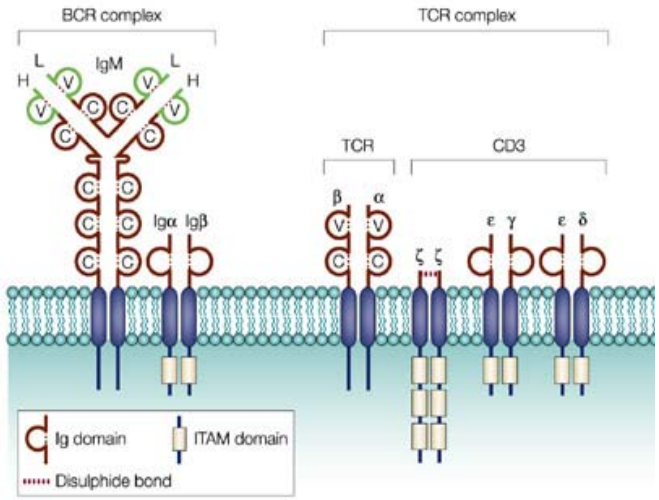
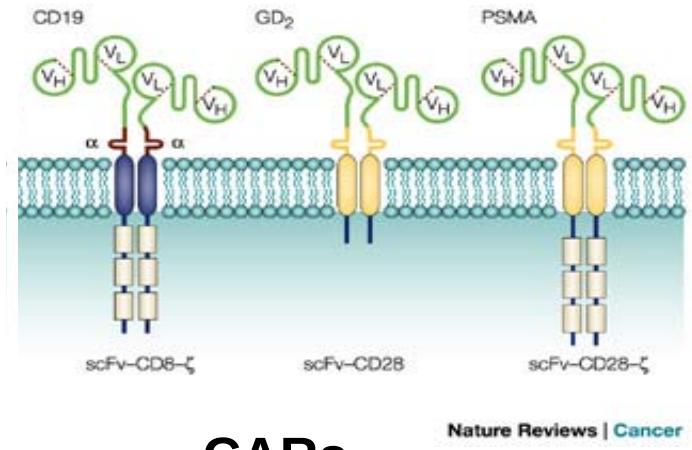
Targeting tumors with genetically enhanced T lymphocytes

- **Redirect T cell specificity towards selected antigens**
  – to overcome self-tolerance and antigen escape
- **Enhance antigen receptor-mediated T cell activation**
  – to increase T cell expansion and survival
- **Increase intra-tumoral T cell activation**
  – to augment tumor rejection

Physiological and chimeric antigen receptors





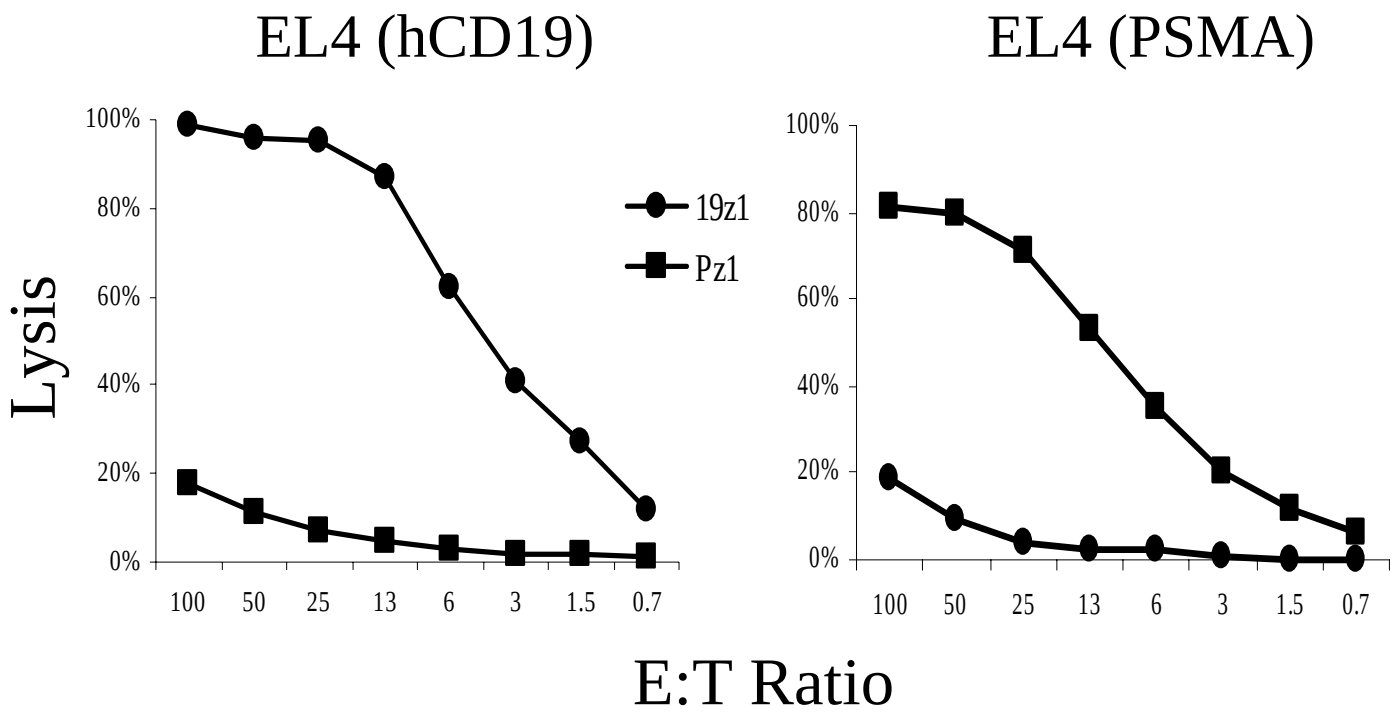
TCRs

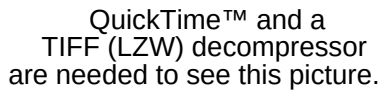
- Extra and intra-cellular antigens
- Adapted to CD3 complex
- Restricted by patient's HLA spec
- No ligand on HLA-negative tumor
- Strength of signal often weak?
- Heterologous pairing

CARs

- Extra-cellular ag only; incl. glycolipids
- No peptide processing required
- Not restricted by patient HLA
- Not limited by HLA down-regulation
- Designer signaling domain
- Immunogenicity?

19z1^{+} and Pz1^{+} T cells specifically lyse CD19^{+} and PSMA^{+} targets, respectively

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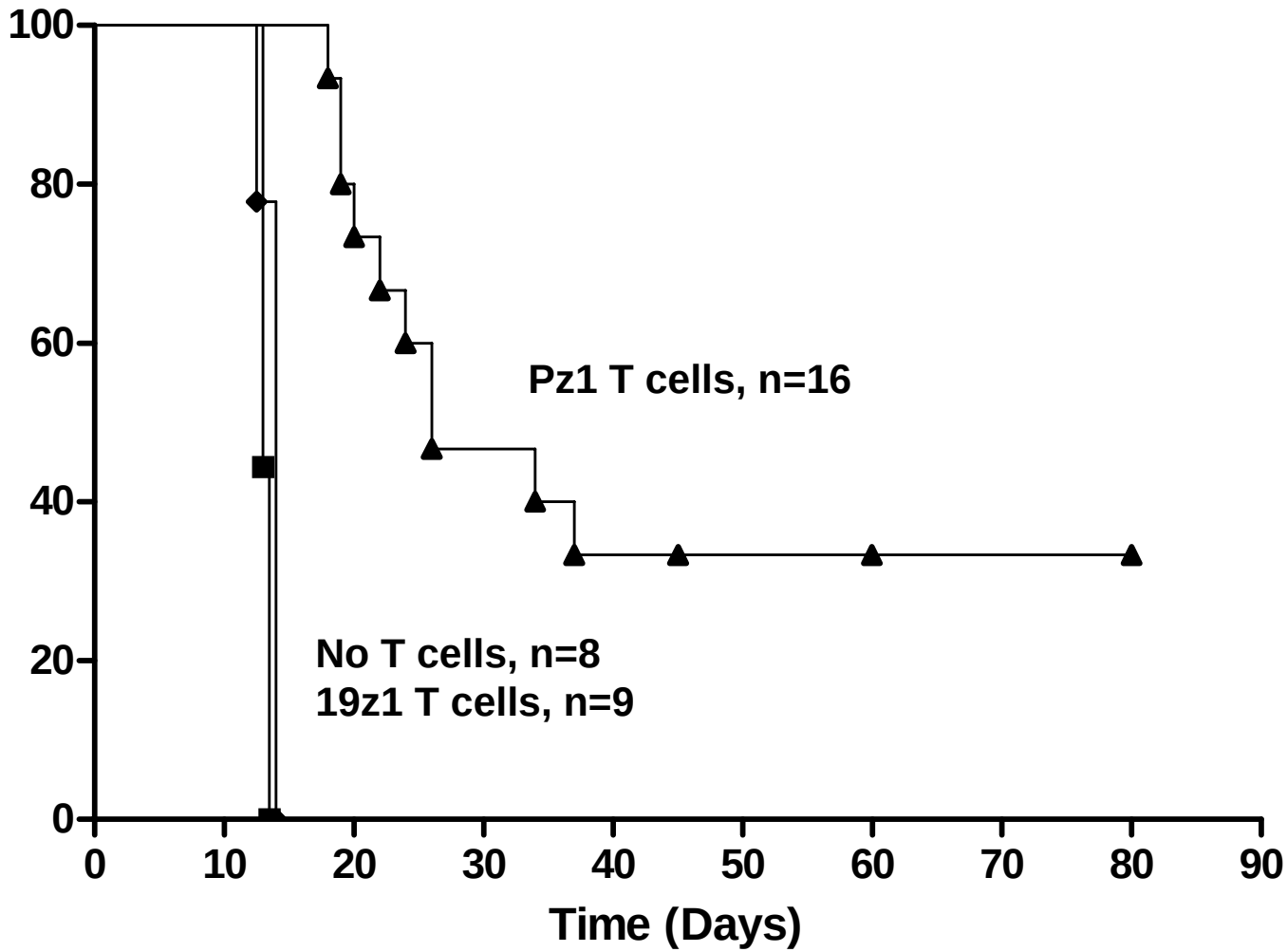
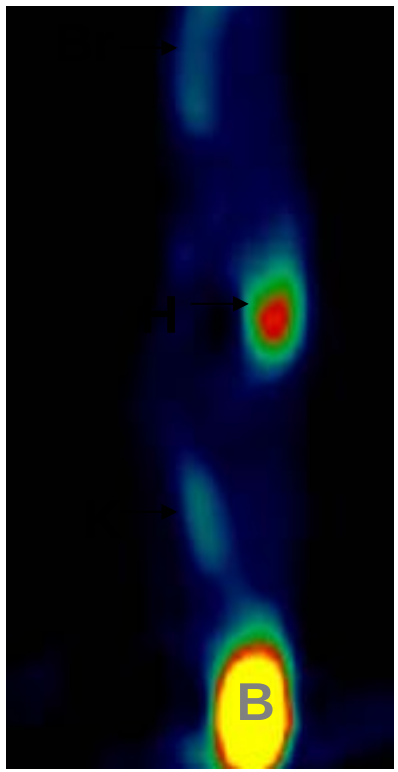
Intravenous administration of Pz1 T cells can eradicate pulmonary RM1.PGLS

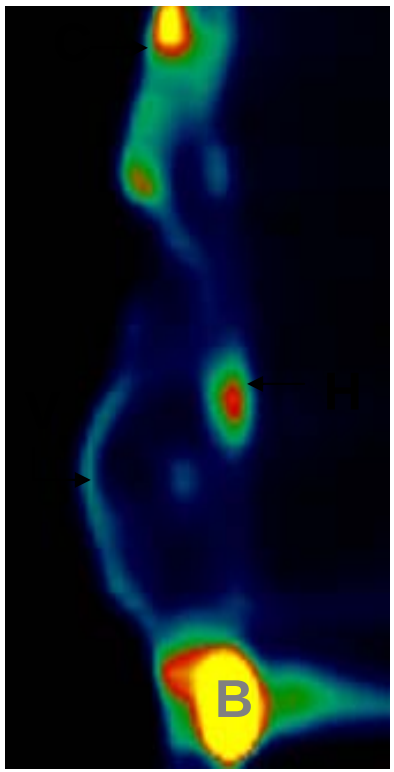


Gade et al., *Cancer Res*, 2005

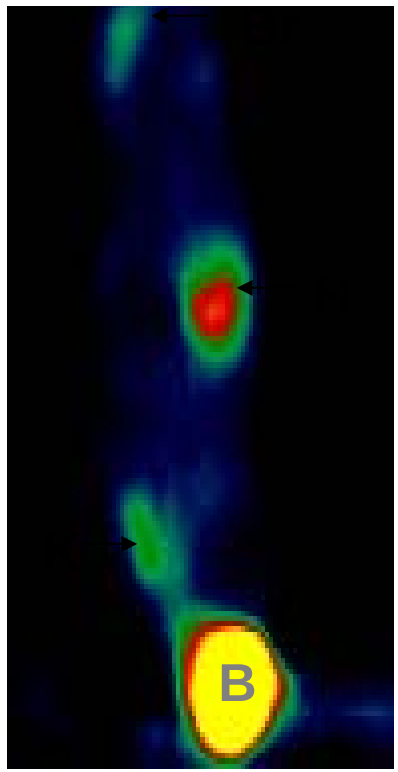
Monitoring tumor burden by positron emission tomography (PET): eradication of established systemic Raji by i.v. 19z1+ T cells



Tumor Free

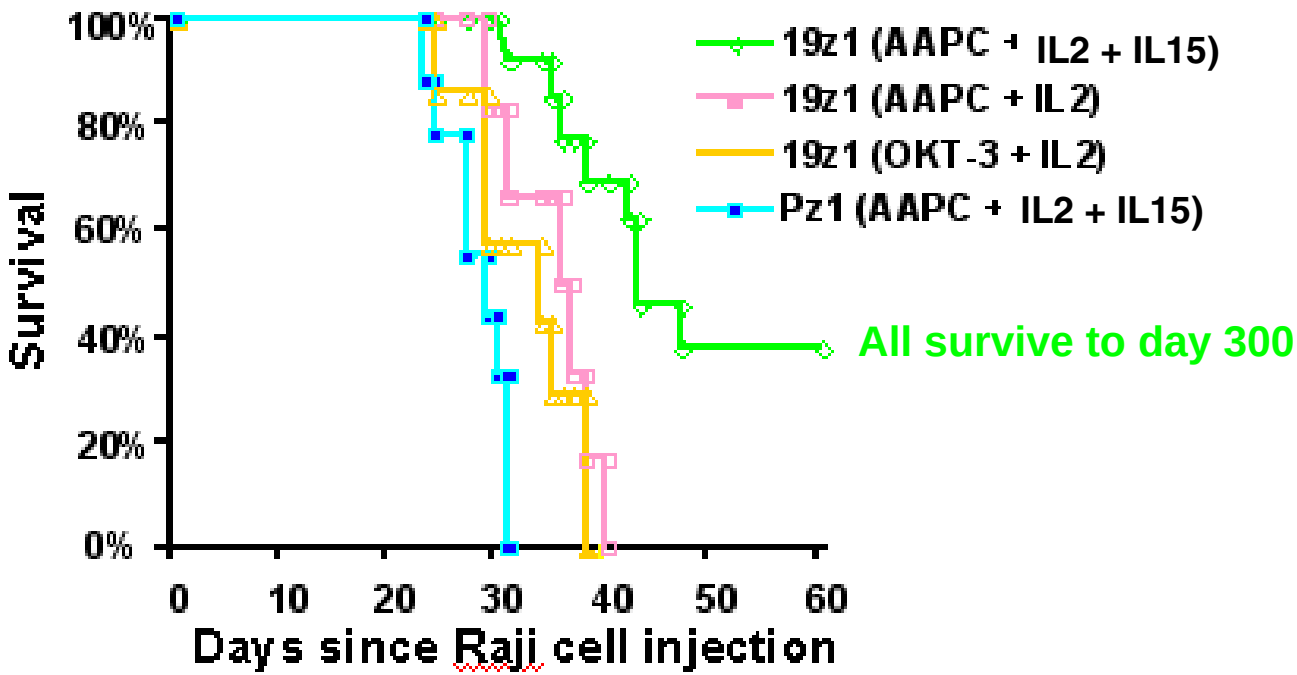


Untreated
4 weeks



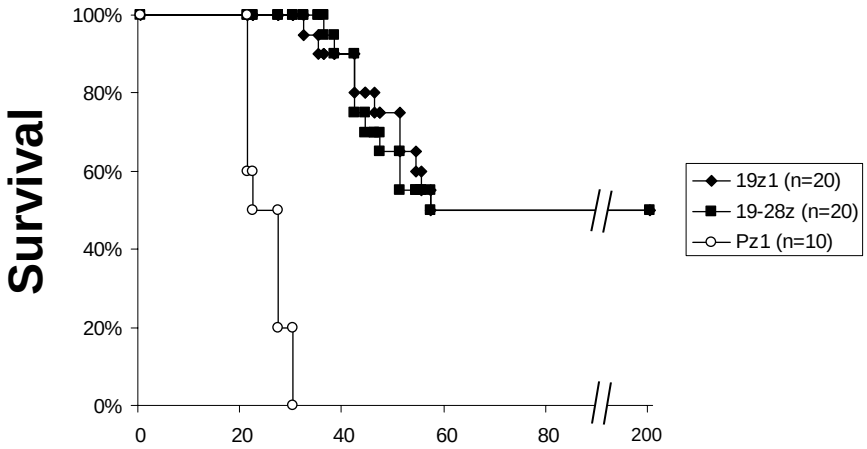
19z1 Treated
16 weeks

T cell expansion conditions determine whether 19z1 T cells eradicate established systemic Raji in SCID-bg mice

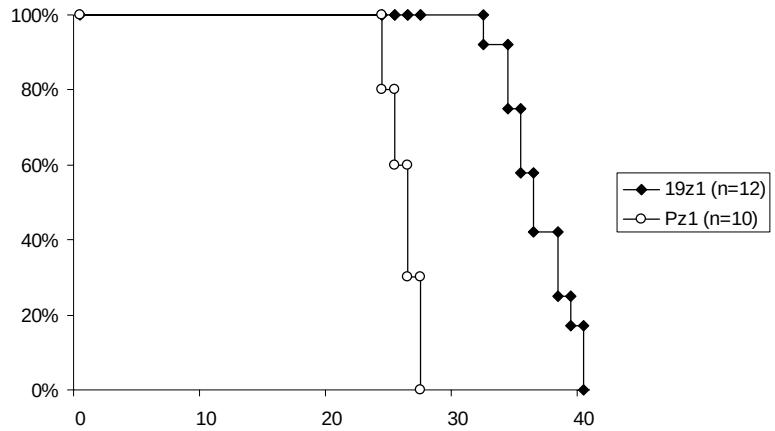


Brentjens et al., *Nature Medicine*, 2003

RAJI

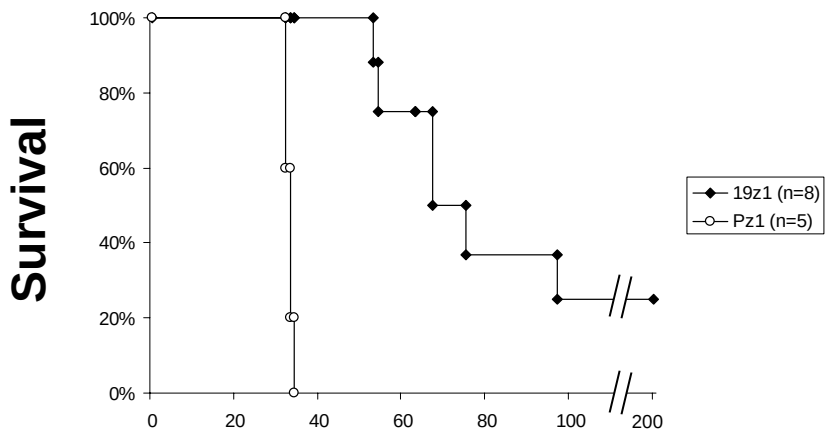


NALM/6



Days since tumor injection

NALM/6-B7

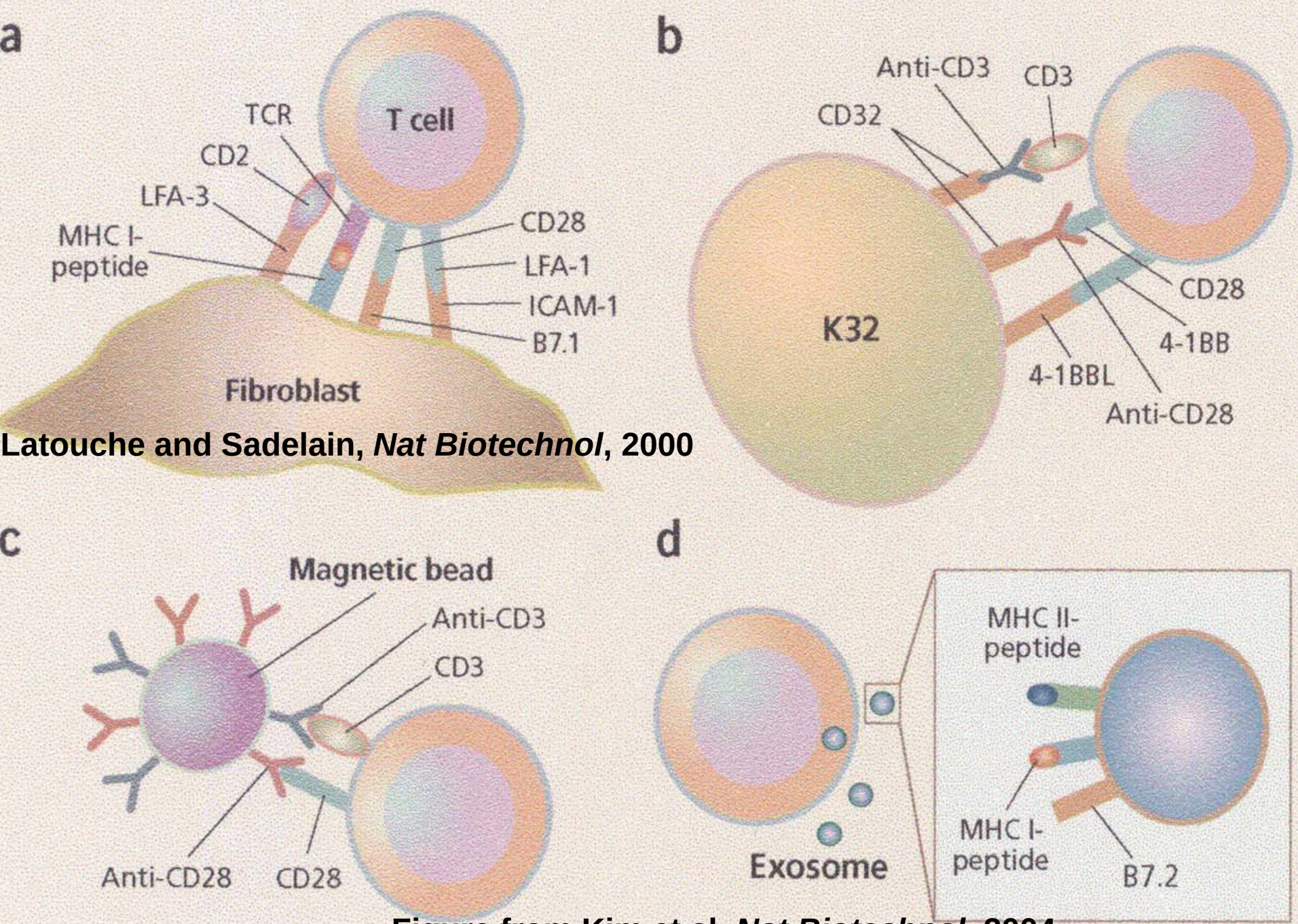


Days since tumor injection

Brentjens et al, *Nat Med*, 2003

What costimulation?
How to safely and effectively provide it?

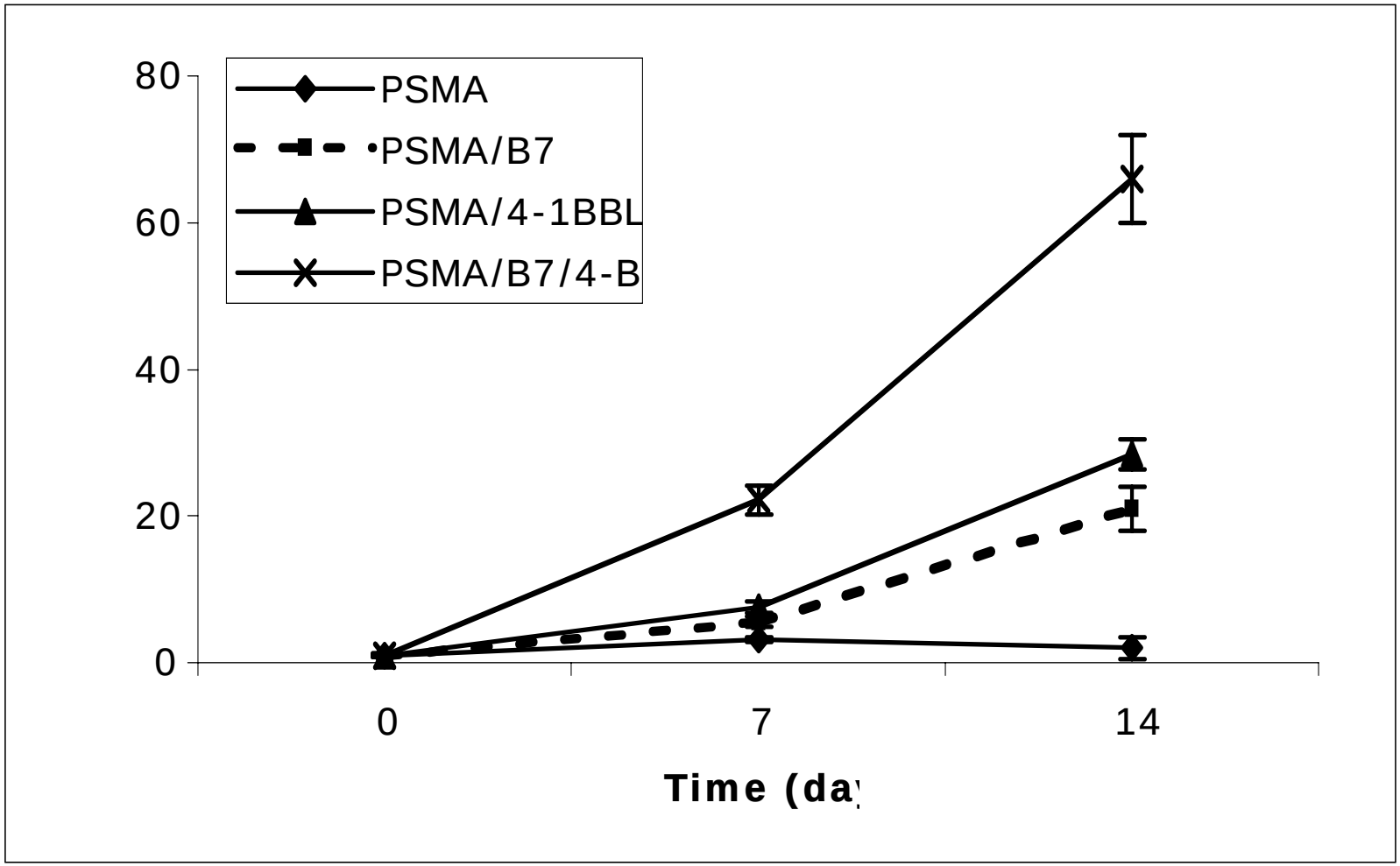
- ***Classic*** **costimulation**
- ***Integrated*** **costimulation**
- ***On-demand*** **costimulation**



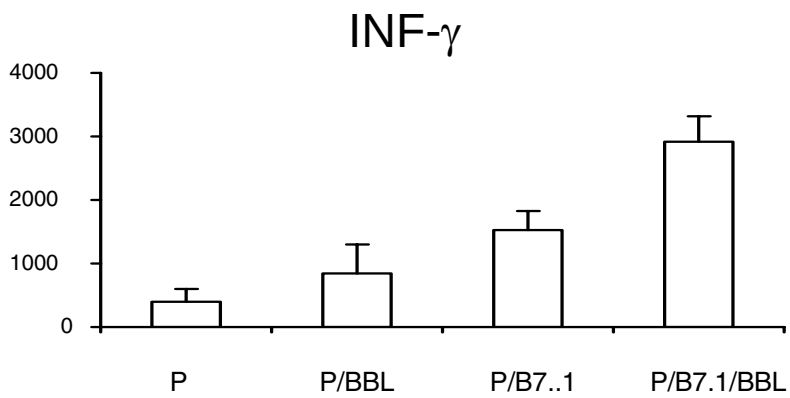
Latouche and Sadelain, *Nat Biotechnol*, 2000

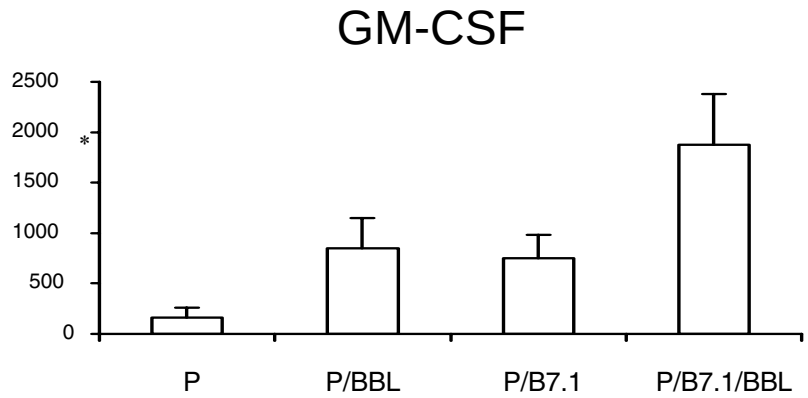
Figure from Kim et al, *Nat Biotechnol*, 2004

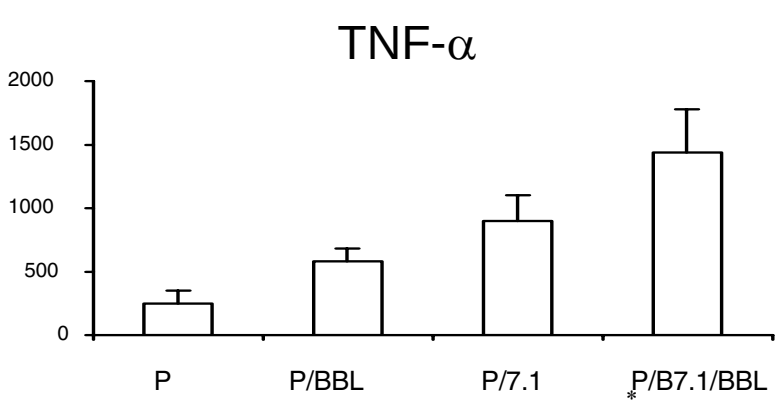
Pz1+ CD8+ PBL proliferation on AAPCs expressing CD80 and/or 4-1BBL

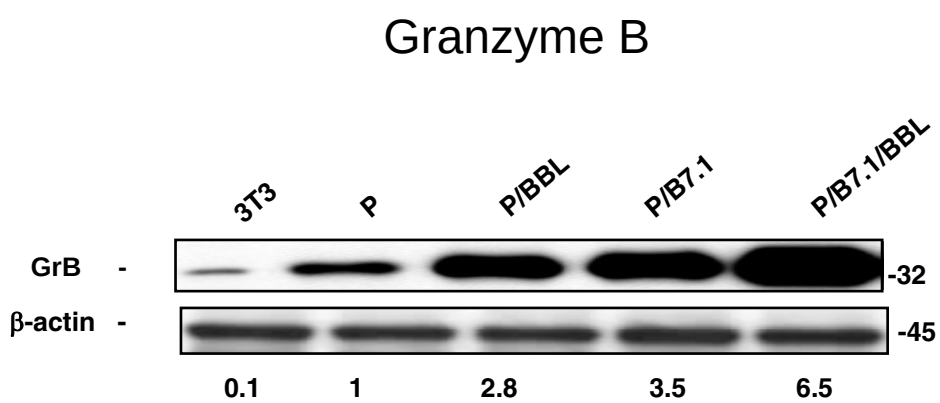


Combined CD80 and 4-1BB signals enhance effector functions





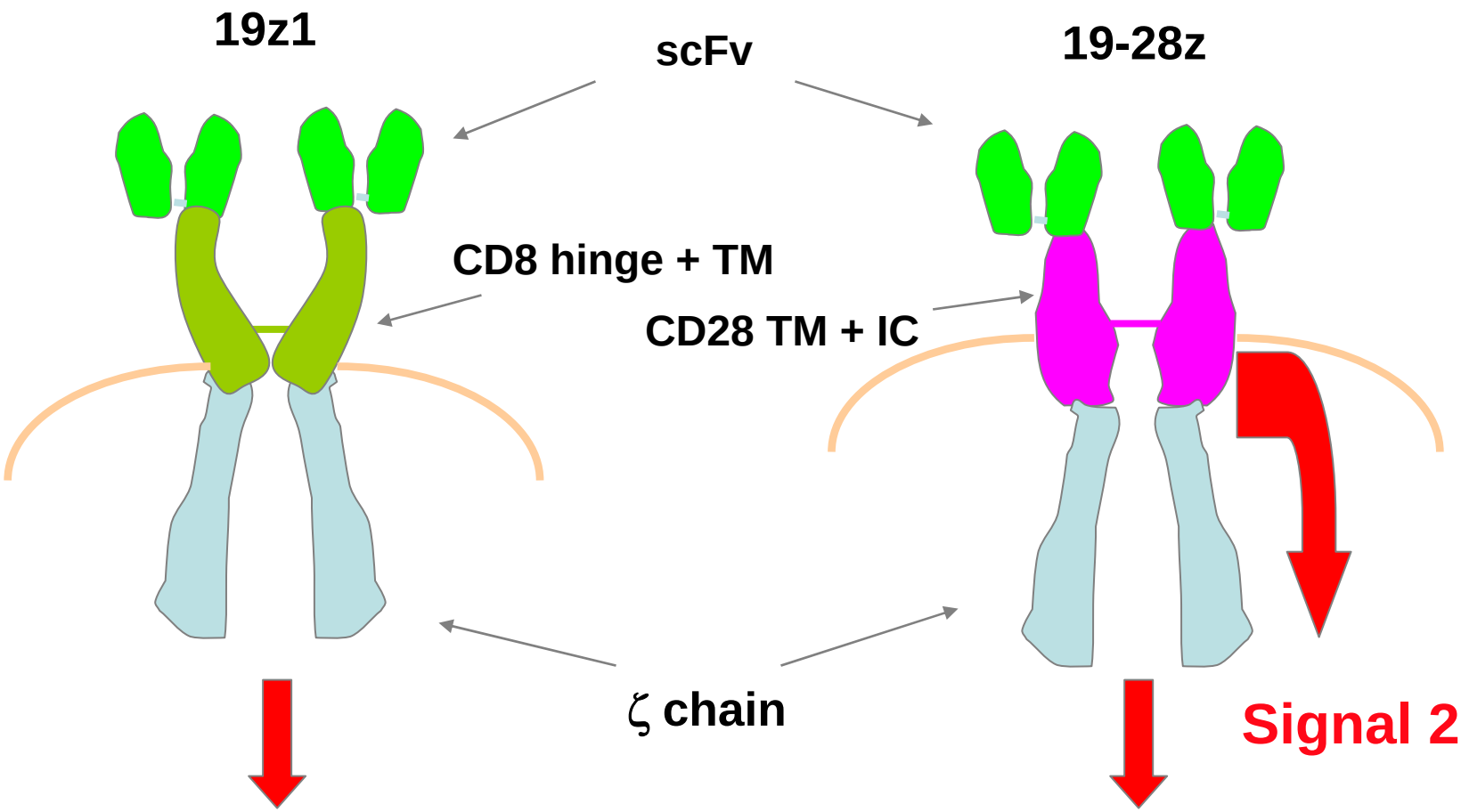




What costimulation and how to safely and effectively provide it

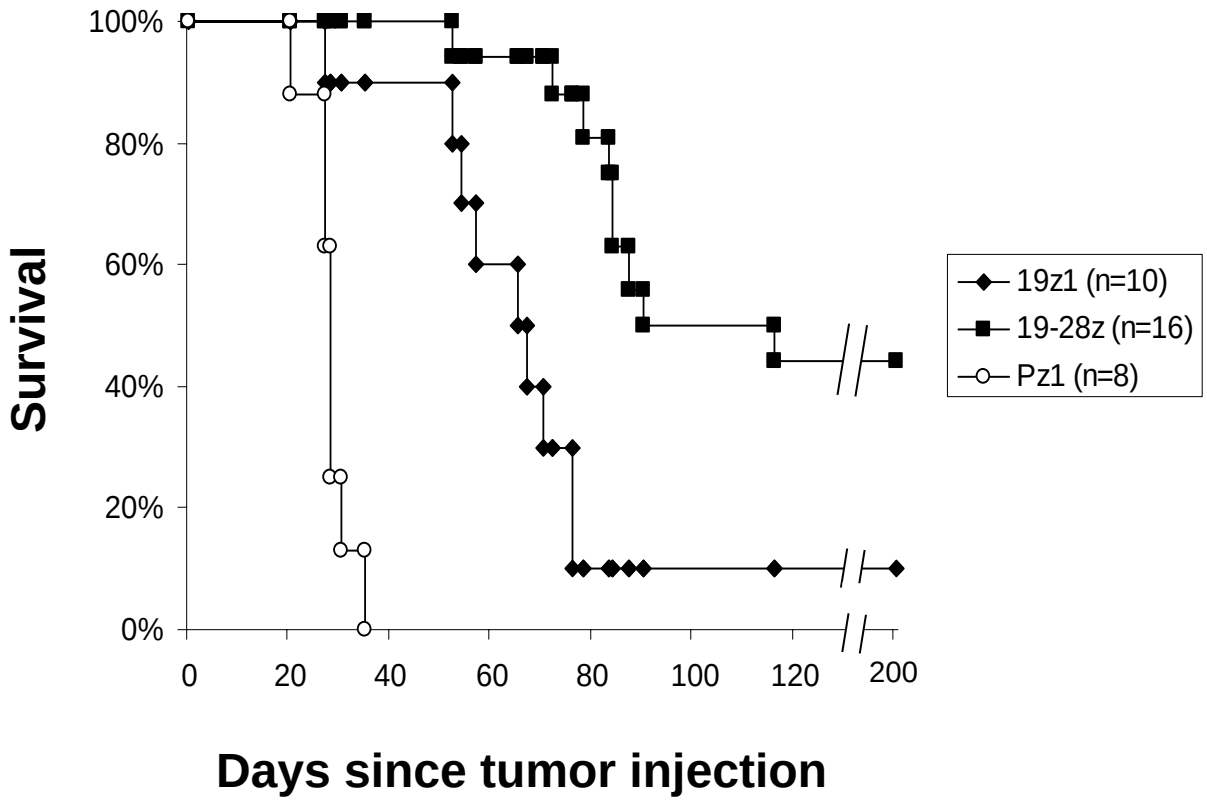
- ***Classic* costimulation**
- ***Built-in* costimulation**
- ***On-demand* costimulation**

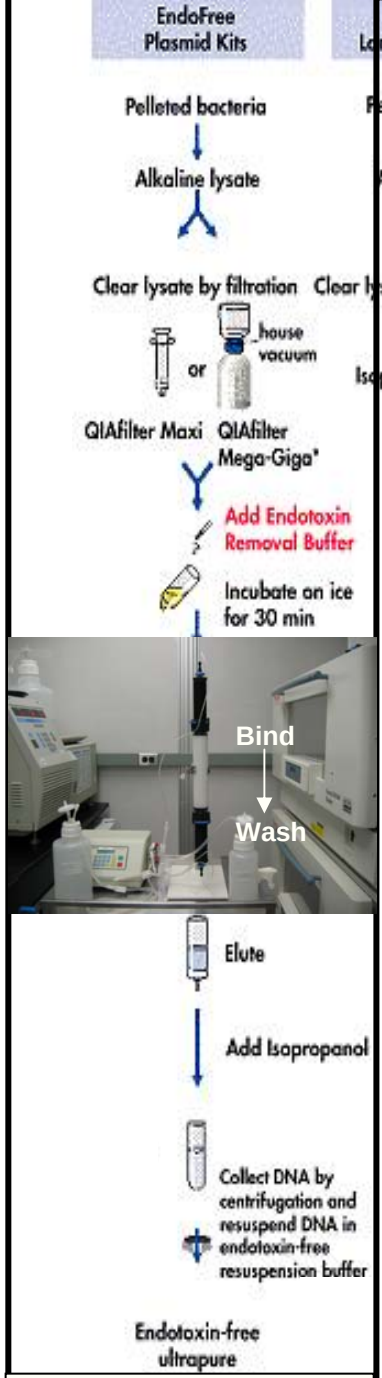
Construction of CD28/CD3ζ receptor



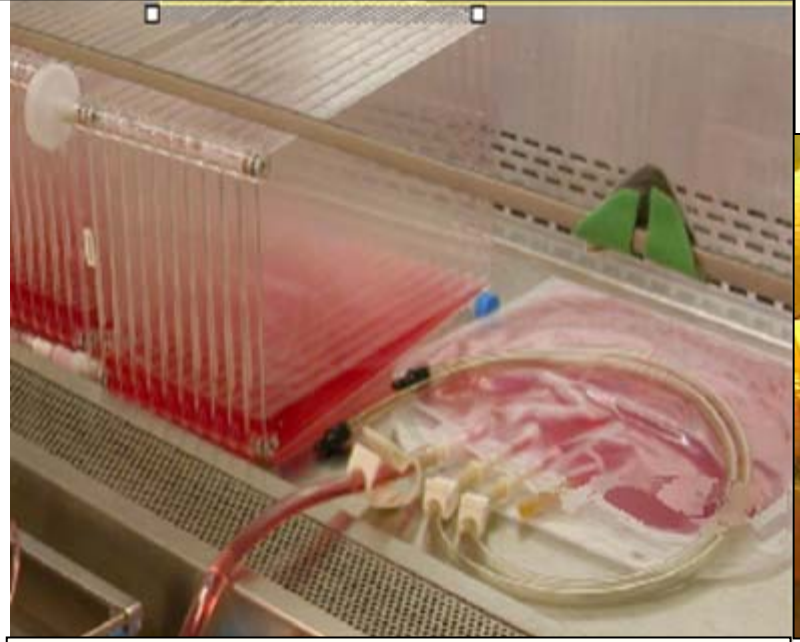
Maher et al, *Nat Biotechnol*, 2002

Four weekly T cell infusions greatly enhance survival (NALM6)





Plasmid DNA



Retroviral vector production

Patient cell transduction

Patient cell expansion - Wave bio

Patient cell selection

A Phase I Trial for the Treatment of Purine Analog-Refractory Chronic Lymphocytic Leukemia using Autologous T cells Genetically Targeted to the B cell Specific Antigen CD19 (IRB 06-138)

- **Enrollment Criteria:**
  Patients with purine analog-refractory CLL disease

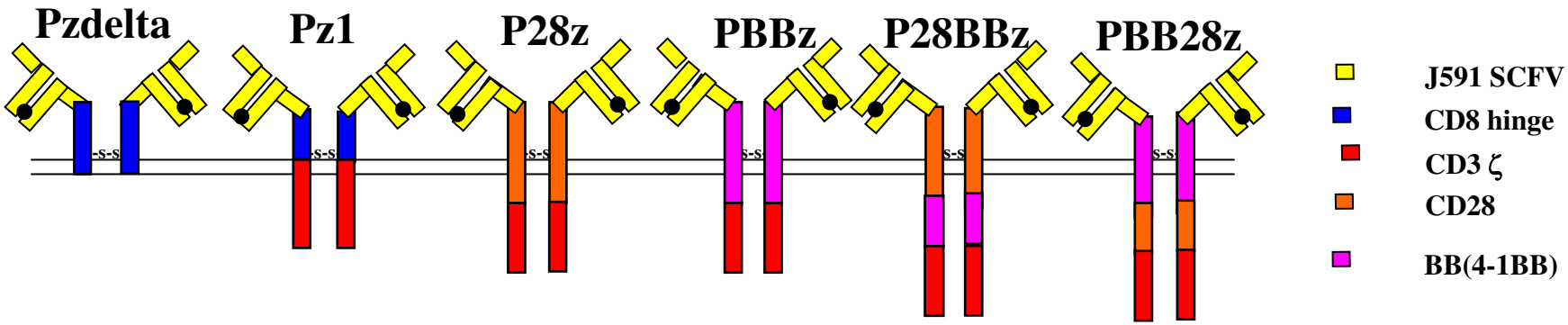
- **Protocol Design:**
  - A 2 step design:
    - Step I: Standard dose escalation of modified T cells
    - Step II: MTD from phase I in combination with dose escalating lympho-depleting cyclophosphamide chemotherapy

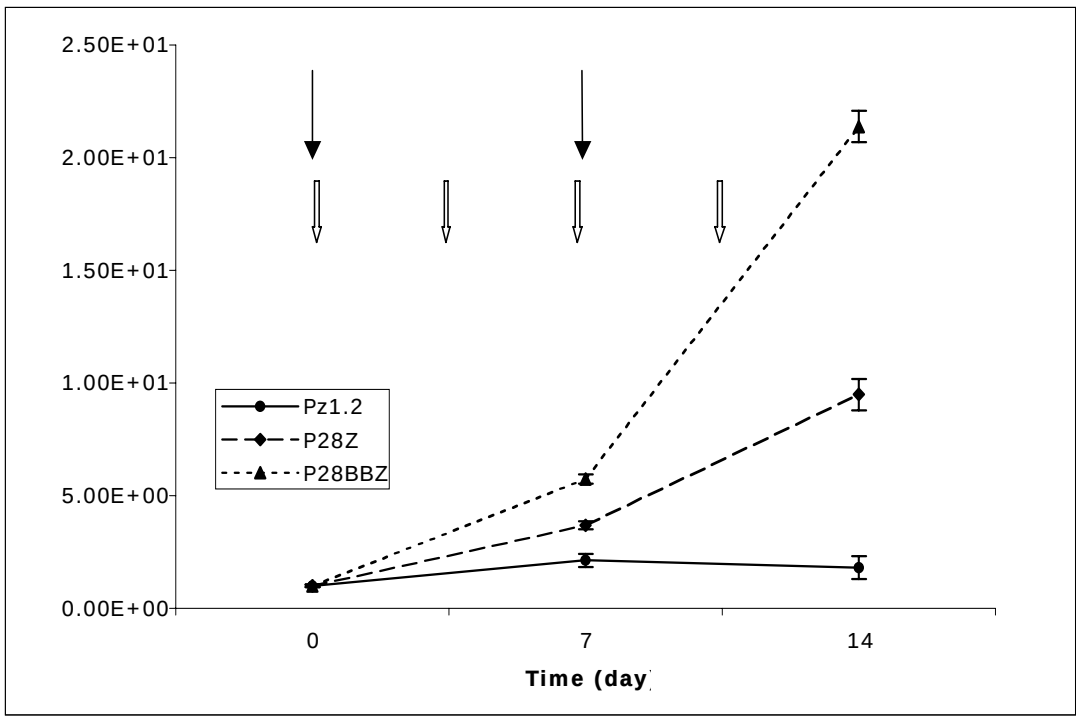
- **Treatment Protocol Phase 1:**
  - Dose escalation of 19-28z T cells
  - 3 dose levels will be studied (3×10^7 T cells/kg, 1×10^8 T cells/kg, and 3×10^8 T cells/kg)

- **Treatment Protocol Phase 2:**
  - Dose escalation of cyclophosphamide prior to MTD dose of T cells established in phase I.
  - 3 dose levels of cyclophosphamide will be tested ($1.5 g/m^2$, $2.2 g/m^2$, and $3.0 g/m^2$)

- **Anticipated patient enrollment**
  - 24-36 patients

“Third generation” chimeric antigen receptors



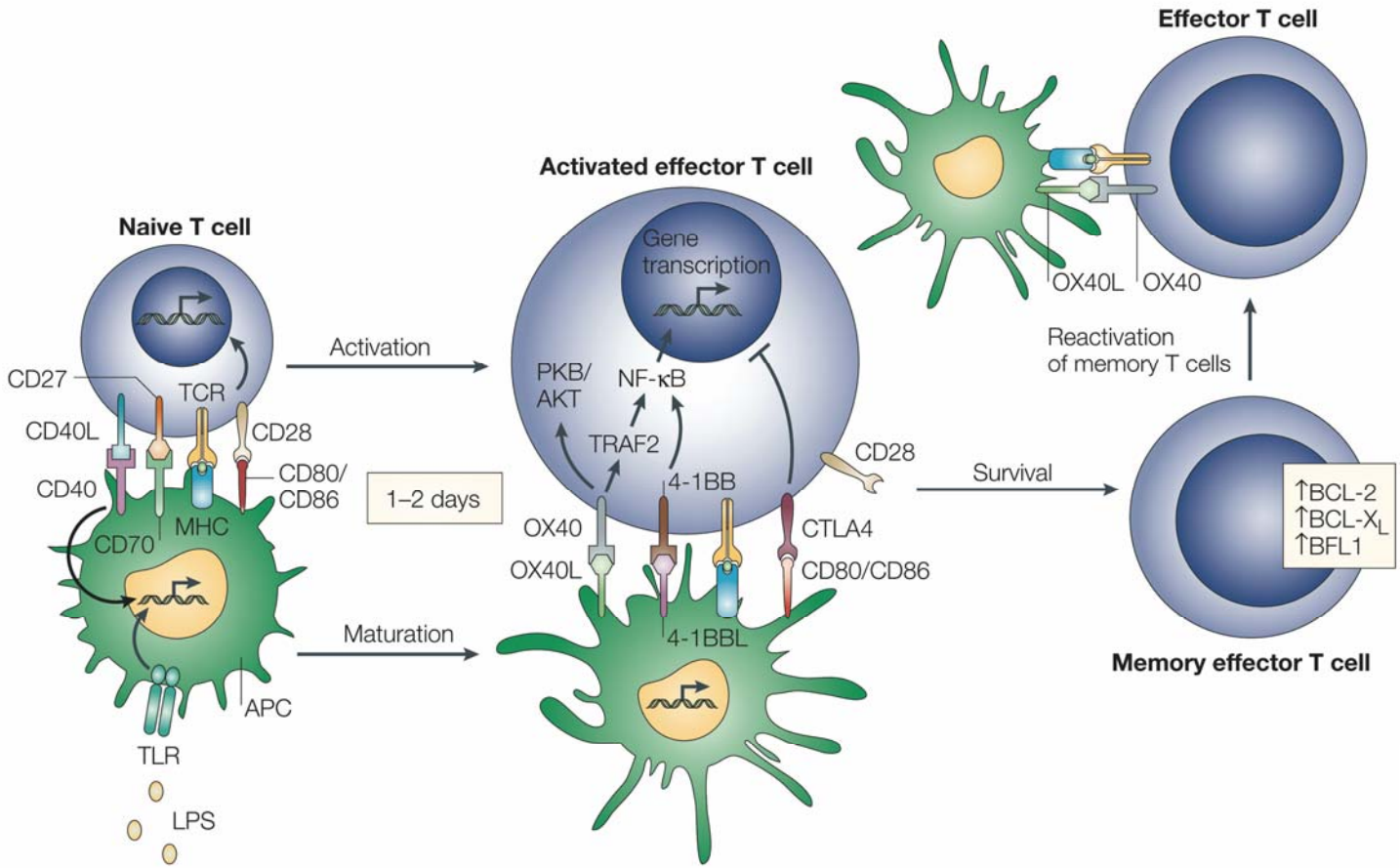


Proliferation of PSMA-redirected CD8+ T cells
(3T3-PSMA AAPC with 20 U/ml IL-2)

What costimulation and how to safely and effectively provide it

- *Classic* costimulation
- *Integrated* costimulation
- ***On-demand*** **costimulation**

ROLES OF COSTIMULATION IN T-CELL FUNCTION



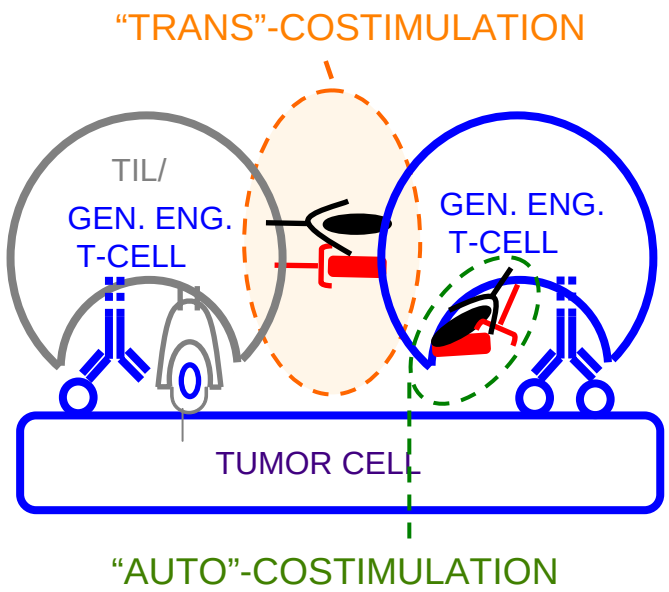
NATURE REVIEW IMMUNOLOGY, JUNE 2004, VOL 4

RESEARCH HYPOTHESIS AND PRIMARY EMPHASIS OF STUDY

1 Tumor antigen-specific T lymphocytes may serve themselves as providers of costimulatory ligands if they constitutively express these ligands and thus offset costimulatory deficiency within the tumor microenvironment

2 Constitutively expressed costimulatory ligands could in principle directly engage their respective receptors eventually following their induced upregulation (*Auto*-costimulation)..

3 … as well as deliver bystander costimulation to other tumor infiltrating lymphocytes. (*Trans*-costimulation)



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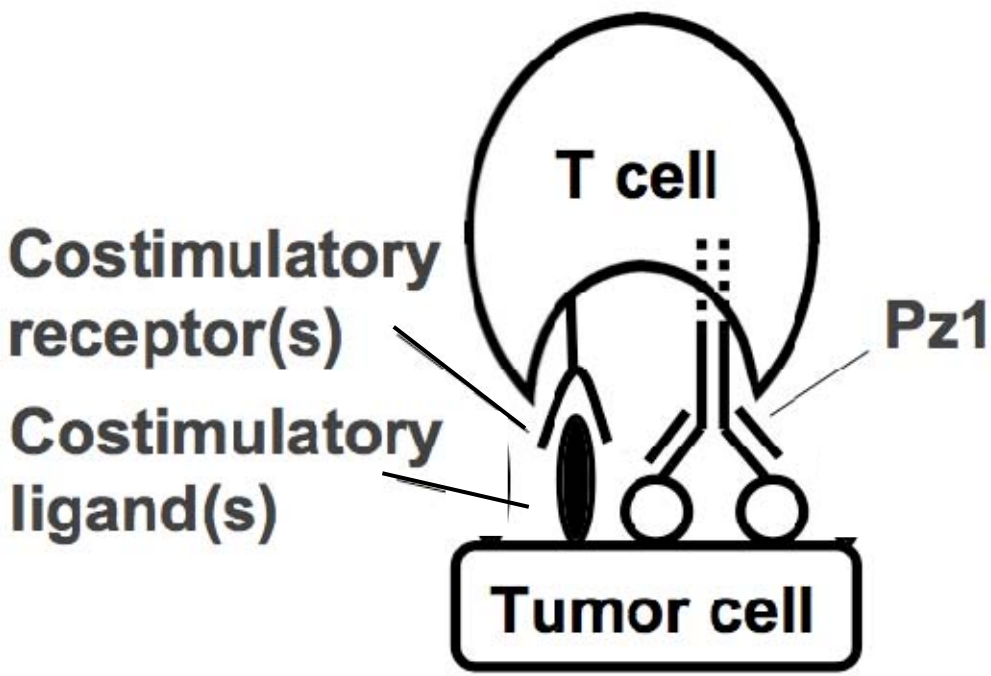
are needed to see this picture.

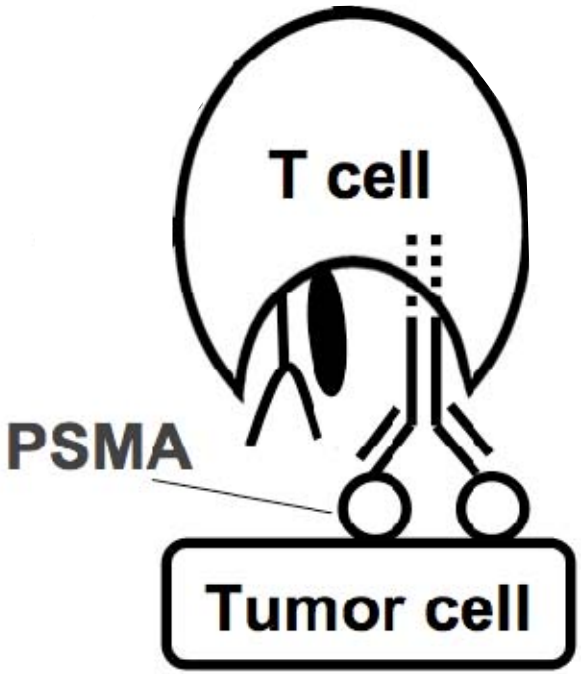
❶

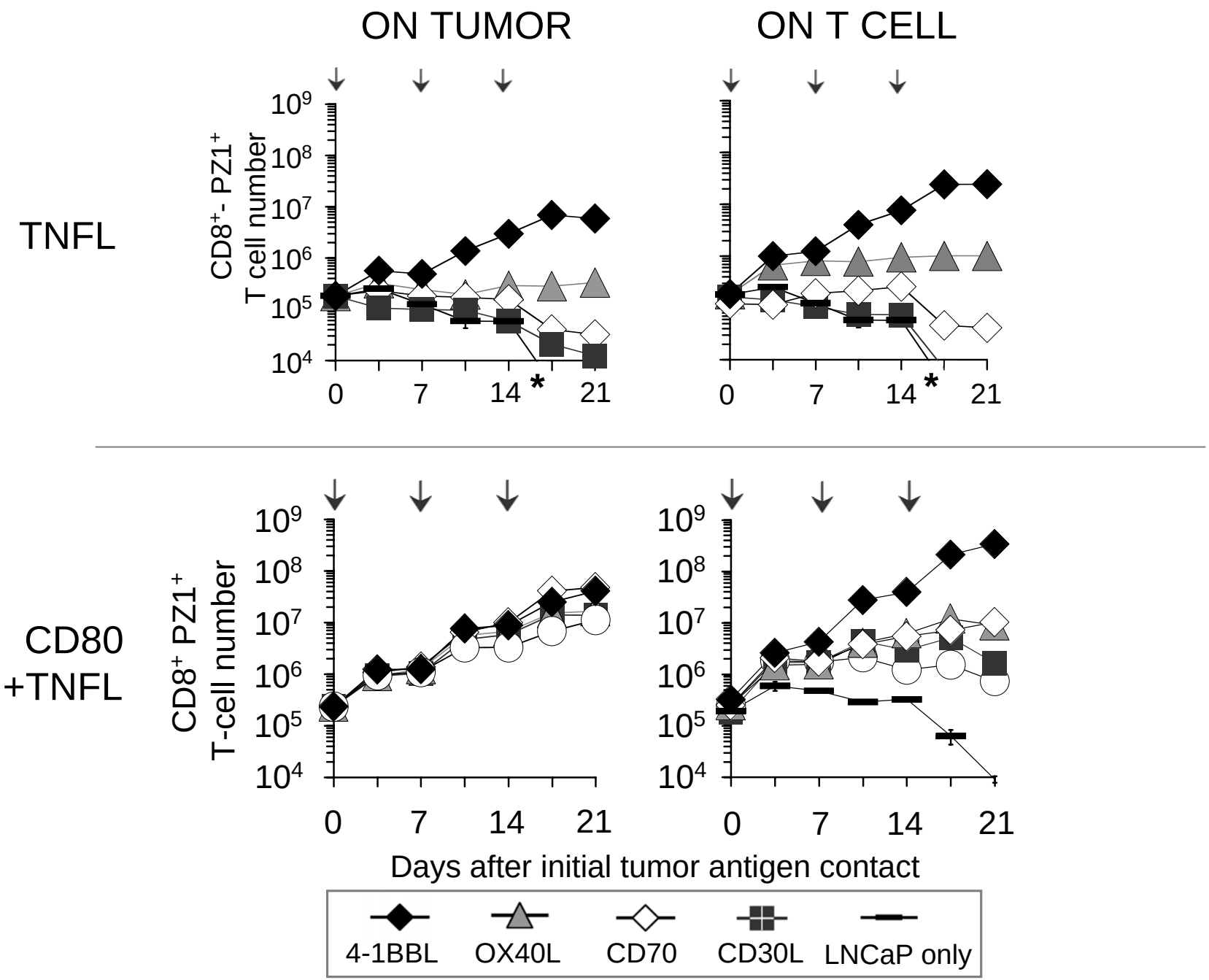
Costimulatory ligands on the Tumor cell

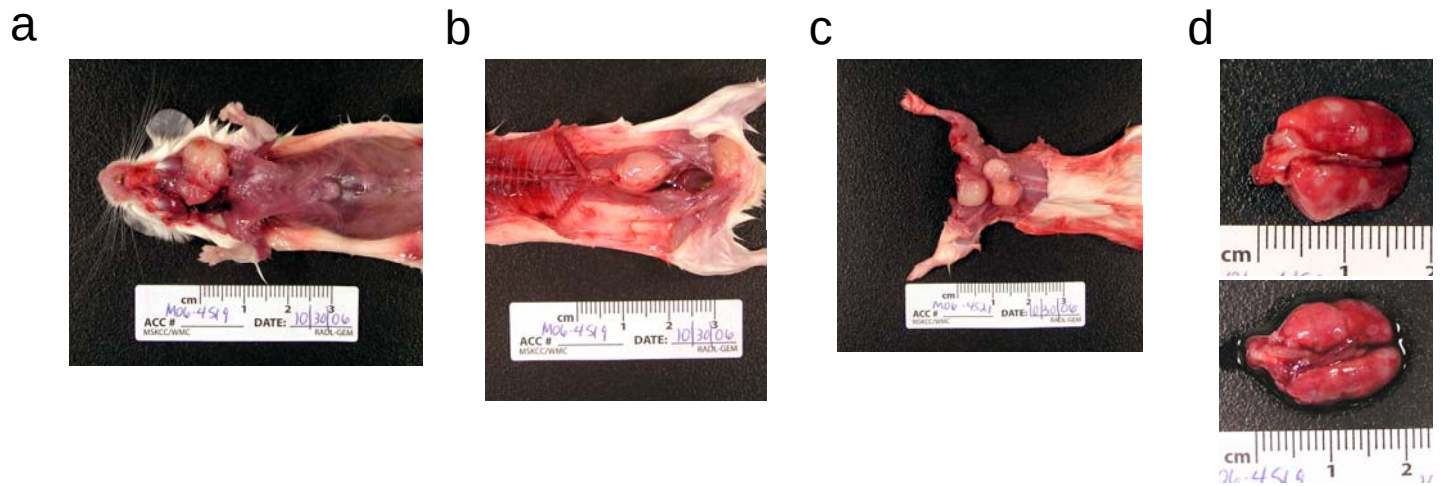
❷

Costimulatory ligands on the T cell





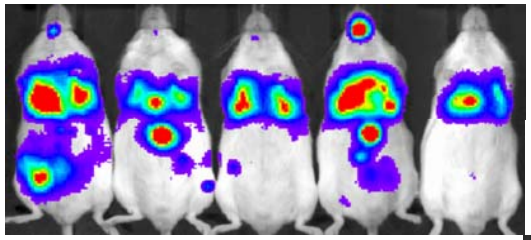

ON TUMOR
ON T CELL
TNFL
CD80 +TNFL
CD8+- PZ1+ T cell number
CD8+ PZ1+ T-cell number
10^9
10^8
10^7
10^6
10^5
10^4
0
7
14
21
*
Days after initial tumor antigen contact
4-1BBL
OX40L
CD70
CD30L
LNCaP only



Supplementary Figure 4

DAY 0

19ζ-CONTROL



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PZ1

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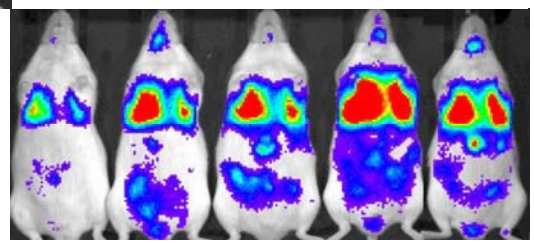

PZ1-CD80

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PZ1-4-1BBL

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PZ1-4-1BBL CD80

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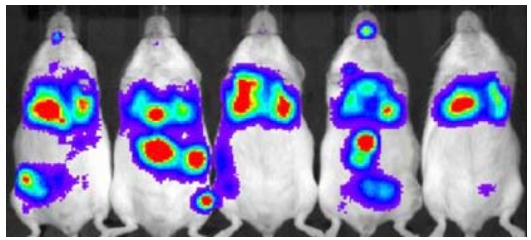
DAY 2

19ζ-CONTROL



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PZ1

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PZ1-CD80

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PZ1-4-1BBL

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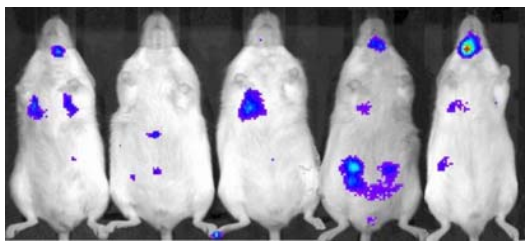
PZ1-4-1BBL CD80



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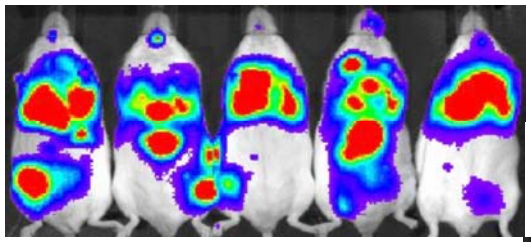
DAY 10

19ζ-CONTROL



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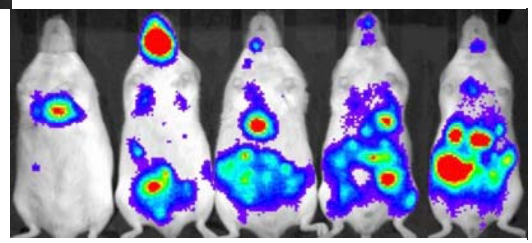

PZ1-CD80

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PZ1-4-1BBL

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QuickTime™ and a
TIFF (Uncompressed) decompressor
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PZ1-4-1BBL CD80

QuickTime™ and a
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TIFF (Uncompressed) decompressor
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DAY 26

19ζ-CONTROL

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QuickTime™ and a
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PZ1

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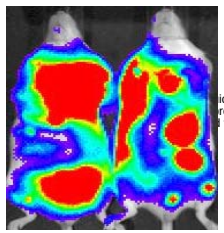
PZ1-CD80



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PZ1-4-1BBL

QuickTime™ and a
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PZ1-4-1BBL CD80

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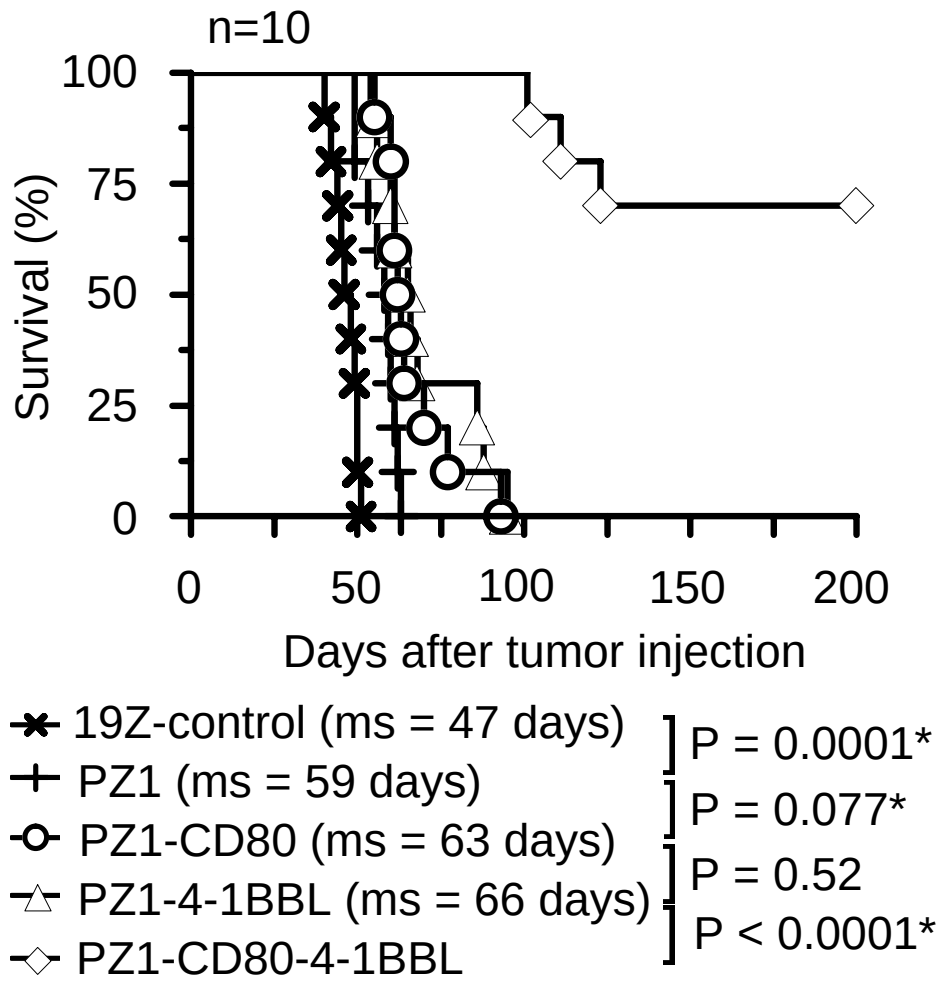
n=10
100
75
50
25
0
Survival (%)
0
50
100
150
200
Days after tumor injection
19Z-control (ms = 47 days)
PZ1 (ms = 59 days)
PZ1-CD80 (ms = 63 days)
PZ1-4-1BBL (ms = 66 days)
PZ1-CD80-4-1BBL
P = 0.0001*
P = 0.077*
P = 0.52
P < 0.0001*

12 hrs

19Z-4-1BBL CD80-CONTROL

QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.

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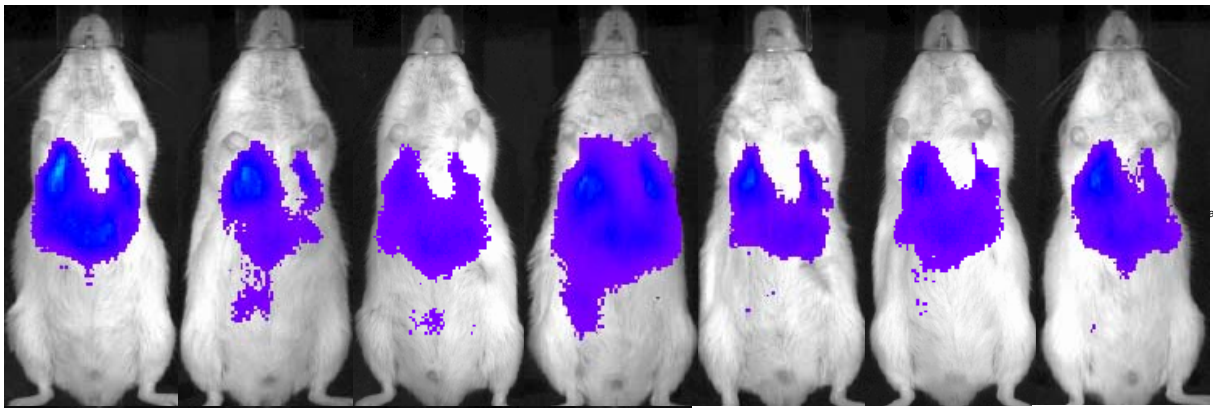
QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.

T-CELL IMAGING

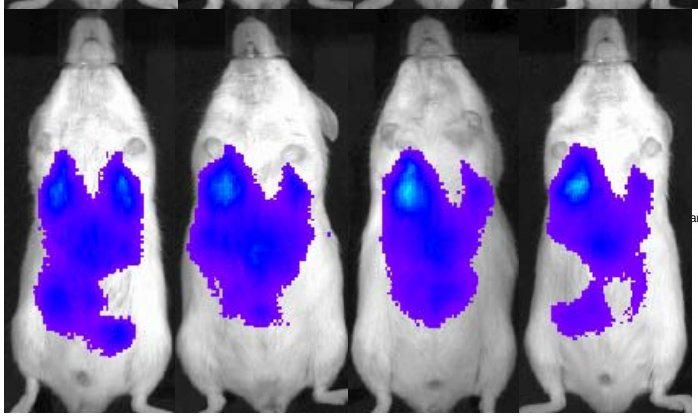
PZ1



QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.

QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.

PZ1-4-1BBL CD80



QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.

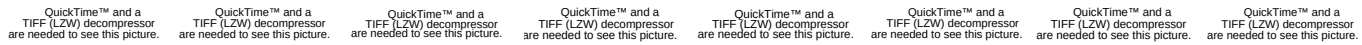
QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.

QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.

QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.

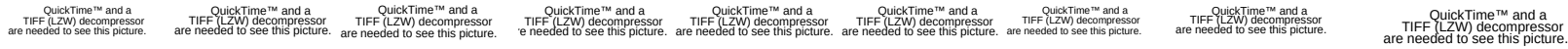
DAY 2

19Z-4-1BBL CD80-CONTROL

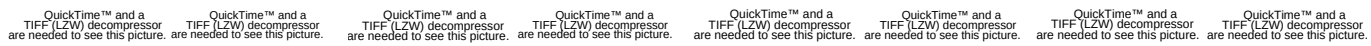


T-CELL IMAGING

PZ1

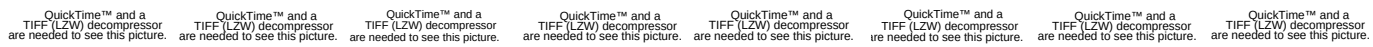


PZ1-4-1BBL CD80



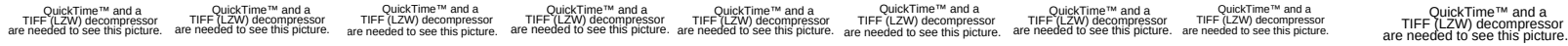
DAY 6

19Z-4-1BBL CD80-CONTROL

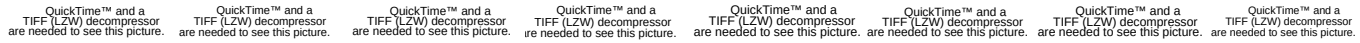


T-CELL IMAGING

PZ1

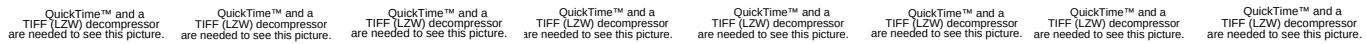


PZ1-4-1BBL CD80



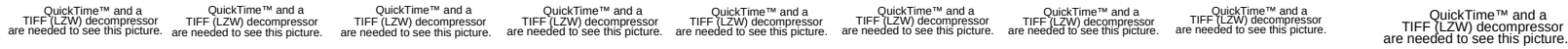
DAY 18

19Z-4-1BBL CD80-CONTROL

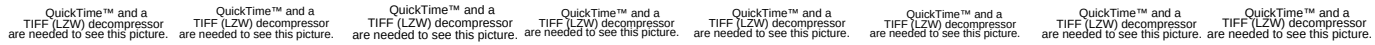


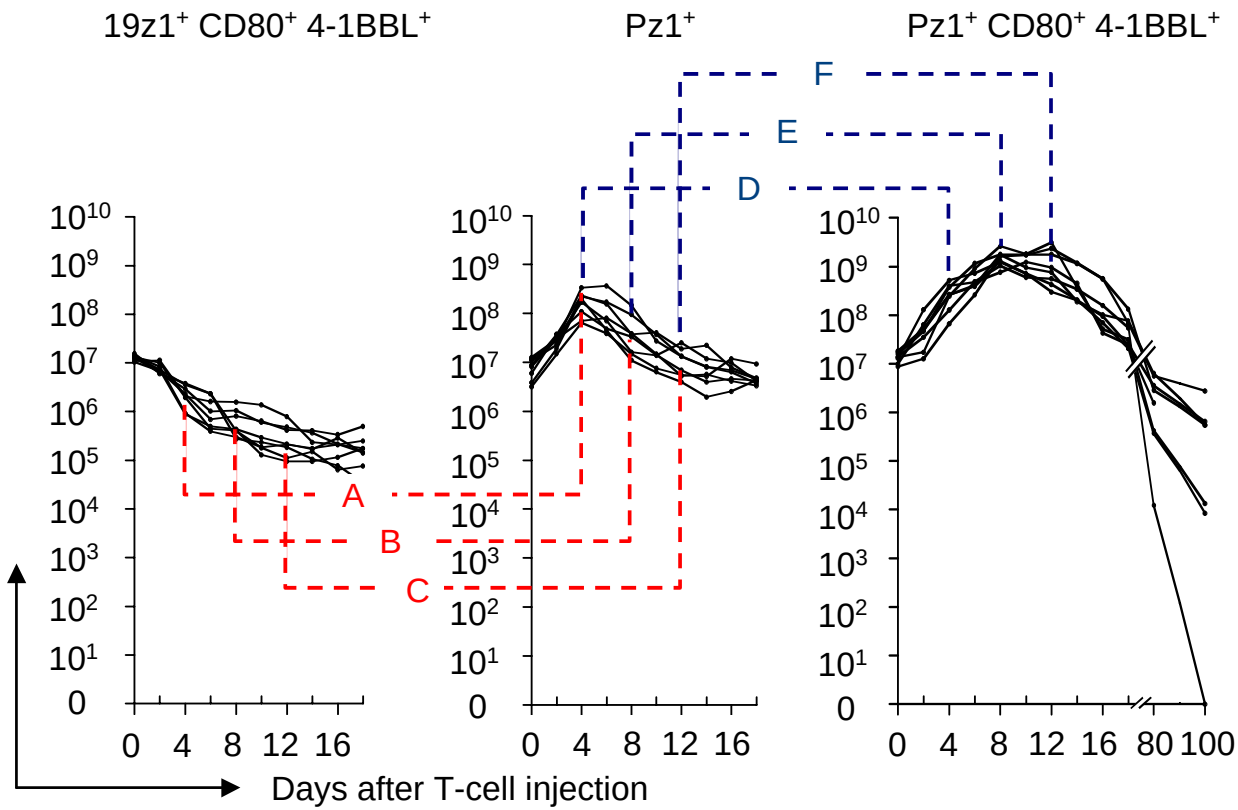
T-CELL IMAGING

PZ1



PZ1-4-1BBL CD80

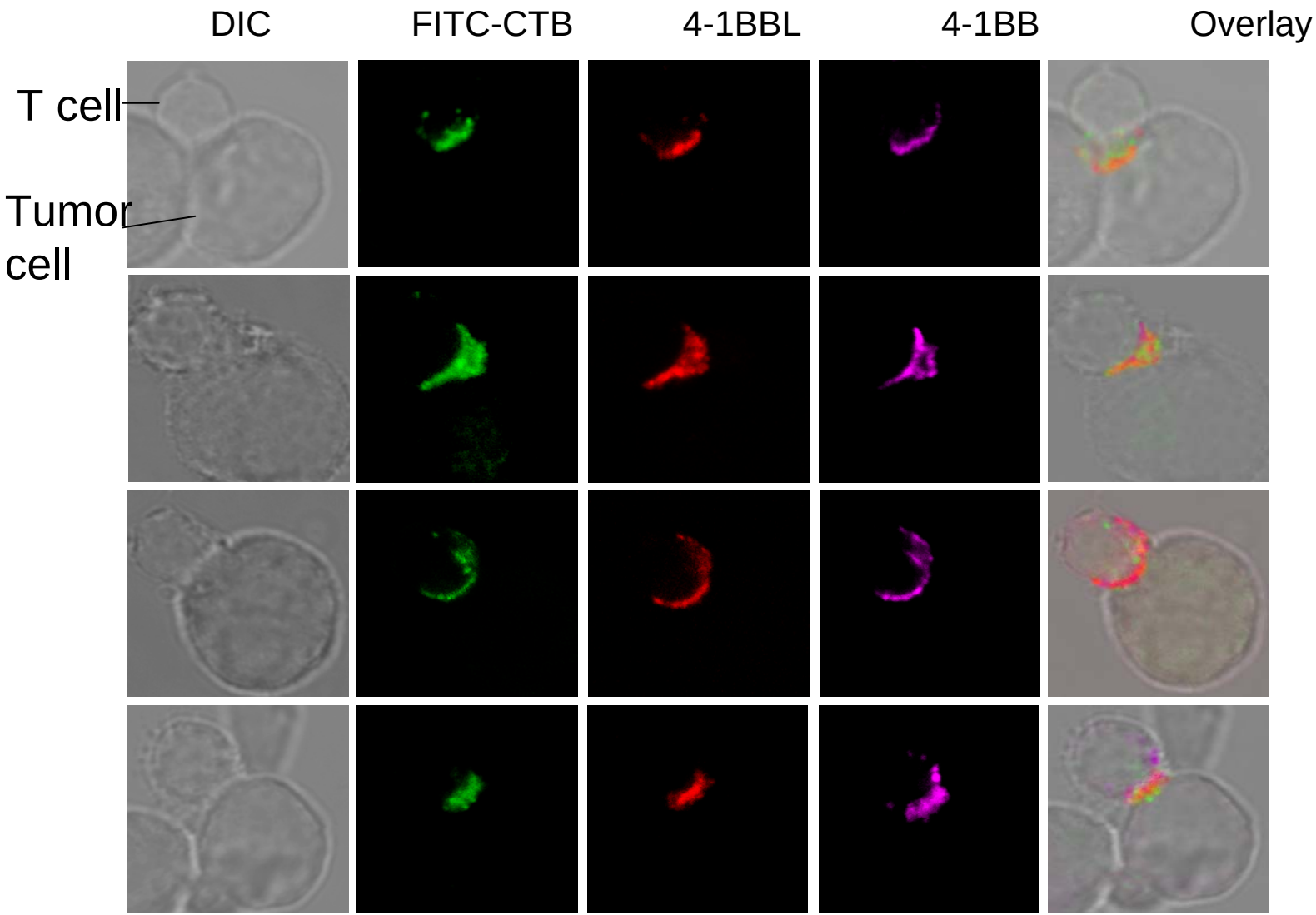


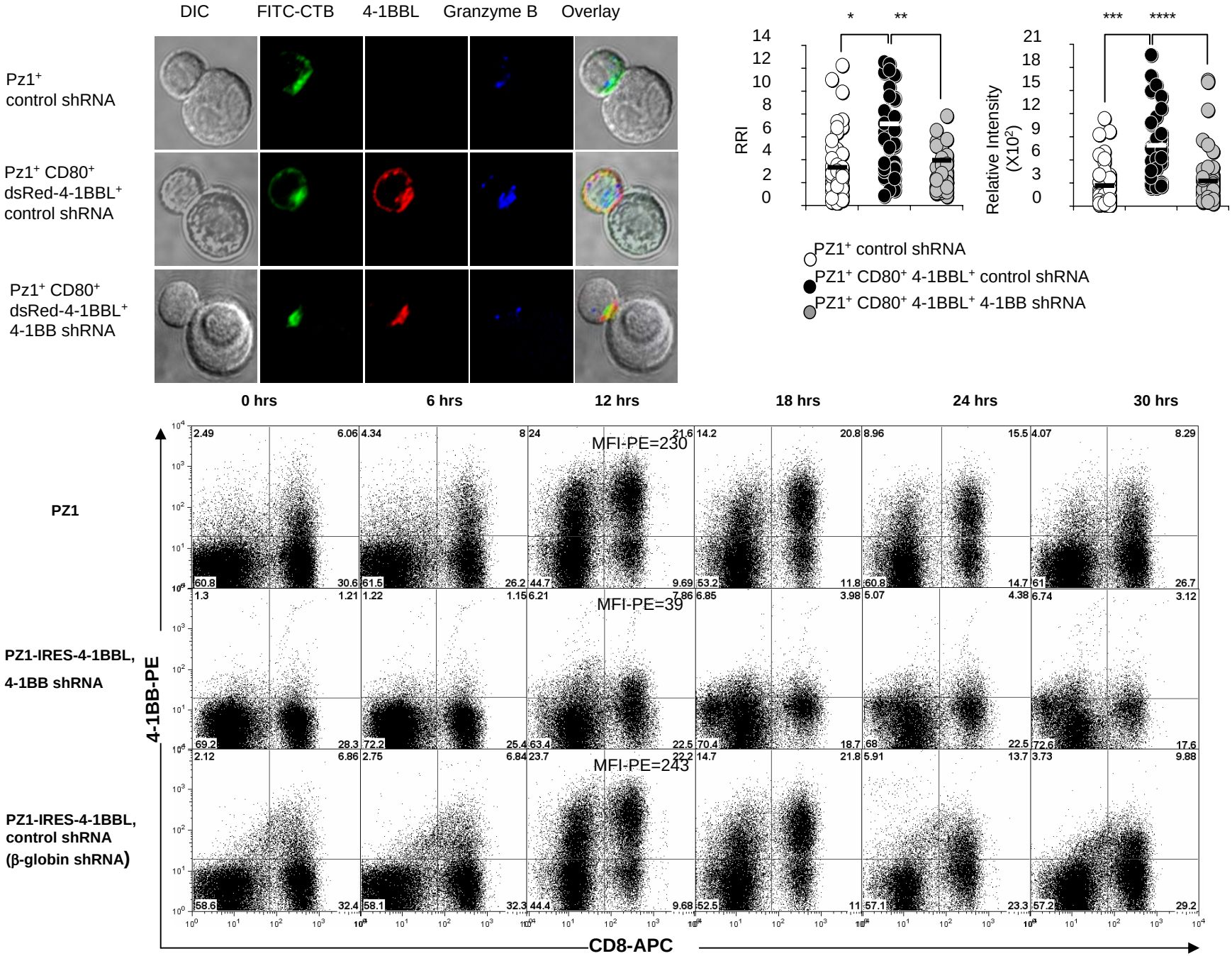


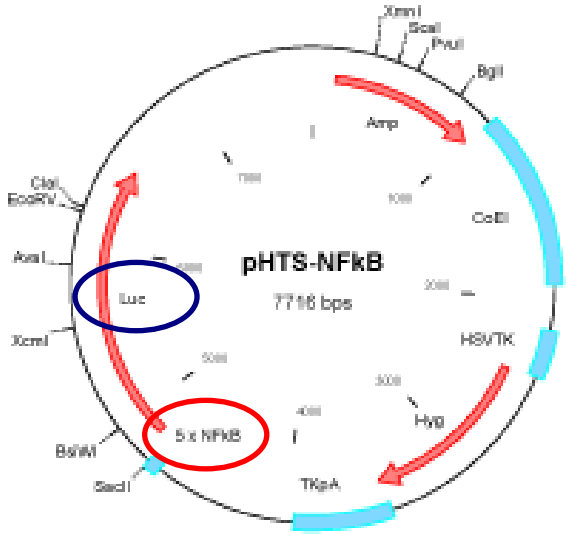
A = median 85.4-fold ↑ (P = 0.0009)
B = median 84.6-fold ↑ (P = 0.0009)
C = median 31.8-fold ↑ (P = 0.0009)

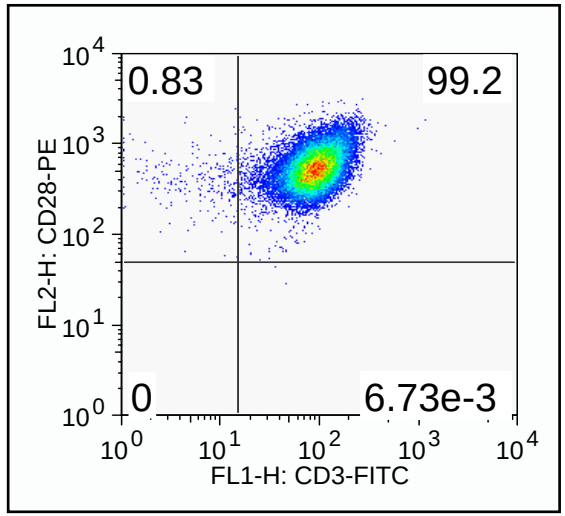
D = median 1.4-fold ↑ (P = 0.0831)
E = median 40.7-fold ↑ (P = 0.0009)
F = median 14.6-fold ↑ (P = 0.0009)

Do CD80+4-1BBL+ T cells auto-costimulate?

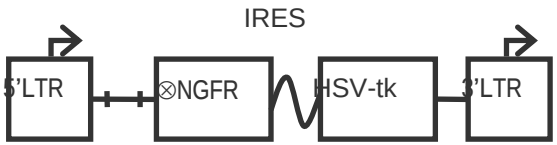



DIC
FITC-CTB
4-1BBL
Granzyme B
Overlay
Pz1+ control shRNA
Pz1+ CD80+ dsRed-4-1BBL+ control shRNA
Pz1+ CD80+ dsRed-4-1BBL+ 4-1BB shRNA
RRI
Relative Intensity (X10²)
PZ1+ control shRNA
PZ1+ CD80+ 4-1BBL+ control shRNA
PZ1+ CD80+ 4-1BBL+ 4-1BB shRNA
0 hrs
6 hrs
12 hrs
18 hrs
24 hrs
30 hrs
PZ1
PZ1-IRES-4-1BBL, 4-1BB shRNA
PZ1-IRES-4-1BBL, control shRNA (β-globin shRNA)
MFI-PE=230
MFI-PE=39
MFI-PE=243
4-1BB-PE
CD8-APC

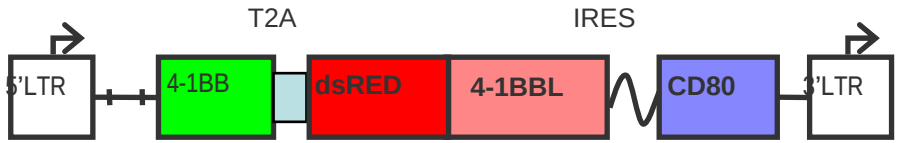


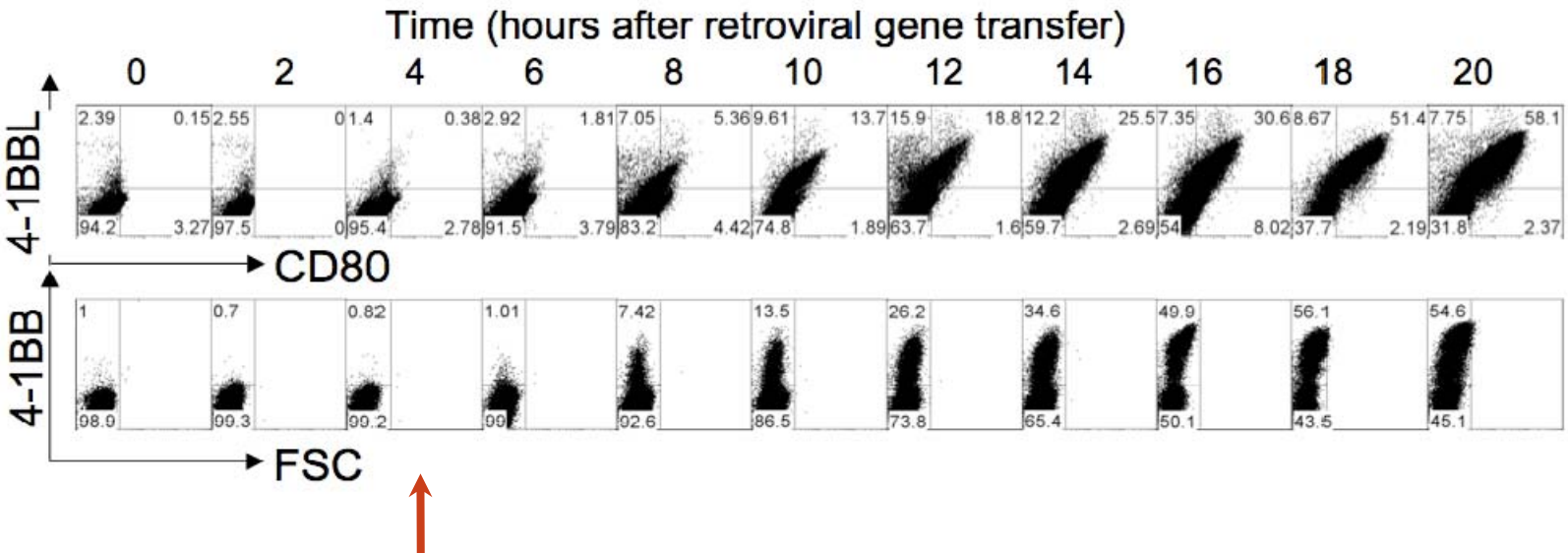


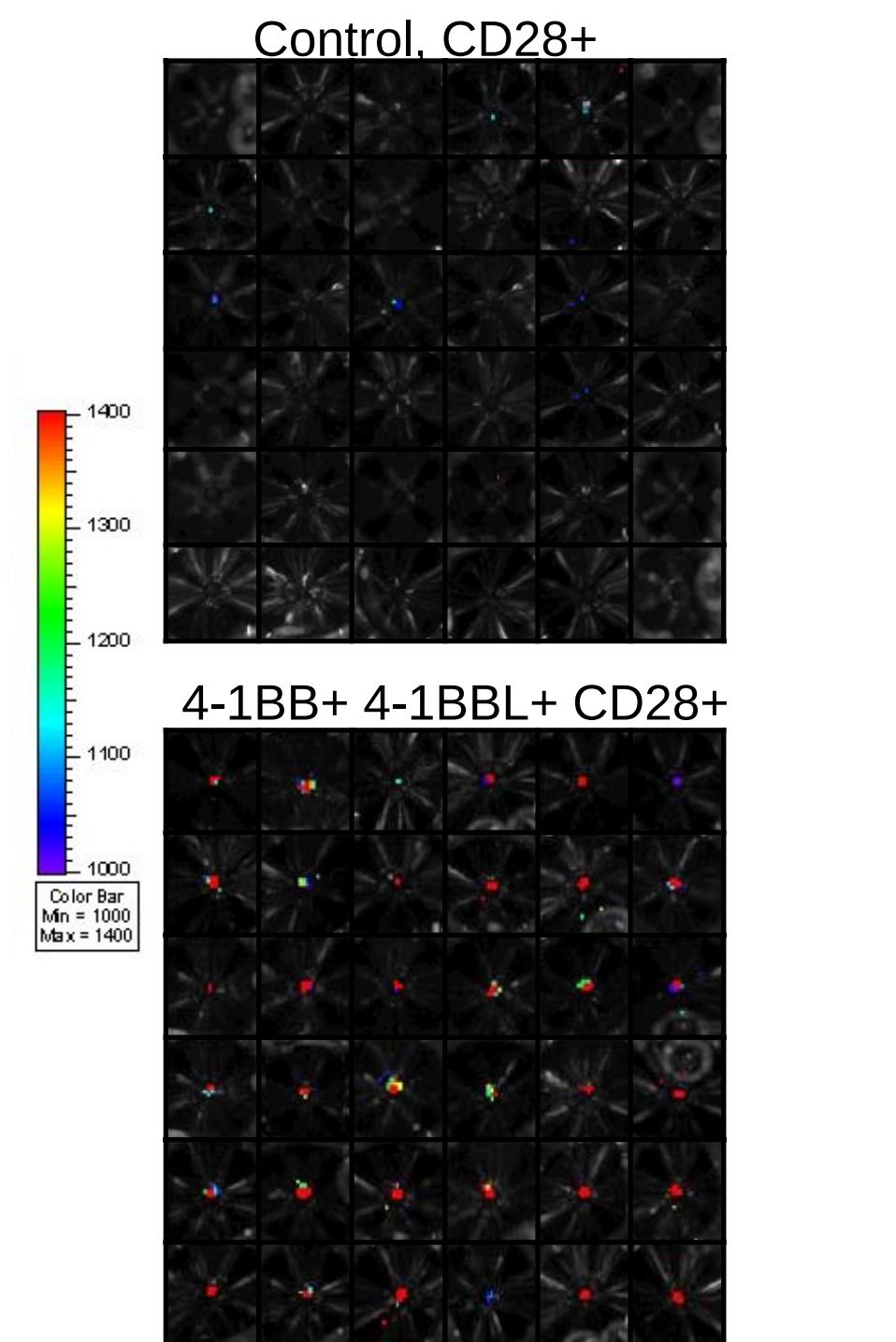
1) SFG-NIT control vector

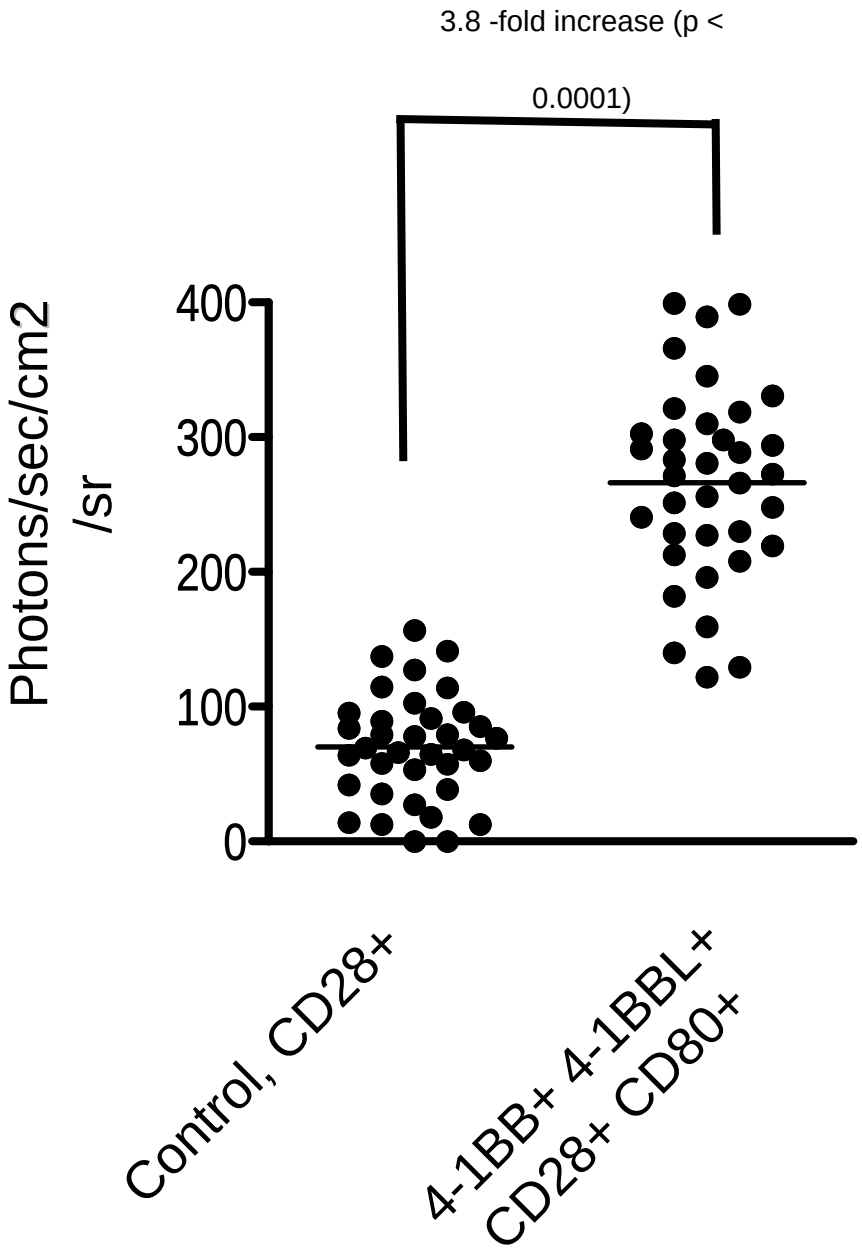


2) SFG-4-1BB-T2A-dsRED-4-1BBL-IRES-CD80






Control, CD28+
4-1BB+ 4-1BBL+ CD28+
1400
1300
1200
1100
1000
Color Bar
Min = 1000
Max = 1400


3.8 -fold increase (p <
0.0001)
Photons/sec/cm2
/sr
400
300
200
100
0
Control, CD28+
4-1BB+ 4-1BBL+
CD28+ CD80+

Can CD80+4-1BBL+ trans-costimulate in the tumor microenvironment?

T cells

Pz1^{+}Click-luc^{+}

19z1^{+}Click-luc^{+}

(all mice)

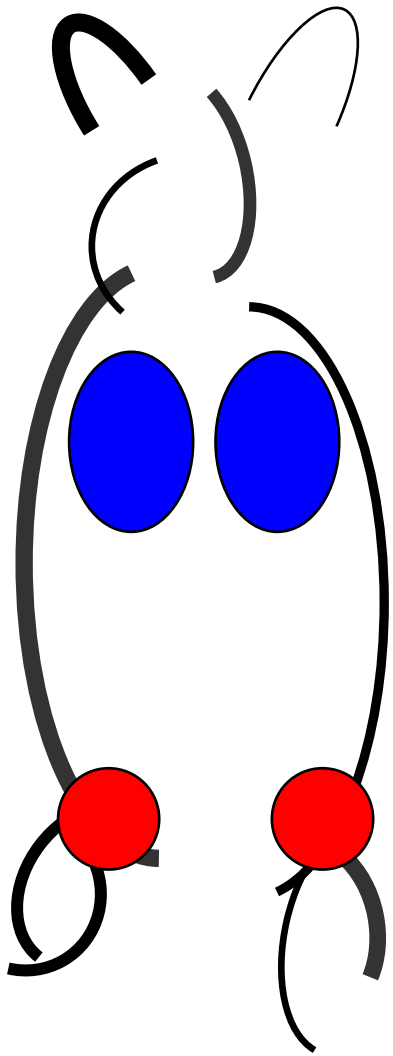
Pz1^{+} T cells

or

Pz1^{+}4-1BBL^{+}CD80^{+}

or

19z1^{+}4-1BBL^{+}CD80^{+}



Tumor targets

RM1.PGLS, Gaussia-luc^{+}

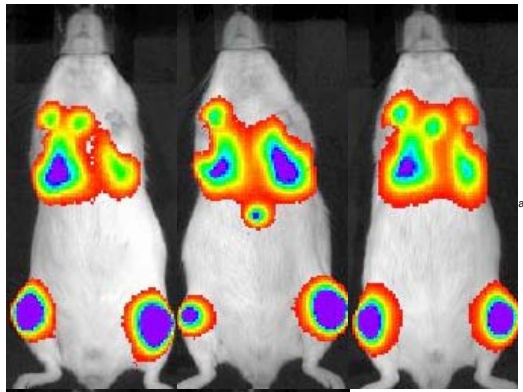
(all mice)

Raji, Gaussia-luc^{+}

(all mice)

DAY 0

PZ1 click
+19Z click
+PZ1

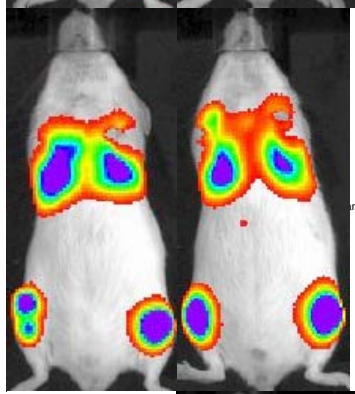


QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

TUMOR IMAGING

PZ1 click
+19Z click
+PZ1-4-1BBL-CD80



QuickTime™ and a
TIFF (LZW) decompressor
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TIFF (LZW) decompressor
are needed to see this picture.

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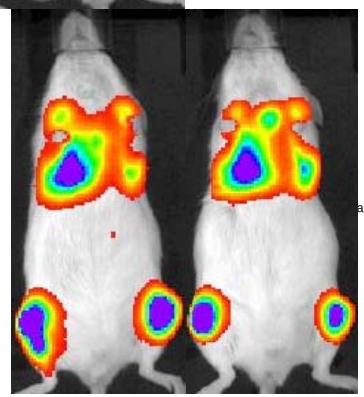
TIFF (LZW) decompressor
are needed to see this picture.

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

PZ1 click
+19Z click
+19Z-4-1BBL-CD80

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.



QuickTime™ and a
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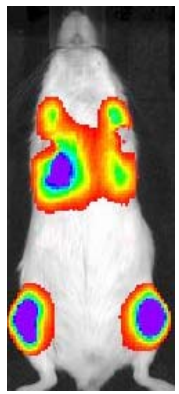

DAY 1

PZ1 click
+19Z click
+PZ1

QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.

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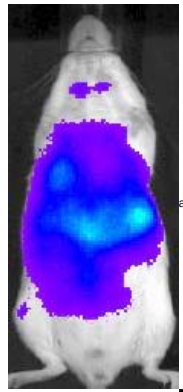
QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.



QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.

T-CELL IMAGING

PZ1 click
+19Z click
+PZ1-4-1BBL-CD80

QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.

QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.

QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.

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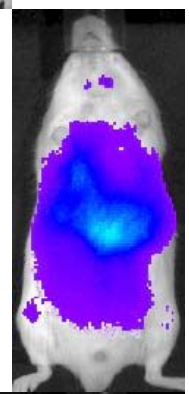
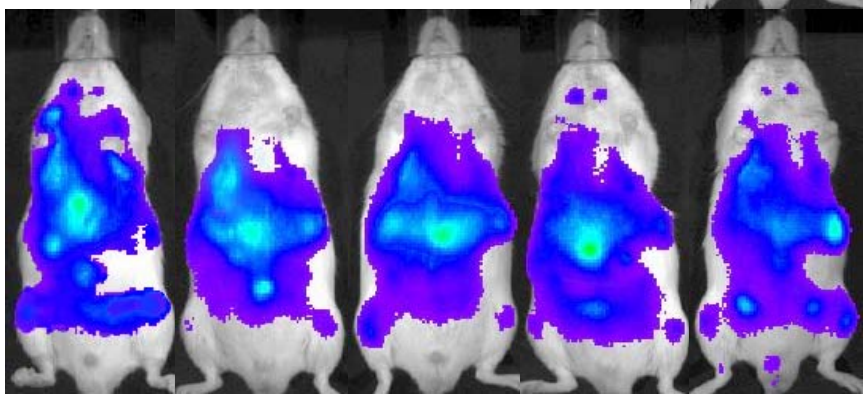


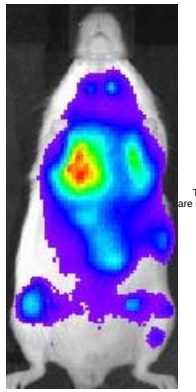
QuickTime™ and a TIFF (LZW) decompressor are needed to see this picture.

PZ1 click
+19Z click
+19Z-4-1BBL-CD80

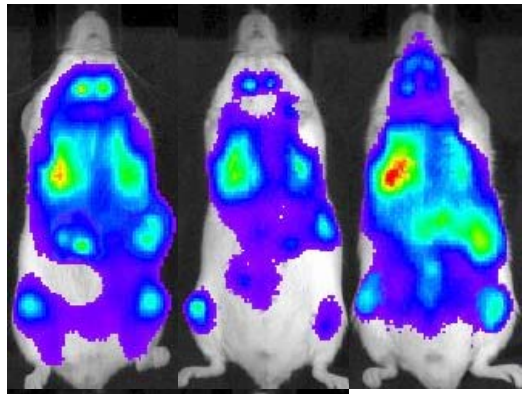


DAY 3

PZ1 click
+19Z click
+PZ1



QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

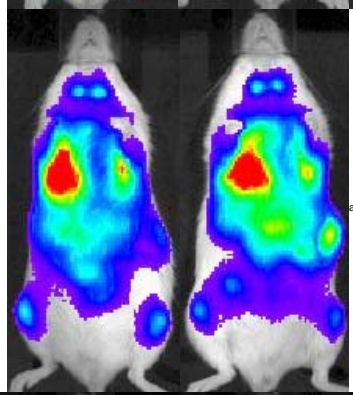


T-CELL IMAGING

PZ1 click
+19Z click
+PZ1-4-1BBL-CD80

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

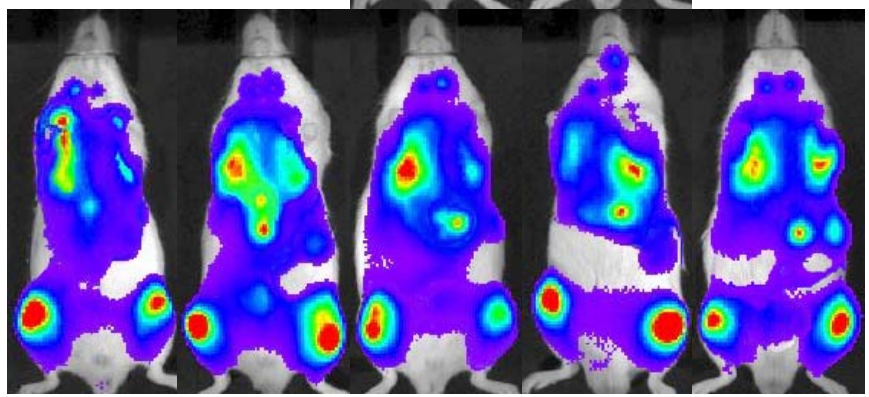
QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.



QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

PZ1 click
+19Z click
+19Z-4-1BBL-CD80



DAY 5

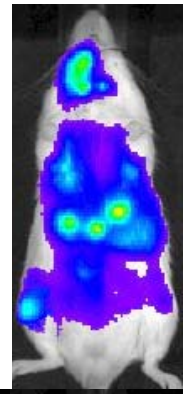
PZ1 click
+19Z click
+PZ1

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

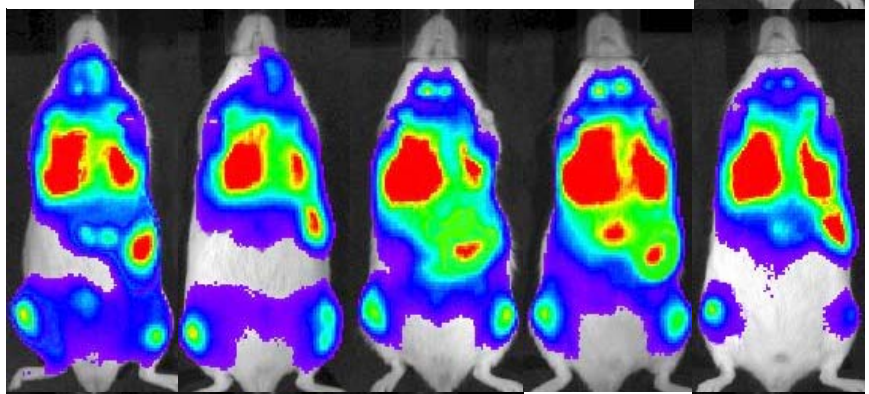
QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.



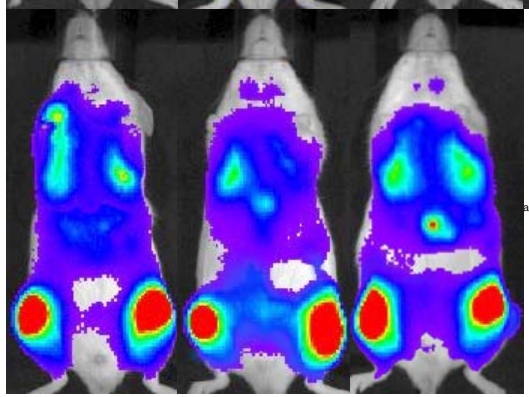
T-CELL IMAGING

PZ1 click
+19Z click
+PZ1-4-1BBL-CD80



QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

PZ1 click
+19Z click
+19Z-4-1BBL-CD80



QuickTime™ and a
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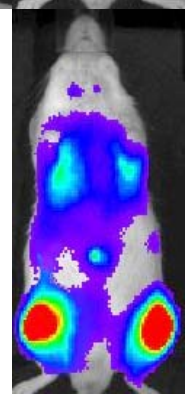
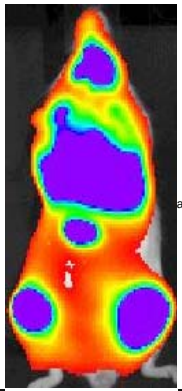


DAY 5 tumor

PZ1 click
+19Z click
+PZ1

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.



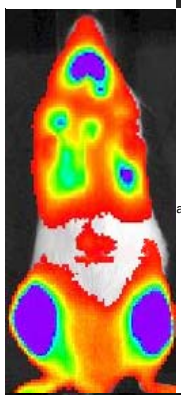
QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

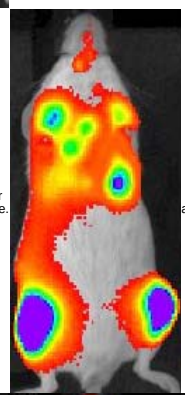
QuickTime™ and a
TIFF (LZW) decompresso
are needed to see this pictu

TUMOR IMAGING

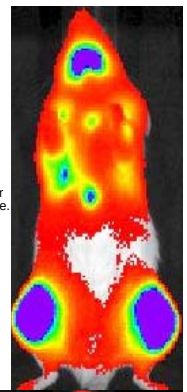
PZ1 click
+19Z click
+PZ1-4-1BBL-CD80



QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

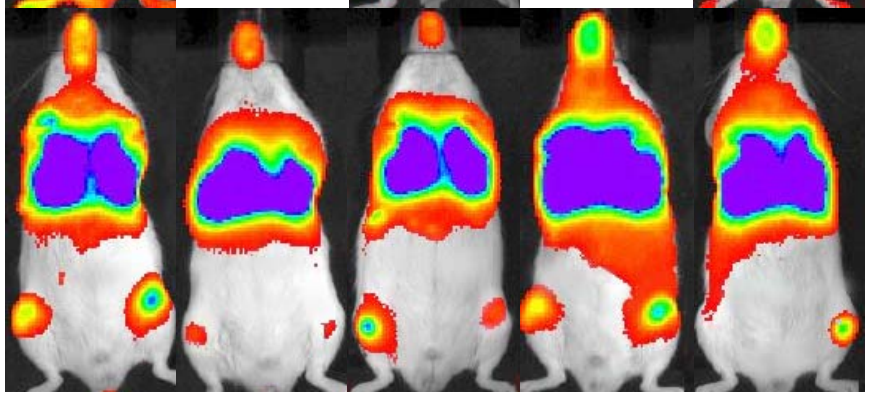


QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.



QuickTime™ and a
TIFF (LZW) decompressor
are needed to see this picture.

PZ1 click
+19Z click
+19Z-4-1BBL-CD80



Conclusions

1. Systemic tumors can be eradicated by CAR-directed human T cells without cytokine support or any other concomitant treatment in SCID mice
2. Xenogeneic tumor models are valuable to assess transduced T cell potency
3. In these models, T cell survival is limiting
4. 4-1BB+CD28 are synergistic in enhancing T cell function and survival
5. T cell potency is proportional to the strength of PI3kinase/Akt activation
- T cell and tumor localizations can be co-registered using BLI
7. T cells expanded under cGMP (i.v. admin) eradicate systemic tumors in mice
8. Have initiated a first trial targeting CD19 in pts with chemorefractory CLL
9. Constitutive expression of costimulatory ligands in T cells sustains antigen-induced T cell survival and function in vitro
10. Auto- and trans-costimulation are promising approaches to enhance T cell function in vivo

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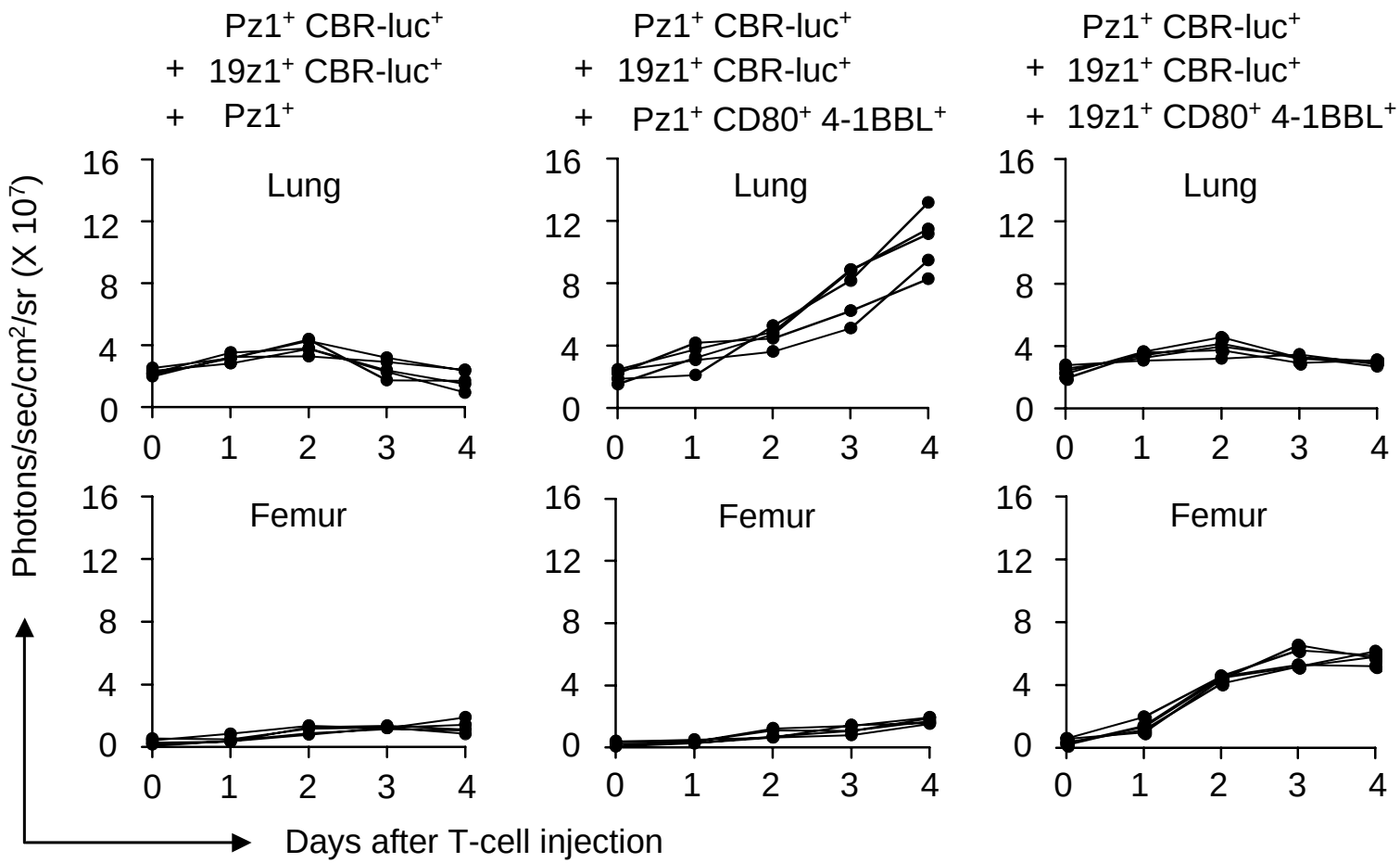
Support

NCI; MSKCC ETC; Alliance for Cancer Gene Therapy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Pz1+ CBR-luc+
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+ 19z1+ CD80+ 4-1BBL+
Lung
Femur
Photons/sec/cm2/sr (X 10^7)
Days after T-cell injection
0
4
8
12
16
0
1
2
3
4