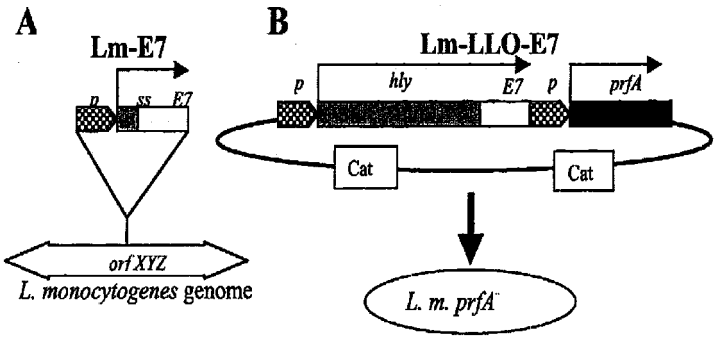


Figure 1

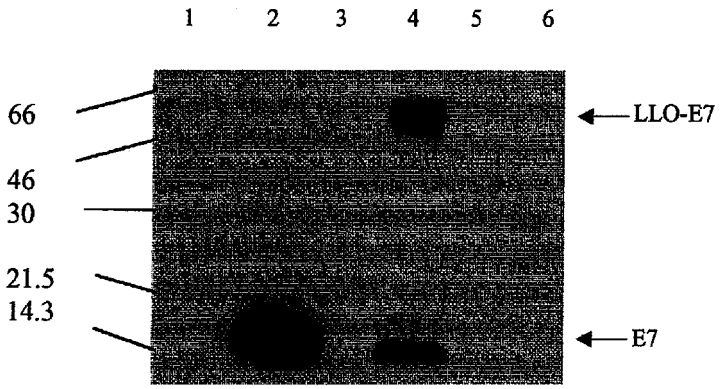


Figure 2

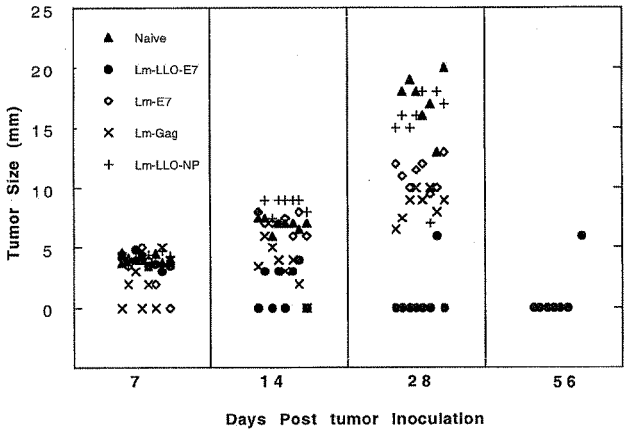

25
20
15
10
5
0
Tumor Size (mm)
Naive
Lm-LLO-E7
Lm-E7
Lm-Gag
Lm-LLO-NP
7
14
28
56
Days Post tumor inoculation

Figure 3

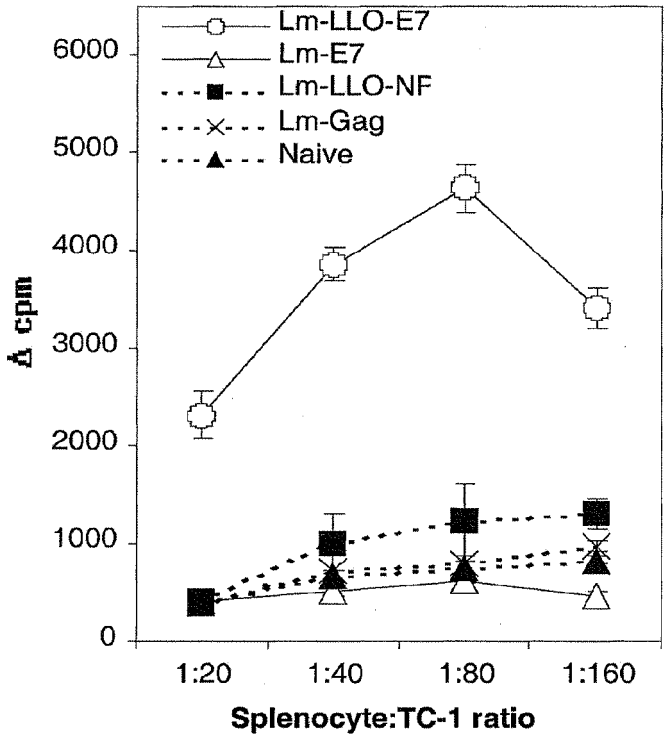

Lm-LLO-E7
Lm-E7
Lm-LLO-NF
Lm-Gag
Naive
6000
5000
4000
3000
2000
1000
0
Δ cpm
1:20
1:40
1:80
1:160
Splenocyte:TC-1 ratio

Figure 4

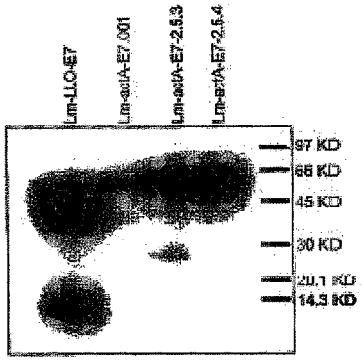


Figure 5A

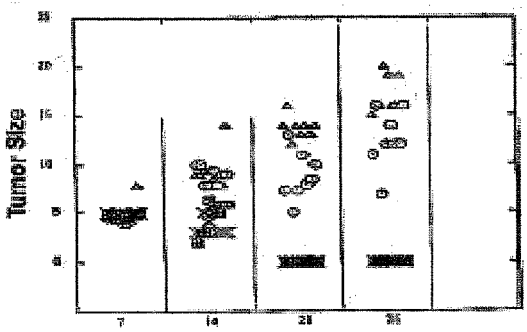


Figure 5B

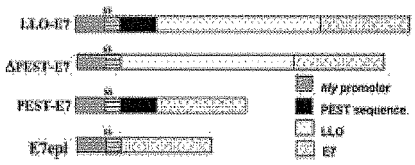
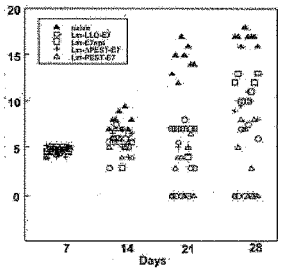


Figure 6A



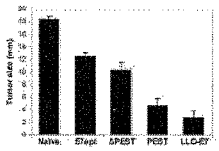


Figure 6B

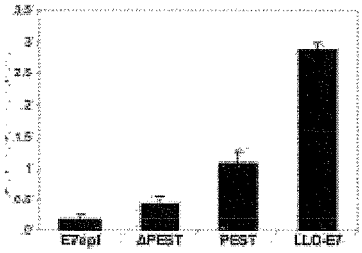


Figure 6C

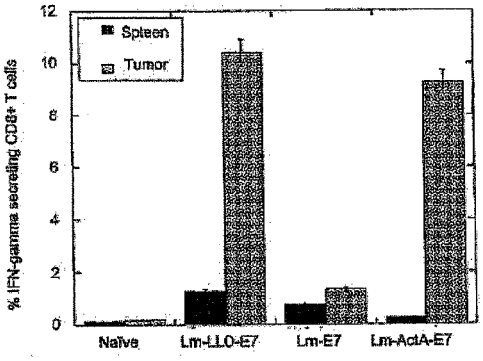


Figure 7A

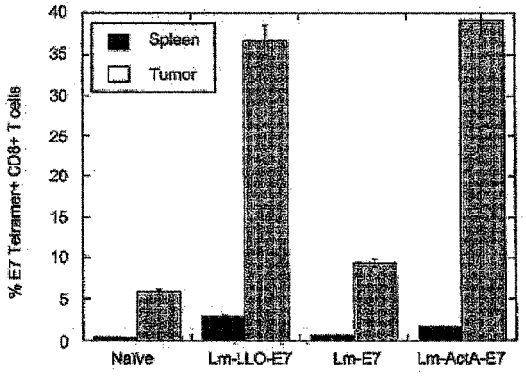


Figure 7B

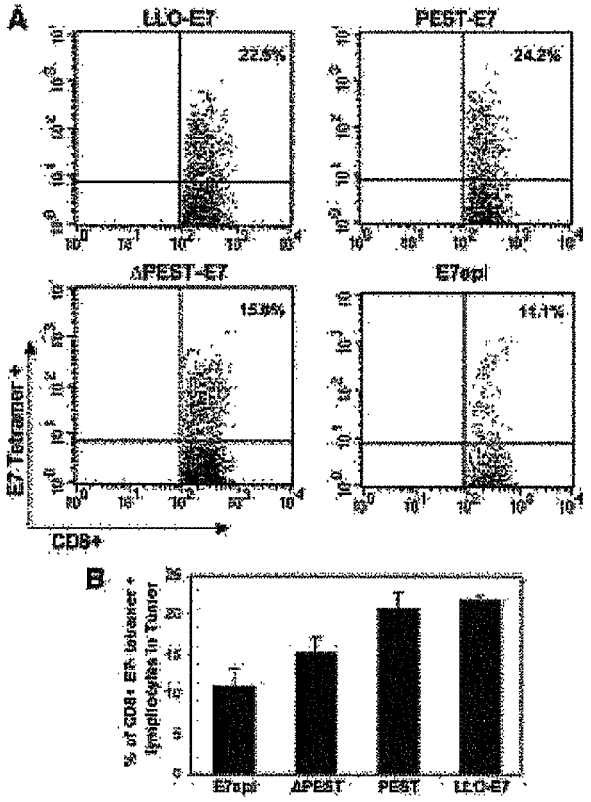

A
LLO-E7
22.5%
PEST-E7
24.2%
ΔPEST-E7
15.8%
E7epi
14.1%
E7 Tetramer +
CD8+
B
% of CD8+ E7- tetramer + lymphocytes in Tumor
E7epi
ΔPEST
PEST
LLO-E7

Figure 8

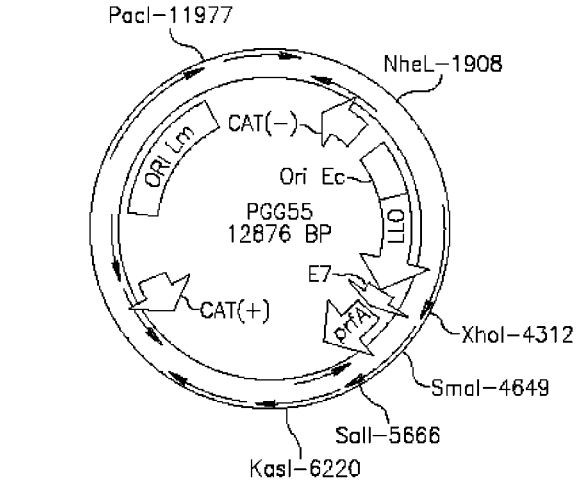


Figure 9A

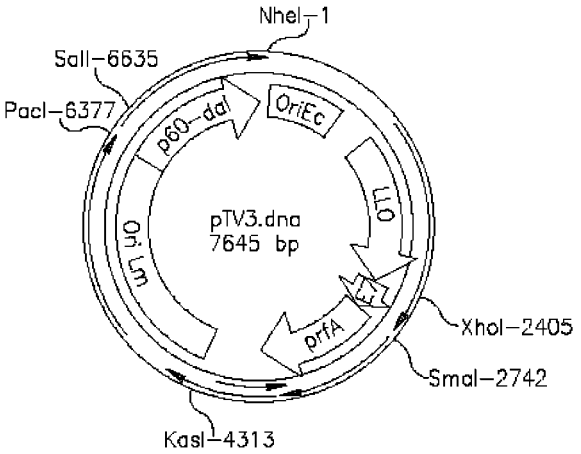


Figure 9B

atggcgcgggatggtatactatacaagcgtatggttcaaaaagatactttgaattaagaagt
acaataaagttaacttcattagacaaaaagaaaaaacaaggaagaatagtacatagttataa
atacttggagagtgaggtgtaatatgggggcagctgatttttgggggtttcatatatgtagtt
tcaagattagccattgttgcggcagtagtttacttcttatacttattgagaaaaattgcaaa
taaatagaaaaaaagccttgtcaaacgaggctttttttatgcaaaaaatacgacgaatgaag
ccatgtgagacaatttggaatagcagacaacaaggaaggtagaacatgttttgaaaaattta
ctgattttcgattattattaacgcttgttaatttaaacatctcttatttttgctaacatata
agtatacaaagggacataaaaaggttaacagcgtttgttaaataggaagtatatgaaaatcc
*tcttttgtgtttctaaatttatttttaaggagtggaga***atgttgaaaaaaaataattggtta**
caaaatgcagtaatagcaatgctagtgttaattgtaggtctgtgcattaatatgggttctgg
aacaaaagtacaagctgagagtattcaacgaccaacgcctattaaccaagtttttccagatc
ccggcctagcgaatgcagtgaaacaaaatttagggaagcaaagtgttacagaccttgtatca
caaaaggaactatctggagtacaaaatttcaatggagataatagcaacattcaatctcttgc
gggaatgcaatttttcactaatttaaaagaacttcatctatcccataatcaaataagtgacc
ttagtcctttaaaggatctaactaagttagaagagctatctgtgaatagaaacagactgaaa
aatttaaacggaattccaagtgcttgtttatctcgcttgtttttagataacaacgaactcag
agatactgactcgcttattcatttgaaaaatctagaaatcttatctattcgtaataataagt
taaaaagtattgtgatgcttggtttttatcaaaactagaggtattagatttgcatggtaat
gaaataacaaatacaggtggactaactagattgaagaaagttaactggatagatttaactgg
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ggttgtgtcctgtgggaattgccagtttatacagatgaagtaagctataagtttagcgaata
tataaacgttggggagactgaggctatatttgatggaacagttacacaacctatcaagaatt
aggacttgtgcacacctgtatactttgagctctcgtataatcacgagagctttttaaaatatg
taagtcttaattatctcttgacaaaaagaacgtttattcgtataaggttaccaagagatgaa
gaaactattttatttacaattcaccttgacaccaaaaactccatatgatatagtaaataagg
ttattaaacaagaaagaagaagcaacccgcttctcgcctcgttaacacgaacgttttcaggc
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cagcccgtttttttatggcctccgaacgaatgagttagcaggccgcagatttgaacagctat
tttctatcttgttgtaacaaaattaagtggaggtggctcaccattagcaaagacatgttggt
aaacgatgggattcgtgcacgtgaagtaagattgatcgaccaagacggtgaacaattaggcg
tgaagagtaaaatcgatgcgcttcaaattgctgaaaaggctaatcttgatctagtgcttgtt
gctccaacagcgaaaccgccagtagctcgta

Figure 10

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

Figure 11

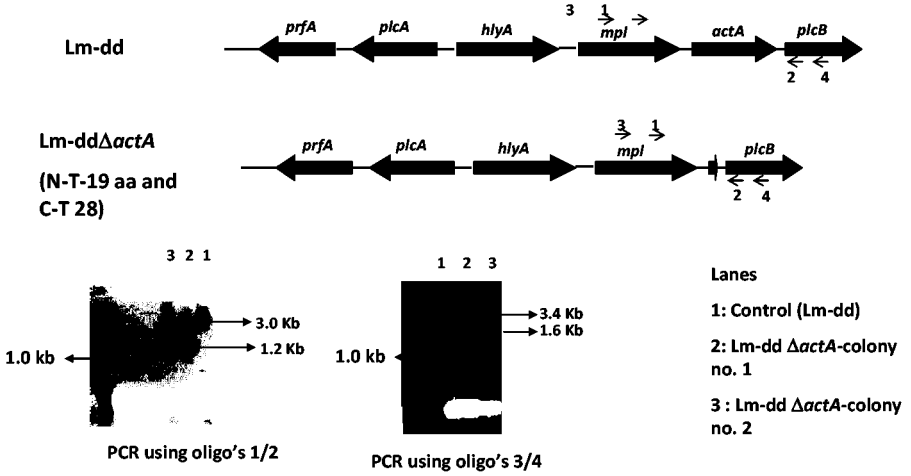

Lm-dd
prfA
plcA
hlyA
mpl
actA
plcB
3
1
2
4
Lm-ddΔactA
(N-T-19 aa and
C-T 28)
3 2 1
3.0 Kb
1.2 Kb
1.0 kb
PCR using oligo's 1/2
1 2 3
3.4 Kb
1.6 Kb
1.0 kb
PCR using oligo's 3/4
Lanes
1: Control (Lm-dd)
2: Lm-dd ΔactA-colony no. 1
3 : Lm-dd ΔactA-colony no. 2

Figure 12

gcgccaaatcattggttgattggtgaggatgtctgtgtgcgtgggtcgcgagatgggcgaataagaagcattaaagatcctgacaaatat
aatcaagcggctcatatgaaagattacgaatcgcttccactcacagaggaaggcgactggggcggagttcattataatagtggtatccc
gaataaagcagcctataatactatcactaaacttggaaaagaaaaaacagaacagctttattttcgcgccttaaagtactatttaacgaaaa
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gggaagcagttggggttaactgattaacaaatgttagagaaaaattaattctccaagtgatattcttaaaataattcatgaatatttttttcttata
ttagctaattaagaagataactaactgctaatccaatttttaacggaacaaattagtgaaaatgaaggccgaattttccttgttctaaaaaggt
tgtattagcgtatcacgaggagggagtataa*gtgggattaaacagatttatgcgtgcgatgatggtggttttcattactgccaattgcatt*
*acgattaaccccgac**gtcgac**ccatacgacgttaattcttgcaatgttagctattggcgtgttctctttaggggcgtttatcaaaattatt*
*caattaagaaaaaatattta*aaaacacagaacgaaagaaaaagtgaggtgaatgatatgaaattcaaaaaggtggttctaggtatgtg
cttgatcgcaagtgttctagtctttccggtaacgataaaagcaaatgcctgttgtgatgaatacttacaaacacccgcagctccgcatgata
ttgacagcaaattaccacataaacttagttggtccgcggataacccgacaaatactgacgtaaatacgcactattggctttttaaacaagc
ggaaaaaatactagctaaagatgtaaatcatatgcgagctaatttaatgaatgaacttaaaaaattcgataaacaaatagctcaaggaata
tatgatgcggatcataaaaatccatattatgatactagtacatttttatctcatttttataatcctgatagagataatacttatttgccgggttttgc
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Figure 13

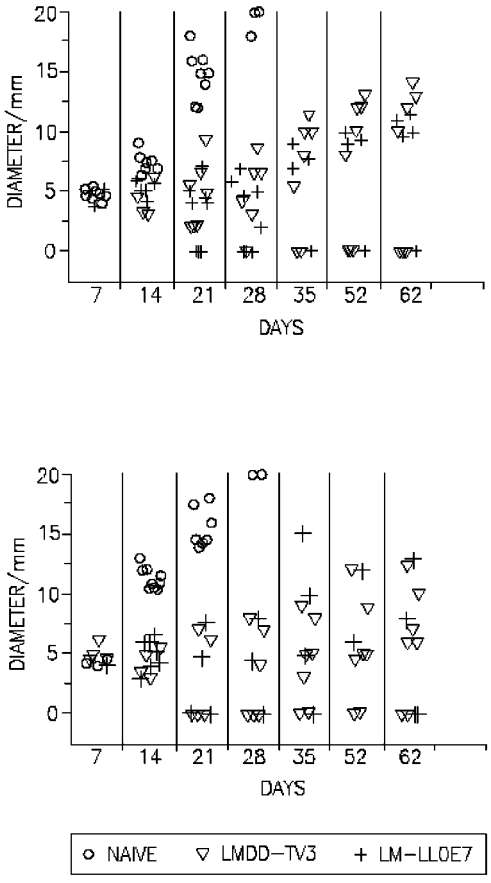

DIAMETER/mm
20
15
10
5
0
7
14
21
28
35
52
62
DAYS
DIAMETER/mm
20
15
10
5
0
7
14
21
28
35
52
62
DAYS
NAIVE
LMDD-TV3
LM-LLOE7

Figure 14

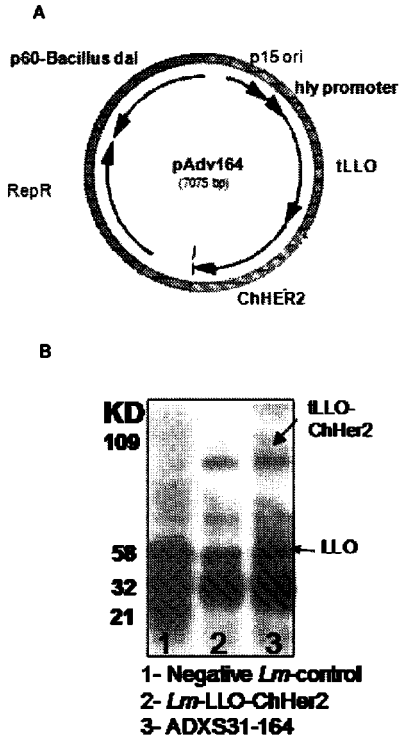
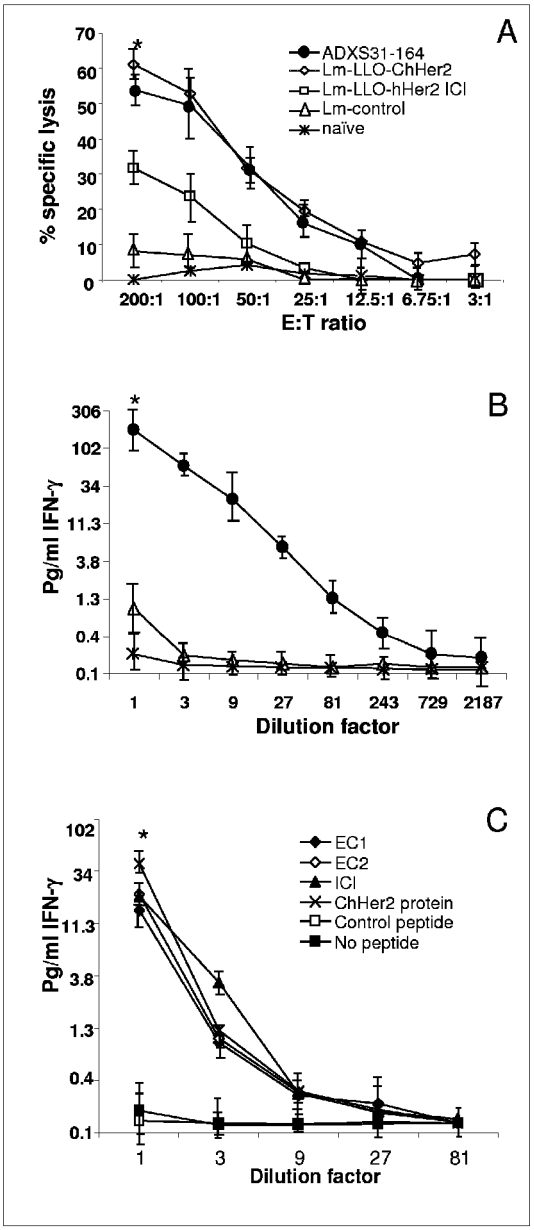

A
p60-Bacillus dal
p15 ori
hly promoter
pAdv164
(7075 bp)
tLLO
RepR
ChHER2
B
KD
109
58
32
21
tLLO-
ChHer2
LLO
1 2 3
1- Negative Lm-control
2- Lm-LLO-ChHer2
3- ADXS31-164

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

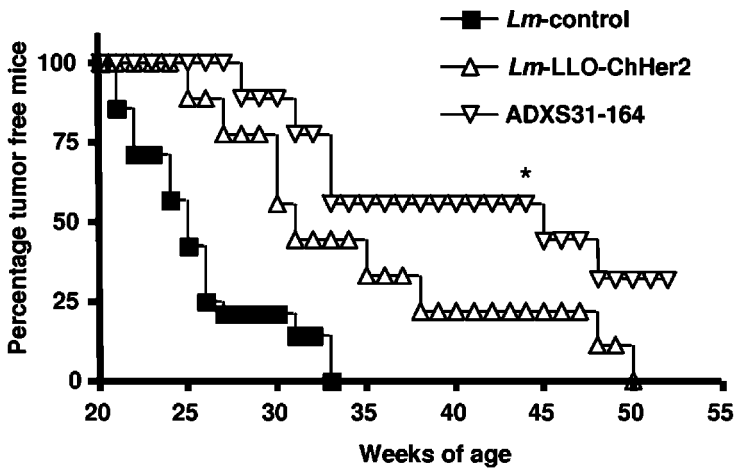

Lm-control
Lm-LLO-ChHer2
ADXS31-164
*
Percentage tumor free mice
100
75
50
25
0
20
25
30
35
40
45
50
55
Weeks of age

Figure 17

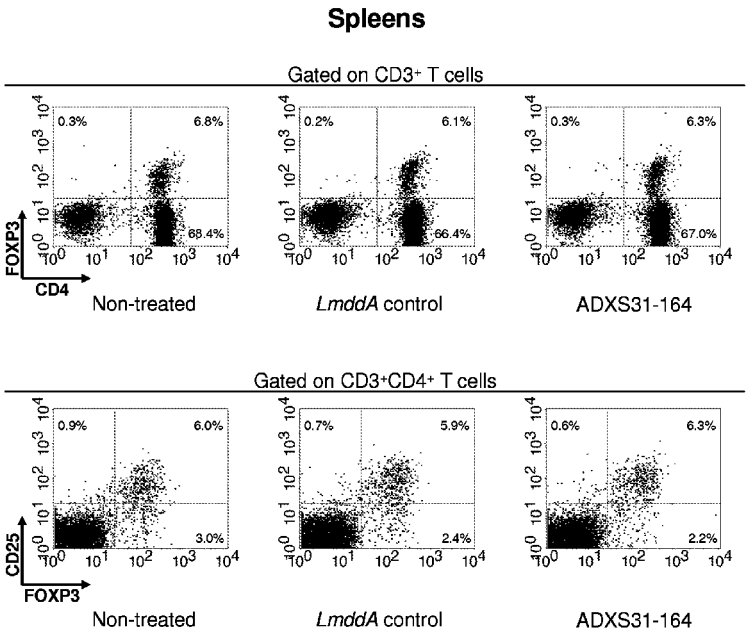

Spleens
Gated on CD3+ T cells
0.3%
6.8%
88.4%
0.2%
6.1%
66.4%
0.3%
6.3%
67.0%
FOXP3
CD4
Non-treated
LmddA control
ADXS31-164
Gated on CD3+CD4+ T cells
0.9%
6.0%
3.0%
0.7%
5.9%
2.4%
0.6%
6.3%
2.2%
CD25
FOXP3
Non-treated
LmddA control
ADXS31-164

Figure 18

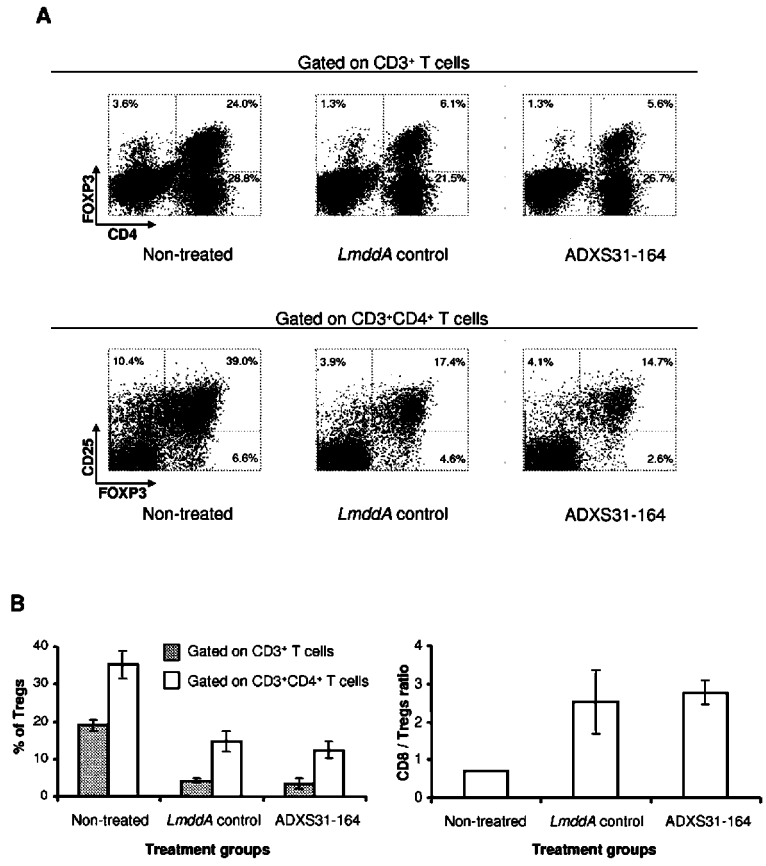

A
Gated on CD3+ T cells
3.6%
24.0%
28.8%
1.3%
6.1%
21.5%
1.3%
5.6%
26.7%
FOXP3
CD4
Non-treated
LmddA control
ADXS31-164
Gated on CD3+CD4+ T cells
10.4%
39.0%
6.6%
3.9%
17.4%
4.6%
4.1%
14.7%
2.6%
CD25
FOXP3
Non-treated
LmddA control
ADXS31-164
B
Gated on CD3+ T cells
Gated on CD3+CD4+ T cells
% of Tregs
40
30
20
10
0
Non-treated
LmddA control
ADXS31-164
Treatment groups
CD8 / Tregs ratio
4
3
2
1
0
Non-treatred
LmddA control
ADXS31-164
Treatment groups

Figure 19

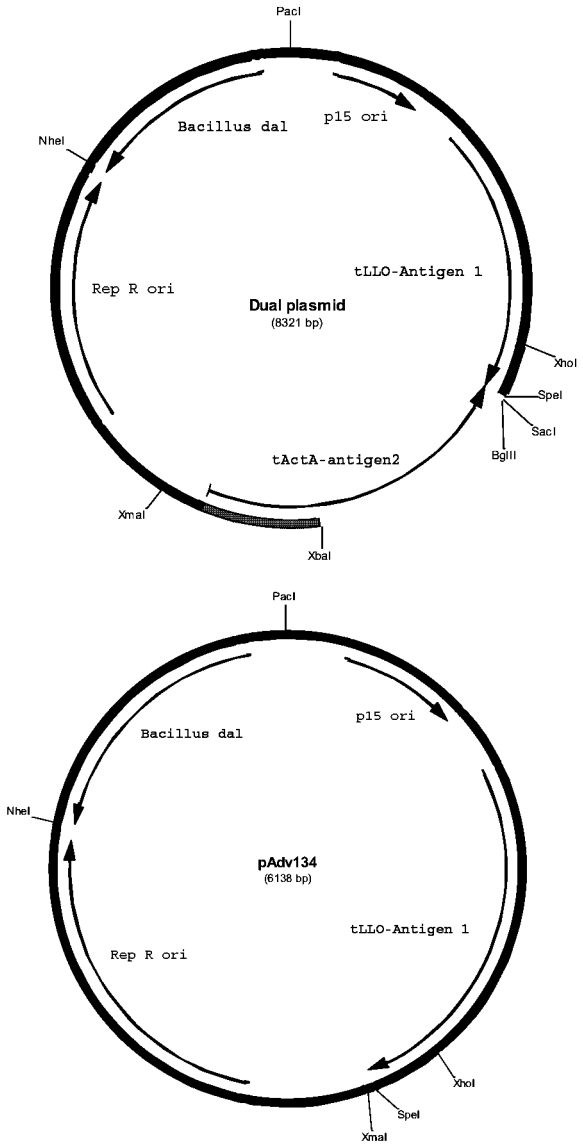

PacI
Bacillus dal
p15 ori
NheI
tLLO-Antigen 1
Rep R ori
Dual plasmid
(8321 bp)
XhoI
SpeI
SacI
BglII
tActA-antigen2
XmaI
XbaI
PacI
p15 ori
Bacillus dal
NheI
pAdv134
(6138 bp)
tLLO-Antigen 1
Rep R ori
XhoI
SpeI
XmaI

Figure 20

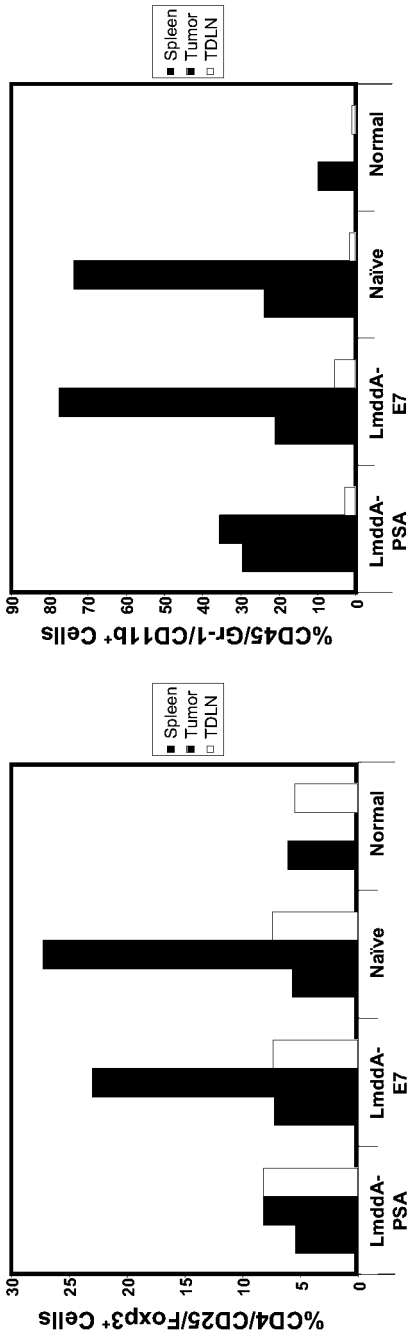

%CD4/CD25/Foxp3+ Cells
0
5
10
15
20
25
30
LmddA-PSA
LmddA-E7
Naïve
Normal
Spleen
Tumor
TDLN
%CD45/Gr-1/CD11b+ Cells
0
10
20
30
40
50
60
70
80
90
LmddA-PSA
LmddA-E7
Naïve
Normal
Spleen
Tumor
TDLN

Figure 21

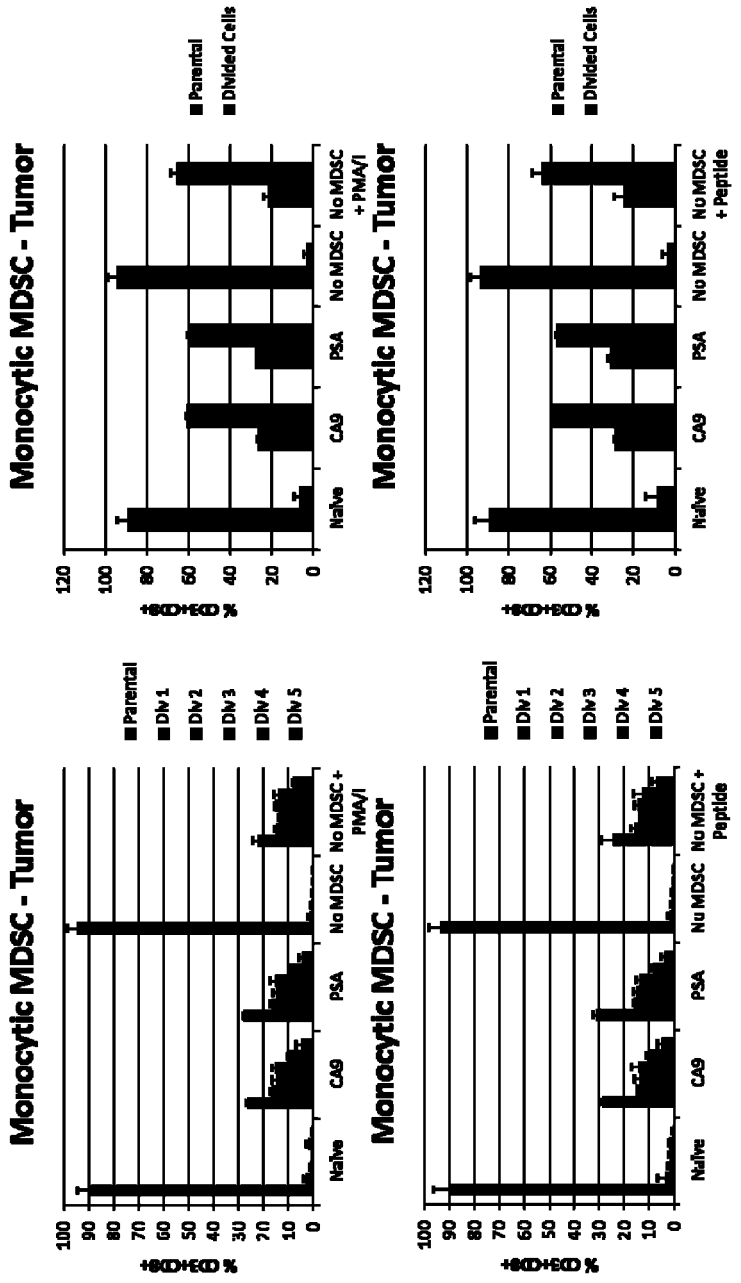

Monocytic MDSC - Tumor
% CD3+CD8+
Parental
Divided Cells
Naïve
CA9
PSA
No MDSC
No MDSC + PMA/I
Monocytic MDSC - Tumor
% CD3+CD8+
Parental
Divided Cells
Naïve
CA9
PSA
No MDSC
No MDSC + Peptide
Monocytic MDSC - Tumor
% CD3+CD8+
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
Naïve
CA9
PSA
No MDSC
No MDSC + PMA/I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

Figure 22

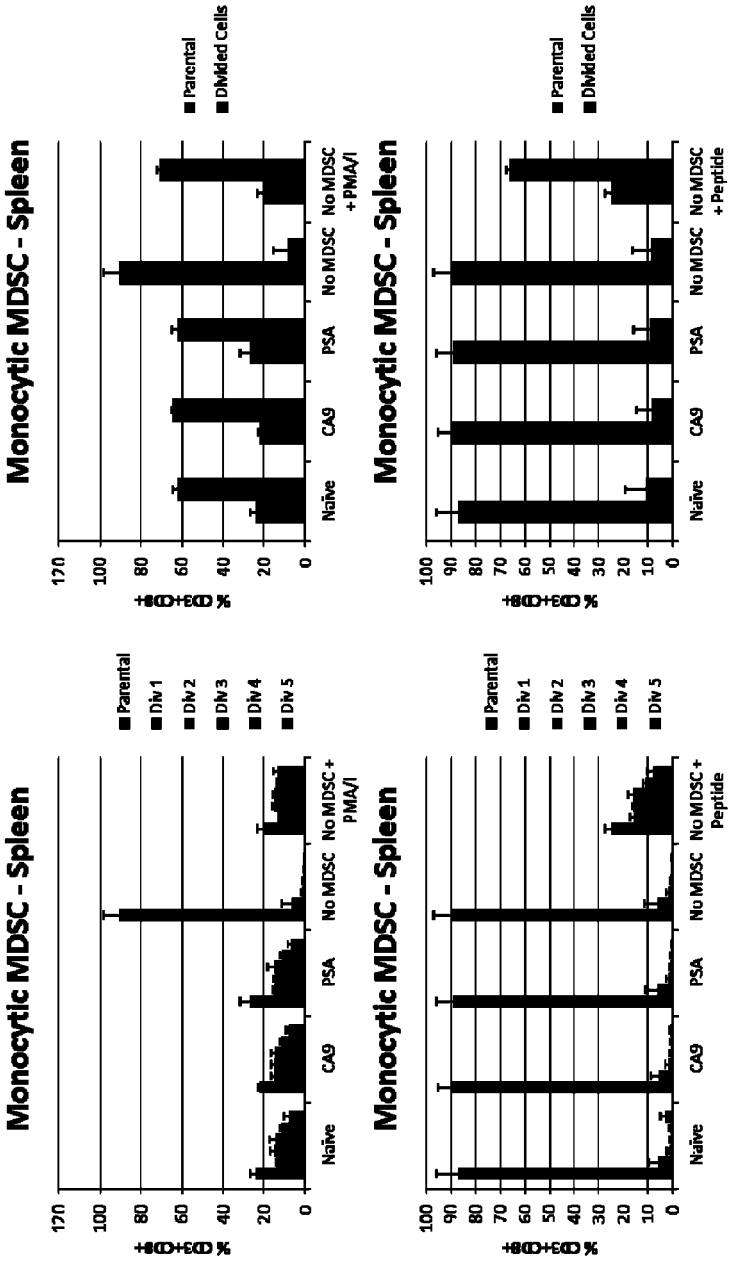

Monocytic MDSC - Spleen
% CD3+CD8+
Parental
Divided Cells
Naive
CA9
PSA
No MDSC
No MDSC + PMA/I
Monocytic MDSC - Spleen
% CD3+CD8+
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
Naive
CA9
PSA
No MDSC
No MDSC + PMA/I
Monocytic MDSC - Spleen
% CD3+CD8+
Parental
Divided Cells
Naive
CA9
PSA
No MDSC
No MDSC + Peptide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

Figure 23

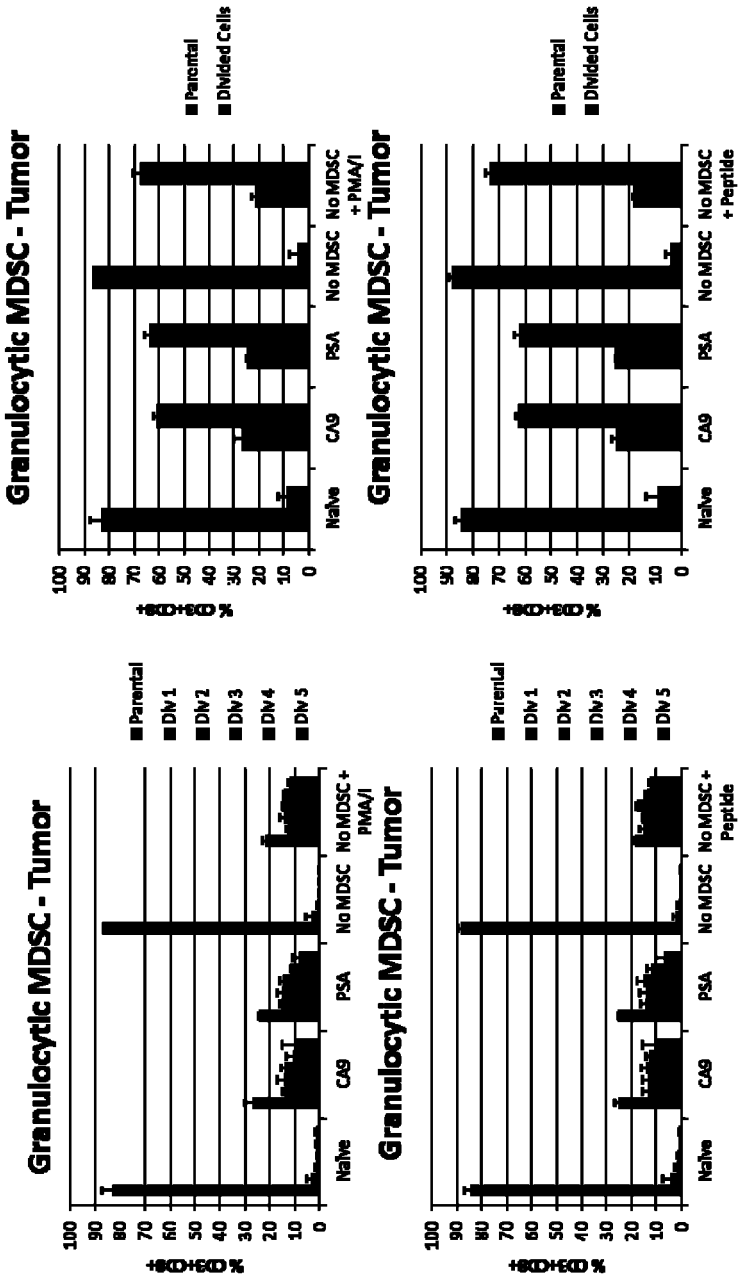

Granulocytic MDSC - Tumor
% CD3+CD8+
Naïve
CA9
PSA
No MDSC
No MDSC + PMA/I
No MDSC + Peptide
Parental
Divided Cells
Div 1
Div 2
Div 3
Div 4
Div 5

Figure 24

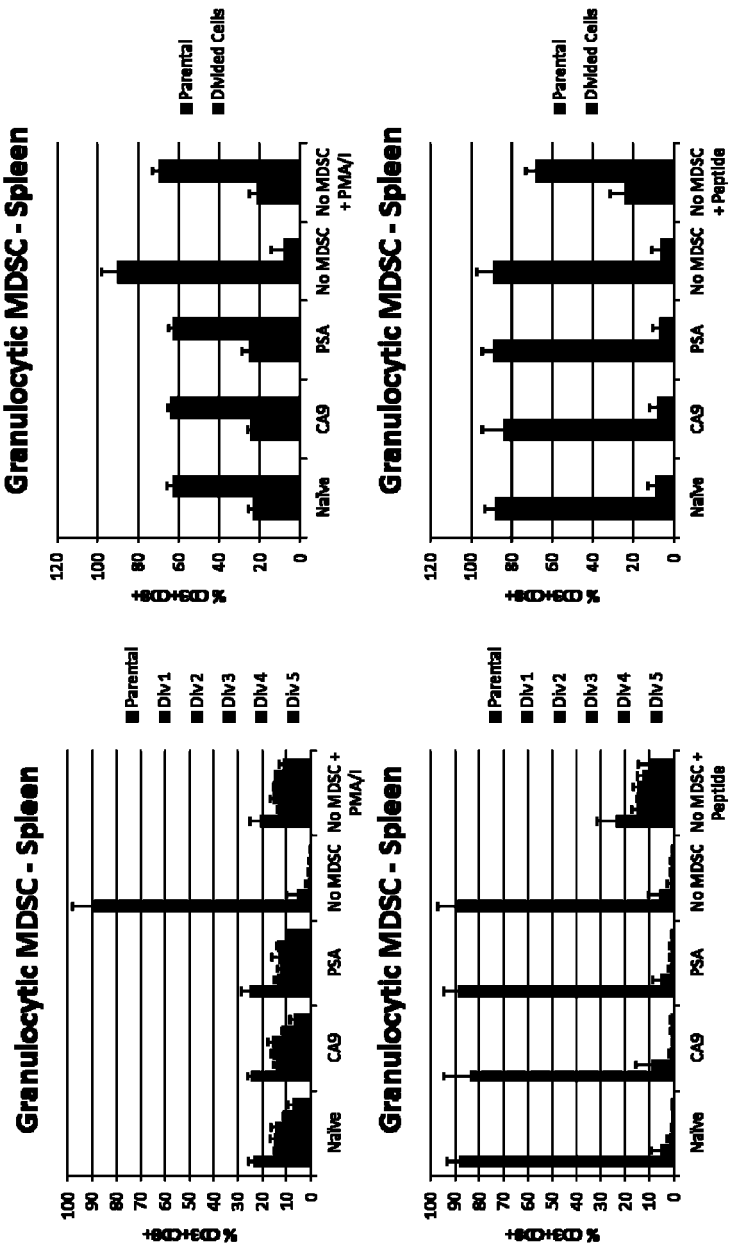

Granulocytic MDSC - Spleen
% CD3+CD8+
Parental
Divided Cells
Naïve
CA9
PSA
No MDSC
No MDSC + PMA/I
Granulocytic MDSC - Spleen
% CD3+CD8+
Parental
Divided Cells
Naïve
CA9
PSA
No MDSC
No MDSC + Peptide
Granulocytic MDSC - Spleen
% CD3+CD8+
Parental
Div1
Div2
Div3
Div4
Div5
Naïve
CA9
PSA
No MDSC
No MDSC + PMA/I
Granulocytic MDSC - Spleen
% CD3+CD8+
Parental
Div1
Div2
Div3
Div4
Div5
Naïve
CA9
PSA
No MDSC
No MDSC + Peptide

Figure 25

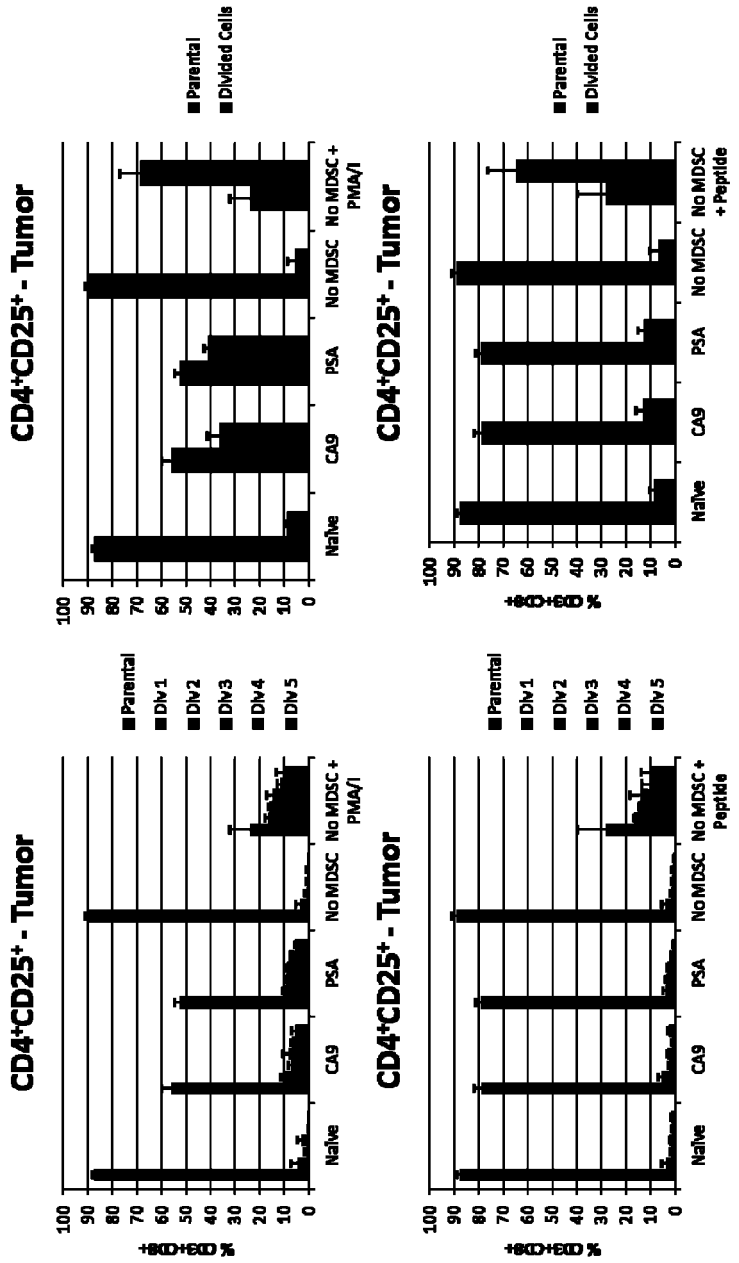

CD4+CD25+ - Tumor
% CD3+CD8+
Parental
Div1
Div2
Div3
Div4
Div5
Naïve
CA9
PSA
No MDSC
No MDSC + PMA/I
Parental
Divided Cells
No MDSC + Peptide

Figure 26

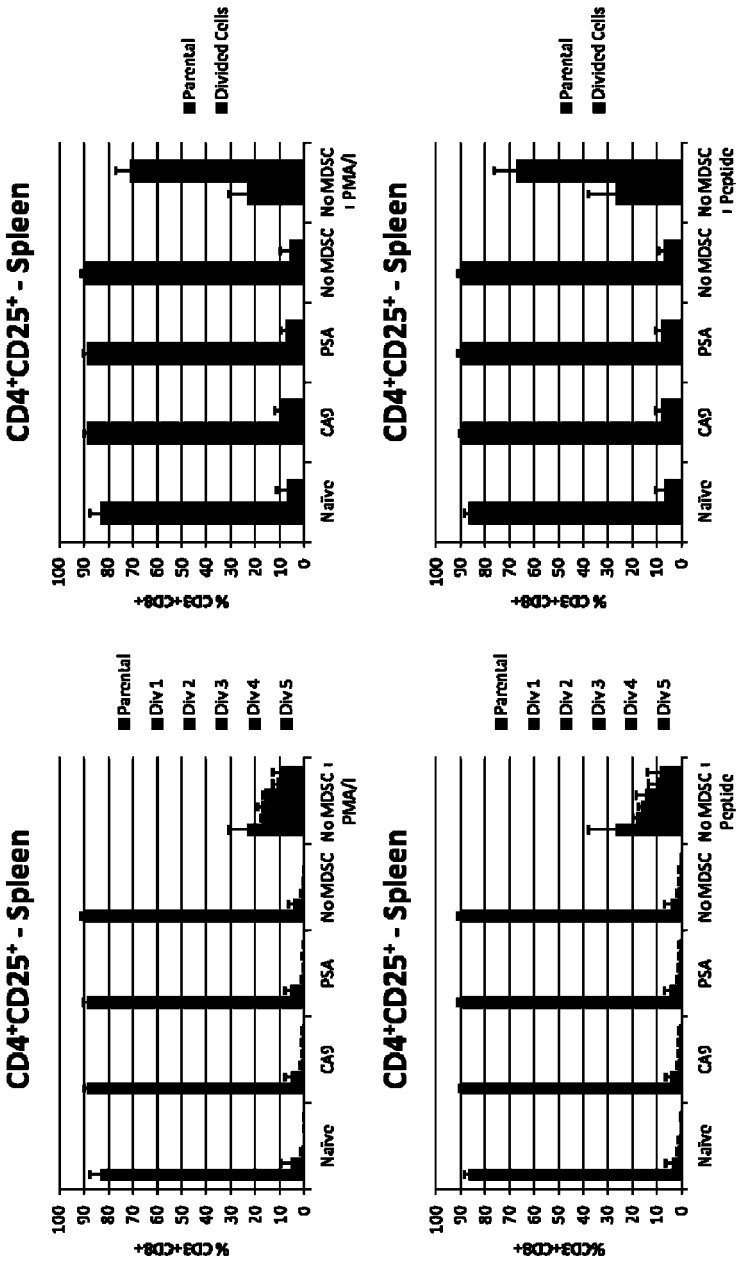

CD4+CD25+ - Spleen
% CD3+CD8+
Naïve
CA9
PSA
No MDSC
No MDSC + PMA/I
No MDSC + Peptide
Parental
Divided Cells
Div 1
Div 2
Div 3
Div 4
Div 5

Figure 27

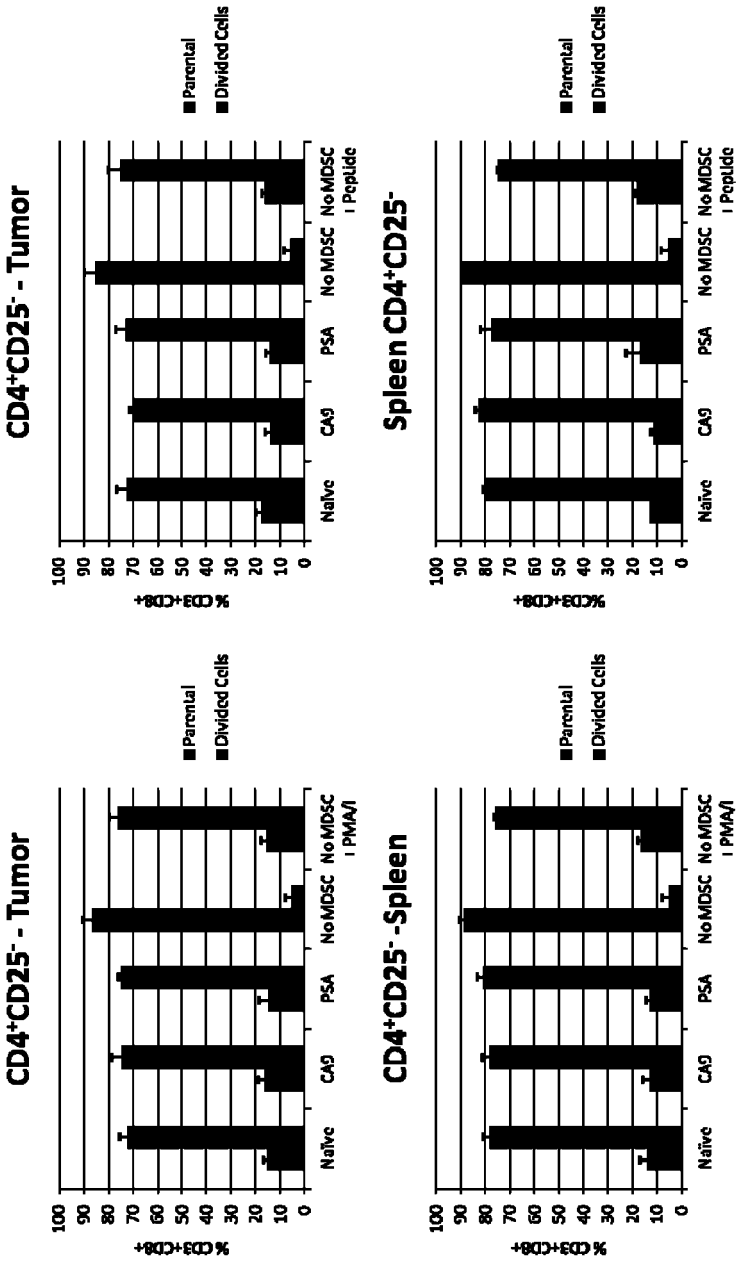

CD4+CD25- - Tumor
CD4+CD25- - Tumor
CD4+CD25- -Spleen
Spleen CD4+CD25-
% CD3+CD8+
Parental
Divided Cells
Naïve
CAG
PSA
No MDSC
No MDSC + Peptide
No MDSC + PMA/I

Figure 28

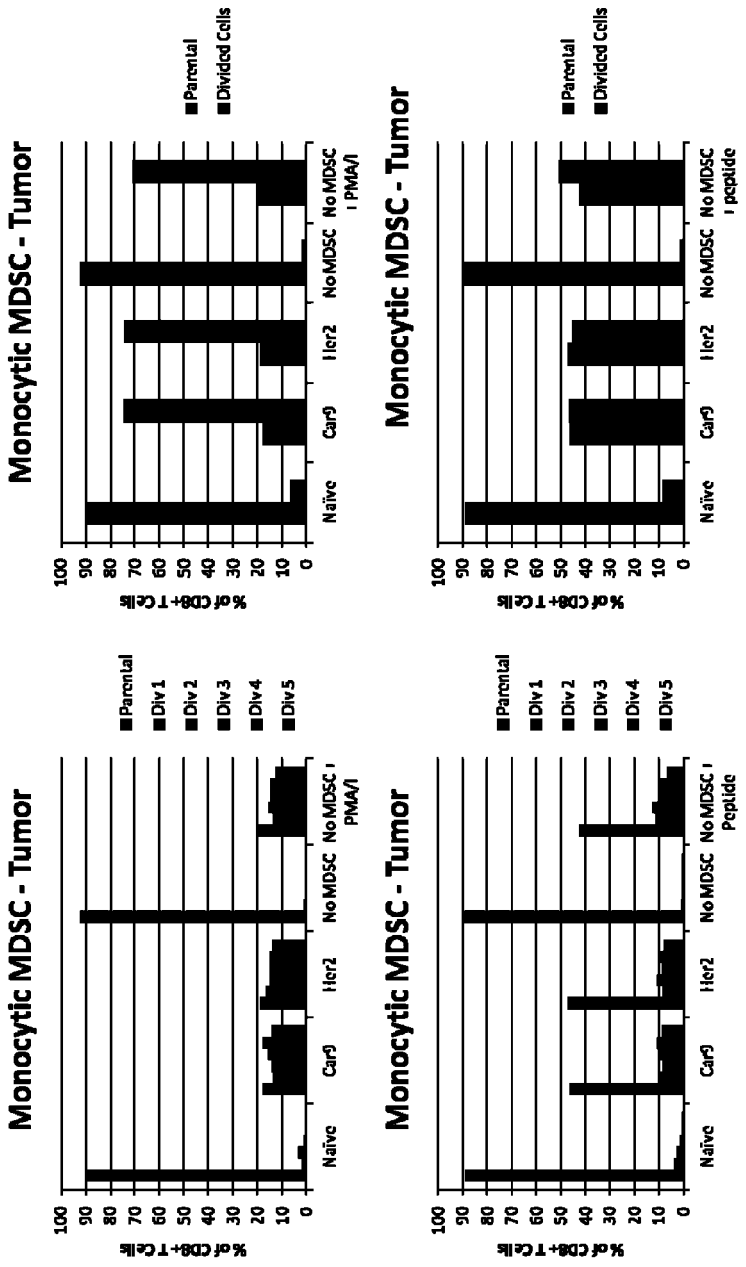

Monocytic MDSC - Tumor
% of CD8+ T Cells
100
90
80
70
60
50
40
30
20
10
0
Naïve
Car9
Her2
No MDSC
No MDSC + PMA/I
Parental
Divided Cells
Monocytic MDSC - Tumor
% of CD8+ T Cells
Naïve
Car9
Her2
No MDSC
No MDSC + peptide
Parental
Divided Cells
Monocytic MDSC - Tumor
% of CD8+ T Cells
Naïve
Car9
Her2
No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

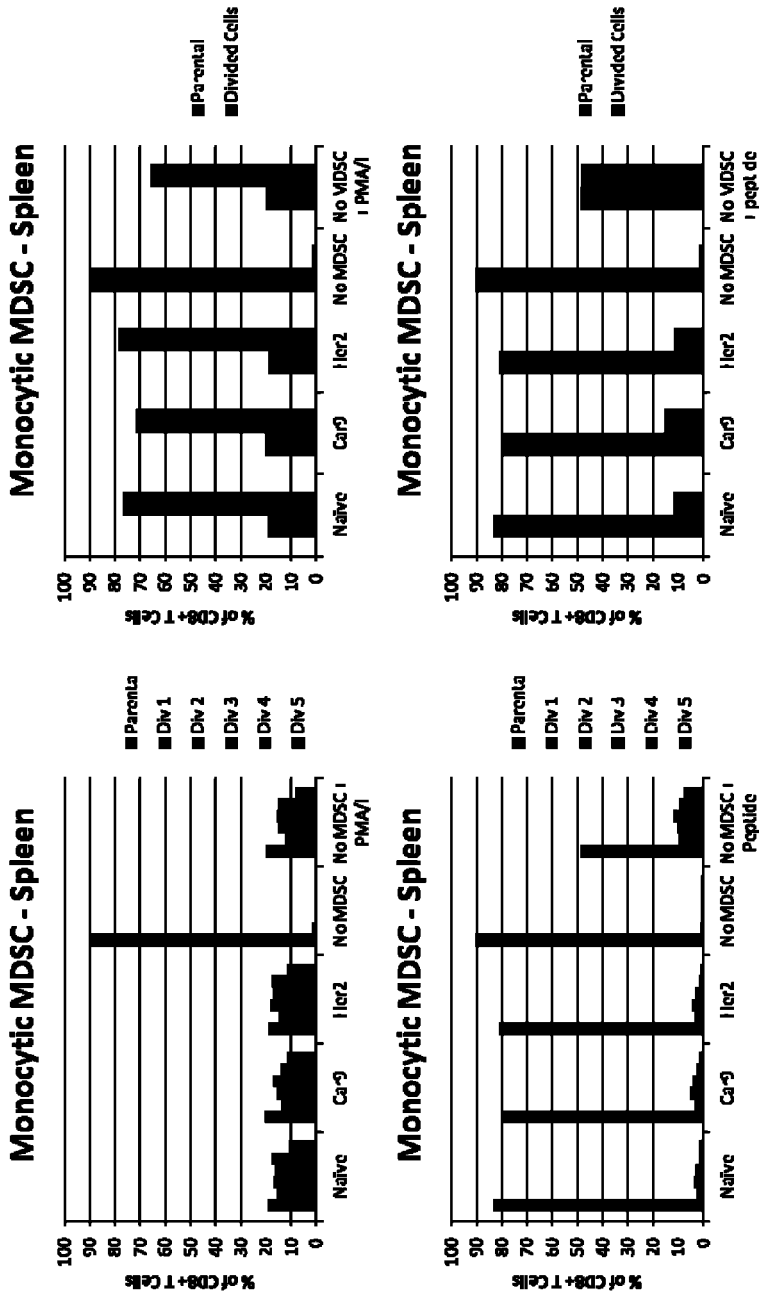

Monocytic MDSC - Spleen
% of CD8+ T Cells
Naive
Car9
Her2
No MDSC
No MDSC + PMA/I
Parental
Divided Cells
Monocytic MDSC - Spleen
% of CD8+ T Cells
Naive
Car9
Her2
No MDSC
No MDSC + PMA/I
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
Monocytic MDSC - Spleen
% of CD8+ T Cells
Naive
Car9
Her2
No MDSC
No MDSC +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

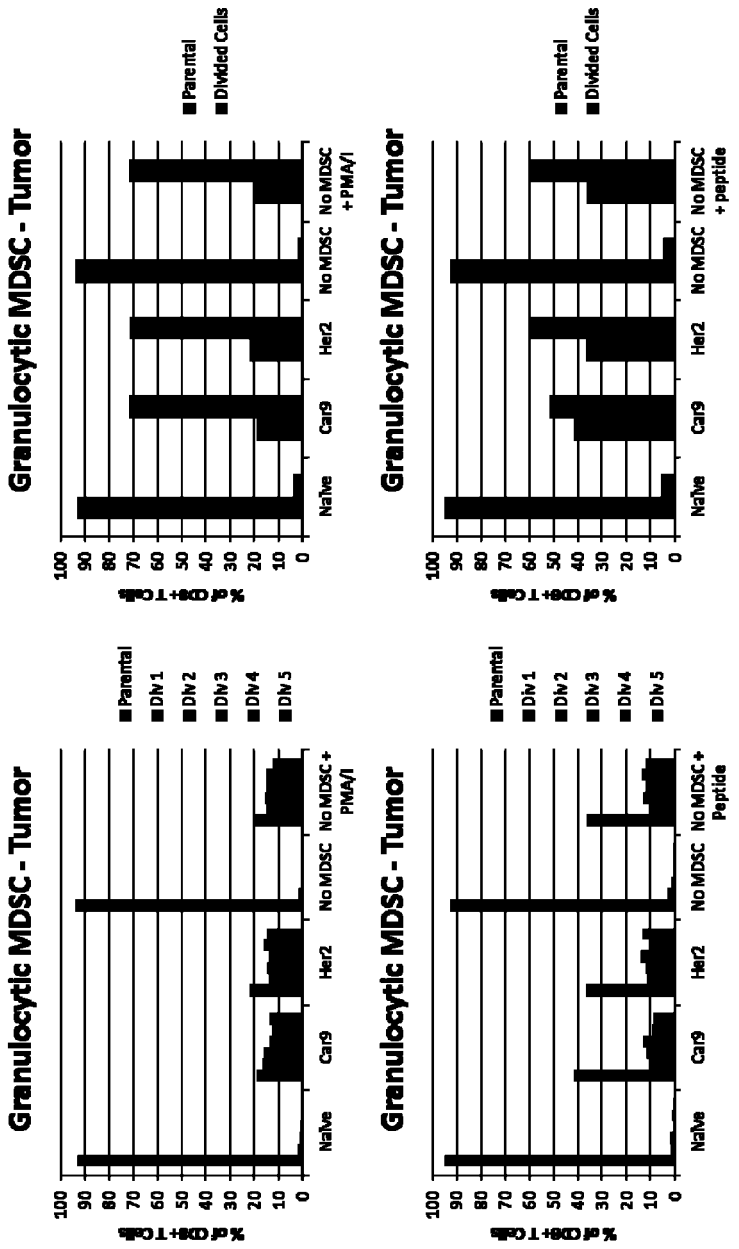

Granulocytic MDSC - Tumor
% of CD8+ T Cells
Parental
Divided Cells
Naïve
Car9
Her2
No MDSC
No MDSC + PMA/I
Granulocytic MDSC - Tumor
% of CD8+ T Cells
Parental
Divided Cells
Naïve
Car9
Her2
No MDSC
No MDSC + peptide
Granulocytic MDSC - Tumor
% of CD8+ T Cells
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
Naïve
Car9
Her2
No MDSC
No MDSC + PMA/I
Granulocytic MDSC - Tumor
% of CD8+ T Cells
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
Naïve
Car9
Her2
No MDSC
No MDSC + Peptide

Figure 31

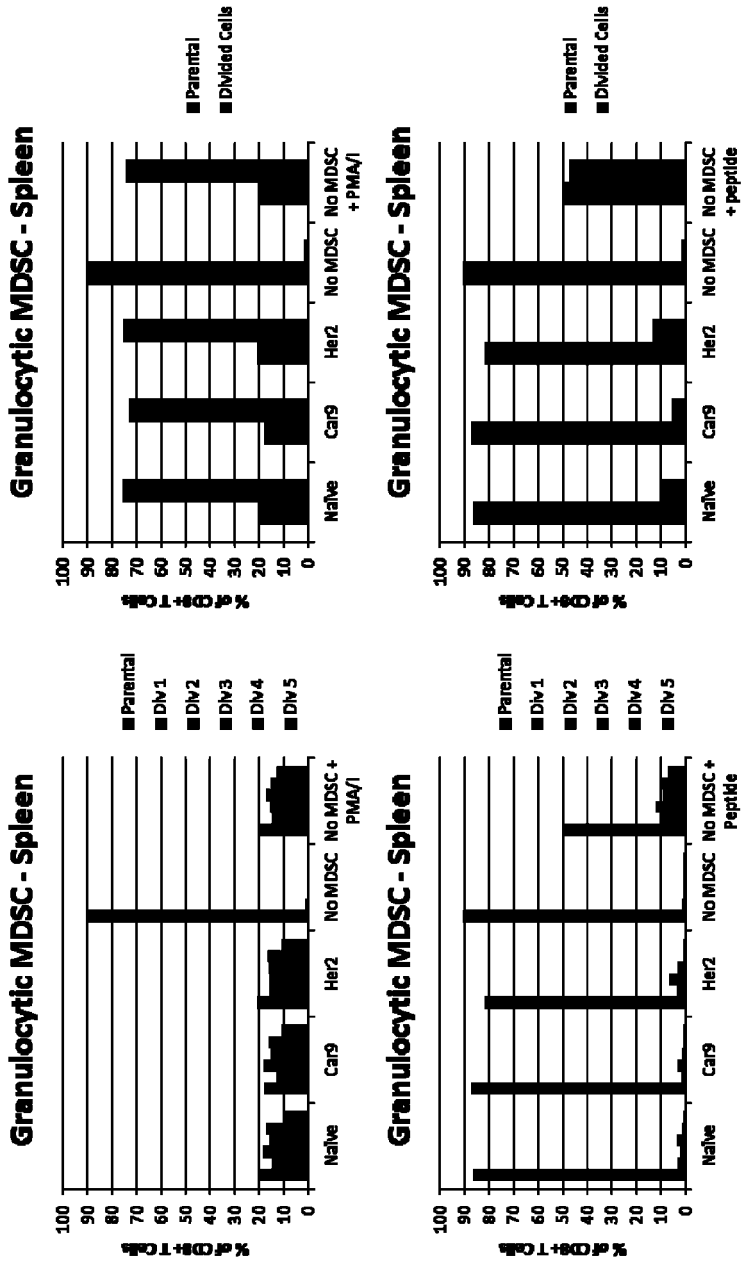

Granulocytic MDSC - Spleen
% of CD8+ T Cells
Naive
Car9
Her2
No MDSC
No MDSC + PMA/I
Parental
Divided Cells
Granulocytic MDSC - Spleen
% of CD8+ T Cells
Naive
Car9
Her2
No MDSC
No MDSC + peptide
Parental
Divided Cells
Granulocytic MDSC - Spleen
% of CD8+ T Cells
Naive
Car9
Her2
No MDSC
No MDSC + PMA/I
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
Granulocytic 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 32

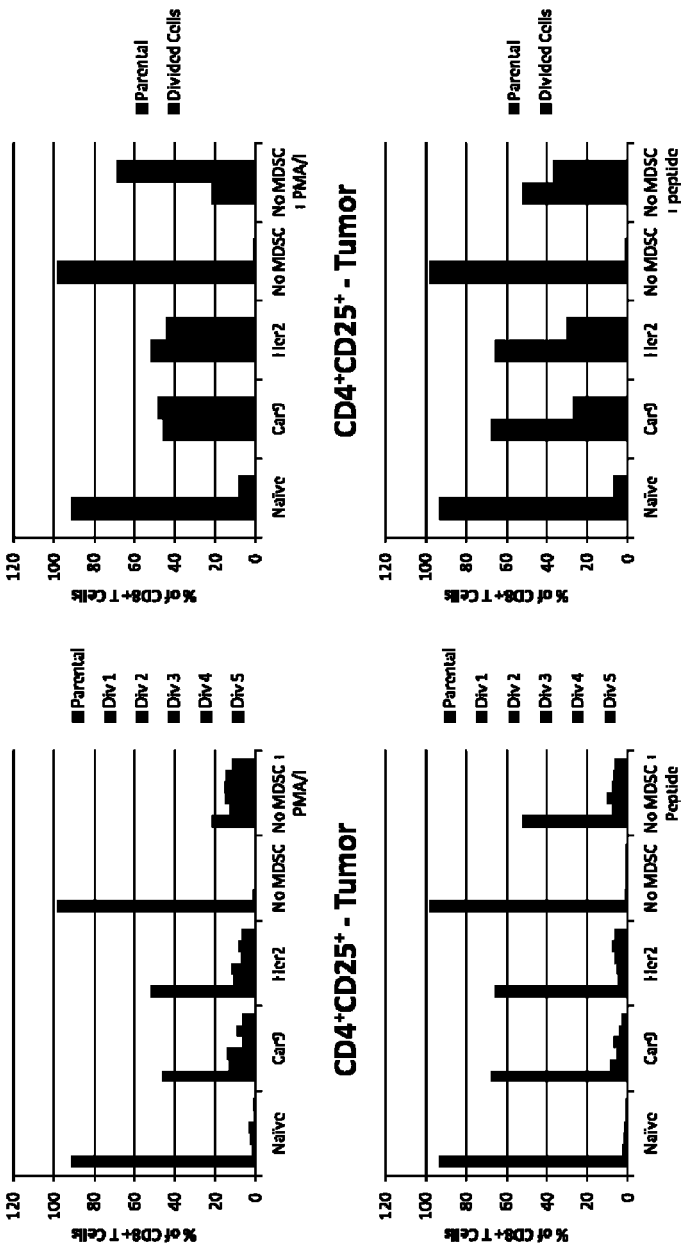

CD4+CD25+ - Tumor
% of CD8+ T Cells
120
100
80
60
40
20
0
Naive
Car9
Her2
No MDSC
No MDSC + PMA/I
No MDSC + Peptide
Parental
Divided Cells
Div 1
Div 2
Div 3
Div 4
Div 5

Figure 33

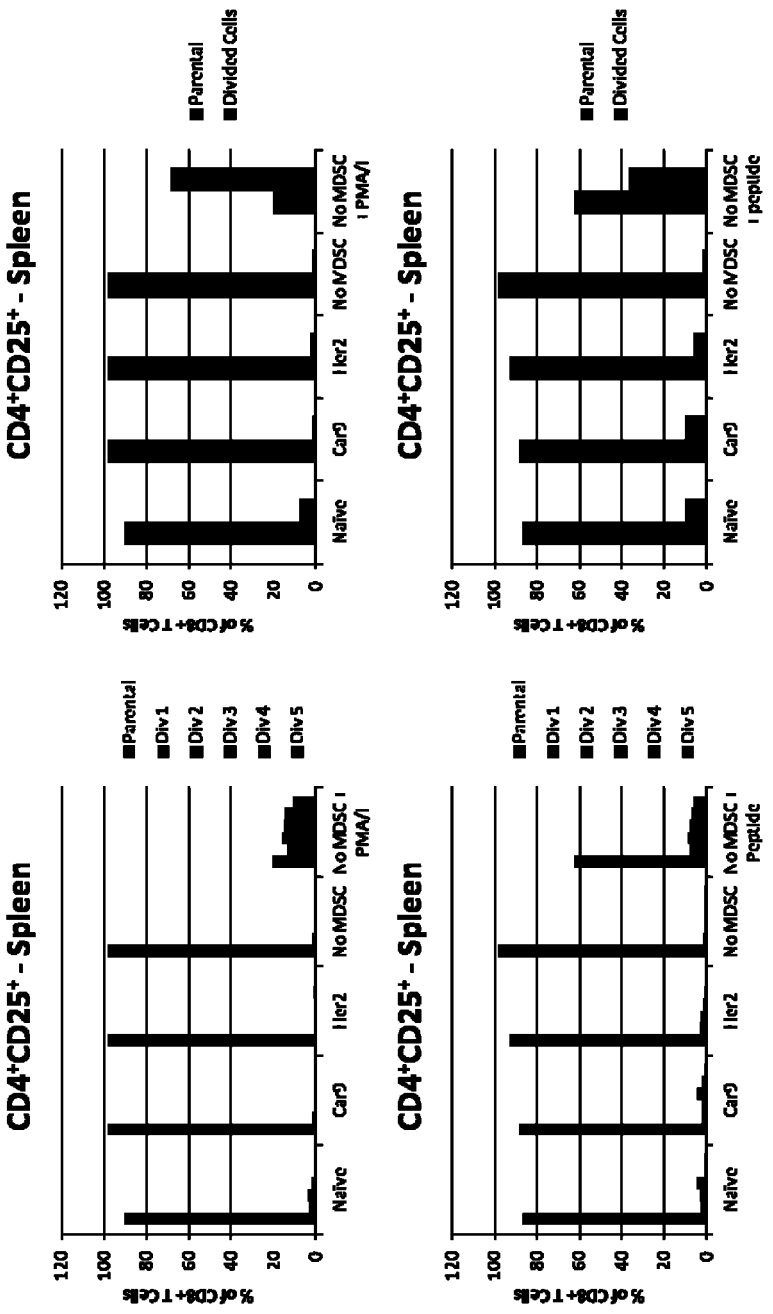

CD4+CD25+ - Spleen
% of CD8+ T Cells
120
100
80
60
40
20
0
Naive
Car9
Iler2
No MDSC
No MDSC + PMA/I
Parental
Divided Cells
CD4+CD25+ - Spleen
% of CD8+ T Cells
Naive
Car9
Iler2
No MDSC
No MDSC + peptide
Parental
Divided Cells
CD4+CD25+ - Spleen
% of CD8+ T Cells
Naive
Car9
Iler2
No MDSC
No MDSC + PMA/I
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
CD4+CD25+ - Spleen
% of CD8+ T Cells
Naive
Car9
Iler2
No MDSC
No MDSC + Peptide
Parental
Div 1
Div 2
Div 3
Div 4
Div 5

Figure 34

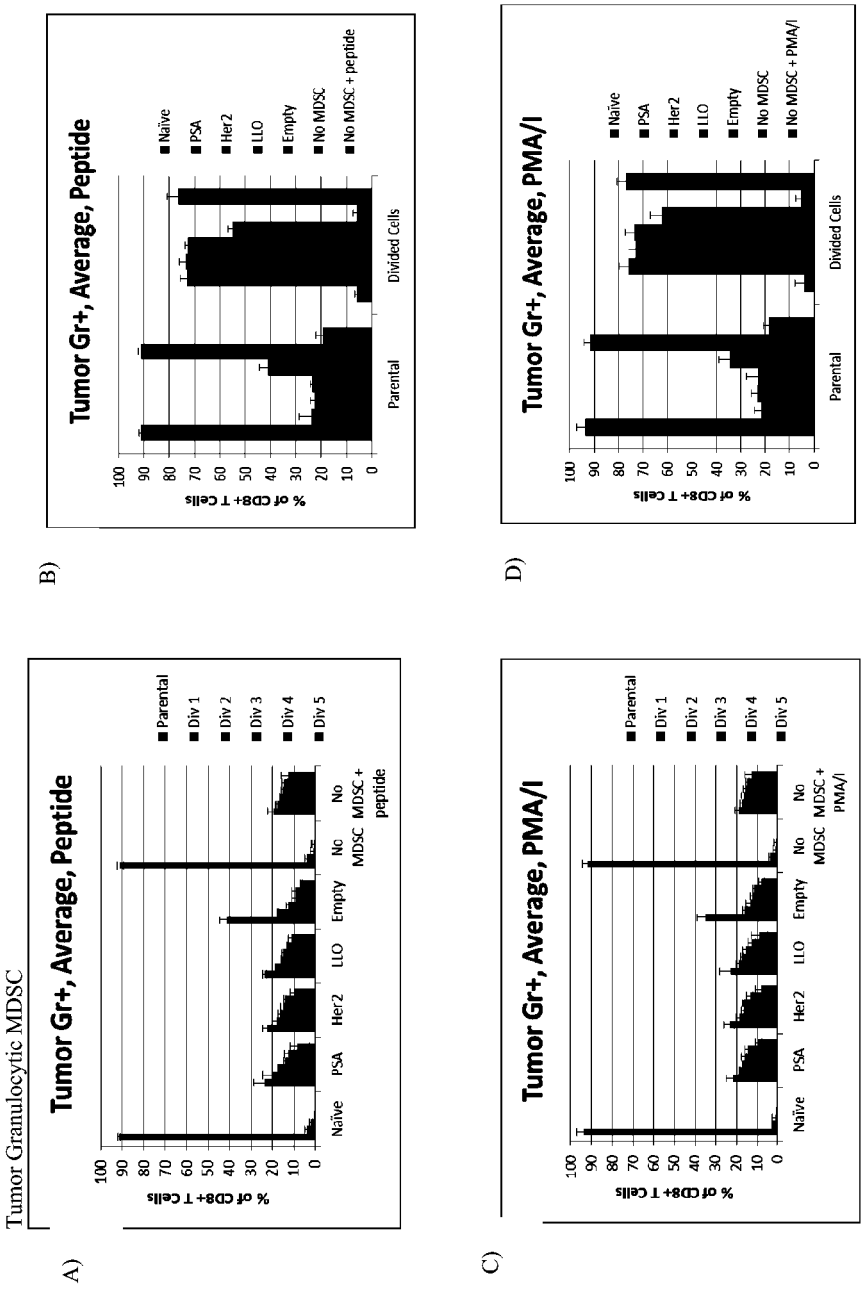

Tumor Granulocytic MDSC
A)
Tumor Gr+, Average, Peptide
% of CD8+ T Cells
Naïve
PSA
Her2
LLO
Empty
No MDSC
No MDSC + peptide
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
B)
Tumor Gr+, Average, Peptide
Naïve
PSA
Her2
LLO
Empty
No MDSC
No MDSC + peptide
Parental
Divided Cells
C)
Tumor Gr+, Average, PMA/I
No MDSC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

Figure 35

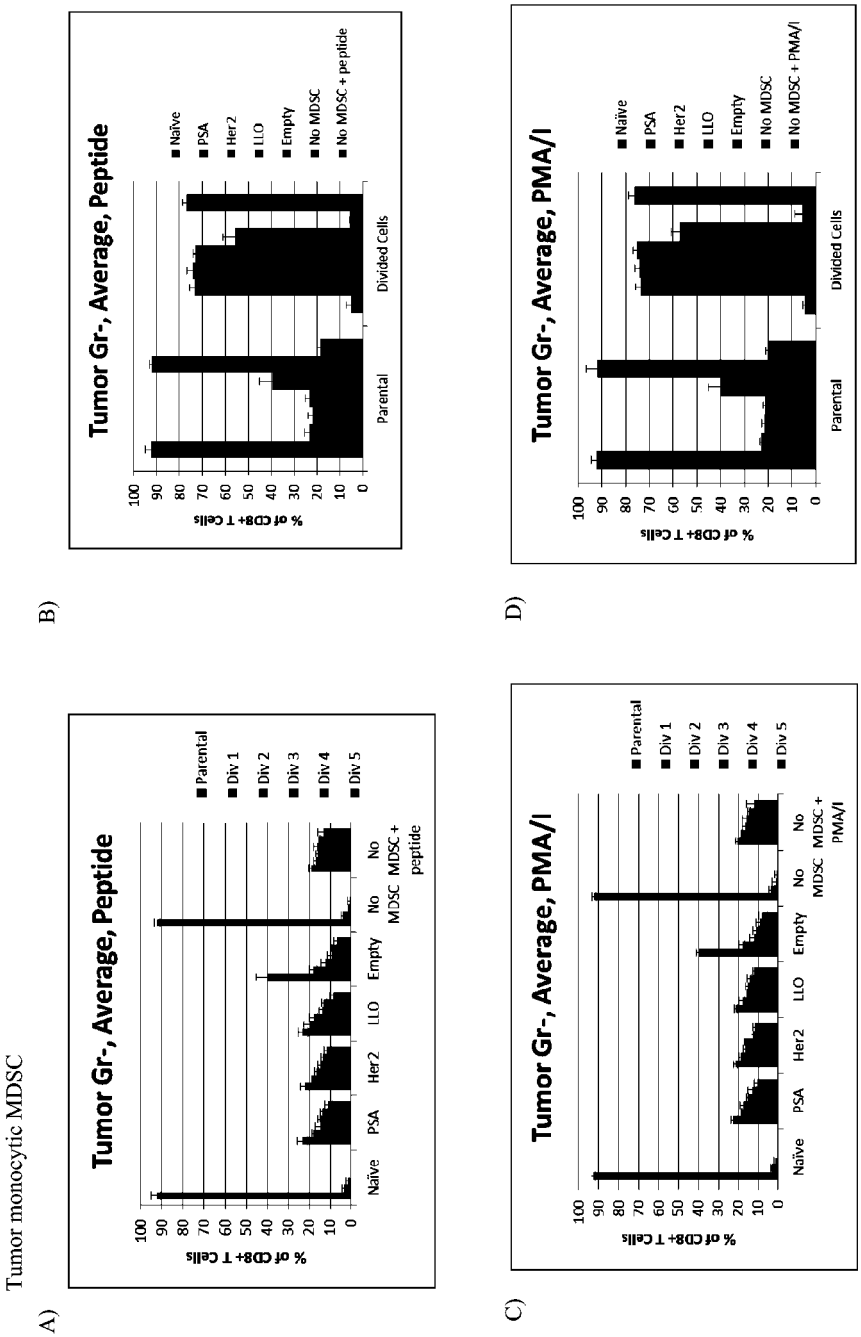

Tumor monocytic MDSC
A)
Tumor Gr-, Average, Peptide
% of CD8+ T cells
Naïve
PSA
Her2
LLO
Empty
No MDSC
No MDSC + peptide
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
B)
Tumor Gr-, Average, Peptide
Divided Cells
C)
Tumor Gr-, Average, PMA/I
No MDSC + PMA/I
D)
Tumor Gr-, Average, PMA/I

Figure 36

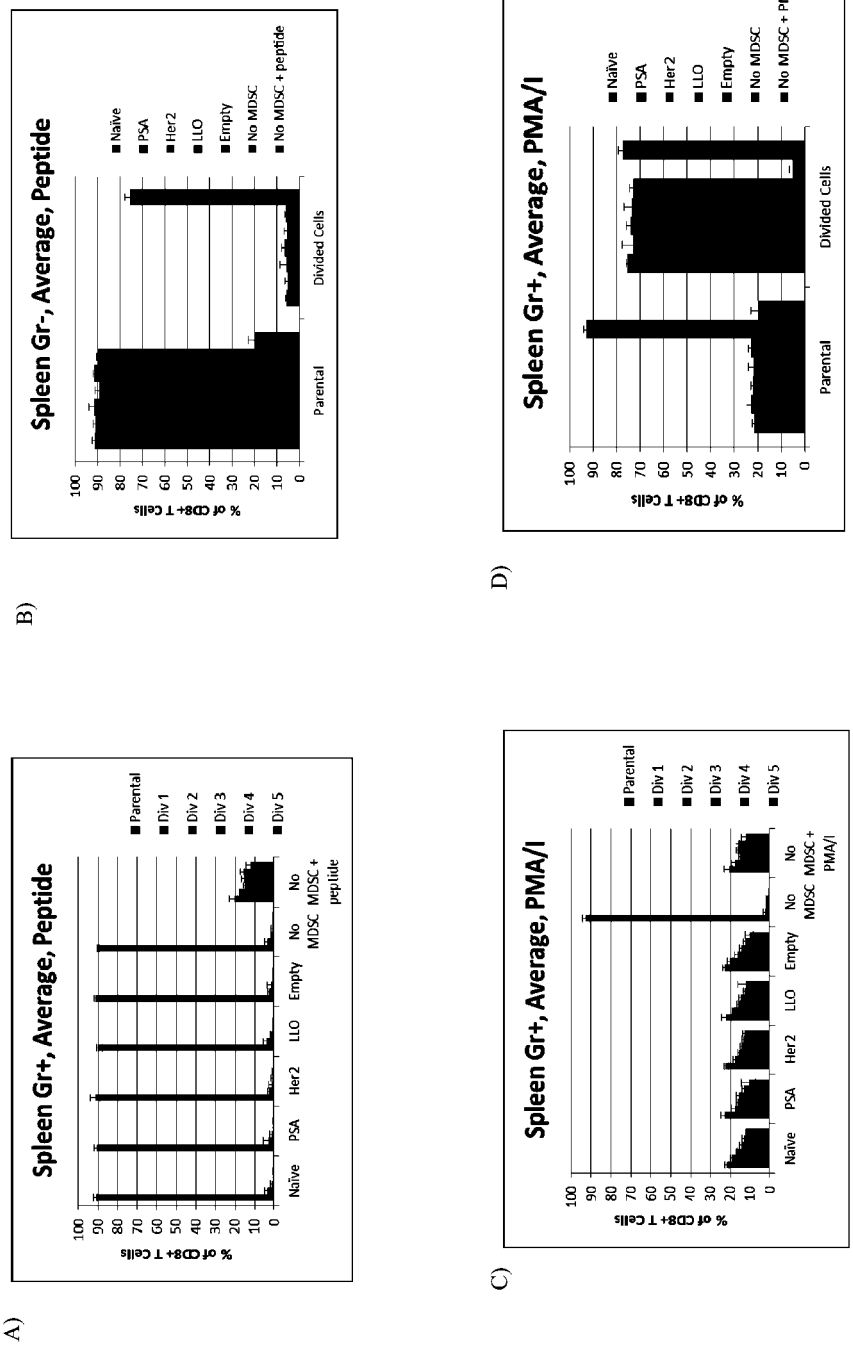
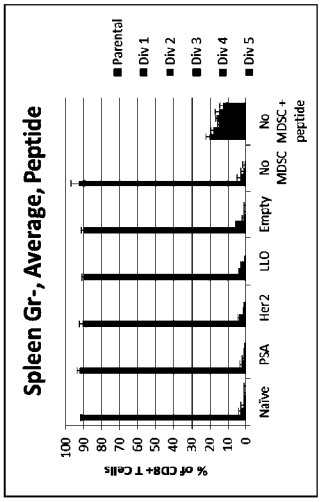

A)
Spleen Granulocytic MDSC
Spleen Gr+, Average, Peptide
% of CD8+ T cells
Naïve
PSA
Her2
LLO
Empty
No MDSC
No MDSC + peptide
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
B)
Spleen Gr-, Average, Peptide
Divided Cells
C)
Spleen Gr+, Average, PMA/I
No MDSC + PMA/I
D)
Spleen Gr+, Average, PMA/I

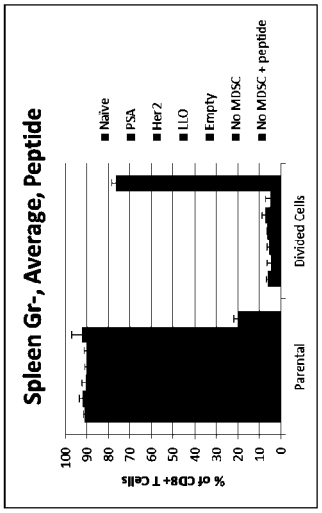
Figure 37

Spleen Monocytic MDSC

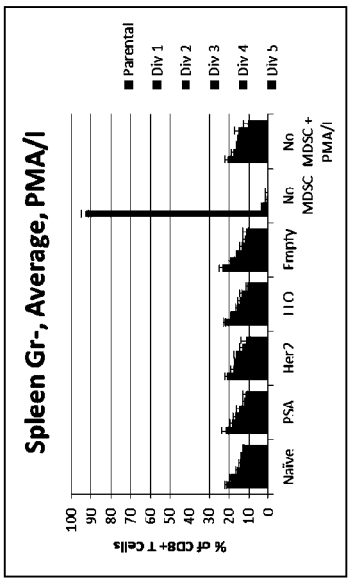
A)



B)



C)



D)

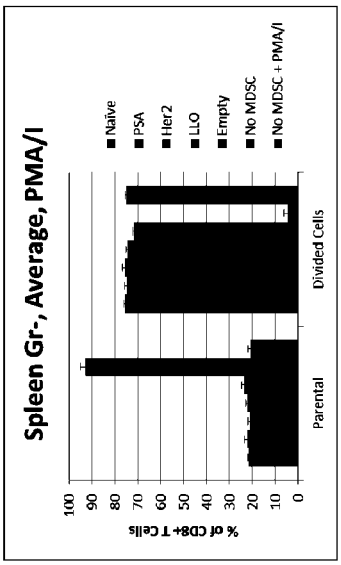


Figure 38

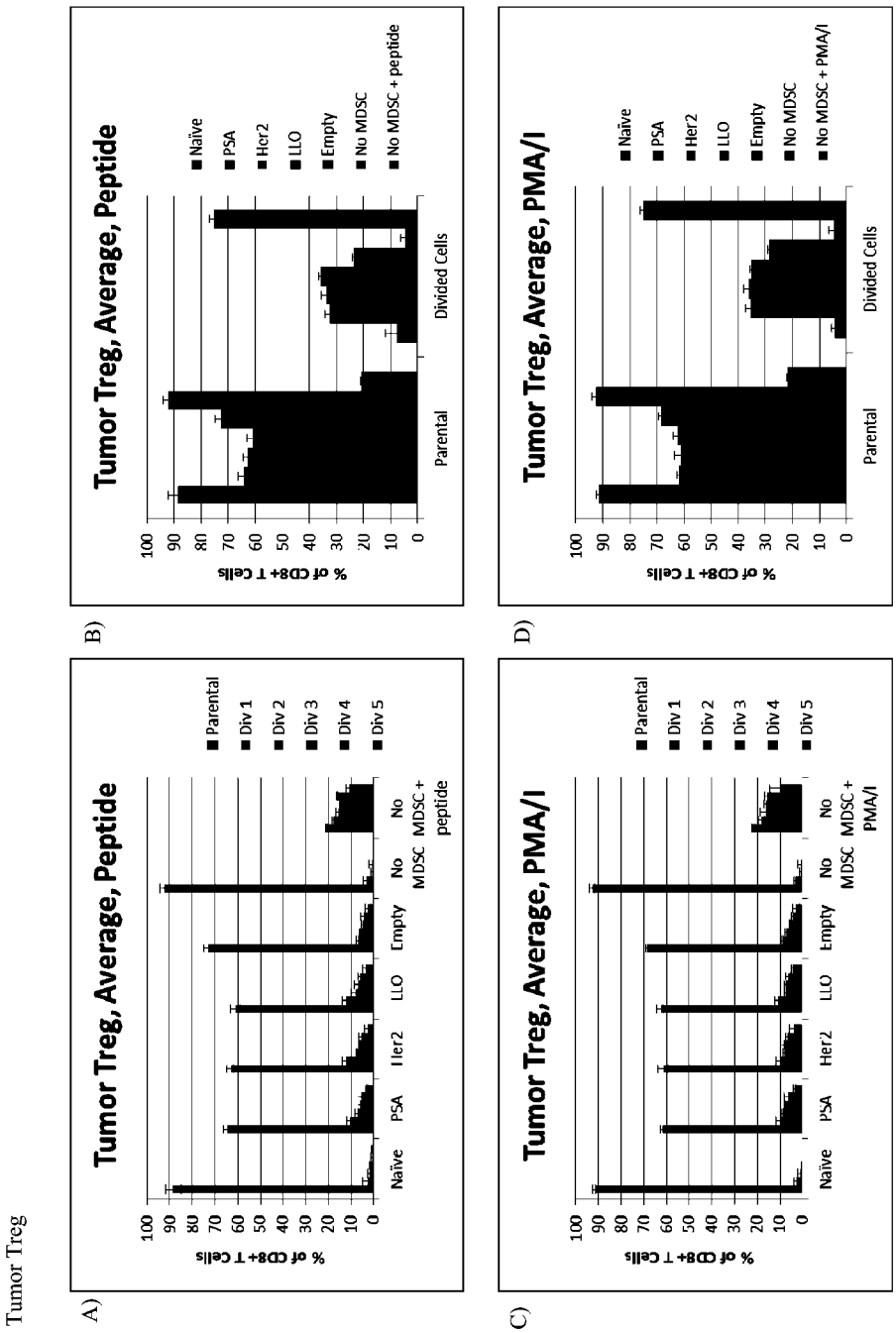

Tumor Treg
A)
Tumor Treg, Average, Peptide
% of CD8+ T cells
Naïve
PSA
Her2
LLO
Empty
No MDSC
No MDSC + peptide
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
B)
Tumor Treg, Average, Peptide
Naïve
PSA
Her2
LLO
Empty
No MDSC
No MDSC + peptide
Parental
Divided Cells
C)
Tumor Treg, Average, PMA/I
No MDSC + PMA/I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

Figure 39

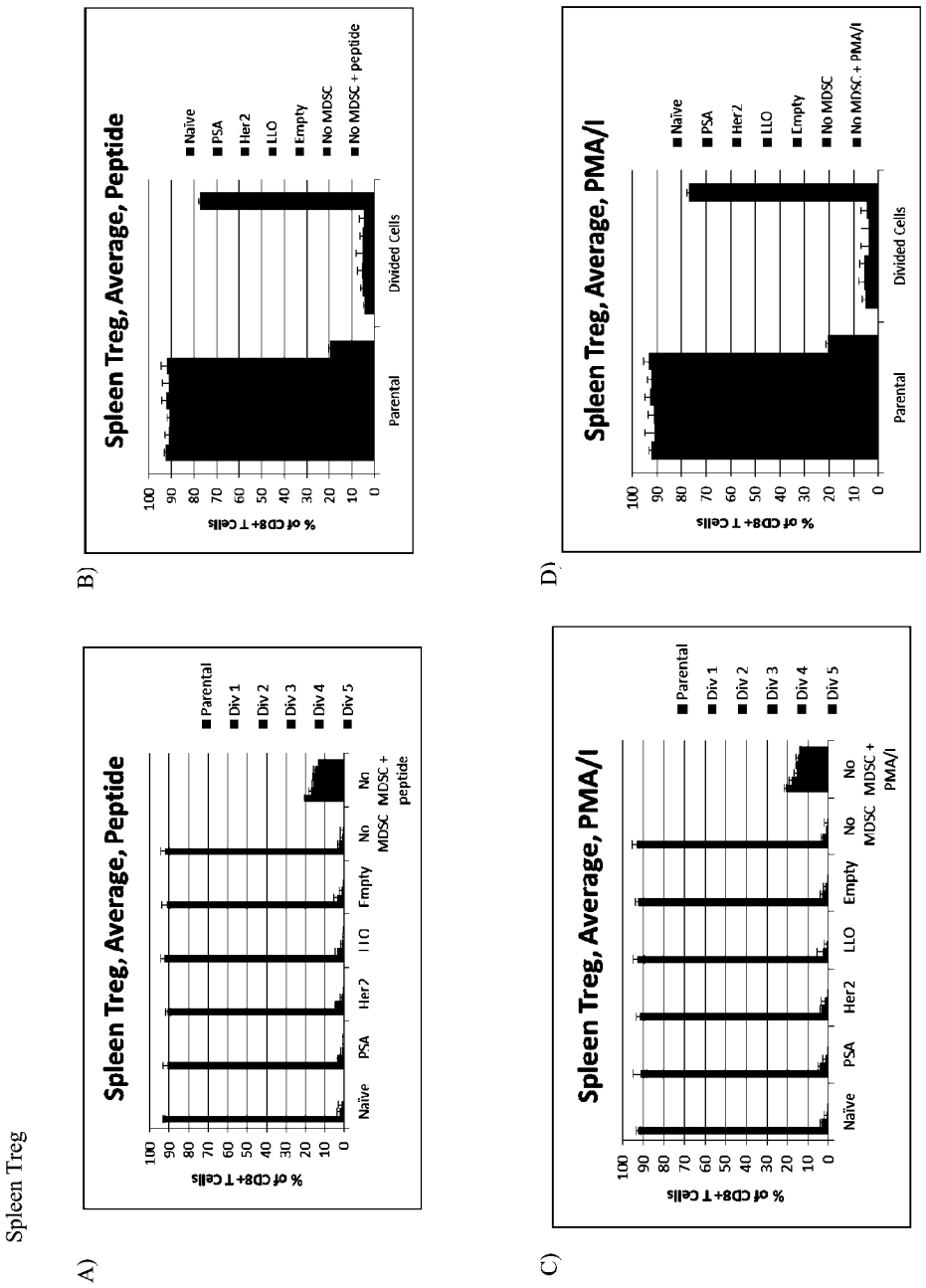

Spleen Treg
A)
Spleen Treg, Average, Peptide
% of CD8+ T cells
Naïve
PSA
Her2
LLO
Empty
No MDSC
No MDSC + peptide
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
B)
Spleen Treg, Average, Peptide
Naïve
PSA
Her2
LLO
Empty
No MDSC
No MDSC + peptide
Parental
Divided Cells
C)
Spleen Treg, Average, PMA/I
No MDSC +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

Figure 40

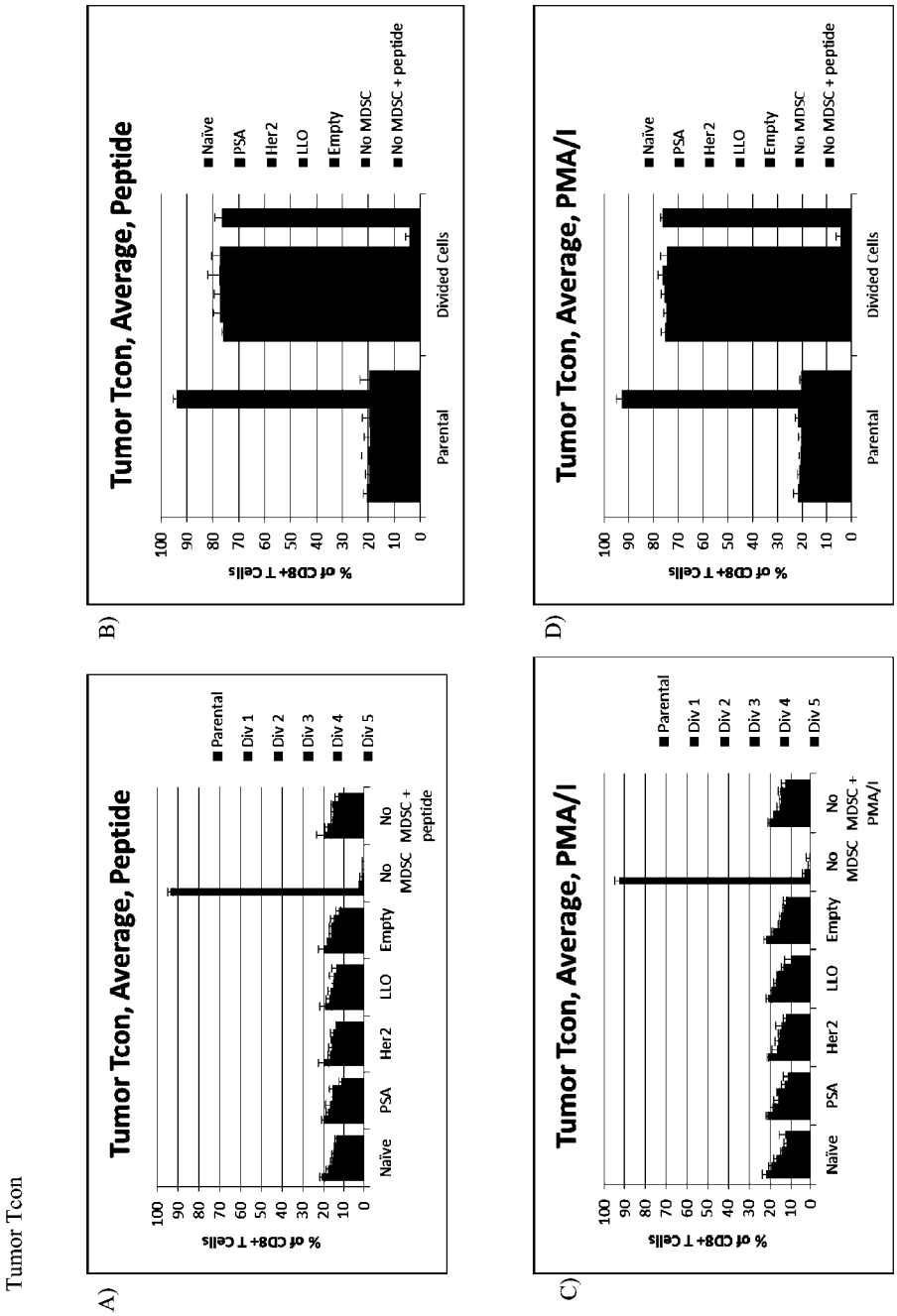

Tumor Tcon
A)
Tumor Tcon, Average, Peptide
% of CD8+ T Cells
Naïve
PSA
Her2
LLO
Empty
No MDSC
No MDSC + peptide
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
B)
Tumor Tcon, Average, Peptide
Naïve
PSA
Her2
LLO
Empty
No MDSC
No MDSC + peptide
Parental
Divided Cells
C)
Tumor Tcon, Average, PMA/I
No MDSC + PMA/I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

Figure 41

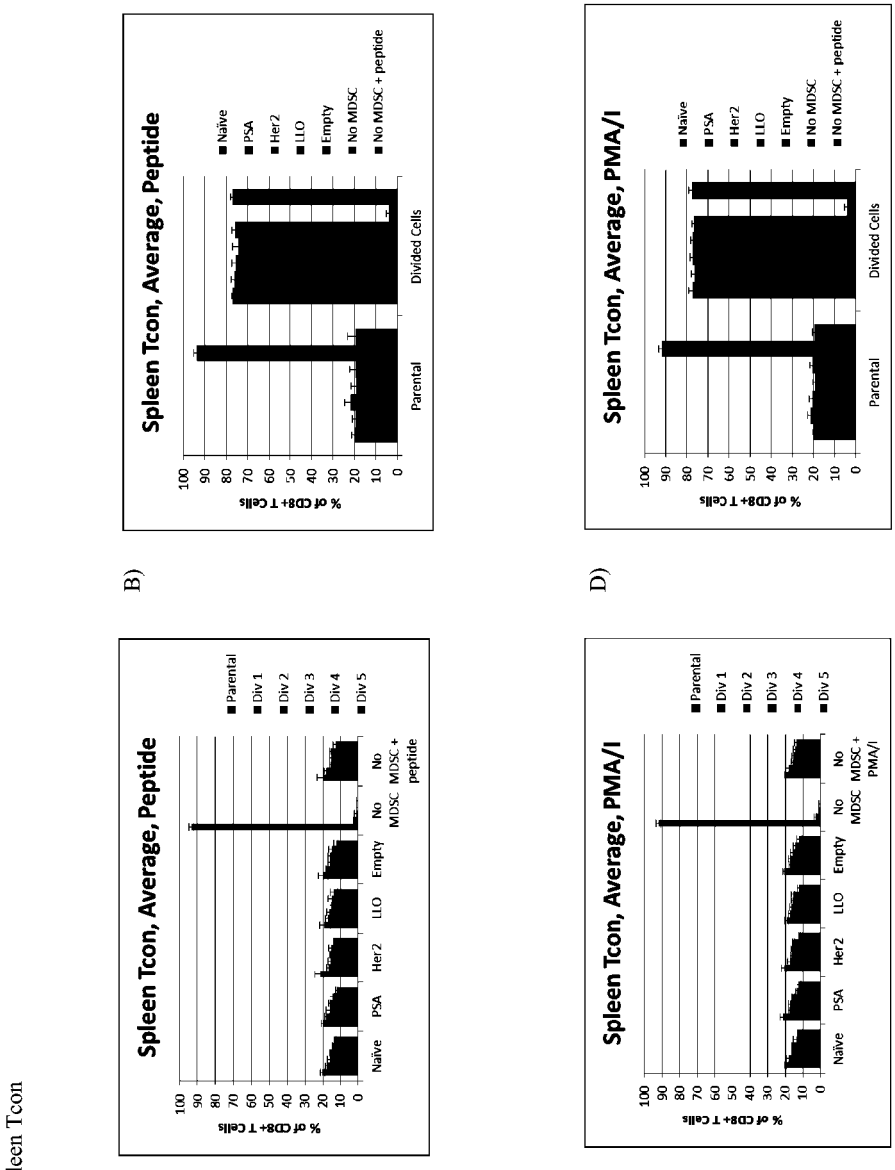

Spleen Tcon
A)
Spleen Tcon, Average, Peptide
% of CD8+ T cells
Naïve
PSA
Her2
LLO
Empty
No MDSC
No MDSC + peptide
Parental
Div 1
Div 2
Div 3
Div 4
Div 5
B)
Spleen Tcon, Average, Peptide
Parental
Divided Cells
Naïve
PSA
Her2
LLO
Empty
No MDSC
No MDSC + peptide
C)
Spleen Tcon, Average, PMA/I
No MDSC +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

Figure 42