Microenvironmental metabolites shaping T cell fate and function in cancer

SITC Tumor Microenvironment Workshop

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Disclosures

Consulting/SAB: BlueSphere Bio, Kalivir, Century Therapeutics, Nanna Therapeutics, Astellas, RemplirBio, and Novasenta

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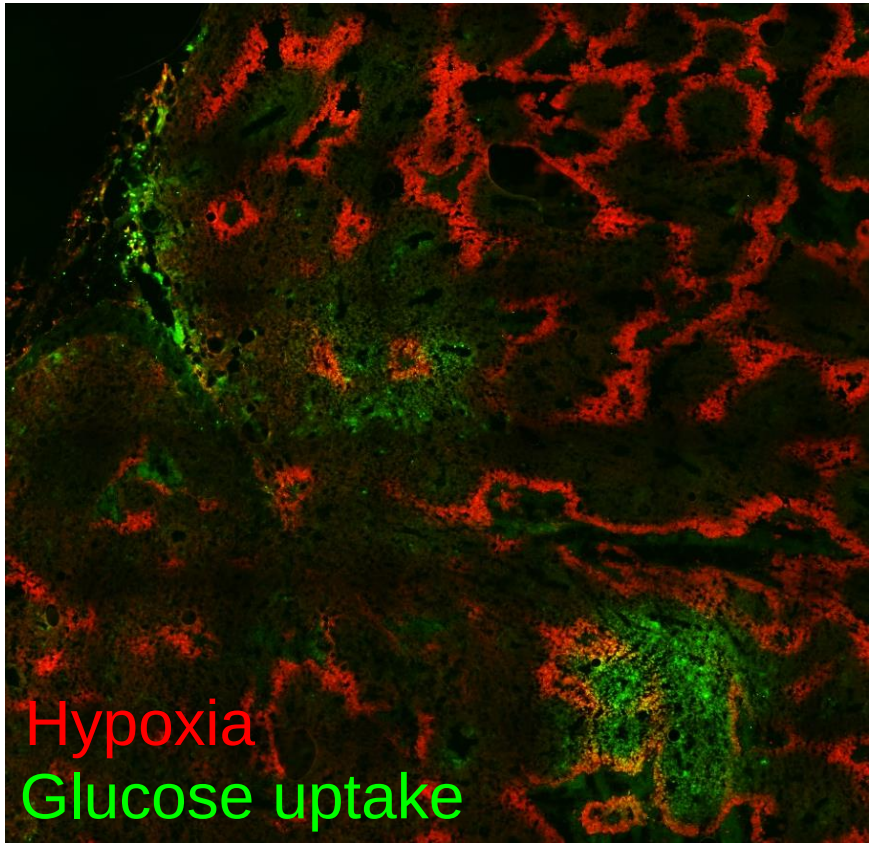
Clinical Trials: Merck, Pfizer, BMS, and Novasenta

Founder and Advisor, Equity: RemplirBio, Novasenta

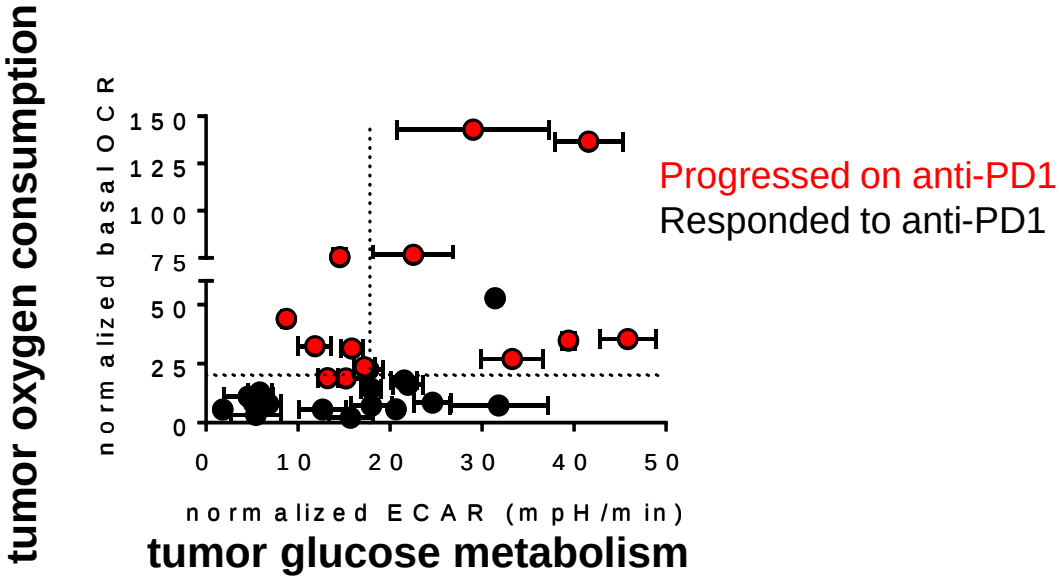
How does the tumor microenvironment's metabolic landscape affect immunotherapy response?

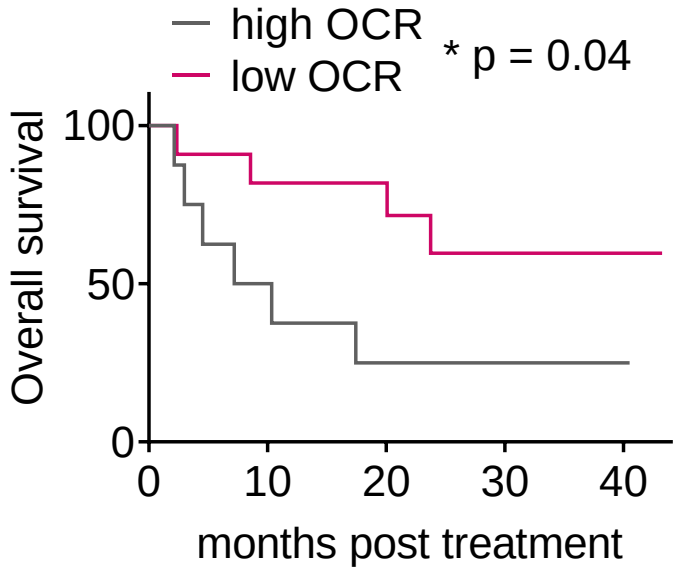


- The tumor microenvironment engages in some very immunosuppressive functions
  - Altering stromal cell function to support tumor growth
  - Changing angiogenesis patterns
  - Providing chronic antigen stimulation
  - Recruitment of immunosuppressive cell types
- However, a common phenotype of cancer is that it is **hungry**
- The tumor microenvironment, driven by the metabolic derangement of tumor cells, generates a **distinct metabolic landscape,** creating both a **sink for essential nutrients** as well as a buildup of **toxic byproducts**
  - Tumors increase oxygen consumption, which creates local zones of hypoxia and production of high concentrations of reactive oxygen species
  - Tumors also become highly glycolytic, creating both a hypoglycemic interstitia but also generating high concentrations of lactic acid
- **A tumor cell's 'appetite' may be variable in the patient population!**

Tumor cell (oxidative) metabolism is associated with immunotherapy response

- We performed metabolic profiling of melanoma patient tumor cells directly from biopsies prior to anti-PD1
- Tumor cell metabolism was highly variable in IO-naïve patients
- Patients with highly oxidative tumor cells were more likely to fail anti-PD1 therapy
- Immune activation and function are extremely energetically demanding!





Najjar, Menk, *JCI Insight* 2019

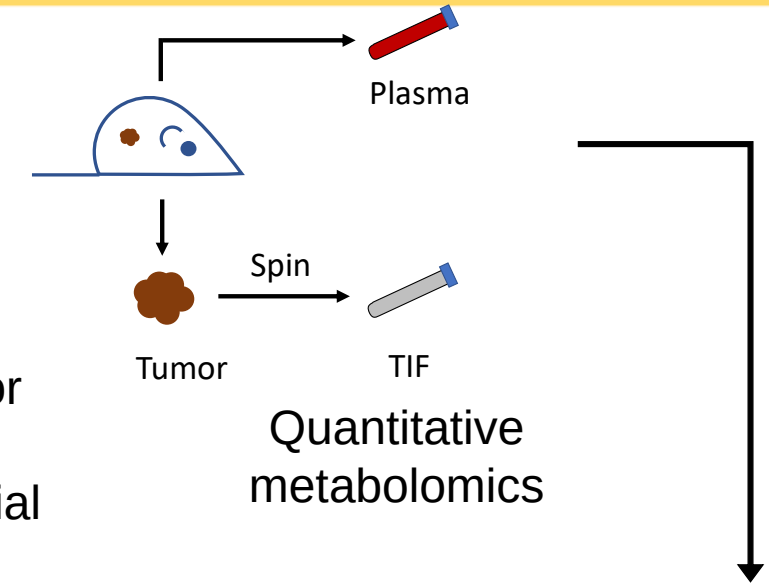
What *is* the metabolic environment in cancer?

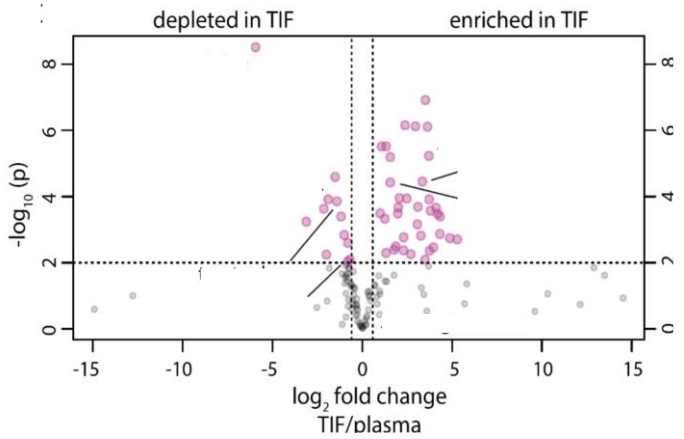
How can you isolate how metabolism *specifically* affects anti-cancer immunity?

- Not only can metabolism act on multiple phases of the immune response, but there are also dozens of other signals T cells endure simultaneously within the tumor microenvironment
  - Oncogenic signals
  - Inflammatory vs suppressive cytokines
  - Physical barriers
  - Immunologic cell-surface signals
- Can we ask how the specific metabolism of the tumor microenvironment acts upon different phases of T cell immunity?

Tumor interstitial fluid: the milieu of the tumor microenvironment

- What do immune cells see metabolically in cancer?
- Enter **Alex Muir**
  - Metabolic regulation of cancer progression, notably in KPC mice – $Kras^{G12D}p53^{f/f}Pdx1^{Cre}$
    - low response to immune checkpoint blockade
    - Highly metabolically deregulated
  - Developed a cost-effective means to generate metabolite libraries for quantitation of metabolomics experiments
- He published a sweeping metabolomic characterization of tumor interstitial fluid (Sullivan et al, *eLife* 2019)






Alex Muir


Chufan Cai

University of Chicago

TIFM
(Tumor Interstitial Fluid Media)

How do T cells respond acutely to TIF media?

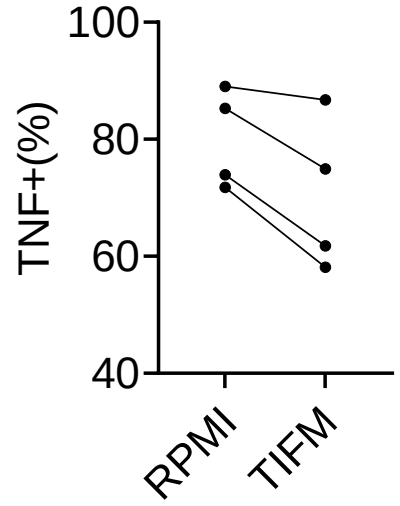
OT-I cells activated with SIINFEKL for 24h

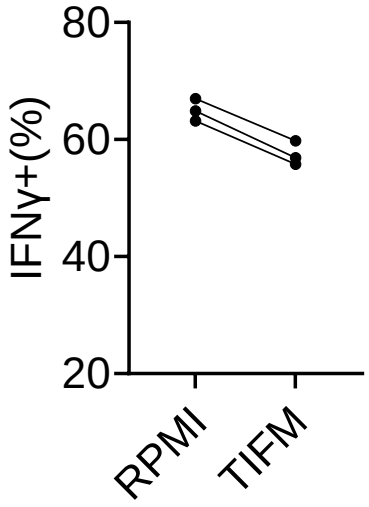
RPMI

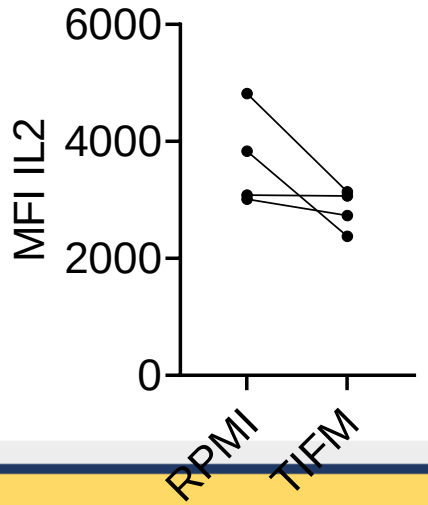
Day 7 Restimulate

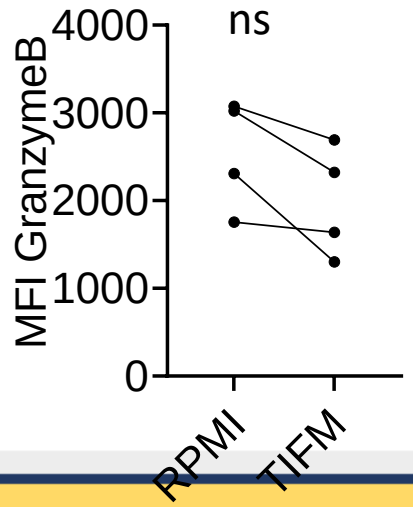
α-CD3/α-CD28 6h

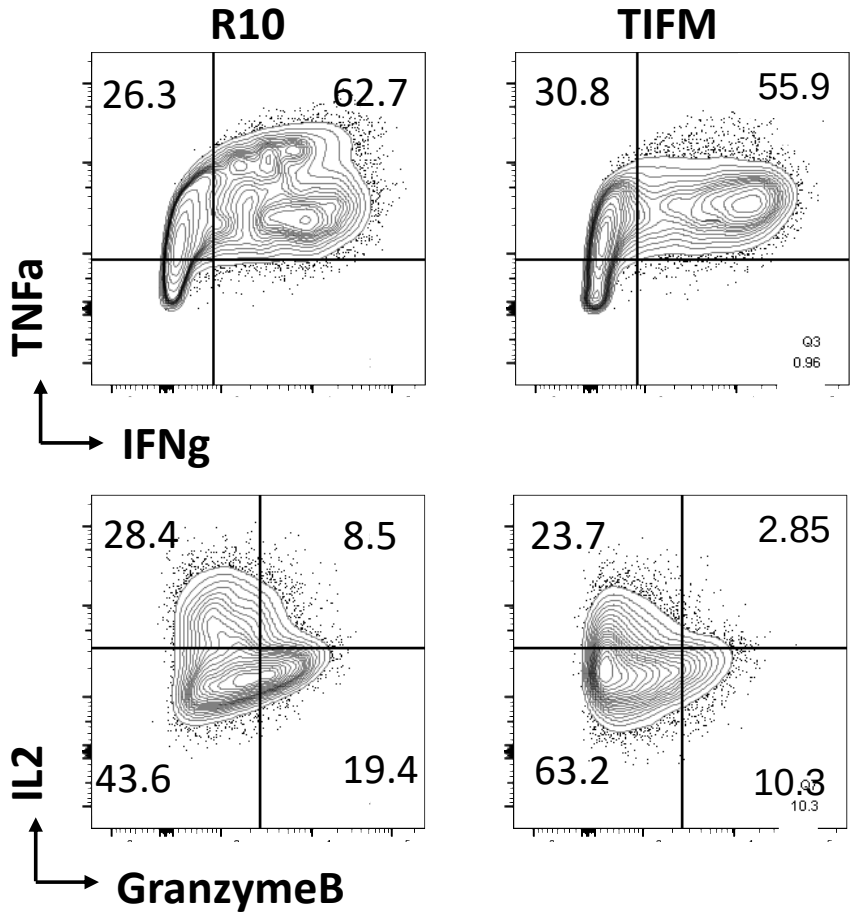
RPMI/TIFM



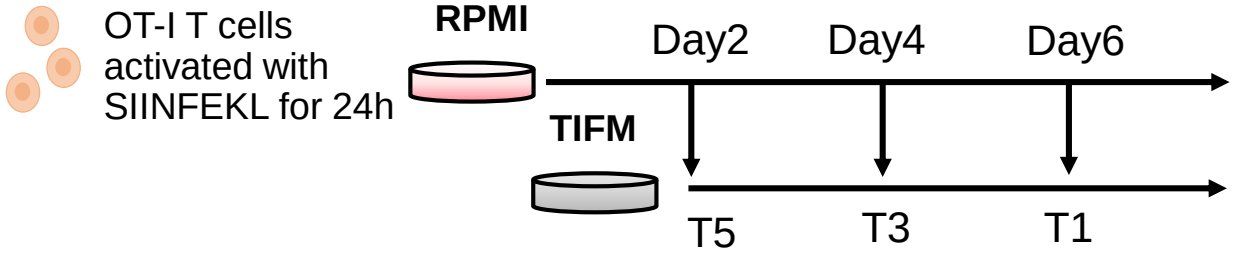


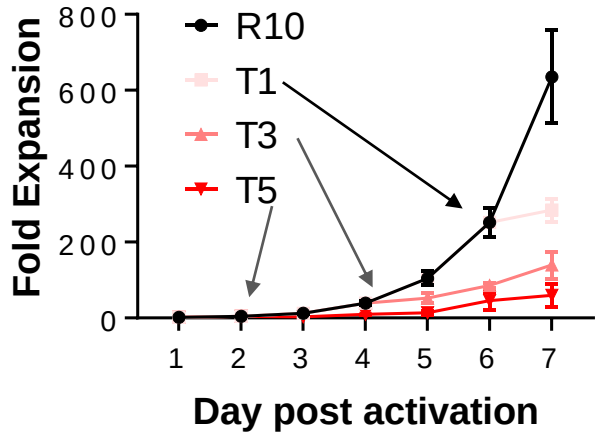


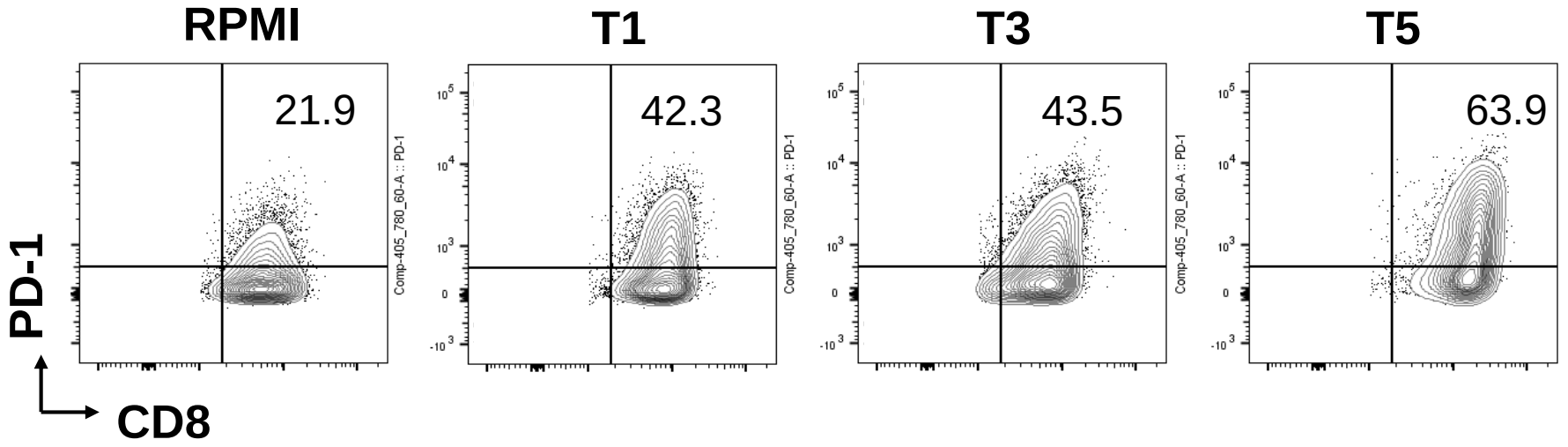


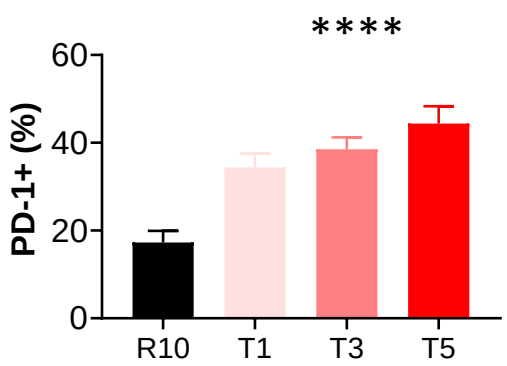


Culture in the TIFM arrests proliferation



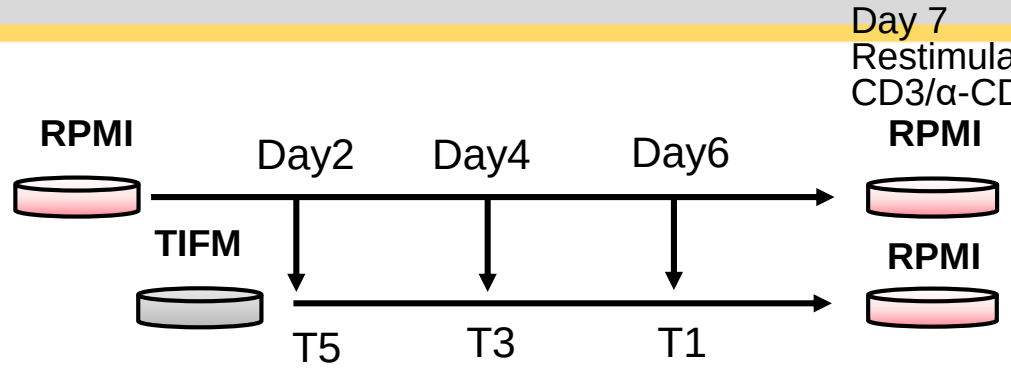


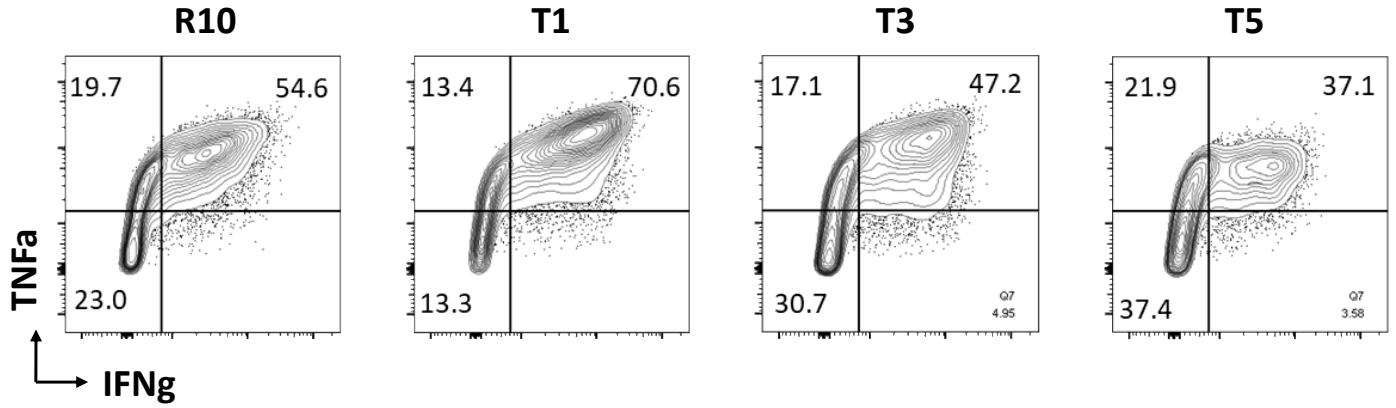


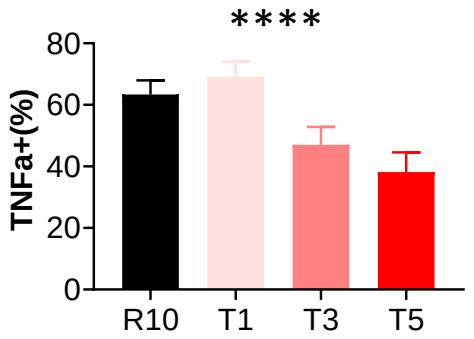


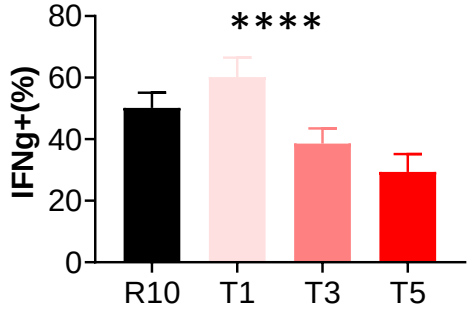
Culture in the TIFM promotes dysfunction

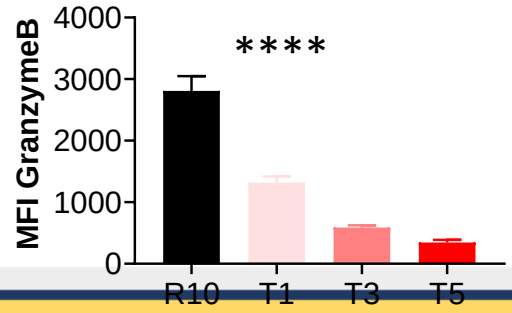
OT-I T cells activated with SIINFEKL for 24h



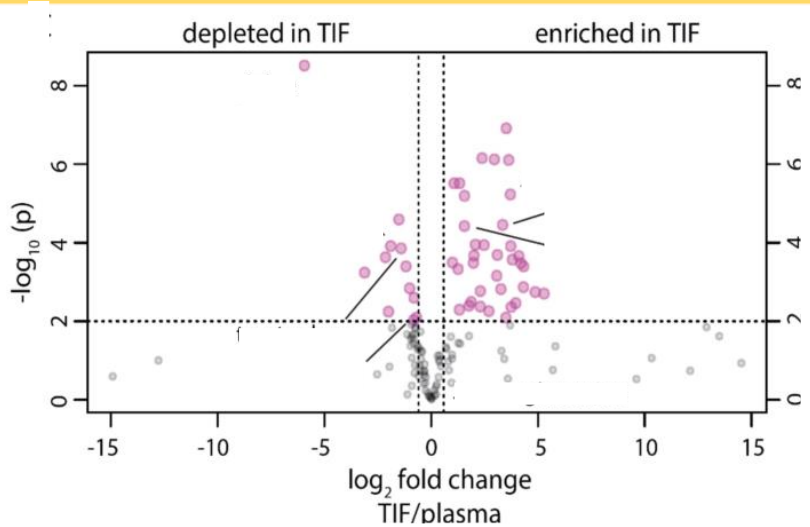




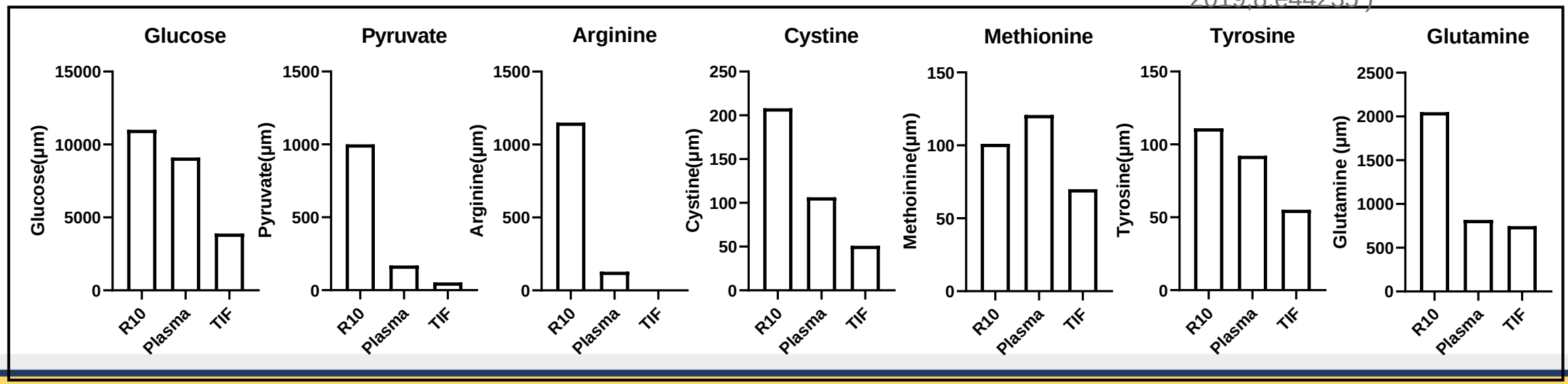




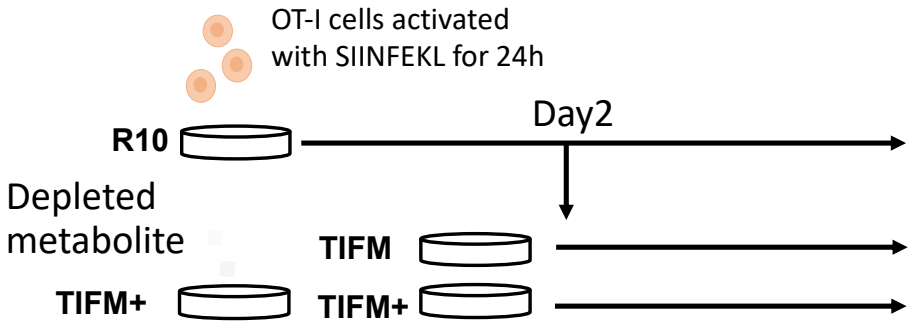
What's missing in TIF media that T cells need?

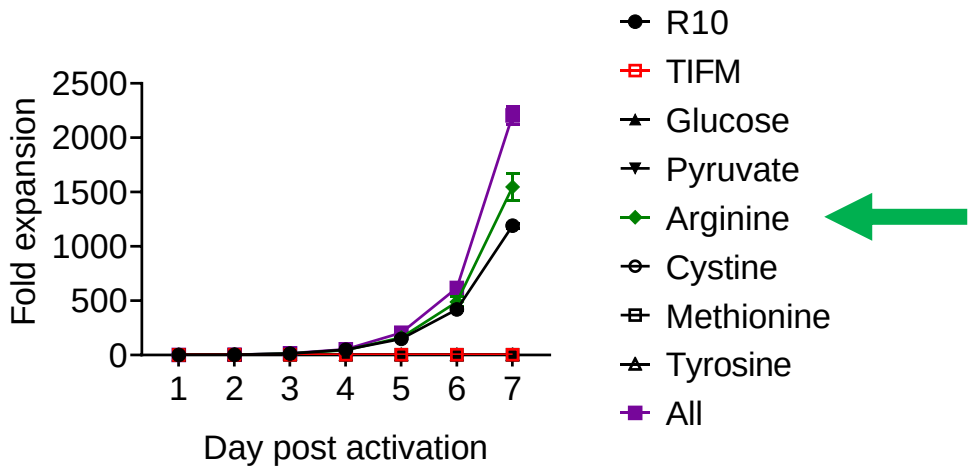


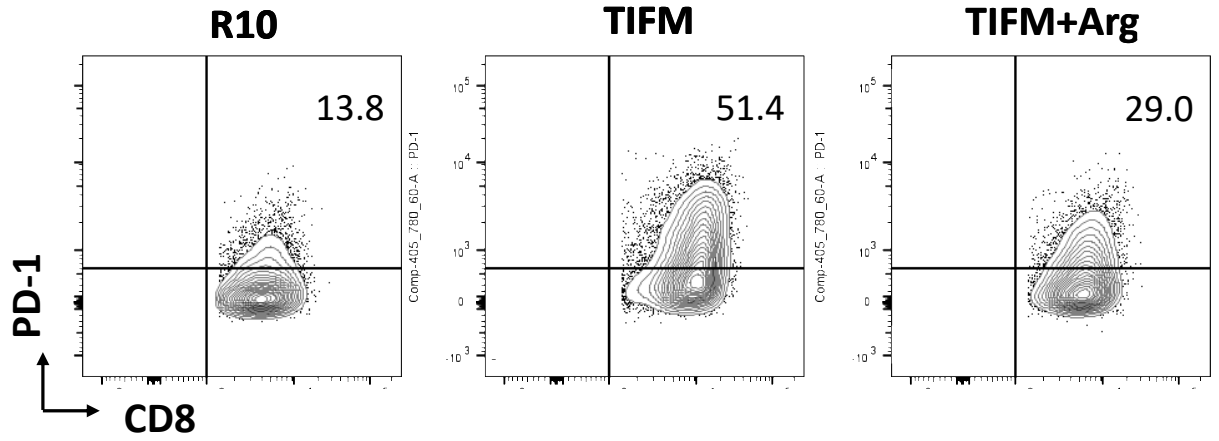
(Sullivan et al. eLife 2019;8:e44235)

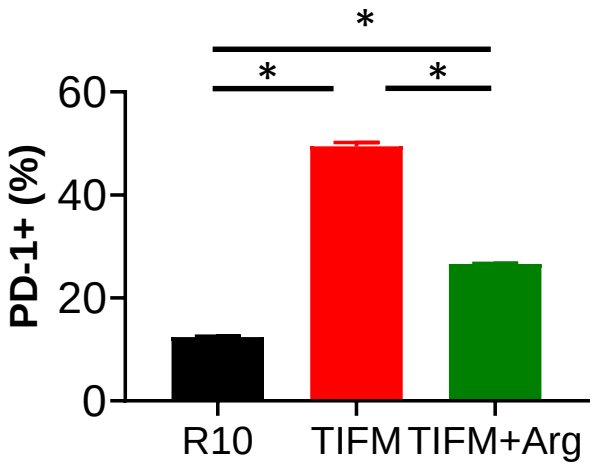


Arginine supplementation of TIFM restores T cell proliferation

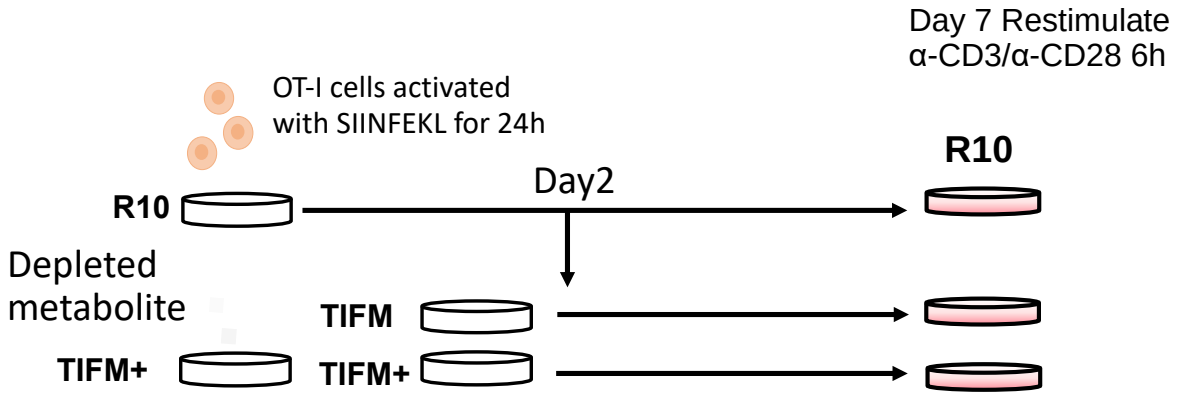


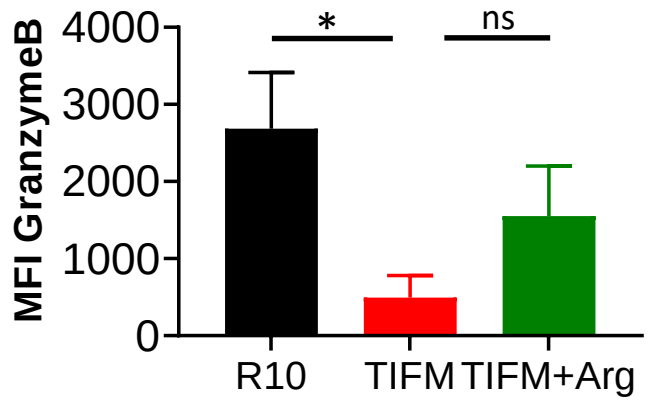


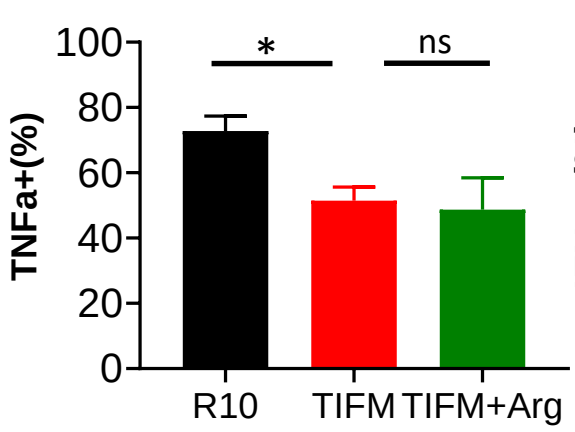


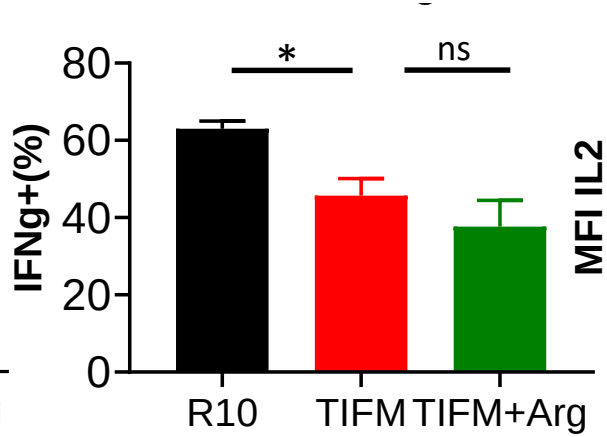


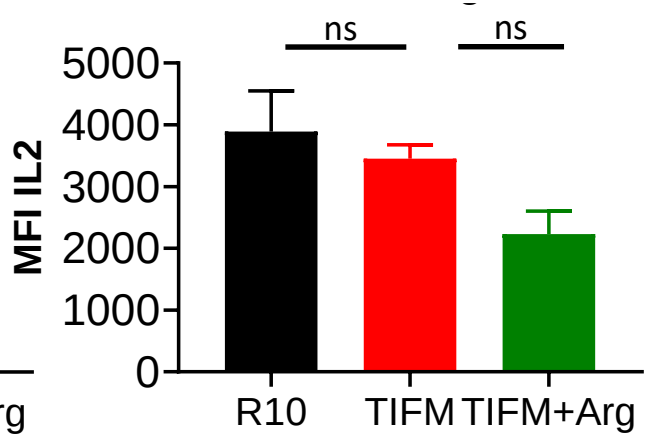
Arginine supplementation of TIFM fails to rescue cytokine production



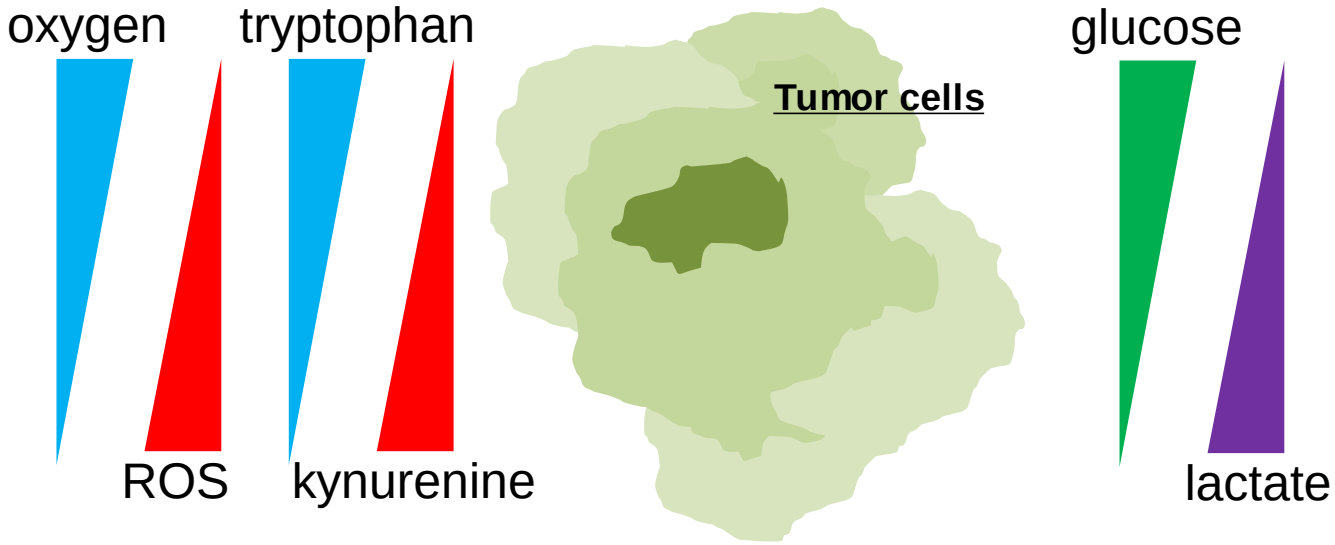






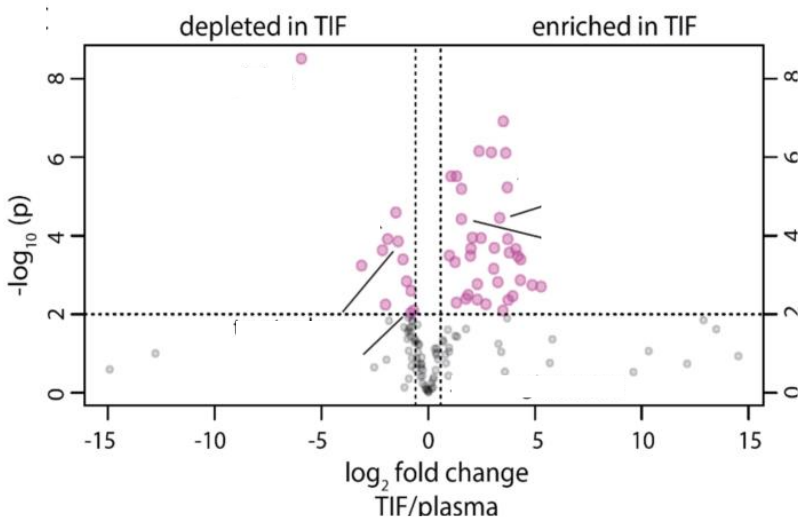


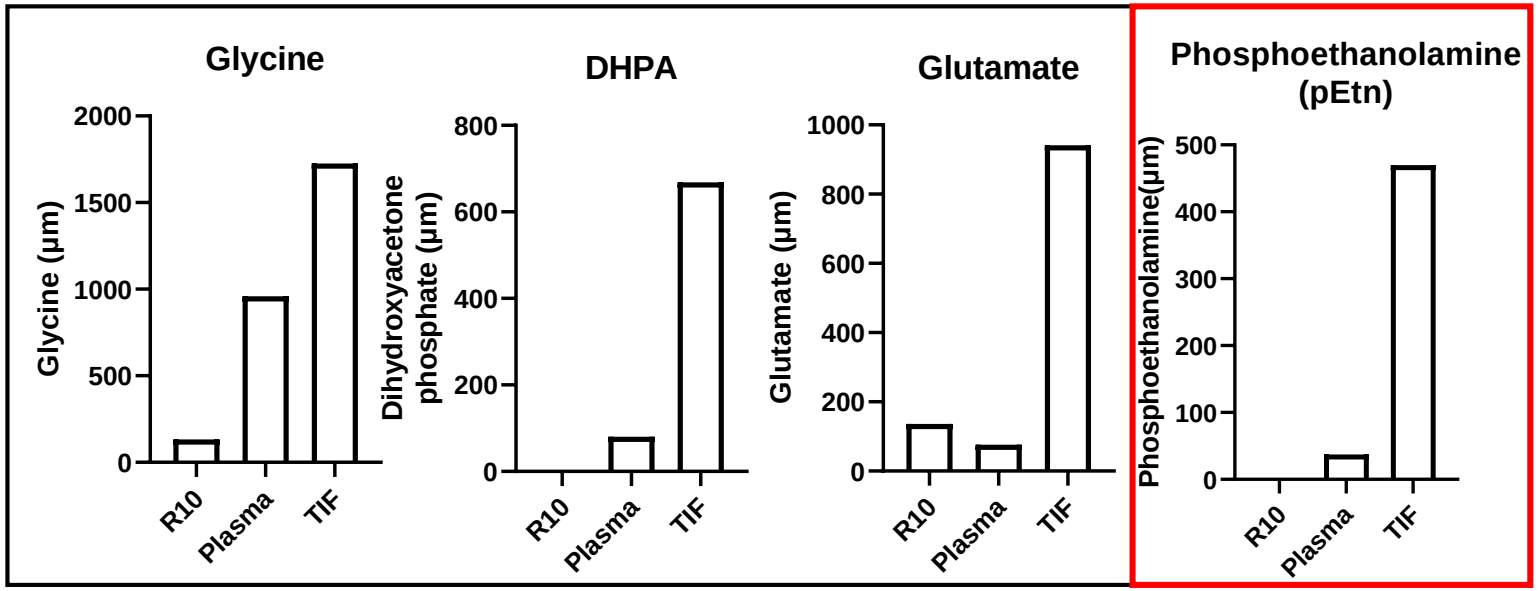
It’s not always about what’s missing…



- The immunometabolic ‘party line’ currently is all about a land grab: tumor cells and T cells are competing for the same nutrients
- A lesser appreciated aspect of the tumor microenvironment is the build-up of metabolic byproducts – as tumors are poorly perfused, these potentially toxic wastes can also act as dominant immunoregulatory molecules
- We are learning these metabolites may be the true culprit in immunoregulation within the TME and beyond…

Are there immunosuppressive metabolites present in TIF which *cause* dysfunction?

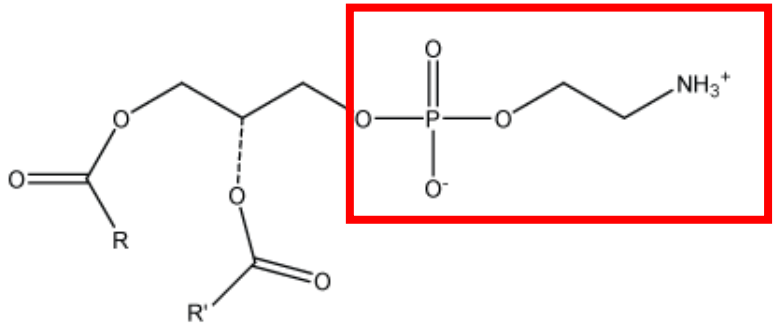




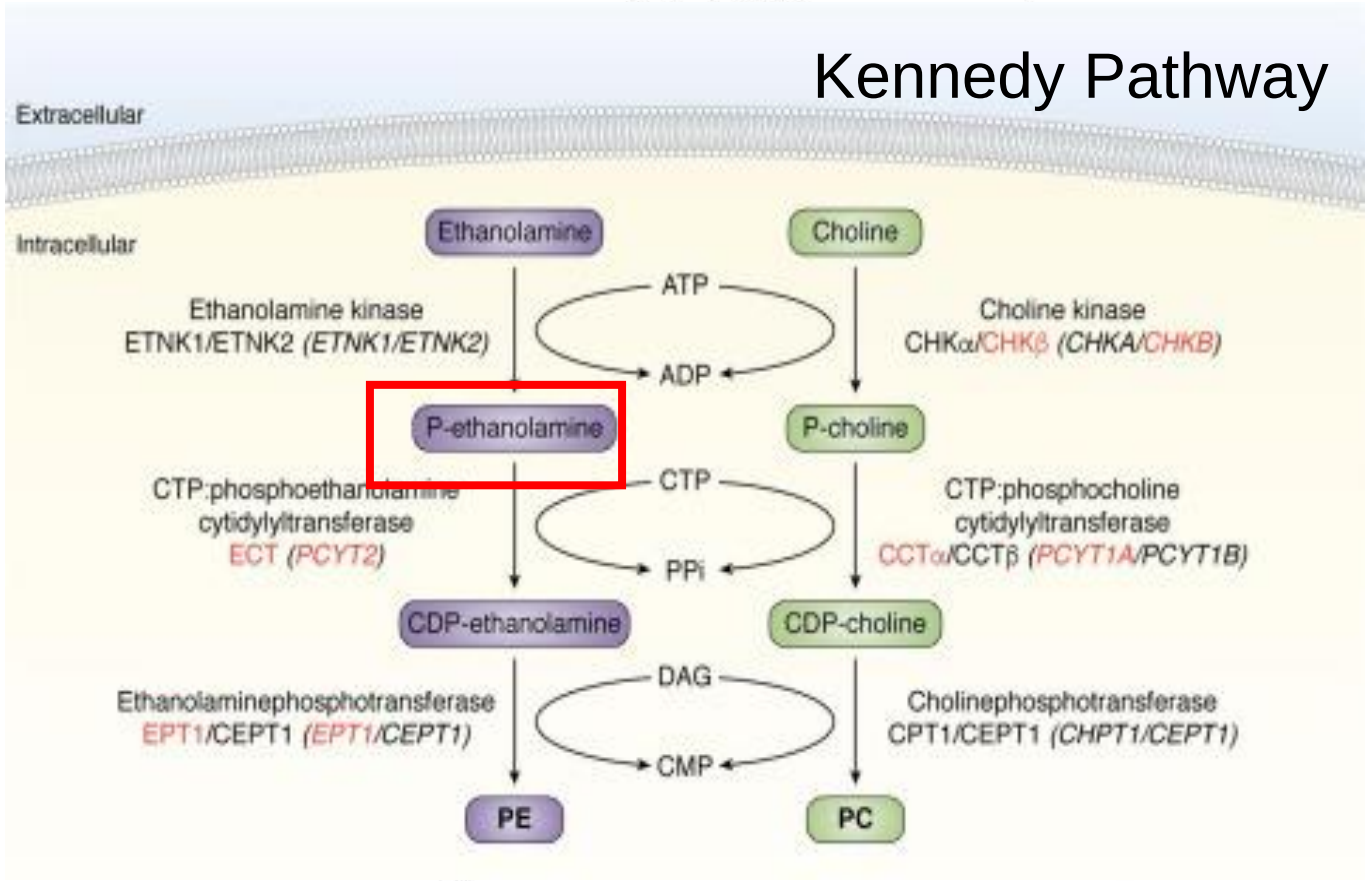
Phosphoethanolamine: a novel oncometabolite?

- pEtn is a commonly upregulated metabolite in TIFs
- pEtn is the head group of phosphatidylethanolamine, a membrane phospholipid
- PE is the second most abundant phospholipid in mammalian cells
  - PC/PE in mice (3:1)
  - PC/PE in flies (1:3)
- pEtn contributes to PE synthesis through the Kennedy pathway

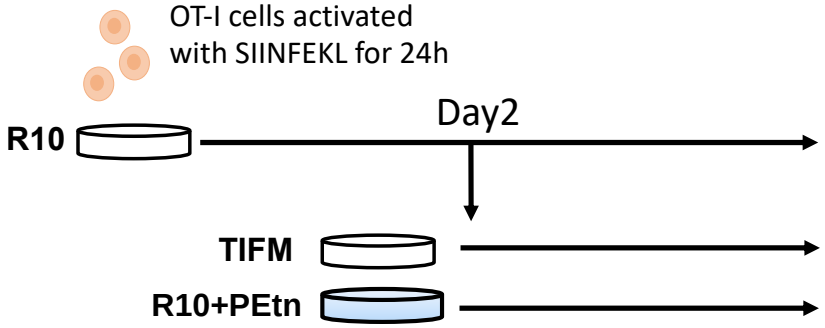
pEtn

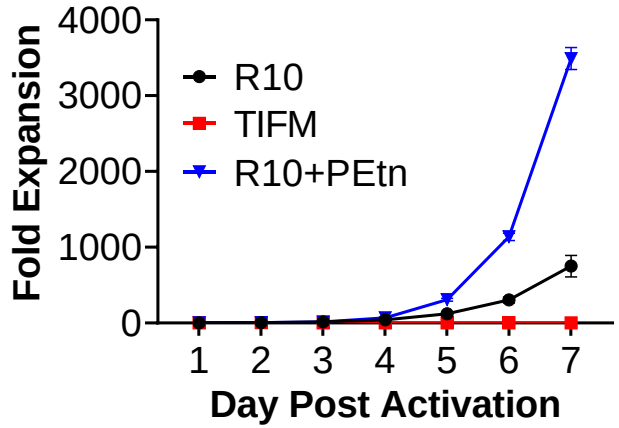


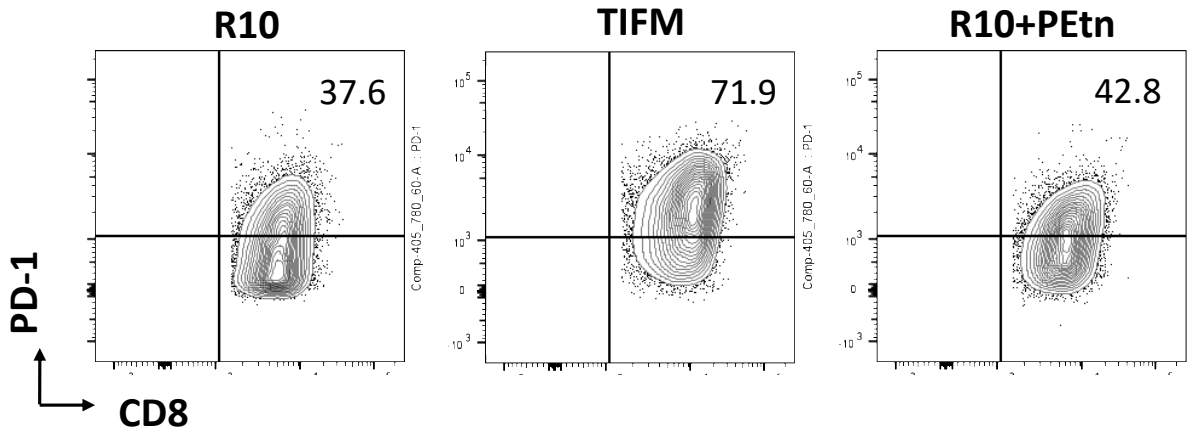
Phosphatidylethanolamine(PE)

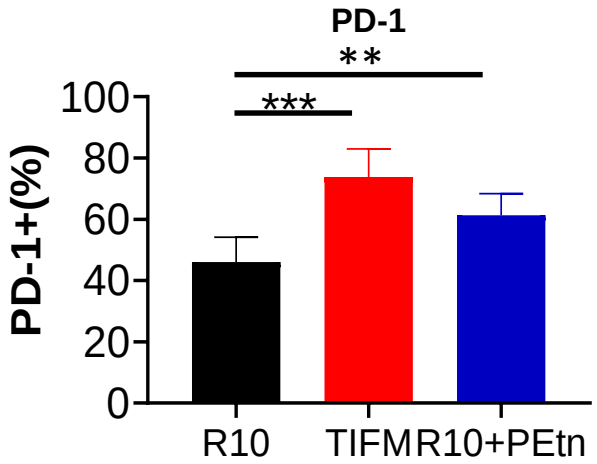


Phosphoethanolamine (pEtn) *supports* T cell proliferation

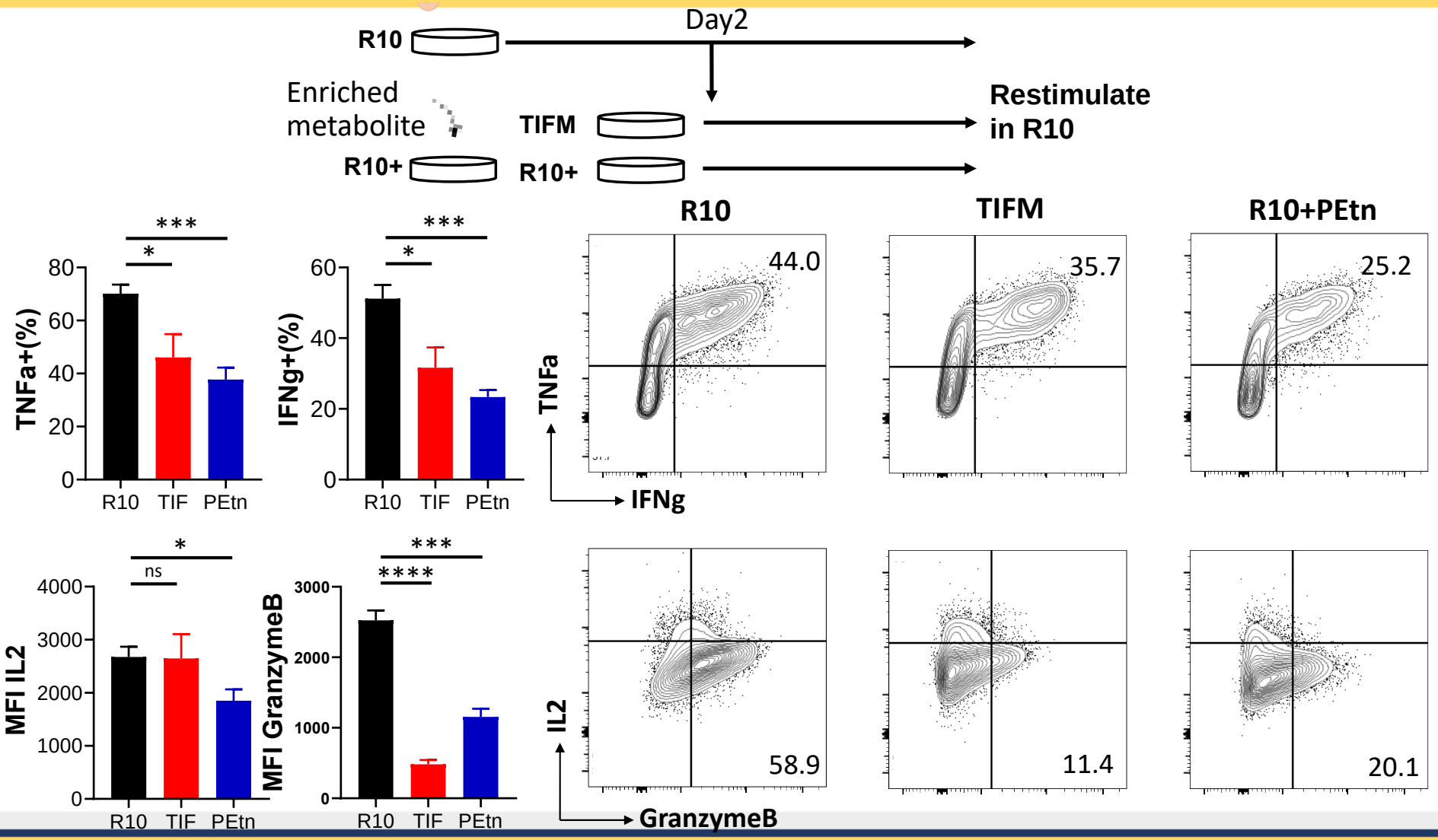




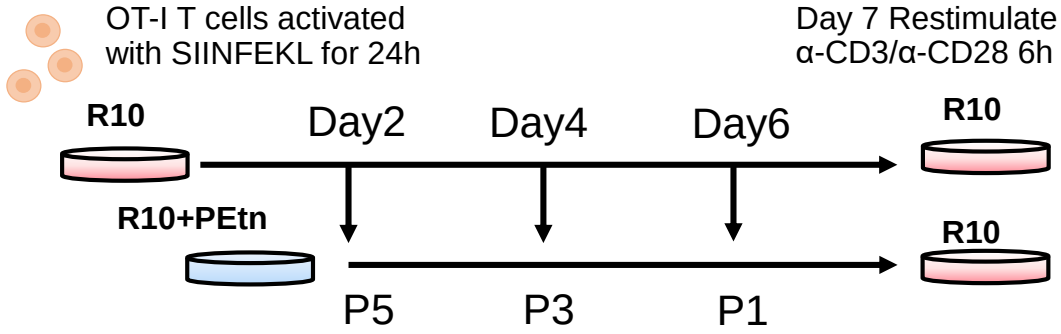


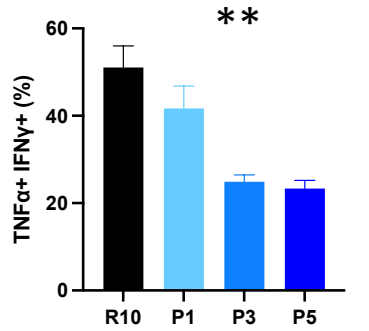


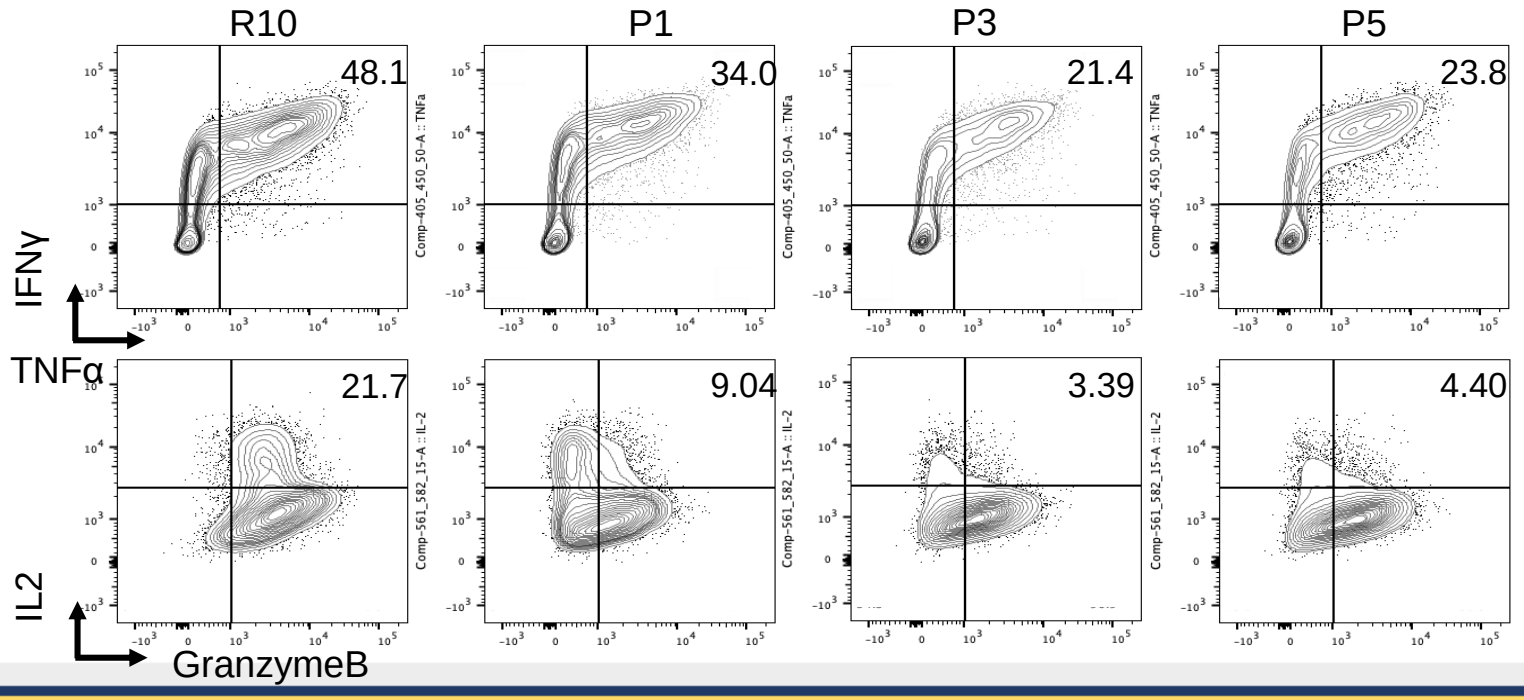
Despite stimulating proliferation, pEtn severely compromises CD8 T cell cytokine production

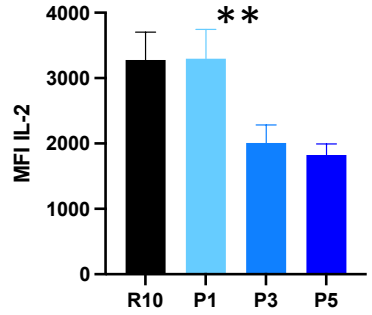


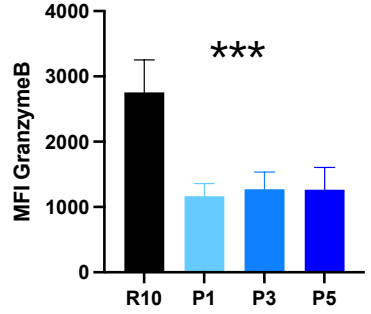
Exposure to pEtn for one day is enough to cause dysfunction





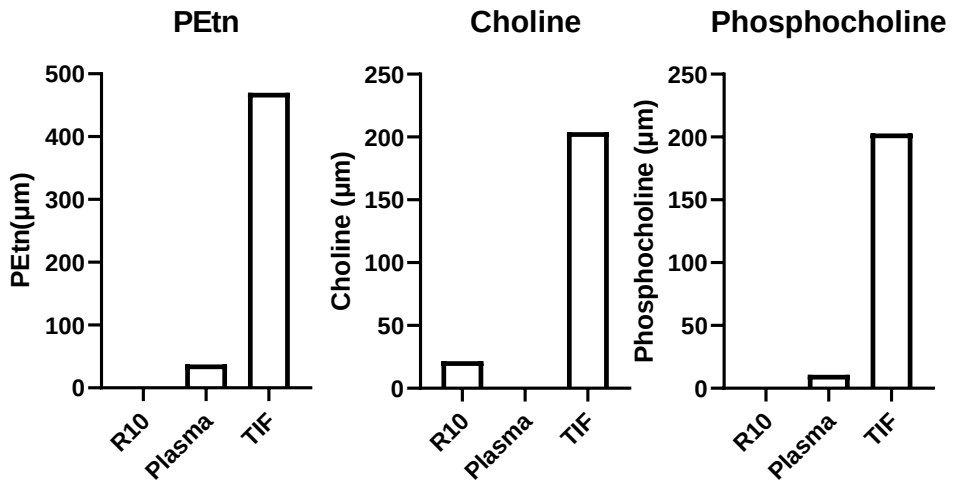






Is the effect driven by pEtn or the Kennedy pathway entirely?

- Choline and phosphocholine in the choline-arm of Kennedy pathway are also enriched in the TIF



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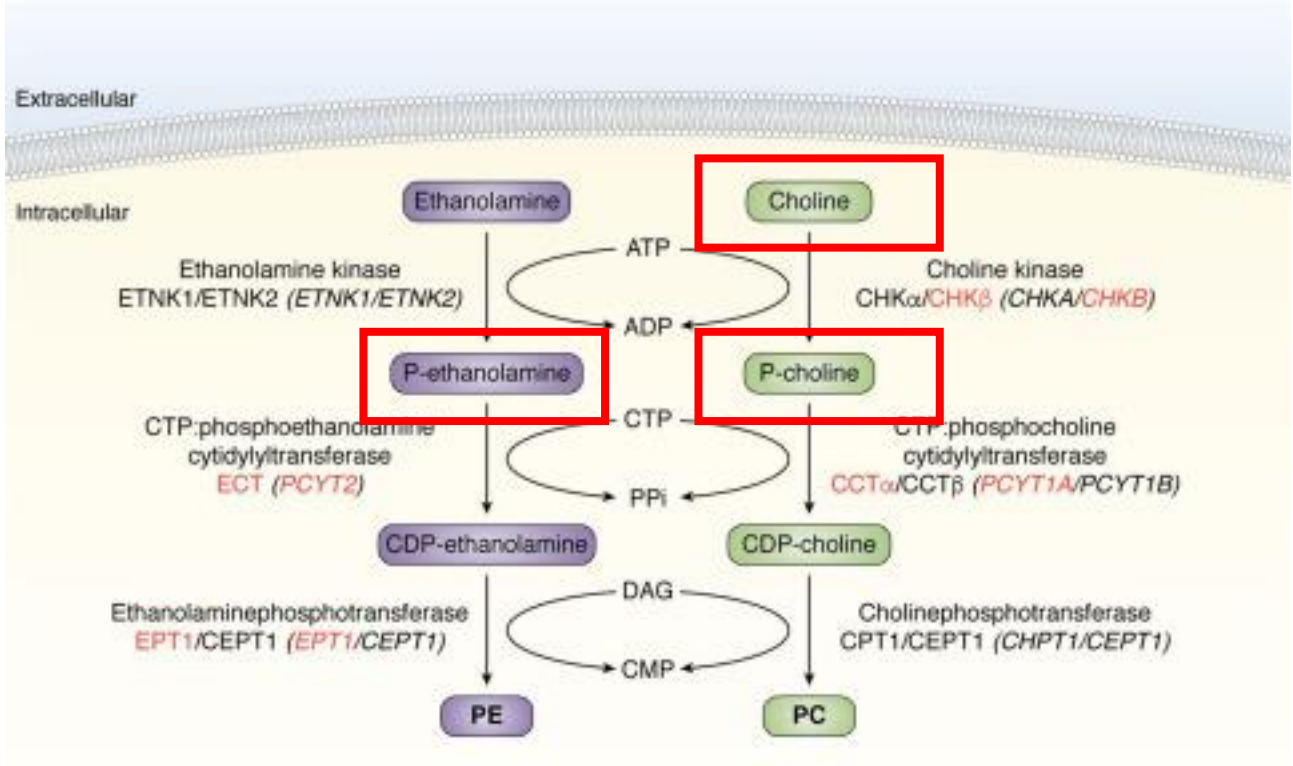
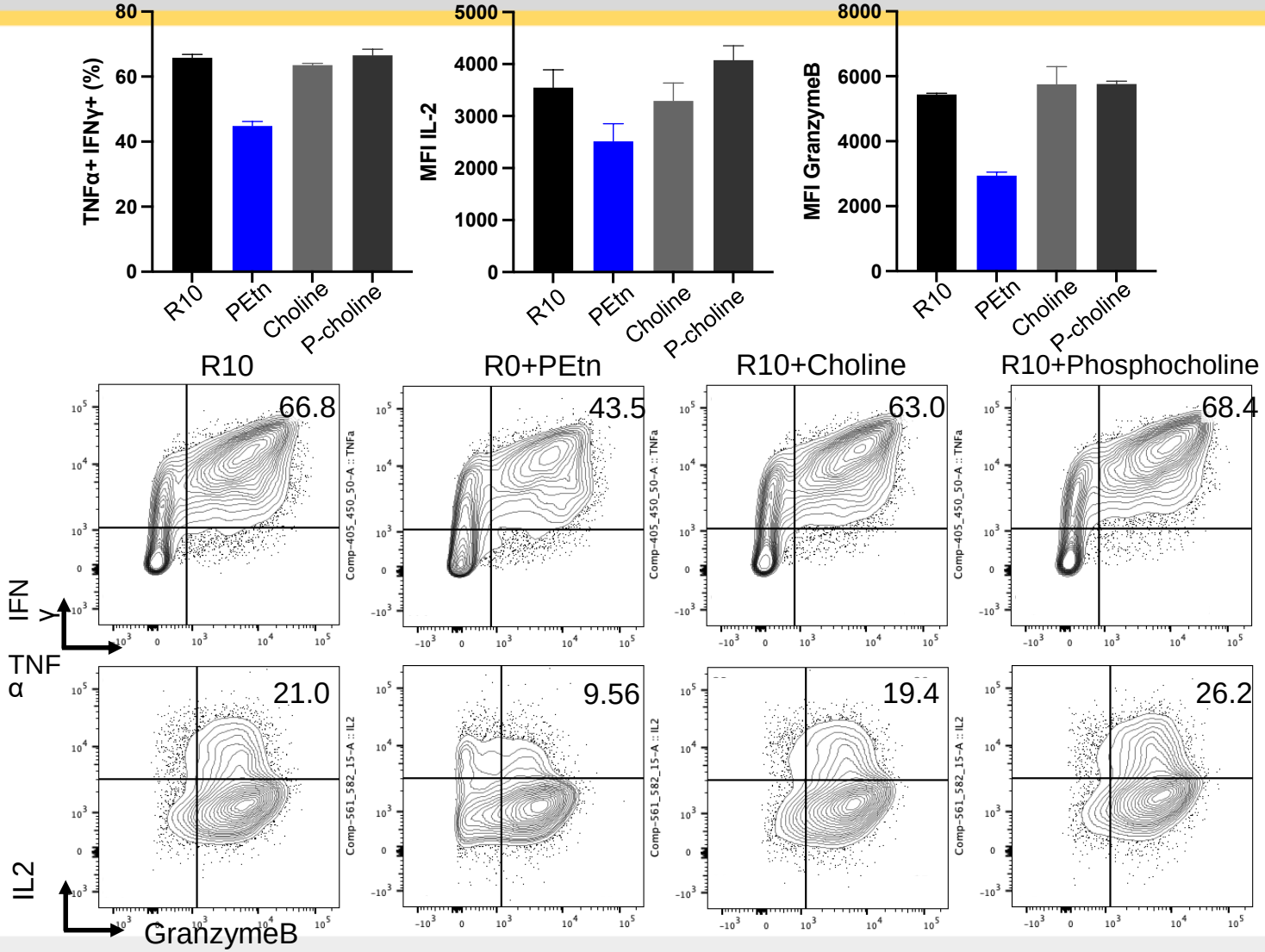


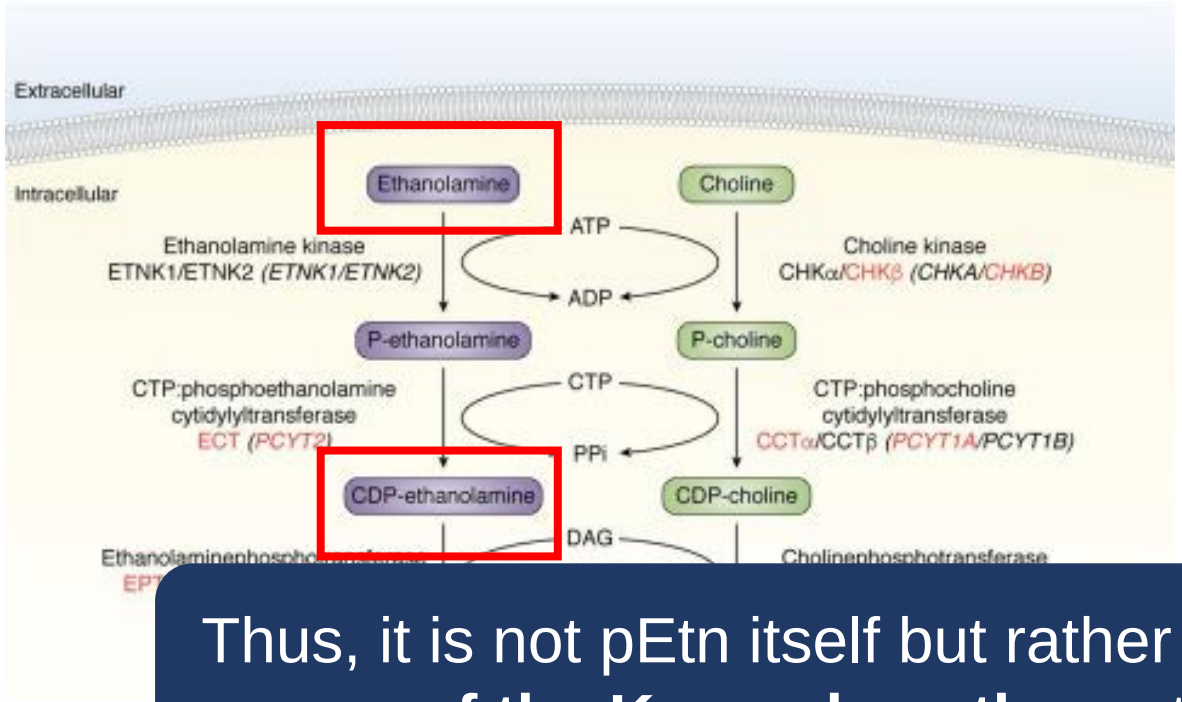
Phosphotidylcholine (PC) precursors do not cause CD8 T cell dysfunction

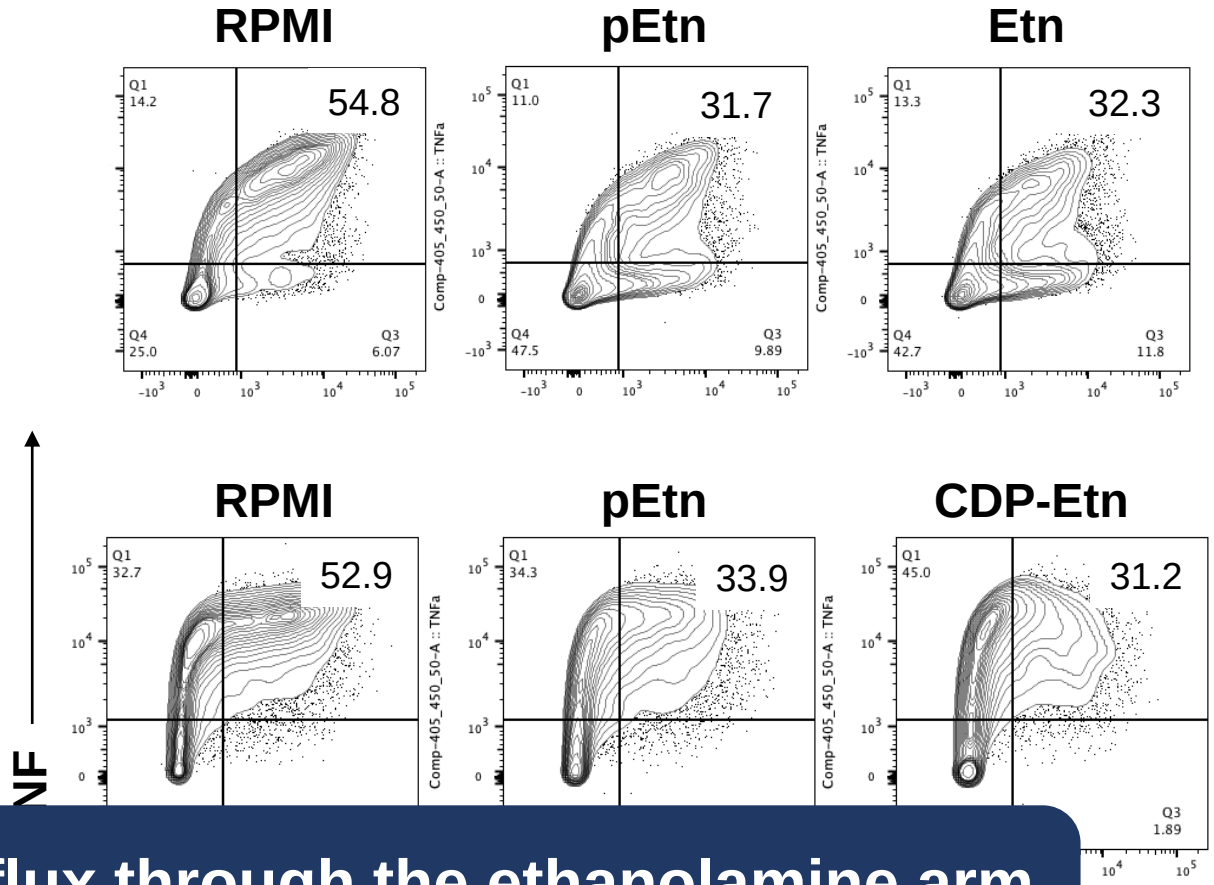
- Repeated experiments with RPMI media cultured with choline or phosphocholine
- The PC arm of the Kennedy pathway **does not** induce T cell dysfunction



Both precursor and product of pEtn metabolism can induce T cell dysfunction

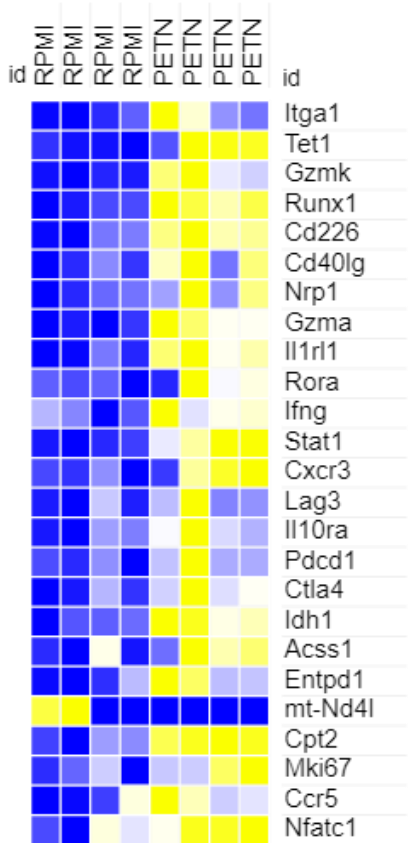
- Our data highlight the importance of the **ethanolamine** arm of the Kennedy pathway
- While Etn and CDP-Etn are **not enriched in TIF**, we asked if a similar treatment regimen would induce T cell dysfunction

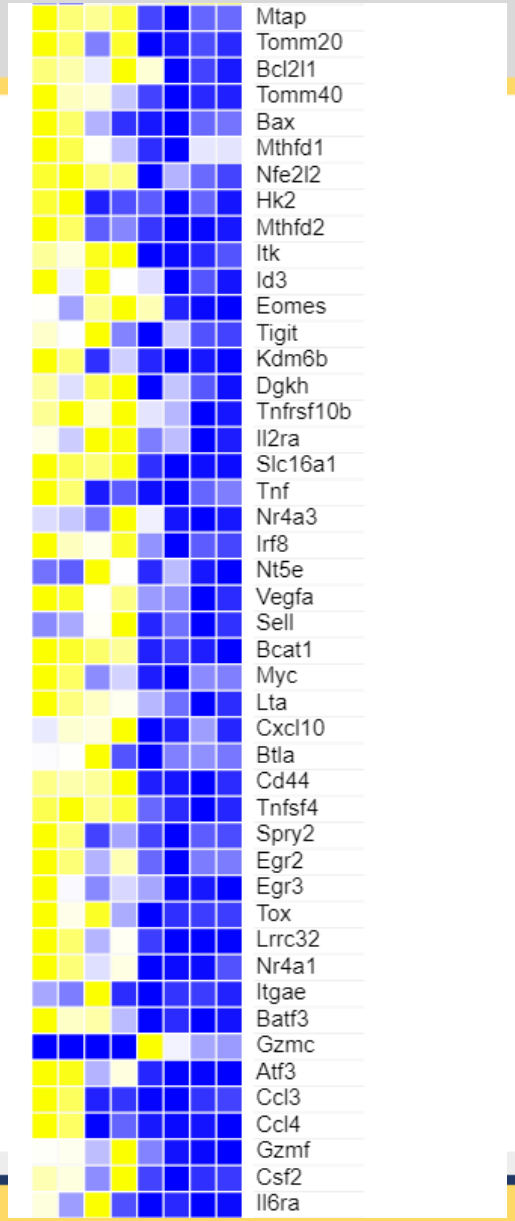




Thus, it is not pEtn itself but rather **flux through the ethanolamine arm of the Kennedy pathway** that induces T cell dysfunction

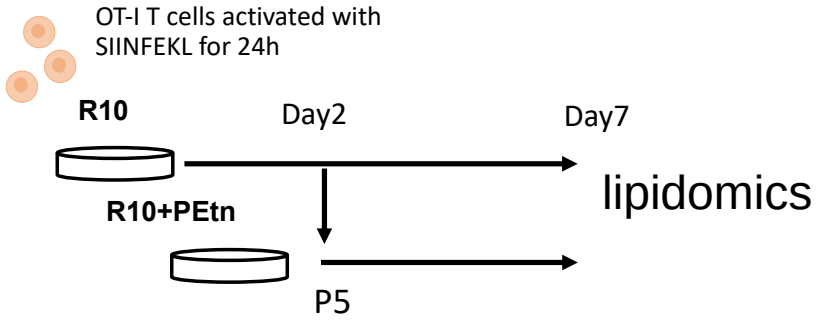
RNAseq identifies some interesting immune or metabolic hits, but no smoking gun

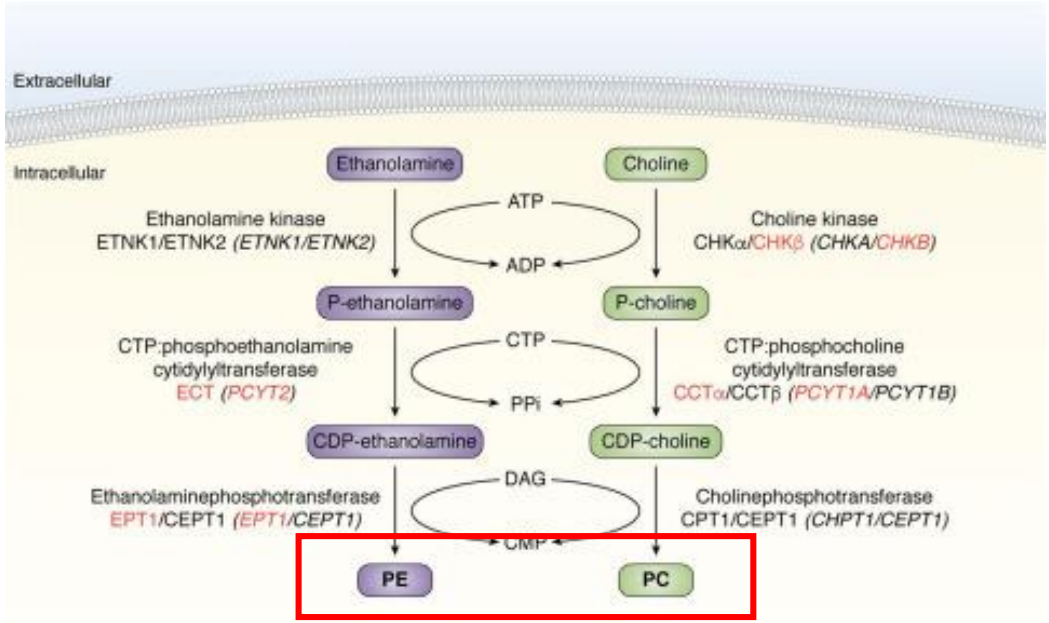


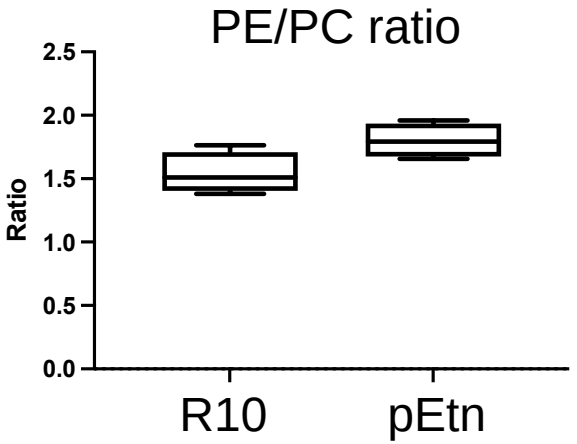


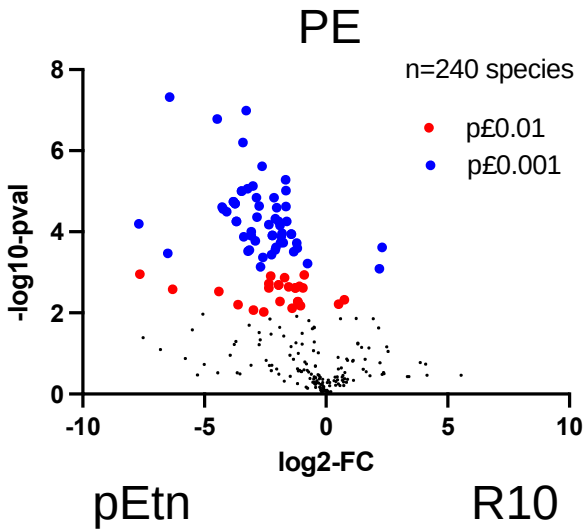
- Performed RNAseq on RPMI or pEtn-expanded T cells
- While these cells had some hints of a dysfunctional status (*Pdcd1, Lag3, Tigit*), the data were certainly not consistent with exhaustion or anergy
- No strong (any?) hits with GSEA/GO analysis
- We had to go beyond transcriptomes to understand this cell-intrinsic immune regulation

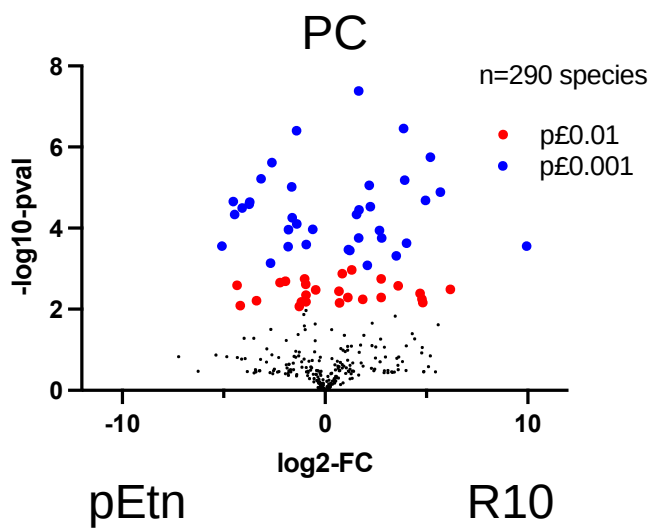
Phosphoethanolamine supplementation dramatically shifts the PE/PC balance of T cells



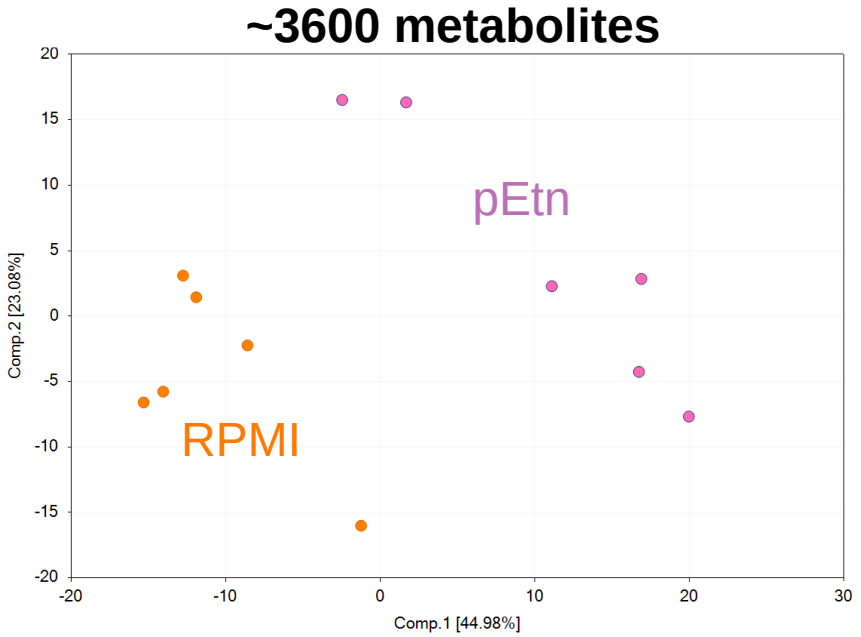




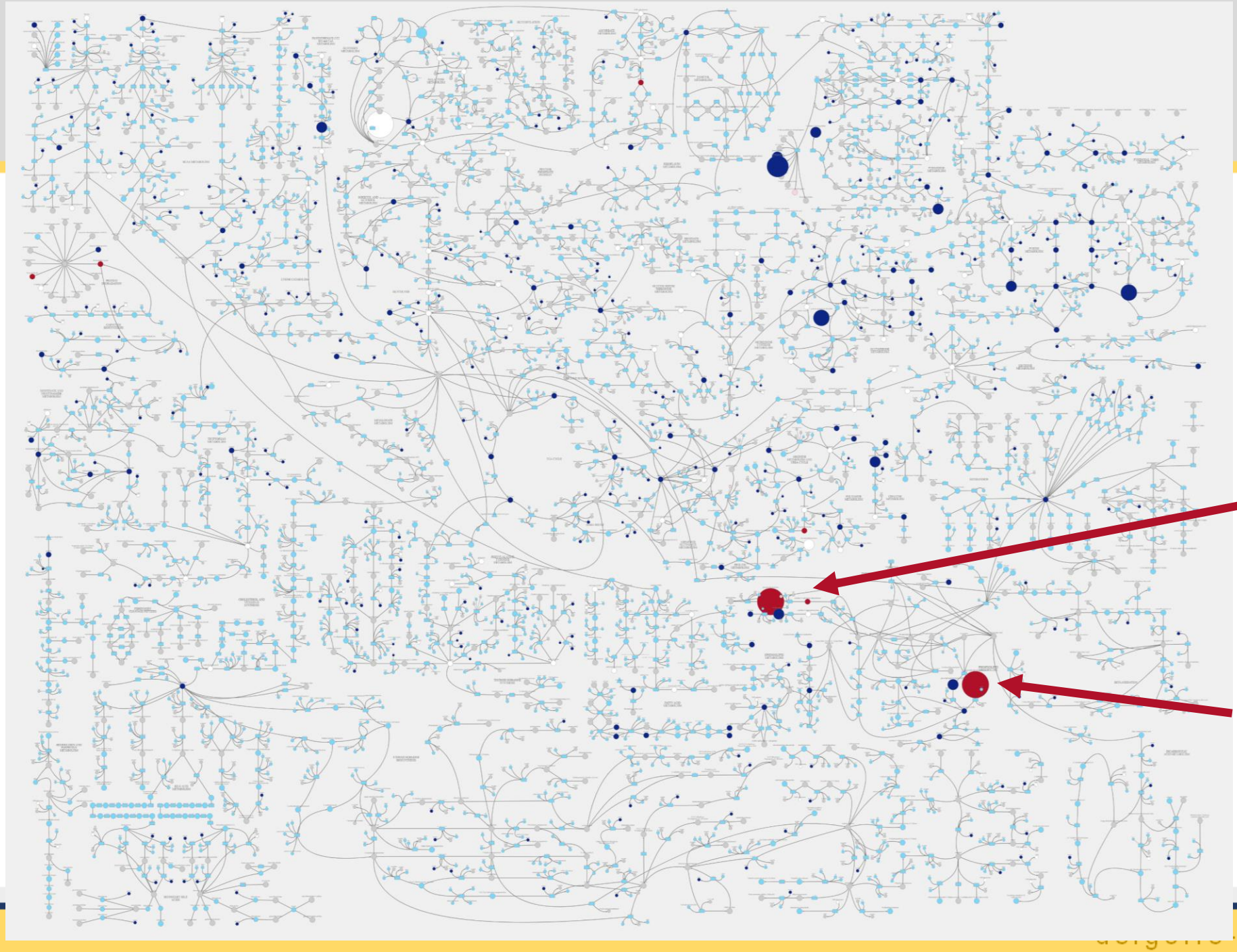




pEtn treatment shifts the entire metabolome of T cells

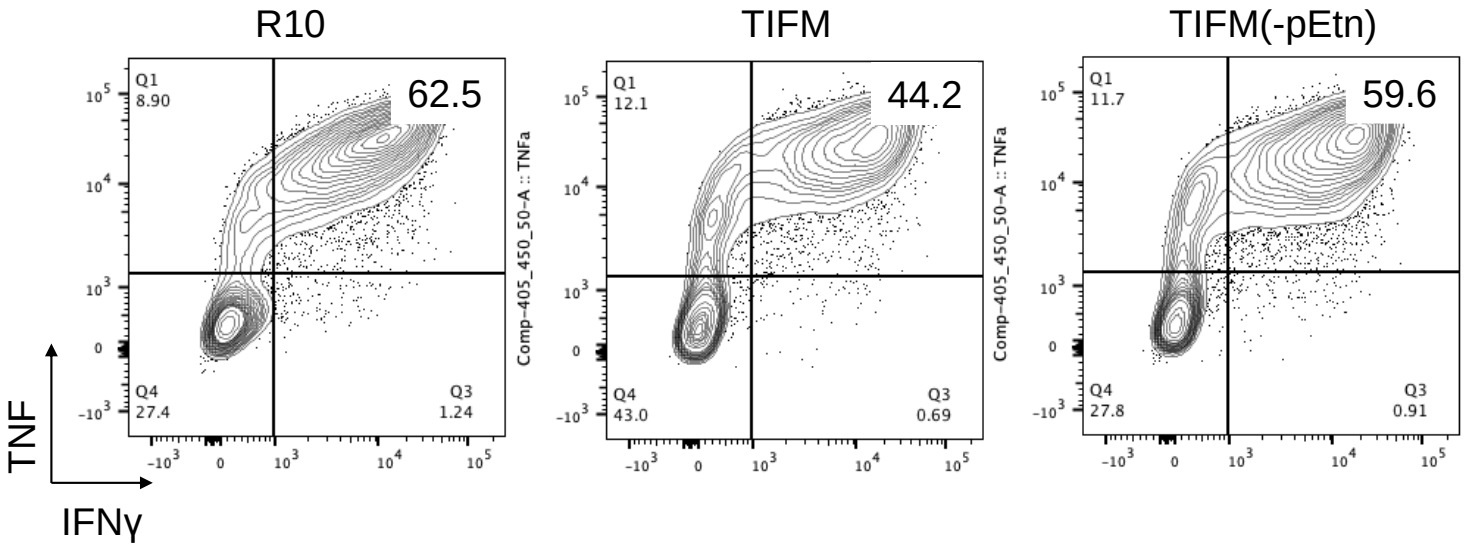


- Global metabolomics profiling revealed pEtn supplementation drove massive ethanolamine flux into membrane and second messenger phospholipids
- The result was a striking reprogramming of the cells' carbohydrate metabolism, and suppression of mitochondrial biogenesis, which we've previously associated with T cell dysfunction

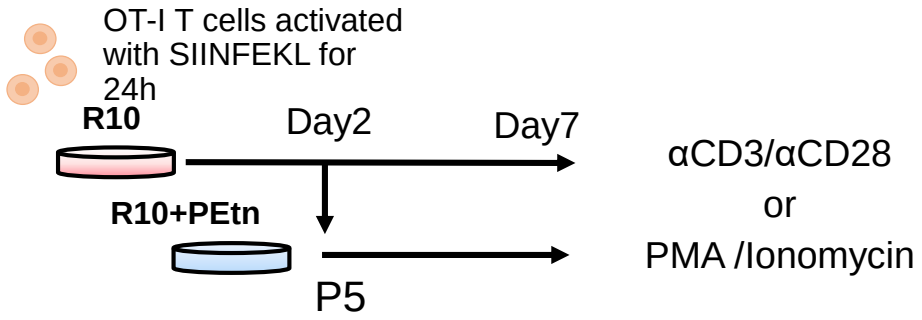

pEtn
Glycerolphosphoethanolamine

How much of the “TIFM” phenotype is pEtn?

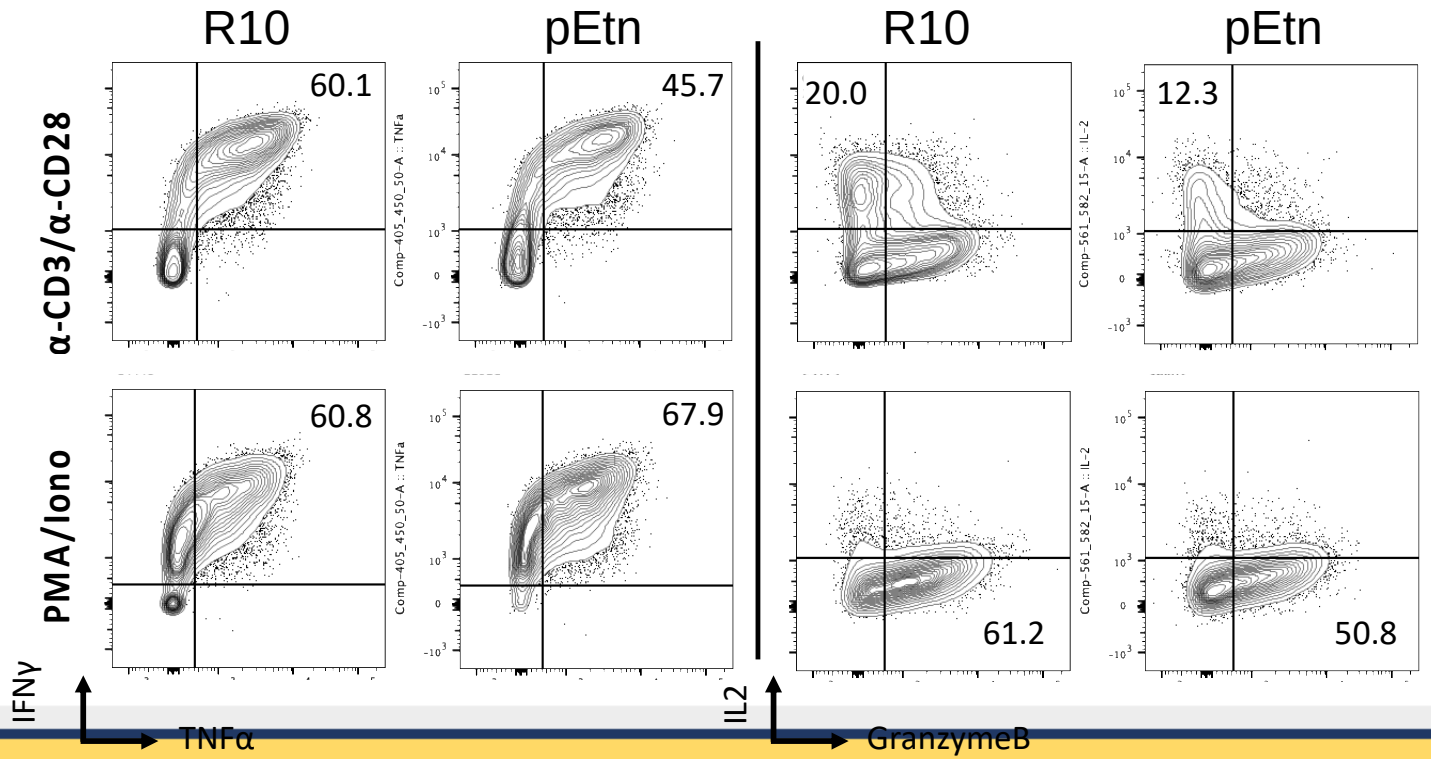
- Formulated TIFM **without** pEtn
- T cells cultured in TIFM still possessed a proliferative defect
- However, T cells cultured in TIFM retain the cytokine production of control cells
- Not a complete rescue, so lots left to do!

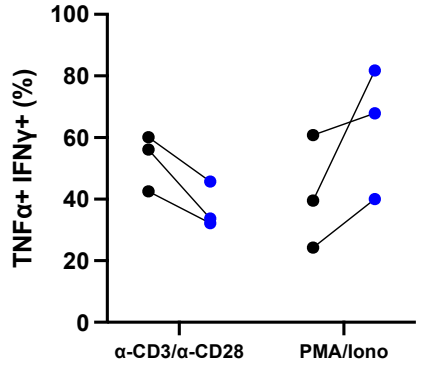


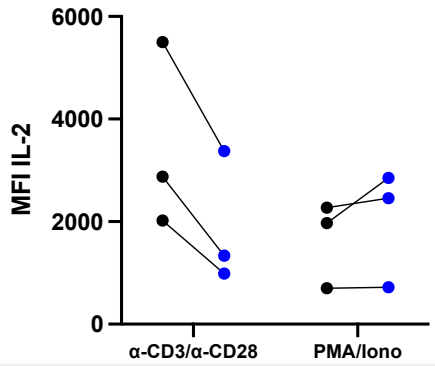
The dysfunction induced by pEtn culture is at the level of TCR and/or CD28 signaling

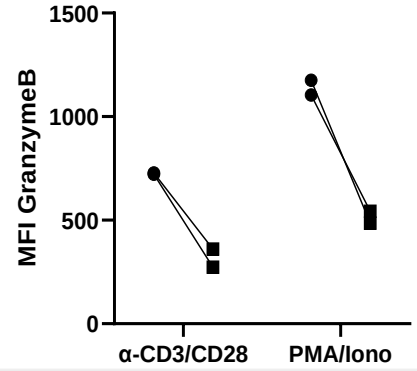


- CD8 T cells cultured in R10+PEtn have lower cytokine production when restimulated with α-CD3/α-CD28
- However, pEtn-cultured T cells have **no cytokine defects** when activated chemically with PMA+ionomycin



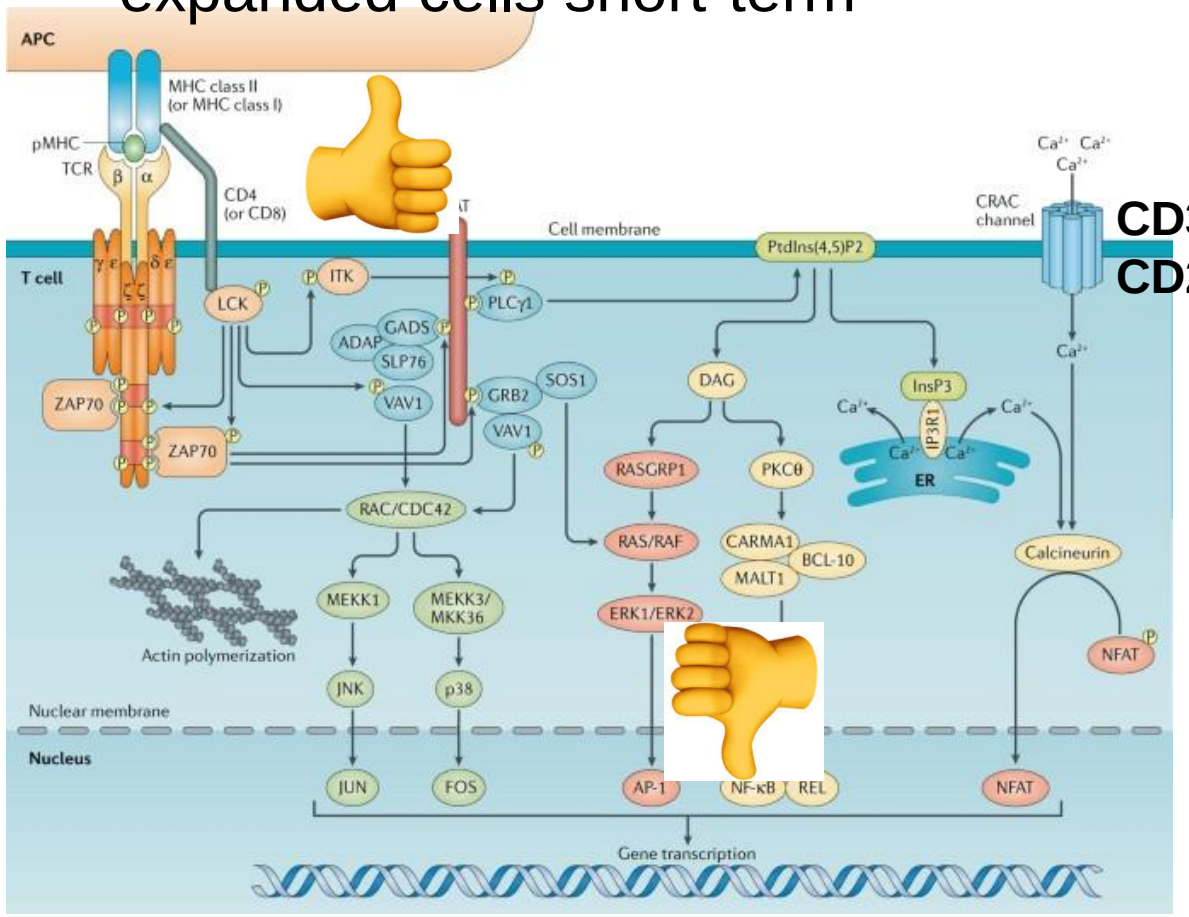


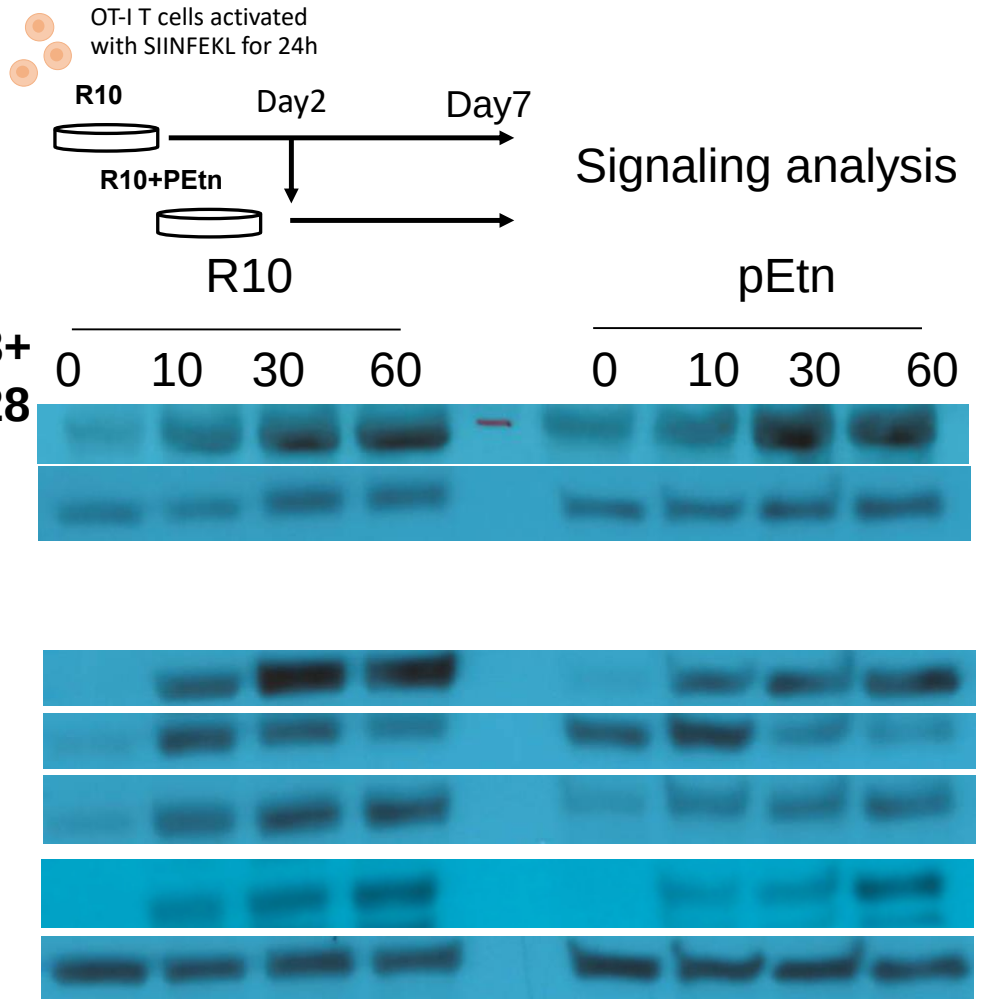




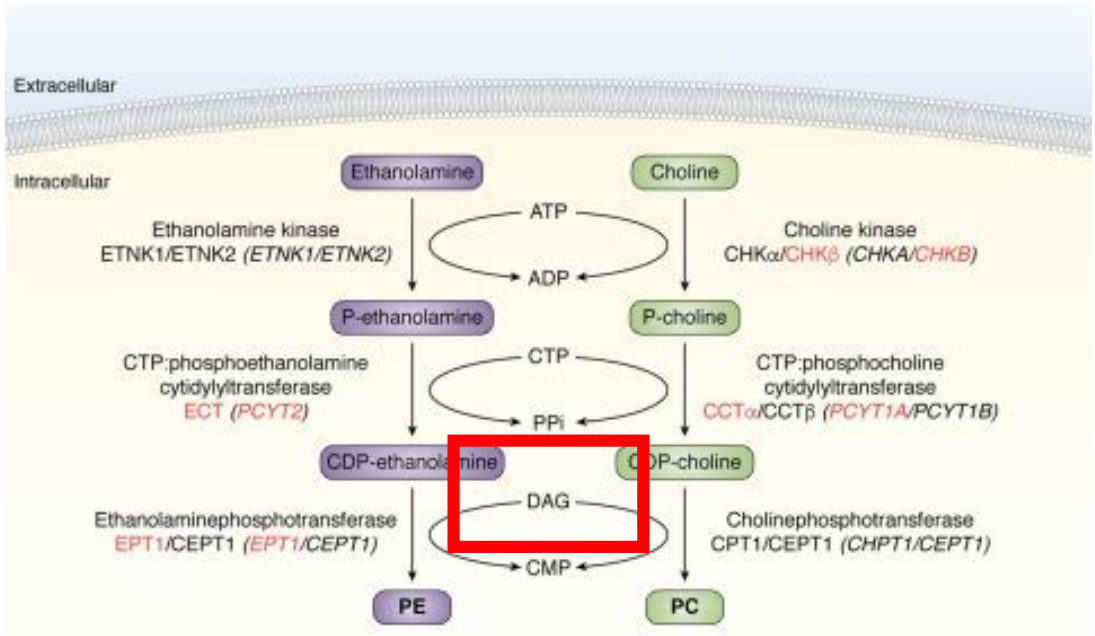
Do pEtn treated cells have defects in TCR signaling?

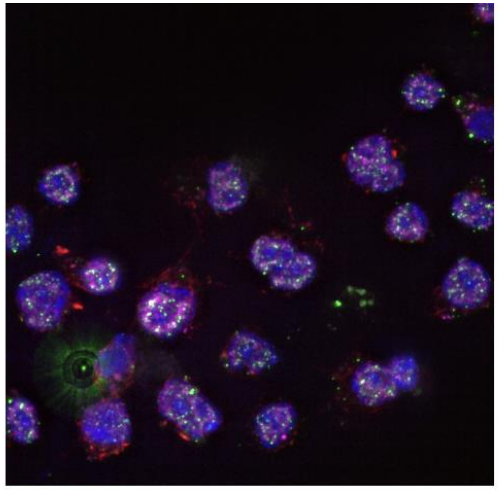
- Restimulated RPMI or pEtn expanded cells short-term



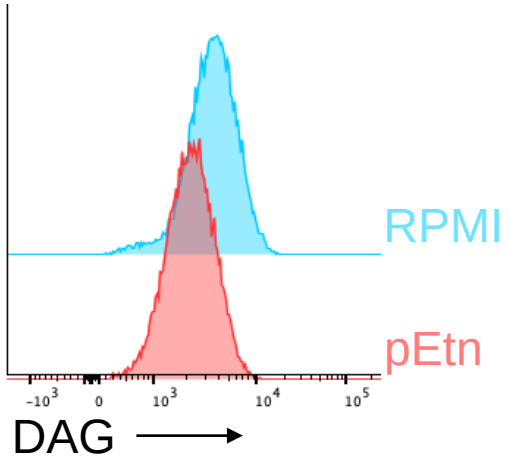


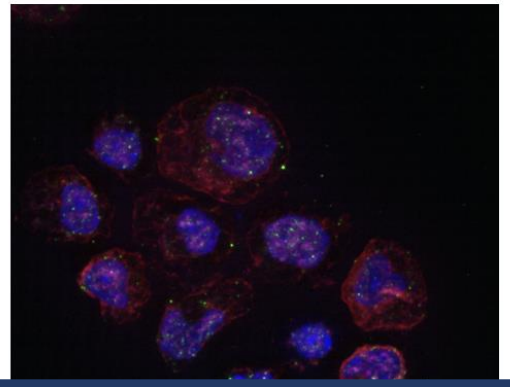
How does pEtn flux reduce distal but not proximal TCR signaling?





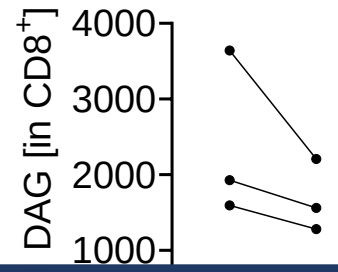
RPMI





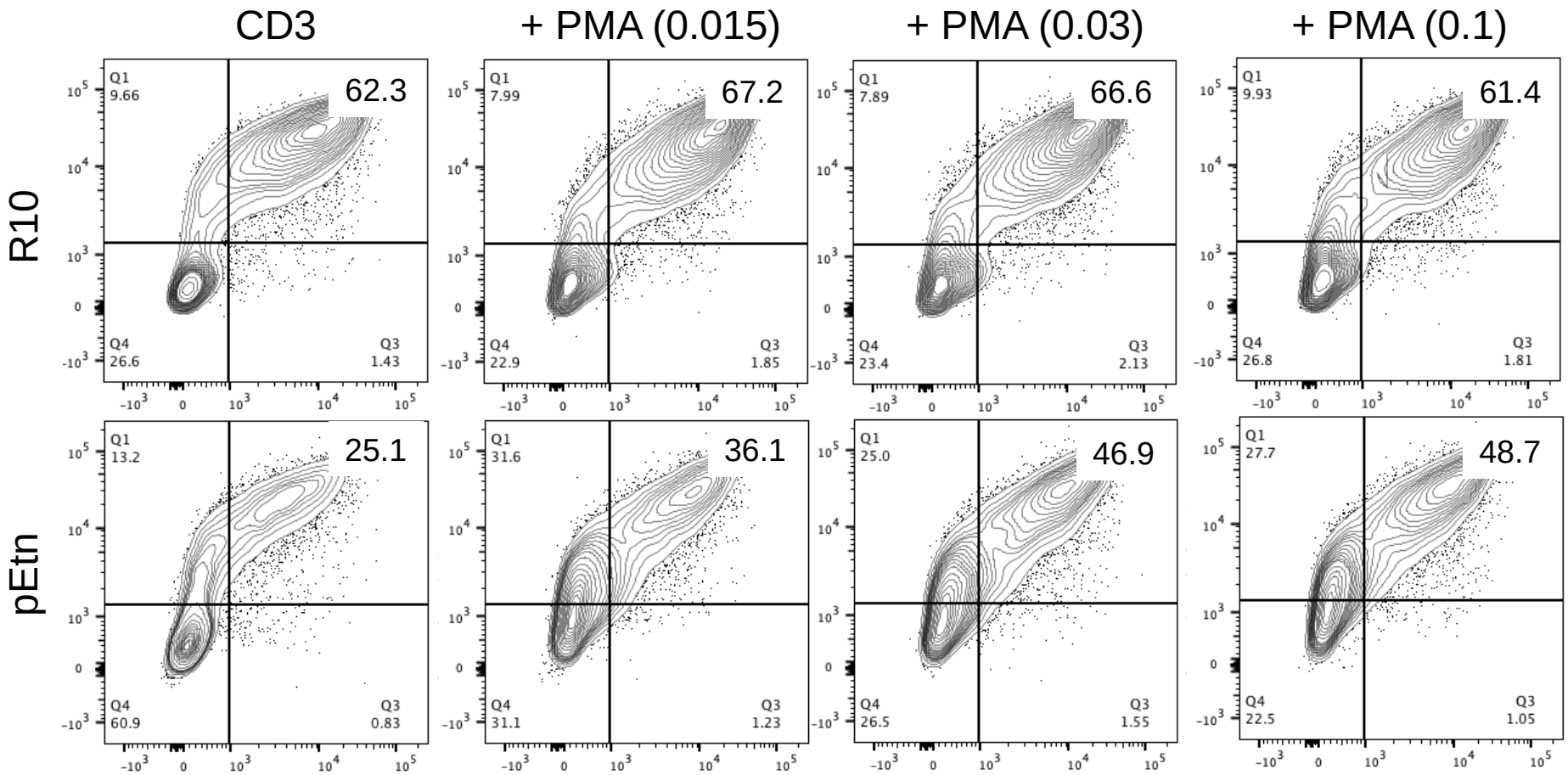
pEtn

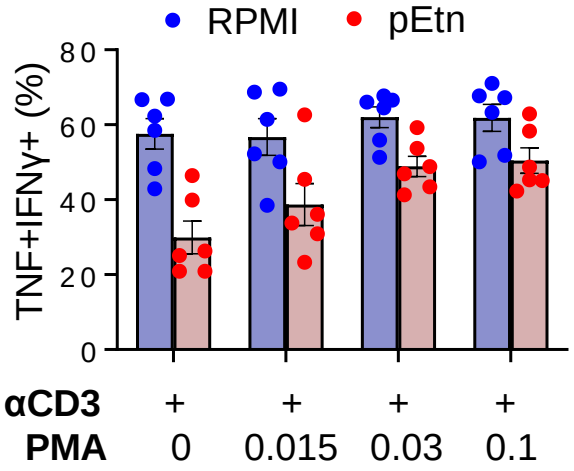
DAPI
Phalloidin



Heightened flux through the Kennedy pathway depletes DAG, a critical second messenger!

Restoration of DAG can rescue pEtn-induced T cell dysfunction





Conclusions

- Media based on tumor interstitial fluid (TIF) allows for a direct view of tumor microenvironment metabolism's role on T cell function and fate
- TIFM induces persistent, heritable dysfunction in CD8+ T cells
- TIFM confirmed the importance of arginine in T cell proliferation, but not T cell dysfunction
- TIFM identified a novel oncometabolite, **phosphoethanolamine**, which drives flux through the ethanolamine arm of the Kennedy pathway, producing PEs
- pEtn treatment paradoxically drives T cell proliferation but inhibits cytokine production
- pEtn induces a distinct form of T cell dysfunction induced through amplified (pathologic?) metabolic flux that depletes critical second messengers, like DAG

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