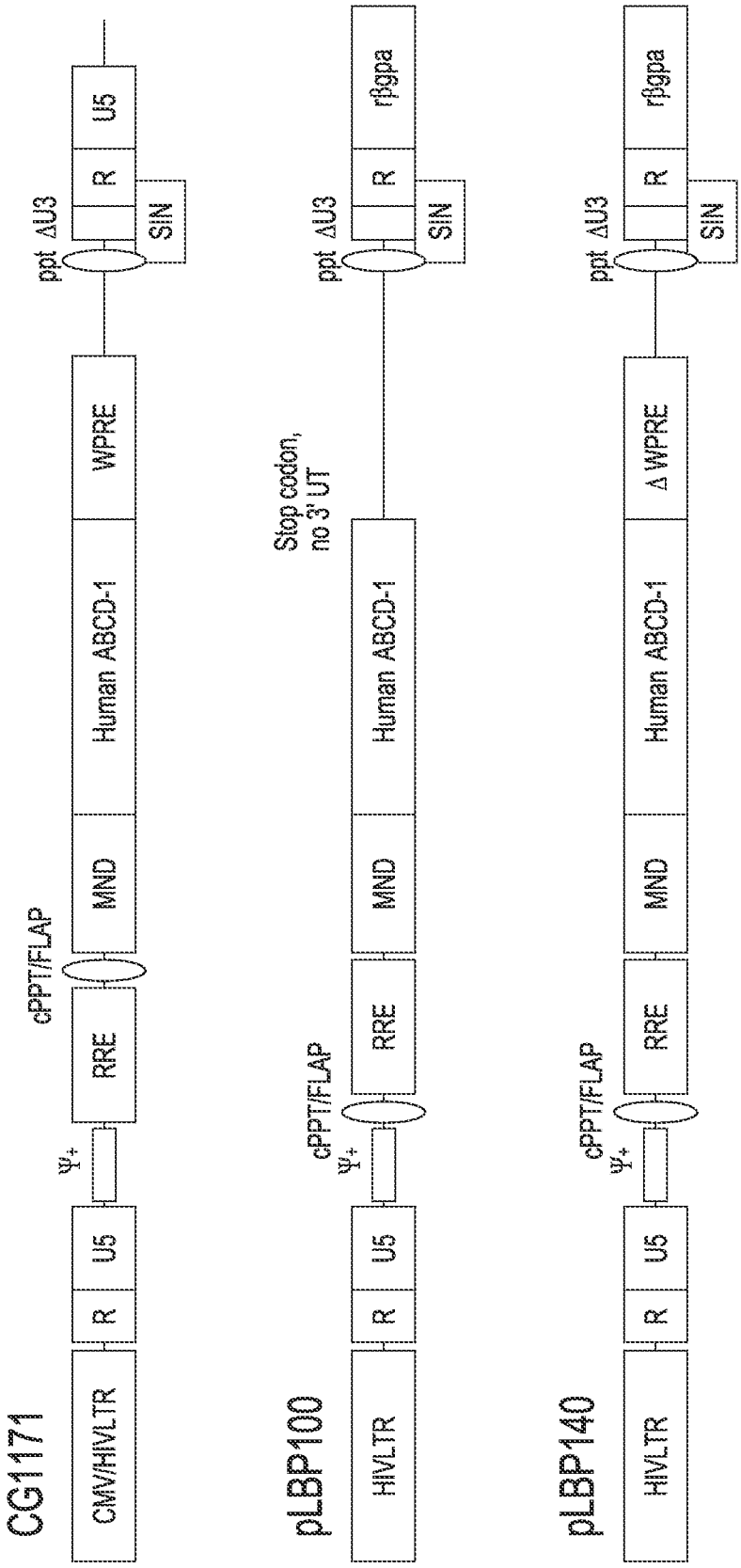

CG1171
CMV/HIVLTR
R
U5
Ψ+
RRE
cPPT/FLAP
MND
Human ABCD-1
WPRE
ppt ΔU3
R
U5
SIN
pLBP100
HIVLTR
R
U5
Ψ+
RRE
cPPT/FLAP
MND
Human ABCD-1
Stop codon, no 3' UT
ppt ΔU3
R
rβgpa
SIN
pLBP140
HIVLTR
R
U5
Ψ+
RRE
cPPT/FLAP
MND
Human ABCD-1
Δ WPRE
ppt ΔU3
R
rβgpa
SIN

FIG. 1

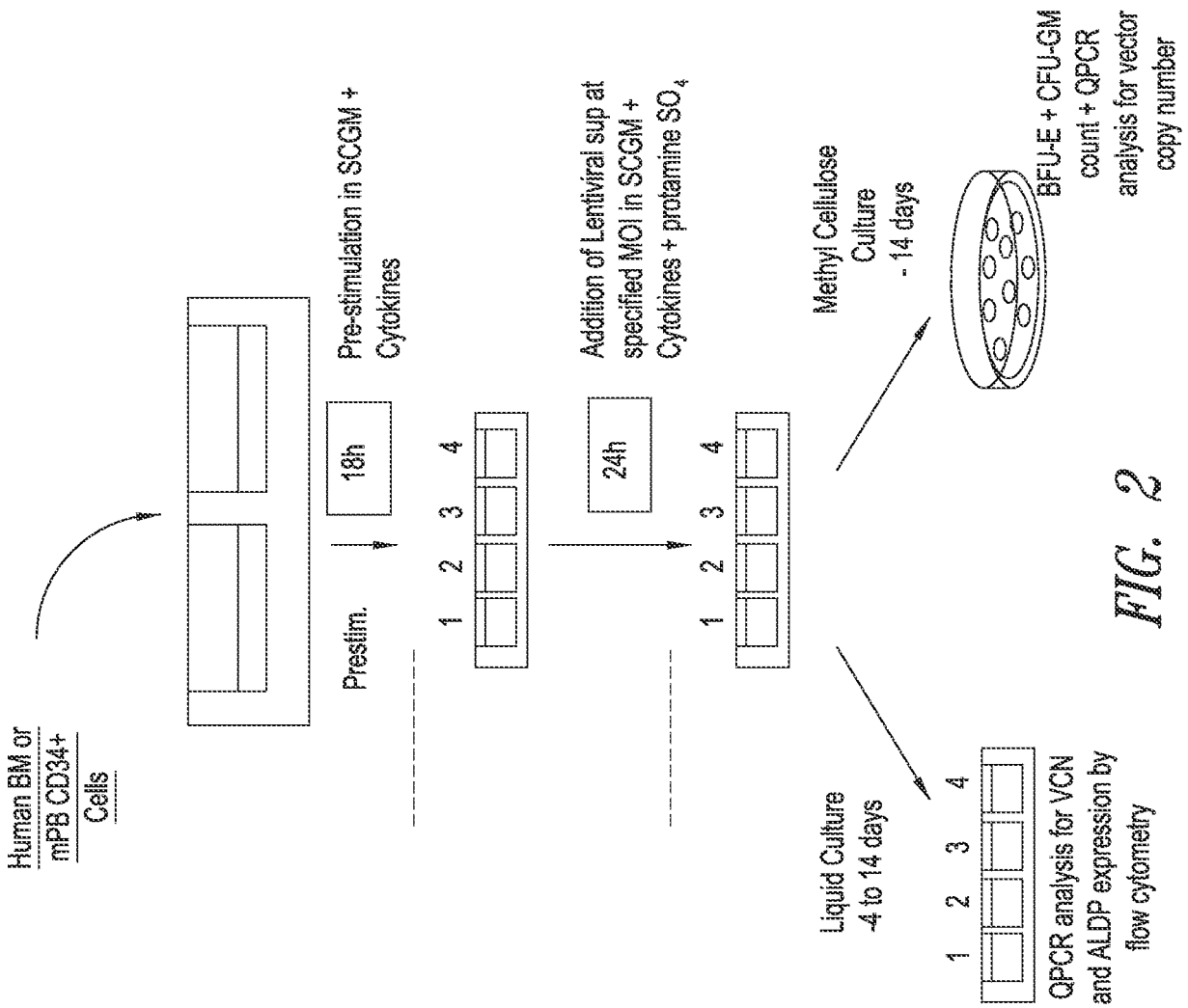

Human BM or mPB CD34+ Cells
Prestim.
18h
Pre-stimulation in SCGM + Cytokines
1 2 3 4
24h
Addition of Lentiviral sup at specified MOI in SCGM + Cytokines + protamine SO_4
1 2 3 4
Methyl Cellulose Culture - 14 days
BFU-E + CFU-GM count + QPCR analysis for vector copy number
Liquid Culture -4 to 14 days
1 2 3 4
QPCR analysis for VCN and ALDP expression by flow cytometry

FIG. 2

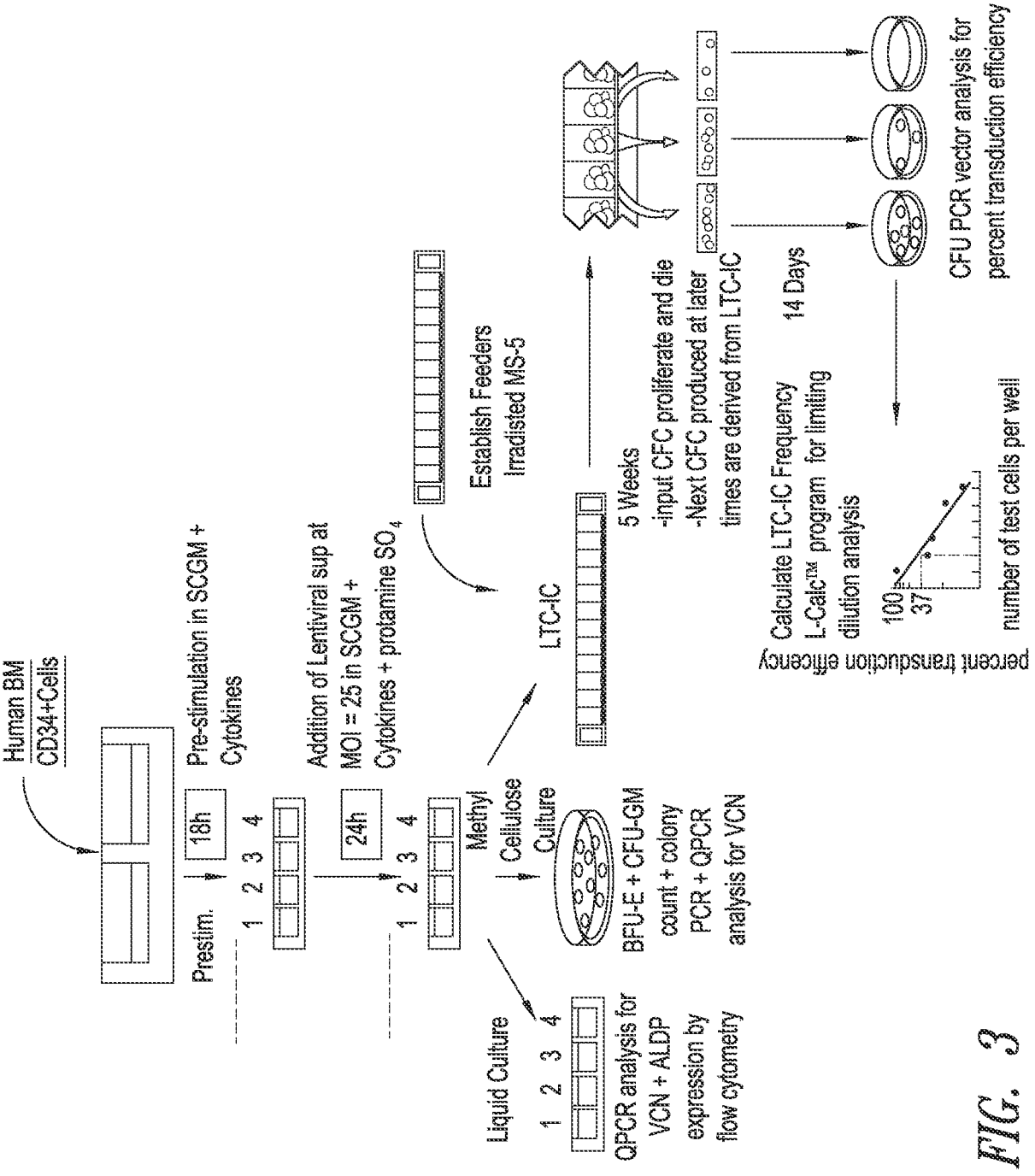

Human BM
CD34+Cells
Prestim.
18h
Pre-stimulation in SCGM + Cytokines
1 2 3 4
Addition of Lentiviral sup at MOI = 25 in SCGM + Cytokines + protamine SO_4
24h
1 2 3 4
Methyl Cellulose Culture
Establish Feeders Irradiated MS-5
LTC-IC
5 Weeks
-input CFC proliferate and die
-Next CFC produced at later times are derived from LTC-IC
Liquid Culture
1 2 3 4
QPCR analysis for VCN + ALDP expression by flow cytometry
BFU-E + CFU-GM count + colony PCR + QPCR analysis for VCN
Calculate LTC-IC Frequency
L-Calc™ program for limiting dilution analysis
14 Days
100
37
percent transduction efficiency
number of test cells per well
CFU PCR vector analysis for percent transduction efficiency

FIG. 3

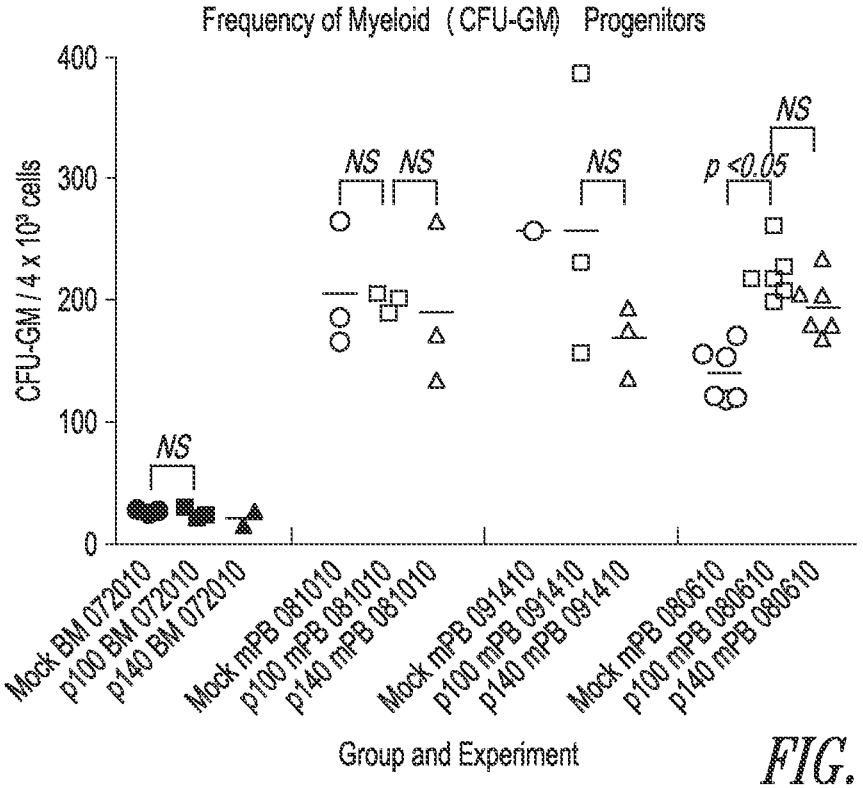

Frequency of Myeloid (CFU-GM) Progenitors
CFU-GM / 4 x 10³ cells
400
300
200
100
0
NS
NS
NS
NS
p <0.05
NS
Mock BM 072010
p100 BM 072010
p140 BM 072010
Mock mPB 081010
p100 mPB 081010
p140 mPB 081010
Mock mPB 091410
p100 mPB 091410
p140 mPB 091410
Mock mPB 080610
p100 mPB 080610
p140 mPB 080610
Group and Experiment

FIG. 4A

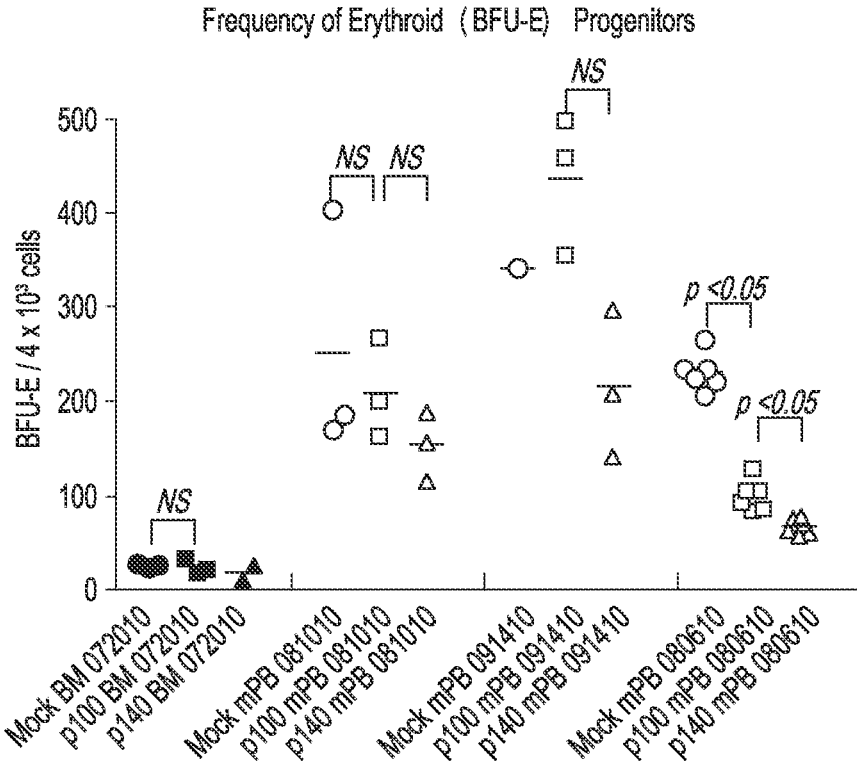

Frequency of Erythroid (BFU-E) Progenitors
BFU-E / 4 x 10³ cells
500
400
300
200
100
0
NS
NS
NS
NS
p <0.05
p <0.05
Mock BM 072010
p100 BM 072010
p140 BM 072010
Mock mPB 081010
p100 mPB 081010
p140 mPB 081010
Mock mPB 091410
p100 mPB 091410
p140 mPB 091410
Mock mPB 080610
p100 mPB 080610
p140 mPB 080610
Group and Experiment

FIG. 4B

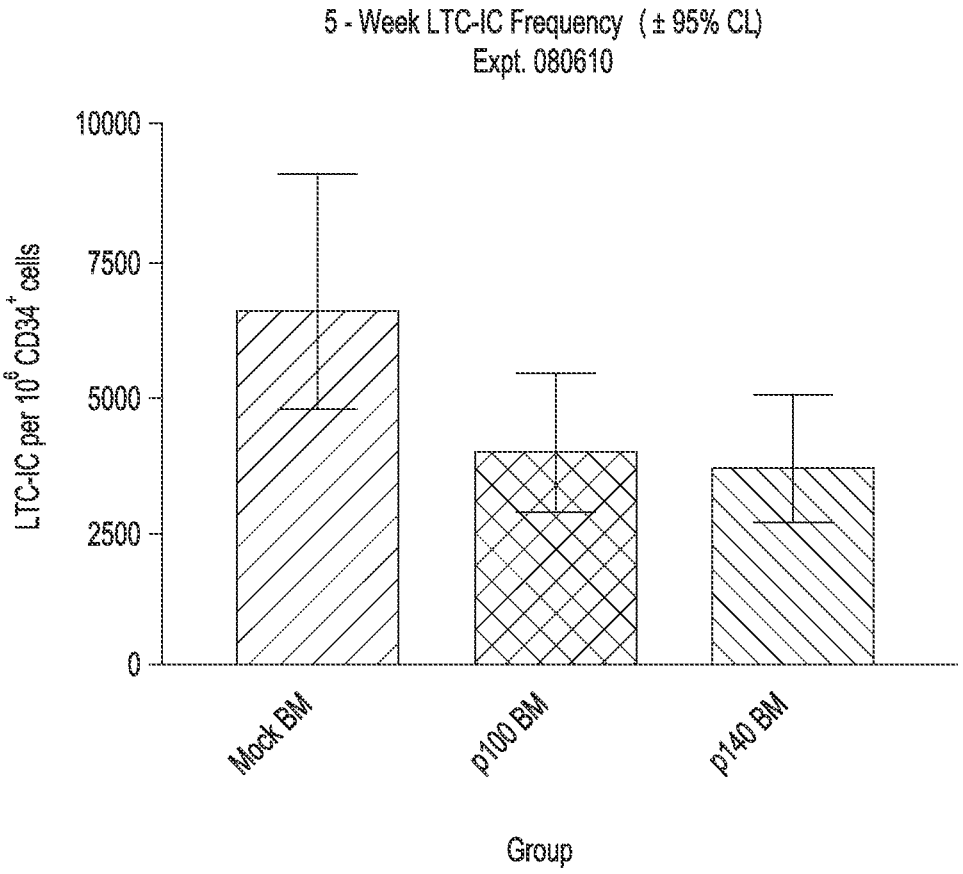

5 - Week LTC-IC Frequency (± 95% CL)
Expt. 080610
LTC-IC per 10^6 CD34^+ cells
10000
7500
5000
2500
0
Mock BM
p100 BM
p140 BM
Group

FIG. 5

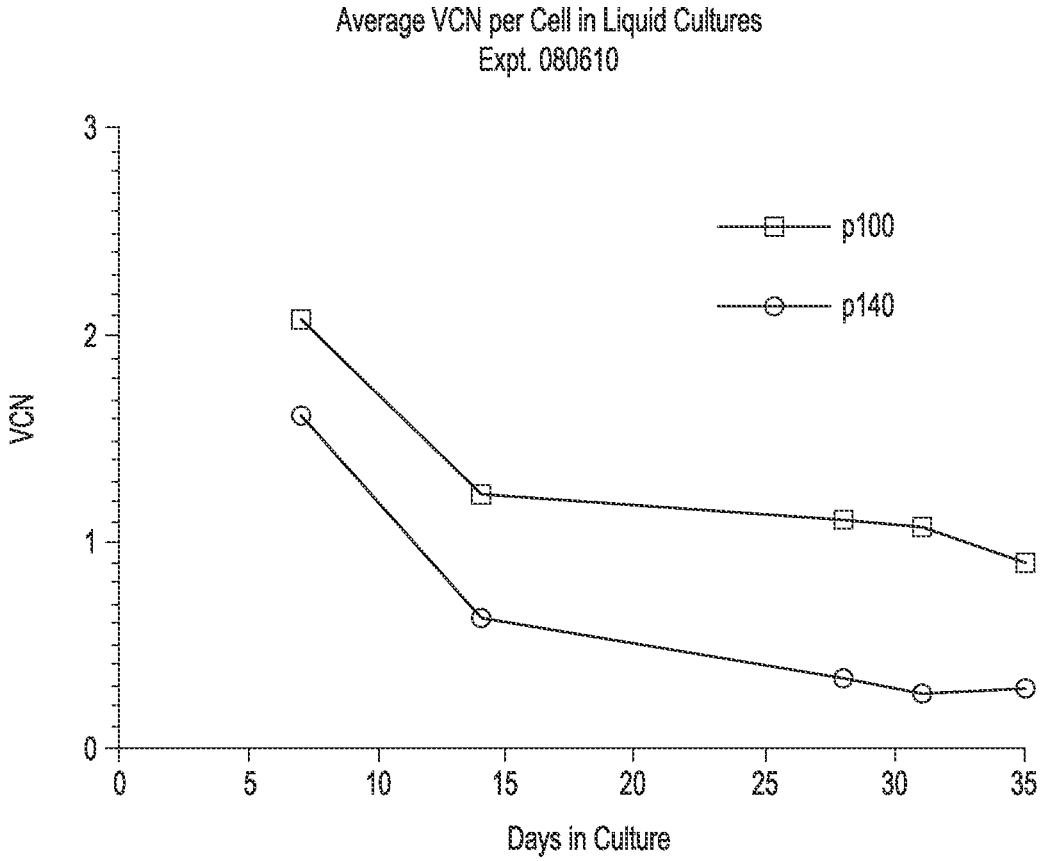

Average VCN per Cell in Liquid Cultures
Expt. 080610
VCN
Days in Culture
p100
p140
0
1
2
3
0
5
10
15
20
25
30
35

FIG. 6A

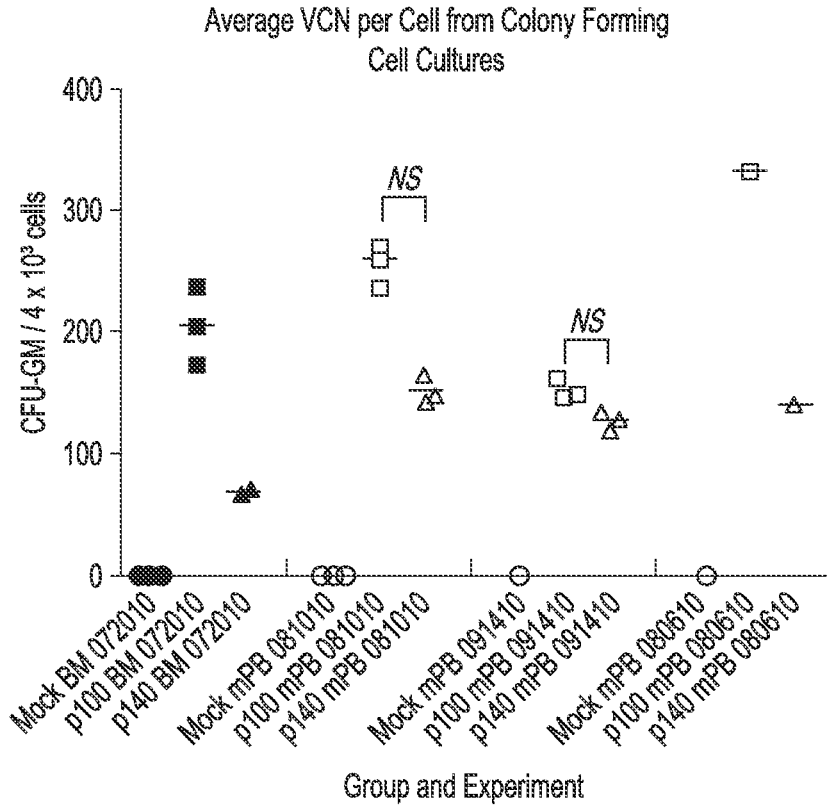

Average VCN per Cell from Colony Forming Cell Cultures
CFU-GM / 4 x 10³ cells
400
300
200
100
0
NS
NS
Mock BM 072010
p100 BM 072010
p140 BM 072010
Mock mPB 081010
p100 mPB 081010
p140 mPB 081010
Mock mPB 091410
p100 mPB 091410
p140 mPB 091410
Mock mPB 080610
p100 mPB 080610
p140 mPB 080610
Group and Experiment

FIG. 6B

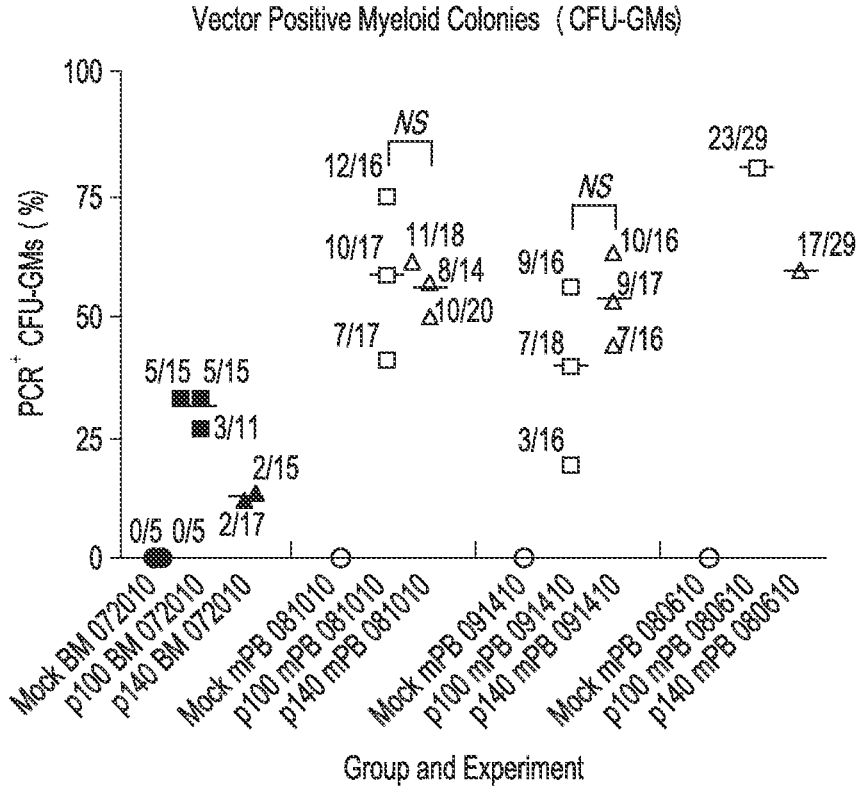

Vector Positive Myeloid Colonies (CFU-GMs)
PCR⁺ CFU-GMs (%)
100
75
50
25
0
NS
NS
12/16
10/17
11/18
8/14
10/20
7/17
5/15 5/15
3/11
2/15
0/5 0/5
2/17
9/16
10/16
9/17
7/18
7/16
3/16
23/29
17/29
Mock BM 072010
p100 BM 072010
p140 BM 072010
Mock mPB 081010
p100 mPB 081010
p140 mPB 081010
Mock mPB 091410
p100 mPB 091410
p140 mPB 091410
Mock mPB 080610
p100 mPB 080610
p140 mPB 080610
Group and Experiment

FIG. 6C

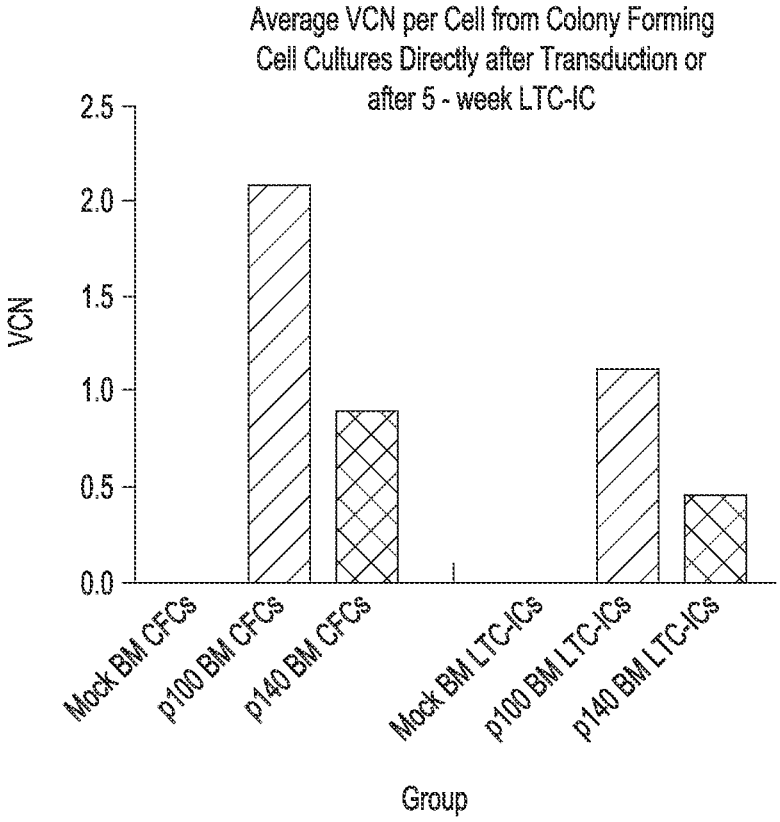

Average VCN per Cell from Colony Forming Cell Cultures Directly after Transduction or after 5 - week LTC-IC
VCN
2.5
2.0
1.5
1.0
0.5
0.0
Mock BM CFCs
p100 BM CFCs
p140 BM CFCs
Mock BM LTC-ICs
p100 BM LTC-ICs
p140 BM LTC-ICs
Group

FIG. 7A

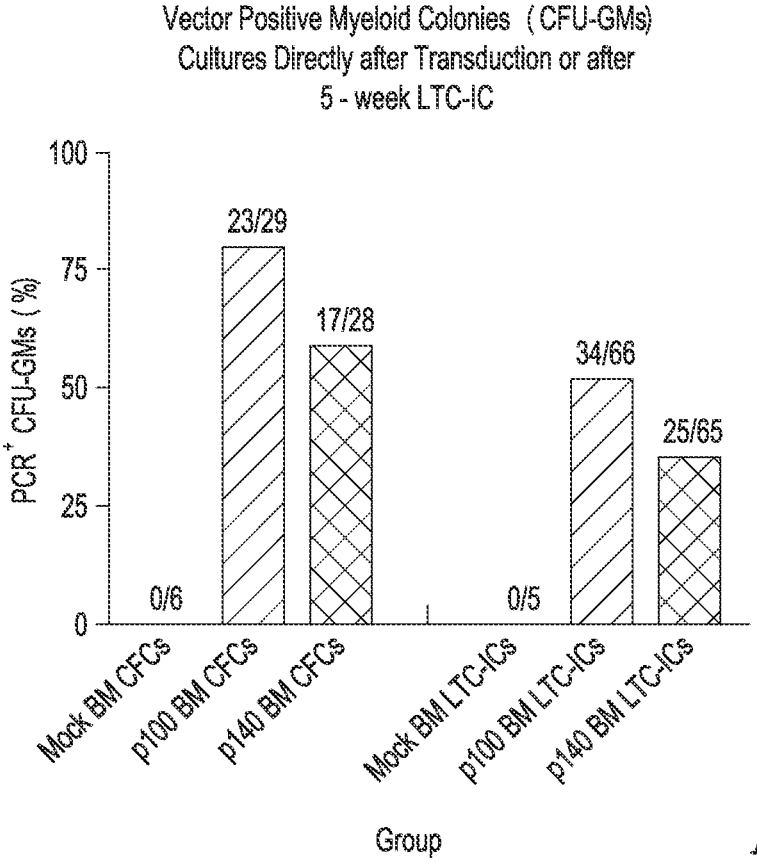

Vector Positive Myeloid Colonies (CFU-GMs) Cultures Directly after Transduction or after 5 - week LTC-IC
PCR+ CFU-GMs (%)
100
75
50
25
0
0/6
23/29
17/28
0/5
34/66
25/65
Mock BM CFCs
p100 BM CFCs
p140 BM CFCs
Mock BM LTC-ICs
p100 BM LTC-ICs
p140 BM LTC-ICs
Group

FIG. 7B

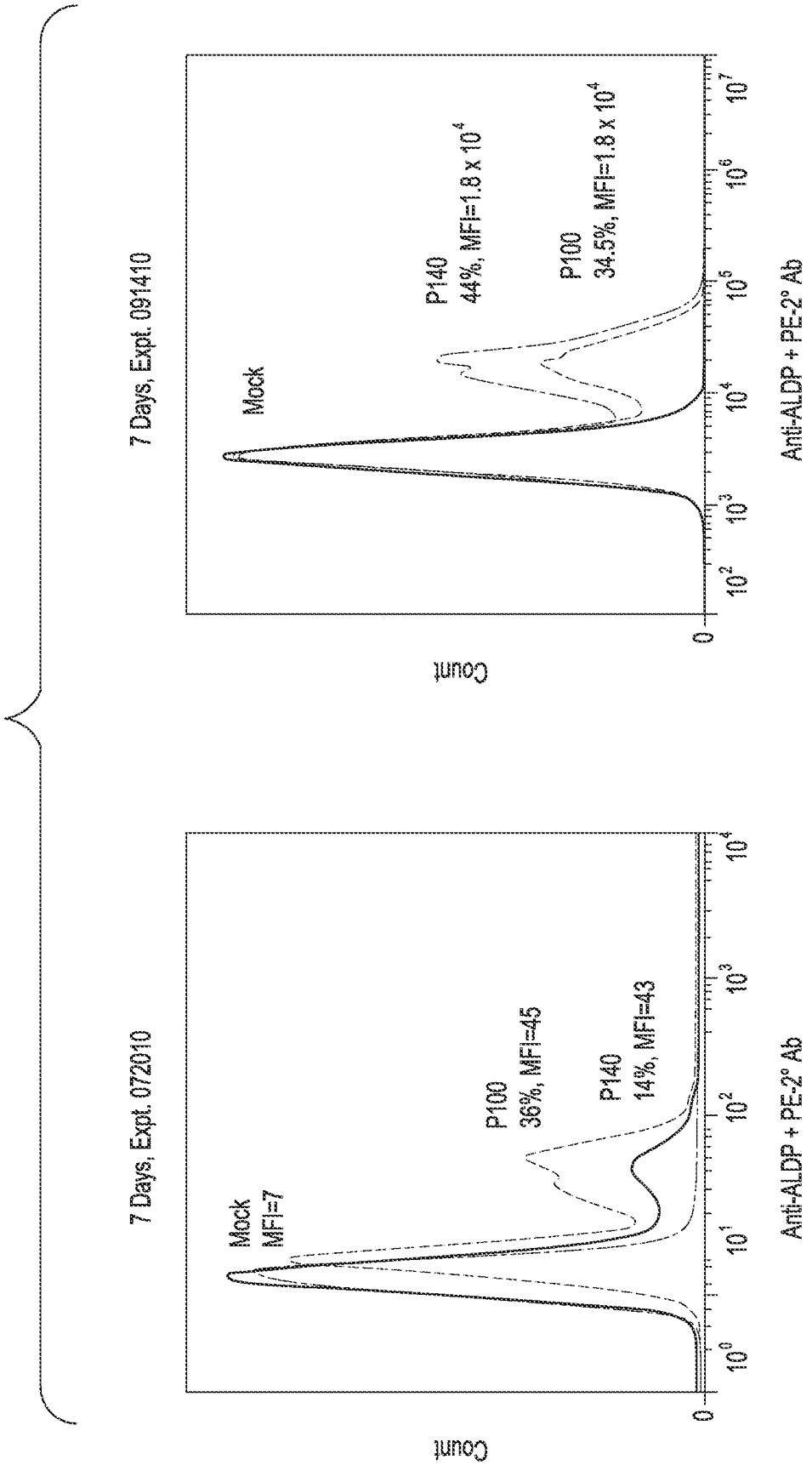

7 Days, Expt. 091410
Mock
P140
44%, MFI=1.8 x 10^4
P100
34.5%, MFI=1.8 x 10^4
Count
Anti-ALDP + PE-2° Ab
7 Days, Expt. 072010
Mock
MFI=7
P100
36%, MFI=45
P140
14%, MFI=43
Count
Anti-ALDP + PE-2° Ab

FIG. 8A1

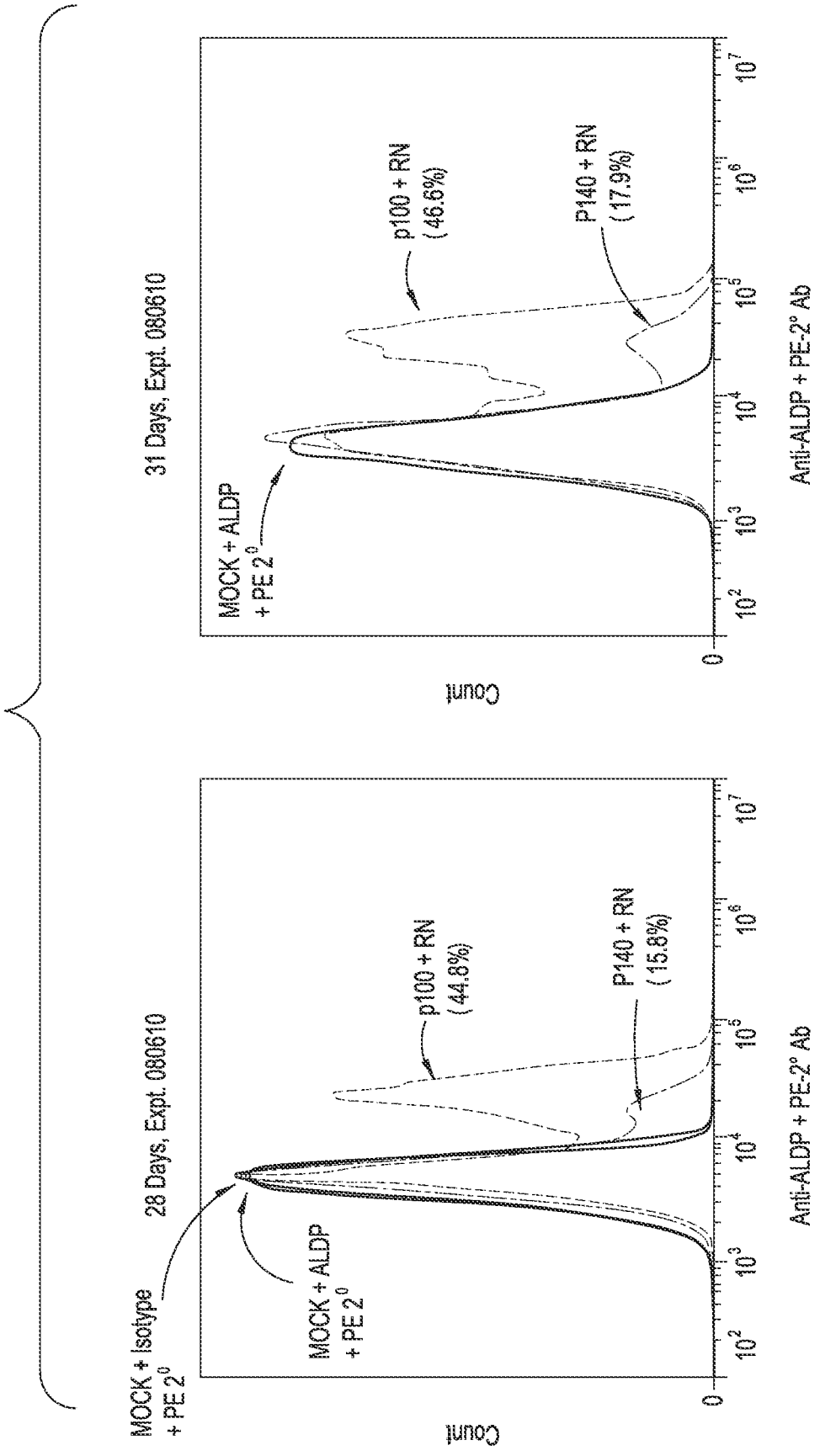

MOCK + Isotype
+ PE 2°
MOCK + ALDP
+ PE 2°
28 Days, Expt. 080610
p100 + RN
(44.8%)
P140 + RN
(15.8%)
Count
Anti-ALDP + PE-2° Ab
MOCK + ALDP
+ PE 2°
31 Days, Expt. 080610
p100 + RN
(46.6%)
P140 + RN
(17.9%)
Count
Anti-ALDP + PE-2° Ab

FIG. 8A2

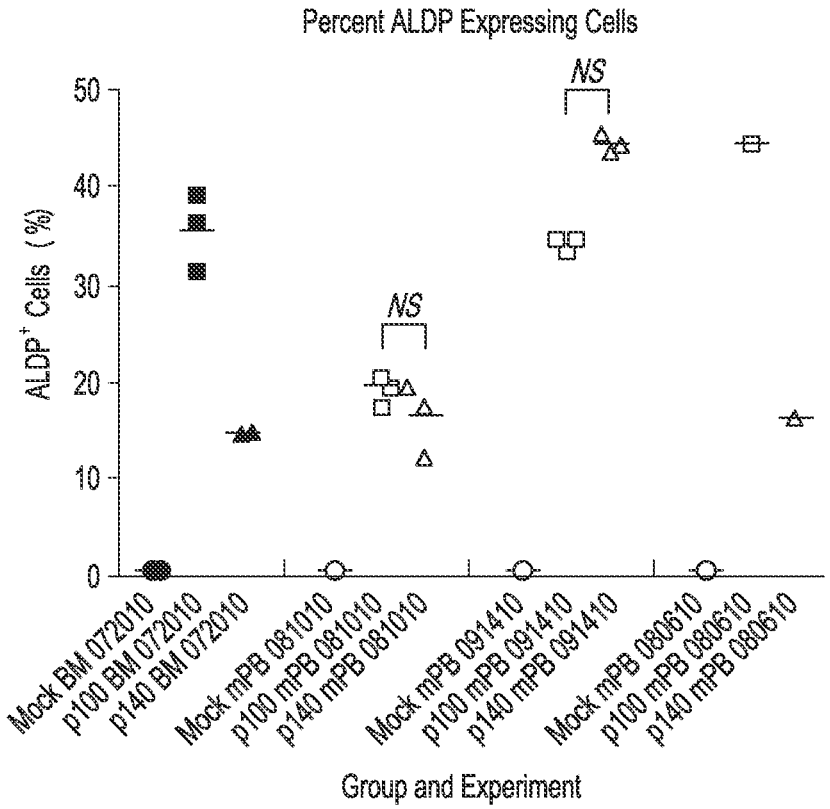

Percent ALDP Expressing Cells
ALDP+ Cells (%)
50
40
30
20
10
0
NS
NS
Mock BM 072010
p100 BM 072010
p140 BM 072010
Mock mPB 081010
p100 mPB 081010
p140 mPB 081010
Mock mPB 091410
p100 mPB 091410
p140 mPB 091410
Mock mPB 080610
p100 mPB 080610
p140 mPB 080610
Group and Experiment

FIG. 8B

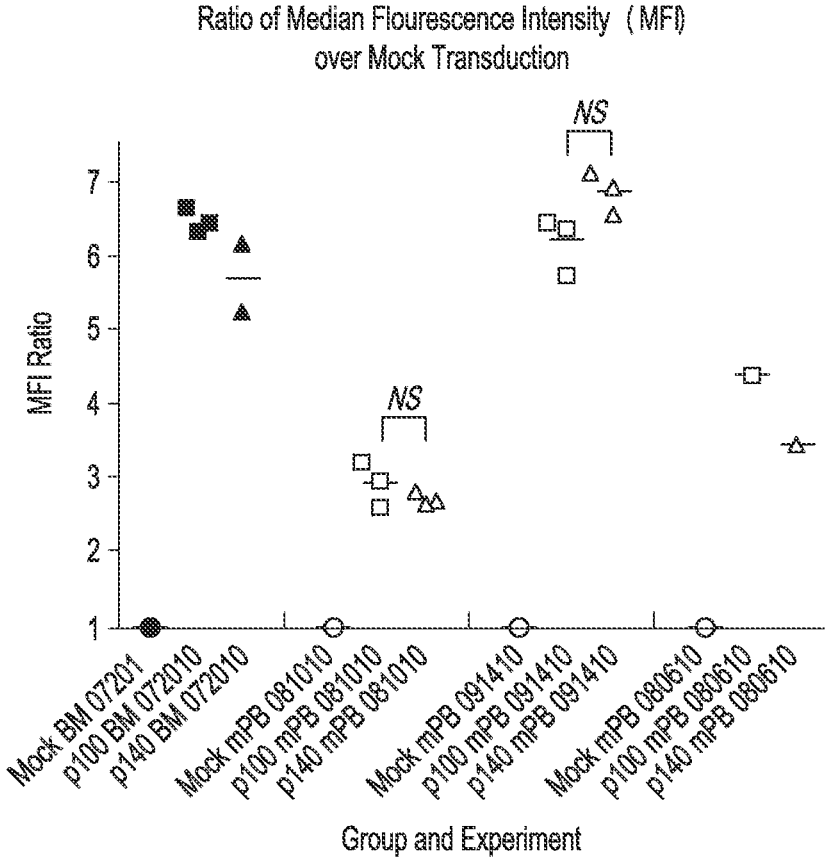

Ratio of Median Flourescence Intensity (MFI)
over Mock Transduction
MFI Ratio
7
6
5
4
3
2
1
NS
NS
Mock BM 07201
p100 BM 072010
p140 BM 072010
Mock mPB 081010
p100 mPB 081010
p140 mPB 081010
Mock mPB 091410
p100 mPB 091410
p140 mPB 091410
Mock mPB 080610
p100 mPB 080610
p140 mPB 080610
Group and Experiment

FIG. 8C

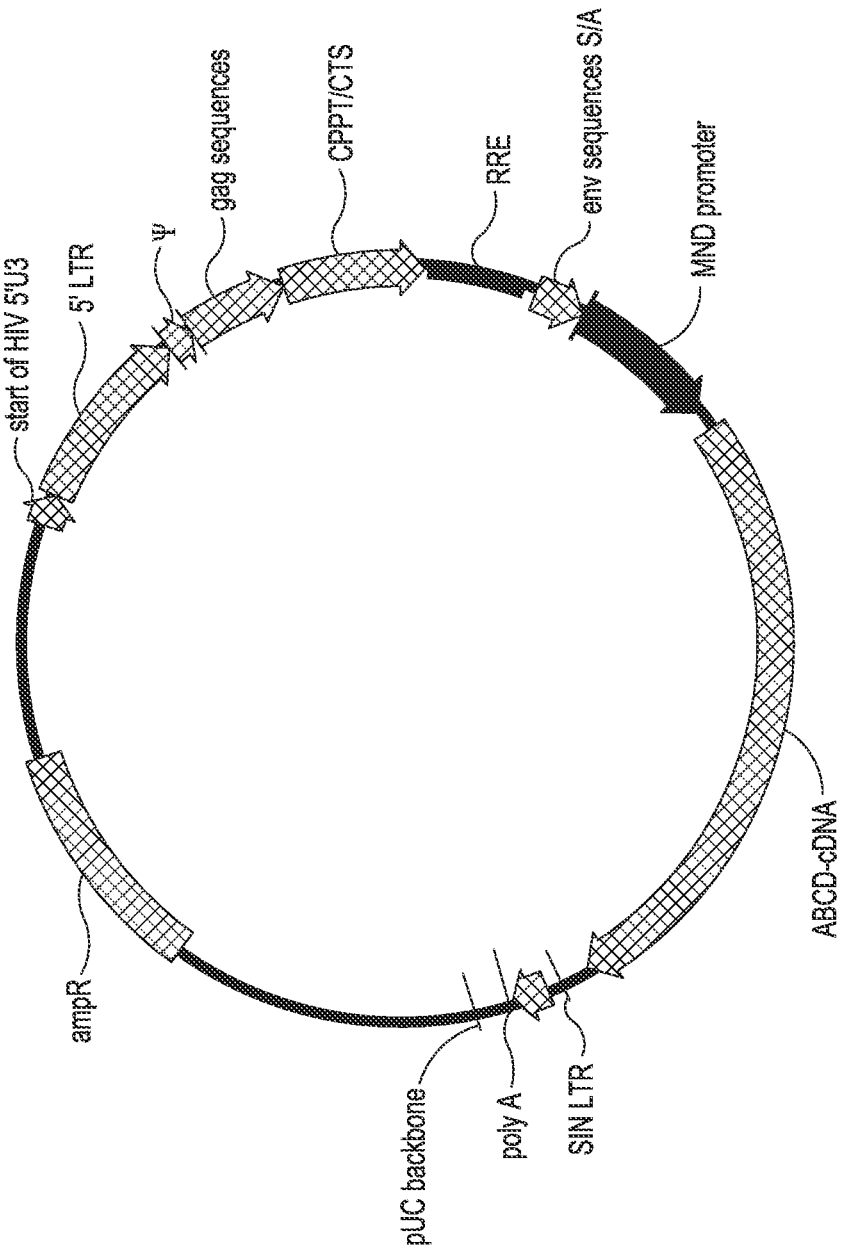

start of HIV 5'U3
5' LTR
Ψ
gag sequences
CPPT/CTS
RRE
env sequences S/A
MND promoter
ABCD-cDNA
SIN LTR
poly A
pUC backbone
ampR

FIG. 9

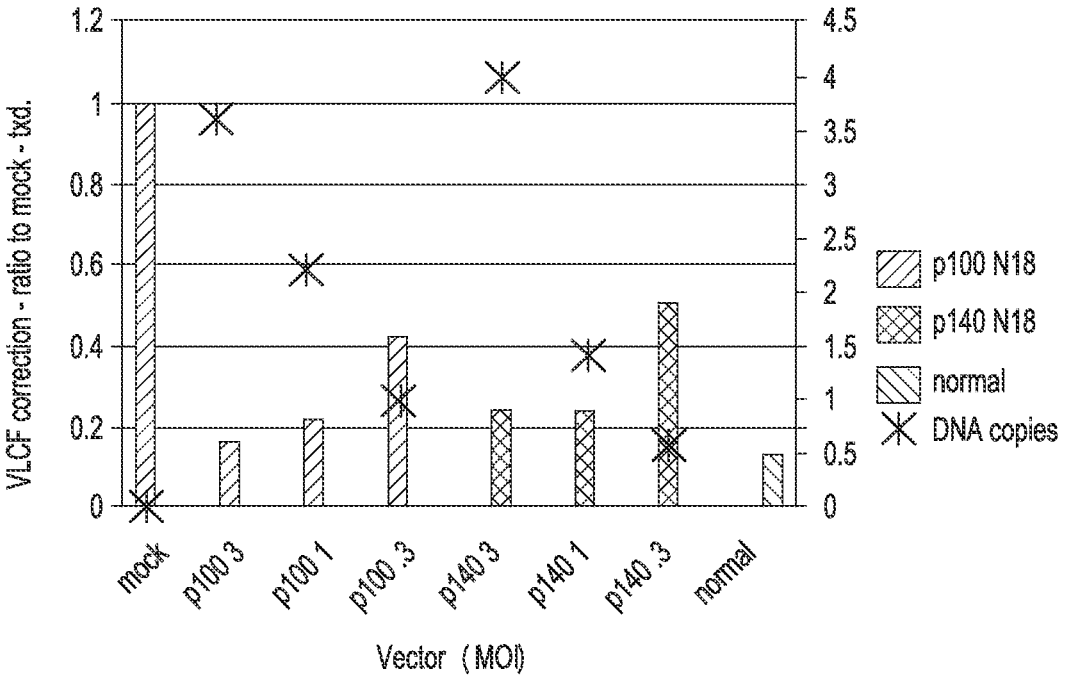

VLCF correction - ratio to mock - txd.
1.2
1
0.8
0.6
0.4
0.2
0
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
mock
p100 3
p100 1
p100 .3
p140 3
p140 1
p140 .3
normal
Vector (MOI)
p100 N18
p140 N18
normal
DNA copies

FIG. 10