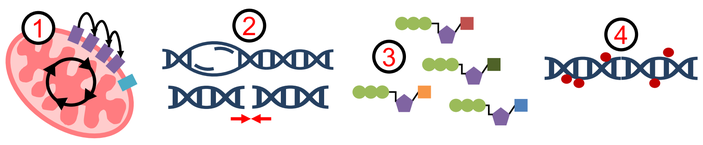
Iron deficient CD8+ T-cells display specific metabolic impairments and sensitivity to aspartate supplementation

Megan Teh[1], Joe Frost[1], Linda Sinclair[2], Nancy Gudgeons[3], Bryan Marzullo[3], Jennie Roberts[3], Christopher Millington[1], Sarah Dimeloe[3], Jan Rehwinkel[1], Andrew Armitage[1], Hal Drakesmith[1]

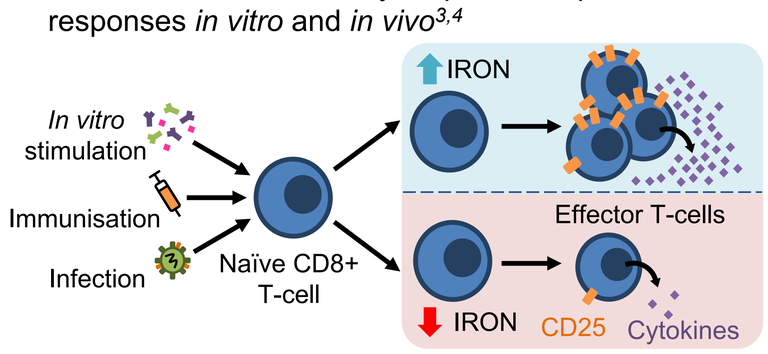
[1]MRC Weatherall Institute of Molecular Medicine, University of Oxford, UK [2]Cell Signalling and Immunology, University of Dundee, UK [3]Institute of Immunology and Immunotherapy, University of Birmingham, UK

Introduction

- Iron is an essential nutrient used for cellular processes including[1]:
  1. Mitochondrial metabolism
  2. DNA replication and repair
  3. dNTP synthesis
  4. Epigenetic modification



- Iron deficiency has widespread global impact, affecting >1.2 billion people[2]
- Reduced iron availability impairs adaptive immune responses *in vitro* and *in vivo*[3,4]



Question: How does iron deficiency affect T-cell biochemistry?

Methods

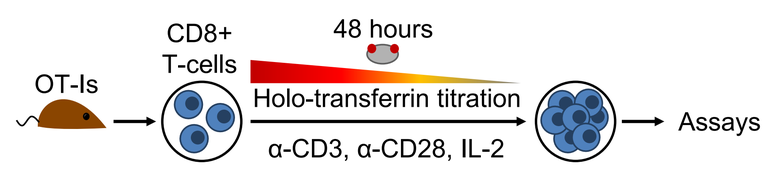


Figure 1. T-cells were cultured for 48h on plates coated with 5 µg/mL α-CD3 in iron free media (RPMI 1640, 10% panexin iron free serum substitute, 1% penicillin/streptomycin, 1% glutamine) with 1 µg/mL α-CD28, 50 U/mL IL-2, 50 µM β-mercaptoethanol and a titration of holotransferrin as the iron source.

Iron starved T-cells have expression profiles indicative of cell cycle arrest and of metabolic rewiring

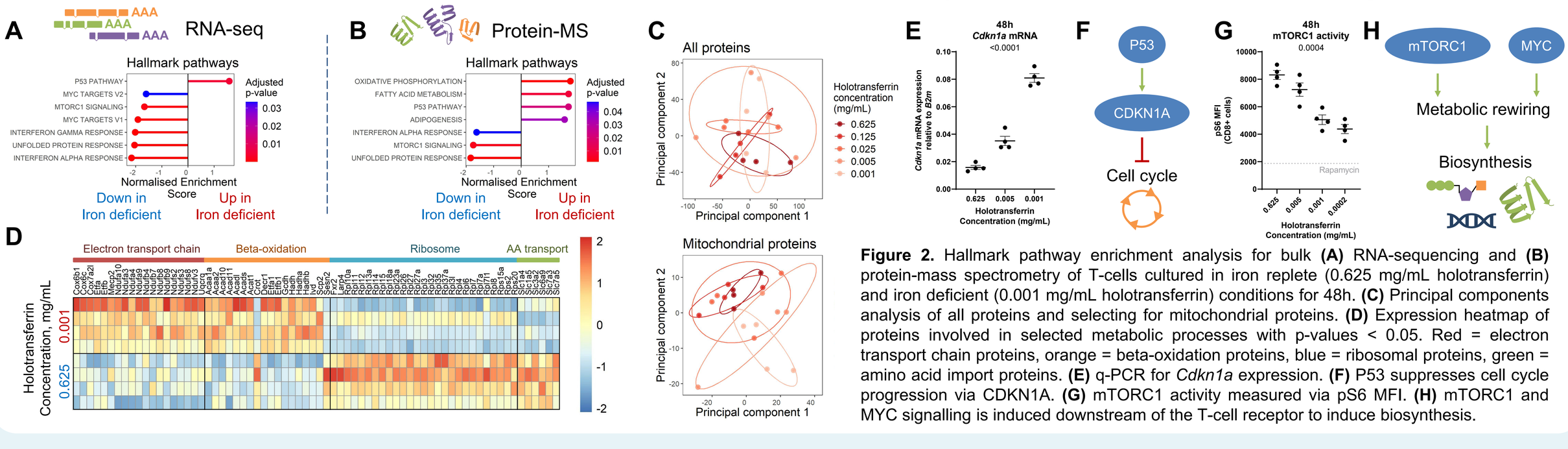


Figure 2. Hallmark pathway enrichment analysis for bulk **(A)** RNA-sequencing and **(B)** protein-mass spectrometry of T-cells cultured in iron replete (0.625 mg/mL holotransferrin) and iron deficient (0.001 mg/mL holotransferrin) conditions for 48h. **(C)** Principal components analysis of all proteins and selecting for mitochondrial proteins. **(D)** Expression heatmap of proteins involved in selected metabolic processes with p-values < 0.05. Red = electron transport chain proteins, orange = beta-oxidation proteins, blue = ribosomal proteins, green = amino acid import proteins. **(E)** q-PCR for *Cdkn1a* expression. **(F)** P53 suppresses cell cycle progression via CDKN1A. **(G)** mTORC1 activity measured via pS6 MFI. **(H)** mTORC1 and MYC signalling is induced downstream of the T-cell receptor to induce biosynthesis.

Iron deprivation alters metabolic routing

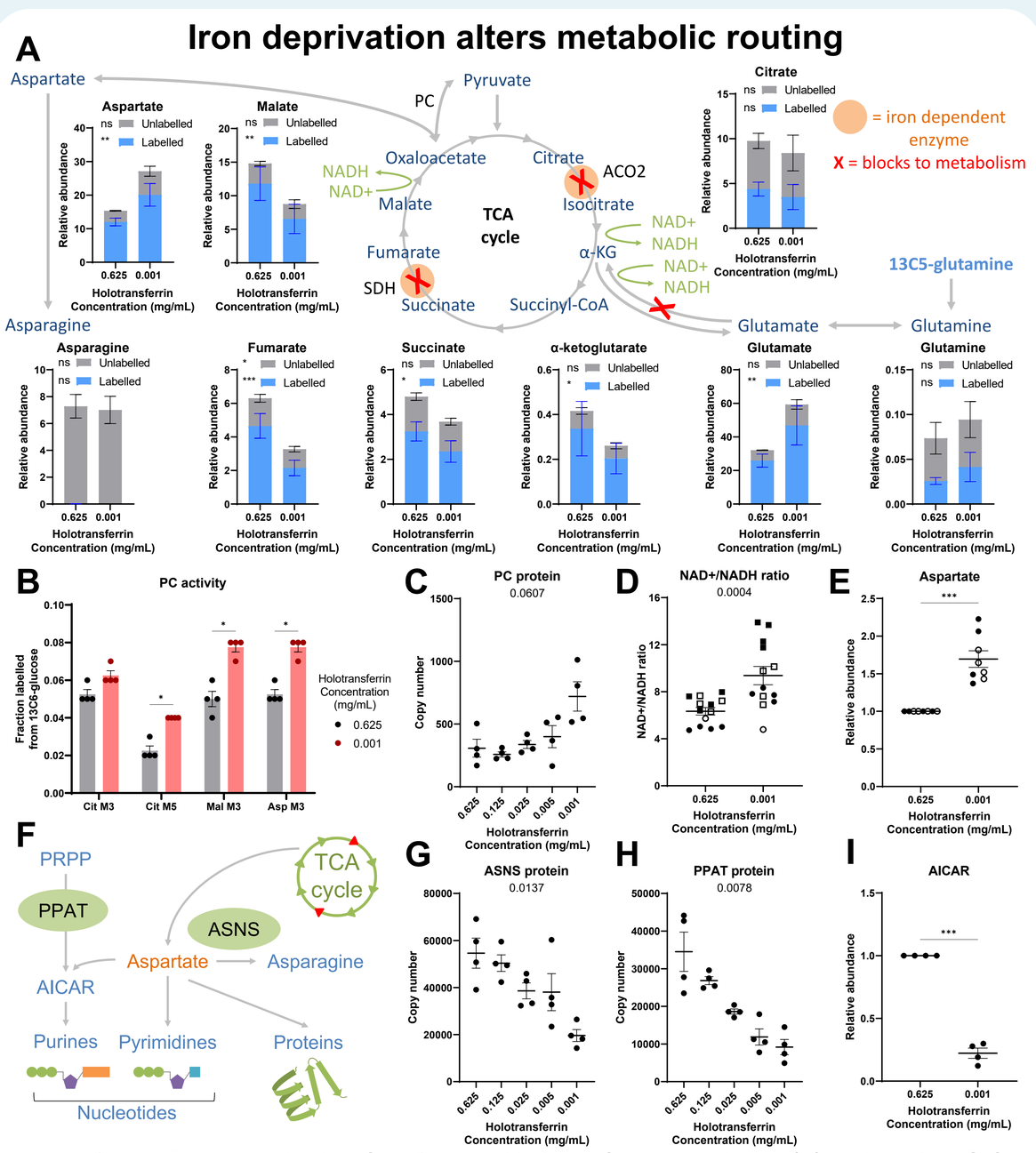


Figure 3. T-cells were activated for 48h in a titration of iron conditions. **(A)** Metabolite-MS for labelled metabolites following 24h pulse with 13C5-glutamine. **(B)** Metabolite isotopomer distribution for labelled metabolites characteristic of pyruvate carboxylase (PC) activity following 24h pulse with 13C6-glucose. **(C)** PC protein expression, **(D)** NAD+/NADH ratio and **(E)** aspartate relative abundance. **(F)** TCA cycle produced aspartate is used in nucleotide, asparagine and protein synthesis. **(G)** ASNS and **(H)** PPAT protein expression. **(I)** AICAR relative expression.

Iron scarcity impairs histone demethylation of H3K27me3

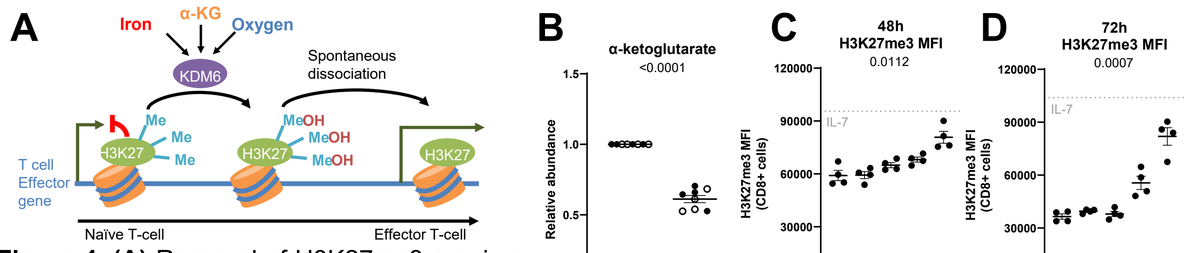


Figure 4. (A) Removal of H3K27me3 requires iron and α-KG dependent KDM6 enzymes. **(B)** α-KG relative abundance and H3K27me3 MFI at **(C)** 48h and **(D)** 72h.

Aspartate supplementation or nucleotide accumulation via SAMHD1-KO partly rescues iron deficient T-cells

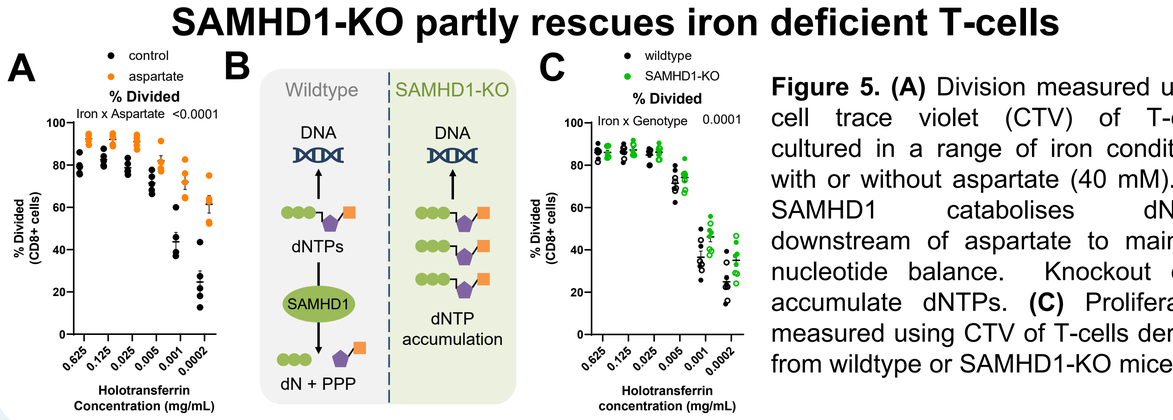


Figure 5. (A) Division measured using cell trace violet (CTV) of T-cells cultured in a range of iron conditions with or without aspartate (40 mM). **(B)** SAMHD1 catabolises dNTPs downstream of aspartate to maintain nucleotide balance. Knockout cells accumulate dNTPs. **(C)** Proliferation measured using CTV of T-cells derived from wildtype or SAMHD1-KO mice.

Summary

Iron deficiency in T-cells results in:

- Induction of the p53 cell cycle arrest program
- Metabolic remodelling featuring reduced MYC and mTORC1 signalling pathways and depletion of amino acid import and ribosomal proteins
- Accumulation of OXPHOS and beta-oxidation proteins
- Suppressed generation of α-ketoglutarate, fumarate and malate which lie downstream of the iron-dependent enzymes aconitase and succinate dehydrogenase
- Increased aspartate production potentially via PC but reduced downstream usage
- Accumulation of the repressive histone mark, H3K27me3

Aspartate or **SAMHD1-KO** rescues proliferation of iron deficient T-cells implying iron starvation may limit aspartate and nucleotide availability due to the iron dependency of these pathways

References

1. Andreini, C., Putignano, V., Rosato, A. & Banci, L. The human iron-proteome. *Metallomics* **10**, 1223-1231, doi:10.1039/c8mt00146d (2018).
2. Vos, T. *et al.* Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 countries, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet* **390**, 1211-1259, doi:https://doi.org/10.1016/S0140-6736(17)32154-2 (2017).
3. Frost, J. N. *et al.* Hepcidin-Mediated Hypoferremia Disrupts Immune Responses to Vaccination and Infection. *Med* **2**, 164-179.e112, doi:10.1016/j.medj.2020.10.004 (2021).
4. Teh, M. R., Frost, J. N., Armitage, A. E. & Drakesmith, H. Analysis of Iron and Iron-Interacting Protein Dynamics During T-Cell Activation. *Frontiers in Immunology* **12**, doi:10.3389/fimmu.2021.714613 (2021).

Acknowledgements

This work was supported by the UK Medical Research Council (MRC Human Immunology Unit core funding, award no. MC_UU_12010/10), with further support from the Clarendon Fund and the Corpus Christi College A. E. Haigh graduate scholarship.