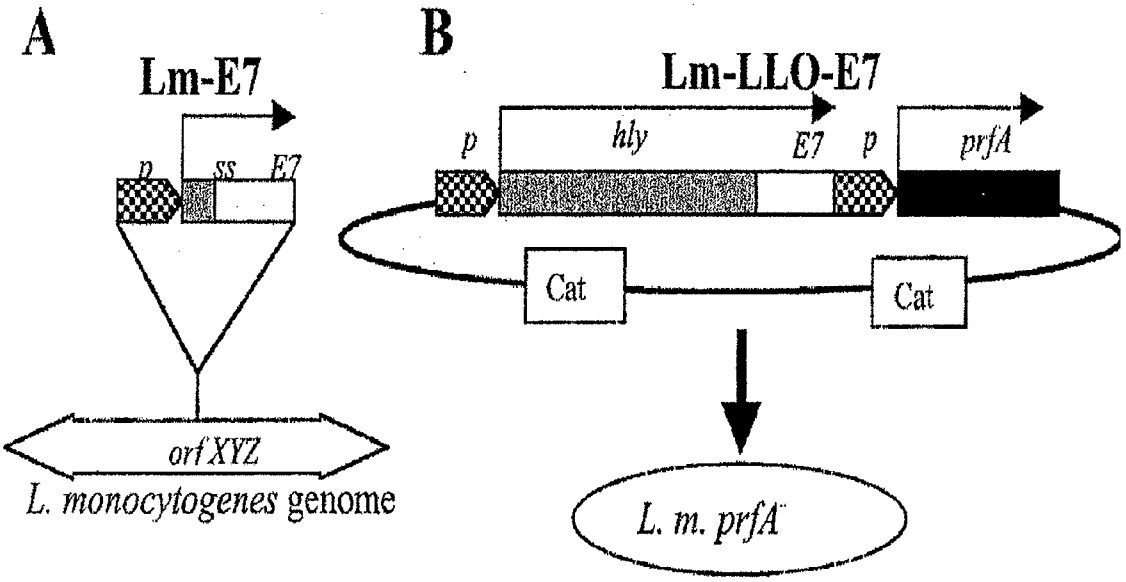


Figure 1

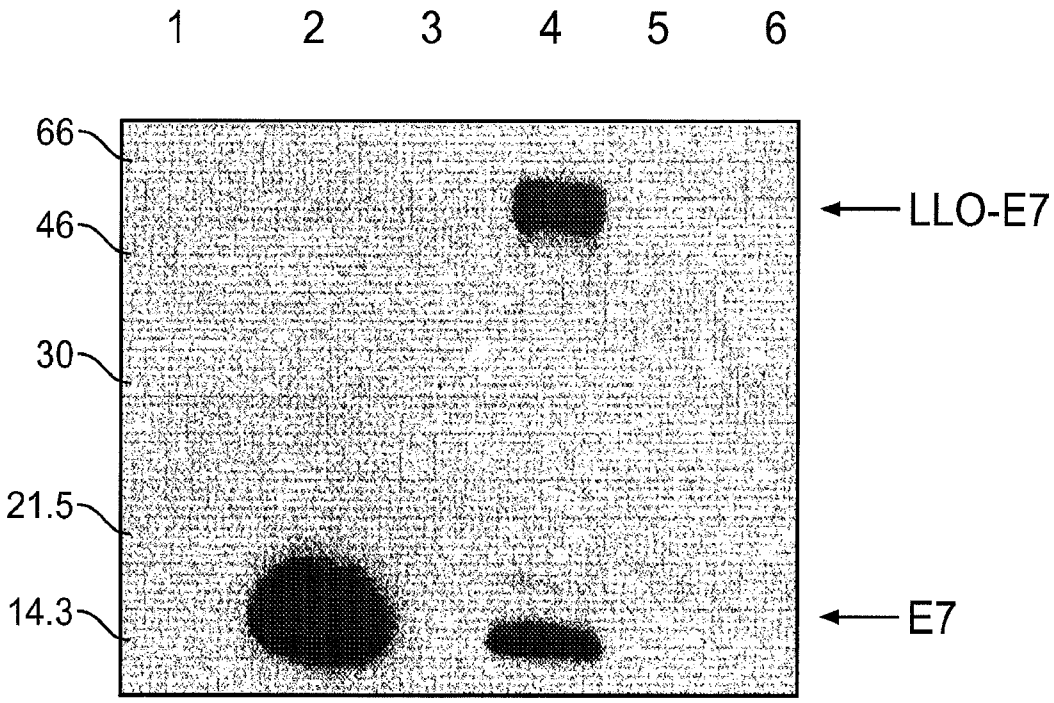


Figure 2

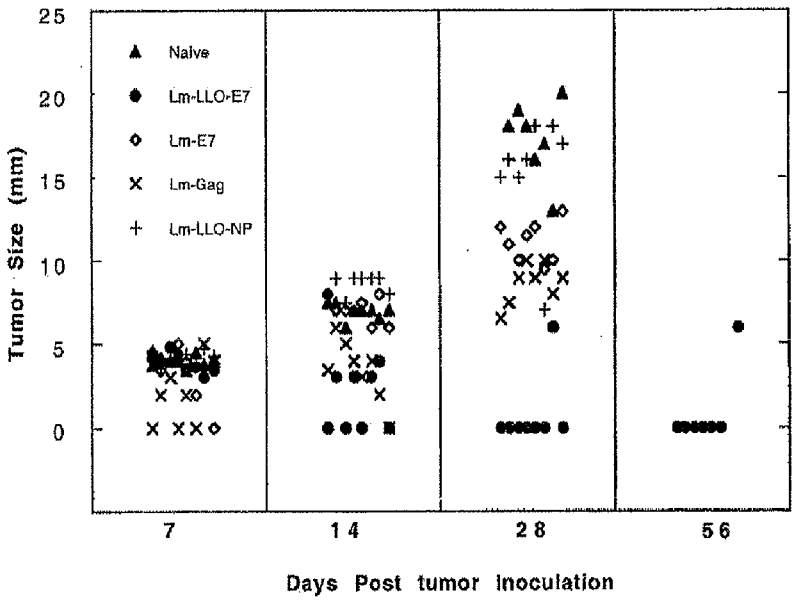

25
20
15
10
5
0
Tumor Size (mm)
Naive
Lm-LLO-E7
Lm-E7
Lm-Gag
Lm-LLO-NP
7
14
28
56
Days Post tumor inoculation

Figure 3

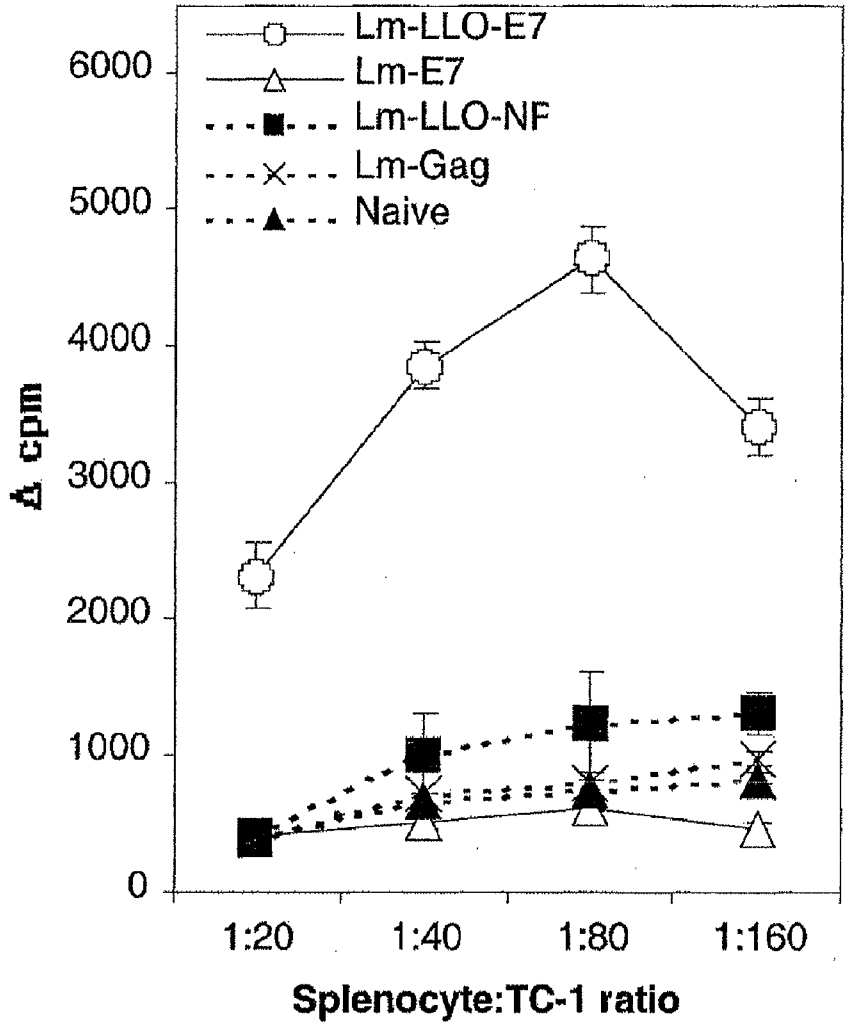

Lm-LLO-E7
Lm-E7
Lm-LLO-NF
Lm-Gag
Naive
6000
5000
4000
3000
2000
1000
0
Δ cpm
1:20
1:40
1:80
1:160
Splenocyte:TC-1 ratio

Figure 4

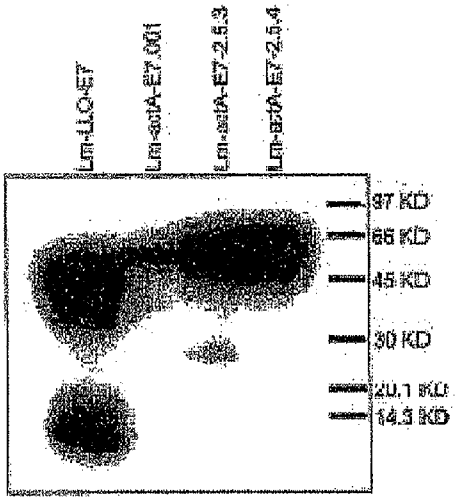


Figure 5A

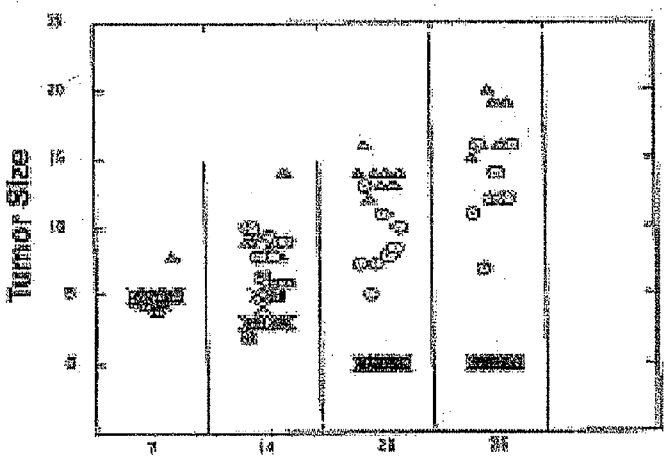


Figure 5B

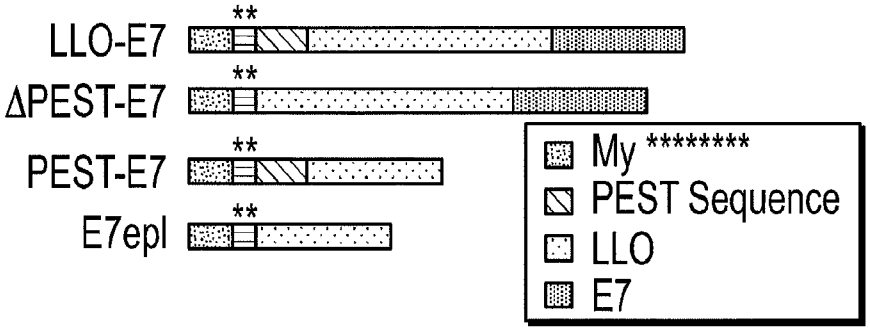
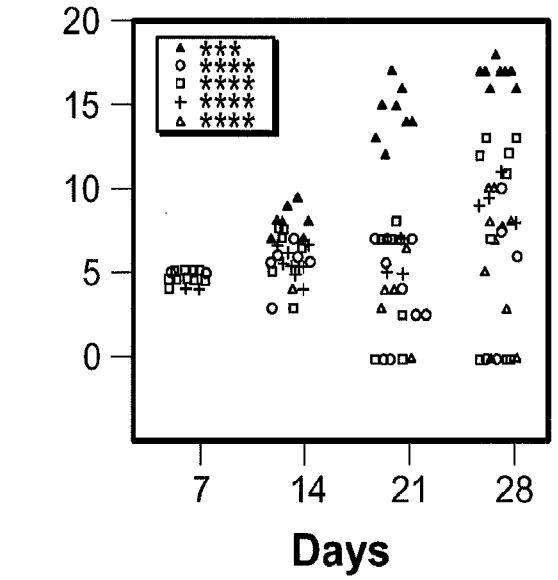


Figure 6A



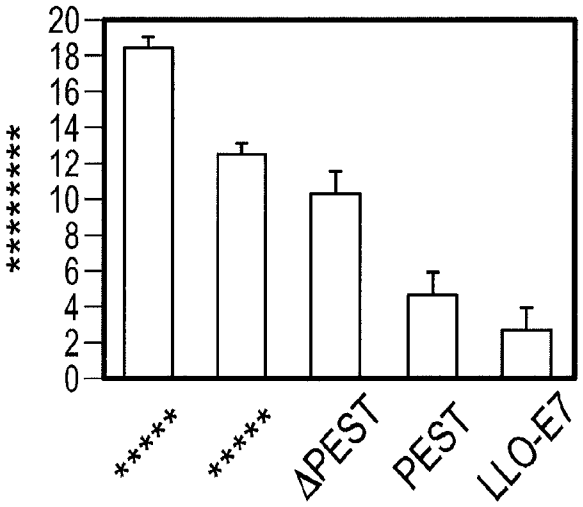


Figure 6B

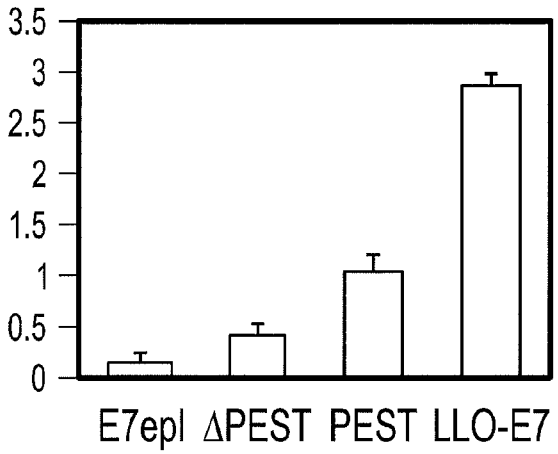


Figure 6C

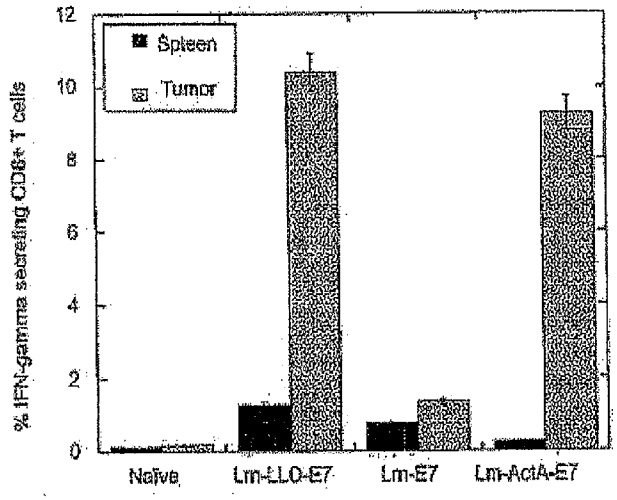


Figure 7A

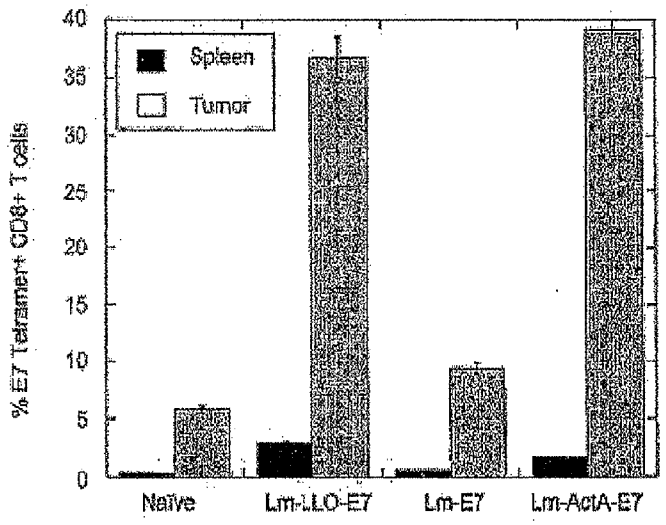


Figure 7B

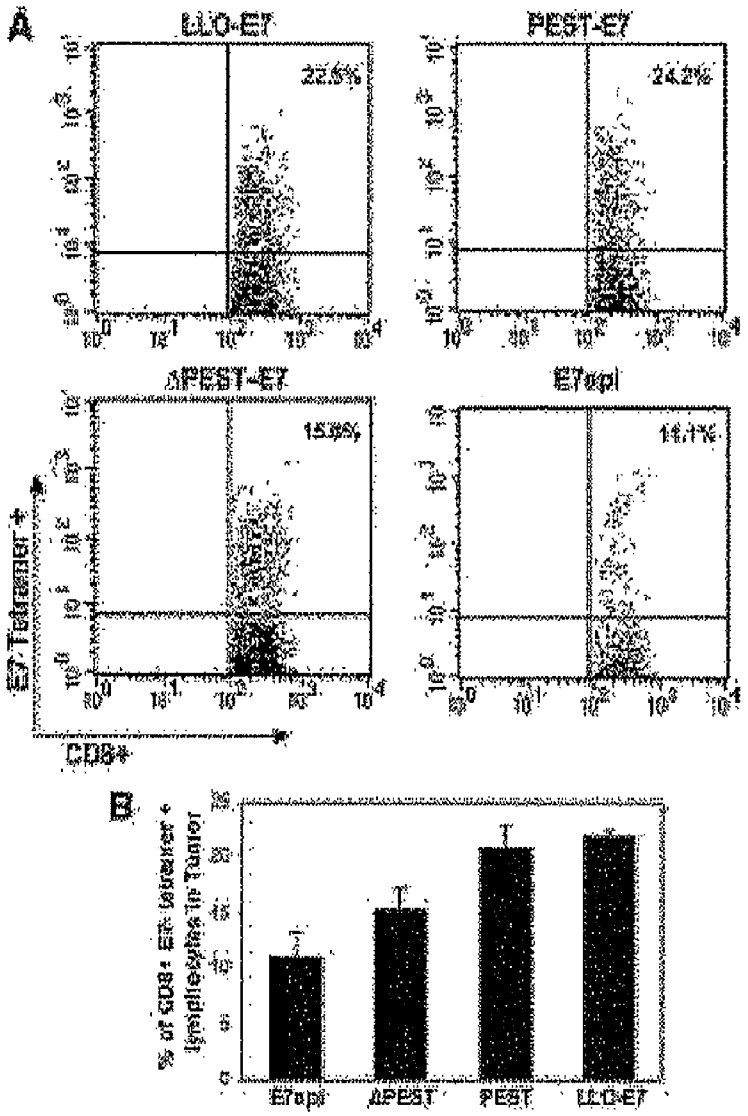

A
LLO-E7
22.6%
PEST-E7
24.2%
ΔPEST-E7
15.8%
E7opt
14.7%
E7 Tetramer+
CD8+
B
% of CD8+ E7 tetramer+ lymphocytes in Tumor
E7opt
ΔPEST
PEST
LLO-E7

Figure 8

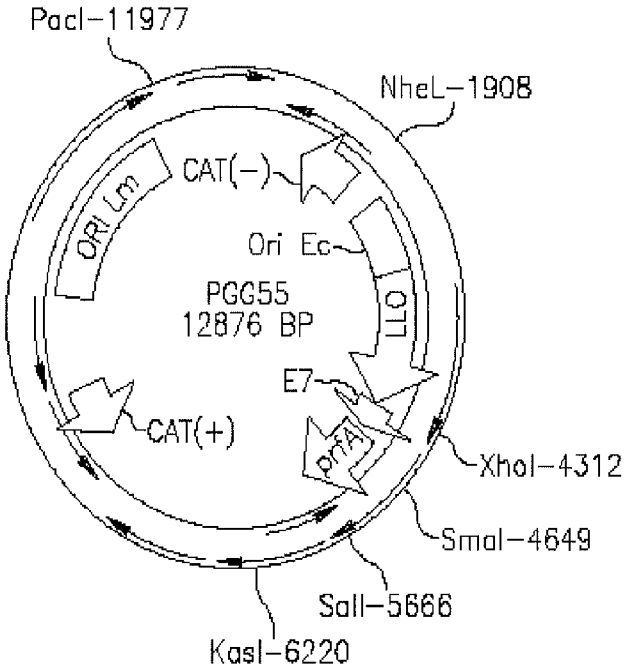


Figure 9A

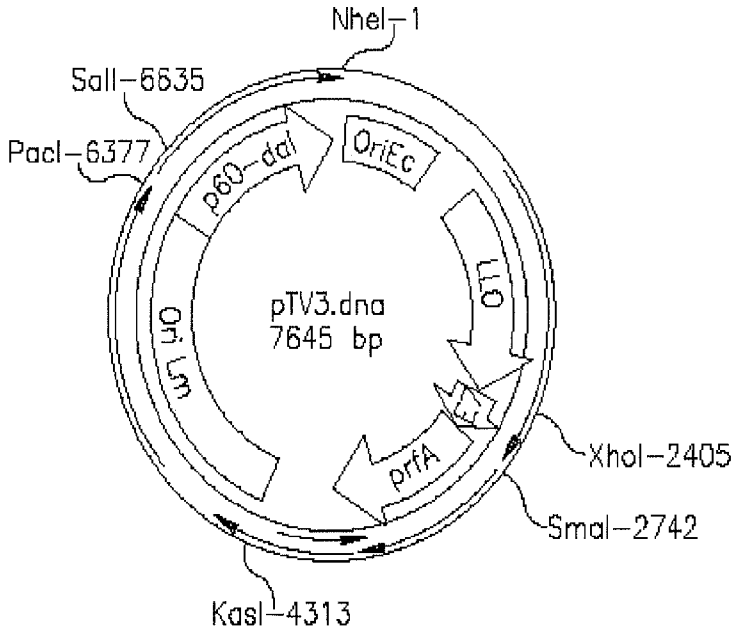


Figure 9B

atggcgcgggatggtatactatacaagcgtatggttcaaaaagatactttgaattaagaagt
acaataaagttaacttcattagacaaaaagaaaaaacaaggaagaatagtacatagttataa
atacttggagagtgaggtgtaatatgggggcagctgatttttggggtttcatatatgtagtt
tcaagattagccattgttgcggcagtagtttacttcttatacttattgagaaaaattgcaaa
taaatagaaaaaaagccttgtcaaacgaggctttttttatgcaaaaaatacgacgaatgaag
ccatgtgagacaatttggaatagcagacaacaaggaaggtagaacatgttttgaaaaattta
ctgattttcgattattattaacgcttgttaatttaaacatctcttatttttgctaacatata
agtatacaaagggacataaaaaggttaacagcgtttgttaaataggaagtatatgaaaatcc
*tcttttgtgtttctaaatttatttttaaggagtggaga***atgttgaaaaaaaataattggtta**
caaaatgcagtaatagcaatgctagtgttaattgtaggtctgtgcattaatatgggttctgg
aacaaaagtacaagctgagagtattcaacgaccaacgcctattaaccaagtttttccagatc
ccggcctagcgaatgcagtgaaacaaaatttagggaagcaaagtgttacagaccttgtatca
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gggaatgcaatttttcactaatttaaaagaacttcatctatcccataatcaaataagtgacc
ttagtcctttaaaggatctaactaagttagaagagctatctgtgaatagaaacagactgaaa
aatttaaacggaattccaagtgcttgtttatctcgcttgtttttagataacaacgaactcag
agatactgactcgcttattcatttgaaaaatctagaaatcttatctattcgtaataataagt
taaaaagtattgtgatgcttggtttttttatcaaaactagaggtattagatttgcatggtaat
gaaataacaaatacaggtggactaactagattgaagaaagttaactggatagatttaactgg
tcagaaatgtgtgaatgaaccagtaaaataccaaccagaattgtatataacaaatactgtca
aagacccagatggaagatggatatctccatattacatcagtaatggtgggagttatgtagat
ggttgtgtcctgtgggaattgccagtttatacagatgaagtaagctataagtttagcgaata
tataaacgttggggagactgaggctatatttgatggaacagttacacaacctatcaagaatt
aggacttgtgcacacctgtatactttgagctctcgtataatcacgagagctttttaaatatg
taagtcttaattatctcttgacaaaaagaacgtttattcgtataaggttaccaagagatgaa
gaaactattttatttacaattcaccttgacaccaaaaactccatatgatatagtaaataagg
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cagcccgtttttttatggcctccgaacgaatgagttagcaggccgcagatttgaacagctat
tttctatcttgttgtaacaaaattaagtggaggtggctcaccattagcaaagacatgttggt
aaacgatgggattcgtgcacgtgaagtaagattgatcgaccaagacggtgaacaattaggcg
tgaagagtaaaatcgatgcgcttcaaattgctgaaaaggctaatcttgatctagtgcttgtt
gctccaacagcgaaaccgccagtagctcgta

Figure 10

*GAATTC*atggcgcgggatggtatactatacaagcgtatggttcaaaaagatactttgaattaa
gaagtacaataaagttaacttcattagacaaaaagaaaaaacaaggaagaatagtacatagtt
ataaatacttggagagtgaggtgtaatatgggggcagctgatttttgggggtttcatatatgta
gtttcaagattagccattgttgcggcagtagtttacttcttatacttattgagaaaaattgca
aataaatagaaaaaaagccttgtcaaacgaggctttttttatgcaaaaaatacgacgaatgaa
gccatgtgagacaatttggaatagcagacaacaaggaaggtagaacatgttttgaaaaattta
ctgattttcgattattattaacgcttgttaatttaaacatctcttatttttgctaacatataa
gtatacaaagggacataaaaaggttaacagcgtttgttaaataggaagtatatgaaaatcctc
ttttgtgtttctaaatttatttttaaggagtggaga*GGATCC*ggacttgtgcacacctgtata
ctttgagctctcgtataatcacgagagctttttaaatatgtaagtcttaattatctcttgaca
aaaagaacgtttattcgtataaggttaccaagagatgaagaaactattttatttacaattcac
cttgacaccaaaaactccatatgatatagtaaataaggttattaaacaagaaagaagaagcaa
cccgcttctcgcctcgttaacacgaacgttttcaggcaaaaaattcaaactttcgtcgcgtag
cttacgcgattttgaatgtgcgggattgctgaaaagcagcccgtttttttatggcctccgaac
gaatgagttagcaggccgcagatttgaacagctattttctatcttgttgtaacaaaattaagt
ggaggtggctcaccattagcaaagacatgttggtaaacgatgggattcgtgcacgtgaagtaa
gattgatcgaccaagacggtgaacaattaggcgtgaagagtaaaatcgatgcgcttcaaattg
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CTGCAG

Figure 11

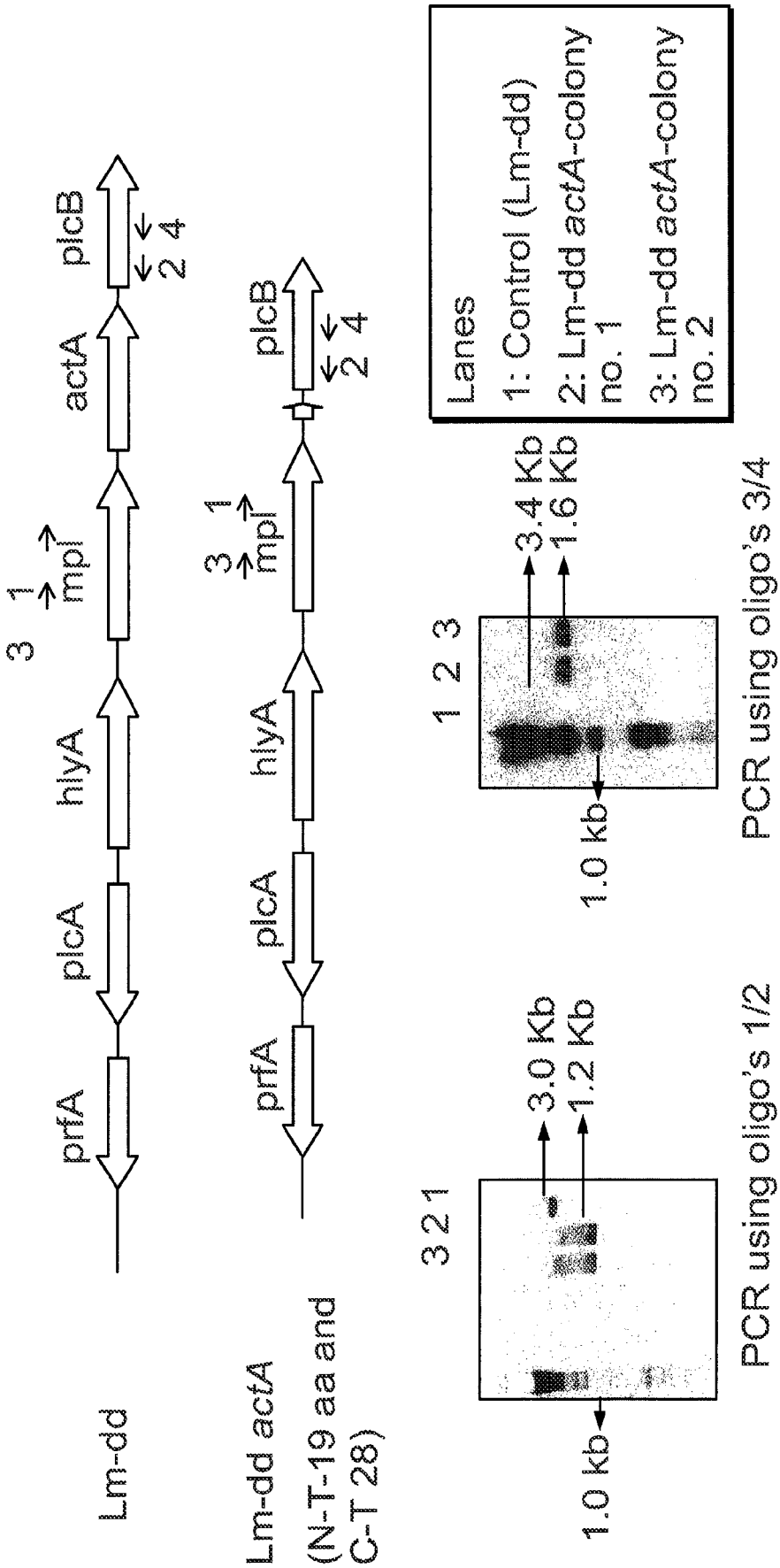

prfA
plcA
hlyA
mpl
actA
plcB
3 1
2 4
Lm-dd
Lm-dd actA
(N-T-19 aa and
C-T 28)
Lanes
1: Control (Lm-dd)
2: Lm-dd actA-colony
no. 1
3: Lm-dd actA-colony
no. 2
1 2 3
3.4 Kb
1.6 Kb
1.0 kb
PCR using oligo's 3/4
3 2 1
3.0 Kb
1.2 Kb
1.0 kb
PCR using oligo's 1/2

Figure 12

gcgccaaatcattggttgattggtgaggatgtctgtgtgcgtgggtcgcgagatgggcgaataagaagcattaaagatcctgacaaatat
aatcaagcggctcatatgaaagattacgaatcgcttccactcacagaggaaggcgactggggcggagttcattataatagtggtatccc
gaataaagcagcctataatactatcactaaacttggaaaagaaaaaacagaacagctttattttcgcgccttaaagtactatttaacgaaaa
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gggaagcagttggggttaactgattaacaaatgttagagaaaaattaattctccaagtgatattcttaaaataattcatgaatatttttttcttata
ttagctaattaagaagataactaactgctaatccaatttttaacggaacaaattagtgaaaatgaaggccgaattttccttgttctaaaaaggt
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*caattaagaaaaaataatta*aaaacacagaacgaaagaaaaagtgaggtgaatgatatgaaattcaaaaaggtggttctaggtatgtg
cttgatcgcaagtgttctagtctttccggtaacgataaaagcaaatgcctgttgtgatgaatacttacaaacacccgcagctccgcatgata
ttgacagcaaattaccacataaacttagttggtccgcggataacccgacaaatactgacgtaaatacgcactattggctttttaaacaagc
ggaaaaaatactagctaaagatgtaaatcatatgcgagctaatttaatgaatgaacttaaaaaattcgataaacaaatagctcaaggaata
tatgatgcggatcataaaaatccatattatgatactagtacatttttatctcatttttataatcctgatagagataatacttatttgccgggttttgc
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Figure 13

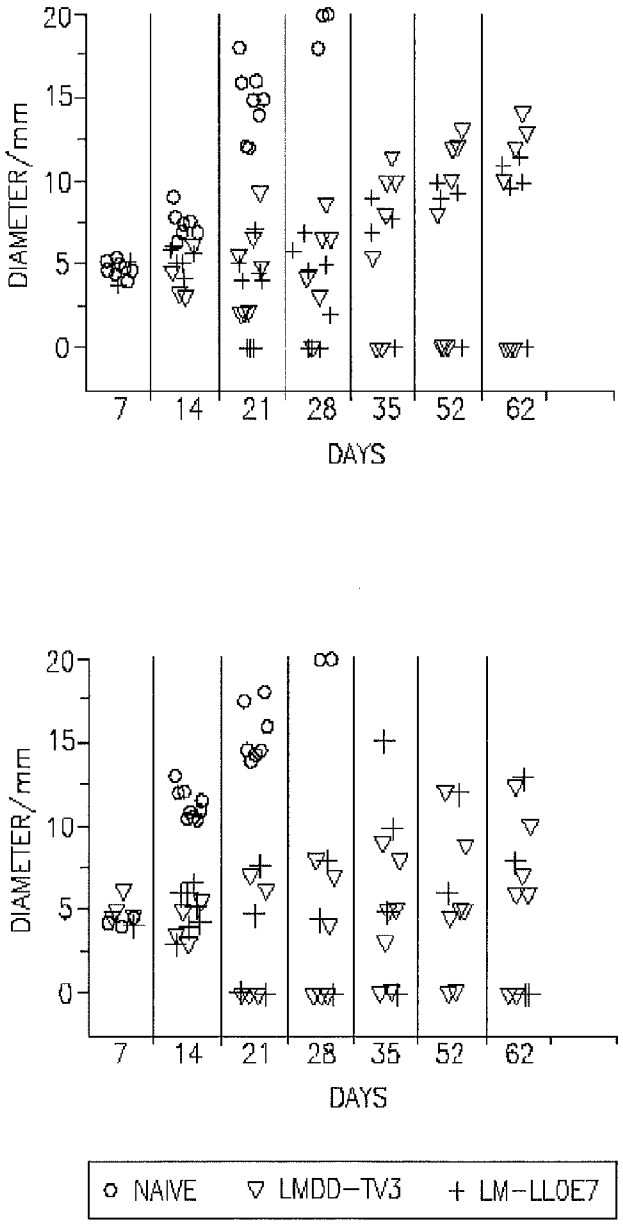

DIAMETER/mm
20
15
10
5
0
7
14
21
28
35
52
62
DAYS
DIAMETER/mm
20
15
10
5
0
7
14
21
28
35
52
62
DAYS
o NAIVE
▽ LMDD-TV3
+ LM-LLOE7

Figure 14

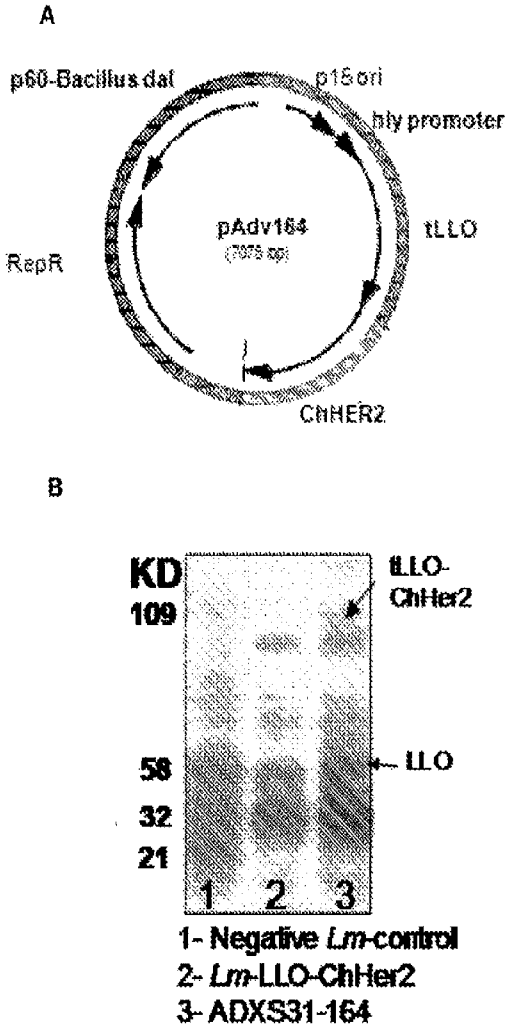
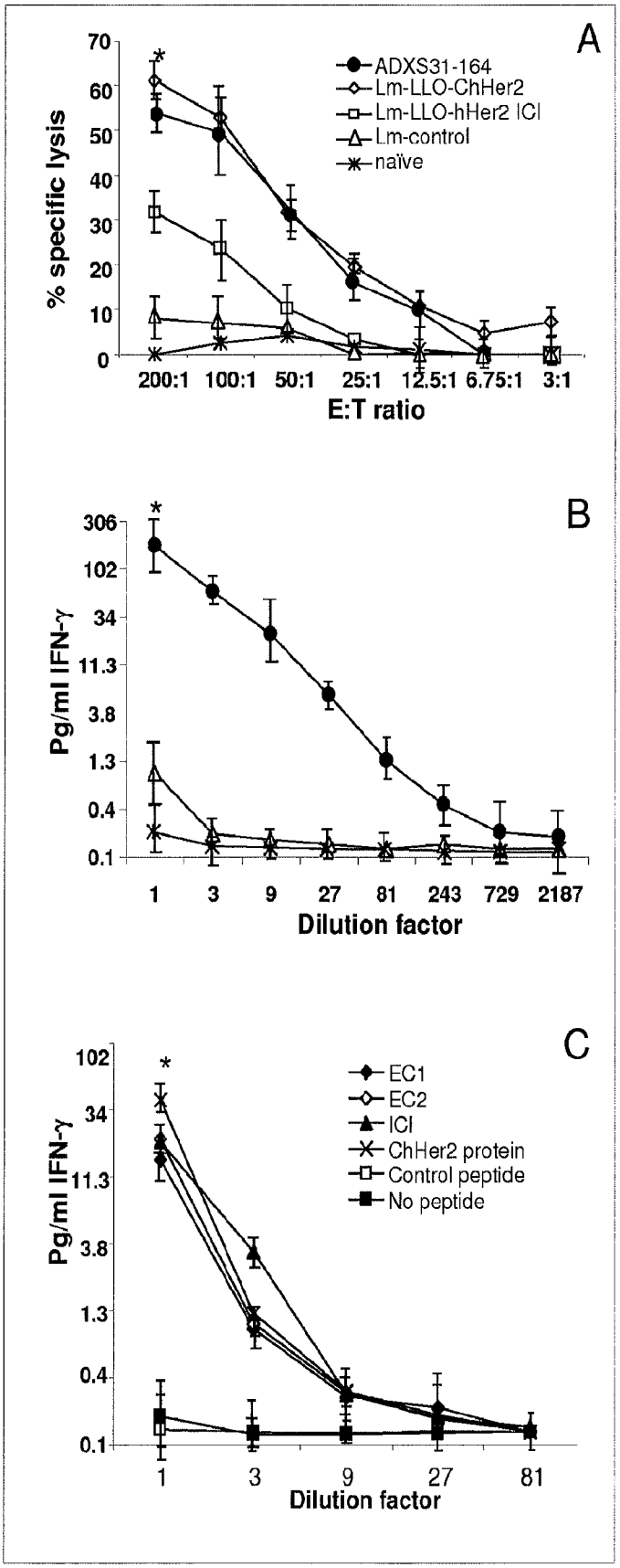

A
p60-Bacillus dal
p15 ori
hly promoter
pAdv164
tLLO
RepR
ChHER2
B
KD
109
58
32
21
tLLO-ChHer2
LLO
1 2 3
1- Negative Lm-control
2- Lm-LLO-ChHer2
3- ADXS31-164

Figure 15

Figure 16


A
% specific lysis
70
60
50
40
30
20
10
0
*
ADXS31-164
Lm-LLO-ChHer2
Lm-LLO-hHer2 ICI
Lm-control
naïve
200:1 100:1 50:1 25:1 12.5:1 6.75:1 3:1
E:T ratio
B
Pg/ml IFN-γ
306
102
34
11.3
3.8
1.3
0.4
0.1
*
1 3 9 27 81 243 729 2187
Dilution factor
C
Pg/ml IFN-γ
102
34
11.3
3.8
1.3
0.4
0.1
*
EC1
EC2
ICI
ChHer2 protein
Control peptide
No peptide
1 3 9 27 81
Dilution factor

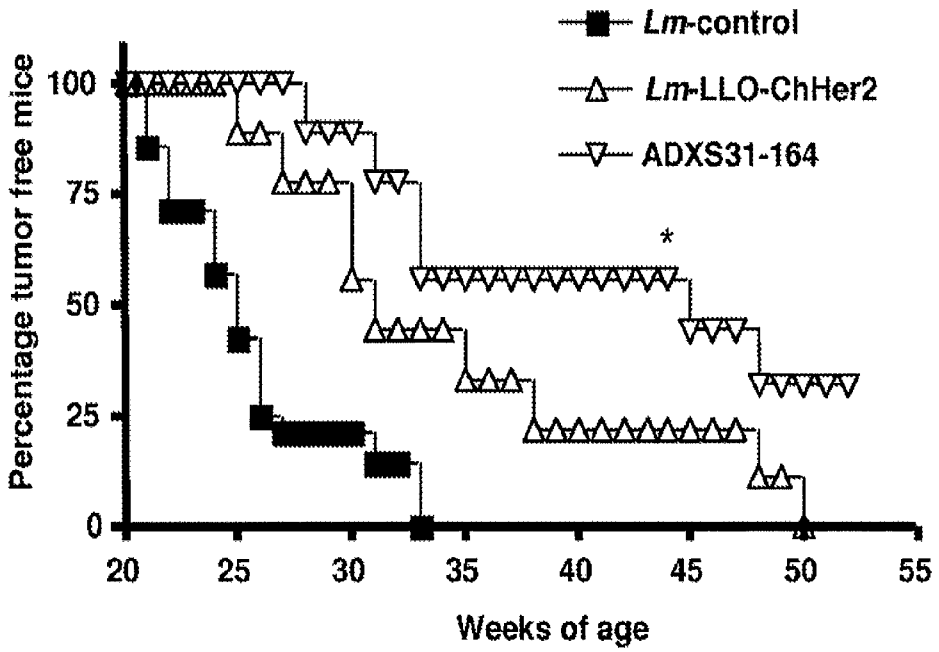

Lm-control
Lm-LLO-ChHer2
ADXS31-164
*
Percentage tumor free mice
100
75
50
25
0
20
25
30
35
40
45
50
55
Weeks of age

Figure 17

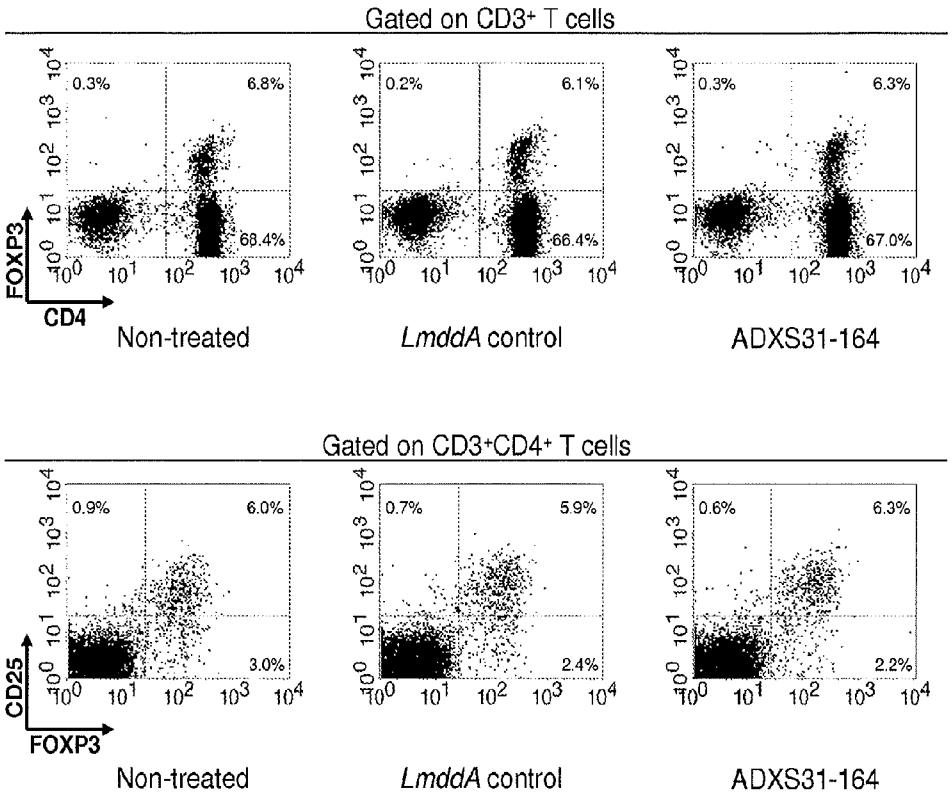

Spleens
Gated on CD3+ T cells
0.3%
6.8%
68.4%
0.2%
6.1%
66.4%
0.3%
6.3%
67.0%
FOXP3
CD4
Non-treated
LmddA control
ADXS31-164
Gated on CD3+CD4+ T cells
0.9%
6.0%
3.0%
0.7%
5.9%
2.4%
0.6%
6.3%
2.2%
CD25
FOXP3
Non-treated
LmddA control
ADXS31-164

Figure 18

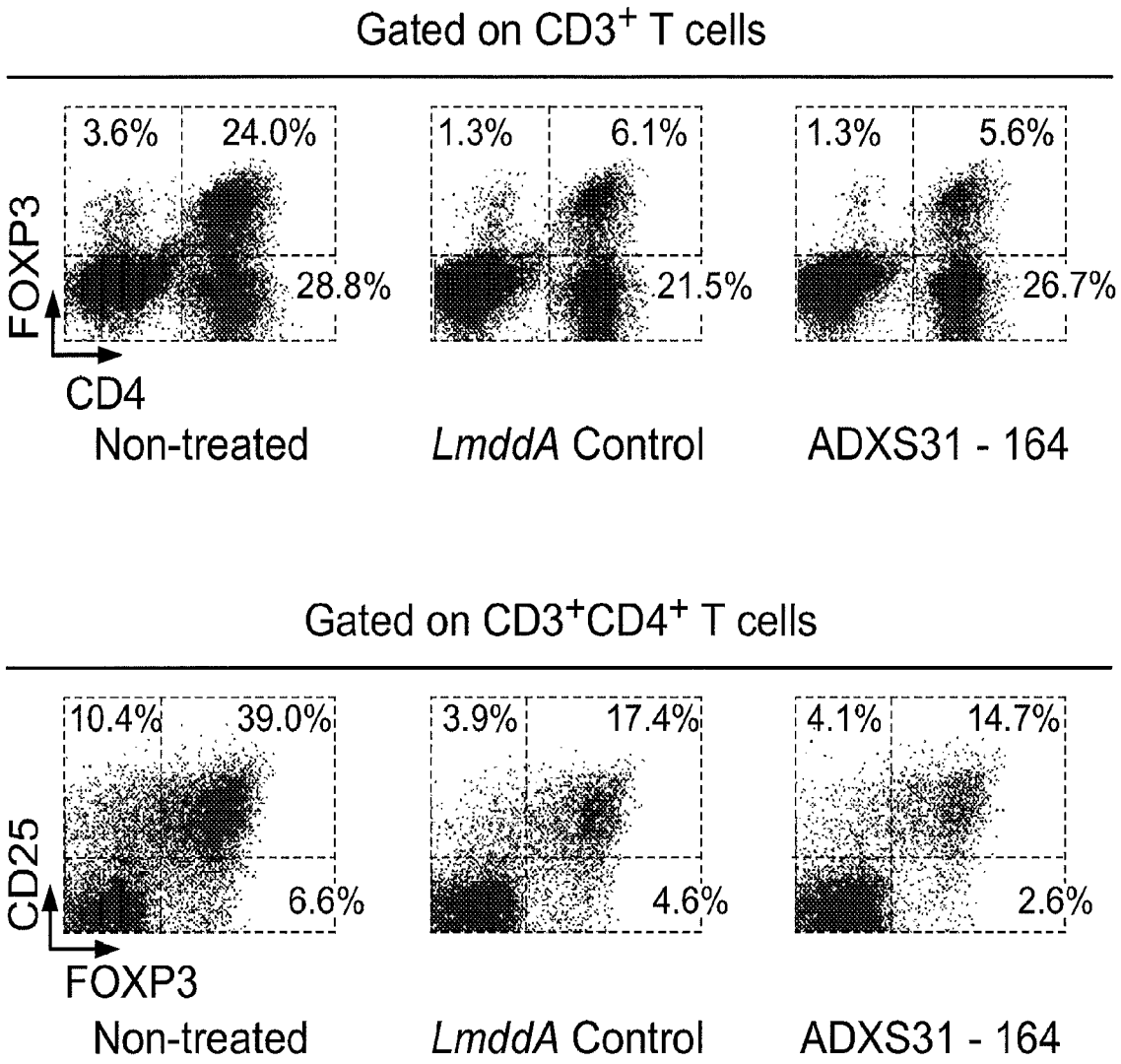
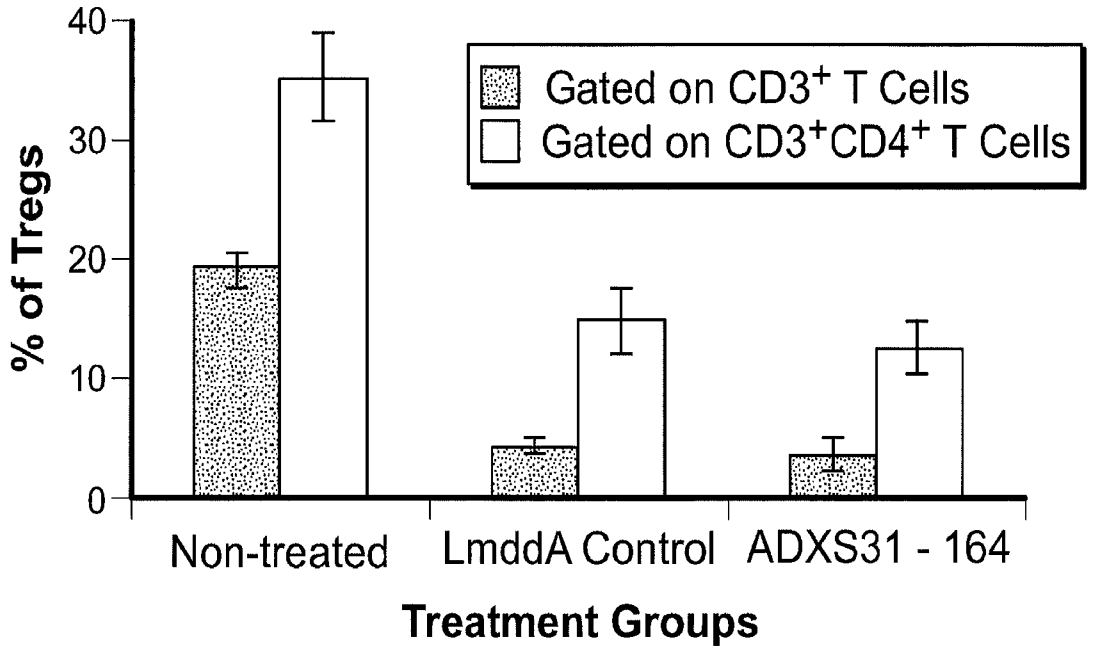

Gated on CD3+ T cells
3.6%
24.0%
28.8%
1.3%
6.1%
21.5%
1.3%
5.6%
26.7%
FOXP3
CD4
Non-treated
LmddA Control
ADXS31 - 164
Gated on CD3+CD4+ T cells
10.4%
39.0%
6.6%
3.9%
17.4%
4.6%
4.1%
14.7%
2.6%
CD25
FOXP3
Non-treated
LmddA Control
ADXS31 - 164

Figure 19A


40
30
20
10
0
% of Tregs
Gated on CD3+ T Cells
Gated on CD3+CD4+ T Cells
Non-treated
LmddA Control
ADXS31 - 164
Treatment Groups

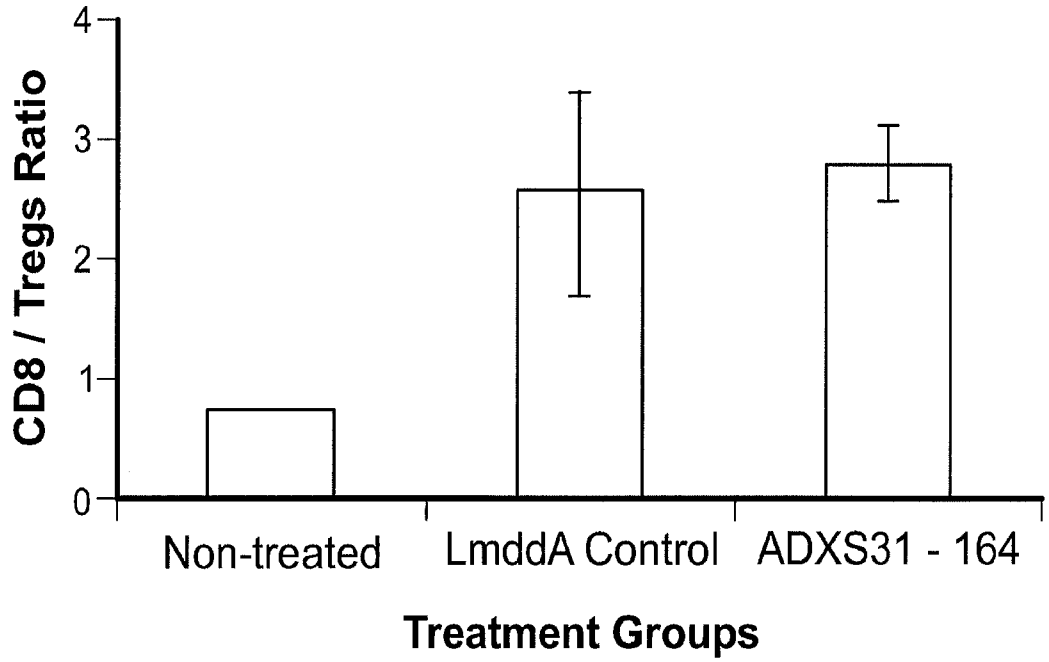

4
3
2
1
0
CD8 / Tregs Ratio
Non-treated
LmddA Control
ADXS31 - 164
Treatment Groups

Figure 19B

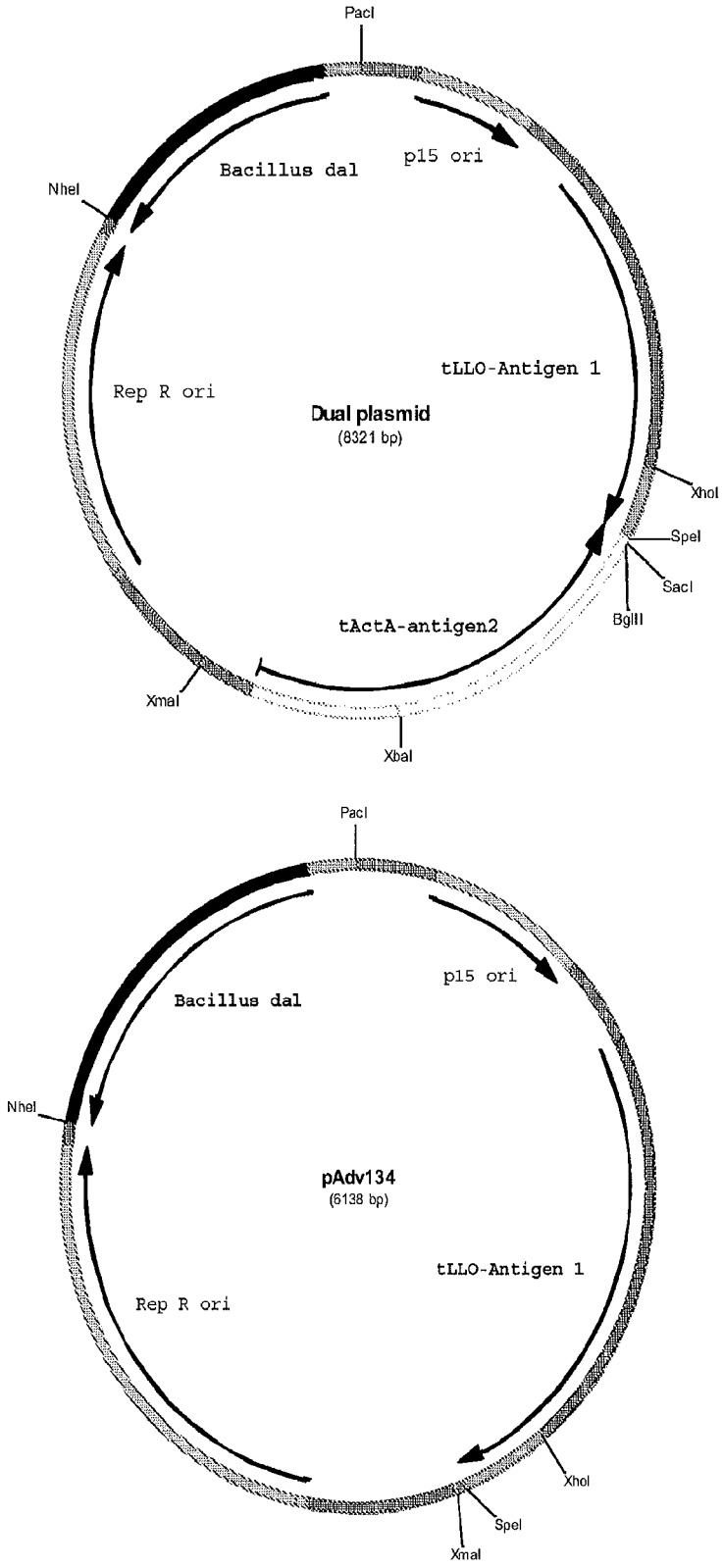
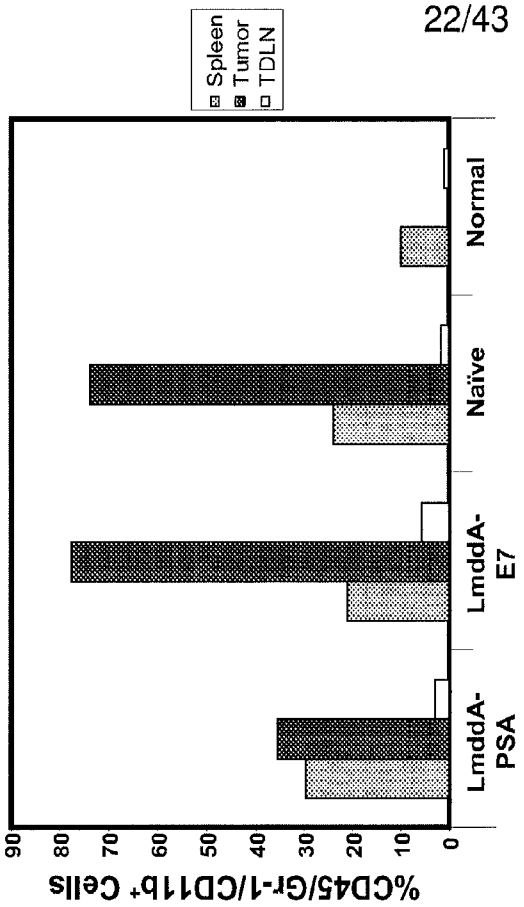

PacI
p15 ori
Bacillus dal
NheI
tLLO-Antigen 1
Rep R ori
Dual plasmid
(8321 bp)
XhoI
SpeI
SacI
BglII
tActA-antigen2
XmaI
XbaI
PacI
p15 ori
Bacillus dal
NheI
pAdv134
(6138 bp)
tLLO-Antigen 1
Rep R ori
XhoI
SpeI
XmaI

Figure 20


Spleen
Tumor
TDLN
%CD45/Gr-1/CD11b+ Cells
0
10
20
30
40
50
60
70
80
90
LmddA-PSA
LmddA-E7
Naïve
Normal

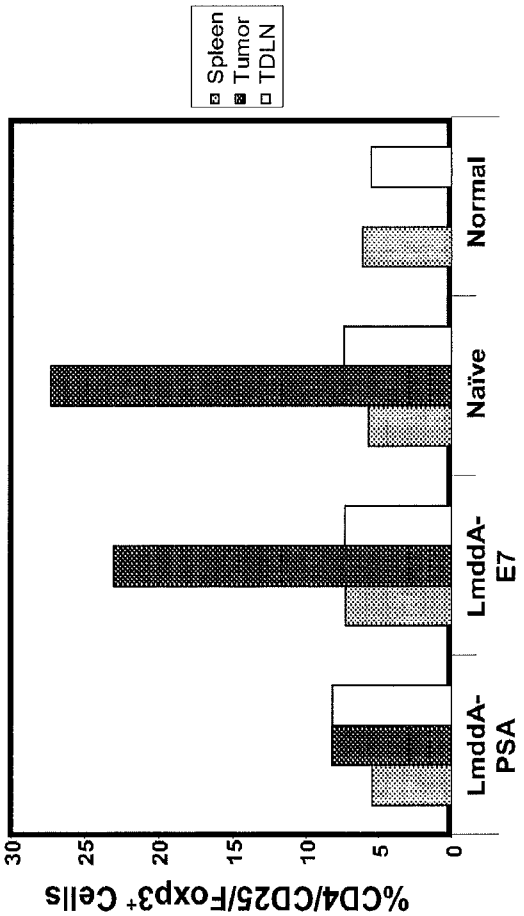

Spleen
Tumor
TDLN
%CD4/CD25/Foxp3+ Cells
0
5
10
15
20
25
30
LmddA-PSA
LmddA-E7
Naïve
Normal

Figure 21

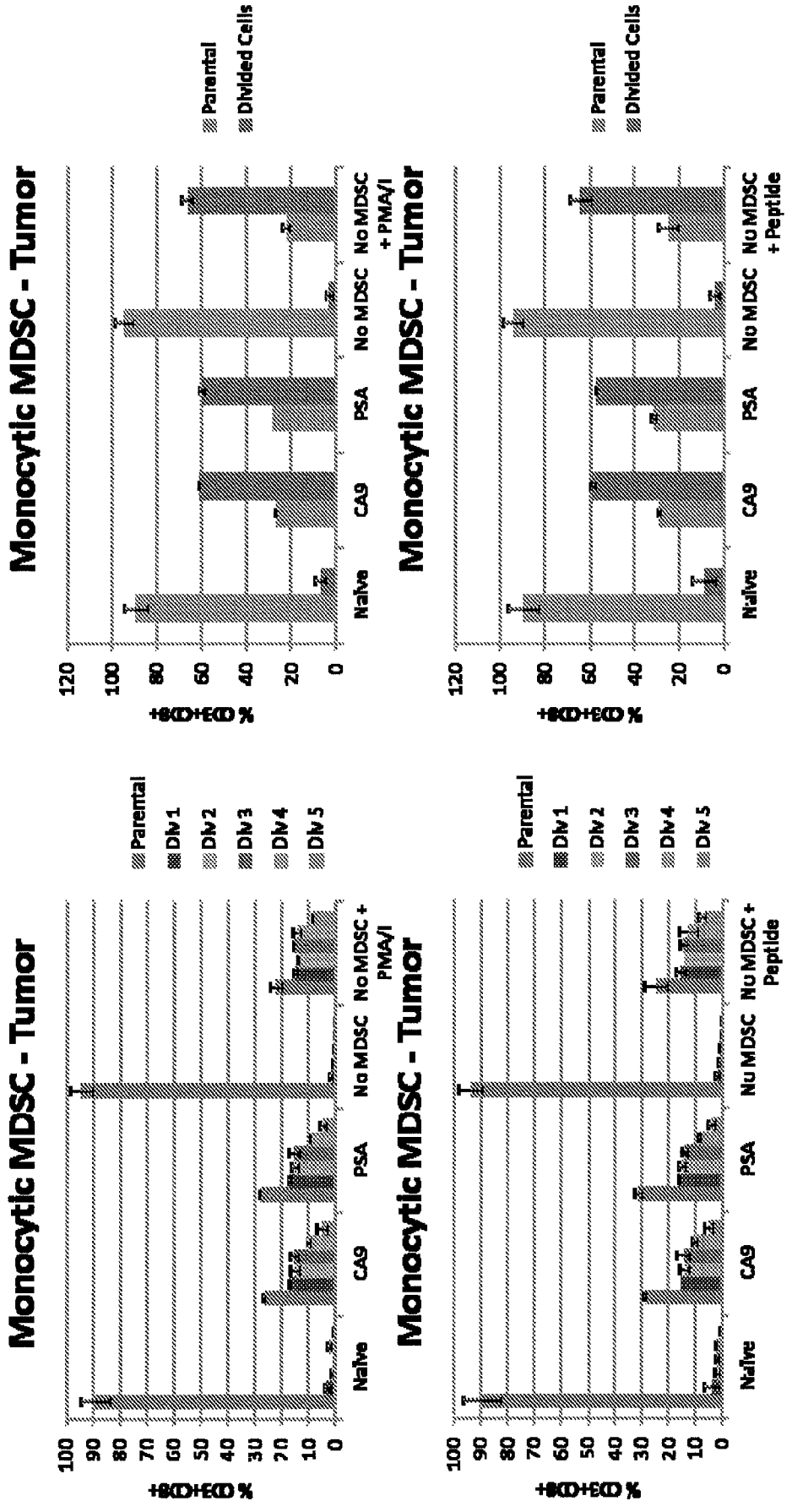

Monocytic MDSC - Tumor
Parental
Divided Cells
% CD3+CD8+
Naïve
CA9
PSA
No MDSC
No MDSC + PMA/I
Nu MDSC
Nu MDSC + Peptide
Div 1
Div 2
Div 3
Div 4
Div 5

Figure 22

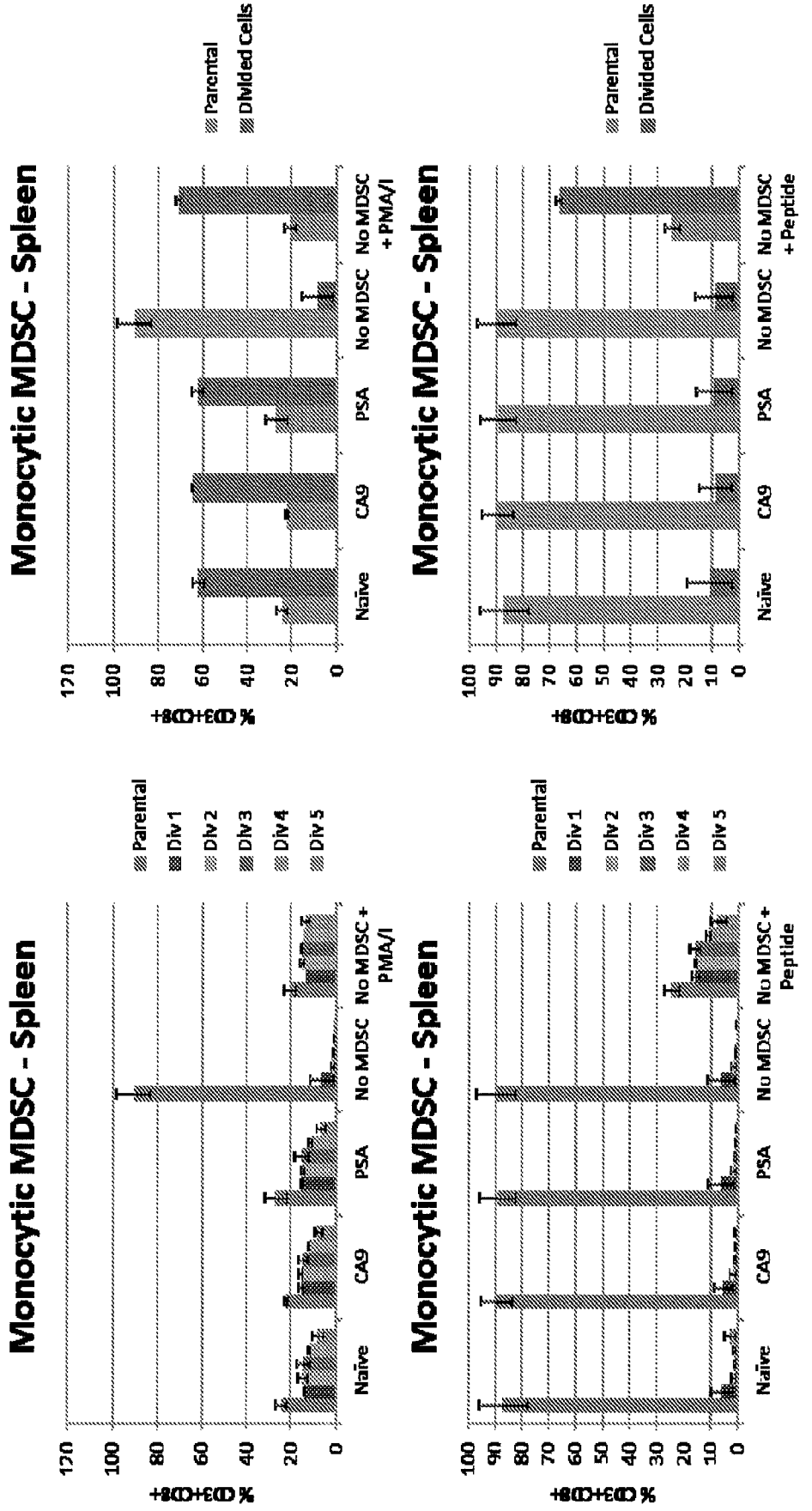

Monocytic MDSC - Spleen
% CD3+CD8+
Parental
Divided Cells
Naïve
CA9
PSA
No MDSC
No MDSC + PMA/I
Monocytic MDSC - Spleen
% CD3+CD8+
Parental
Divided Cells
Naïve
CA9
PSA
No MDSC
No MDSC + Peptide
Monocytic MDSC - Spleen
% CD3+CD8+
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
Naïve
CA9
PSA
No MDSC
No MDSC + PMA/I
Monocytic MDSC - Spleen
% CD3+CD8+
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
Naïve
CA9
PSA
No MDSC
No MDSC + Peptide

Figure 23

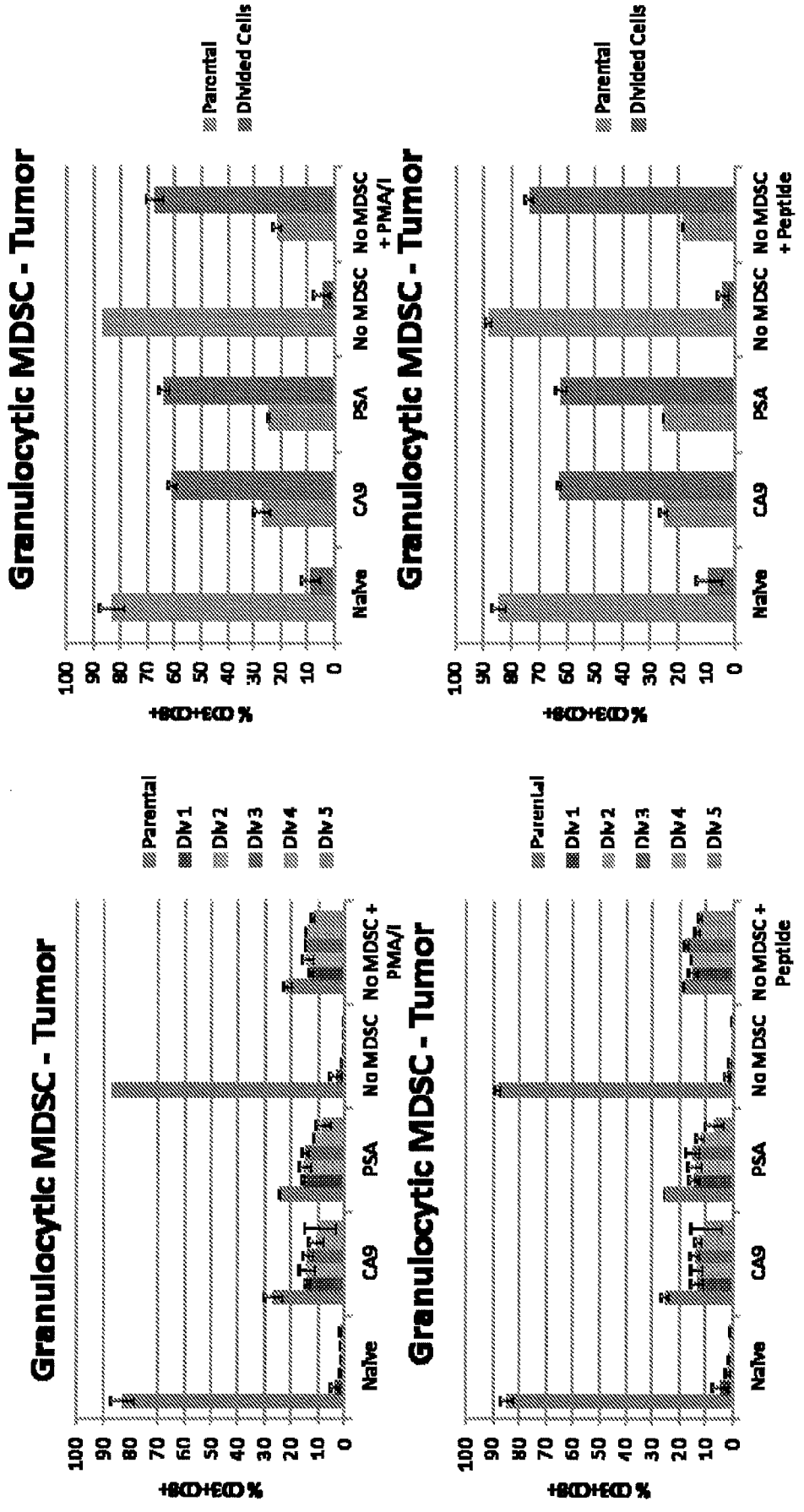

Granulocytic MDSC - Tumor
Parental
Divided Cells
% CD3+CD8+
100
90
80
70
60
50
40
30
20
10
0
Naïve
CA9
PSA
No MDSC
No MDSC + PMA/I
No MDSC + Peptide
Granulocytic MDSC - Tumor
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
% CD3+CD8+
Naïve
CA9
PSA
No MDSC
No MDSC + PMA/I
No MDSC + Peptide

Figure 24

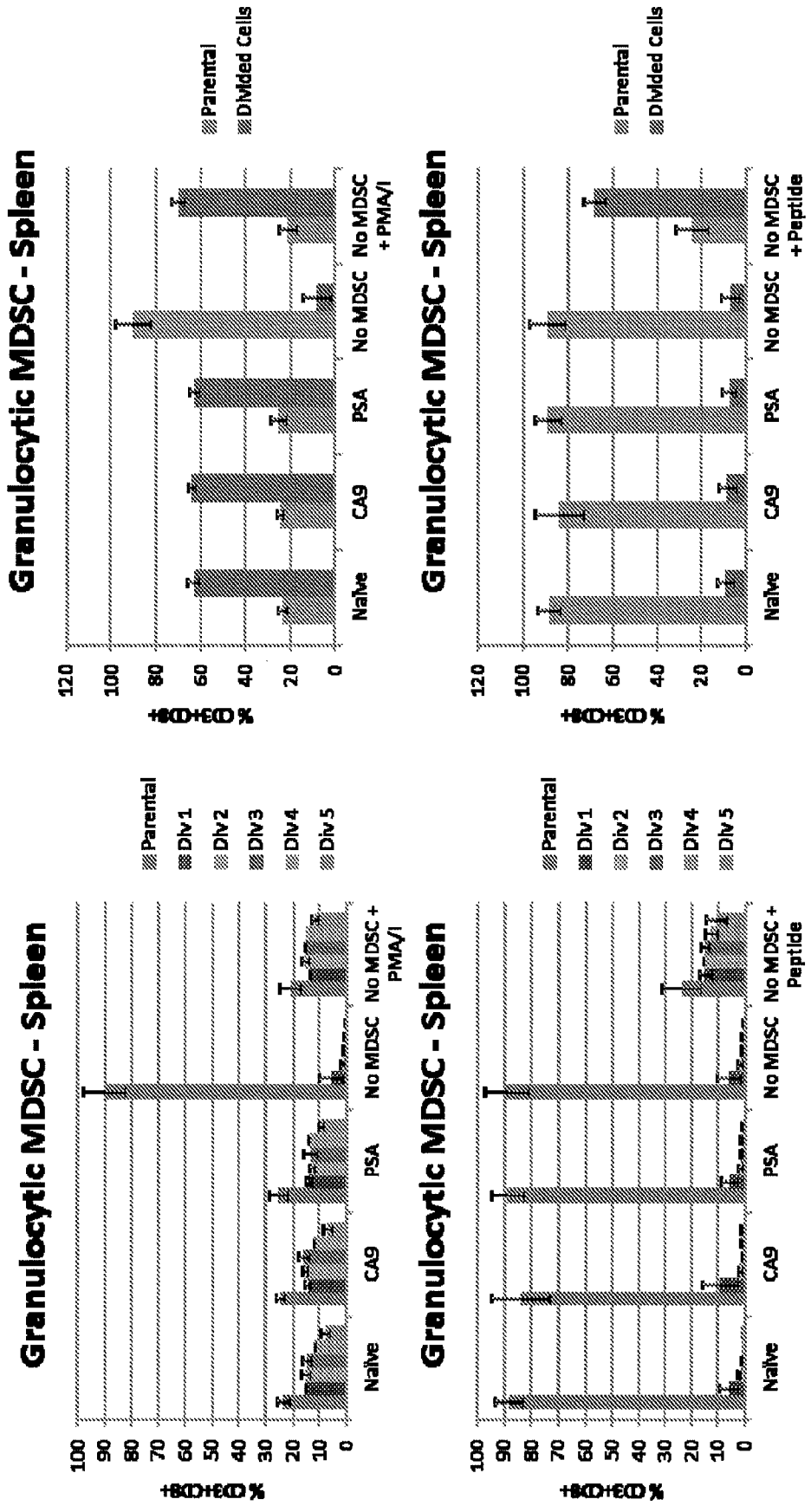

Granulocytic MDSC - Spleen
% CD3+CD8+
Parental
Divided Cells
Naïve
CA9
PSA
No MDSC
No MDSC + PMA/I
Granulocytic MDSC - Spleen
% CD3+CD8+
Parental
Divided Cells
Naïve
CA9
PSA
No MDSC
No MDSC +Peptide
Granulocytic MDSC - Spleen
% CD3+CD8+
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
Naïve
CA9
PSA
No MDSC
No MDSC + PMA/I
Granulocytic MDSC - Spleen
% CD3+CD8+
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
Naïve
CA9
PSA
No MDSC
No MDSC + Peptide

Figure 25

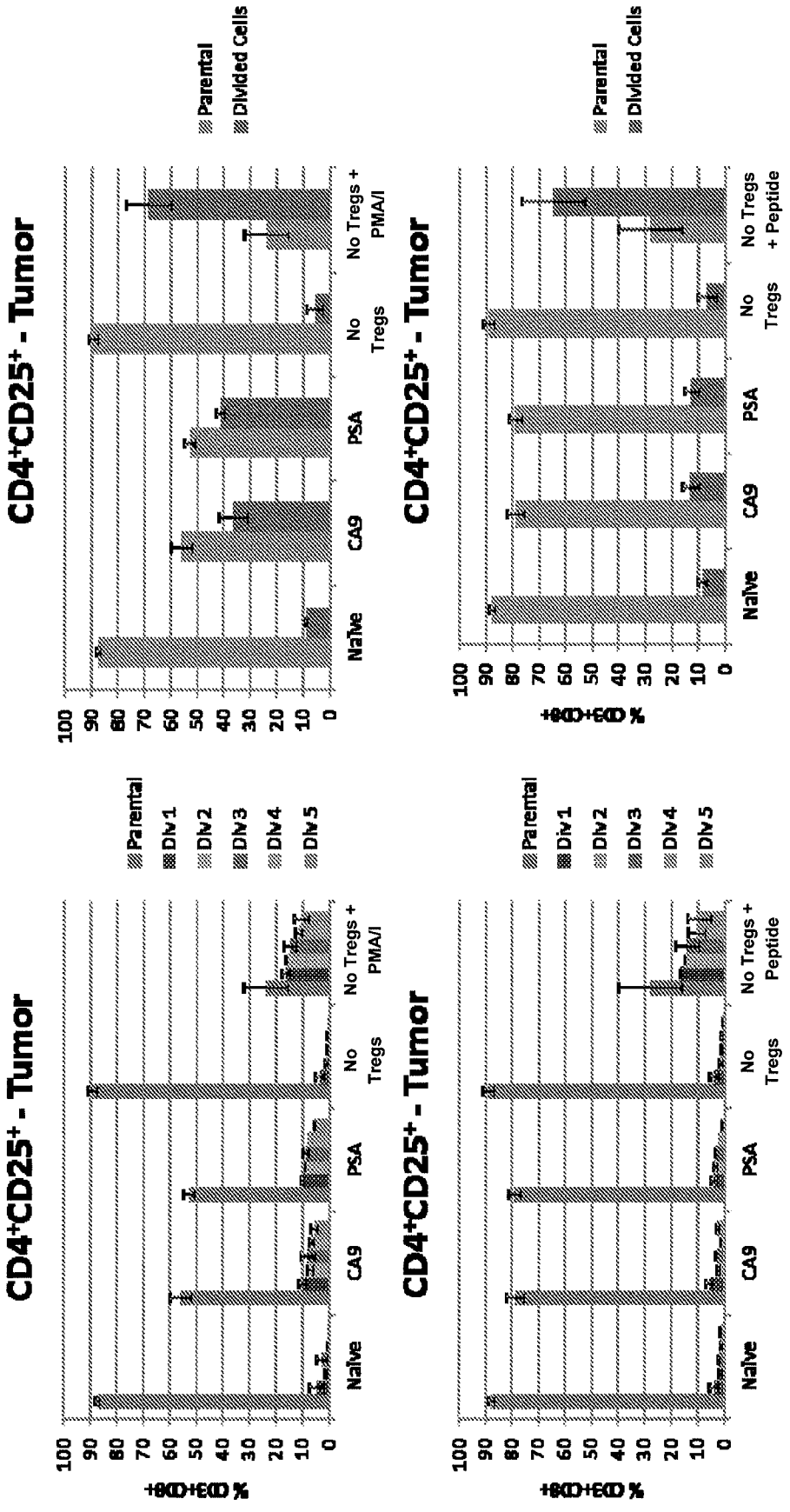

CD4+CD25+ - Tumor
% CD3+CD8+
Parental
Divided Cells
Naïve
CA9
PSA
No Tregs
No Tregs + PMA/I
CD4+CD25+ - Tumor
% CD3+CD8+
Parental
Divided Cells
Naïve
CA9
PSA
No Tregs
No Tregs + Peptide
CD4+CD25+ - Tumor
% CD3+CD8+
Parental
Div1
Div2
Div3
Div4
Div5
Naïve
CA9
PSA
No Tregs
No Tregs + PMA/I
CD4+CD25+ - Tumor
% CD3+CD8+
Parental
Div1
Div2
Div3
Div4
Div5
Naïve
CA9
PSA
No Tregs
No Tregs + Peptide

Figure 26

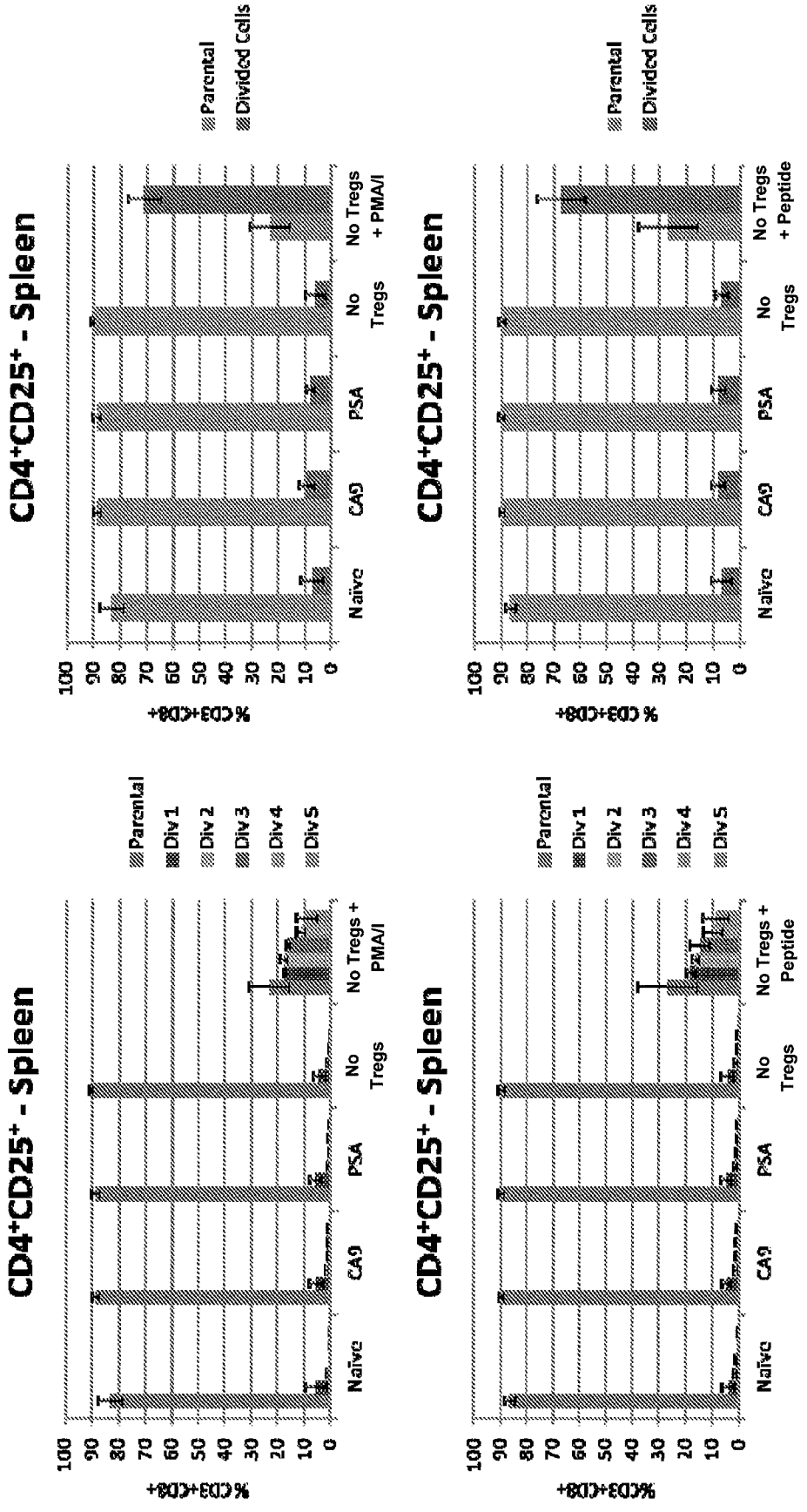

CD4+CD25+ - Spleen
% CD3+CD8+
Parental
Divided Cells
Div 1
Div 2
Div 3
Div 4
Div 5
Naïve
CAD
PSA
No Tregs
No Tregs + PMA/I
No Tregs + Peptide

Figure 27

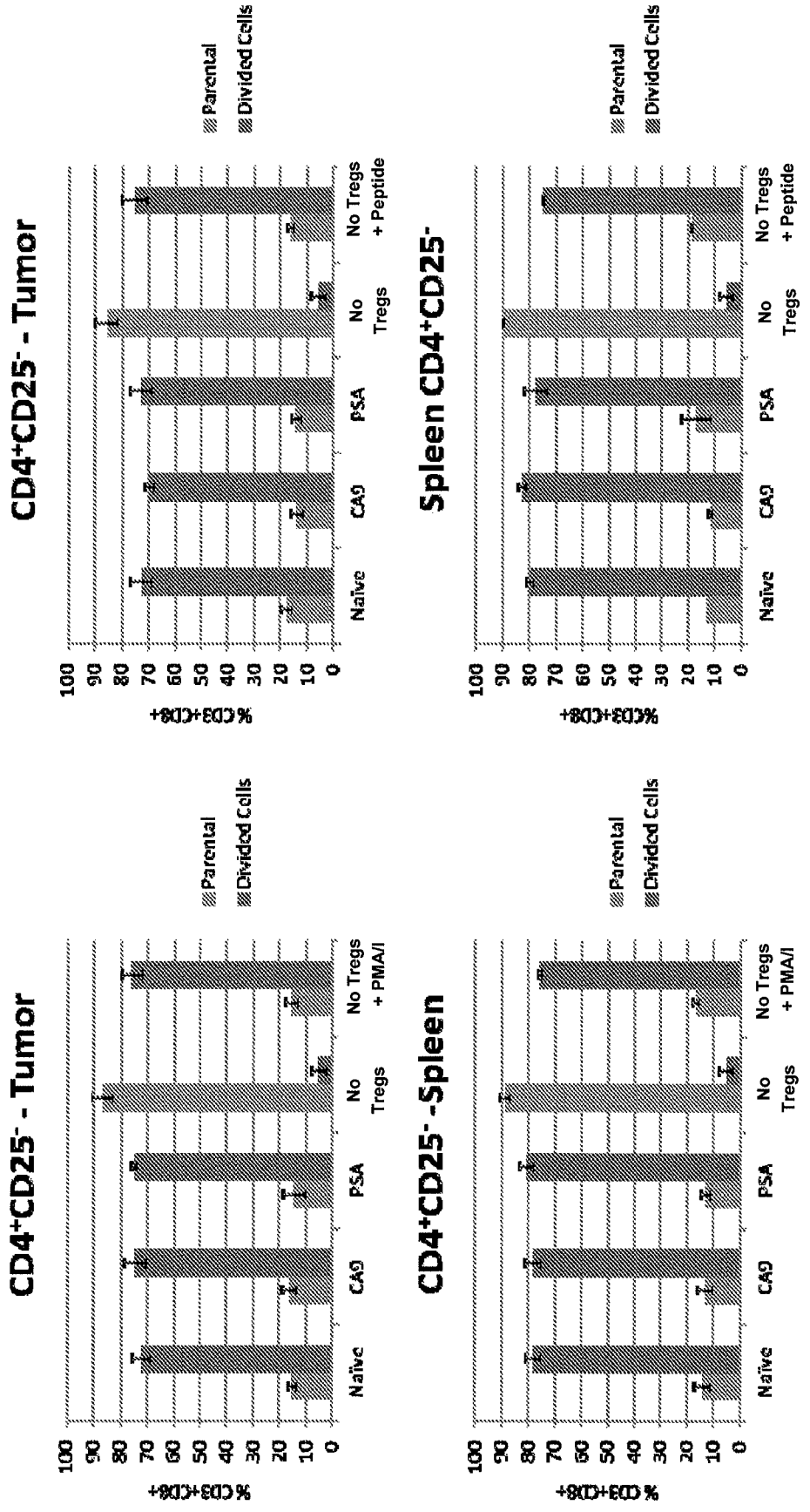

CD4+CD25- - Tumor
Spleen CD4+CD25-
CD4+CD25- - Tumor
CD4+CD25- -Spleen
% CD3+CD8+
Parental
Divided Cells
Naïve
CA9
PSA
No Tregs
No Tregs + Peptide
No Tregs + PMA/I

Figure 28

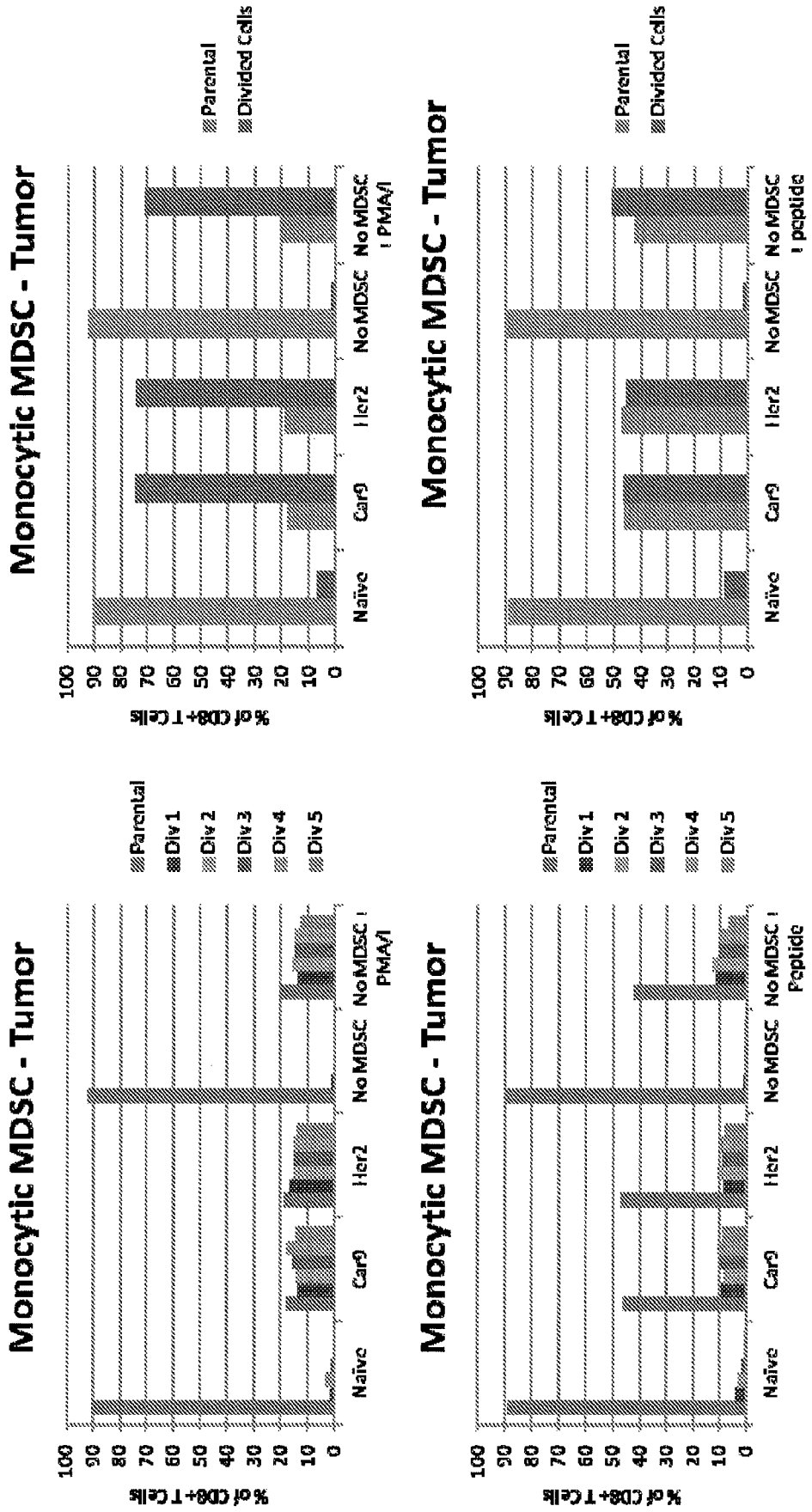

Monocytic MDSC - Tumor
% of CD8+ T Cells
100
90
80
70
60
50
40
30
20
10
0
Naïve
Car9
Her2
No MDSC
No MDSC + PMA/I
Parental
Divided Cells
Monocytic MDSC - Tumor
% of CD8+ T Cells
Naïve
Car9
Her2
No MDSC
No MDSC + peptide
Parental
Divided Cells
Monocytic MDSC - Tumor
% of CD8+ T Cells
Naïve
Car9
Her2
No MDSC
No MDSC + PMA/I
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
Monocytic MDSC - Tumor
% of CD8+ T Cells
Naïve
Car9
Her2
No MDSC
No MDSC + Peptide
Parental
Div 1
Div 2
Div 3
Div 4
Div 5

Figure 29

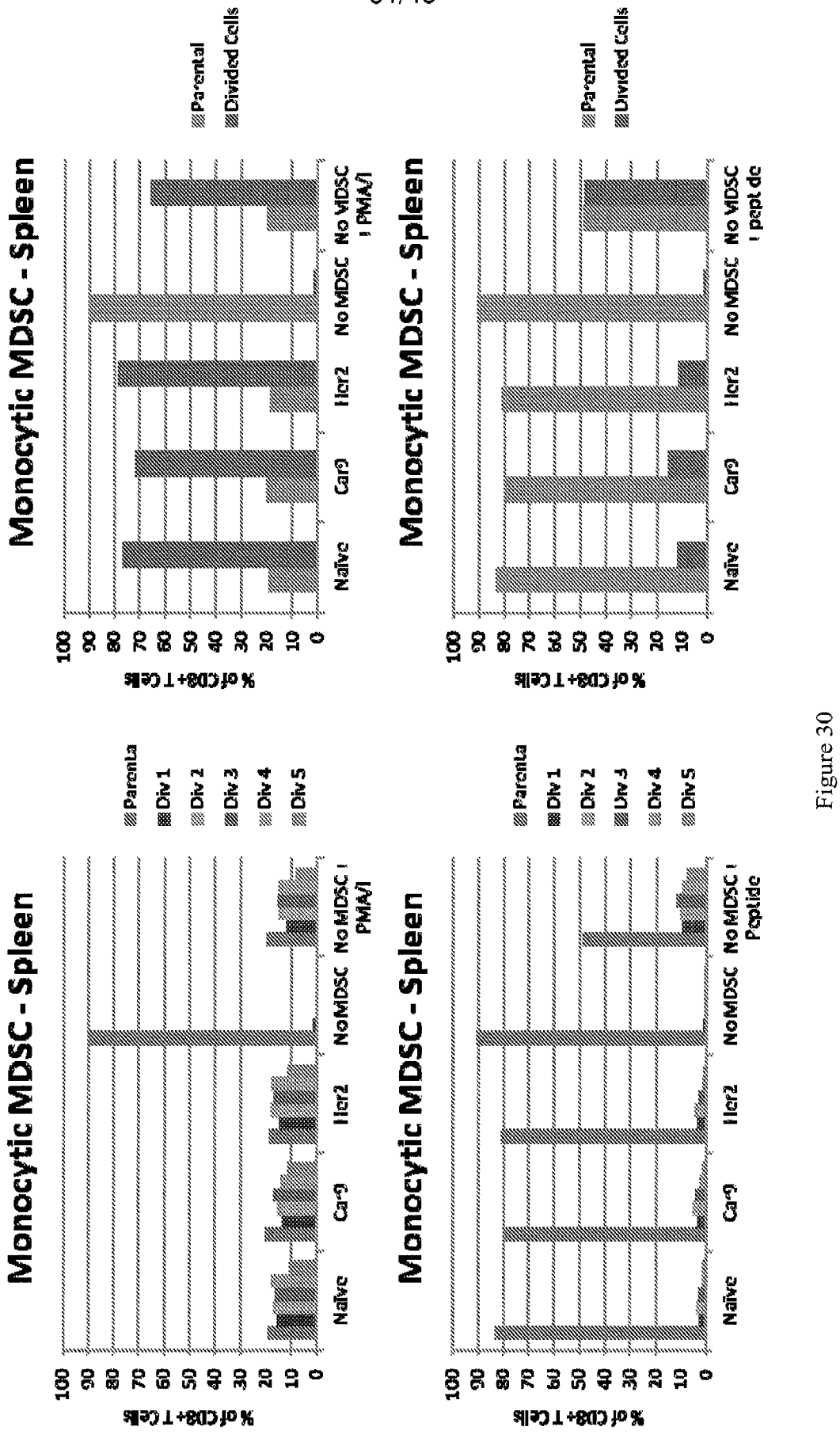

Monocytic MDSC - Spleen
% of CD8+ T Cells
Naive
Car9
Her2
No MDSC
No MDSC + PMA/I
Parental
Divided Cells
Monocytic MDSC - Spleen
% of CD8+ T Cells
Naive
Car9
Her2
No MDSC
No MDSC + pept de
Parental
Divided Cells
Monocytic MDSC - Spleen
% of CD8+ T Cells
Naive
Car9
Her2
No MDSC
No MDSC + PMA/I
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
Monocytic MDSC - Spleen
% of CD8+ T Cells
Naive
Car9
Her2
No MDSC
No MDSC + Peptide
Parental
Div 1
Div 2
Div 3
Div 4
Div 5

Figure 30

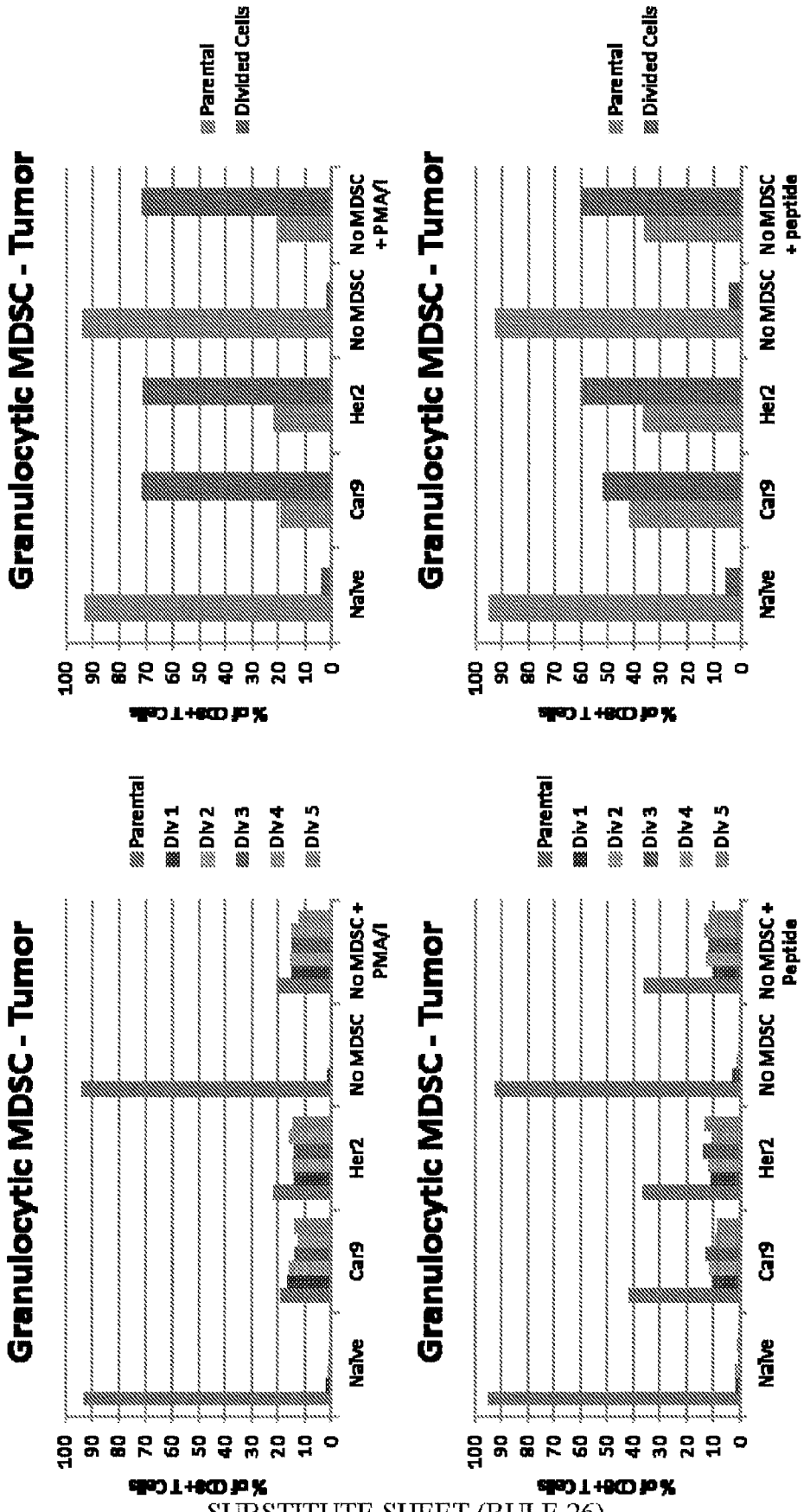

Granulocytic MDSC - Tumor
% of CD8+ T Cells
100
90
80
70
60
50
40
30
20
10
0
Naïve
Car9
Her2
No MDSC
No MDSC + PMA/I
Parental
Divided Cells
Granulocytic MDSC - Tumor
% of CD8+ T Cells
Naïve
Car9
Her2
No MDSC
No MDSC + peptide
Parental
Divided Cells
Granulocytic MDSC - Tumor
% of CD8+ T Cells
Naïve
Car9
Her2
No MDSC
No MDSC + PMA/I
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
Granulocytic MDSC - Tumor
% of CD8+ T Cells
Naïve
Car9
Her2
No MDSC
No MDSC + Peptide
Parental
Div 1
Div 2
Div 3
Div 4
Div 5

Figure 31

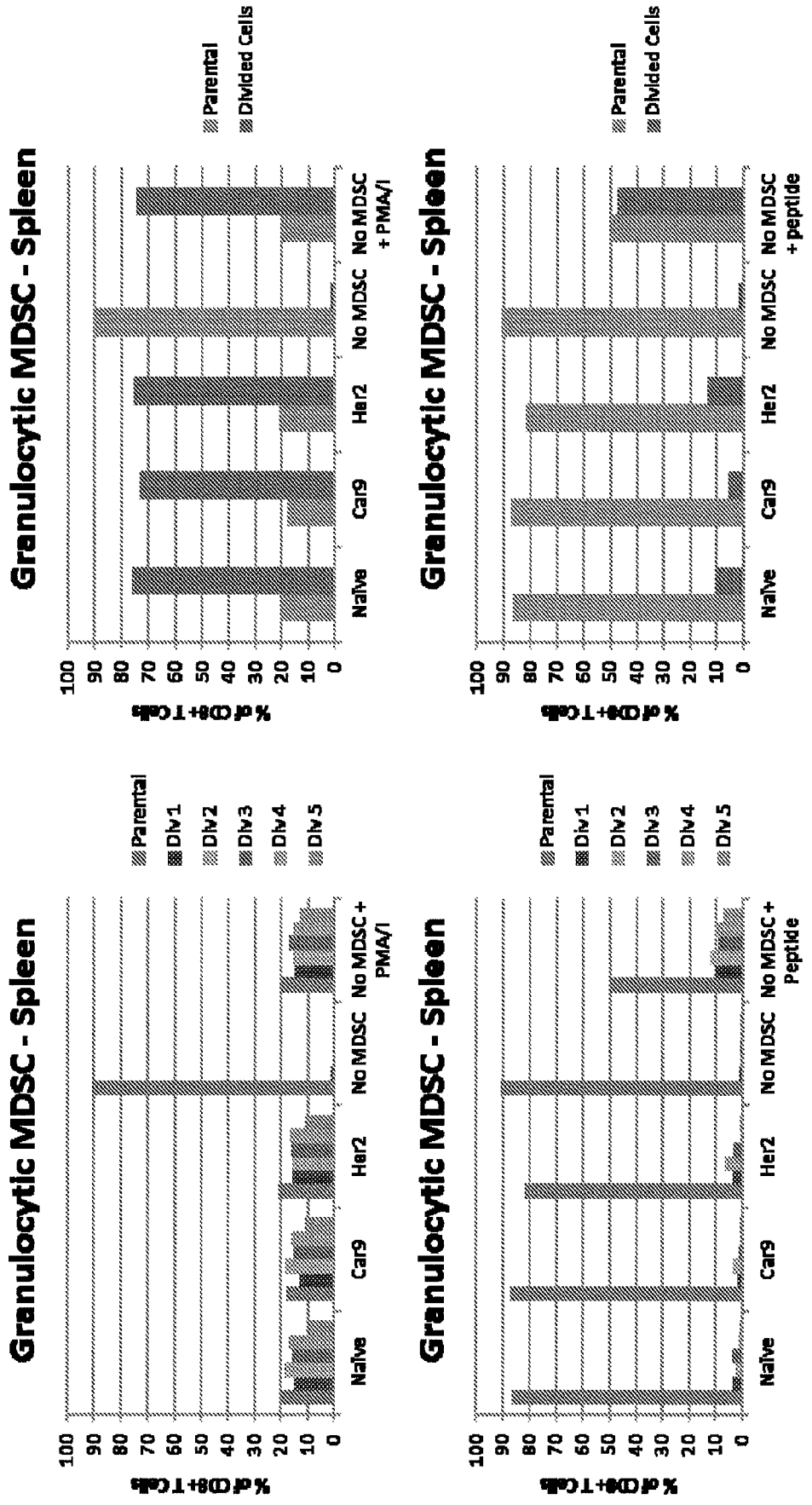

Granulocytic MDSC - Spleen
% of CD8+ T Cells
100
90
80
70
60
50
40
30
20
10
0
Parental
Divided Cells
Naïve
Car9
Her2
No MDSC
No MDSC + PMA/I
Granulocytic MDSC - Spleen
% of CD8+ T Cells
Parental
Divided Cells
Naïve
Car9
Her2
No MDSC
No MDSC + peptide
Granulocytic MDSC - Spleen
% of CD8+ T Cells
Parental
Div1
Div2
Div3
Div4
Div5
Naïve
Car9
Her2
No MDSC
No MDSC + PMA/I
Granulocytic MDSC - Spleen
% of CD8+ T Cells
Parental
Div1
Div2
Div3
Div4
Div5
Naïve
Car9
Her2
No MDSC
No MDSC + Peptide

Figure 32

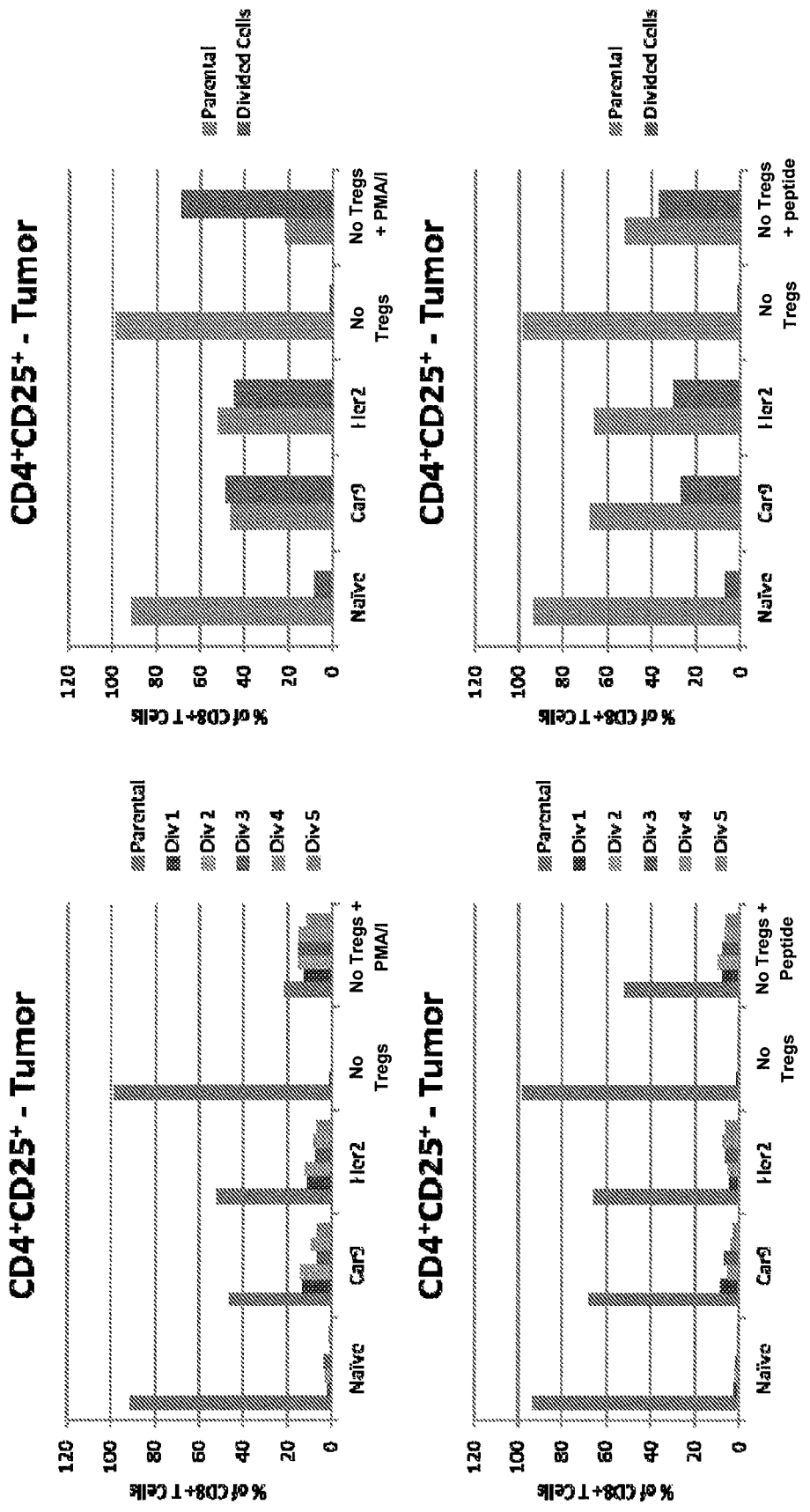

CD4+CD25+ - Tumor
% of CD8+ T cells
120
100
80
60
40
20
0
Parental
Divided Cells
Naive
Car9
Her2
No Tregs
No Tregs + PMA/I
CD4+CD25+ - Tumor
% of CD8+ T cells
Parental
Divided Cells
Naive
Car9
Her2
No Tregs
No Tregs + peptide
CD4+CD25+ - Tumor
% of CD8+ T cells
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
Naive
Car9
Her2
No Tregs
No Tregs + PMA/I
CD4+CD25+ - Tumor
% of CD8+ T cells
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
Naive
Car9
Her2
No Tregs
No Tregs + Peptide

Figure 33

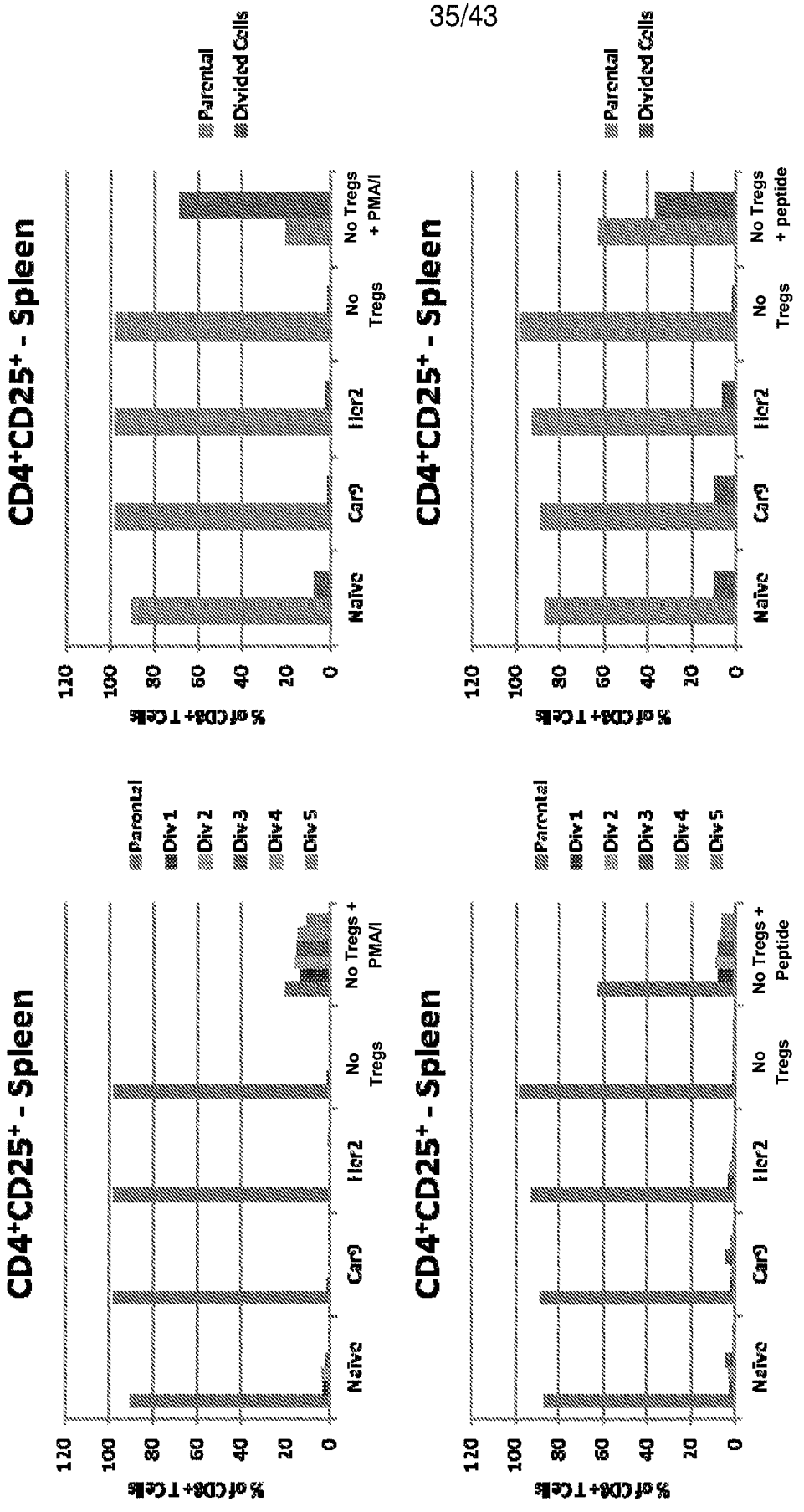

CD4+CD25+ - Spleen
% of CD8+ T Cells
120
100
80
60
40
20
0
Naive
Car9
Her2
No Tregs
No Tregs + PMA/I
No Tregs + peptide
Parental
Divided Cells
Div 1
Div 2
Div 3
Div 4
Div 5

Figure 34

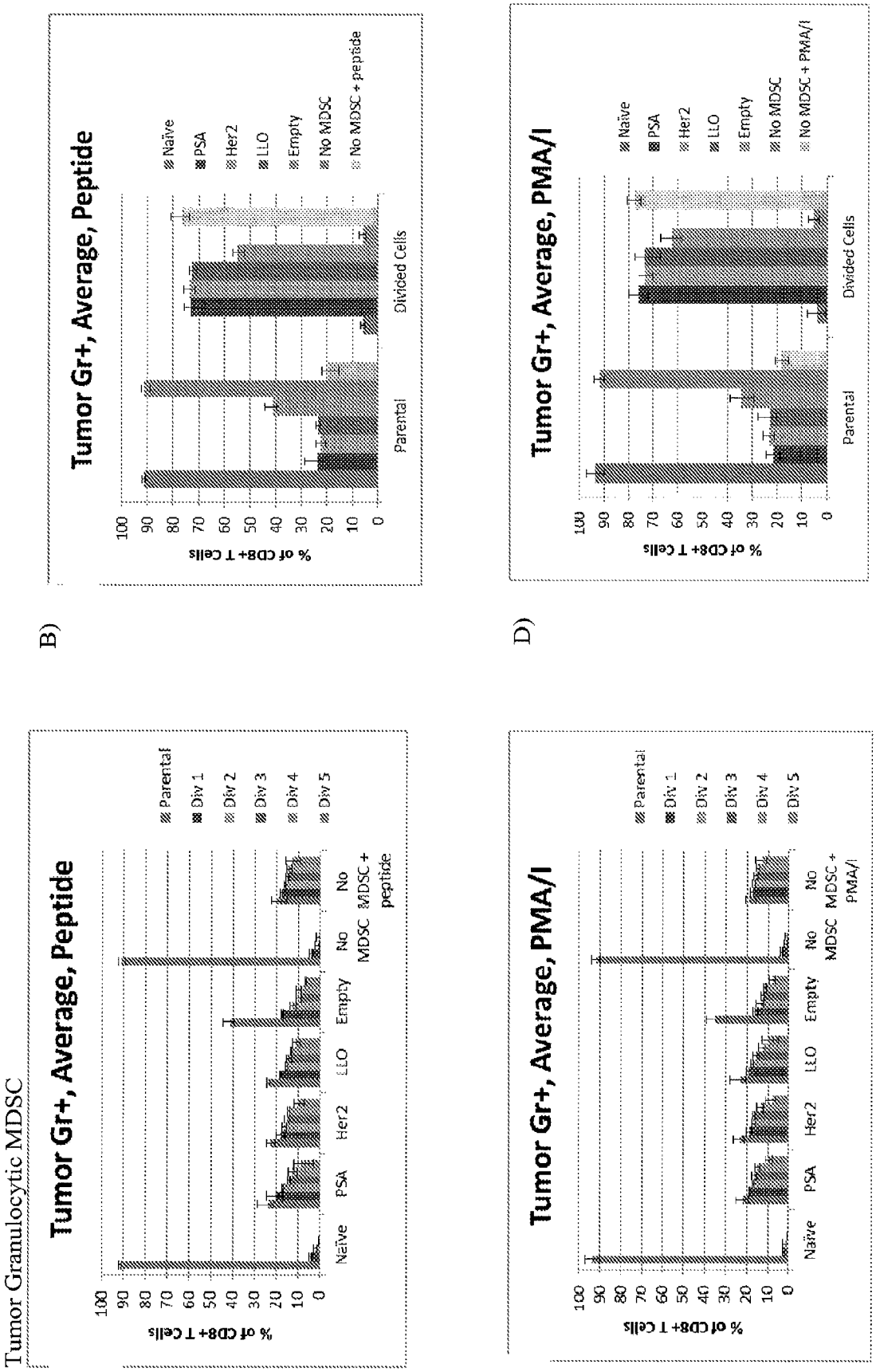
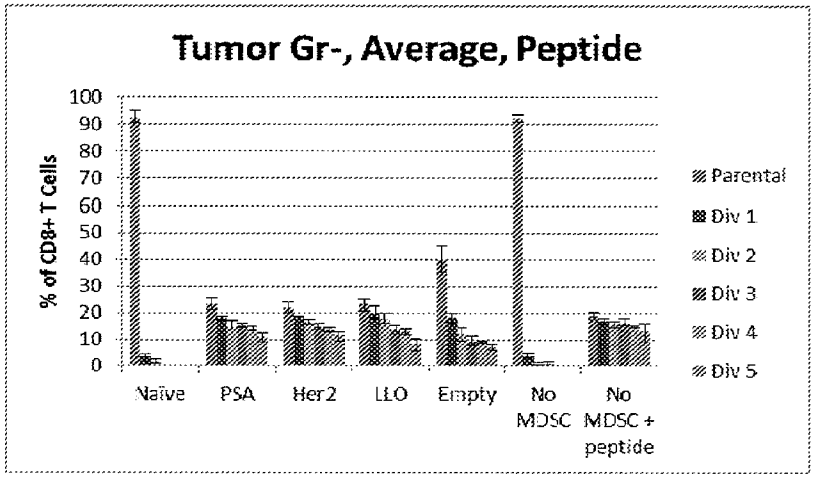

Tumor Granulocytic MDSC
A)
Tumor Gr+, Average, Peptide
% of CD8+ T cells
Naïve
PSA
Her2
LLO
Empty
No MDSC
No MDSC + peptide
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
B)
Tumor Gr+, Average, Peptide
Parental
Divided Cells
C)
Tumor Gr+, Average, PMA/I
No MDSC + PMA/I
D)
Tumor Gr+, Average, PMA/I

Figure 35

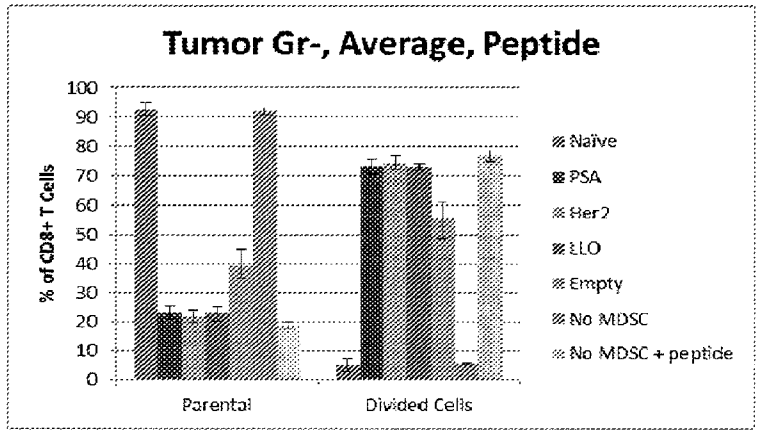
SUBSTITUTE SHEET (RULE 26)

Tumor monocytic MDSC

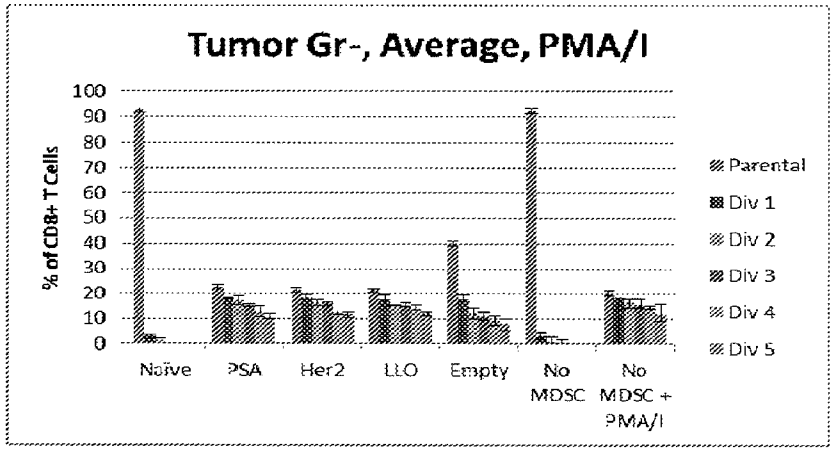
A)



B)



C)



D)

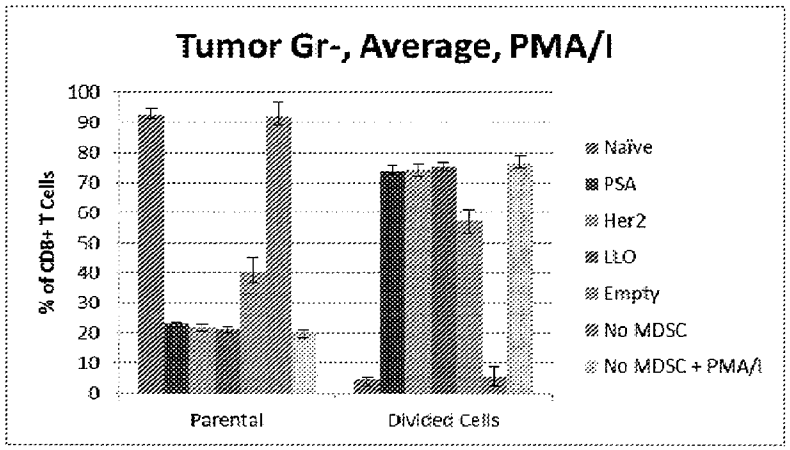
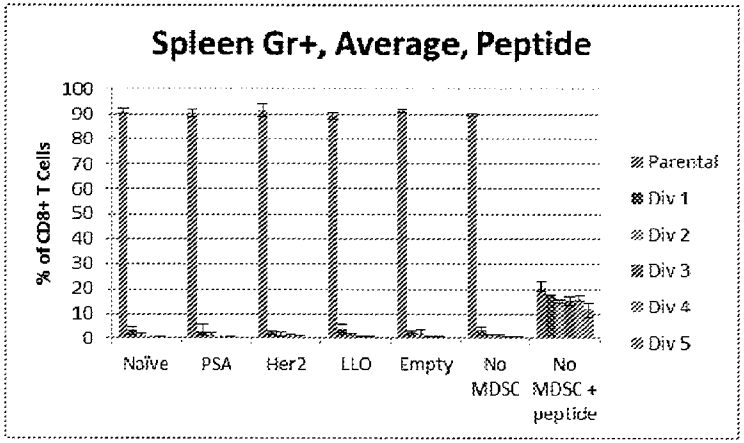


Figure 36

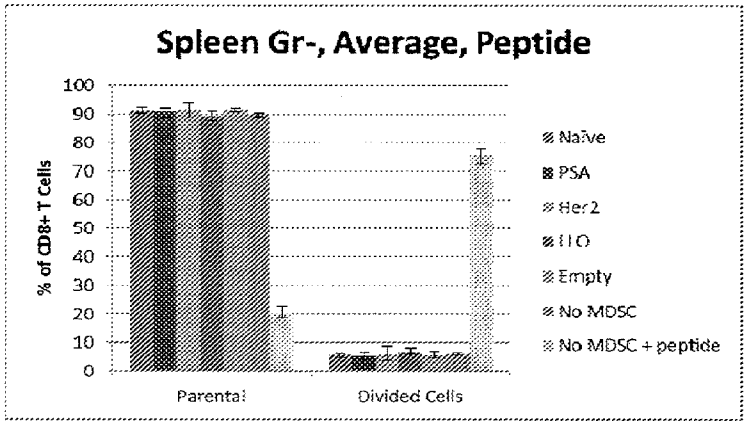
SUBSTITUTE SHEET (RULE 26)

Spleen Granulocytic MDSC

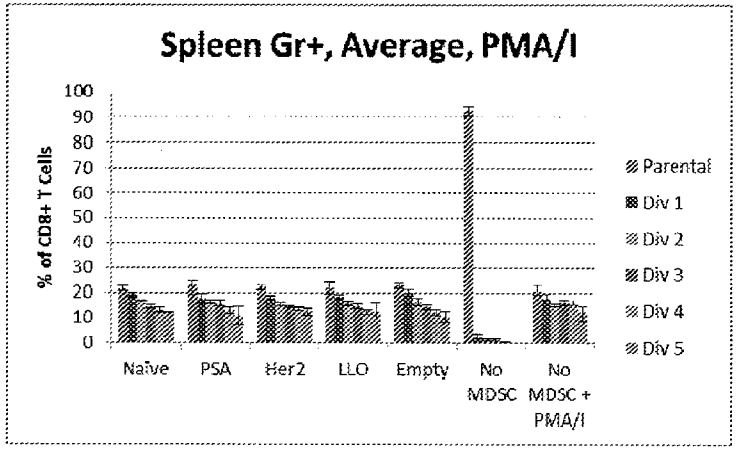
A)



B)



C)



D)

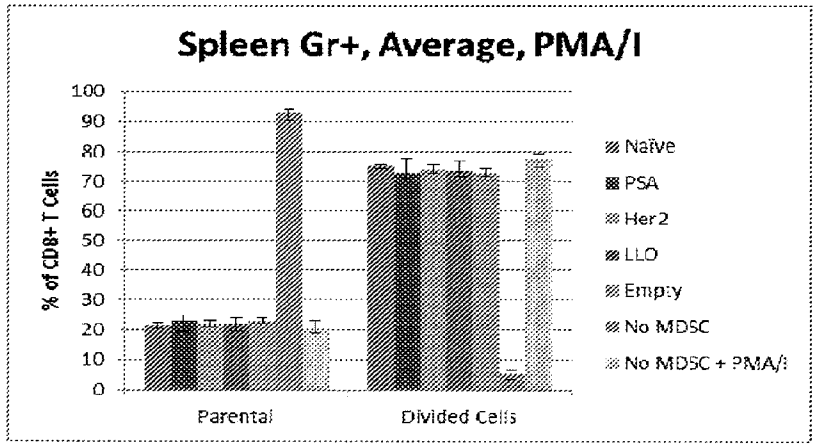
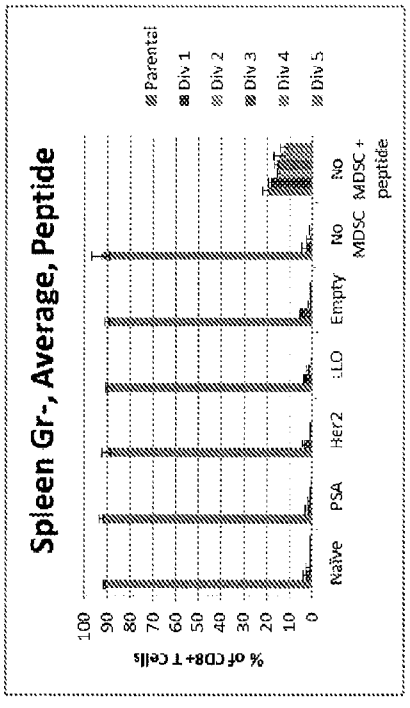


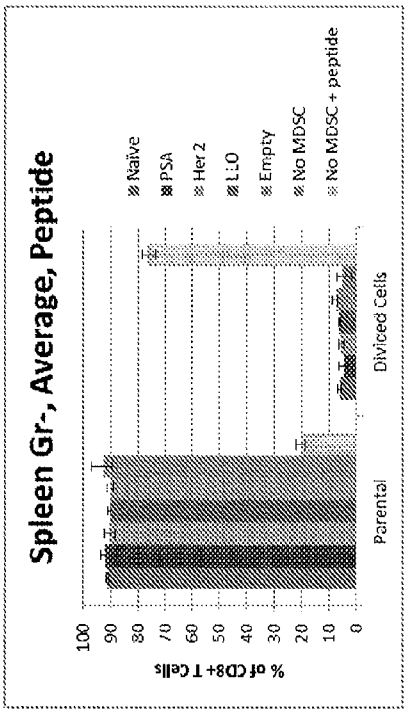
Figure 37

Spleen Monocytic MDSC

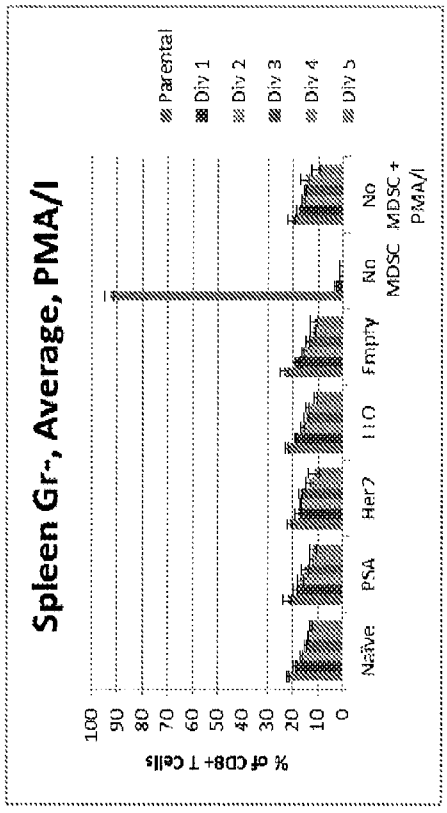
A)



B)



C)



D)

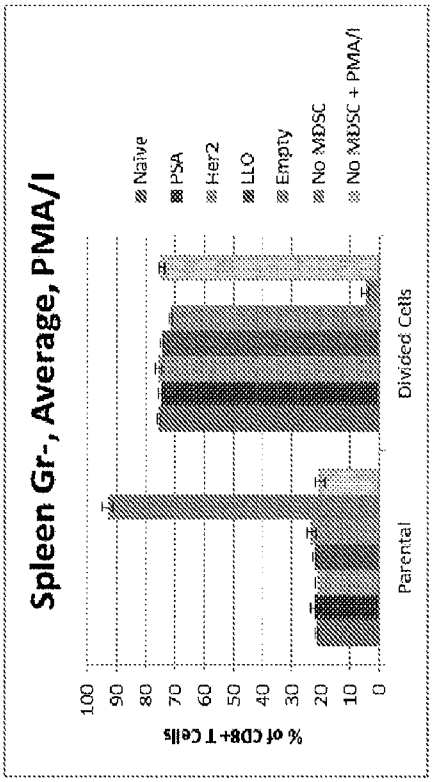
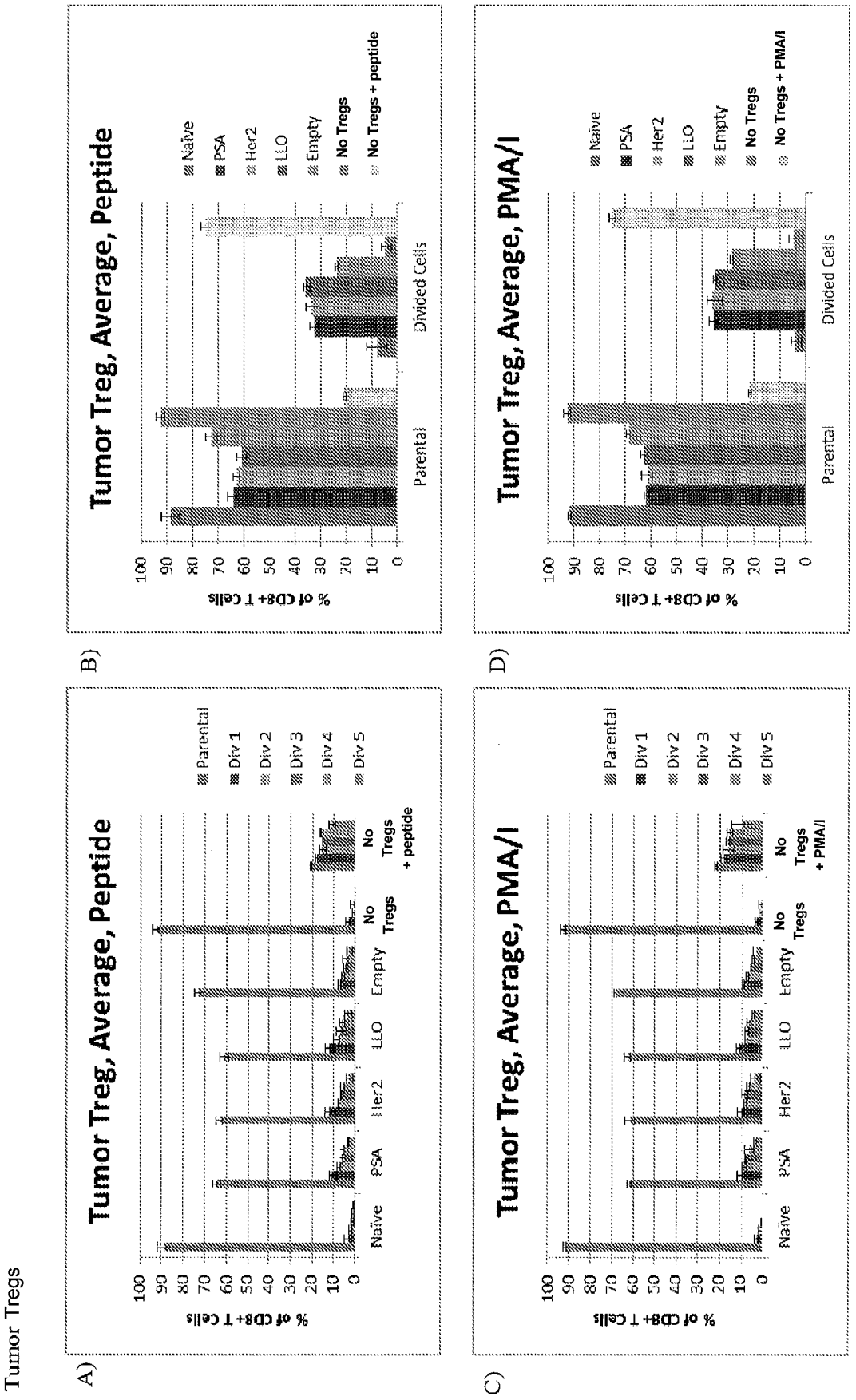


Figure 38



Tumor Tregs

Figure 39

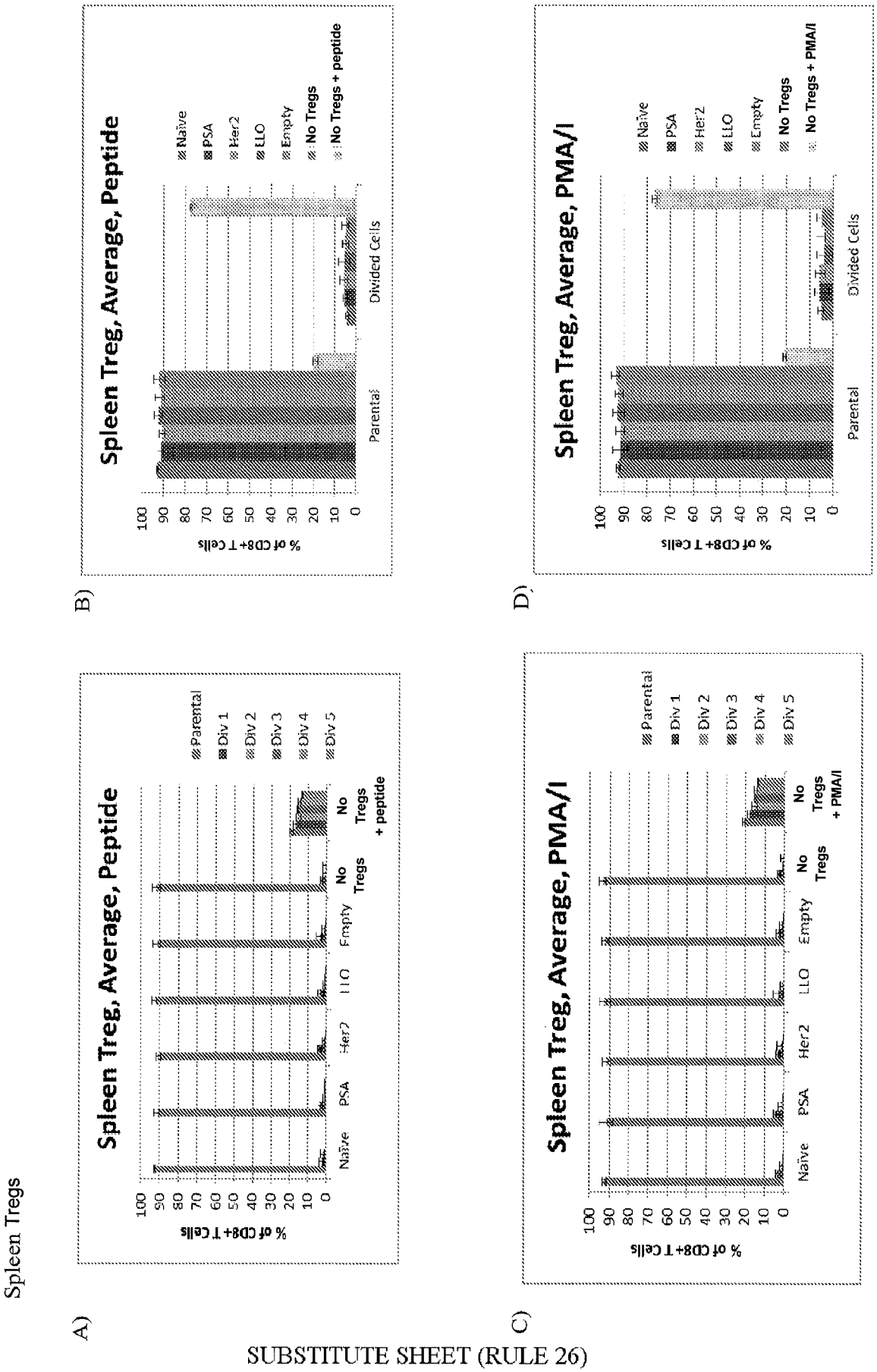
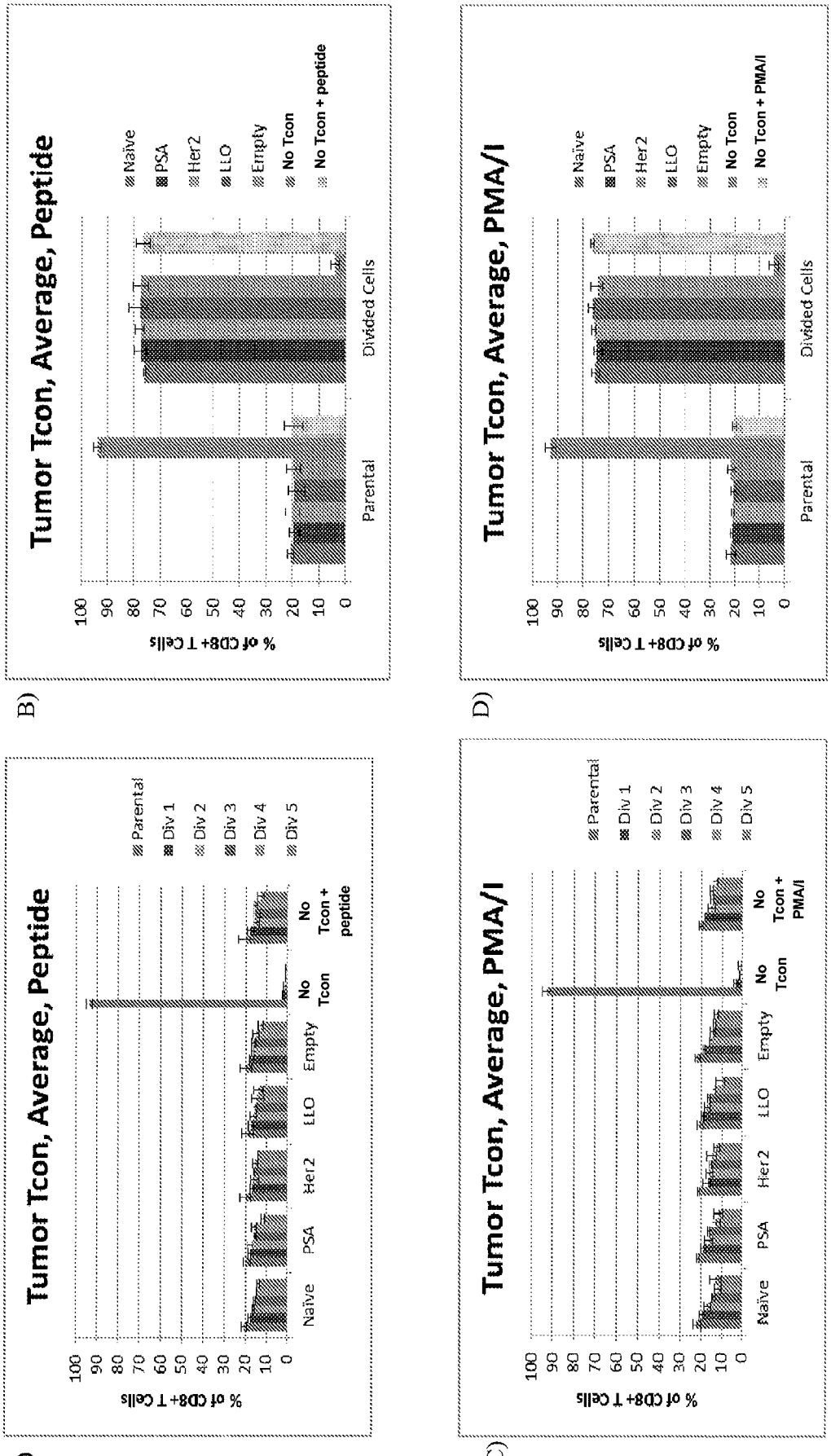

Spleen Tregs
A)
Spleen Treg, Average, Peptide
% of CD8+ T cells
Naive
PSA
Her2
LLO
Empty
No Tregs
No Tregs + peptide
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
B)
Spleen Treg, Average, Peptide
% of CD8+ T cells
Parental
Divided Cells
Naive
PSA
Her2
LLO
Empty
No Tregs
No Tregs + peptide
C)
Spleen Treg, Average, PMA/I
% of CD8+ T cells
Naive
PSA
Her2
LLO
Empty
No Tregs
No Tregs + PMA/I
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
D)
Spleen Treg, Average, PMA/I
% of CD8+ T cells
Parental
Divided Cells
Naive
PSA
Her2
LLO
Empty
No Tregs
No Tregs + PMA/I

Figure 40



Tumor Tcon

Figure 41

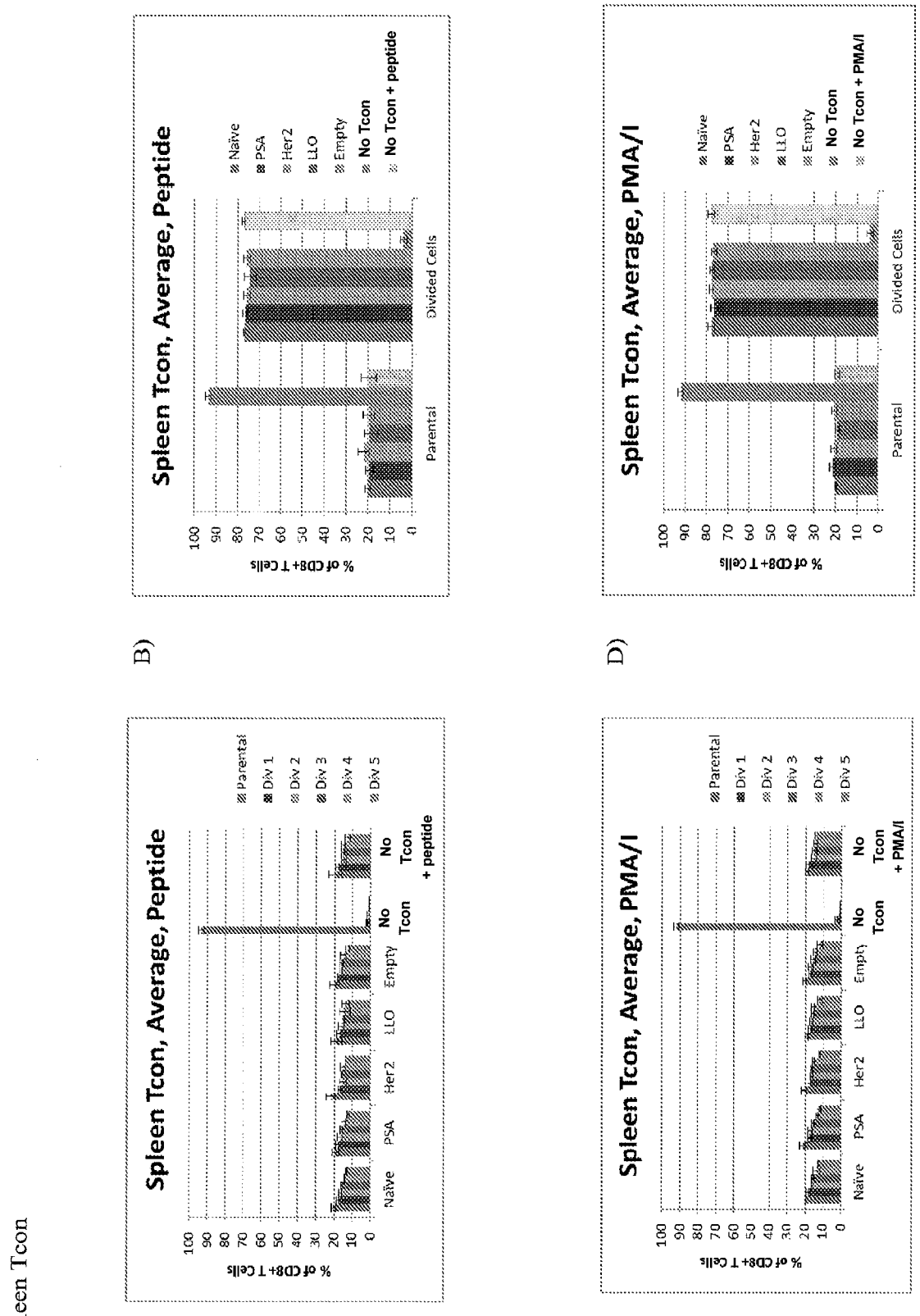

Spleen Tcon
A)
Spleen Tcon, Average, Peptide
% of CD8+ T Cells
Naive
PSA
Her2
LLO
Empty
No Tcon
No Tcon + peptide
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
B)
Spleen Tcon, Average, Peptide
Parental
Divided Cells
C)
Spleen Tcon, Average, PMA/I
No Tcon + PMA/I
D)
Spleen Tcon, Average, PMA/I
Parental
Divided Cells

Figure 42