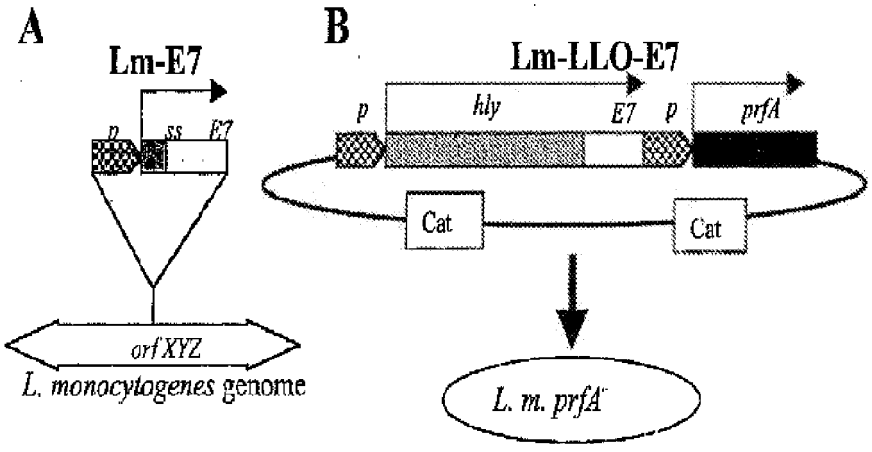


Figure 1

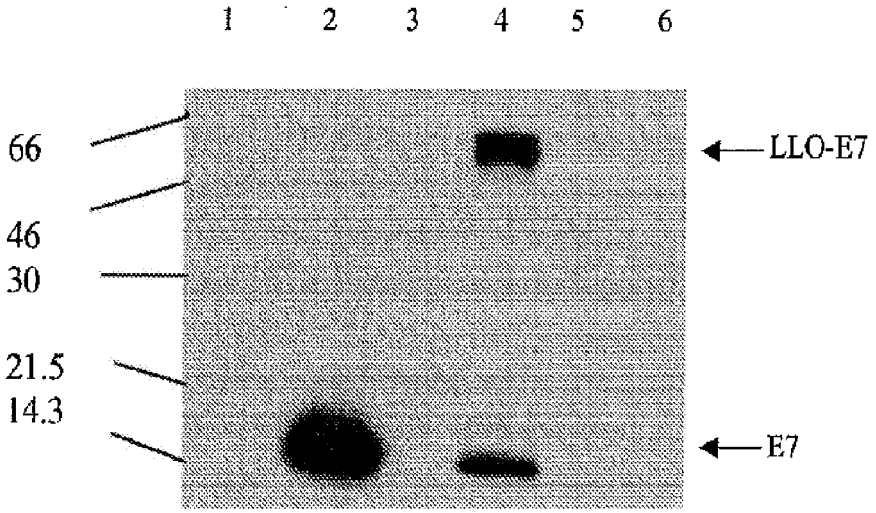


Figure 2

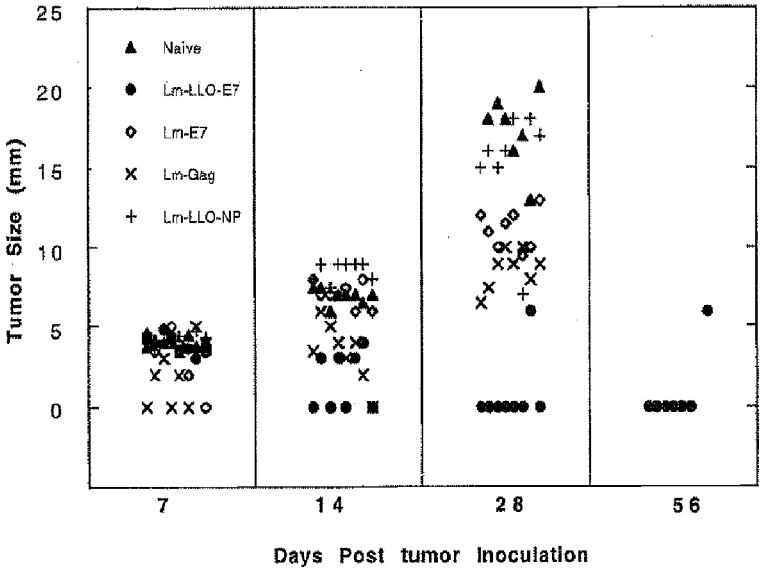

25
20
15
10
5
0
Tumor Size (mm)
Naive
Lm-LLO-E7
Lm-E7
Lm-Gag
Lm-LLO-NP
7
14
28
56
Days Post tumor Inoculation

Figure 3

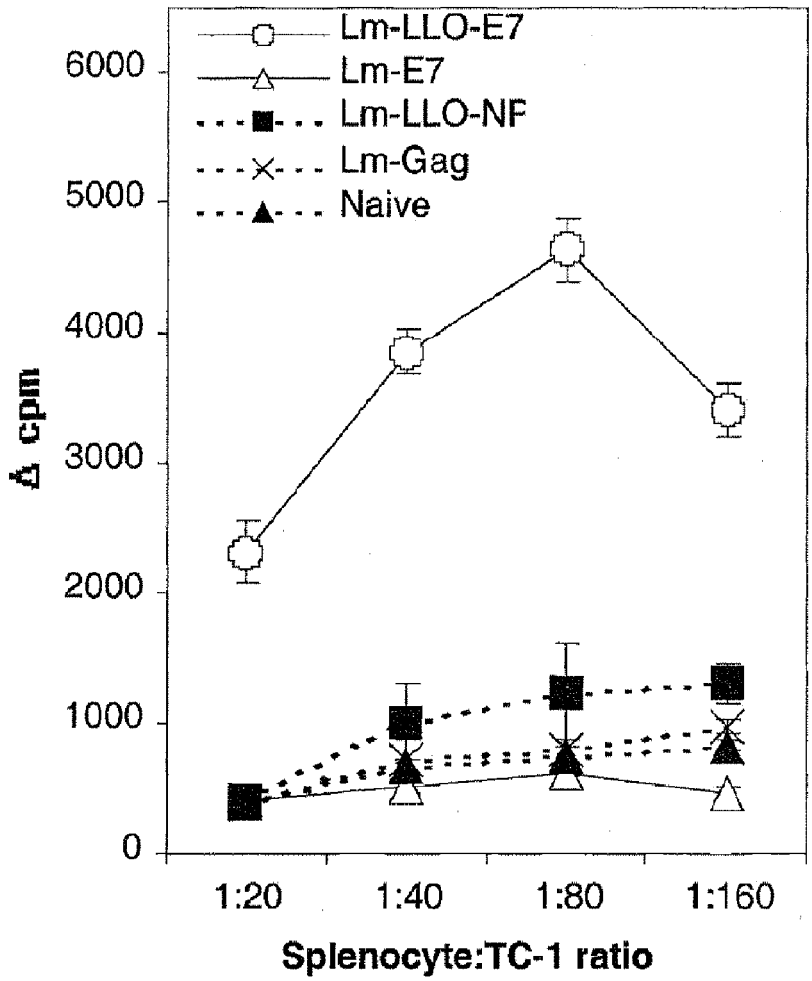

Lm-LLO-E7
Lm-E7
Lm-LLO-NF
Lm-Gag
Naive
6000
5000
4000
3000
2000
1000
0
Δ cpm
1:20
1:40
1:80
1:160
Splenocyte:TC-1 ratio

Figure 4

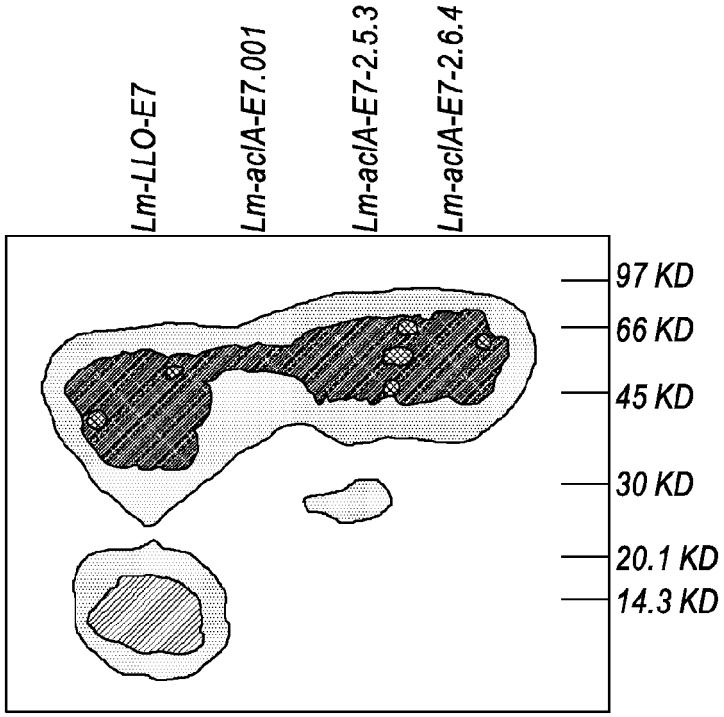


Figure 5A

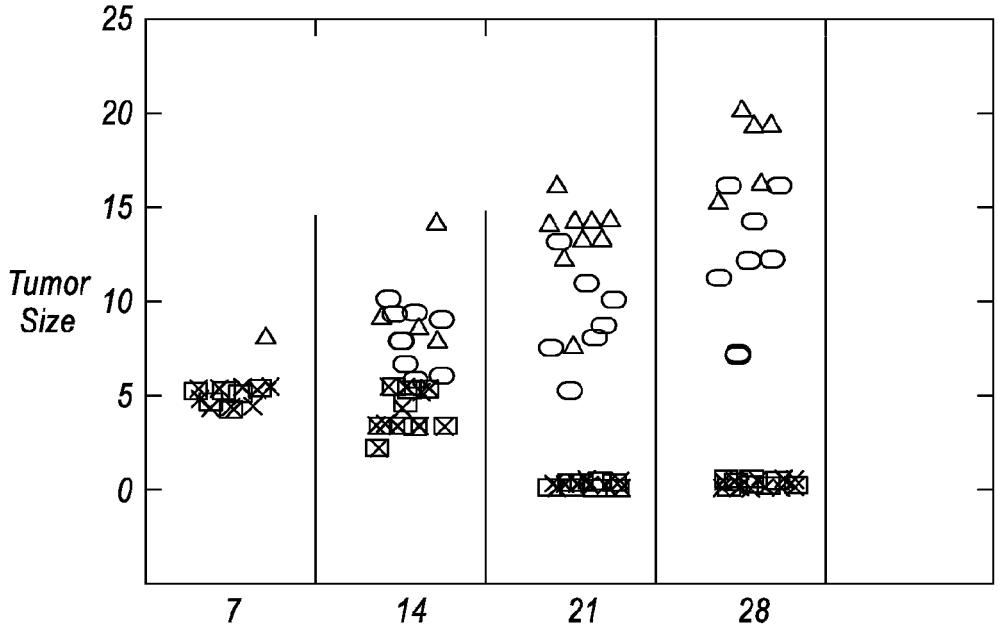


Figure 5B

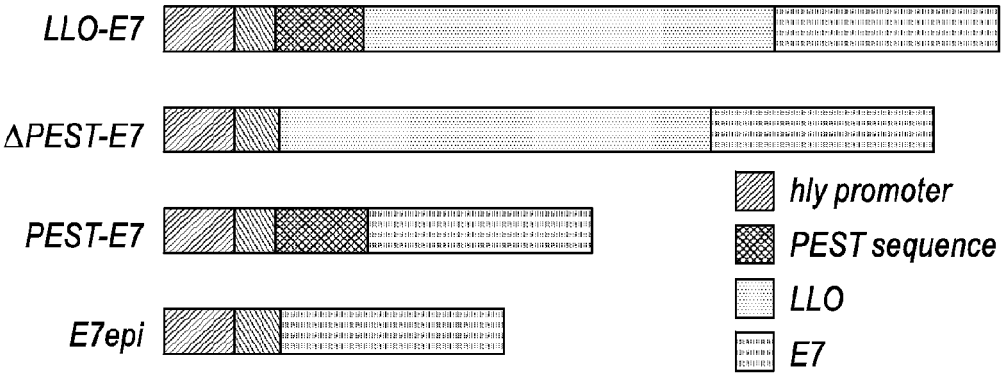


Figure 6A

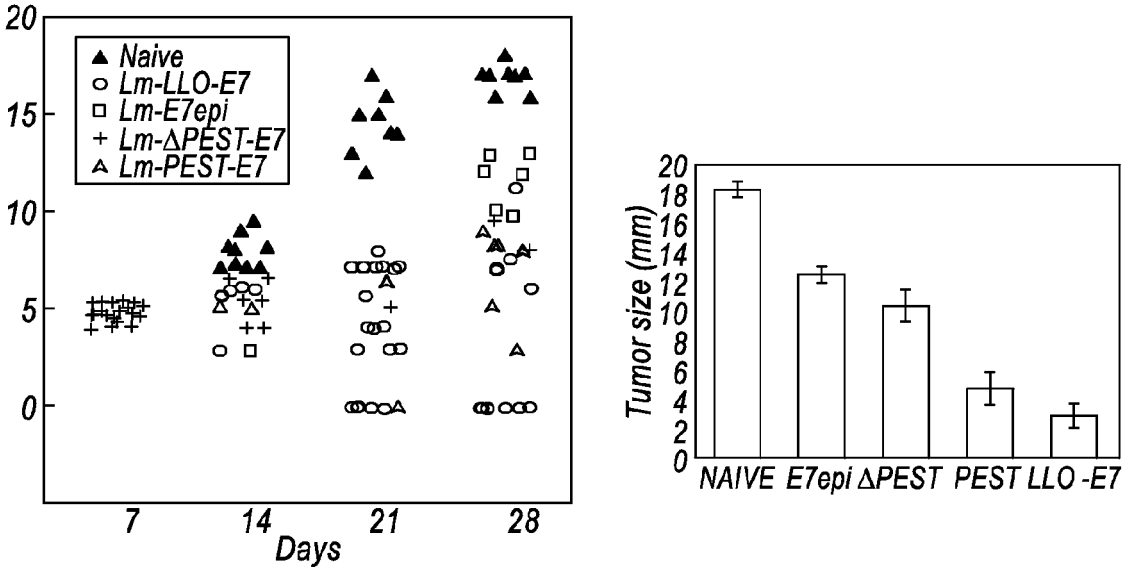


Figure 6B

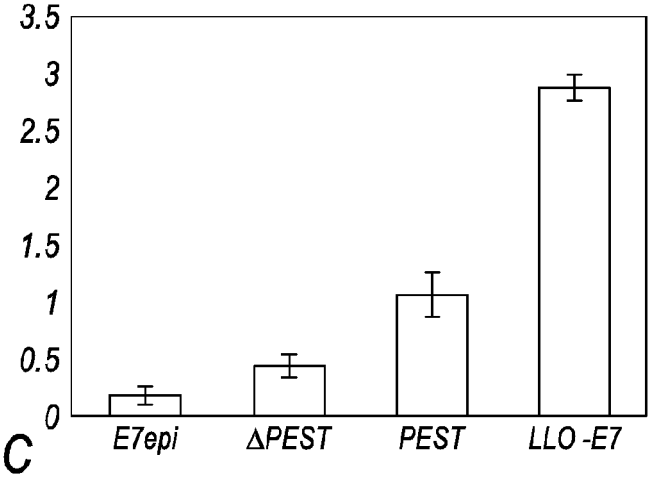


Figure 6C

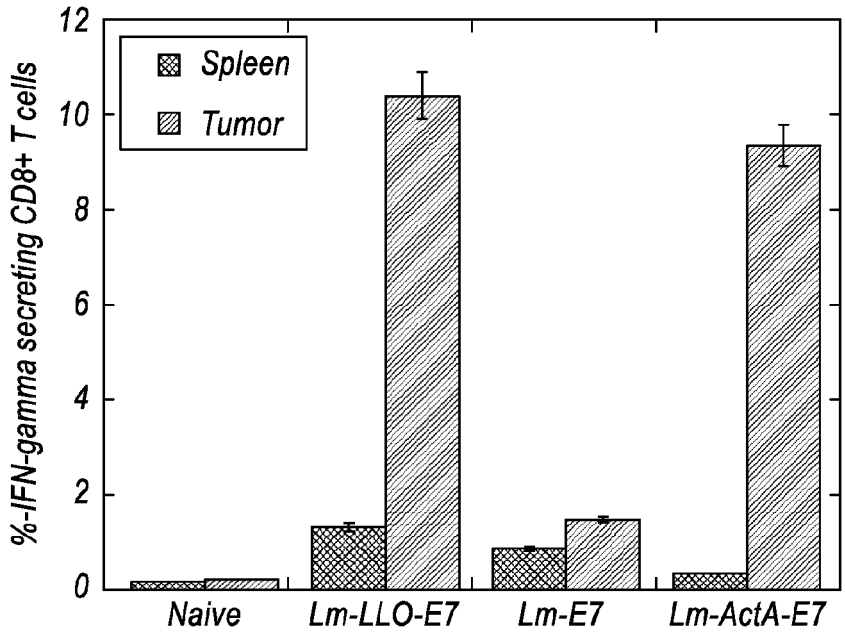


Figure 7A

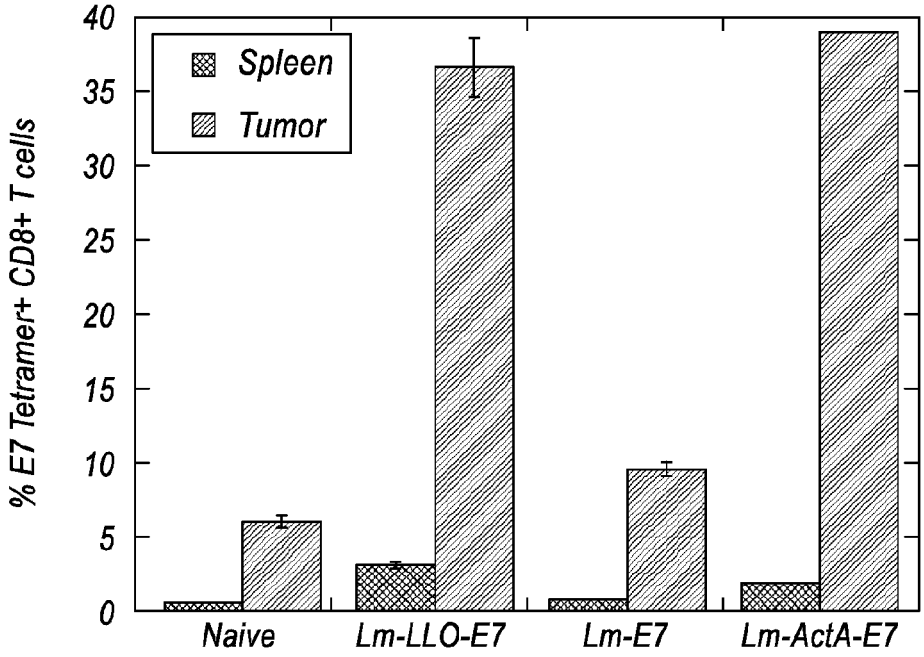


Figure 7B

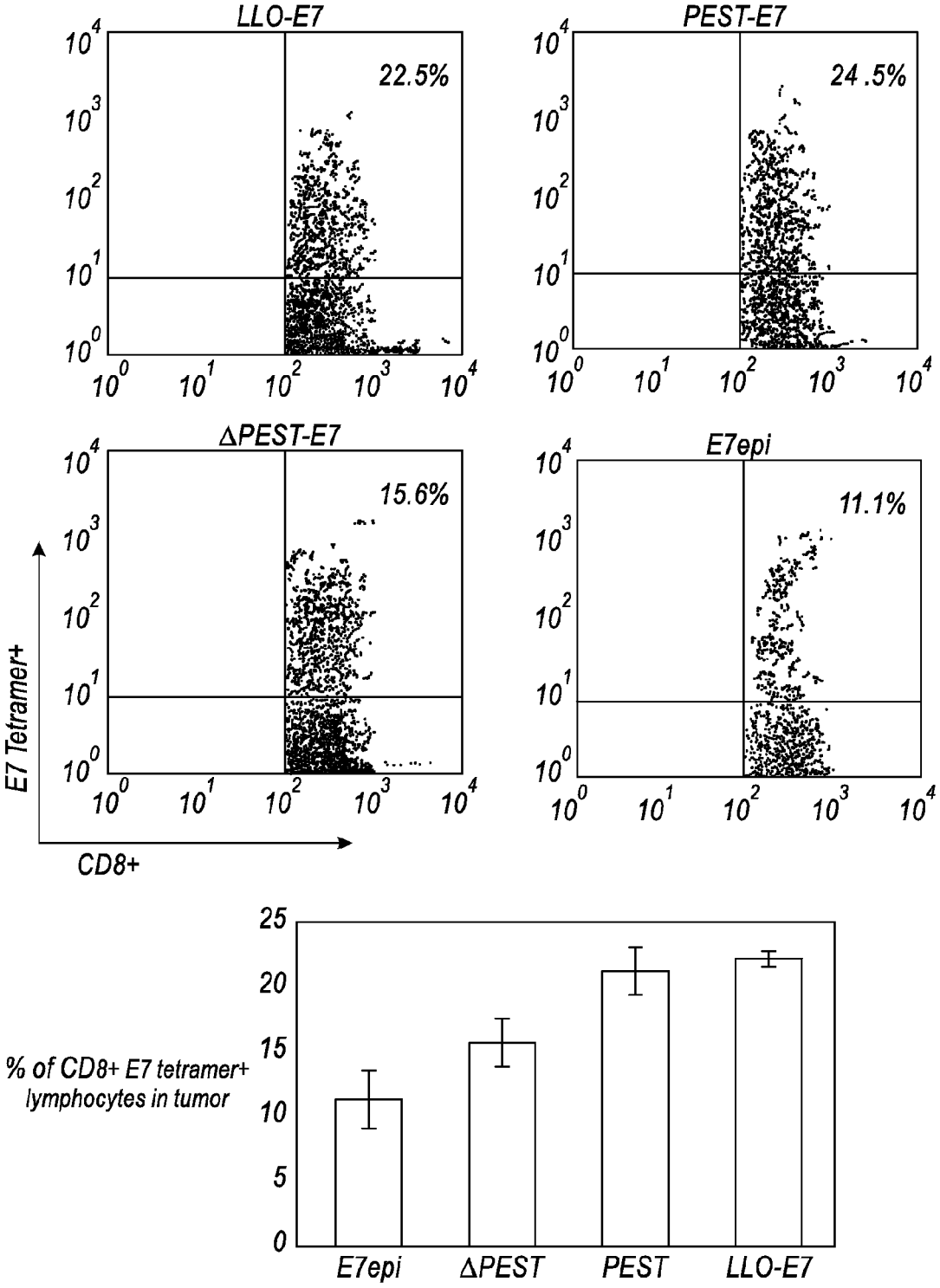

LLO-E7
22.5%
PEST-E7
24.5%
ΔPEST-E7
15.6%
E7epi
11.1%
E7 Tetramer+
CD8+
% of CD8+ E7 tetramer+ lymphocytes in tumor
25
20
15
10
5
0
E7epi
ΔPEST
PEST
LLO-E7

Figure 8

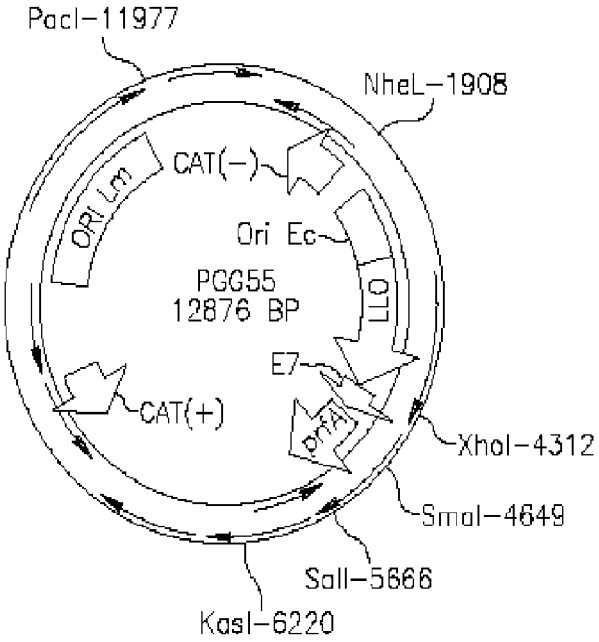


Figure 9A

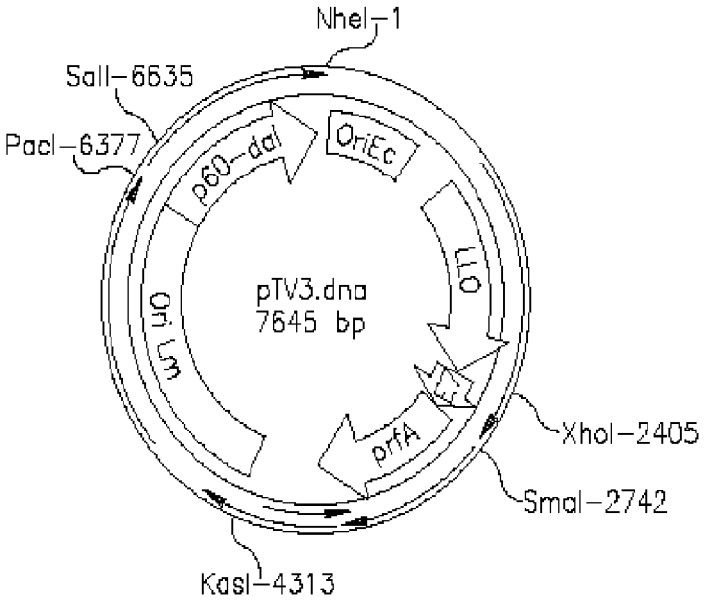


Figure 9B

atggcgcgggatggtatactatacaagcgtatggttcaaaaagatactttgaattaagaagt
acaataaagttaacttcattagacaaaaagaaaaaacaaggaagaatagtacatagttataa
atacttggagagtgaggtgtaatatgggggcagctgatttttggggtttcatatatgtagtt
tcaagattagccattgttgcggcagtagtttacttcttatacttattgagaaaaattgcaaa
taaatagaaaaaaagccttgtcaaacgaggctttttttatgcaaaaaatacgacgaatgaag
ccatgtgagacaatttggaatagcagacaacaaggaaggtagaacatgttttgaaaaattta
ctgattttcgattattattaacgcttgttaatttaaacatctcttatttttgctaacatata
agtatacaaagggacataaaaaggttaacagcgtttgttaaataggaagtatatgaaaatcc
*tcttttgtgtttctaaatttatttttaaggagtggaga***atgttgaaaaaaaataattggtta**
caaaatgcagtaatagcaatgctagtgttaattgtaggtctgtgcattaatatgggttctgg
aacaaaagtacaagctgagagtattcaacgaccaacgcctattaaccaagtttttccagatc
ccggcctagcgaatgcagtgaaacaaaatttagggaagcaaagtgttacagaccttgtatca
caaaaggaactatctggagtacaaaatttcaatggagataatagcaacattcaatctcttgc
gggaatgcaatttttcactaatttaaaagaacttcatctatcccataatcaaataagtgacc
ttagtcctttaaaggatctaactaagttagaagagctatctgtgaatagaaacagactgaaa
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agatactgactcgcttattcatttgaaaaatctagaaatcttatctattcgtaataataagt
taaaaagtattgtgatgcttggtttttatcaaaactagaggtattagatttgcatggtaat
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ggttgtgtcctgtgggaattgccagtttatacagatgaagtaagctataagtttagcgaata
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aggacttgtgcacacctgtatactttgagctctcgtataatcacgagagcttttaaatatg
taagtcttaattatctcttgacaaaaagaacgtttattcgtataaggttaccaagagatgaa
gaaactattttatttacaattcaccttgacaccaaaaactccatatgatatagtaaataagg
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cagcccgttttttatggcctccgaacgaatgagttagcaggccgcagatttgaacagctat
tttctatcttgttgtaacaaaattaagtggaggtggctcaccattagcaaagacatgttggt
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tgaagagtaaaatcgatgcgcttcaaattgctgaaaaggctaatcttgatctagtgcttgtt
gctccaacagcgaaaccgccagtagctcgta

Figure 10

*GAATTC*atggcgcgggatggtatactatacaagcgtatggttcaaaaagatactttgaattaa
gaagtacaataaagttaacttcattagacaaaaagaaaaaacaaggaagaatagtacatagtt
ataaatacttggagagtgaggtgtaatatgggggcagctgatttttggggtttcatatatgta
gtttcaagattagccattgttgcggcagtagtttacttcttatacttattgagaaaaattgca
aataaatagaaaaaaagccttgtcaaacgaggctttttttatgcaaaaaatacgacgaatgaa
gccatgtgagacaatttggaatagcagacaacaaggaaggtagaacatgttttgaaaaattta
ctgattttcgattattattaacgcttgttaatttaaacatctcttatttttgctaacatataa
gtatacaaagggacataaaaaggttaacagcgtttgttaaataggaagtatatgaaaatcctc
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ctttgagctctcgtataatcacgagagctttttaaatatgtaagtcttaattatctcttgaca
aaaagaacgtttattcgtataaggttaccaagagatgaagaaactattttatttacaattcac
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cttacgcgattttgaatgtgcgggattgctgaaaagcagcccgtttttttatggcctccgaac
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ggaggtggctcaccattagcaaagacatgttggtaaacgatgggattcgtgcacgtgaagtaa
gattgatcgaccaagacggtgaacaattaggcgtgaagagtaaaatcgatgcgcttcaaattg
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CTGCAG

Figure 11

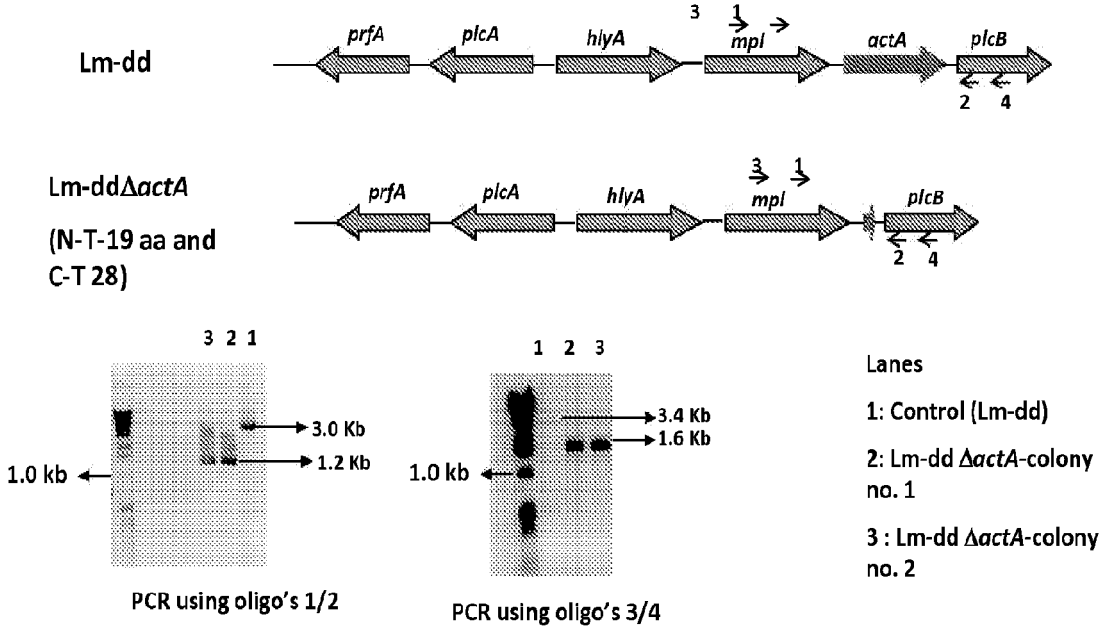

Lm-dd
prfA
plcA
hlyA
3
1
mpl
actA
plcB
2
4
Lm-ddΔactA
(N-T-19 aa and
C-T 28)
3 2 1
3.0 Kb
1.2 Kb
1.0 kb
PCR using oligo's 1/2
1 2 3
3.4 Kb
1.6 Kb
1.0 kb
PCR using oligo's 3/4
Lanes
1: Control (Lm-dd)
2: Lm-dd ΔactA-colony no. 1
3 : Lm-dd ΔactA-colony no. 2

Figure 12

gcgccaaatcattggttgattggtgaggatgtctgtgtgcgtgggtcgcgagatgggcgaataagaagcattaaagatcctgacaaatat
aatcaagcggctcatatgaaagattacgaatcgcttccactcacagaggaaggcgactggggcggagttcattataatagtggtatccc
gaataaagcagcctataatactatcactaaacttggaaaagaaaaaacagaacagctttattttcgcgccttaaagtactatttaacgaaaa
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*acgattaaccccgac**gtcgac**ccatacgacgttaattcttgcaatgttagctattggcgtgttctctttaggggcgtttatcaaaattatt*
*caattaagaaaaaataatta*aaaacacagaacgaaagaaaaagtgaggtgaatgatatgaaattcaaaaaggtggttctaggtatgtg
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ggaaaaaatactagctaaagatgtaaatcatatgcgagctaatttaatgaatgaacttaaaaaattcgataaacaaatagctcaaggaata
tatgatgcggatcataaaaatccatattatgatactagtacatttttatctcatttttataatcctgatagagataatacttatttgccgggttttgc
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Figure 13

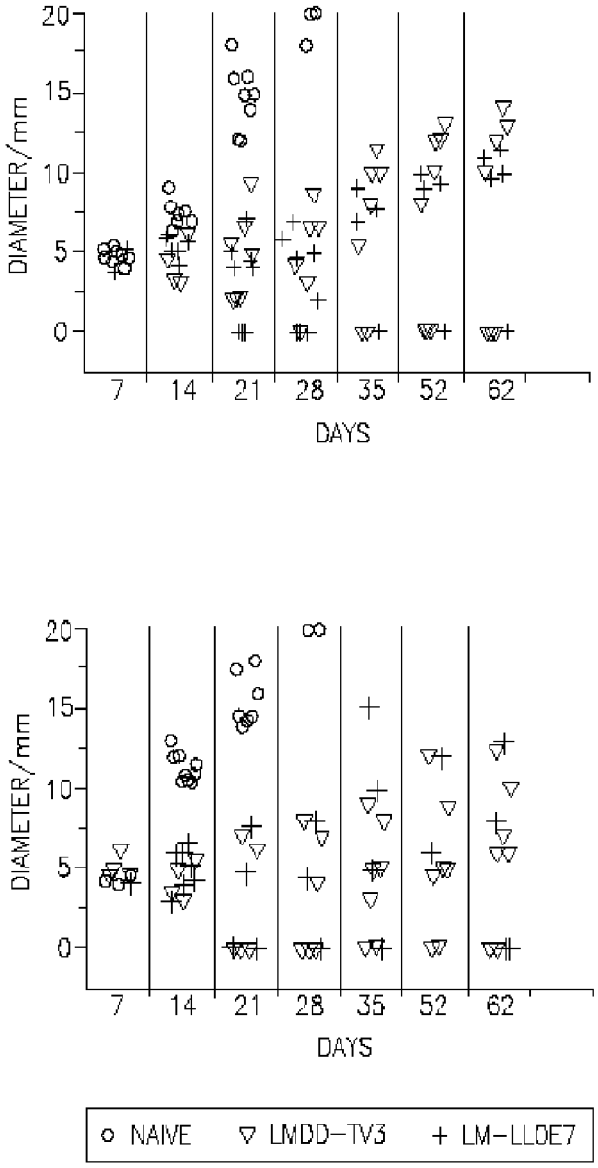
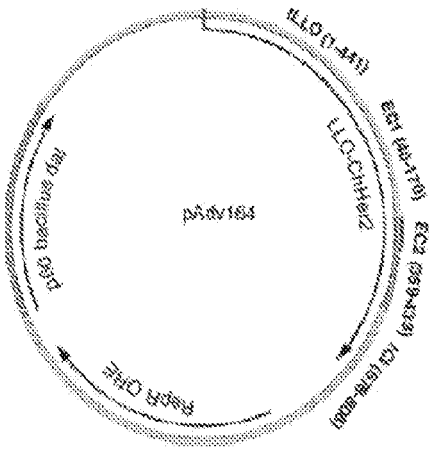

DIAMETER/mm
20
15
10
5
0
7
14
21
28
35
52
62
DAYS
NAIVE
LMDD-TV3
LM-LLDE7

Figure 14

A.


pAdv164
LLO-ChiHer2
Amp R
pUC ori

B.

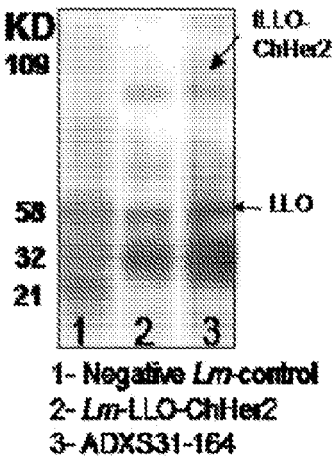
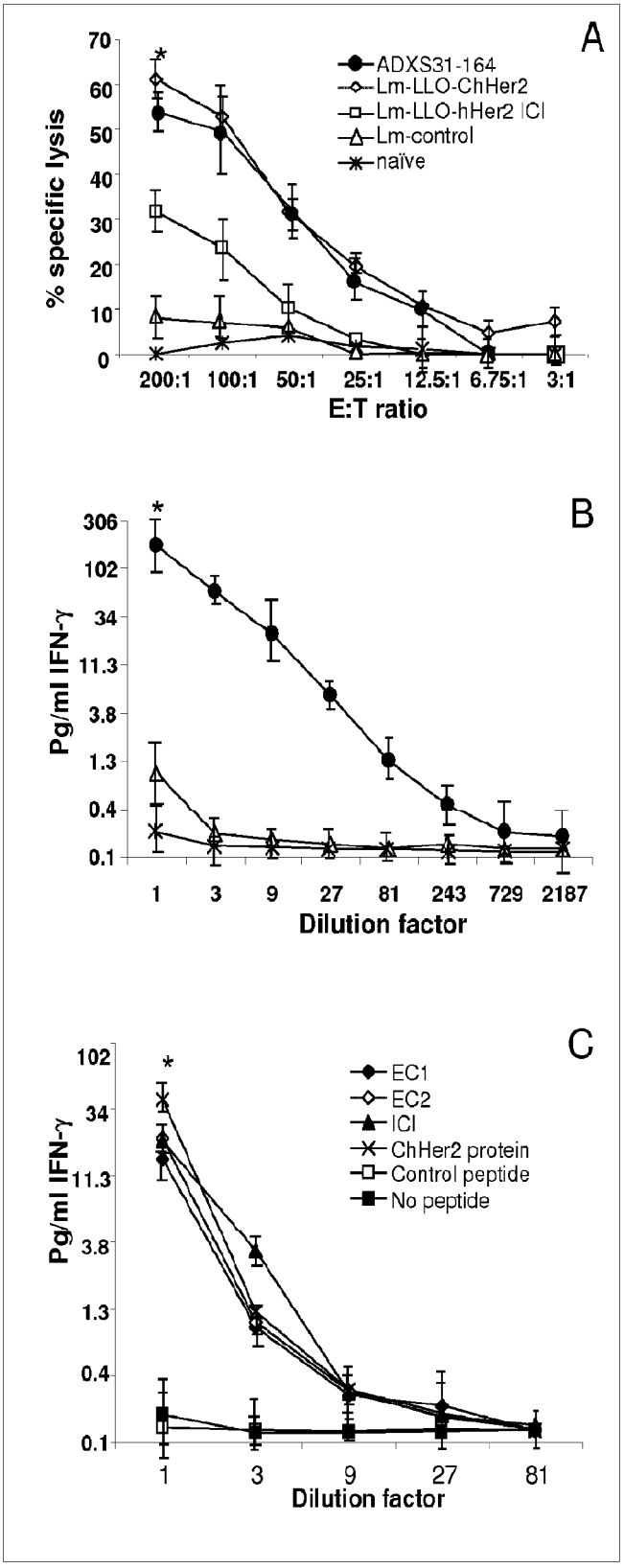

KD
109
58
32
21
fLLO-ChiHer2
LLO
1 2 3
1- Negative Lm-control
2- Lm-LLO-ChiHer2
3- ADXS31-164

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

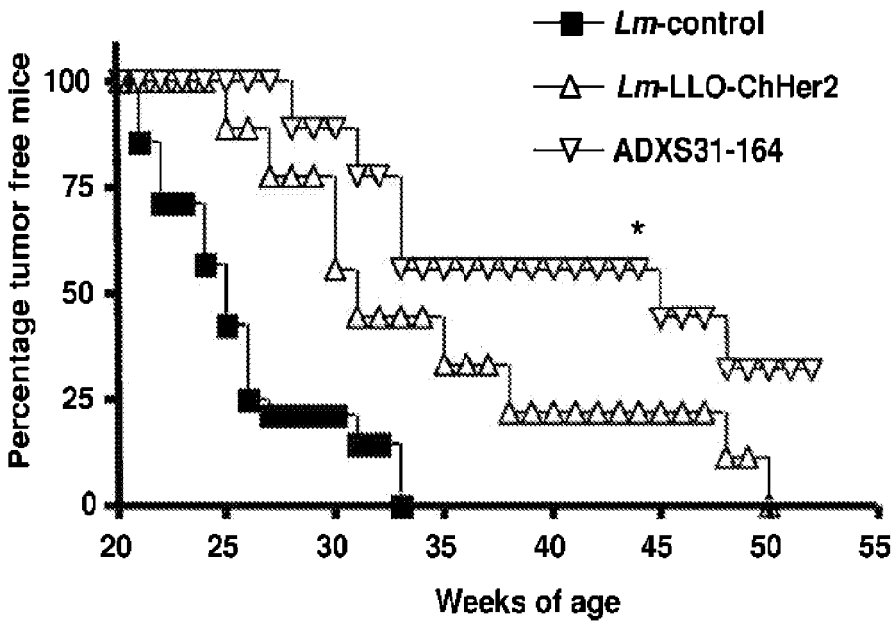

Lm-control
Lm-LLO-ChHer2
ADXS31-164
Percentage tumor free mice
100
75
50
25
0
20
25
30
35
40
45
50
55
Weeks of age
*

Figure 17

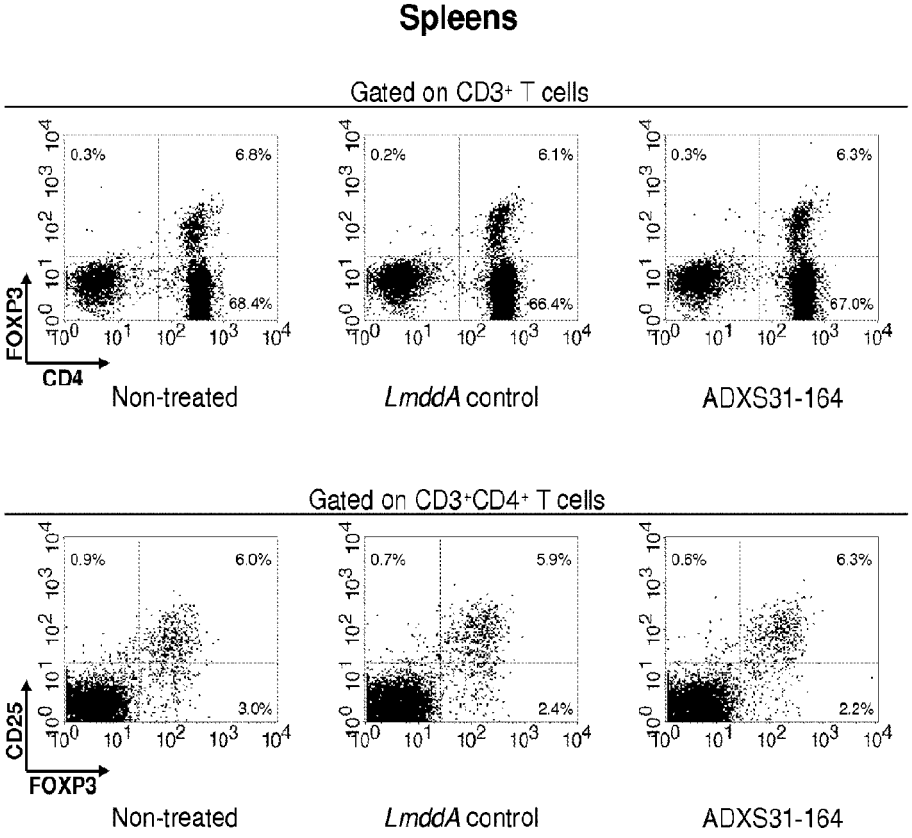

Spleens
Gated on CD3+ T cells
0.3%
6.8%
68.4%
0.2%
6.1%
66.4%
0.3%
6.3%
67.0%
FOXP3
CD4
Non-treated
LmddA control
ADXS31-164
Gated on CD3+CD4+ T cells
0.9%
6.0%
3.0%
0.7%
5.9%
2.4%
0.6%
6.3%
2.2%
CD25
FOXP3
Non-treated
LmddA control
ADXS31-164

Figure 18

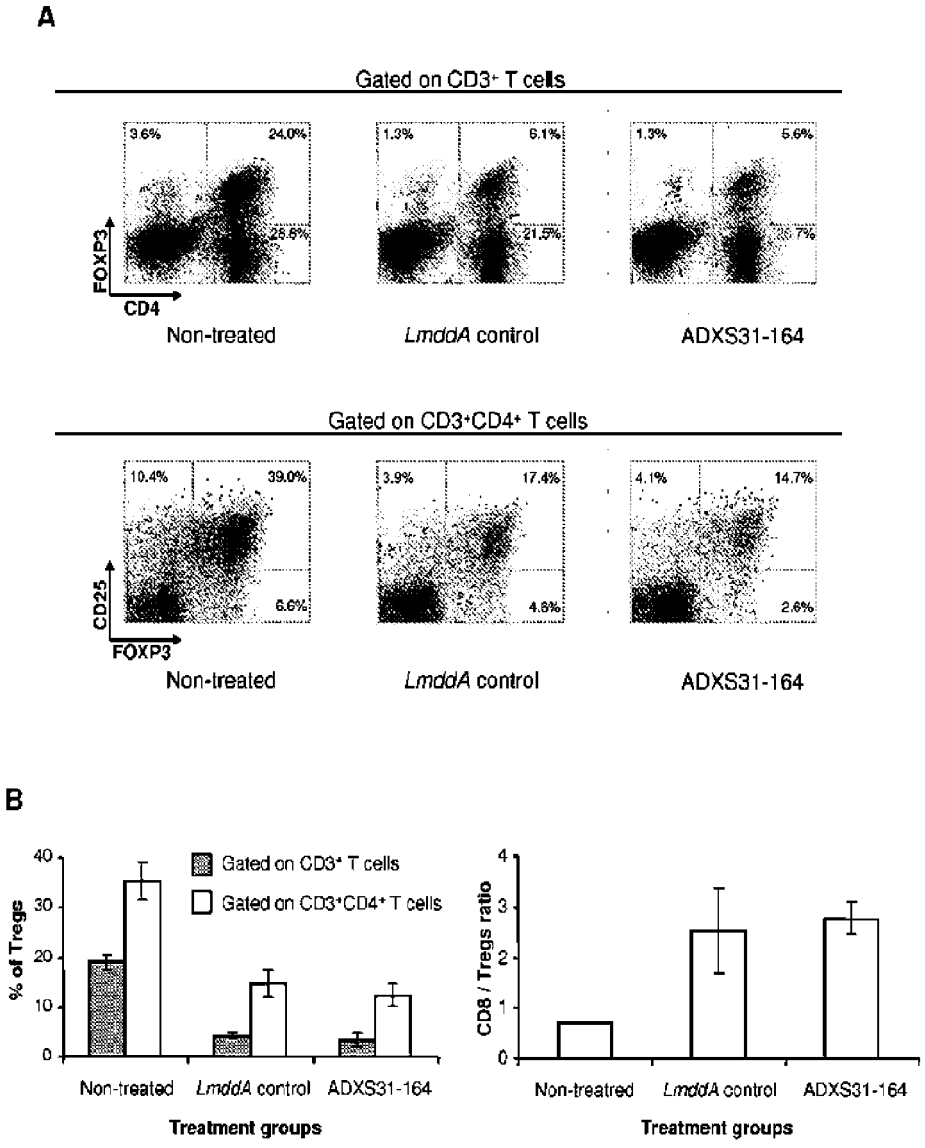

A
Gated on CD3+ T cells
3.6%
24.0%
28.6%
1.3%
6.1%
21.5%
1.3%
5.6%
7%
FOXP3
CD4
Non-treated
LmddA control
ADXS31-164
Gated on CD3+CD4+ T cells
10.4%
39.0%
6.6%
3.9%
17.4%
4.6%
4.1%
14.7%
2.6%
CD25
FOXP3
Non-treated
LmddA control
ADXS31-164
B
Gated on CD3+ T cells
Gated on CD3+CD4+ T cells
% of Tregs
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

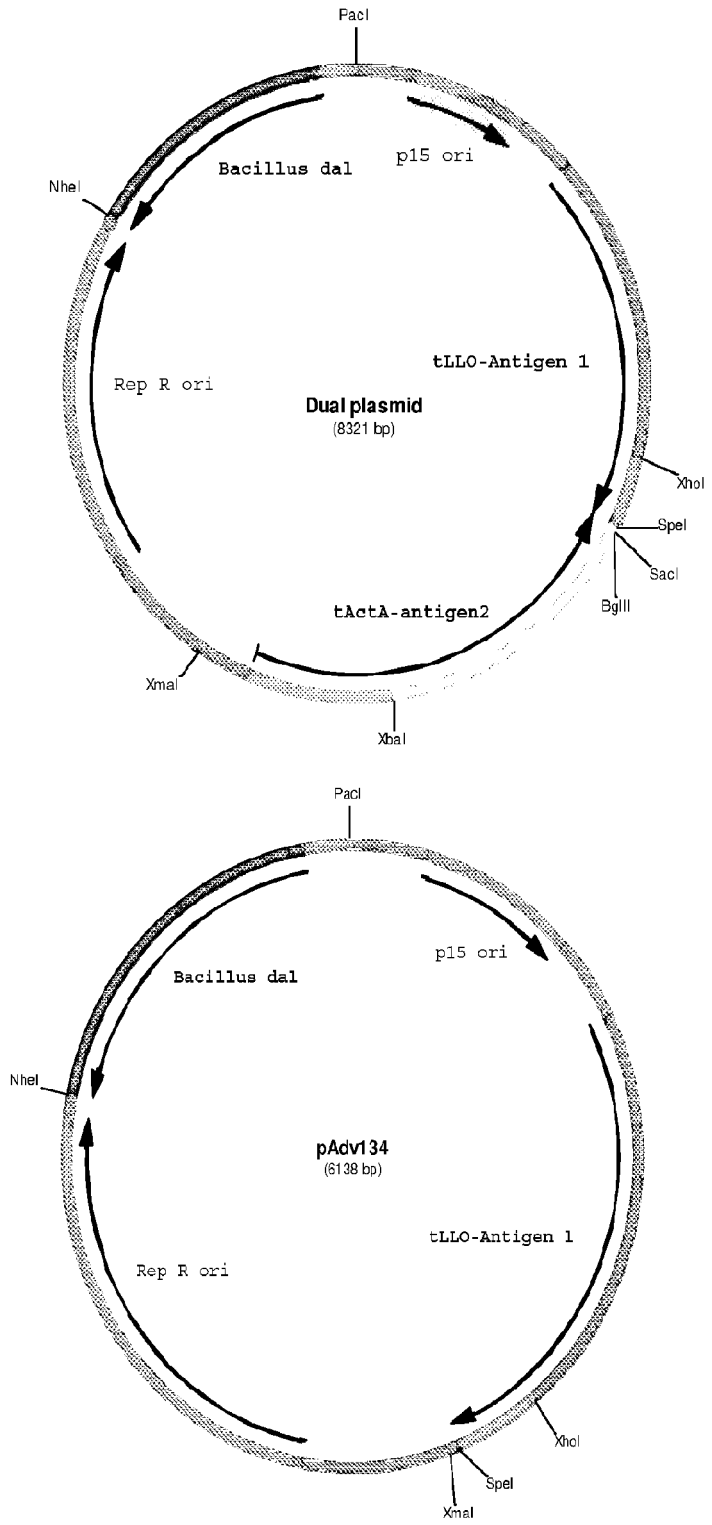

PacI
p15 ori
Bacillus dal
NheI
tLLO-Antigen 1
Rep R ori
Dual plasmid
(8321 bp)
XhoI
SpeI
SacI
BglII
tActA-antigen2
XmaI
XbaI
PacI
p15 ori
Bacillus dal
NheI
pAdv134
(6138 bp)
tLLO-Antigen 1
Rep R ori
XhoI
SpeI
XmaI

Figure 20

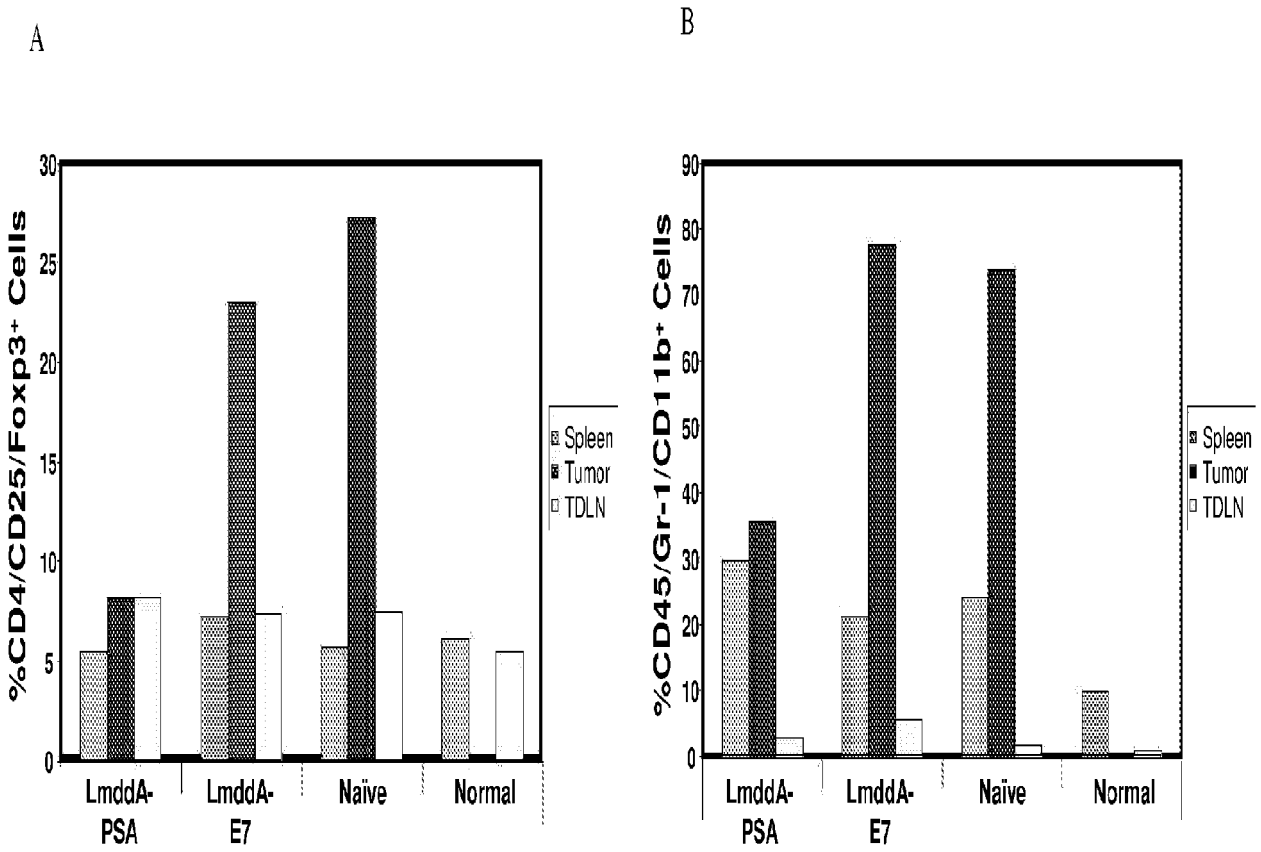

A
B
%CD4/CD25/Foxp3+ Cells
%CD45/Gr-1/CD11b+ Cells
Spleen
Tumor
TDLN
LmddA-PSA
LmddA-E7
Naïve
Normal

Figure 21

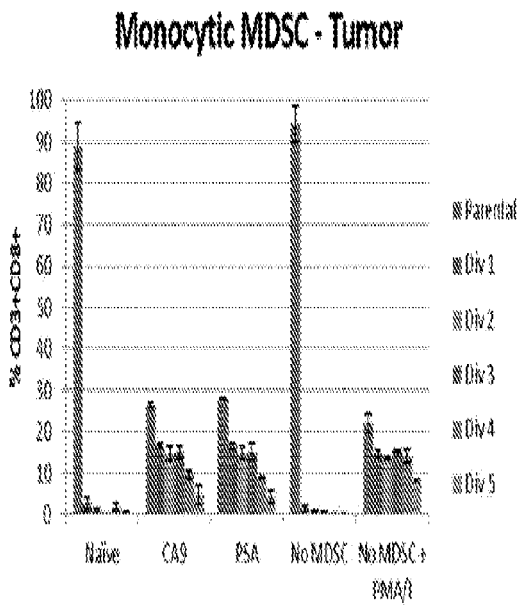


Figure 22A

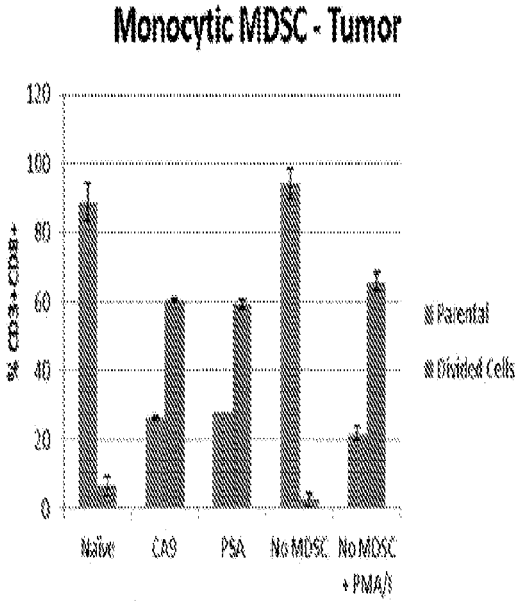


Figure 22B

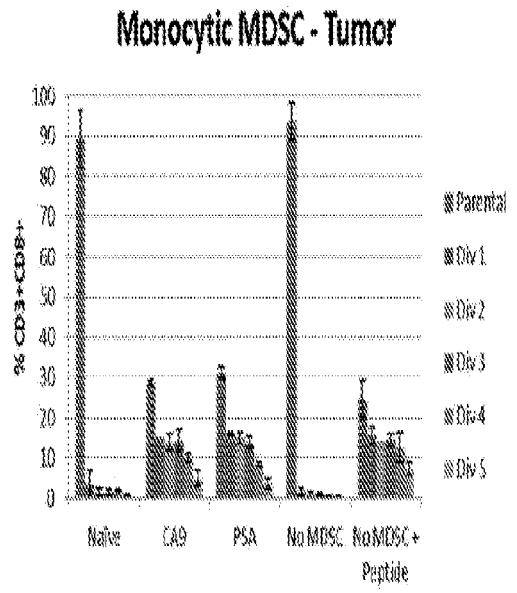


Figure 22C

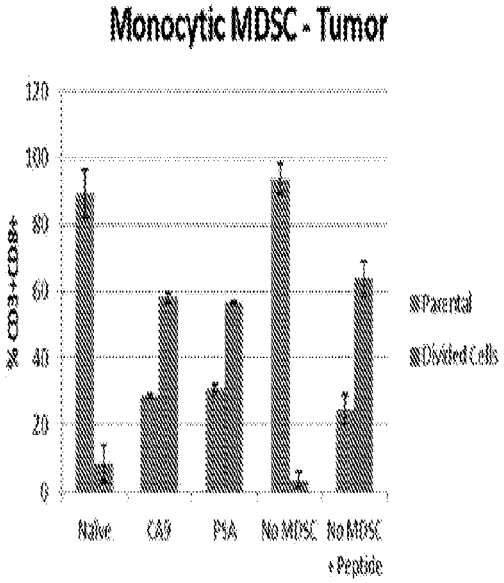


Figure 22D

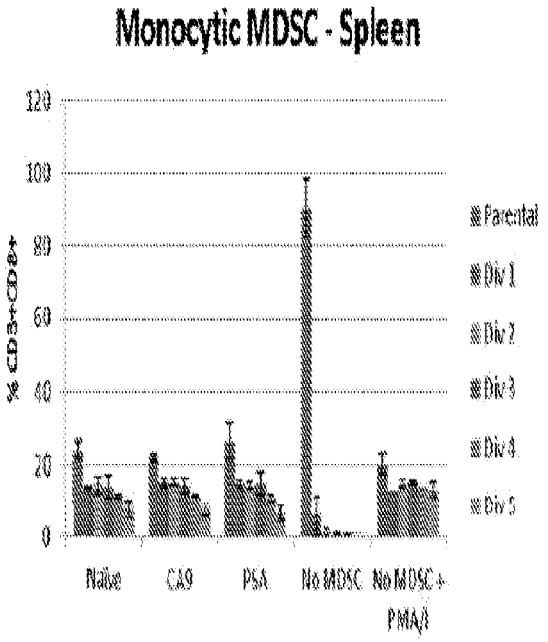


Figure 23A

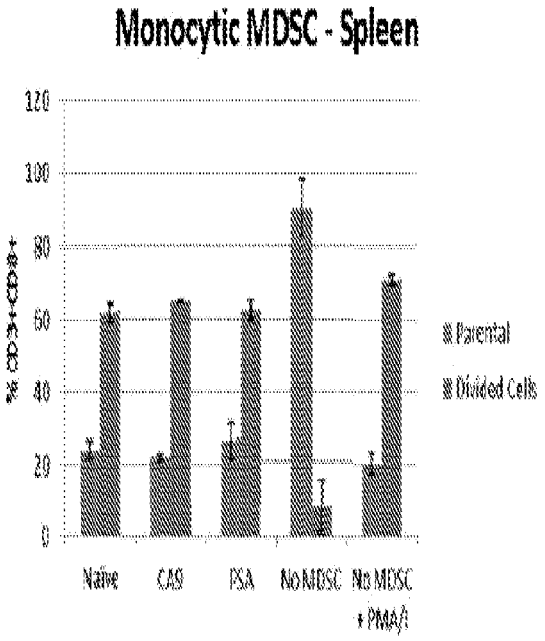


Figure 23B

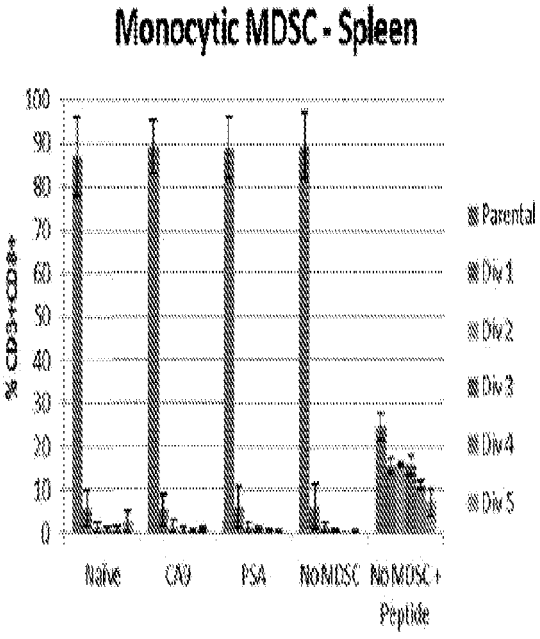


Figure 23C

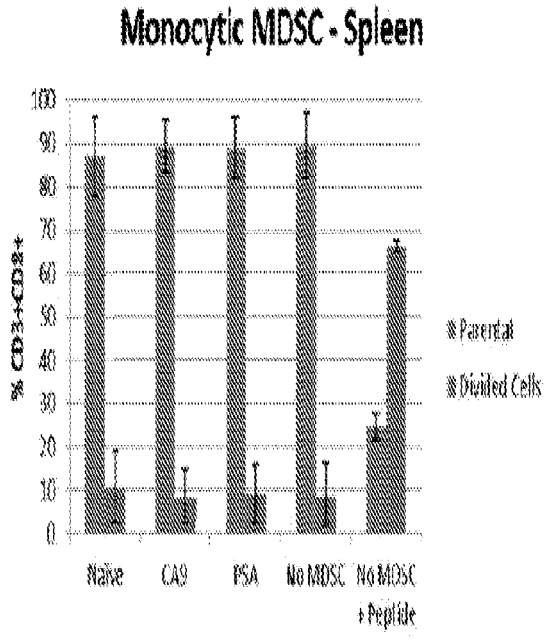


Figure 23D

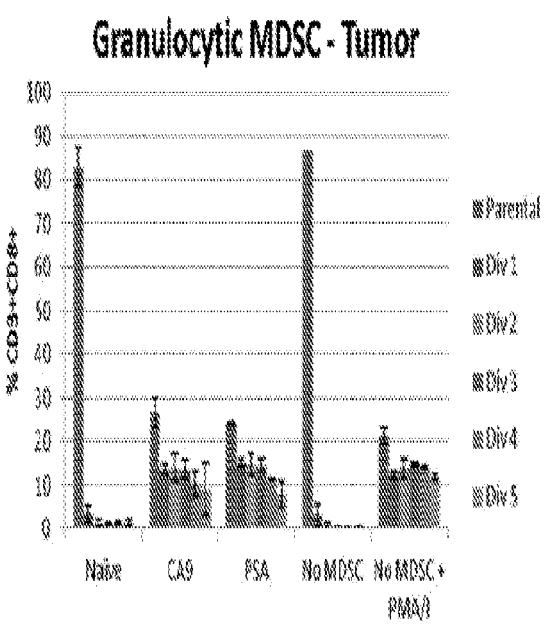


Figure 24A

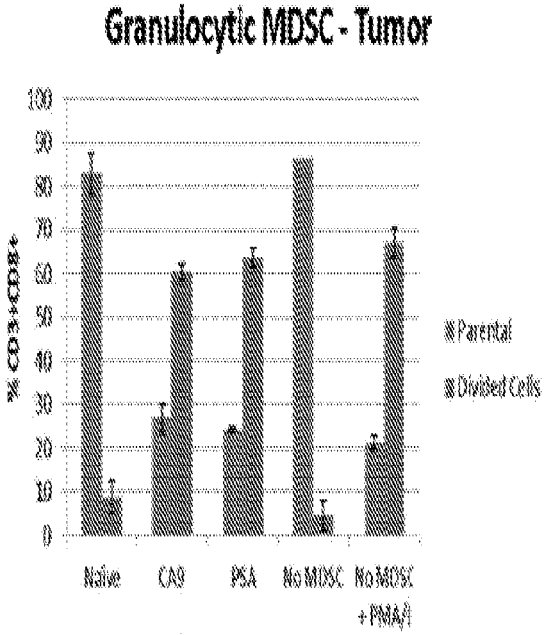


Figure 24B

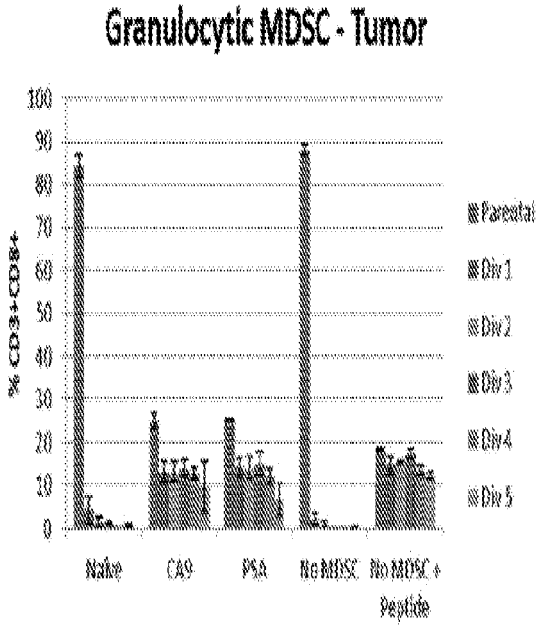


Figure 24C

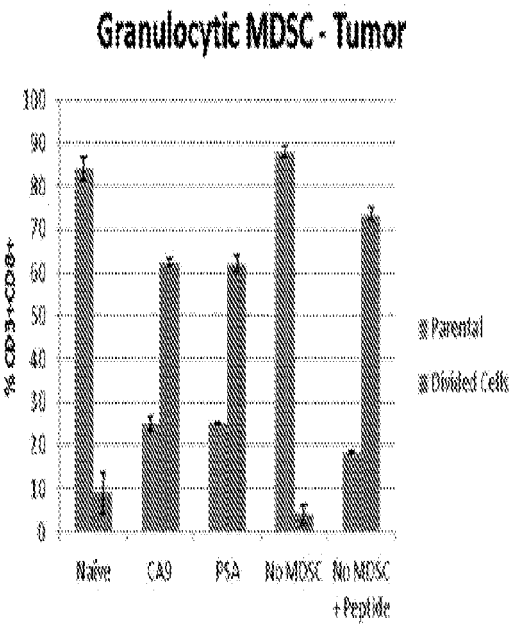


Figure 24D

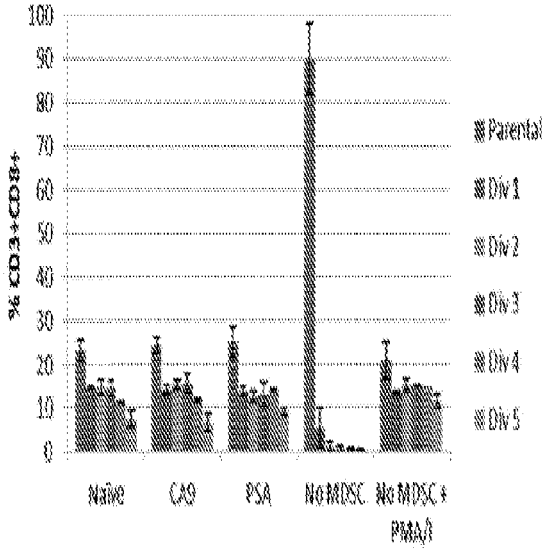


Figure 25A

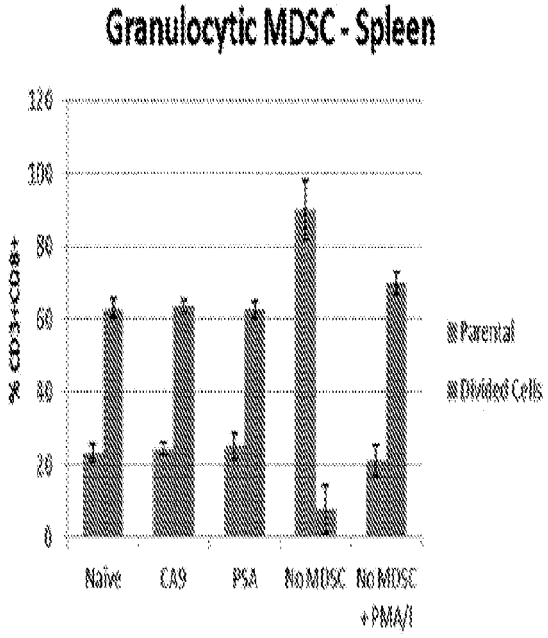


Figure 25B

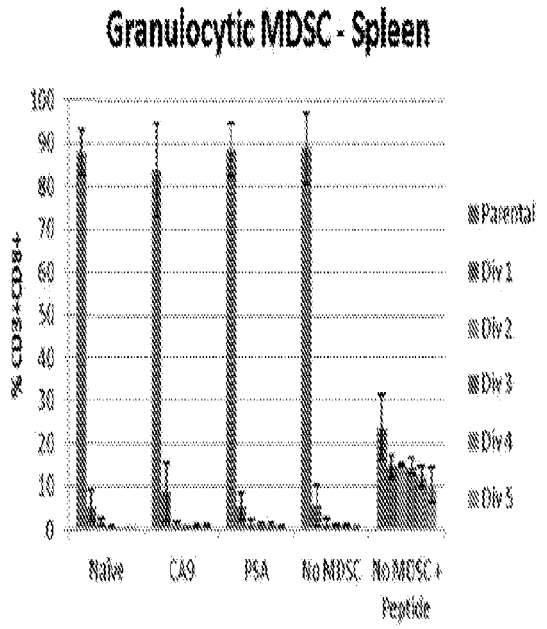


Figure 25C

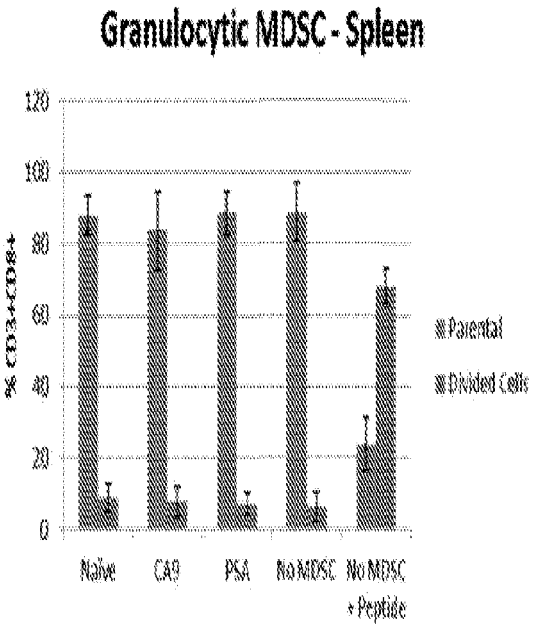


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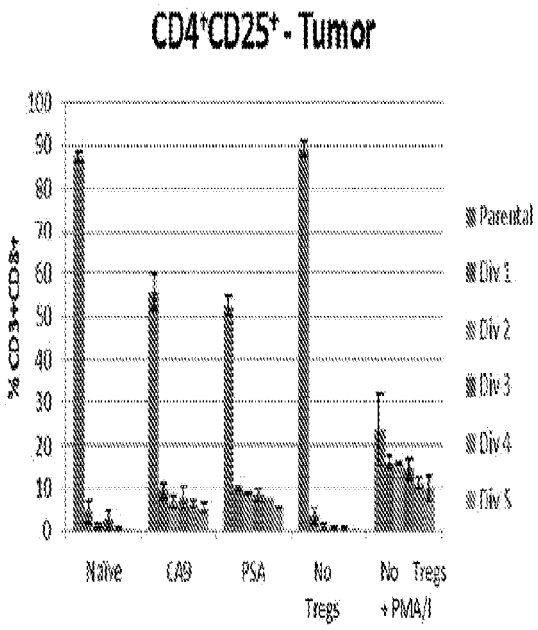


Figure 26A

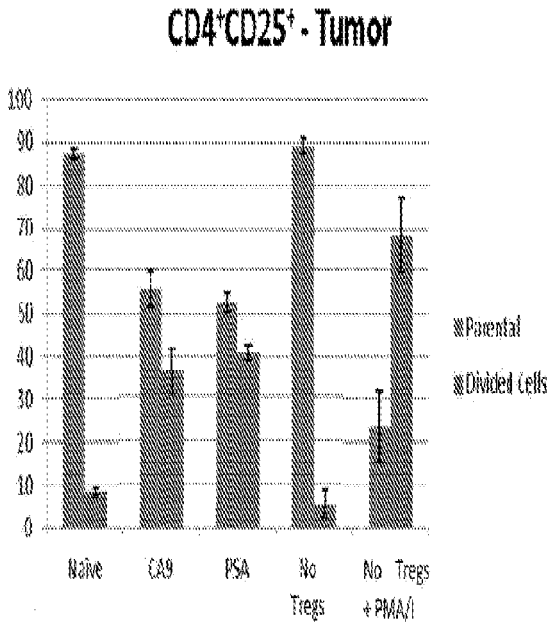


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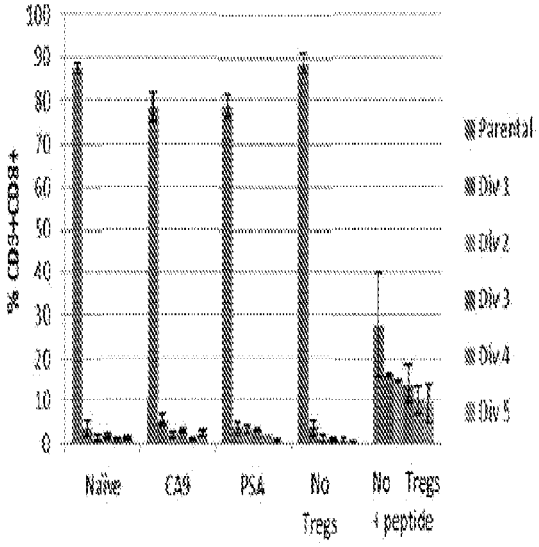


Figure 26C

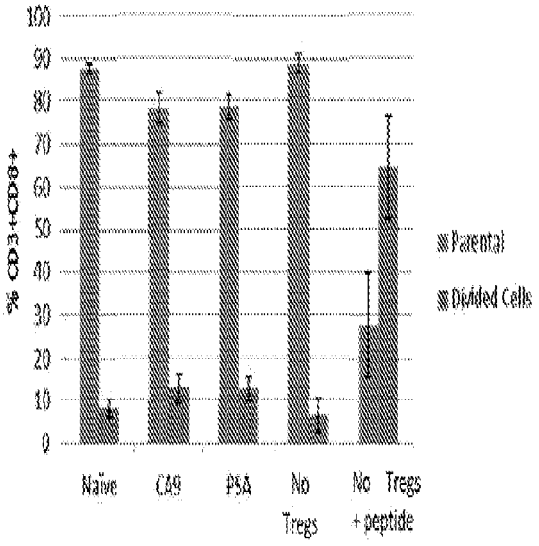


Figure 26D

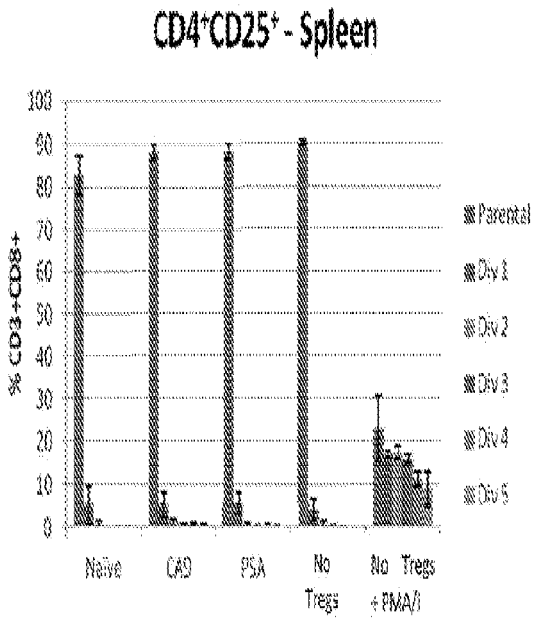


Figure 27A

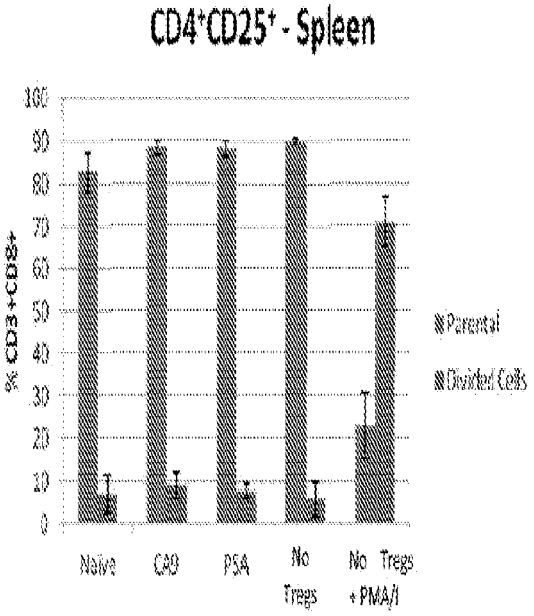


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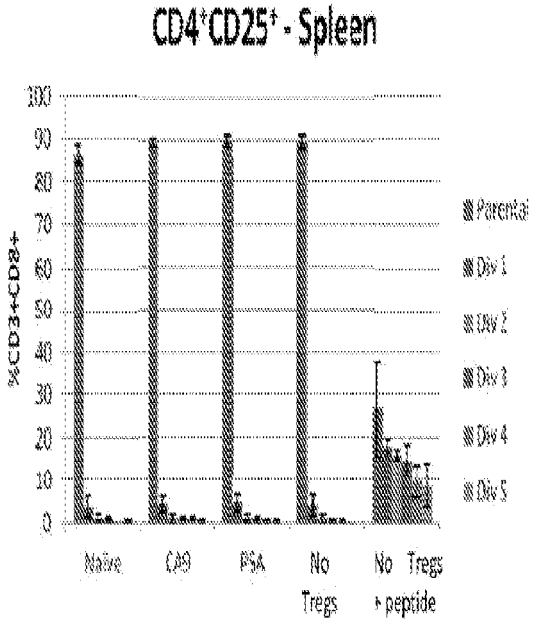


Figure 27C

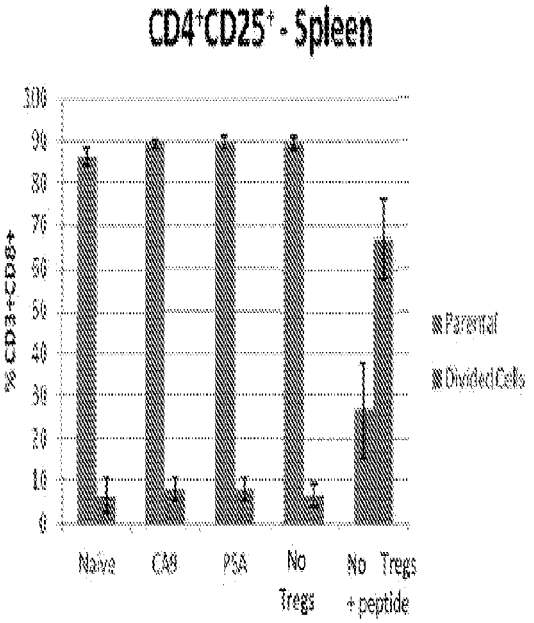


Figure 27D

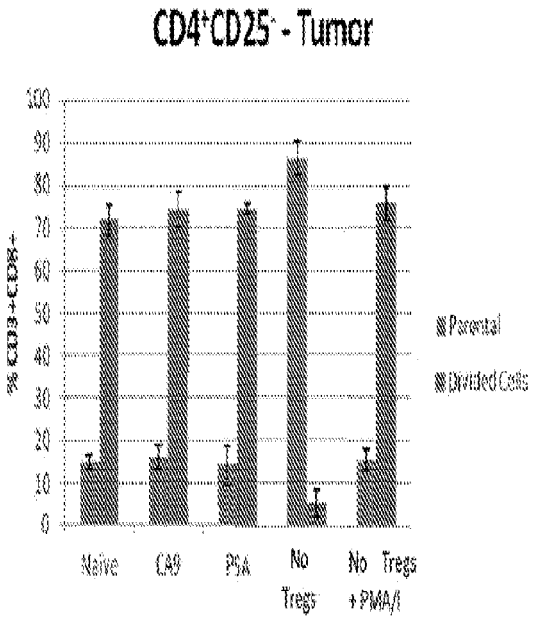


Figure 28A

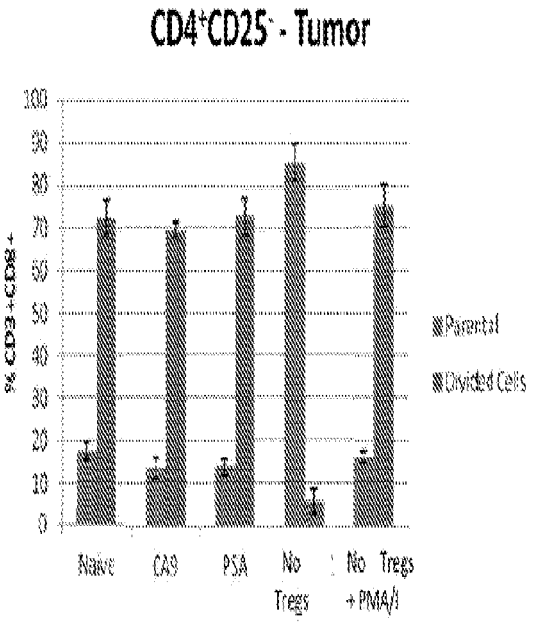


Figure 28B

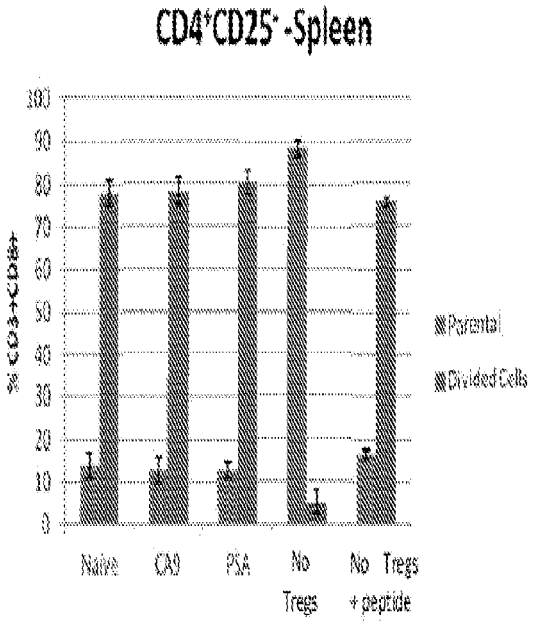


Figure 28C

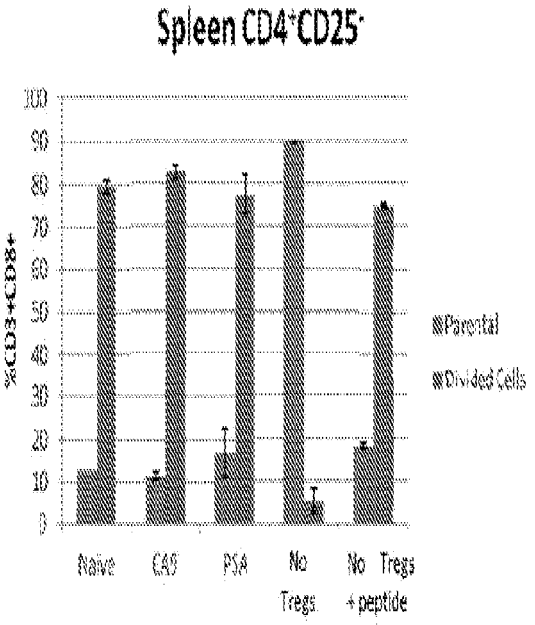


Figure 28D

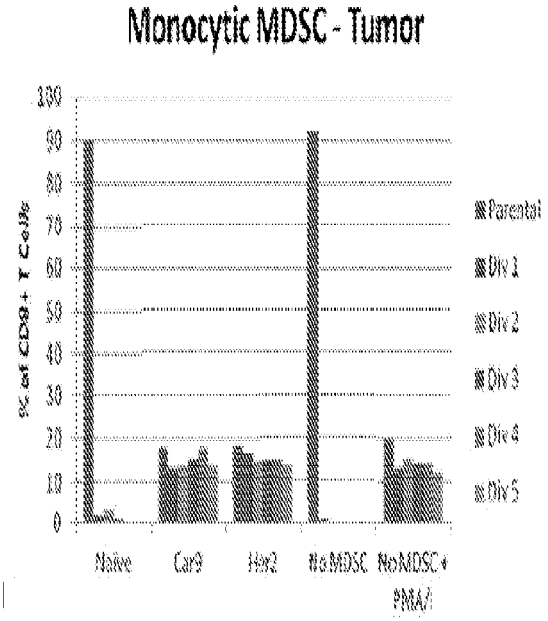


Figure 29A

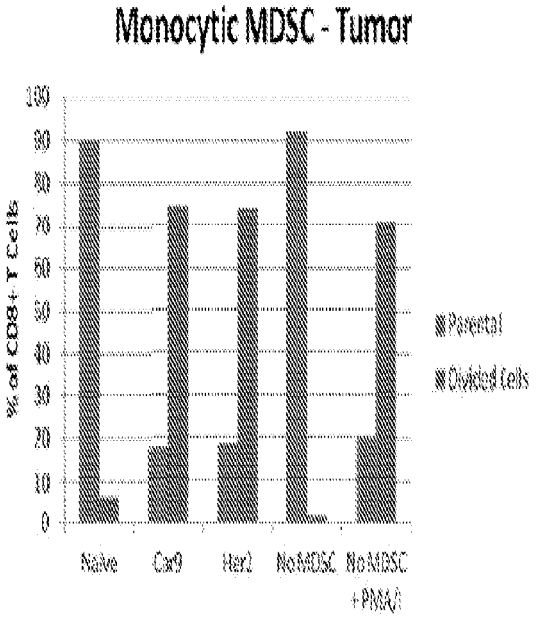


Figure 29B

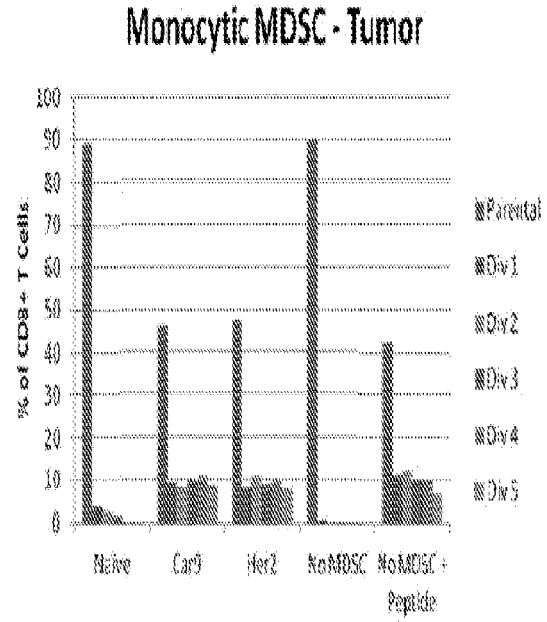


Figure 29C

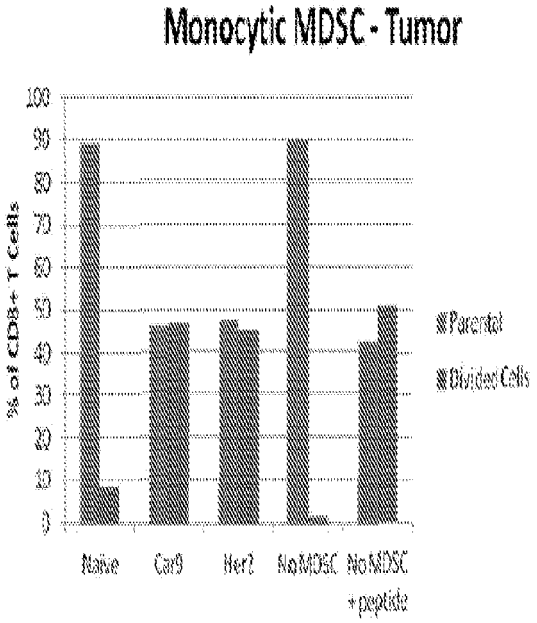


Figure 29D

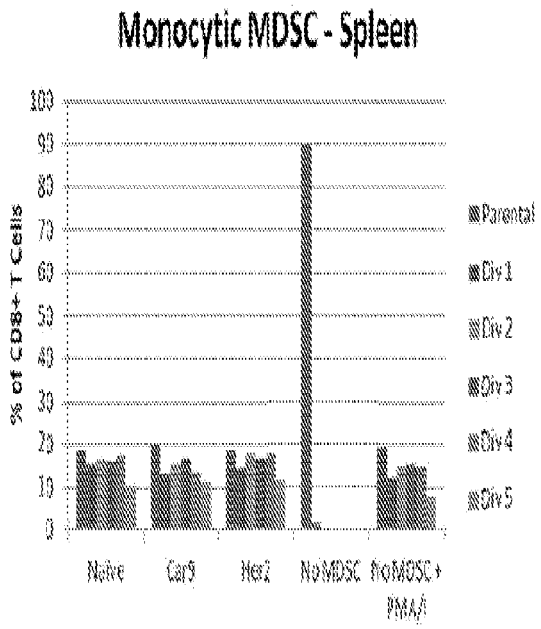


Figure 30A

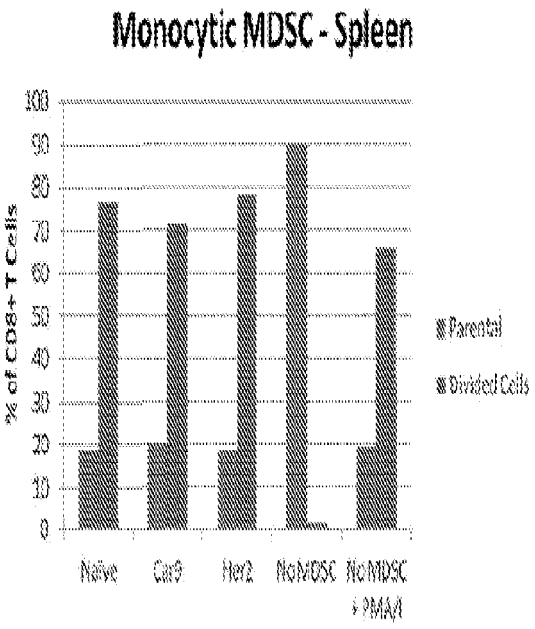


Figure 30B

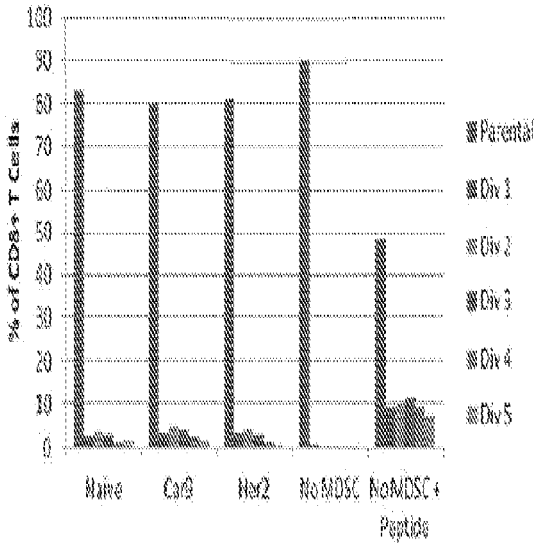


Figure 30C

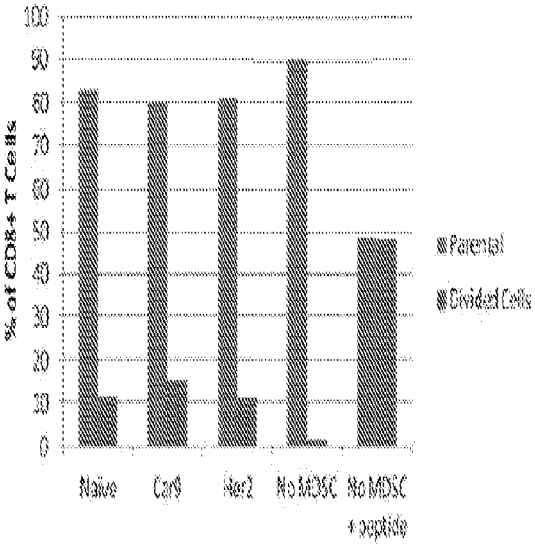


Figure 30D

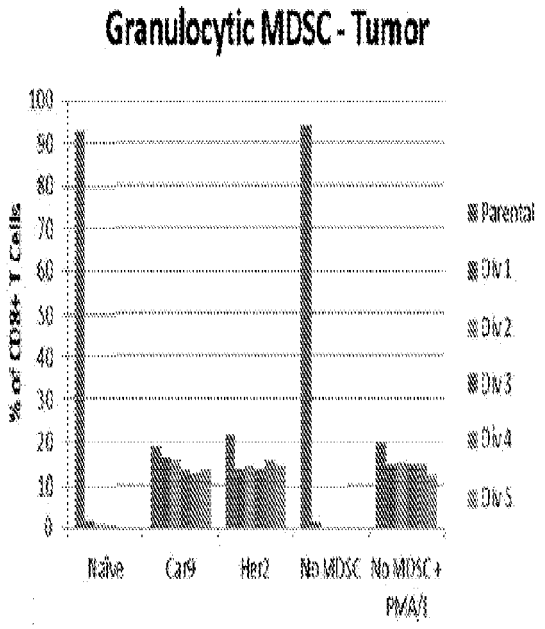


Figure 31A

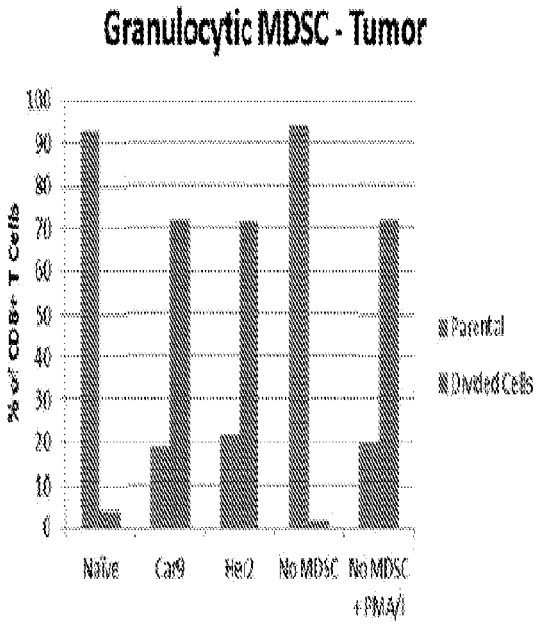


Figure 31B

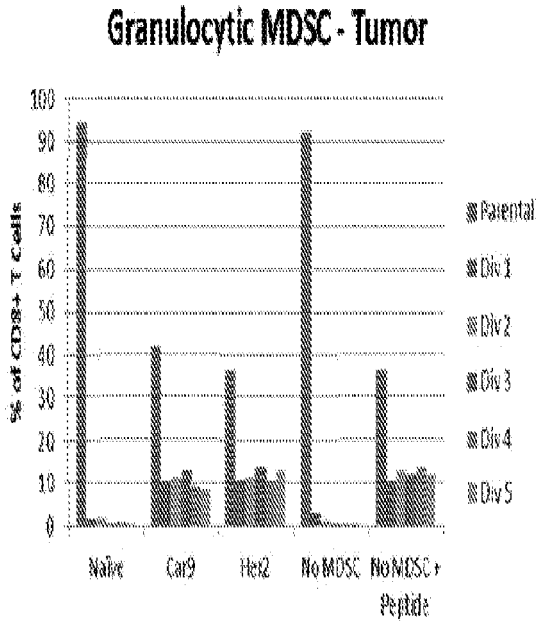


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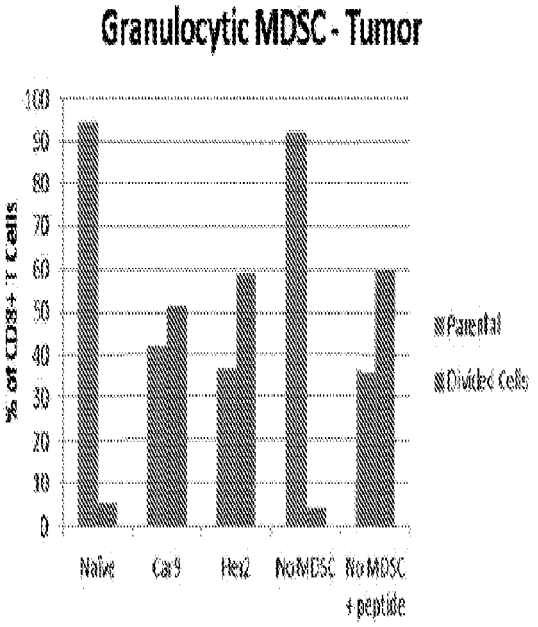


Figure 31D

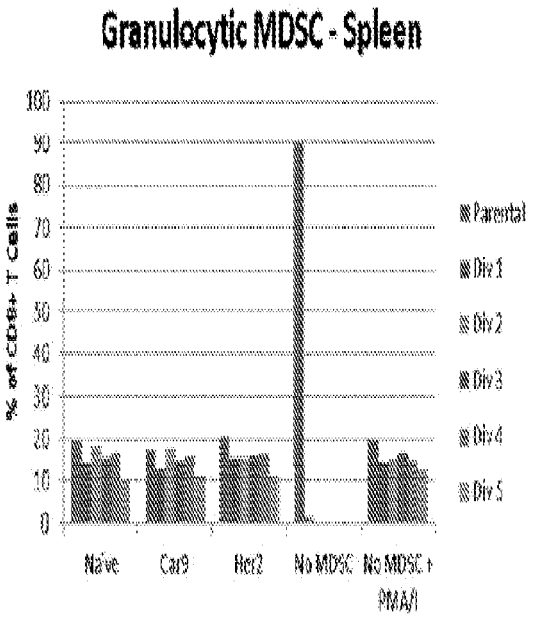


Figure 32A

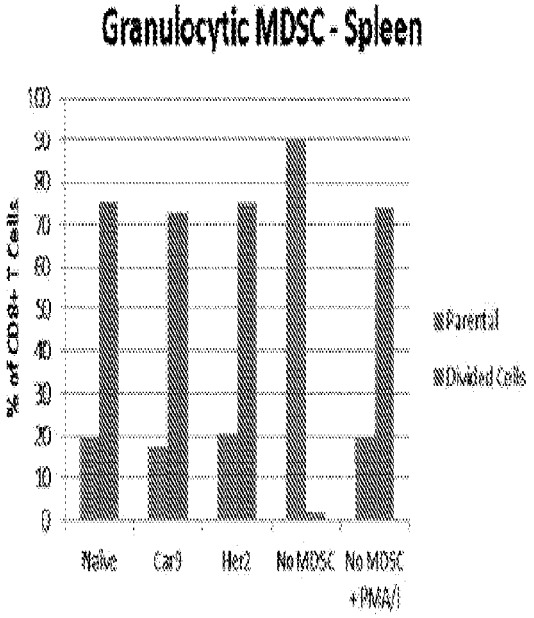


Figure 32B

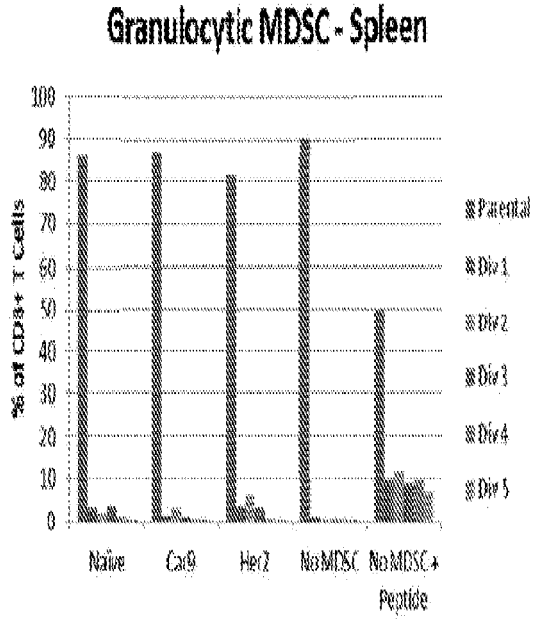


Figure 32C

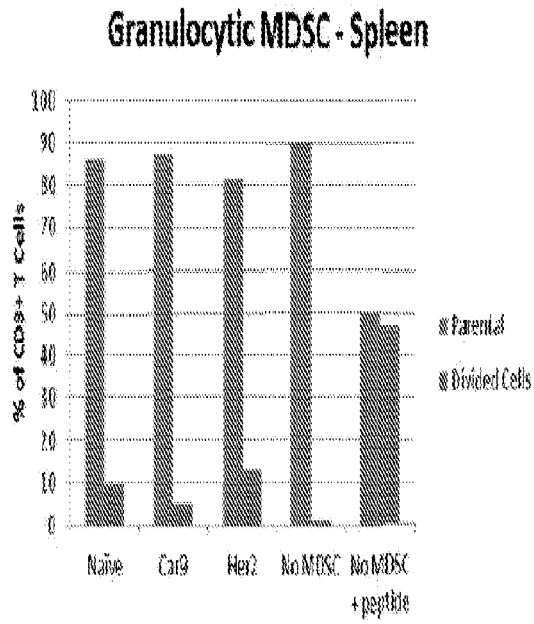


Figure 32D

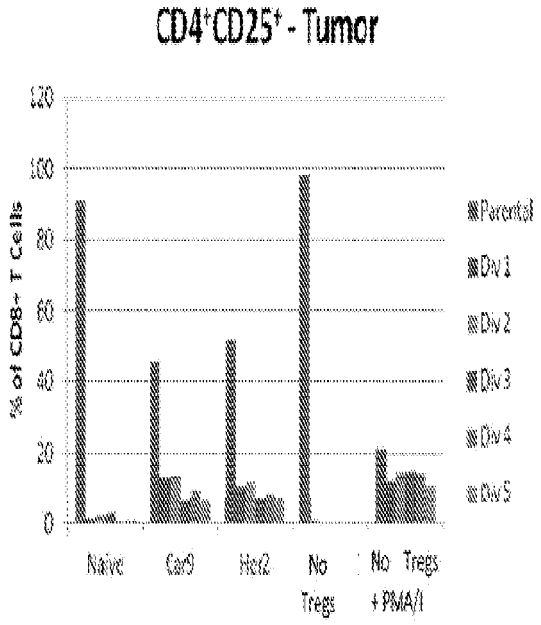


Figure 33A

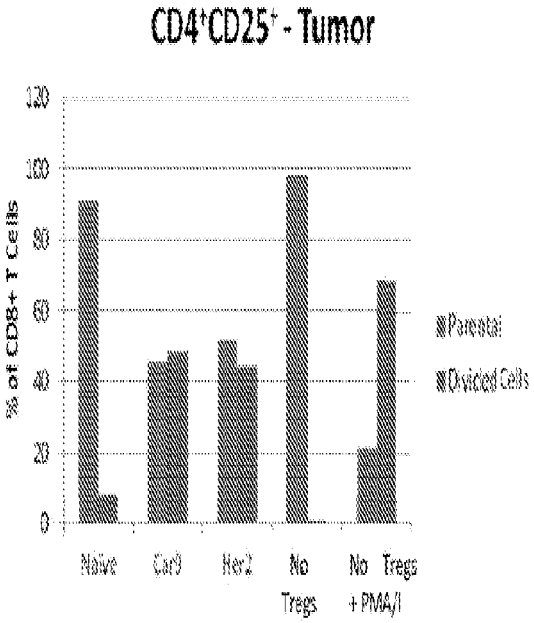


Figure 33B

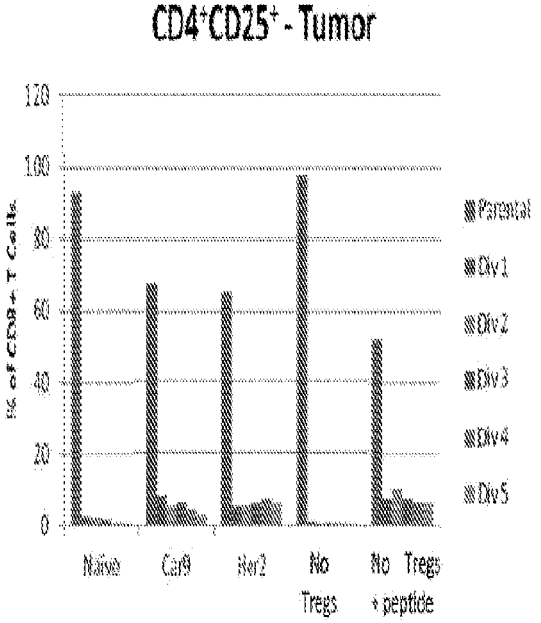


Figure 33C

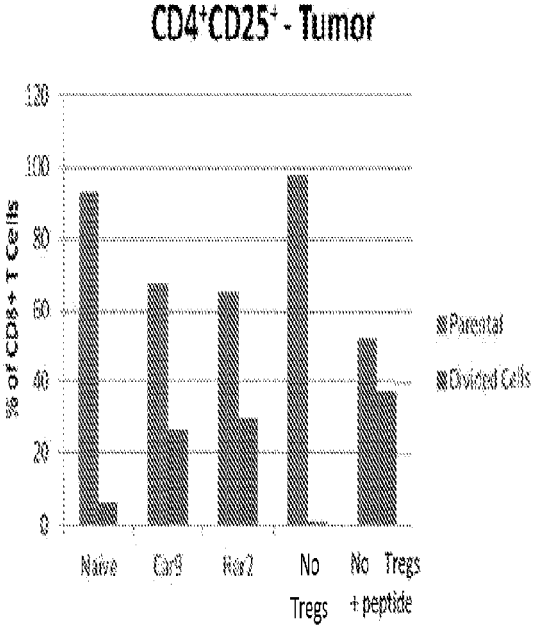


Figure 33D

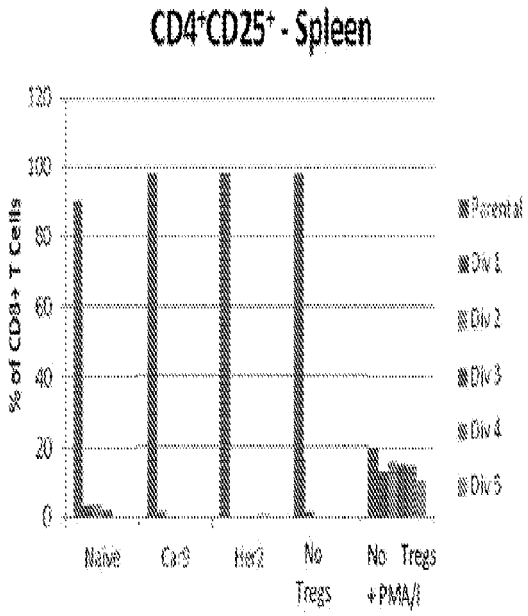


Figure 34A

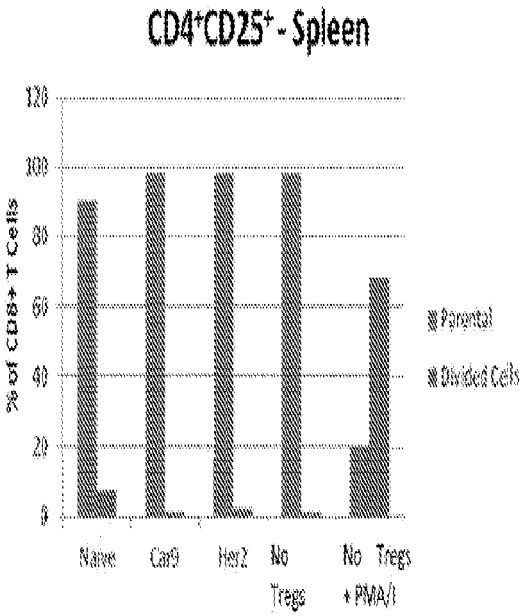


Figure 34B

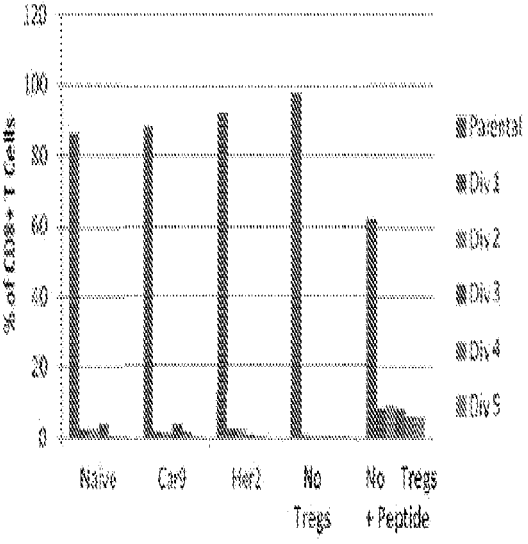


Figure 34C

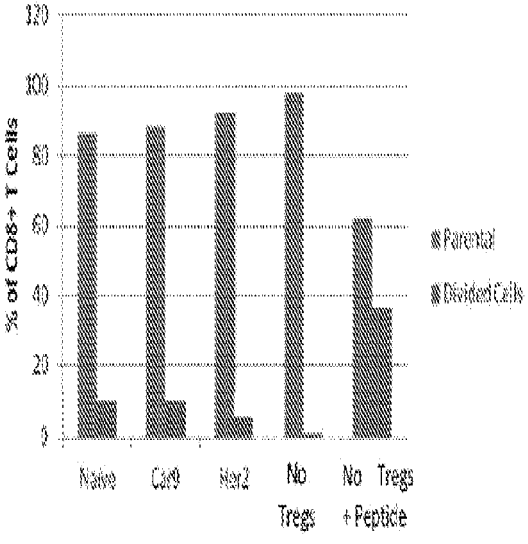


Figure 34D

Tumor Granulocytic MDSC

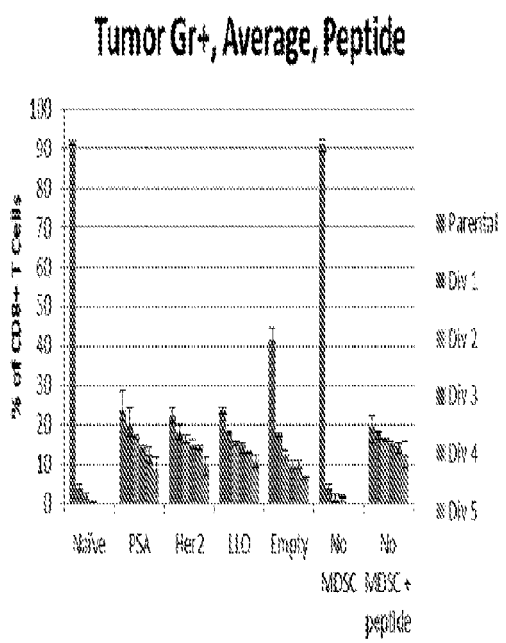


Figure 35A

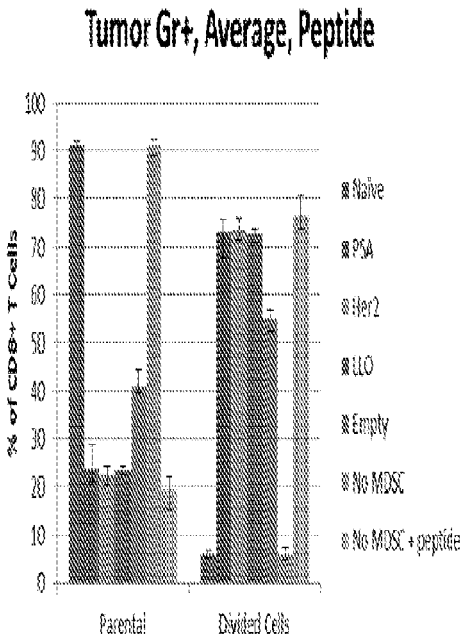


Figure 35B

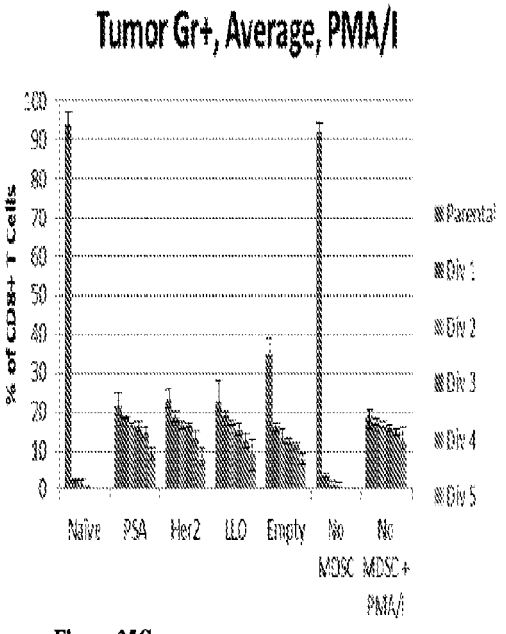


Figure 35C

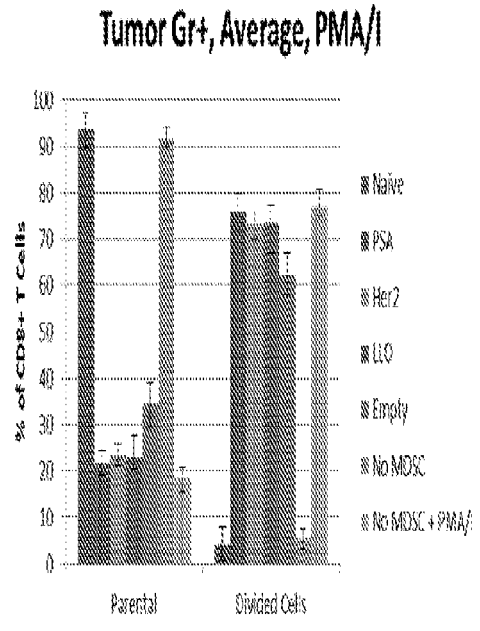


Figure 35D

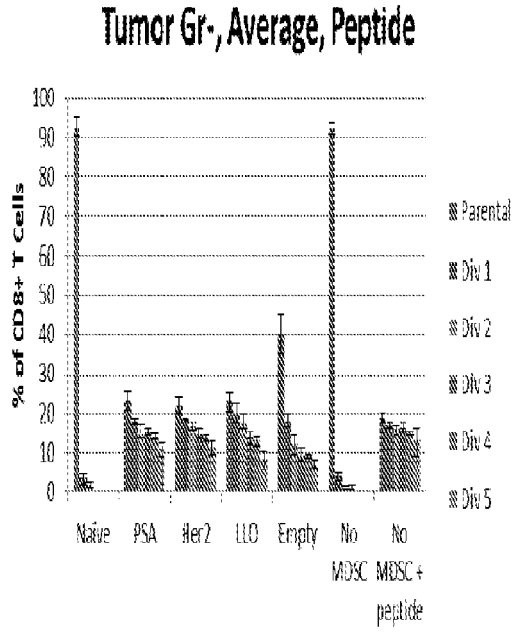


Figure 36A

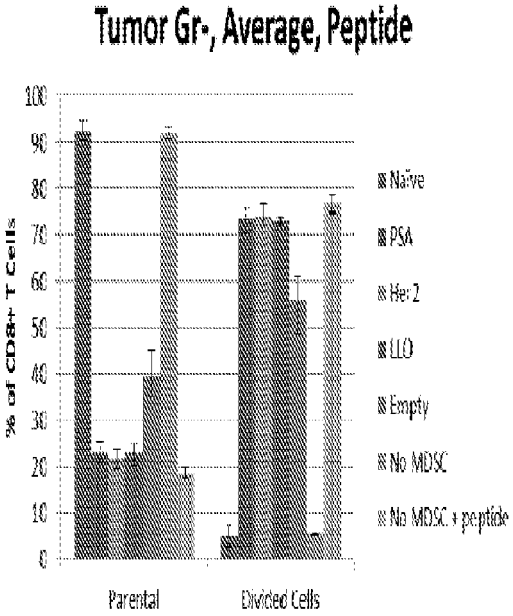


Figure 36B

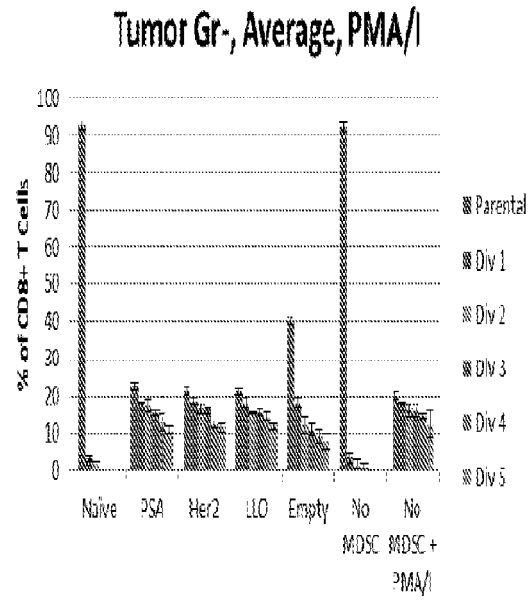


Figure 36C

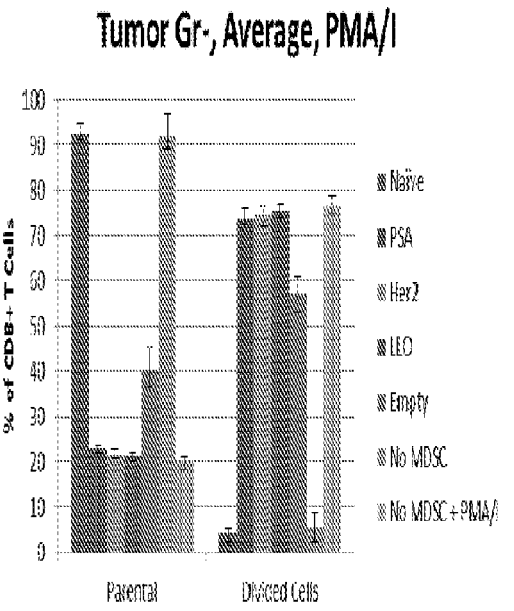


Figure 36D

Spleen Granulocytic MDSC

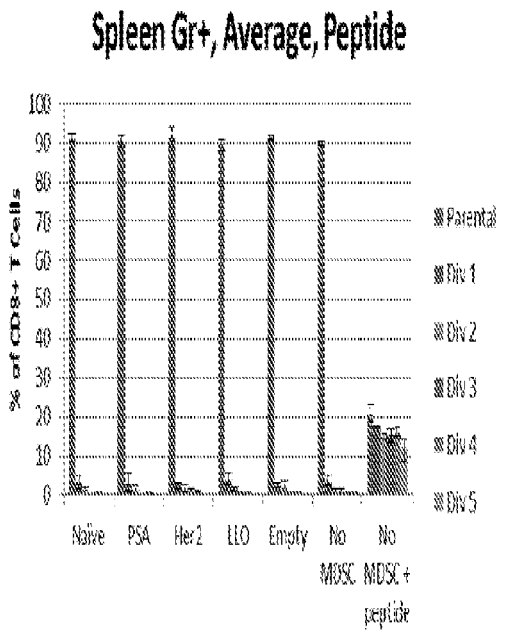


Figure 37A

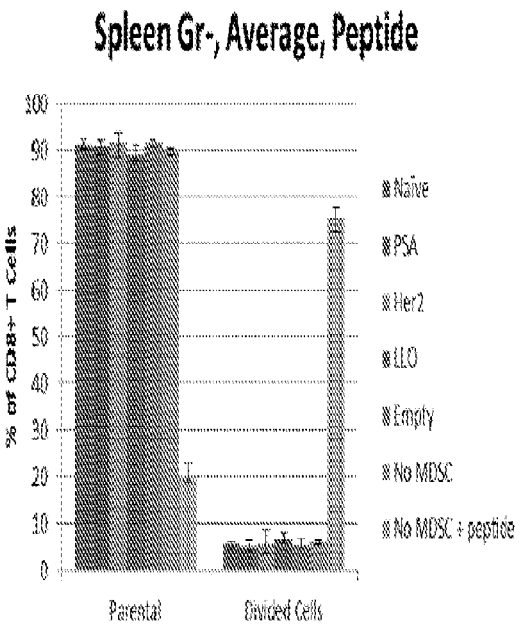


Figure 37B

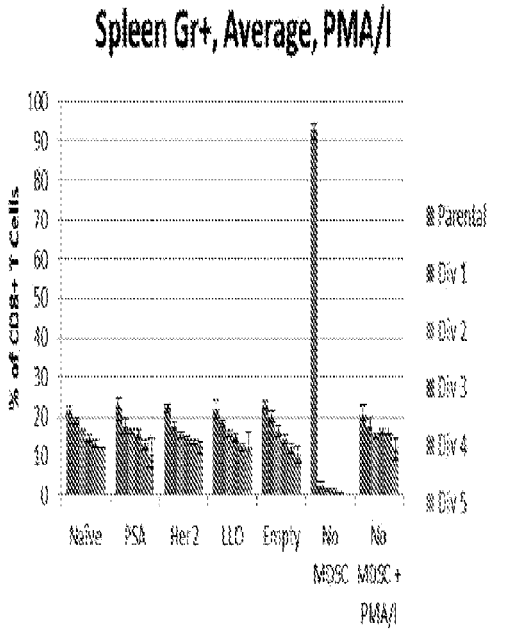


Figure 37C

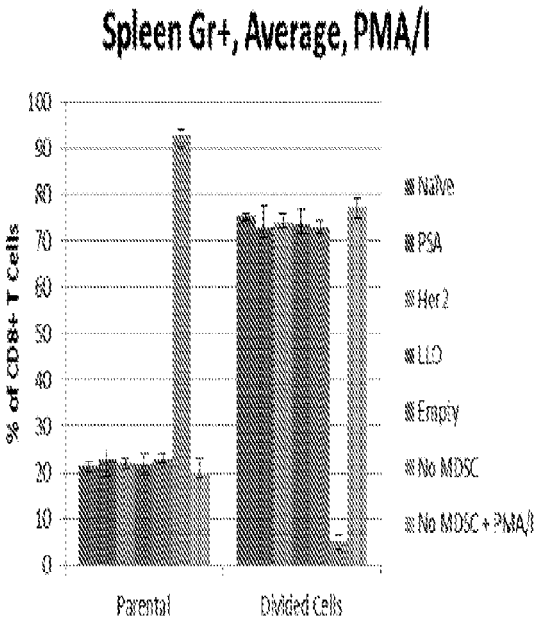


Figure 37D

Spleen Monocytic MDSC

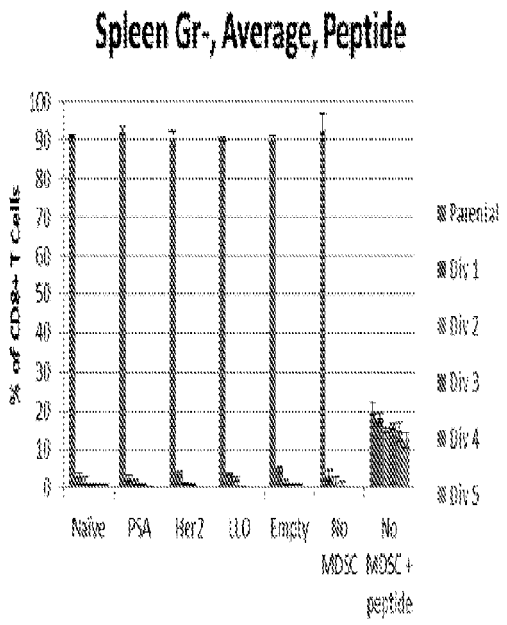


Figure 38A

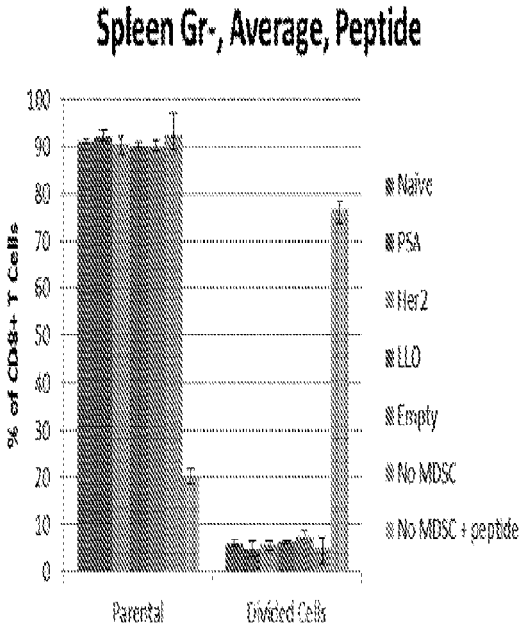


Figure 38B

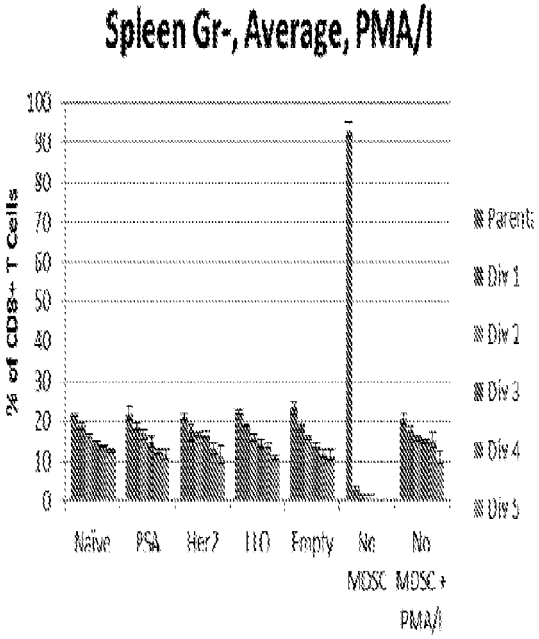


Figure 38C

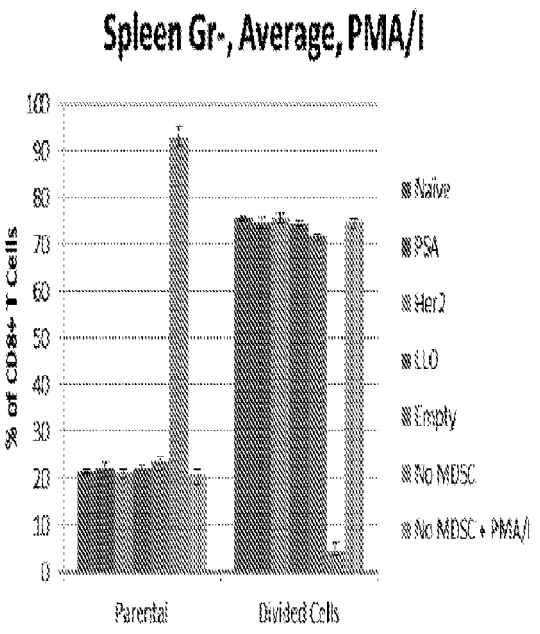


Figure 38D

Tumor Tregs

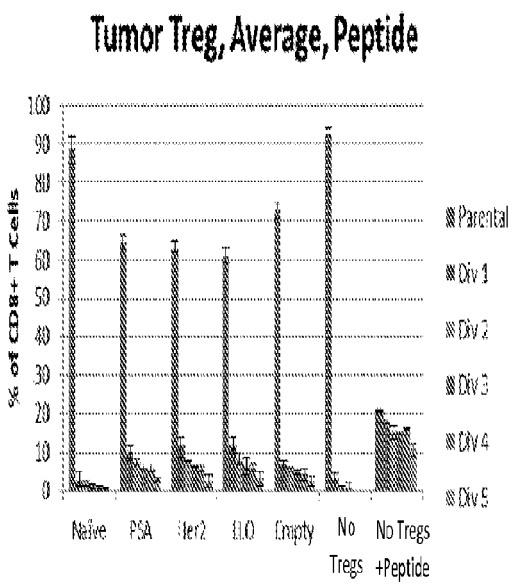


Figure 39A

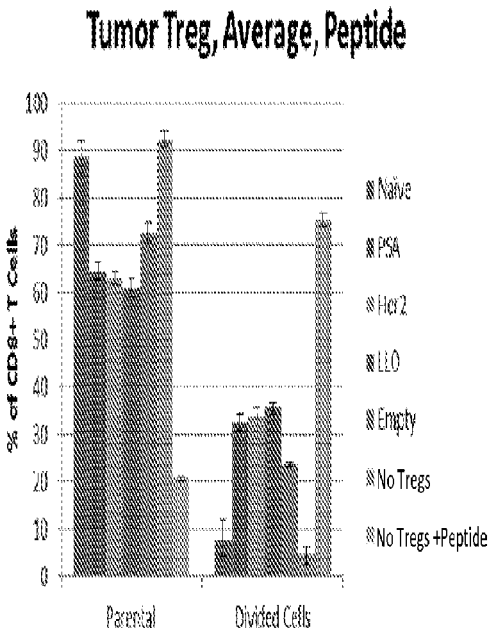


Figure 39B

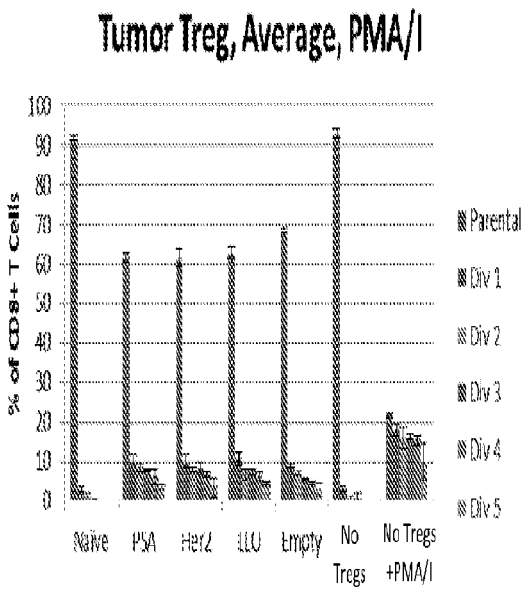


Figure 39C

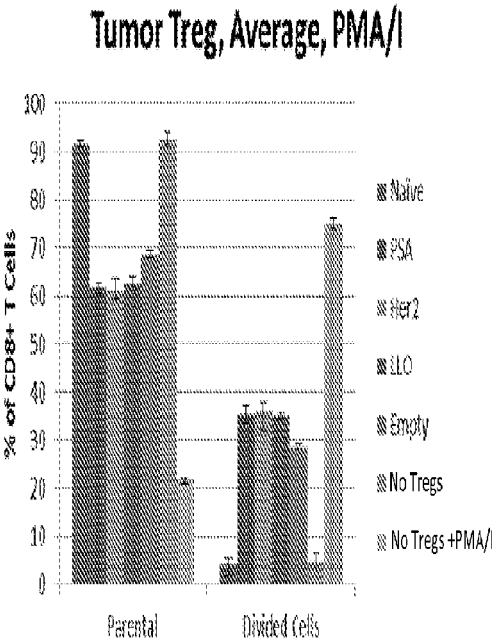


Figure 39D

Spleen Tregs

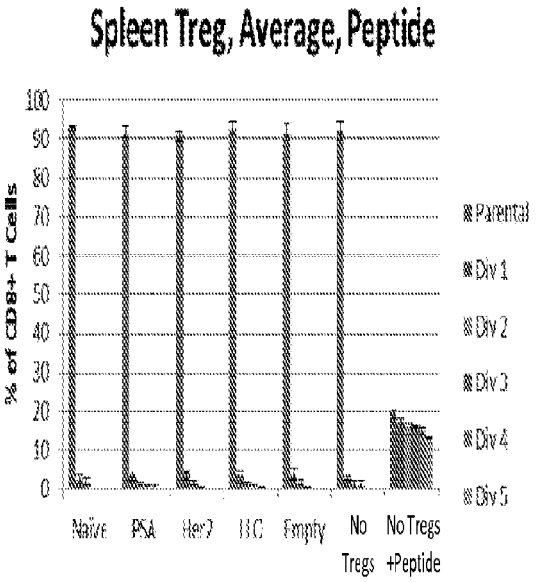


Figure 40A

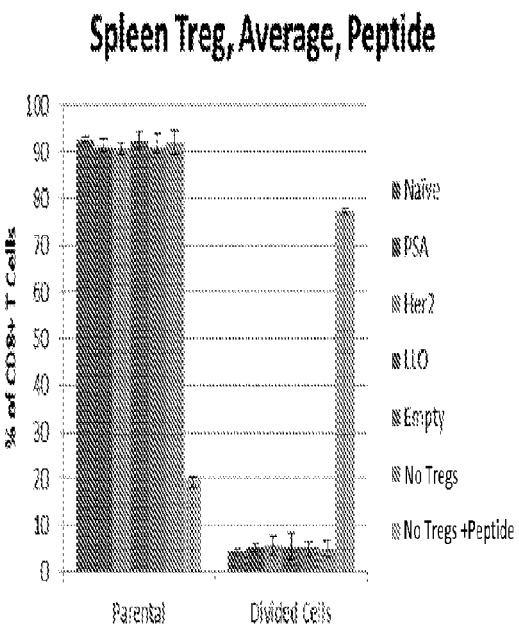


Figure 40B

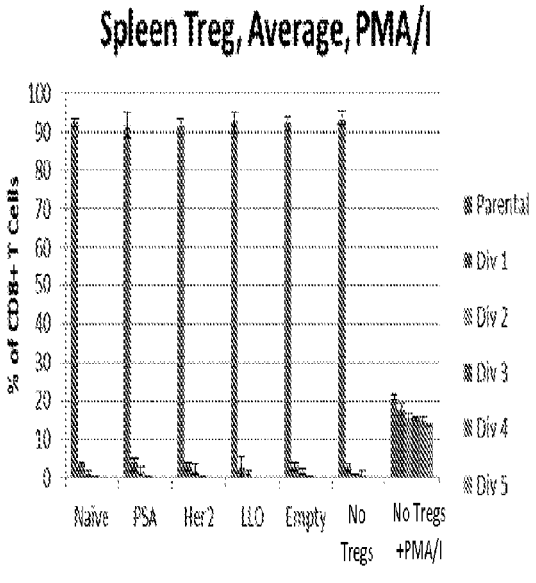


Figure 40C

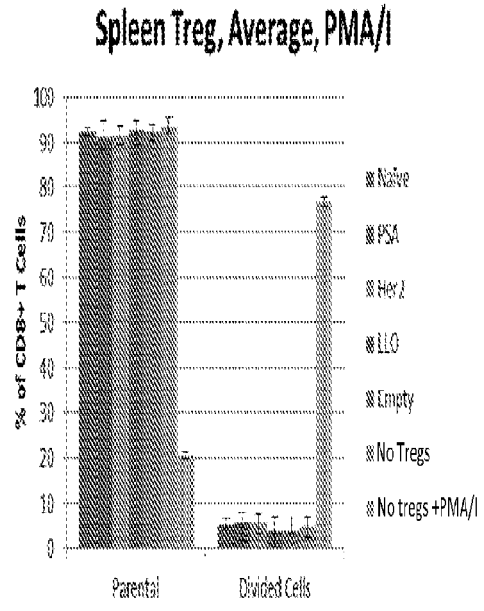


Figure 40D

Tumor Tcon

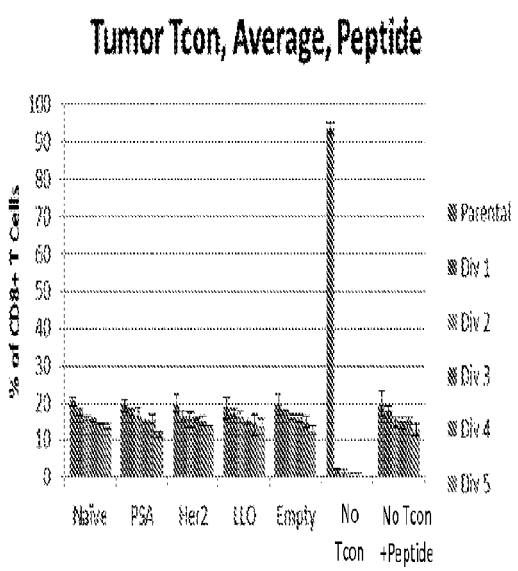


Figure 41A

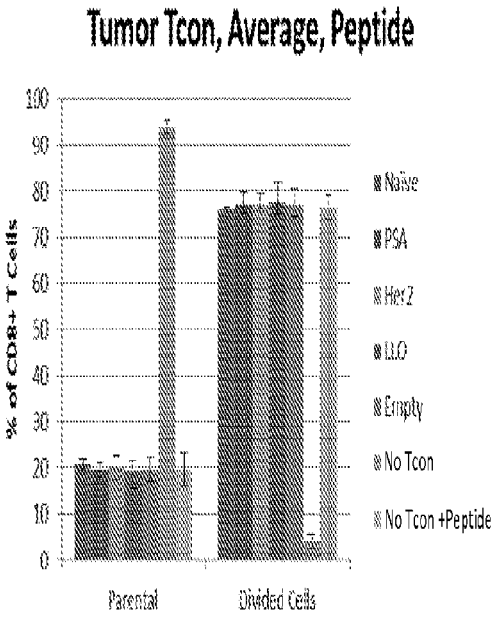


Figure 41B

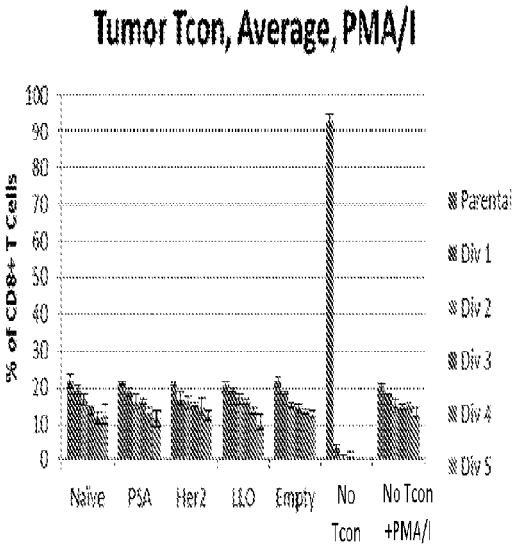


Figure 41C

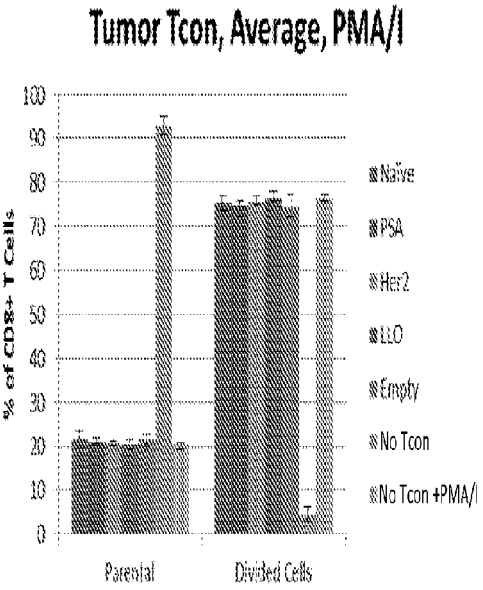


Figure 41D

Spleen Tcon

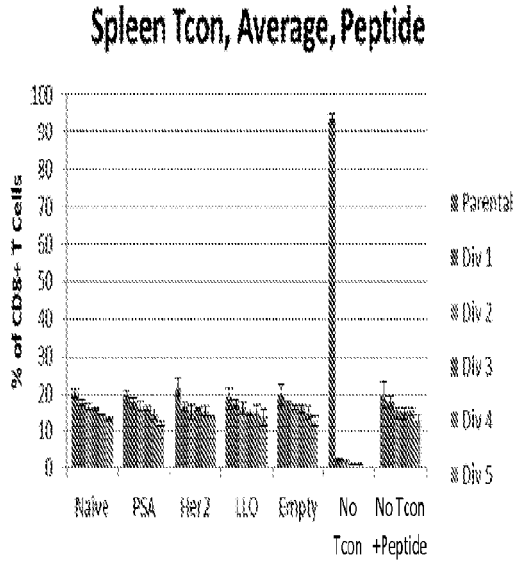


Figure 42A

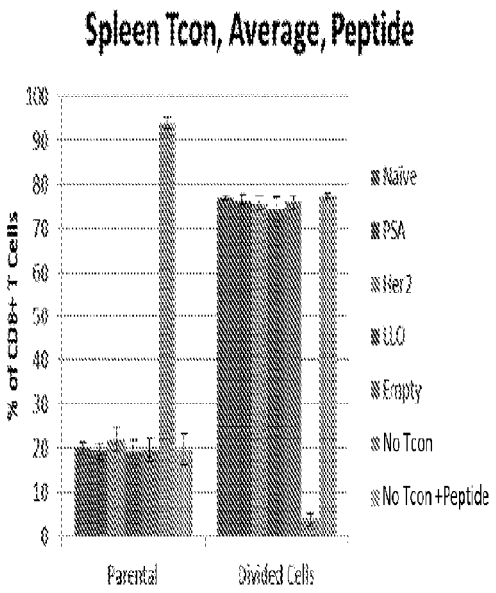


Figure 42B

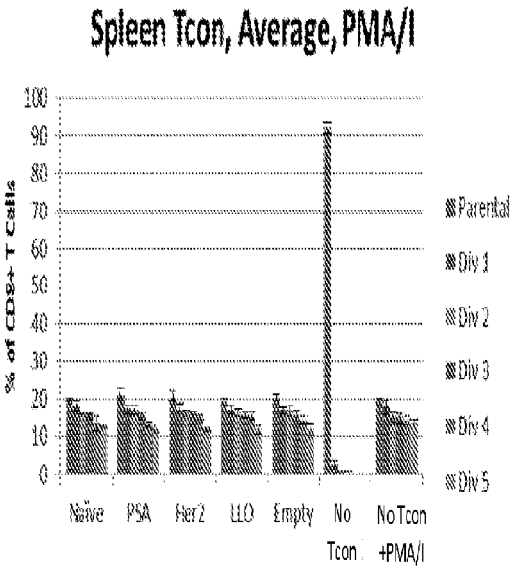


Figure 42C

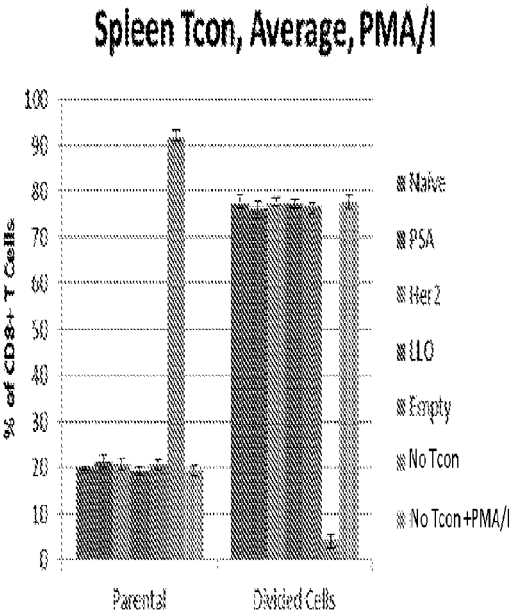


Figure 42D

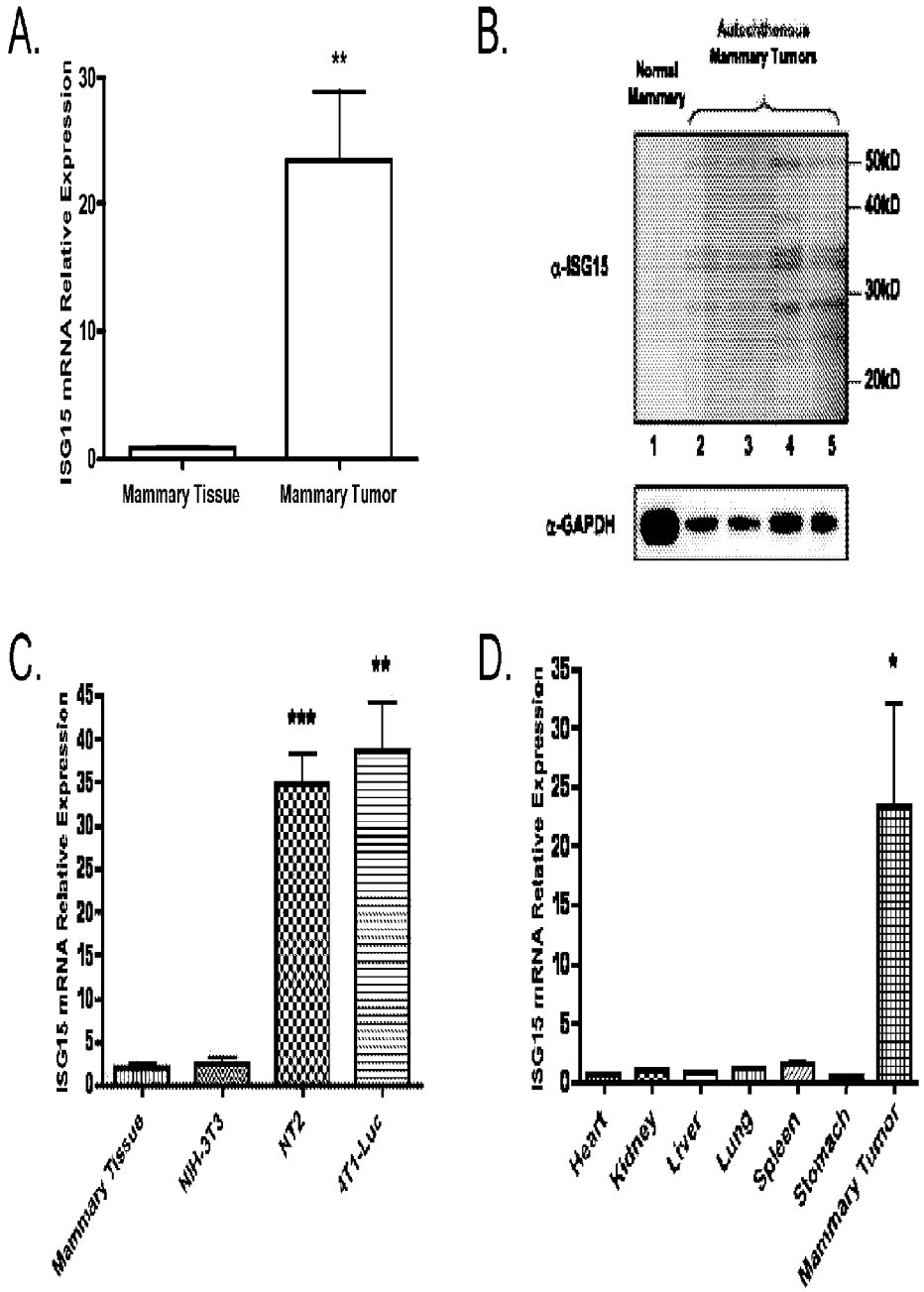

A.
ISG15 mRNA Relative Expression
30
20
10
0
**
Mammary Tissue
Mammary Tumor
B.
Autochthonous Mammary Tumors
Normal Mammary
α-ISG15
50kD
40kD
30kD
20kD
1 2 3 4 5
α-GAPDH
C.
ISG15 mRNA Relative Expression
45
40
35
30
25
20
15
10
5
0
***
**
Mammary Tissue
NIH-3T3
NT2
4T1-Luc
D.
ISG15 mRNA Relative Expression
35
30
25
20
15
10
5
0
*
Heart
Kidney
Liver
Lung
Spleen
Stomach
Mammary Tumor

Figure 43

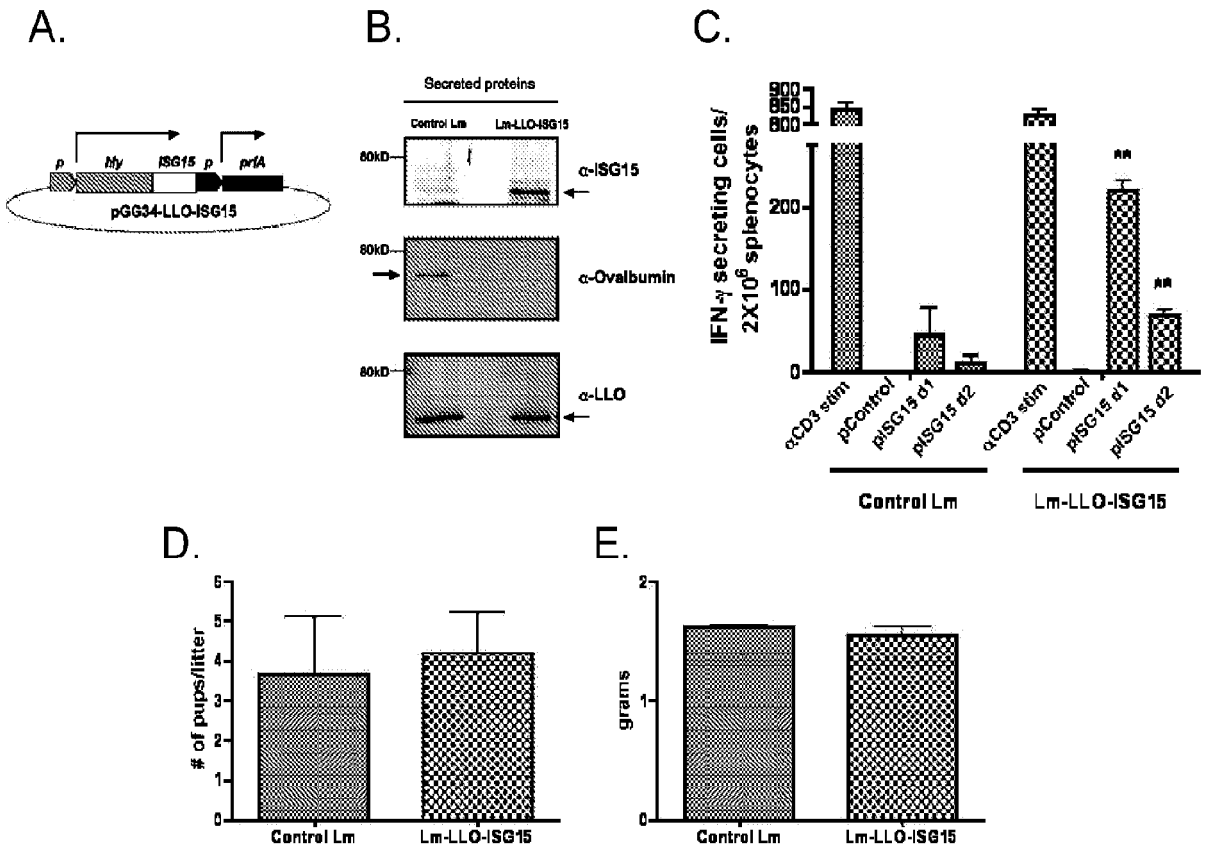

A.
B.
C.
p
hly
ISG15
p
prfA
pGG34-LLO-ISG15
Secreted proteins
Control Lm
Lm-LLO-ISG15
80kD
α-ISG15
α-Ovalbumin
α-LLO
IFN-γ secreting cells/ 2X10^6 splenocytes
αCD3 stim
pControl
pISG15 d1
pISG15 d2
Control Lm
Lm-LLO-ISG15
D.
# of pups/litter
E.
grams

Figure 44

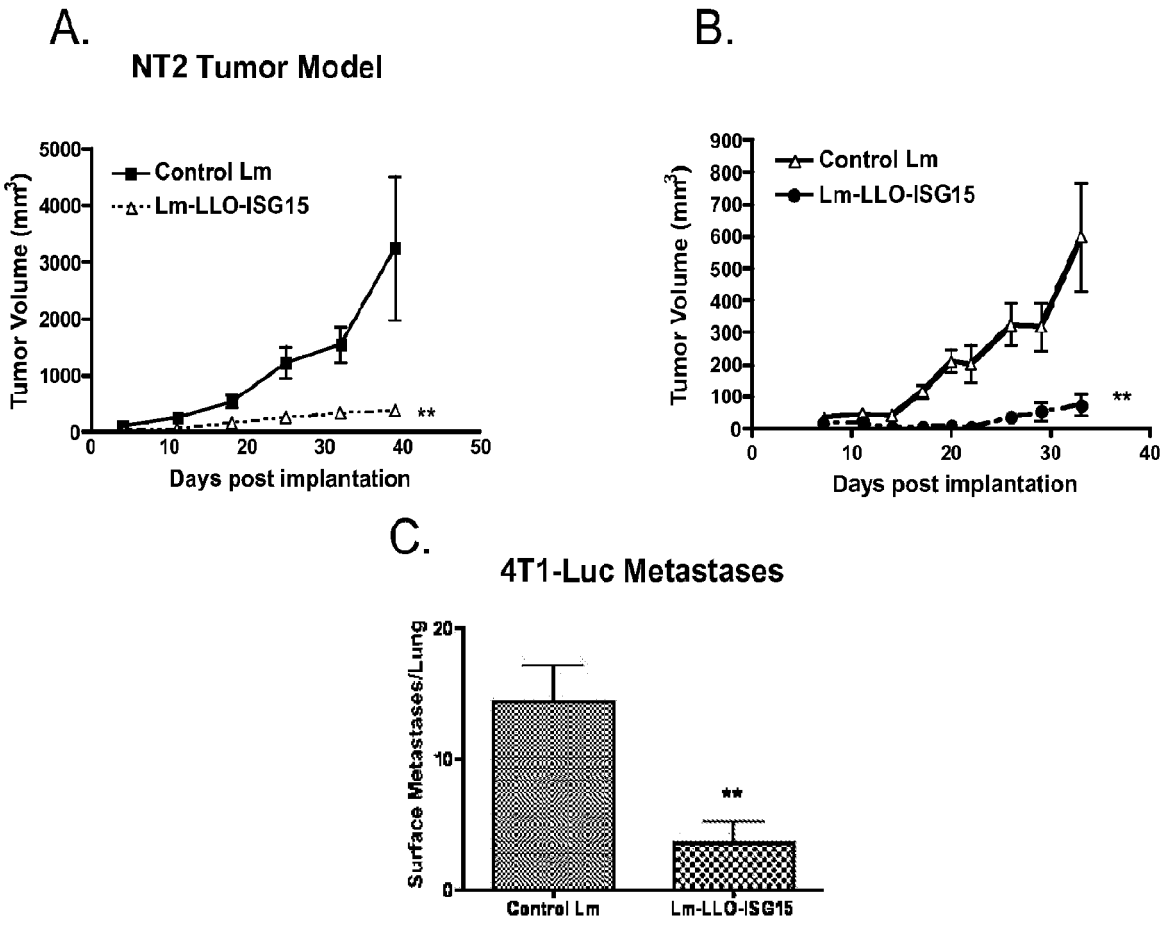

A.
NT2 Tumor Model
Tumor Volume (mm3)
Control Lm
Lm-LLO-ISG15
**
Days post implantation
B.
Tumor Volume (mm3)
Control Lm
Lm-LLO-ISG15
**
Days post implantation
C.
4T1-Luc Metastases
Surface Metastases/Lung
**
Control Lm
Lm-LLO-ISG15

Figure 45

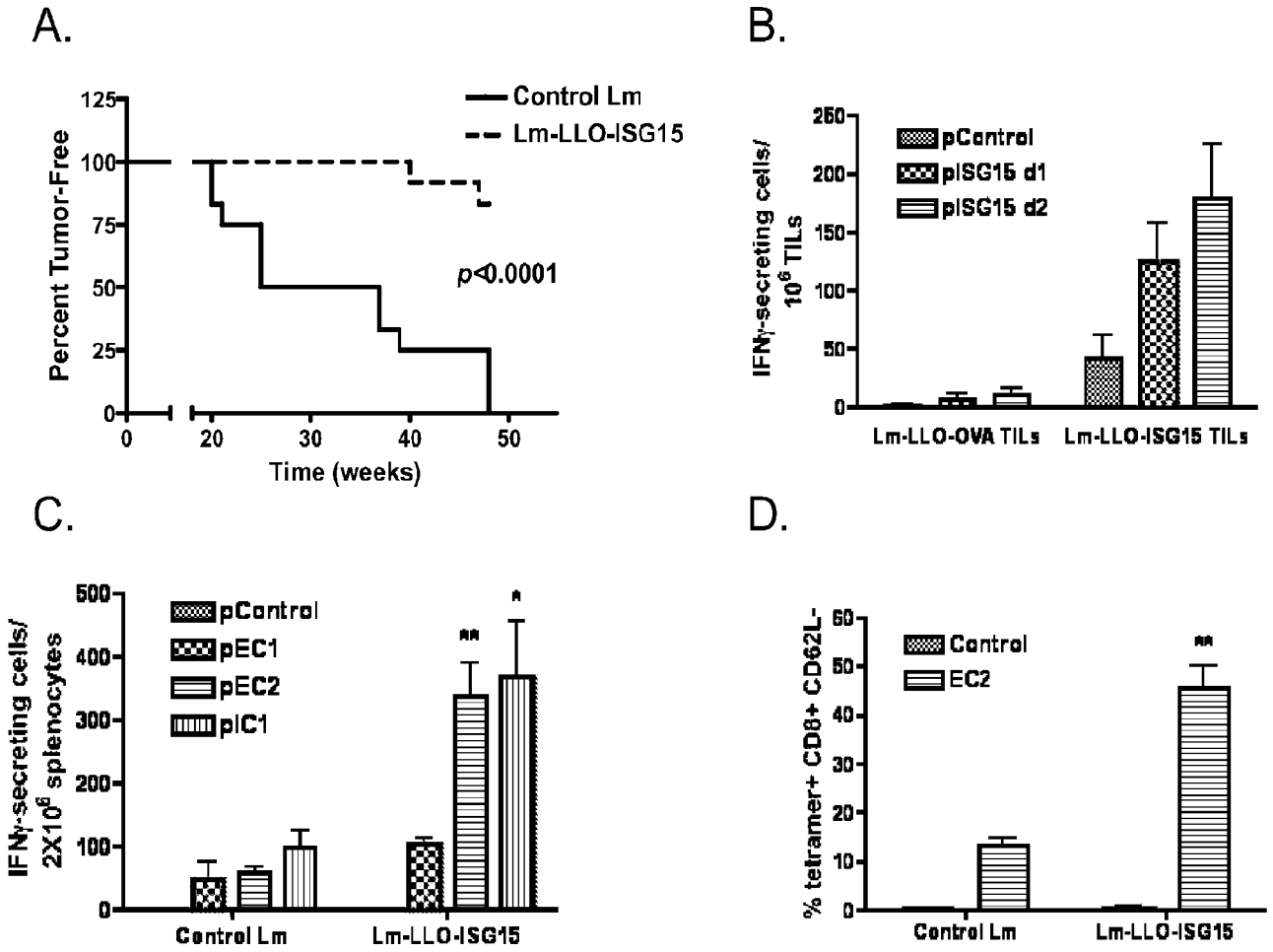

A.
Control Lm
Lm-LLO-ISG15
Percent Tumor-Free
125
100
75
50
25
0
0
20
30
40
50
Time (weeks)
p<0.0001
B.
pControl
pISG15 d1
pISG15 d2
IFNγ-secreting cells/ 10^6 TILs
250
200
150
100
50
0
Lm-LLO-OVA TILs
Lm-LLO-ISG15 TILs
C.
pControl
pEC1
pEC2
pIC1
IFNγ-secreting cells/ 2X10^6 splenocytes
500
400
300
200
100
0
**
*
Control Lm
Lm-LLO-ISG15
D.
Control
EC2
% tetramer+ CD8+ CD62L-
60
50
40
30
20
10
0
**
Control Lm
Lm-LLO-ISG15

Figure 46

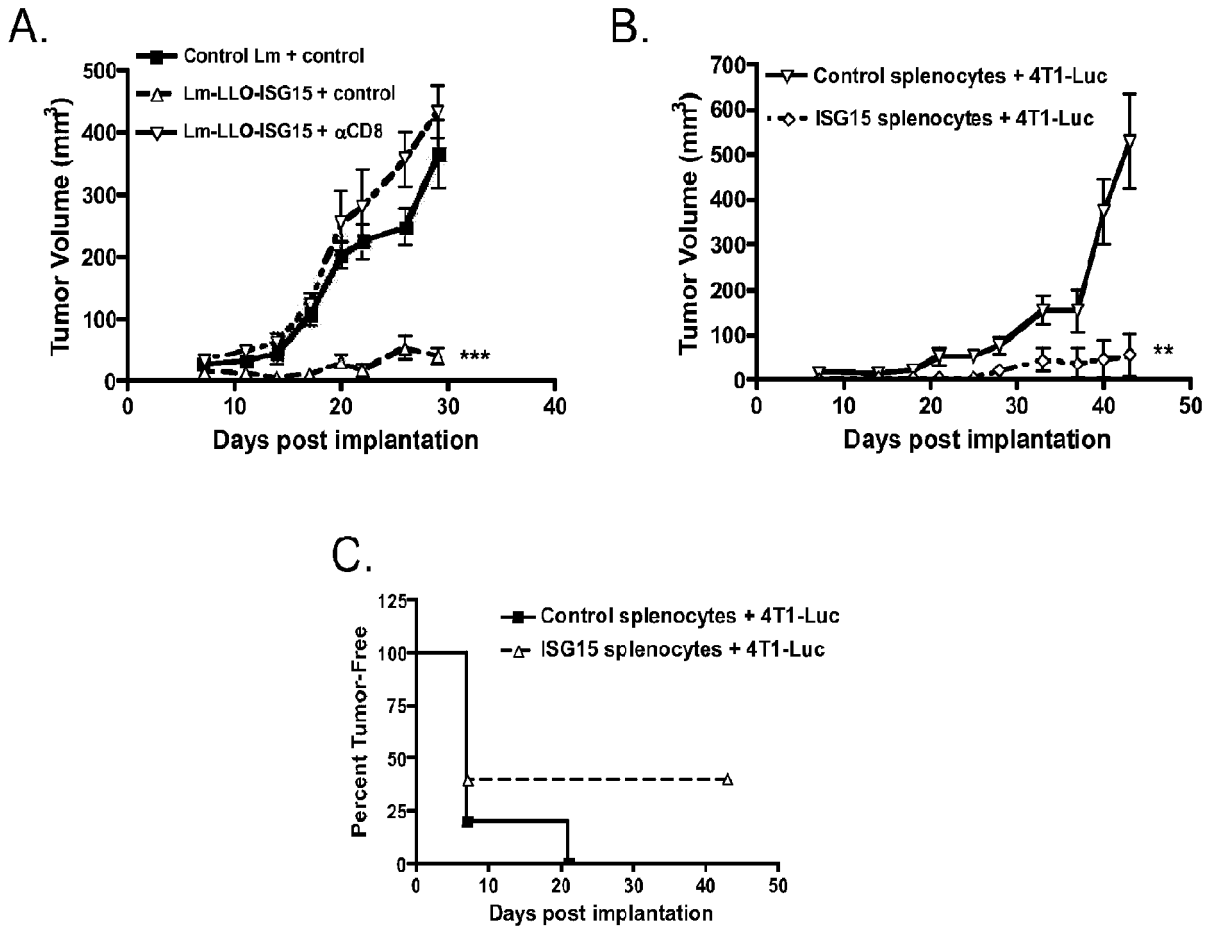

A.
Control Lm + control
Lm-LLO-ISG15 + control
Lm-LLO-ISG15 + αCD8
Tumor Volume (mm3)
Days post implantation
***
B.
Control splenocytes + 4T1-Luc
ISG15 splenocytes + 4T1-Luc
Tumor Volume (mm3)
Days post implantation
**
C.
Control splenocytes + 4T1-Luc
ISG15 splenocytes + 4T1-Luc
Percent Tumor-Free
Days post implantation

Figure 47

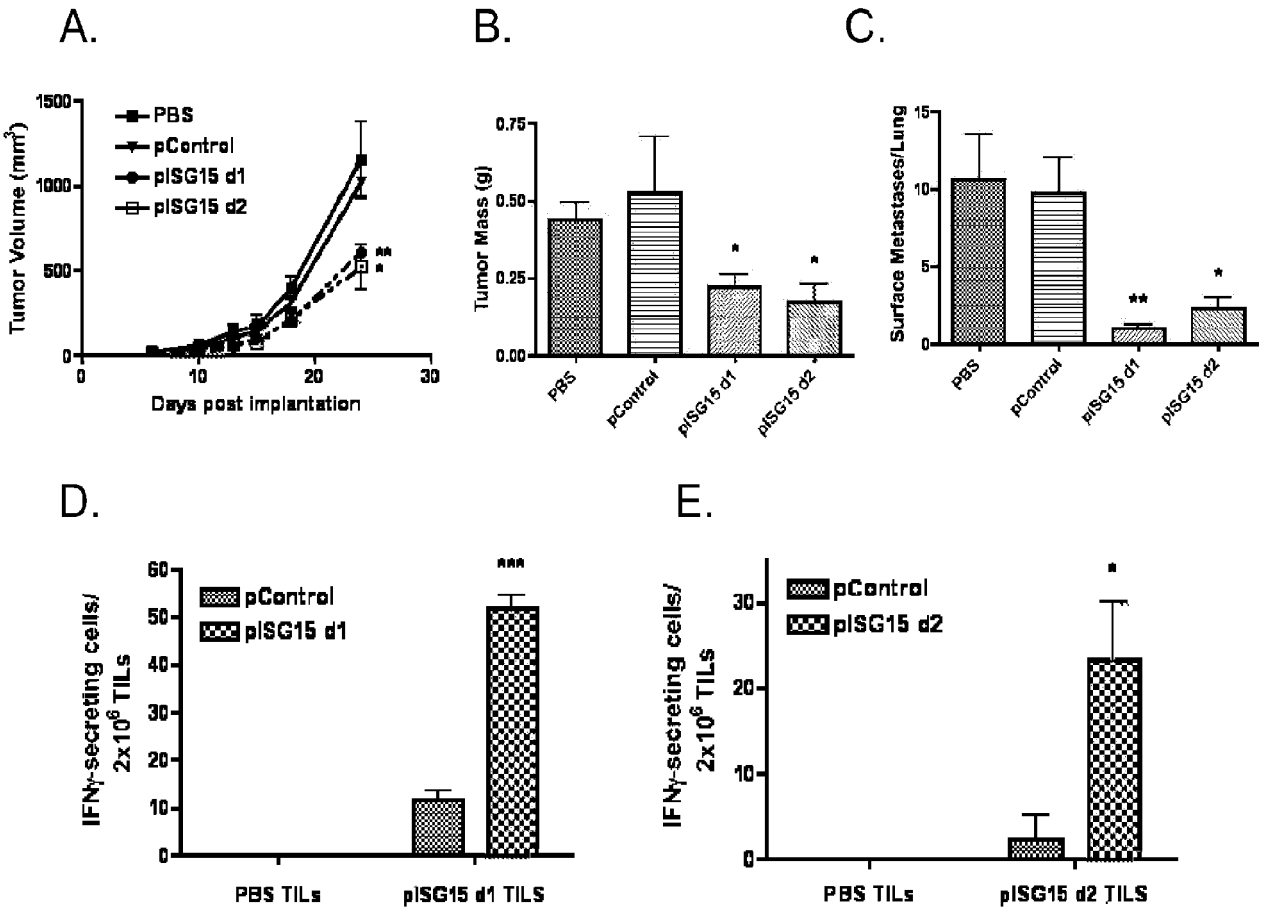

A.
B.
C.
Tumor Volume (mm3)
PBS
pControl
pISG15 d1
pISG15 d2
Days post implantation
Tumor Mass (g)
Surface Metastases/Lung
D.
E.
IFNγ-secreting cells/ 2x10^6 TILs
PBS TILs
pISG15 d1 TILs
pISG15 d2 TILs

Figure 48

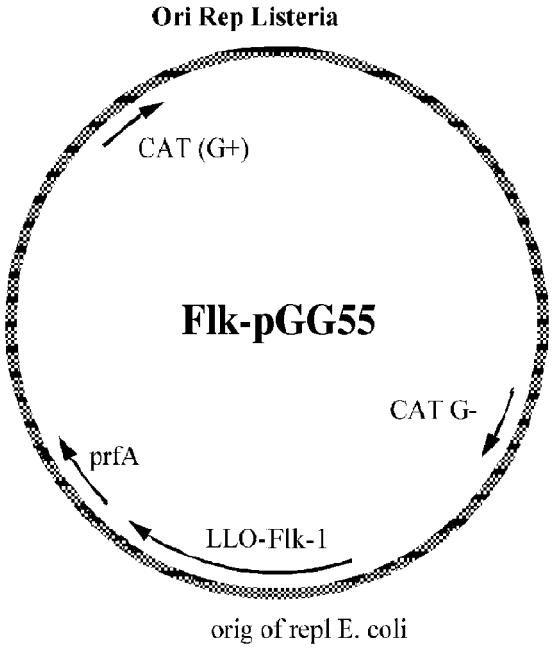


Figure 49A

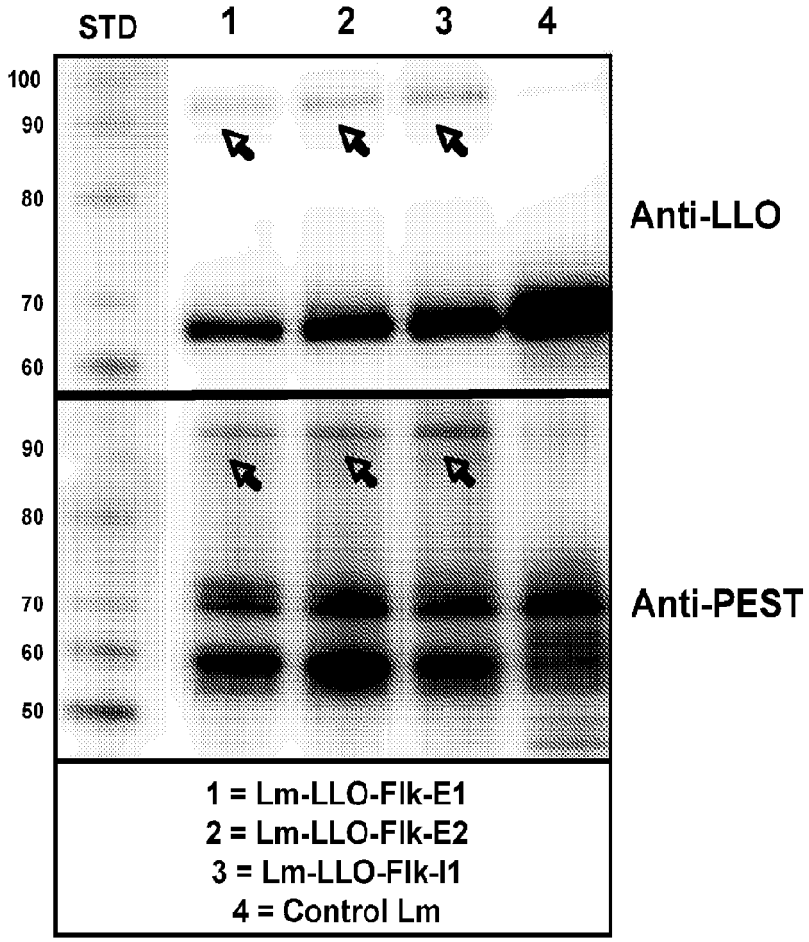


Figure 49B

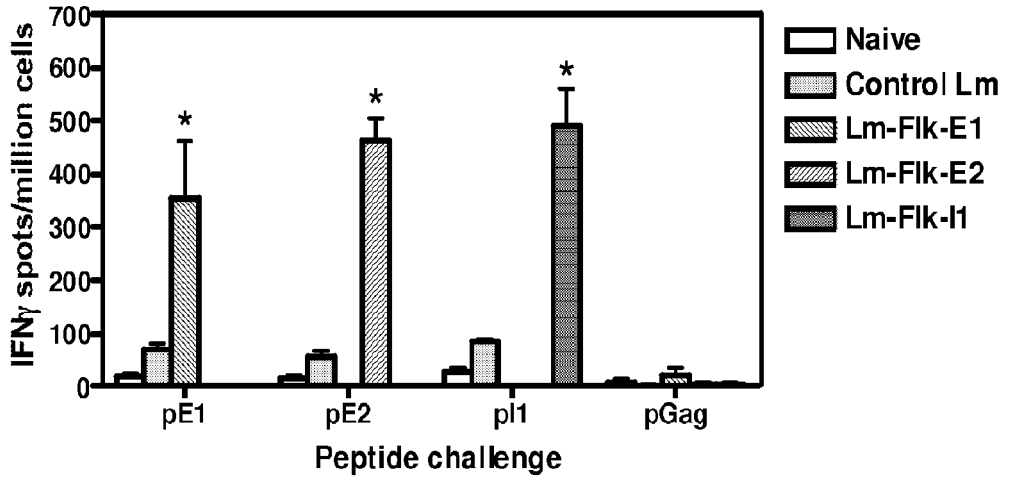


Figure 49C

MESKALLAVALWFCVETRAASVGLPGDFLHPPKLSTQKDILTILANTTLQITCRGQRDLDWLWPN
AQRDSEERVLVTECGGGGDSIFCKTLTIPRVVGNDTGAYKCSYRDVDIASTVYVYVRDYRSPFIAS
VSDQHGIVYITENKNKTVVIPCRGSISNLNVSLCARYPEKRFVPDGNRISWDSEIGFTLPSYMISYA
GMVFCEAKINDETYQSIMYIVVVVGYRIYDVILSPPHEIELSAGEKLVLNCTARTELNVGLDFTWHS
PPSKSHHKKIVNRDVKPFPGTVAKMFLSTLTIESVTKSDQGEYTCVASSGRMIKRNRTFVRVHTK
PFIAFGSGMKSLVEATVGSQVRIPVKYLSYPAPDIKWYRNGRPIESNYTMIVGDELTIMEVTERDA
GNYTVILTNPISMEKQSHMVSLVVNVPPQIGEKALISPMDSYQYGTMQTLTCTVYANPPLHHIQW
YWQLEEACSYRPGQTSPYACKEWRHVEDFQGGNKIEVTKNQYALIEGKNKTVSTLVIQAANVSA
LYKCEAINKAGRGERVISFHVIRGPEITVQPAAQPTEQESVSLLCTADRNTFENLTWYKLGSQATS
VHMGESLTPVCKNLDALWKLNGTMFSNSTNDILIVAFQNASLQDQGDYVCSAQDKKTKKRHCLV
KQLIILERMAPMITGNLENQTTTIGETIEVTCPASGNPTPHITWFKDNETLVEDSGIVLRDGNRNLTI
RRVRKEDGGLYTCQACNVLGCARAETLFIIEGAQEKTNLEVIILVGTAVIAMFFWLLLVIVLRTVKR
ANEGELKTGYLSIVMDPDELPLDERCERLPYDASKWEFPRDRLKLGKPLGRGAFGQVIEADAFGI
DKTATCKTVAVKMLKEGATHSEHRALMSELKILIHIGHHLNVVNLLGACTKPGGPLMVIVEFCKFG
NLSTYLRGKRNEFVPYKSKGARFRQGKDYVGELSVDLKRRLDSITSSQSSASSGFVEEKSLSDV
EEEEASEELYKDFLTLEHLICYSFQVAKGMEFLASRKCIHRDLAARNILLSEKNVVKICDFGLARDI
YKDPDYVRKGDARLPLKWMAPETIFDRVYTIQSDVWSFGVLLWEIFSLGASPYPGVKIDEEFCRR
LKEGTRMRAPDYTTPEMYQTMLDCWHEDPNQRPSFSELVEHLGNLLQANAQQDGKDYIVLPMS
ETLSMEEDSGLSLPTSPVSCMEEEEVCDPKFHYDNTAGISHYLQNSKRKSRPVSVKTFEDIPLEE
PEVKVIPDDSQTDSGMVLASEELKTLEDRNKLSPSFGGMMPSKSRESVASEGSNQTSGYQSGY
HSDDTDTTVYSSDEAGLLKMVDAAVHADSGTTLRSPPV

Flk-E1
Flk-E2
Flk-I1

Figure 50A

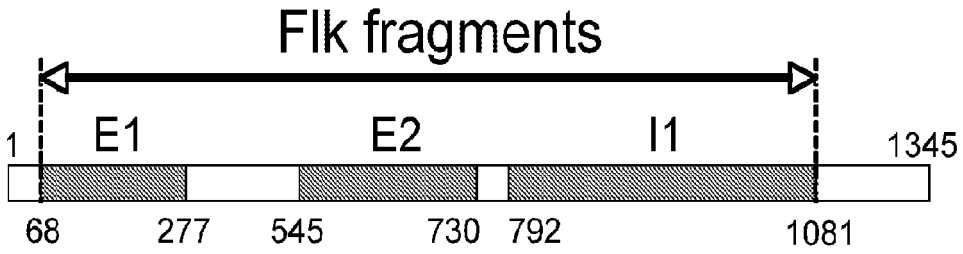


Figure 50B

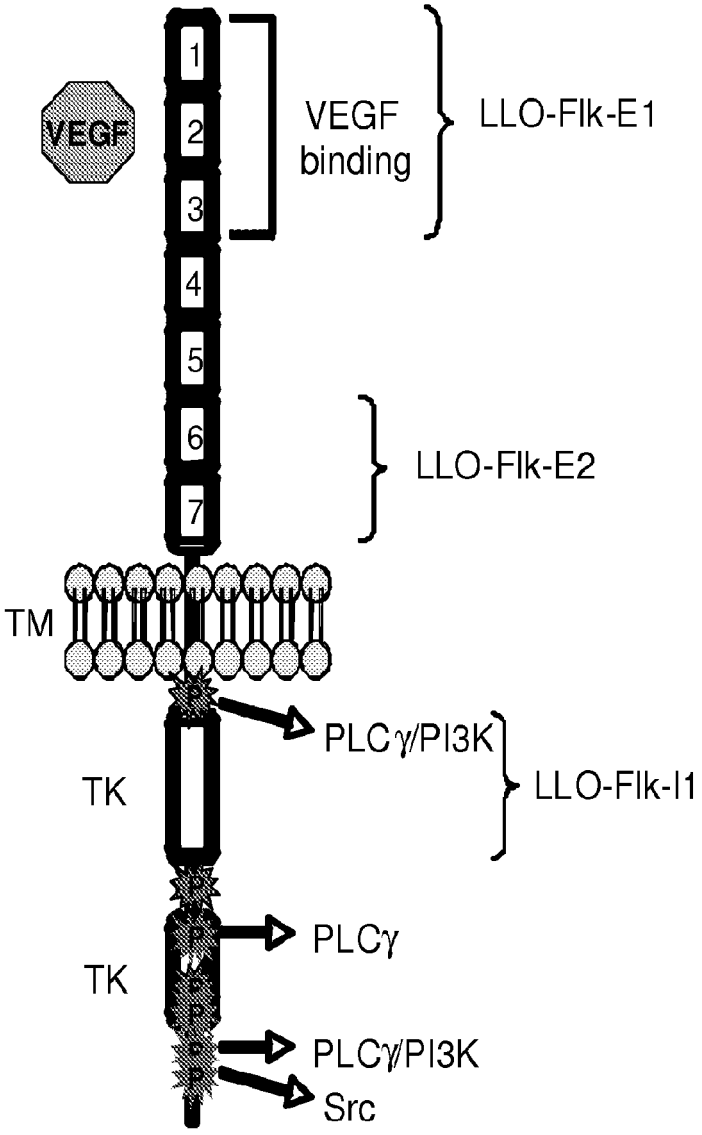


Figure 50C

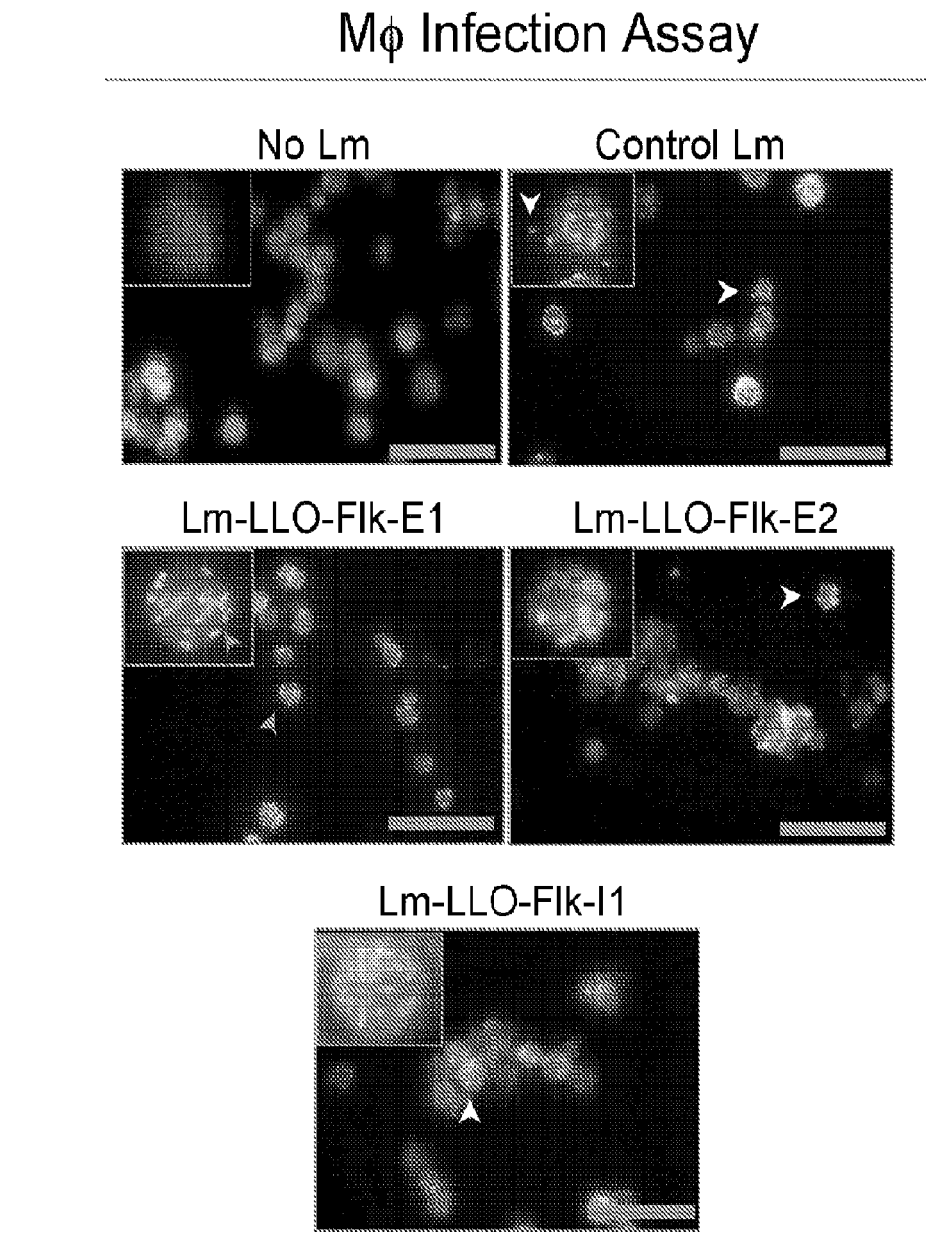

Mϕ Infection Assay
No Lm
Control Lm
Lm-LLO-Flk-E1
Lm-LLO-Flk-E2
Lm-LLO-Flk-I1
Listeria-Alexa488/CD11b-PE/Nucleus-DAPI

Figure 50D

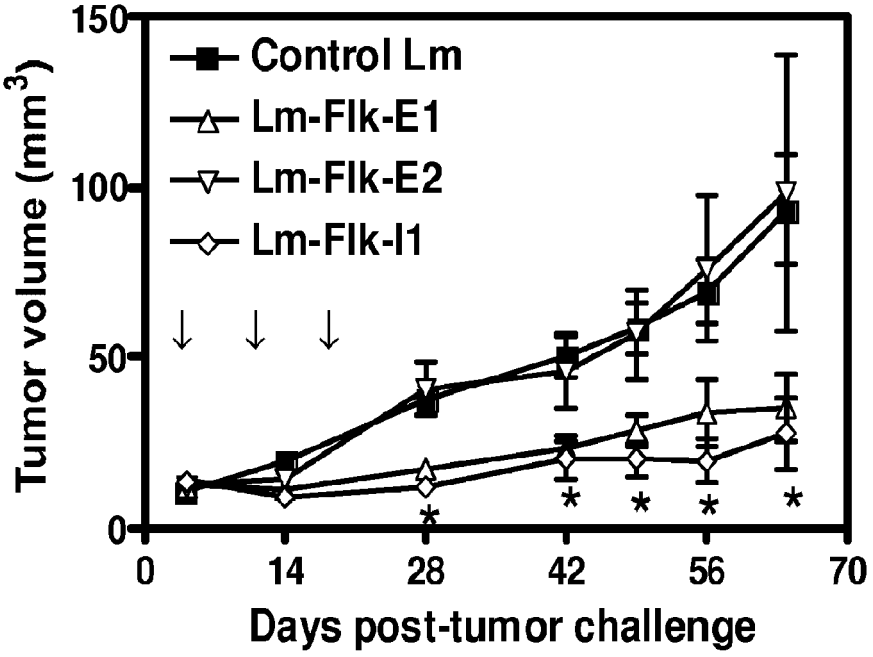


Figure 51A

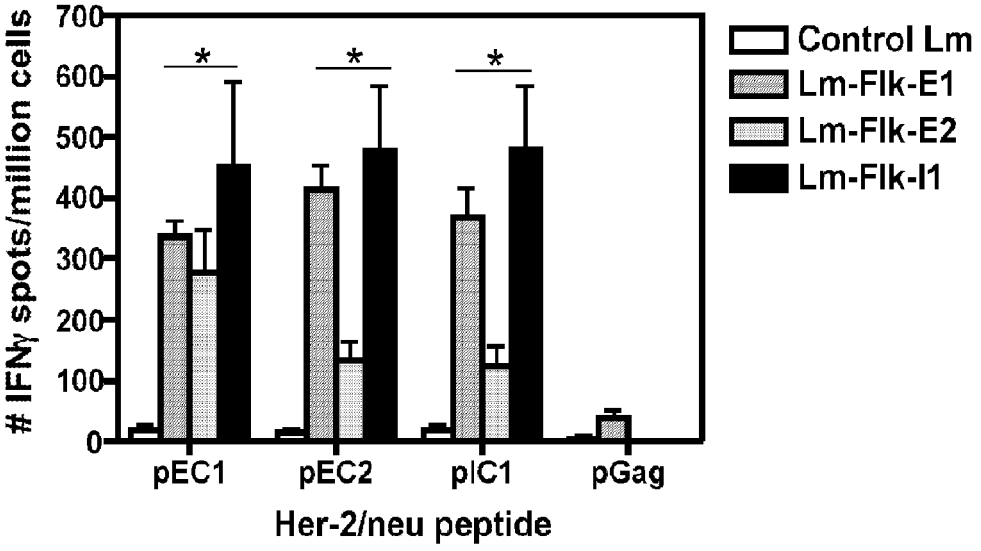
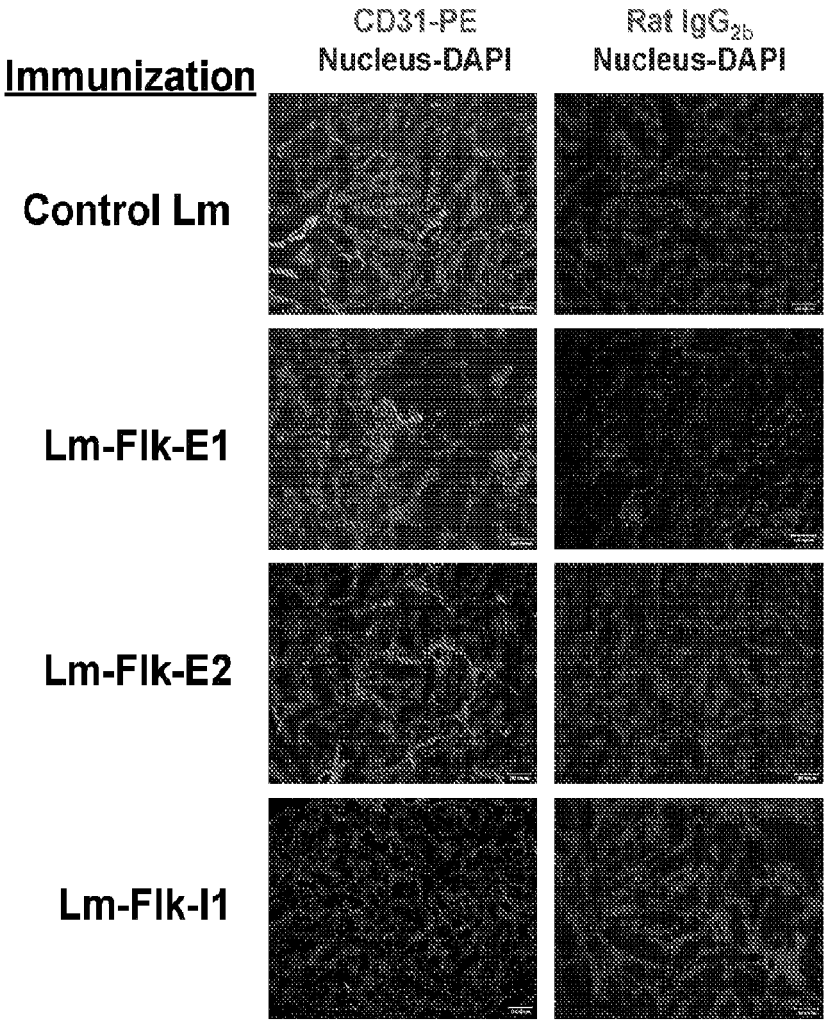


Figure 51B


Immunization
CD31-PE
Nucleus-DAPI
Rat IgG2b
Nucleus-DAPI
Control Lm
Lm-Flk-E1
Lm-Flk-E2
Lm-Flk-I1

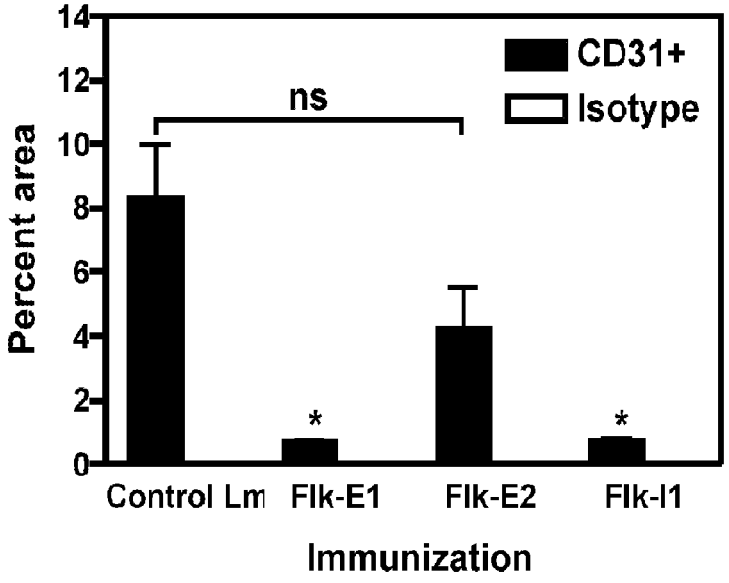

14
12
10
8
6
4
2
0
Percent area
ns
CD31+
Isotype
*
*
Control Lm
Flk-E1
Flk-E2
Flk-I1
Immunization

Figure 51C

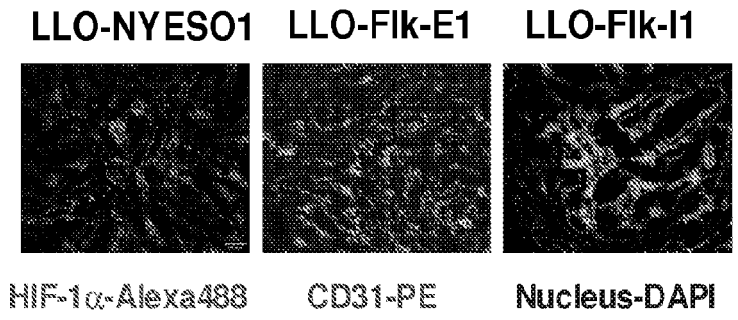


Figure 51D

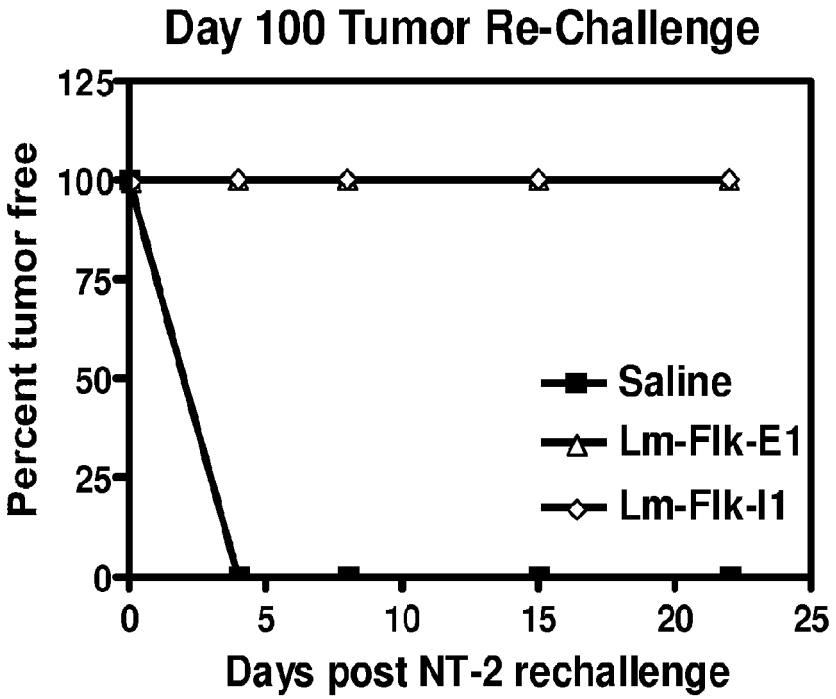


Figure 52A

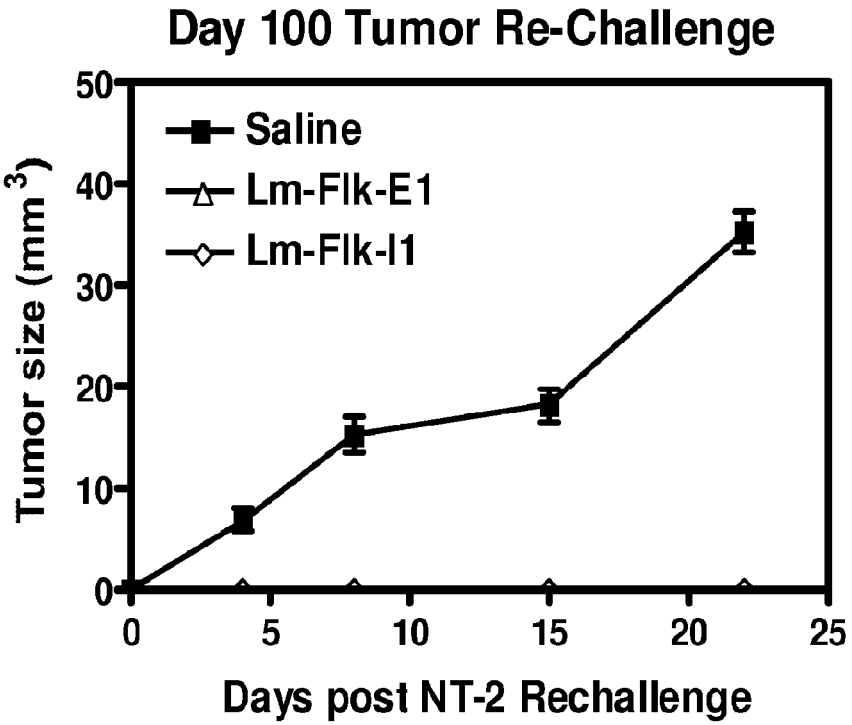


Figure 52B

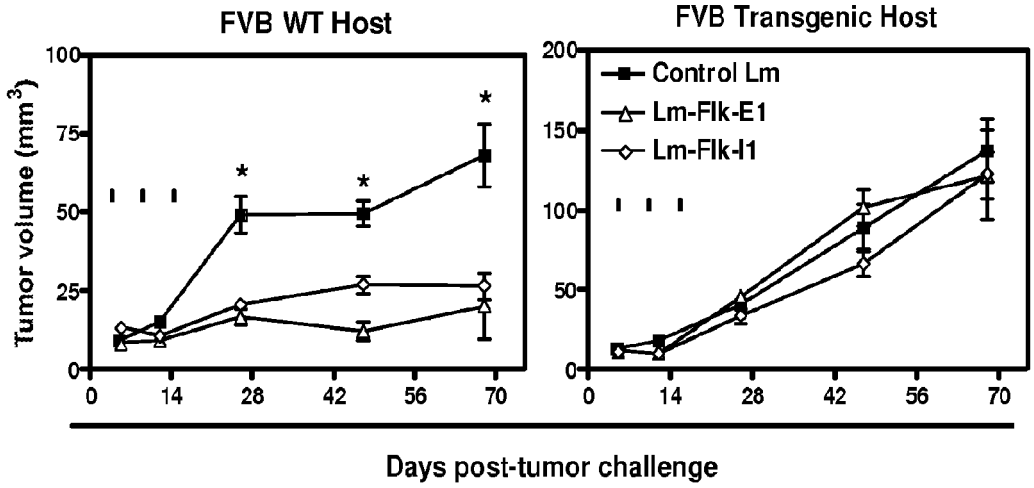


Figure 53A

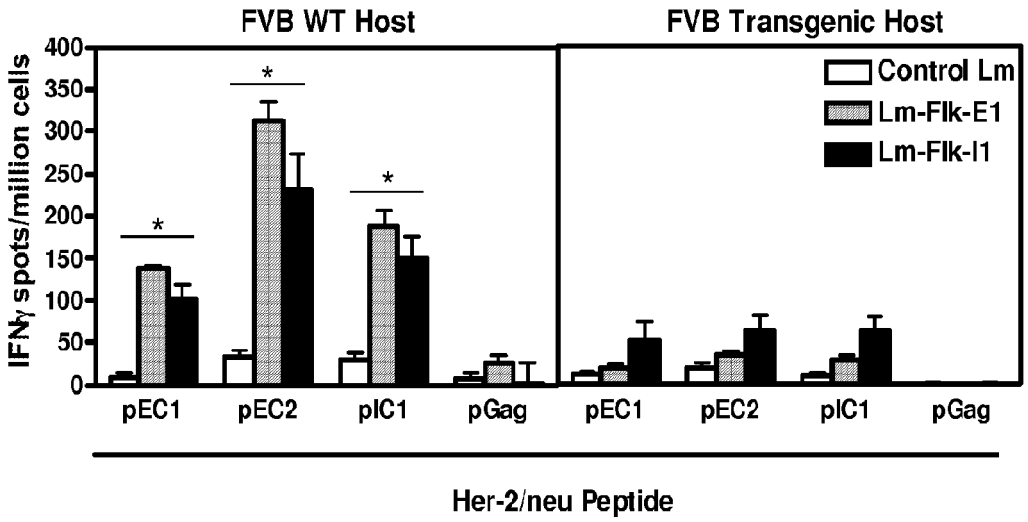

FVB WT Host
FVB Transgenic Host
Control Lm
Lm-Flk-E1
Lm-Flk-I1
IFNγ spots/million cells
400
350
300
250
200
150
100
50
0
*
*
*
pEC1
pEC2
pIC1
pGag
pEC1
pEC2
pIC1
pGag
Her-2/neu Peptide

Figure 53B

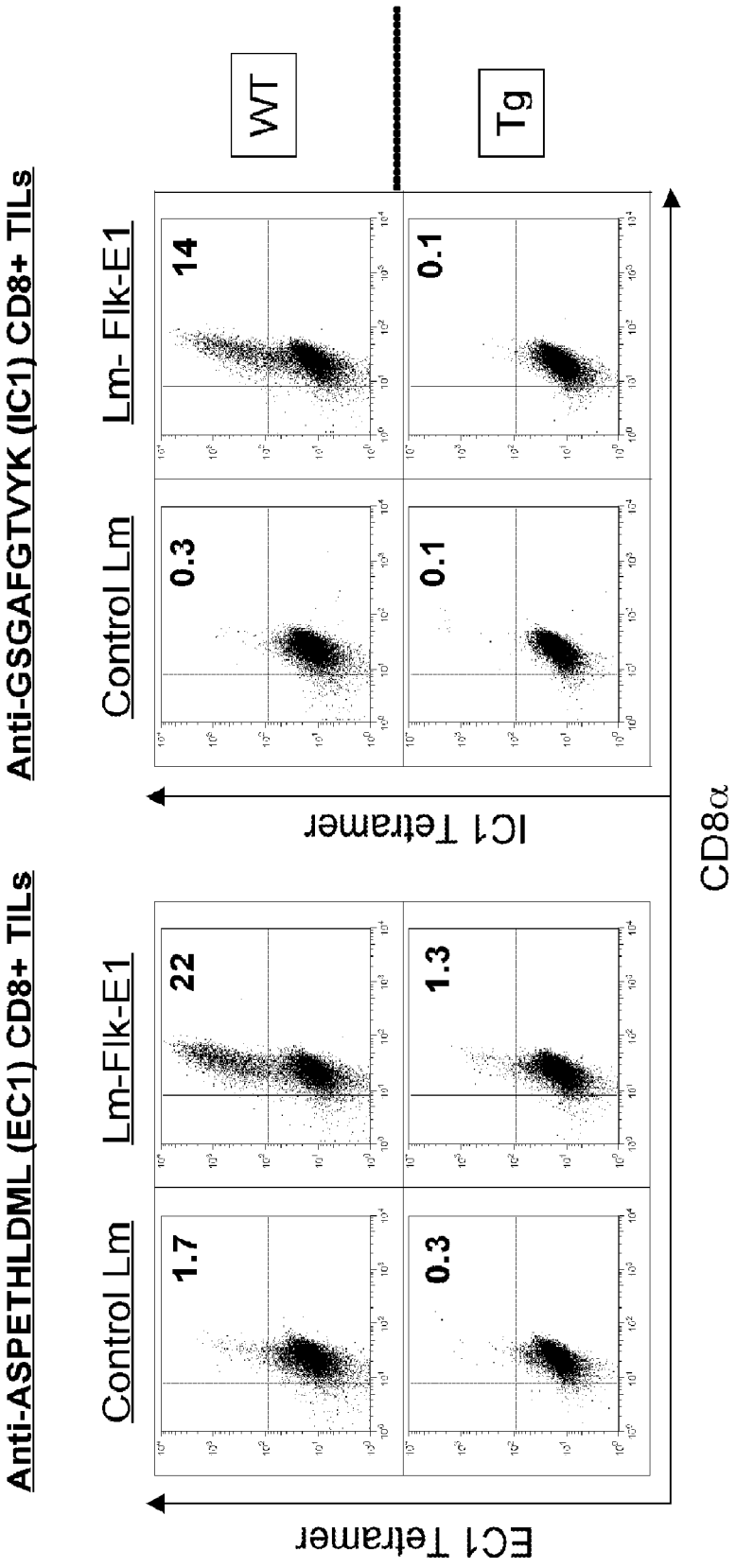

Anti-ASPETHLDML (EC1) CD8+ TILs
Anti-GSGAFGTVYK (IC1) CD8+ TILs
Control Lm
Lm-Flk-E1
Control Lm
Lm- Flk-E1
WT
Tg
1.7
22
0.3
1.3
0.3
0.1
14
0.1
EC1 Tetramer
IC1 Tetramer
CD8α

Figure 53C

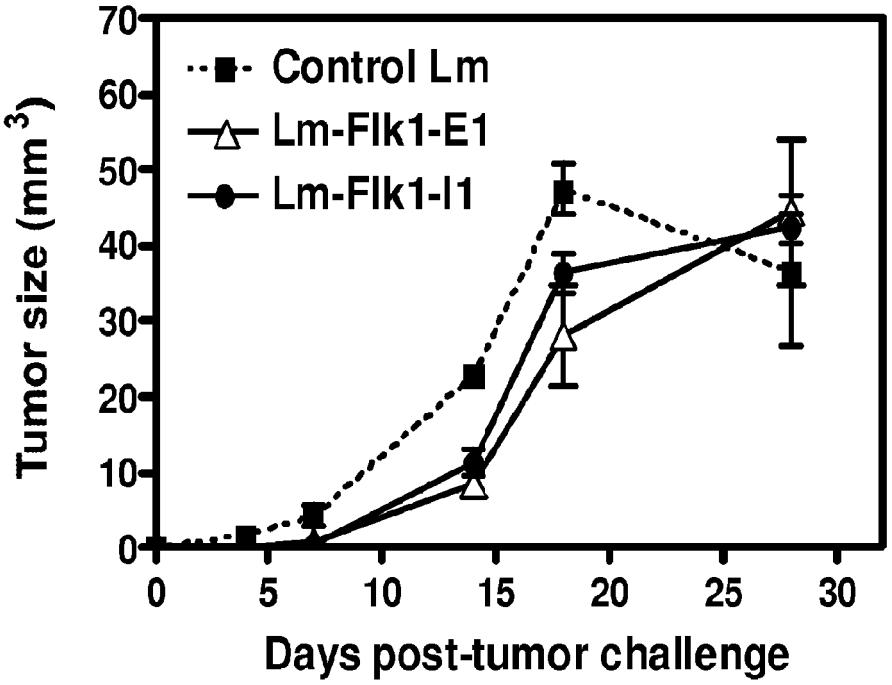


Figure 54A

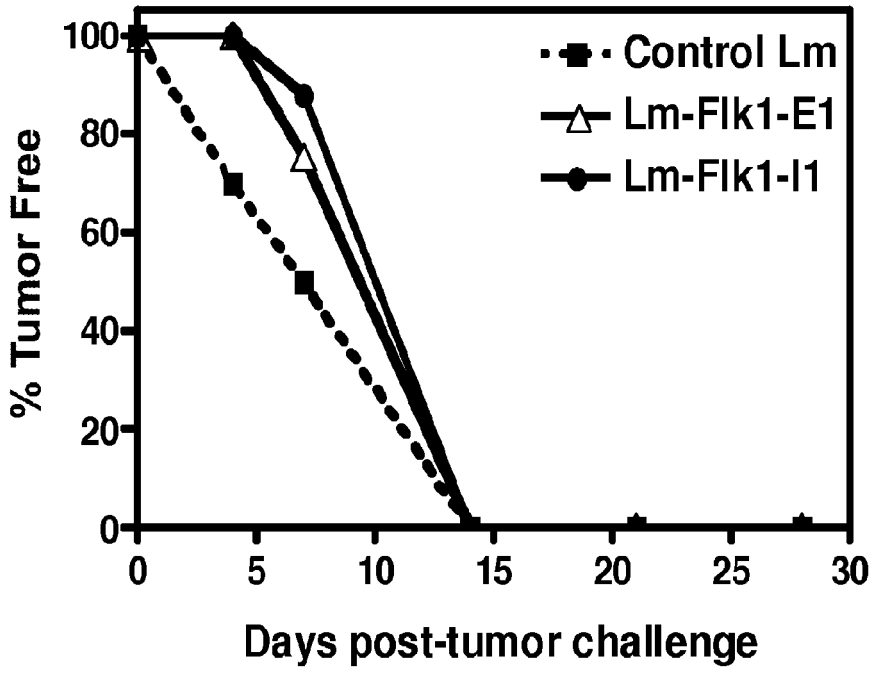


Figure 54B

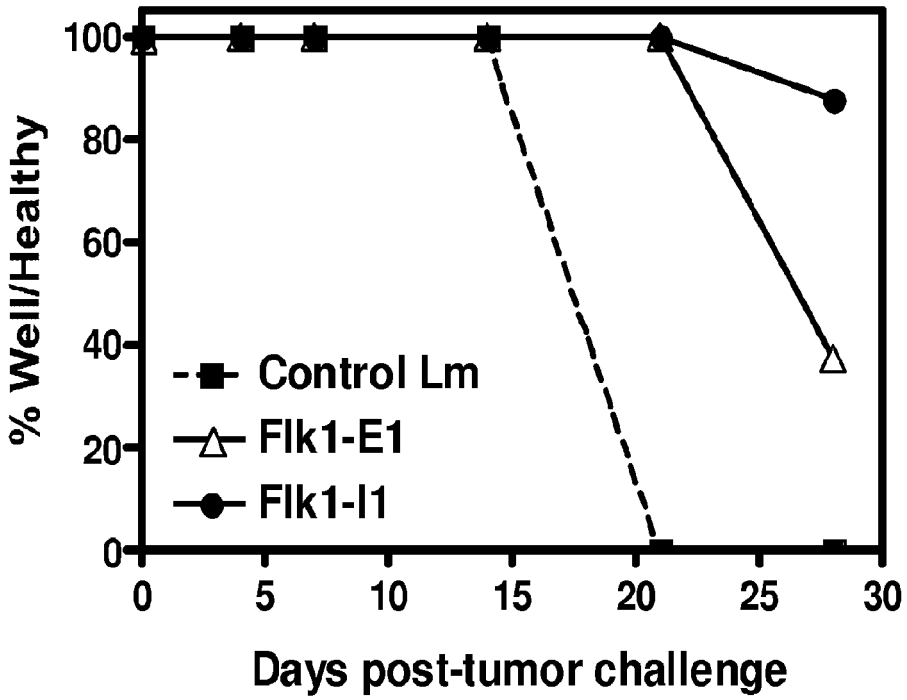


Figure 54C

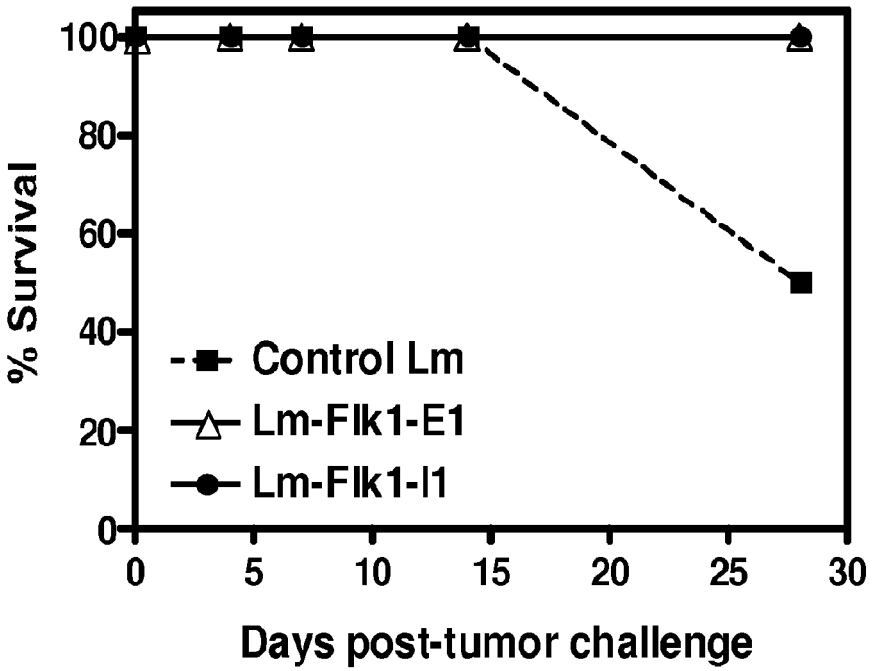
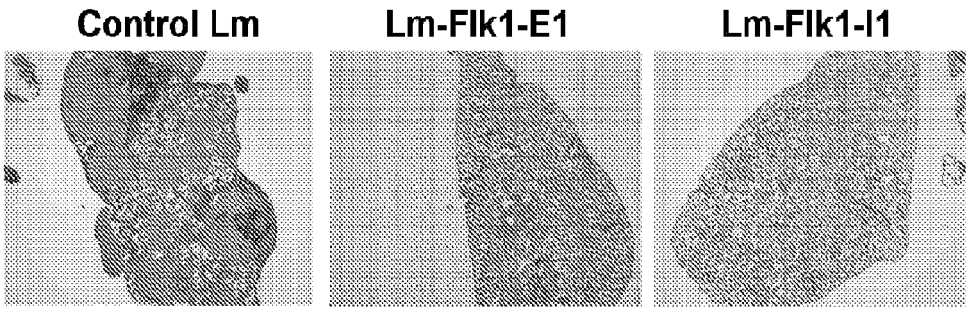


Figure 54D


Control Lm
Lm-Flk1-E1
Lm-Flk1-l1

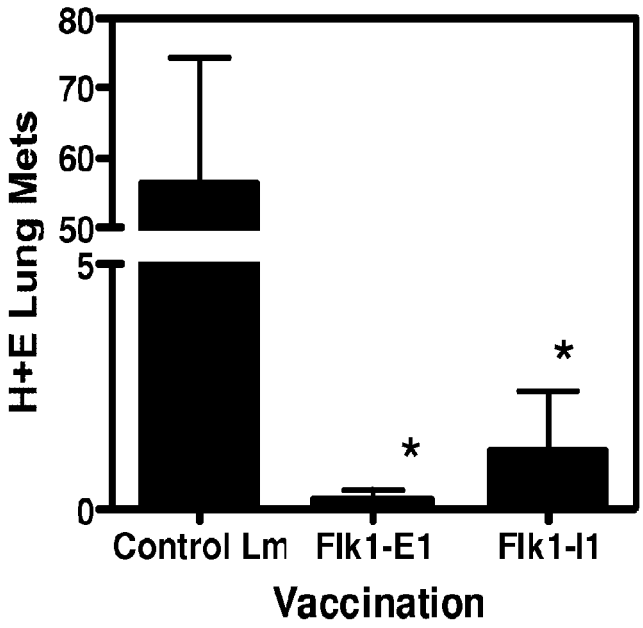

80
70
60
50
5
0
H+E Lung Mets
*
*
Control Lm
Flk1-E1
Flk1-l1
Vaccination

Figure 55A

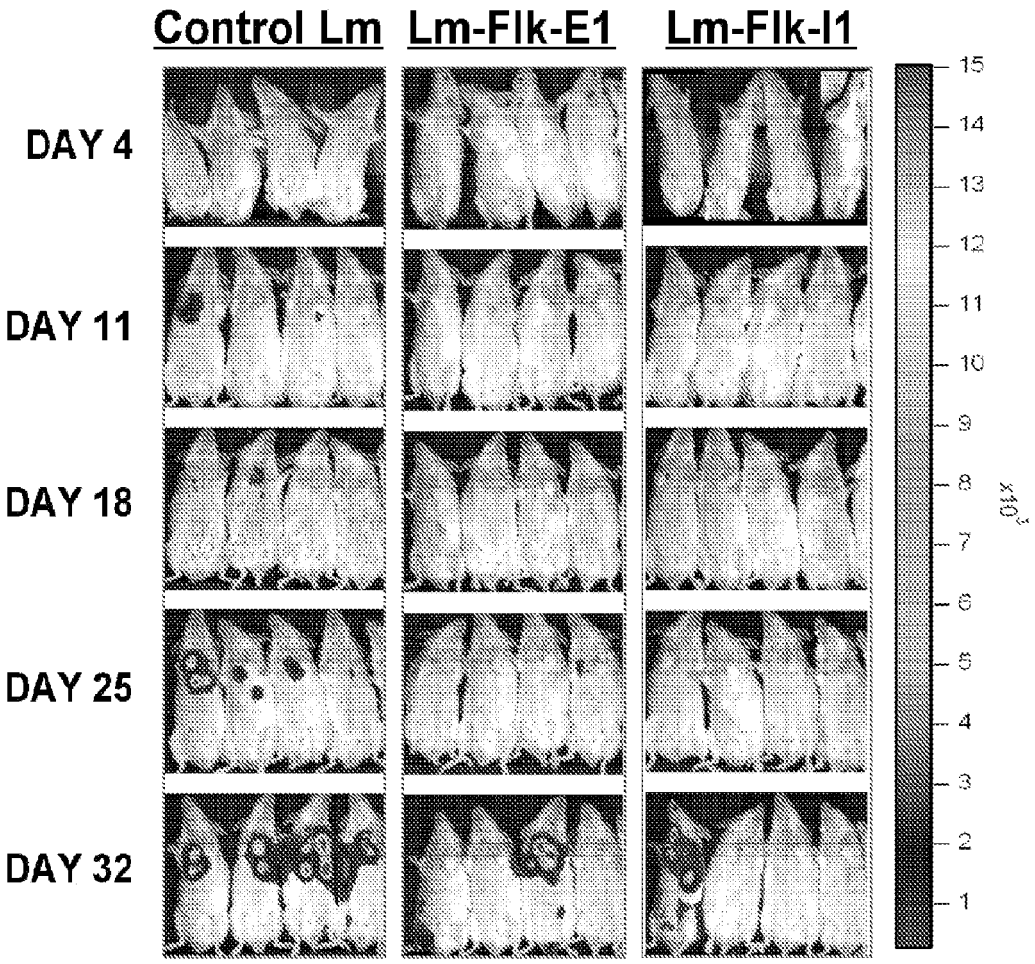

Control Lm
Lm-Flk-E1
Lm-Flk-l1
DAY 4
DAY 11
DAY 18
DAY 25
DAY 32
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
x10³

Figure 55B

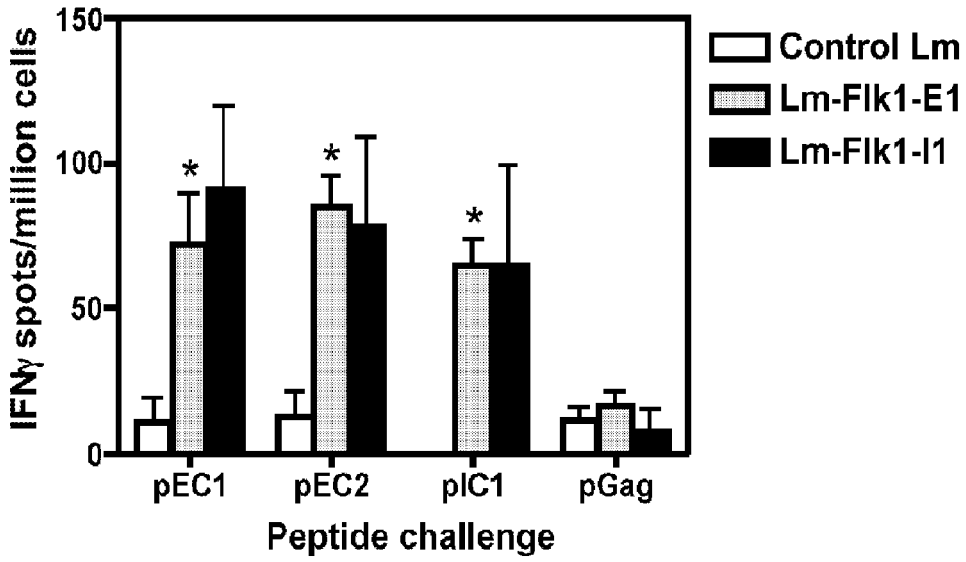


Figure 55C

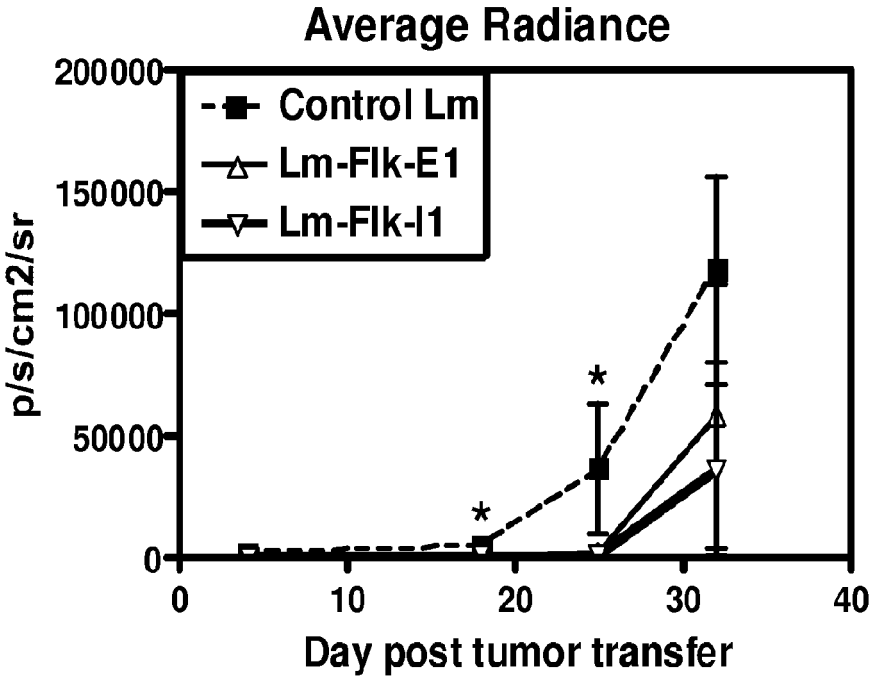


Figure 55D

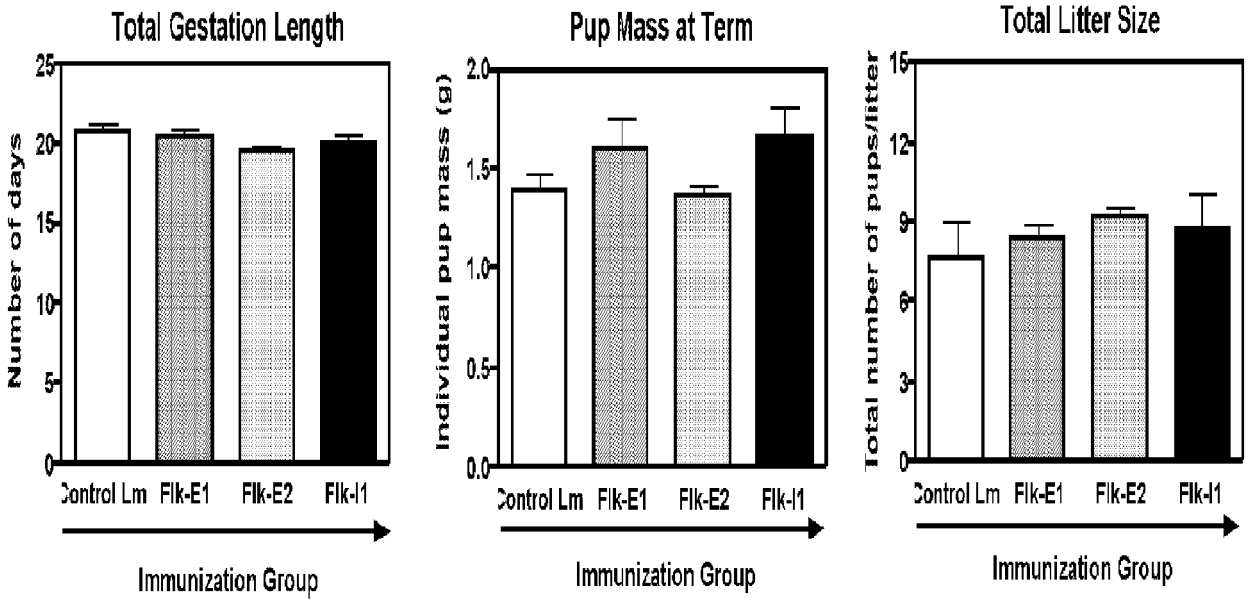
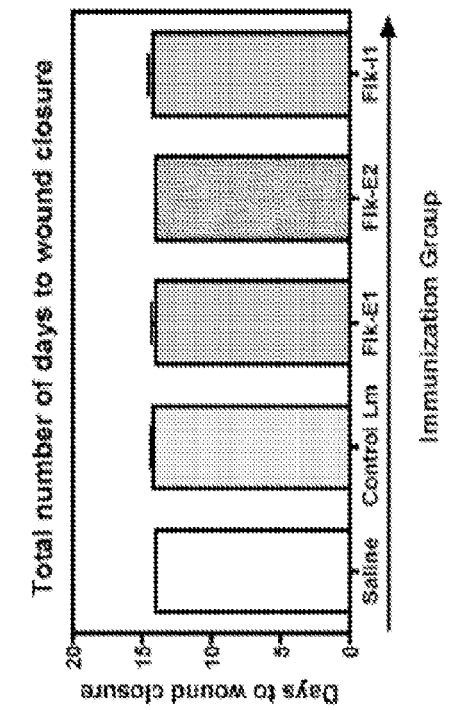

Total Gestation Length
Number of days
25
20
15
10
5
0
Control Lm
Flk-E1
Flk-E2
Flk-I1
Immunization Group
Pup Mass at Term
Individual pup mass (g)
2.0
1.5
1.0
0.5
0.0
Control Lm
Flk-E1
Flk-E2
Flk-I1
Immunization Group
Total Litter Size
Total number of pups/litter
15
12
9
6
3
0
Control Lm
Flk-E1
Flk-E2
Flk-I1
Immunization Group

Figure 56A


Total number of days to wound closure
Days to wound closure
20
15
10
5
0
Saline
Control Lm
Flk-E1
Flk-E2
Flk-I1
Immunization Group

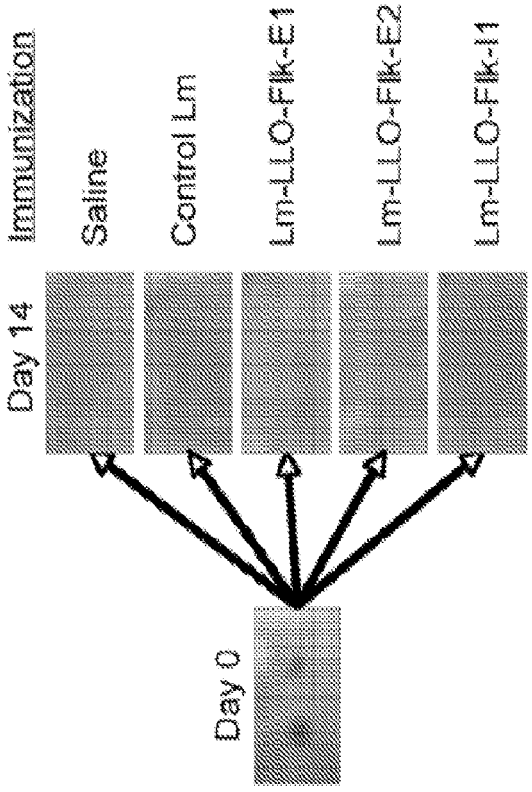

Immunization
Saline
Control Lm
Lm-LLO-Flk-E1
Lm-LLO-Flk-E2
Lm-LLO-Flk-I1
Day 14
Day 0

Figure 56B

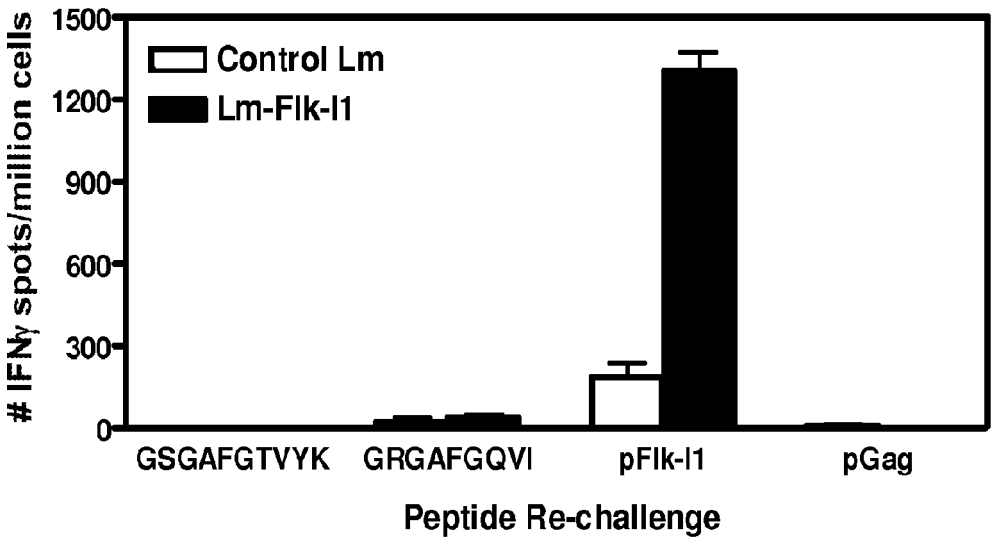

1500
1200
900
600
300
0
IFNγ spots/million cells
Control Lm
Lm-Flk-l1
GSGAFGTVYK
GRGAFGQVI
pFlk-l1
pGag
Peptide Re-challenge

Figure 57

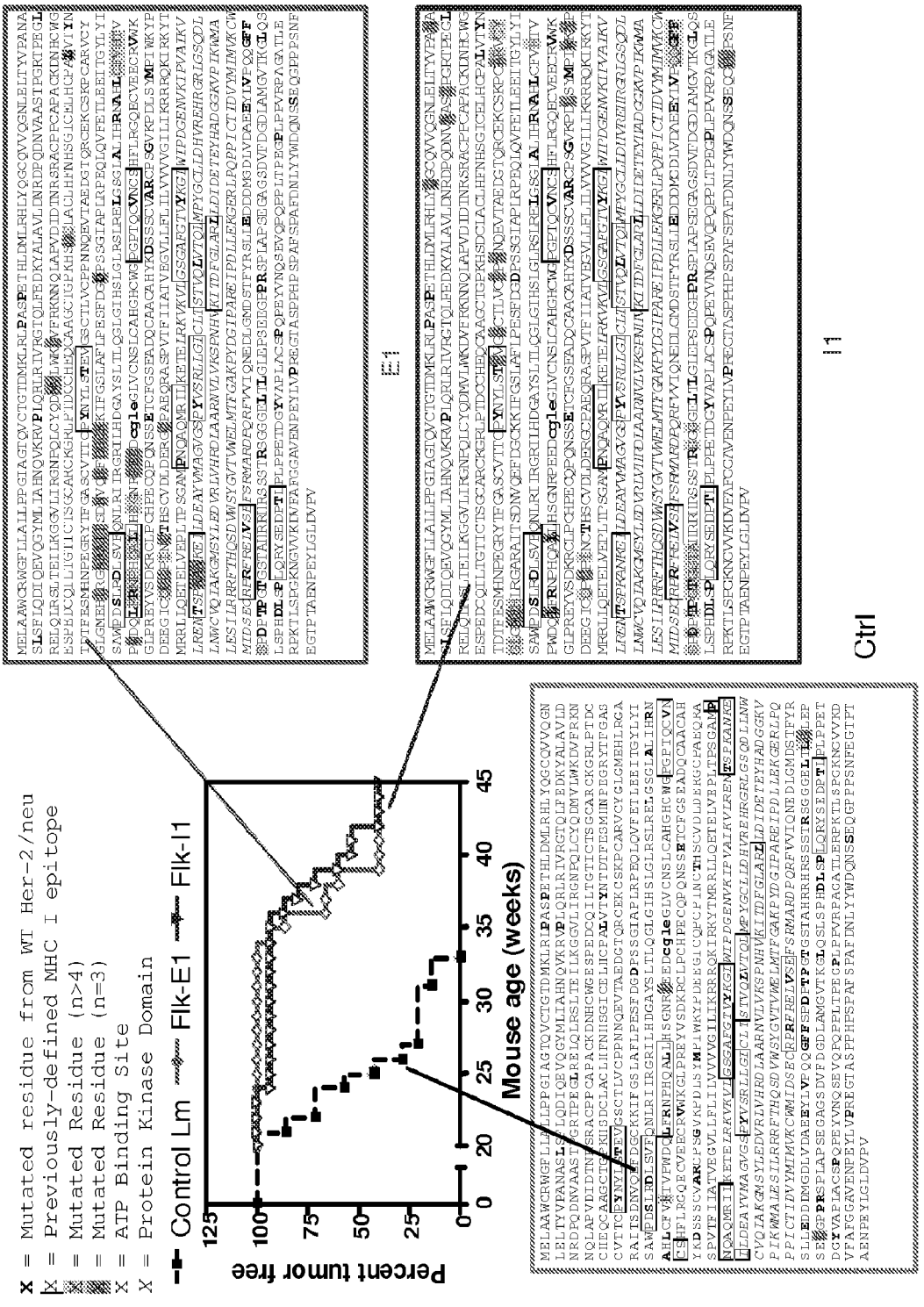

X = Mutated residue from WT Her-2/neu
X = Previously-defined MHC I epitope
= Mutated Residue (n>4)
= Mutated Residue (n=3)
X = ATP Binding Site
X = Protein Kinase Domain
Control Lm
Flk-E1
Flk-I1
Percent tumor free
Mouse age (weeks)
E1
I1
Ctrl

Figure 58A

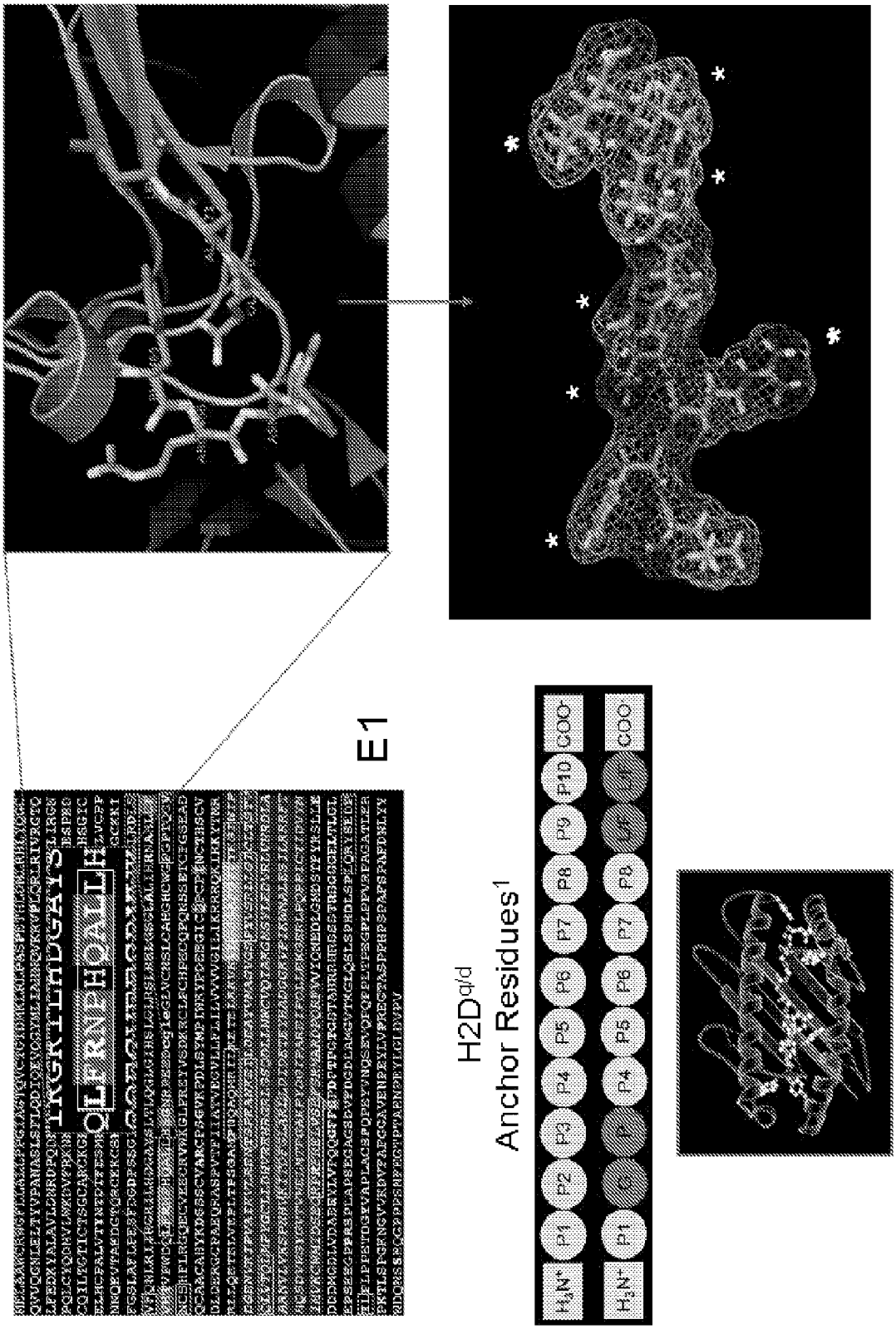


Figure 58B

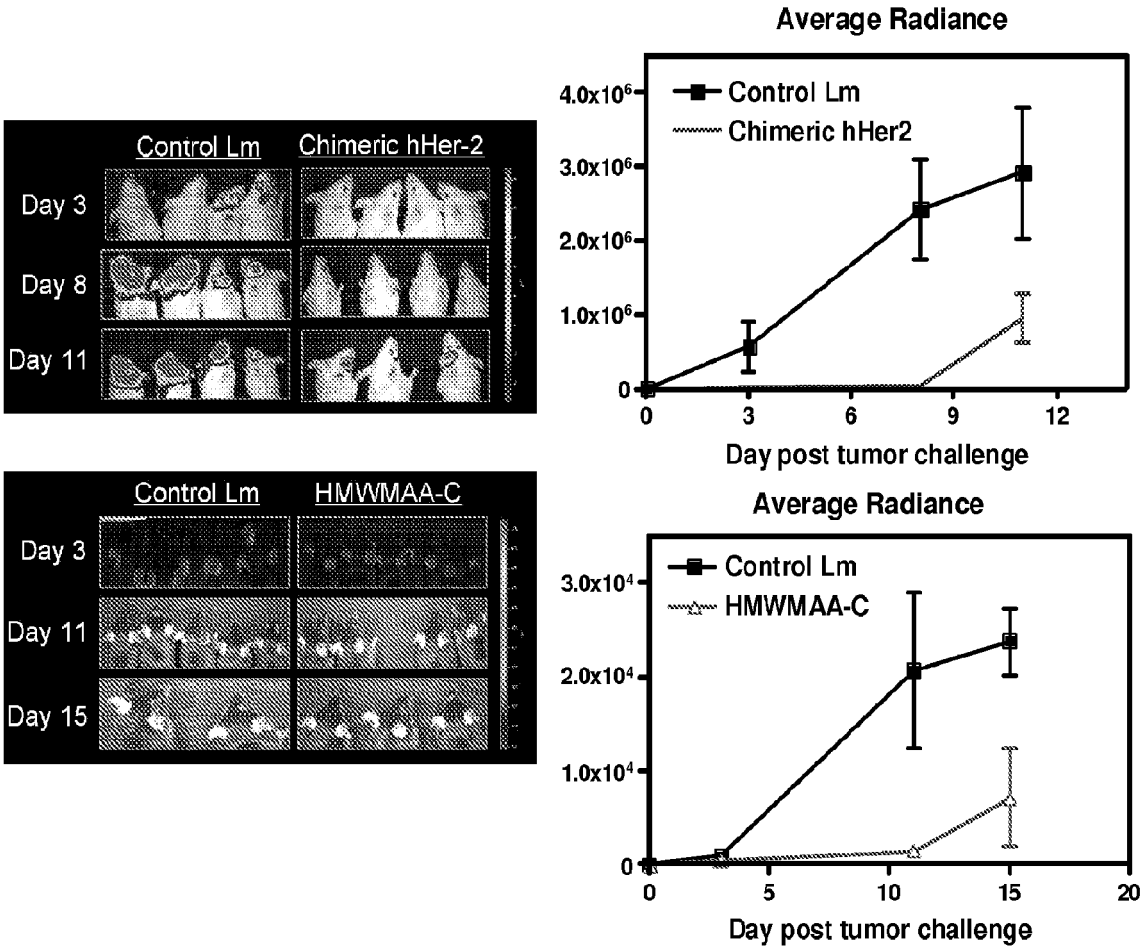

Control Lm
Chimeric hHer-2
Day 3
Day 8
Day 11
Average Radiance
Control Lm
Chimeric hHer2
4.0x10⁶
3.0x10⁶
2.0x10⁶
1.0x10⁶
0
0 3 6 9 12
Day post tumor challenge
Control Lm
HMWMAA-C
Day 3
Day 11
Day 15
Average Radiance
Control Lm
HMWMAA-C
3.0x10⁴
2.0x10⁴
1.0x10⁴
0
0 5 10 15 20
Day post tumor challenge

Figure 59

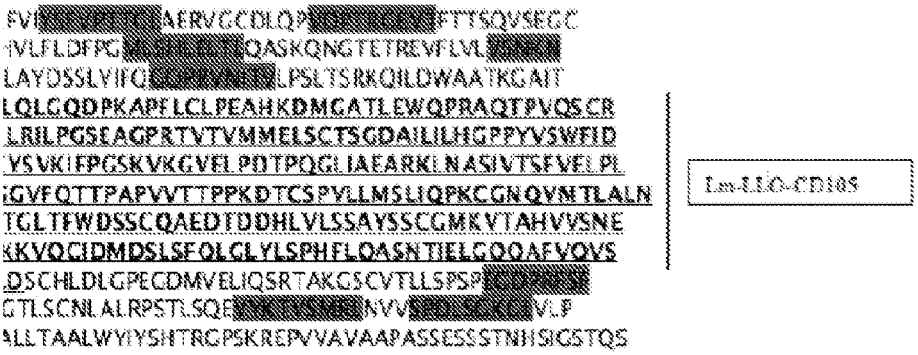


Figure 60

A)

MDRGVLPLPTLLFVTYSFVPTTGLAERVGCDLQPVDPTRGEVTFTTSQVSEGCVAQAA
NAVREVHVLFLDFPGMLSHLELTLQASKQNGTETREVFLVLVSNKNVFVKFQAPEHP Lm-LLO-CI
LHLAYDSSLVIFQGQPRVNITVLPSLTSRKQELDWAATKGAITSIAALDDFQSIVLQLG
QDPKAPFLCLPEAHKDMGATLEWQPRAQTPVQSCRLEGVSGHKEAYLRHLPGSEAG
PRTVTVMMELSCTSGDAILIHGPPYVSWFIDLNHSMQILTTGEYSVKIFPGSKVKGV
ELPDTPQGLIAEARKLNASIVTSFVELPLVSNVSLRASSCGGVFQFTPAPVVTPPKDTCS
PVLLMSLIQPKCGNQVMTLALNKKHVQTLQCTITGLTPWDSSCQAEDTDDHLVLSS
AYSSCGMKVTAHVVSNEVIISFPSGSPPLRKKVQCDMDSLSFQLGLYLSPHFLQASNT
IELGQQAFVQVSVSPLTSEVTVQLDSCHLDLGPEGDMVELIQSRTAKGSCVTLSPSP Lm-LLO-CI
EGDPRFSFLLRVYMVPTPTAGTLSCNLALRPSTLSQEVYKTVSMRLNVVSPDLSGKG
LVLPSVLGITFGAFLIGALLTAALWYIYSHTRGPSKREPVVAVAAPASSESSSTNHSGSTQ
STPCSTSSMA

In CD105A: AGPRTVTVM (D^{4})
In CD105B AYSSCGMKV (K^{5})

B)

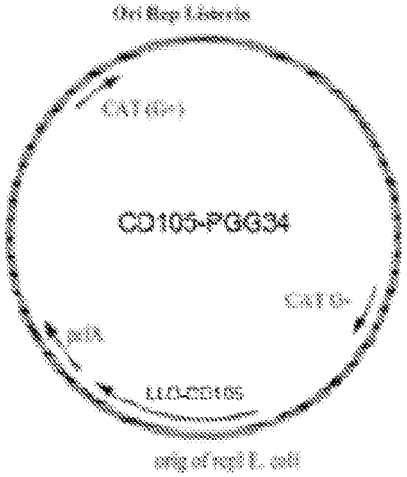


Figure 61

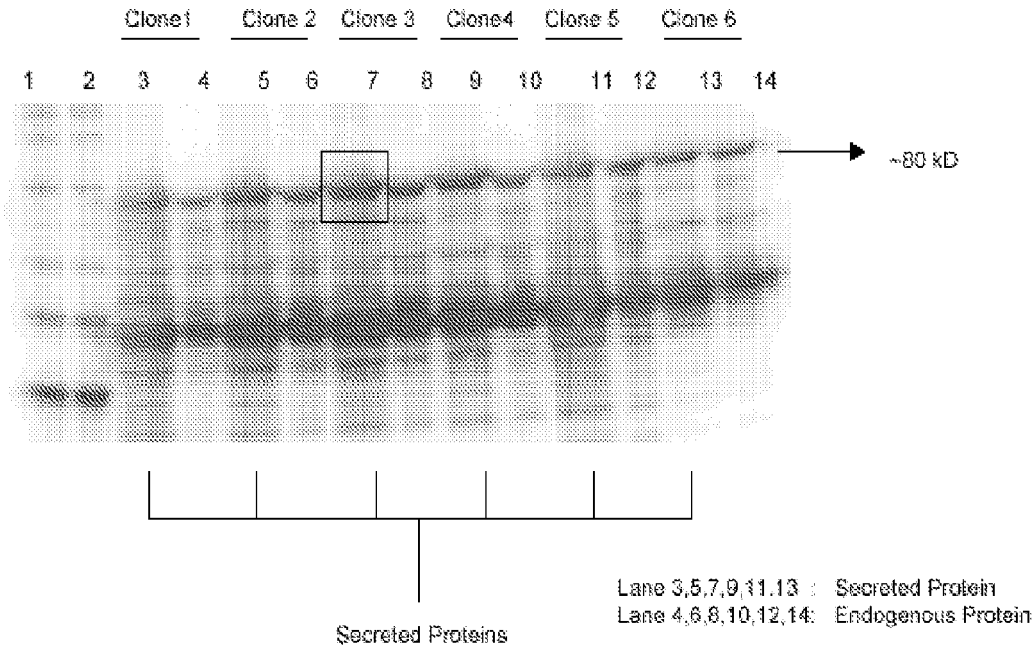

Clone1
Clone 2
Clone 3
Clone4
Clone 5
Clone 6
1 2 3 4 5 6 7 8 9 10 11 12 13 14
~80 kD
Secreted Proteins
Lane 3,5,7,9,11,13 : Secreted Protein
Lane 4,6,8,10,12,14: Endogenous Protein

Figure 62

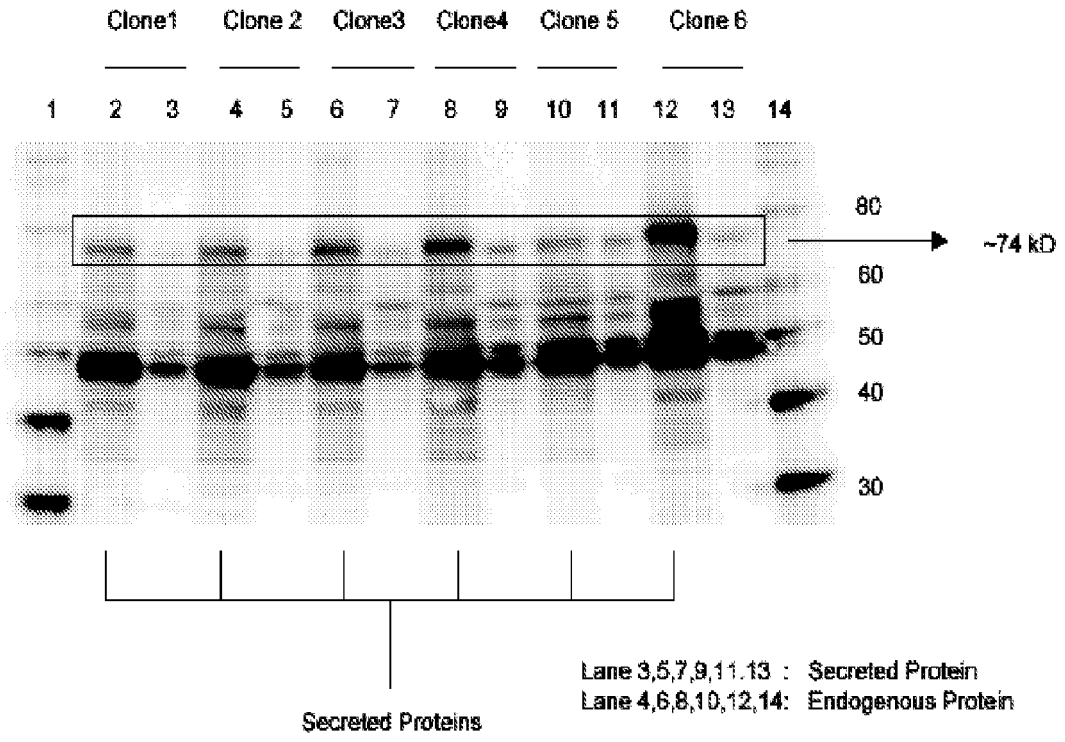
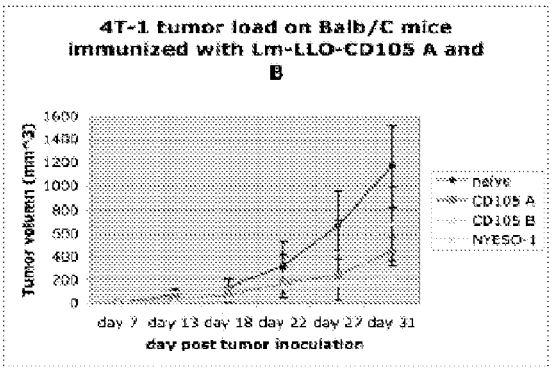

Clone1
Clone 2
Clone3
Clone4
Clone 5
Clone 6
1 2 3 4 5 6 7 8 9 10 11 12 13 14
80
60
50
40
30
~74 kD
Secreted Proteins
Lane 3,5,7,9,11.13 : Secreted Protein
Lane 4,6,8,10,12,14: Endogenous Protein

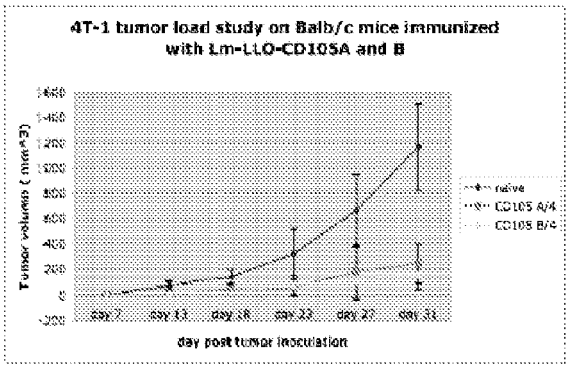
Figure 63

A. 1st immunization on Day 1



Immunized on day
1, 8 and 15

B. 1st immunization on Day 4



Immunized on day
4, 11 and18

Figure 64

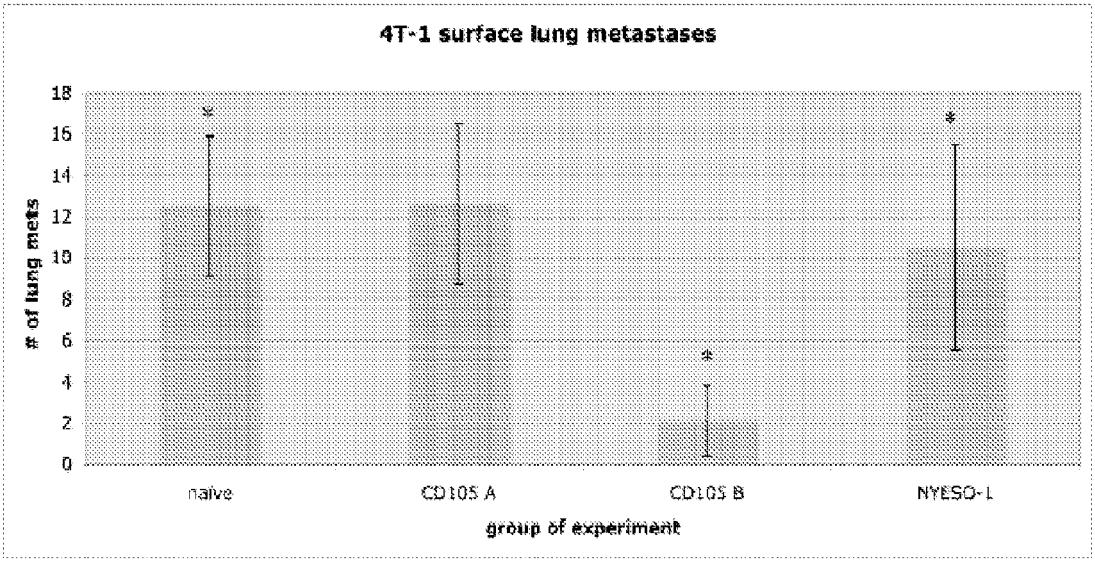

4T-1 surface lung metastases
# of lung mets
18
16
14
12
10
8
6
4
2
0
*
*
*
naive
CD105 A
CD105 B
NYESO-1
group of experiment

Figure 65

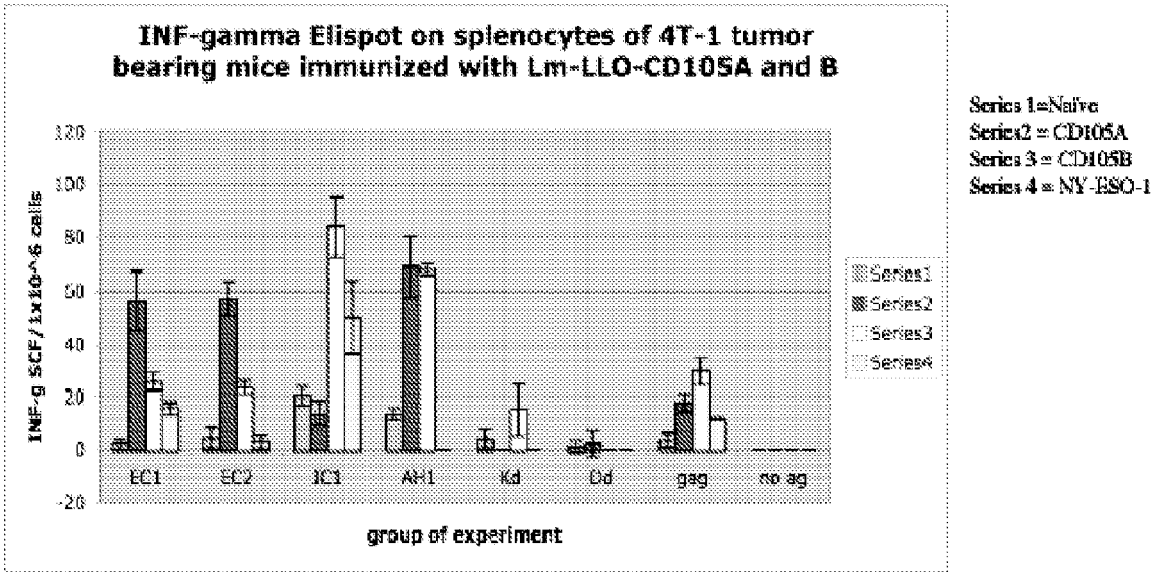

INF-gamma Elispot on splenocytes of 4T-1 tumor bearing mice immunized with Lm-LLO-CD105A and B
INF-g SCF/1x10^6 cells
120
100
80
60
40
20
0
-20
EC1
EC2
IC1
AH1
Kd
Dd
gag
no ag
group of experiment
Series1
Series2
Series3
Series4
Series 1=Naïve
Series2 = CD105A
Series 3 = CD105B
Series 4 = NY-ESO-1

Figure 66

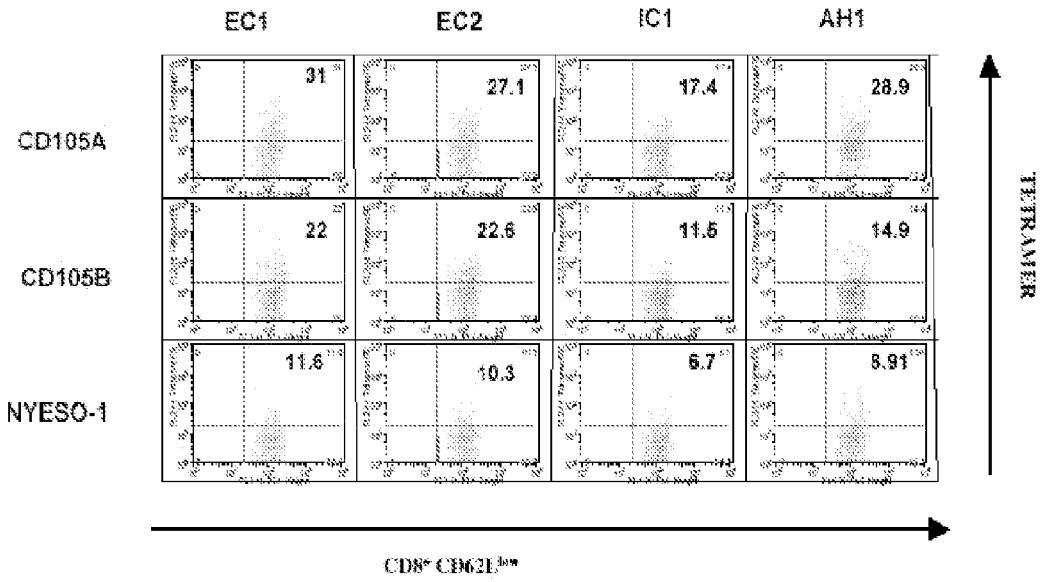
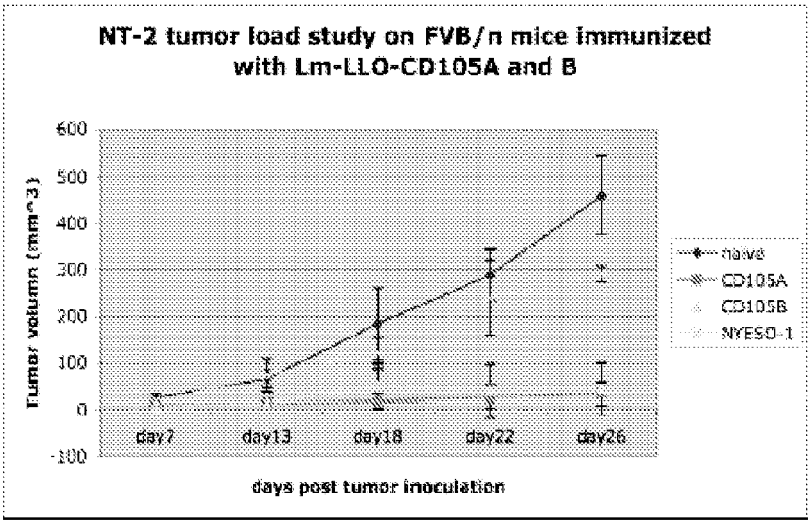

EC1
EC2
IC1
AH1
CD105A
CD105B
NYESO-1
31
27.1
17.4
28.9
22
22.6
11.5
14.9
11.6
10.3
6.7
8.91
TETRAMER
CD8+ CD62L

Figure 67



Tumor free on day 60
Naïve 0/8
CD105A 4/8
CD105B 2/8
NYESO-1 0/8

Figure 68

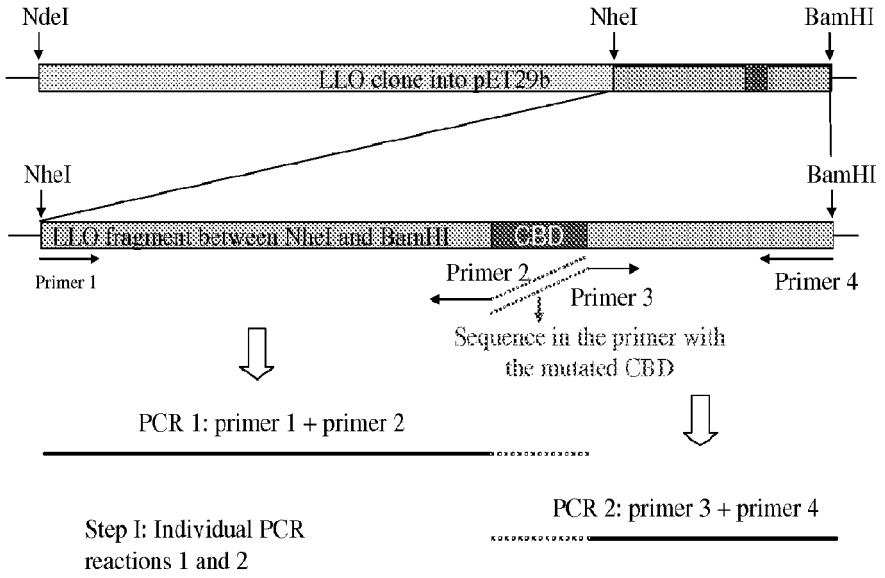
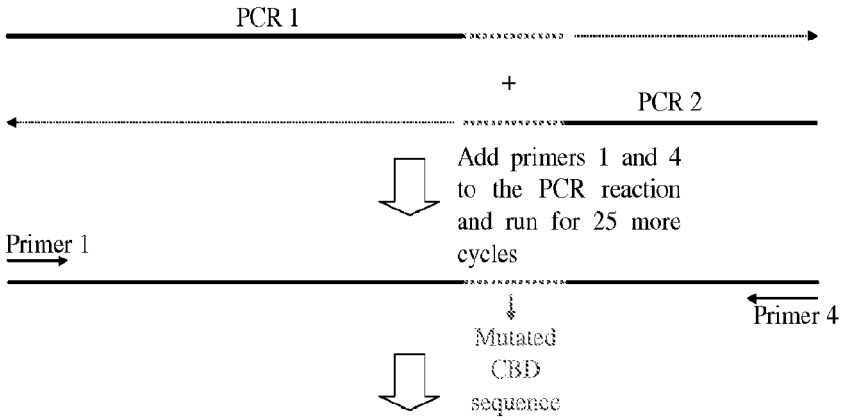


Figure 69A

Step II: mix the products of PCR 1 and PCR 2 and allow the annealing of the complementary region (mutated CBD) between the products from PCR 1 and 2



Clone PCR product into the pCR2.1 vector (Invitrogen)
Verify the sequence of LLO and the mutated CBD

Figure 69B

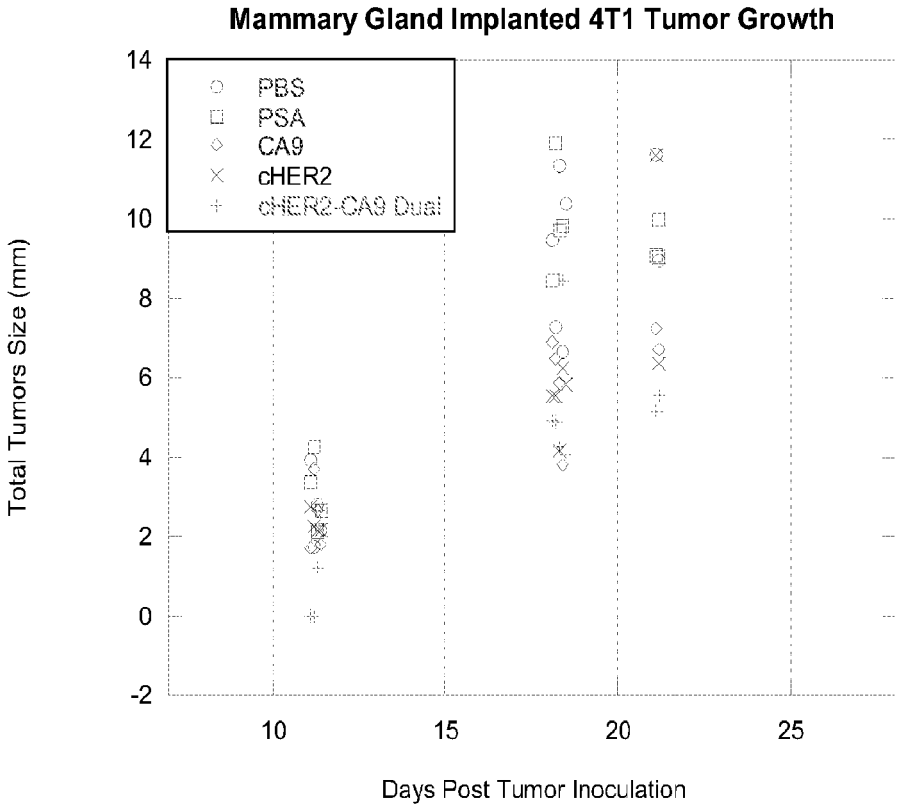

Mammary Gland Implanted 4T1 Tumor Growth
PBS
PSA
CA9
cHER2
cHER2-CA9 Dual
Total Tumors Size (mm)
14
12
10
8
6
4
2
0
-2
10
15
20
25
Days Post Tumor Inoculation

Figure 70

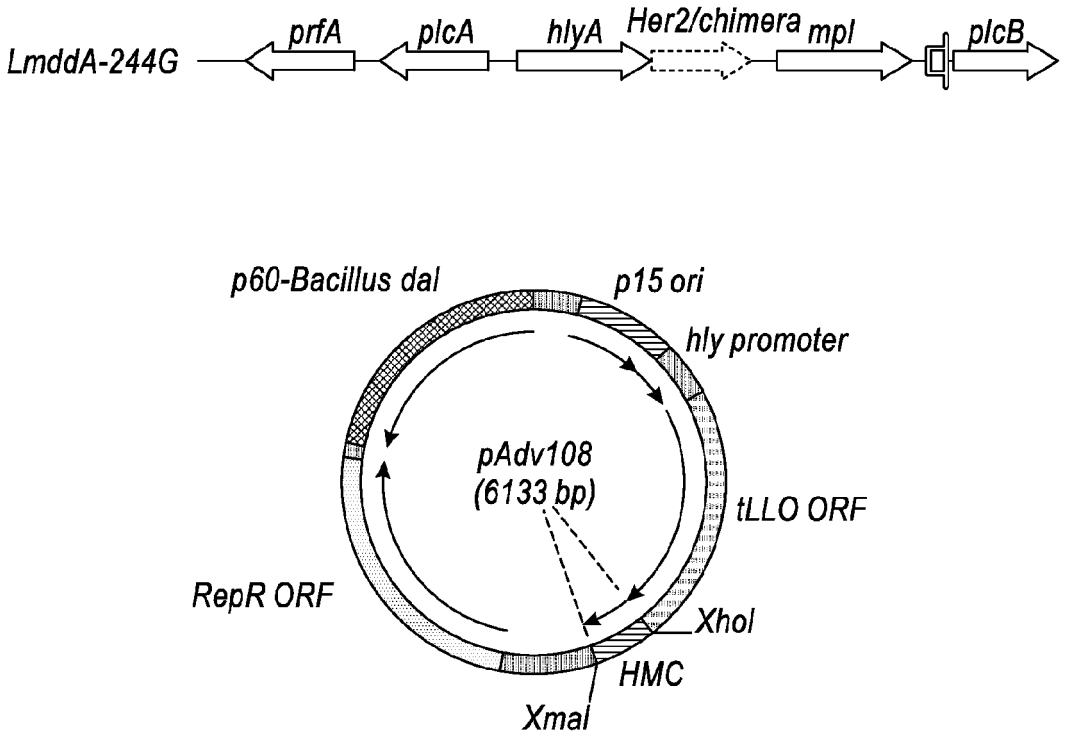


Figure 71A

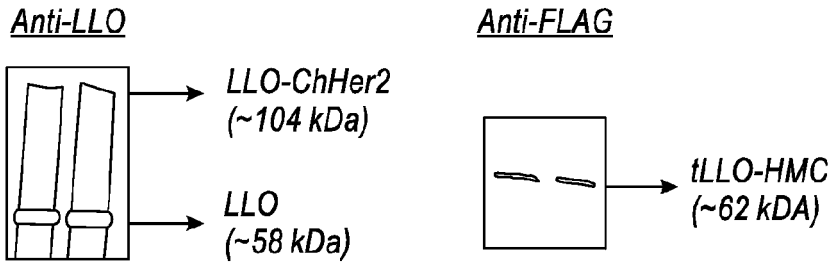


Figure 71B

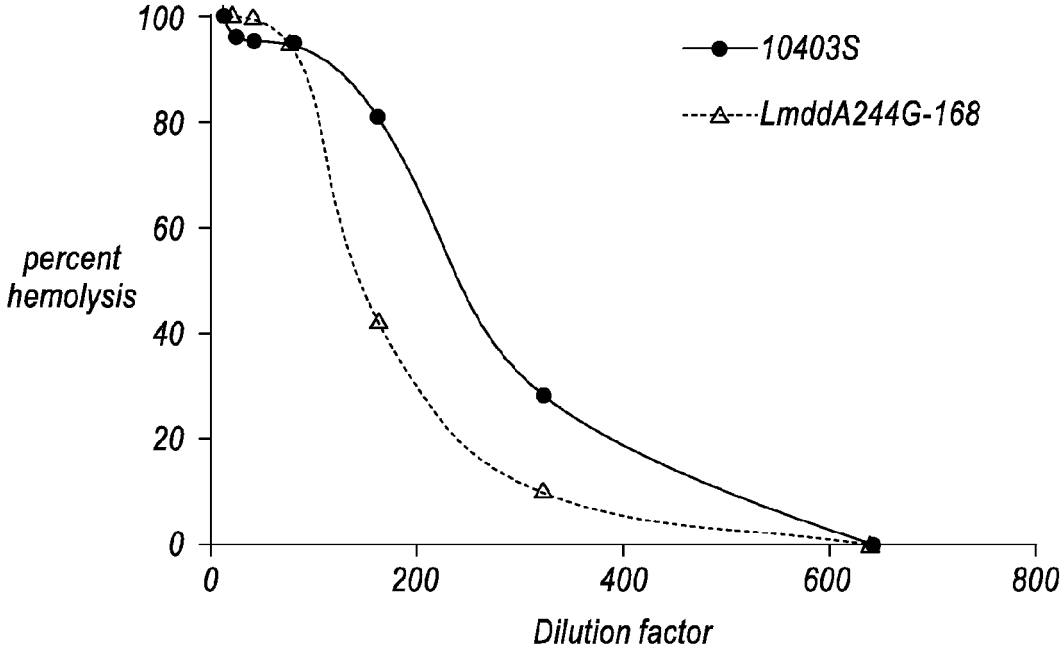


Figure 72A

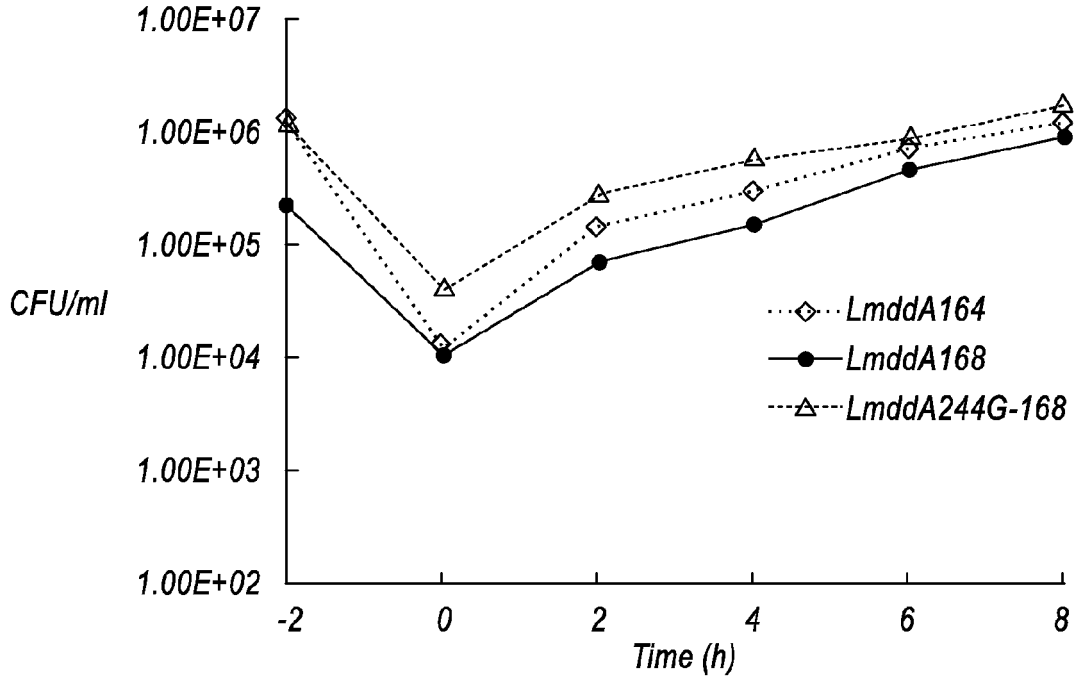


Figure 72B

Figure 73A

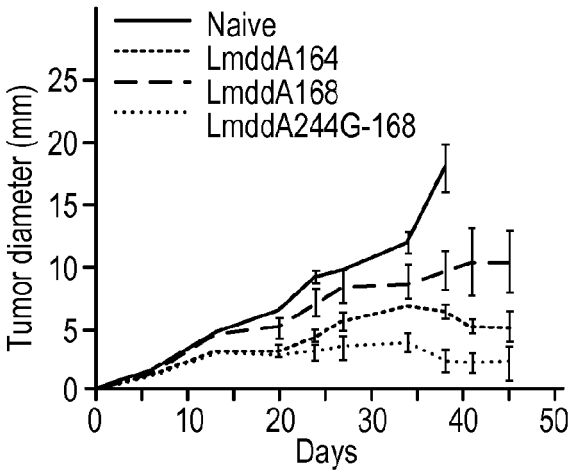


Figure 73B

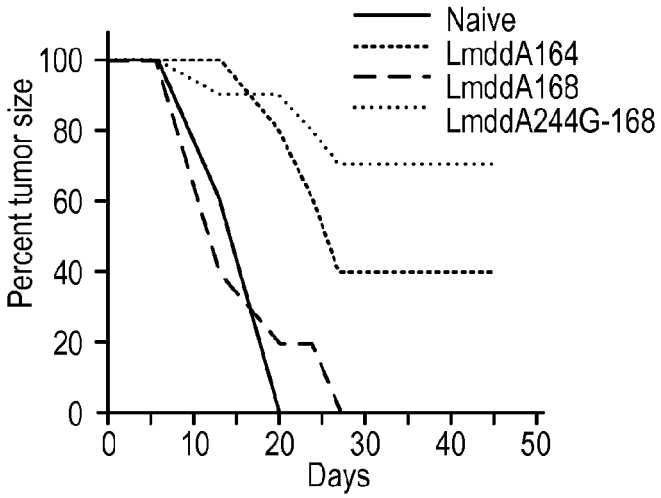
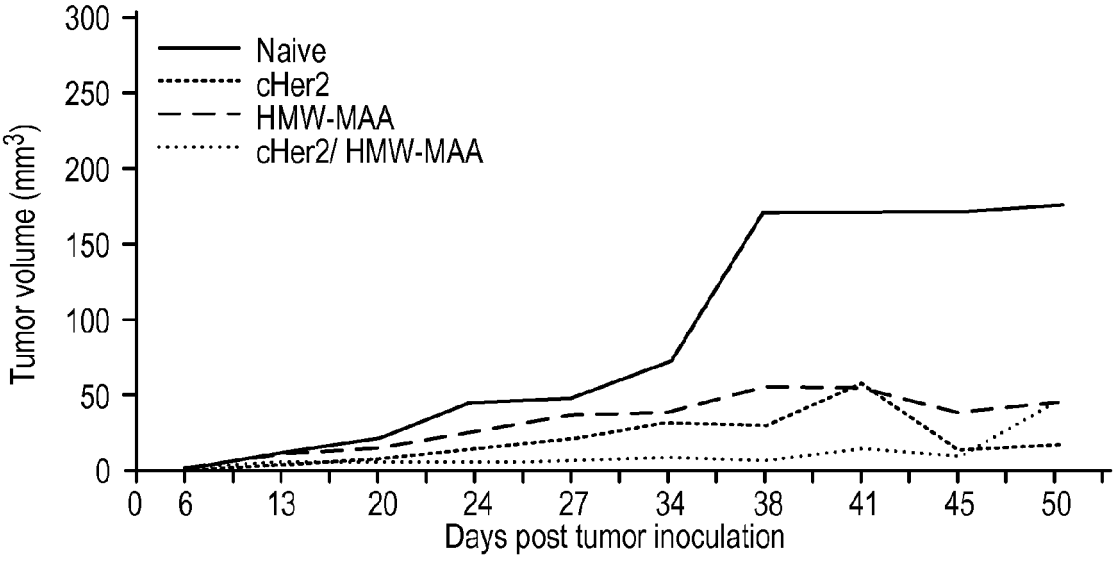


Figure 73C



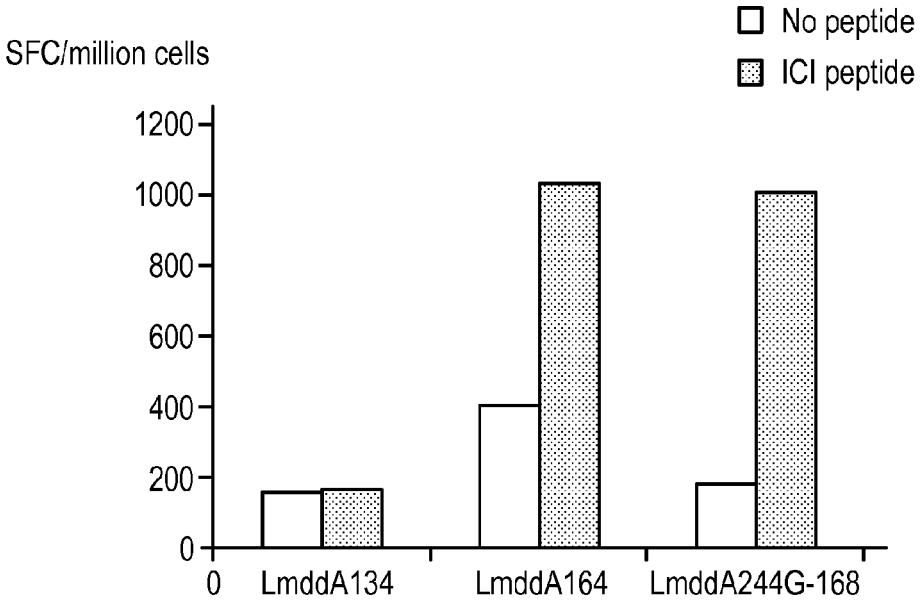


Figure 74A

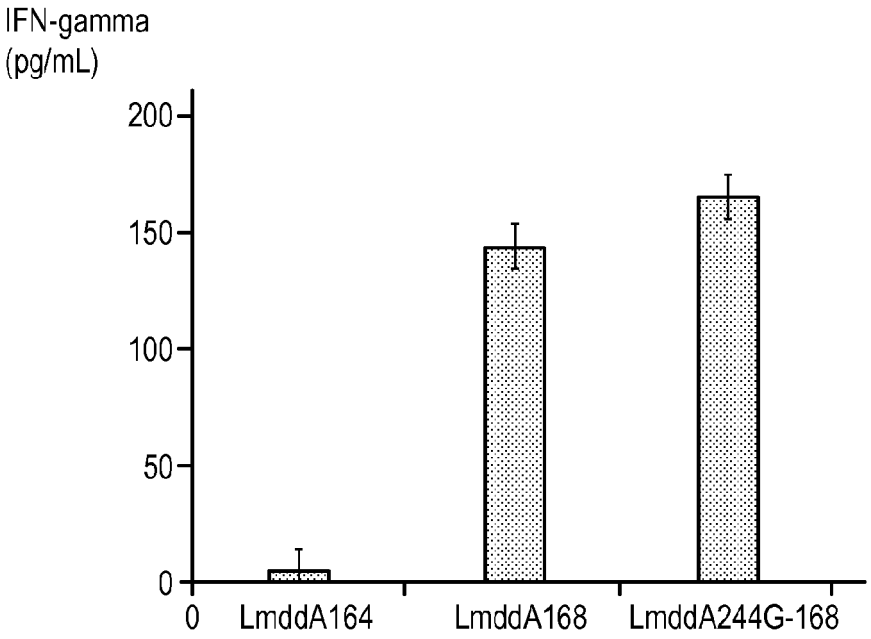


Figure 74B

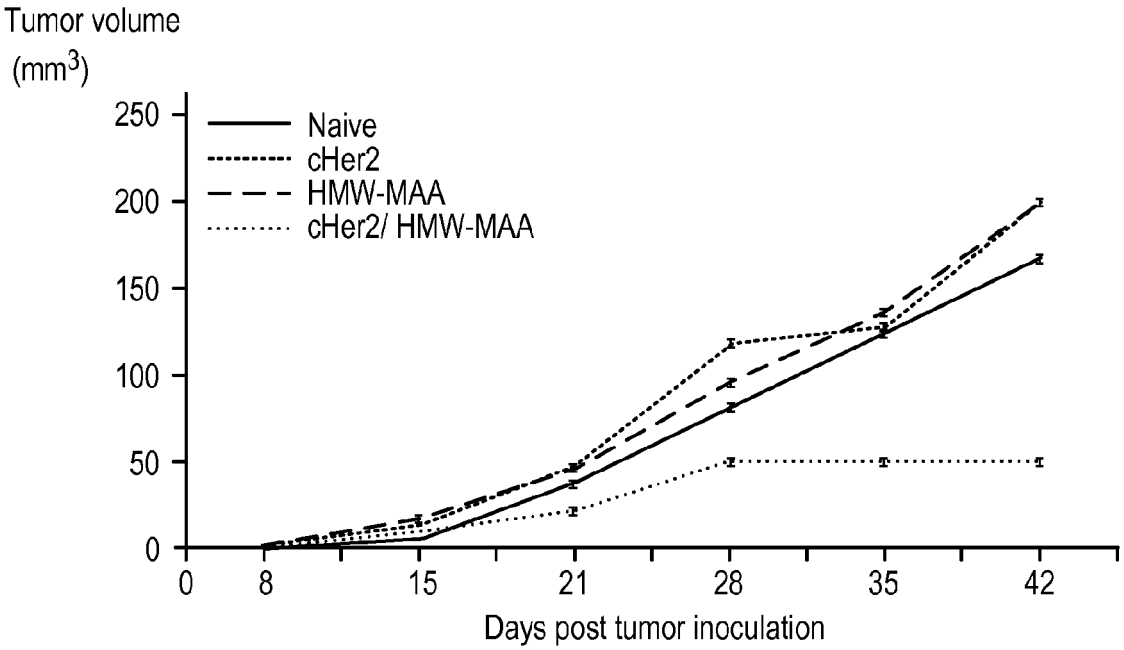

Tumor volume
(mm3)
250
200
150
100
50
0
Naive
cHer2
HMW-MAA
cHer2/ HMW-MAA
0
8
15
21
28
35
42
Days post tumor inoculation

Figure 75

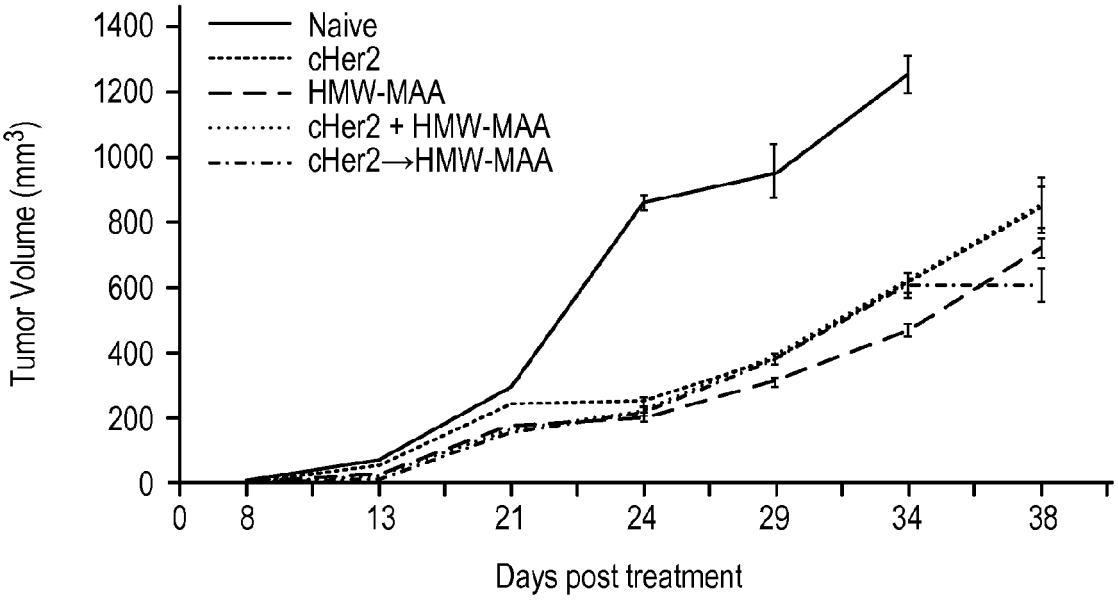

Naive
cHer2
HMW-MAA
cHer2 + HMW-MAA
cHer2→HMW-MAA
Tumor Volume (mm3)
1400
1200
1000
800
600
400
200
0
0
8
13
21
24
29
34
38
Days post treatment

Figure 76