Metabolic engineering and immunity

Mathias Wenes, PhD

Assistant professor
Laboratory for metabolic regulation of immunity
University of Geneva

mathias.wenes@unige.ch

Objectives

1) Understand core metabolic pathways in immune cells.
   - Glycolysis, oxidative phosphorylation, fatty acid oxidation.
   - Why do immune cells depend on a specific metabolic pathway?

2) Learn strategies to metabolically engineer immune cells for cancer therapy.
   - Metabolic gene deletion/overexpression
   - Small molecule inhibitors of metabolic processes
   - Nutrient supplementation

Lecture overview

1) Introduction to immunology and cellular metabolism

2) Metabolism is closely connected to immune cell phenotype and function
  1) Innate immunity: Macrophages
  2) Adaptive immunity: T cells
      1) CD4 T cells (helper T cells)
      2) CD8 T cells (cytotoxic T cells)
      3) T cell exhaustion

3) Immunotherapy of cancer: targeting metabolism
      1) Dealing with metabolic factors that exhaust anti-tumor immunity
      2) Metabolic engineering to enhance antitumor immunity
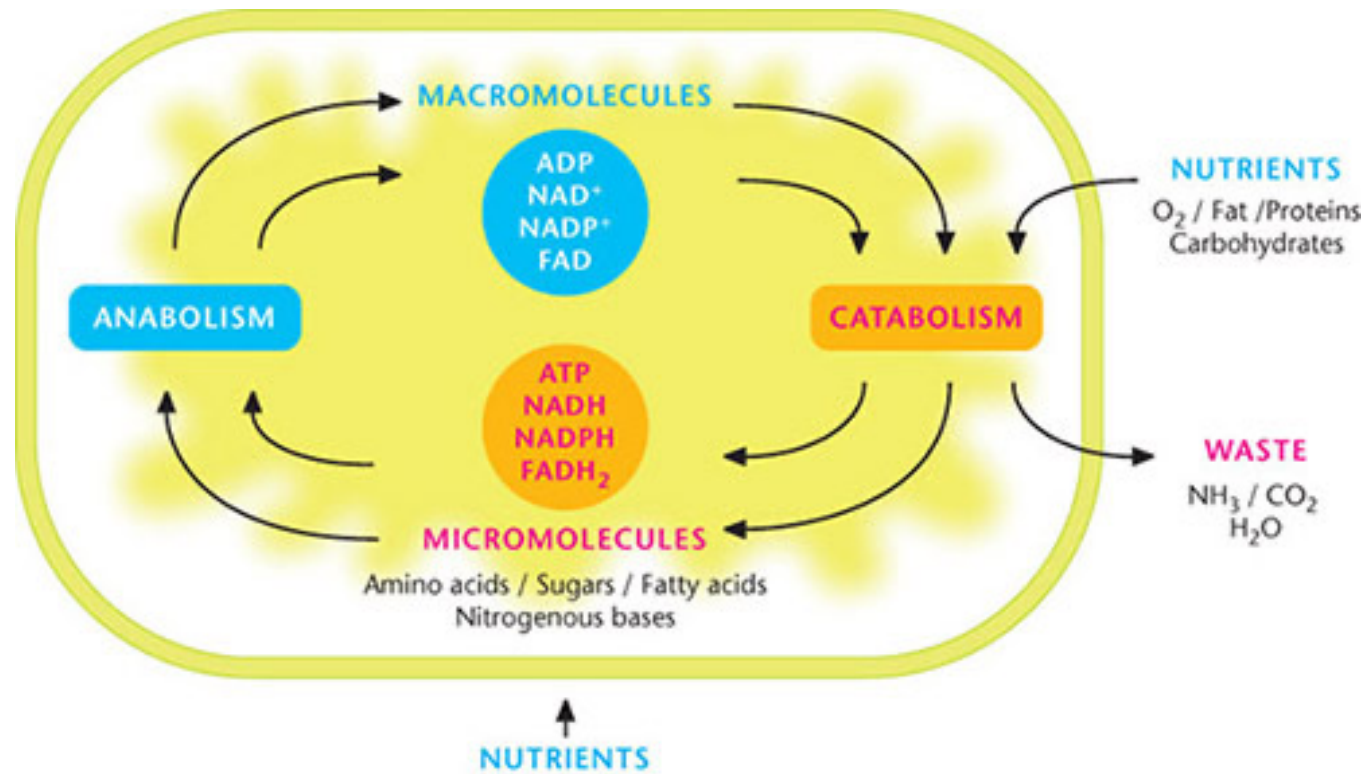
Overview

1) **Introduction to immunology and cellular metabolism**

   1) How does metabolism contribute to cell function?
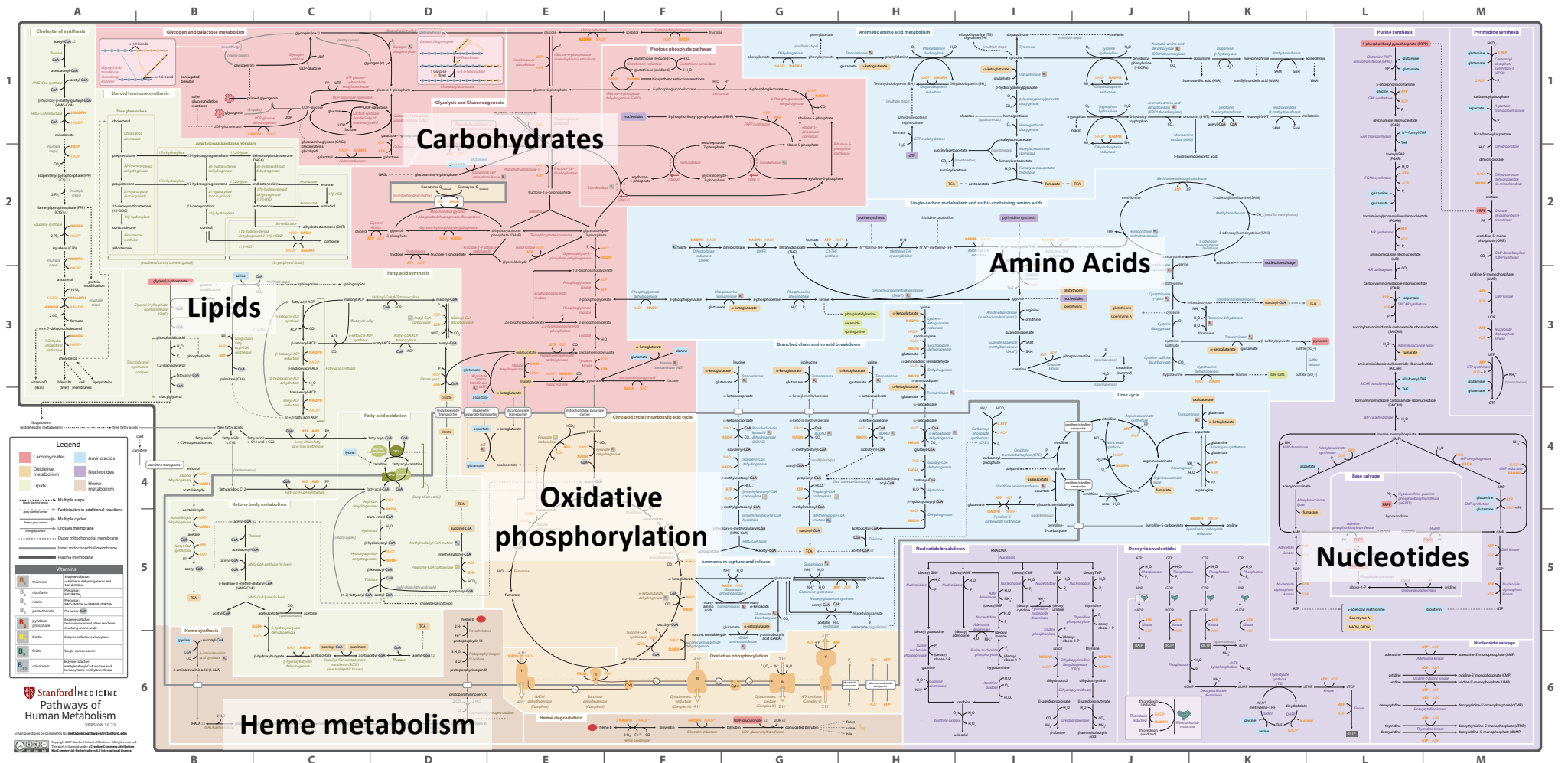   1) Energy
      2) Building blocks for cellular function and proliferation (Lipids, proteins, nucleic acids)
      3) Signaling function (e.g. epigenetics)

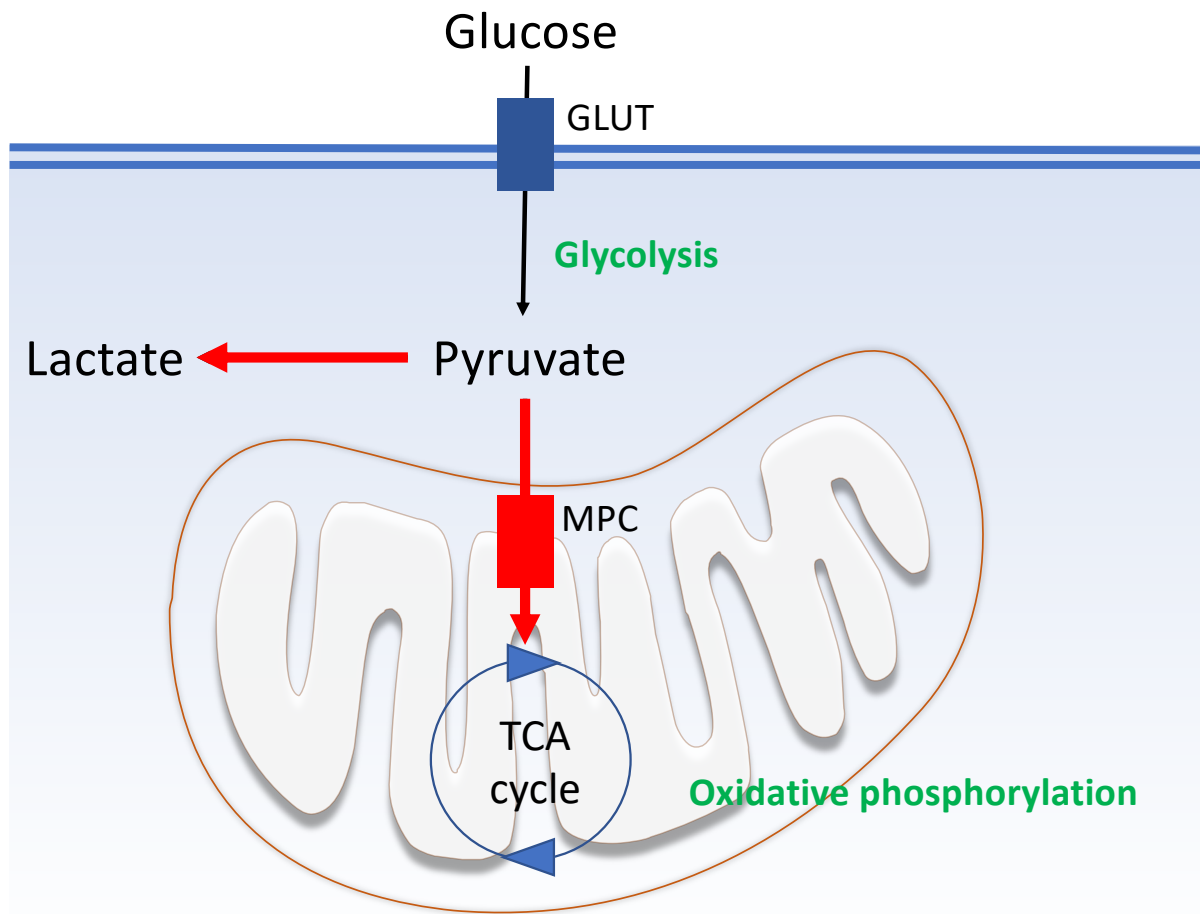   2) Overview of immune cell types

What is cellular metabolism?

Overview of major metabolic pathways in cells

Source: https://metabolicpathways.stanford.edu/

Energy production through glucose fermentation versus oxidation

Glucose

GLUT

Glycolysis

Lactate ← Pyruvate

MPC

TCA cycle
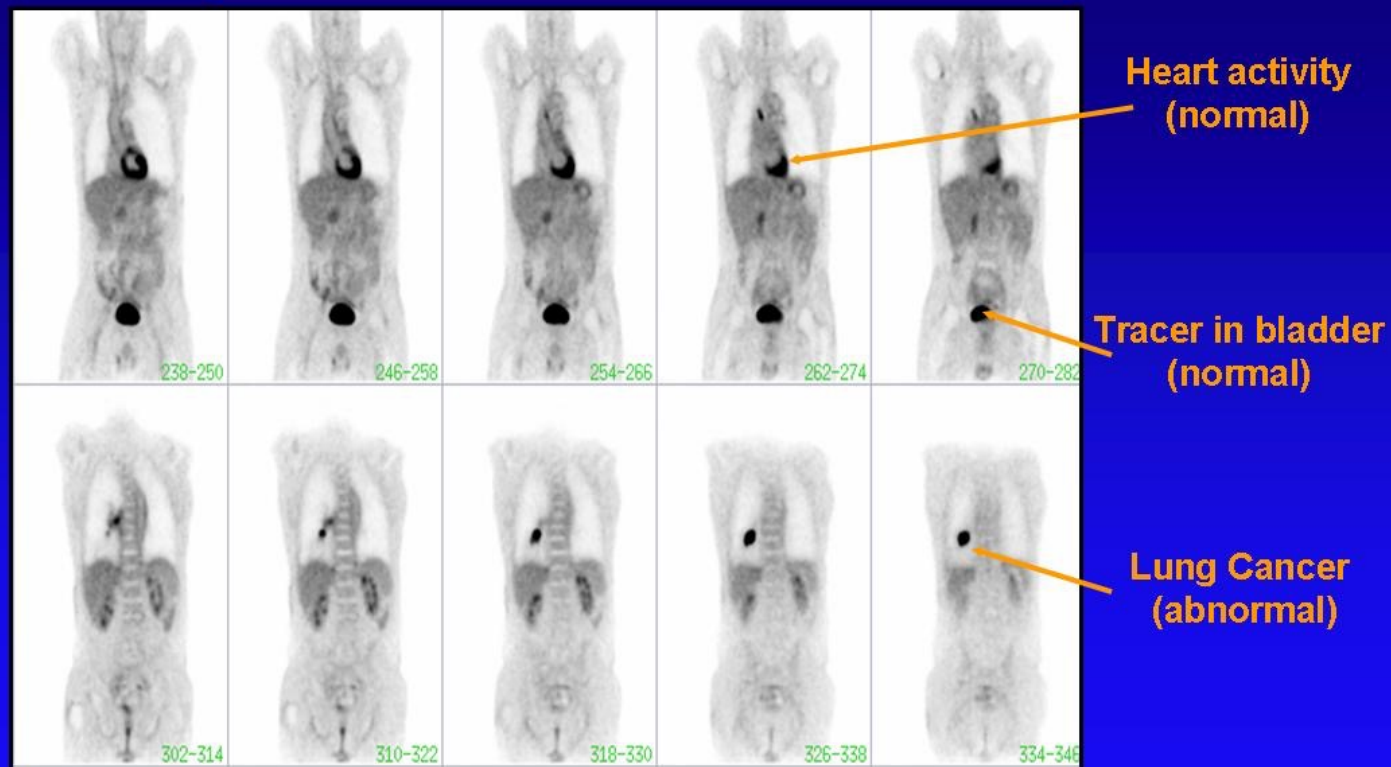
Oxidative phosphorylation

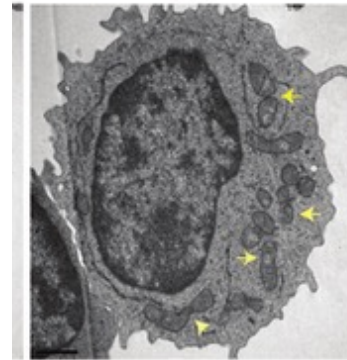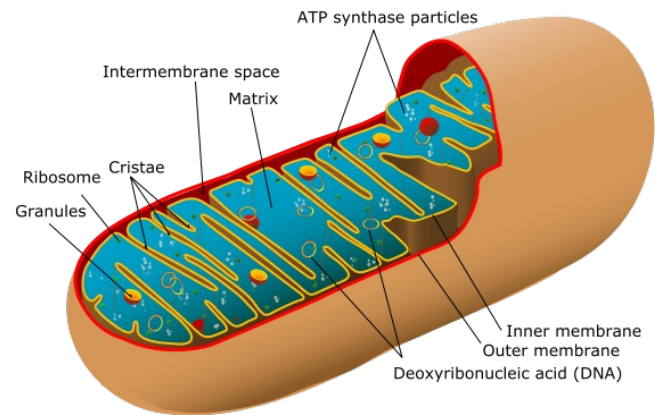| Glycolysis	Oxphos
2 molecules ATP	30 molecules ATP
Faster	Slower
NAD+ regenerated through lactate (fermentation)	NAD+ regenerated through electron transport chain (oxidation)
Anaerobic	Aerobic

Glycolysis

Chandel N., Navigating Metabolism 2014 (book)
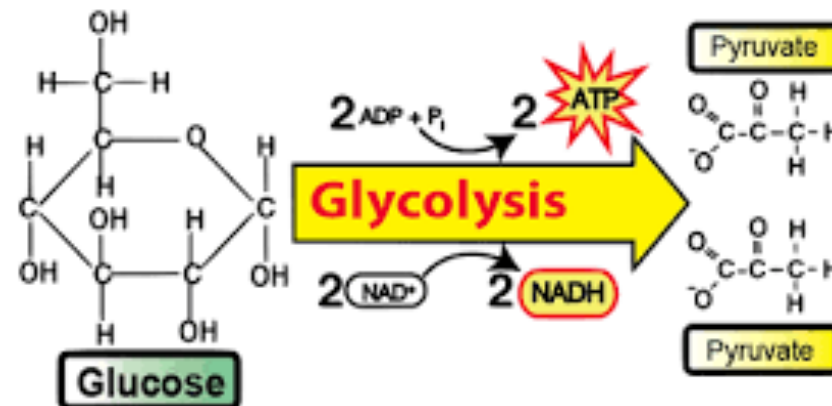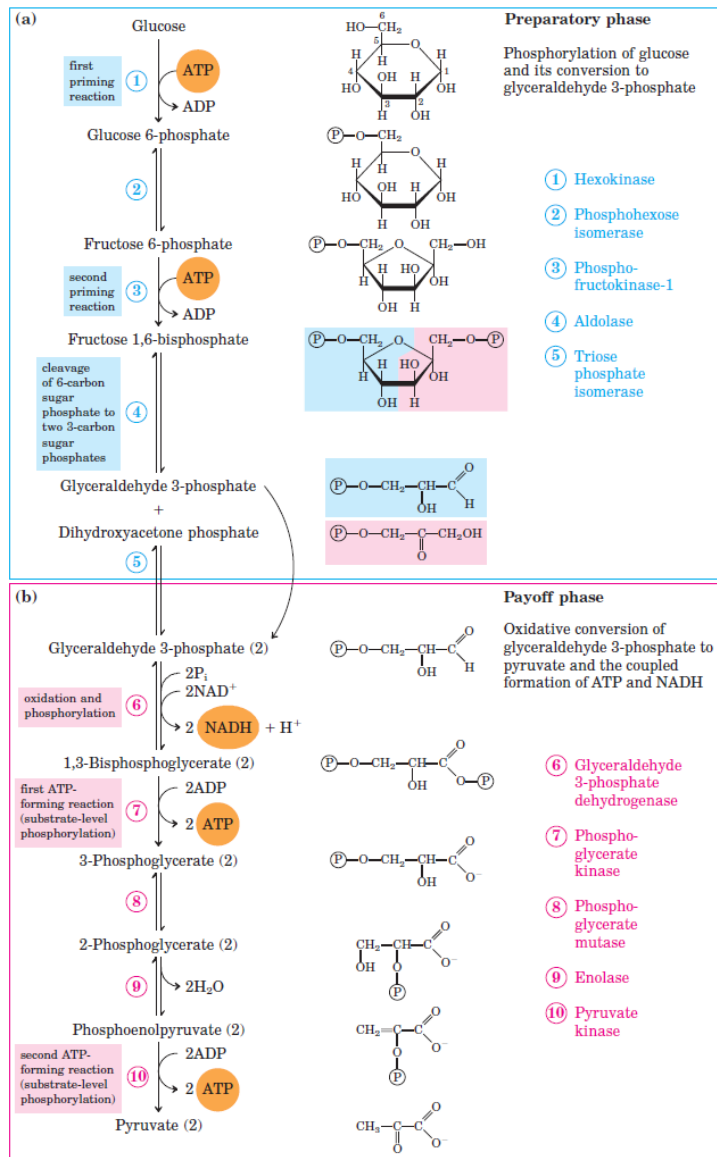
PET scan (Positron Emission Tomography) as evidence of glycolytic bias in tumors

Mitochondria: the energy powerhouse

ATP synthase particles
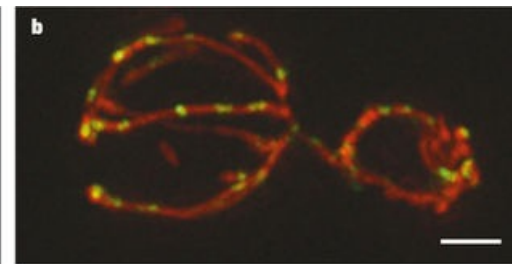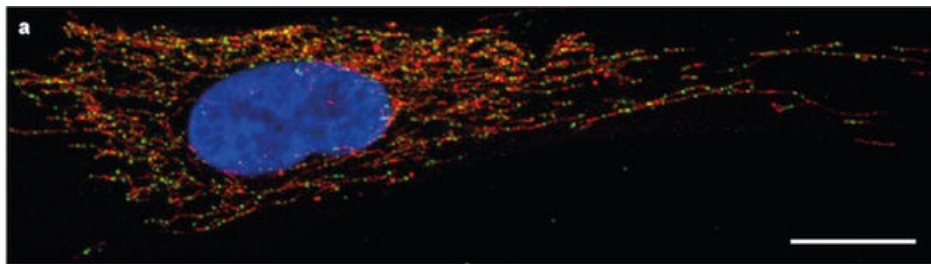Intermembrane space
Matrix
Cristae
Ribosome
Granules
Inner membrane
Outer membrane
Deoxyribonucleic acid (DNA)

Henson et al., JCI 2014
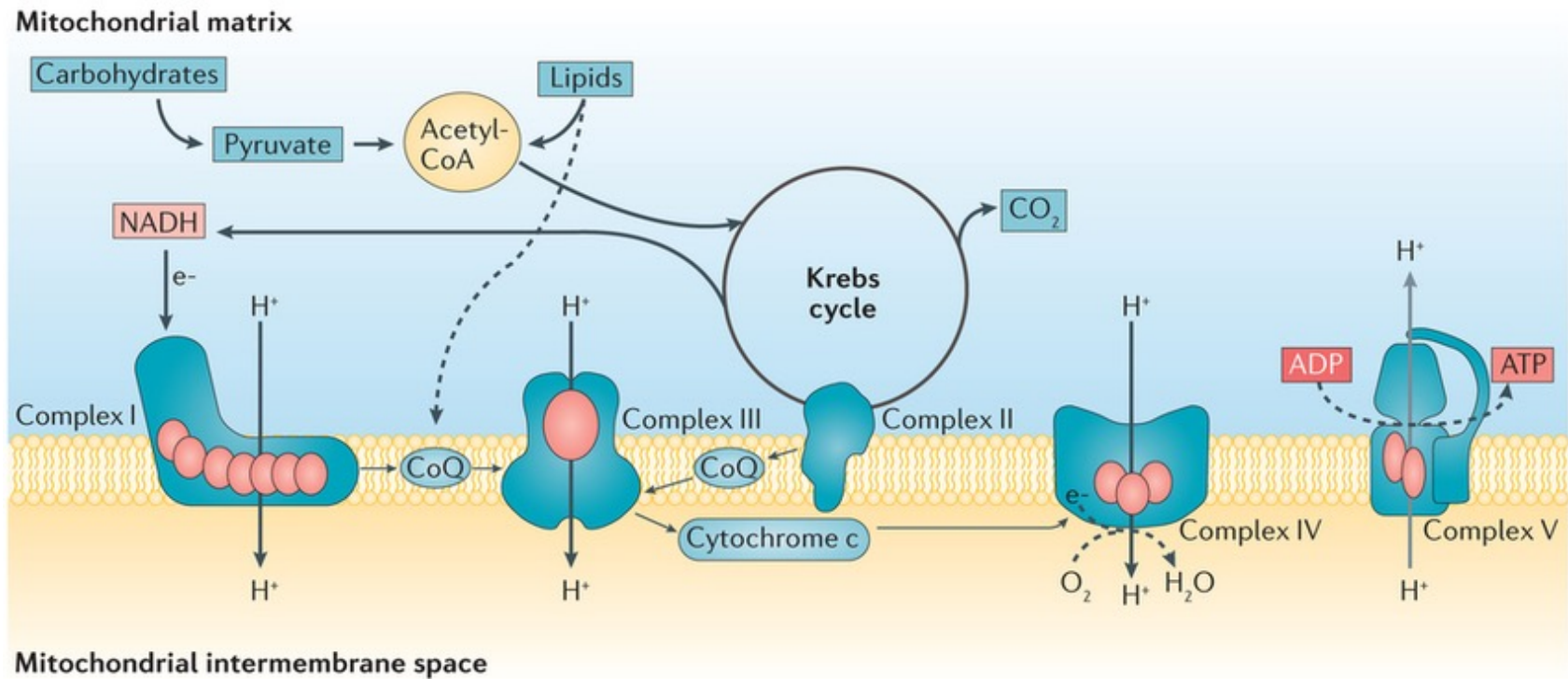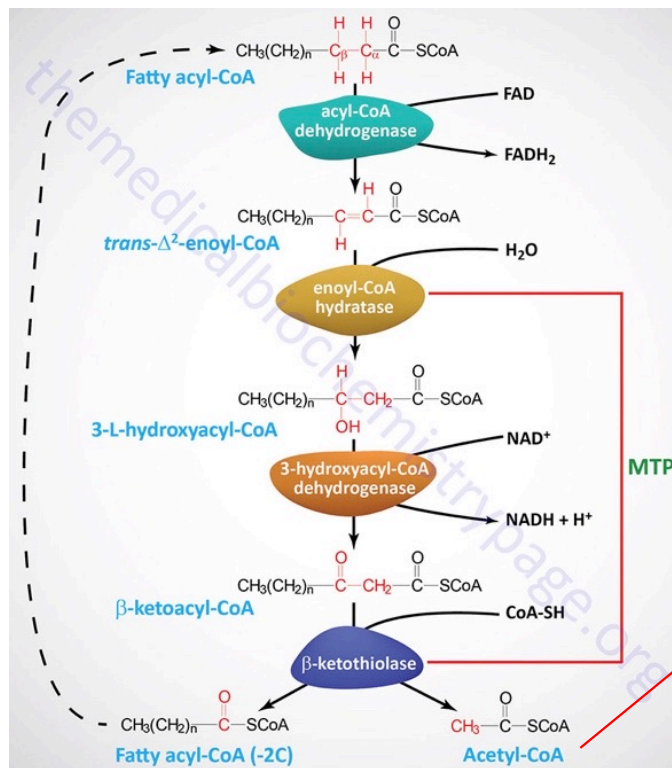
Mitochondrial form and function

Jonathan R. Friedman[1] & Jodi Nunnari[1]

Electron transport chain and oxidative phosphorylation
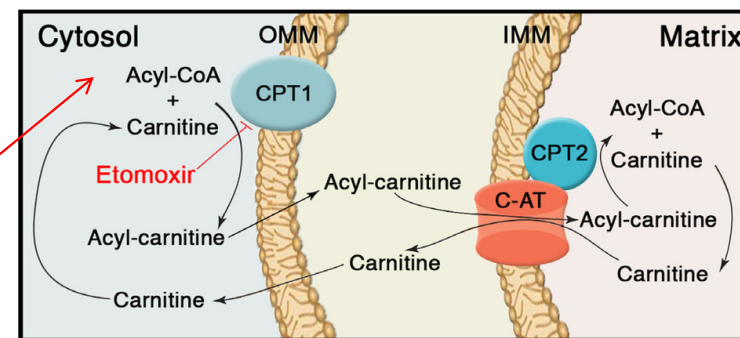
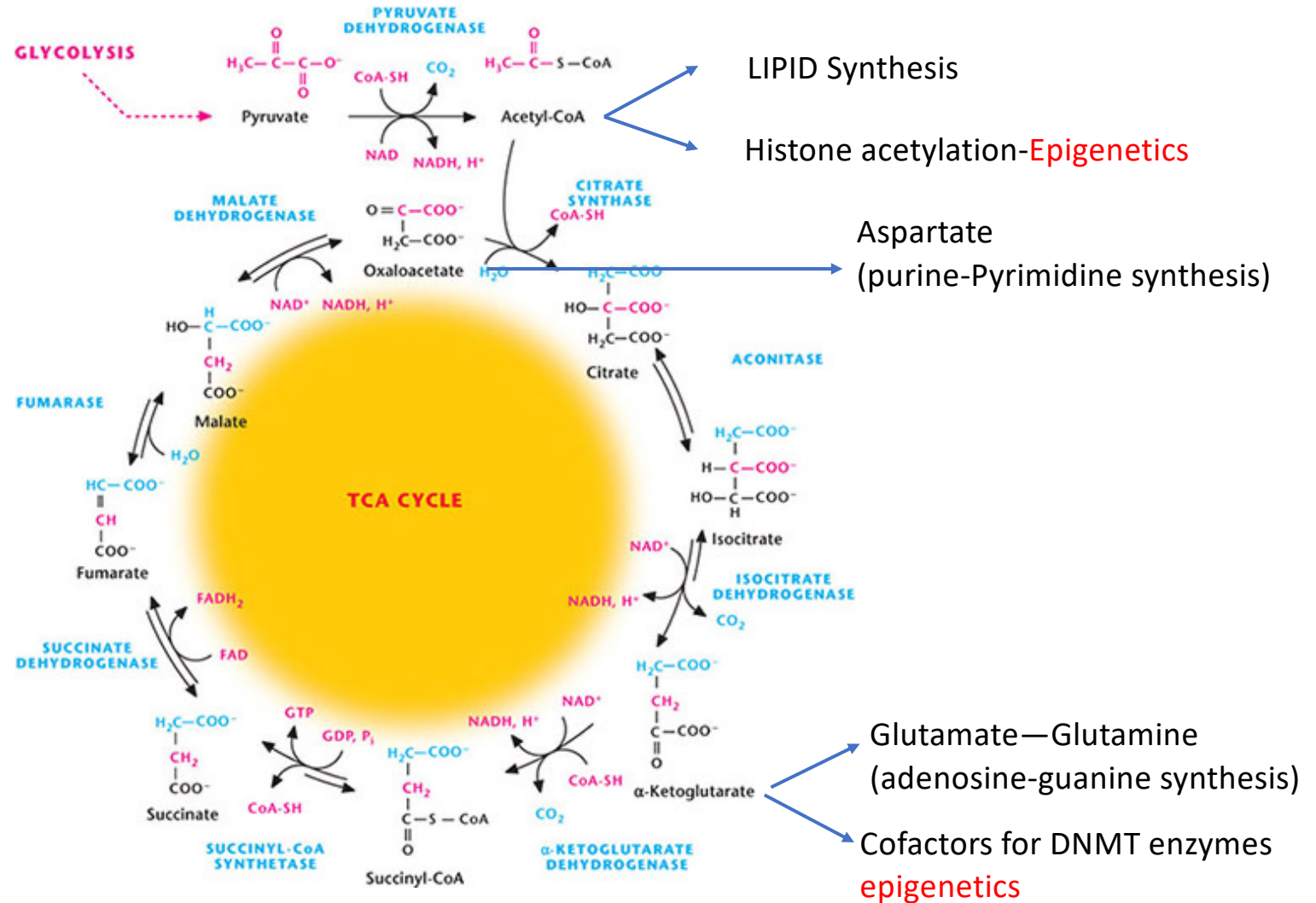Emma F., et al., Nature Rev Neph, 2016

Fatty acid β-oxidation

Fatty acid oxidation produces **Acetyl-CoA** in the **cytosol**, which is imported by **CPT enzymes** into the **mitochondria** for oxidation

Nomura M. et. al., (2016) Nat. Immunol.

The TCA cycle is a major producer of cellular **building blocks**

The TCA cycle produces:
- **three NADH,**
- **one FADH$_2$**
- **one GTP**

LIPID Synthesis

Histone acetylation-Epigenetics

Aspartate
(purine-Pyrimidine synthesis)

Glutamate—Glutamine
(adenosine-guanine synthesis)

Cofactors for DNMT enzymes
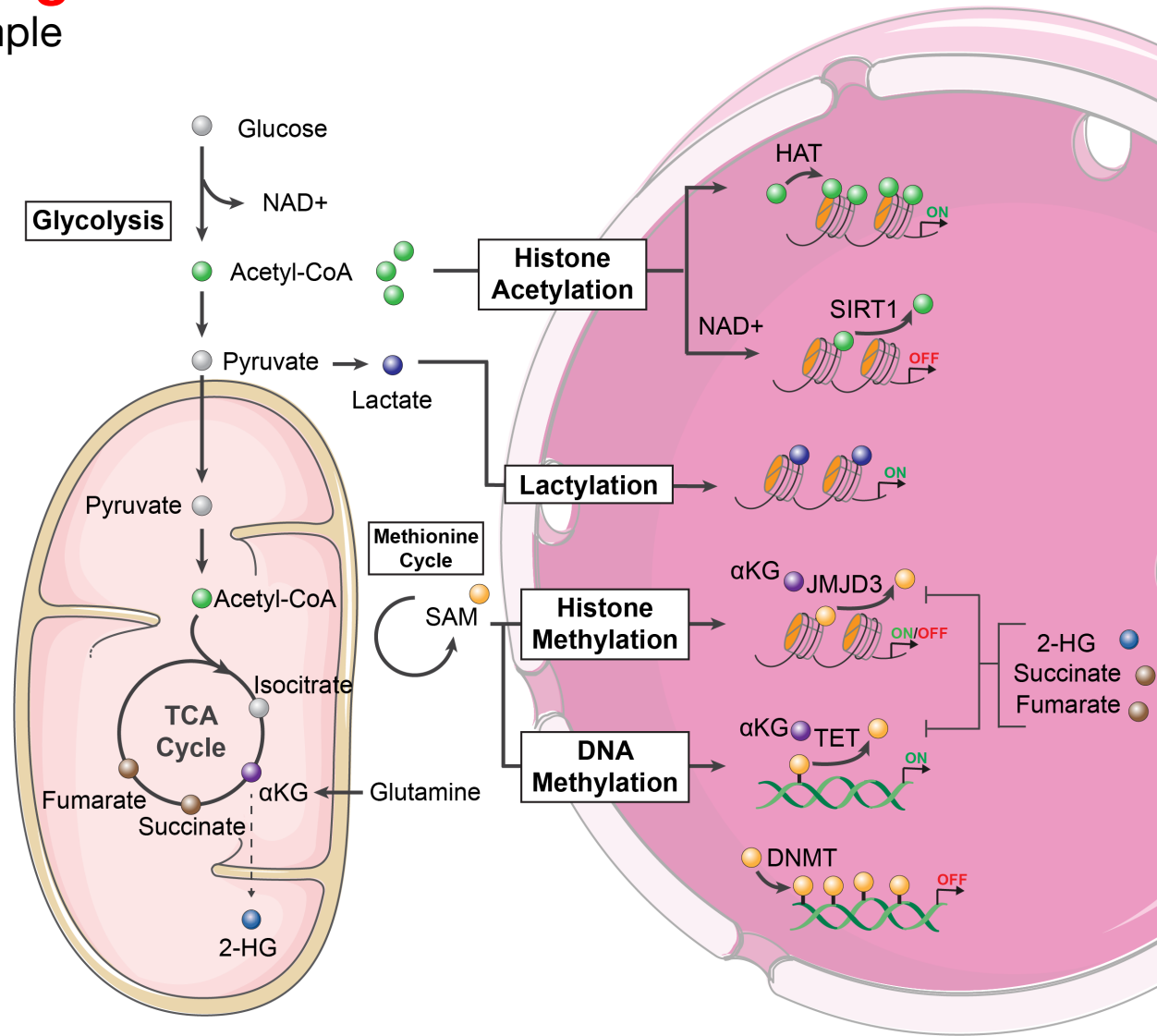epigenetics

Chandel N., Navigating Metabolism 2014 (book)

Metabolism in **cellular signaling**
Regulation of gene expression as an example
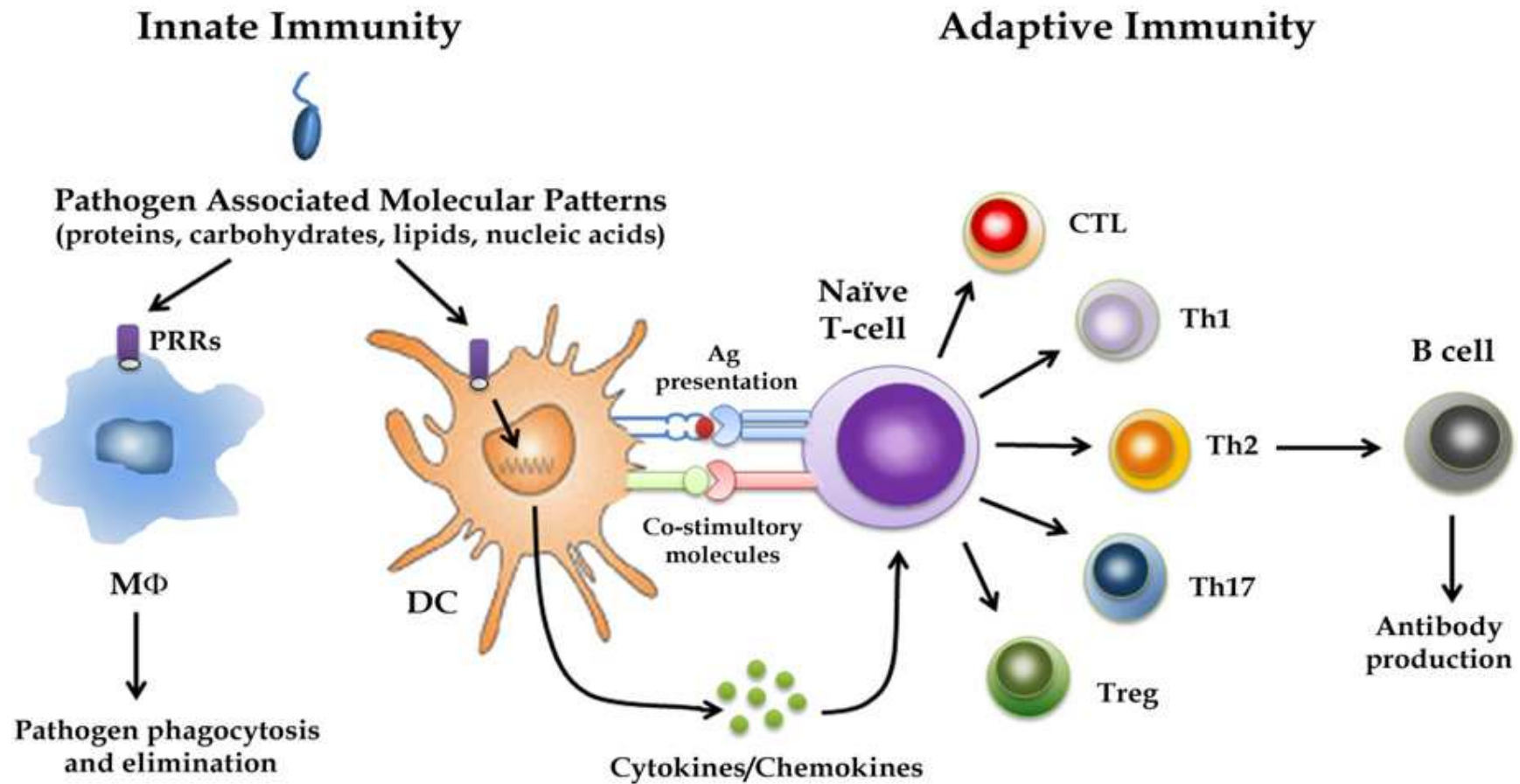
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    2) Overview of immune cell types

Innate and adaptive immune response

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        1) Dealing with metabolic factors that exhaust anti-tumor immunity
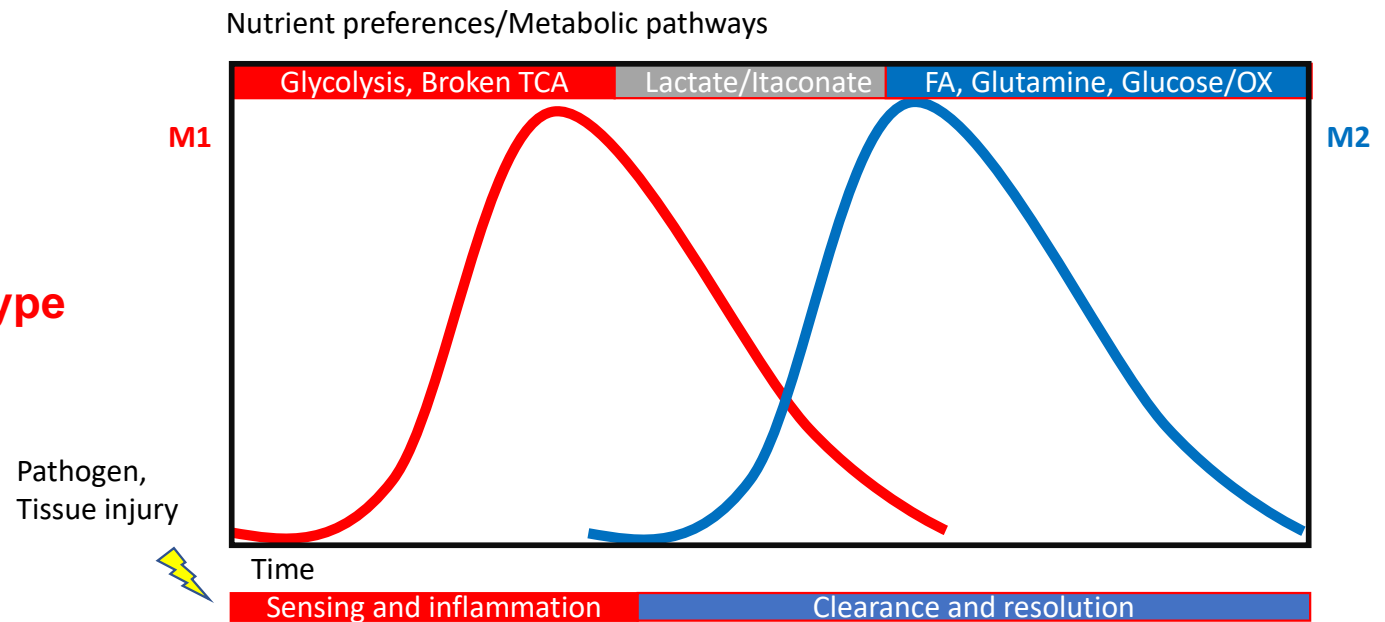   2) Metabolic engineering to enhance antitumor immunity

μακρός φαγεῖν-Big eaters

<u>PRO-INFLAMMATORY **M1**</u>

- **Bacteria**
- **Damaged/infected cells**

- **Antigen presentation**
- **Activation towards pro-inflammatory phenotype**

<u>ANTI-INFLAMMATORY **M2**</u>

- **Dead cells**
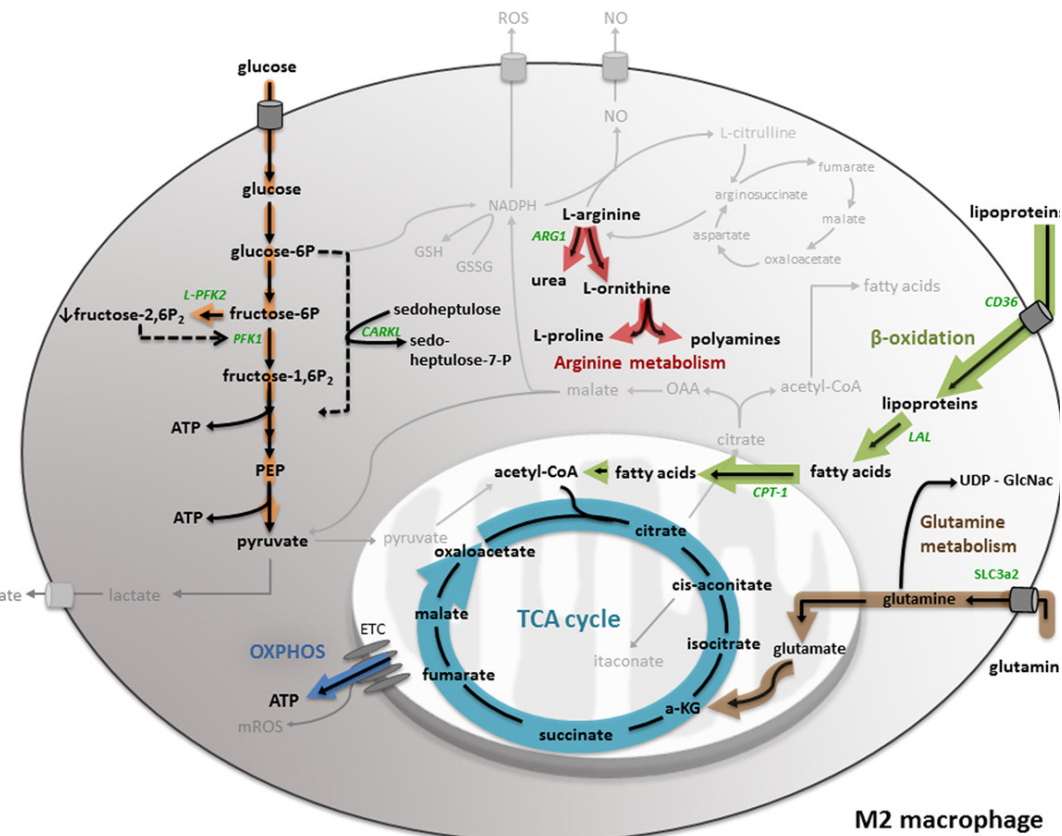- **Debris**

- **Resolution of inflammation**
- **Wound healing**

Nutrient preferences/Metabolic pathways

| Glycolysis, Broken TCA | Lactate/Itaconate | FA, Glutamine, Glucose/OX |

M1

M2

Pathogen,
Tissue injury

Time

| Sensing and inflammation | Clearance and resolution |

Macrophage polarization is accomplished by engagement of specific metabolic pathways
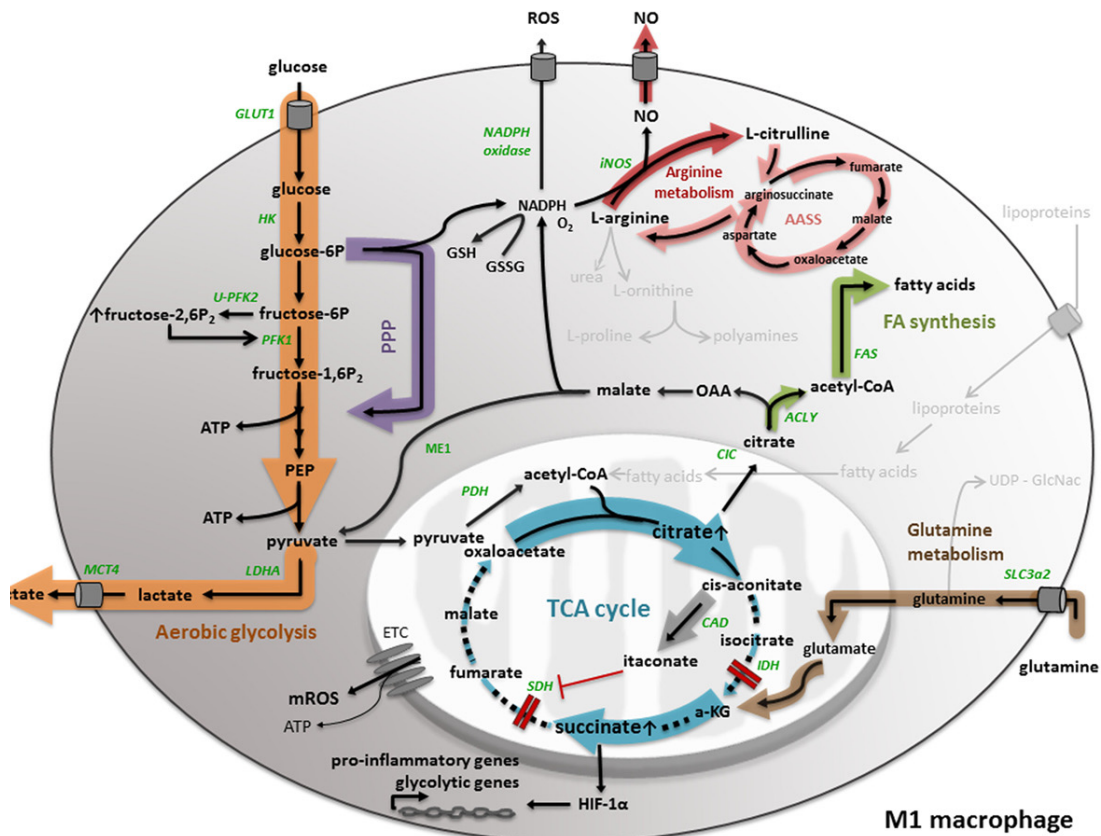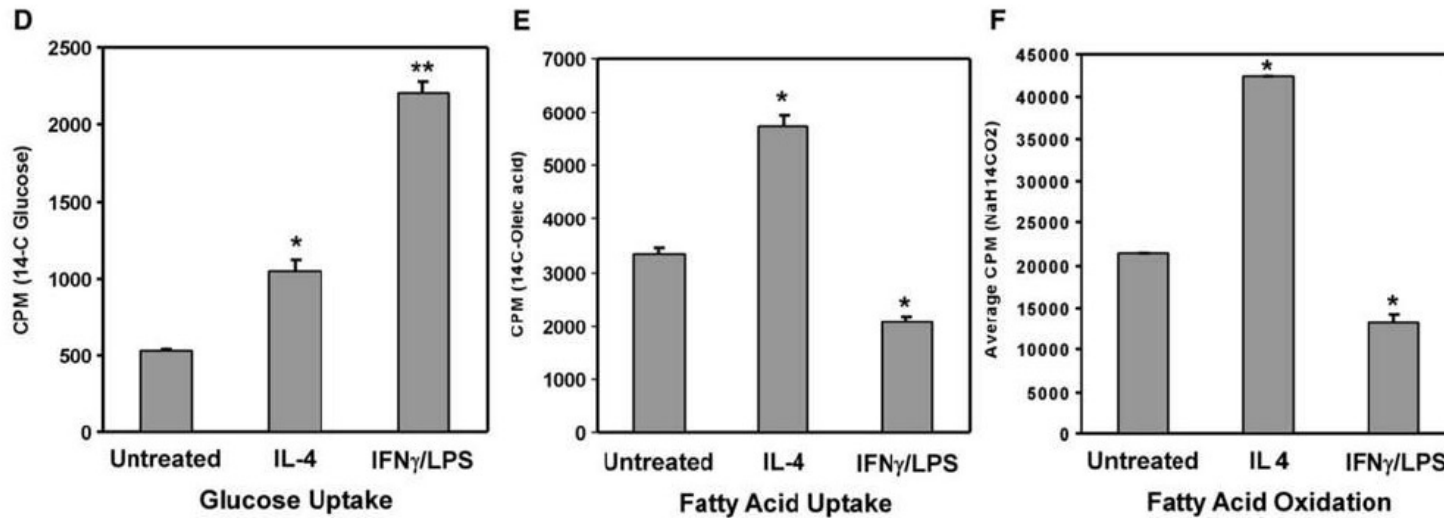

Pro-inflammatory and immunosupportive

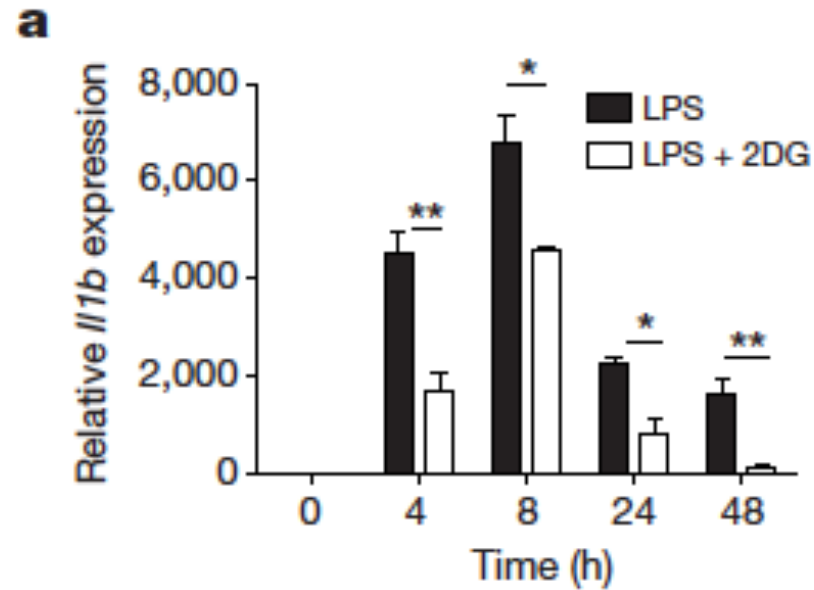Anti-inflammatory and immunosuppressive

Geeraerts et al., Front in Imm, 2017

Metabolic preference in activated macrophages
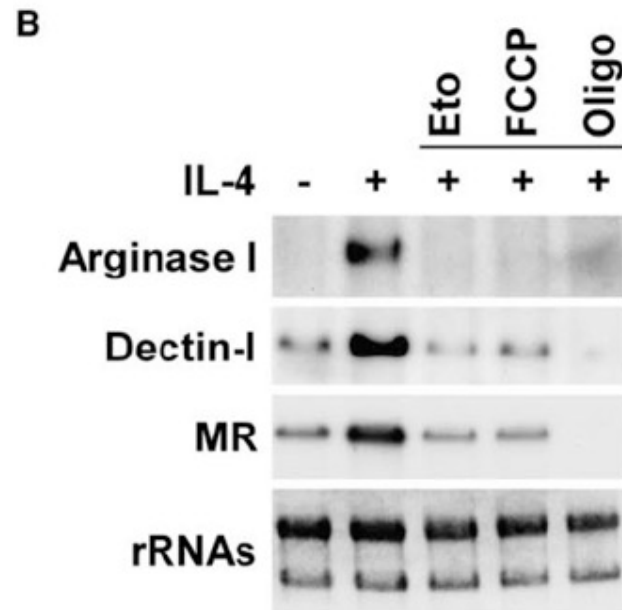

Vats D. et. al., (2006) CeLl Metab.

Warburg glycolysis is essential for M1 activation

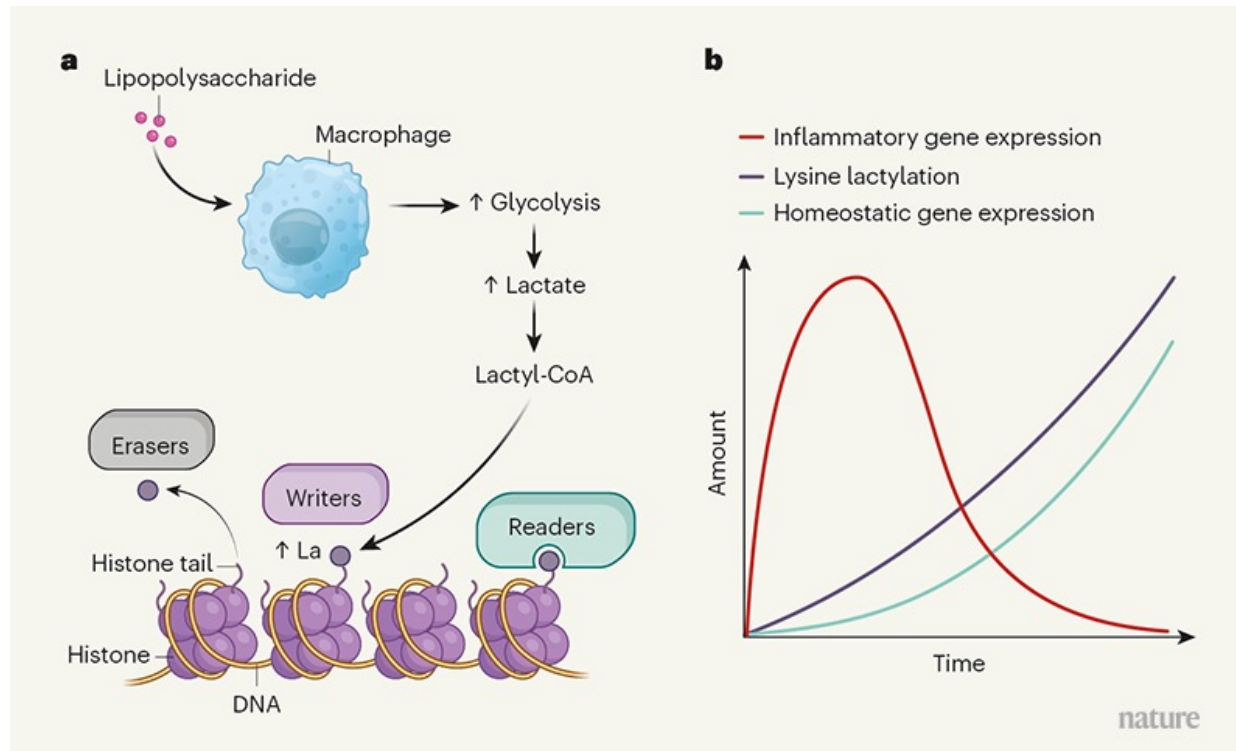2DG: 2-deoxyGlucose (glucose competitor-inhibits glycolysis

Tannahill G. et. al., (2013) Nature

Fatty acid oxidation is essential for M2 activation

Vats D. et. al., (2006) Cell Metab.

Histone lactylation, a new epigenetic modification

Izzo and Wellen, Nature 2019

Zhang et al., Nature 2019

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3) Immunotherapy of cancer: targeting metabolism
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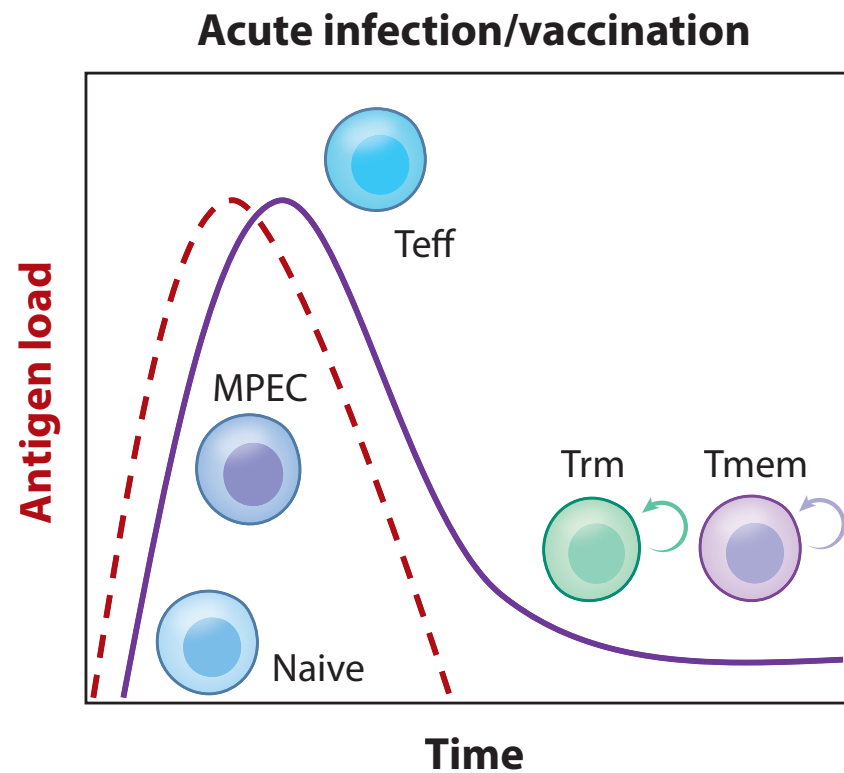   2) Metabolic engineering to enhance antitumor immunity

Innate and adaptive immune response

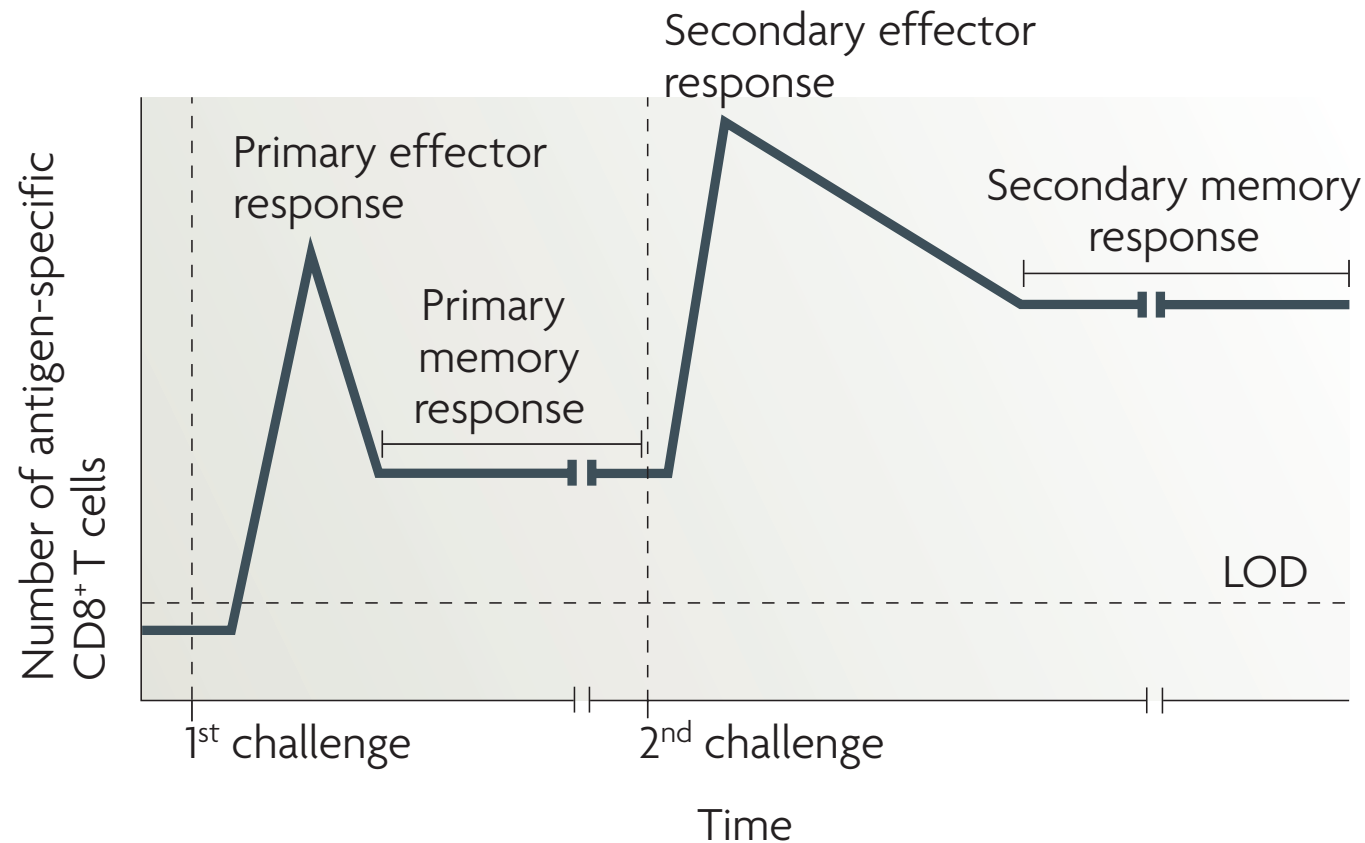# 3 signals are required to fully activate a T cell

Neves *et al.*, IntechOpen, 2012
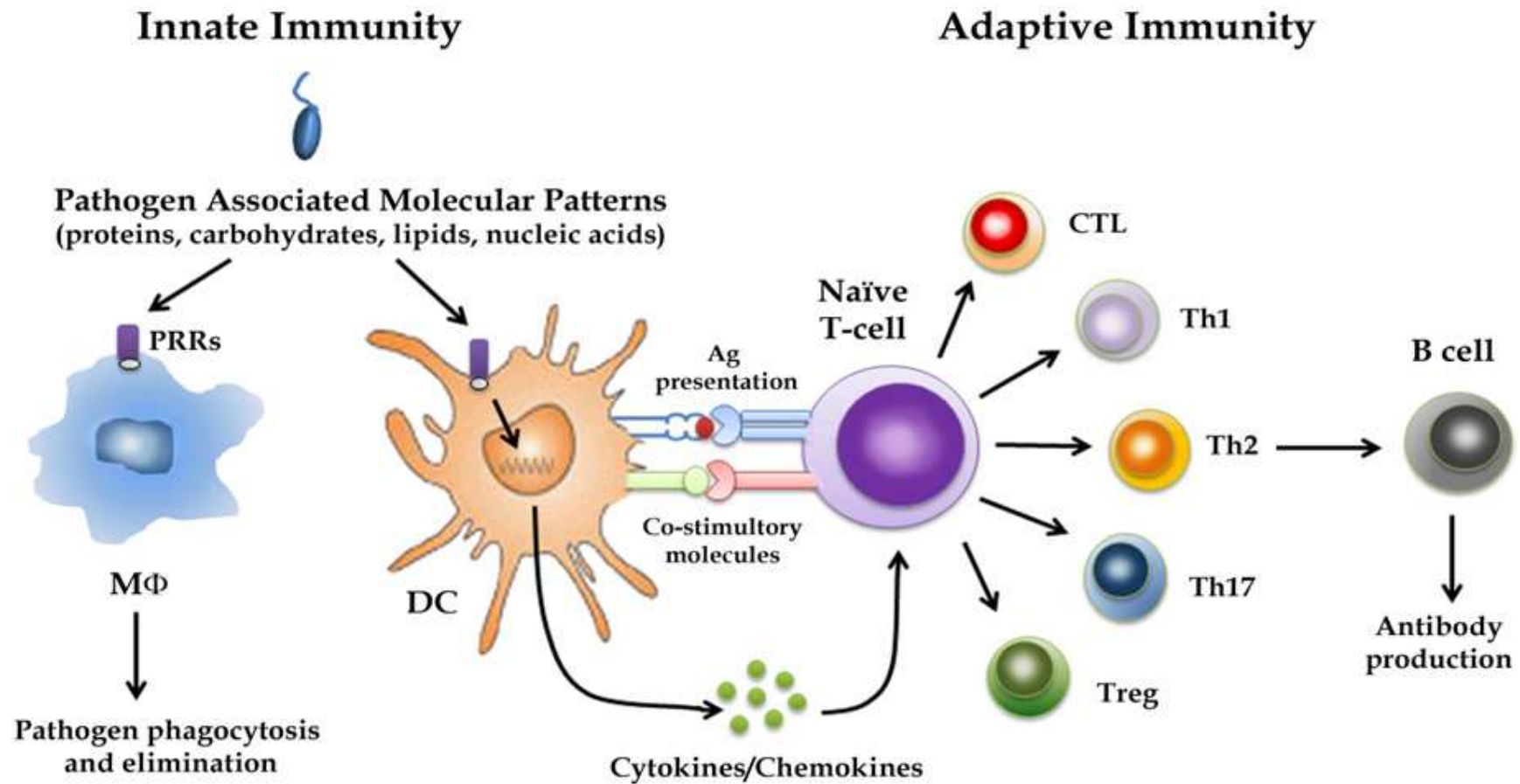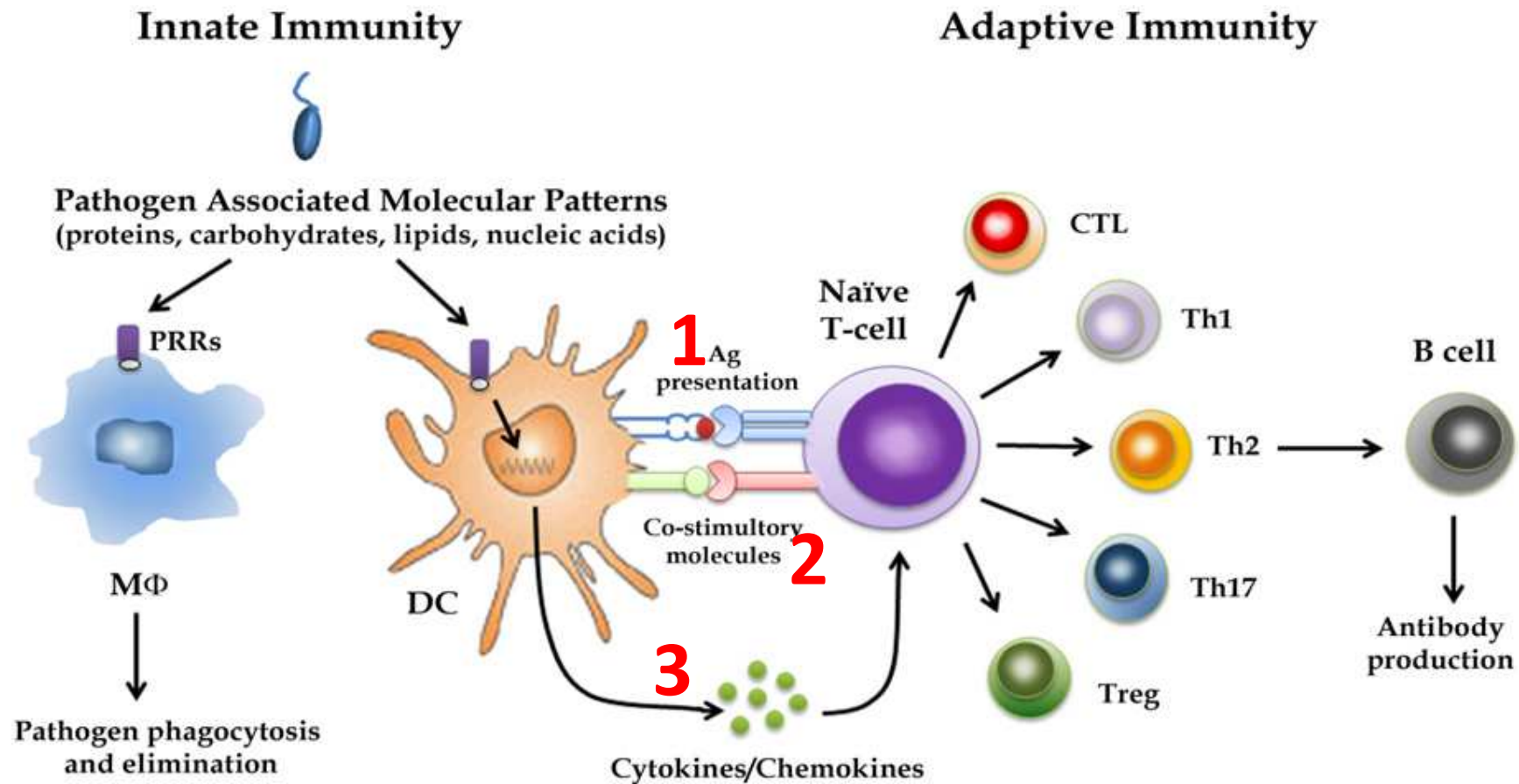
The T cell response to acute infection and vaccination

Acute infection/vaccination

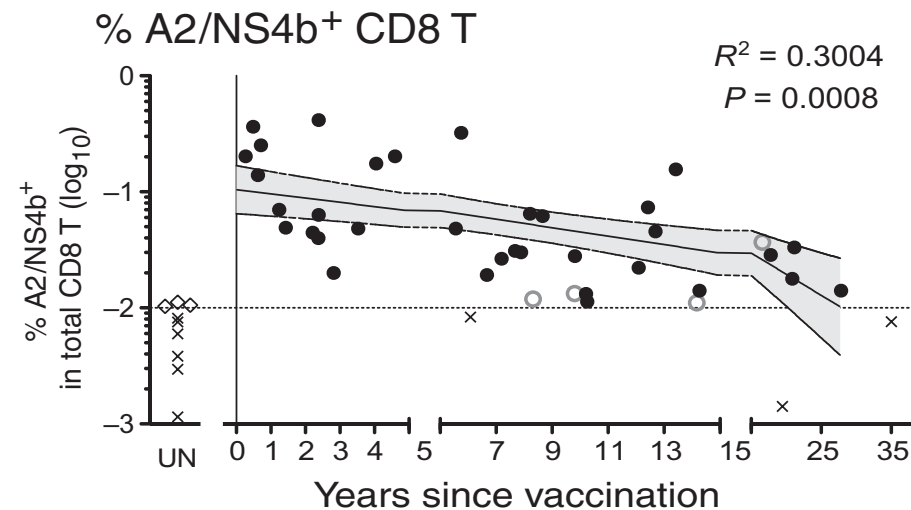Antigen load / Time

Teff, MPEC, Naive, Trm, Tmem

McLane *et al.*, Annu. Rev. Immunol., 2019

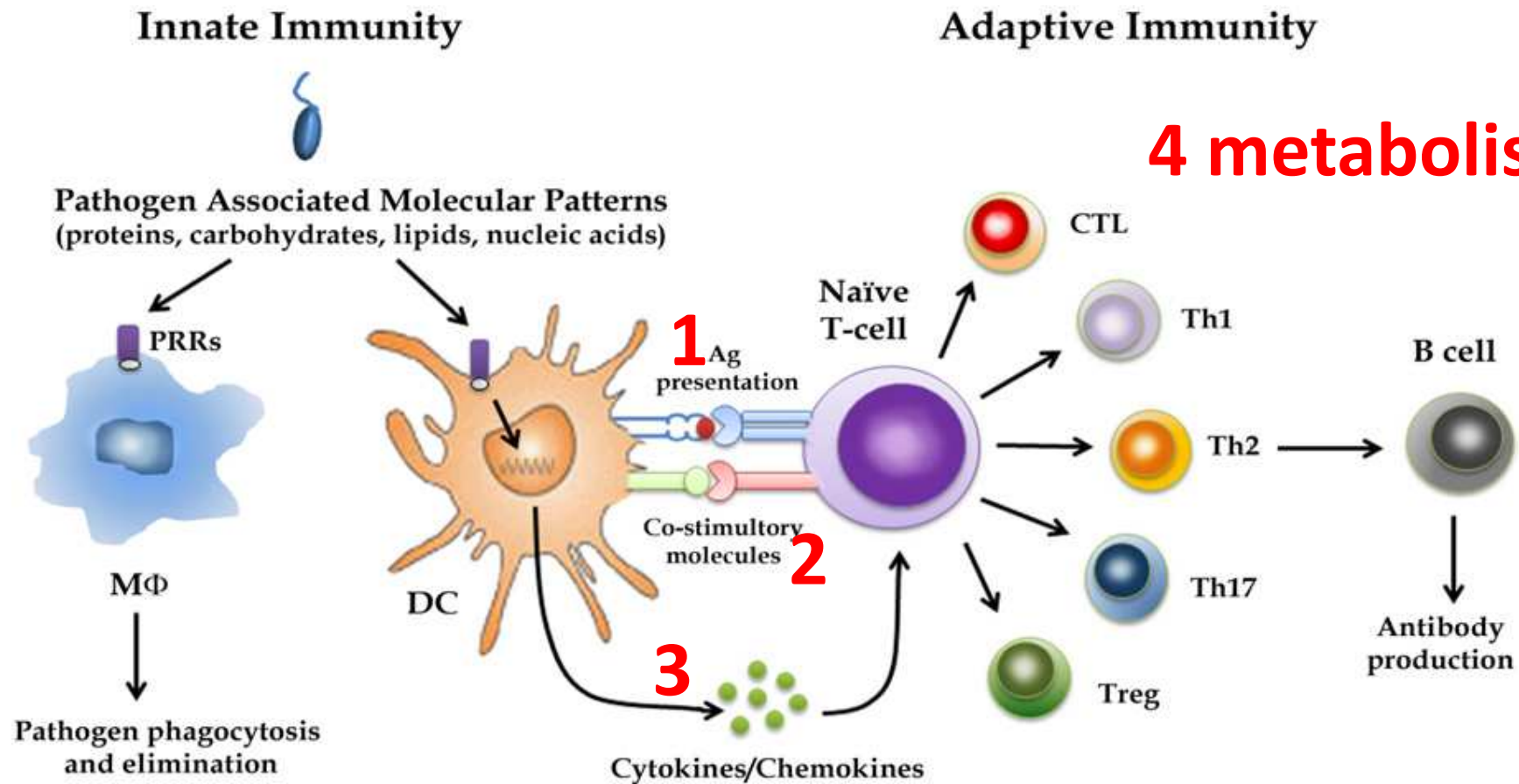# Memory T cells ensure long-lasting immune protection


Harty *et al.*, Nat. Rev. Immunol., 2008

# Long-lasting stem cell–like memory CD8+ T cells with a naïve-like profile upon yellow fever vaccination

Fuertes Marraco, Speiser, *et al.*, Sci. Transl. Med., 2015

~~3~~ 4 signals are required to fully activate a T cell
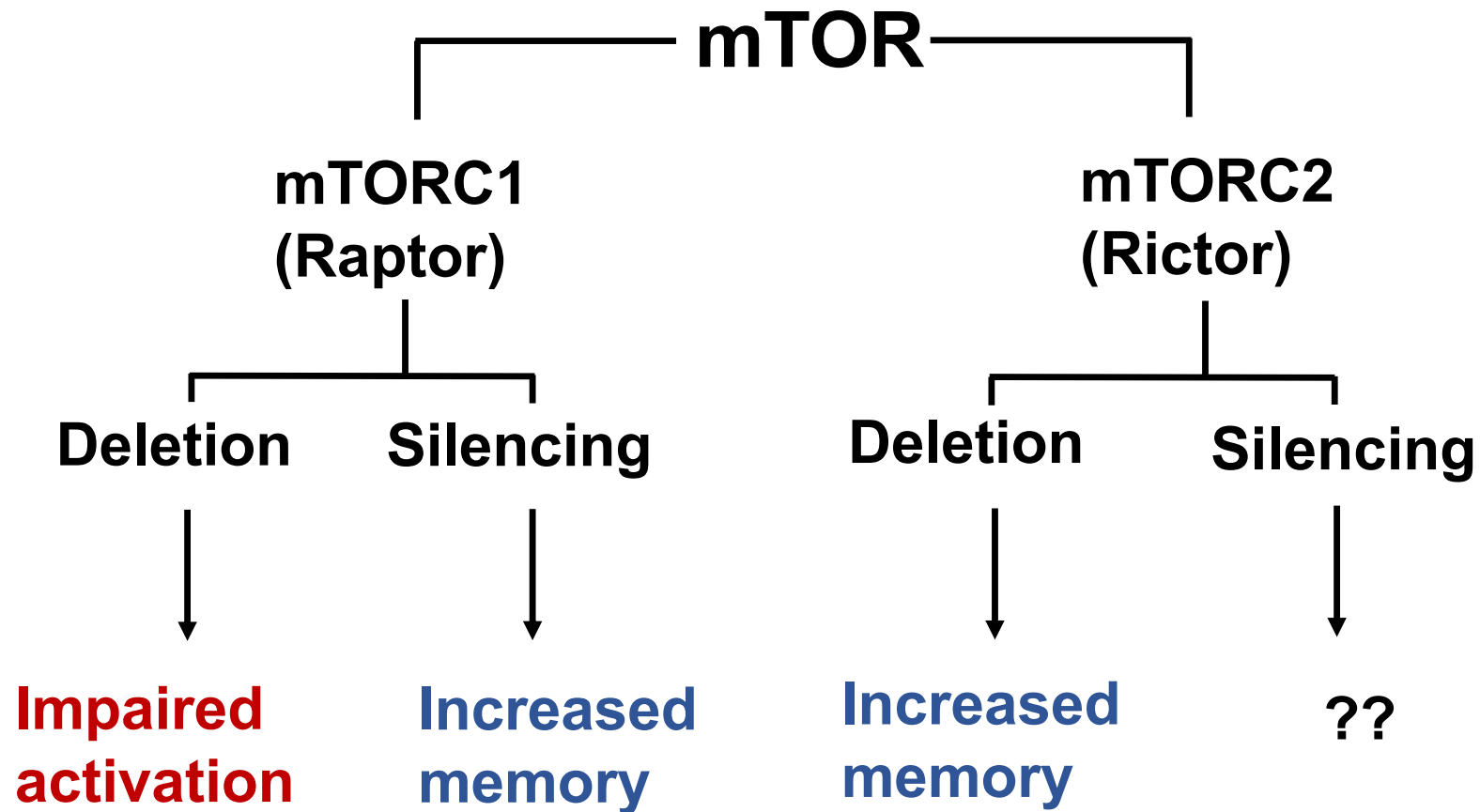

Neves *et al.*, IntechOpen, 2012

T cell clonal expansion and differentiation requires a massive activation of specific metabolic pathways

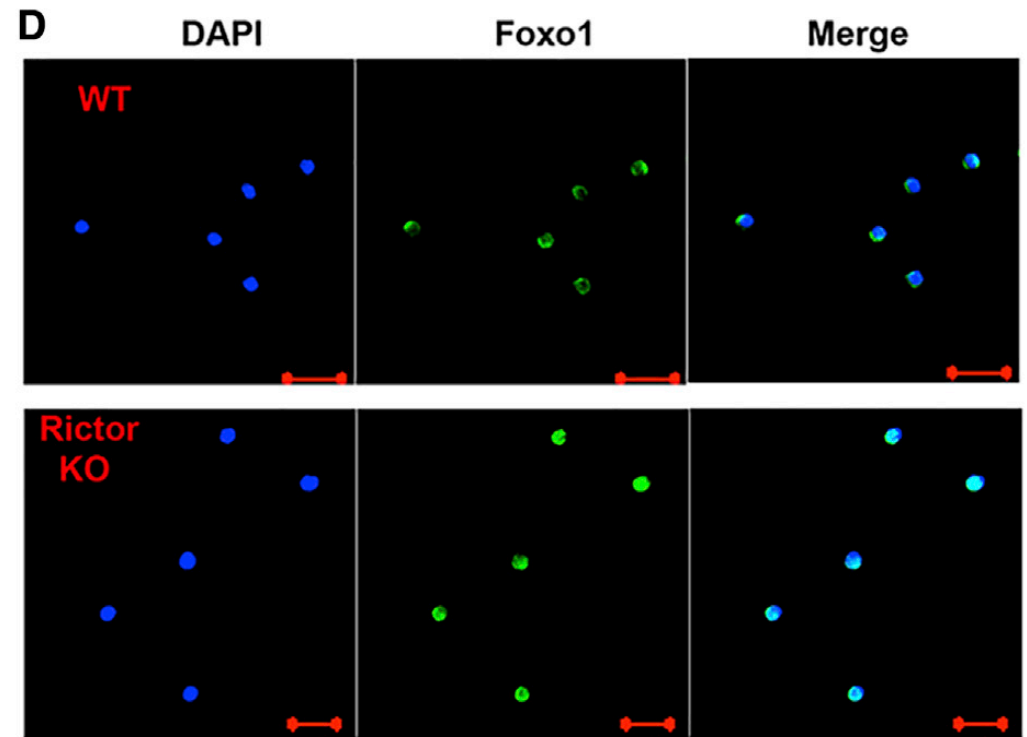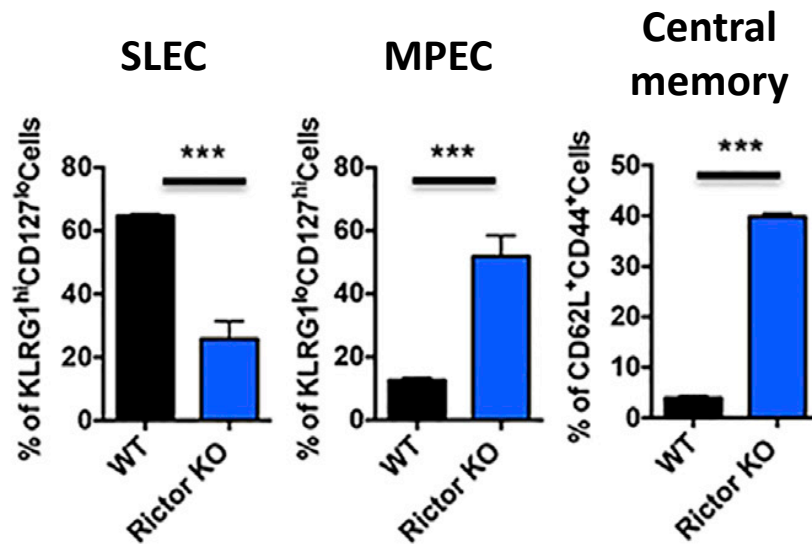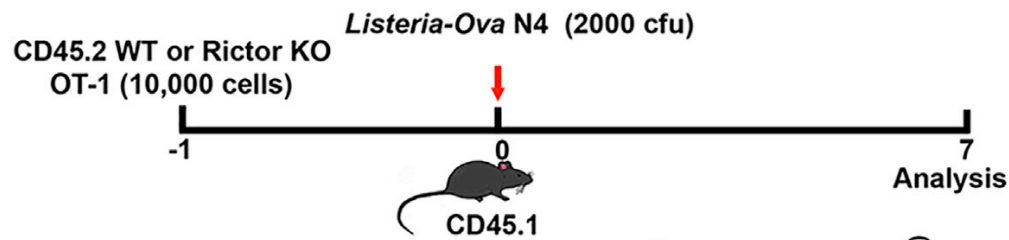Kaech *et al.*, Nat. Rev. Imm. 2012
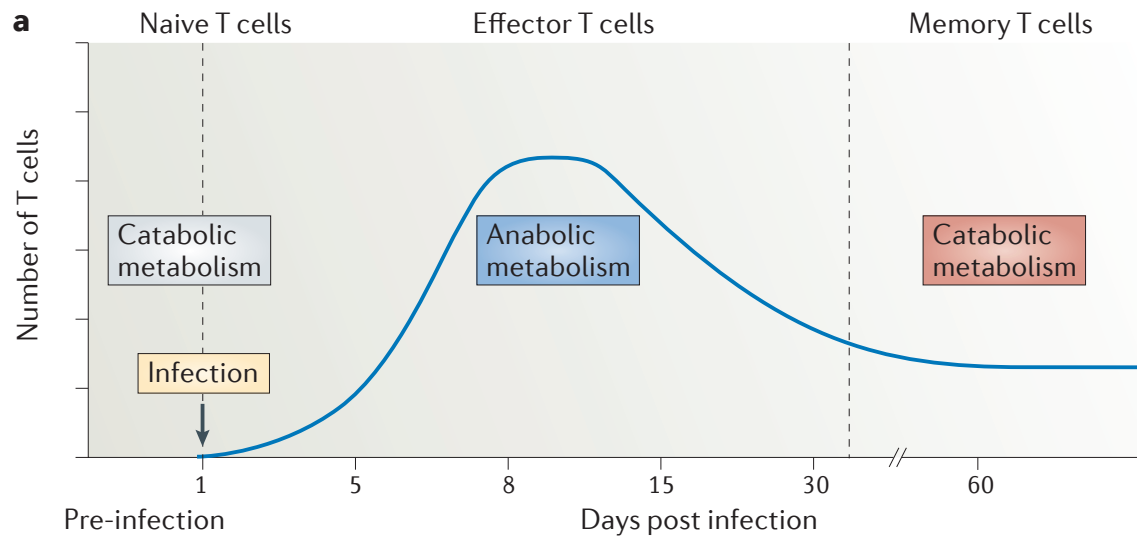
Energy demands and supply change during acute and resolving phases
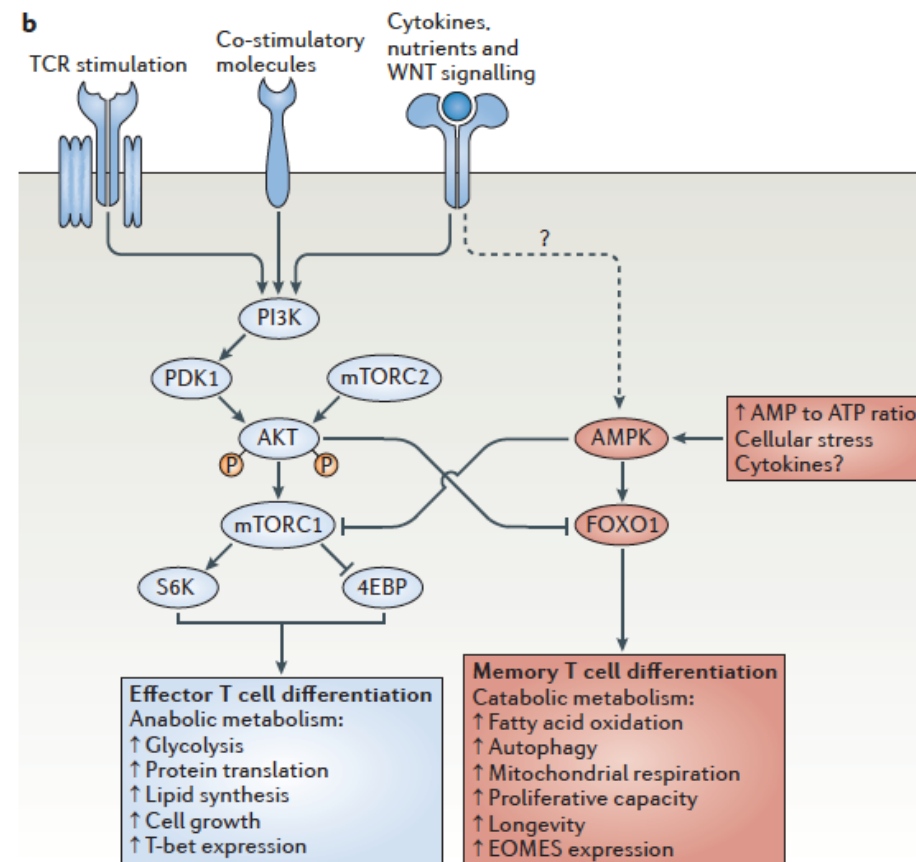
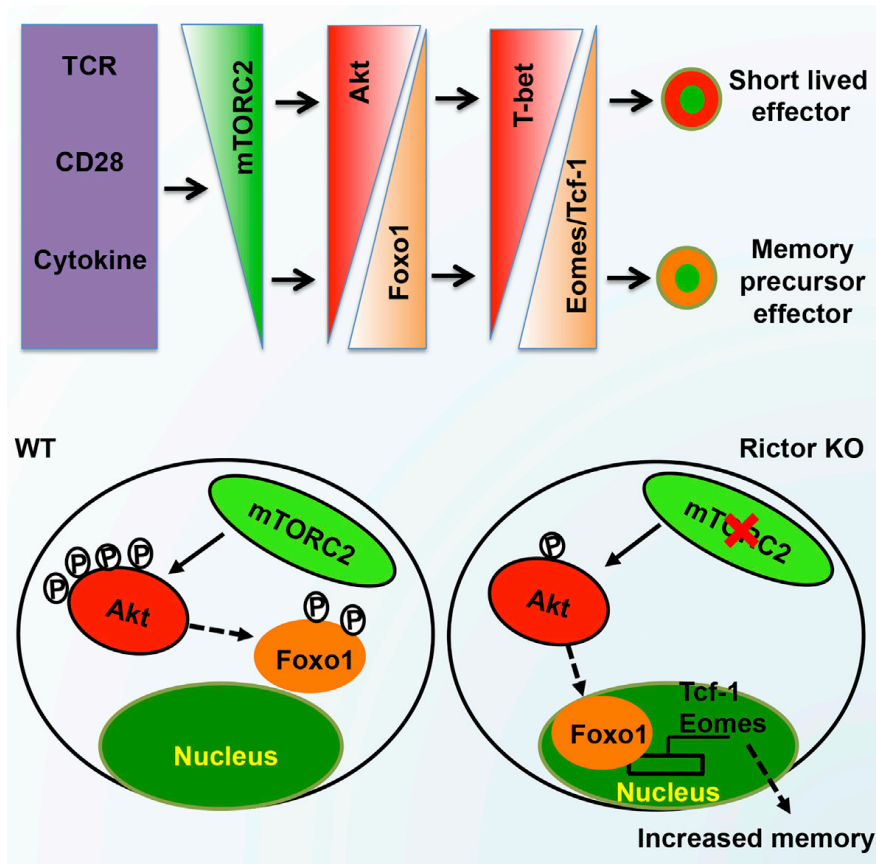# mTOR plays a central role in CD8 T cell activation and differentiation

mTORC2 controls CD8 T cell memory differentiation in a FOXO1-dependent manner
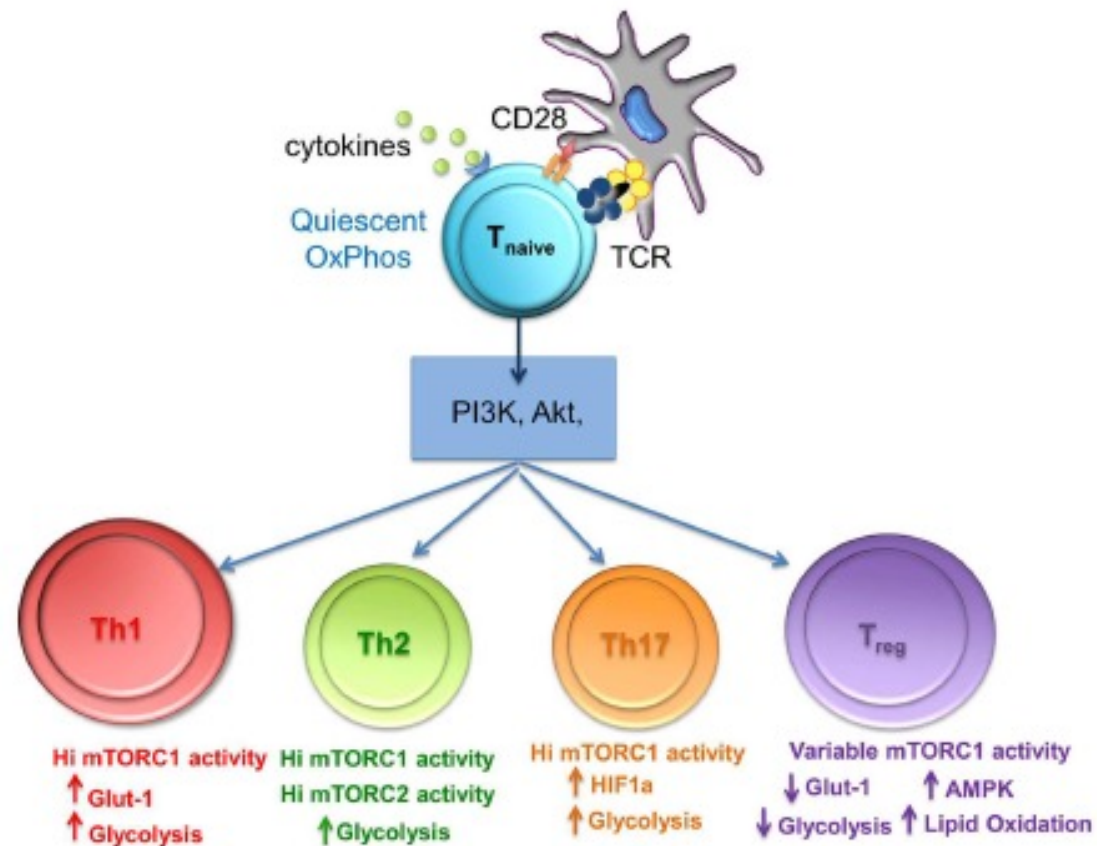

Zhang, Romero *et al.*, Cell Rep. 2016

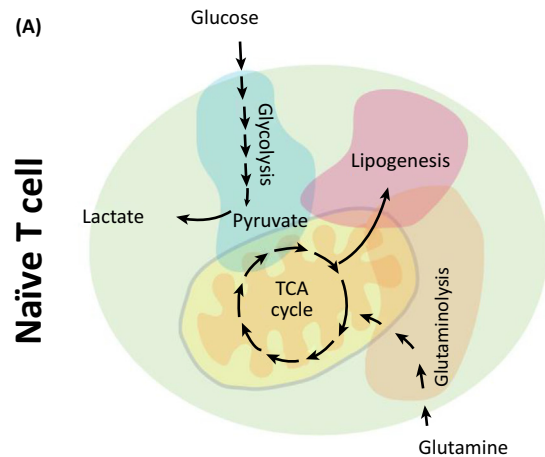# mTORC2 controls CD8 T cell memory differentiation in a FOXO1-dependent manner

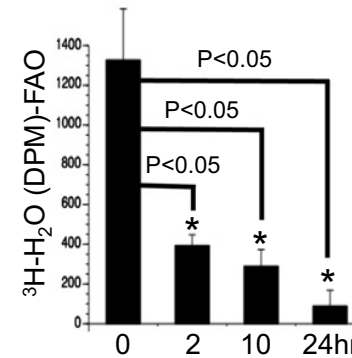# Helper T cell differentiation is also controlled by mTOR

Coe D. J. et. al., (2014) Frontier in Immunol.

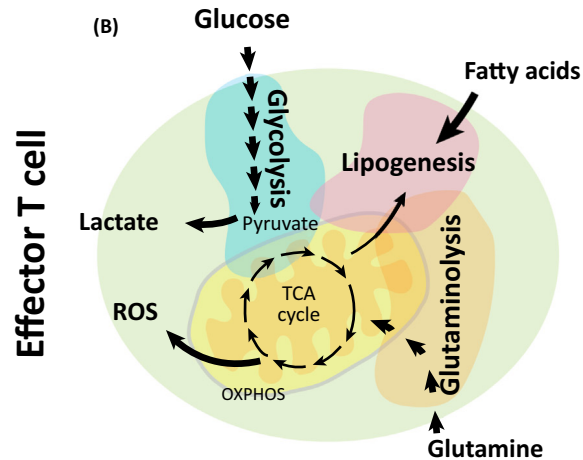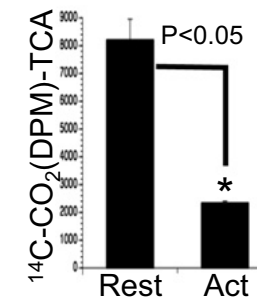# T cell clonal expansion requires a massive activation of specific metabolic pathways
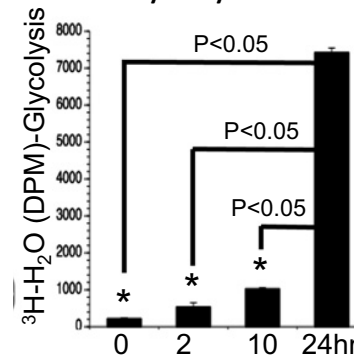

(A) Naïve T cell

(B) Effector T cell

Fatty acid oxidation

Pyruvate oxidation

Glycolysis

Glutamine oxidation
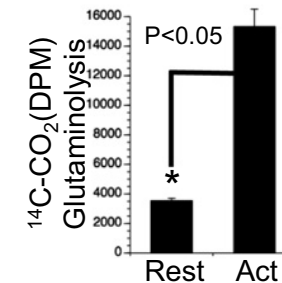
Pentose phosphate pathway
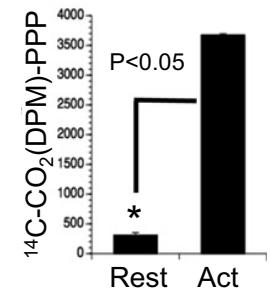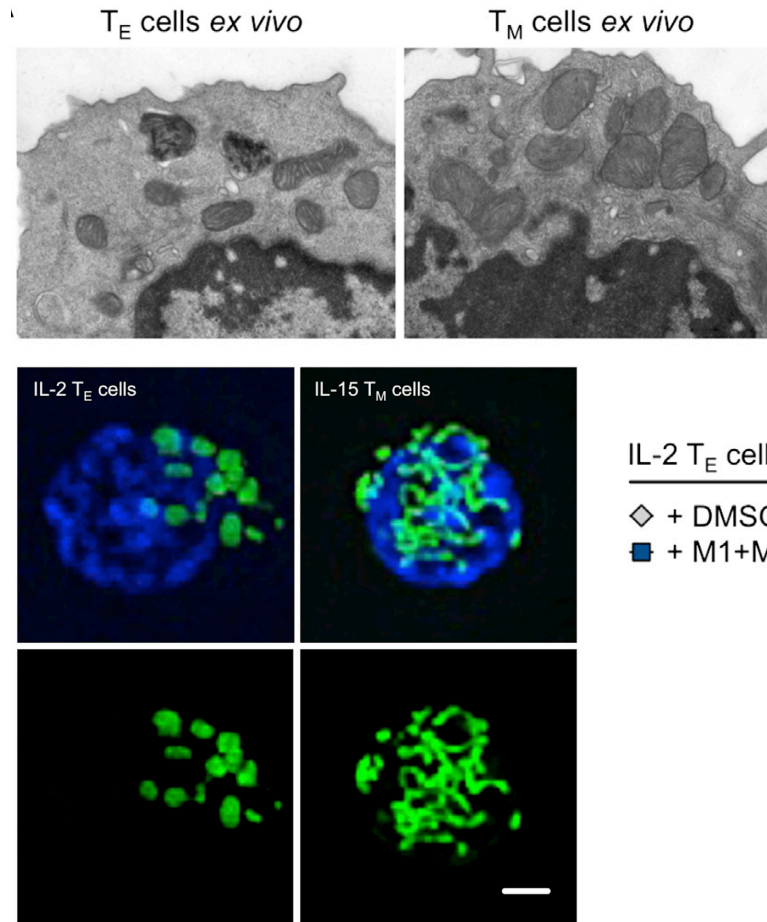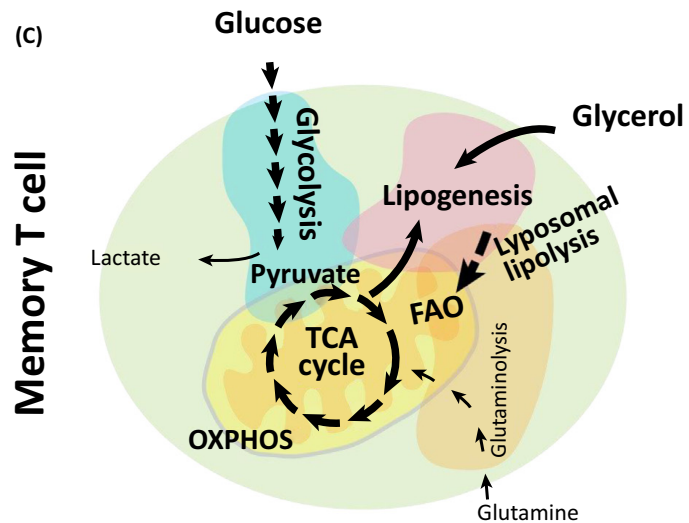
Zhang, Romero *et al.*, Trends Mol.Med. 2018

Wang *et al.*, Immunity 2011

Memory T cells focus on mitochondrial metabolism

(C)

Memory T cell

Glucose

Glycolysis

Lactate

Pyruvate

Glycerol

Lipogenesis

Lysosomal lipolysis

FAO

TCA cycle

OXPHOS

Glutaminolysis

Glutamine

Zhang, Romero *et al.*, Trends Mol.Med. 2018

T_E cells *ex vivo*

T_M cells *ex vivo*

IL-2 T_E cells

IL-15 T_M cells

IL-2 T_E cells
◇ + DMSO
◼ + M1+Mdivi-1

Donor cells (x10^4)

Spleen

***

Buck *et al.*, Cell 2016

Memory T cells focus on mitochondrial metabolism

Zhang, Romero *et al.*, Trends Mol.Med. 2018

Cui *et al.*, Cell 2015

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Tumor-infiltrating T cells: prototype of T cell exhaustion
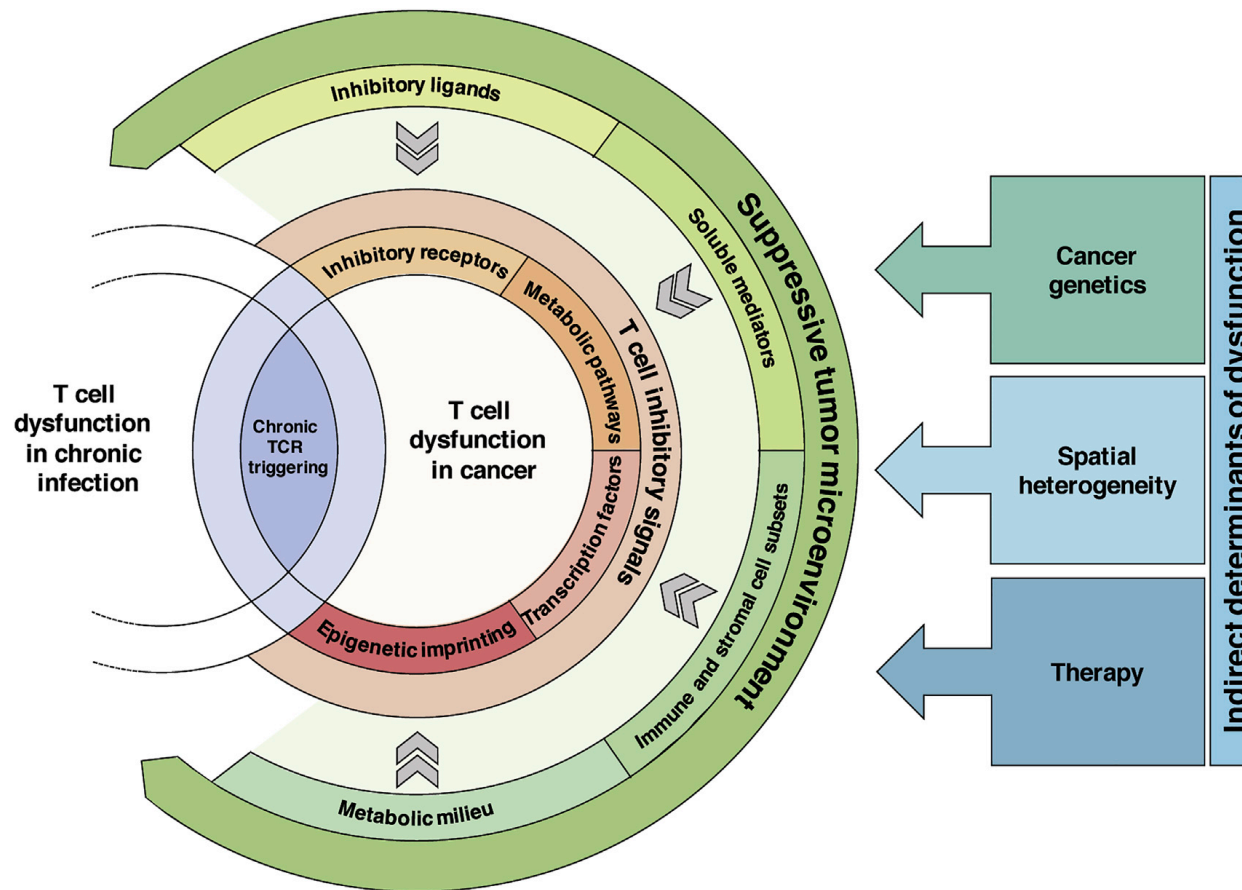
Chronic viral infection and cancer are characterized by antigen persistence and T cell exhaustion

McLane *et al.*, Annu. Rev. Immunol., 2019

3 signals model to induce T cell exhaustion
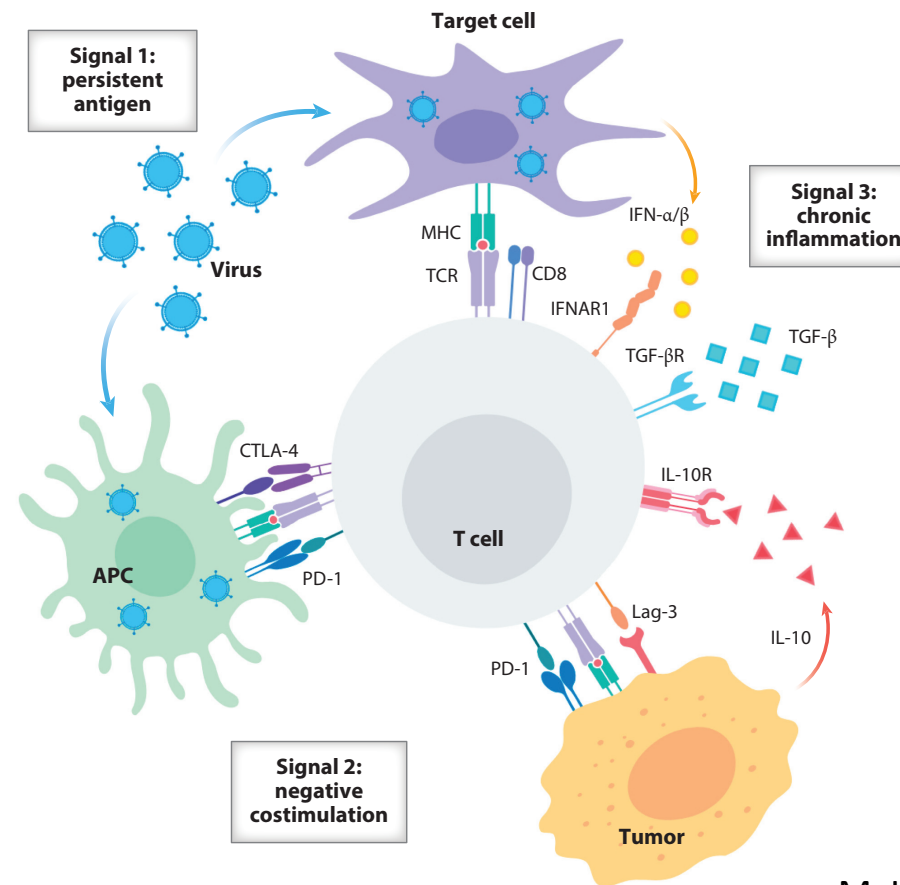
McLane *et al.*, Annu. Rev. Immunol., 2019

Metabolism in exhaustion

Zhang, Romero *et al.*, Trends Mol.Med. 2018

Patsoukis *et al.*, Nat.Comm. 2015

Metabolism in exhaustion
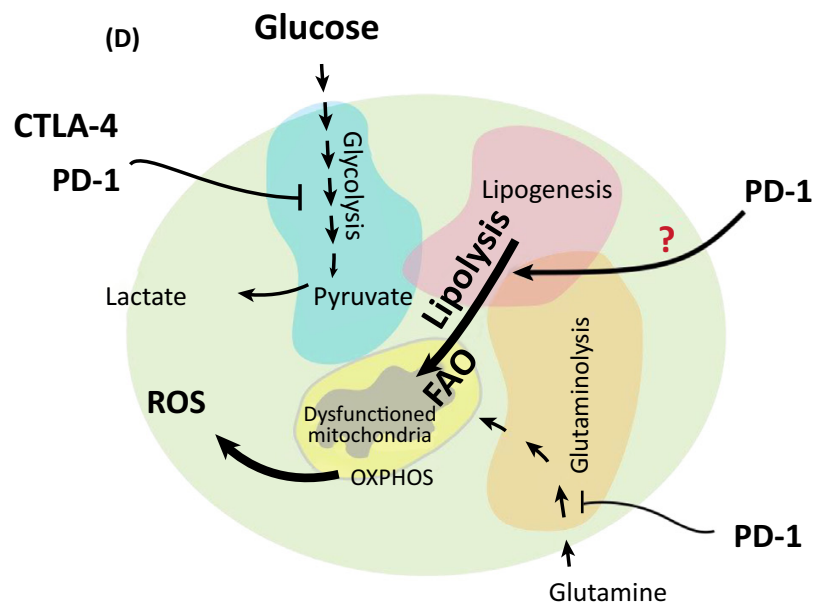
Zhang, Romero *et al.*, Trends Mol.Med. 2018
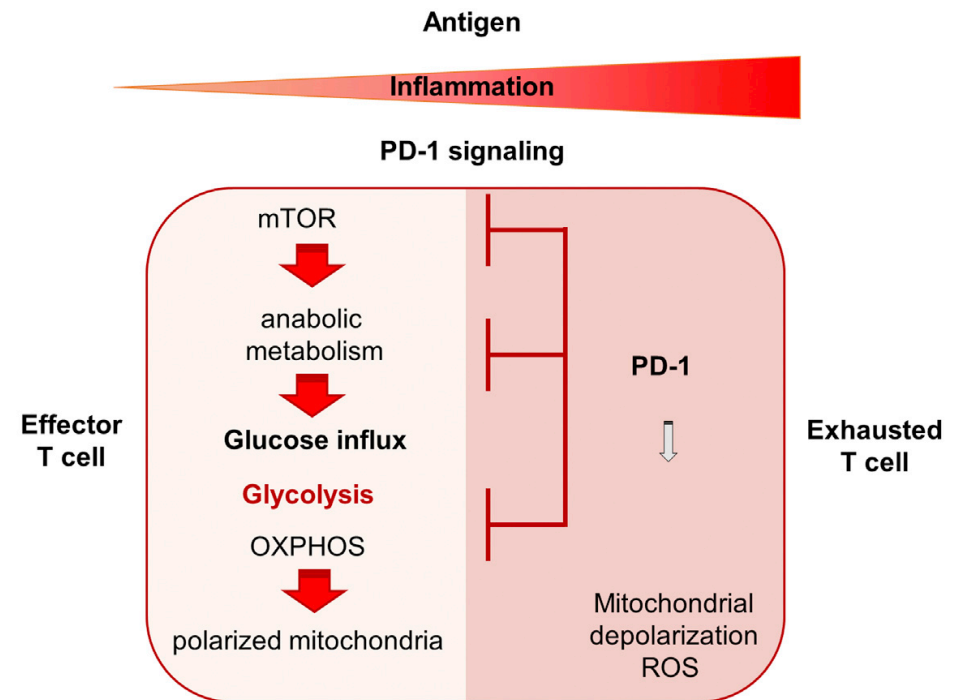
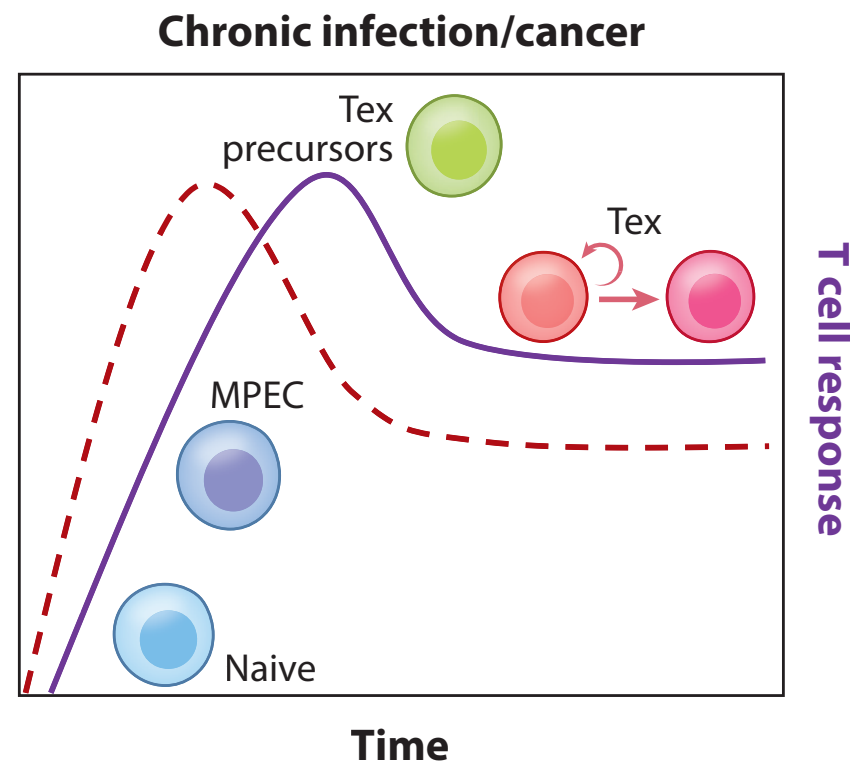Bengsch *et al.*, Immunity 2016

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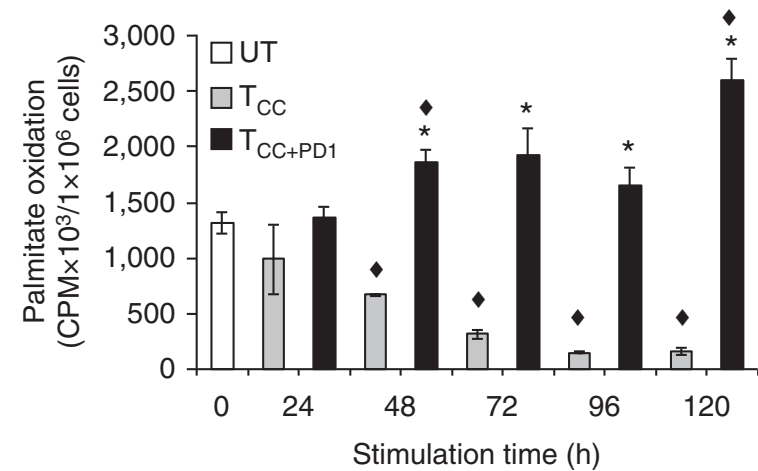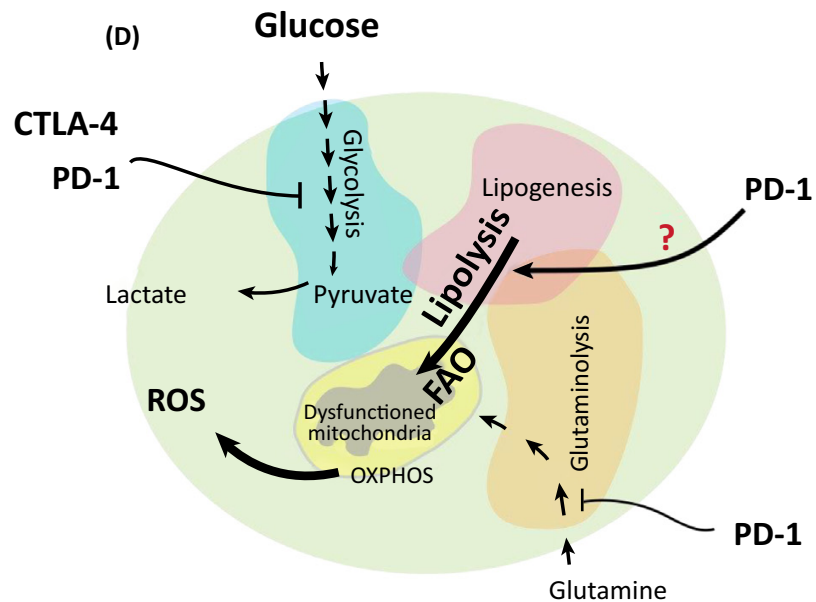 2) Metabolic engineering to enhance antitumor immunity

Cancer cells steal glucose from T cells

Glucose levels
are low in tumors

Glucose is required for
effector T cell function

High glycolytic tumors
(hexokinase 2 overexpression, HK2-OE)
limit anti-tumor T cell function

Ho *et al.*, Cell 2015

Metabolic stress in the tumor microenvironment and its impact on antitumor immunity
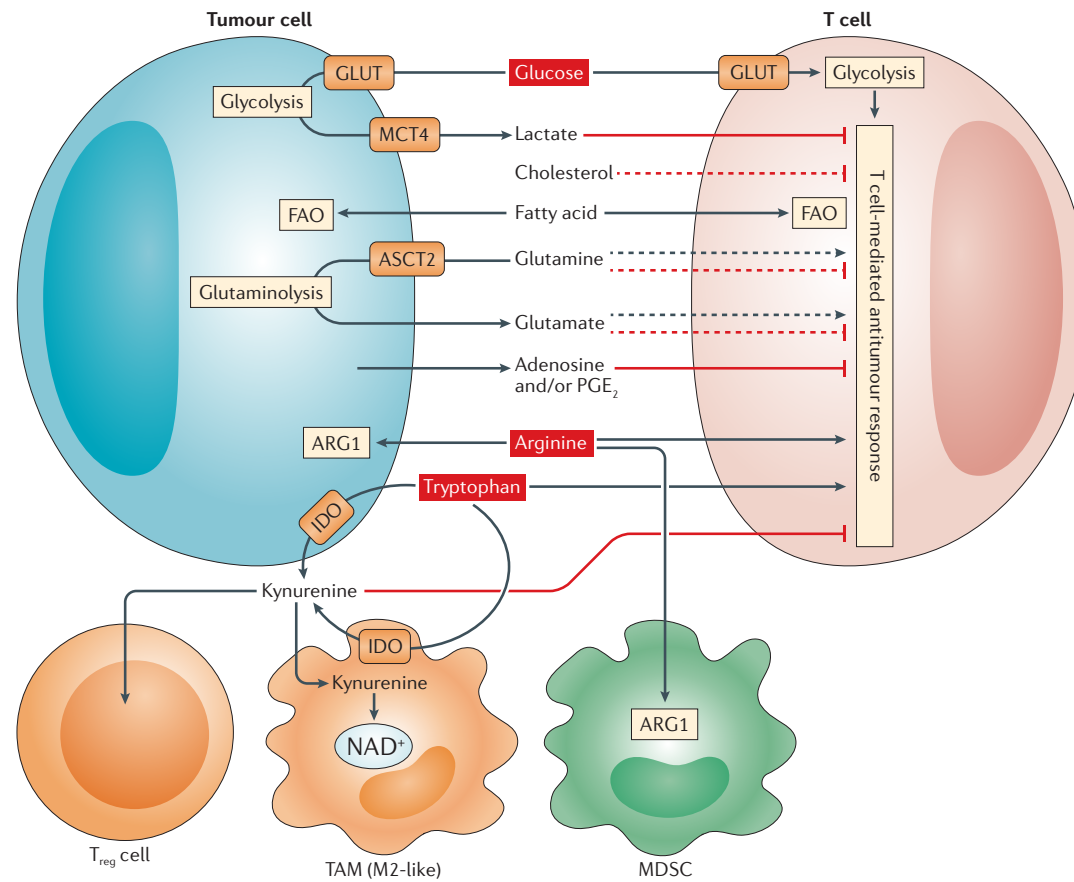
Li, Wenes, Romero *et al.*, Nat.Rev.Clin.Onc. 2019

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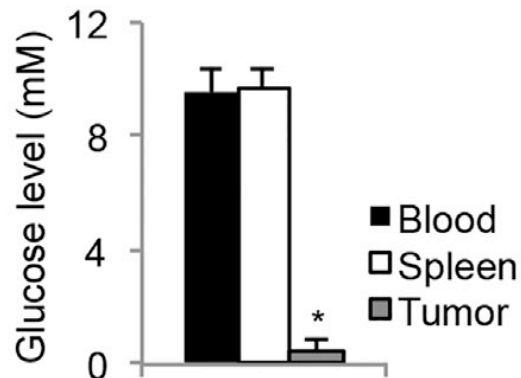2) Metabolism is closely connected to immune cell phenotype and function
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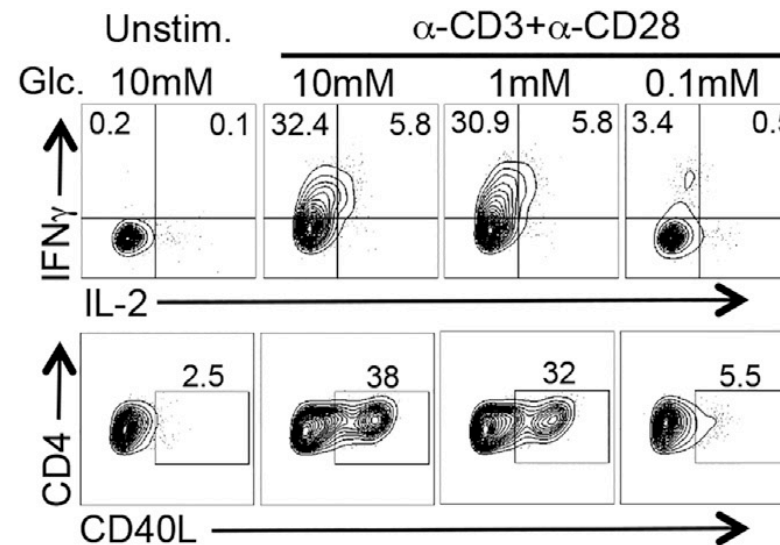
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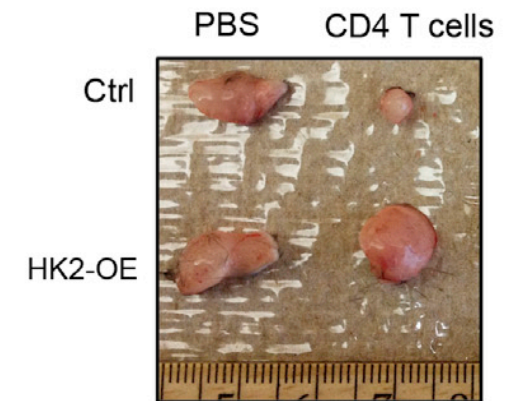   2) Metabolic engineering to enhance antitumor immunity

Cancer immunotherapy strategies

1. Vaccines

- **Provenge**
- **Neo-antigens**
- **Tumor associated-antigens**

Vaccine Therapy — Vaccine, Lymph nodes stimulated, Lymph node, Lymphocytes

Cell-Transfer Therapy — Antitumor lymphocytes grown in culture, Cultured lymphocytes, Tumor excised

Lymphocytes reach tumor — Lymphocyte-mediated lysis and apoptosis of tumor cells — Lymphocytes
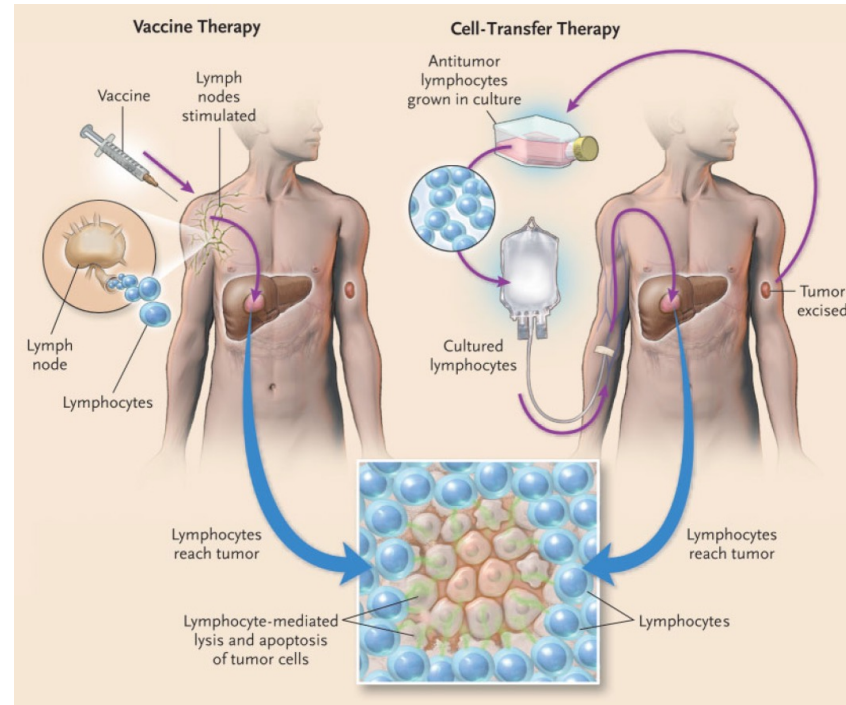
2. Adoptive cell transfer

- **Kymriah**
- **Yescarta**

3. Immunomodulating agents

- **Ipilimumab (α-CTLA-4)**
- **Nivolumab, Pembrolizumab (α-PD-1)**
- **Atezolizumab, Avelumab (α-PD-L1)**

Adapted from Rosenberg, NEJM, 2004

Immunotherapy is enhanced by nutrient supplementation through metabolically engineered bacteria
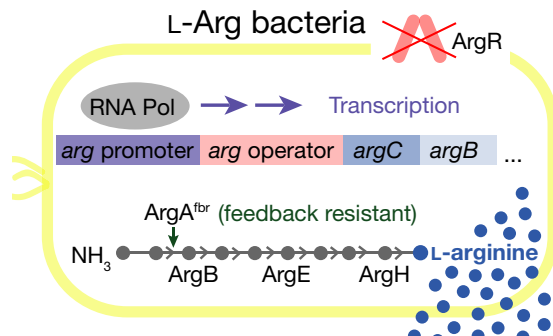
Arginine supply synergizes with checkpoint blockade immunotherapy
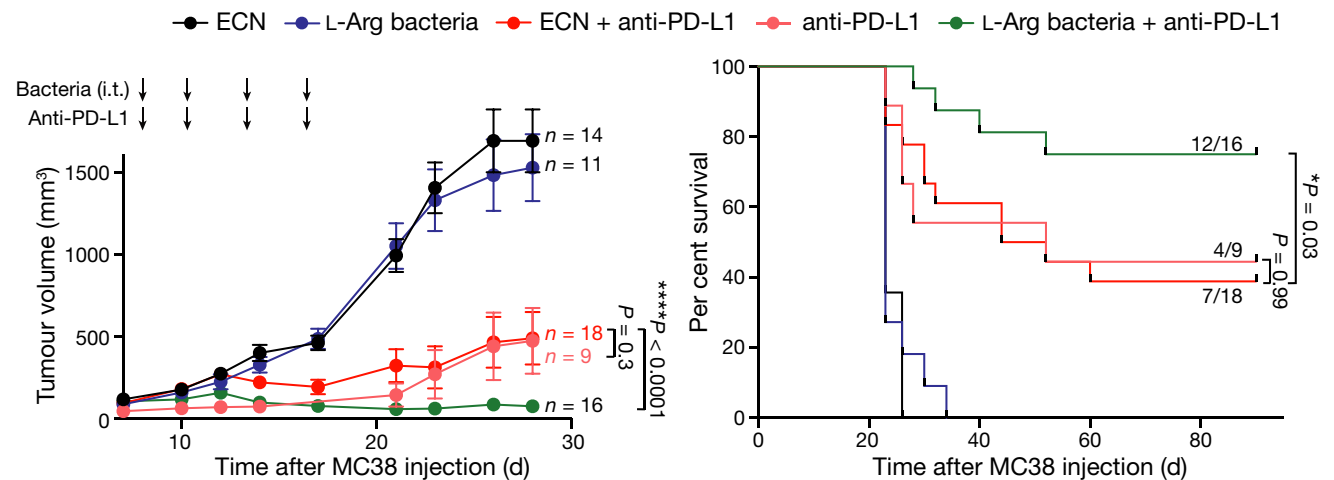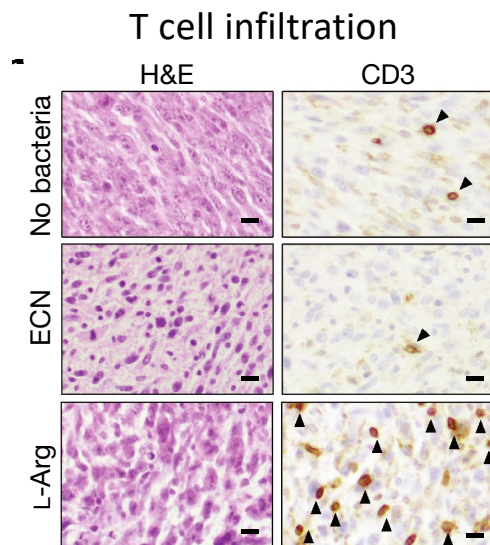
Canale *et al.,* 2021 Nature

Tumor infiltrating lymphocytes have defective mitochondria

Enhancing mitochondrial biogenesis allows T cells to maintain anti-tumor function

CD8+ T_eff   CD8+ TIL

25000X

50000X

Day 23 Tumor Frequencies of transferred cells

Day 21 Tumor Mitochondrial respiration

Spleen

SCR   PGC-1α

Scharping *et al.*, Immunity 2016

Dumauthioz, Wenes, Romero *et al.*, Cell.Mol.Immunol. 2020
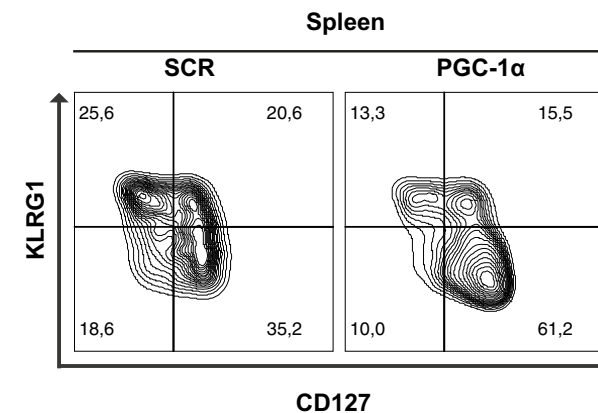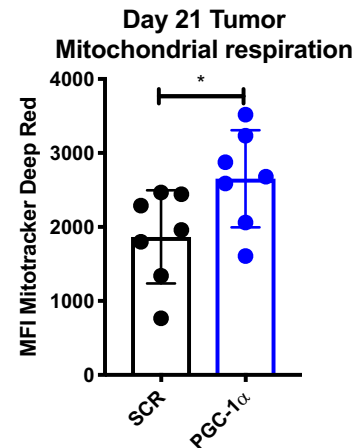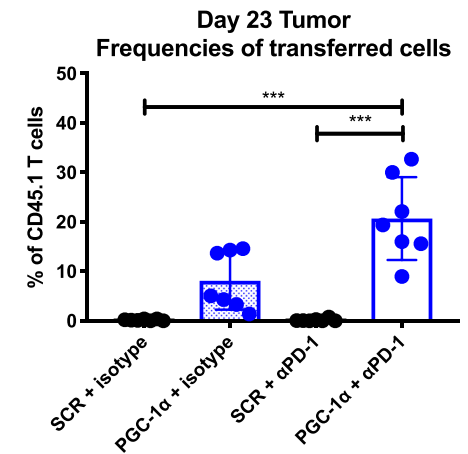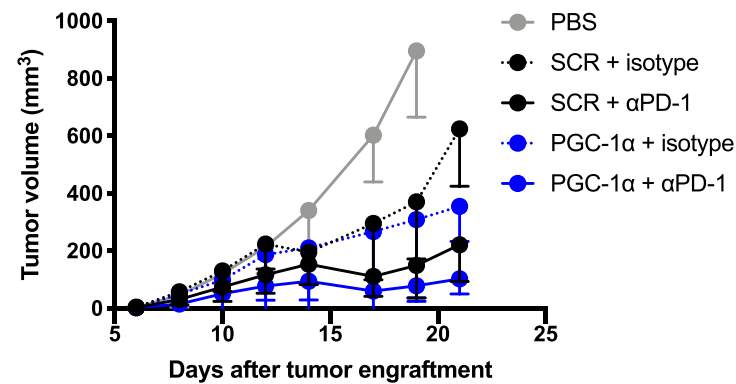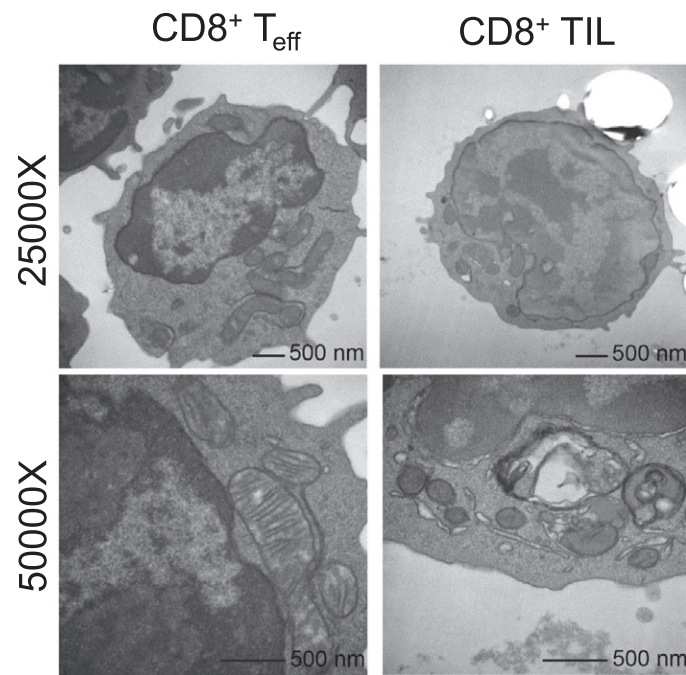
Metabolic reprogramming of terminally exhausted CD8+ T cells by IL-10 enhances anti-tumor immunity

d

YUMM1.7-OVA

- ○— OT-I + PBS
- ○— OT-I + IL-10–Fc

Tumor area (mm^2) vs Time post inoculation (d)

$P < 0.001$

Metabolic pathway diagram:
- ATP
- Lactate ← Glycolysis ← Glucose ⊥ 2-DG
- Pyruvate → MPC ⊥ UK5099
- Glutamine → ⊥ BPTES → OXPHOS
- Fatty acid → ETO ⊥ FAO
- OXPHOS → Oligomycin ⊥ ATP

d

OXPHOS

Enrichment score

FDR $Q = 0.15$
NES = 1.32

IL-10–Fc — PBS

Tumor area (mm^2) vs Time post inoculation (d)

- ○— WT OT-I + PBS
- ○— WT OT-I + IL-10–Fc
- ○— MPC1-KO OT-I + PBS
- ○— MPC1-KO OT-I + IL-10–Fc

$P < 0.001$
NS
$P < 0.001$

Guo, Xie et al., Nature Immunology 2021

A T cell memory phenotype is a good prognostic factor for adoptive cell transfer immunotherapy in cancer

Cancer patient

Tumor-antigen specific T cell or CAR T cell

In vitro expansion

Terminally differentiated
Short lived
Exhausted

Reinfusion in patient

Weak anti-tumor response

A T cell memory phenotype is a good prognostic factor for adoptive cell transfer immunotherapy in cancer

Cancer patient

Tumor-antigen specific T cell or CAR T cell

In vitro expansion

Can we metabolically drive memory differentiation for adoptive immunotherapy?

...sion
Long lived

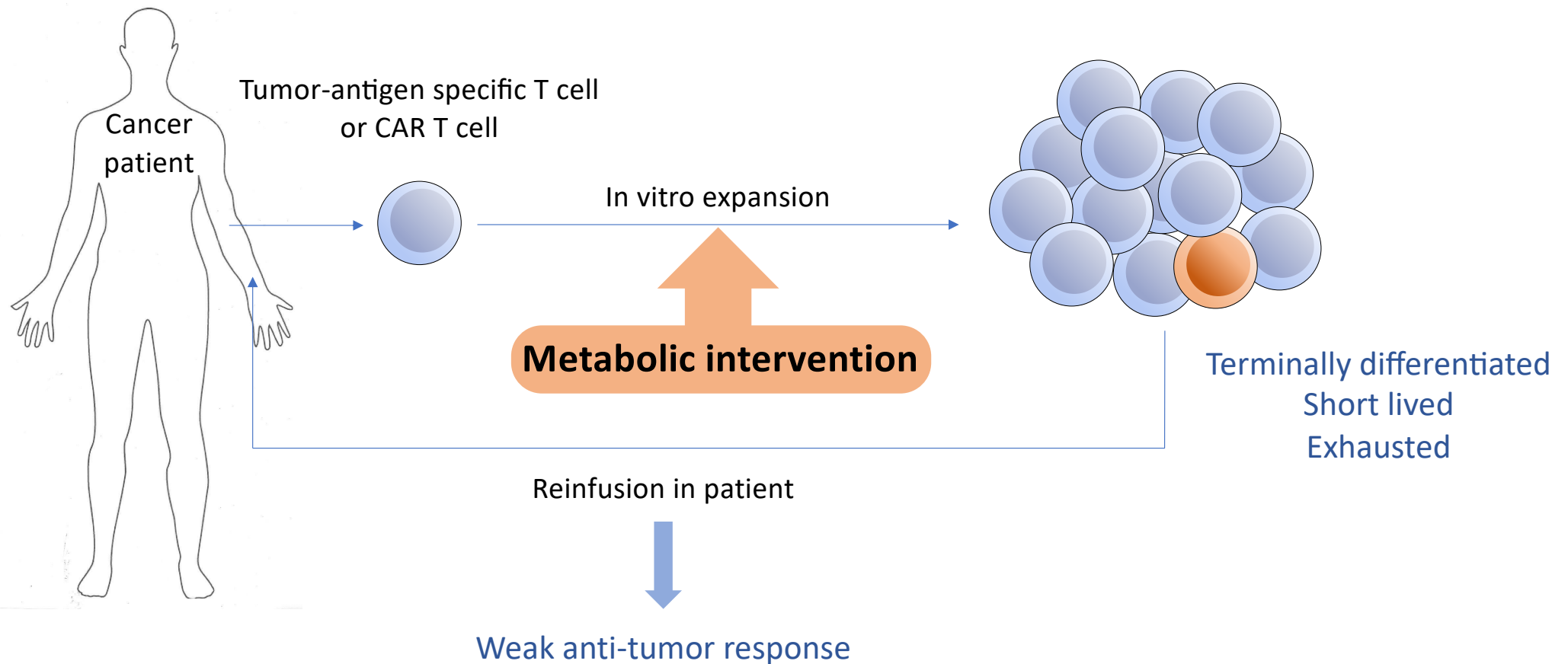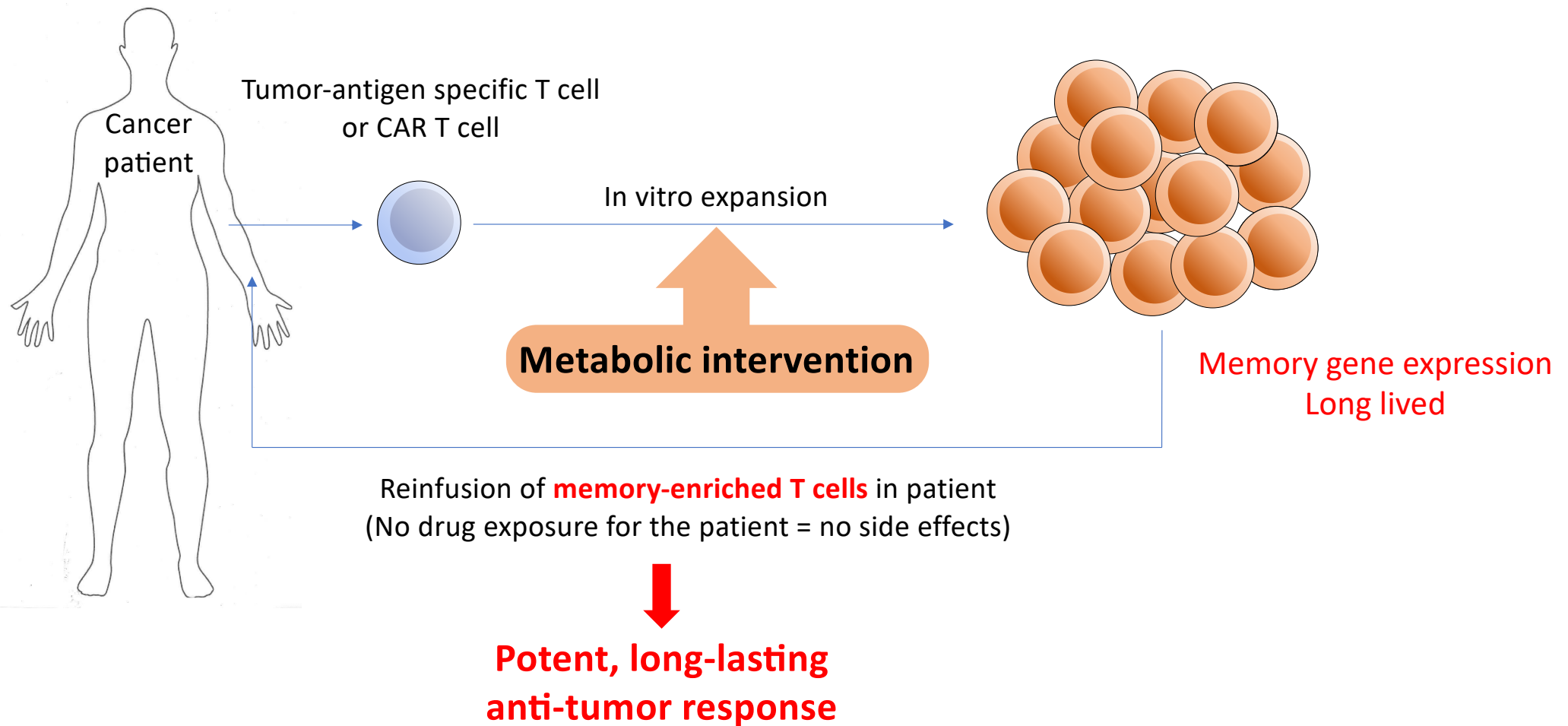Reinfusion in patient

Potent, long-lasting anti-tumor response

Metabolic intervention during ex vivo expansion for adoptive cell immunotherapy

Cancer patient

Tumor-antigen specific T cell or CAR T cell

In vitro expansion

Metabolic intervention

Terminally differentiated
Short lived
Exhausted

Reinfusion in patient

Weak anti-tumor response

Metabolic intervention during ex vivo expansion for adoptive cell immunotherapy

Cancer patient

Tumor-antigen specific T cell
or CAR T cell

In vitro expansion

Metabolic intervention

Memory gene expression
Long lived

Reinfusion of **memory-enriched T cells** in patient
(No drug exposure for the patient = no side effects)

**Potent, long-lasting
anti-tumor response**

Reductive carboxylation instructs effector T cell differentiation
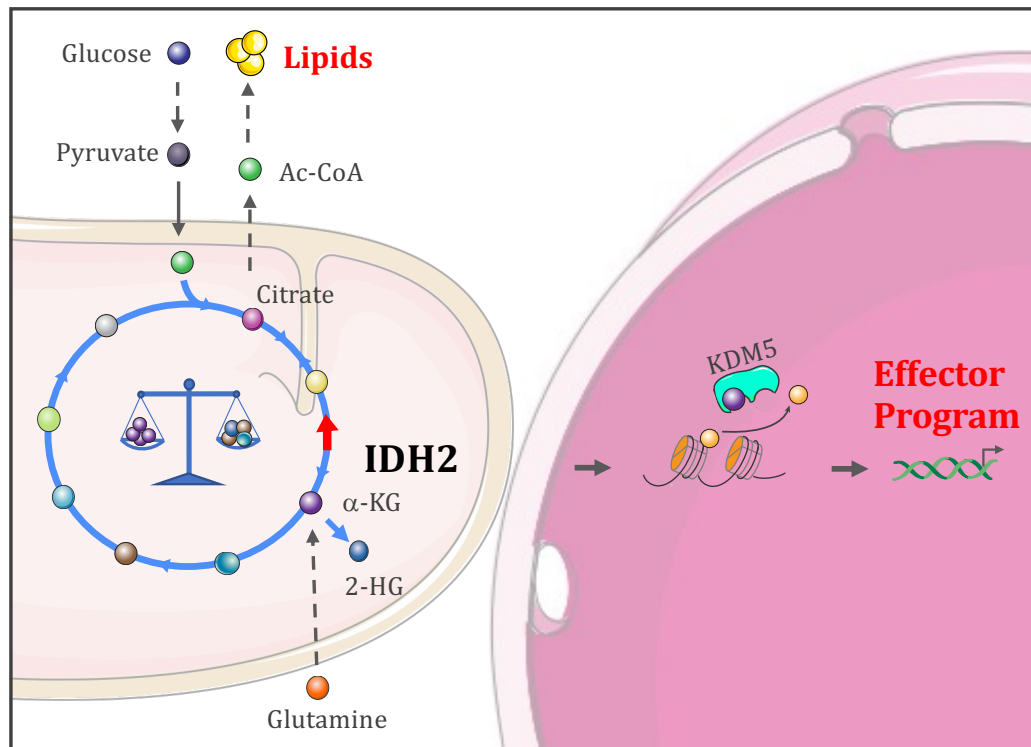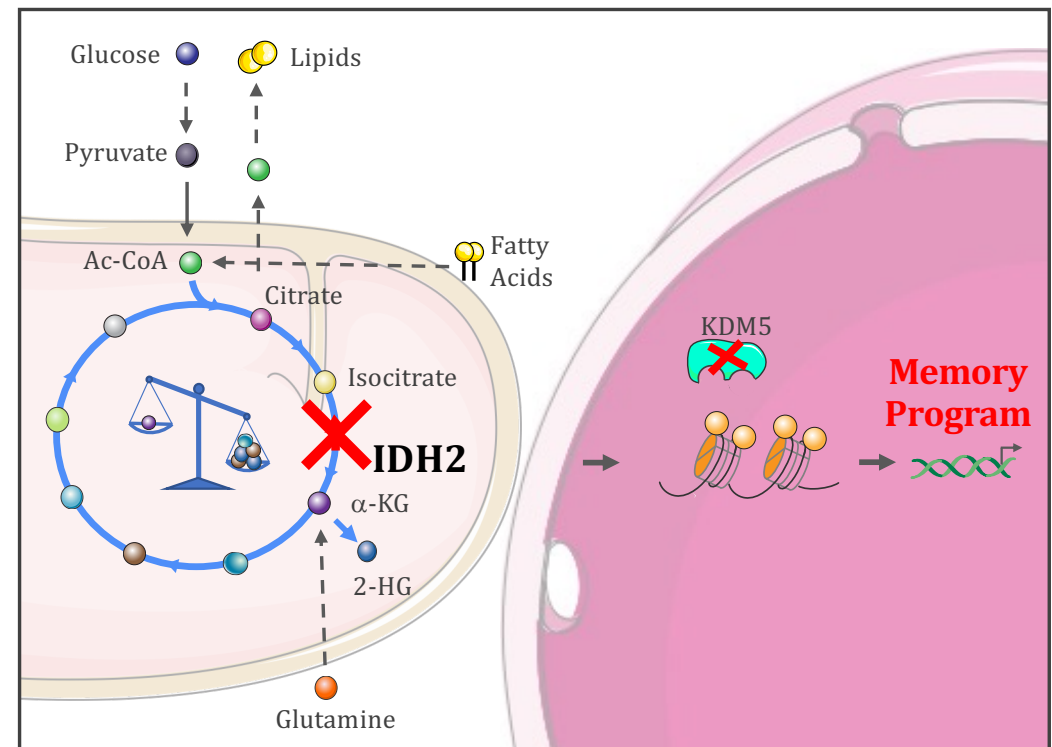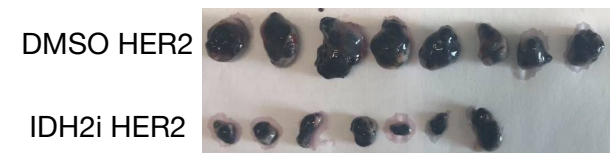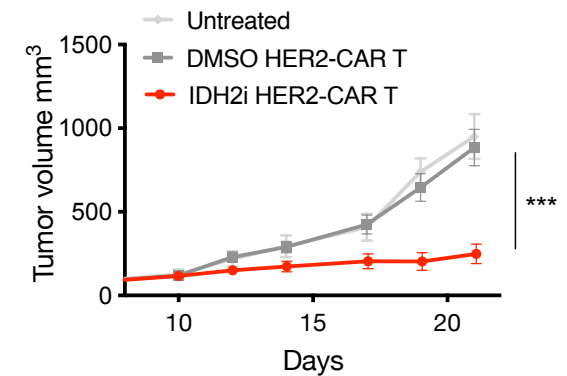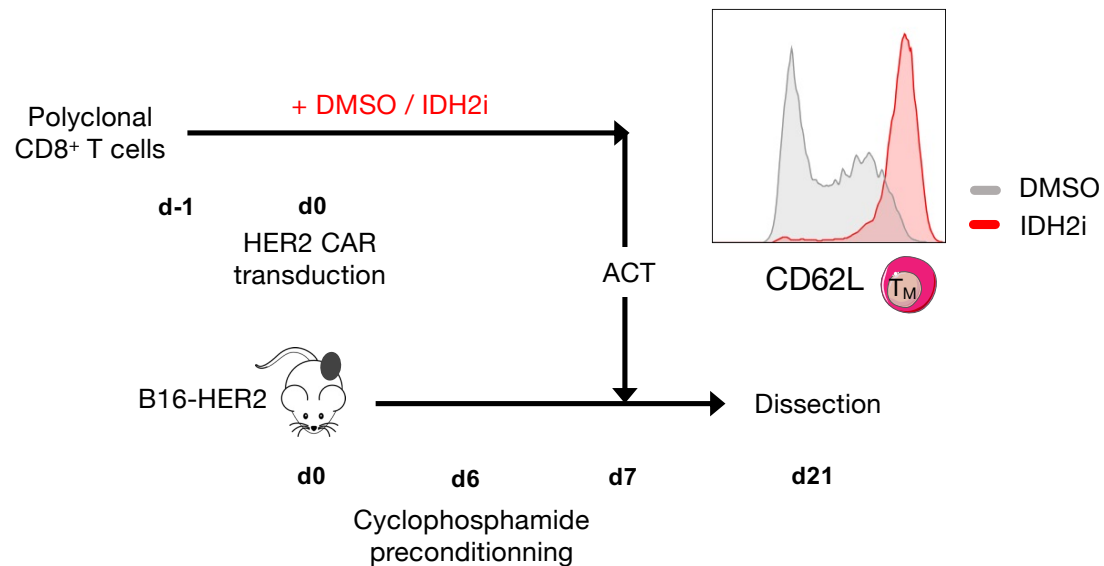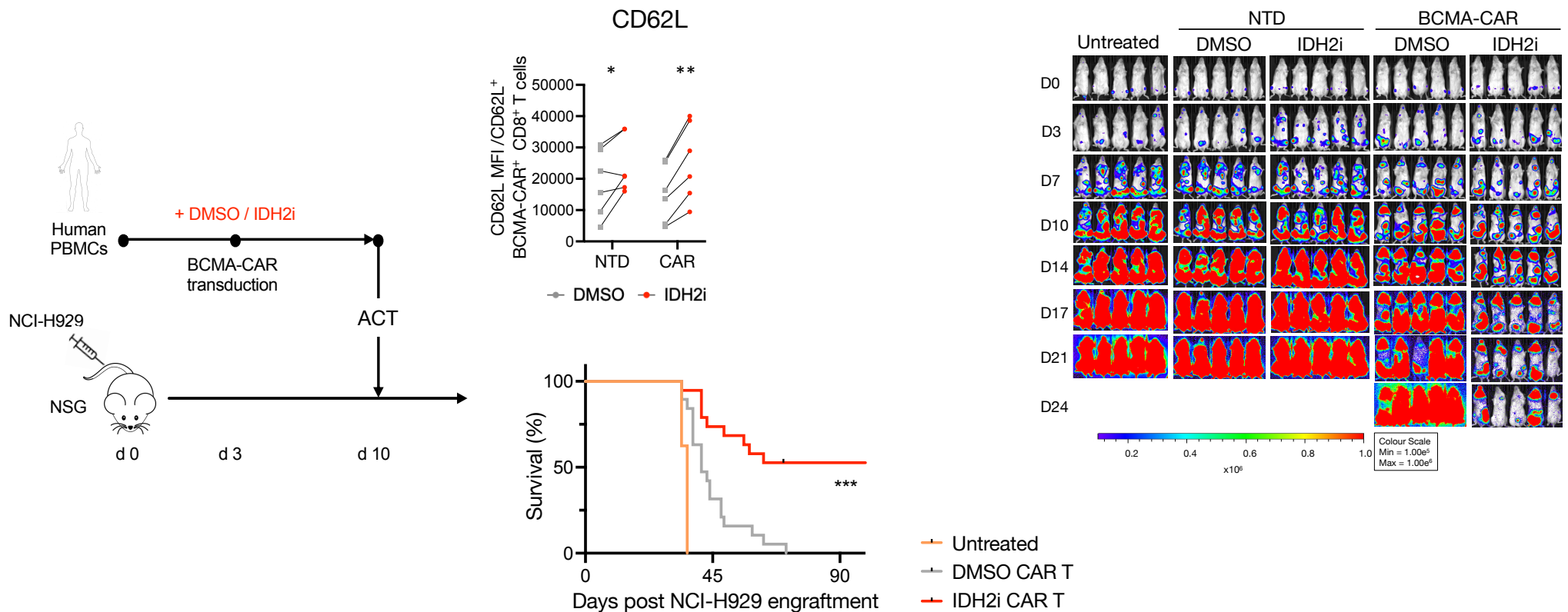

Jaccard, … Wenes, Nature 2023

IDH2 inhibition improves tumour control in a model of murine HER2-CAR T cell ACT against melanoma
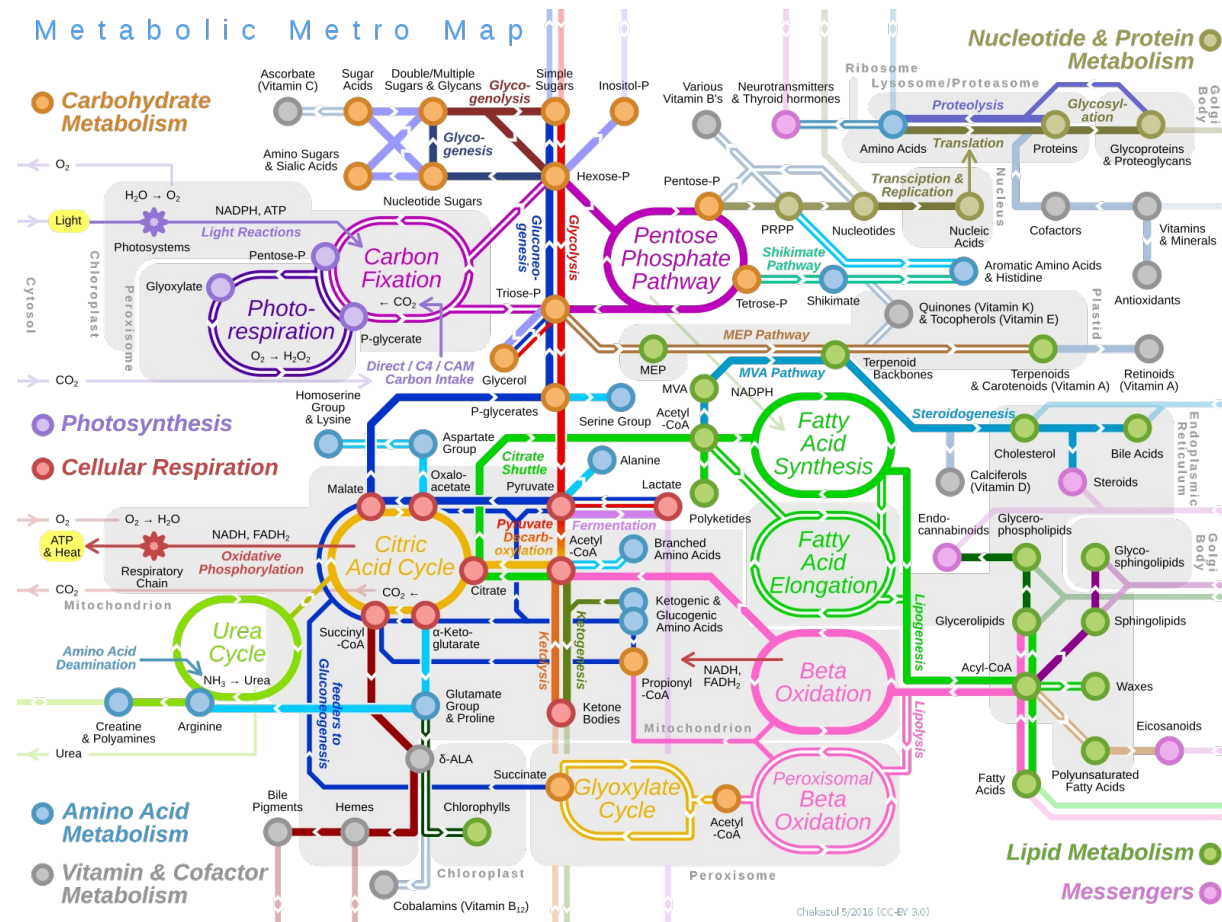
IDH2 inhibition improves tumour control in a model of human BCMA-CAR T cell ACT against multiple myeloma

IDH2i-conditioned CAR T cells show superior antitumour efficacy in murine melanoma and human xenograft models

By identifying the metabolic metro map of our immune cells will help to understand better their functions

Metabolic Metro Map

Suggested reviews (to read for interest)

A guide to immunometabolism for immunologists

Luke A. J. O'Neill[1], Rigel J. Kishton[2] and Jeff Rathmell[2]

Nature Rev Immunology, 2016

Review

Metabolic adaptation of lymphocytes in immunity and disease

Nicole M. Chapman[1] and Hongbo Chi[1,*]
[1]Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN 38105, USA
*Correspondence: hongbo.chi@stjude.org
https://doi.org/10.1016/j.immuni.2021.12.012

Immunity, 2022

Immunity
Review

Metabolic Reprogramming of Immune Cells in Cancer Progression

Subhra K. Biswas[1,*]
[1]Singapore Immunology Network (SIgN), Agency for Science, Technology & Research (A*STAR), #04-06 Immunos, 8A Biomedical Grove, Singapore 138648, Singapore
*Correspondence: subhra_biswas@immunol.a-star.edu.sg
http://dx.doi.org/10.1016/j.immuni.2015.09.001

Immunity, 2015

Immunity