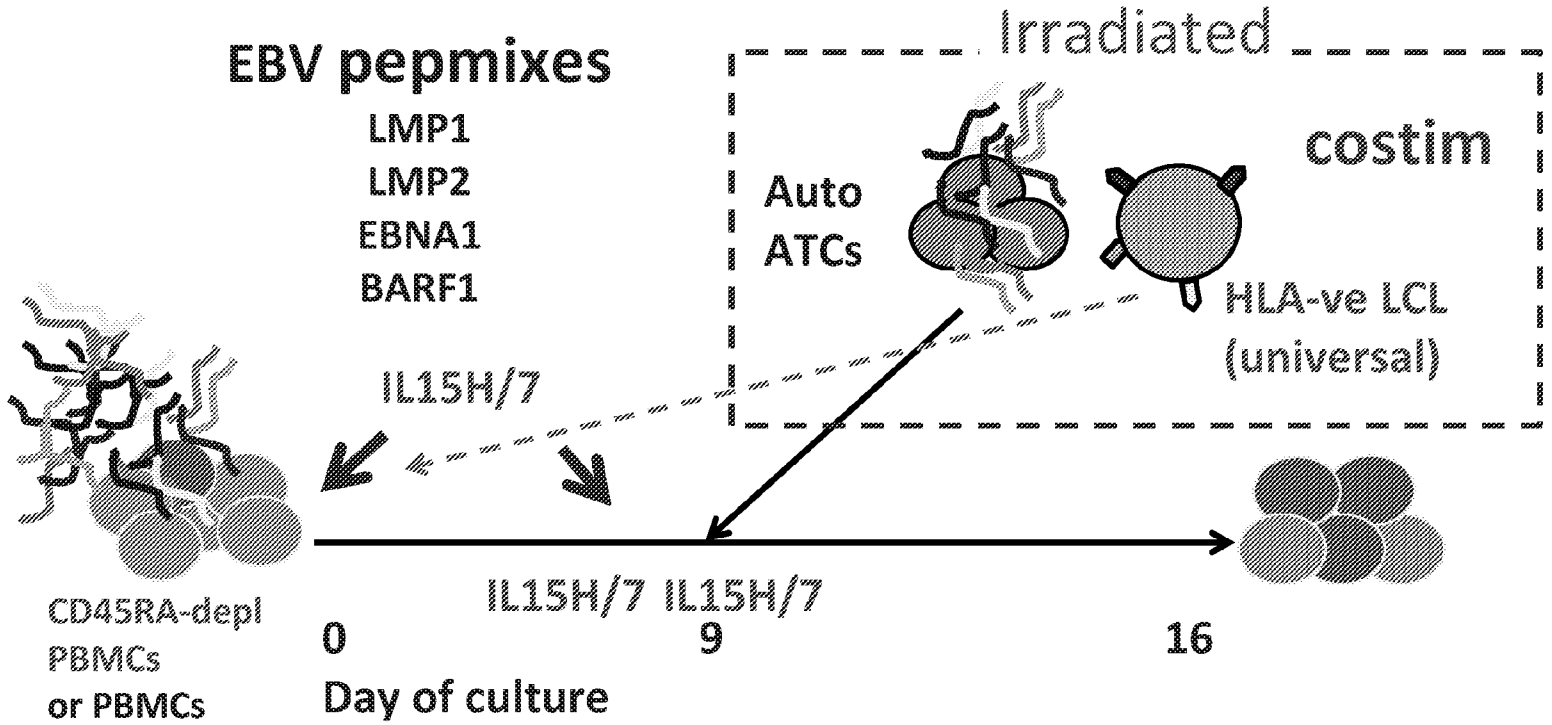


Day 0: Optional: Deplete CD45RA+ T-cells from PBMC
Pulse PBMCs or CD45RA-depleted PBMCs with viral peptides
Optional: Use irradiated HLA-ve LCL in first stimulation
Day 9: 2nd stimulation with peptide-pulsed irradiated autologous ATCs and HLA-ve LCLs
Day 16: Cryopreserve

FIG. 1

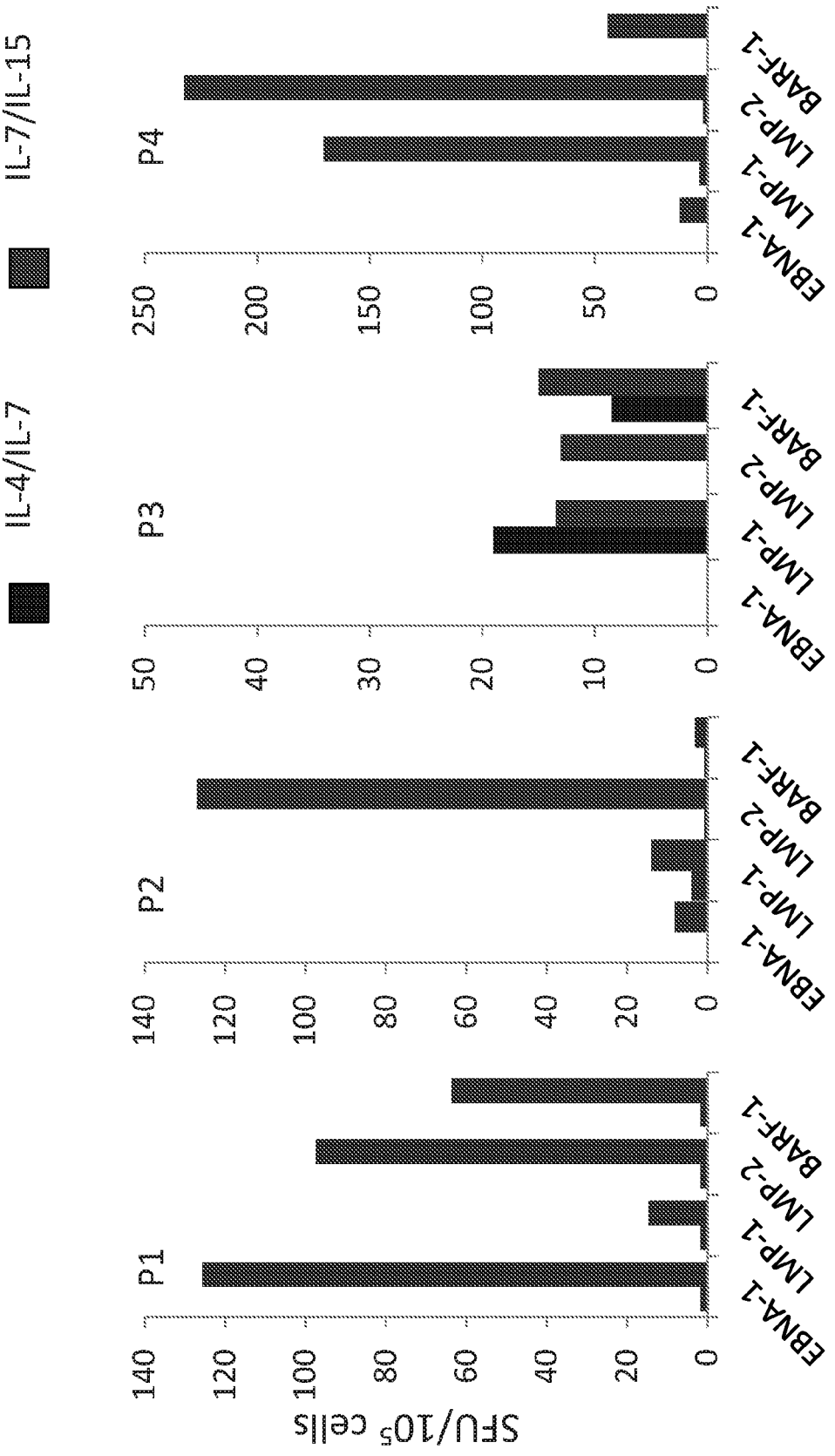

IL-4/IL-7
IL-7/IL-15
SFU/10^5 cells
P1
P2
P3
P4
EBNA-1
LMP-1
LMP-2
BARF-1
0
20
40
60
80
100
120
140
10
30
50
150
200
250

FIG. 2

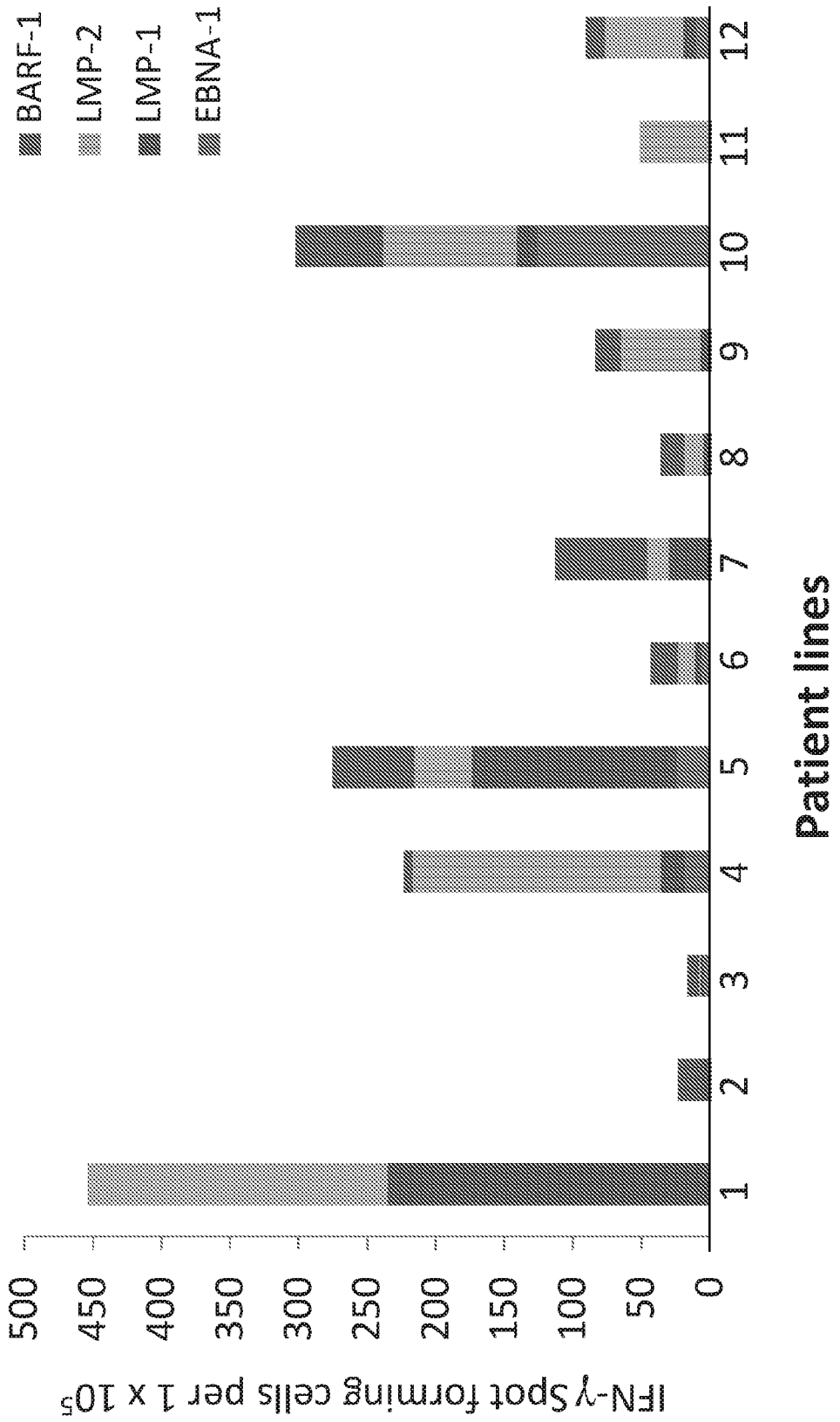

BARF-1
LMP-2
LMP-1
EBNA-1
IFN-γ Spot forming cells per 1 x 10⁵
0
50
100
150
200
250
300
350
400
450
500
1
2
3
4
5
6
7
8
9
10
11
12
Patient lines

FIG. 3

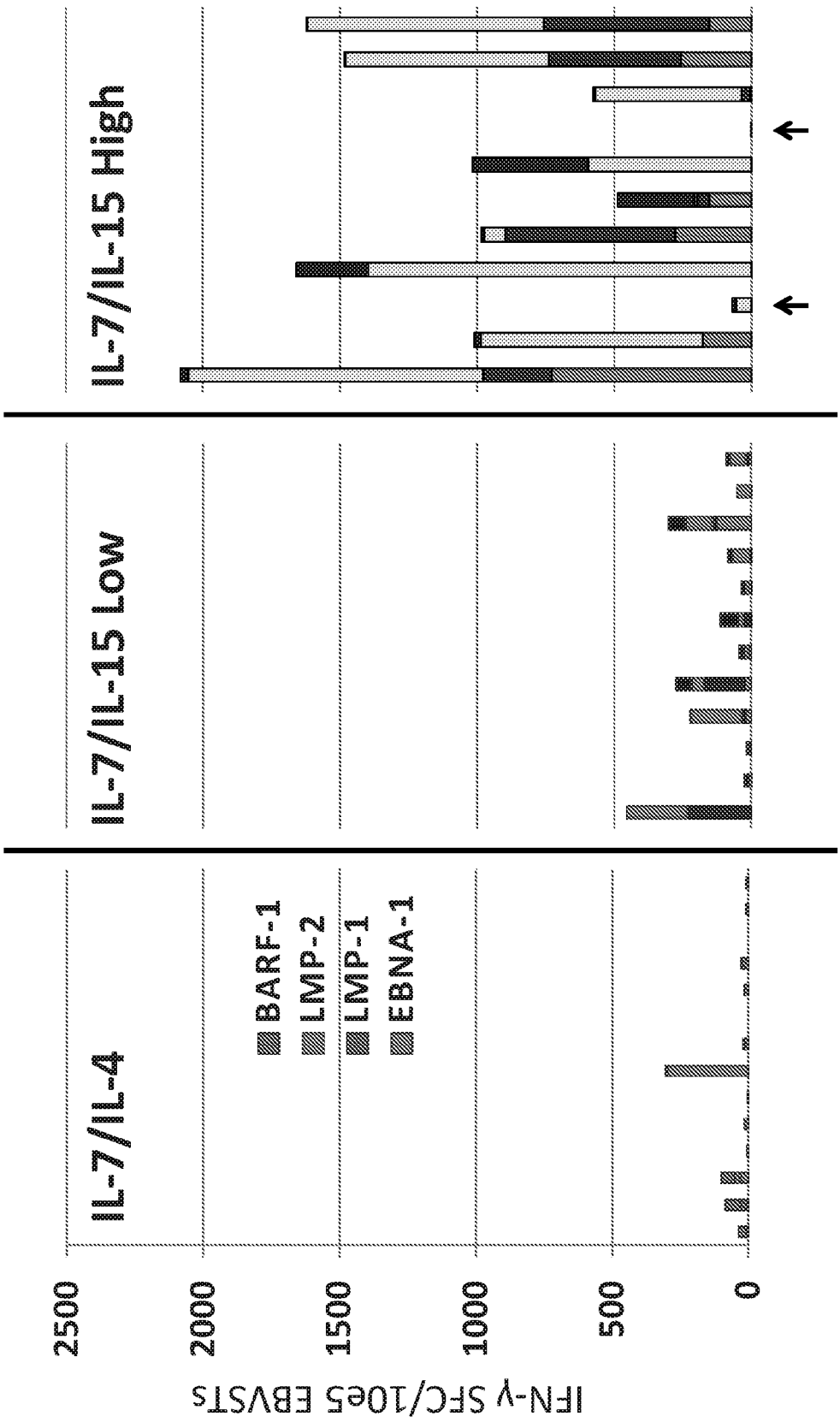

IL-7/IL-4
IL-7/IL-15 Low
IL-7/IL-15 High
BARF-1
LMP-2
LMP-1
EBNA-1
IFN-γ SFC/10e5 EBVSTs
2500
2000
1500
1000
500
0

FIG. 4

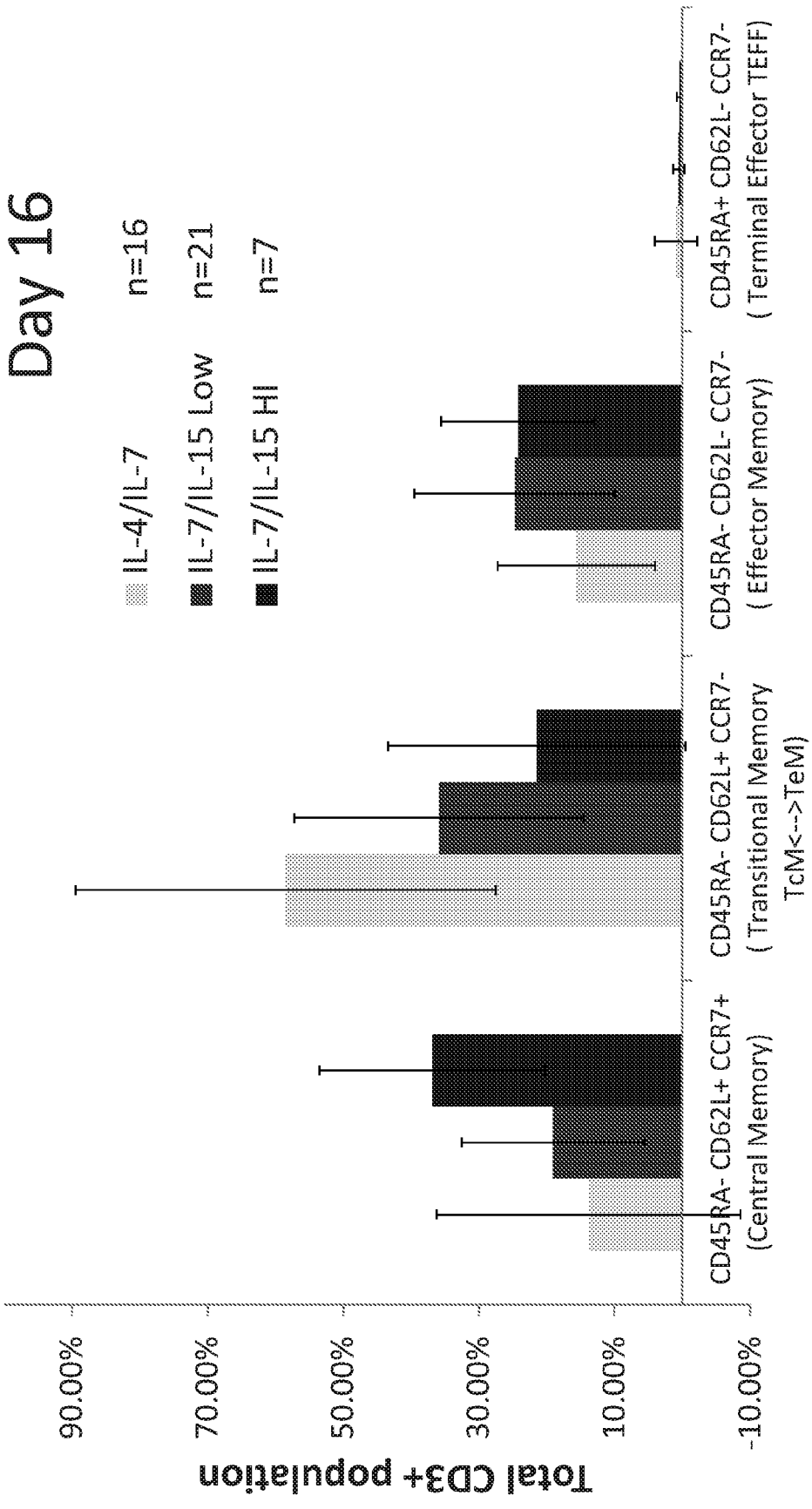

Day 16
IL-4/IL-7 n=16
IL-7/IL-15 Low n=21
IL-7/IL-15 HI n=7
Total CD3+ population
90.00%
70.00%
50.00%
30.00%
10.00%
-10.00%
CD45RA- CD62L+ CCR7+ (Central Memory)
CD45RA- CD62L+ CCR7- (Transitional Memory TcM<-->TeM)
CD45RA- CD62L- CCR7- (Effector Memory)
CD45RA+ CD62L- CCR7- (Terminal Effector TEFF)

FIG. 5

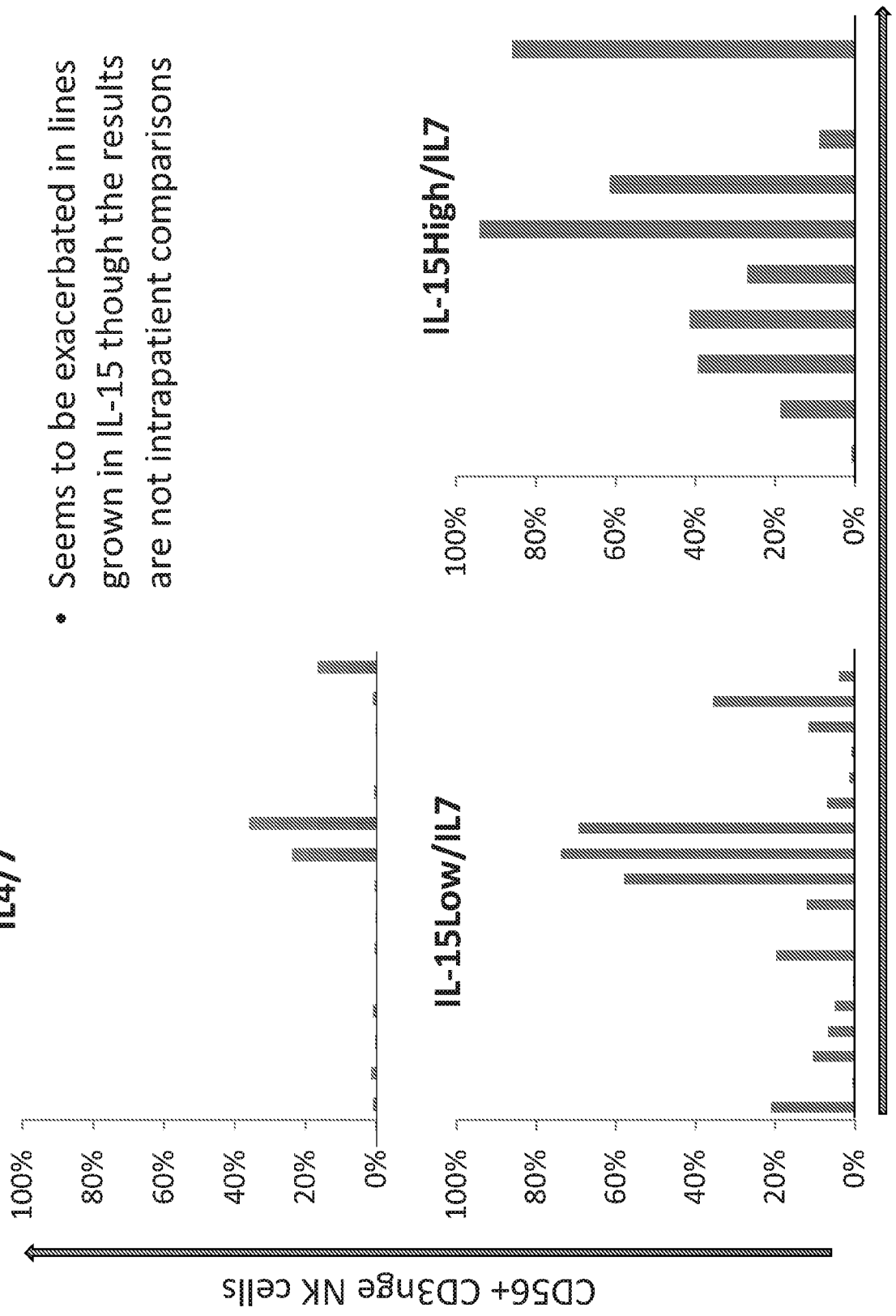

IL4/7
IL-15Low/IL7
IL-15High/IL7
• Seems to be exacerbated in lines grown in IL-15 though the results are not intrapatient comparisons
100%
80%
60%
40%
20%
0%
CD56+ CD3nge NK cells
Patient Lines

FIG. 6

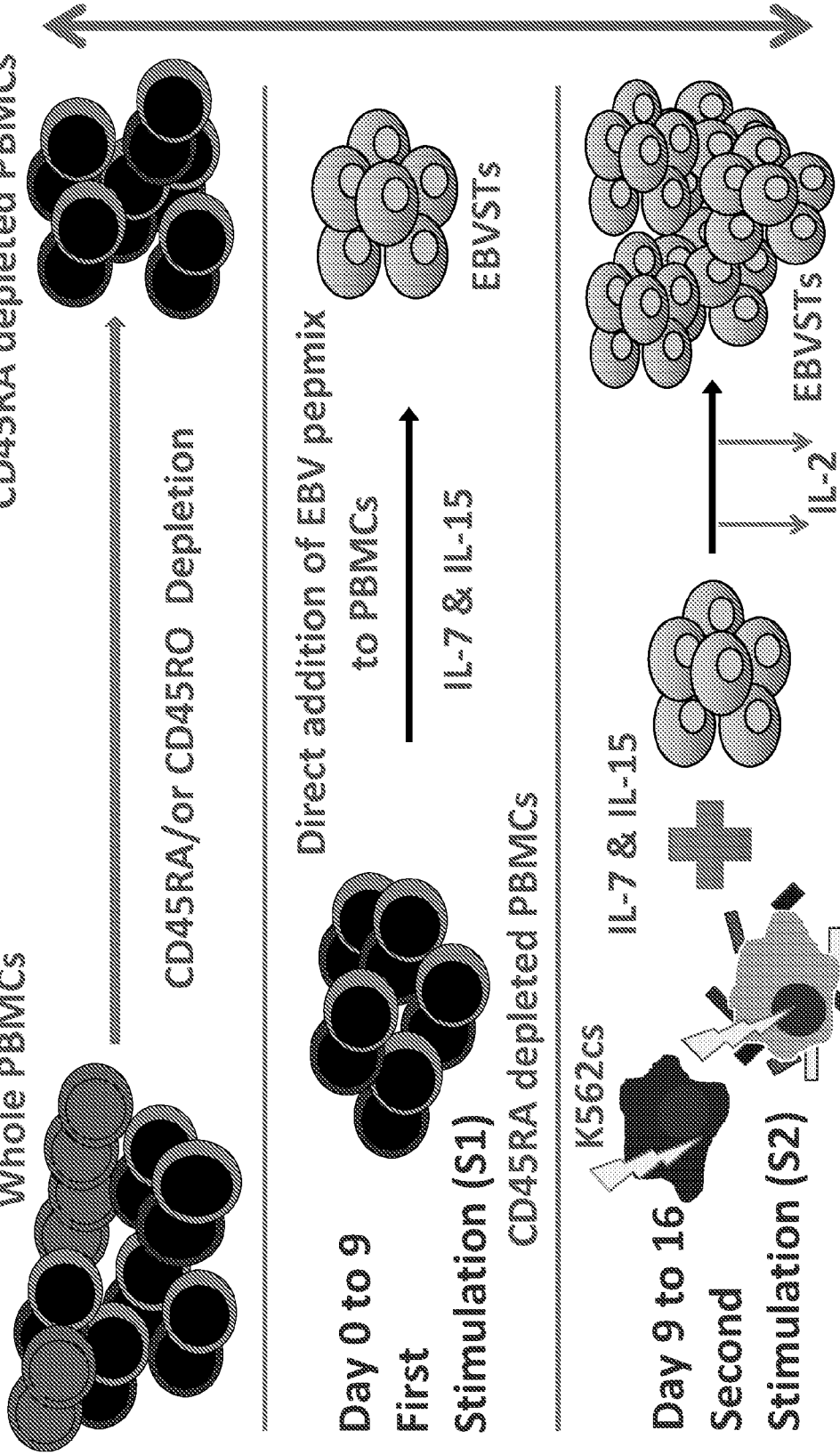

Whole PBMCs
CD45RA depleted PBMCs
CD45RA/or CD45RO Depletion
Day 0 to 9
First
Stimulation (S1)
CD45RA depleted PBMCs
Direct addition of EBV pepmix
to PBMCs
IL-7 & IL-15
EBVSTs
Day 9 to 16
Second
Stimulation (S2)
K562cs
IL-7 & IL-15
EBV-Pepmix pulsed ATCs
IL-2
EBVSTs

FIG. 7

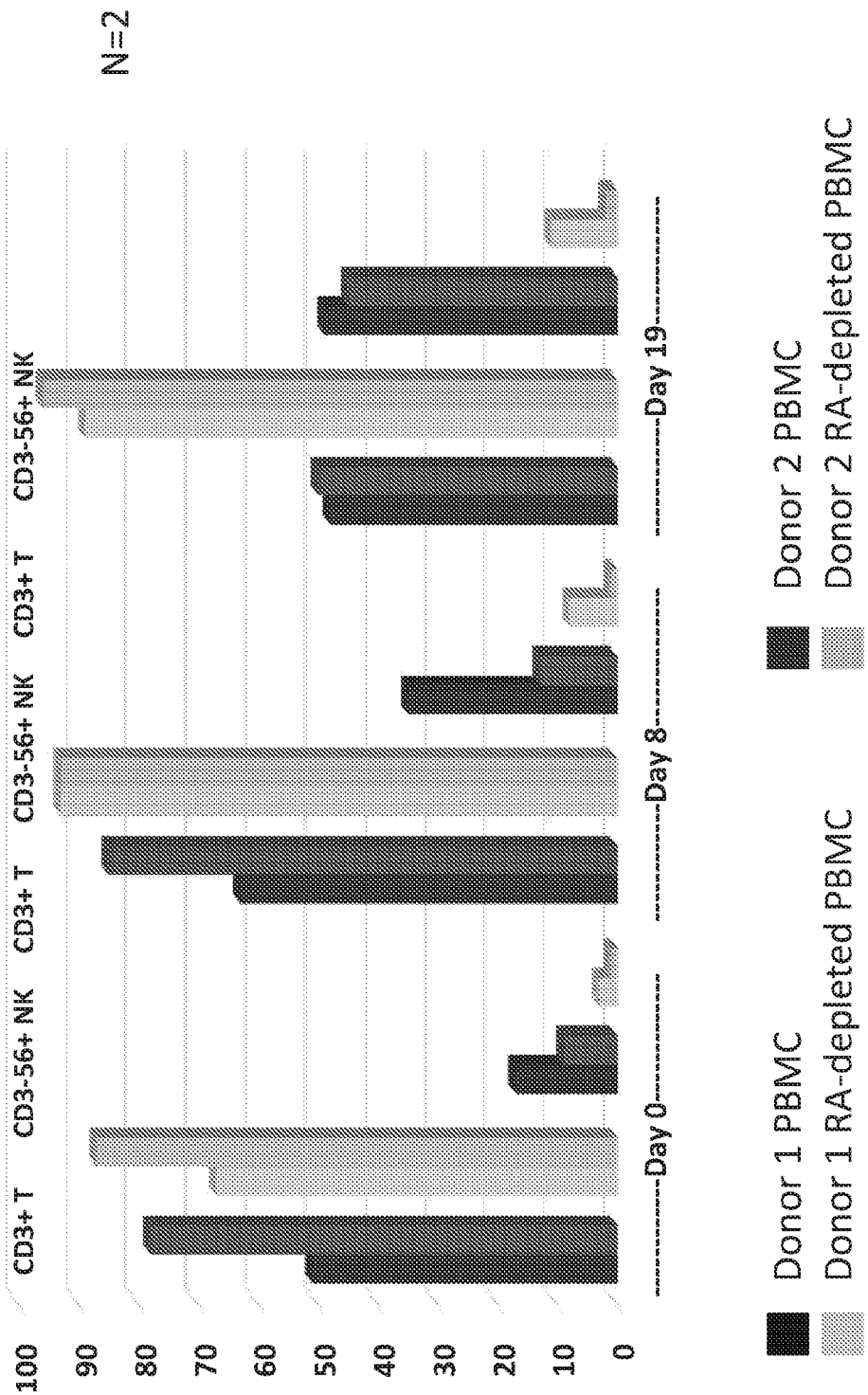

N=2
100
90
80
70
60
50
40
30
20
10
0
CD3+ T
CD3-56+ NK
CD3+ T
CD3-56+ NK
CD3+ T
CD3-56+ NK
Day 0
Day 8
Day 19
Donor 1 PBMC
Donor 1 RA-depleted PBMC
Donor 2 PBMC
Donor 2 RA-depleted PBMC

FIG. 8

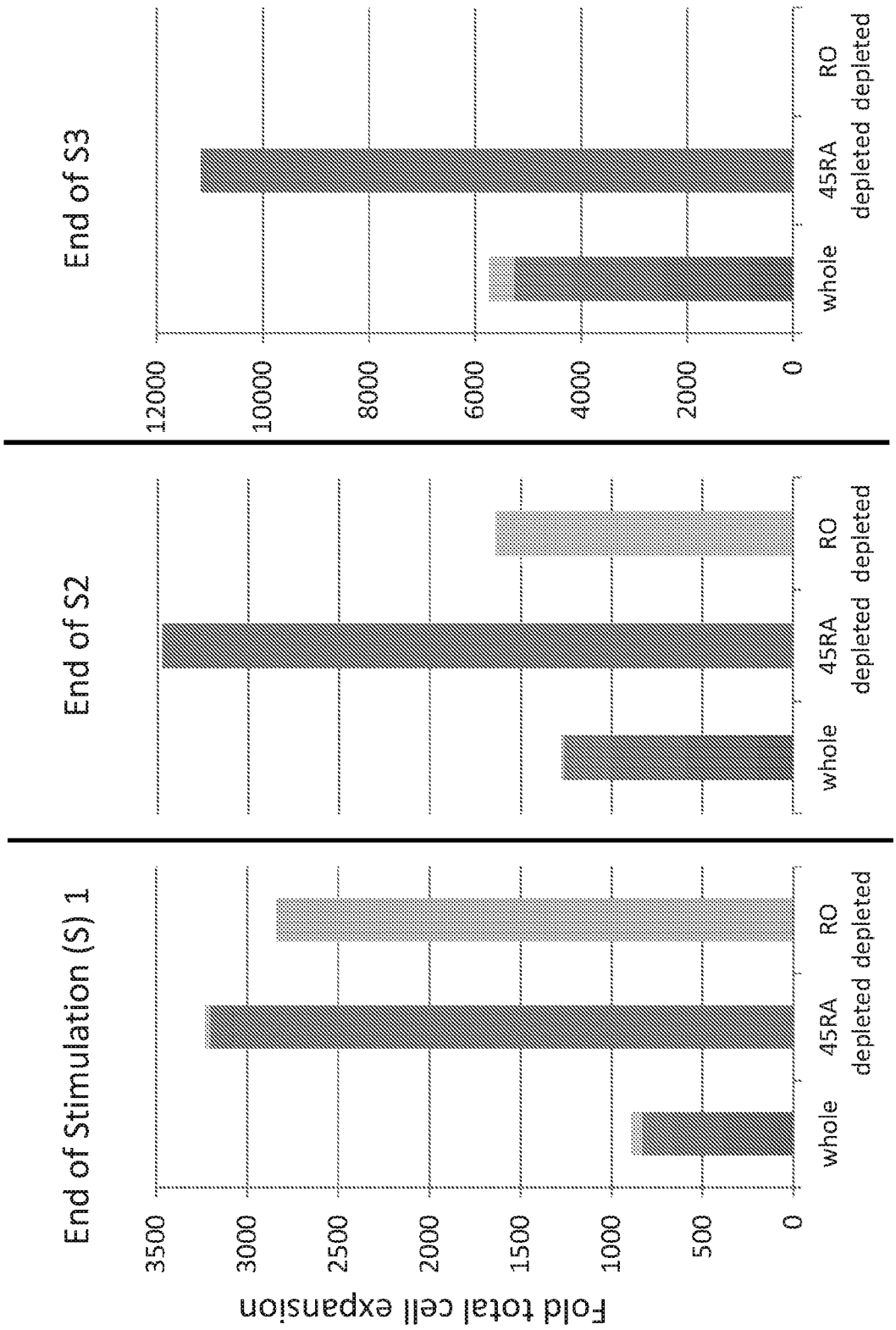

Fold total cell expansion
End of Stimulation (S) 1
End of S2
End of S3
0
500
1000
1500
2000
2500
3000
3500
2000
4000
6000
8000
10000
12000
whole
45RA depleted
RO depleted

FIG. 9

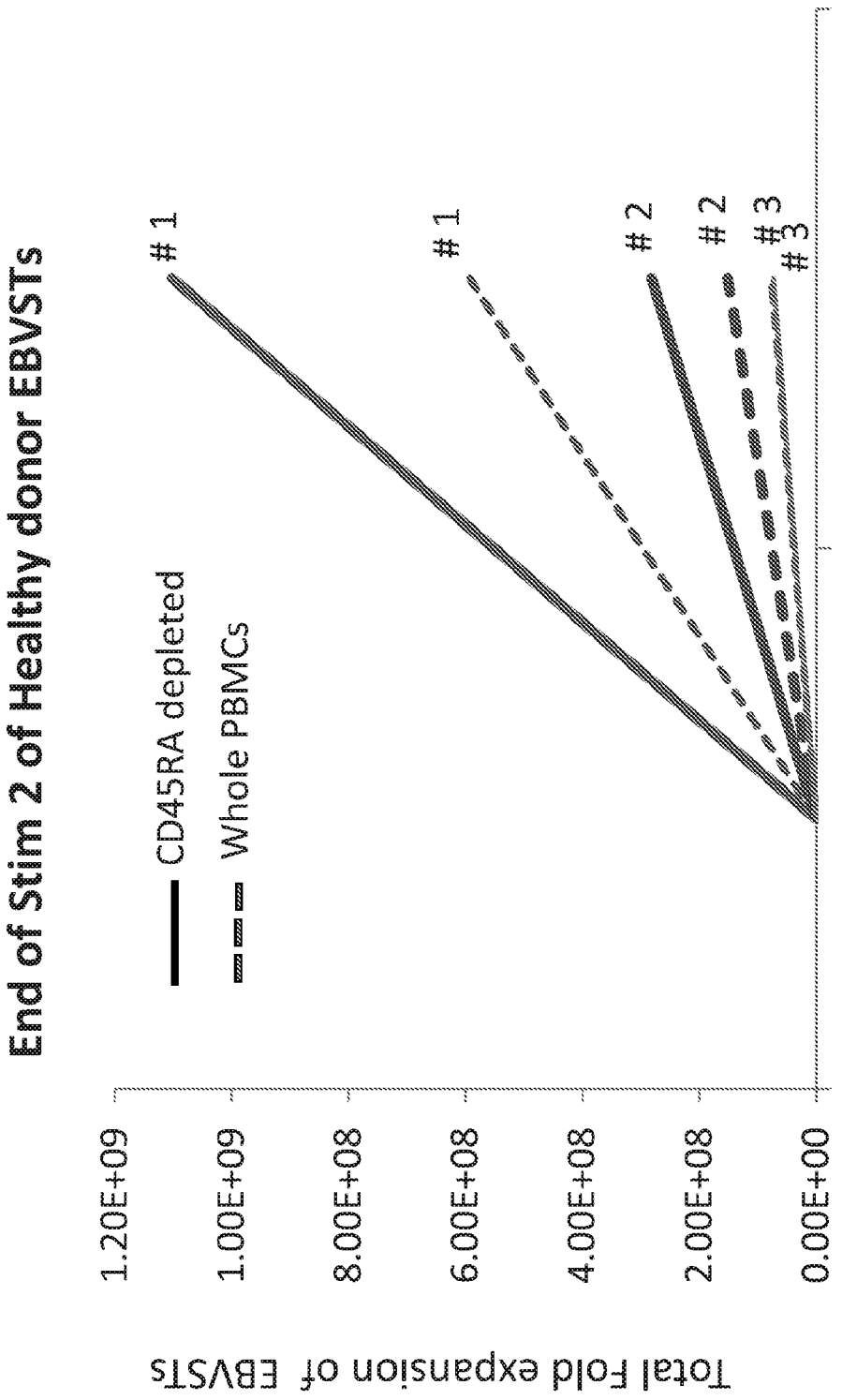

End of Stim 2 of Healthy donor EBVSTs
CD45RA depleted
Whole PBMCs
Total Fold expansion of EBVSTs
1.20E+09
1.00E+09
8.00E+08
6.00E+08
4.00E+08
2.00E+08
0.00E+00
Day 0
S2 D7
1
1
2
2
3
3

FIG. 10

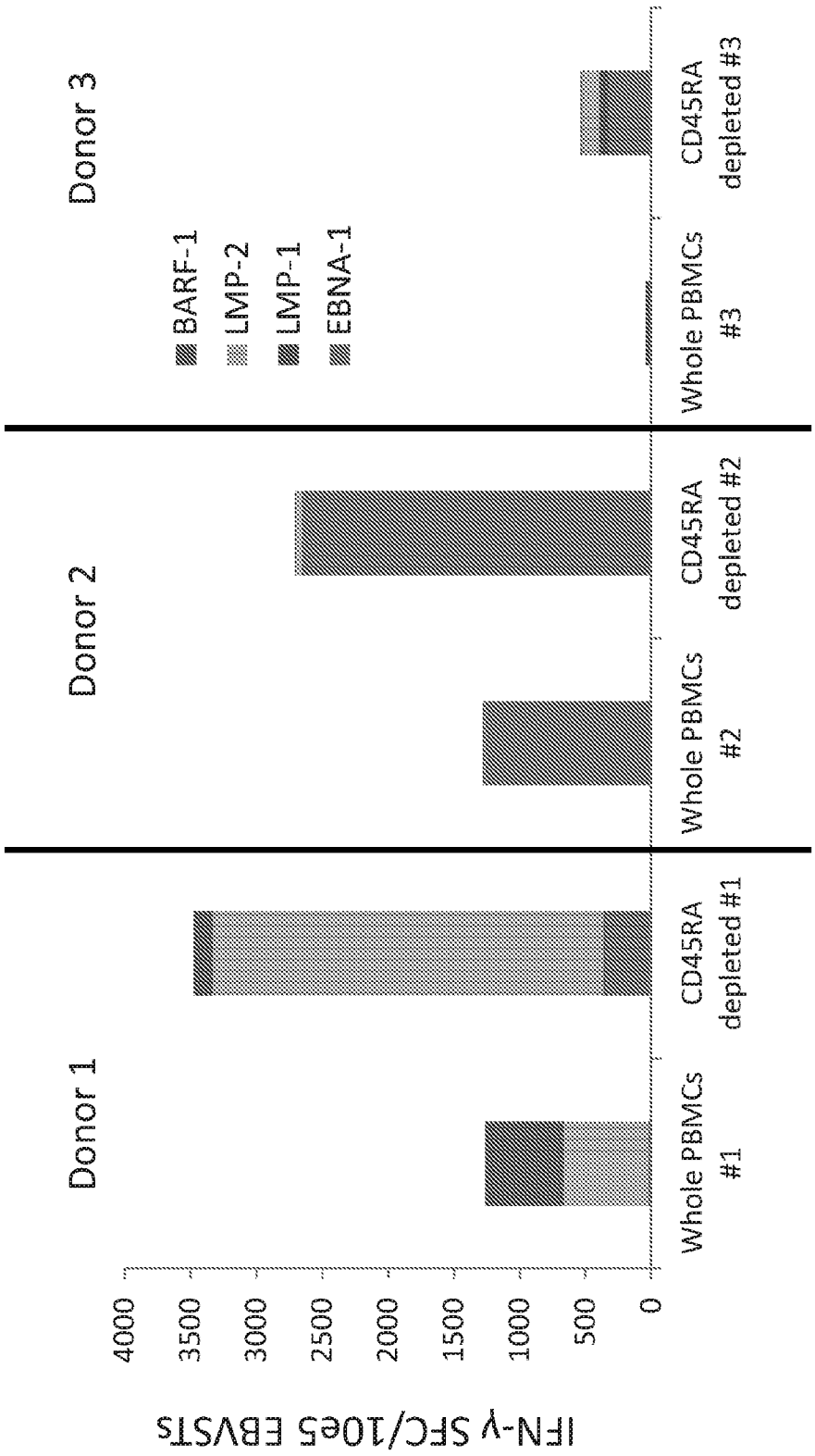

IFN-γ SFC/10e5 EBVSTs
4000
3500
3000
2500
2000
1500
1000
500
0
Donor 1
Donor 2
Donor 3
BARF-1
LMP-2
LMP-1
EBNA-1
Whole PBMCs #1
CD45RA depleted #1
Whole PBMCs #2
CD45RA depleted #2
Whole PBMCs #3
CD45RA depleted #3
Healthy donor lines

FIG. 11

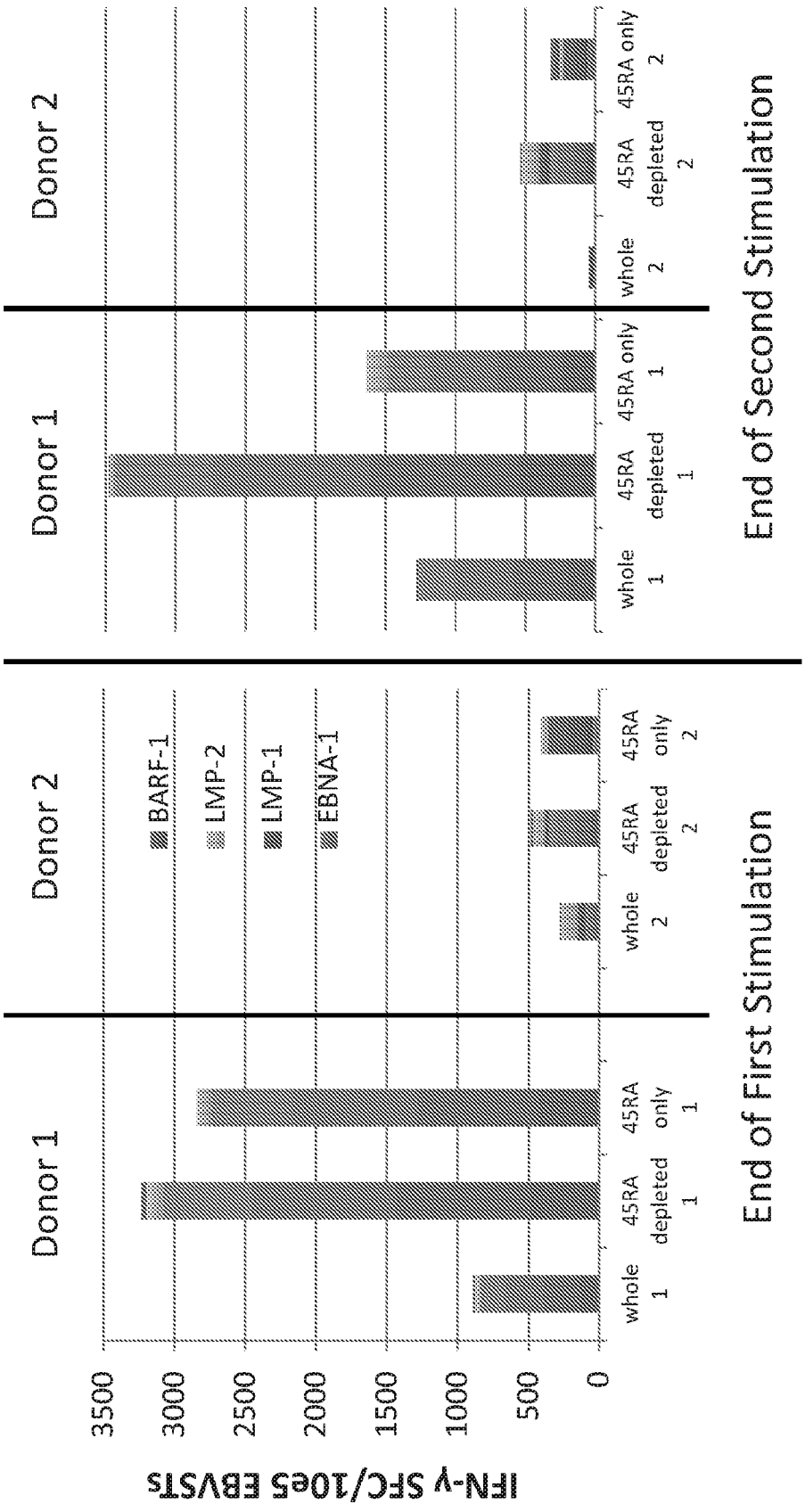

IFN-γ SFC/10e5 EBVSTs
3500
3000
2500
2000
1500
1000
500
0
BARF-1
LMP-2
LMP-1
EBNA-1
Donor 1
Donor 2
whole 1
45RA depleted 1
45RA only 1
whole 2
45RA depleted 2
45RA only 2
End of First Stimulation
End of Second Stimulation

FIG. 12

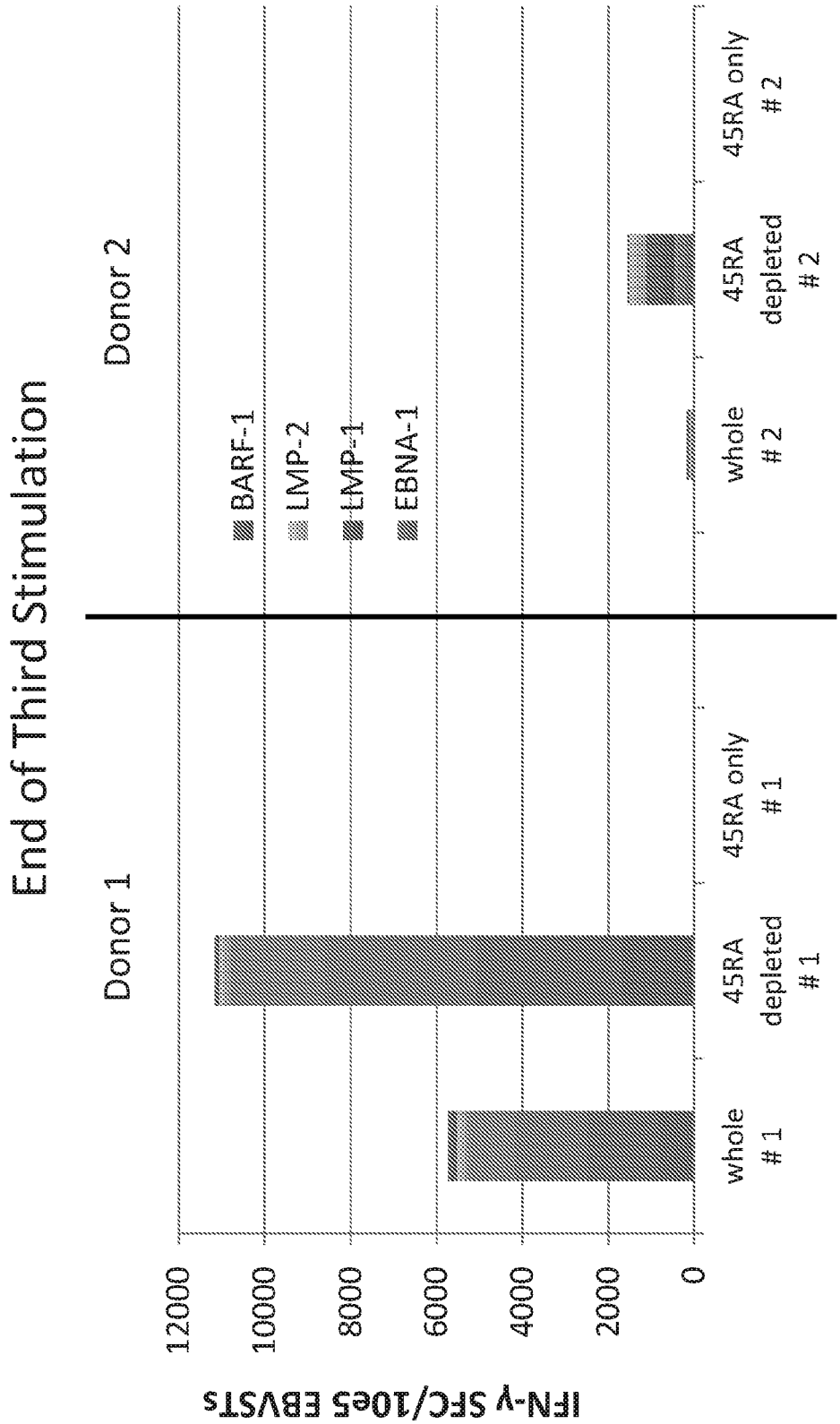

End of Third Stimulation
Donor 1
Donor 2
BARF-1
LMP-2
LMP-1
EBNA-1
IFN-γ SFC/10e5 EBVSTs
12000
10000
8000
6000
4000
2000
0
whole # 1
45RA depleted # 1
45RA only # 1
whole # 2
45RA depleted # 2
45RA only # 2
Note: CD45RA+ cells fail to expand beyond second stimulation

FIG. 13

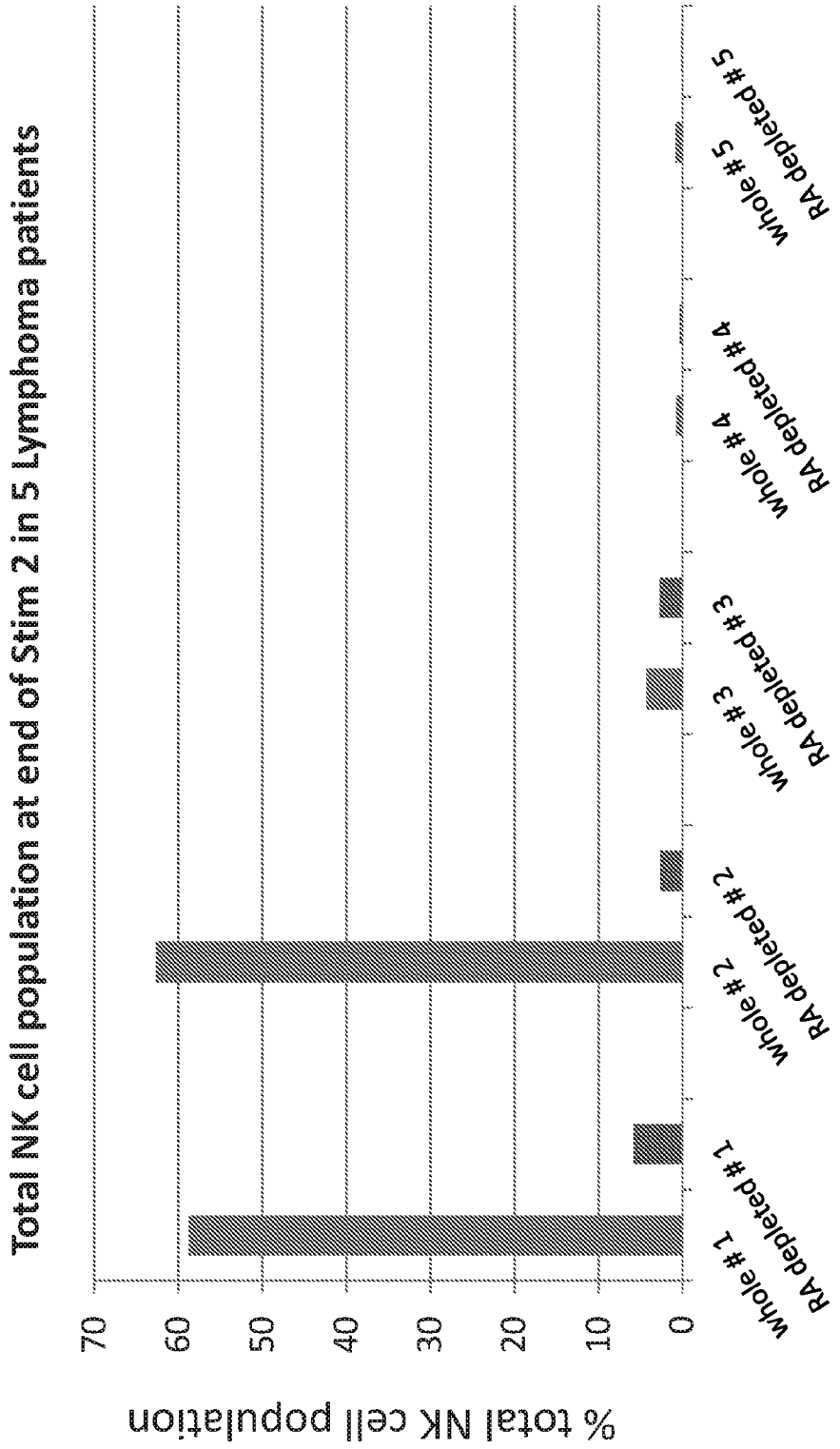

Total NK cell population at end of Stim 2 in 5 Lymphoma patients
% total NK cell population
70
60
50
40
30
20
10
0
Whole #1
RA depleted #1
Whole #2
RA depleted #2
Whole #3
RA depleted #3
Whole #4
RA depleted #4
Whole #5
RA depleted #5

FIG. 14

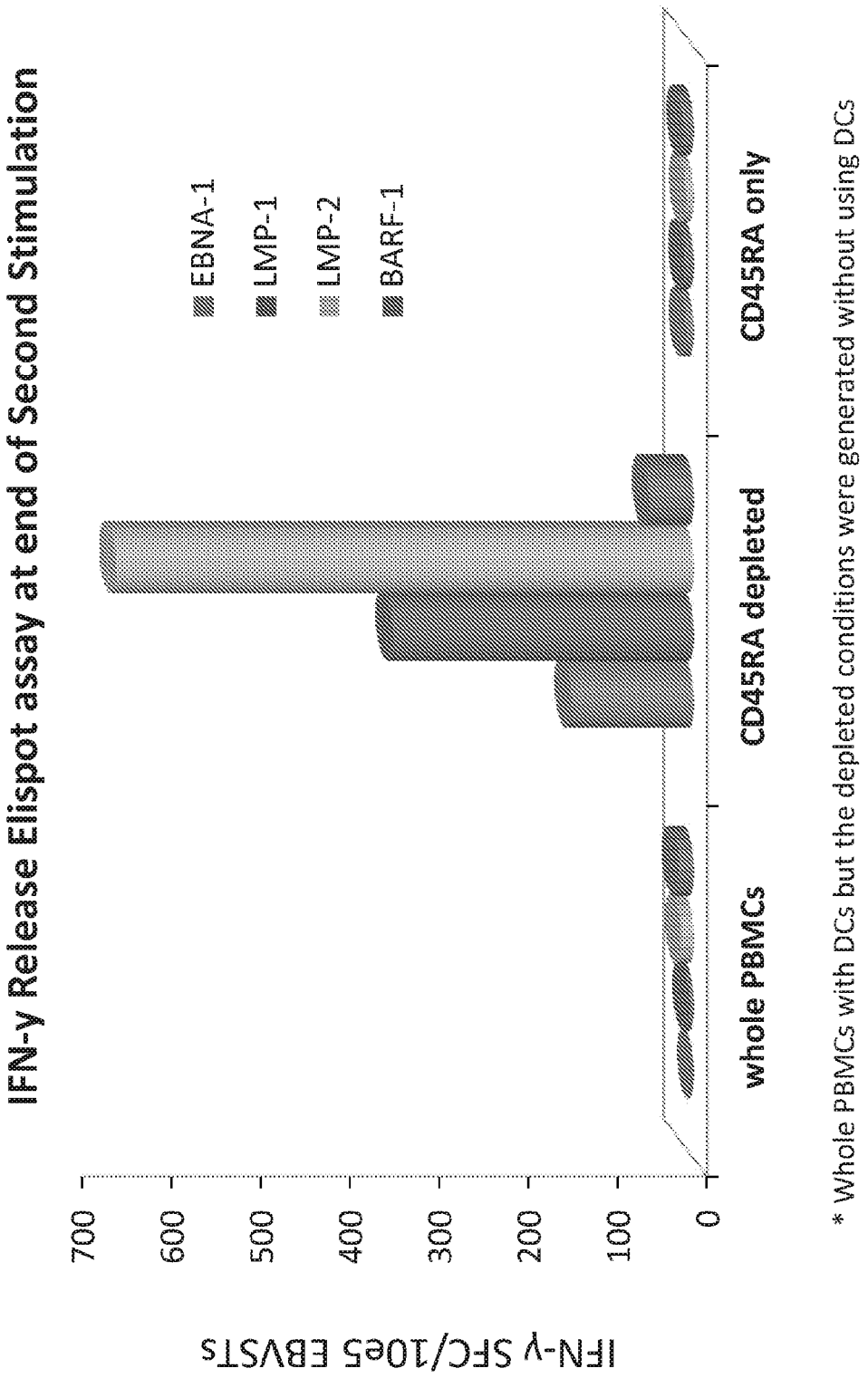

IFN-γ Release Elispot assay at end of Second Stimulation
EBNA-1
LMP-1
LMP-2
BARF-1
IFN-γ SFC/10e5 EBVSTs
700
600
500
400
300
200
100
0
whole PBMCs
CD45RA depleted
CD45RA only
* Whole PBMCs with DCs but the depleted conditions were generated without using DCs

FIG. 15

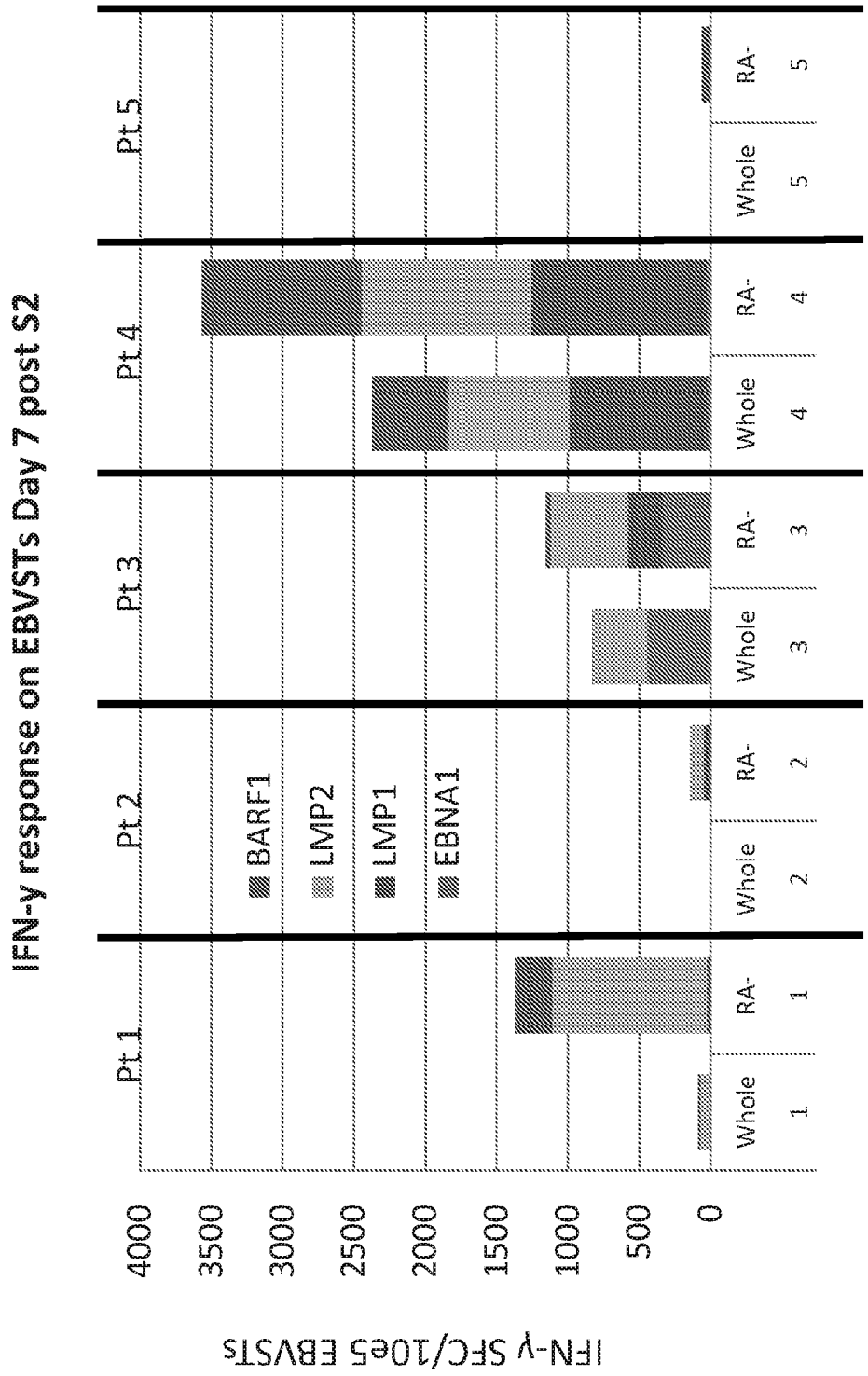

IFN-γ response on EBVSTs Day 7 post S2
IFN-γ SFC/10e5 EBVSTs
4000
3500
3000
2500
2000
1500
1000
500
0
BARF1
LMP2
LMP1
EBNA1
Pt.1
Pt.2
Pt.3
Pt.4
Pt.5
Whole
RA-
1
2
3
4
5

FIG. 16

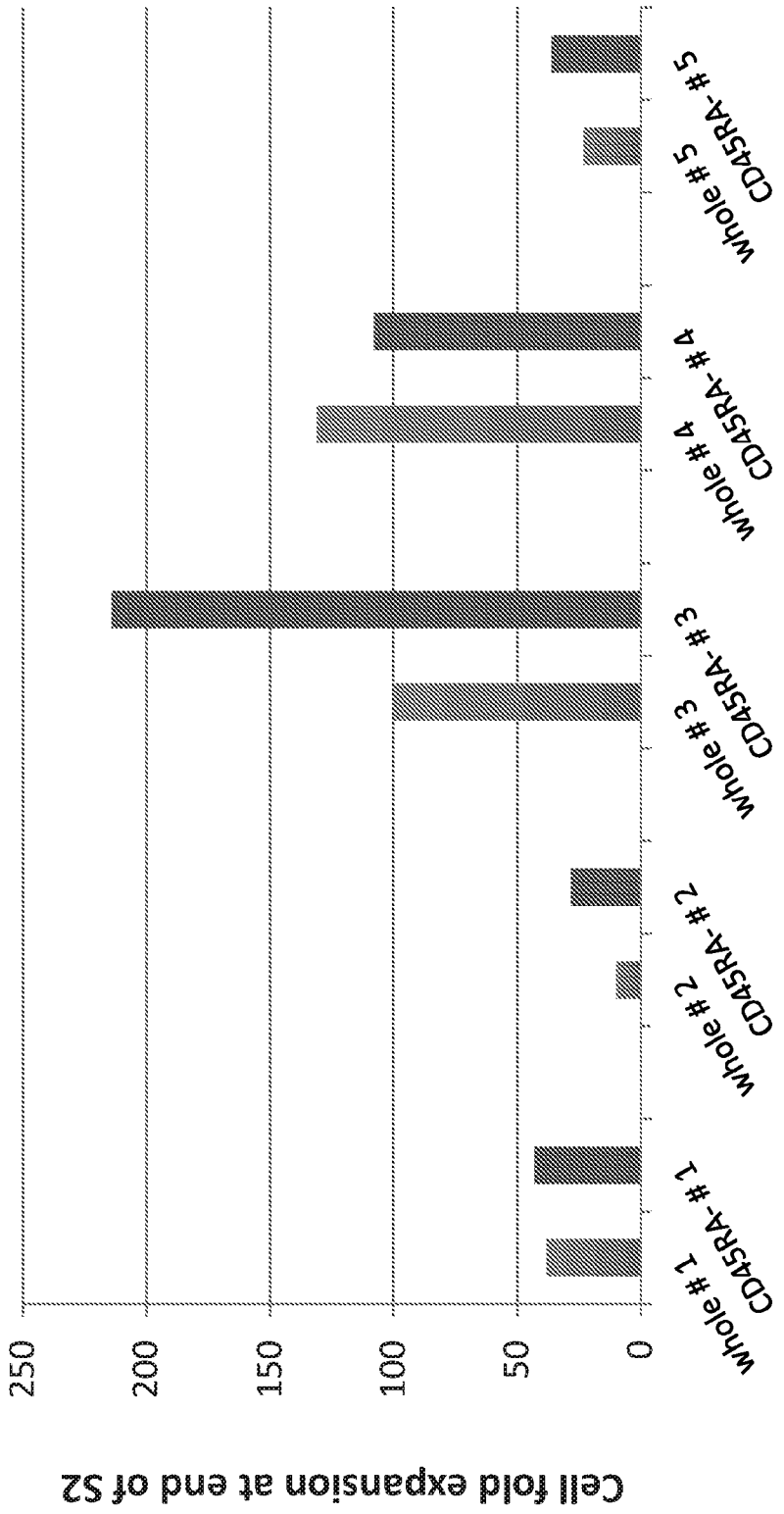

Cell fold expansion at end of S2
250
200
150
100
50
0
Whole #1
CD45RA-#1
Whole #2
CD45RA-#2
Whole #3
CD45RA-#3
Whole #4
CD45RA-#4
Whole #5
CD45RA-#5

FIG. 17

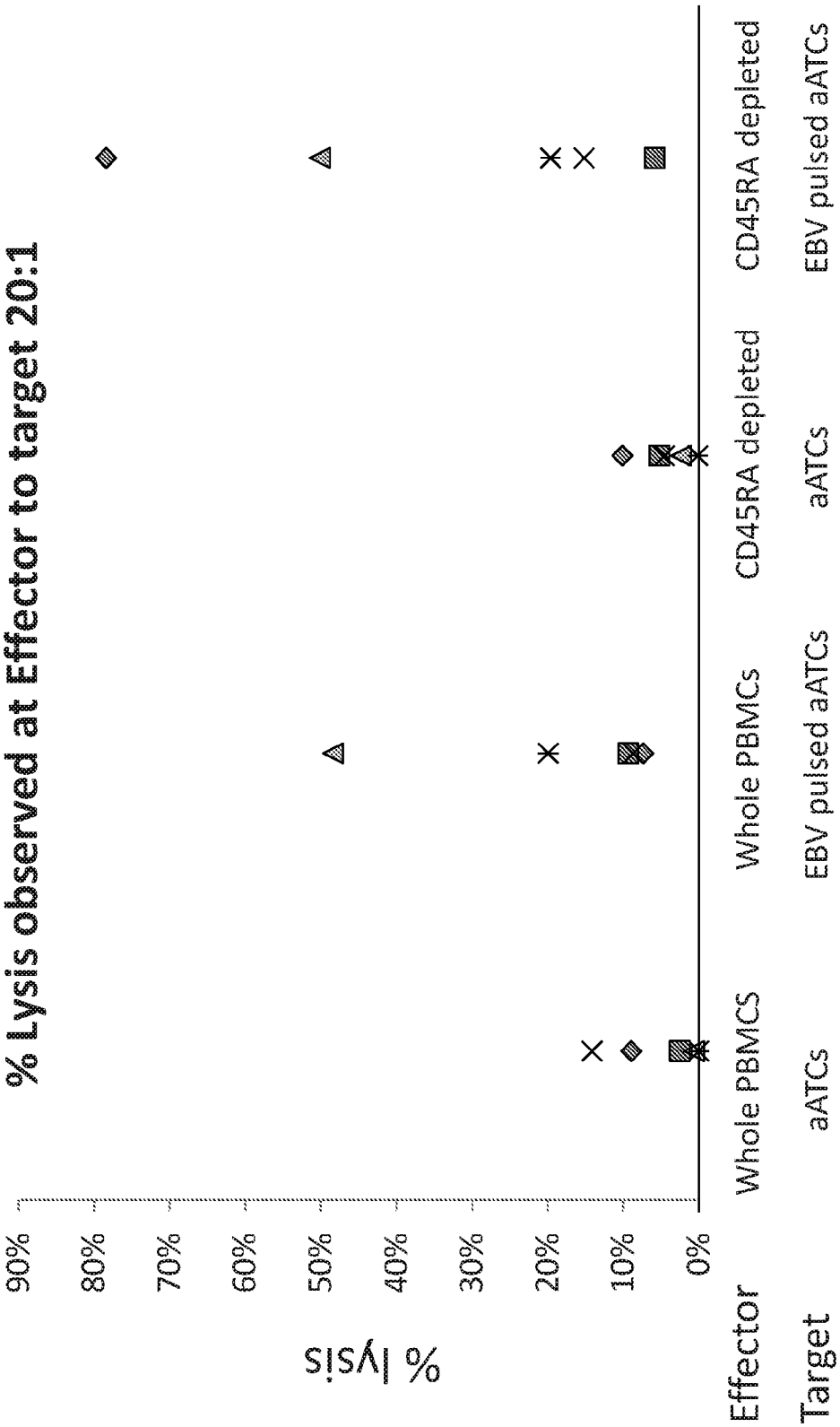

% Lysis observed at Effector to target 20:1
% lysis
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Effector
Target
Whole PBMCS
aATCs
Whole PBMCs
EBV pulsed aATCs
CD45RA depleted
aATCs
CD45RA depleted
EBV pulsed aATCs

FIG. 18

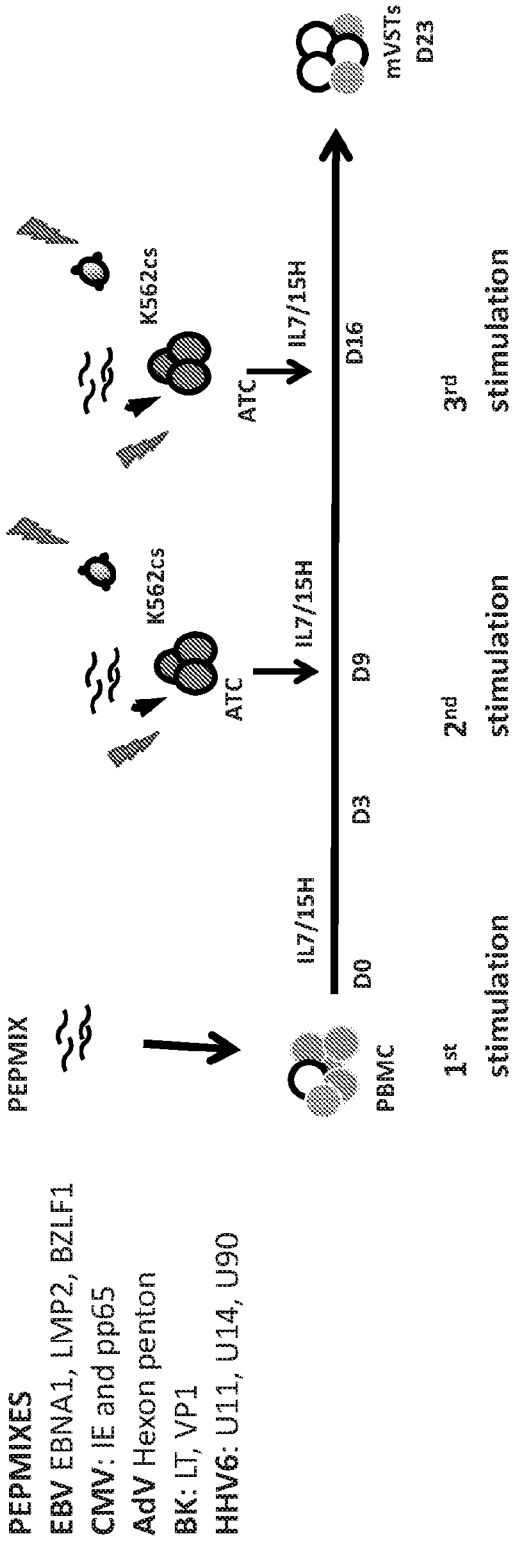

PEPMIX
PBMC
D0
IL7/15H
D3
ATC
K562cs
D9
IL7/15H
ATC
K562cs
D16
IL7/15H
mVSTs
D23
1st stimulation
2nd stimulation
3rd stimulation
PEPMIXES
EBV EBNA1, LMP2, BZLF1
CMV: IE and pp65
AdV Hexon penton
BK: LT, VP1
HHV6: U11, U14, U90

FIG. 19

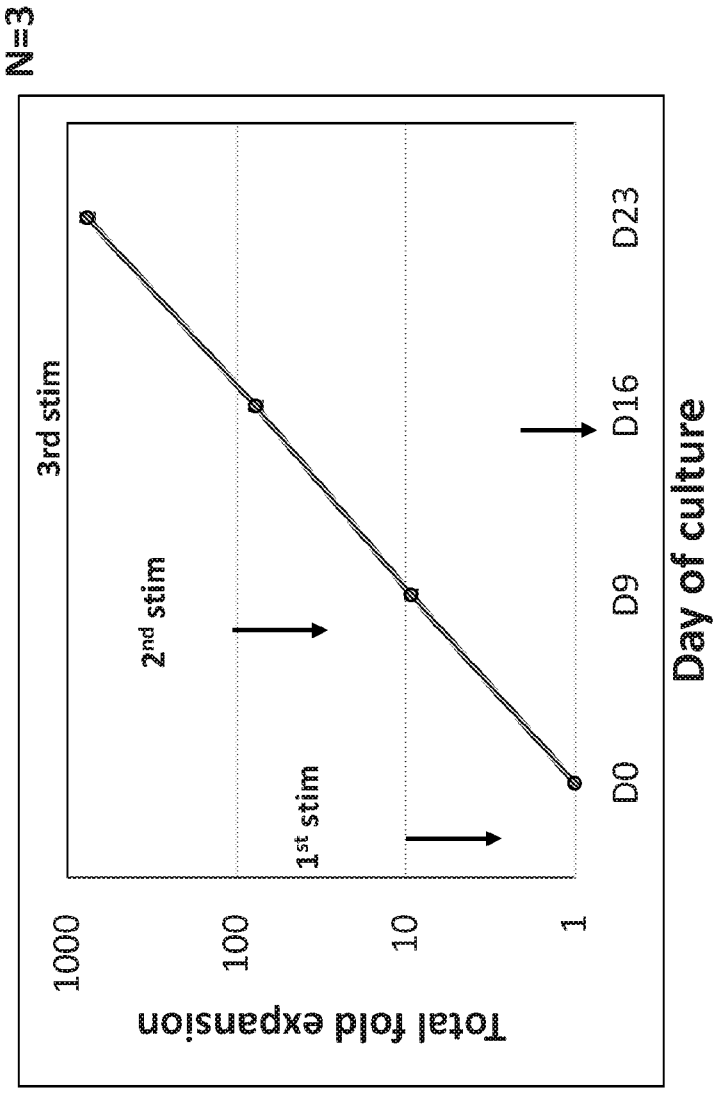

N=3
Total fold expansion
1000
100
10
1
Day of culture
D0
D9
D16
D23
1st stim
2nd stim
3rd stim

FIG. 20

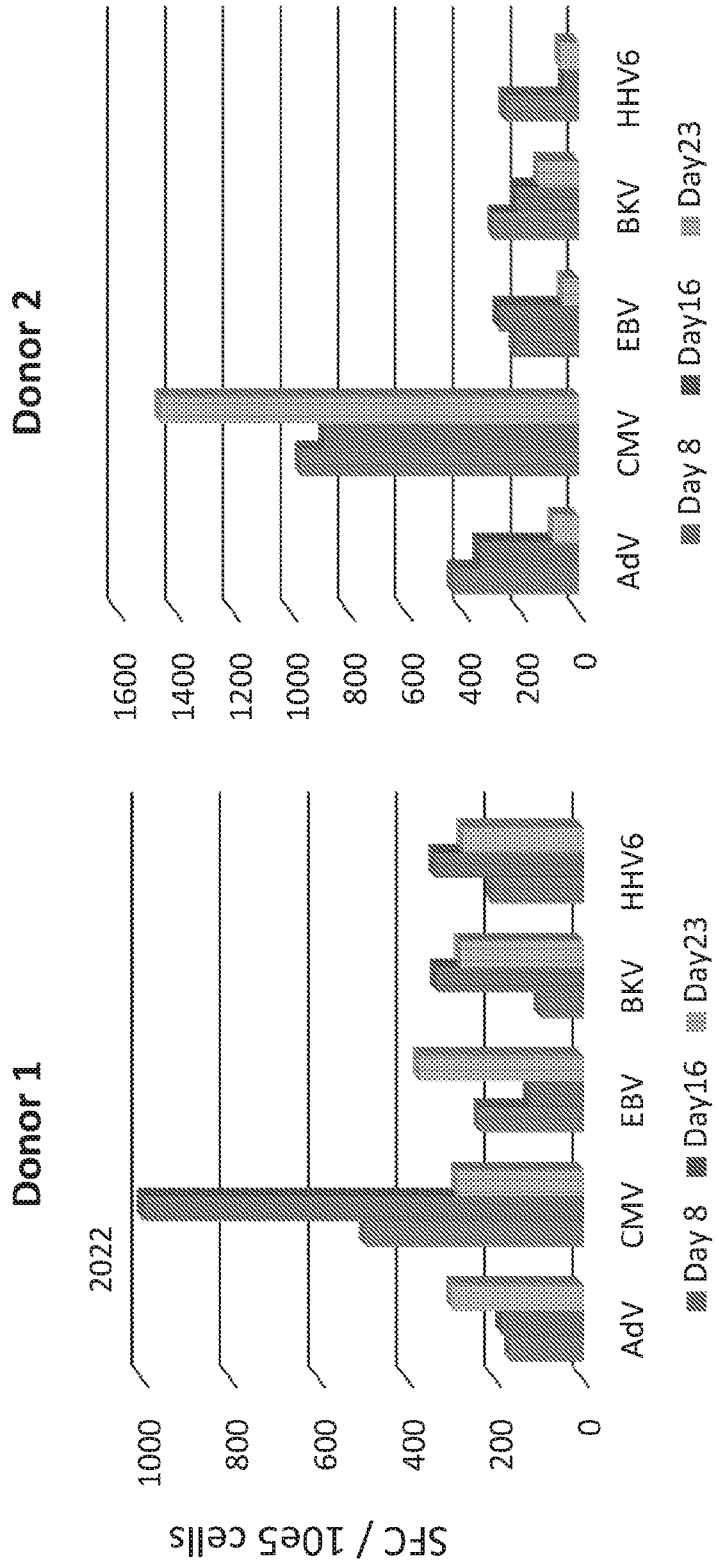
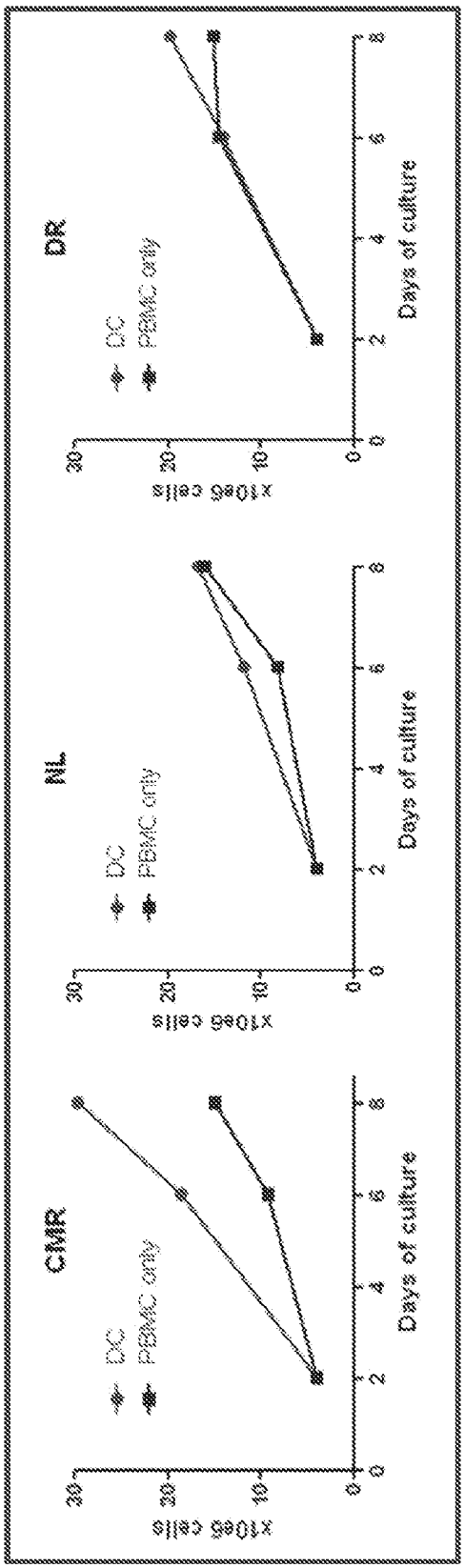

Donor 1
Donor 2
SFC / 10e5 cells
2022
1600
1400
1200
1000
800
600
400
200
0
AdV
CMV
EBV
BKV
HHV6
Day 8
Day16
Day23

FIG. 21



No significant difference (p=0.25) in proliferation between DC and PBMC initiated VSTs

FIG. 22

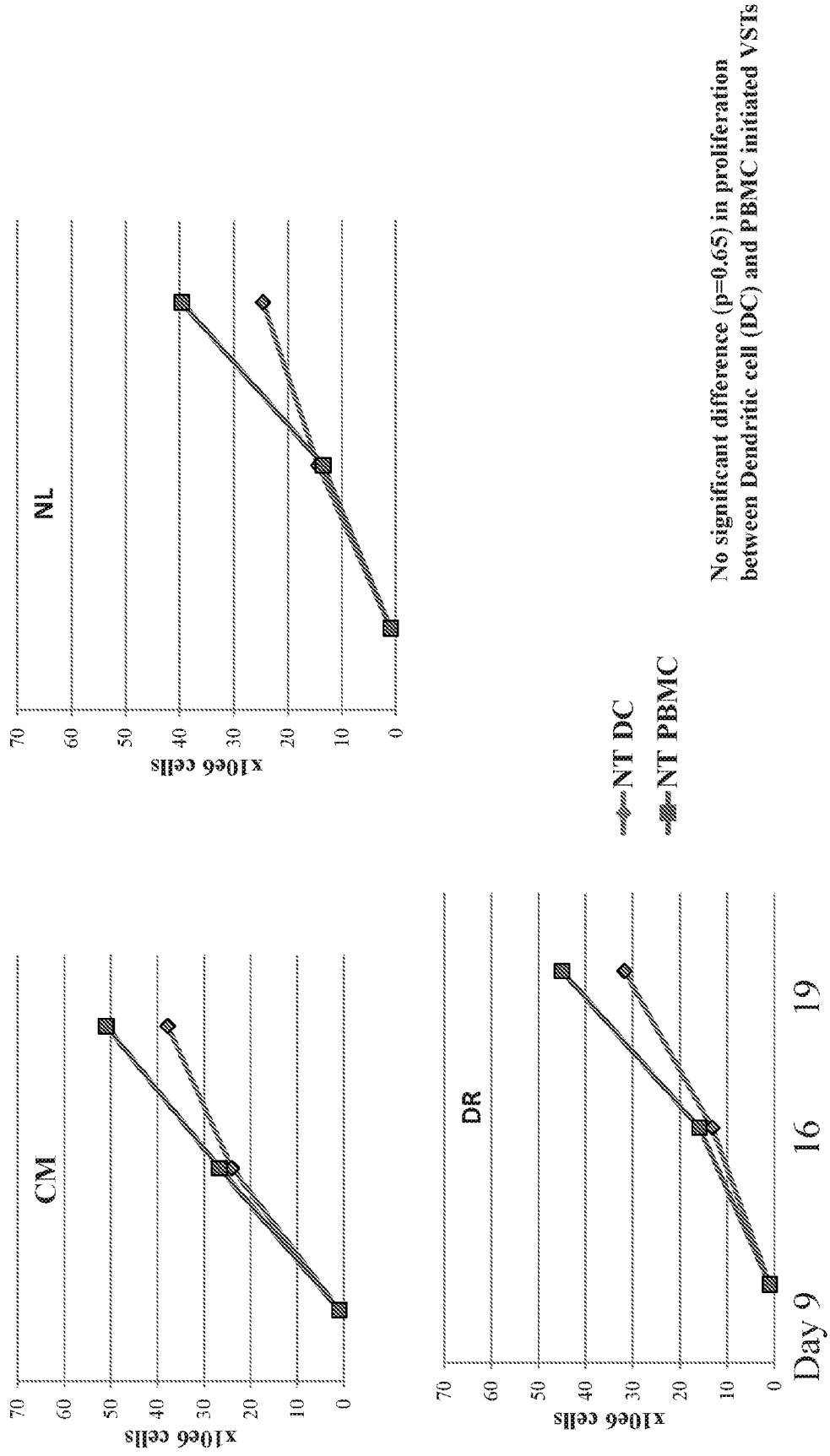

NL
CM
DR
x10e6 cells
0
10
20
30
40
50
60
70
Day 9
16
19
NT DC
NT PBMC
No significant difference (p=0.65) in proliferation between Dendritic cell (DC) and PBMC initiated VSTs

FIG. 23

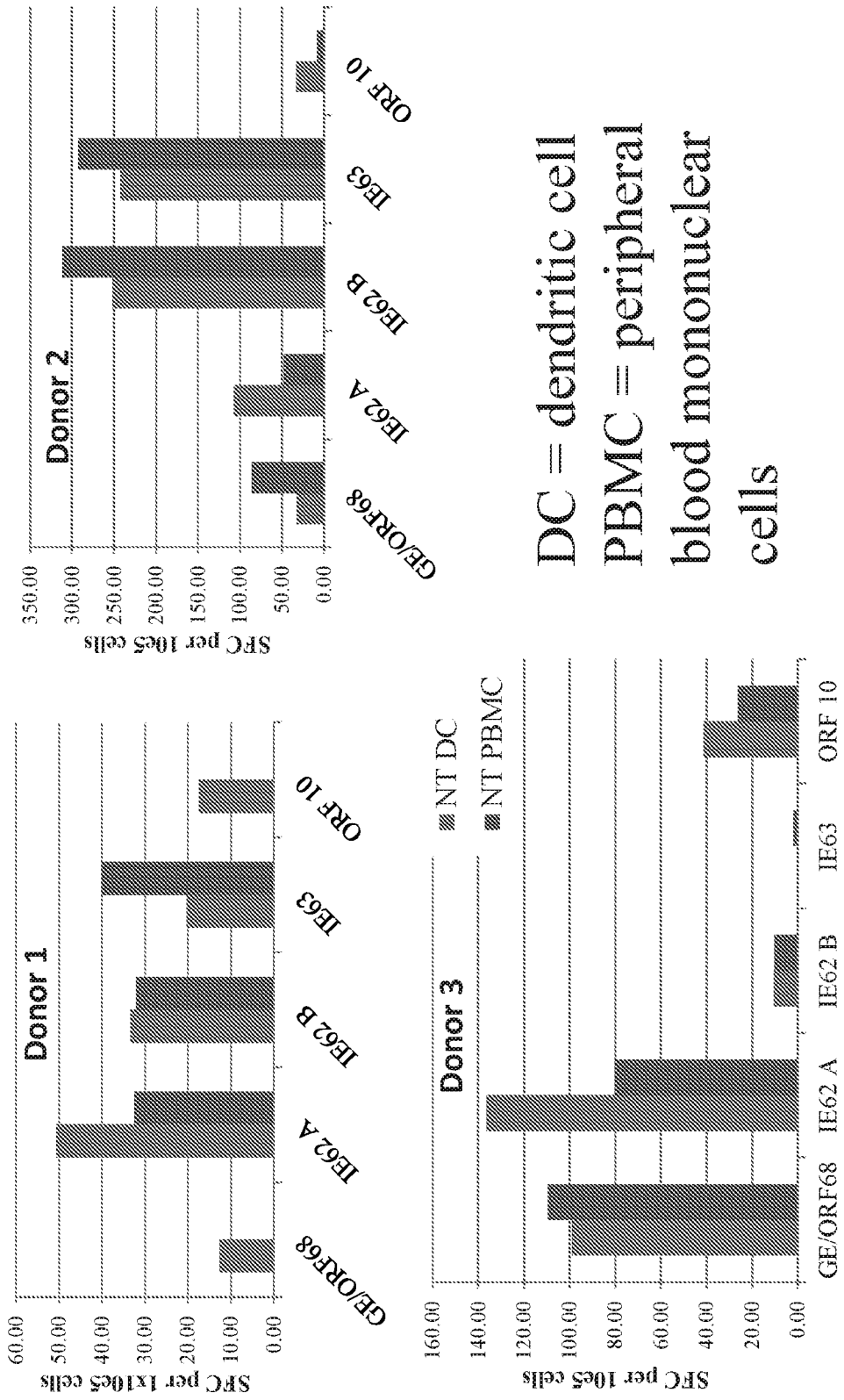

Donor 1
SFC per 1x10e5 cells
60.00
50.00
40.00
30.00
20.00
10.00
0.00
GE/ORF68
IE62 A
IE62 B
IE63
ORF 10
Donor 2
SFC per 10e5 cells
350.00
300.00
250.00
200.00
150.00
100.00
50.00
0.00
GE/ORF68
IE62 A
IE62 B
IE63
ORF 10
Donor 3
SFC per 10e5 cells
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
NT DC
NT PBMC
GE/ORF68
IE62 A
IE62 B
IE63
ORF 10
DC = dendritic cell
PBMC = peripheral blood mononuclear cells

FIG. 24

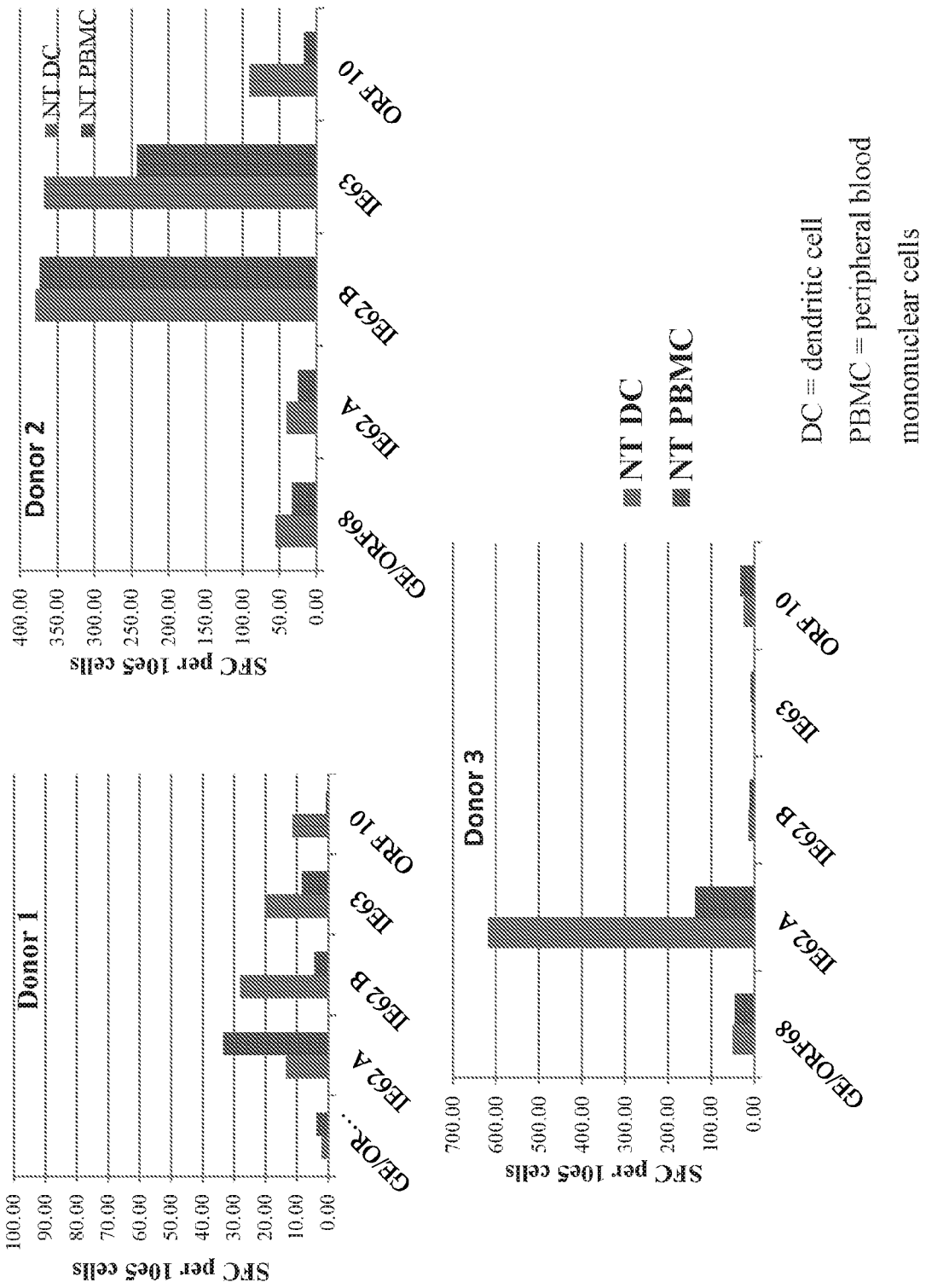

Donor 1
SFC per 10e5 cells
100.00
90.00
80.00
70.00
60.00
50.00
40.00
30.00
20.00
10.00
0.00
GE/OR...
IE62 A
IE62 B
IE63
ORF 10
Donor 2
SFC per 10e5 cells
400.00
350.00
300.00
250.00
200.00
150.00
100.00
50.00
0.00
NT DC
NT PBMC
GE/ORF68
IE62 A
IE62 B
IE63
ORF 10
Donor 3
SFC per 10e5 cells
700.00
600.00
500.00
400.00
300.00
200.00
100.00
0.00
NT DC
NT PBMC
GE/ORF68
IE62 A
IE62 B
IE63
ORF 10
DC = dendritic cell
PBMC = peripheral blood mononuclear cells

FIG. 25

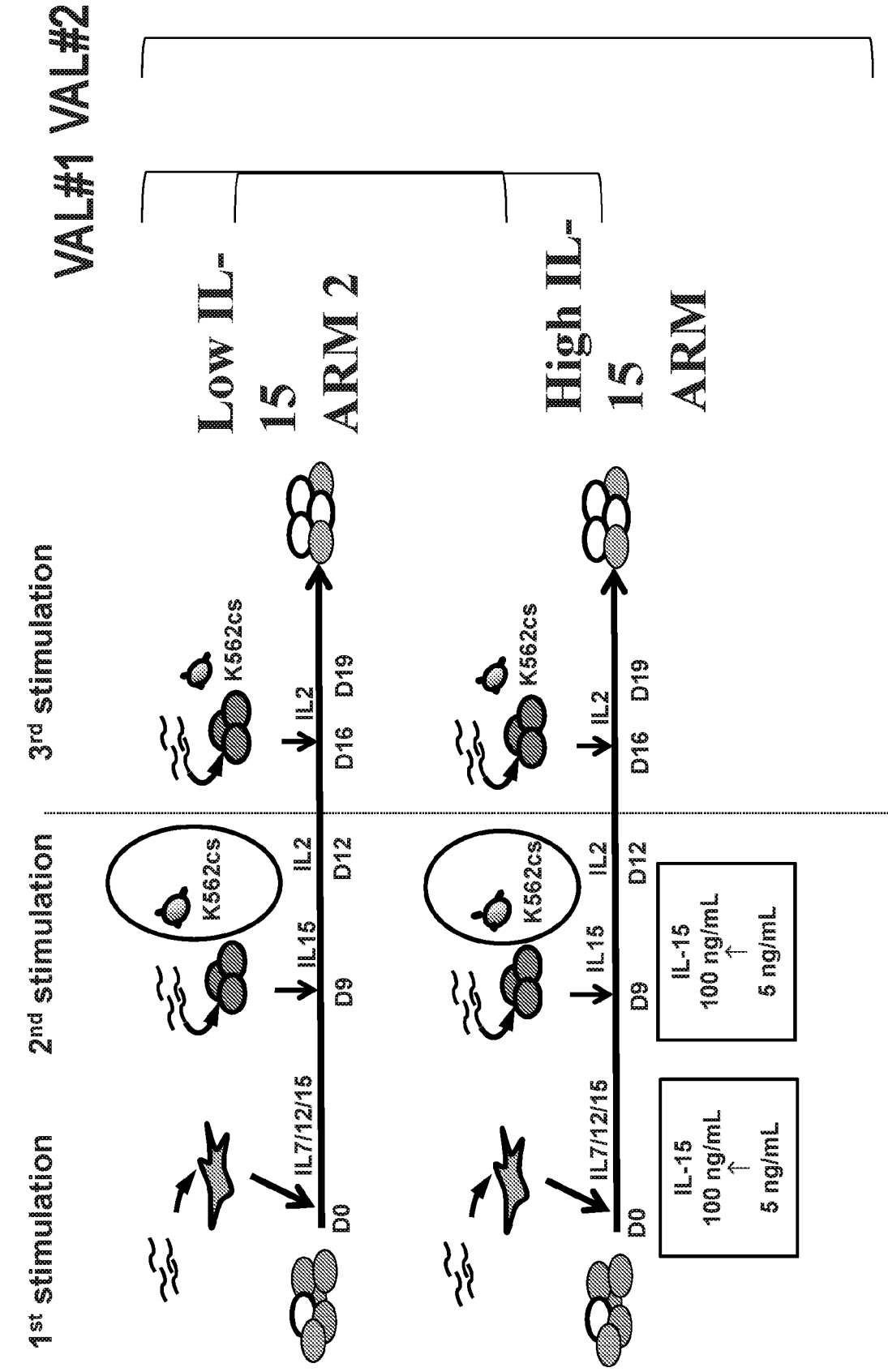

VAL#1 VAL#2
Low IL-15 ARM 2
High IL-15 ARM
1st stimulation
2nd stimulation
3rd stimulation
IL7/12/15
D0
IL15
D9
IL2
D12
K562cs
IL2
D16
D19
IL-15 100 ng/mL → 5 ng/mL

FIG. 26

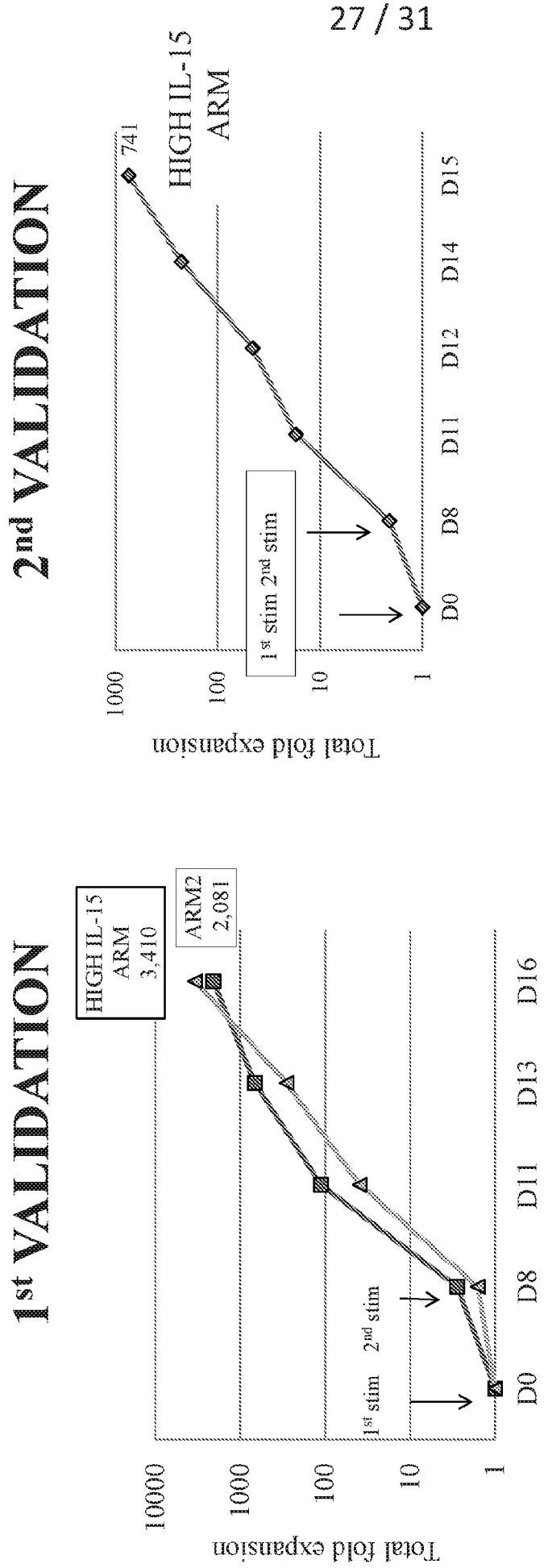

1st VALIDATION
HIGH IL-15 ARM 3,410
ARM2 2,081
Total fold expansion
10000
1000
100
10
1
1st stim
2nd stim
D0
D8
D11
D13
D16
2nd VALIDATION
HIGH IL-15 ARM
741
Total fold expansion
1000
100
10
1
1st stim 2nd stim
D0
D8
D11
D12
D14
D15

FIG. 27

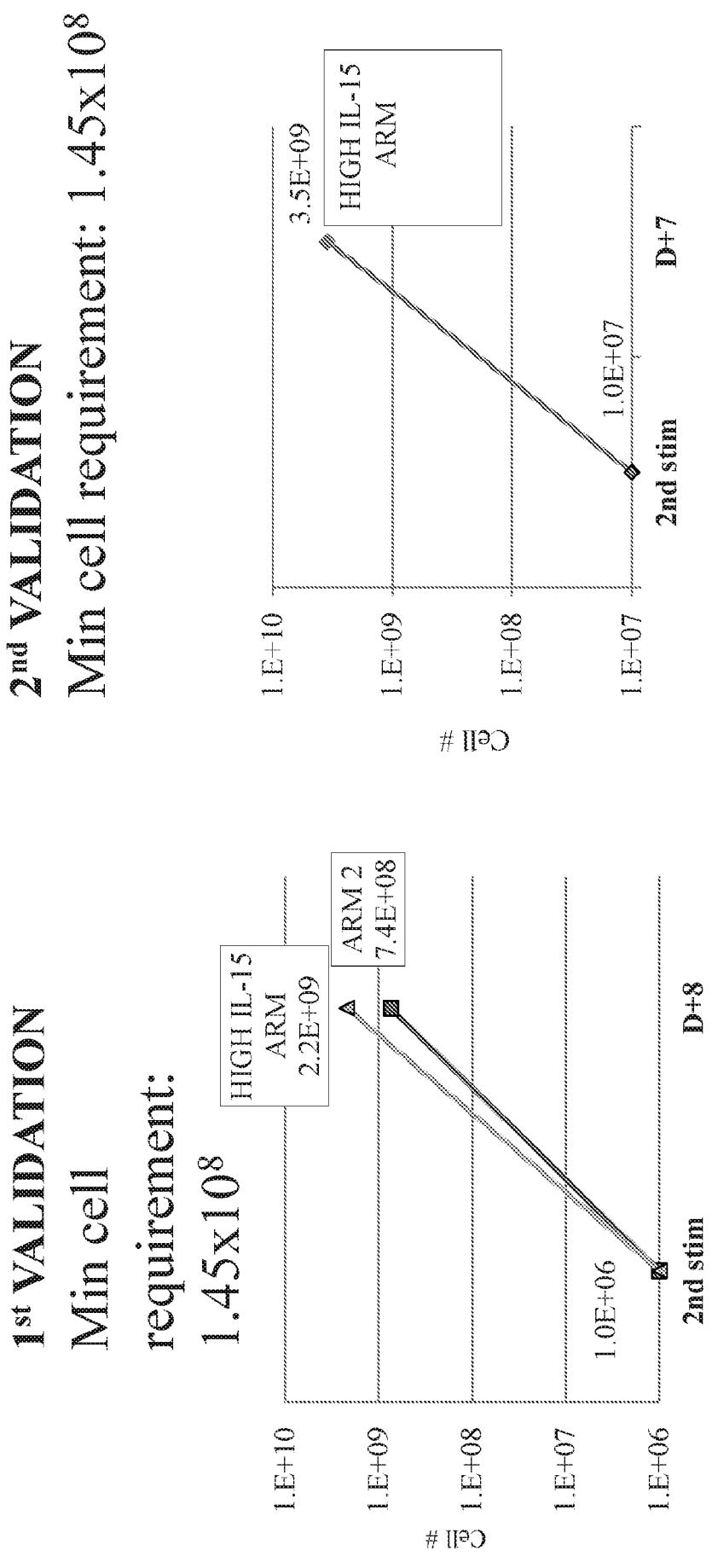
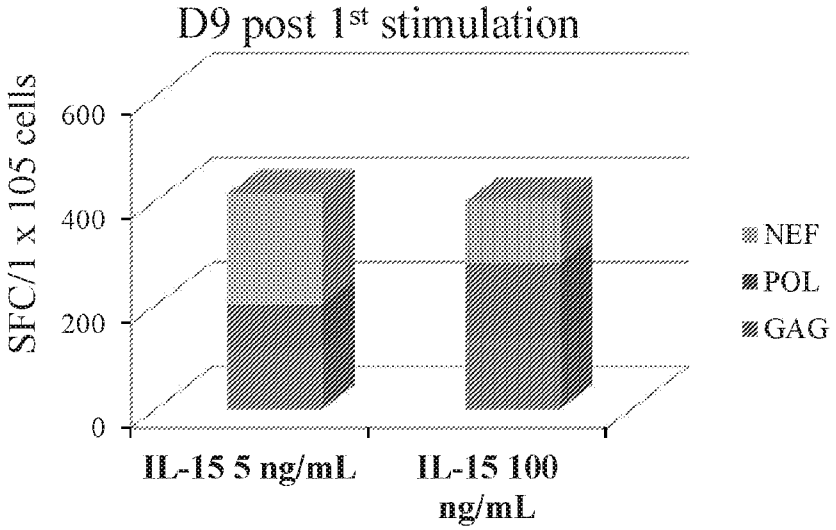
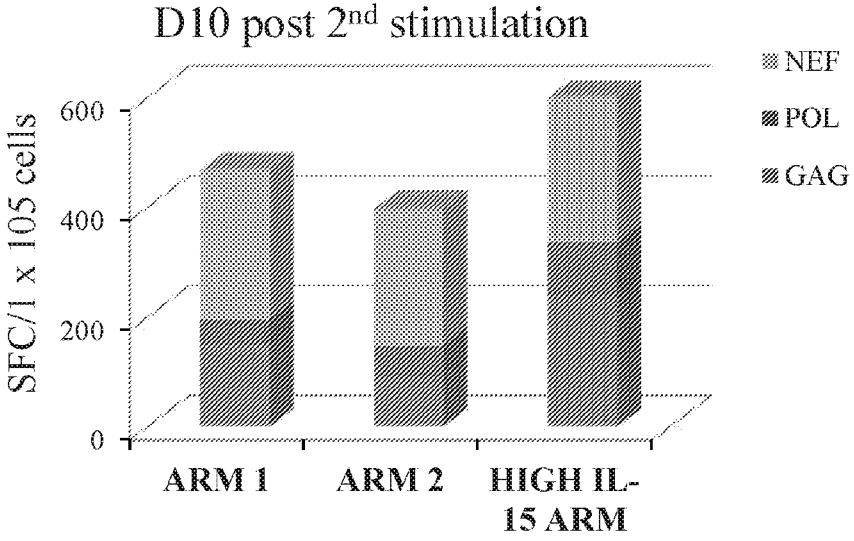

2nd VALIDATION
Min cell requirement: 1.45x10^8
HIGH IL-15 ARM
3.5E+09
1.0E+07
2nd stim
D+7
1.E+10
1.E+09
1.E+08
1.E+07
Cell #
1st VALIDATION
Min cell requirement: 1.45x10^8
HIGH IL-15 ARM 2.2E+09
ARM 2 7.4E+08
1.0E+06
2nd stim
D+8
1.E+10
1.E+09
1.E+08
1.E+07
1.E+06
Cell #

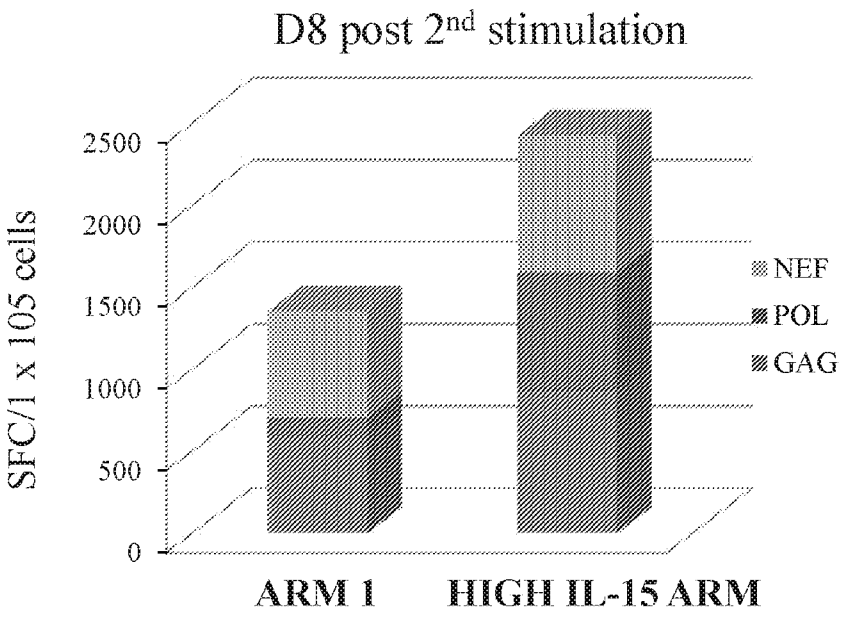
FIG. 28

1st VALIDATION





2nd VALIDATION



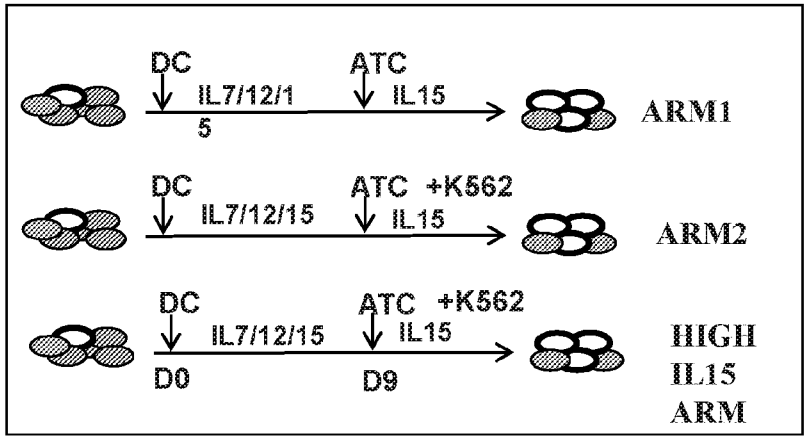
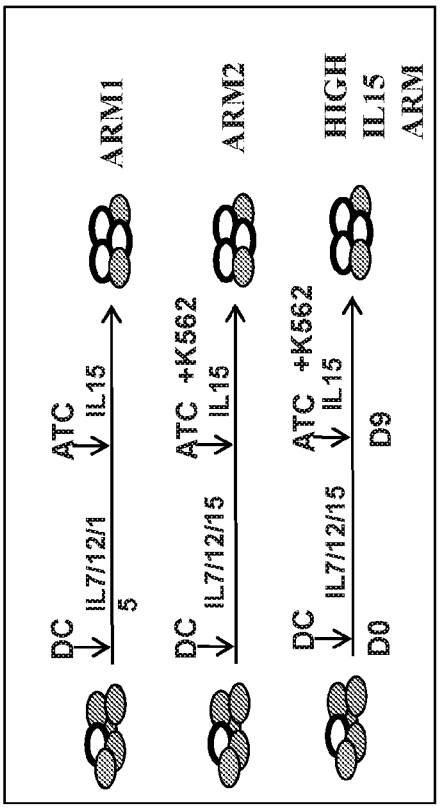


FIG. 29


DC
IL7/12/1
5
ATC
IL15
ARM1
DC
IL7/12/15
ATC +K562
IL15
ARM2
DC
IL7/12/15
D0
ATC +K562
IL15
D9
HIGH IL15 ARM

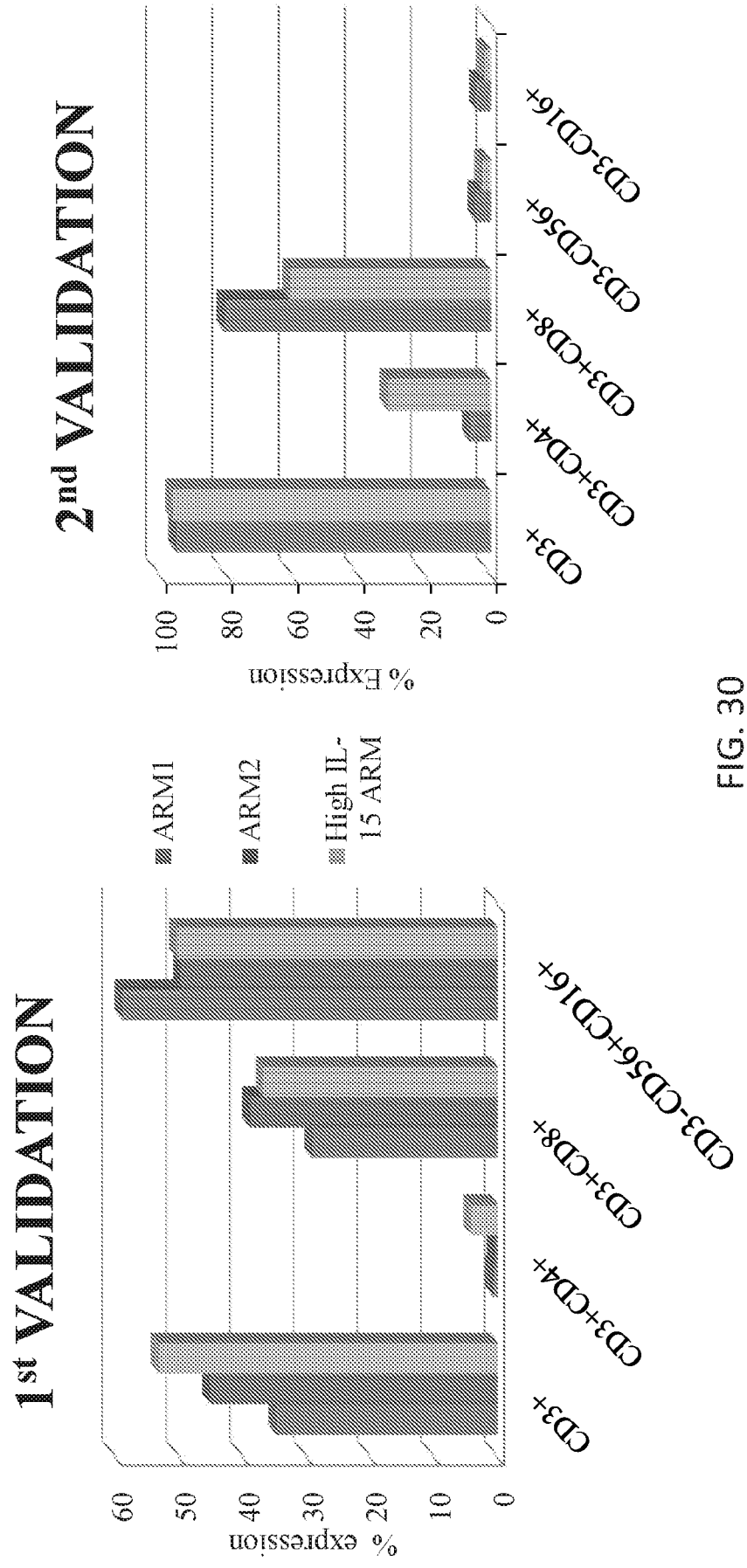

1st VALIDATION
% expression
0
10
20
30
40
50
60
ARM1
ARM2
High IL-15 ARM
CD3+
CD3+CD4+
CD3+CD8+
CD3-CD56+CD16+
2nd VALIDATION
% Expression
0
20
40
60
80
100
CD3+
CD3+CD4+
CD3+CD8+
CD3-CD56+
CD3-CD16+

FIG. 30

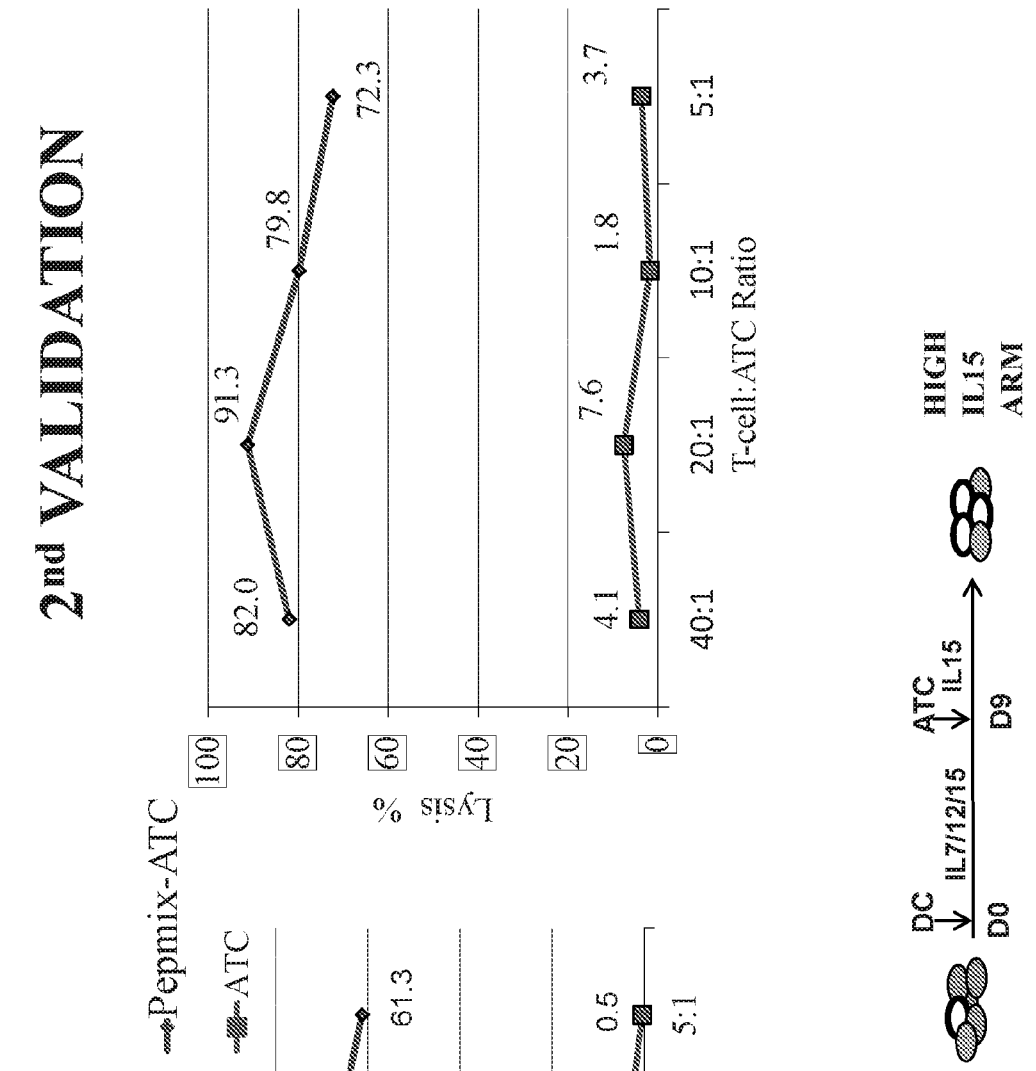

1st VALIDATION
2nd VALIDATION
Pepmix-ATC
ATC
% Lysis
T-cell:ATC Ratio
40:1
20:1
10:1
5:1
79.1
90.7
69.2
61.3
3.4
2.9
5.0
0.5
82.0
91.3
79.8
72.3
4.1
7.6
1.8
3.7
DC
IL7/12/15
D0
ATC
IL15
D9
HIGH
IL15
ARM

FIG. 31