Armored CAR T cells enhance antitumor efficacy and overcome the tumor microenvironment

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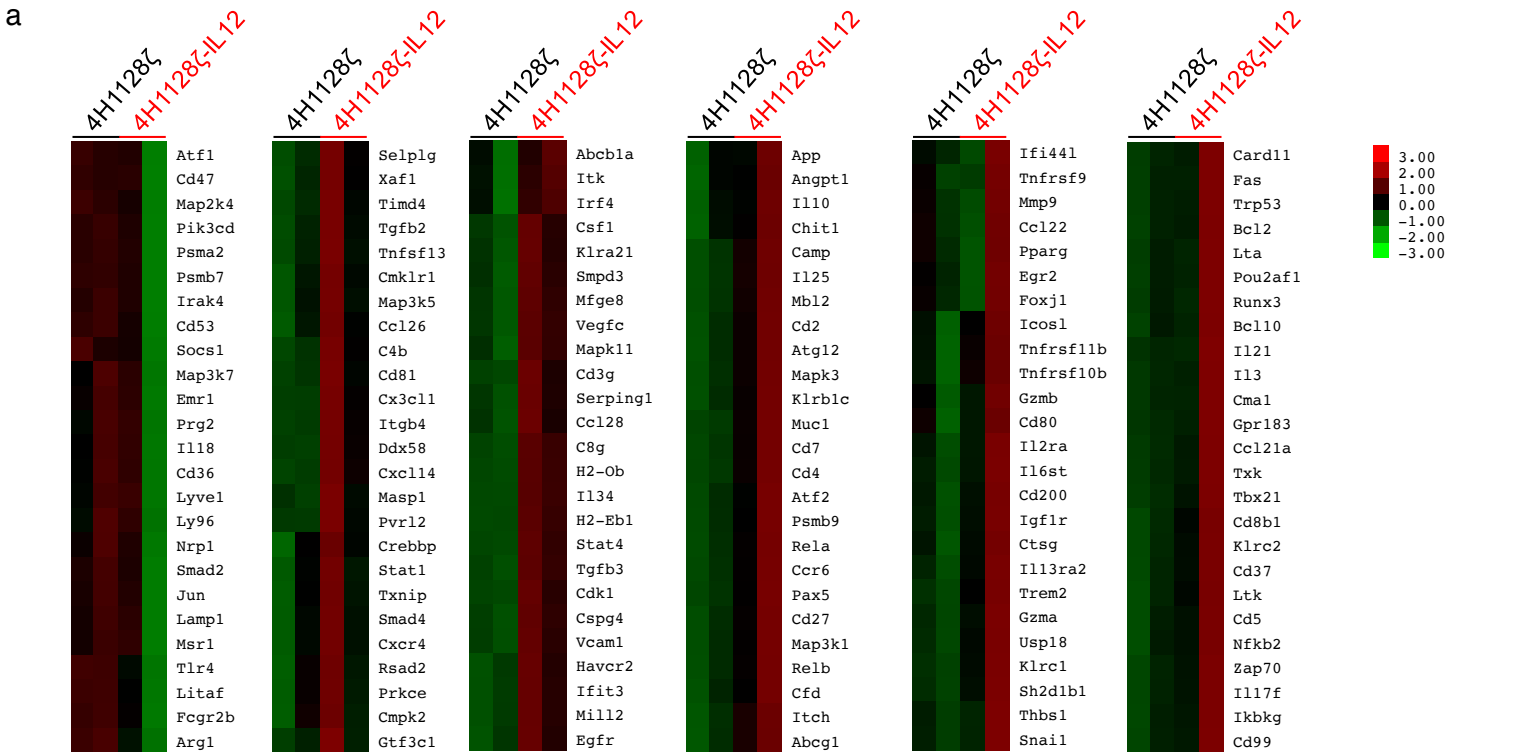
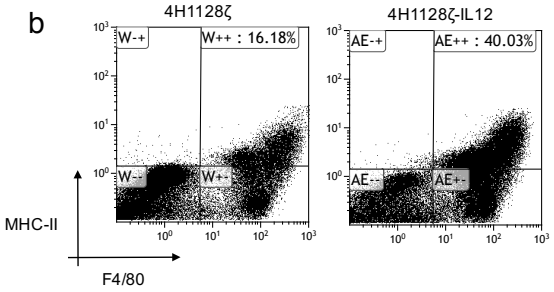
Supplementary figure 1. Transcriptome differences and functional assay on TAMs recovered from animals treated with 4H1128ζ-IL12 and 4H1128ζ CAR T cells. (a). Heatmap representations of differentially expressed genes from pooled recovered TAMs from 4H1128ζ-IL12 and 4H1128ζ- treated mice. **(b).** MHC-II expression on recovered TAMs 48 hr after treatment with 4H1128ζ-IL12 or 4H1128ζ T cells. **(c).** Intracellular and secreted IL-6 and IL-10 levels from TAMs recovered from mice treated with either 4H1128ζ-IL12 and 4H1128ζ T cells. *$p < 0.05$. Data are plotted as mean ± SEM. **(d)**. Arginase activity in recovered TAMs from 4H1128ζ-IL12 and 4H1128ζ- treated mice. Data are plotted as mean ± SEM.

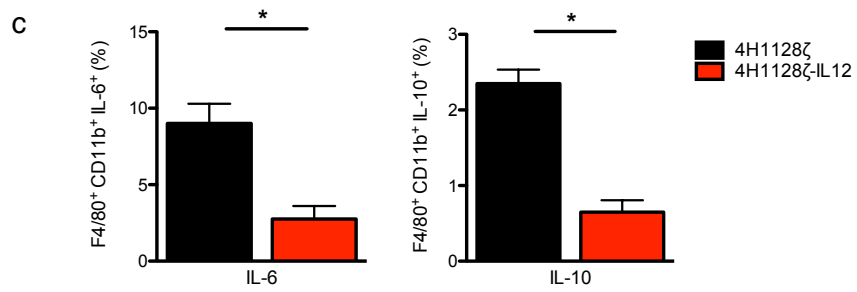
Supplementary figure 2. Endogenous effectors are not required for IL-12 armored CAR T cell efficacy. (a). Female C57BL/6 Ly5.1 mice between 6-8 weeks old were injected i.p with $1x10^7$ ID8-$Muc16^{ecto}$ cells and treated with 4H1128ζ or 4H1128ζ-IL12 CAR T cells derived from C57BL/6 Thy1.2 splenocytes. 48 hr after CAR T cell infusion, peritoneal washes were performed and stained for endogenous Ly5.1 T cells. Recovered Ly5.1 T cells were gated on the $Muc16^-$ $F4/80^-$ population of peritoneal cells, *p = 0.04. Data are plotted as mean ± SEM. Data shown are representative results from 3 independent experiments. **(b)**. Female IFN-γ knockout (IFN-$\gamma^{-/-}$) or CD8 knockout ($CD8^{-/-}$) mice between 6-8 weeks old were treated with $2x10^6$ 4H1128ζ or 4H1128ζ-IL12 CAR T cells derived from WT splenocytes 35 days after i.p tumor inoculation, *p < 0.001, #p = 0.001. n= 5 mice per group. Statistical analysis performed using a log-rank (Mantel-Cox) test.

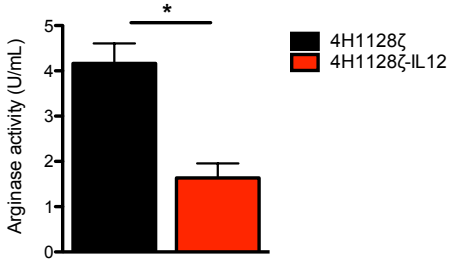
Supplementary figure 3. Ascites-derived IFN-γ induces upregulation of PD-L1 on ID8-Muc16^{ecto} cells. ID8-Muc16^{ecto} cells cultured for 16 hr in the presence of PBS, pooled ascites with undetectable IFN-γ or pooled ascites with IFN-γ. IFN-γ levels from PBS and asities also shown.

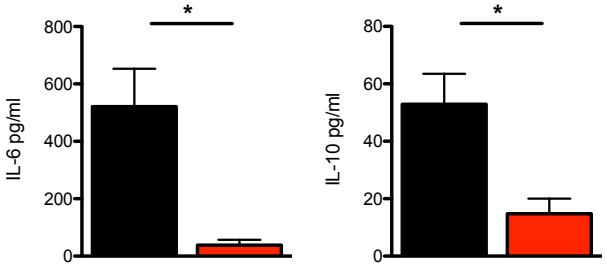
Supplementary figure 4. 4H1128ζ-IL12 CAR T does not lead to increased toxicity in mice. (a). Serum cytokine levels obtained from tumor-bearing WT mice pre- and 7 days post infusion of 1928ζ**,** 1928ζ-IL12, 4H1128ζ and 4H1128ζ-IL12 T cells. *p < 0.05. Data are plotted as mean ± SEM. **(b)**. Non-tumor (NT) and tumor (T) bearing mice were treated with $2x10^6$ i.v 4H1128ζ-IL12 T cells on D0 and $2x10^6$ i.p 4H1128ζ-IL12 T cells on D1 (i.v/i.p) and subsequently weighed. **(c)**. Following i.v/i.p infusion of 4H1128ζ-IL12, blood chemistries, hematologic, liver function and renal function parameters were measured at day 3 and day 16. *p < 0.05, lymphocytes *p < 0.05. Data are plotted as mean ± SEM.

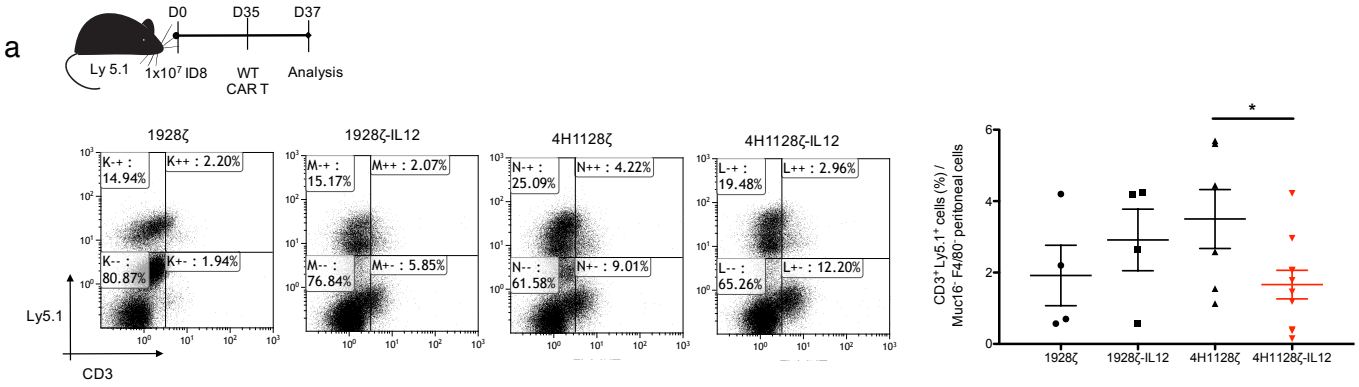

a
4H1128ζ
4H1128ζ-IL12
Atf1
Cd47
Map2k4
Pik3cd
Psma2
Psmb7
Irak4
Cd53
Socs1
Map3k7
Emr1
Prg2
Il18
Cd36
Lyve1
Ly96
Nrp1
Smad2
Jun
Lamp1
Msr1
Tlr4
Litaf
Fcgr2b
Arg1
Selplg
Xaf1
Timd4
Tgfb2
Tnfsf13
Cmklr1
Map3k5
Ccl26
C4b
Cd81
Cx3cl1
Itgb4
Ddx58
Cxcl14
Masp1
Pvrl2
Crebbp
Stat1
Txnip
Smad4
Cxcr4
Rsad2
Prkce
Cmpk2
Gtf3c1
Abcb1a
Itk
Irf4
Csf1
Klra21
Smpd3
Mfge8
Vegfc
Mapk11
Cd3g
Serping1
Ccl28
C8g
H2-Ob
Il34
H2-Eb1
Stat4
Tgfb3
Cdk1
Cspg4
Vcam1
Havcr2
Ifit3
Mill2
Egfr
App
Angpt1
Il10
Chit1
Camp
Il25
Mbl2
Cd2
Atg12
Mapk3
Klrb1c
Muc1
Cd7
Cd4
Atf2
Psmb9
Rela
Ccr6
Pax5
Cd27
Map3k1
Relb
Cfd
Itch
Abcg1
Ifi44l
Tnfrsf9
Mmp9
Ccl22
Pparg
Egr2
Foxj1
Icosl
Tnfrsf11b
Tnfrsf10b
Gzmb
Cd80
Il2ra
Il6st
Cd200
Igf1r
Ctsg
Il13ra2
Trem2
Gzma
Usp18
Klrc1
Sh2d1b1
Thbs1
Snai1
Card11
Fas
Trp53
Bcl2
Lta
Pou2af1
Runx3
Bcl10
Il21
Il3
Cma1
Gpr183
Ccl21a
Txk
Tbx21
Cd8b1
Klrc2
Cd37
Ltk
Cd5
Nfkb2
Zap70
Il17f
Ikbkg
Cd99
3.00
2.00
1.00
0.00
−1.00
−2.00
−3.00

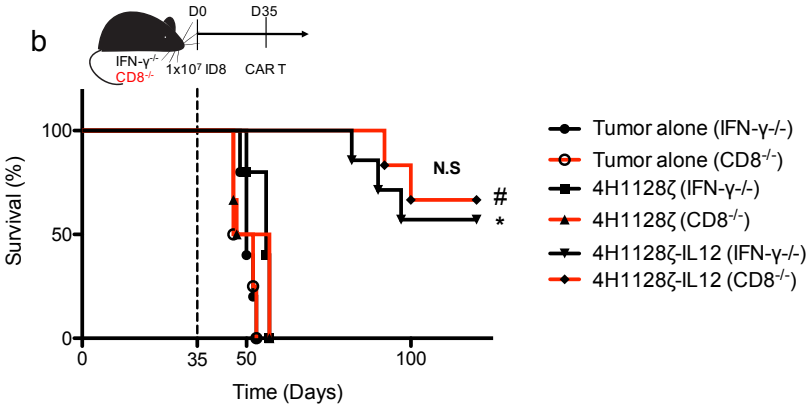

b
4H1128ζ
4H1128ζ-IL12
W-+
W++ : 16.18%
W--
W+-
AE-+
AE++ : 40.03%
AE--
AE+-
MHC-II
F4/80

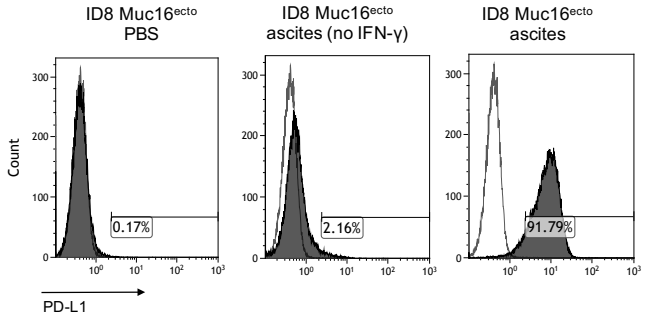

c
F4/80+ CD11b+ IL-6+ (%)
IL-6
F4/80+ CD11b+ IL-10+ (%)
IL-10
4H1128ζ
4H1128ζ-IL12

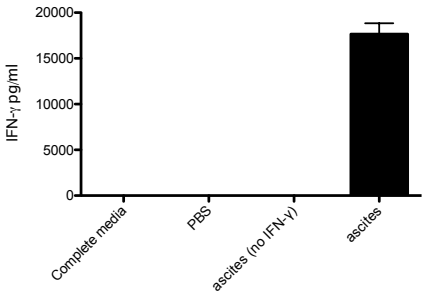

d
Arginase activity (U/mL)
4H1128ζ
4H1128ζ-IL12

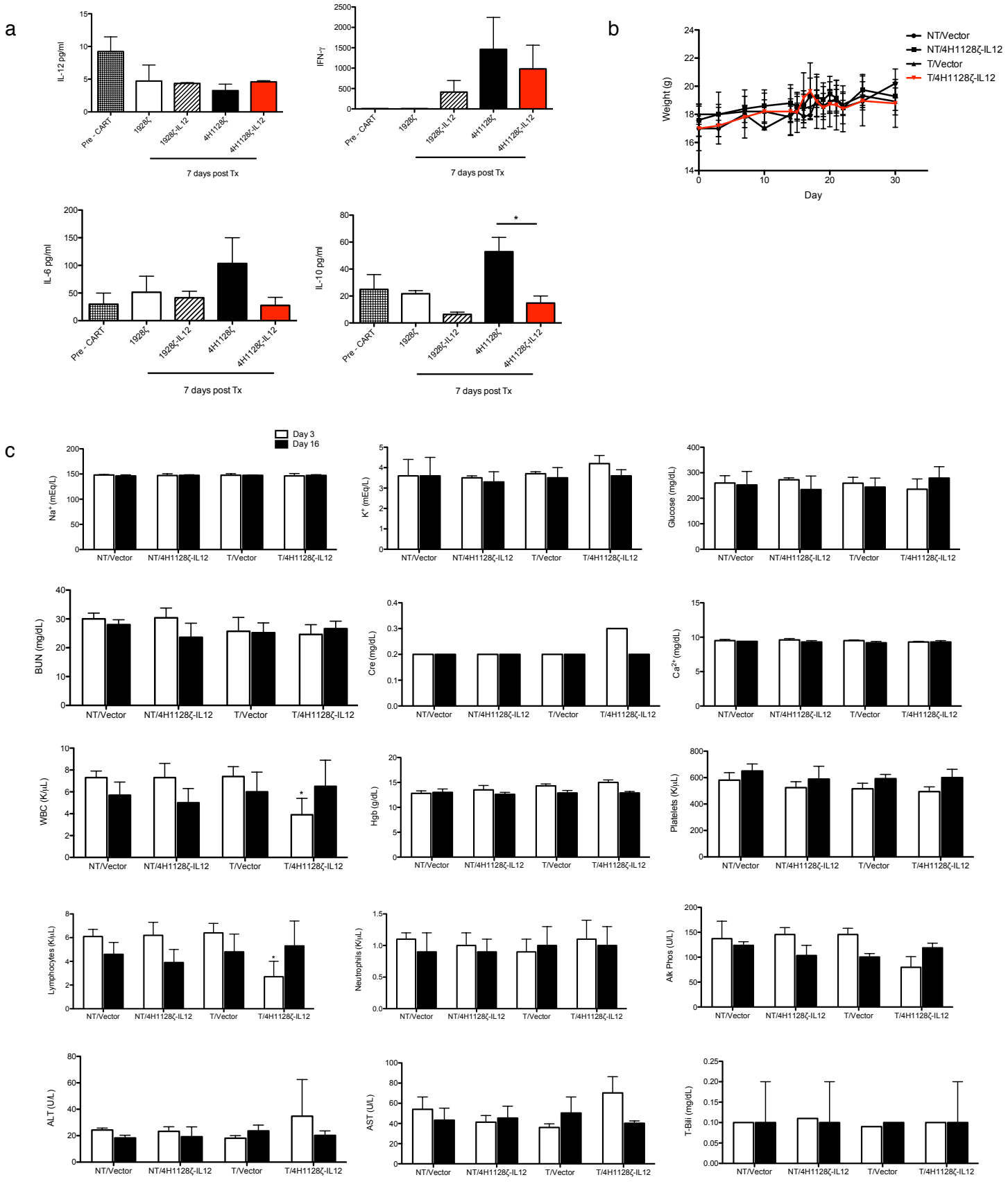

IL-6 pg/ml
IL-10 pg/ml


a
D0
D35
D37
Ly 5.1
1x10⁷ ID8
WT CAR T
Analysis
1928ζ
1928ζ-IL12
4H1128ζ
4H1128ζ-IL12
K-+ : 14.94%
K++ : 2.20%
K-- : 80.87%
K+- : 1.94%
M-+ : 15.17%
M++ : 2.07%
M-- : 76.84%
M+- : 5.85%
N-+ : 25.09%
N++ : 4.22%
N-- : 61.58%
N+- : 9.01%
L-+ : 19.48%
L++ : 2.96%
L-- : 65.26%
L+- : 12.20%
Ly5.1
CD3
CD3⁺Ly5.1⁺ cells (%) / Mucl6⁻F4/80⁻ peritoneal cells
*
1928ζ
1928ζ-IL12
4H1128ζ
4H1128ζ-IL12


b
D0
D35
IFN-γ-/-
CD8-/-
1x10⁷ ID8
CAR T
Survival (%)
Time (Days)
N.S
#
*
Tumor alone (IFN-γ-/-)
Tumor alone (CD8-/-)
4H1128ζ (IFN-γ-/-)
4H1128ζ (CD8-/-)
4H1128ζ-IL12 (IFN-γ-/-)
4H1128ζ-IL12 (CD8-/-)


ID8 Muc16ecto
PBS
ID8 Muc16ecto
ascites (no IFN-γ)
ID8 Muc16ecto
ascites
Count
0.17%
2.16%
91.79%
PD-L1


IFN-γ pg/ml
20000
15000
10000
5000
0
Complete media
PBS
ascites (no IFN-γ)
ascites


a
IL-12 pg/ml
IFN-γ
IL-6 pg/ml
IL-10 pg/ml
Pre - CART
1928ζ
1928ζ-IL12
4H1128ζ
4H1128ζ-IL12
7 days post Tx
b
Weight (g)
Day
NT/Vector
NT/4H1128ζ-IL12
T/Vector
T/4H1128ζ-IL12
c
Day 3
Day 16
Na+ (mEq/L)
K+ (mEq/L)
Glucose (mg/dL)
BUN (mg/dL)
Cre (mg/dL)
Ca2+ (mg/dL)
WBC (K/μL)
Hgb (g/dL)
Platelets (K/μL)
Lymphocytes (K/μL)
Neutrophils (K/μL)
Alk Phos (U/L)
ALT (U/L)
AST (U/L)
T-Bili (mg/dL)