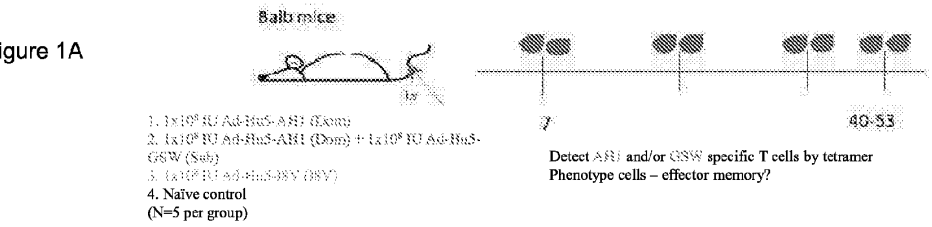
Figure 1A


Balb mice
1. 1x10⁸ IU Ad-Hu5-AH1 (Dom)
2. 1x10⁸ IU Ad-Hu5-AH1 (Dom) + 1x10⁸ IU Ad-Hu5-GSW (Sub)
3. 1x10⁸ IU Ad-Hu5-IBV (IBV)
4. Naïve control
(N=5 per group)
7
40-53
Detect AH1 and/or GSW specific T cells by tetramer
Phenotype cells – effector memory?

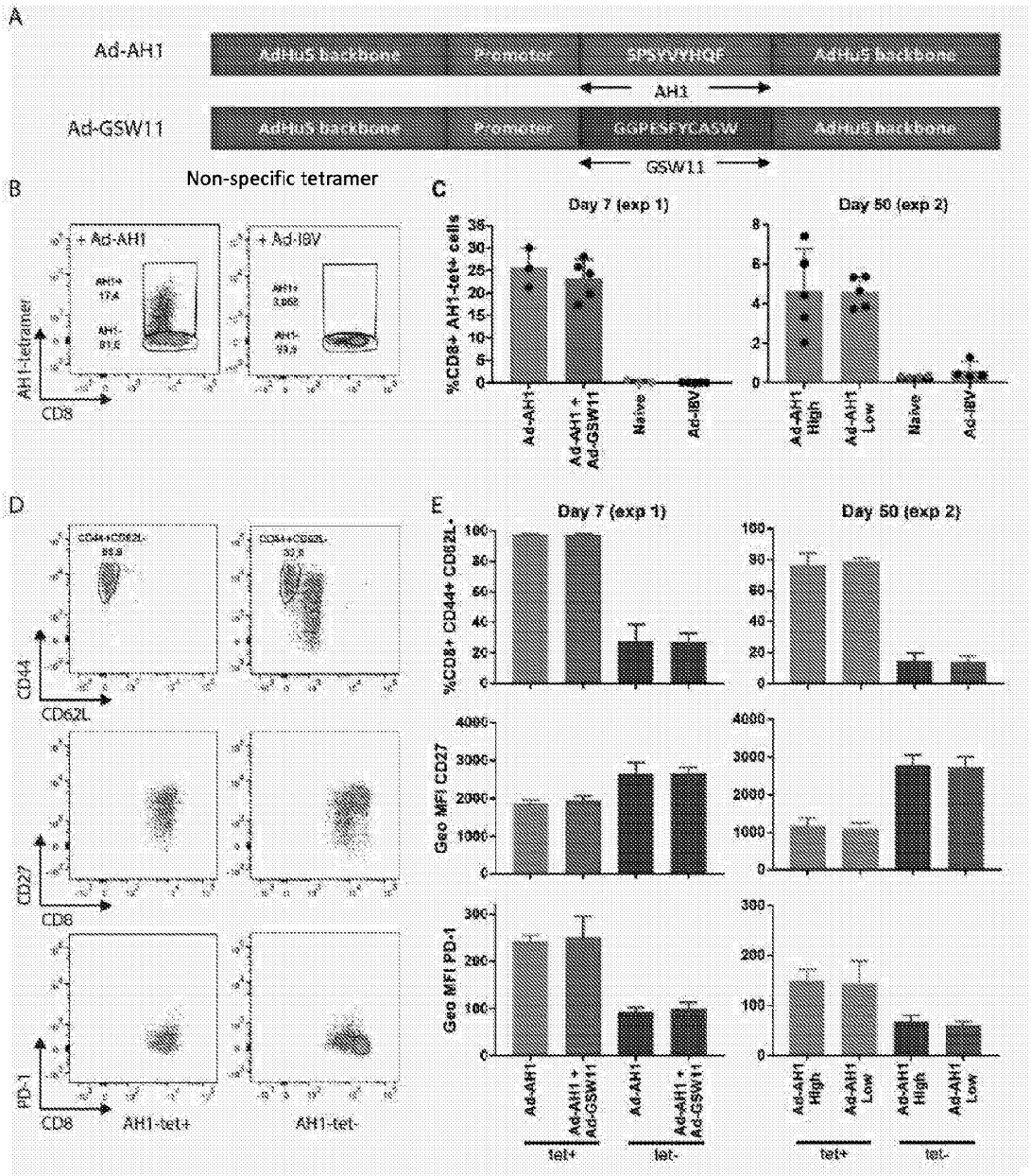
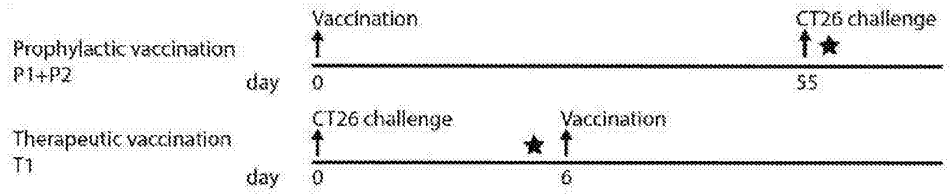
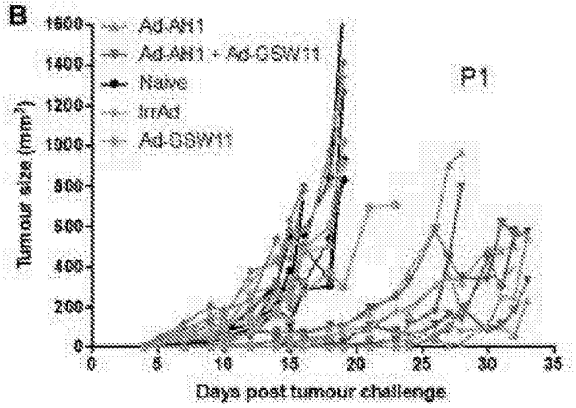

A
Ad-AH1
AdHu5 backbone
Promoter
SPSYVYHQF
AdHu5 backbone
AH1
Ad-GSW11
AdHu5 backbone
Promoter
GGPESFYEAGW
AdHu5 backbone
GSW11
B
Non-specific tetramer
+ Ad-AH1
+ Ad-IBV
AH1-tetramer
CD8
C
Day 7 (exp 1)
Day 50 (exp 2)
%CD8+ AH1-tet+ cells
Ad-AH1
Ad-AH1 + Ad-GSW11
Naive
Ad-IBV
Ad-AH1 High
Ad-AH1 Low
D
CD44
CD62L
CD27
CD6
PD-1
CD8
AH1-tet+
AH1-tet-
E
Day 7 (exp 1)
Day 50 (exp 2)
%CD8+ CD44+ CD62L-
Geo MFI CD27
Geo MFI PD-1
tet+
tet-

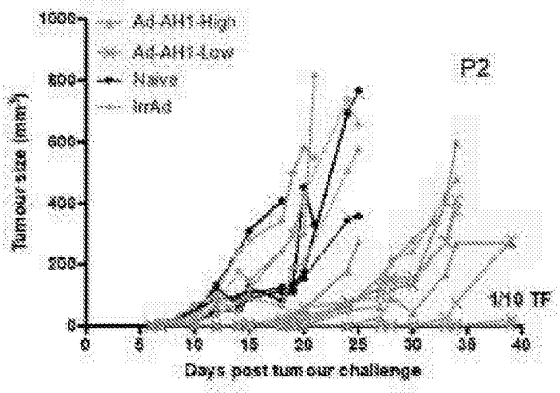
Figure 2A



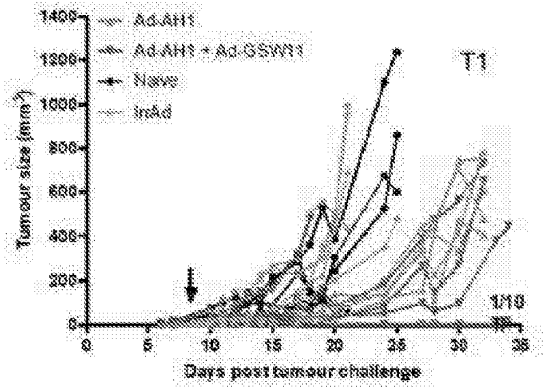
2 B



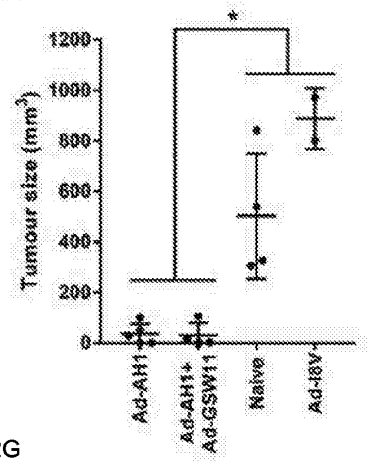
2C



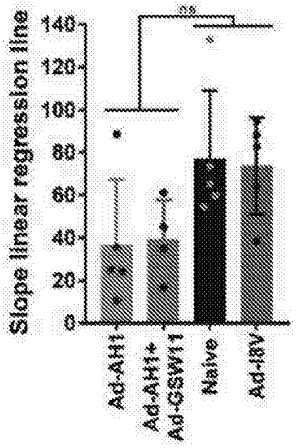
2D



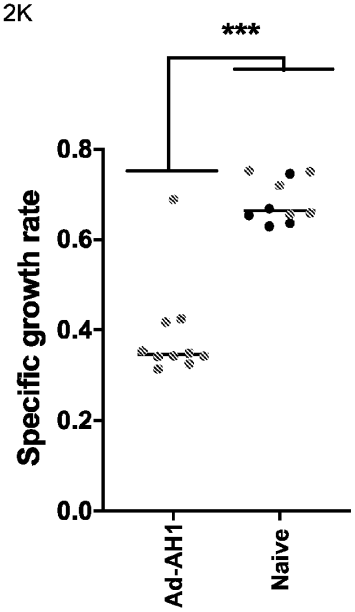
2E



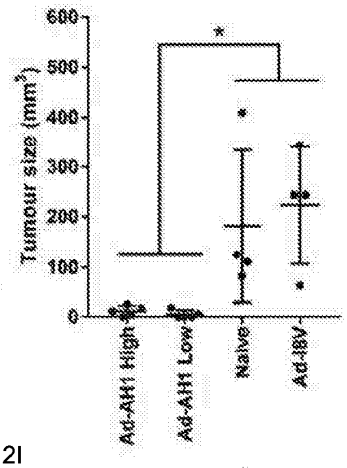
2F



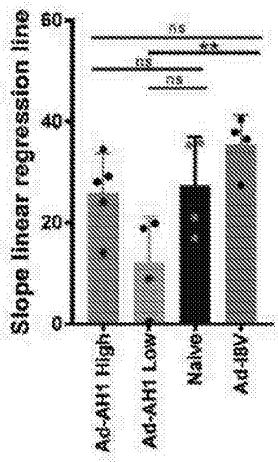
2K



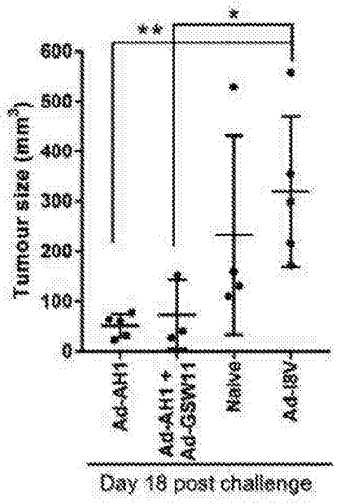
2G



2H



2I



2J

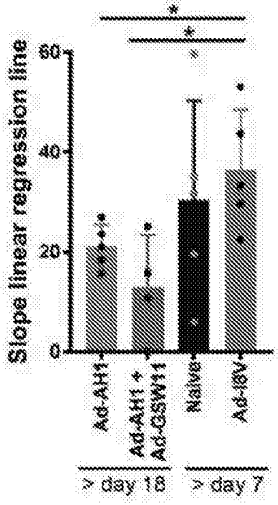
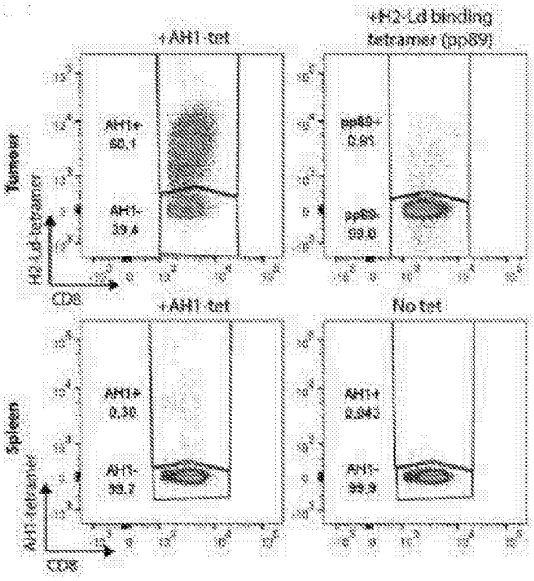
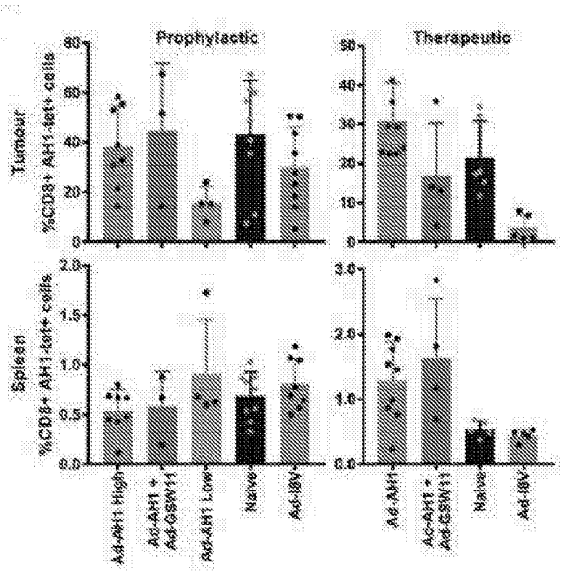


Figure 3A



3B



3C

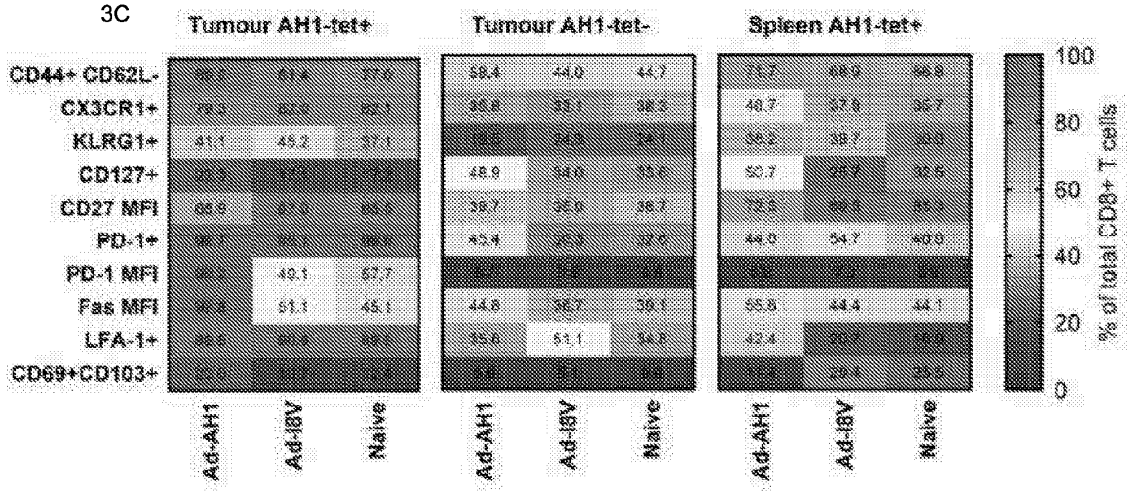
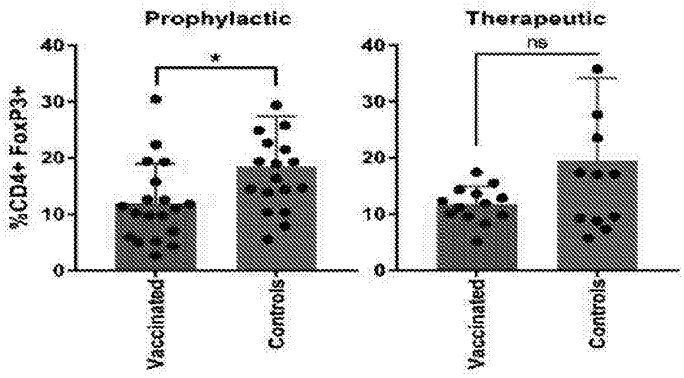


Figure 4A


Prophylactic
Therapeutic
%CD4+ FoxP3+
40
30
20
10
0
*
ns
Vaccinated
Controls

4B

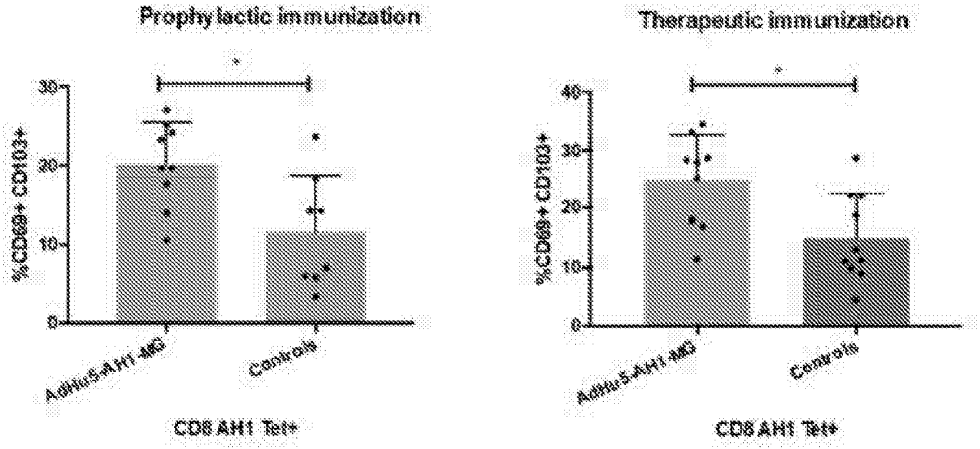
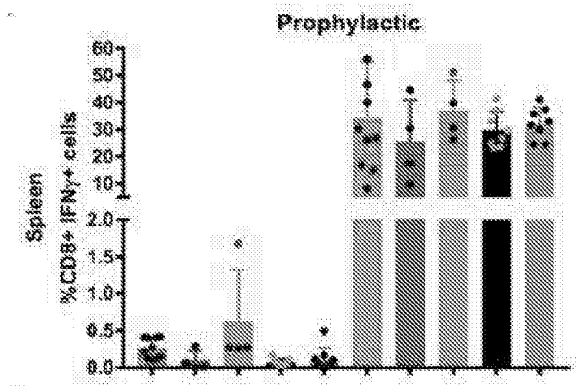
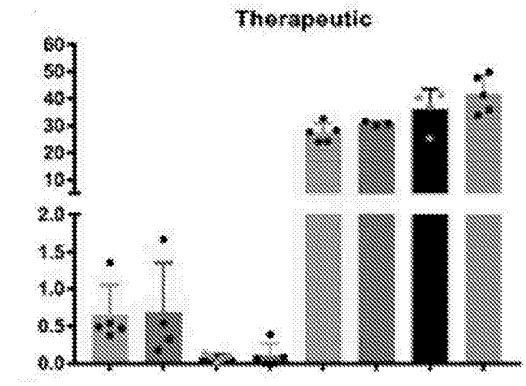

Prophylactic immunization
Therapeutic immunization
%CD69+ CD103+
30
20
10
0
40
*
AdHu5-AH1-MG
Controls
CD8 AH1 Tet+

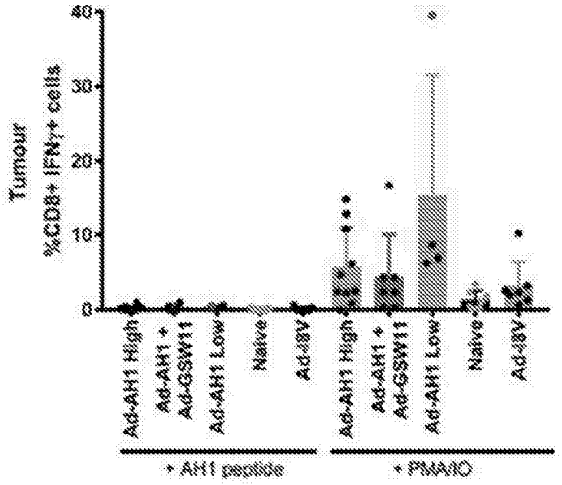
Figure 5A



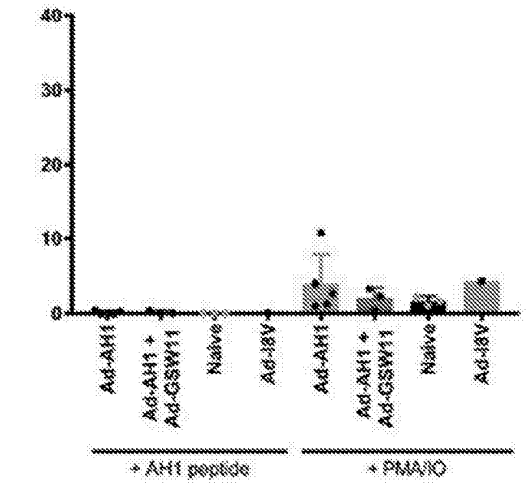
5B



5C

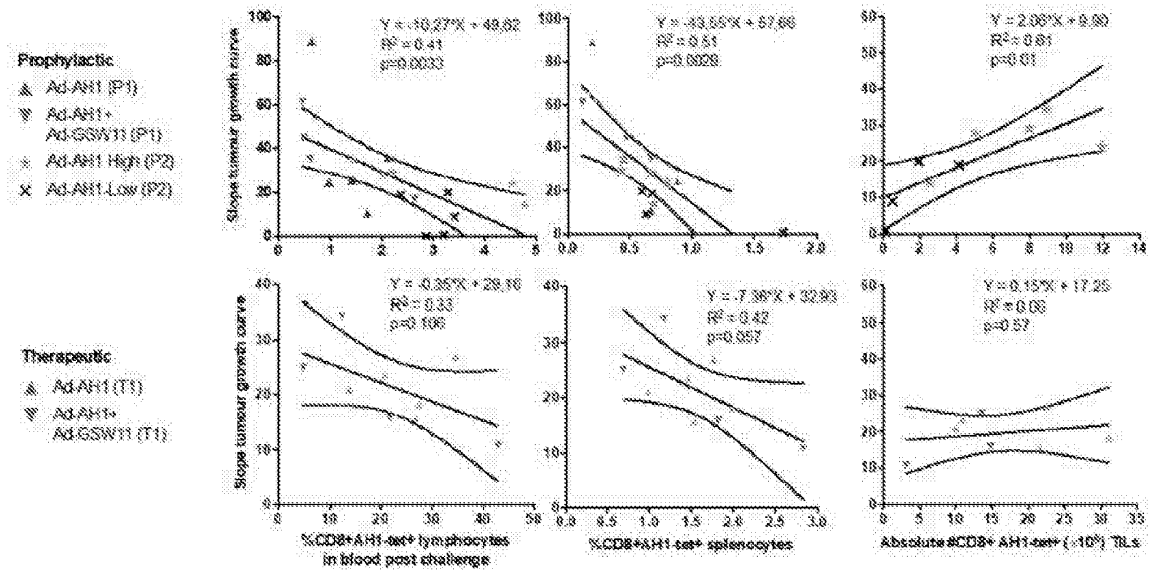


5D

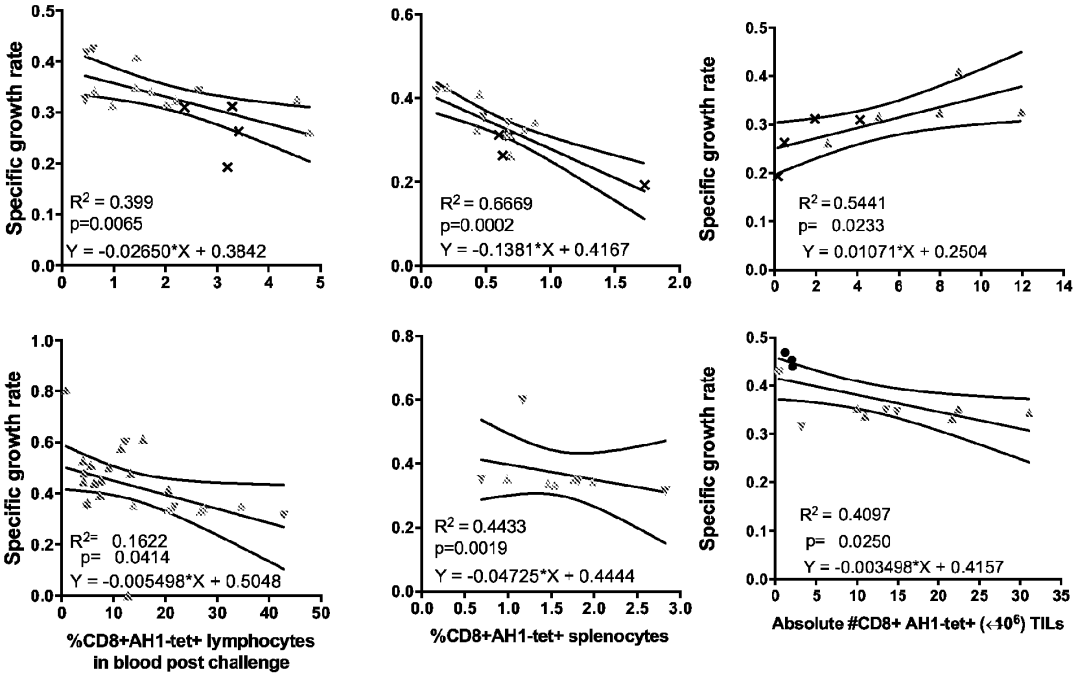


SUBSTITUTE SHEET (RULE 26)

Figure 6A

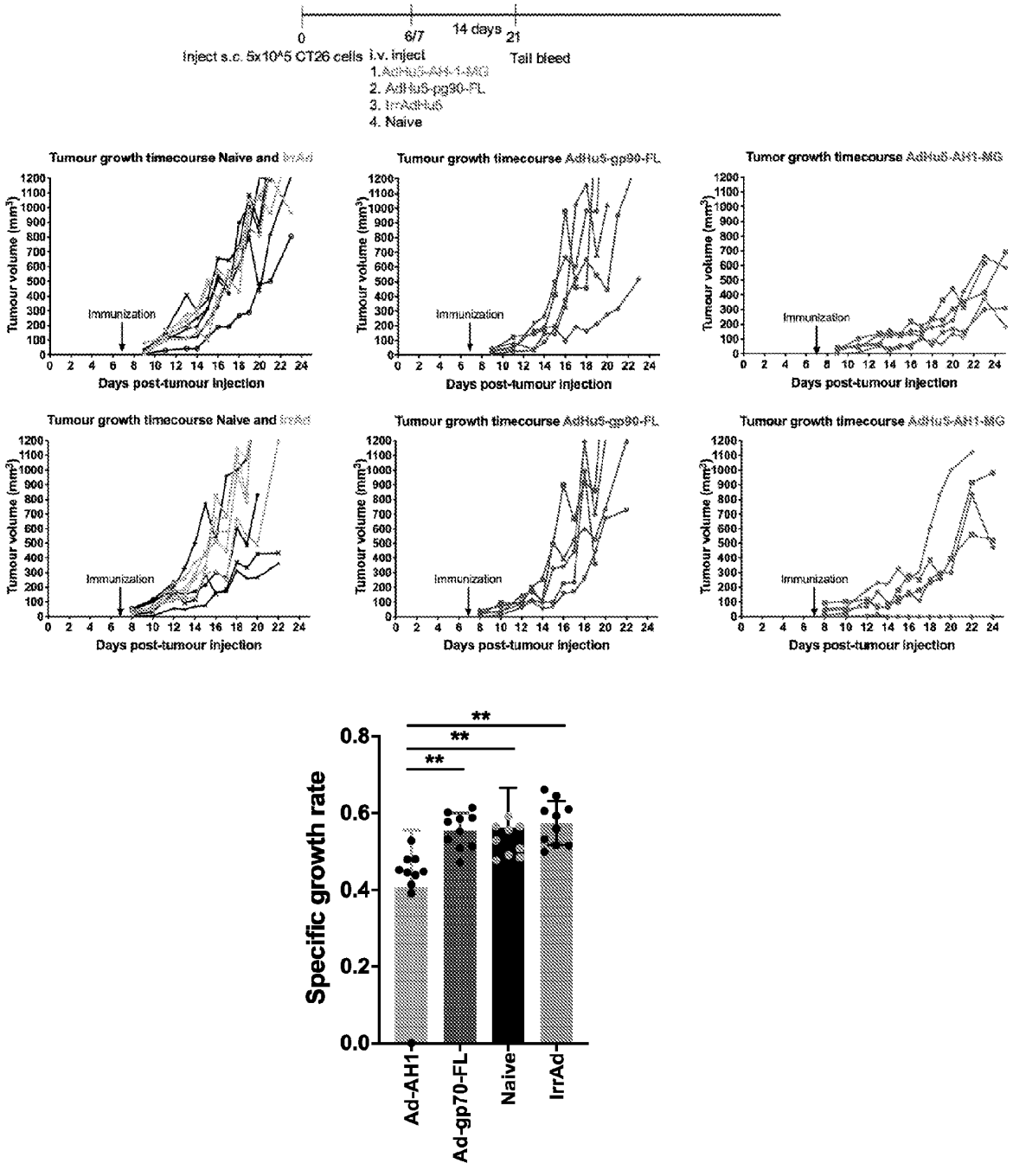


6B



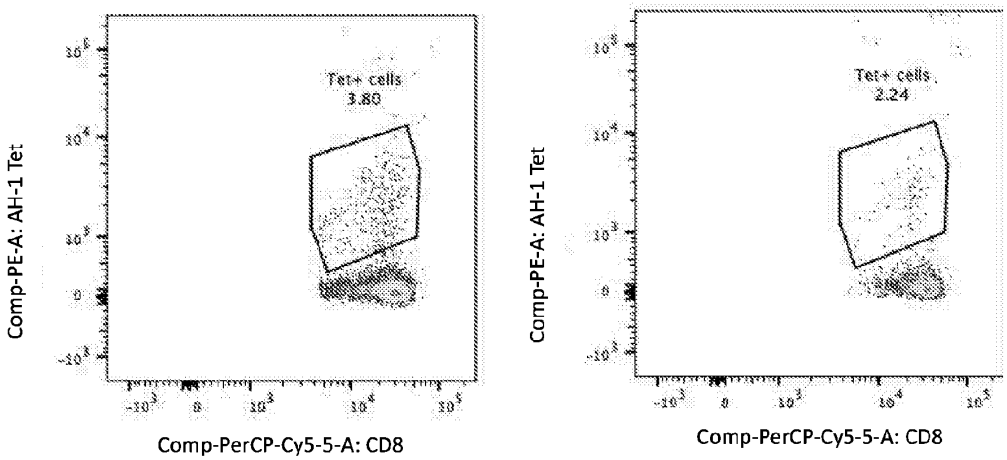
SUBSTITUTE SHEET (RULE 26)

Figure 7A



SUBSTITUTE SHEET (RULE 26)

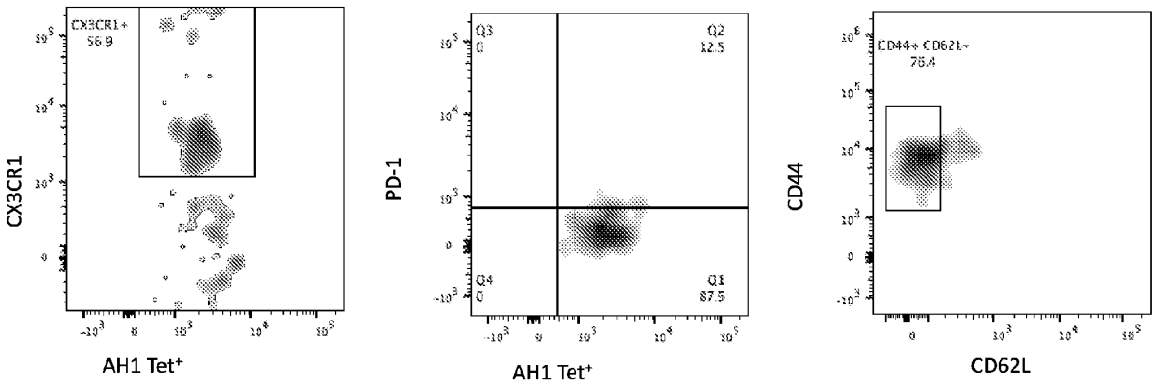
Figure 7B



Long term protected_1L 1R.fcs
CD8+
8706

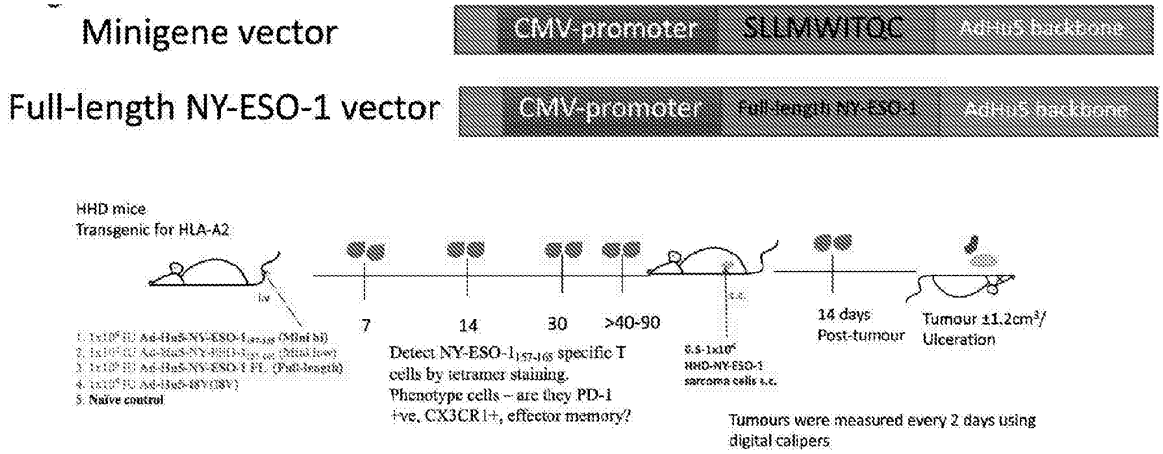
Long term protected_2R 1L.fcs
CD8+
3209

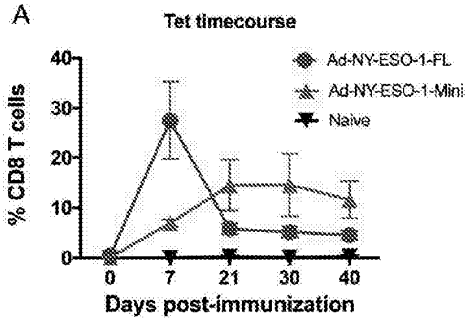
Phenotype of the antigen-specific cells in long-term protected mice



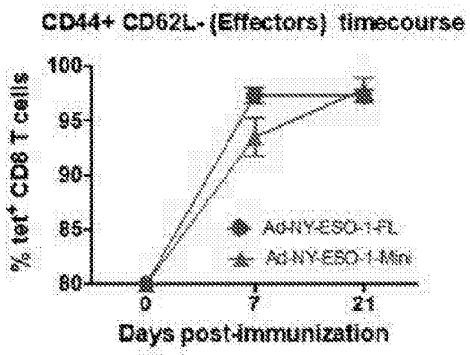
SUBSTITUTE SHEET (RULE 26)

Figure 8A

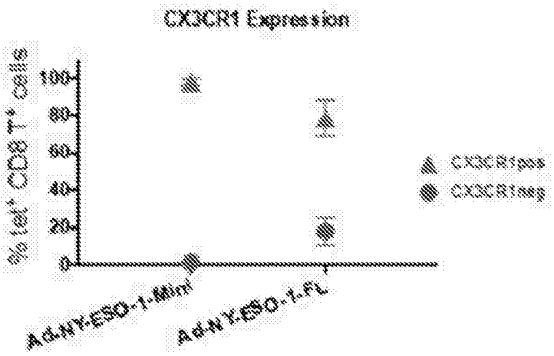




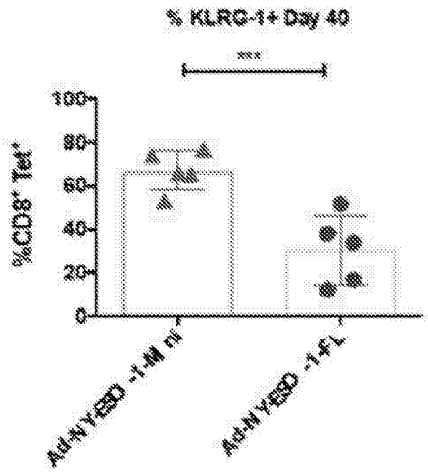
8B



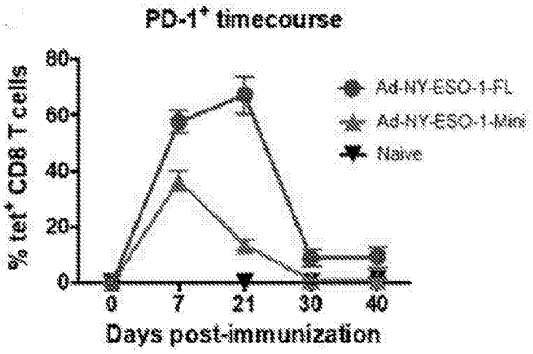
8C



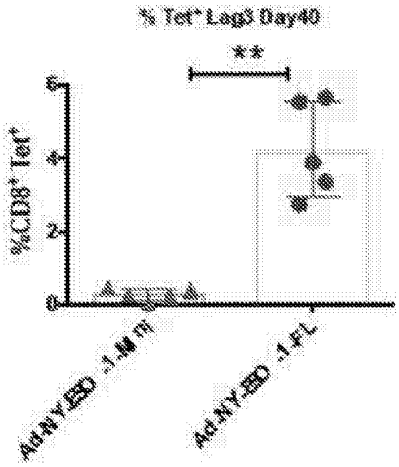
8D


% KLRG-1+ Day 40
***
%CD8+ Tet+
100
80
60
40
20
0
Ad-NY-ESO -1-Mini
Ad-NY-ESO -1-FL

8E


PD-1+ timecourse
% tet+ CD8 T cells
80
60
40
20
0
0
7
21
30
40
Days post-immunization
Ad-NY-ESO-1-FL
Ad-NY-ESO-1-Mini
Naive

8F


% Tet+ Lag3 Day40
**
%CD8+ Tet+
6
4
2
0
Ad-NY-ESO -1-Mini
Ad-NY-ESO -1-FL

8G

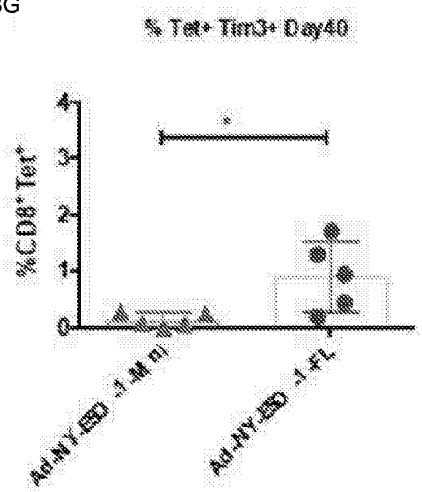
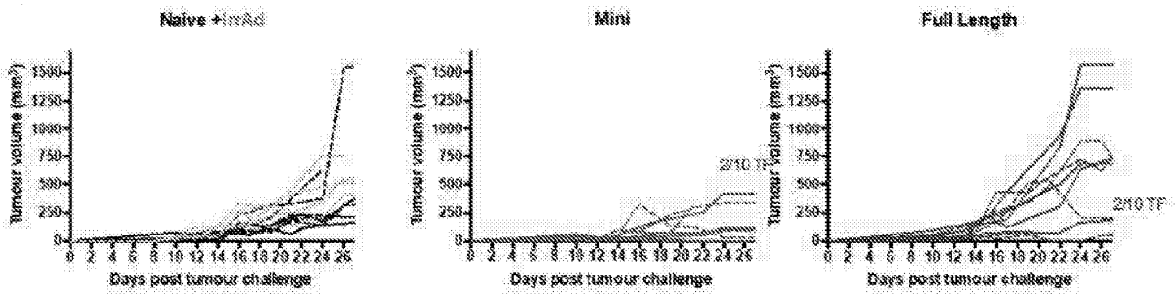
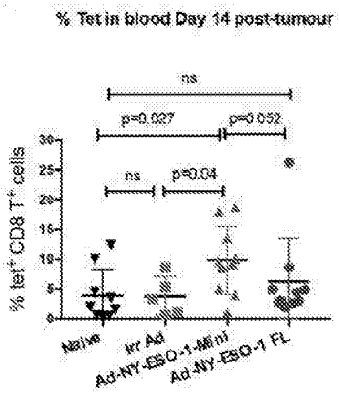

% Tet+ Tim3+ Day40
*
%CD8+ Tet+
4
3
2
1
0
Ad-NY-ESO -1-Mini
Ad-NY-ESO -1-FL

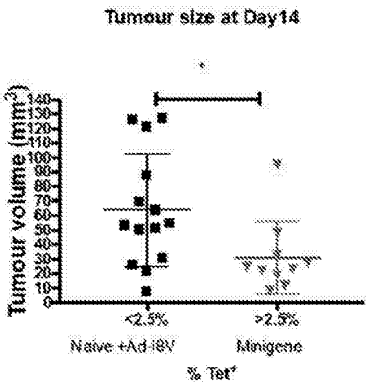
Figure 9A



9B



9C



9D

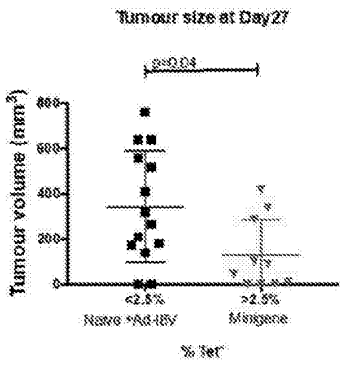
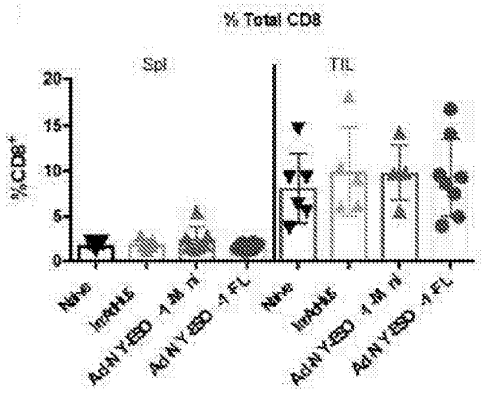
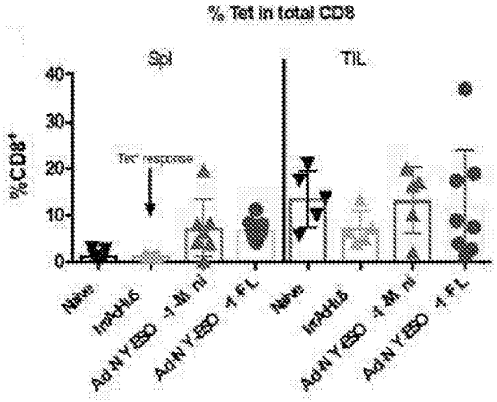


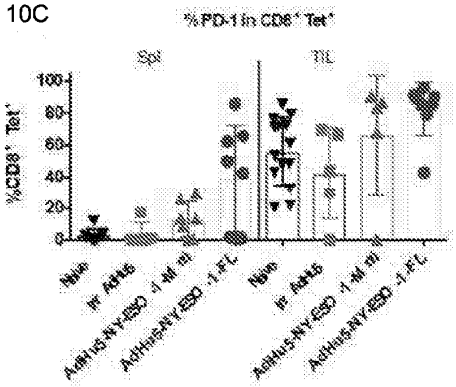
Figure 10A



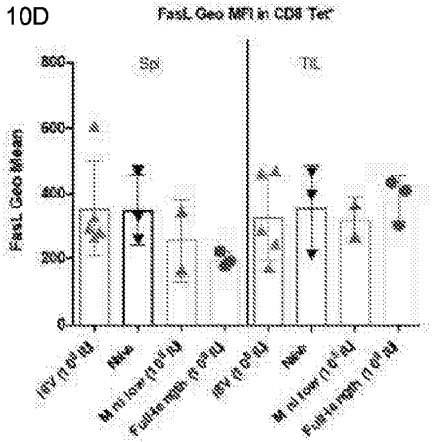
10B



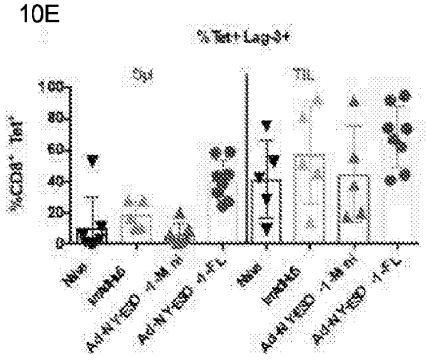
10C



10D



10E



10F

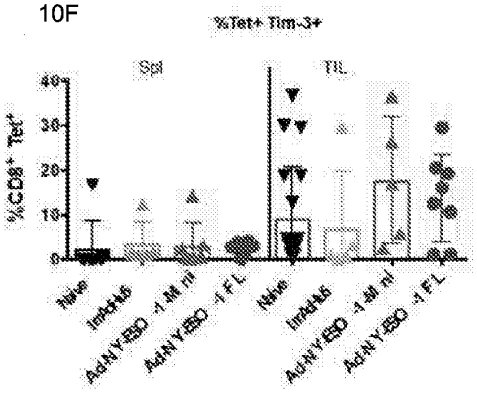
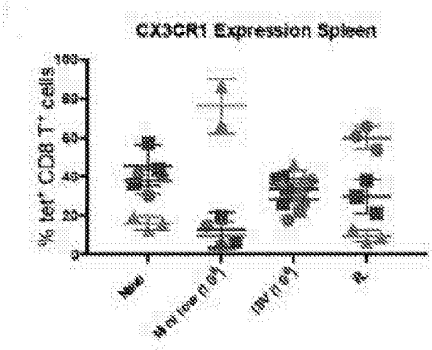
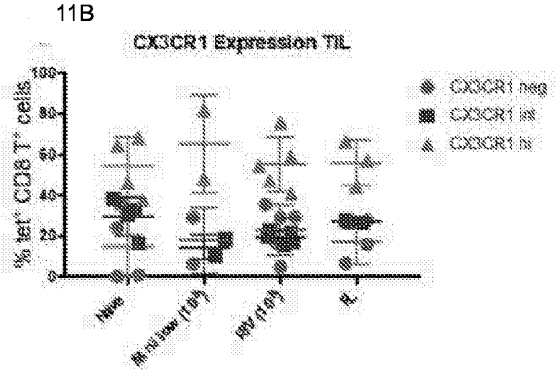


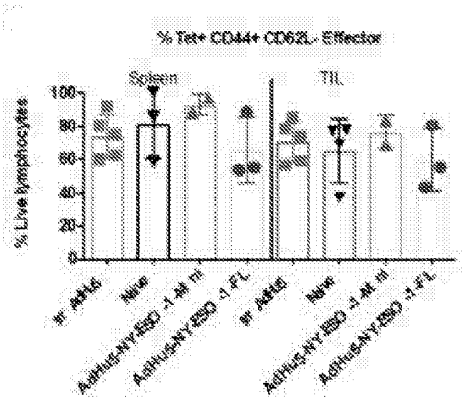
Figure 11A



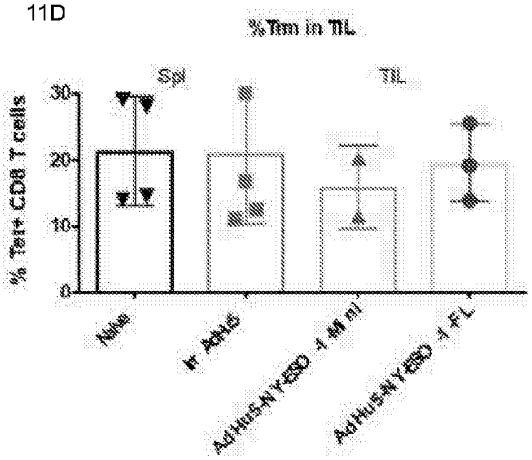
11B



11C



11D



11E

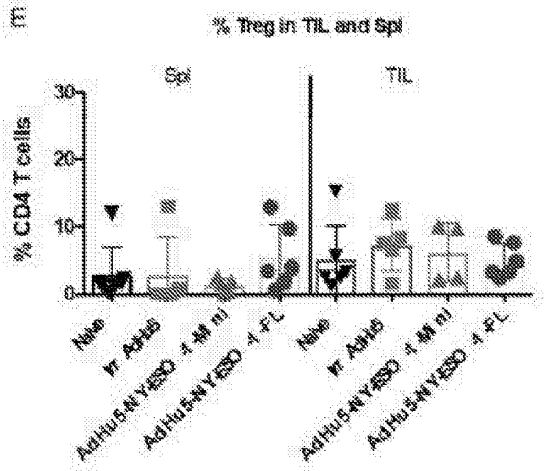
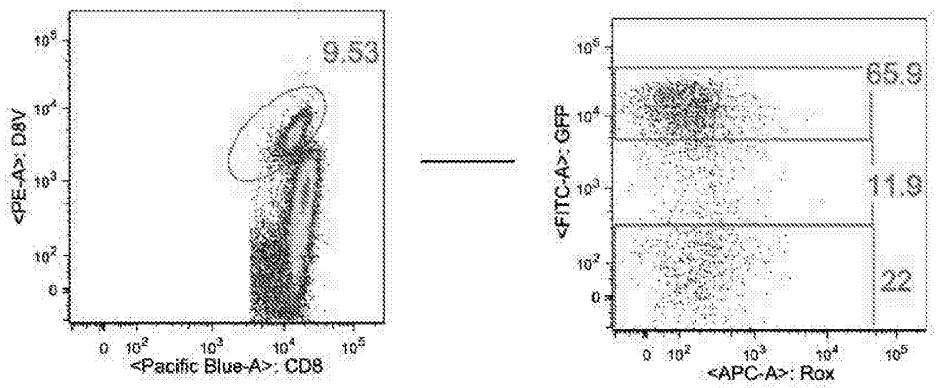
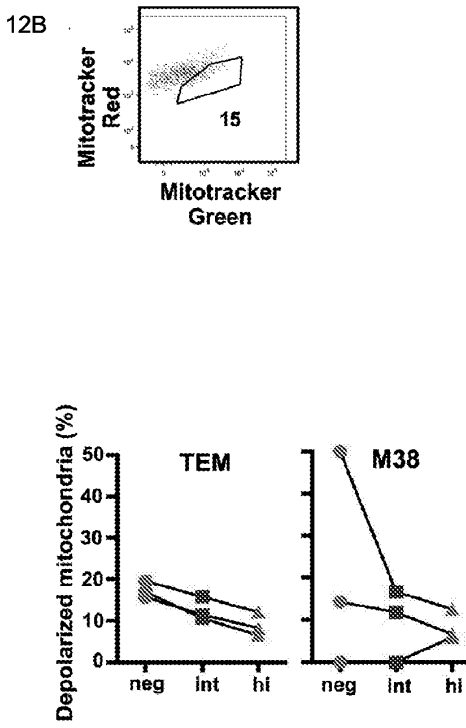


Figure 12A



12B



12C

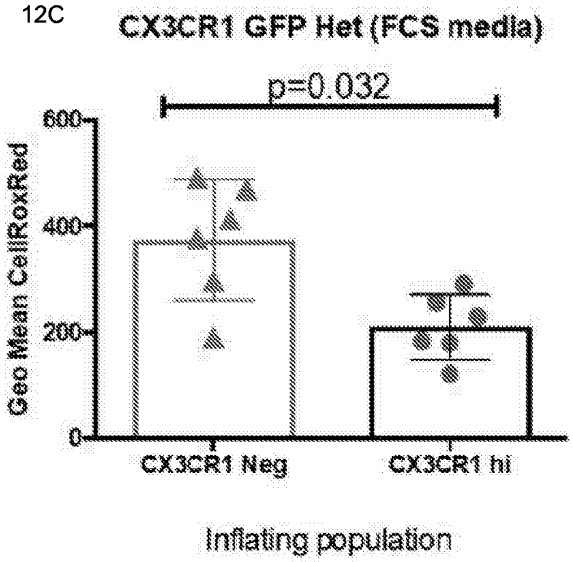
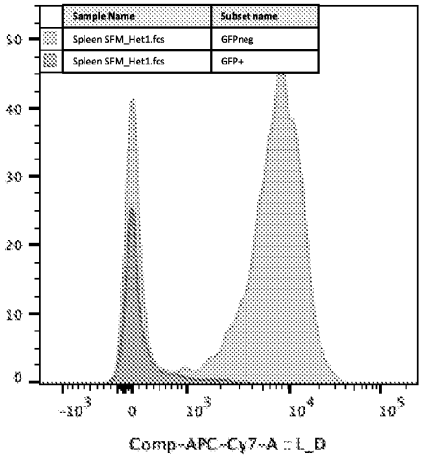
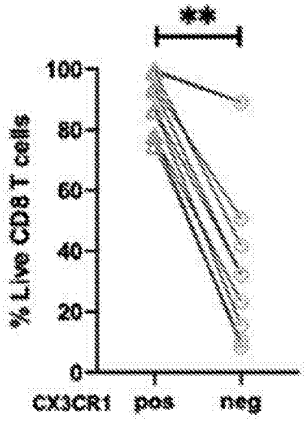
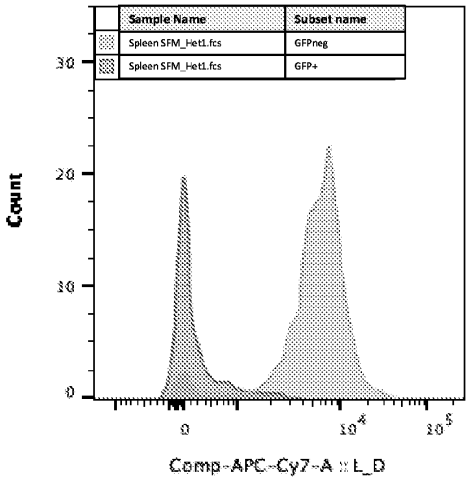


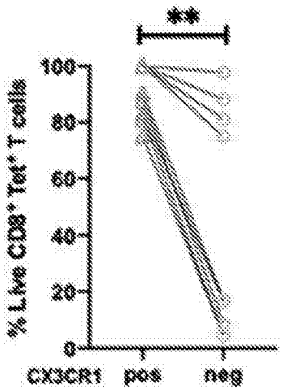
Figure 12D



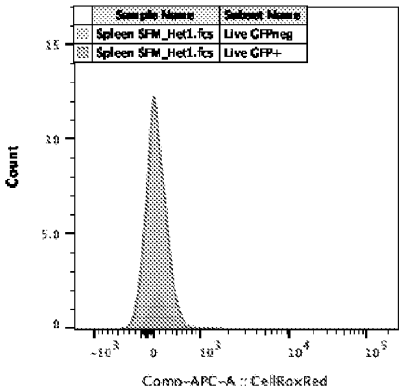


12E





12F



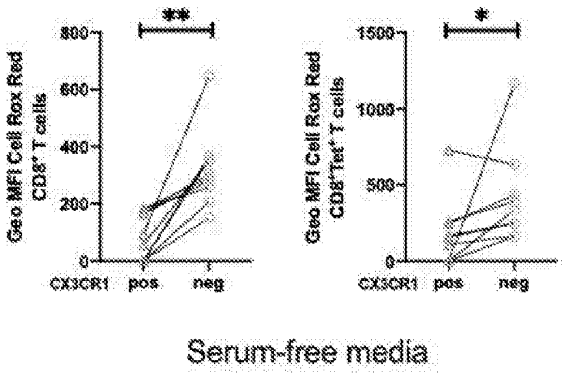


Figure 13

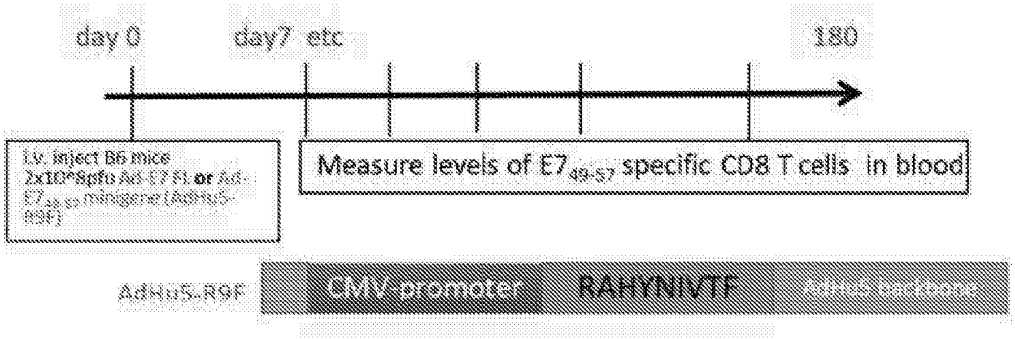
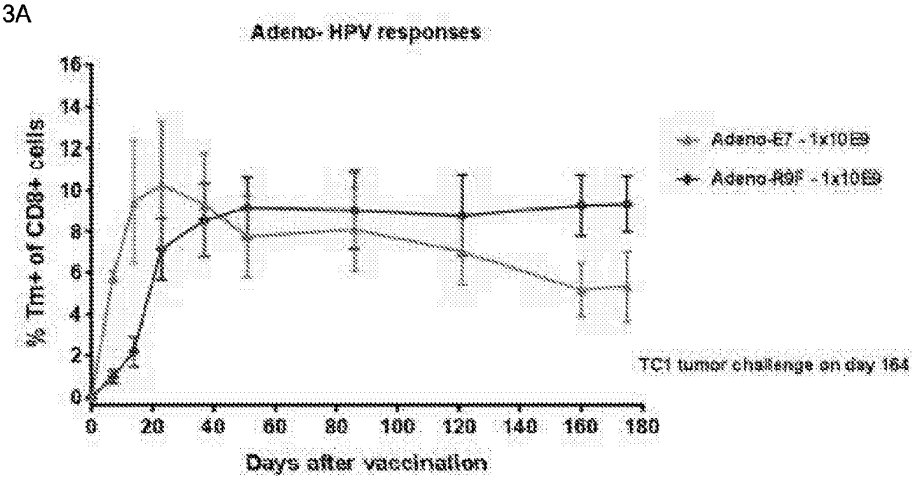


Figure 13A



- Large, durable population of epitope-specific cells
- Challenge with TC1-HPV E6/E7 (cervical carcinoma model) s.c at day >180 post-immunization

Figure 13B

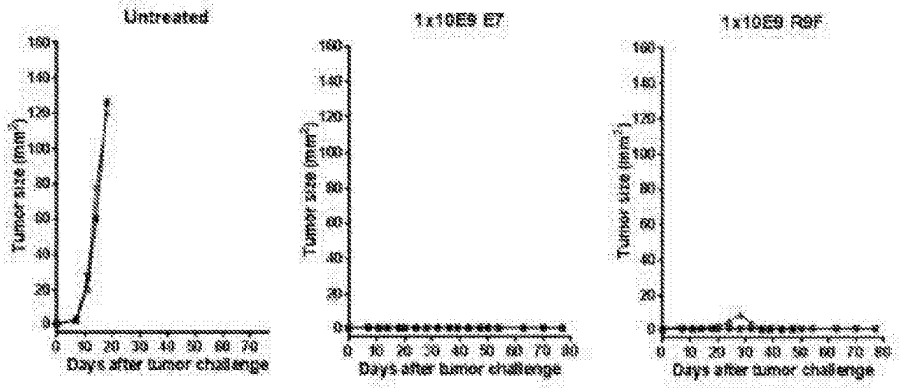


Figure 14

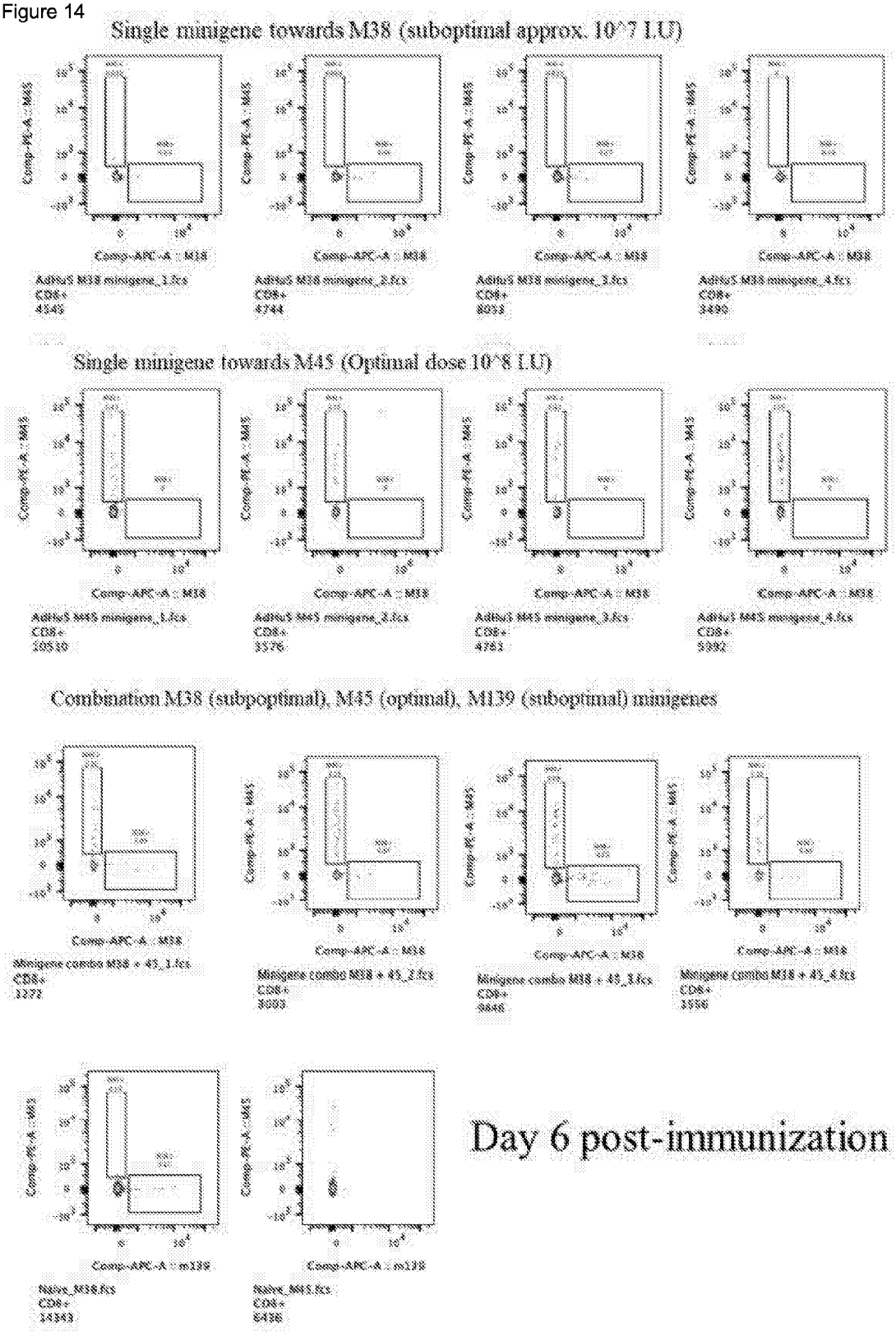
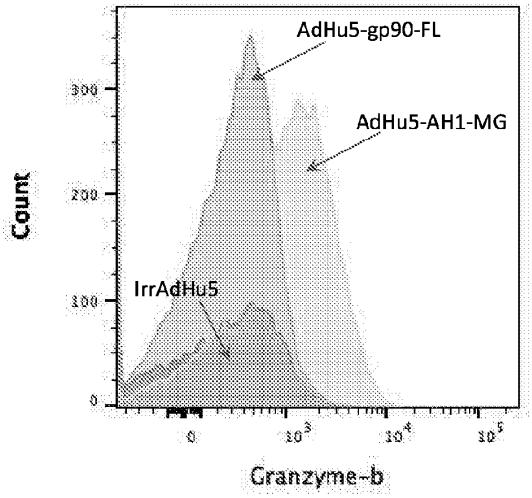

Single minigene towards M38 (suboptimal approx. 10^7 I.U)
Comp-PE-A :: M45
Comp-APC-A :: M38
AdHu5 M38 minigene_1.fcs
CD8+
4145
AdHu5 M38 minigene_2.fcs
CD8+
4744
AdHu5 M38 minigene_3.fcs
CD8+
8053
AdHu5 M38 minigene_4.fcs
CD8+
3490
Single minigene towards M45 (Optimal dose 10^8 I.U)
AdHu5 M45 minigene_1.fcs
CD8+
10510
AdHu5 M45 minigene_2.fcs
CD8+
3576
AdHu5 M45 minigene_3.fcs
CD8+
4781
AdHu5 M45 minigene_4.fcs
CD8+
5382
Combination M38 (subpoptimal), M45 (optimal), M139 (suboptimal) minigenes
Minigene combo M38 + 45_1.fcs
CD8+
3272
Minigene combo M38 + 45_2.fcs
CD8+
8003
Minigene combo M38 + 45_3.fcs
CD8+
9848
Minigene combo M38 + 45_4.fcs
CD8+
3550
Comp-APC-A :: m139
Naive_M38.fcs
CD8+
14343
Naive_M45.fcs
CD8+
8438
Day 6 post-immunization

Figure 15A



15B

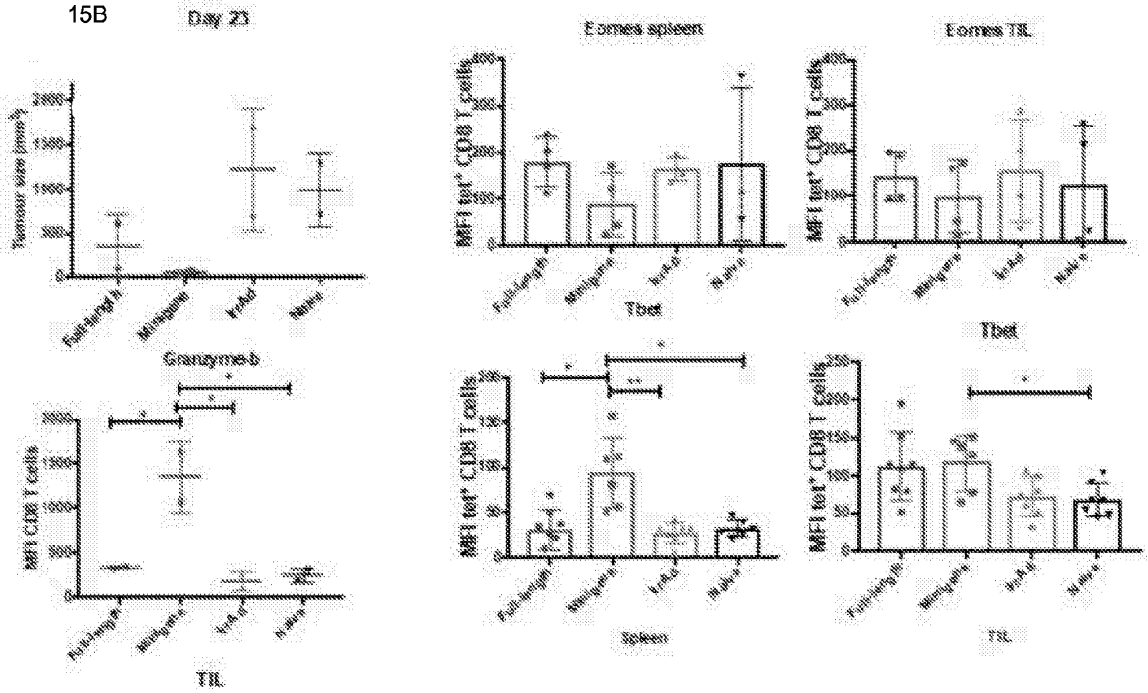
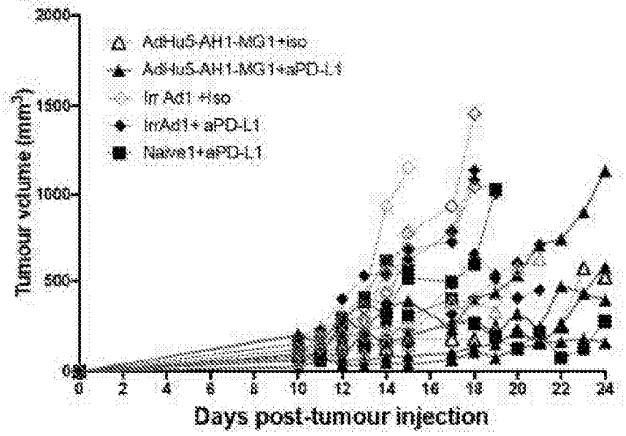
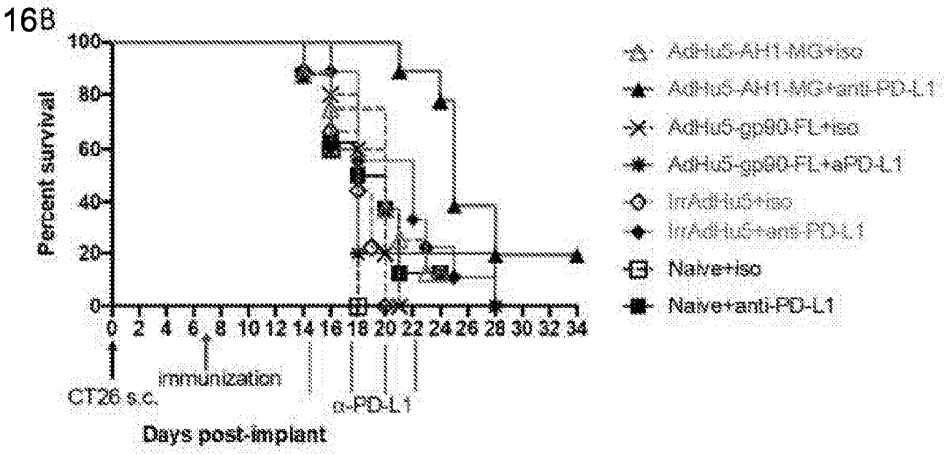


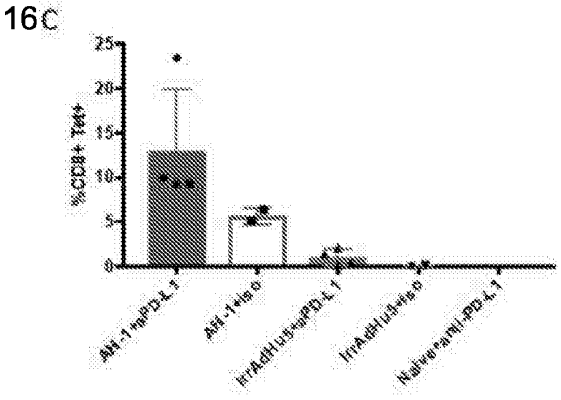
Figure 16A



16B



16C



16D

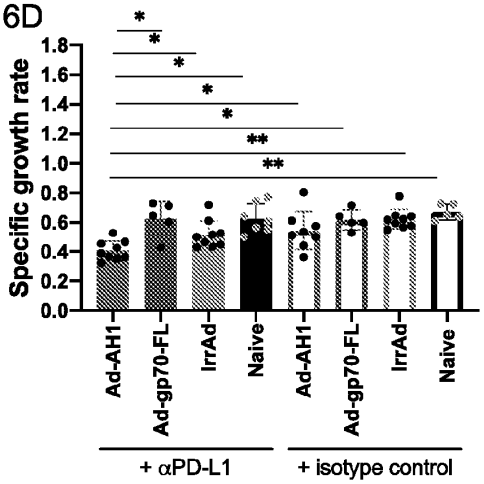
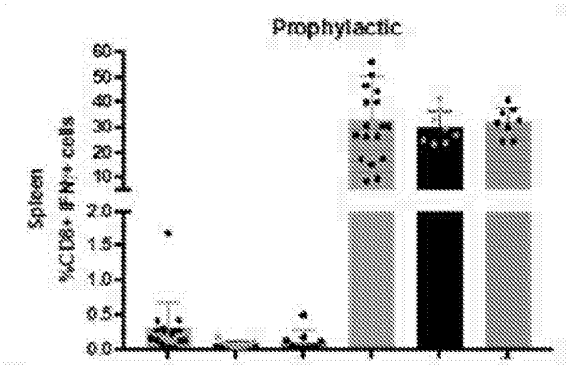
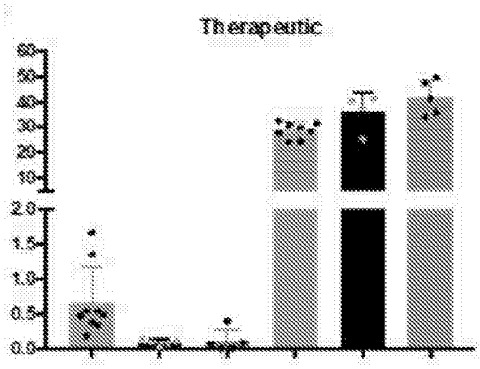


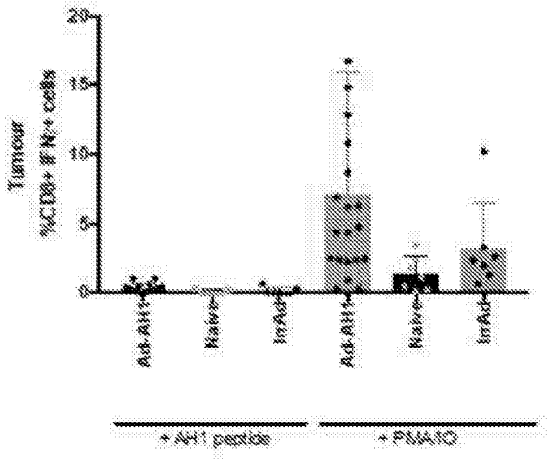
Figure 17A



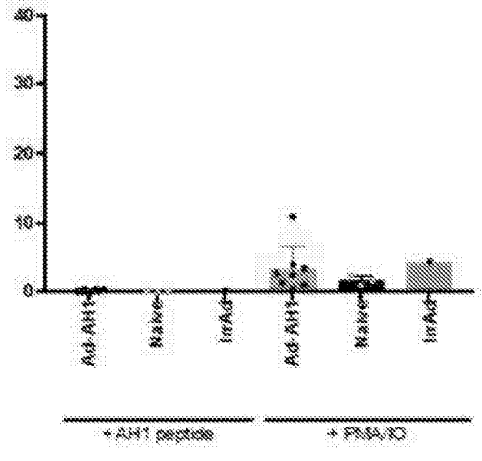
17B



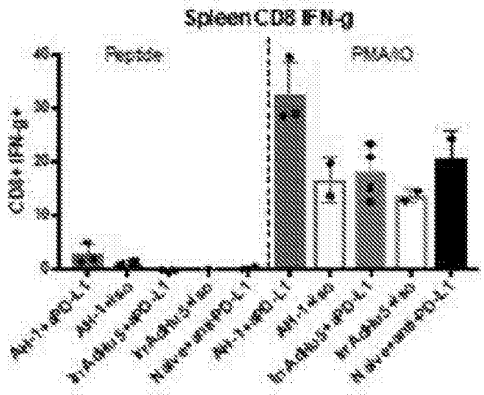
17C



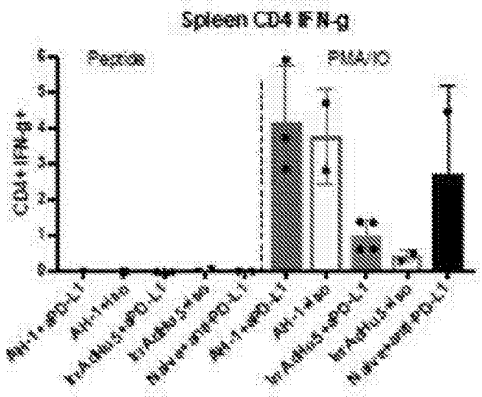
17D



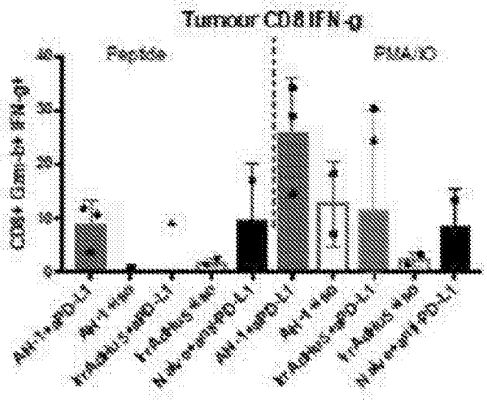
17E



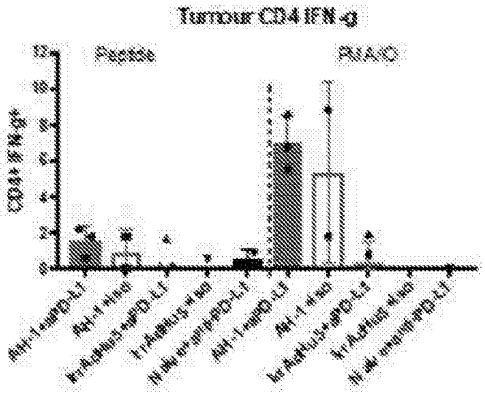
17F



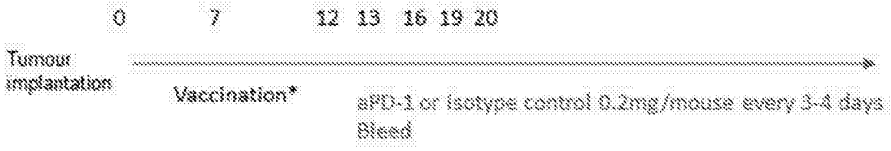
17G



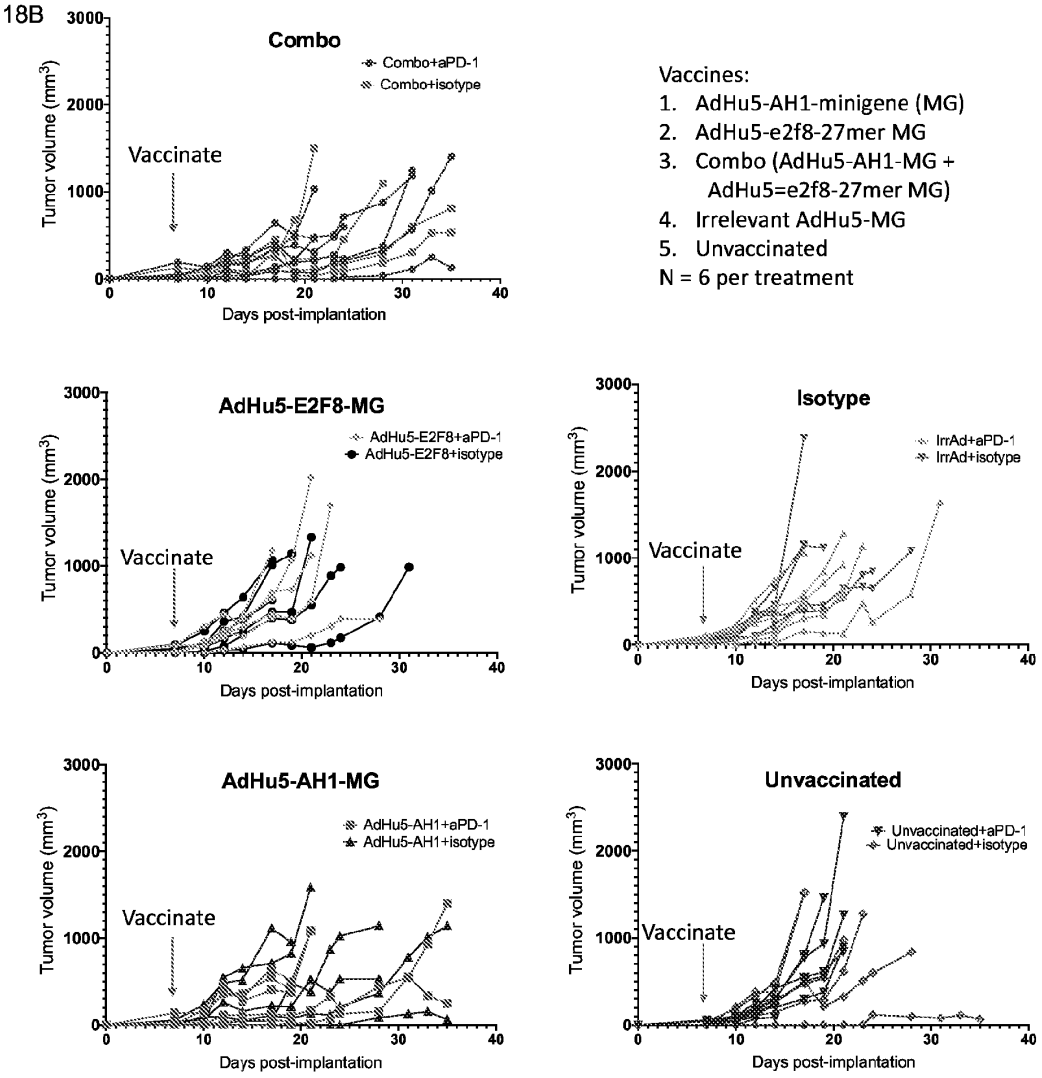
17H



18A



18B

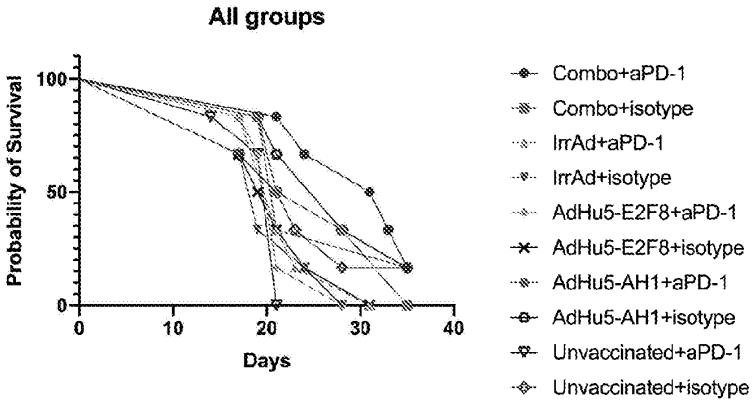


Vaccines:

1. AdHu5-AH1-minigene (MG)
2. AdHu5-e2f8-27mer MG
3. Combo (AdHu5-AH1-MG + AdHu5=e2f8-27mer MG)
4. Irrelevant AdHu5-MG
5. Unvaccinated

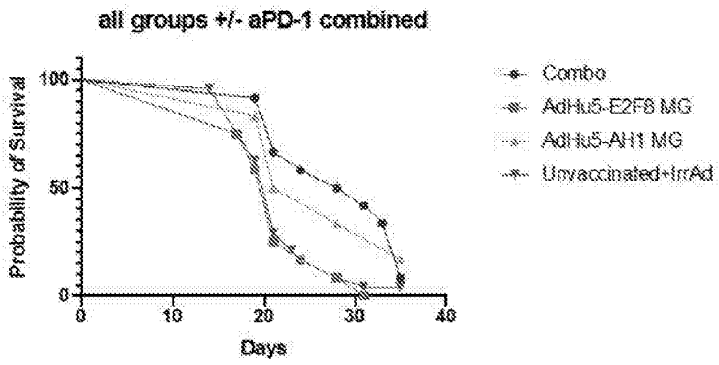
N = 6 per treatment

Figure 19A



	Median survival
Combo+aPD-1	32
Combo+isotype	24.5
IrrAd+aPD-1	21
IrrAd+isotype	19
AdHu5-E2F8+aPD-1	21
AdHu5-E2F8+isotype	20
AdHu5-AH1+aPD-1	21
AdHu5-AH1+isotype	28
Unvaccinated+aPD-1	21
Unvaccinated+isotype	22

19B



	Combo	AdHu5-E2F8 MG	AdHu5-AH1 MG	Unvaccinated+IrrAd
Median survival	29.5	21	24.5	21

Figure 20

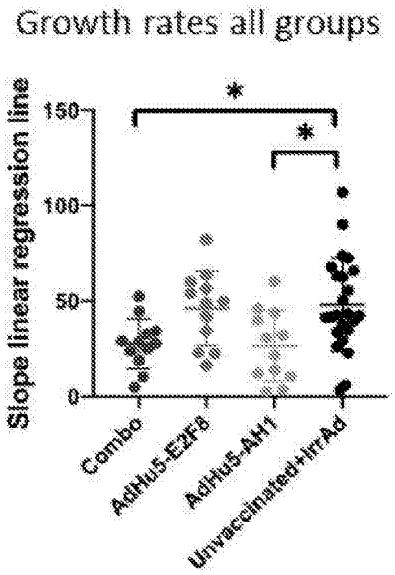


Figure 21

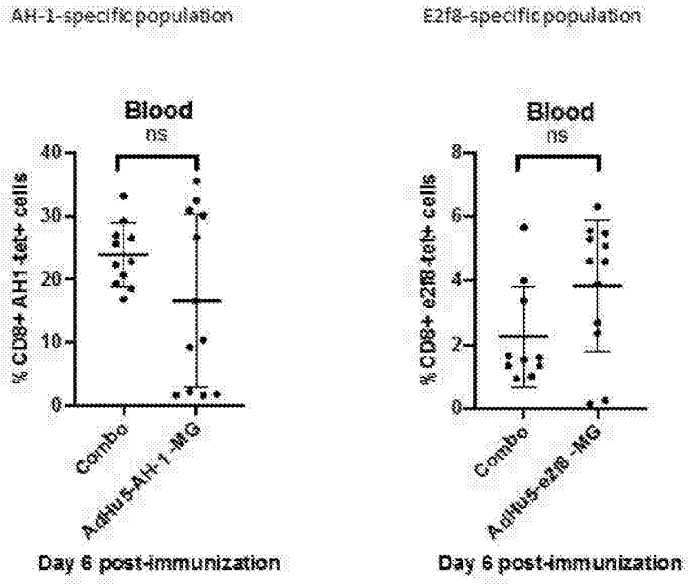


Figure 22

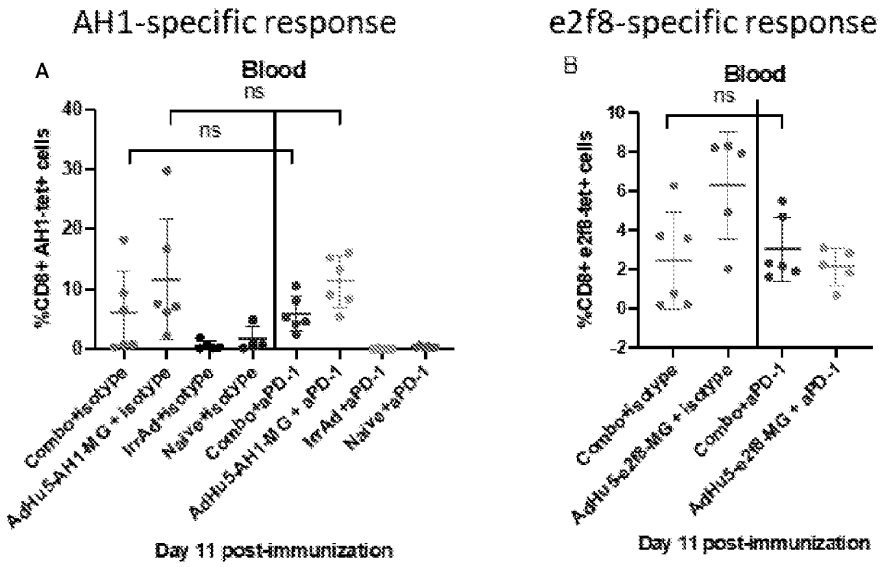

AH1-specific response
A
Blood
ns
ns
%CD8+ AH1-tet+ cells
40
30
20
10
0
Combo+Isotype
AdHu5-AH1-MG + Isotype
IrrAd+Isotype
Naïve+Isotype
Combo+aPD-1
AdHu5-AH1-MG + aPD-1
IrrAd +aPD-1
Naïve+aPD-1
Day 11 post-immunization
e2f8-specific response
B
Blood
ns
%CD8+ e2f8-tet+ cells
10
8
6
4
2
0
-2
Combo+Isotype
AdHu5-e2f8-MG + Isotype
Combo+aPD-1
AdHu5-e2f8-MG + aPD-1
Day 11 post-immunization

Figure 22

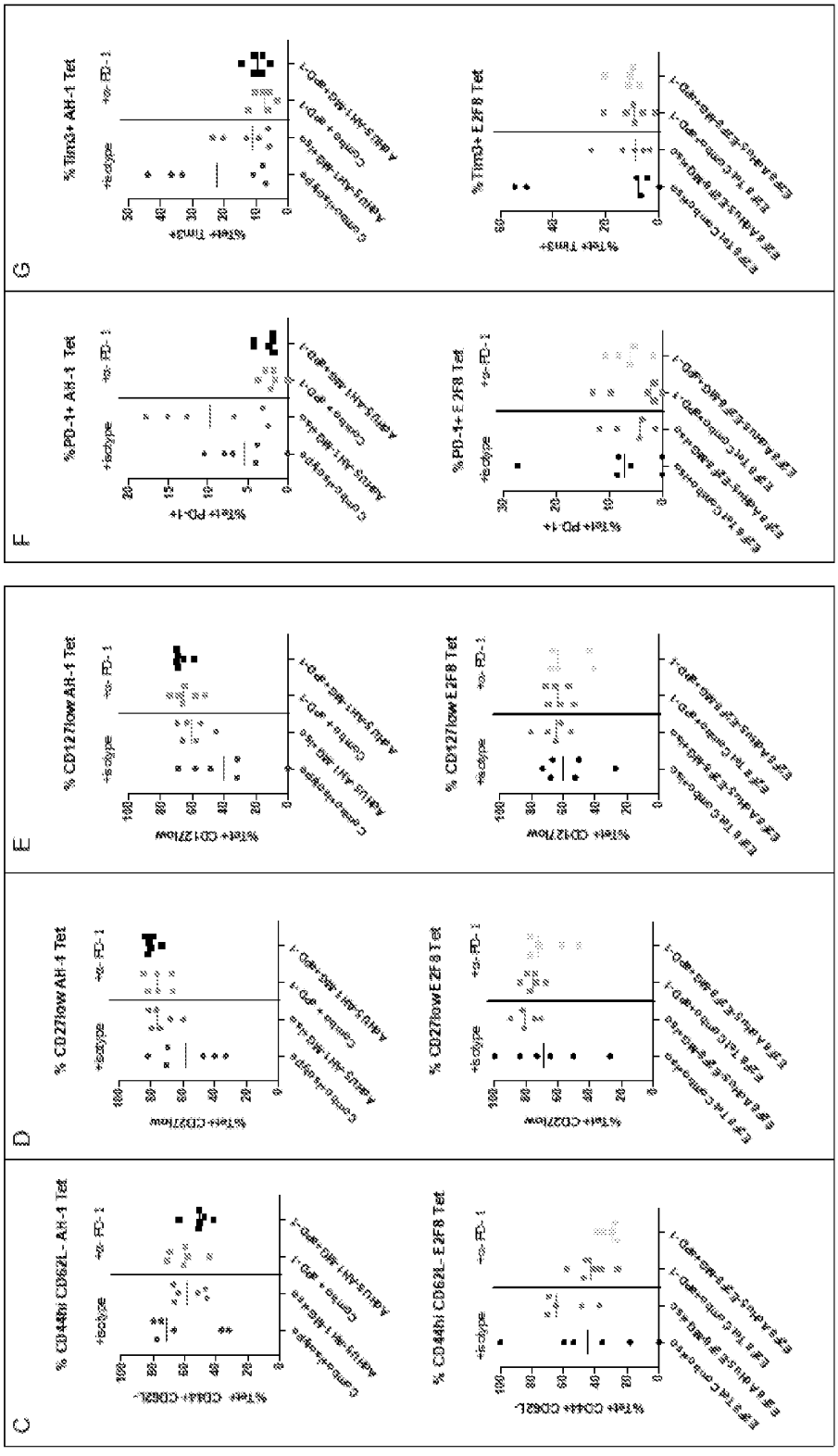

C
% CD44hi CD62L- AH-1 Tet
D
% CD27low AH-1 Tet
E
% CD127low AH-1 Tet
F
% PD-1+ AH-1 Tet
G
% Tim3+ AH-1 Tet
% CD44hi CD62L- E2F8 Tet
% CD27low E2F8 Tet
% CD127low E2F8 Tet
% PD-1+ E2F8 Tet
% Tim3+ E2F8 Tet

Figure 23

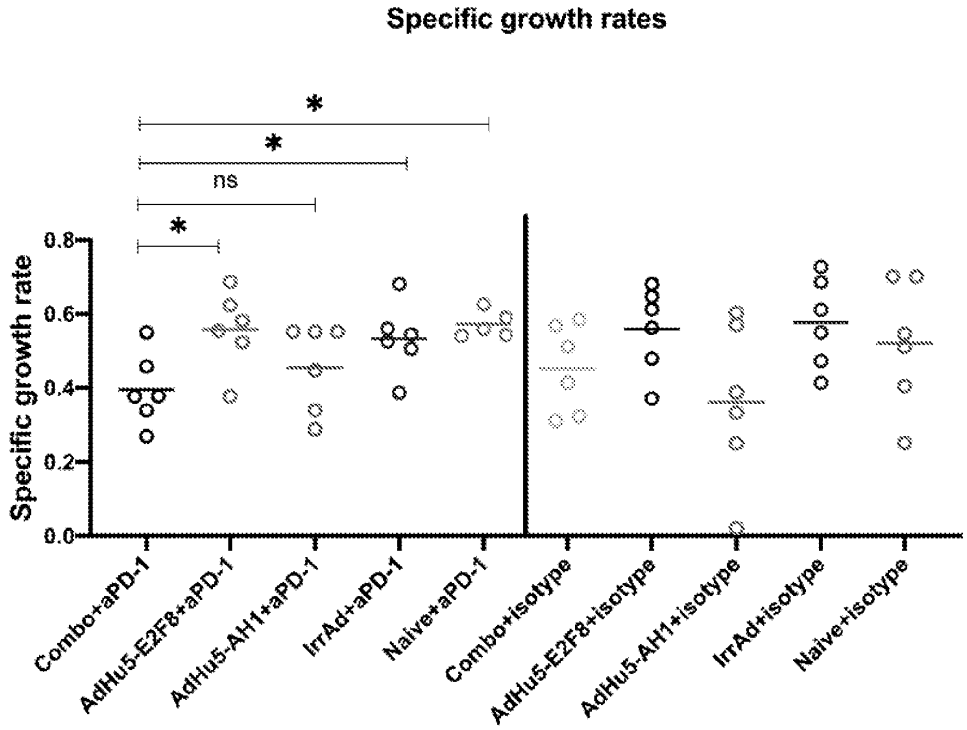

Specific growth rates
Specific growth rate
0.0
0.2
0.4
0.6
0.8
*
*
ns
*
Combo+aPD-1
AdHu5-E2F8+aPD-1
AdHu5-AH1+aPD-1
IrrAd+aPD-1
Naive+aPD-1
Combo+isotype
AdHu5-E2F8+isotype
AdHu5-AH1+isotype
IrrAd+isotype
Naive+isotype

Figure 24

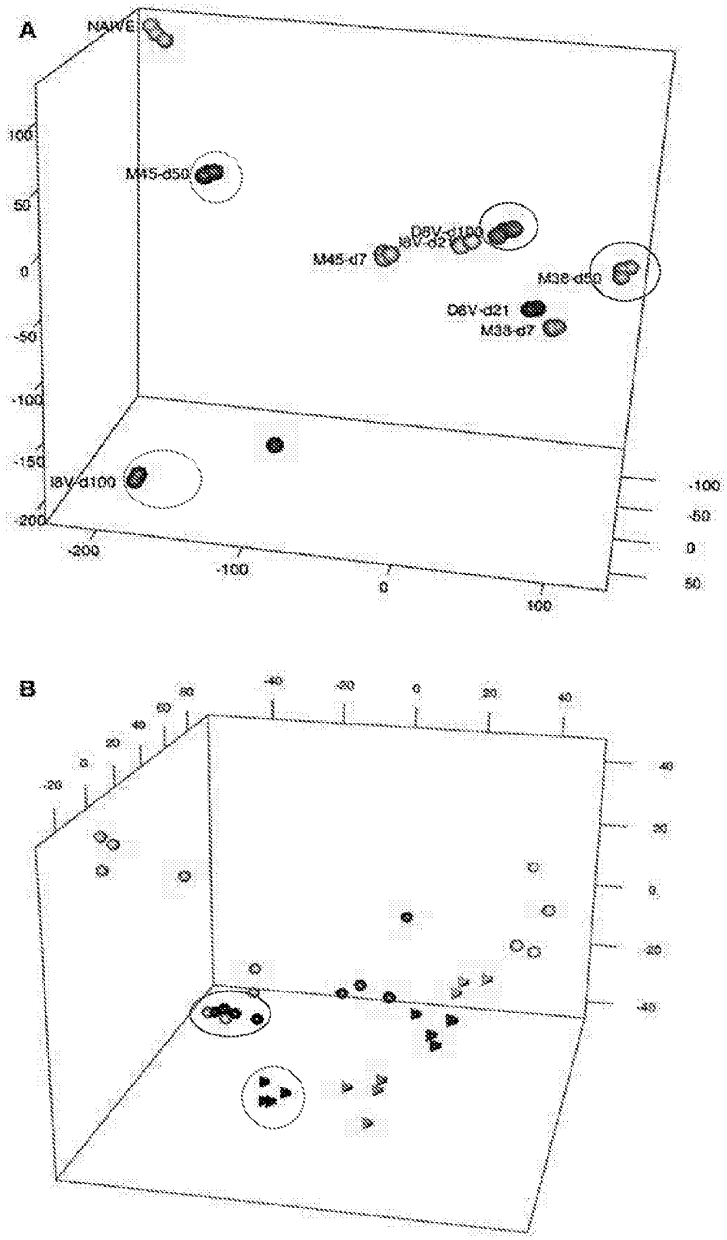

A
NAIVE
M45-d50
D8V-d100
D8V-d21
M45-d7
M38-d50
D8V-d21
M38-d7
D8V-d100
100
50
0
-50
-100
-150
-200
-200
-100
0
100
-100
-50
0
50
B

Figure 25

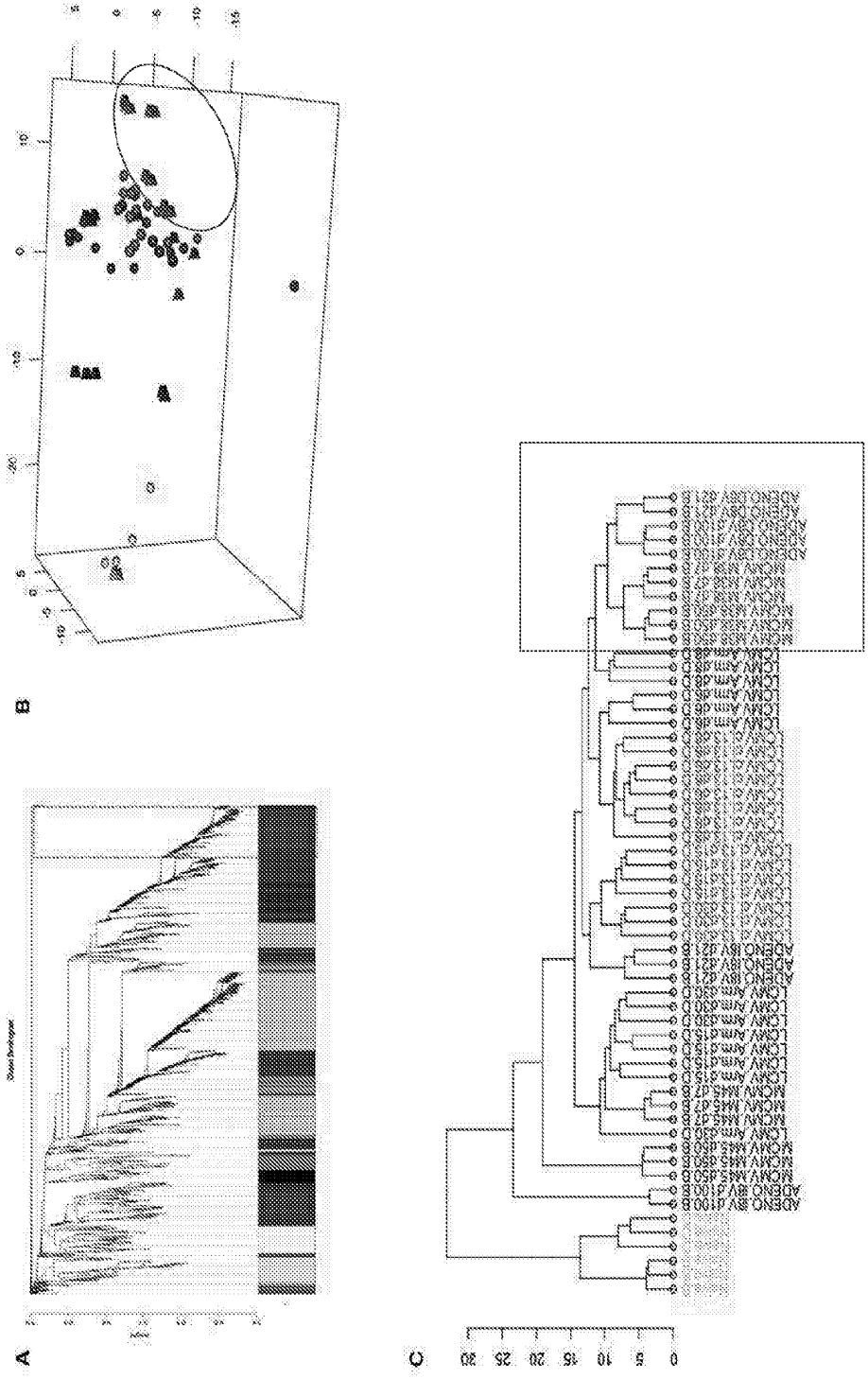


Figure 27A

1. Layout of AdHu5 adenovirus with minigene immunogen cassette:

2. Layout of minigene immunogen cassette:

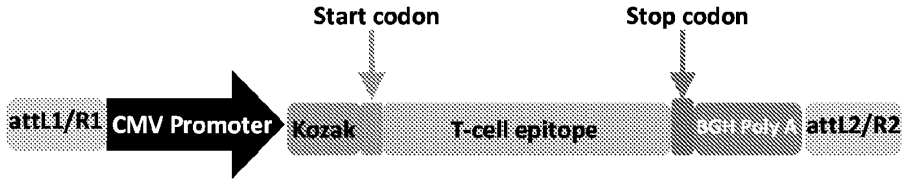
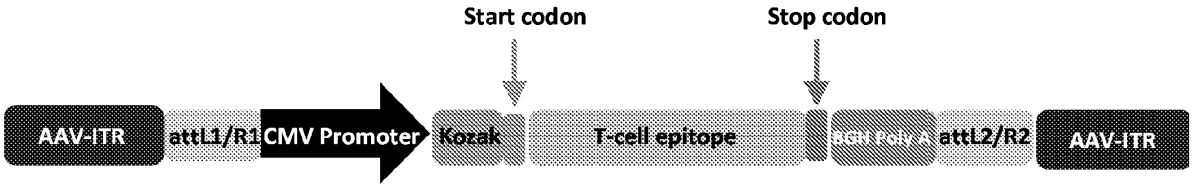
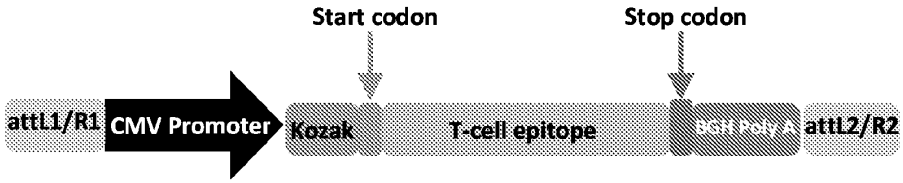


Figure 27B

1. Layout of AAV-ITR-minigene immunogen cassette:



2. Layout of minigene immunogen cassette: