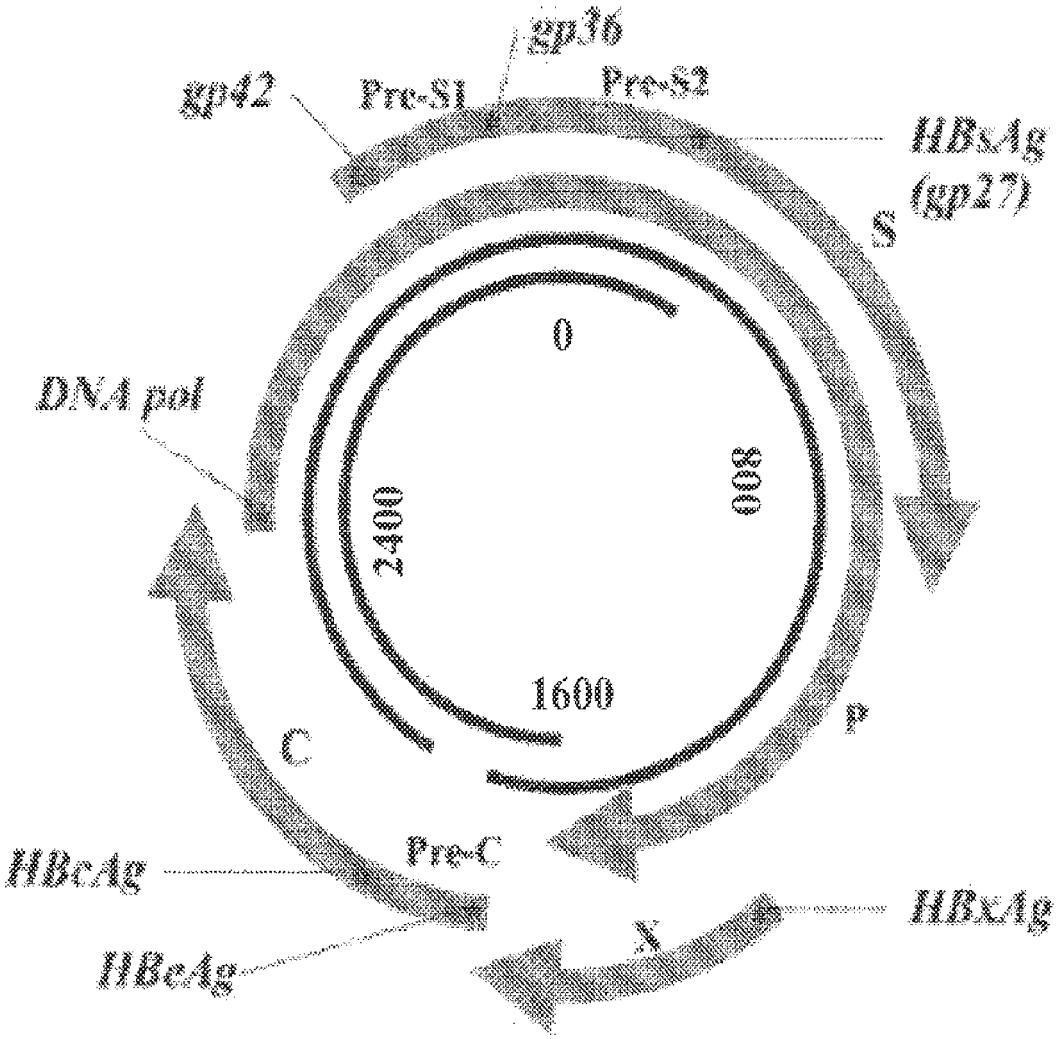

gp36
gp42
Pre-S1
Pre-S2
HBsAg
(gp27)
S
0
DNA pol
800
2400
1600
P
C
Pre-C
HBcAg
HBeAg
HBxAg
X

FIGURE 1

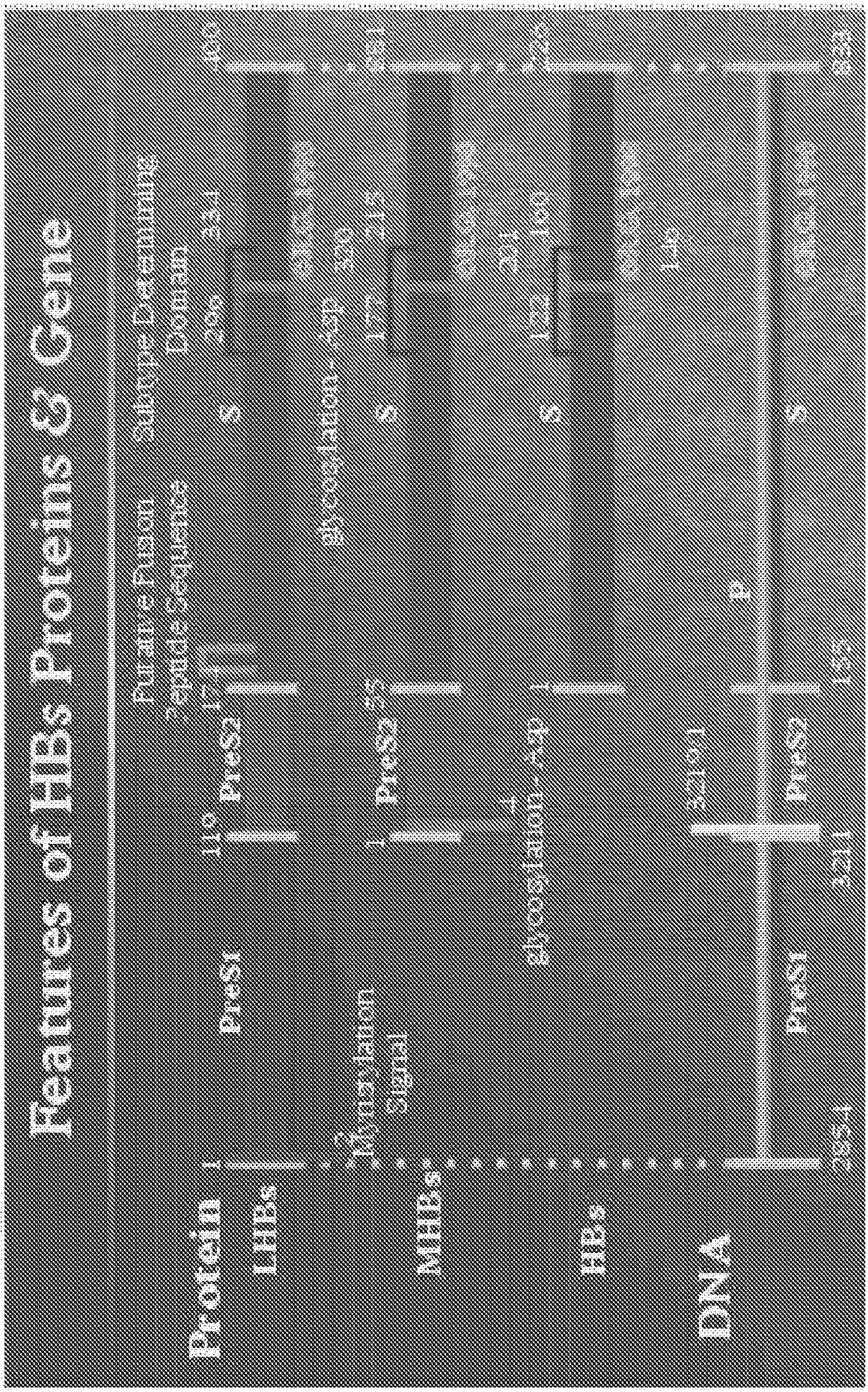

Features of HBs Proteins & Gene
Protein
LHBs
MHBs
HBs
DNA
PreS1
PreS2
S
P
Myristylation Signal
Putative Fusion Peptide Sequence
Subtype Determining Domain
glycosylation-Asp

FIGURE 2

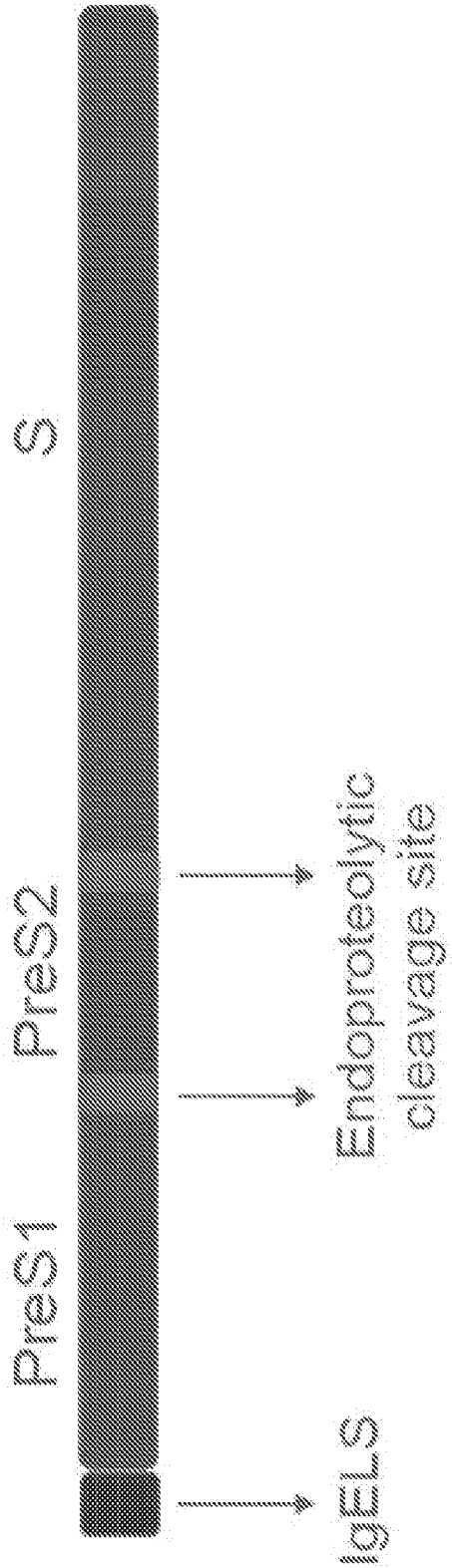


FIGURE 3

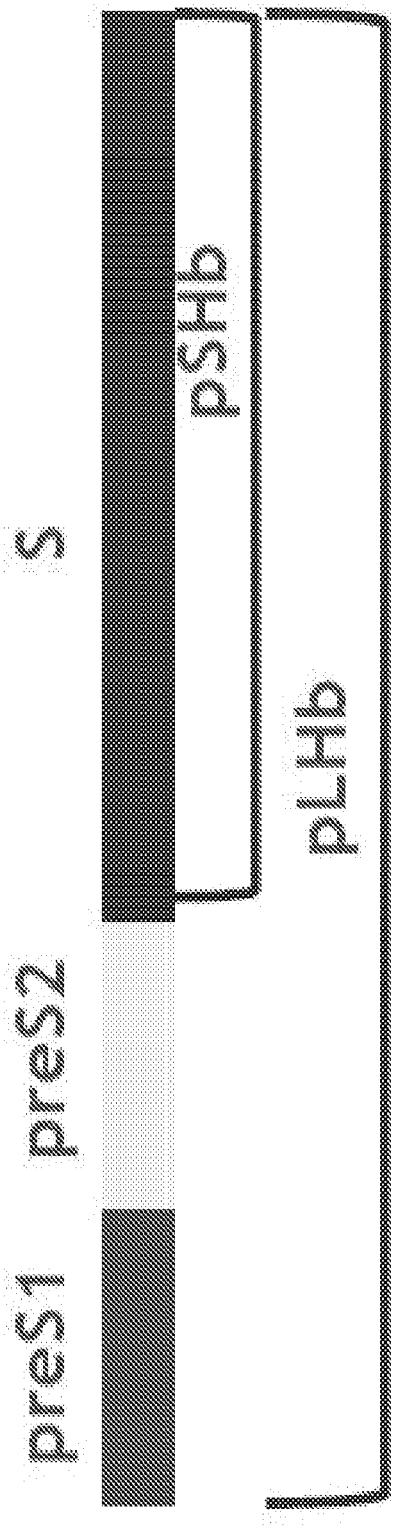


FIGURE 4

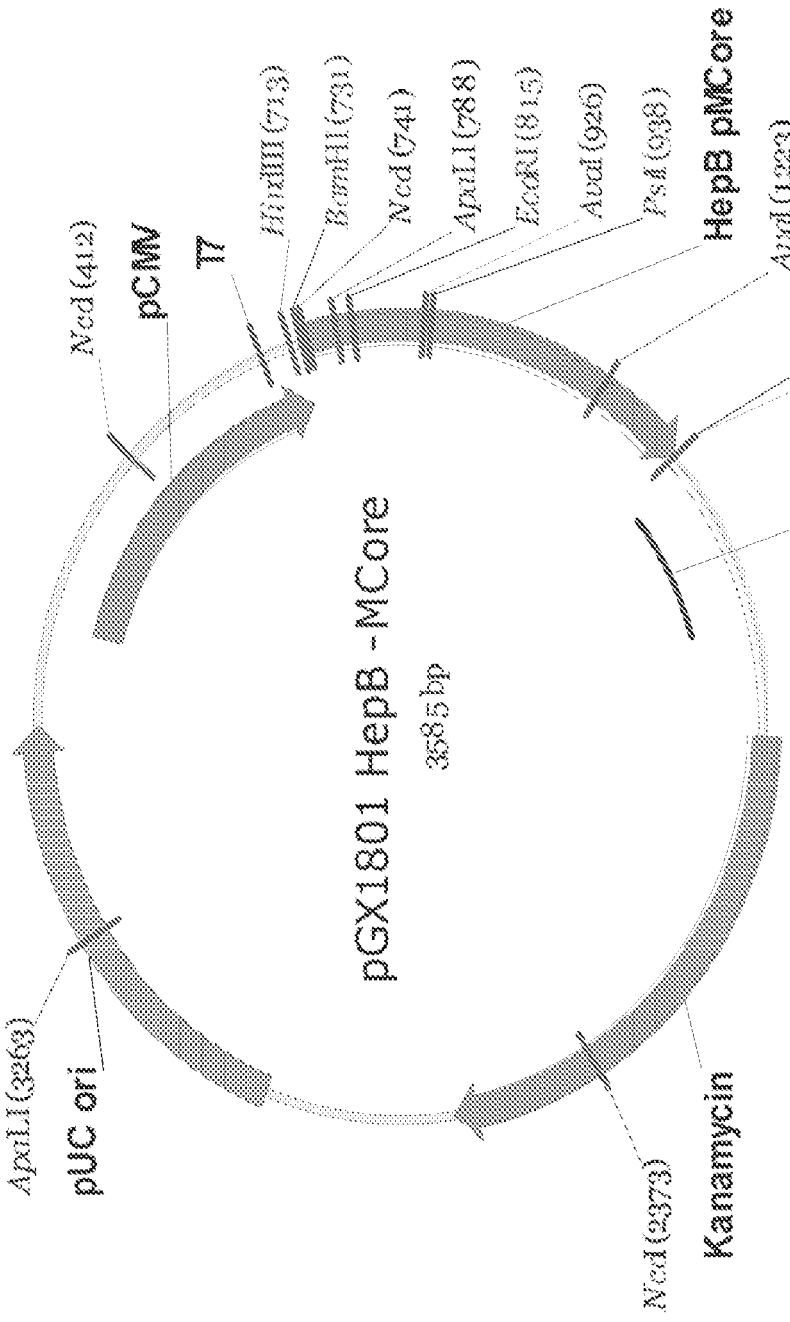

pGX1801 HepB - MCore
3585 bp
pCMV
T7
HepB pMCore
BGH pA
pUC ori
Kanamycin
NcoI (412)
HindIII (713)
BamHI (731)
NcoI (741)
ApaLI (788)
EcoRI (815)
AvaI (926)
PstI (938)
AvaI (1223)
XhoI (1373)
AvaI (1373)
ApaLI (3269)
NcoI (2373)

FIGURE 5

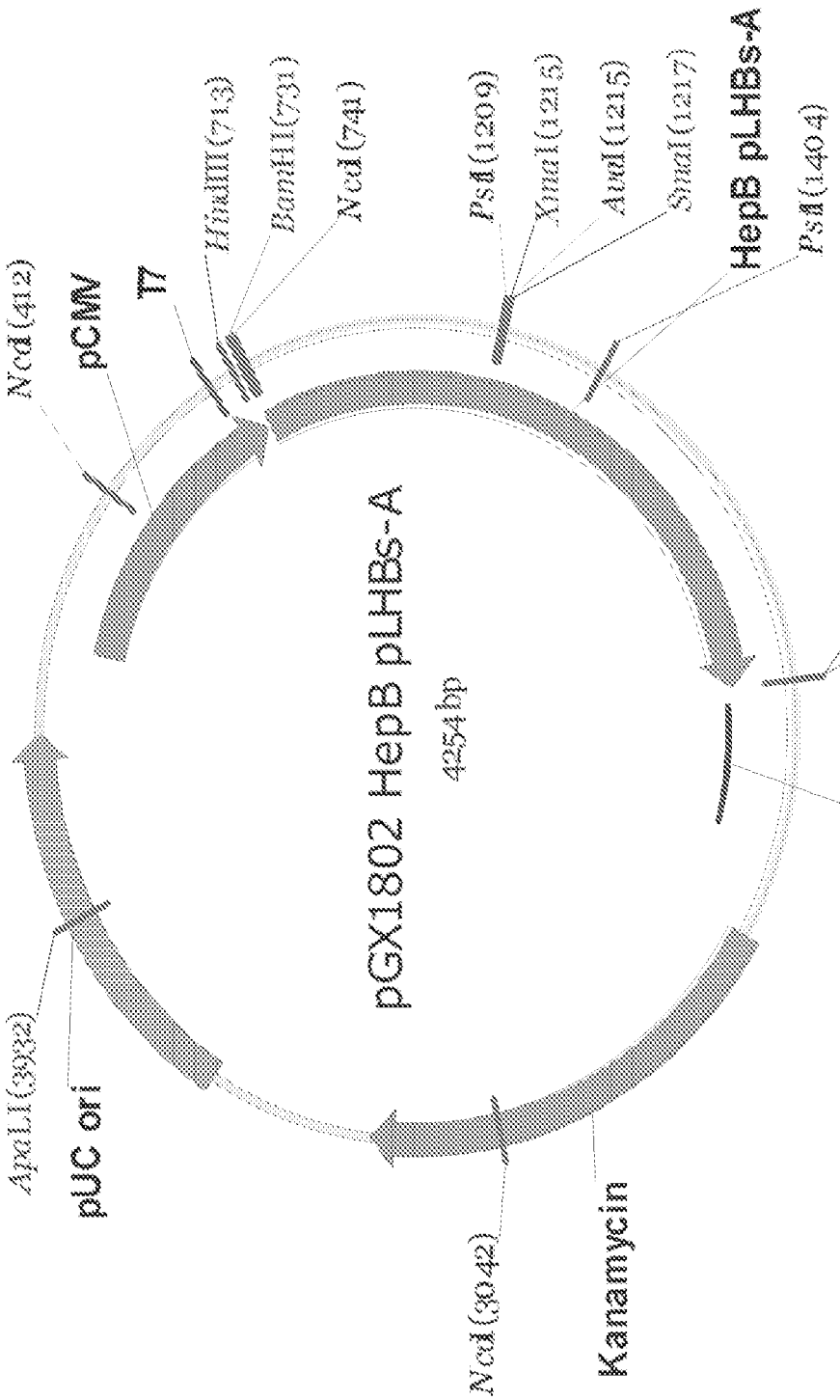

pGX1802 HepB pLHBs-A
4254 bp
HepB pLHBs-A
pCMV
T7
pUC ori
Kanamycin
BGH pA
NcoI (412)
HindIII (713)
BamHI (731)
NcoI (741)
PstI (1209)
XmaI (1213)
AvaI (1215)
SmaI (1217)
PstI (1404)
XhoI (2042)
AvaII (2042)
NcoI (3042)
ApaLI (3932)

FIGURE 6

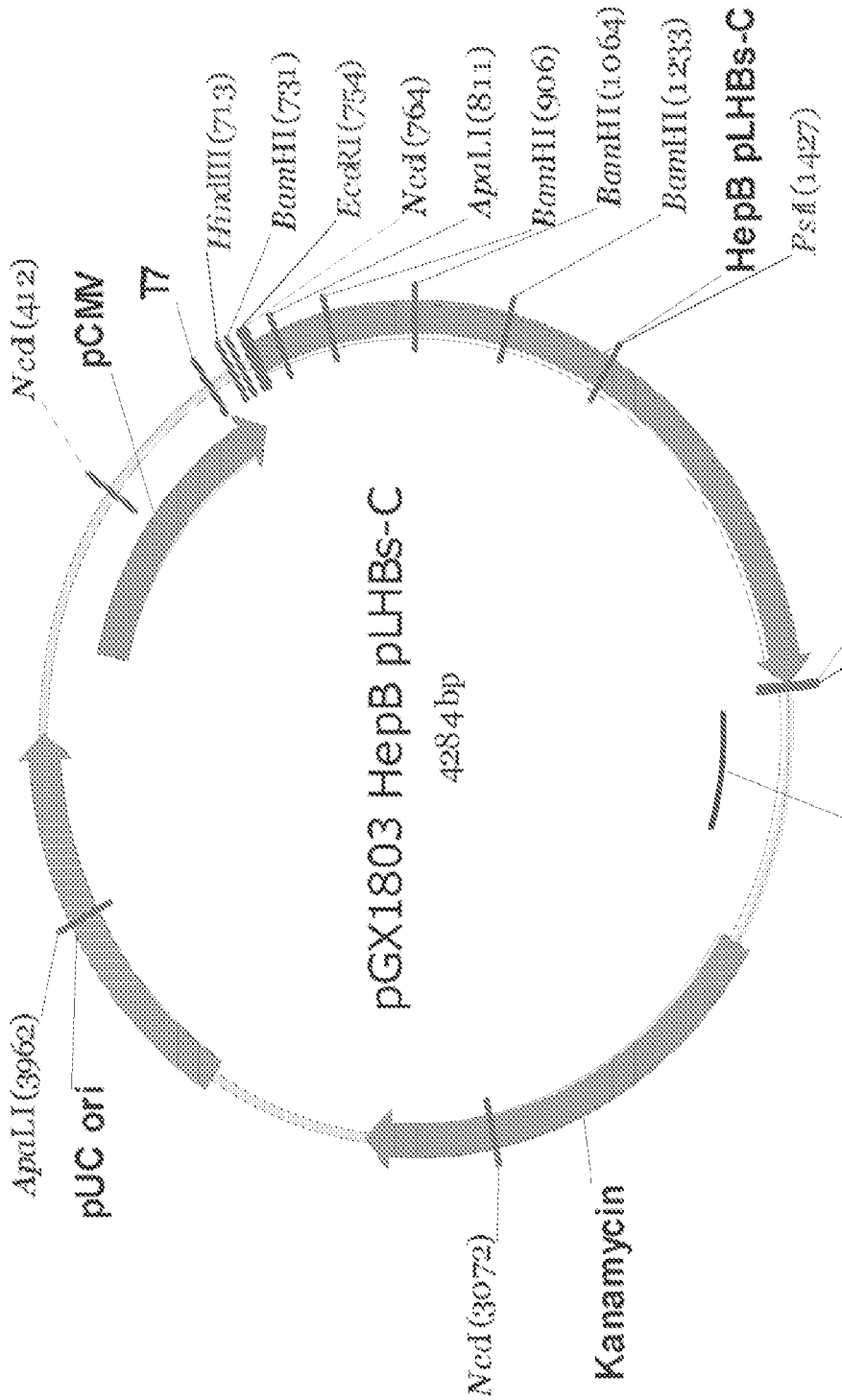

pGX1803 HepB pLHBs-C
4284 bp
pCMV
T7
HepB pLHBs-C
pUC ori
Kanamycin
BGH pA
NcoI (412)
HindIII (713)
BamHI (731)
EcoRI (754)
NcoI (764)
ApaLI (811)
BamHI (906)
BamHI (1064)
BamHI (1233)
PstI (1427)
NotI (2066)
AvaI (2072)
NcoI (3072)
ApaLI (3962)

FIGURE 7

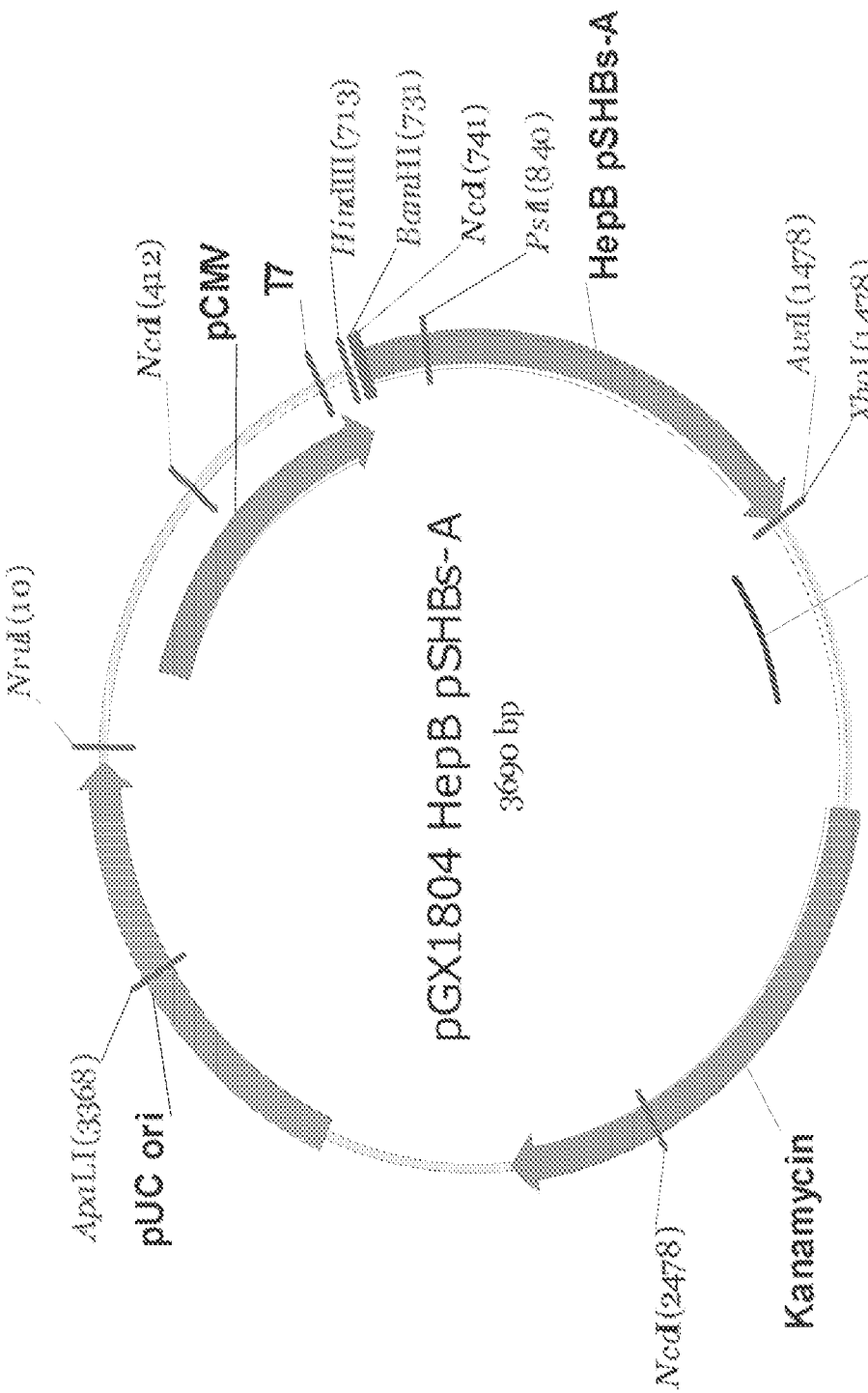

pGX1804 HepB pSHBs-A
3690 bp
NcoI (412)
pCMV
T7
HindIII (713)
BamHI (731)
NcoI (741)
PstI (840)
HepB pSHBs-A
AseI (1478)
XhoI (1478)
BGH pA
NcoI (2478)
Kanamycin
ApaLI (3368)
pUC ori
NruI (10)

FIGURE 8

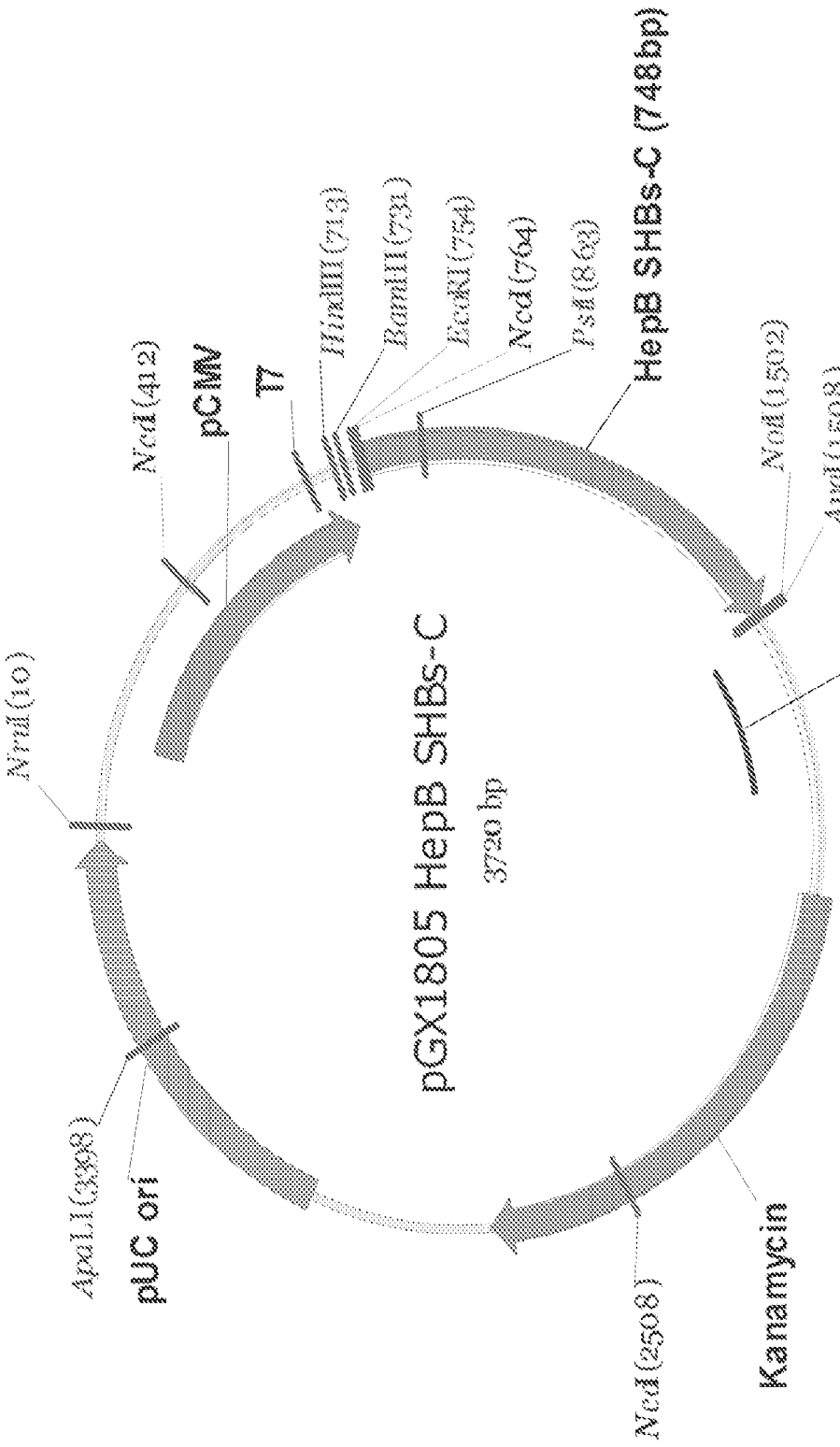

pGX1805 HepB SHBs-C
3720 bp
HepB SHBs-C (748bp)
pCMV
T7
pUC ori
BGH pA
Kanamycin
NcoI (412)
HindIII (713)
BamHI (731)
EcoNI (754)
NcoI (764)
PstI (863)
NotI (1502)
AseI (1508)
NruI (10)
ApaLI (3368)
NcoI (2508)

FIGURE 9

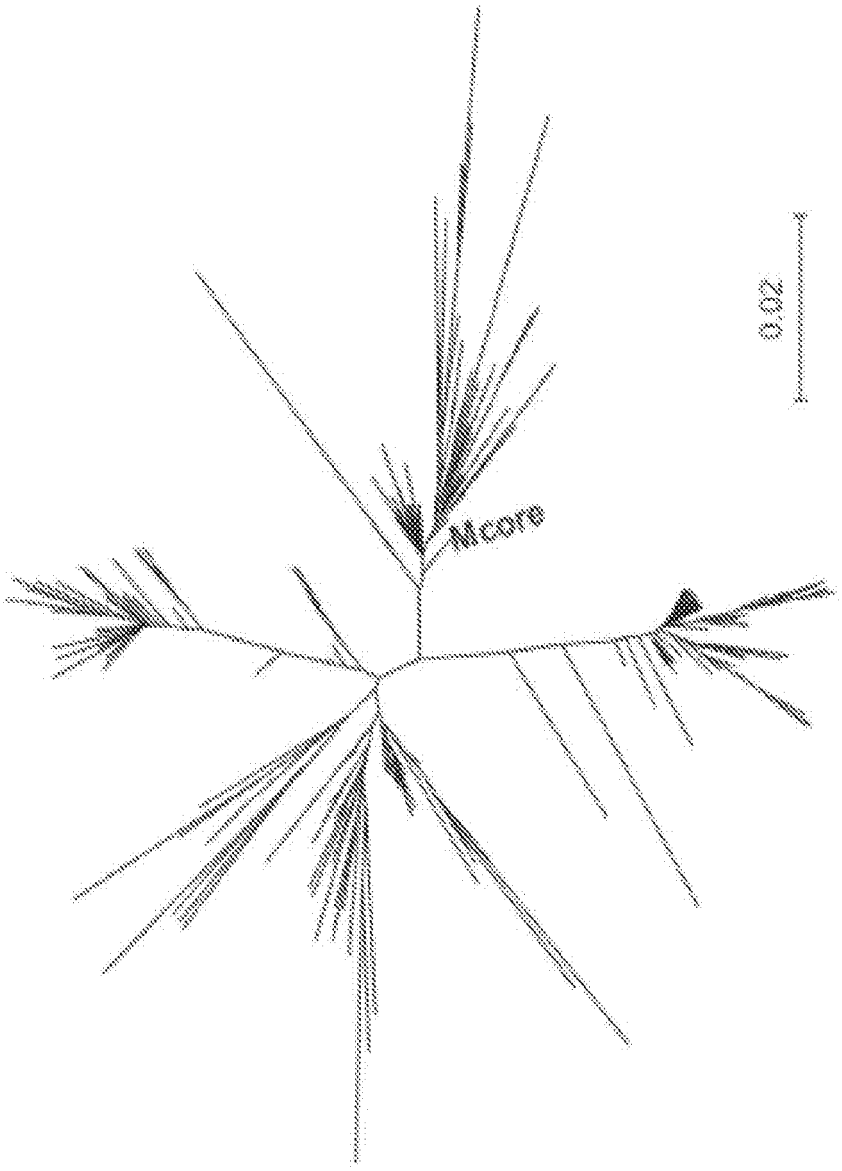


FIGURE 10

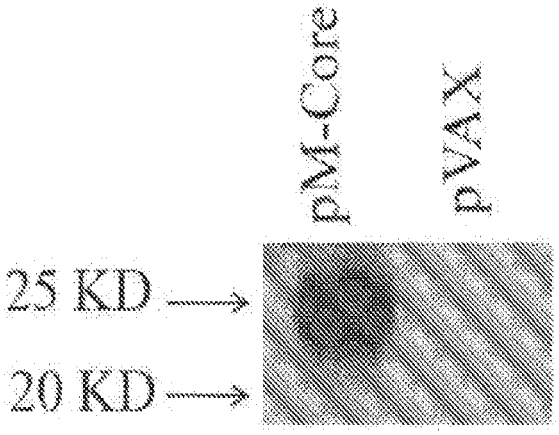


FIGURE 11A

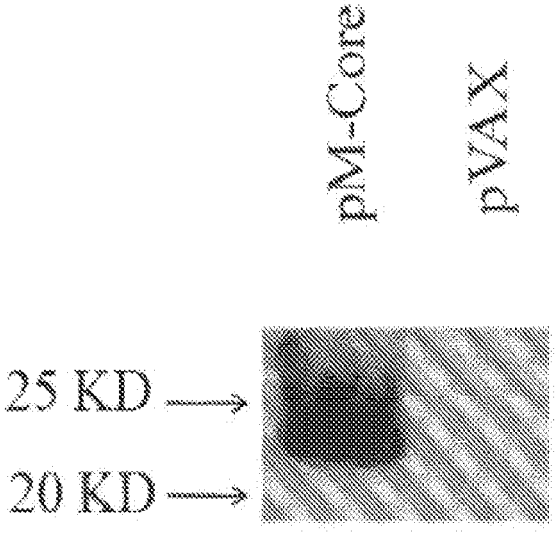


FIGURE 11B

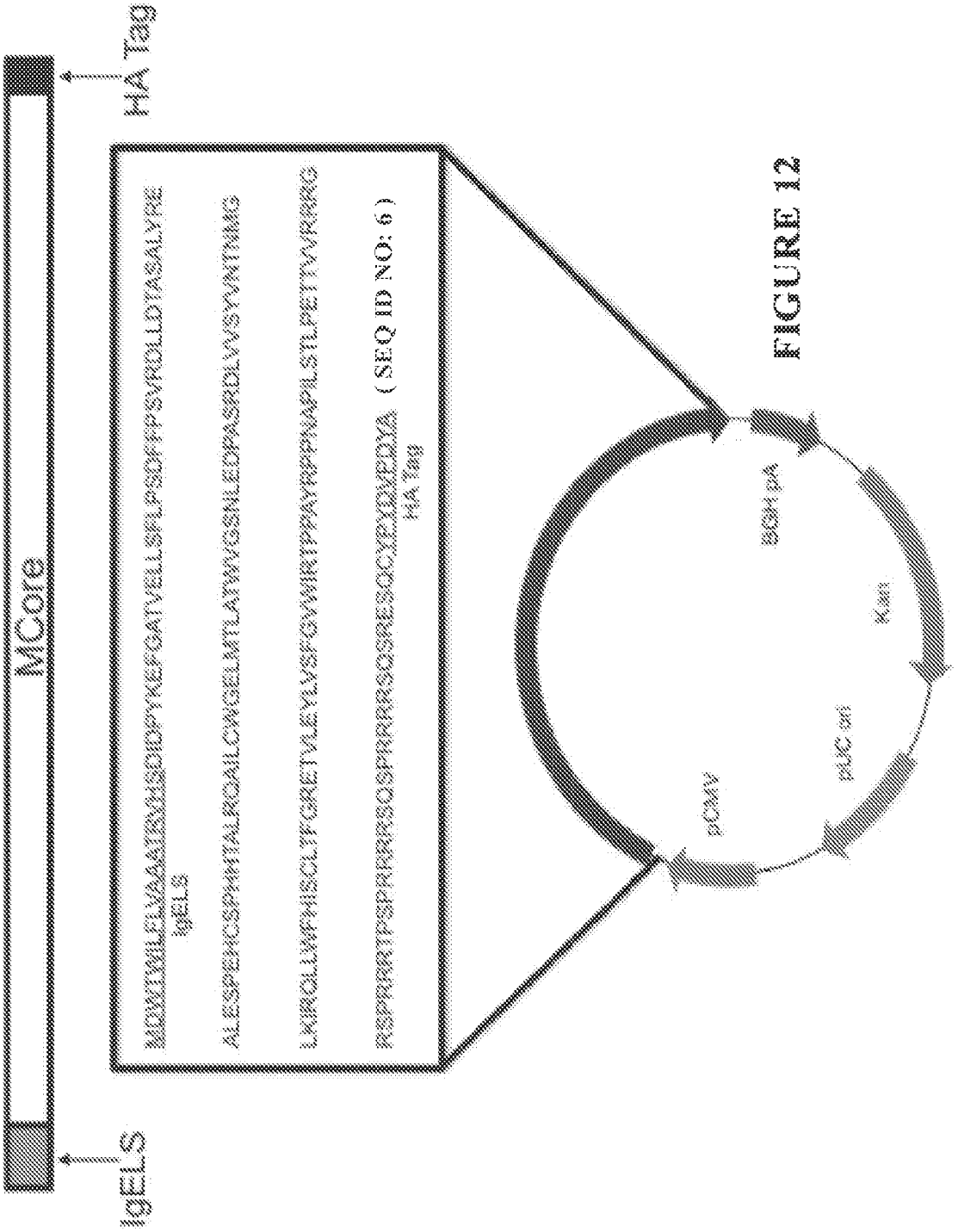

MCore
HA Tag
IgELS
MDWTWILFLVAAATRVHSQIDPYKEFGATVELLSFLPSDFFPSVRDLLDTASALYRE
IgELS
ALESPEHCSPHHTALRQAILCWGELMTLATWVGSNLEDPASRDLVVSYVNTNMG
LKRQLLWFHISCLTFGRETVLEYLVSFGVWIRTPPAYRPPNAPILSTLPETTVVRRRG
RSPRRRTPSPRRRRSQSPRRRRSQSRESQCYEDQVPEDYA (SEQ ID NO: 6)
HA Tag
pCMV
pUC ori
SGH pA
Kan

FIGURE 12

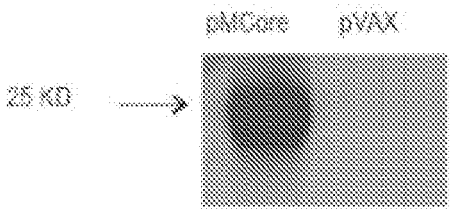


FIGURE 13

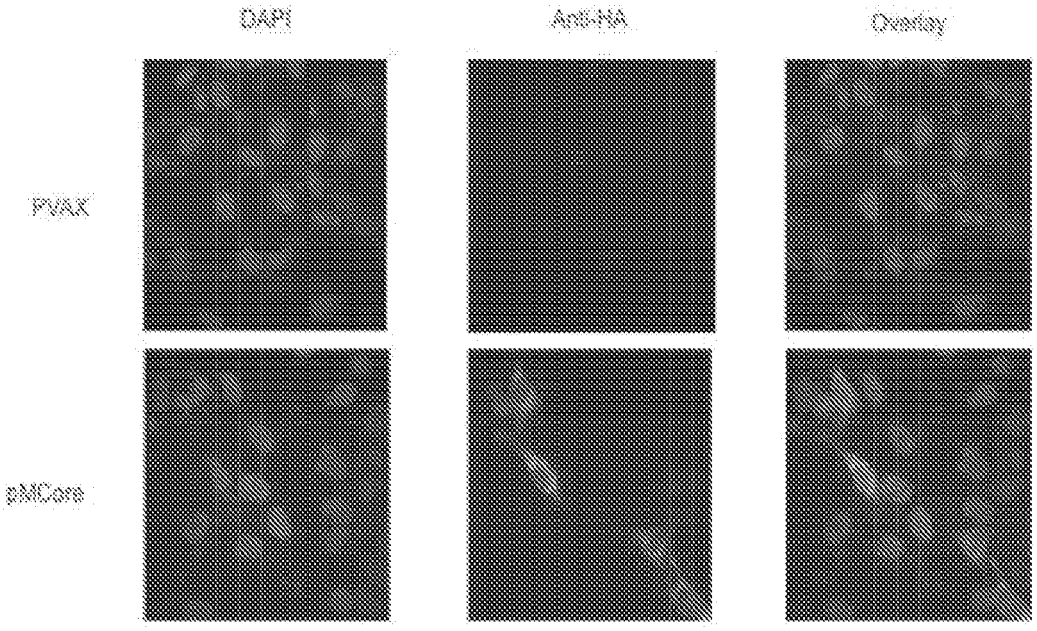


FIGURE 14

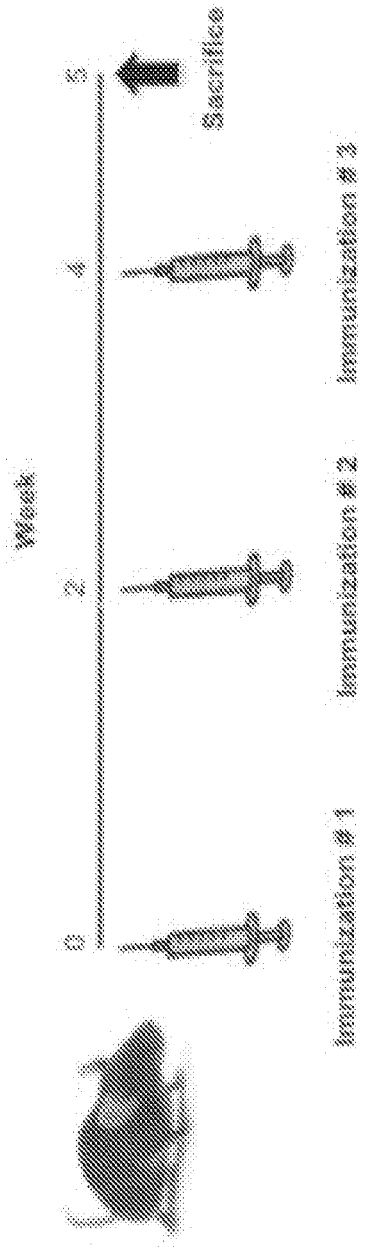

Week
0
2
4
5
Immunization # 1
Immunization # 2
Immunization # 3
Sacrifice

FIGURE 15

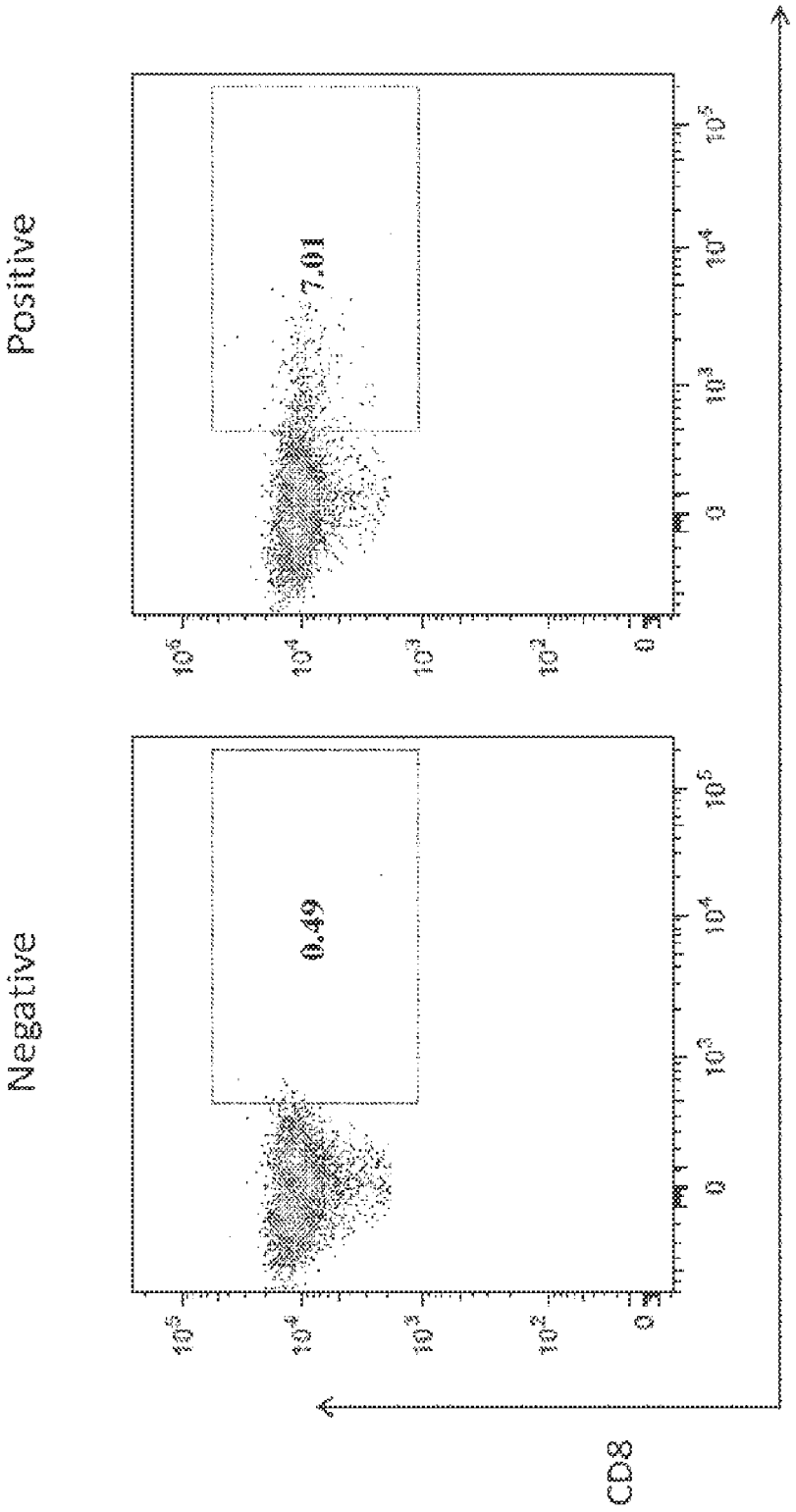


FIGURE 16
(Page 1 of 2)

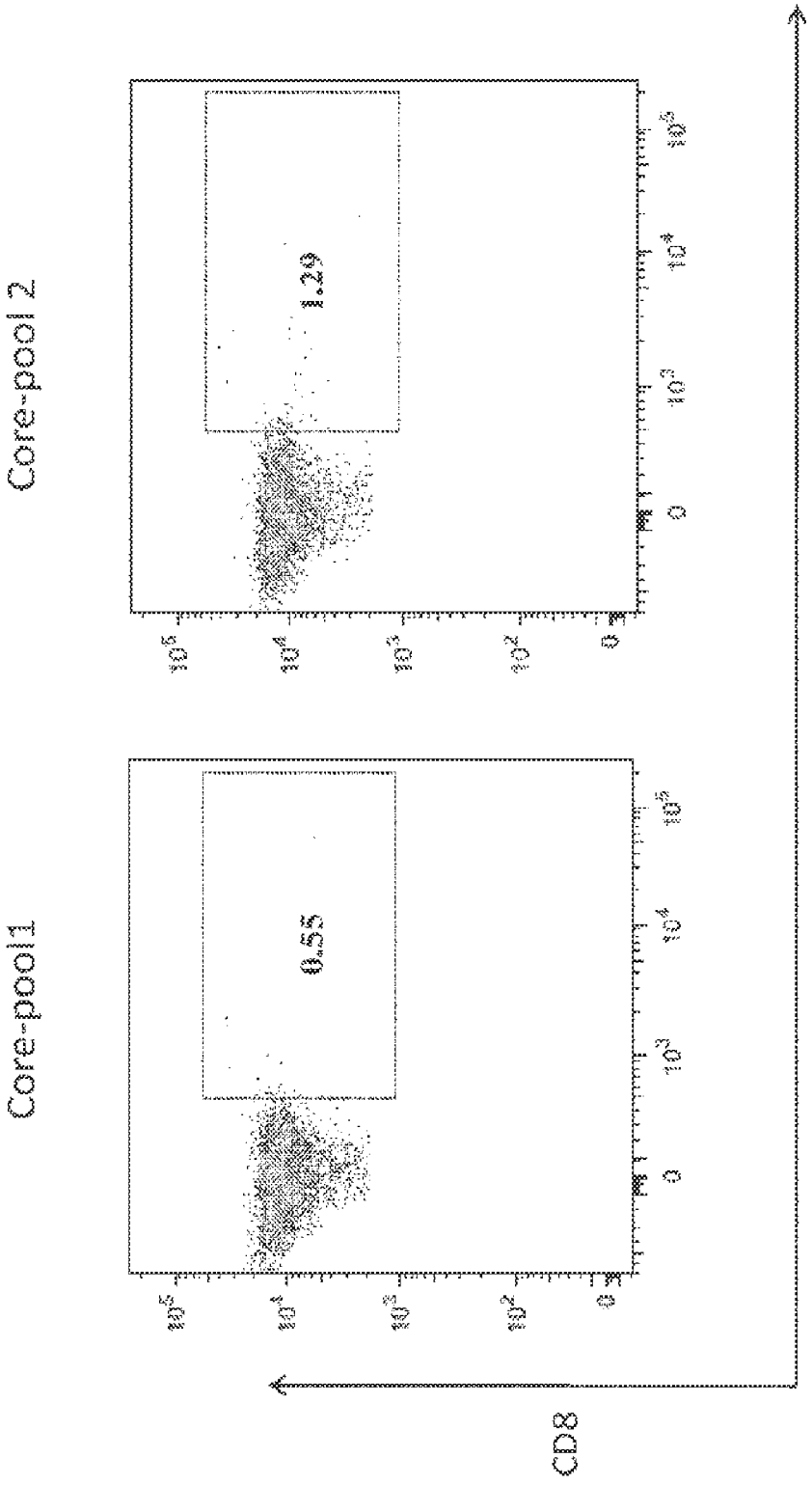
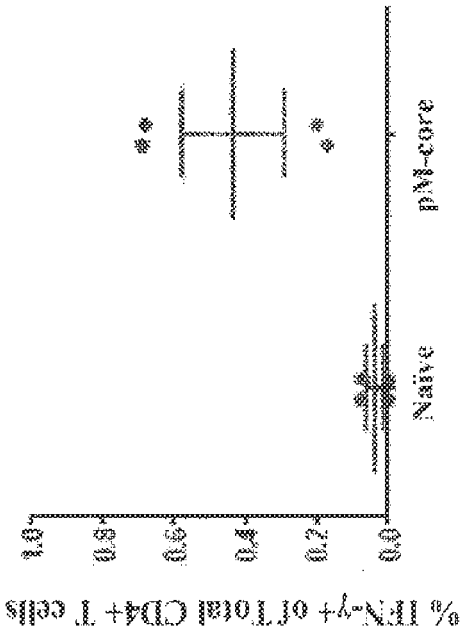


FIGURE 16, continued
(Page 2 of 2)


% IFN-γ+ of Total CD4+ T cells
1.0
0.8
0.6
0.4
0.2
0.0
Naive
pM-core
**
**

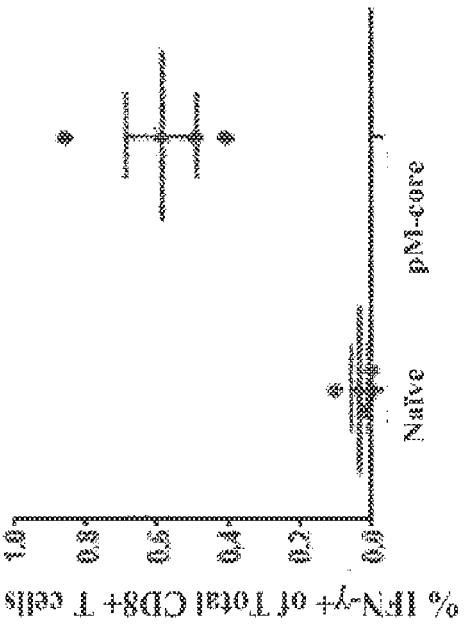

% IFN-γ+ of Total CD8+ T cells
1.0
0.8
0.6
0.4
0.2
0.0
Naive
pM-core
*
*

FIGURE 17

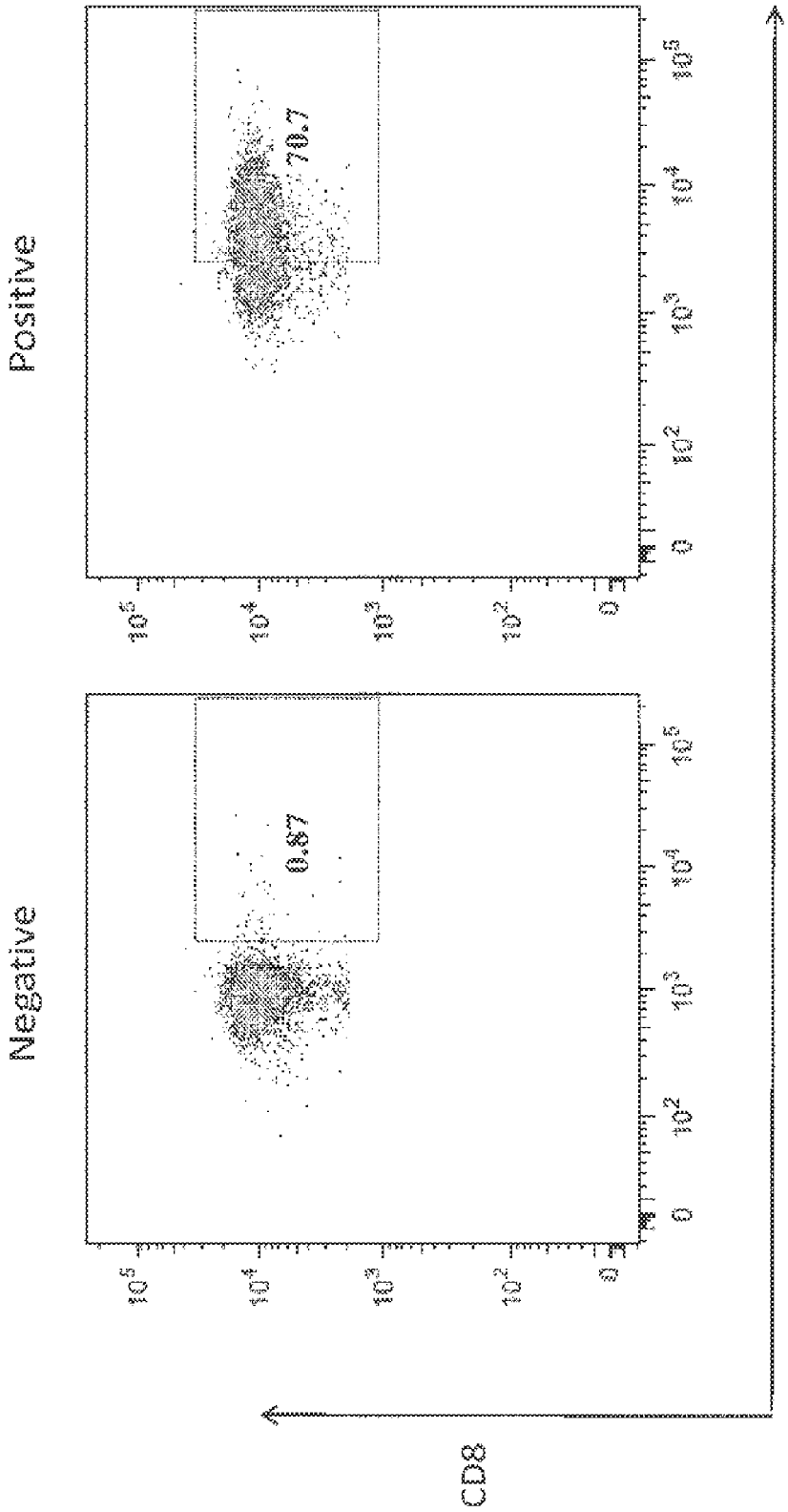


FIGURE 18
(Page 1 of 2)

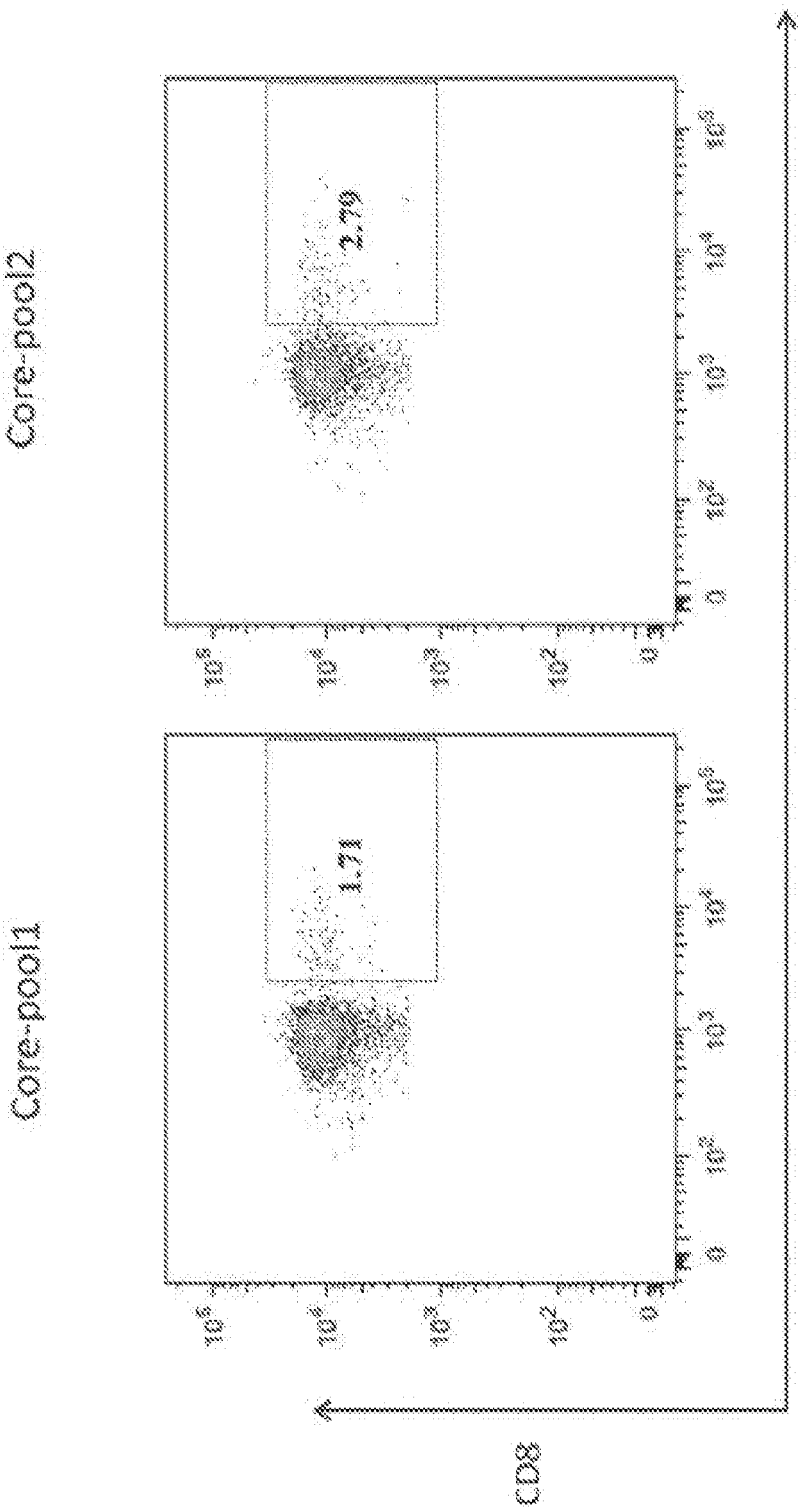


FIGURE 18, continued
(Page 2 of 2)

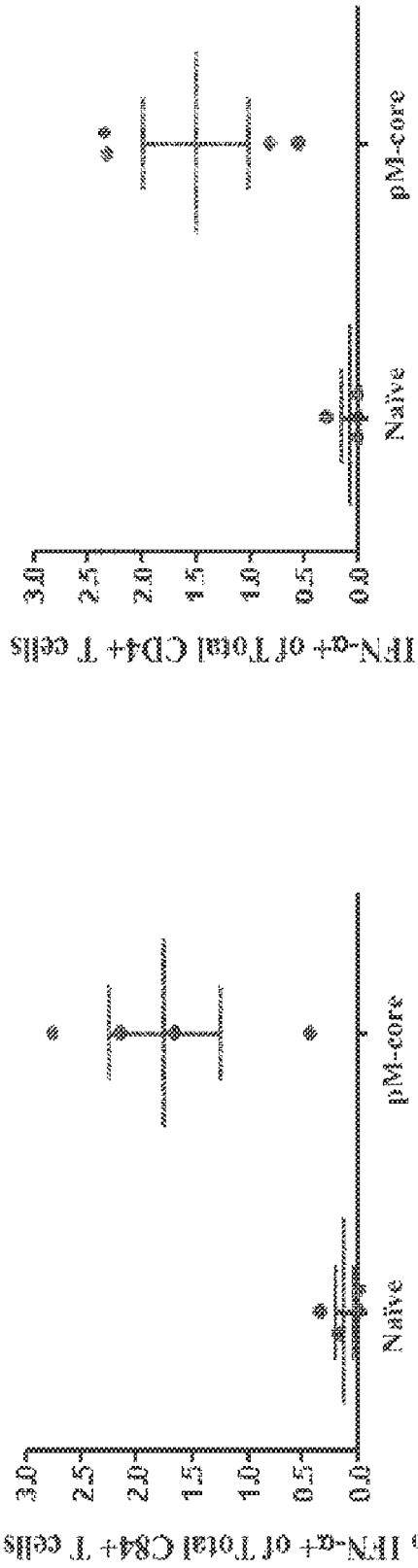

% IFN-g+ of Total CD4+ T cells
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Naive
pM-core
% IFN-g+ of Total CD4+ T cells
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Naive
pM-core

FIGURE 19

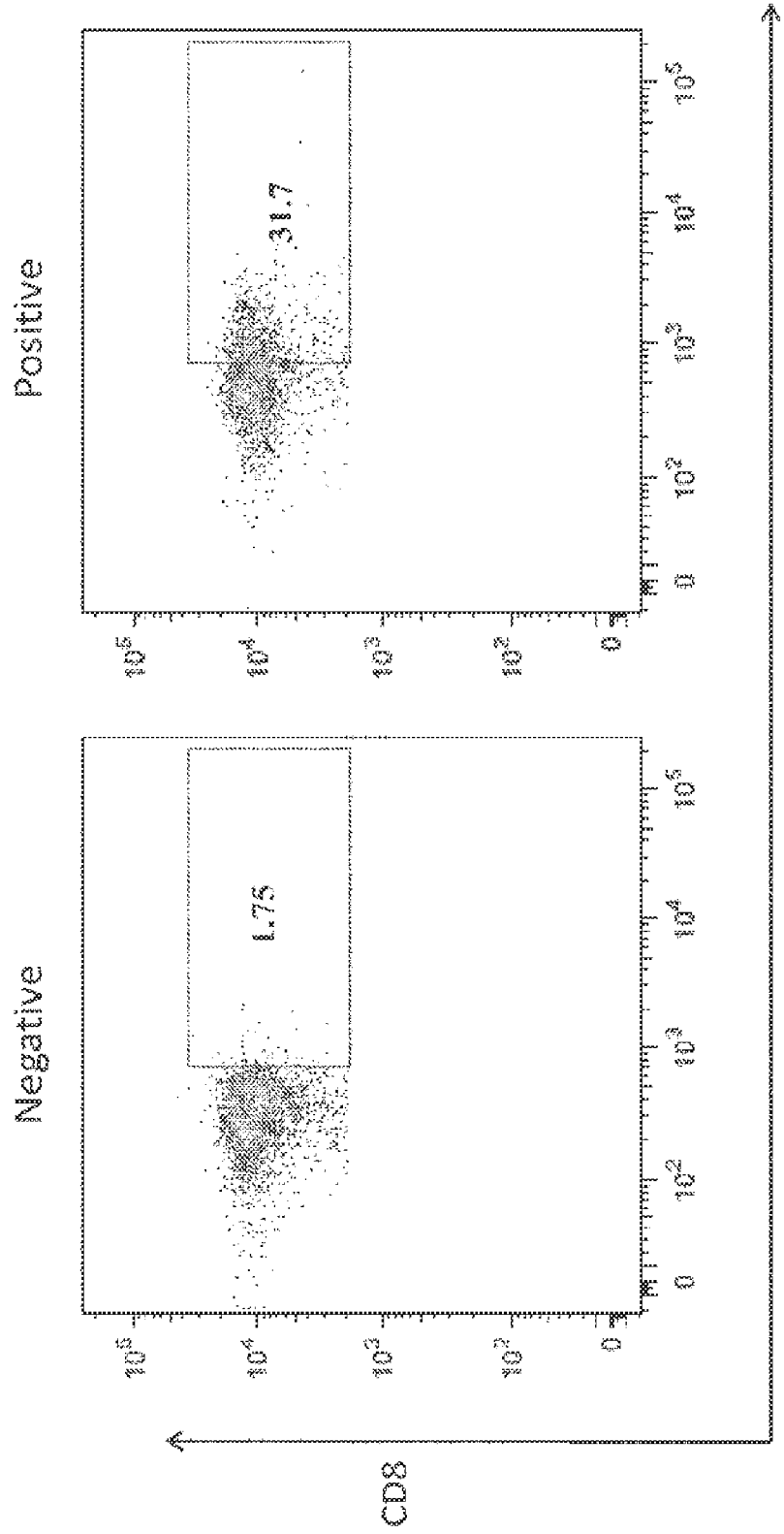


FIGURE 20
(Page 1 of 2)

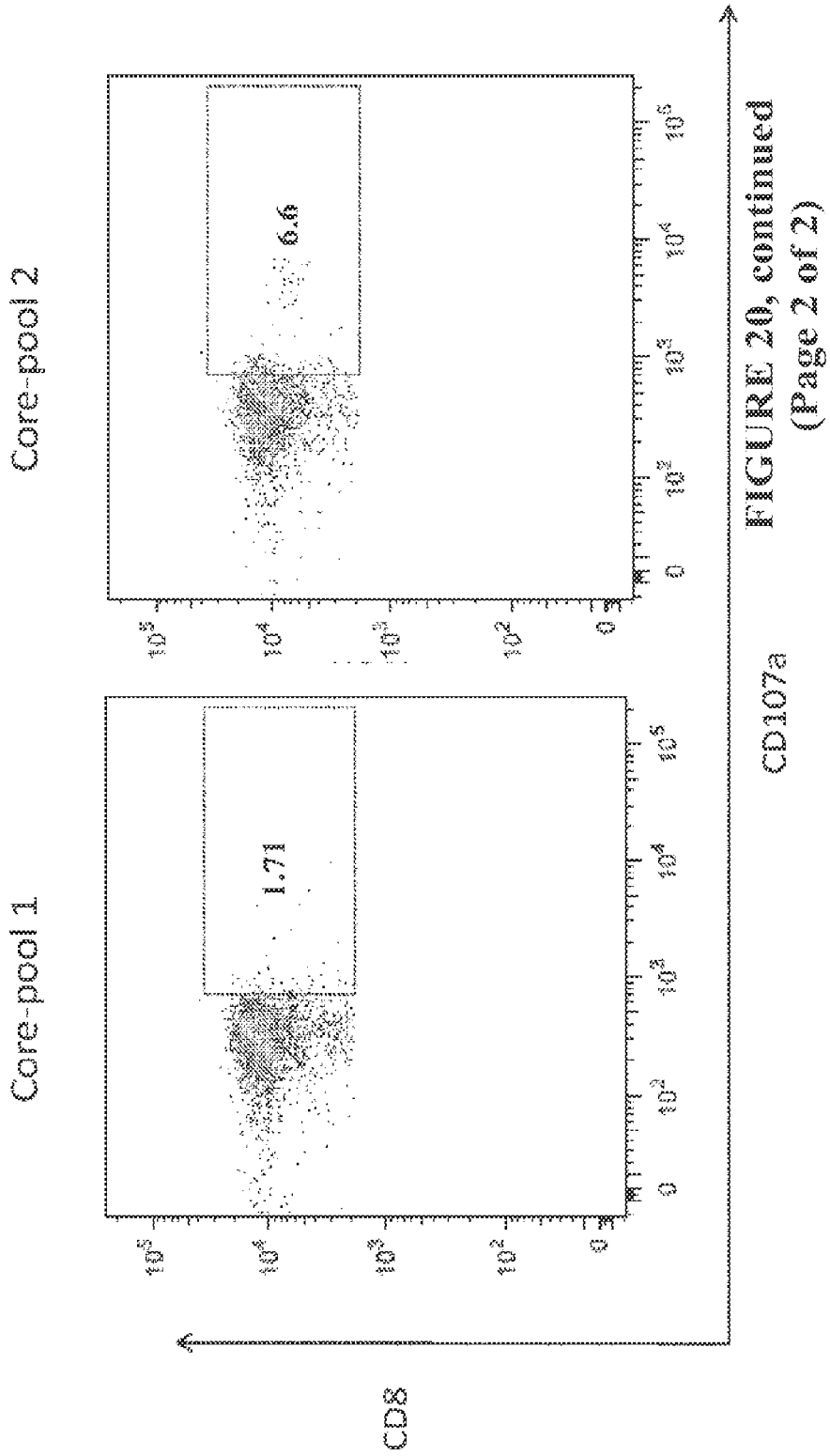


FIGURE 2B, continued
(Page 2 of 2)

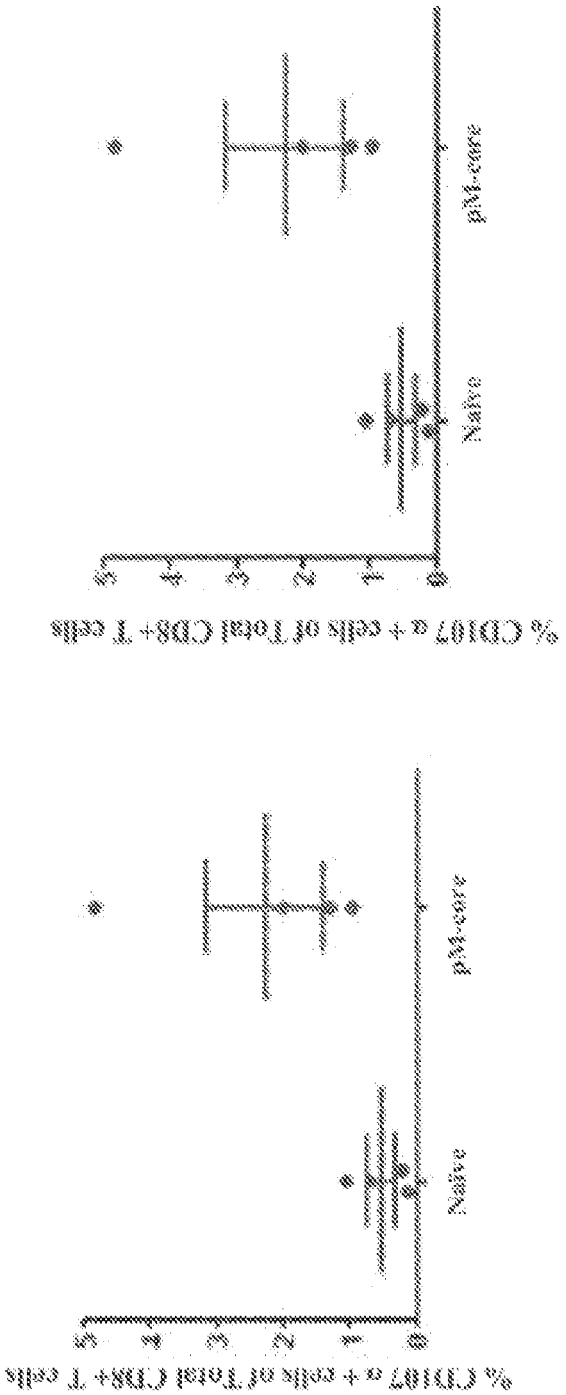

% CD107 α + cells of Total CD8+ T cells
0
1
2
3
4
5
Naïve
p34-core
% CD107 α + cells of Total CD8+ T cells
0
1
2
3
4
5
Naïve
p34-core

FIGURE 21

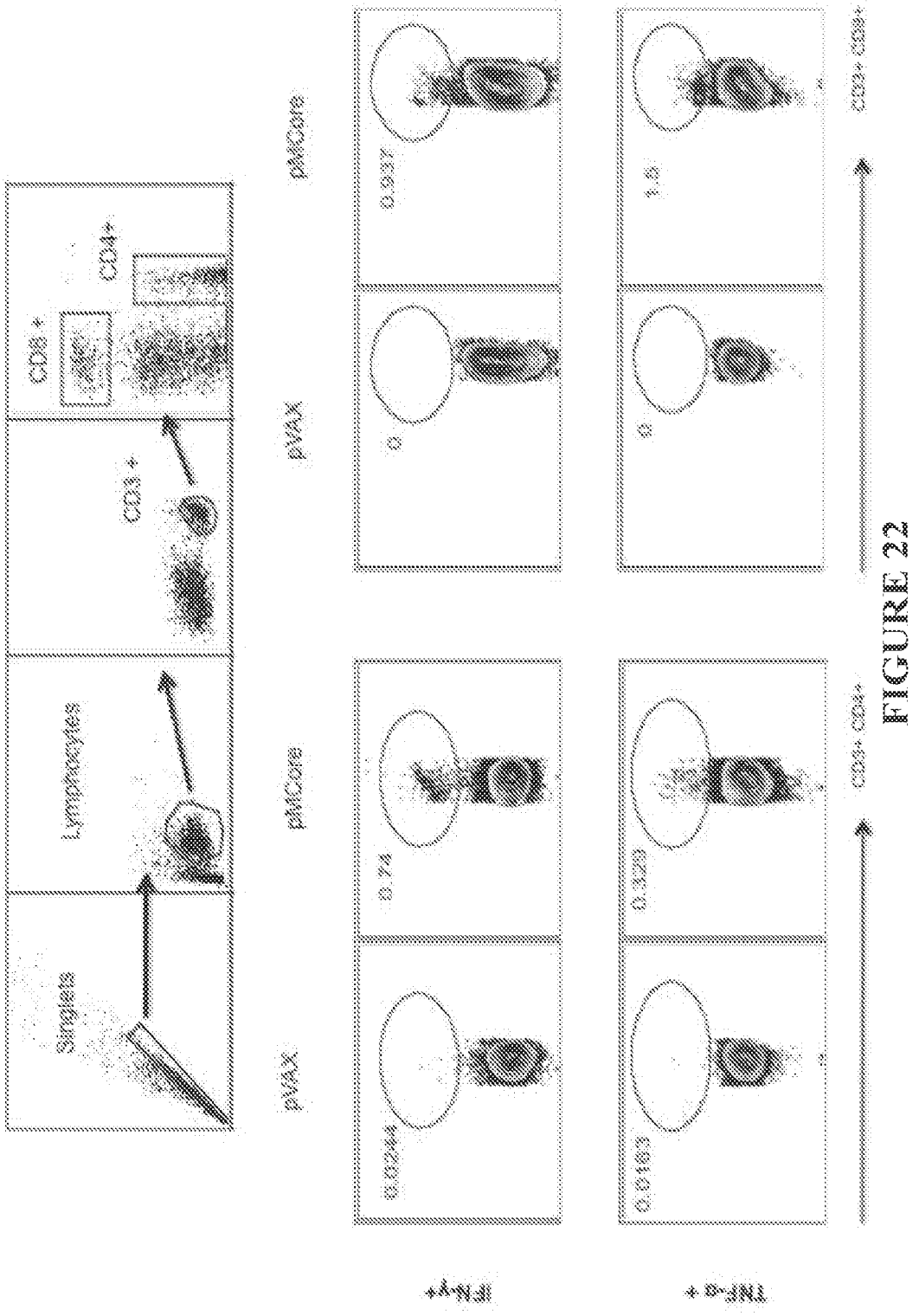

Singlets
Lymphocytes
CD3 +
CD8 +
CD4+
pVAX
pMCore
pVAX
pMCore
0.0244
0.74
0.0163
0.329
0
0.937
0
1.5
IFN-γ+
TNF-α +
CD3+ CD4+
CD3+ CD8+

FIGURE 22

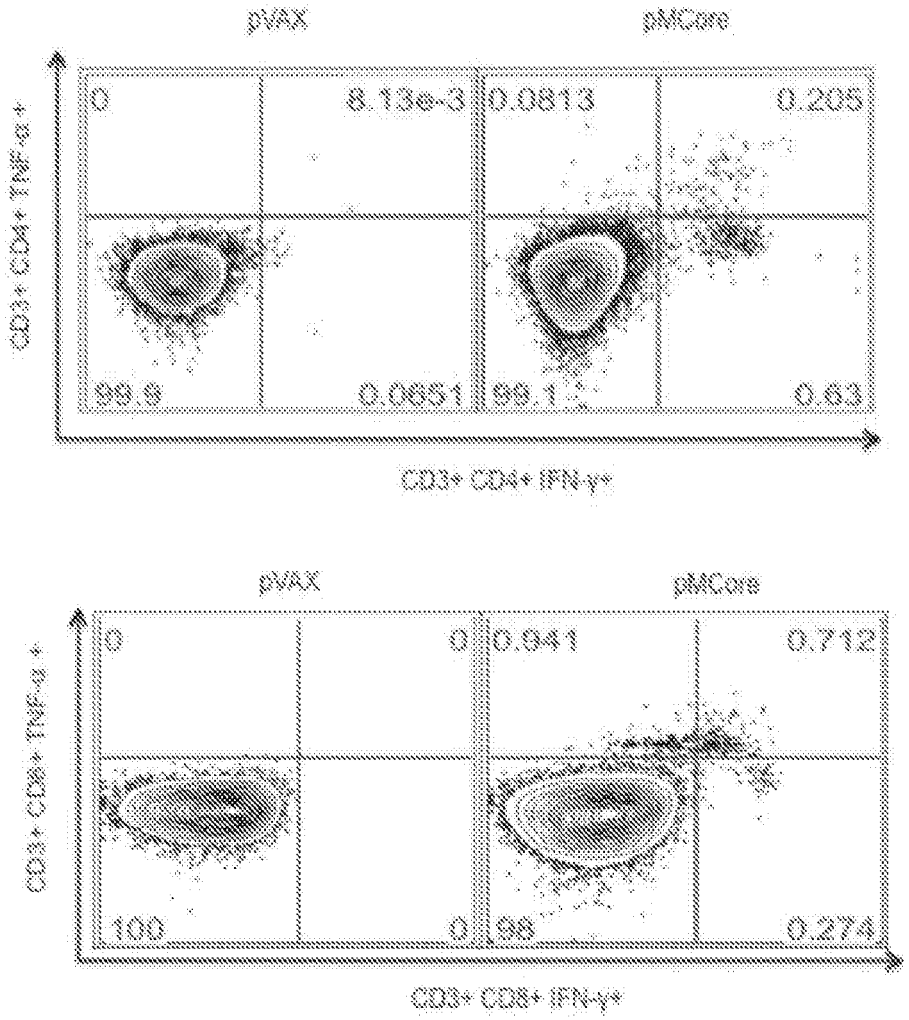

pVAX
pMCore
0
8.13e-3
0.0813
0.205
99.9
0.0651
99.1
0.63
CD3+ CD4+ TNF-α+
CD3+ CD4+ IFN-γ+
pVAX
pMCore
0
0
0.941
0.712
100
0
98
0.274
CD3+ CD8+ TNF-α+
CD3+ CD8+ IFN-γ+

FIGURE 23

pVAX

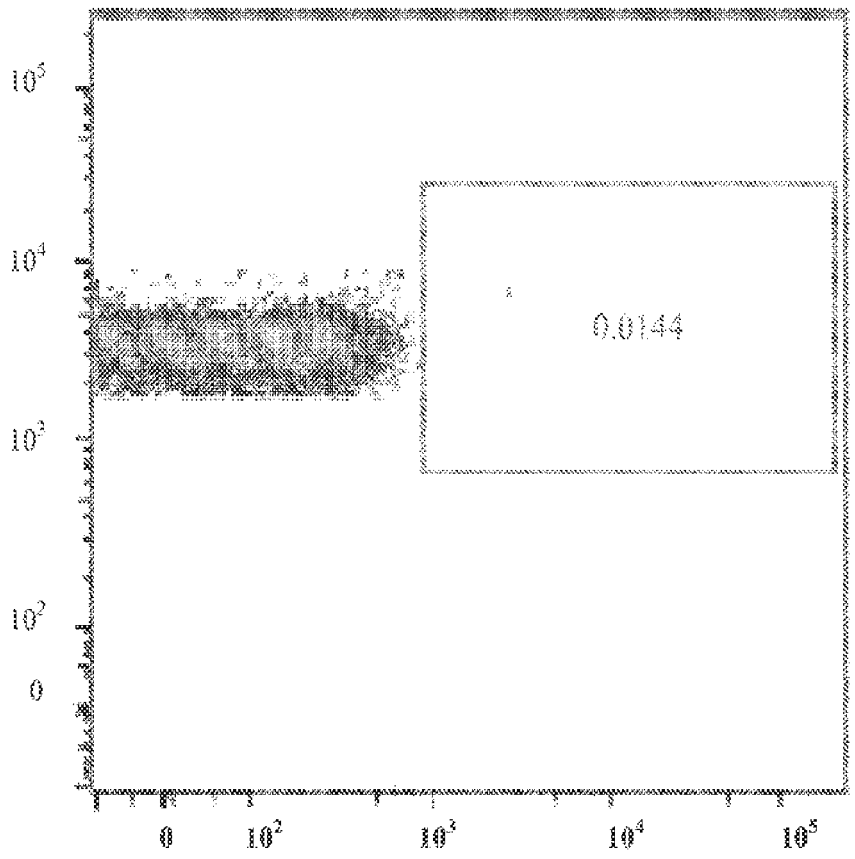


FIGURE 24
(Page 1 of 3)

pMCore

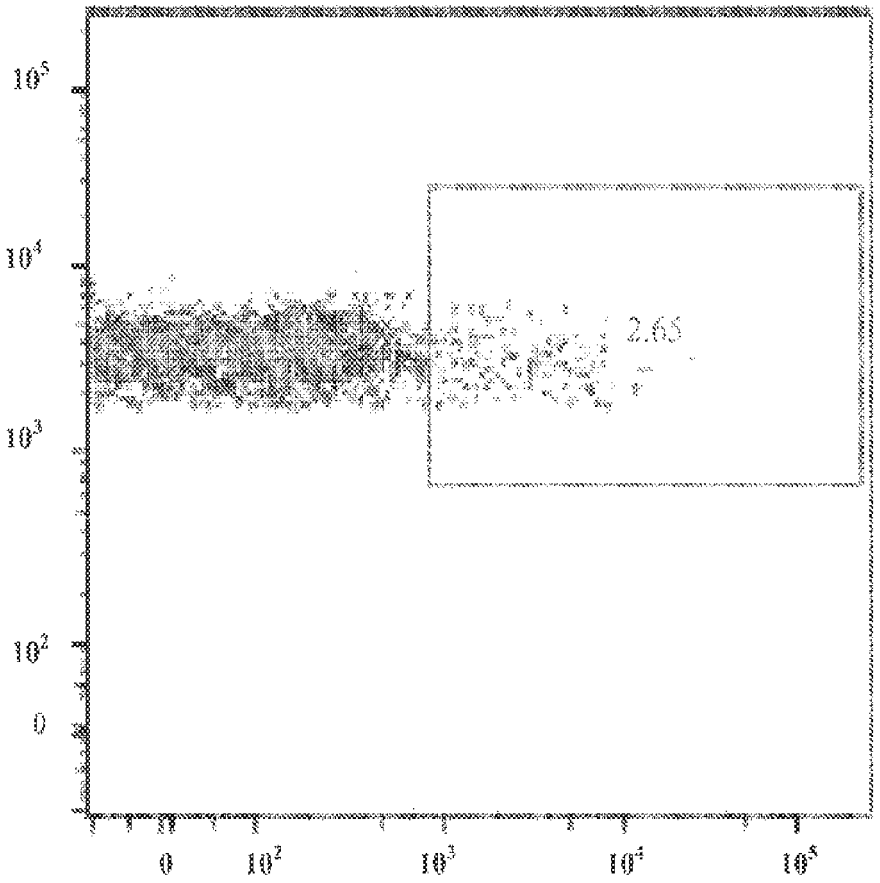


FIGURE 24, continued
(Page 2 of 3)

Pos. Control

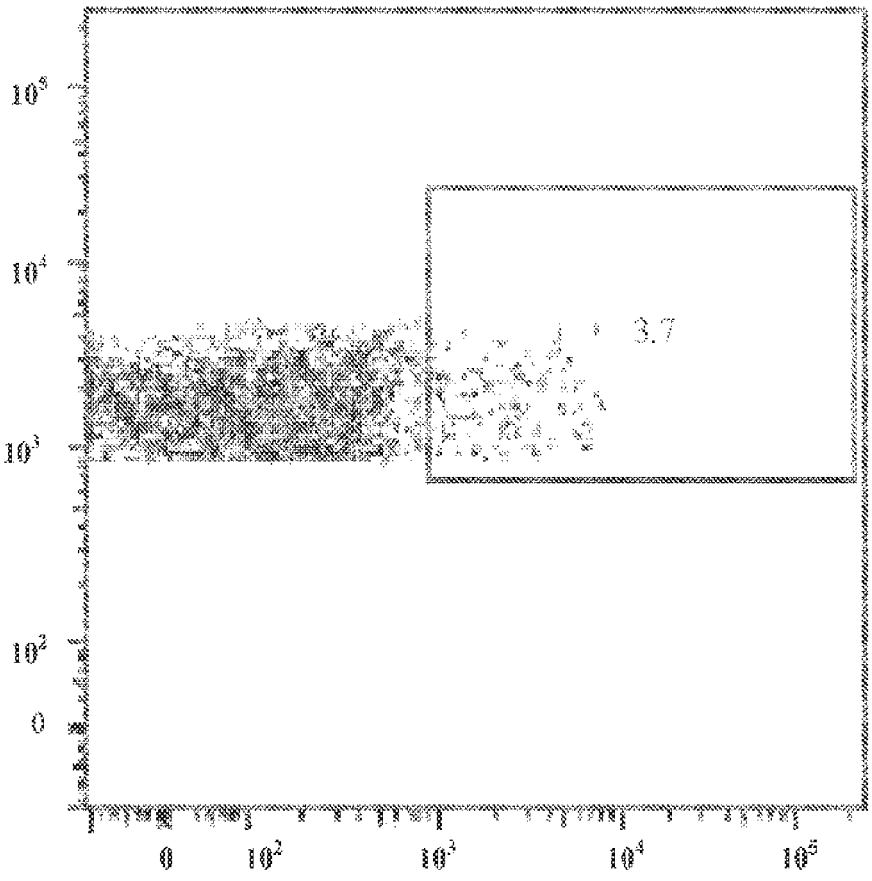


FIGURE 24, continued
(Page 3 of 3)

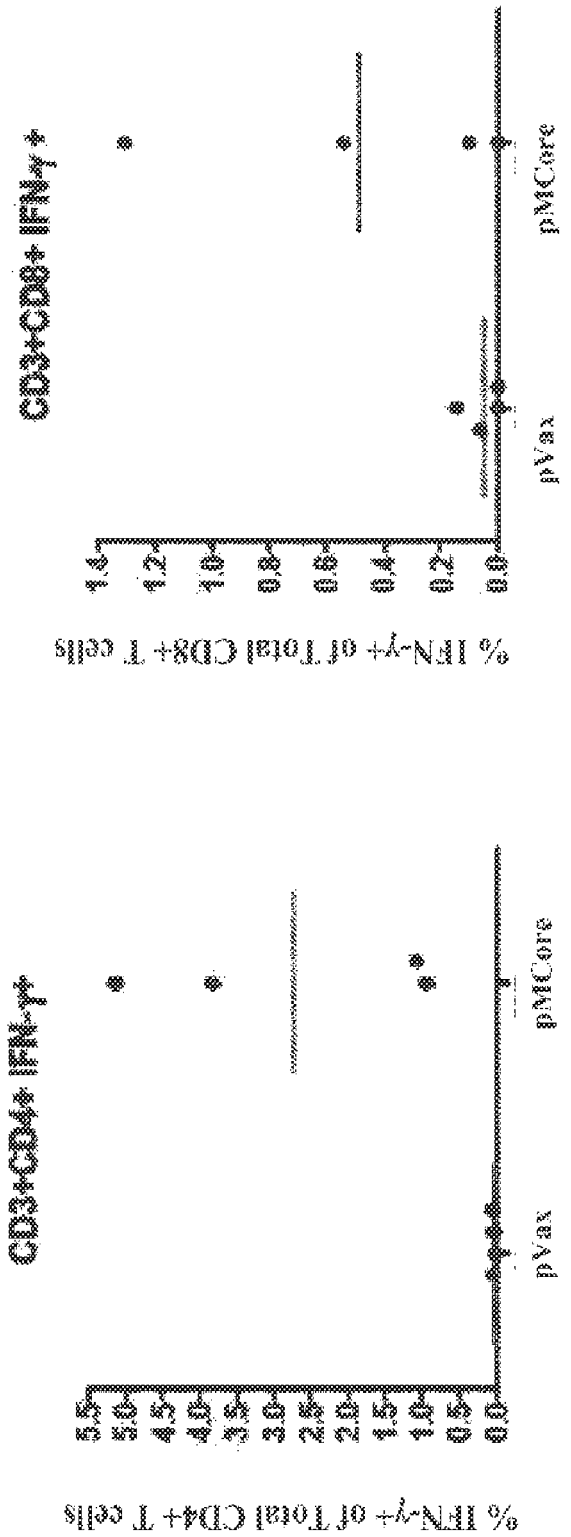

CD3+CD4+ IFN-γ+
% IFN-γ+ of Total CD4+ T cells
55
50
45
40
35
30
25
20
15
10
5
0
pVax
pMCore
CD3+CD8+ IFN-γ+
% IFN-γ+ of Total CD8+ T cells
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
pVax
pMCore

FIGURE 25

pVAX

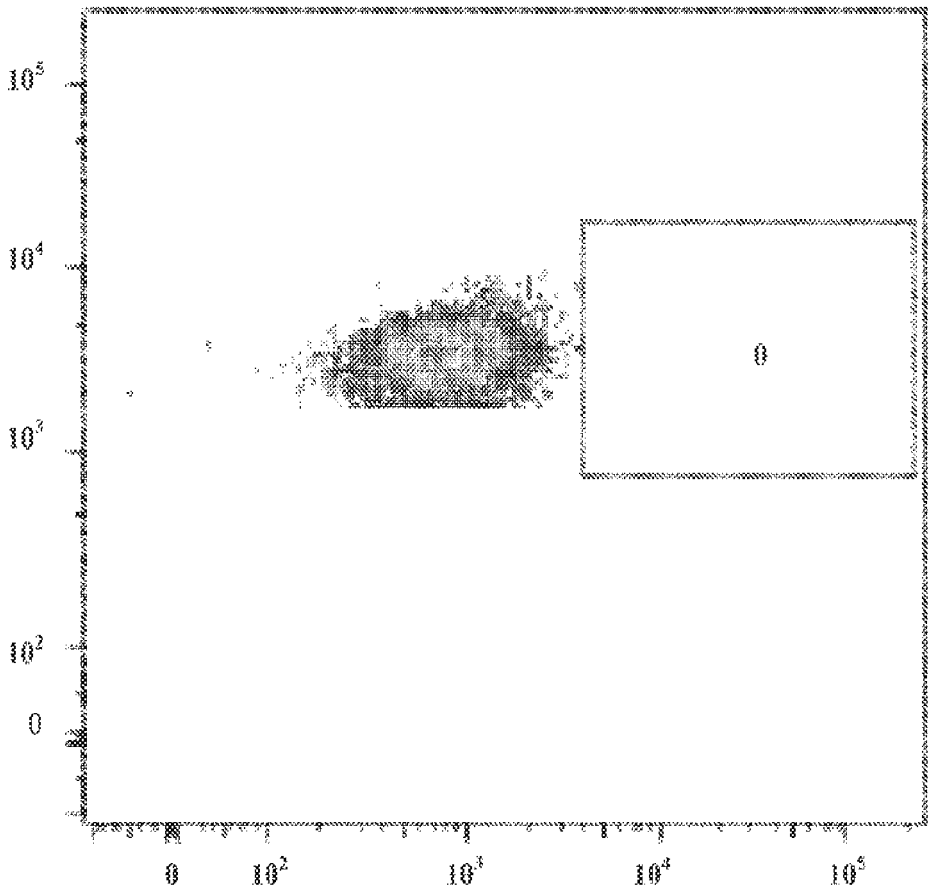


FIGURE 26
(Page 1 of 3)

pMCore

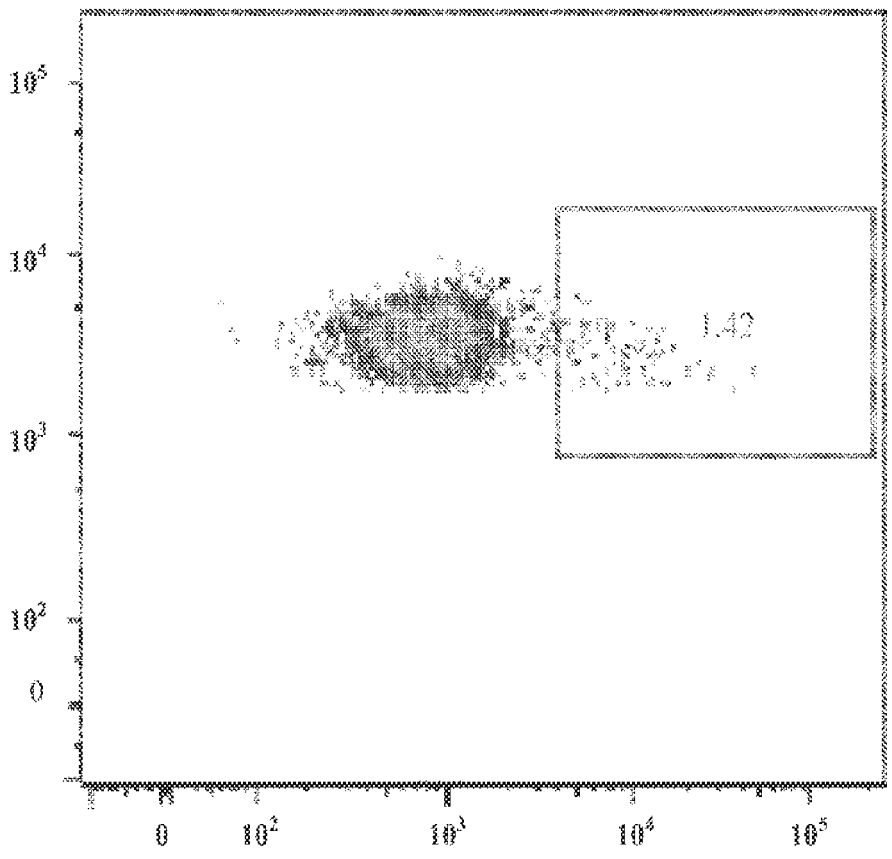


FIGURE 26, continued
(Page 2 of 3)

Pos. Control

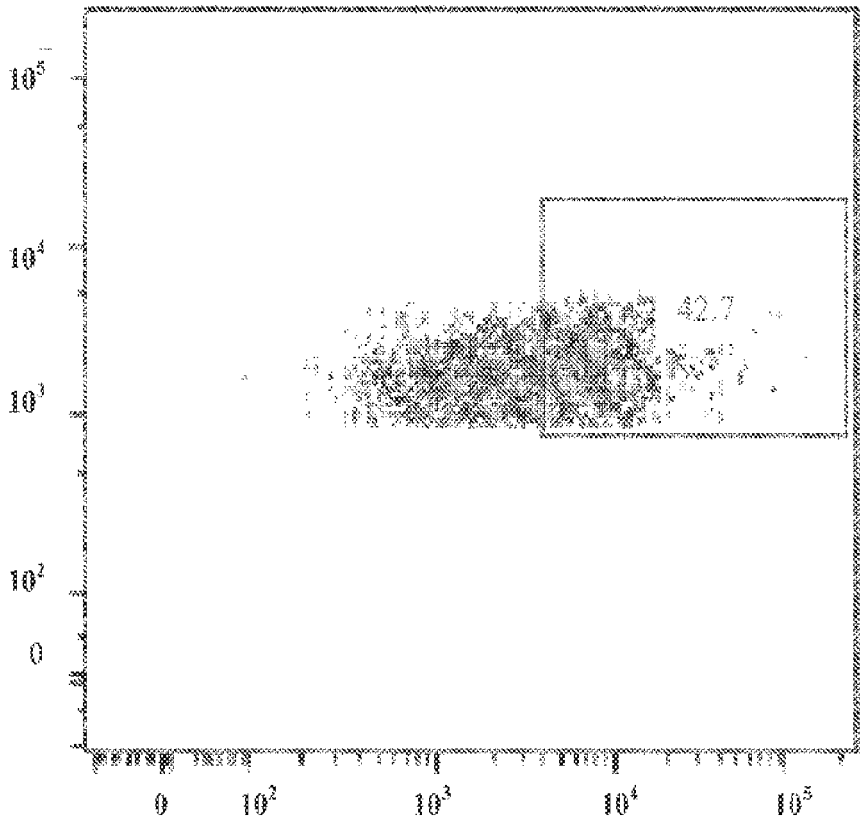


FIGURE 26, continued
(Page 3 of 3)

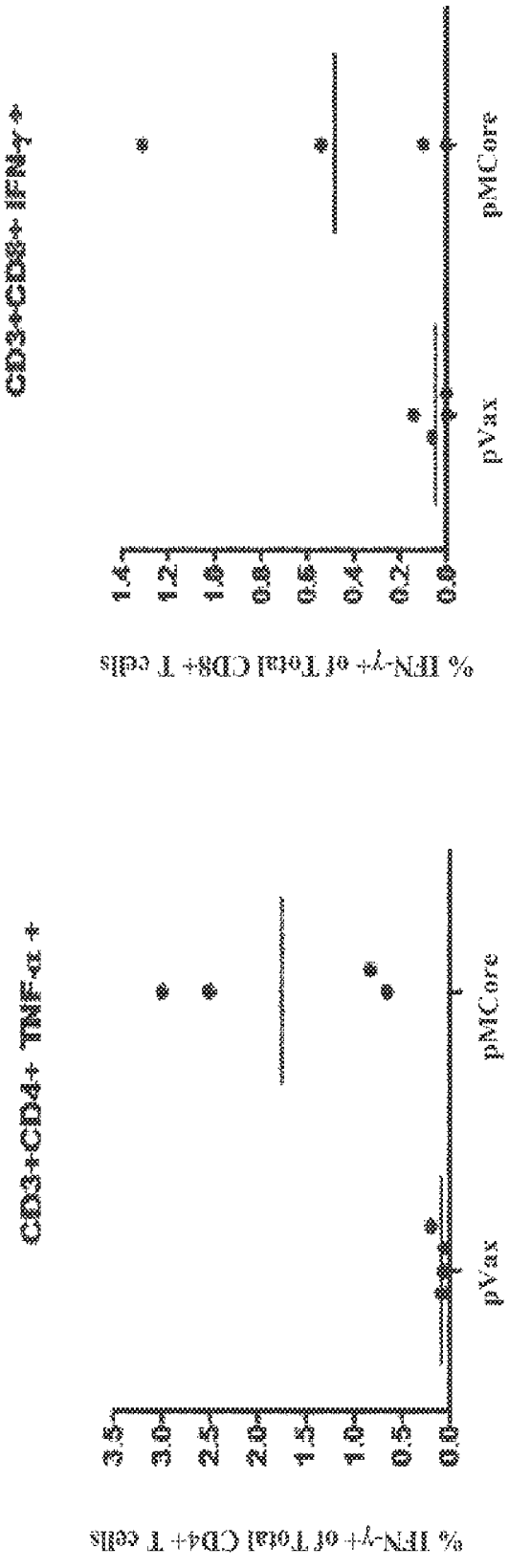

CD3+CD4+ TNF-α +
% IFN-γ+ of Total CD4+ T cells
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
pVax
pMCore
CD3+CD8+ IFN-γ +
% IFN-γ+ of Total CD8+ T cells
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
pVax
pMCore

FIGURE 27

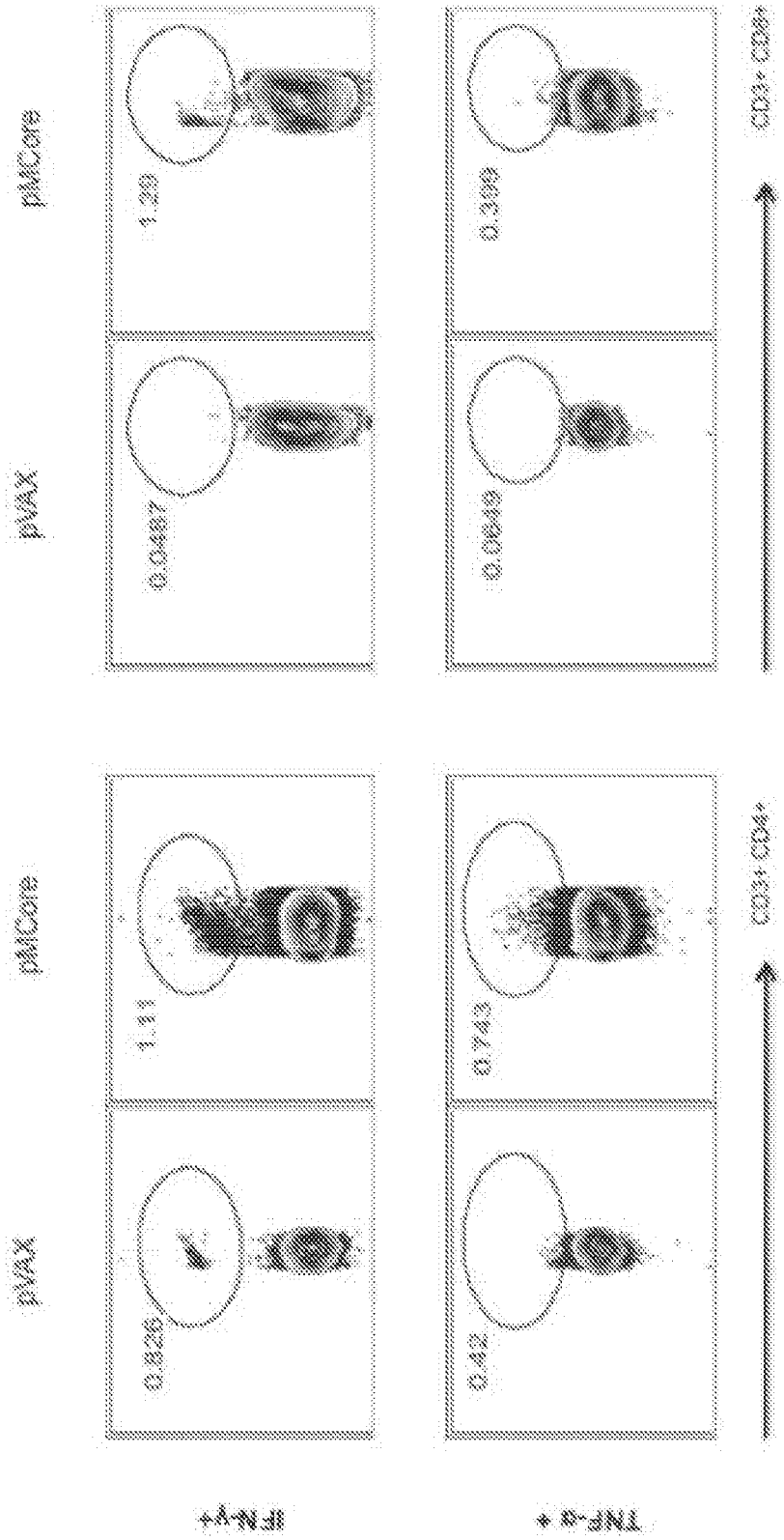

IFN-γ+
TNF-α +
pVAX
pMCore
pVAX
pMCore
0.826
1.11
0.42
0.743
0.0467
1.29
0.0649
0.399
CD3+ CD4+
CD3+ CD8+

FIGURE 28

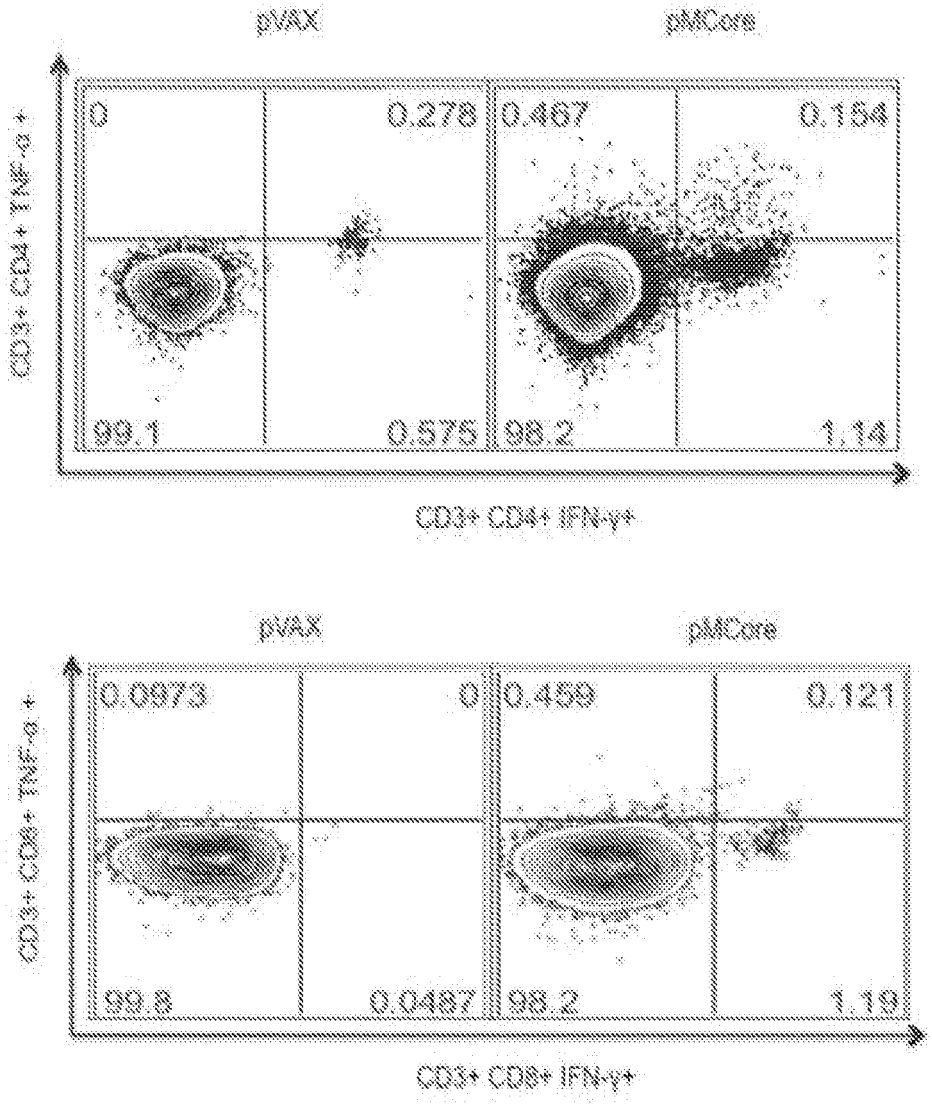

pVAX
pMCore
0
0.278
0.467
0.154
99.1
0.575
98.2
1.14
CD3+ CD4+ TNF-α +
CD3+ CD4+ IFN-γ+
pVAX
pMCore
0.0973
0
0.459
0.121
99.8
0.0487
98.2
1.19
CD3+ CD8+ TNF-α +
CD3+ CD8+ IFN-γ+

FIGURE 29

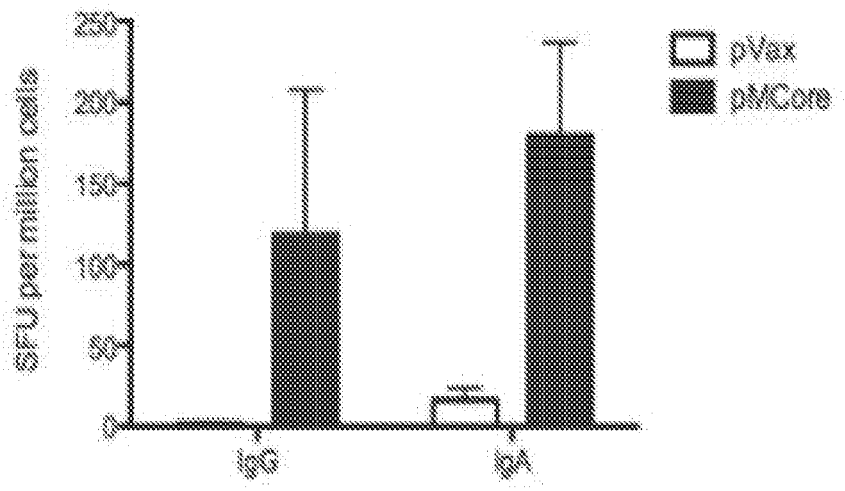


FIGURE 30

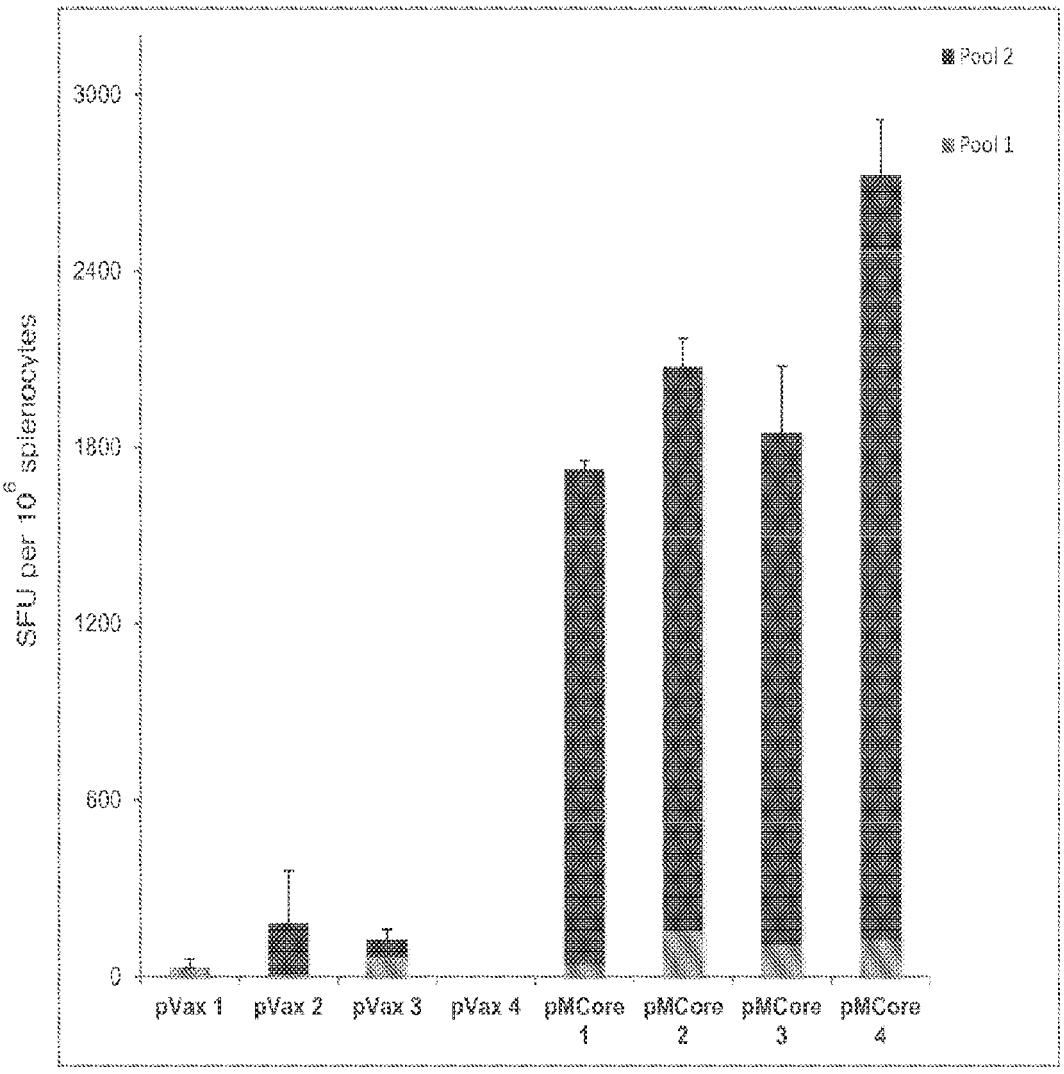

Pool 2
Pool 1
3000
2400
1800
1200
600
0
SFU per 10^6 splenocytes
pVax 1
pVax 2
pVax 3
pVax 4
pMCore 1
pMCore 2
pMCore 3
pMCore 4

FIGURE 31

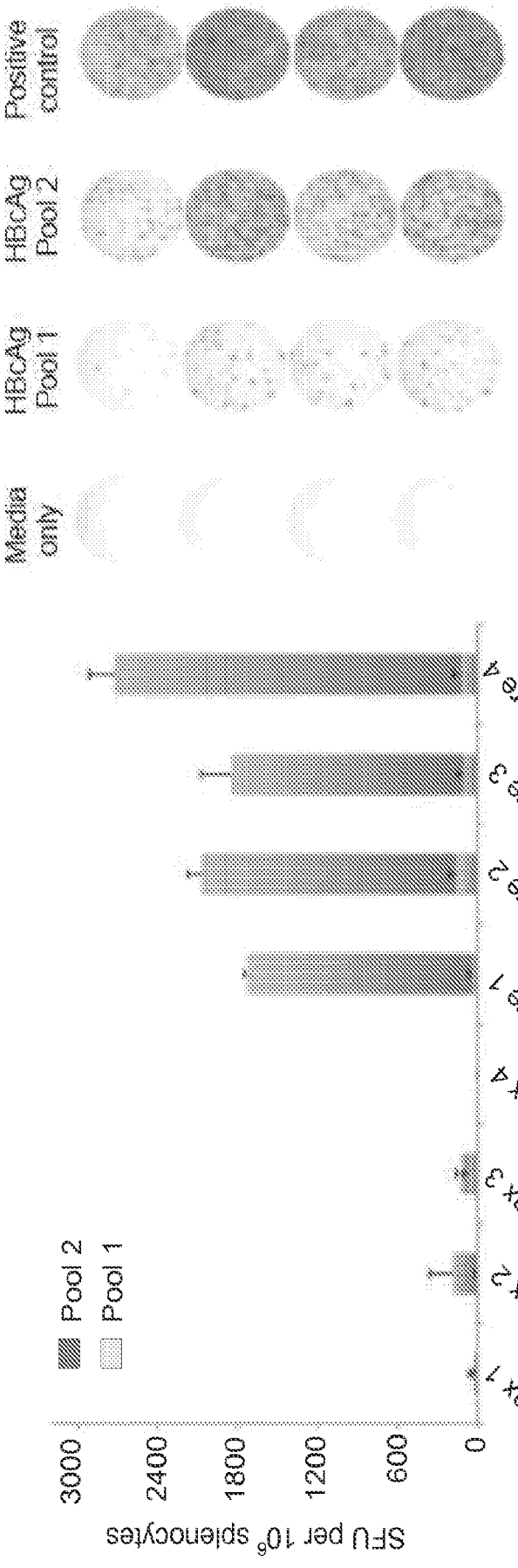

Positive control
HBcAg Pool 2
HBcAg Pool 1
Media only
SFU per 10^6 splenocytes
3000
2400
1800
1200
600
0
Pool 2
Pool 1
pVax 1
pVax 2
pVax 3
pVax 4
pMCore 1
pMCore 2
pMCore 3
pMCore 4

FIGURE 32

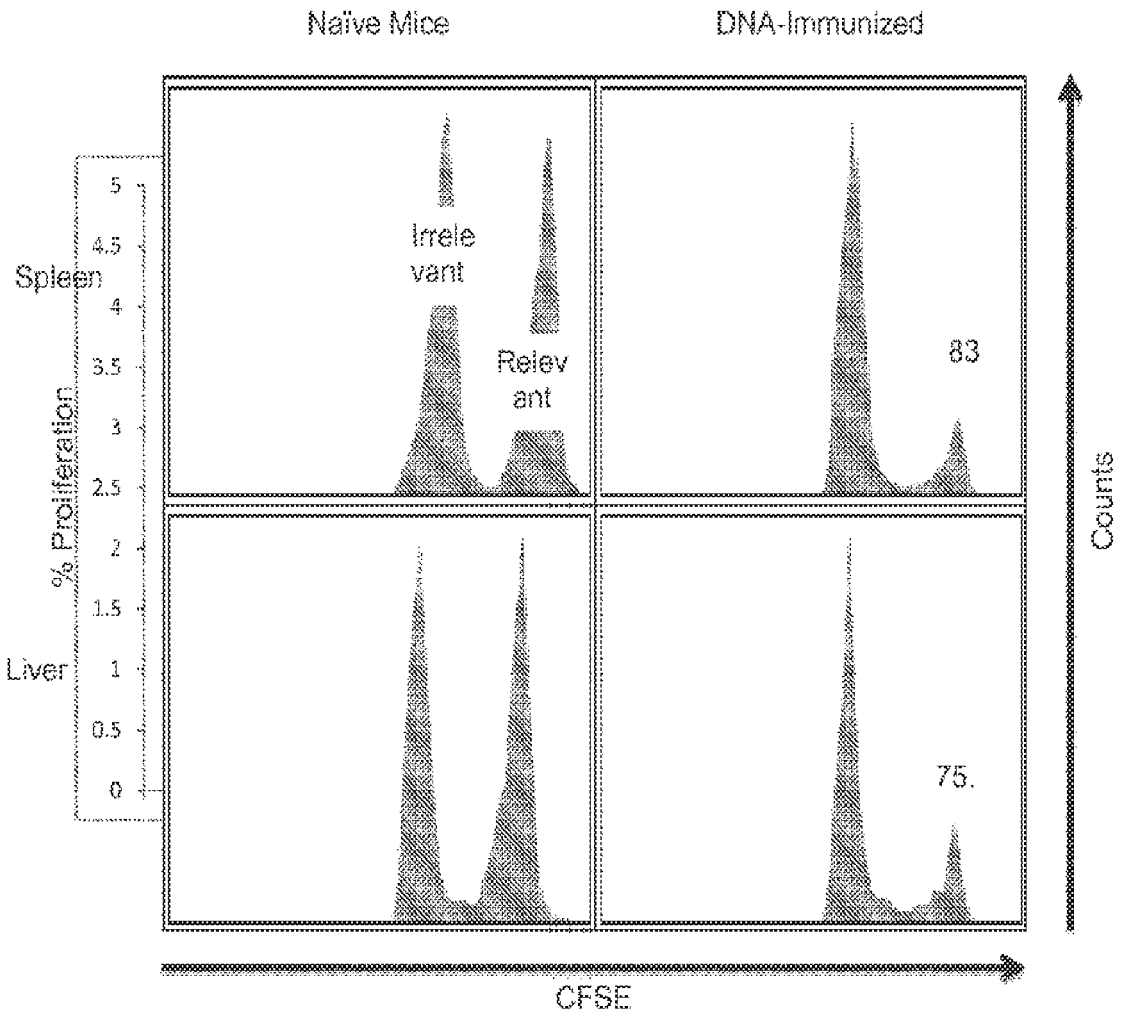

Naïve Mice
DNA-Immunized
Spleen
Liver
% Proliferation
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Irrele vant
Relev ant
83
75.
Counts
CFSE

FIGURE 33

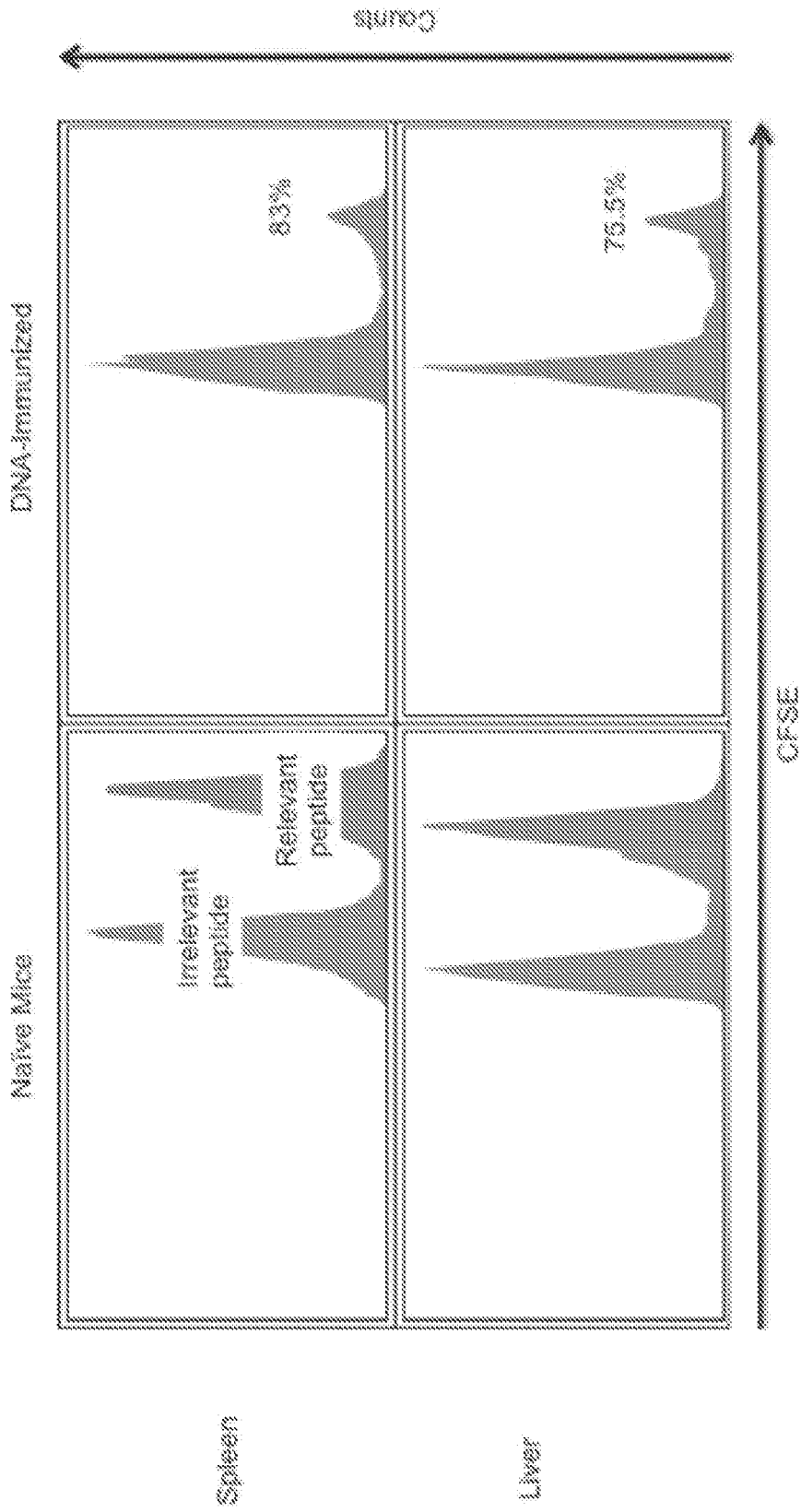

Counts
DNA-Immunized
Naïve Mice
83%
76.5%
Irrelevant peptide
Relevant peptide
CFSE
Spleen
Liver

FIGURE 34

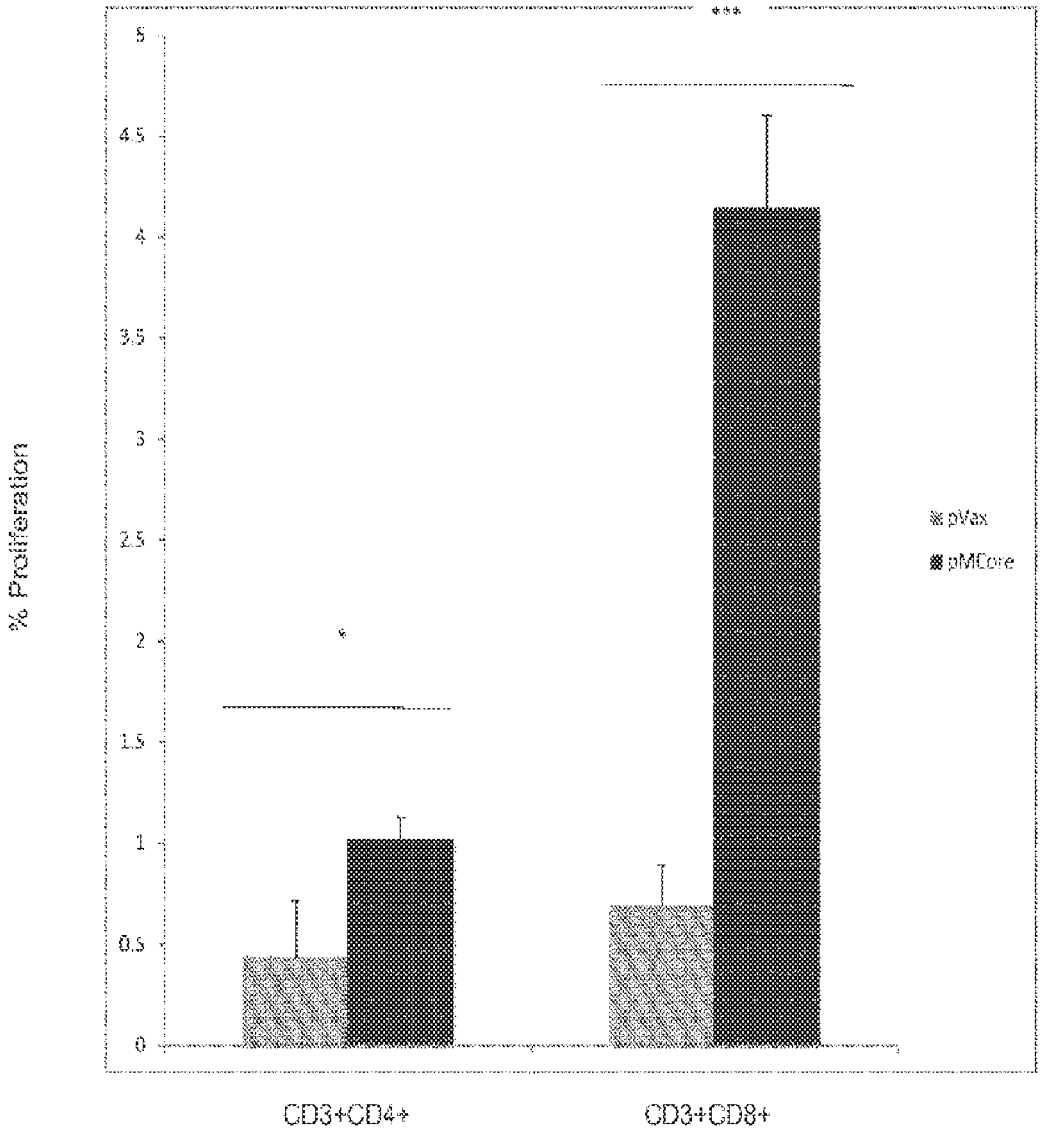

% Proliferation
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
*
***
pVax
pMCore
CD3+CD4+
CD3+CD8+

FIGURE 35

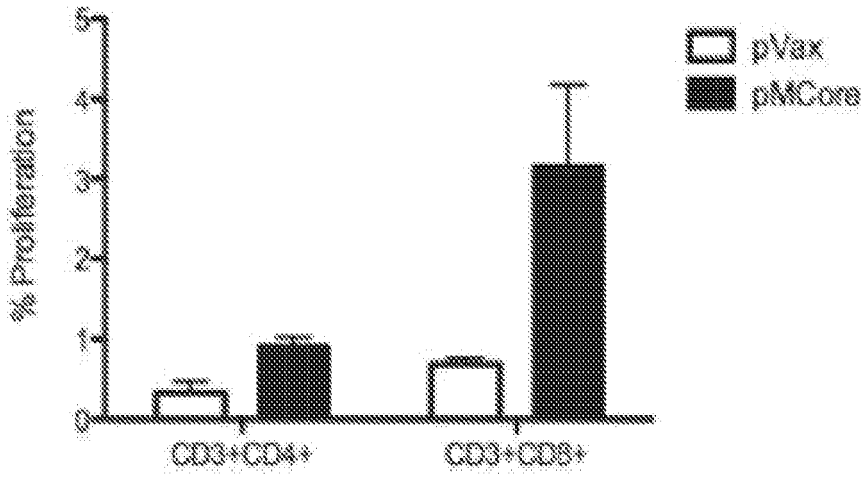


FIGURE 36

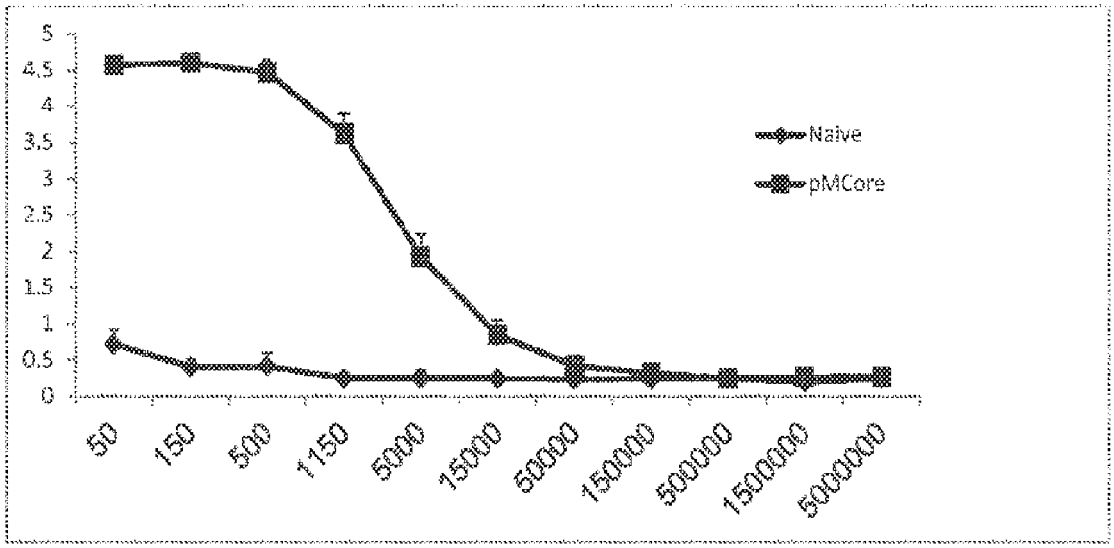


FIGURE 37A

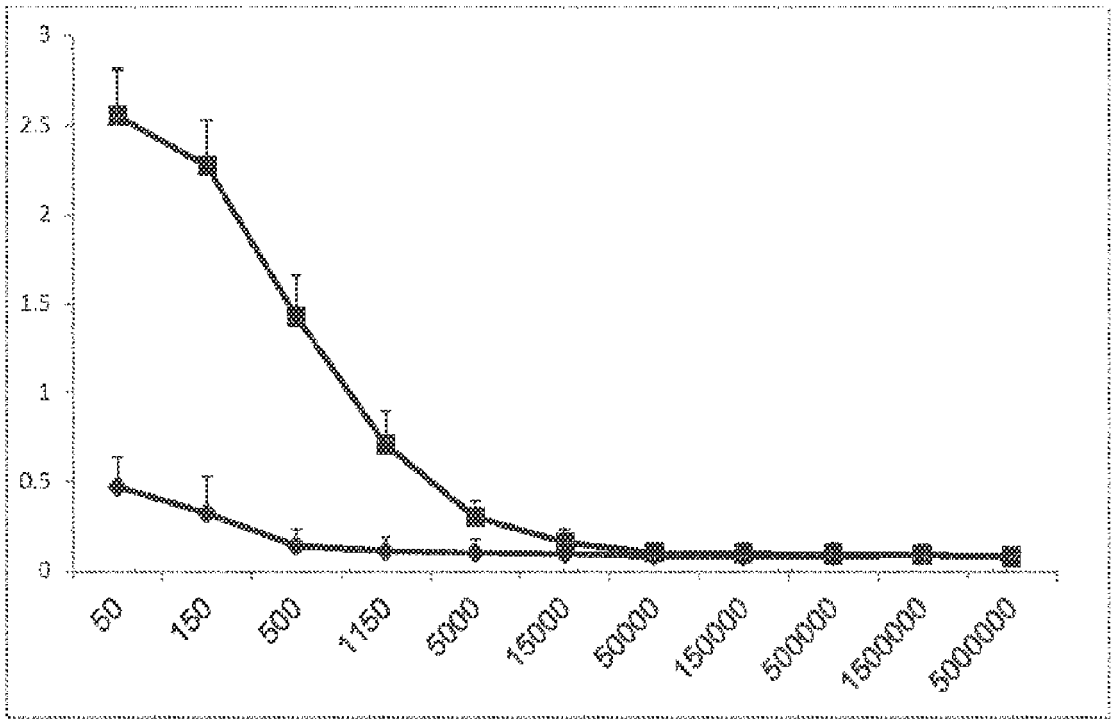


FIGURE 37B

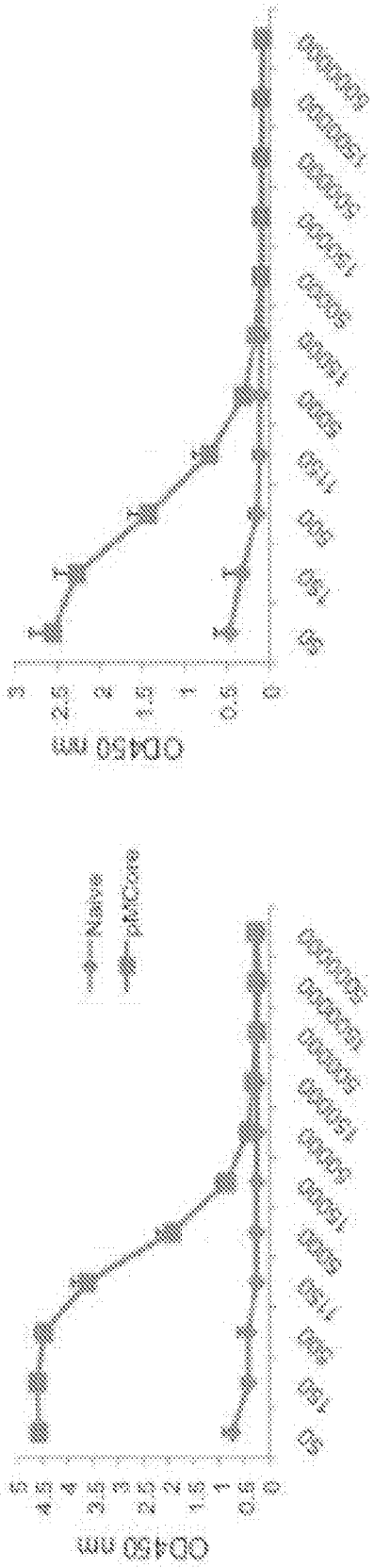


FIGURE 38

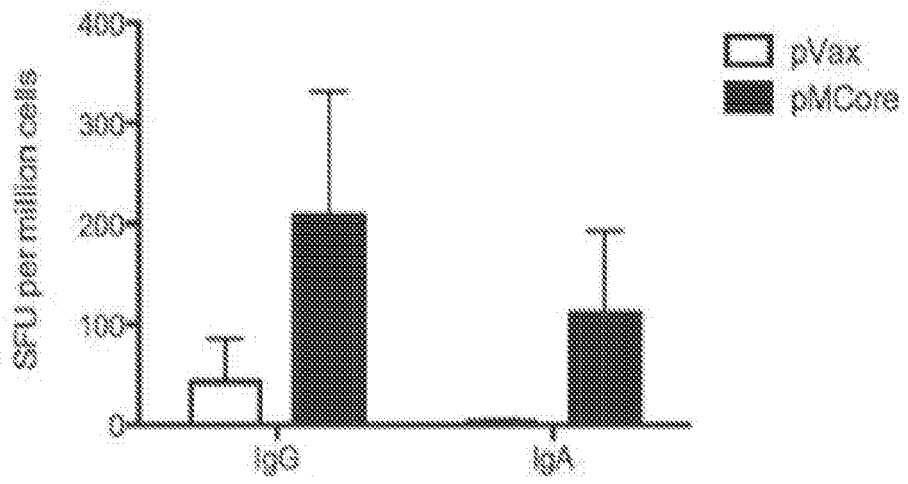


FIGURE 39

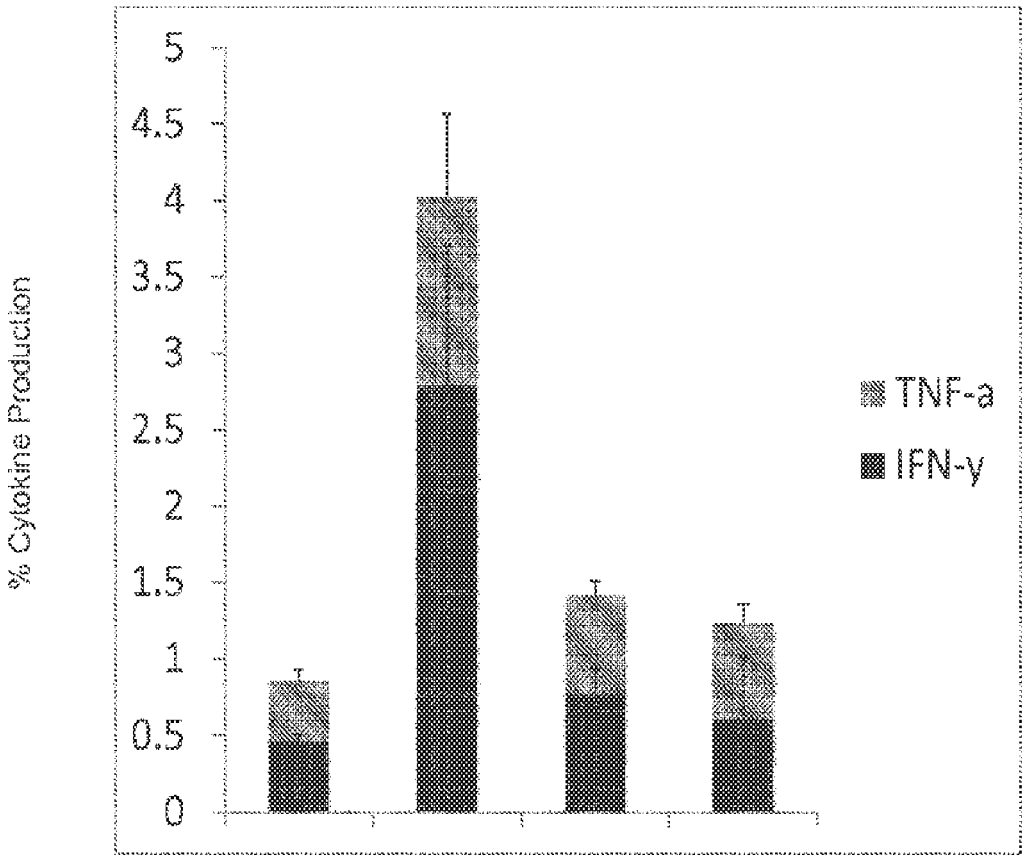


FIGURE 40

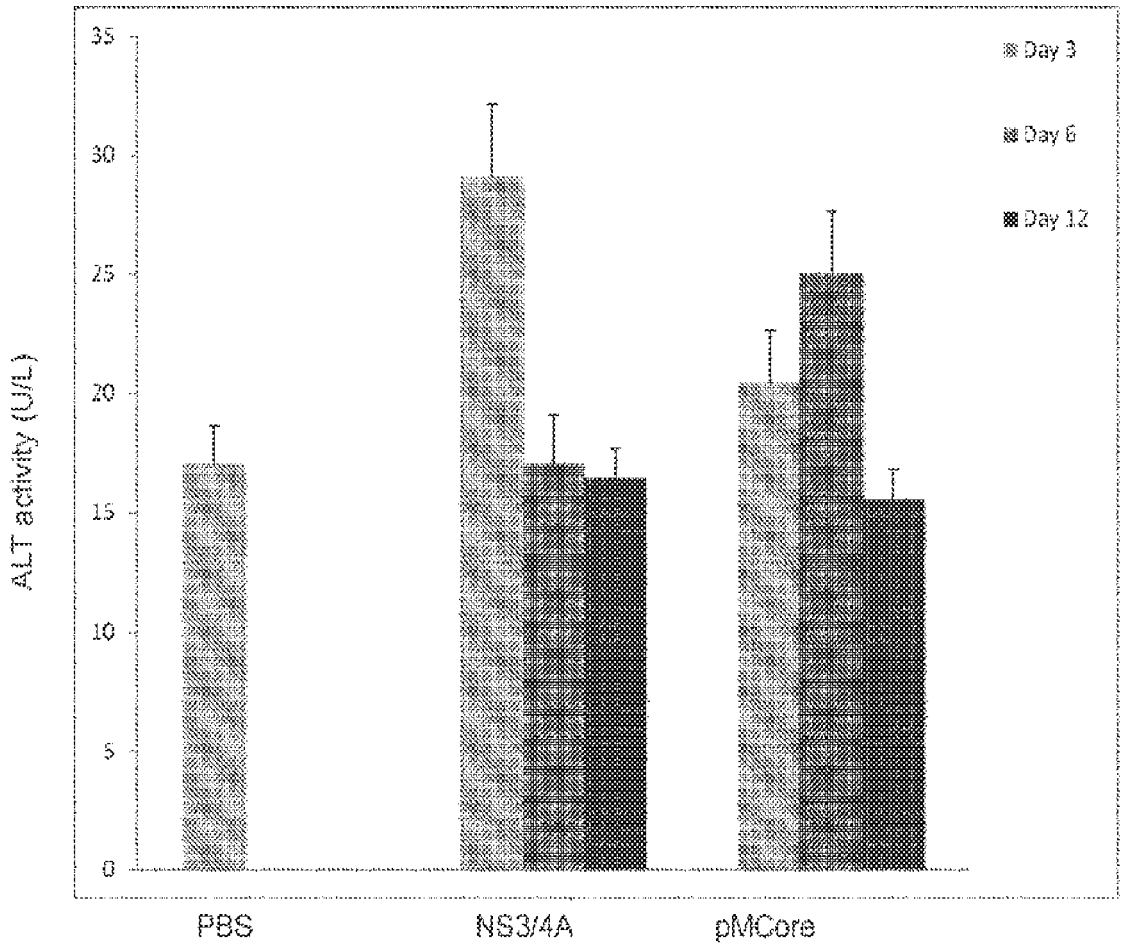

ALT activity (U/L)
35
30
25
20
15
10
5
0
Day 3
Day 6
Day 12
PBS
NS3/4A
pMCore

FIGURE 41

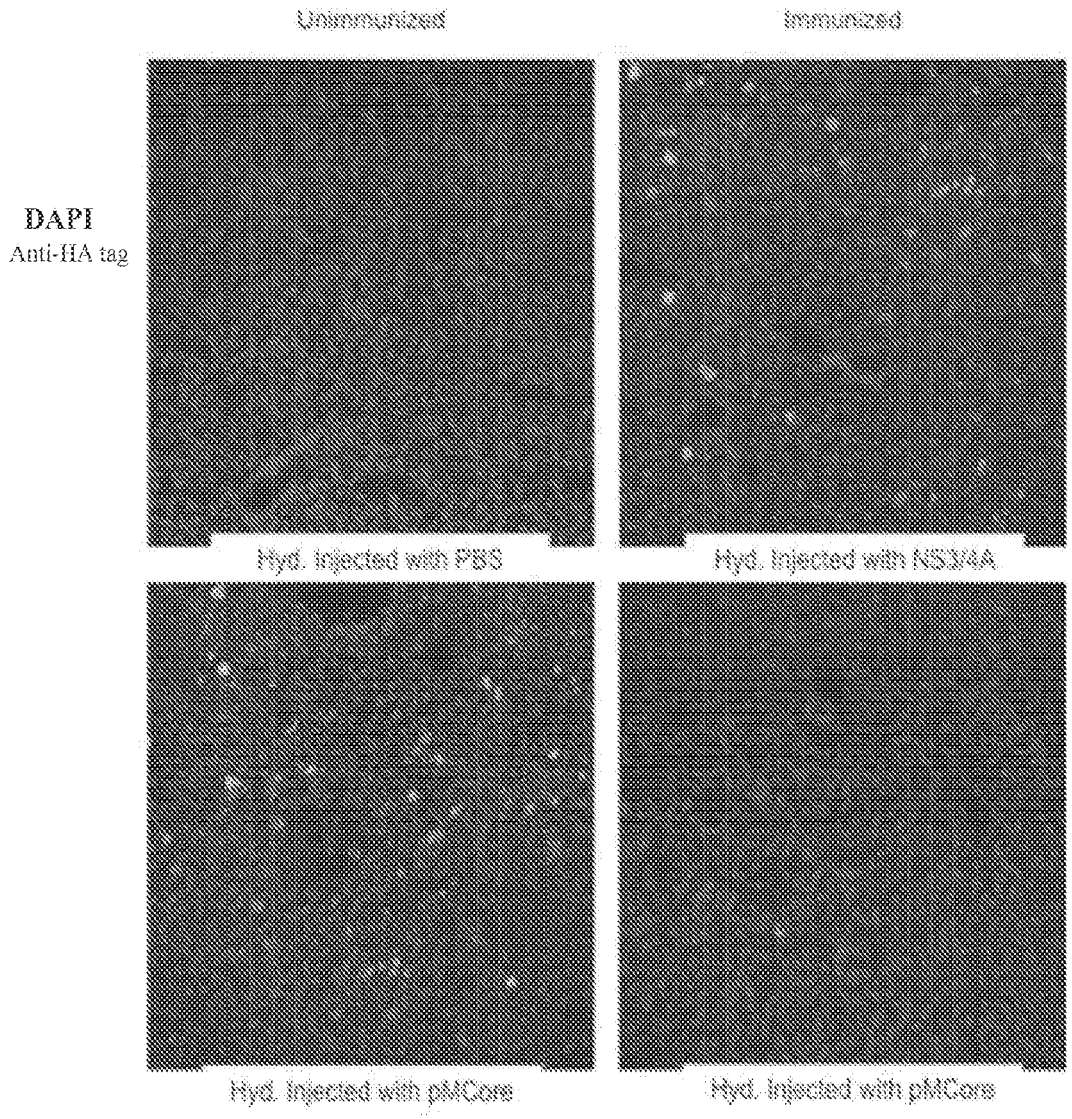

Unimmunized
Immunized
DAPI
Anti-HA tag
Hyd. injected with PBS
Hyd. injected with NS3/4A
Hyd. injected with pMCore
Hyd. injected with pMCore

FIGURE 42

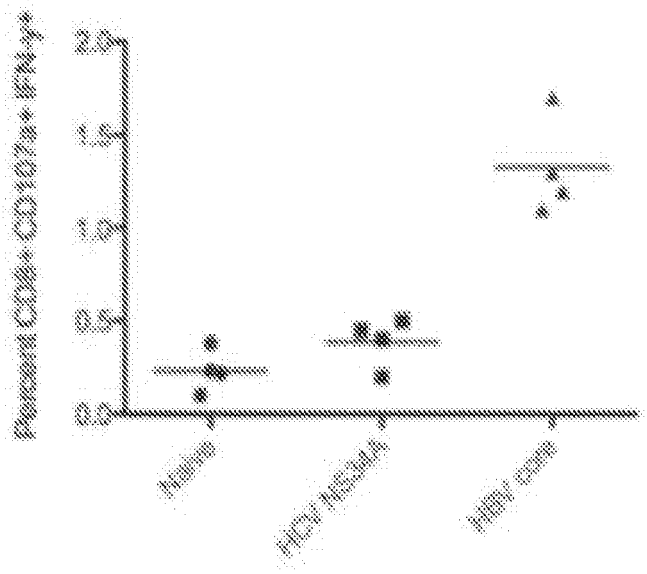


FIGURE 43

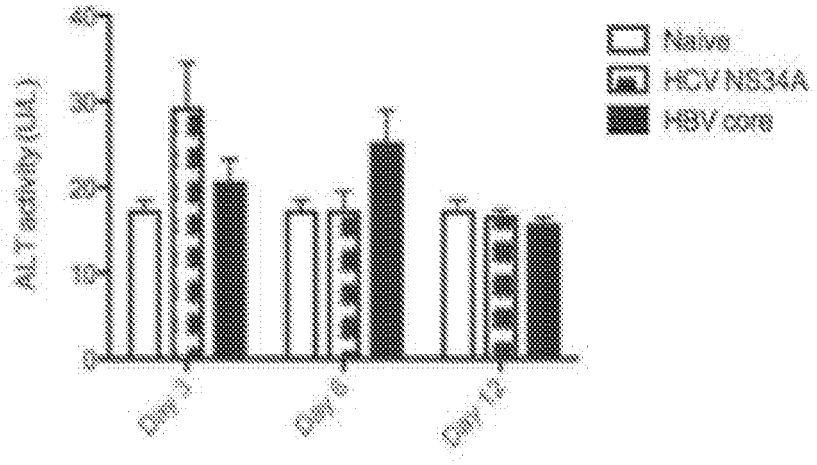


FIGURE 44

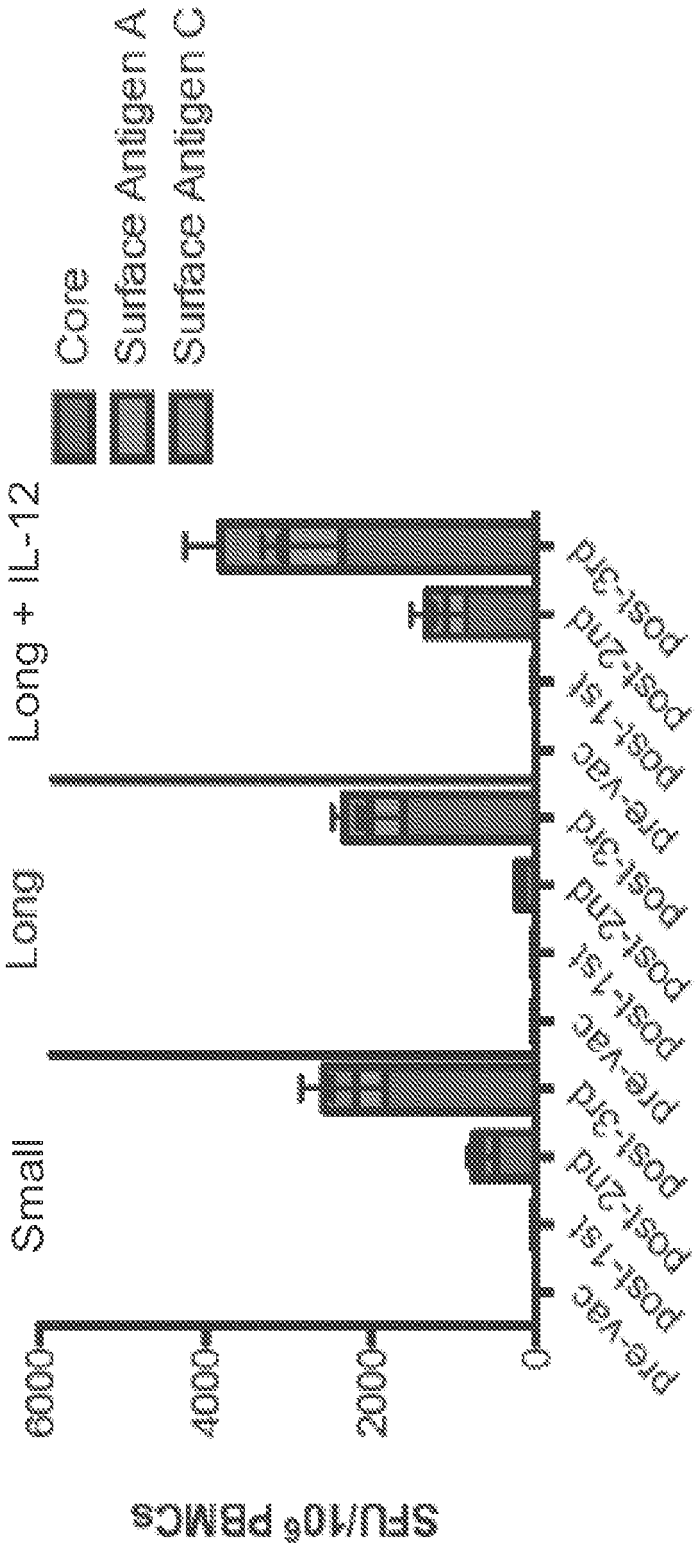

Core
Surface Antigen A
Surface Antigen C
Long + IL-12
Long
Small
SFU/10^6 PBMCs
6000
4000
2000
0
pre-vac
post-1st
post-2nd
post-3rd
pre-vac
post-1st
post-2nd
post-3rd
pre-vac
post-1st
post-2nd
post-3rd
pre-vac
post-1st
post-2nd
post-3rd

FIGURE 45

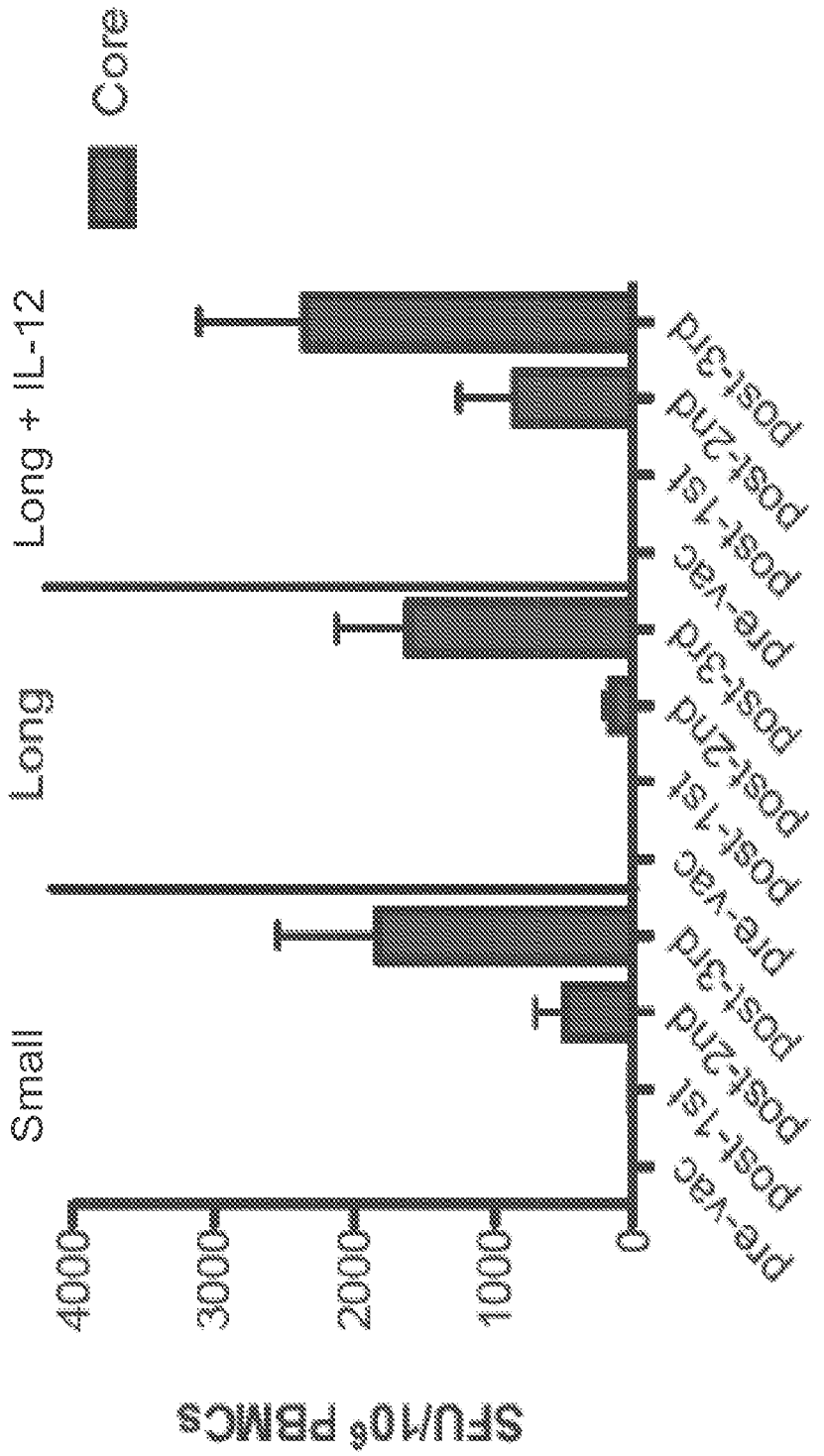

SFU/10^6 PBMCs
4000
3000
2000
1000
0
Small
Long
Long + IL-12
Core
pre-vac
post-1st
post-2nd
post-3rd

FIGURE 46

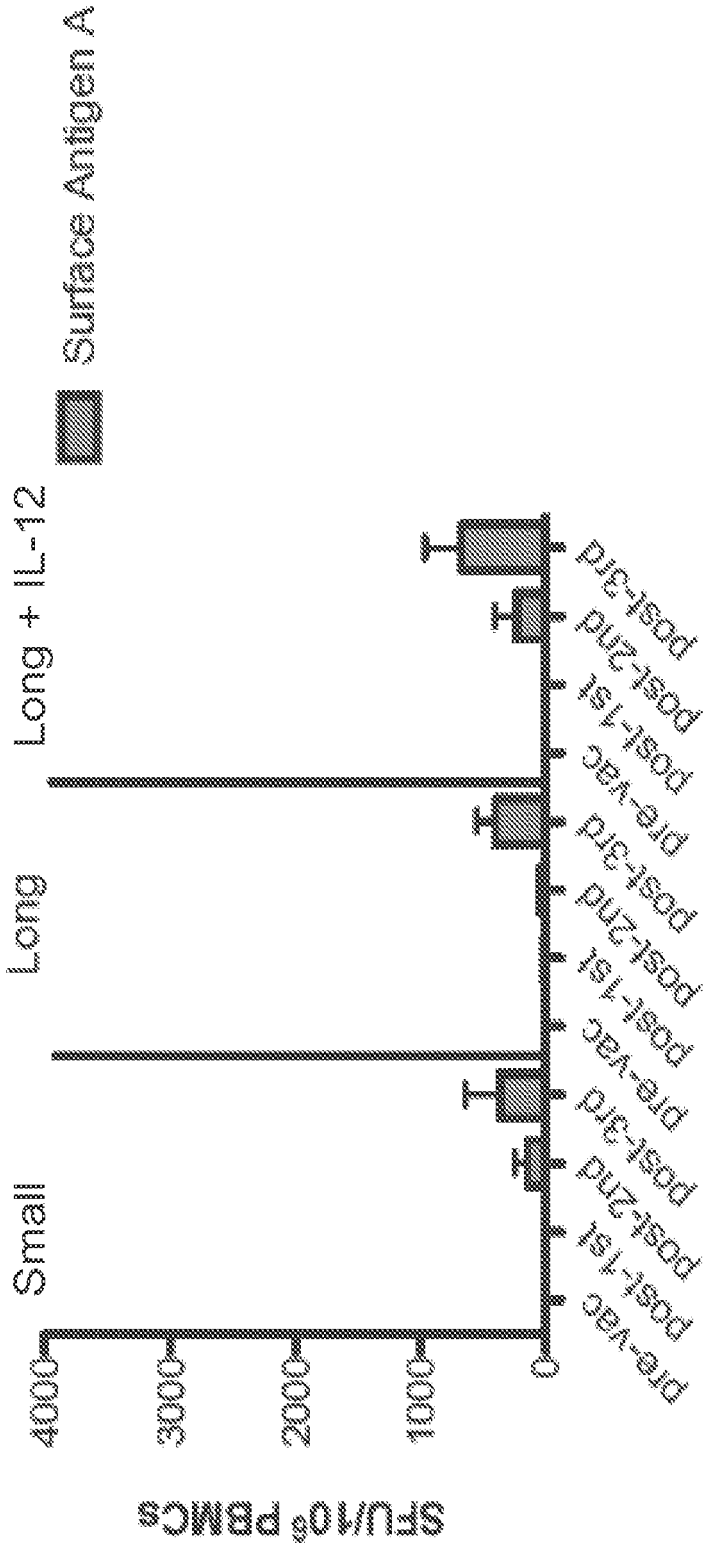

Surface Antigen A
Long + IL-12
Long
Small
SFU/10^6 PBMCs
4000
3000
2000
1000
0
Pre-Vac
Post-1st
Post-2nd
Post-3rd
Pre-Vac
Post-1st
Post-2nd
Post-3rd
Pre-Vac
Post-1st
Post-2nd
Post-3rd

FIGURE 47

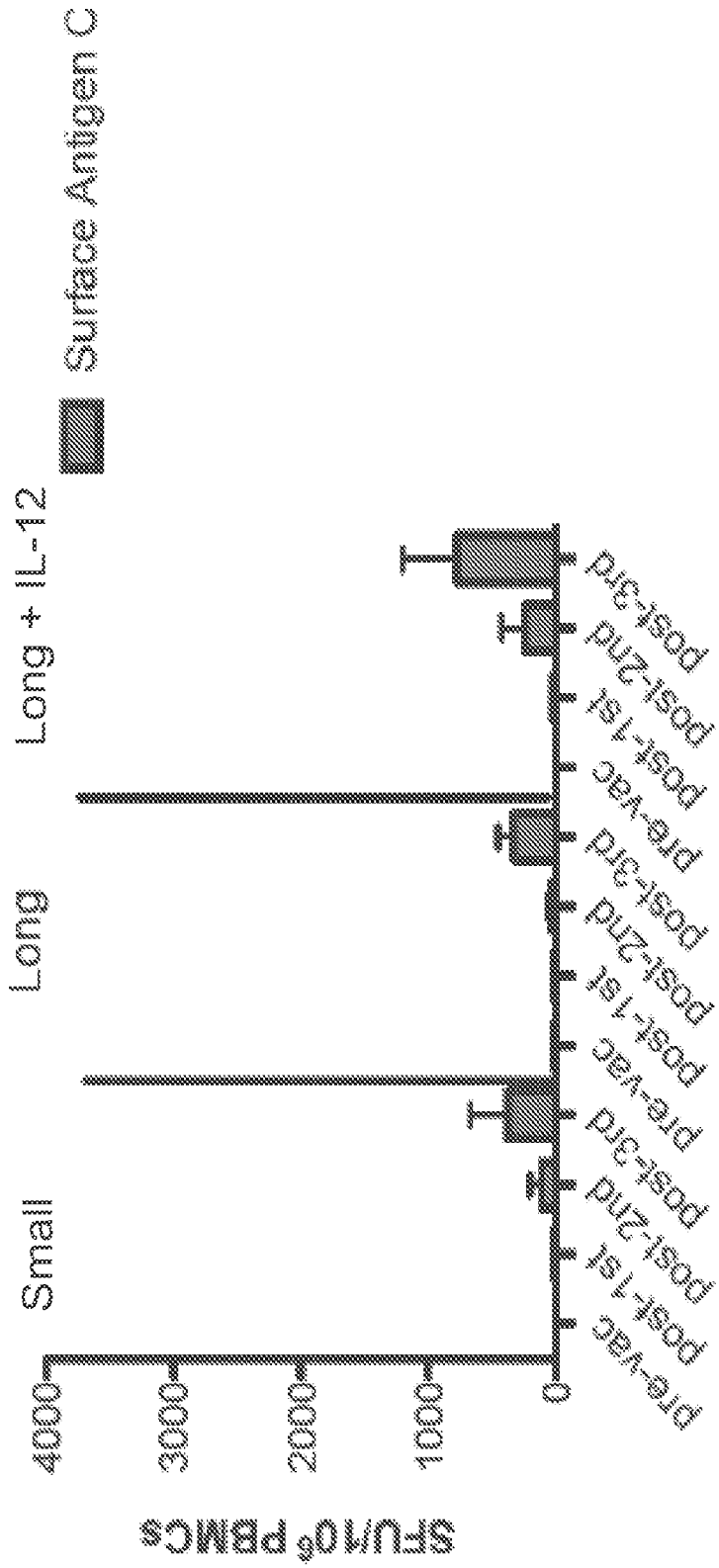

Surface Antigen C
Long + IL-12
Long
Small
SFU/10^6 PBMCs
4000
3000
2000
1000
0
Pre-Vac
Post-1st
Post-2nd
Post-3rd
Pre-Vac
Post-1st
Post-2nd
Post-3rd
Pre-Vac
Post-1st
Post-2nd
Post-3rd

FIGURE 48

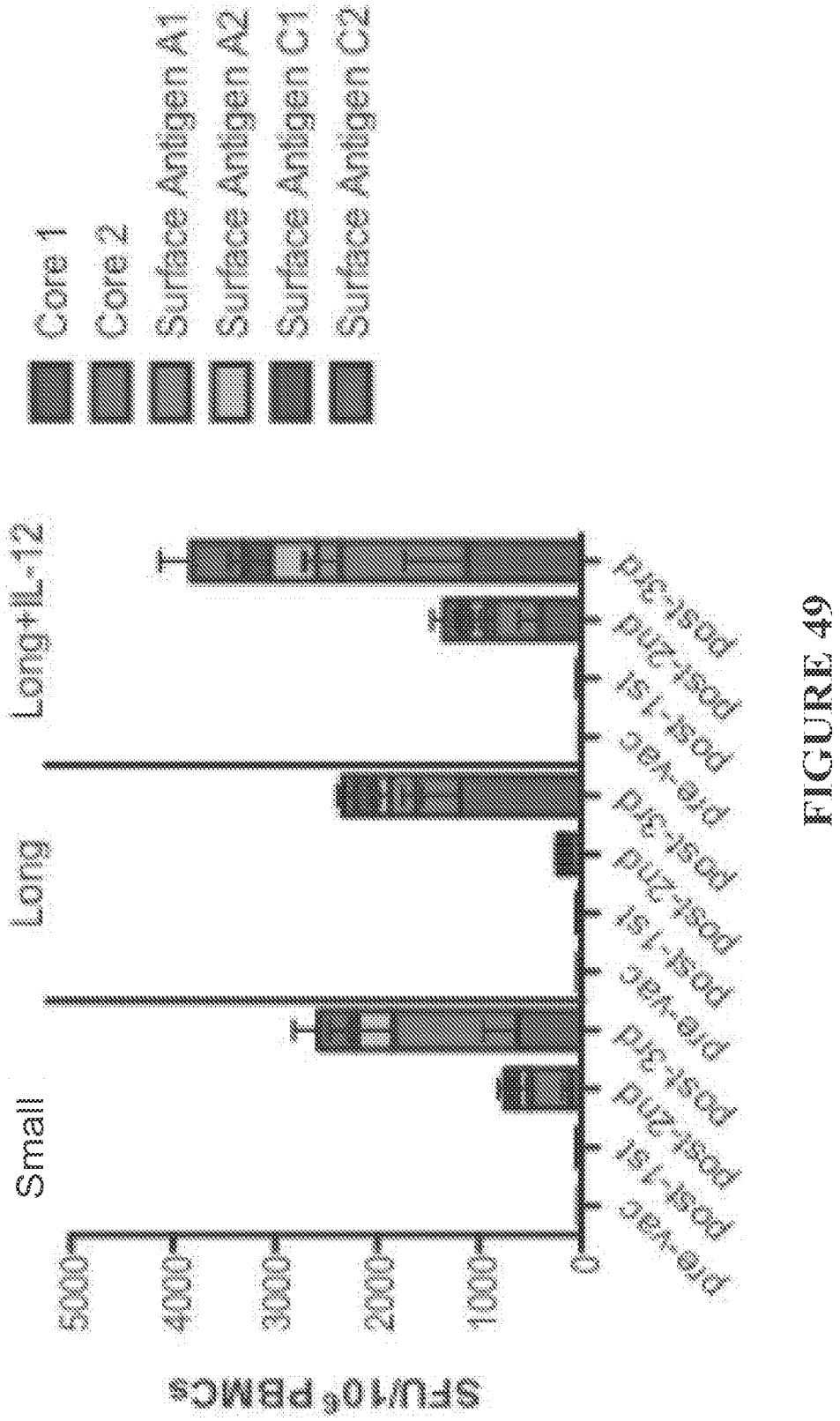

Core 1
Core 2
Surface Antigen A1
Surface Antigen A2
Surface Antigen C1
Surface Antigen C2
Long+IL-12
Long
Small
5000
4000
3000
2000
1000
0
SFU/10^6 PBMCs
pre-vac
pvz-150d
191-150d
pvc-150d
pre-vac
pvz-150d
191-150d
pvc-150d
pre-vac
pvz-150d
191-150d
pvc-150d

FIGURE 49

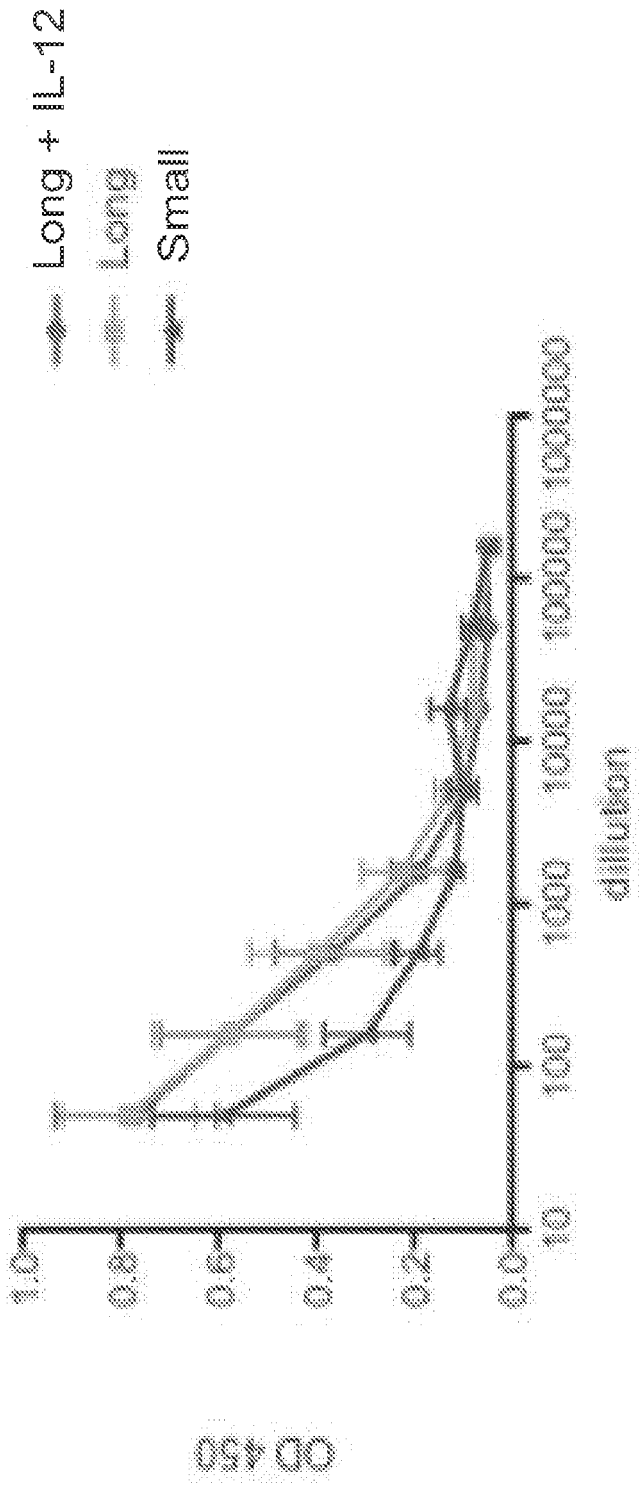

Long + IL-12
Long
Small
post 3rd OD
OD 450
1.0
0.8
0.6
0.4
0.2
0.0
10
100
1000
10000
100000
1000000
dilution

FIGURE 50

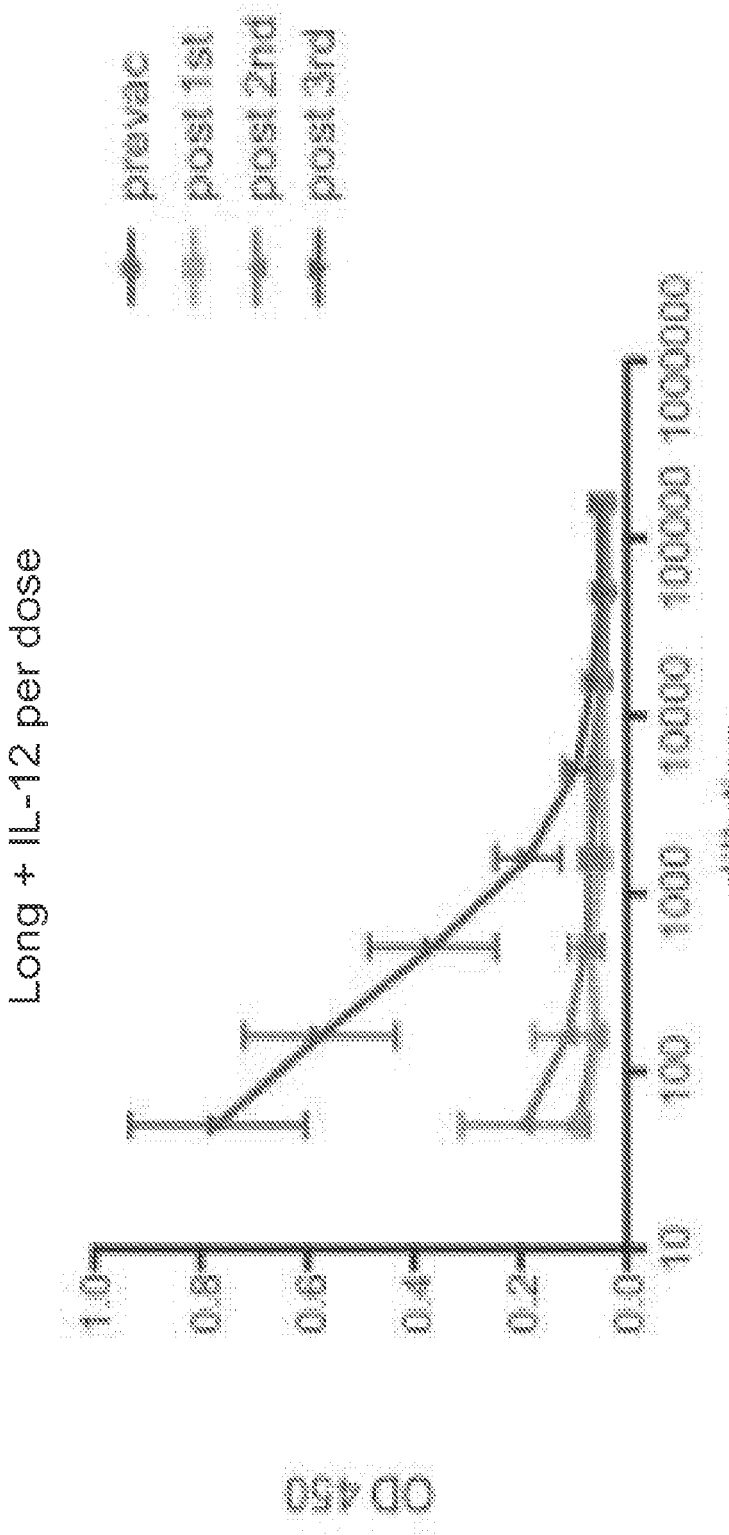

Long + IL-12 per dose
prevac
post 1st
post 2nd
post 3rd
OD 450
1.0
0.8
0.6
0.4
0.2
0.0
10
100
1000
10000
100000
1000000
dilution

FIGURE 51

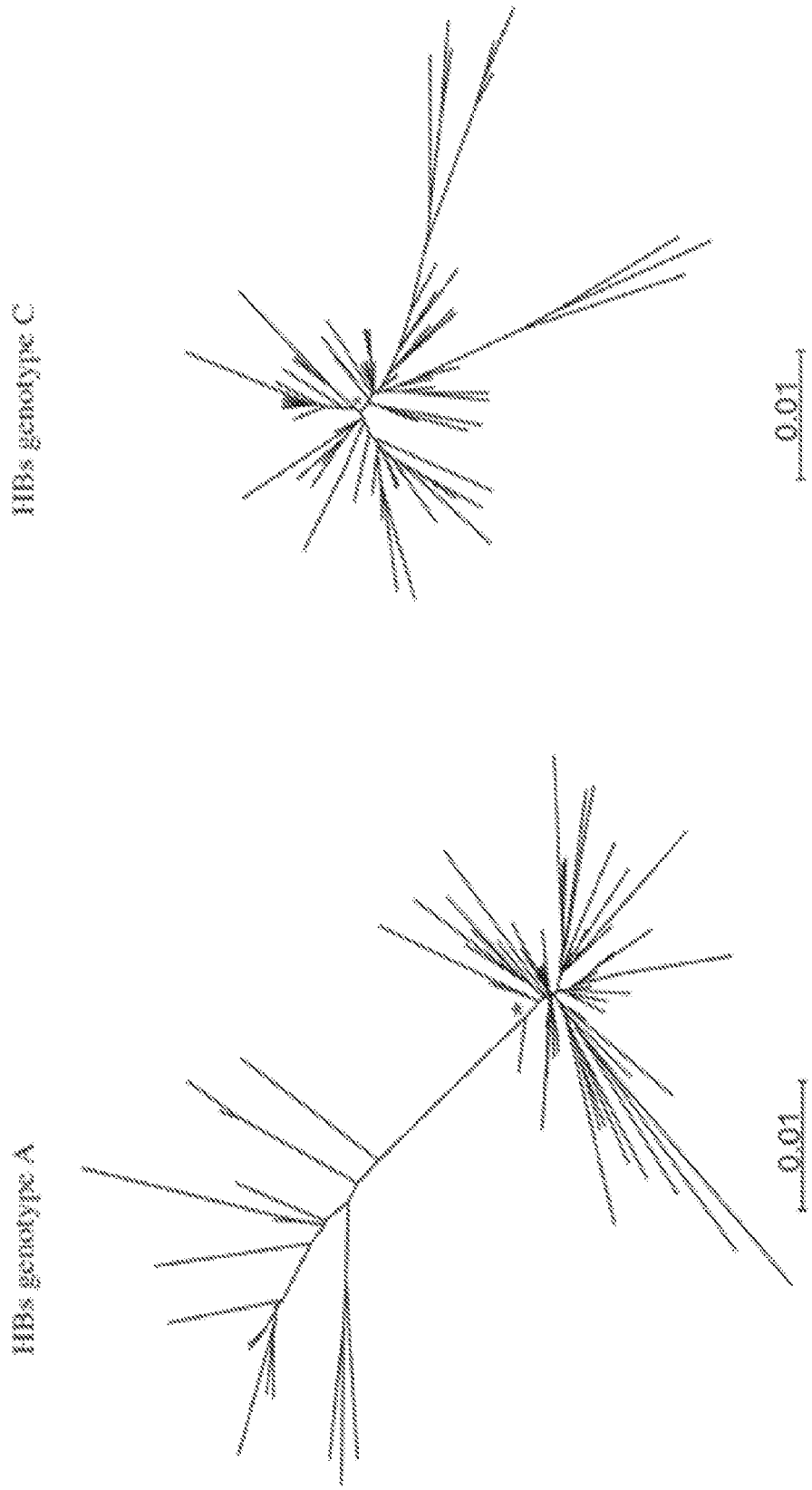


Figure 52A

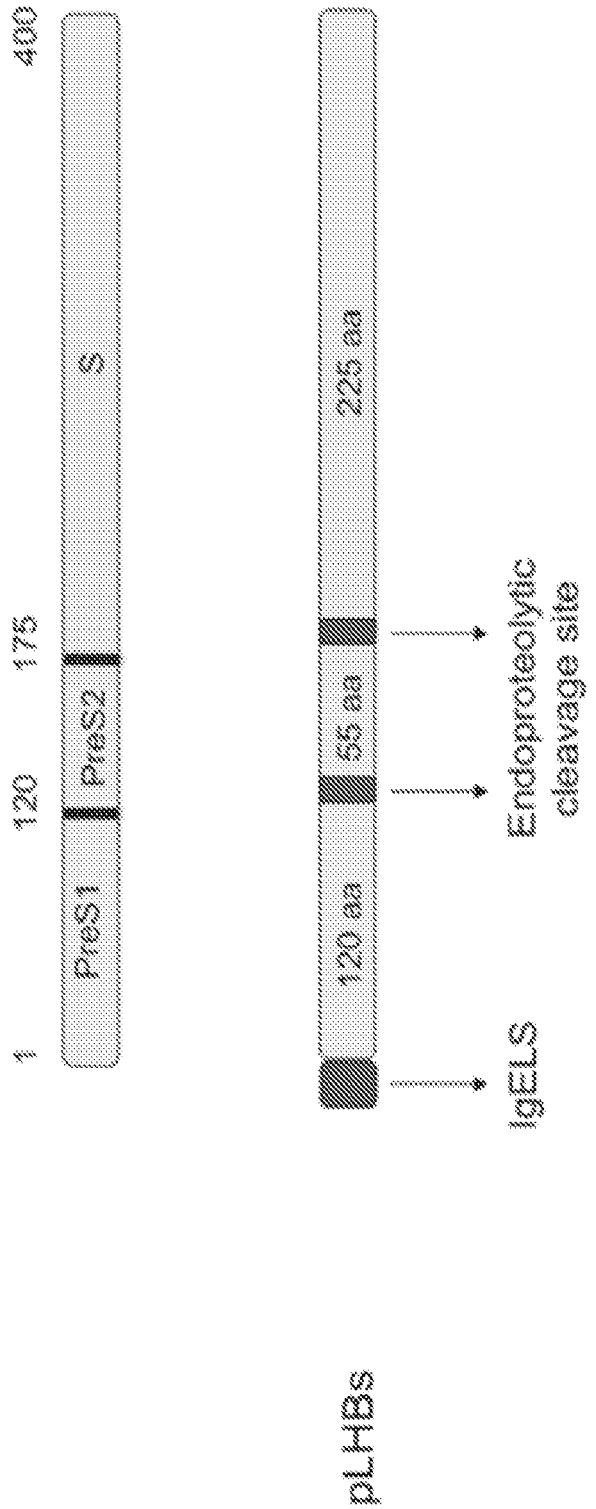

1
120
175
400
PreS1
PreS2
S
120 aa
55 aa
225 aa
IgELS
Endoproteolytic cleavage site
225 aa
pLHBs
pSHBs

Figure 52B

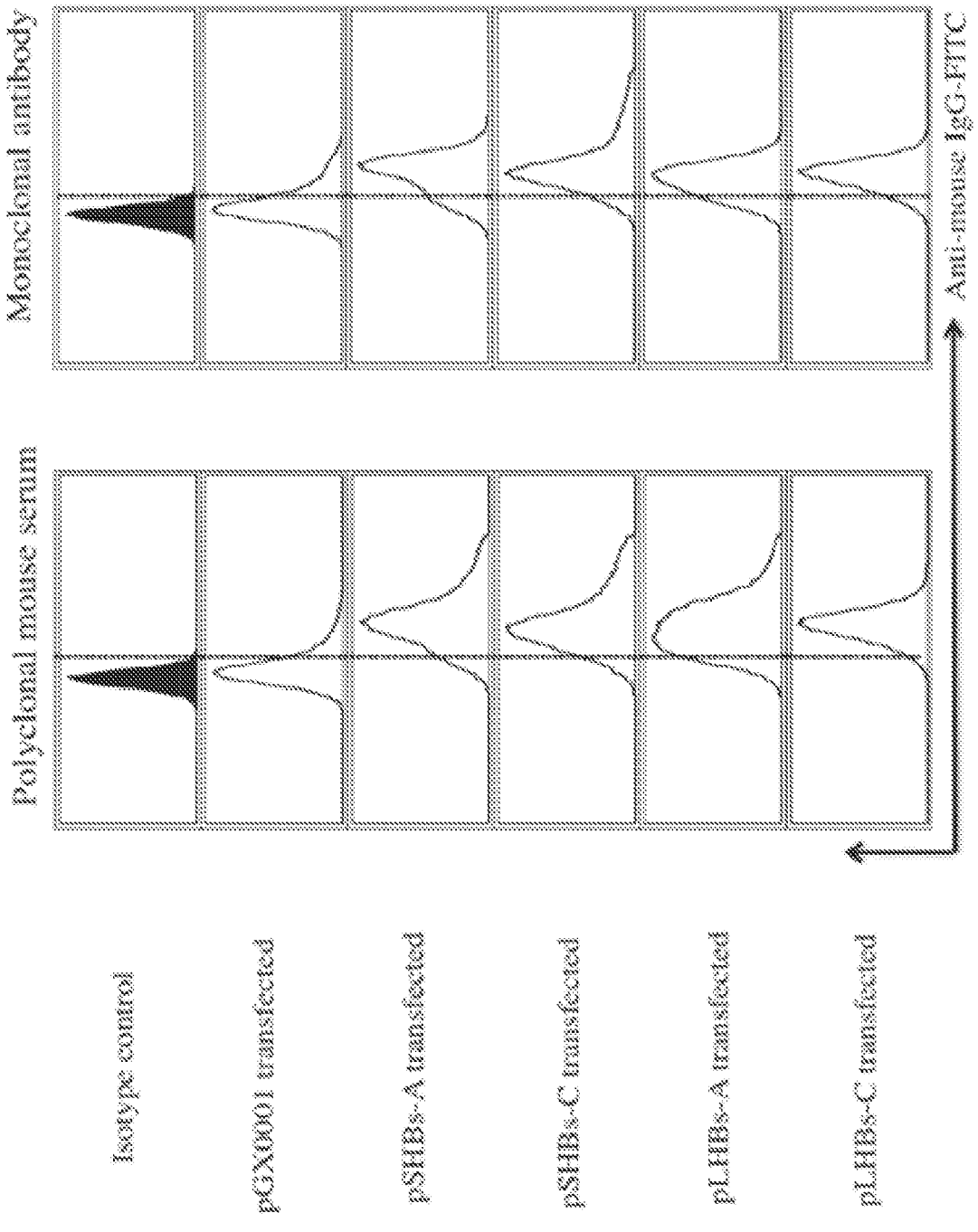

Monoclonal antibody
Polyclonal mouse serum
Anti-mouse IgG-FITC
Isotype control
pGX8001 transfected
pSHBs-A transfected
pSHBs-C transfected
pLHBs-A transfected
pLHBs-C transfected

Figure 52C

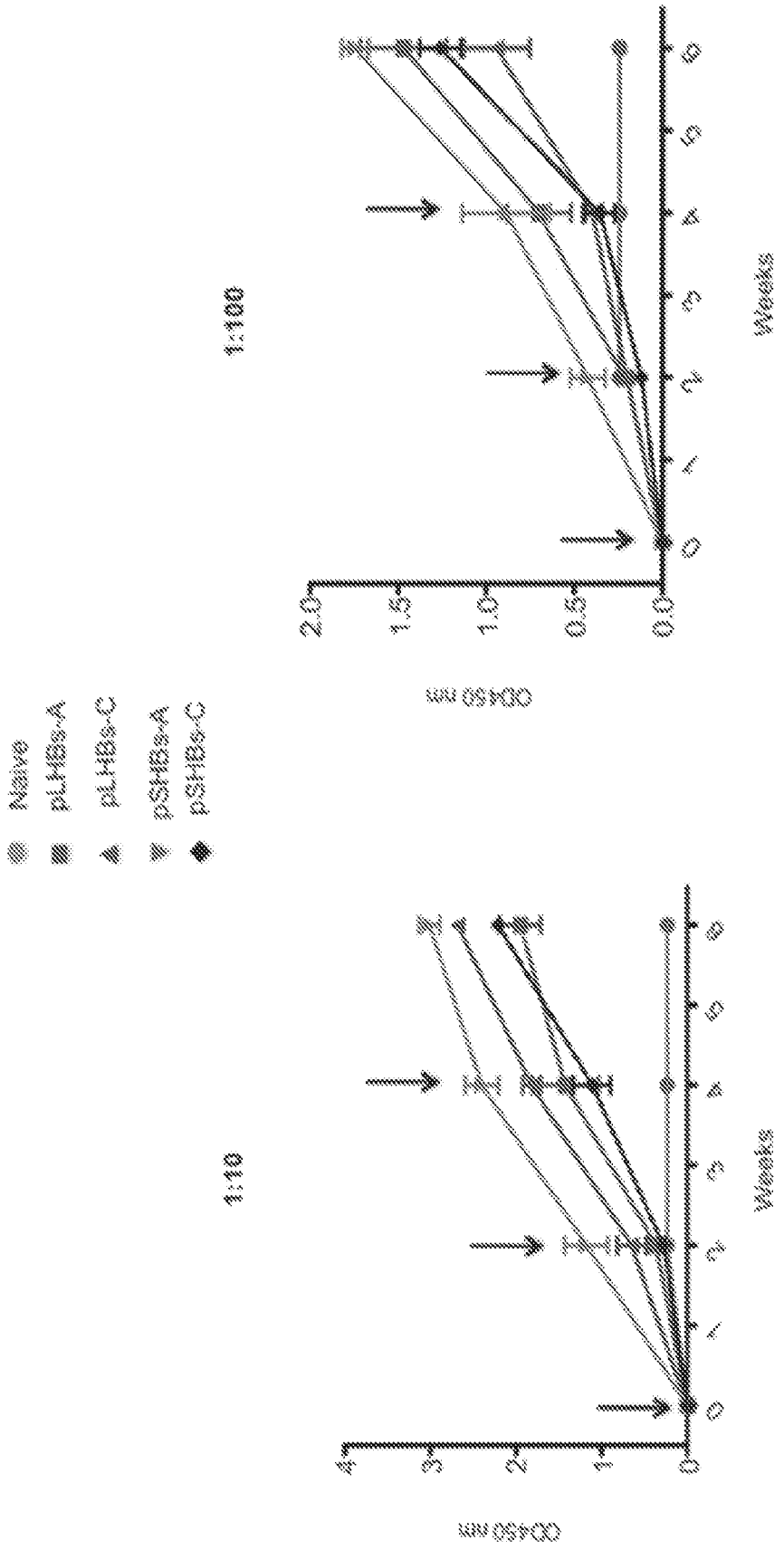

Naive
pLHBs-A
pLHBs-C
pSHBs-A
pSHBs-C
1:100
1:10
OD450 nm
Weeks
0.0
0.5
1.0
1.5
2.0
0
1
2
3
4
0
1
2
4
5
6

Figure 53A

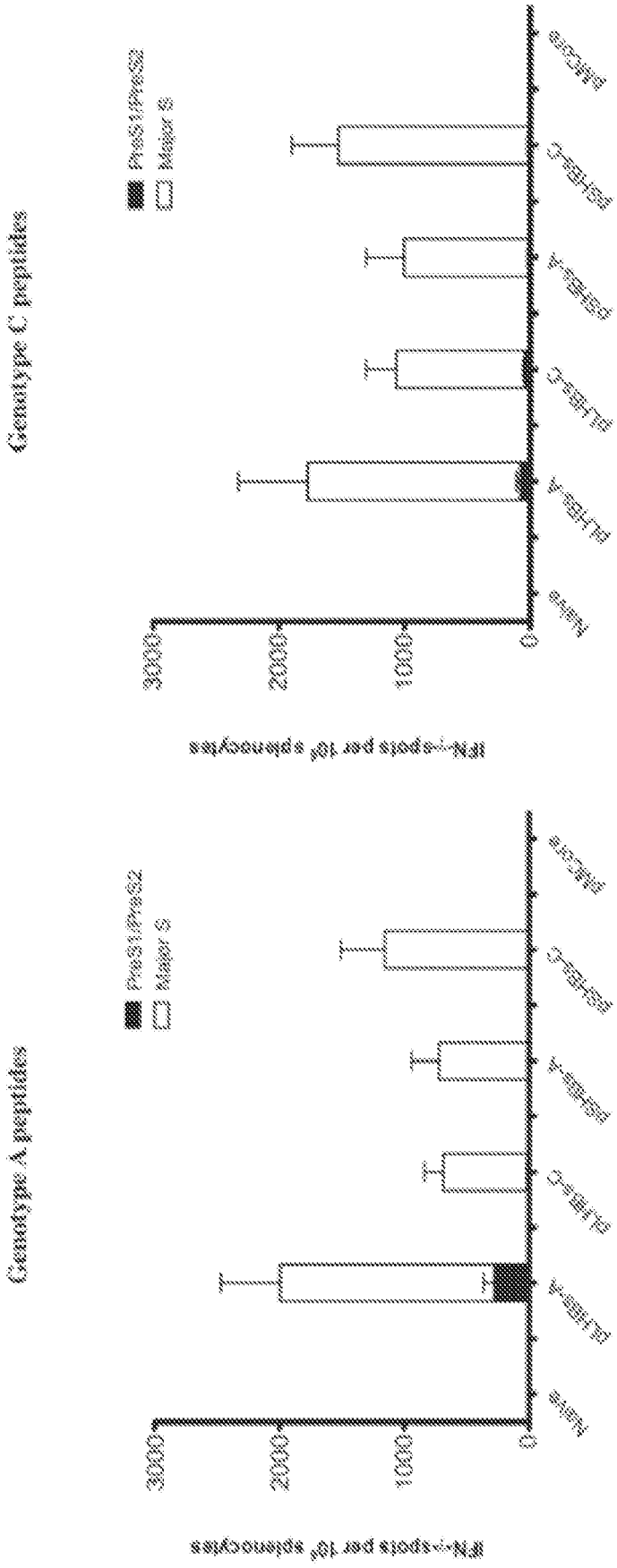

Genotype C peptides
PreS1/PreS2
Major S
IFN-γ spots per 10⁶ splenocytes
3000
2000
1000
0
Naïve
HBsAg-A
HBsAg-B
HBsAg-C
HBsAg-D
HBsAg-E
Genotype A peptides
PreS1/PreS2
Major S
IFN-γ spots per 10⁶ splenocytes
3000
2000
1000
0
Naïve
HBsAg-A
HBsAg-B
HBsAg-C
HBsAg-D
HBsAg-E

Figure 53B

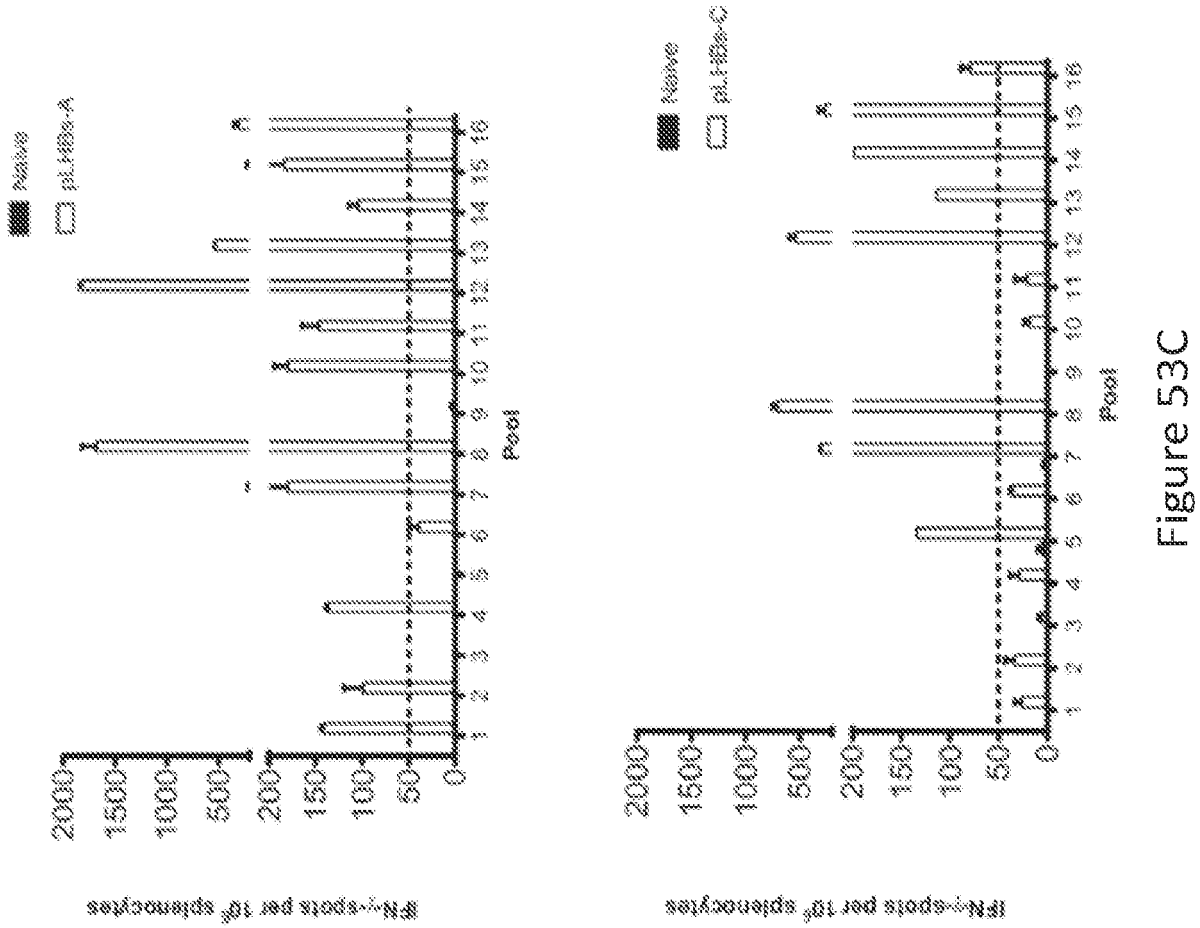

Naive
pLHBs-A
pLHBs-C
IFN-γ-spots per 10^6 splenocytes
Pool
2000
1500
1000
500
200
150
100
50
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16

Figure 53C

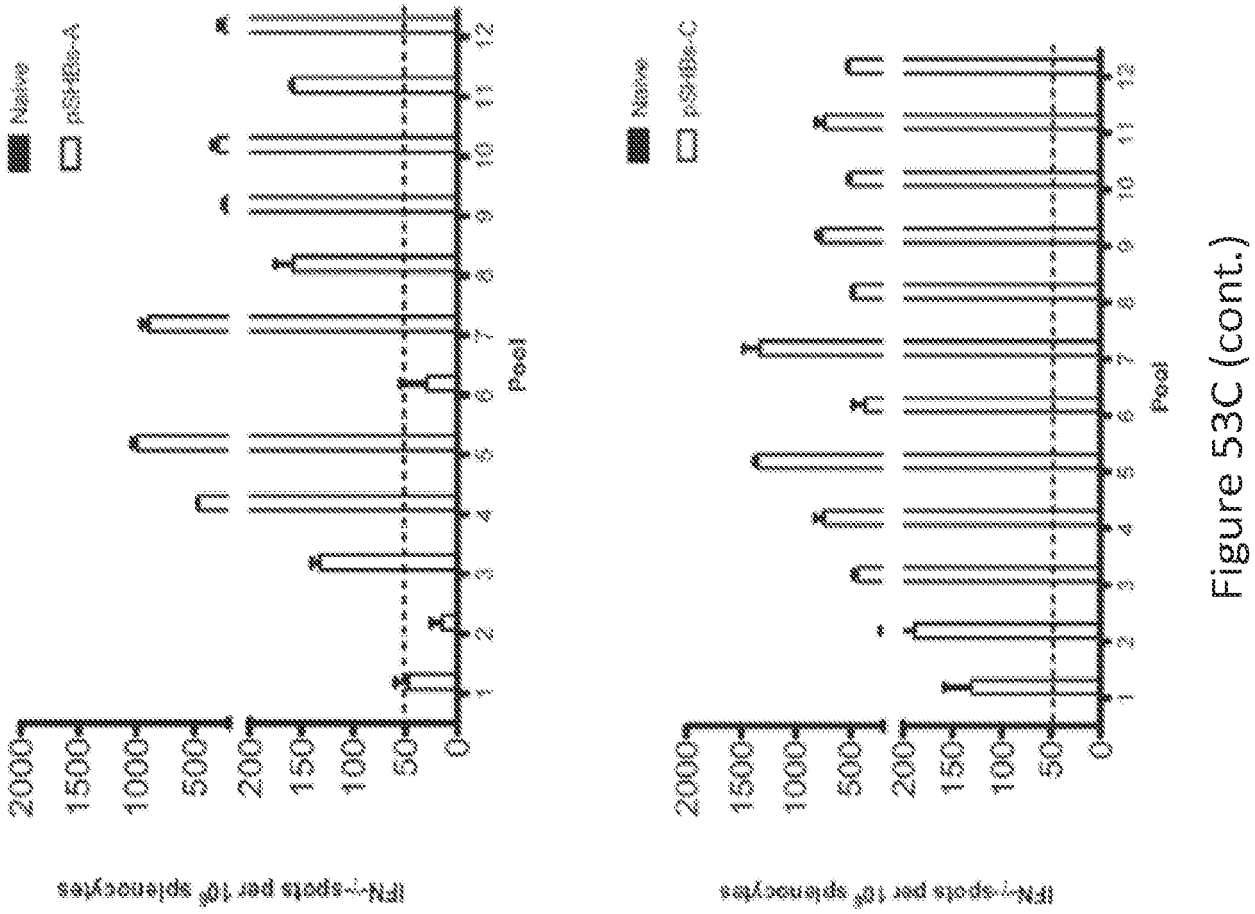


Figure 53C (cont.)

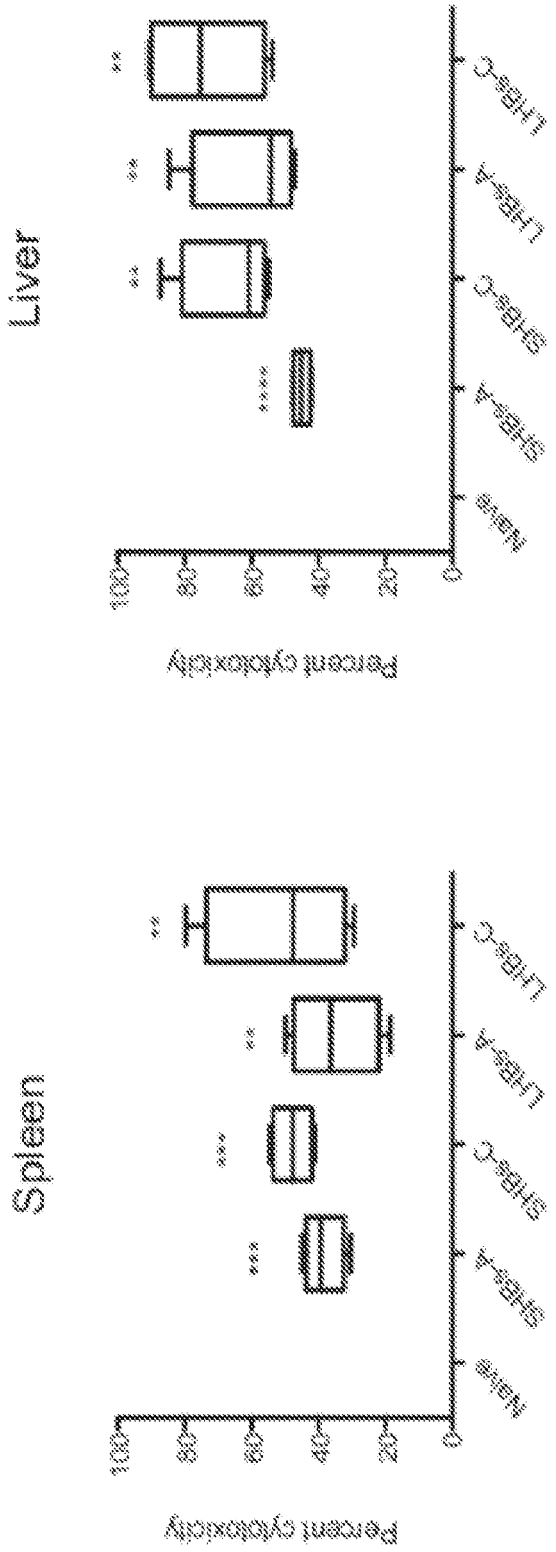

Liver
Spleen
Percent cytotoxicity
100
80
60
40
20
0
None
Sphk1-A
Sphk2-A
Sphk1-C
Sphk2-C

Figure 54

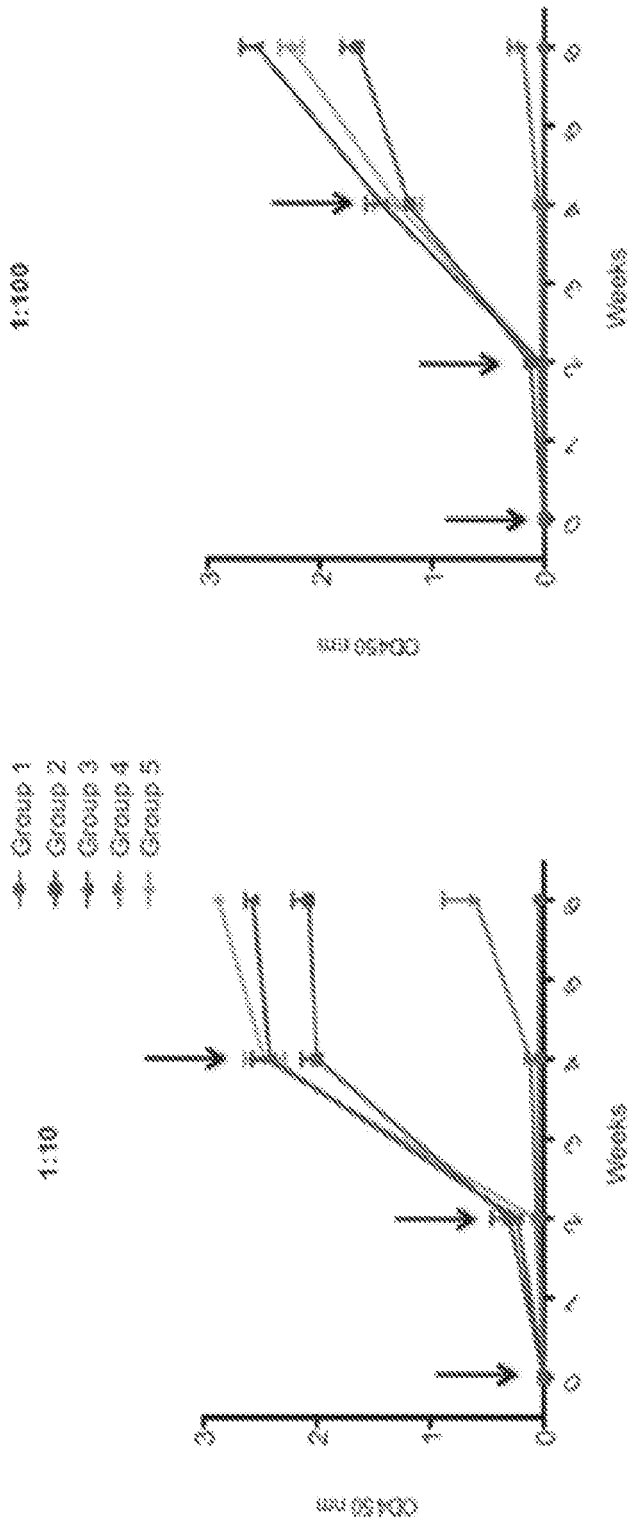

1:100
1:10
Group 1
Group 2
Group 3
Group 4
Group 5
Weeks
0
1
2
3
4
6
8
10

Figure 55A

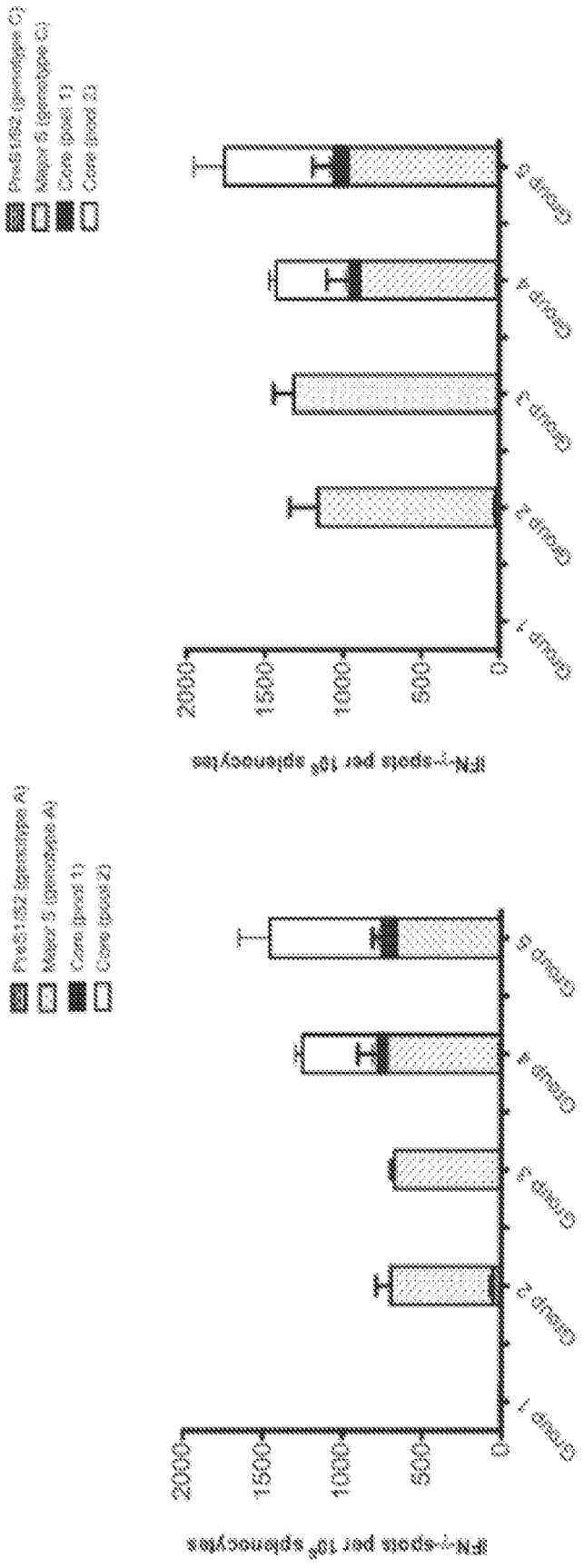

Peptide pool (group C)
Mage-b (group C)
Core (gag) (C)
Core (pol) (C)
Peptide pool (group A)
Mage-b (group A)
Core (gag) (A)
Core (pol) (A)
IFN-γ-spots per 10^6 splenocytes
2000
1500
1000
500
0
Group 1
Group 2
Group 3
Group 4
Group 5

Figure 55B

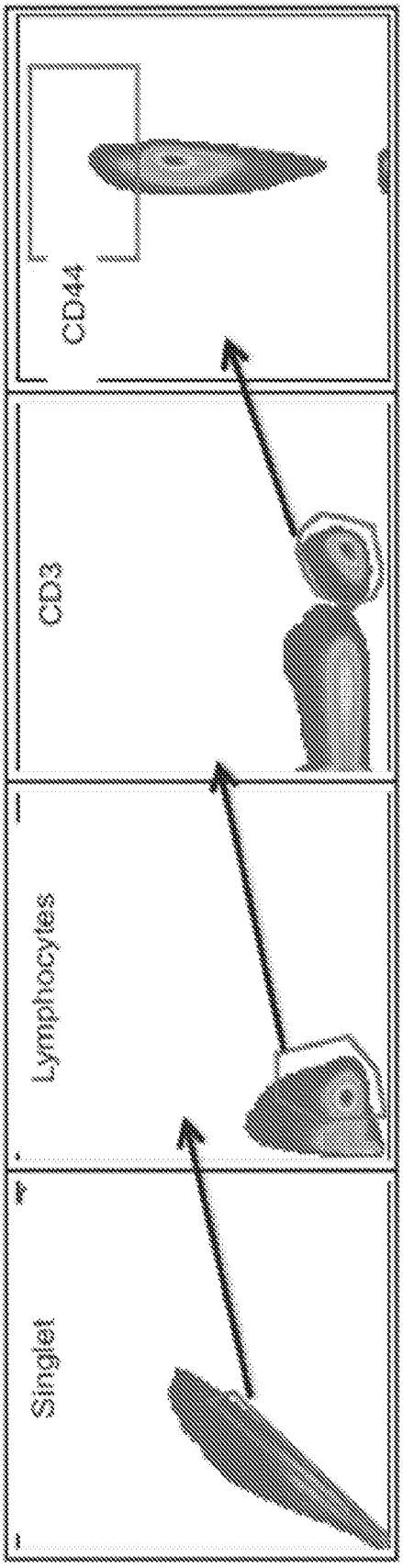


Figure 56A

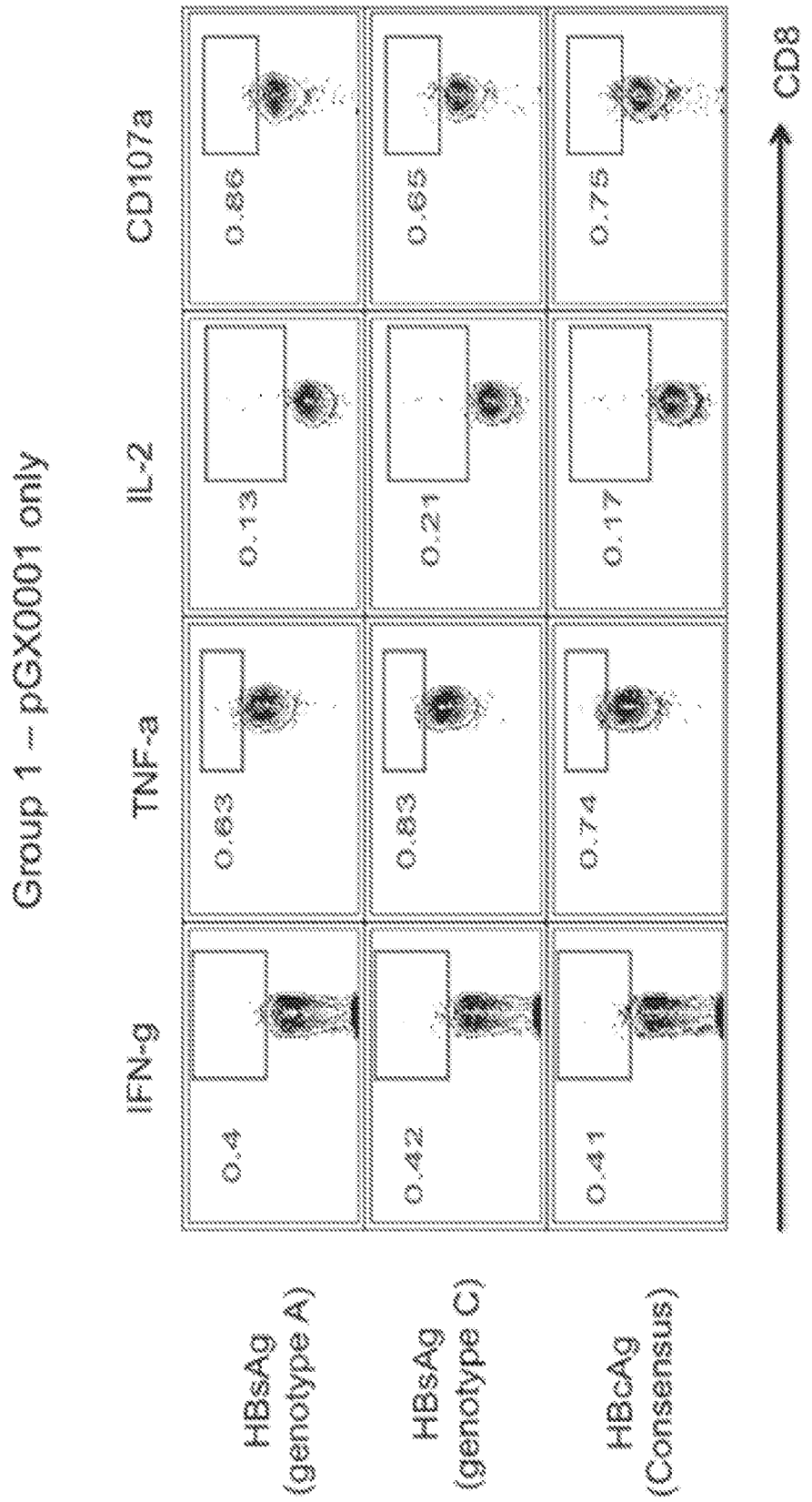

Group 1 – pGX0001 only
IFN-g
TNF-a
IL-2
CD107a
0.4
0.83
0.13
0.86
0.42
0.83
0.21
0.65
0.41
0.74
0.17
0.75
HBsAg (genotype A)
HBsAg (genotype C)
HBcAg (Consensus)
CD8

Figure 56B

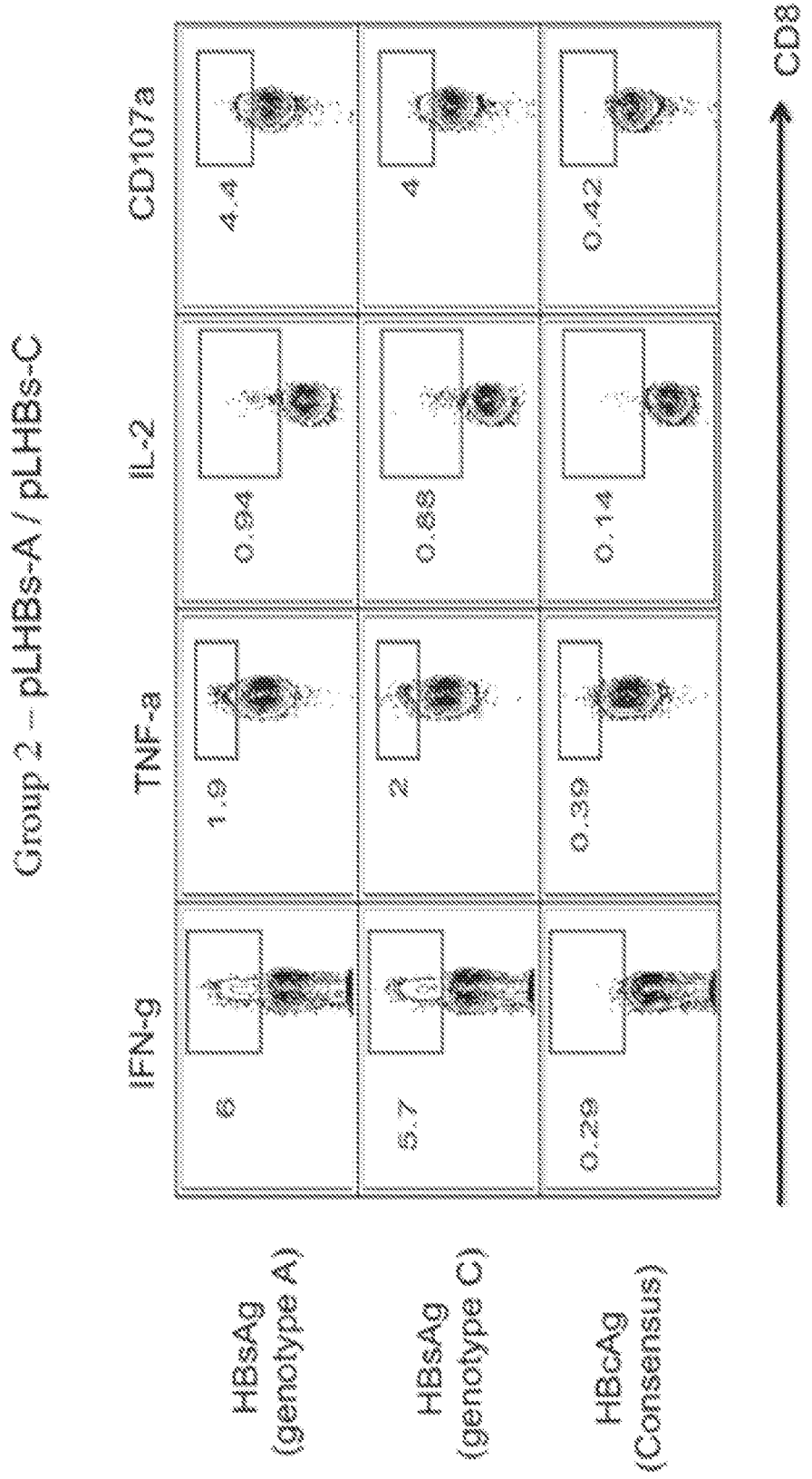

Group 2 – pLHBs-A / pLHBs-C
IFN-g
TNF-a
IL-2
CD107a
6
1.9
0.94
4.4
5.7
2
0.88
4
0.28
0.39
0.14
0.42
HBsAg (genotype A)
HBsAg (genotype C)
HBcAg (Consensus)
CD8

Figure 56C

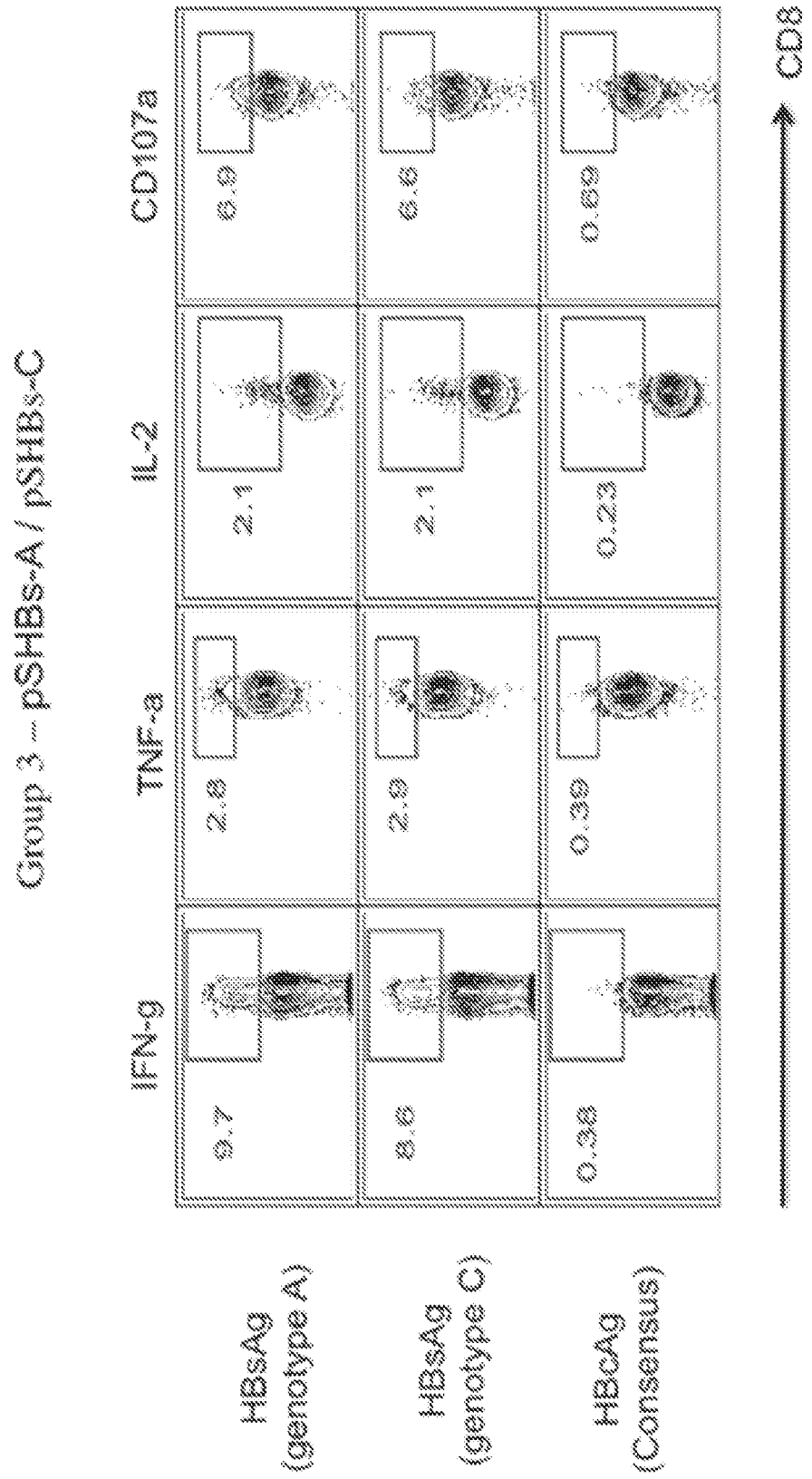

Group 3 – pSHBs-A / pSHBs-C
IFN-g
TNF-a
IL-2
CD107a
HBsAg (genotype A)
HBsAg (genotype C)
HBcAg (Consensus)
9.7
2.8
2.1
6.9
8.6
2.9
2.1
6.6
0.38
0.39
0.23
0.89
CD8

Figure 56D

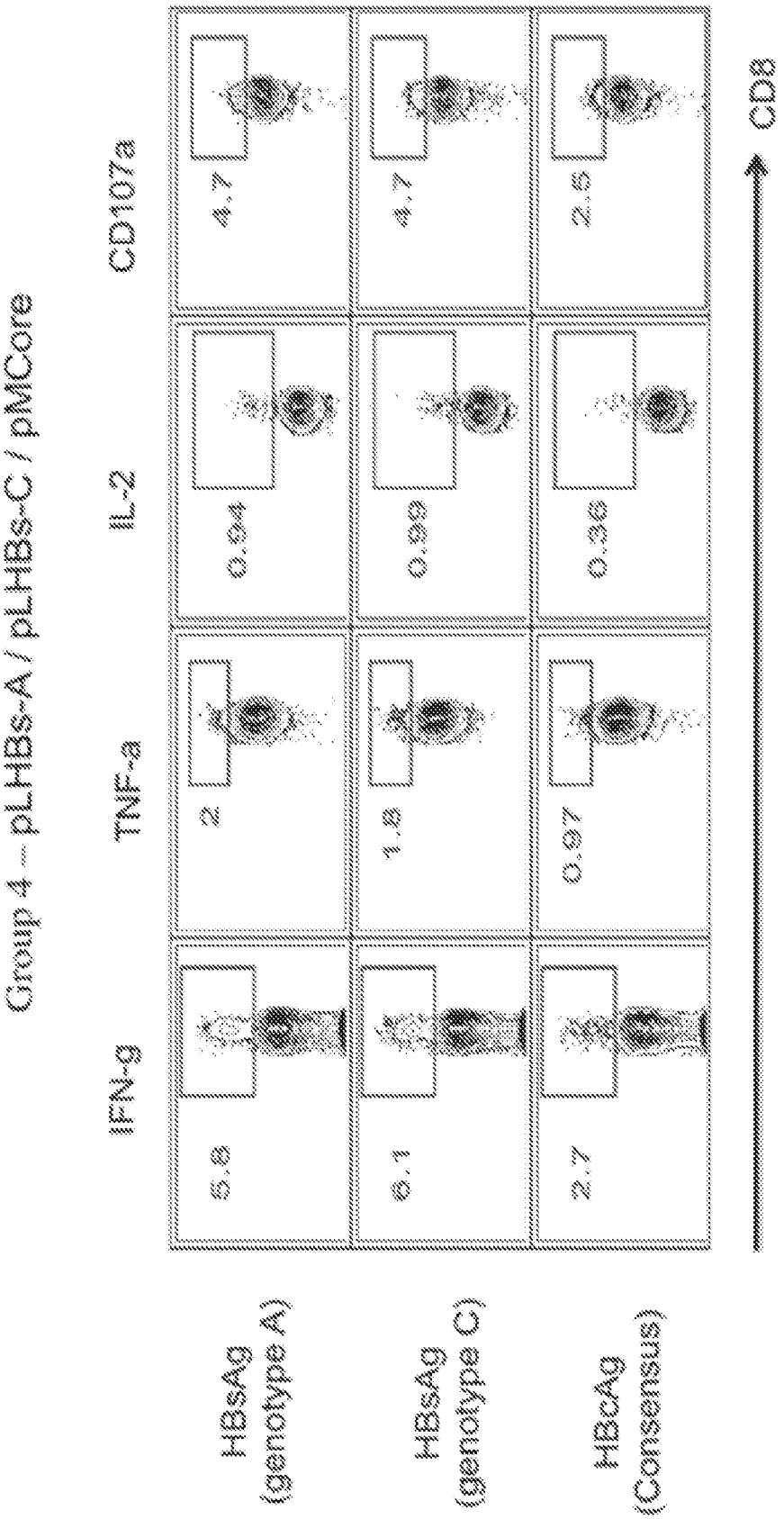

Group 4 – pLHBs-A / pLHBs-C / pMCore
IFN-g
TNF-a
IL-2
CD107a
5.8
2
0.64
4.7
6.1
1.8
0.99
4.7
2.7
0.97
0.36
2.6
HBsAg (genotype A)
HBsAg (genotype C)
HBcAg (Consensus)
CD8

Figure 56E

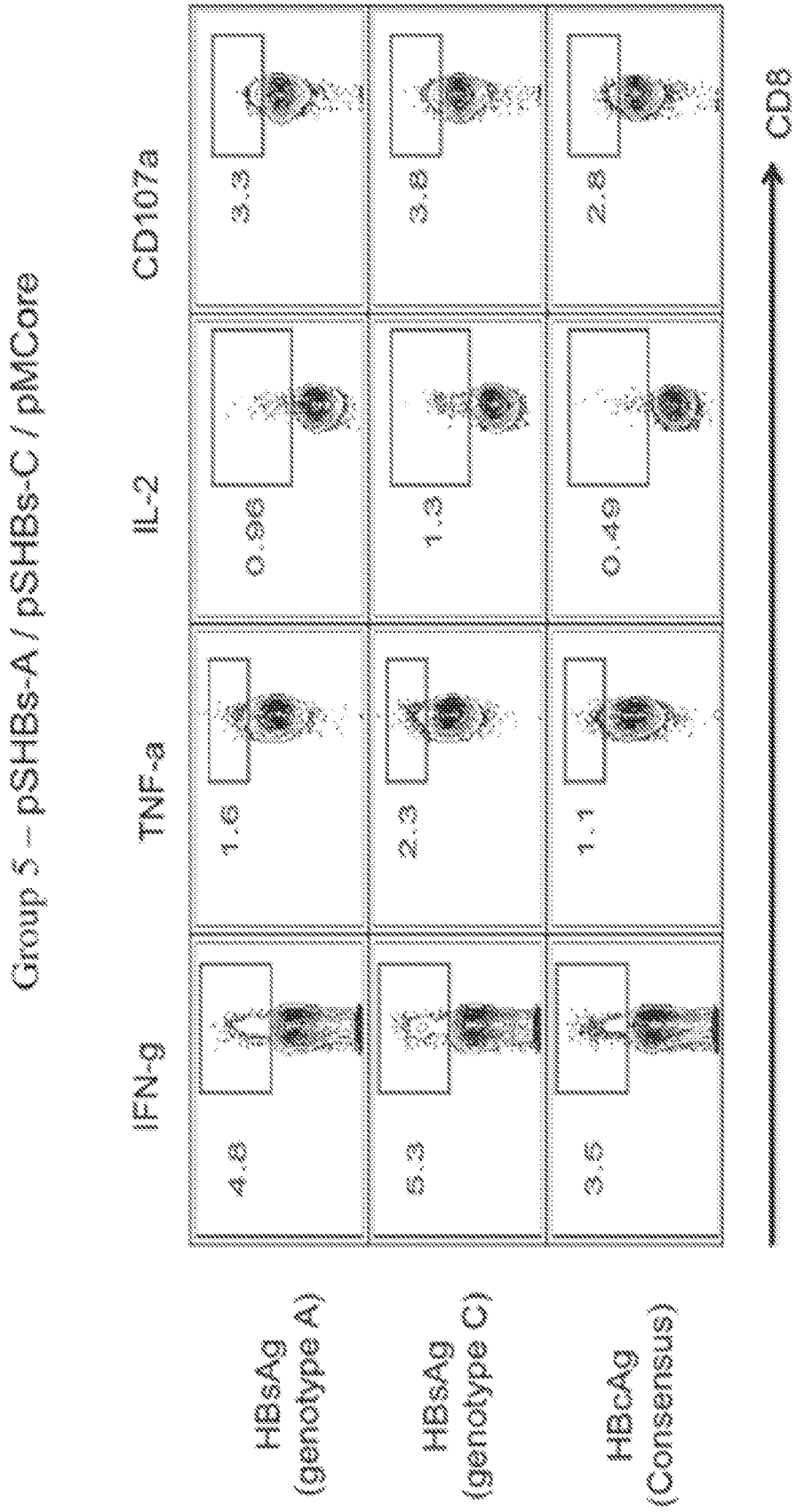

Group 5 – pSHBs-A / pSHBs-C / pMCore
IFN-g
TNF-a
IL-2
CD107a
4.8
1.6
0.98
3.3
5.3
2.3
1.3
3.8
3.5
1.1
0.49
2.8
HBsAg (genotype A)
HBsAg (genotype C)
HBcAg (Consensus)
CD8

Figure 56F

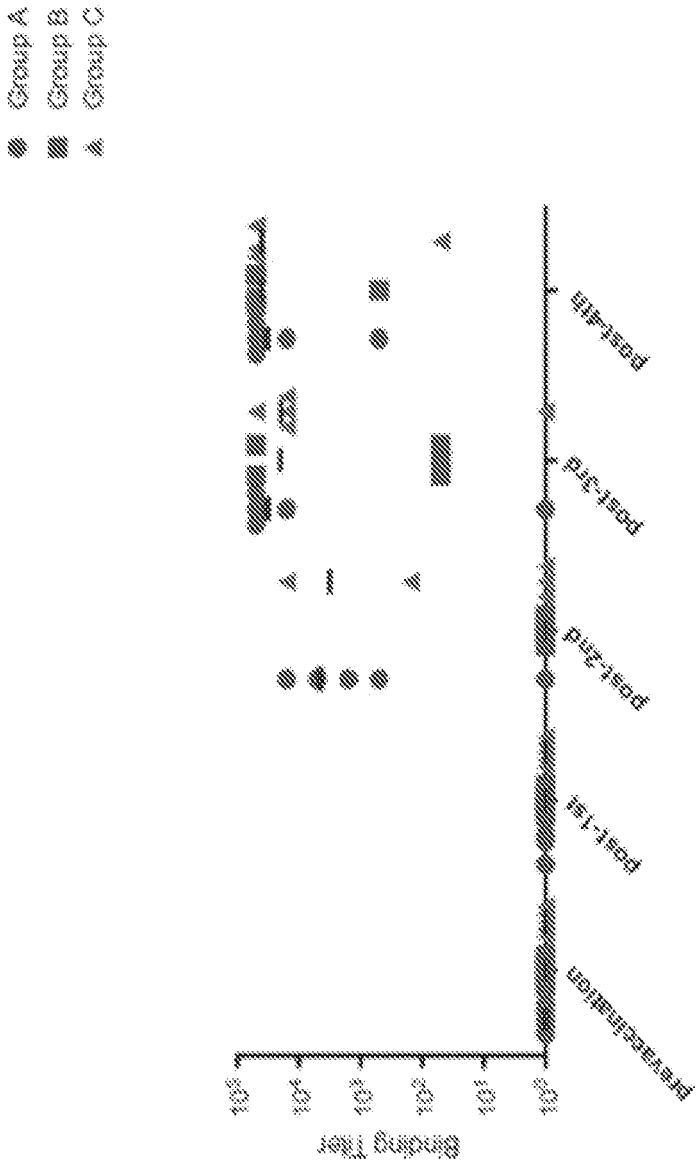

Group A
Group B
Group C
Binding Titer
1e0
1e1
1e2
1e3
1e4
1e5
Pre-vaccination
post-1st
post-2nd
post-3rd
post-4th

Figure 57A

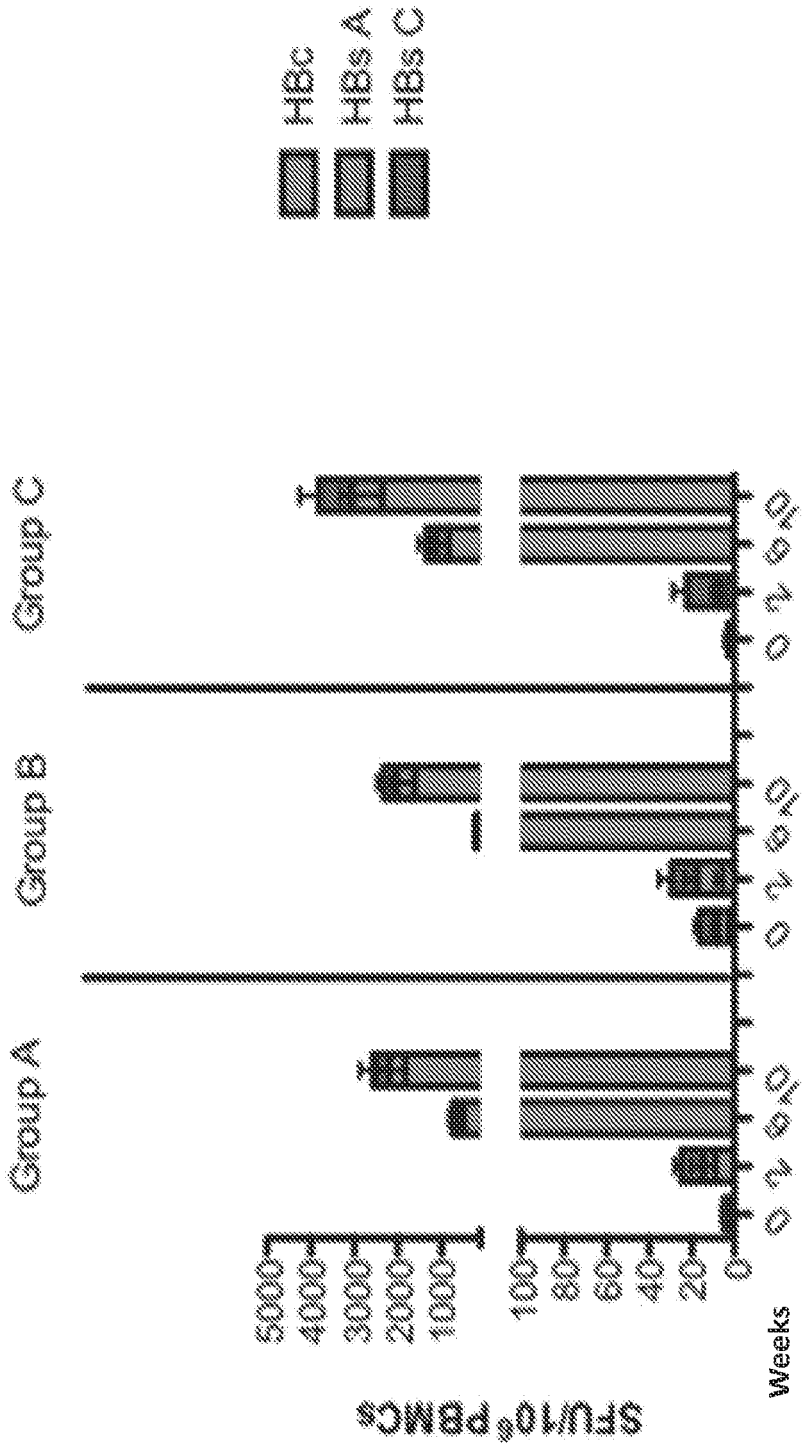
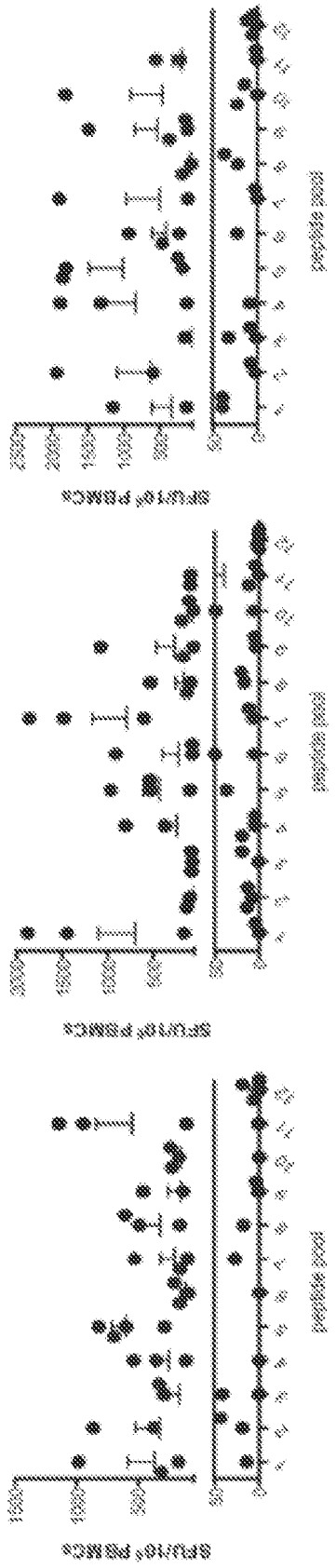

HBc
HBs A
HBs C
Group A
Group B
Group C
SFU/10^6 PBMCs
Weeks
0
2
6
10
20
40
60
80
100
1000
2000
3000
4000
5000

Figure 57B



Group	Monkey ID	Positive Matrix Pools	Positive Peptide Epitopes
Group A	4924	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11	20
	5022	2, 4, 6, 6, 7, 8, 9, 10	16
	4943	1, 2, 3, 6, 7, 8, 11	14
	4902	1, 2, 4, 5, 10, 11	10
	4904	2, 8, 10	4
Group B	4928	1, 4, 5, 6, 7, 8, 9, 10, 11	28
	5016	1, 2, 3, 7, 10	6
	4941	5, 6, 8, 11	4
	4936	1, 2, 3, 4, 5, 6, 8, 11	12
	4939	5, 6, 8	2
Group C	4929	1, 2, 3, 4, 5, 6, 10, 11	16
	4945	1, 2, 3, 4, 5, 6, 8, 10, 11	18
	4946	5, 6, 7, 8, 9	8
	5015	1, 2, 4, 5, 6, 7, 8, 9, 11	20
	5018	4, 5, 8	3

Figure 57C

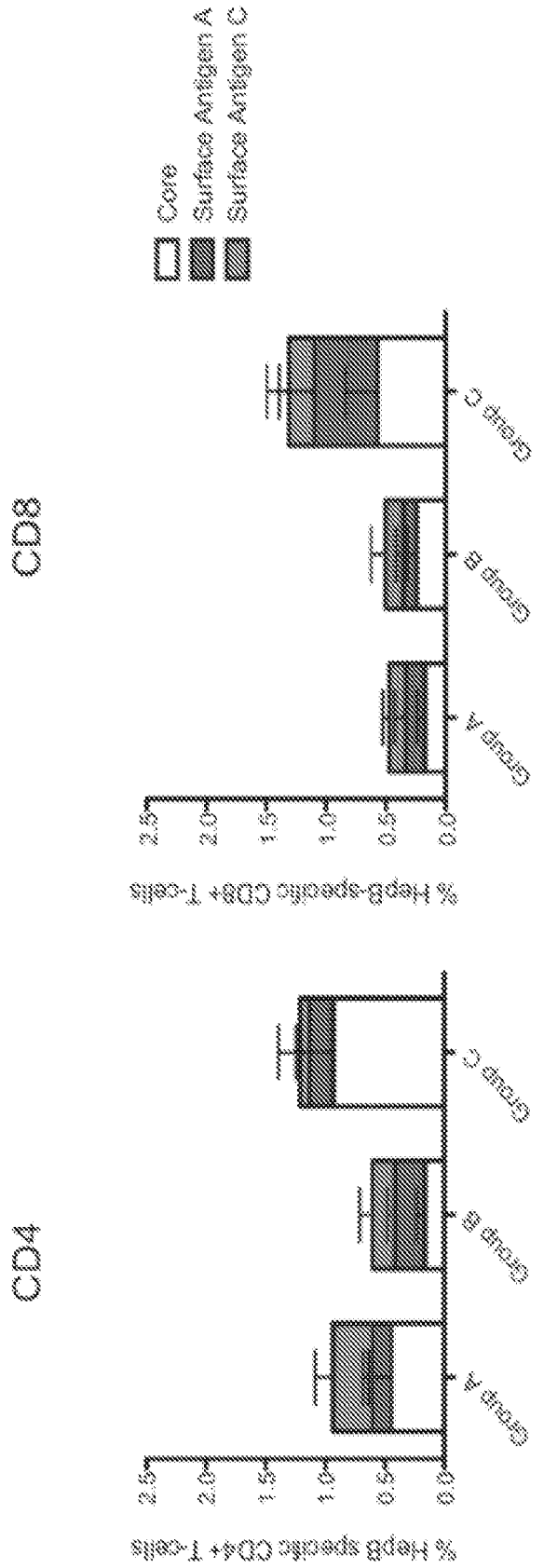

Core
Surface Antigen A
Surface Antigen C
CD8
% HepB-specific CD8+ T-cells
2.5
2.0
1.5
1.0
0.5
0.0
Group A
Group B
Group C
CD4
% HepB specific CD4+ T-cells
2.5
2.0
1.5
1.0
0.5
0.0
Group A
Group B
Group C

Figure 57D

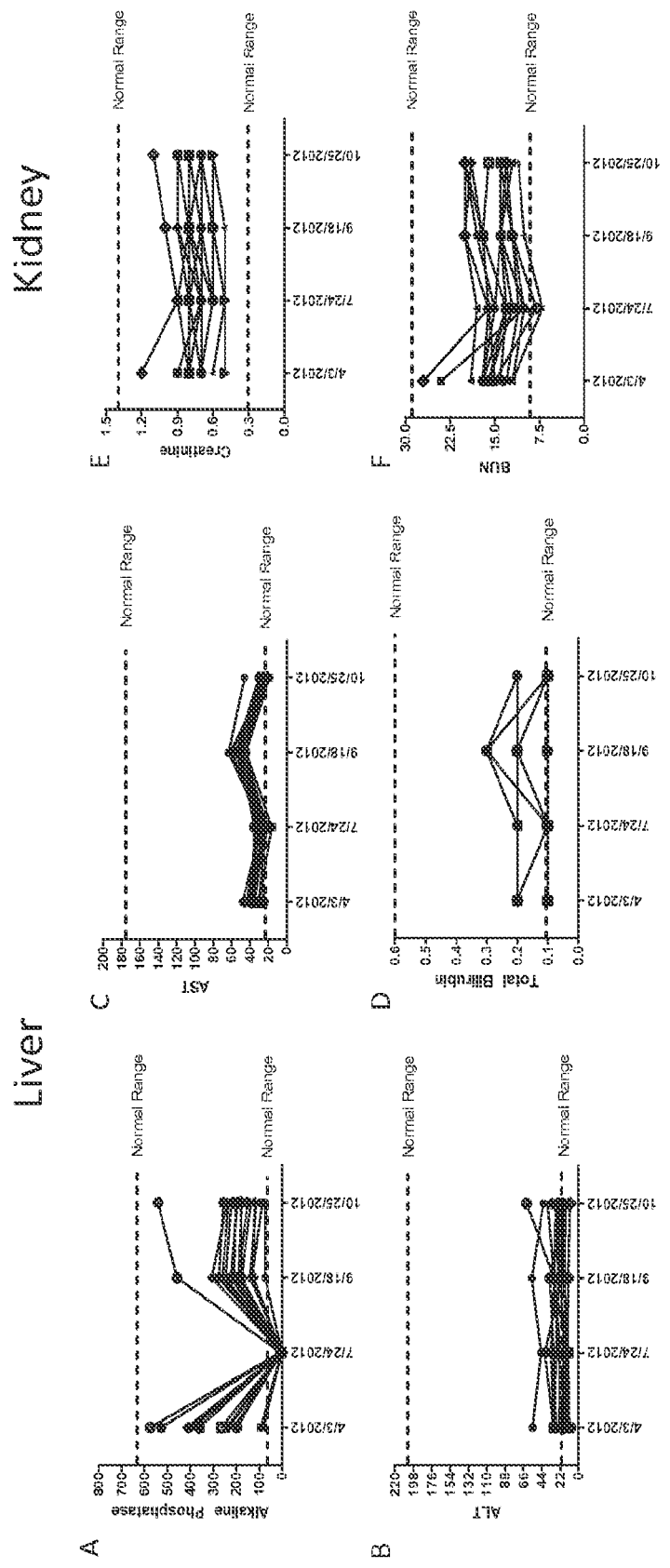

Liver
Kidney
A
Alkaline Phosphatase
B
ALT
C
AST
D
Total Bilirubin
E
Creatinine
F
BUN
Normal Range
4/3/2012
7/24/2012
9/18/2012
10/25/2012

Figure 58

PreS1
IgELS
1 MDWTWILFLVAAATRVHSGG WSSKPRKGMG TNLSVPNPLG FFPDHQLDPA FGANSNNPDW
61 DFNPIKDHWP AANQVGVGAF GPGLTPPHGG ILGWSPQAQG ILTTVSTIPP PASTNRQSGR
PreS2
Cleavage site
121 QPTPISPPLR DSHPQARGRK RRSMQWNSTA FHQALQDPRV RGLYFPAGGS SSGTVNPAPN
S
Cleavage site
181 IASHISSISA RTGDPVTNRG RKRRSMENIT SGFLGPLLVL QAGFFLLTRI LTIPQSLDSW
241 WTSLNFLGGS PVCLGQNSQS PTSNHSPTSC PPICPGYRWM CLRRFIIFLF ILLLCLIFLL
301 VLLDYQGMLP VCPLIPGSTT TSTGPCKTCT TPAQGNSMFP SCCCTKPTDG NCTCIPIPSS
361 WAFAKYLWEW ASVRFSWLSL LVPFVQWFVG LSPTVWLSAI WMMWYWGPSL YSIVSPFIPL
421 LPIFFCLWVY I (SEQ ID NO: 10)

Figure 59A

IgELS
S
1 MDWTWILFLVAAATRVHSEN ITSGFLGPLL VLQAGFFLLT RILTIPQSLD SWWTSLNFLG
61 GSPVCLGQNS QSPTSNHSPT SCPPICPGYR WMCLRRFIIF LFILLLCLIF LLVLLDYQGM
121 LPVCPLIPGS TTTSTGPCKT CTTPAQGNSM FPSCCCTKPT DGNCTCIPIP SSWAFAKYLW
181 EWASVRFSWL SLLVPFVQWF VGLSPTVWLS AIWMMWYWGP SLYSIVSPFI PLLPIFFCLW
241 VYI (SEQ ID NO: 14)

Figure 59B

IgELS PreS1

1 MDWTWILFLVAAATRVHSGG WSSKPRQGMG TNLSVPNPLG FFPDHQLDPA FGANSNNPDW

61 DFNPNKDHWP EANQVGAGAF GPGFTPPHGG LLGWSPQAQG ILTTVPAAPP PASTNRQSGR

Cleavage site PreS2

121 QPTPISPPLR DSHPQA**RGRK RRS**MQWNSTT FHQALLDPRV RGLYFPAGGS SSGTVNPVPT

Cleavage site S

181 TASPISSIFS RTGDPAPN**RG RKRRS**MESTT SGFLGPLLVL QAGFFLLTRI LTIPQSLDSW

241 WTSLNFLGGA PTCPGQNSQS PTSNHSPTSC PPICPGYRWM CLRRFIIFLF ILLLCLIFLL

301 VLLDYQGMLP VCPLLPGTST TSTGPCKTCT IPAQGTSMFP SCCCTKPSDG NCTCIPIPSS

361 WAFARFLWEW ASVRFSWLSL LVPFVQWFVG LSPTVWLSVI WMMWYWGPSL YNILSPFLPL

421 LPIFFCLWVY I (SEQ ID NO: 12)

Figure 59C

IgELS
S
1 MDWVTWILFLVAAATRVHSES TTSGFLGPLL VLQAGFFLLT RILTIPQSLD SWWTSLNFLG
61 GAPTCPGQNS QSPTSNHSPT SCPPICPGYR WMCLRRFIIF LFILLLCLIF LLVLLDYQGM
121 LPVCPLLPGT STTSTGPCKT CTIPAQGTSM FPSCCCTKPS DGNCTCIPIP SSWAFARFLW
181 EWASVRFSWL SLLVPFVQWF VGLSPTVWLS VIWMMWYWGP SLYNILSPFL PLLPIFFCLW
241 VYI (SEQ ID NO: 16)

Figure 59D