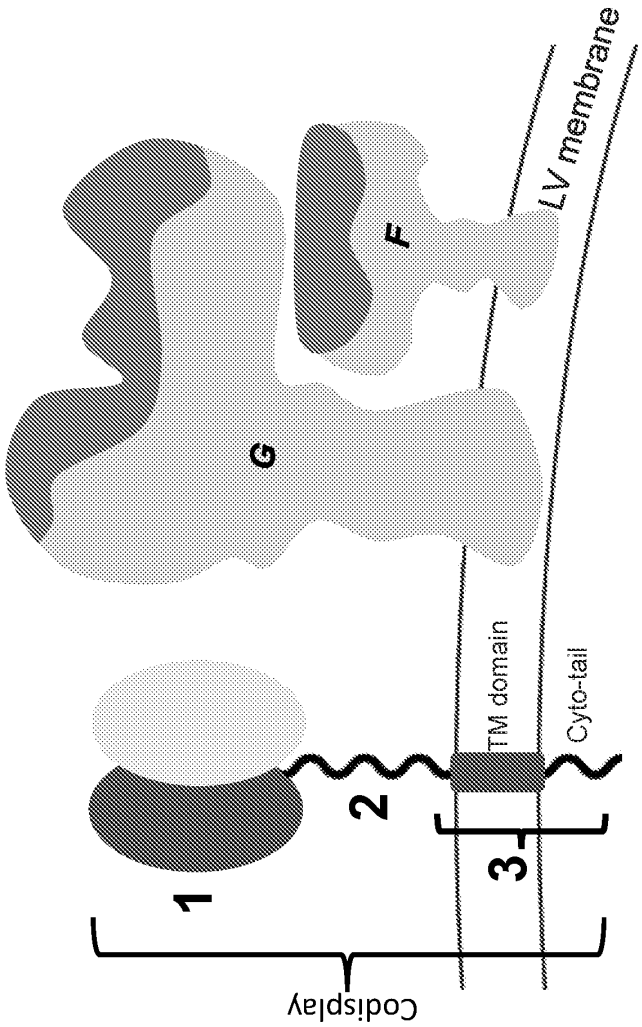


FIG. 1

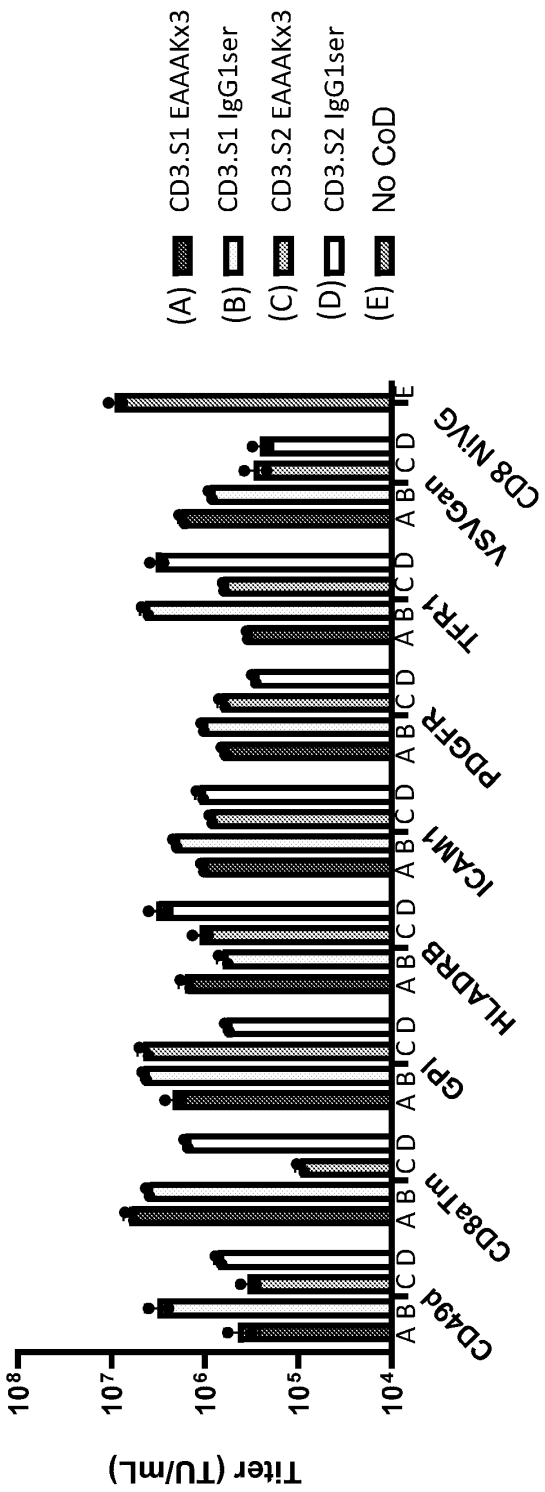

(A) CD3.S1 EAAAKx3
(B) CD3.S1 IgG1ser
(C) CD3.S2 EAAAKx3
(D) CD3.S2 IgG1ser
(E) No CoD
Titer (TU/mL)
10^8
10^7
10^6
10^5
10^4
CD49d
CD8aTm
GPI
HLADRB
ICAM1
PDGFR
TFR1
VSVGan
CD8 NiVG
A B C D

FIG. 2A

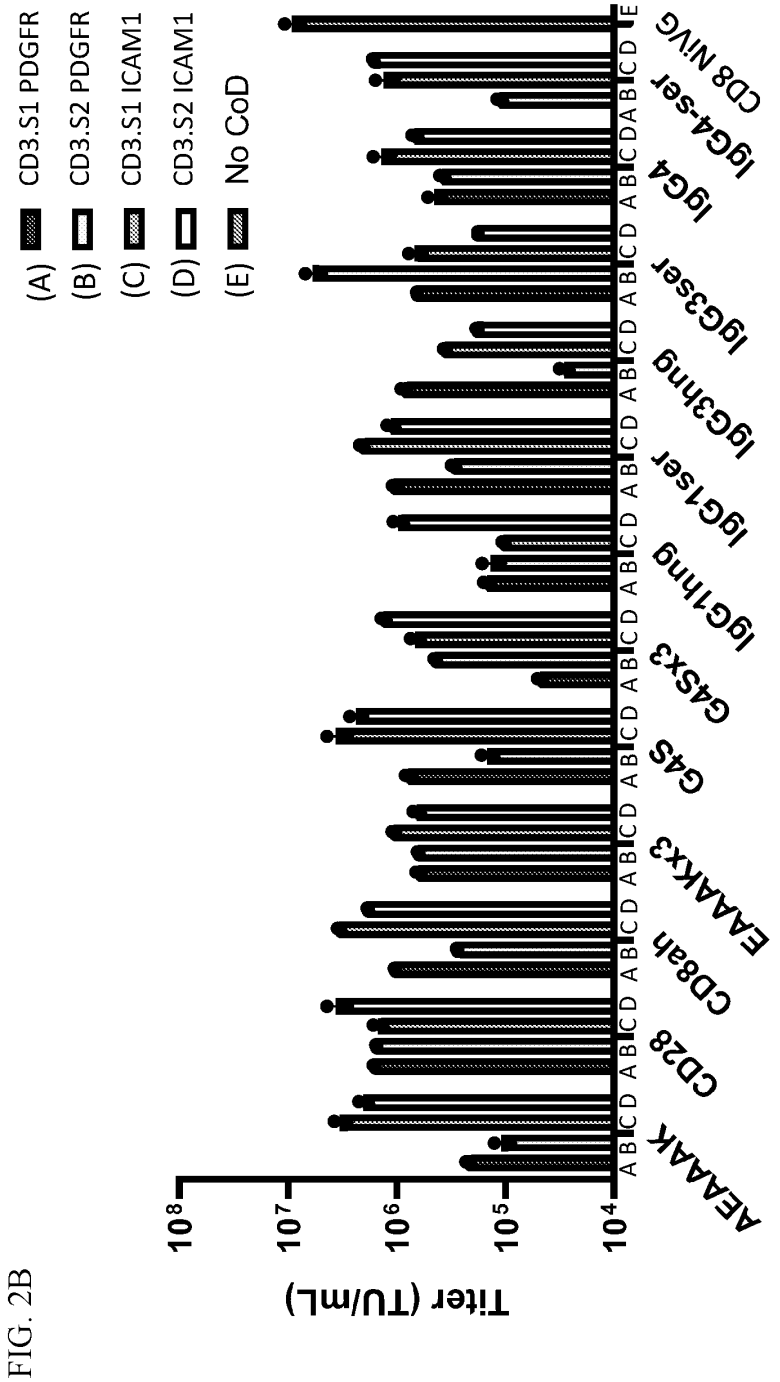

(A) CD3.S1 PDGFR
(B) CD3.S2 PDGFR
(C) CD3.S1 ICAM1
(D) CD3.S2 ICAM1
(E) No CoD
Titer (TU/mL)
10^8
10^7
10^6
10^5
10^4
AEAAAK
CD28
CD8ah
EAAAK3
G4S
G4Sx3
IgG1hng
IgG1ser
IgG3hng
IgG3ser
IgG4
IgG4-ser
CD8 N/VG

FIG. 2B

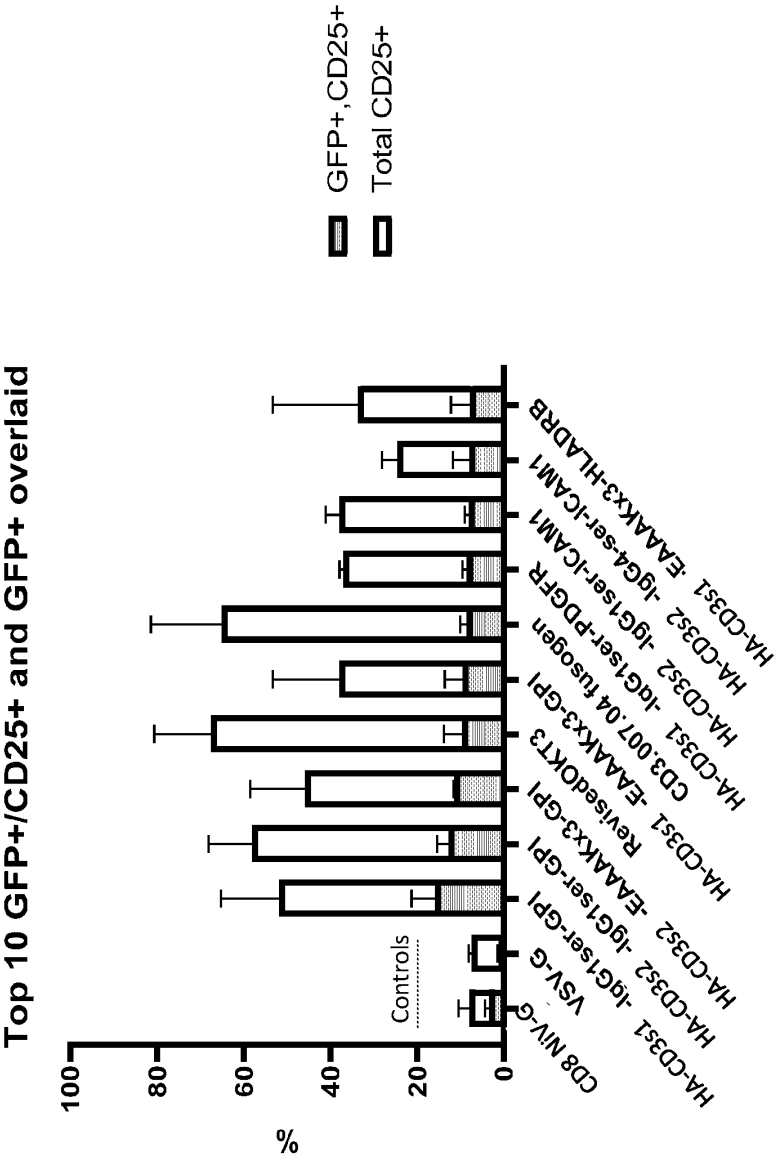

Top 10 GFP+/CD25+ and GFP+ overlaid
GFP+,CD25+
Total CD25+
%
100
80
60
40
20
0
Controls
CD8 NiV-G
VSV-G
HA-CD3s1 -IgG1ser-GPI
HA-CD3s2 -IgG1ser-GPI
HA-CD3s2 -EAAAKx3-GPI
HA-CD3s1 -EAAAKx3-GPI
CD3.007.04 fusogen
HA-CD3s1 -IgG1ser-PDGFR
HA-CD3s2 -IgG1ser-PDGFR
HA-CD3s2 -IgG4-ser-ICAM1
HA-CD3s1 -EAAAKx3-HLADRB

FIG. 3A

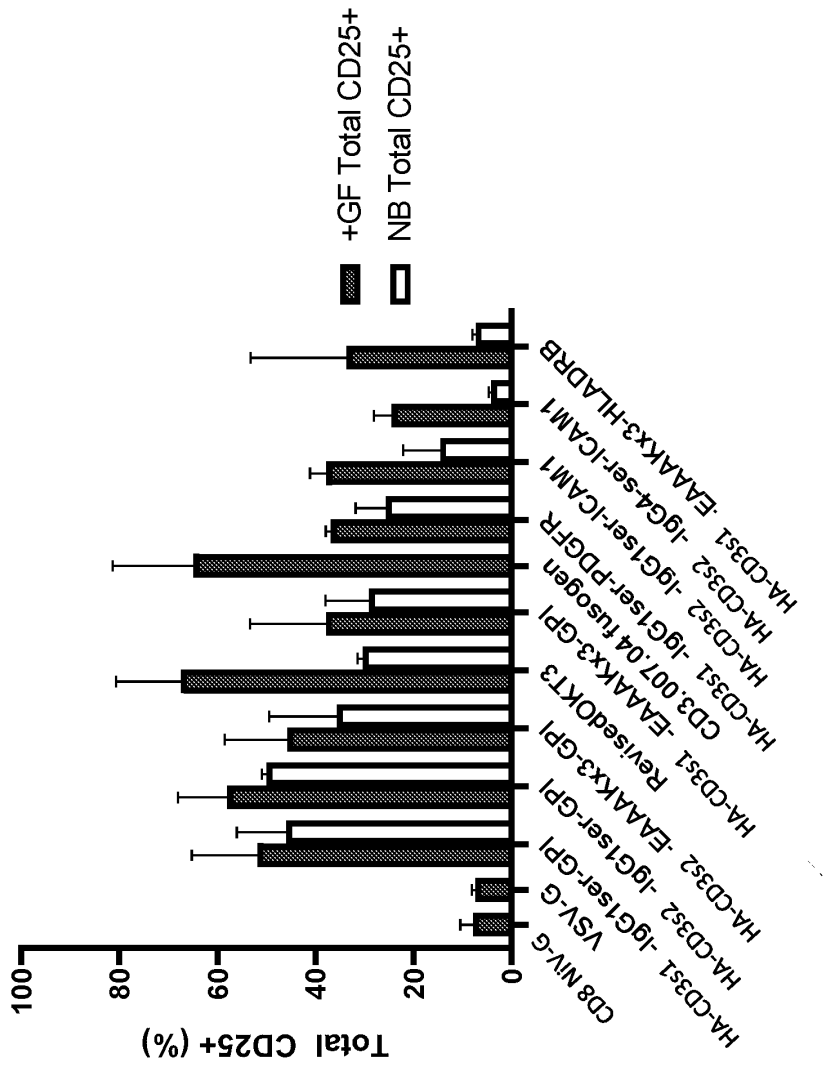

+GF Total CD25+
NB Total CD25+
Total CD25+ (%)
100
80
60
40
20
0
CD8 NiV-G
VSV-G
HA-CD3s1 -IgG1ser-GPI
HA-CD3s2 -IgG1ser-GPI
HA-CD3s2 -EAAAKx3-GPI
HA-CD3s1 -EAAAKx3-GPI
CD3.007.04 fusogen
HA-CD3s1 -IgG1ser-PDGFR
HA-CD3s2 -IgG1ser-PDGFR
HA-CD3s2 -IgG4-ser-ICAM1
HA-CD3s1 -EAAAKx3-ICAM1
HA-CD3s1 -EAAAKx3-HLADRB

FIG. 3B

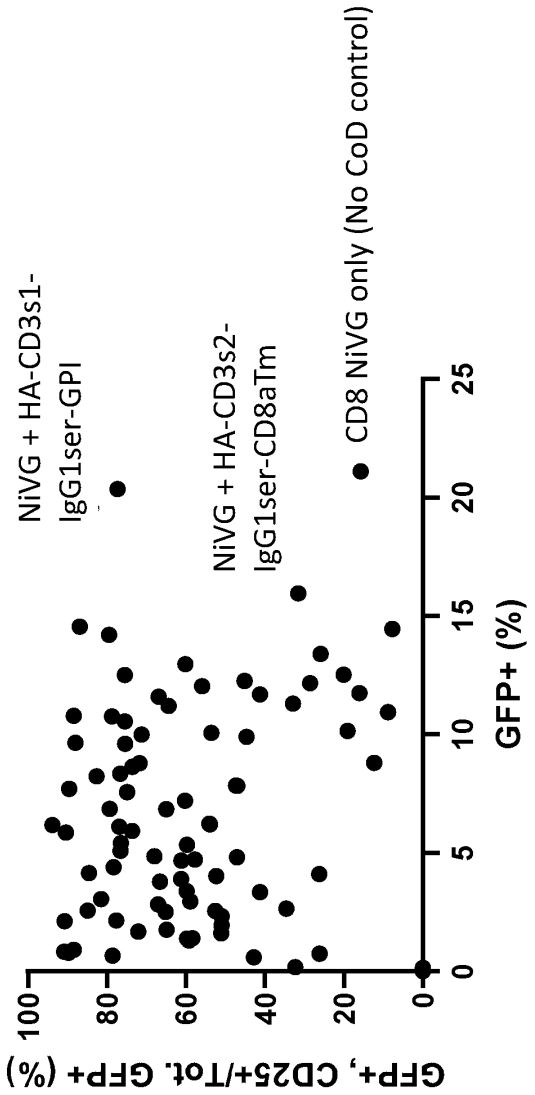

GFP+, CD25+/Tot. GFP+ (%)
100
80
60
40
20
0
0
5
10
15
20
25
GFP+ (%)
NiVG + HA-CD3s1-IgG1ser-GPI
NiVG + HA-CD3s2-IgG1ser-CD8aTm
CD8 NiVG only (No CoD control)

FIG. 3C

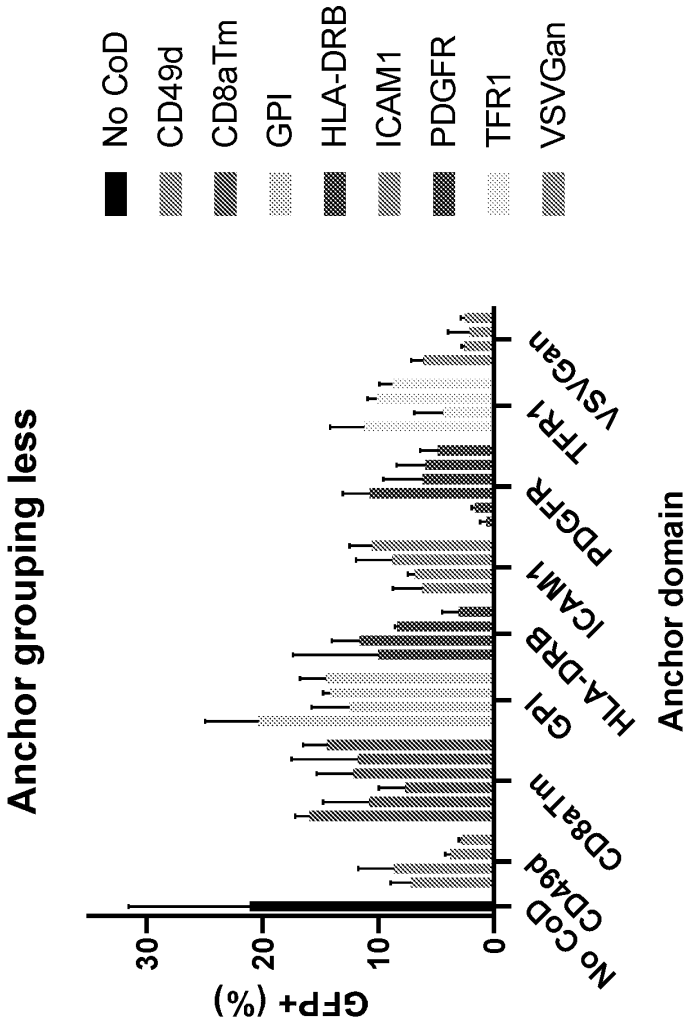

Anchor grouping less
No CoD
CD49d
CD8aTm
GPI
HLA-DRB
ICAM1
PDGFR
TFR1
VSVGan
GFP+ (%)
0
10
20
30
Anchor domain

FIG. 4A

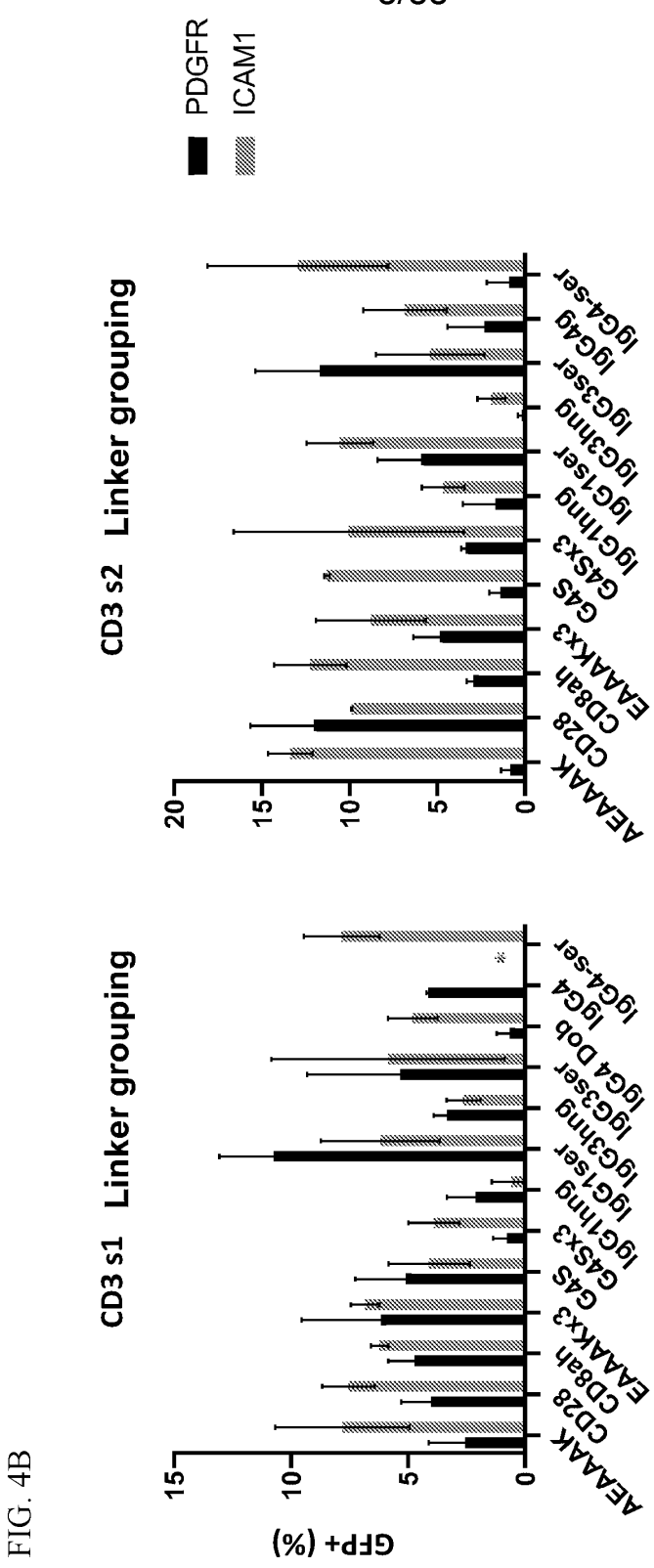

PDGFR
ICAM1
CD3 s2 Linker grouping
CD3 s1 Linker grouping
GFP+ (%)
0
5
10
15
20
AEAAAK
CD28
CD8ah
EAAAKx3
G4S
G4Sx3
IgG1hng
IgG1ser
IgG3hng
IgG3ser
IgG4 Dob
IgG4
IgG4-ser

FIG. 4B

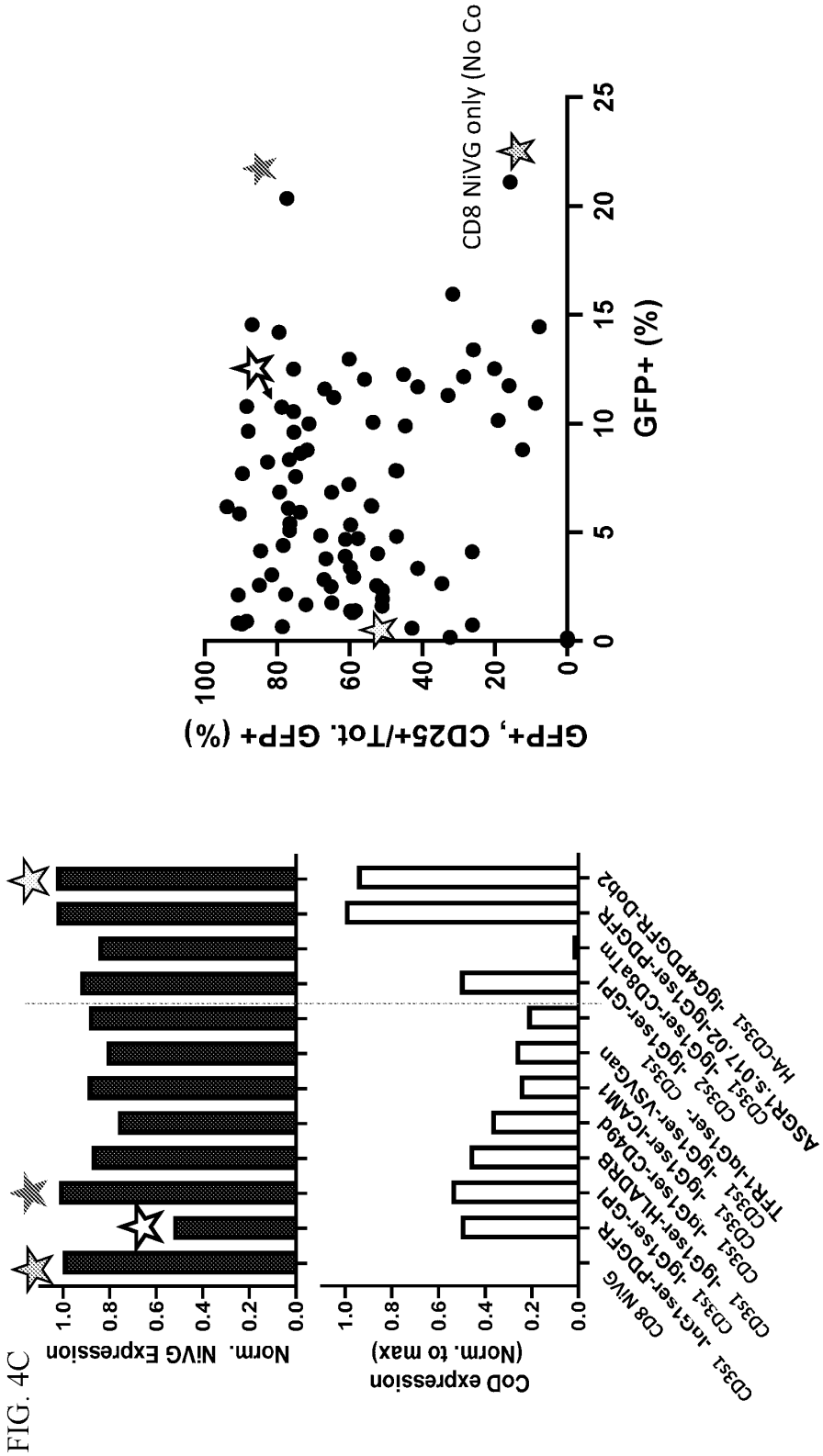

GFP+, CD25+/Tot. GFP+ (%)
GFP+ (%)
CD8 NiVG only (No Co
Norm. NiVG Expression
CoD expression (Norm. to max)
CD3s1 -1gG1ser-PDGFR
CD3s1 -1gG1ser-GPI
CD3s1 -1gG1ser-HLADRB
CD3s1 -1gG1ser-CD49d
CD3s1 -1gG1ser-ICAM1
TFR1-1gG1ser- CD3s2 -1gG1ser-VSVGan
ASGR1.s.017.02-1gG1ser-CD8aTm
HA-CD3s1 -1gG4PDGFR-Dob2

FIG. 4C

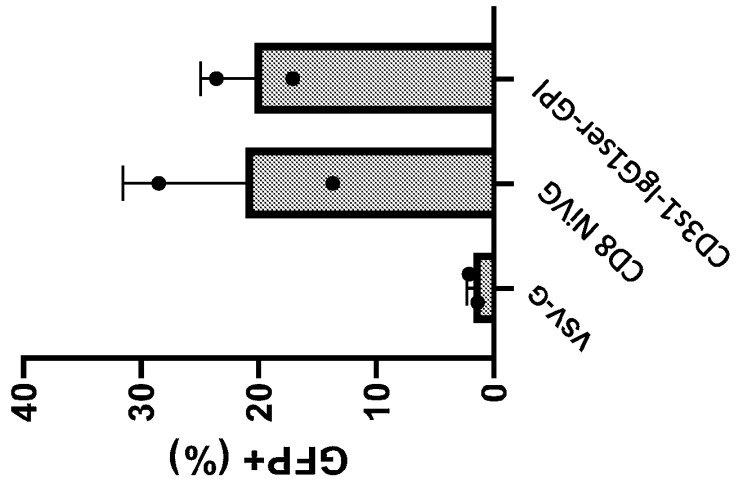


FIG. 5A

FIG. 5B

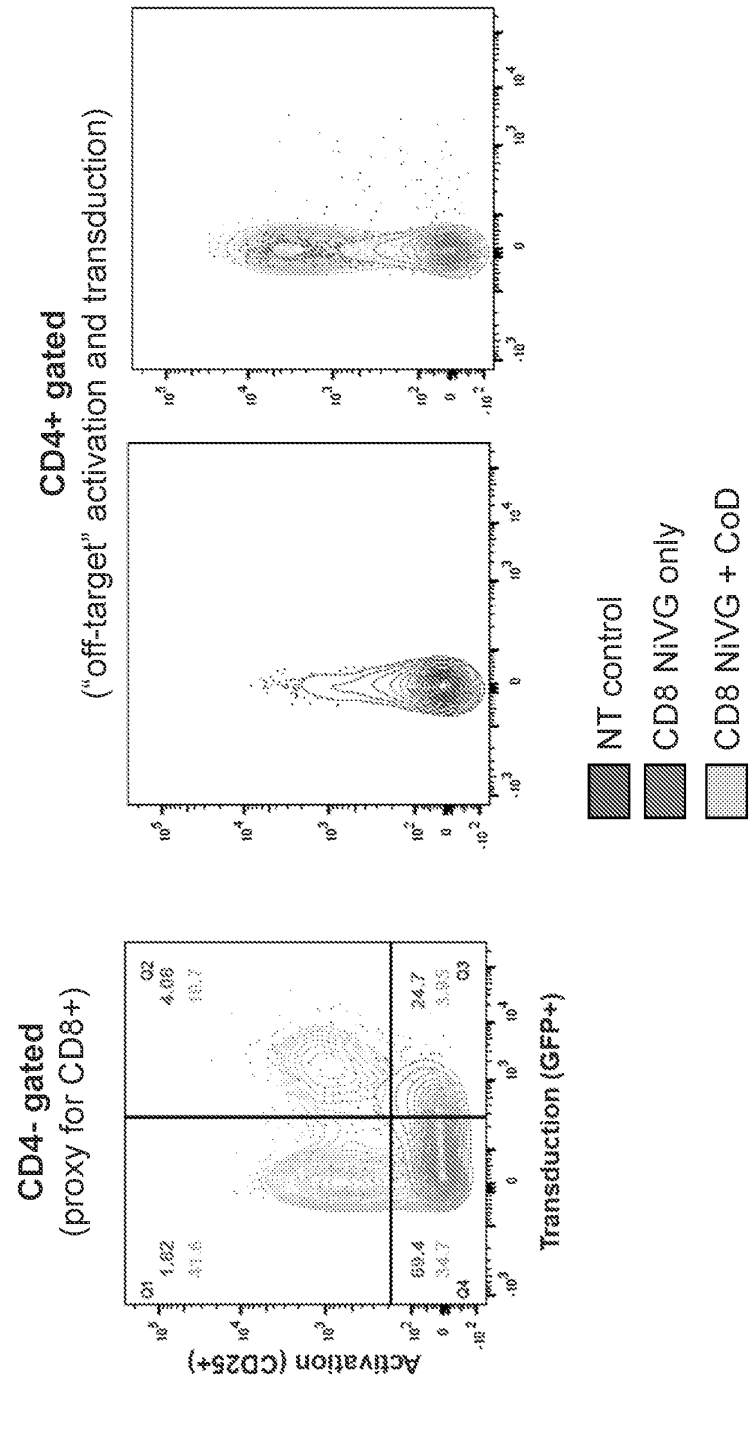
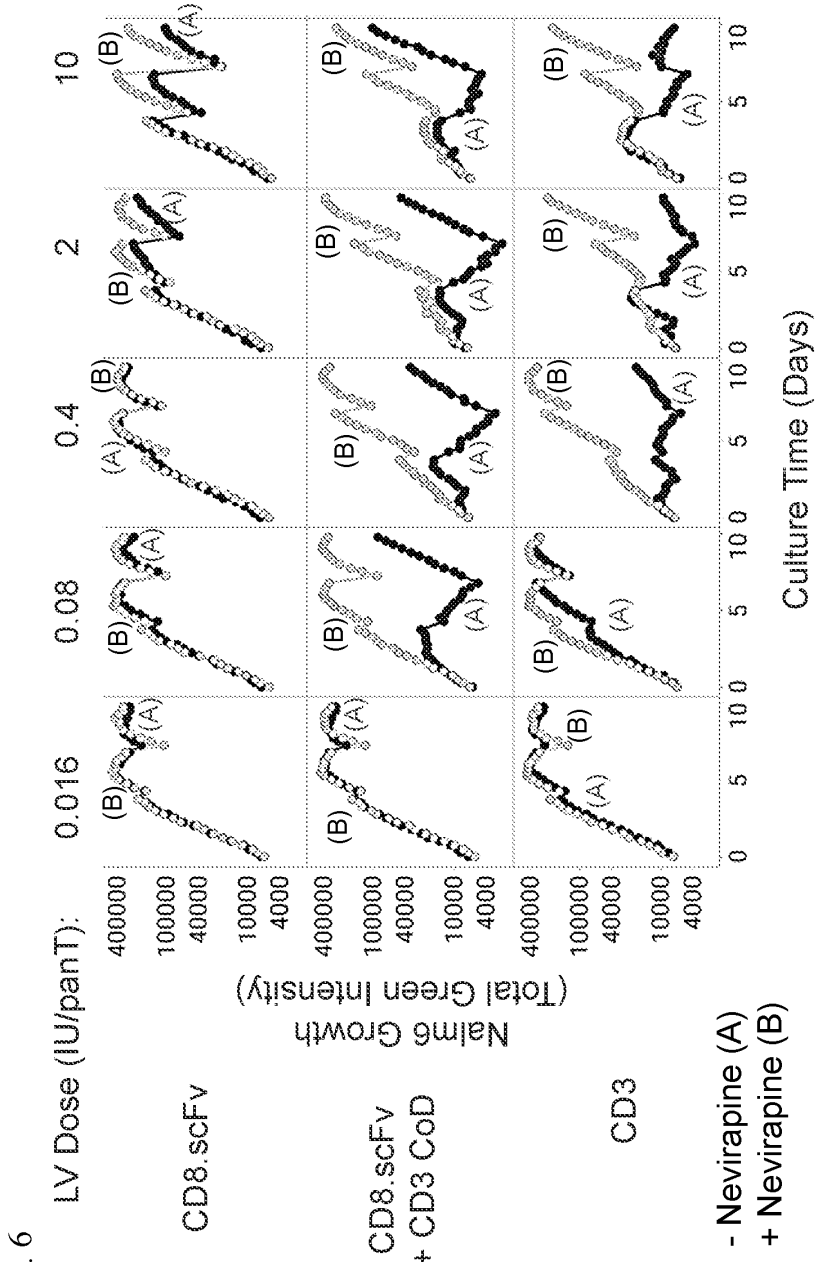

CD4- gated
(proxy for CD8+)
CD4+ gated
("off-target" activation and transduction)
Activation (CD25+)
Transduction (GFP+)
Q1
1.62
Q2
4.86
Q3
24.7
Q4
69.4
NT control
CD8 NiVG only
CD8 NiVG + CoD

FIG. 6


LV Dose (IU/panT):
0.016
0.08
0.4
2
10
CD8.scFv
CD8.scFv
+ CD3 CoD
CD3
Nalm6 Growth
(Total Green Intensity)
400000
100000
40000
10000
4000
0
5
10 0
5
10 0
5
10 0
5
10
Culture Time (Days)
- Nevirapine (A)
+ Nevirapine (B)

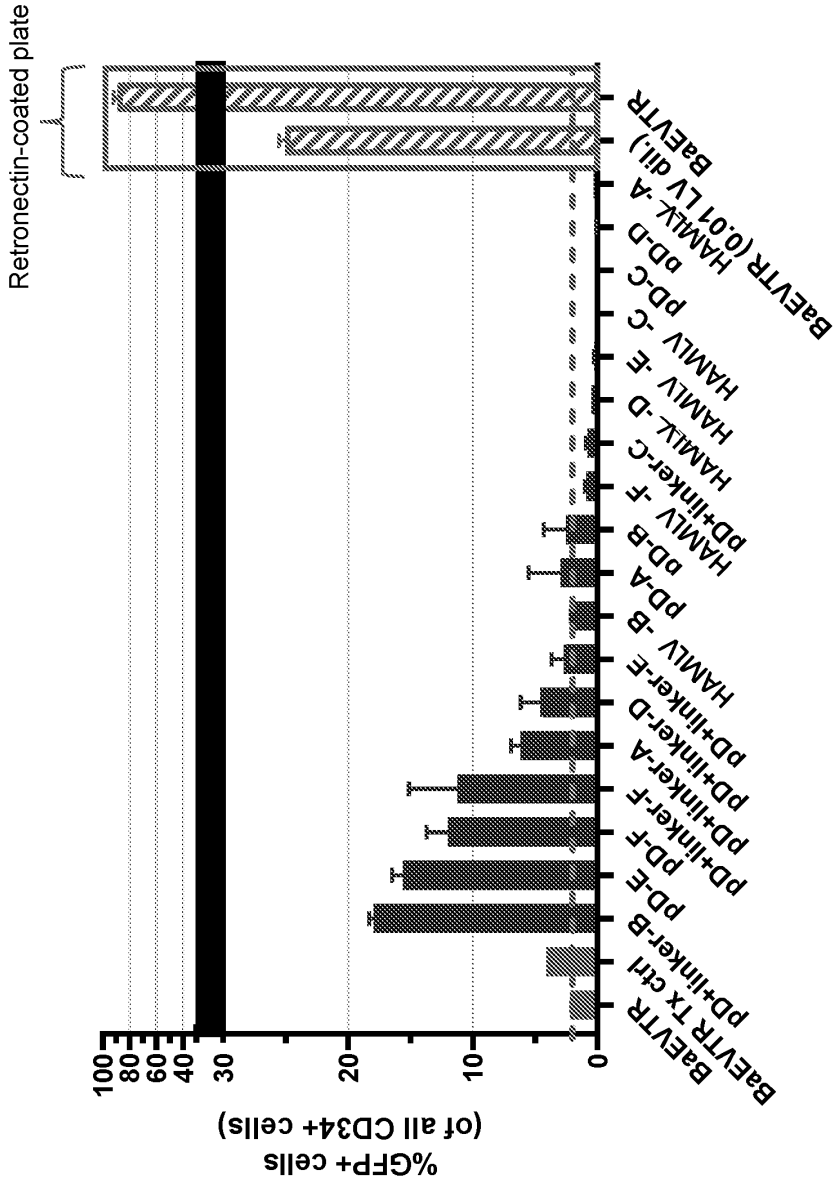

Retronectin-coated plate
%GFP+ cells
(of all CD34+ cells)
100
80
60
40
30
20
10
0
BaEVTR
BaEVTR Tx ctrl
PD+linker-B
PD-E
PD-F
PD+linker-F
PD+linker-A
PD+linker-D
HAMLV -E
PD-A
PD-B
HAMLV -F
PD+linker-C
HAMLV -D
HAMLV -E
HAMLV -C
PD-C
PD-D
HAMLV -A
BaEVTR (0.01 LV dil.)
BaEVTR

FIG. 7A

FIG. 7B

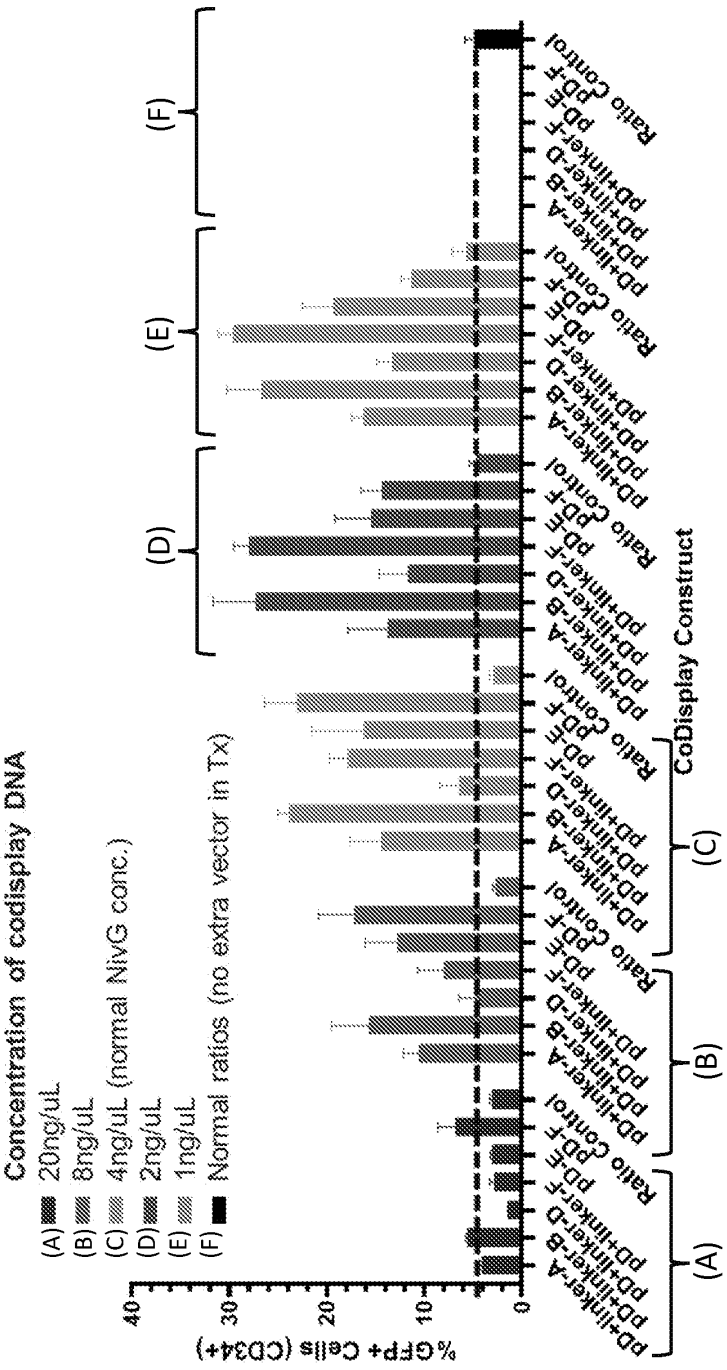

Concentration of codisplay DNA
(A) 20ng/uL
(B) 8ng/uL
(C) 4ng/uL (normal NivG conc.)
(D) 2ng/uL
(E) 1ng/uL
(F) Normal ratios (no extra vector in Tx)
%GFP+ Cells (CD34+)
0
10
20
30
40
pD+linker-A
pD+linker-B
pD+linker-D
pD+linker-F
pD-F
Ratio Control
(A)
(B)
(C)
(D)
(E)
(F)
CoDisplay Construct

FIG. 7D

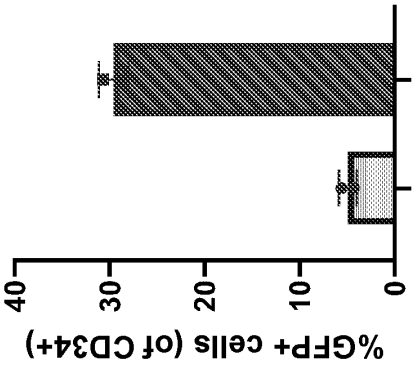
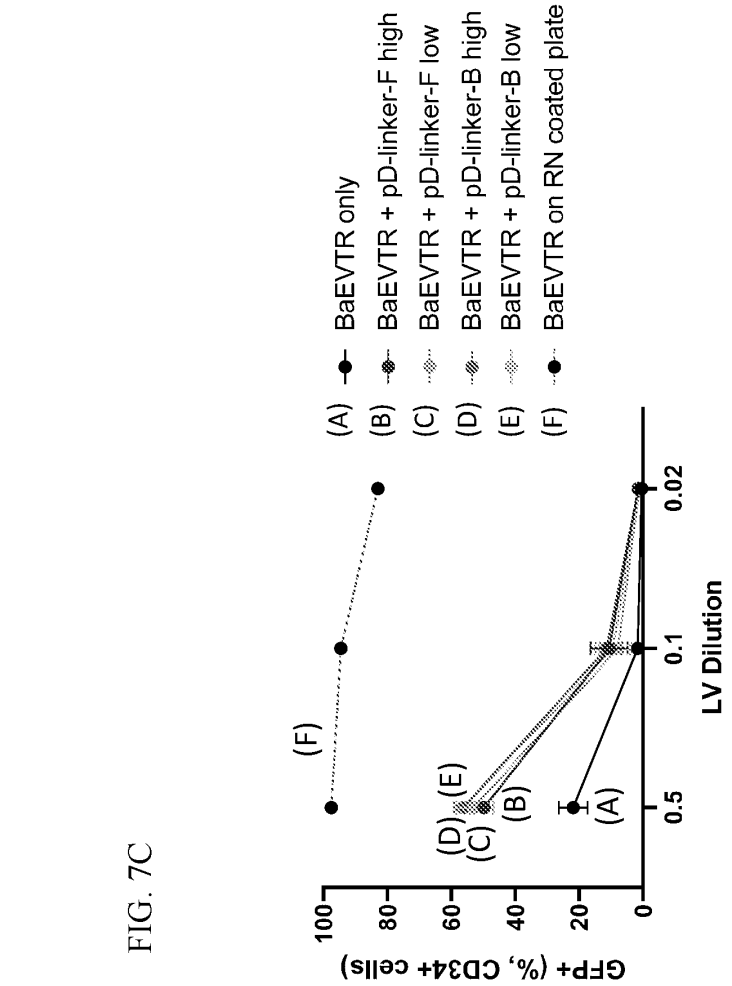

%GFP+ cells (of CD34+)
40
30
20
10
0

FIG. 7C


GFP+ (%, CD34+ cells)
100
80
60
40
20
0
0.5
0.1
0.02
LV Dilution
(A)
(B)
(C)
(D)
(E)
(F)
(A) BaEVTR only
(B) BaEVTR + pD-linker-F high
(C) BaEVTR + pD-linker-F low
(D) BaEVTR + pD-linker-B high
(E) BaEVTR + pD-linker-B low
(F) BaEVTR on RN coated plate

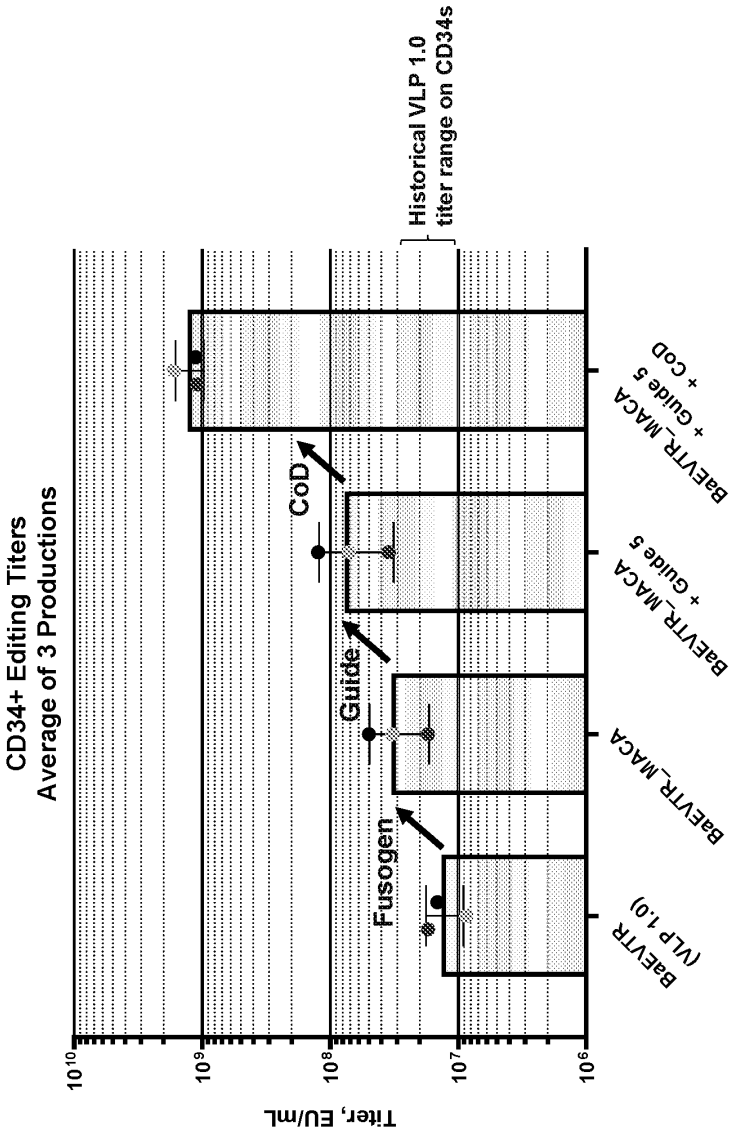

CD34+ Editing Titers
Average of 3 Productions
Historical VLP 1.0
titer range on CD34s
Titer, EU/mL
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
Fusogen
Guide
CoD
BaEVTR (VLP 1.0)
BaEVTR_MACA
BaEVTR_MACA + Guide 5
BaEVTR_MACA + Guide 5 + CoD

FIG. 7E

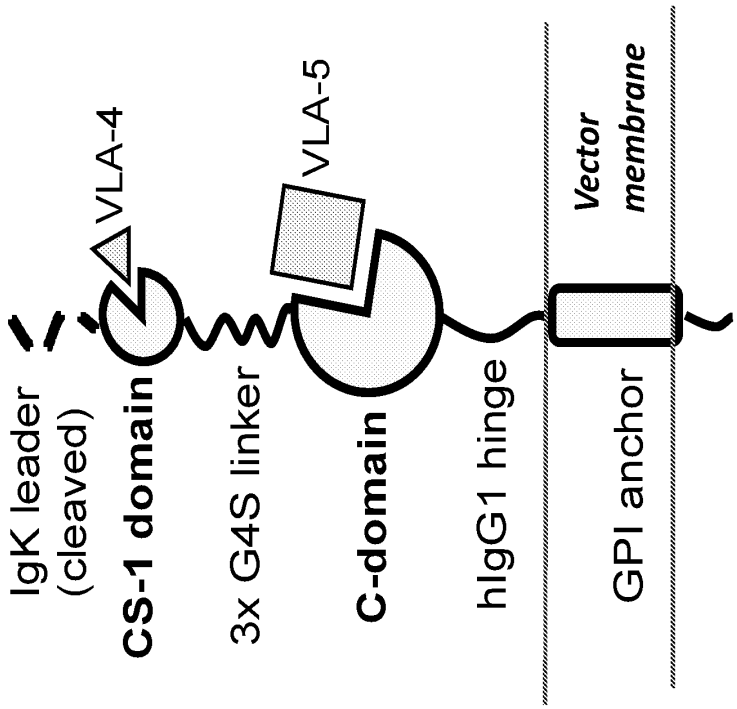

IgK leader
(cleaved)
CS-1 domain
VLA-4
3x G4S linker
VLA-5
C-domain
hIgG1 hinge
Vector
membrane
GPI anchor

FIG. 7F

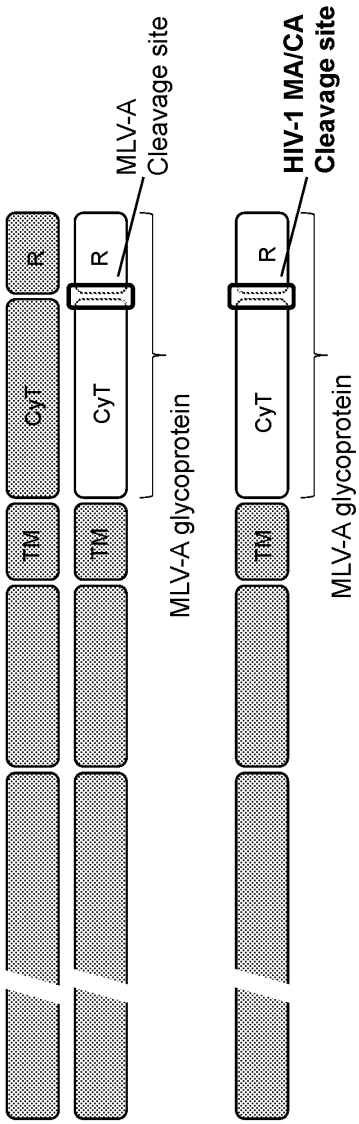

BaEV wt
BaEVTR
BaEVTR MACA
R
CyT
TM
MLV-A Cleavage site
HIV-1 MA/CA Cleavage site
MLV-A glycoprotein

FIG. 8A

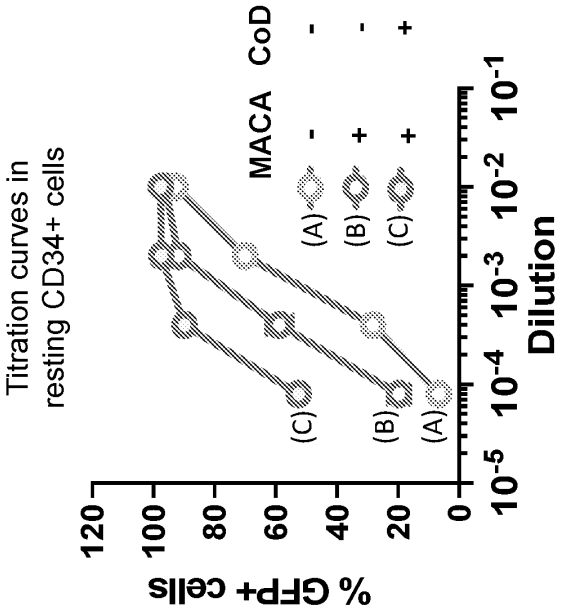

Titration curves in
resting CD34+ cells
% GFP+ cells
120
100
80
60
40
20
0
10-5
10-4
10-3
10-2
10-1
Dilution
MACA CoD
(A) -
(B) +
(C) +
-
+
(A)
(B)
(C)

FIG. 8C

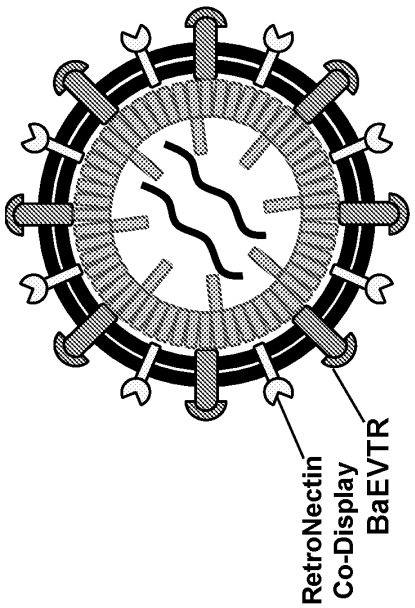

RetroNectin
Co-Display
BaEVTR

FIG. 8B

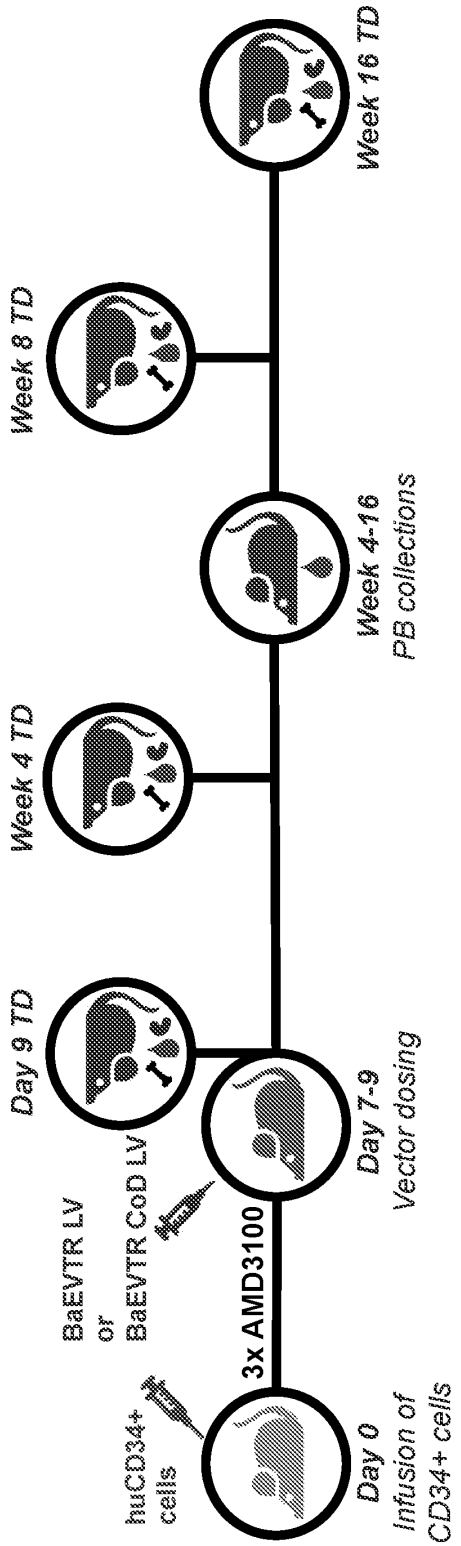
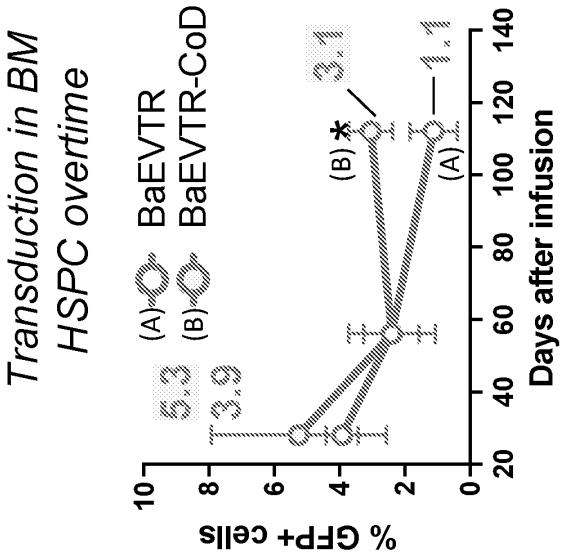

Day 0
Infusion of CD34+ cells
huCD34+ cells
3x AMD3100
Day 7-9
Vector dosing
BaEVTR LV
or
BaEVTR CoD LV
Day 9 TD
Week 4 TD
Week 4-16
PB collections
Week 8 TD
Week 16 TD

FIG. 8D


Transduction in BM
HSPC overtime
(A) BaEVTR
(B) BaEVTR-CoD
6.3
3.9
3.1
1.1
(B)
(A)
% GFP+ cells
Days after infusion
0
2
4
6
8
10
20
40
60
80
100
120
140

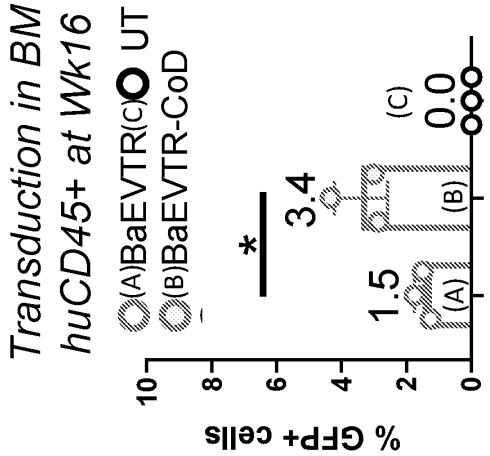

Transduction in BM
huCD45+ at Wk16
(A)BaEVTR
(B)BaEVTR-CoD
(C) UT
*
3.4
1.5
2.1
0.0
(A)
(B)
(C)
% GFP+ cells
0
2
4
6
8
10

FIG. 8E

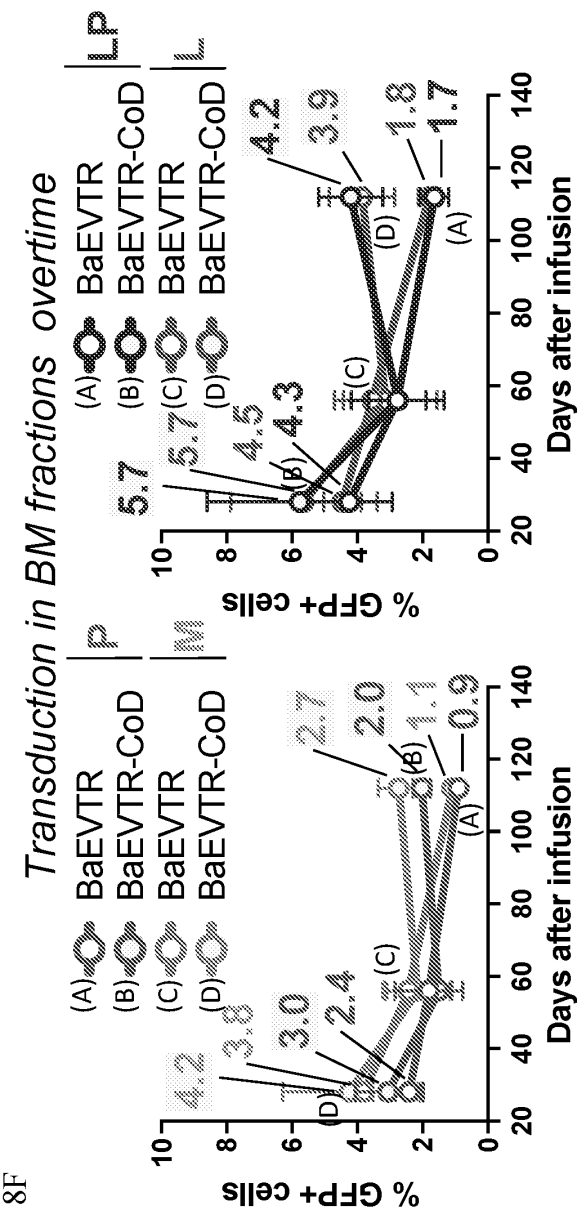

Transduction in BM fractions overtime
(A) BaEVTR
(B) BaEVTR-CoD
(C) BaEVTR
(D) BaEVTR-CoD
P
M
LP
L
% GFP+ cells
Days after infusion
0
2
4
6
8
10
20
40
60
80
100
120
140
4.2
3.8
3.0
2.4
2.7
2.0
1.1
0.9
5.7
5.7
4.5
4.3
4.2
3.9
1.8
1.7

FIG. 8F

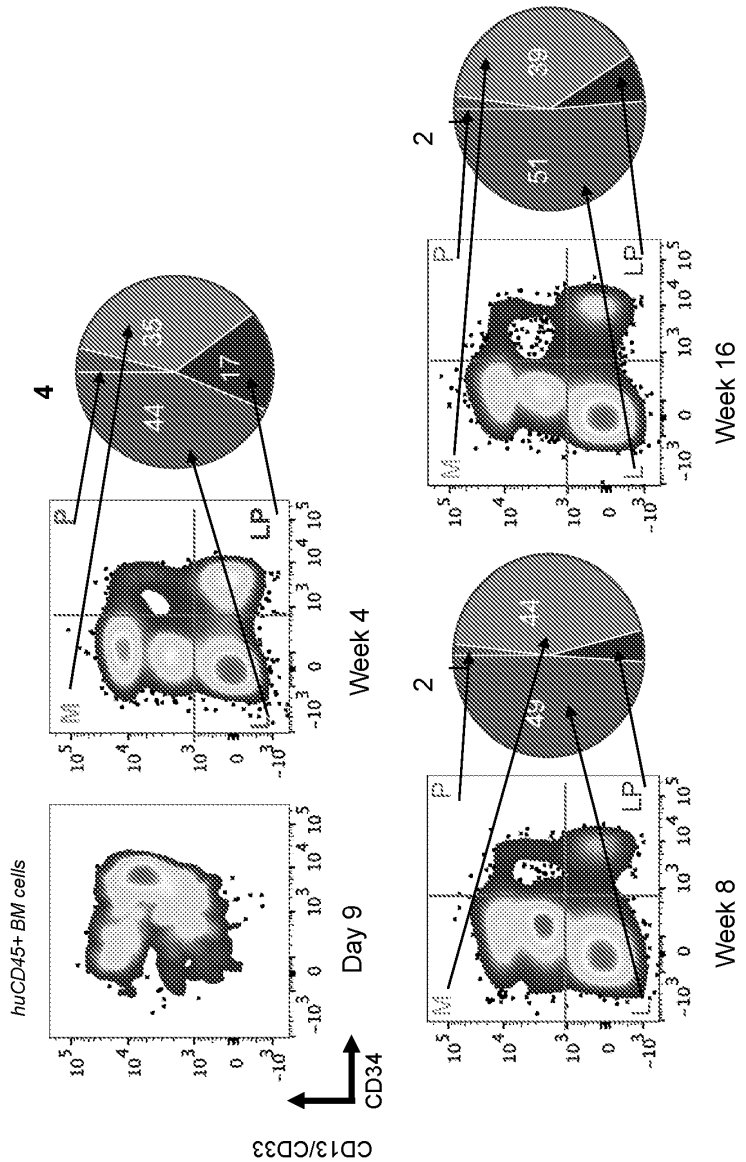

huCD45+ BM cells
CD13/CD33
CD34
Day 9
Week 4
Week 8
Week 16
4
2
2
44
35
17
49
44
51
39
M
P
LP
L

FIG. 8G

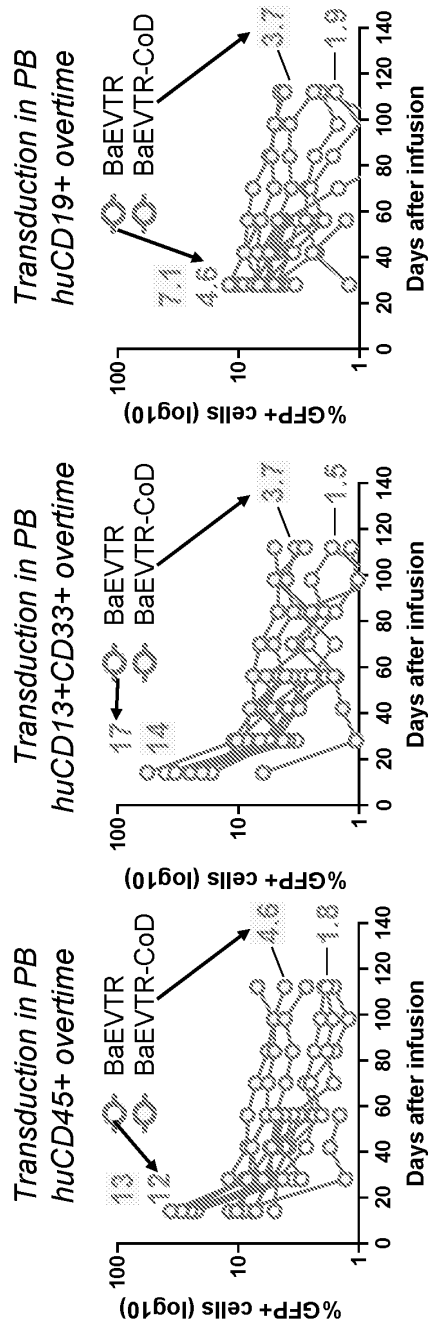

Transduction in PB
huCD45+ overtime
BaEVTR
BaEVTR-CoD
13
12
4.6
1.8
%GFP+ cells (log10)
Days after infusion
Transduction in PB
huCD13+CD33+ overtime
17
14
3.7
1.6
Transduction in PB
huCD19+ overtime
7.1
4.6
3.7
1.9

FIG. 8H

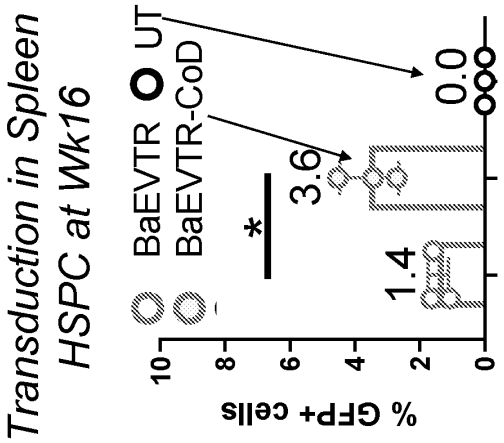

Transduction in Spleen
HSPC at Wk16
BaEVTR
BaEVTR-CoD
UT
% GFP+ cells
10
8
6
4
2
0
3.6
1.4
0.0
*

FIG. 8J

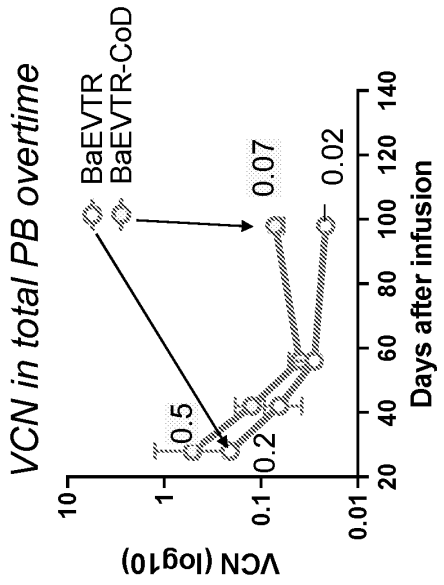

VCN in total PB overtime
BaEVTR
BaEVTR-CoD
VCN (log10)
10
1
0.1
0.01
0.5
0.2
0.07
0.02
20
40
60
80
100
120
140
Days after infusion

FIG. 8I

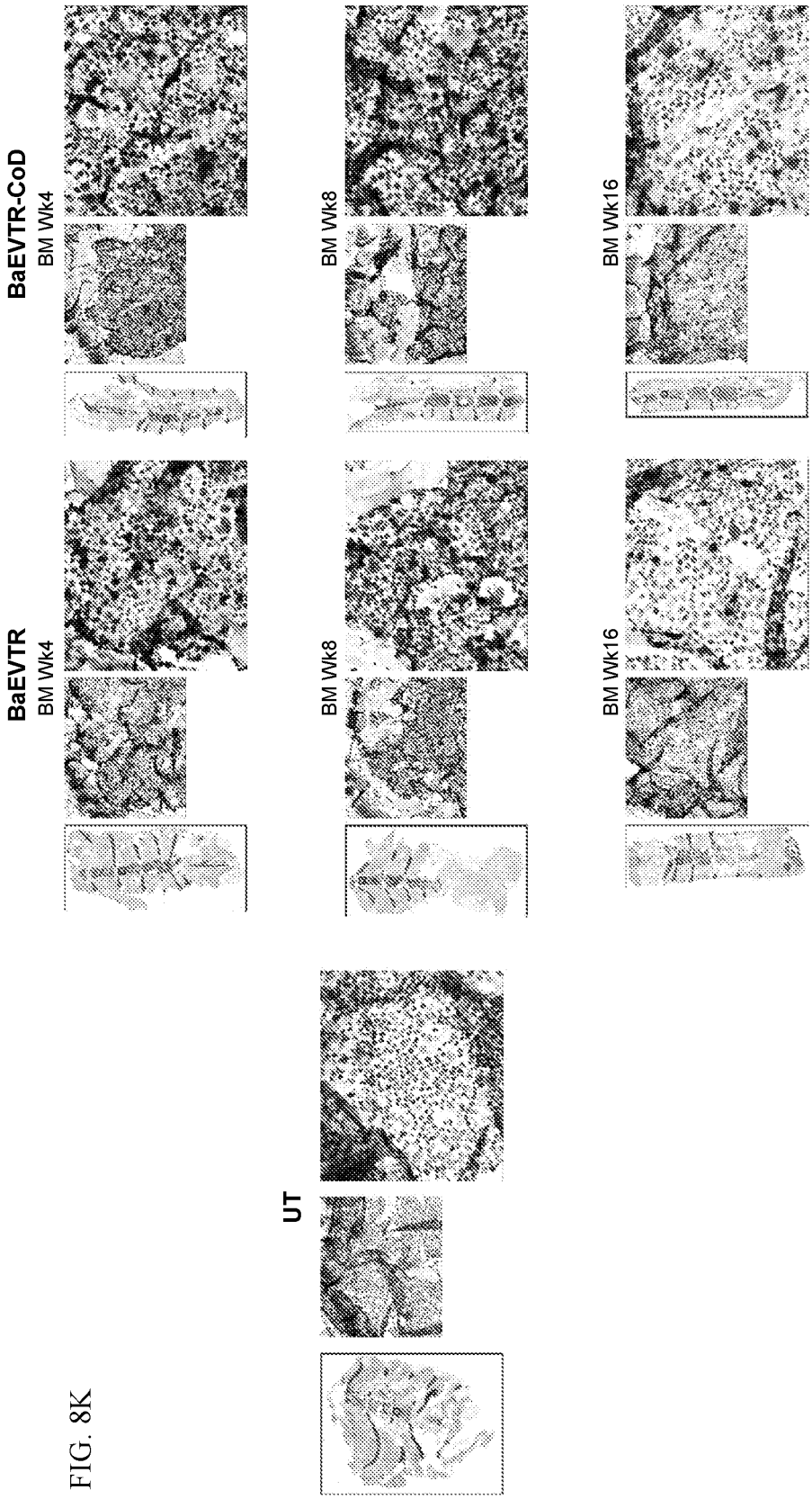


FIG. 8K

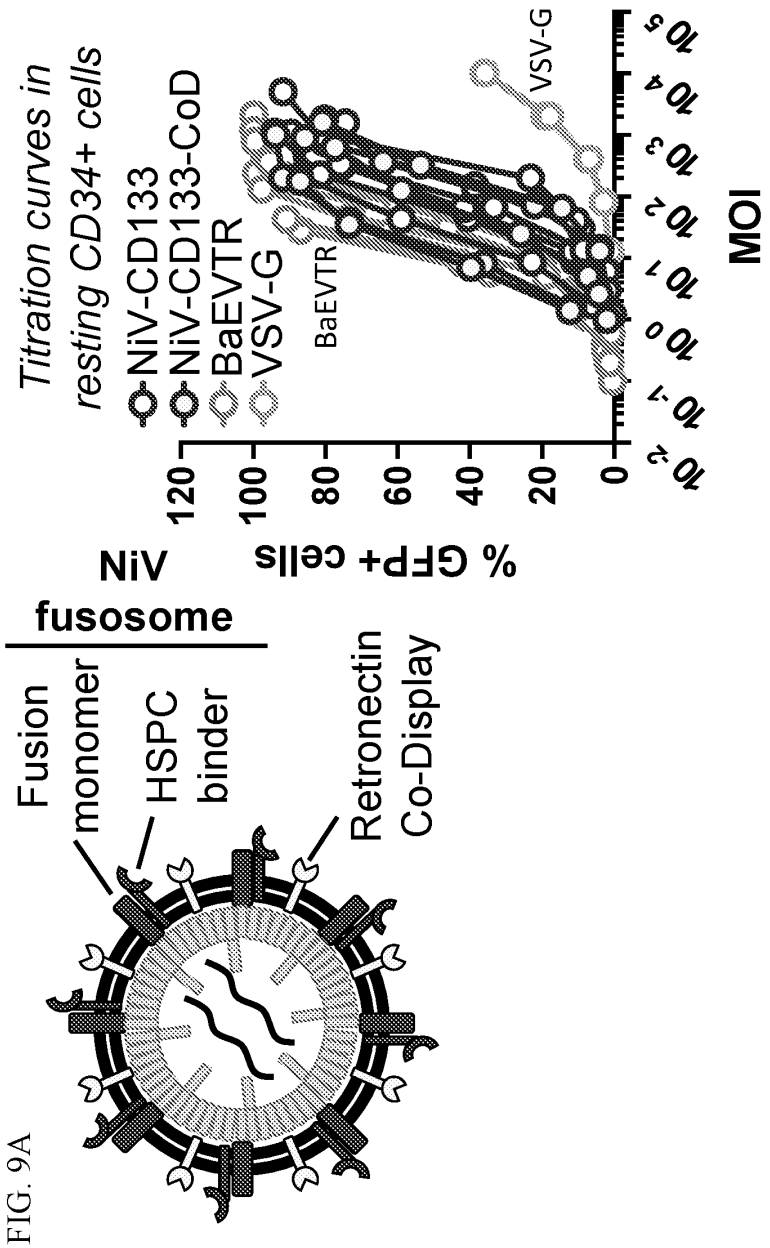

NiV
fusosome
Fusion monomer
HSPC binder
Retronectin Co-Display
% GFP+ cells
120
100
80
60
40
20
0
MOI
10 -2
10 -1
10 0
10 1
10 2
10 3
10 4
10 5
Titration curves in resting CD34+ cells
NiV-CD133
NiV-CD133-CoD
BaEVTR
VSV-G
BaEVTR
VSV-G

FIG. 9A

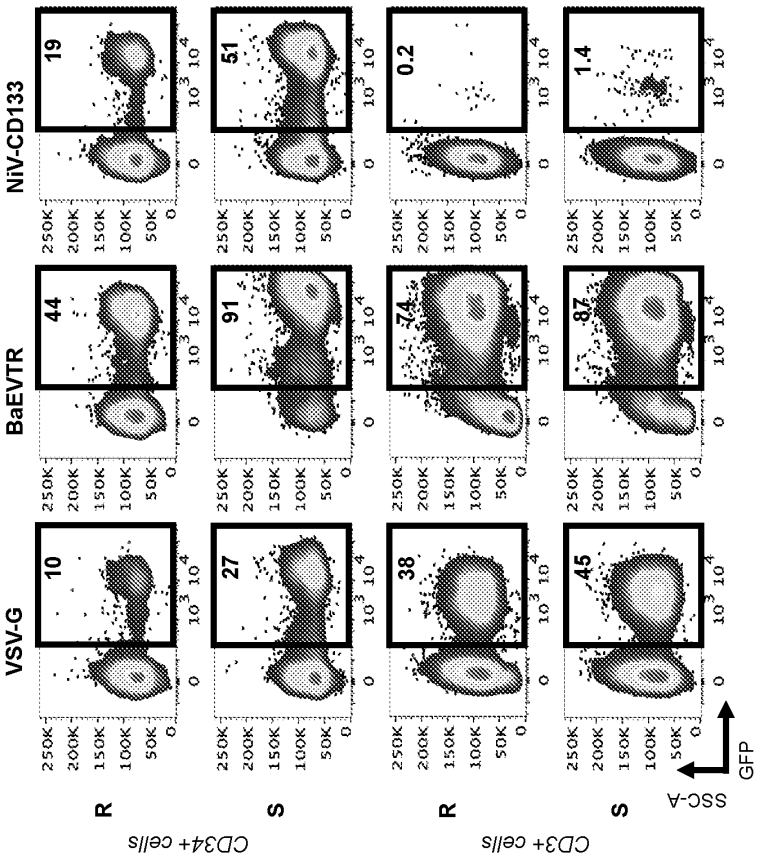

VSV-G
BaEVTR
NiV-CD133
10
27
44
91
19
51
38
45
74
87
0.2
1.4
R
S
CD34+ cells
CD3+ cells
SSC-A
GFP

FIG. 9B

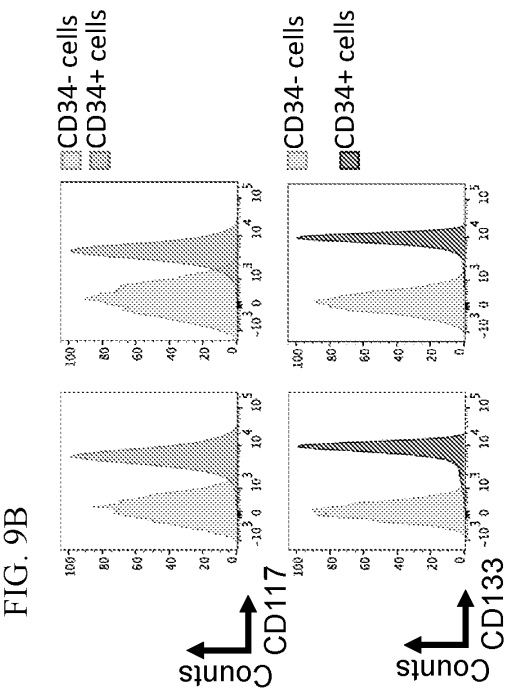
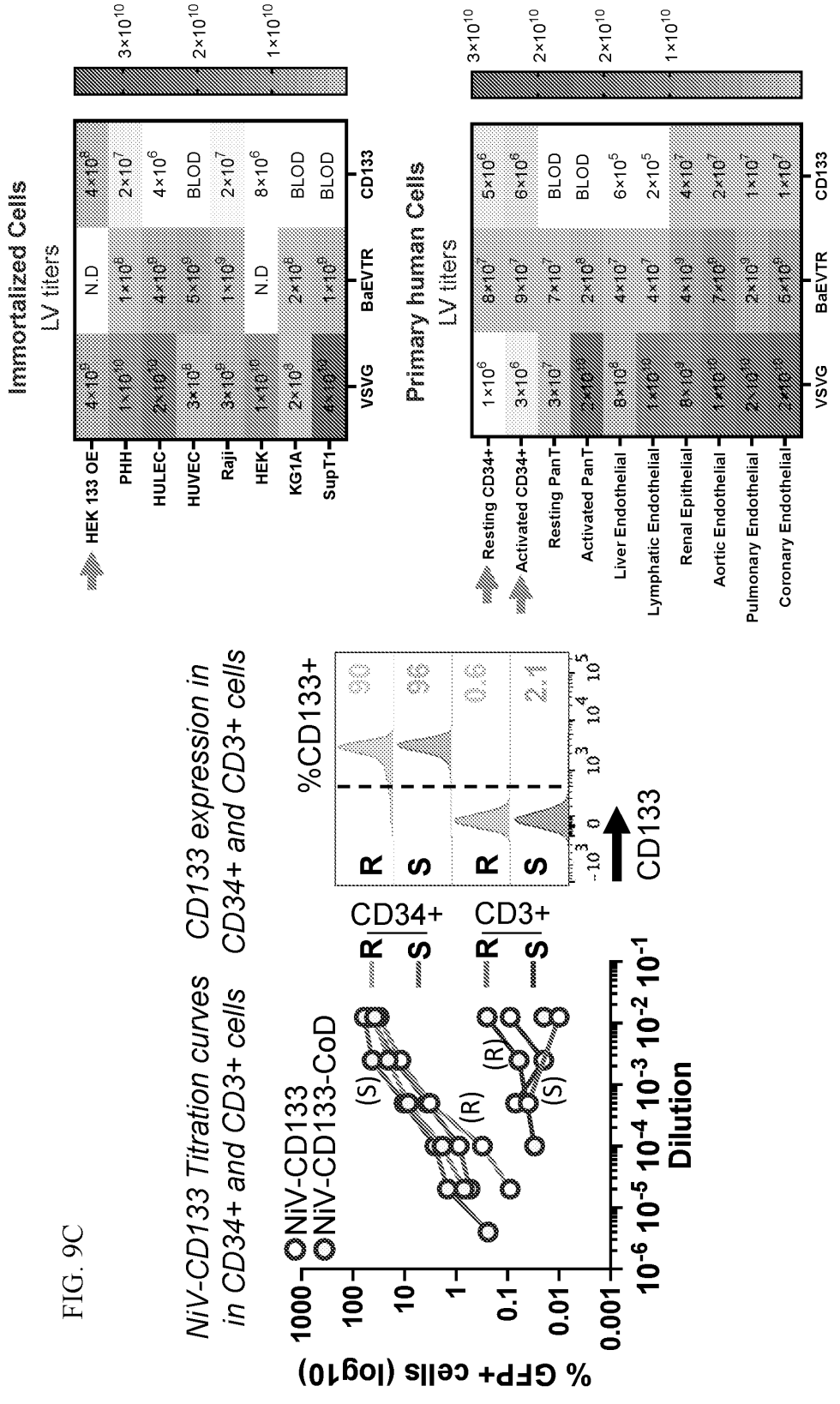
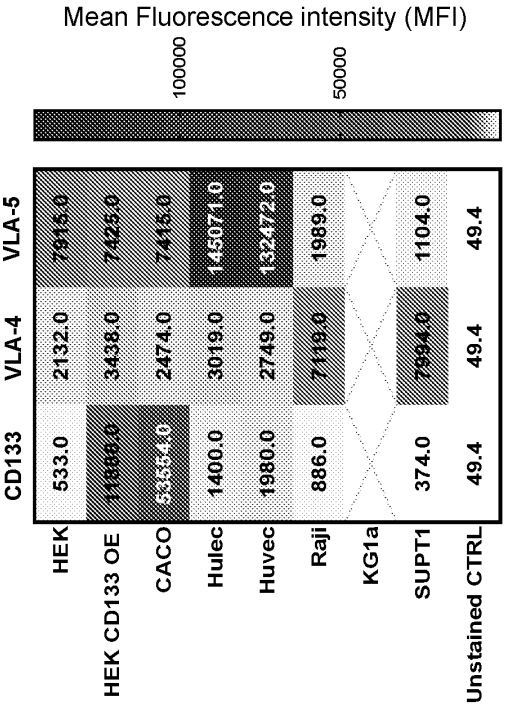

CD34- cells
CD34+ cells
Counts
CD117
CD133

FIG. 9C


Immortalized Cells
LV titers
HEK 133 OE
PHH
HULEC
HUVEC
Raji
HEK
KG1A
SupT1
VSVG
BaEVTR
CD133
N.D
BLOD
Primary human Cells
LV titers
Resting CD34+
Activated CD34+
Resting PanT
Activated PanT
Liver Endothelial
Lymphatic Endothelial
Renal Epithelial
Aortic Endothelial
Pulmonary Endothelial
Coronary Endothelial
NiV-CD133 Titration curves CD133 expression in
in CD34+ and CD3+ cells CD34+ and CD3+ cells
NiV-CD133
NiV-CD133-CoD
% GFP+ cells (log10)
Dilution
CD34+
CD3+
R
S
%CD133+
CD133


Mean Fluorescence intensity (MFI)
100000
50000
CD133
VLA-4
VLA-5
HEK
533.0
2132.0
7815.0
HEK CD133 OE
11666.0
3438.0
7425.0
CACO
53554.0
2474.0
7416.0
Hulec
1400.0
3019.0
115071.0
Huvec
1980.0
2749.0
132472.0
Raji
886.0
7119.0
1989.0
KG1a
SUPT1
374.0
7994.0
1104.0
Unstained CTRL
49.4
49.4
49.4

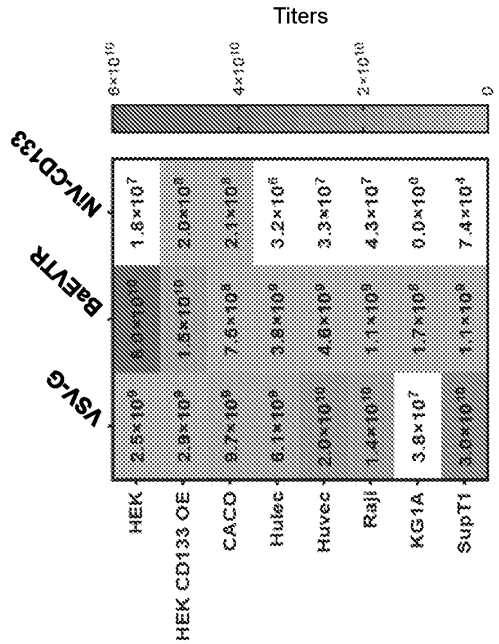

Titers
VSV-G
BaEVTR
NiV-CD133
HEK
HEK CD133 OE
CACO
Hulec
Huvec
Raji
KG1A
SupT1

FIG. 9D

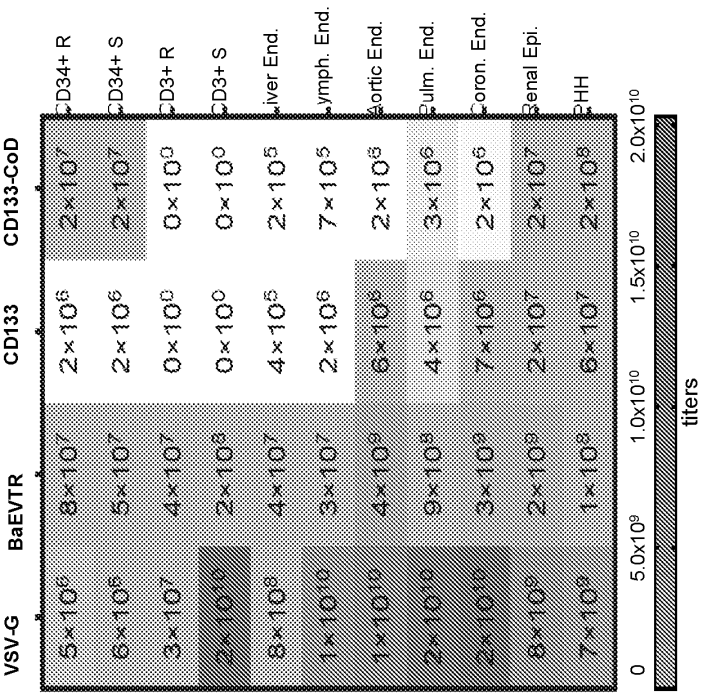

VSV-G
BaEVTR
NiV CD133
NiV CD133-CoD
CD34+ R
CD34+ S
CD3+ R
CD3+ S
Liver End.
Lymph. End.
Aortic End.
Pulm. End.
Coron. End.
Renal Epi.
HHH
0
5.0x10^9
1.0x10^10
1.5x10^10
2.0x10^10
titers

FIG. 9E

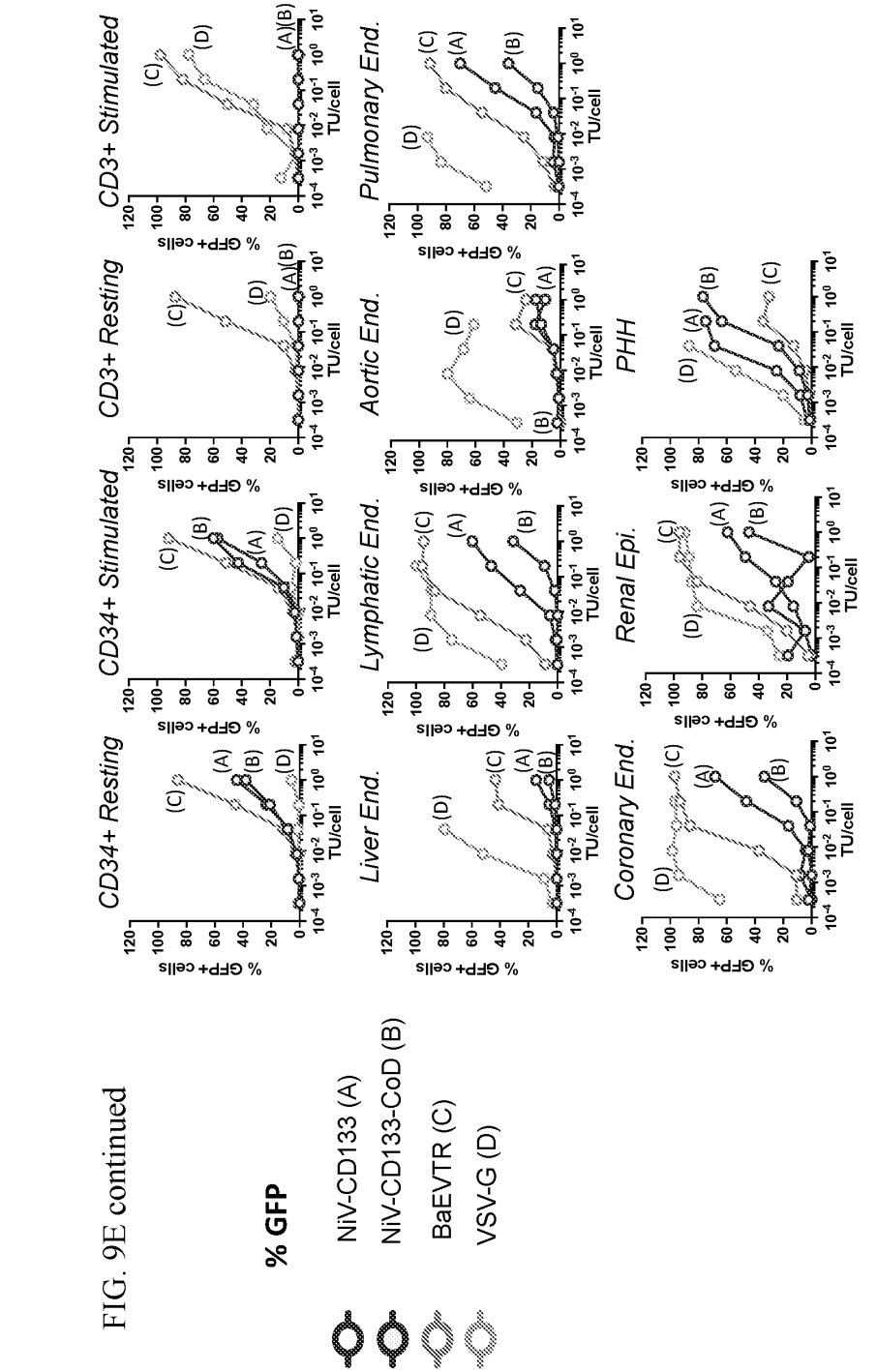


FIG. 9E continued

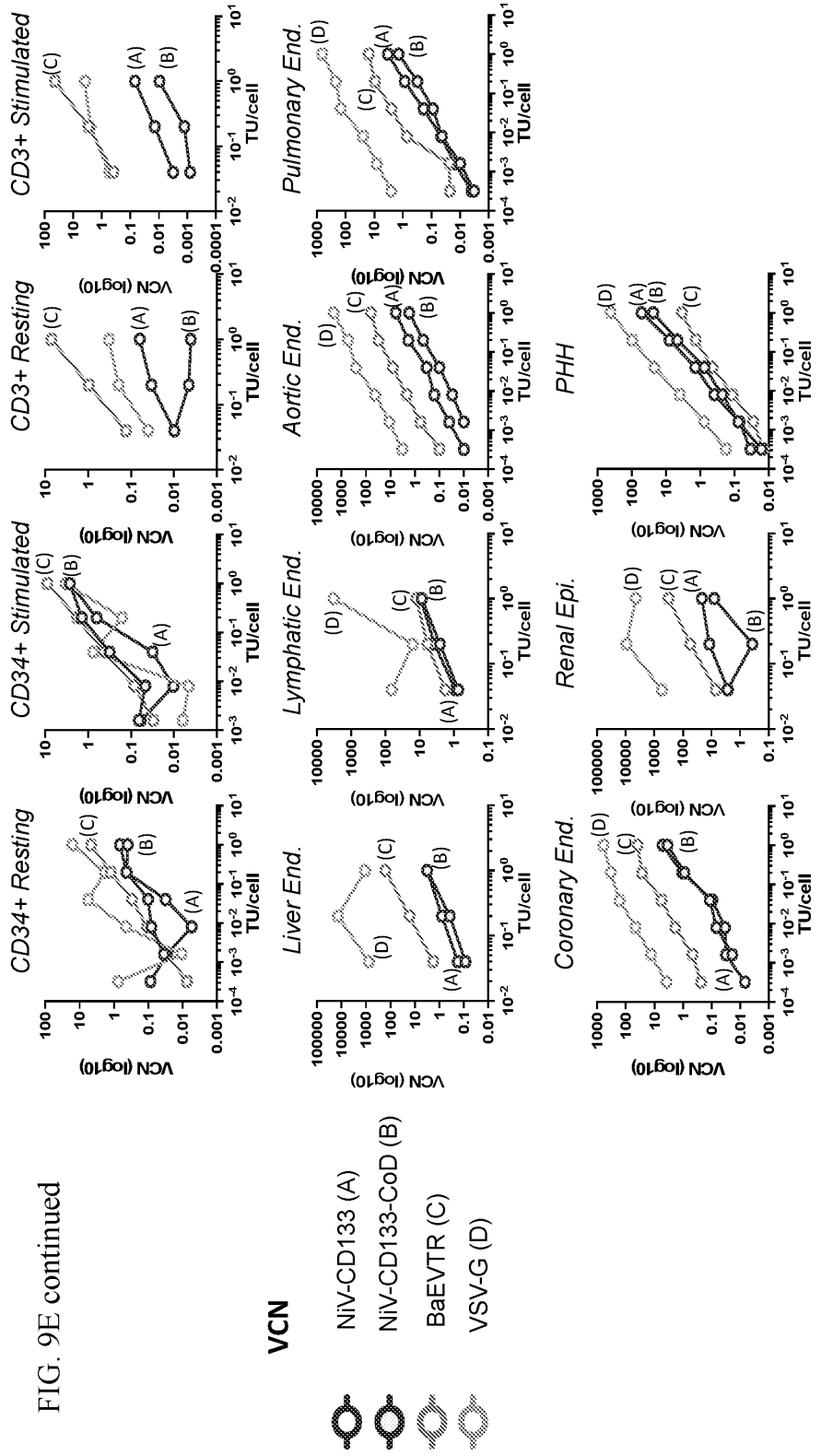


FIG. 9E continued

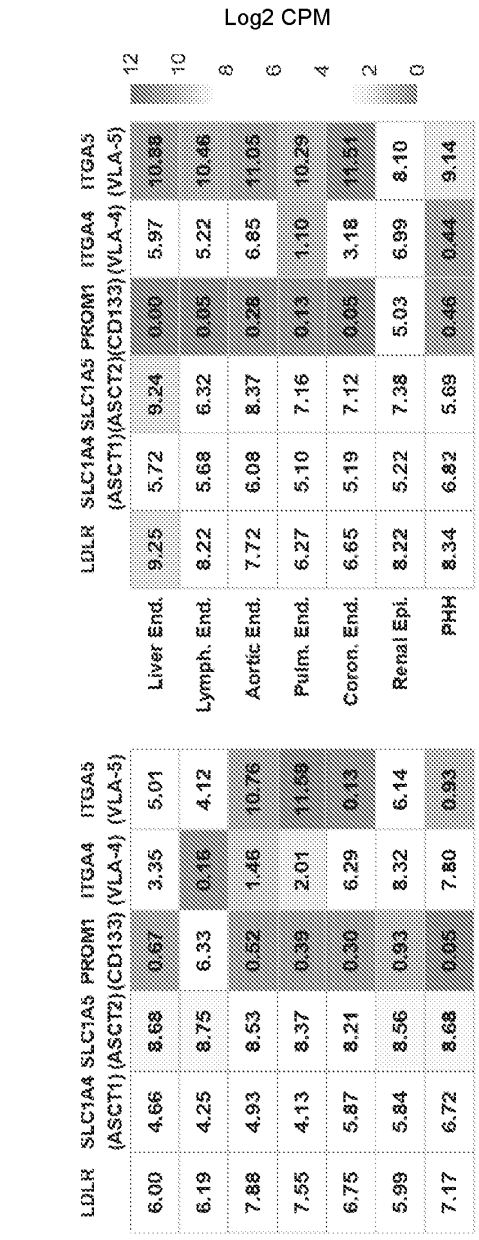

Log2 CPM
12
10
8
6
4
2
0
LDLR
SLC1A4 (ASCT1)
SLC1A5 (ASCT2)
PROM1 (CD133)
ITGA4 (VLA-4)
ITGA5 (VLA-5)
HEK293LX 6.00 4.66 8.68 0.67 3.35 5.01
Caco2 6.19 4.25 8.75 6.33 0.16 4.12
Hulec 7.88 4.93 8.53 0.52 1.46 10.76
Huvec 7.55 4.13 8.37 0.39 2.01 11.58
Raji 6.75 5.87 8.21 0.30 6.29 0.13
KG1A 5.99 5.84 8.56 0.93 8.32 6.14
SupT1 7.17 6.72 8.68 0.05 7.80 0.93
Liver End. 9.25 5.72 9.24 0.08 5.97 10.38
Lymph. End. 8.22 5.68 6.32 0.05 5.22 10.46
Aortic End. 7.72 6.08 8.37 0.28 6.85 11.05
Pulm. End. 6.27 5.10 7.16 0.13 1.10 10.29
Coron. End. 6.65 5.19 7.12 0.05 3.18 11.51
Renal Epi. 8.22 5.22 7.38 5.03 6.99 8.10
PHH 8.34 6.82 5.69 0.46 0.44 9.14

FIG. 9F

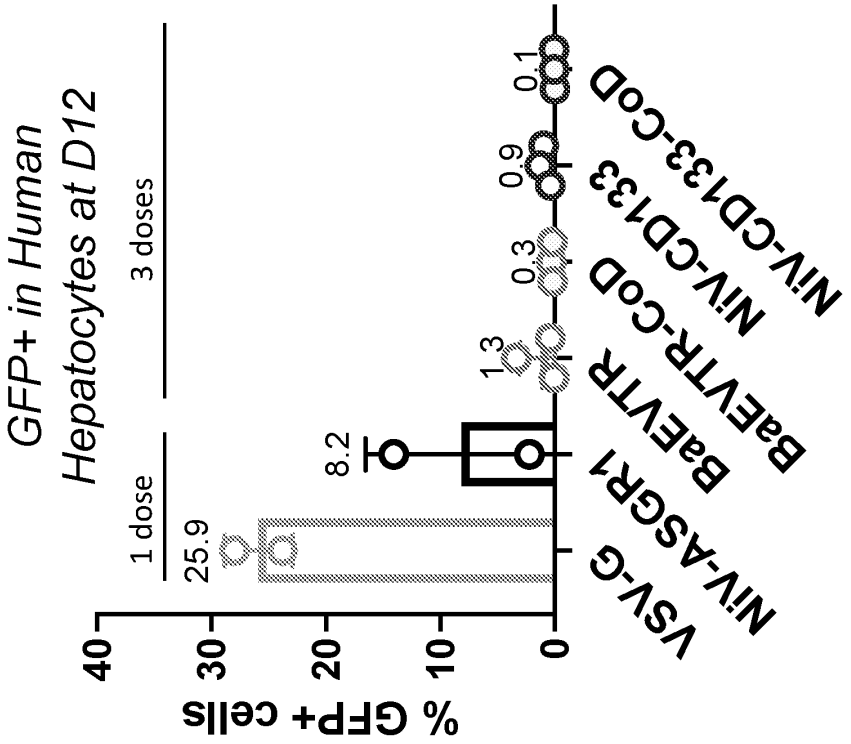

GFP+ in Human Hepatocytes at D12
1 dose
3 doses
% GFP+ cells
40
30
20
10
0
25.9
8.2
1.3
0.3
0.9
0.1
VSV-G
NiV-ASGR1
BaEVTR
BaEVTR-CoD
NiV-CD133
NiV-CD133-CoD

FIG. 9G

FIG. 10B

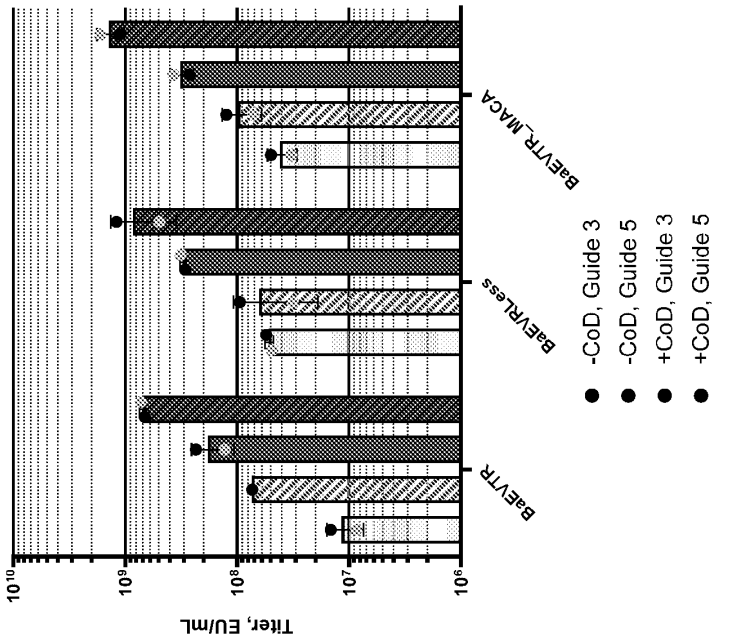
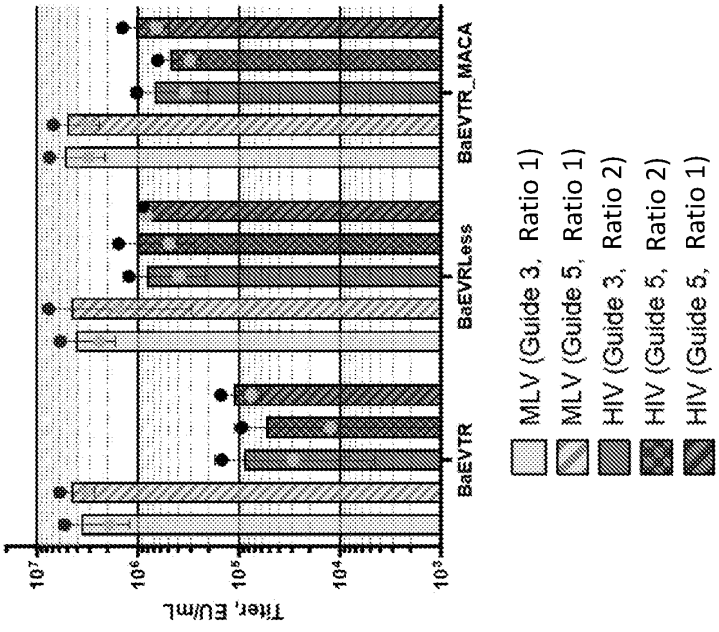


FIG. 10A



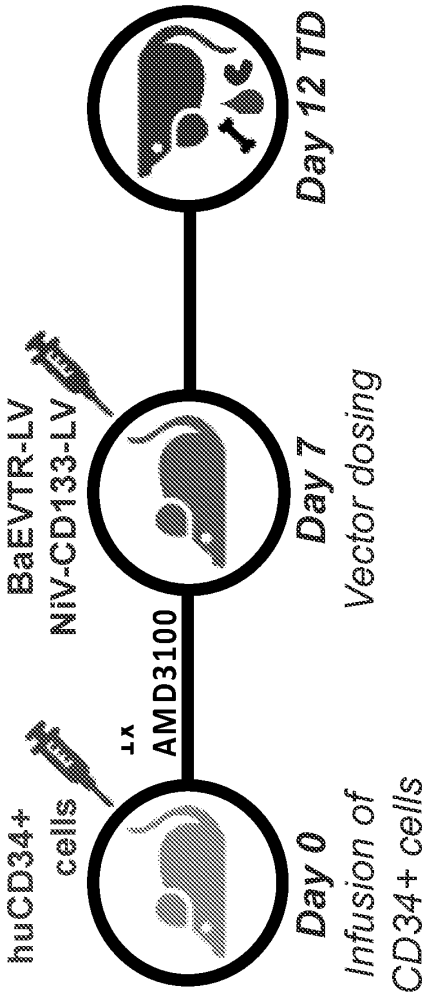

huCD34+
cells
x1
AMD3100
BaEVTR-LV
NiV-CD133-LV
Day 0
Infusion of
CD34+ cells
Day 7
Vector dosing
Day 12 TD

FIG. 11A

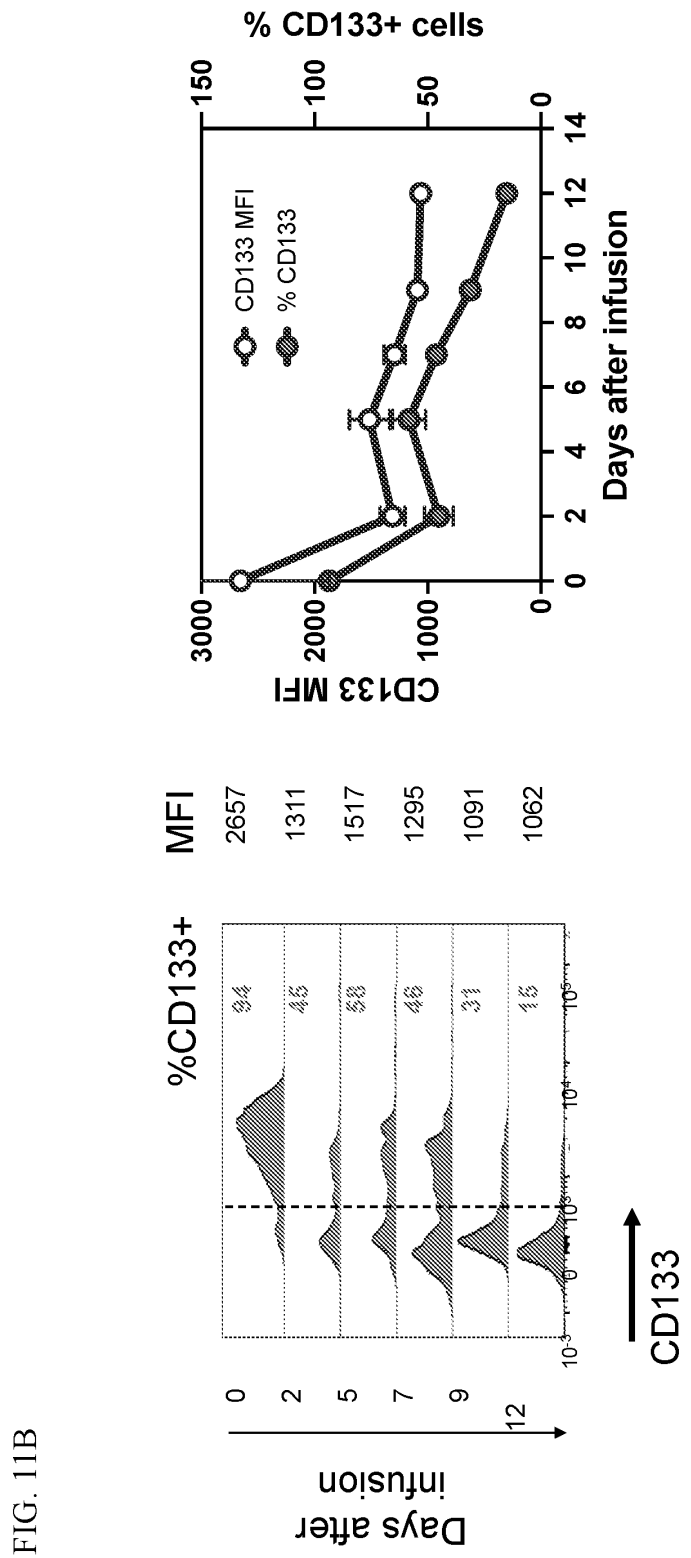

% CD133+ cells
150
100
50
0
CD133 MFI
% CD133
3000
2000
1000
0
0 2 4 6 8 10 12 14
Days after infusion
%CD133+
MFI
94
2657
45
1311
58
1517
46
1295
31
1091
16
1062
0
2
5
7
9
12
Days after infusion
CD133

FIG. 11B

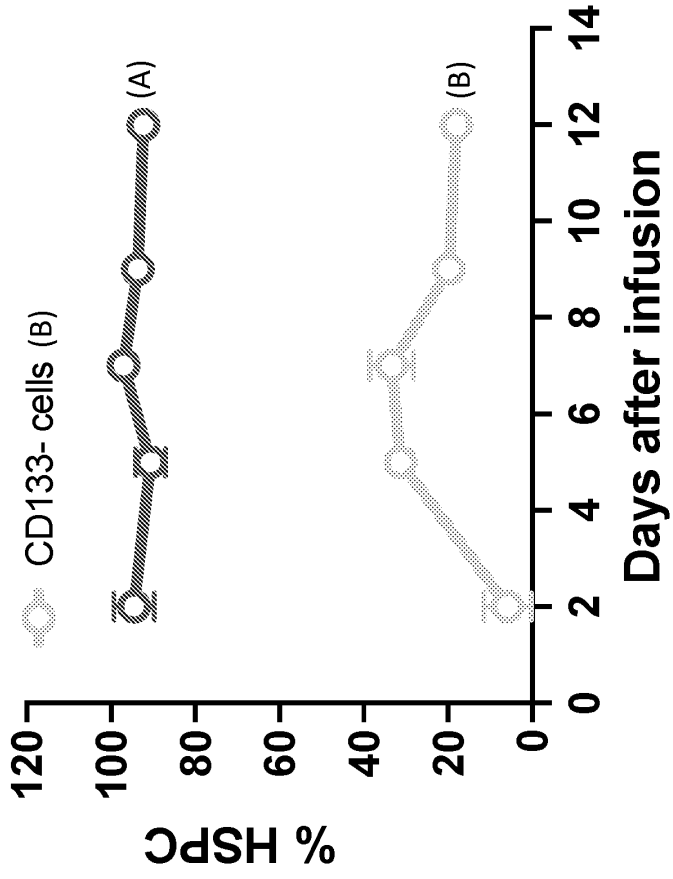

CD133+ cells (A)
CD133- cells (B)
% HSPC
120
100
80
60
40
20
0
0
2
4
6
8
10
12
14
Days after infusion
(A)
(B)

FIG. 11C

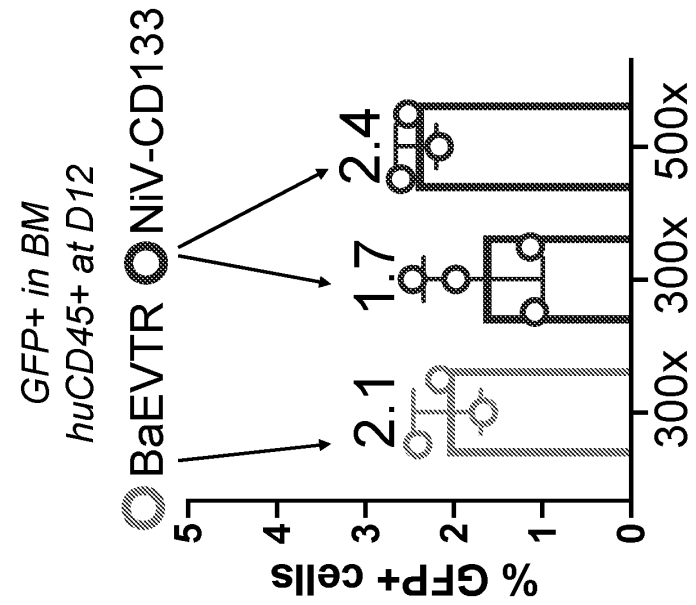

GFP+ in BM
huCD45+ at D12
BaEVTR
NiV-CD133
% GFP+ cells
0
1
2
3
4
5
2.1
1.7
2.4
300x
300x
500x

FIG. 11D

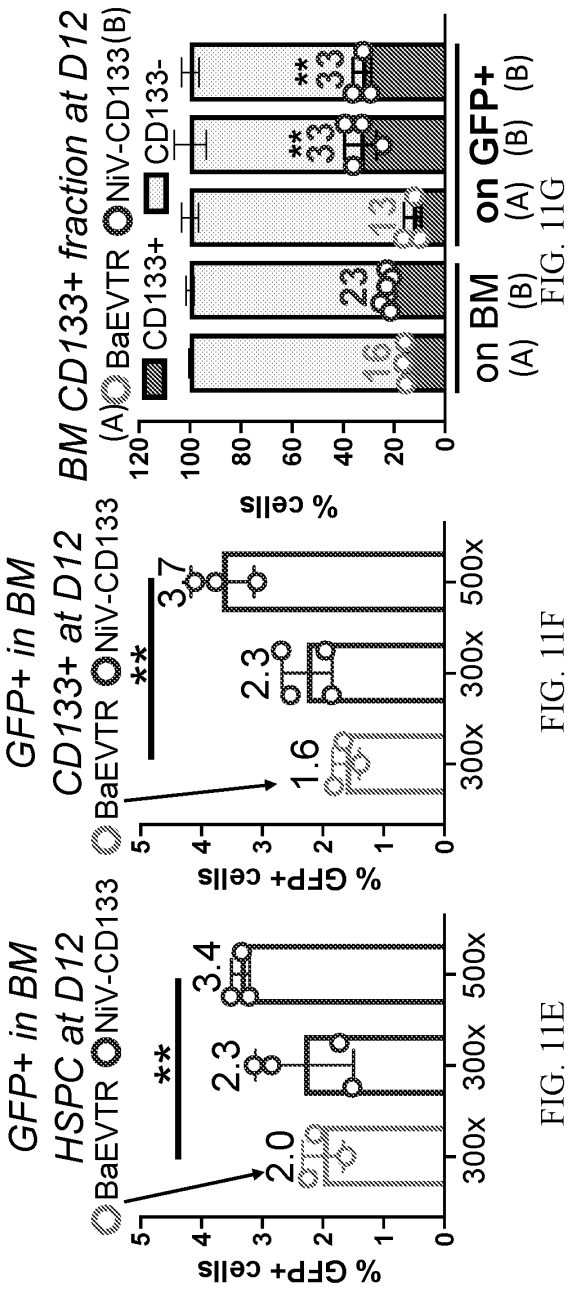


FIG. 11E

FIG. 11F

FIG. 11G

FIG. 11H

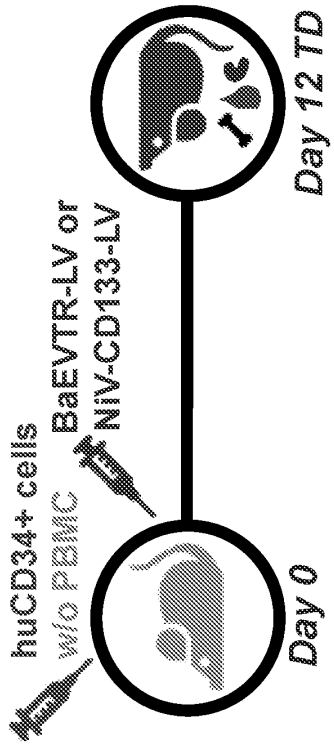
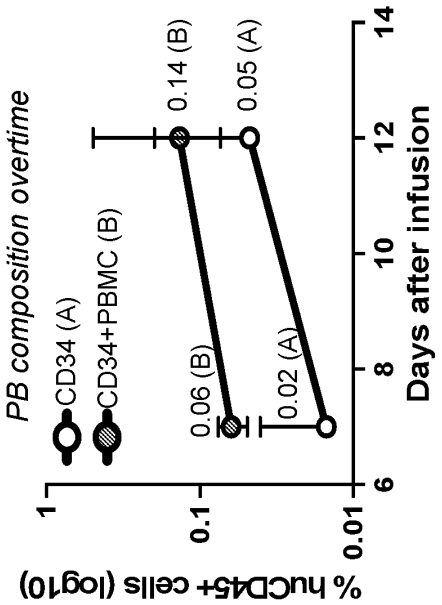


FIG. 11I



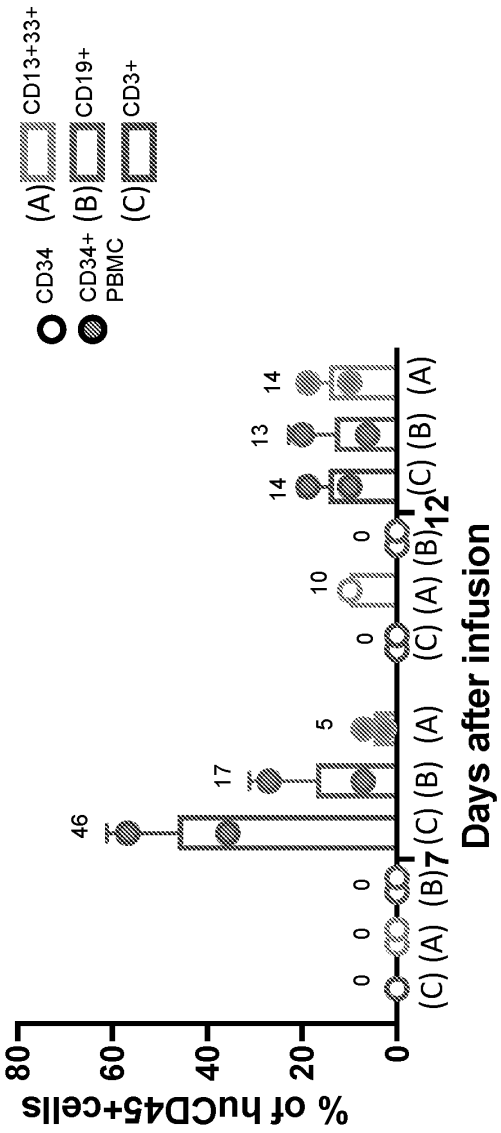

PB huCD45+ composition
% of huCD45+cells
Days after infusion
CD34
CD34+ PBMC
(A) CD13+33+
(B) CD19+
(C) CD3+
0
20
40
60
80
0 0 0
46
17
5
0
10
0
14
13
14
(C) (A) (B) 7 (C) (B) (A) (C) (A) (B) 12 (C) (B) (A)

FIG. 11J

FIG. 11K

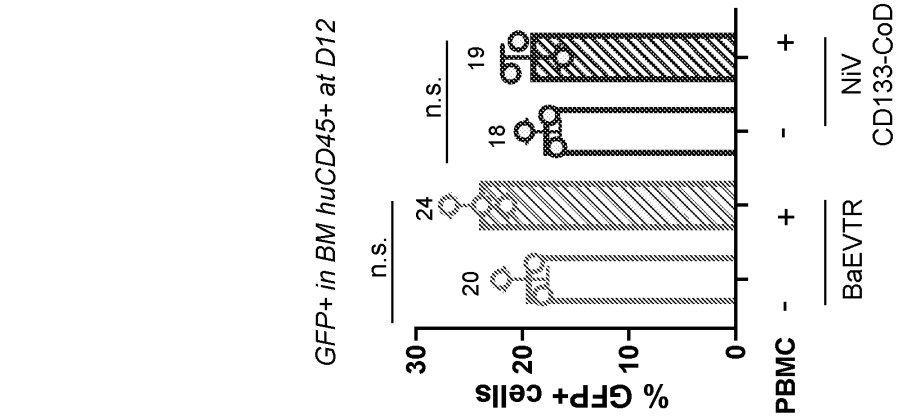
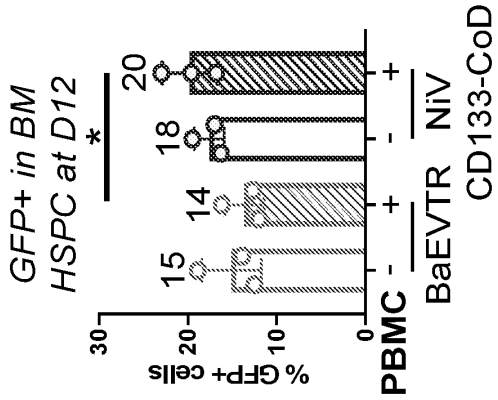


FIG. 11L



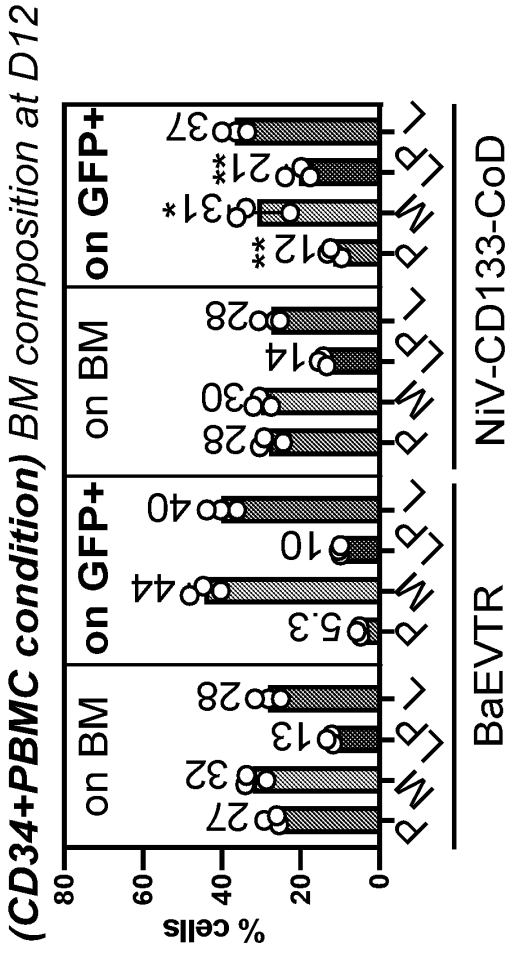

(CD34+PBMC condition) BM composition at D12
% cells
0
20
40
60
80
on BM
on GFP+
on BM
on GFP+
27
32
13
28
5.3
44
10
40
28
30
14
28
12**
31*
21**
37
LT
M
P
LT
M
P
LT
M
P
LT
M
P
BaEVTR
NiV-CD133-CoD

FIG. 11M

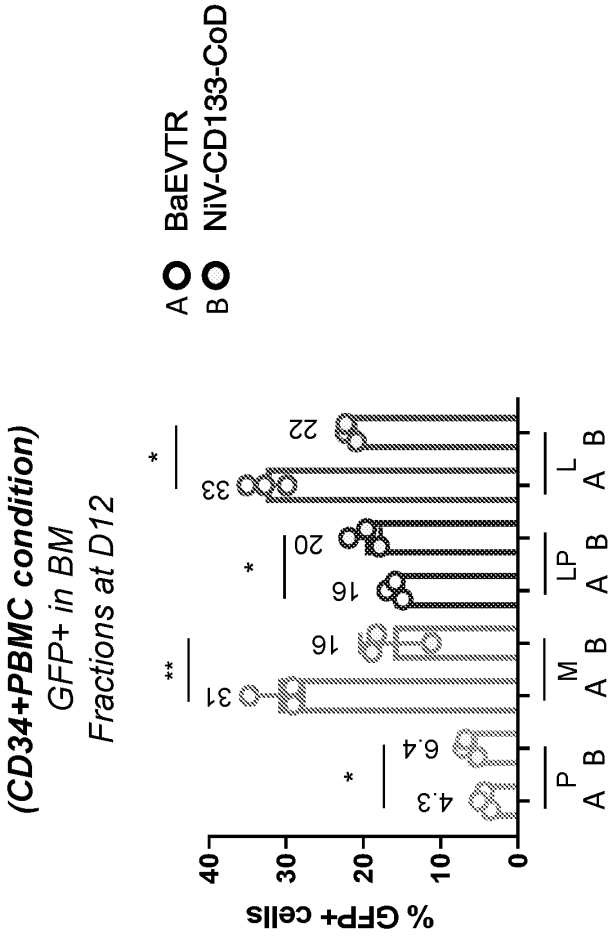

(CD34+PBMC condition)
GFP+ in BM
Fractions at D12
A BaEVTR
B NiV-CD133-CoD
% GFP+ cells
0
10
20
30
40
4.3
6.4
31
16
16
20
33
22
*
**
*
*
P
M
LP
L
A B A B A B A B

FIG. 11N

FIG. 11O

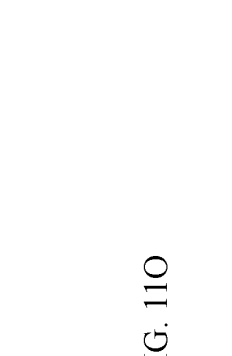


FIG. 11P

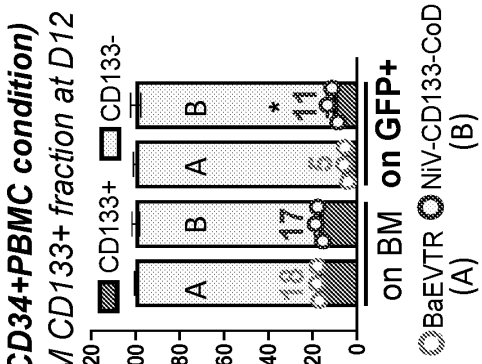


FIG. 11Q

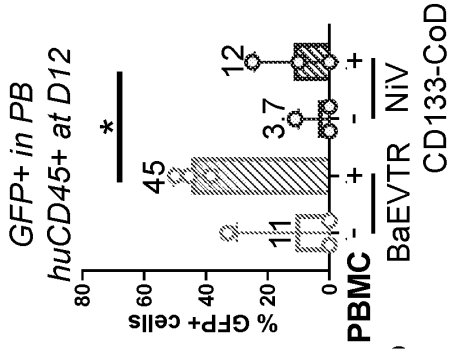


FIG. 11S

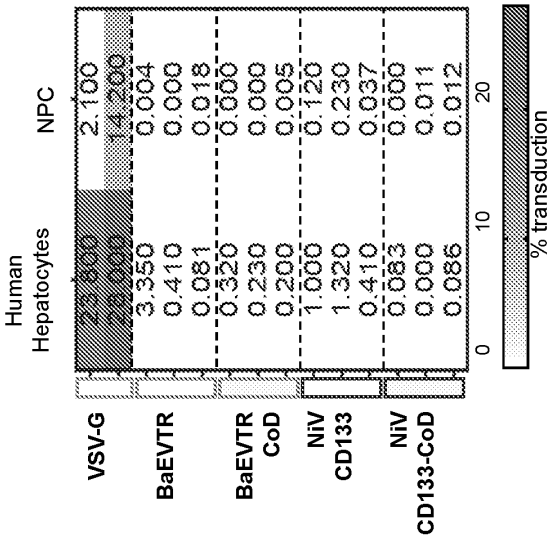
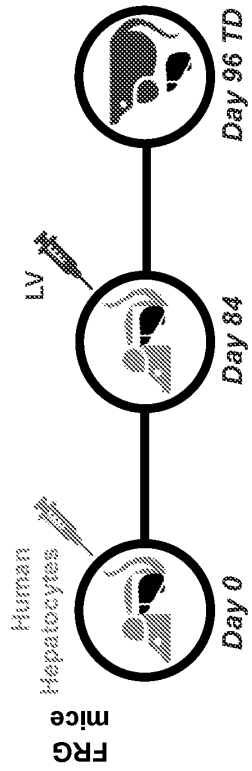


FIG. 11R



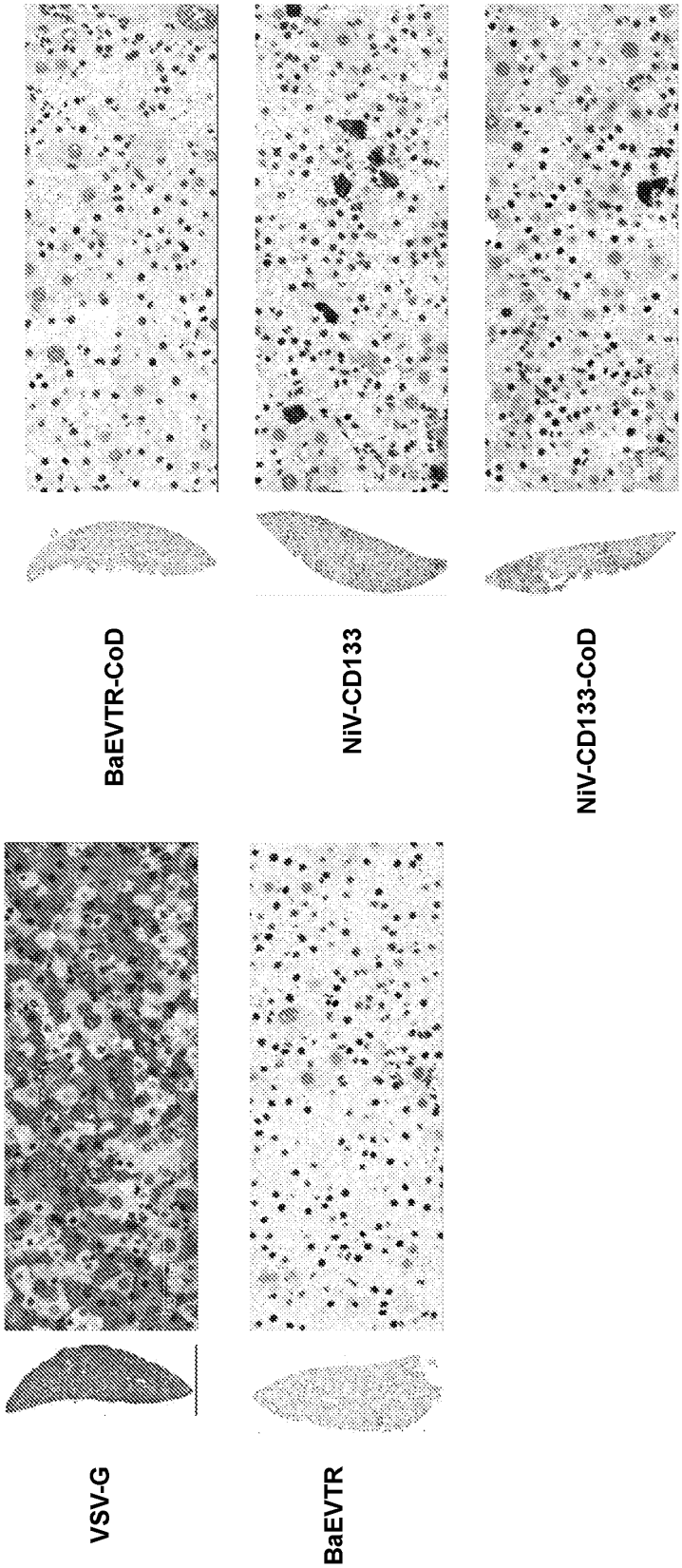
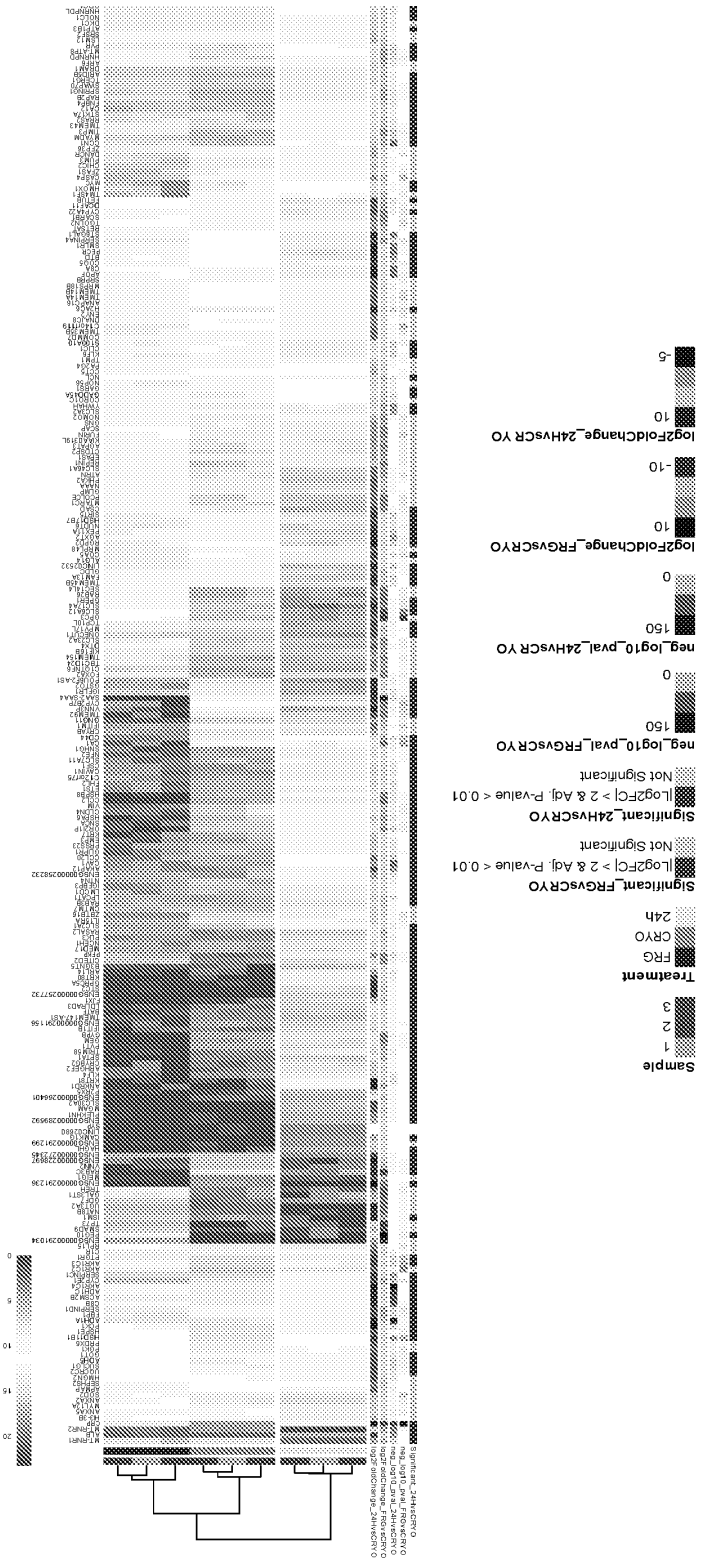


FIG. 11T

FIG. 11U


Sample
1
2
3
Treatment
FRG
CRYO
24h
Significant_FRGvsCRYO
|Log2FC| > 2 & Adj. P-value < 0.01
Not Significant
Significant_24HvsCRYO
|Log2FC| > 2 & Adj. P-value < 0.01
Not Significant
neg_log10_pval_FRGvsCRYO
150
0
neg_log10_pval_24HvsCRYO
150
0
log2FoldChange_FRGvsCRYO
10
-10
log2FoldChange_24HvsCRYO
10
-5

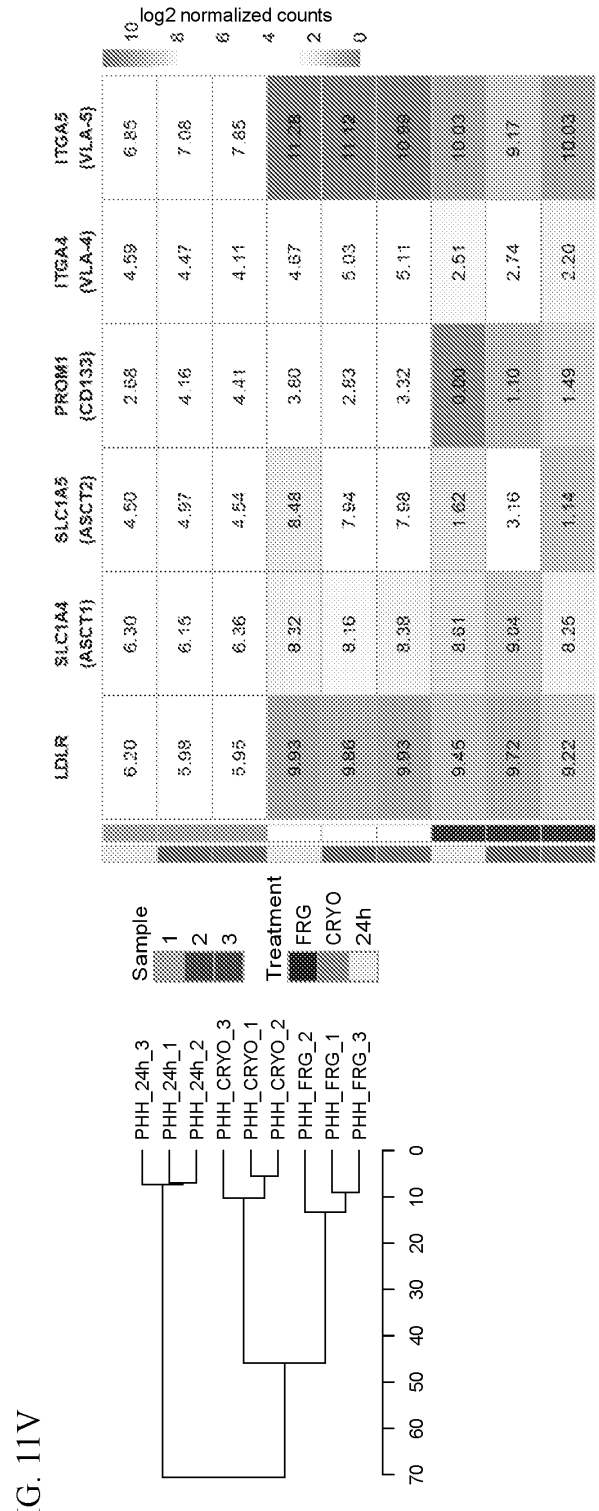

log2 normalized counts
10
8
6
4
2
0
LDLR
SLC1A4 (ASCT1)
SLC1A5 (ASCT2)
PROM1 (CD133)
ITGA4 (VLA-4)
ITGA5 (VLA-5)
6.20 6.30 4.50 2.68 4.59 6.85
5.98 6.15 4.97 4.16 4.47 7.08
5.95 6.36 4.54 4.41 4.11 7.85
9.93 8.32 8.48 3.80 4.67 11.28
9.86 8.16 7.94 2.83 5.03 11.12
9.83 8.38 7.68 3.32 5.11 10.99
9.45 8.61 1.62 0.89 2.51 10.03
9.72 9.03 3.16 1.10 2.74 9.17
9.22 8.25 1.14 1.49 2.20 10.03
Sample
1
2
3
Treatment
FRG
CRYO
24h
PHH_24h_3
PHH_24h_1
PHH_24h_2
PHH_CRYO_3
PHH_CRYO_1
PHH_CRYO_2
PHH_FRG_2
PHH_FRG_1
PHH_FRG_3
70 60 50 40 30 20 10 0

FIG. 11V

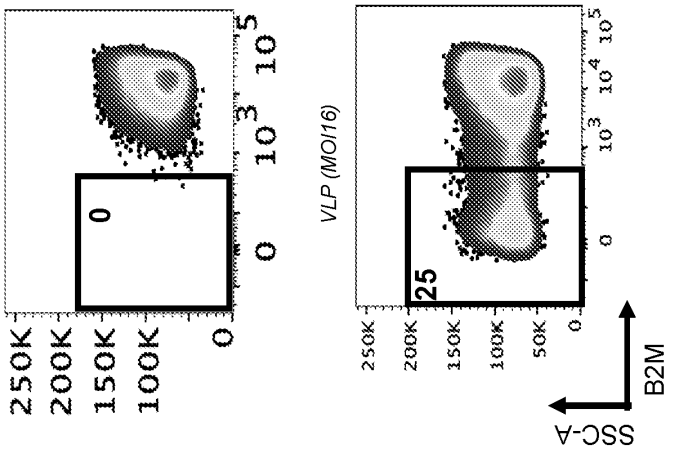


FIG. 12B

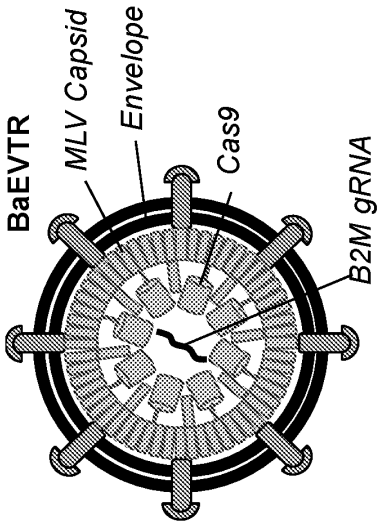
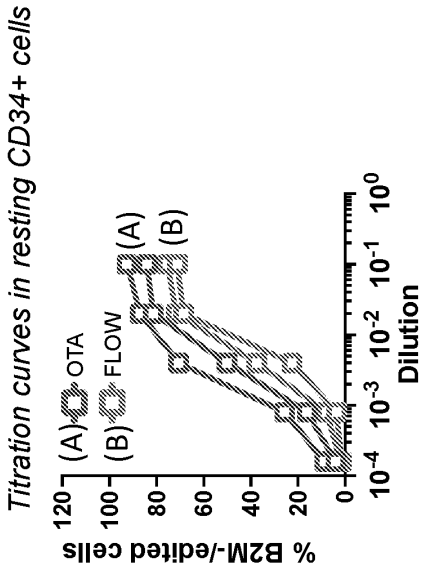


FIG. 12A


Titration curves in resting CD34+ cells
% B2M-/edited cells
120
100
80
60
40
20
0
(A) OTA
(B) FLOW
(A)
(B)
10^{-4} 10^{-3} 10^{-2} 10^{-1} 10^{0}
Dilution

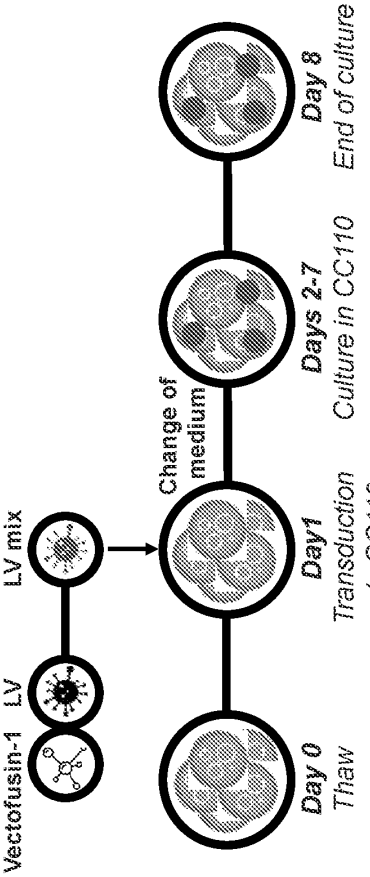

Vectofusin-1
LV
LV mix
Day 0
Thaw
Day1
Transduction
w/o CC110
Change of medium
Days 2-7
Culture in CC110
Day 8
End of culture

FIG. 12C

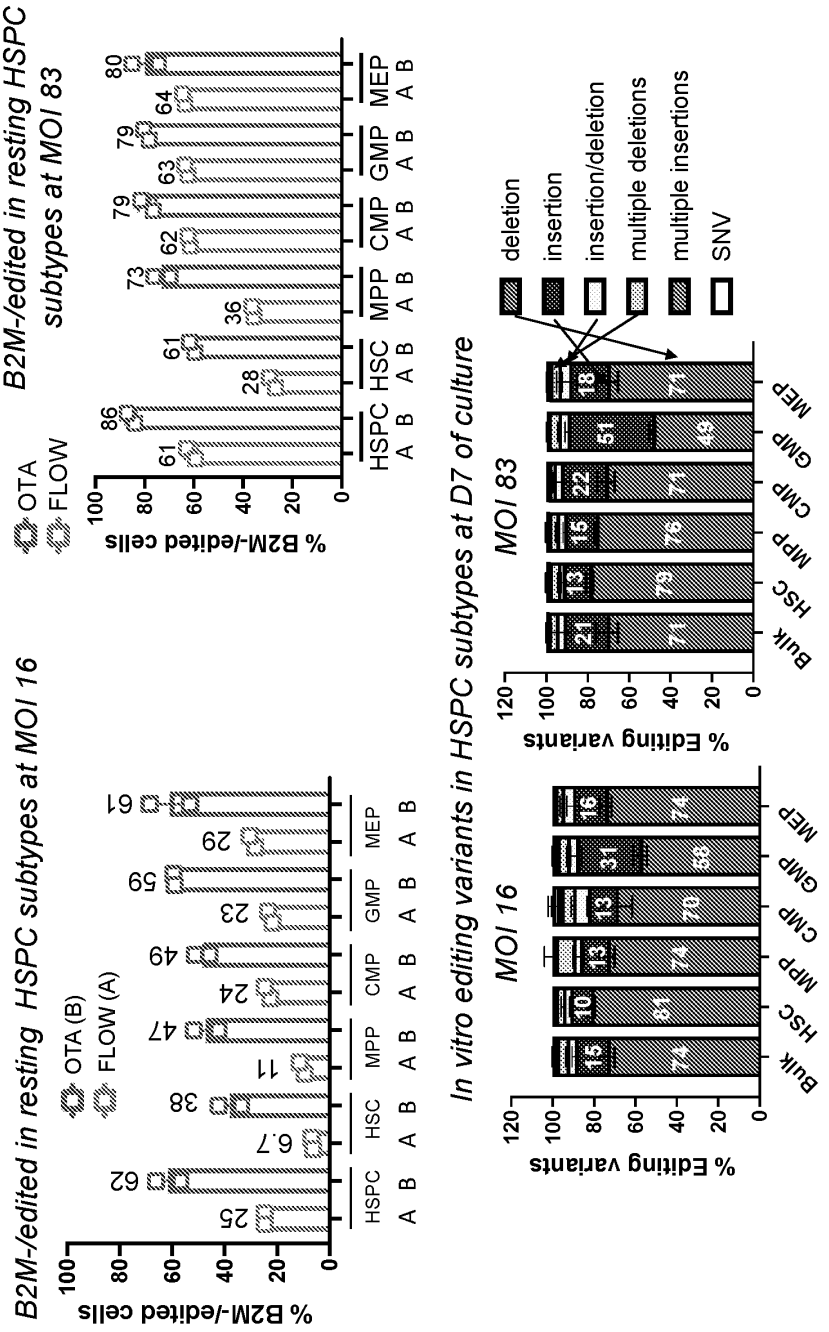

B2M-/edited in resting HSPC subtypes at MOI 16
OTA (B)
FLOW (A)
% B2M-/edited cells
HSPC HSC MPP CMP GMP MEP
A B A B A B A B A B A B
25 62 6.7 38 11 47 24 49 23 59 29 61
B2M-/edited in resting HSPC subtypes at MOI 83
OTA
FLOW
% B2M-/edited cells
HSPC HSC MPP CMP GMP MEP
A B A B A B A B A B A B
86 61 28 61 36 73 62 79 63 79 64 80
In vitro editing variants in HSPC subtypes at D7 of culture
MOI 16
% Editing variants
Bulk HSC MPP CMP GMP MEP
15 10 61 13 74 13 70 31 68 16 74
MOI 83
% Editing variants
Bulk HSC MPP CMP GMP MEP
21 13 78 15 76 22 71 51 48 18 71
deletion
insertion
insertion/deletion
multiple deletions
multiple insertions
SNV

FIG. 12D

FIG. 12E

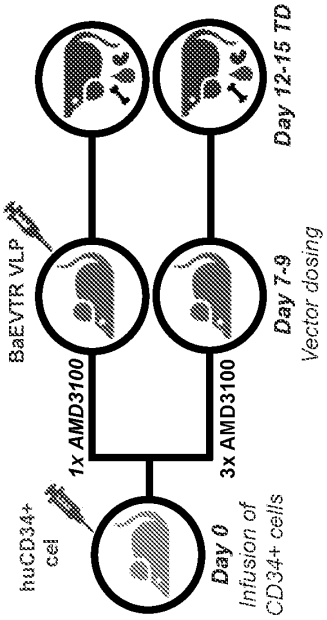


FIG. 12F

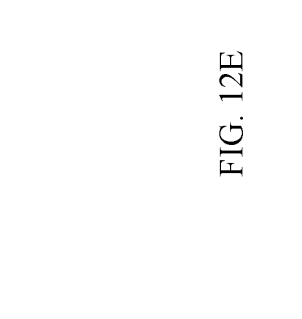
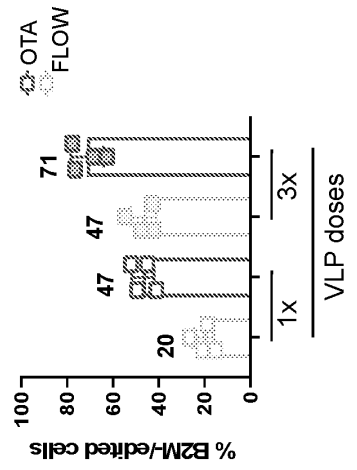


FIG. 12G

FIG. 12H



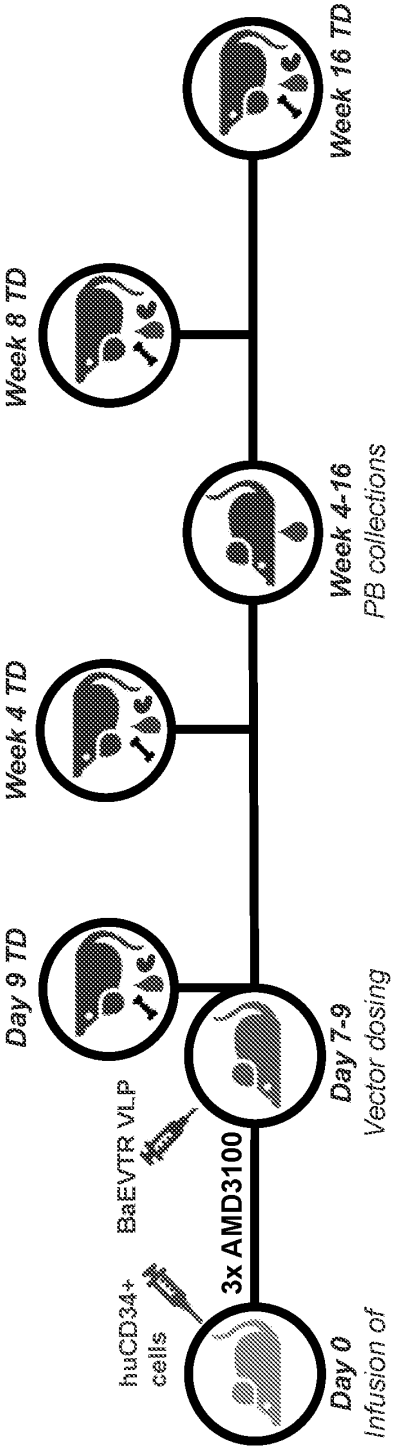

Day 0
Infusion of CD34+ cells
huCD34+ cells
3x AMD3100
BaEVTR VLP
Day 7-9
Vector dosing
Day 9 TD
Week 4 TD
Week 4-16
PB collections
Week 8 TD
Week 16 TD

FIG. 12I

FIG. 12J

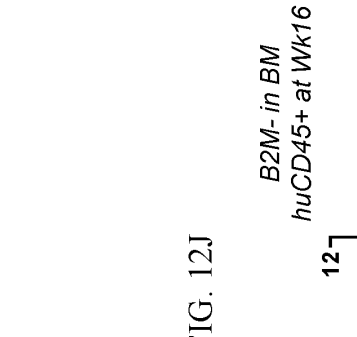


FIG. 12K

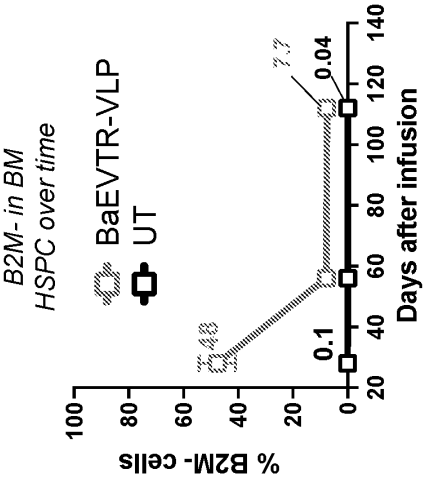
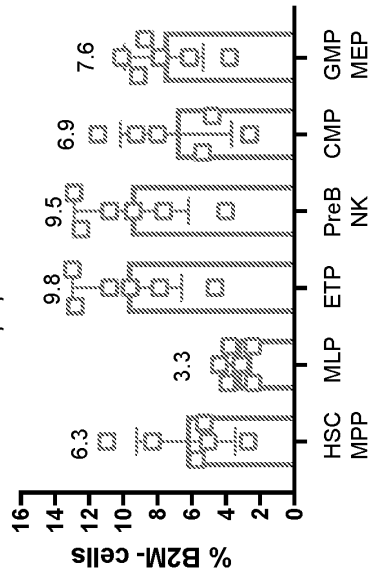


FIG. 12L



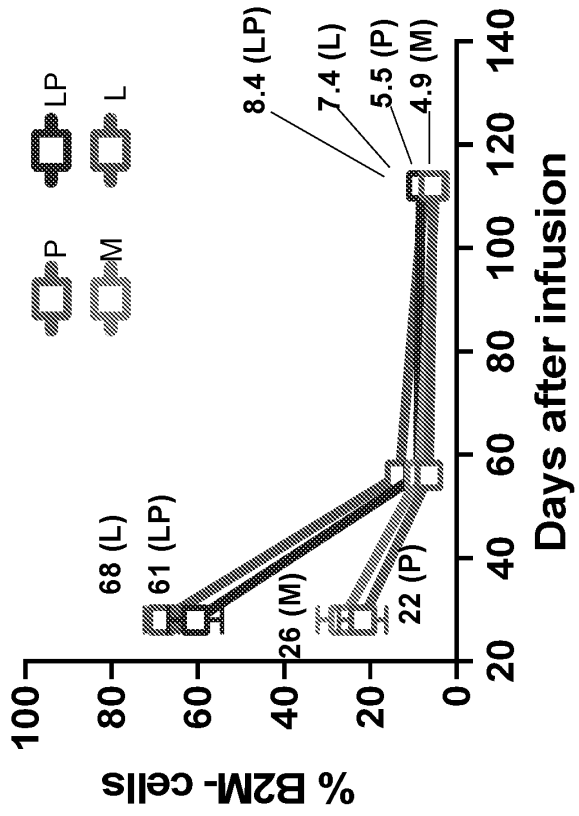

% B2M- cells
Days after infusion
100
80
60
40
20
0
20
40
60
80
100
120
140
LP
L
P
M
8.4 (LP)
7.4 (L)
5.5 (P)
4.9 (M)
68 (L)
61 (LP)
26 (M)
22 (P)

FIG. 12M

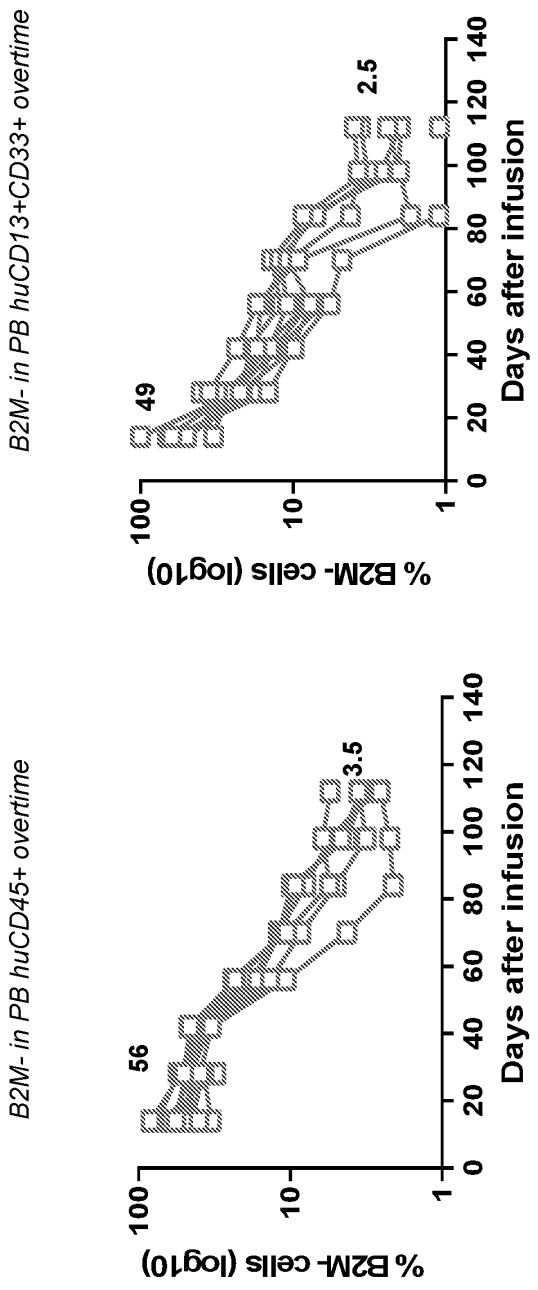

B2M- in PB huCD13+CD33+ overtime
% B2M- cells (log10)
100
10
1
0
20
40
60
80
100
120
140
Days after infusion
49
2.5
B2M- in PB huCD45+ overtime
% B2M- cells (log10)
100
10
1
0
20
40
60
80
100
120
140
Days after infusion
56
3.5

FIG. 12N

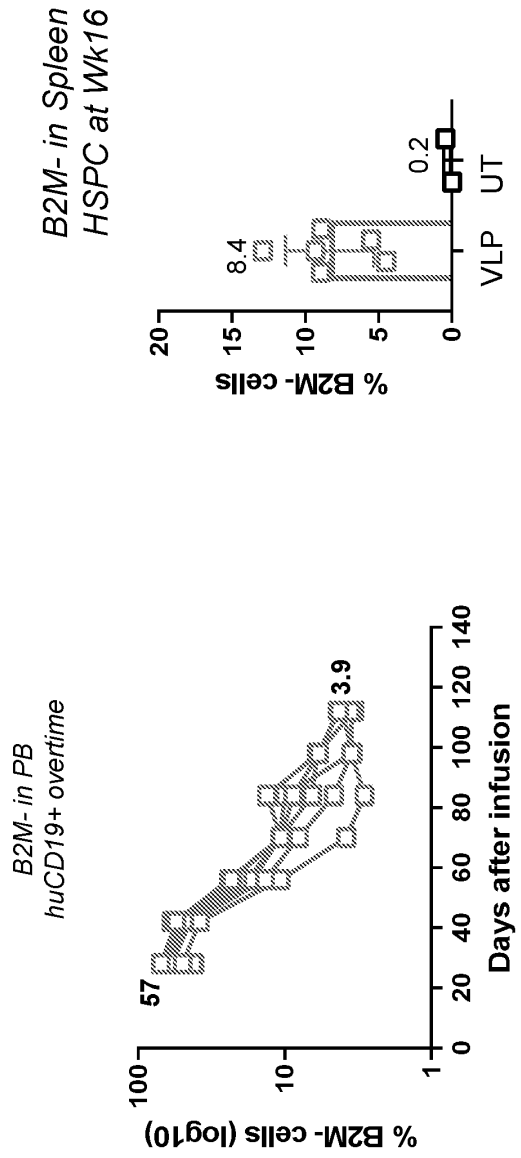

B2M- in PB
huCD19+ overtime
% B2M- cells (log10)
100
10
1
0
20
40
60
80
100
120
140
Days after infusion
57
3.9
B2M- in Spleen
HSPC at Wk16
% B2M- cells
20
15
10
5
0
8.4
0.2
VLP
UT

FIG. 12O

FIG. 12Q

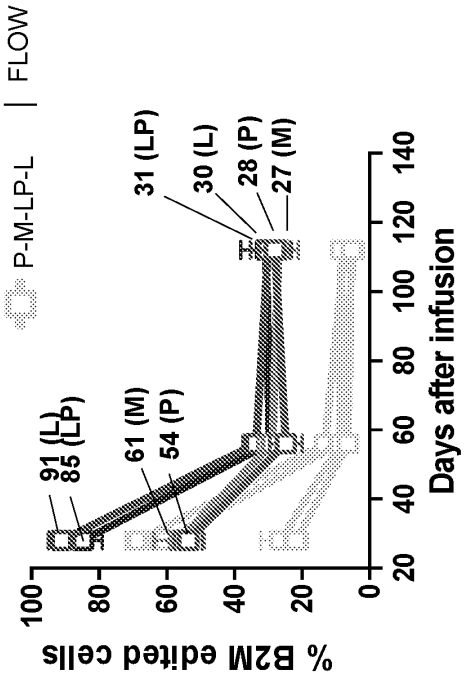


FIG. 12P

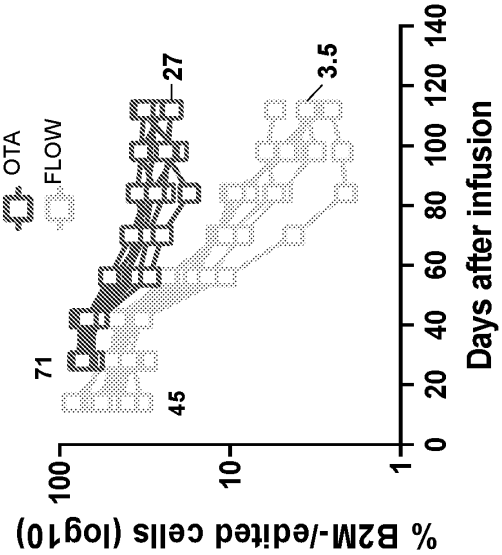


FIG. 12S

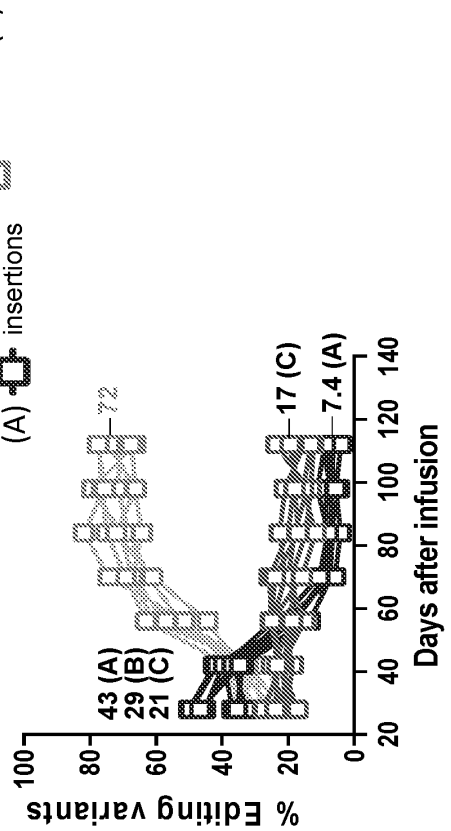
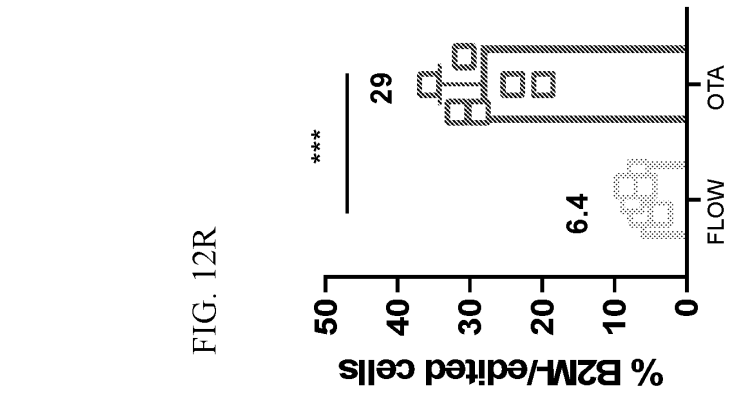


FIG. 12R



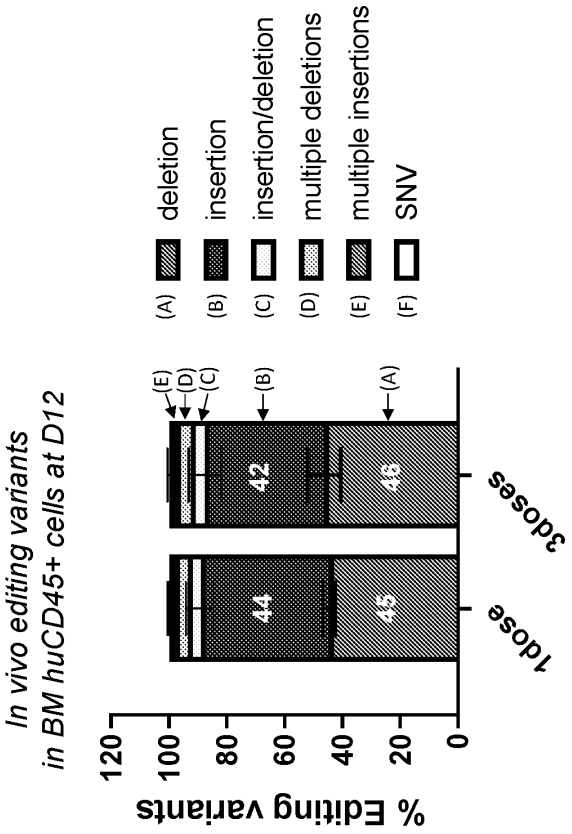

In vivo editing variants
in BM huCD45+ cells at D12
% Editing variants
120
100
80
60
40
20
0
1dose
3doses
44
45
42
46
(A)
(B)
(C)
(D)
(E)
(F)
deletion
insertion
insertion/deletion
multiple deletions
multiple insertions
SNV

FIG. 12T

FIG. 12U

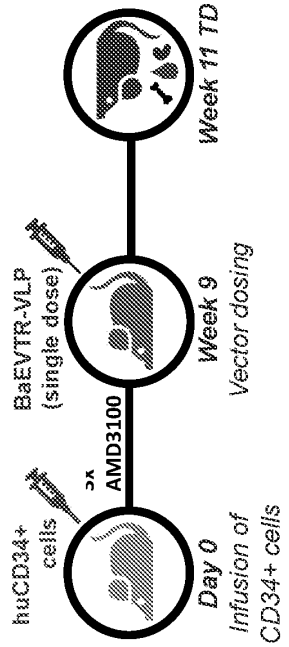


FIG. 12V

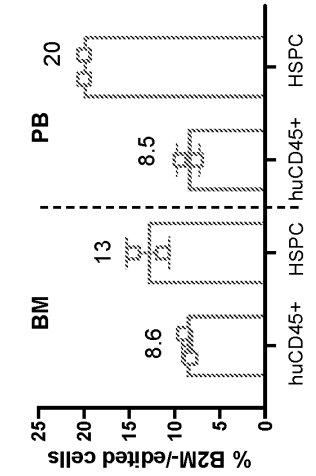


FIG. 12W

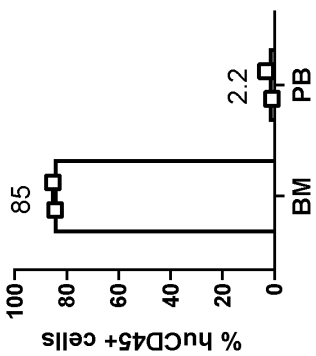


FIG. 12X

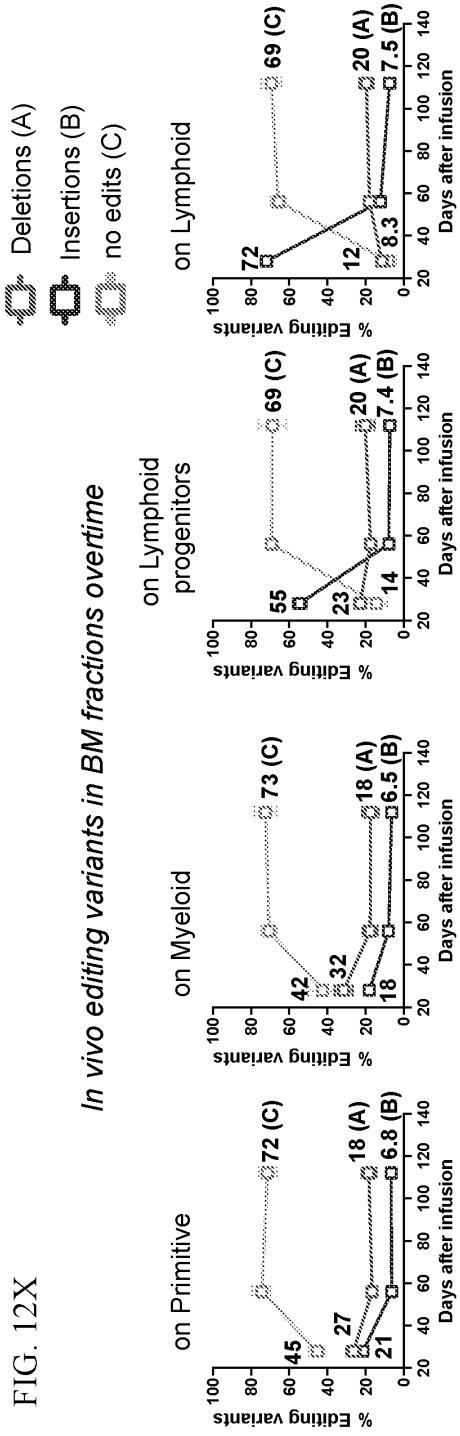

In vivo editing variants in BM fractions overtime
Deletions (A)
Insertions (B)
no edits (C)
on Primitive
on Myeloid
on Lymphoid progenitors
on Lymphoid
% Editing variants
Days after infusion
45
27
21
72 (C)
18 (A)
6.8 (B)
42
32
18
73 (C)
18 (A)
6.5 (B)
55
23
14
69 (C)
20 (A)
7.4 (B)
72
12
8.3
69 (C)
20 (A)
7.5 (B)

FIG. 13A

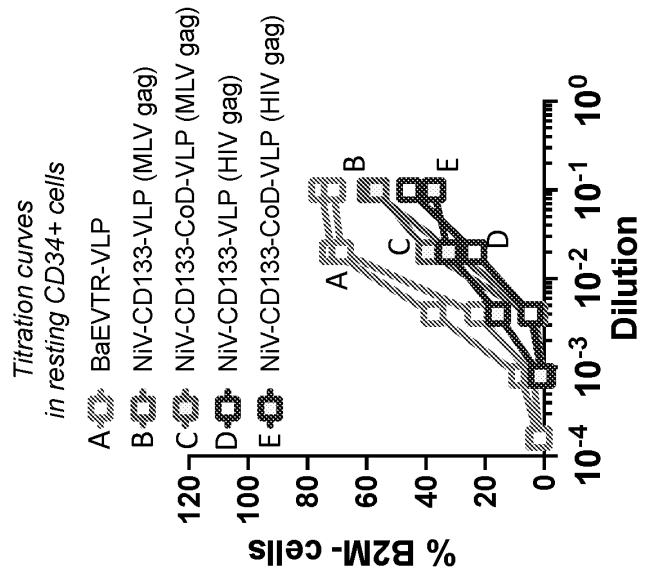
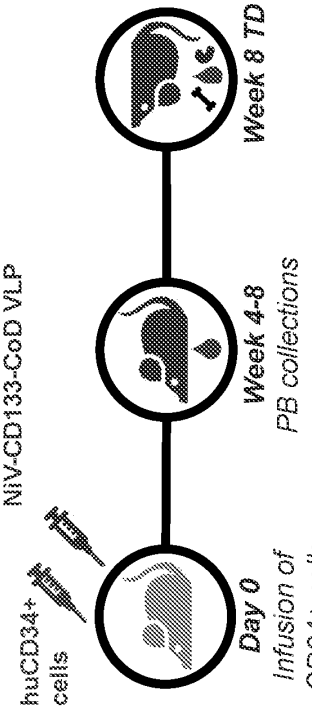


FIG. 13B



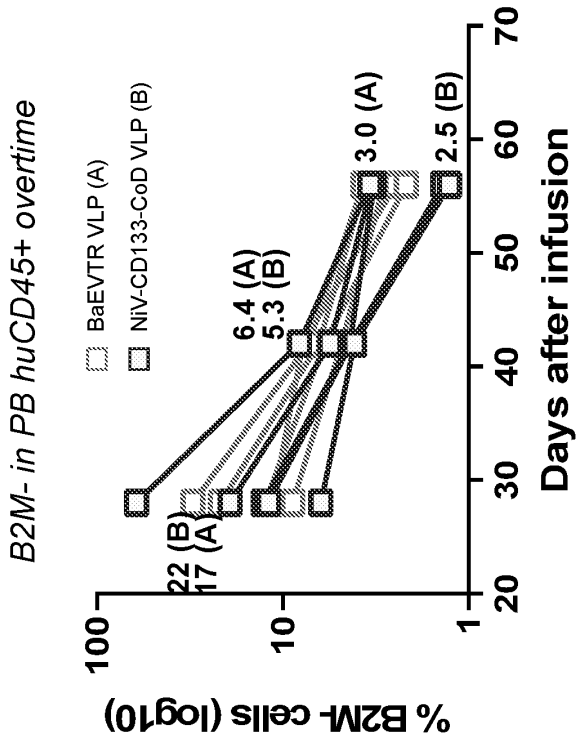
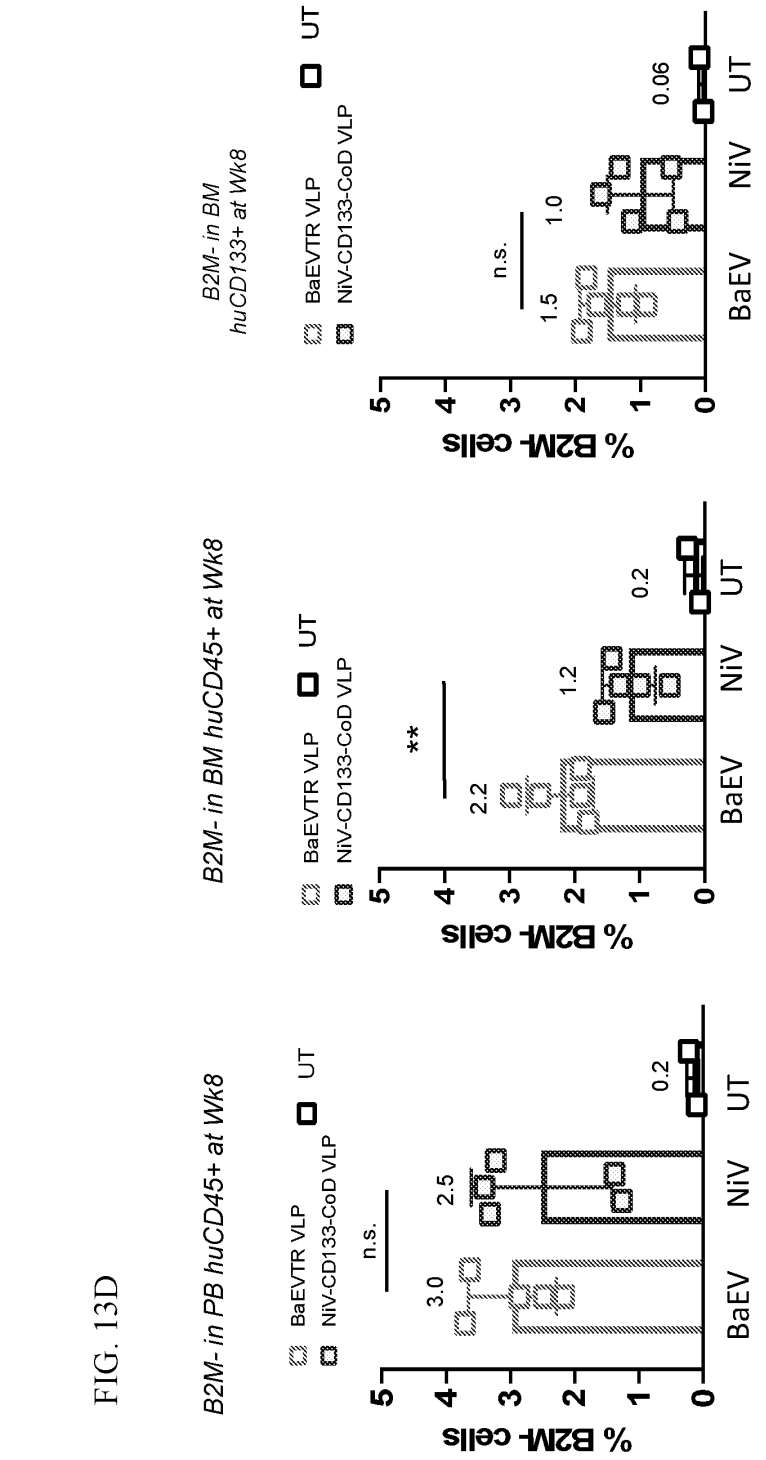

B2M- in PB huCD45+ overtime
BaEVTR VLP (A)
NiV-CD133-CoD VLP (B)
% B2M- cells (log10)
100
10
1
20
30
40
50
60
70
Days after infusion
22 (B)
17 (A)
6.4 (A)
5.3 (B)
3.0 (A)
2.5 (B)

FIG. 13C

FIG. 13D


B2M- in PB huCD45+ at Wk8
B2M- in BM huCD45+ at Wk8
B2M- in BM huCD133+ at Wk8
BaEVTR VLP
NiV-CD133-CoD VLP
UT
% B2M- cells
n.s.
**
3.0
2.5
0.2
2.2
1.2
0.2
1.5
1.0
0.06
BaEV
NiV
UT

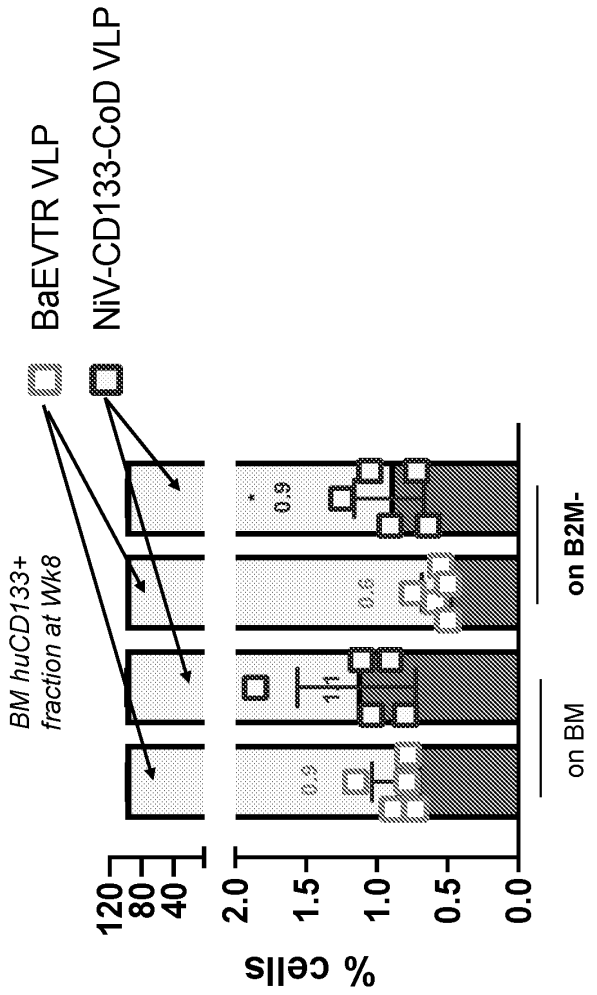

BaEVTR VLP
NiV-CD133-CoD VLP
BM huCD133+ fraction at Wk8
% cells
120
80
40
2.0
1.5
1.0
0.5
0.0
0.9
1.1
0.8
0.9
on BM
on B2M-

FIG. 13E

FIG. 13F

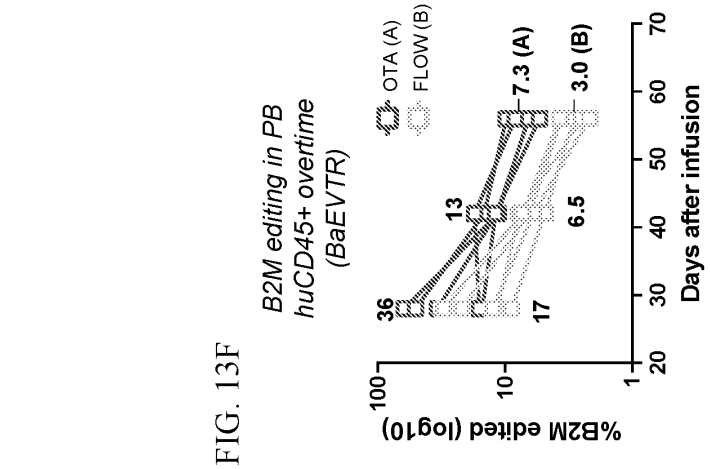
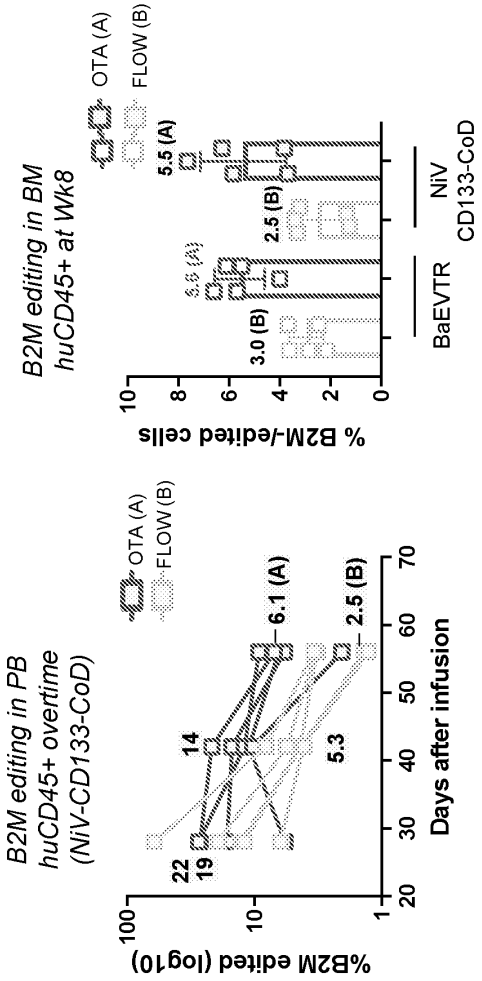


FIG. 13G



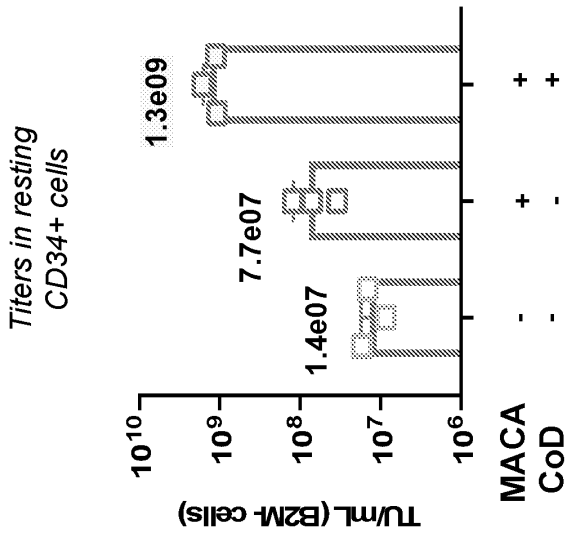
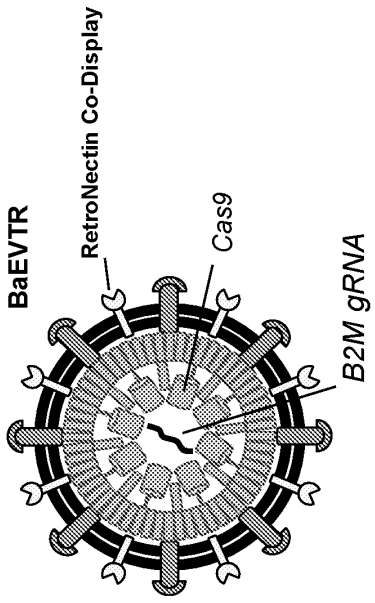

Titers in resting
CD34+ cells
1.3e09
7.7e07
1.4e07
TU/mL (B2M- cells)
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
MACA
CoD
-
+
-
+
+

FIG. 13H


BaEVTR
RetroNectin Co-Display
Cas9
B2M gRNA

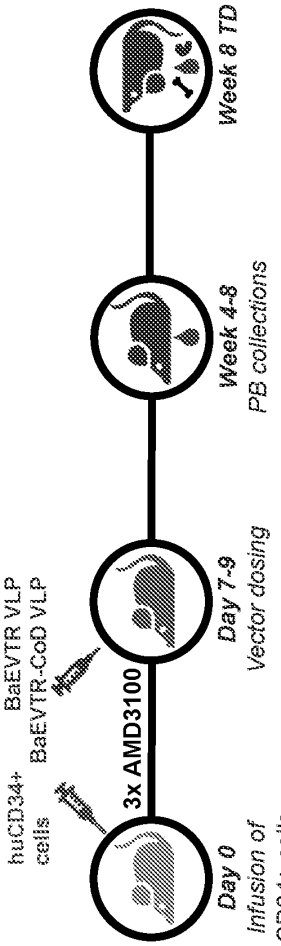


FIG. 13J

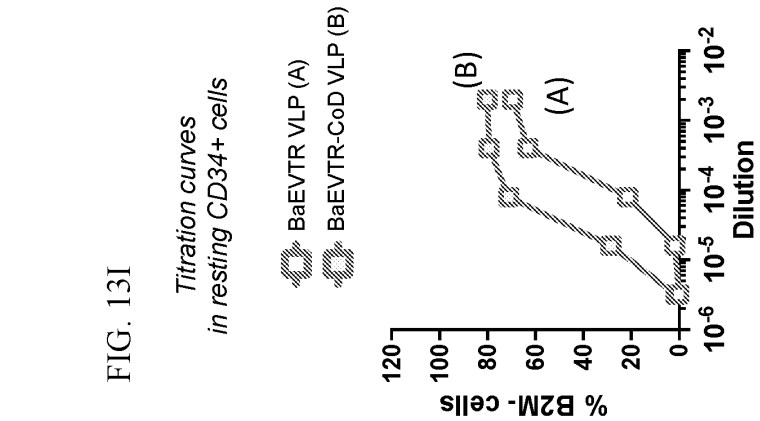


FIG. 13I

FIG. 13L

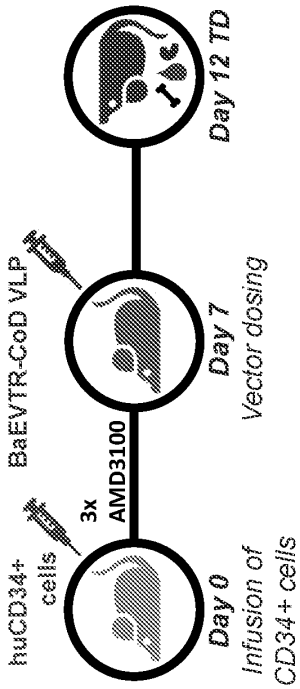
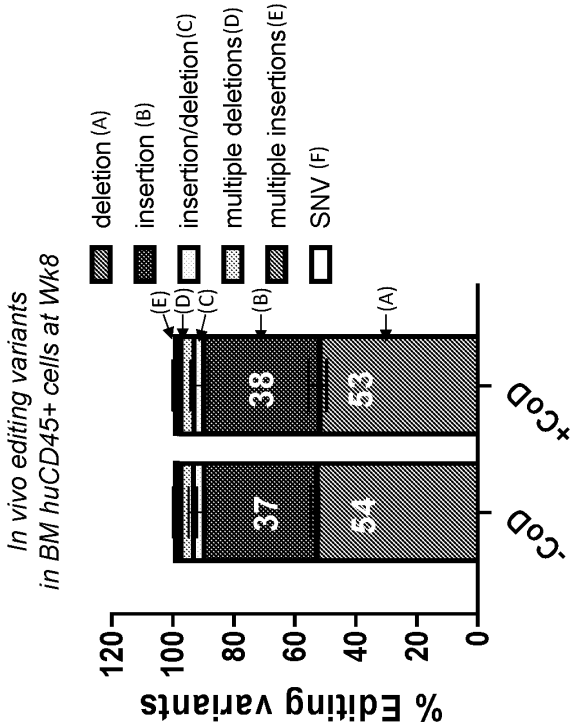


FIG. 13K



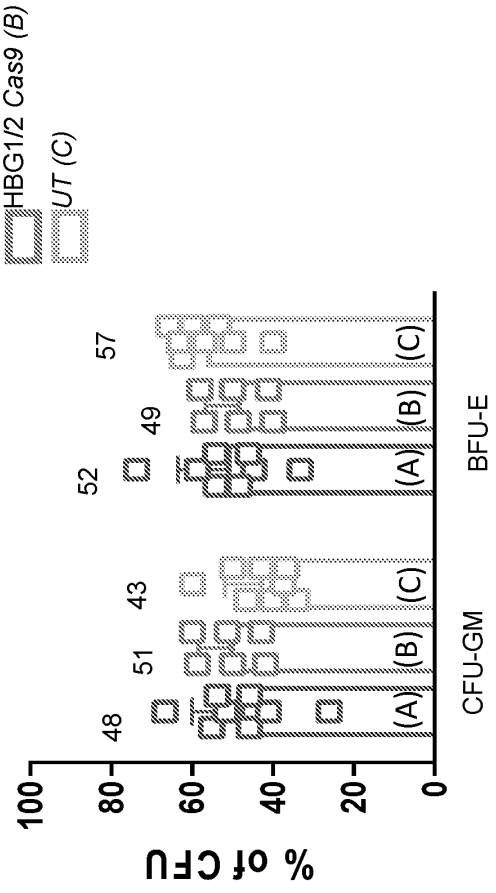

BCL11a ABE (A)
HBG1/2 Cas9 (B)
UT (C)
% of CFU
100
80
60
40
20
0
48
51
43
52
49
57
(A)
(B)
(C)
(A)
(B)
(C)
CFU-GM
BFU-E

FIG. 13M

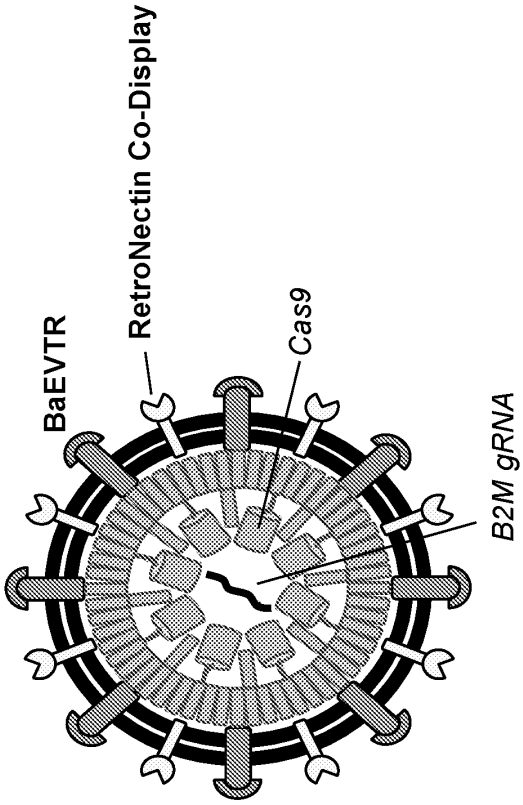


FIG. 14A

FIG. 14B

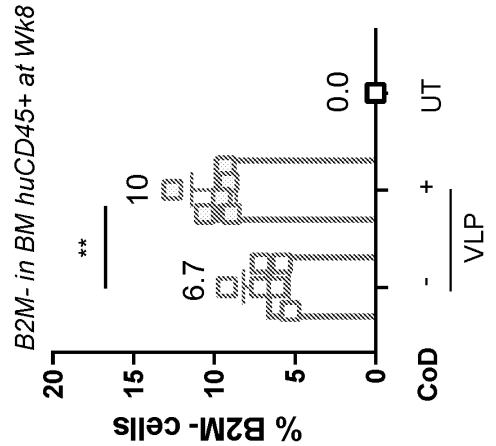


FIG. 14C

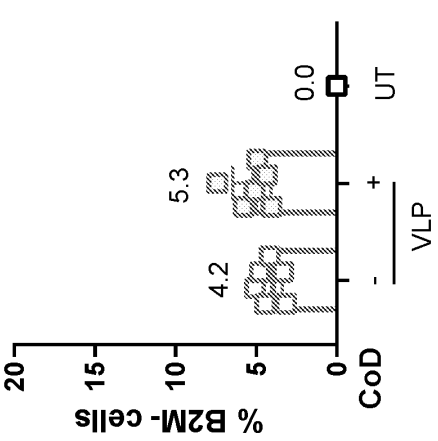
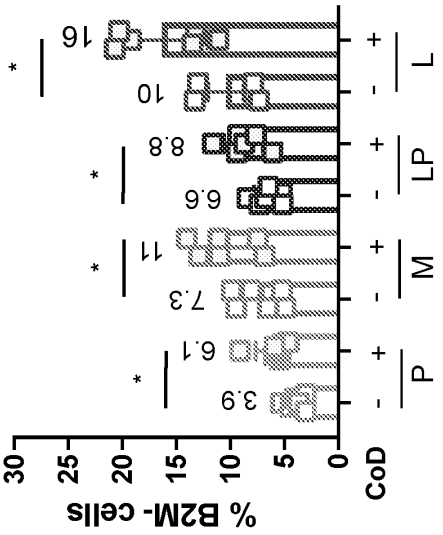
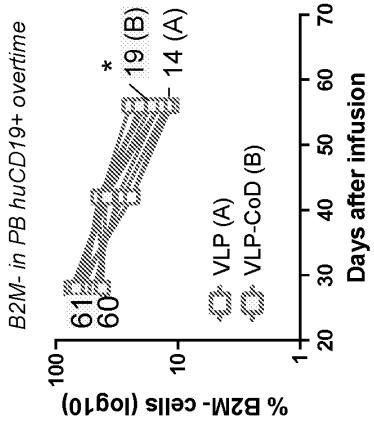
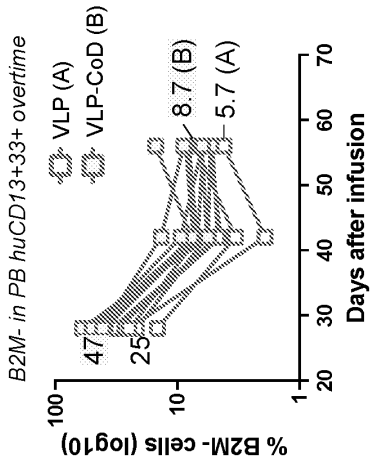


FIG. 14D




B2M- in PB huCD19+ overtime
% B2M- cells (log10)
100
10
1
20
30
40
50
60
70
Days after infusion
61
60
*
19 (B)
14 (A)
VLP (A)
VLP-CoD (B)


B2M- in PB huCD13+33+ overtime
% B2M- cells (log10)
100
10
1
20
30
40
50
60
70
Days after infusion
47
25
VLP (A)
VLP-CoD (B)
8.7 (B)
5.7 (A)

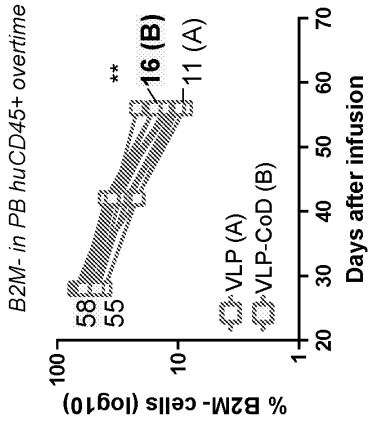

B2M- in PB huCD45+ overtime
% B2M- cells (log10)
100
10
1
20
30
40
50
60
70
Days after infusion
58
55
**
16 (B)
11 (A)
VLP (A)
VLP-CoD (B)

FIG. 14E

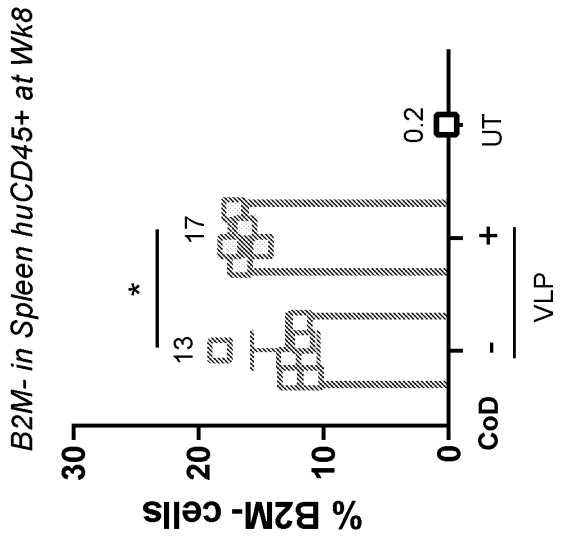
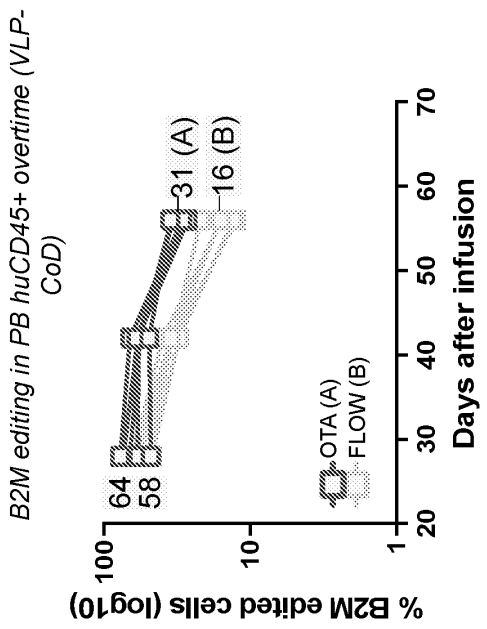

B2M- in Spleen huCD45+ at Wk8
% B2M- cells
30
20
10
0
CoD
-
+
VLP
UT
0.2
13
17
*

FIG. 14F


B2M editing in PB huCD45+ overtime (VLP-CoD)
% B2M edited cells (log10)
100
10
1
64
58
31 (A)
16 (B)
OTA (A)
FLOW (B)
20
30
40
50
60
70
Days after infusion

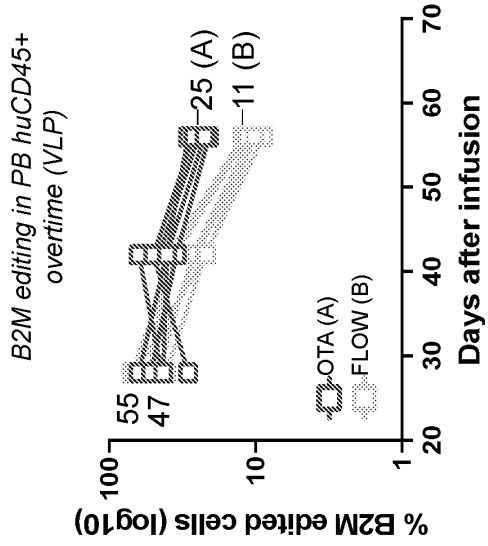

B2M editing in PB huCD45+ overtime (VLP)
% B2M edited cells (log10)
100
10
1
55
47
25 (A)
11 (B)
OTA (A)
FLOW (B)
20
30
40
50
60
70
Days after infusion

FIG. 14G

FIG. 14I

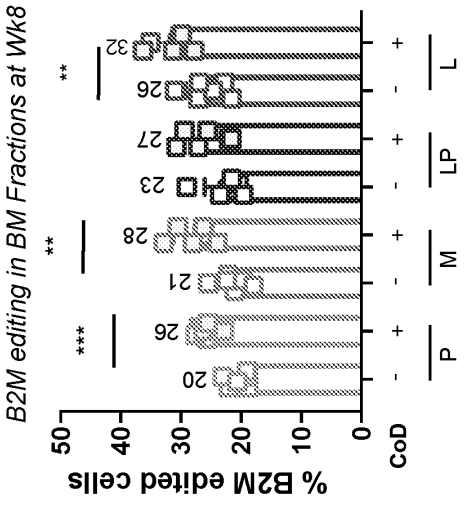
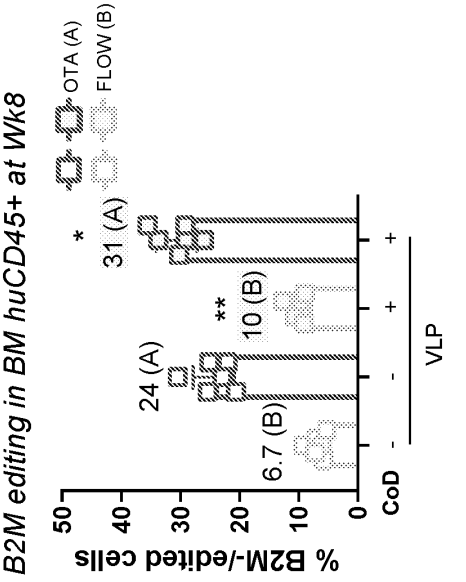


FIG. 14H



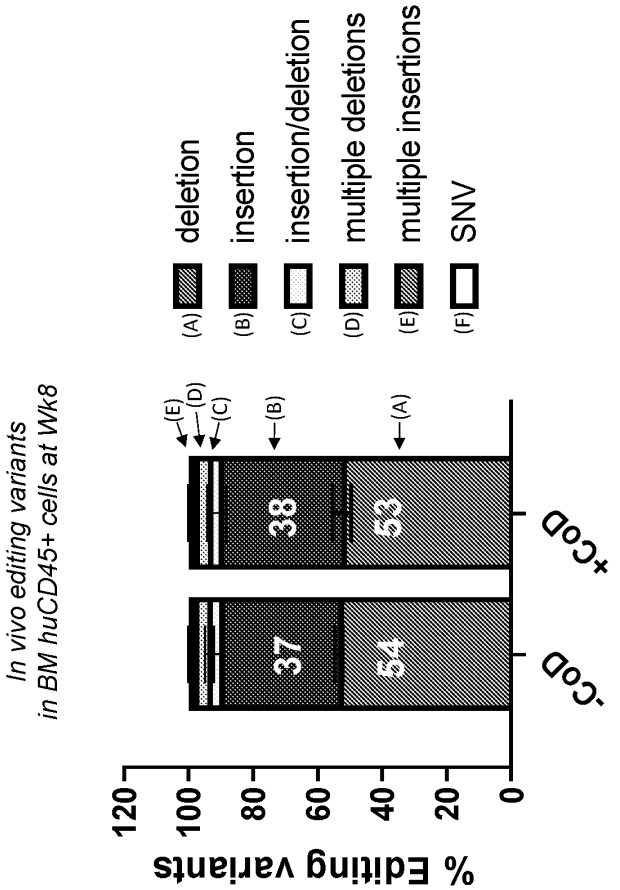

In vivo editing variants
in BM huCD45+ cells at Wk8
% Editing variants
120
100
80
60
40
20
0
-CoD
+CoD
37
54
38
53
(A)
(B)
(C)
(D)
(E)
(F)
deletion
insertion
insertion/deletion
multiple deletions
multiple insertions
SNV

FIG. 14J

FIG. 15B

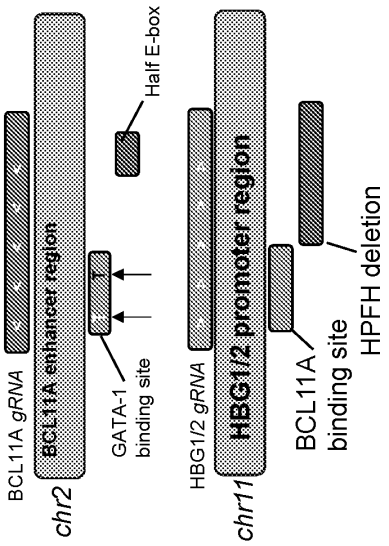
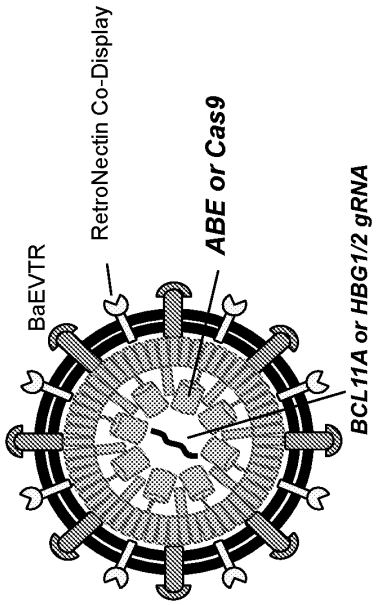


FIG. 15A



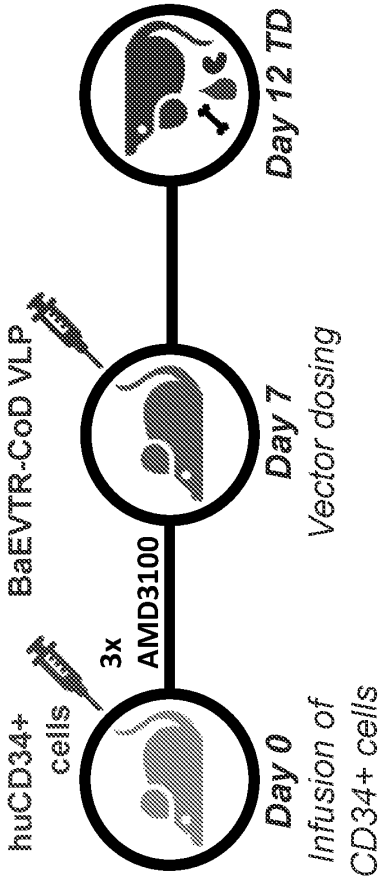

huCD34+ cells
3x
AMD3100
BaEVTR-CoD VLP
Day 0
Infusion of CD34+ cells
Day 7
Vector dosing
Day 12 TD

FIG. 15C

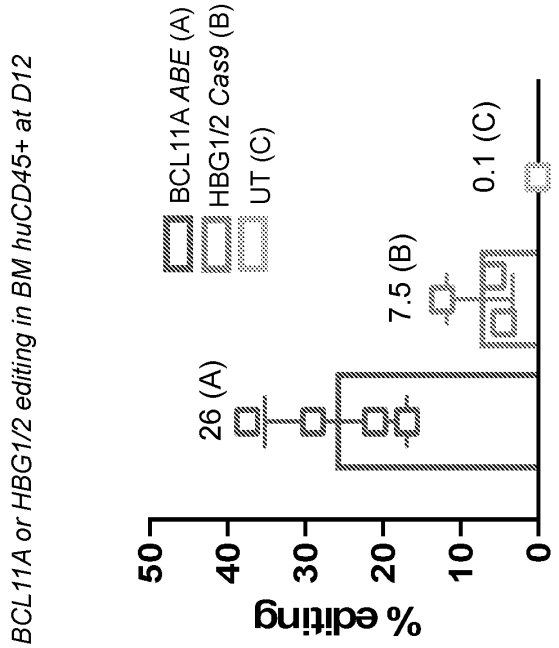
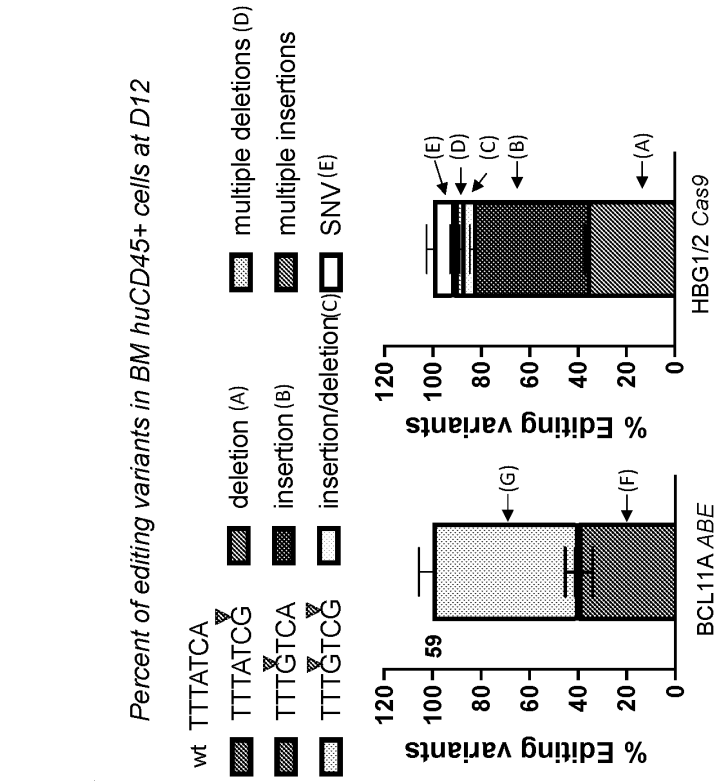

BCL11A or HBG1/2 editing in BM huCD45+ at D12
% editing
50
40
30
20
10
0
BCL11A ABE (A)
HBG1/2 Cas9 (B)
UT (C)
26 (A)
7.5 (B)
0.1 (C)

FIG. 15D

FIG. 15E


Percent of editing variants in BM huCD45+ cells at D12
wt TTTATCA
(F) TTTATCG
TTTGTCA
(G) TTTGTCG
deletion (A)
multiple deletions (D)
insertion (B)
multiple insertions
insertion/deletion(C)
SNV (E)
% Editing variants
120
100
80
60
40
20
0
59
(G)
(F)
BCL11A ABE
(E)
(D)
(C)
(B)
(A)
HBG1/2 Cas9

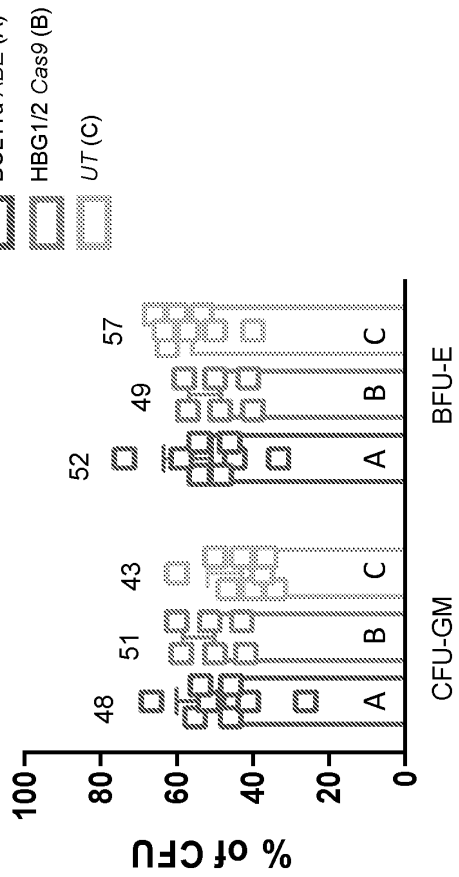

BCL11a ABE (A)
HBG1/2 Cas9 (B)
UT (C)
% of CFU
100
80
60
40
20
0
48
51
43
52
49
57
A
B
C
A
B
C
CFU-GM
BFU-E

FIG. 15F

FIG. 15G

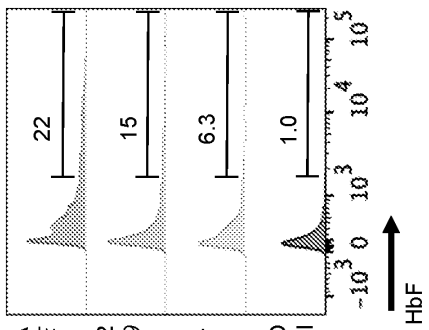
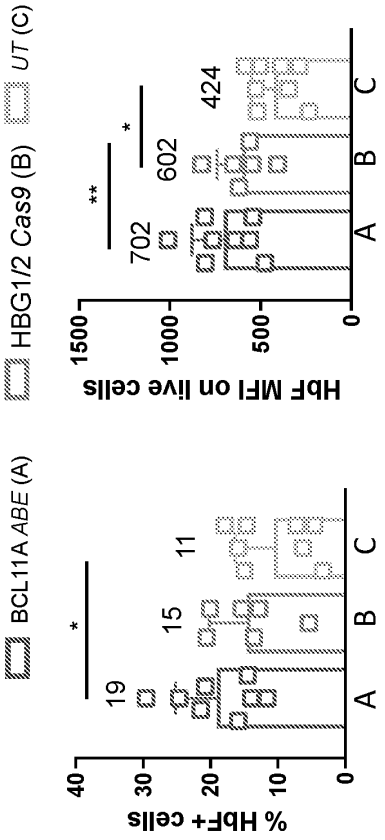


FIG. 15H



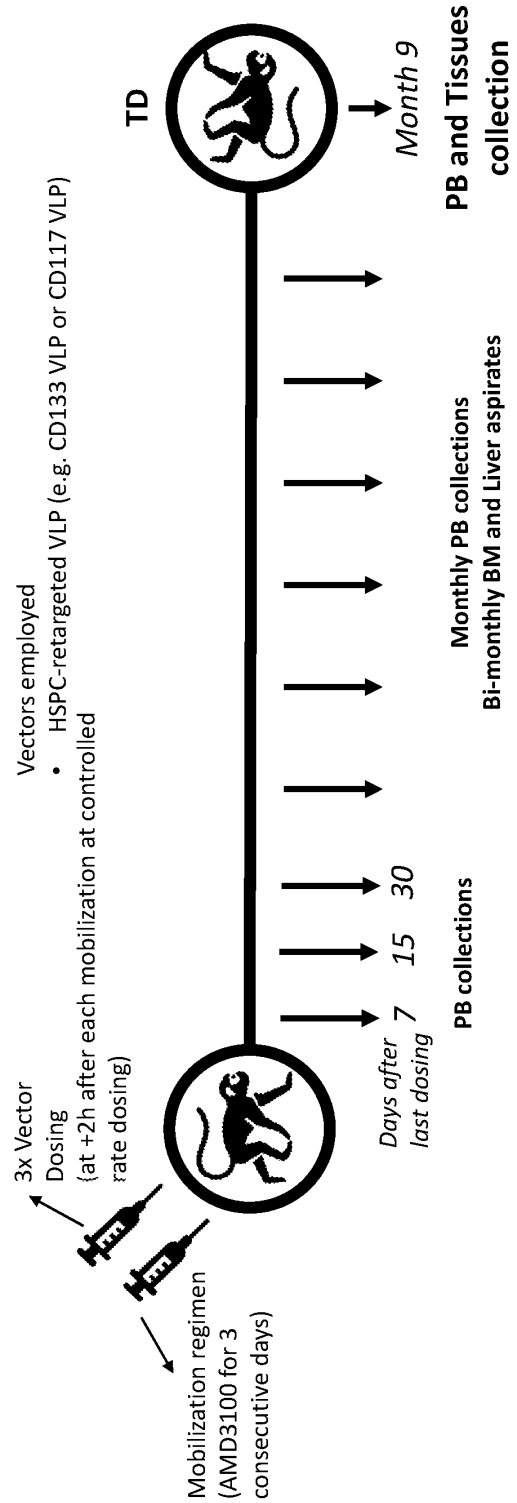

Vectors employed
• HSPC-retargeted VLP (e.g. CD133 VLP or CD117 VLP)
3x Vector Dosing
(at +2h after each mobilization at controlled rate dosing)
Mobilization regimen (AMD3100 for 3 consecutive days)
Days after last dosing
7
15
30
PB collections
Monthly PB collections
Bi-monthly BM and Liver aspirates
TD
Month 9
PB and Tissues collection

FIG. 16