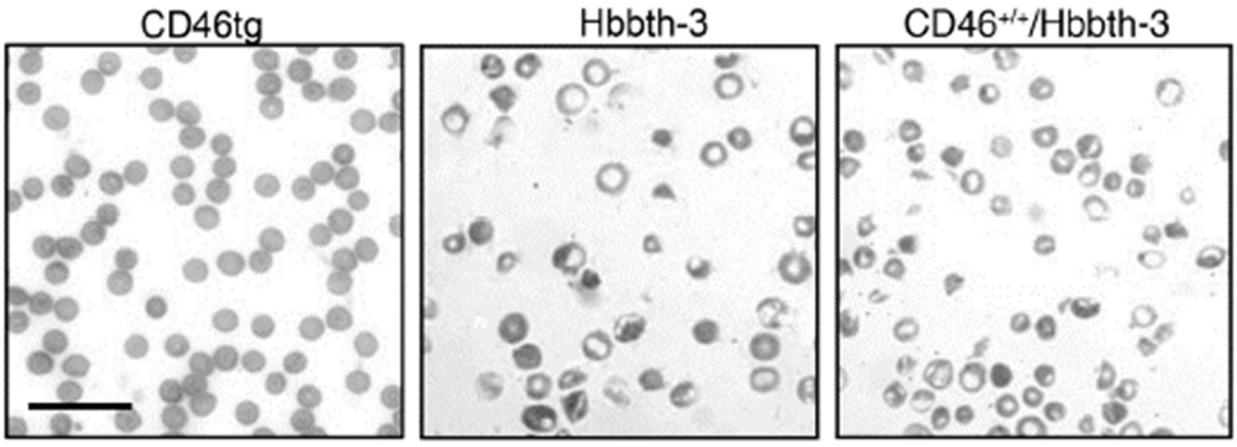

CD46tg
Hbbth-3
CD46+/+/Hbbth-3

FIG. 9C

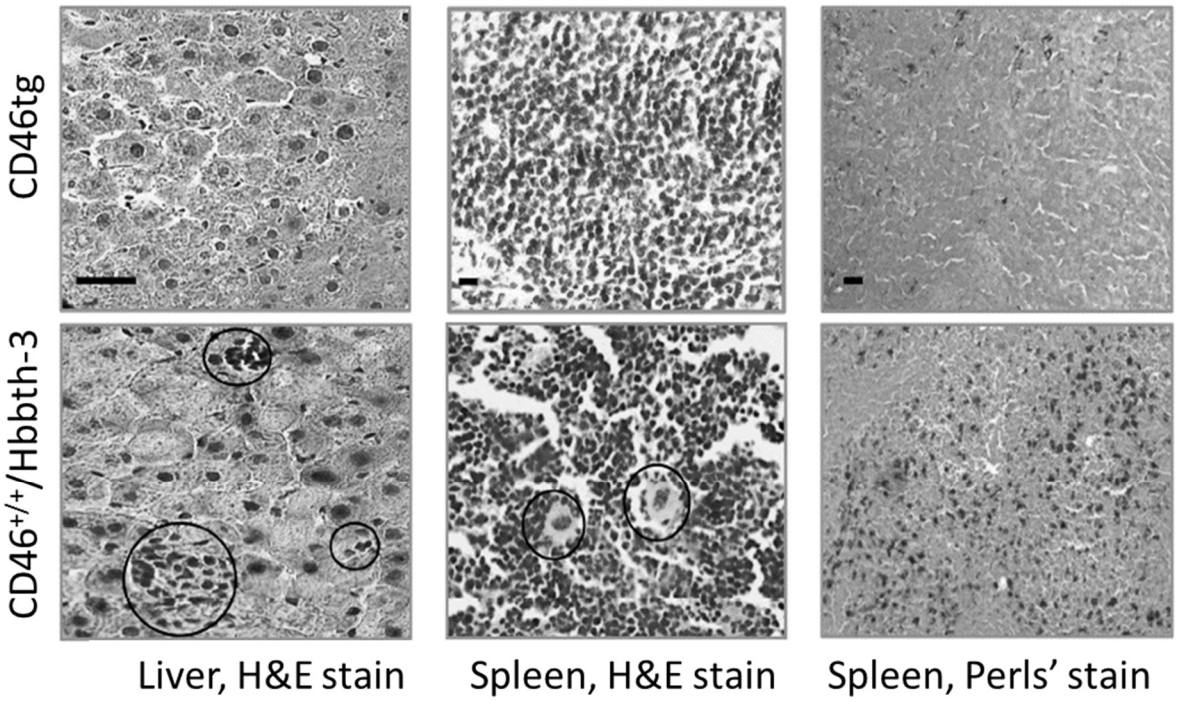

CD46tg
CD46+/+/Hbbth-3
Liver, H&E stain
Spleen, H&E stain
Spleen, Perls' stain

FIG. 10

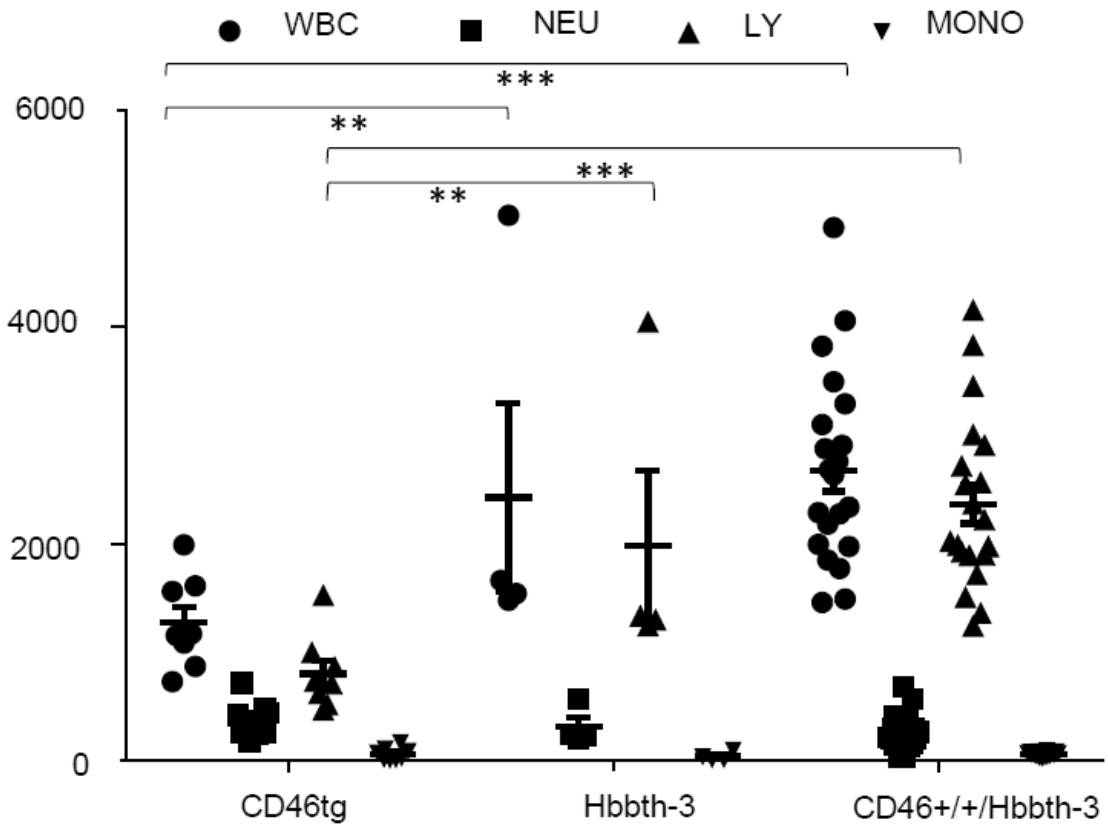

WBC
NEU
LY
MONO
***
**
***
**
6000
4000
2000
0
CD46tg
Hbbth-3
CD46+/+/Hbbth-3

FIG. 11

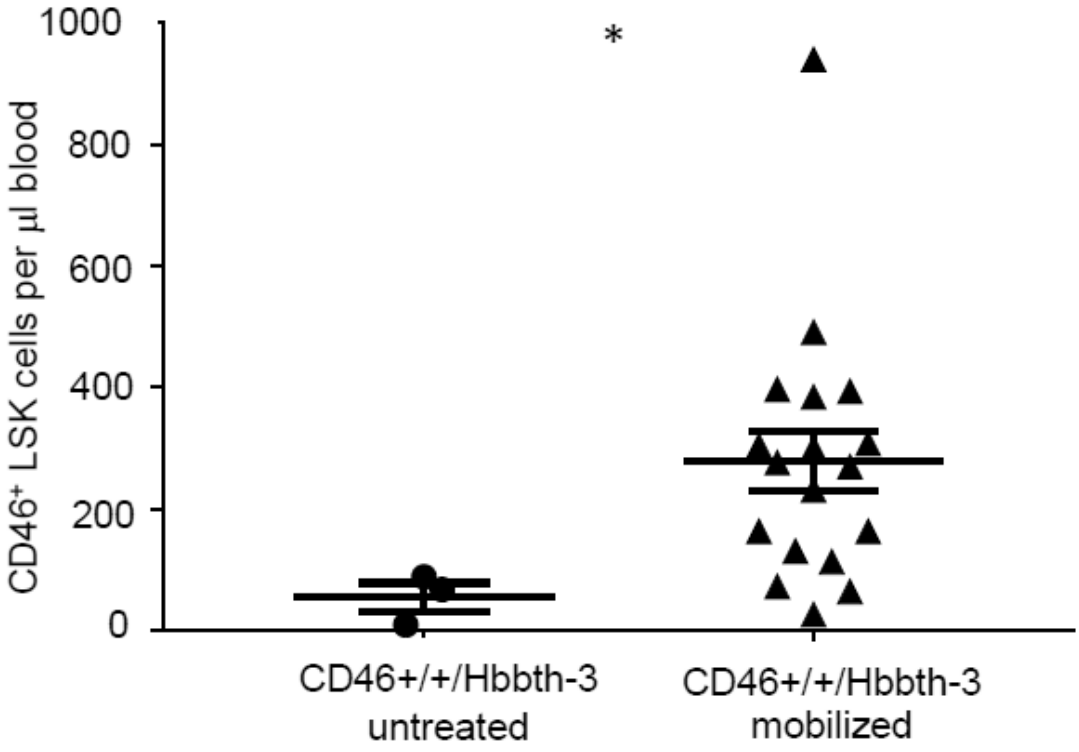

1000
800
600
400
200
0
CD46+ LSK cells per µl blood
*
CD46+/+/Hbbth-3
untreated
CD46+/+/Hbbth-3
mobilized

FIG. 12

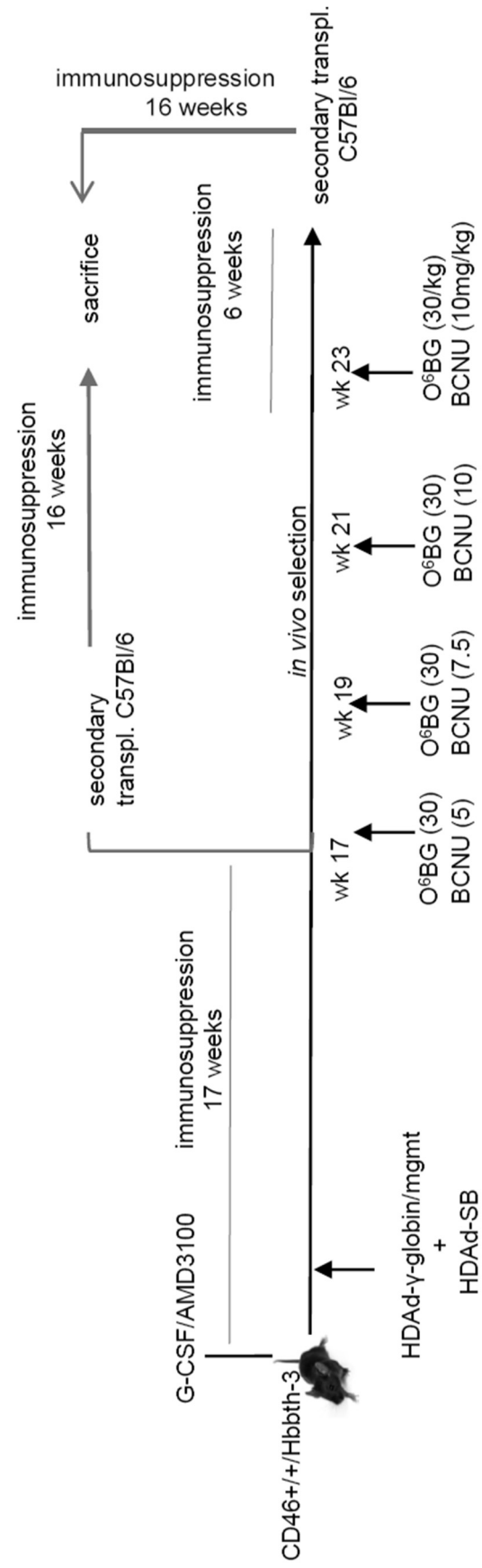

immunosuppression
16 weeks
secondary transpl.
C57Bl/6
immunosuppression
6 weeks
immunosuppression
16 weeks
sacrifice
secondary
transpl. C57Bl/6
in vivo selection
immunosuppression
17 weeks
G-CSF/AMD3100
CD46+/+/Hbbth-3
HDAd-γ-globin/mgmt
+
HDAd-SB
(2x10^10 vp, twice
IV
wk 17
O6BG (30)
BCNU (5)
wk 19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

FIG. 13A

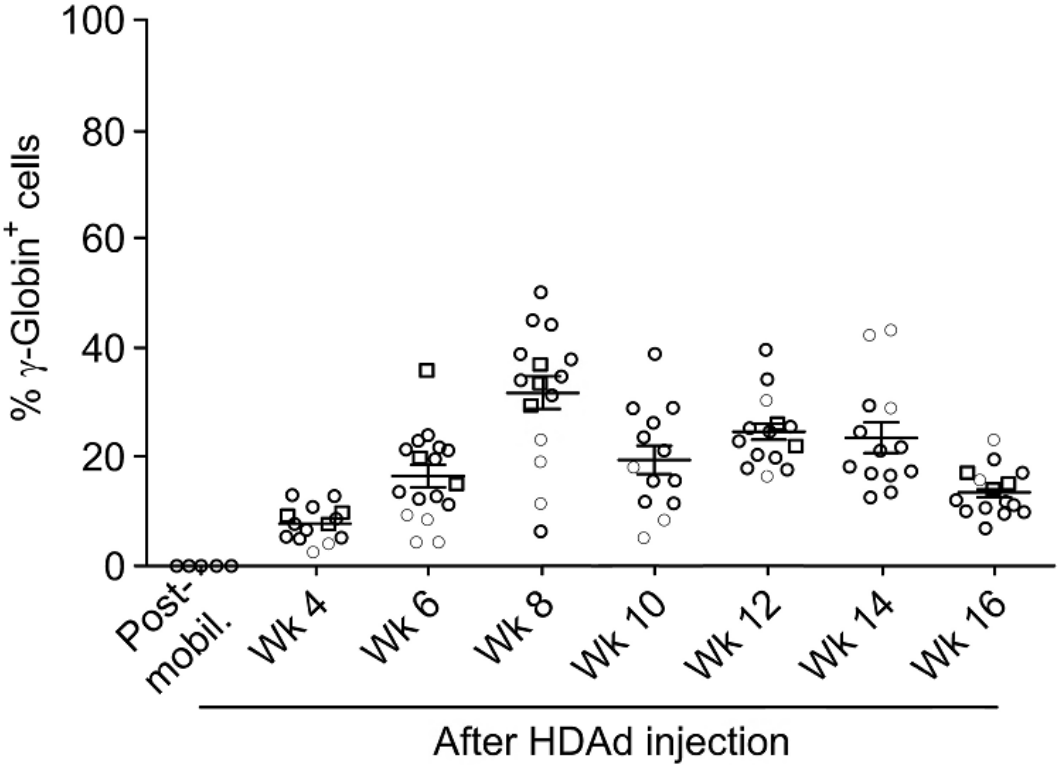


FIG. 13B

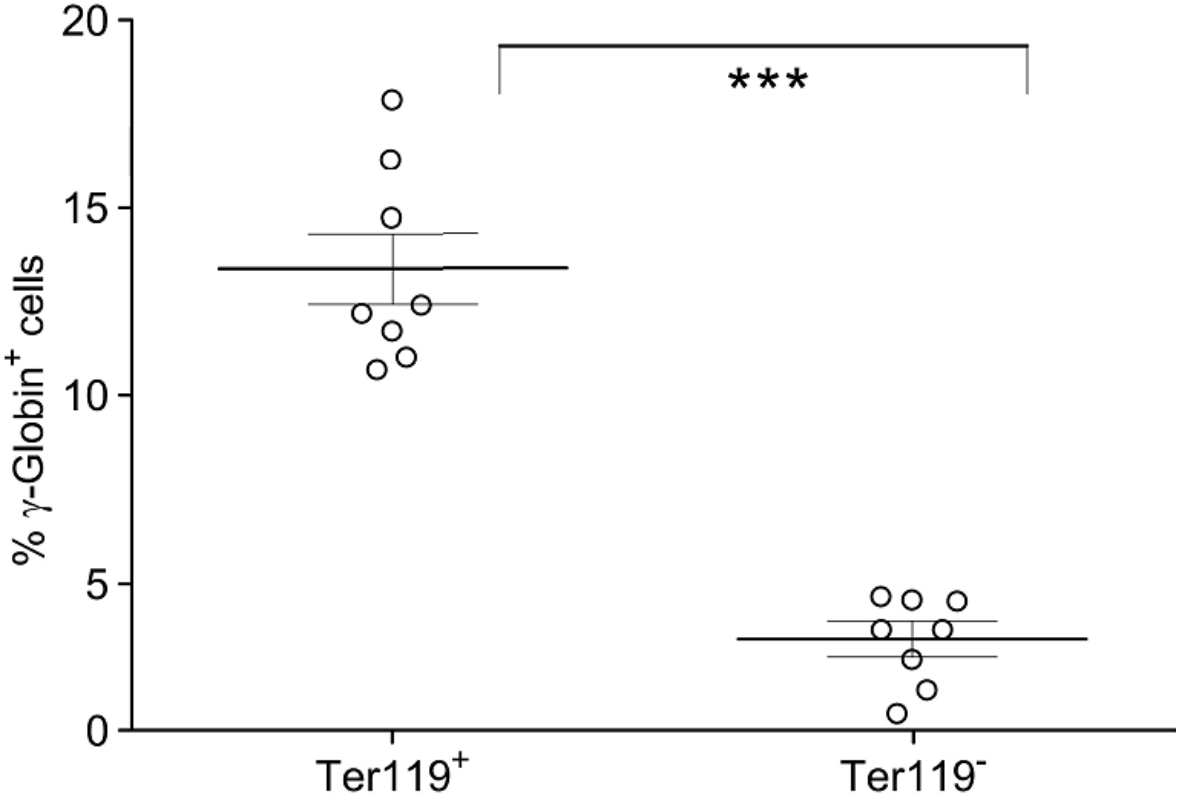


FIG. 13C

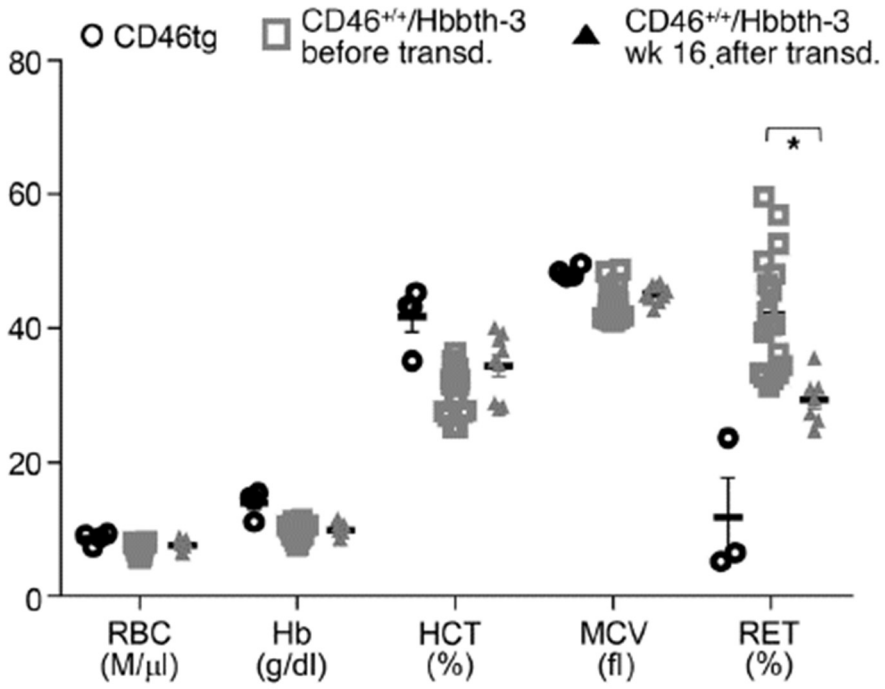


FIG. 13D

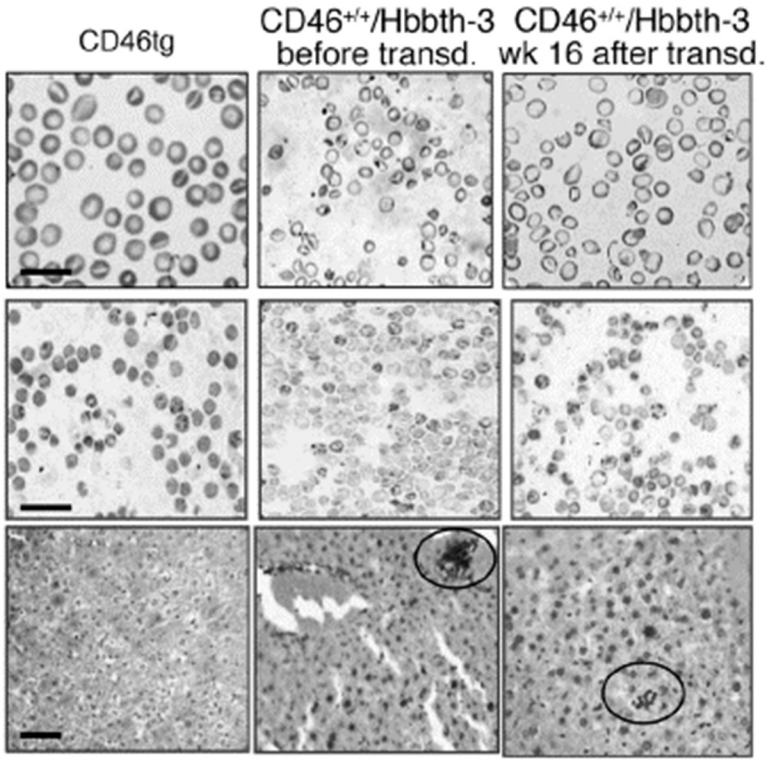


FIG. 13E

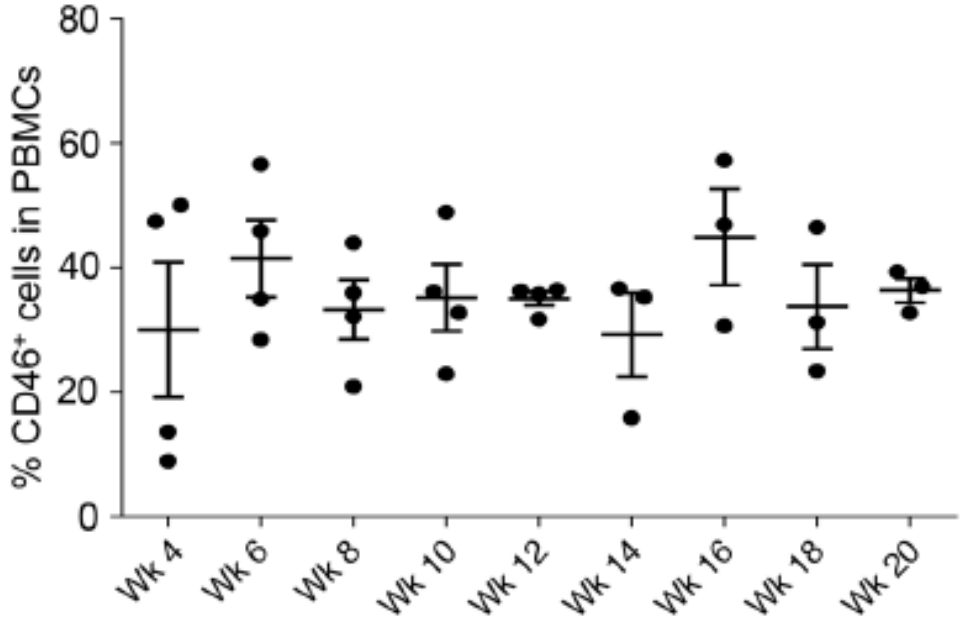


FIG. 13F

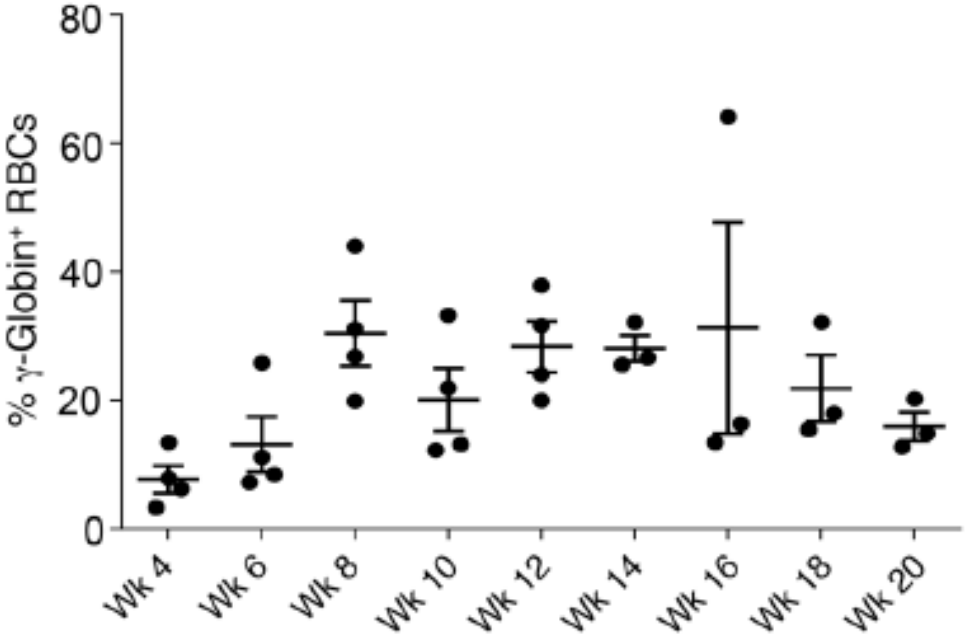


FIG. 14A

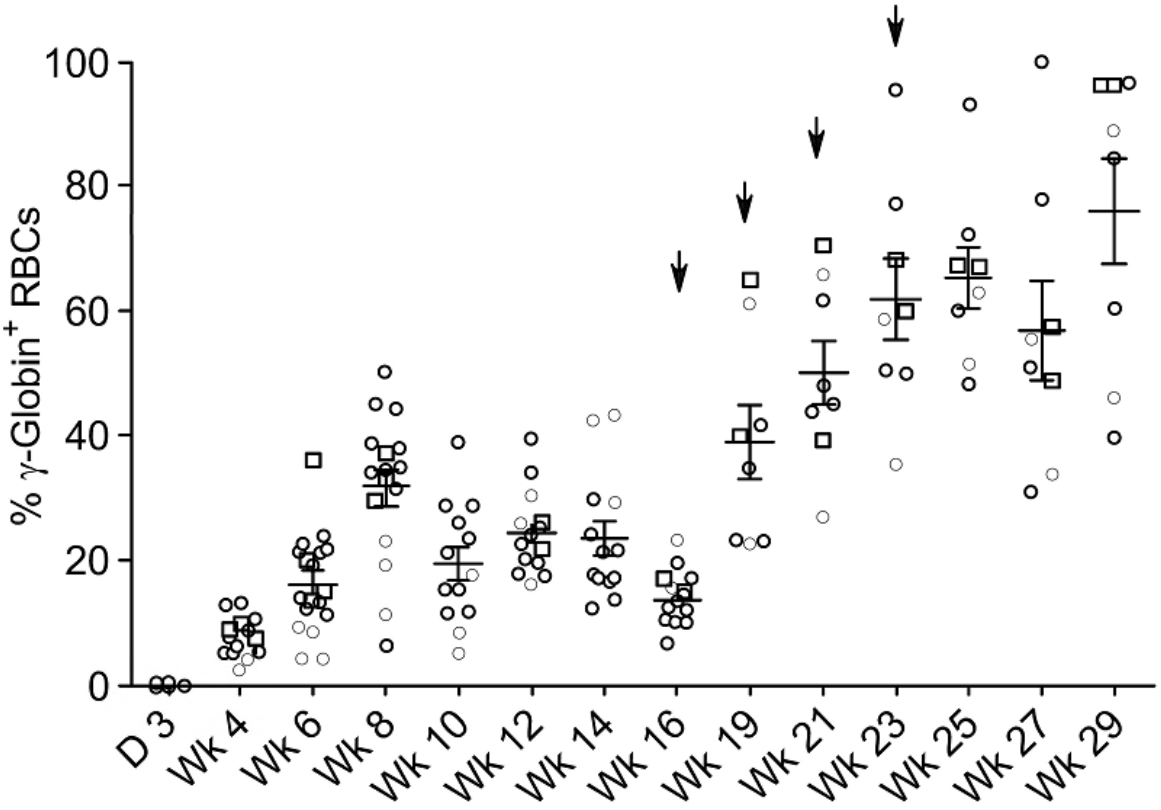


FIG. 14B

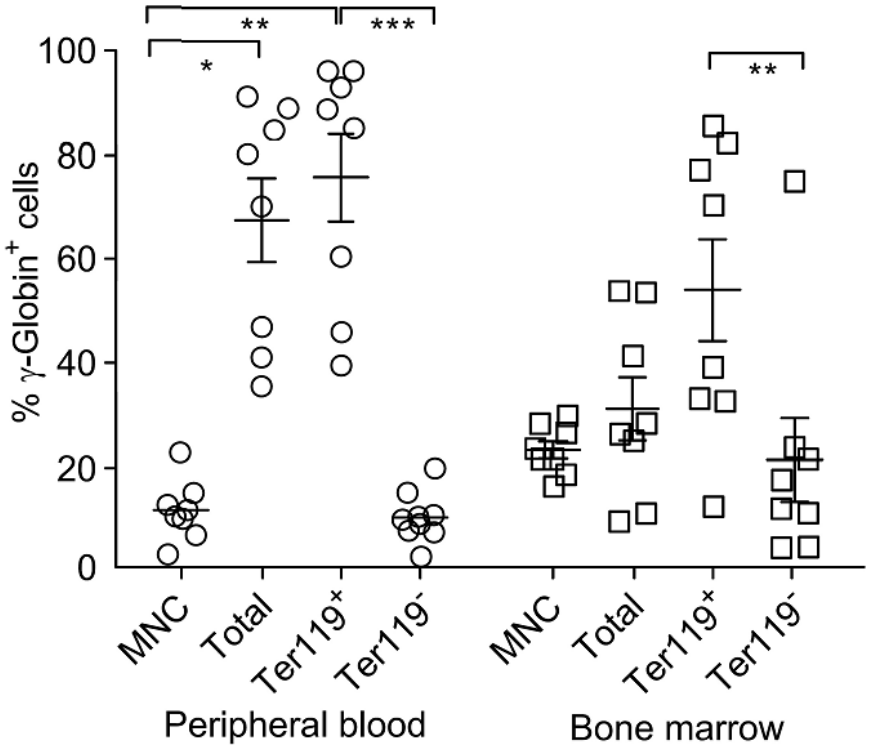


FIG. 14C

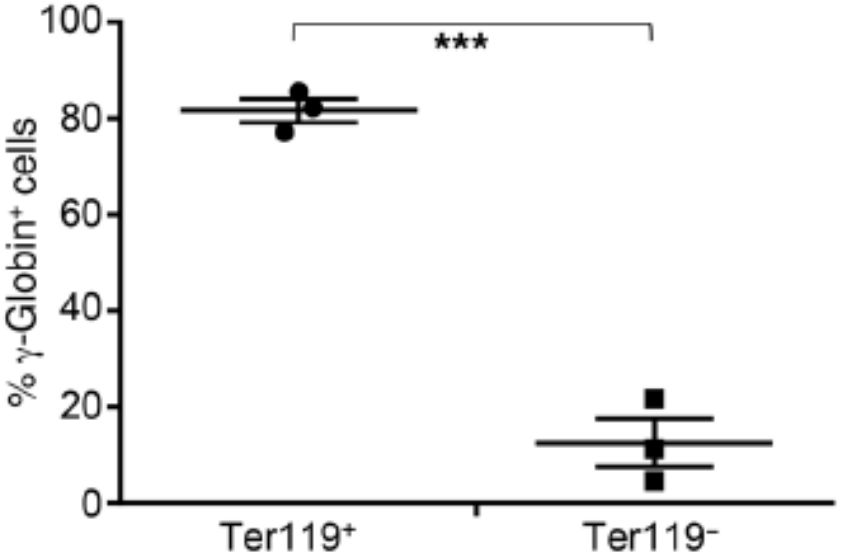


FIG. 14D

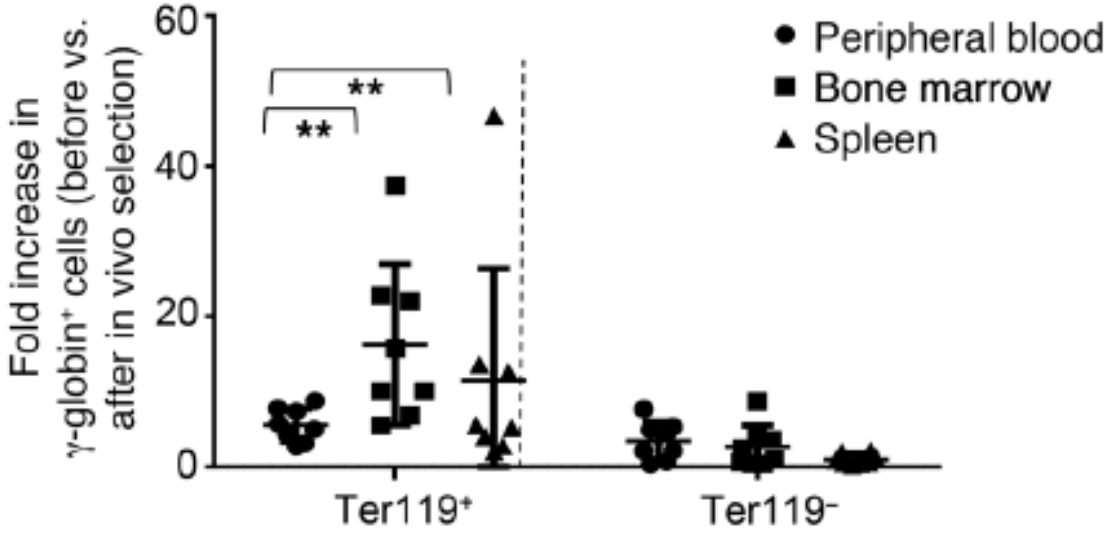


FIG. 14E

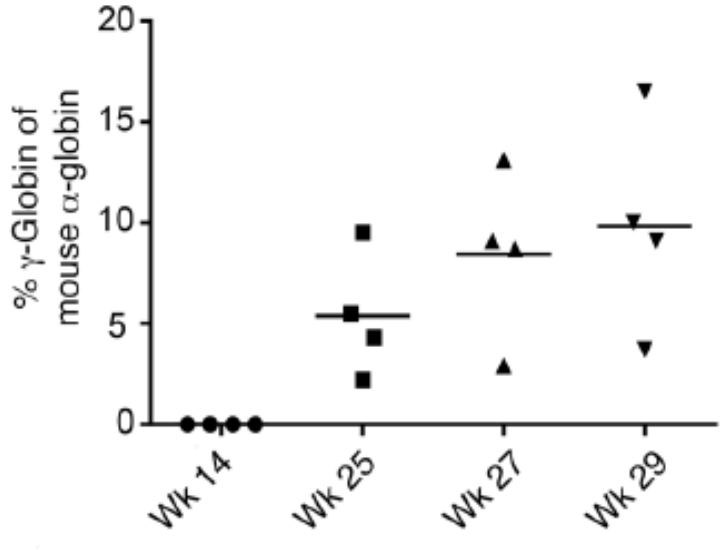


FIG. 14F

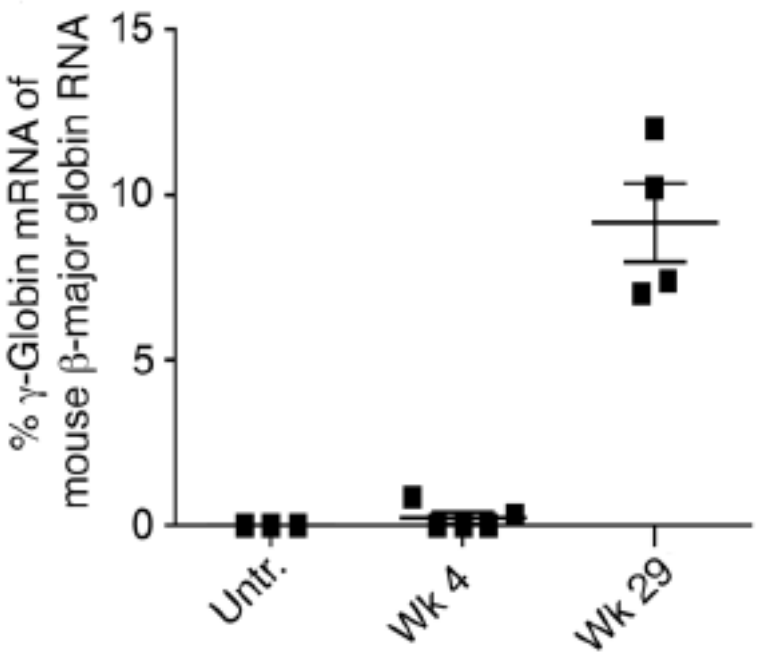


FIG. 15A

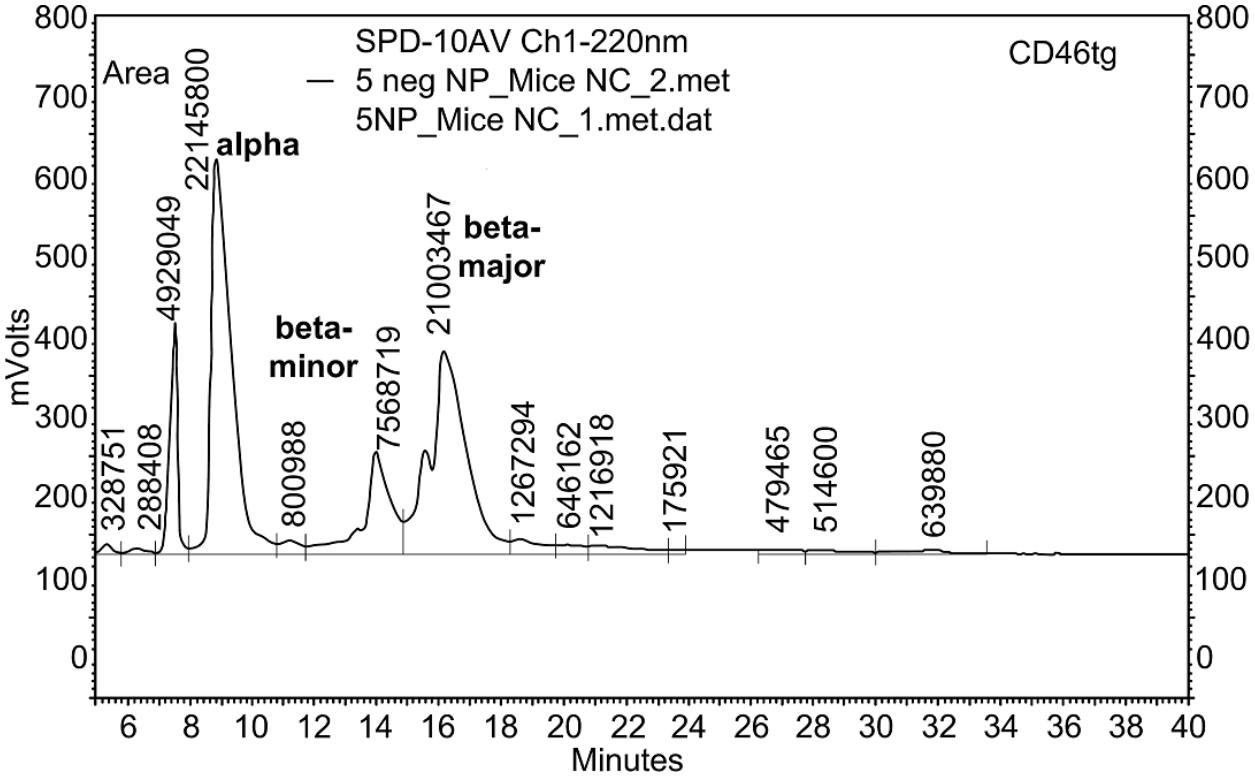


FIG. 15B

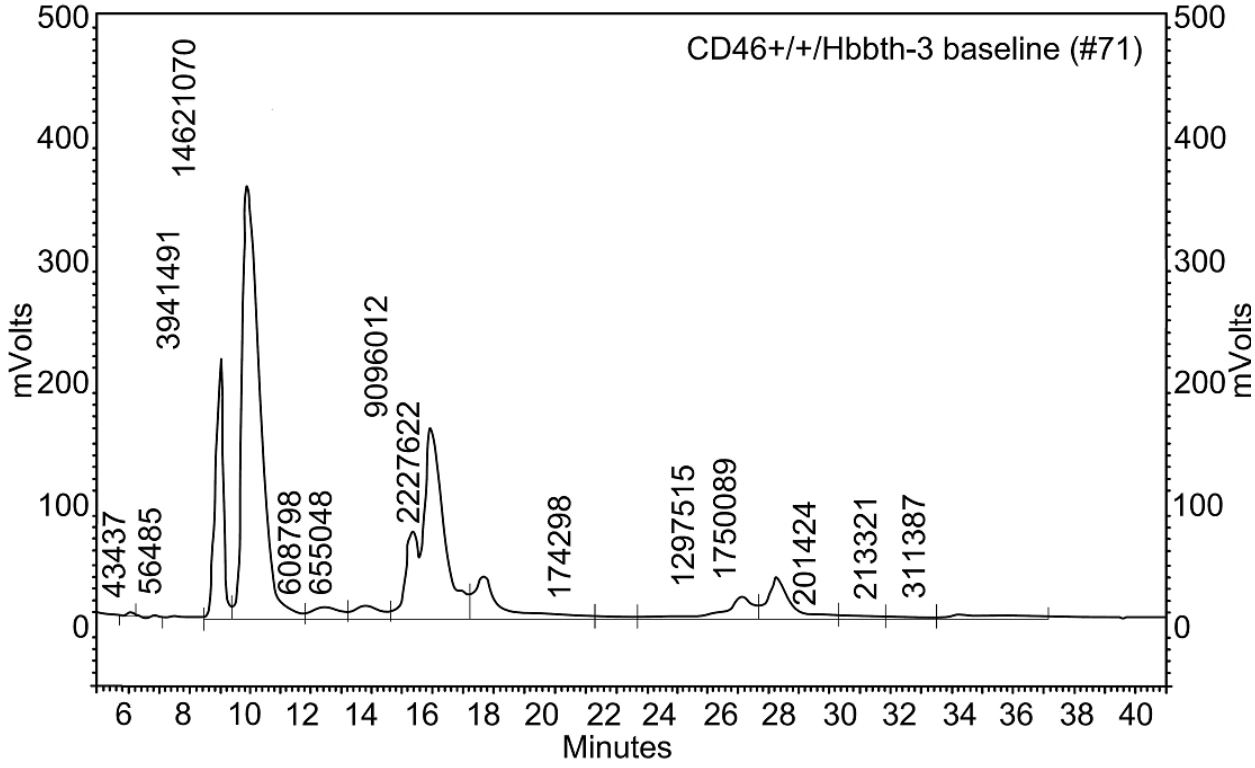


FIG. 15C

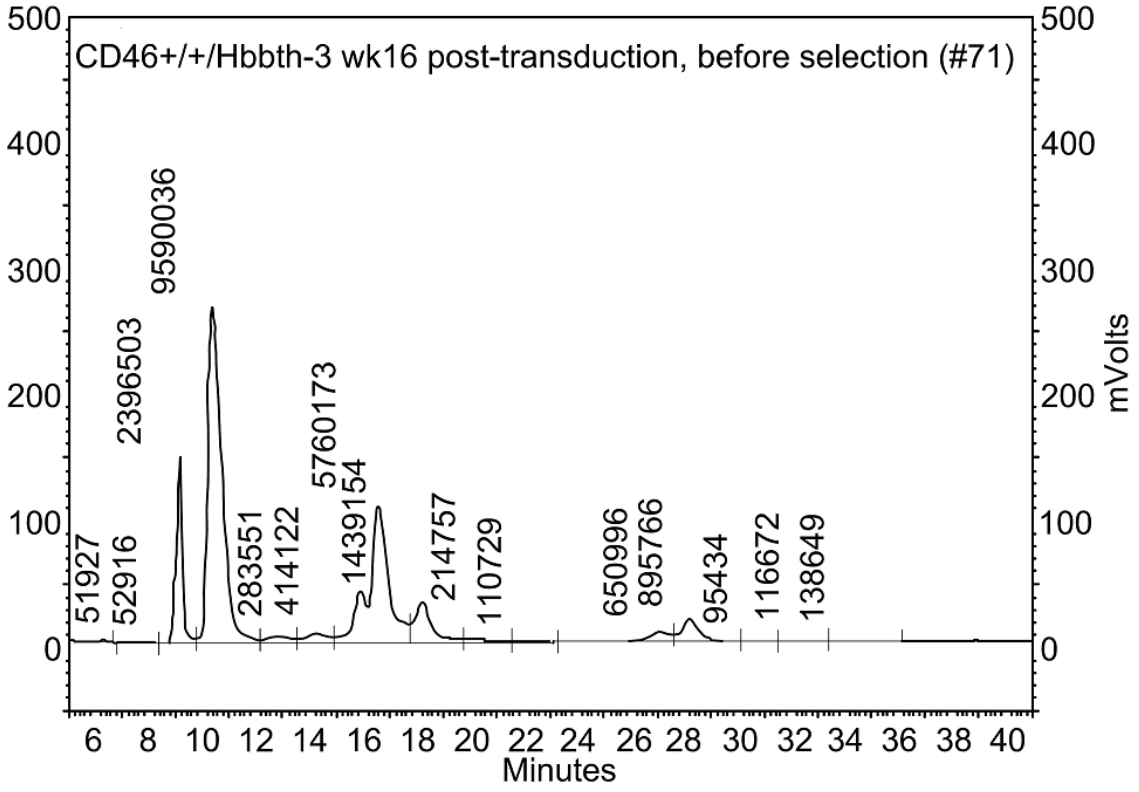


FIG. 15D

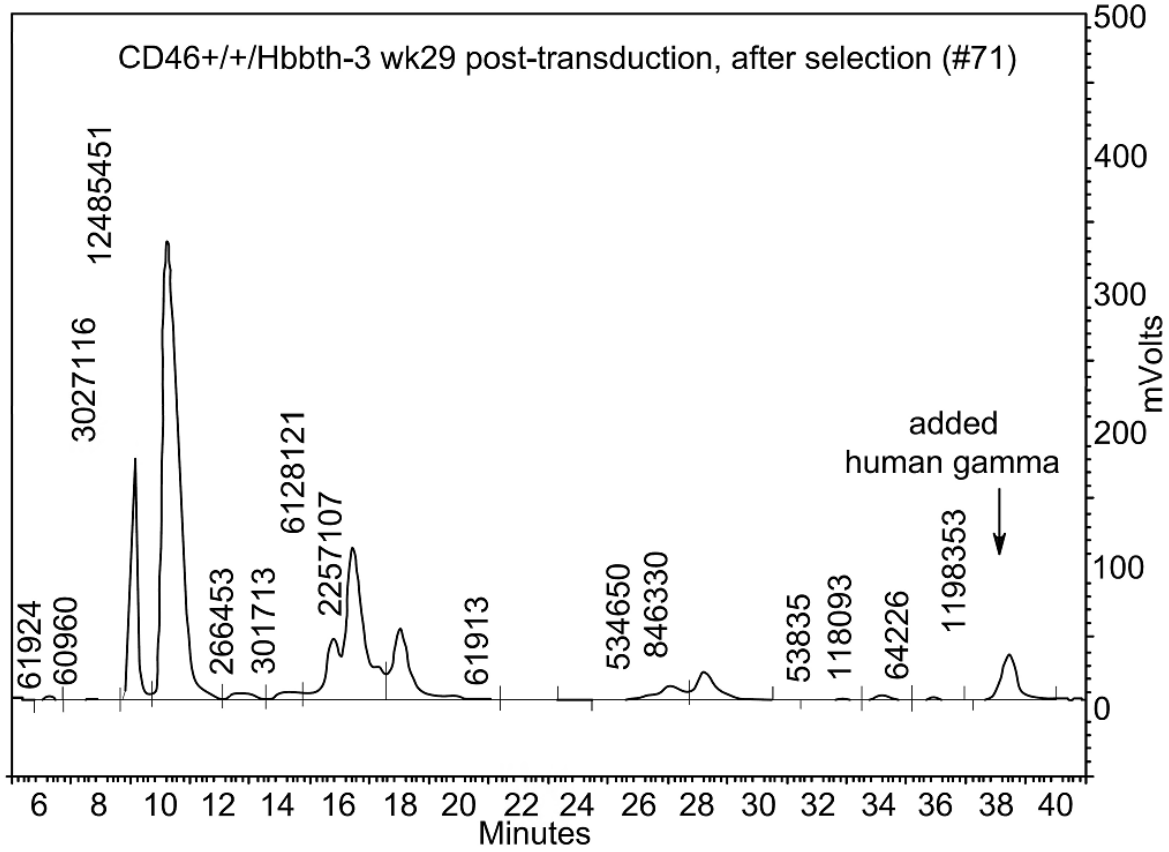


FIG. 16

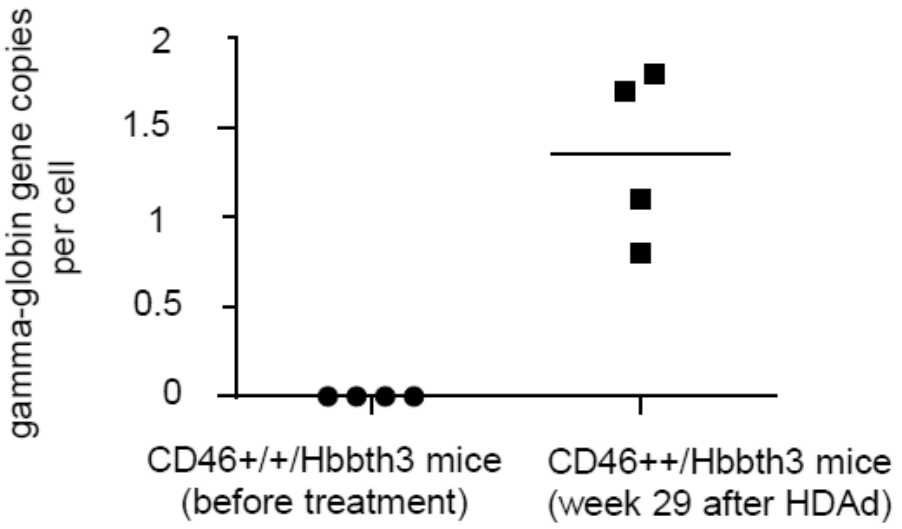


FIG. 17A

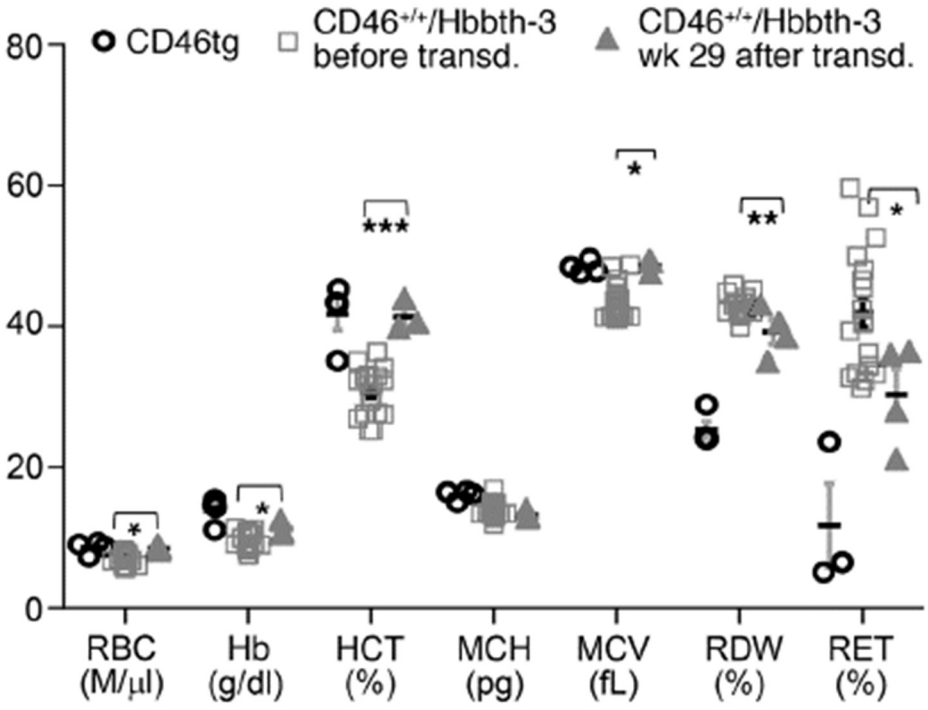


FIG. 17B

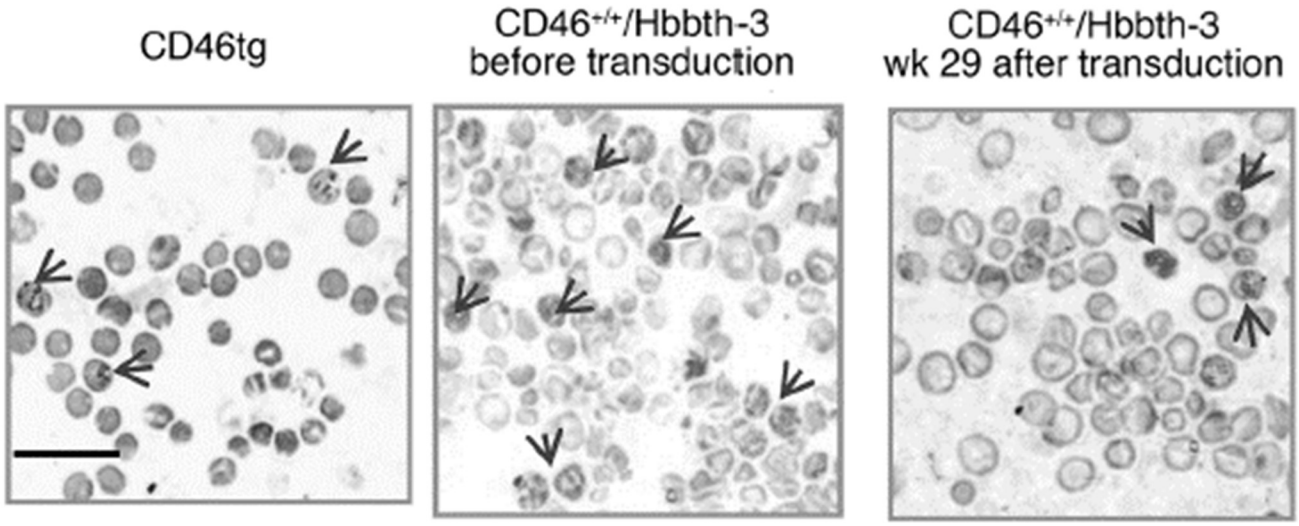


FIG. 17C

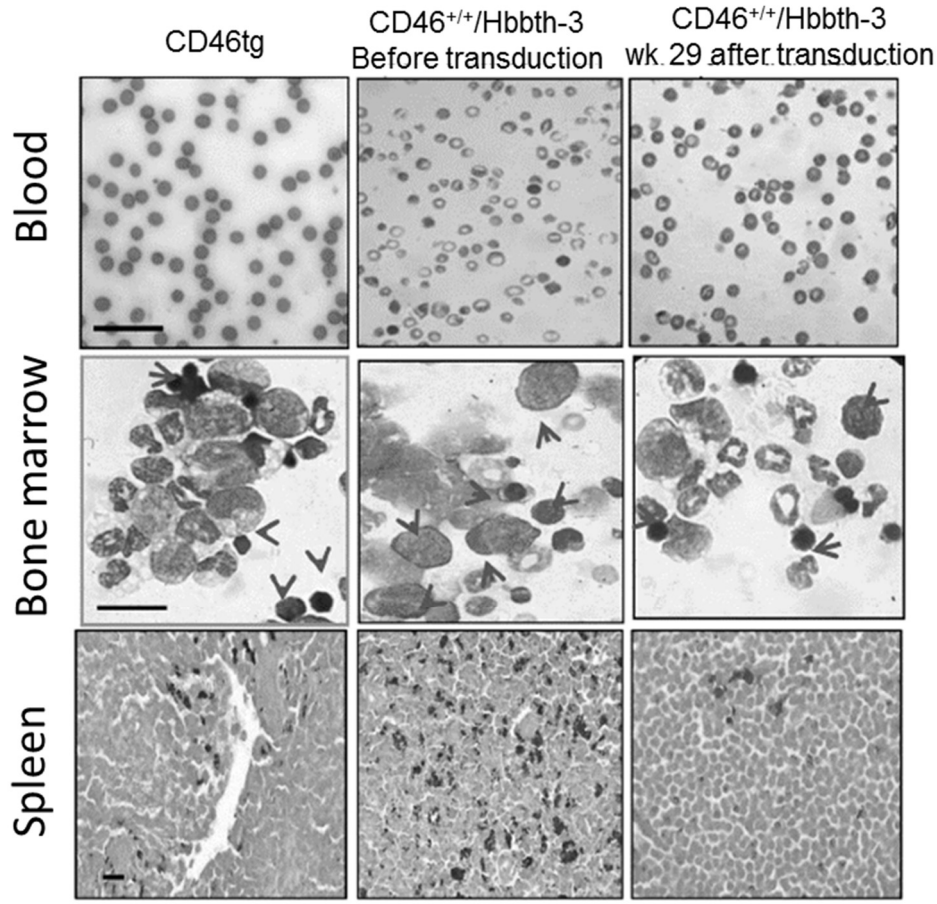


FIG. 17D

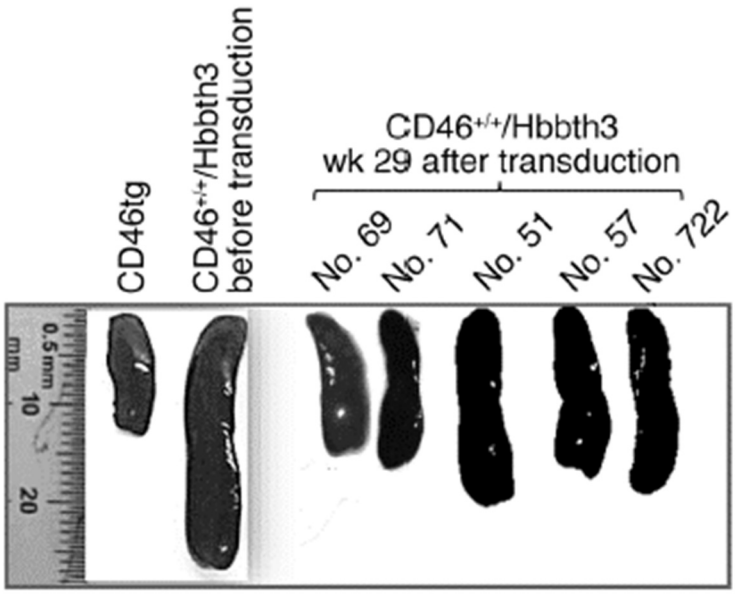


FIG. 17E

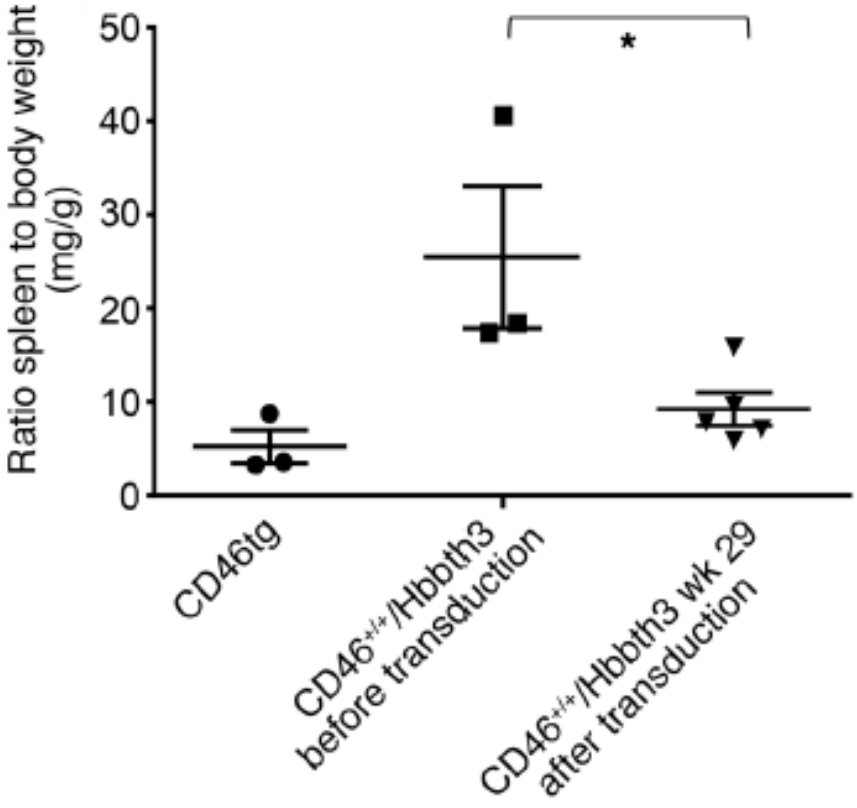


FIG. 18A

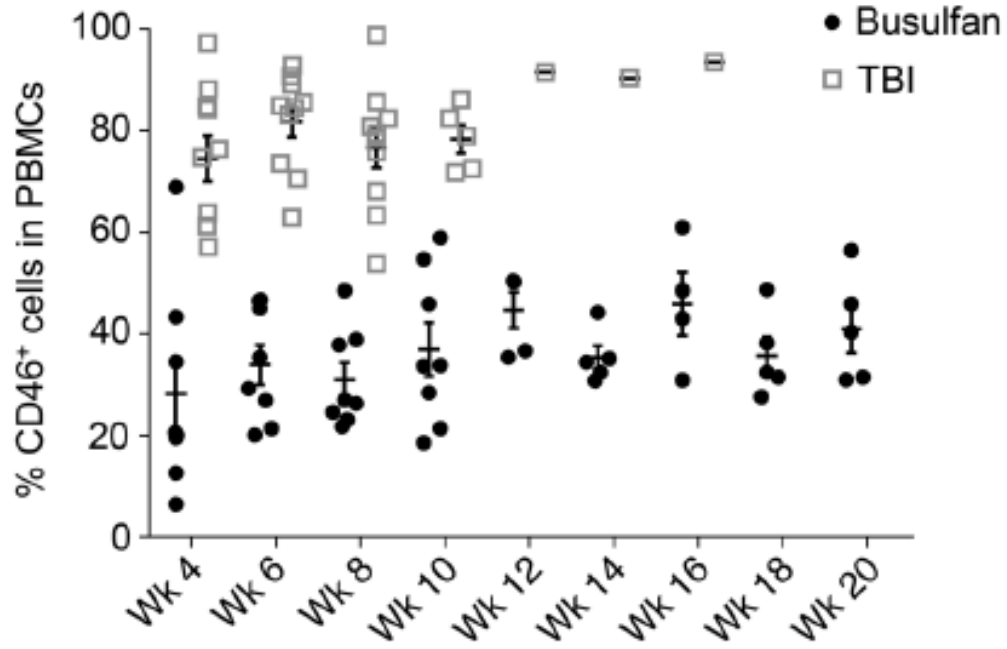


FIG. 18B

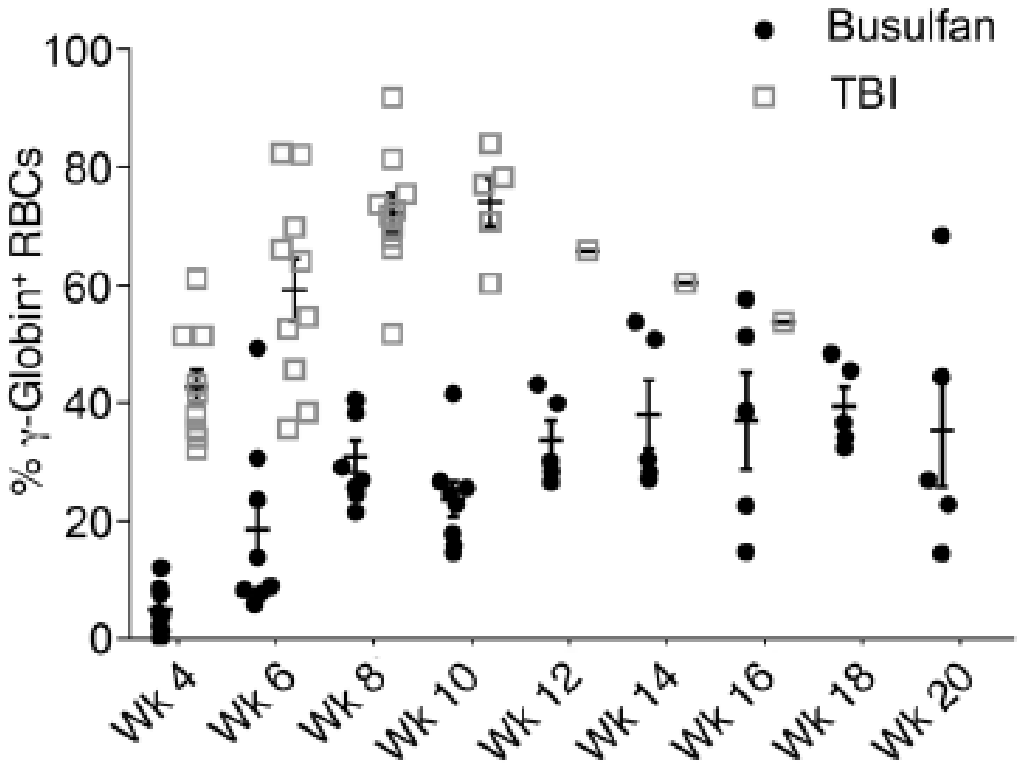


FIG. 18C

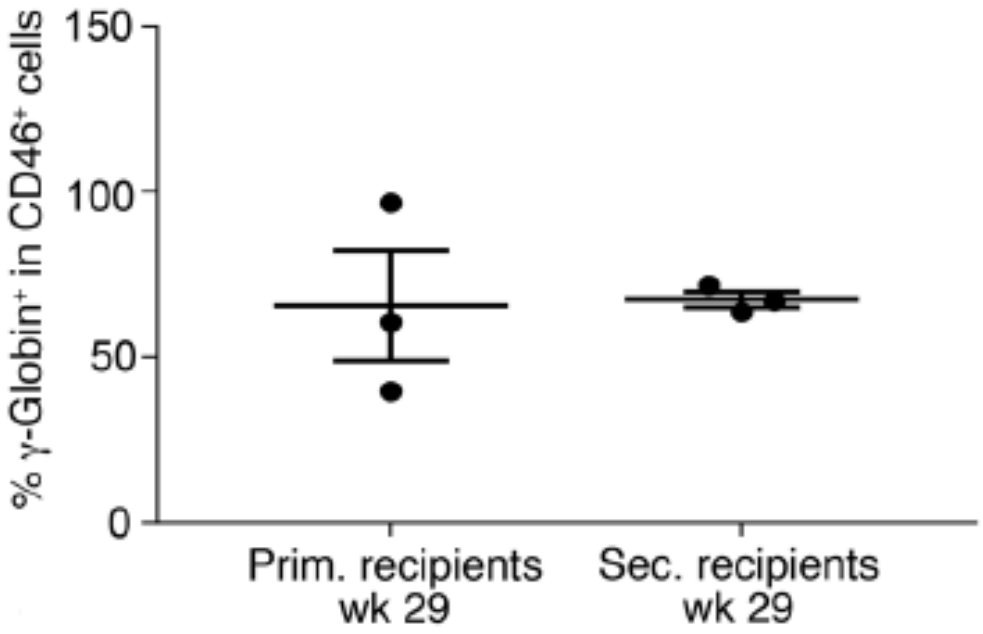


FIG. 18D

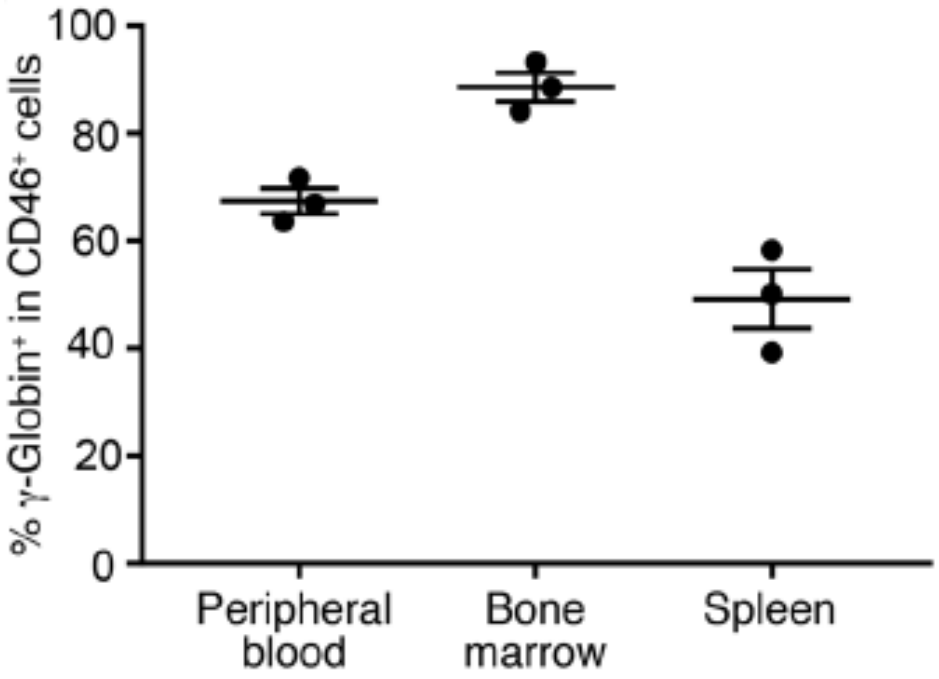


FIG. 18E

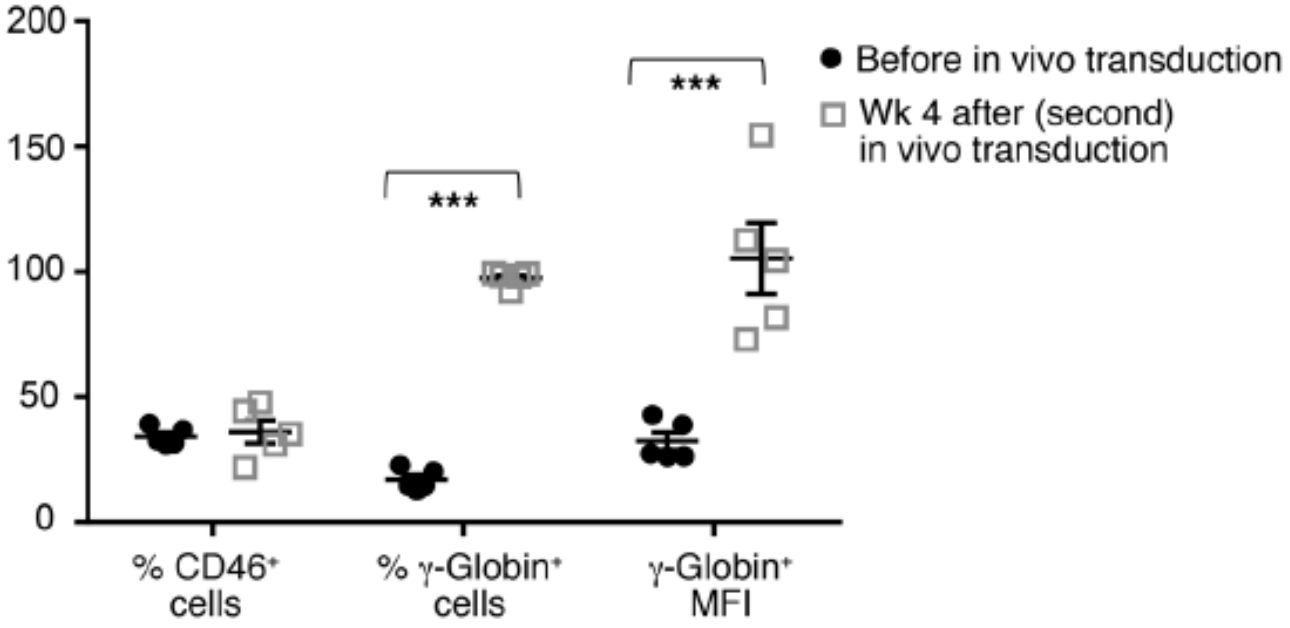


FIG. 19A

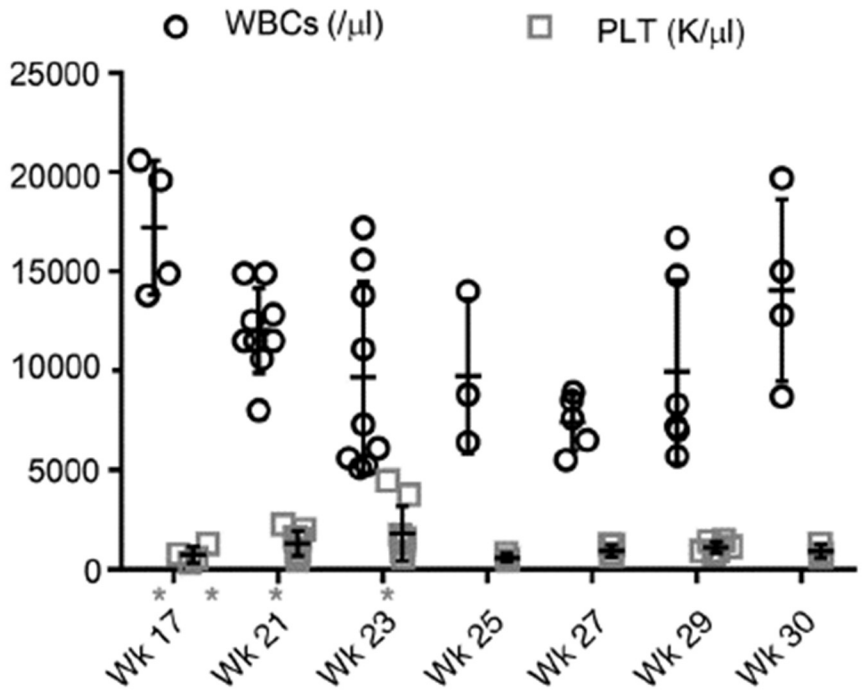


FIG. 19B

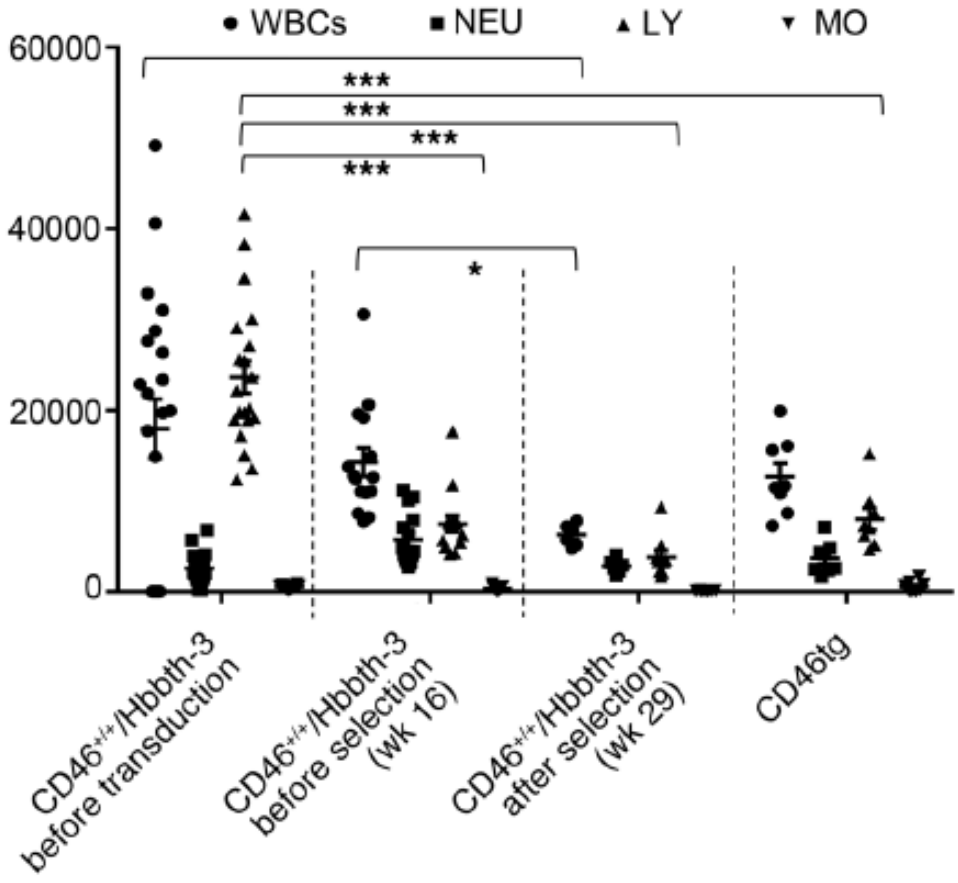


FIG. 19C

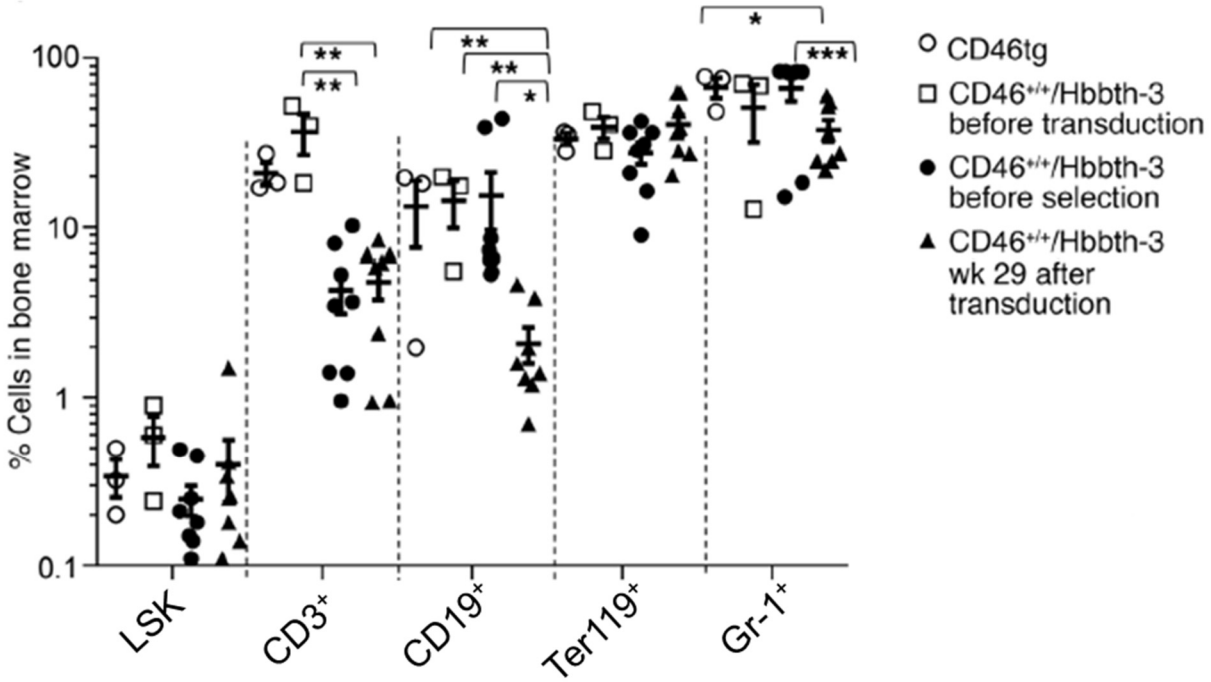


FIG. 19D

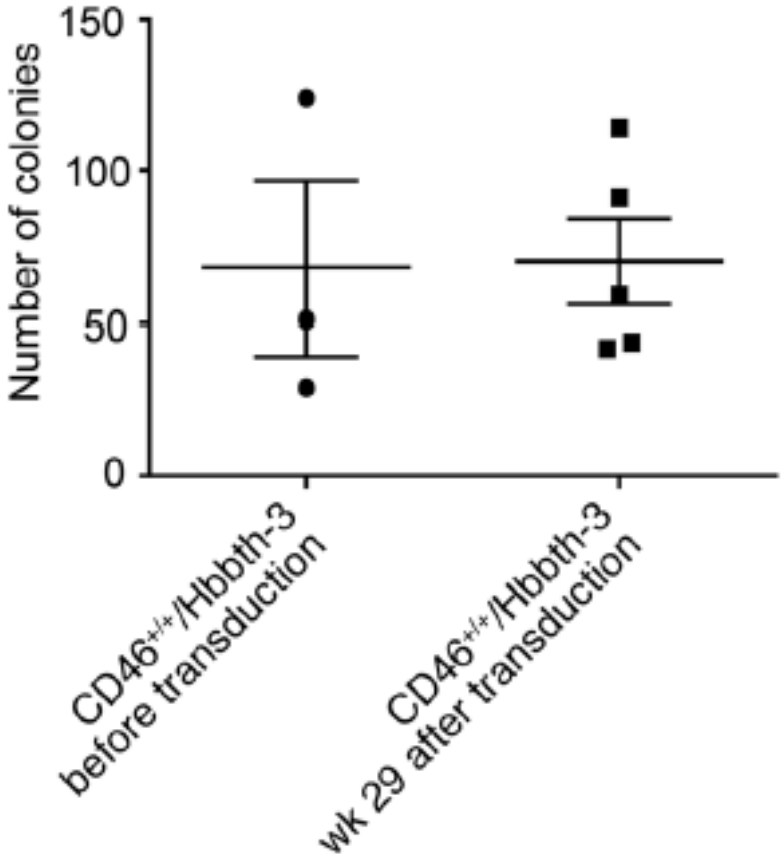

150
100
50
0
Number of colonies
CD46+/+/Hbbth-3
before transduction
CD46+/+/Hbbth-3
wk 29 after transduction

FIG. 20A

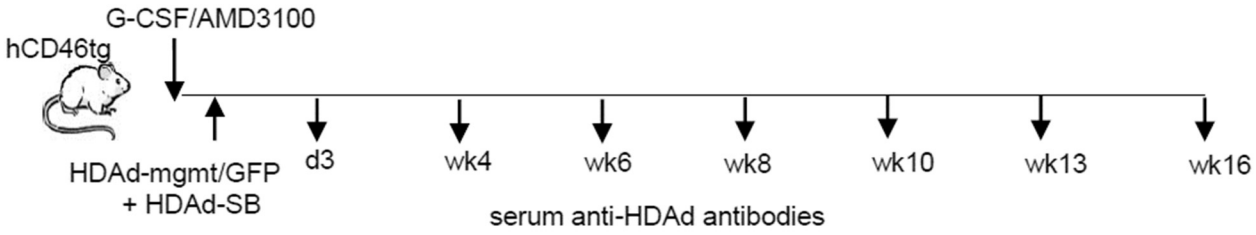


FIG. 20B

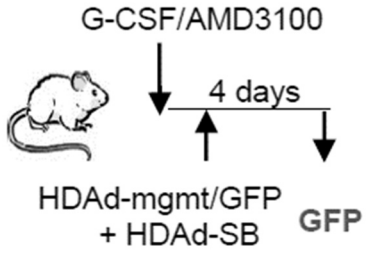


FIG. 20C

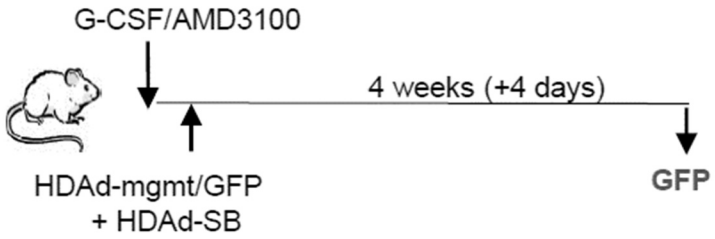


FIG. 20D



FIG. 20E

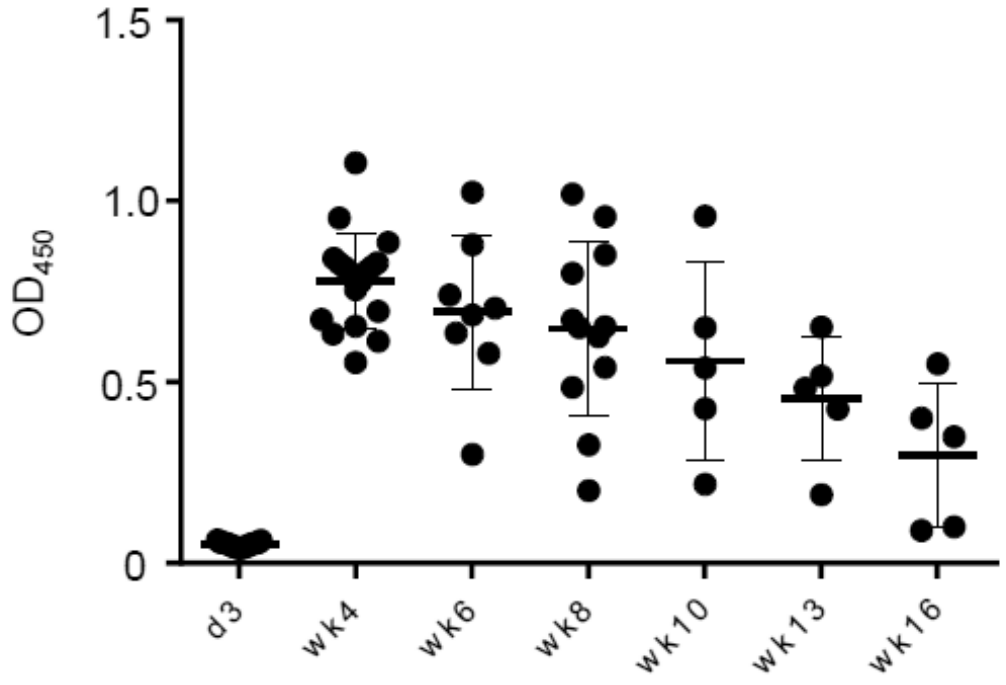


FIG. 20F

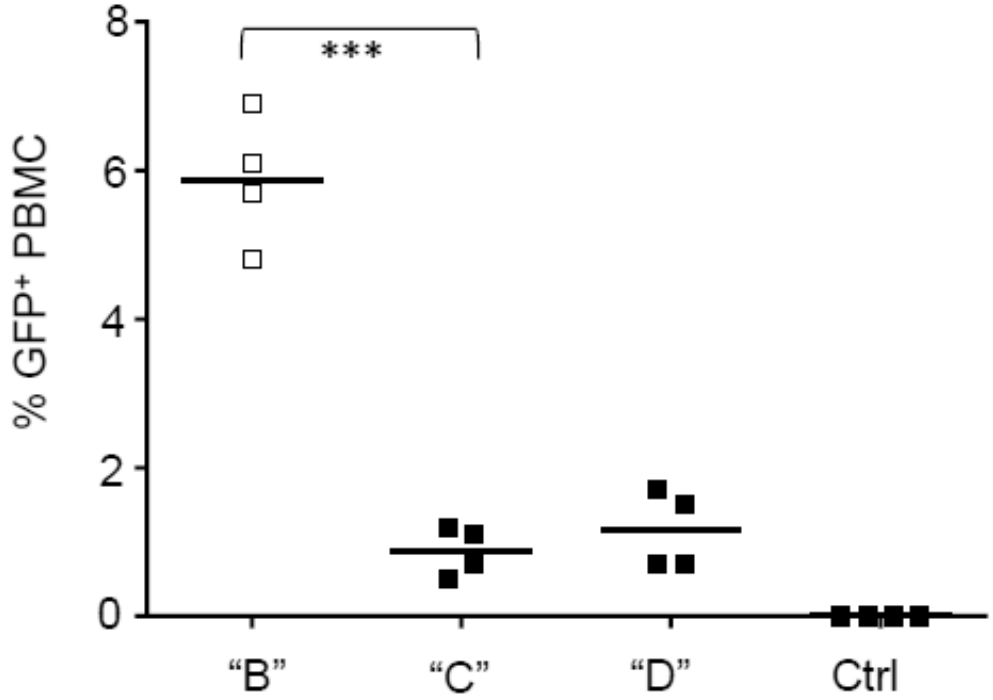


FIG. 21A

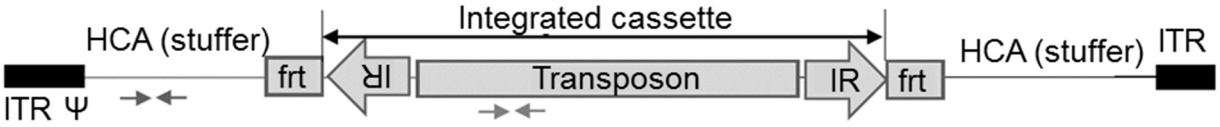


FIG. 21B

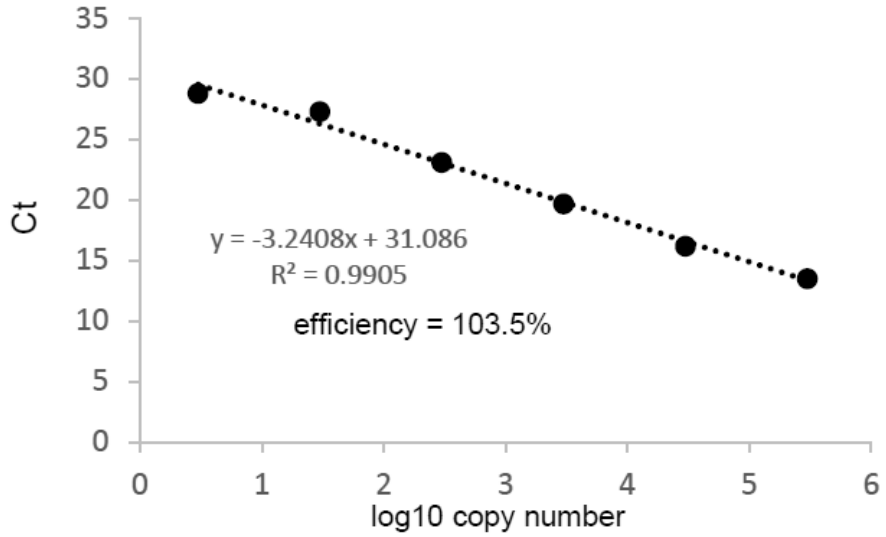


FIG. 21C

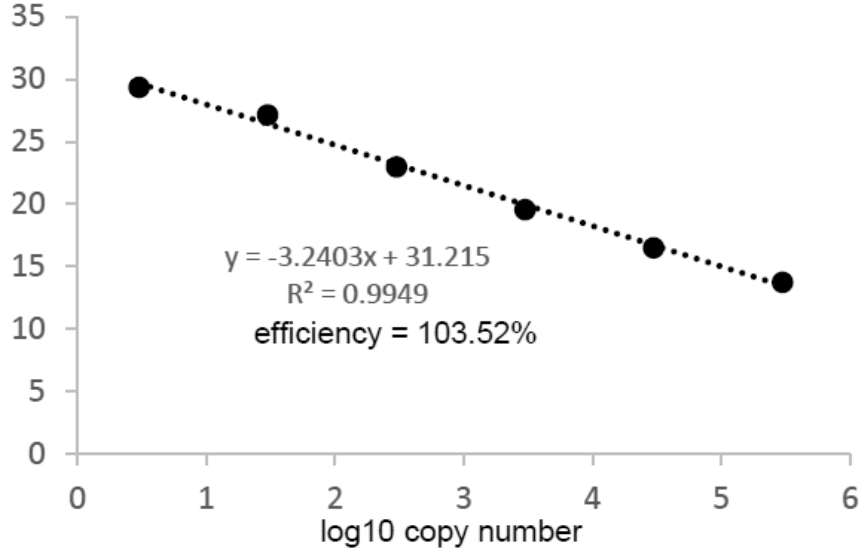


FIG. 21D

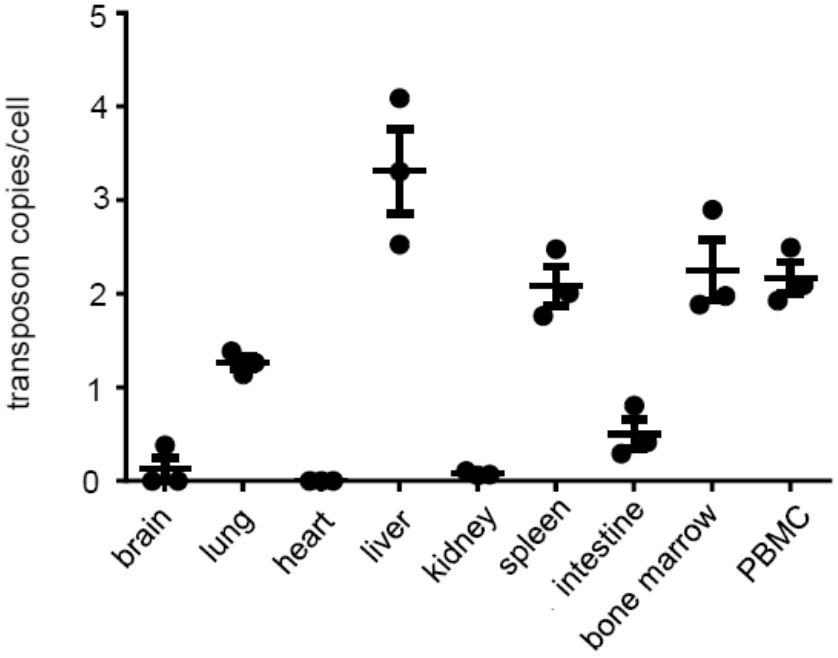

transposon copies/cell
5
4
3
2
1
0
brain
lung
heart
liver
kidney
spleen
intestine
bone marrow
PBMC

FIG. 22A

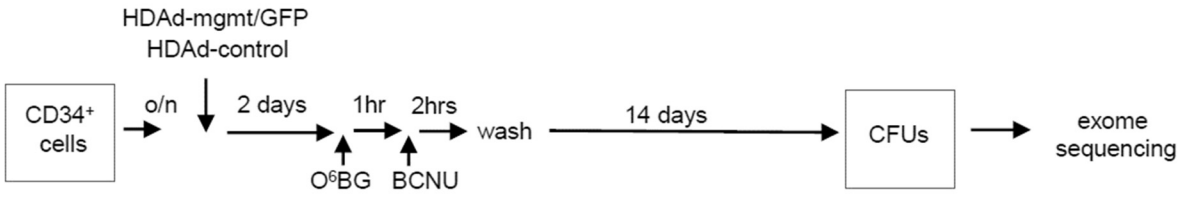


FIG. 22B

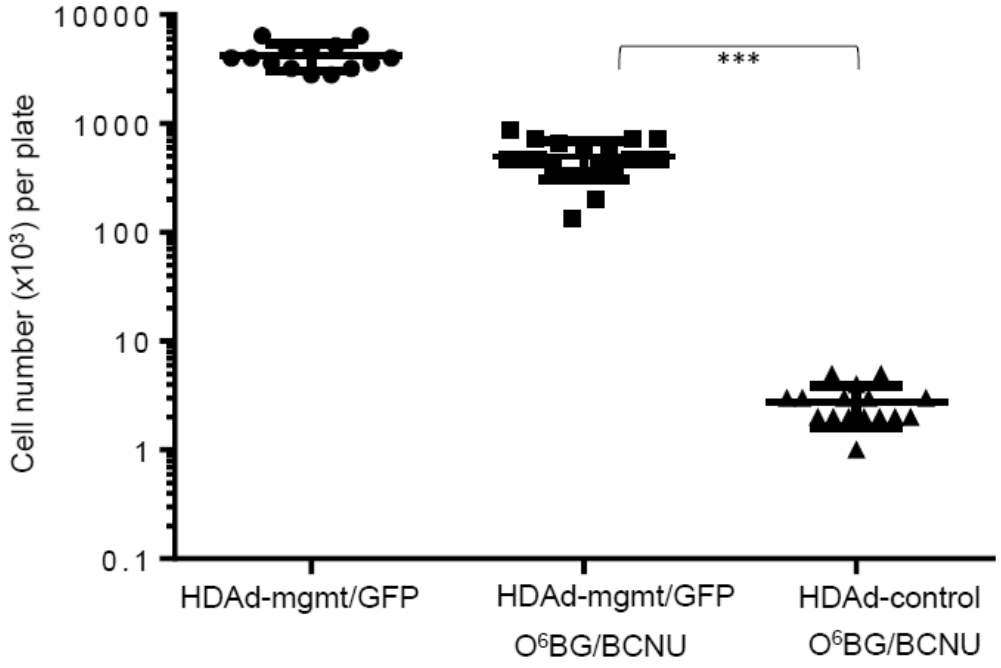


FIG. 22C

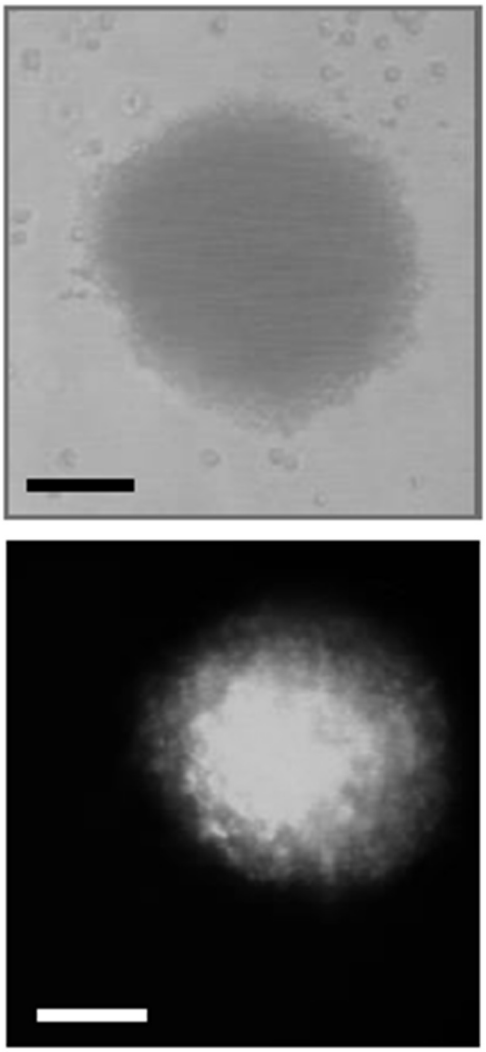


FIG. 23

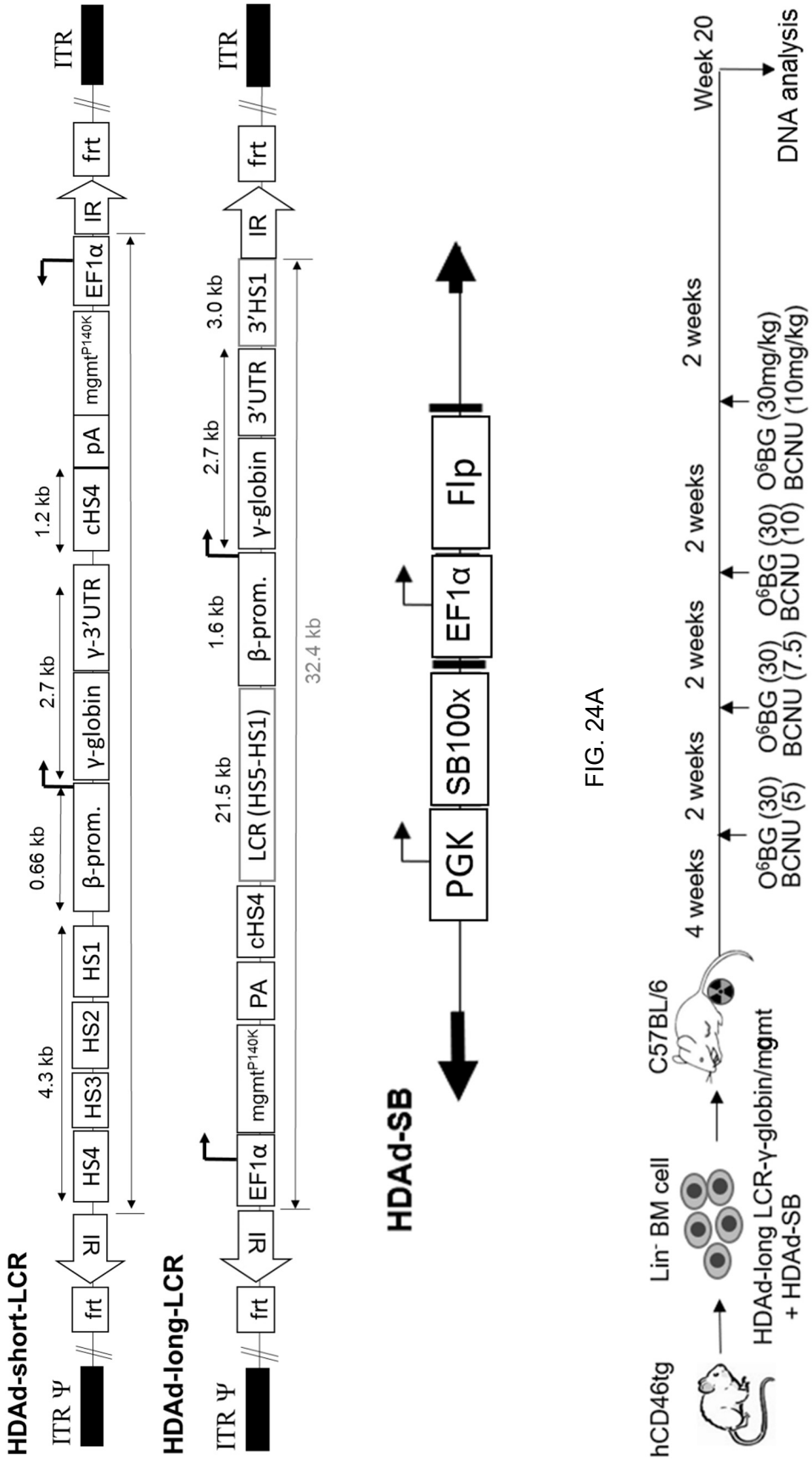


FIG. 24A

FIG. 24C

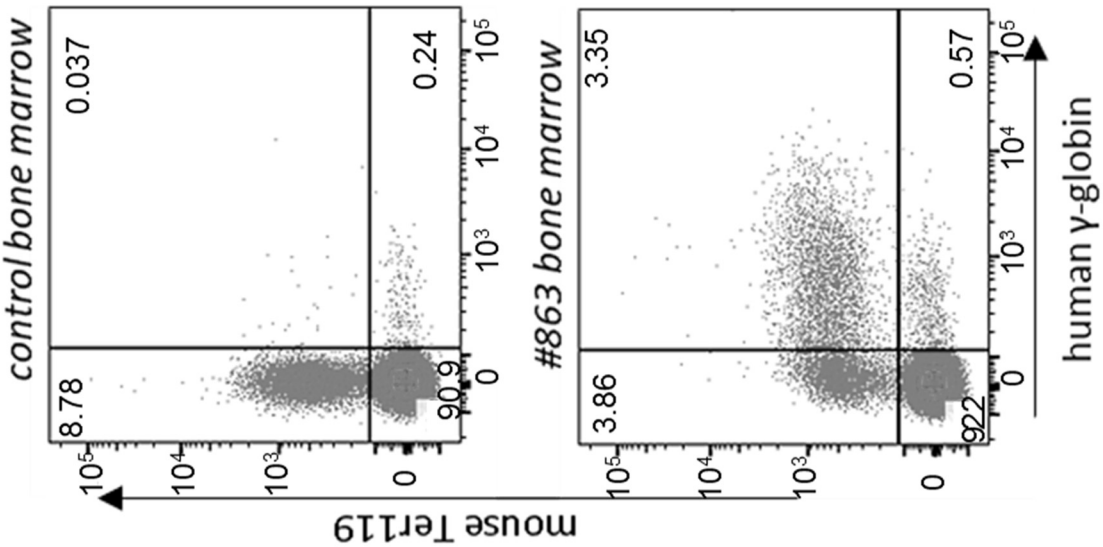


FIG. 24B

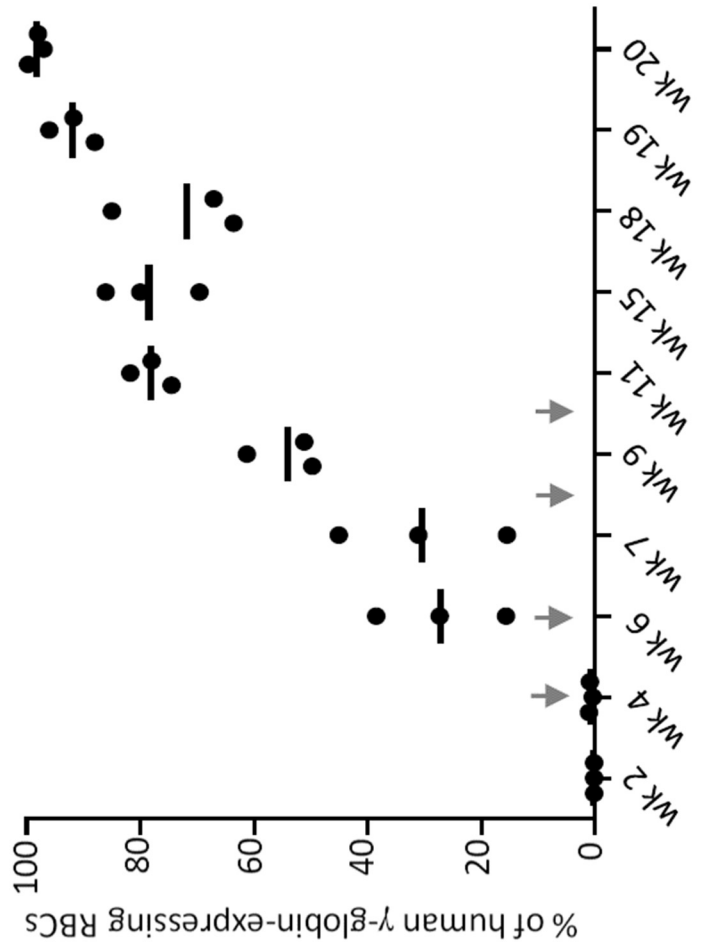


FIG. 24D

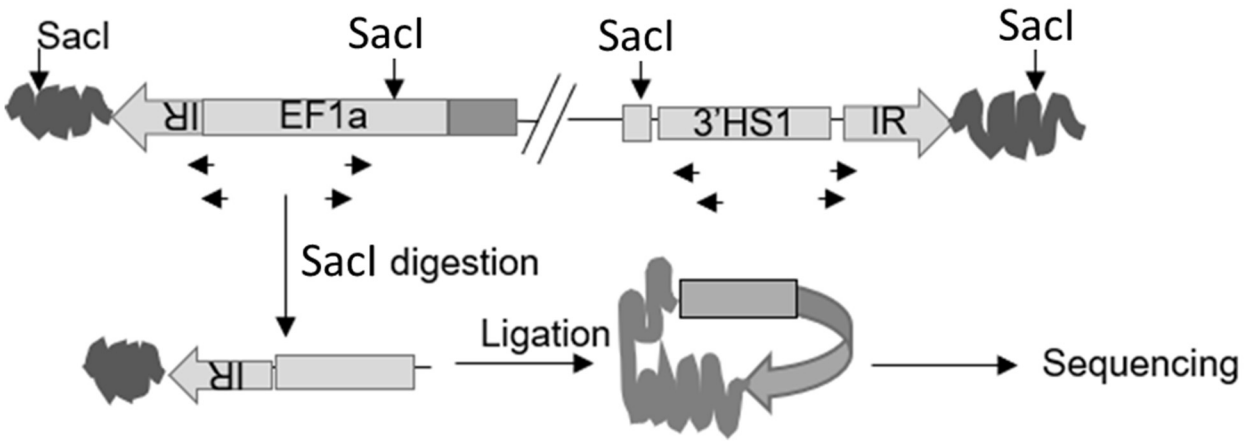


FIG. 24E

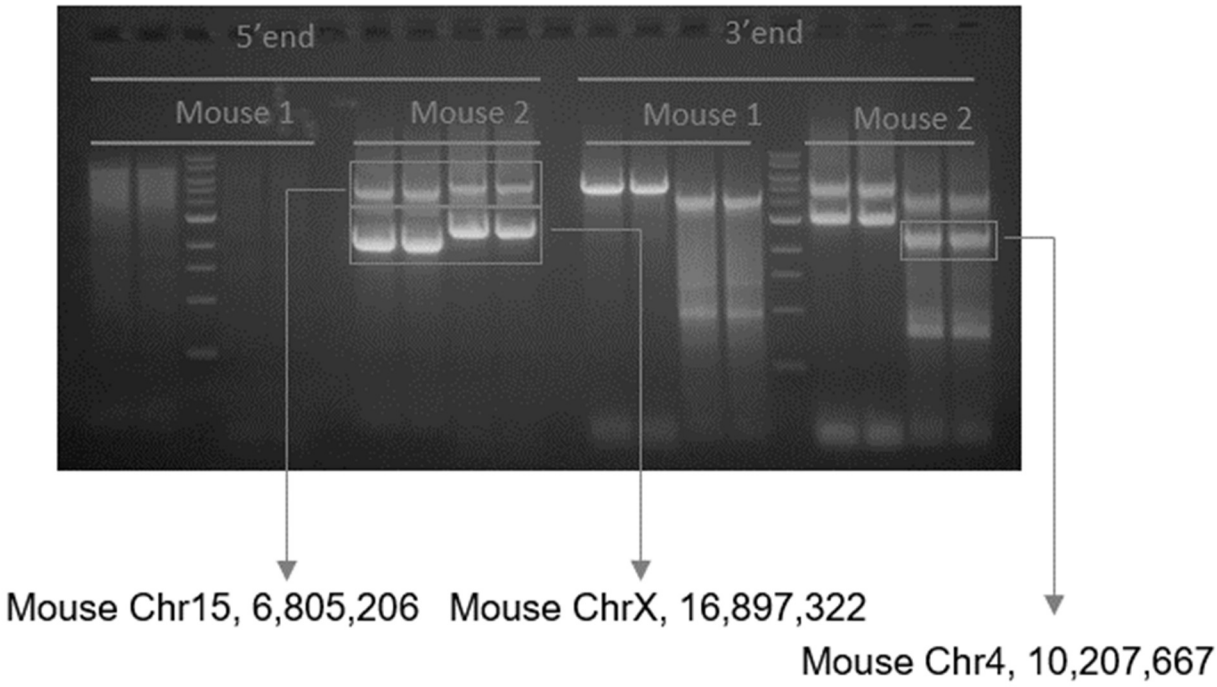


FIG. 24F

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

FIG. 25B

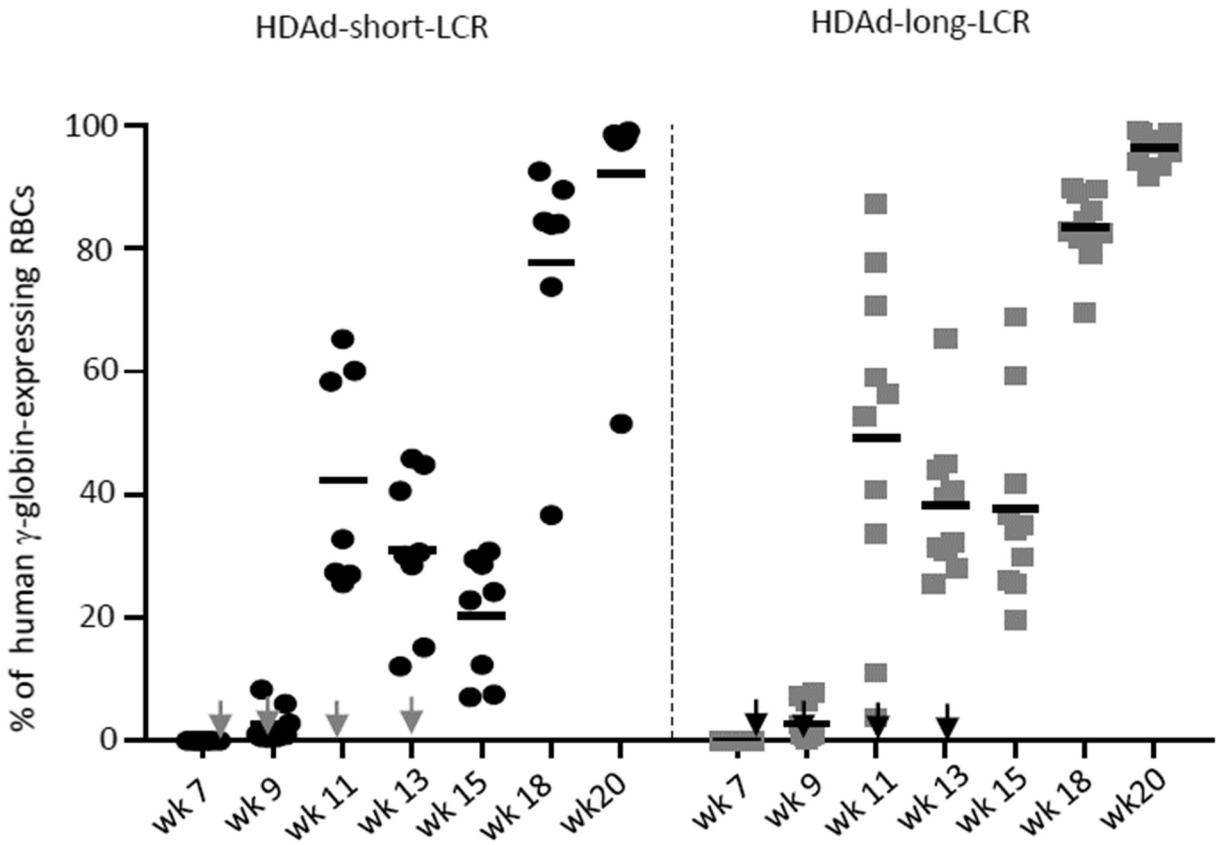


FIG. 25C

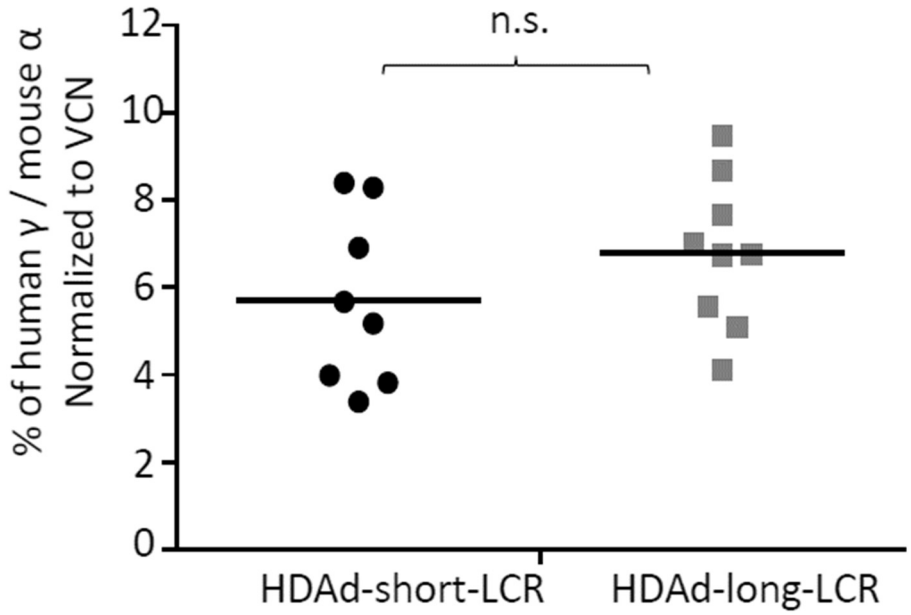


FIG. 25D

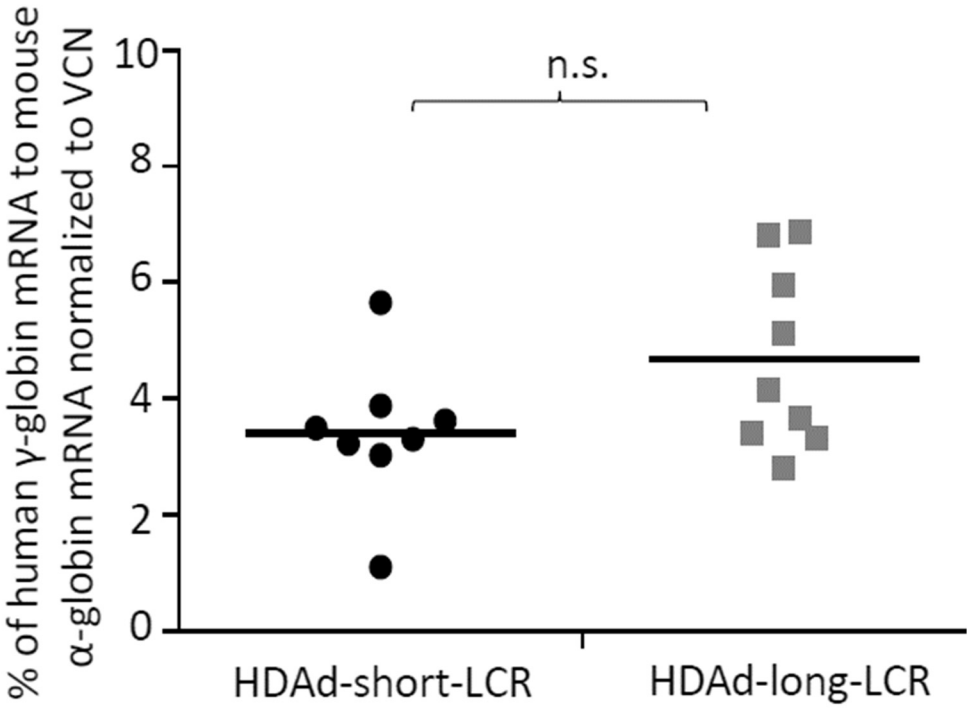


FIG. 25E

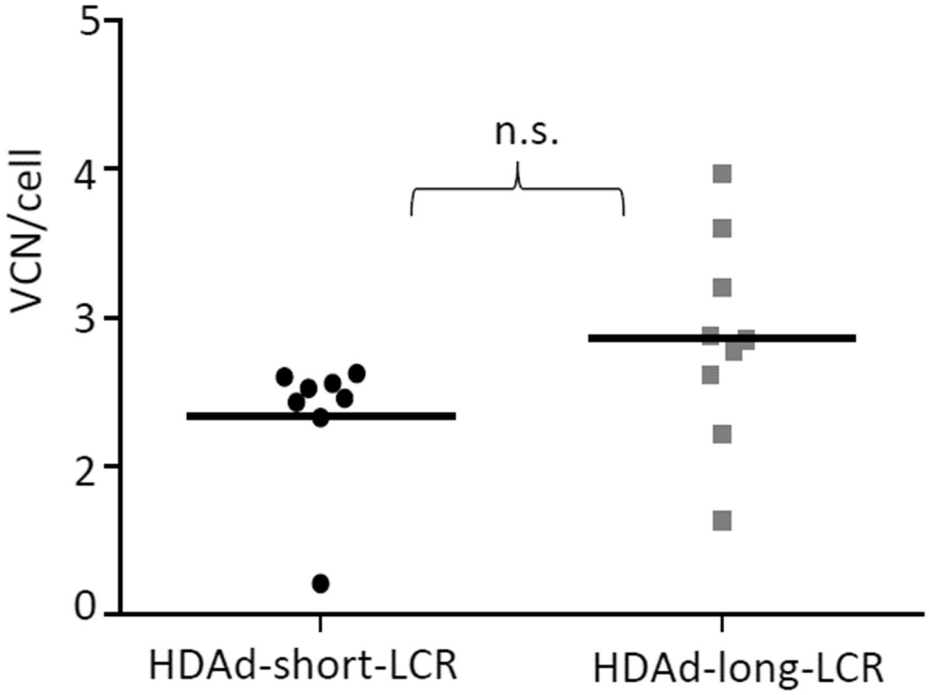


FIG. 26A

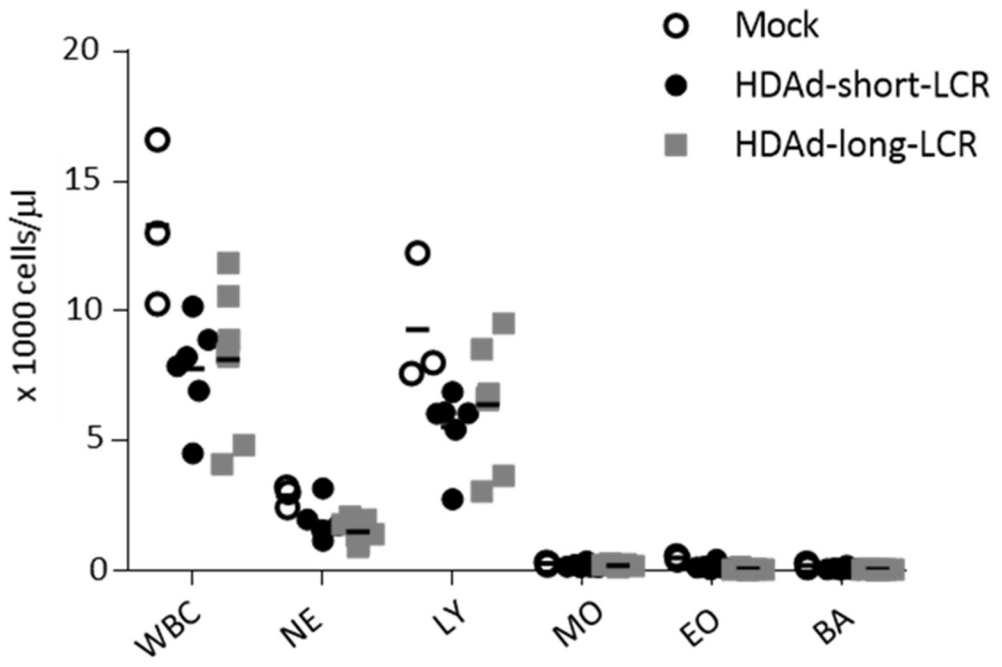


FIG. 26B

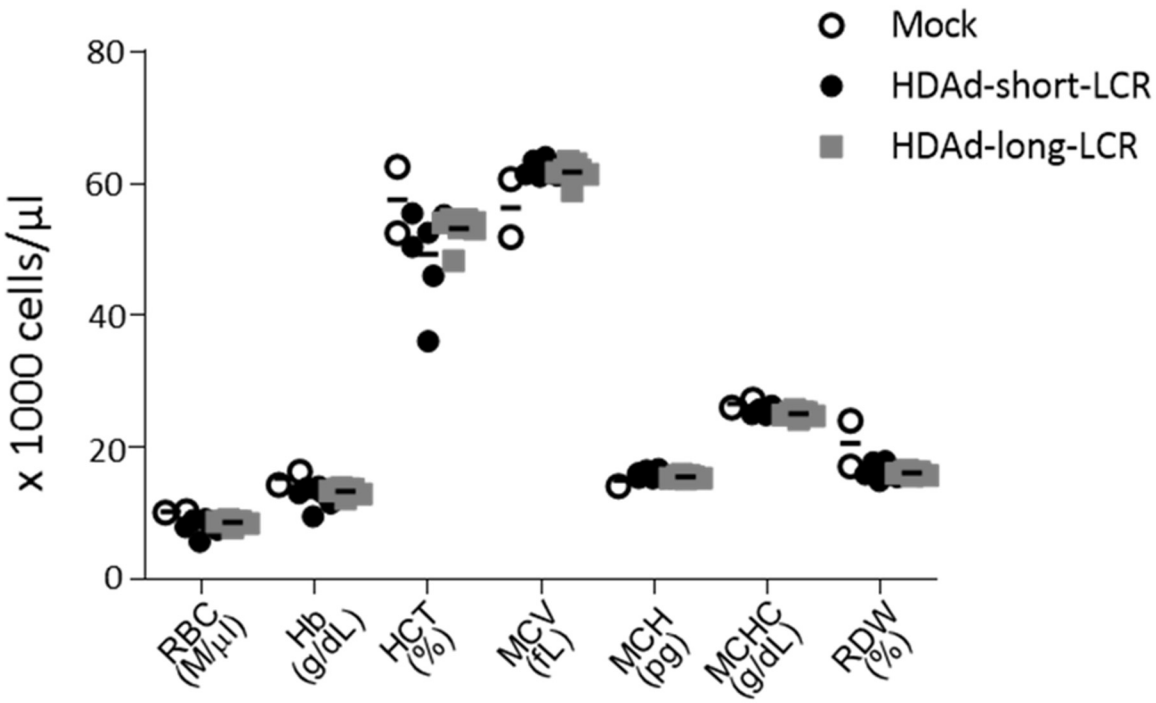


FIG. 26C

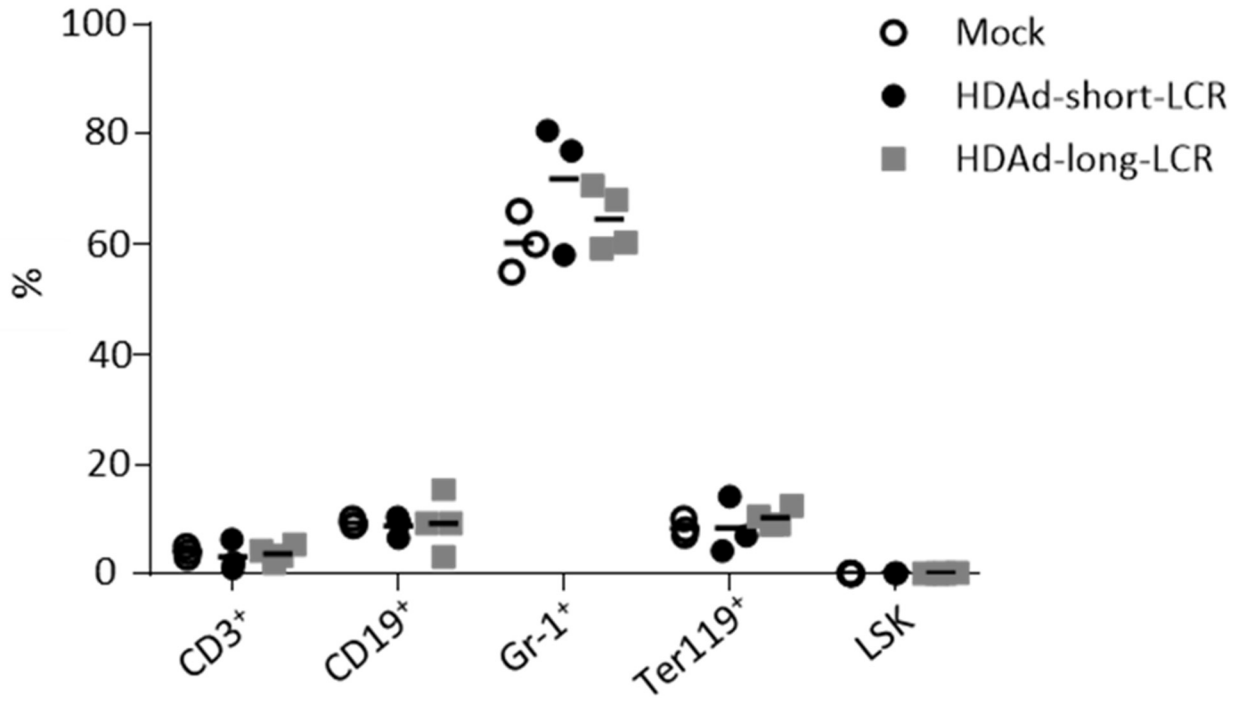


FIG. 26D

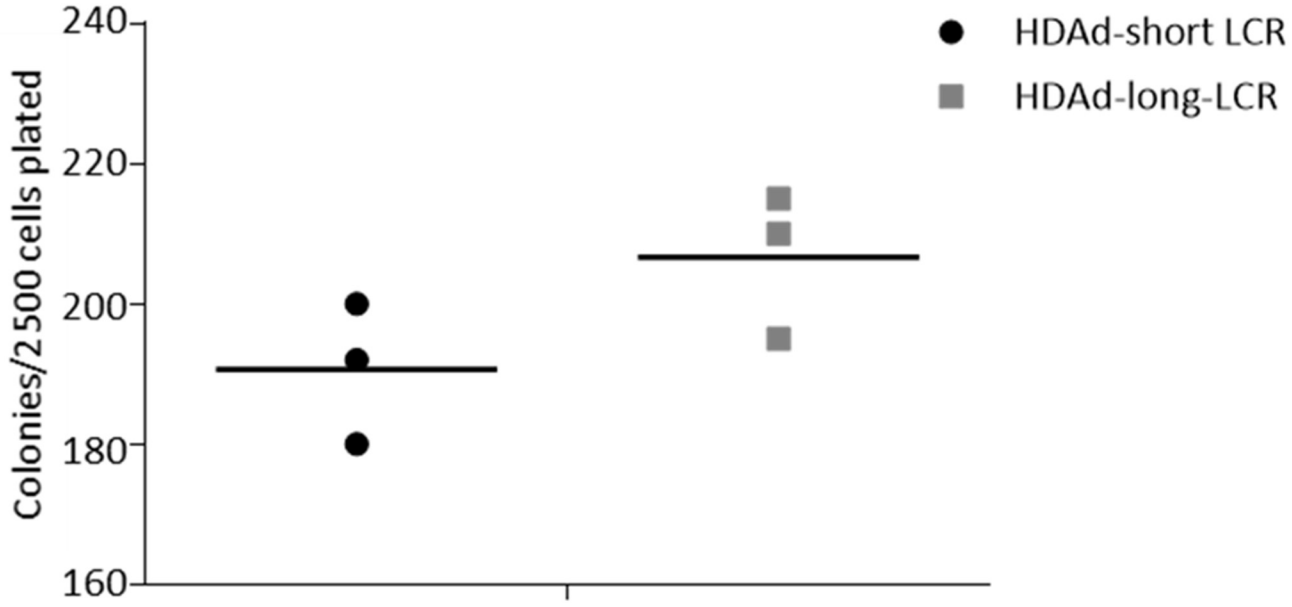


FIG. 27

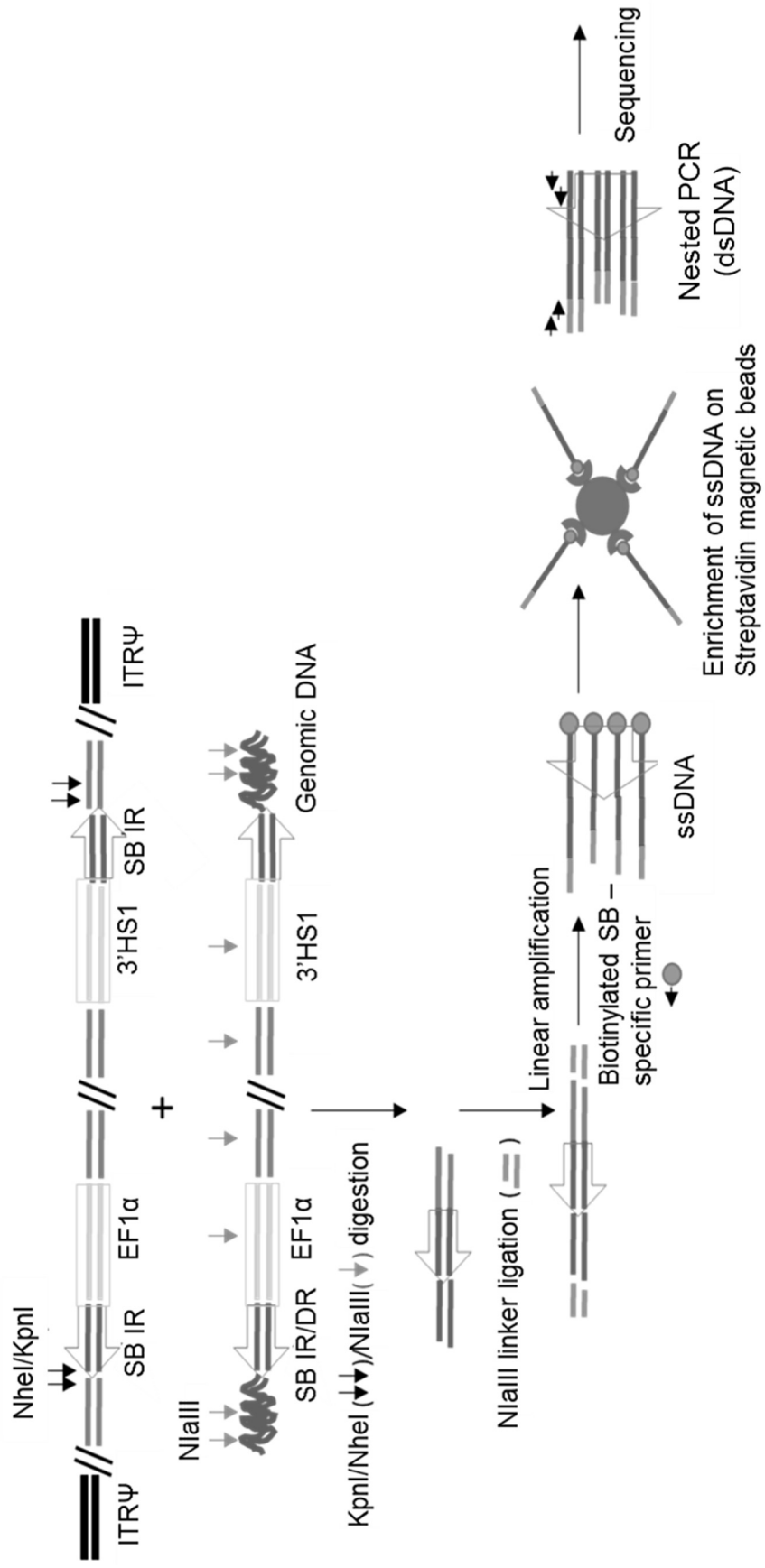

NheI/KpnI
ITRΨ
SB IR
EF1α
3'HS1
SB IR
ITRΨ
+
NlaIII
SB IR/DR
EF1α
3'HS1
Genomic DNA
KpnI/NheI (▼▼)/NlaIII(▼) digestion
NlaIII linker ligation (═)
Linear amplification
Biotinylated SB – specific primer
ssDNA
Enrichment of ssDNA on Streptavidin magnetic beads
Nested PCR (dsDNA)
Sequencing

FIG. 28A

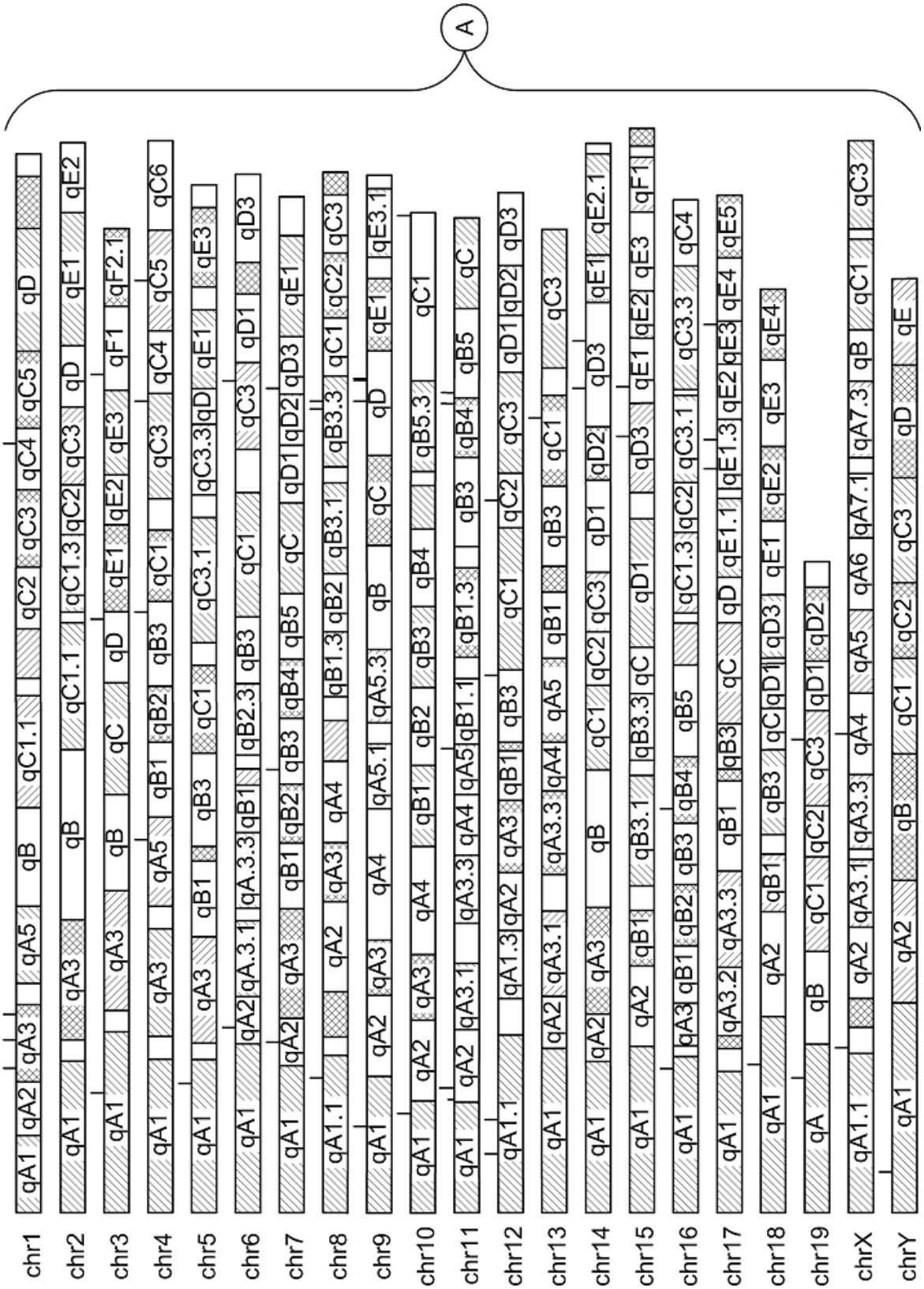

A
chr1
chr2
chr3
chr4
chr5
chr6
chr7
chr8
chr9
chr10
chr11
chr12
chr13
chr14
chr15
chr16
chr17
chr18
chr19
chrX
chrY

FIG. 28A cont'd.

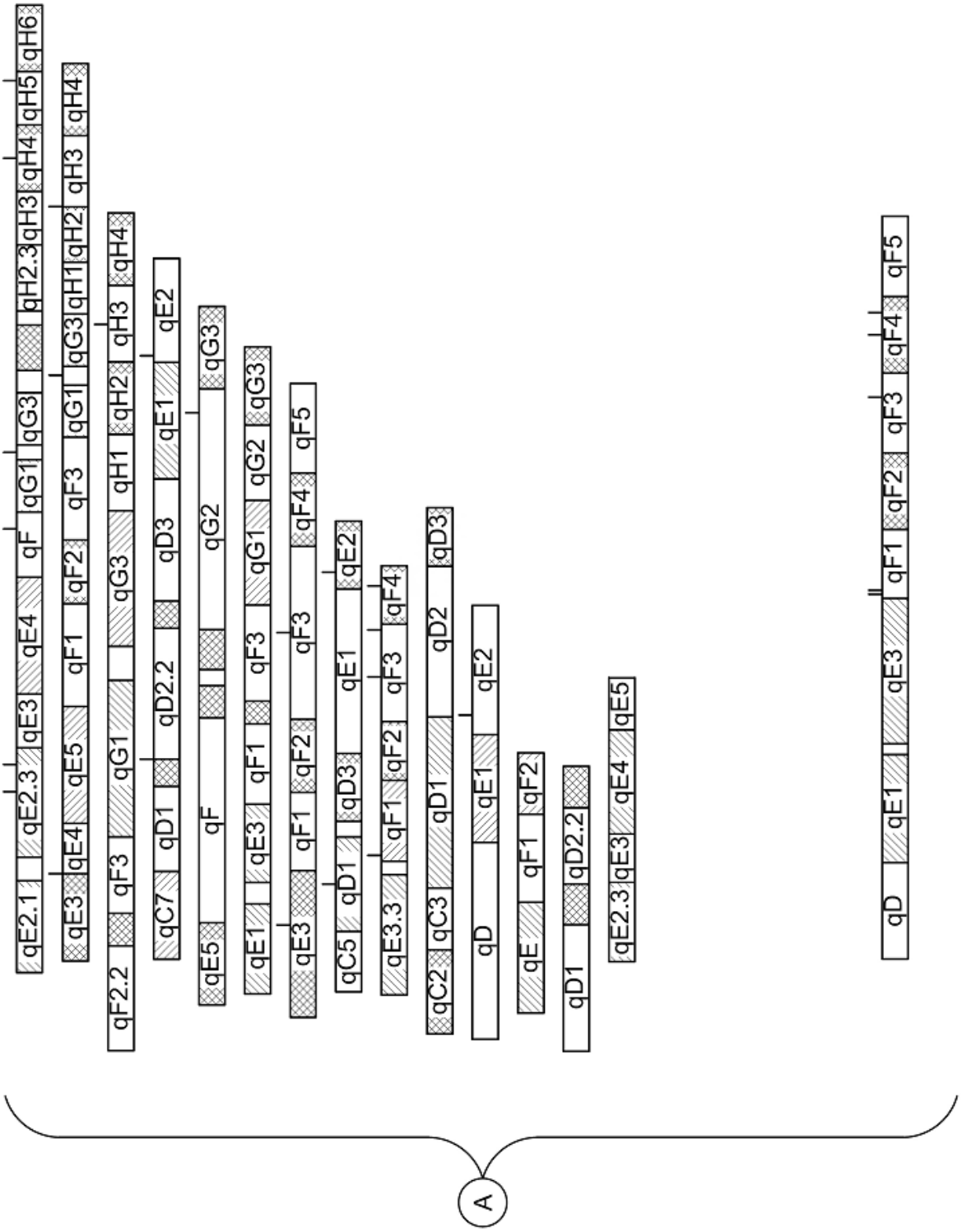


FIG 28B

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

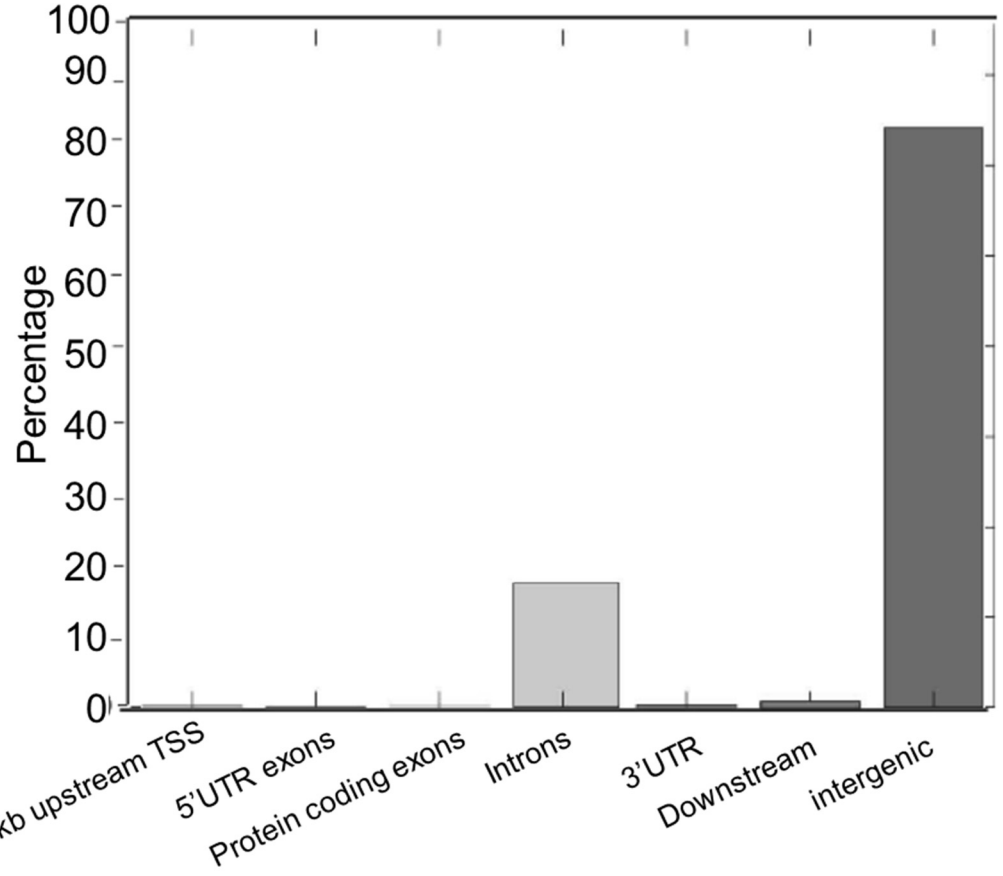


FIG. 28D

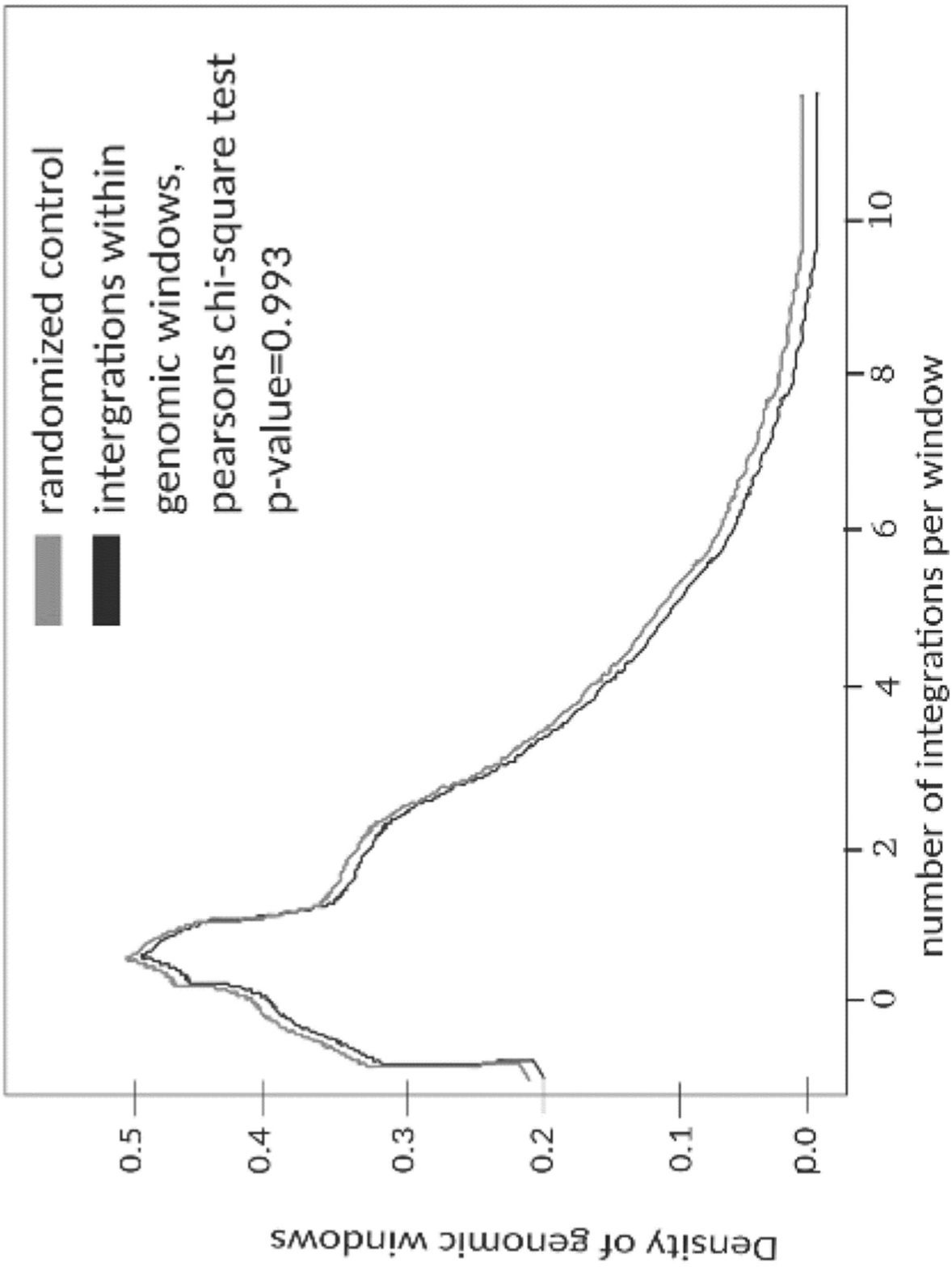

randomized control
intergrations within genomic windows,
pearsons chi-square test
p-value=0.993
Density of genomic windows
0.0
0.1
0.2
0.3
0.4
0.5
0
2
4
6
8
10
number of integrations per window

FIG. 29A

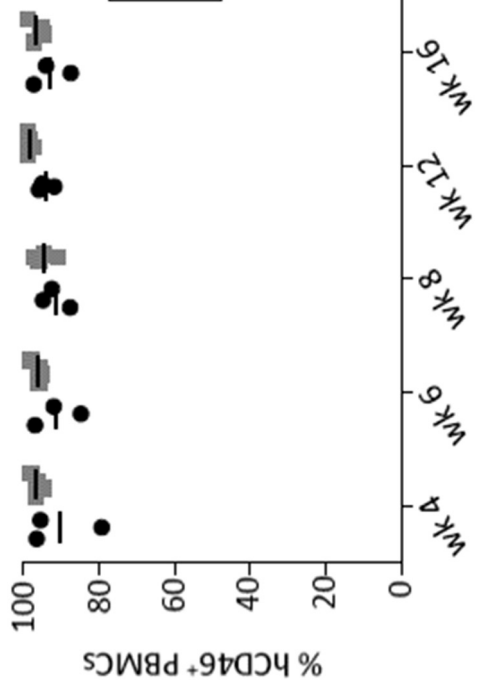


FIG. 29B

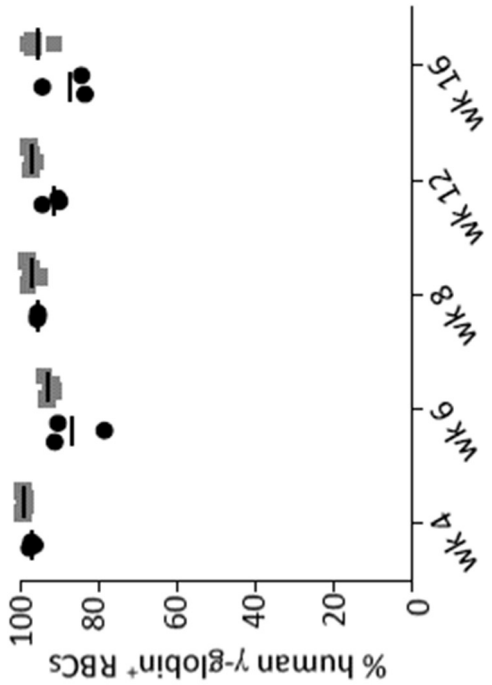
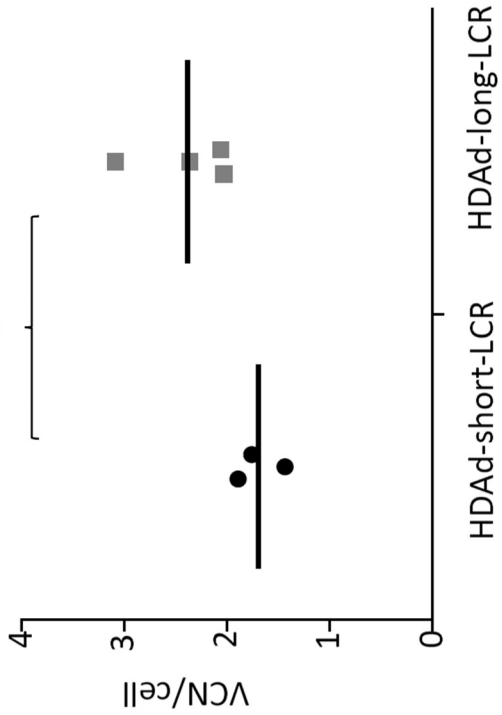


FIG. 29C



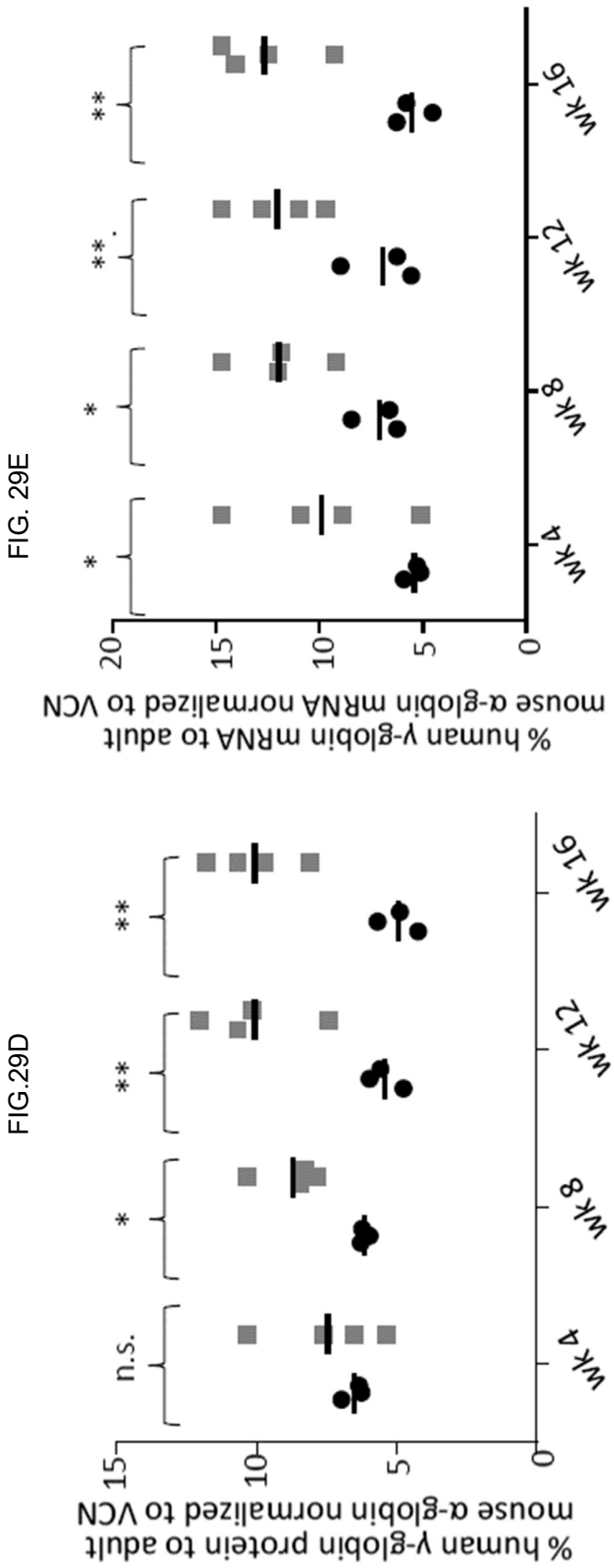


FIG.29D

FIG. 29E

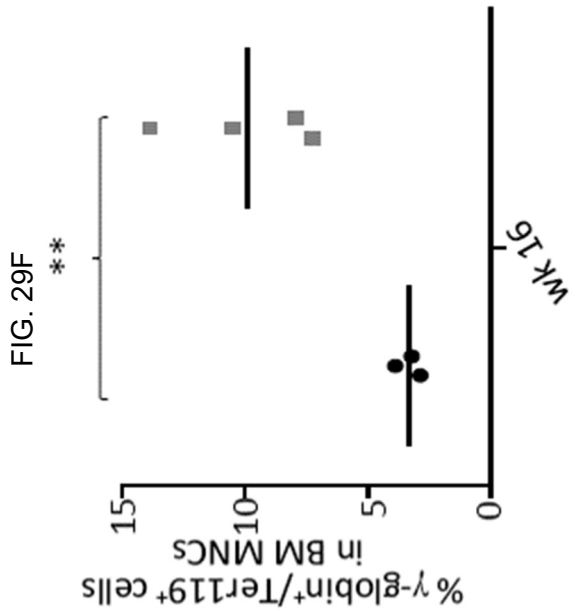


FIG. 29F

FIG. 29G

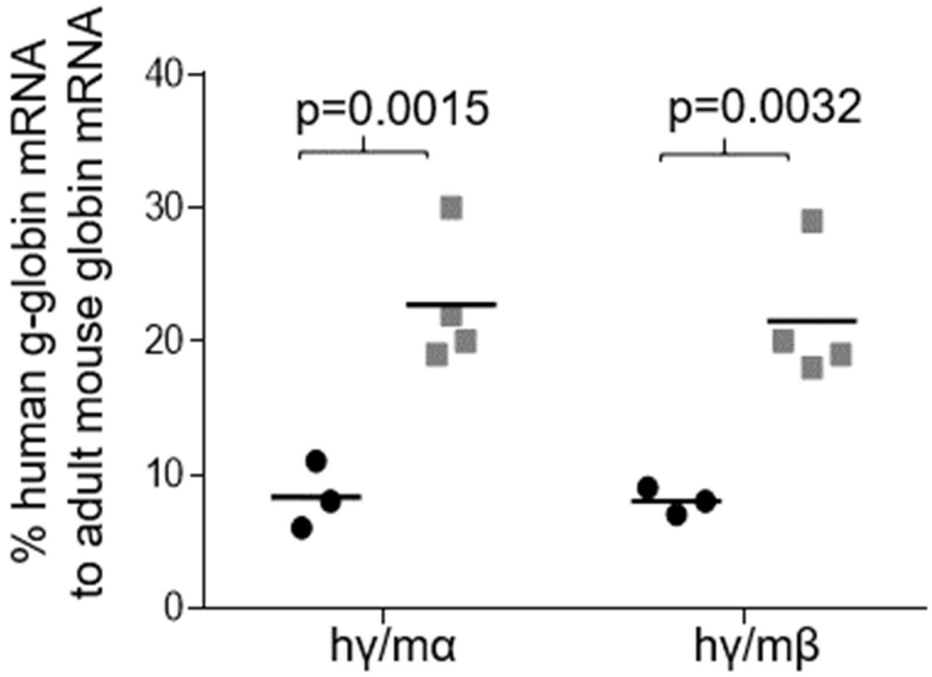

% human g-globin mRNA
to adult mouse globin mRNA
40
30
20
10
0
p=0.0015
p=0.0032
hγ/mα
hγ/mβ

FIG. 29H

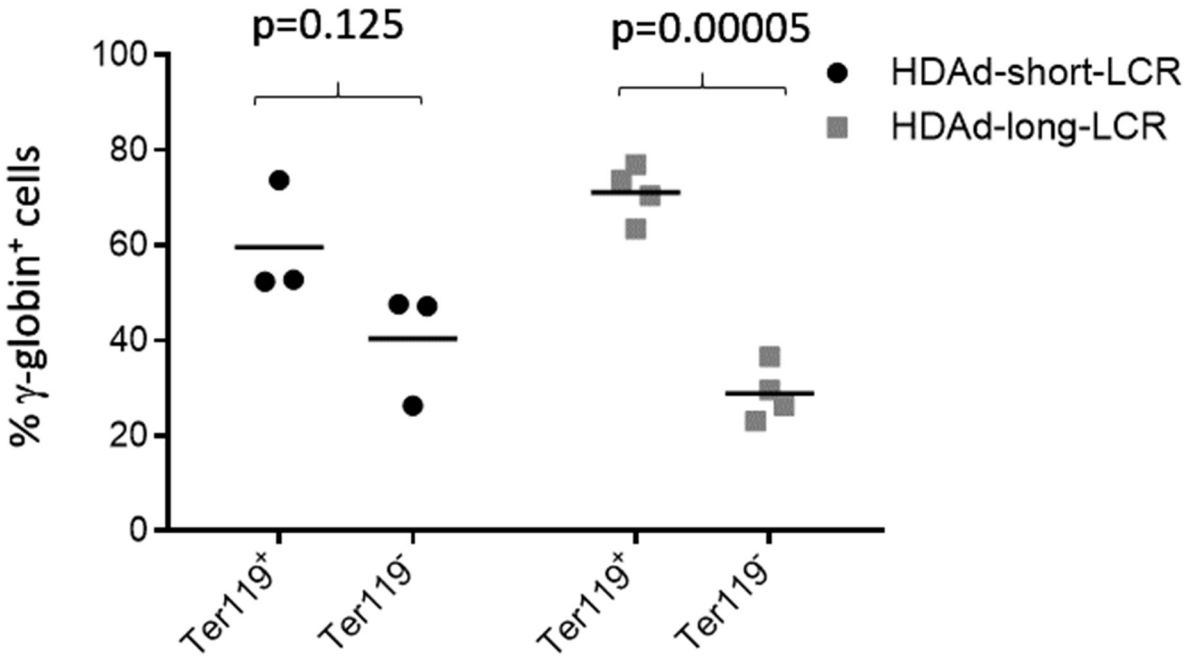


FIG. 29I

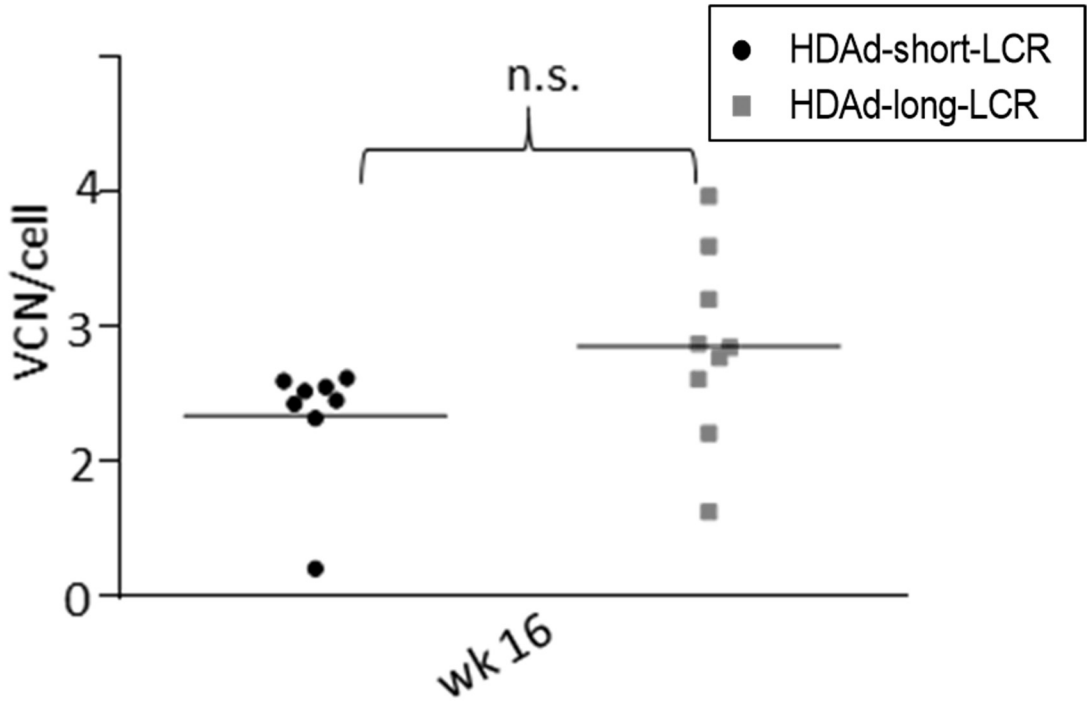


FIG. 30A

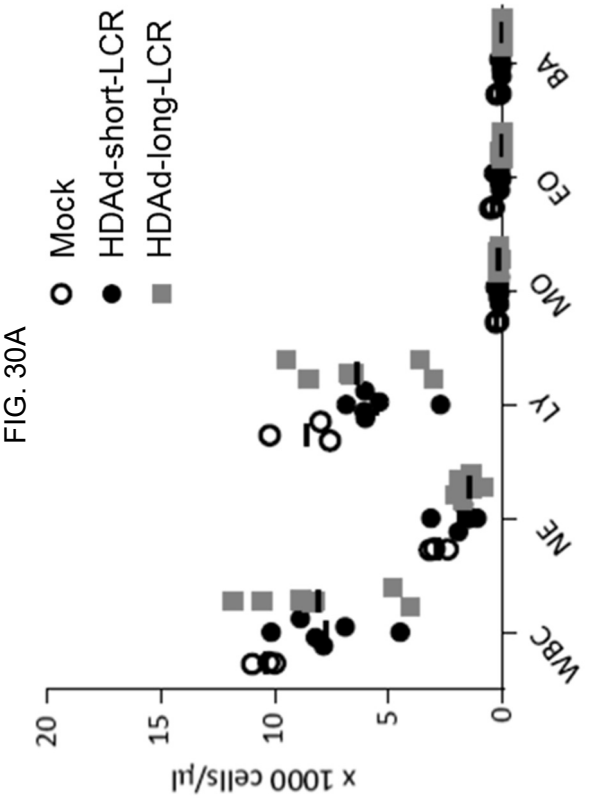


FIG. 30B

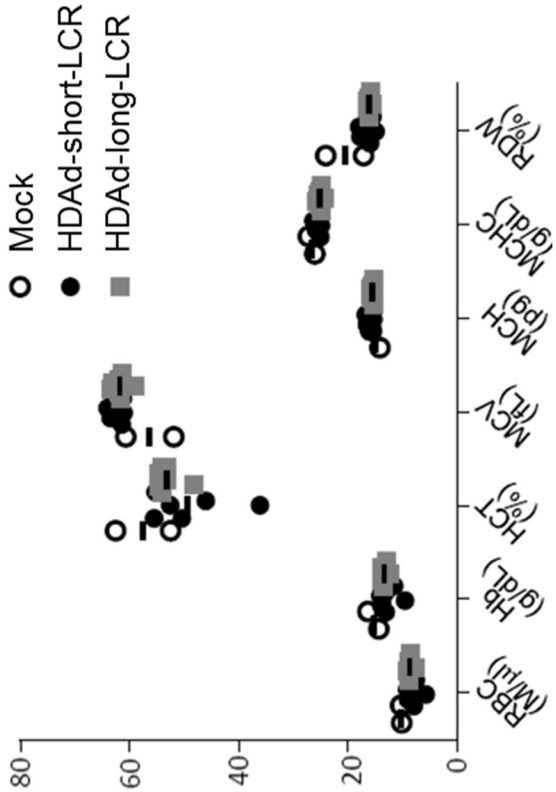


FIG. 30C

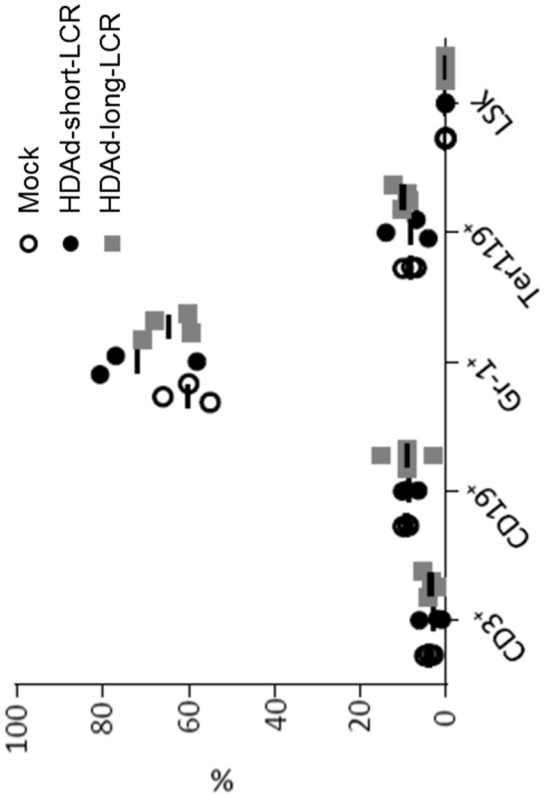


FIG. 30D

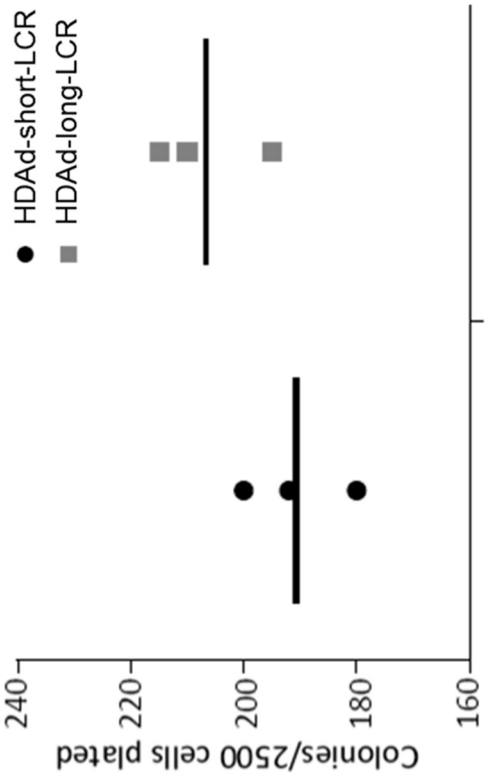


FIG. 31A

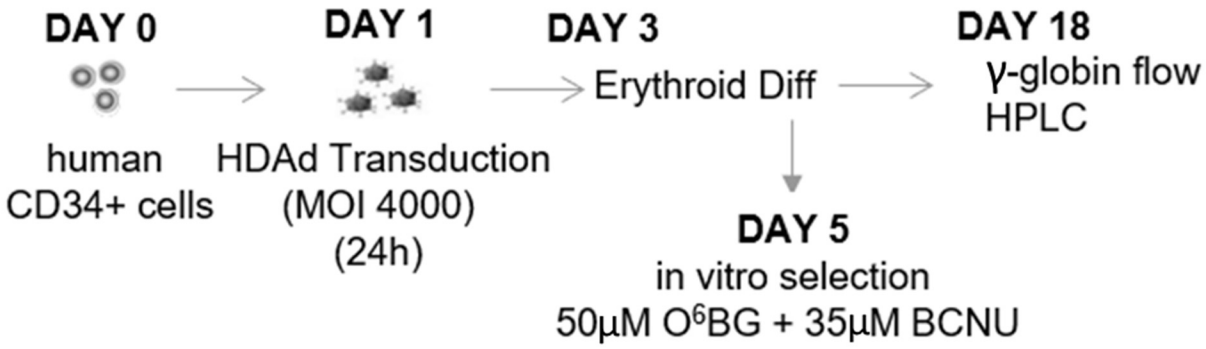


FIG. 31B

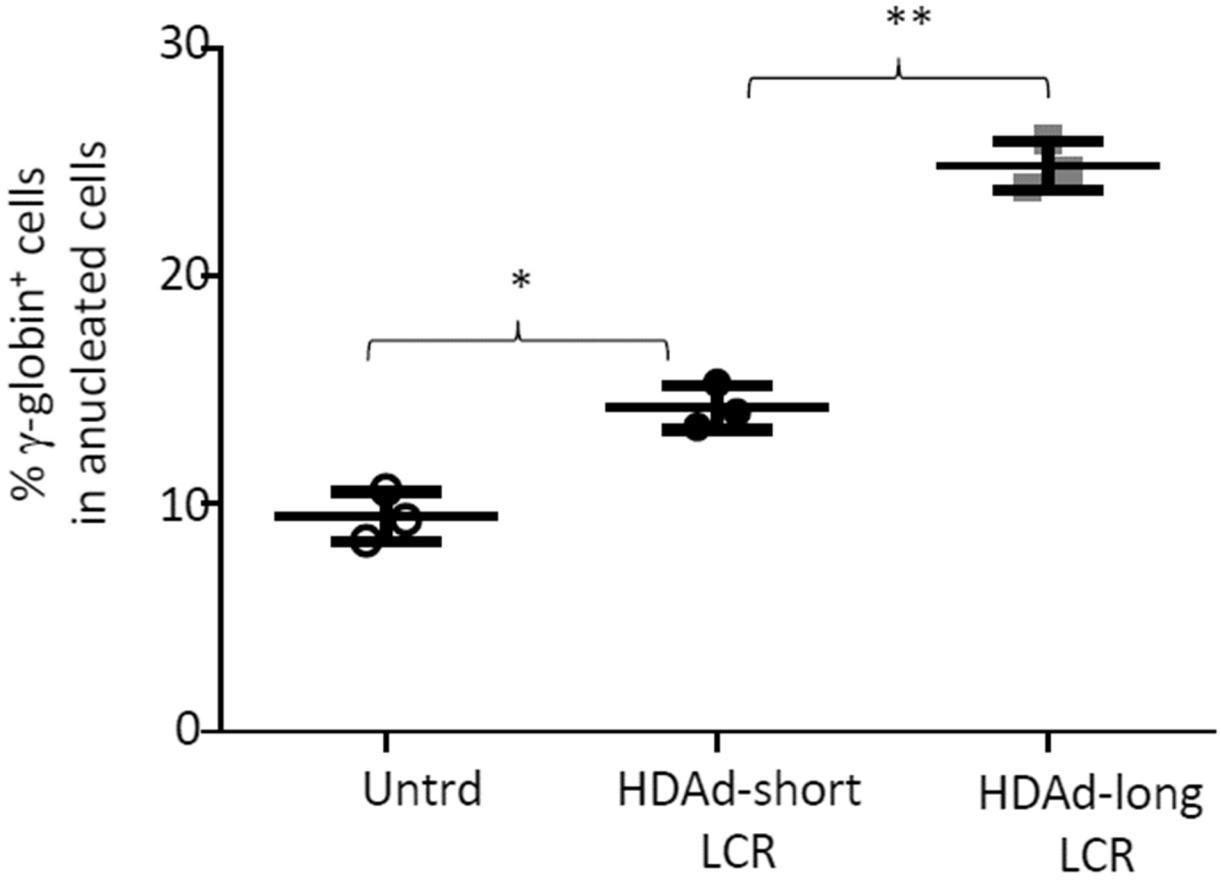


FIG. 31C

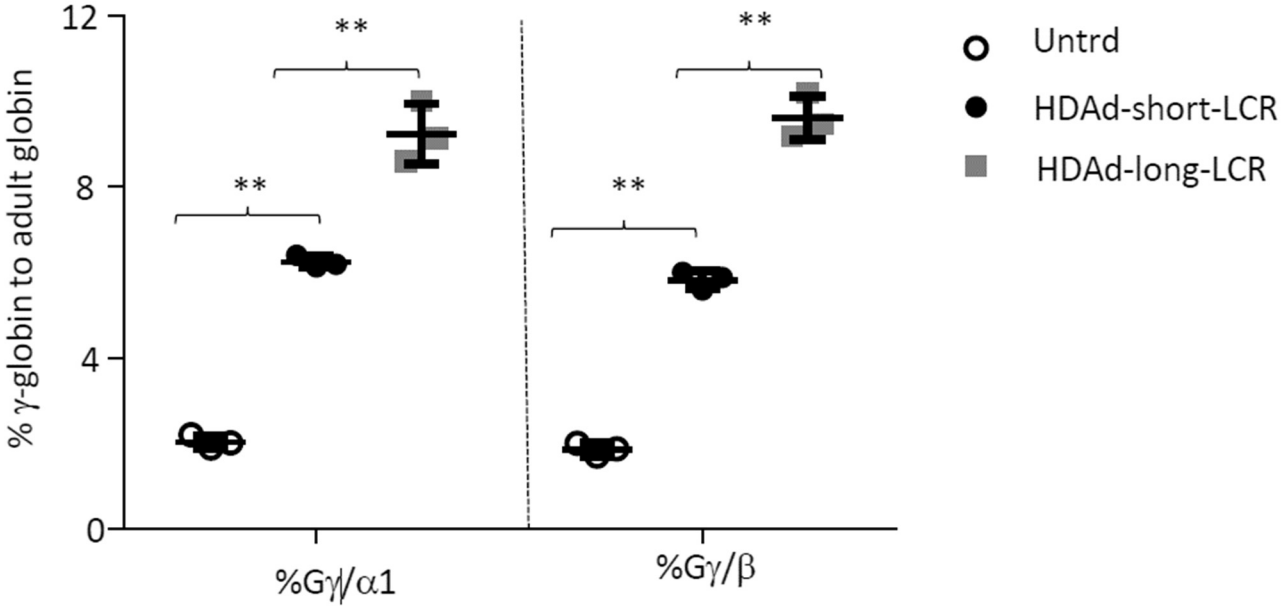


FIG. 31D

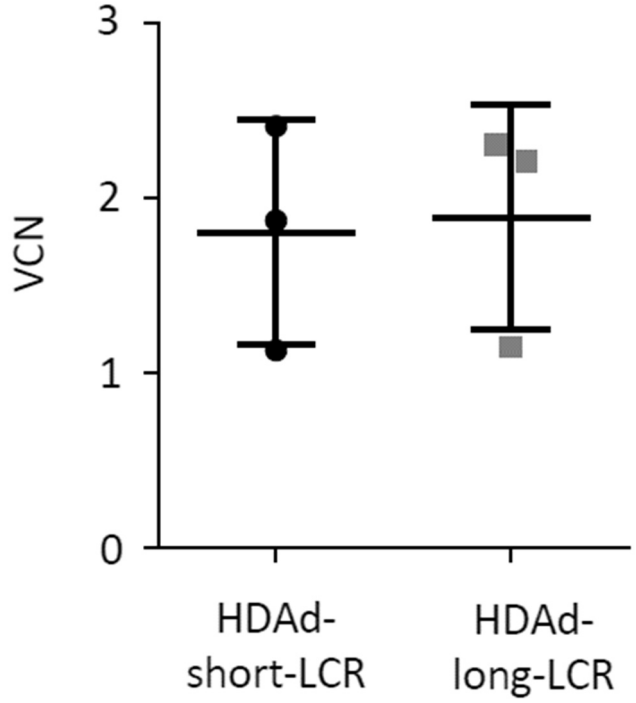


FIG. 32A

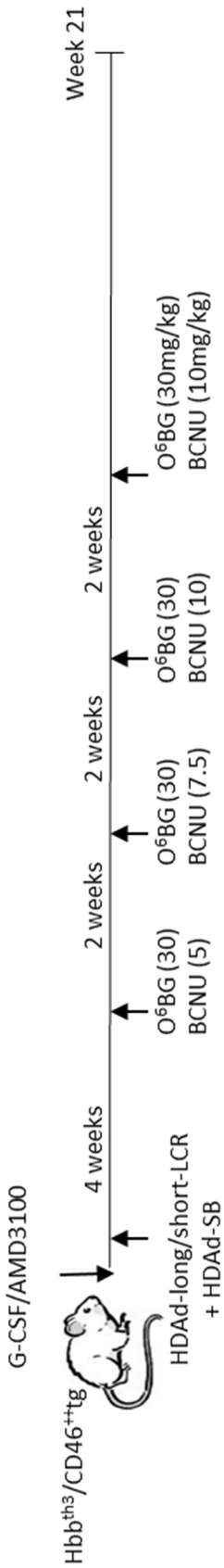


FIG. 32B

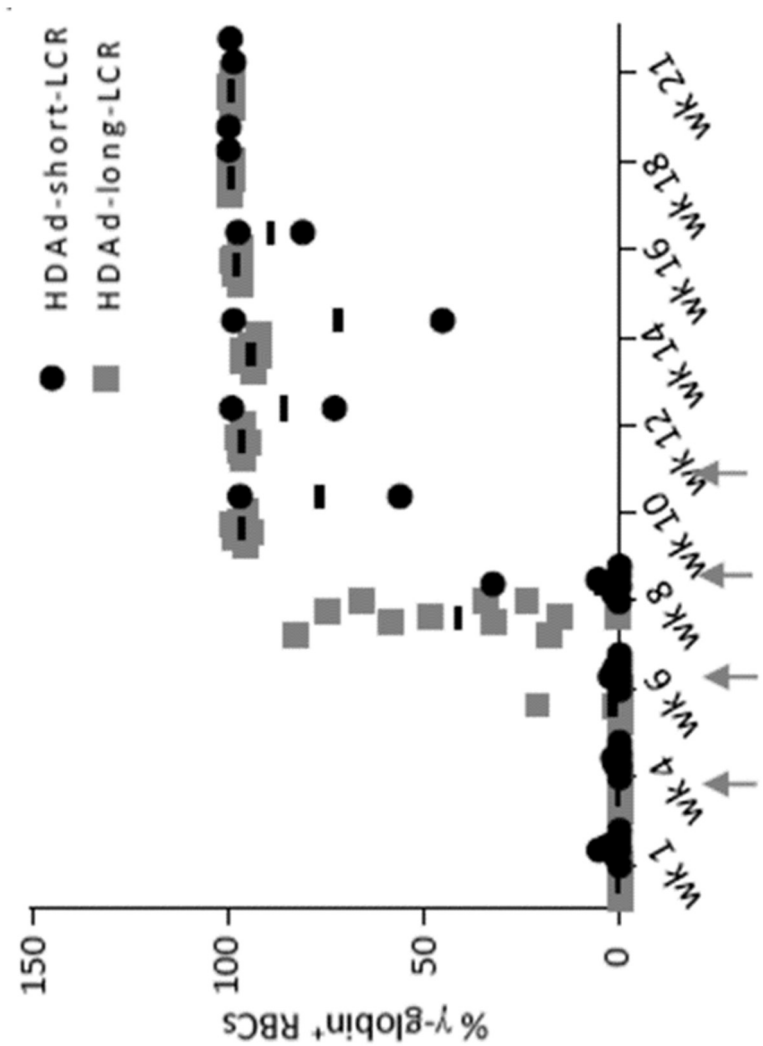


FIG. 32C

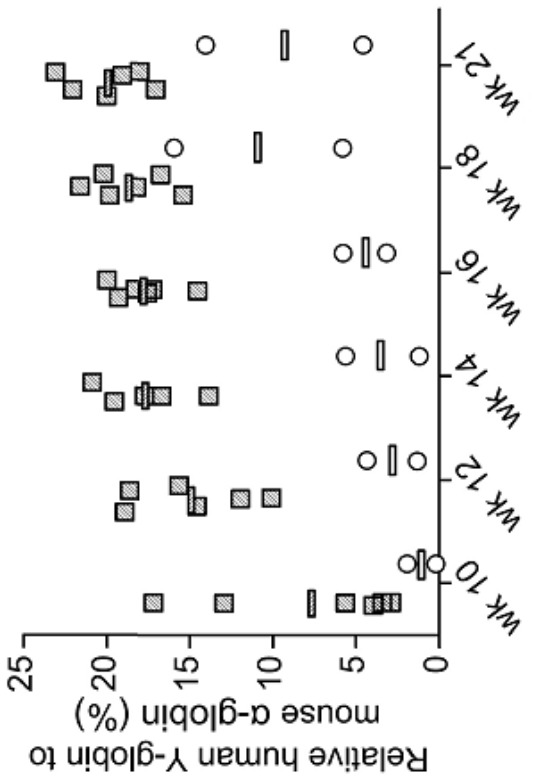


FIG. 32D

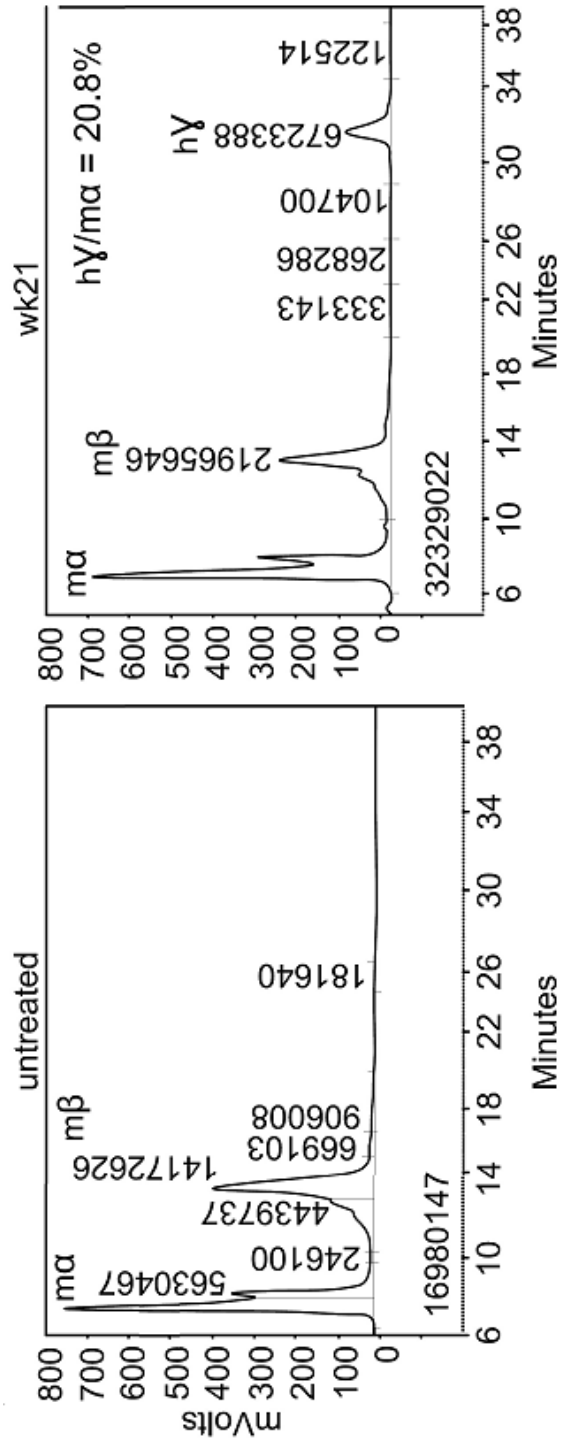


FIG. 32E

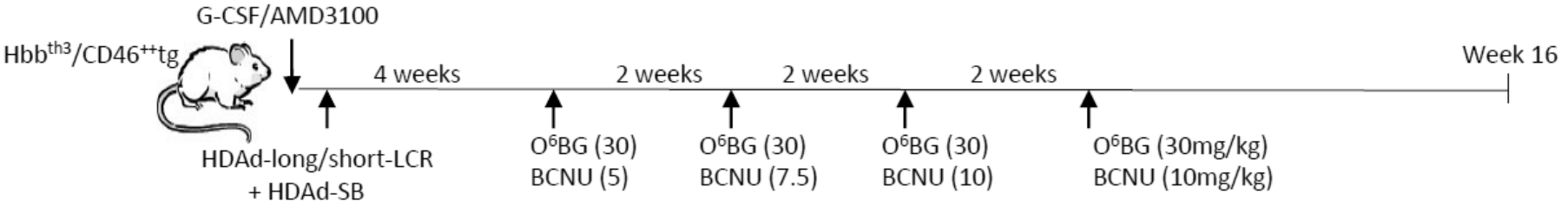


FIG. 32F

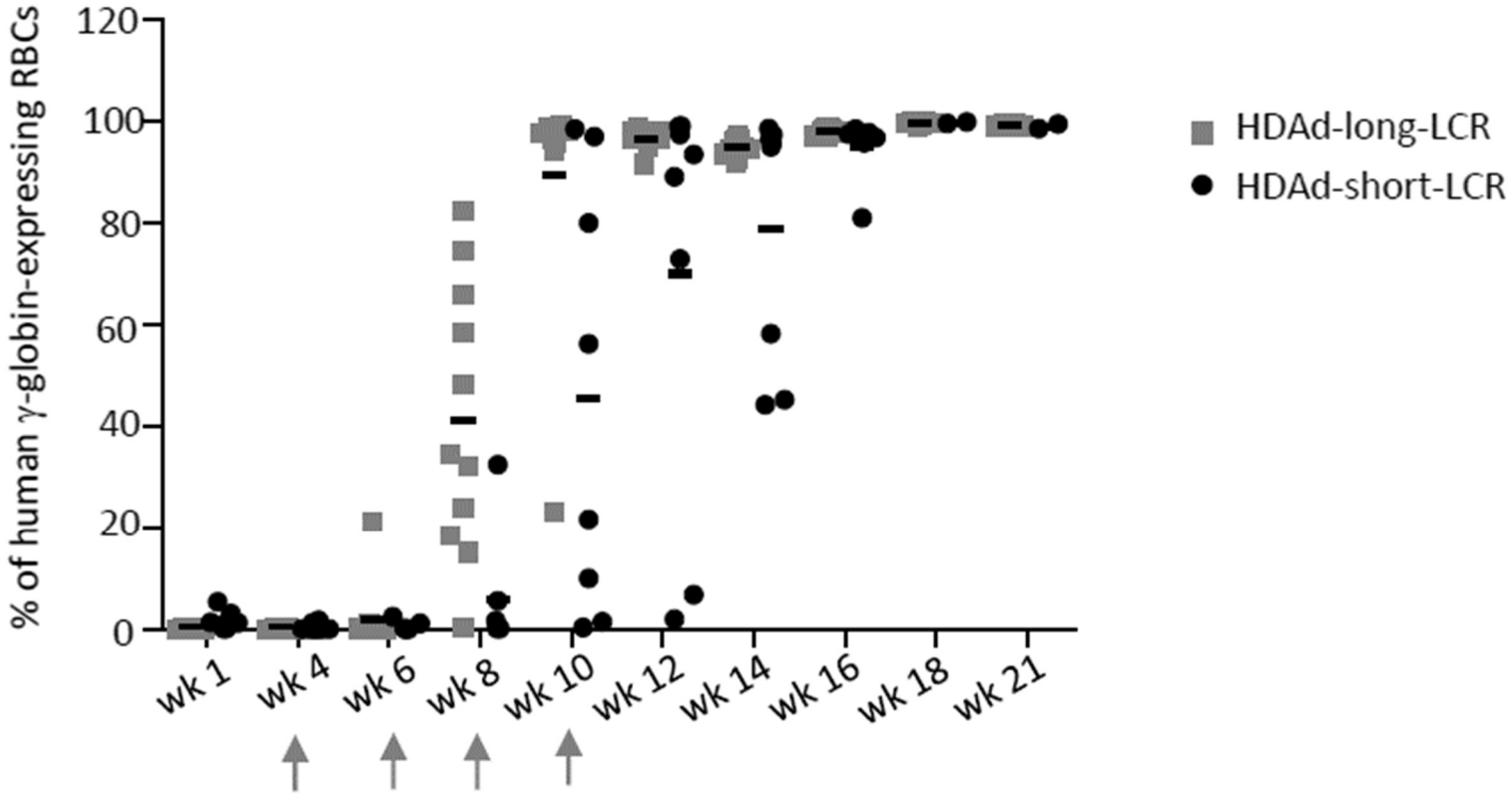


FIG. 32G

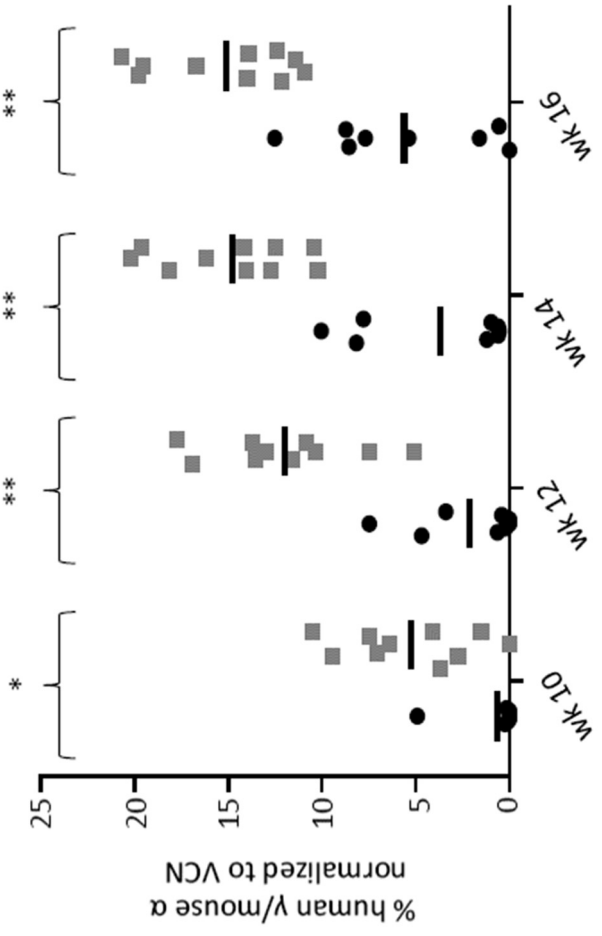


FIG. 32H

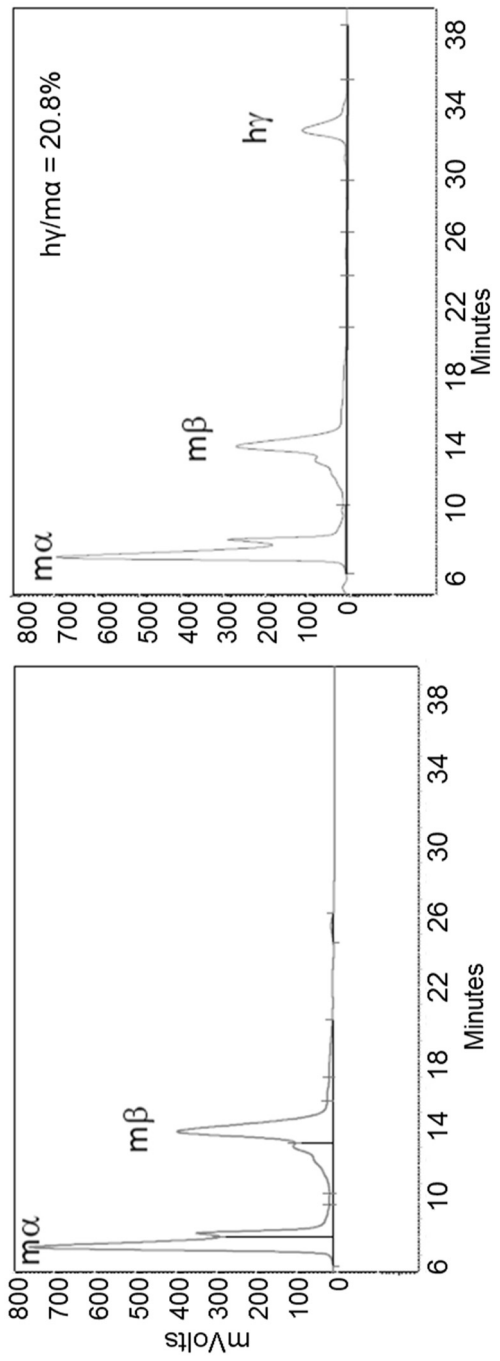


FIG. 33A

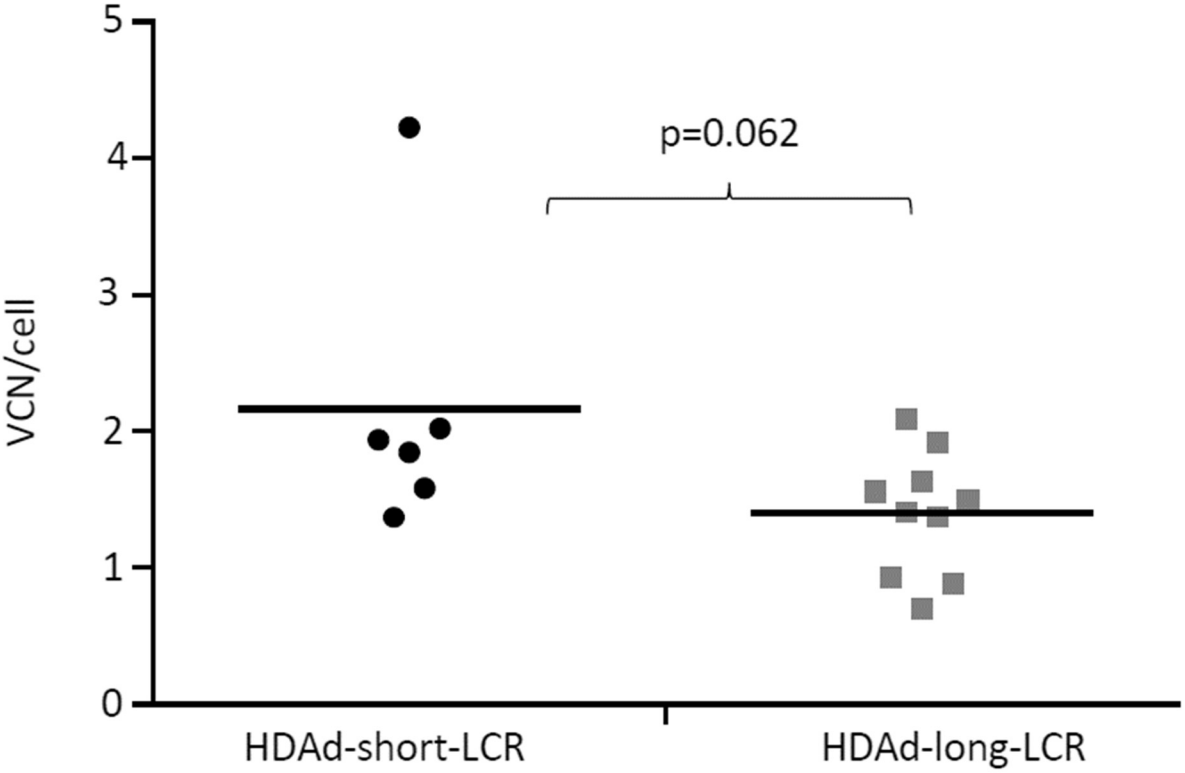


FIG. 33B

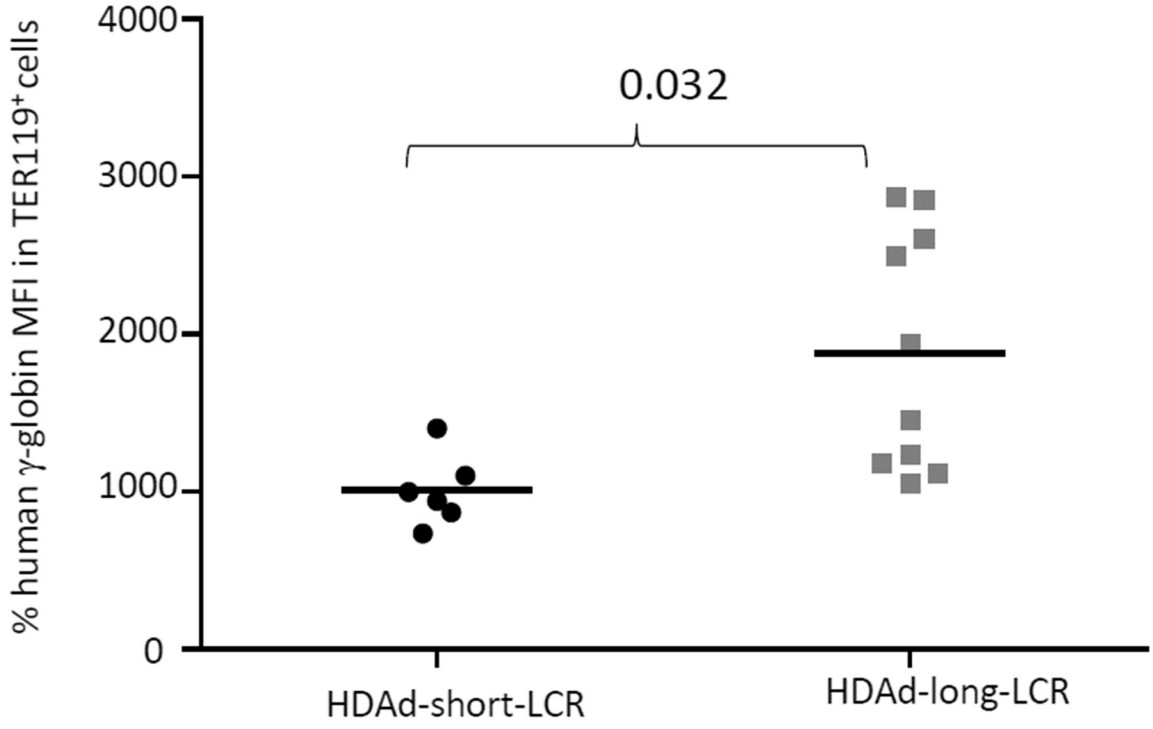
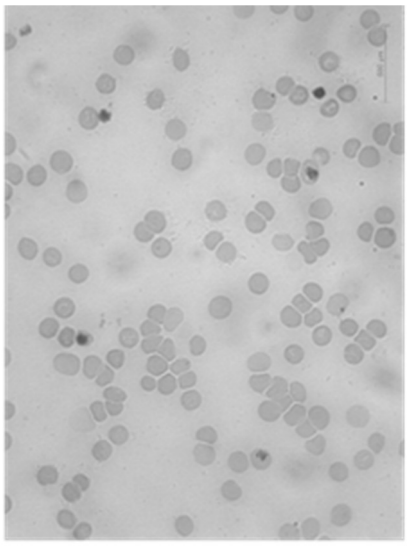


FIG. 34


Week 10 after treatment

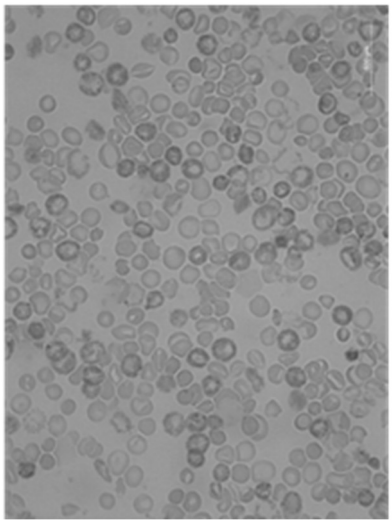
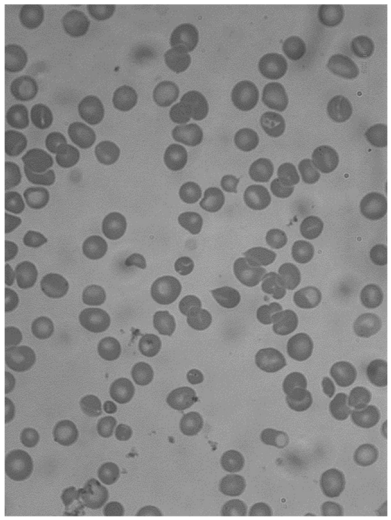
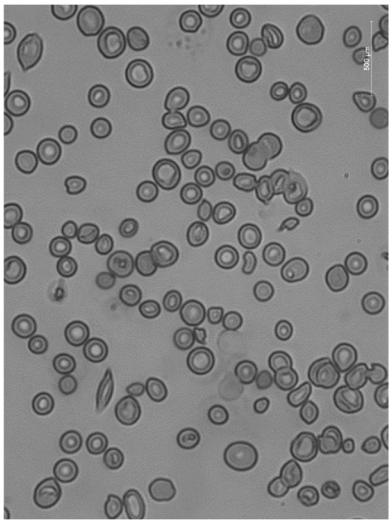

Before treatment

FIG. 35


Week 10 after treatment-long LCR


Before treatment

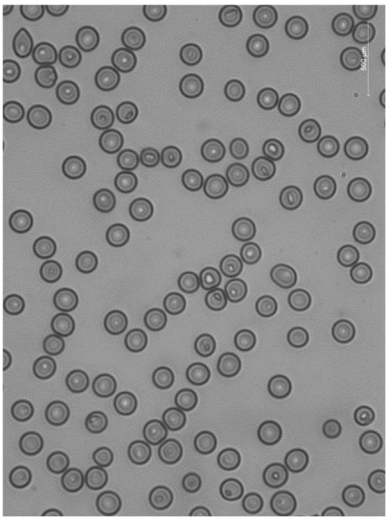

C57BL6 (Normal mice)

FIG. 36A

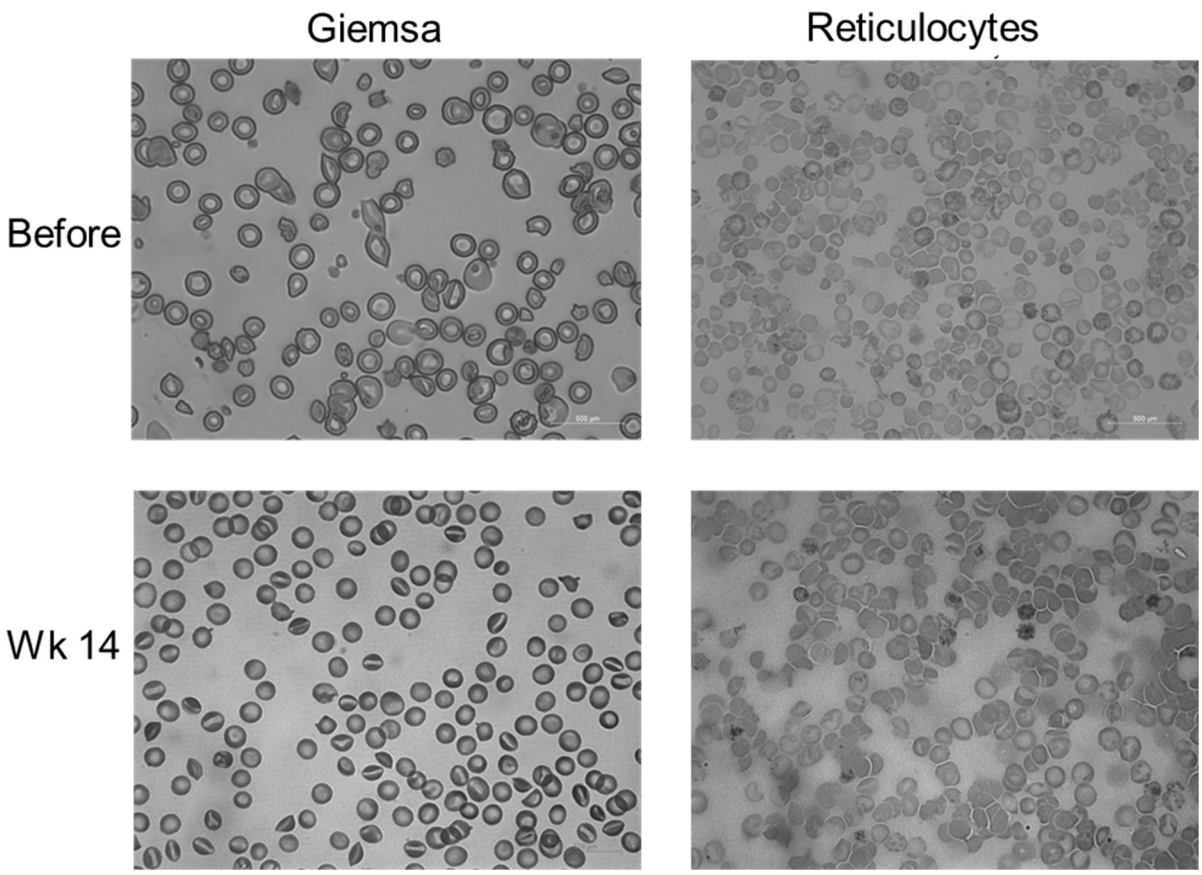


FIG. 36B

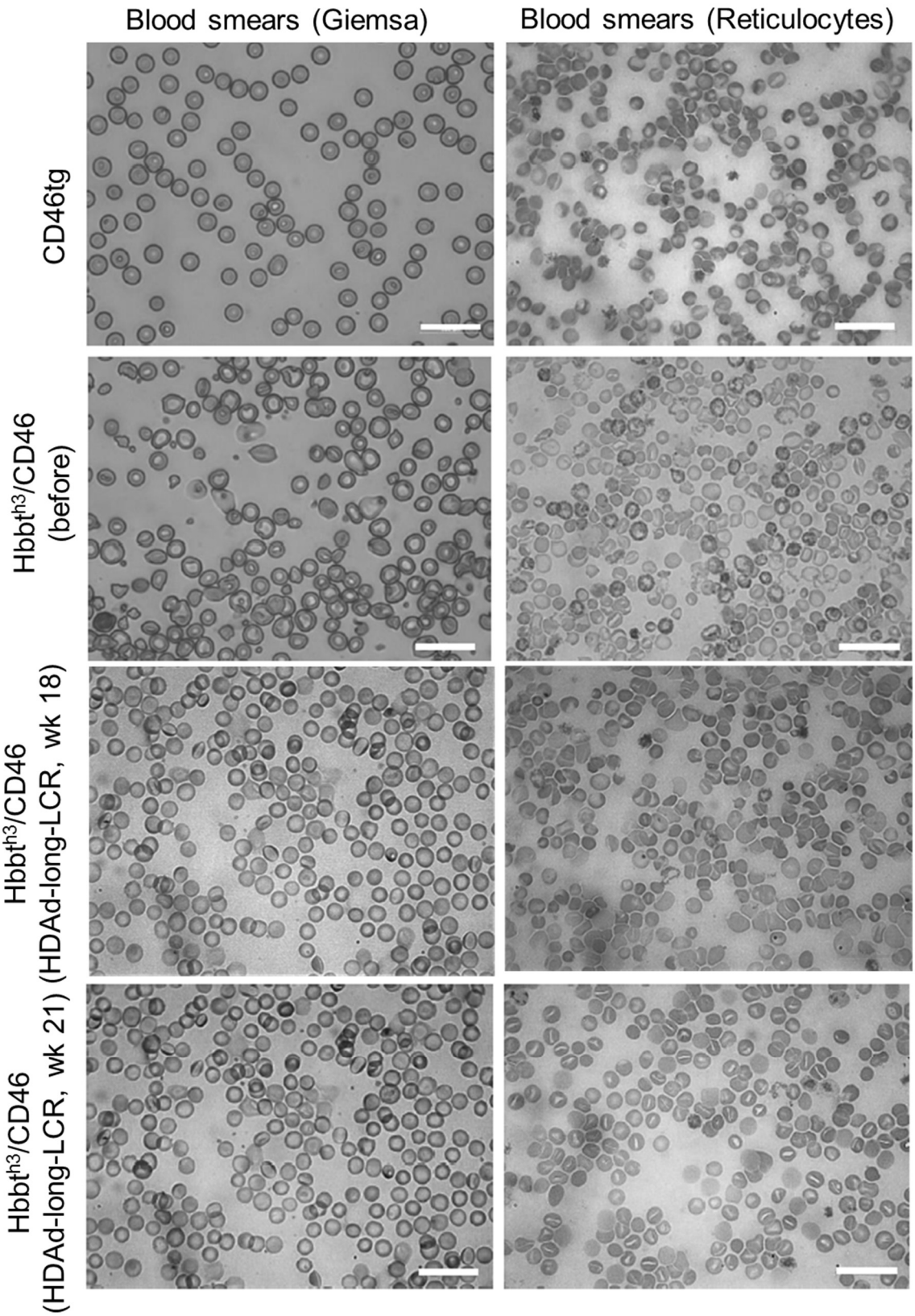

Blood smears (Giemsa)
Blood smears (Reticulocytes)
CD46tg
Hbbth3/CD46 (before)
Hbbth3/CD46 (HDAd-long-LCR, wk 18)
Hbbth3/CD46 (HDAd-long-LCR, wk 21)

FIG. 36C

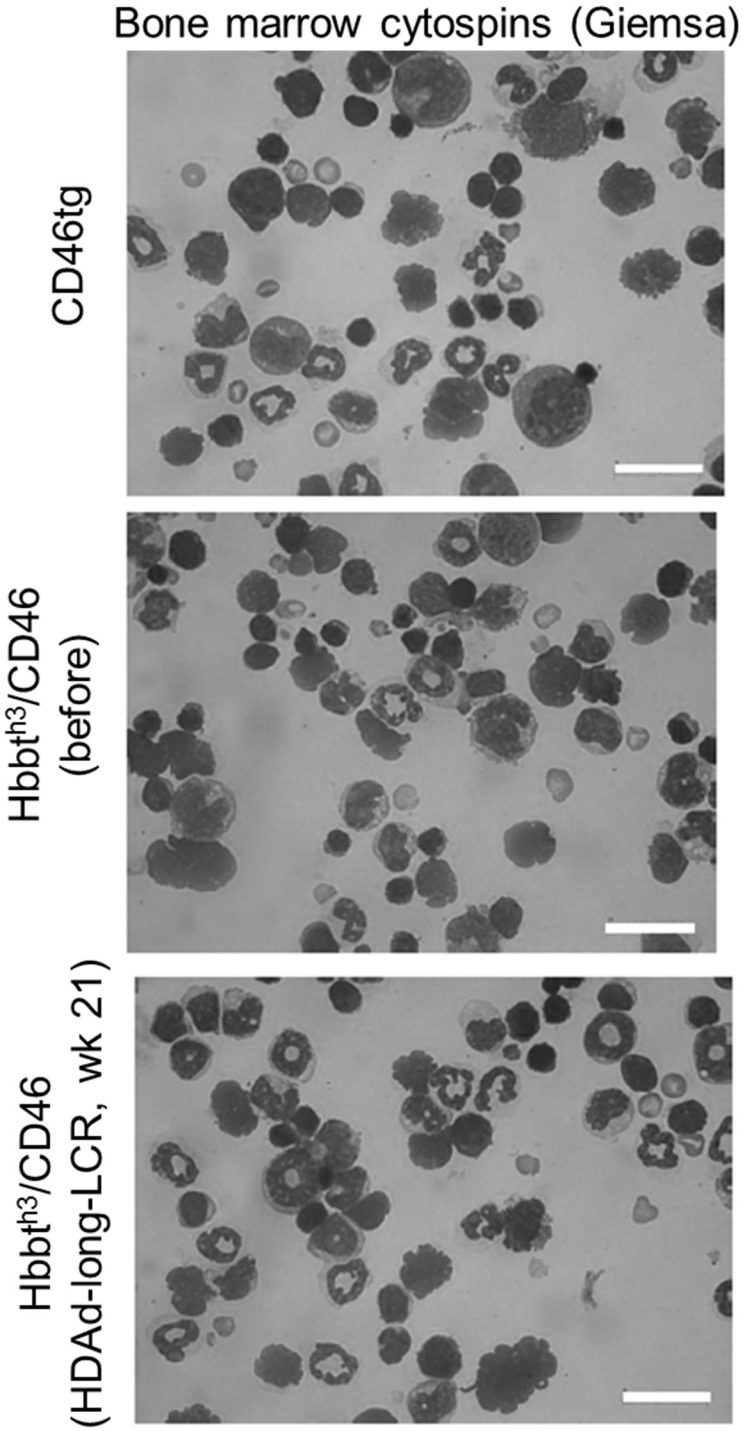

Bone marrow cytospins (Giemsa)
CD46tg
Hbbth3/CD46 (before)
Hbbth3/CD46 (HDAd-long-LCR, wk 21)

FIG. 37A

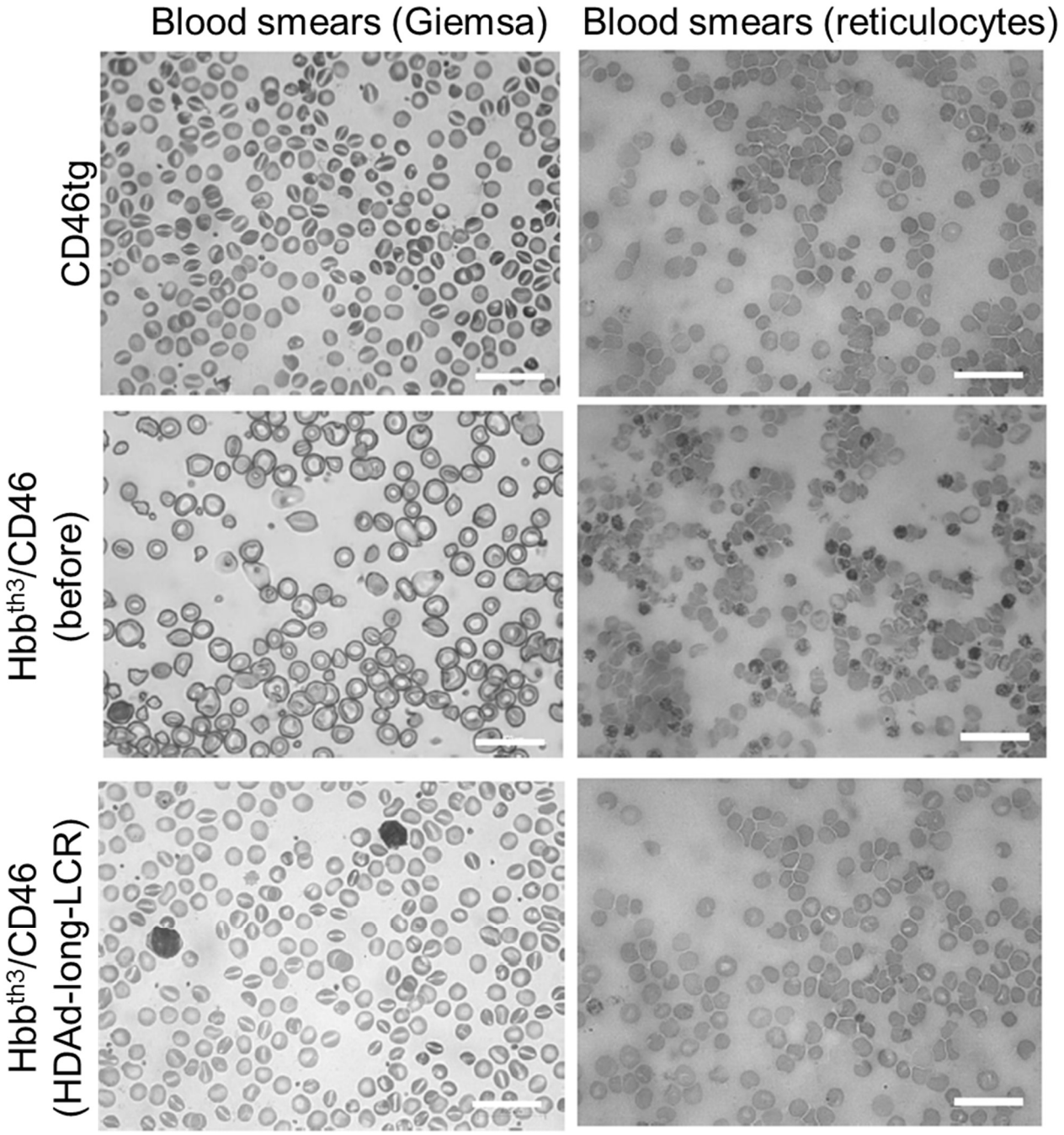

Blood smears (Giemsa)
Blood smears (reticulocytes)
CD46tg
Hbb^{th3}/CD46 (before)
Hbb^{th3}/CD46 (HDAd-long-LCR)

FIG. 37B

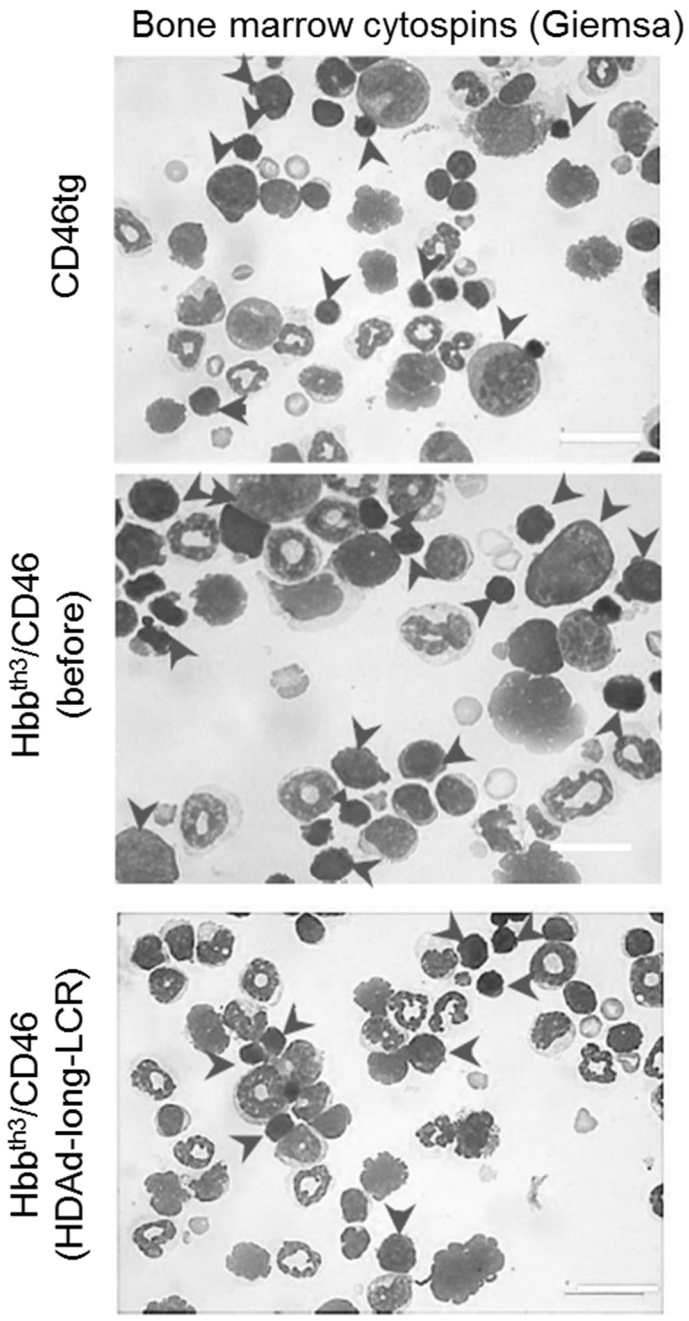

Bone marrow cytospins (Giemsa)
CD46tg
Hbb^th3/CD46 (before)
Hbb^th3/CD46 (HDAd-long-LCR)

FIG. 38

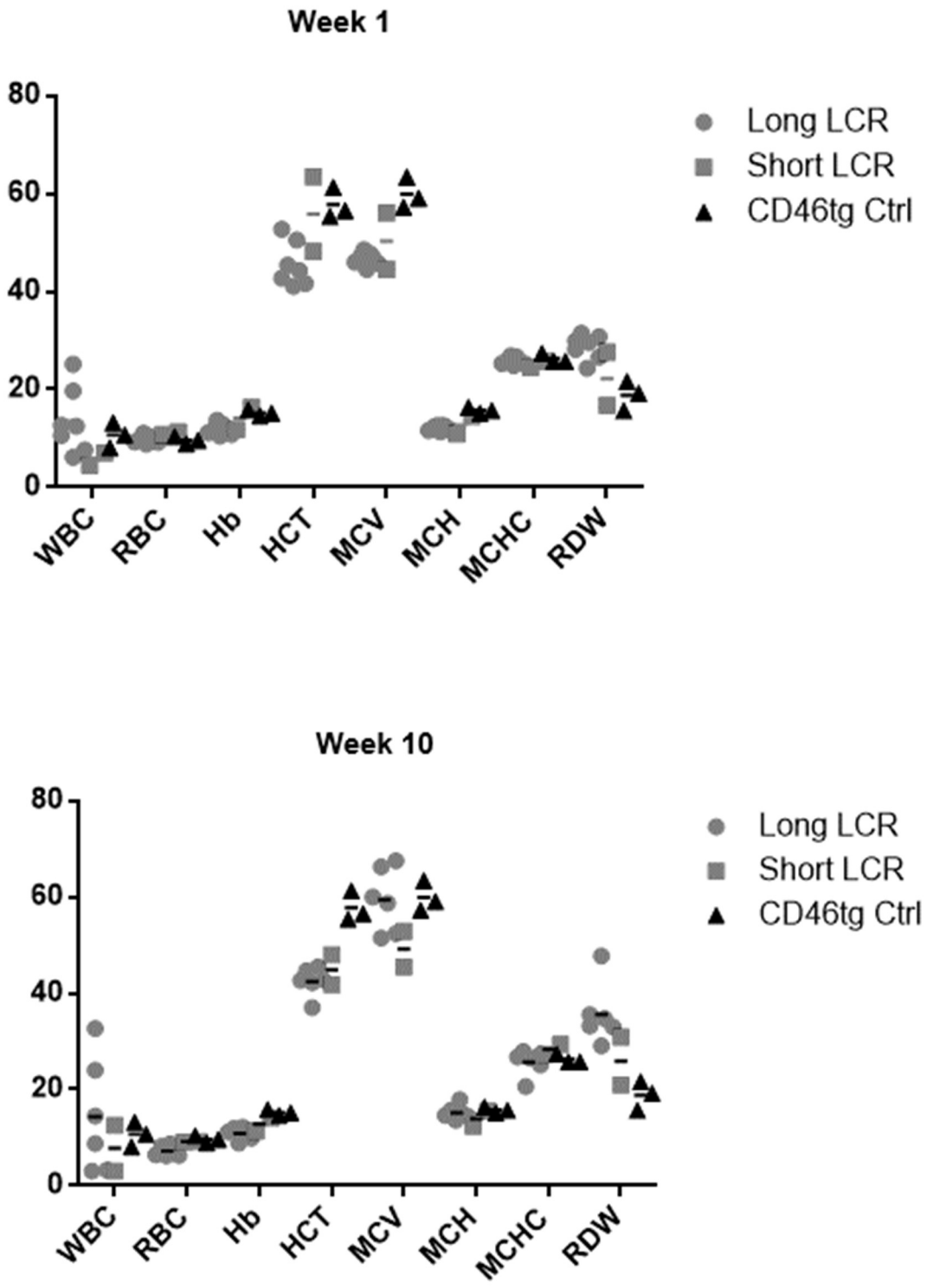

Week 1
80
60
40
20
0
WBC
RBC
Hb
HCT
MCV
MCH
MCHC
RDW
Long LCR
Short LCR
CD46tg Ctrl
Week 10
80
60
40
20
0
WBC
RBC
Hb
HCT
MCV
MCH
MCHC
RDW
Long LCR
Short LCR
CD46tg Ctrl

FIG. 39A

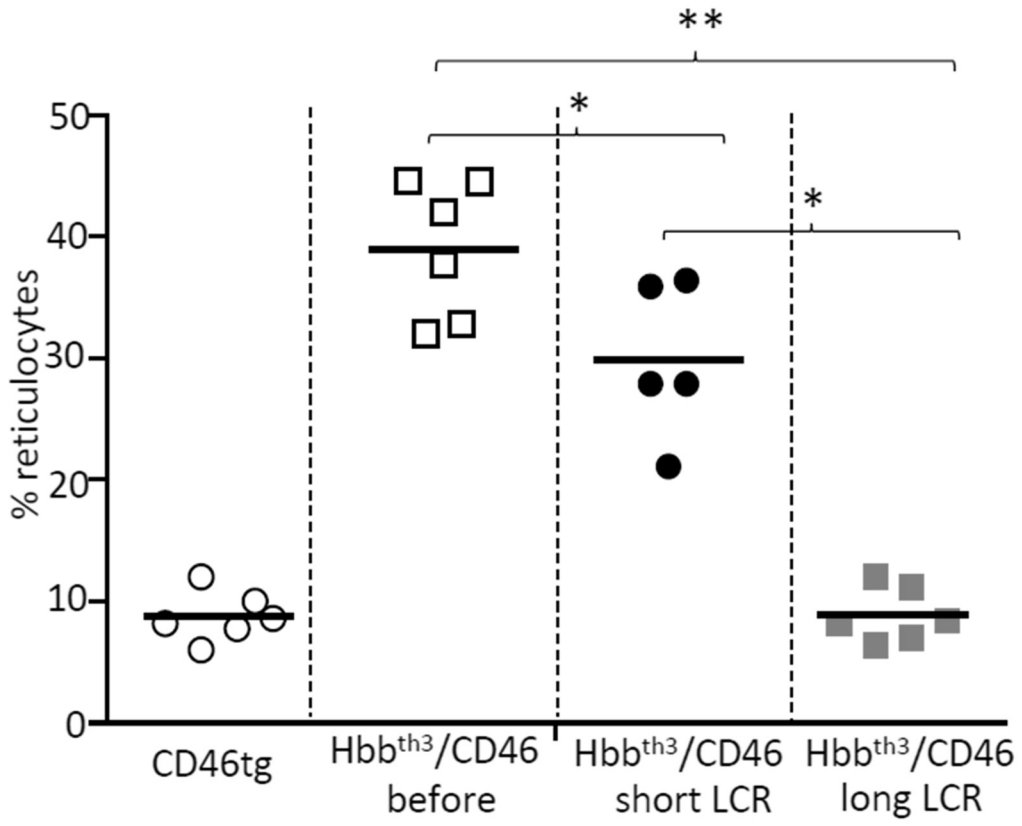

**
*
*
50
40
30
20
10
0
% reticulocytes
CD46tg
Hbb^th3/CD46 before
Hbb^th3/CD46 short LCR
Hbb^th3/CD46 long LCR

FIG. 39B

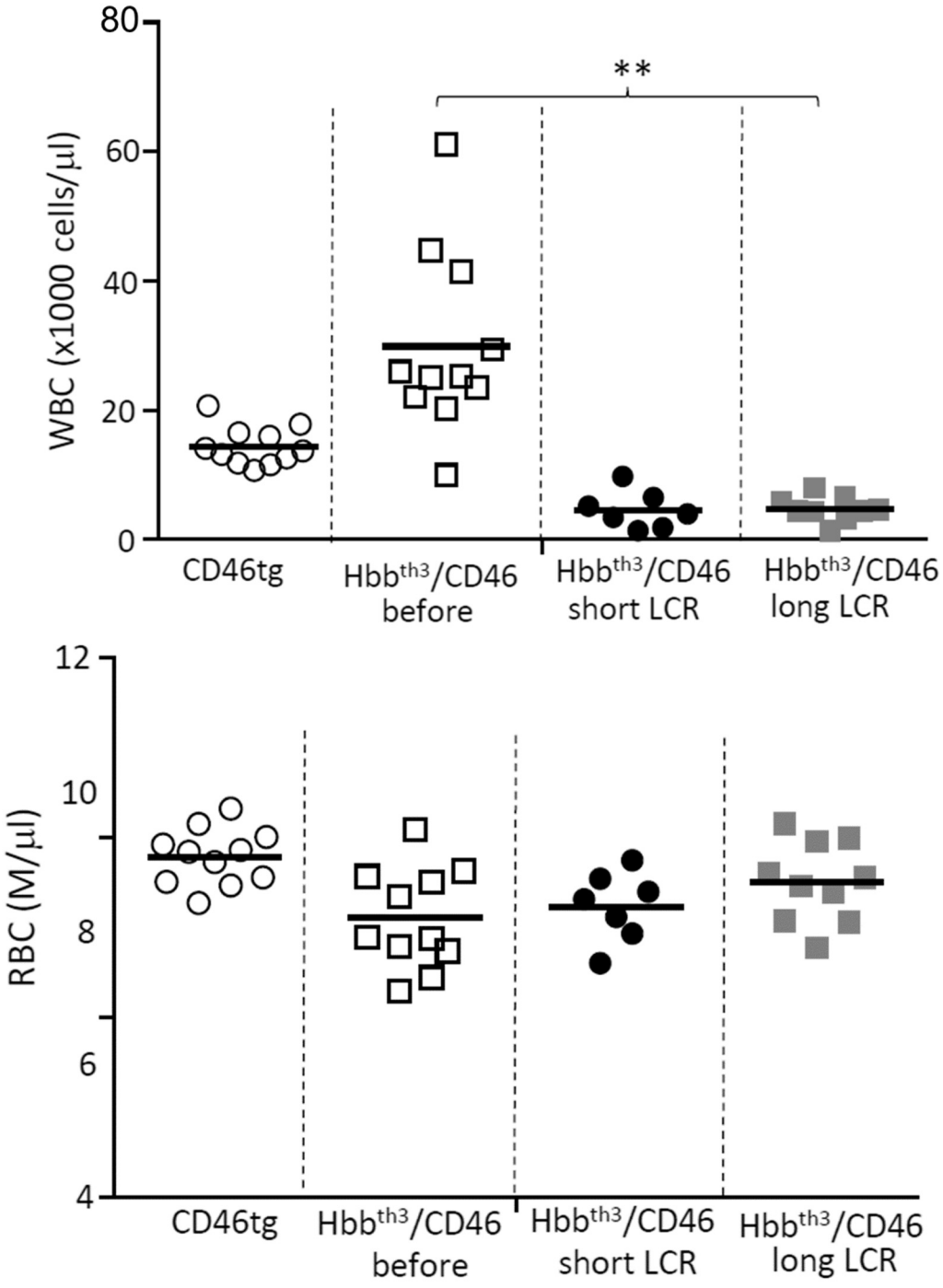

80
60
40
20
0
WBC (x1000 cells/µl)
**
CD46tg
Hbb^th3/CD46 before
Hbb^th3/CD46 short LCR
Hbb^th3/CD46 long LCR
12
10
8
6
4
RBC (M/µl)
CD46tg
Hbb^th3/CD46 before
Hbb^th3/CD46 short LCR
Hbb^th3/CD46 long LCR

FIG. 39B cont'd

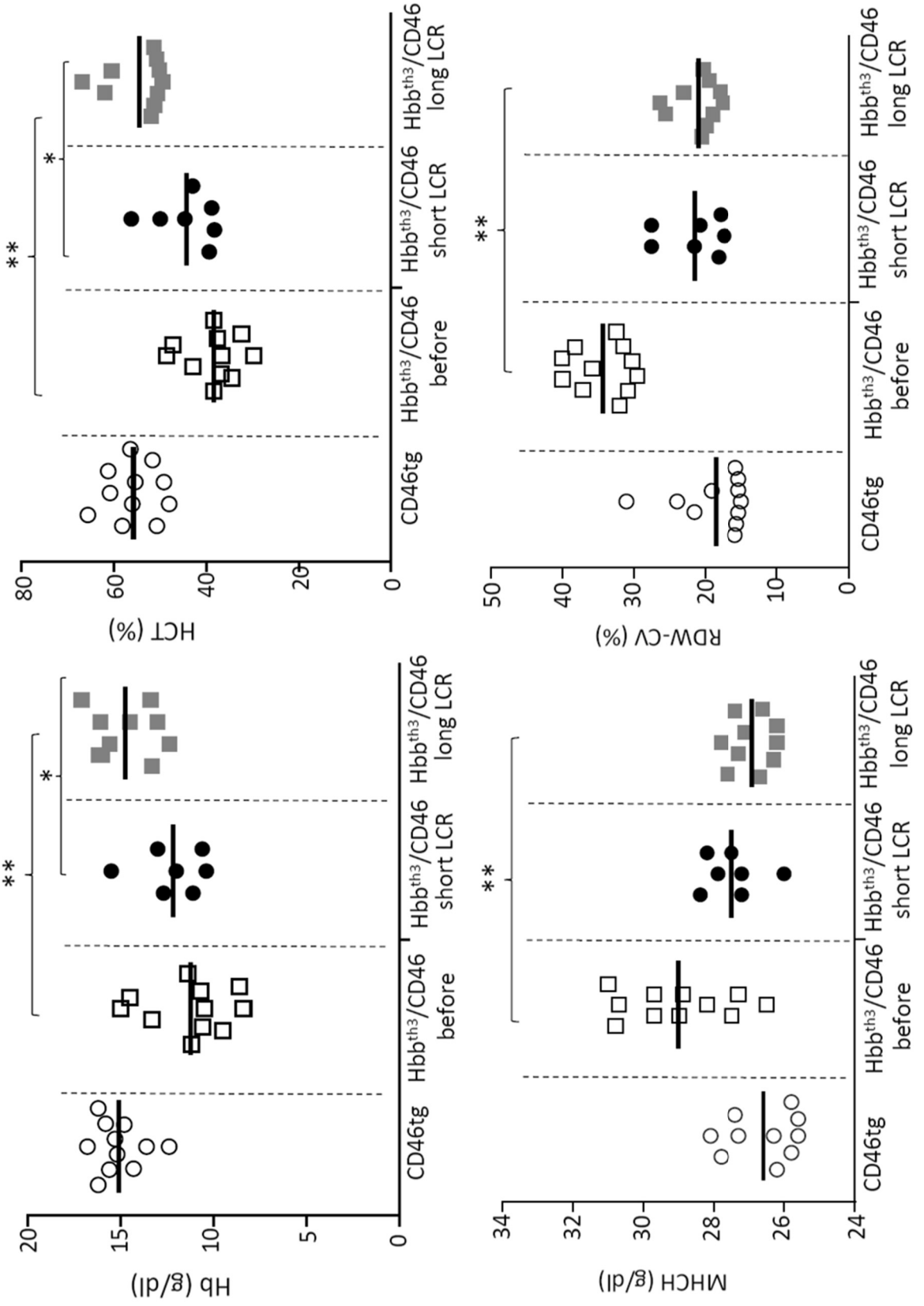
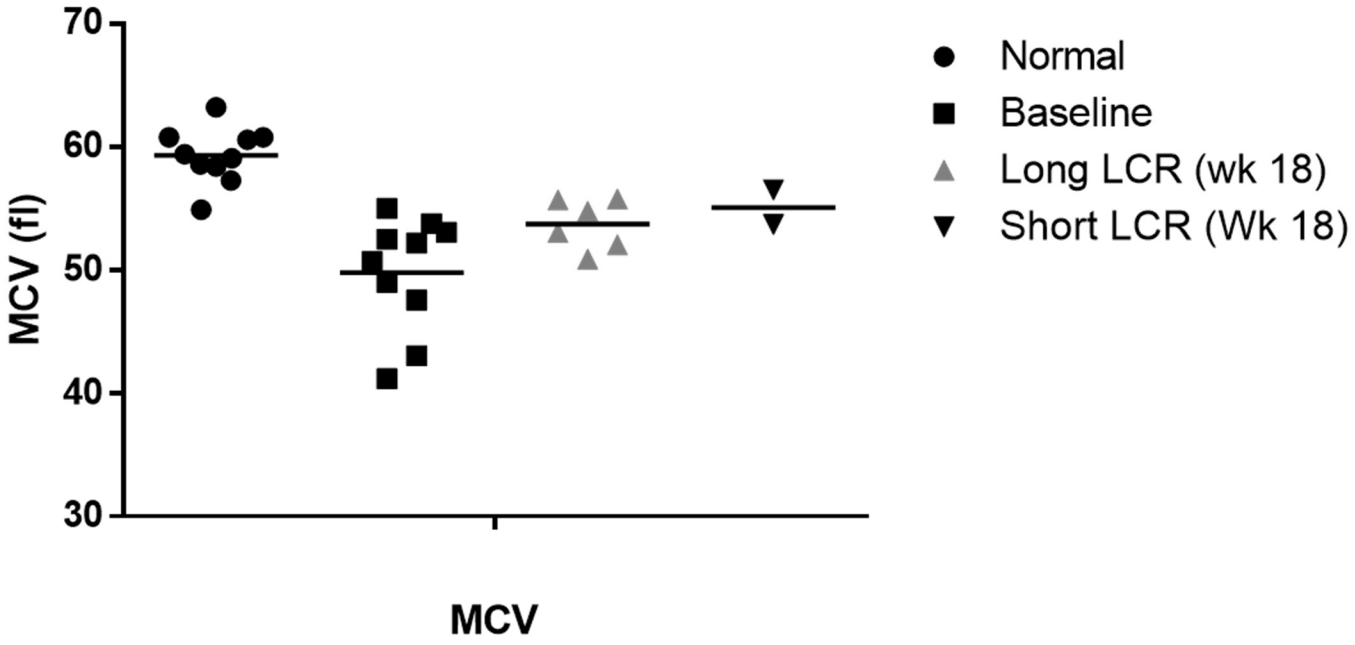


FIG. 39B cont'd



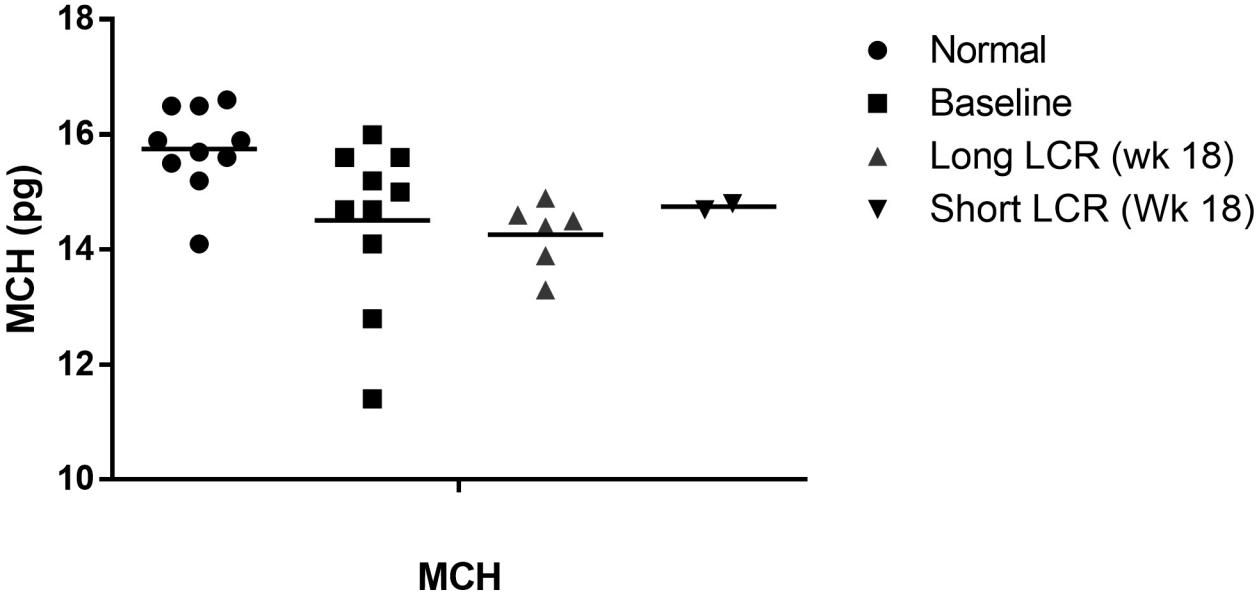
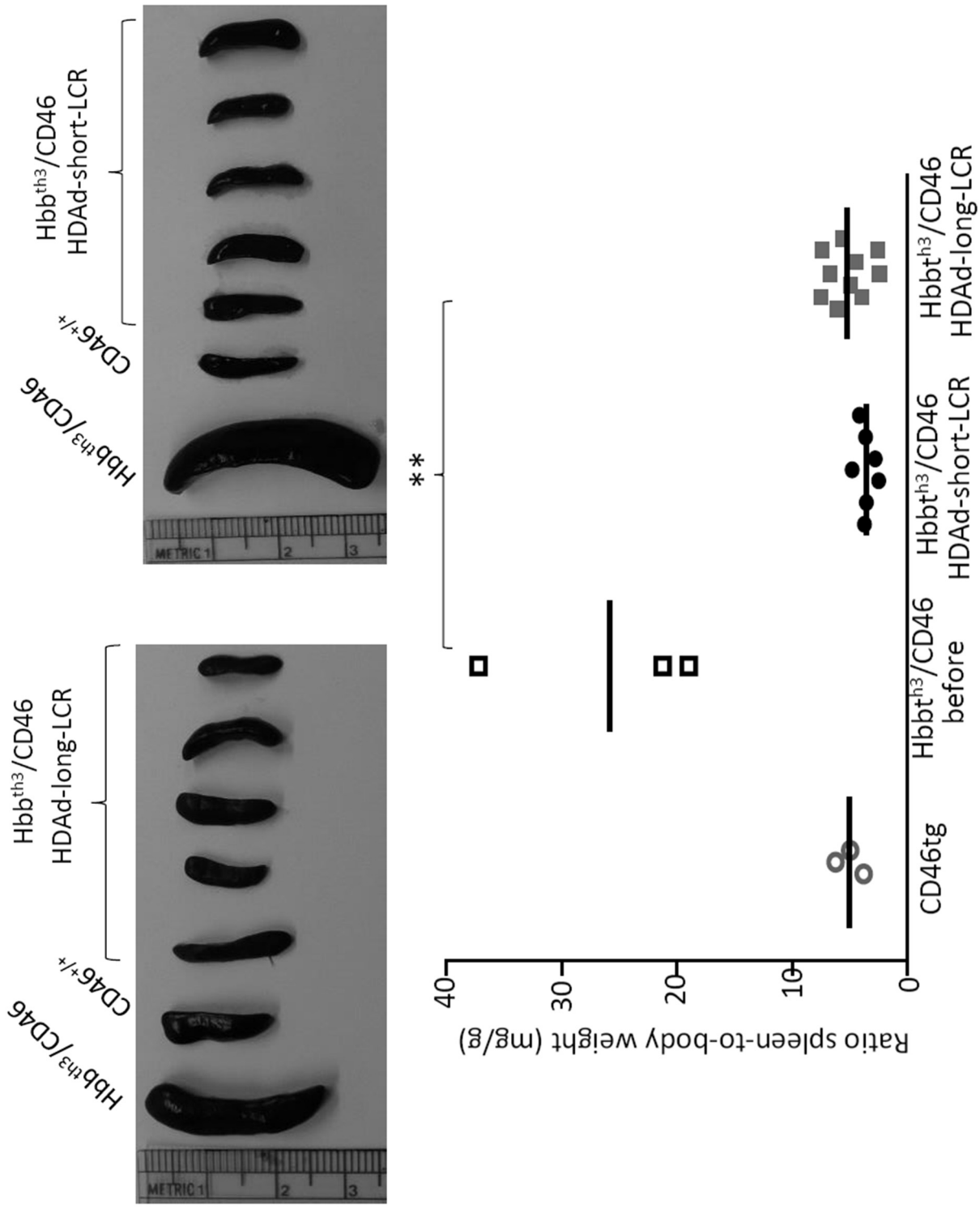


FIG. 40A


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

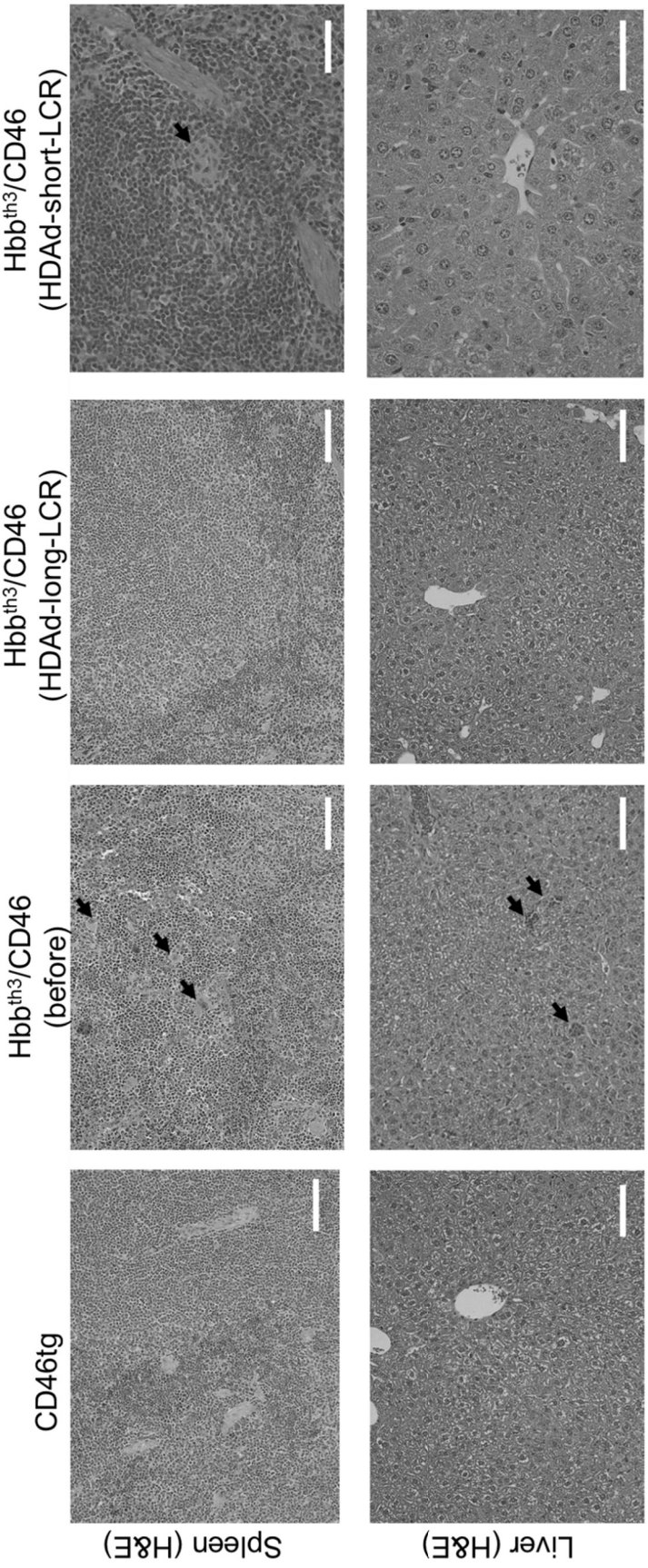

CD46tg
Hbb^{th3}/CD46 (before)
Hbb^{th3}/CD46 (HDAd-long-LCR)
Hbb^{th3}/CD46 (HDAd-short-LCR)
Spleen (H&E)
Liver (H&E)

FIG. 40B

FIG. 41

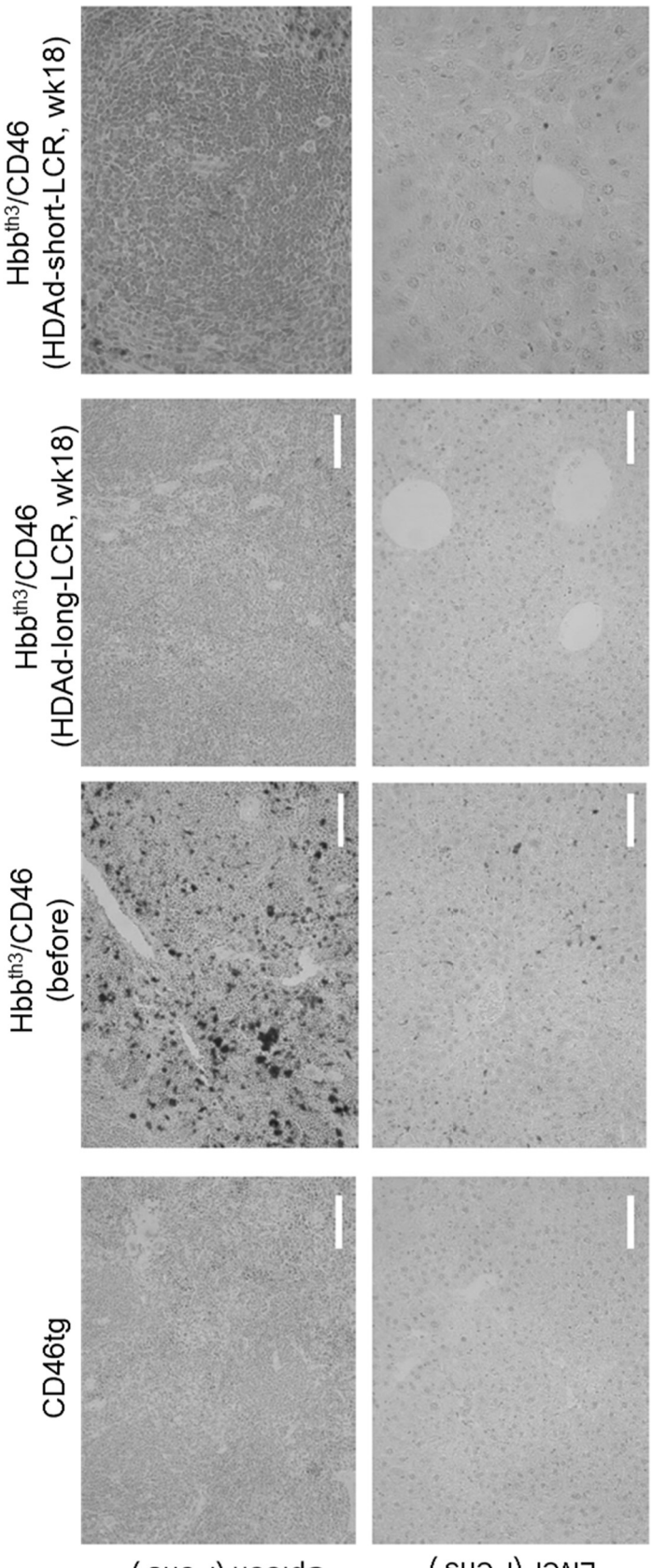

CD46tg
Hbb^{th3}/CD46 (before)
Hbb^{th3}/CD46 (HDAd-long-LCR, wk18)
Hbb^{th3}/CD46 (HDAd-short-LCR, wk18)
Spleen (Perls')
Liver (Perls')

FIG. 42A

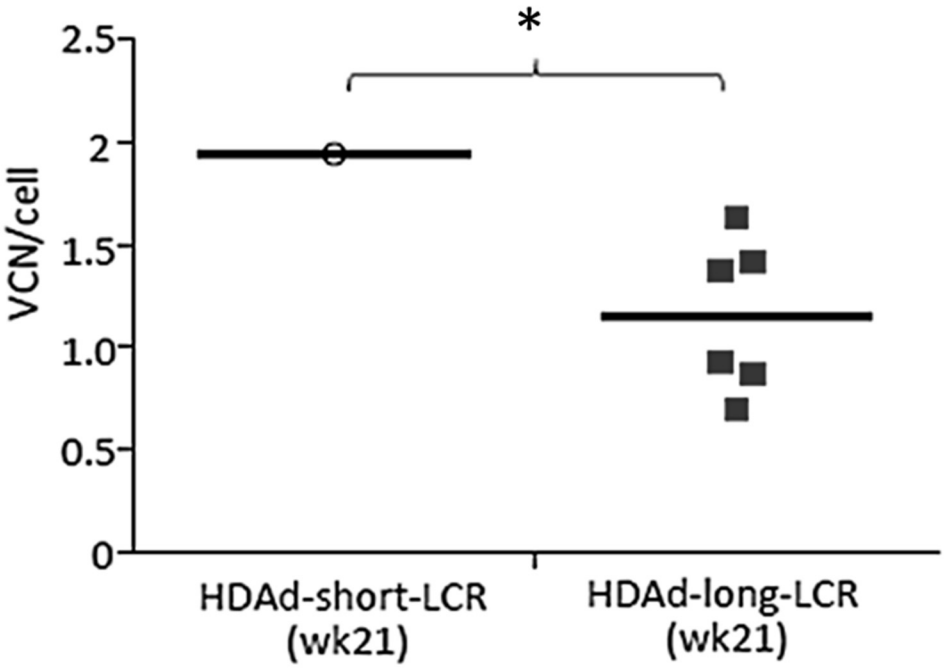


FIG. 42B

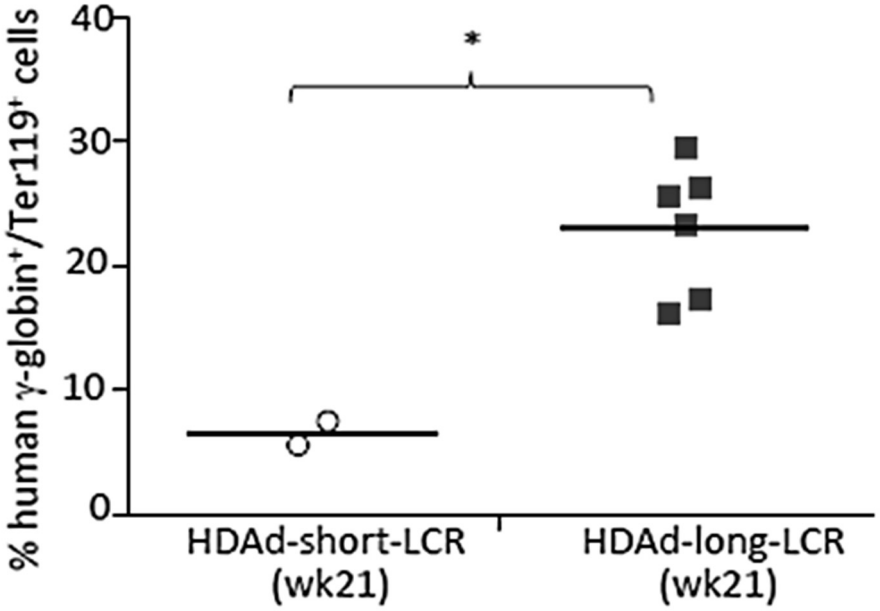


FIG. 42C

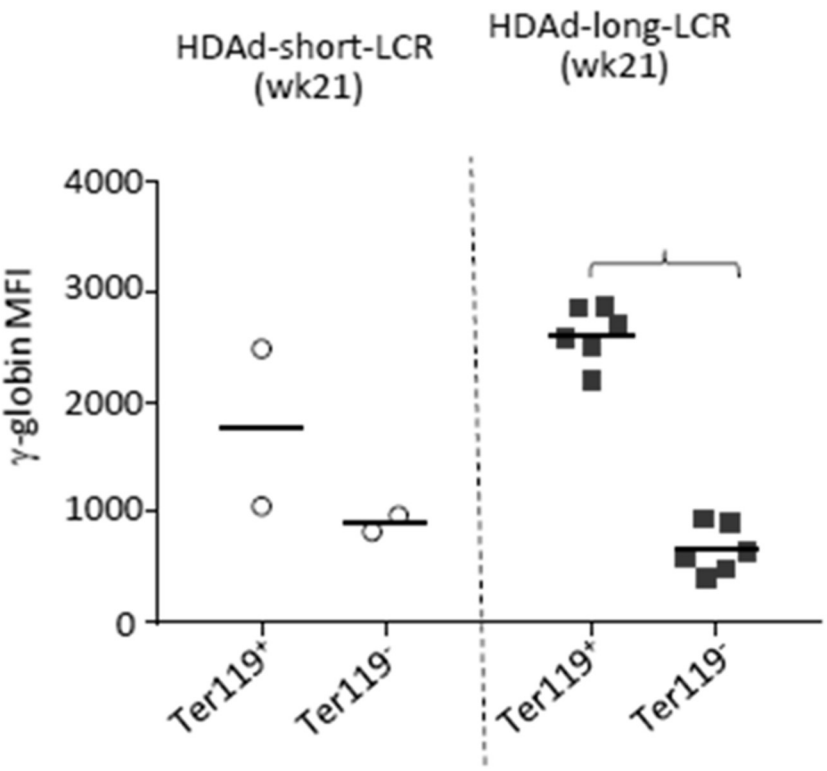

HDAd-short-LCR
(wk21)
HDAd-long-LCR
(wk21)
γ-globin MFI
4000
3000
2000
1000
0
Ter119+
Ter119-
Ter119+
Ter119-

FIG. 43

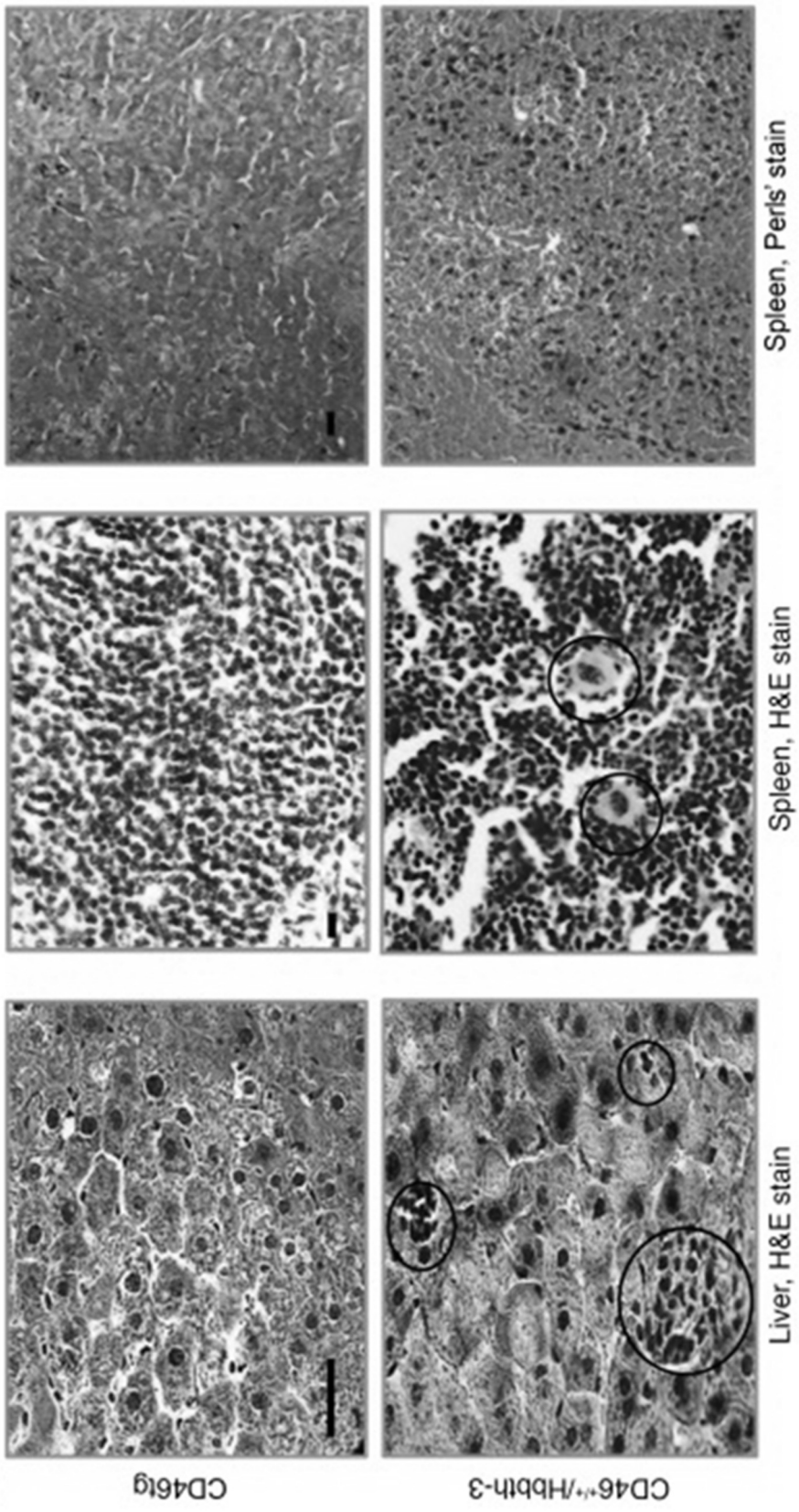

Before
CD46tg
CD46-/-/Hbb1h-3
Liver, H&E stain
Spleen, H&E stain
Spleen, Perls' stain

FIG. 44A

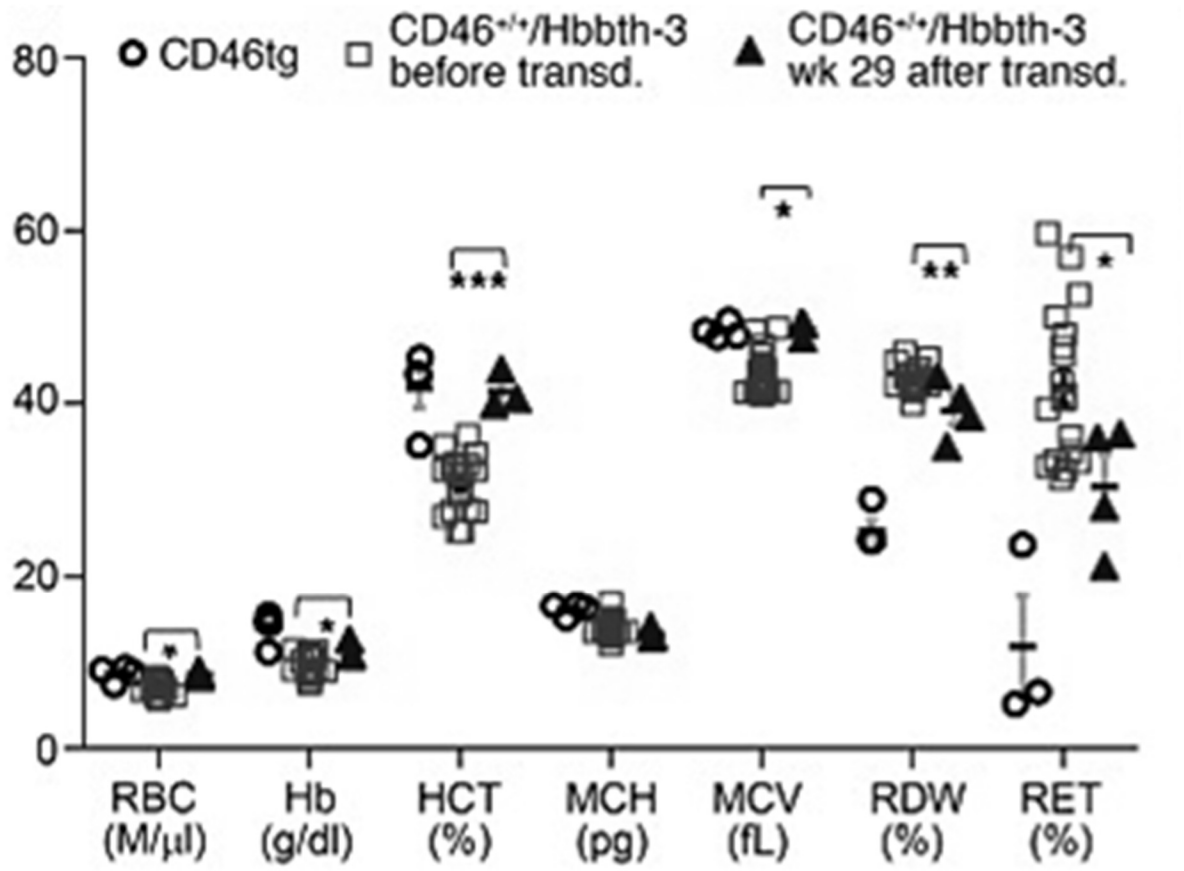


FIG. 44B

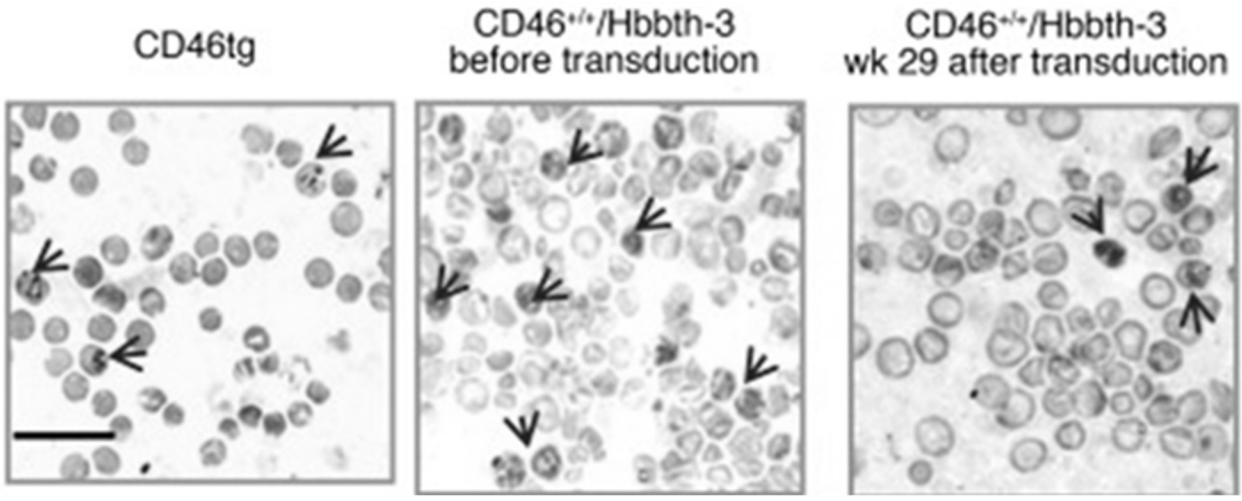


FIG. 44C

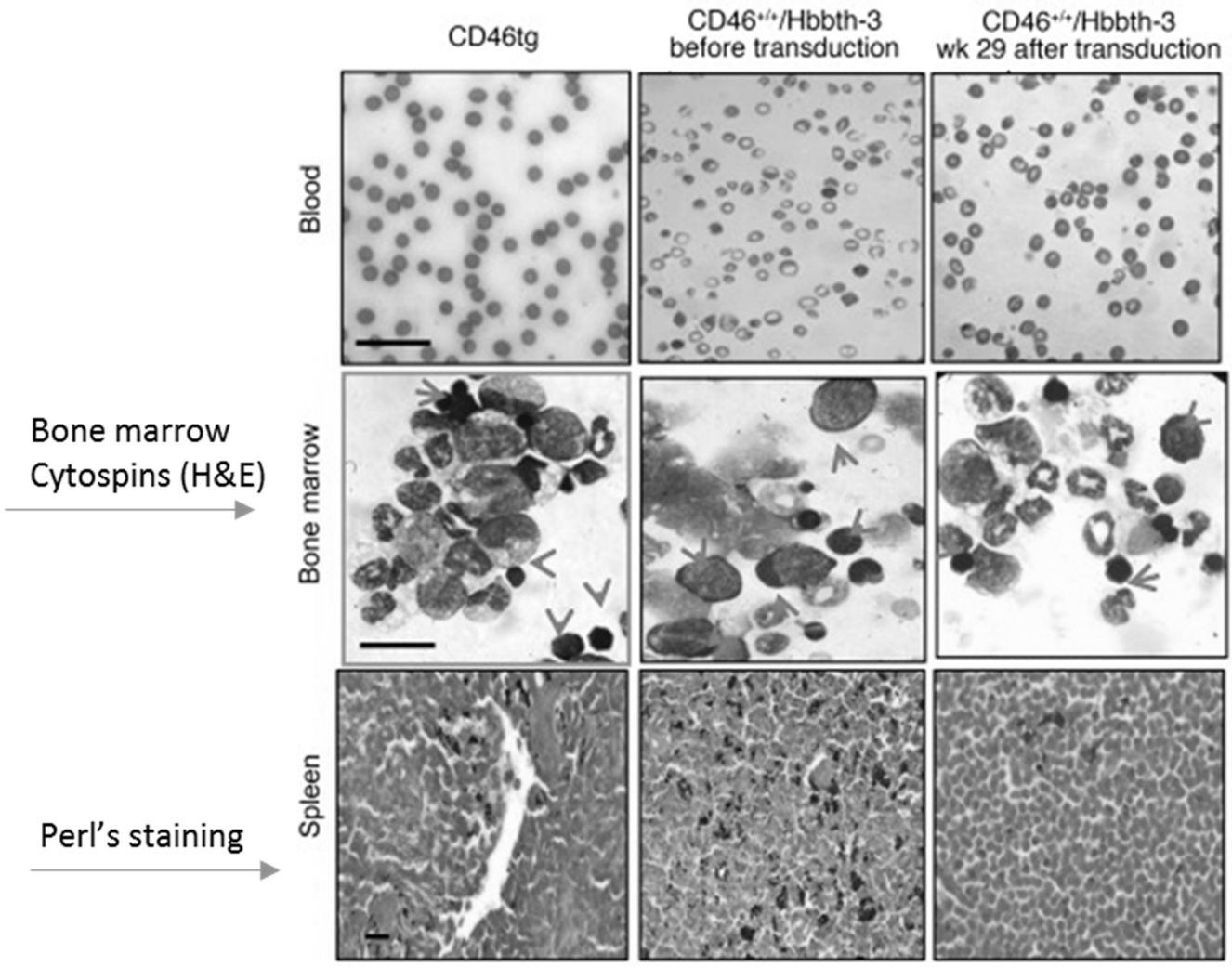

CD46tg
CD46+/+/Hbbth-3 before transduction
CD46+/+/Hbbth-3 wk 29 after transduction
Blood
Bone marrow
Spleen
Bone marrow Cytospins (H&E)
Perl's staining

FIG. 44D

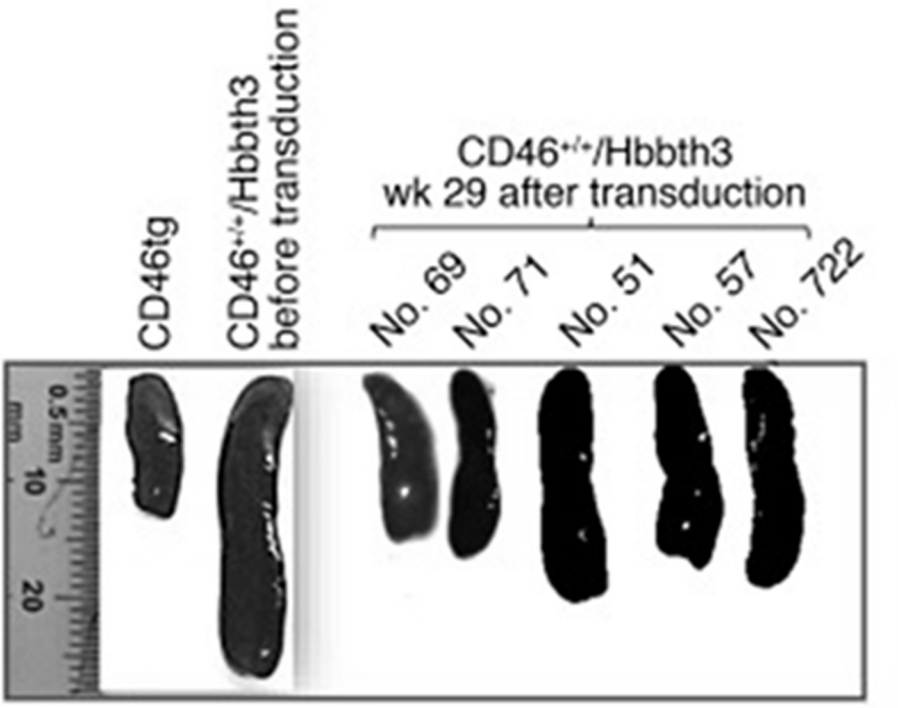


FIG. 44E

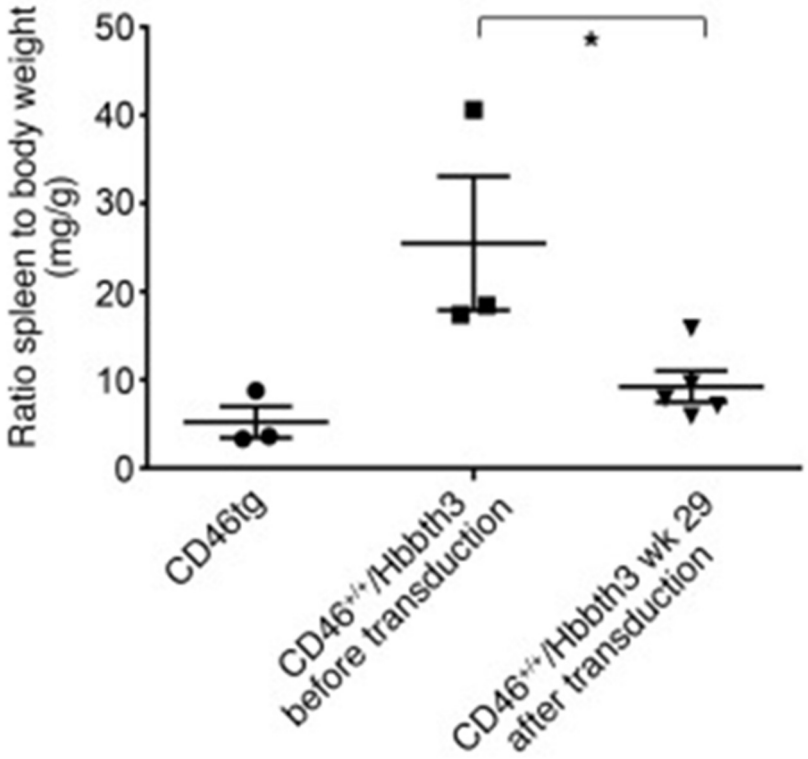


FIG. 45

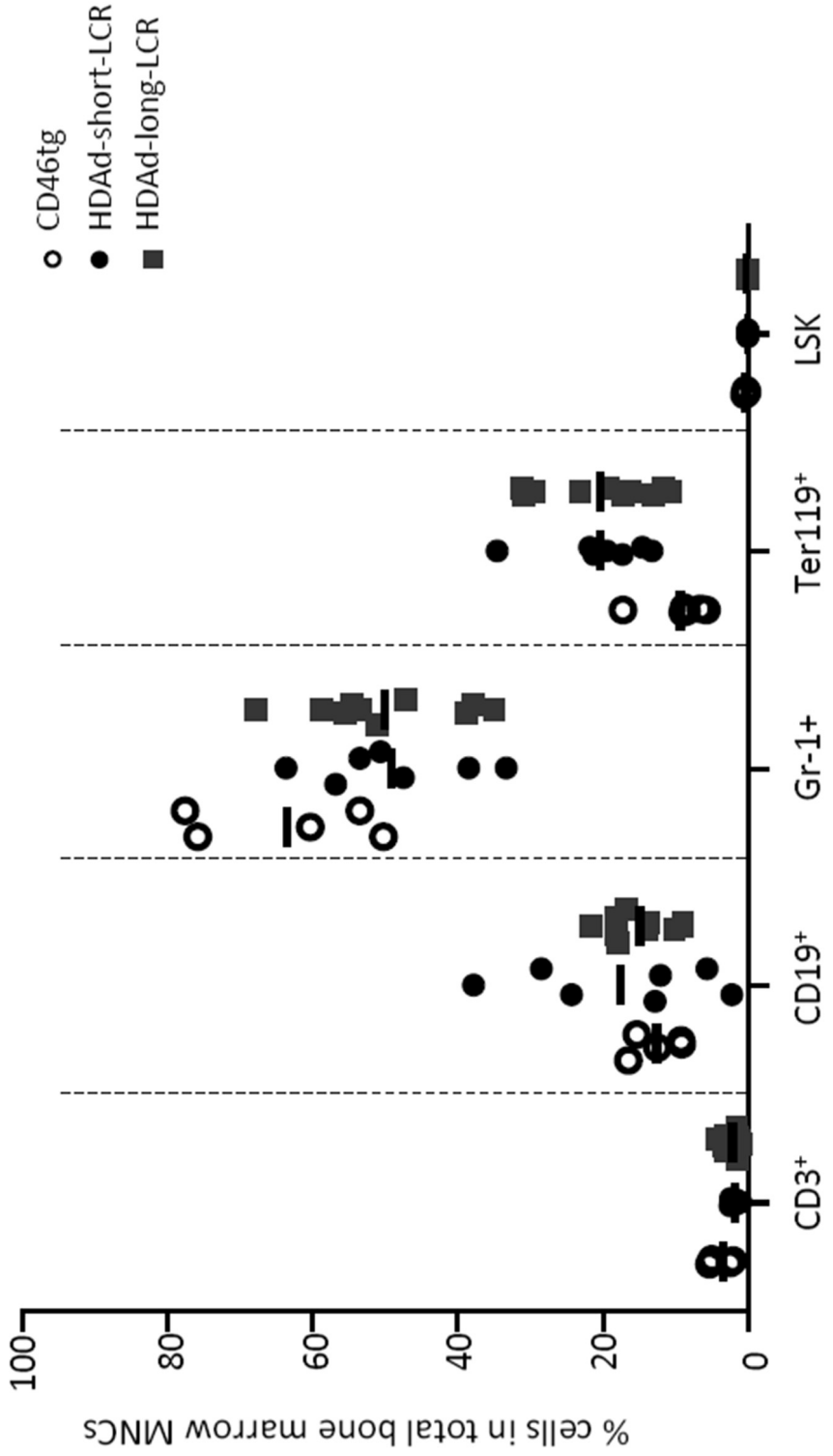

CD46tg
HDAd-short-LCR
HDAd-long-LCR
% cells in total bone marrow MNCs
100
80
60
40
20
0
CD3+
CD19+
Gr-1+
Ter119+
LSK

FIG. 46

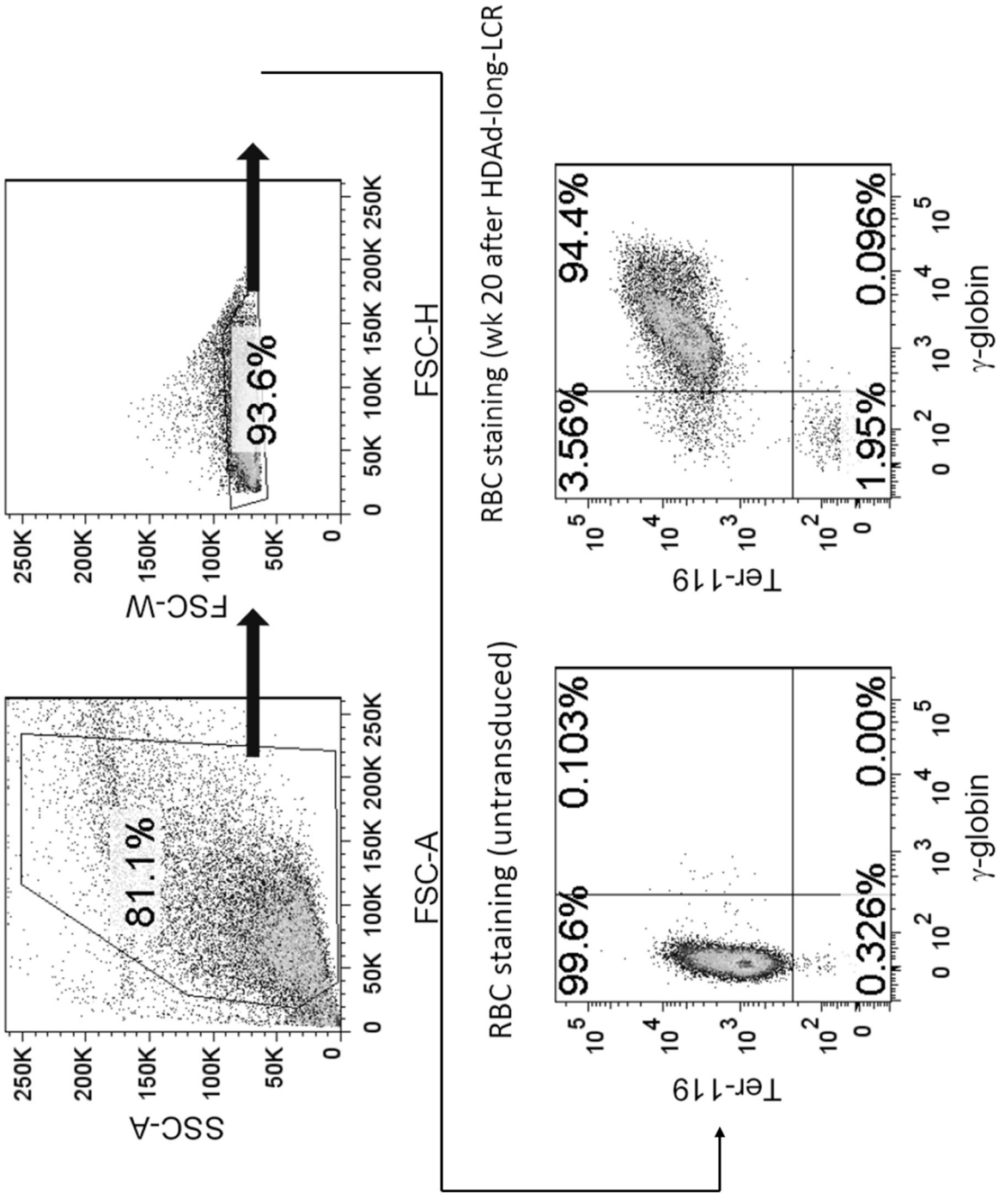

SSC-A
FSC-A
81.1%
FSC-W
FSC-H
93.6%
RBC staining (untransduced)
Ter-119
γ-globin
99.6%
0.103%
0.326%
0.00%
RBC staining (wk 20 after HDAd-long-LCR
3.56%
94.4%
1.95%
0.096%

FIG. 47A

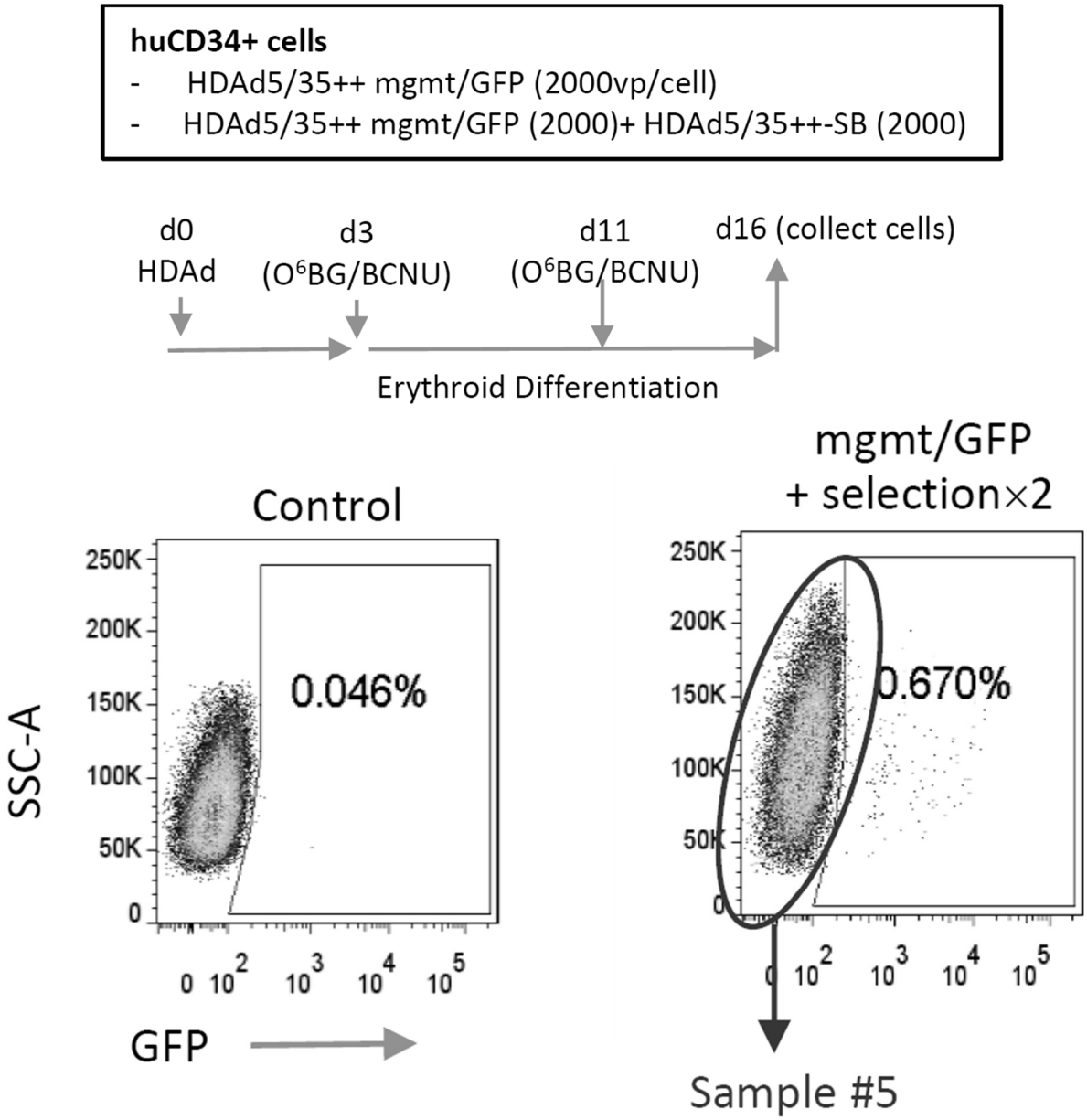

huCD34+ cells
- HDAd5/35++ mgmt/GFP (2000vp/cell)
- HDAd5/35++ mgmt/GFP (2000)+ HDAd5/35++-SB (2000)
d0
HDAd
d3
(O6BG/BCNU)
d11
(O6BG/BCNU)
d16 (collect cells)
Erythroid Differentiation
Control
mgmt/GFP
+ selection×2
SSC-A
250K
200K
150K
100K
50K
0
0.046%
0.670%
0 10^2 10^3 10^4 10^5
GFP
Sample #5

FIG. 47A cont'd

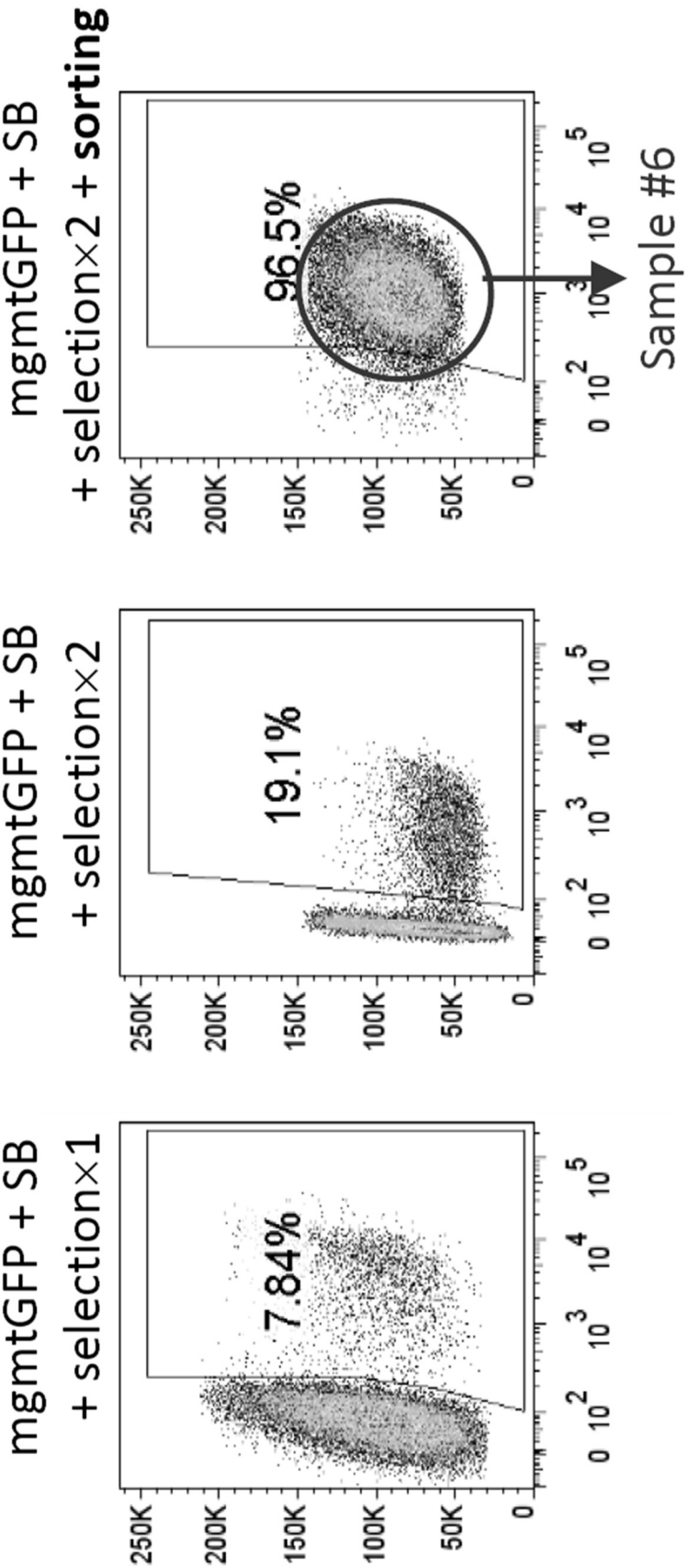


FIG. 47B

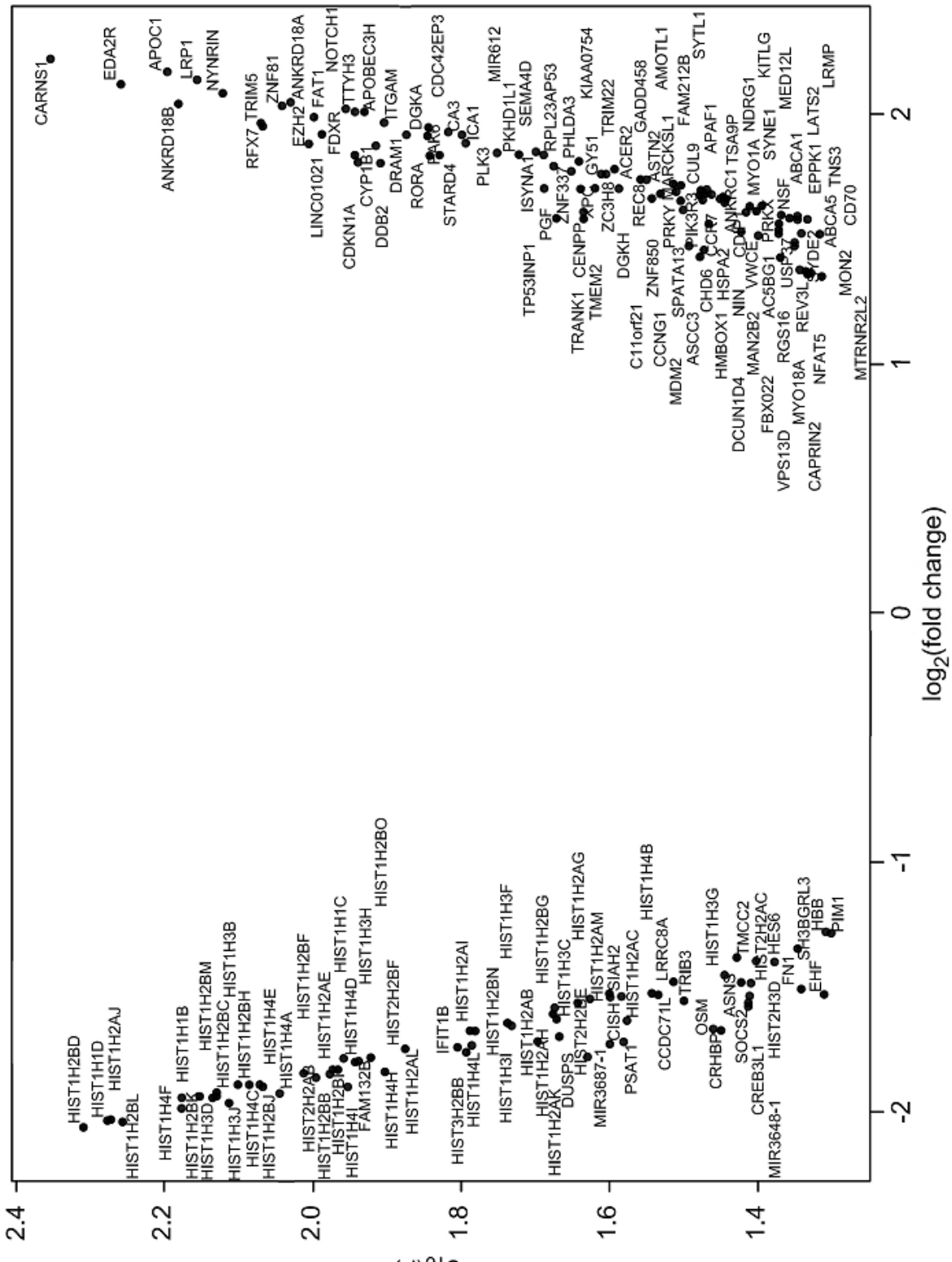

-log10(p)
log2(fold change)
CARNS1
EDA2R
APOC1
LRP1
NYNRIN
ANKRD18B
RFX7
TRIM5
ZNF81
EZH2
ANKRD18A
FAT1
NOTCH1
FDXR
TTYH3
APOBEC3H
ITGAM
LINC01021
CDKN1A
CYP1B1
DDB2
DRAM1
DGKA
RORA
CDC42EP3
STARD4
CA3
CA1
PLK3
PKHD1L1
MIR612
SEMA4D
TP53INP1
ISYNA1
RPL23AP53
PGF
ZNF33A
PHLDA3
TRANK1
CENPP
GY51
KIAA0754
TMEM2
ZC3H8
TRIM22
DGKH
ACER2
GADD458
C11orf21
REC8
ASTN2
AMOTL1
CCNG1
ZNF850
PRKY
ARCKSL1
FAM212B
MDM2
SPATA13
IK3R3
CUL9
SYTL1
ASCC3
CHD6
CCR7
APAF1
HMBOX1
HSPA2
ANKRC1
TSA9P
DCUN1D4
NIN
CD4
MYO1A
NDRG1
MAN2B2
VWCE
KITLG
FBX022
AC5BG1
PRKX
SYNE1
VPS13D
RGS16
USP
NSF
MED12L
MYO18A
REV3L
ABCA1
CAPRIN2
NFAT5
PDE4
EPPK1
LATS2
ABCA5
TNS3
LRMP
MON2
CD70
MTRNR2L2
HIST1H2BD
HIST1H1D
HIST1H2AJ
HIST1H2BL
HIST1H4F
HIST1H1B
HIST1H2BK
HIST1H2BM
HIST1H3D
HIST1H2BC
HIST1H3B
HIST1H3J
HIST1H2BH
HIST1H4C
HIST1H4E
HIST1H2BJ
HIST1H4A
HIST2H2AB
HIST1H2BF
HIST1H2AE
HIST1H2BB
HIST1H1C
HIST1H4I
HIST1H4D
HIST1H3H
FAM132B
HIST1H2BO
HIST1H4H
HIST2H2BF
HIST1H2AL
IFIT1B
HIST3H2BB
HIST1H4L
HIST1H2AI
HIST1H2BN
HIST1H3I
HIST1H3F
HIST1H2AB
HIST1H2BG
HIST1H2AH
HIST1H3C
HIST1H2AK
DUSP5
HIST2H2BE
HIST1H2AG
MIR3687-1
HIST1H2AM
CISH
SIAH2
PSAT1
HIST1H2AC
HIST1H4B
CCDC71L
LRRC8A
TRIB3
OSM
HIST1H3G
CRHBP
ASNS
TMCC2
SOCS2
HIST2H2AC
CREB3L1
HES6
HIST2H3D
FN1
SH3BGRL3
MIR3648-1
EHF
HBB
PIM1

FIG. 48

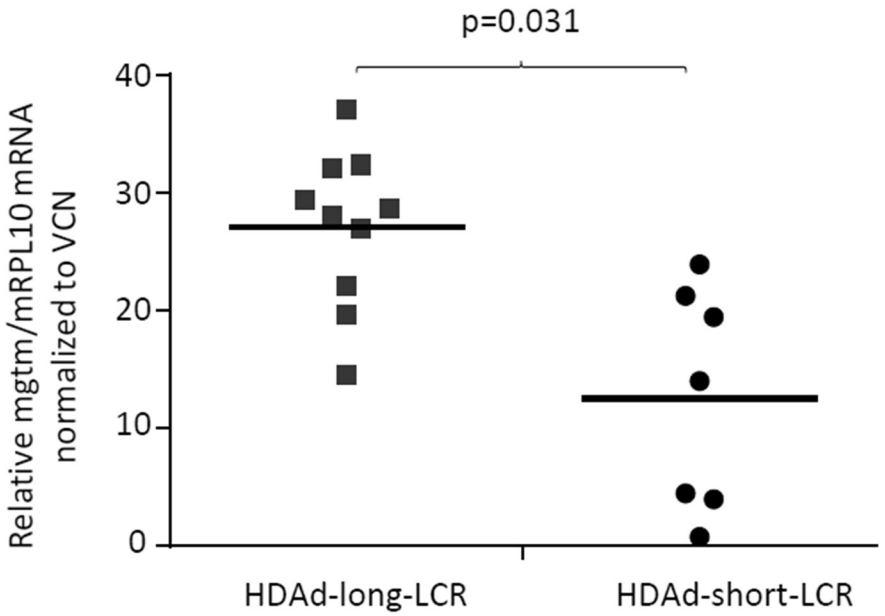

p=0.031
40
30
20
10
0
Relative mgtm/mRPL10 mRNA normalized to VCN
HDAd-long-LCR
HDAd-short-LCR

FIG. 49

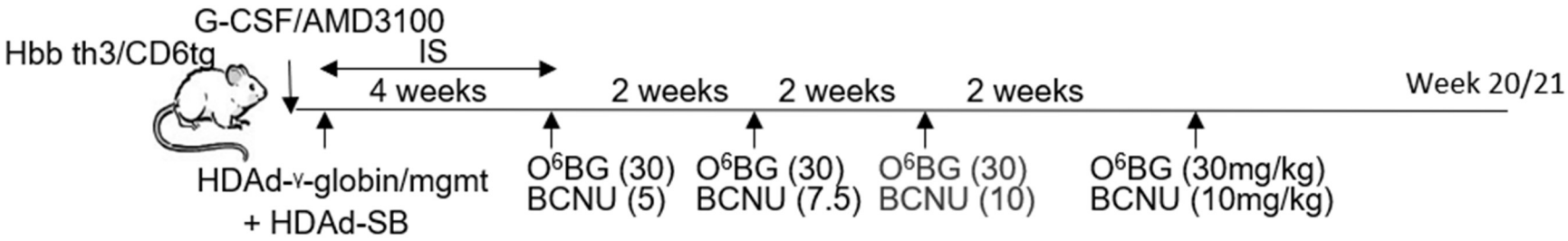

G-CSF/AMD3100
IS
Hbb th3/CD6tg
4 weeks
2 weeks
2 weeks
2 weeks
Week 20/21
HDAd-γ-globin/mgmt
+ HDAd-SB
O6BG (30)
BCNU (5)
O6BG (30)
BCNU (7.5)
O6BG (30)
BCNU (10)
O6BG (30mg/kg)
BCNU (10mg/kg)

FIG. 50

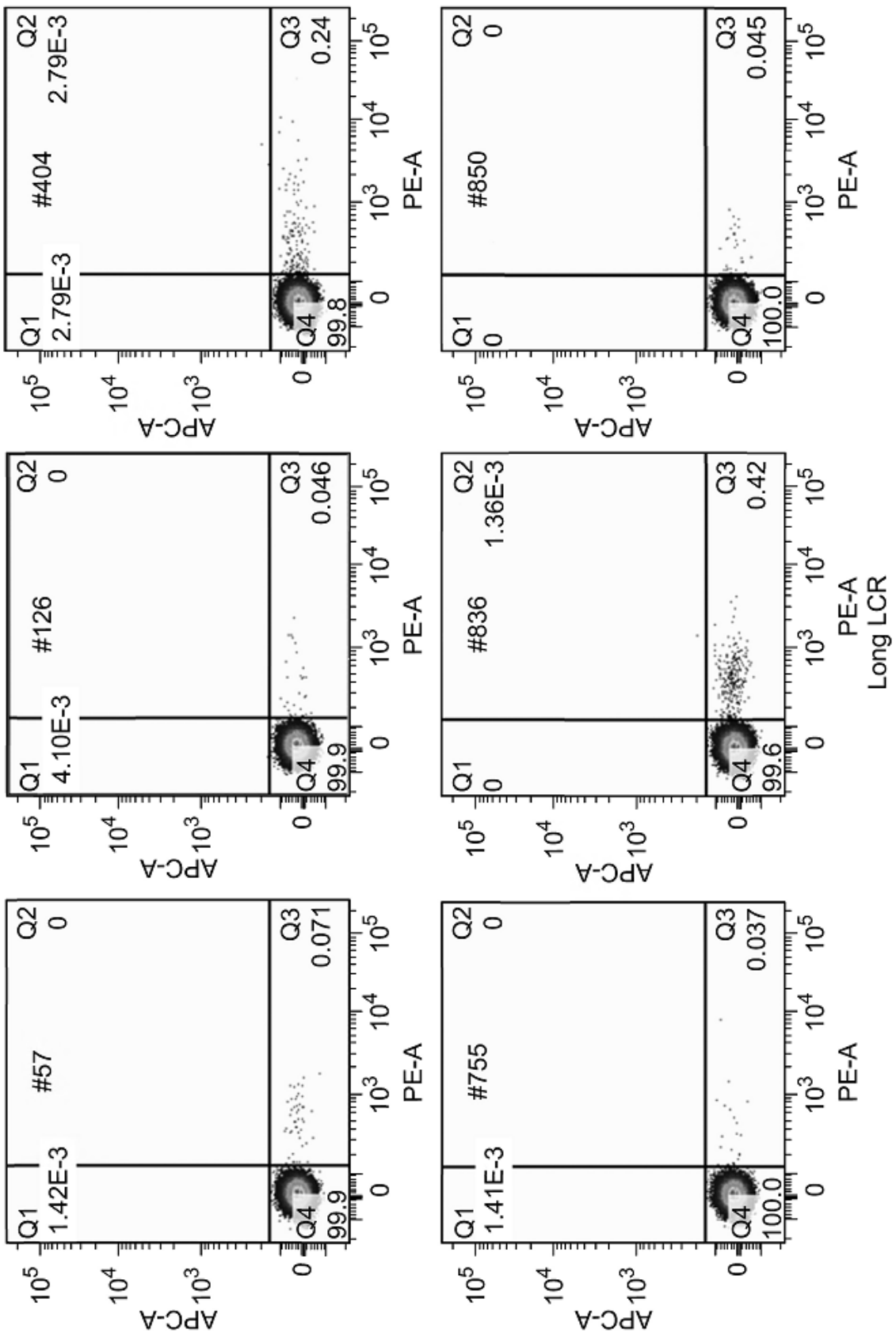
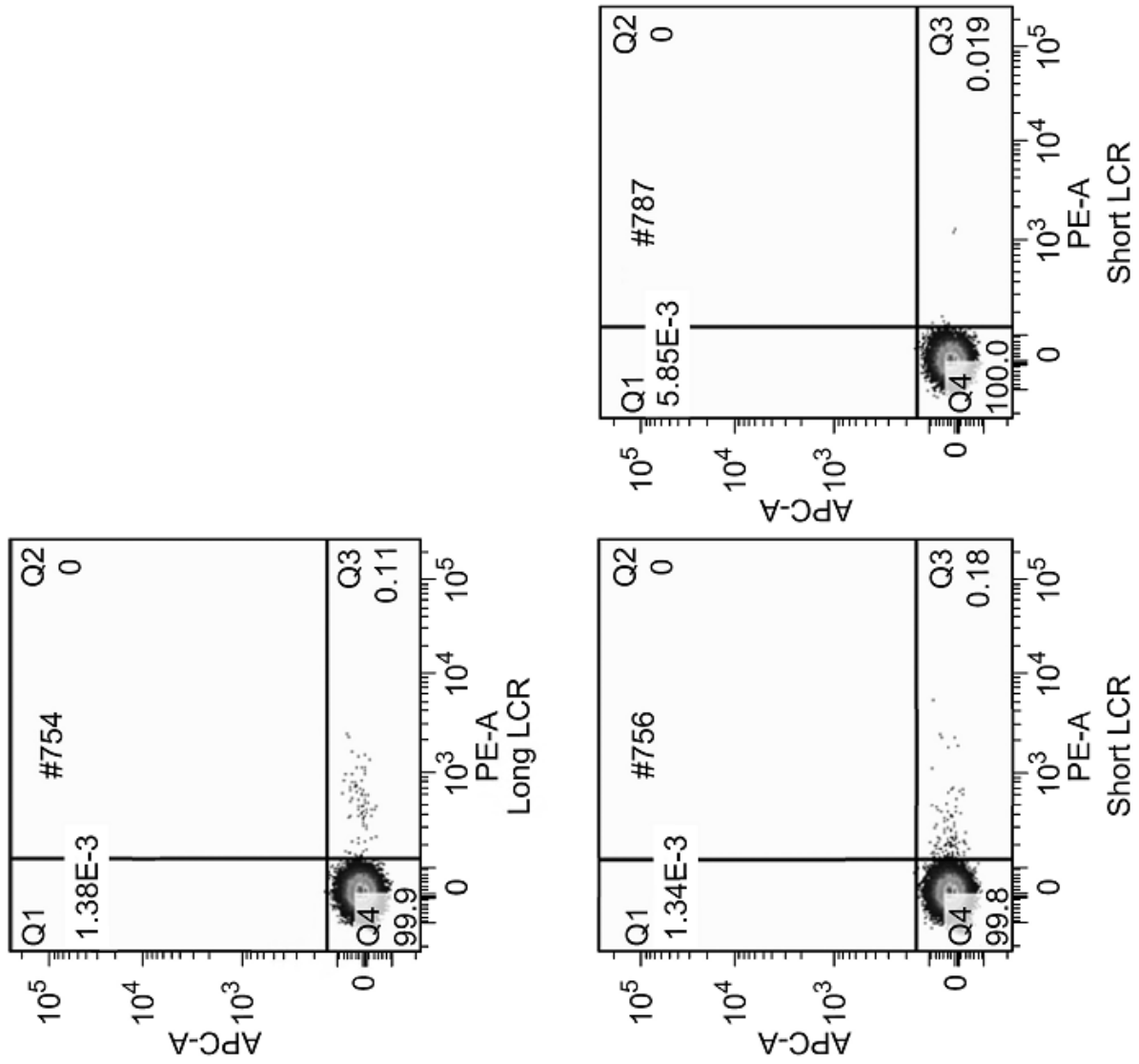

Q1
1.42E-3
#57
Q2
0
Q4
99.9
Q3
0.071
Q1
4.10E-3
#126
Q2
0
Q4
99.9
Q3
0.046
Q1
2.79E-3
#404
Q2
2.79E-3
Q4
99.8
Q3
0.24
Q1
1.41E-3
#755
Q2
0
Q4
100.0
Q3
0.037
Q1
0
#836
Q2
1.36E-3
Q4
99.6
Q3
0.42
Q1
0
#850
Q2
0
Q4
100.0
Q3
0.045
APC-A
PE-A
Long LCR

FIG. 50 cont'd.


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

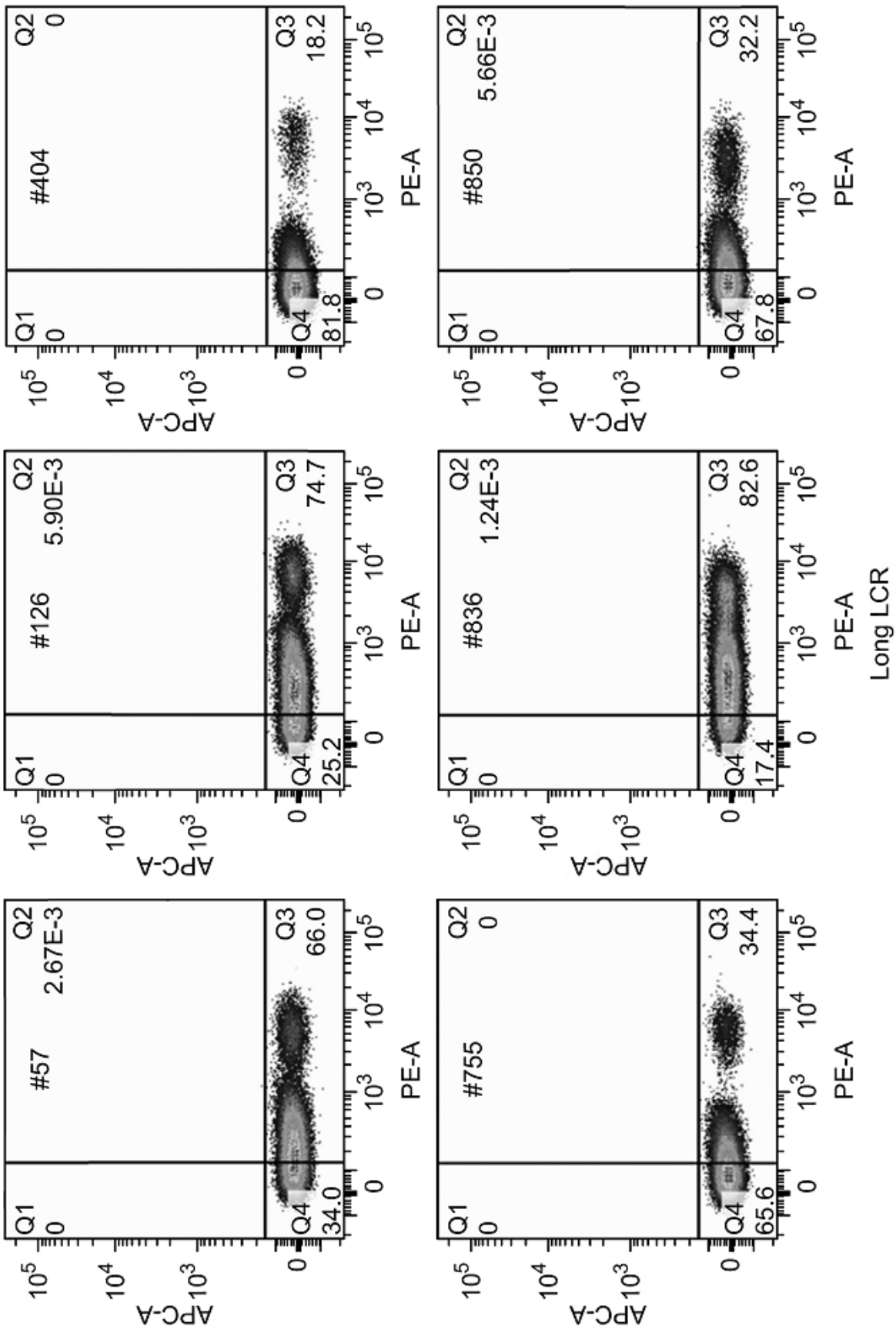

#57
Q1 0
Q2 2.67E-3
Q4 34.0
Q3 66.0
#126
Q1 0
Q2 5.90E-3
Q4 25.2
Q3 74.7
#404
Q1 0
Q2 0
Q4 81.8
Q3 18.2
#755
Q1 0
Q2 0
Q4 65.6
Q3 34.4
#836
Q1 0
Q2 1.24E-3
Q4 17.4
Q3 82.6
#850
Q1 0
Q2 5.66E-3
Q4 67.8
Q3 32.2
APC-A
PE-A
Long LCR

FIG. 51

FIG. 51 cont'd.

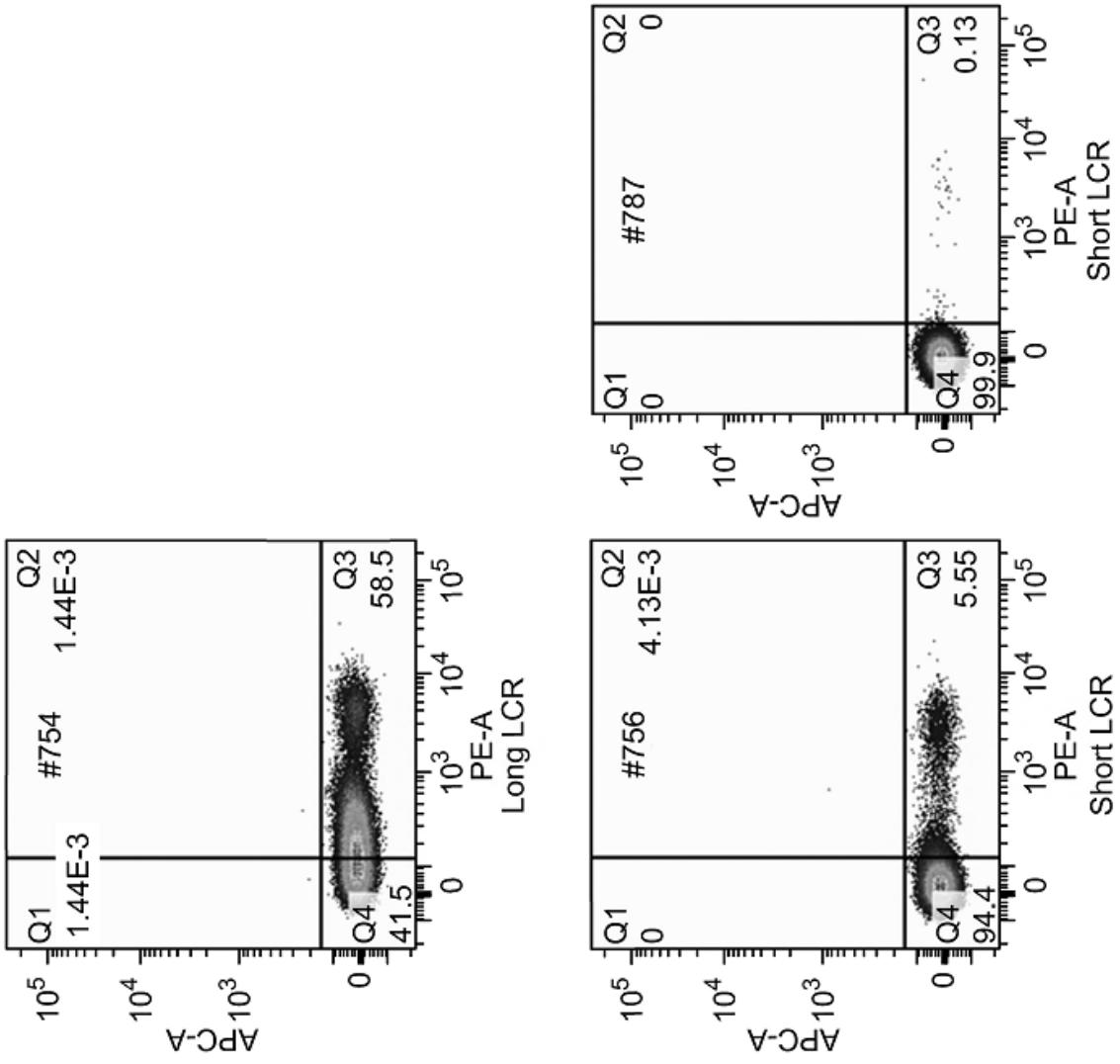

Q1
1.44E-3
#754
Q2
1.44E-3
Q4
41.5
Q3
58.5
APC-A
PE-A
Long LCR
Q1
0
#787
Q2
0
Q4
99.9
Q3
0.13
Short LCR
Q1
0
#756
Q2
4.13E-3
Q4
94.4
Q3
5.55
Short LCR

FIG. 52

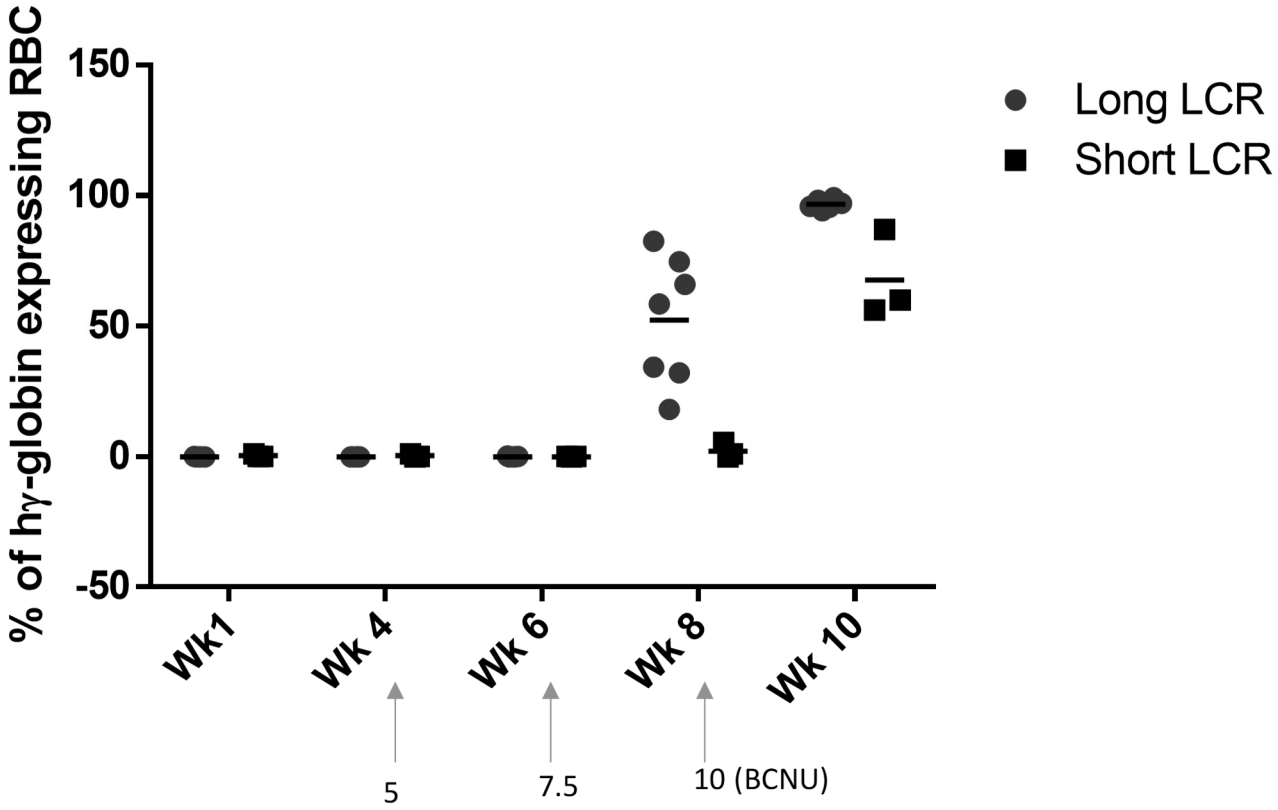

% of hγ-globin expressing RBC
150
100
50
0
-50
Wk1
Wk 4
Wk 6
Wk 8
Wk 10
5
7.5
10 (BCNU)
Long LCR
Short LCR

FIG. 53

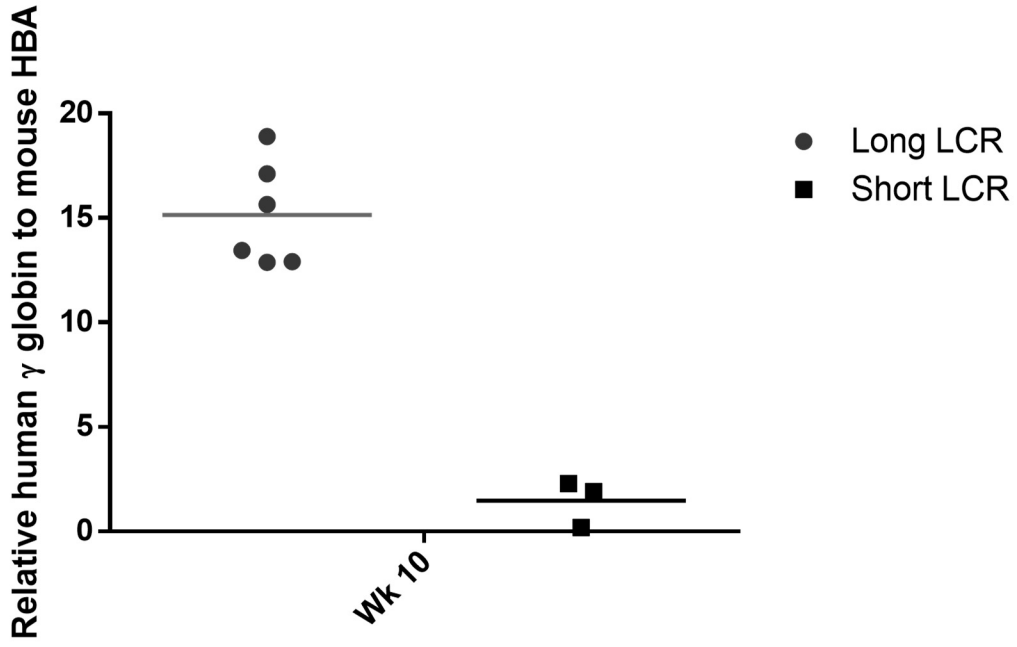

Relative human γ globin to mouse HBA
20
15
10
5
0
Wk 10
Long LCR
Short LCR

FIG. 54

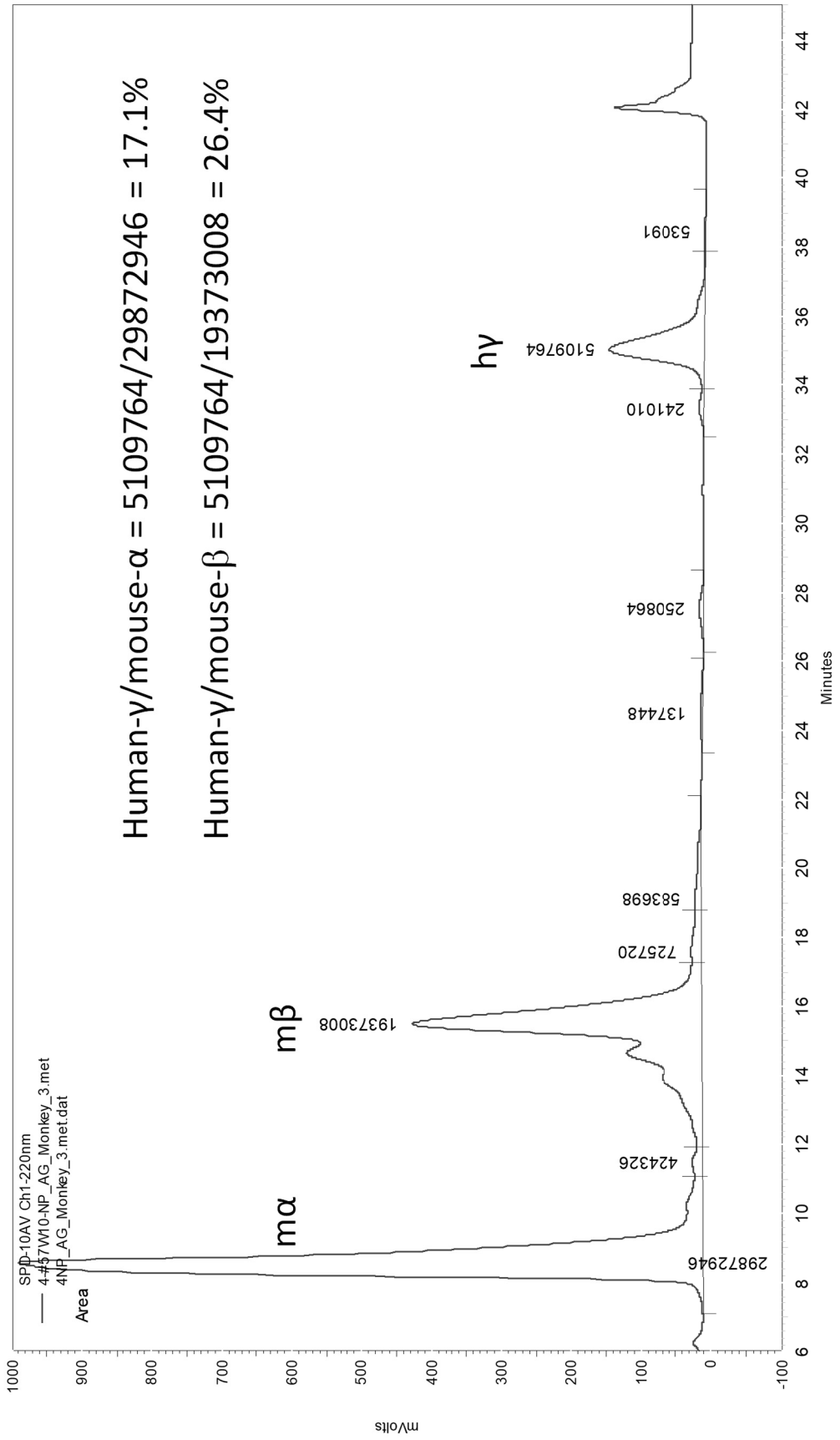

Human-γ/mouse-α = 5109764/29872946 = 17.1%
Human-γ/mouse-β = 5109764/19373008 = 26.4%
mα
mβ
hγ
SPD-10AV Ch1-220nm
4457W10-NP_AG_Monkey_3.met
4NP_AG_Monkey_3.met.dat
Area
29872946
424326
19373008
725720
583698
137448
250864
5109764
241010
53091
mVolts
Minutes

FIG. 55A

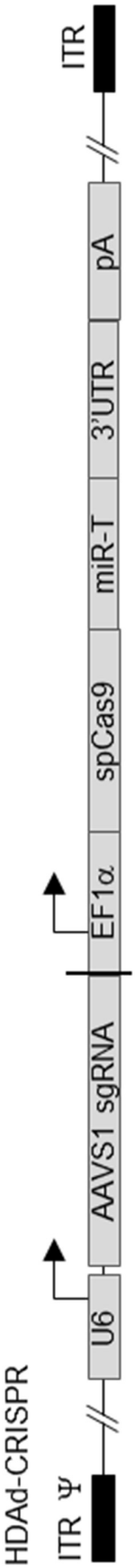


FIG. 55B

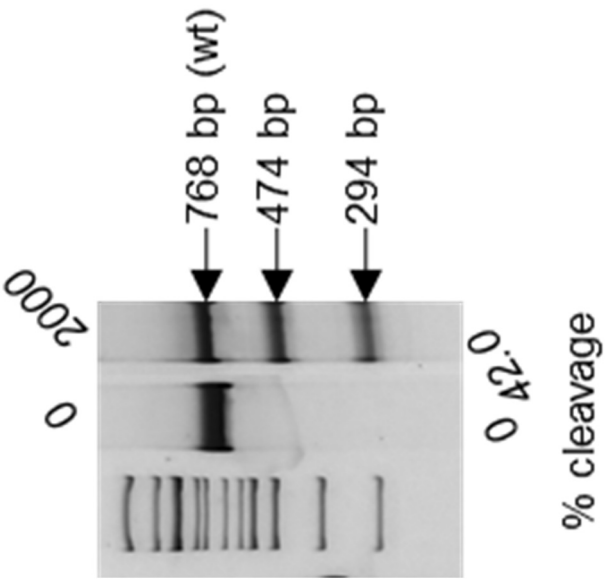


FIG. 55C



(SEQ ID NOs: 6-18)

FIG. 55D

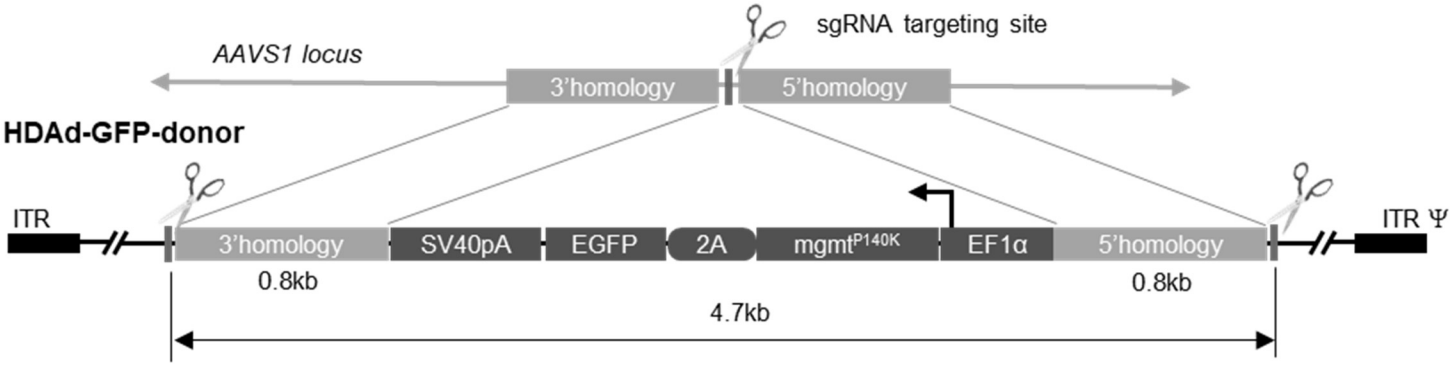


FIG. 55E

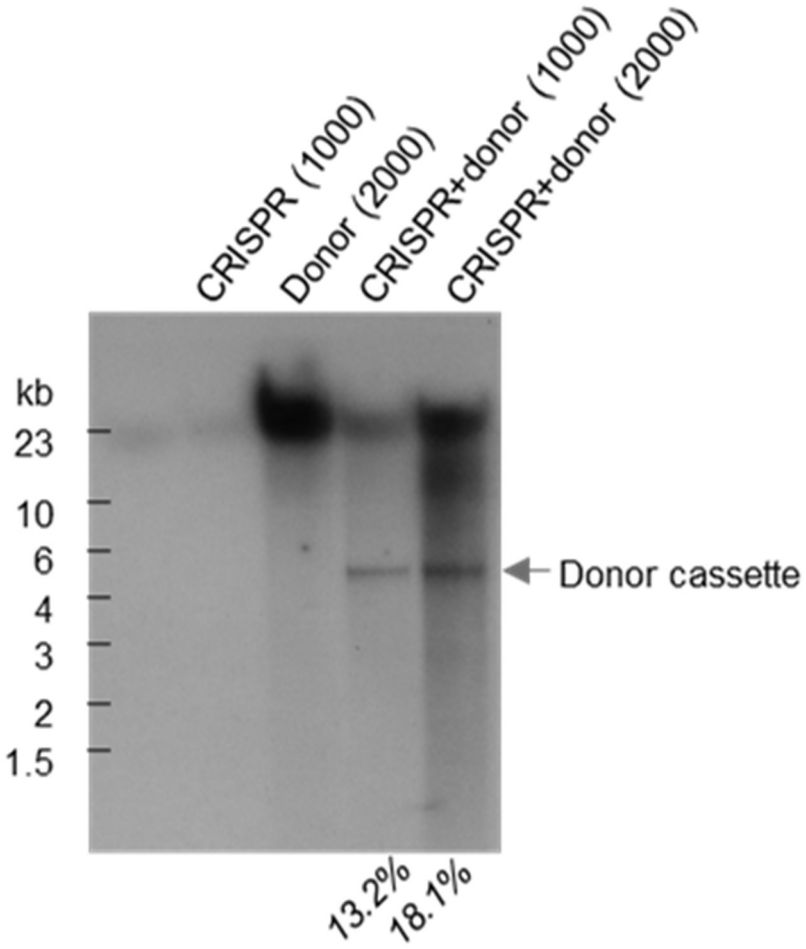


FIG. 56A

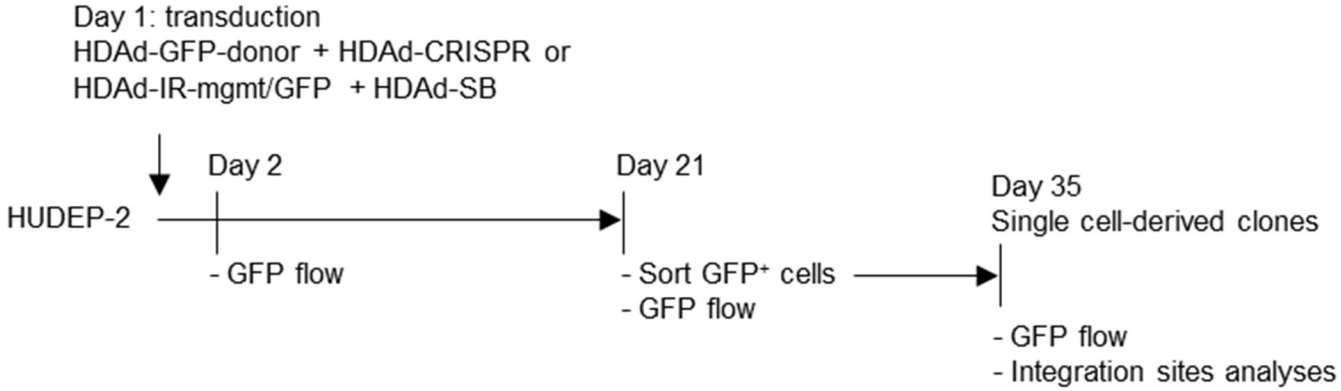


FIG. 56B

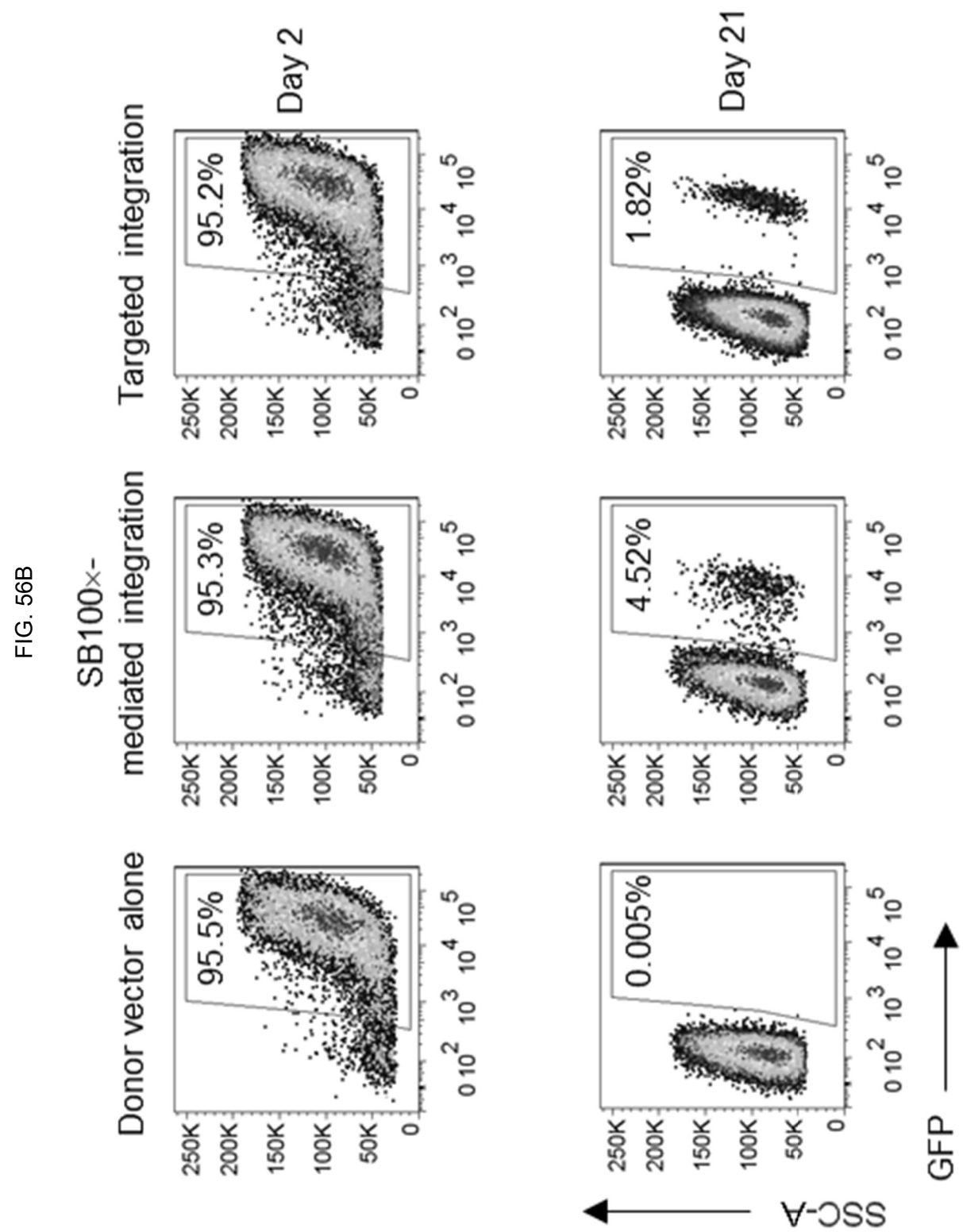

Donor vector alone
SB100×-mediated integration
Targeted integration
Day 2
Day 21
95.5%
95.3%
95.2%
0.005%
4.52%
1.82%
250K
200K
150K
100K
50K
0
0 10^2 10^3 10^4 10^5
SSC-A
GFP

FIG. 56C

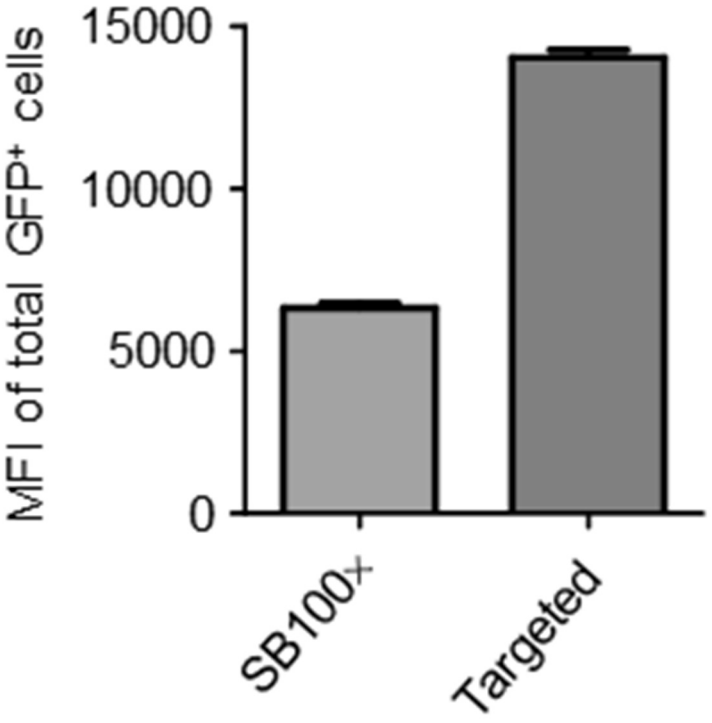


FIG. 56D

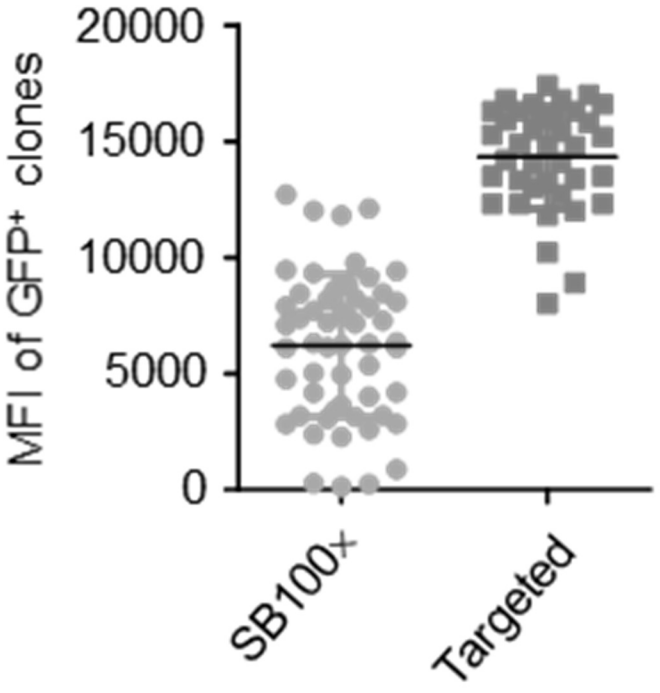


FIG. 56E

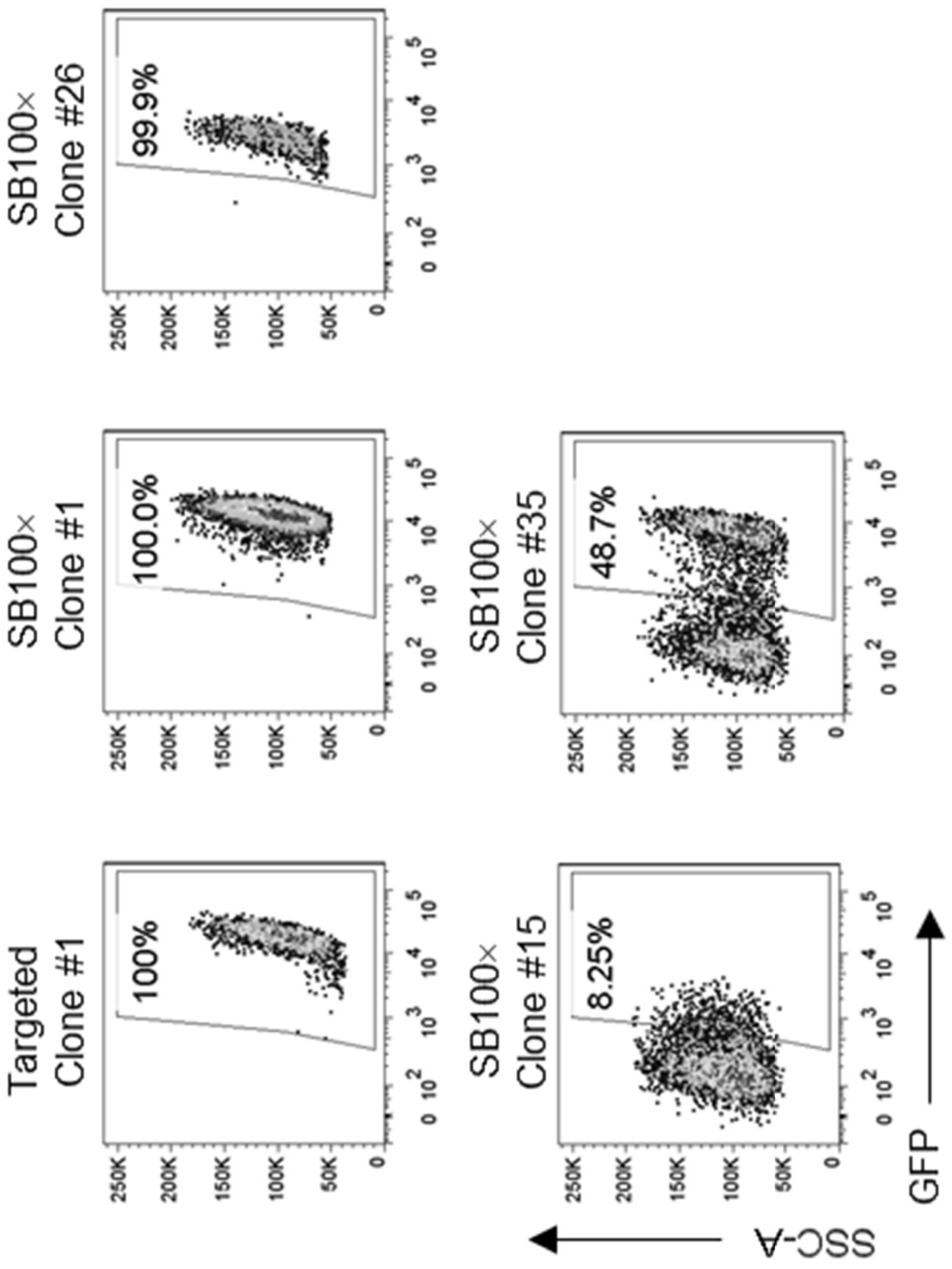

Targeted
Clone #1
100%
SB100×
Clone #1
100.0%
SB100×
Clone #26
99.9%
SB100×
Clone #15
8.25%
SB100×
Clone #35
48.7%
SSC-A
GFP
250K
200K
150K
100K
50K
0
0 10^2 10^3 10^4 10^5

FIG. 56F

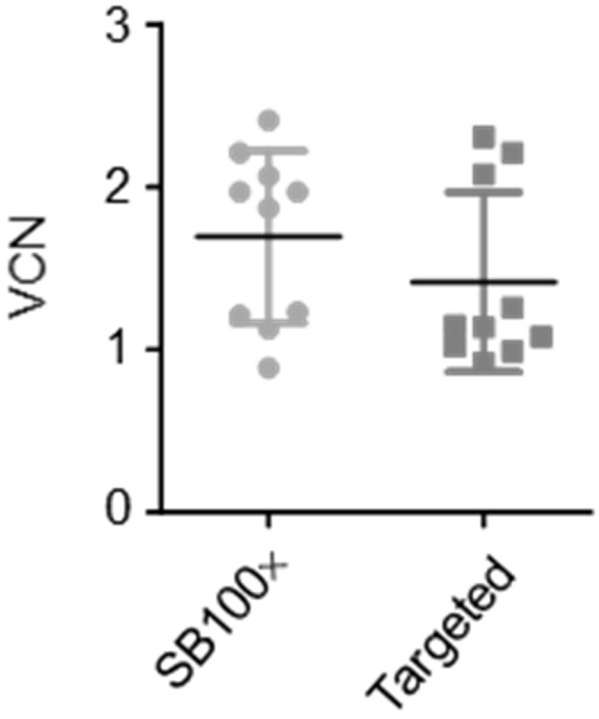


FIG. 57A

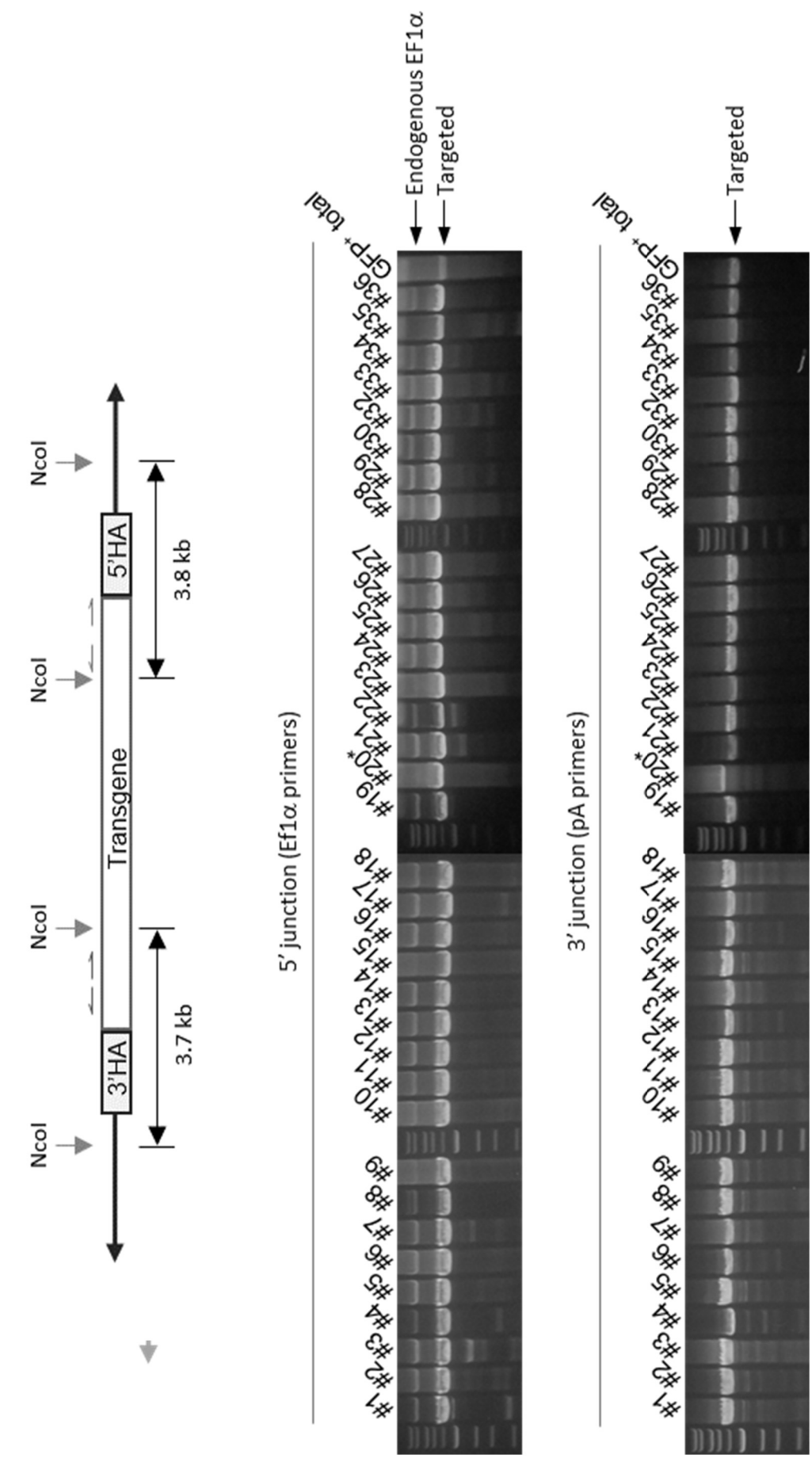

NcoI
NcoI
NcoI
NcoI
3'HA
Transgene
5'HA
3.7 kb
3.8 kb
5' junction (Ef1α primers)
3' junction (pA primers)
Endogenous EF1α
Targeted
Targeted
total
GFP+
#1
#2
#3
#4
#5
#6
#7
#8
#9
#10
#11
#12
#13
#14
#15
#16
#17
#18
#19*
#20*
#21
#22
#23
#24
#25
#26
#27
#28
#29
#30
#31
#32
#33
#34
#35
#36

FIG. 57B

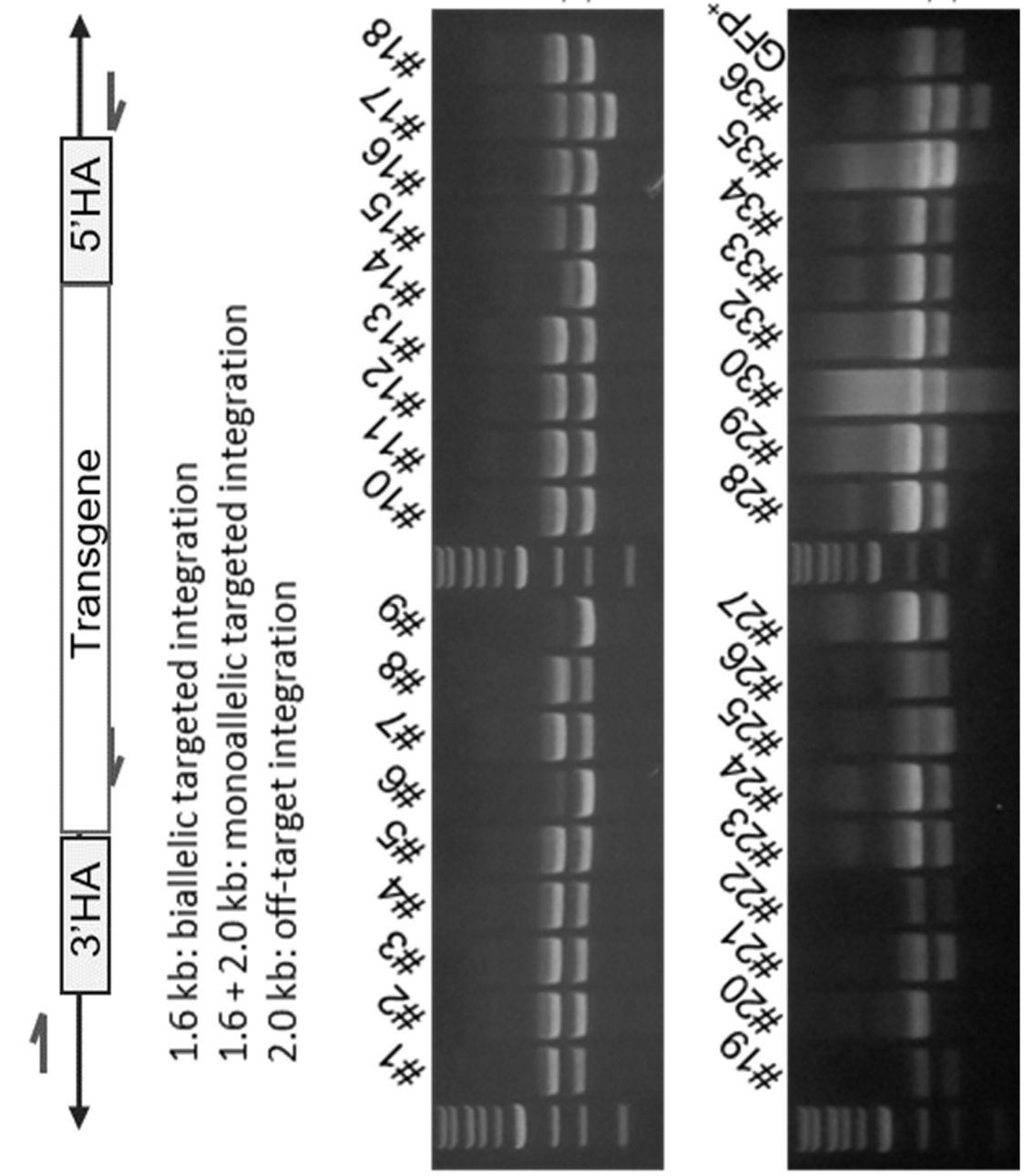

3'HA
Transgene
5'HA
1.6 kb: biallelic targeted integration
1.6 + 2.0 kb: monoallelic targeted integration
2.0 kb: off-target integration
#1 #2 #3 #4 #5 #6 #7 #8 #9 #10 #11 #12 #13 #14 #15 #16 #17 #18
#19 #20 #21 #22 #23 #24 #25 #26 #27 #28 #29 #30 #31 #32 #33 #34 #35 #36
GFP+ total
2.0 kb
1.6 kb
2.0 kb
1.6 kb

FIG. 58A

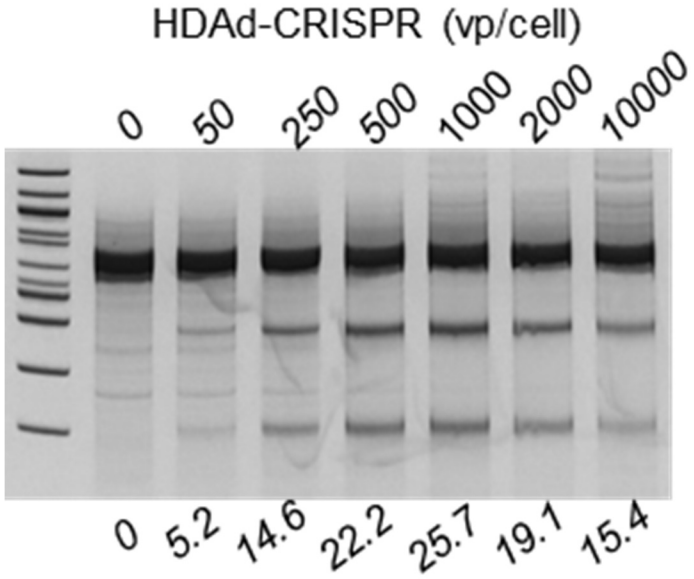


FIG. 58B

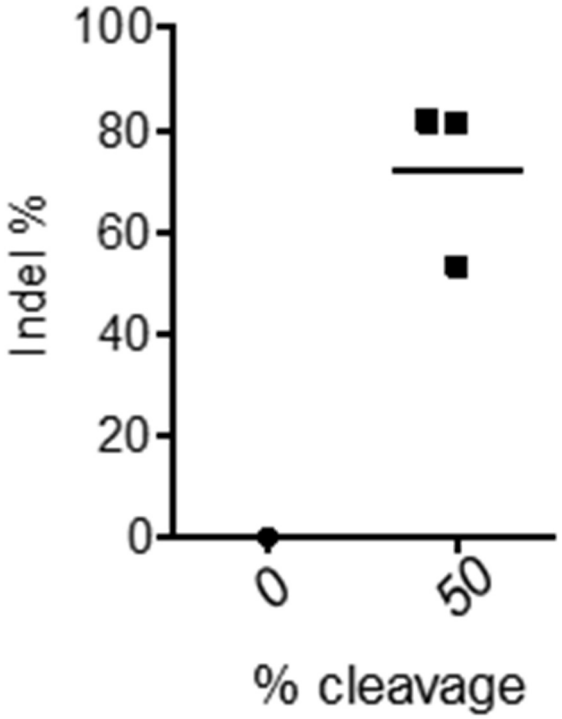
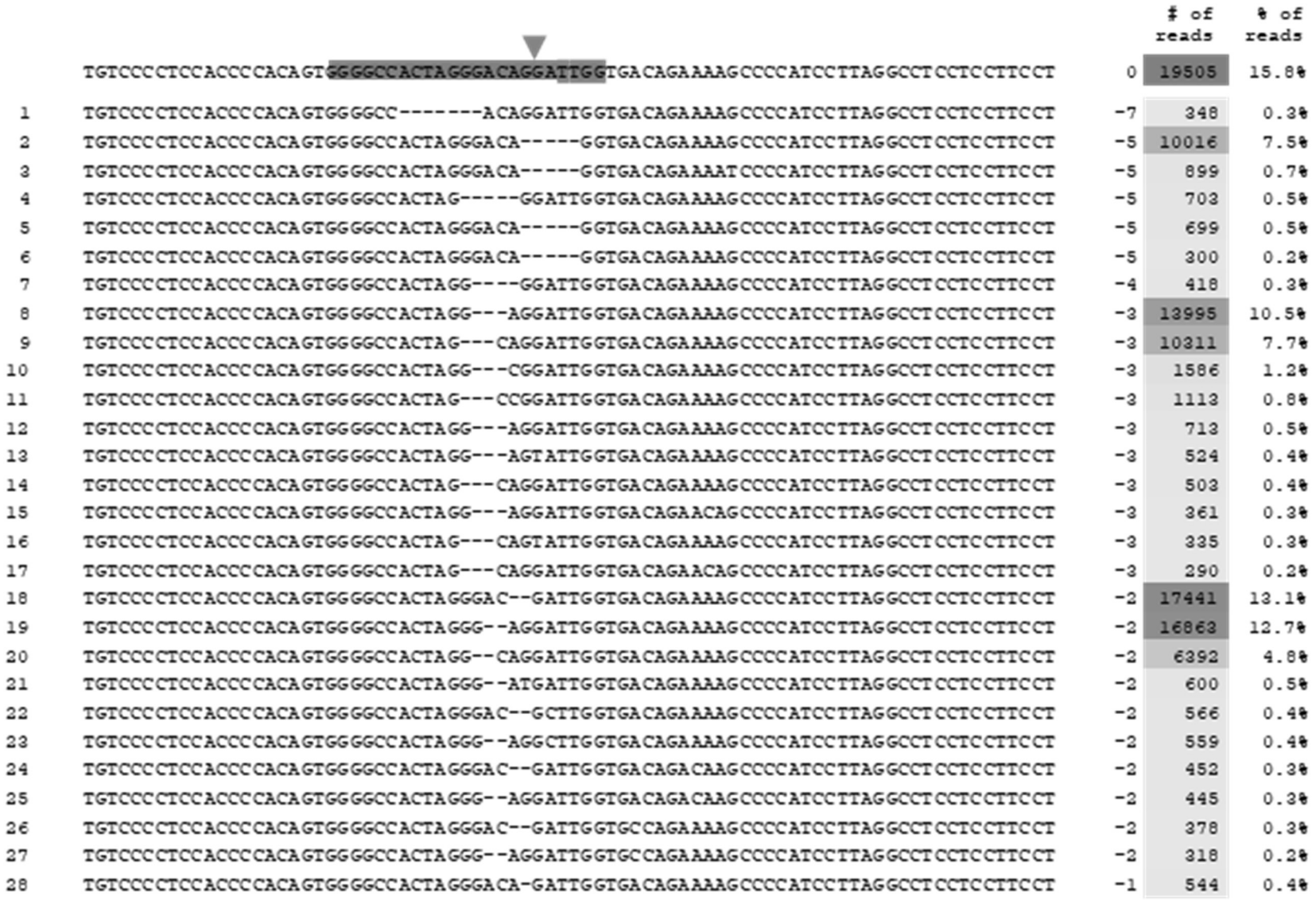


FIG. 58C



			# 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

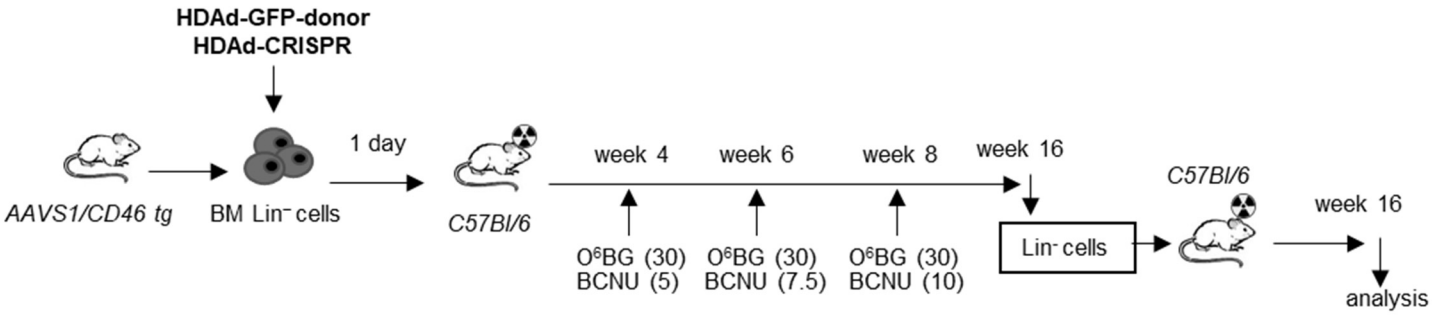


FIG. 59B

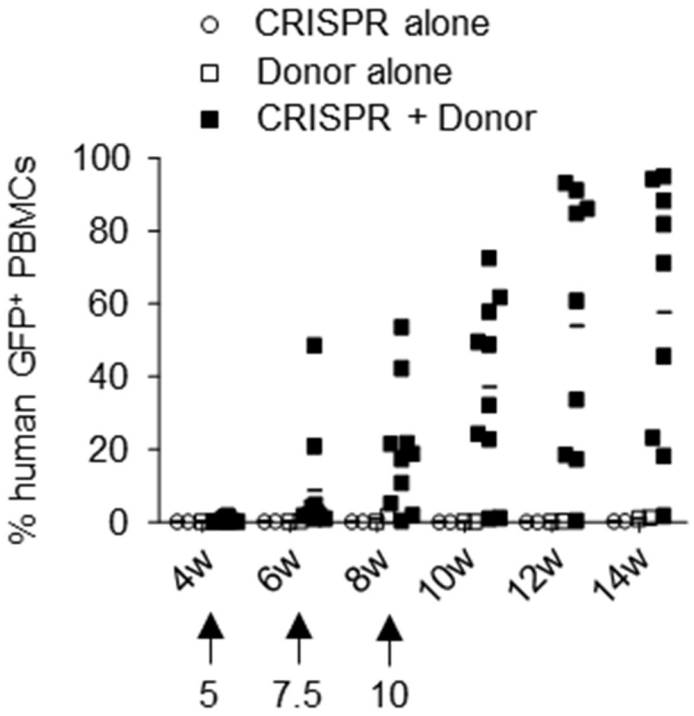


FIG. 59C

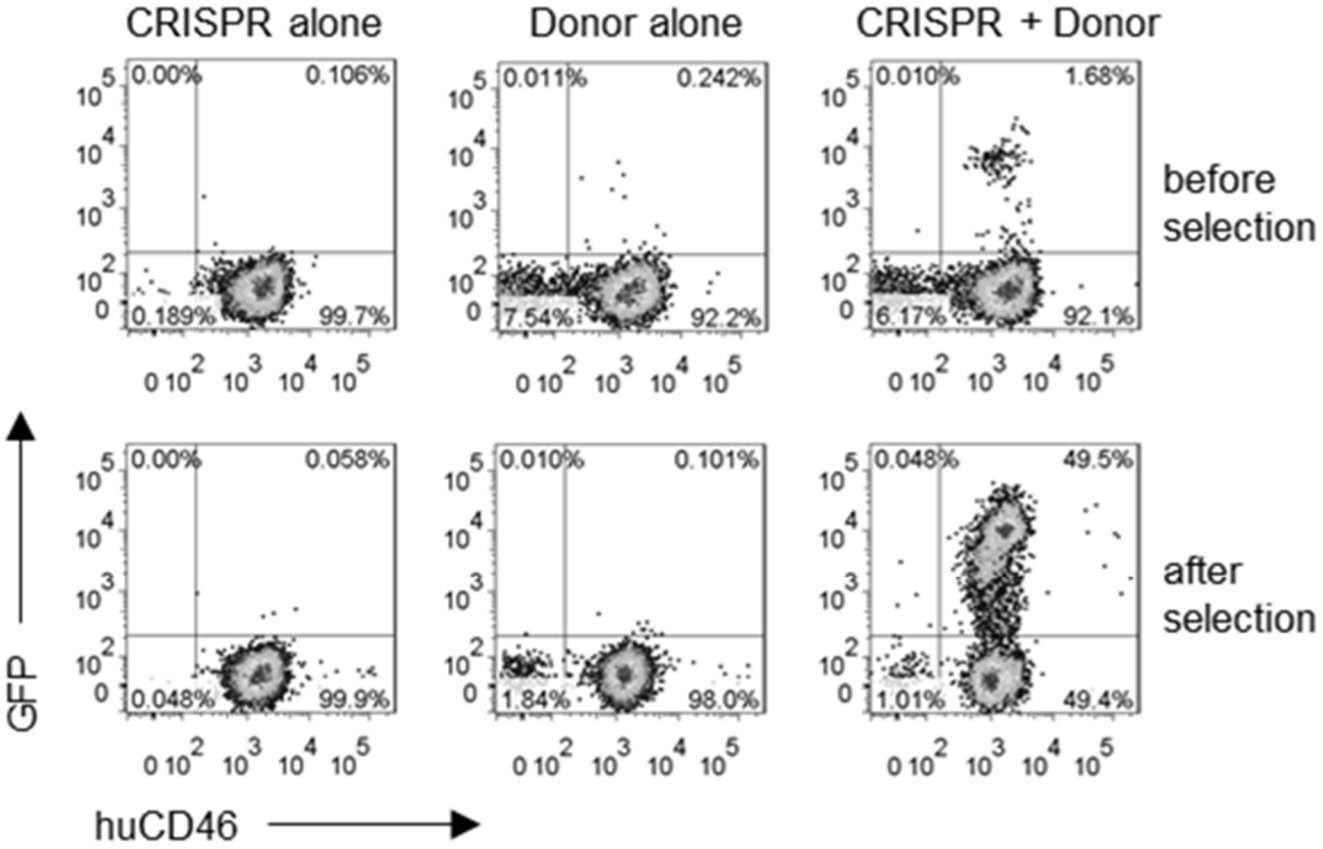


FIG. 59D

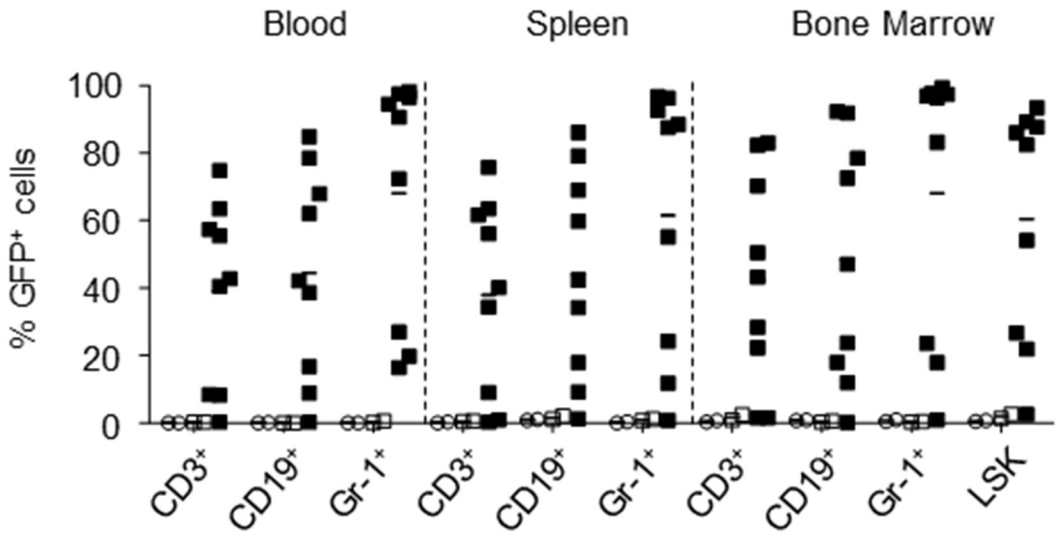


FIG. 60A

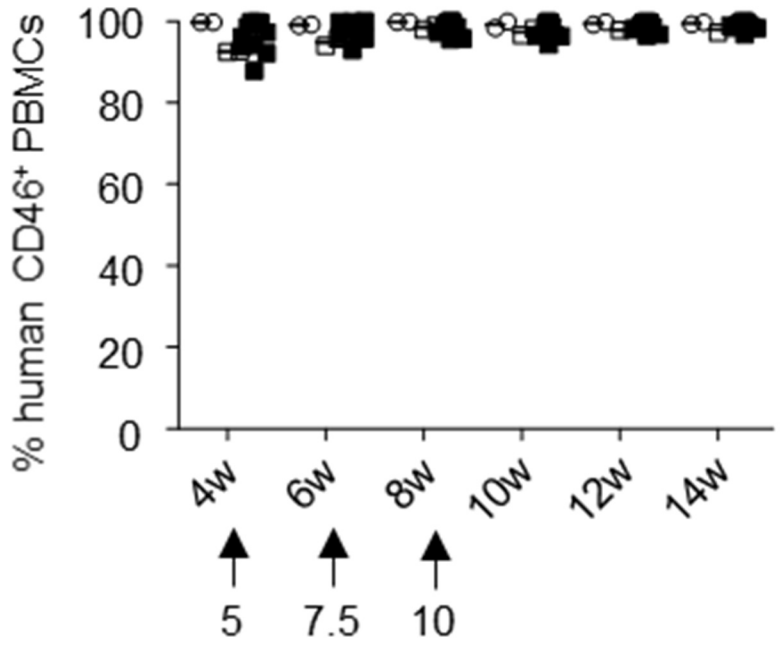


FIG. 60B

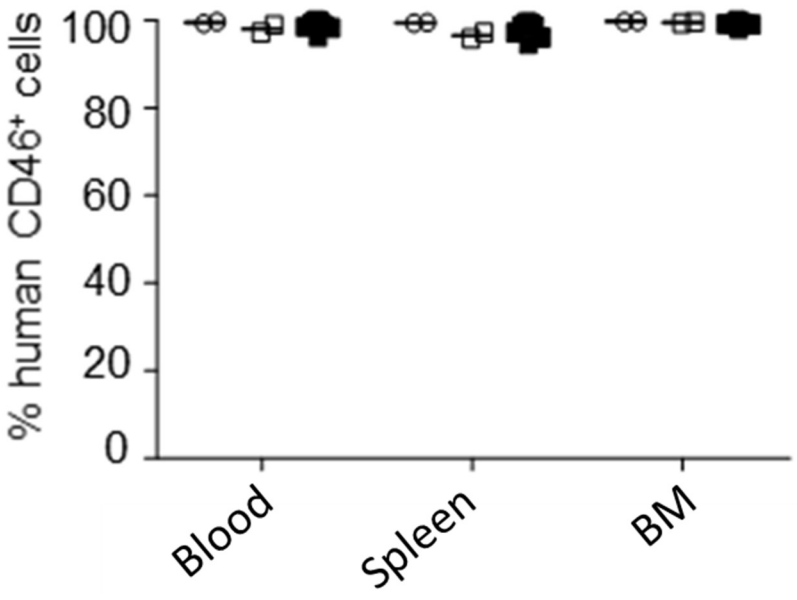


FIG. 60C

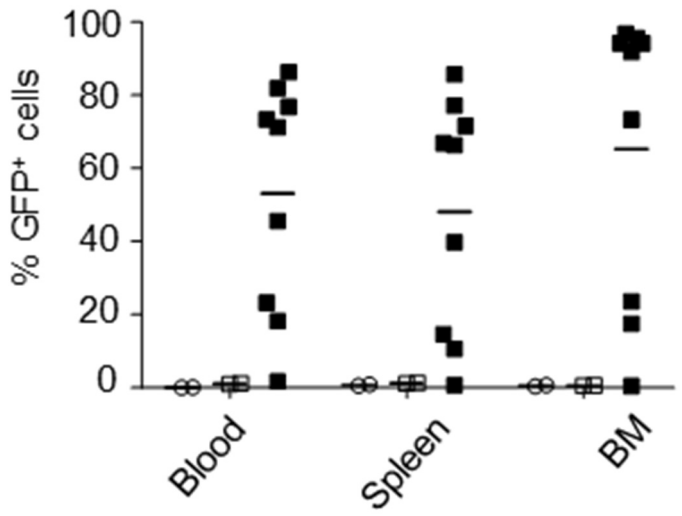


FIG. 60D

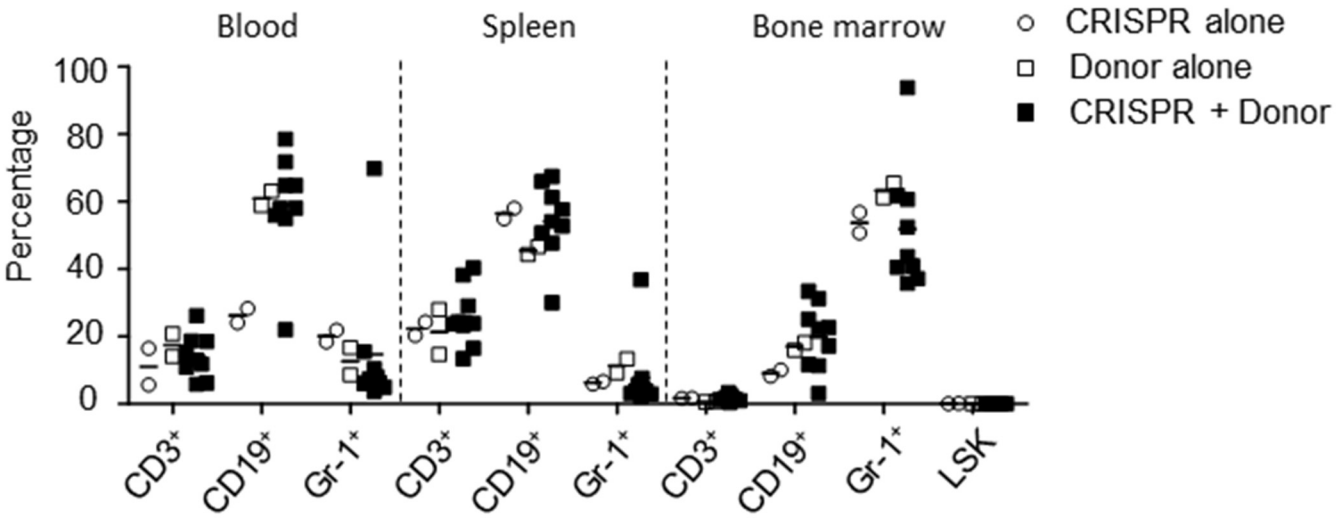


FIG. 60E

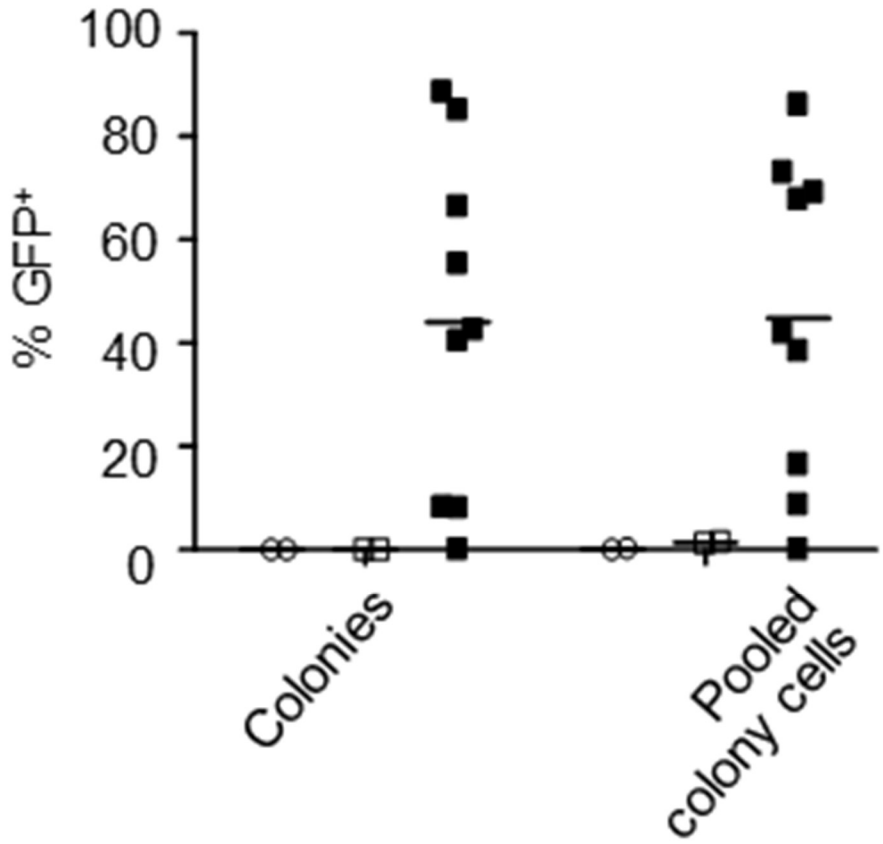


FIG. 61A

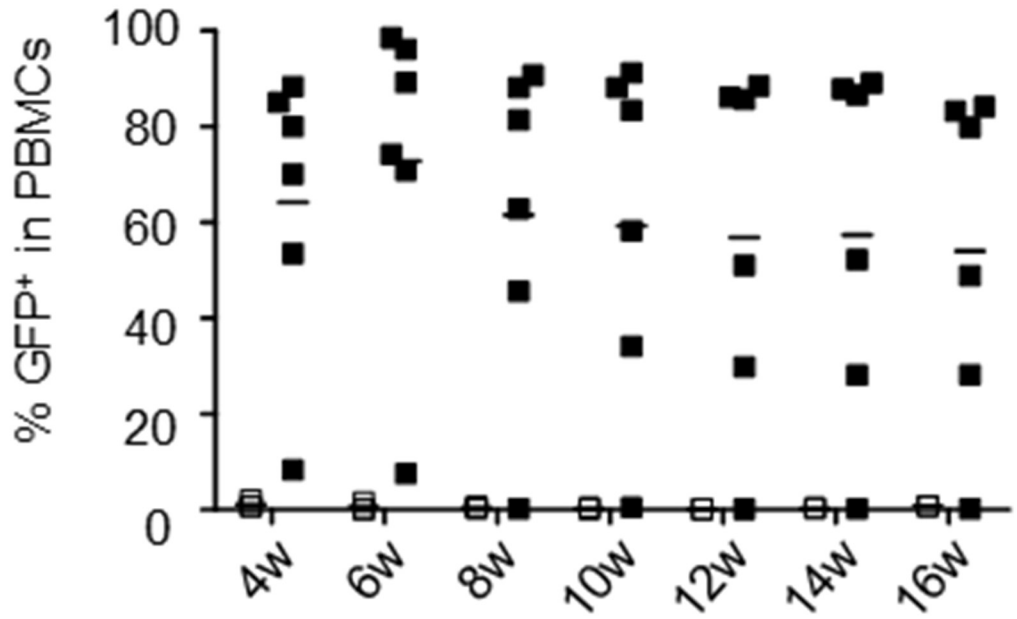


FIG. 61B

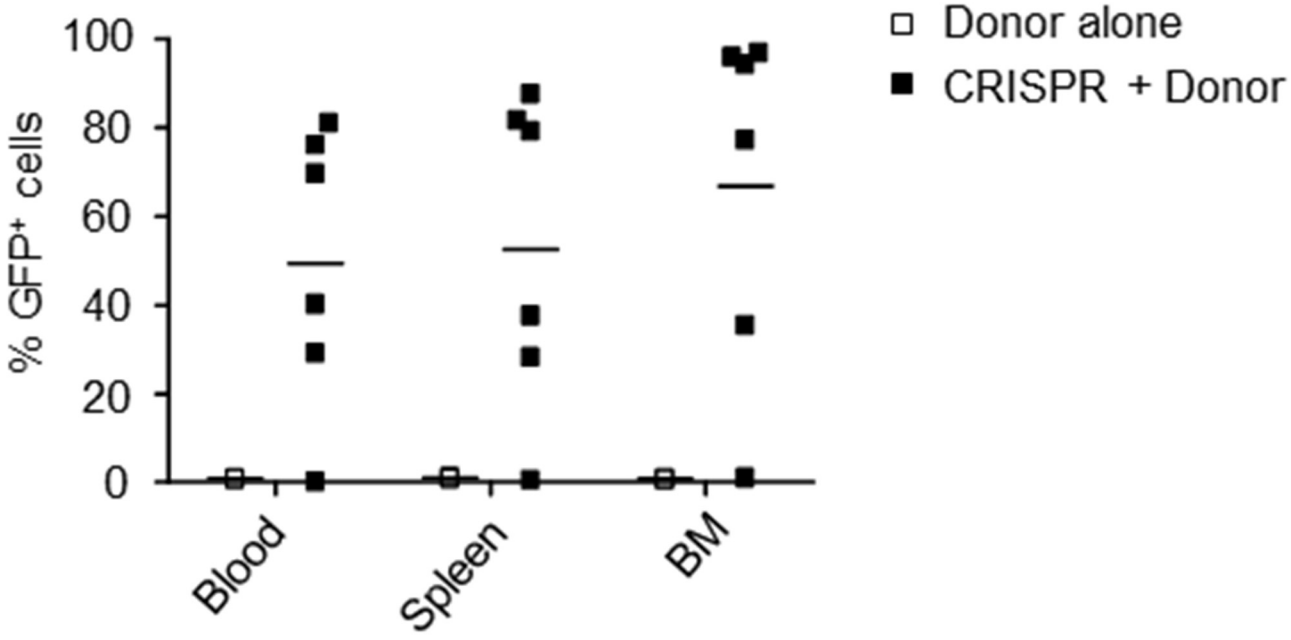


FIG. 61C

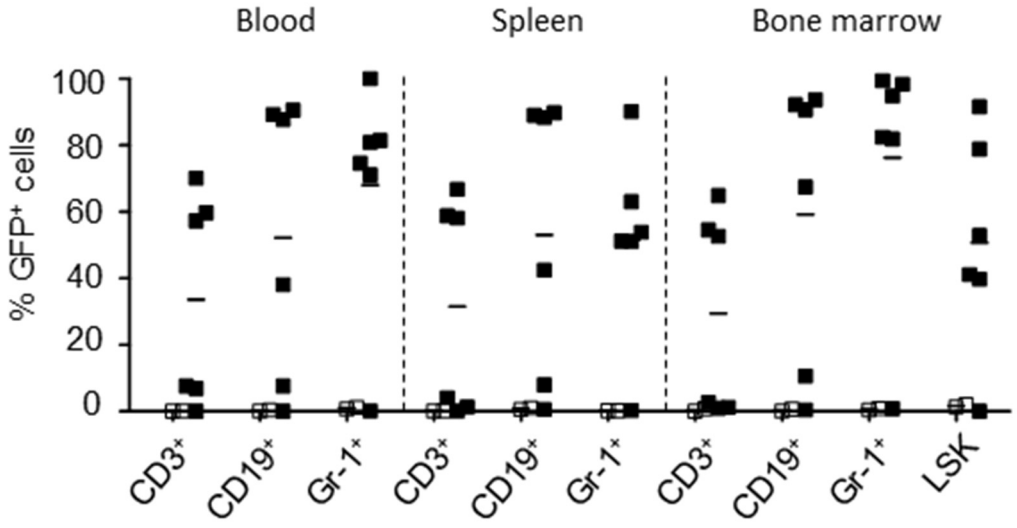


FIG. 61D

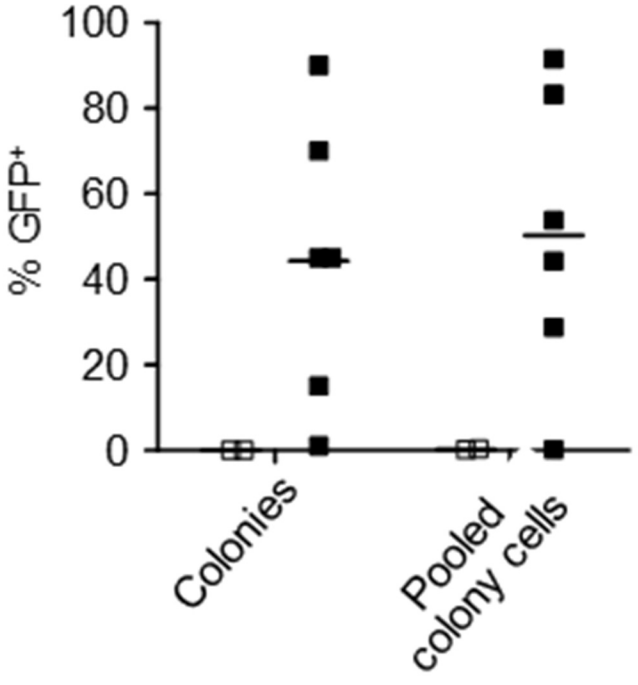


FIG. 61E

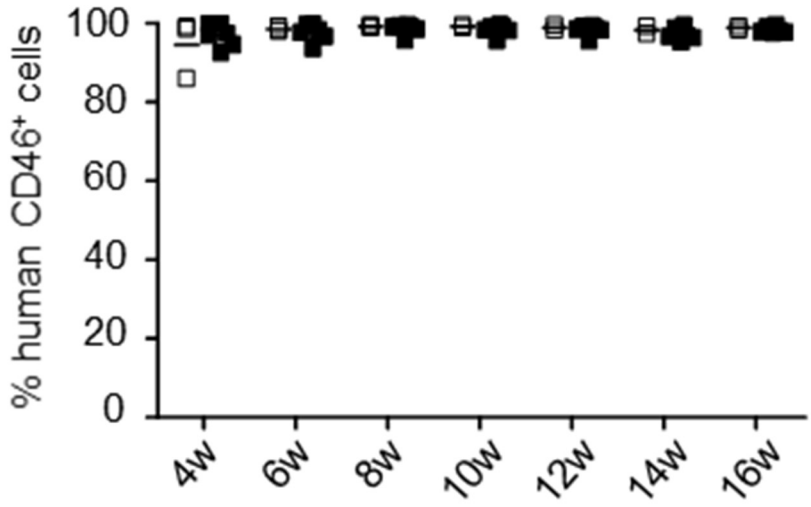


FIG. 61F

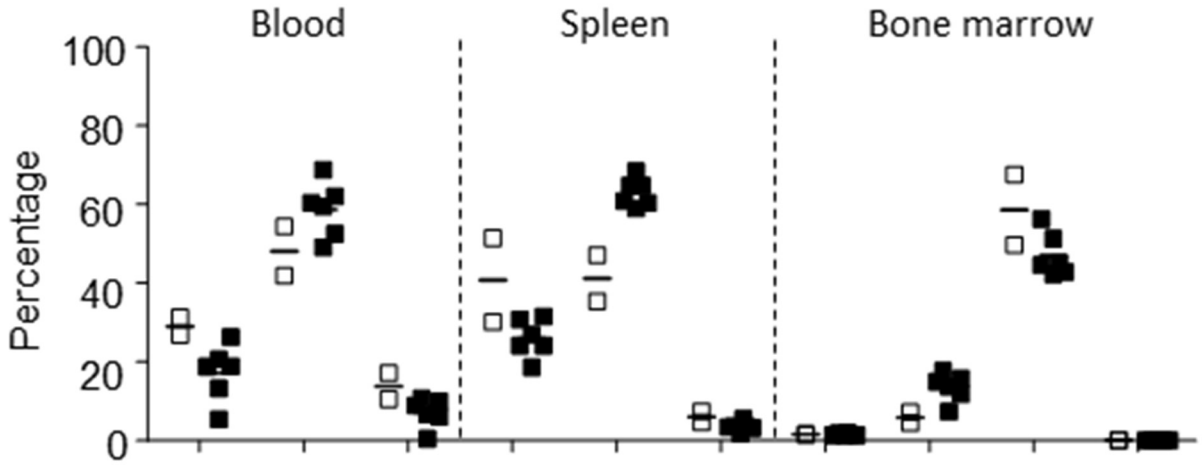


FIG. 62A

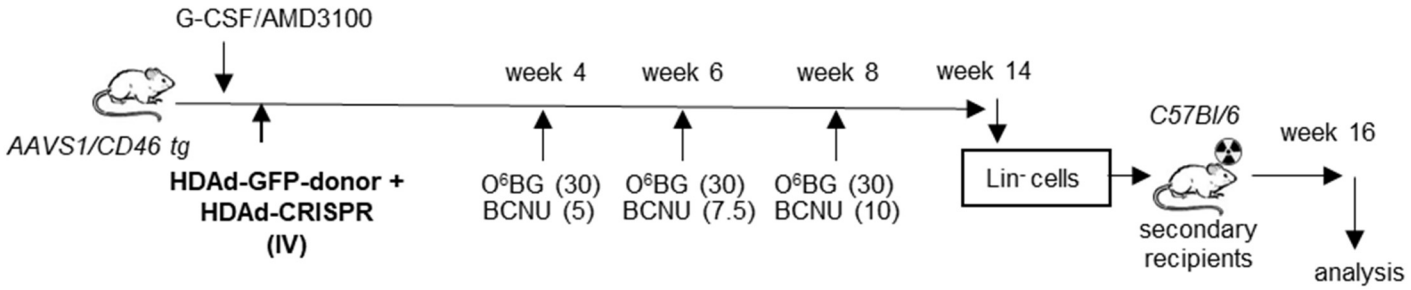


FIG. 62B

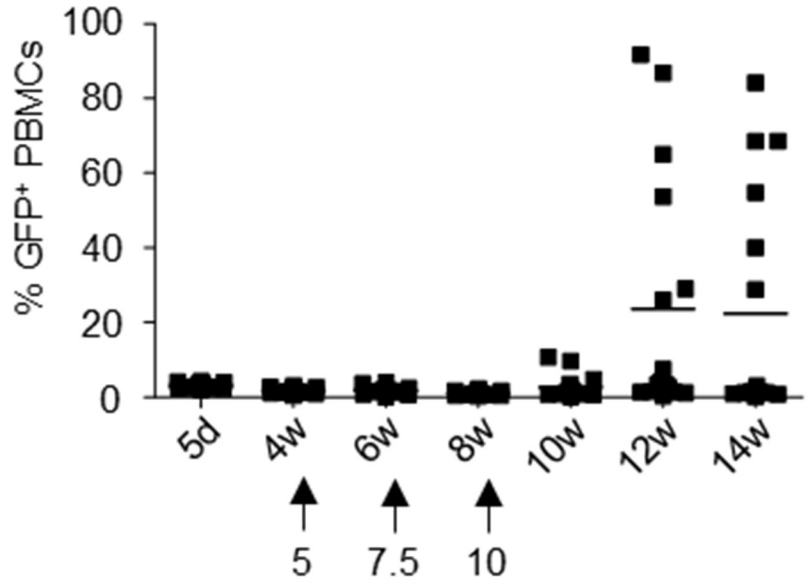


FIG. 62C

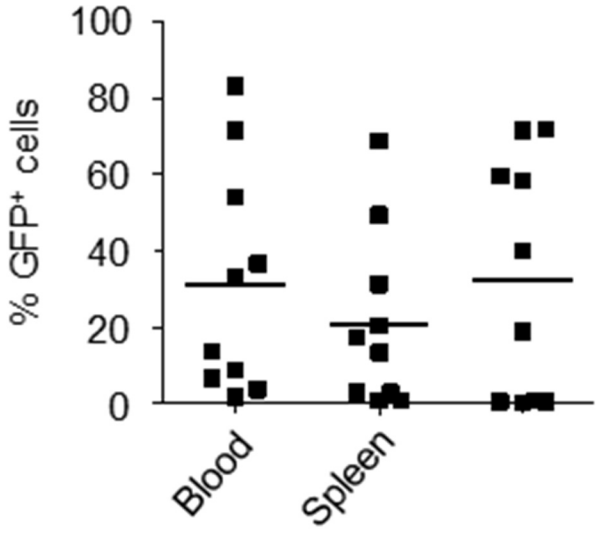


FIG. 62D

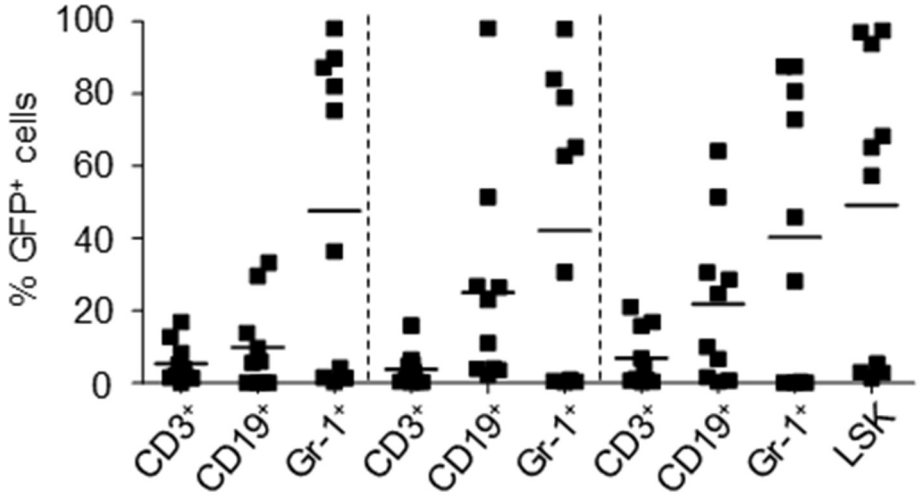


FIG. 62E

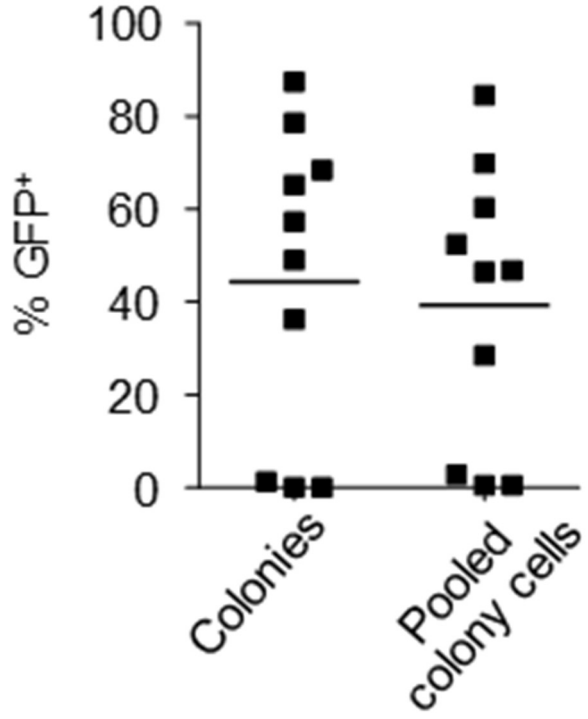


FIG. 62F

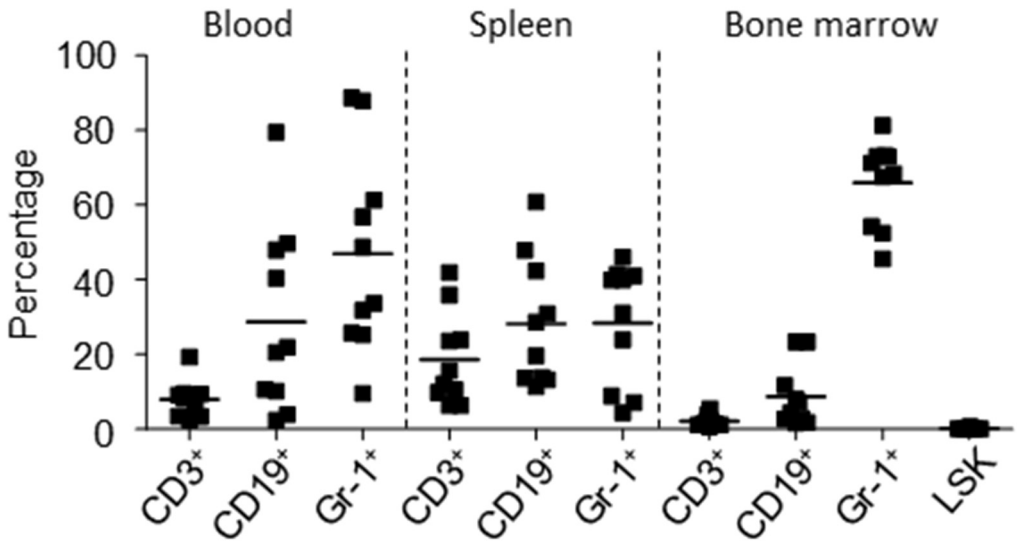


FIG. 63A

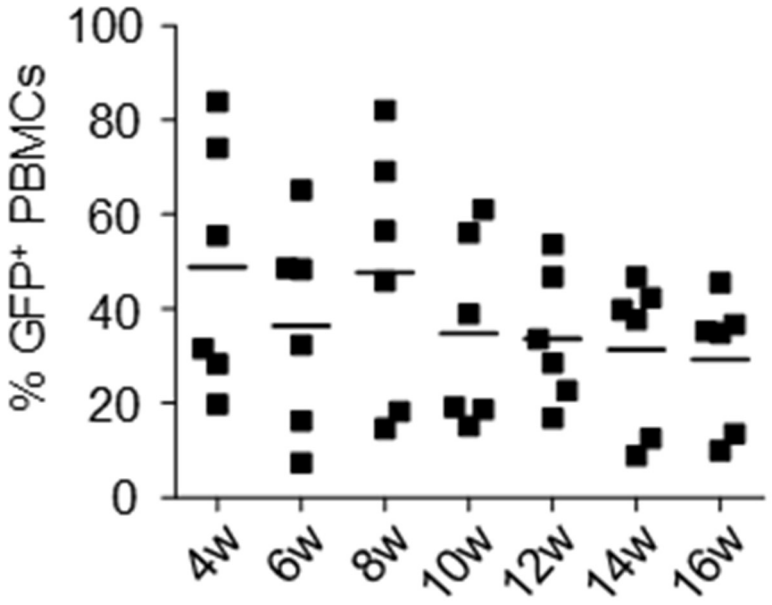


FIG. 63B

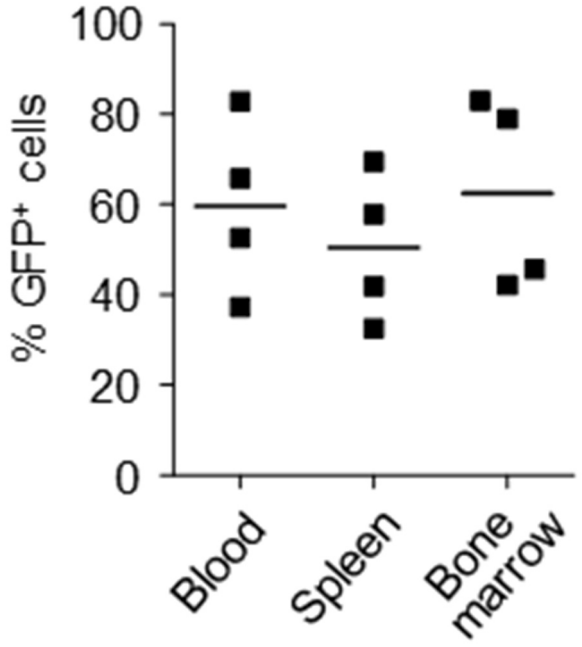


FIG. 63C

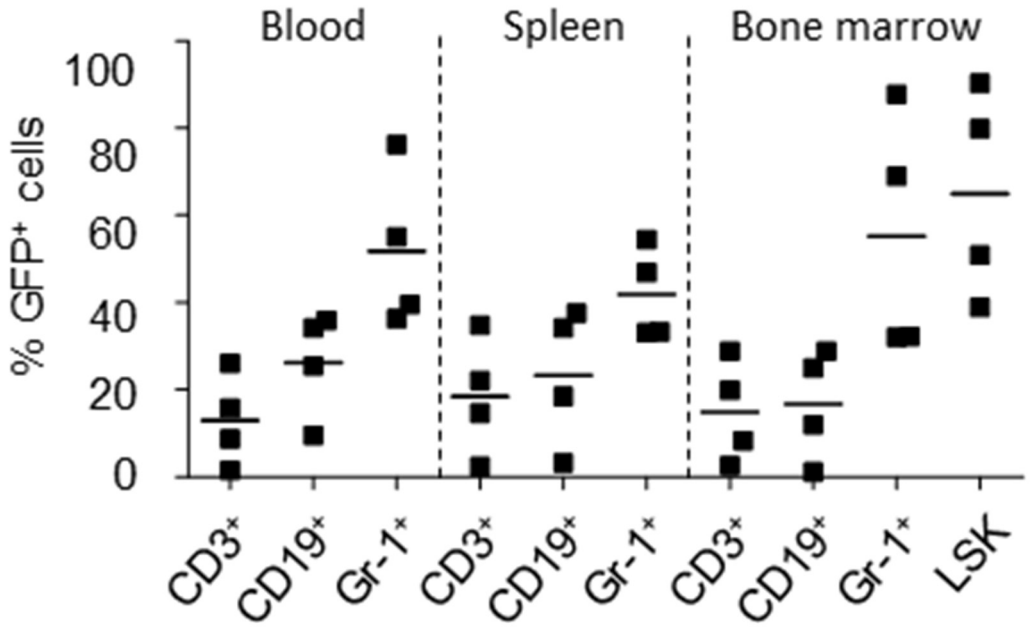


FIG. 63D

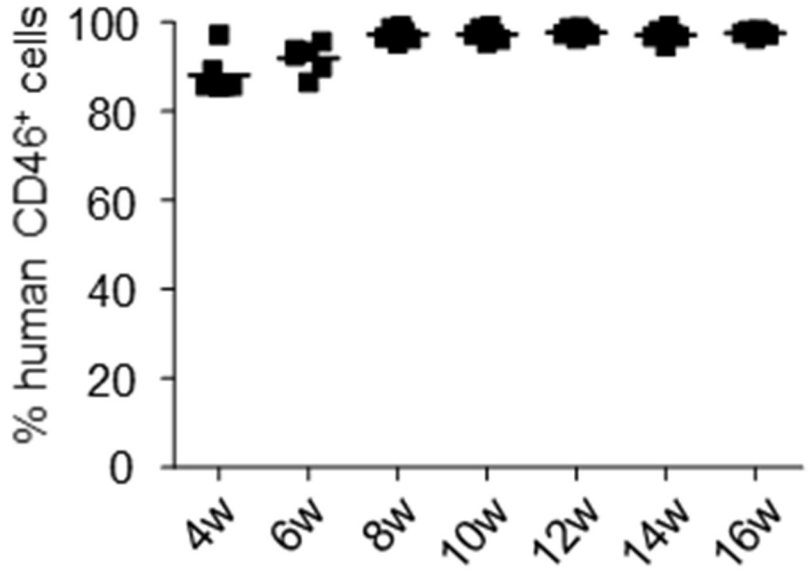


FIG. 63E

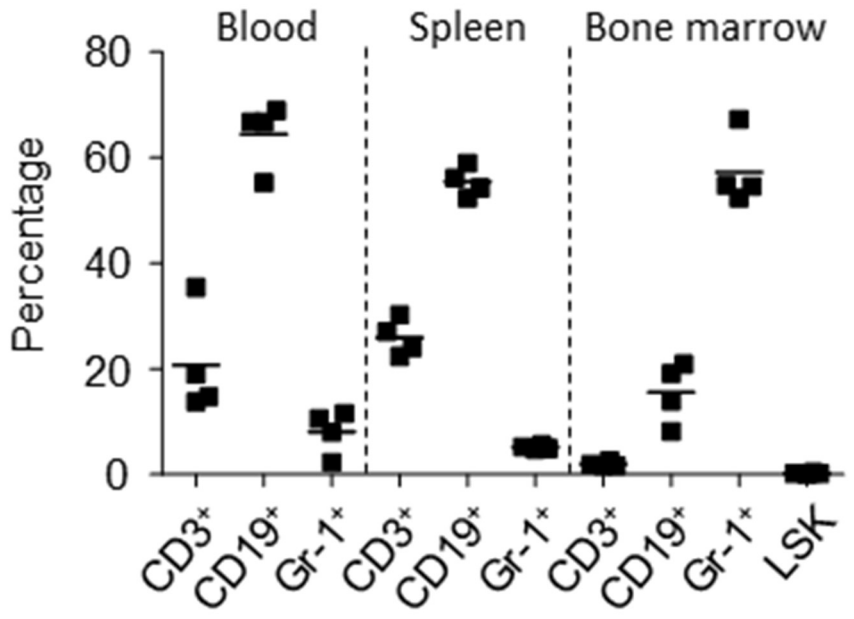


FIG. 64A

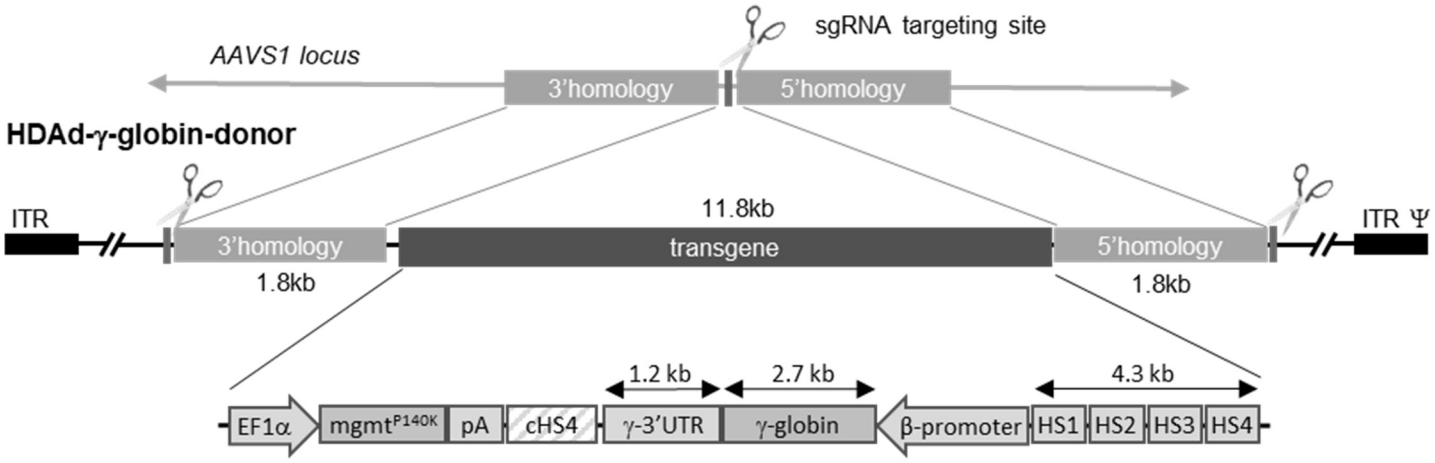


FIG. 64B

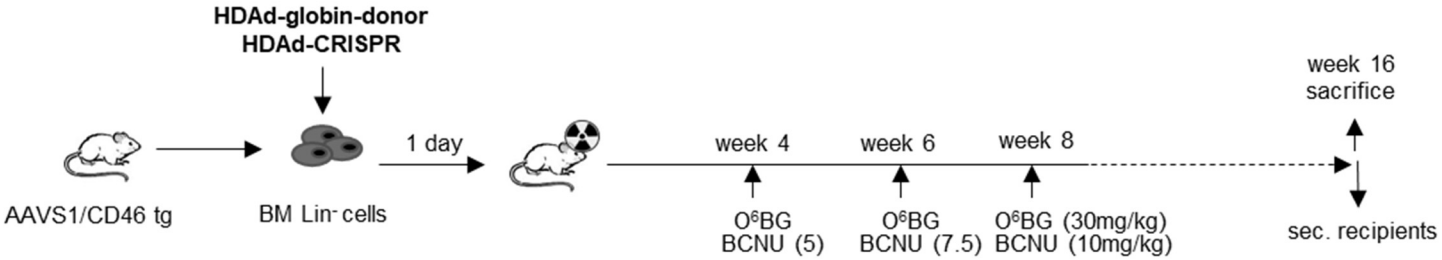


FIG. 64C

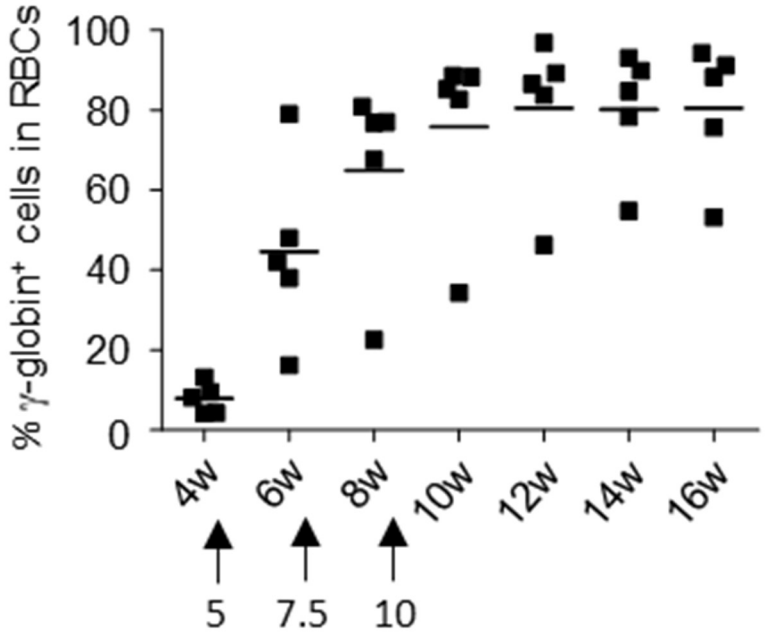


FIG. 64D

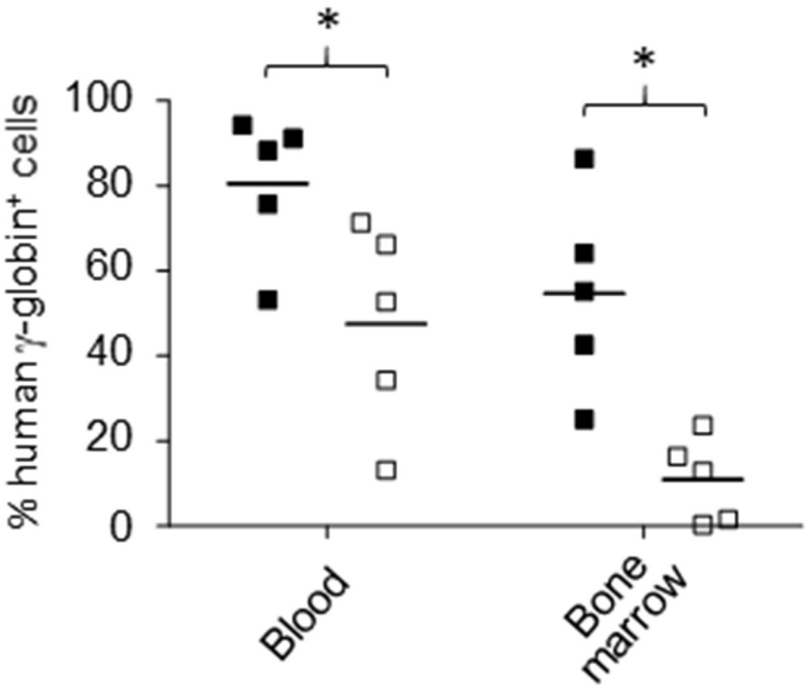


FIG. 64E

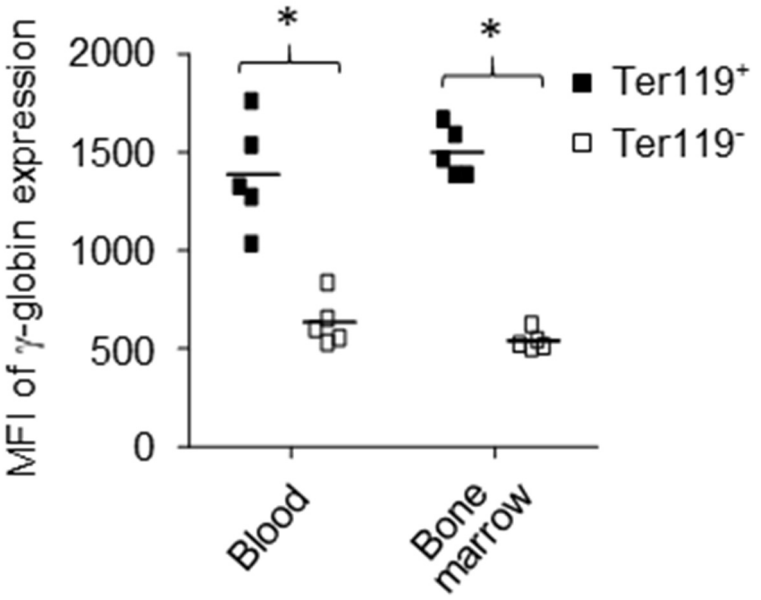


FIG. 64F

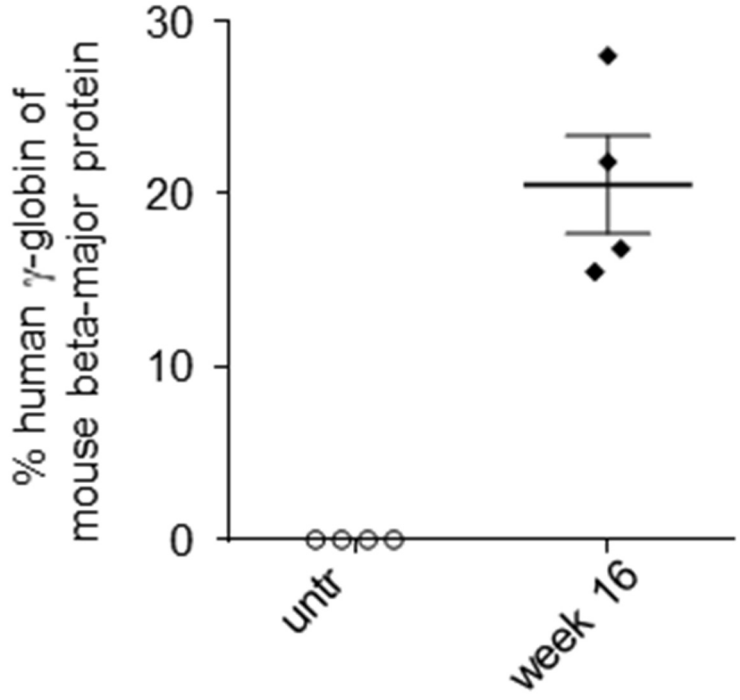


FIG. 64G

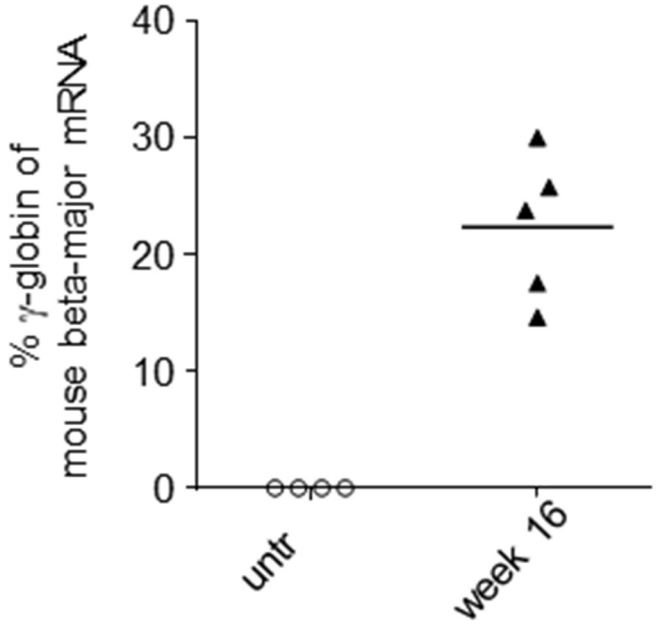


FIG. 64H

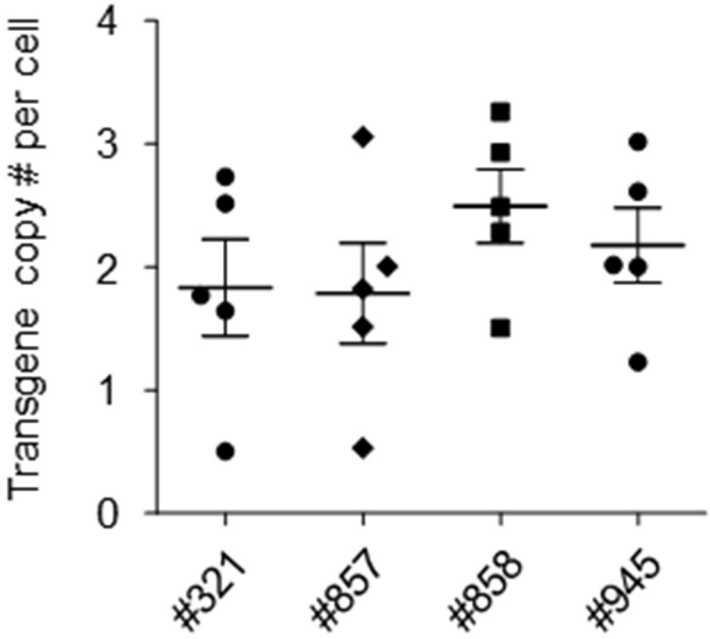


FIG. 65A

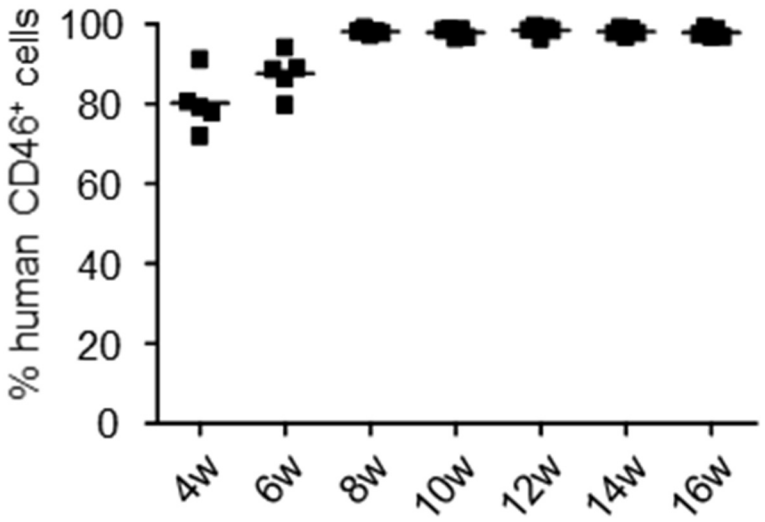


FIG. 65B

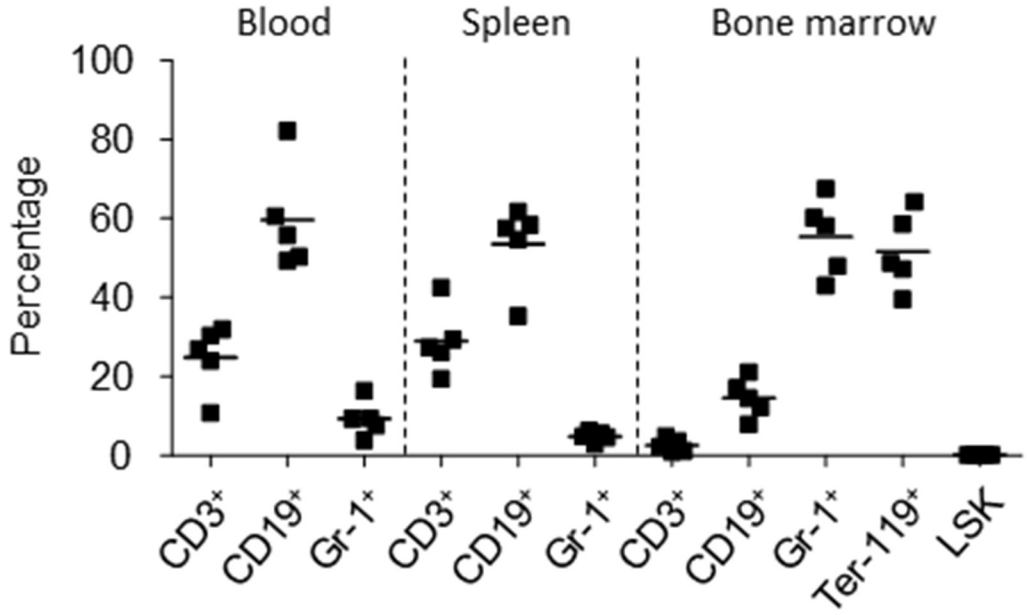


FIG. 66A

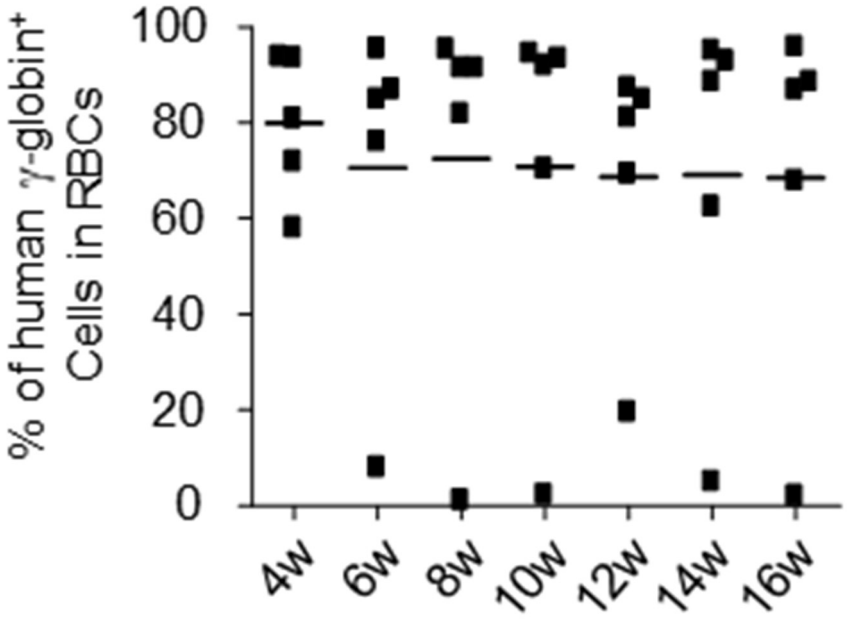


FIG. 66B

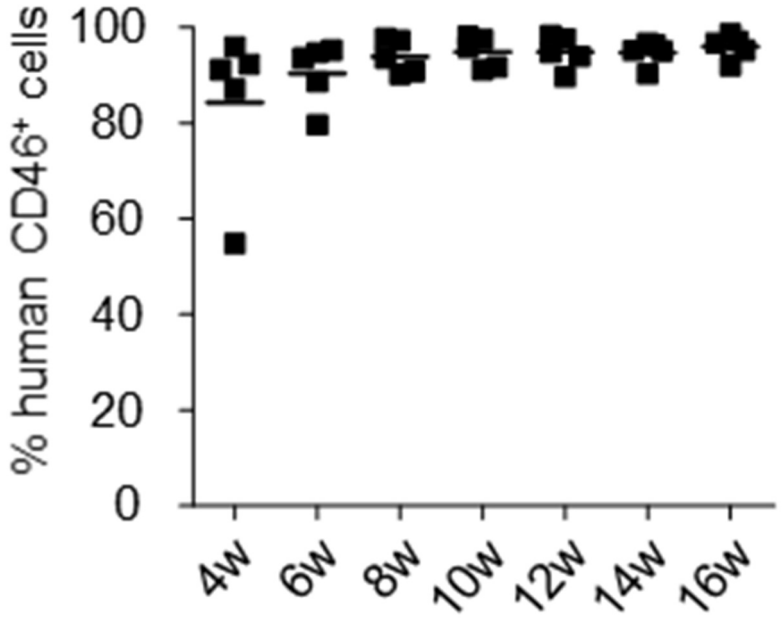


FIG. 66C

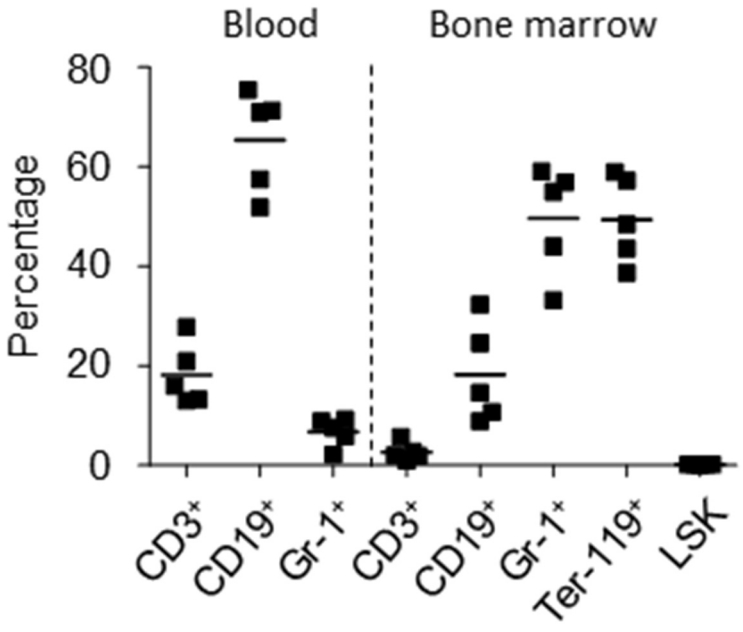

Blood
Bone marrow
Percentage
80
60
40
20
0
CD3+
CD19+
Gr-1+
CD3+
CD19+
Gr-1+
Ter-119+
LSK

FIG. 67A

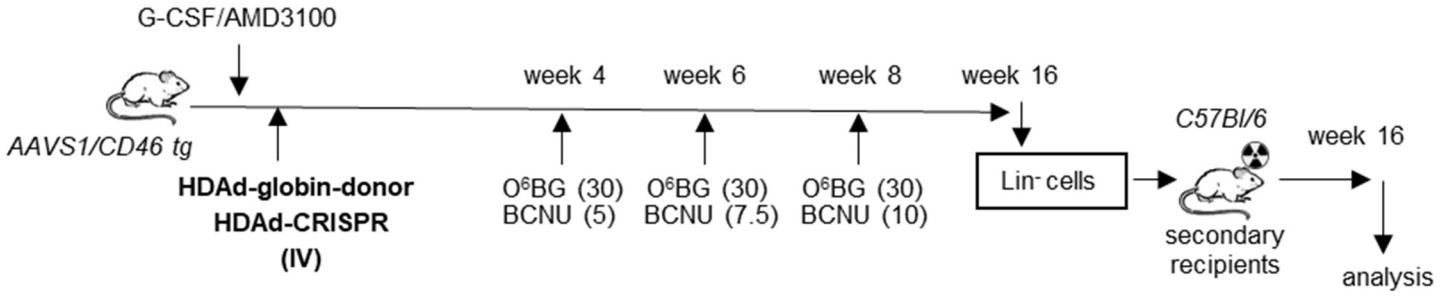


FIG. 67B

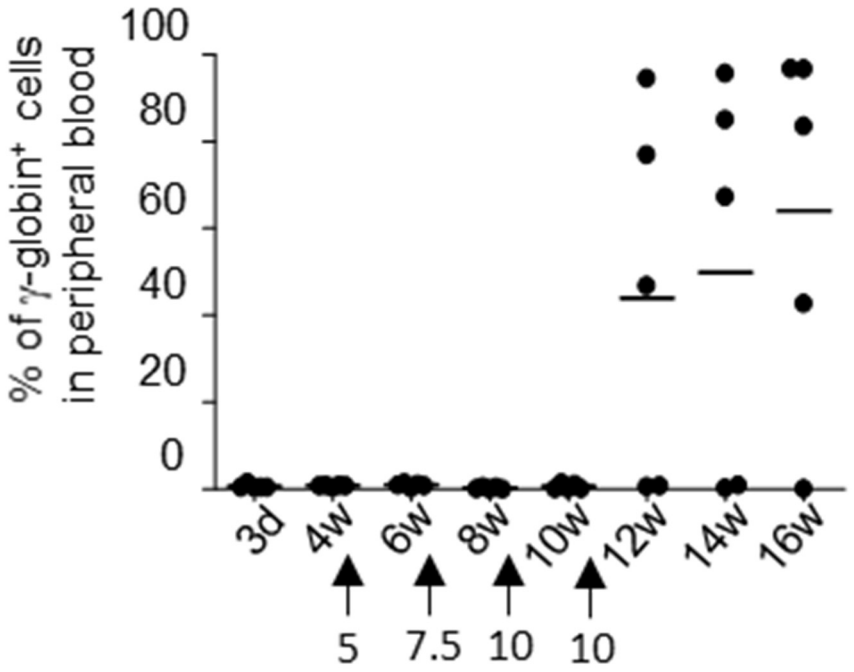


FIG. 67C

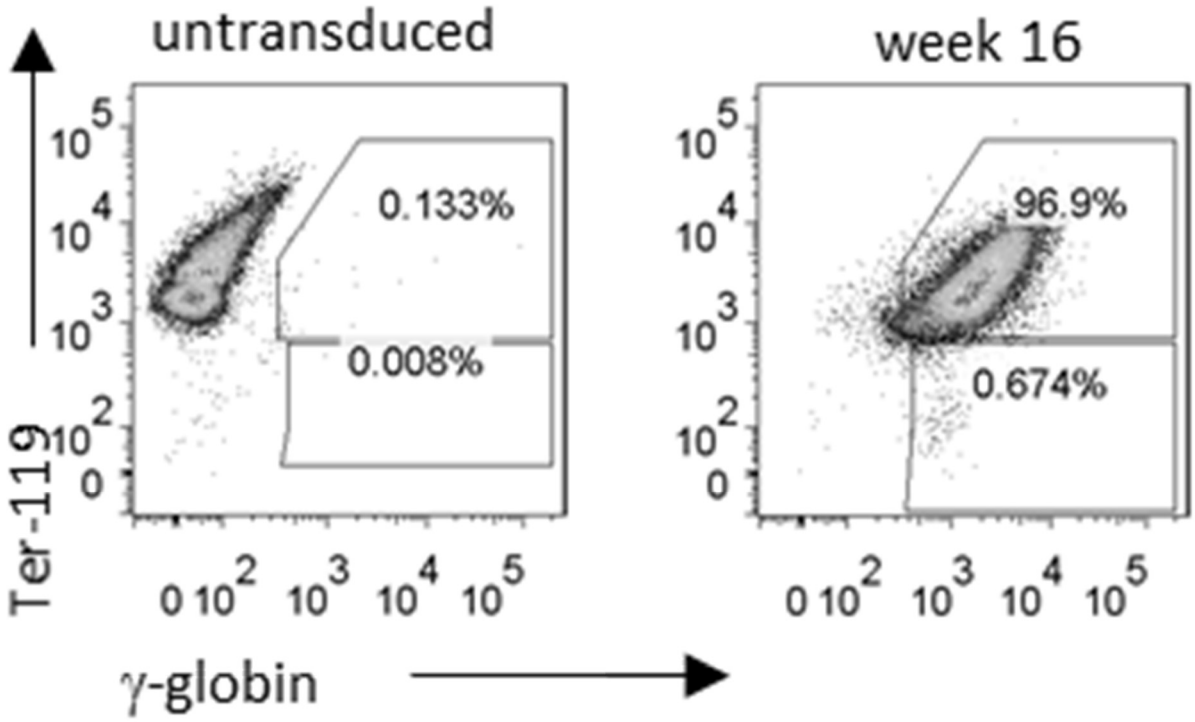


FIG. 67D

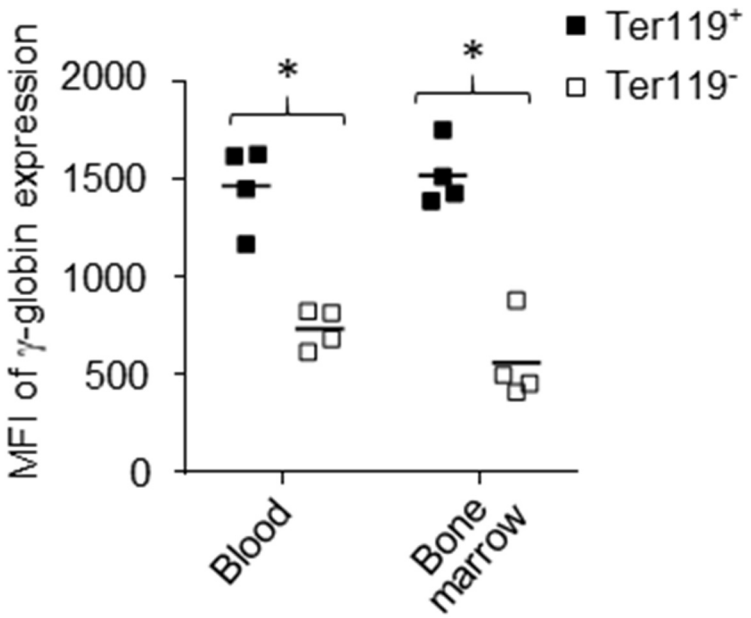


FIG. 67E

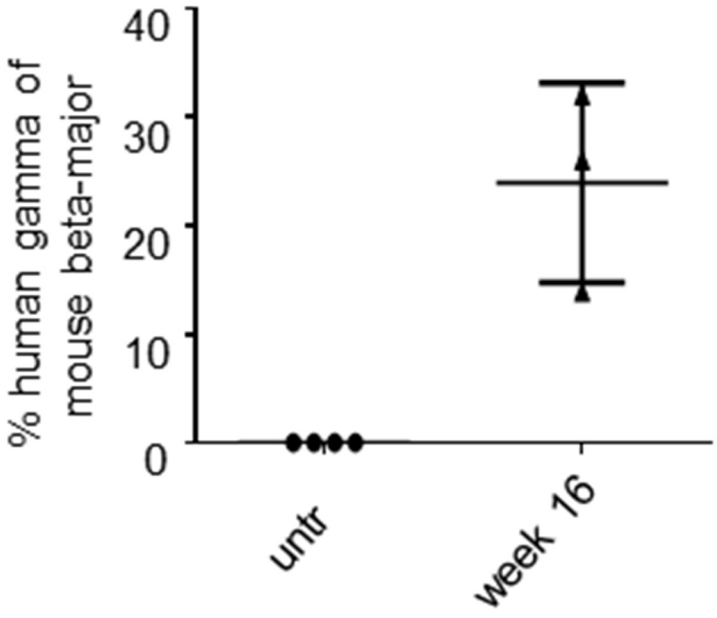


FIG. 67F

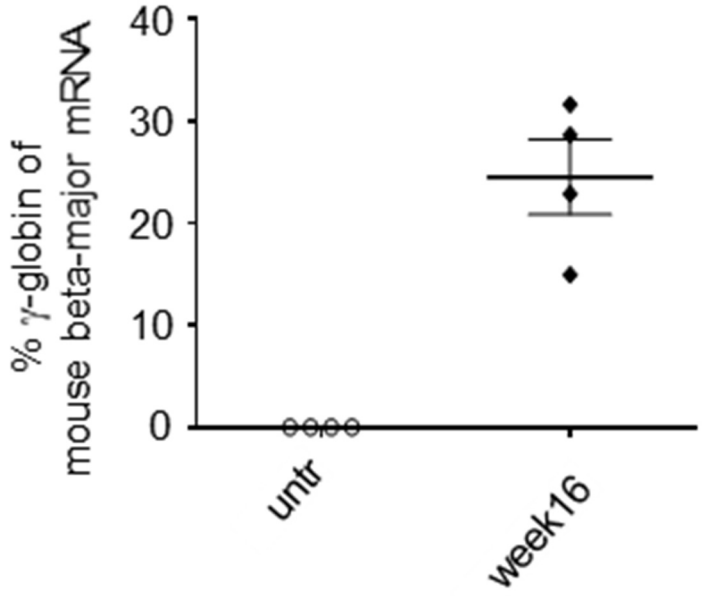


FIG. 67G

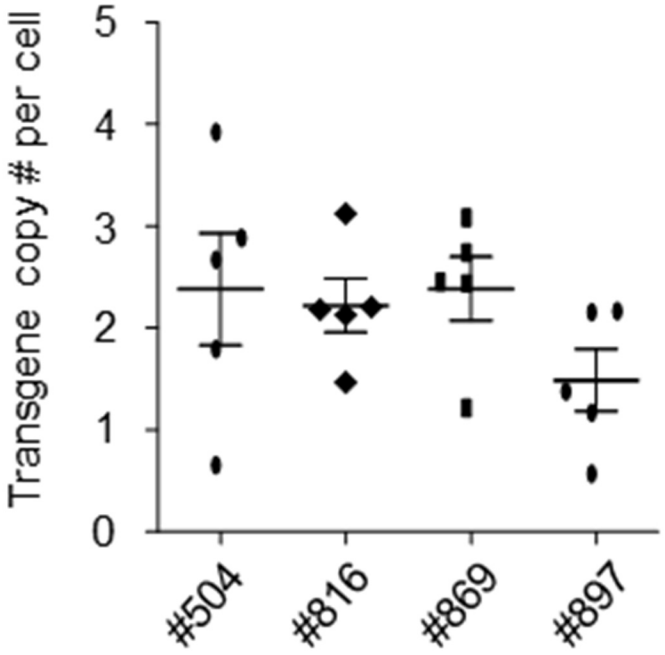


FIG. 67H

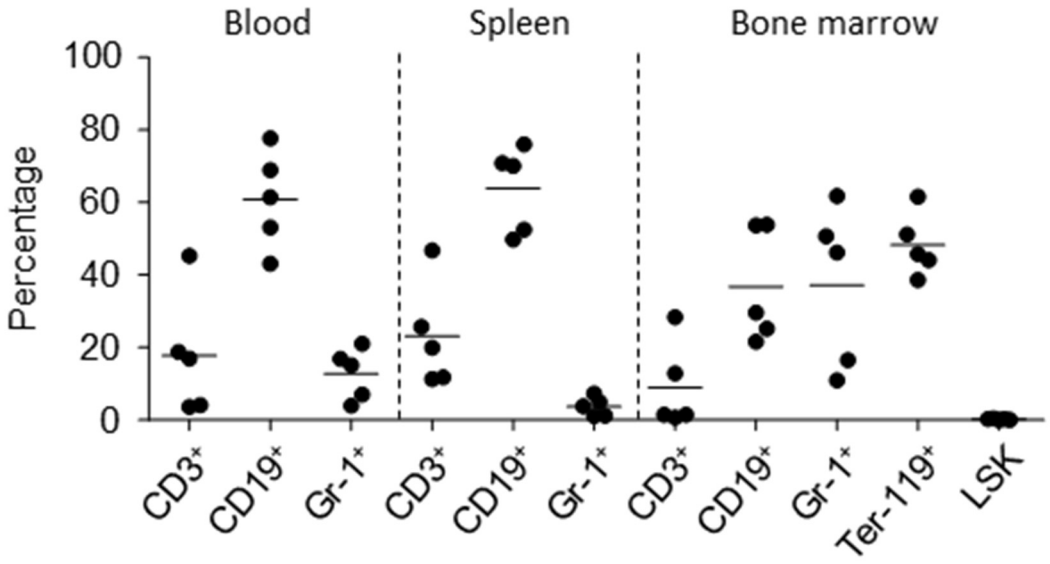


FIG. 68A

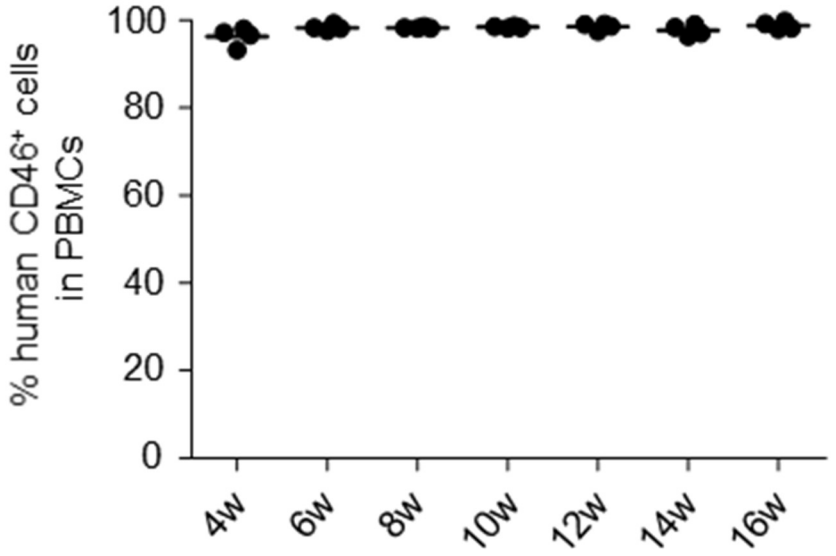


FIG. 68B

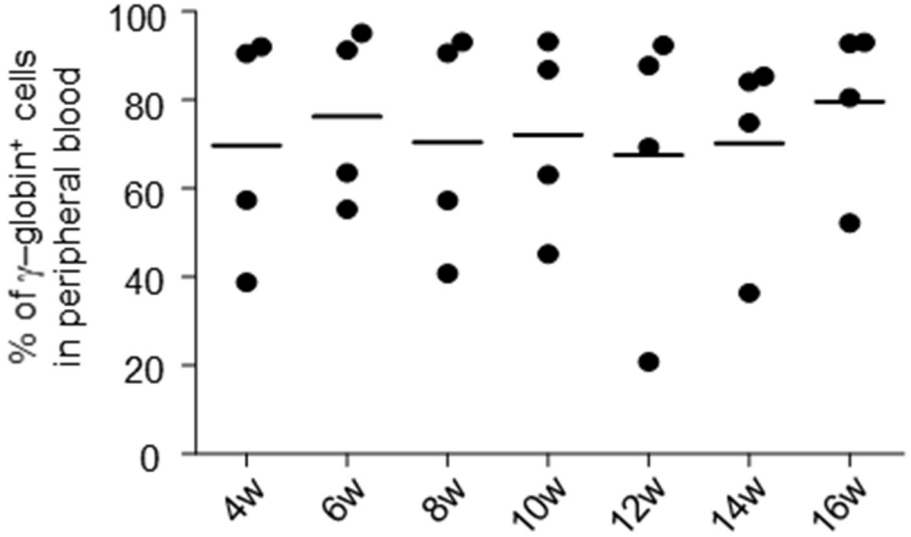


FIG. 68C

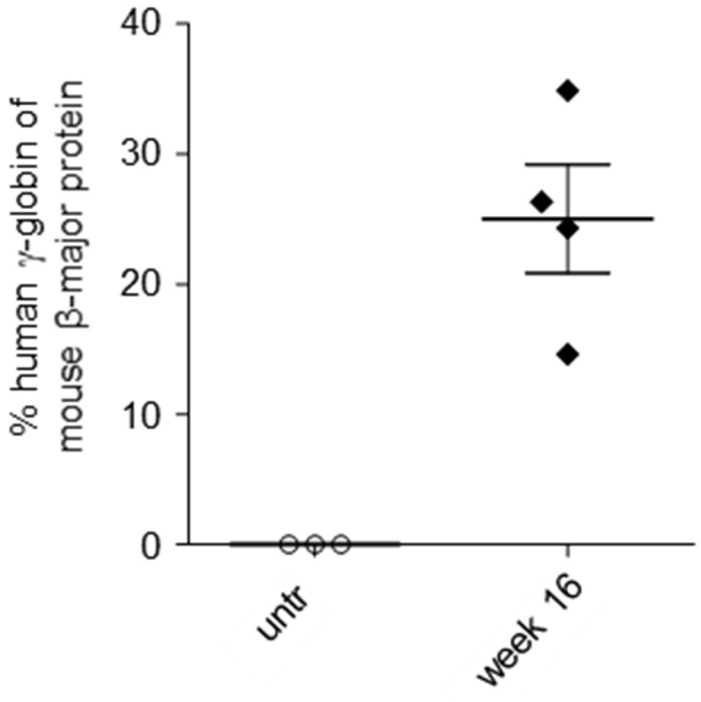


FIG. 68D

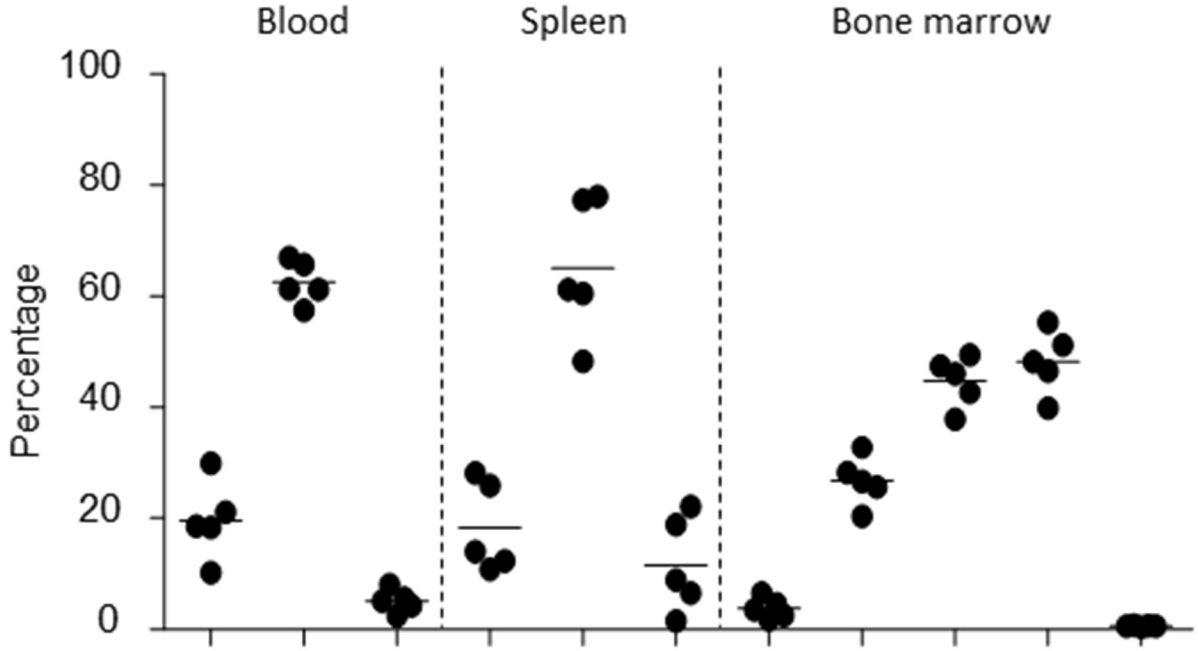


FIG. 69A

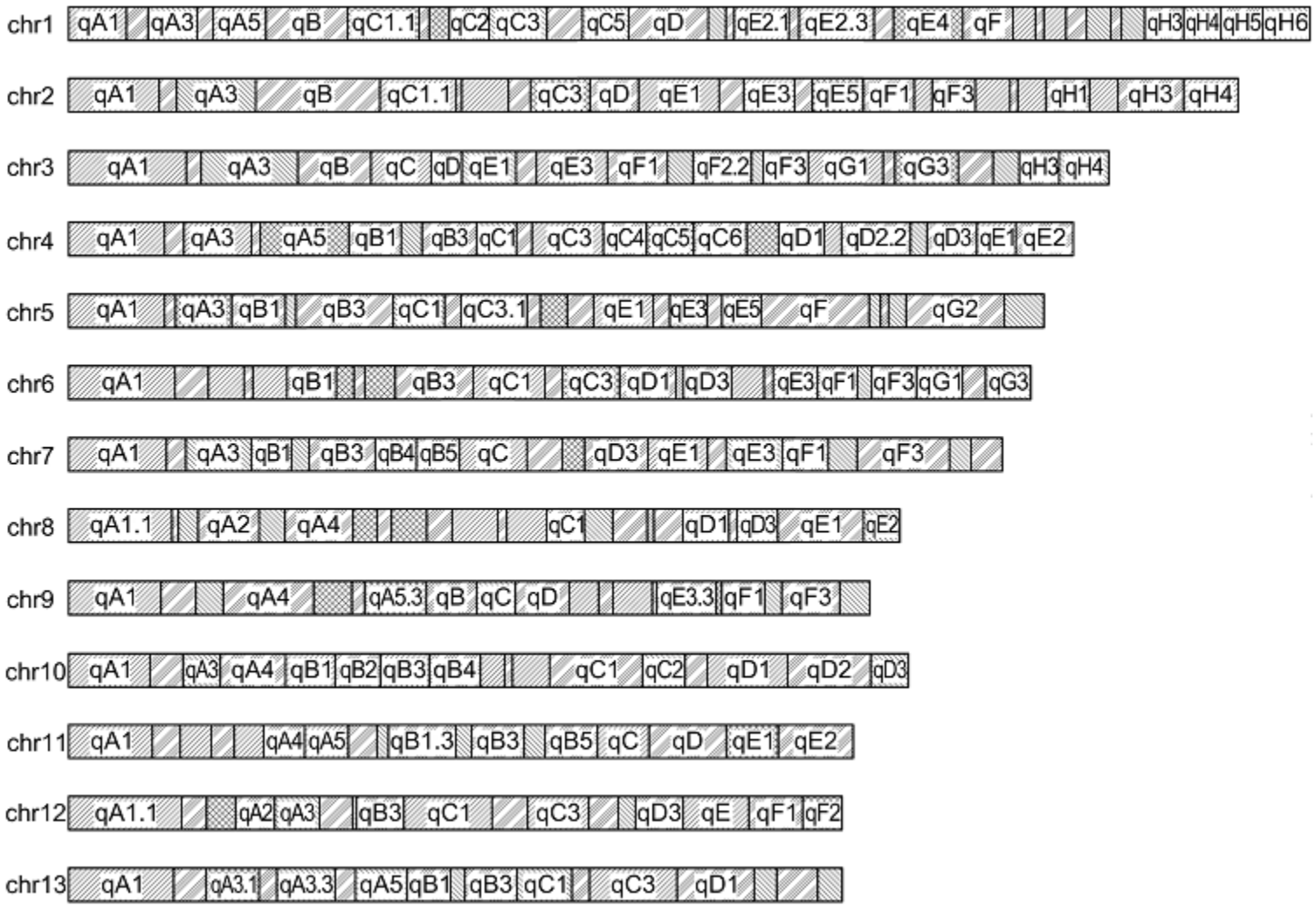

chr1
qA1 qA3 qA5 qB qC1.1 qC2 qC3 qC5 qD qE2.1 qE2.3 qE4 qF qH3 qH4 qH5 qH6
chr2
qA1 qA3 qB qC1.1 qC3 qD qE1 qE3 qE5 qF1 qF3 qH1 qH3 qH4
chr3
qA1 qA3 qB qC qD qE1 qE3 qF1 qF2.2 qF3 qG1 qG3 qH3 qH4
chr4
qA1 qA3 qA5 qB1 qB3 qC1 qC3 qC4 qC5 qC6 qD1 qD2.2 qD3 qE1 qE2
chr5
qA1 qA3 qB1 qB3 qC1 qC3.1 qE1 qE3 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

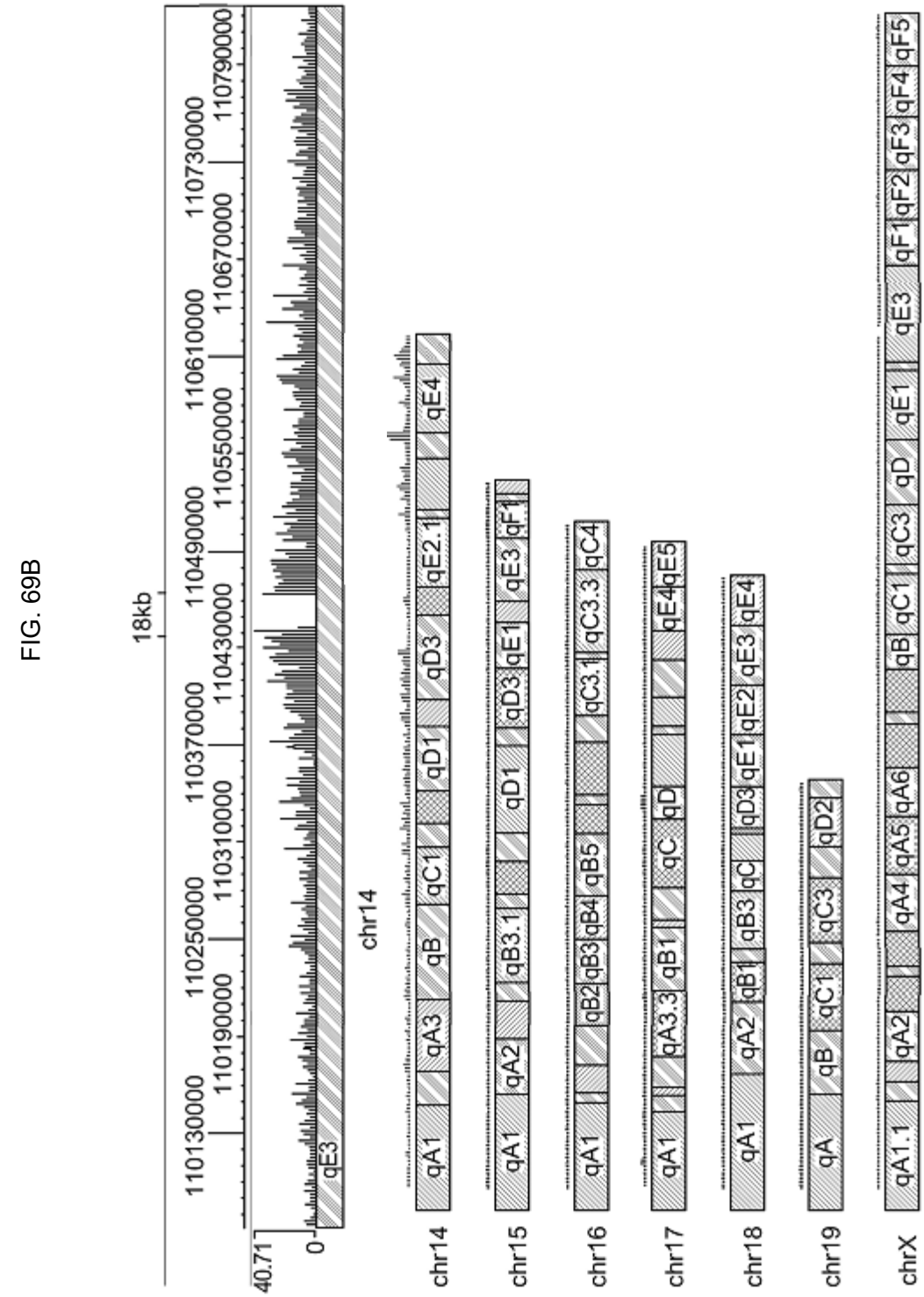

18kb
110130000
110190000
110250000
110310000
110370000
110430000
110490000
110550000
110610000
110670000
110730000
110790000
40.71
0
qE3
chr14
chr14
qA1
qA3
qB
qC1
qD1
qD3
qE2.1
qE4
chr15
qA1
qA2
qB3.1
qD1
qD3
qE1
qE3
qF1
chr16
qA1
qB2
qB3
qB4
qB5
qC3.1
qC3.3
qC4
chr17
qA1
qA3.3
qB1
qC
qD
qE4
qE5
chr18
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

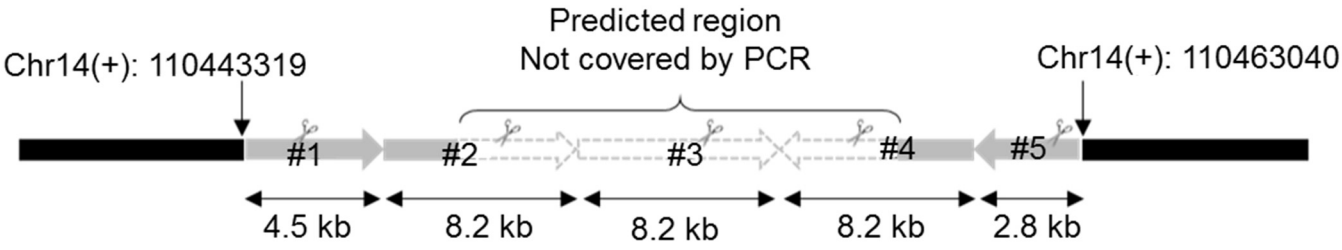


FIG. 71A

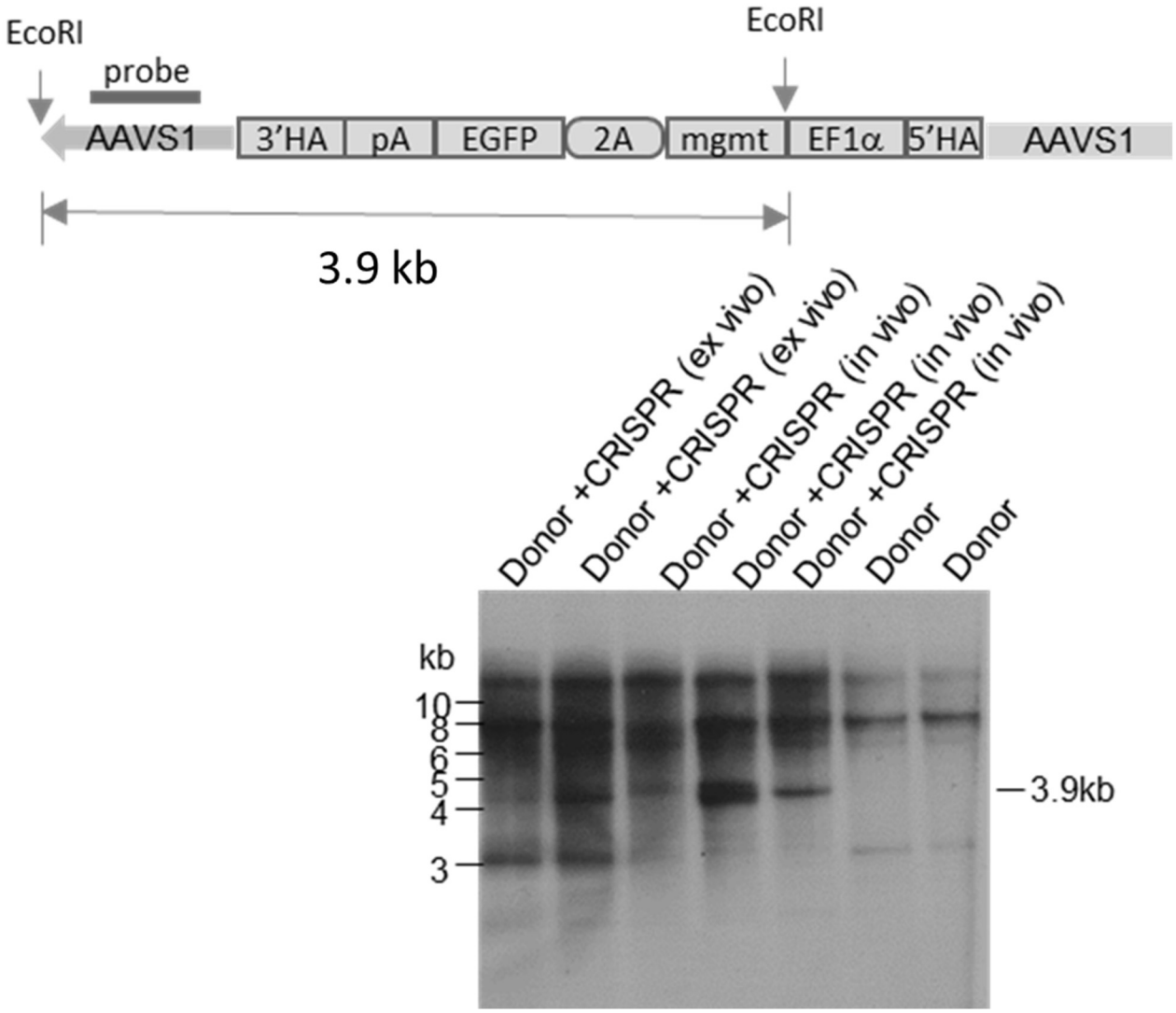


FIG. 71B

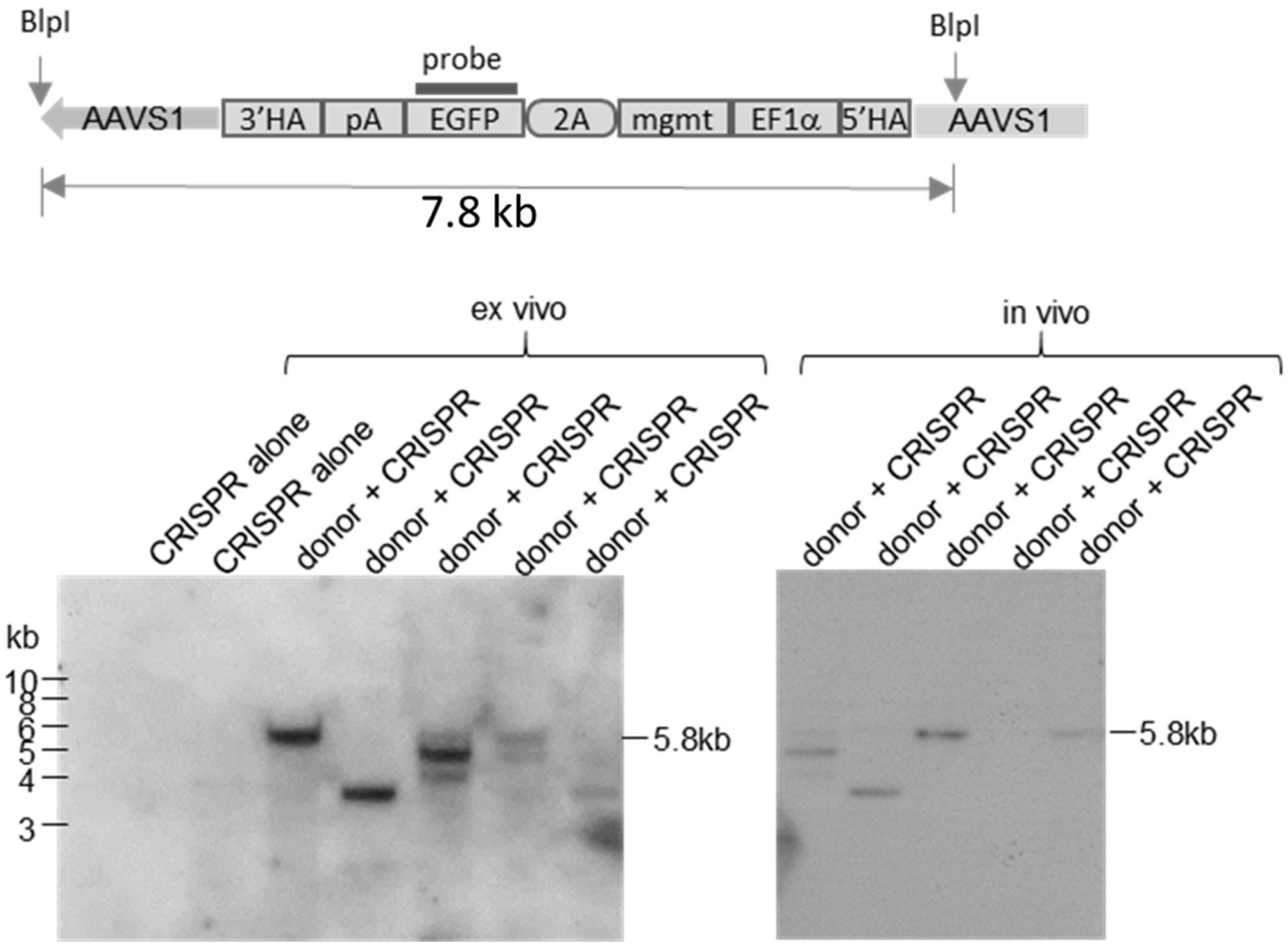

BlpI
probe
BlpI
AAVS1
3'HA
pA
EGFP
2A
mgmt
EF1α
5'HA
AAVS1
7.8 kb
ex vivo
in vivo
CRISPR alone
CRISPR alone
donor + CRISPR
donor + CRISPR
donor + CRISPR
donor + CRISPR
donor + CRISPR
donor + CRISPR
donor + CRISPR
donor + CRISPR
donor + CRISPR
kb
10
8
6
5
4
3
5.8kb
5.8kb

FIG. 72A

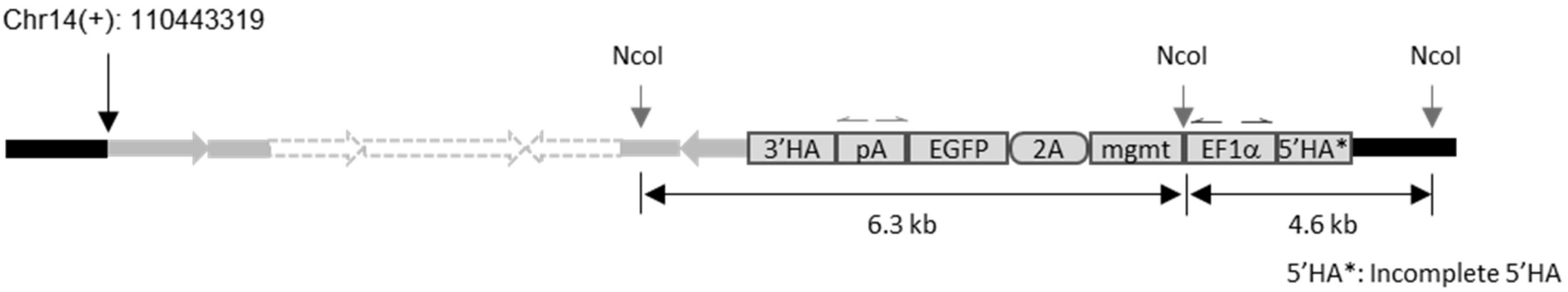


FIG. 72B

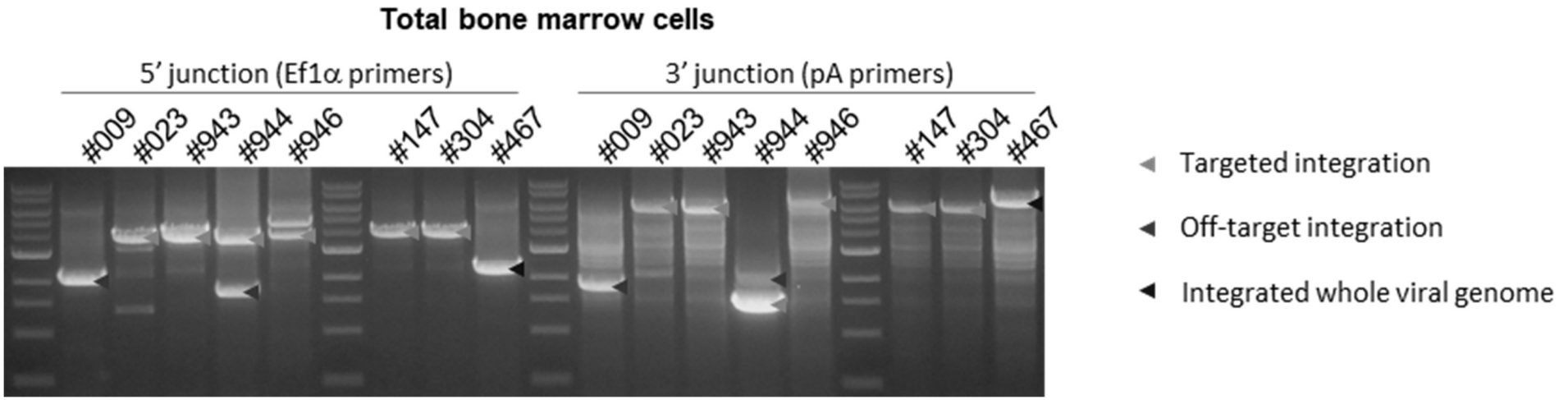


FIG. 72C

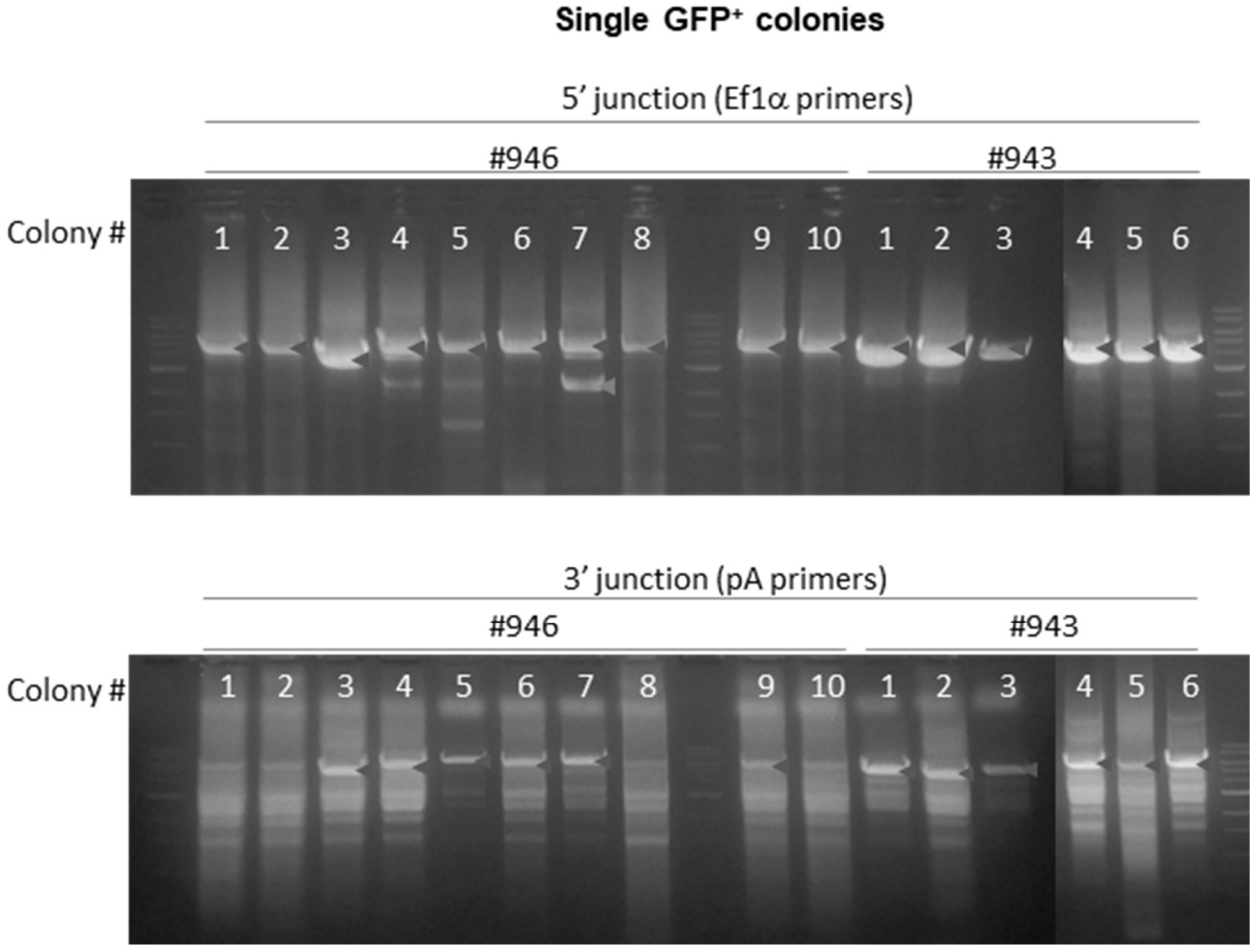

Single GFP+ colonies
5' junction (Ef1α primers)
#946
#943
Colony #
1 2 3 4 5 6 7 8 9 10 1 2 3 4 5 6
3' junction (pA primers)
#946
#943
Colony #
1 2 3 4 5 6 7 8 9 10 1 2 3 4 5 6

FIG. 73A

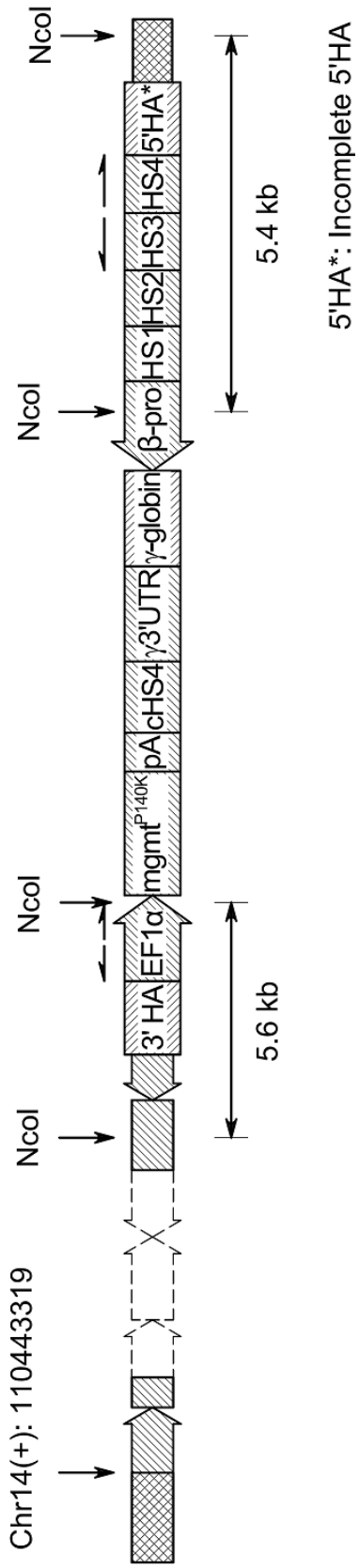


FIG. 73B

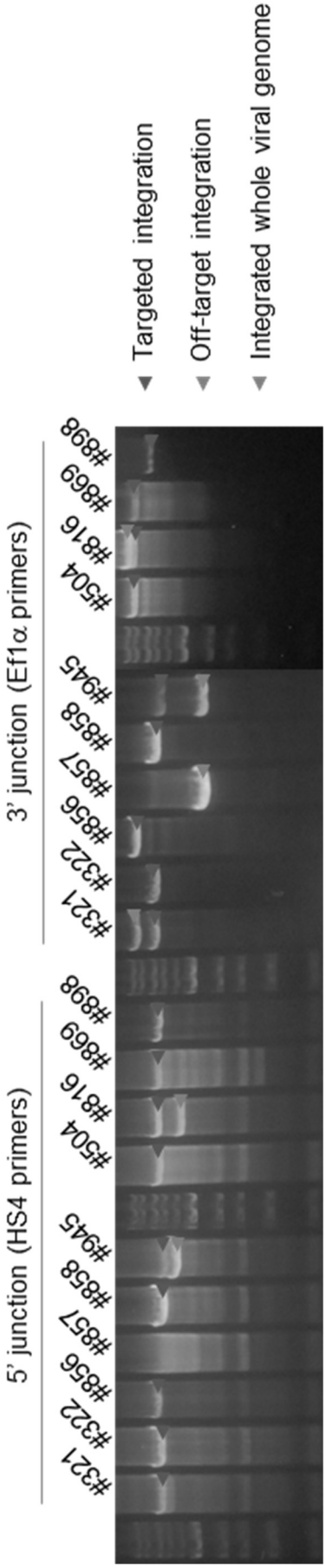


FIG. 74A

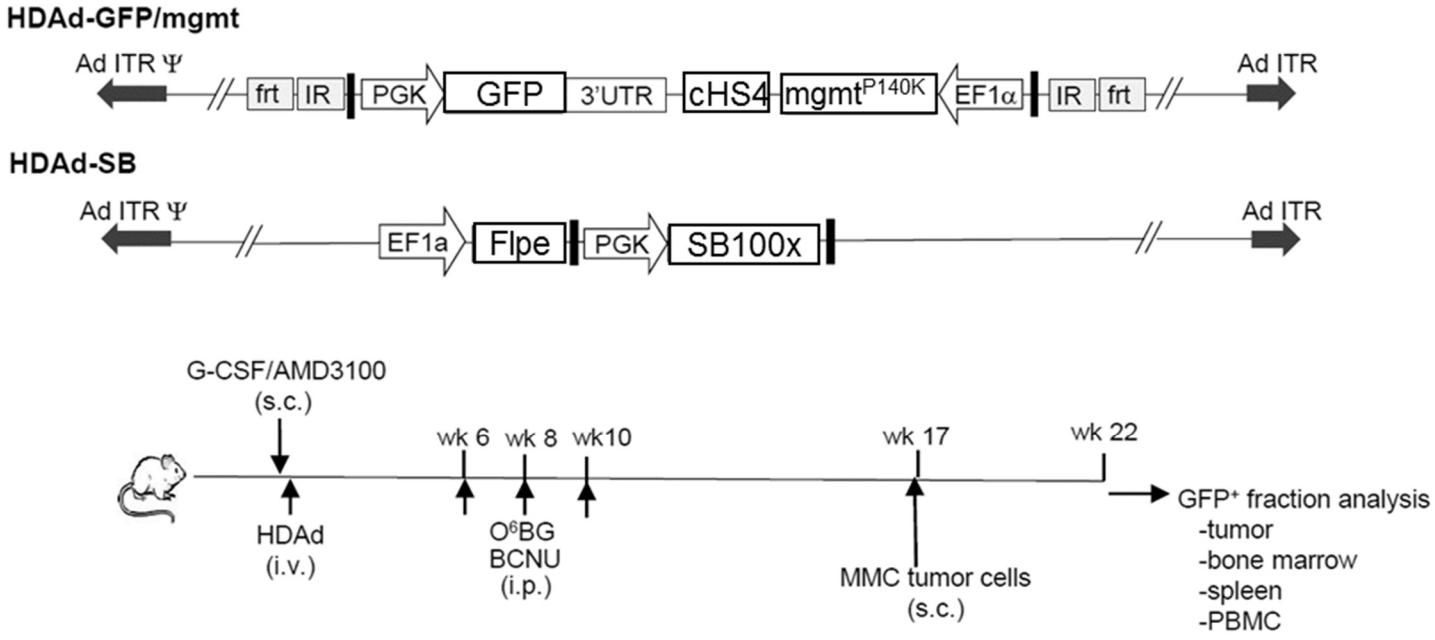


FIG. 74B

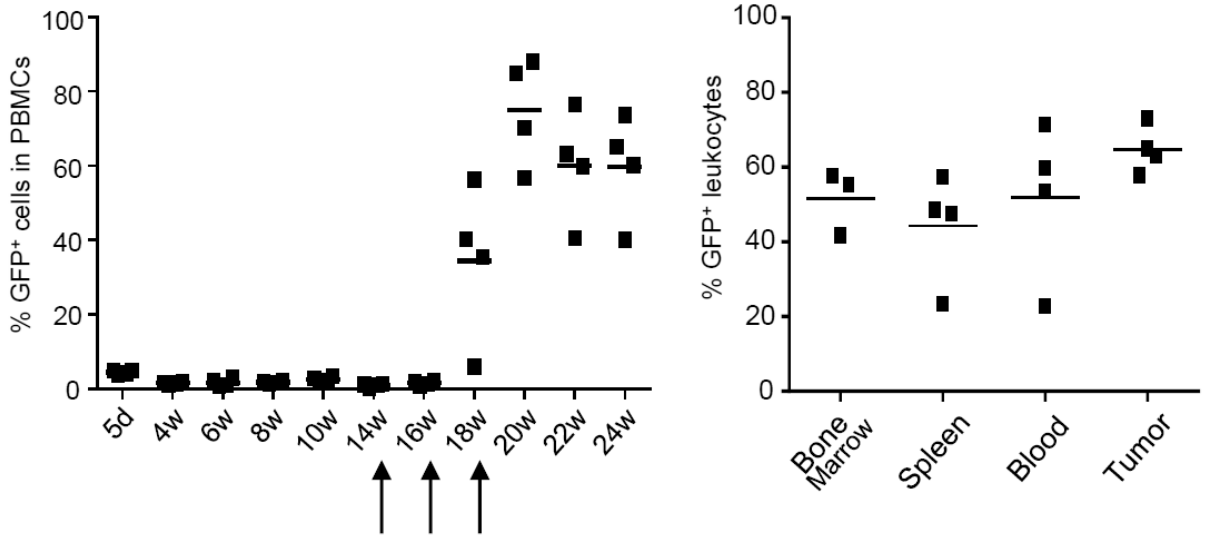
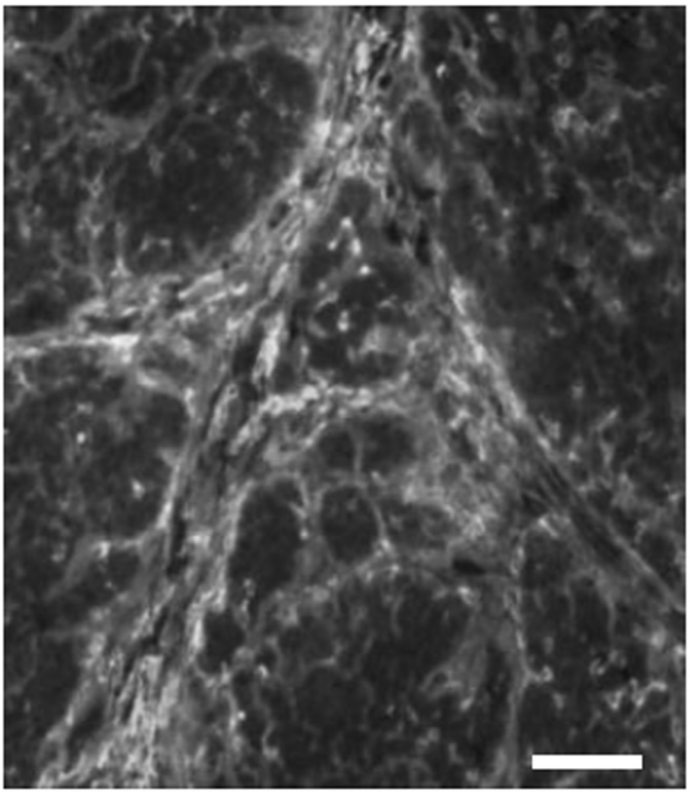


FIG. 74C



GFP/laminin

FIG. 74D

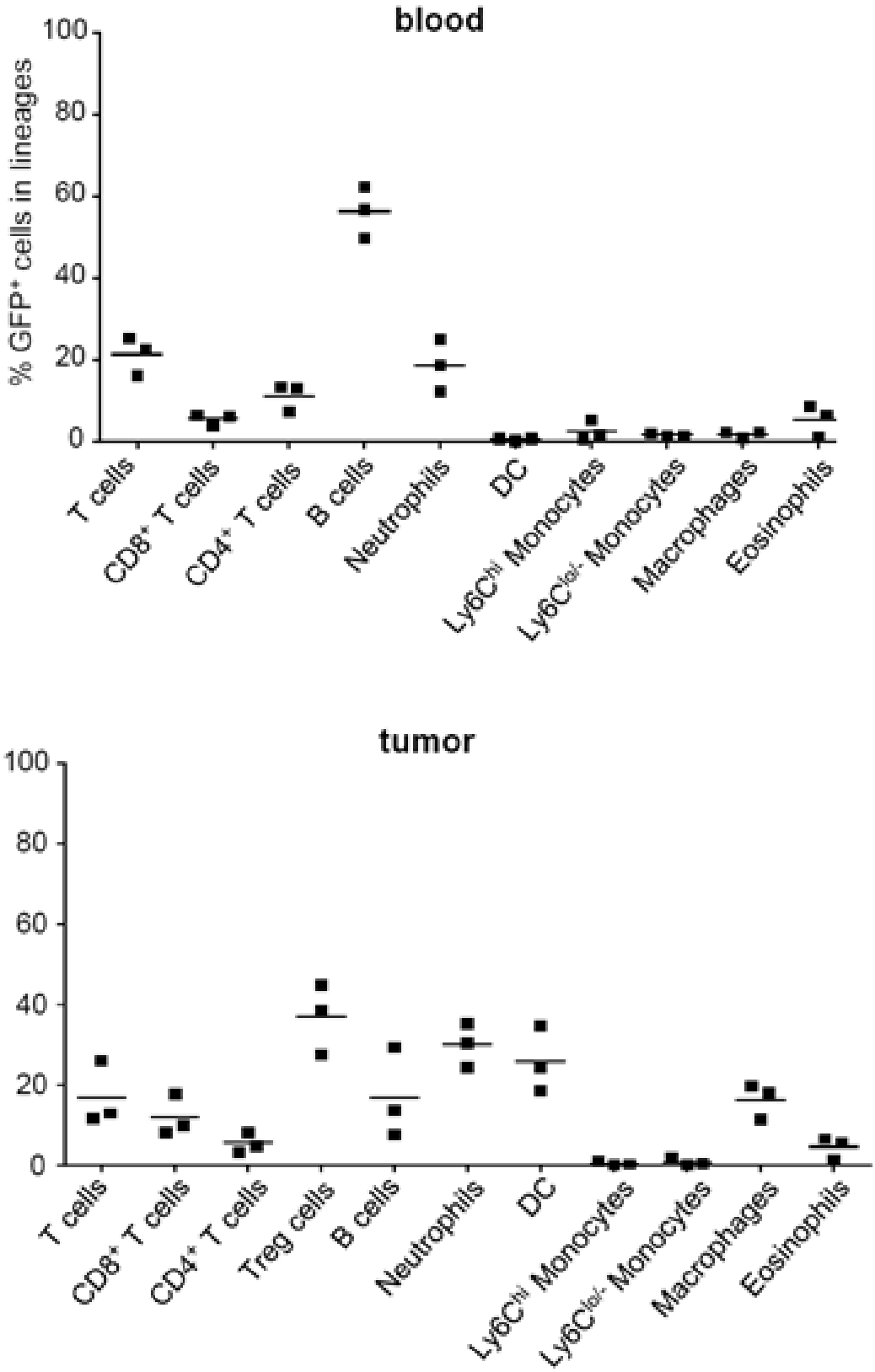

blood
% GFP+ cells in lineages
100
80
60
40
20
0
T cells
CD8+ T cells
CD4+ T cells
B cells
Neutrophils
DC
Ly6Chi Monocytes
Ly6Clo/- Monocytes
Macrophages
Eosinophils
tumor
100
80
60
40
20
0
T cells
CD8+ T cells
CD4+ T cells
Treg cells
B cells
Neutrophils
DC
Ly6Chi Monocytes
Ly6Clo/- Monocytes
Macrophages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

FIG. 75

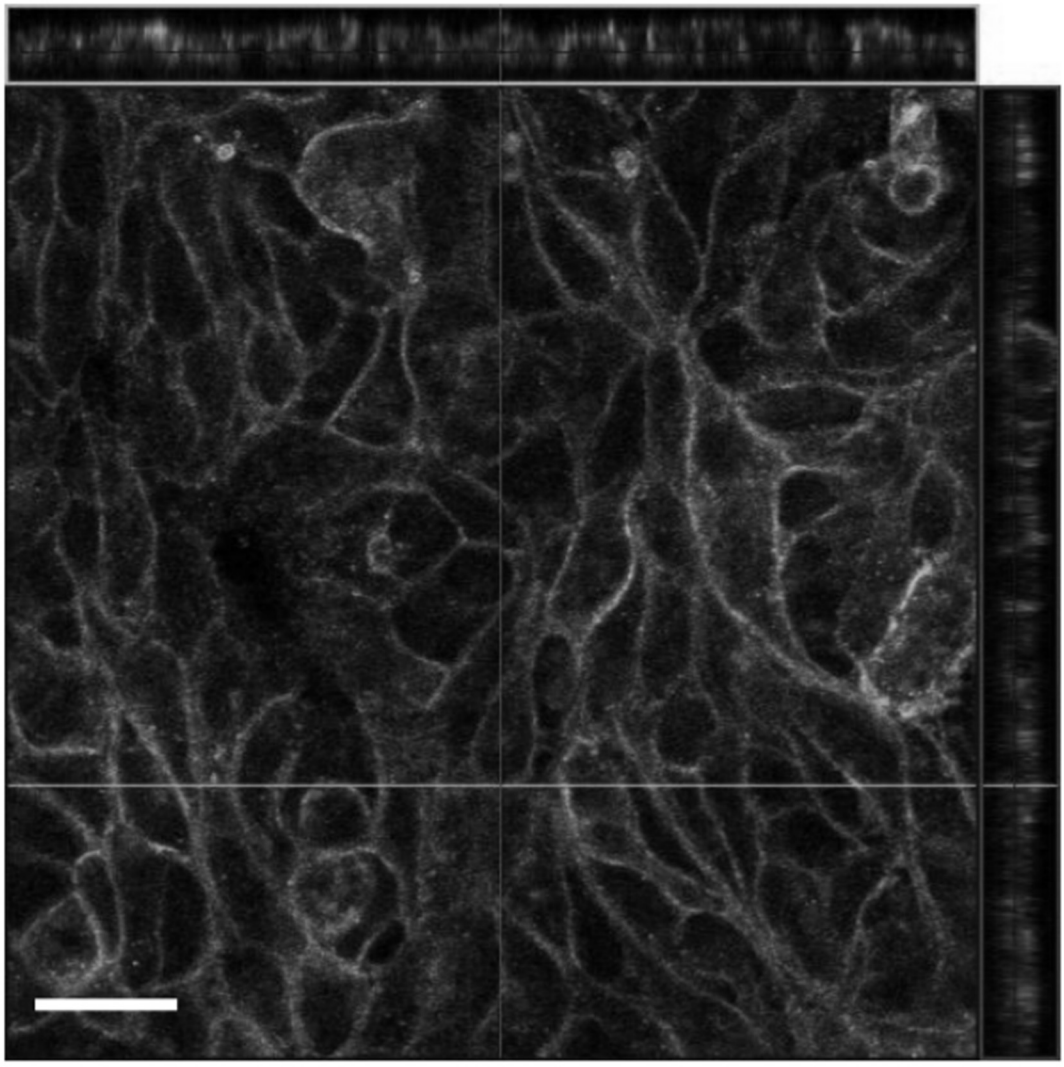


FIG. 76

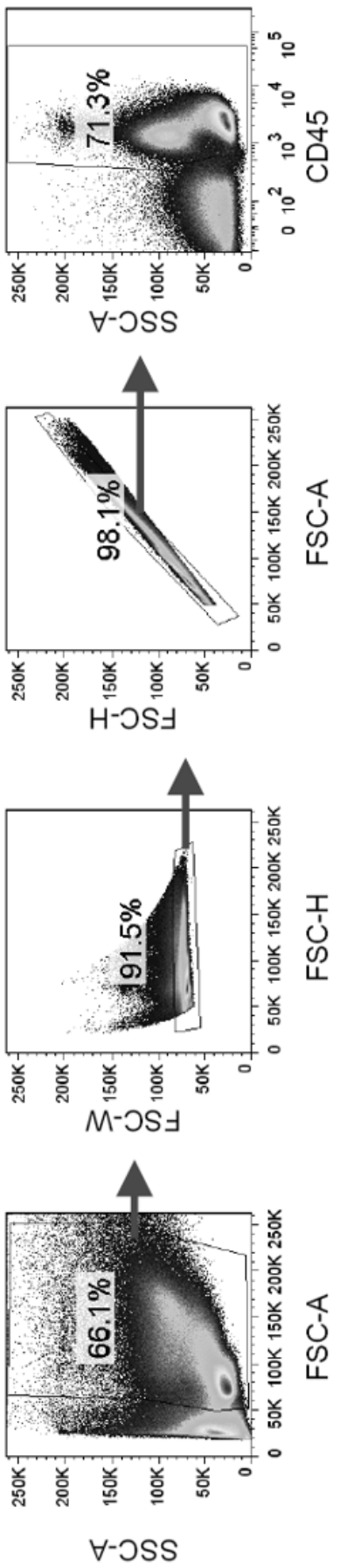


FIG. 76 cont'd

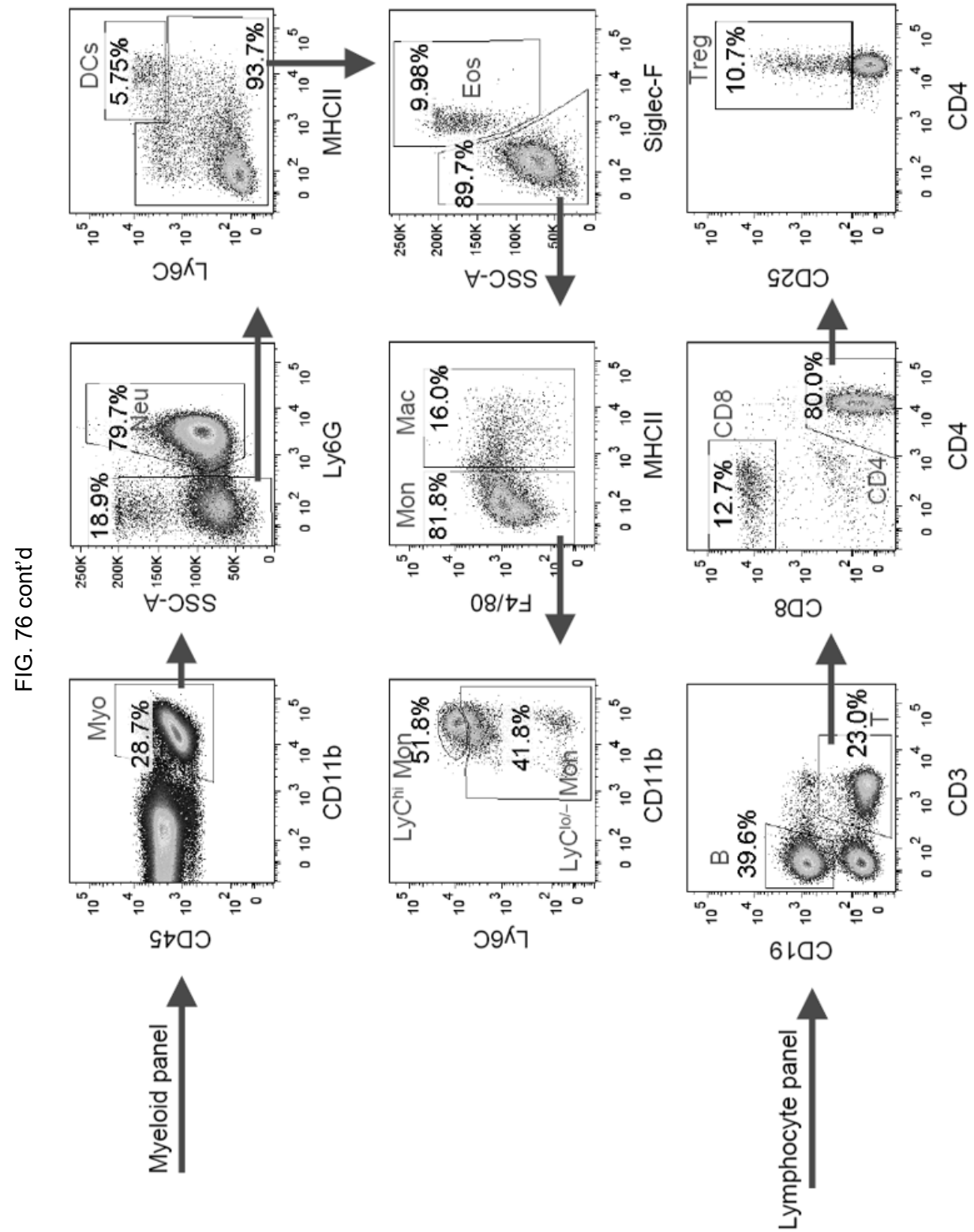

Myeloid panel
Lymphocyte panel
CD45
CD11b
Myo
28.7%
SSC-A
Ly6G
18.9%
79.7%
Neu
Ly6C
MHCII
DCs
5.75%
93.7%
Ly6C
CD11b
LyChi Mon
51.8%
41.8%
LyClo/- Mon
F4/80
MHCII
Mon
Mac
81.8%
16.0%
SSC-A
Siglec-F
89.7%
9.98%
Eos
CD19
CD3
B
39.6%
T
23.0%
CD8
CD4
12.7%
CD8
80.0%
CD4
CD25
CD4
Treg
10.7%

FIG. 77

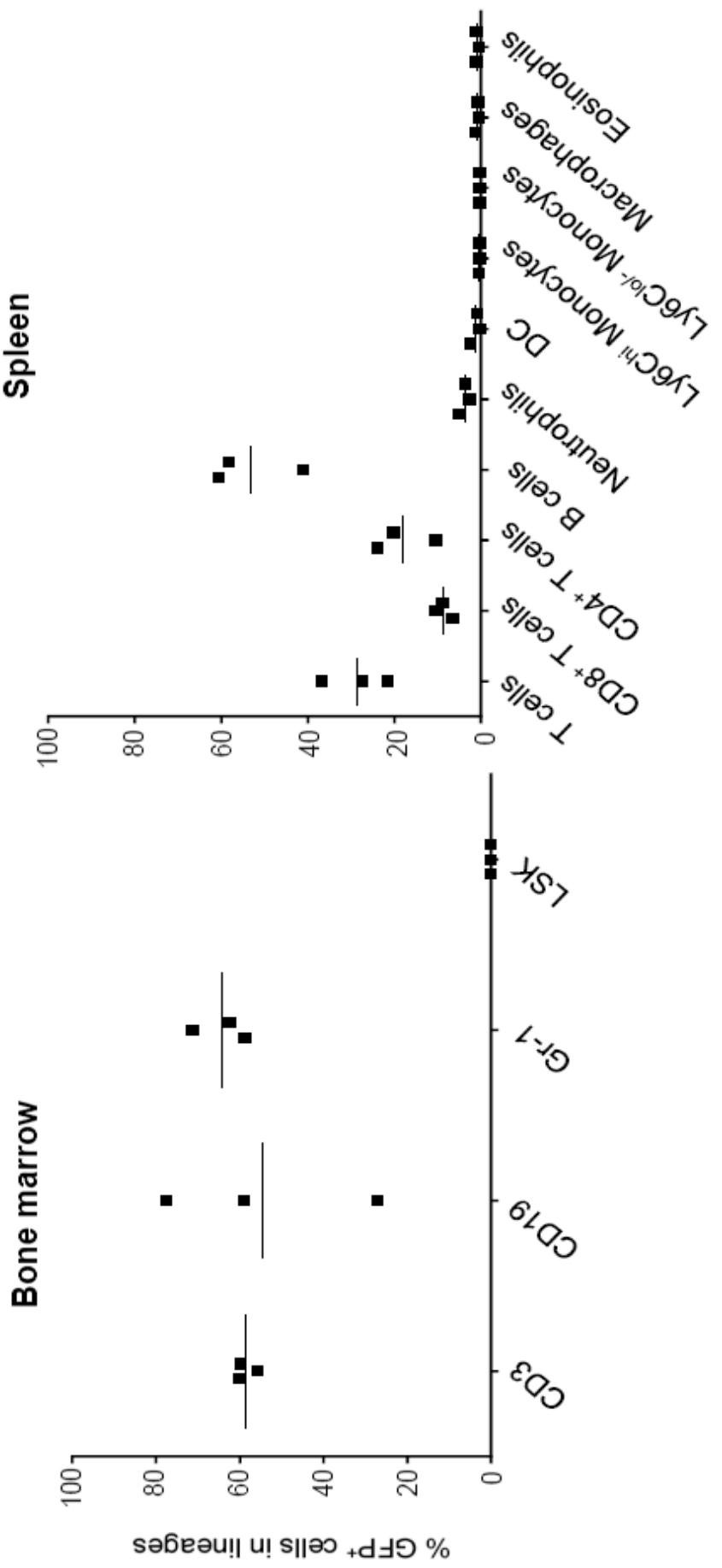

% GFP+ cells in lineages
Bone marrow
CD3
CD19
Gr-1
LSK
Spleen
T cells
CD8+ T cells
CD4+ T cells
B cells
Neutrophils
DC
Ly6Chi Monocytes
Ly6Clo- Monocytes
Macrophages
Eosinophils
0
20
40
60
80
100

FIG. 78A

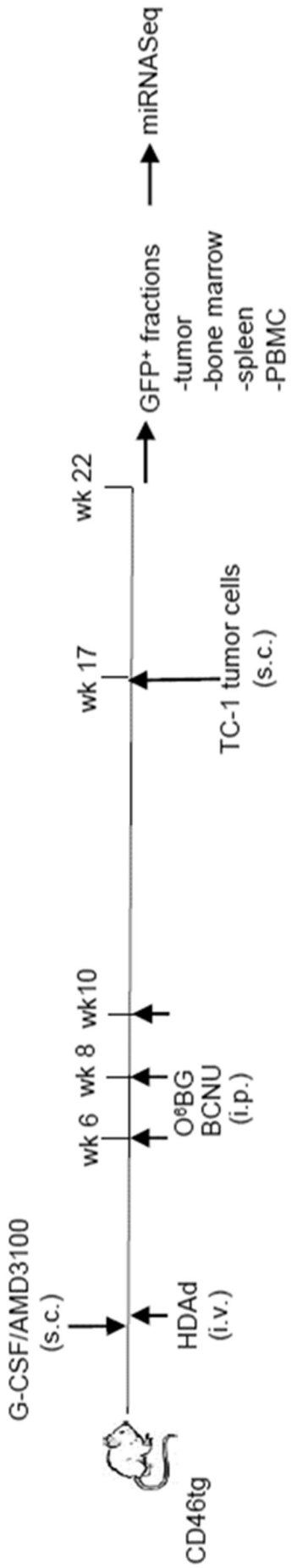


FIG. 78B

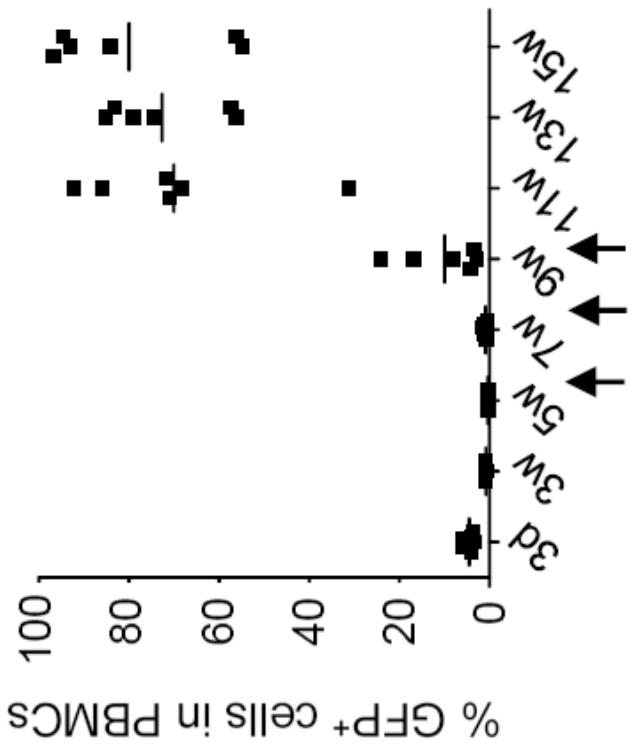


FIG. 78C

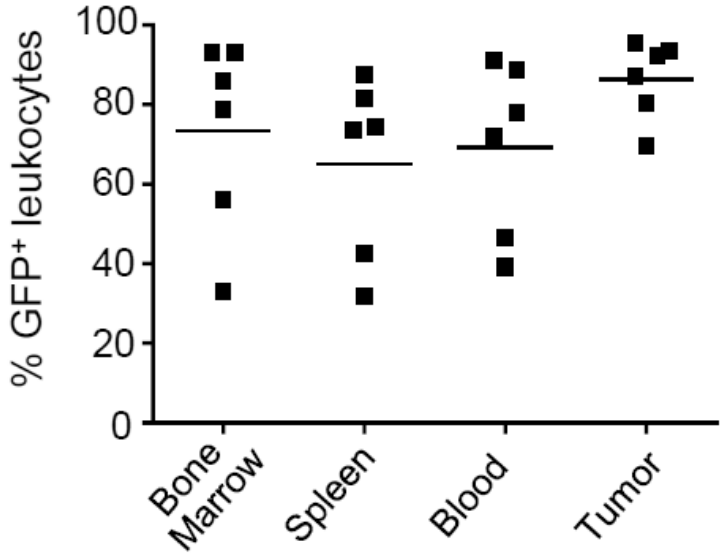


FIG. 78D

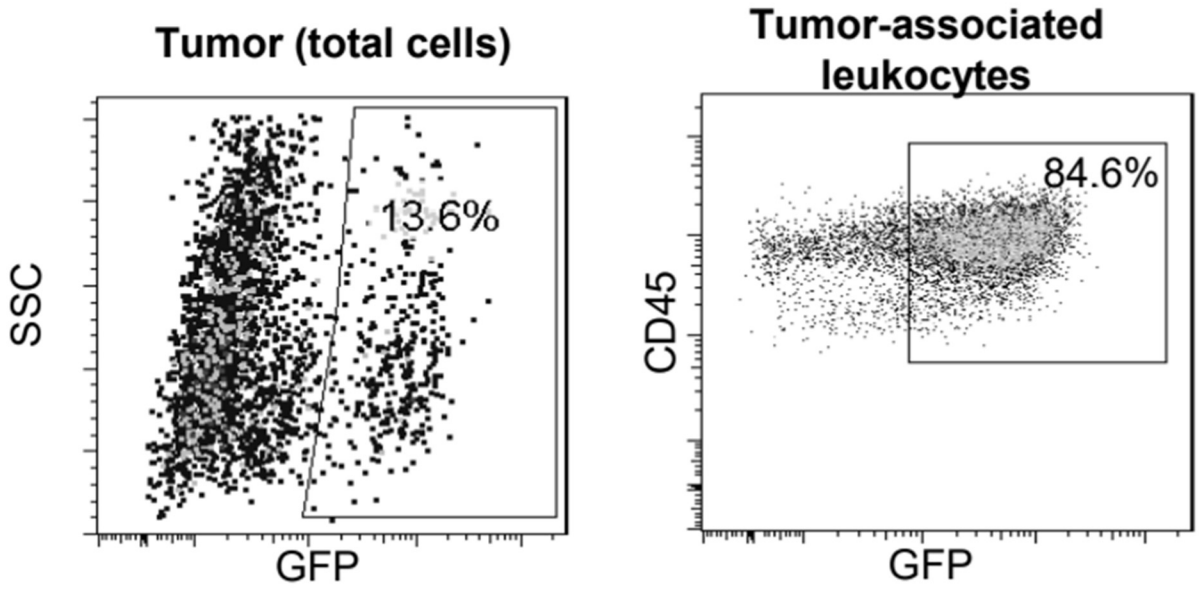


FIG. 78E

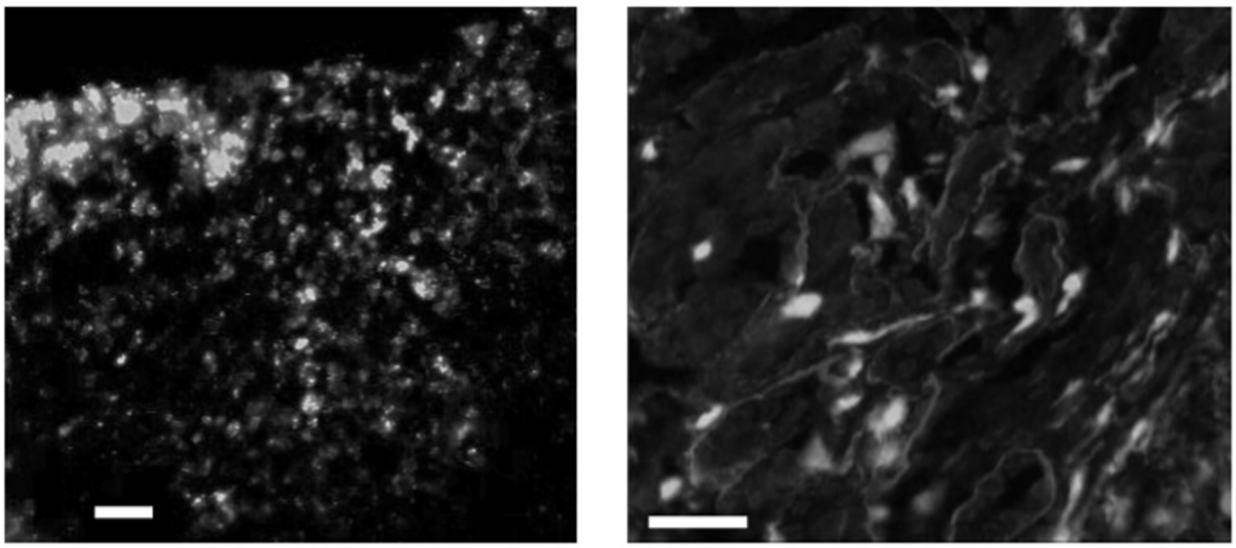


FIG. 78F

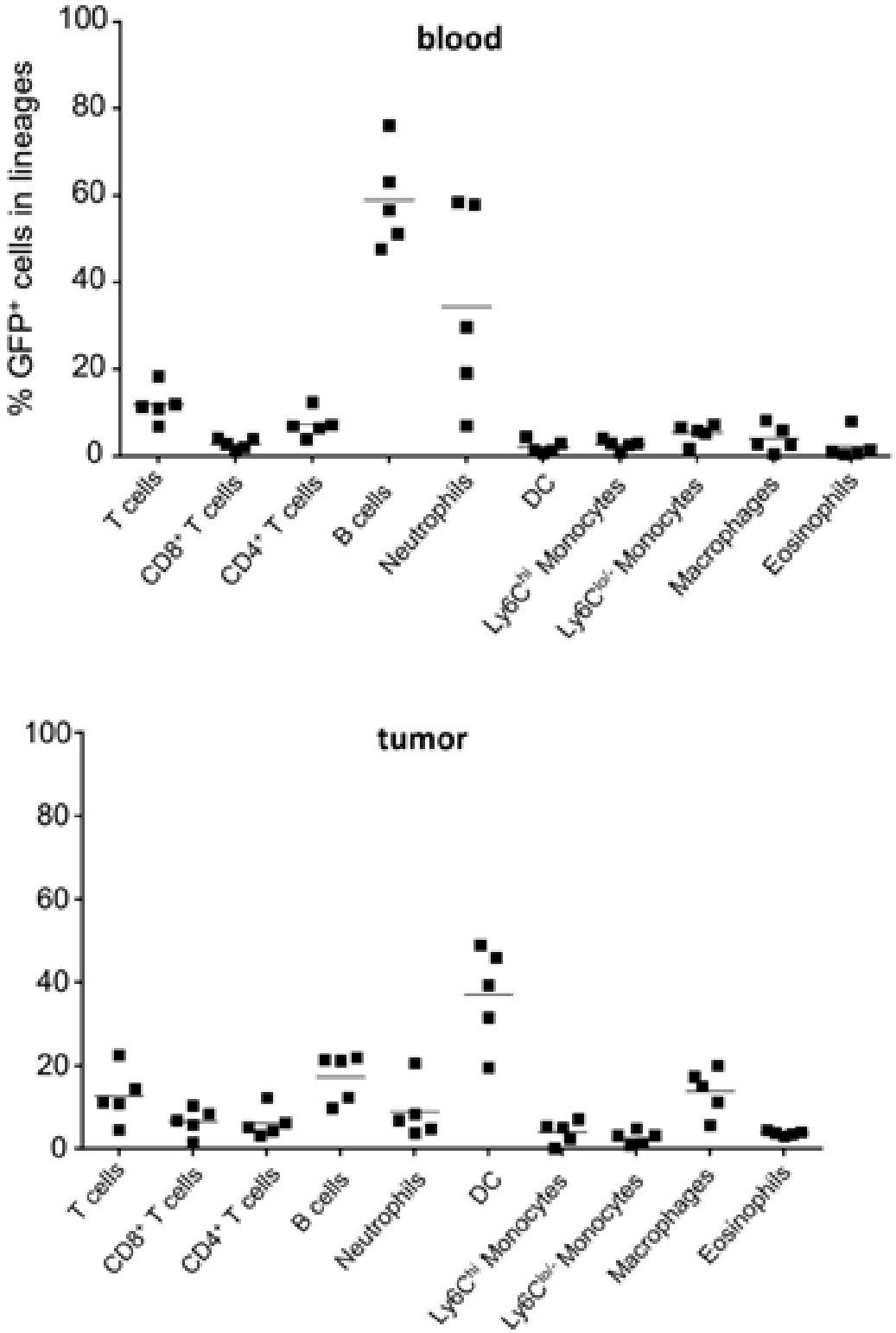

blood
% GFP+ cells in lineages
100
80
60
40
20
0
T cells
CD8+ T cells
CD4+ T cells
B cells
Neutrophils
DC
Ly6Chi Monocytes
Ly6Clo/- Monocytes
Macrophages
Eosinophils
tumor
100
80
60
40
20
0
T cells
CD8+ T cells
CD4+ T cells
B cells
Neutrophils
DC
Ly6Chi Monocytes
Ly6Clo/- Monocytes
Macrophages
Eosinophils

FIG. 79A

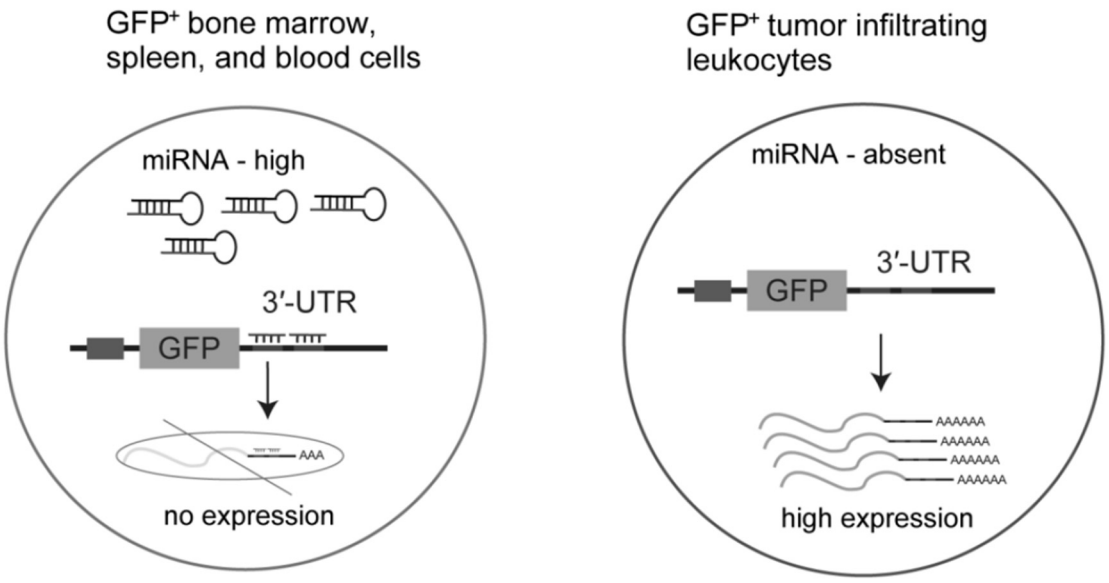


FIG. 79B

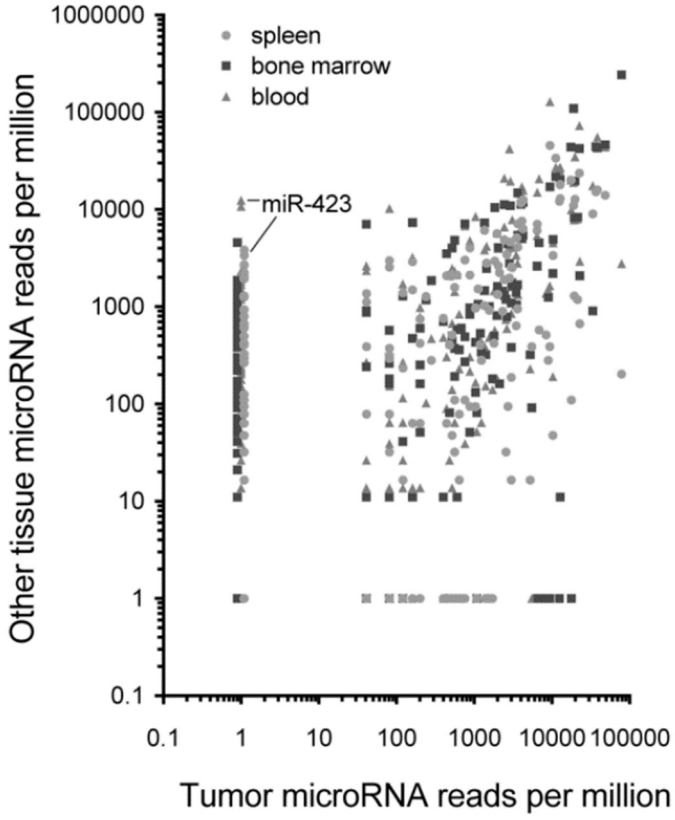


FIG. 79C

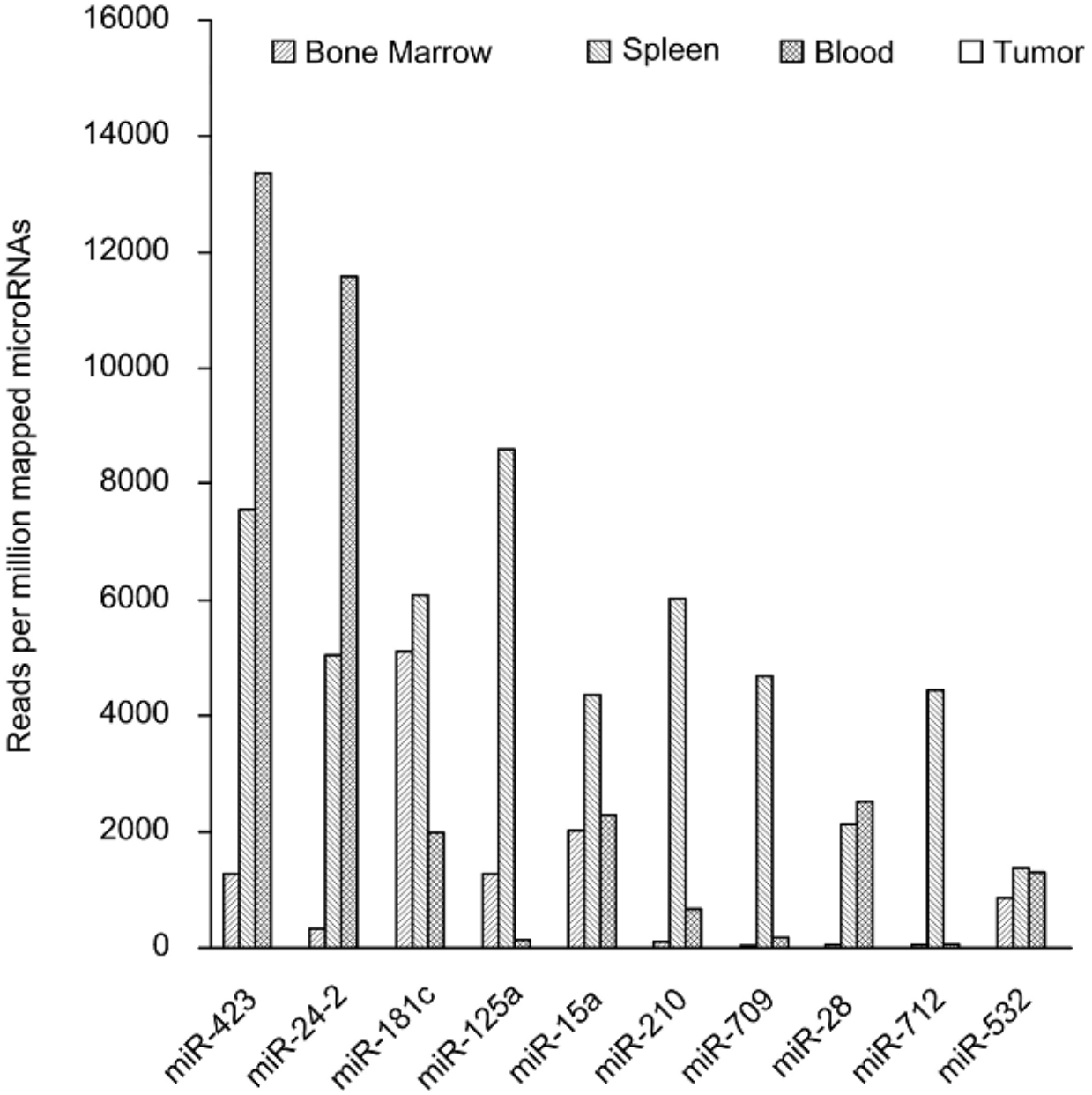

16000
14000
12000
10000
8000
6000
4000
2000
0
Reads per million mapped microRNAs
Bone Marrow
Spleen
Blood
Tumor
miR-423
miR-24-2
miR-181c
miR-125a
miR-15a
miR-210
miR-709
miR-28
miR-712
miR-532

FIG. 80A

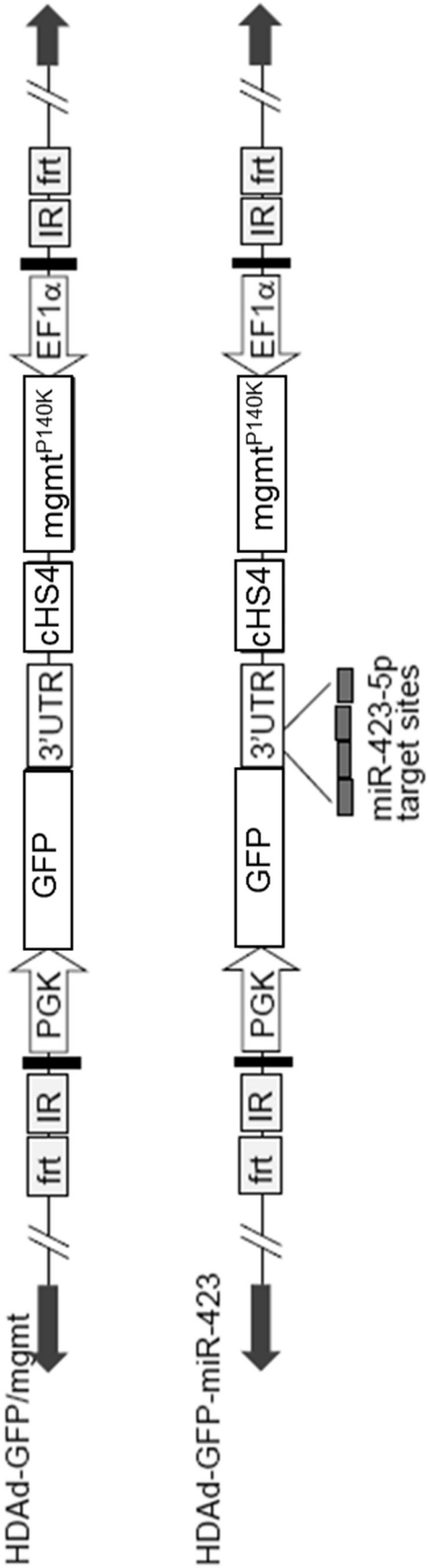

HDAd-GFP/mgmt
frt
IR
PGK
GFP
3'UTR
cHS4
mgmt^{P140K}
EF1α
IR
frt
HDAd-GFP-miR-423
frt
IR
PGK
GFP
3'UTR
cHS4
mgmt^{P140K}
EF1α
IR
frt
miR-423-5p
target sites

FIG. 80B

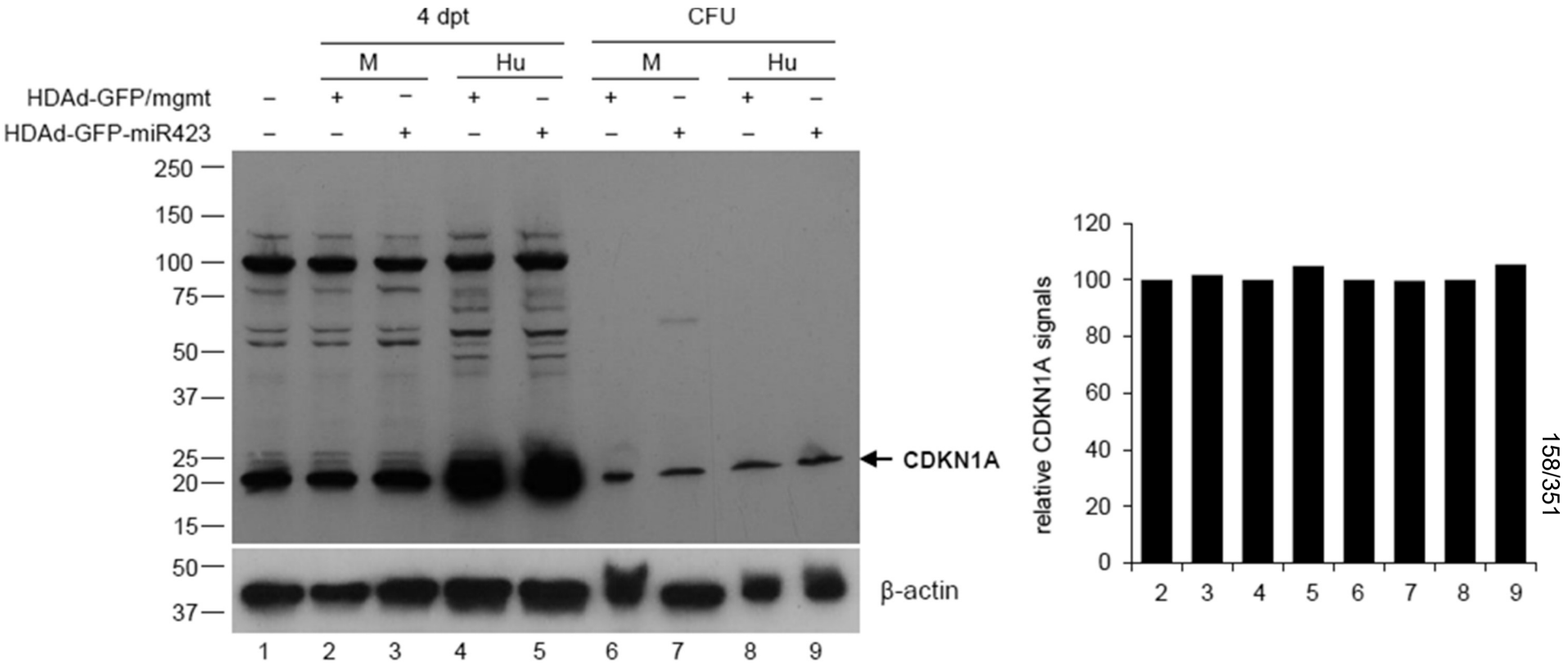

4 dpt
CFU
M
Hu
M
Hu
HDAd-GFP/mgmt
HDAd-GFP-miR423
250
150
100
75
50
37
25
20
15
50
37
CDKN1A
β-actin
1 2 3 4 5 6 7 8 9
relative CDKN1A signals
120
100
80
60
40
20
0
2 3 4 5 6 7 8 9
158/351

FIG. 80C

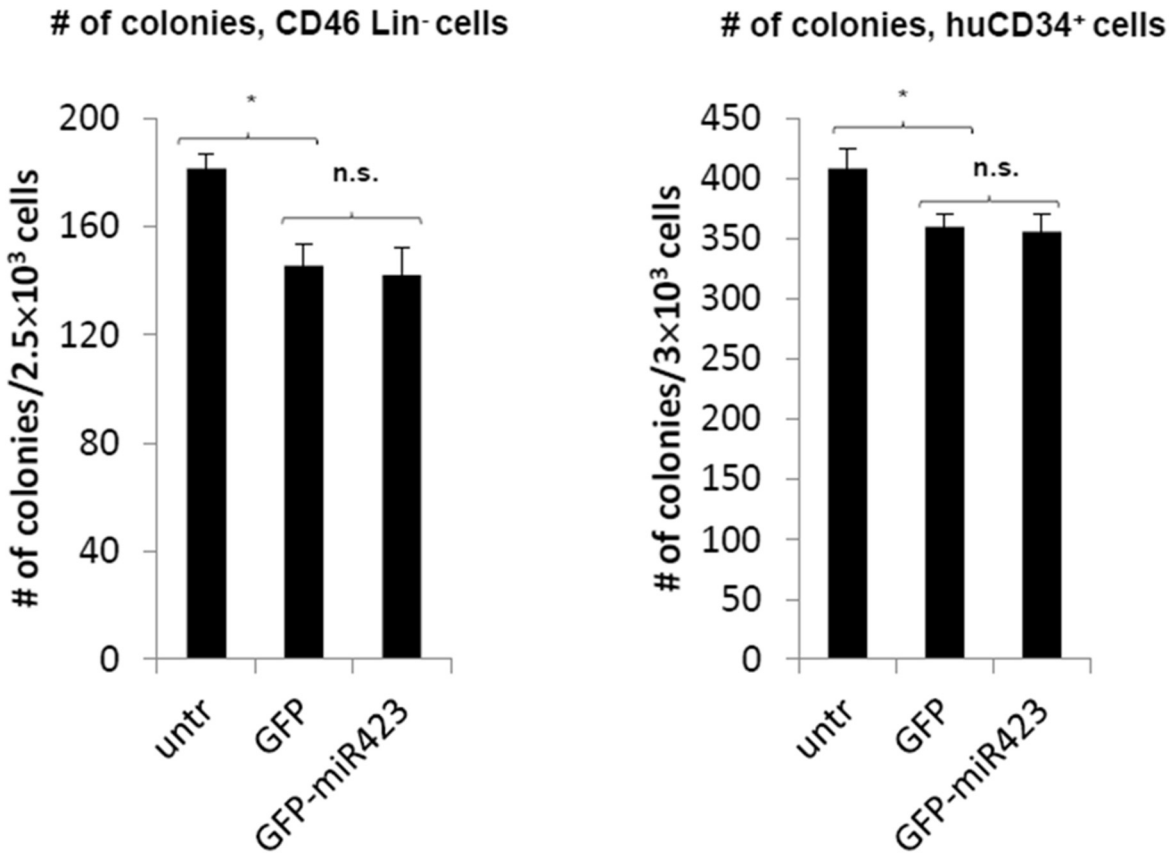
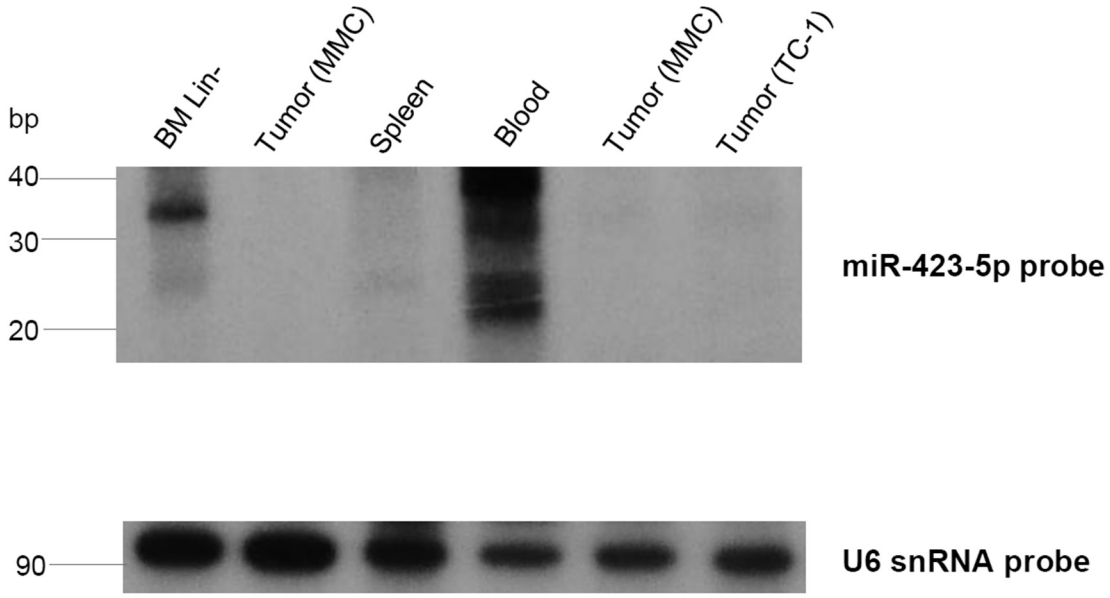


FIG. 81



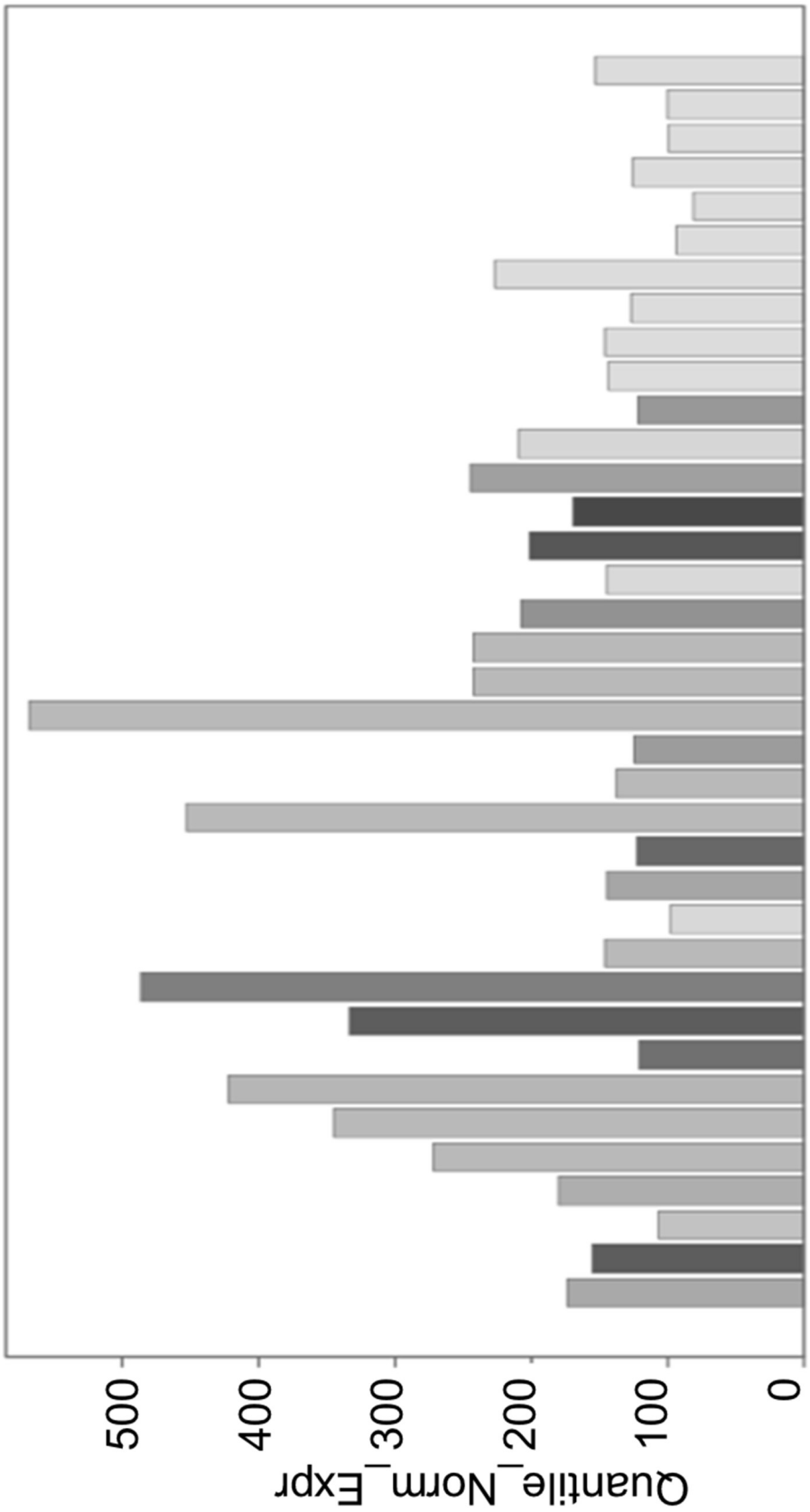


FIG. 82A

FIG. 82B

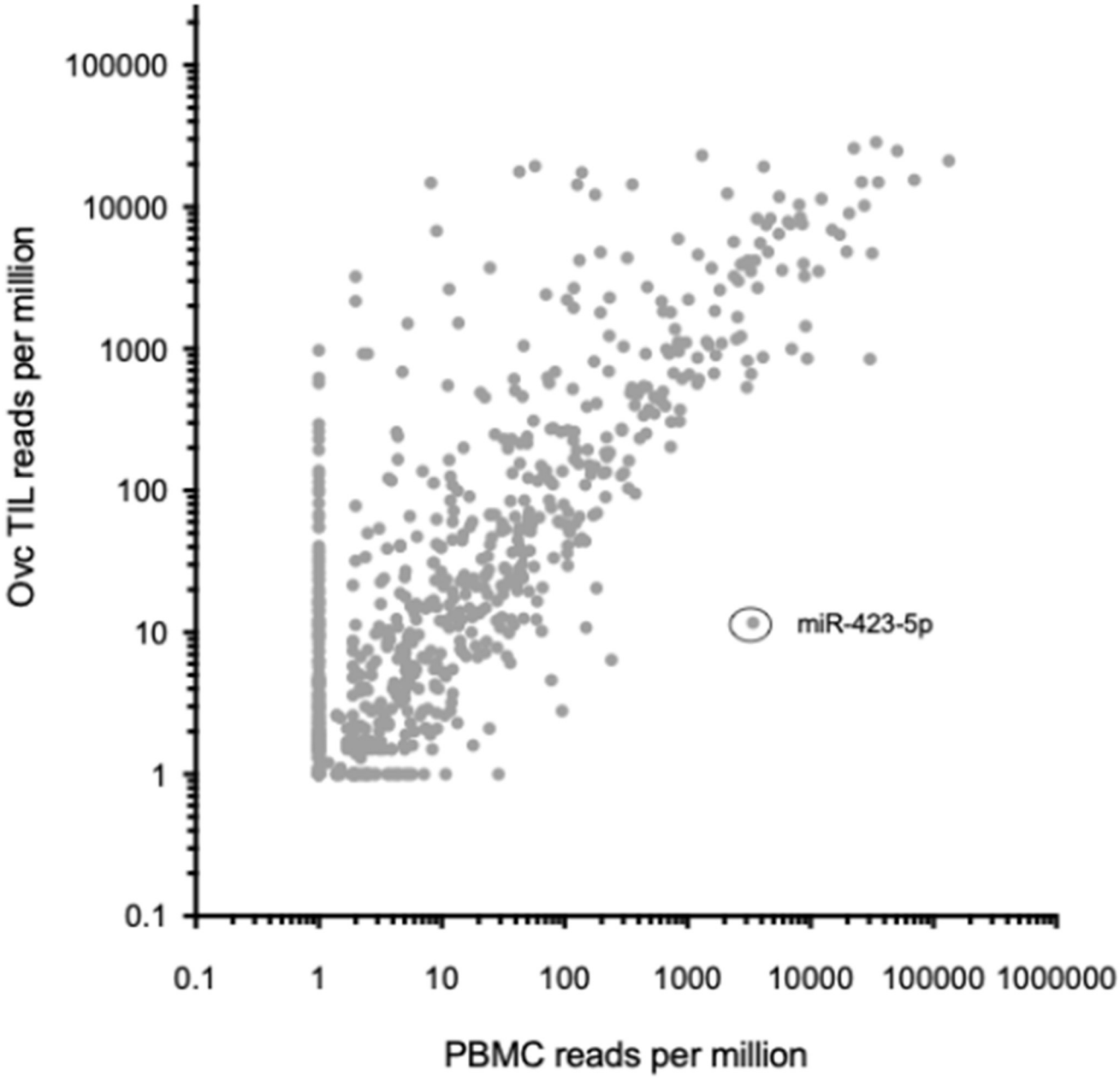

100000
10000
1000
100
10
1
0.1
Ovc TIL reads per million
0.1
1
10
100
1000
10000
100000
1000000
PBMC reads per million
miR-423-5p

FIG. 83A

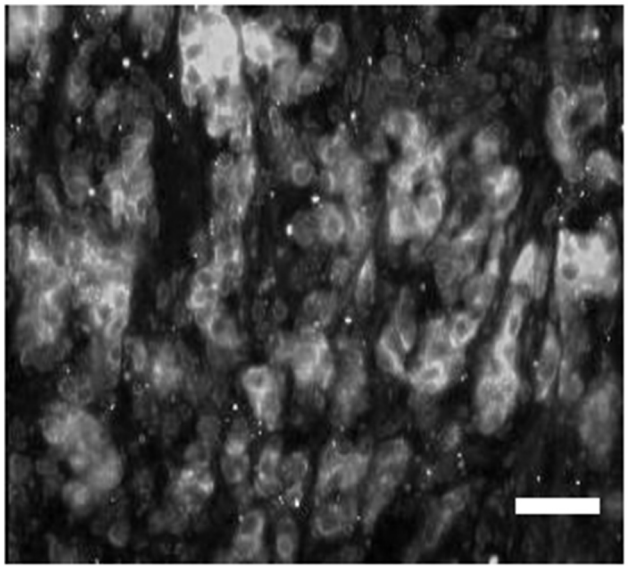


FIG. 83B

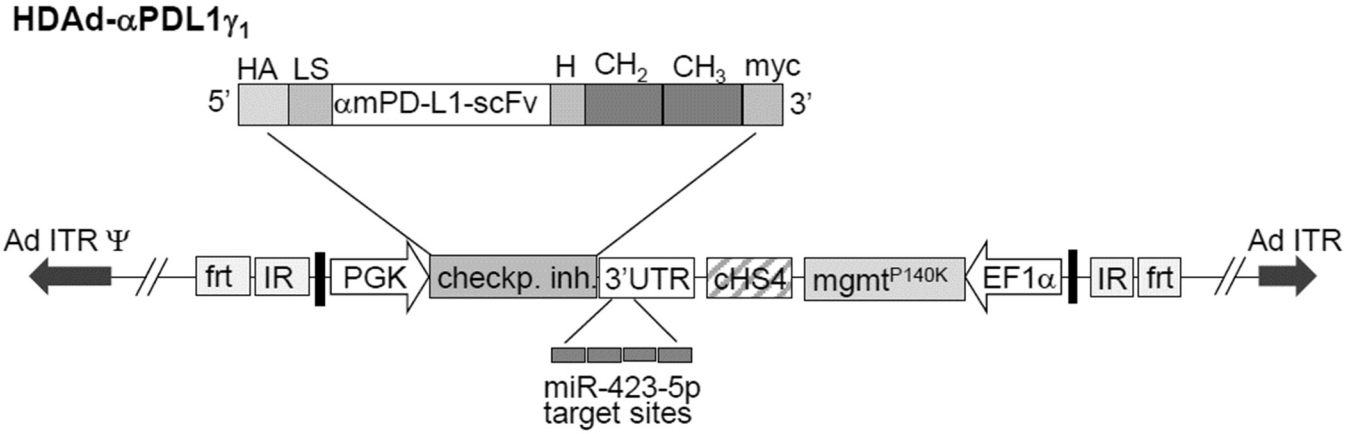


FIG. 83C

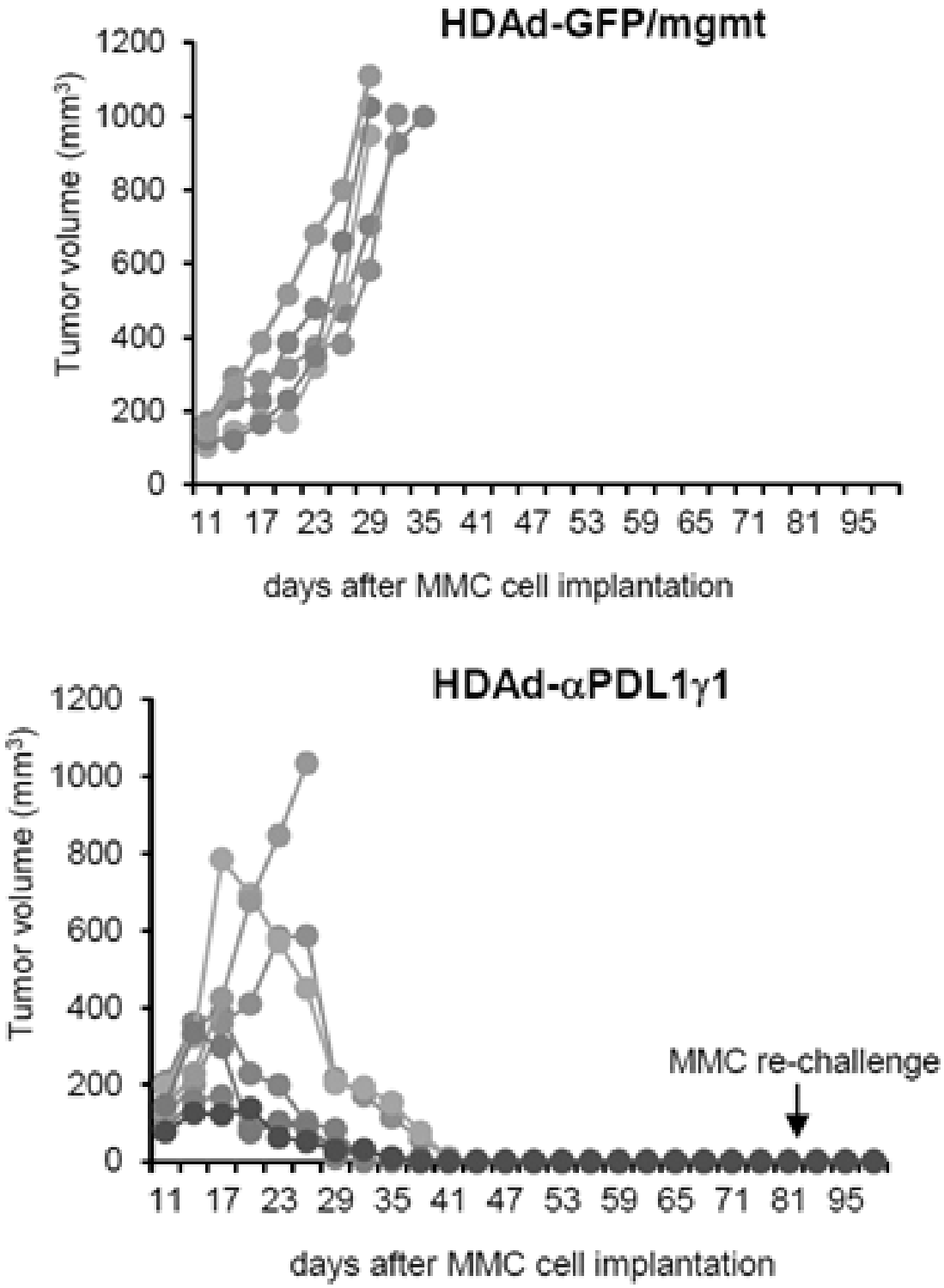

HDAd-GFP/mgmt
Tumor volume (mm3)
1200
1000
800
600
400
200
0
11 17 23 29 35 41 47 53 59 65 71 81 95
days after MMC cell implantation
HDAd-αPDL1γ1
Tumor volume (mm3)
1200
1000
800
600
400
200
0
MMC re-challenge
11 17 23 29 35 41 47 53 59 65 71 81 95
days after MMC cell implantation

FIG. 83D

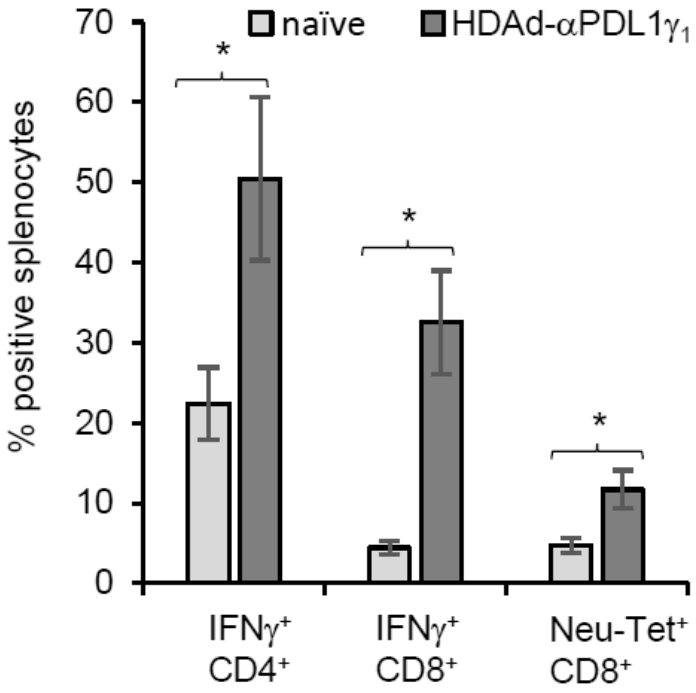


FIG. 83E

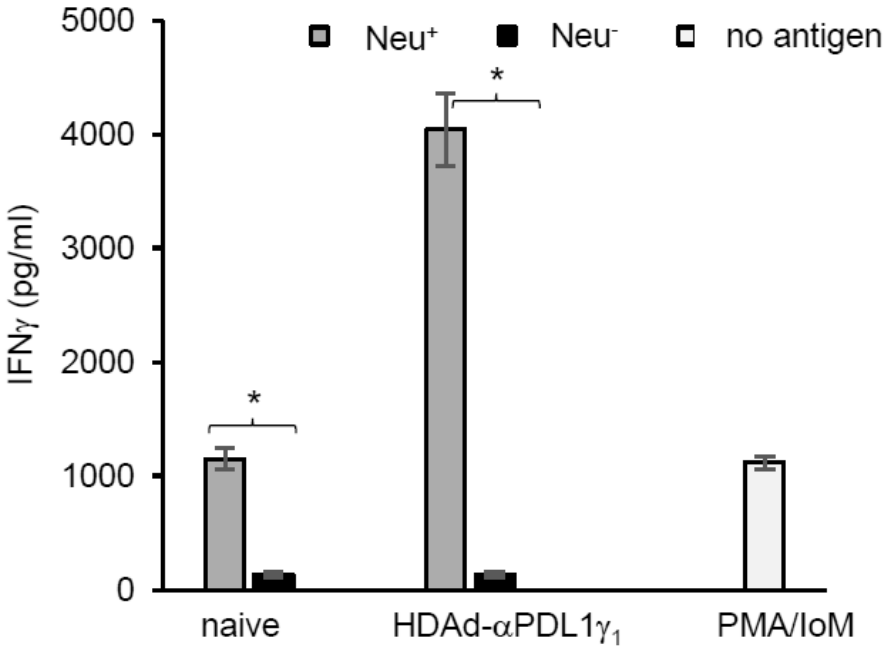
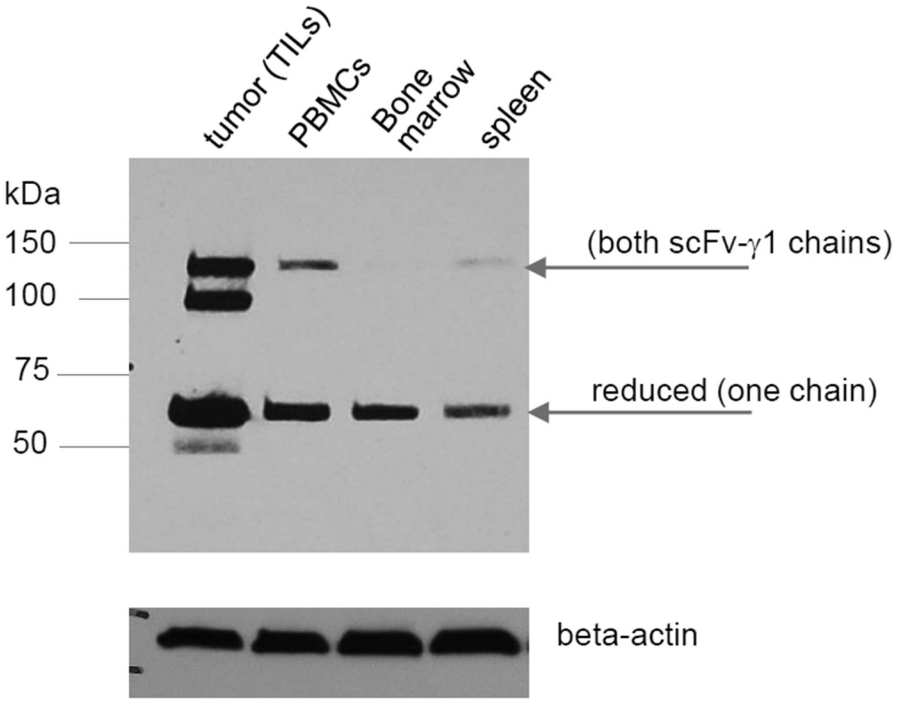


FIG. 84A


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

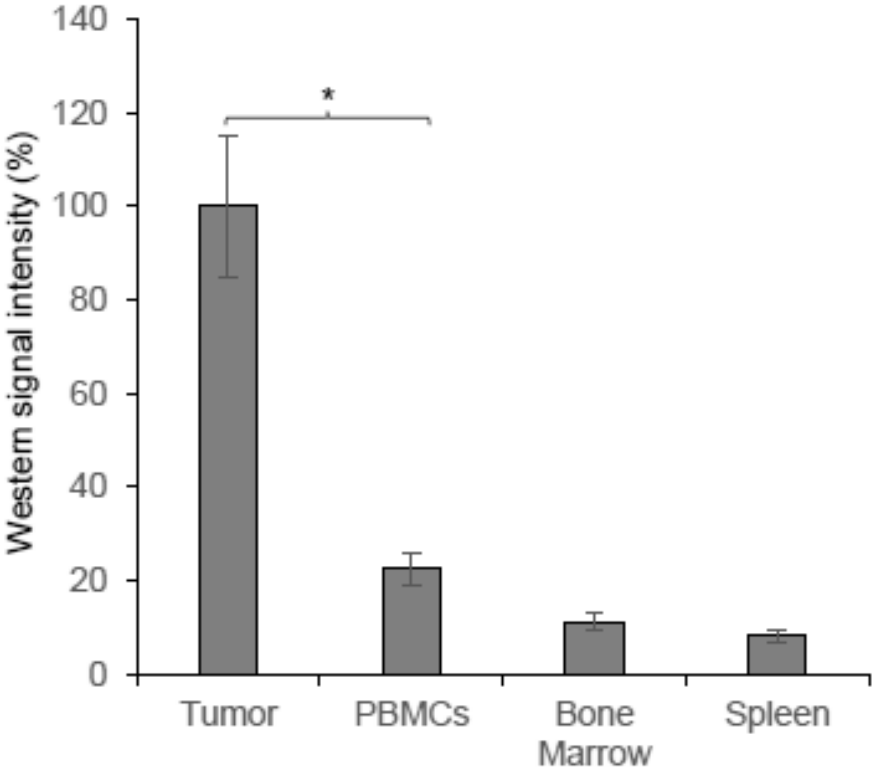

Western signal intensity (%)
140
120
100
80
60
40
20
0
*
Tumor
PBMCs
Bone Marrow
Spleen

FIG. 84B

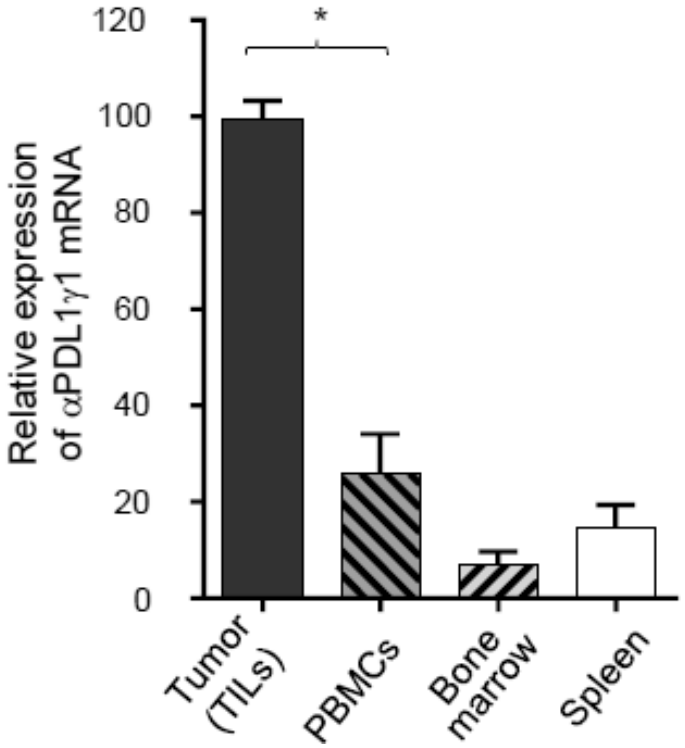


FIG. 84C

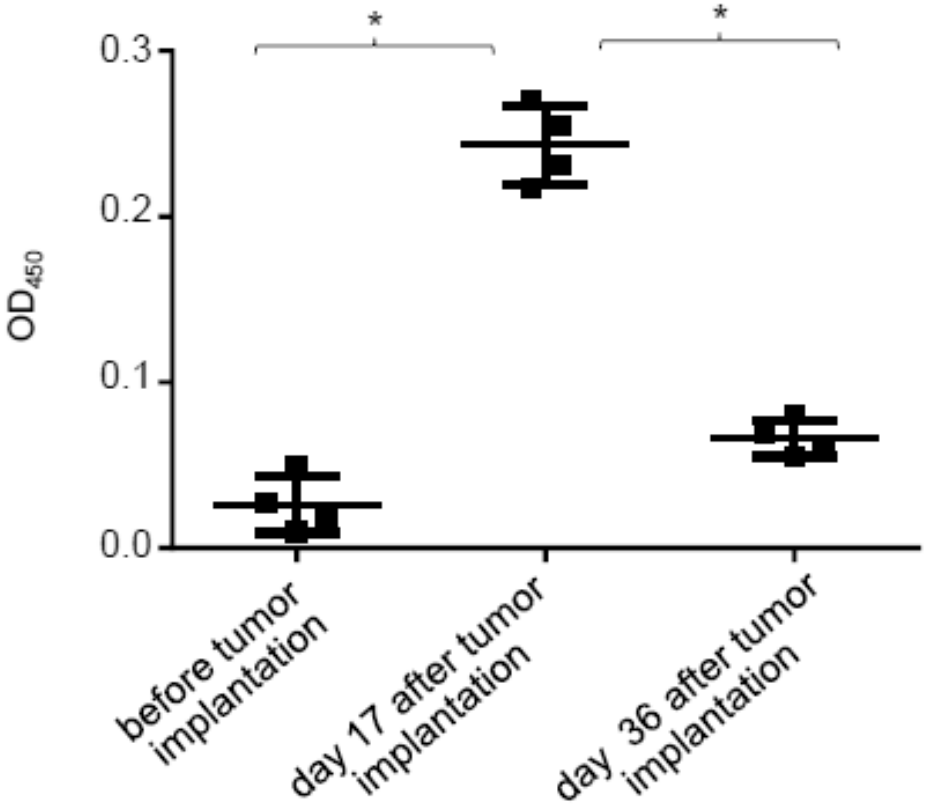


FIG. 85A

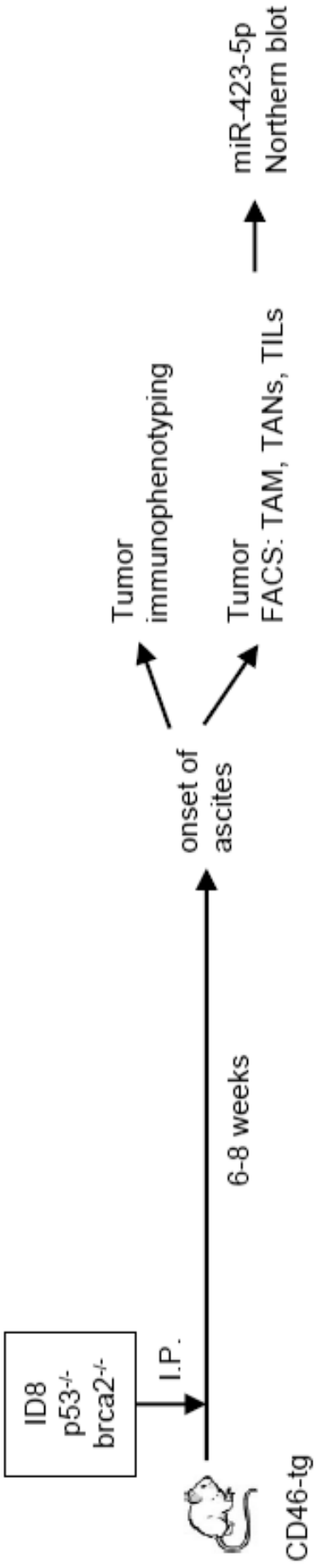

ID8
p53-/-
brca2-/-
I.P.
CD46-tg
6-8 weeks
onset of
ascites
Tumor
immunophenotyping
Tumor
FACS: TAM, TANs, TILs
miR-423-5p
Northern blot

FIG. 85B

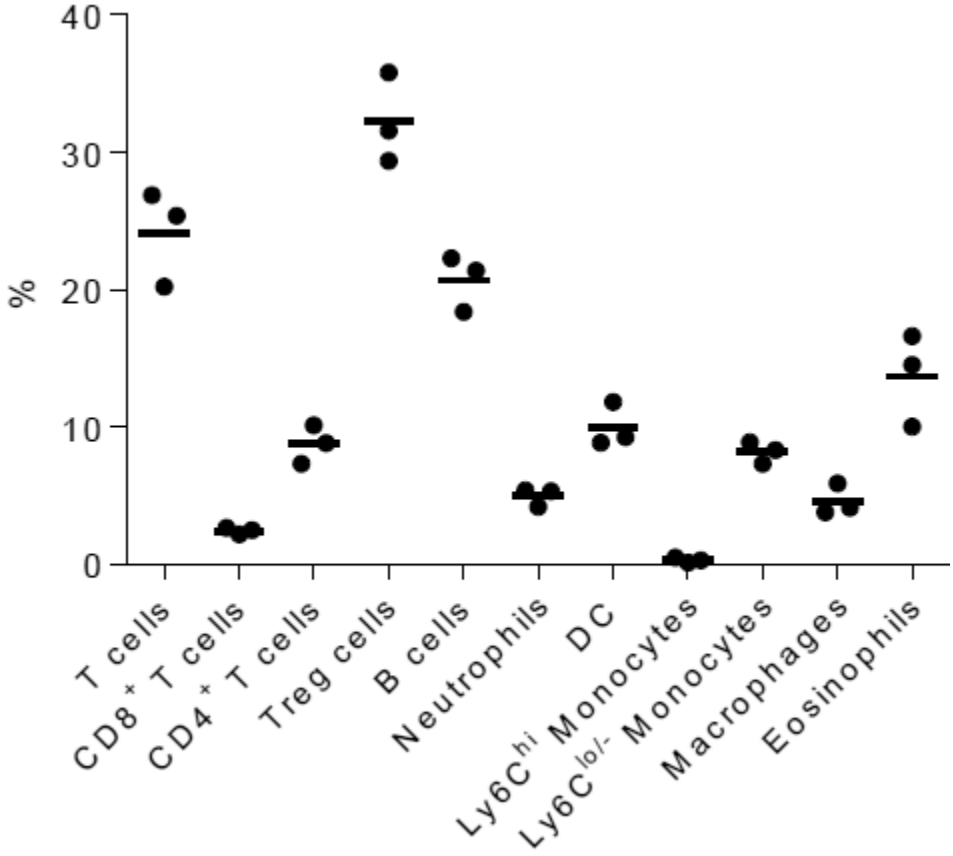

40
30
20
10
0
%
T cells
CD8+ T cells
CD4+ T cells
Treg cells
B cells
Neutrophils
DC
Ly6Chi Monocytes
Ly6Clo/- Monocytes
Macrophages
Eosinophils

FIG. 85C

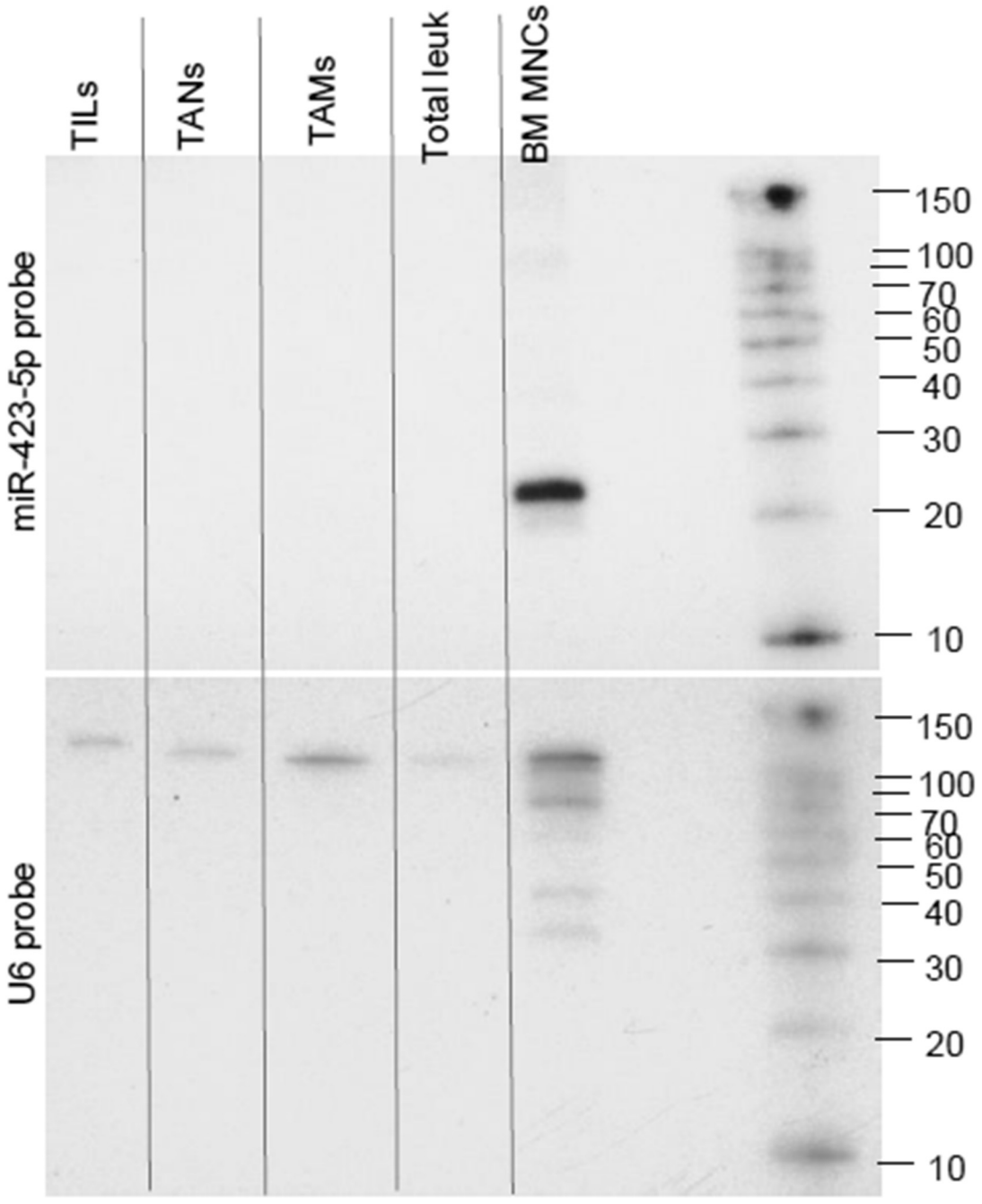

TILs
TANs
TAMs
Total leuk
BM MNCs
miR-423-5p probe
U6 probe
150
100
70
60
50
40
30
20
10

FIG. 85D

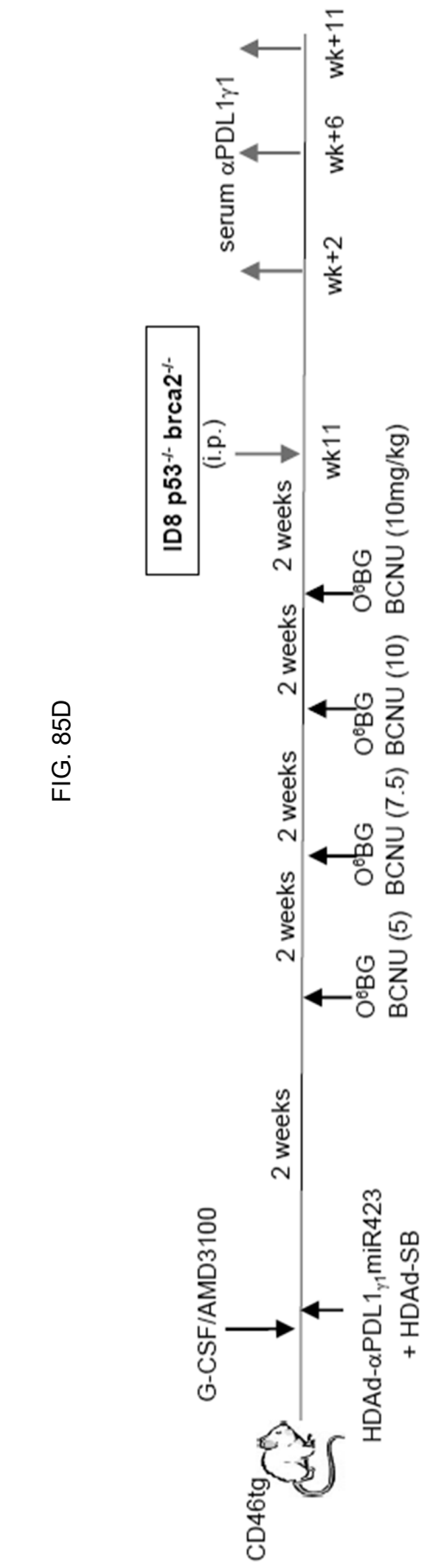


FIG. 85E

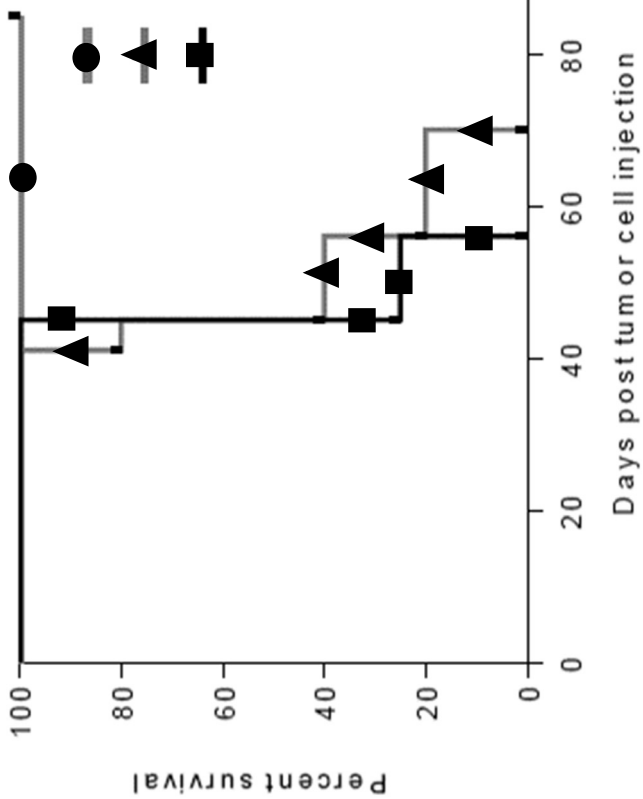


FIG. 85F

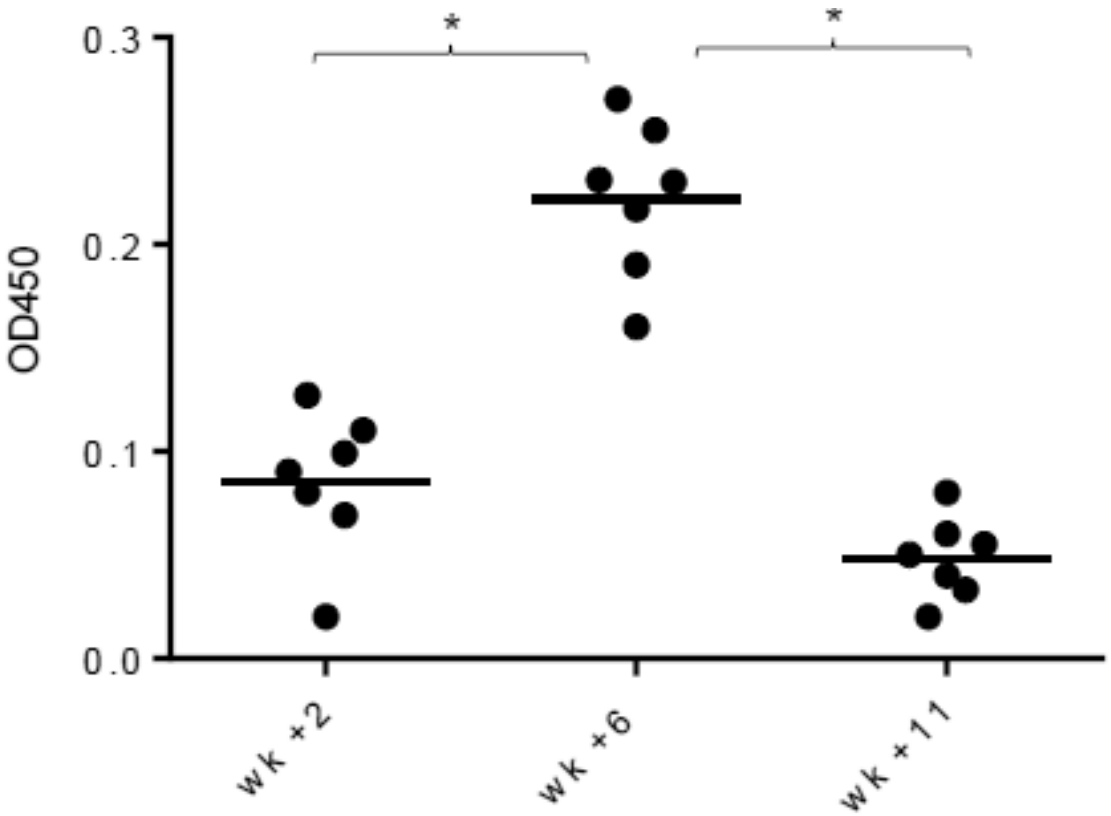


FIG. 86A

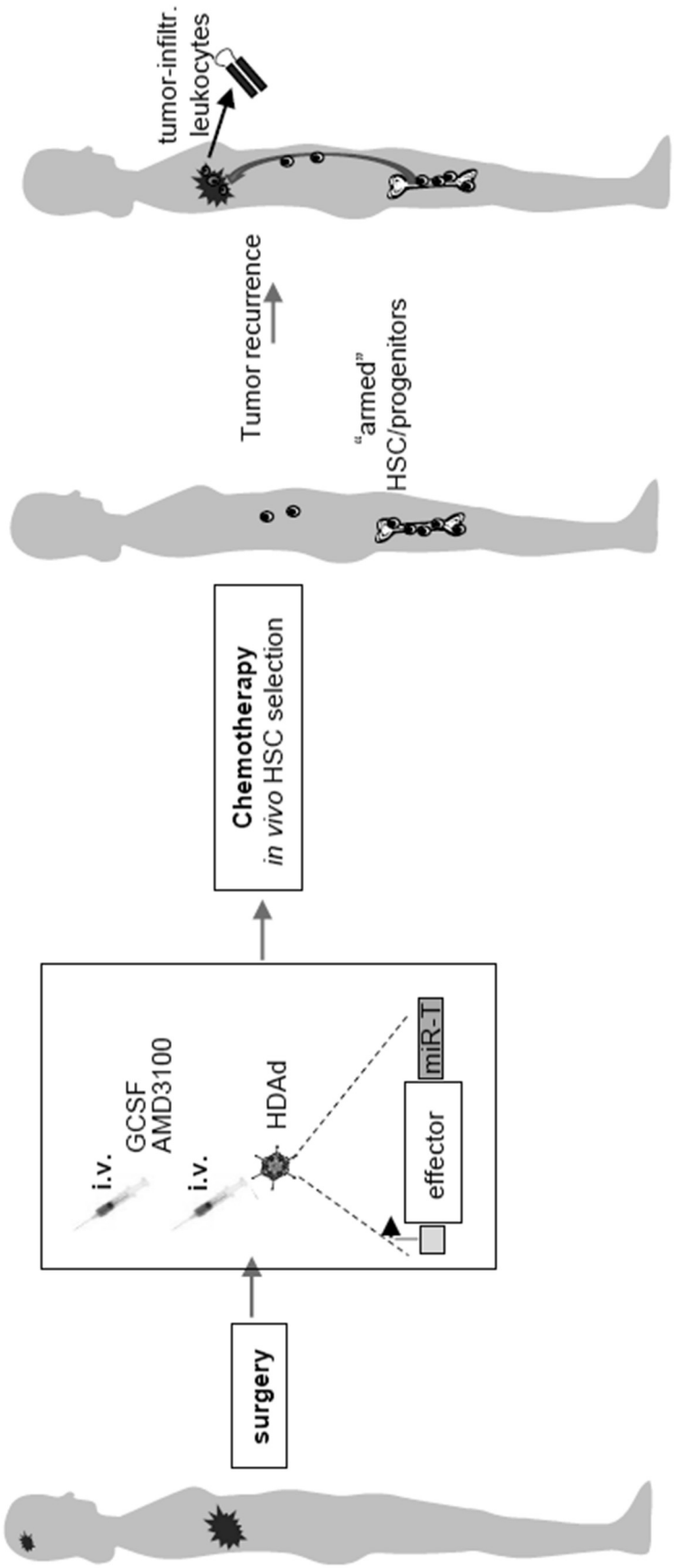

surgery
i.v.
GCSF
AMD3100
i.v.
HDAd
effector
miR-T
Chemotherapy
in vivo HSC selection
"armed"
HSC/progenitors
Tumor recurrence
tumor-infiltr.
leukocytes

FIG. 86B

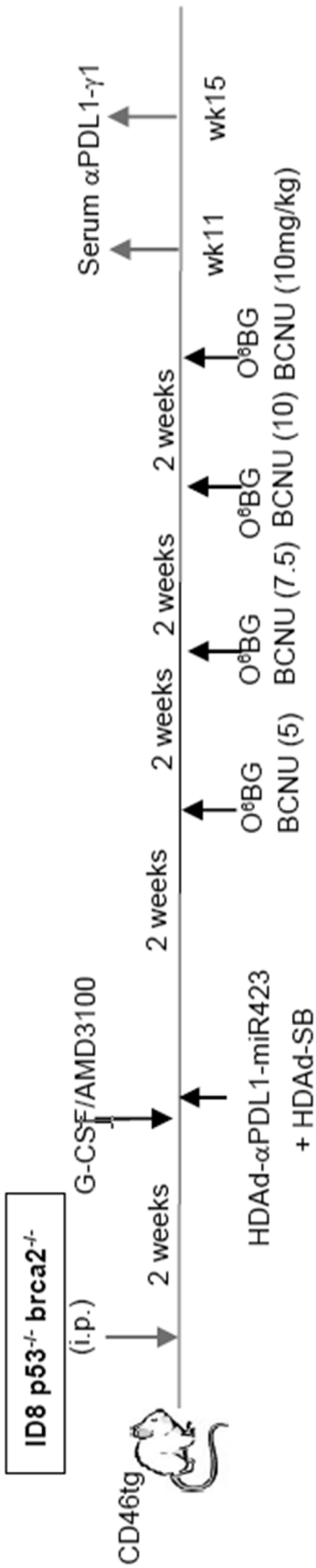

ID8 p53-/- brca2-/-
(i.p.)
CD46tg
2 weeks
G-CSF/AMD3100
2 weeks
HDAd-αPDL1-miR423
+ HDAd-SB
2 weeks
O6BG
BCNU (5)
2 weeks
O6BG
BCNU (7.5)
2 weeks
O6BG
BCNU (10)
2 weeks
O6BG
BCNU (10mg/kg)
wk11
Serum αPDL1-γ1
wk15

FIG. 86C

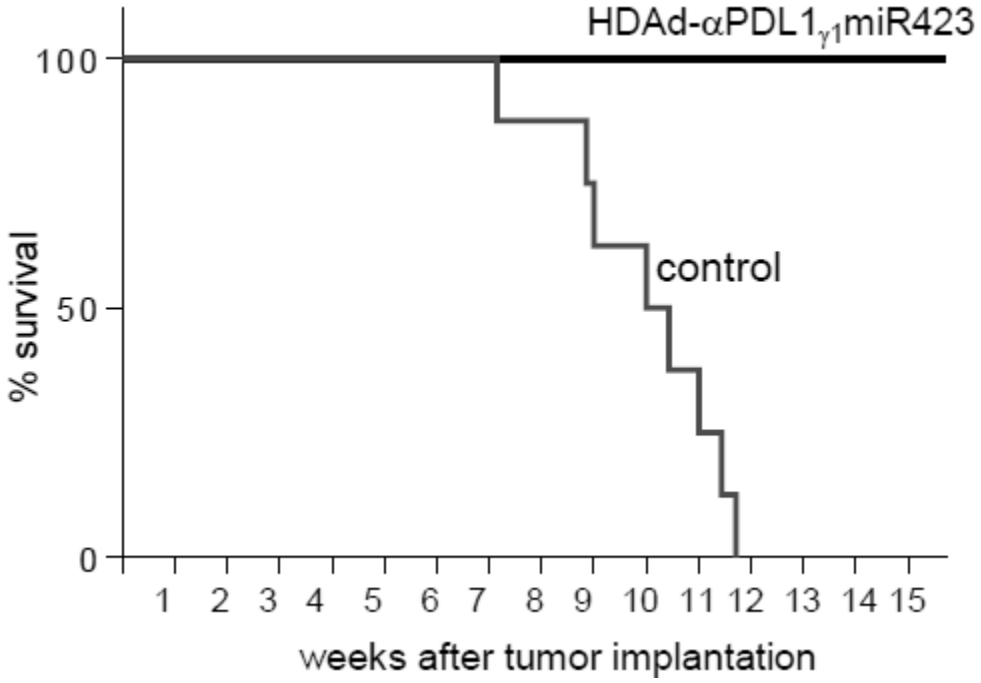


FIG. 86D

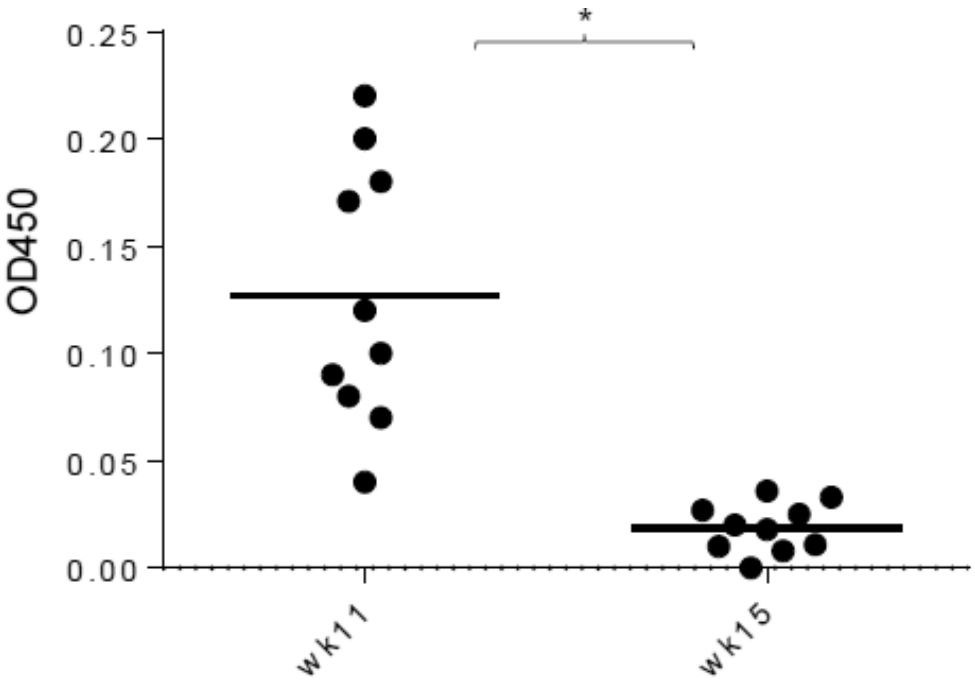


FIG. 87A

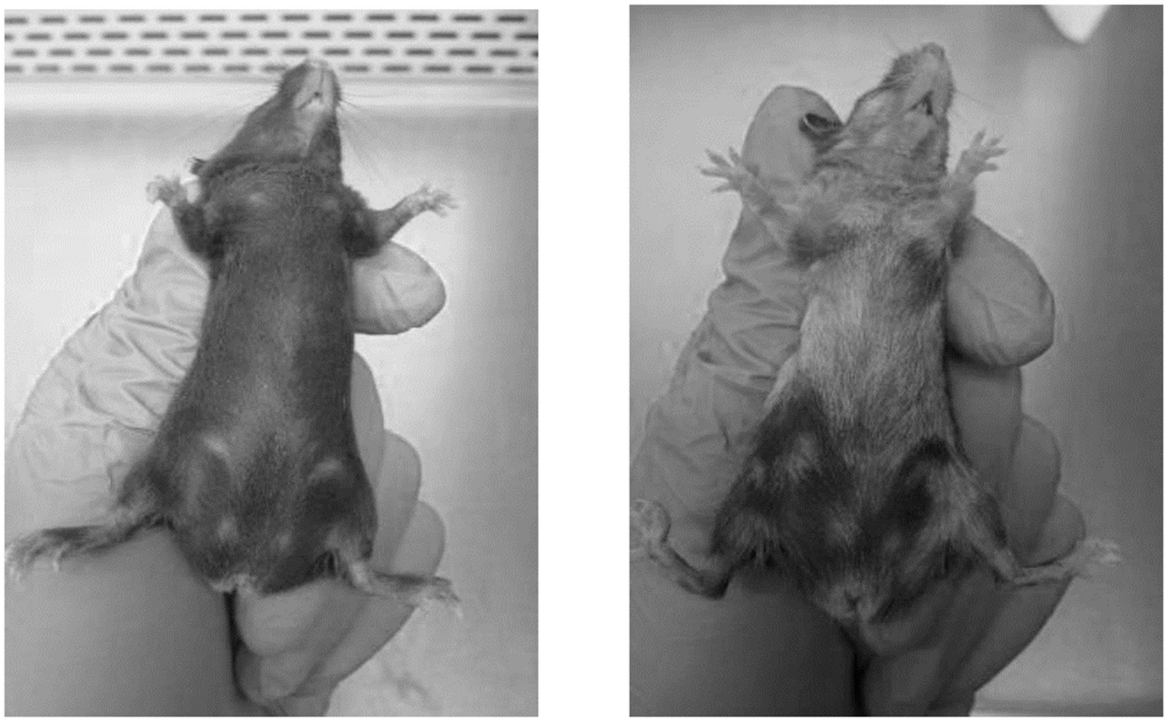


FIG. 87B

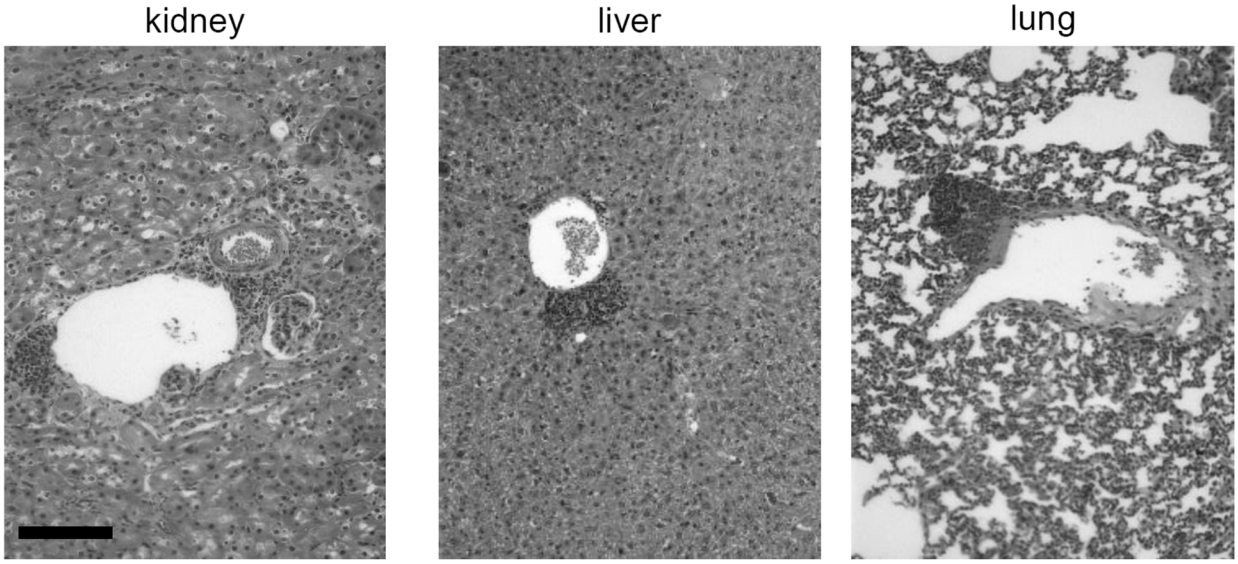


FIG. 88A

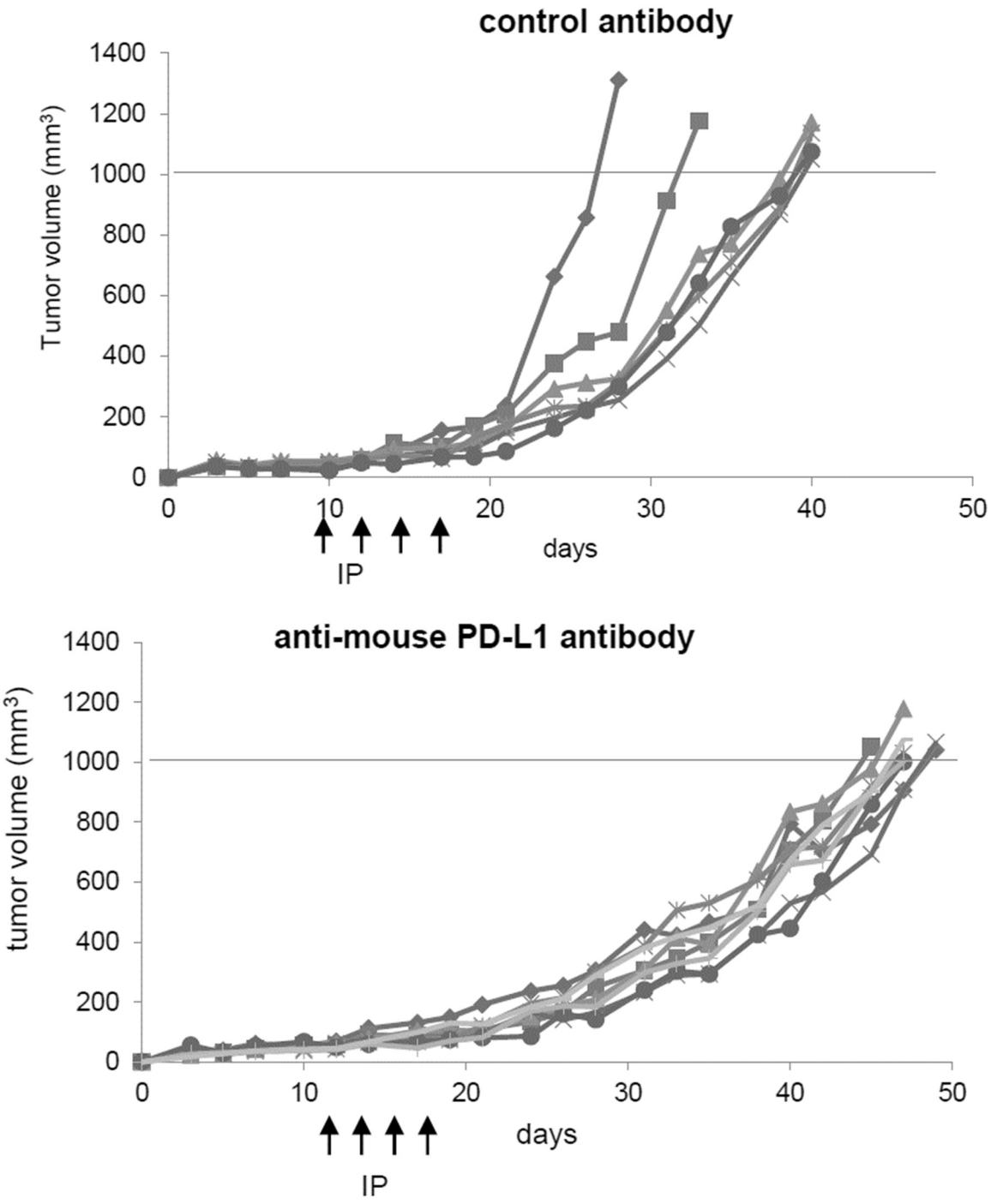

control antibody
Tumor volume (mm³)
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
days
IP
anti-mouse PD-L1 antibody
tumor volume (mm³)
1400
1200
1000
800
600
400
200
0
0
10
20
30
40
50
days
IP

FIG. 88B

FIG. 88B

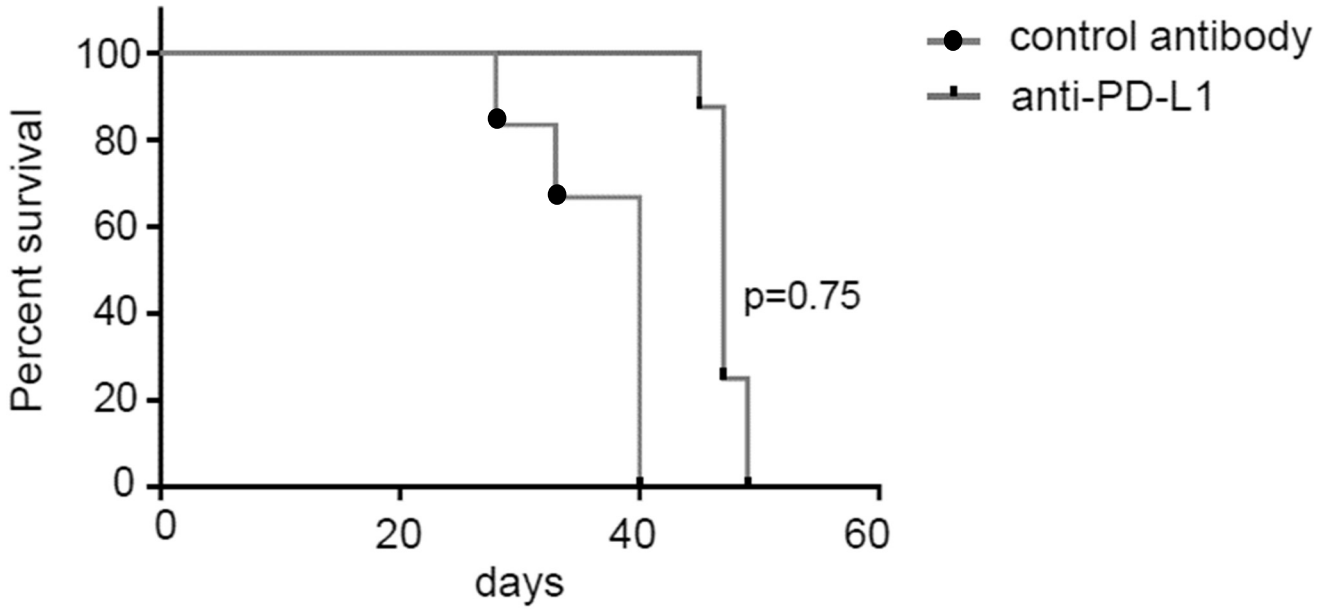


FIG. 88C

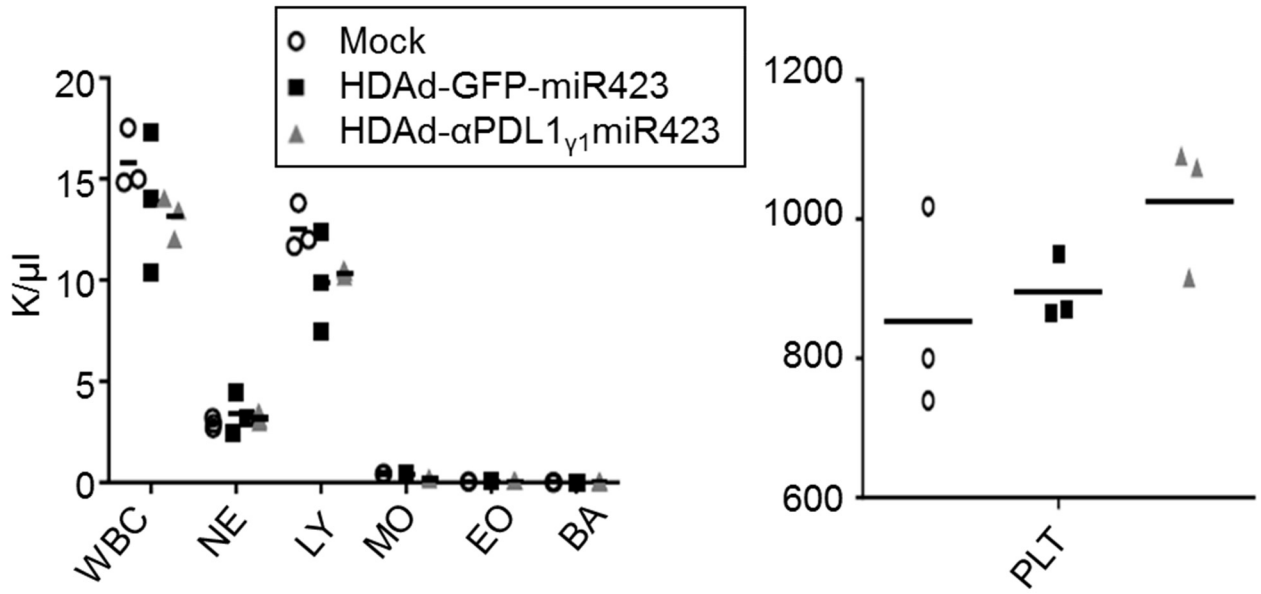


FIG. 88D

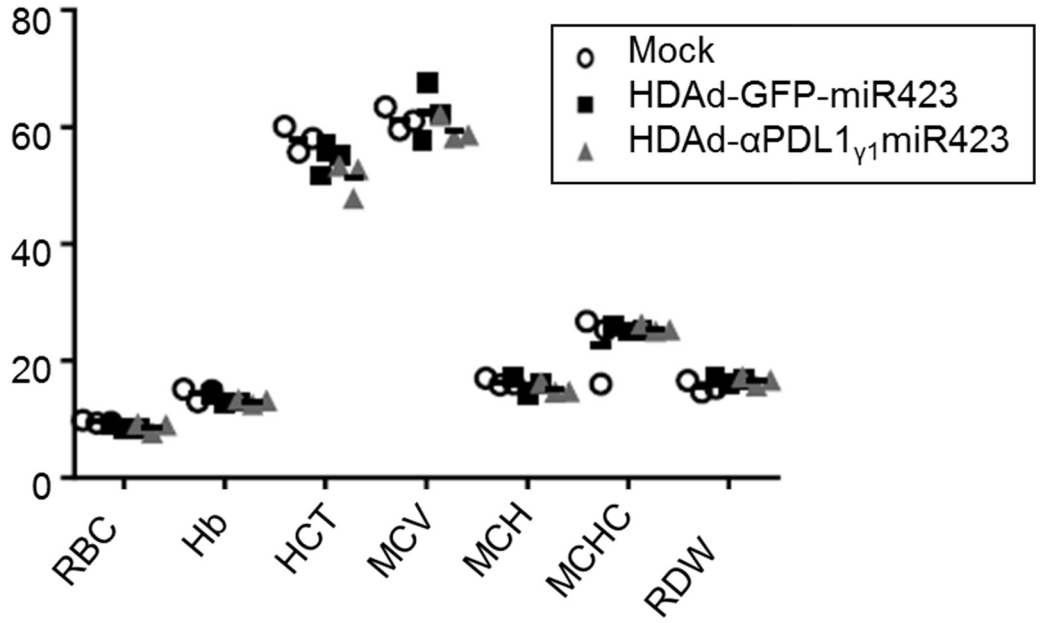


FIG. 88E

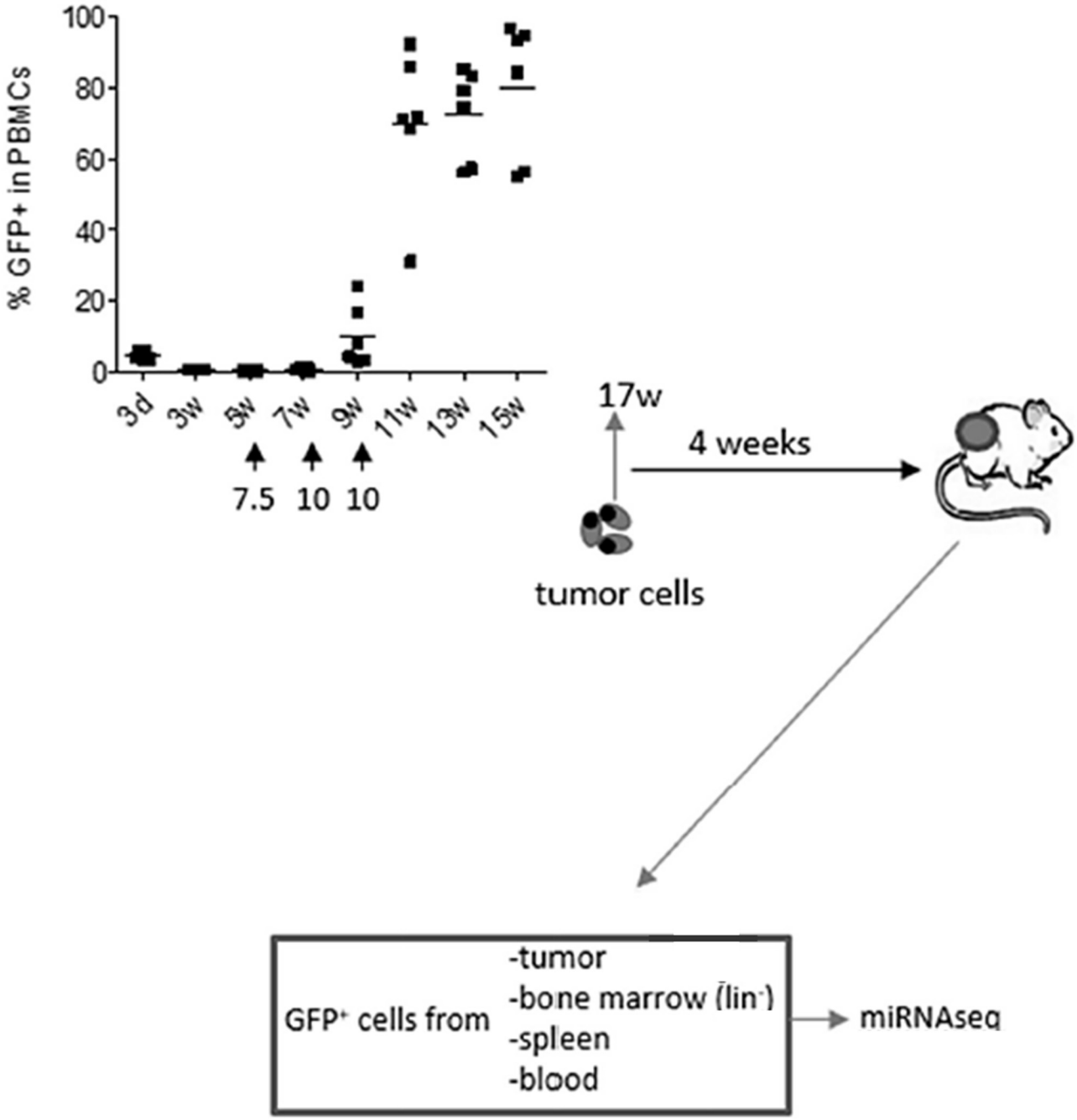
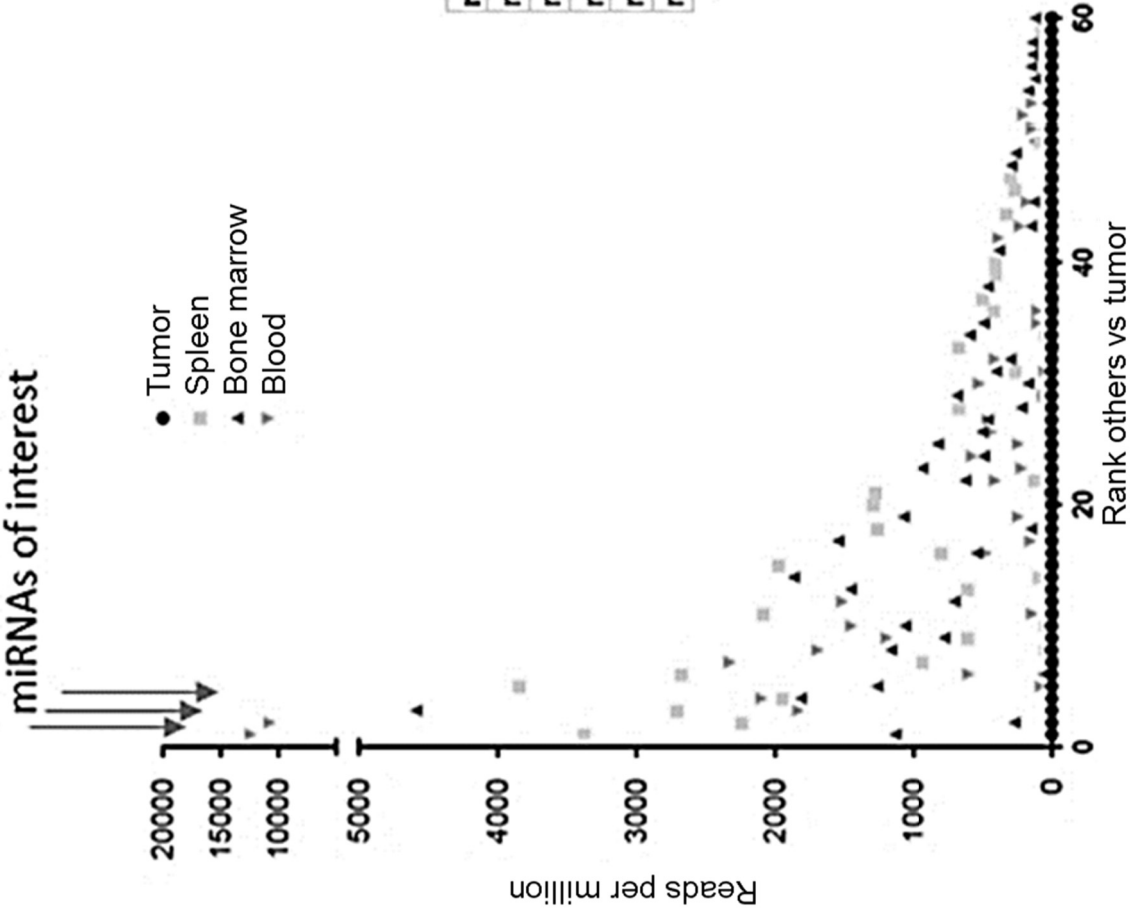

% GFP+ in PBMCs
100
80
60
40
20
0
3d
3w
5w
7w
9w
11w
13w
15w
7.5
10
10
17w
4 weeks
tumor cells
GFP+ cells from
-tumor
-bone marrow (lin-)
-spleen
-blood
miRNAseq

FIG. 88E cont'd



miRNA	Tumor	Spleen	BM	Blood
mir-423	100	7563.82	1263.47	13350.56
mir-24-2	100	5019.65	302.65	11586.12
mir-181c	100	6065.20	5106.75	1970.61
mir-125a	100	8609.37	1408.71	83.07
mir-15a	100	4357.46	2023.19	2257.84

FIG. 88F

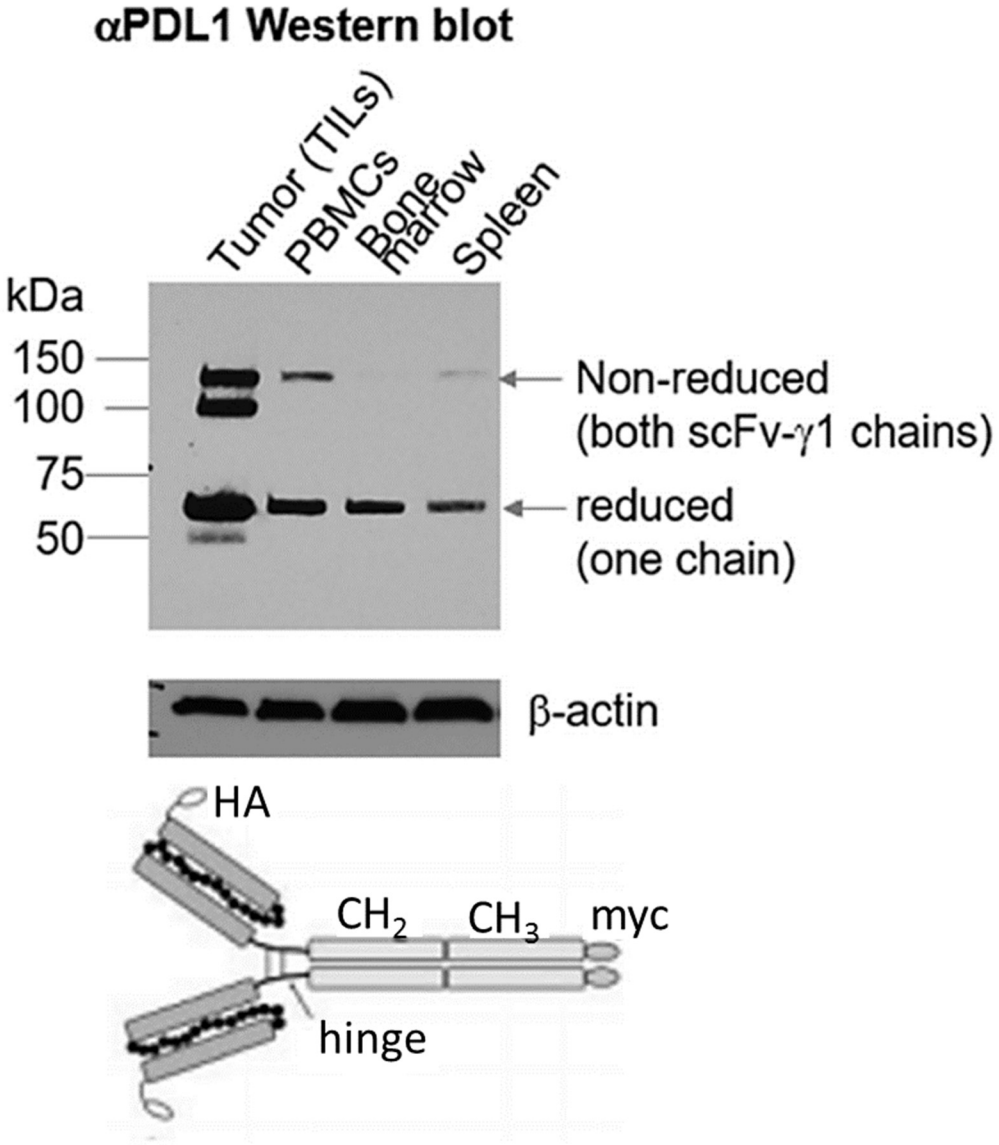
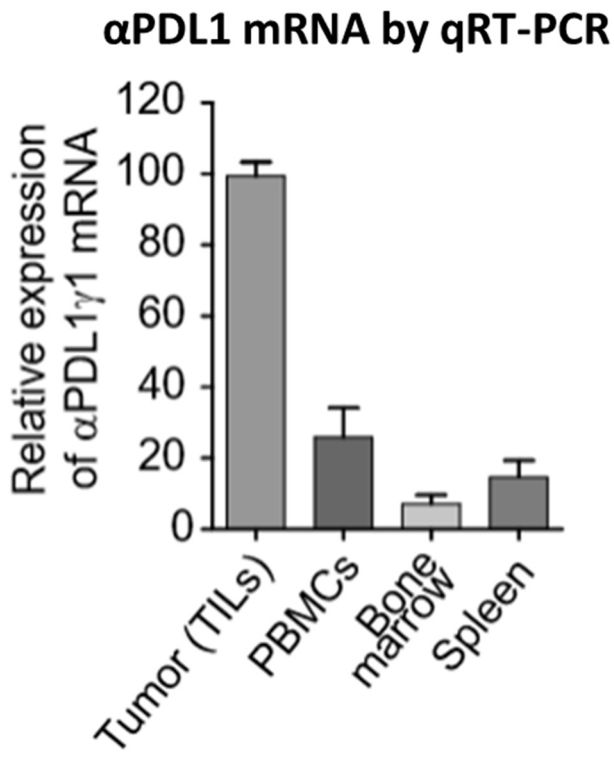

αPDL1 Western blot
Tumor (TILs)
PBMCs
Bone marrow
Spleen
kDa
150
100
75
50
Non-reduced
(both scFv-γ1 chains)
reduced
(one chain)
β-actin
HA
CH2
CH3
myc
hinge

FIG. 88F cont'd



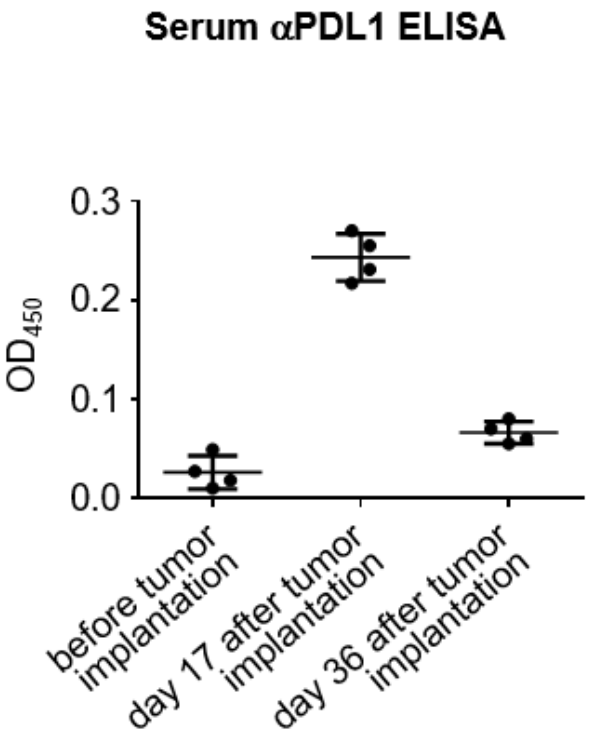


FIG. 88G

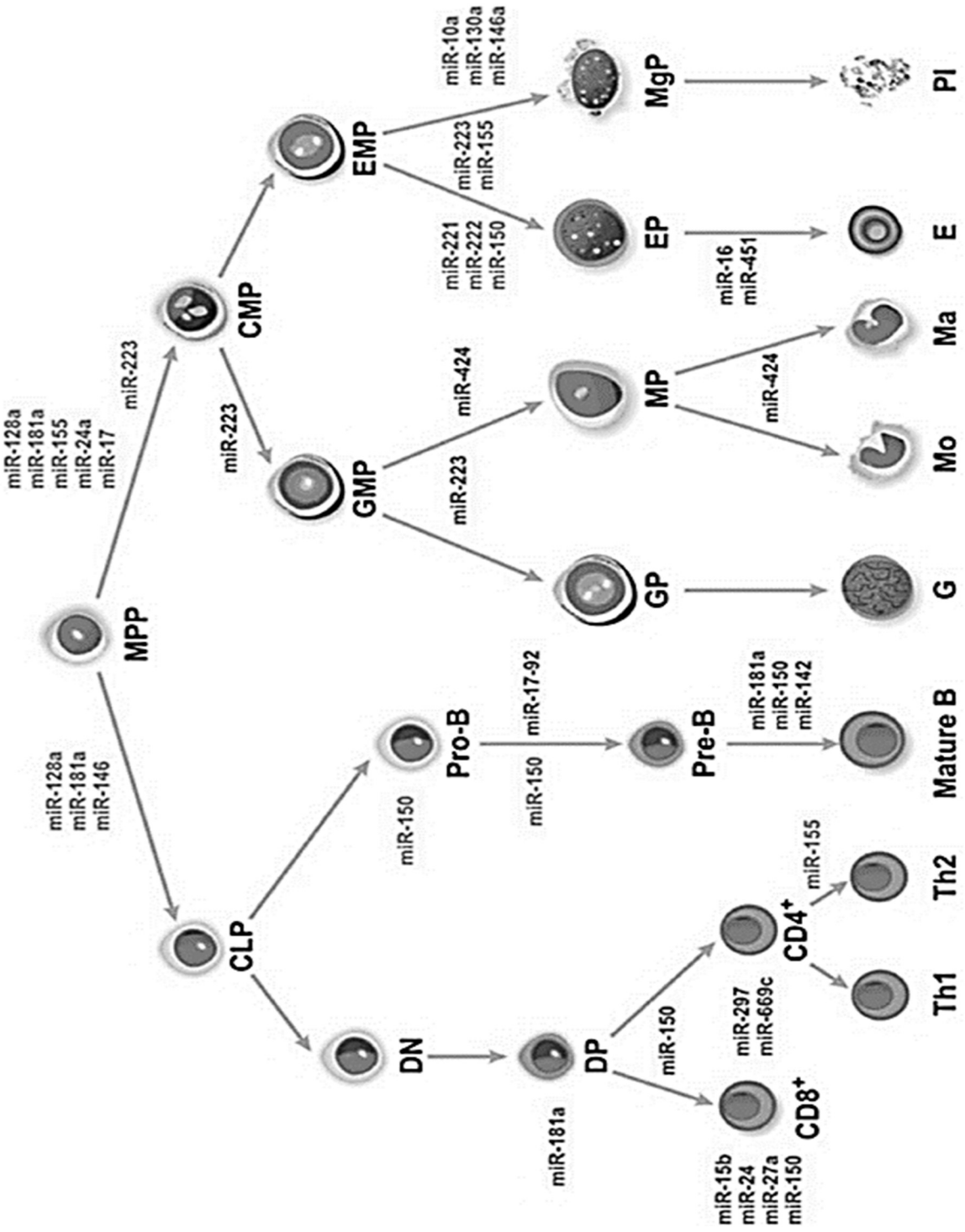

MPP
CLP
CMP
GMP
EMP
DN
DP
CD4+
CD8+
Th1
Th2
Pro-B
Pre-B
Mature B
GP
MP
EP
MgP
G
Mo
Ma
E
Pl
miR-128a
miR-181a
miR-155
miR-24a
miR-17
miR-223
miR-128a
miR-181a
miR-146
miR-223
miR-223
miR-424
miR-223
miR-424
miR-10a
miR-130a
miR-146a
miR-223
miR-155
miR-221
miR-222
miR-150
miR-16
miR-451
miR-150
miR-17-92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

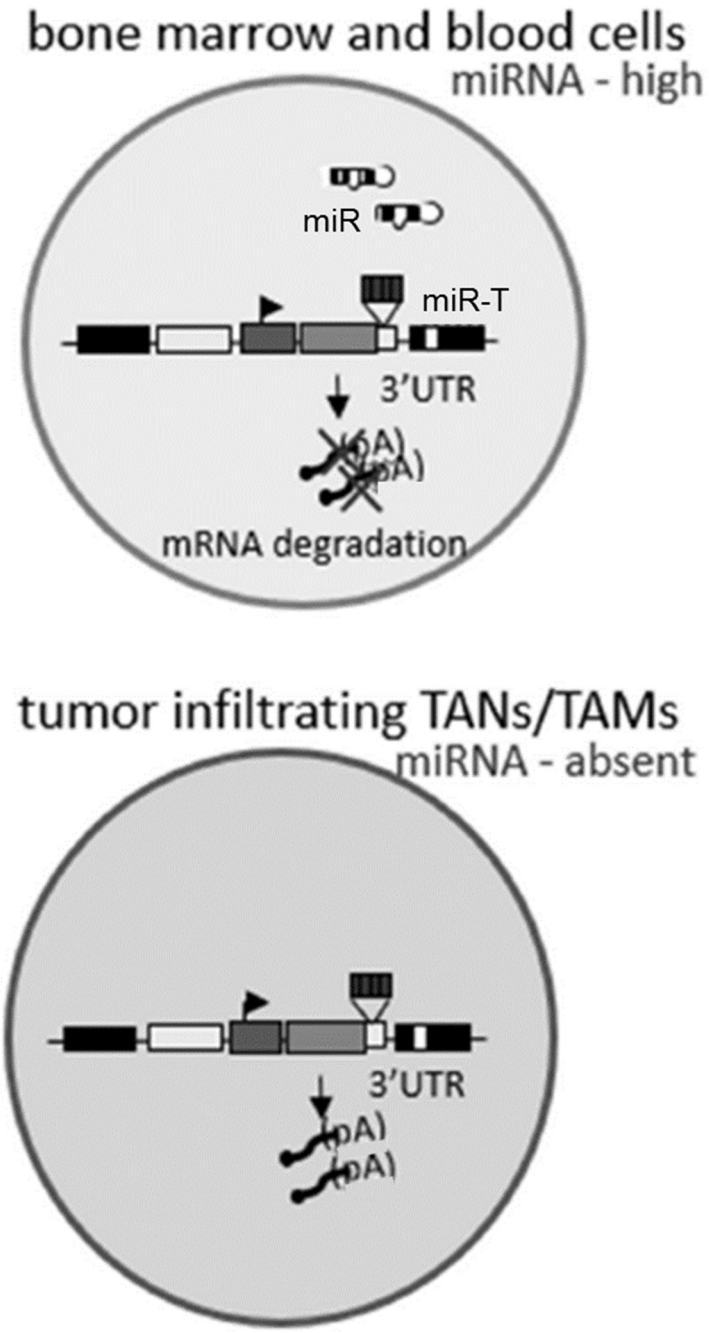


FIG. 88H

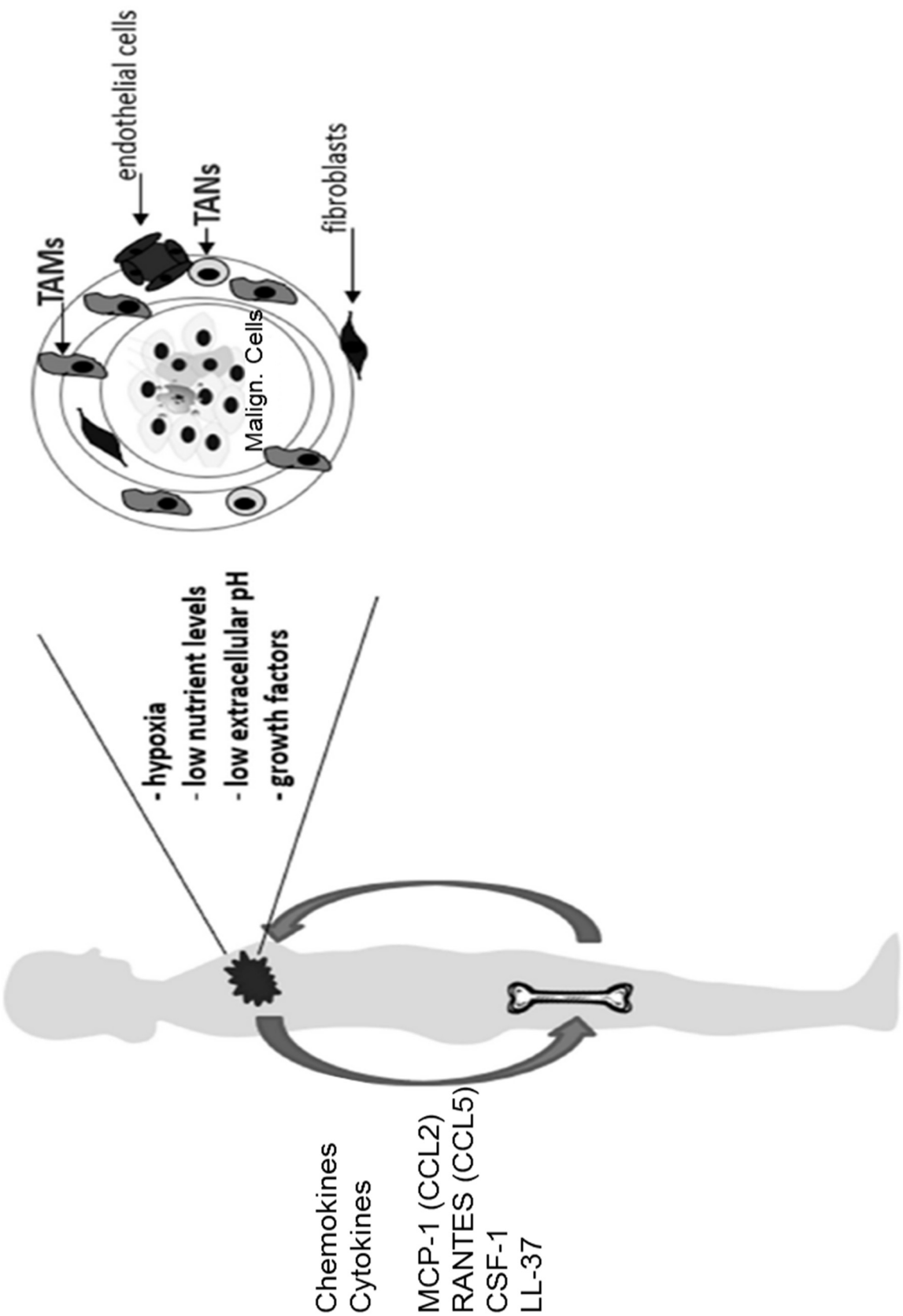

endothelial cells
TANs
TAMs
fibroblasts
Malign. Cells
- hypoxia
- low nutrient levels
- low extracellular pH
- growth factors
Chemokines
Cytokines
MCP-1 (CCL2)
RANTES (CCL5)
CSF-1
LL-37

FIG. 88H cont'd

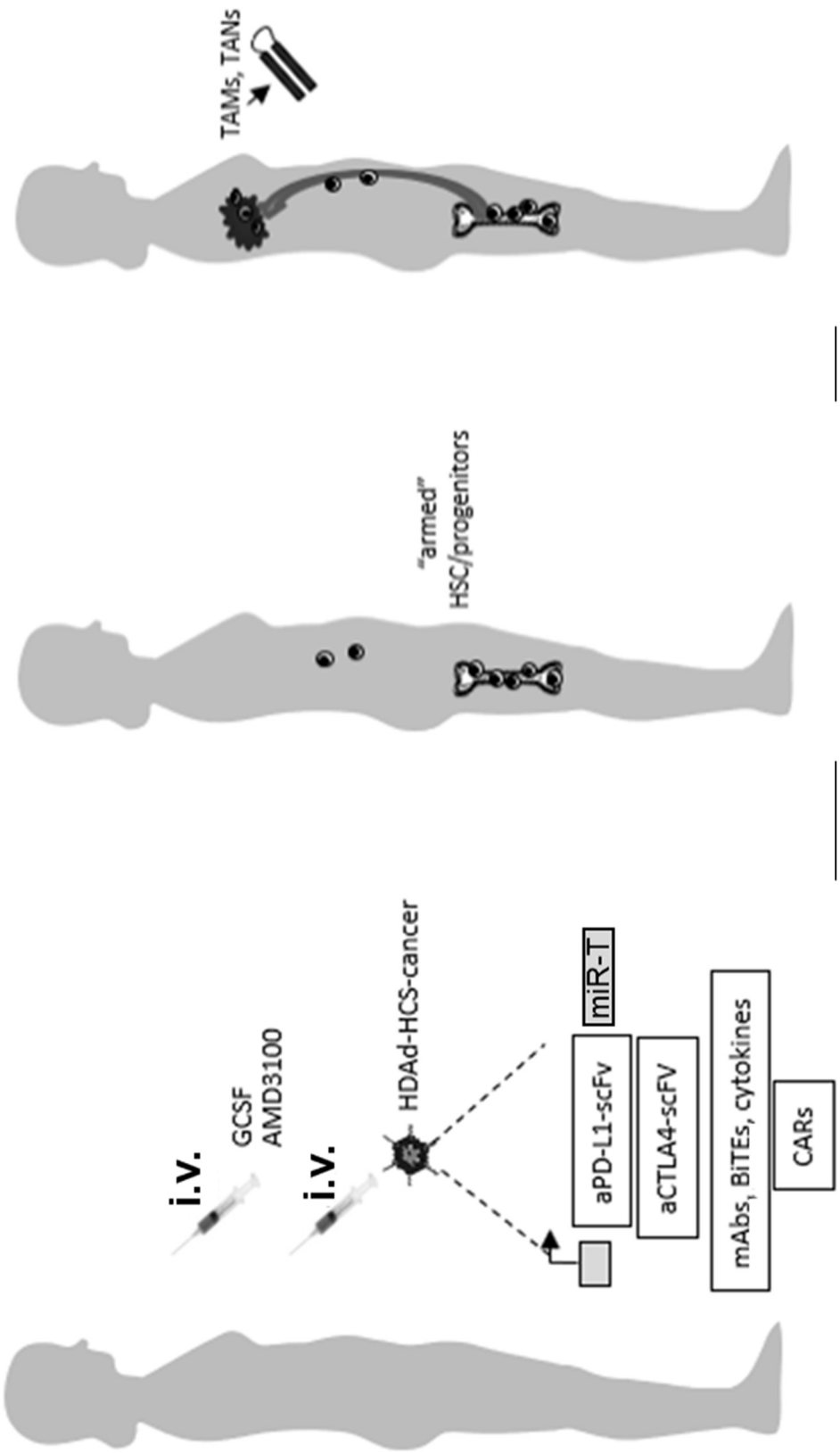
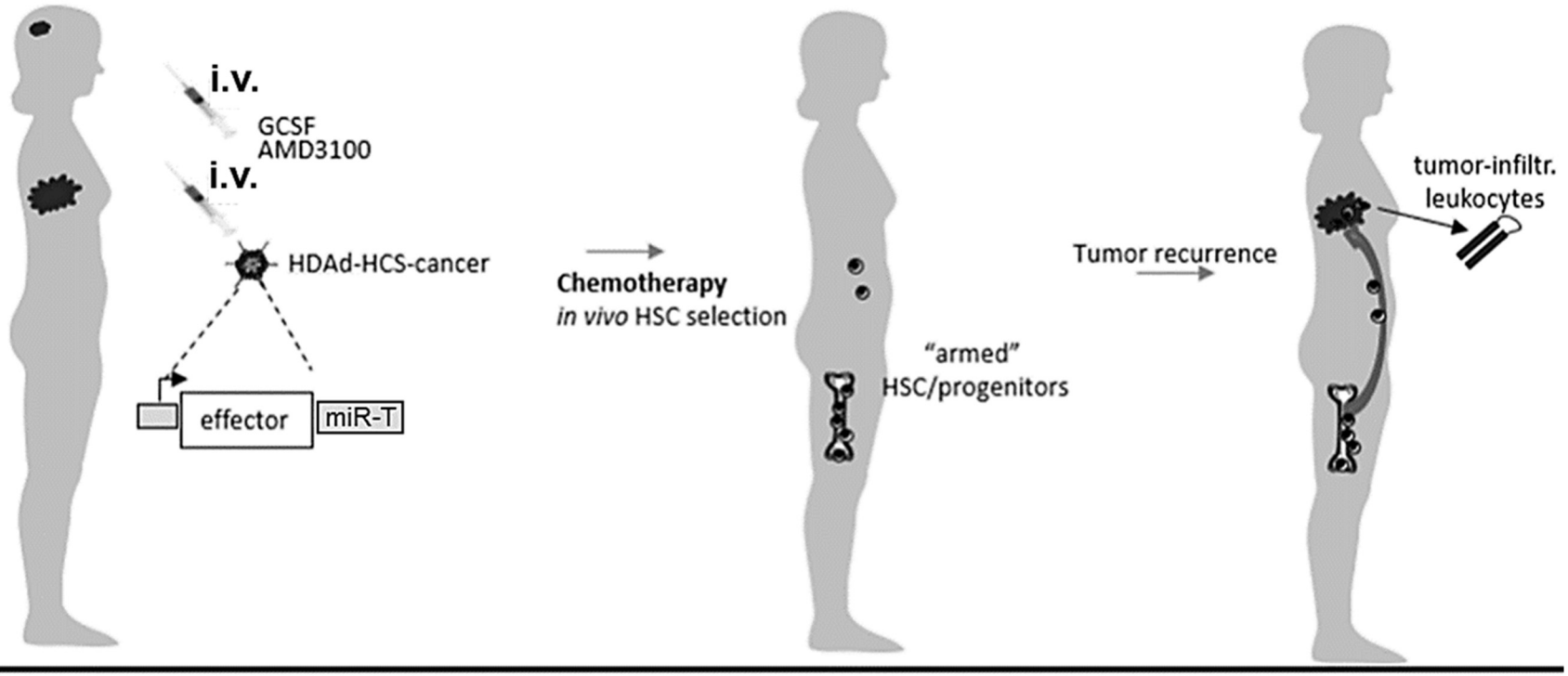


FIG. 88H cont'd



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

-difficult to treat full-fledged tumors

FIG. 89A

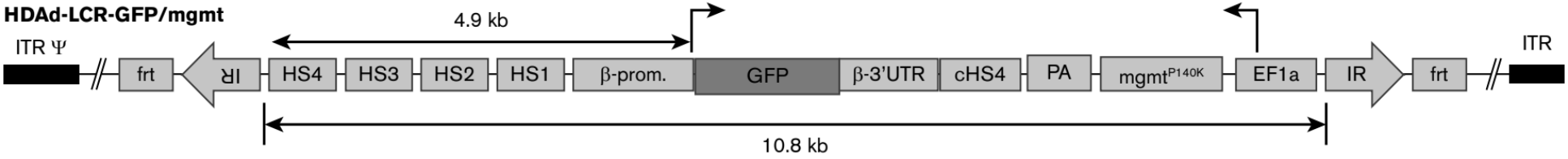


FIG. 89B

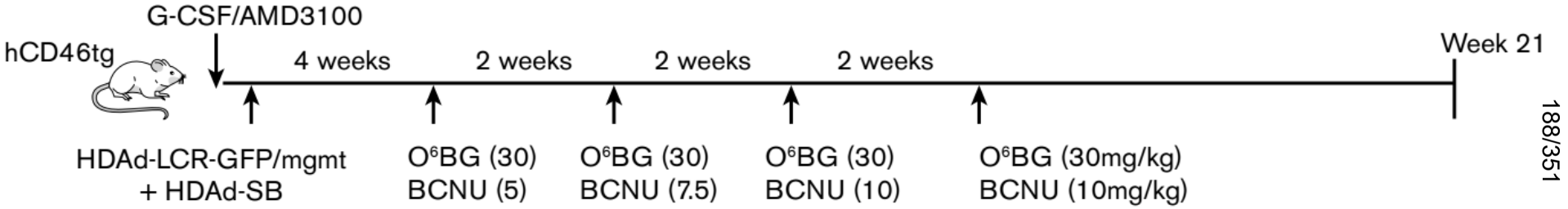


FIG. 89C

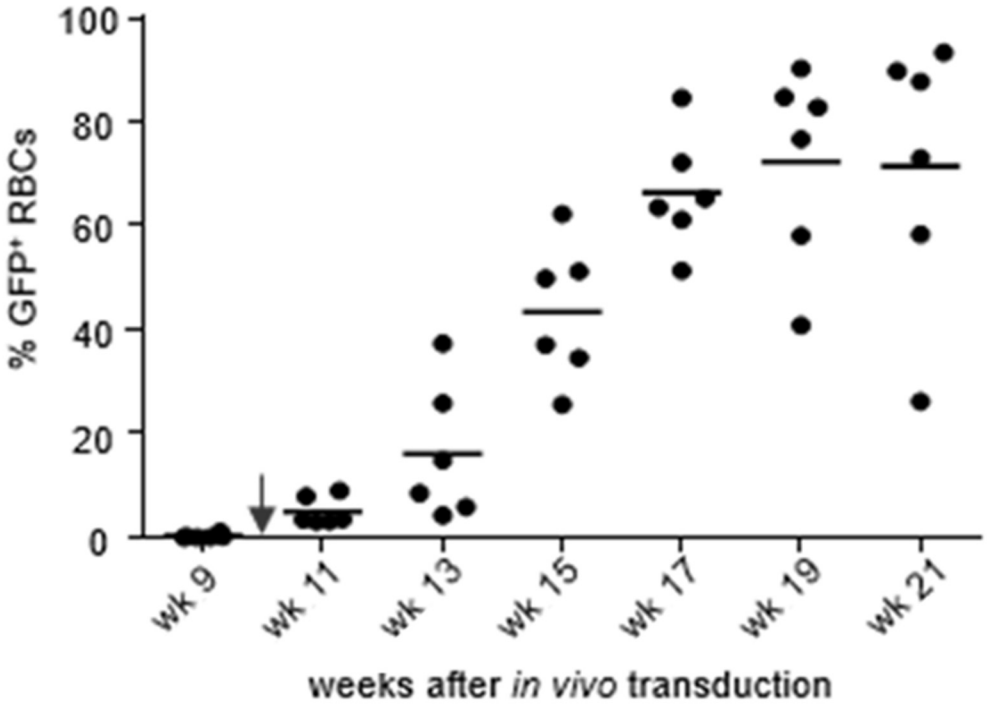


FIG. 89D

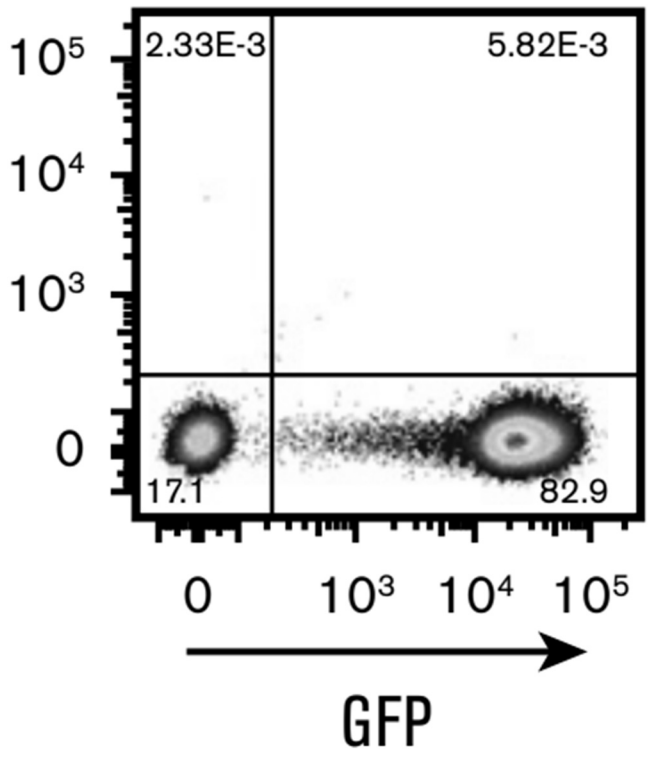


FIG. 89E

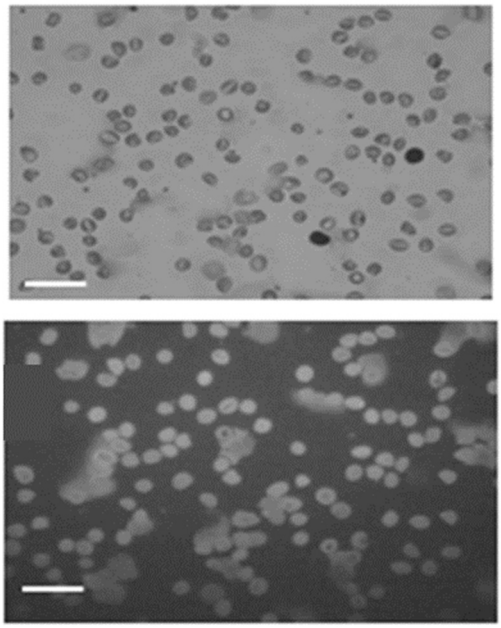


FIG. 89F

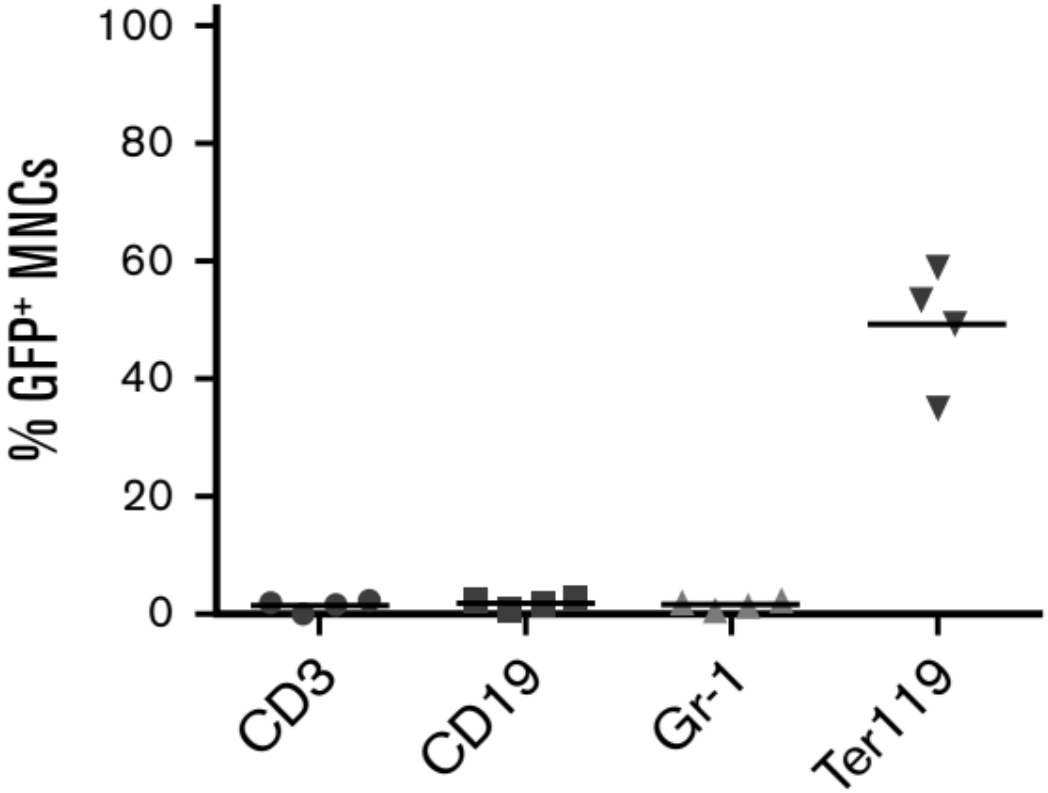


FIG. 89G

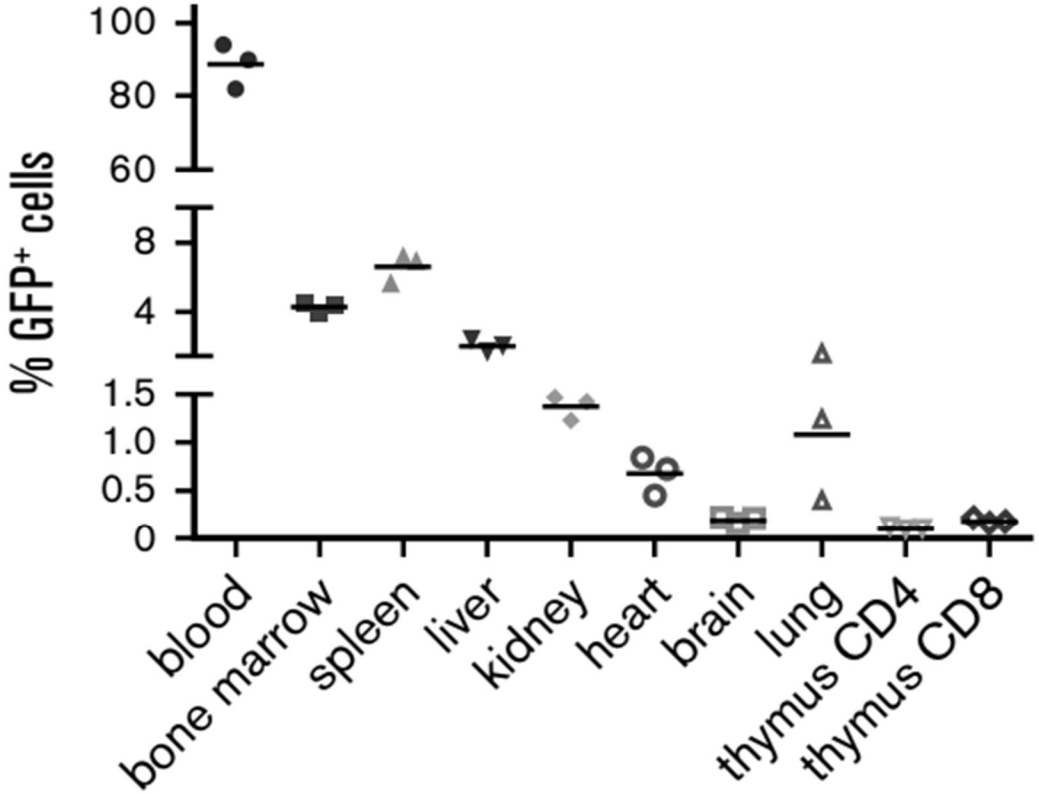


FIG. 89H

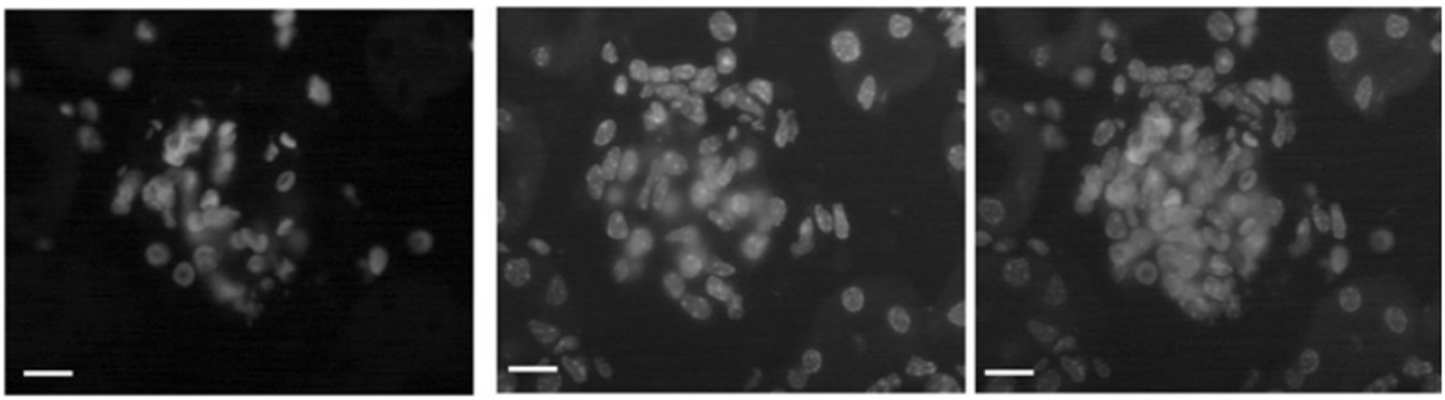


FIG. 90A

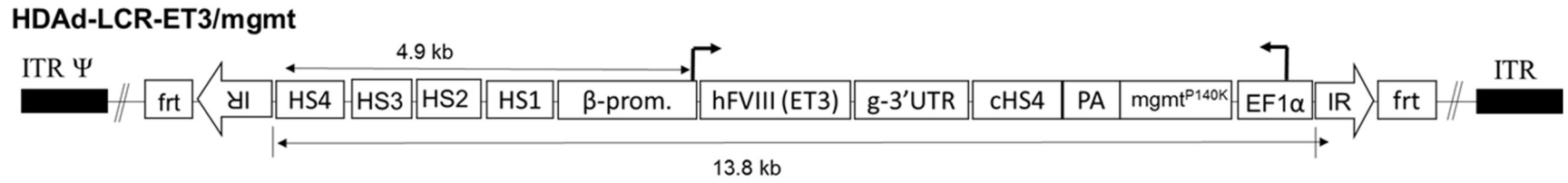


FIG. 90B

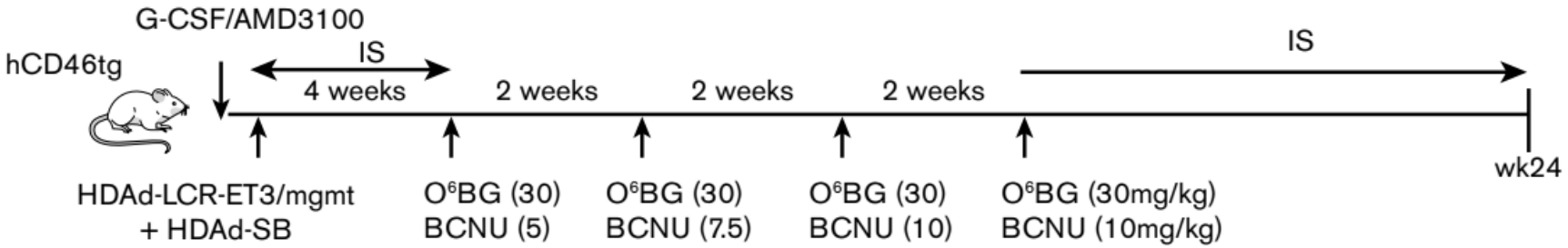


FIG. 90C

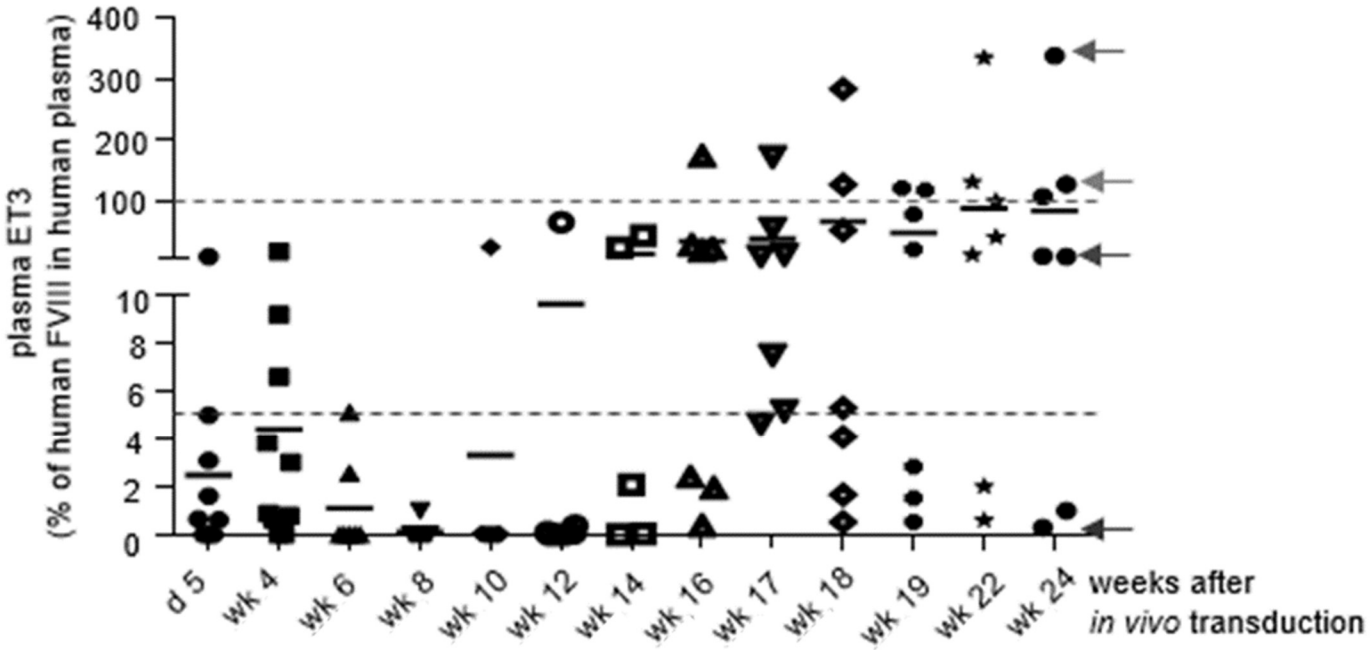


FIG. 90D

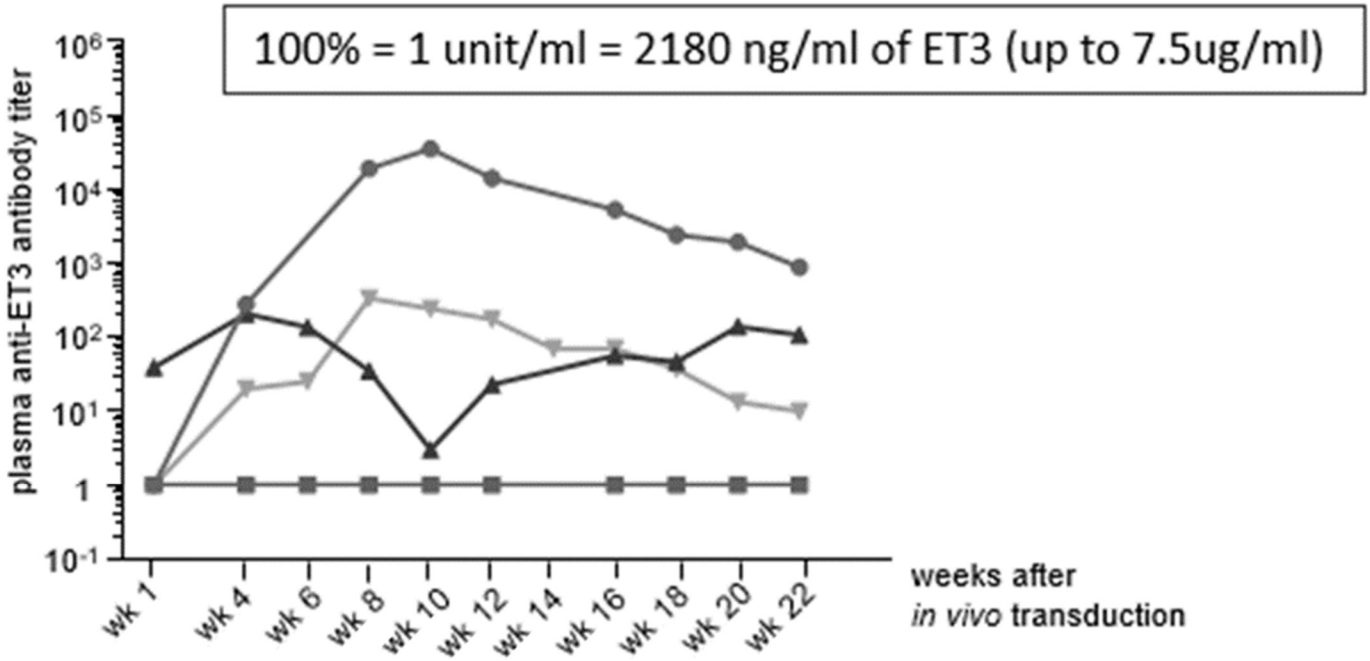


FIG. 90E

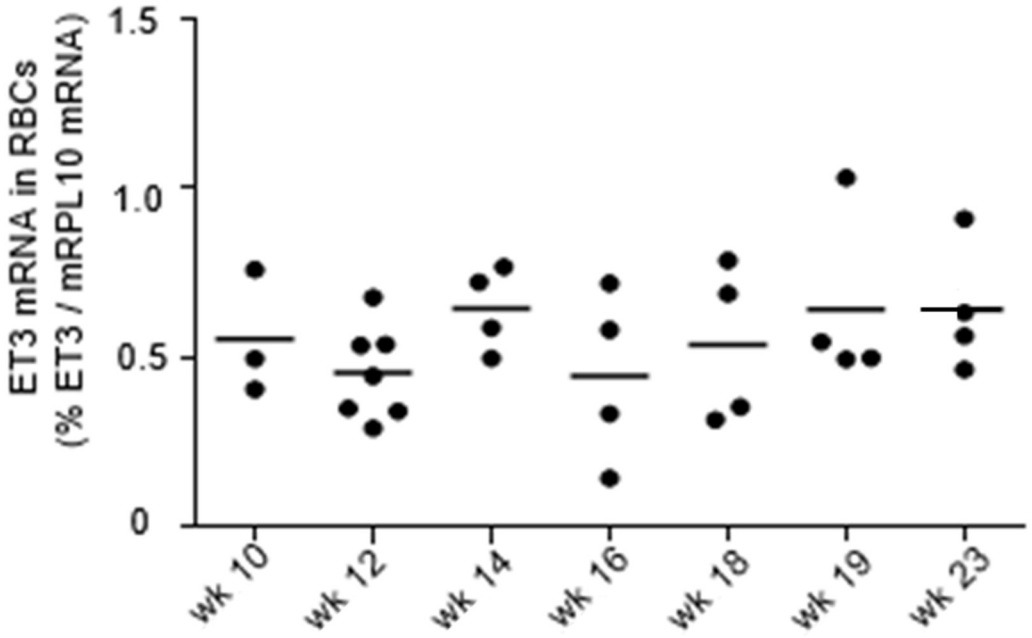


FIG. 90F

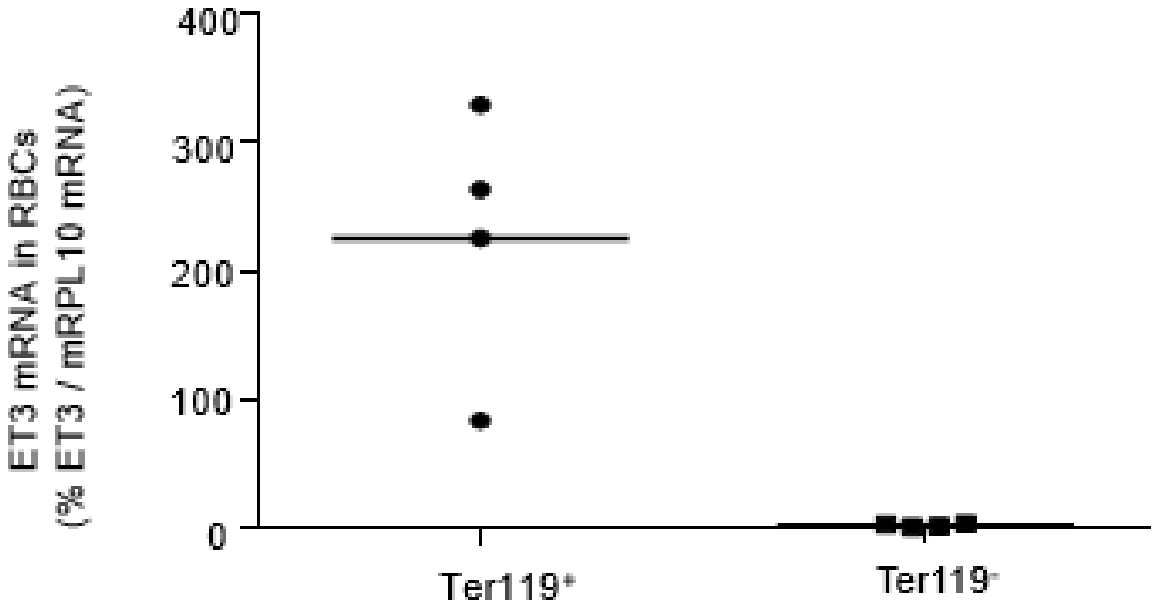


FIG. 90G

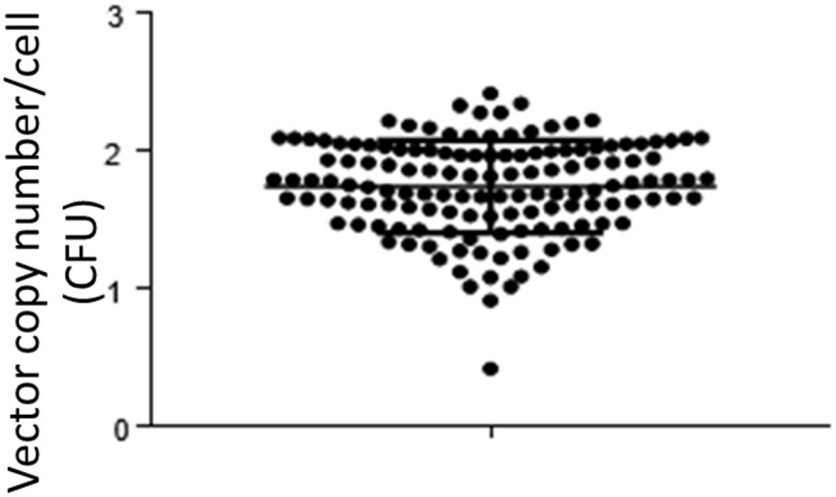


FIG. 90H

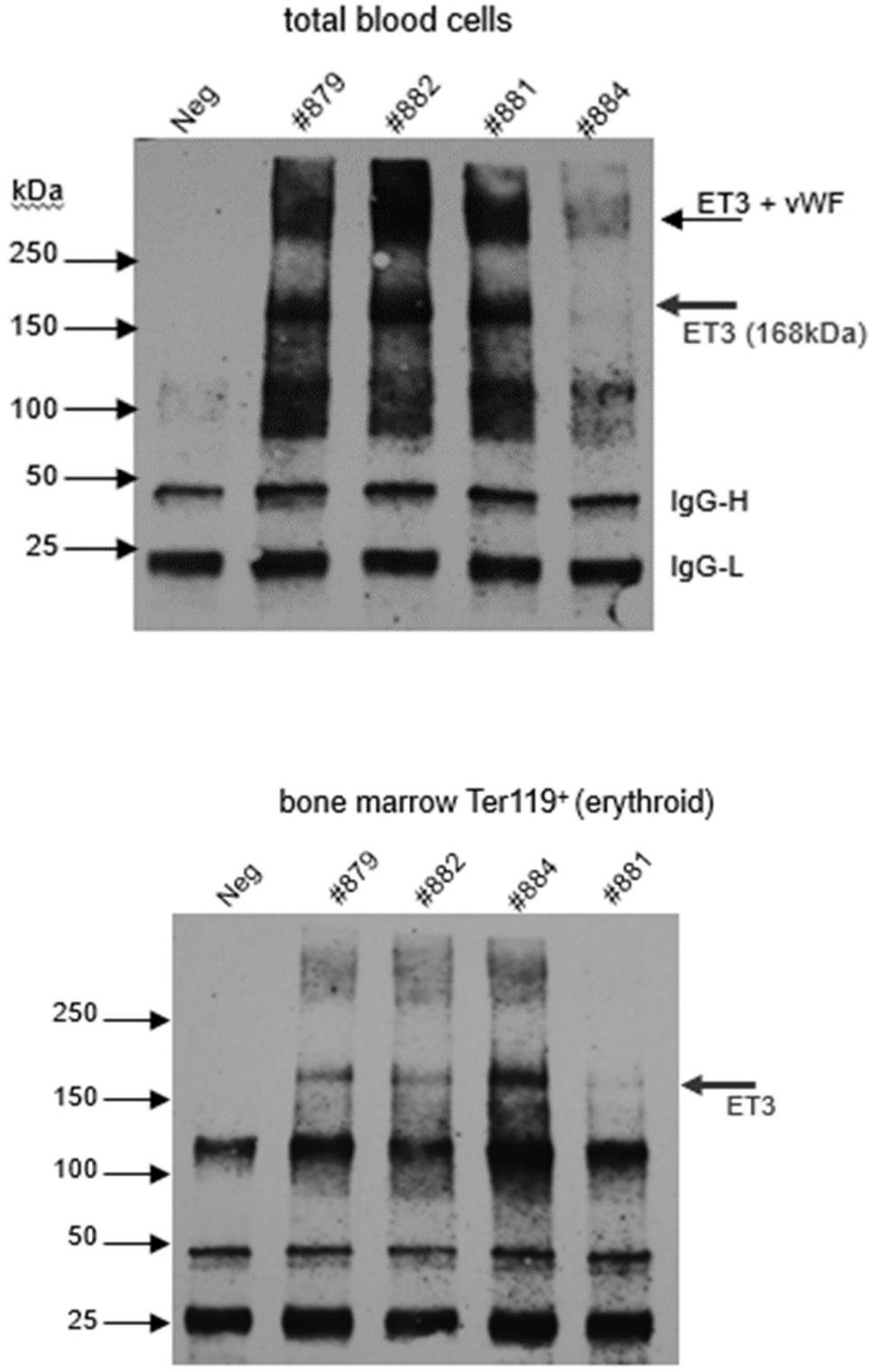

total blood cells
Neg
#879
#882
#881
#884
kDa
250
150
100
50
25
ET3 + vWF
ET3 (168kDa)
IgG-H
IgG-L
bone marrow Ter119+ (erythroid)
Neg
#879
#882
#884
#881
250
150
100
50
25
ET3

FIG. 90H cont'd

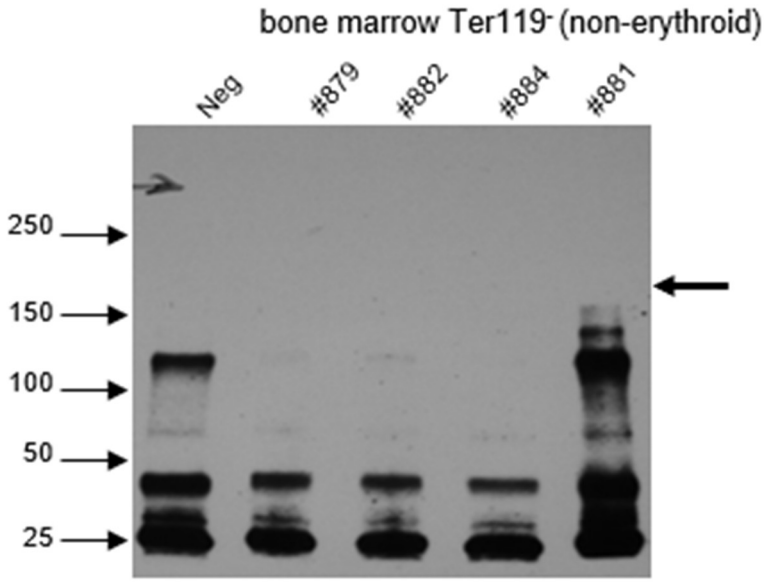


FIG. 90I

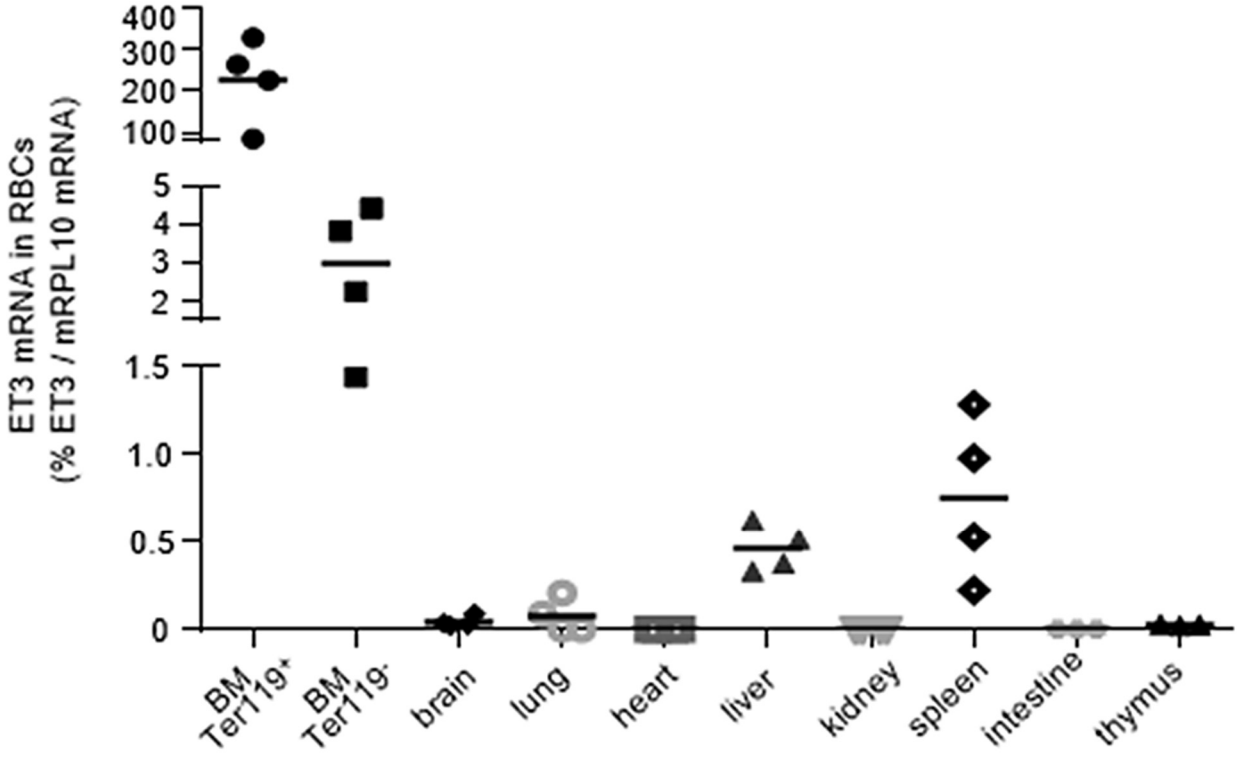


FIG. 91A

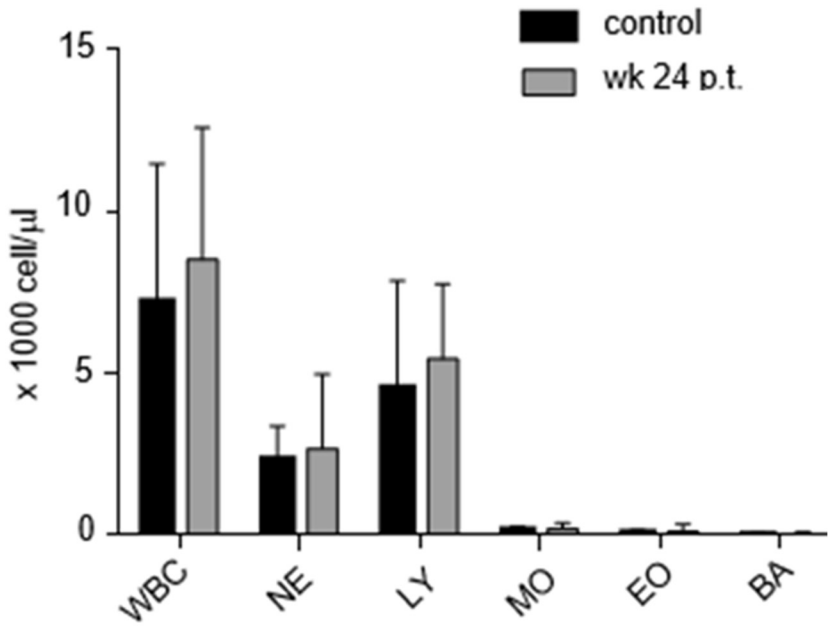


FIG. 91B

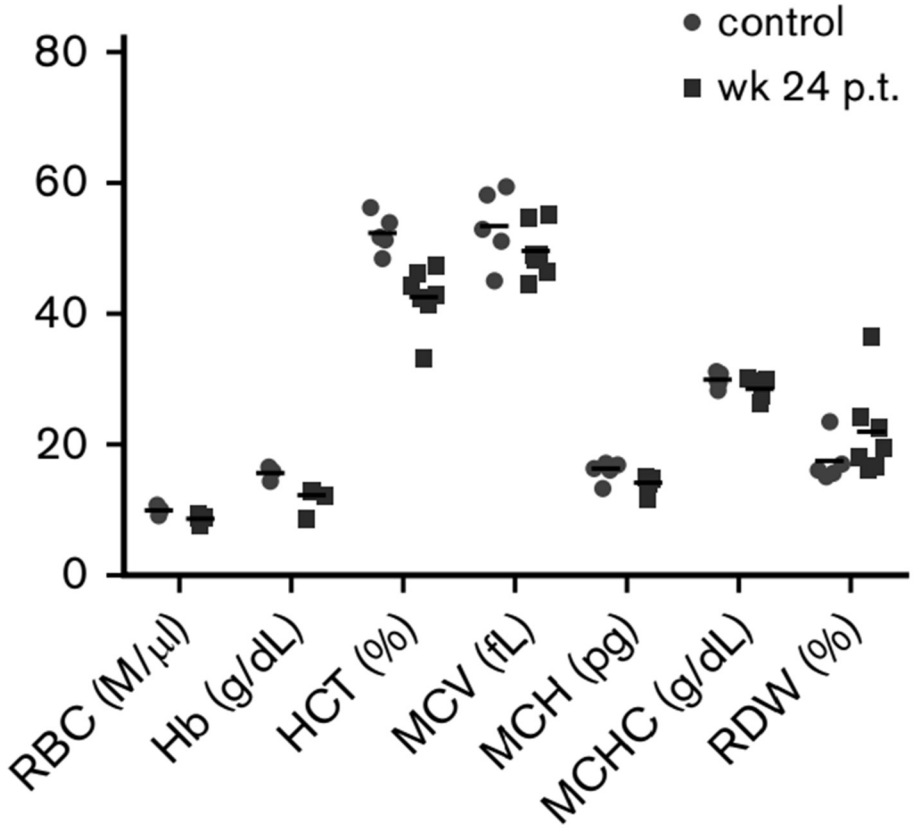


FIG. 91C

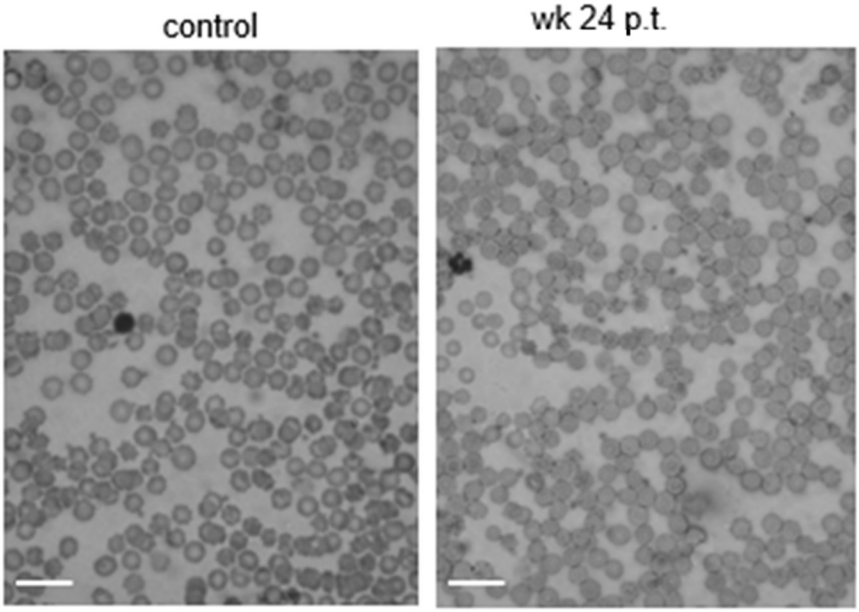


FIG. 91D

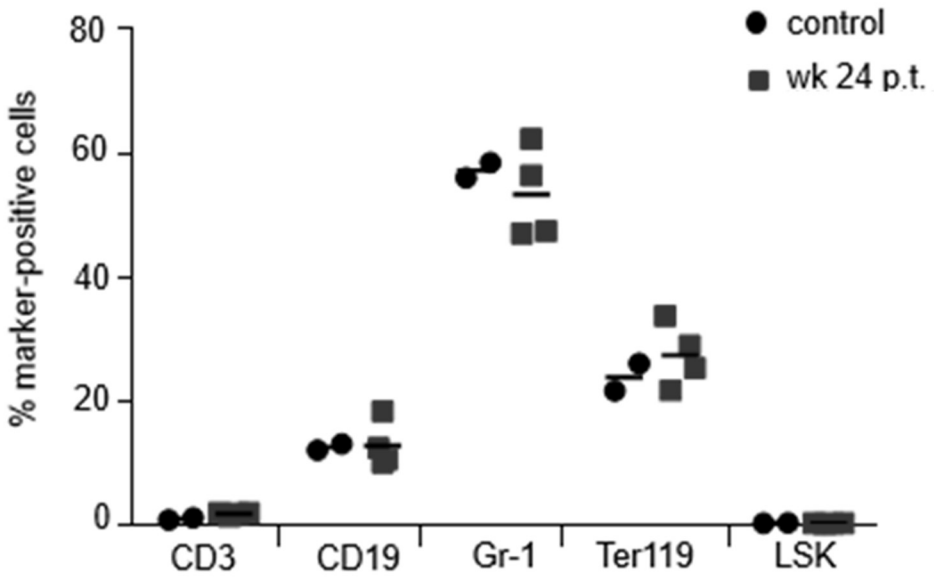


FIG. 92A

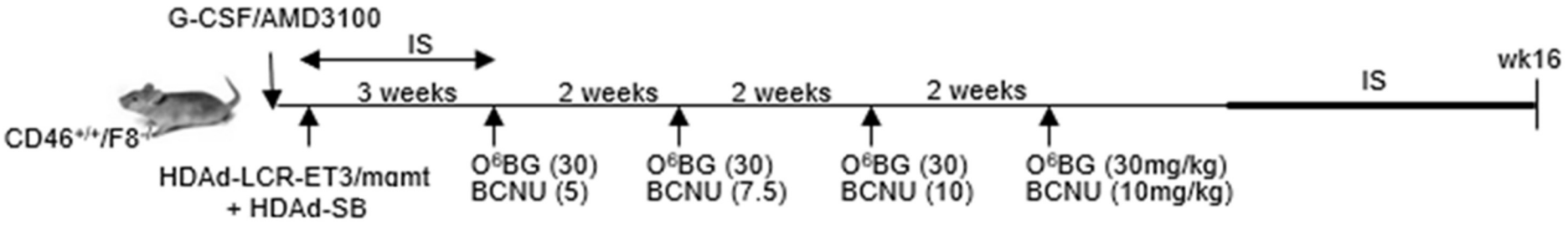


FIG. 92B

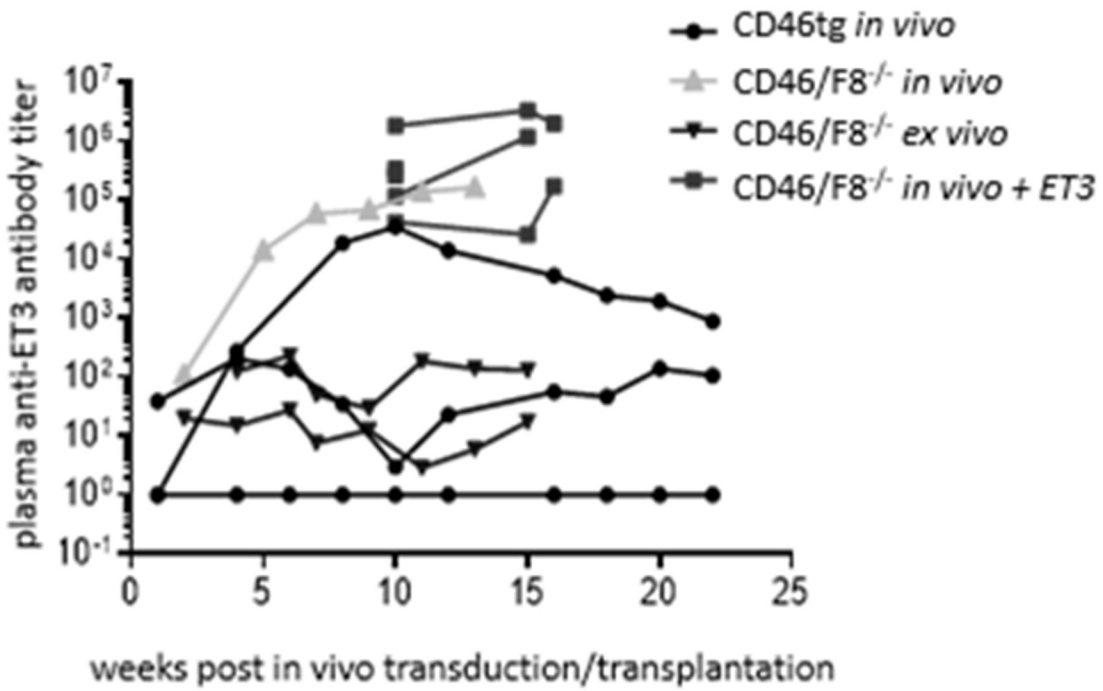


FIG. 92C

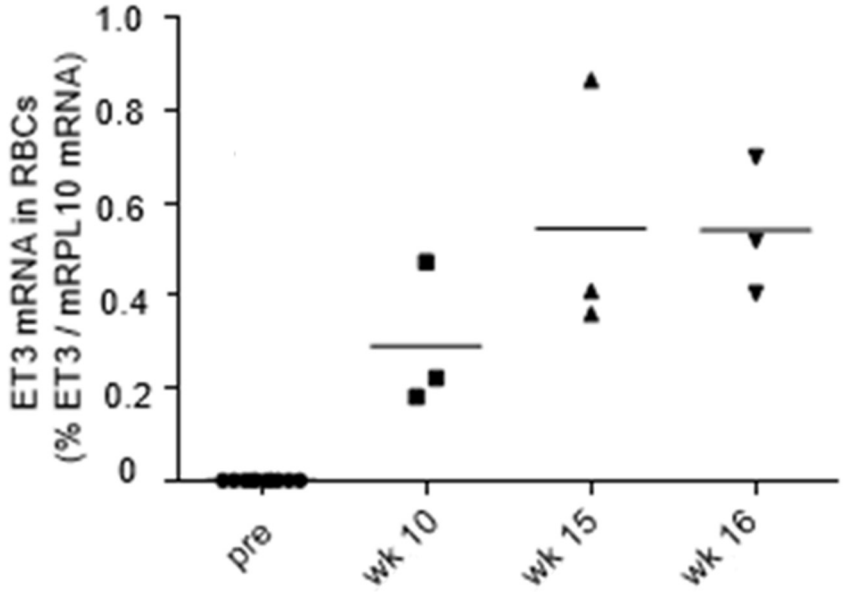


FIG. 92D

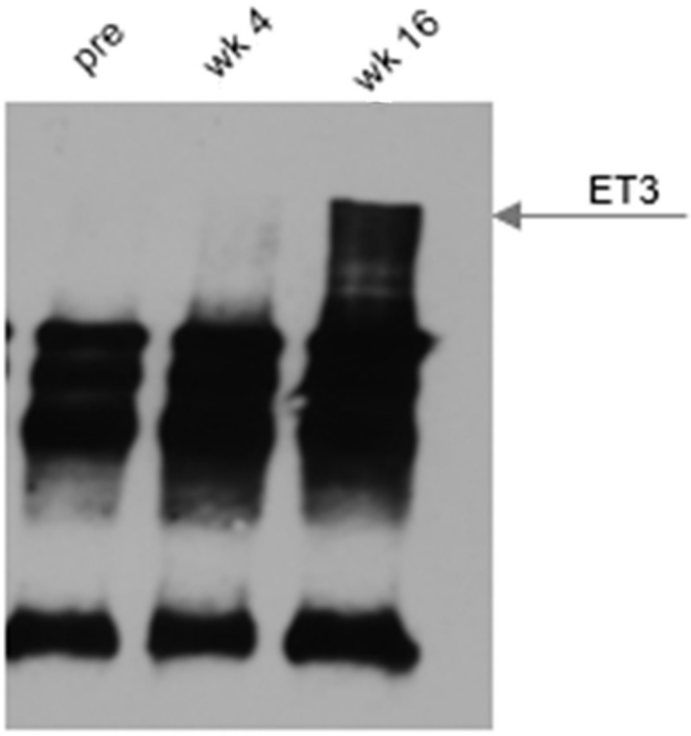


FIG. 92E

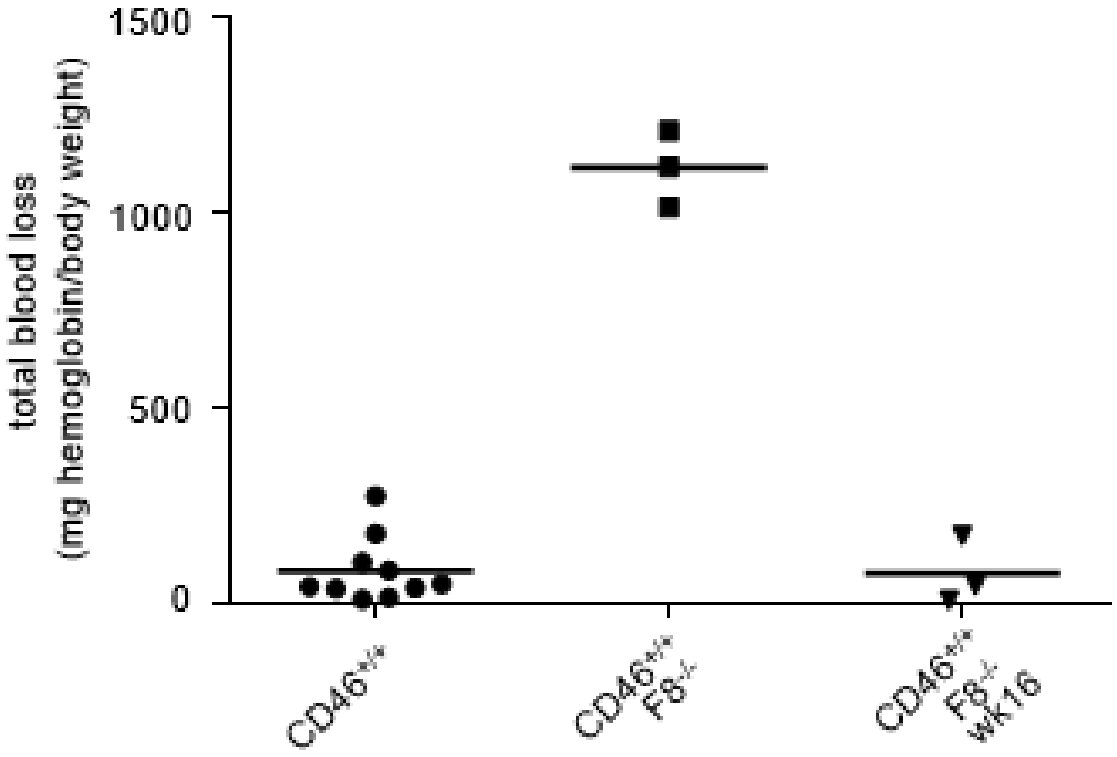


FIG. 92F

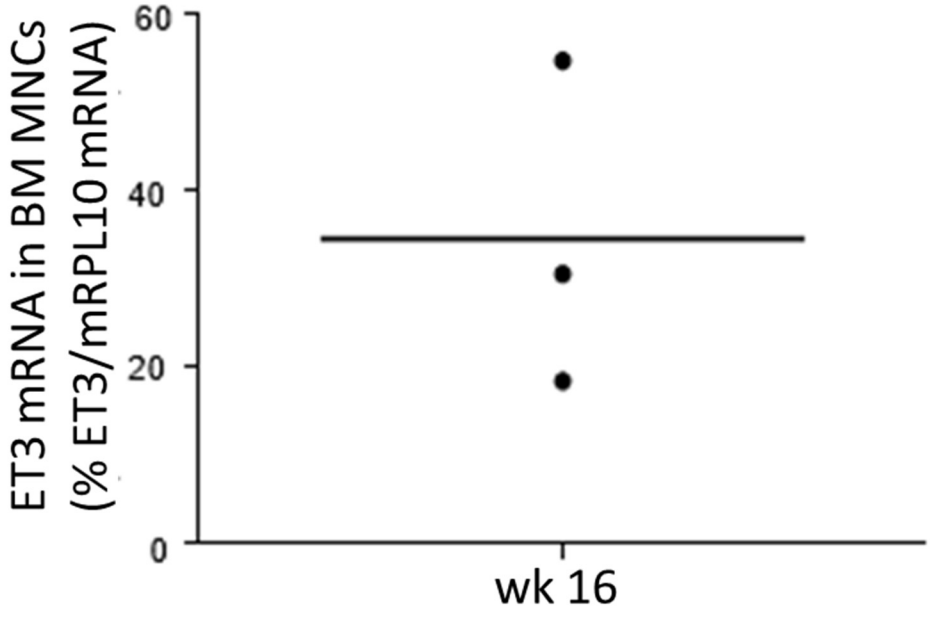


FIG. 92G

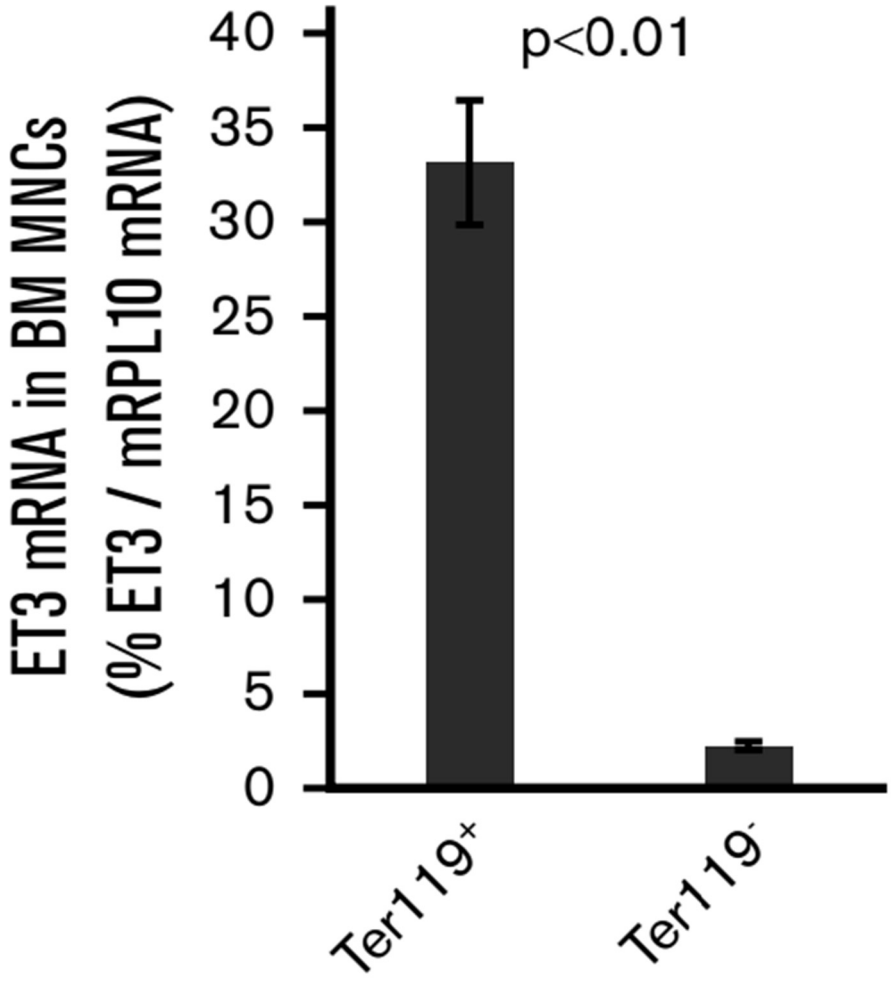

ET3 mRNA in BM MNCs
(% ET3 / mRPL10 mRNA)
40
35
30
25
20
15
10
5
0
p<0.01
Ter119+
Ter119-

FIG. 93A

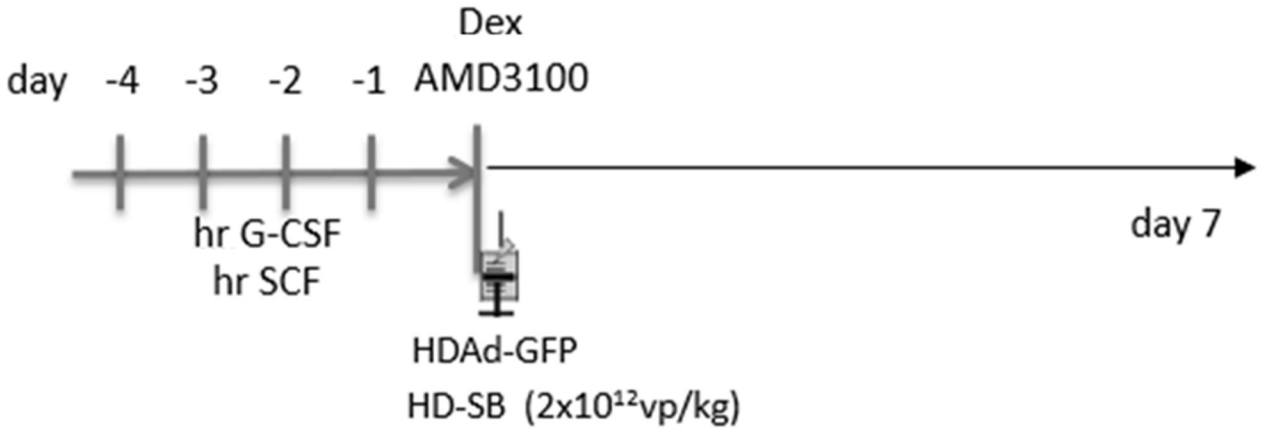


FIG. 93B

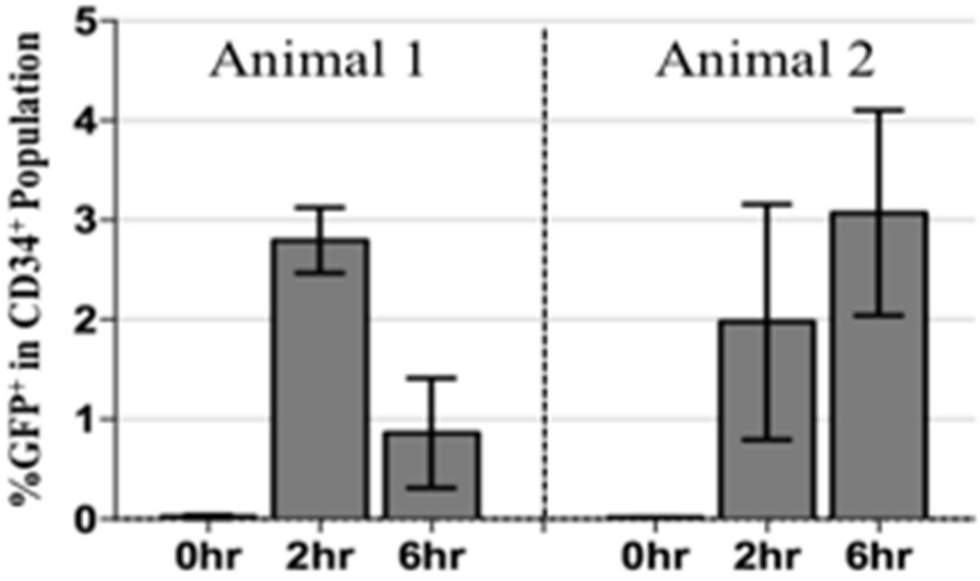


FIG. 93C

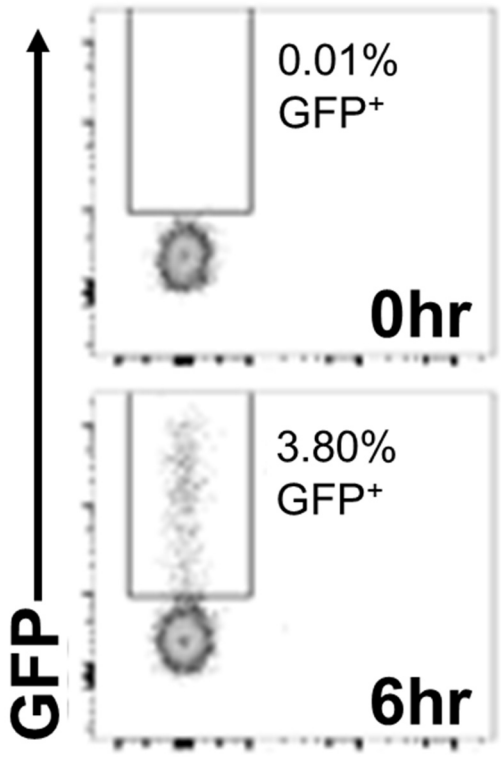


FIG. 93D

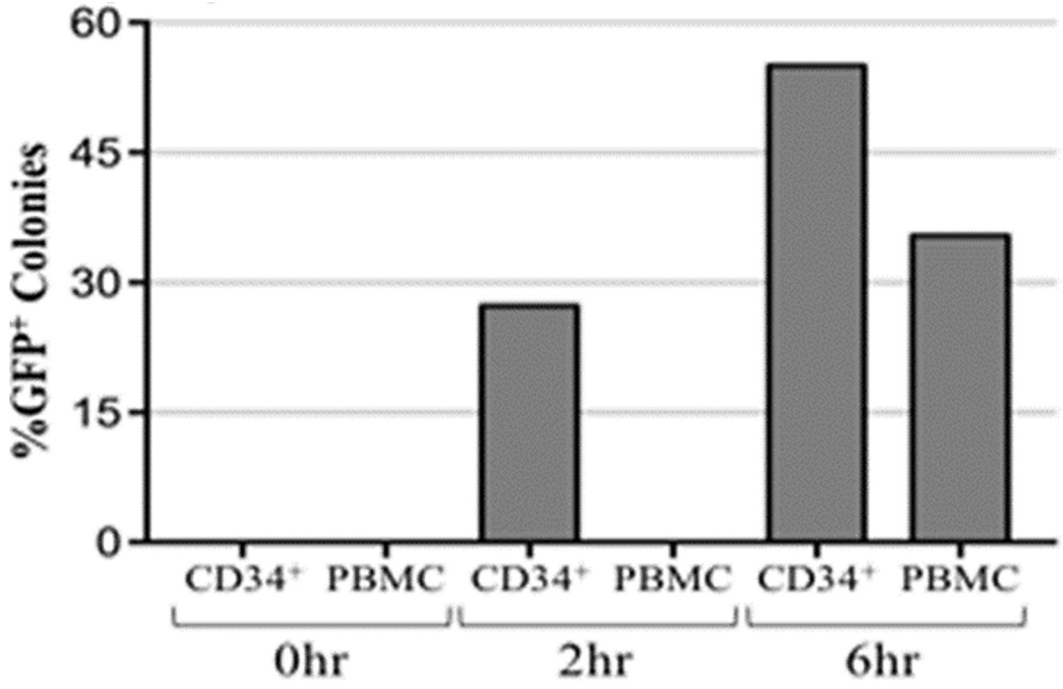


FIG. 93E

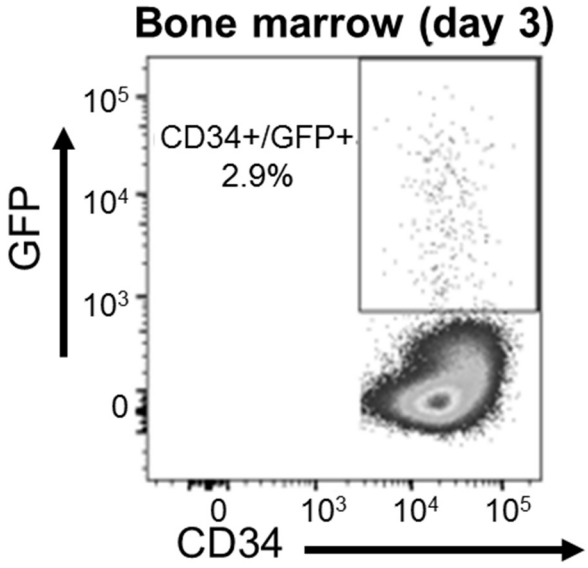

Bone marrow (day 3)
CD34+/GFP+
2.9%
GFP
10^5
10^4
10^3
0
0
10^3
10^4
10^5
CD34

FIG. 94A

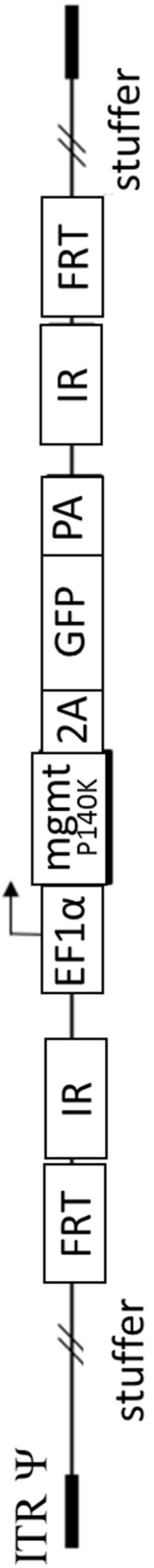


FIG. 94B

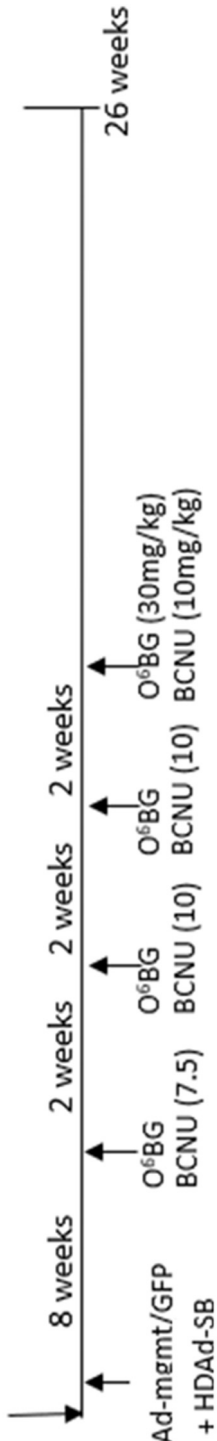


FIG. 94C

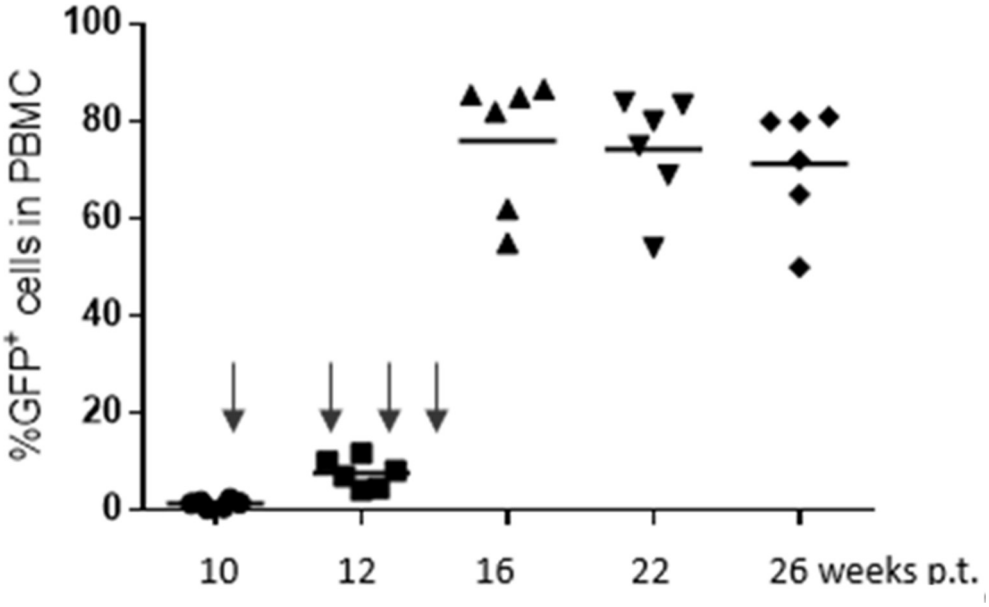


FIG. 94D

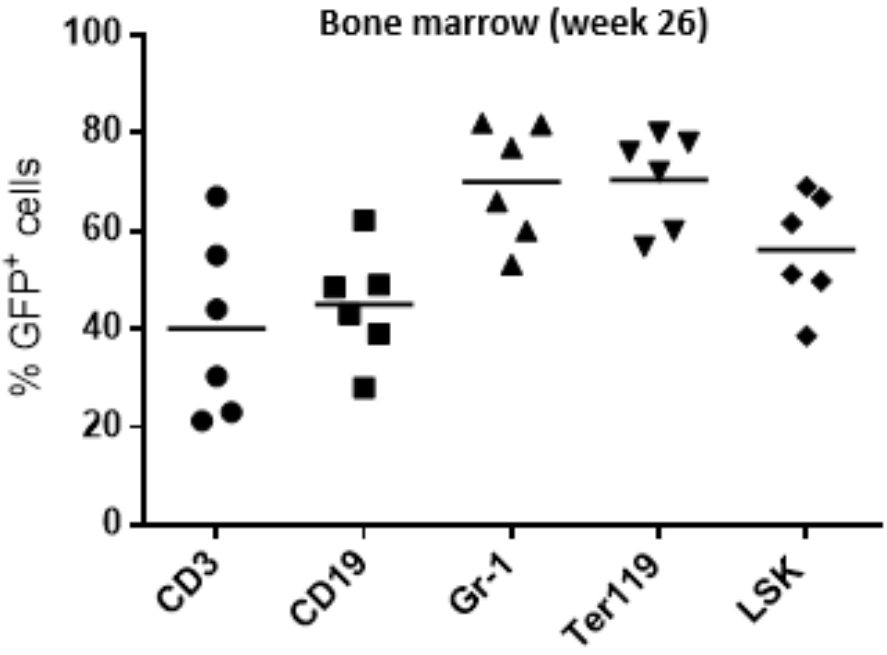


FIG. 94E

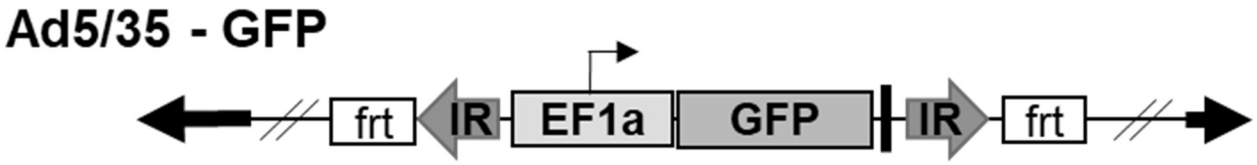


FIG. 94F

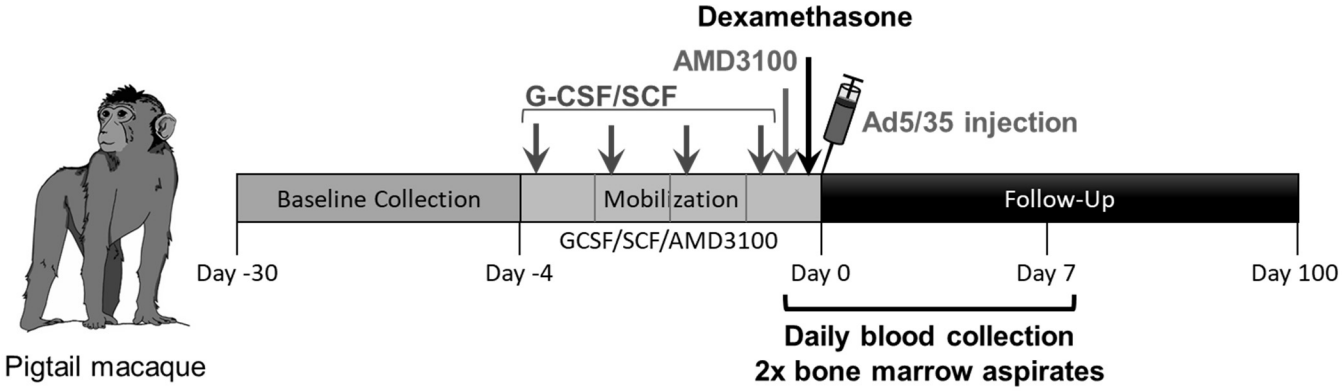


FIG. 94G

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

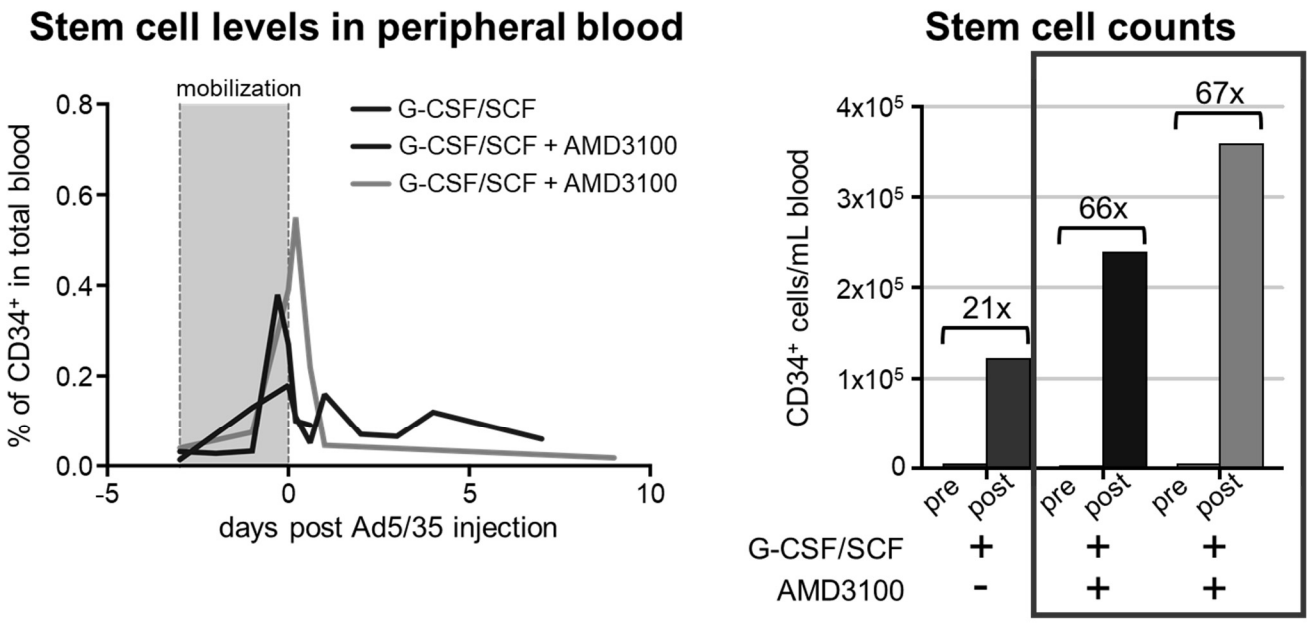


FIG. 94I

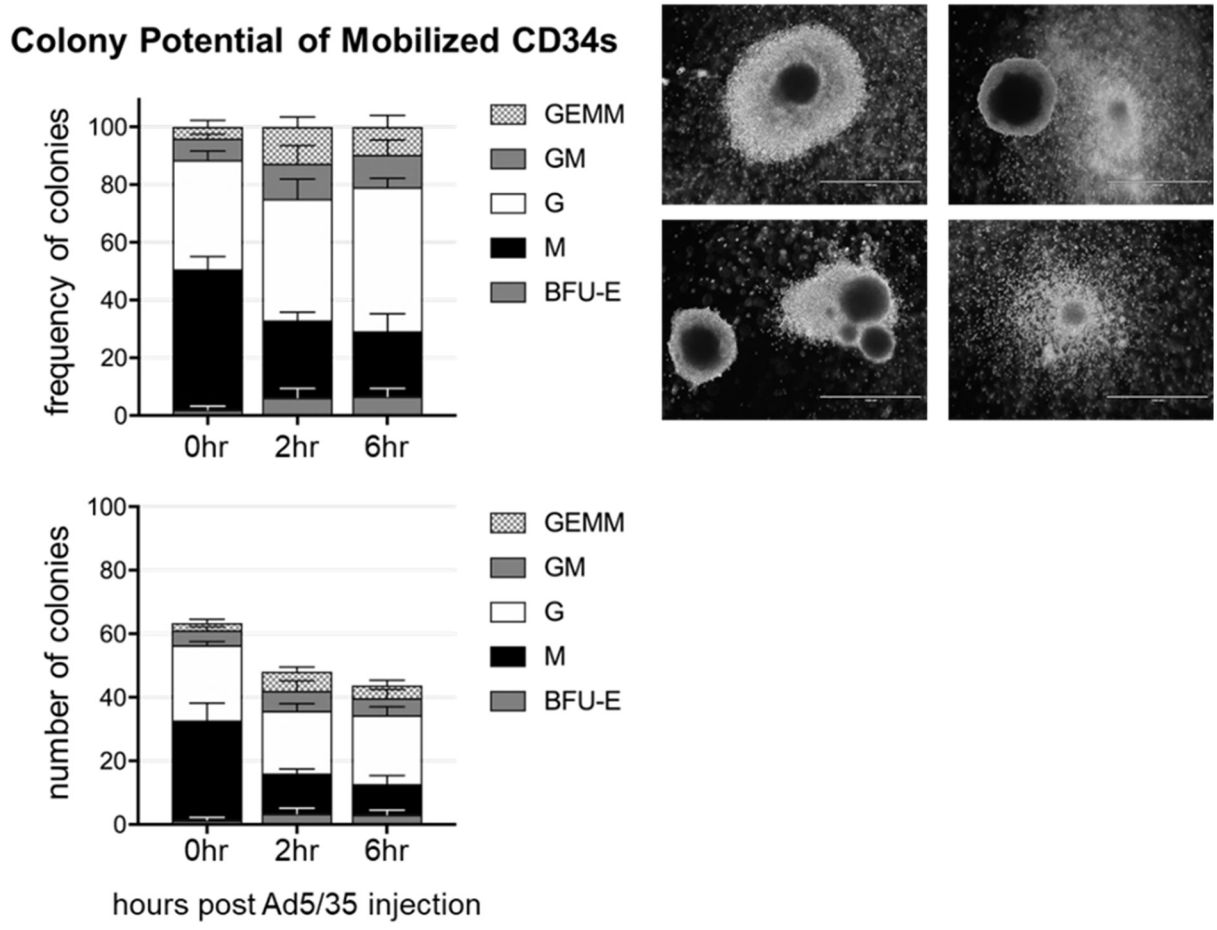


FIG. 94J

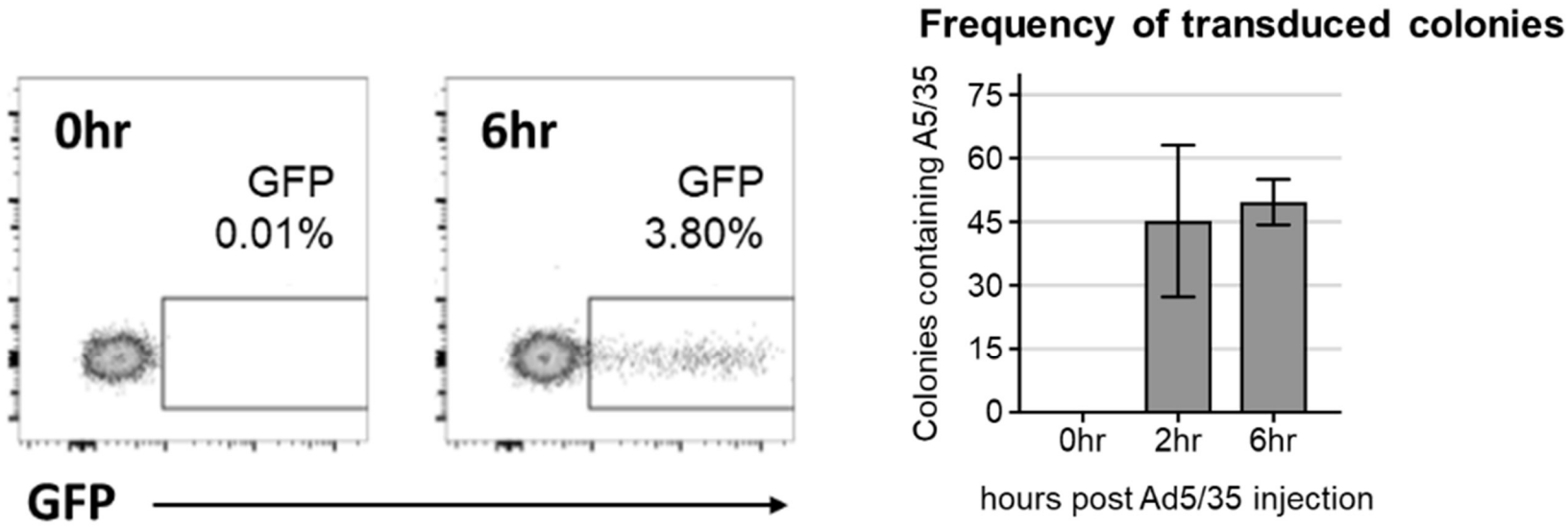


FIG. 94K

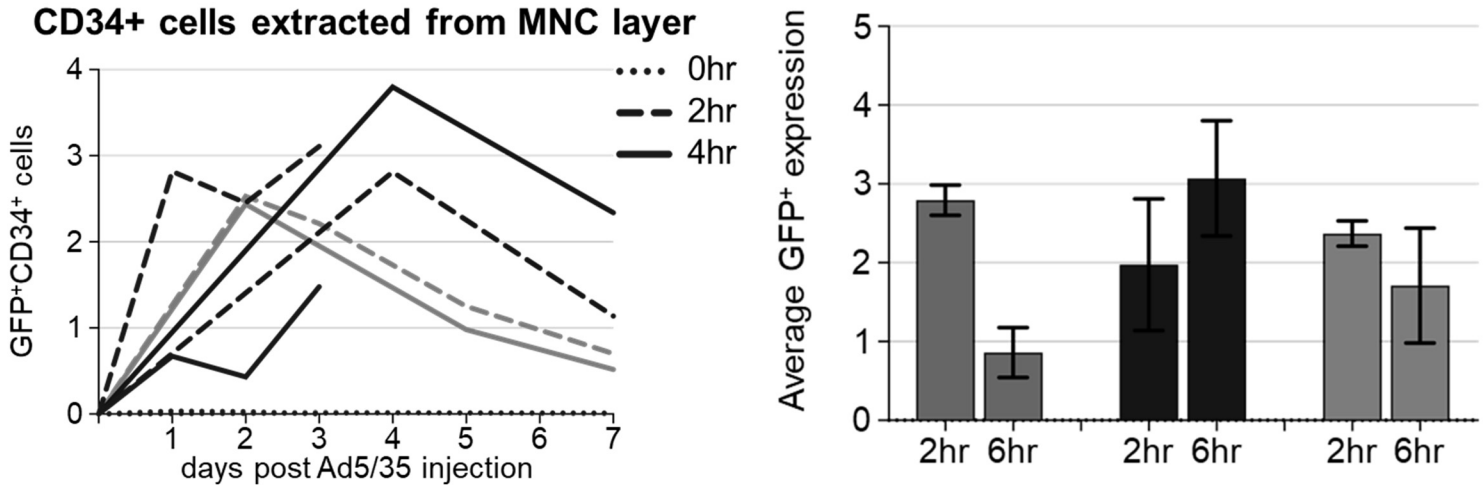


FIG. 94L

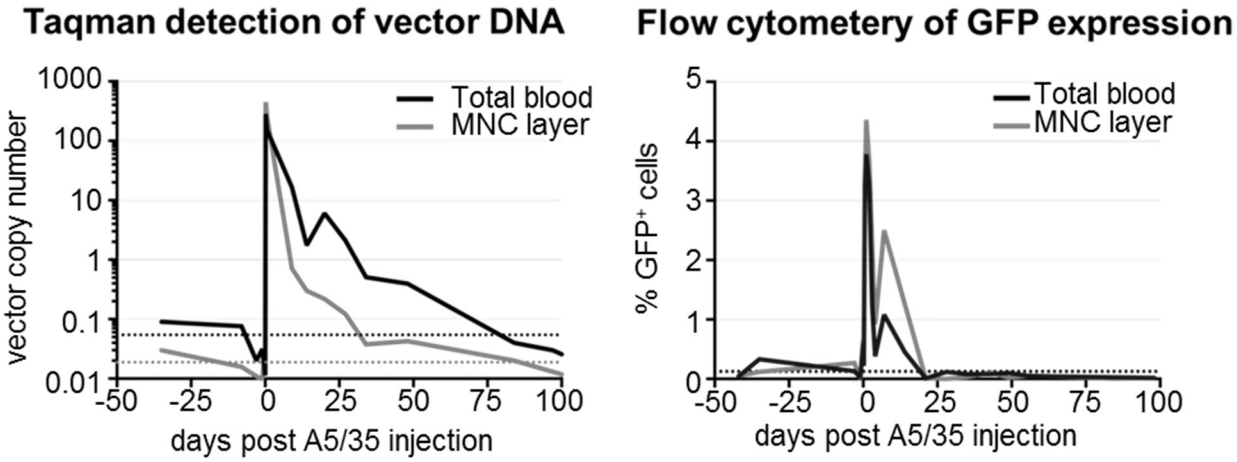


FIG. 94M

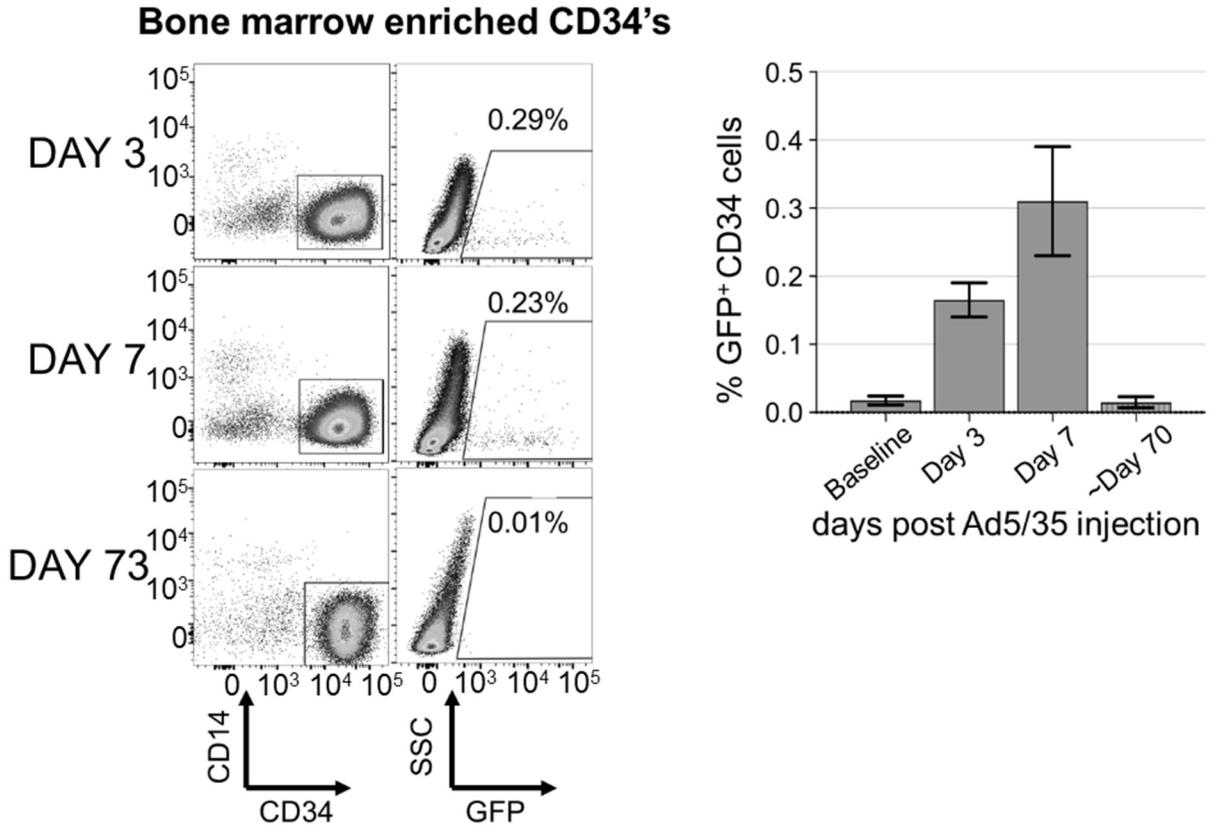


FIG. 95

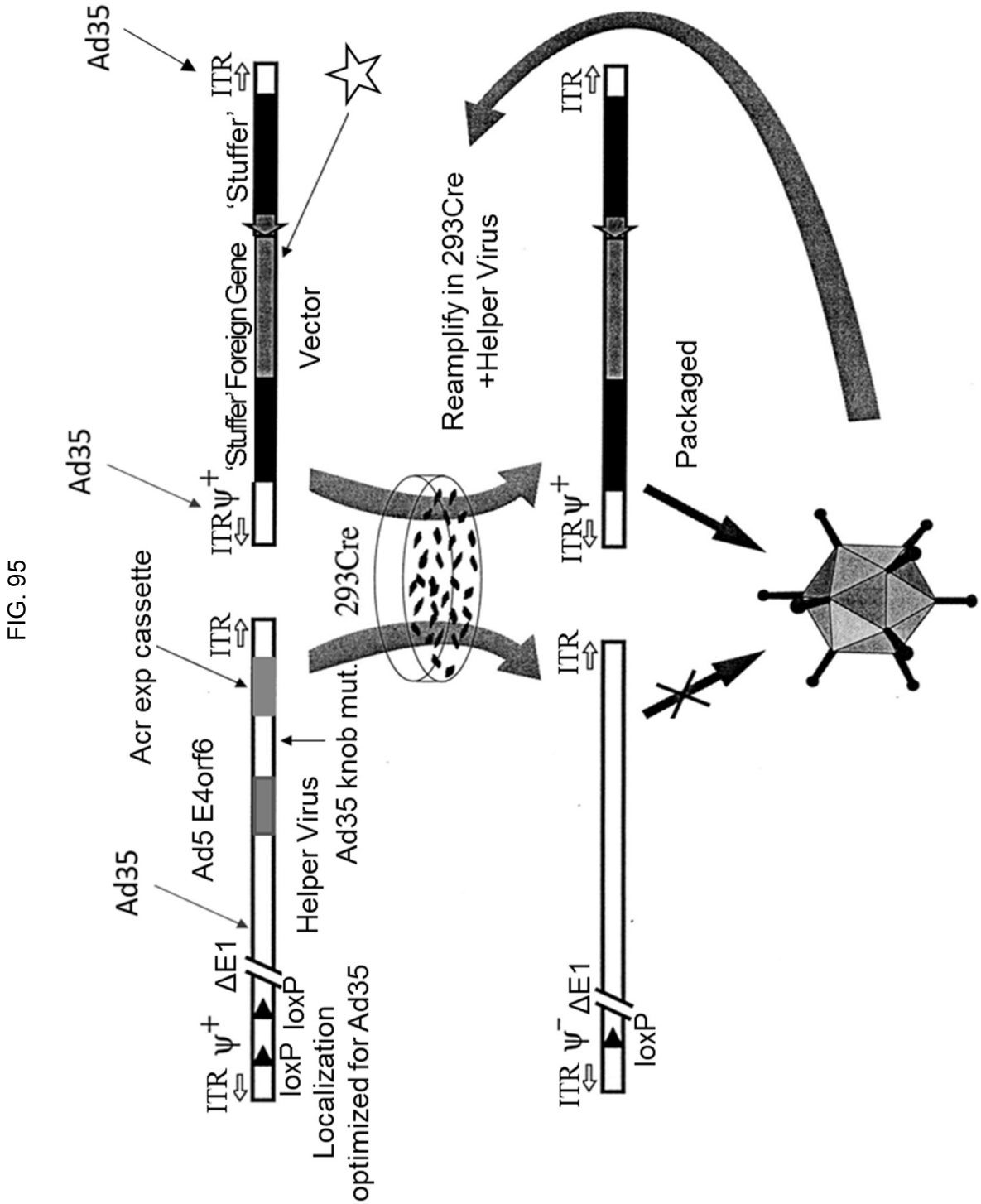

Ad35
ITR
'Stuffer'
Ad35
ITR ψ+
'Stuffer' Foreign Gene
Vector
Reamplify in 293Cre
+Helper Virus
ITR
Packaged
Ad35
Acr exp cassette
ITR
293Cre
ITR ψ+
Ad5 E4orf6
Helper Virus
Ad35 knob mut.
Ad35
ITR ψ+ ΔE1
loxP loxP
Localization
optimized for Ad35
ITR
ITR ψ- ΔE1
loxP

FIG. 96

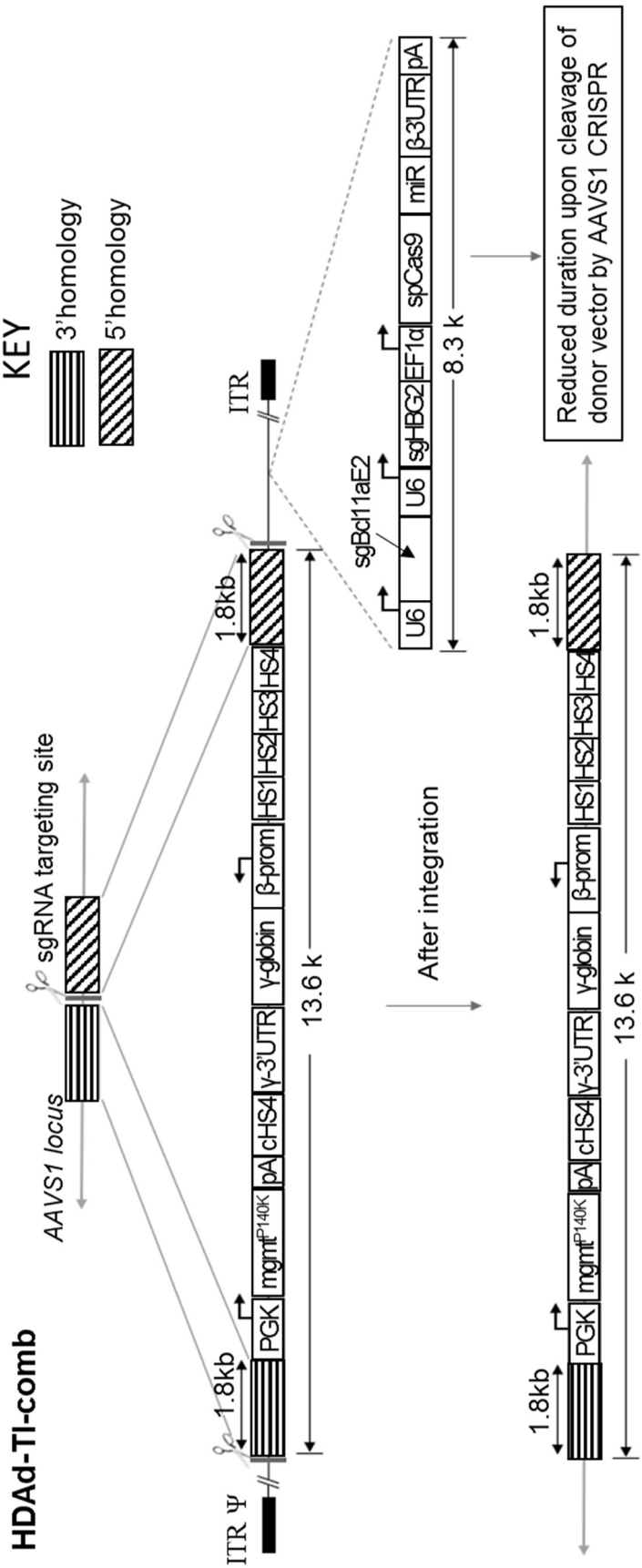

KEY
3'homology
5'homology
HDAd-TI-comb
AAVS1 locus
sgRNA targeting site
ITR Ψ
1.8kb
PGK
mgmt^P140K
pA
cHS4
γ-3'UTR
γ-globin
β-prom
HS1
HS2
HS3
HS4
13.6 k
ITR
sgBcl11aE2
U6
U6
sgHBG2
EF1d
spCas9
miR
β-3'UTR
pA
8.3 k
After integration
Reduced duration upon cleavage of donor vector by AAVS1 CRISPR

FIG. 97A

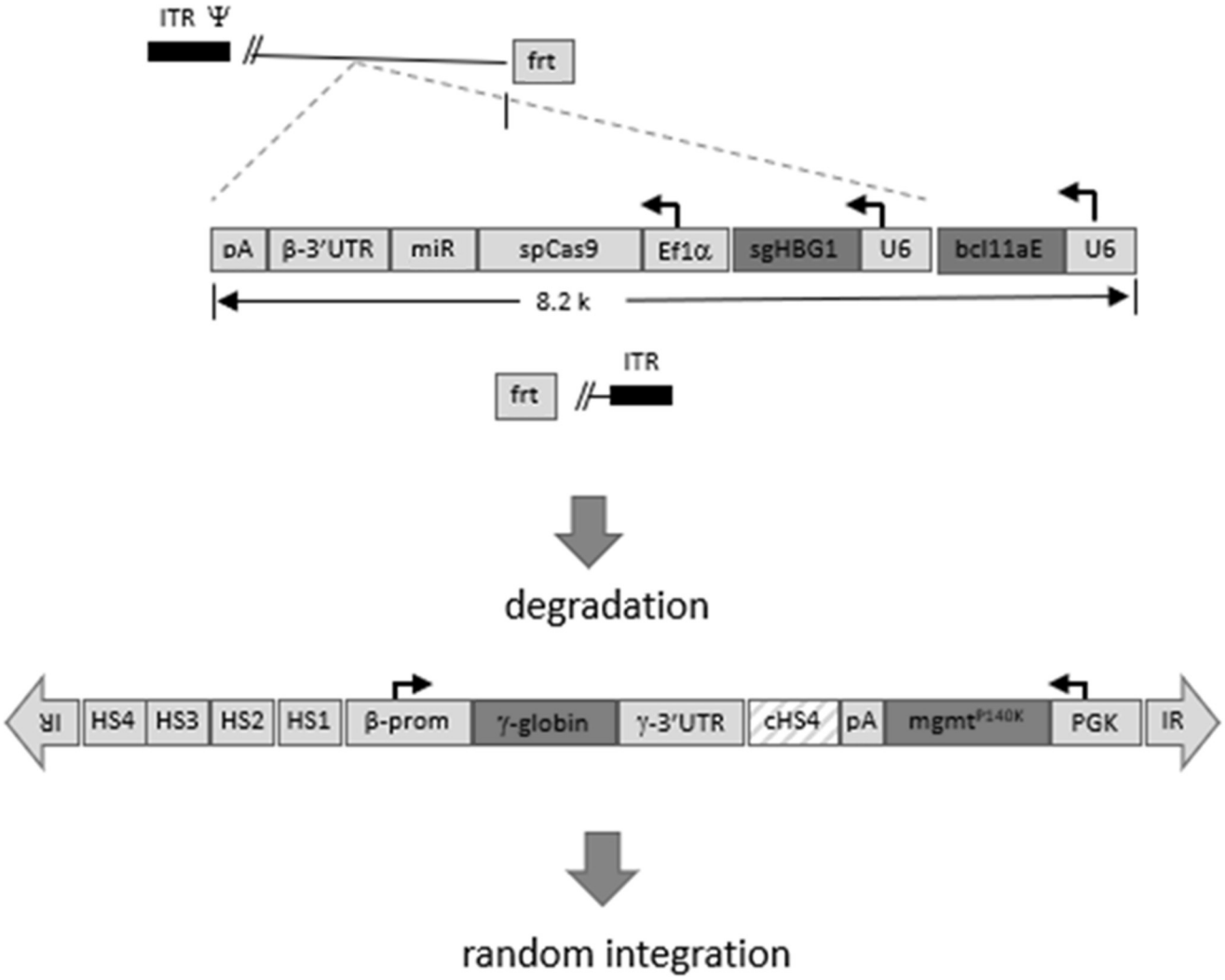

ITR Ψ
frt
pA
β-3'UTR
miR
spCas9
Ef1α
sgHBG1
U6
bcl11aE
U6
8.2 k
frt
ITR
degradation
IR
HS4
HS3
HS2
HS1
β-prom
γ-globin
γ-3'UTR
cHS4
pA
mgmt^{P140K}
PGK
IR
random integration

FIG. 97B

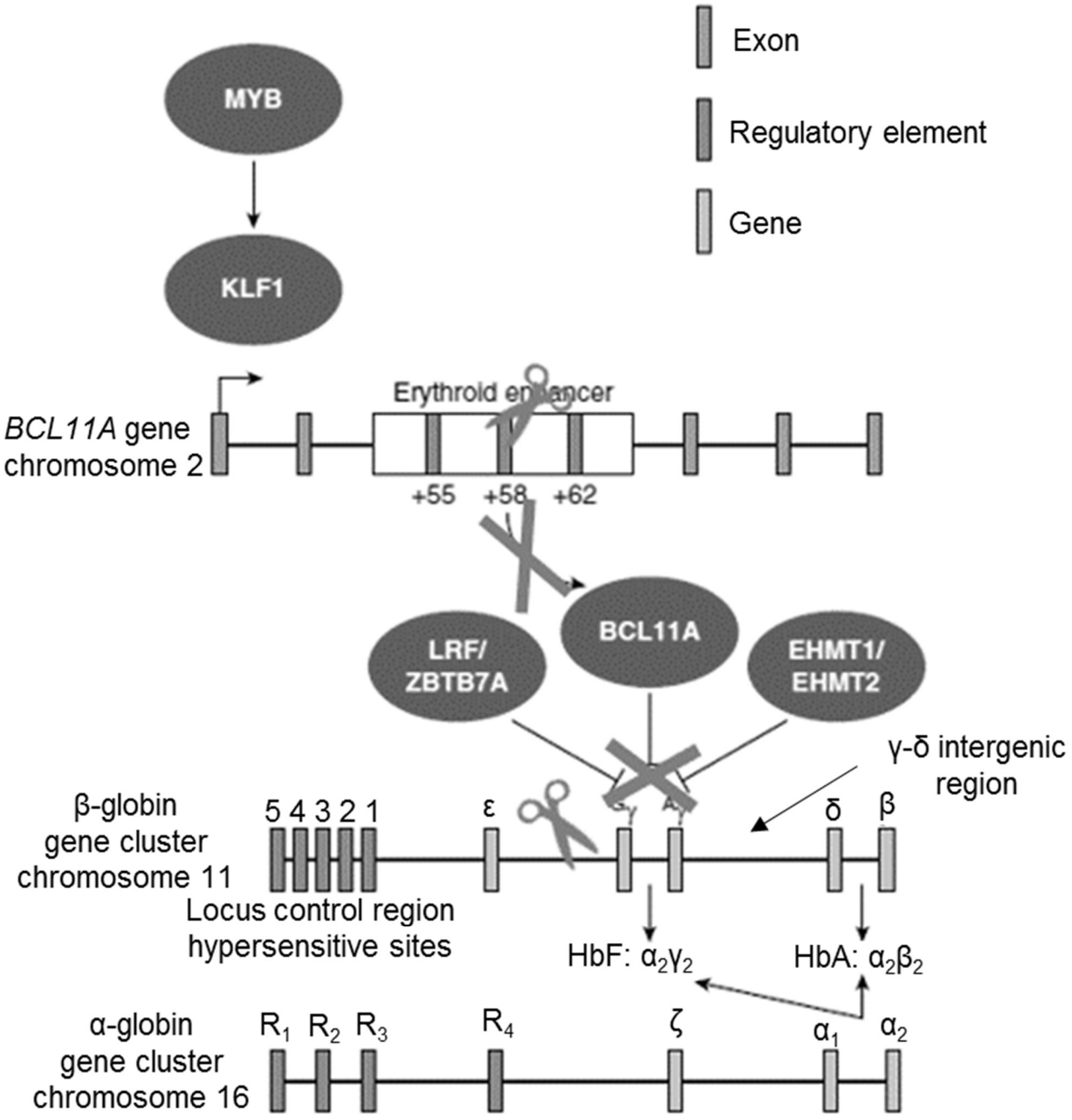

Exon
Regulatory element
Gene
MYB
KLF1
BCL11A gene
chromosome 2
Erythroid enhancer
+55
+58
+62
LRF/
ZBTB7A
BCL11A
EHMT1/
EHMT2
γ-δ intergenic
region
β-globin
gene cluster
chromosome 11
5 4 3 2 1
ε
δ
β
Locus control region
hypersensitive sites
HbF: α2γ2
HbA: α2β2
α-globin
gene cluster
chromosome 16
R1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

FIG. 97C

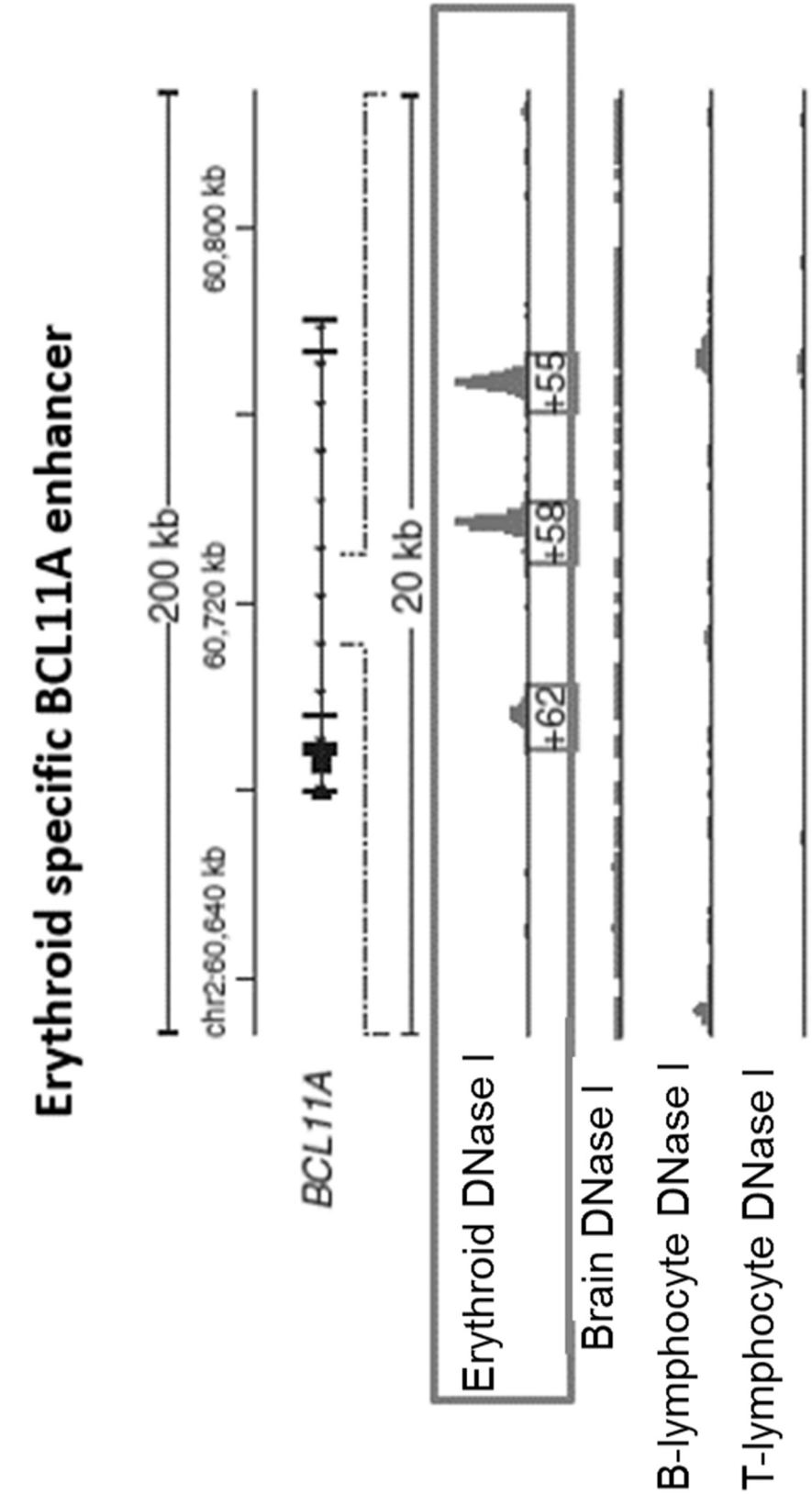

Erythroid specific BCL11A enhancer
200 kb
chr2:60,640 kb
60,720 kb
60,800 kb
BCL11A
20 kb
+62
+58
+55
Erythroid DNase I
Brain DNase I
B-lymphocyte DNase I
T-lymphocyte DNase I

FIG. 97D

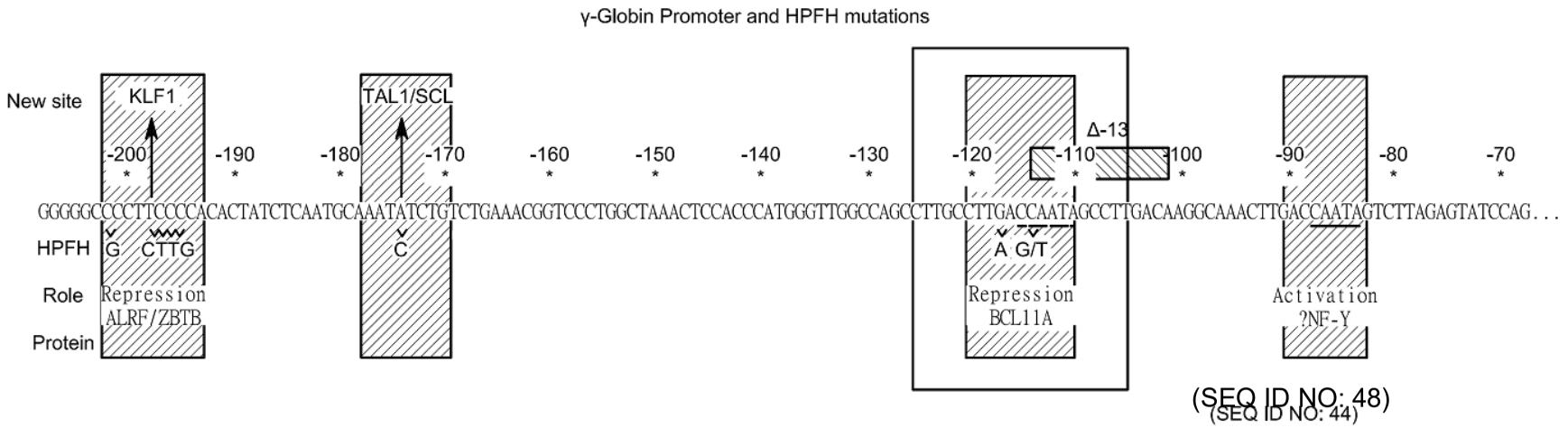


FIG. 98A

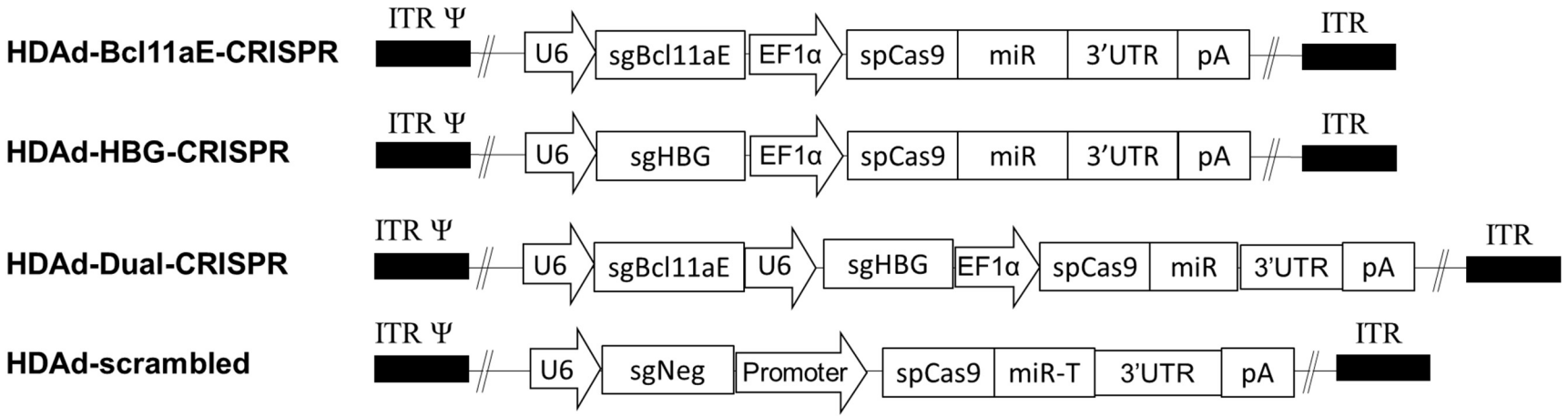


FIG. 98B

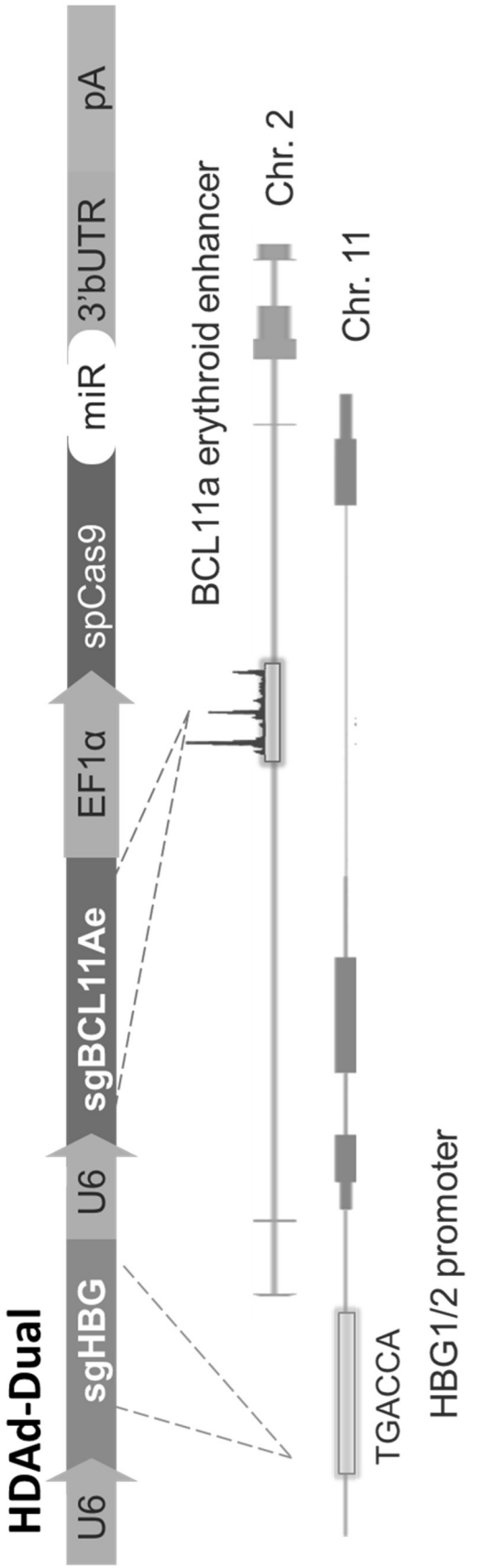


FIG. 98C

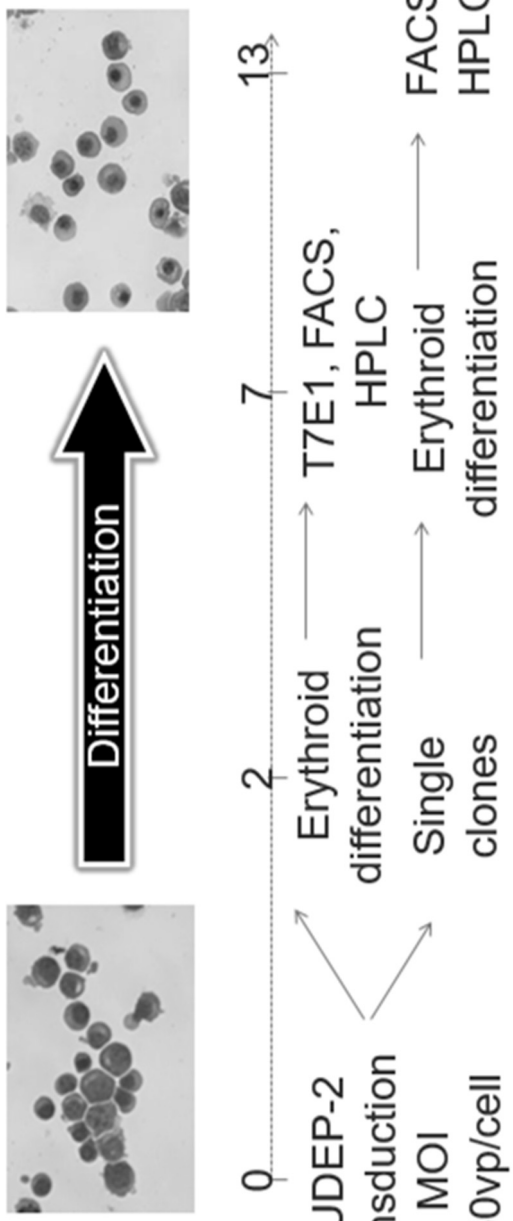


FIG. 98D

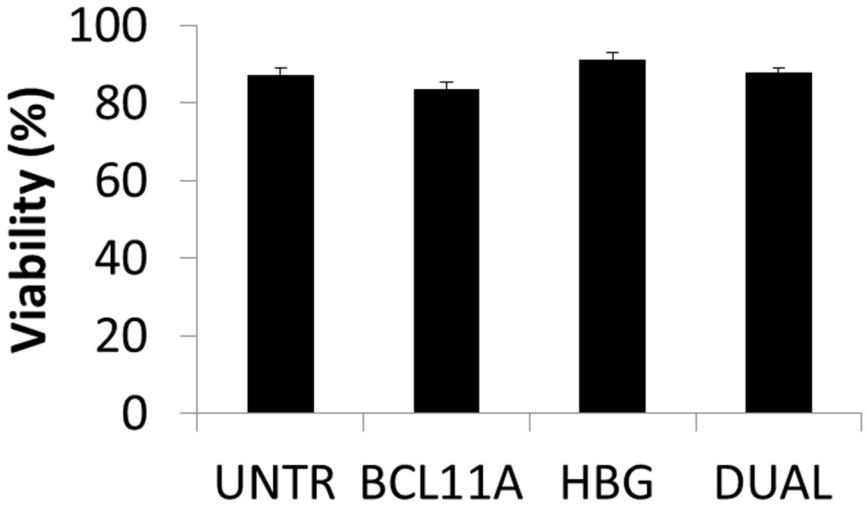


FIG. 98E

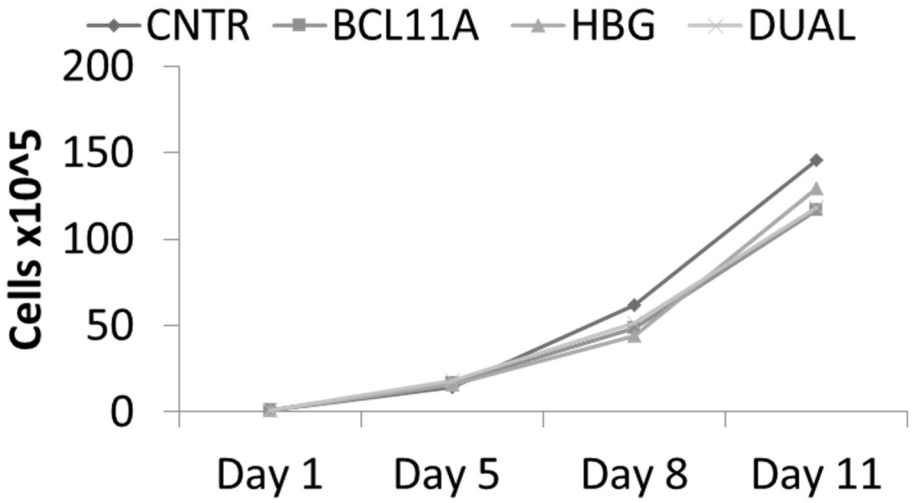


FIG. 98F

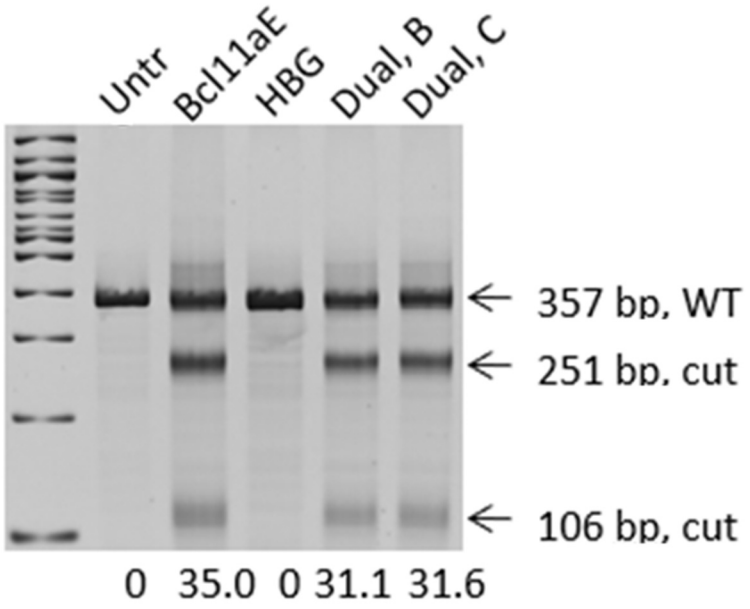


FIG. 98G

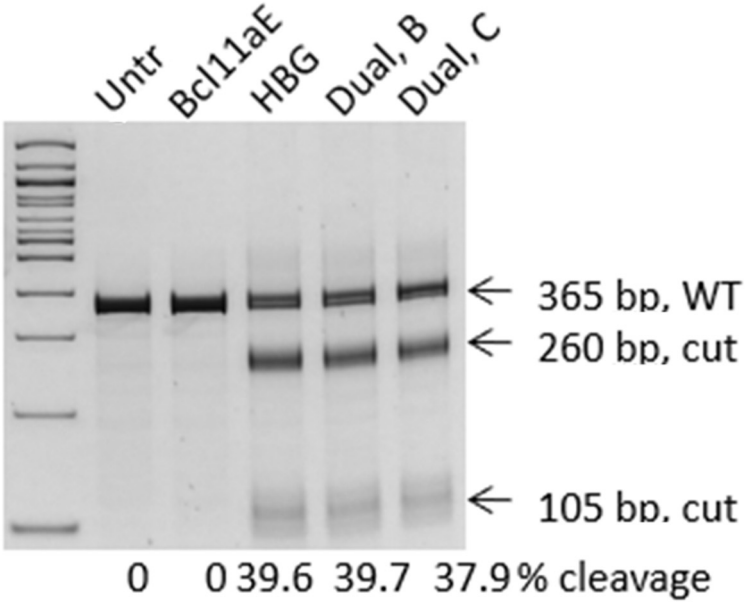


FIG. 98H

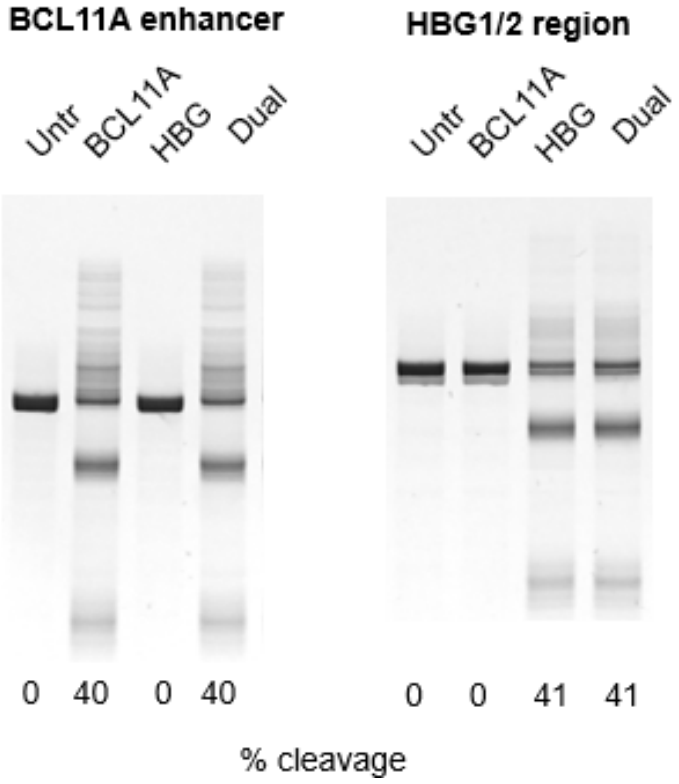
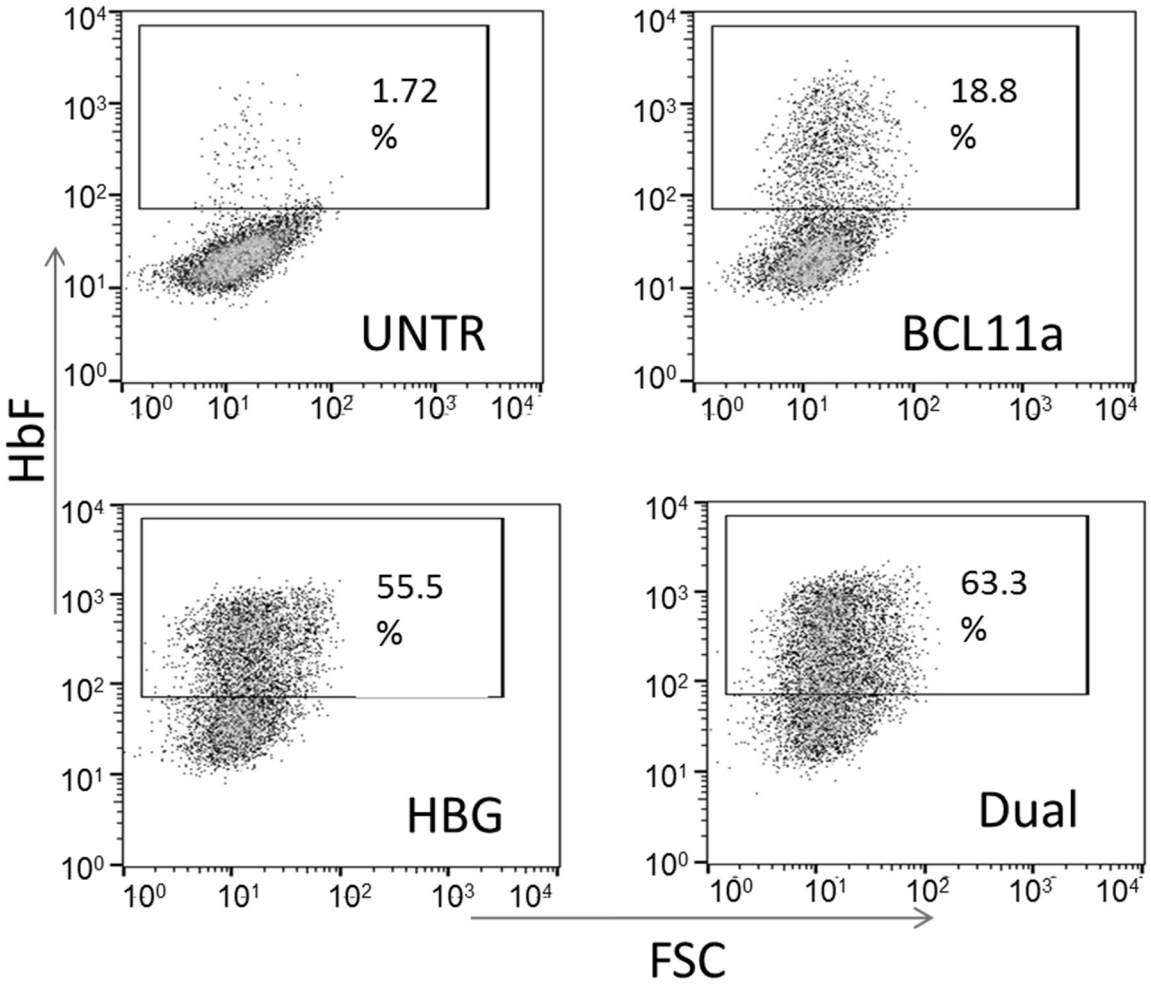

BCL11A enhancer
HBG1/2 region
Untr
BCL11A
HBG
Dual
Untr
BCL11A
HBG
Dual
0 40 0 40
0 0 41 41
% cleavage

FIG. 98I


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

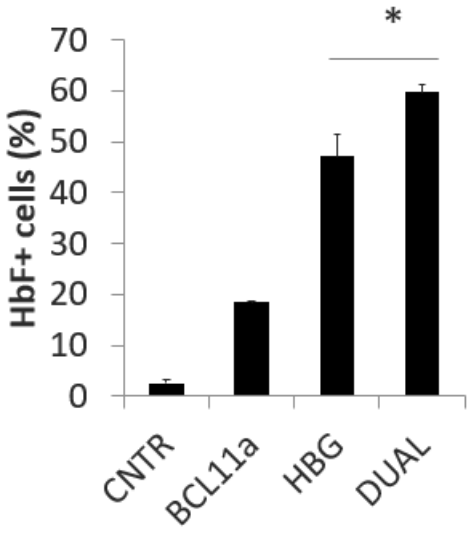

*
HbF+ cells (%)
70
60
50
40
30
20
10
0
CNTR
BCL11a
HBG
DUAL

FIG. 98J

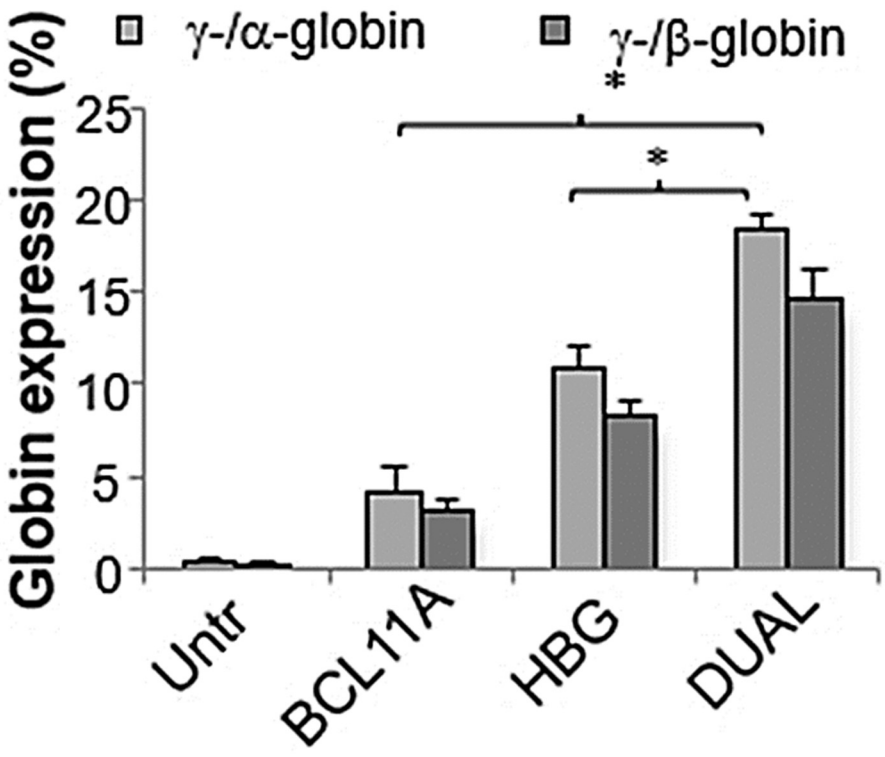

γ-/α-globin
γ-/β-globin
Globin expression (%)
25
20
15
10
5
0
*
*
Untr
BCL11A
HBG
DUAL

FIG. 98K

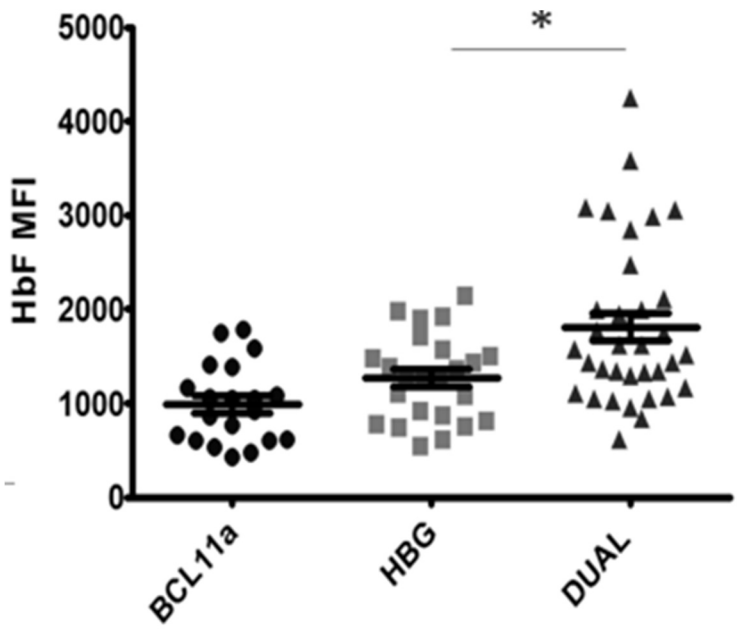
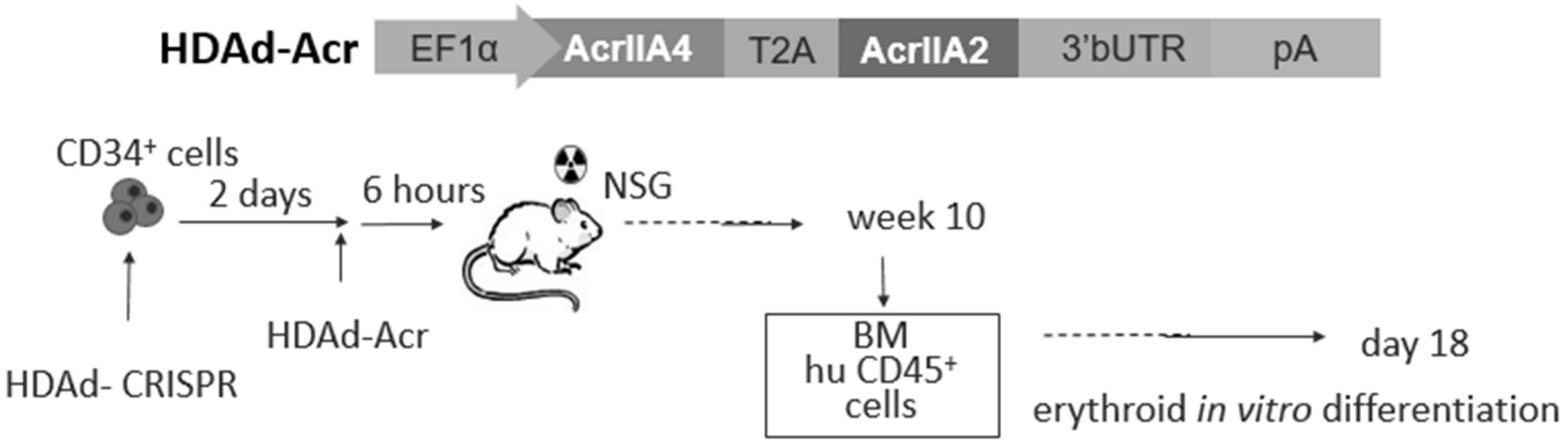


FIG. 98L



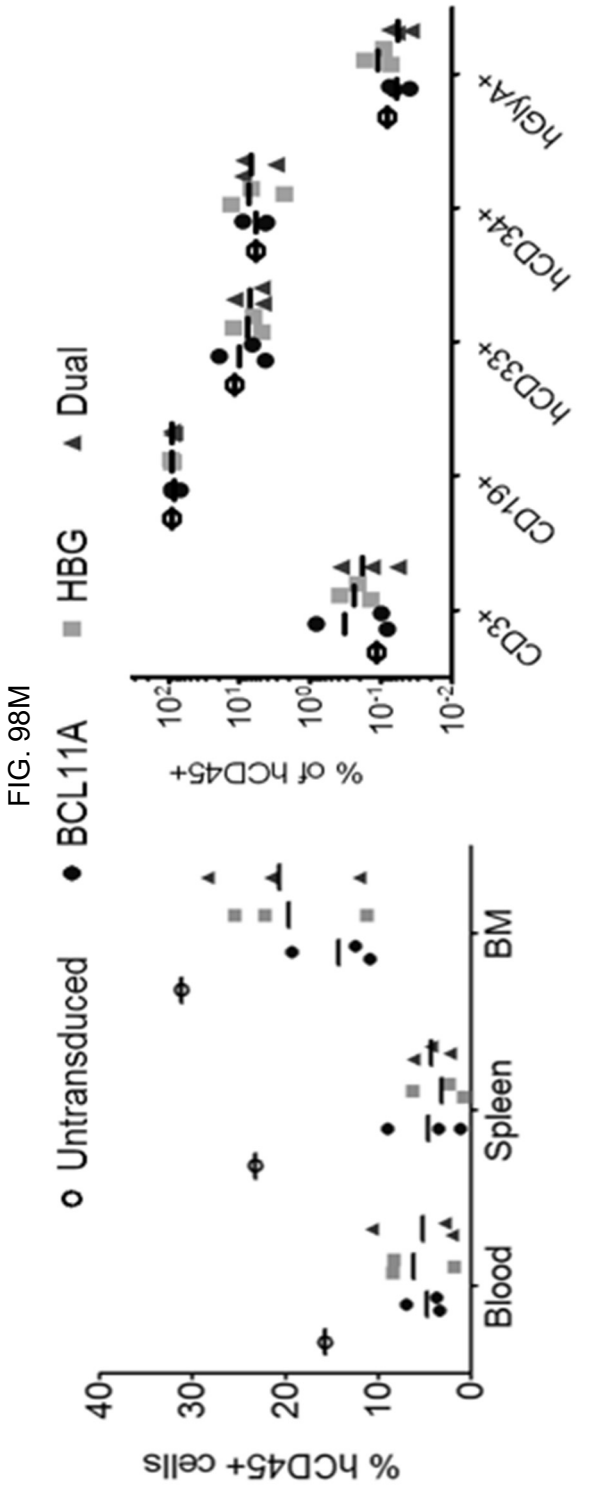


FIG. 98M

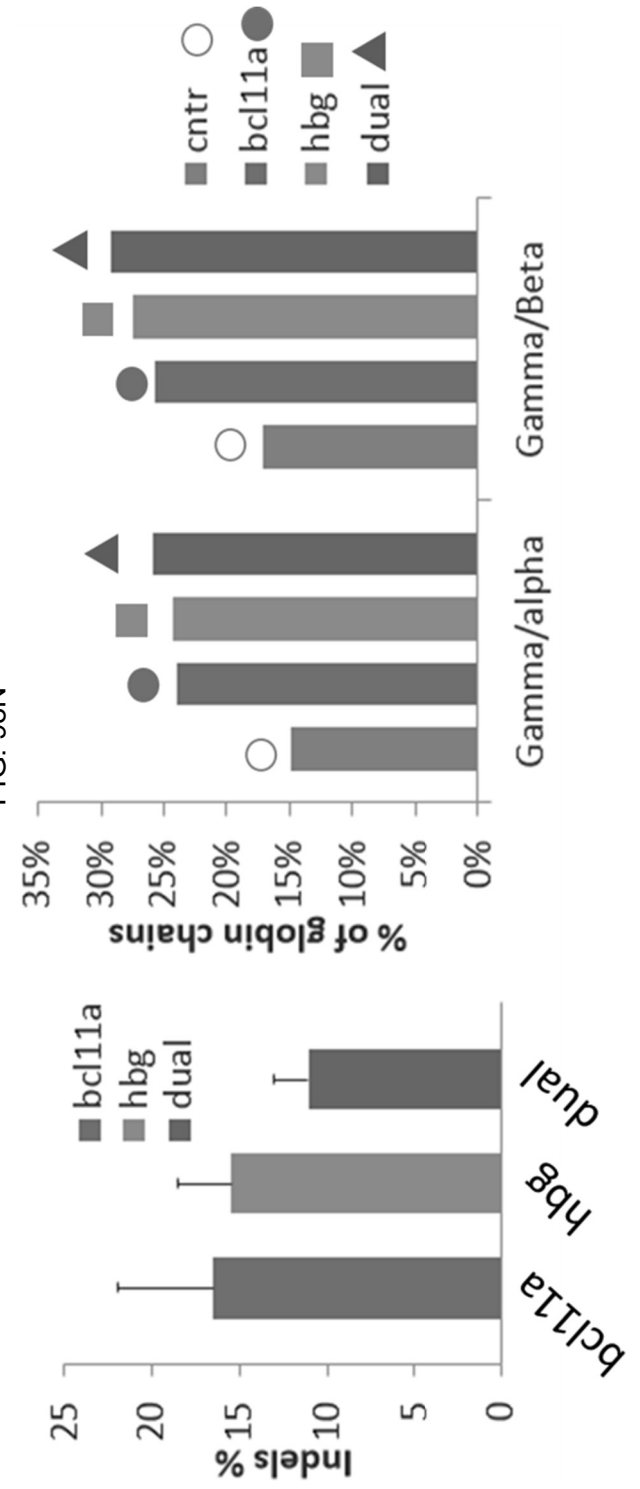


FIG. 98N

FIG. 99A

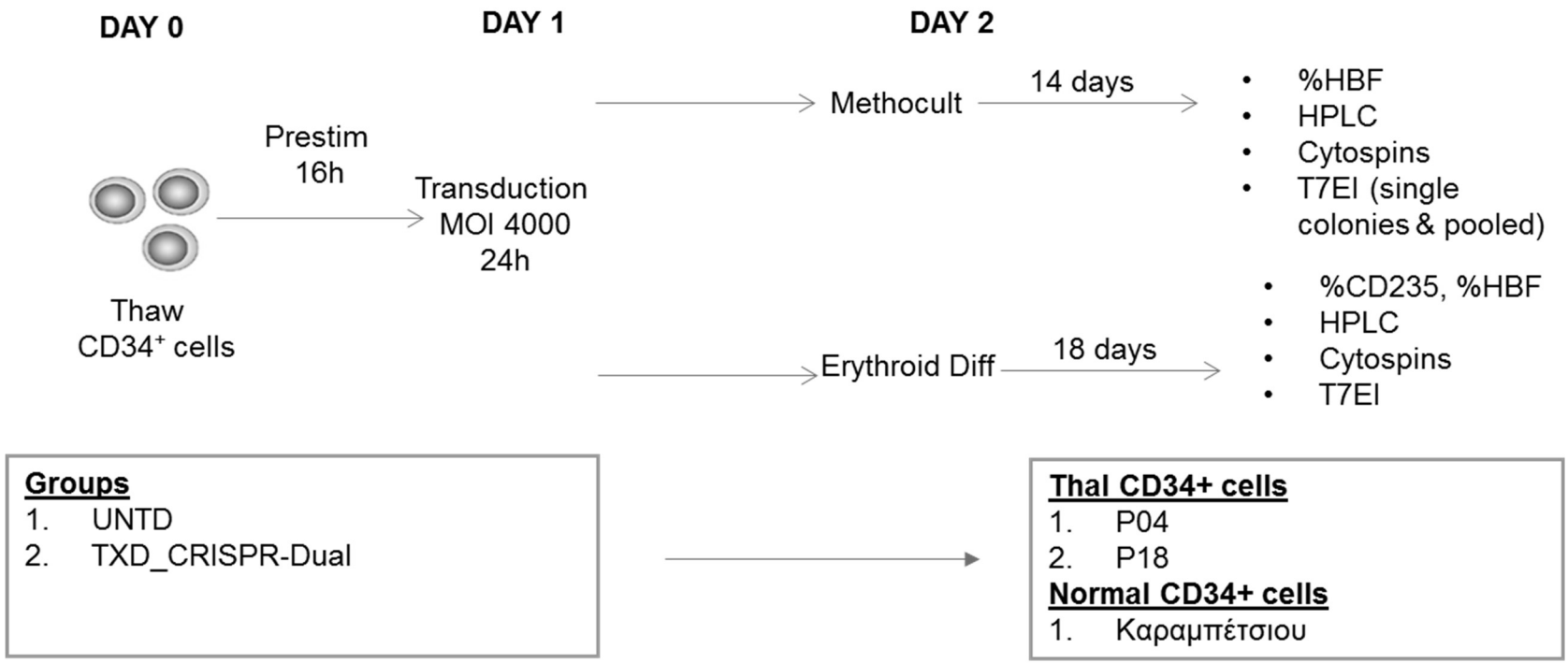

DAY 0
DAY 1
DAY 2
Thaw
CD34+ cells
Prestim
16h
Transduction
MOI 4000
24h
Methocult
14 days
%HBF
HPLC
Cytospins
T7EI (single colonies & pooled)
Erythroid Diff
18 days
%CD235, %HBF
HPLC
Cytospins
T7EI
Groups
1. UNTD
2. TXD_CRISPR-Dual
Thal CD34+ cells
1. P04
2. P18
Normal CD34+ cells
1. Καραμπέτσιου

FIG. 99B

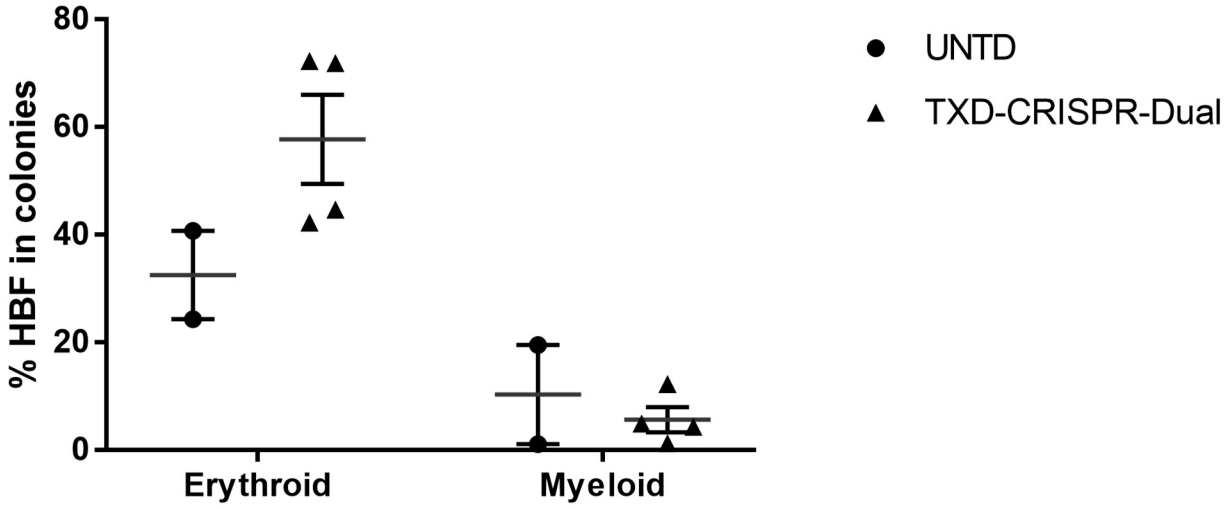


FIG. 99C

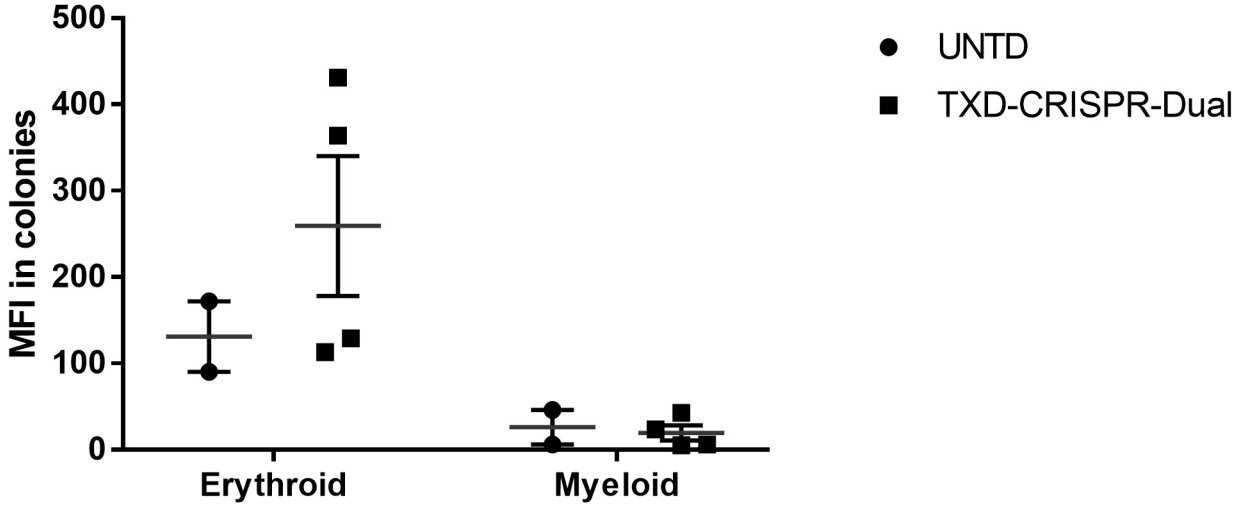


FIG. 99D

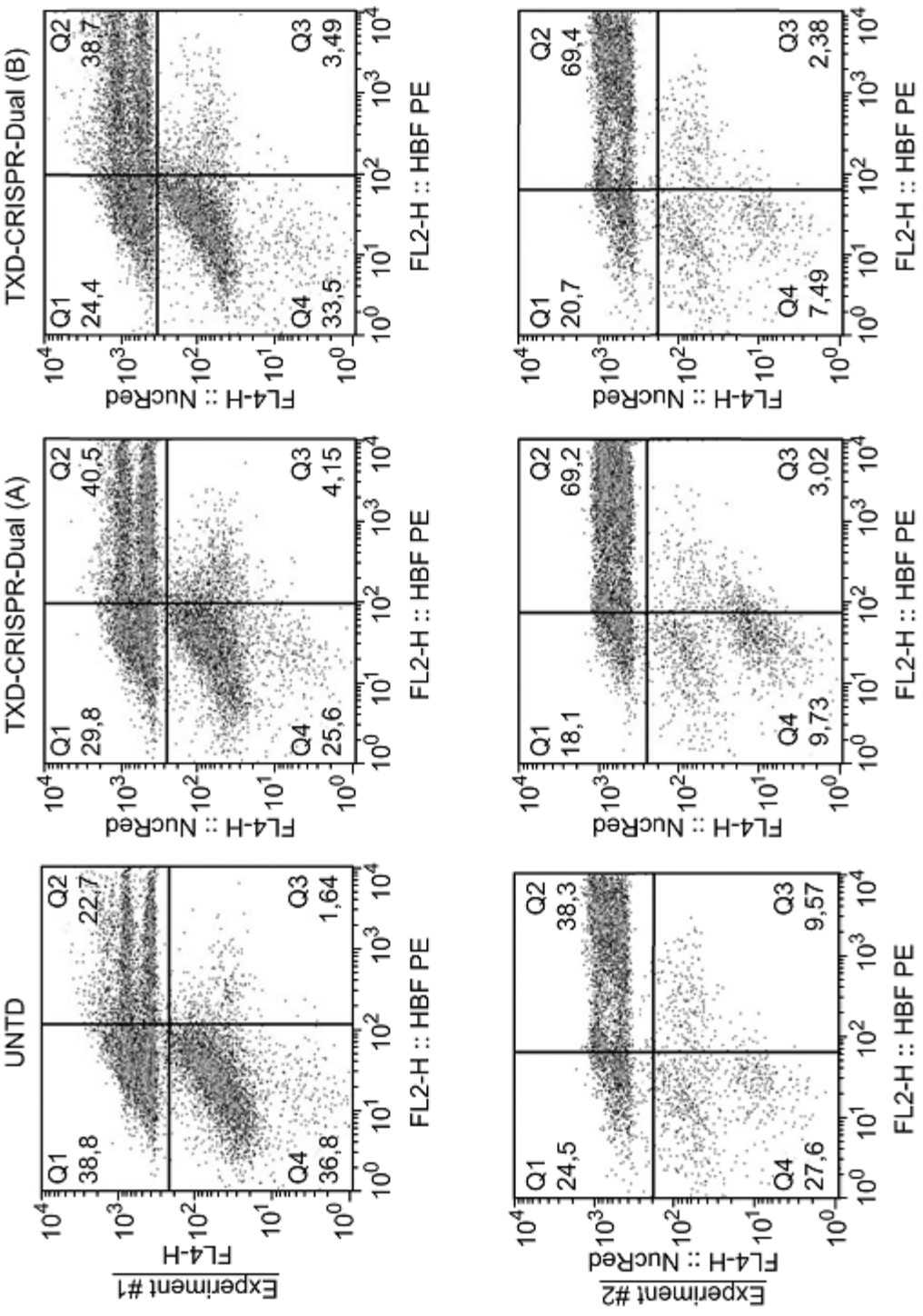

UNTD
TXD-CRISPR-Dual (A)
TXD-CRISPR-Dual (B)
Experiment #1
Experiment #2
FL4-H
FL4-H :: NucRed
FL2-H :: HBF PE
Q1 38,8
Q2 22,7
Q3 1,64
Q4 36,8
Q1 29,8
Q2 40,5
Q3 4,15
Q4 25,6
Q1 24,4
Q2 38,7
Q3 3,49
Q4 33,5
Q1 24,5
Q2 38,3
Q3 9,57
Q4 27,6
Q1 18,1
Q2 69,2
Q3 3,02
Q4 9,73
Q1 20,7
Q2 69,4
Q3 2,38
Q4 7,49

FIG. 99E

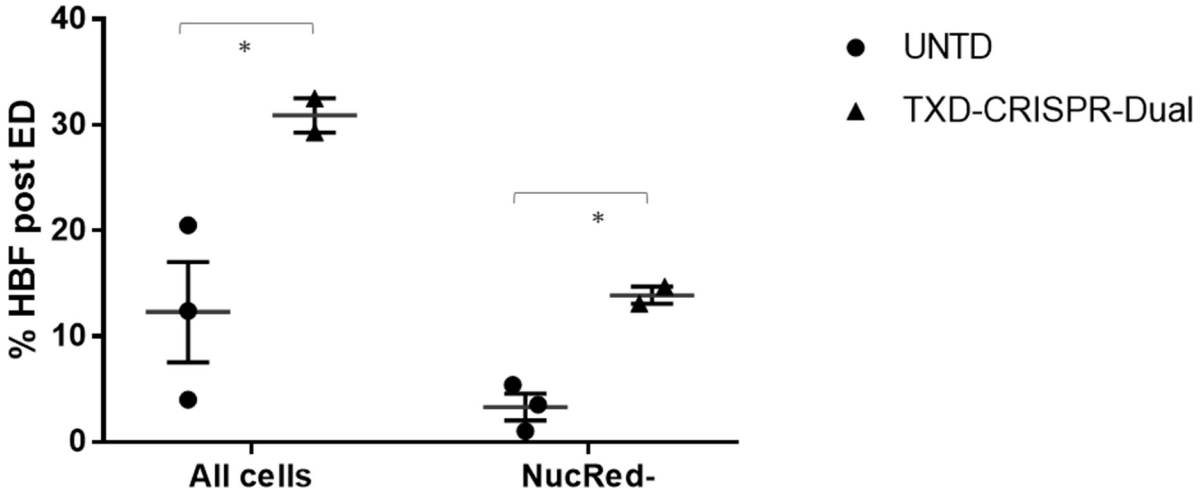


FIG. 99F

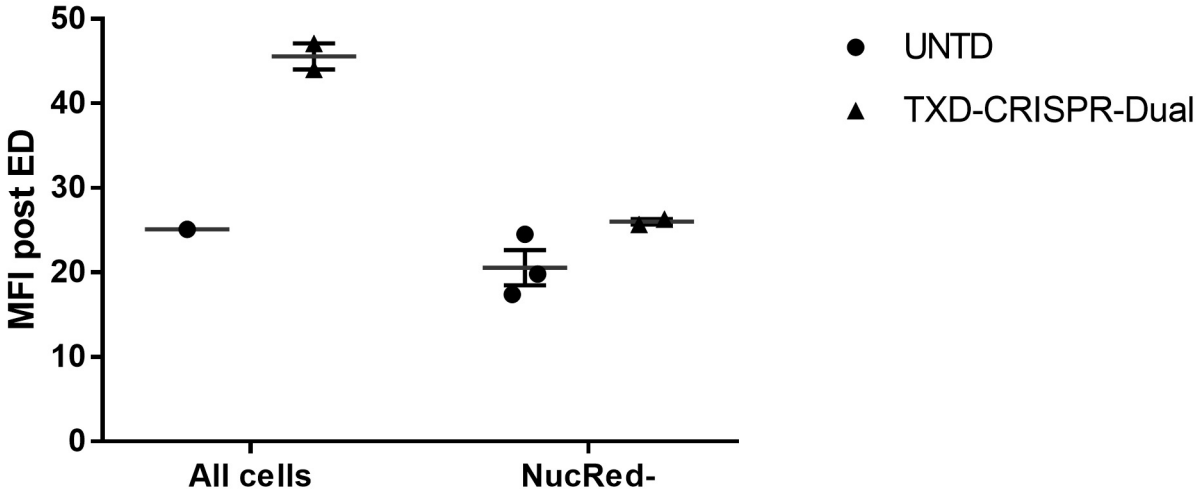


FIG. 99G

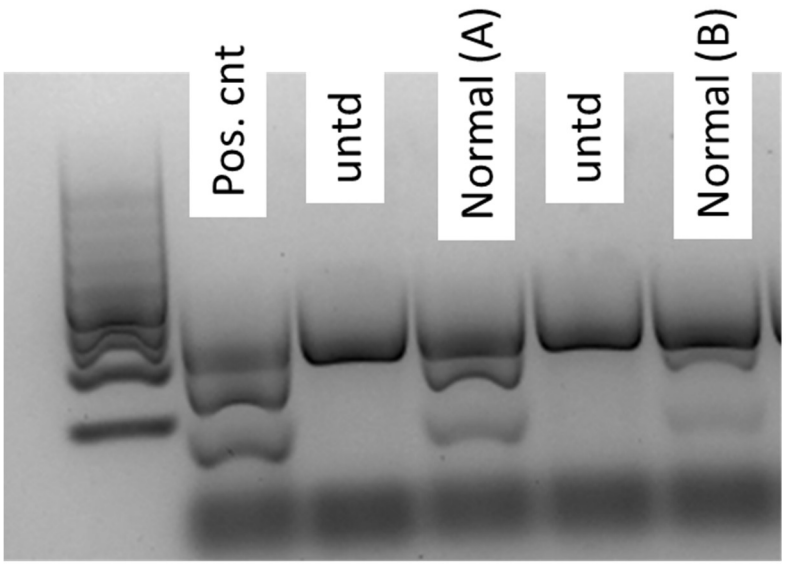


FIG. 99H

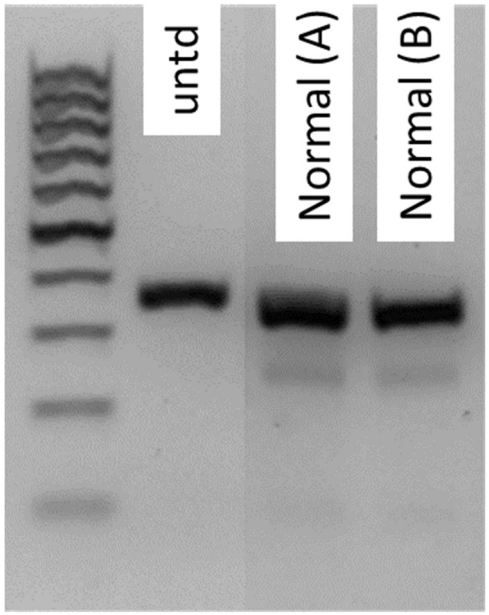


FIG. 99I

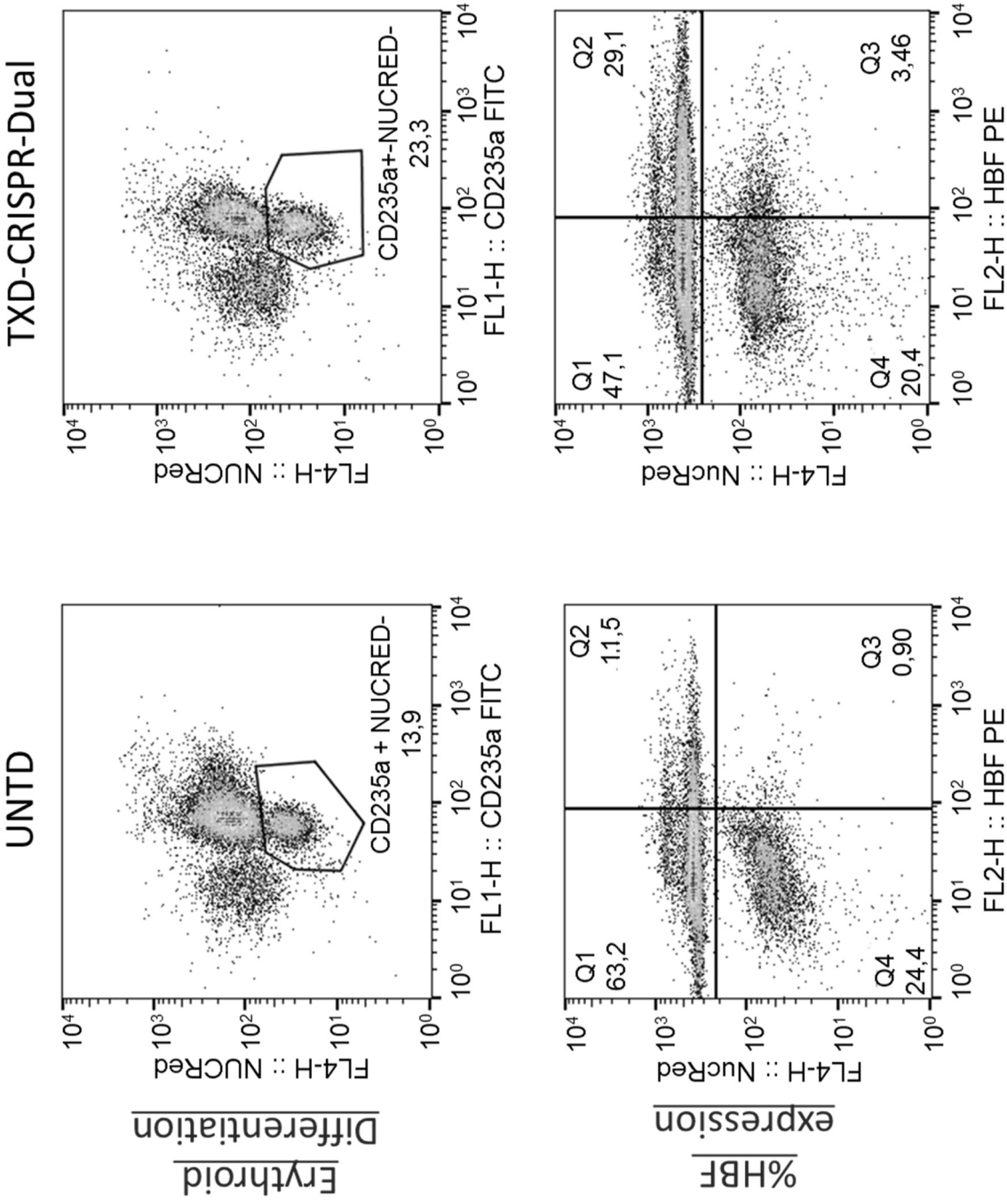

UNTD
TXD-CRISPR-Dual
Erythroid Differentiation
%HBF expression
FL4-H :: NUCRed
FL1-H :: CD235a FITC
FL2-H :: HBF PE
CD235a + NUCRED- 13,9
CD235a+-NUCRED- 23,3
Q1 63,2
Q2 11,5
Q3 0,90
Q4 24,4
Q1 47,1
Q2 29,1
Q3 3,46
Q4 20,4

FIG. 99J

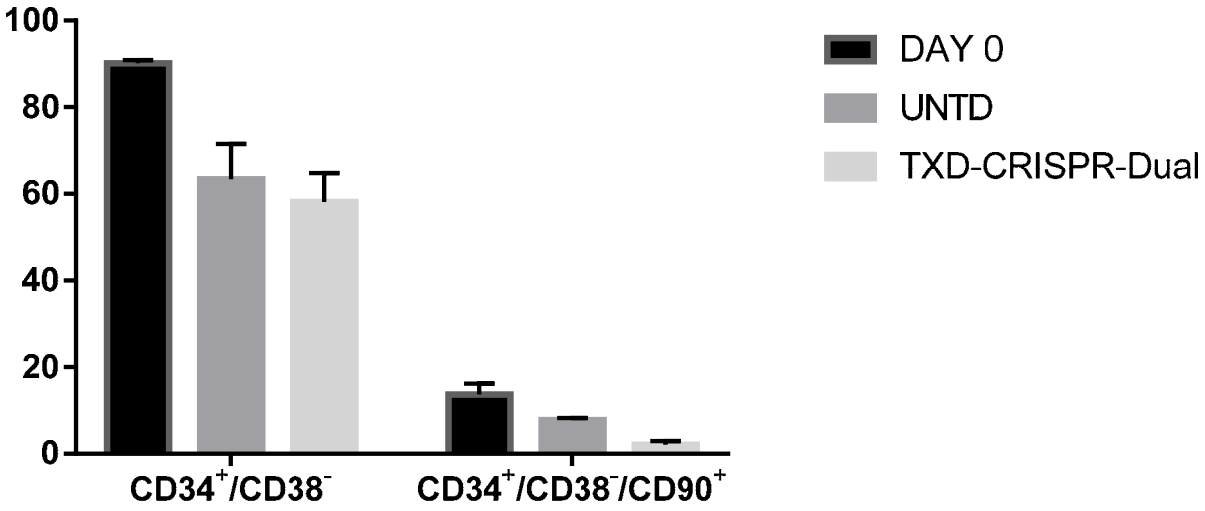


FIG. 99 K

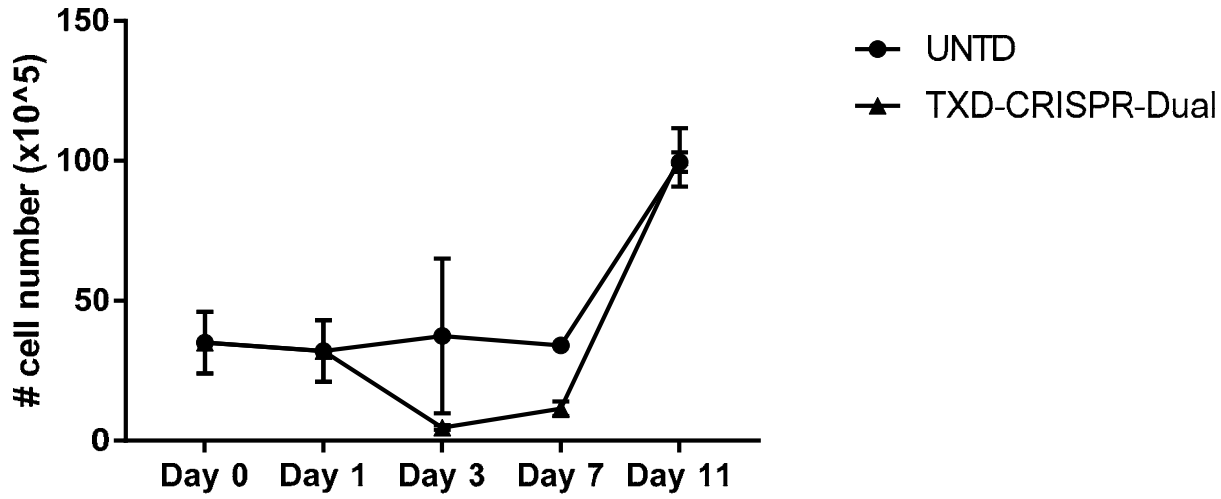


FIG. 99L

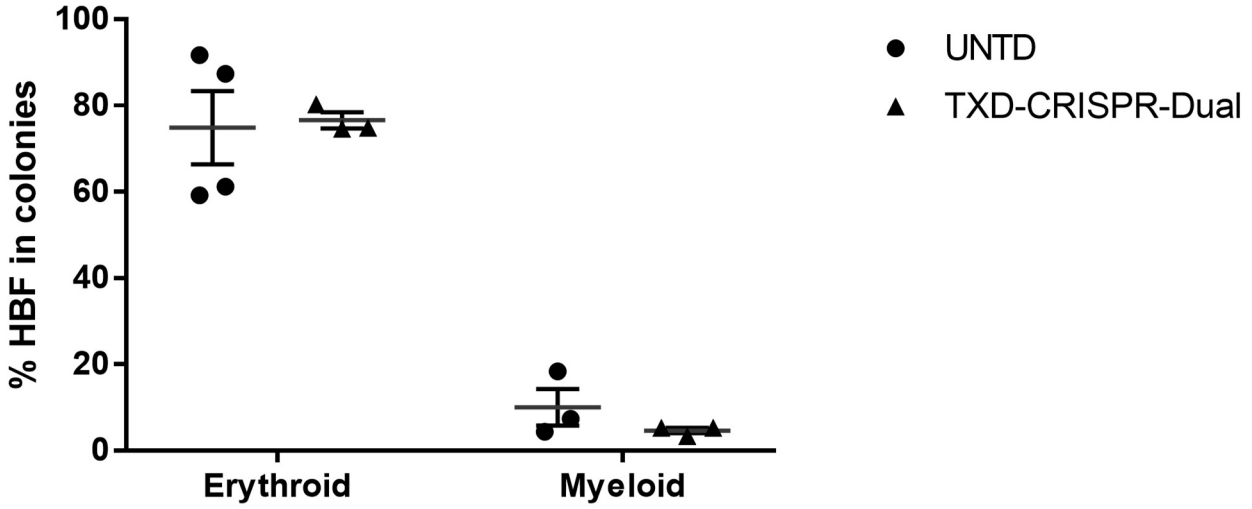


FIG. 99M

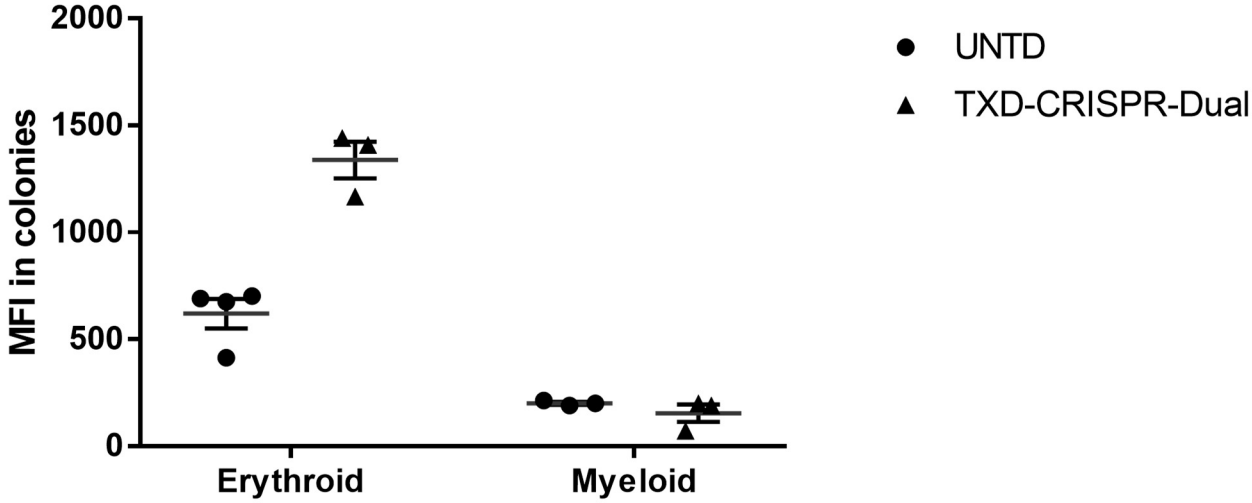


FIG. 99N

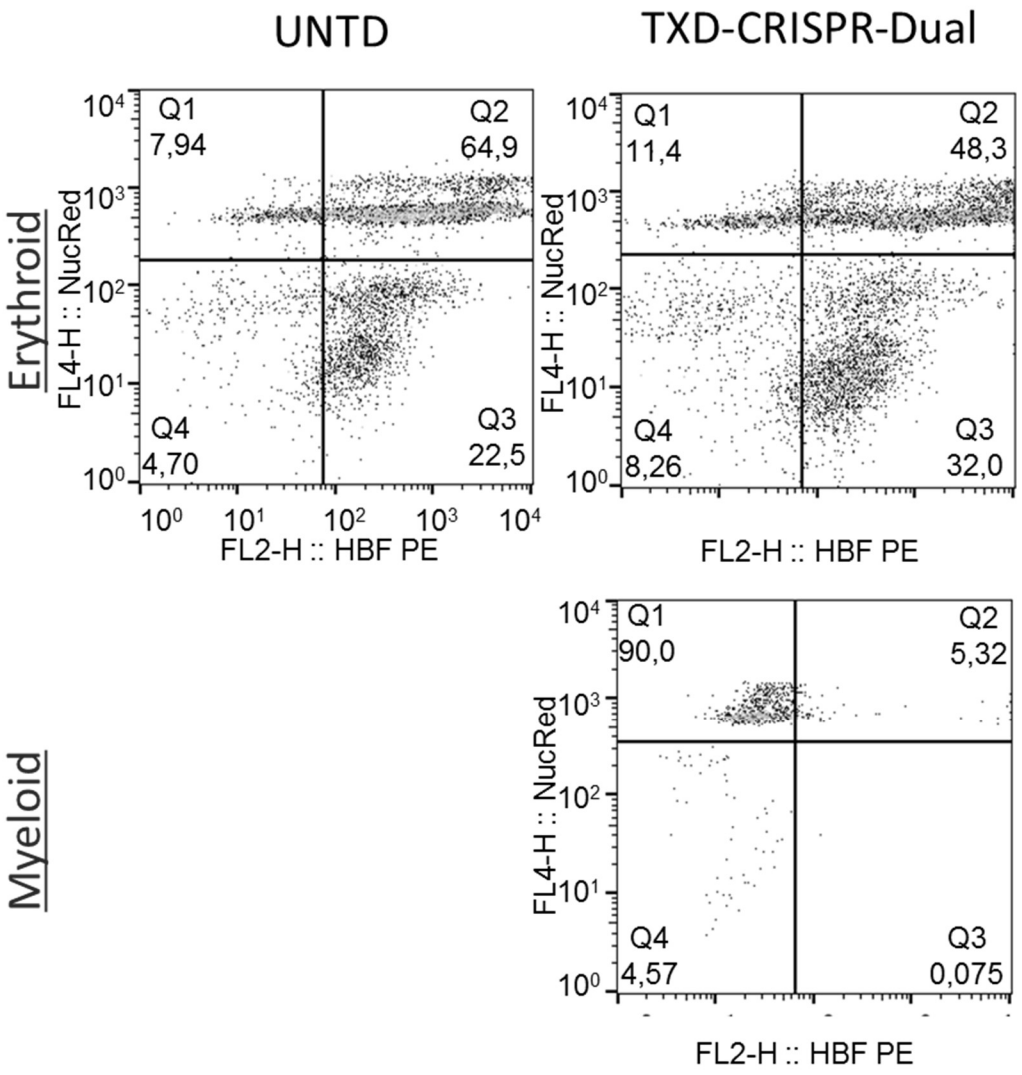

UNTD
TXD-CRISPR-Dual
Erythroid
Myeloid
FL4-H :: NucRed
FL2-H :: HBF PE
Q1
7,94
Q2
64,9
Q4
4,70
Q3
22,5
Q1
11,4
Q2
48,3
Q4
8,26
Q3
32,0
Q1
90,0
Q2
5,32
Q4
4,57
Q3
0,075

FIG. 99O

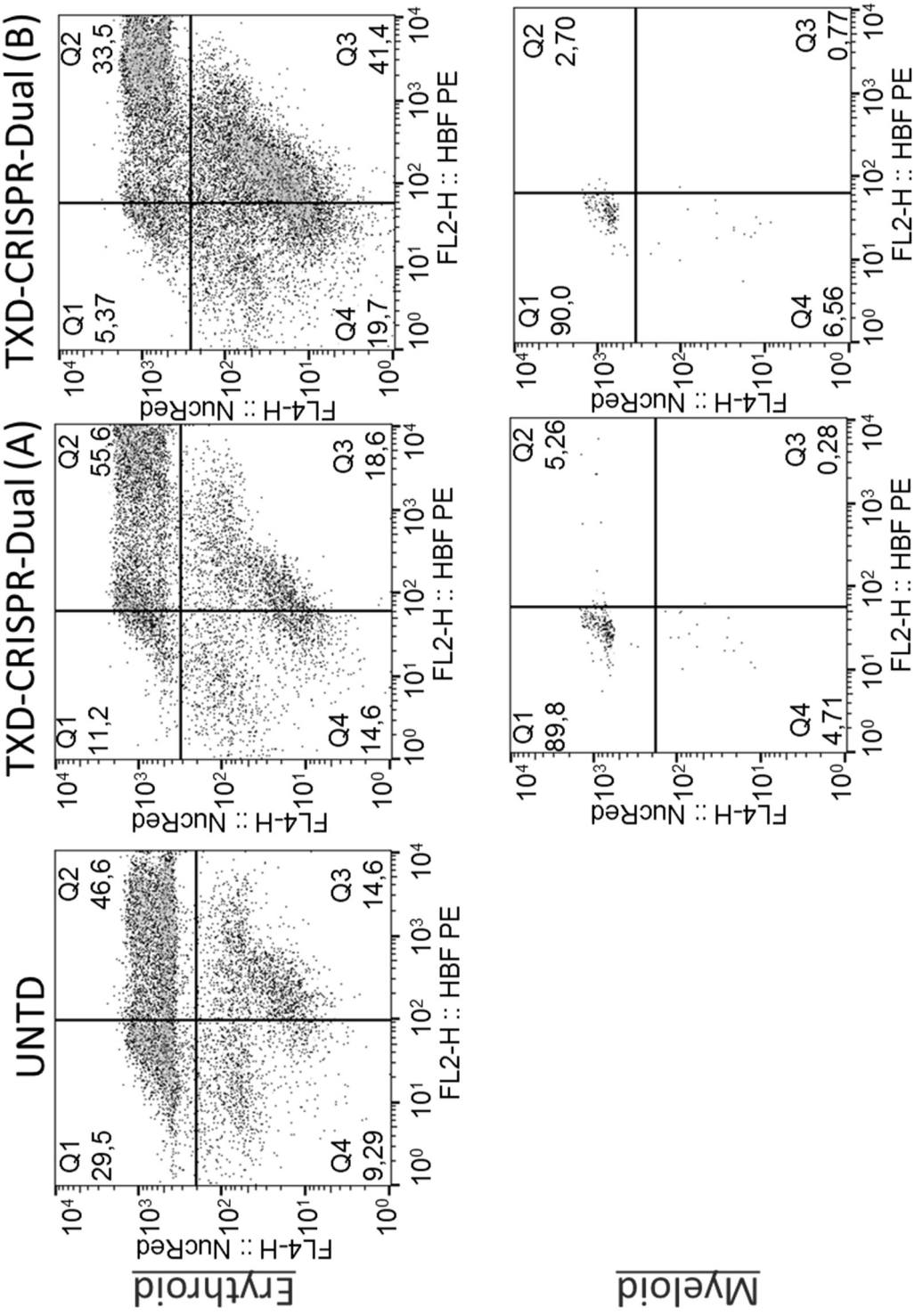

UNTD
TXD-CRISPR-Dual (A)
TXD-CRISPR-Dual (B)
Erythroid
Myeloid
FL4-H :: NucRed
FL2-H :: HBF PE
Q1 29,5
Q2 46,6
Q4 9,29
Q3 14,6
Q1 11,2
Q2 55,6
Q4 14,6
Q3 18,6
Q1 5,37
Q2 33,5
Q4 19,7
Q3 41,4
Q1 89,8
Q2 5,26
Q4 4,71
Q3 0,28
Q1 90,0
Q2 2,70
Q4 6,56
Q3 0,77

FIG. 99P

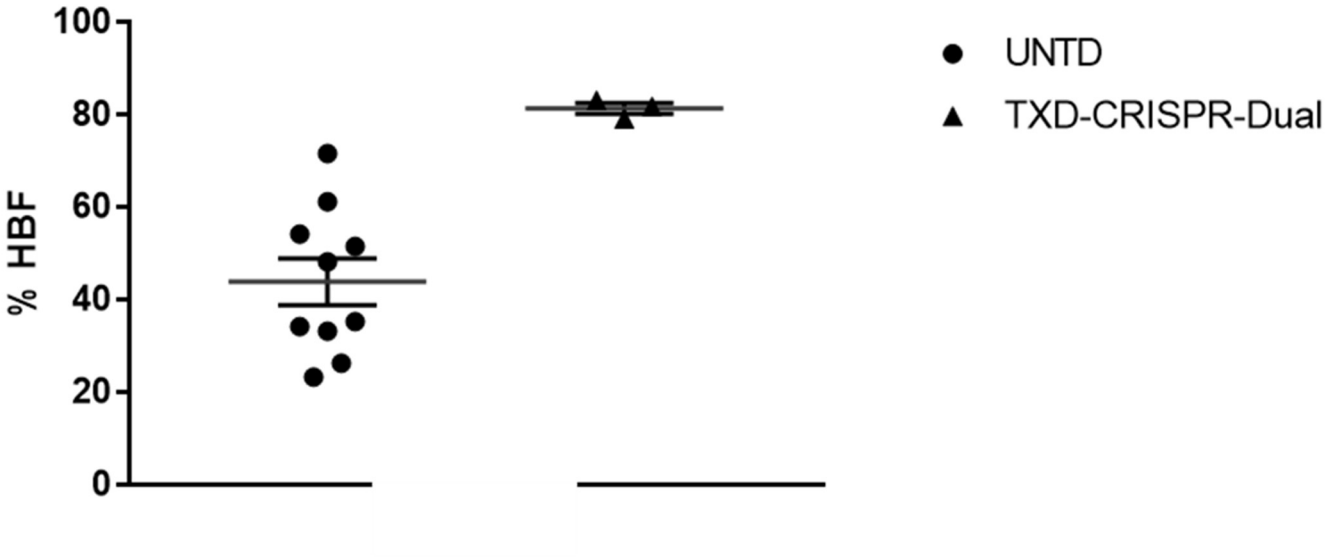


FIG. 99Q

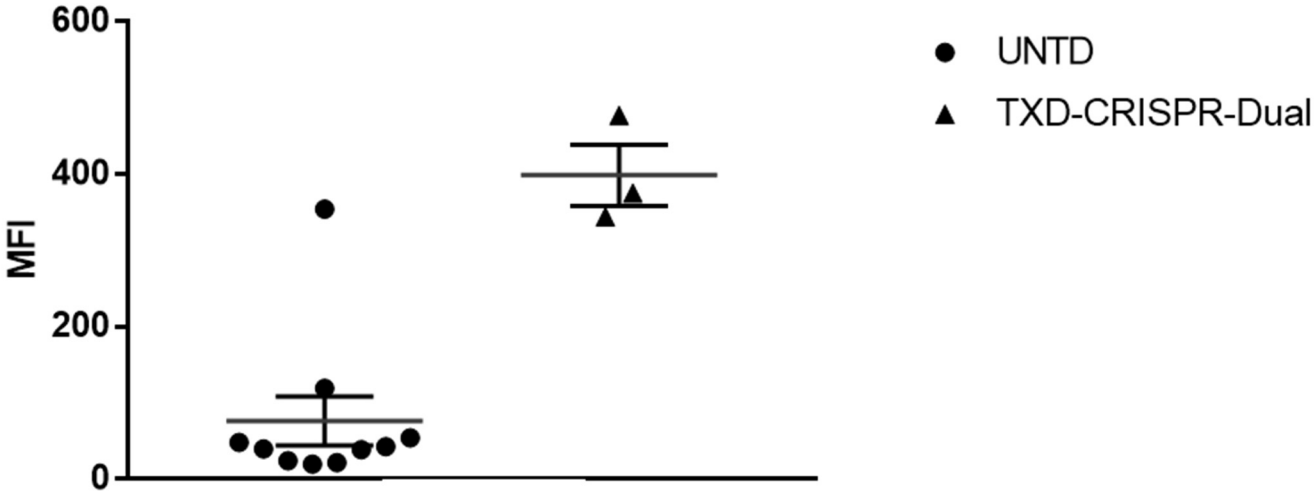


FIG. 99R

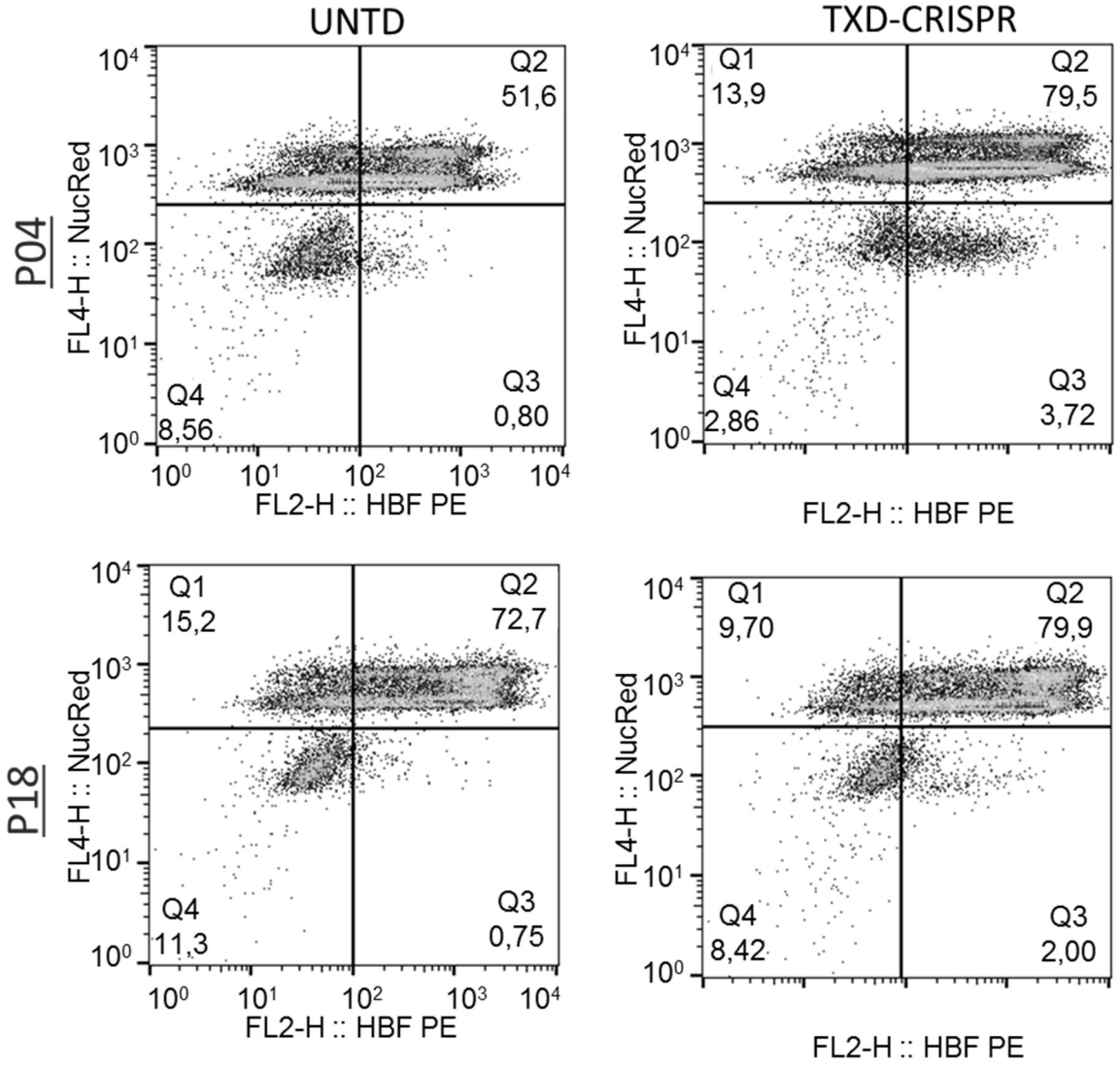

UNTD
TXD-CRISPR
P04
P18
FL4-H :: NucRed
FL2-H :: HBF PE
Q1
Q2
Q3
Q4
51,6
0,80
8,56
13,9
79,5
3,72
2,86
15,2
72,7
0,75
11,3
9,70
79,9
2,00
8,42

FIG. 99S

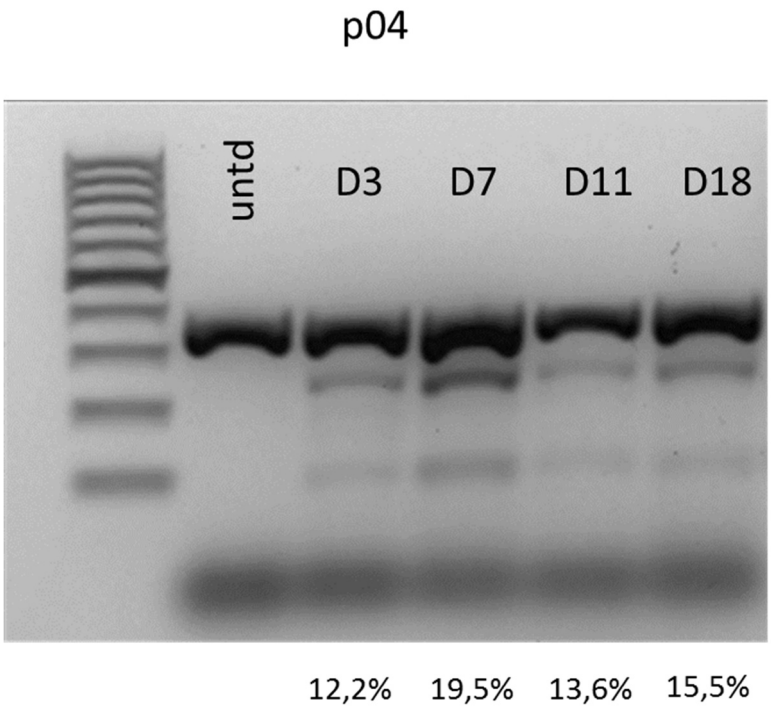


FIG. 99T

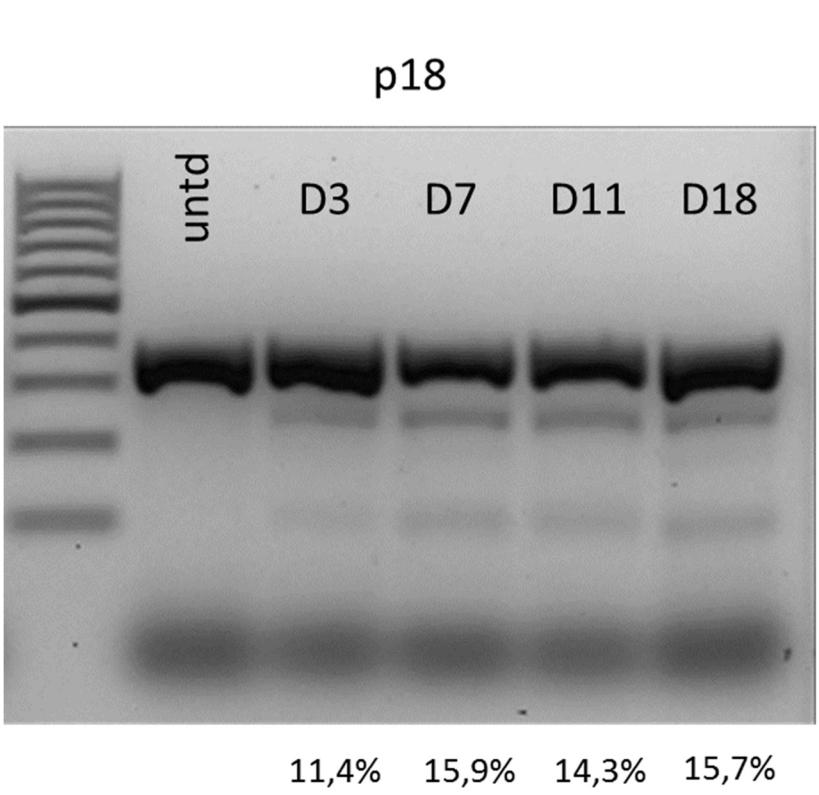


FIG. 99U

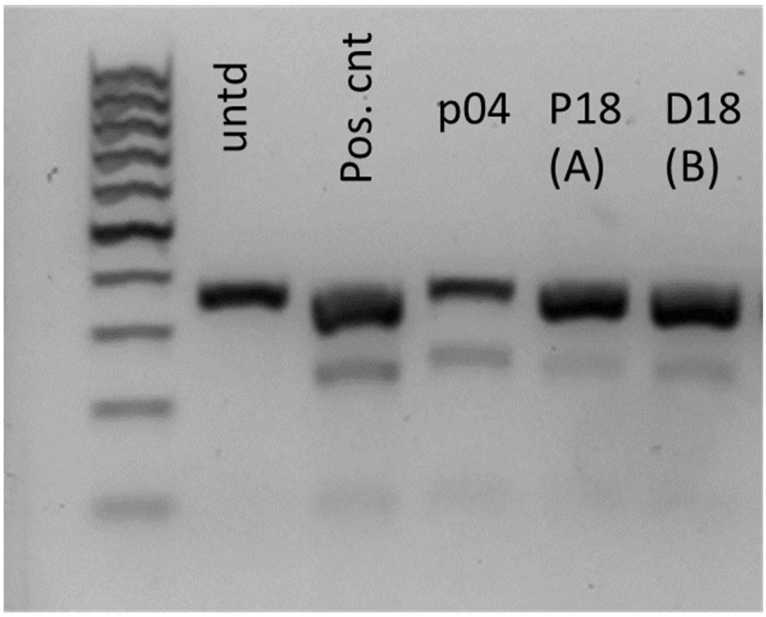


FIG. 100

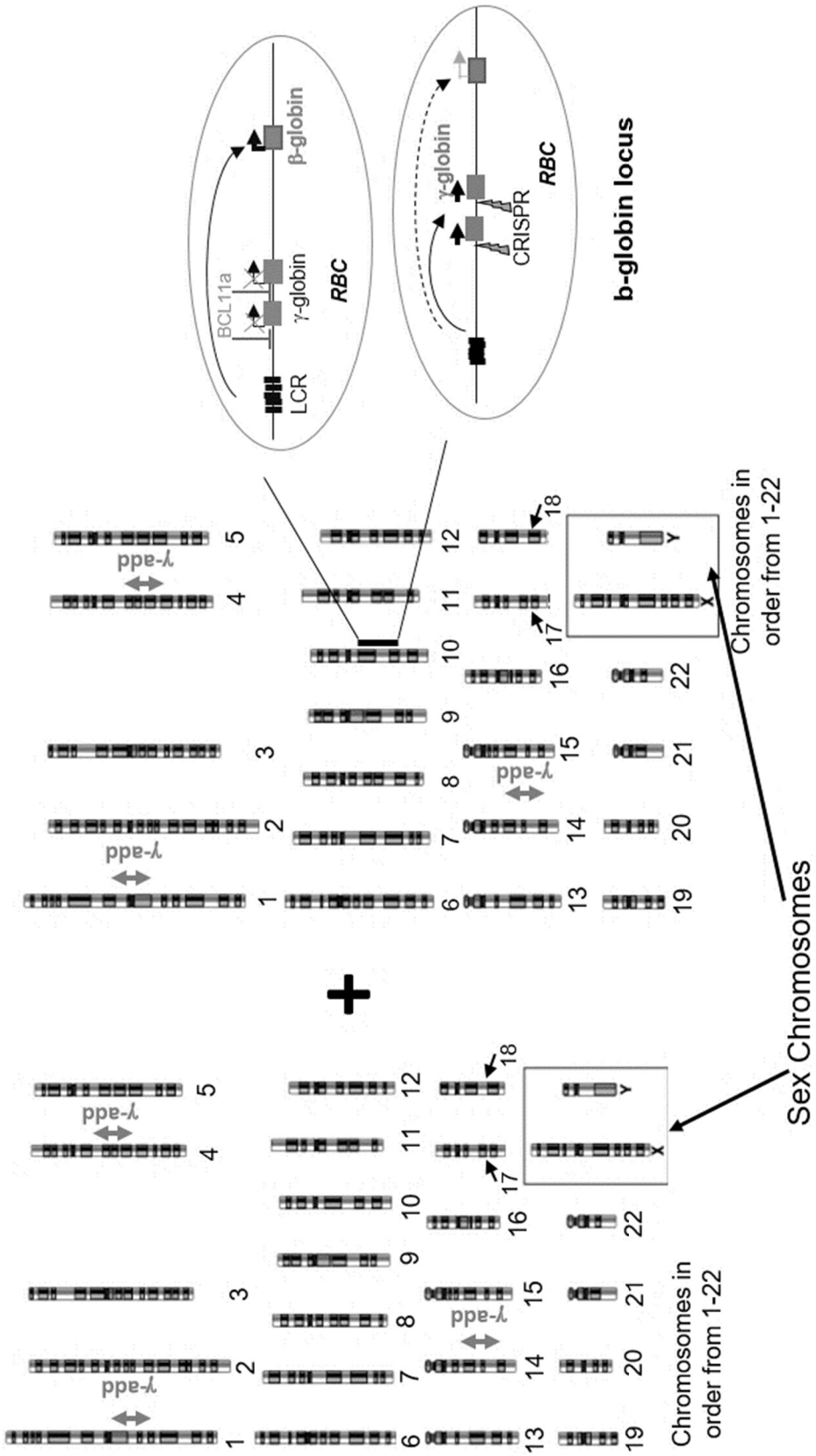

Combination of g-globin gene addition and re-activation of endogenous g-globin
SB100x-mediated
g-globin gene integration
CRISPR-Cas9-mediated
reactivation of fetal g-globin
γ-add
BCL11a
LCR
γ-globin
RBC
β-globin
γ-globin
CRISPR
RBC
b-globin locus
Chromosomes in order from 1-22
Sex Chromosomes
Chromosomes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

FIG. 101

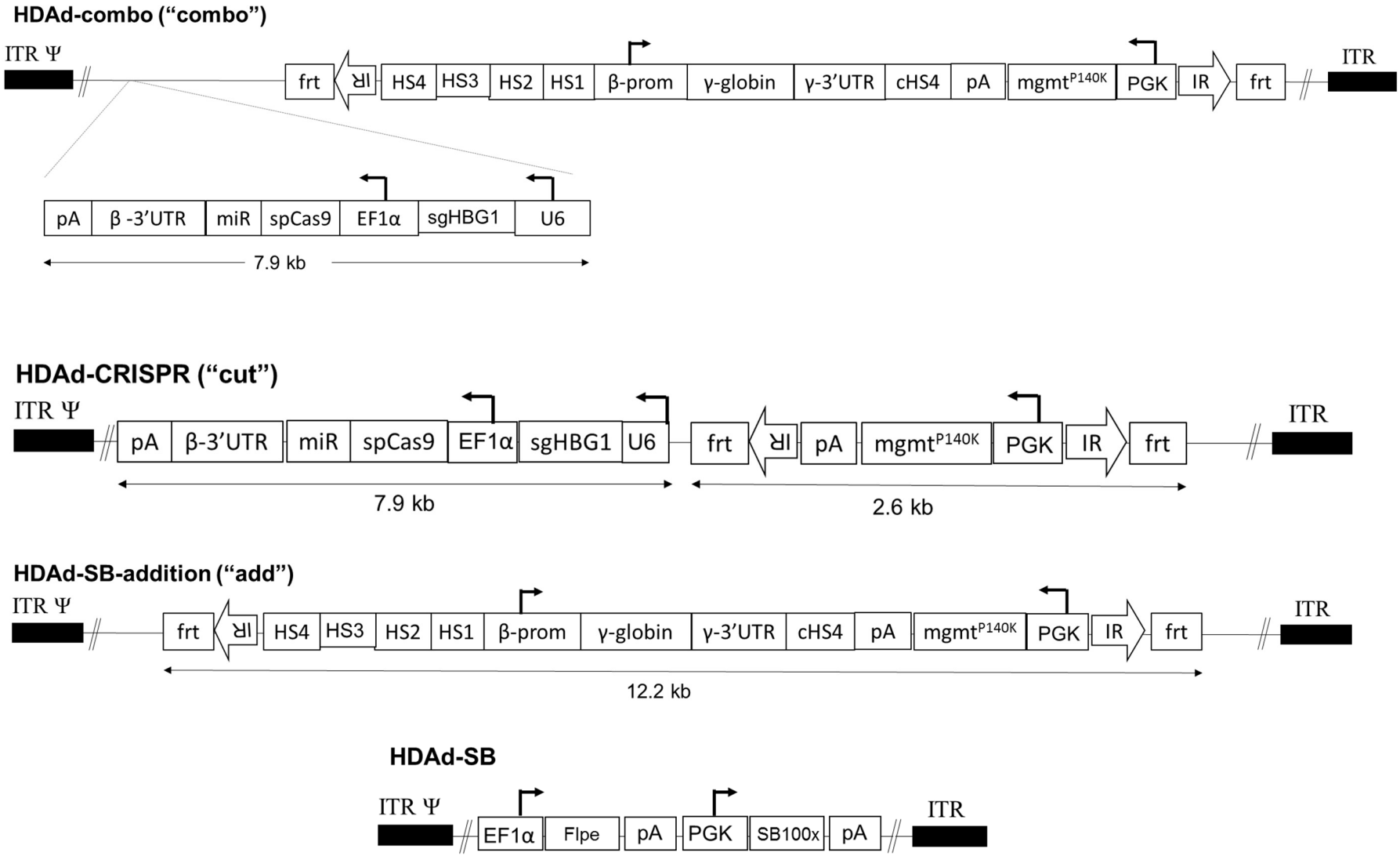

HDAd-combo ("combo")
ITR Ψ
frt
IR
HS4
HS3
HS2
HS1
β-prom
γ-globin
γ-3'UTR
cHS4
pA
mgmt^{P140K}
PGK
IR
frt
ITR
pA
β -3'UTR
miR
spCas9
EF1α
sgHBG1
U6
7.9 kb
HDAd-CRISPR ("cut")
ITR Ψ
pA
β-3'UTR
miR
spCas9
EF1α
sgHBG1
U6
7.9 kb
frt
IR
pA
mgmt^{P140K}
PGK
IR
frt
2.6 kb
ITR
HDAd-SB-addition ("add")
ITR Ψ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

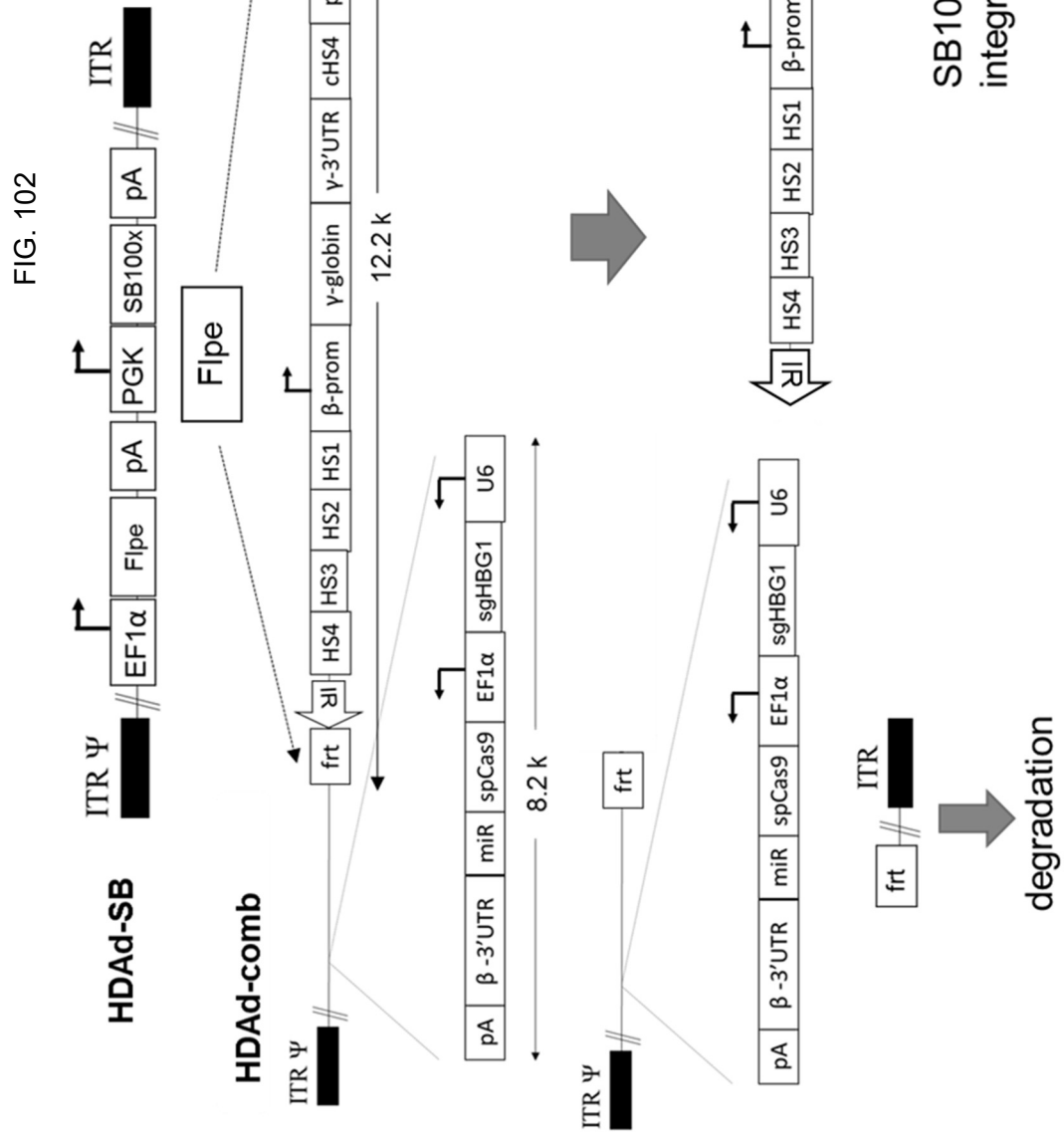

HDAd-SB
ITR Ψ
EF1α
Flpe
pA
PGK
SB100x
pA
ITR
HDAd-comb
ITR Ψ
frt
IR
HS4
HS3
HS2
HS1
β-prom
γ-globin
γ-3'UTR
cHS4
pA
mgmt^{P140K}
PGK
IR
frt
ITR
12.2 k
Flpe
pA
β -3'UTR
miR
spCas9
EF1α
sgHBG1
U6
8.2 k
frt
ITR Ψ
IR
HS4
HS3
HS2
HS1
β-prom
γ-globin
γ-3'UTR
cHS4
pA
mgmt^{P140K}
PGK
IR
SB100x-mediated random integration via IRs
pA
β -3'UTR
miR
spCas9
EF1α
sgHBG1
U6
frt
ITR
degradation

FIG. 103A

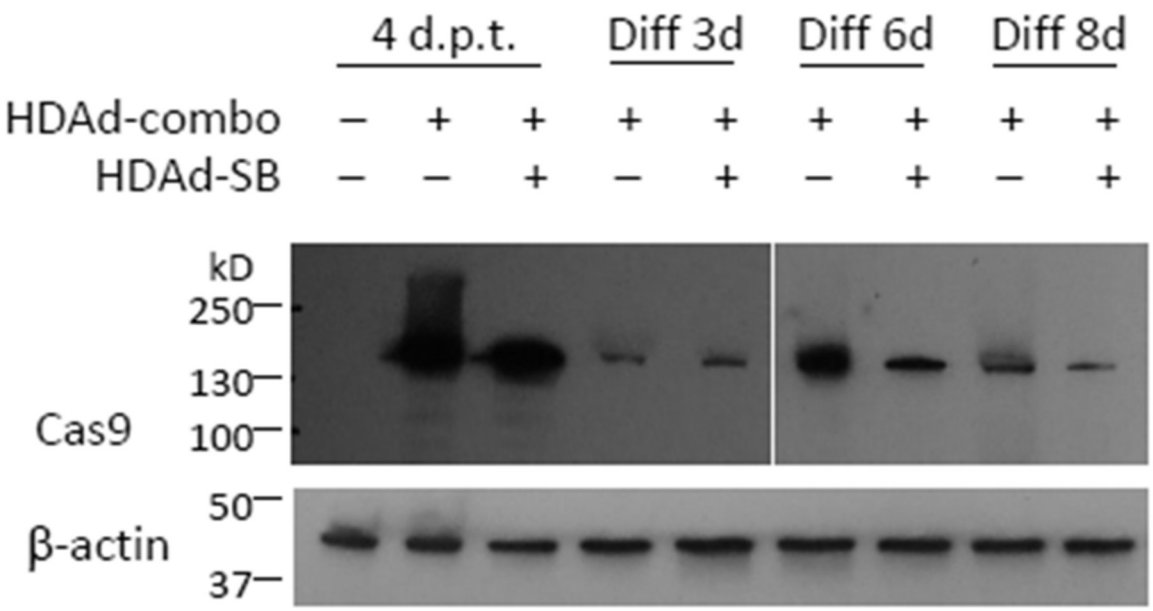


FIG. 103B

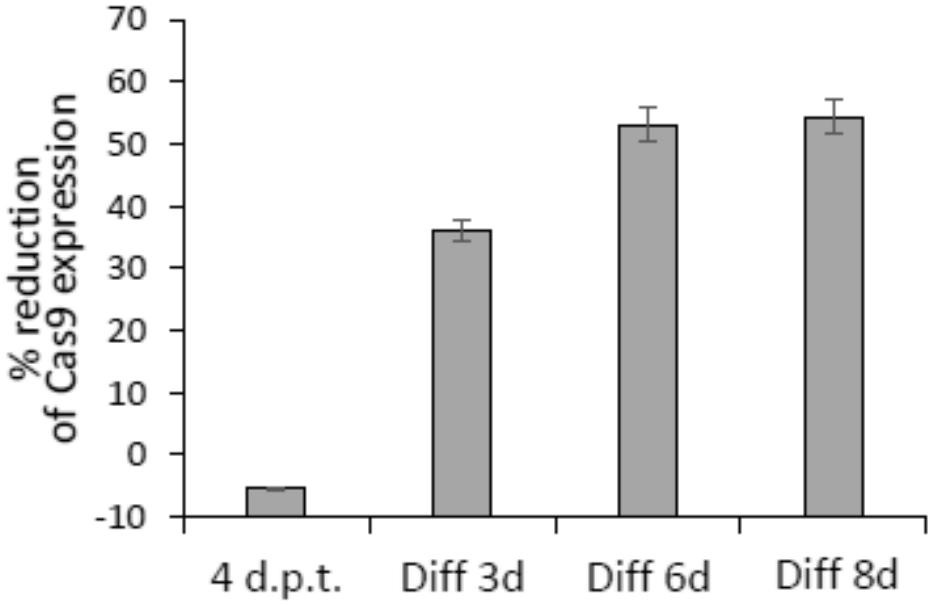


FIG. 103C

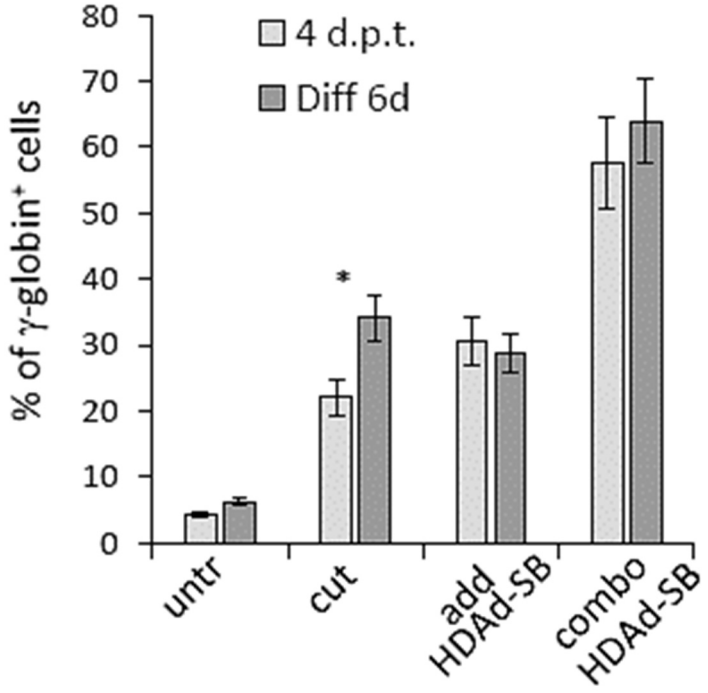


FIG. 103D

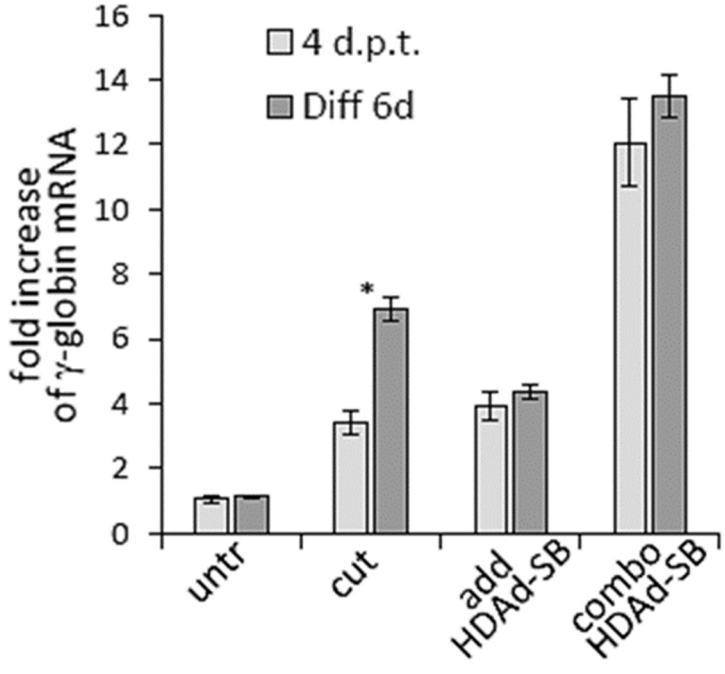


FIG. 104A

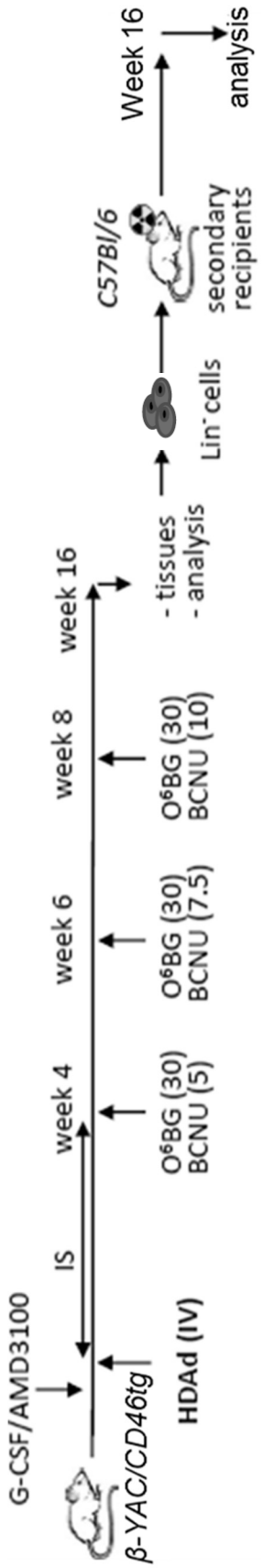


FIG. 104B

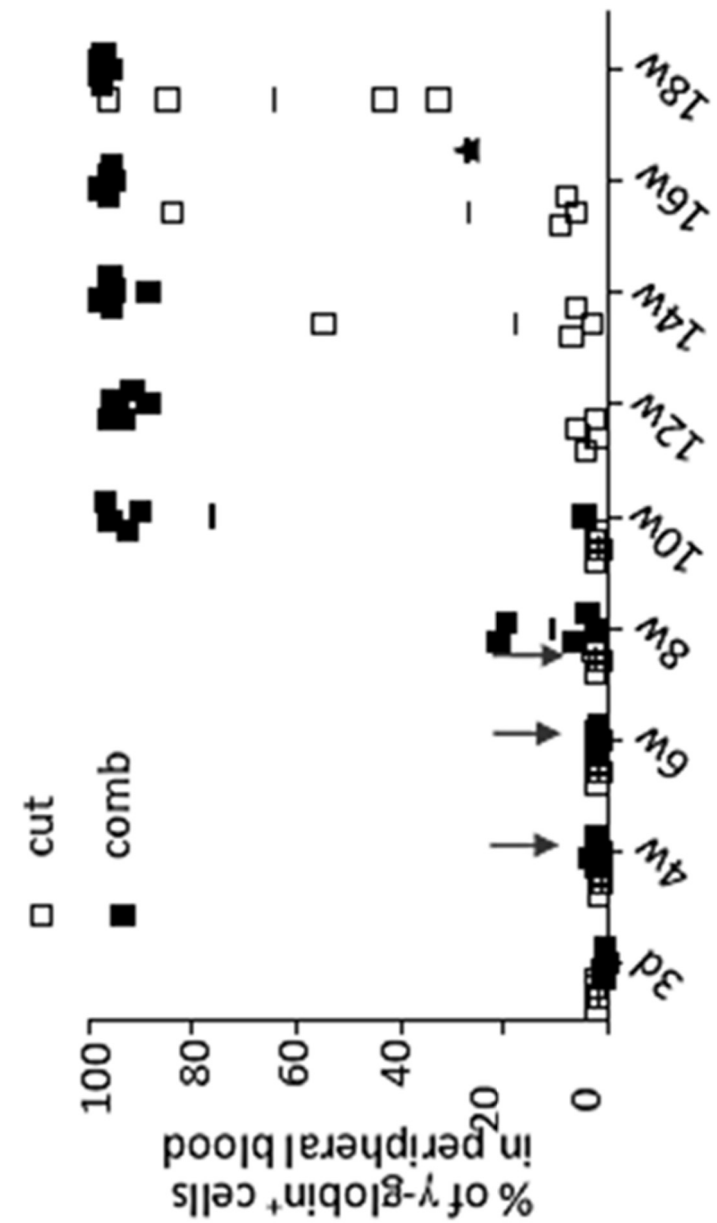


FIG. 104C

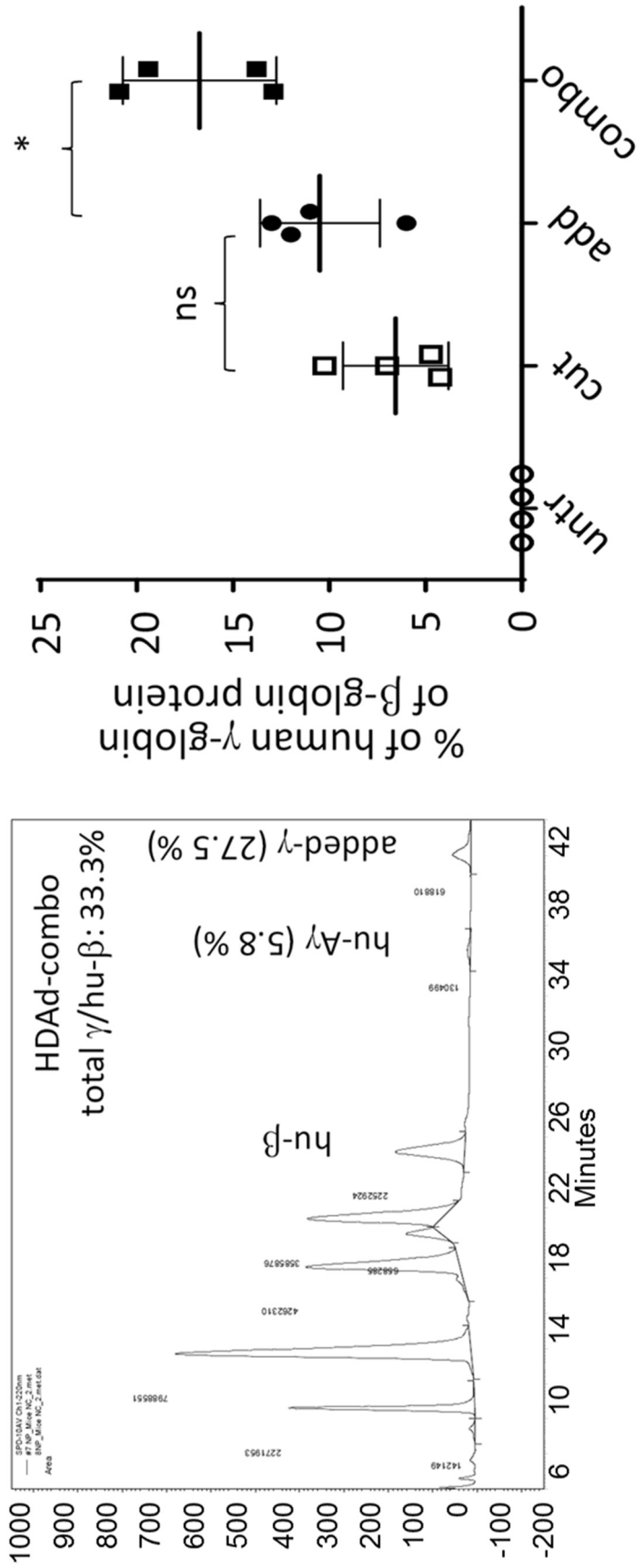

% of human γ-globin
of β-globin protein
25
20
15
10
5
0
untr
cut
add
combo
ns
*
HDAd-combo
total γ/hu-β: 33.3%
hu-β
hu-Aγ (5.8 %)
added-γ (27.5 %)
1000
900
800
700
600
500
400
300
200
100
0
-100
-200
6
10
14
18
22
26
30
34
38
42
Minutes

FIG. 104D

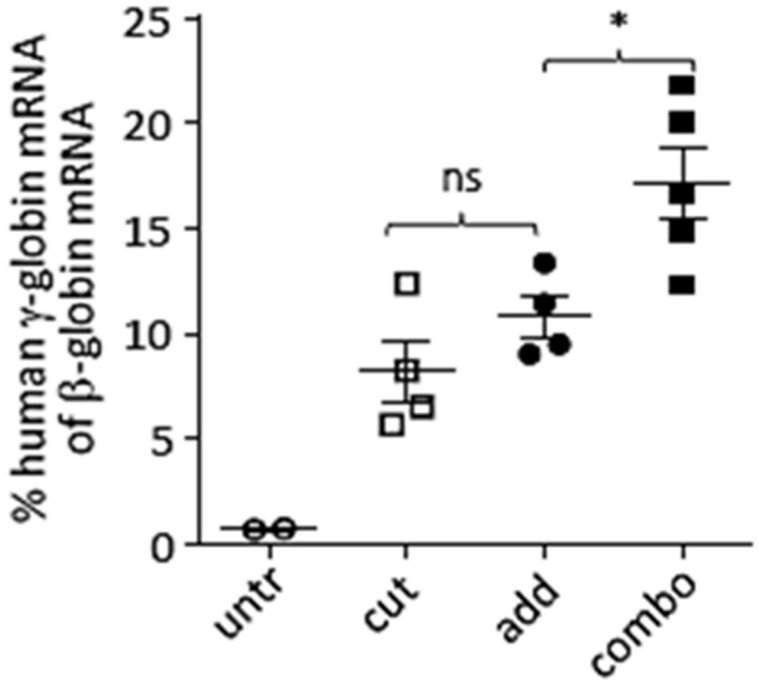


FIG. 104E

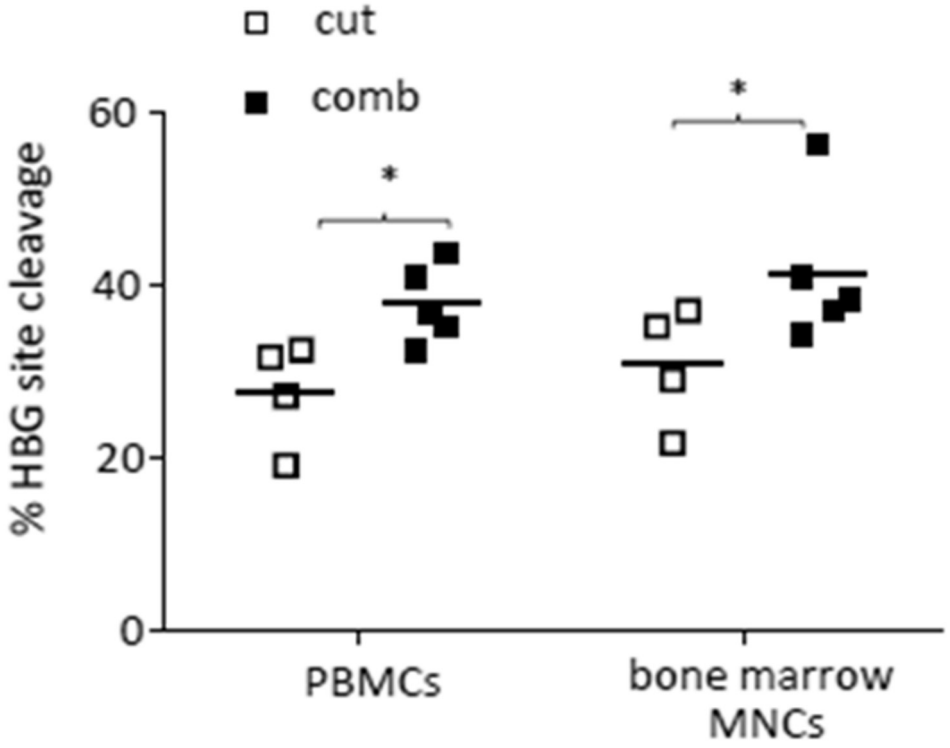


FIG. 104F

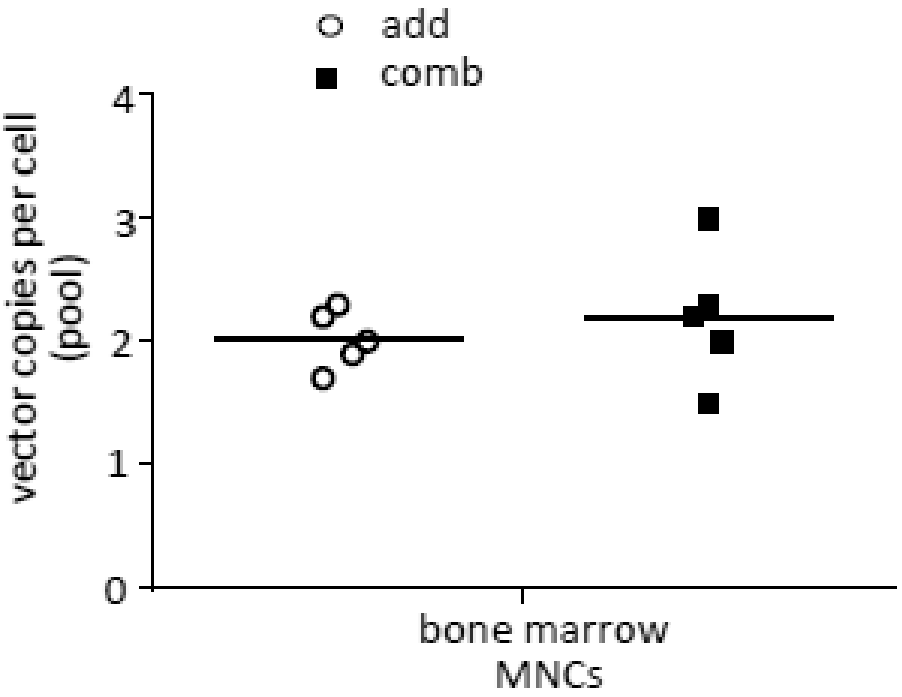


FIG. 104G

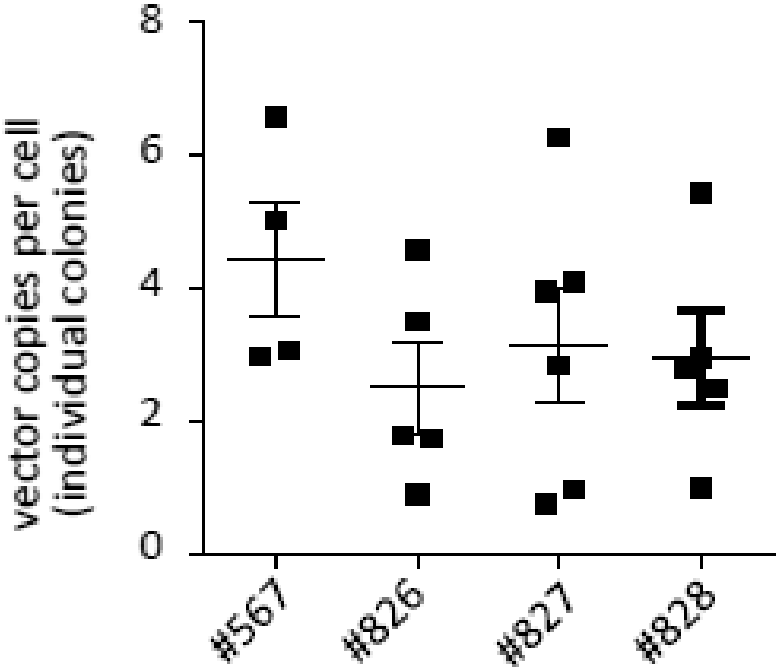


FIG. 104H

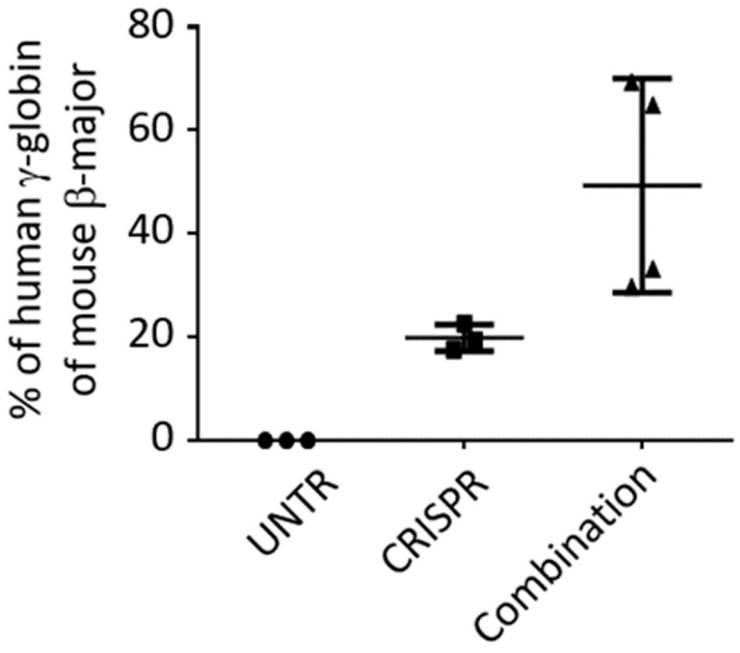

% of human γ-globin
of mouse β-major
80
60
40
20
0
UNTR
CRISPR
Combination

FIG. 105

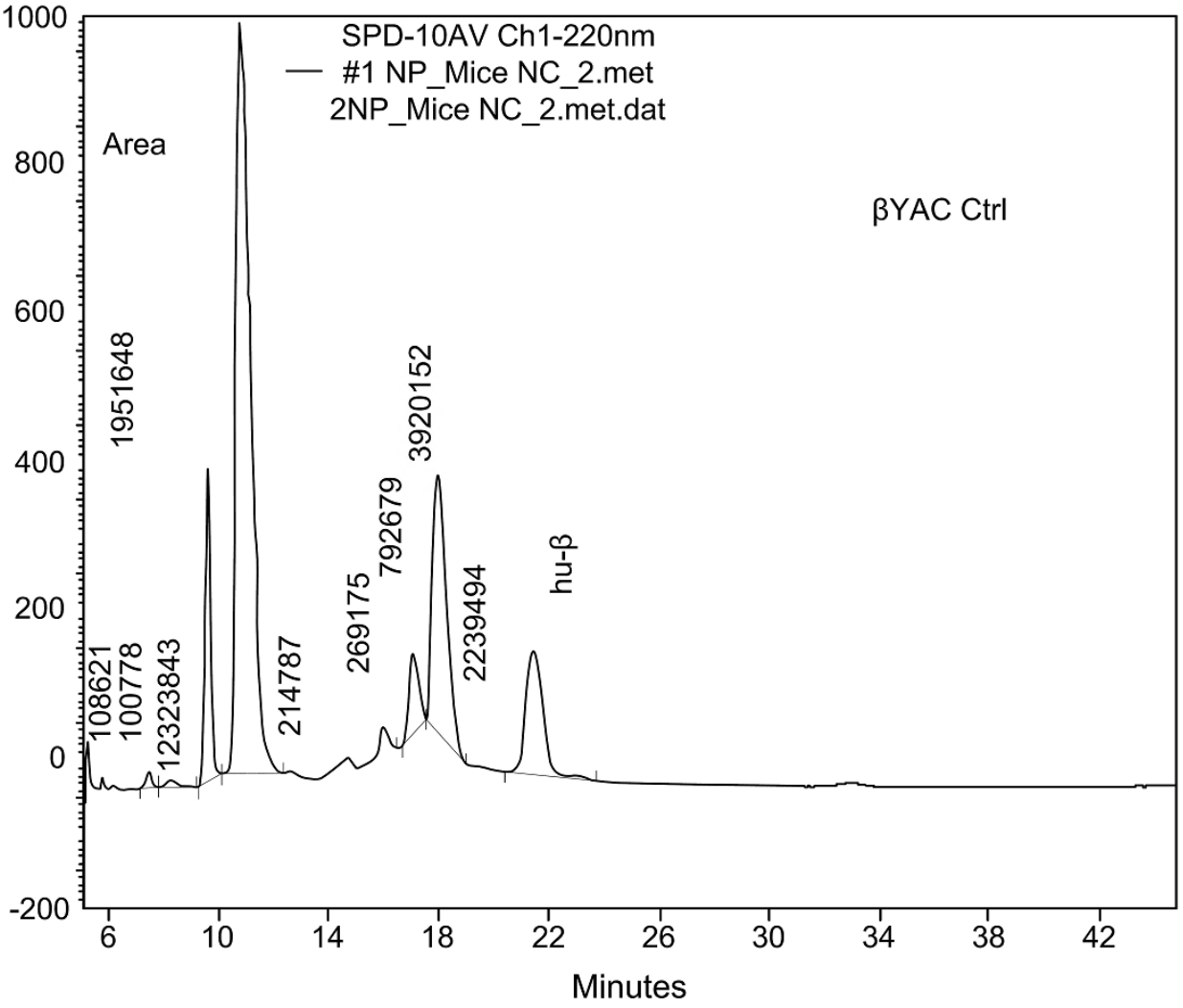

SPD-10AV Ch1-220nm
#1 NP_Mice NC_2.met
2NP_Mice NC_2.met.dat
Area
βYAC Ctrl
1951648
108621
100778
12323843
214787
269175
792679
3920152
2239494
hu-β
1000
800
600
400
200
0
-200
6
10
14
18
22
26
30
34
38
42
Minutes

FIG. 105 cont'd

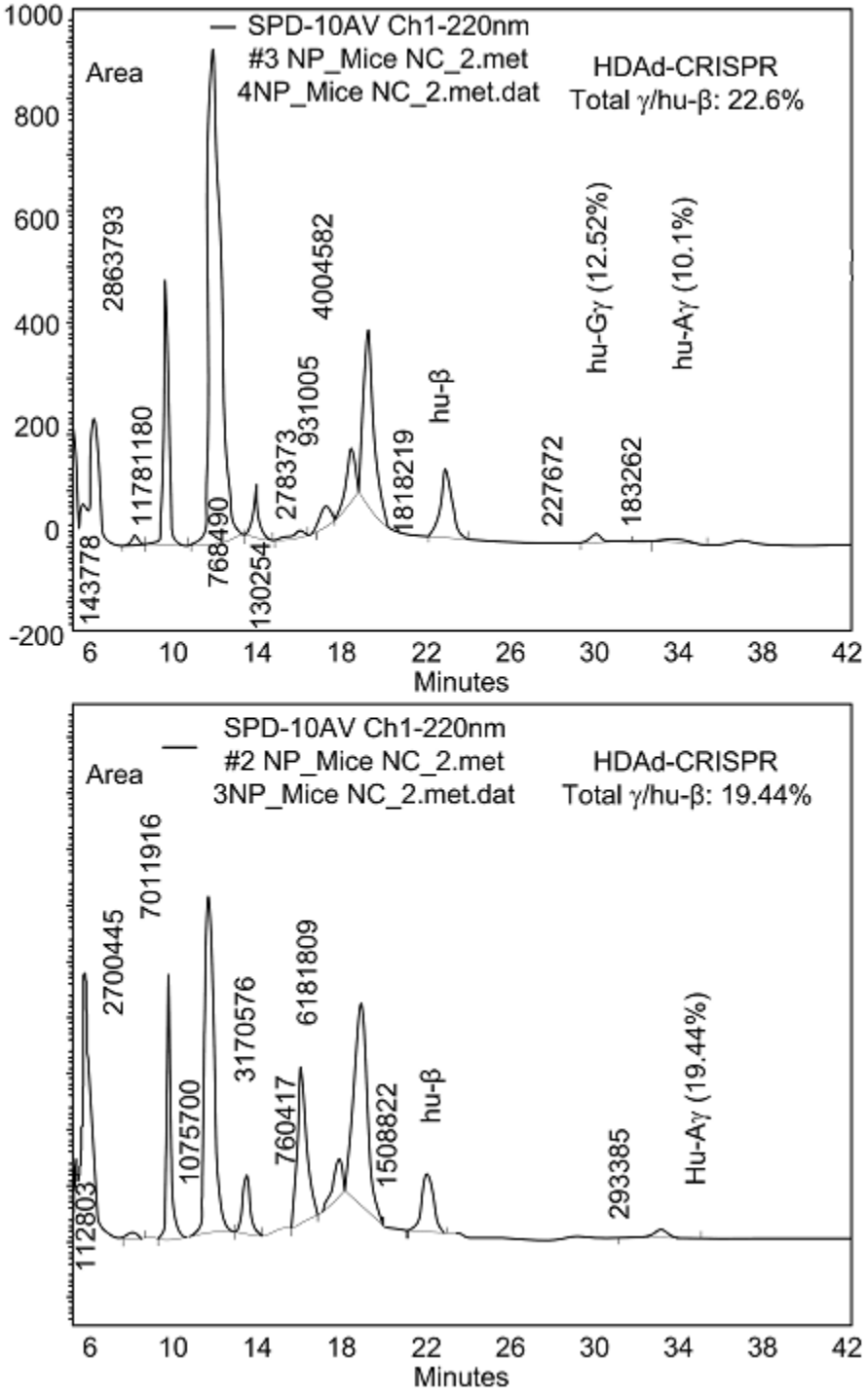


FIG. 105 cont'd

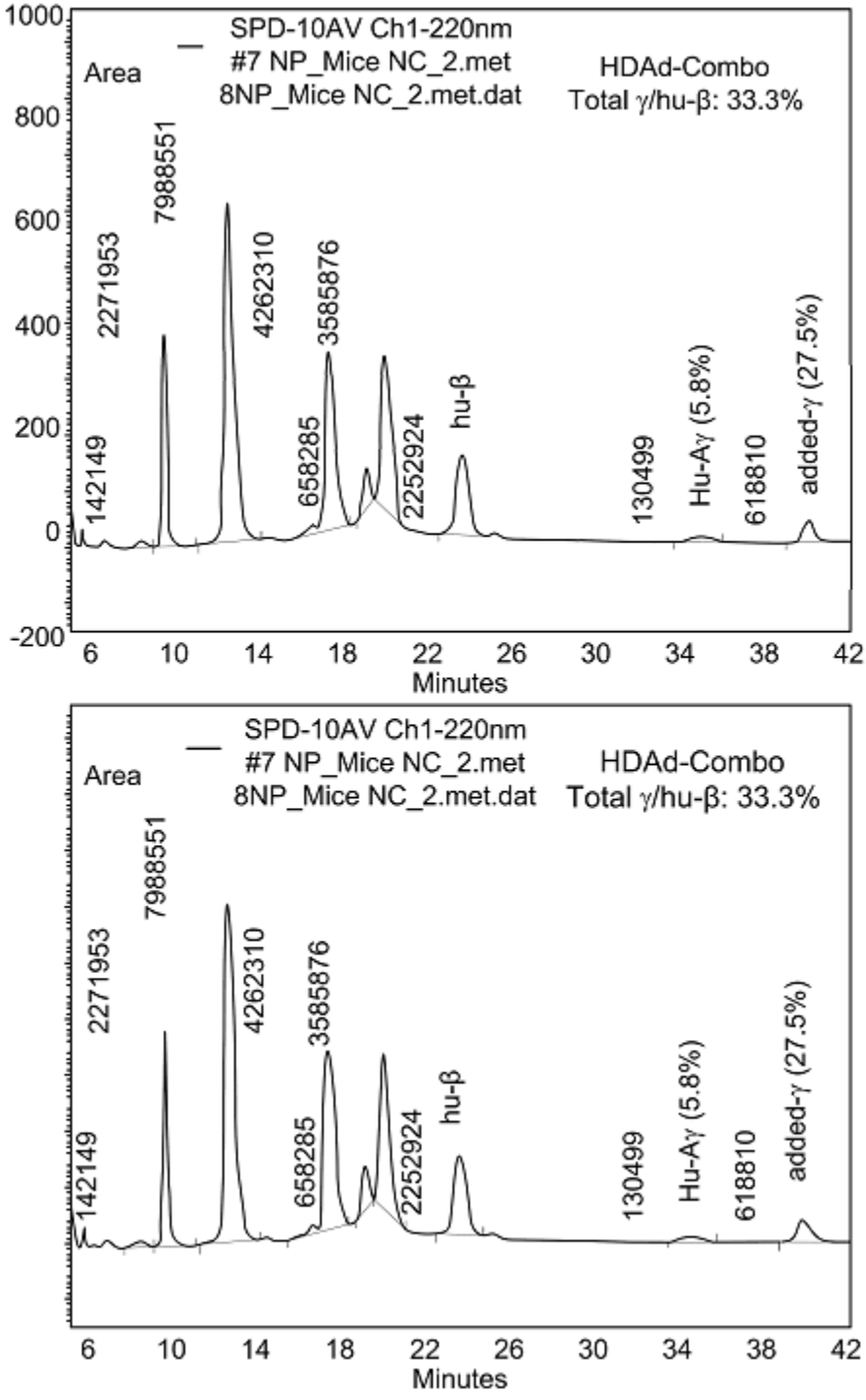


FIG. 106

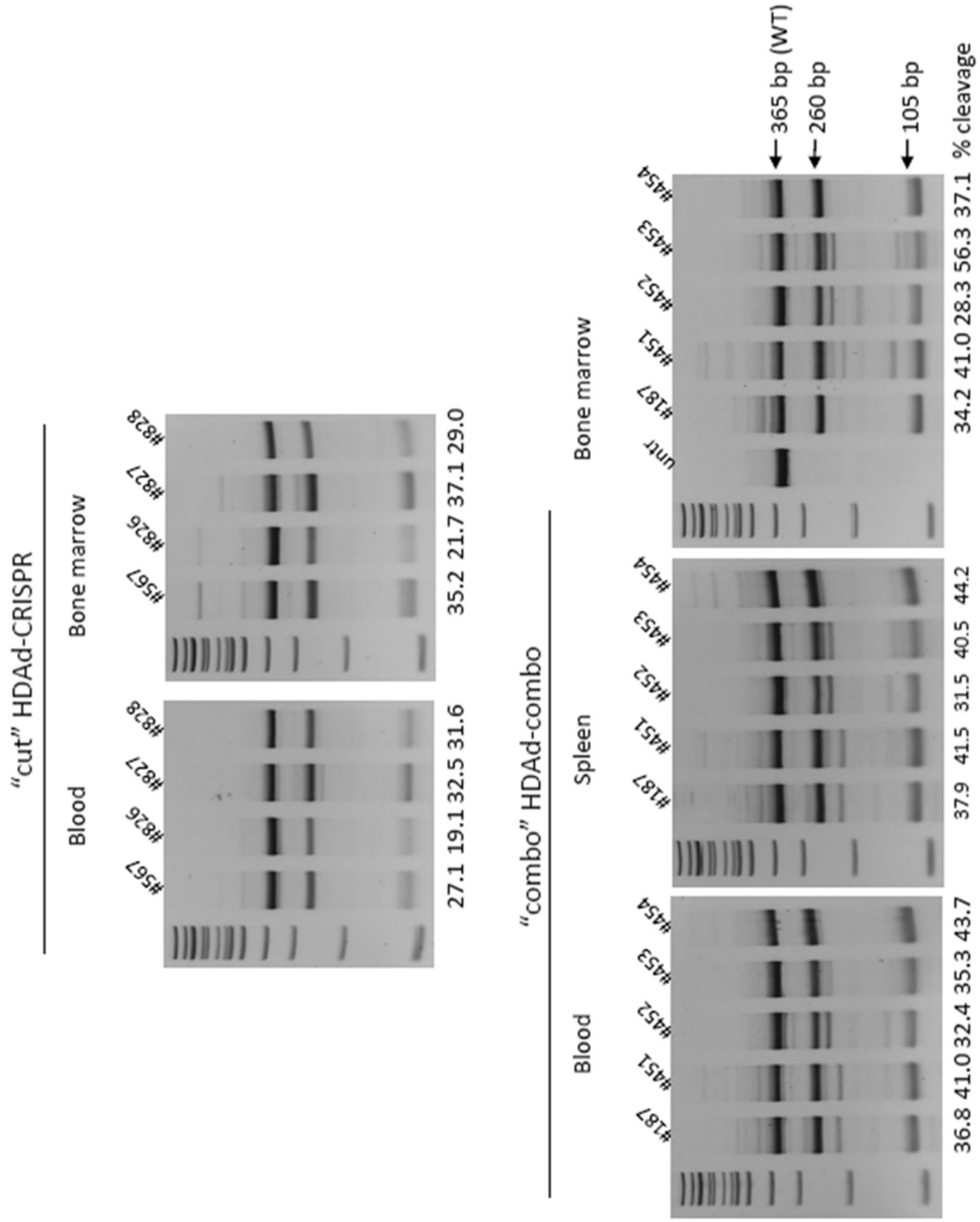

"cut" HDAd-CRISPR
Blood
Bone marrow
#567
#826
#827
#828
27.1 19.1 32.5 31.6
35.2 21.7 37.1 29.0
"combo" HDAd-combo
Blood
Spleen
Bone marrow
#187
#451
#452
#453
#454
unt
36.8 41.0 32.4 35.3 43.7
37.9 41.5 31.5 40.5 44.2
34.2 41.0 28.3 56.3 37.1
365 bp (WT)
260 bp
105 bp
% cleavage

FIG. 107A

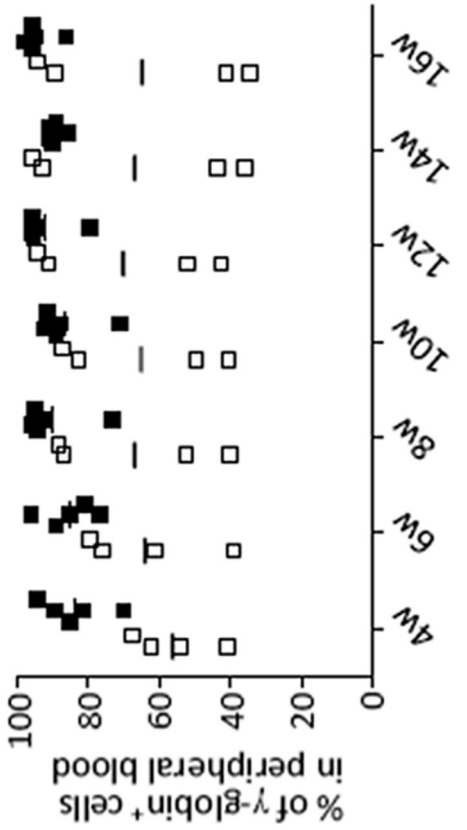


FIG. 107B

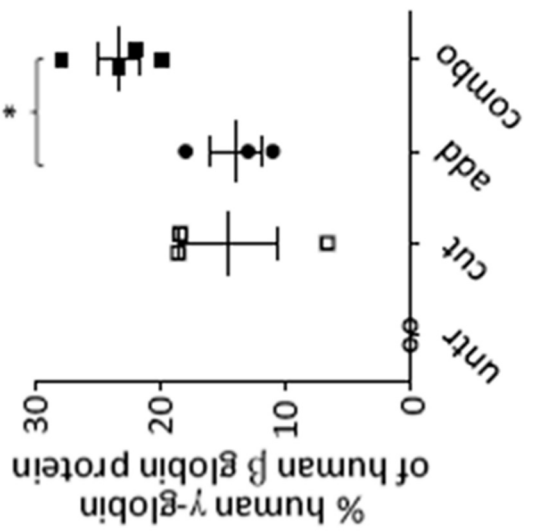


FIG. 107C

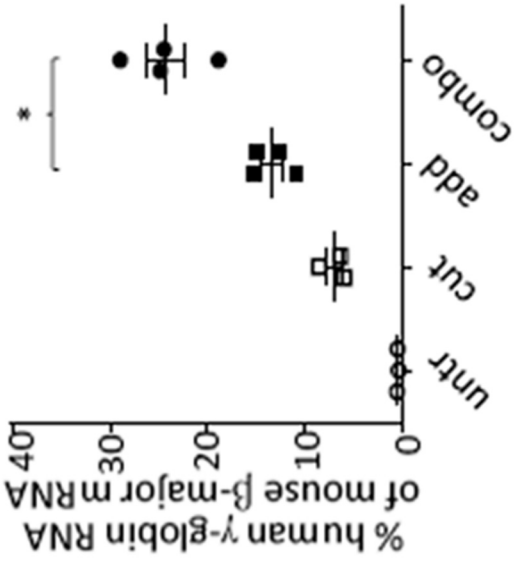


FIG. 107D

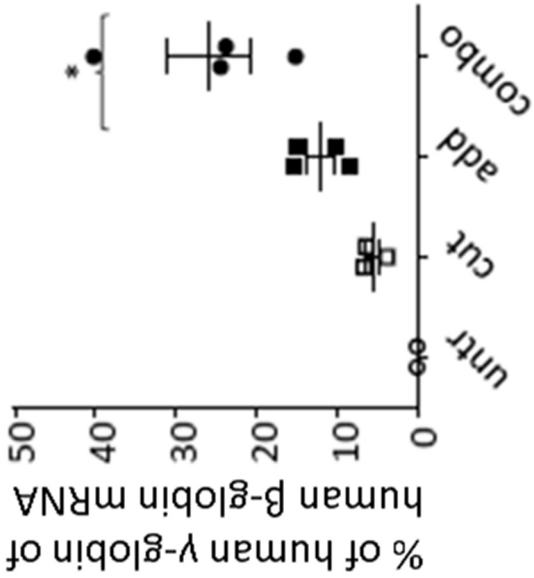


FIG. 107E

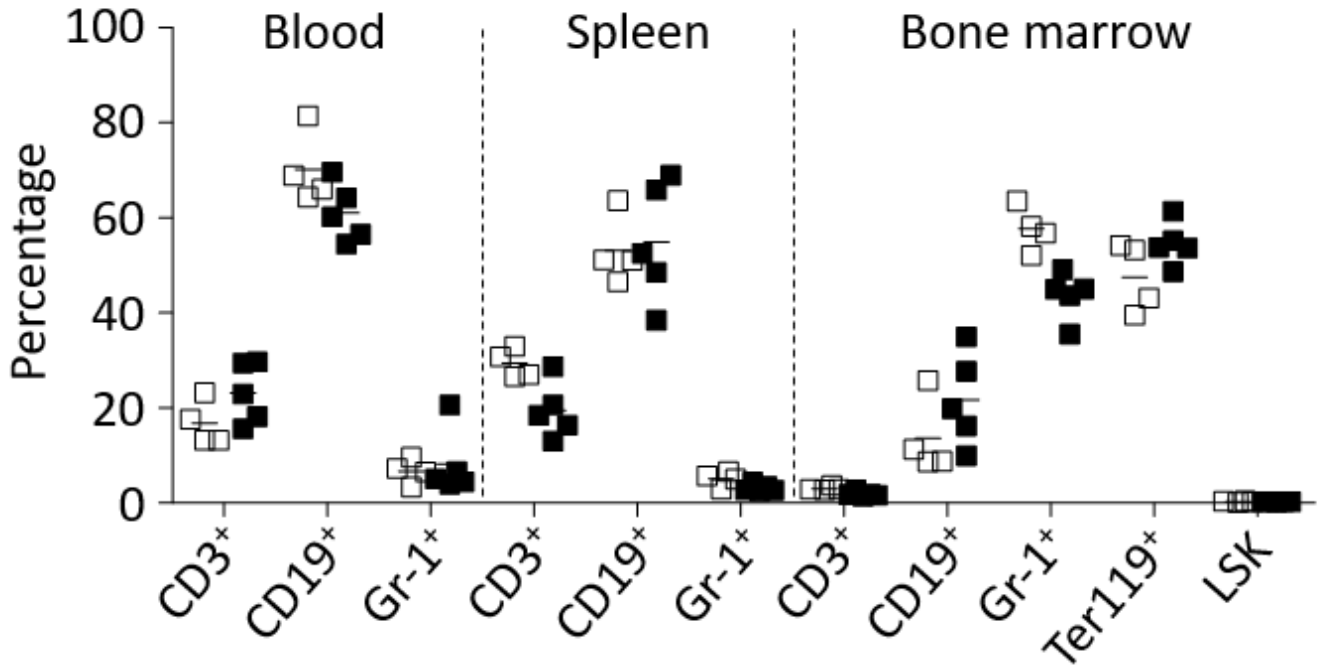


FIG. 107F

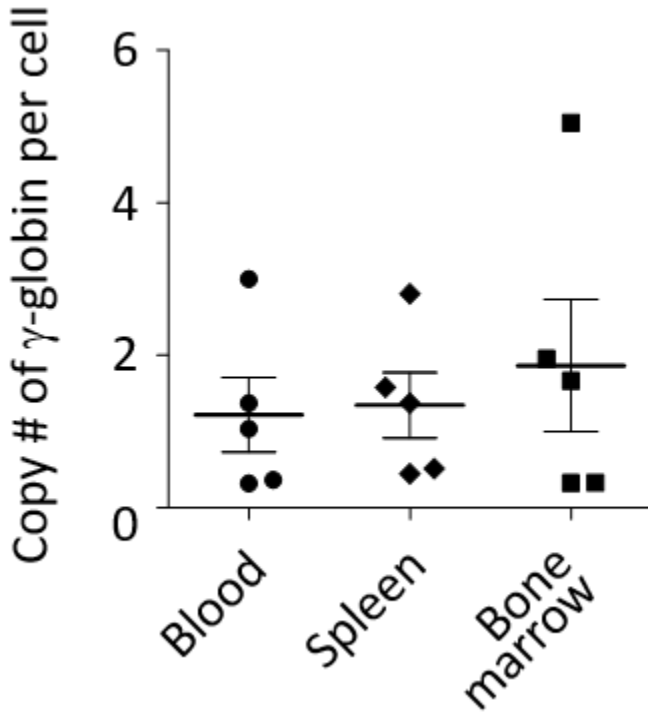


FIG. 108A

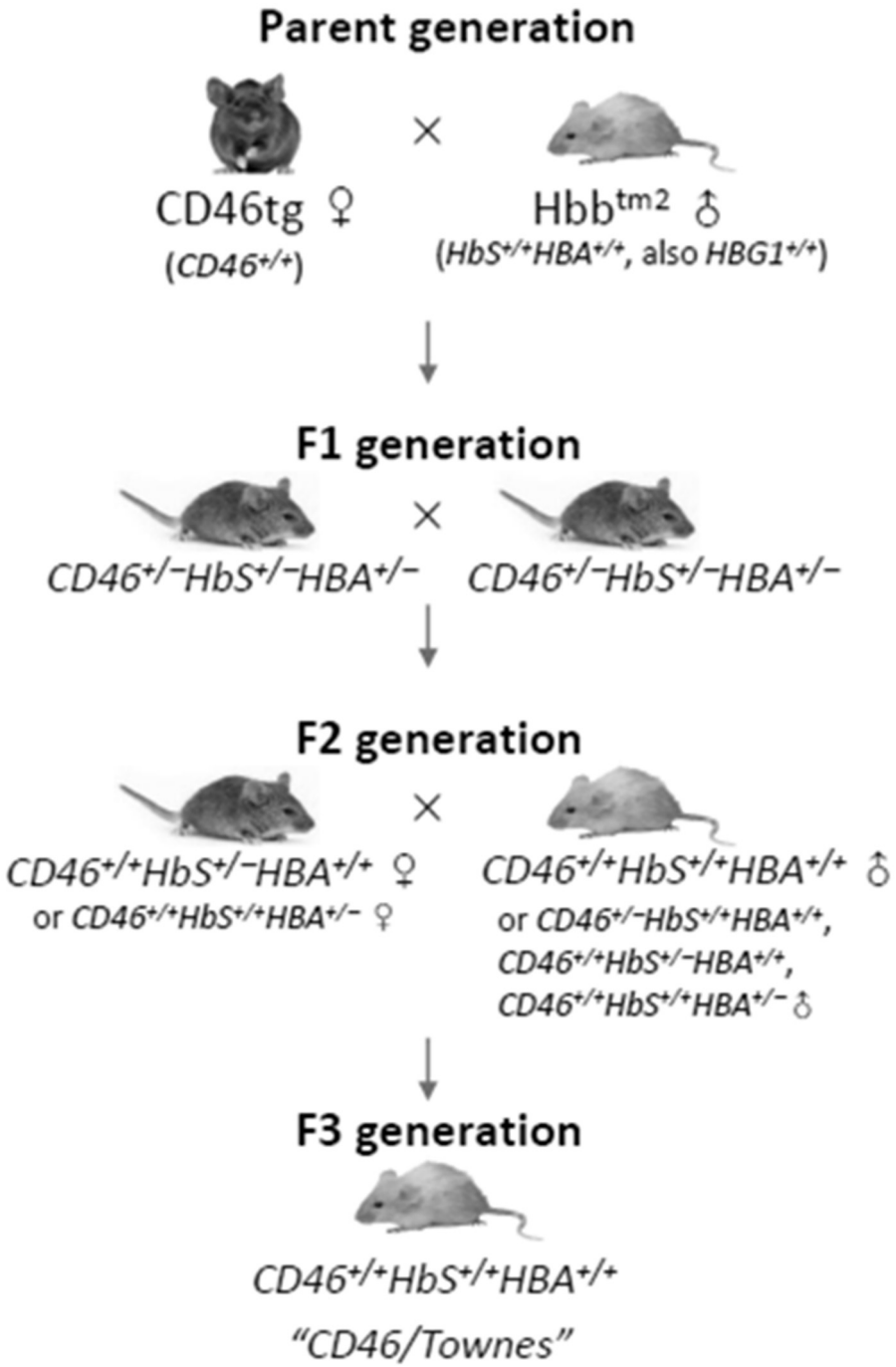

Parent generation
CD46tg ♀
(CD46+/+)
×
Hbbtm2 ♂
(HbS+/+HBA+/+, also HBG1+/+)
F1 generation
CD46+/−HbS+/−HBA+/−
×
CD46+/−HbS+/−HBA+/−
F2 generation
CD46+/+HbS+/−HBA+/+ ♀
or CD46+/+HbS+/+HBA+/− ♀
×
CD46+/+HbS+/+HBA+/+ ♂
or CD46+/−HbS+/+HBA+/+,
CD46+/+HbS+/−HBA+/+,
CD46+/+HbS+/+HBA+/− ♂
F3 generation
CD46+/+HbS+/+HBA+/+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

FIG. 108B

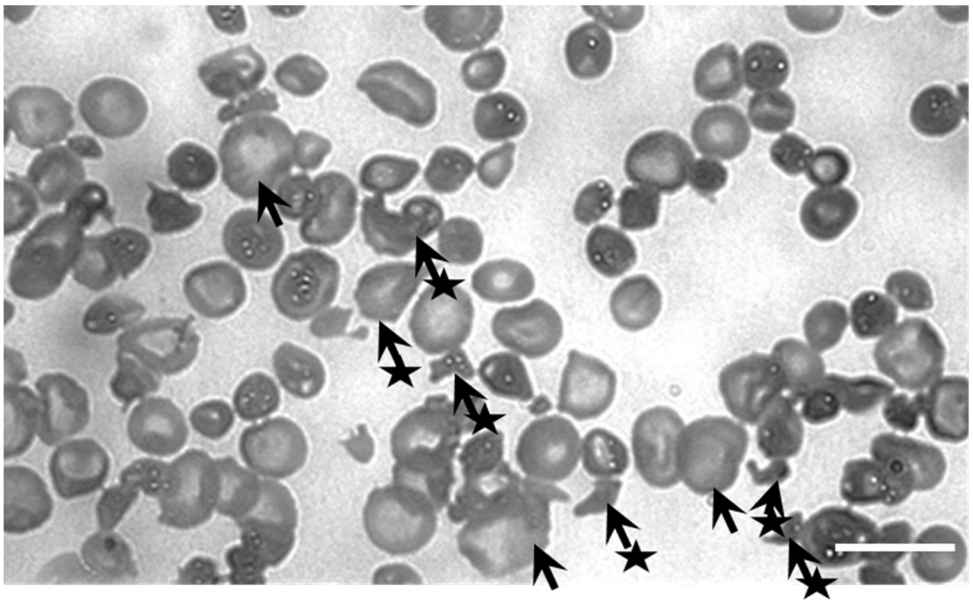


FIG. 108C

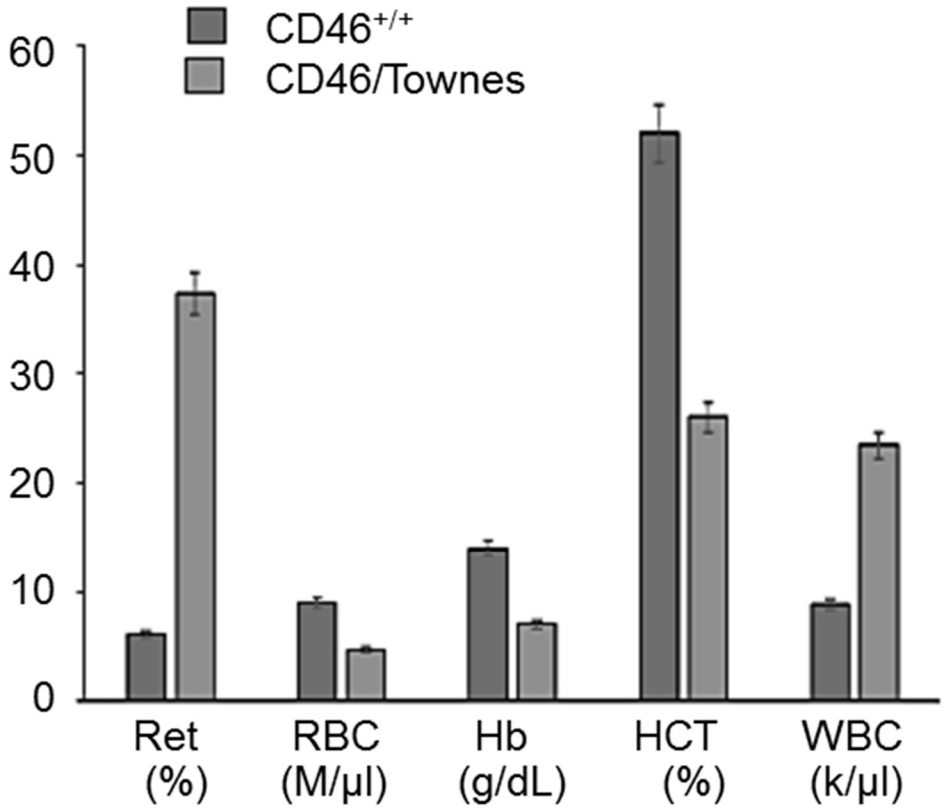


FIG. 108D

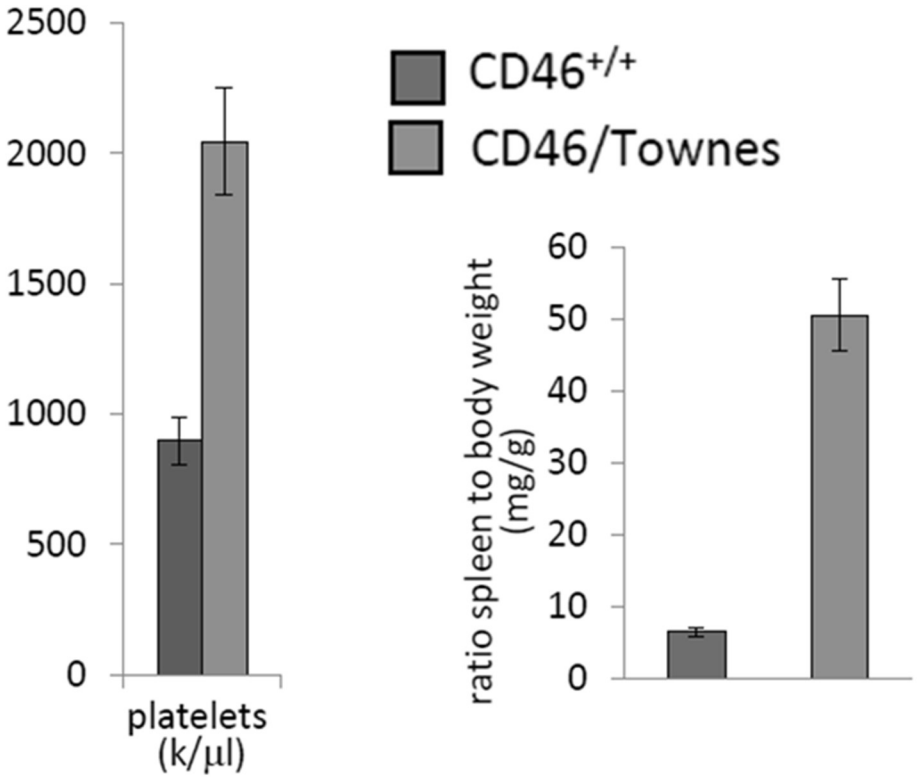


FIG. 109A

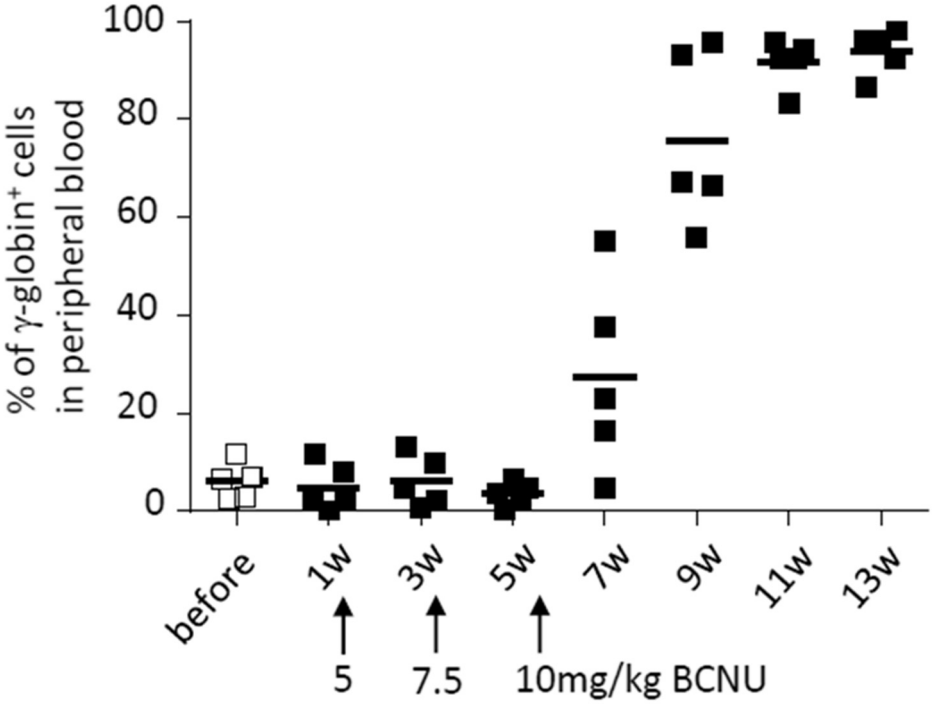


FIG. 109B

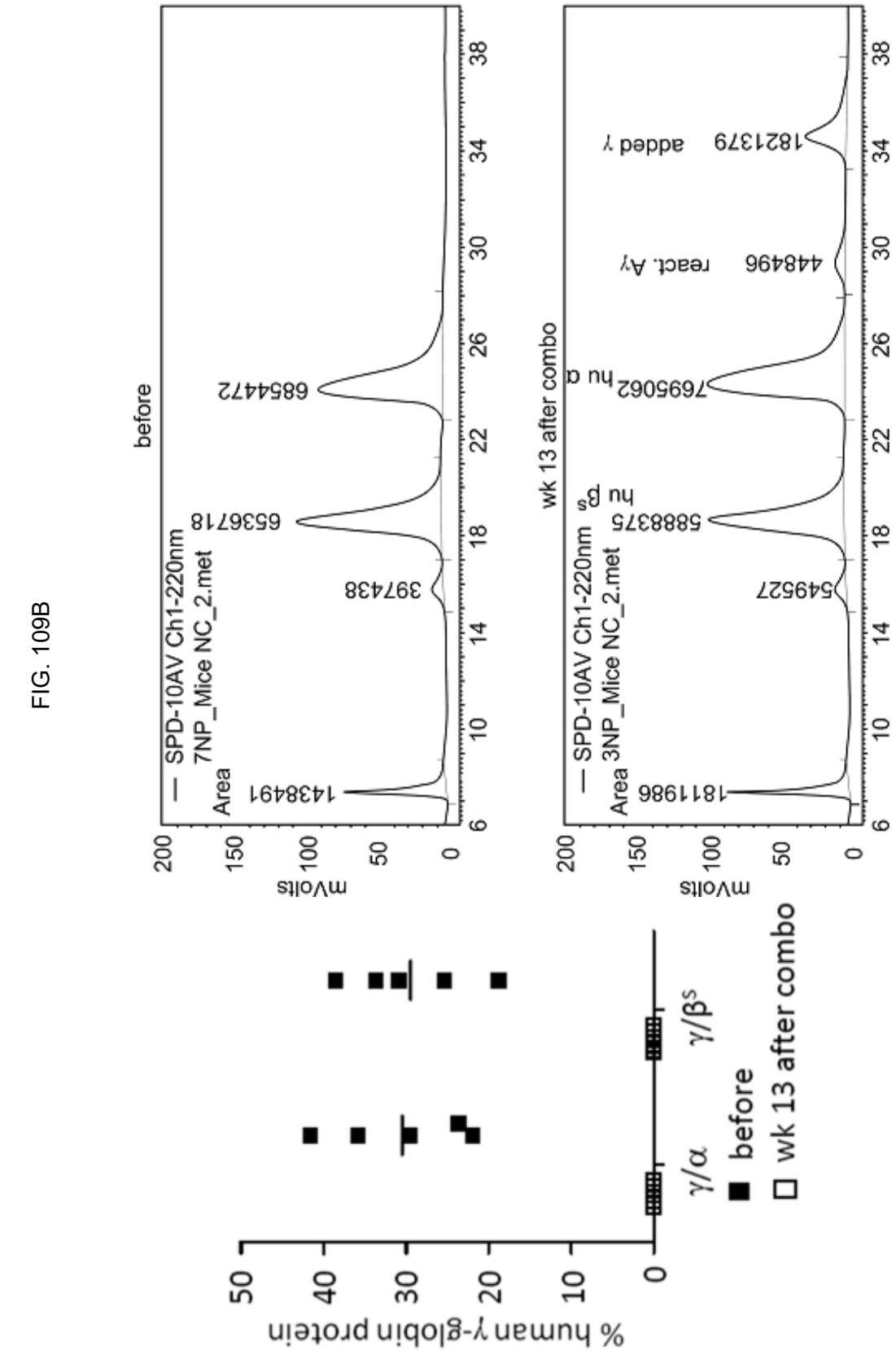

before
SPD-10AV Ch1-220nm
7NP_Mice NC_2.met
Area
1438491
397438
653718
685472
mVolts
wk 13 after combo
SPD-10AV Ch1-220nm
3NP_Mice NC_2.met
Area
1811986
549527
588375
hu βS
769506
hu α
448496
react. Aγ
1821379
added γ
mVolts
% human γ-globin protein
γ/α
γ/βS
before
wk 13 after combo

FIG. 109C

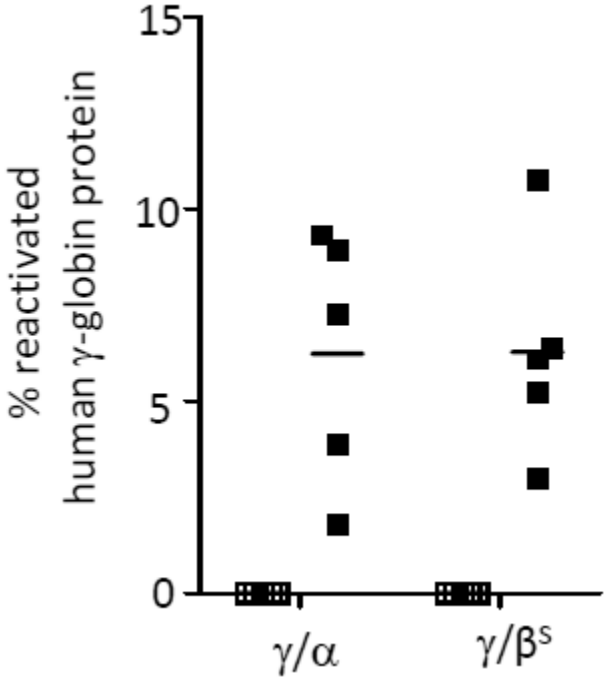


FIG. 109D

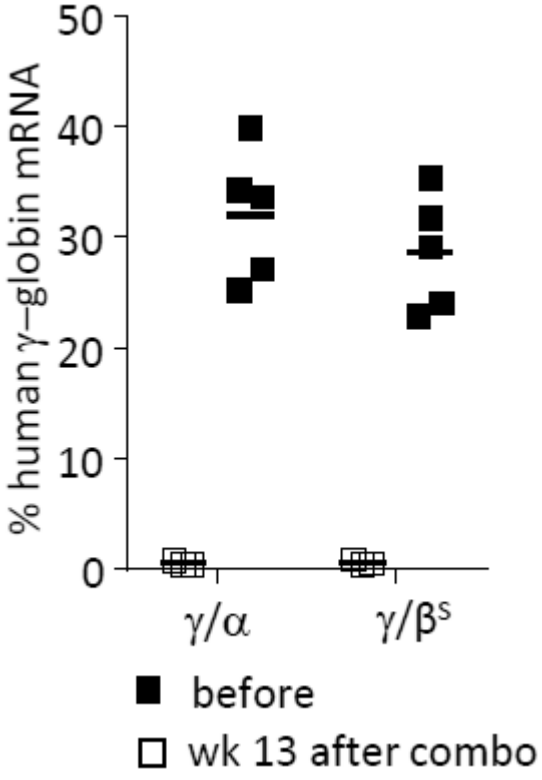


FIG. 109E

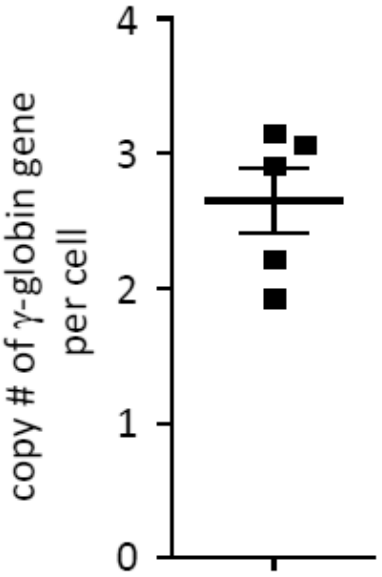


FIG. 109F

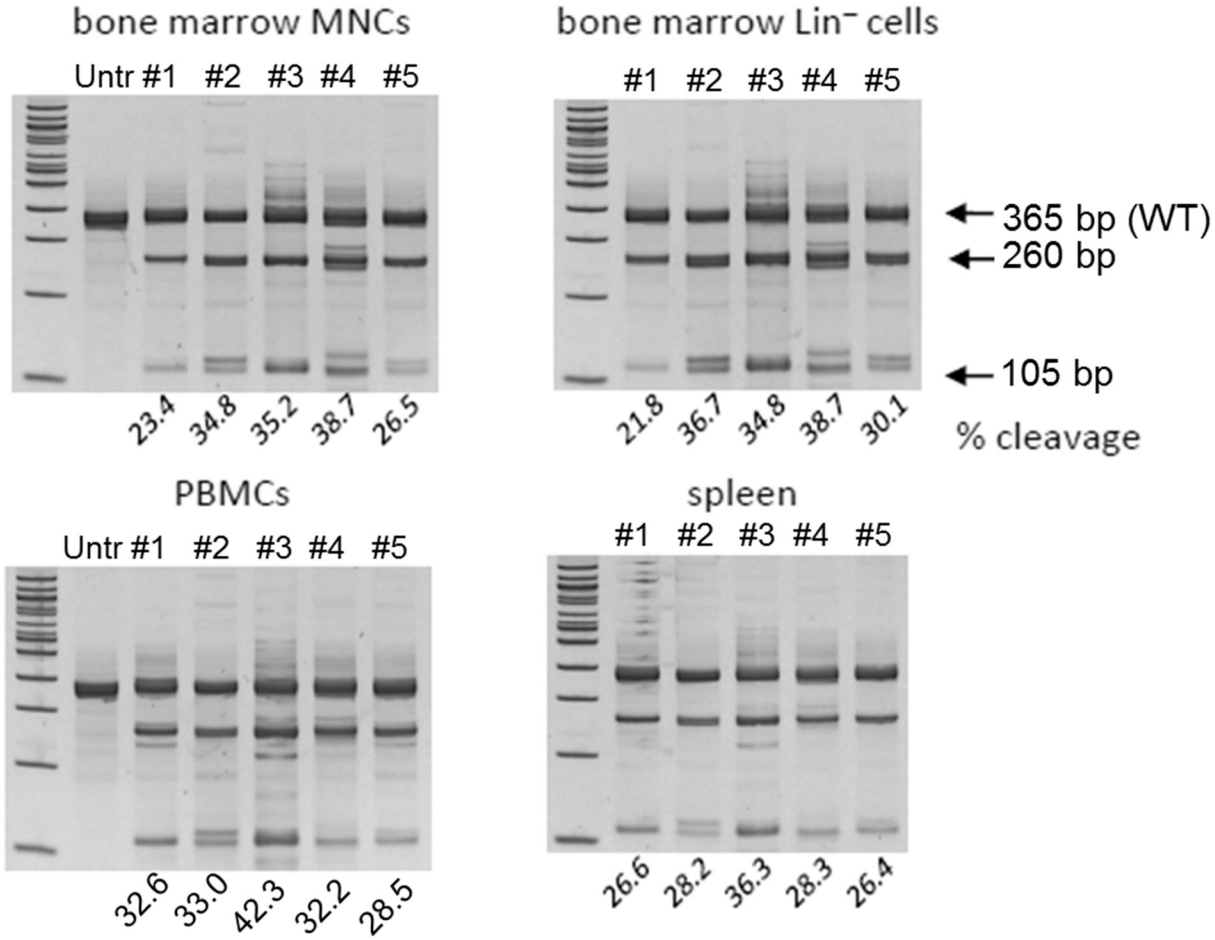


FIG. 110A

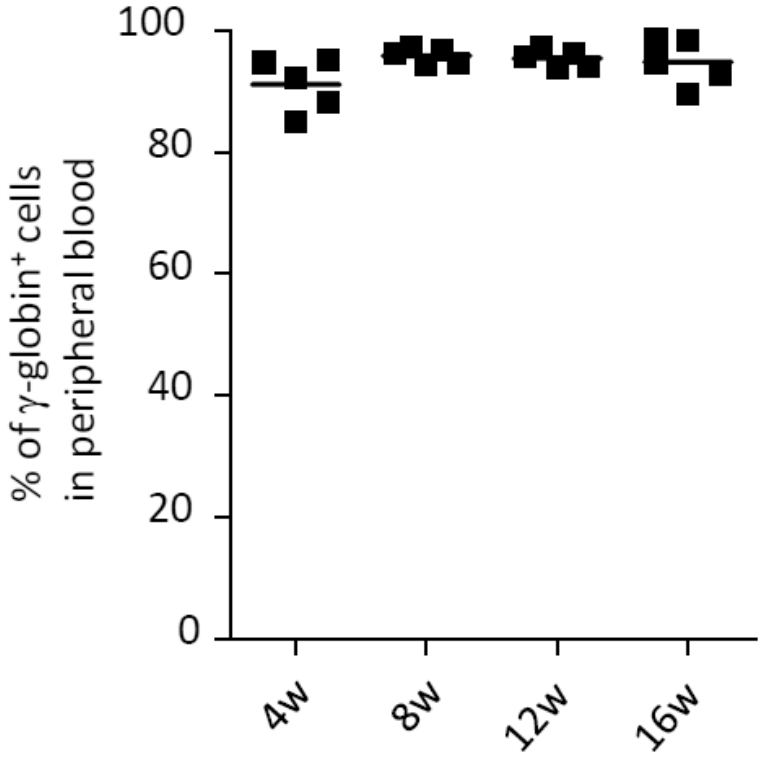


FIG. 110B

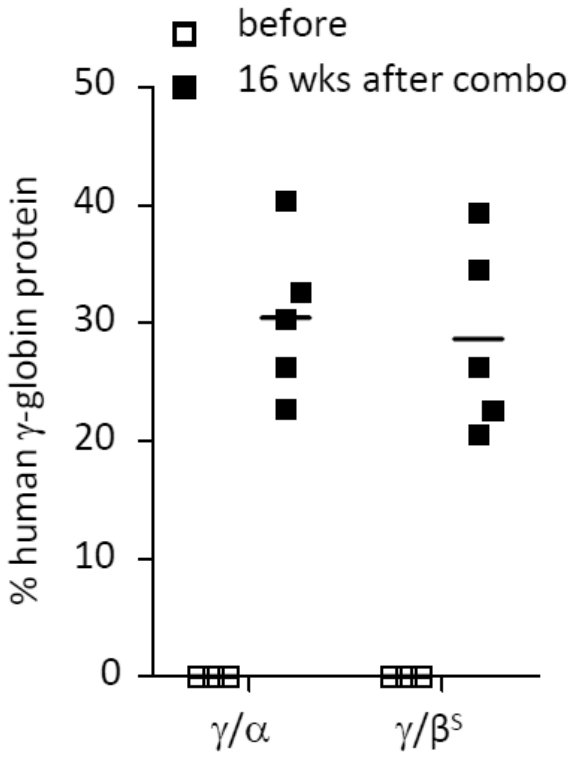


FIG. 111A

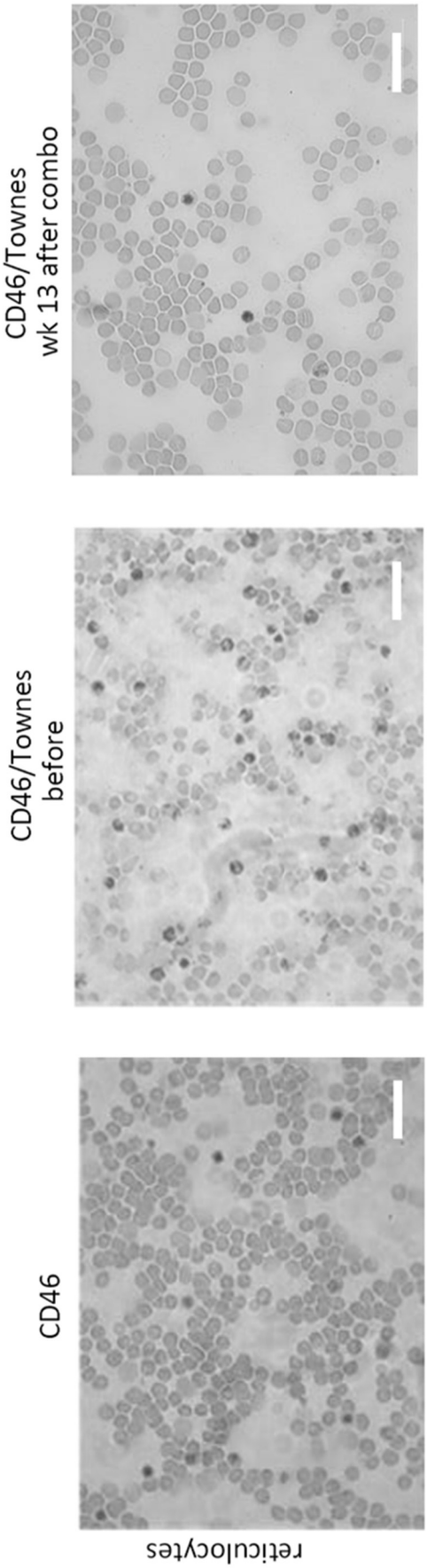


FIG. 111B

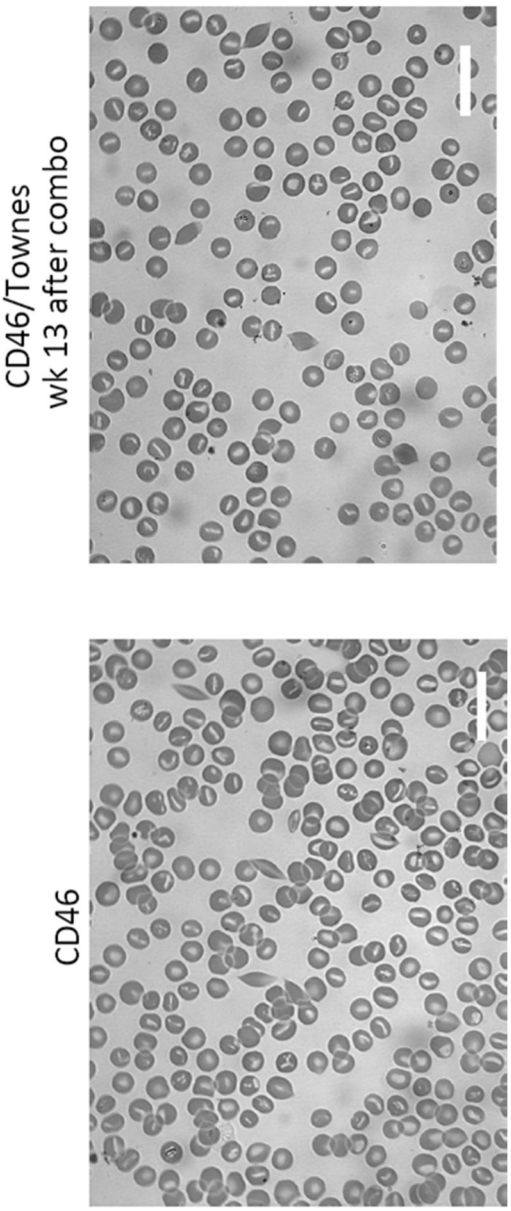


FIG. 111C

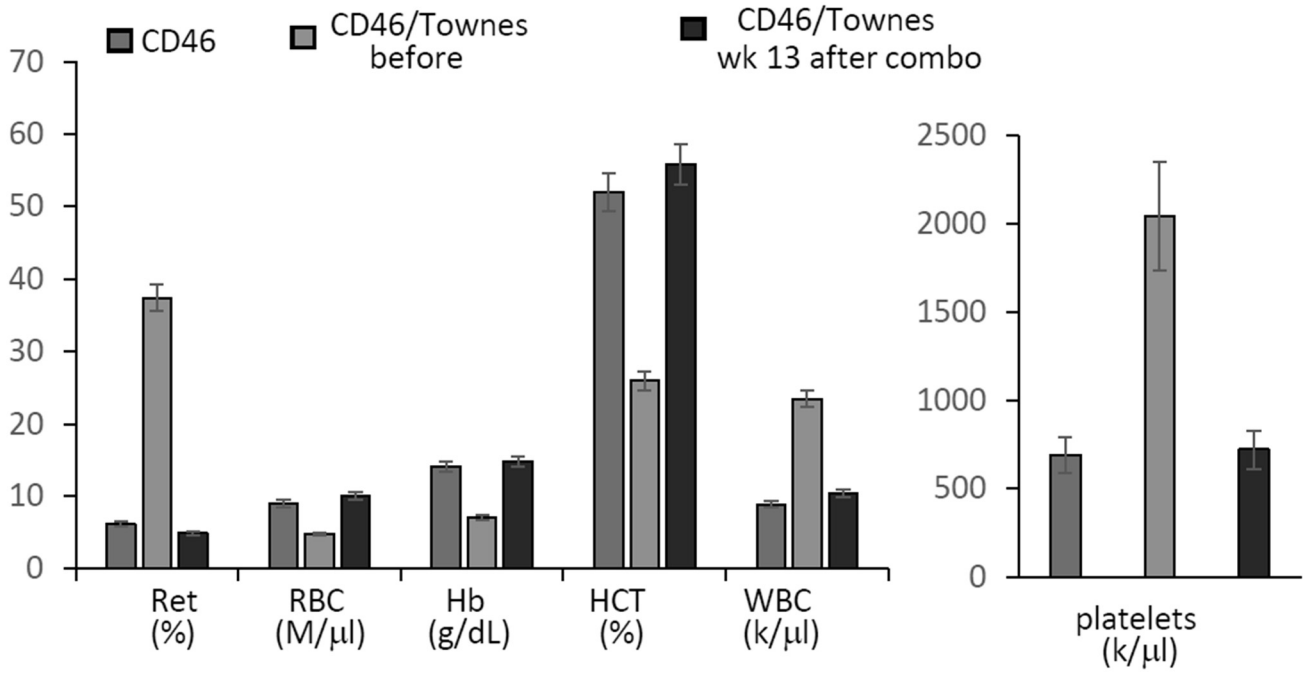

CD46
CD46/Townes before
CD46/Townes wk 13 after combo
70
60
50
40
30
20
10
0
Ret (%)
RBC (M/µl)
Hb (g/dL)
HCT (%)
WBC (k/µl)
2500
2000
1500
1000
500
0
platelets (k/µl)

FIG. 112A

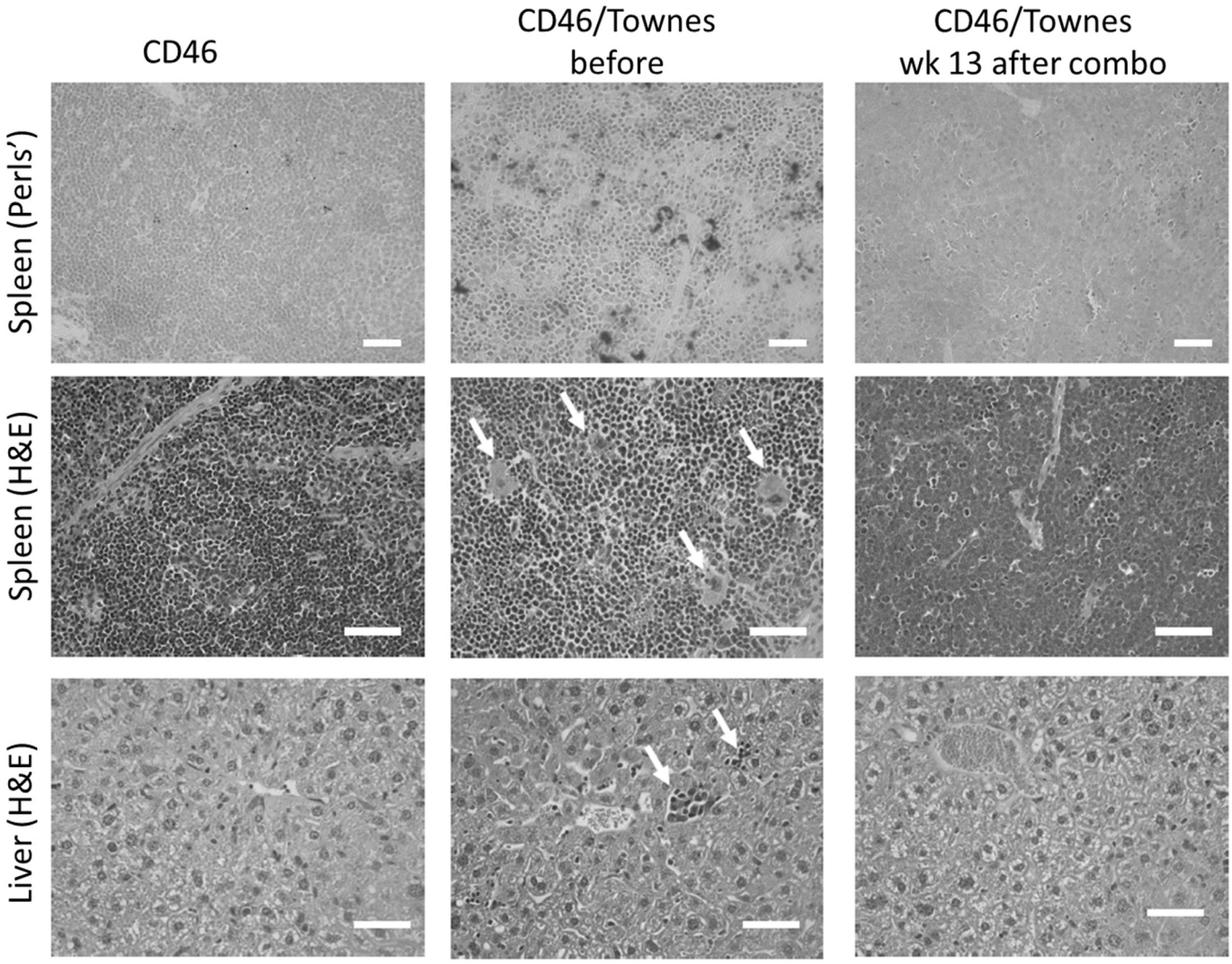
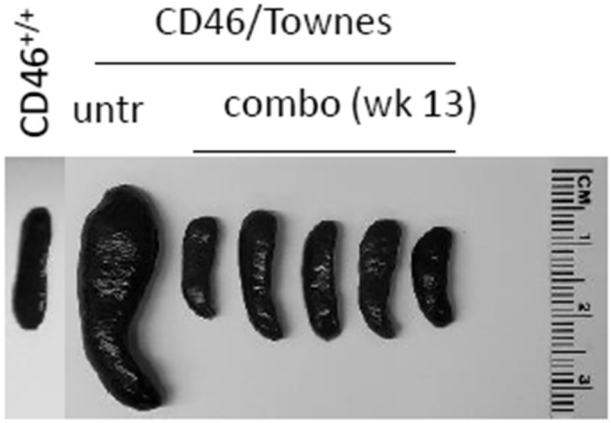


FIG. 112B



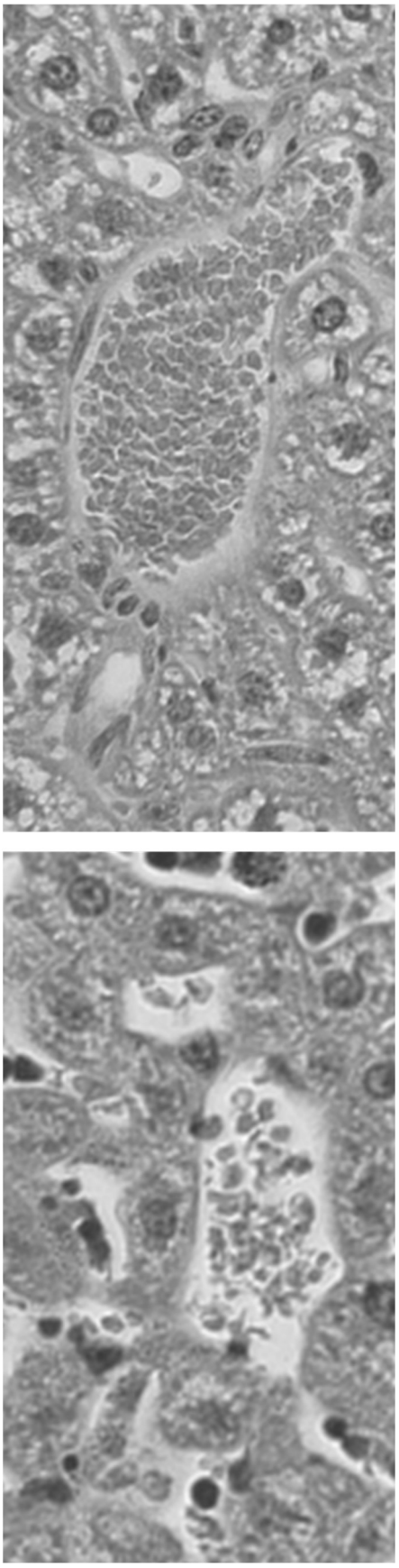
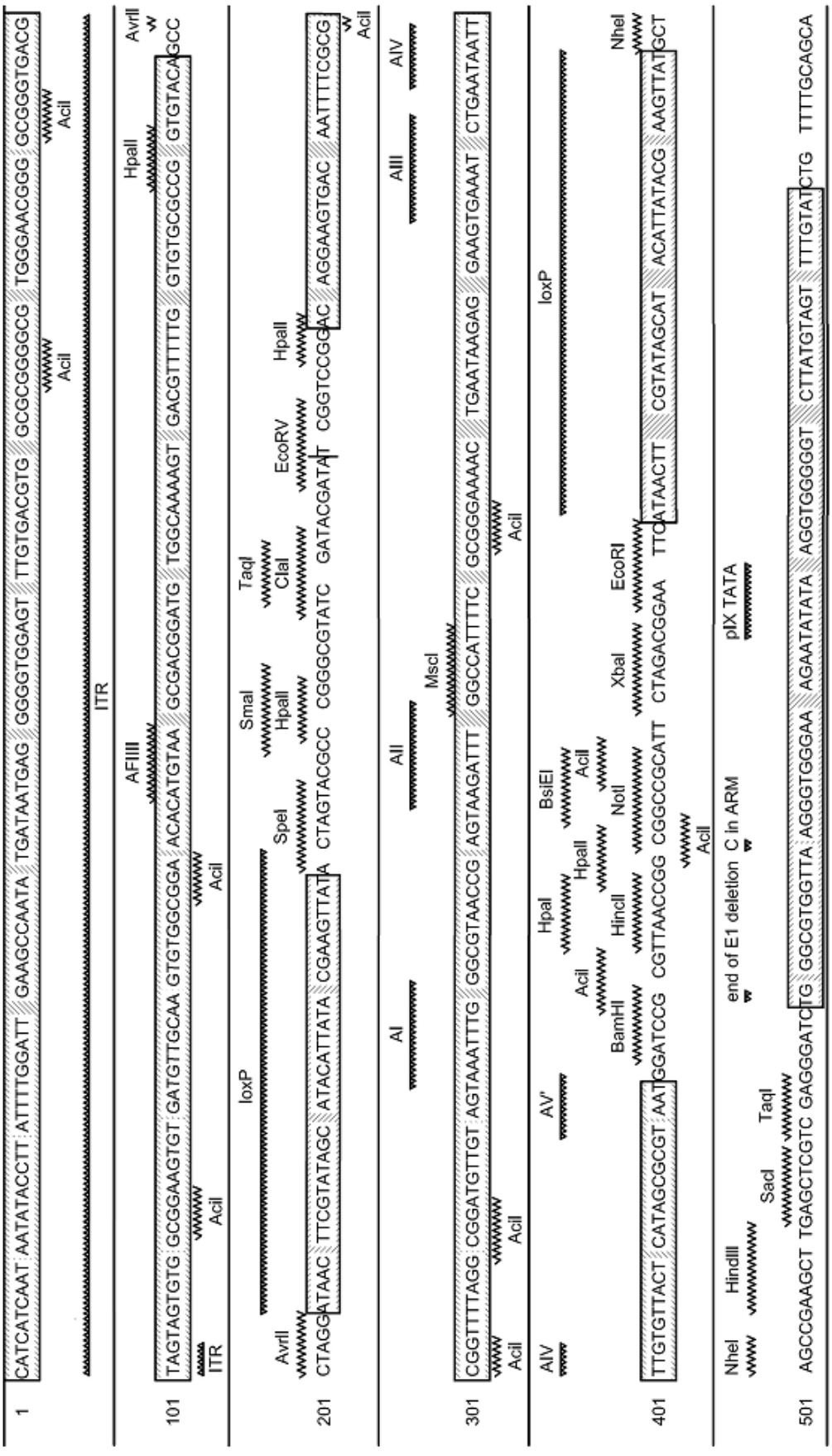


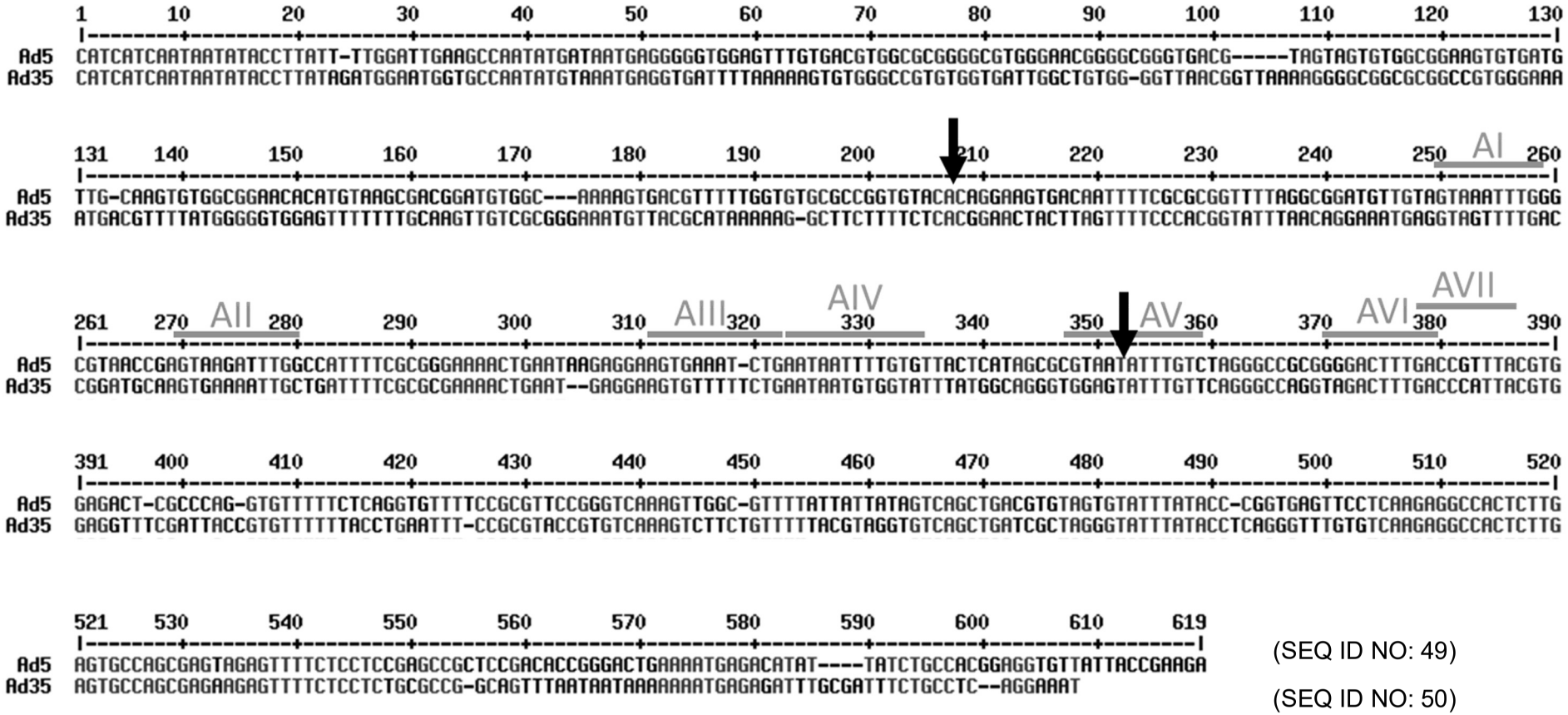
FIG. 112C

FIG. 113



SEQ ID NO: 262

FIG. 114



(SEQ ID NO: 49)

(SEQ ID NO: 50)

FIG. 115

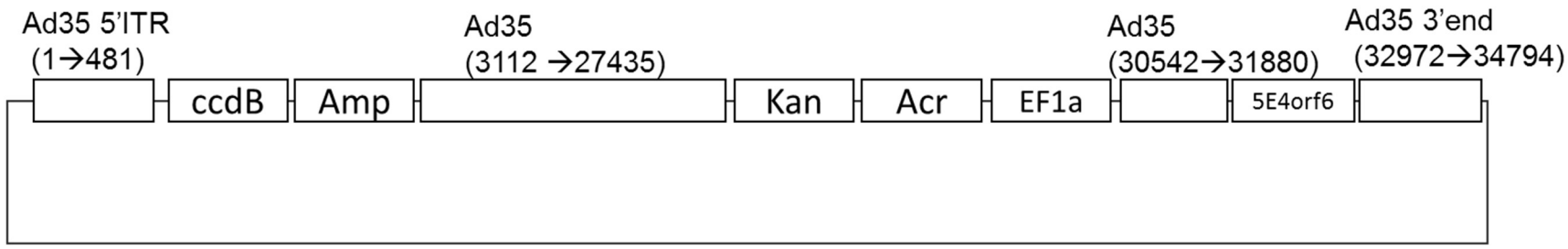


FIG. 116

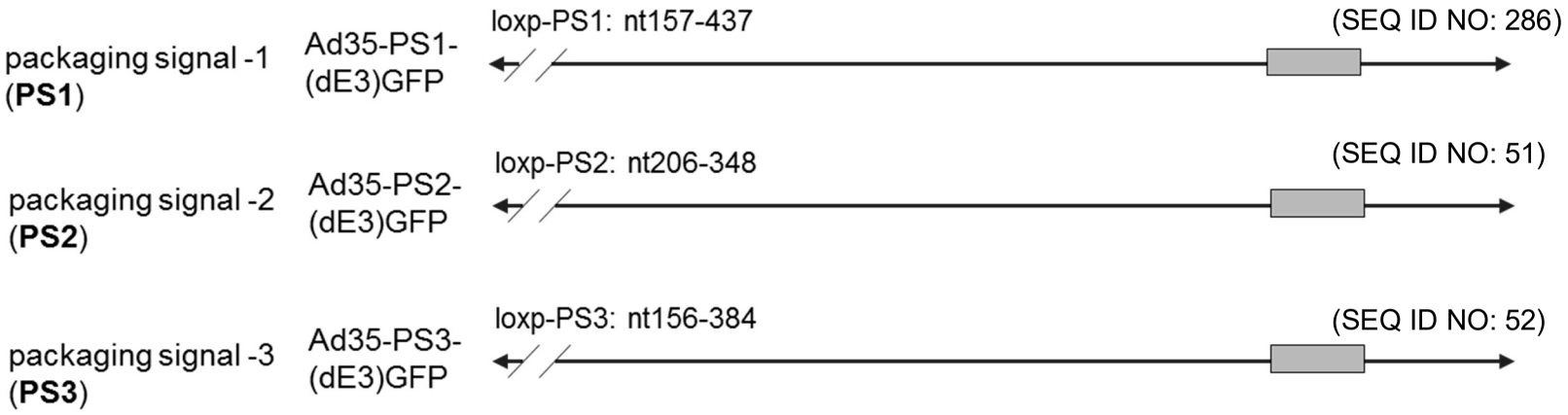


FIG. 117

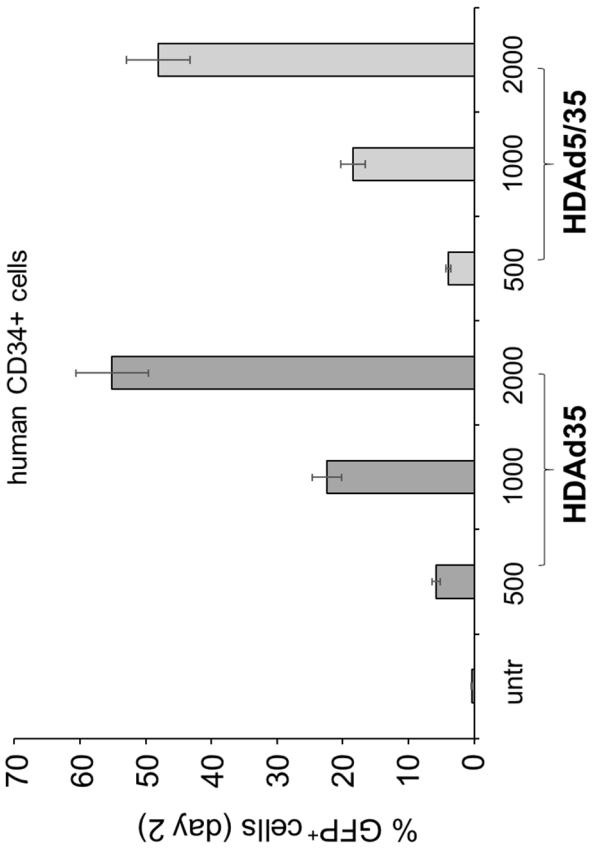


FIG. 118

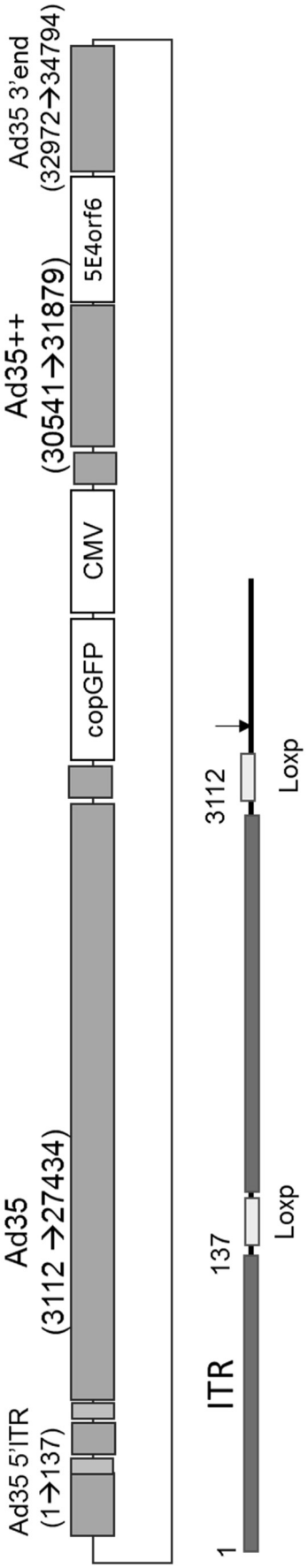


FIG. 119

CATCATCAATAATATACCTTATAGATGGAATGGTGCCAATATGTAAATGAGGTGATTTTAAAAAGTGTGGGCCGTGTGGTGATTGG
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

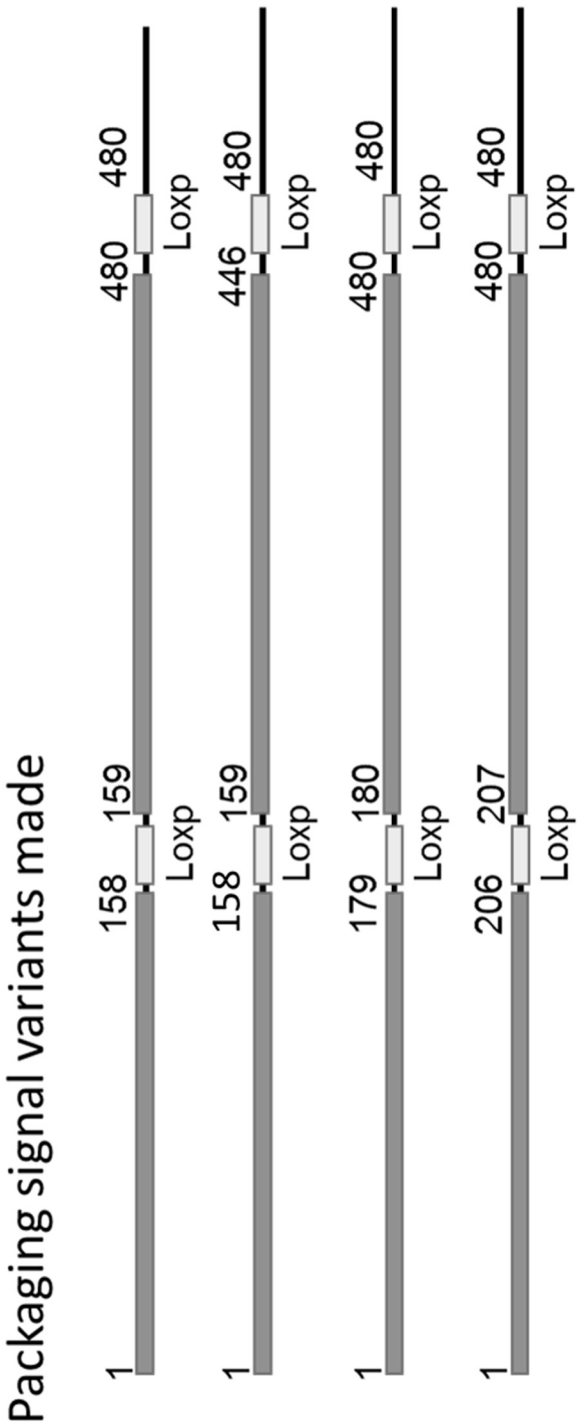

Packaging signal variants made
1
158
159
Loxp
480
480
1
158
159
Loxp
446
480
1
179
180
Loxp
480
480
1
206
207
Loxp
480
480

FIG. 120B

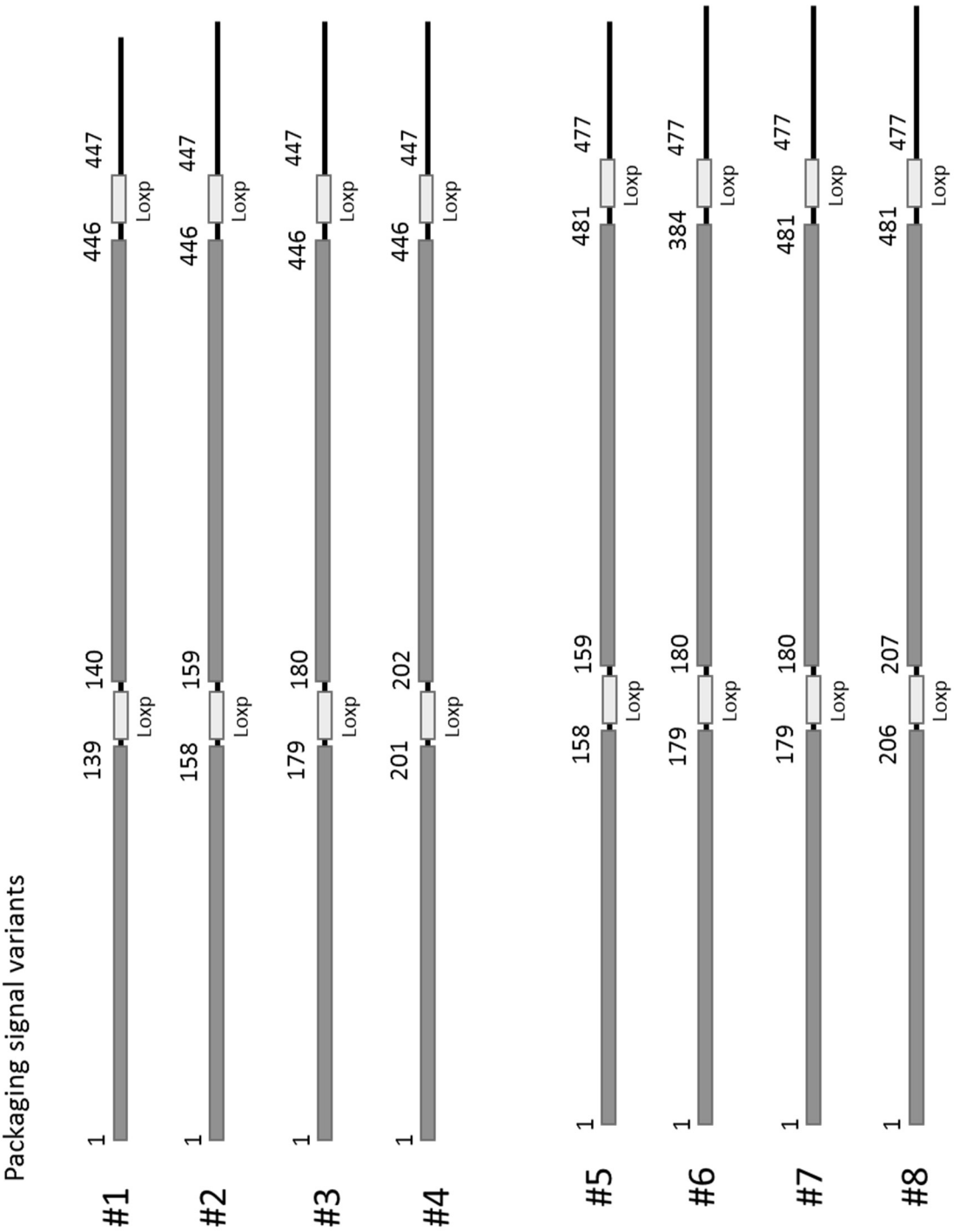

Packaging signal variants
#1
1
139
140
Loxp
446
447
Loxp
#2
1
158
159
Loxp
446
447
Loxp
#3
1
179
180
Loxp
446
447
Loxp
#4
1
201
202
Loxp
446
447
Loxp
#5
1
158
159
Loxp
481
477
Loxp
#6
1
179
180
Loxp
384
477
Loxp
#7
1
179
180
Loxp
481
477
Loxp
#8
1
206
207
Loxp
481
477
Loxp

FIG. 121

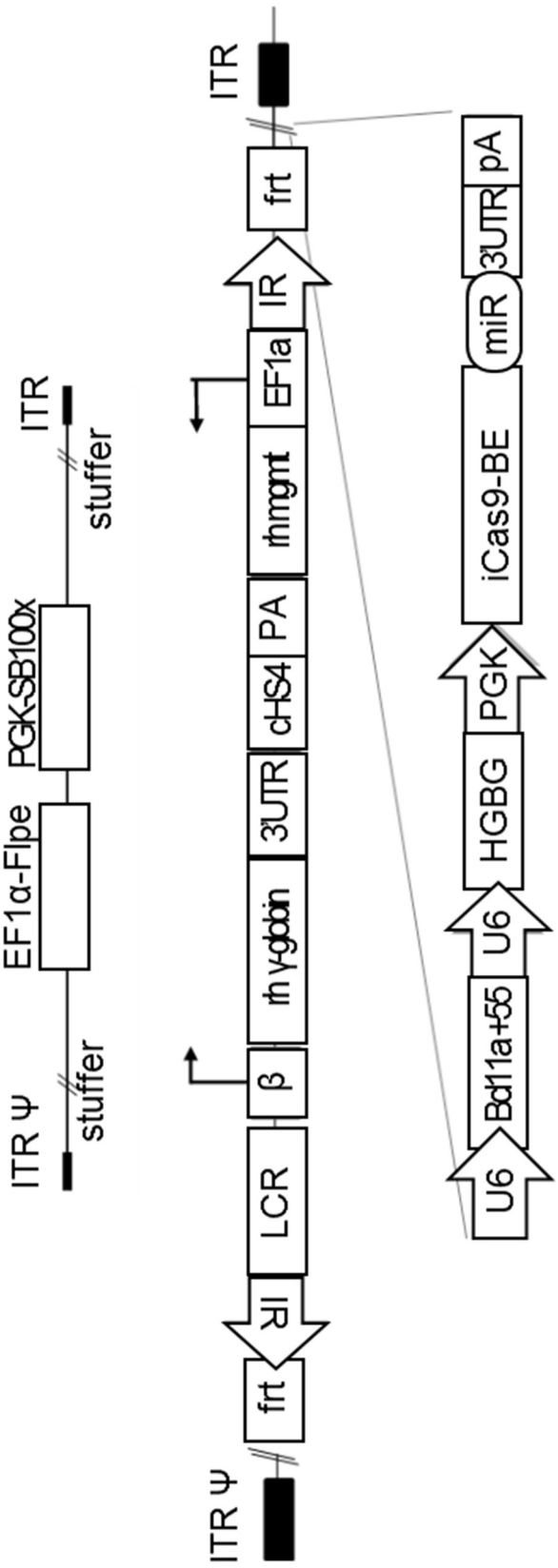

ITR Ψ
stuffer
EF1α-Flpe
PGK-SB100x
ITR
stuffer
ITR Ψ
frt
IR
LCR
β
rh γ-globin
3'UTR
cHS4
PA
rhmgmt
EF1a
IR
frt
ITR
U6
Bcl11a+55
U6
HGBG
PGK
iCas9-BE
miR
3'UTR
pA

FIG. 122

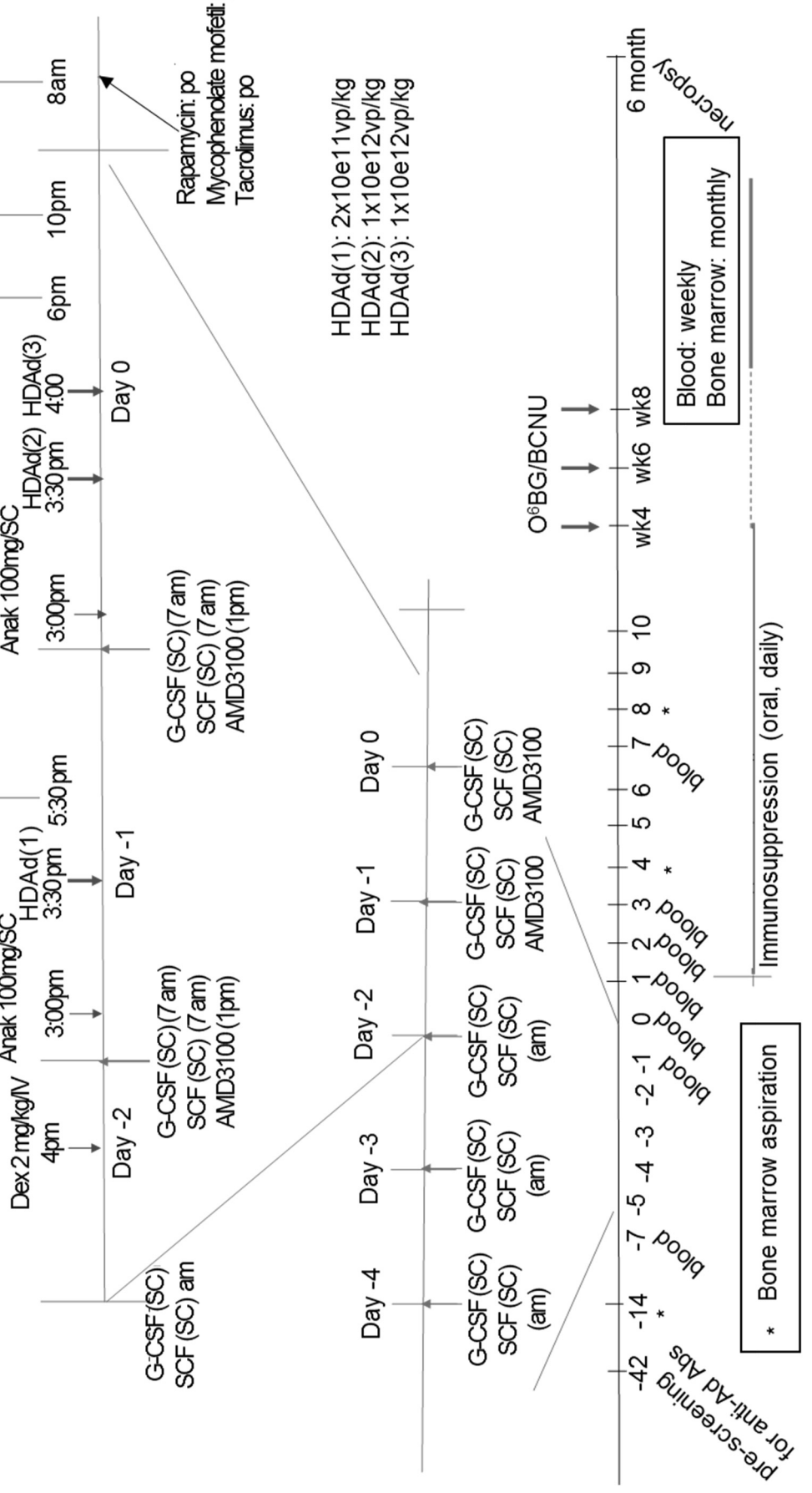

Dex 2 mg/kg/IV
4pm
Day -2
Dex 2 mg/kg/IV
Pred 10mg/kg/IV
Anak 100mg/SC
3:00pm
HDAd(1)
3:30pm
Day -1
blood
5:30pm
Dex 2 mg/kg/IV
Pred 10mg/kg/IV
Anak 100mg/SC
3:00pm
HDAd(2)
3:30pm
HDAd(3)
4:00
Day 0
blood
6pm
blood
10pm
blood
8am
Rapamycin: po
Mycophenolate mofetil: po
Tacrolimus: po
G-CSF (SC)
SCF (SC)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

FIG. 123

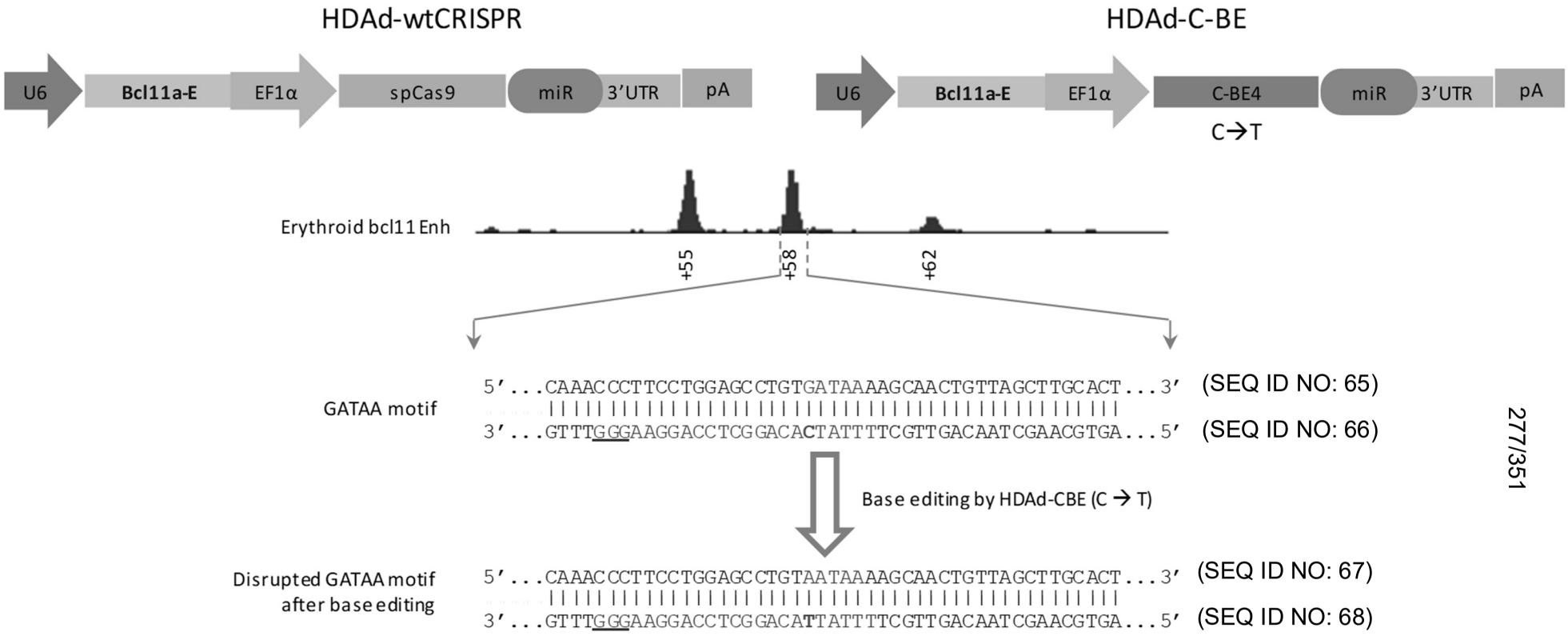

HDAd-wtCRISPR
U6
Bcl11a-E
EF1α
spCas9
miR
3'UTR
pA
HDAd-C-BE
U6
Bcl11a-E
EF1α
C-BE4
miR
3'UTR
pA
C→T
Erythroid bcl11 Enh
+55
+58
+92
GATAA motif
5'...CAAACCCTTCCTGGAGCCTGTGATAAAAGCAACTGTTAGCTTGCACT...3' (SEQ ID NO: 65)
3'...GTTTGGGAAGGACCTCGGACACTATTTTCGTTGACAATCGAACGTGA...5' (SEQ ID NO: 66)
Base editing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

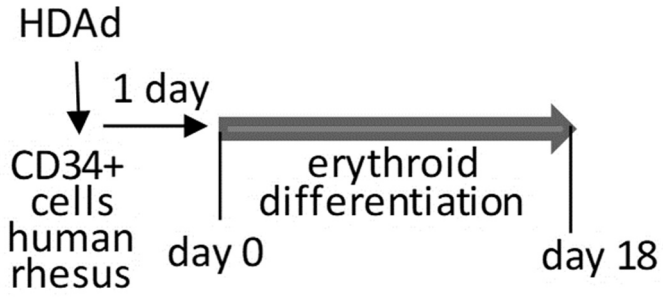


FIG. 124B

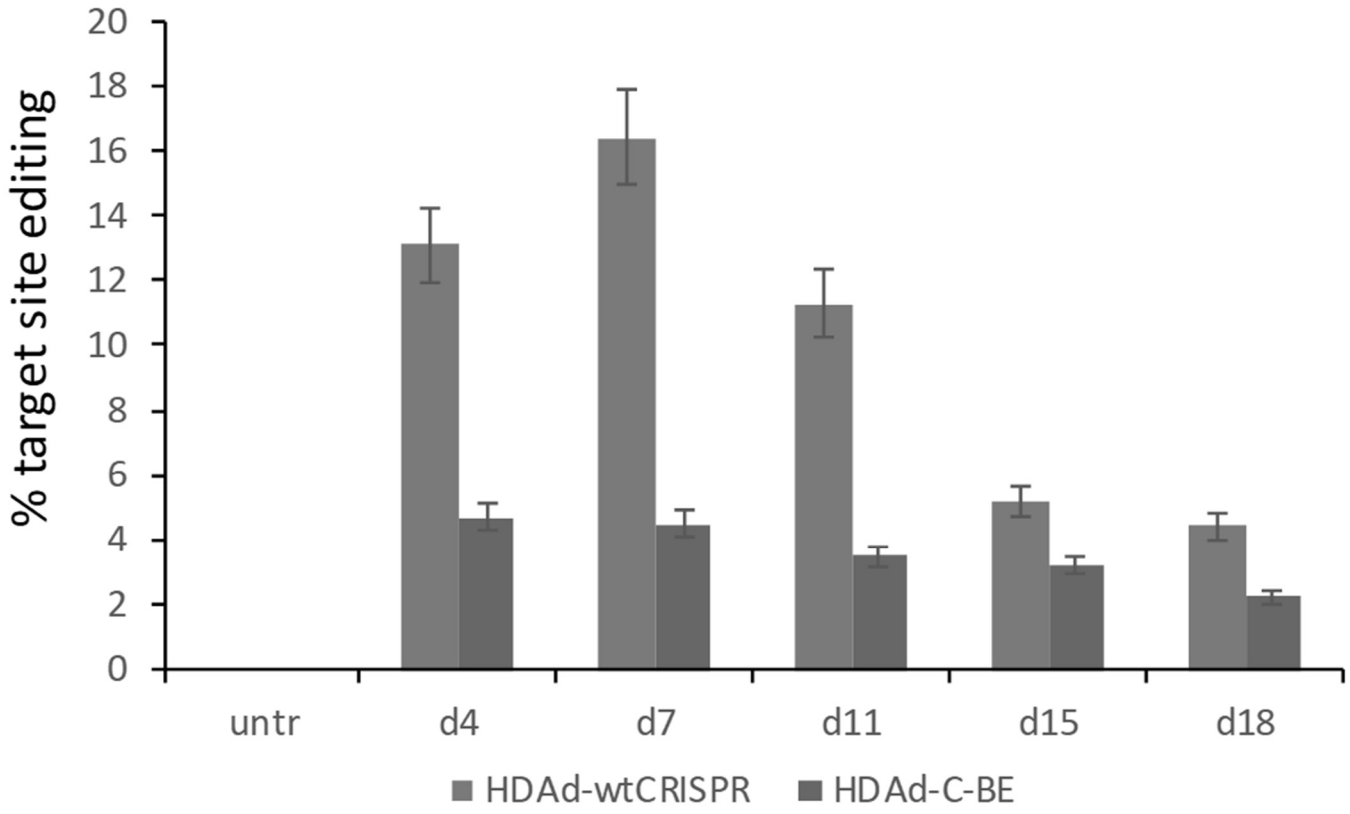


FIG. 124C

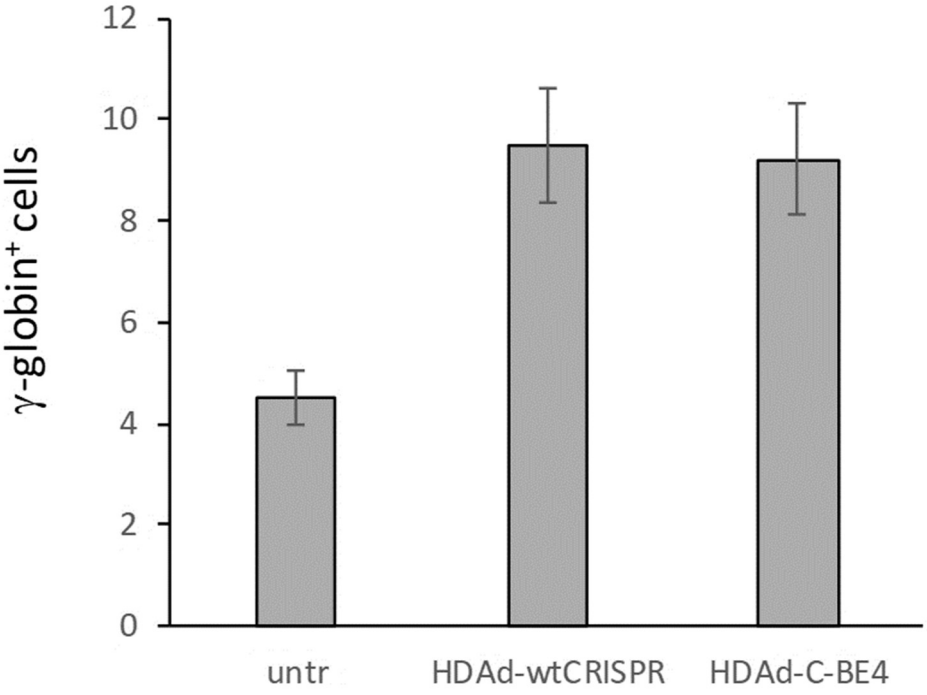


FIG. 125

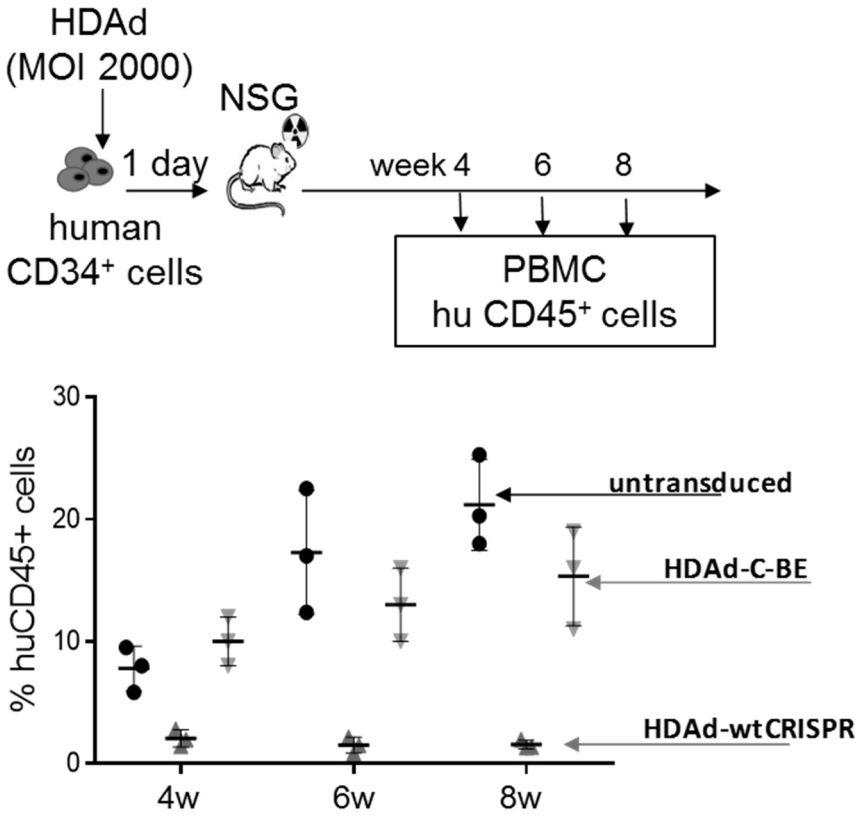


FIG. 126

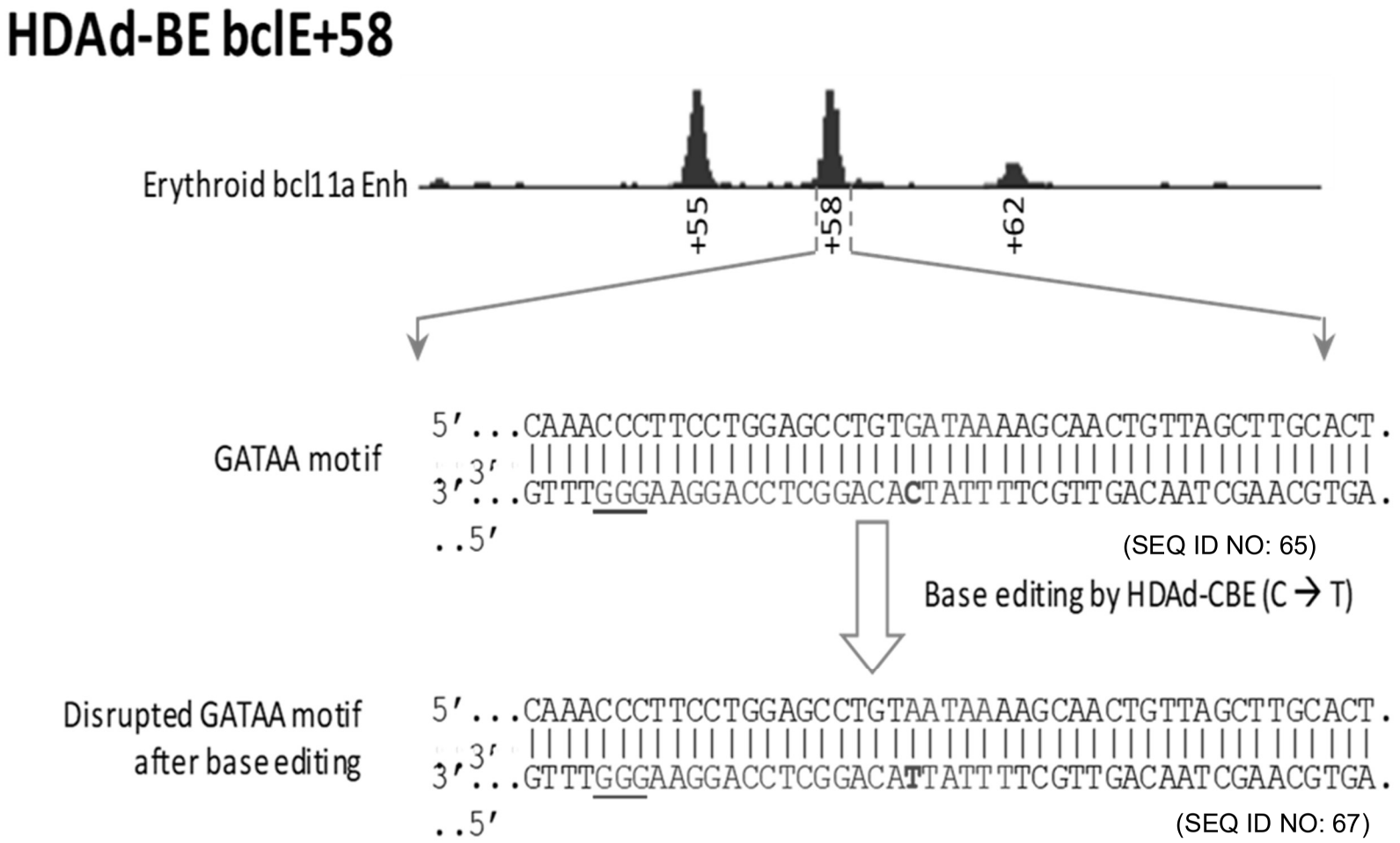

HDAd-BE bclE+58
Erythroid bcl11a Enh
+55
+58
+62
GATAA motif
5′...CAAACCCTTCCTGGAGCCTGTGATAAAAGCAACTGTTAGCTTGCACT.
3′...GTTTGGGAAGGACCTCGGACACTATTTCGTTGACAATCGAACGTGA.
..5′
(SEQ ID NO: 65)
Base editing by HDAd-CBE (C → T)
Disrupted GATAA motif
after base editing
5′...CAAACCCTTCCTGGAGCCTGTAATAAAAGCAACTGTTAGCTTGCACT.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

FIG. 126 cont'd

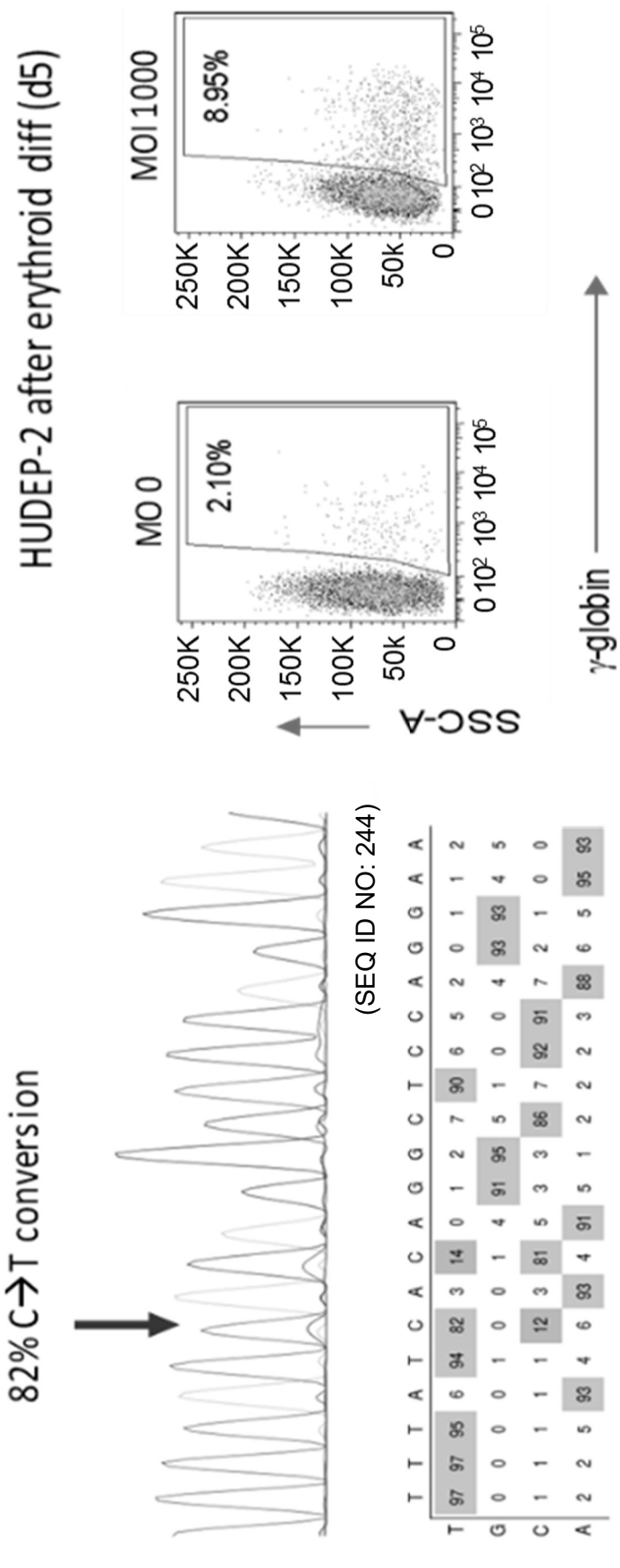


FIG. 126 cont'd

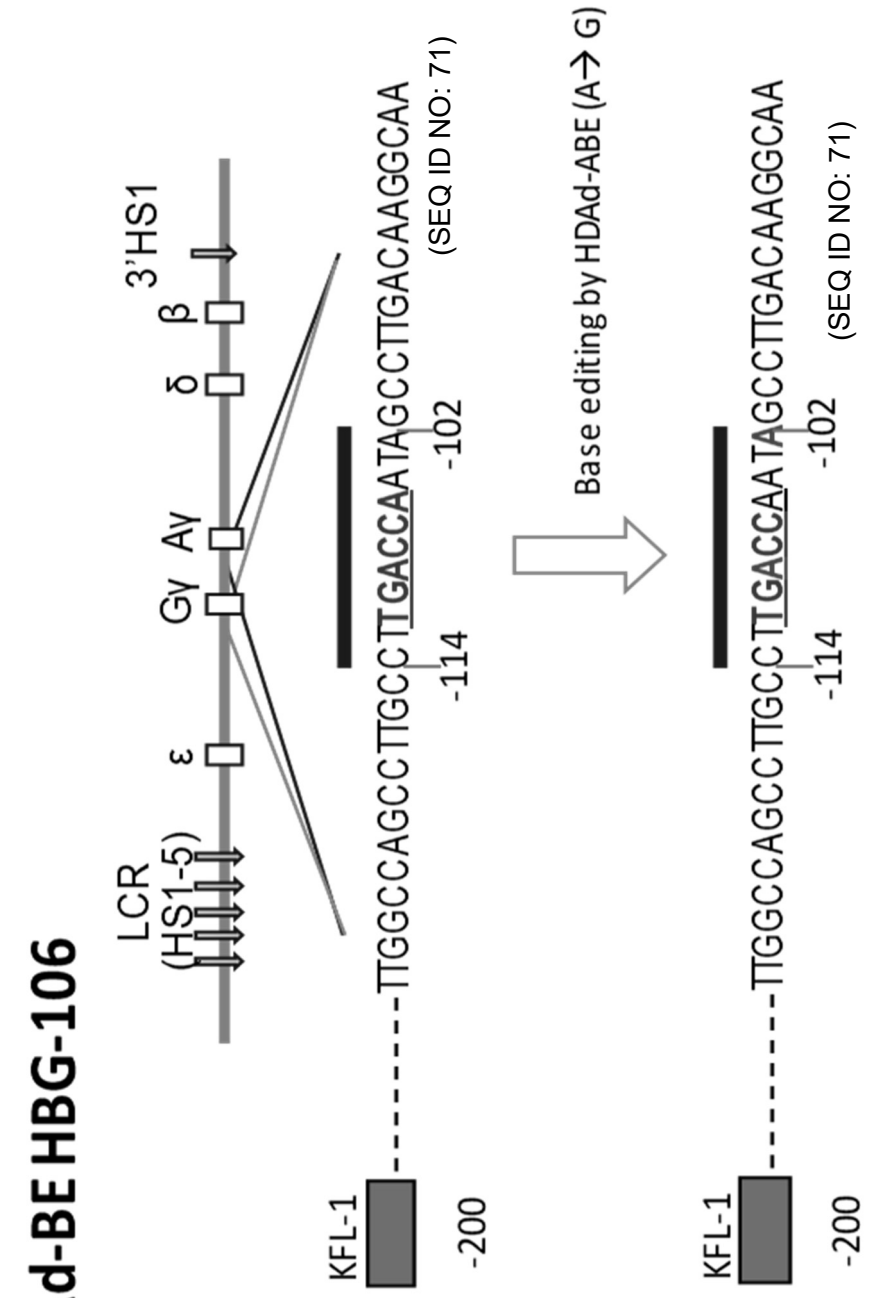
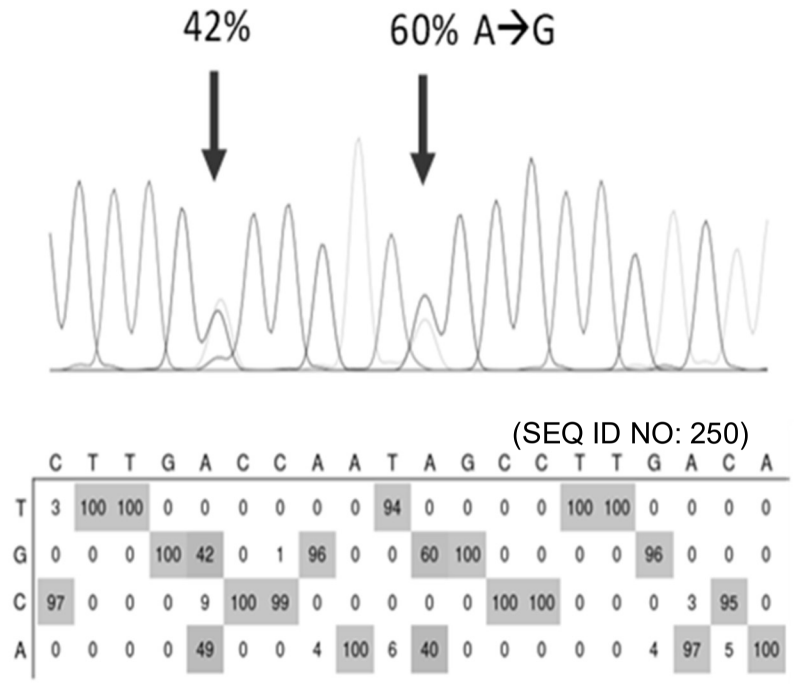


FIG. 126 cont'd



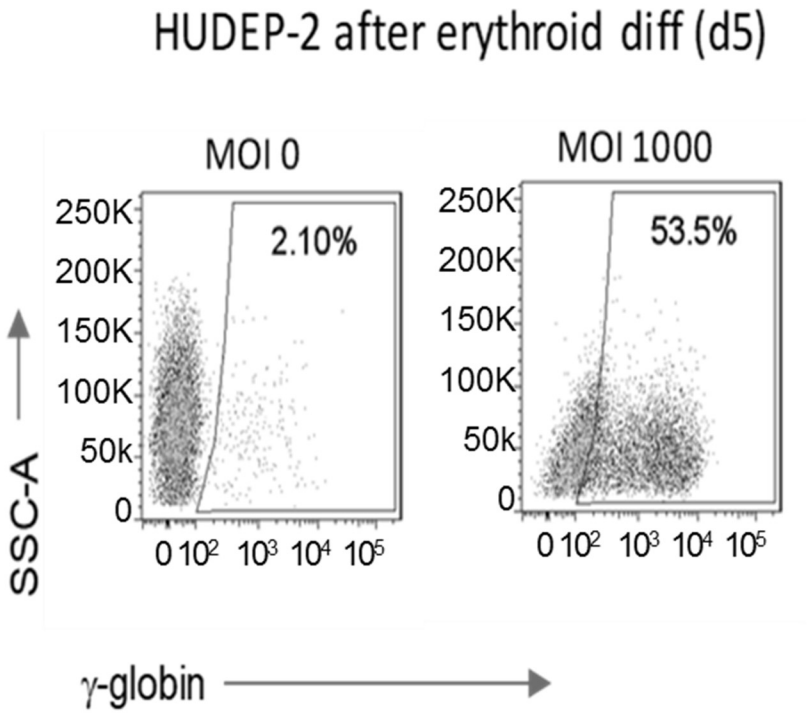


FIG.127

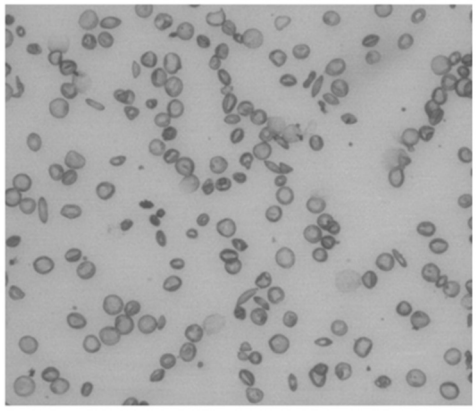


FIG. 127B

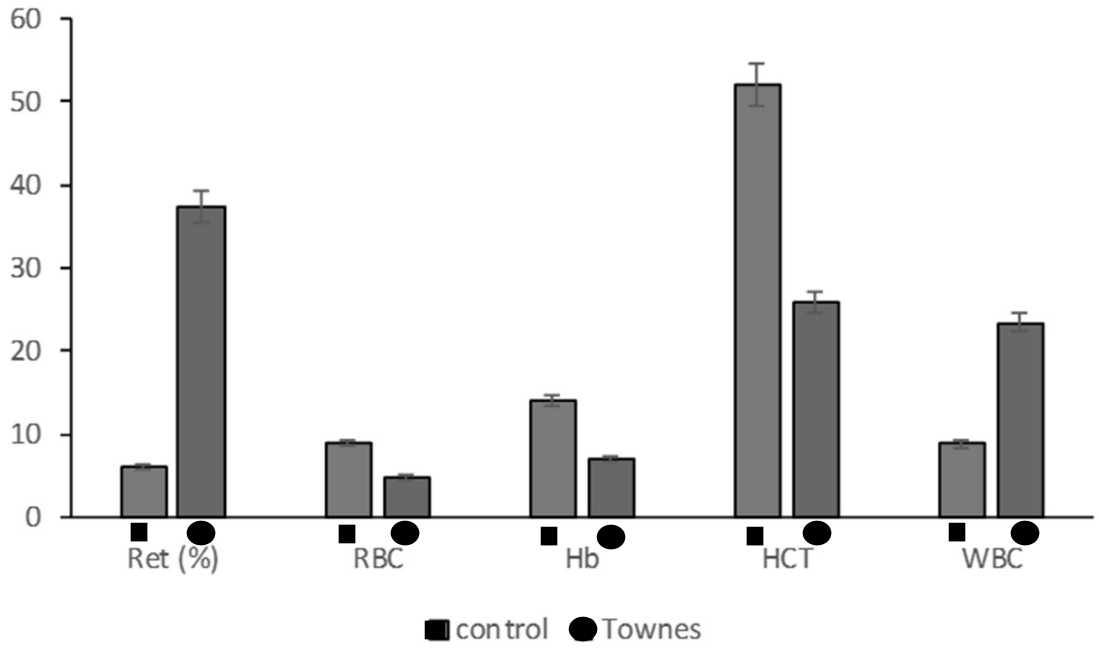


FIG. 128A

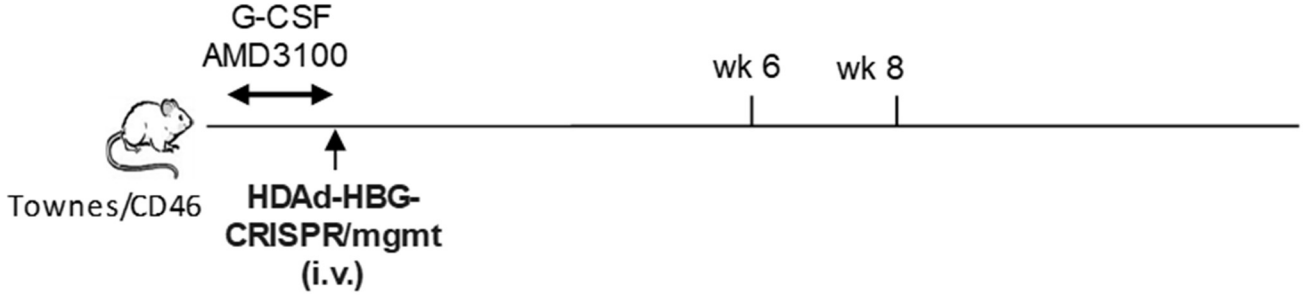


FIG. 128B

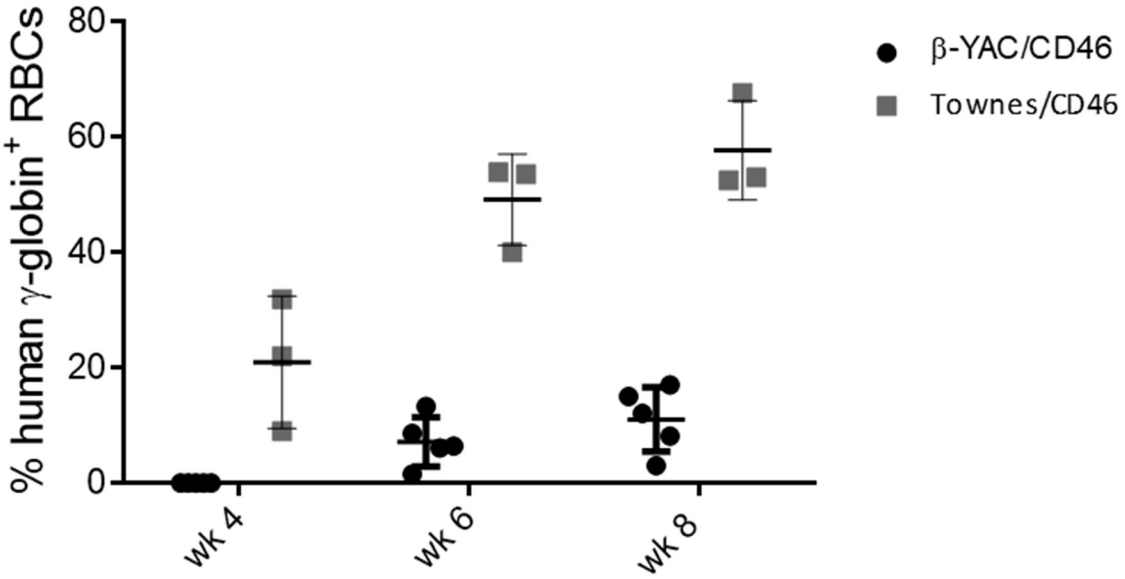


FIG. 128C

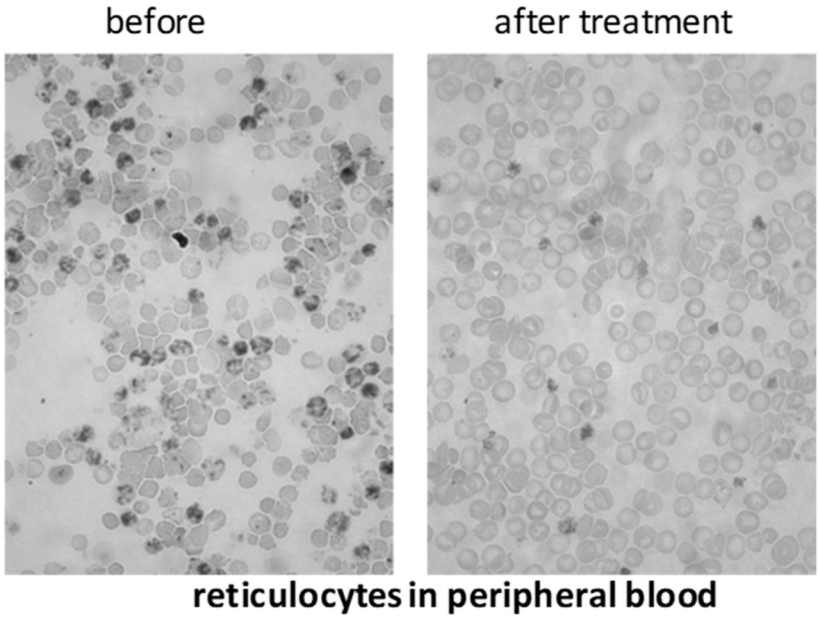


FIG. 129A

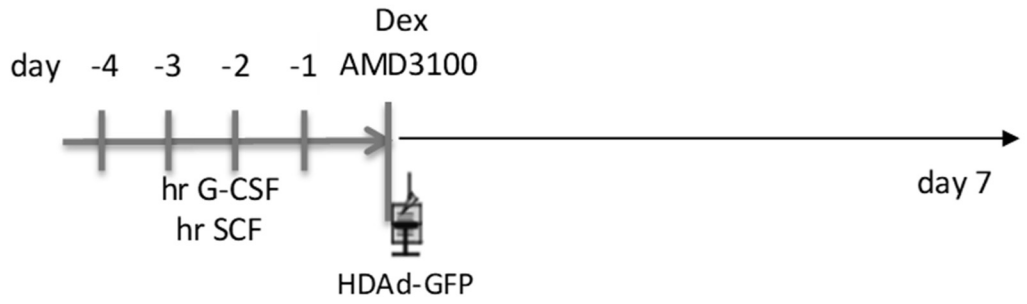


FIG.129B

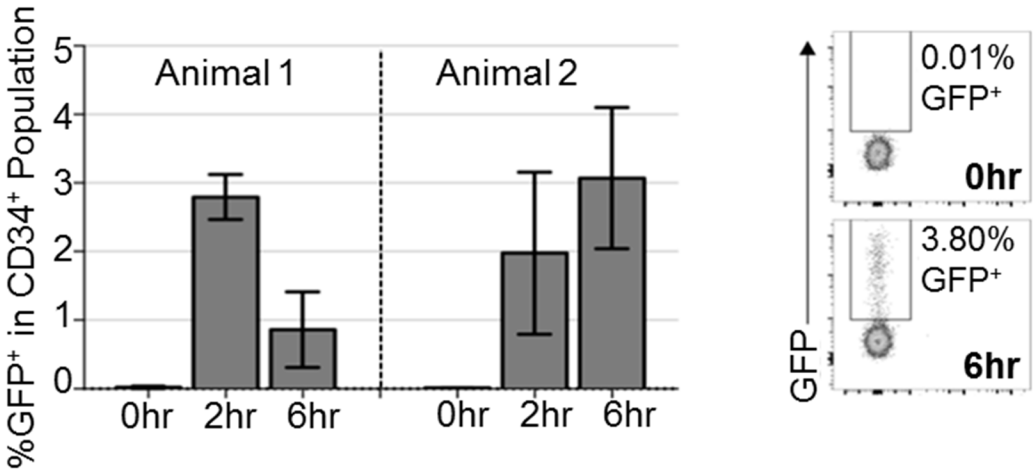


FIG. 129C

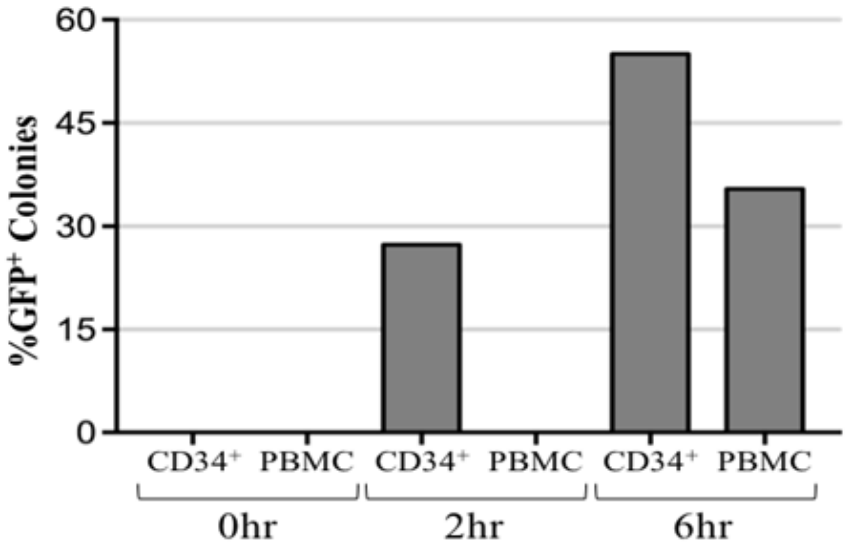


FIG. 129D

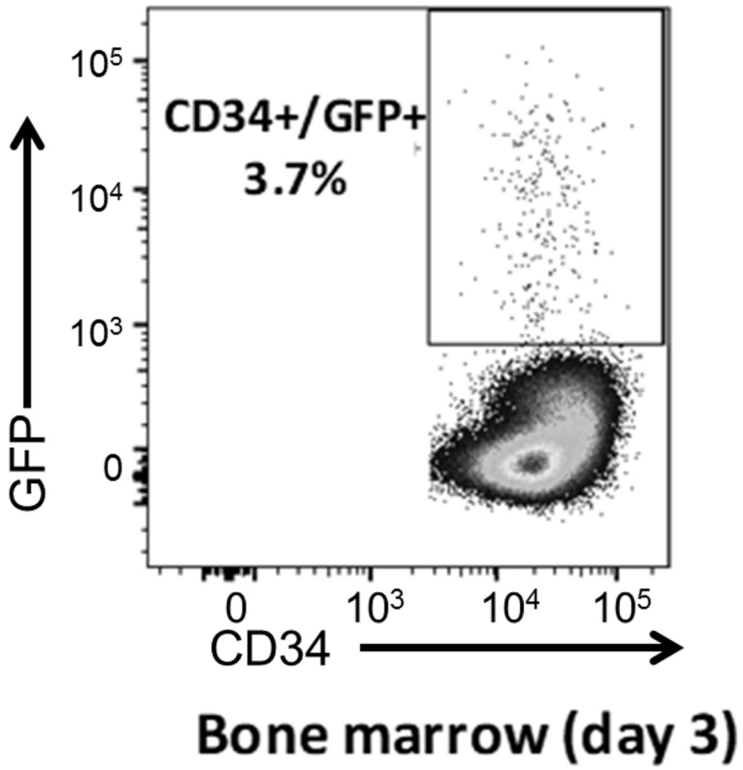


FIG. 130

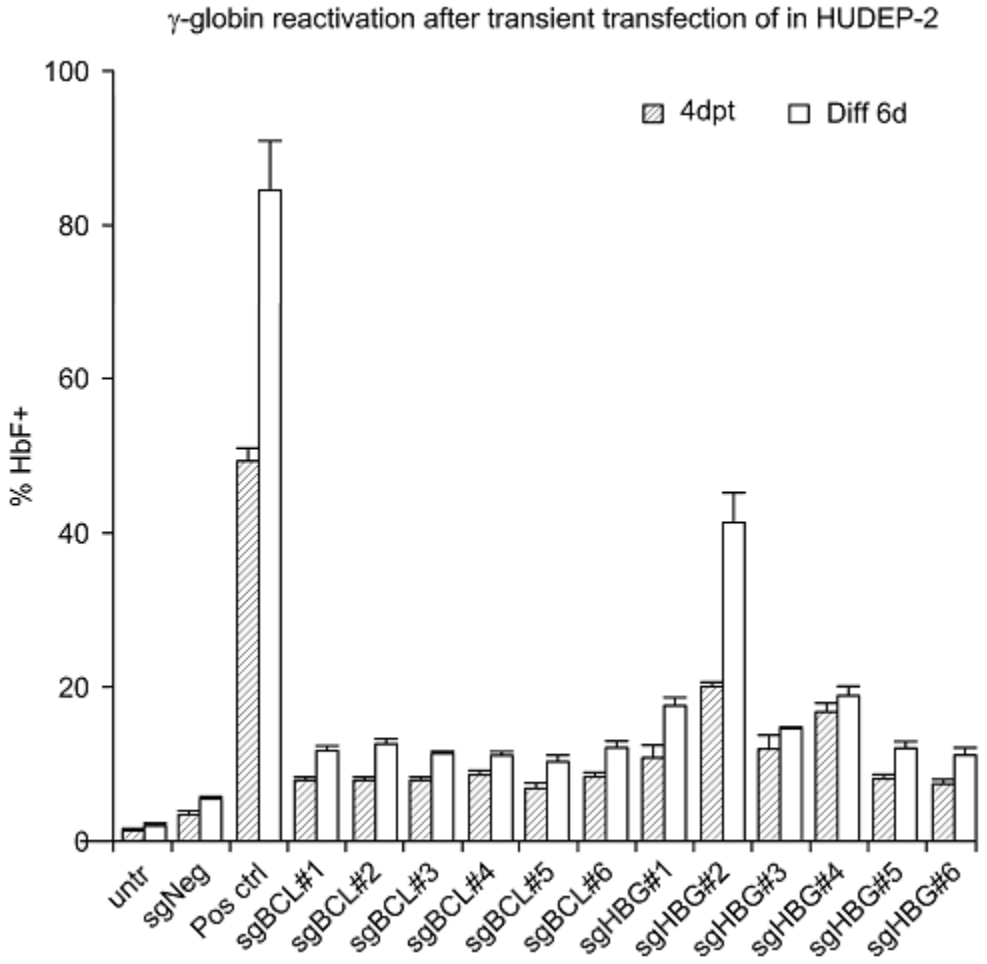


FIG. 131A

FIG. 131B

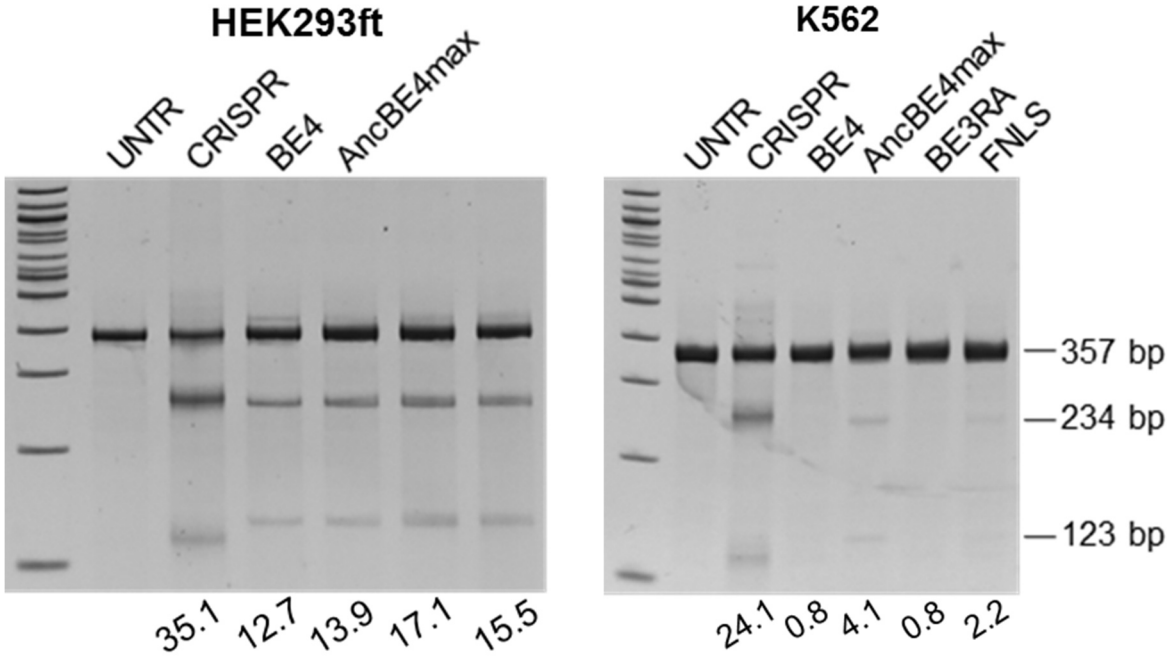


FIG. 132A

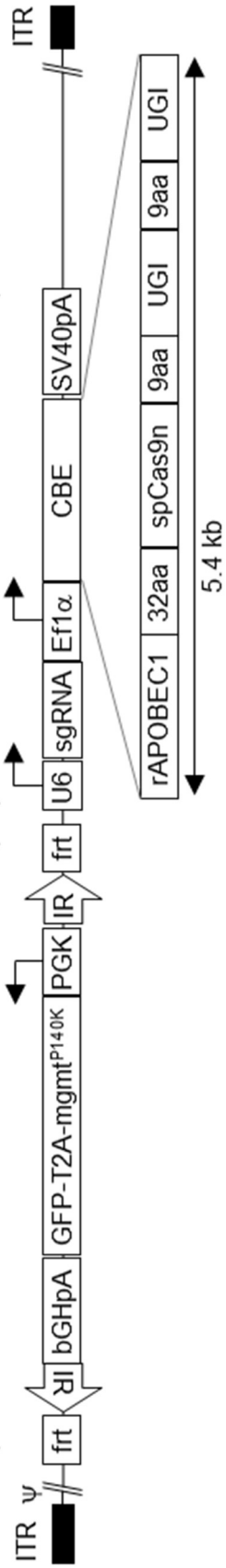


FIG. 132B

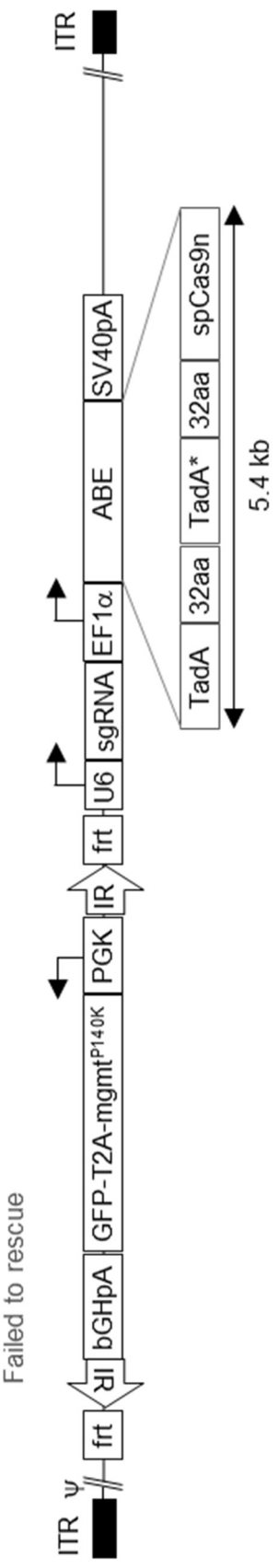


FIG. 132C

```
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 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

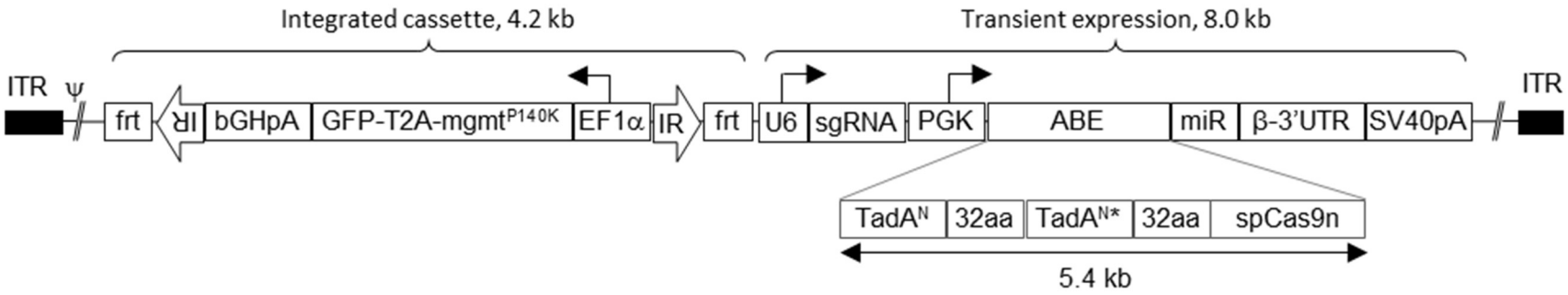


FIG. 133B

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

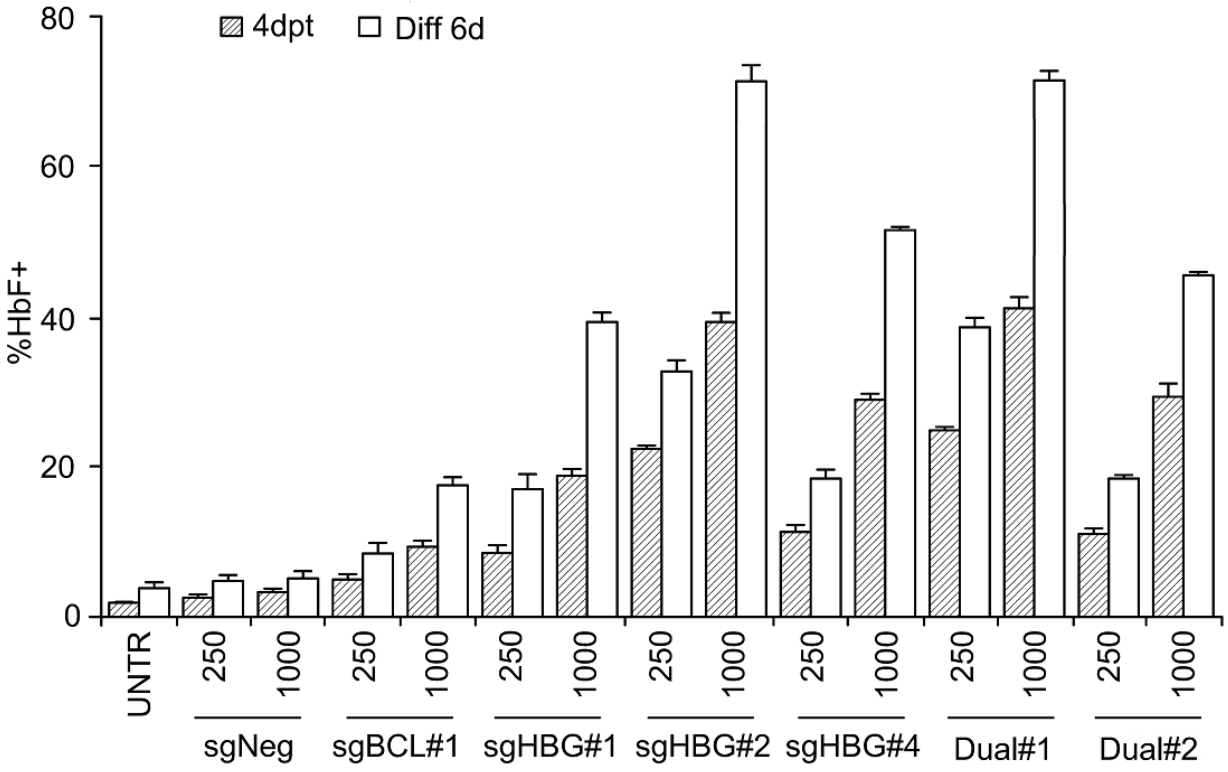
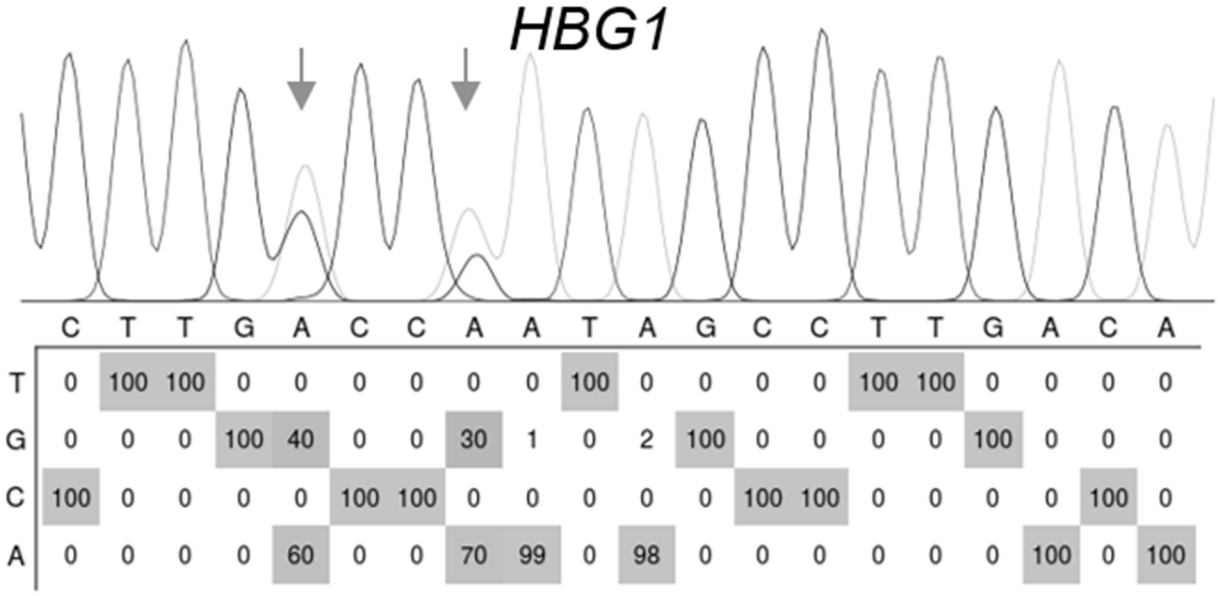

80
60
40
20
0
%HbF+
4dpt
Diff 6d
UNTR
250
1000
sgNeg
sgBCL#1
sgHBG#1
sgHBG#2
sgHBG#4
Dual#1
Dual#2

FIG. 133D

sgHBG#2

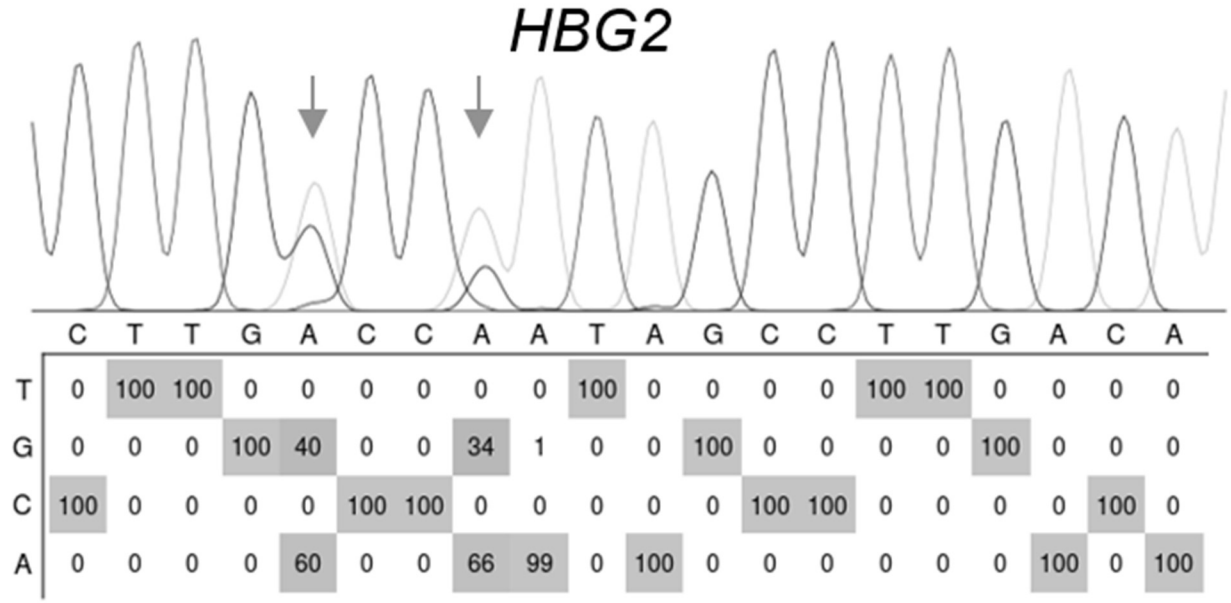
HBG1



	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

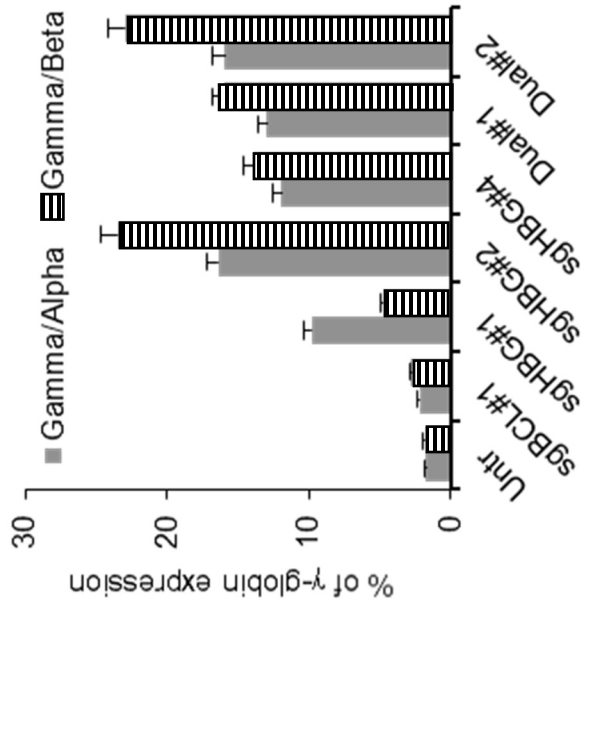


FIG. 133F

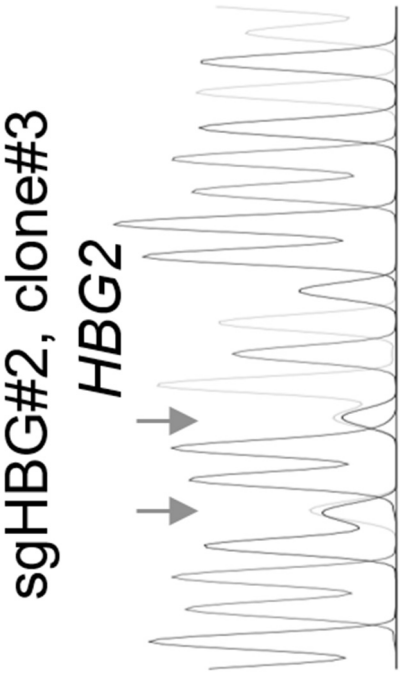


FIG. 133G

FIG. 133H

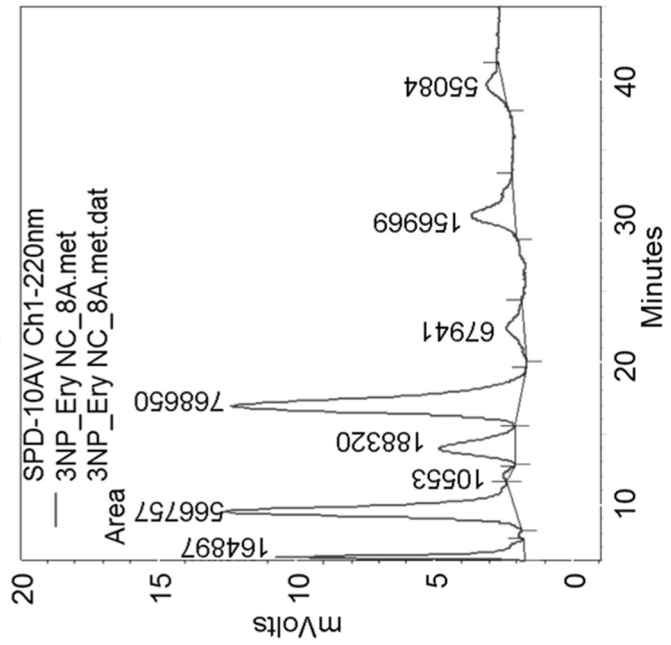
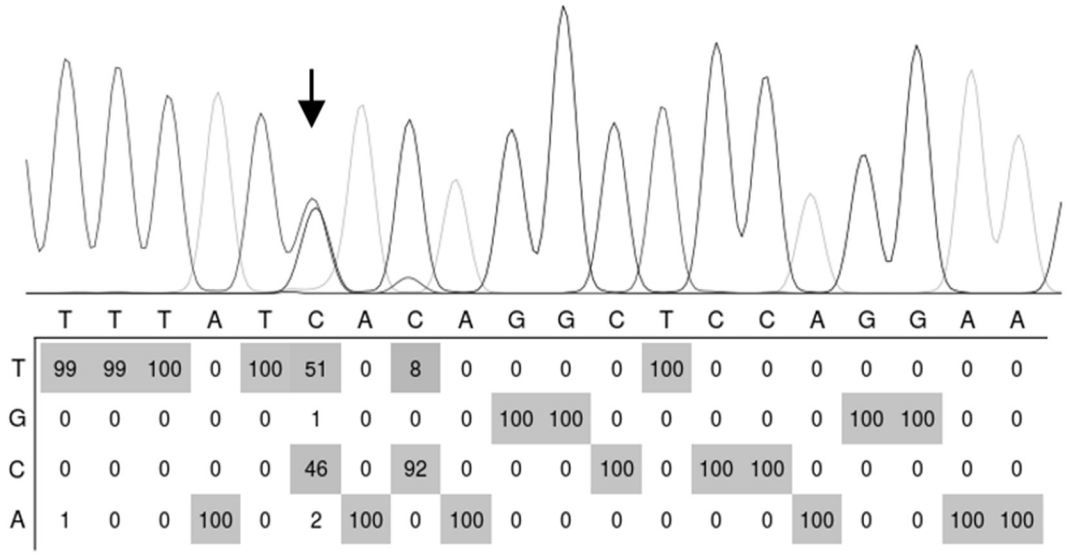


FIG. 134A

sgBCL#1

Bcl11A

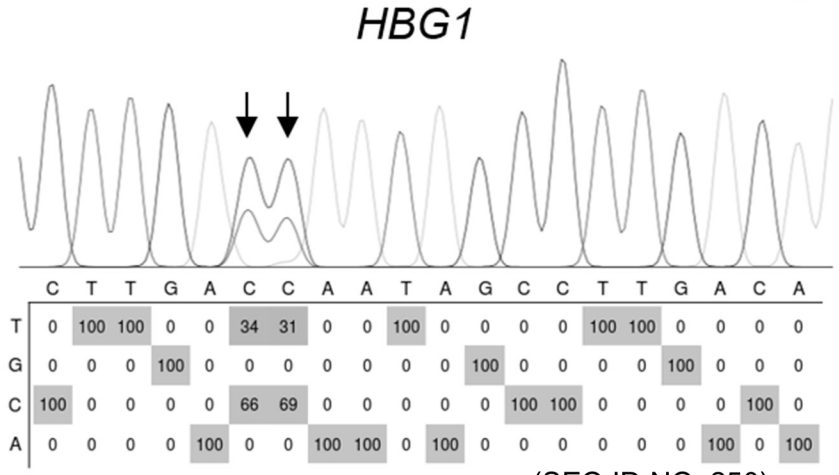


	T	T	T	A	T	C	A	C	A	G	G	C	T	C	C	A	G	G	A	A
T	99	99	100	0	100	51	0	8	0	0	0	0	100	0	0	0	0	0	0	0
G	0	0	0	0	0	1	0	0	0	100	100	0	0	0	0	0	100	100	0	0
C	0	0	0	0	0	46	0	92	0	0	0	100	0	100	100	0	0	0	0	0
A	1	0	0	100	0	2	100	0	100	0	0	0	0	0	0	100	0	0	100	100

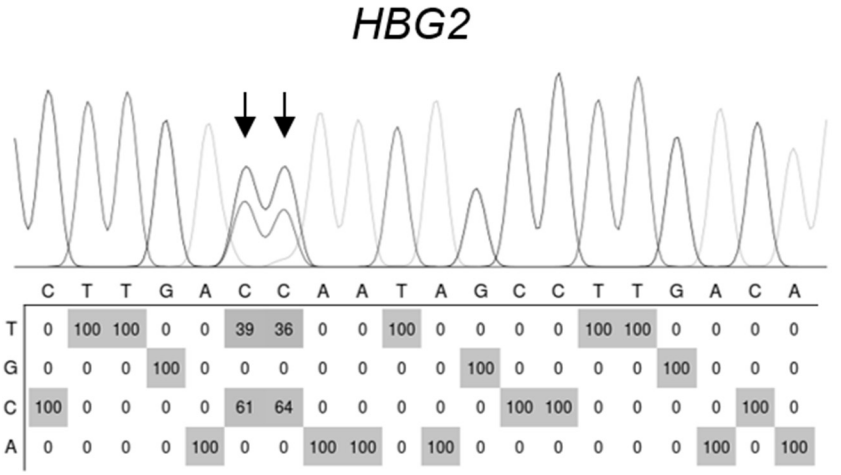
(SEQ ID NO: 244)

FIG. 134A cont'd

sgHBG#1

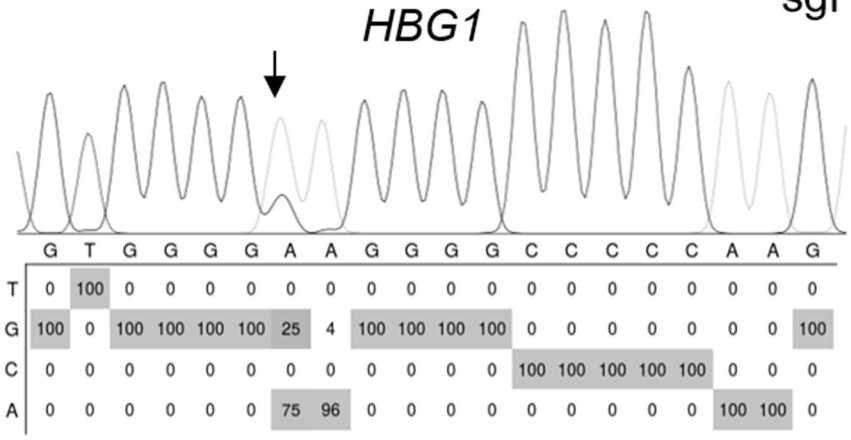


(SEQ ID NO: 250)

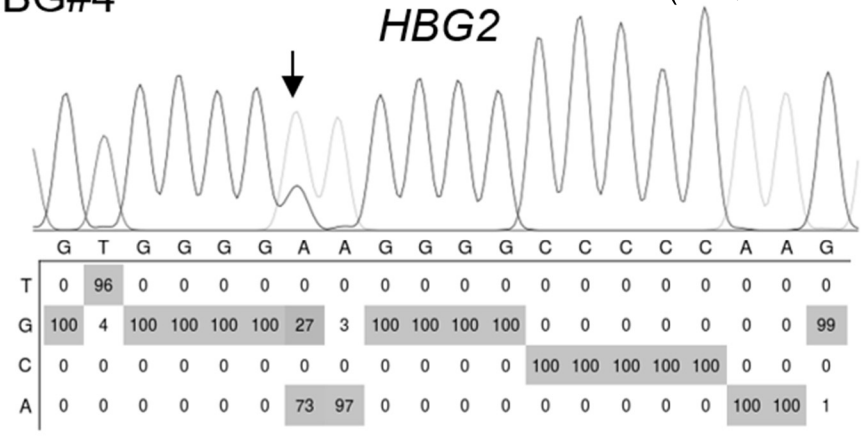


(SEQ ID NO: 250)

sgHBG#4



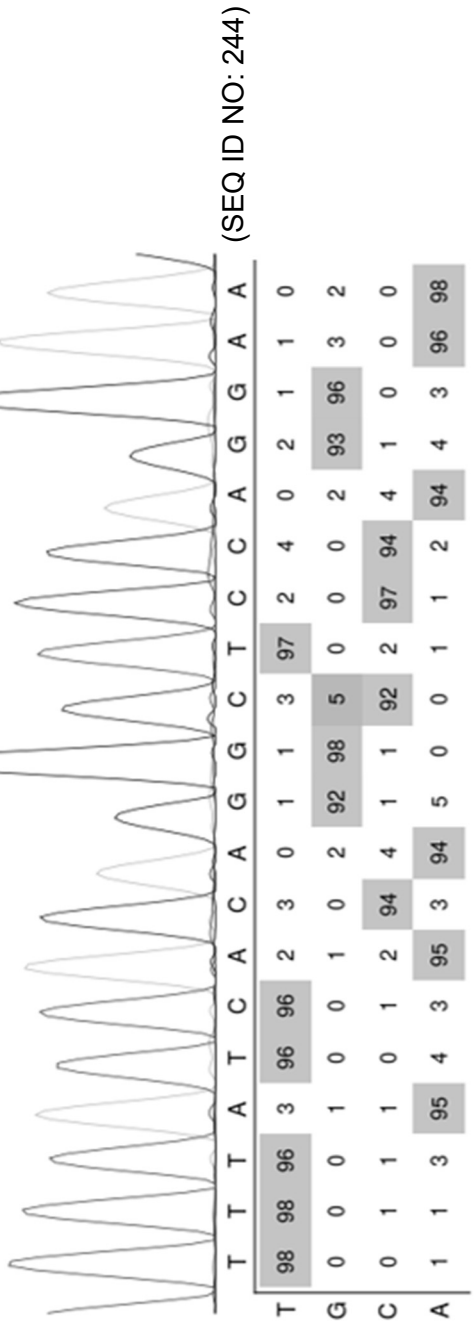
(SEQ ID NO: 253)



(SEQ ID NO: 253)

FIG. 134B

sgBCL#1, Clone#5
Bcl11A



(SEQ ID NO: 244)

FIG. 134B cont'd

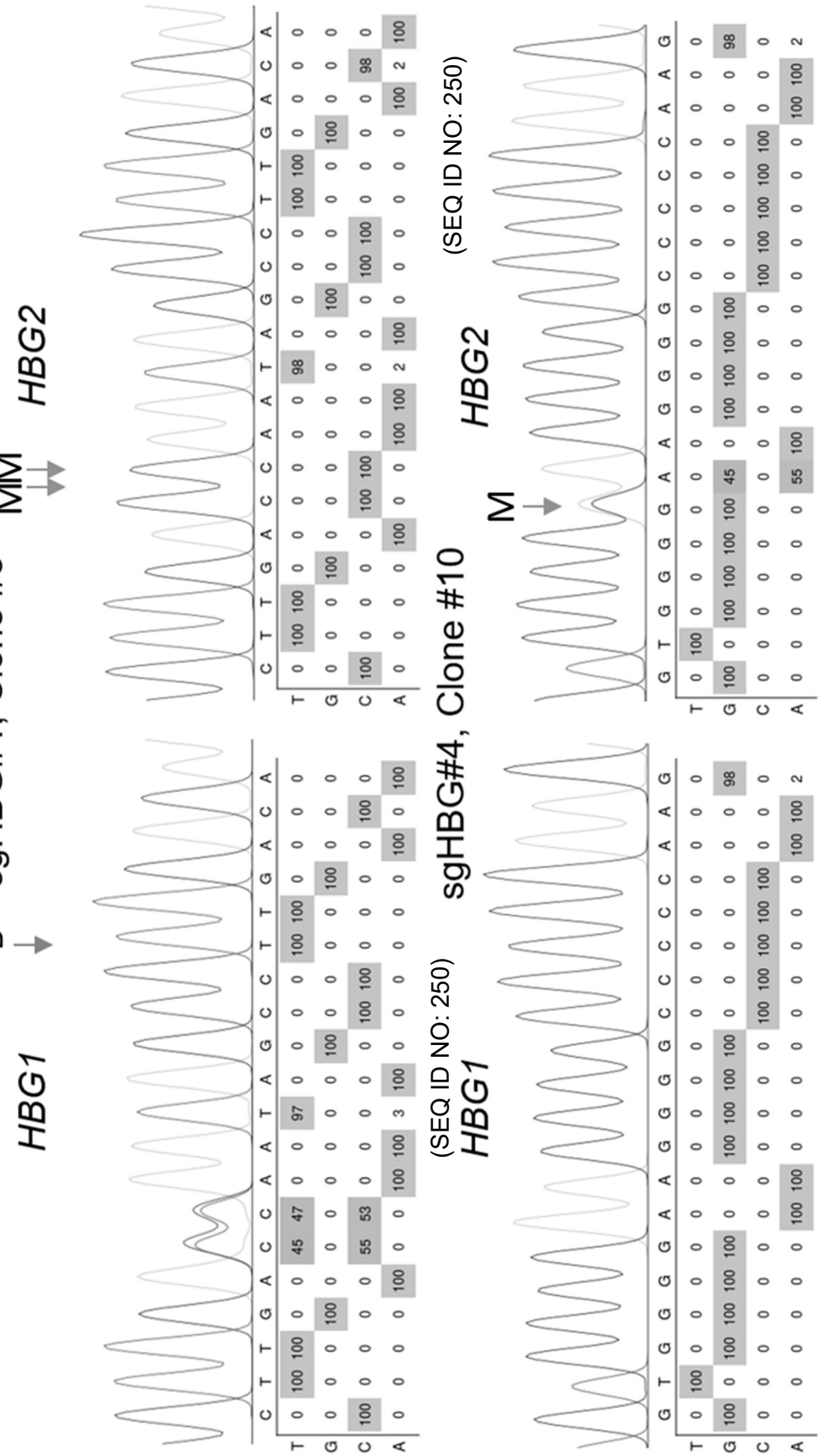


FIG. 134C

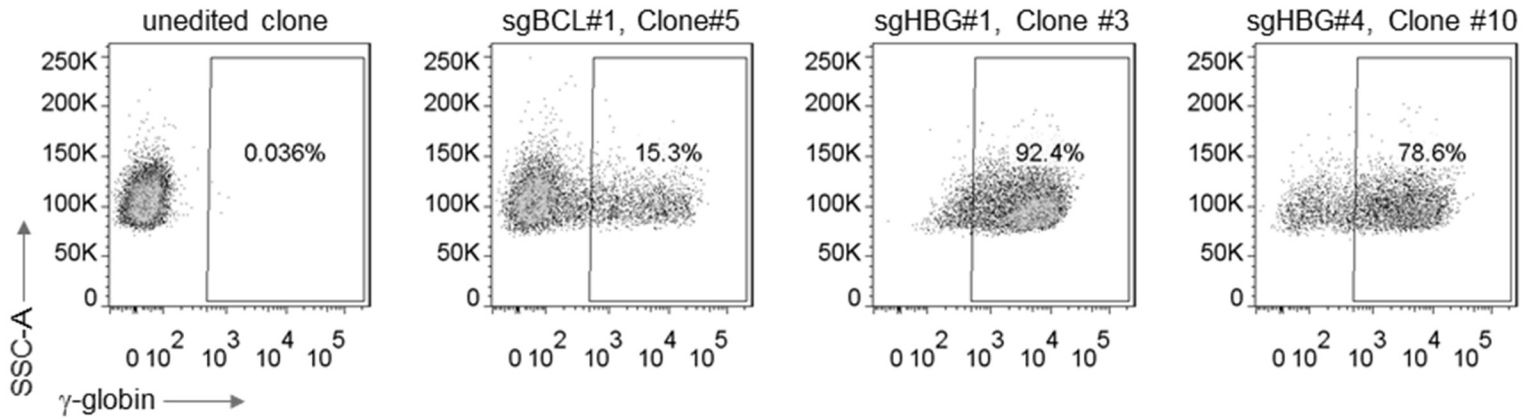


FIG. 135A

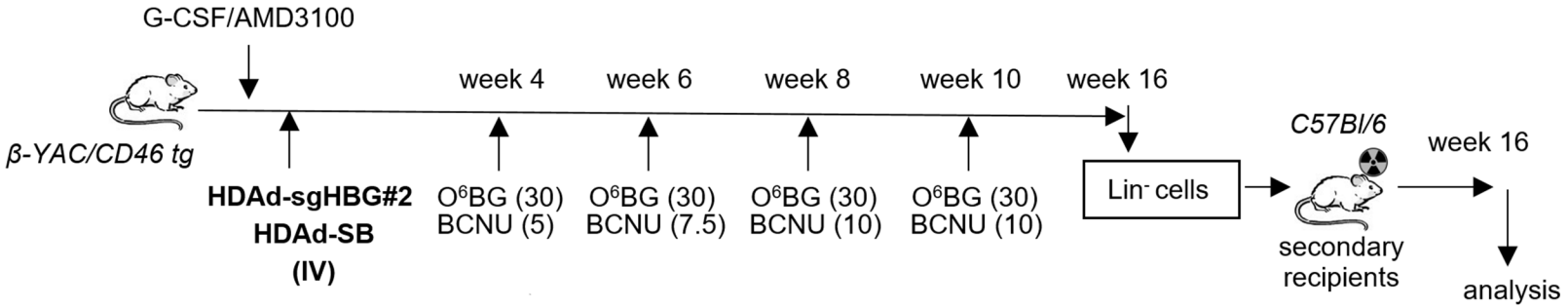


FIG. 135B

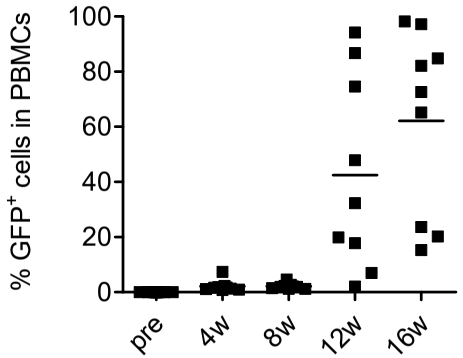


FIG. 135C

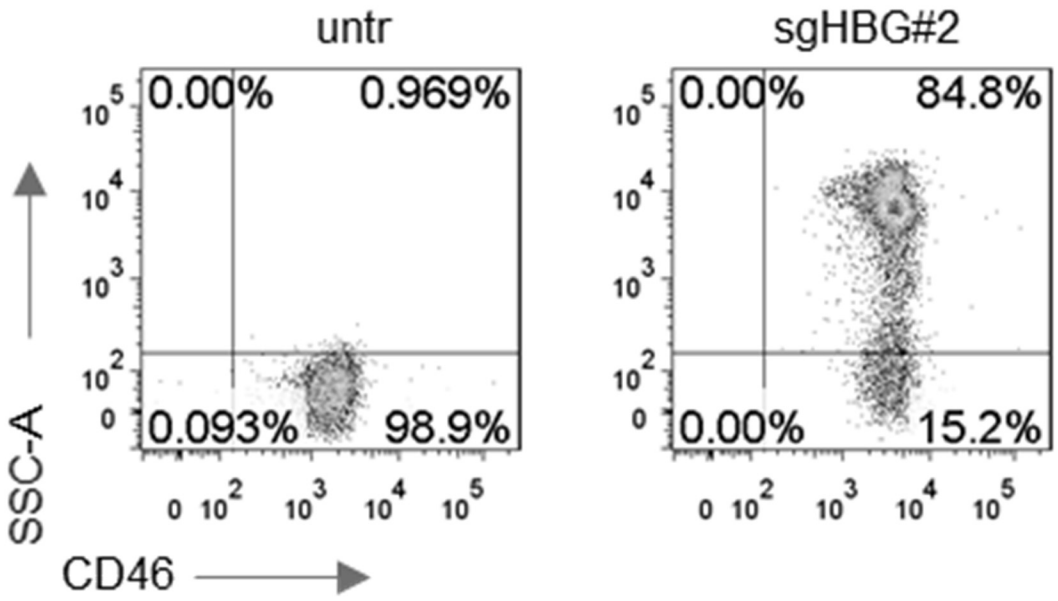


FIG. 135D

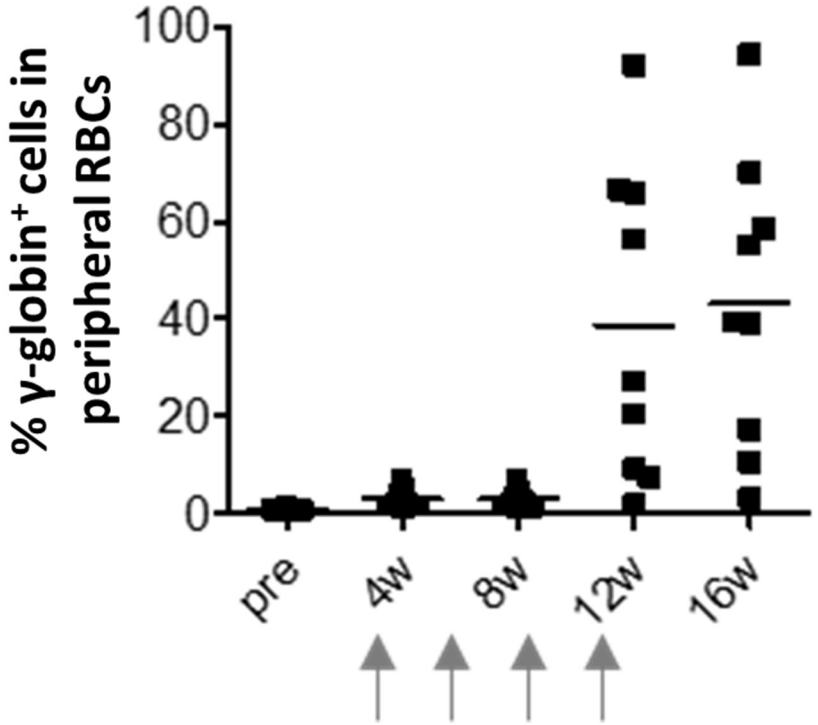


FIG. 135E

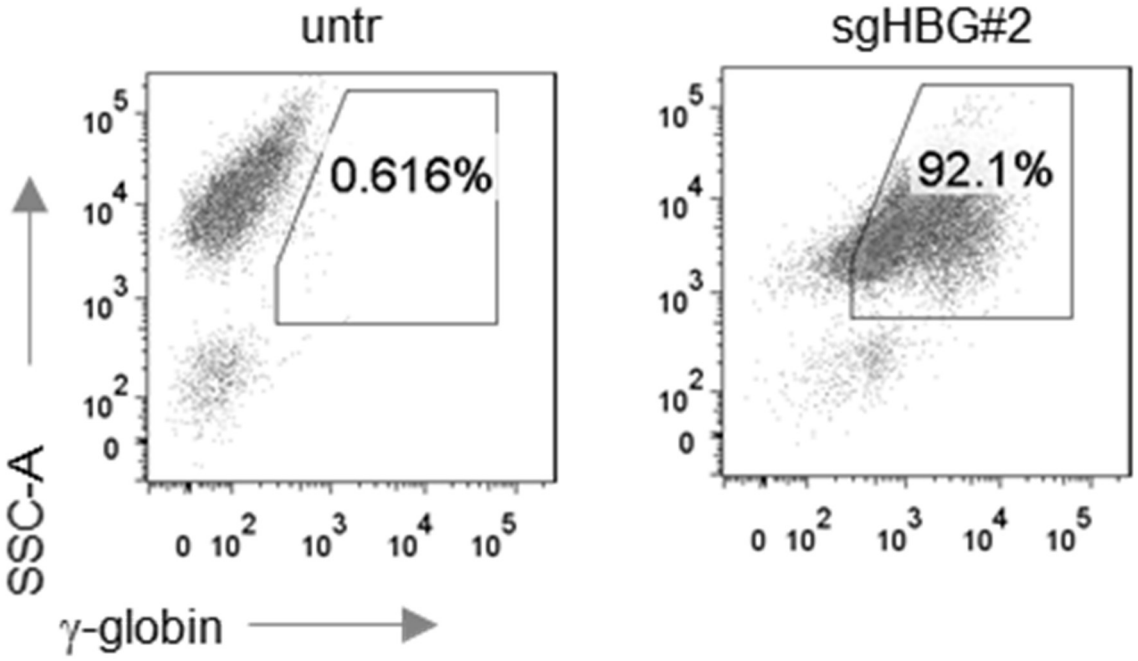


FIG. 135F

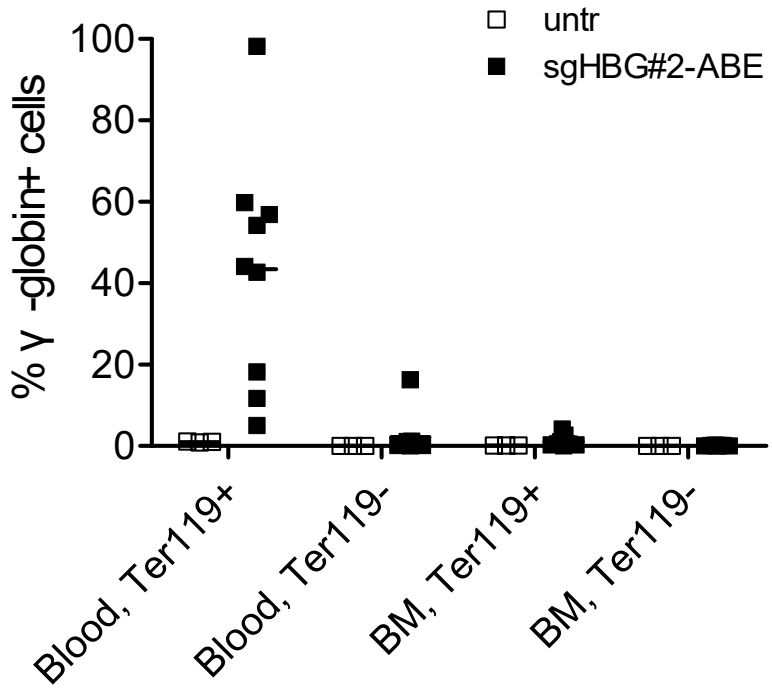


FIG. 135G

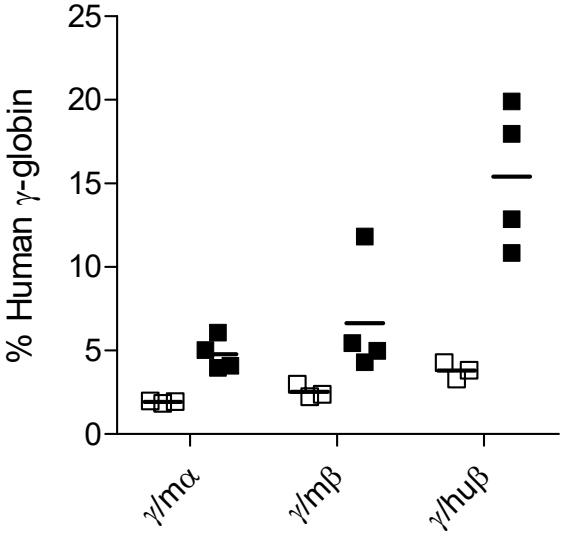


FIG. 135H

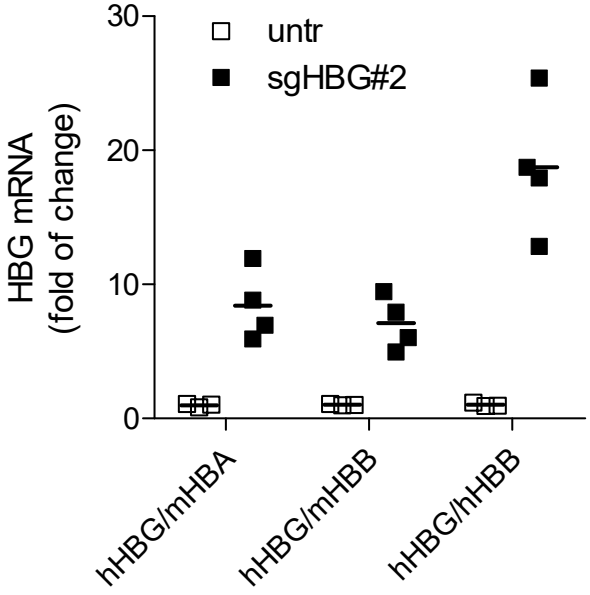


FIG. 135I

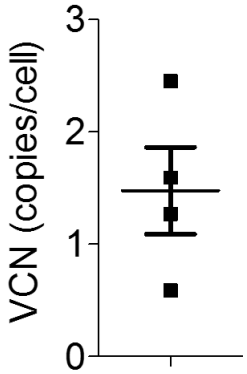


FIG. 136

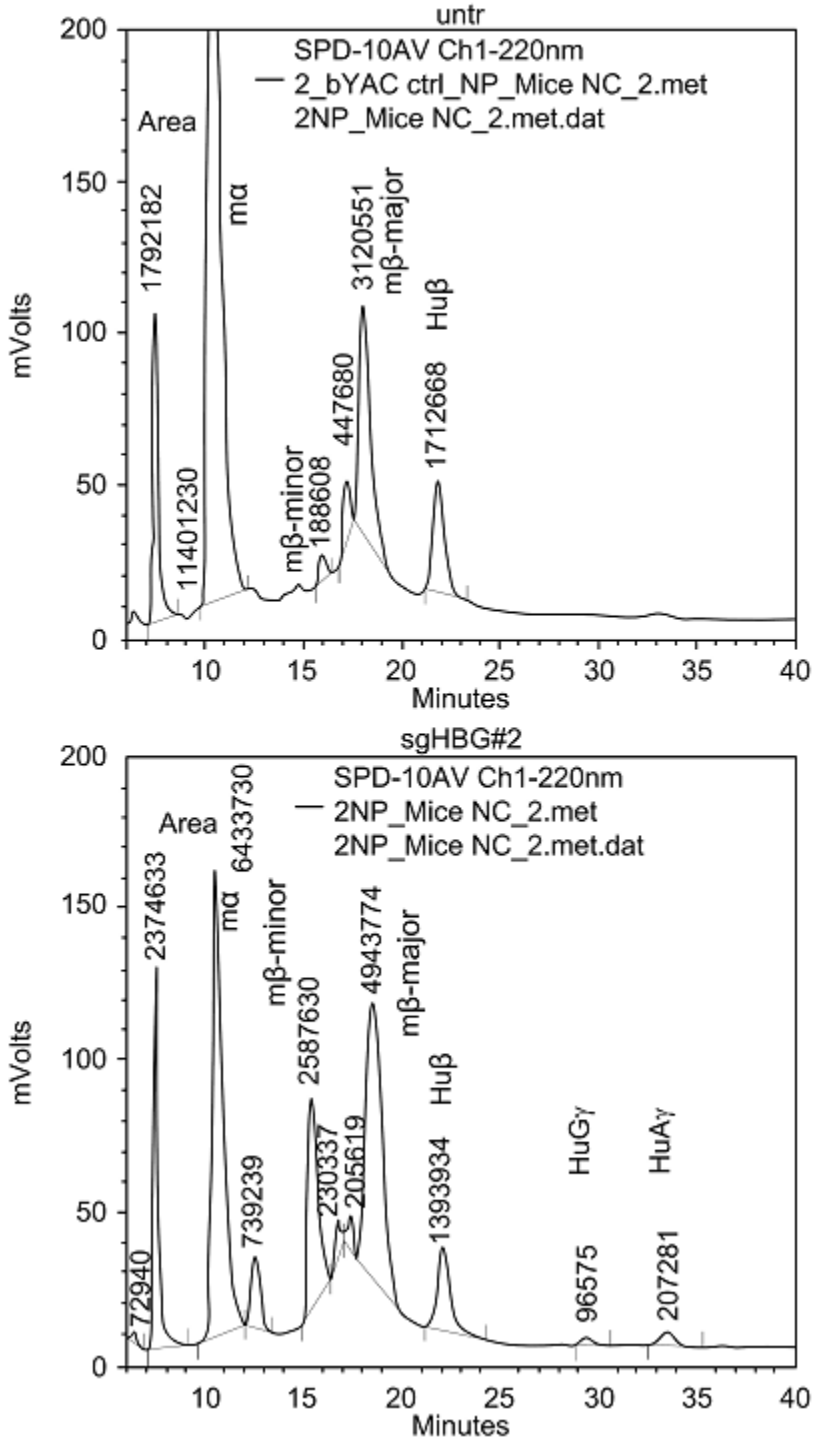
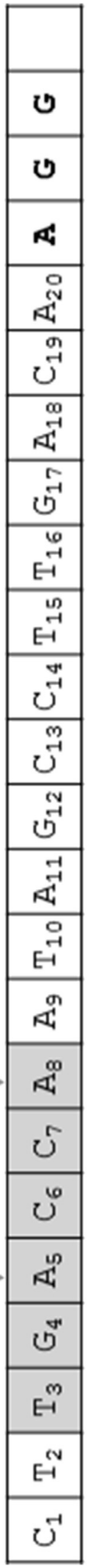

untr
SPD-10AV Ch1-220nm
2_bYAC ctrl_NP_Mice NC_2.met
2NP_Mice NC_2.met.dat
Area
1792182
11401230
mα
mβ-minor
188608
447680
3120551
mβ-major
Huβ
1712668
mVolts
Minutes
sgHBG#2
SPD-10AV Ch1-220nm
2NP_Mice NC_2.met
2NP_Mice NC_2.met.dat
Area
2374633
6433730
mα
mβ-minor
2587630
4943774
mβ-major
Huβ
72940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

FIG. 137A



(SEQ ID NO: 276)

FIG. 137B

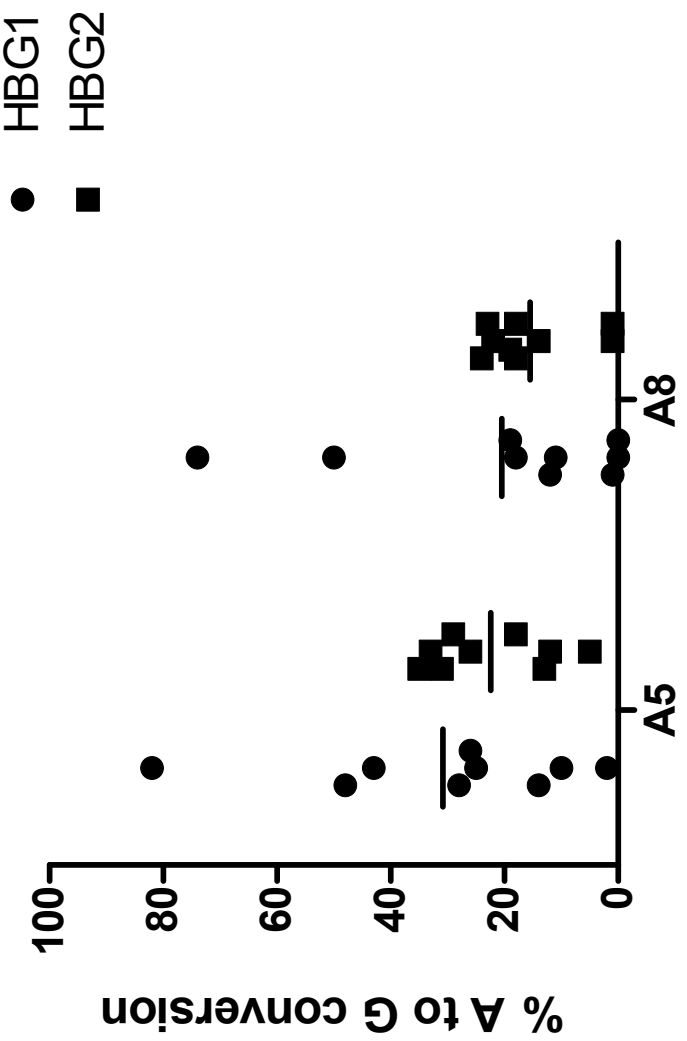


FIG. 137C

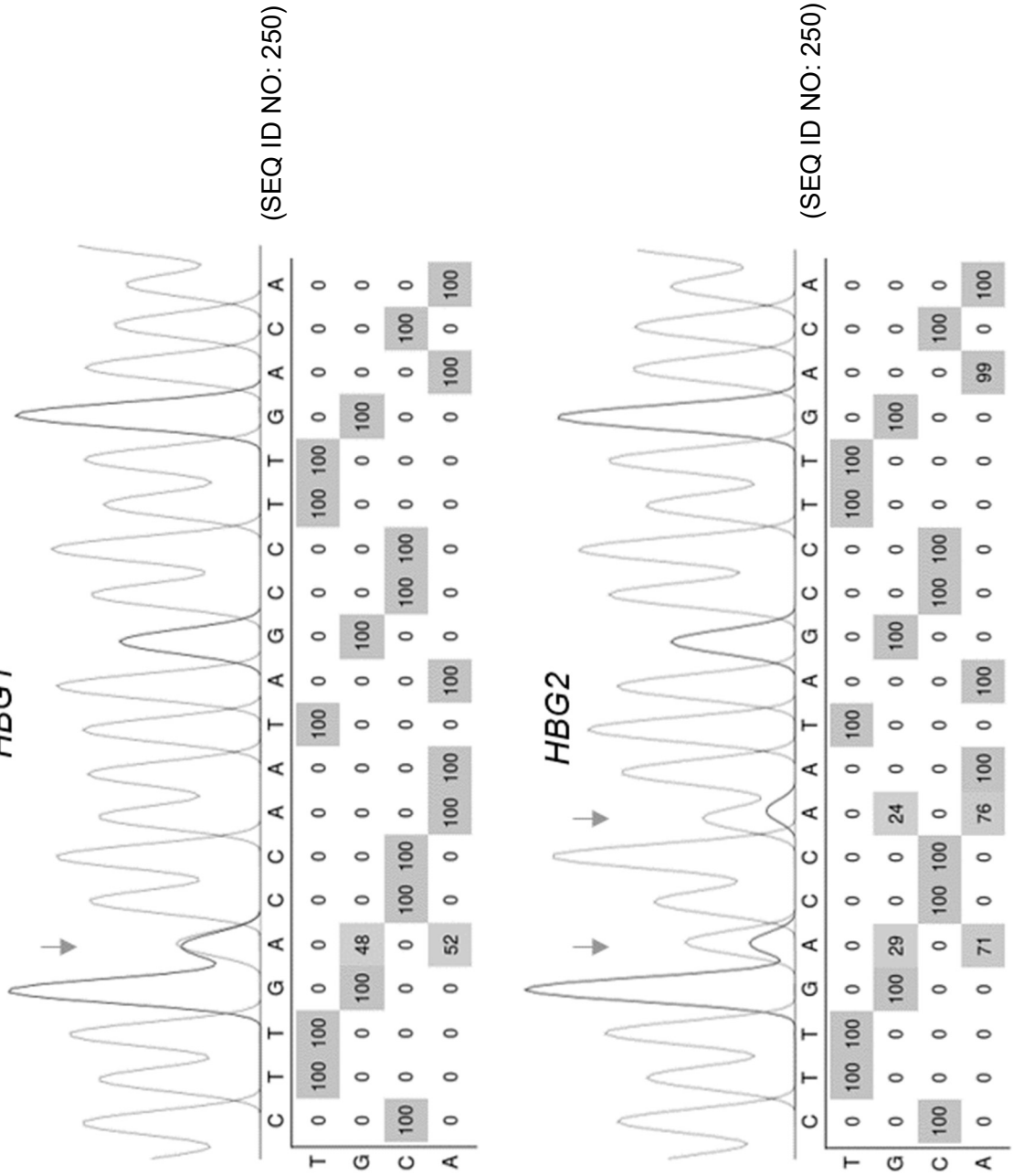

HBG1
(SEQ ID NO: 250)
C T T G A C C A A T A G C C T T G A C A
T 0 100 100 0 0 0 0 0 0 100 0 0 0 0 100 100 0 0 0 0
G 0 0 0 100 48 0 0 0 0 0 100 0 0 0 0 0 100 0 0 0
C 100 0 0 0 0 100 100 0 0 0 0 100 100 0 0 0 0 0 100 0
A 0 0 0 0 52 0 0 100 100 0 100 0 0 0 0 0 0 100 0 100
HBG2
(SEQ ID NO: 250)
C T T G A C C A A T A G C C T T G A C A
T 0 100 100 0 0 0 0 0 0 100 0 0 0 0 100 100 0 0 0 0
G 0 0 0 100 29 0 0 24 0 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 71 0 0 76 100 0 100 0 0 0 0 0 0 99 0 100

FIG. 137D

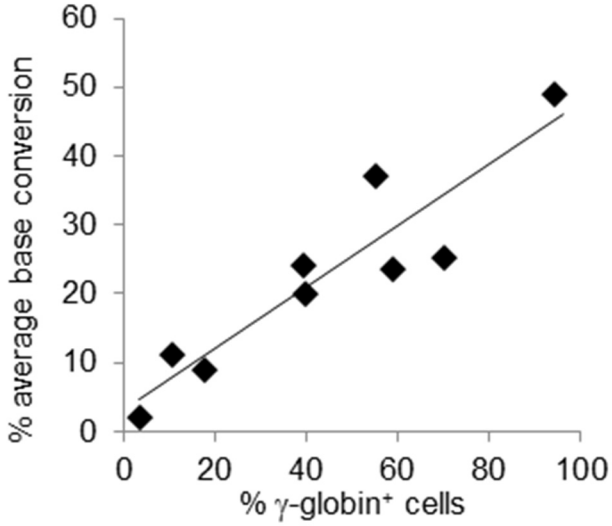


FIG. 137E

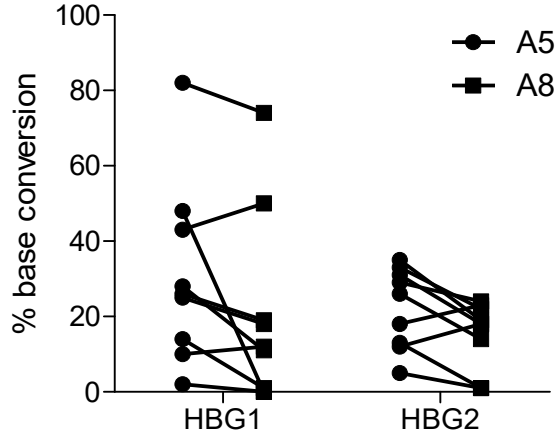


FIG. 137F

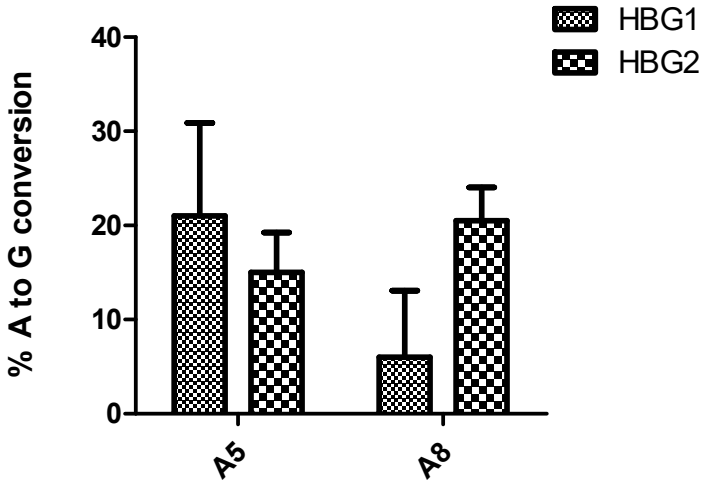


FIG. 137G

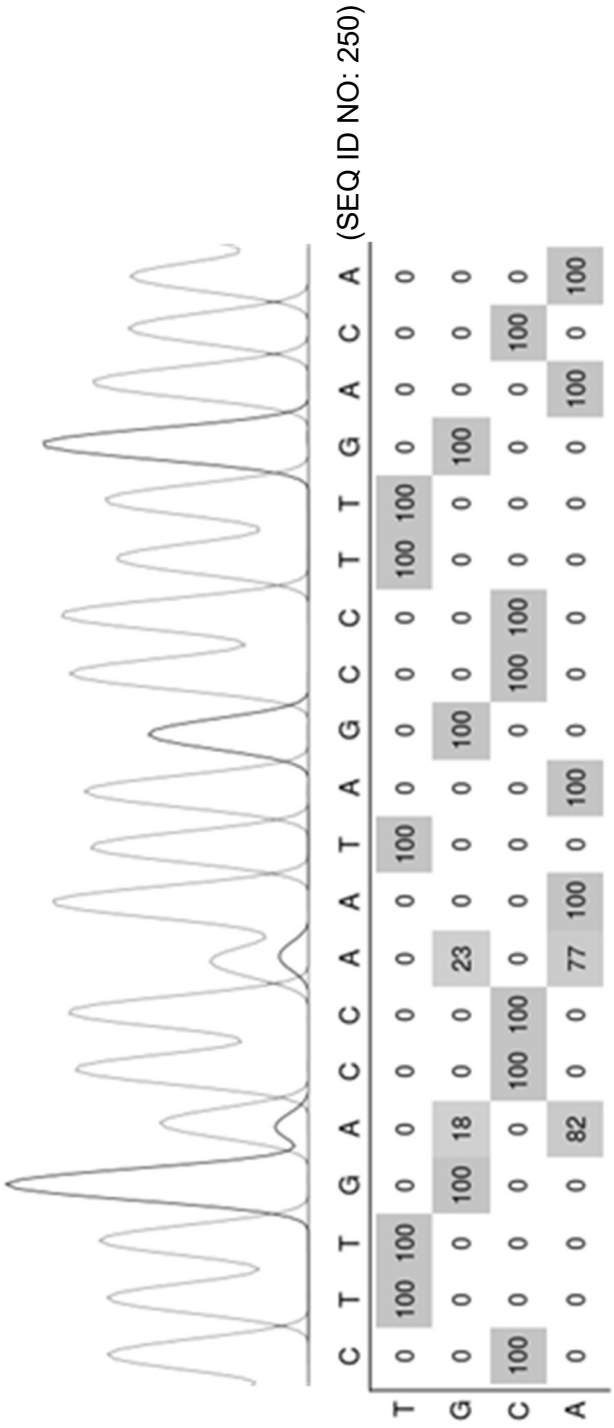

(SEQ ID NO: 250)
C T T G A C C A A T A G C C T T G A C A
T 0 100 100 0 0 0 0 100 0 0 0 100 100 0 0 0 0
G 0 0 0 100 18 0 0 23 0 0 0 100 0 0 100 0 0 0
C 100 0 0 0 0 100 100 0 0 0 0 0 100 100 0 0 100 0
A 0 0 0 0 82 0 0 77 100 0 100 0 0 0 0 100 0 100

FIG. 138A

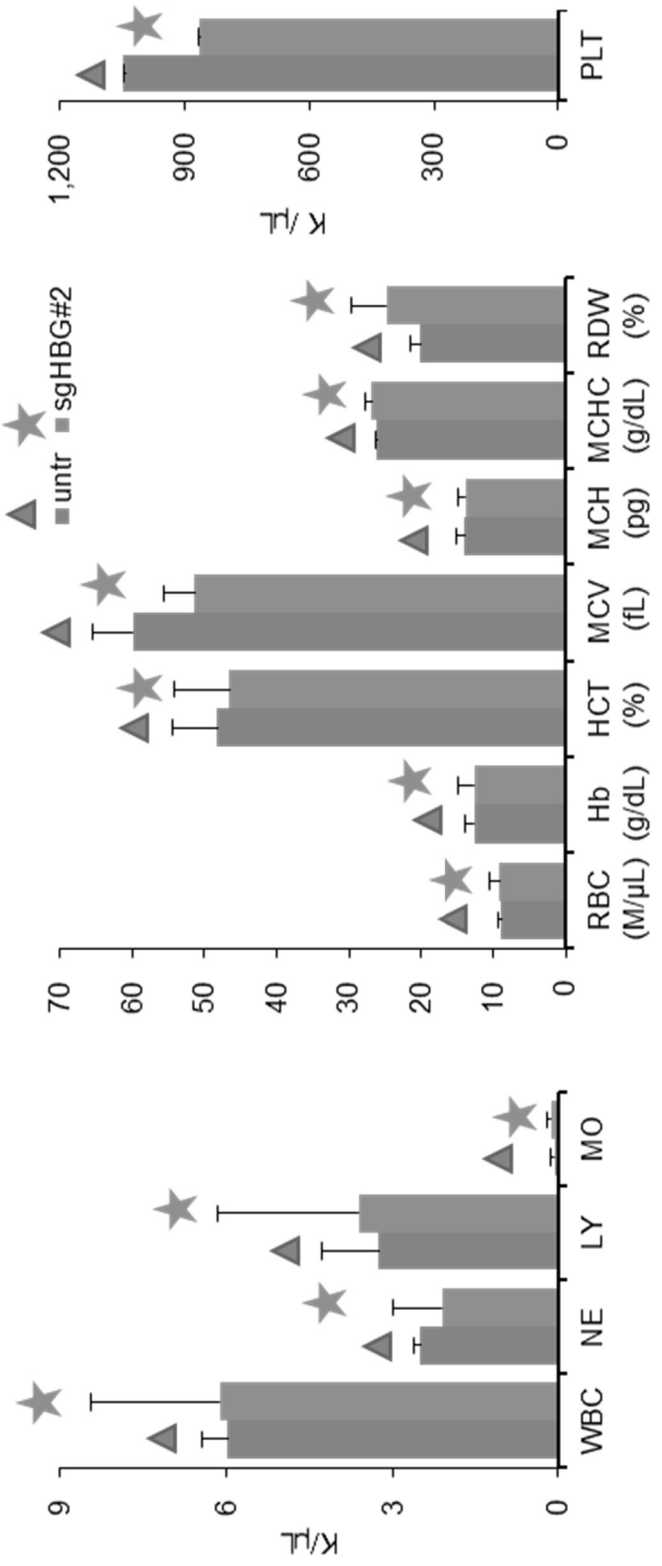

untr
sgHBG#2
K/μL
9
6
3
0
WBC
NE
LY
MO
70
60
50
40
30
20
10
0
RBC (M/μL)
Hb (g/dL)
HCT (%)
MCV (fL)
MCH (pg)
MCHC (g/dL)
RDW (%)
K /μL
1,200
900
600
300
0
PLT

FIG. 138B

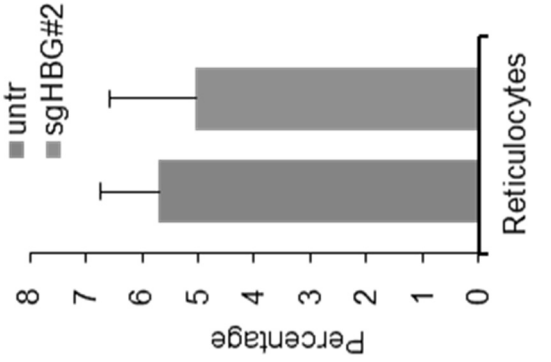


FIG. 138C

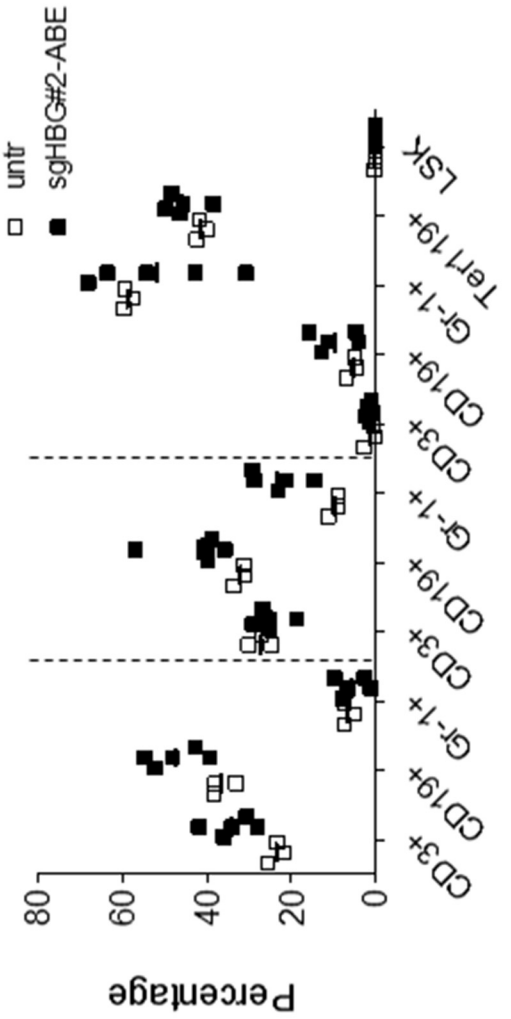


FIG. 138D

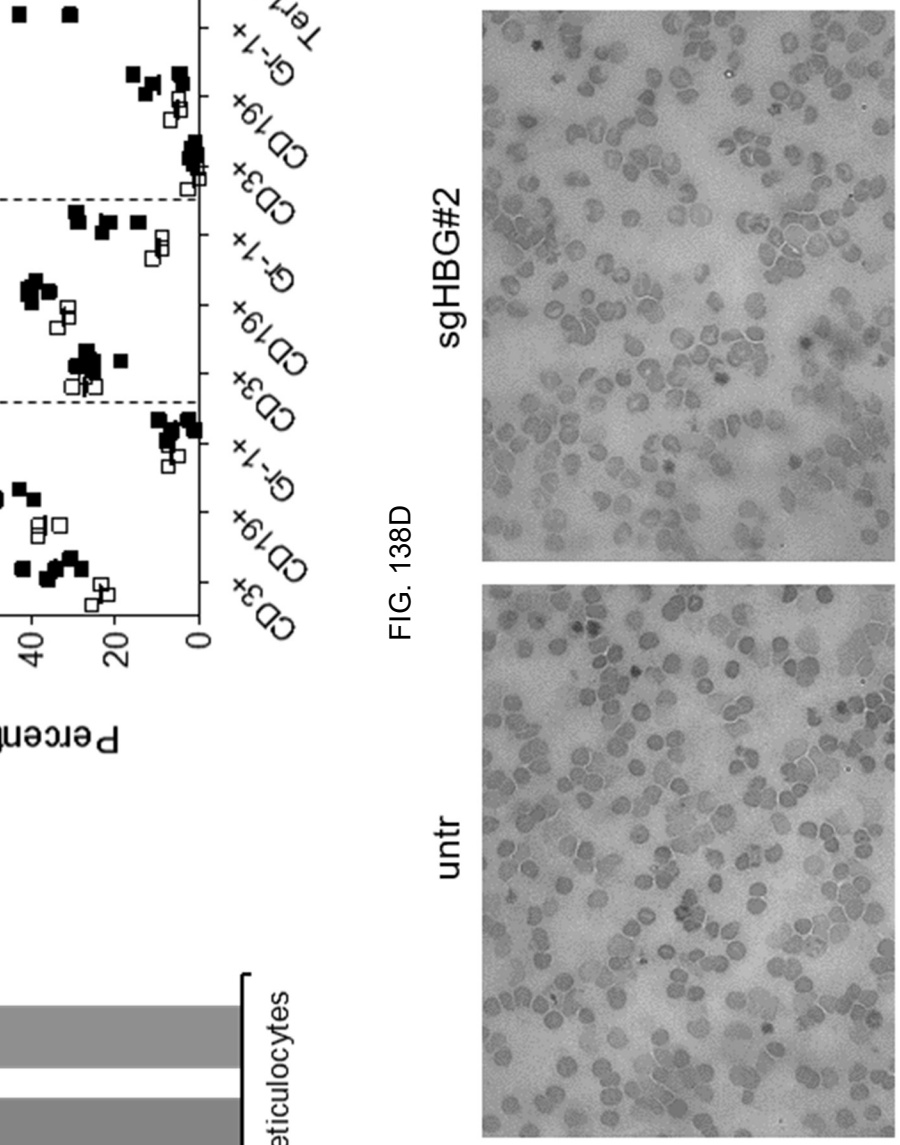


FIG. 139A

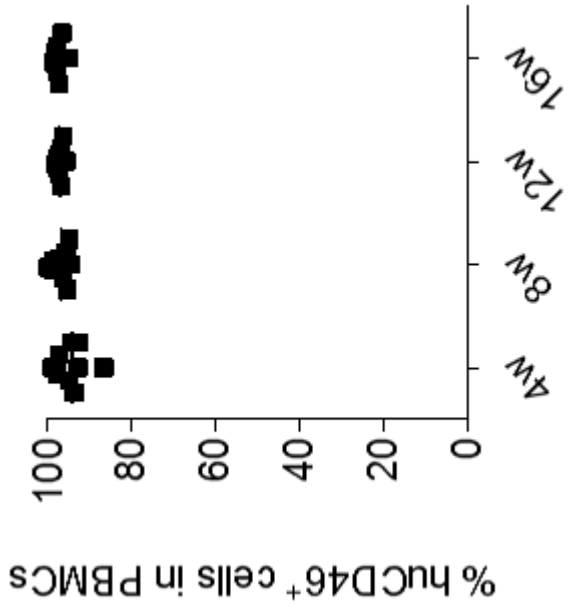


FIG. 139B

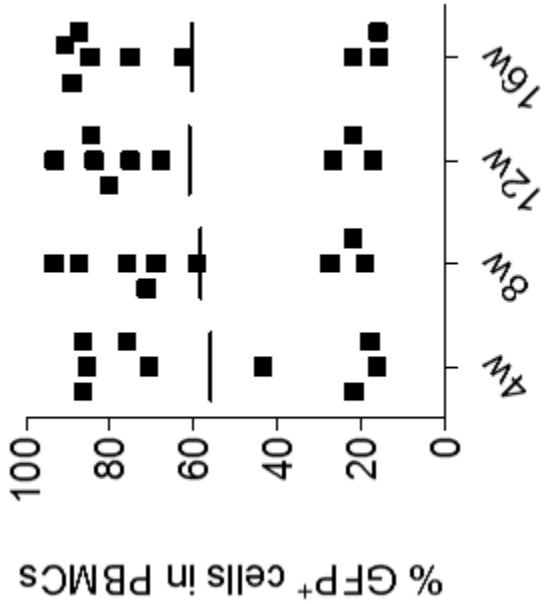


FIG. 139C

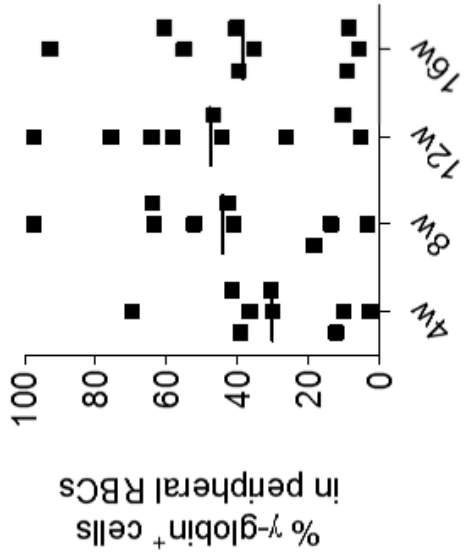


FIG. 140A

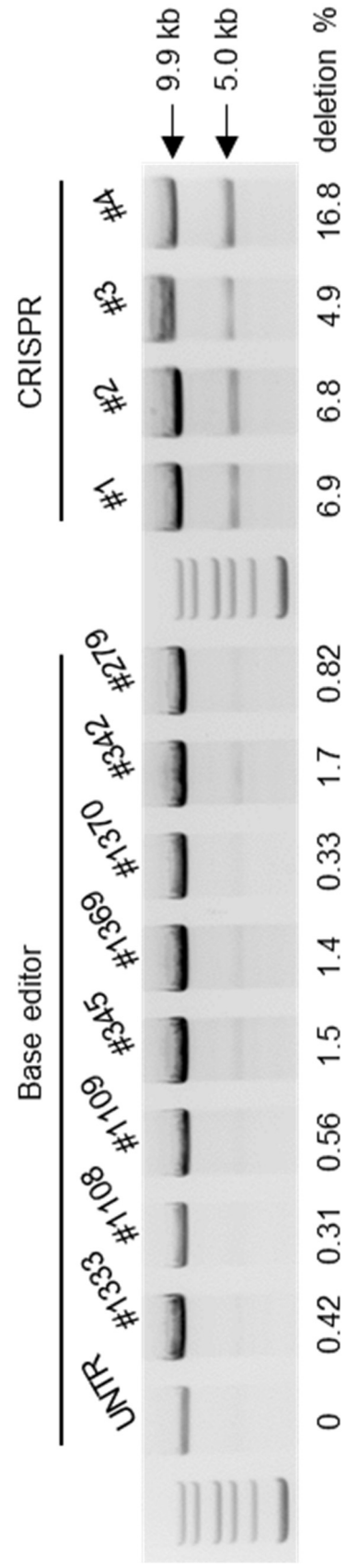


FIG. 140B

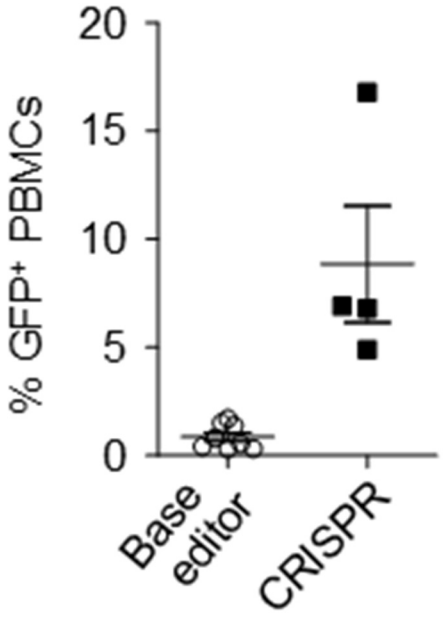


FIG. 141

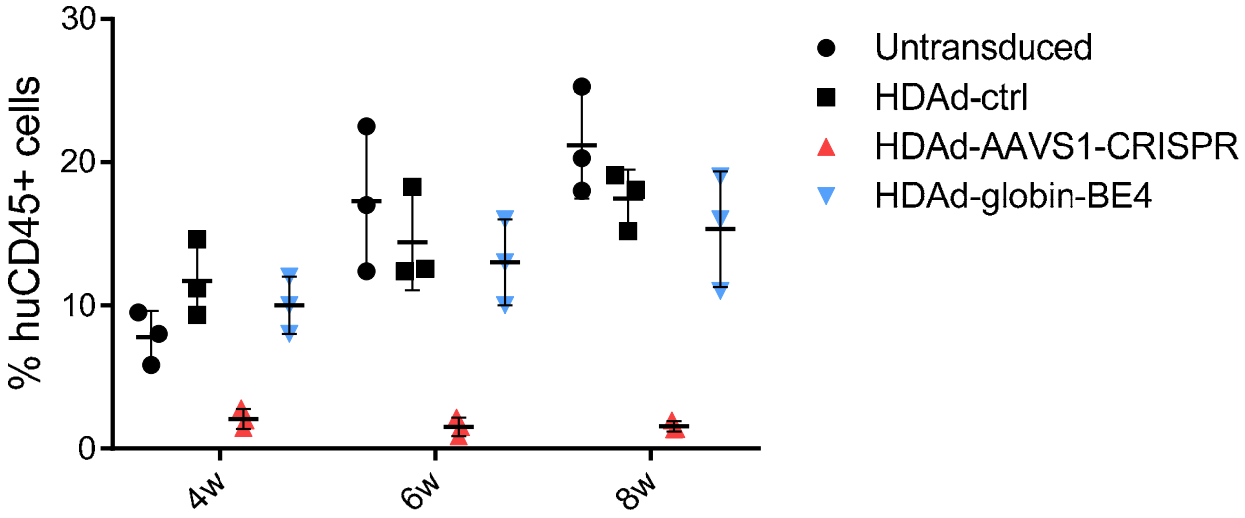


FIG. 142

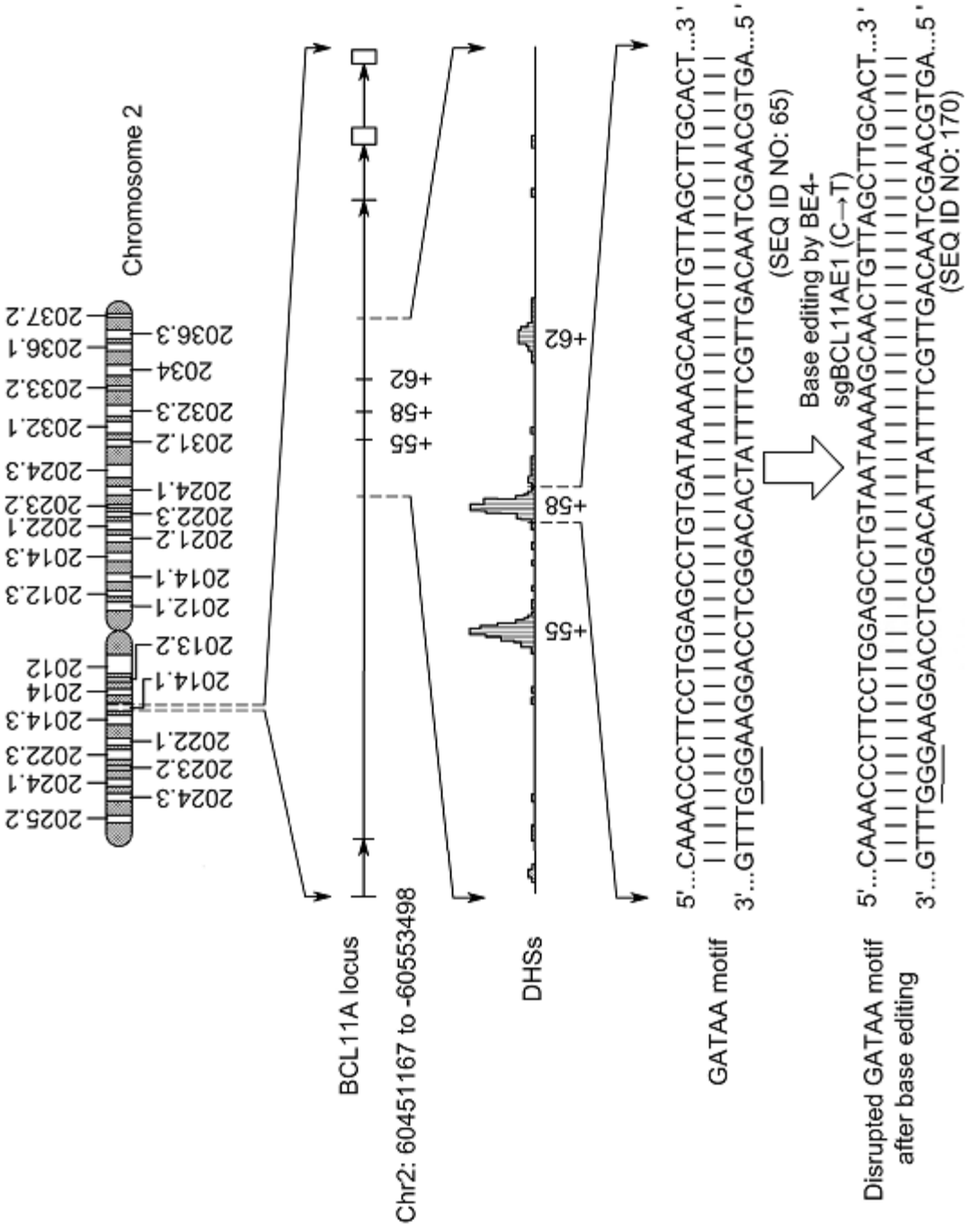

Chromosome 2
2037.2
2036.3
2036.1
2034
2033.2
2032.3
2032.1
2031.2
2024.3
2024.1
2023.2
2022.3
2022.1
2021.2
2014.3
2014.1
2012.3
2012.1
2013.2
2014.1
2012
2014
2014.3
2022.1
2022.3
2023.2
2024.1
2024.3
2025.2
BCL11A locus
Chr2: 60451167 to -60553498
+55
+58
+62
DHSs
+55
+58
+62
GATAA motif
5'...CAAACCCTTCCTGGAGCCTGTGATAAAAGCAACTGTTAGCTTGCACT...3'
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

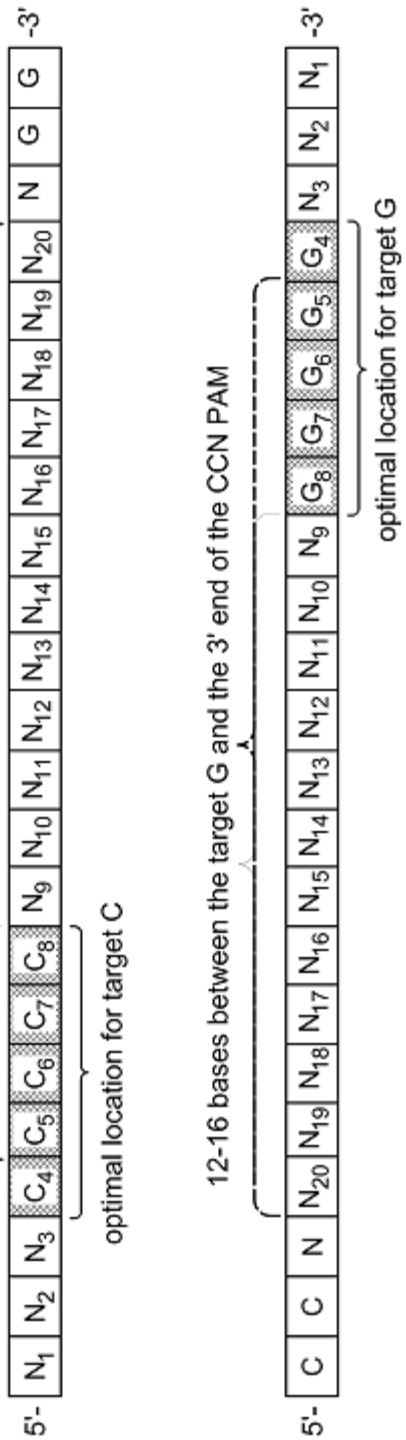

12-16 bases between the target C and the 5' end of the NGG PAM
5'- N1 N2 N3 C4 C5 C6 C7 C8 N9 N10 N11 N12 N13 N14 N15 N16 N17 N18 N19 N20 N G G -3'
optimal location for target C
12-16 bases between the target G and the 3' end of the CCN PAM
5'- C C N N20 N19 N18 N17 N16 N15 N14 N13 N12 N11 N10 N9 G8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

FIG. 144

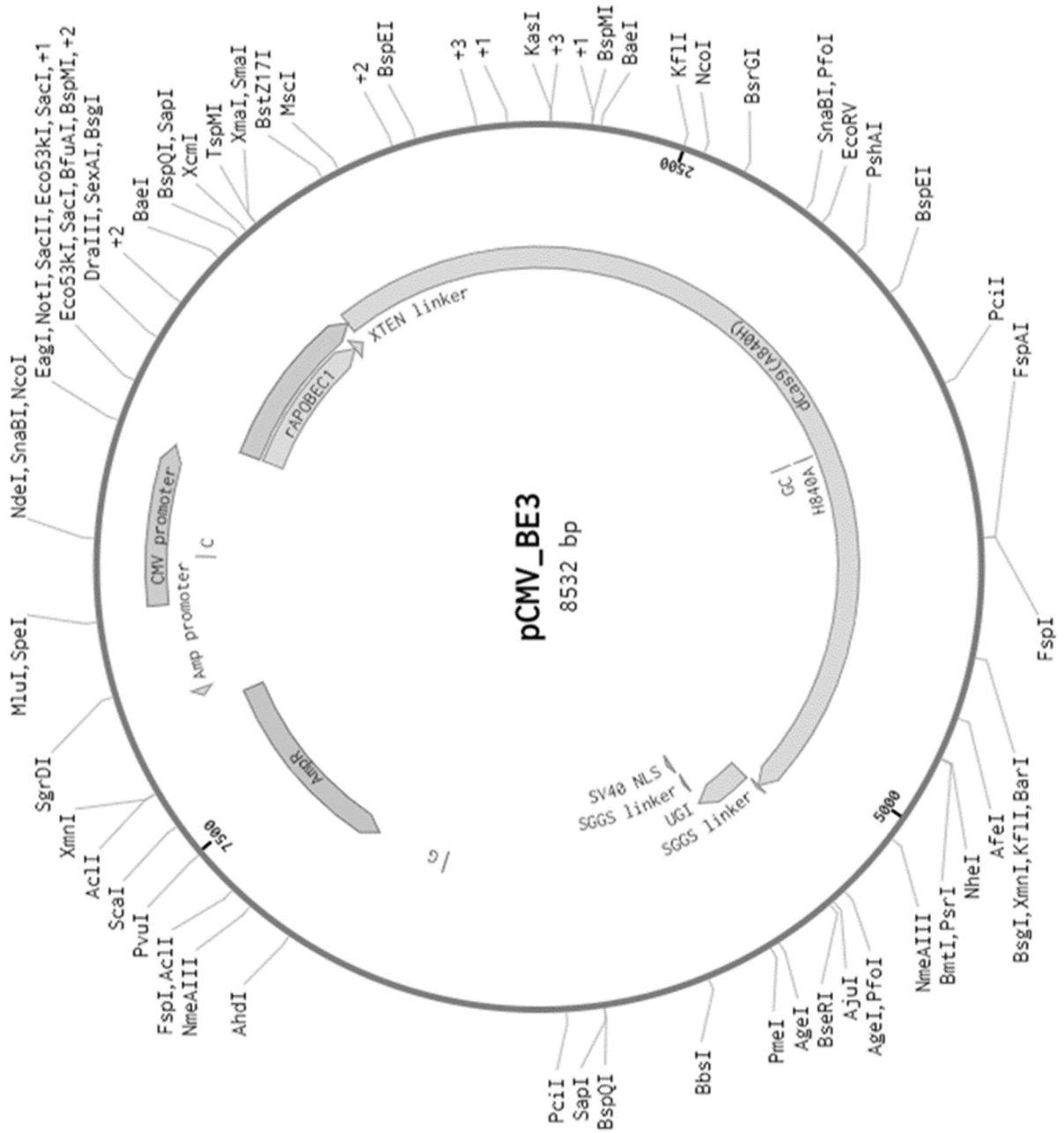

pCMV_BE3
8532 bp
CMV promoter
T7 promoter
rAPOBEC1
XTEN linker
dCas9 (A840H)
nCas9 (A840H)
UGI
SV40 NLS
SGGS linker
SGGS linker
AmpR
ori
2500
5000
7500
NdeI, SnaBI, NcoI
EagI, NotI, SacII, Eco53kI, SacI, +1
Eco53kI, SacI, BfuAI, BspMI, +2
DraIII, SexAI, BsgI
+2
BaeI
BspQI, SapI
XcmI
TspMI
XmaI, SmaI
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

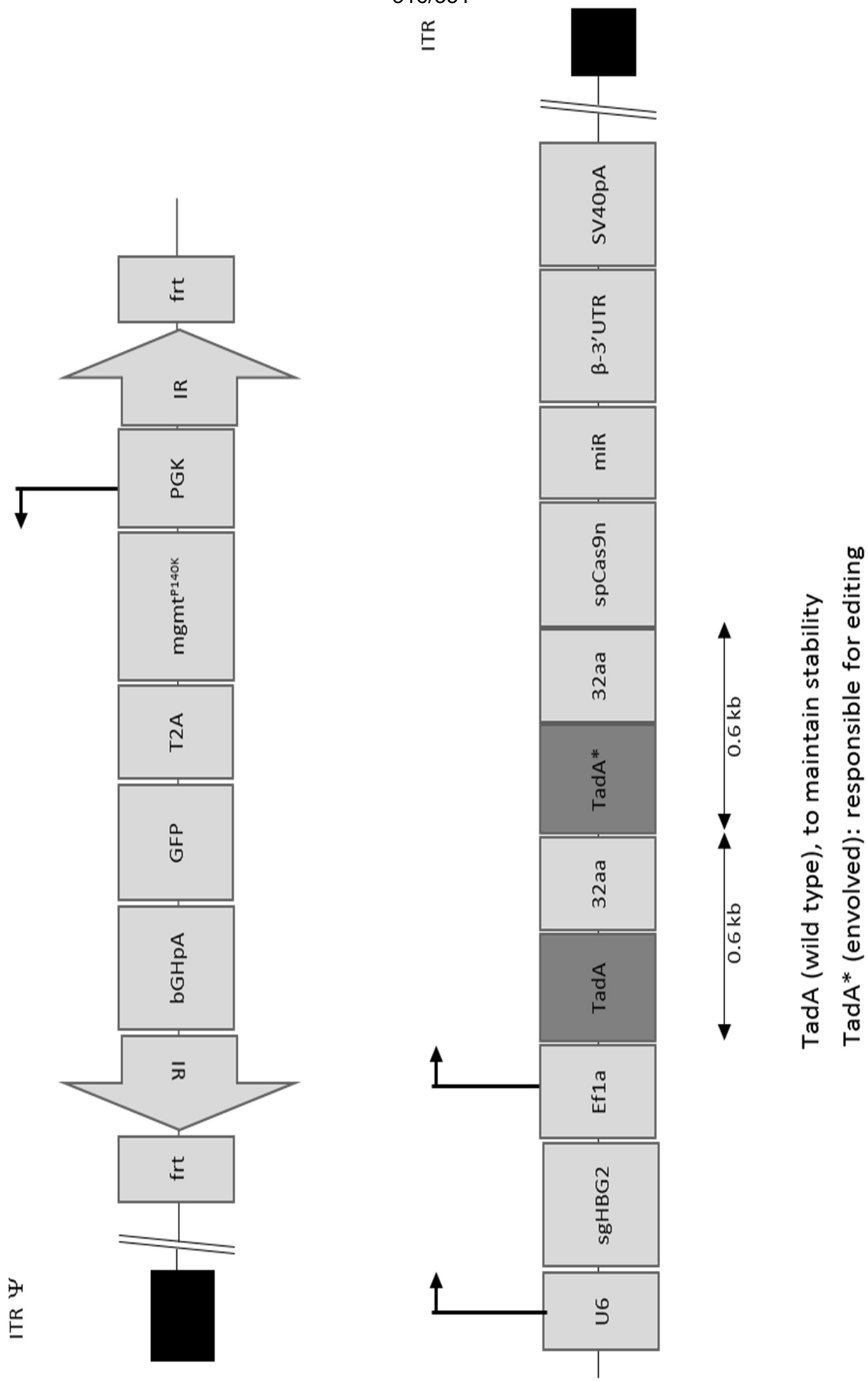

ITR Ψ
frt
IR
PGK
mgmt^{P140K}
T2A
GFP
bGHpA
IR
frt
ITR
U6
sgHBG2
Ef1a
TadA
32aa
TadA*
32aa
spCas9n
miR
β-3'UTR
SV40pA
0.6 kb
0.6 kb
TadA (wild type), to maintain stability
TadA* (envolved): responsible for editing

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.....

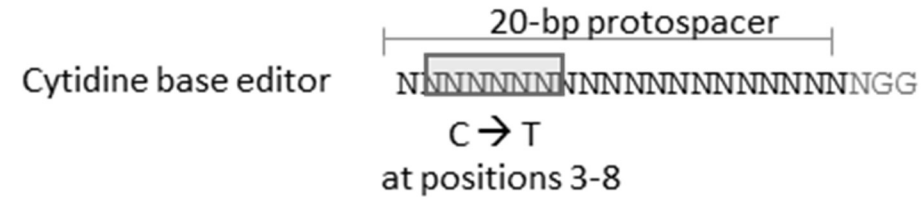
   361 caccaccccggcatgaaccaccgggtggagatcacagagggaatcctggcagacgagtgcgccgccctgctgagcgatttctttagaatgcggagacaggagatcaaggcccagaagaag
   361 ...t.....t.....c..c..a..t..c.....t..a..t.....t..t.....c.g....ct.....t.t....t..t.....

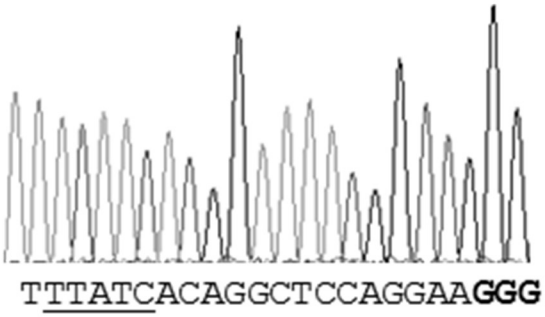
   481 gcacagagctccaccgactctggaggatctagcggaggatcctctggaagcgagacaccaggcacaagcgagtccgccacaccagagagctccggcggctcctccggaggatcc
   481 ..c.....c.....c.....ctct.....t.....ag...a.....t..a...ag...g...ag.ag...g..g..a
```

(SEQ ID NO: 267)

(SEQ ID NO: 268)

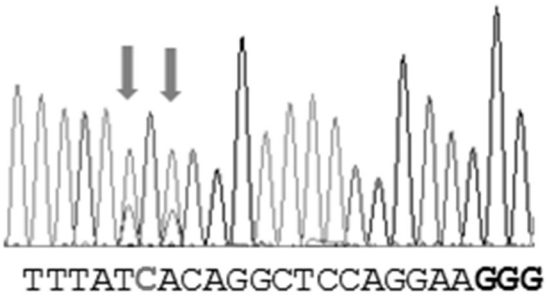
FIG. 147





Wild type sequence
Underlined: GATAA motif
Bold: PAM motif

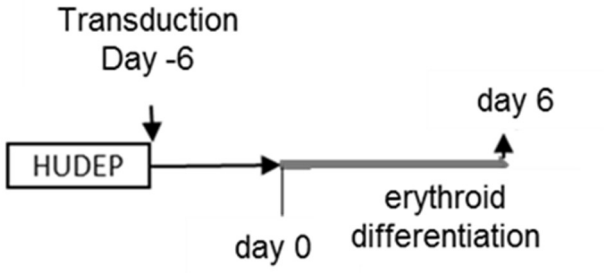
(SEQ ID NO: 269)

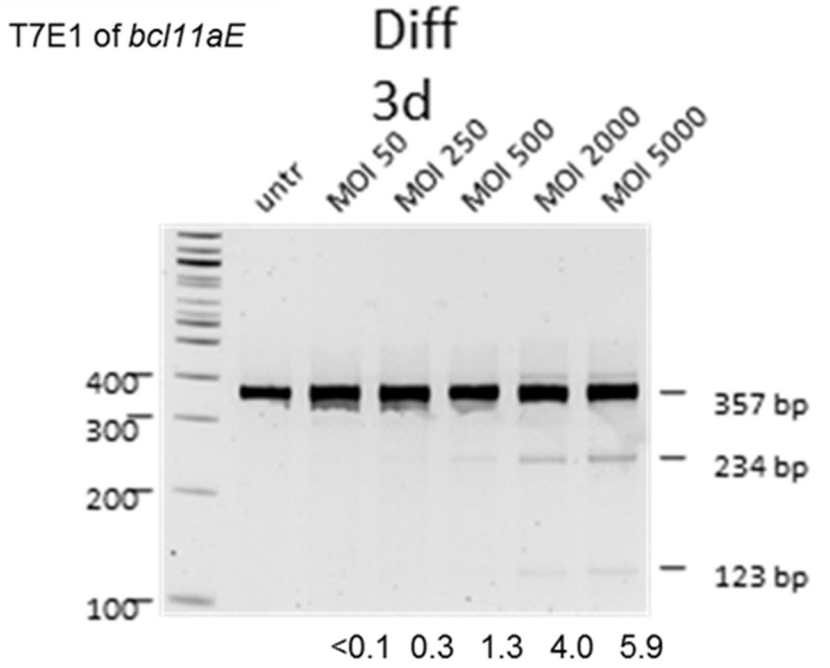


Base edited sequence
Arrows: edited bases
Purple: disrupted base of GATAA motif

(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

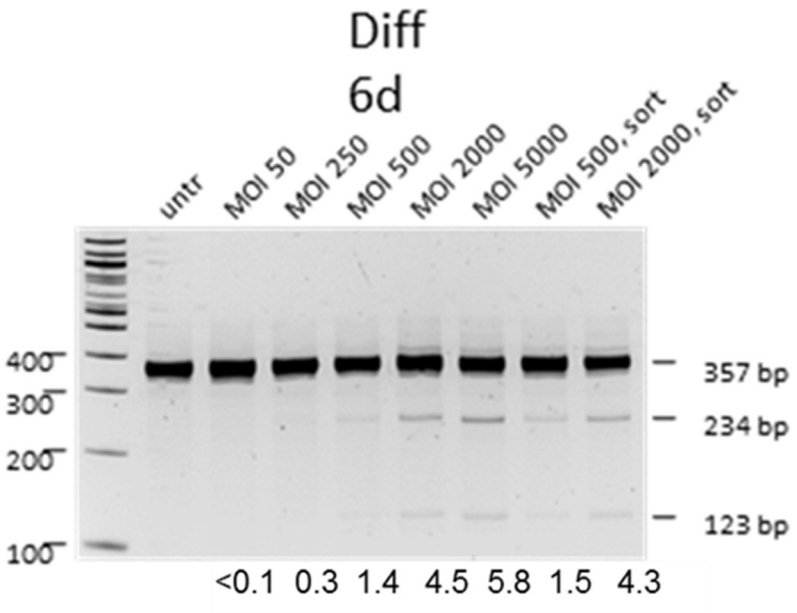

Diff
6d
untr
MOI 50
MOI 250
MOI 500
MOI 2000
MOI 5000
MOI 500, sort
MOI 2000, sort
400
300
200
100
357 bp
234 bp
123 bp
<0.1 0.3 1.4 4.5 5.8 1.5 4.3

FIG. 149

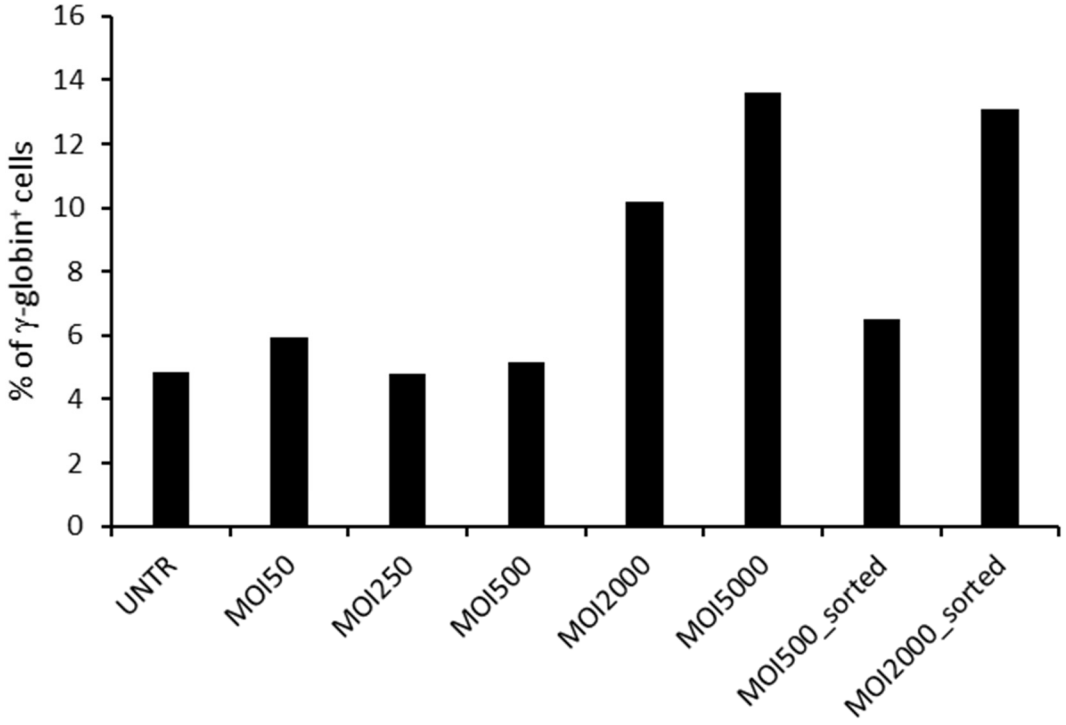


FIG. 150

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

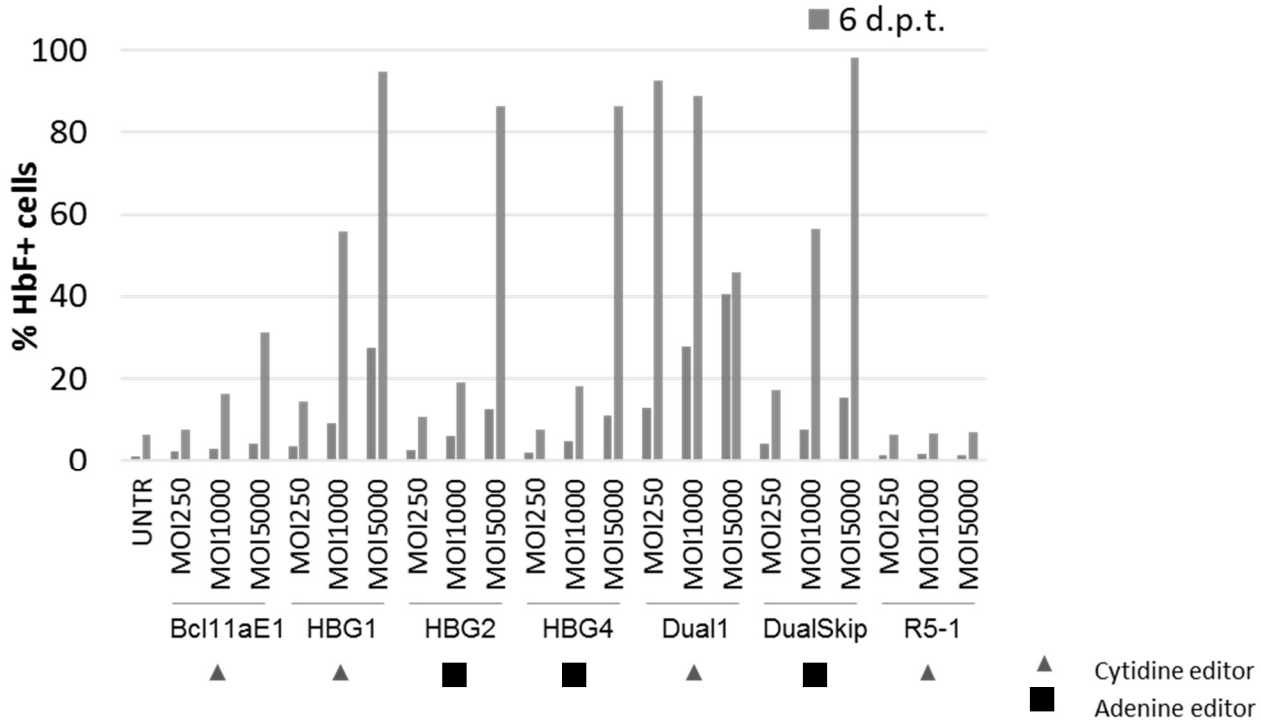


FIG. 152

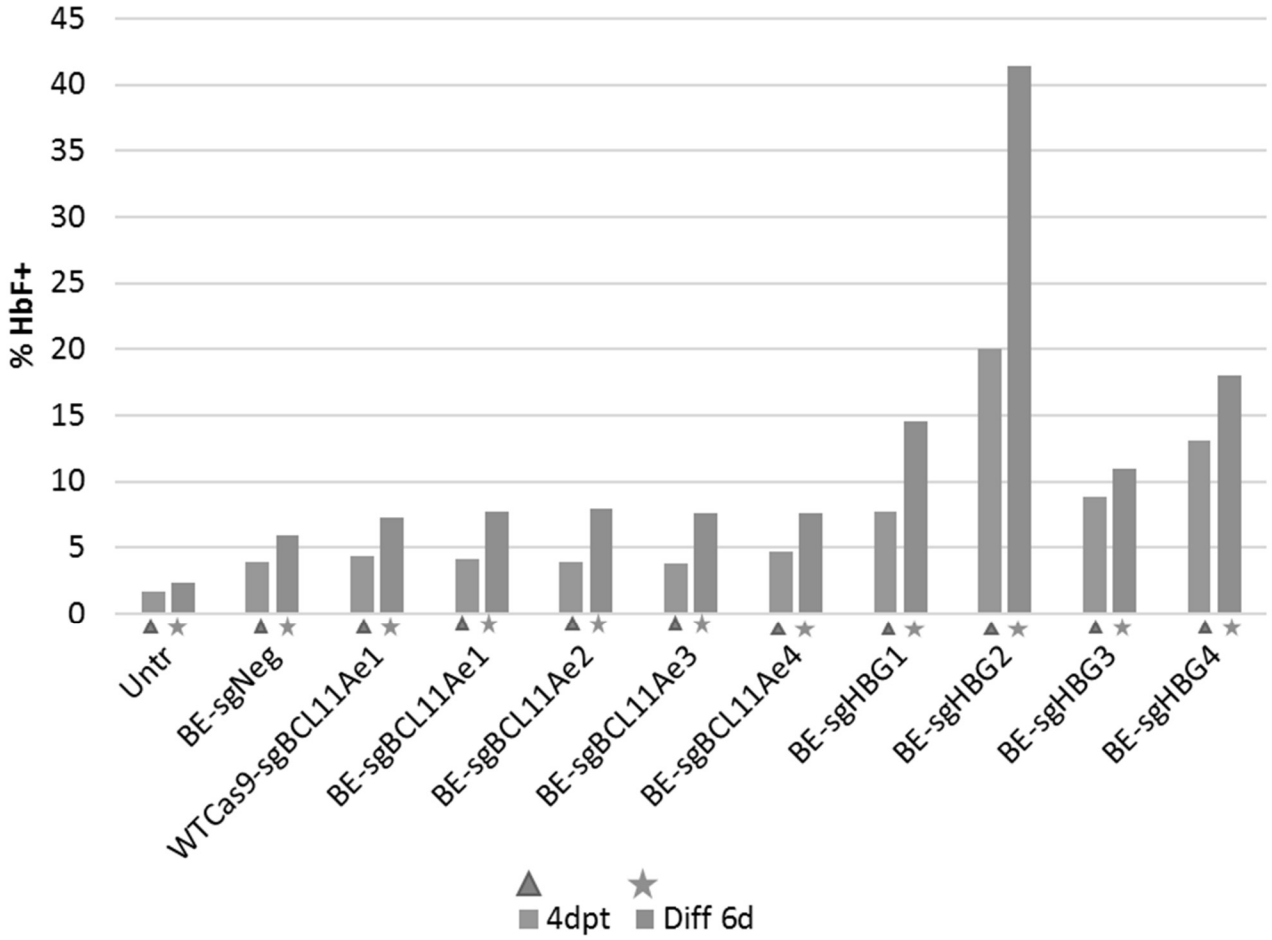

45
40
35
30
25
20
15
10
5
0
% HbF+
Untr
BE-sgNeg
WTCas9-sgBCL11Ae1
BE-sgBCL11Ae1
BE-sgBCL11Ae2
BE-sgBCL11Ae3
BE-sgBCL11Ae4
BE-sgHBG1
BE-sgHBG2
BE-sgHBG3
BE-sgHBG4
4dpt
Diff 6d

FIG. 153

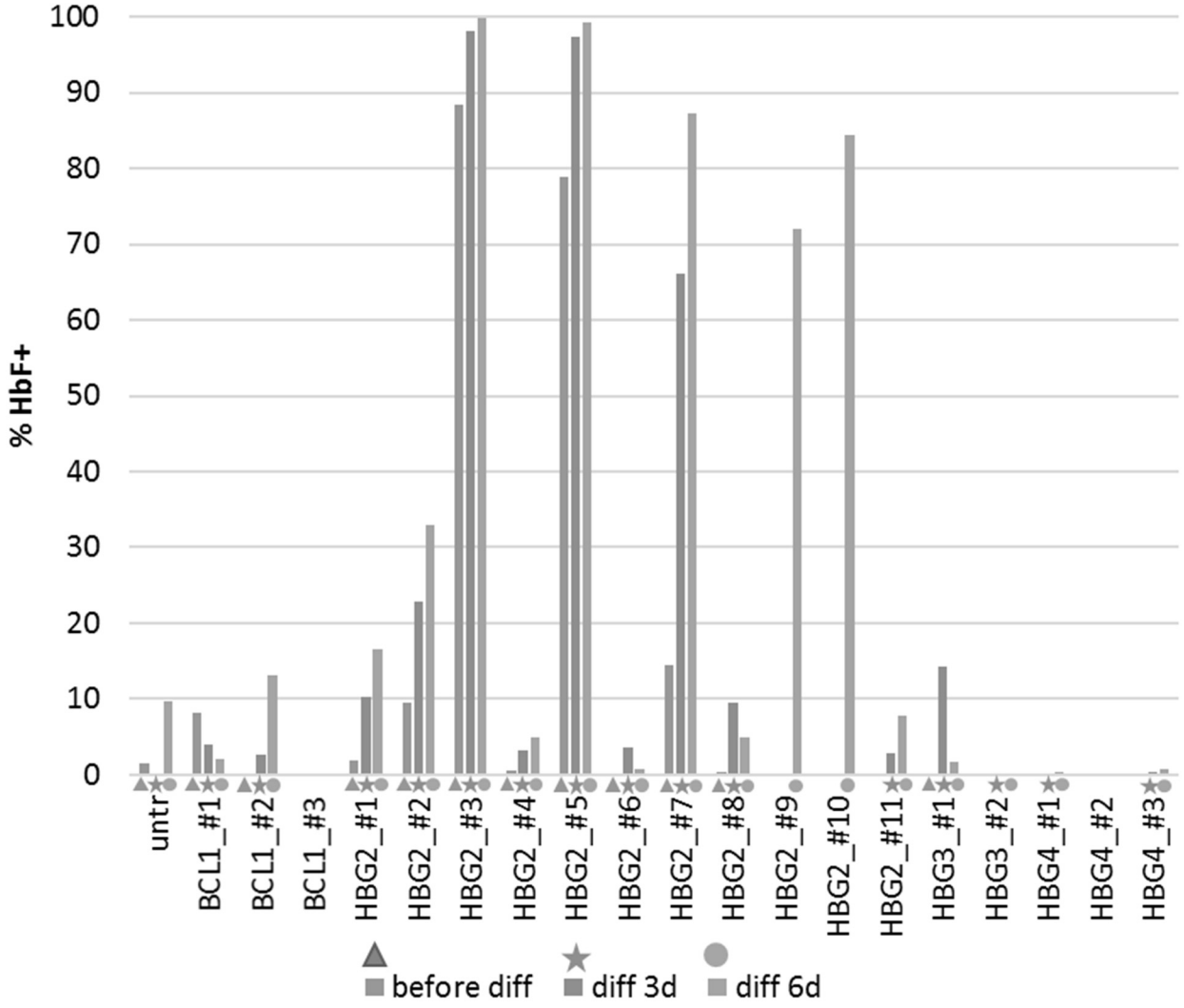
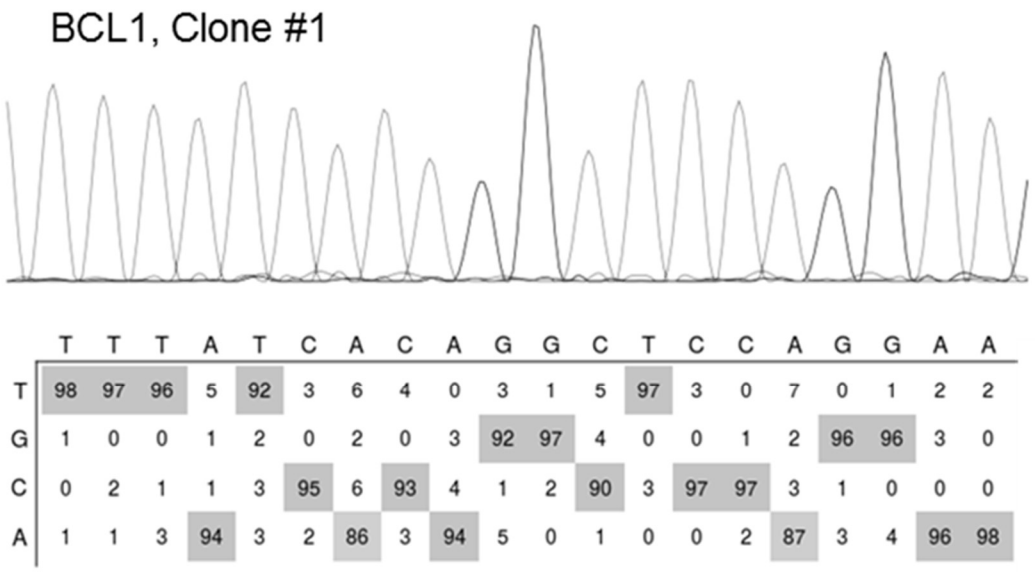

100
90
80
70
60
50
40
30
20
10
0
% HbF+
untr
BCL1_#1
BCL1_#2
BCL1_#3
HBG2_#1
HBG2_#2
HBG2_#3
HBG2_#4
HBG2_#5
HBG2_#6
HBG2_#7
HBG2_#8
HBG2_#9
HBG2_#10
HBG2_#11
HBG3_#1
HBG3_#2
HBG4_#1
HBG4_#2
HBG4_#3
before diff
diff 3d
diff 6d

FIG. 154A

Single cell clones



(SEQ ID NO: 271)

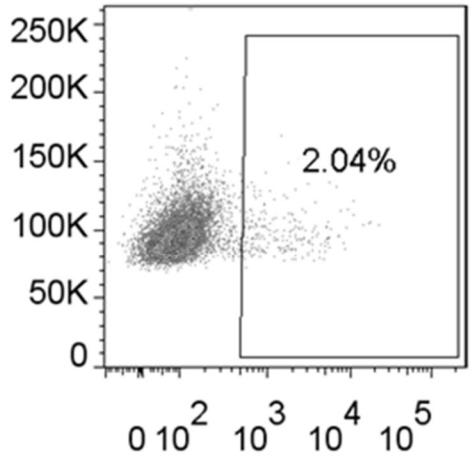
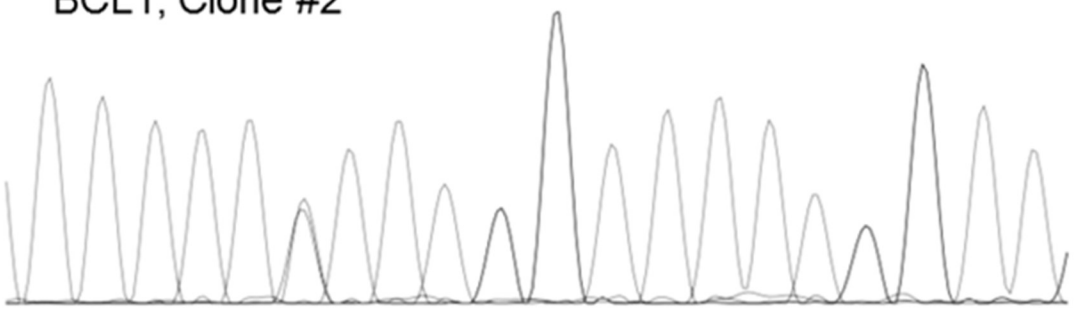


FIG. 154B

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)

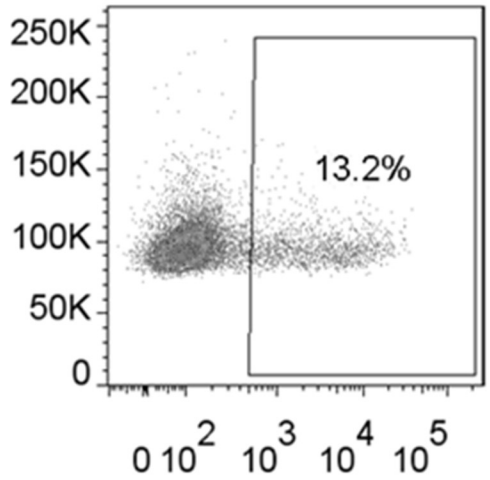
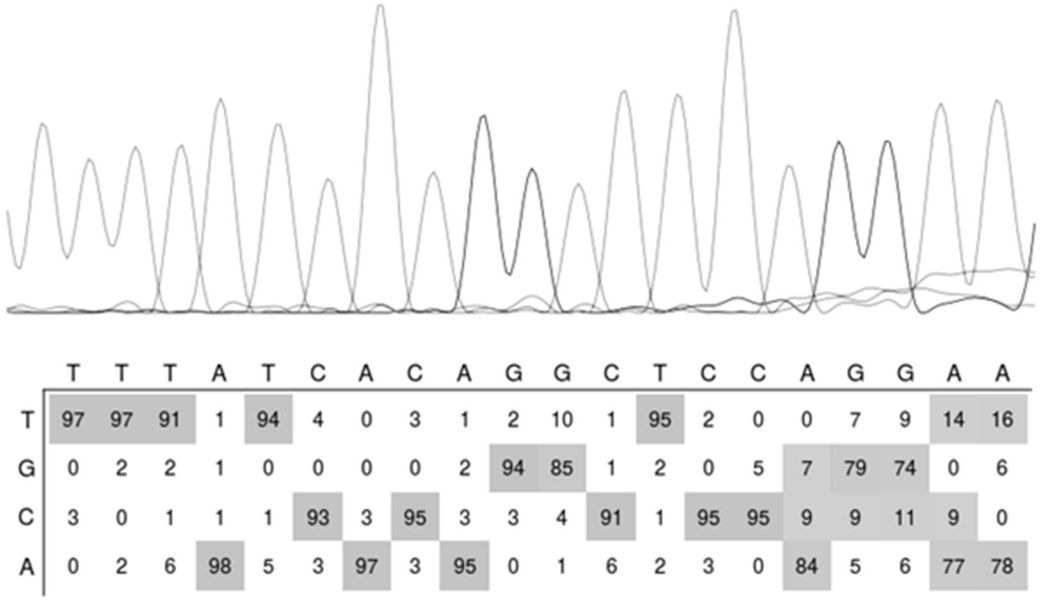


FIG. 154C

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)

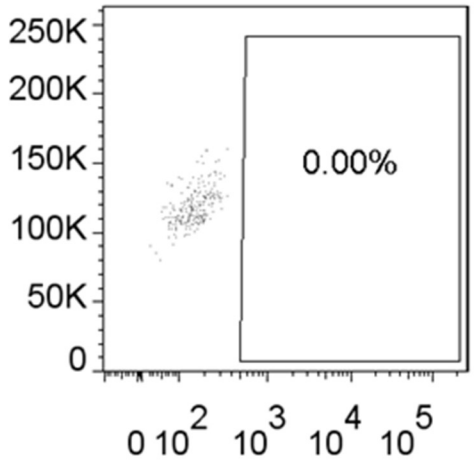
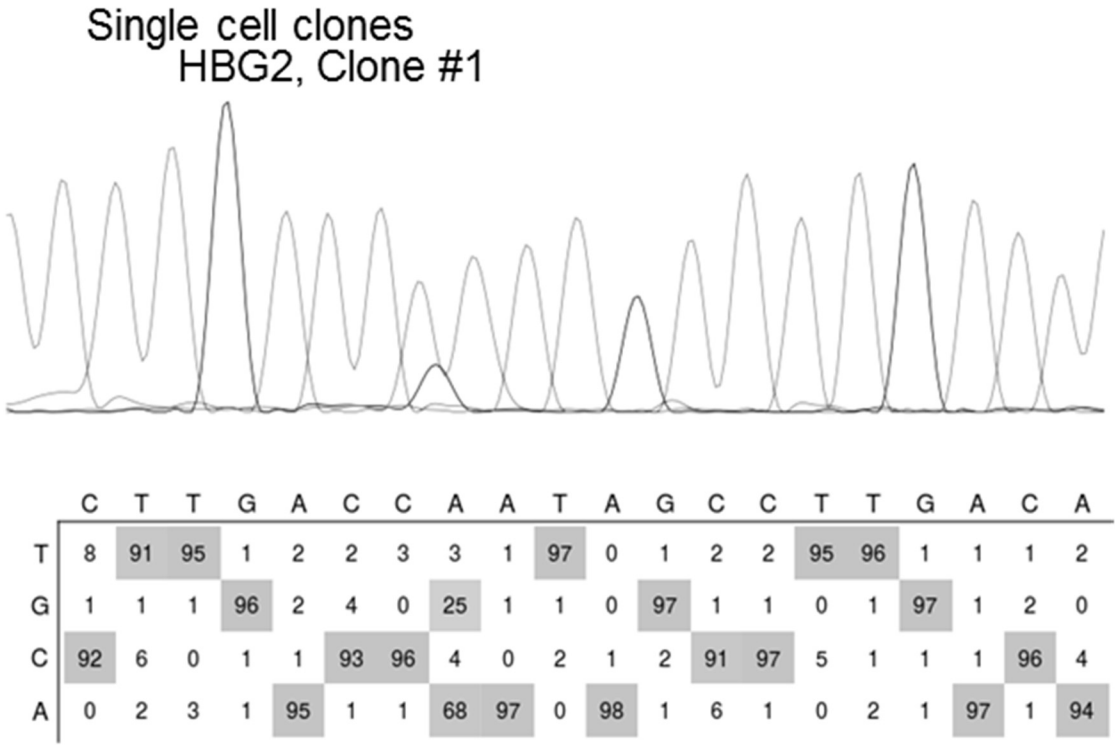
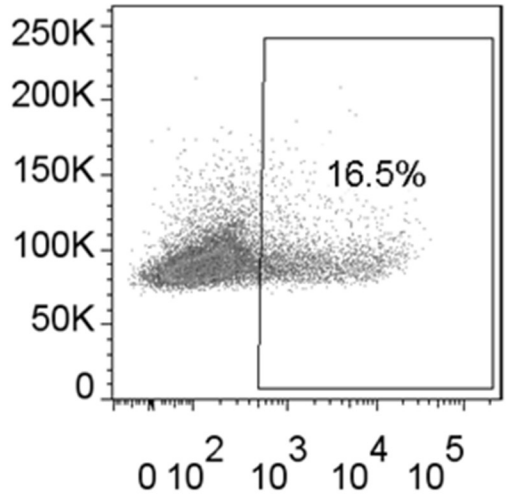
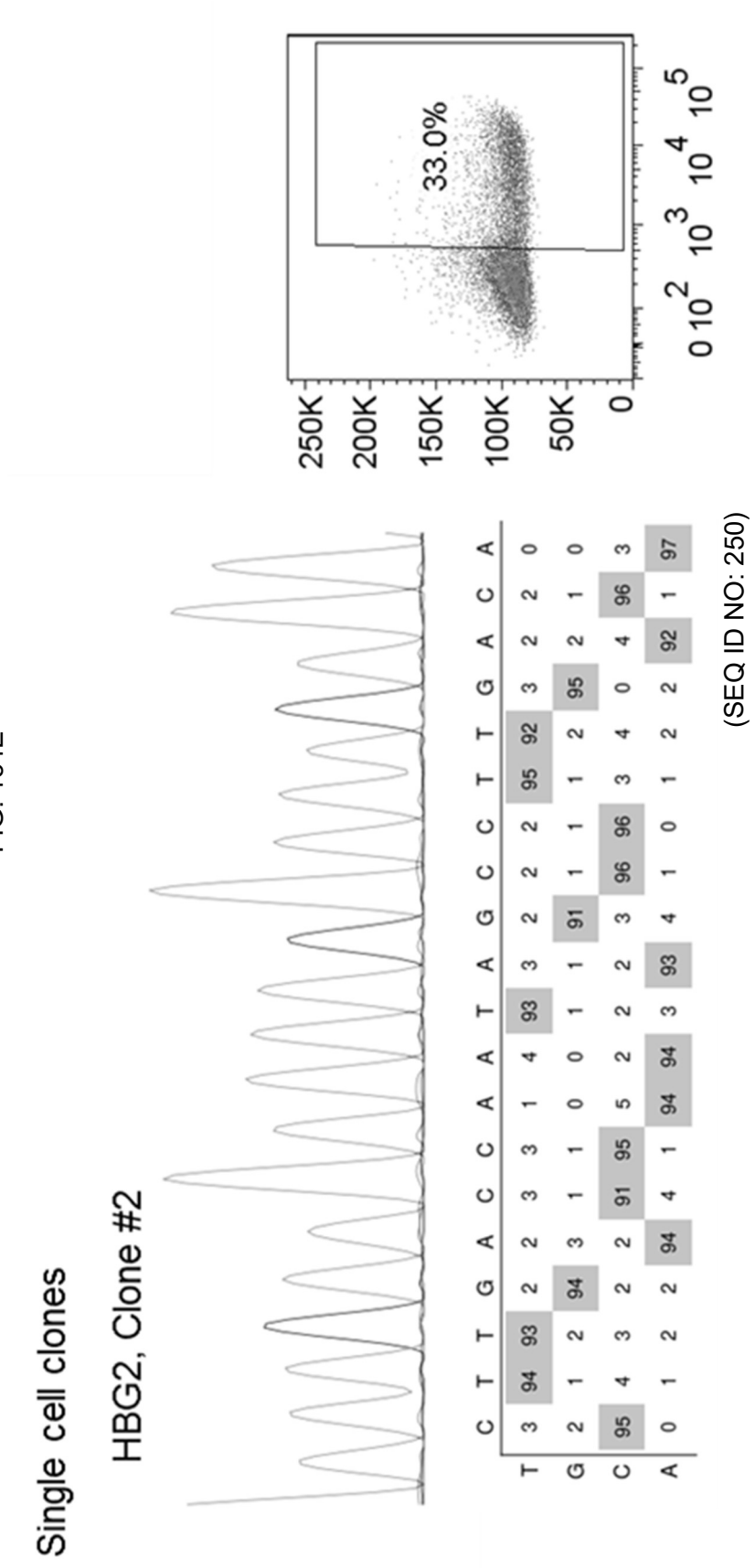


FIG. 154D



(SEQ ID NO: 250)





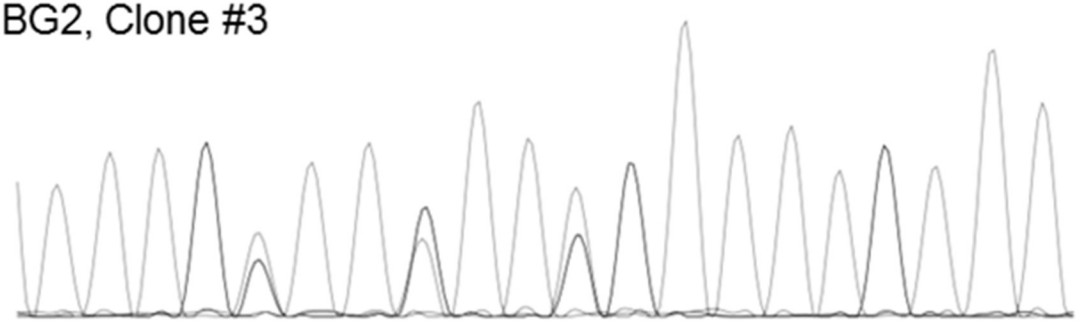
(SEQ ID NO: 250)

FIG. 154E

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)

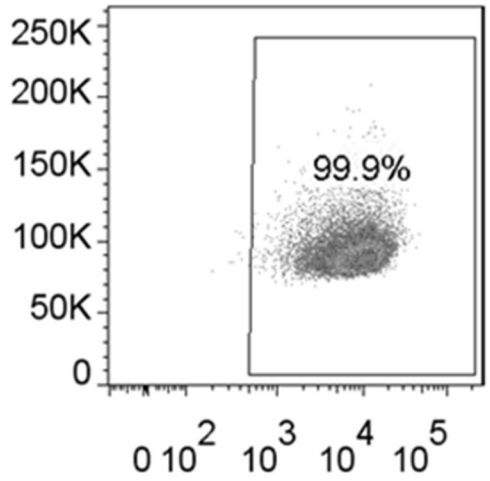
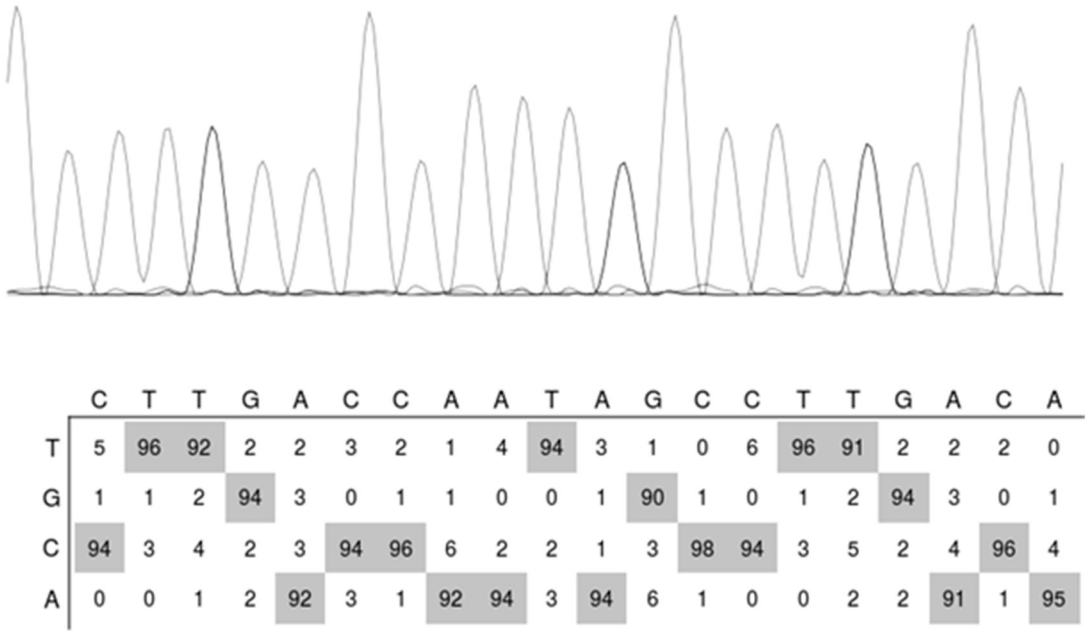


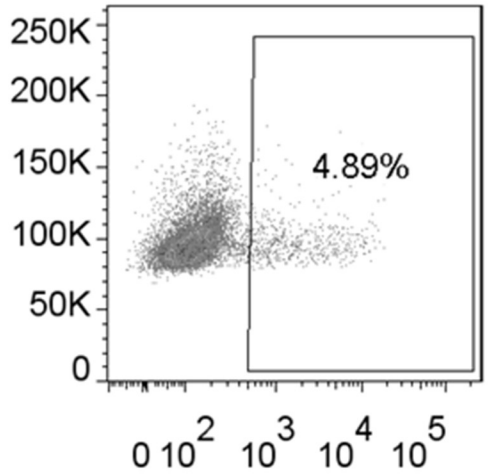
FIG. 154G

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

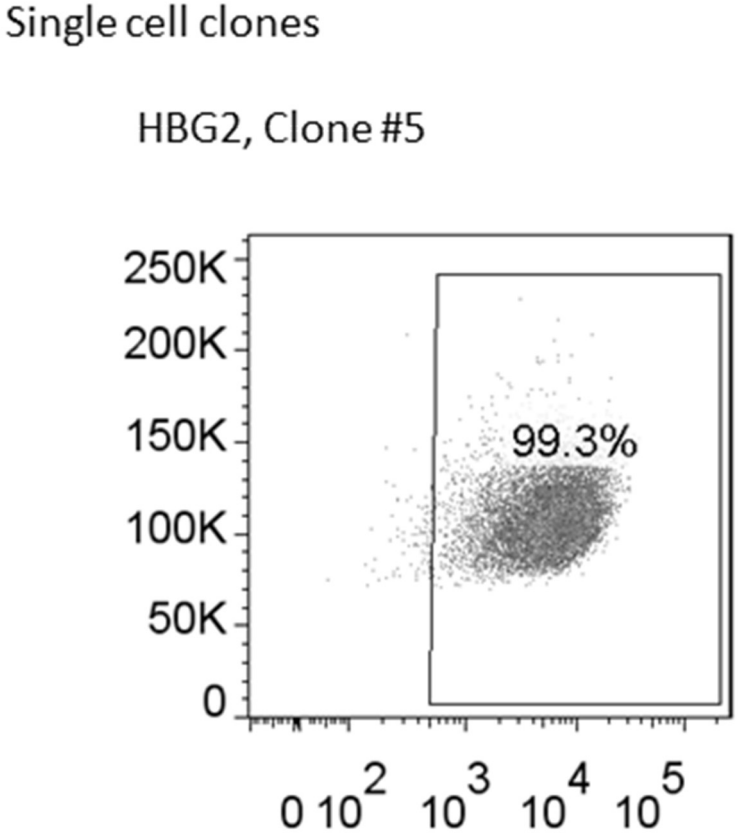
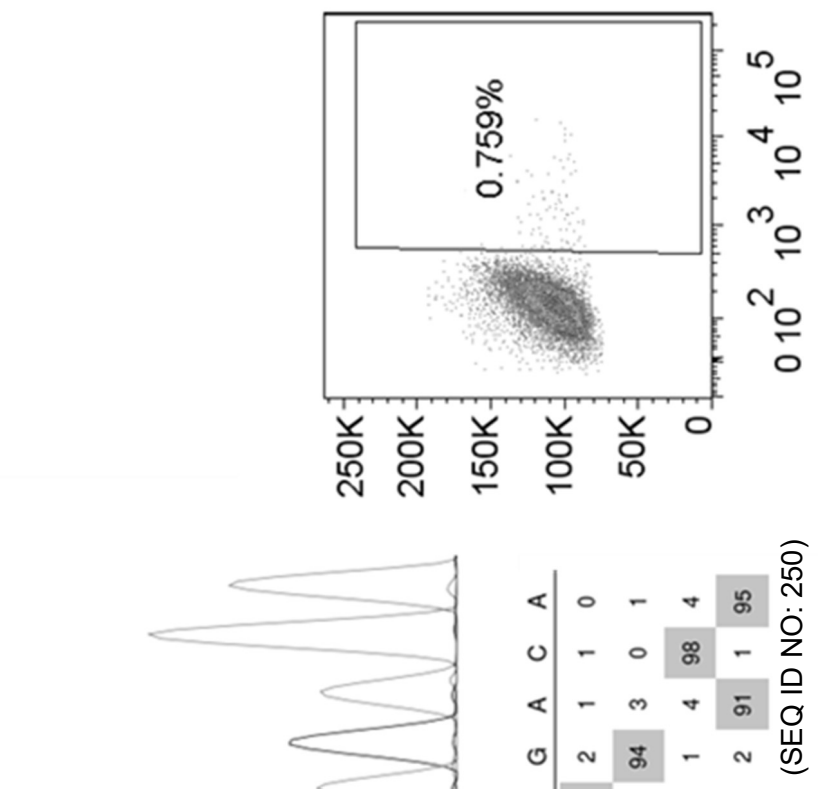

Single cell clones
HBG2, Clone #5
250K
200K
150K
100K
50K
0
99.3%
0 10^2 10^3 10^4 10^5

FIG. 154I



(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)

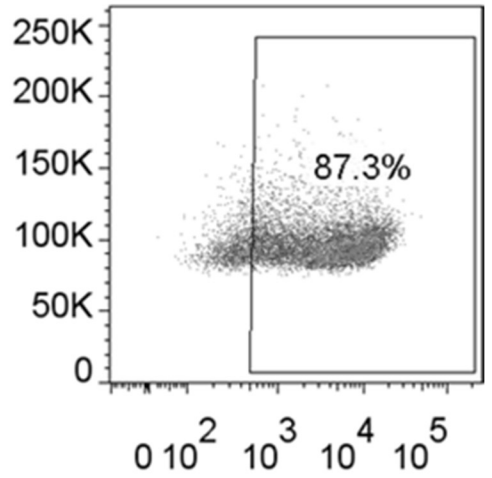
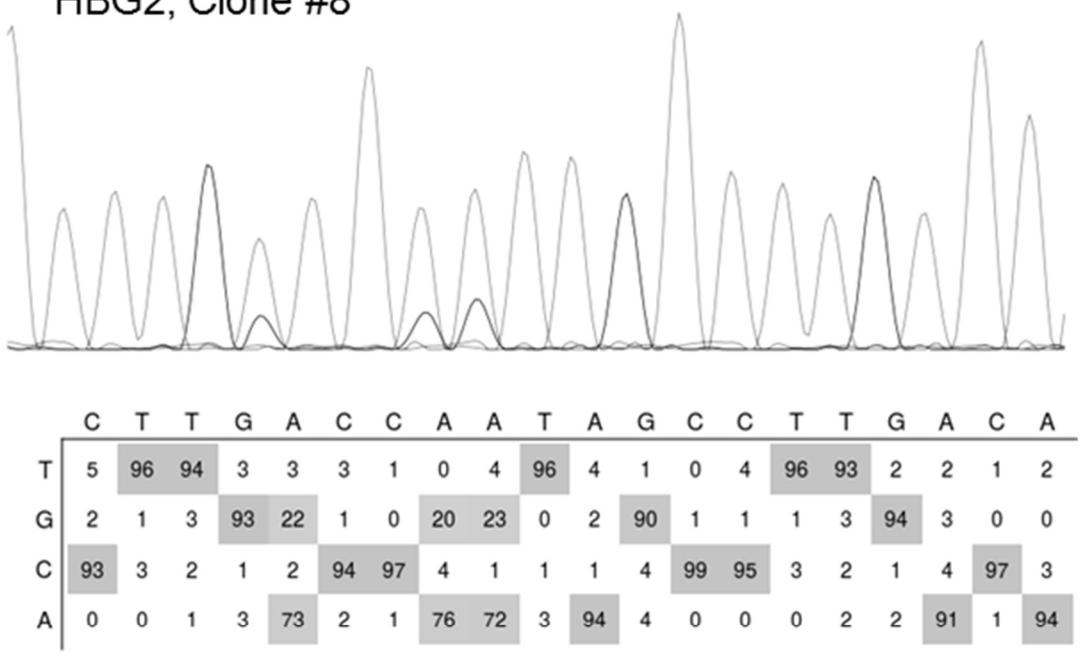


FIG. 154K

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)

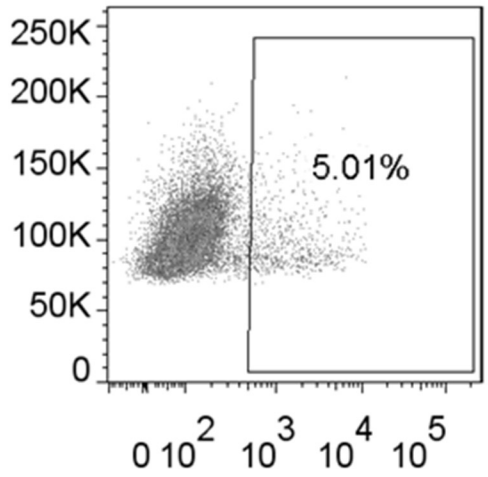


FIG. 154L

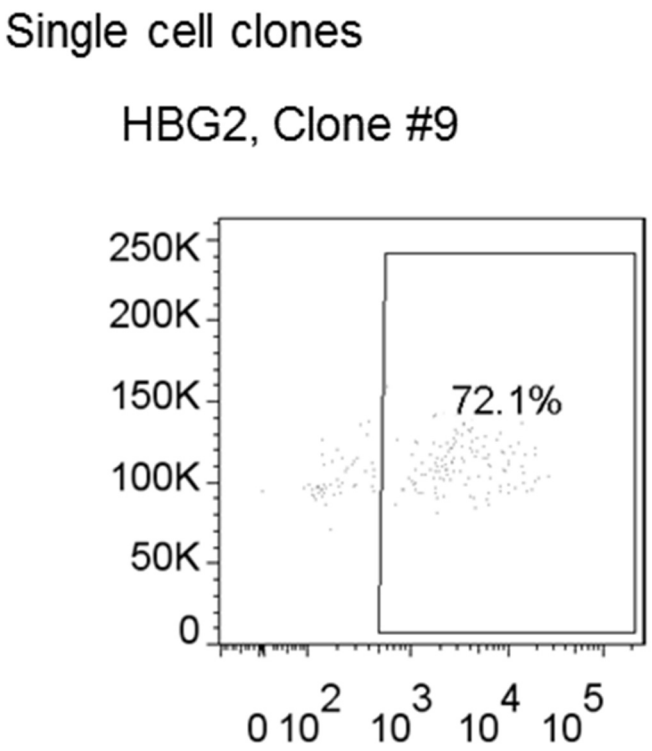


FIG. 154M

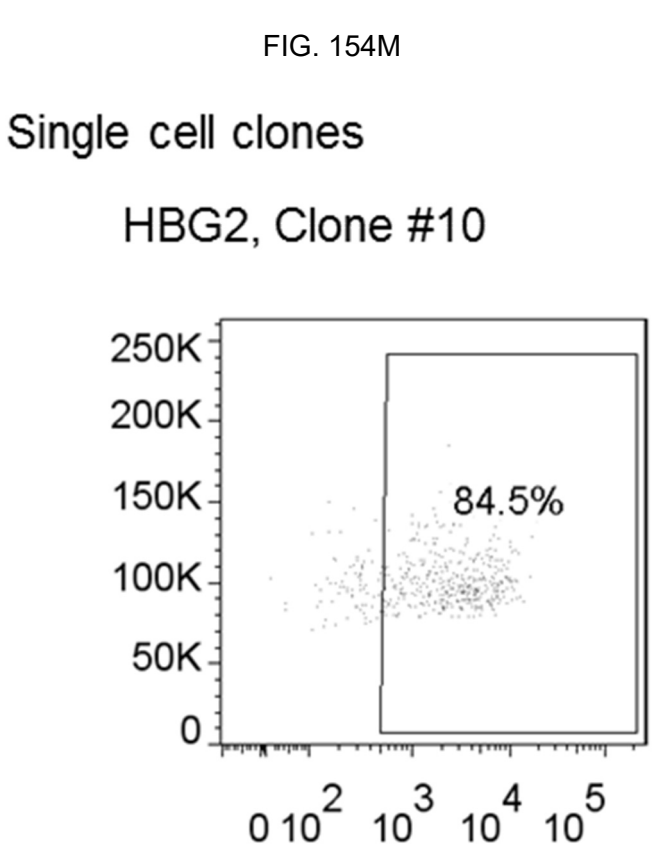


FIG. 154N

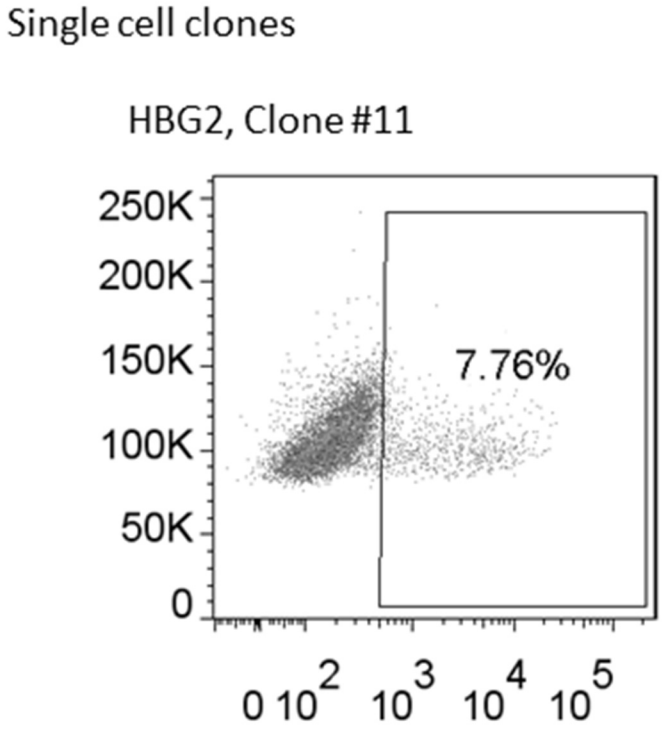
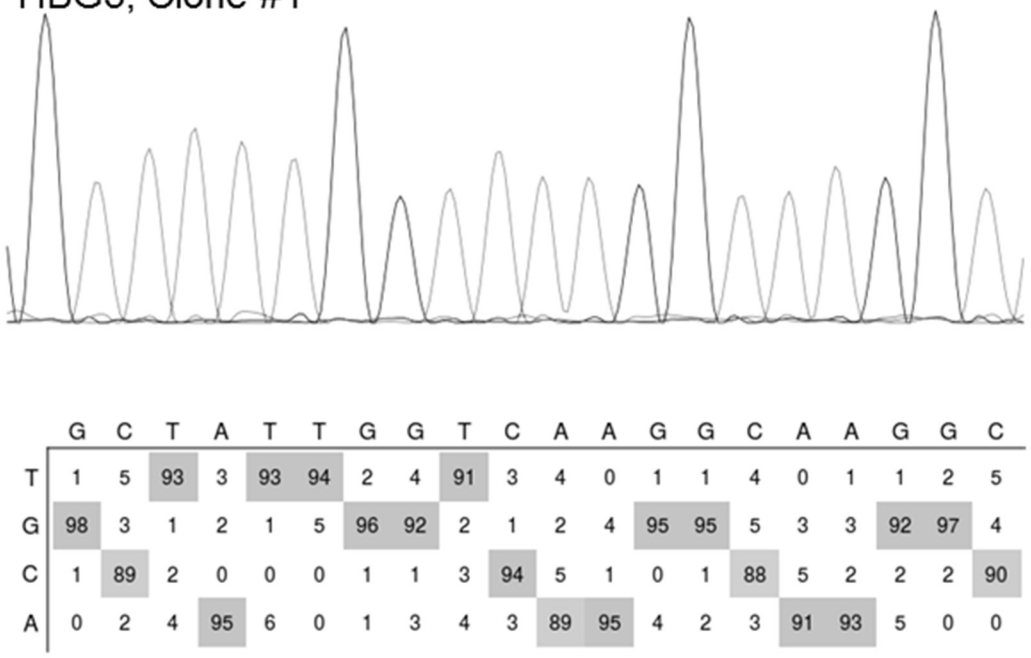

Single cell clones
HBG2, Clone #11
250K
200K
150K
100K
50K
0
7.76%
0 10^2 10^3 10^4 10^5

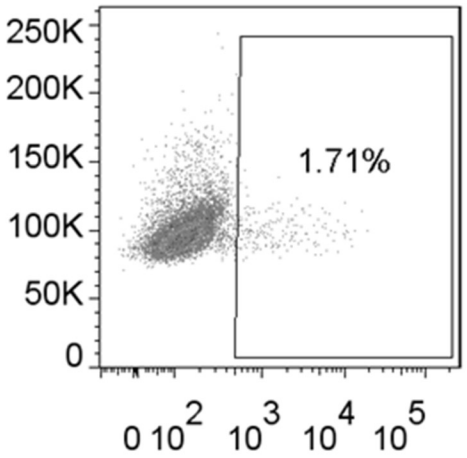
FIG. 154O

Single cell clones

HBG3, Clone #1

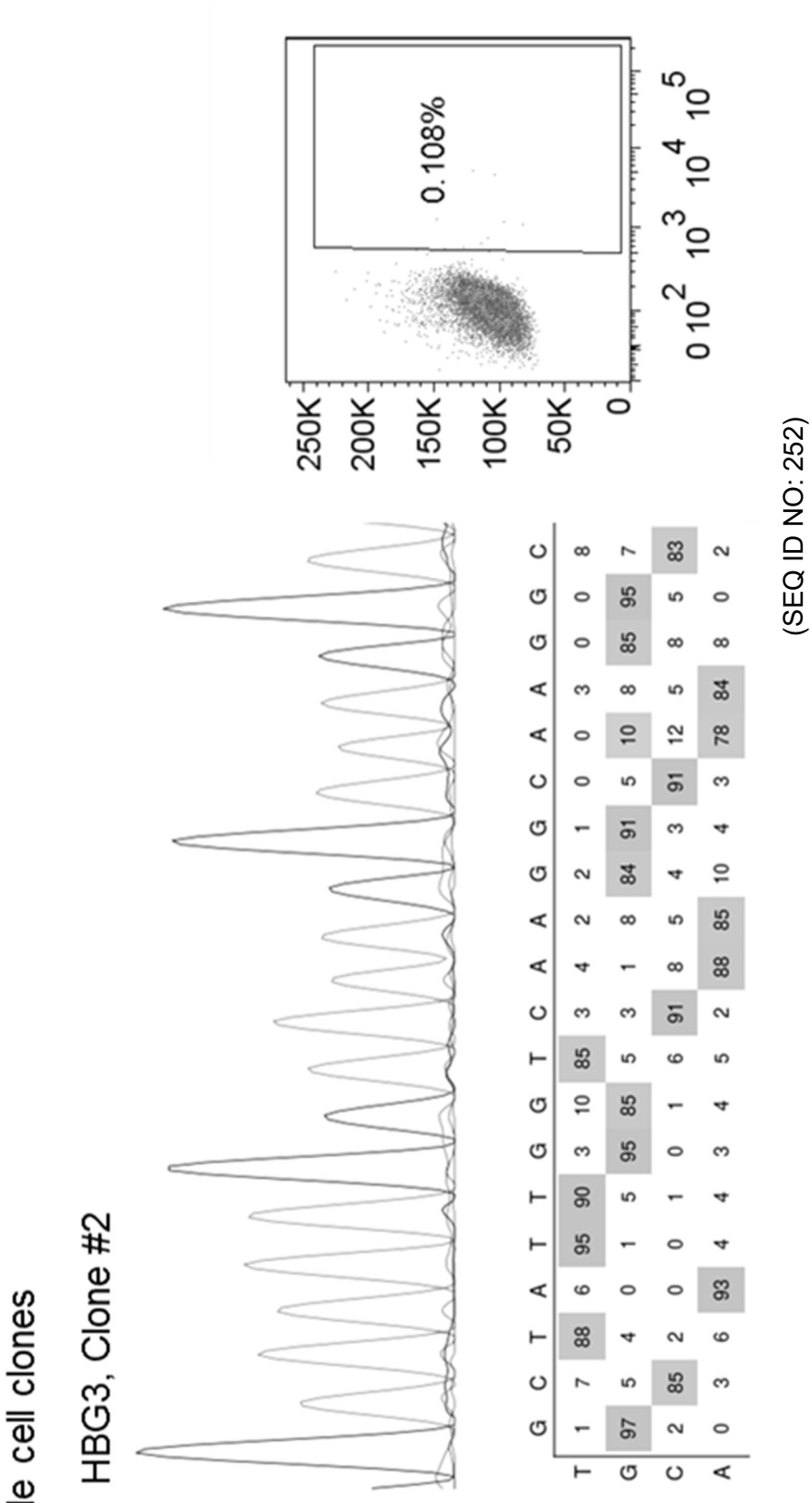


	G	C	T	A	T	T	G	G	T	C	A	A	G	G	C	A	A	G	G	C
T	1	5	93	3	93	94	2	4	91	3	4	0	1	1	4	0	1	1	2	5
G	98	3	1	2	1	5	96	92	2	1	2	4	95	95	5	3	3	92	97	4
C	1	89	2	0	0	0	1	1	3	94	5	1	0	1	88	5	2	2	2	90
A	0	2	4	95	6	0	1	3	4	3	89	95	4	2	3	91	93	5	0	0



(SEQ ID NO: 252)

FIG. 154P



(SEQ ID NO: 252)

FIG. 154Q

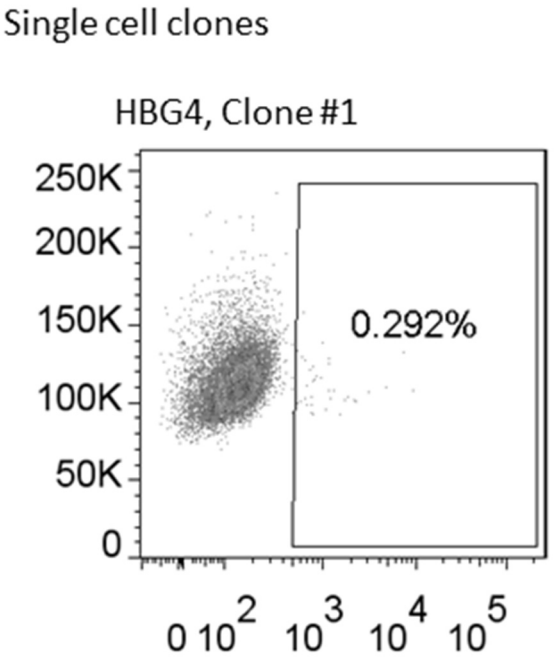


FIG. 154R

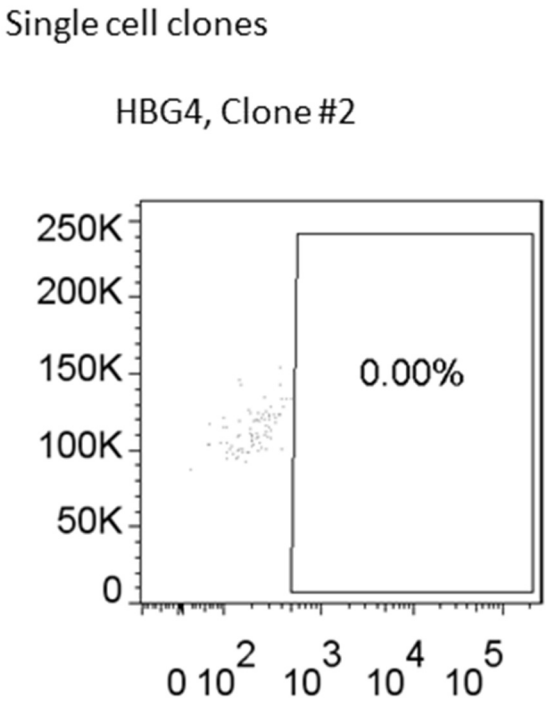


FIG. 154S

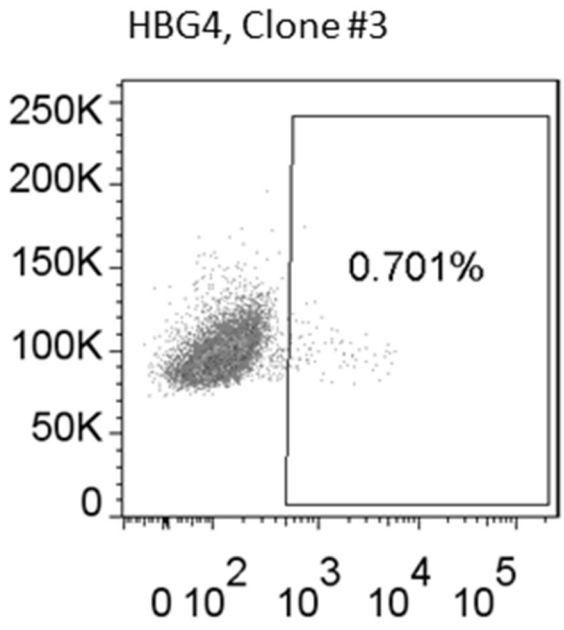


FIG. 155

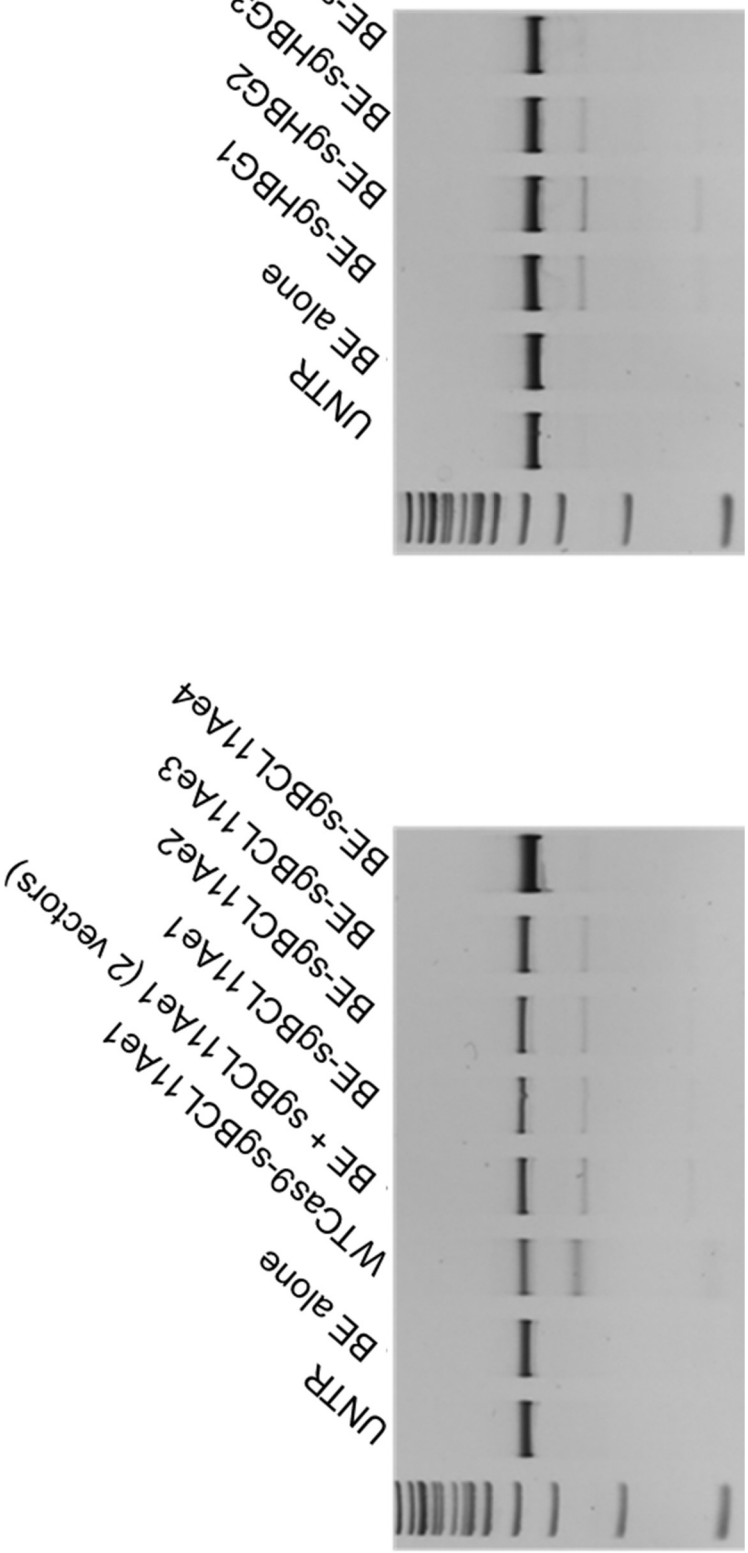

UNTR
BE alone
WTCas9-sgBCL11Ae1
BE + sgBCL11Ae1 (2 vectors)
BE-sgBCL11Ae1
BE-sgBCL11Ae2
BE-sgBCL11Ae3
BE-sgBCL11Ae4
UNTR
BE alone
BE-sgHBG1
BE-sgHBG2
BE-sgHBG3
BE-sgHBG4

FIG. 156A

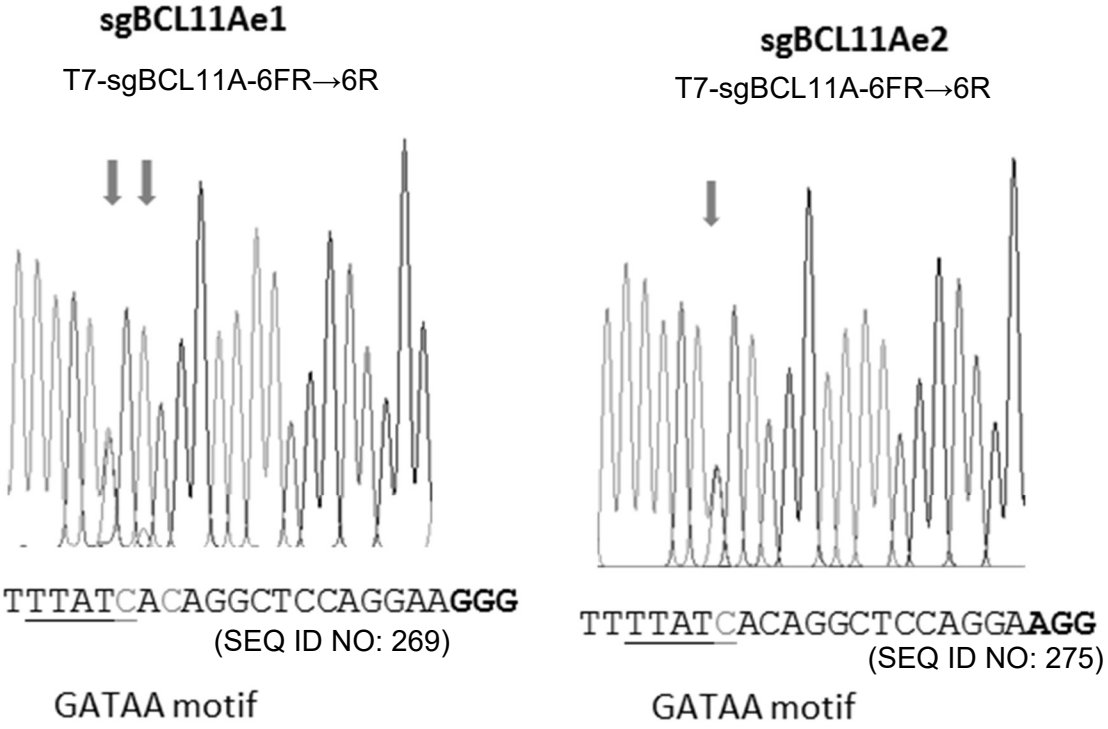


FIG. 156B

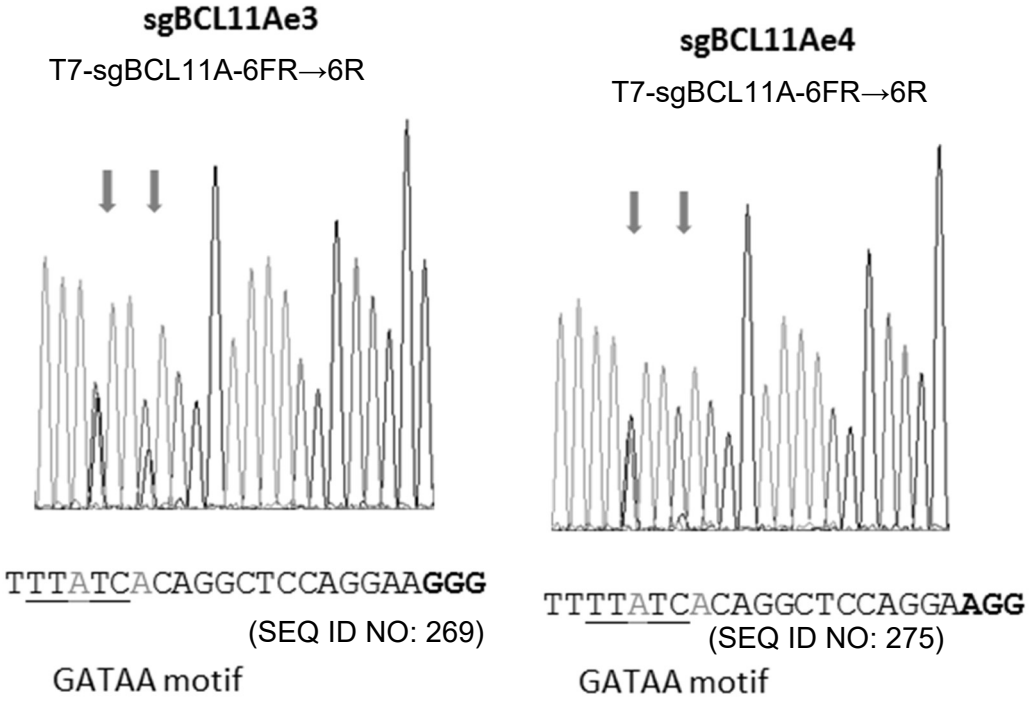


FIG. 156C

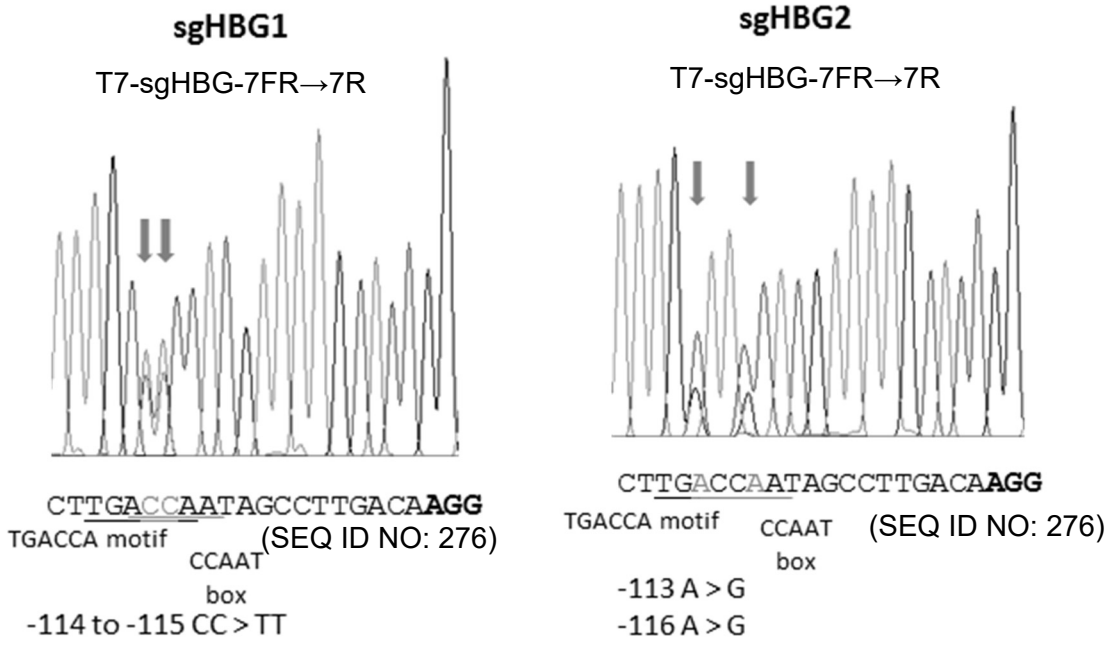


FIG. 156D

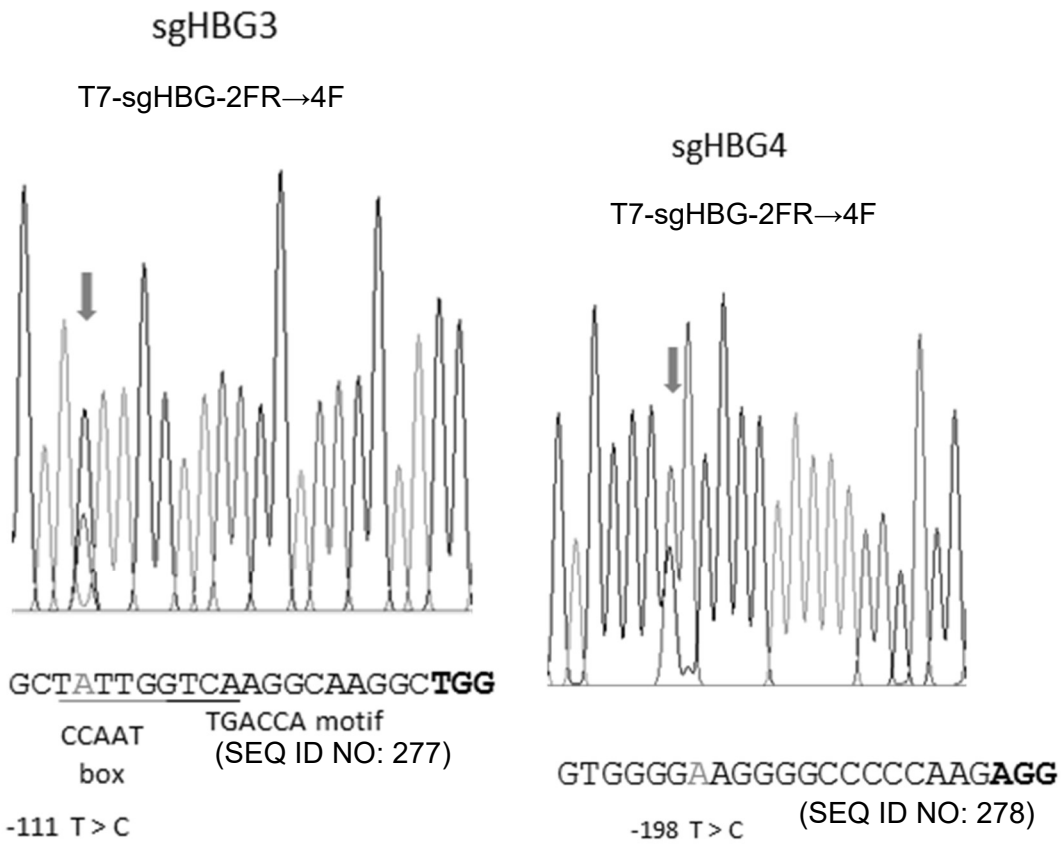


FIG. 157

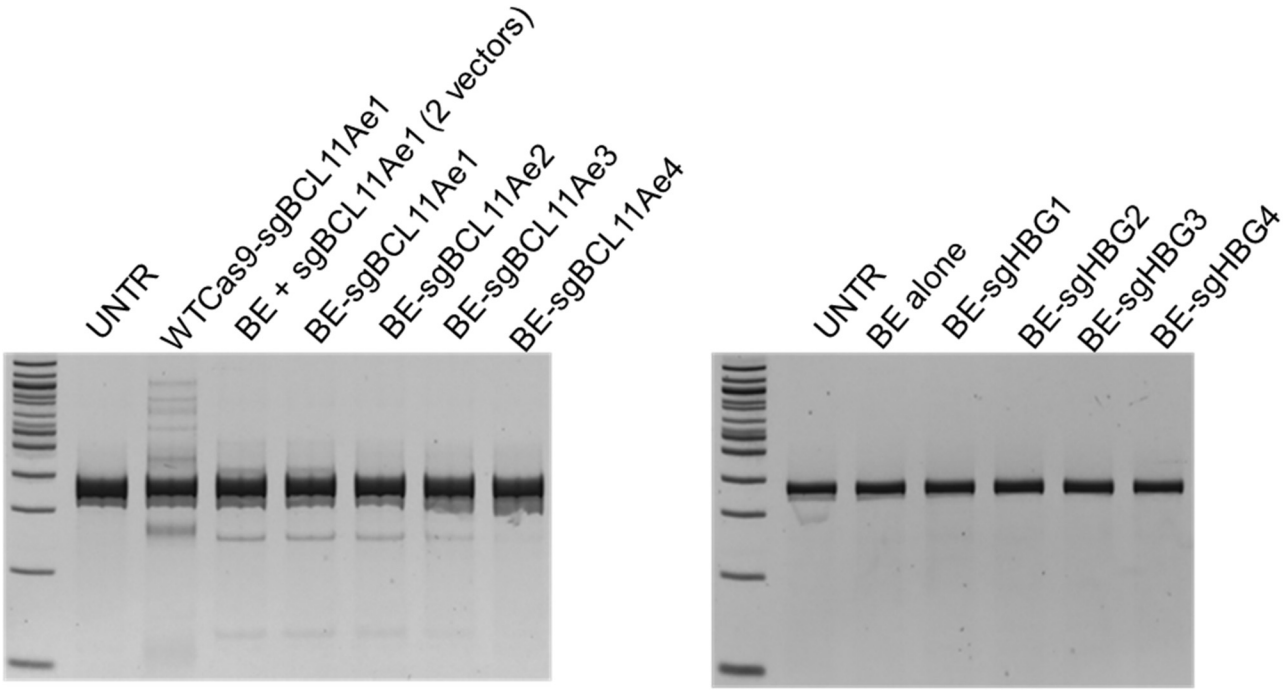


FIG. 158

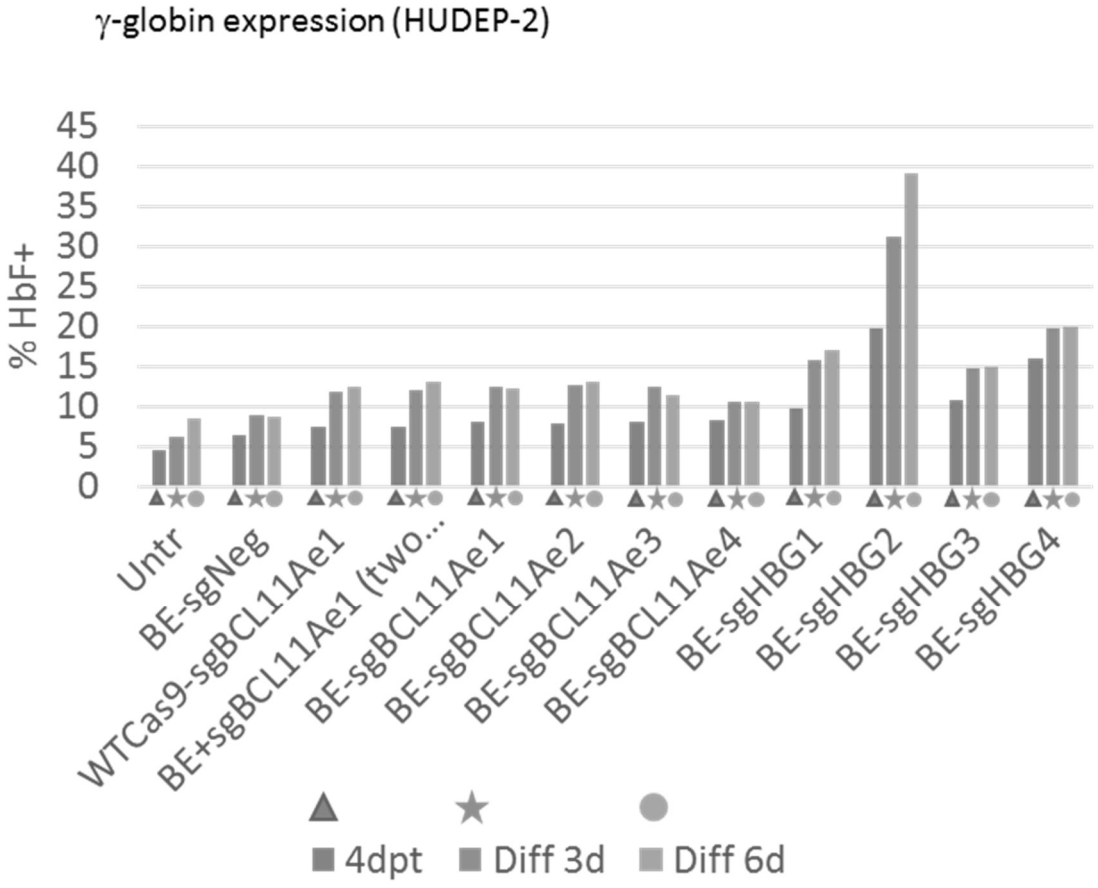


FIG. 159A

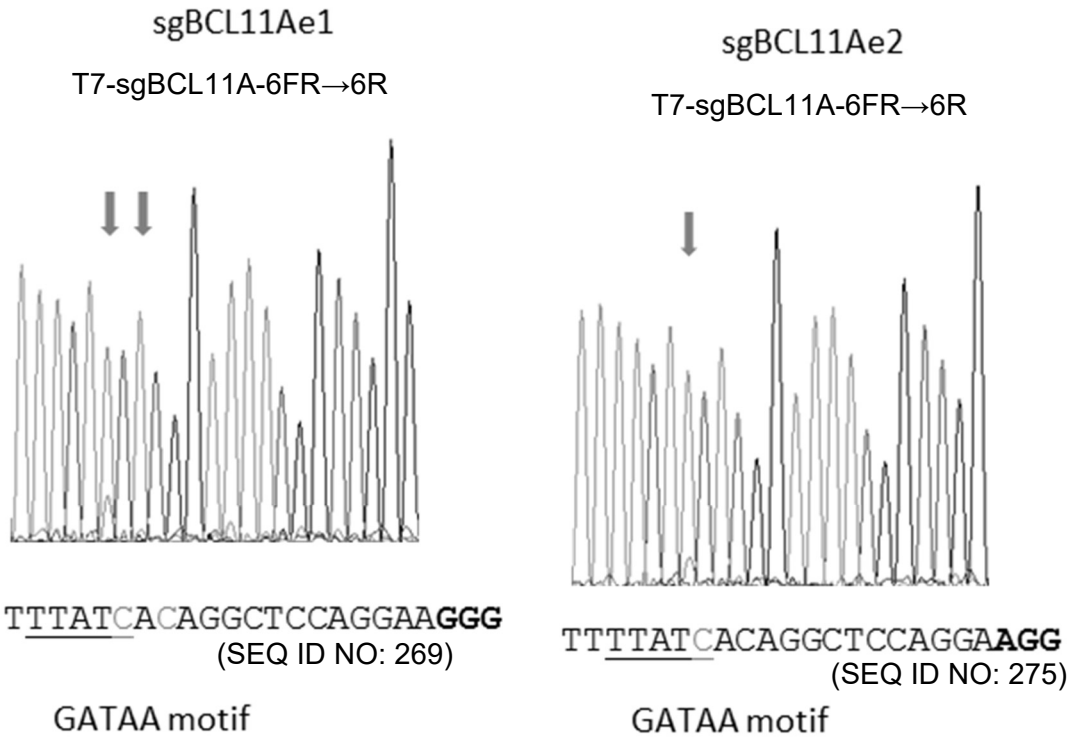


FIG. 159B

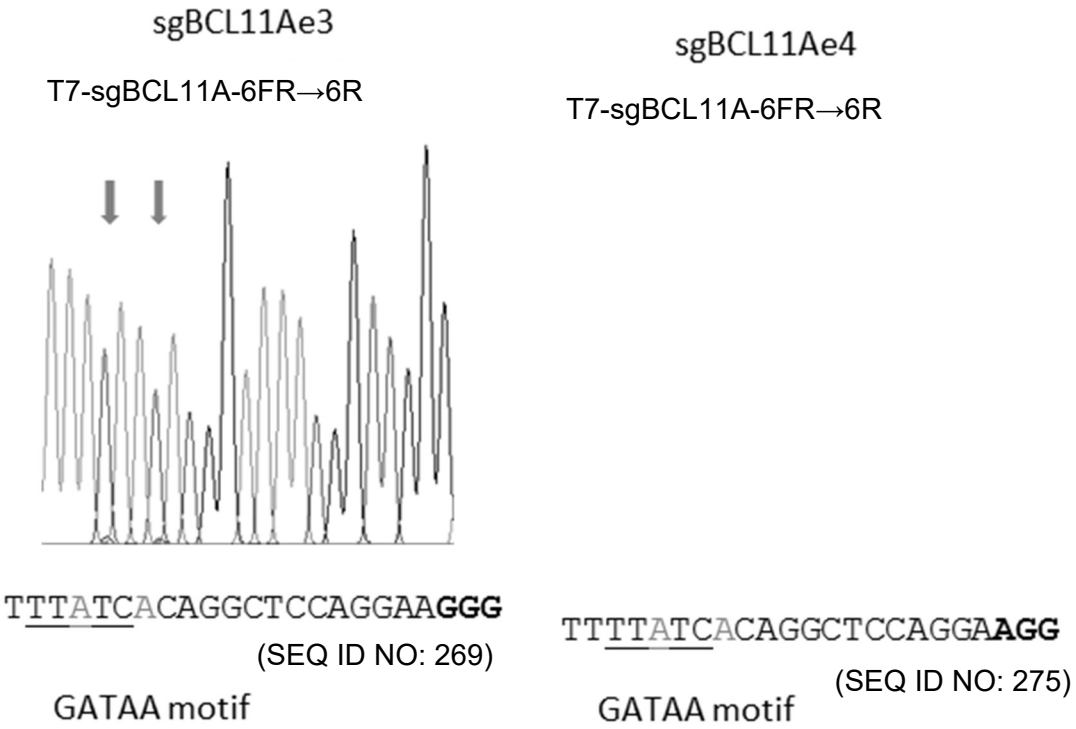


FIG. 159C

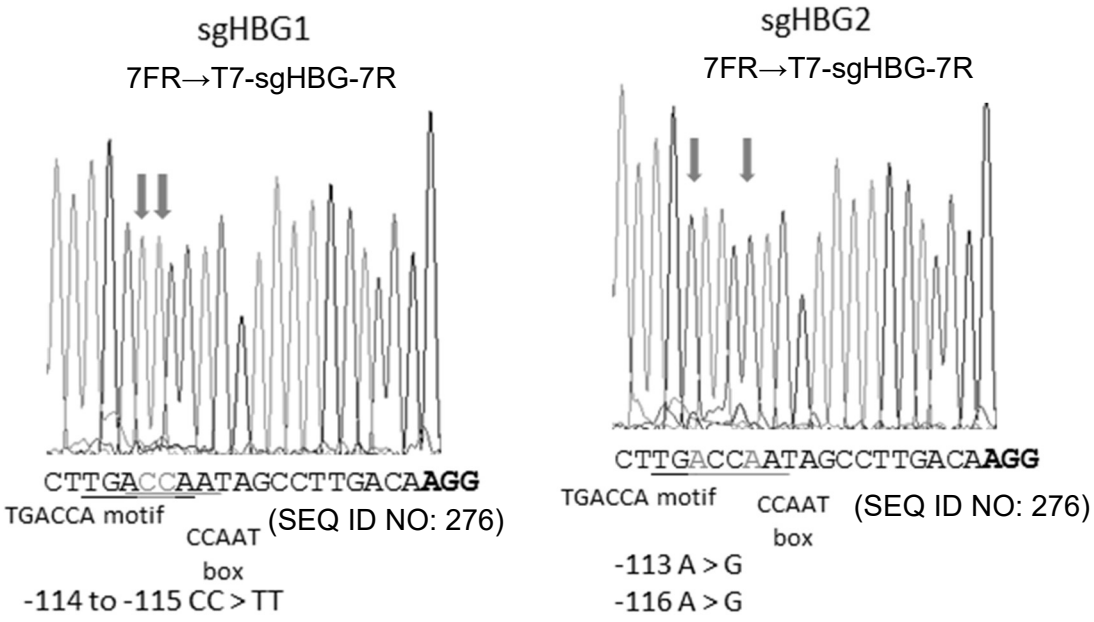


FIG. 159D

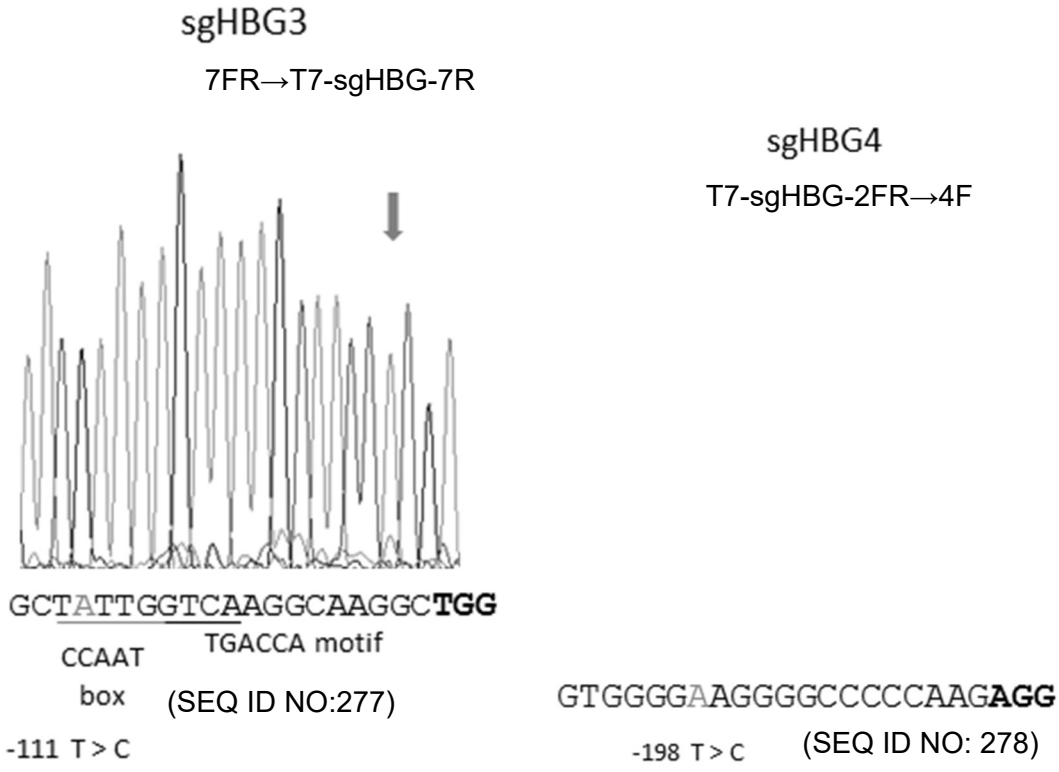


FIG. 160

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

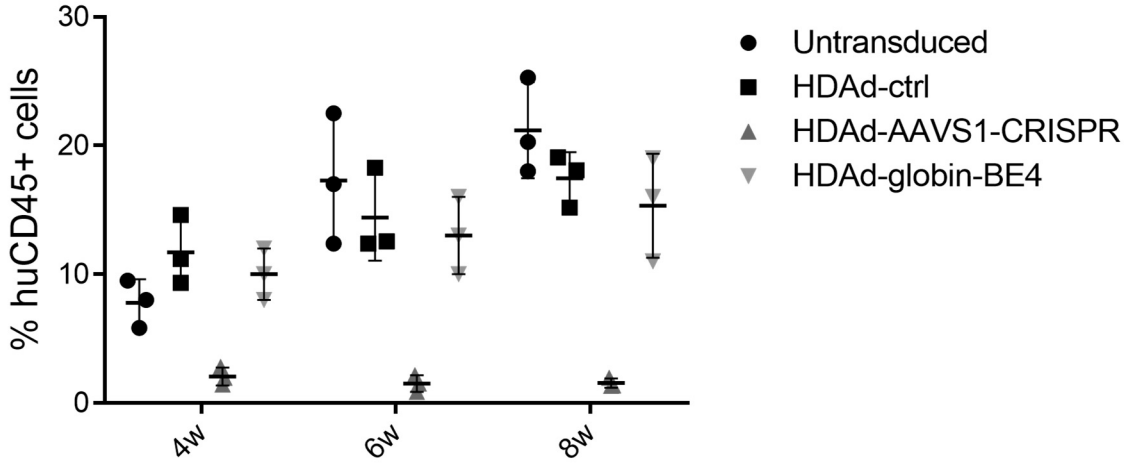
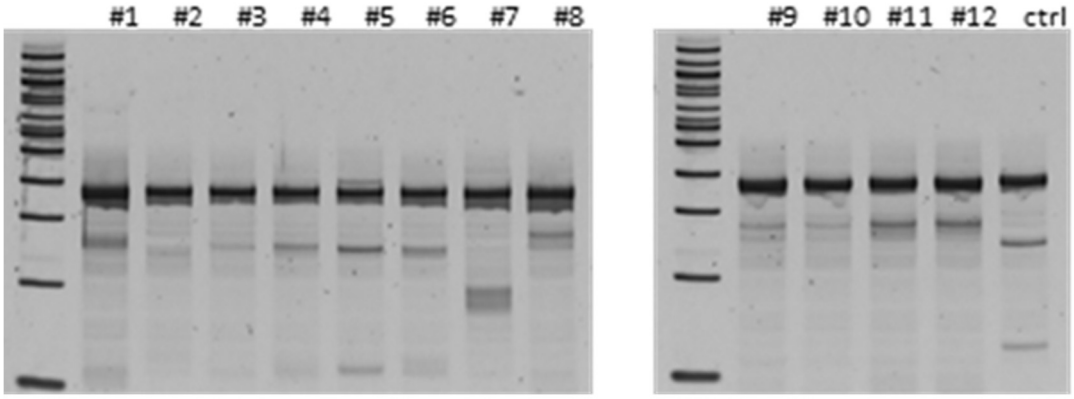


FIG. 162

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

Bcl11a enhancer



HBG promoter

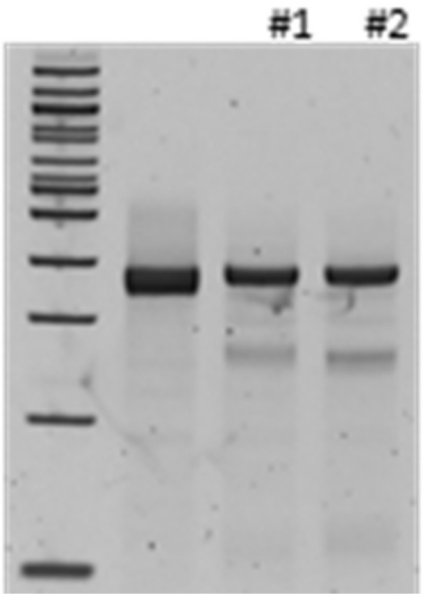


FIG. 163

Dual base editing vector application

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

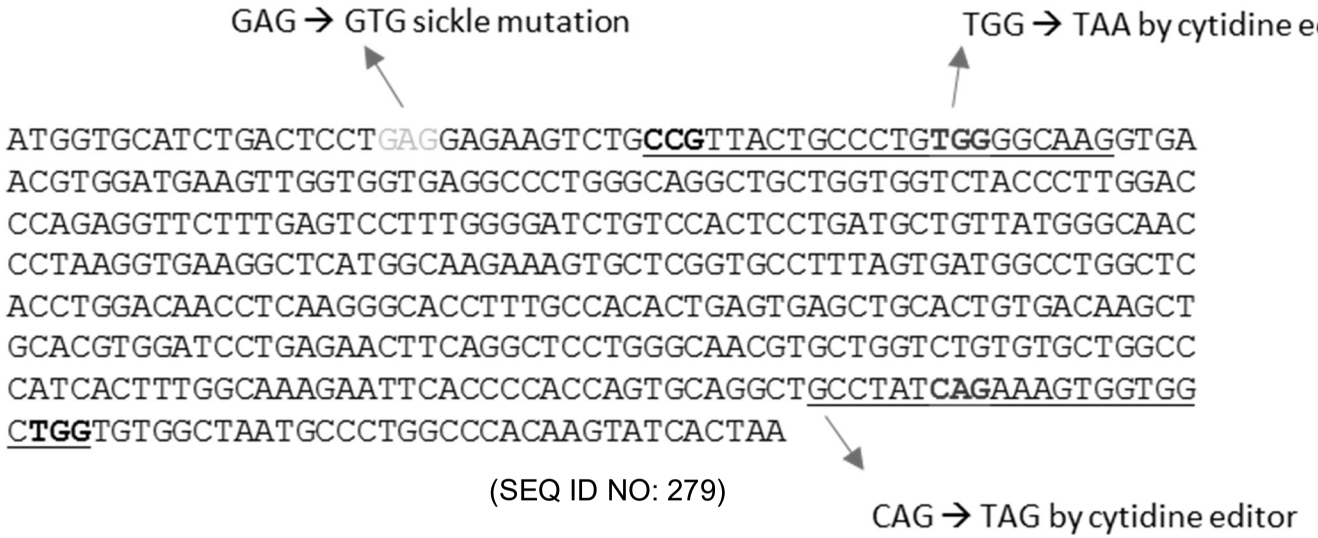


FIG. 164

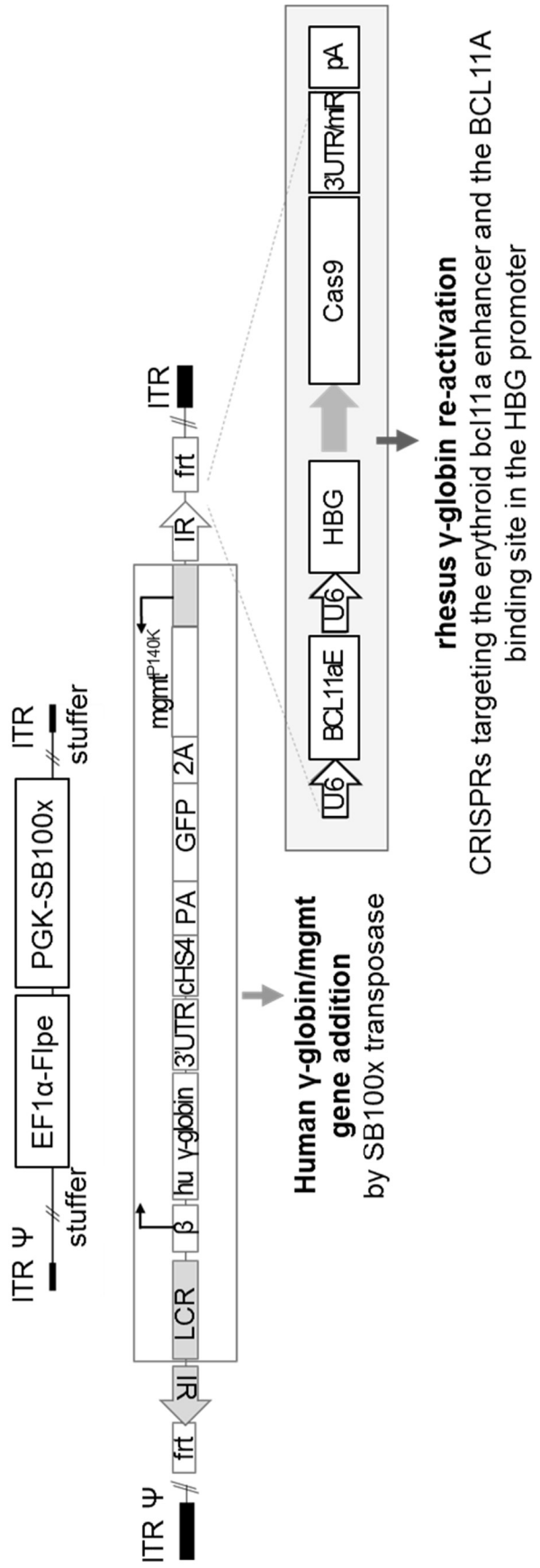

ITR Ψ
stuffer
EF1α-Flpe
PGK-SB100x
ITR
stuffer
ITR Ψ
frt
IR
LCR
β
hu γ-globin
3'UTR
cHS4
PA
GFP
2A
mgmt P140K
IR
frt
ITR
Human γ-globin/mgmt gene addition
by SB100x transposase
U6
BCL11aE
U6
HBG
Cas9
3'UTR
miR
pA
rhesus γ-globin re-activation
CRISPRs targeting the erythroid bcl11a enhancer and the BCL11A binding site in the HBG promoter

FIG. 165

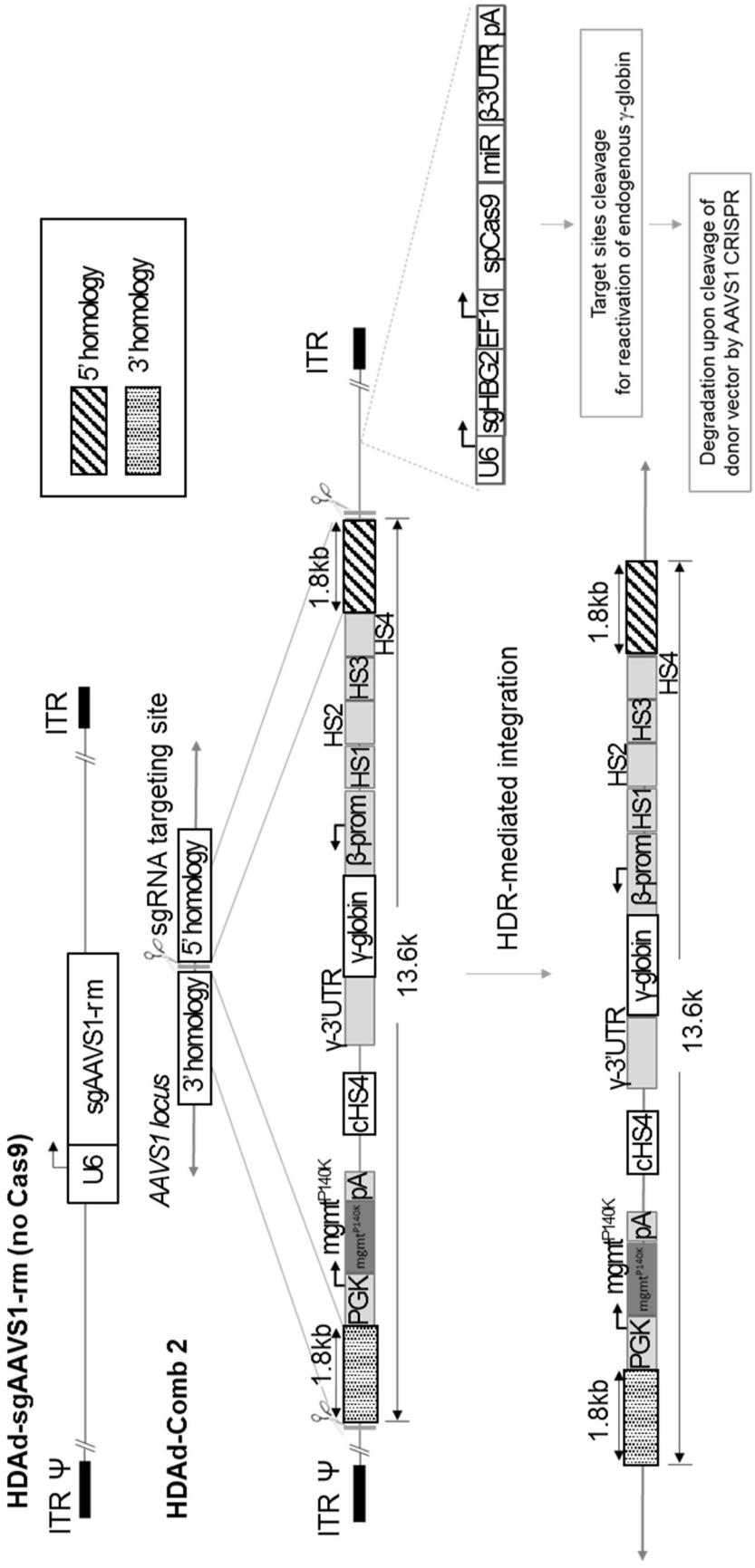
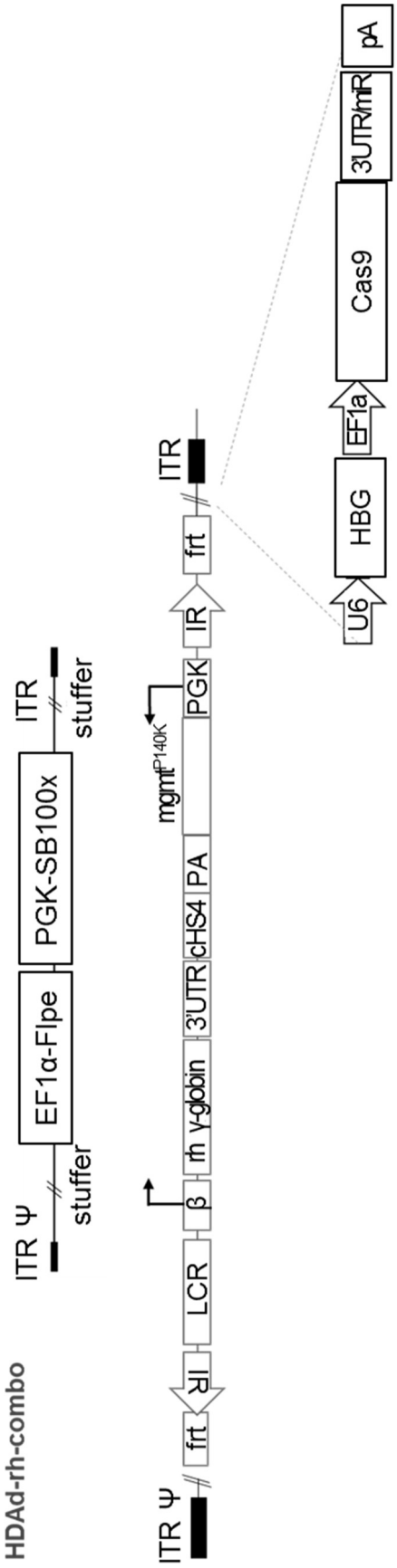

HDAd-sgAAVS1-rm (no Cas9)
ITR Ψ
U6
sgAAVS1-rm
ITR
HDAd-Comb 2
AAVS1 locus
sgRNA targeting site
3' homology
5' homology
5' homology
3' homology
ITR Ψ
1.8kb
PGK
mgmtP140K
pA
cHS4
γ-3'UTR
γ-globin
β-prom
HS1
HS2
HS3
HS4
13.6k
ITR
1.8kb
HDR-mediated integration
U6
sgHBG2
EF1α
spCas9
miR
β-3UTR
pA
Target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

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'UTR/mIR
pA