Tumor Immune Microenvironment: A Holistic Approach Workshop

April 21-22, 2022 • San Diego and Virtually

#SITCworkshop

sitc

Society for Immunotherapy of Cancer

The role of metabolites in the tumor microenvironment

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Department of Cell Biology
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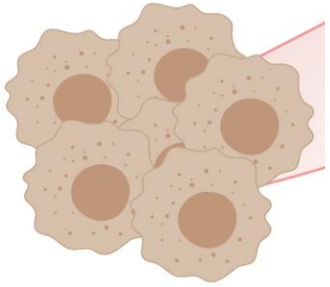
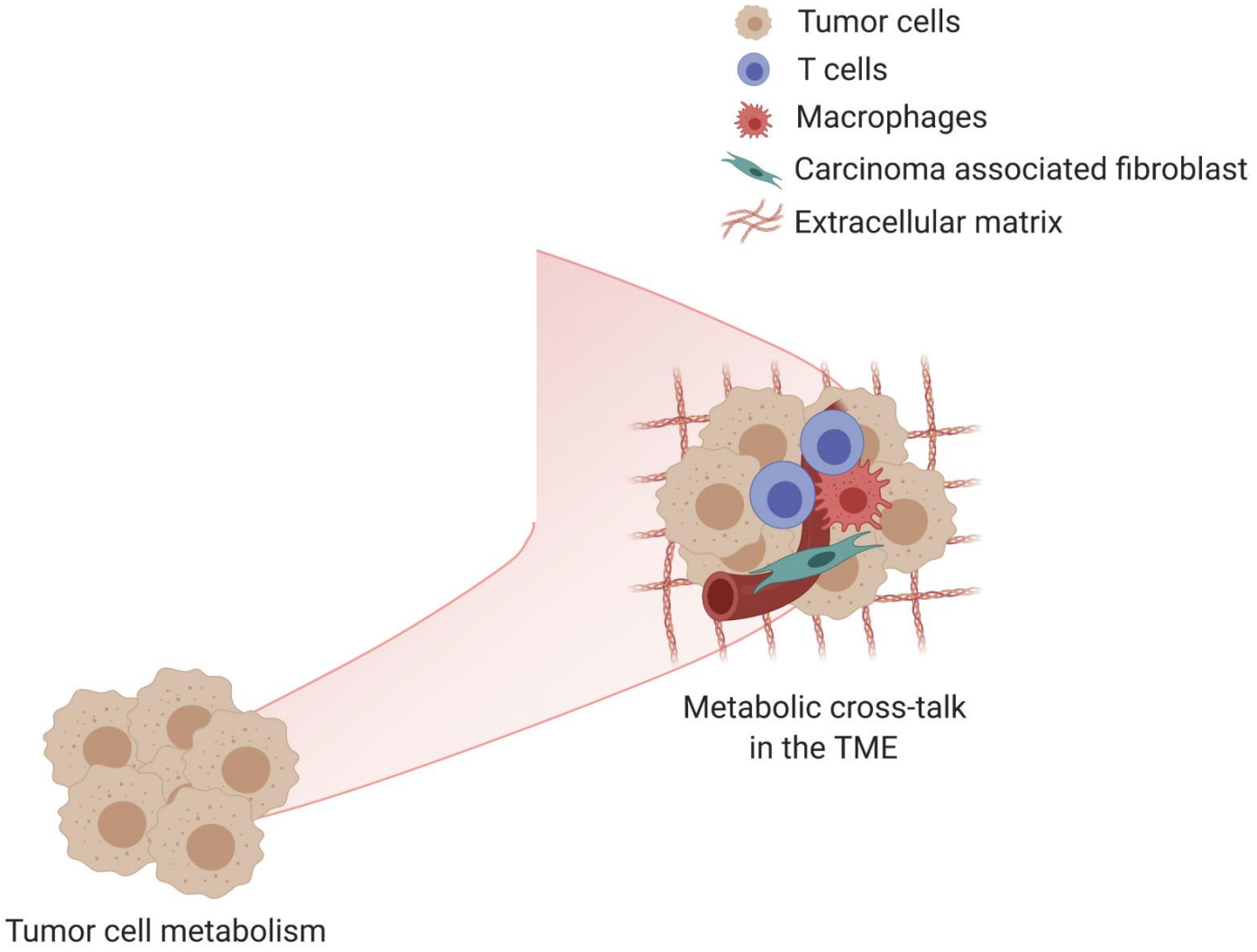
2022

Tiered view of tumor metabolism

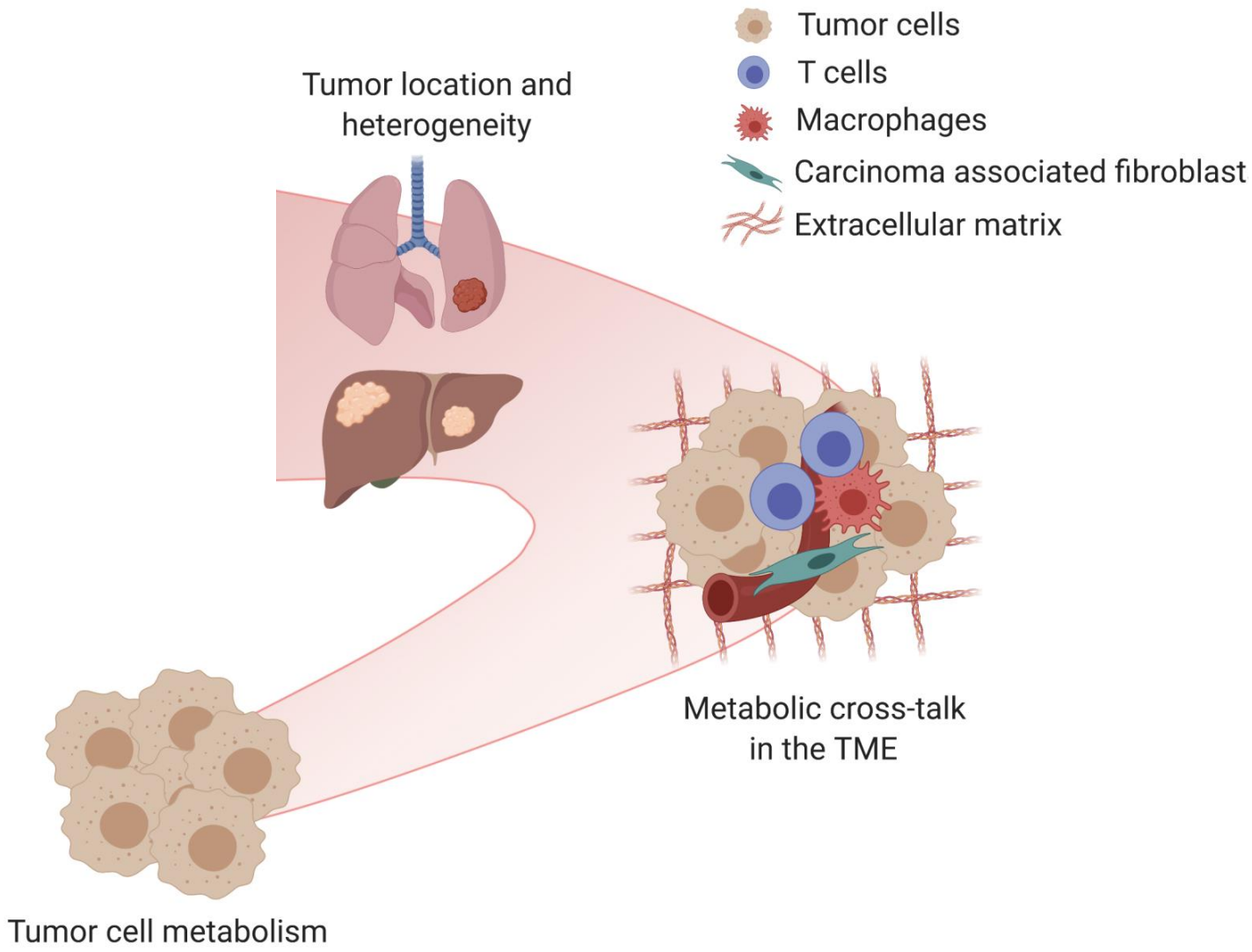


Tumor cell metabolism

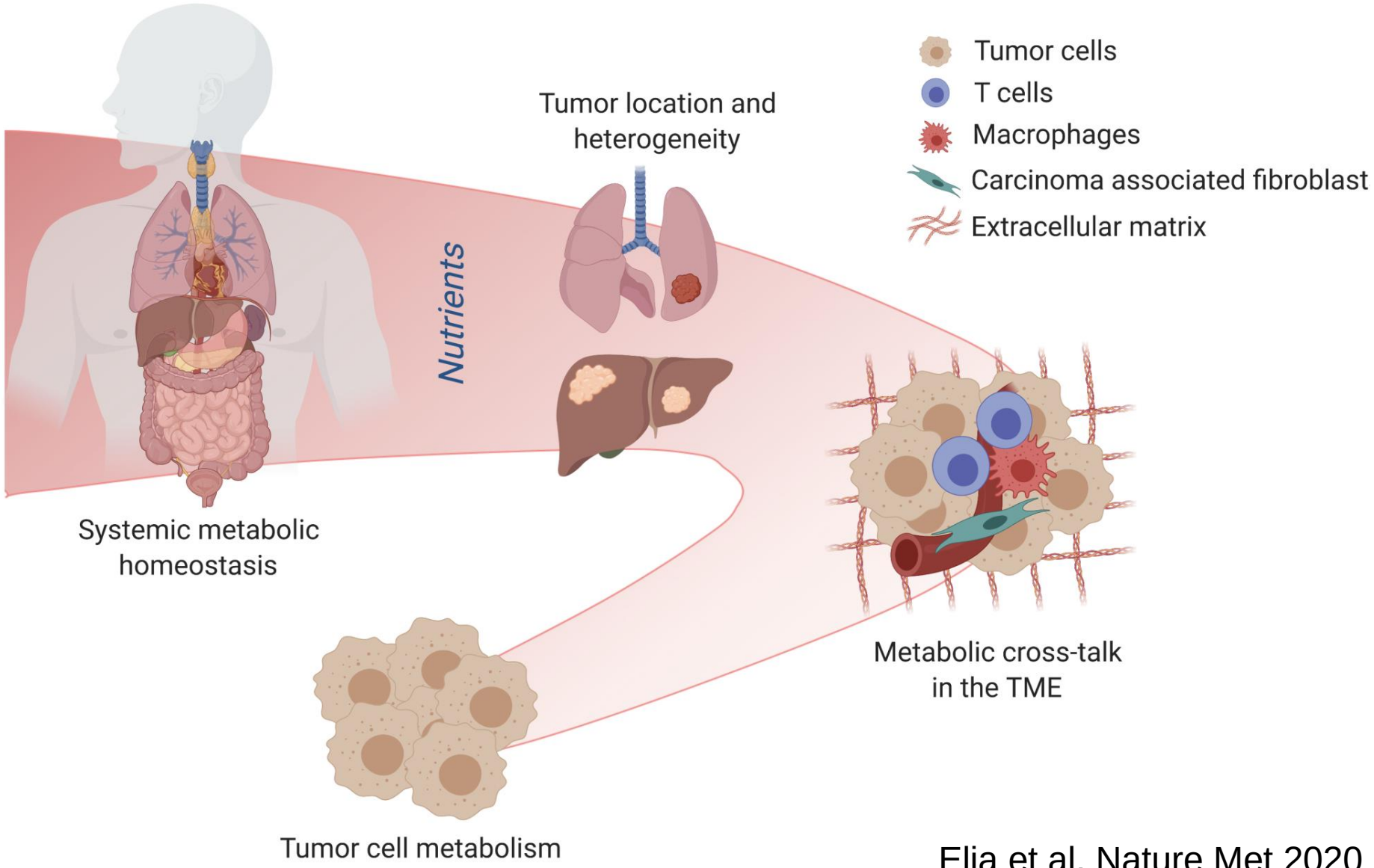
Tiered view of tumor metabolism



Tiered view of tumor metabolism

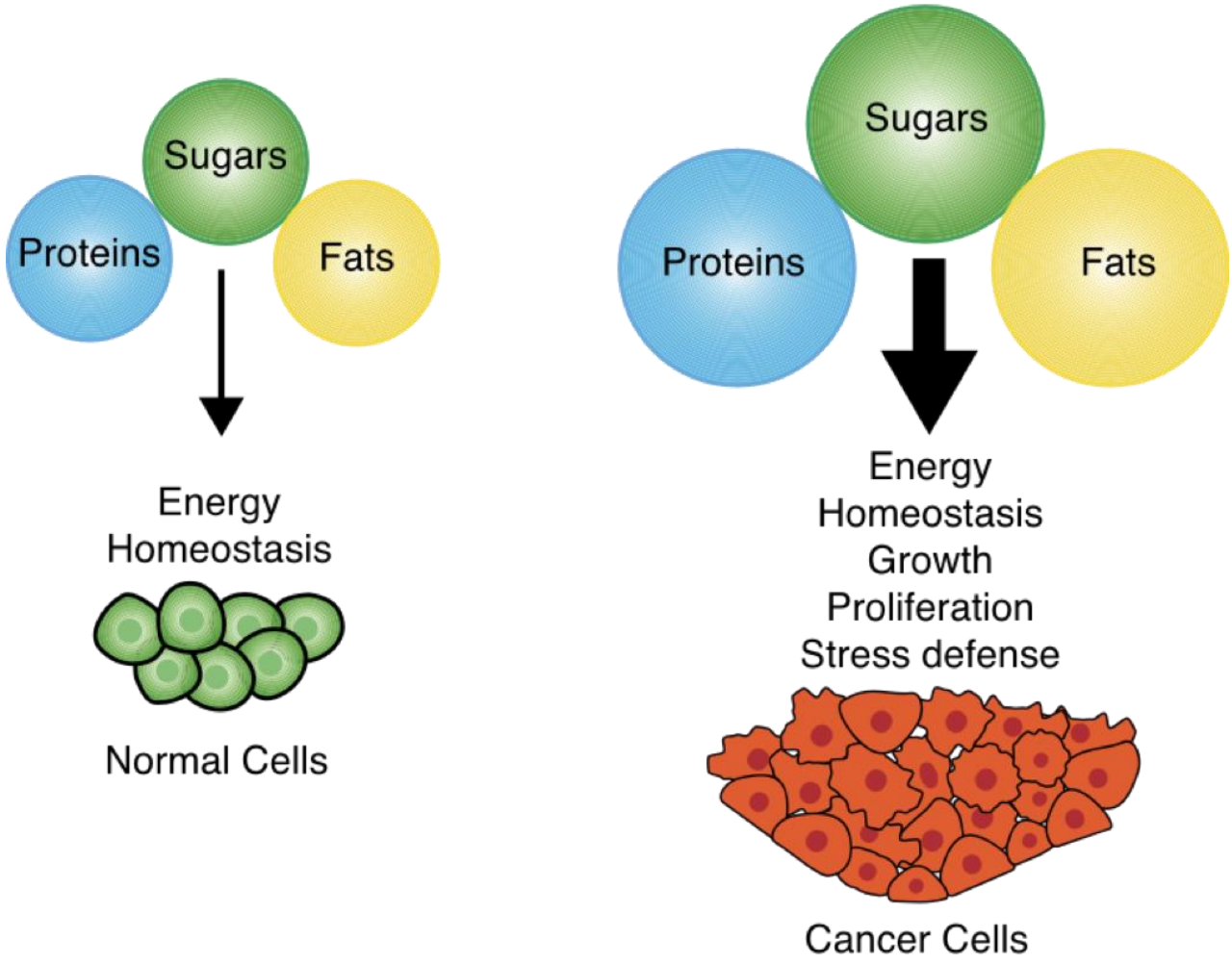


Tiered view of tumor metabolism

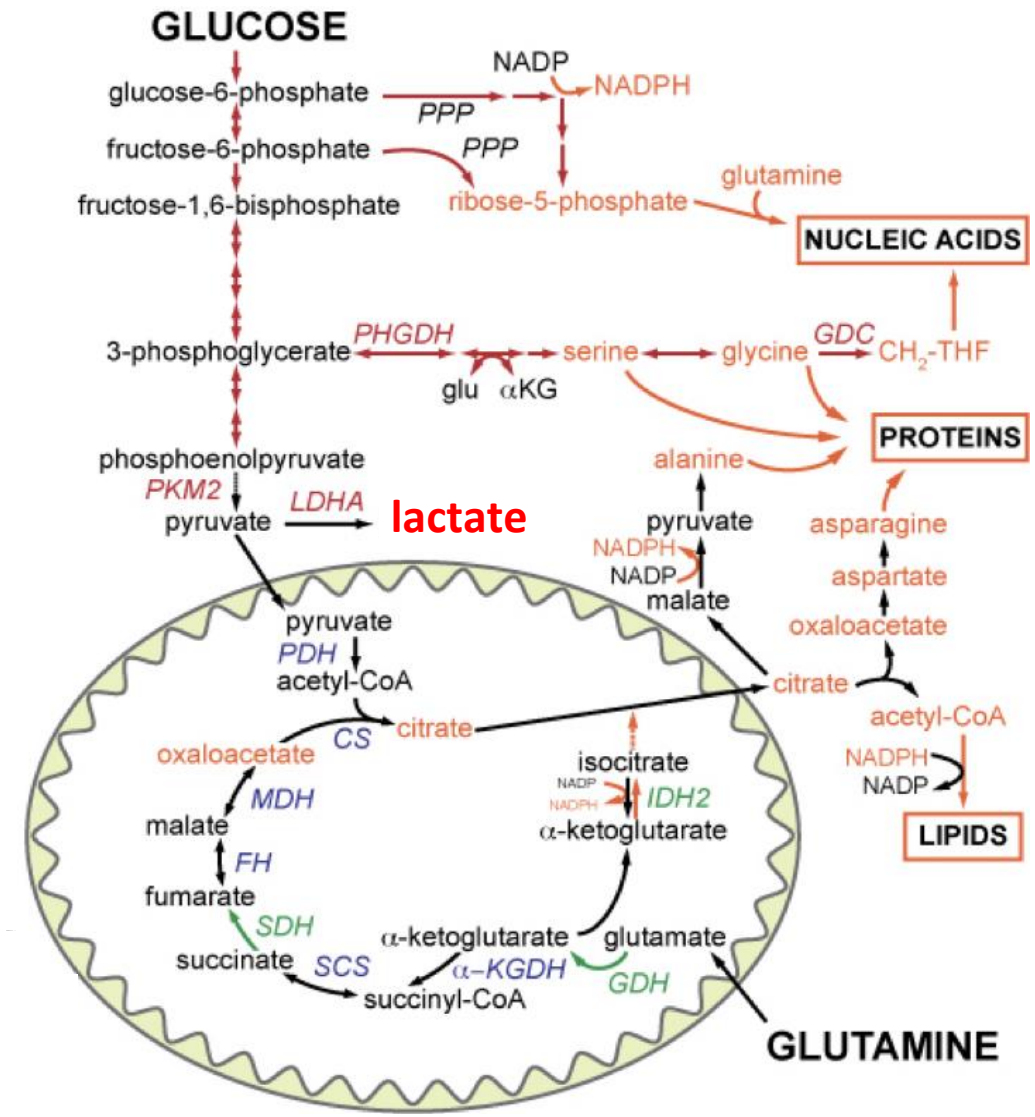


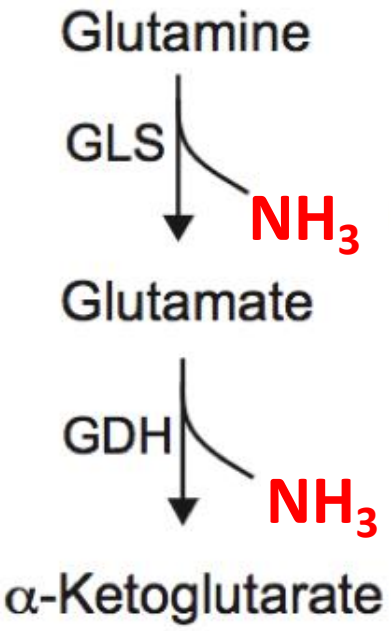
Elia et al, Nature Met 2020

Nutrient use is altered in cancers

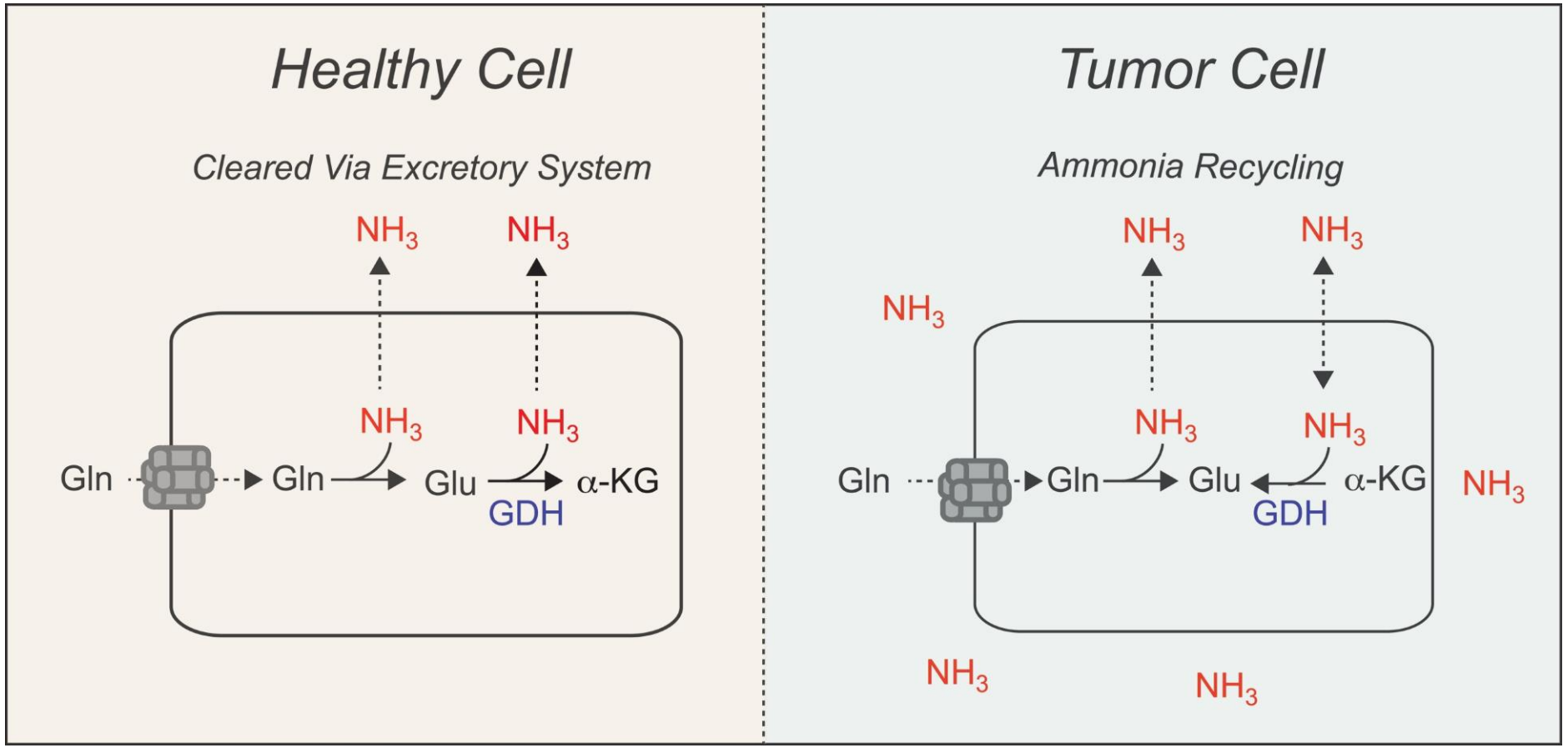


Cell proliferation is linked to metabolism





Ammonia promotes tumor proliferation

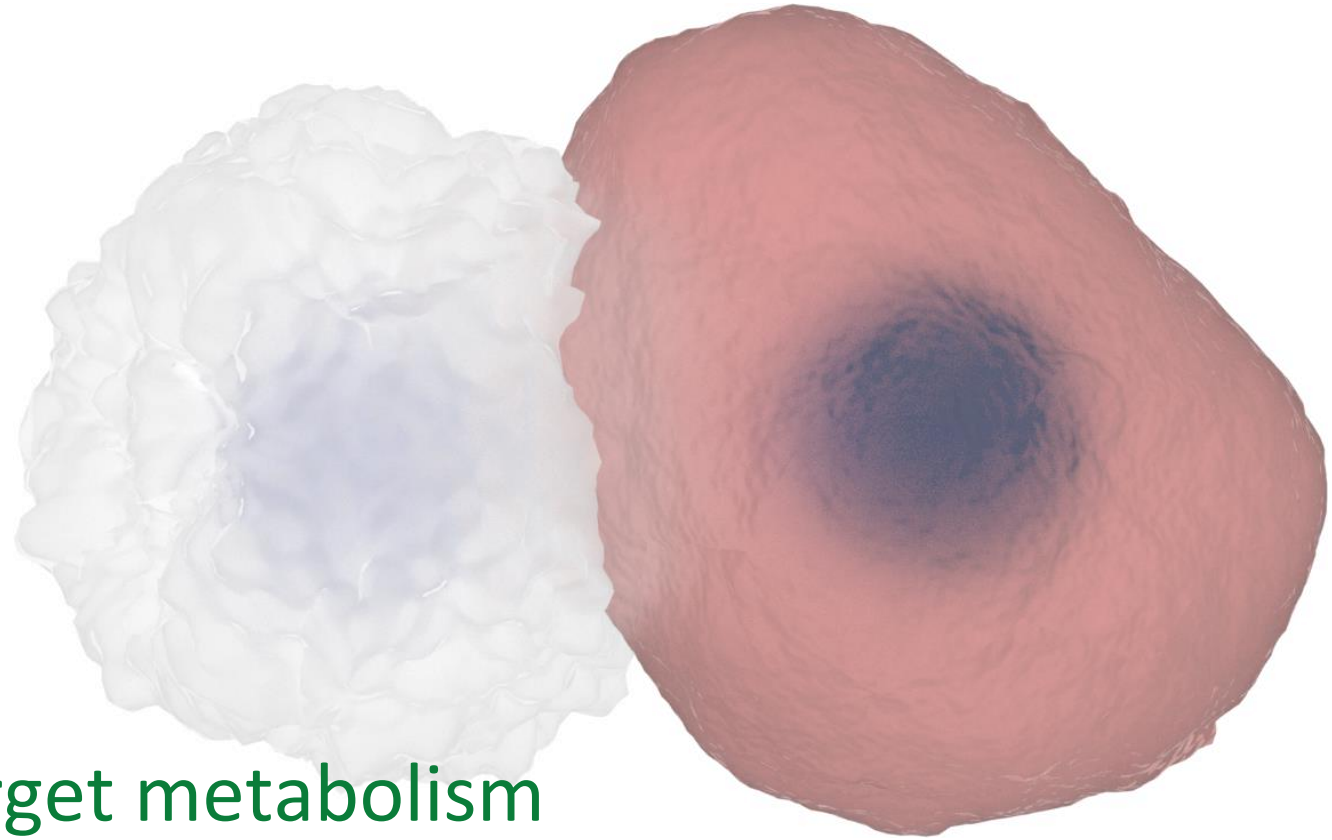


Spinelli et al, 2017, Science

The TME is a unique niche

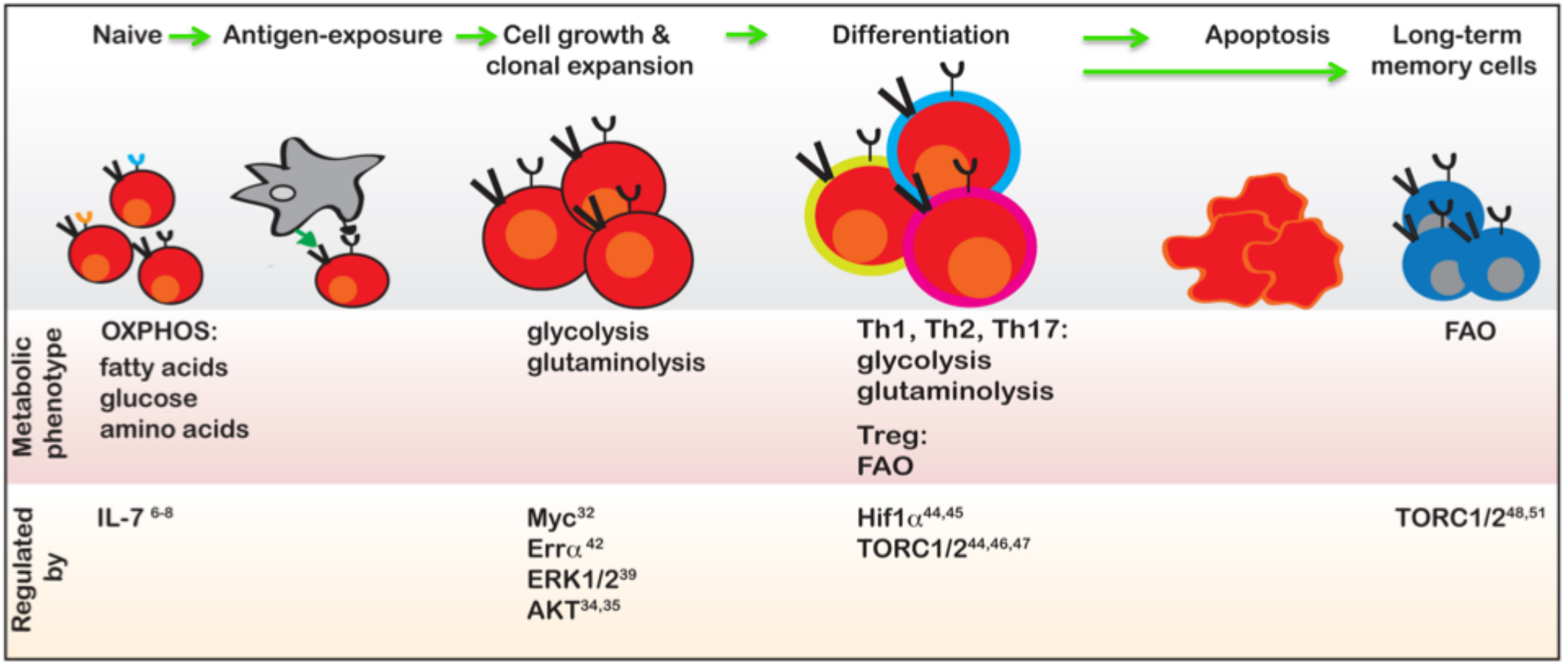
Altered levels of Fuels

Accumulation of inhibitory metabolites: lactate, ammonia



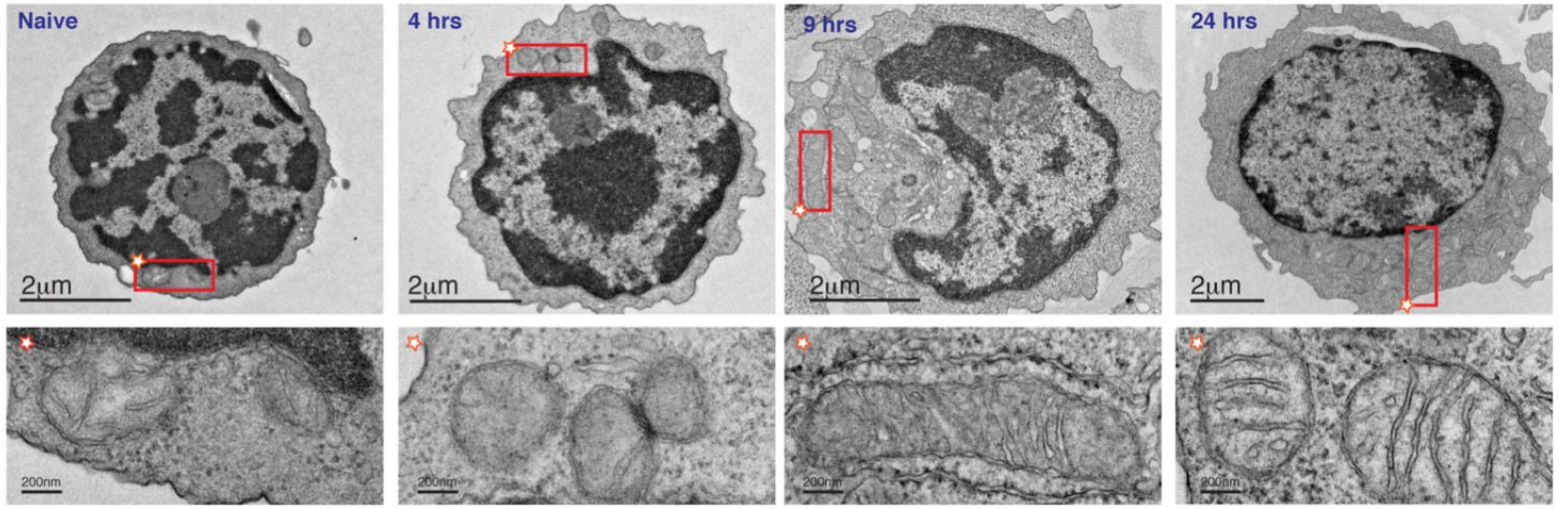
Challenge: How do we target metabolism specifically in the TME?

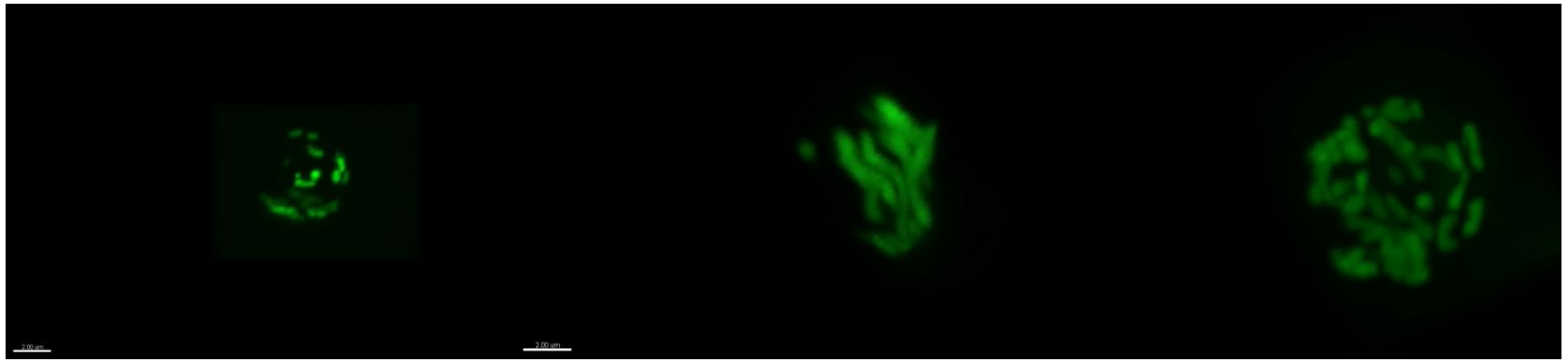
Metabolism in the T cell life cycle



Ron Harel et. al. Gerontology 2015

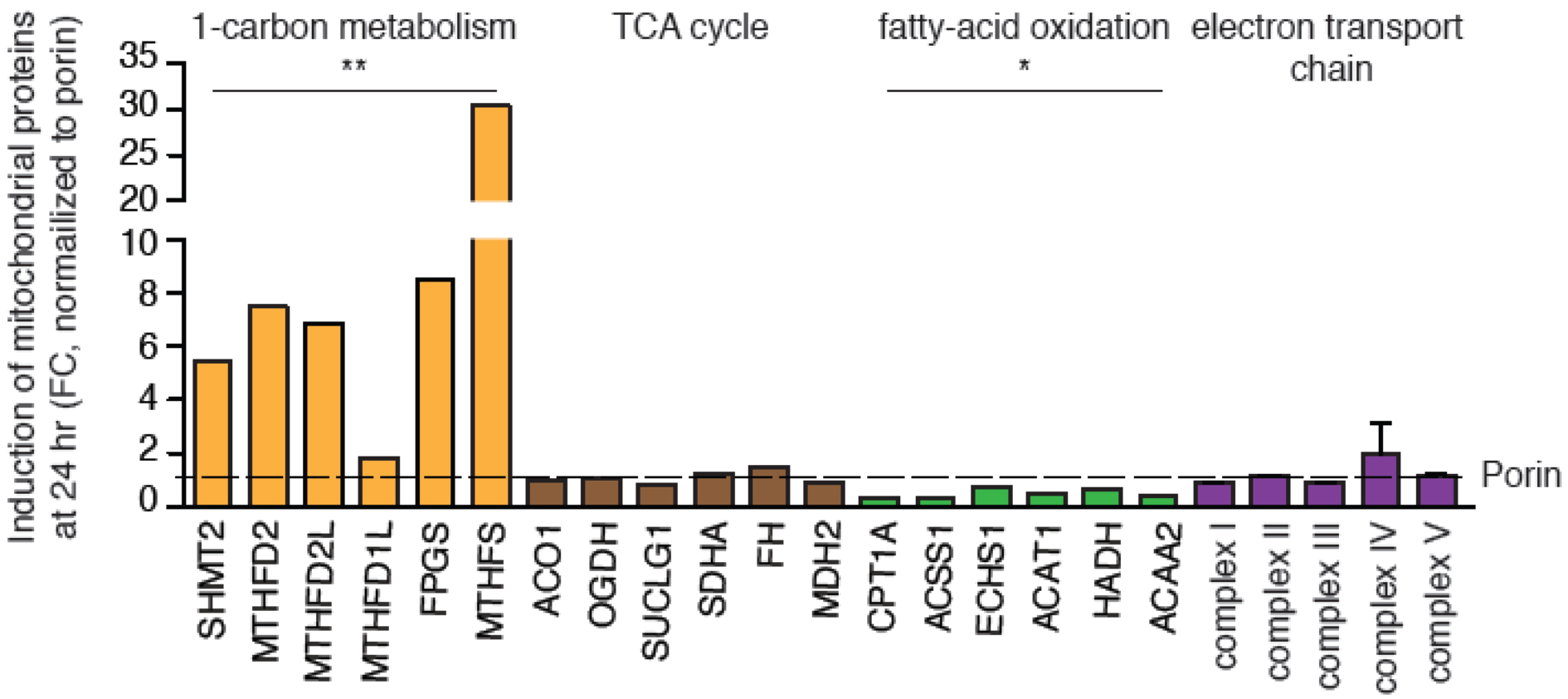
T Cell activation initiates mitochondrial biogenesis





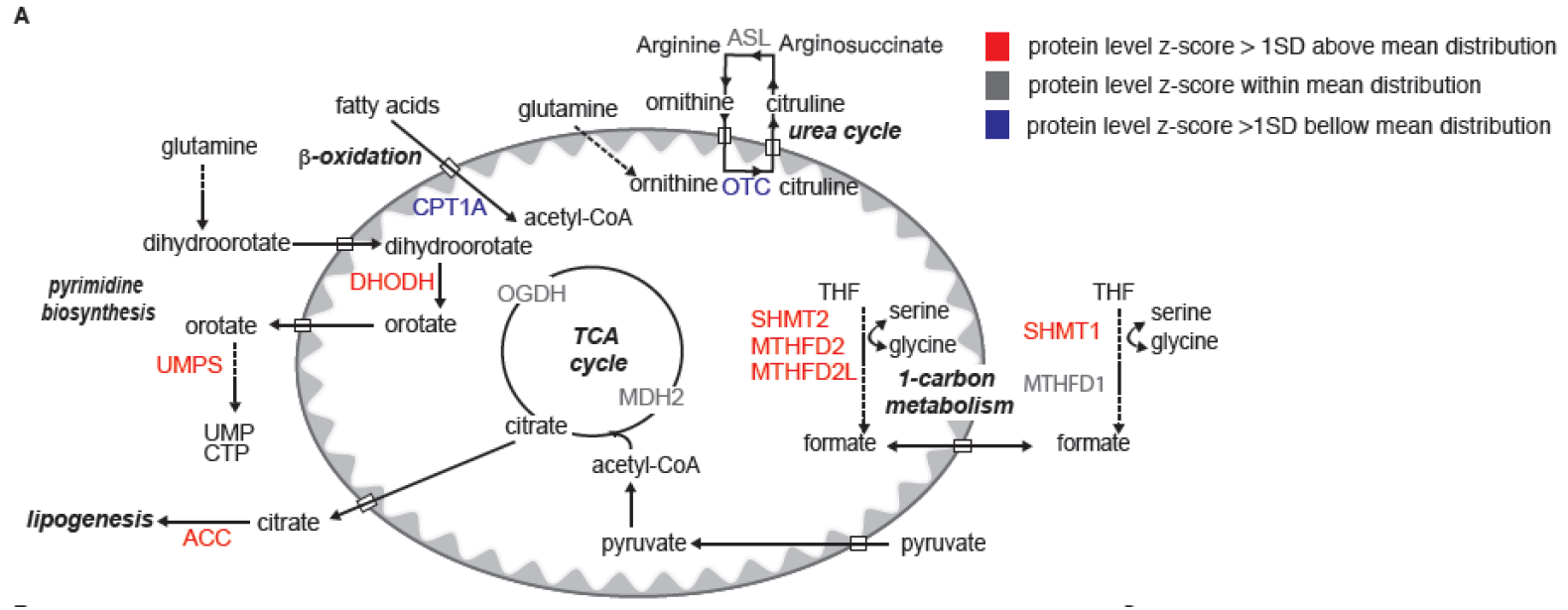
Ron Harel et al, Cell Met, 2016
Ron Harel et al, PNAS, 2018

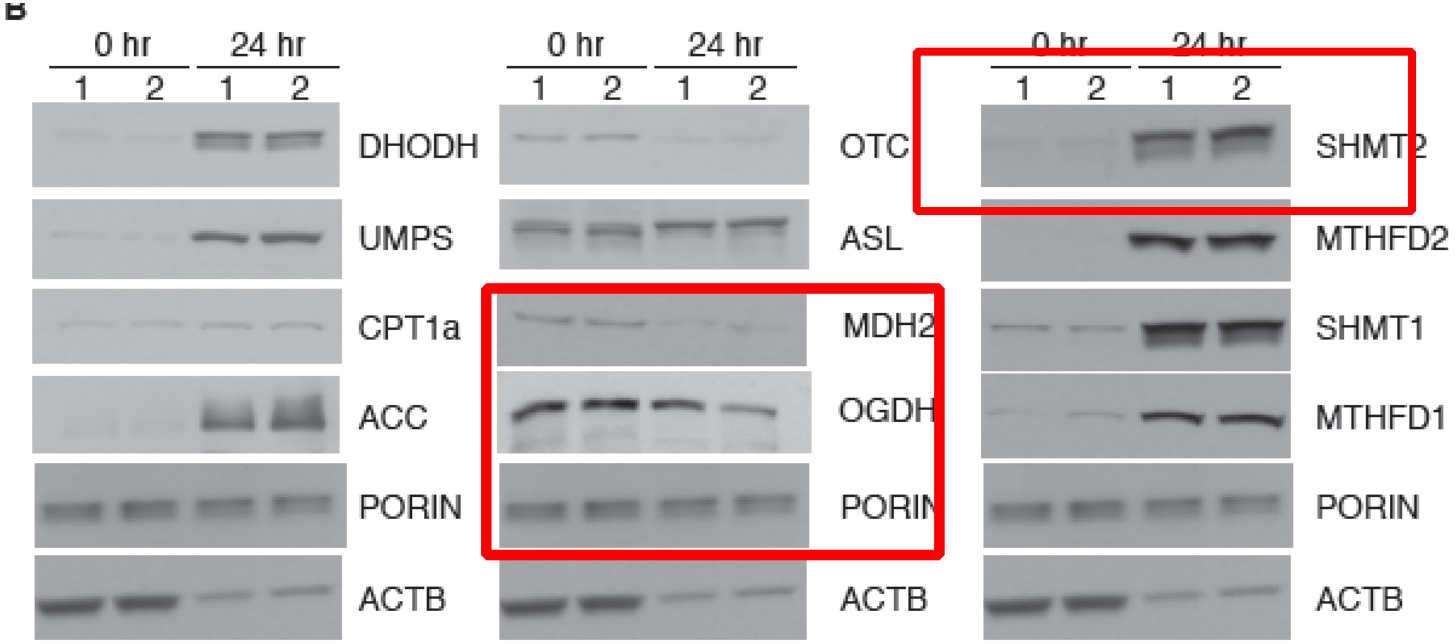
Mitochondrial One Carbon Metabolism is induced with T cell activation



Steve Gygi

Not a typical biogenesis profile

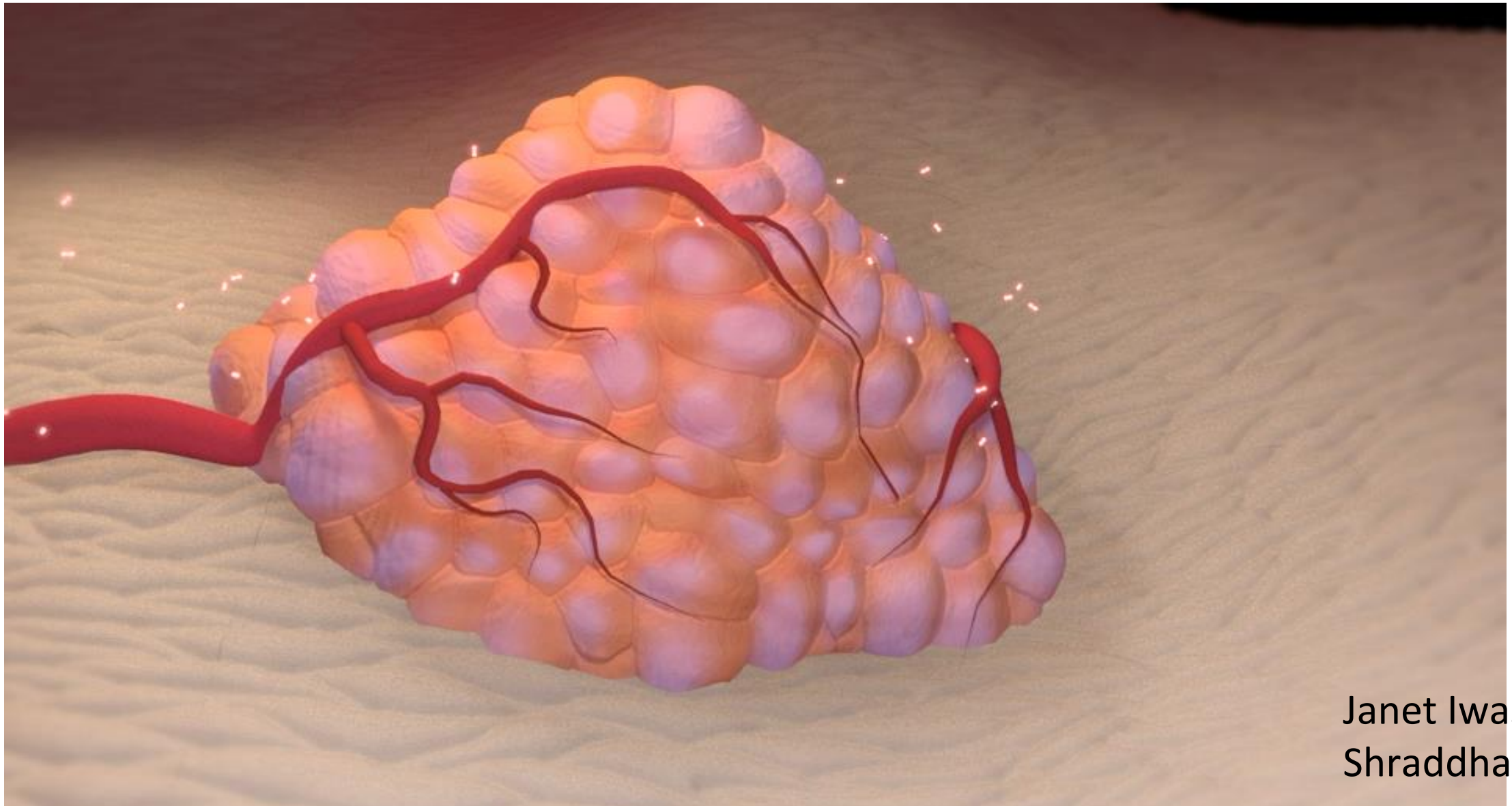




Concepts

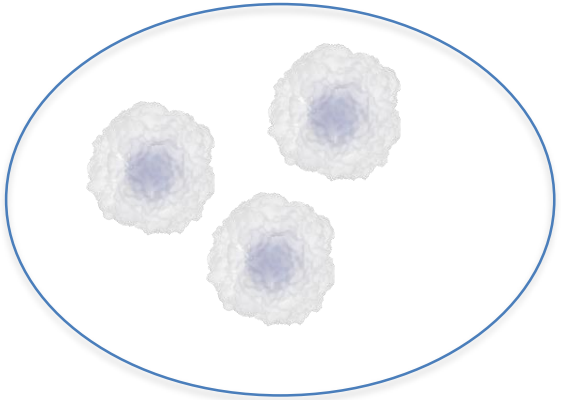
- T cells use similar fuels and signaling networks as many cancer cells.
- T cells become anabolic engines when activated.
- Not a typical biogenesis profile - suited for the anabolic demands of T cells.

What is the role of metabolism in the tumor microenvironment?

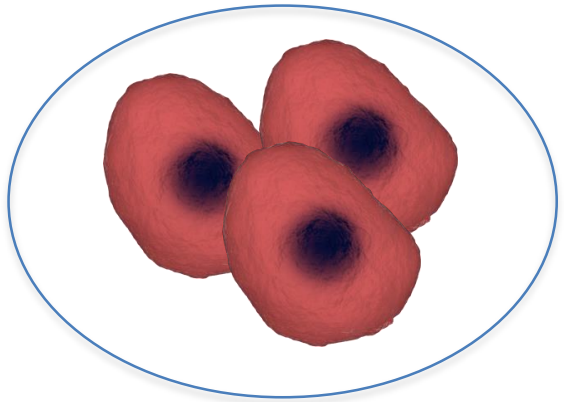


Janet Iwasa
Shraddha Nayak

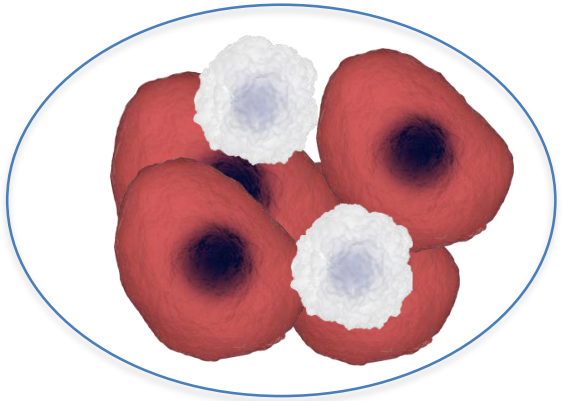
Can we identify tumor versus T cell dependencies?



OT1 T cells



Ova+/- tumor cells



T cells + tumor cells
Co-culture

Drijvers et al, 2020

What is the metabolism of a cytotoxic T cell?

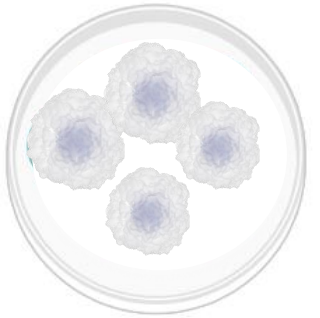
cytotoxic

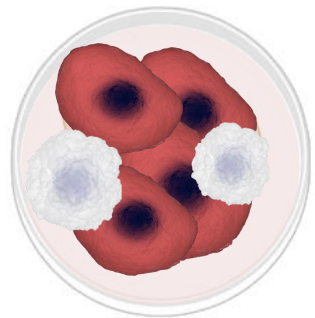
less cytotoxic

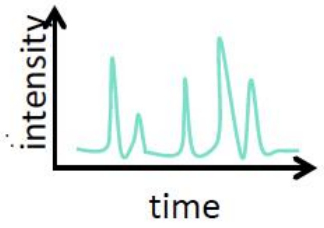
T cells

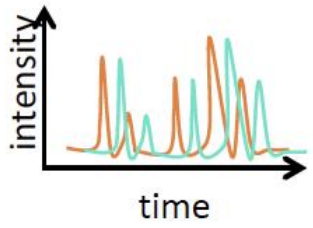
B16 OVA co-culture

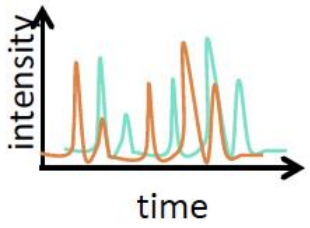
co-culture + CM from Tumors







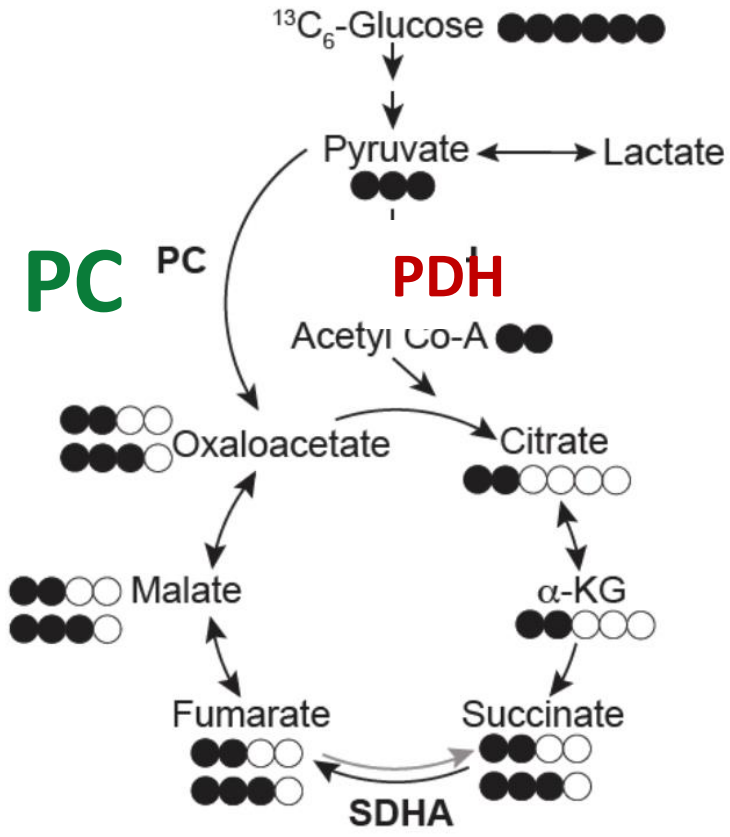


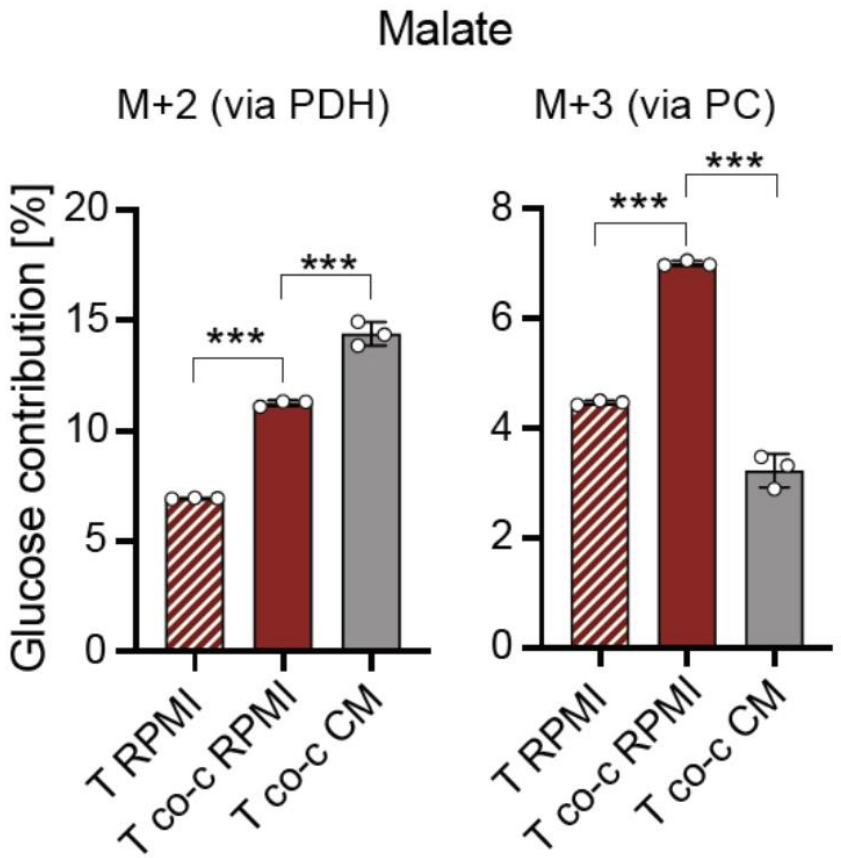


Rapid separation
Metabolite profiling

Elia et al, in revision

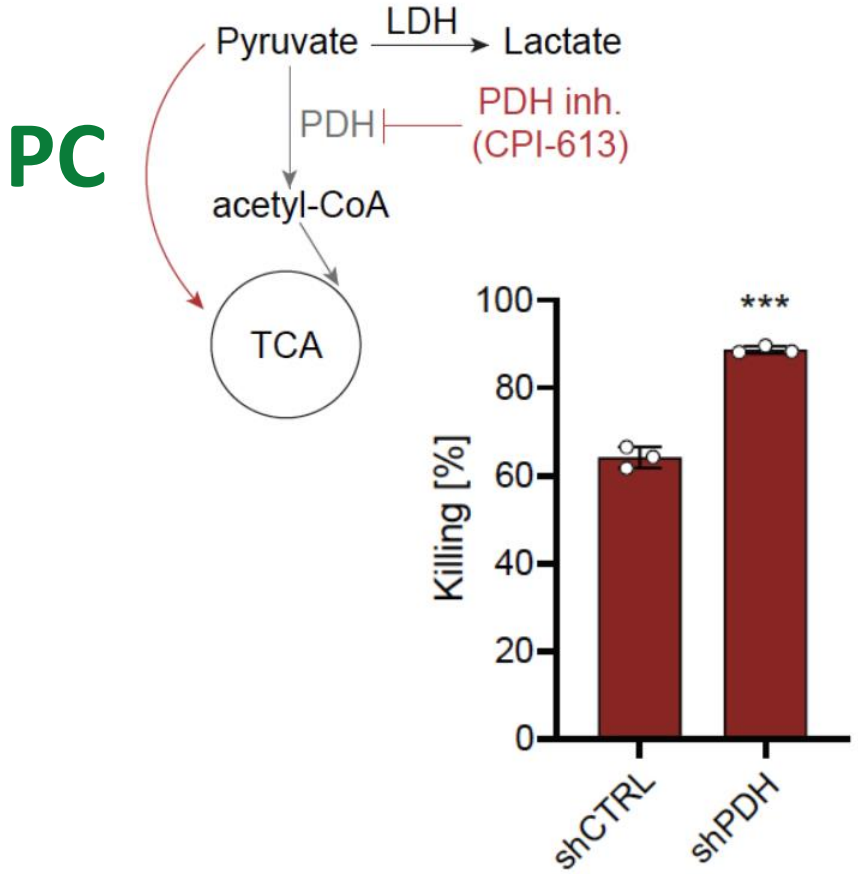
Cytotoxic T cells switch pyruvate utilization by increasing PC activity



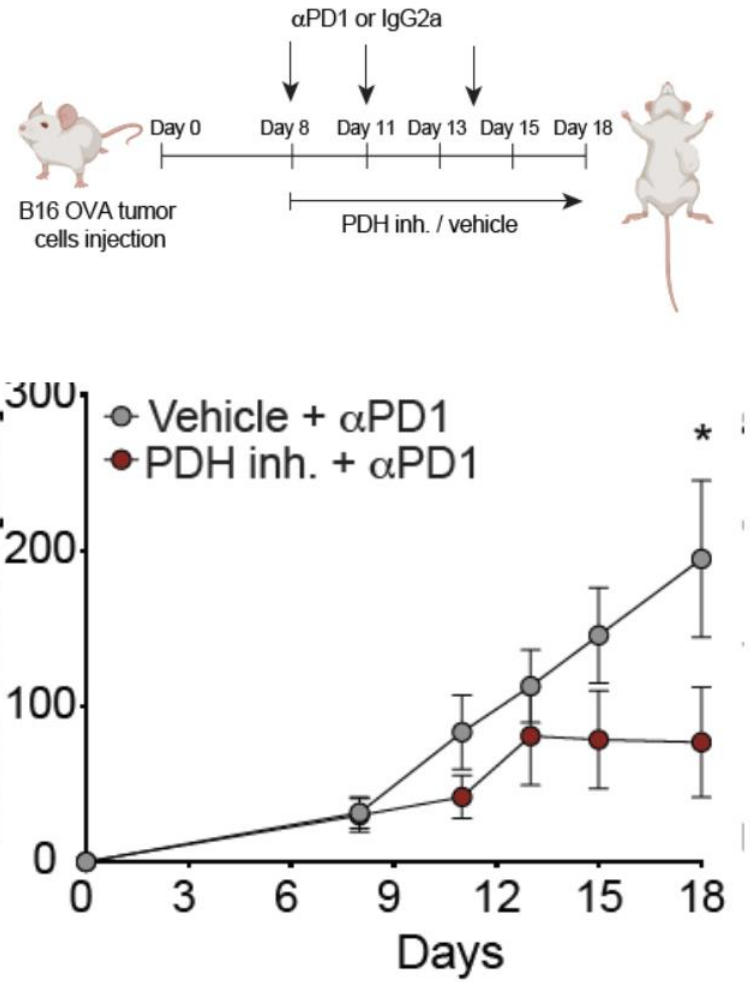


Is PDH inhibition sufficient to increase anti-tumor cytotoxicity?

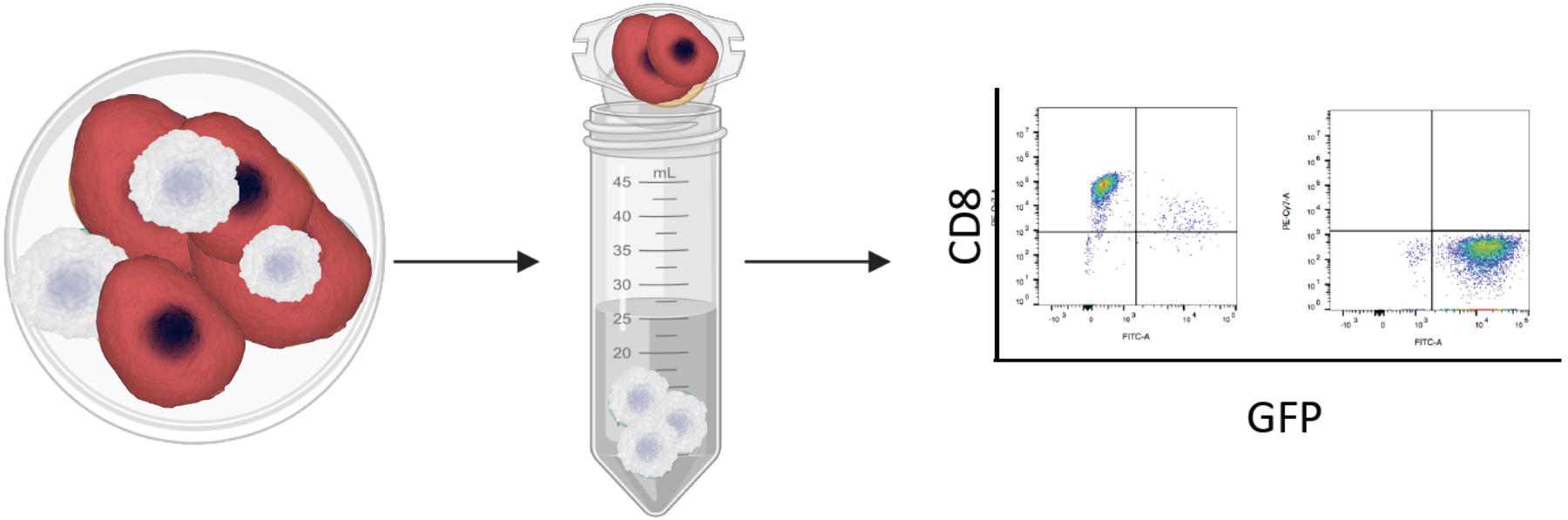
Co-culture



In vivo

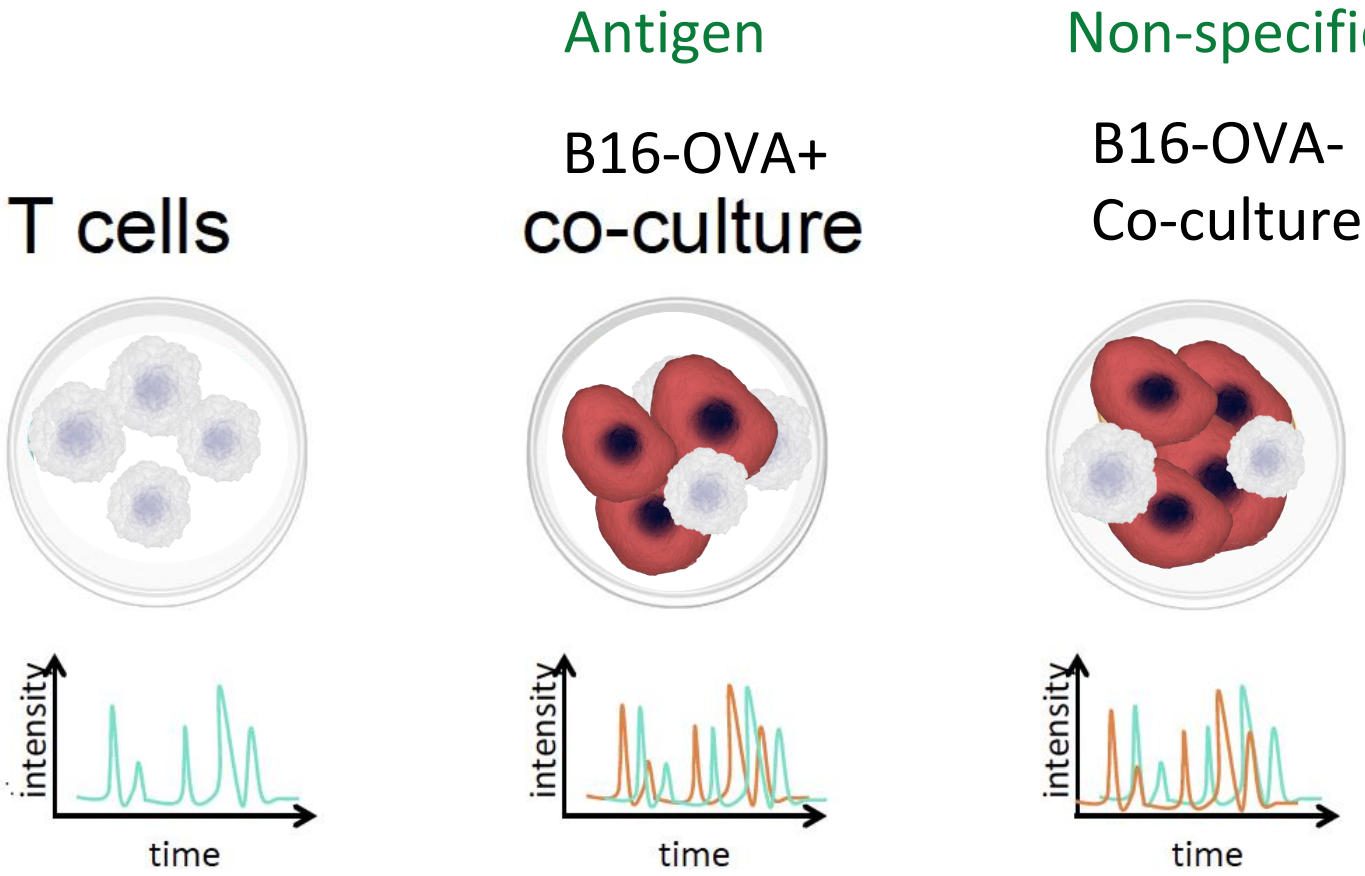


Can we develop methods to rapidly separate co-cultures to study signaling and metabolism?



Elia et al, in revision

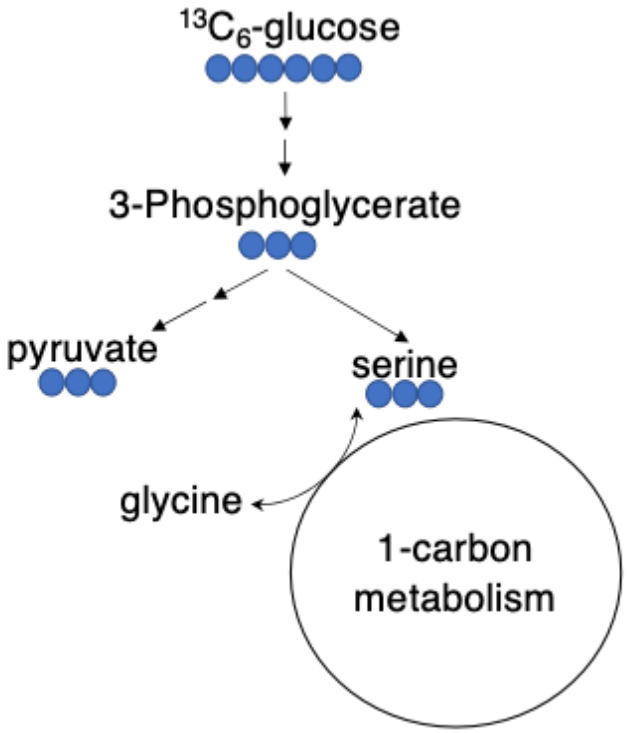
What is the metabolism of antigen-specific T cell-mediated killing of tumors?

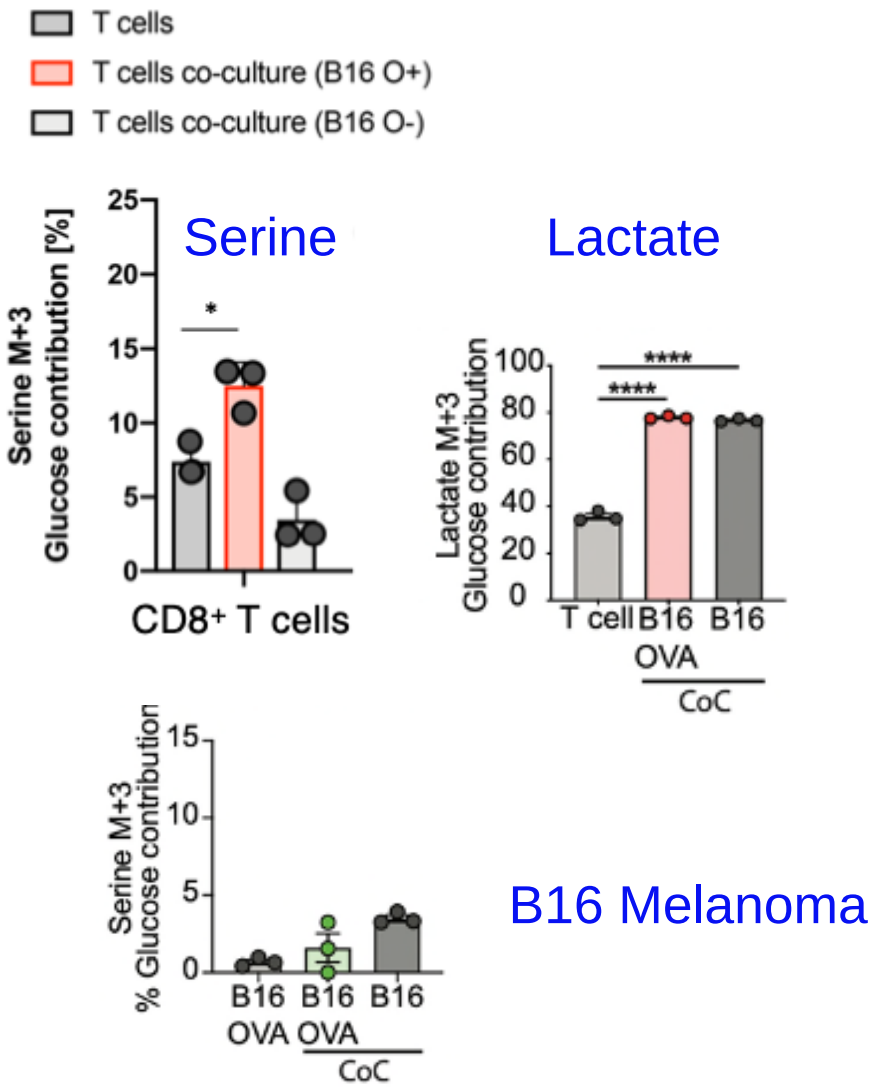


Rapid separation
Metabolite profiling

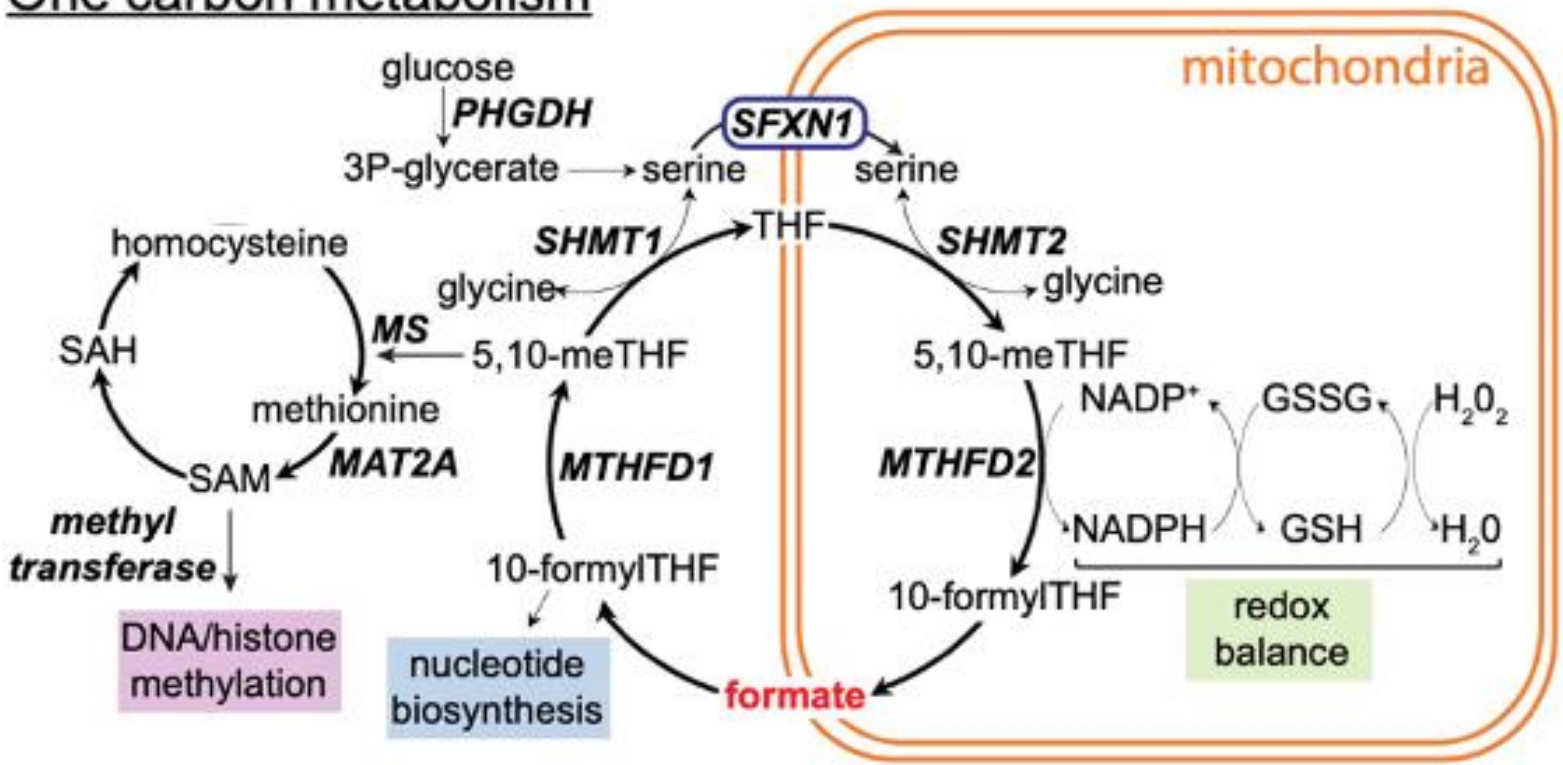
Rowe et al, in preparation

Tumor cell contact *increases* CD8⁺ T cell serine biosynthesis



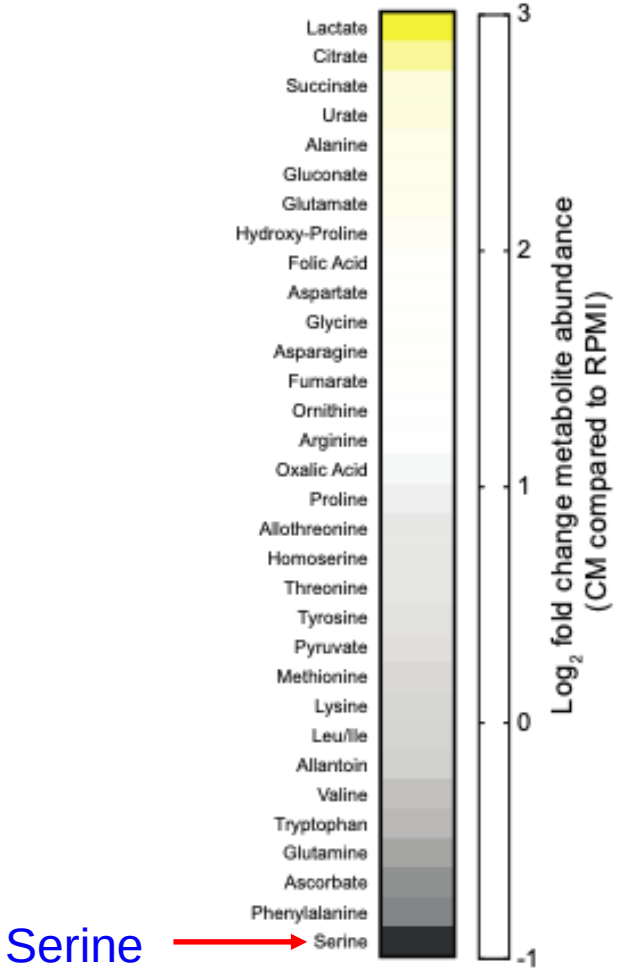


One carbon metabolism



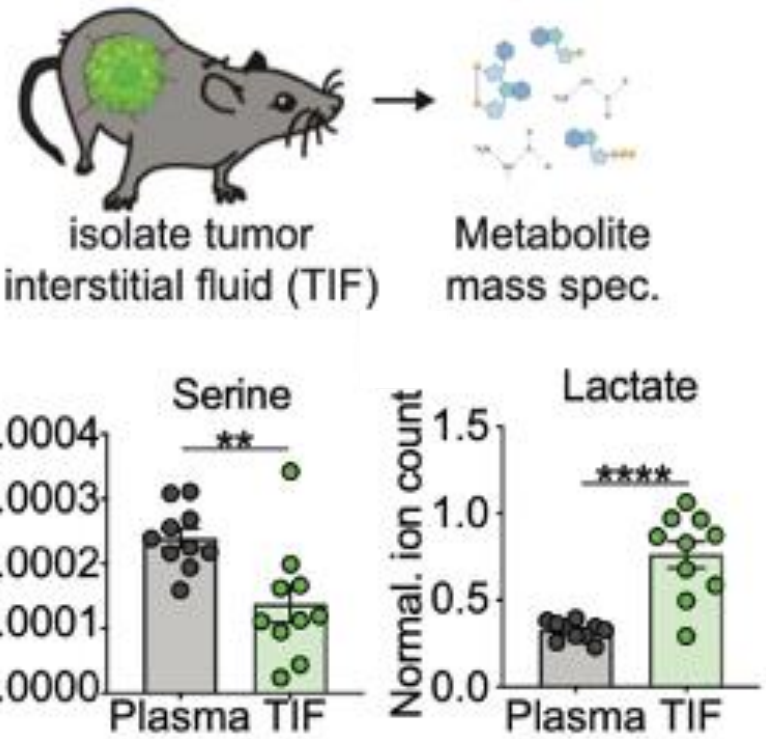
Serine is limiting in the TME

Conditioned Media vs RPMI

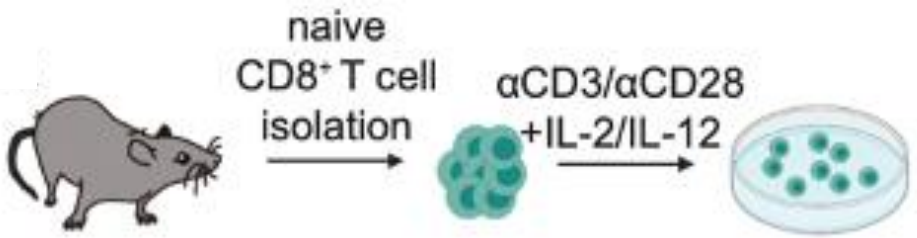


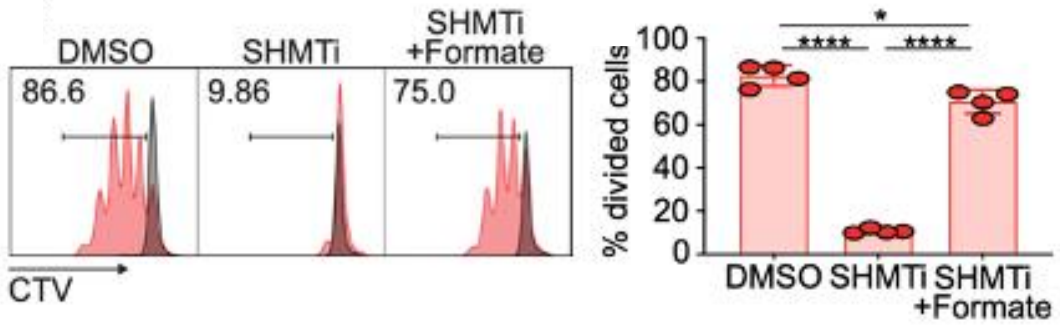
Serine →

Tumor Interstitial fluid (In vivo)

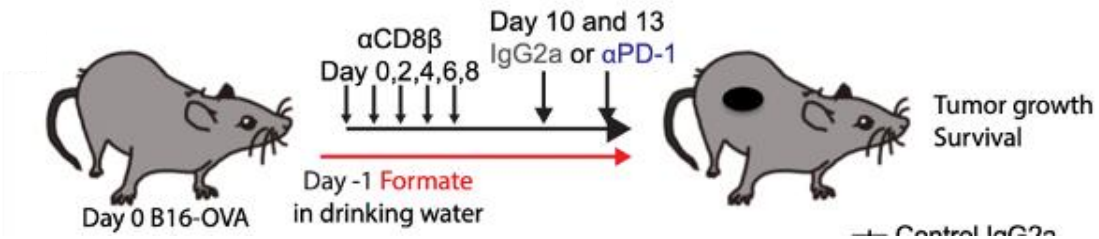


Formate rescues diminished One Carbon metabolism in CD8+ T cells



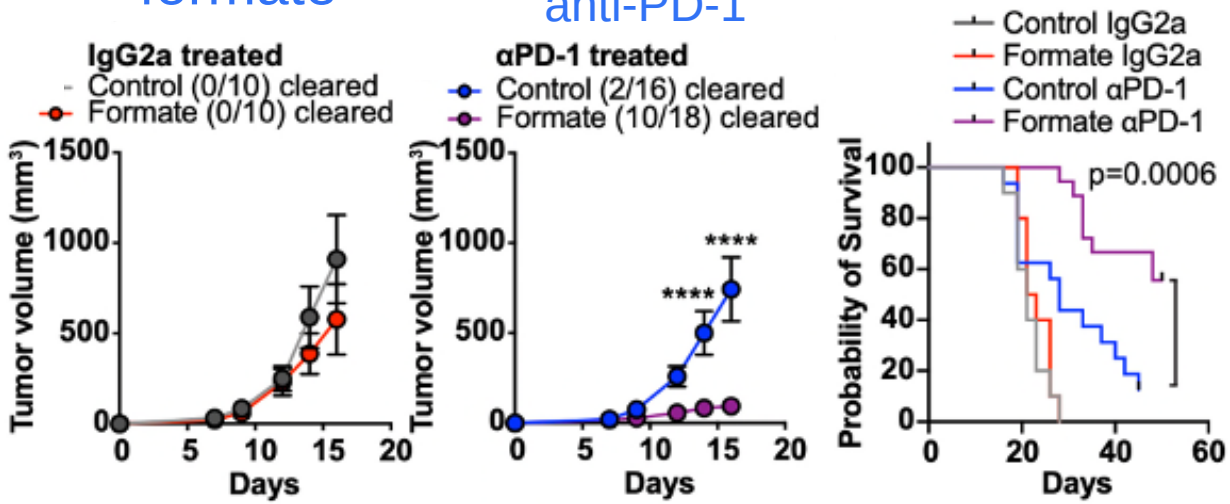


Formate treatment synergizes with anti-PD-1



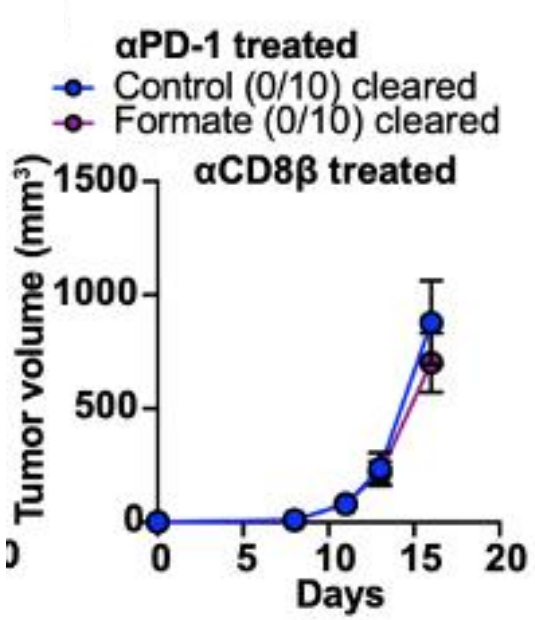
formate

formate
anti-PD-1

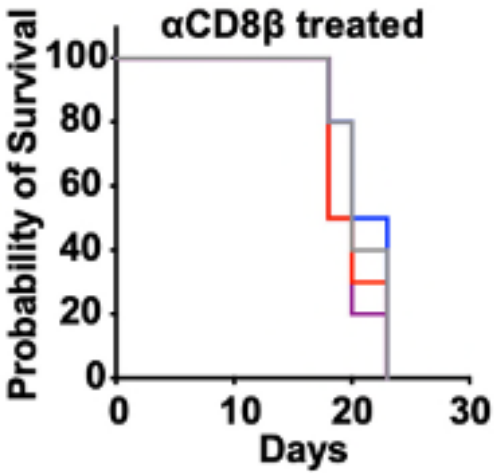


CD8+ T cells are required for benefits of formate

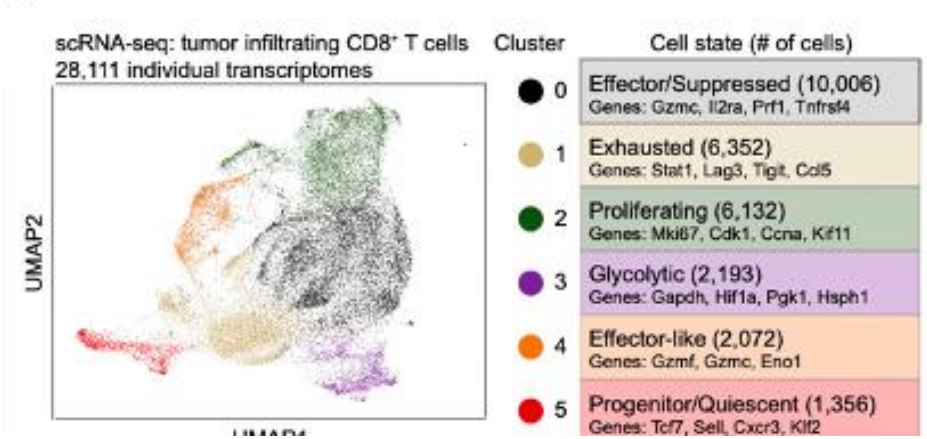
Tumor growth

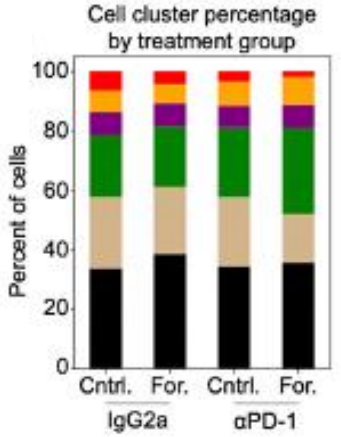


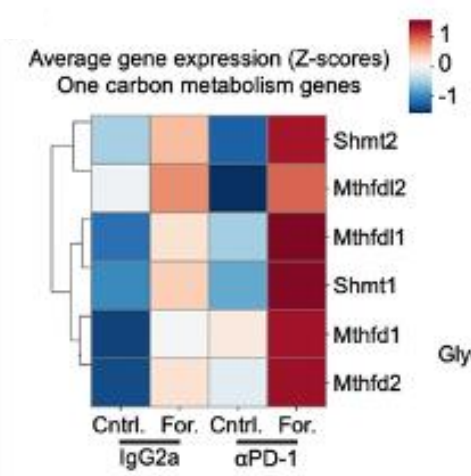
Survival

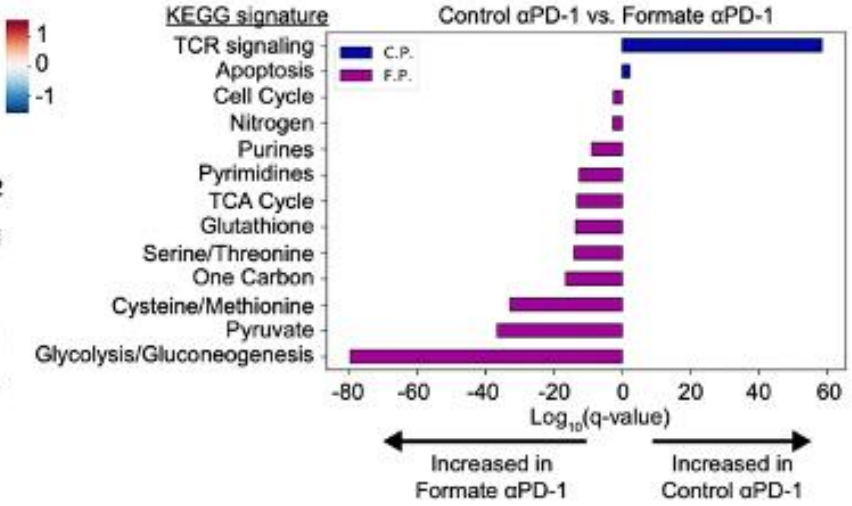


Formate treatment impacts T cells and metabolism

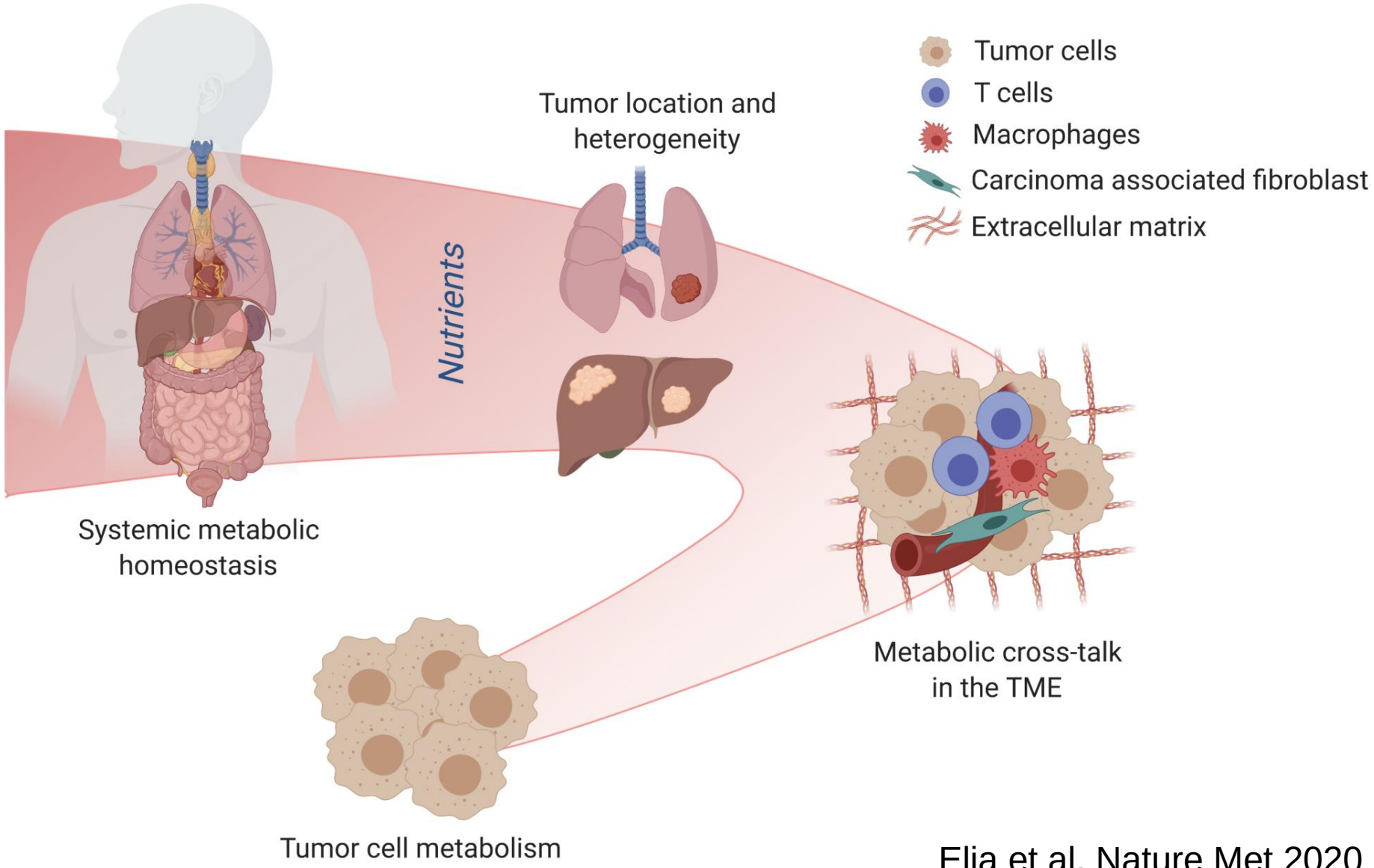






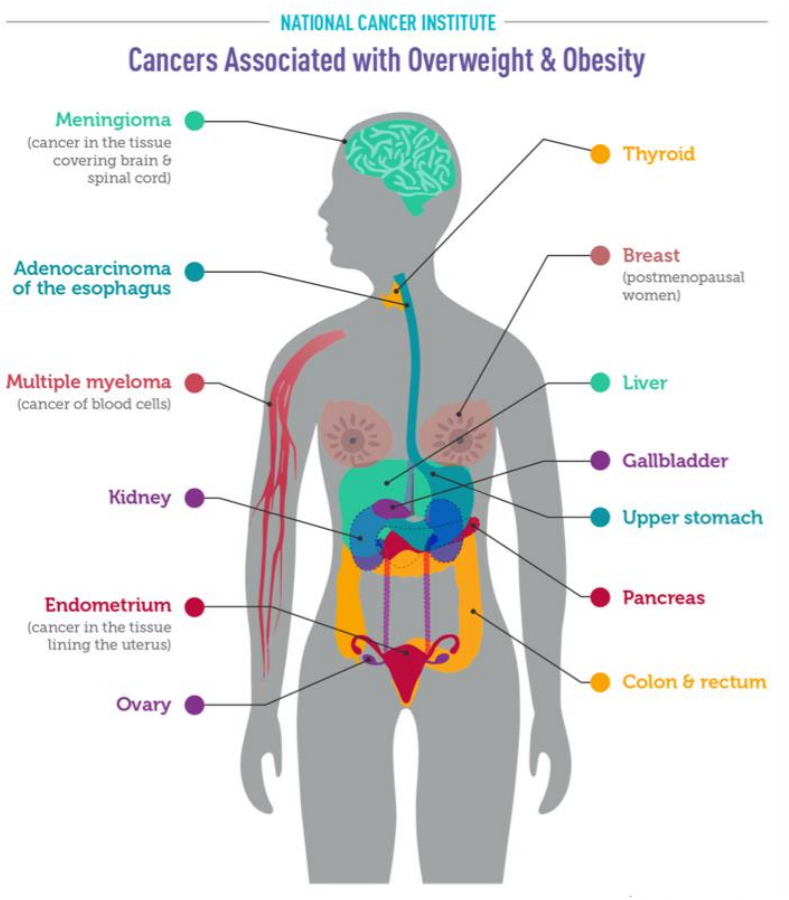


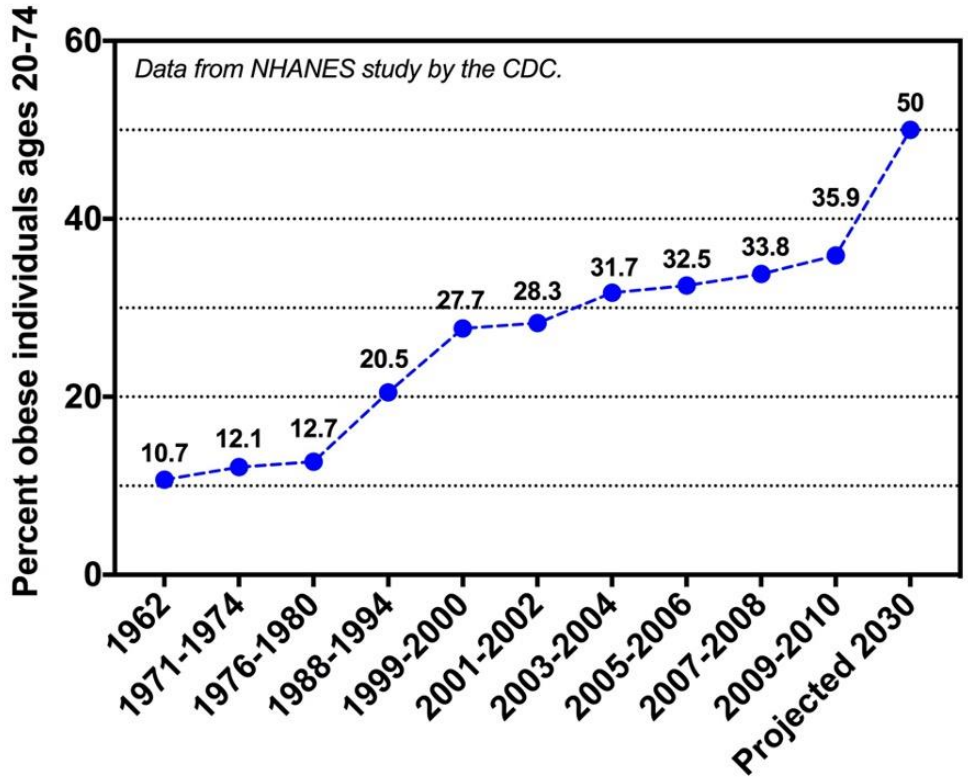
Tiered view of tumor metabolism



Elia et al, Nature Met 2020

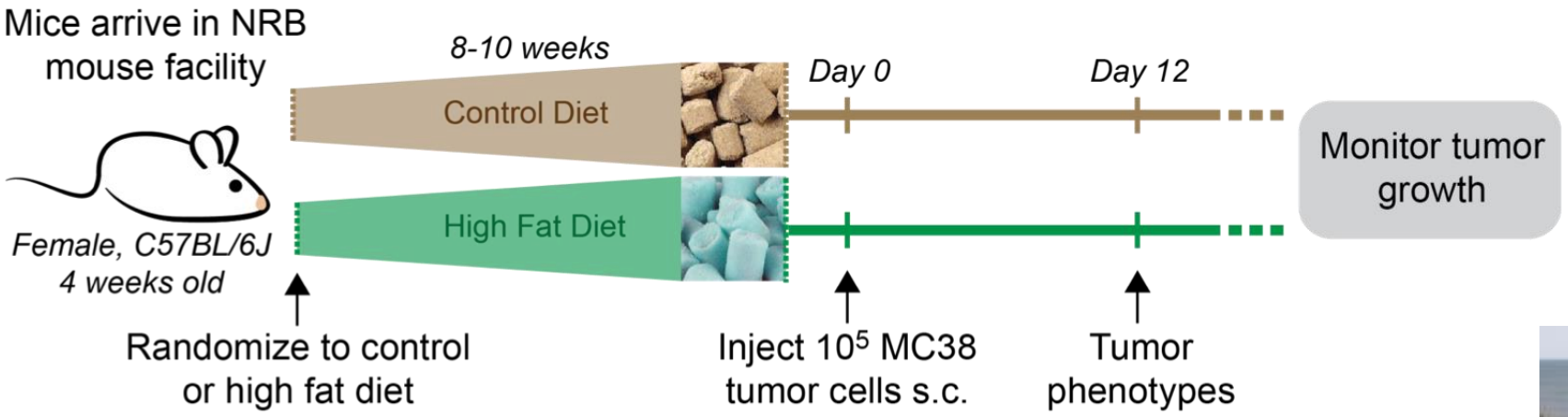
Obesity is a cancer risk factor





"Body Fatness and Cancer – Viewpoint of the IARC Working Group". NEJM (2016)

Using Mice to study tumor-immune mechanisms

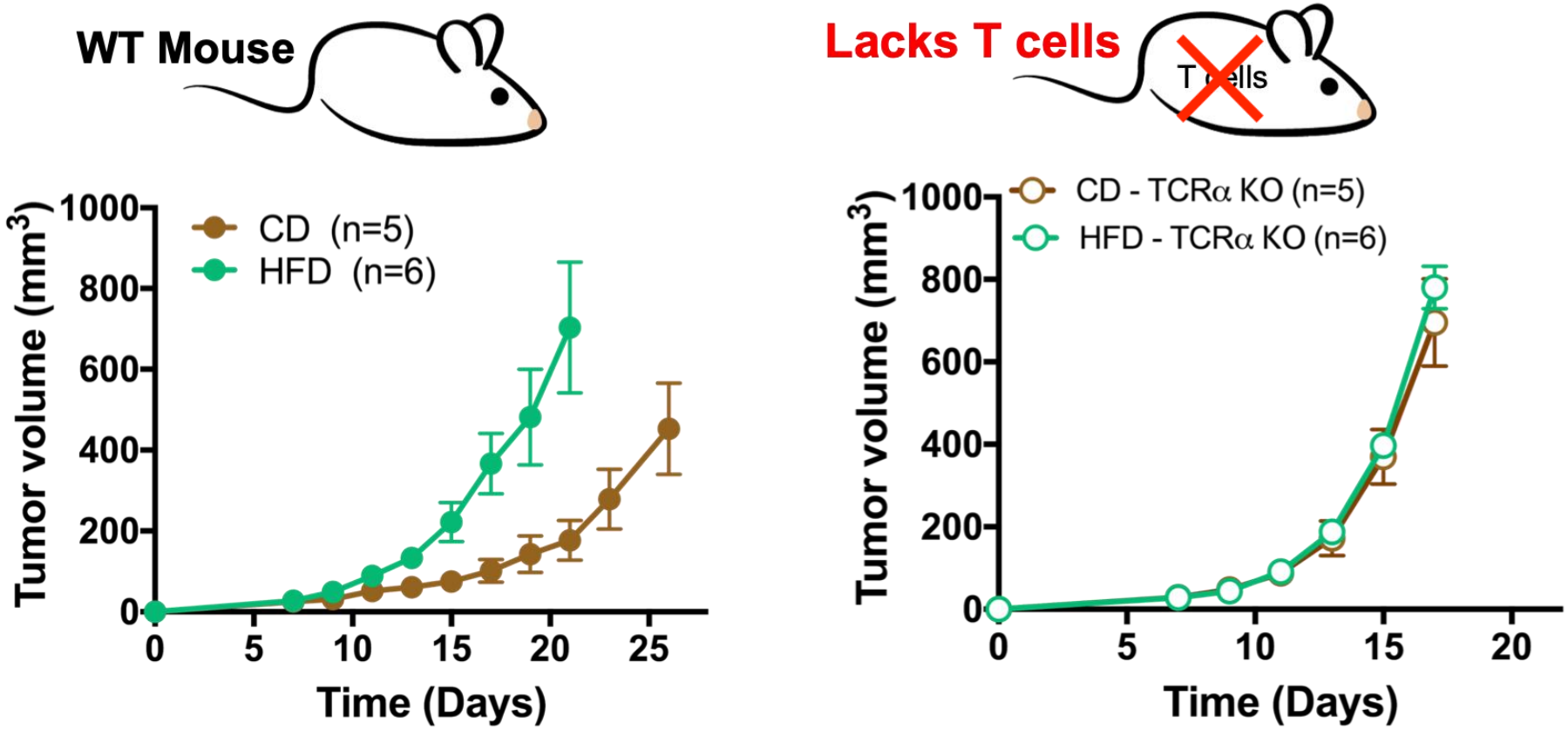


Alison Ringel
MIT

<u>System benefits</u>
antigen-specificity
Tumors carry a reporter
immune contributions
Identify mechanisms
Study causal mechanisms

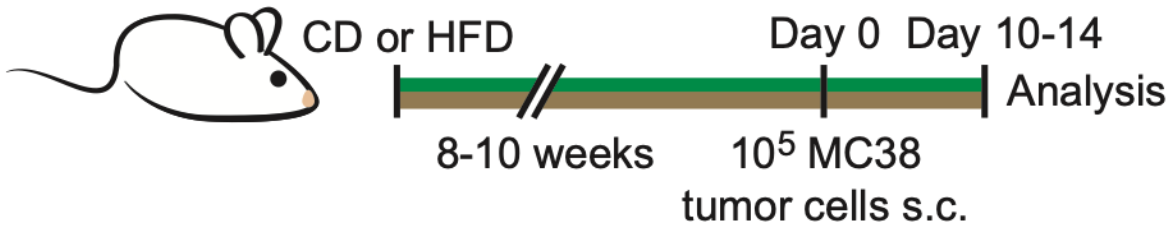
Ringel et al, Cell, 2020

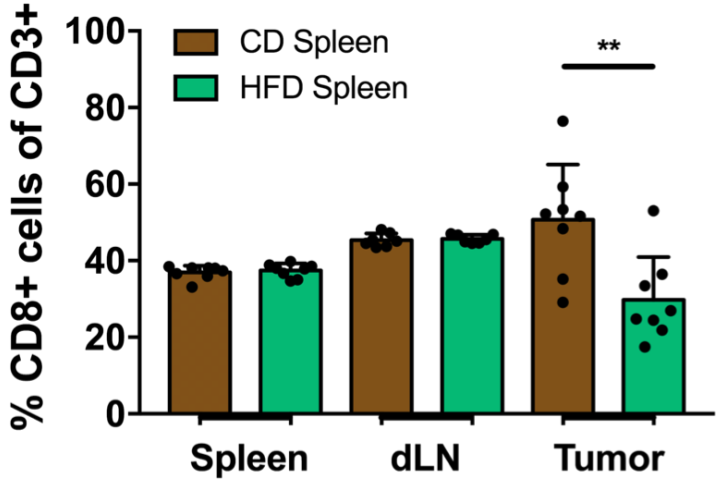
T cells are required for increased tumor growth on high fat diet

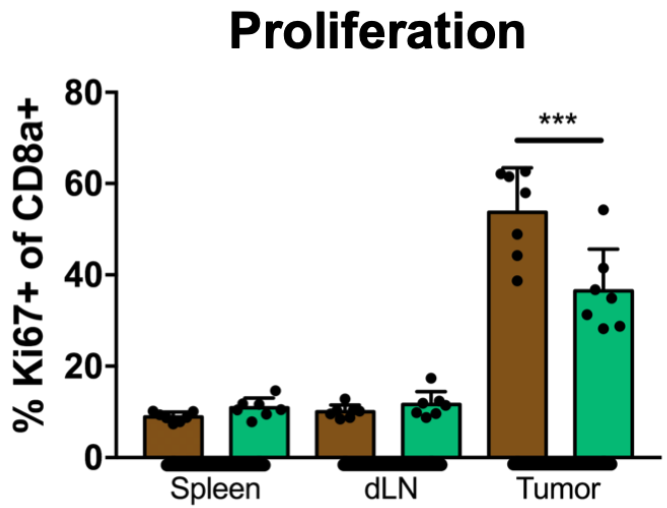


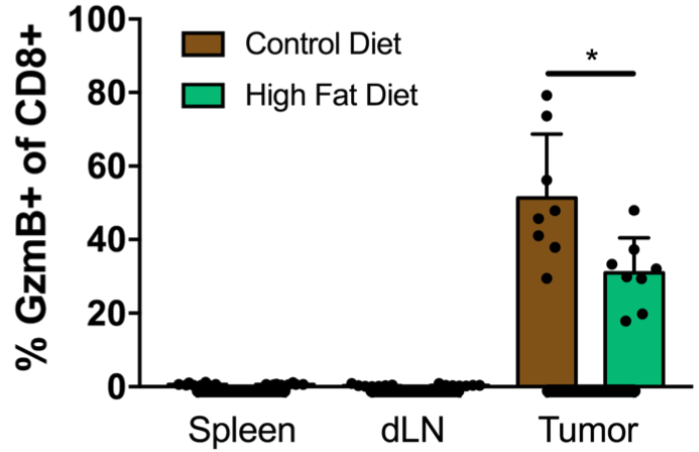
*phenocopy with CD8+ T cell depletion but not with CD4+ T cell depletion

High fat diet reduces the proportion of intratumoral CD8⁺ T cells







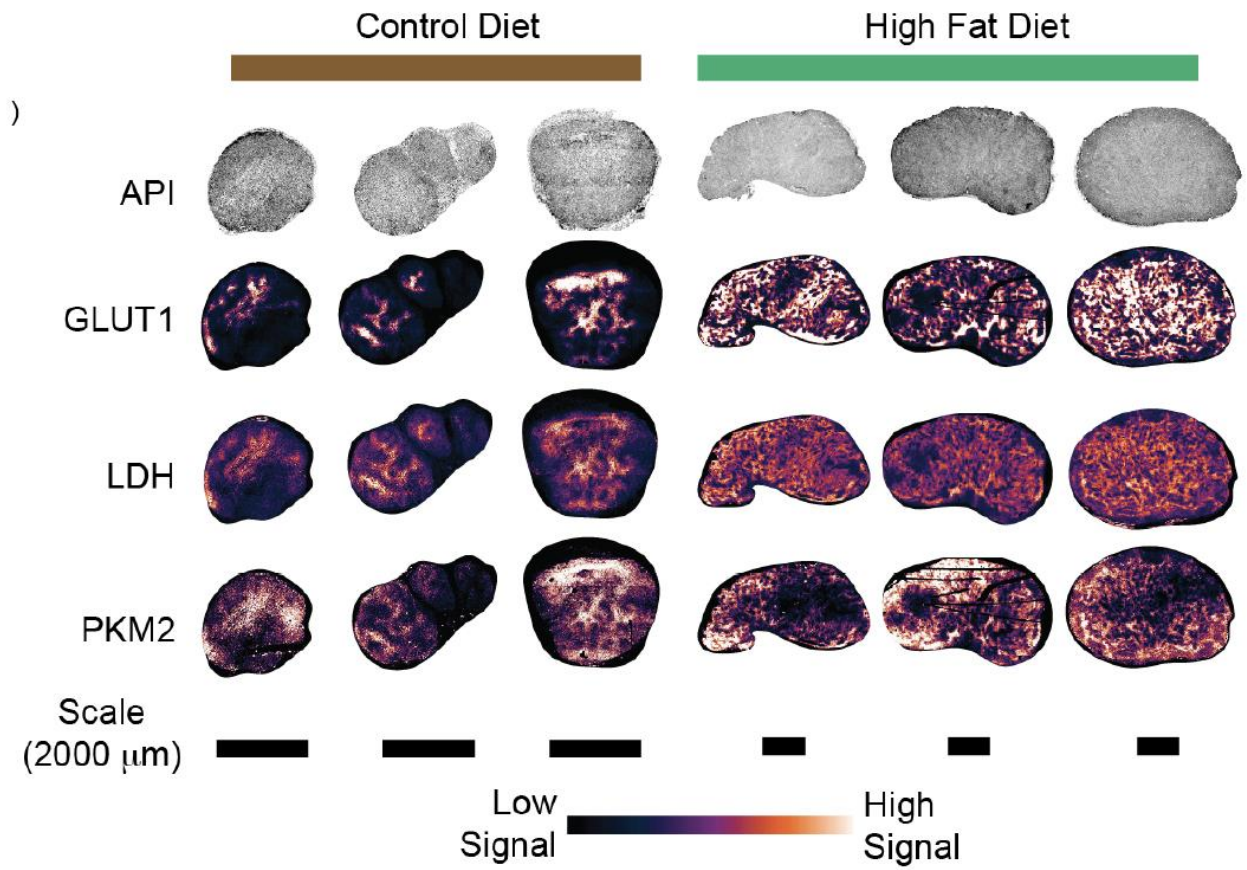


Day 12 Analysis of Tumor Immune Infiltrate

Challenges:

1. Many metabolic alterations are not reflected by transcription.
2. What are the metabolic states in an intact tissue?
3. What are the spatial relationships between immune cells and tumor cells?

CyCIF revealed a pattern of glycolytic proteins that differ with diet



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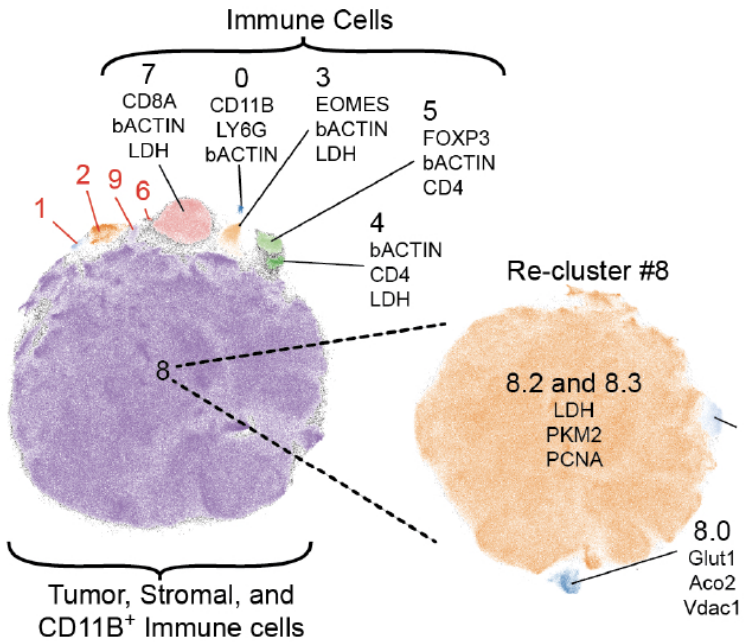
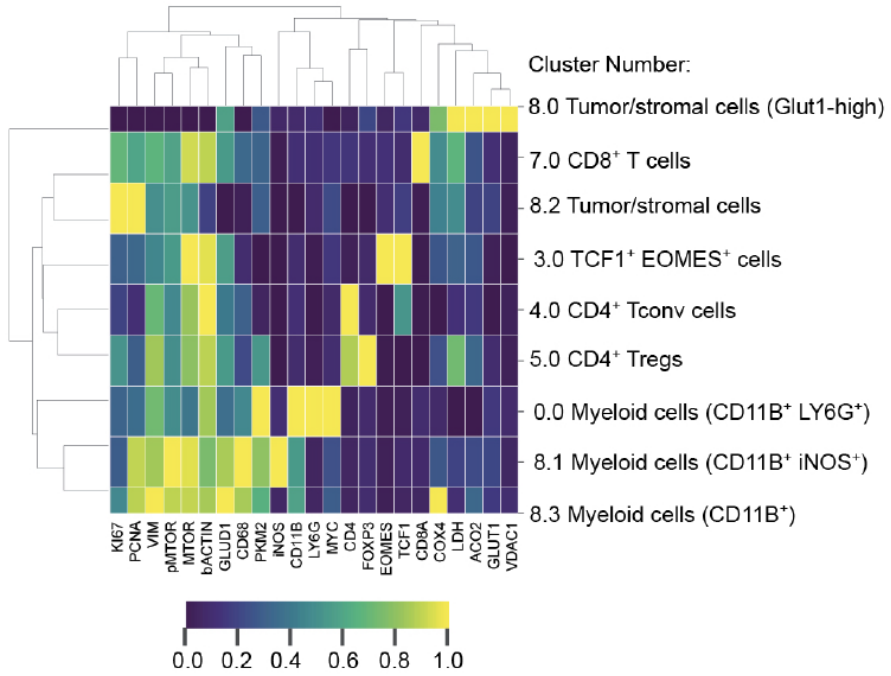
Zoltan Maliga

Dimensional reduction and density-based clustering reveals core cell phenotypes





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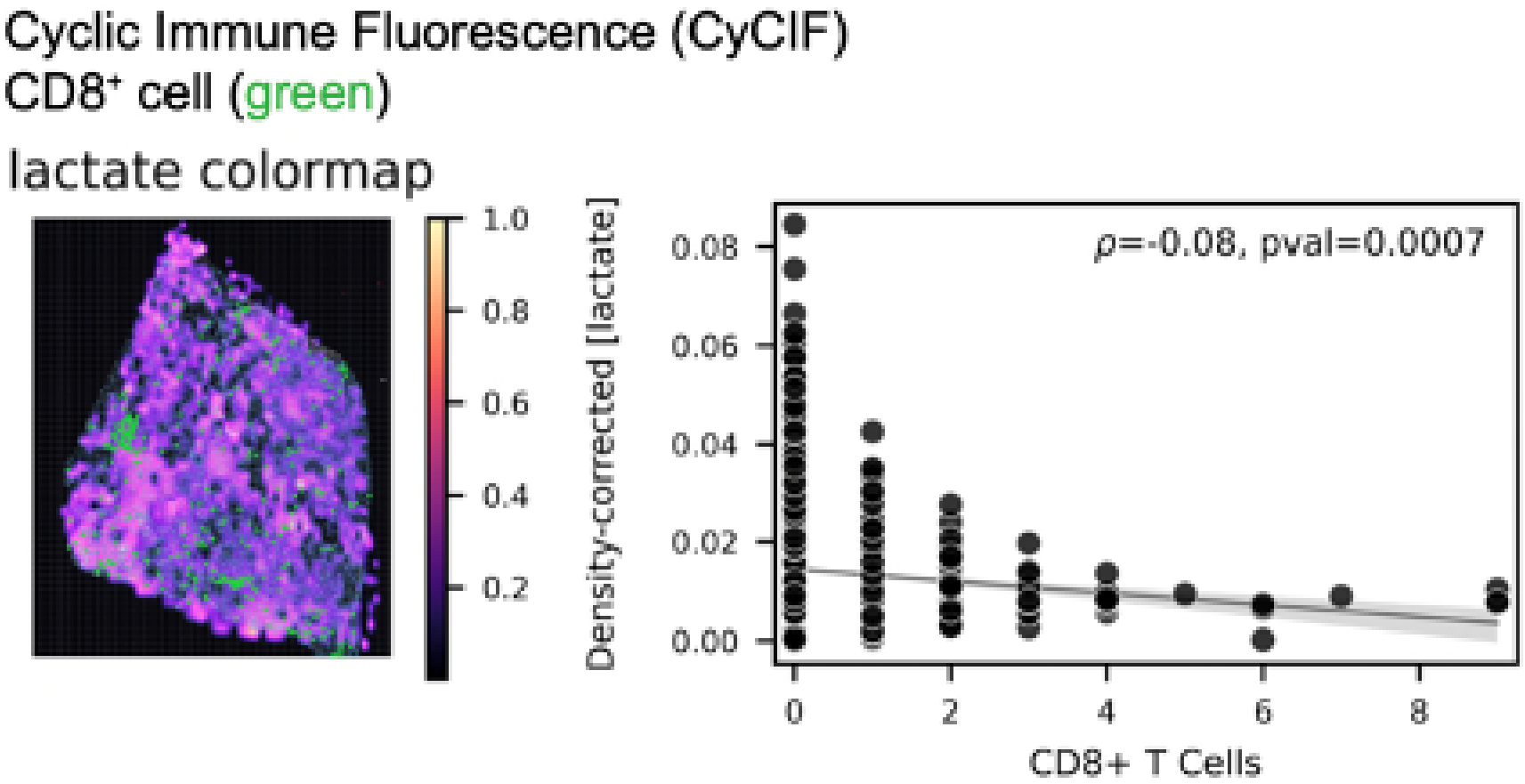
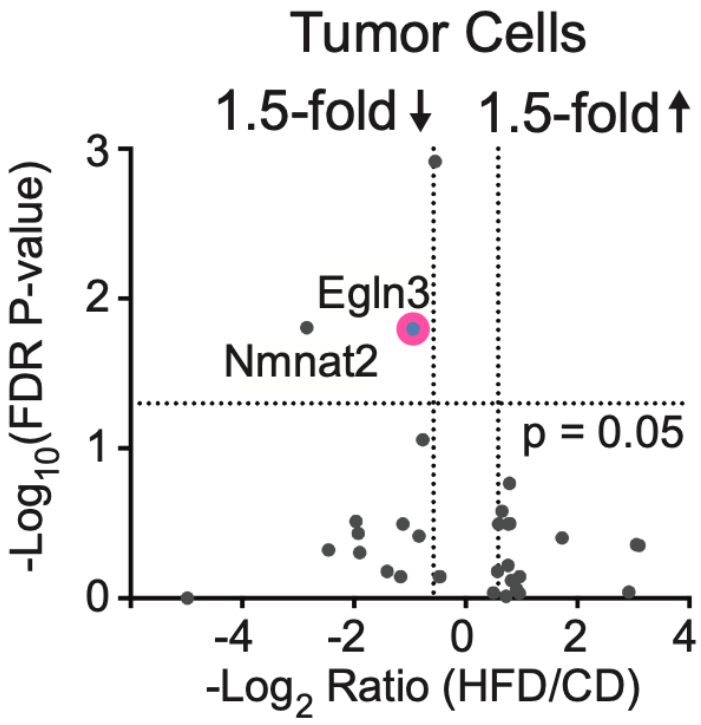
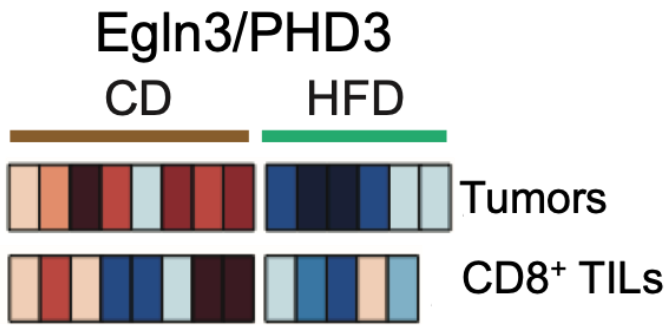
Greg Baker

Combining metabolite profiles with CyCIF cell reporters



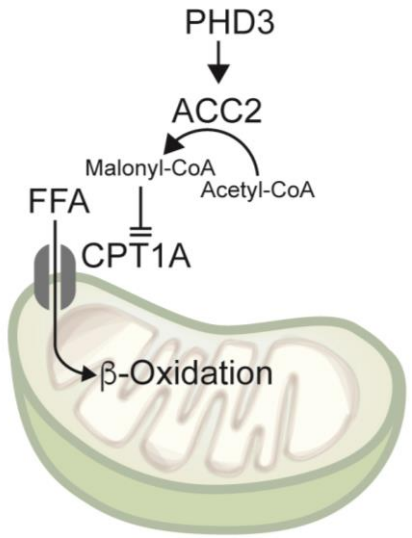
MC38 tumors downregulate PHD3 expression with high fat diet





MC38-GFP RNA-seq (Day 12 Tumors, top metabolic genes)

PHD3 represses fat metabolism



Mitochondrial FAO **Repressed**

German N. J. Yoon H. et al., Mol Cell. 2016

PHD3 loss increases fat oxidation

Mitochondrial FAO **Repressed**

Control Diet

PHD3 ↑

Less Fat Oxidation

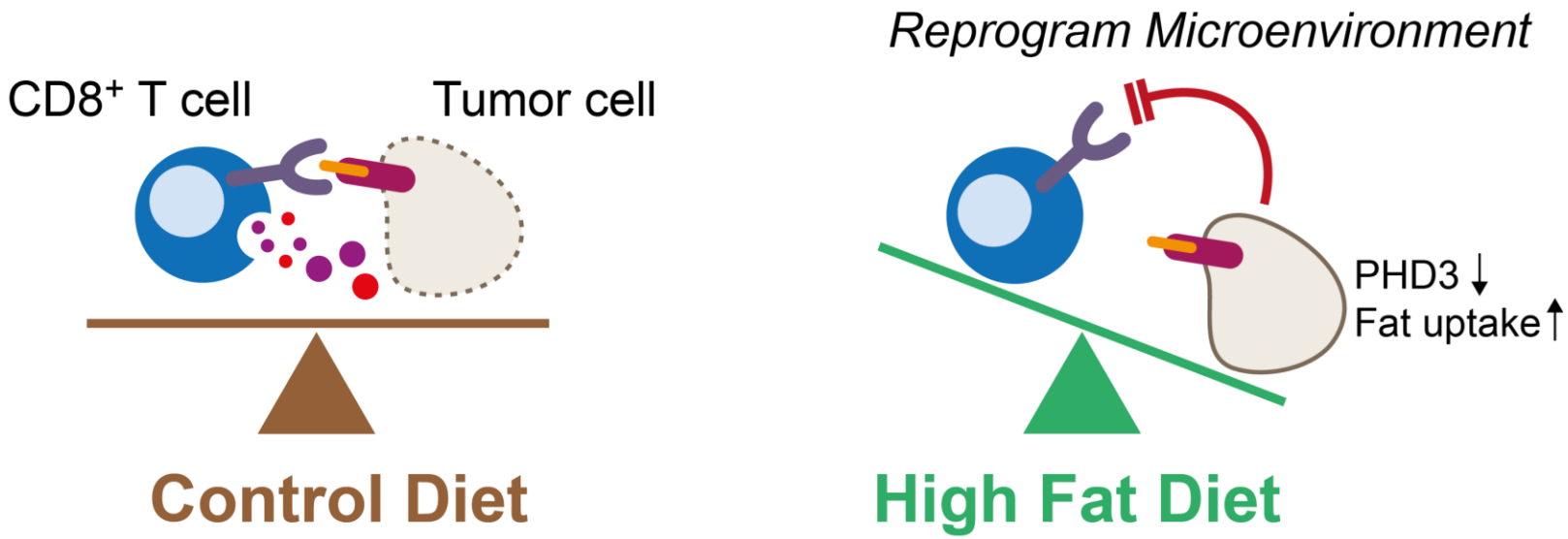
High-Fat Diet

PHD3 ↓

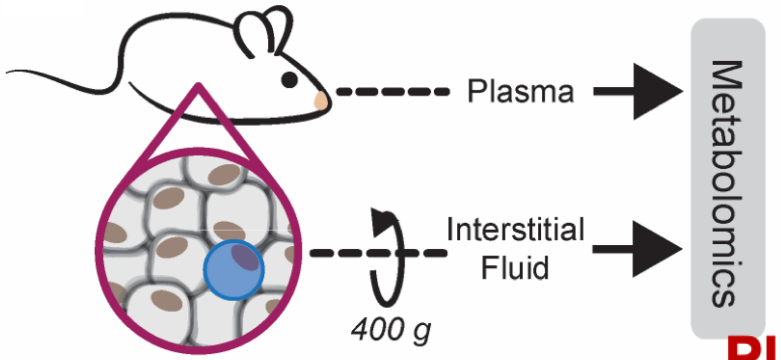
More Fat Oxidation

German N. J. Yoon H. et al., Mol Cell. 2016

Does diet-induced obesity cause a metabolic tug of war in tumors?

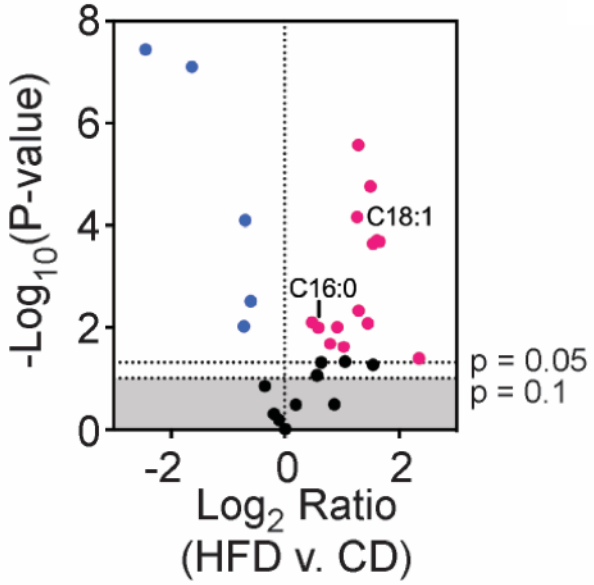


Surprise- obesity alters fatty acid profiles systemically vs. the tumor



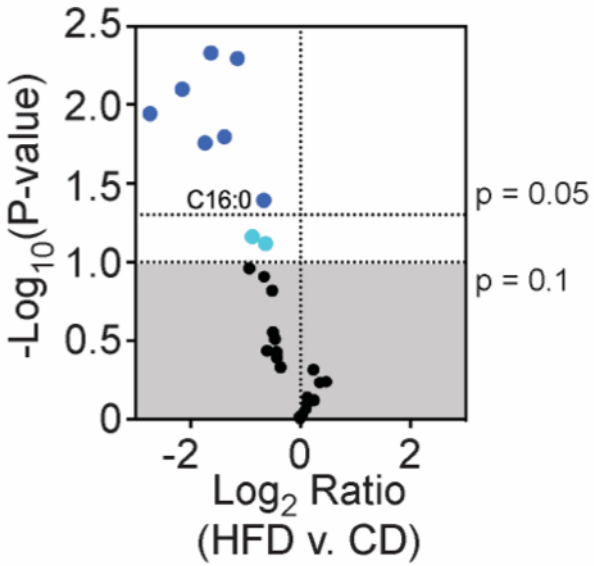
Plasma

(Systemic Metabolism

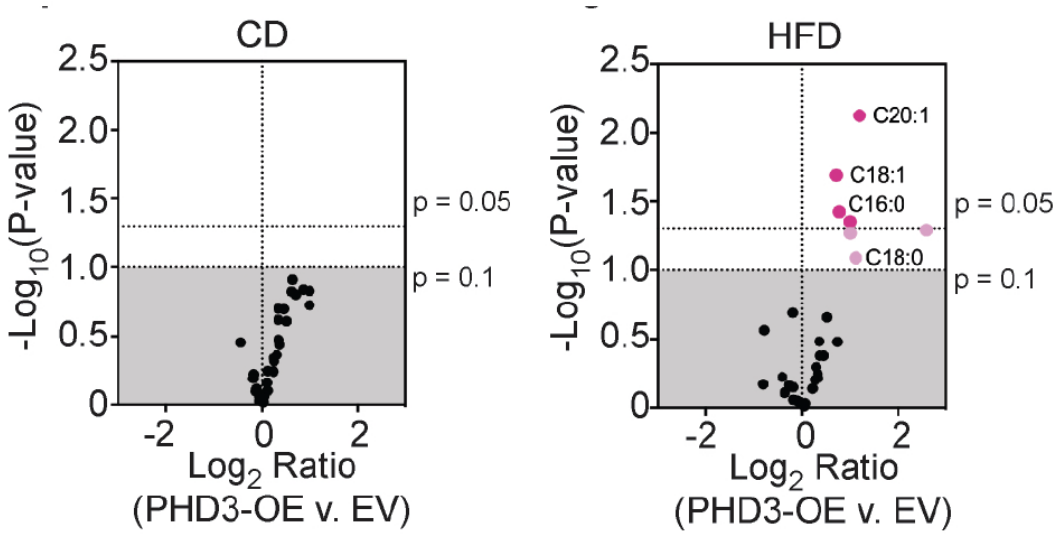


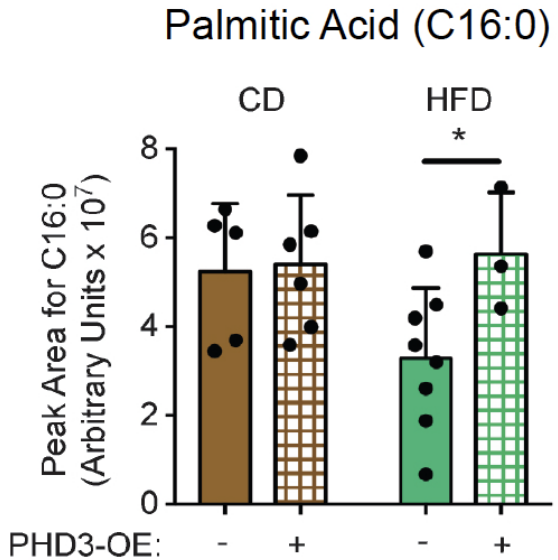
Tumor

(Local Metabolism

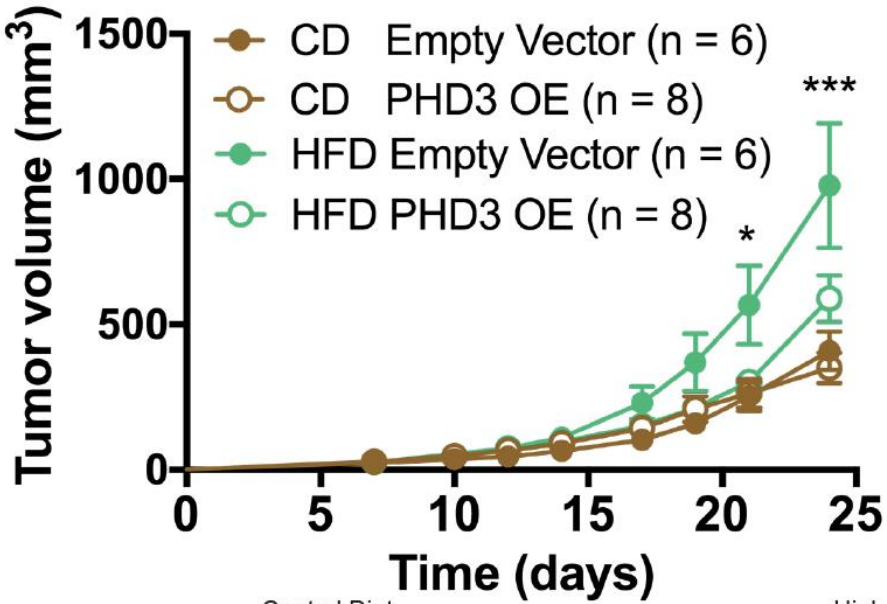


PHD3 overexpression increases free fatty acids in the tumor microenvironment

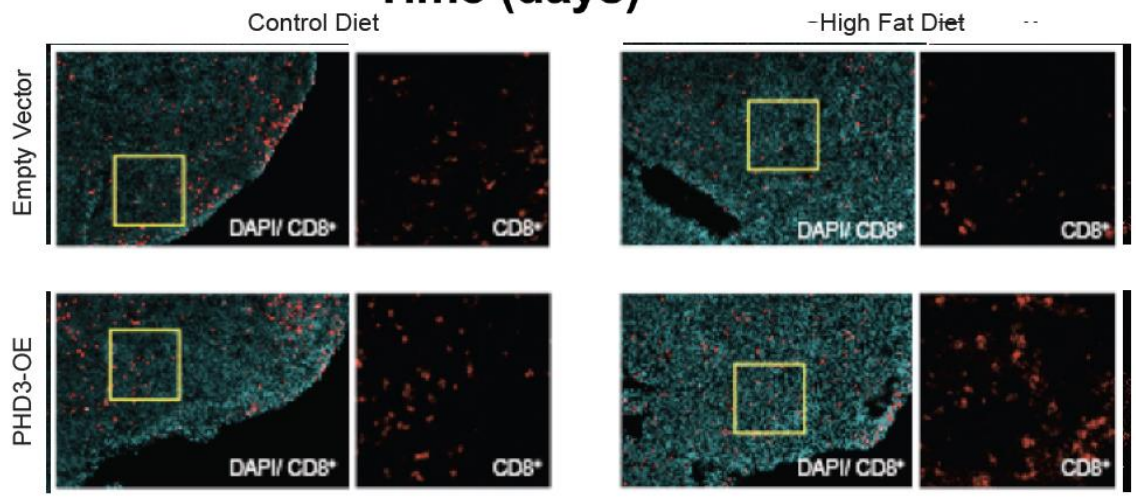


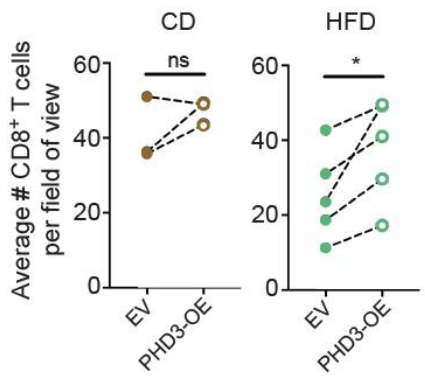


PHD3 OE is sufficient to reduce tumor growth on HFD

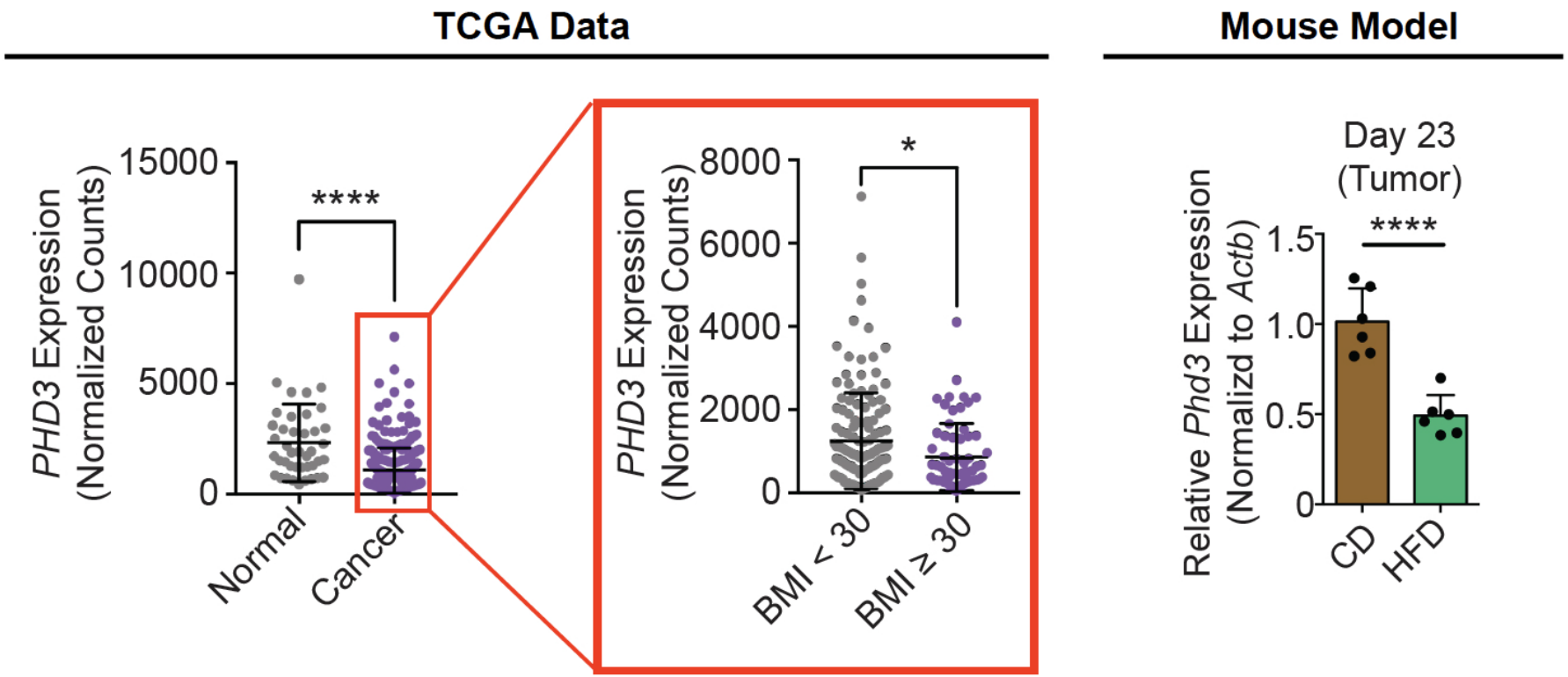


**CD8+T cell-dependent





Does PHD3 stratify with obesity or immune activation markers in patients?



In colorectal cancer patients, PHD3-low samples are immunologically “cold”

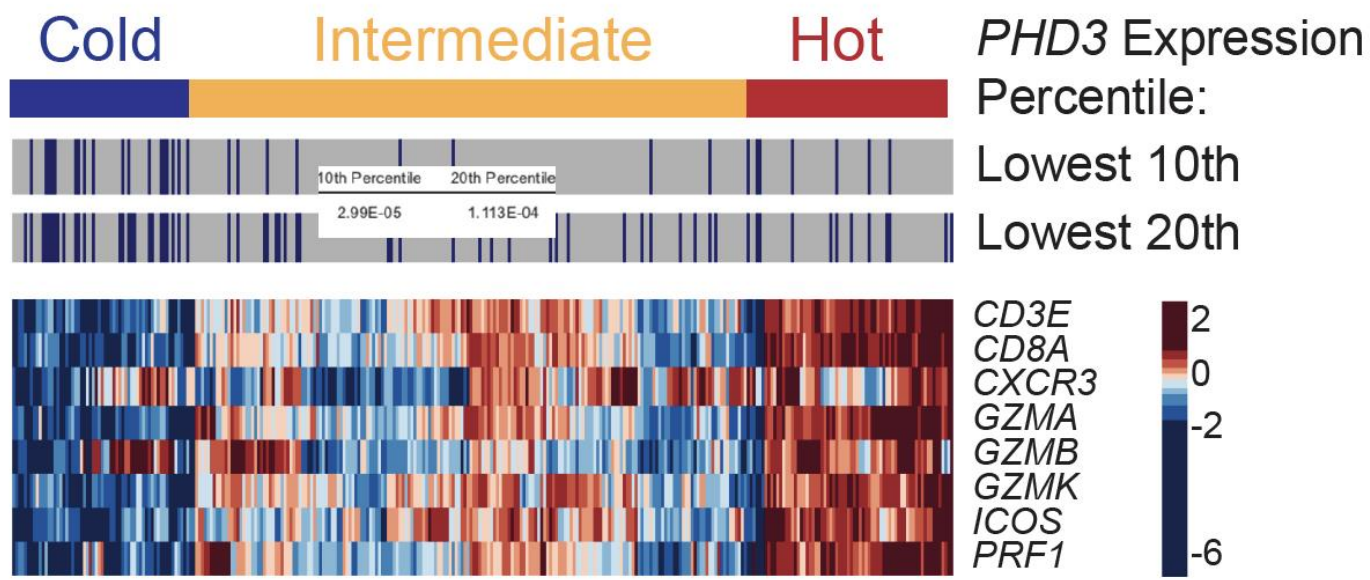
Cluster tumors by CD8^{+} immune signature expression levels

↓

Identify immunologically “cold” tumors

↓

Calculate PHD3 quantiles and score enrichment in “cold” patient samples



PHD3 Expression Percentile:

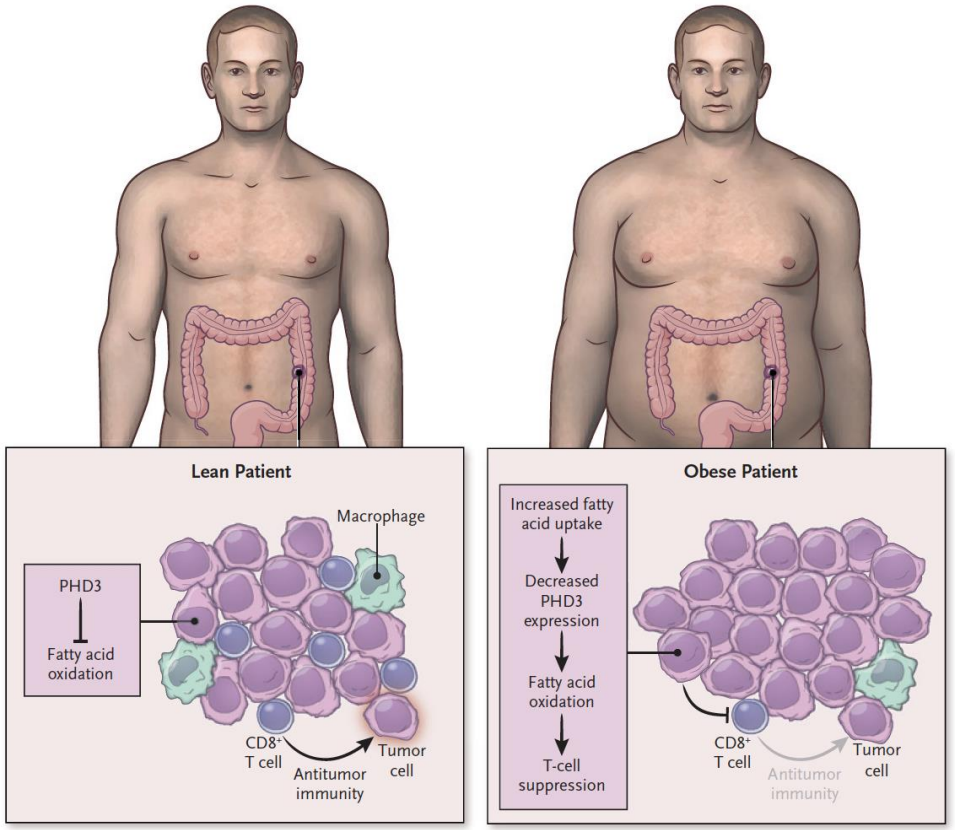
Lowest 10th

Lowest 20th

Fisher’s Exact Test: p = 2.99E-05 (10th)
p = 1.11E-4 (20th)

How does obesity affect anti-tumor immunity?

- Obesity causes tumors to burn more fats.
- This depletes T cells of fats and impairs their function.
- Restore anti-tumor immunity with obesity by a metabolic gene manipulation in cancer.



Rathmell, NEJM, 2021
Ringel et al, Cell, 2020

Ongoing studies

- Identify additional mechanisms that improve anti-tumor immunity.
- Understand whether these mechanisms are important in other states, ages, metabolic conditions and diets.

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