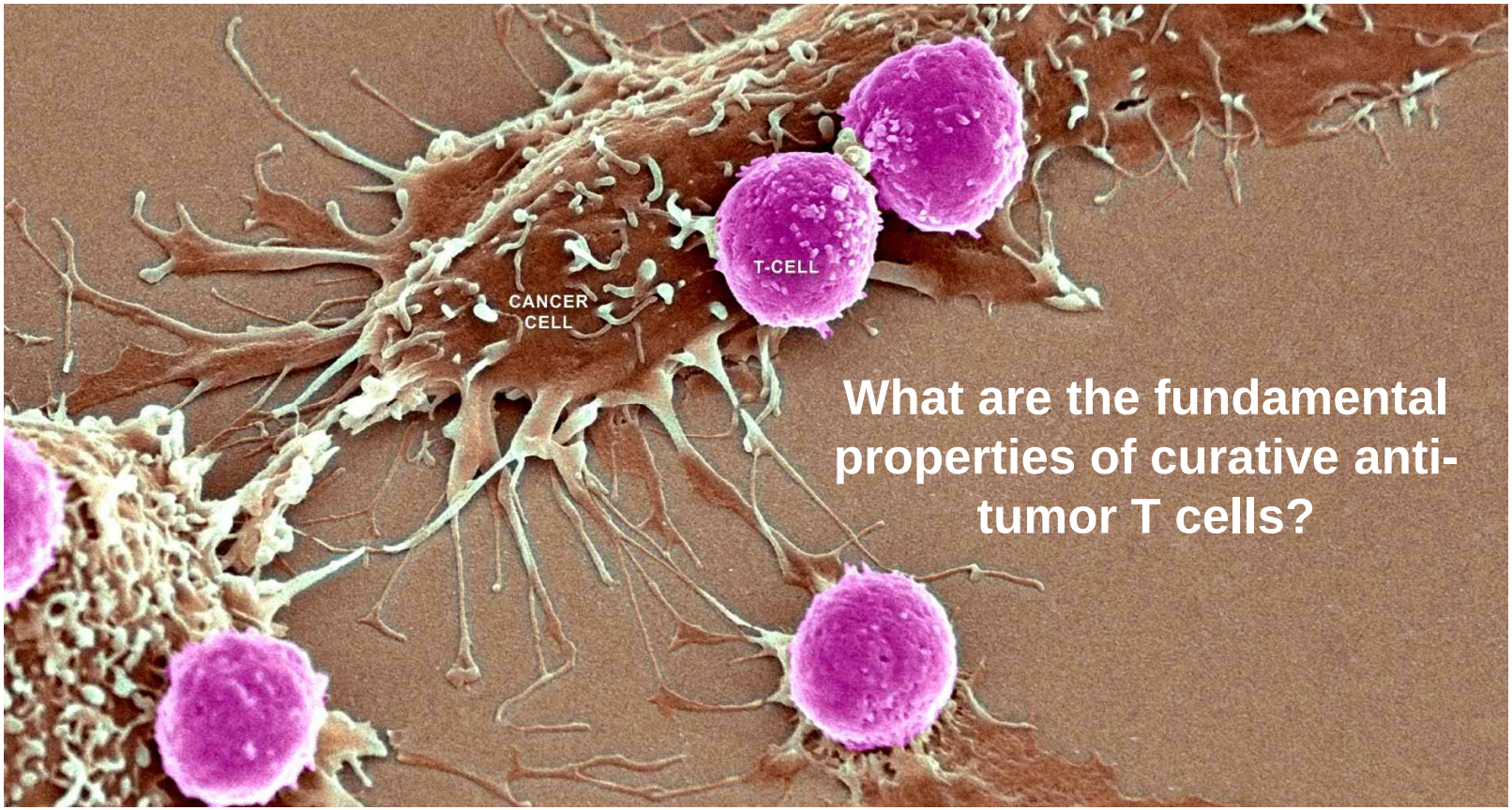


What are the fundamental properties of curative anti-tumor T cells?

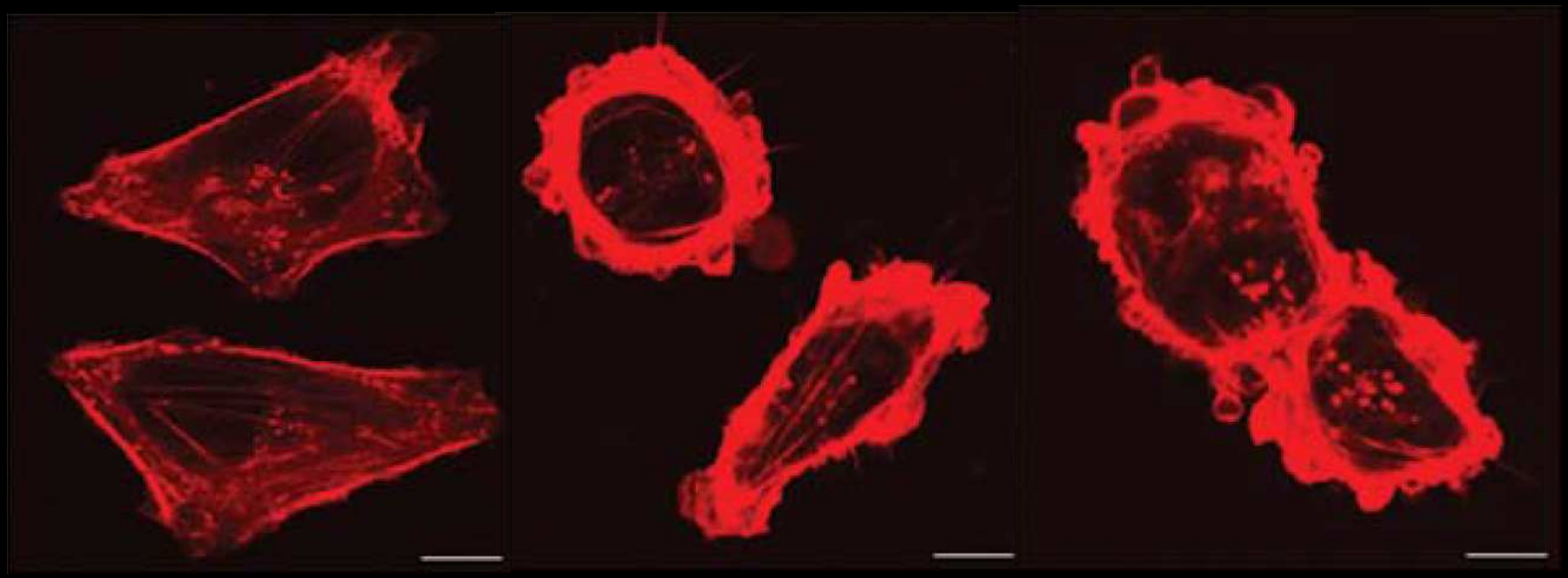
NIH Center for Regenerative Medicine

Nicholas P. Restifo

NATIONAL CANCER INSTITUTE

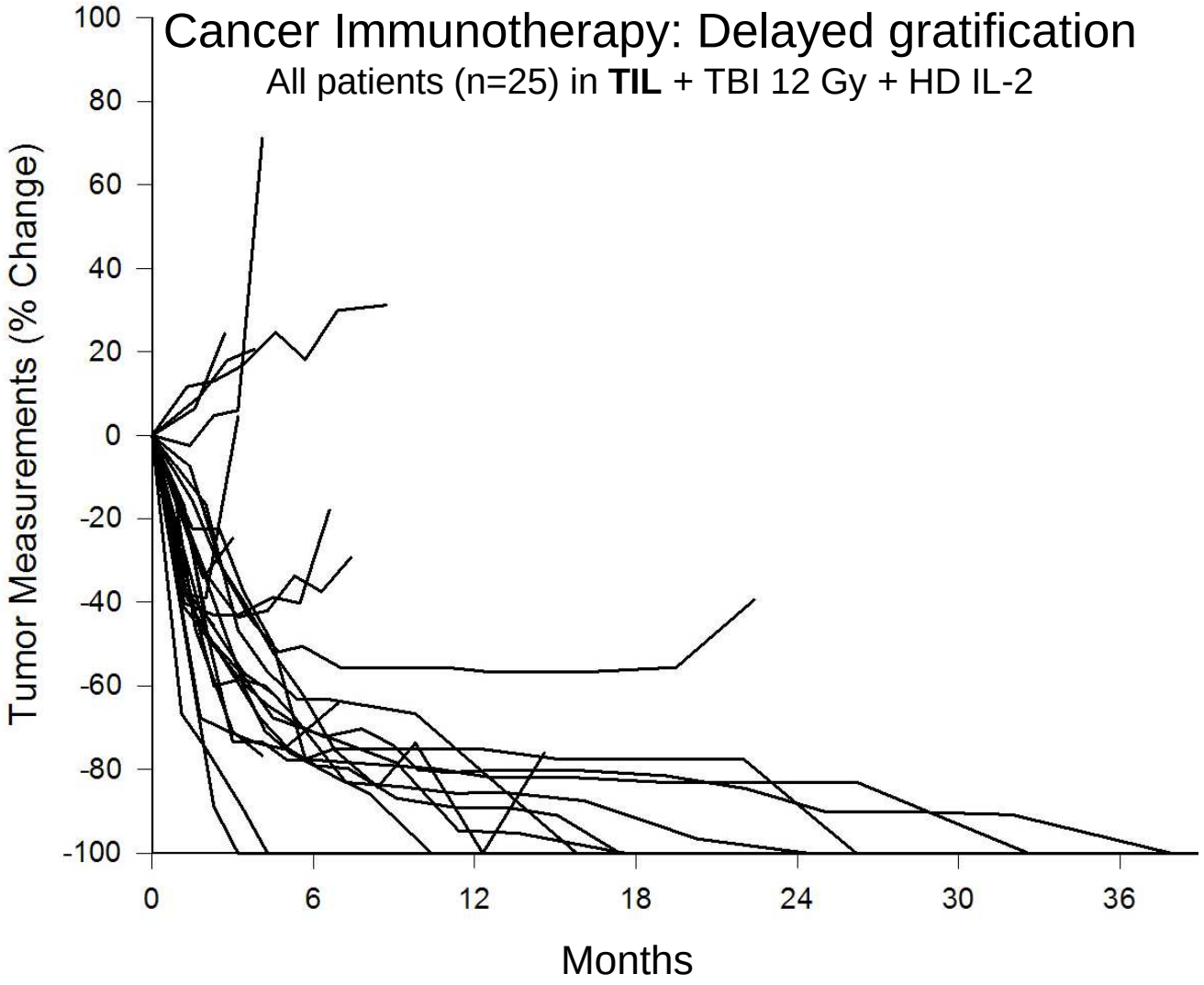
SITC

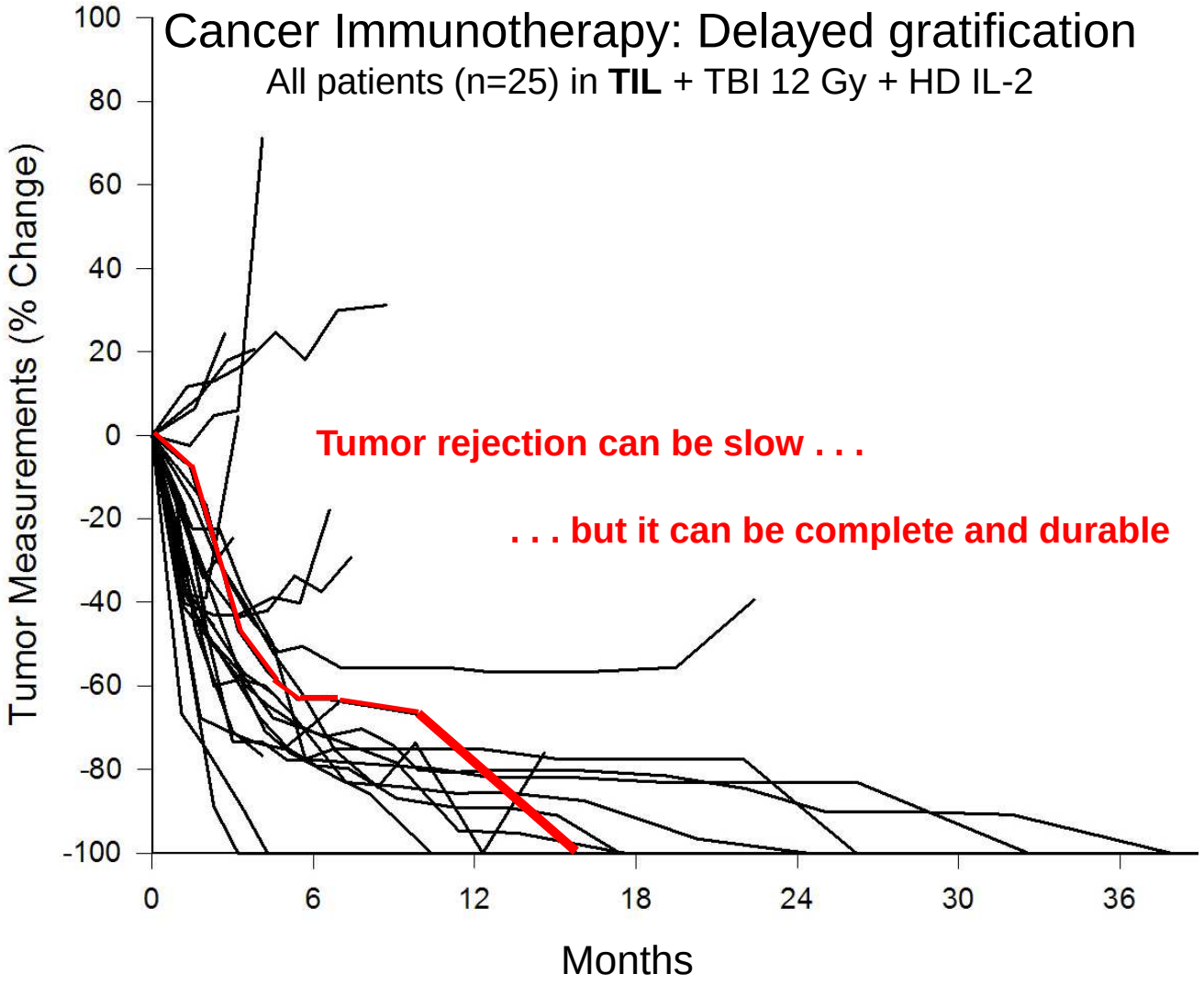
November 6, 2015



Khajah, Int J Oncol, 2015

Surgery, radiation, and chemotherapy / targeted therapy can rapidly kill tumor cells but these modalities can fail to cure in the setting of metastatic solid tumors . . .


Cancer Immunotherapy: Delayed gratification
All patients (n=25) in **TIL** + TBI 12 Gy + HD IL-2
Tumor Measurements (% Change)
100
80
60
40
20
0
-20
-40
-60
-80
-100
0
6
12
18
24
30
36
Months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100
80
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20
0
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-60
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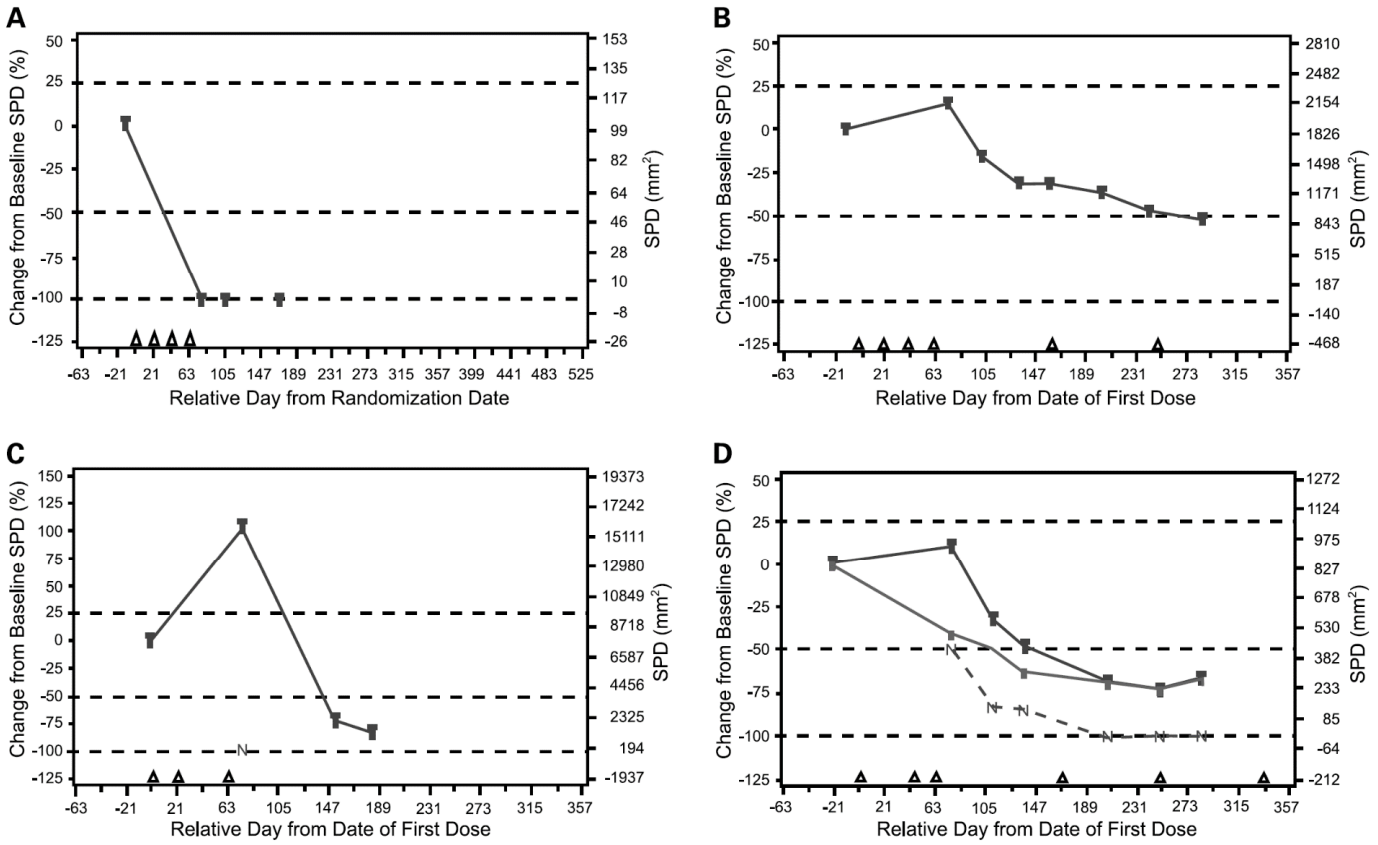
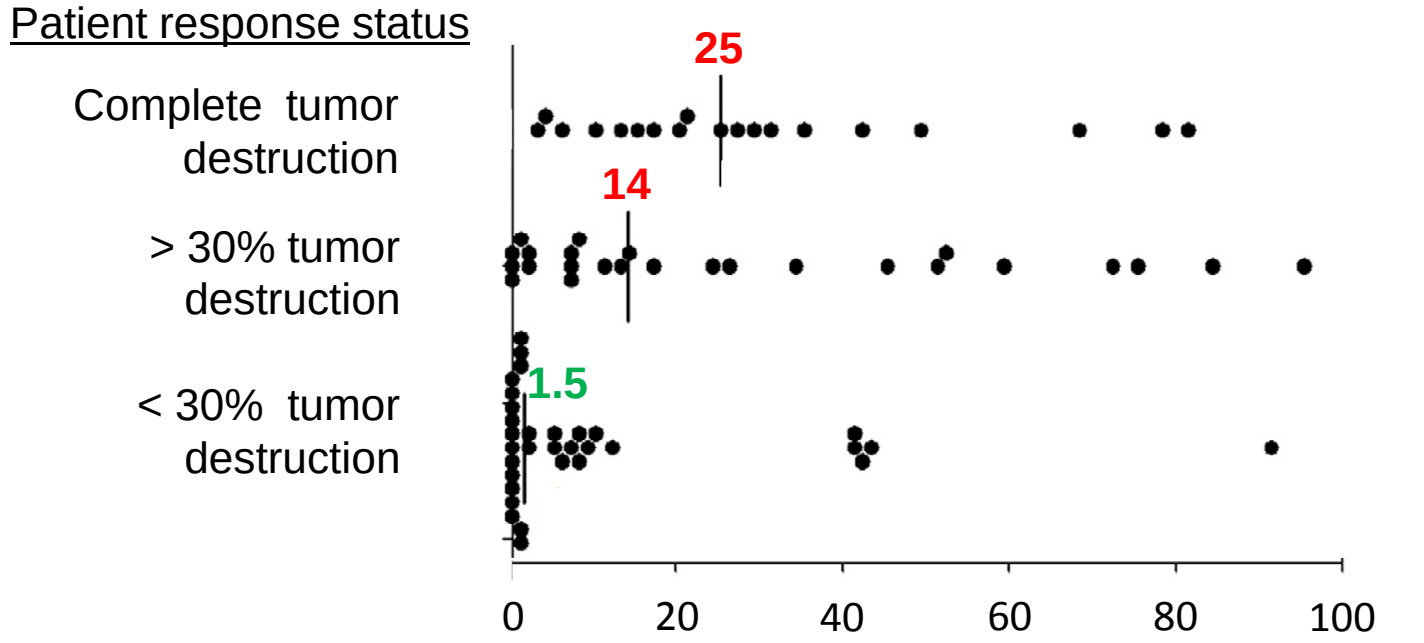
0
6
12
18
24
30
36
Months
Tumor rejection can be slow . . .
. . . but it can be complete and durable

Patterns of response to Ipilumumab in 4 patients with melanoma



Wolchok, et al, Clin Cancer Res, 2009

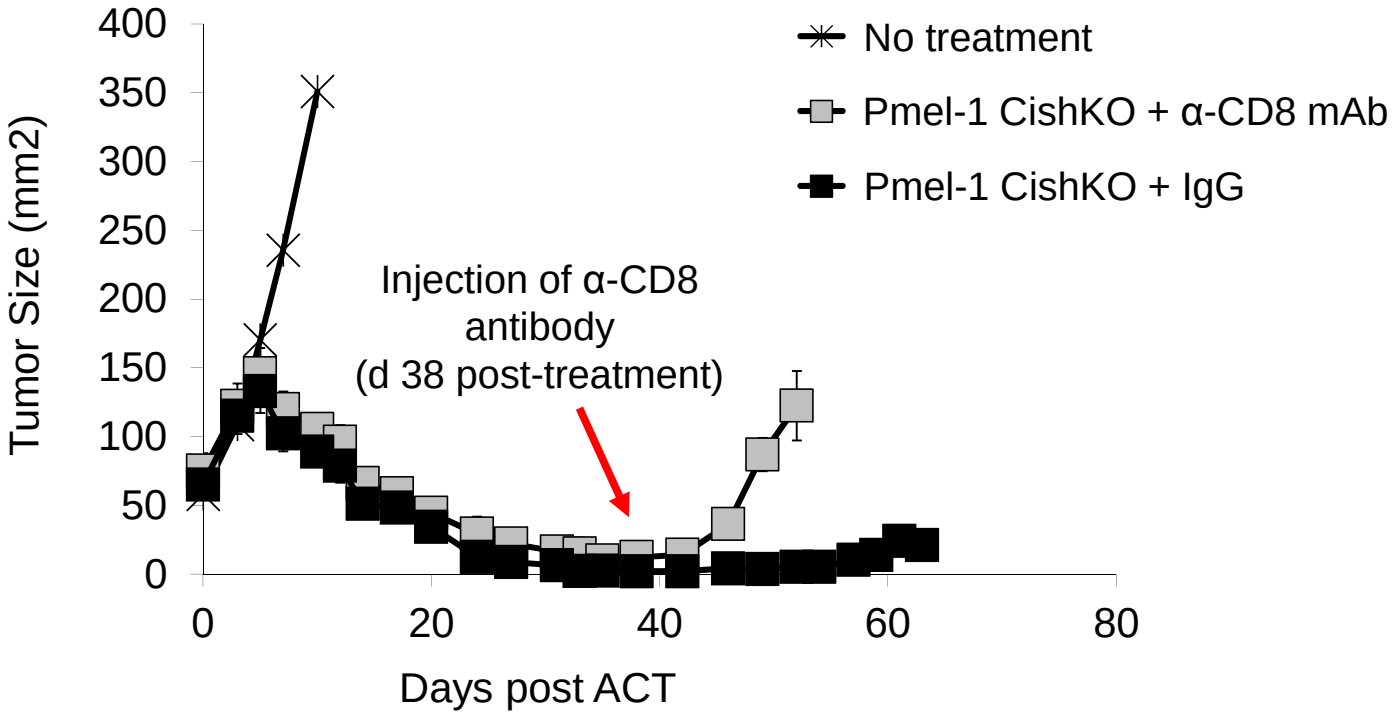
T cell persistence at 1 month is highly correlated with objective clinical response



Rosenberg SA et al. *Clin Cancer Res,* 2011

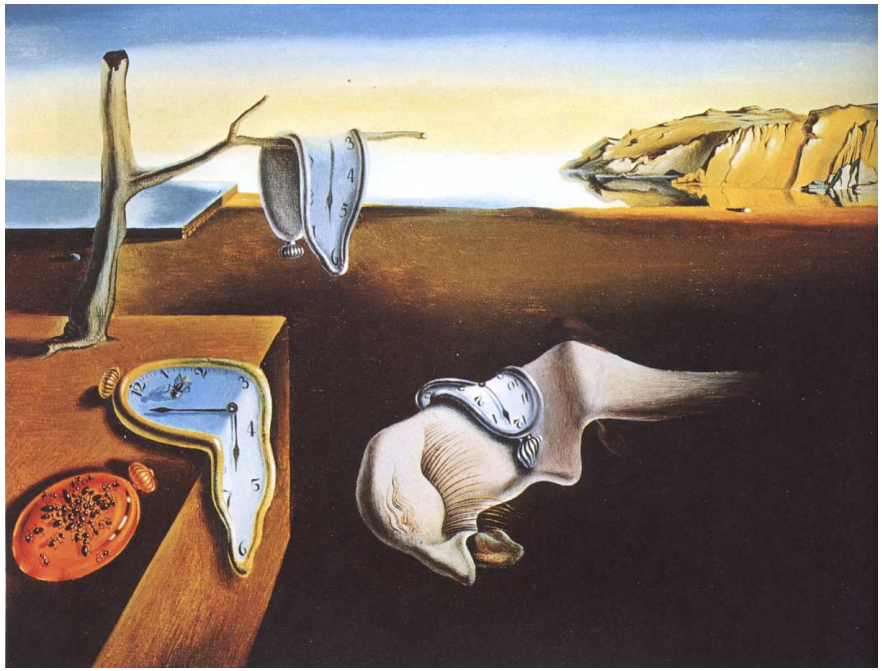
* CR + PR vs. NR < 0.001

Prolonged tumor regression is mediated by ongoing activity of living CD8^{+} T cells



Palmer, et al, *J Exp Med, Online ahead of print* Nov 2, 2015

How do we achieve the persistence of memory?



PNAS

Memory T and memory B cells share a transcriptional program of self-renewal with long-term hematopoietic stem cells

Chance John Luckey*†, Deepta Bhattacharya*†‡, Ananda W. Goldrath*†§, Irving L. Weissman‡, Christophe Benoist*¶, and Diane Mathis*¶

*Joslin Diabetes Center; Departments of Pathology and Medicine, Brigham and Women's Hospital, Harvard Medical School, 1 Joslin Place, Boston, MA 02215; and ‡Stanford Institute for Stem Cell Biology and Regenerative Medicine, Department of Pathology, Stanford University School of Medicine, Stanford, CA 94305-5323

Contributed by Diane Mathis, December 23, 2005

ARTICLES

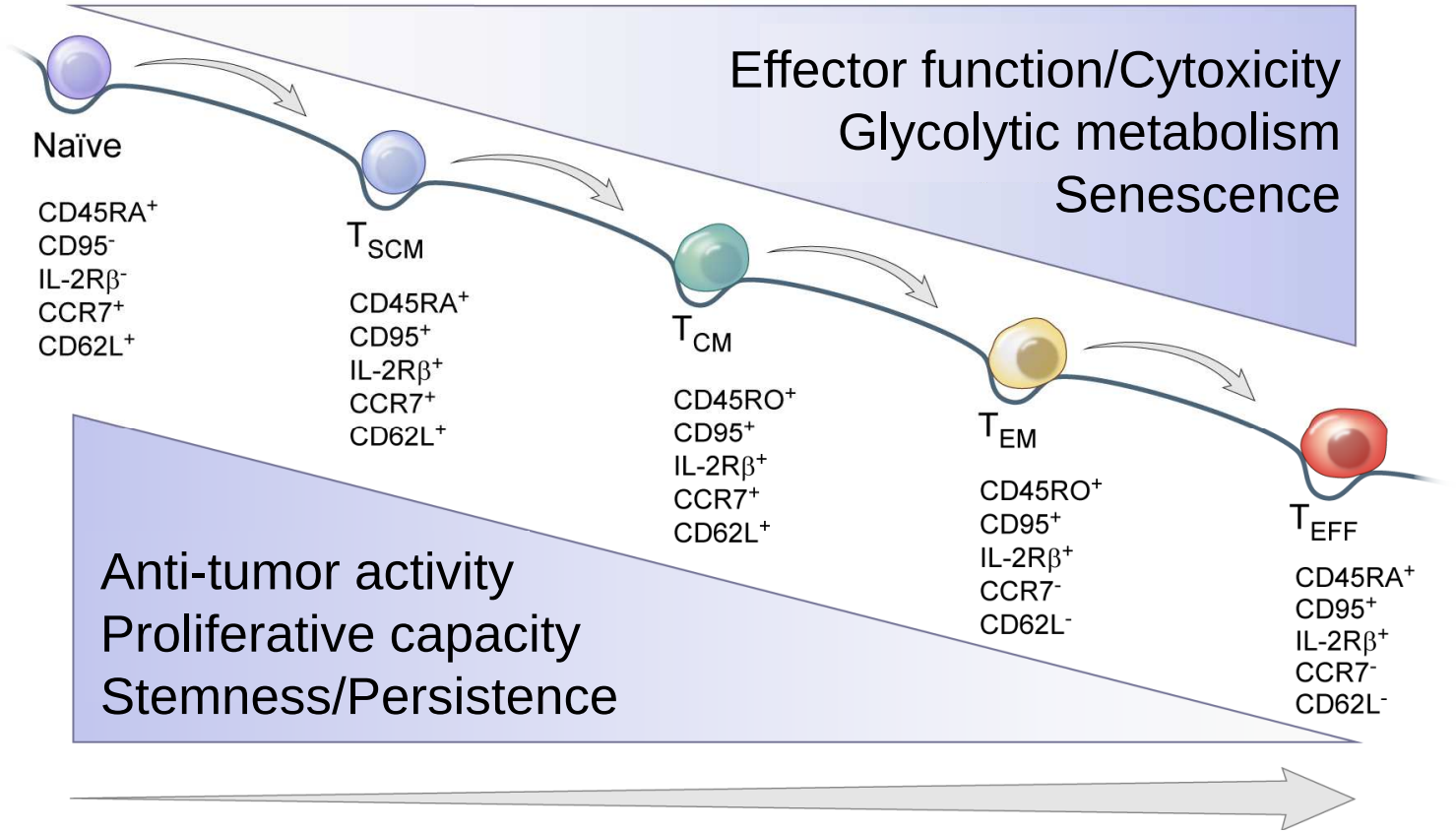
nature medicine

A human memory T cell subset with stem cell–like properties

Luca Gattinoni[1,9], Enrico Lugli[2,9], Yun Ji[1], Zoltan Pos[3,4], Chrystal M Paulos[5,6], Máire F Quigley[7,8], Jorge R Almeida[8], Emma Gostick[7], Zhiya Yu[1], Carmine Carpenito[5,6], Ena Wang[3,4], Daniel C Douek[8], David A Price[7,8], Carl H June[5,6], Francesco M Marincola[3,4], Mario Roederer[2,9] & Nicholas P Restifo[1,9]

VOLUME 17 | NUMBER 10 | OCTOBER 2011

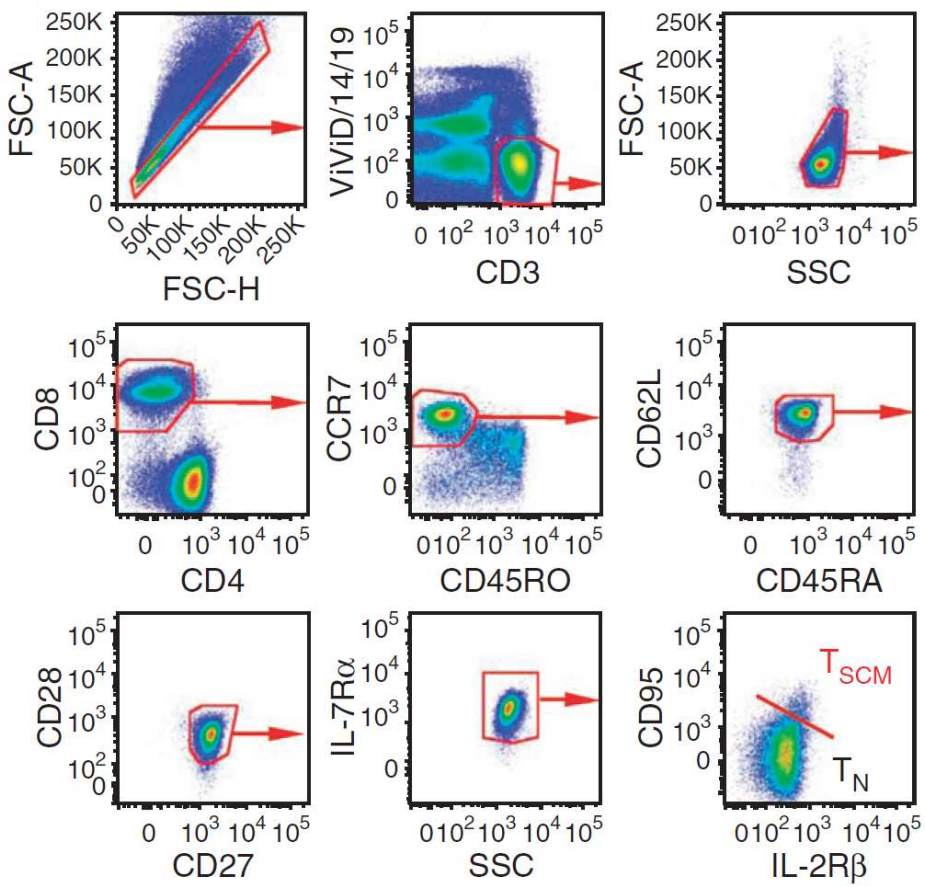
Stem cell-like capacity for each persisting T cell clonotype



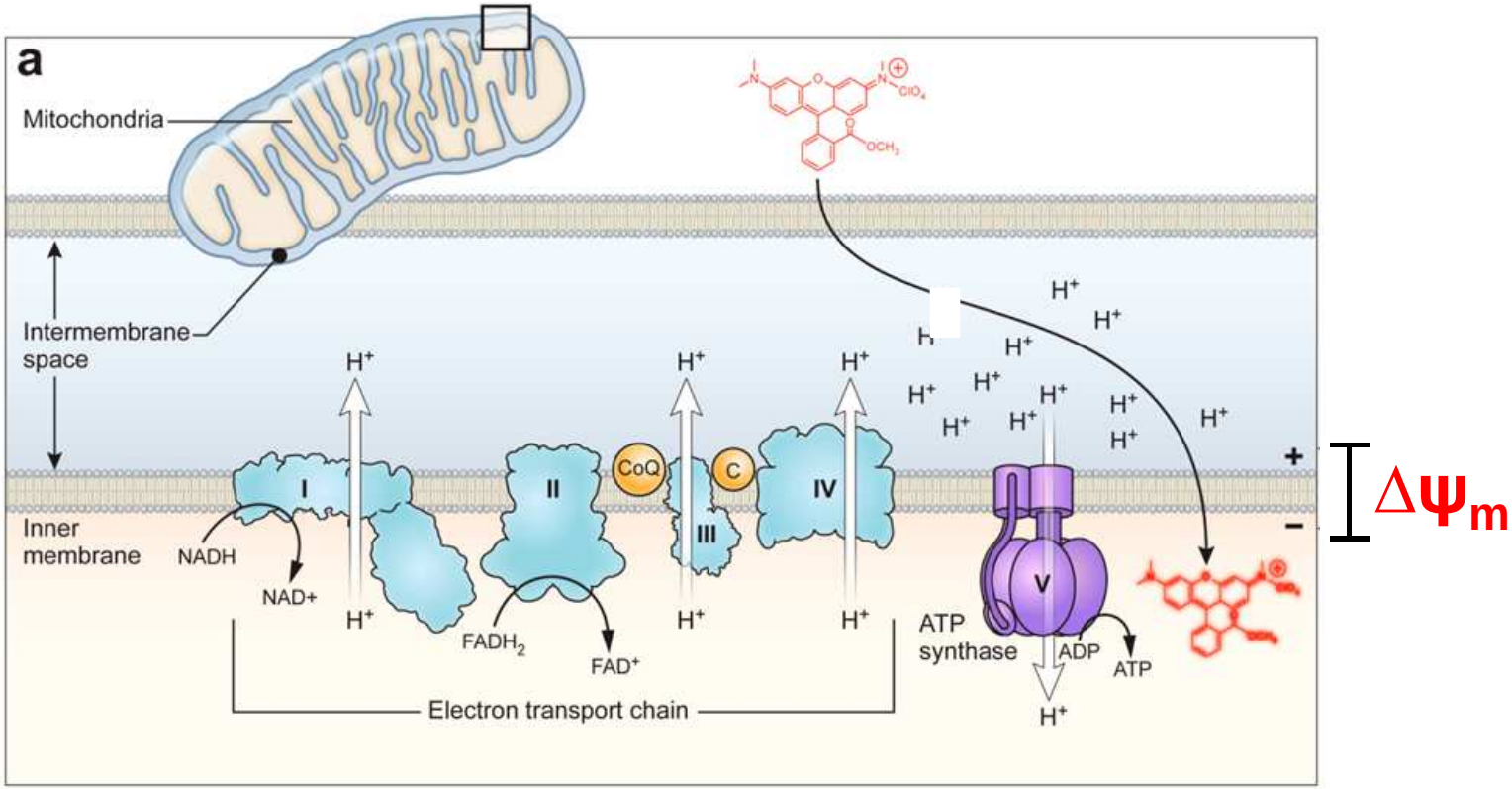
Increasing cellular ness after ACT

Adapted from Restifo, *Blood*, 2014; Gattinoni, et al, Nat Med, 2009 & 2011

Identification of human memory stem T cells (Tscm)

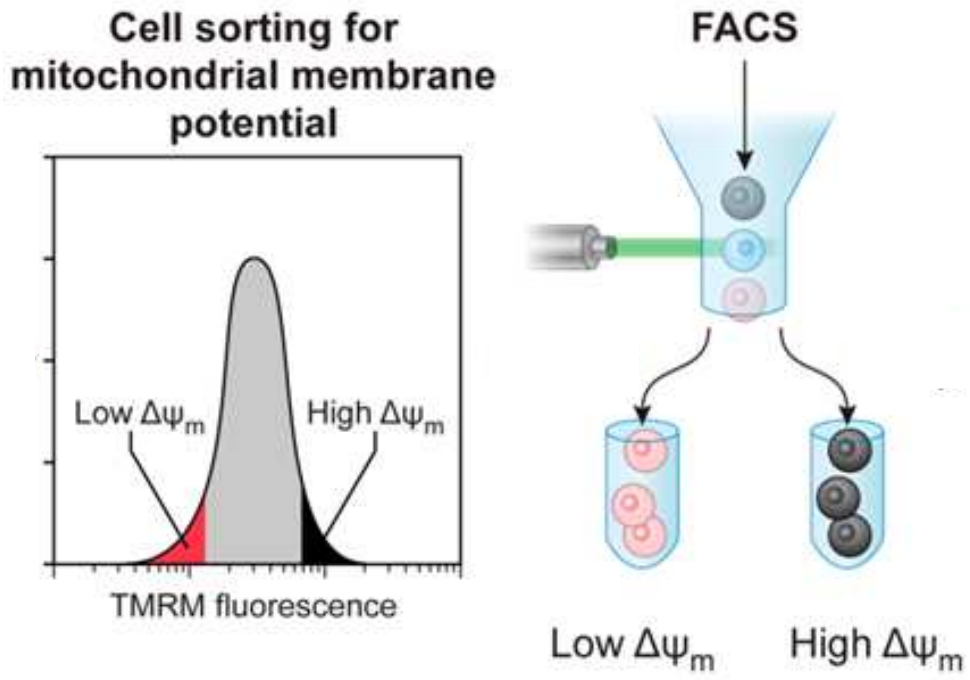


Measuring mitochondrial membrane potential ($\Delta\psi_m$) in individual living cells using tetramethyl rhodamine methyl ester (TMRM)



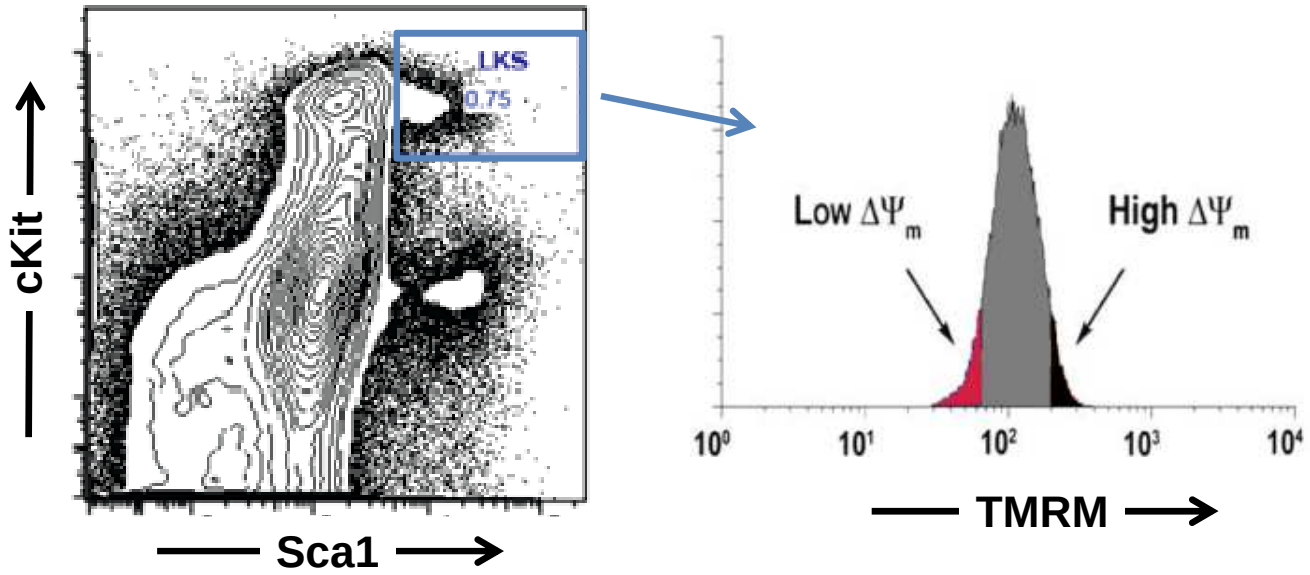
Sukumar, *Cell Metabolism, In Press*

Measuring mitochondrial membrane potential ($\Delta\psi_m$) in individual living cells using tetramethyl rhodamine methyl ester (TMRM)



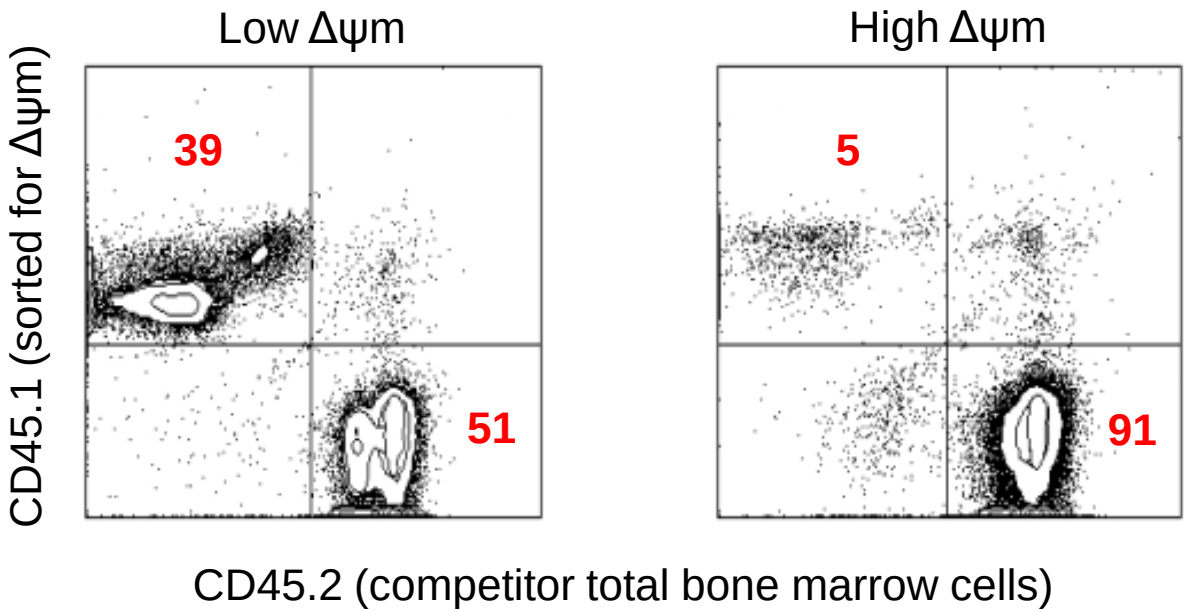
A low mitochondrial membrane potential marks self-renewing hematopoietic stem cells

LT-HSC (Lin$^-$ c-kit$^+$ Sca$^+$) (0.75% of population)
Congenically labeled with CD45.1



Long-term reconstitution
In lethally irradiated hosts

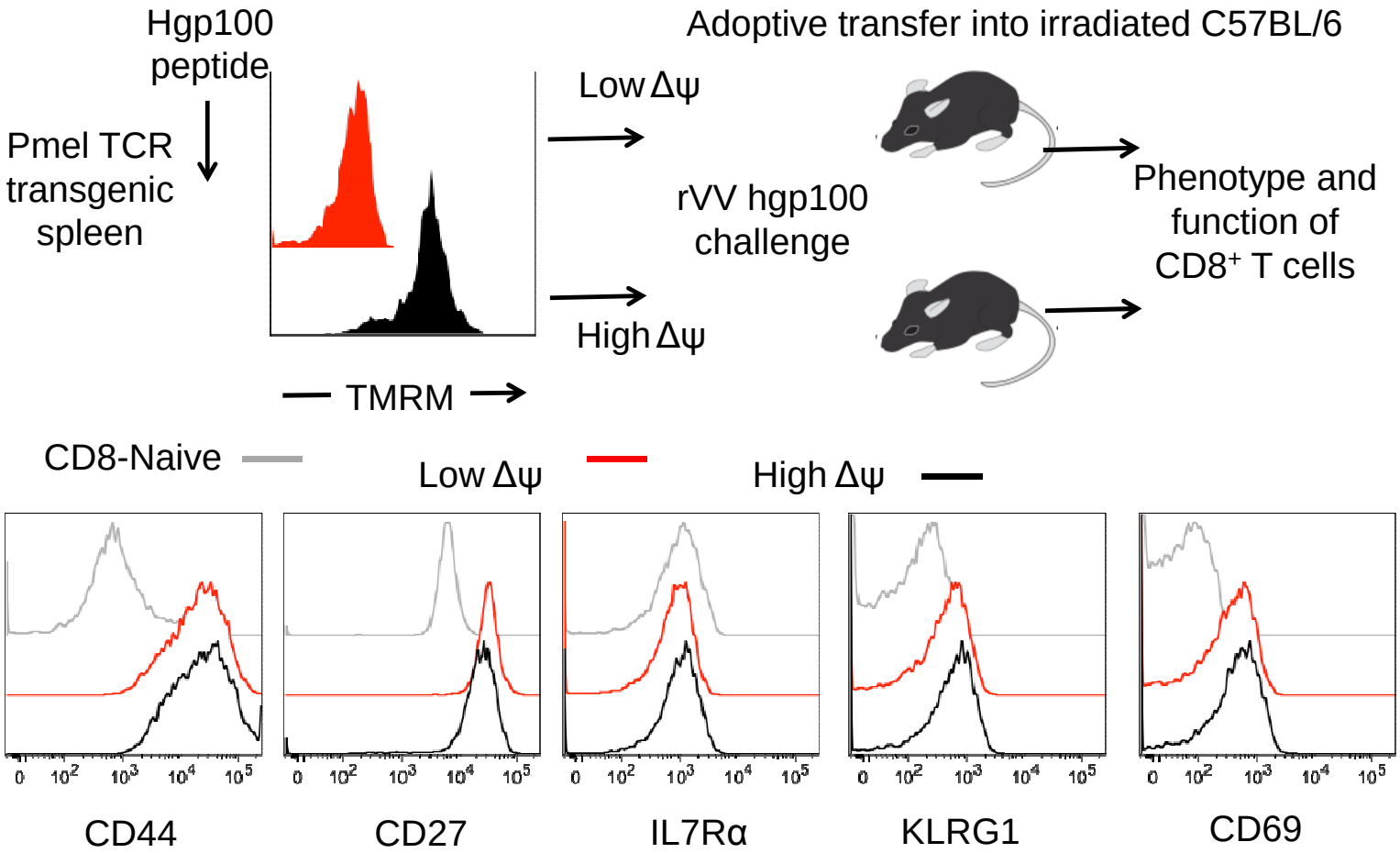
Low mitochondrial membrane potential marks self-renewing hematopoietic stem cells



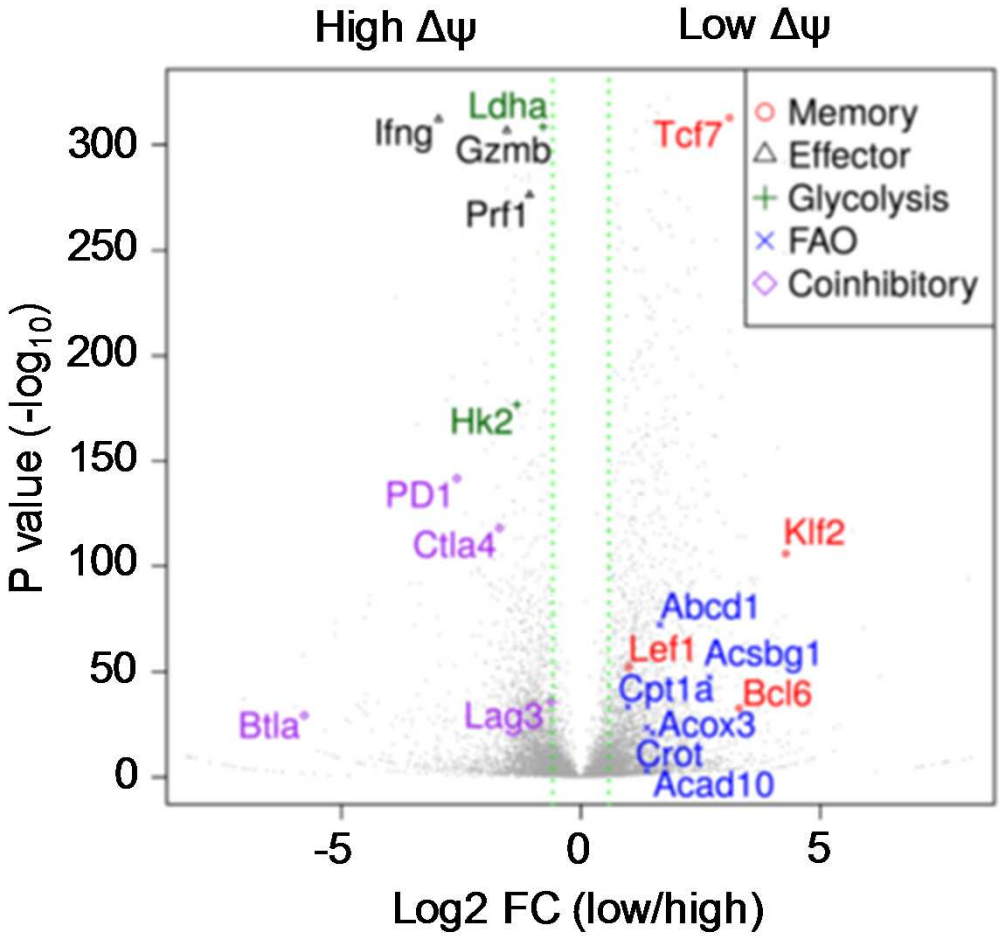
Reconstitution of host lymphocytes

Transplantation into lethally irradiated recipients (CD45.2) using:
-- 300 CD45.1 (low or high membrane potential) Lin^- $Sca1^+$ c-Kit^+ cells along with
-- 3 $X10^5$ CD45.2 competitor total bone marrow cells (CD45.2)

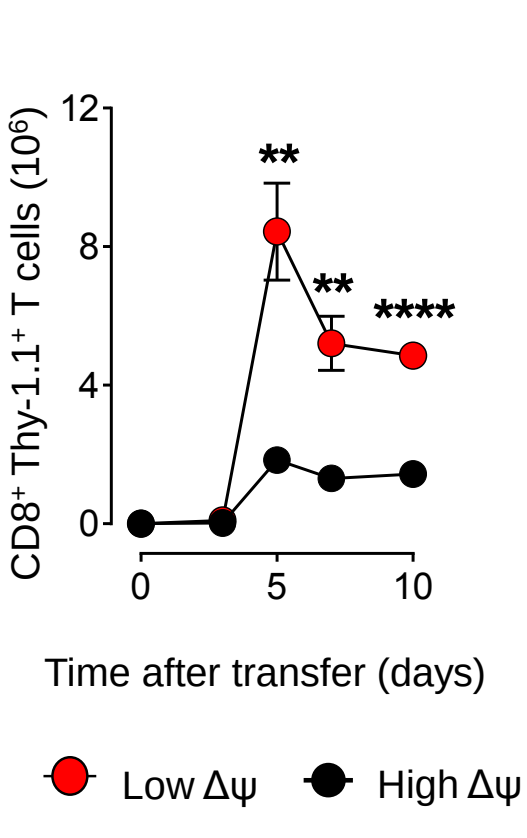
Characterization of CD8$^+$ T cells sorted based on ΔΨm

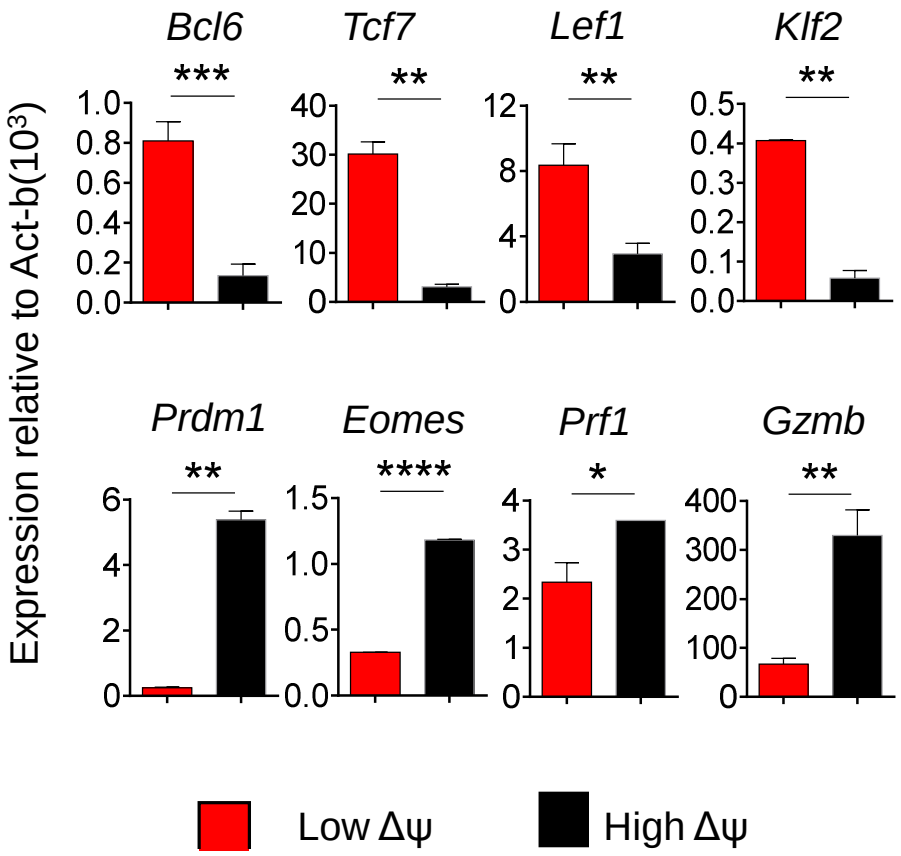


RNA-seq ‘volcano plot’ of cells sorted based on mitochondrial membrane potential

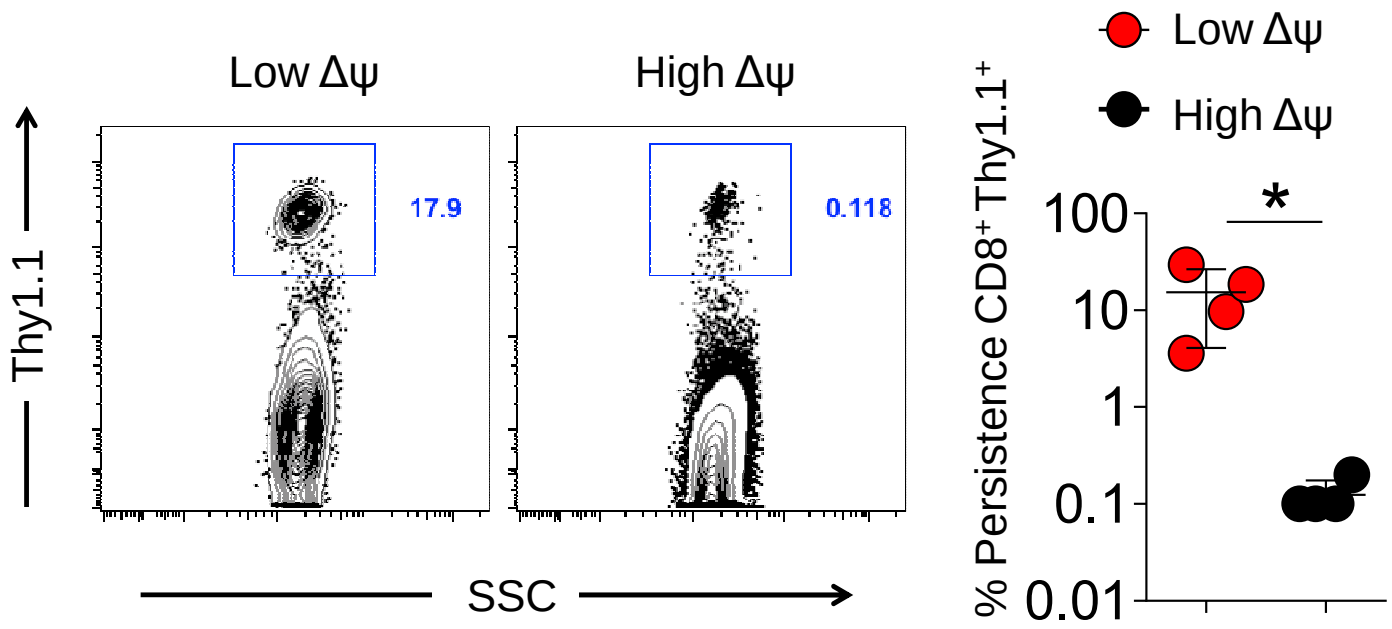


Mitochondrial membrane potential (Δψm) segregates short-lived effector from memory T cell precursors

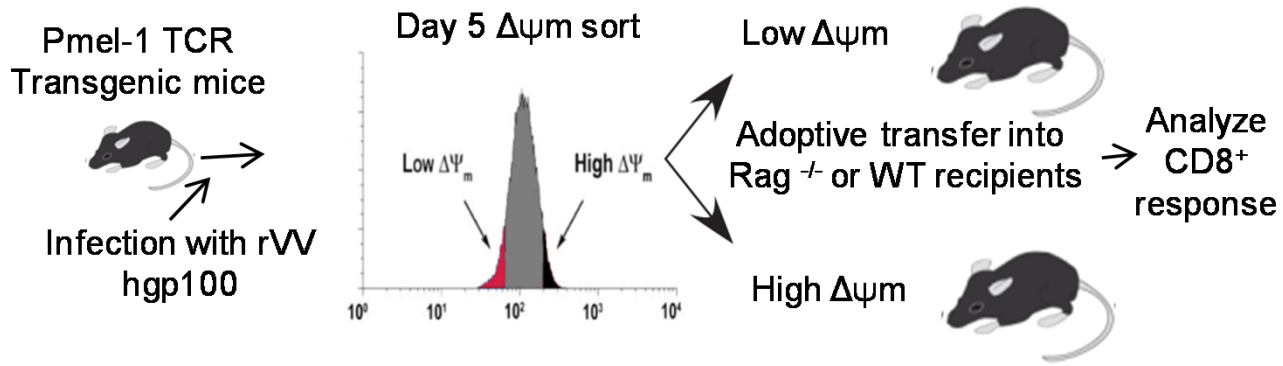


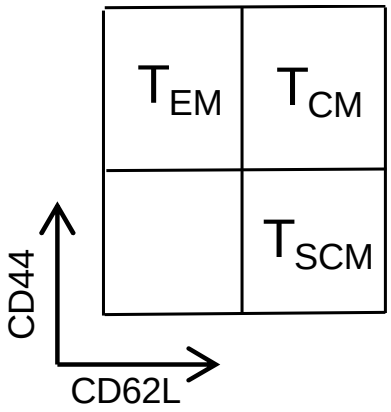


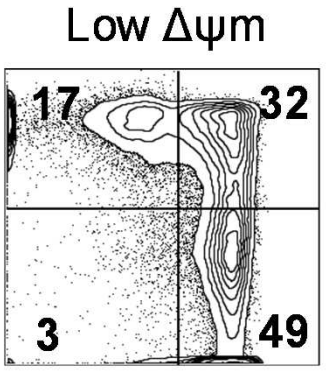
Low Δψm CD8$^+$ T cells demonstrate increased long-term *in vivo* persistence (300 days)

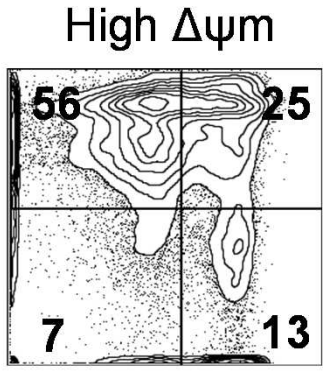


Δψm segregates long-lived memory T cells from short-lived effectors *in vivo* after infection

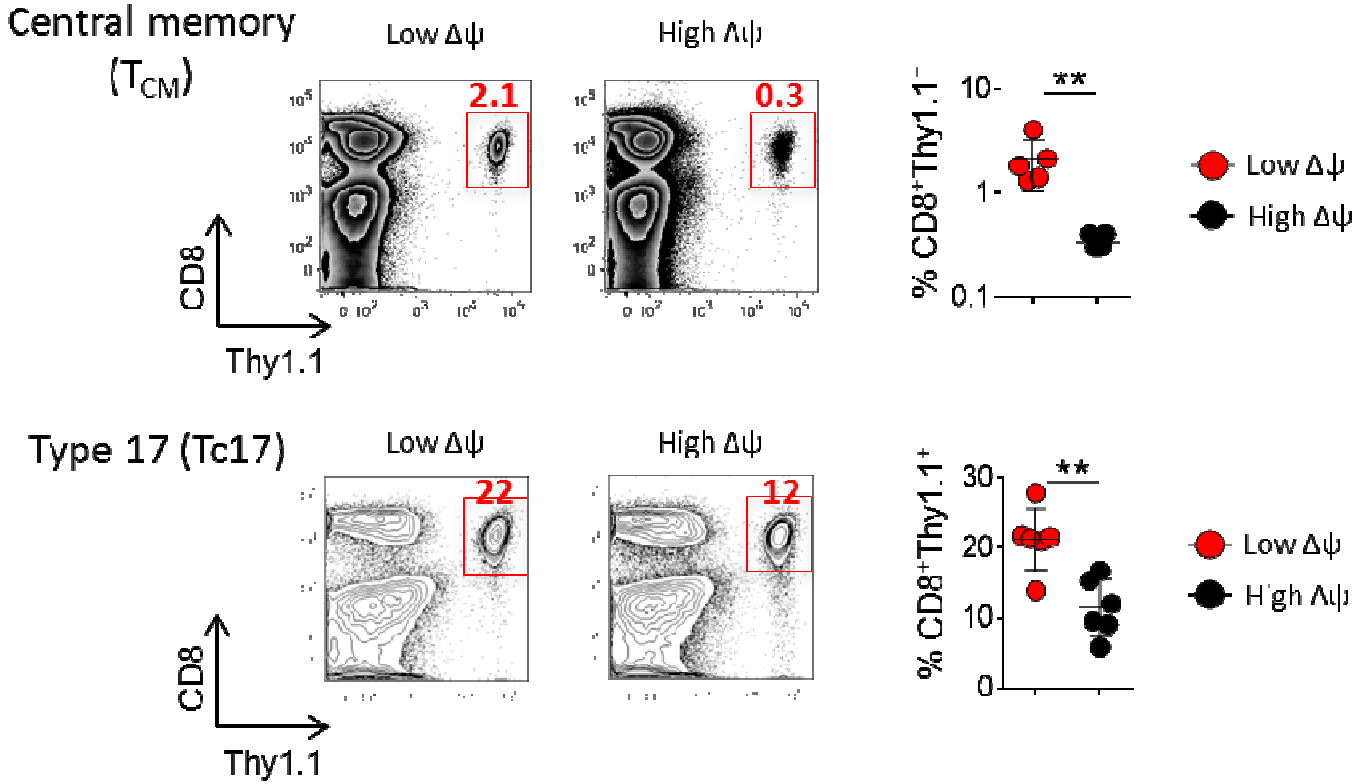




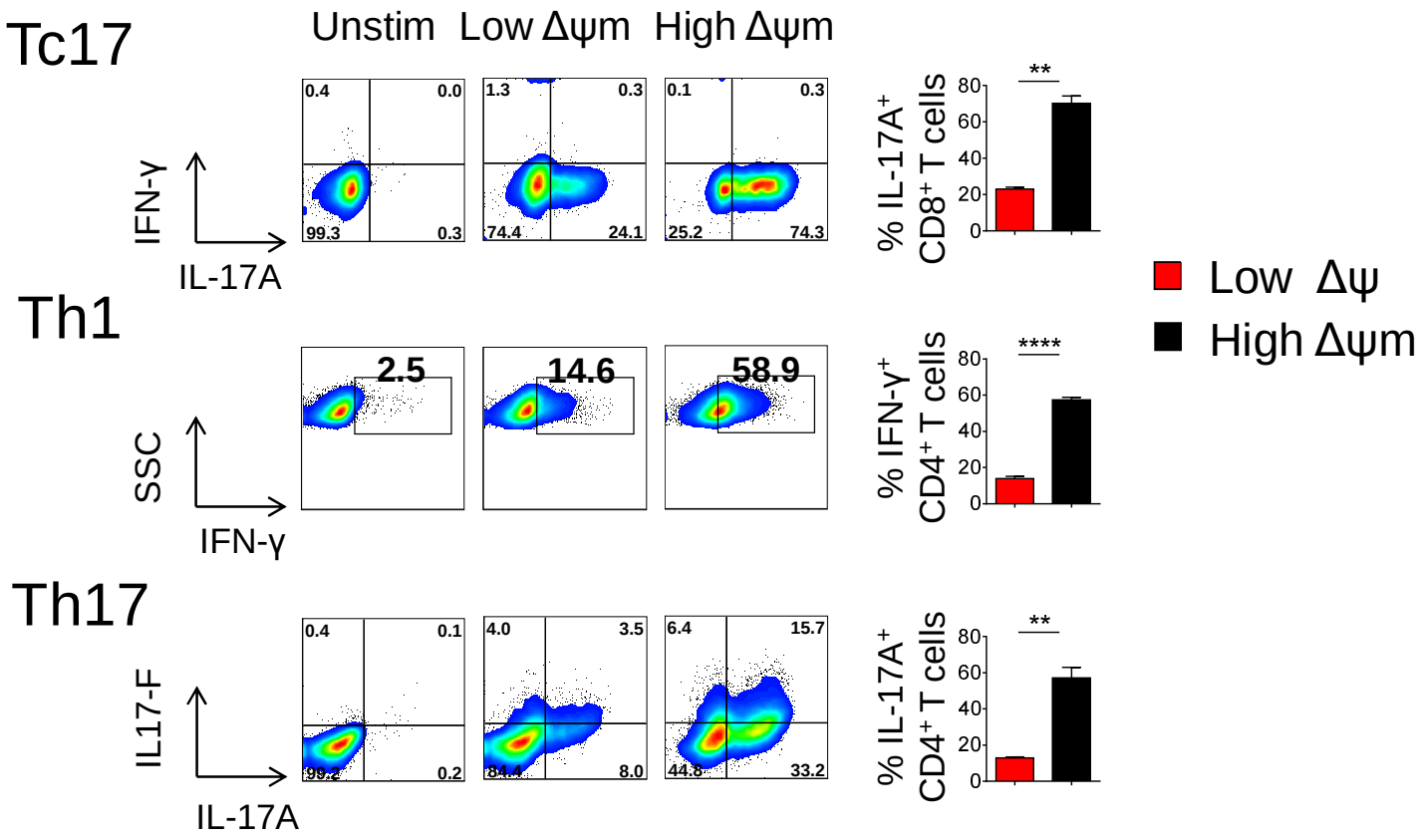


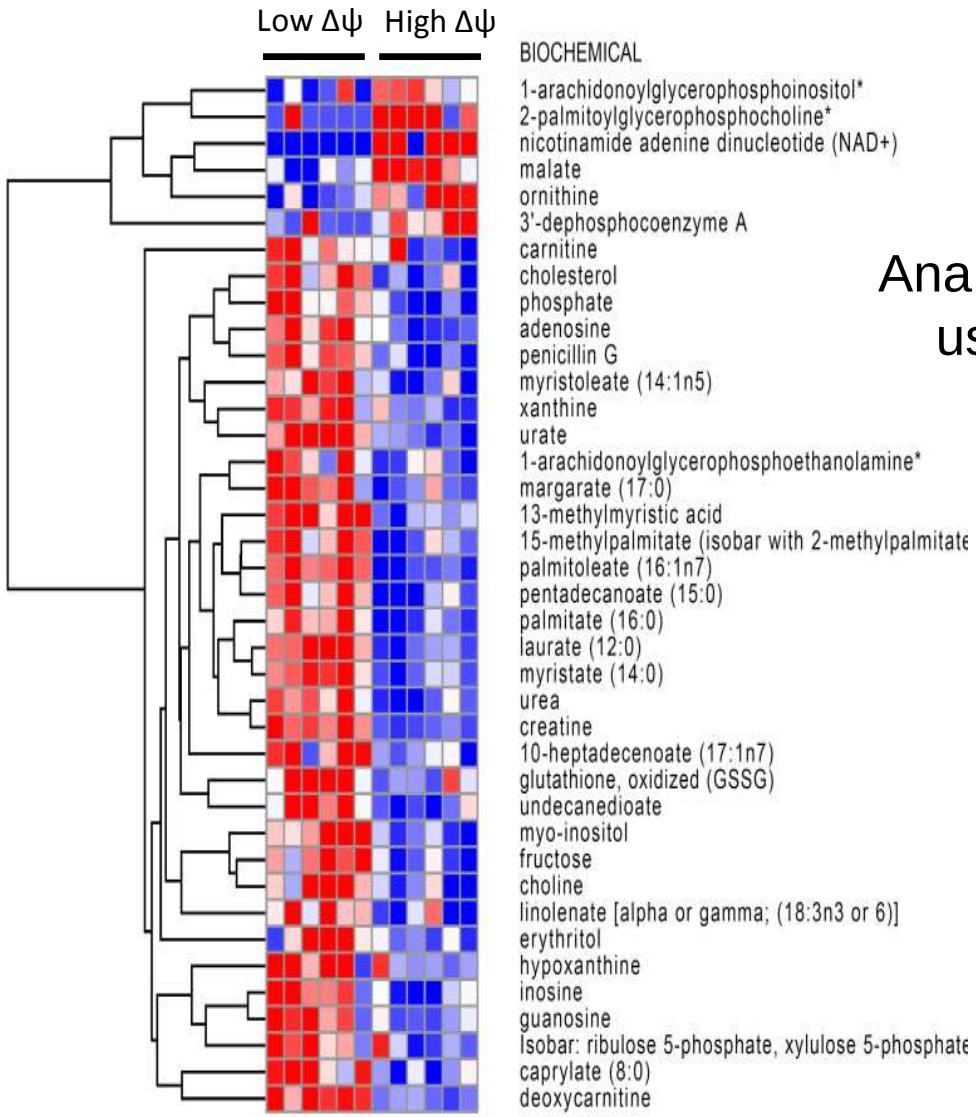


Low Δψm cells identify ‘metabolically fit’ T cells within sorted populations of T_{CM} and Tc17



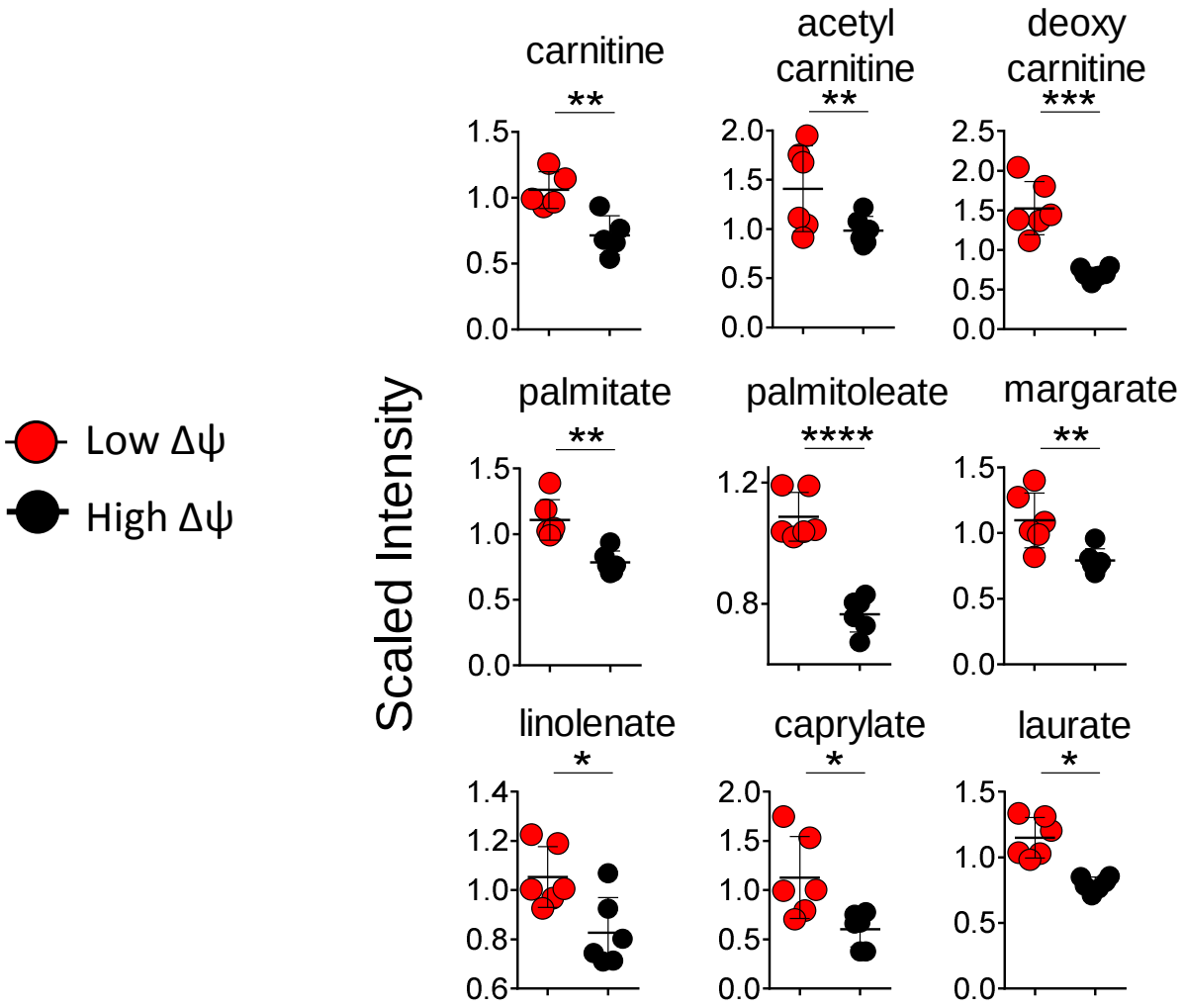
High-ΔΨm is associated with effector cytokine production in T cells



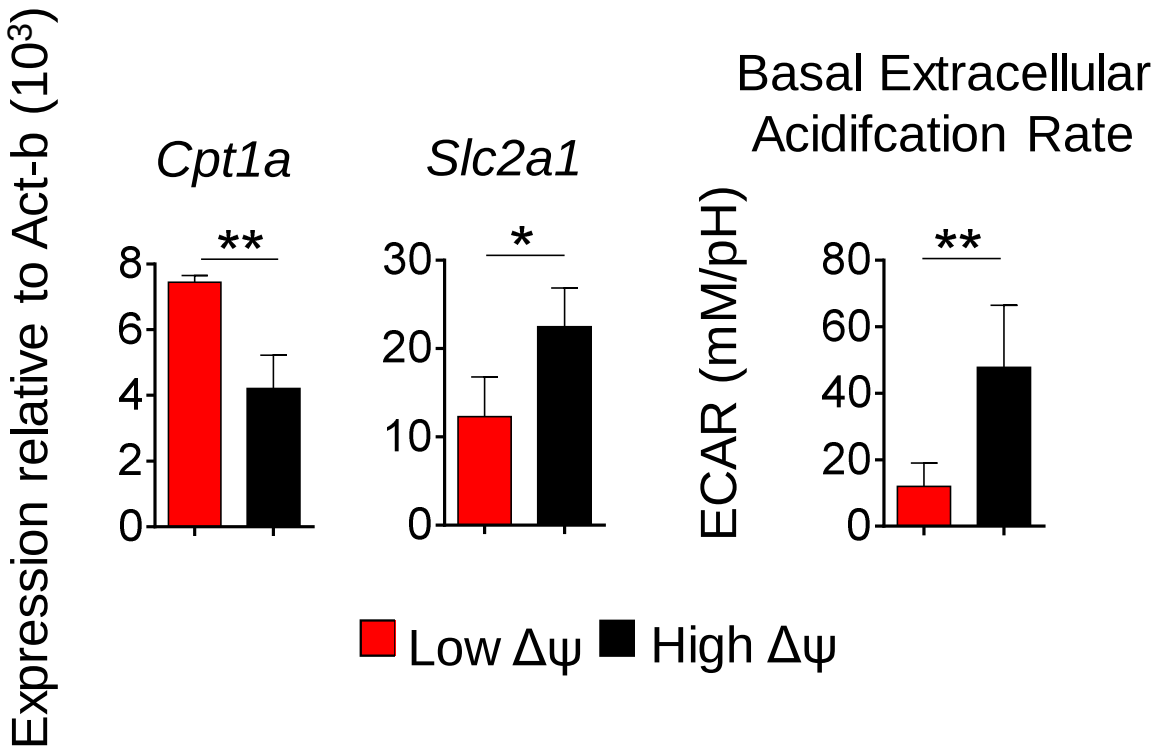


Analysis of metabolites using HLPC-Mass Spectrometry

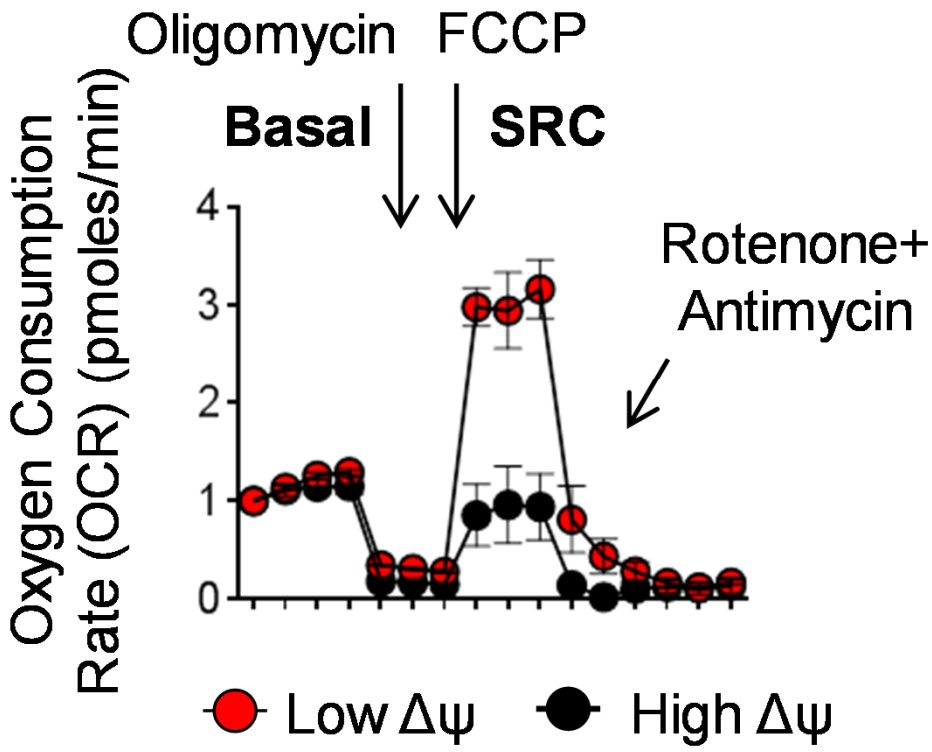
Low Δψm cells display increased fatty acid metabolites



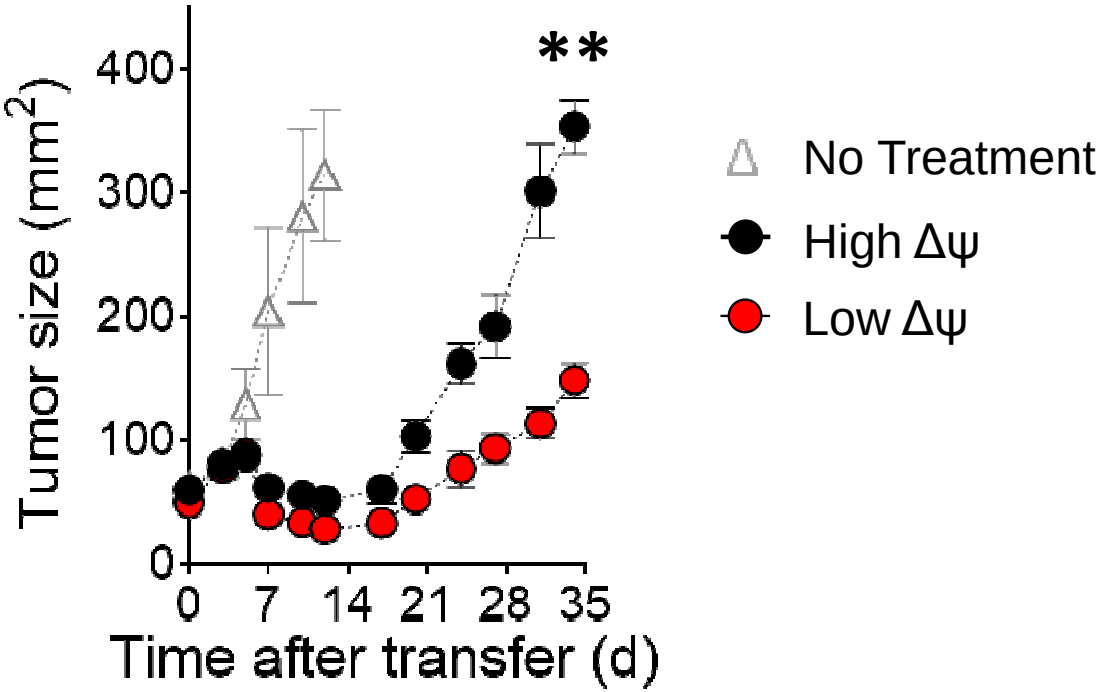
Low Δψm cells display a metabolic profile of memory CD8^{+} T cells



Low Δψm cells display a metabolic profile of memory CD8^{+} T cells



Low Δψm CD8$^+$ T cells control established tumor even when sorted from an established T cell culture



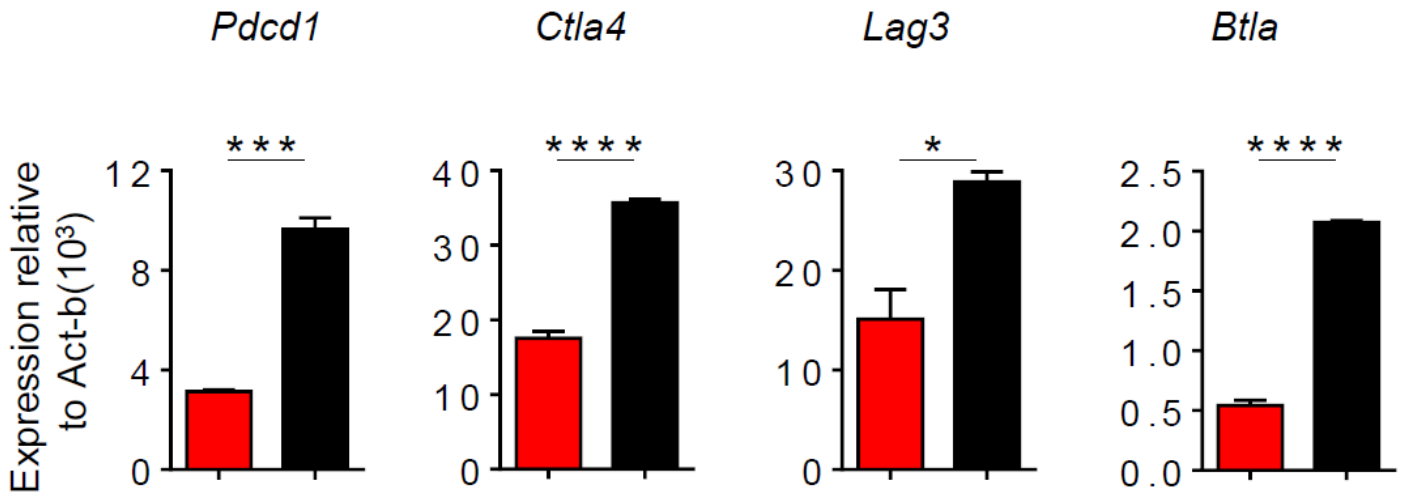
Low Δψm $CD8^+$ T cells demonstrate increased autoimmune vitiligo

Low Δψm cells:

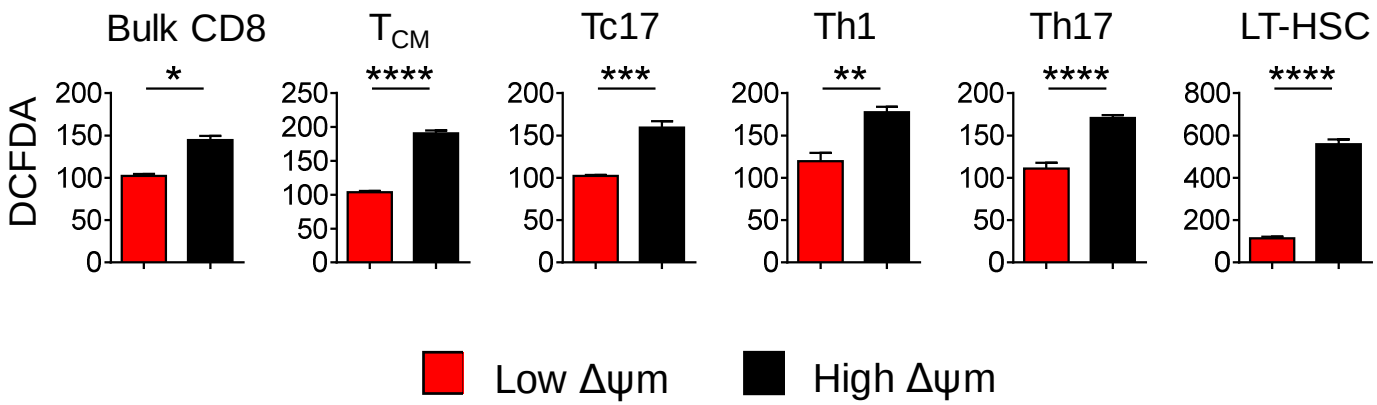
1. *Are more stem cell-like*
2. *They burn fats not glucose*
3. *They have more spare respiratory capacity*
4. *The persist longer*
5. *They control established tumor better*

High Δψm cells make more cytokines, but why do they die?

CD8^{+} T cells with low Δψm have decreased checkpoint

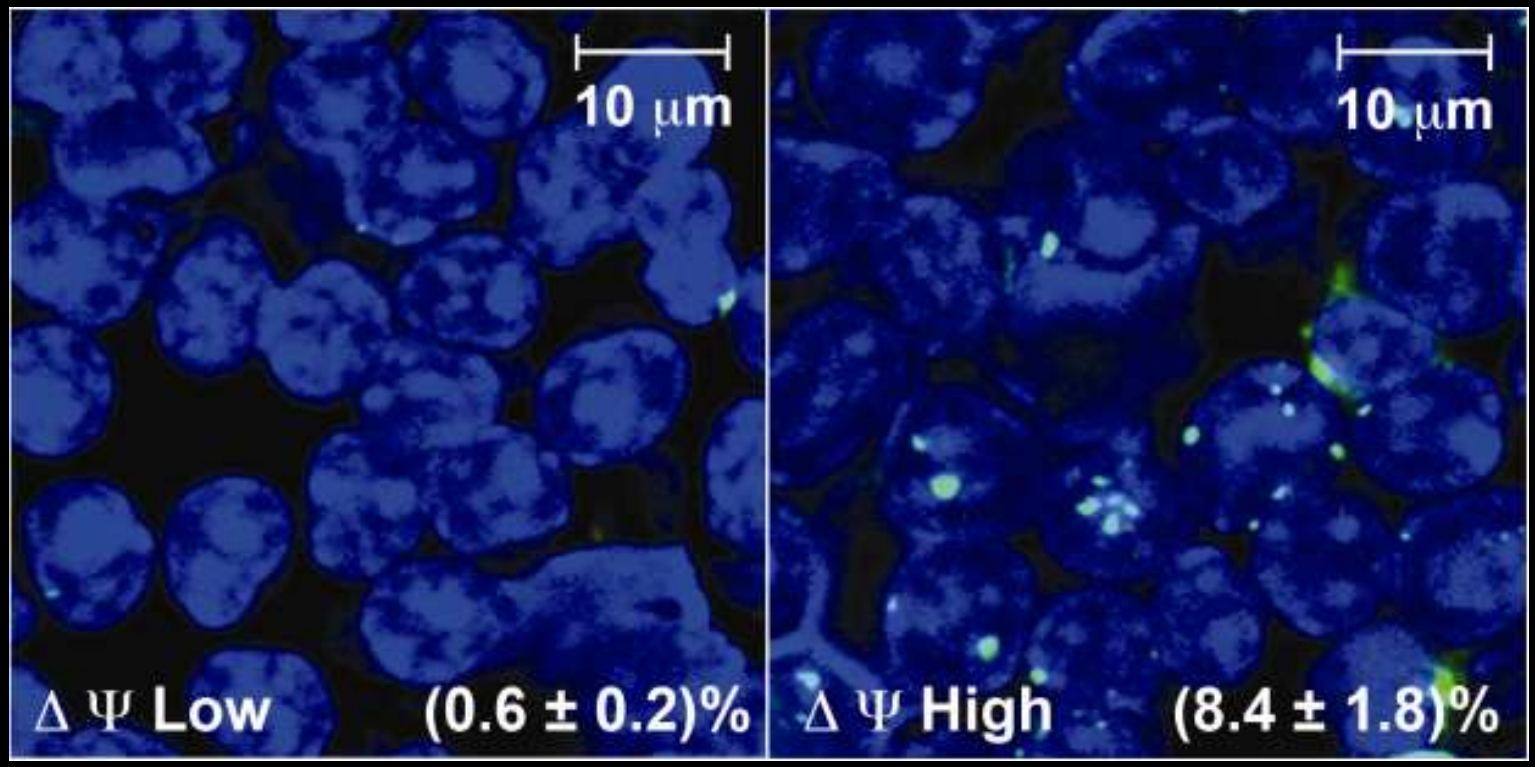


CD8^{+} T cells with low Δψm have decreased levels of reactive oxygen species (ROS)



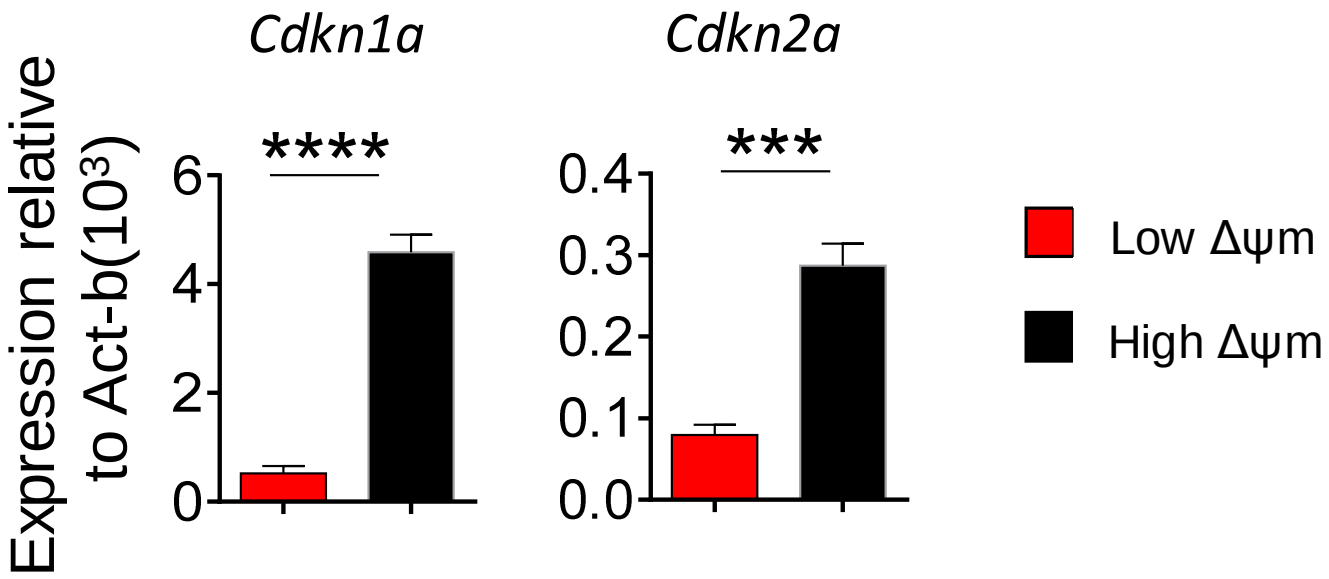
DCFDA is a cell-permeable non-fluorescent probe. 2′,7′-Dichlorofluorescin diacetate is de-esterified intracellularly and turns to highly fluorescent 2′,7′-dichlorofluorescein upon oxidation.

High Δψm CD8^{+} T cells display increased DNA damage

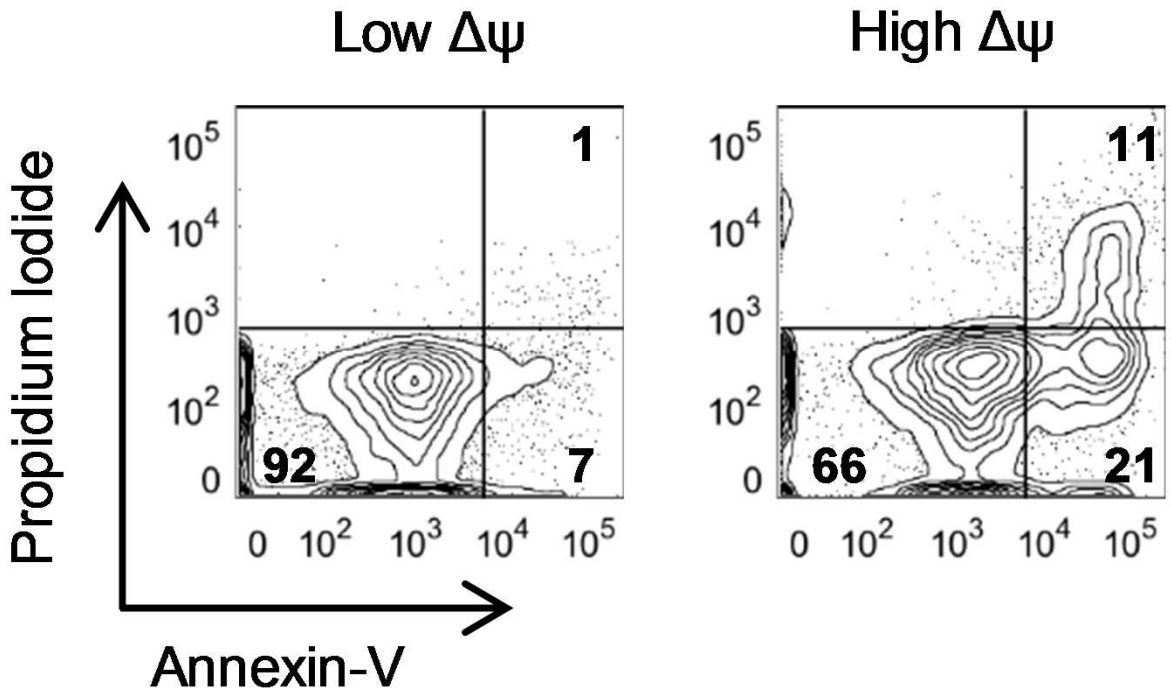


Stain for γ-H2AX, a marker for dsDNA breaks

High Δψm CD8^{+} T cells elevated levels of biomarkers of ‘physiological age’



$CD8^+$ T cells with high Δψm die by apoptosis



SUMMARY

- ❑ Low ΔΨm $CD8^+$ T cells demonstrate long-term *in vivo* persistence and superior anti-tumor activity

- ❑ Low ΔΨm T cells display metabolic signature of memory $CD8^+$ T cells

- ❑ High-ΔΨm is associated with effector cytokine production in T cells, followed by DNA damage, senescence and death.

- ❑ Low ΔΨm identifies metabolically fit cells among HSC and $CD8^+$ T_{CM} subsets

THE VISION: What is required to bring cell-based therapies to the many patients who need them?

1. Concerted commitment to basic science.
2. Concerted effort to create vector and cell production laboratories for patients.
3. Robust technology transfer: open sourcing & industrial partners.

National Cancer Institute

Restifo Lab:
Past and present

Madhu Sukumar
Rahul Roychoudhuri
Douglas C Palmer
Christopher A. Klebanoff
Nick Acquavella
Joe Crompton
Nick Klemen
Tori Yamamoto
Zhiya Yu
Robert Eil
Jenny Pan
Shashank Patel
David Clever
Gautam Mehta
Raul Vizcardo
Linda Tran
Devi Gurusamy

John O'Shea:
Golnaz Vahedi
Vittorio Sartorelli

Francis Collins:
Stephen C. J. Parker

Richard Siegel:
Madhu Ramaswamy
Anthony C. Cruz

David Stroncek:
Franco Marincola
Ena Wang

Luca Gattinoni:
Luca Gattinoni
Yun Ji

Rosenberg Lab:
Eric Tran
Alena Gross

Leonard Lab:
Warren Leonard
Rosanne Spolksi
Peng Li

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