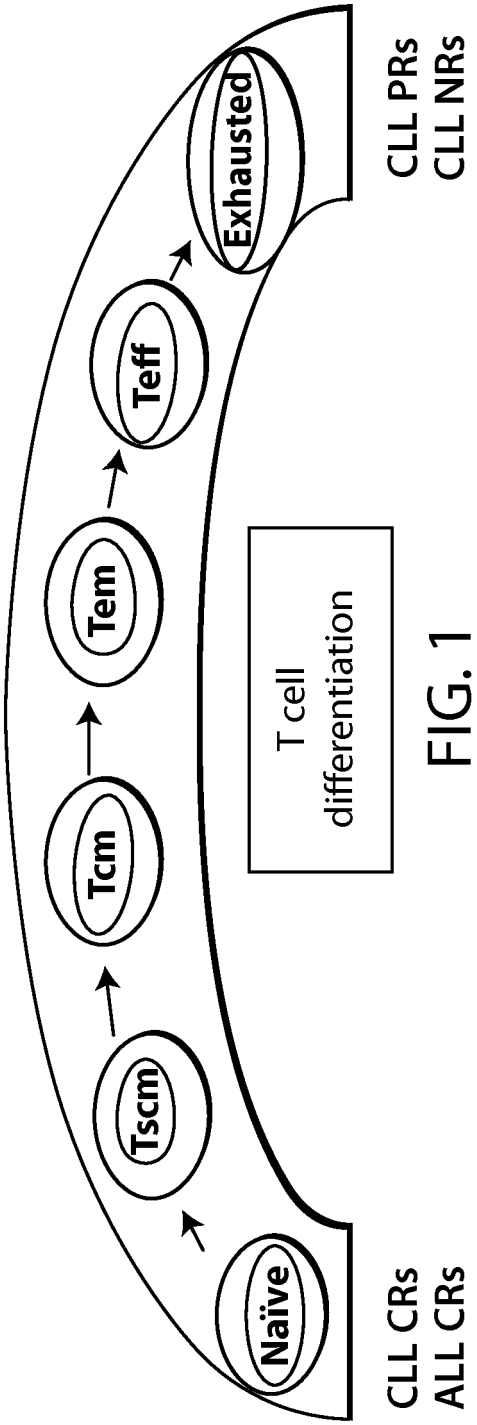

Naïve
Tscm
Tcm
Tem
Teff
Exhausted
T cell differentiation
CLL CRs
ALL CRs
CLL PRs
CLL NRs

FIG. 1

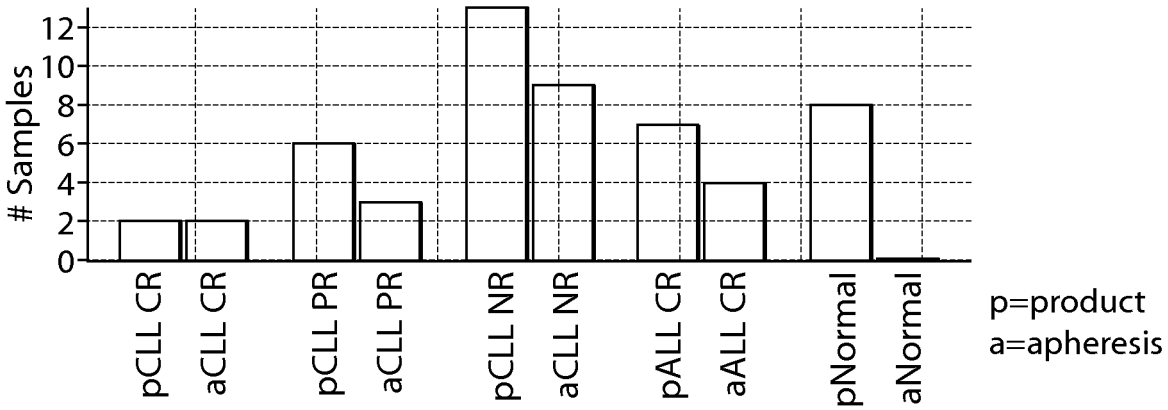

Samples
12
10
8
6
4
2
0
pCLL CR
aCLL CR
pCLL PR
aCLL PR
pCLL NR
aCLL NR
pALL CR
aALL CR
pNormal
aNormal
p=product
a=apheresis

FIG 2A

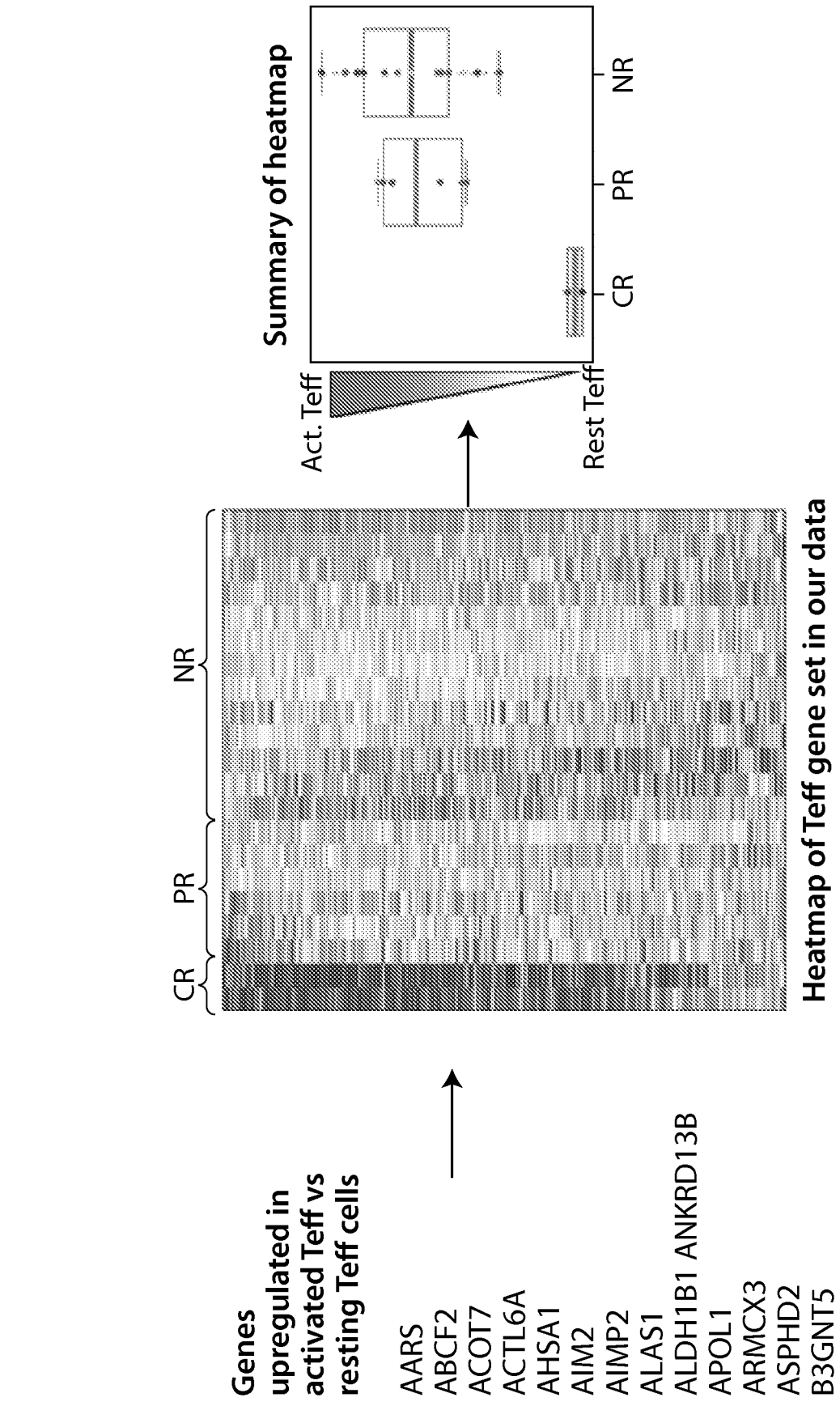

Genes upregulated in activated Teff vs resting Teff cells
AARS
ABCF2
ACOT7
ACTL6A
AHSA1
AIM2
AIMP2
ALAS1
ALDH1B1 ANKRD13B
APOL1
ARMCX3
ASPHD2
B3GNT5
CR
PR
NR
Heatmap of Teff gene set in our data
Act. Teff
Rest Teff
Summary of heatmap
CR
PR
NR

FIG. 2B

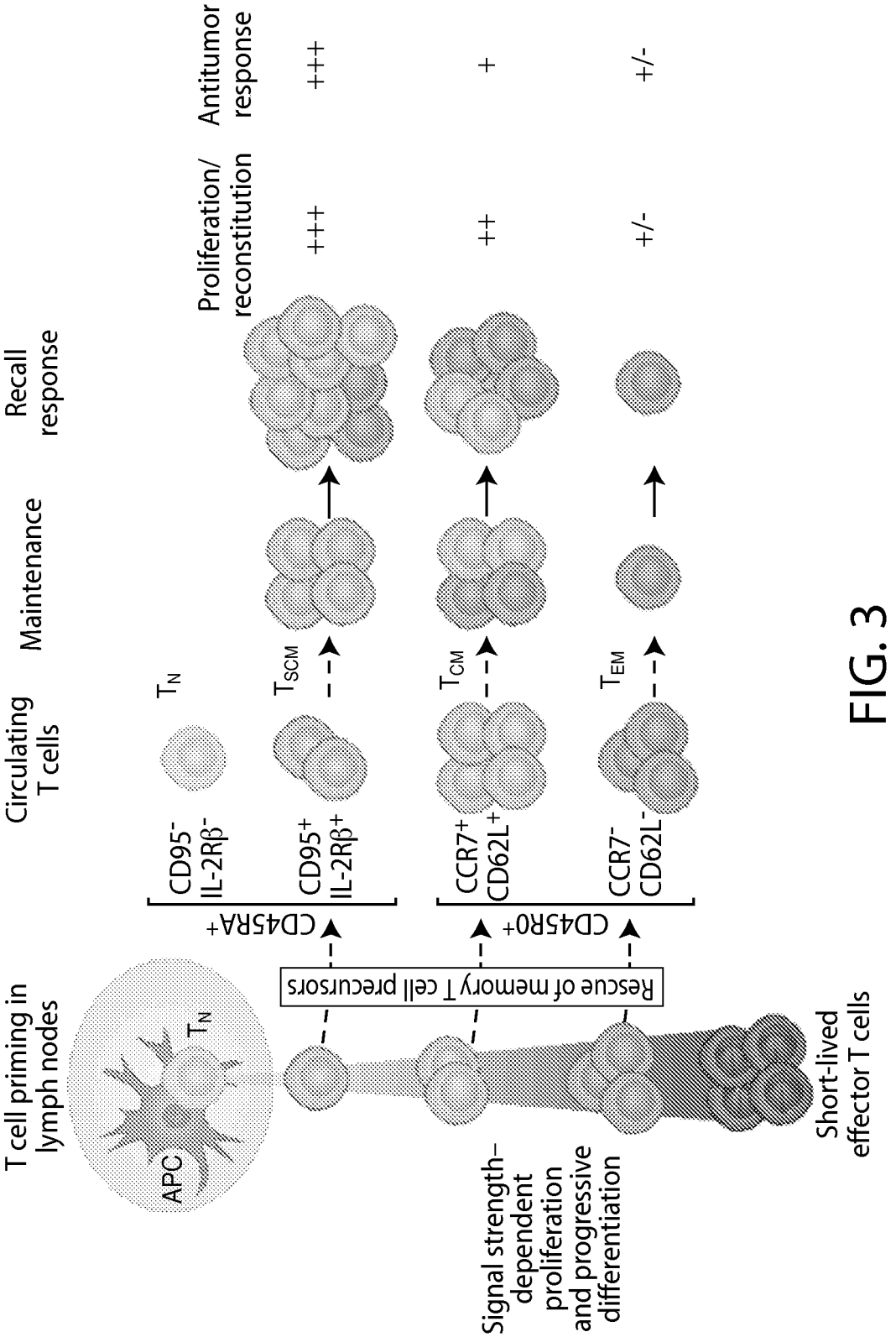

T cell priming in lymph nodes
APC
T_N
Rescue of memory T cell precursors
Signal strength–dependent proliferation and progressive differentiation
Short-lived effector T cells
Circulating T cells
Maintenance
Recall response
Proliferation/ reconstitution
Antitumor response
CD45RA+
CD45RO+
CD95- IL-2Rβ-
CD95+ IL-2Rβ+
CCR7+ CD62L+
CCR7- CD62L-
T_N
T_{SCM}
T_{CM}
T_{EM}
+++
++
+/-
+++
+
+/-

FIG. 3

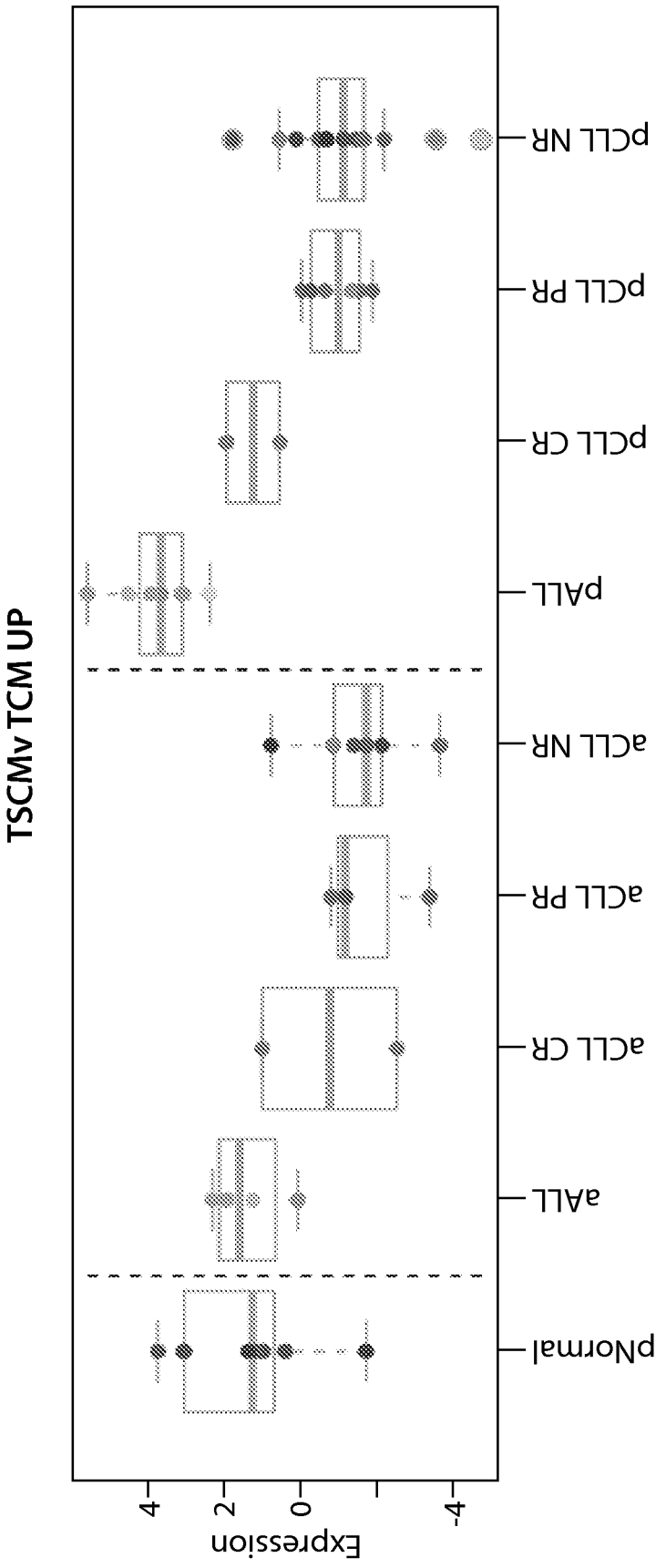

TSCMv TCM UP
Expression
4
2
0
-4
pNormal
aALL
aCLL CR
aCLL PR
aCLL NR
pALL
pCLL CR
pCLL PR
pCLL NR

FIG. 4

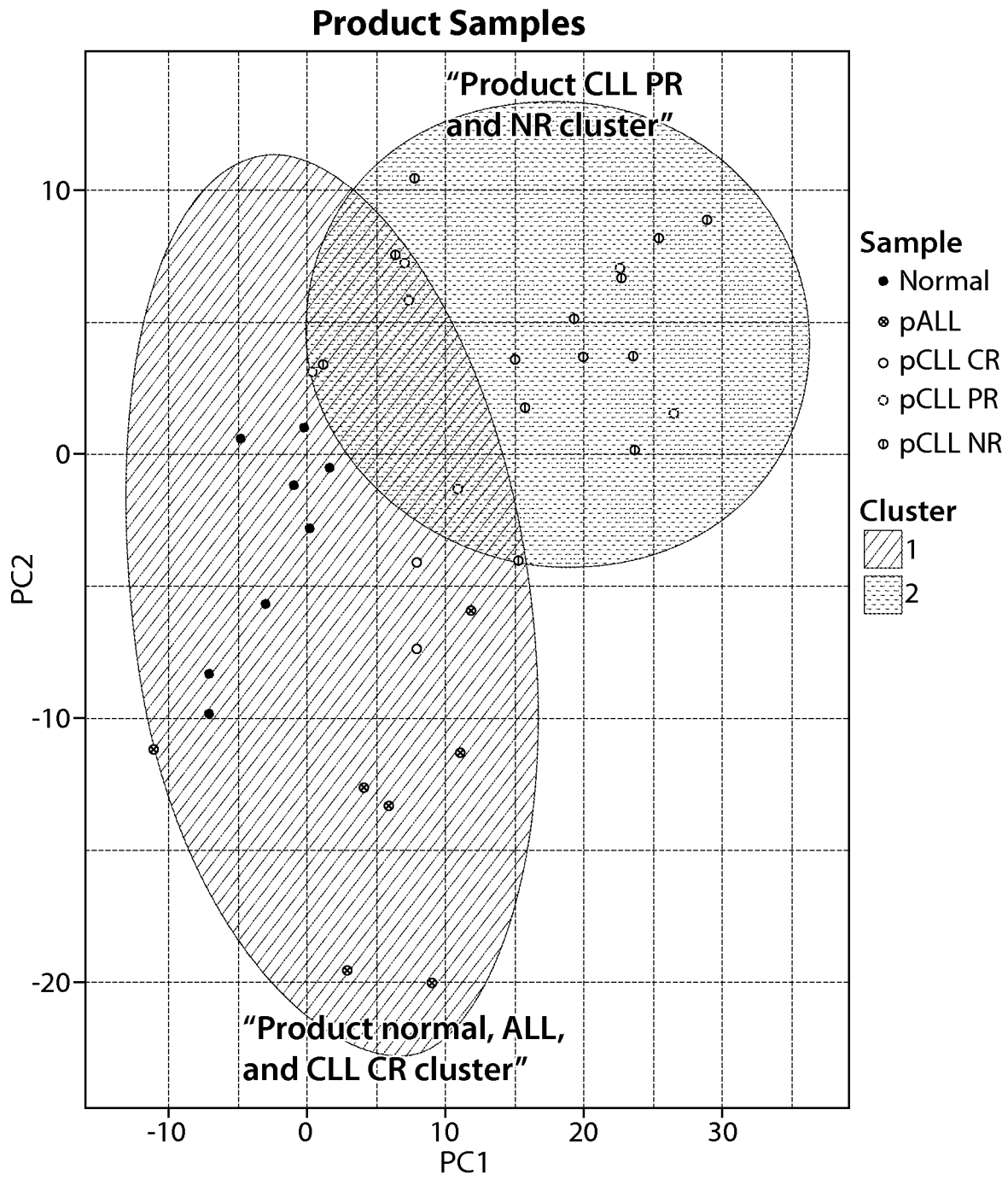

Product Samples
"Product CLL PR and NR cluster"
"Product normal, ALL, and CLL CR cluster"
PC2
PC1
10
0
-10
-20
-10
0
10
20
30
Sample
Normal
pALL
pCLL CR
pCLL PR
pCLL NR
Cluster
1
2

FIG. 5A

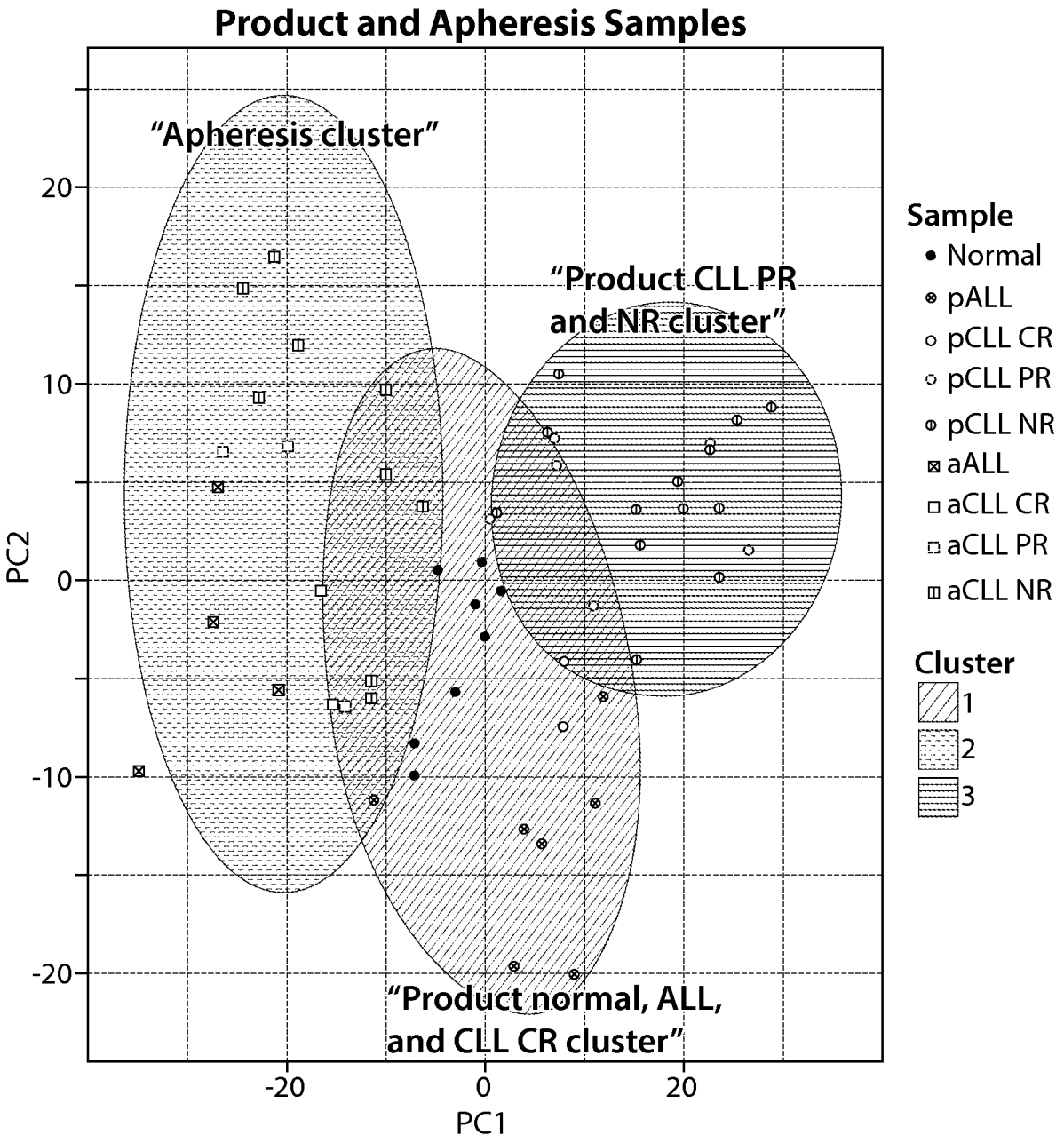

Product and Apheresis Samples
"Apheresis cluster"
"Product CLL PR and NR cluster"
"Product normal, ALL, and CLL CR cluster"
PC2
PC1
20
10
0
-10
-20
-20
0
20
Sample
Normal
pALL
pCLL CR
pCLL PR
pCLL NR
aALL
aCLL CR
aCLL PR
aCLL NR
Cluster
1
2
3

FIG. 5B

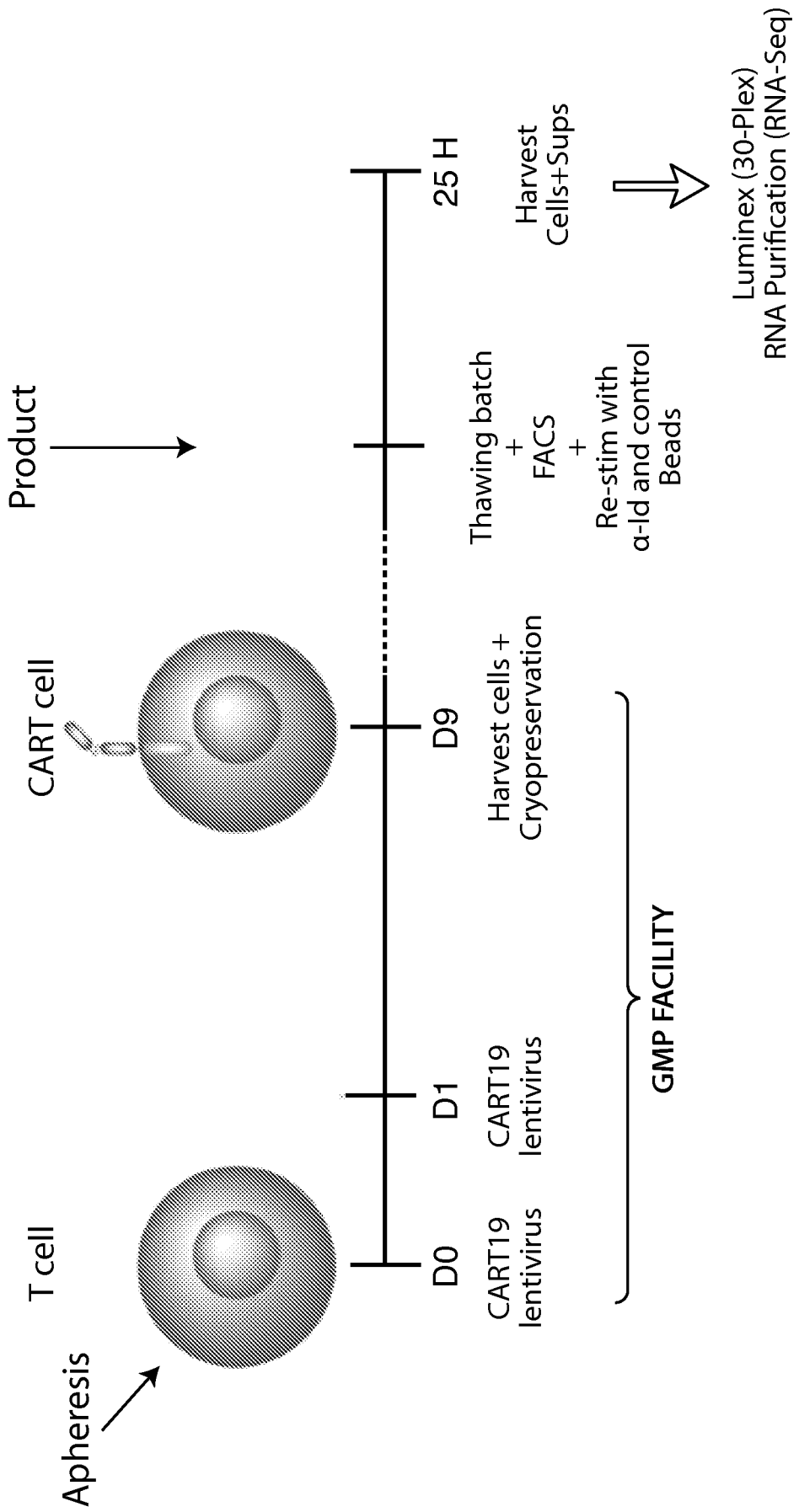

Apheresis
T cell
CART cell
Product
D0
CART19 lentivirus
D1
CART19 lentivirus
D9
Harvest cells + Cryopreservation
25 H
Thawing batch + FACS + Re-stim with α-Id and control Beads
Harvest Cells+Sups
Luminex (30-Plex) RNA Purification (RNA-Seq)
GMP FACILITY

FIG. 6

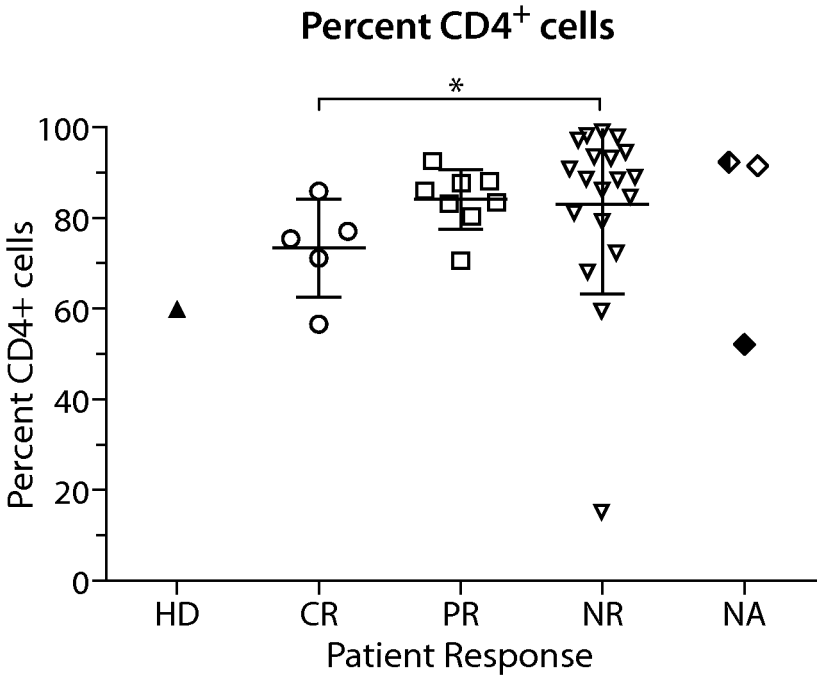

Percent CD4+ cells
*
100
80
60
40
20
0
Percent CD4+ cells
HD
CR
PR
NR
NA
Patient Response

FIG. 7A

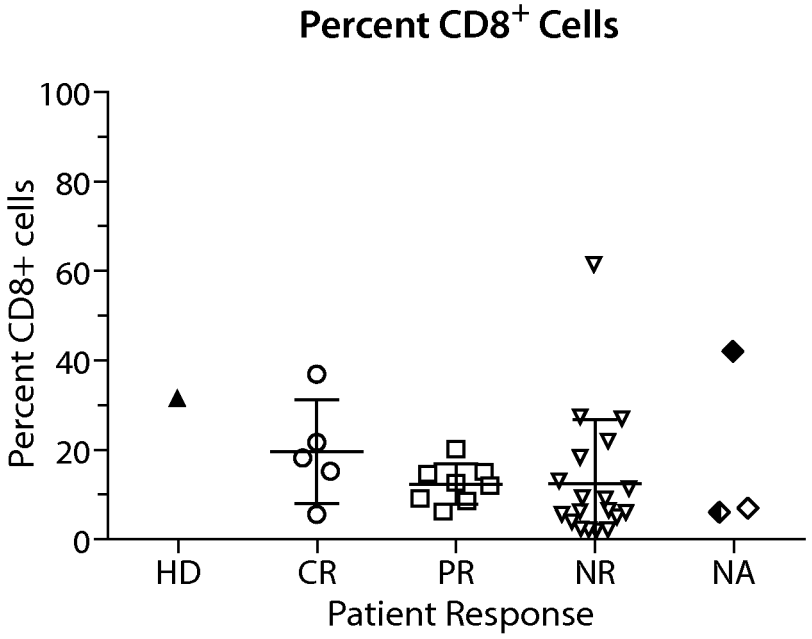

Percent CD8+ Cells
100
80
60
40
20
0
Percent CD8+ cells
HD
CR
PR
NR
NA
Patient Response

FIG. 7B

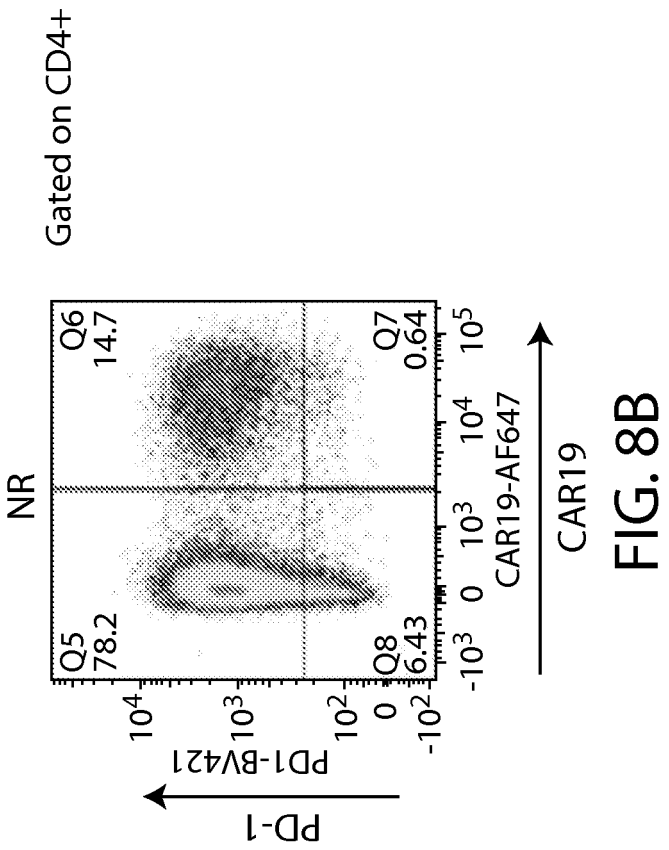

Gated on CD4+
NR
Q5
78.2
Q6
14.7
Q8
6.43
Q7
0.64
PD-1
PD1-BV421
CAR19
CAR19-AF647

FIG. 8B

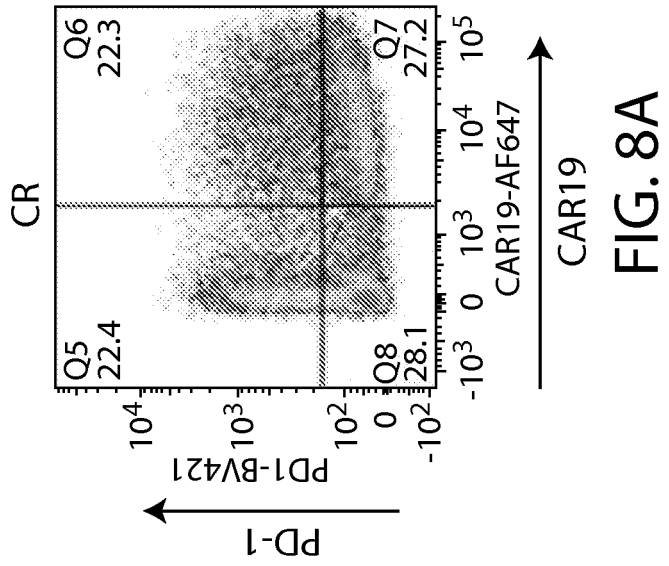

CR
Q5
22.4
Q6
22.3
Q8
28.1
Q7
27.2
PD-1
PD1-BV421
CAR19
CAR19-AF647

FIG. 8A

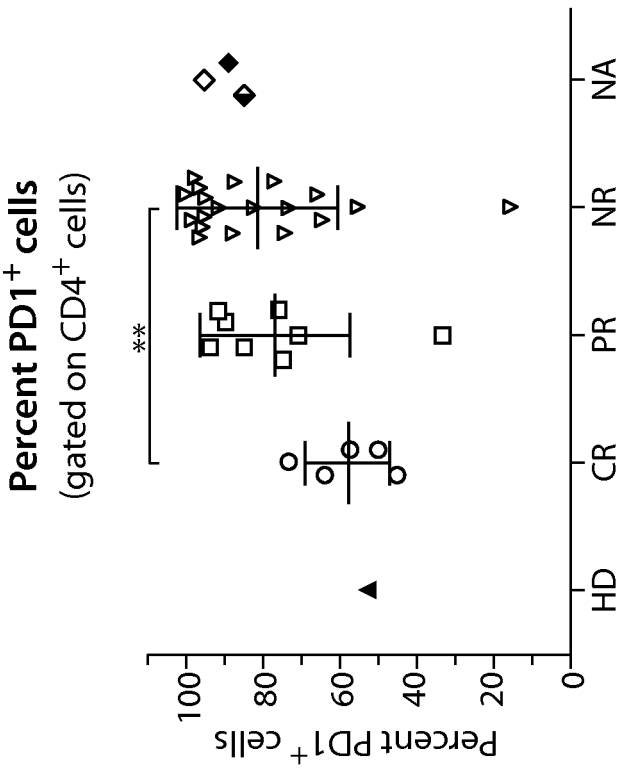

Percent PD1+ cells
(gated on CD4+ cells)
Percent PD1+ cells
0
20
40
60
80
100
**
HD
CR
PR
NR
NA
Patient Response

FIG. 8C

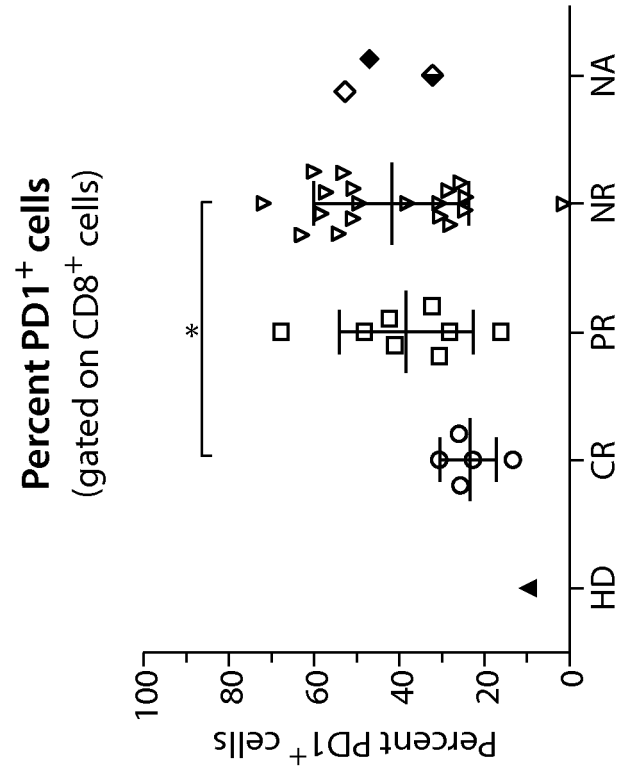

Percent PD1+ cells
(gated on CD8+ cells)
Percent PD1+ cells
0
20
40
60
80
100
*
HD
CR
PR
NR
NA
Patient Response

FIG. 8D

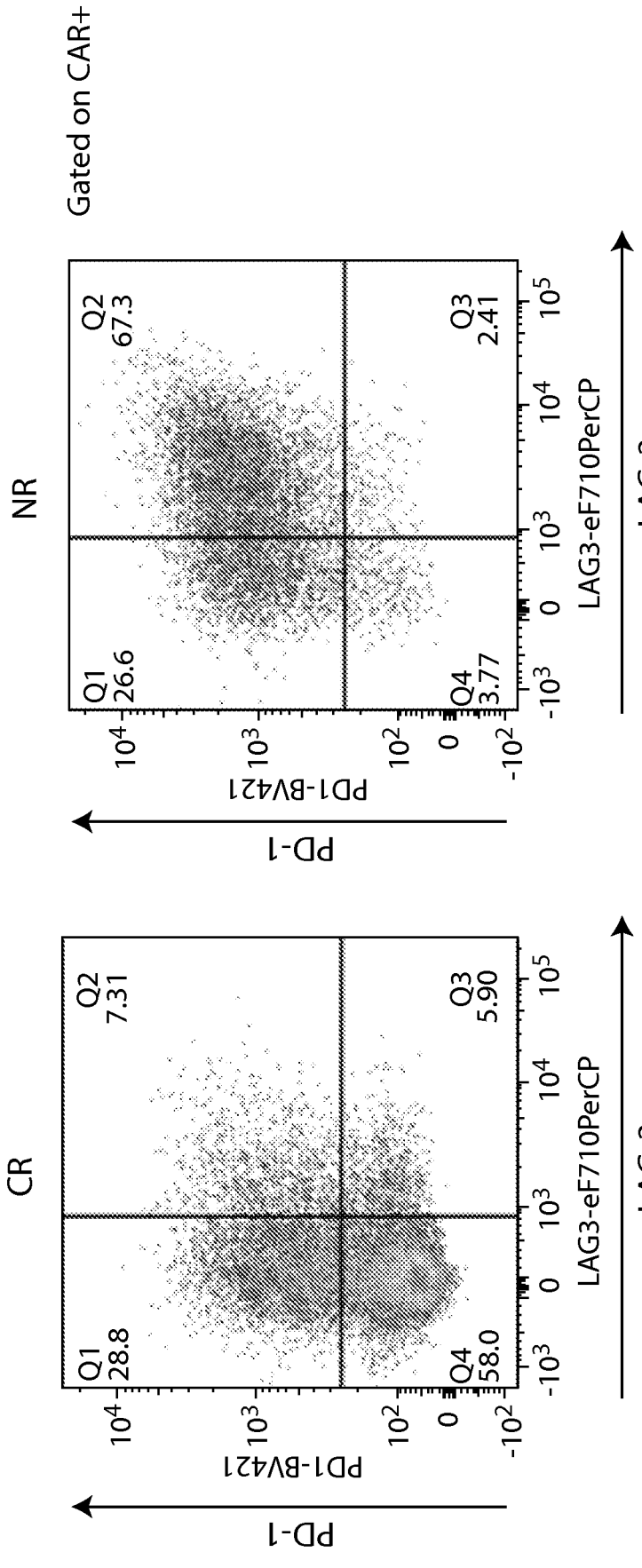

Gated on CAR+
NR
CR
PD-1
PD1-BV421
LAG-3
LAG3-eF710PerCP
Q1
26.6
Q2
67.3
Q3
2.41
Q4
3.77
Q1
28.8
Q2
7.31
Q3
5.90
Q4
58.0

FIG. 9B

FIG. 9A

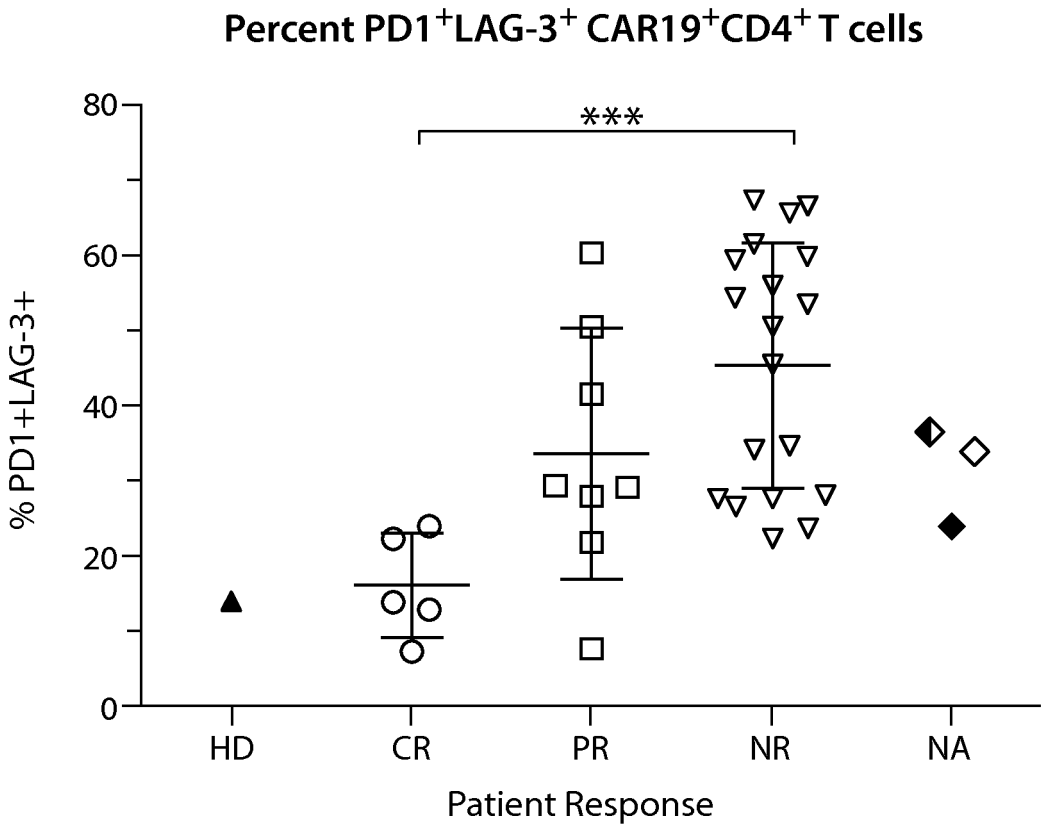

Percent PD1+LAG-3+ CAR19+CD4+ T cells
***
% PD1+LAG-3+
80
60
40
20
0
HD
CR
PR
NR
NA
Patient Response

FIG. 9C

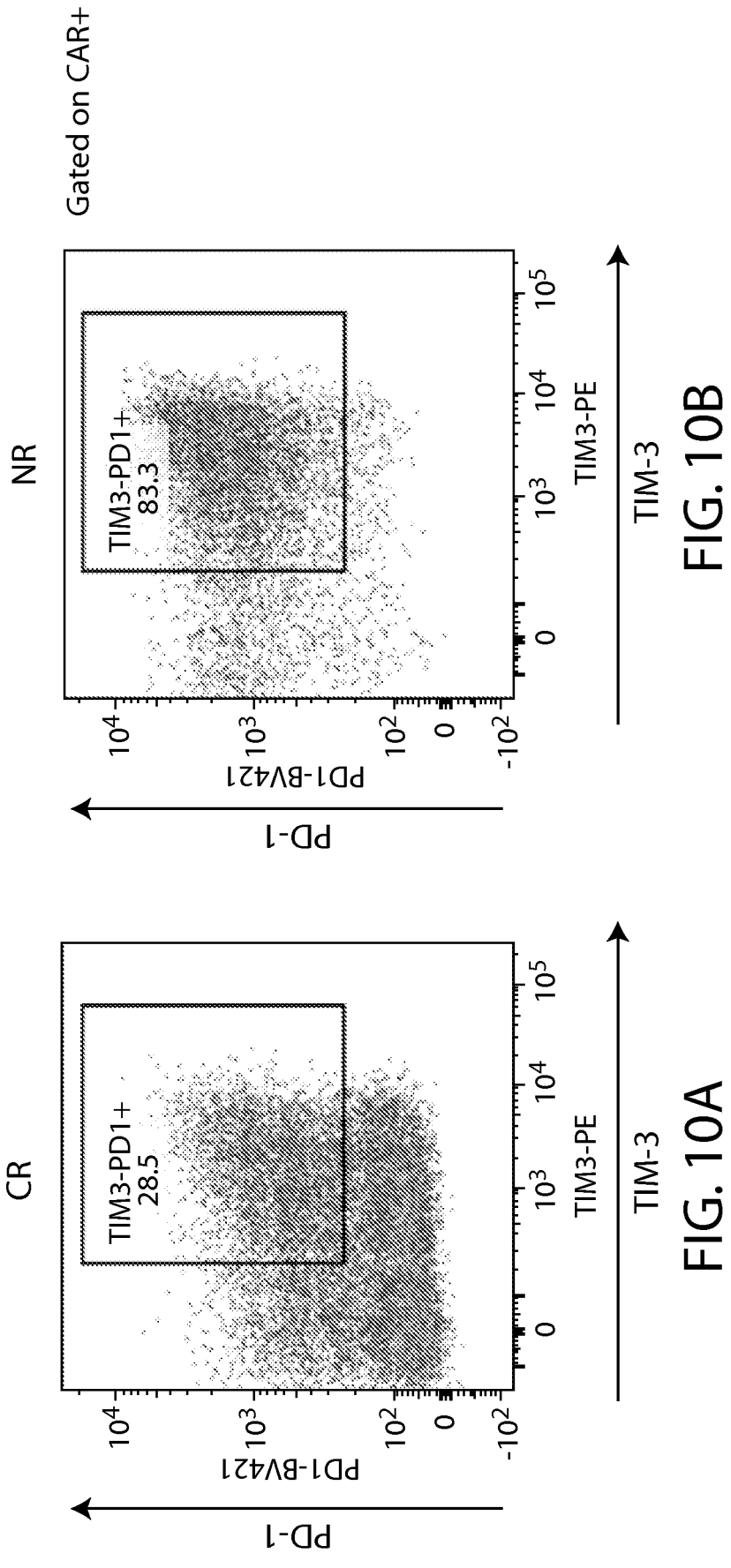


FIG. 10B

FIG. 10A

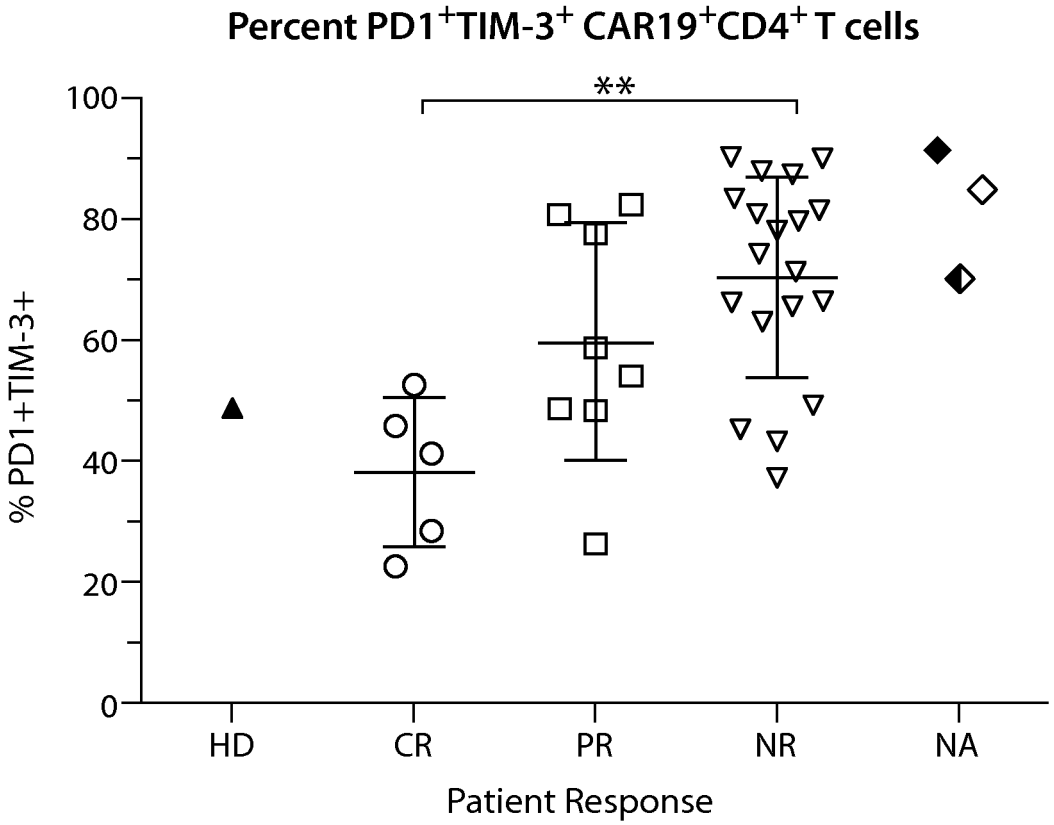

Percent PD1+TIM-3+ CAR19+CD4+ T cells
**
100
80
60
40
20
0
% PD1+TIM-3+
HD
CR
PR
NR
NA
Patient Response

FIG. 10C

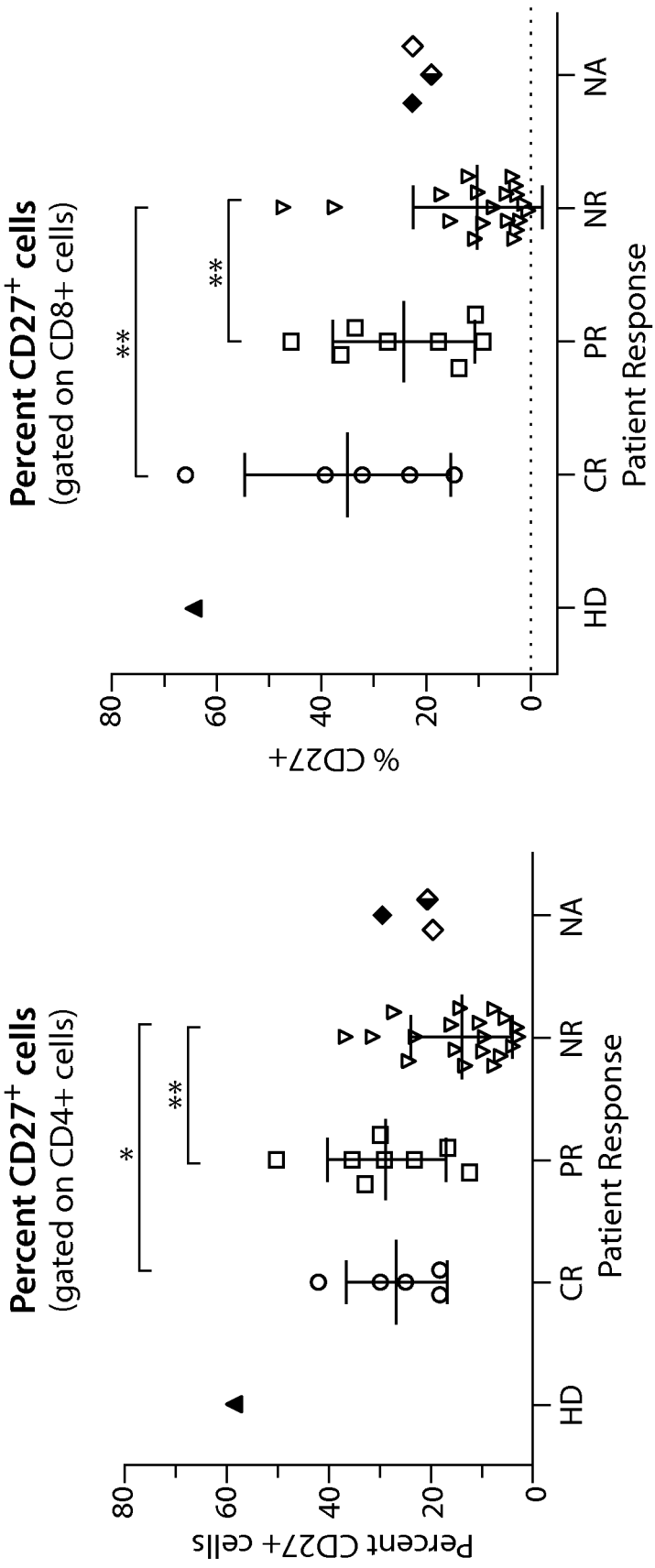

Percent CD27+ cells
(gated on CD4+ cells)
Percent CD27+ cells
(gated on CD8+ cells)
Patient Response
HD
CR
PR
NR
NA
*
**
**
**
0
20
40
60
80

FIG. 11

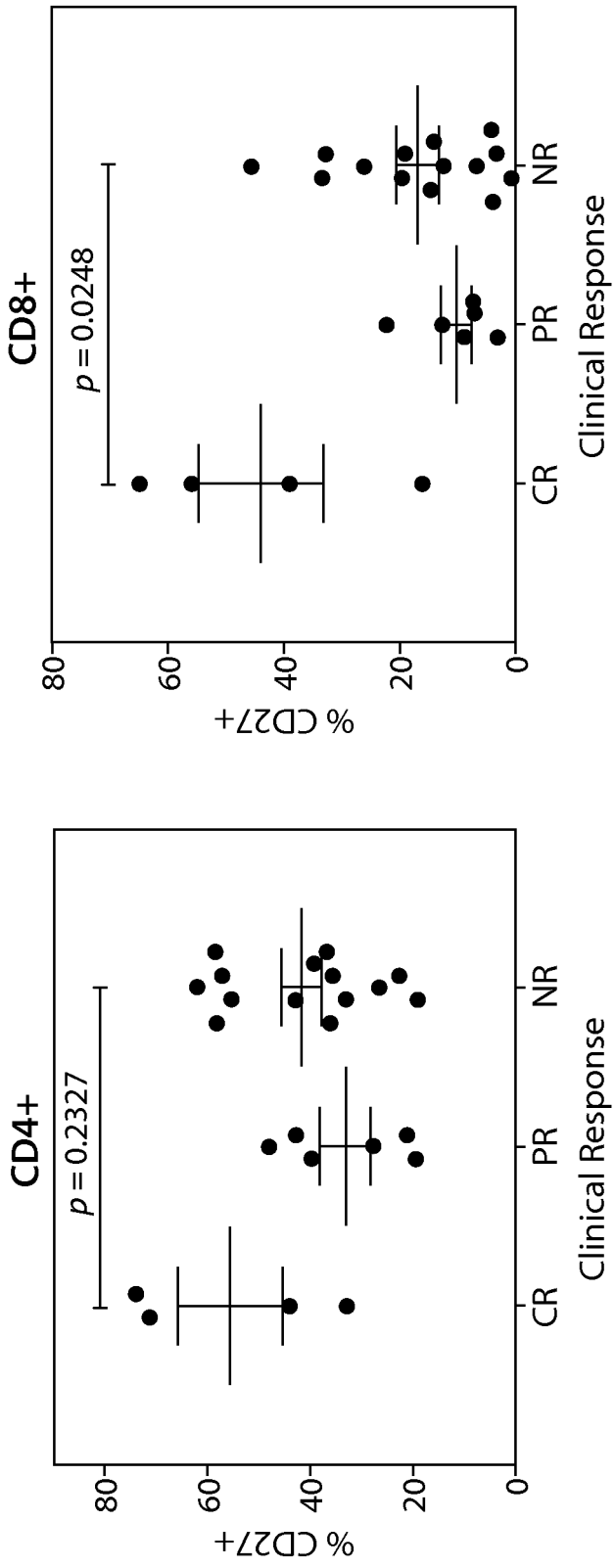

CD8+
p = 0.0248
% CD27+
0
20
40
60
80
CR
PR
NR
Clinical Response
CD4+
p = 0.2327
% CD27+
0
20
40
60
80
CR
PR
NR
Clinical Response

FIG. 12

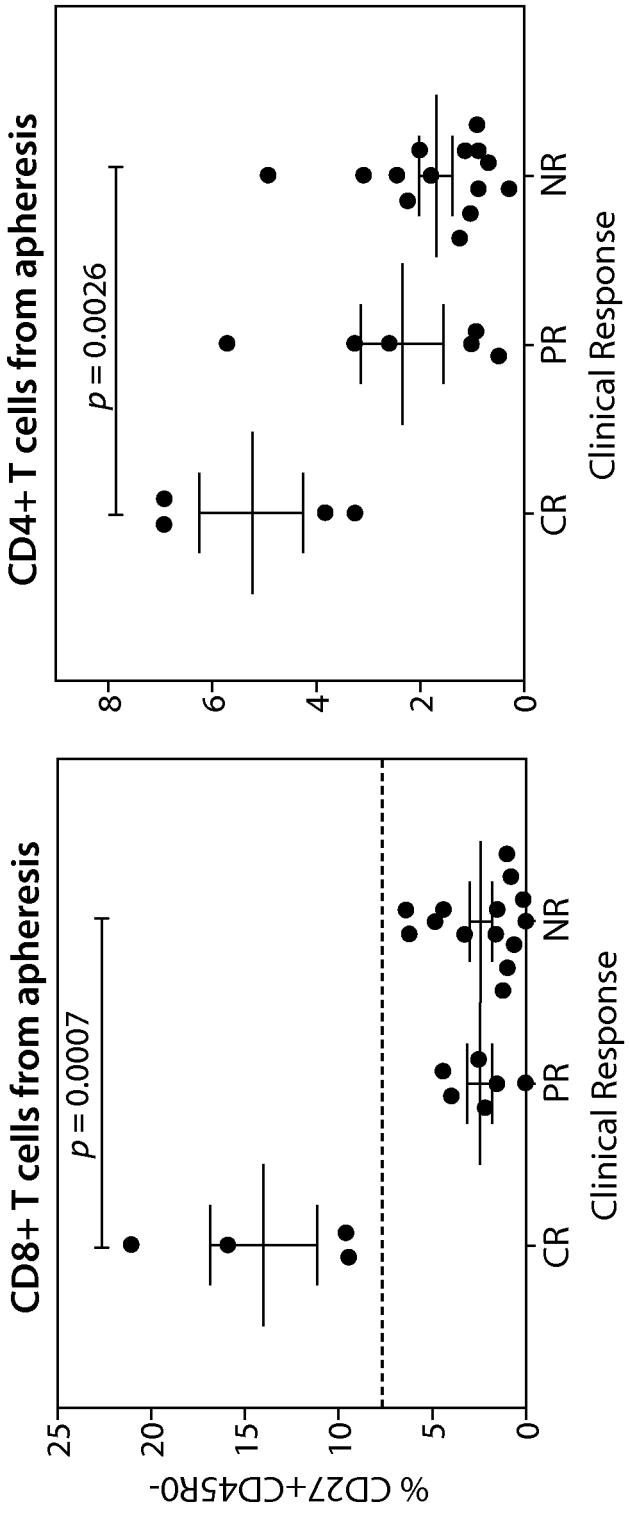

CD8+ T cells from apheresis
p = 0.0007
% CD27+CD45R0-
25
20
15
10
5
0
CR
PR
NR
Clinical Response
CD4+ T cells from apheresis
p = 0.0026
8
6
4
2
0
CR
PR
NR
Clinical Response

FIG. 13

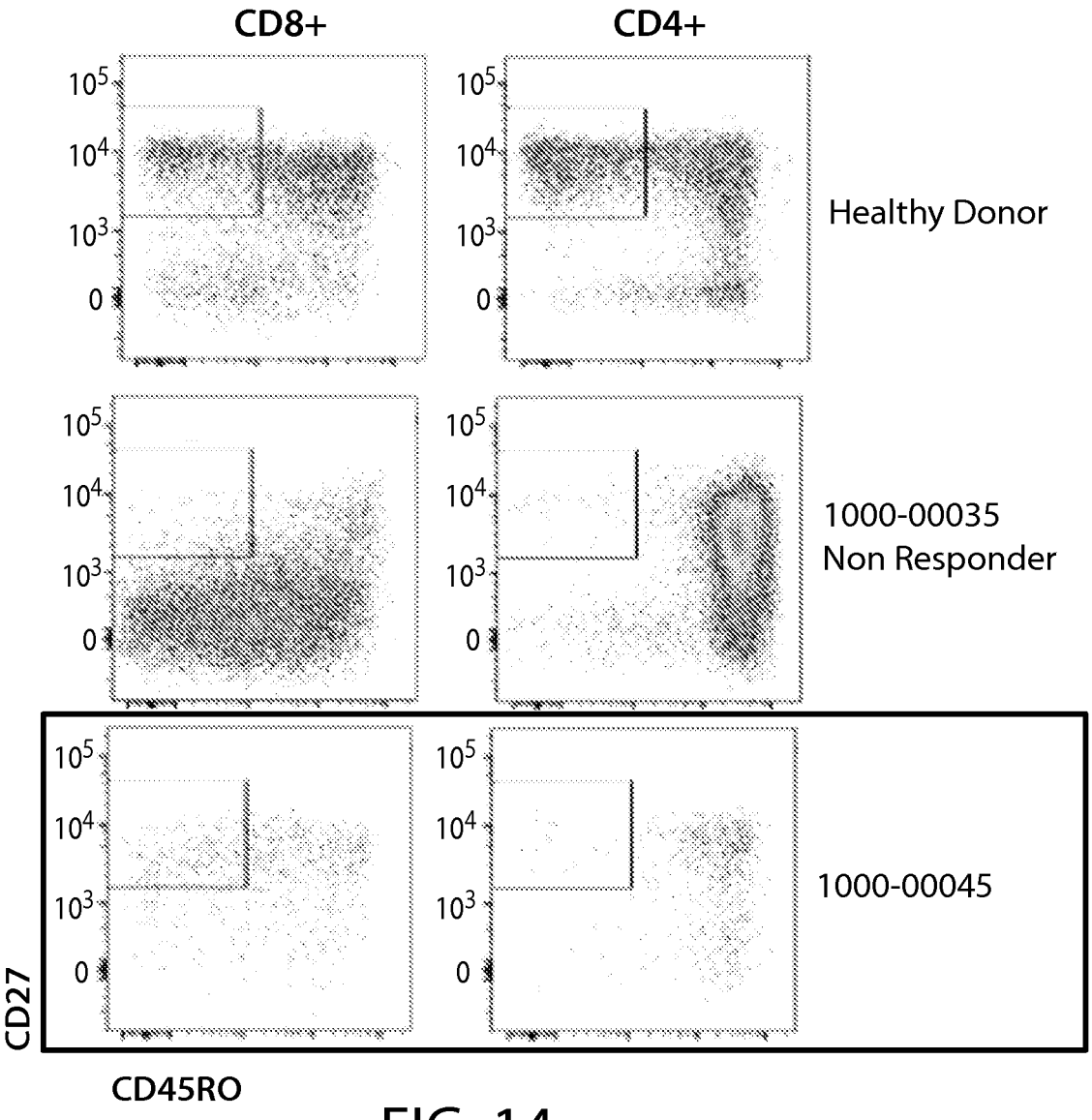

CD8+
CD4+
10^5
10^4
10^3
0
Healthy Donor
1000-00035
Non Responder
1000-00045
CD27
CD45RO

FIG. 14

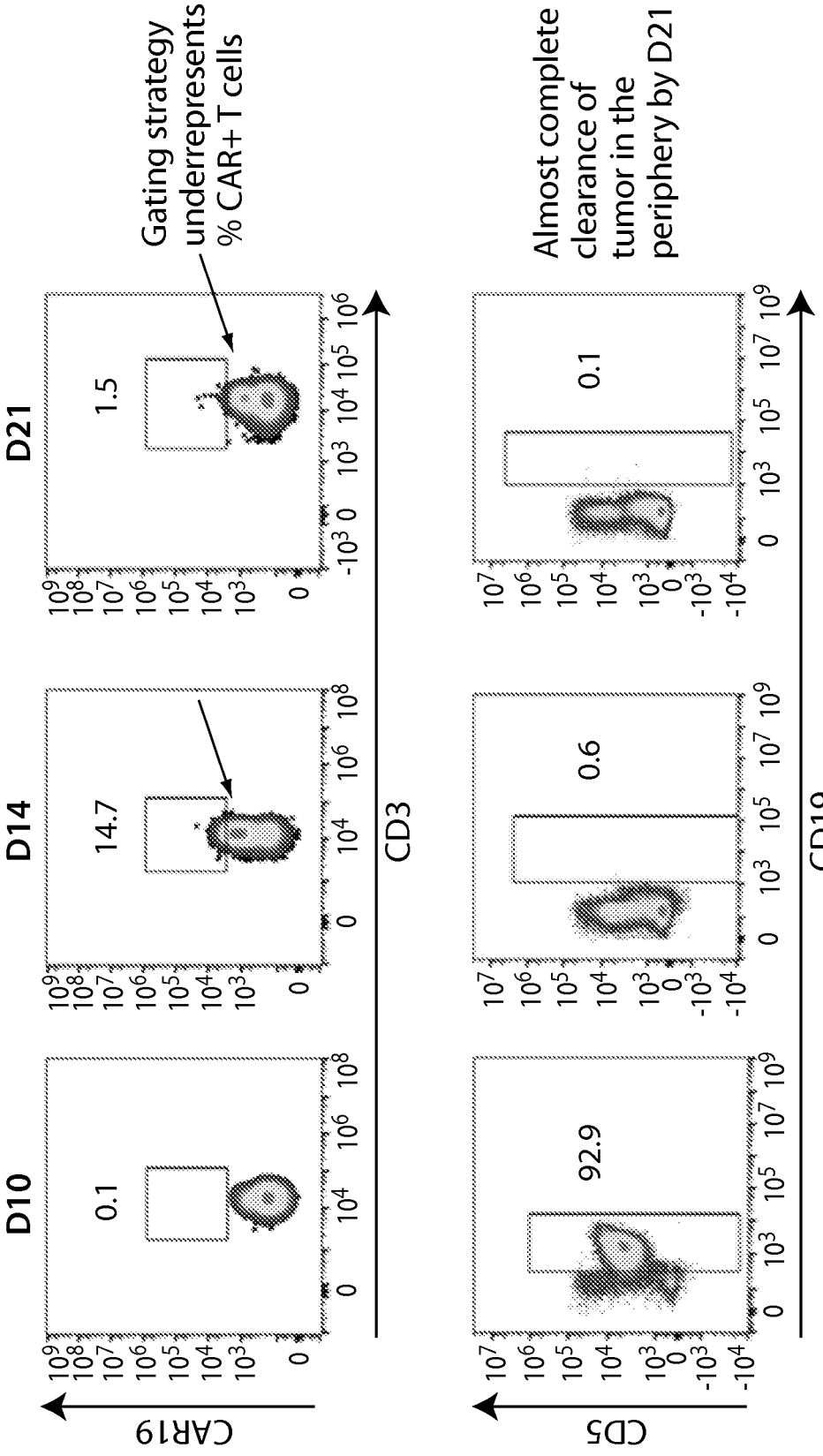

D10
D14
D21
0.1
14.7
1.5
92.9
0.6
0.1
CAR19
CD3
CD5
CD19
Gating strategy underrepresents % CAR+ T cells
Almost complete clearance of tumor in the periphery by D21

FIG. 15

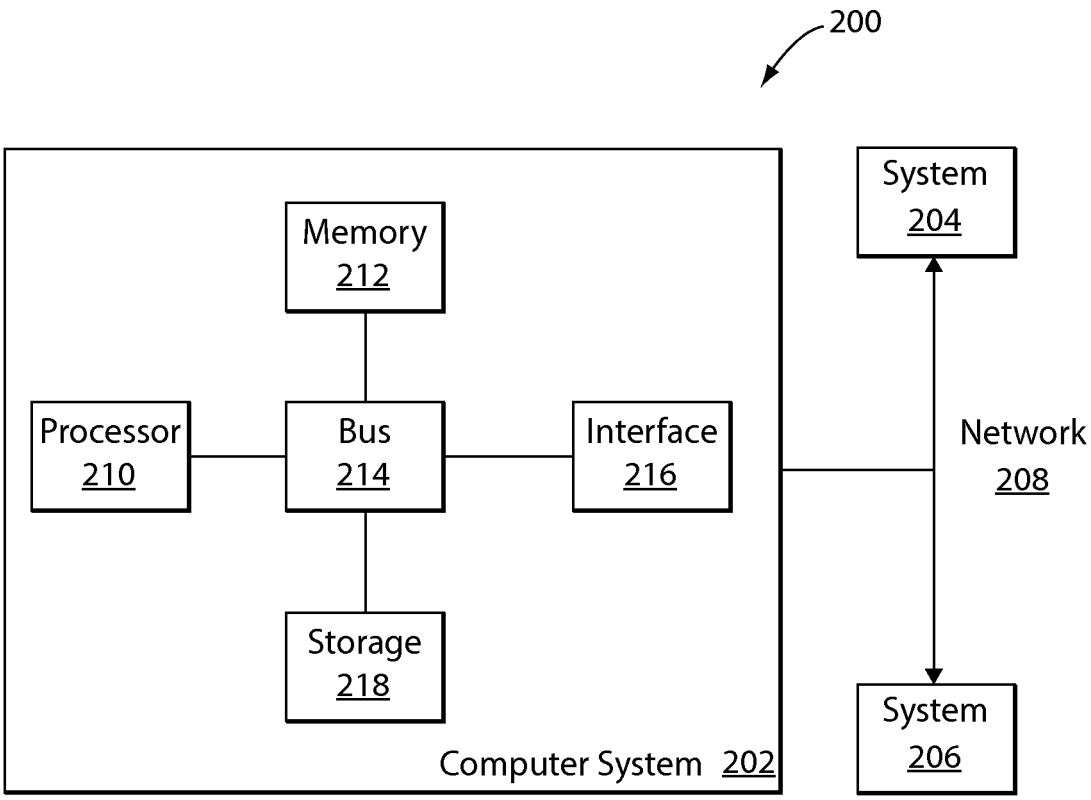

200
System
204
Memory
212
Processor
210
Bus
214
Interface
216
Network
208
Storage
218
System
206
Computer System 202

FIG. 16

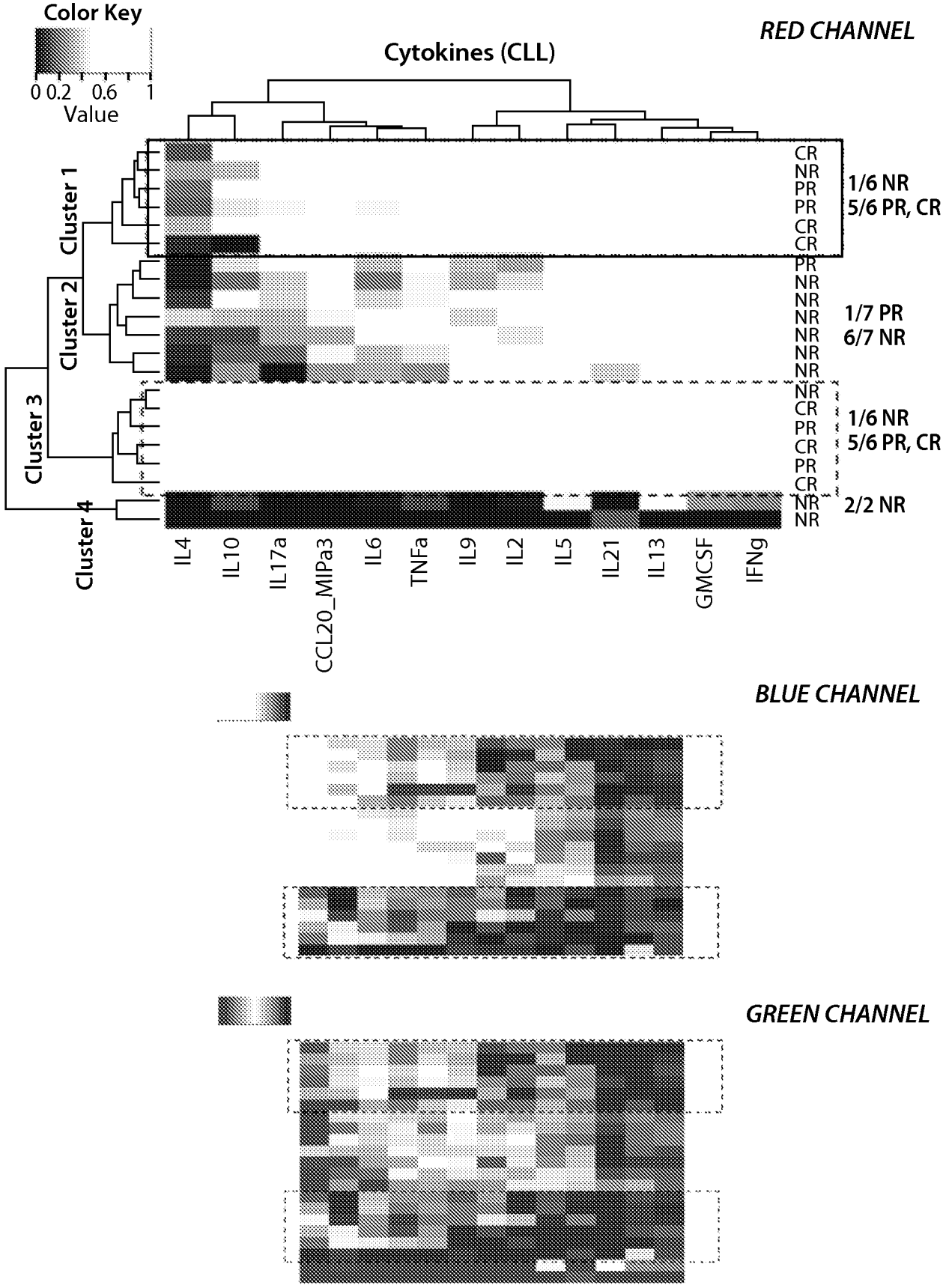

Color Key
0 0.2 0.6 1
Value
RED CHANNEL
Cytokines (CLL)
Cluster 1
Cluster 2
Cluster 3
Cluster 4
CR
NR
PR
PR
CR
CR
PR
NR
NR
NR
NR
NR
NR
CR
PR
CR
PR
CR
NR
NR
1/6 NR
5/6 PR, CR
1/7 PR
6/7 NR
1/6 NR
5/6 PR, CR
2/2 NR
IL4
IL10
IL17a
CCL20_MIPa3
IL6
TNFa
IL9
IL2
IL5
IL21
IL13
GMCSF
IFNg
BLUE CHANNEL
GREEN CHANNEL

FIG. 17

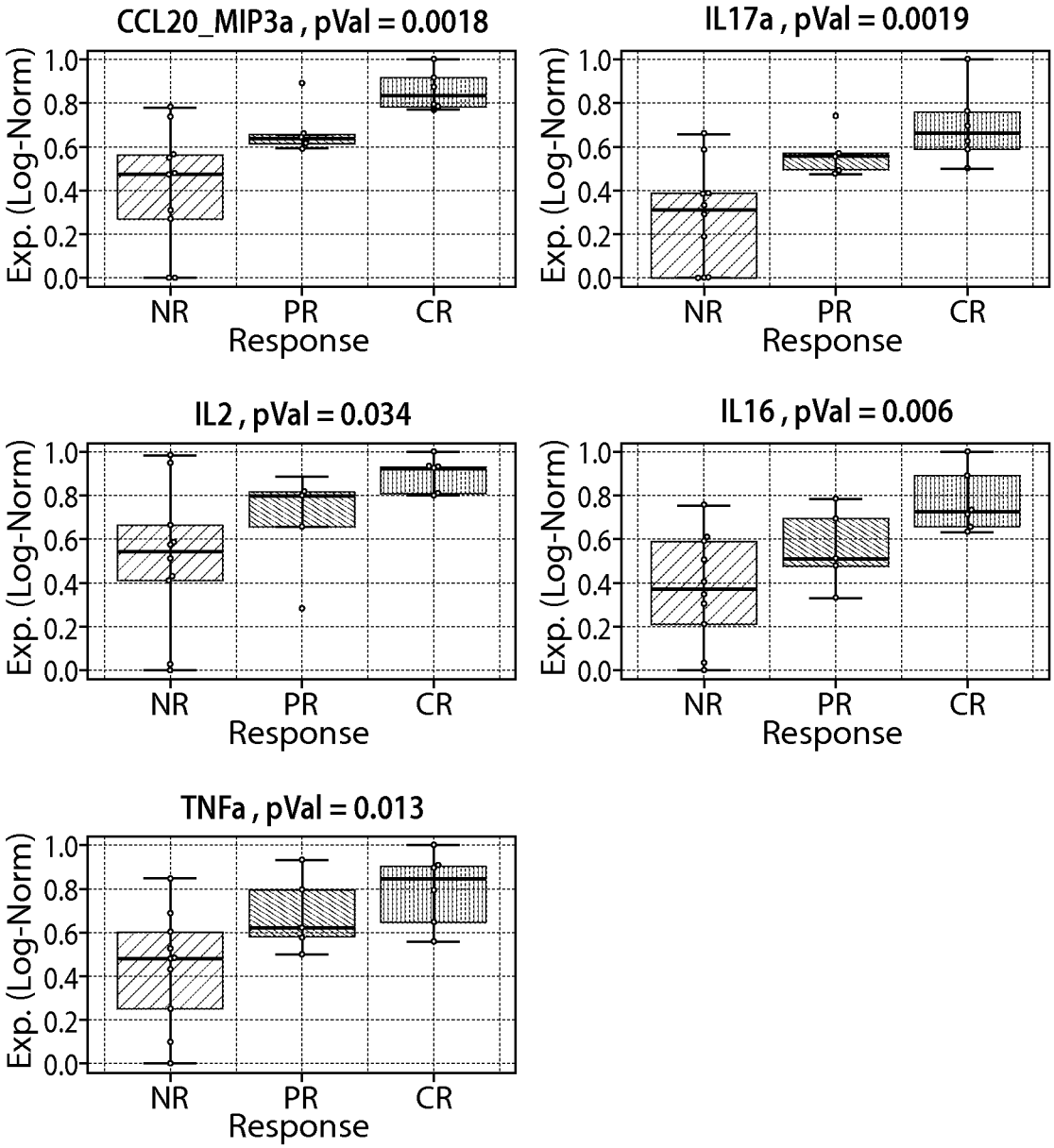

CCL20_MIP3a , pVal = 0.0018
IL17a , pVal = 0.0019
IL2 , pVal = 0.034
IL16 , pVal = 0.006
TNFa , pVal = 0.013
Exp. (Log-Norm)
1.0
0.8
0.6
0.4
0.2
0.0
NR
PR
CR
Response

FIG. 18

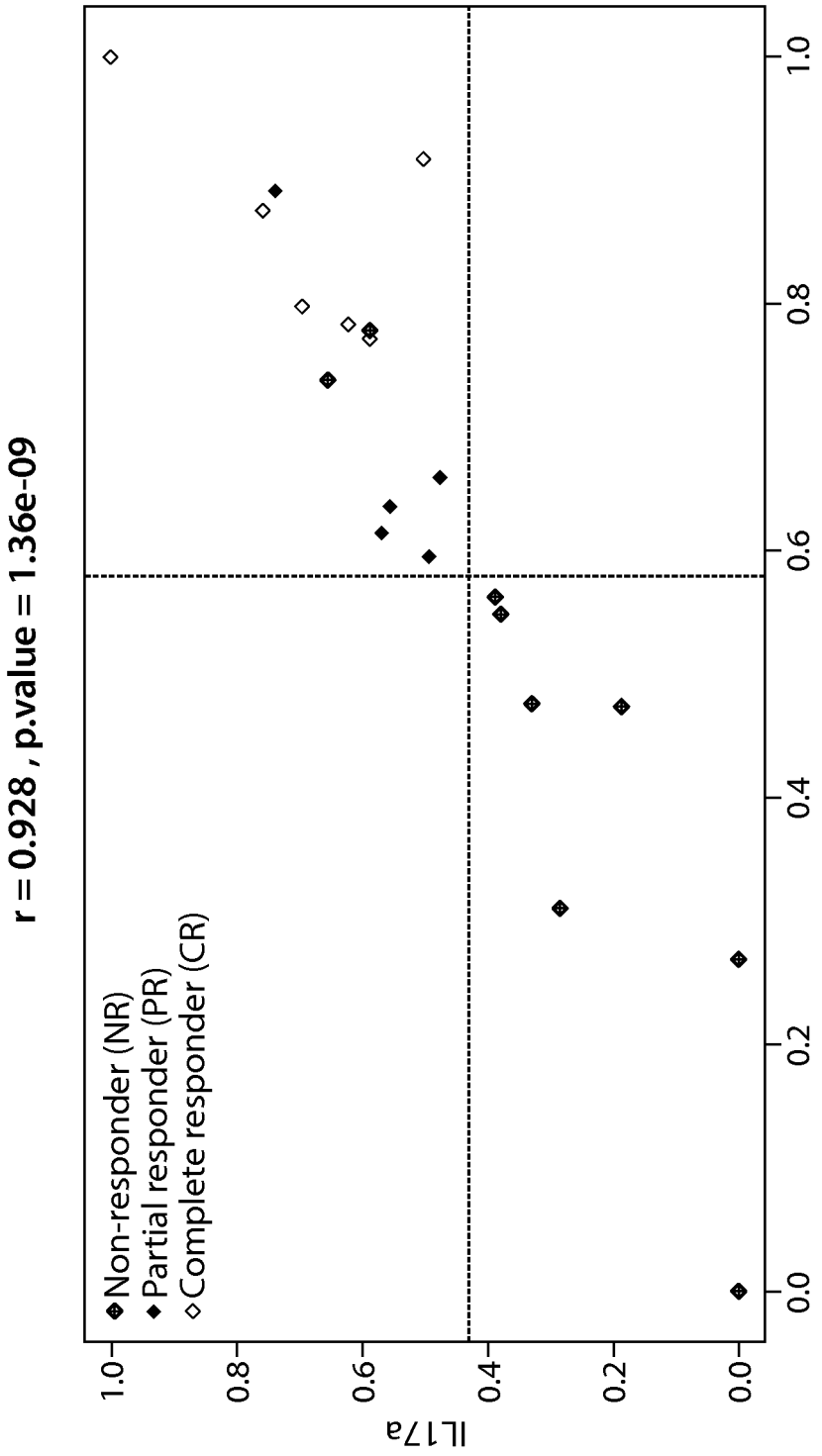

r = 0.928 , p.value = 1.36e-09
Non-responder (NR)
Partial responder (PR)
Complete responder (CR)
IL17a
CCL20_MIP3a
0.0
0.2
0.4
0.6
0.8
1.0

FIG. 19A

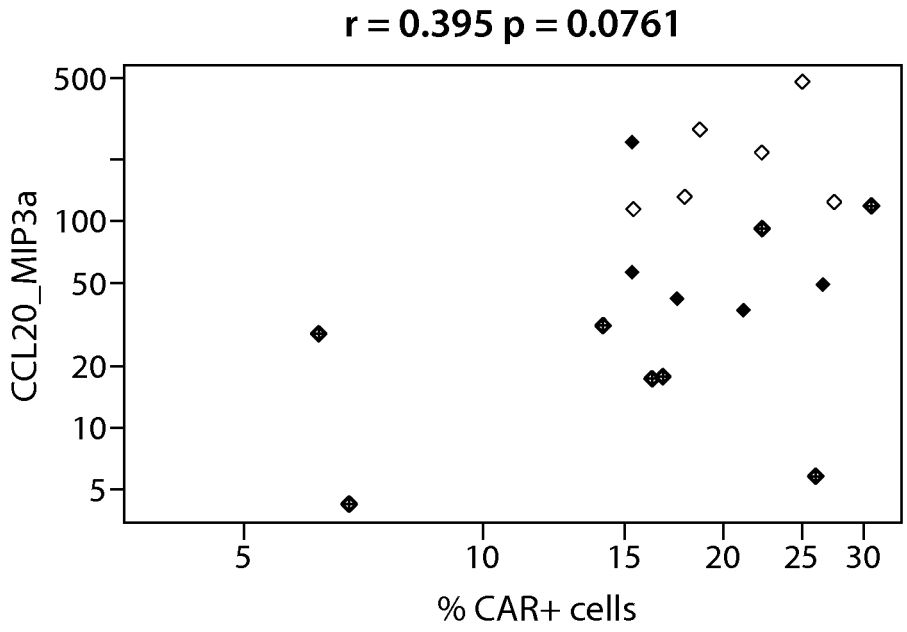


FIG. 19B

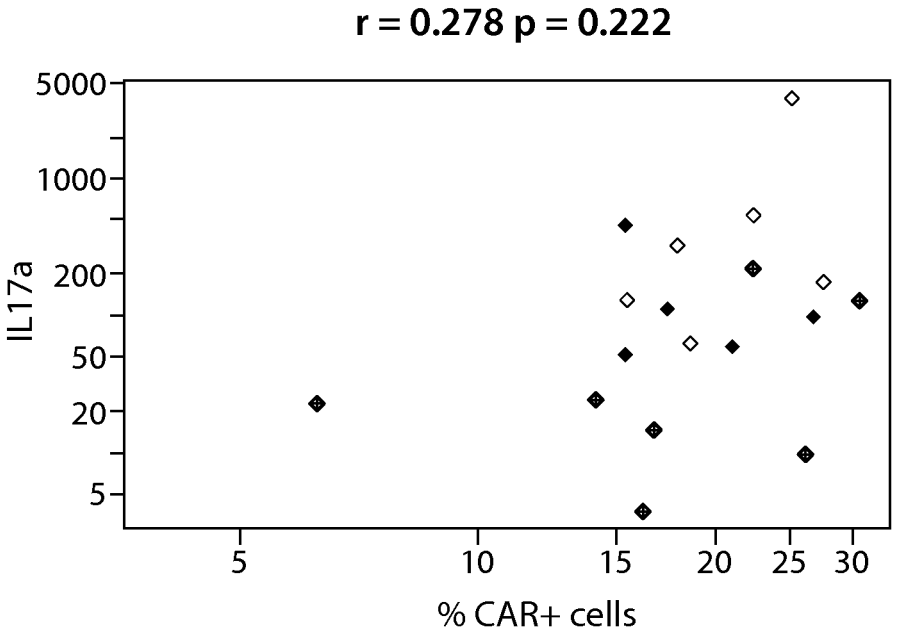
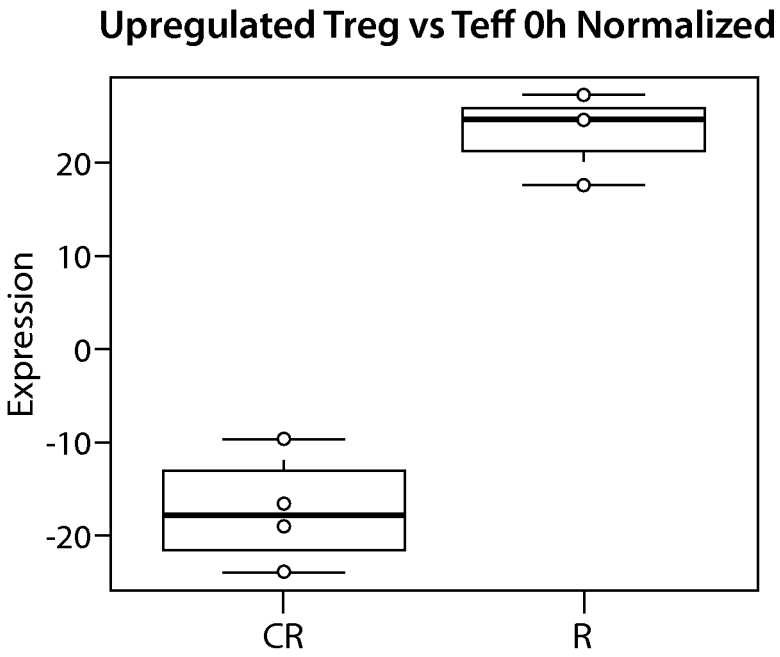


FIG. 19C



p=0.000215

FIG. 20

In CLL, CAR+% cells at Pre-harvest level distinguishes responders
Transduction rate may be indicative of response in CLL

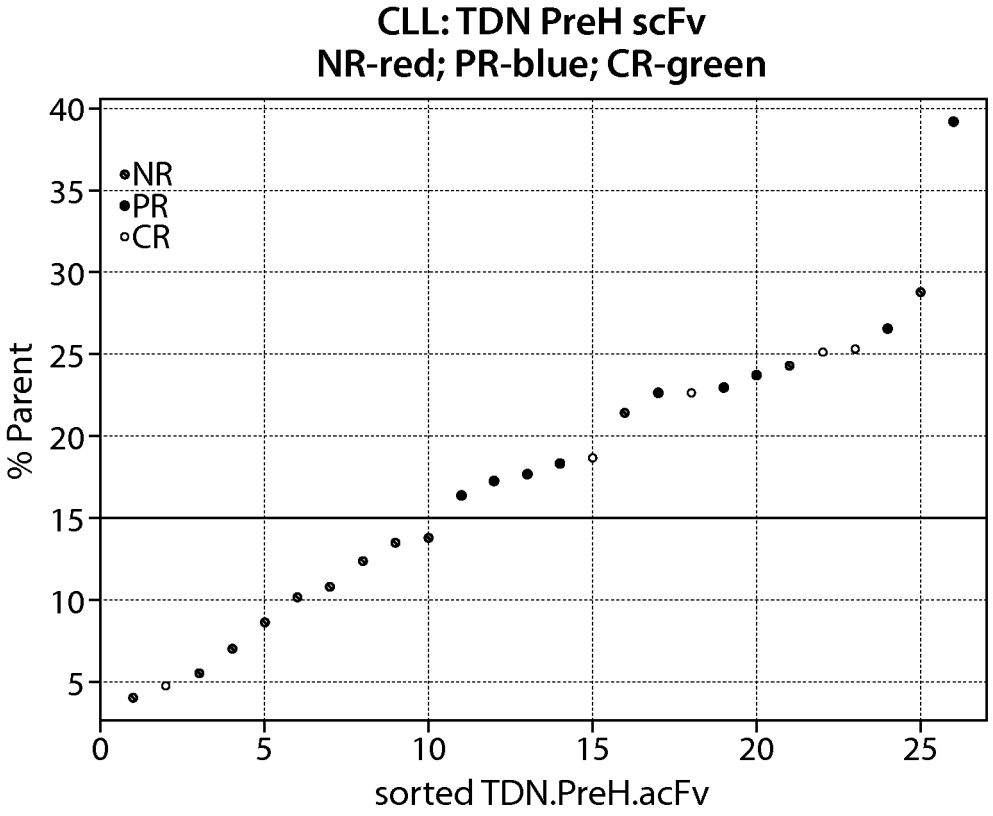


FIG. 21

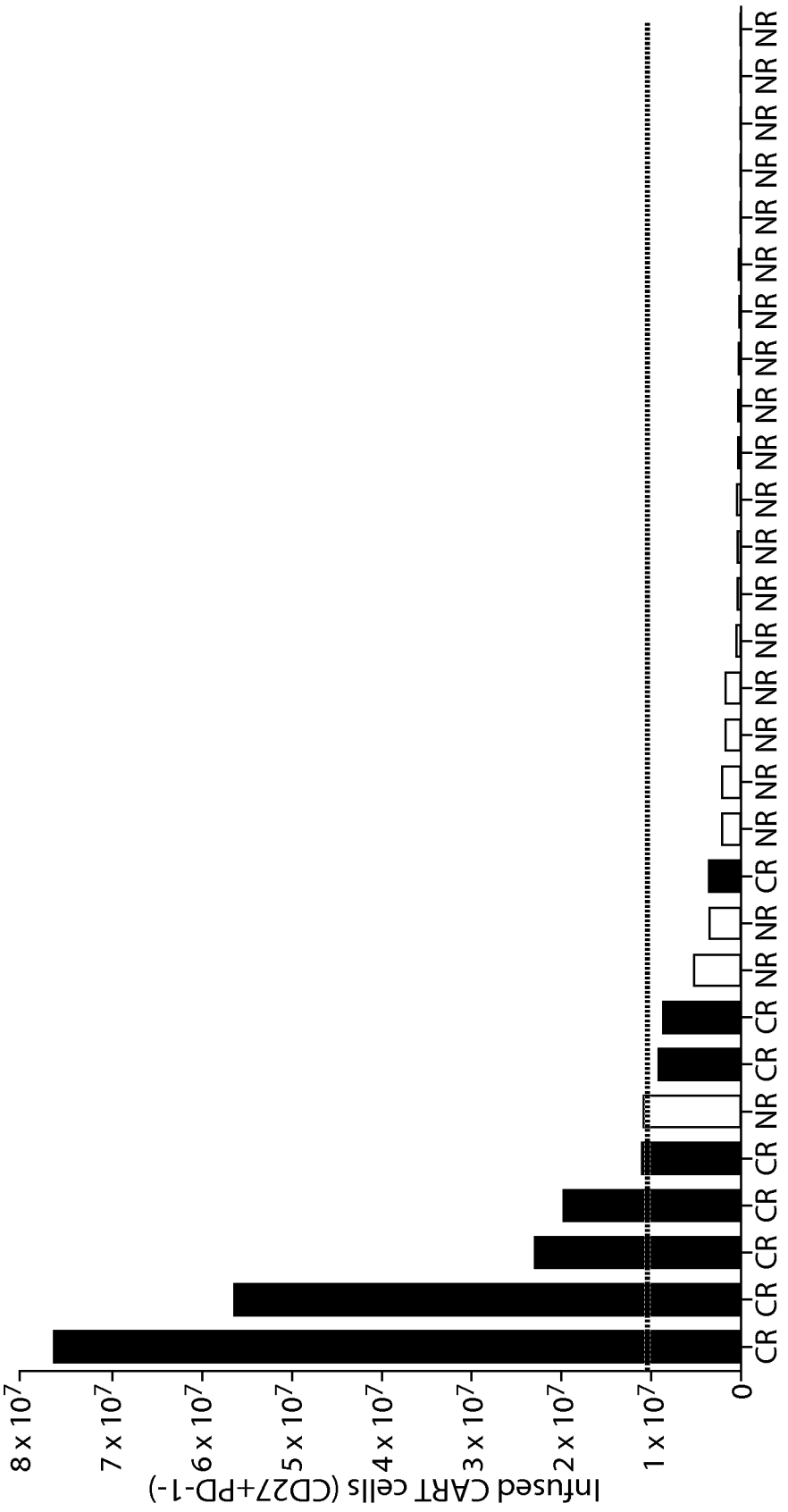

Infused CART cells (CD27+PD-1-)
8 x 10^7
7 x 10^7
6 x 10^7
5 x 10^7
4 x 10^7
3 x 10^7
2 x 10^7
1 x 10^7
0
CR CR CR CR CR NR CR CR NR NR CR NR NR NR NR NR NR NR NR NR NR NR NR NR
Clinical Response

FIG. 22

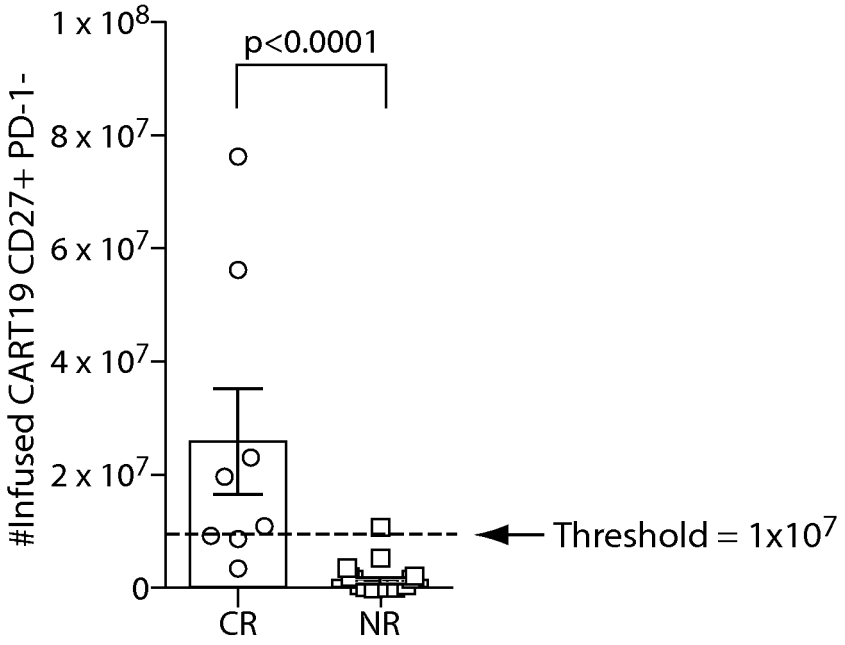

1 x 10^8
8 x 10^7
6 x 10^7
4 x 10^7
2 x 10^7
0
#Infused CART19 CD27+ PD-1-
p<0.0001
Threshold = 1x10^7
CR
NR

FIG. 23