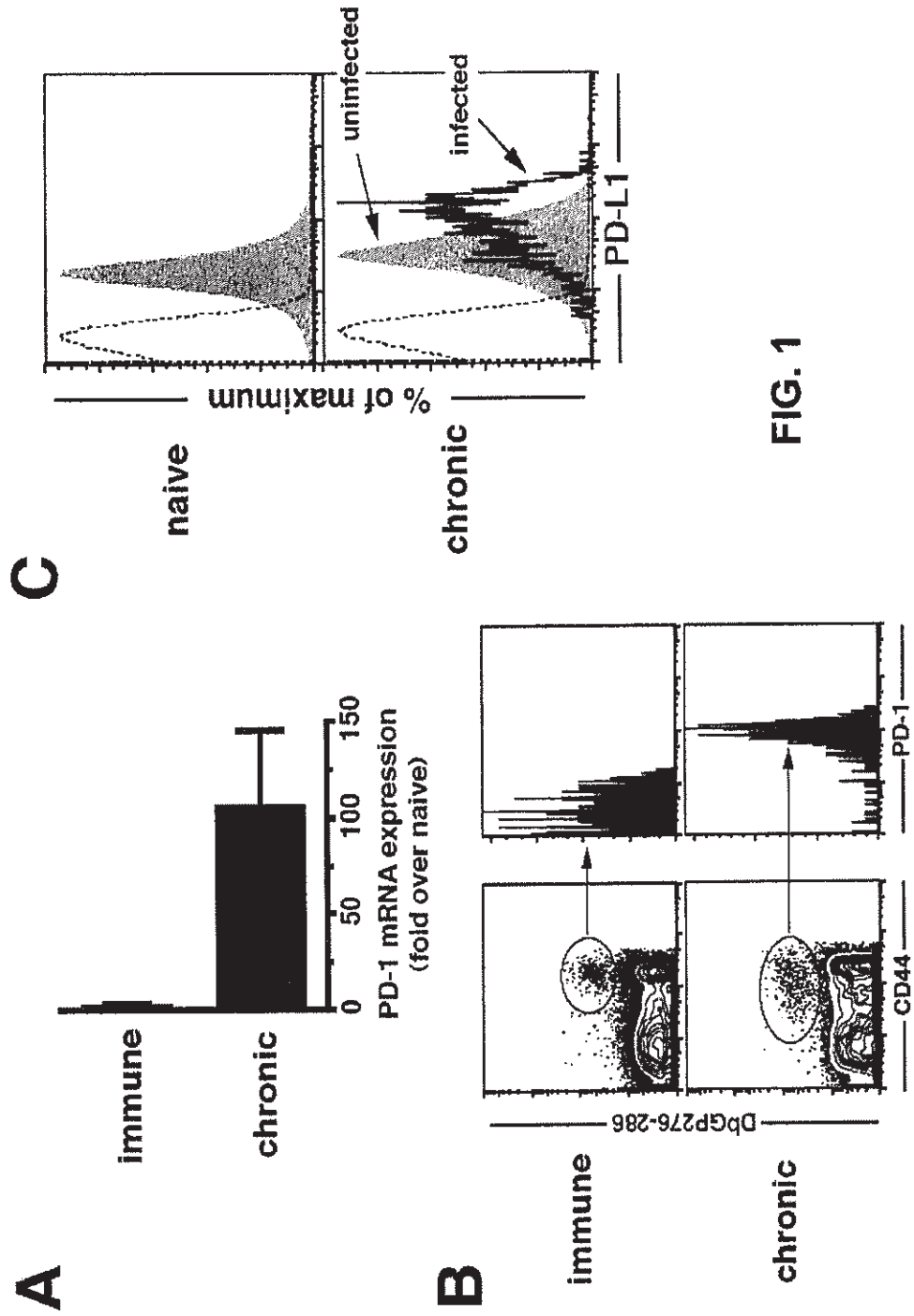

A
immune
chronic
0
50
100
150
PD-1 mRNA expression
(fold over naive)
B
immune
chronic
DbGP276-286
CD44
PD-1
C
naive
chronic
% of maximum
uninfected
infected
PD-L1

FIG. 1

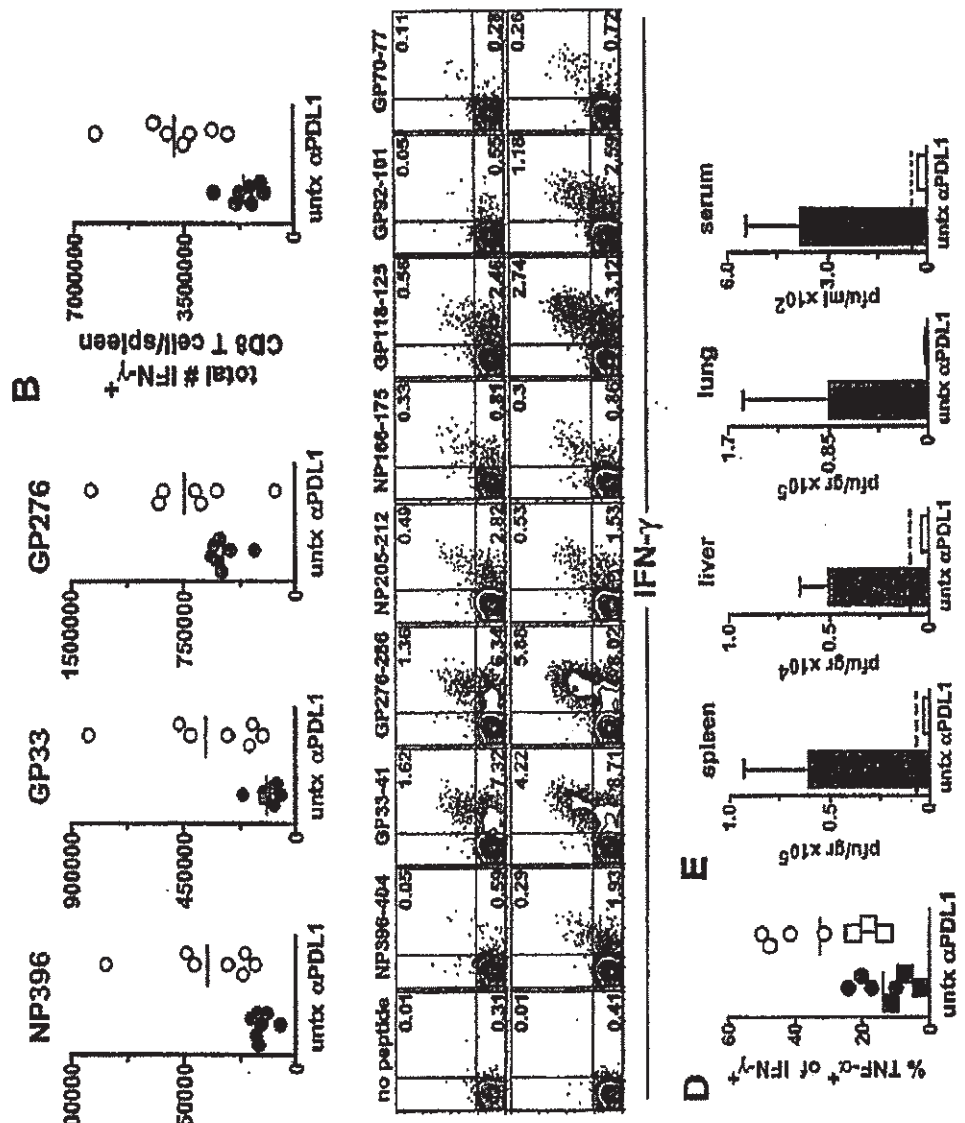

A
NP396
GP33
GP276
Ag-specific CD8 T cells/spleen
300000
150000
900000
450000
1500000
750000
0
untx
αPDL1
B
total # IFN-γ+ CD8 T cell/spleen
7000000
3500000
C
untx
αPDL1
TNF-α
IFN-γ
no peptide
NP396-404
GP33-41
GP276-286
NP205-212
NP166-175
GP118-125
GP32-181
GP70-77
D
% TNF-α+ of IFN-γ+
60
40
20
E
spleen
liver
lung
serum
pfu/gr x10^5
pfu/gr x10^4
pfu/ml x10^2
1.0
0.5
1.7
0.85
6.0
3.0

FIG. 2

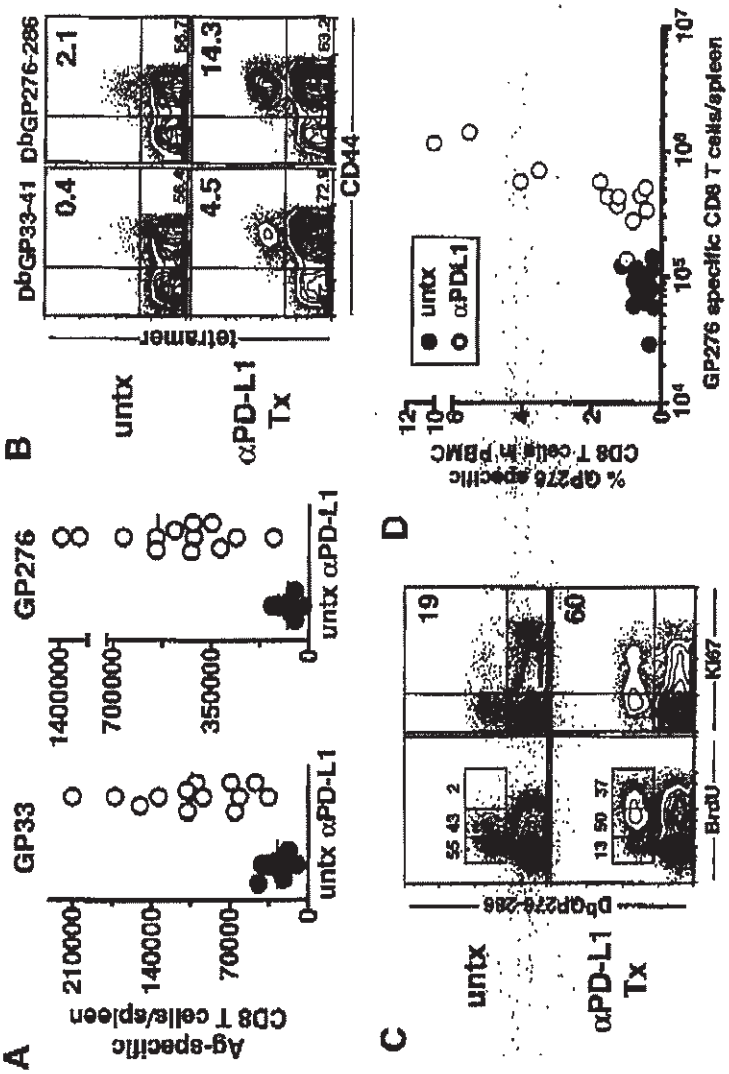

A
Ag-specific CD8 T cells/spleen
GP33
GP276
untx αPD-L1
untx αPD-L1
B
untx
αPD-L1 Tx
DbGP33-41
DbGP276-286
0.4
4.5
2.1
14.3
tetramer
CD44
C
untx
αPD-L1 Tx
DbGP276-286
BrdU
Ki67
55 43 2
13 50 37
19
60
D
% GP276 specific CD8 T cells in PBMC
untx
αPDL1
GP276 specific CD8 T cells/spleen

FIG. 3

FIG. 4

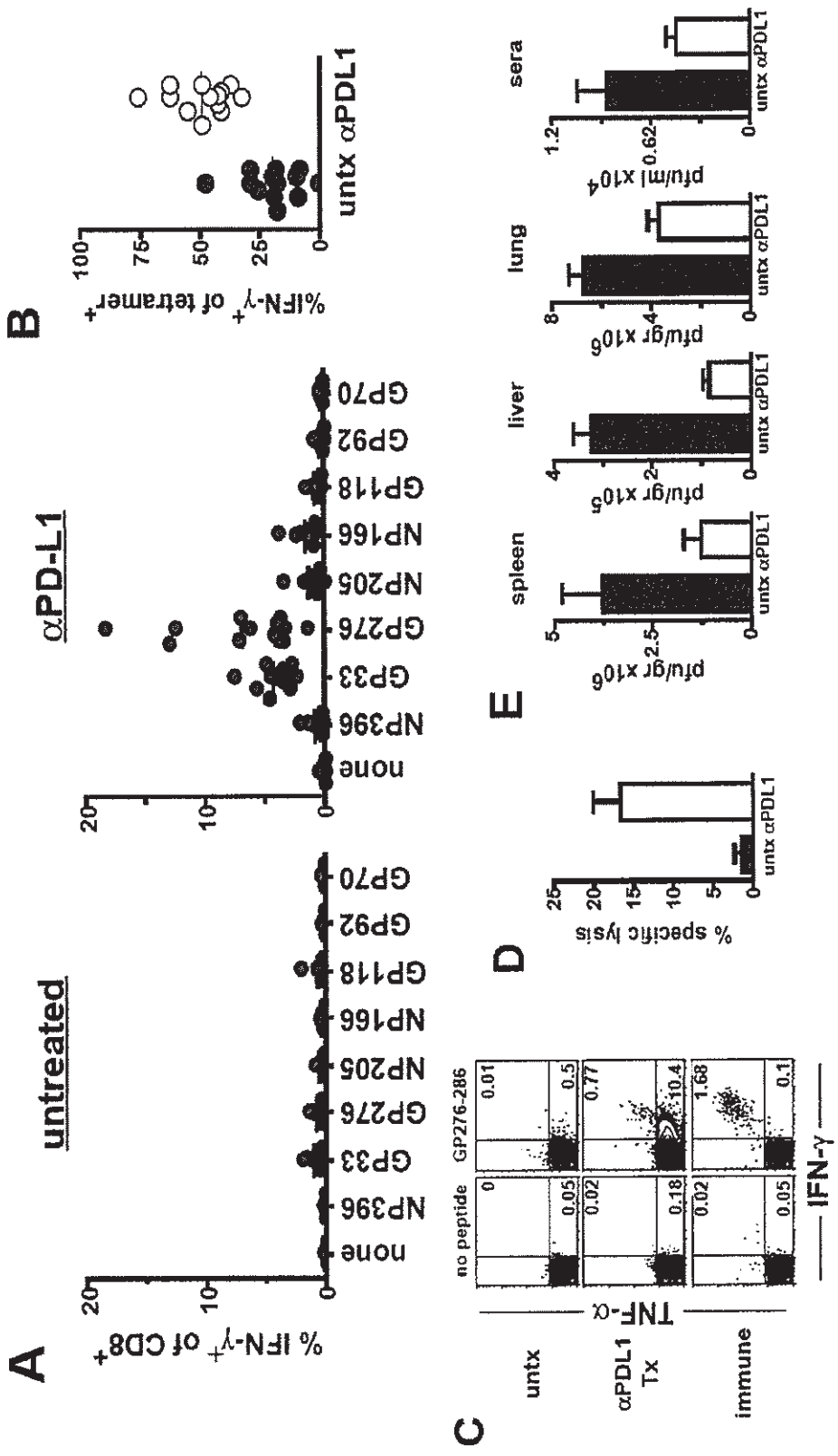

A
untreated
αPD-L1
% IFN-γ+ of CD8+
none
NP396
GP33
GP276
NP205
NP166
GP118
GP92
GP70
B
%IFN-γ+ of tetramer+
untx αPDL1
C
untx
αPDL1 Tx
immune
no peptide
GP276-286
TNF-α
IFN-γ
D
% specific lysis
untx αPDL1
E
spleen
liver
lung
sera
pfu/gr x10^6
pfu/gr x10^5
pfu/ml x10^4
untx αPDL1

FIG. 5

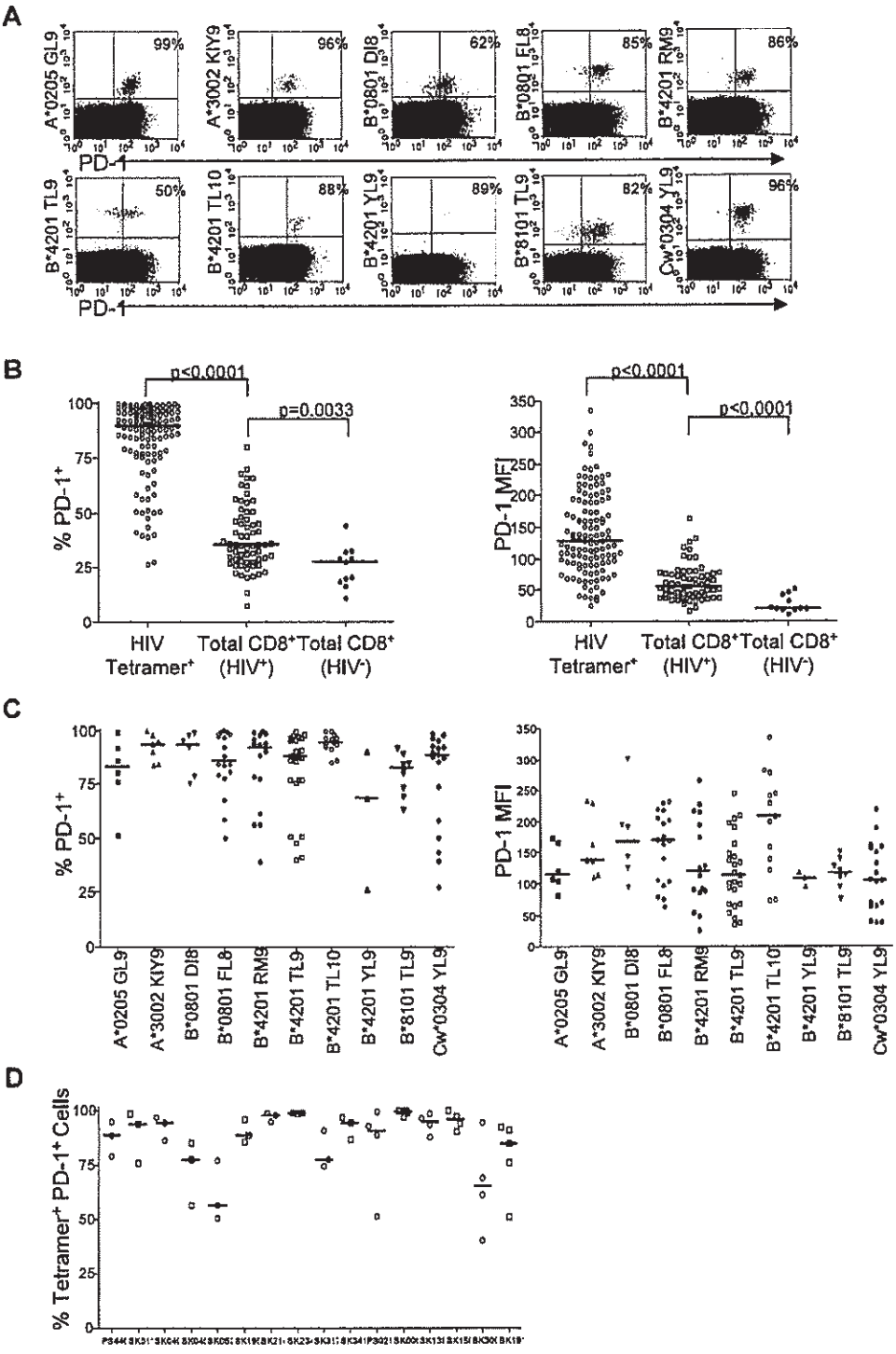

A
A*0205 GL9
99%
A*3002 KIY9
96%
B*0801 DI8
62%
B*0801 FL8
85%
B*4201 RM9
86%
PD-1
B*4201 TL9
50%
B*4201 TL10
88%
B*4201 YL9
89%
B*8101 TL9
82%
Cw*0304 YL9
96%
PD-1
B
p<0.0001
p=0.0033
% PD-1+
HIV Tetramer+
Total CD8+ (HIV+)
Total CD8+ (HIV-)
p<0.0001
p<0.0001
PD-1 MFI
C
% PD-1+
PD-1 MFI
A*0205 GL9
A*3002 KIY9
B*0801 DI8
B*0801 FL8
B*4201 RM9
B*4201 TL9
B*4201 TL10
B*4201 YL9
B*8101 TL9
Cw*0304 YL9
D
% Tetramer+ PD-1+ Cells

FIG. 6

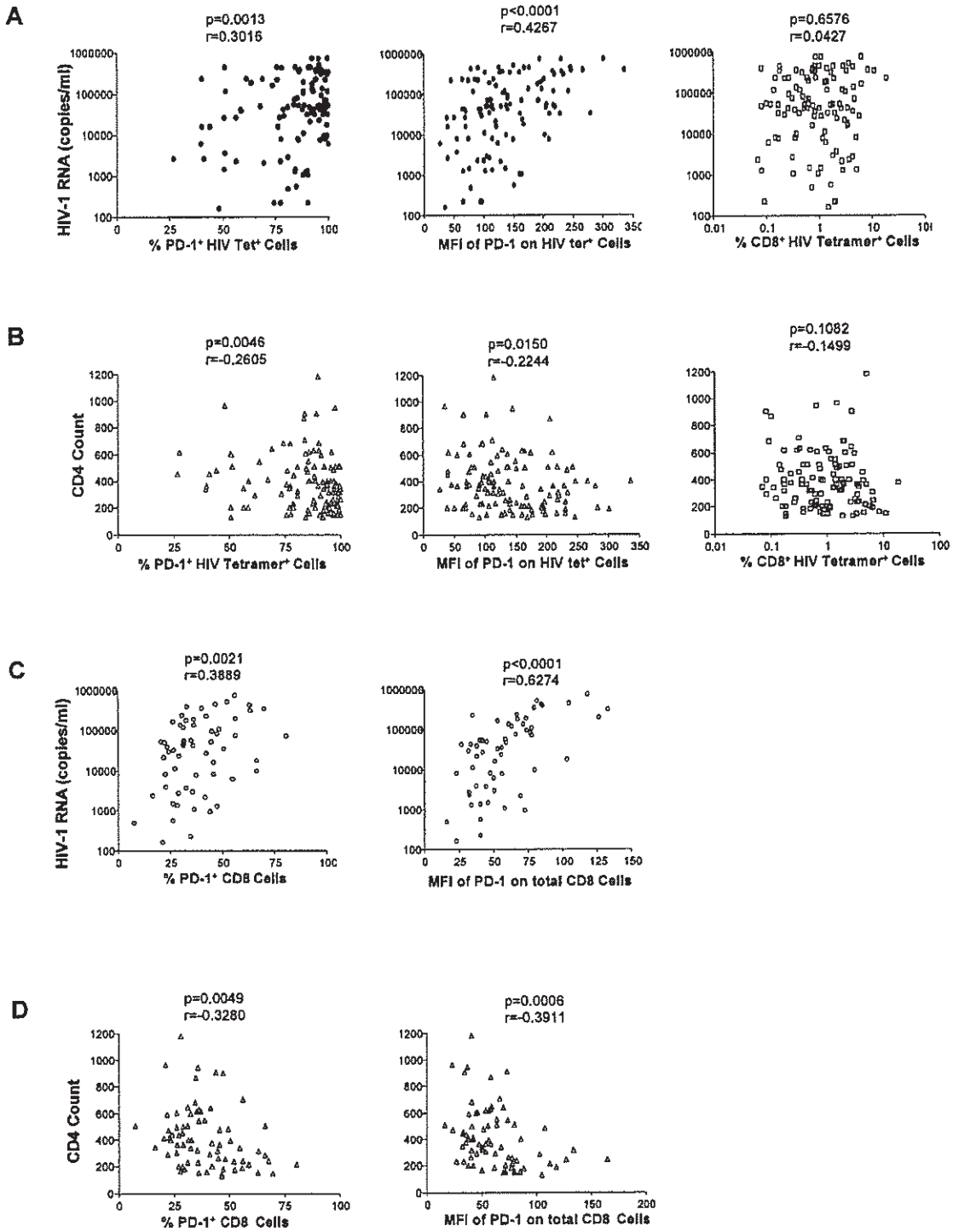

A
p=0.0013
r=0.3016
HIV-1 RNA (copies/ml)
% PD-1+ HIV Tet+ Cells
p<0.0001
r=0.4267
MFI of PD-1 on HIV tet+ Cells
p=0.6576
r=0.0427
% CD8+ HIV Tetramer+ Cells
B
p=0.0046
r=-0.2605
CD4 Count
% PD-1+ HIV Tetramer+ Cells
p=0.0150
r=-0.2244
MFI of PD-1 on HIV tet+ Cells
p=0.1082
r=-0.1499
% CD8+ HIV Tetramer+ Cells
C
p=0.0021
r=0.3889
HIV-1 RNA (copies/ml)
% PD-1+ CD8 Cells
p<0.0001
r=0.6274
MFI of PD-1 on total CD8 Cells
D
p=0.0049
r=-0.3280
CD4 Count
% PD-1+ CD8 Cells
p=0.0006
r=-0.3911
MFI of PD-1 on total CD8 Cells

FIG. 7

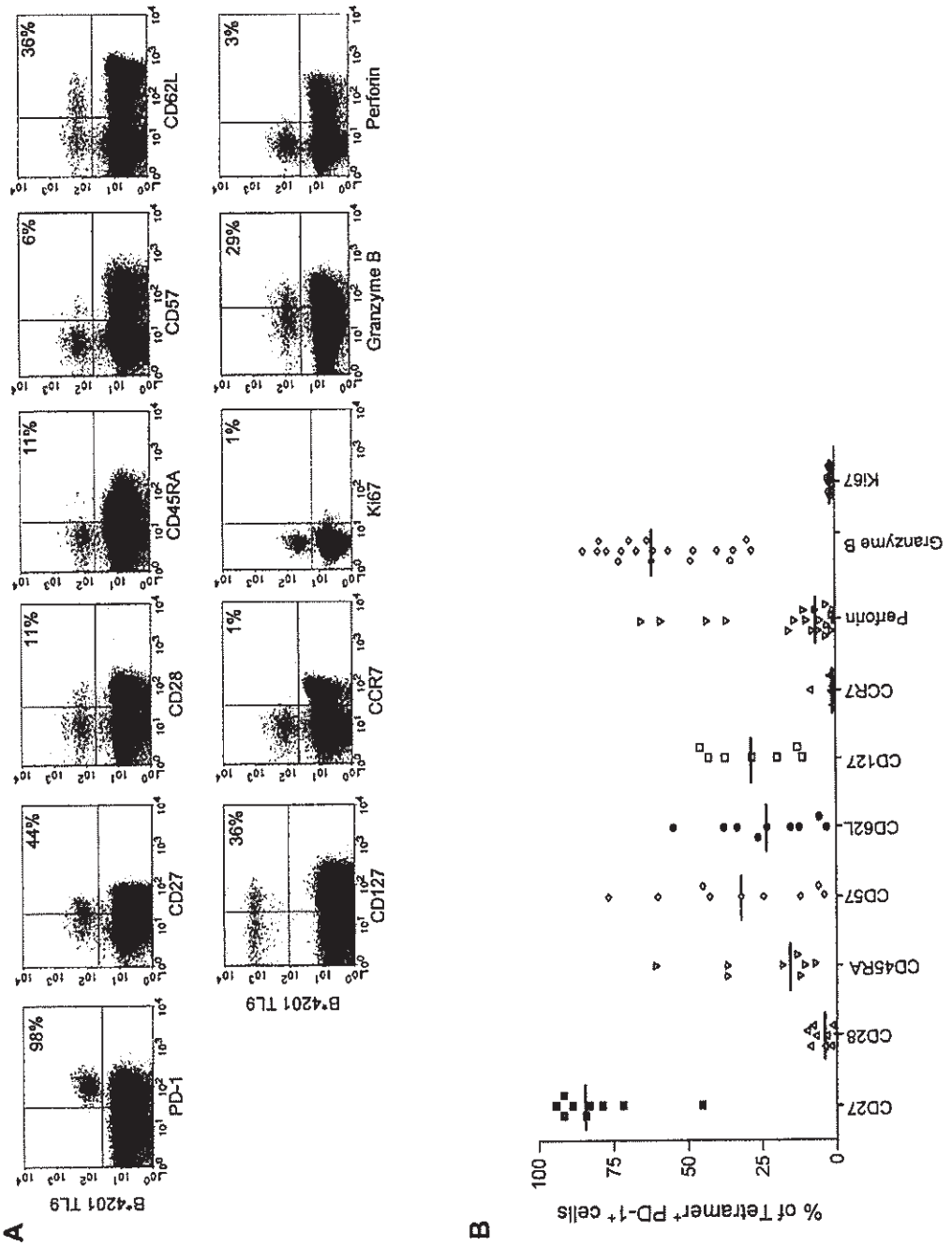

A
B*4201 TL9
98%
PD-1
44%
CD27
11%
CD28
11%
CD45RA
6%
CD57
36%
CD62L
B*4201 TL9
36%
CD127
1%
CCR7
1%
Ki67
29%
Granzyme B
3%
Perforin
B
% of Tetramer+ PD-1+ cells
100
75
50
25
0
CD27
CD28
CD45RA
CD57
CD62L
CD127
CCR7
Perforin
Granzyme B
Ki67

FIG. 8

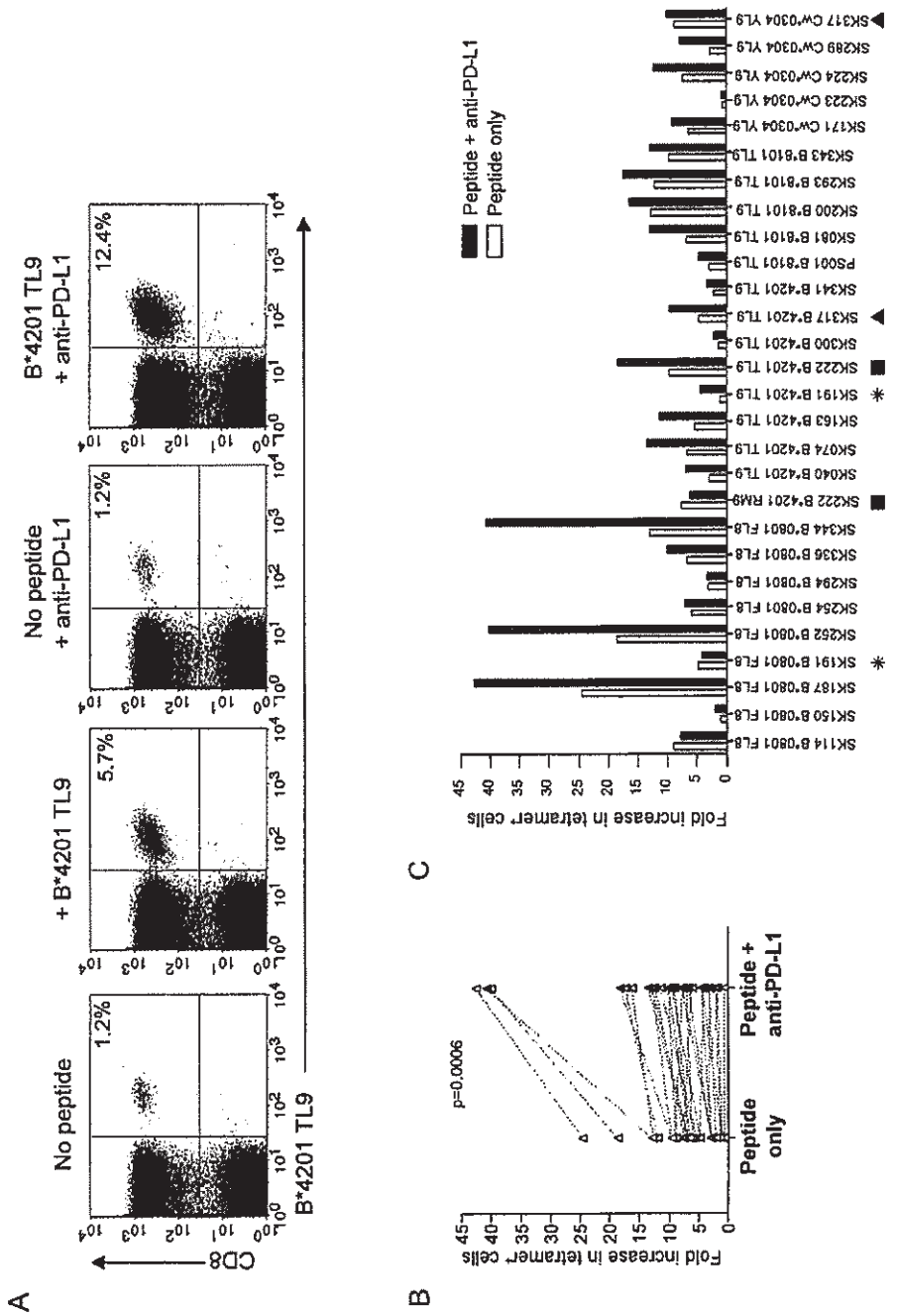

A
No peptide
1.2%
+ B*4201 TL9
5.7%
No peptide
+ anti-PD-L1
1.2%
B*4201 TL9
+ anti-PD-L1
12.4%
CD8
B*4201 TL9
B
p=0.0006
Fold increase in tetramer cells
Peptide only
Peptide + anti-PD-L1
C
Peptide + anti-PD-L1
Peptide only
Fold increase in tetramer cells
SK114 B*0801 FLR
SK150 B*0801 FLR
SK187 B*0801 FLR
SK191 B*0801 FLR
SK262 B*0801 FLR
SK264 B*0801 FLR
SK294 B*0801 FLR
SK336 B*0801 FLR
SK344 B*0801 FLR
SK322 B*4201 RM9
SK040 B*4201 TL9
SK074 B*4201 TL9
SK163 B*4201 TL9
SK191 B*4201 TL9
SK322 B*4201 TL9
SK300 B*4201 TL9
SK317 B*4201 TL9
SK341 B*4201 TL9
PS001 B*8101 TL9
SK081 B*8101 TL9
SK200 B*8101 TL9
SK293 B*8101 TL9
SK343 B*8101 TL9
SK171 Cw*0304 YL9
SK223 Cw*0304 YL9
SK224 Cw*0304 YL9
SK289 Cw*0304 YL9
SK317 Cw*0304 YL9

FIG. 9

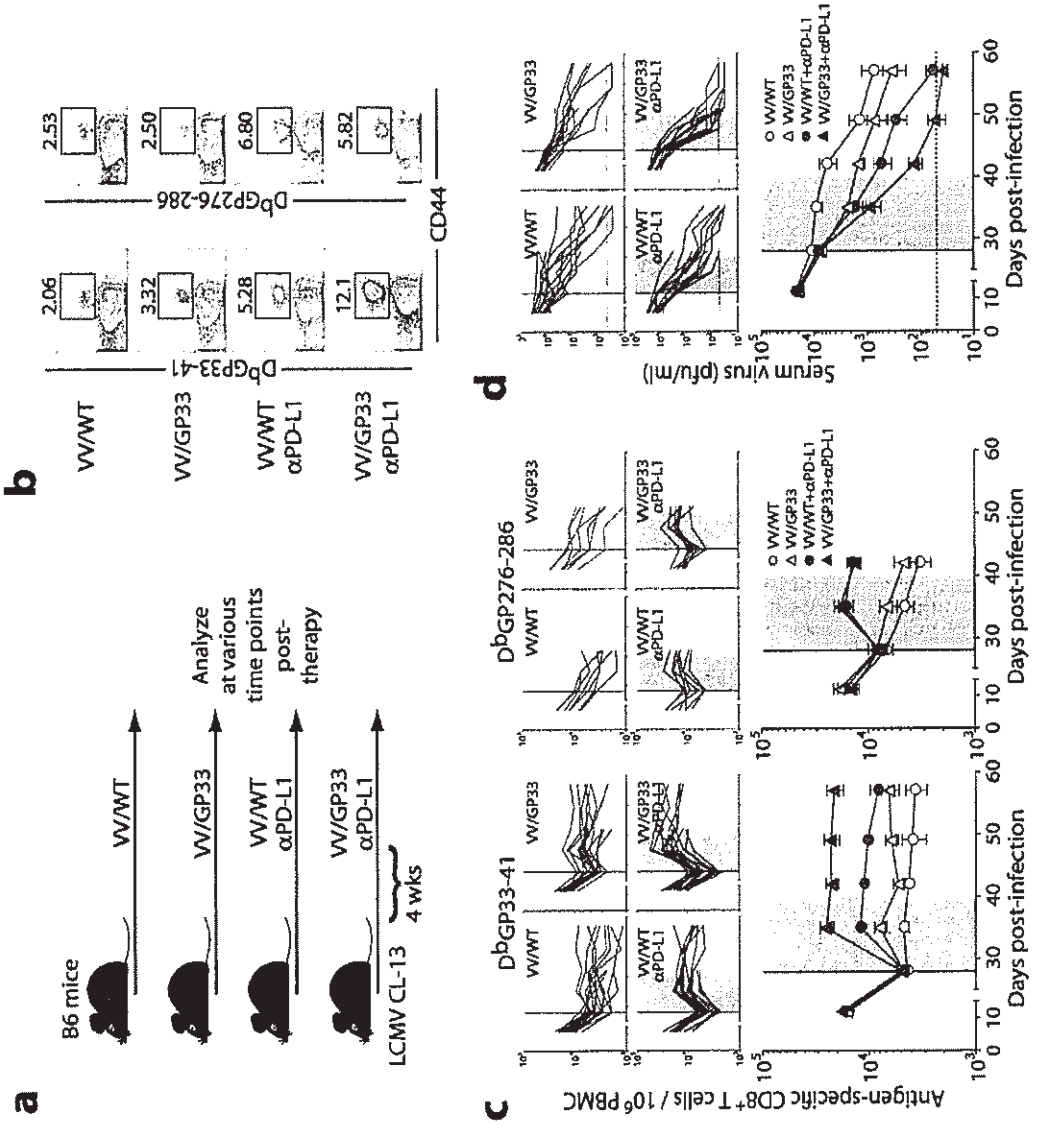

a
B6 mice
VV/WT
VV/GP33
VV/WT αPD-L1
VV/GP33 αPD-L1
LCMV CL-13
4 wks
Analyze at various time points post-therapy
b
VV/WT
VV/GP33
VV/WT αPD-L1
VV/GP33 αPD-L1
DbGP33-41
DbGP276-286
CD44
2.06
3.32
5.28
12.1
2.53
2.50
6.80
5.82
c
DbGP33-41
DbGP276-286
Antigen-specific CD8+ T cells / 10^6 PBMC
Days post-infection
d
Serum Virus (pfu/ml)
Days post-infection
VV/WT
VV/GP33
VV/WT+αPD-L1
VV/GP33+αPD-L1

FIG. 10

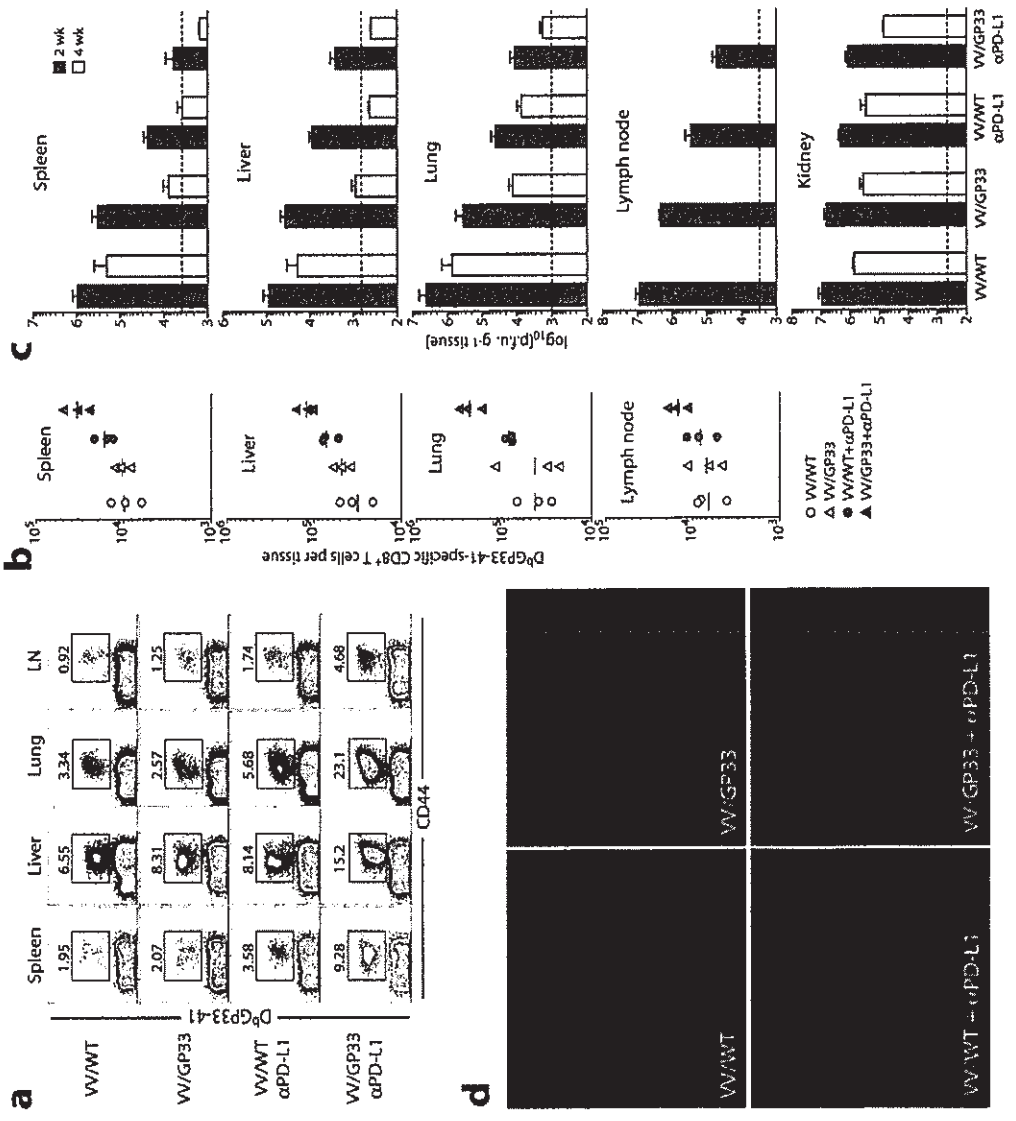

a
Spleen
Liver
Lung
LN
VV/WT
VV/GP33
VV/WT αPD-L1
VV/GP33 αPD-L1
1.95
6.55
3.34
0.92
2.07
8.31
2.57
1.25
3.58
8.14
5.68
1.74
9.28
15.2
23.1
4.68
DbGP33-41
CD44
b
DbGP33-41-specific CD8+ T cells per tissue
Spleen
Liver
Lung
Lymph node
VV/WT
VV/GP33
VV/WT+αPD-L1
VV/GP33+αPD-L1
c
log10(p.f.u. g-1 tissue)
2 wk
4 wk
Spleen
Liver
Lung
Lymph node
Kidney
VV/WT
VV/GP33
VV/WT αPD-L1
VV/GP33 αPD-L1
d
VV/WT
VV/GP33
VV/WT + αPD-L1
VV/GP33 + αPD-L1

FIG. 11

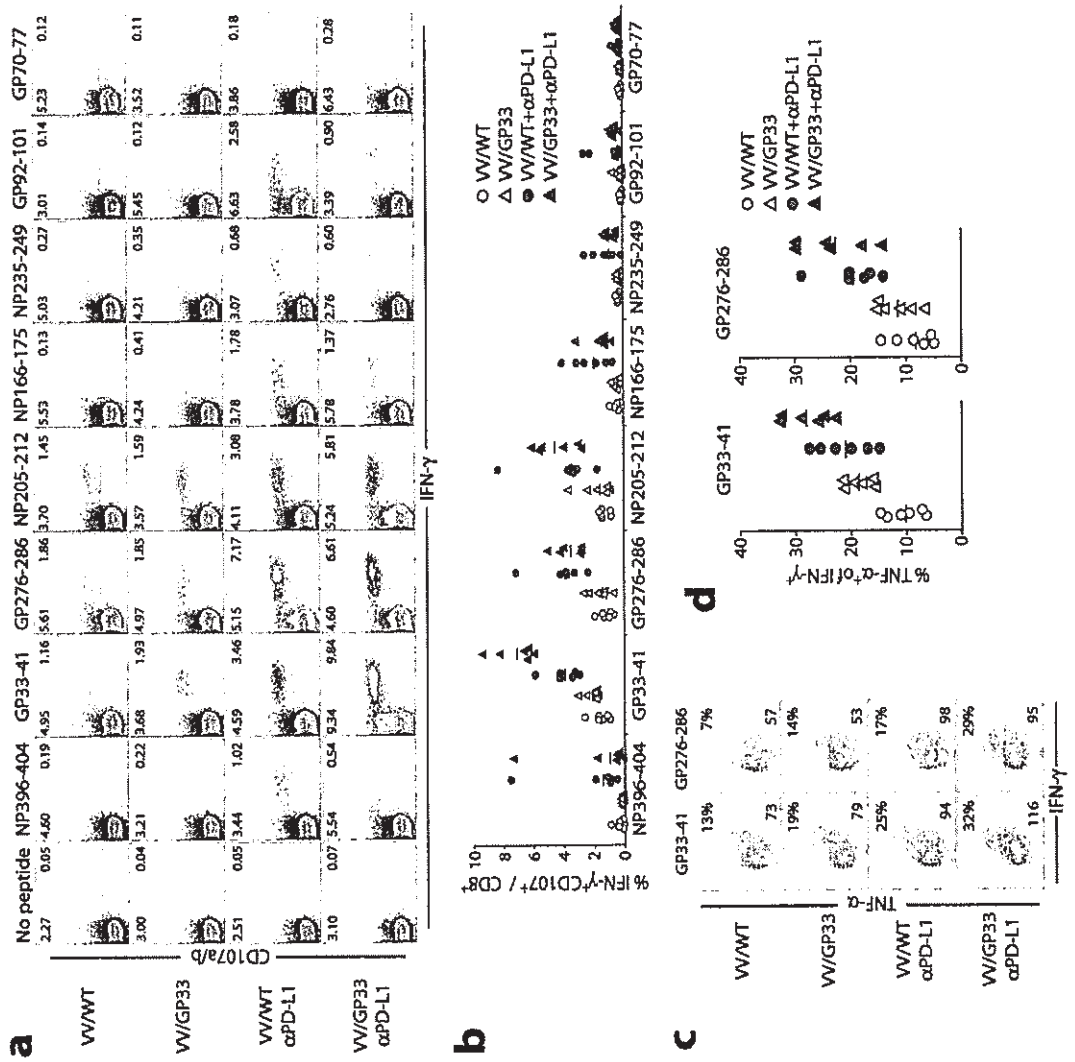

a
No peptide
NP396-404
GP33-41
GP276-286
NP205-212
NP166-175
NP235-249
GP92-101
GP70-77
VV/WT
VV/GP33
VV/WT αPD-L1
VV/GP33 αPD-L1
CD107a/b
IFN-γ
b
VV/WT
VV/GP33
VV/WT+αPD-L1
VV/GP33+αPD-L1
% IFN-γ+CD107+ / CD8+
c
GP33-41
GP276-286
TNF-α
IFN-γ
d
GP33-41
GP276-286
% TNF-α+of IFN-γ+

FIG. 12

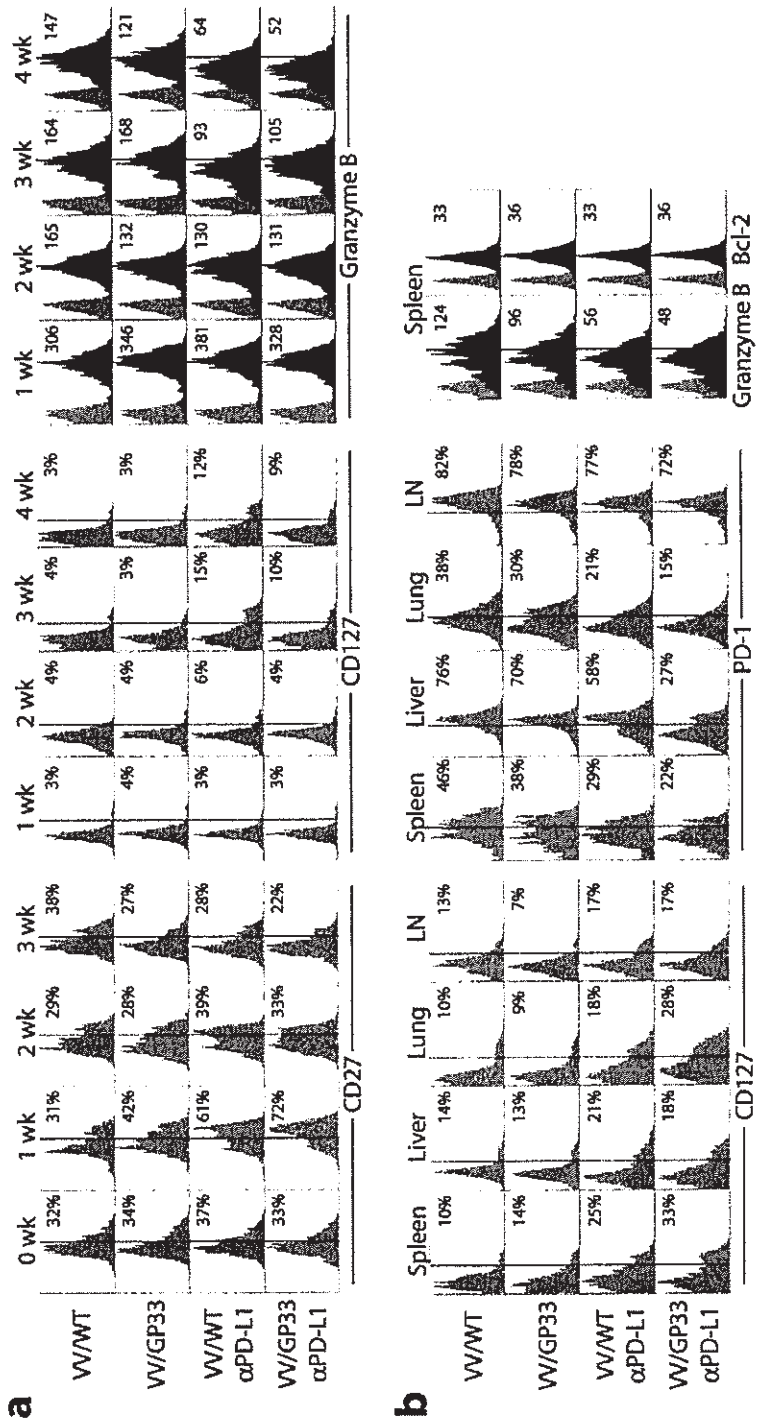
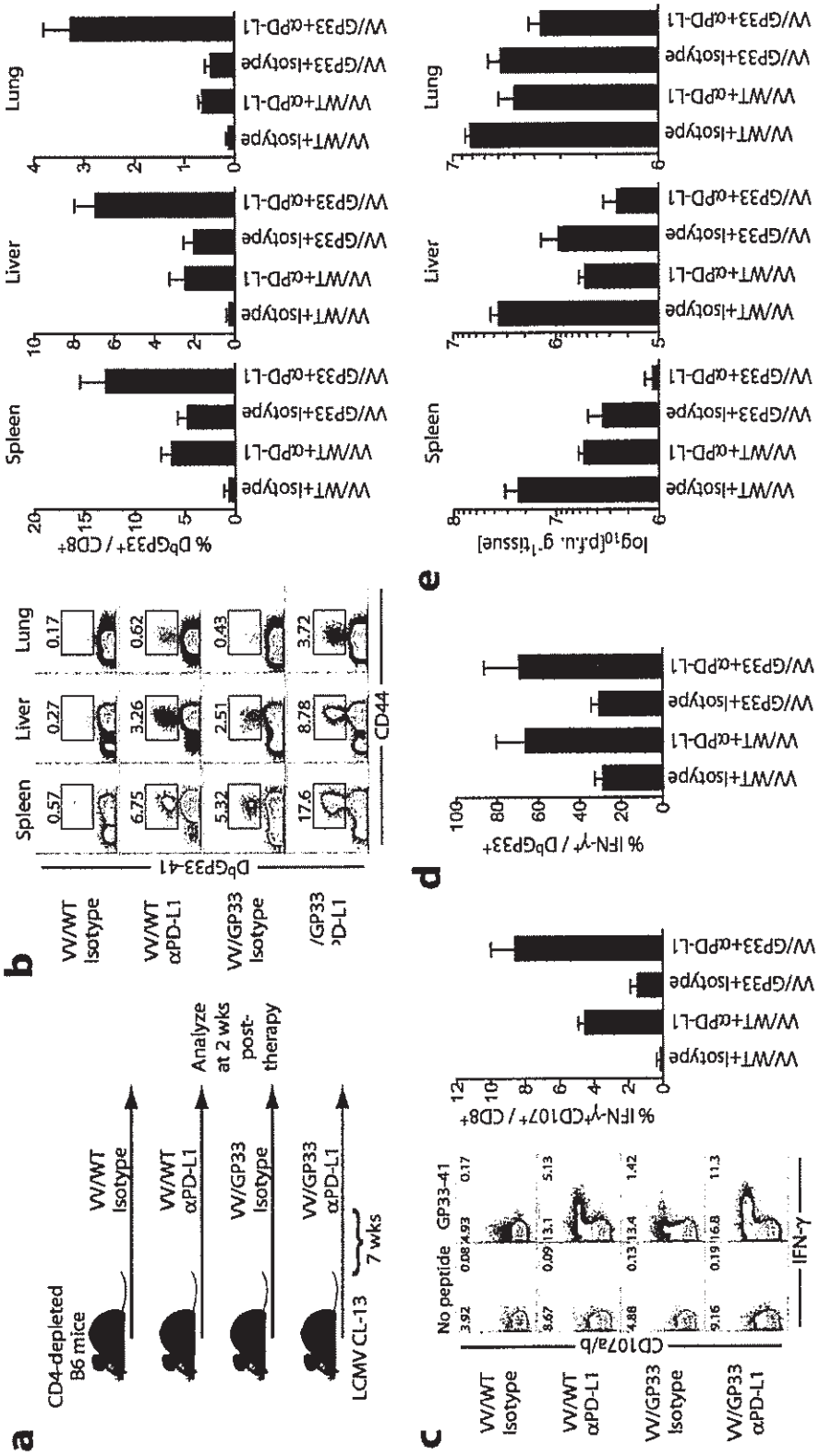

a
VV/WT
VV/GP33
VV/WT αPD-L1
VV/GP33 αPD-L1
0 wk
1 wk
2 wk
3 wk
4 wk
CD27
CD127
Granzyme B
b
Spleen
Liver
Lung
LN
PD-1
Granzyme B
Bcl-2

FIG. 13


a
CD4-depleted B6 mice
LCMV CL-13
7 wks
VV/WT Isotype
VV/WT αPD-L1
VV/GP33 Isotype
VV/GP33 αPD-L1
Analyze at 2 wks post-therapy
b
Spleen
Liver
Lung
DbGP33-41
CD44
VV/WT Isotype
VV/WT αPD-L1
VV/GP33 Isotype
VV/GP33 αPD-L1
0.57
6.75
5.32
17.6
0.27
3.26
2.51
8.78
0.17
0.62
0.43
3.72
c
No peptide
GP33-41
CD107a/b
IFN-γ
VV/WT Isotype
VV/WT αPD-L1
VV/GP33 Isotype
VV/GP33 αPD-L1
3.92
0.08
4.93
0.17
3.67
0.09
13.1
5.13
4.88
0.13
13.4
1.42
9.16
0.19
16.8
11.3
d
% IFN-γ+ / DbGP33+
% IFN-γ+CD107+ / CD8+
VV/WT+Isotype
VV/WT+αPD-L1
VV/GP33+Isotype
VV/GP33+αPD-L1
e
Spleen
Liver
Lung
log10[p.f.u. g-1tissue]
% DbGP33+ / CD8+
VV/WT+Isotype
VV/WT+αPD-L1
VV/GP33+Isotype
VV/GP33+αPD-L1

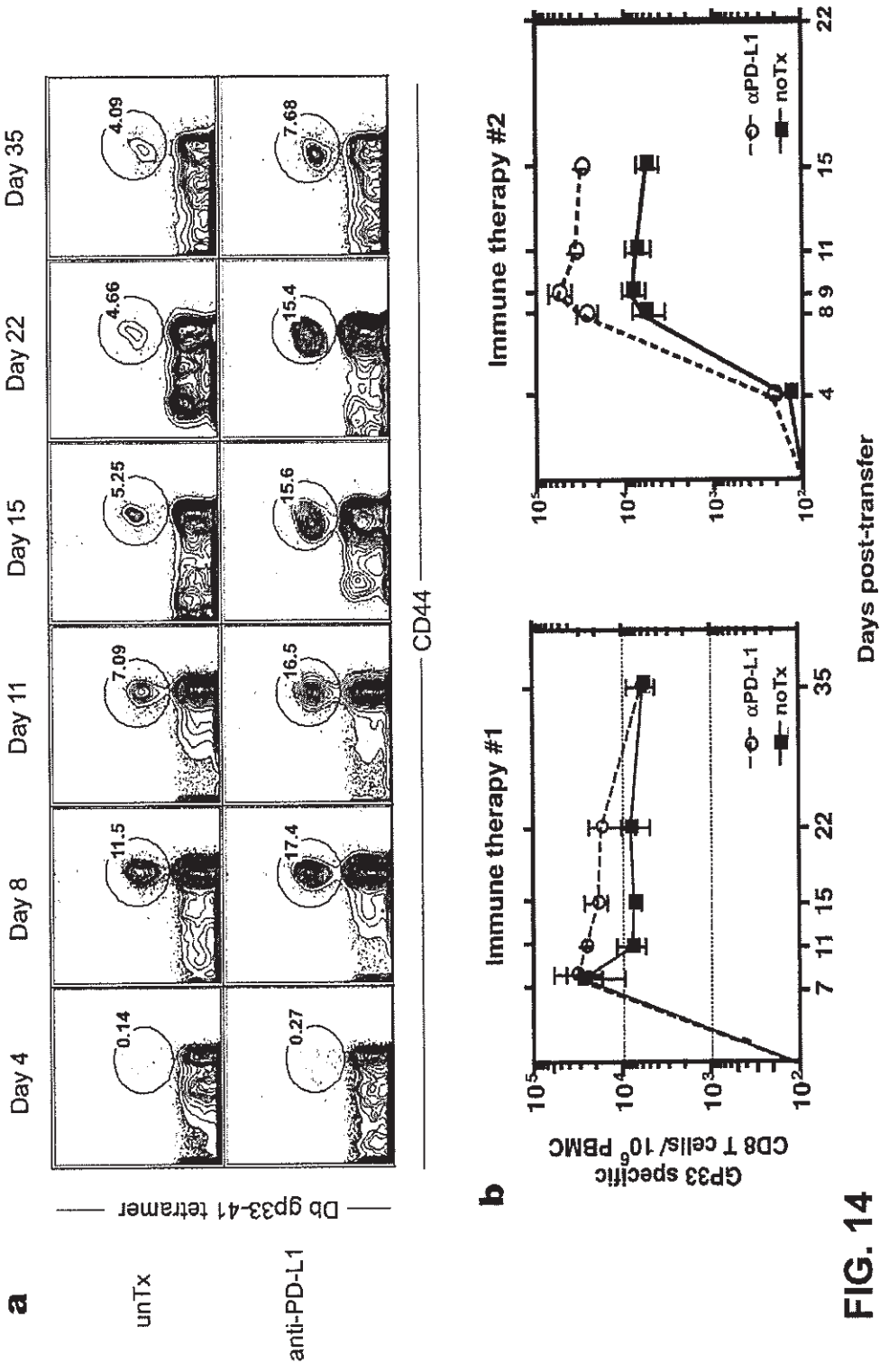

a
Day 4
Day 8
Day 11
Day 15
Day 22
Day 35
unTx
anti-PD-L1
0.14
11.5
7.09
5.25
4.66
4.09
0.27
17.4
16.5
15.6
15.4
7.68
Db gp33-41 tetramer
CD44
b
Immune therapy #1
Immune therapy #2
αPD-L1
noTx
GP33 specific CD8 T cells/ 10^6 PBMC
Days post-transfer

FIG. 14

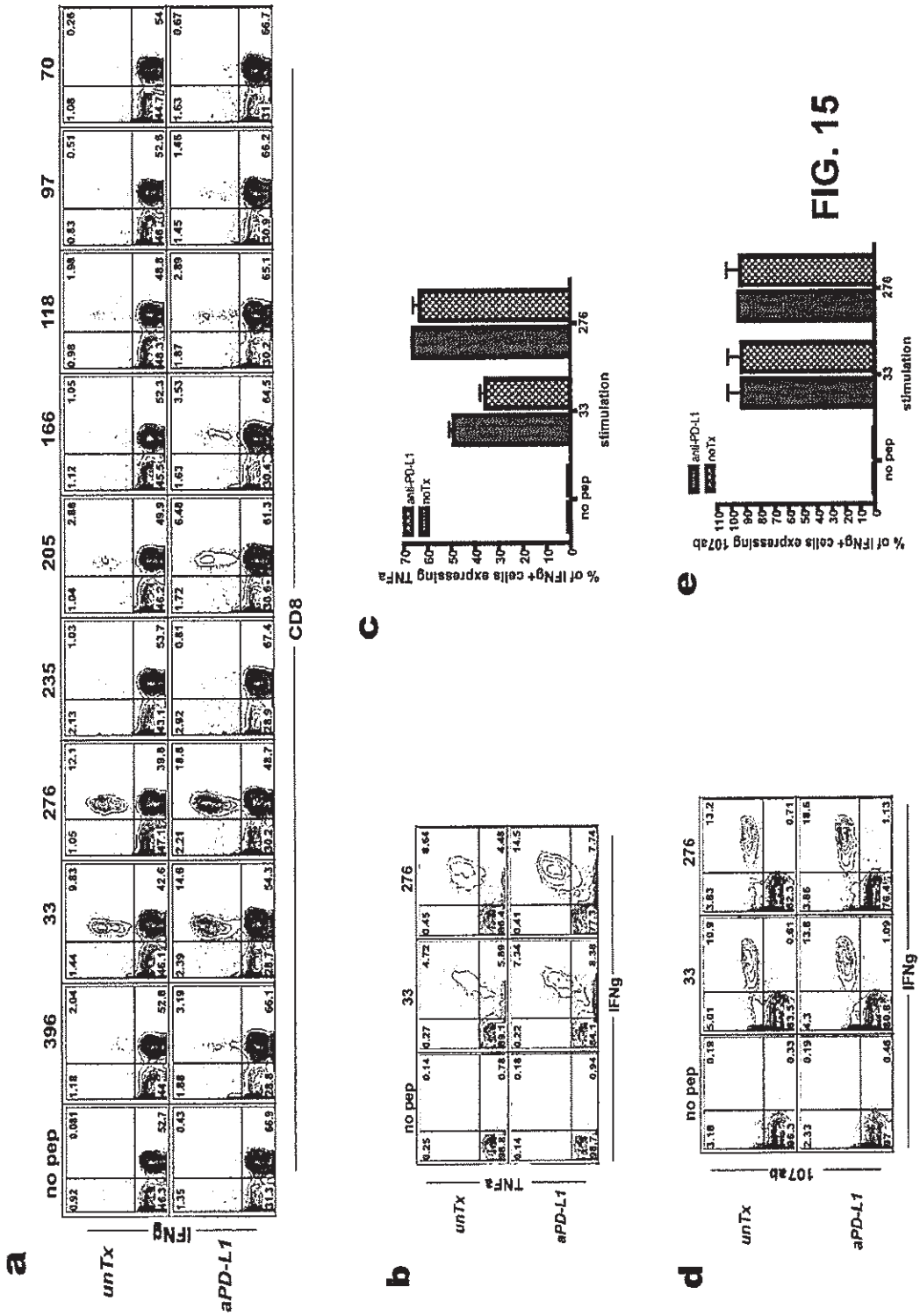

a
no pep
396
33
276
235
205
166
118
97
70
unTx
aPD-L1
IFNg
CD8
b
no pep
33
276
unTx
aPD-L1
TNFa
IFNg
c
anti-PD-L1
noTx
% of IFNg+ cells expressing TNFa
no pep
33
276
stimulation
d
no pep
33
276
unTx
aPD-L1
107ab
IFNg
e
anti-PD-L1
noTx
% of IFNg+ cells expressing 107ab
no pep
33
276
stimulation

FIG. 15

FIG. 16

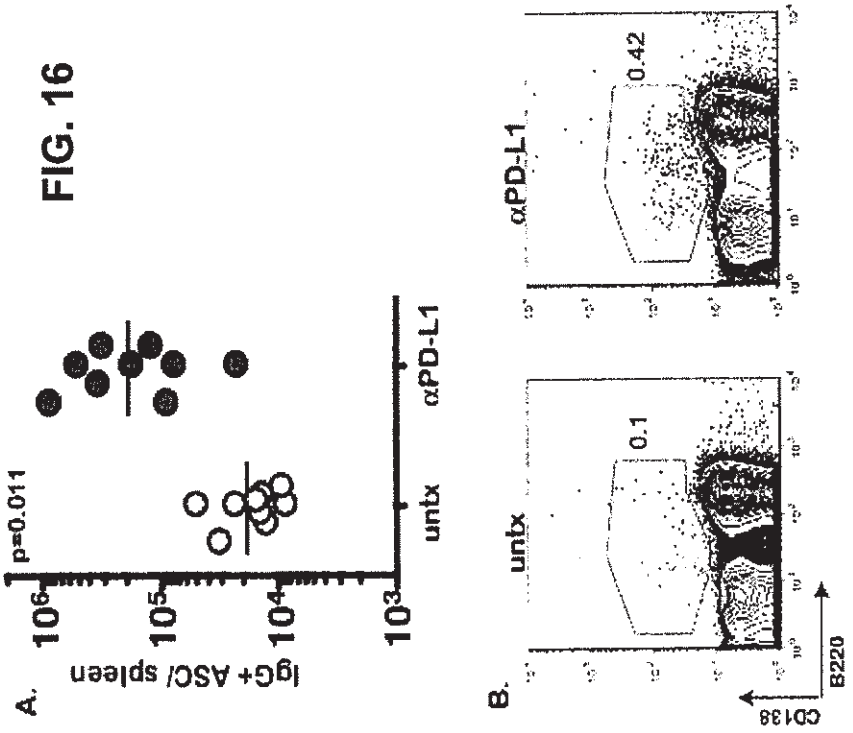

A.
IgG+ ASC/ spleen
p=0.011
unbx
αPD-L1
B.
unbx
0.1
αPD-L1
0.42
CD138
B220

FIG. 17

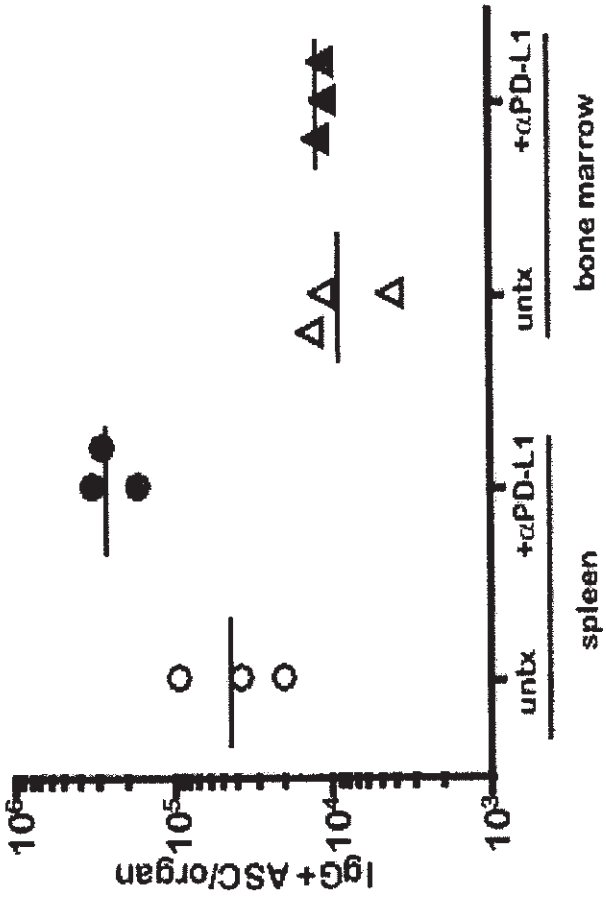

IgG+ ASC/organ
10^6
10^5
10^4
10^3
untx
+αPD-L1
untx
+αPD-L1
spleen
bone marrow

FIG. 18

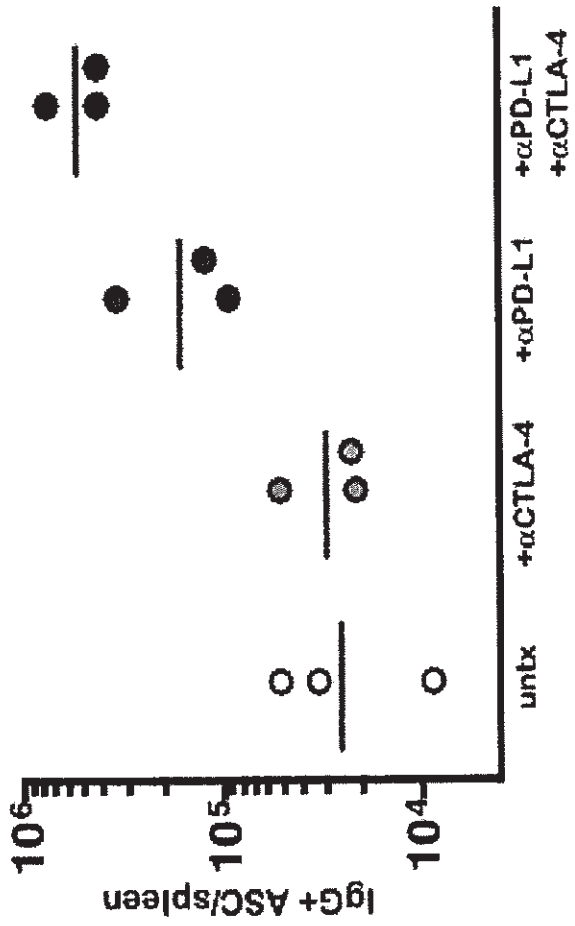


FIG. 19

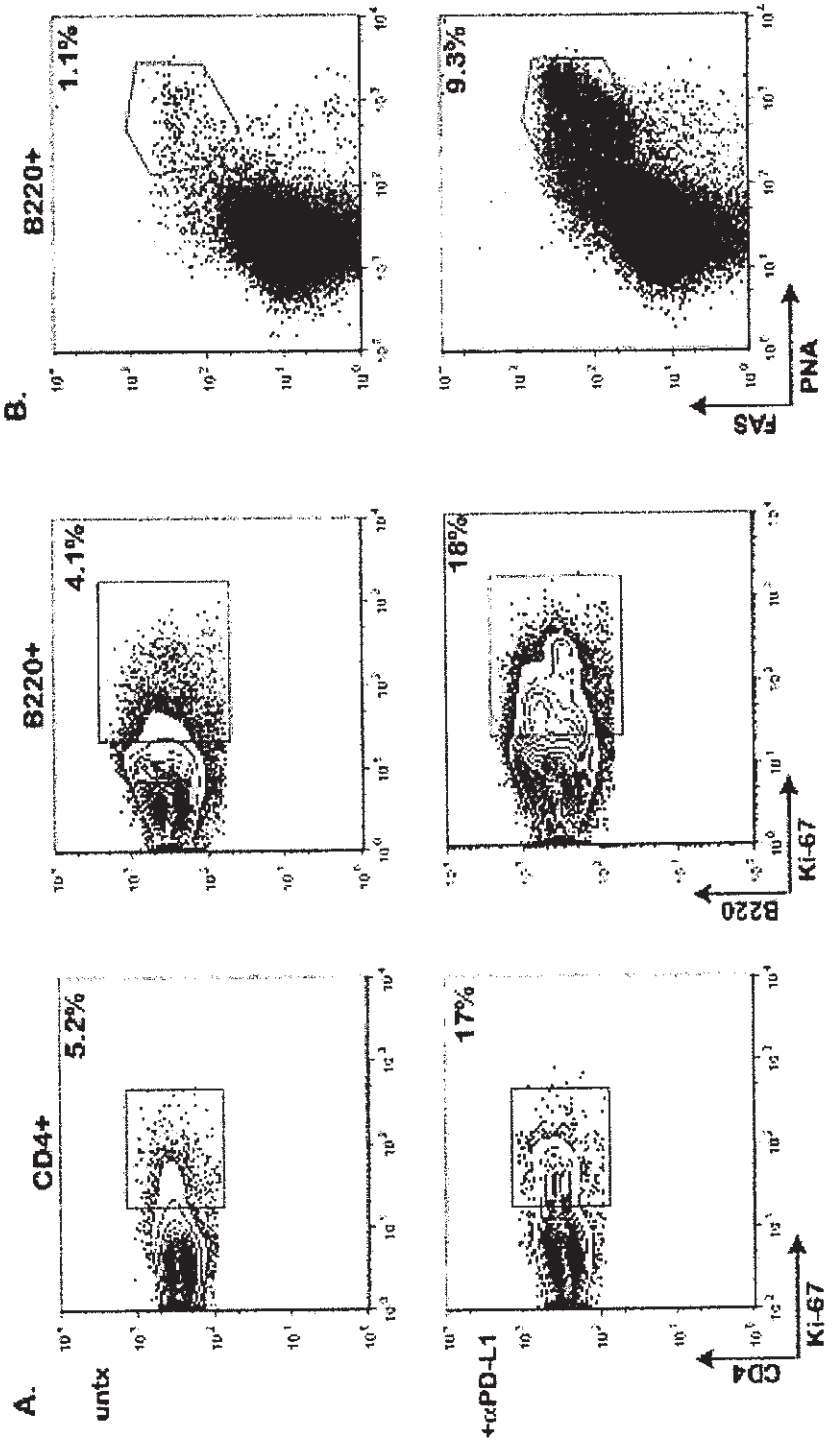

A.
CD4+
5.2%
untx
17%
+αPD-L1
CD4
Ki-67
B.
B220+
4.1%
18%
B220
Ki-67
B220+
1.1%
9.3%
FAS
PNA

FIG. 20

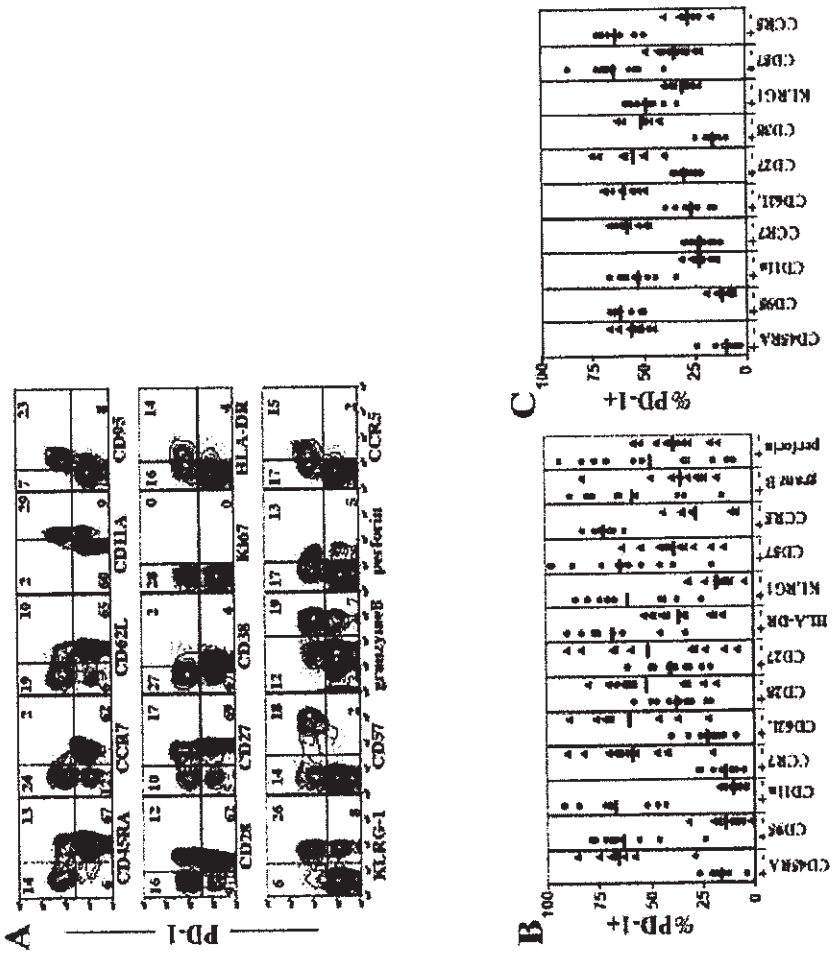

A
PD-1
CD45RA
CCR7
CD62L
CD95
CD28
CD27
CD38
Ki67
HLA-DR
KLRG-1
CD57
granzyme B
perforin
CCR5
B
%PD-1+
CD45RA
CD8
CD11a
CCR7
CD62L
CD27
CD28
HLA-DR
KLRG1
CD57
CCR5
gran B
perforin
C
%PD-1+
CD45RA
CD8
CD11a
CCR7
CD62L
CD27
CD28
KLRG1
CD57
CCR5

FIG. 21

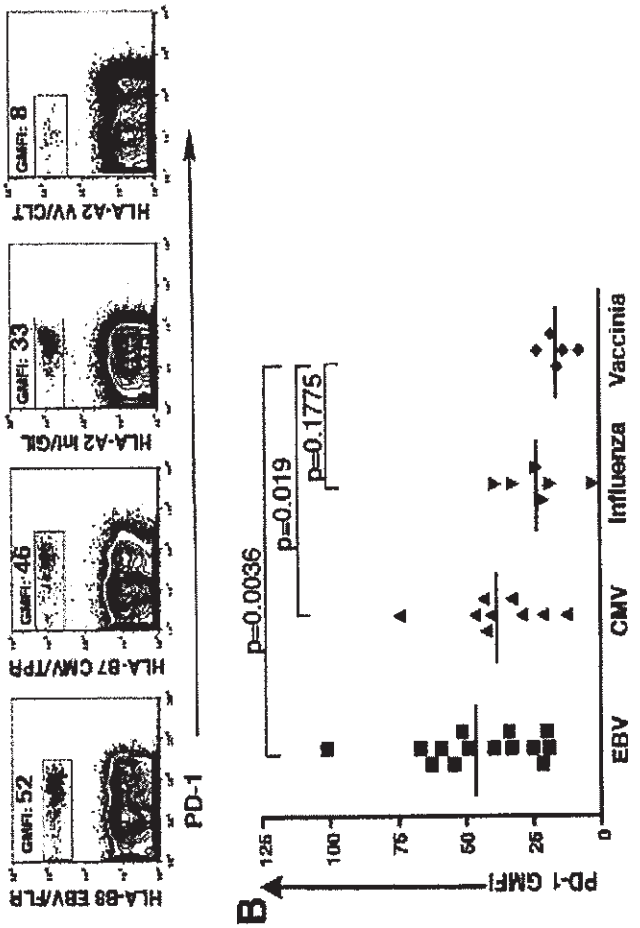
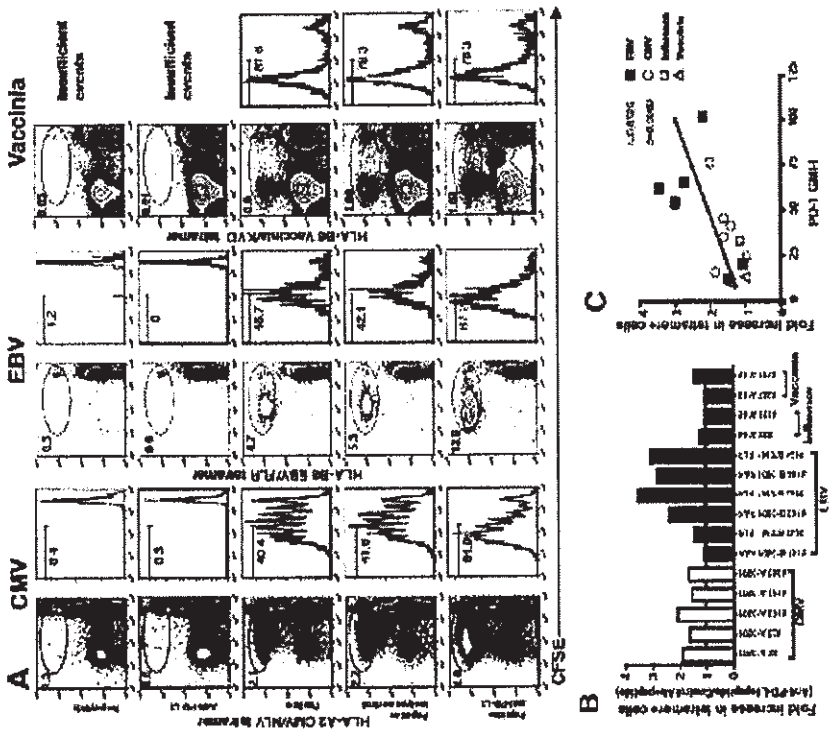

A
HLA-B8 EBV/FLR
GMFI: 52
HLA-B7 CMV/TPR
GMFI: 46
HLA-A2 Inf/GIL
GMFI: 33
HLA-A2 VV/CLT
GMFI: 8
PD-1
B
PD-1 GMFI
125
100
75
50
25
0
p=0.0036
p=0.019
p=0.1775
EBV
CMV
Influenza
Vaccinia

FIG. 22


A
CMV
EBV
Vaccinia
Insufficient events
HLA-A2 CMVNLV tetramer
HLA-B8 EBVFLR tetramer
HLA-B8 VacciniaVD tetramer
CFSE
B
Fold increase in tetramer+ cells
C
Fold increase in tetramer+ cells
PD-1 GMFI

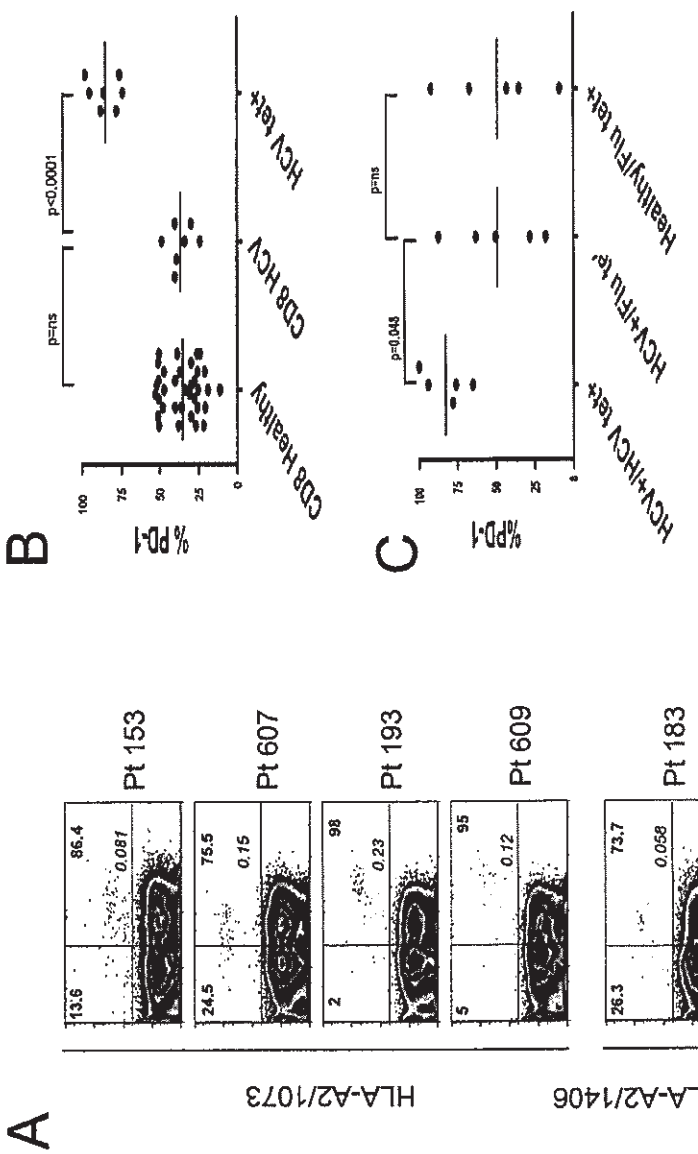

A
HLA-A2/1073
HLA-A2/1406
Pt 153
Pt 607
Pt 193
Pt 609
Pt 183
86.4
0.081
13.6
75.5
0.15
24.5
98
0.23
2
95
0.12
5
73.7
0.058
26.3
PD-1
B
% PD-1
p=ns
p<0.0001
CD8 Healthy
CD8 HCV
HCV tet+
C
% PD-1
p=ns
p=0.048
HCV+/HCV tet+
HCV+/Flu tet+
Healthy/Flu tet+

FIG. 23

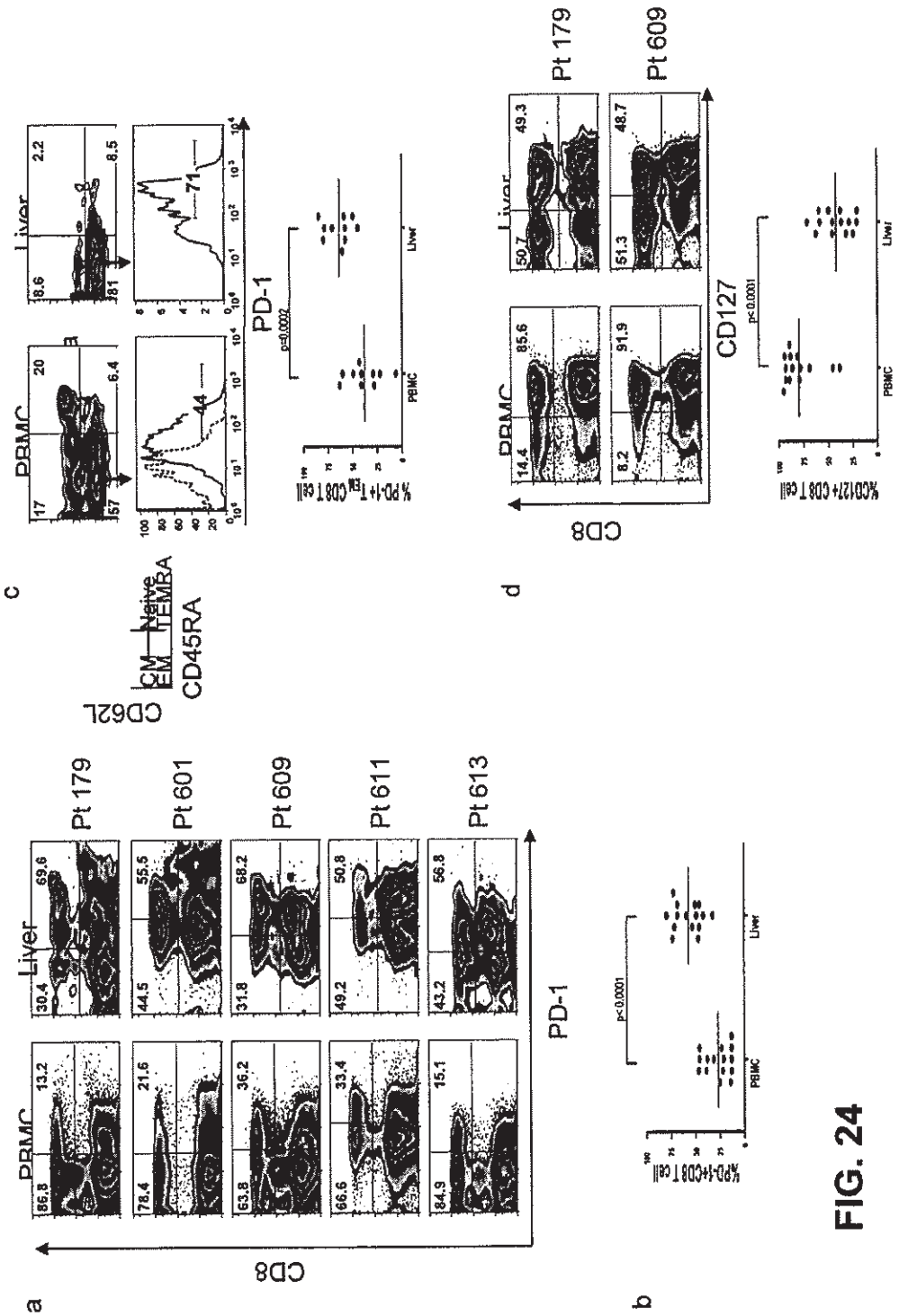

a
PBMC
Liver
Pt 179
Pt 601
Pt 609
Pt 611
Pt 613
CD8
PD-1
b
%PD-1+CD8 T cell
p<0.0001
PBMC
Liver
c
CD62L
CD45RA
CM
Naive
EM
TEMRA
PD-1
%PD-1+ TEM CD8 T cell
p=0.0002
d
CD127
%CD127+ CD8 T cell
p<0.0001

FIG. 24

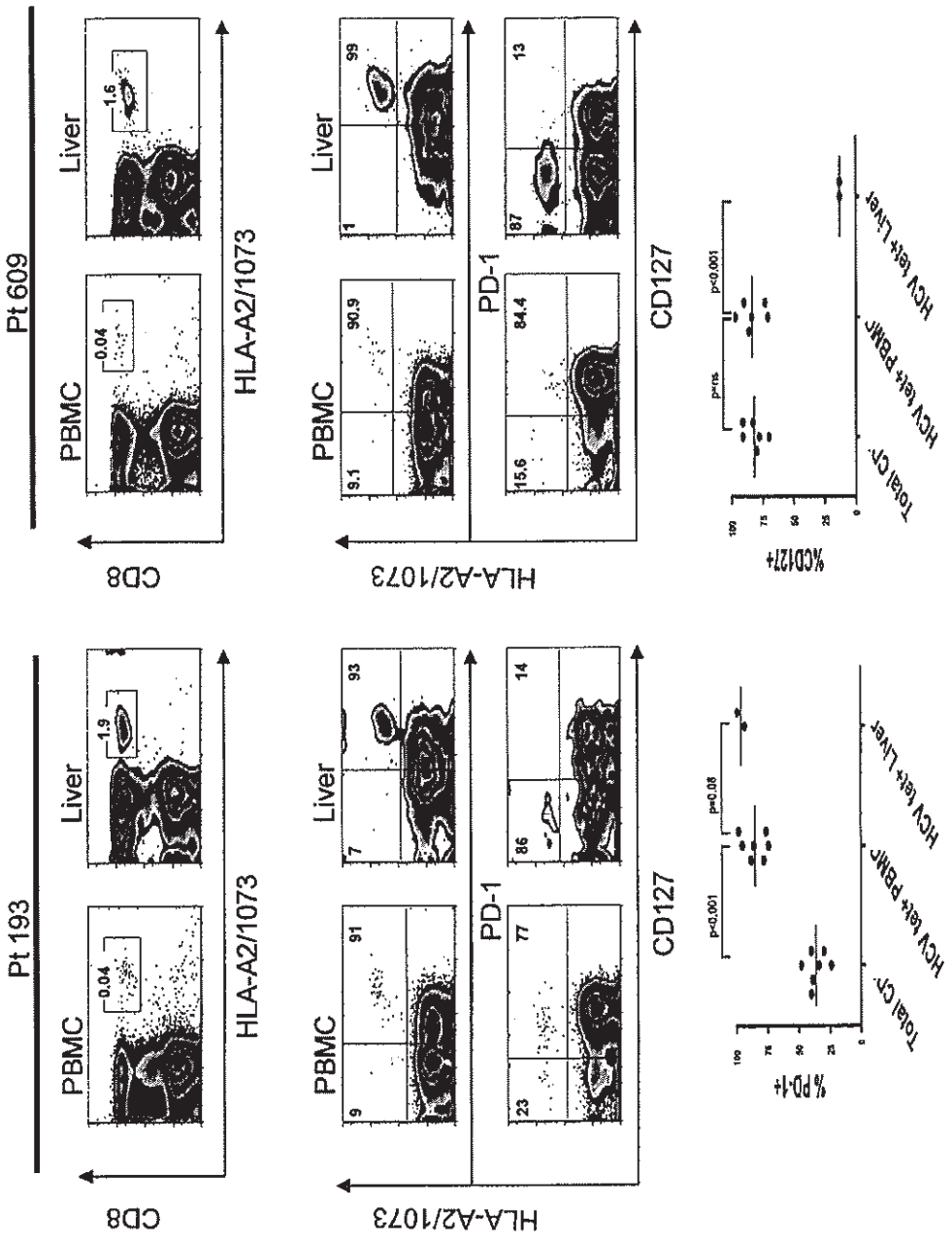

Pt 609
PBMC
Liver
0.04
1.6
CD8
HLA-A2/1073
99
1
90.9
9.1
87
13
84.4
15.6
PD-1
CD127
%CD127+
p=ns
p<0.001
Total CP
HCV tet+ PBMP
HCV tet+ Liver
Pt 193
1.9
93
7
91
9
86
14
77
23
p<0.001
p=0.08
% PD-1+

FIG. 25

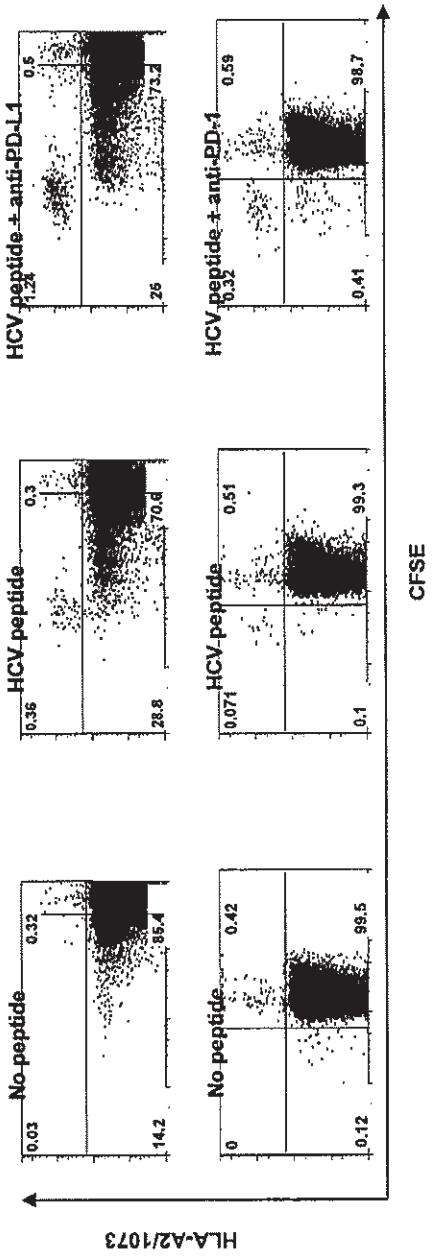

HCV peptide + anti-PD-L1
HCV peptide + anti-PD-1
HCV peptide
HCV peptide
No peptide
No peptide
1.24
0.5
26
73.2
0.32
0.59
0.41
98.7
0.36
0.3
28.8
70.6
0.071
0.51
0.1
99.3
0.03
0.32
14.2
85.4
0
0.42
0.12
99.5
CFSE
HLA-A2/1073

FIG. 26

FIG. 27

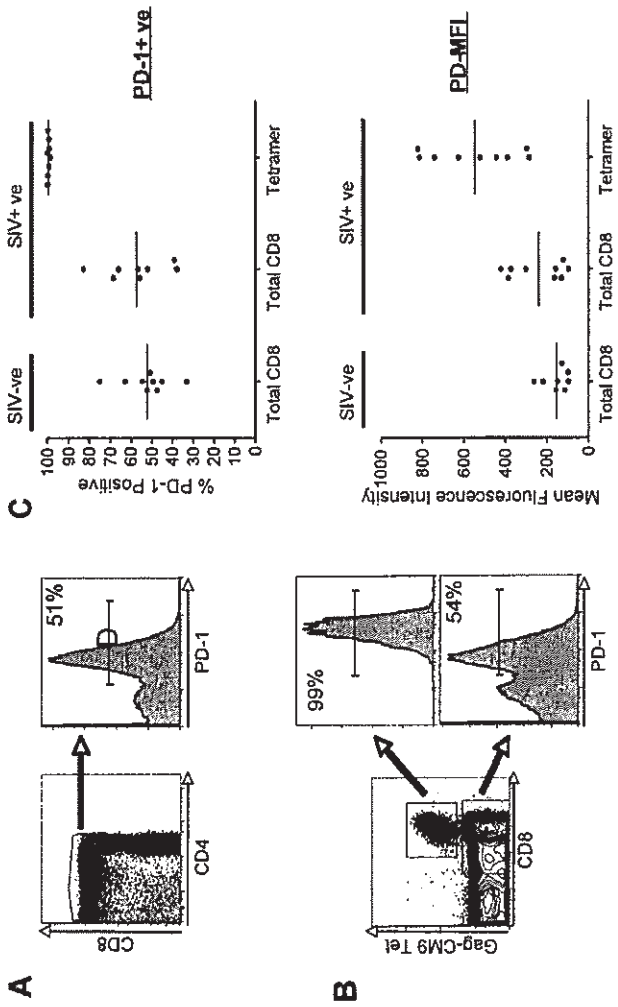
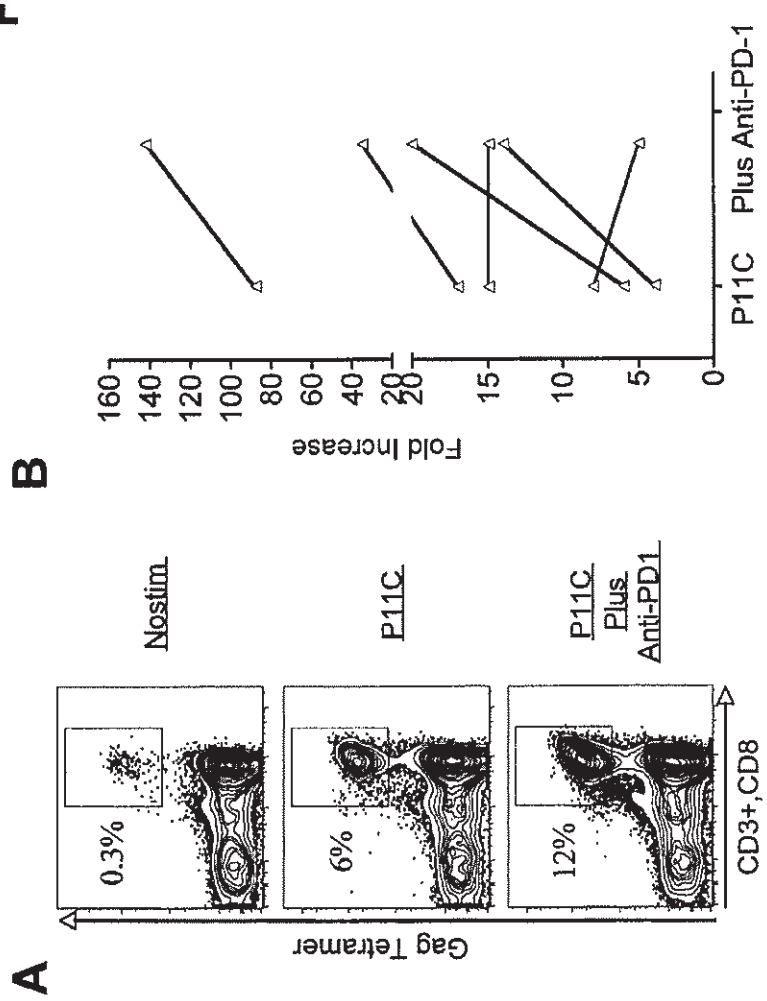

A
CD8
CD4
51%
PD-1
B
Gag-CM9 Tet
CD8
99%
54%
PD-1
C
SIV-ve
SIV+ ve
PD-1+ ve
% PD-1 Positive
Total CD8
Total CD8
Tetramer
SIV-ve
SIV+ ve
PD-MFI
Mean Fluorescence Intensity
Total CD8
Total CD8
Tetramer

FIG. 28


A
Nostim
P11C
P11C Plus Anti-PD1
0.3%
6%
12%
Gag Tetramer
CD3+,CD8
B
Fold Increase
160
140
120
100
80
60
40
20
20
15
10
5
0
P11C
Plus Anti-PD-1

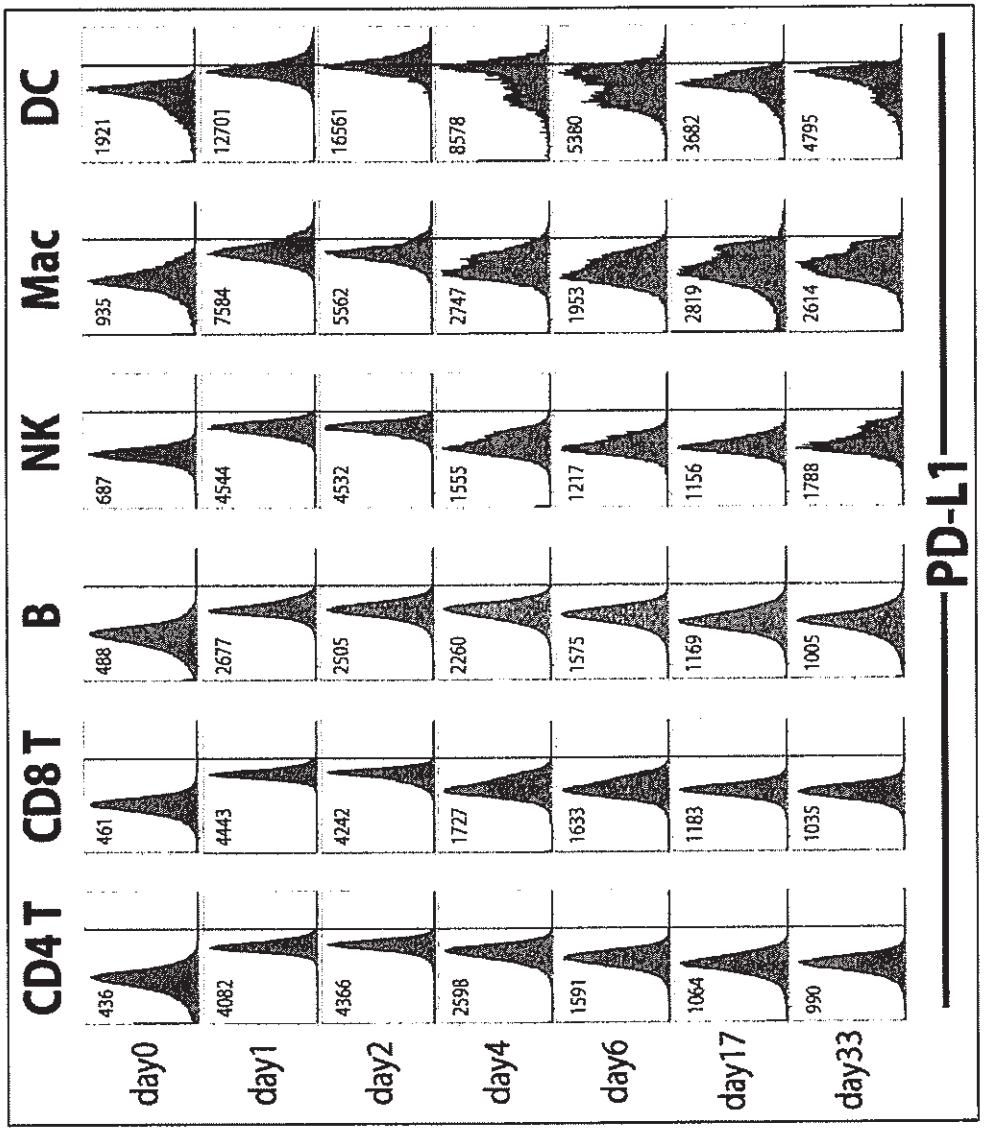

CD4T
CD8T
B
NK
Mac
DC
day0
day1
day2
day4
day6
day17
day33
436
461
488
687
935
1921
4082
4443
2677
4544
7584
12701
4366
4242
2505
4532
5562
16561
2398
1727
2260
1555
2747
8578
1591
1633
1575
1217
1953
5380
1064
1183
1169
1156
2819
3682
990
1035
1005
1788
2614
4795
PD-L1

FIG. 29