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Article

Keywords:

Posted Date: August 26th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10438987/v1>

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Additional Declarations: **Yes** there is potential Competing Interest. KHV is on the board of directors and shareholder of Bristol Myers Squibb and on the science advisory board (with stock options) of PMV Pharma, RAZE Therapeutics, Volastra Pharmaceuticals and Kovina Therapeutics. She is on the SAB of Ludwig Cancer and a co-founder and consultant of Faeth Therapeutics. She has been in receipt of research funding from Astex Pharmaceuticals and AstraZeneca and contributed to CRUK Cancer Research Technology filing of patent application WO/2017/144877. The other authors declare no competing interests.

TIGAR coordinates polyol and glutamine metabolism to regulate CD4⁺ T cells

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Abstract

CD4⁺ T cells undergo extensive metabolic reprogramming during activation and differentiation, yet how metabolic enzymes coordinate metabolite utilisation with lineage commitment remains poorly understood. Here, we identify the metabolic enzyme TP53-induced glycolysis and apoptosis regulator (TIGAR) as a critical regulator of CD4⁺ T-cell fate. Although classically linked to glycolysis, TIGAR unexpectedly regulated CD4⁺ T-cell differentiation independently of these canonical functions. We found that TIGAR deficiency impaired glutamine utilisation, enhanced Th1 differentiation, and suppressed Th17 and regulatory T-cell generation in CD4⁺ T cells. Mechanistically, TIGAR deficiency upregulated aldose reductase, a key enzyme of polyol metabolism, and both pharmacological inhibition of aldose reductase and sorbitol supplementation recapitulated key aspects of the TIGAR-deficient phenotype, establishing the polyol pathway as a metabolic regulator of CD4⁺ T-cell differentiation and migration. *In vivo*, T cell-specific TIGAR ablation enhanced inflammatory IFN γ -producing CD4⁺ T-cell responses during *Citrobacter rodentium* infection and exacerbated T cell-mediated colitis. Consistent with these findings, Mendelian randomisation identified genetically predicted aldose reductase expression as a causal risk factor for inflammatory bowel disease in humans. Together, our findings uncover a TIGAR–polyol metabolic axis linking mitochondrial metabolism to CD4⁺ T-cell fate and intestinal inflammation, establishing polyol metabolism as a previously unrecognised metabolic checkpoint and potential therapeutic target in immune-mediated disease.

Introduction

CD4⁺ T cells are central orchestrators of adaptive immunity, tailoring their responses to the type of pathogen and the surrounding tissue environment. In response to distinct cytokine cues, naïve CD4⁺ T cells differentiate into inflammatory T helper (Th) subsets, including Th1 and Th17 cells, or into immunoregulatory populations such as regulatory T cells (Tregs). Beyond cytokine signalling, emerging evidence highlights cellular metabolism as a key determinant of CD4⁺ T cell fate and function¹. Specific metabolic programmes, including glycolysis, mitochondrial metabolism, and lipid utilisation, are differentially engaged among CD4⁺ T cell subsets, thereby linking metabolic state to function^{2,3}.

Enhanced glycolytic metabolism is a defining feature of Th1, Th2 and Th17 cells⁴. Glycolysis feeds multiple branching pathways, such as the pentose phosphate pathway (PPP), to support the biosynthesis of macromolecules. Besides the PPP, glucose can be diverted through the polyol pathway, in which glucose is reduced to sorbitol by aldose reductase (AR) and subsequently oxidised to fructose by sorbitol dehydrogenase. This pathway is typically activated under conditions of excess glucose and is associated with osmotic stress and an altered redox balance⁵. AR is expressed in the stroma of colorectal cancers, particularly in anti-inflammatory M2-like macrophages, and also promotes LPS-driven inflammation in macrophages⁶⁻⁸. Studies in *Drosophila* have also linked polyol metabolism to systemic gut inflammatory signalling⁹; however, its contribution to CD4⁺ T cell function remains largely unexplored. Emerging evidence suggests that glycolytic branching pathways modulate T cell activation and differentiation¹⁰⁻¹⁴, positioning the polyol pathway as a potential regulator of these processes.

Glycolytic control is mediated in part by the phosphofructokinase-2/fructose-2,6-bisphosphatase (PFKFB) family, which regulates intracellular levels of fructose-2,6-bisphosphate and, thereby, the glycolytic rate¹⁵. PFKFBP3 has been found to support T cell proliferation and is strongly upregulated in CD4⁺ T cells from patients with rheumatoid arthritis^{16,17}. The TP53-induced glycolysis and apoptosis regulator (TIGAR) is also a unidirectional fructose-2,6-bisphosphatase that redirects glucose flux toward the pentose phosphate pathway¹⁸. TIGAR has been implicated in redox homeostasis across various cancer cell types, in metastatic cancer dissemination, and in modulating inflammatory responses during sepsis via a TIGAR-TAK1 interaction in macrophages¹⁹⁻²¹. While TIGAR's role in transformed cancer cells is well characterised²², its role in untransformed cells, particularly primary immune cells such as T cells, remains uncharted.

Here, we discovered that TIGAR regulates CD4⁺ T cell fate and function via metabolic reprogramming of the glutamine and polyol pathways. We identify a previously unappreciated link between TIGAR-dependent metabolic reprogramming, sorbitol metabolism, and T cell migration, uncovering a metabolic axis that coordinates CD4⁺ T cell differentiation and functional behaviour in several models. These findings provide new insight into how metabolic pathways integrate to shape immune responses in health and disease.

Results

TIGAR regulates survival and activation, but not glycolysis, in T cells.

Given that TIGAR is expressed in immune organs and is upregulated during mitogen-induced human lymphocyte proliferation^{23,24}, we sought to test whether TIGAR expression is regulated in a T-cell receptor (TCR) and co-stimulation-dependent manner in human and mouse CD4⁺ T cells. Indeed, in both species, T cell activation downstream of CD3 and CD28 engagement induced robust TIGAR expression (**Fig. 1a, b**). To investigate the role of TIGAR in T cells, we generated T-cell-specific TIGAR-null mice by crossing *Tigar*^{fl} mice²⁵ with CD4-Cre mice²⁶. TIGAR deletion in activated T cells was validated by immunoblot (**Fig. 1b**). We also performed extensive immune profiling of lymph nodes and spleen from WT (WT-T) and TIGAR-T cell-specific KO (KO-T) female and male mice and found no effects on frequencies of immune cell subsets (**Extended Data Fig. 1a**).

Given that TIGAR is upregulated upon activation, we next performed T cell proliferation assays with TIGAR WT and null CD4⁺ T cells. These data revealed no effect of TIGAR on T cell proliferation (**Fig. 1c**). We also did not detect differences between WT and TIGAR-null T cells during homeostatic proliferation in lymphopenic Rag2-null recipients (**Extended Data Fig. 1b and c**). However, in line with TIGAR loss increasing sensitivity to apoptosis in cancer cells¹⁸, we observed a significant increase in apoptotic cells, as indicated by Annexin V and propidium iodide staining, in TIGAR-null T cells compared to controls after 24 hours of activation (**Fig. 1d, Extended Data Fig. 1d**). During early T cell activation, T cells upregulate the activation markers CD69 and CD44, while downregulate the naïve marker CD62L. TIGAR ablation retained the expression of the naïve marker CD62L while limiting the upregulation of the activation markers CD69 and CD44, indicating that TIGAR contributes to T cell activation (**Extended Data Fig. 1e**).

Since TIGAR has been identified as a regulator of glycolysis in various cell types^{18,27,28}, we sought to determine whether it regulates glycolysis in primary CD4⁺ T cells after 24 hours of activation. 2-NBD-glucose uptake, measured by flow cytometry, showed no differences between WT and TIGAR-ablated CD4⁺ T cells (**Extended Data Fig. 1f**). Next, we performed Seahorse analysis to assess glycolytic rate and glycolytic stress responses, measuring glucose utilisation in real time and changes in the extracellular acidification rates (ECAR) as a proxy of glycolysis. Unexpectedly, WT and TIGAR-null CD4⁺ T cells responded similarly (**Fig. 1e, f**). We further employed an

orthogonal approach to assess glycolysis using stable isotope tracing analysis with $^{13}\text{C}_6$ -glucose in WT and KO T cells, which showed no significant effects on glycolysis-associated metabolites, such as the pentose phosphate pathway (PPP: ribose-5-phosphate, UTP), serine biosynthesis (glycine), pyruvate, and lactate (**Fig. 1g, h**).

To distinguish TIGAR-dependent functions from its canonical role in glycolytic regulation, we inhibited PFKFB3, the principal F2,6BP-generating enzyme in T cells (**Fig. 1i**)²⁹. Using two PFKFB3 inhibitors, AZ67 and 3PO, we performed T-cell proliferation assays. In contrast to TIGAR deficiency, PFKFB3 inhibition markedly reduced T-cell proliferation (**Fig. 1j**), suggesting that TIGAR regulates T-cell function through a distinct mechanism. Molecular docking of fructose-6-phosphate into the fructose biphosphate binding pockets of PFKFB3 and TIGAR revealed favourable binding within the PFKFB3 catalytic pocket, with multiple stabilising interactions. In contrast, docking to TIGAR was less favourable and predicted a steric clash with Tyr92 (**Fig. 1k**), suggesting that catalytic activity would be observed only at high intracellular F-2,6-P levels. We next performed structural similarity analysis using Distance-matrix ALIgnment (DALI)³⁰ for protein structure comparisons to TIGAR and identified phosphoglycerate mutase and alpha-ribazole phosphatase as the closest homologs to TIGAR (**Extended Data Fig. 2a**). Molecular docking further revealed that TIGAR may share enzymatic activity with α -ribozole-5'-phosphate phosphatase and bisphosphoglycerate mutase (**Fig. 1l**). Taken together, these findings suggest that TIGAR plays a distinct role in T cell activation, independent of its metabolism of fructose-2,6-bisphosphate and regulation of the PPP.

TIGAR shapes CD4⁺ T cell fate via regulation of mitochondrial and glutamine metabolism

We next assessed whether TIGAR modulates CD4⁺ T cell mitochondrial metabolic reprogramming, a key metabolic switch for differentiation³¹. Seahorse analysis revealed an increased oxygen consumption rate (OCR) in TIGAR-null CD4⁺ T cells, without major differences in ECAR, as compared to WT littermates (**Fig. 2a**). Increased mitochondrial activity typically requires enhanced tricarboxylic acid (TCA) cycle activity, which can be fuelled by multiple metabolites (**Fig. 2b**). Initially, we measured the contribution of alanine using $^{13}\text{C}_3$ -alanine. Here, no significant differences were observed between WT and KO CD4⁺ T cells in ^{13}C incorporation (**Extended Data Fig. 2b**). We next evaluated the contribution of glutamine, a conditionally essential amino acid in proliferating T cells³²⁻³⁴, through glutaminolysis. While we observed no

difference in glutamine uptake (**Extended Data Fig. 2c**), we observed a significant reduction in glutamine metabolism, as evidenced by reduced incorporation into glutamate, α -ketoglutarate (α KG), and citrate (**Fig. 2c**). We also observed lower glutamine-derived contributions to aspartate and glutathione biosynthesis (**Fig. 2d**), overall suggesting that TIGAR supports glutamine metabolism in activated CD4⁺ T cells. Specifically, stable isotope tracing revealed that TIGAR deficiency primarily impairs the initial step of glutamine catabolism, catalysed by glutaminase (GLS), as evidenced by reduced incorporation of ¹³C₅-glutamine into glutamate (**Fig. 2c**). To further confirm this, we performed a proliferation assay in the presence or absence of the GLS1 inhibitor CB-839. As expected, WT T cells were more sensitive to CB-839 compared to TIGAR-deficient, further suggesting that TIGAR supports glutaminolysis (**Fig. 2e**). Using ¹³C₅-glutamine tracing, we further confirmed that TIGAR-ablated CD4⁺ T cells metabolise glutamine similarly with or without the GLS1 inhibitor, while WT CD4⁺ T cells reduced glutamine catabolism in the presence of CB-839, to levels equivalent to TIGAR loss (**Fig. 2f**). This reduction of glutaminolysis is independent of changes in GLS expression between KO-T and WT counterparts (**Extended Data Fig. 2d**). Given that GLS1 modulates CD4⁺ T cell fate³⁴, we assessed T helper lineages Th1 and Th17 and the anti-inflammatory inducible Treg lineage. Under Th1-polarising conditions, TIGAR-deficient CD4⁺ T cells exhibited increased differentiation toward the Th1 lineage, as indicated by enhanced IFN- γ production (**Fig. 2g, Extended Data Fig. 2e**). Similarly, knockout of *TIGAR* in naive human CD4⁺ T cells preferentially induced the production and secretion of IFN- γ (**Fig. 2h, i**), suggesting a role for TIGAR-mediated metabolism in regulating the Th1 lineage. Conversely, TIGAR loss significantly ablated Th17 differentiation in cell culture, as evidenced by reduced IL-17A production (**Fig. 2j, Extended Data Fig. 2f**). These data align with previous observations that GLS1 loss promotes Th1 differentiation while limiting Th17 polarisation. However, unlike GLS1 ablation, TIGAR deletion under iTreg-polarising conditions impaired FOXP3 induction (**Extended Data Fig. 2g, h**), suggesting that TIGAR may regulate other metabolic pathways involved in CD4⁺ T cell fate. These data underpin a role for TIGAR in modulating cellular metabolism, in part through glutamine catabolism, and CD4⁺ T cell fate.

TIGAR is a negative regulator of the polyol pathway in CD4⁺ T cells

To gain further mechanistic insight into TIGAR's role in CD4⁺ T cells, we performed proteomic analysis of CD4⁺ T cells activated for 24 hours. Interestingly, we observed an upregulation in

myosin heavy chain proteins and aldose reductase (AR) (**Fig. 3a, b**), which converts glucose to the polyol sorbitol in a NADPH-dependent reaction (**Fig. 3c**). Therefore, we next explored how sorbitol directly affects CD4⁺ T cell differentiation. Under Th1-polarising conditions, exogenous sorbitol favoured IFN- γ production (**Fig. 3d, Extended Data Fig. 3a**), while a significant yet mild reduction was observed during Th17 differentiation, as demonstrated by lower IL-17A expression (**Fig. 3e, Extended Data Fig. 3b**). Therefore, increasing intracellular sorbitol recapitulated the effect of TIGAR deletion on Th1 and Th17 differentiation. However, under iTreg conditions, sorbitol supplementation did not impact differentiation, in contrast to TIGAR deletion (**Extended Data Fig. 3c, d**). To further demonstrate the relevance of AR in CD4⁺ T cell polarisation in wild-type conditions, we next sought to test how AR inhibition modulates this process. Using the AR inhibitor Epalrestat, which is used for Type II diabetes to limit sorbitol accumulation in tissues³⁵, we found that, in a dose-dependent manner, it significantly ablates differentiation towards the Th1 lineage, as demonstrated by lower IFN γ production (**Fig. 3f, Extended Data Fig. 3e**) as well as Th17 polarisation, evidenced by lower IL-17A production (**Fig. 3g, Extended Data Fig. 3f**). While the TIGAR Th1 phenotype may be explained in part by the induction of AR, the effect of AR inhibition is in stark contrast with the TIGAR Th17 phenotype, which highlights the complexity of the polyol pathway in modulating Th1-Th17 cell dynamics. While glutamine metabolism did not account for the reduced iTreg differentiation capacity of TIGAR-deficient CD4⁺ T cells *in vitro*, AR inhibition increased iTreg differentiation (**Extended Data Fig. 3g, h**), supporting a role for the TIGAR–polyol pathway in suppressing Treg polarisation.

Given the marked upregulation of AR in TIGAR-deficient CD4⁺ T cells, we next asked whether increased polyol pathway activity altered intracellular redox homeostasis, as seen in other cell types and disease states^{36–38}, since the polyol pathway both consumes NADPH and regenerates NADH. We therefore quantified intracellular NAD(P)H levels via spectral flow cytometry³⁹ between TIGAR WT and null CD4⁺ T cells. Interestingly, no differences were detected, suggesting that other pathways may be engaged to maintain this balance (**Extended Data Fig. 4a**). We further examined total intracellular ROS levels (CellROX) and mitochondrial ROS (MitoSOX) and found no difference between WT and TIGAR-ablated CD4⁺ T cells activated for 24 h (**Extended Data Fig. 4b and c**). Key pathways that play a role in mitigating oxidative stress include the TCA cycle and fatty acid oxidation (FAO)^{40,41} (**Extended Data Fig. 4d**). Using ¹³C₆-glucose tracing, we

observed an increase in the contribution of glucose to citrate and α -KG (**Extended Data Fig. 4e**), suggesting an increase in NADPH through the isocitrate dehydrogenase (IDH) reaction. Given that TIGAR-null CD4⁺ T cells exhibit higher oxygen consumption (**Fig. 2a**), we next evaluated FAO as an additional source of TCA cycle intermediates, thereby supporting IDH activity. We next evaluated fatty acid usage in CD4⁺ T cells with and without TIGAR. Interestingly, TIGAR deficiency favoured the absorption of medium-chain fatty acids (MCFAs) relative to long-chain fatty acids (LCFAs) in CD4⁺ T cells activated for 24 h (**Fig. 3h, i**). Seahorse FAO Stress Test analysis demonstrated that TIGAR-deficient CD4⁺ T cells rely on MCFA oxidation to support mitochondrial function, as indicated by reduced OCR and spare respiratory capacity following FAO inhibition with etomoxir (**Fig. 3j**). Overall, these findings suggest that TIGAR deficiency – potentially through upregulated AR – reprograms mitochondrial metabolism. To directly test whether the polyol pathway is sufficient to modulate mitochondrial function, we pharmacologically and metabolically manipulated AR activity and sorbitol levels in WT CD4⁺ T cells. Interestingly, modulation of the polyol pathway through either sorbitol supplementation or AR inhibition increased mitochondrial activity while only modestly affecting glycolysis, as reflected by an elevated OCR/ECAR ratio measured by Seahorse bioenergetic analysis (**Extended Data Fig. 4f and g**). In addition, manipulating sorbitol metabolism reduced spare respiratory capacity, suggesting that mitochondria may already be operating near maximal capacity at baseline in WT CD4⁺ T cells, thereby limiting their ability to respond to additional metabolic stress (**Extended Data Fig. 4h and i**). Taken together, these data identify a TIGAR–polyol metabolic axis that shapes CD4⁺ T cell differentiation, mitochondrial metabolism, and flexibility.

TIGAR modulates T cell motility

In cancer cells, TIGAR loss enhances cell migration and metastasis²¹. In line with this, our proteomics dataset (**Fig. 3a,b**) revealed upregulation of myosin heavy chain proteins, which are involved in cell motility^{42,43}. Therefore, we next assessed the cytoskeleton in WT and TIGAR-ablated CD4⁺ T cells post 24-hour activation. Consistently, we observed enhanced cytoskeletal protrusions in the absence of TIGAR (**Fig. 4a**) compared with WT counterparts, suggesting an impact on cell movement. To assess T cell migratory dynamics, we performed epifluorescence live-cell imaging of V450-labelled WT CD4⁺ T cells and CTFR-labelled TIGAR-null CD4⁺ T cells interacting with LPS-matured bone marrow–derived dendritic cells (BMDCs) (**Fig. 4b**). Single-

cell migratory dynamics were analysed to quantify average speed, meandering index, outreach ratio, and relative turning angle (**Fig. 4c, d**). The higher average speed and turning angle, as well as the lower meandering index and outreach ratio, suggest TIGAR loss leads to increased scanning behaviour and improved confined exploration (**Fig. 4e**). These data suggest that TIGAR ablation in CD4⁺ T cells promotes a highly dynamic migratory phenotype characterised by elevated cell speed and more tortuous, less persistent migration, suggesting increased environmental scanning and confined exploration. Interestingly, sorbitol metabolism has been linked to increased metastasis in colorectal cancer⁴⁴. Inhibition of AR with Epalrestat resulted in the opposite movement behaviour to TIGAR loss: reduced average speed, reduced confined exploration (higher outreach ratio and meandering index), and lower distance coverage (reduced turning angle) (**Fig. 4f**), suggesting that AR, in addition to increased myosins in TIGAR-null CD4⁺ T cells, is involved in promoting cell speed and scanning. However, exogenous sorbitol at 2 and 5 mM did not affect T-cell migratory behaviour (**Extended Data Fig. 5a-d**); Collectively, these data indicate that the AR sorbitol-generating reaction is required for CD4⁺ T-cell speed and scanning behaviour, and that this dependency may be controlled by TIGAR

T cell-specific TIGAR loss promotes IFN γ -inflammatory responses during *Citrobacter* infection

Thus far, our *in vitro* data indicate that TIGAR regulates the balance between Th1 and Th17-driven inflammation. *Citrobacter rodentium* is an extracellular Gram-negative enteric pathogen that causes colitis. *C. rodentium* induces pathogenic Th17 cells that exhibit plasticity toward IFN- γ production, both locally and systemically, at two weeks post-infection^{45,46}. Therefore, we examined T-cell-specific TIGAR depletion in the Th17-induced *C. rodentium* model on day 13 of infection (**Fig. 5a**), during the late-acute/early resolution phase, which is dependent on CD4⁺ T cells⁴⁵. Bacterial load was equivalent at days 7, 12, and 13 in mice lacking TIGAR in the T-cell compartment and WT controls (**Fig. 5b, c**). Analyses of ROR γ t expression in CD4⁺ T cells in the colon and mesenteric lymph nodes (LN) were unchanged between the two genotypes (**Extended Data Fig. 6a**). However, assessment of CD4⁺ T cell cytokine production for IFN γ and IL-17A in the colonic lamina propria (cLP) and the spleen revealed differences in polarisation states. Interestingly, TIGAR loss in CD4⁺ T cells promoted differentiation towards IFN- γ production, as

indicated by increased IFN γ ⁺IL-17A⁻ and IFN γ ⁺IL17⁺ cells in the cLP (**Fig. 5d, e**) compared to controls. Systemically, TIGAR deletion also induced CD4⁺ T cells producing IFN γ in the spleen on day 13 post-infection (**Fig. 5f**), suggesting increased *Citrobacter*-induced inflammation. To evaluate the luminal inflammatory milieu, we next measured cytokine abundances in stool collected at day 12 and observed a significant reduction in several cytokines involved in Th17-mediated responses: IL-21 (produced by Th17 cells and sustains lineage commitment)⁴⁷, IL-23 (involved in Th17 maintenance and expansion⁴⁸) and a modest decrease in IL-31 (a regulator of the epithelial barrier⁴⁹) (**Fig. 5g**). However, no differences were observed in other cytokines and inflammatory biomarkers, including lipocalin, IL-22, IL-27, IL-1BB, and MIP-3 α (**Extended Data Fig. 5b, c**). These data underpin a role for TIGAR in altering the Th17-related cytokine landscape within the gut in response to enteric pathogens. Taken together, TIGAR restrains inflammatory CD4⁺ T-cell polarisation during *Citrobacter* infection, limiting the shift toward IFN γ -producing Th17 cells and modulating, through cellular metabolism, the host-microbiota interface

TIGAR deletion in CD4 T cells exacerbates colitis by driving Th1 polarisation and limiting *de novo* Tregs

Whole-body deletion of TIGAR has been associated with increased susceptibility to colitis²³. This prompted us to test the contribution of TIGAR in the differentiation of inflammatory CD4⁺ Th1 T cells using a T-cell adoptive transfer model of colitis (**Fig. 6a**). Recipient mice with TIGAR-null donor T cells depleted for Tregs exhibited a significant reduction in survival compared with recipient mice receiving TIGAR-expressing donor CD4⁺ T cells (**Fig. 6b**). CD4⁺ T cell frequencies were unchanged in the colonic lamina propria (cLP), spleen, and peripheral lymph nodes (pLN) (**Extended Data Fig. 7a**). However, assessment of *de novo* differentiation of Th1 cells in immune compartments revealed significant induction of CD4⁺ IFN γ ⁺ Th1 cells in the cLP, spleen and pLN (**Fig. 6c, Extended Data Fig. 7b**) in mice harbouring TIGAR-deleted CD4⁺ T cells. Increased circulating IFN γ was also detected in the plasma of recipient mice adoptively transferred with TIGAR-null CD4⁺ T cells compared to controls (**Extended Data Fig. 7c**). Given that we observed lower iTreg differentiation *in vitro*, we examined *de novo* polarisation of Tregs in this model. Strikingly, Tregs were significantly fewer in the spleen and mesenteric LN, with no notable differences in the cLP (**Fig. 6d, Extended Data Fig. 7d**). We next assessed the suppressive

behaviour of peripheral (p)Tregs isolated from WT and TIGAR KO-T mice in an *in vivo* suppression assay. Here, WT and KO-T pTregs were co-transferred with WT CD4⁺CD25⁻ T cells in Rag2-deficient recipients (**Extended Data Fig. 7e**). pTregs from both genotypes prevented autoimmunity and inflammation in immune compartments (**Extended Data Fig. 7f, g**) with mild effects on CD4⁺ T cell expansion, maintenance and induction of CD4⁺ FOXP3⁺ T cells in the spleen, mLN and pLN (**Extended Data Fig. h, i**). Overall, this suggests that TIGAR is most likely involved in the *de novo* induction of Tregs rather than their suppressive function. Collectively, these data show that TIGAR regulates intestinal and systemic inflammation in colitis by maintaining the balance of the CD4⁺ Th1/Treg axis.

Aldose reductase as a downstream effector associated with colonic disease in humans

To assess whether genes altered by TIGAR loss in CD4⁺ T cells (**Fig. 3a**) may causally influence the risk of inflammatory bowel disease (IBD), we performed Mendelian randomisation (MR) analyses using summary-level genetic data. MR estimates causal relationships using genetic variants as instrumental variables, assuming these variants are associated with the exposure, independent of confounders, and affect the outcome only through the exposure^{50,51}. *Cis*-expression quantitative trait loci (*cis*-eQTLs) were obtained from the eQTLGen Consortium meta-analysis of blood gene expression (n = 31,684)⁵². After harmonisation with outcome datasets, three genes encoding proteins that were upregulated in TIGAR-null CD4⁺ T cells as identified by proteomics (**Fig. 6e**) had valid genetic instruments available for analysis: *AKR1B1* (which encodes AR), *RAPGEF2* (which encodes a guanine nucleotide exchange factor), and *MFSD1* (which encodes a lysosomal transporter). Outcome summary statistics were obtained from a large GWAS of IBD comprising 86,640 individuals of predominantly European ancestry⁵³, including 42,950 IBD cases and 53,536 controls. MR analyses were conducted for overall IBD and the two major clinical subtypes, ulcerative colitis (20,417 cases, 52,230 controls) and Crohn's disease (22,575 cases, 46,693 controls). Strikingly, evidence was found for an effect of genetically predicted *AKR1B1* expression on IBD risk (OR = 1.40, 95% CI 1.10–1.77, P = 0.005), ulcerative colitis (OR = 1.50, 95% CI 1.11–2.01, P = 0.008), and Crohn's disease (OR = 1.50, 95% CI 1.09–1.93, P = 0.011) in humans (**Fig. 6e**), while no statistically significant associations were observed for *RAPGEF2* or *MFSD1* (**Fig. 6e**). These data align with the T-cell-induced colitis model, in which TIGAR loss increases AR expression and drives pathogenic Th1 cell differentiation. These data position the

polyol pathway as a positive regulator of developing immune-related bowel disease, including IBD, ulcerative colitis and Crohn's disease.

Discussion

Cellular metabolism shapes immune cell function, and our findings build on this, specifically how glucose-alternative pathways govern CD4⁺ T cell fate and migratory behaviour. Here, we demonstrate that TIGAR rewires CD4⁺ T cell differentiation by regulating the balance between Th1 and Th17 cells in both cell culture and mouse models. While TIGAR can function as a fructose biphosphatase in enzymatic assays⁵⁴, our data indicate that in primary CD4⁺ T cells, TIGAR may not be a potent regulator of glycolysis. TIGAR has been shown to catalyse the phosphorylation of other phospho-esters, such as 3-bisphosphoglycerate, suggesting that it may have additional substrates⁵⁵. New methods for identifying novel and physiological substrates have been developed, such as MIDAS⁵⁶, which may shed light on the breadth of TIGAR's enzymatic function. Mechanistically, we found that TIGAR loss increased AR expression, which converts glucose to the sugar alcohol sorbitol, the first step of the polyol pathway. AR has been associated with the regulation of macrophage polarisation, inflammatory responses and susceptibility to *Clostridium difficile* infection^{8,9,57-59}. However, it is unclear if AR influences other immune cell types. Our data strongly position the polyol pathway as an essential regulator between inflammatory and anti-inflammatory CD4⁺ T cell lineages, favouring inflammation while limiting Tregs. AR can also regulate glucose flux via *de novo* sugar nucleotide synthesis and glycosylation. AR inhibition increased GDP-mannose, a building block for mannosylation and N-linked glycoproteins, thereby affecting protein function⁶⁰. We found that TIGAR deficiency and upregulation of AR reduced glutaminolysis via GLS1, without affecting its expression. Since post-translational modification is known to affect GLS1 activity⁶¹, AR-driven changes in glycosylation may represent an additional mechanism by which AR regulates GLS1 function and glutamine utilisation in CD4⁺ T cells.

TIGAR deficiency has often been associated with increased ROS, often attributed to reduced NADPH generation via the PPP¹⁸. However, increased AR activity may also drive redox imbalance by consuming NADPH, thereby elevating ROS independently of PPP flux. In contrast, TIGAR loss in CD4⁺ T cells did not alter total or mitochondrial ROS levels, suggesting that redox

homeostasis may be more tightly regulated in CD4 T cells than transformed cancer cells. This could reflect compensatory NADPH-generating pathways, such as enhanced IDH activity, which may buffer redox stress in T cells compared to other cell types. Redox state has also been shown to play a key role in cancer cell migration. Indeed, TIGAR is a negative regulator of metastasis in pancreatic cancer, and enhanced reverse SORD activity (sorbitol production from fructose) similarly promotes liver metastasis in colorectal cancer^{21,44}. Interestingly, our findings identify the TIGAR-polyol axis as a molecular rheostat that fine-tunes CD4⁺ T-cell migratory dynamics. TIGAR deficiency, associated with AR upregulation, enhances cell speed and exploratory scanning behaviour, whereas pharmacological inhibition of AR in WT CD4⁺ T cells elicits the opposite phenotype.

Modulating sorbitol and glutamine metabolism through TIGAR has profound effects on disease. While TIGAR loss in the T-cell compartment did not affect *C. rodentium* bacterial load, it enhanced IFN γ production in a Th17-driven response as well as a constrained Th17 response in the gut luminal milieu. Modulating host cellular metabolism may profoundly influence metabolic crosstalk with enteric pathogens. For example, whole-body loss of AR, which reduces sorbitol production by the host, confers resistance to *C. difficile* infection, as this pathogen relies on sorbitol as a metabolic substrate⁵⁹. This raises the possibility that modulating host-derived metabolites of the polyol pathway can directly influence pathogen metabolism while simultaneously dictating CD4⁺ T cell fate. Dysregulated IFN γ production is a mediator of colitis⁶², and we have found that in TIGAR-deficient CD4⁺ T cells, which polarise more efficiently towards the Th1 lineage, exacerbate T cell-mediated colitis compared to control counterparts. Taken together, these data indicate that TIGAR is a negative regulator of IFN γ -mediated responses, in part mediated by the polyol pathway. The role of AR as a rheostat of pathogenic inflammation is further supported by Mendelian randomisation in humans, which indicates that genetically predicted increased expression of AKR1B1, the gene encoding AR, causally increases the risk of IBD, Crohn's disease and ulcerative colitis.

We have identified several metabolic axes, including the polyol pathway, glutamine and mitochondrial metabolism, through which TIGAR may regulate CD4⁺ T cell fate. However, further studies are required to uncover the precise mechanisms linking metabolic adaptation to T cell fate. Our data suggest that TIGAR-null CD4⁺ T cells undergo metabolic rewiring that

promotes mitochondrial metabolism, in which TCA cycle intermediates have an established role as epigenetic regulators in immune cells. This suggests that TIGAR-dependent metabolic adaptations may influence chromatin remodelling and gene expression, ultimately shaping CD4⁺ T cell differentiation. Consequently, epigenetic modifications have been shown to modulate the expression of essential CD4⁺ T cell polarisation transcription factors such as ROR γ t and FOXP3⁶³⁻⁶⁵. Thus, TIGAR-driven metabolic reprogramming may influence the epigenetic landscape, shaping CD4⁺ T cell fate.

Materials and Methods

Mice and *in vivo* models

For all *in vivo* models, at least four mice per group were used. C57BL/6 CD4 Cre transgenic mice were purchased from the Jackson Laboratories and crossed with TIGAR^{lox/lox} mice to generate T-cell specific TIGAR knock-out mice. Mice were bred and housed at the Maisonneuve Rosemont Hospital Research Center animal facility. All animal experiments were approved by the Centre de Recherche de l'Hôpital Maisonneuve Rosemont Animal Welfare and Ethical Review Body (protocol 2023-3145) and conducted in accordance with the Canadian Council on Animal Care guidelines. *Rag* 2^{-/-} and *CD4*⁺;*Tigar*^{ff} mice were bred and housed at the Francis Crick Institute animal facility. Animal experiments were subject to ethical review by the Francis Crick Animal Welfare and Ethical Review Body and regulation by the UK Home Office project licence P319AE968. All procedures were performed in accordance with the Animals (Scientific Procedures) Act 1986 and the EU Directive 2010. Mice were maintained under a 12-h day:night cycle from 07:00 to 19:00, with access to food and water ad libitum, at 21 °C and 55% humidity.

Homeostatic proliferation

CFSE-stained CD4⁺ T cells from TIGAR WT-T and KO-T C57BL/6J donors T cells (stained according to manufacturer's instructions, BioLegend) were intravenously injected (1 × 10⁶ cells/mouse in PBS) into C57BL/6J *Rag*2^{-/-} mice. At day 3 post-injection, spleens were collected, and the CFSE dilution of donor CD4⁺ T cells was measured by flow cytometry.

T cell-induced colitis model

Six- to eight-week-old male Rag2^{-/-} mice were injected with TIGAR WT-T or KO-T CD4⁺CD25⁻ T cells (0.5×10^6 cells/mouse), isolated from donor C57BL/6J mice. Overall survival was monitored, and inflammation in the colonic lamina propria, spleen, and peripheral lymph nodes was assessed on day 21 by intracellular cytokine staining followed by flow cytometry. *In vivo* Treg suppression was performed by adoptive transfer of Tregs (0.2×10^6) and Tconv (0.8×10^6) cells into Rag^{-/-} recipient C57BL/6 mice. Humane endpoints were defined as >15% weight loss or chronic diarrhea, as approved under UK Home Office project license P319AE968. None of these limits was exceeded.

Citrobacter rodentium infection

Six- to eight-week-old female C57BL/6J TIGAR WT-T or KO-T mice were infected with *Citrobacter rodentium* strain DBS100 ($2-3 \times 10^8$ CFU/mouse) by oral gavage. Bacterial load was measured using CFU (colony forming units), from the faeces at Day 7 and 12 and from the cecum at Day 13 of the infection by homogenization, dilution and plating on MacConkey agar plates, which are selective and differential for *C. rodentium*. At day 13 post-infection, cLP and spleen were collected to assess pathogenic Th17 cells using flow cytometry, followed by PMA/Ionomycin/Golgi Stop stimulation and intracellular cytokine staining. Lipocalin (R&D Biosystems, Cat#DY1857) and cytokine multiplexing (Eve Technologies) were also assessed from faecal extracts of infected mice at day 12 post-infection.

Murine T cell isolation and cell culture

CD4⁺ T cells were isolated from spleens and peripheral lymph nodes, prepared into single-cell suspensions, and counted with methylene blue. CD4⁺ T cells were isolated using negative isolation kits (StemCell Technologies, Miltenyi Biotec) according to the manufacturer's protocol. T cells were then activated and cultured as previously described⁶⁶ using plate-bound anti-CD3 ($5 \mu\text{g.mL}^{-1}$, BioLegend, 145-2C11, 1015436) and anti-CD28 ($2 \mu\text{g.mL}^{-1}$, BioLegend, 37.51, 14-0281-85) antibodies in T cell medium (TCM) (IMDM, 10% fetal bovine serum (FBS), 1% penicillin-streptomycin and $50 \mu\text{M}$ β -mercaptoethanol).

T cell differentiation assays

Th1 cells. Naive CD4⁺ T cells were isolated using the manufacturer's instructions and activated by seeding 0.5×10^6 cells per well in a 48-well tissue culture plate coated with anti-CD3 ($5 \mu\text{g} \cdot \text{ml}^{-1}$) and anti-CD28 ($2 \mu\text{g} \cdot \text{ml}^{-1}$). TCM was supplemented with IL-2 ($10 \text{ ng} \cdot \text{ml}^{-1}$, Miltenyi Biotec, Cat#130-120-662), IL-12 ($20 \text{ ng} \cdot \text{ml}^{-1}$, BioLegend, Cat#577004), and anti-IL-4 (BioLegend, clone 11B11, Cat# 57430). CD4⁺ T cells were restimulated with the PMA–ionomycin–GolgiStop cocktail, followed by surface and intracellular cytokine staining for IFN γ (clone XMG1.2, Thermo Fisher Scientific, Cat# 11-7311-82) and TBET (clone 4B10, BioLegend, Cat# 644824), 3 days post-differentiation.

Th17 cells. Total lymph node cells were activated by seeding 0.2×10^6 cells per well in a 96-well tissue culture plate and stimulating with plate-bound anti-CD3 ($2 \mu\text{g}/\text{mL}$) and anti-CD28 ($2 \mu\text{g}/\text{mL}$) antibodies. TCM was supplemented with rhTGF- β 1 ($2 \text{ ng} \cdot \text{ml}^{-1}$, Miltenyi Biotec, Cat# 130-095-067) and rmIL-6 ($25 \text{ ng} \cdot \text{ml}^{-1}$, Miltenyi Biotec, Cat# 130-096-682). CD4⁺ T cells were restimulated with the PMA–ionomycin–GolgiStop cocktail, followed by surface and intracellular cytokine staining for ROR γ T (clone B2D, Cat#17-6981-82, ThermoFisher Scientific) and IL17A (clone 4B10, Cat#560666, BD BioSciences), 3 days post-differentiation.

iT_{reg} cells. Naive CD4⁺ T cells were isolated according to the manufacturer's instructions and activated by seeding 0.5×10^6 cells per well in a 48-well tissue culture plate coated with anti-CD3 ($5 \mu\text{g}/\text{ml}$) and anti-CD28 ($2 \mu\text{g}/\text{ml}$). TCM was supplemented with rhTGF- β 1 ($2.5\text{-}5 \text{ ng ml}^{-1}$, Miltenyi Biotec, Cat# 130-095-067), IL-2 (10 ng ml^{-1} , Miltenyi Biotec, Cat#130-120-662), and anti-IFN γ (BioLegend, clone XMG1.2, Cat#505802). CD4⁺ T cells were stained for FOXP3 (clone MF-14, BioLegend, Cat#505031) and CD25 (clonePC61, BioLegend, 102012), 3 days post differentiation.

Immunofluorescence

13 mm borosilicate glass coverslips (thickness 1.5 mm, VWR) were placed in a 24-well plate and coated with 50 mg/mL poly-d-lysine (Sigma) for 30 min at room temperature, followed by one water wash, and then allowed to air-dry. Two million activated WT-T or KOT CD4⁺ T cells were gently placed over the coated slides and allowed to attach in a humidified 37 °C incubator for 30 min. Unattached cells were aspirated, and 4% PFA was added, and cells were fixed for 10 minutes at room temperature, followed by one PBS wash and aspiration. Samples were blocked

with 0.4% Triton X-100/PBS/10% bovine serum albumin solution. Antibody Phalloidin (ThermoFisher Scientific, A12380) was diluted to 1/400 in PBS. After a PBS wash, samples were incubated with DAPI solution (1:10 000 dilution in PBS; BD Pharmingen) for 5 minutes at room temperature. Slides were washed once with PBS, inverted and placed on antifade mounting media (VECTASHIELD) on superfrost microscope slides (Thermo Scientific, 12372098). Slides were sealed with CoverGrip coverslip sealant. Images were taken on an Inverted confocal super-resolution Leica Stellaris STED imaging system with a 63× oil objective and 1.4 NA. The 405nm (Blue) and 561nm (Red/Orange) lasers were used for excitation and z-stacks were collected as 16 bits per pixel (average size of 240×240 pixels). Data was analyzed using the Leica software.

Bone marrow-derived dendritic cells and LPS stimulation *in vitro*

Bone marrow cells were isolated from the femurs and tibias of mice by flushing with cRPMI (RPMI 1640 supplemented with 10% FBS, 1% Pen/Strep, 2 mM L-glutamine, 2 mM sodium pyruvate, nonessential amino acids, and HEPES). Red blood cells were lysed using ACK lysis buffer, and cells were resuspended in cRPMI. Bone marrow cells were cultured at 1.5×10^6 cells/well in 6-well plates in cRPMI supplemented with GM-CSF (50 ng.ml⁻¹) and IL-4 (20 ng.ml⁻¹). On Days 2, 3, and 6 of culture, half of the medium was replaced with fresh GM-CSF- and IL-4-supplemented medium. LPS stimulation (1 µg/ml) was performed on day 6 of culture for an overnight incubation. On day 7, BMDCs were harvested and isolated using 14.7% Histodenz. Cells from the interface were washed and used for co-culture with activated CD4⁺ T cells and live imaging.

Flow cytometry

Single-cell suspensions were stained for surface markers in PBS for 20 min at 4°C. Intracellular proteins (cytokines) were assessed using the FOXP3/Transcription staining buffer set (Invitrogen, eBioscience) following the manufacturer's instructions. Cells were permeabilized for 30 min and stained for intracellular proteins for a minimum of 1 hour at 4 °C. Fluorochromes were purchased from BioLegend, eBioscience (ThermoFisher Scientific), BD Pharmingen, or BioLegend. All surface fluorescent antibodies were used at a dilution of 1:500. Re-stimulation was performed using PMA (Millipore Sigma), Ionomycin (Cayman Chemical) and GolgiStop (BD Biosciences) for 4 hours as previously described⁶⁷. All intracellular antibodies were used at a dilution of 1:200.

Samples were acquired on the BD LSRFortessa X-20 and FACSCelesta (BD Biosciences). Data were analysed using FlowJo (version 10.10.0).

Apoptosis Assay

Apoptosis was measured by Annexin V/PI staining by Flow Cytometry. Annexin-V APC (BD Biosciences, Cat# 550474) was used at a 1/100 dilution and PI at a 12.5 $\mu\text{g/mL}$ final concentration in homemade Annexin V Binding Buffer.

Activation markers expression

24-hour activated CD4^+ WT-T or KO-T cells were stained for naive CD62L (BioLegend, Cat# 104406) marker, as well as canonical activation markers for 30 minutes with CD69 (BioLegend, Cat# 104541) and CD44 (BioLegend, Cat#103012).

Lipid and glucose uptake

Lipid uptake was assessed in anti- CD3 (5 $\mu\text{g.mL}^{-1}$) and anti- CD28 (2 $\mu\text{g.mL}^{-1}$)- activated WT-T or KO-T CD4^+ T cells for 24 hours, followed by incubation with BODIPY dyes for 1 hour at a final concentration of 1 μM . (BODIPY Red C12 and FL C16). Glucose uptake was measured by incubating activated CD4^+ T cells with glucose analog 2-NBDG (N13195, ThermoFisher) for 1 hour at 50 μM final concentration. Mean fluorescence intensity was measured by Flow Cytometry.

NAD(P)H quantification

NAD(P)H was measured using autofluorescence NAD(P)H signals in TIGAR WT-T and KO-T activated CD4^+ T cells for 24 hours using the UV-5 detector channel (~450 nm) using spectral flow cytometry. Emission corresponds to the natural fluorescence of reduced NADH and NADPH in cells.

Immunoblotting

Cells were lysed using RIPA buffer (Millipore SIGMA) supplemented with 1% SDS and phosphatase and protease inhibitor cocktail (Cell Signaling, Cat# 5872), denatured at 95 $^{\circ}\text{C}$, and

resolved on 10% polyacrylamide homemade gels. Gels were transferred onto nitrocellulose or PVDF membranes (Bio-Rad, Cat#1620115). Working dilution of primary and secondary antibodies are listed in the antibodies section.

Proteomics

Proteomic analysis was performed at the University of Sherbrooke platform including protein extraction with S-Trap micro-spin column digestion, followed by mass spectrometry and DIA-NN analysis. Volcano plot and lollipop plot of Upregulated and Downregulated proteins were created using Rstudio, version 2025.09.01+401.

S-Trap micro-spin column digestion

The S-Trap™ micro-spin column protocol (ProtiFi) was used with minor modifications for protein digestion. Cell pellets were resuspended in 92 µL of 1x lysis buffer (5% SDS, 50 mM TEAB, pH 8.5), heated at 95°C for 3 min, followed by sonication to minimise viscosity. Prior to processing, lysates were centrifuged at 16,000 x g for 5 min to clarify. BCA colorimetric assay was used to quantify protein concentration. Samples (up to 50 µg in 92 µL of 1x lysis buffer) were reduced with 4 µL of 120 mM tris(2-carboxyethyl) phosphine (TCEP, final concentration 5 mM), and incubated for 15 min at 55 °C. Extracts were alkylated by the addition of 4 µL of 500 mM chloroacetamide (CIAA) (20 mM final concentration), for 10 minutes in the dark at room temperature. Next, 5 µL of 27.5% phosphoric acid (PA) were introduced to the extracts, upon which 690 µL of wash buffer (90% methanol in 100 mM TEAB, pH 8.5) was added. Samples were then passed through Monarch® Spin PCR & DNA Cleanup columns (New England Biolabs), followed by 3 washes (150 µL of wash buffer). For in-column digestion overnight at 37 °C, a digestion buffer (20 µL of 200 mM TEAB including 5 µg of Trypsin MS-grade (Pierce)) was used. Digested peptides were eluted with 60 µL 50 mM TEAB, pH 8.5, after which 60 µL of 0.2% formic acid (FA) was added, and lastly 60 µL of 50% acetonitrile (CH₃CN). Eluates (3) were combined, transferred to 1.5 mL LoBind tubes, and dried with a SpeedVac. 50-300 µL of 1% FA in MS-grade water was used to resuspend the peptides, followed by quantification (NanoDrop). Peptides were stored at -80 °C until mass spectrometry analysis.

Mass spectrometry analysis

375 ng of Digested peptides were inserted into an HPLC (nanoElute, Bruker Daltonics, Burlington, ON, Canada) comprising a trap column (Acclaim PepMap100 C18, 0.3 mm id × 5 mm, Dionex Corporation, Sunnyvale, CA, USA), and an analytical C18 column (1.9 µm beads, 75 µm × 25 cm, PepSep, Bruker Daltonics, Burlington, ON, Canada). Peptide separation occurred for 2 hours at a 400 nL.min⁻¹ flow rate, and a linear gradient of 5–37% CH₃CN in 0.1% FA, while injected into an ion mobility mass spectrometer (TimsTOF Pro) via a Captive Spray nano electrospray source (Bruker Daltonics). The target intensity was set at 20 000 with an intensity threshold at 2500, and samples were acquired in diaPASEF mode. Each TIMS scan (100 ms) consisted of one ion mobility window and 27 m/z isolation steps (m/z 114–1,414, 50 Da width) per cycle (1.27 s duty cycle) with a collision energy of 42.0 eV. These steps span the diagonal acquisition range for +2 and +3 charged peptides in the m/z–ion mobility space.

DIA-NN analysis

DIA-NN software (version 1.9.2) and the Uniprot mouse proteome database (27/01/2024, 54,825 entries) were used to analyse the raw files, as previously performed⁶⁸. FASTA digestion was enabled for library-free search and library generation, along with Deep learning-based spectra, retention times, and ion mobility prediction. Analysis settings were the following: 2 miscleavages, 2 variable modifications; fixed modifications were carbamidomethylation on cysteine and N-terminal methionine excision; variable modifications were protein N-terminal acetylation and methionine oxidation; enzyme was trypsin; 7-30 amino acids peptide length; 2-4 as precursor charge range; 100-1700 as precursor m/z range; 110-1500 as fragment ion m/z range; 20 ppm of mass tolerance was fixed for both precursor and fragment ions; 5% of precursor FDR; match between runs, as well as enabling protein inference and peptidoform scoring options

The mass spectrometry proteomics data have been deposited with the ProteomeXchange Consortium via the PRIDE⁶⁹ partner repository under the dataset identifier PXD081095.

Enzyme-linked immunosorbent assay

ELISAs were performed on IFNγ from the plasma of colitis-induced mice using the Mouse IFNγ ELISA kit (eBioscience Thermo Fisher Scientific, Cat#KMC4021). Lipocalin was measured using the Mouse Lipocalin-2/NGAL DuoSet ELISA kit (R&D biosystems, Cat# DY1857) from

fecal extracts from *Citrobacter rodentium* infected mice. ELISAs were performed following the manufacturer's procedures.

Human CD4⁺ T cell TIGAR knock-down

Peripheral blood was collected from healthy, non-fasted individuals. Peripheral blood mononuclear cells (PBMCs) were isolated via density gradient centrifugation using Lymphoprep™ (STEMCELL Technologies). Human CD4⁺ T-cells (130-096-533) were isolated by magnetic separation using the autoMACS® Pro Separator as per the manufacturer's instructions (Miltenyi). T-cells were activated with plate-bound anti-CD3 (2 µg.ml⁻¹; OKT3; BioLegend) and soluble anti-CD28 (20 µg.ml⁻¹; CD28.2; BioLegend) at 37 °C in 5% CO₂-in-air for 24 hours as per previously described methods⁷⁰. To prevent impaired T-cell activation, culture media were supplemented with 10% dialysed fetal bovine serum (FBS; Fisher Scientific) after 3 hours. Gene editing of TIGAR in human CD4⁺ T cells was performed using CRISPR/Cas9. Pan CD4⁺ T cells were isolated by negative selection using the autoMACS isolation kit (Miltenyi Biotec), and stimulated with anti-CD3/CD28 for 48 hours. Concerning nucleofection, ribonucleoprotein complexes (RNPs) were generated by assembling 60 pmol of HiFi Cas9 nuclease (IDT) with 180 pmol of sgRNA (3:1 sgRNA:Cas9 ratio). Three distinct TIGAR-targeting sgRNAs (AA, AC, and AD) obtained from IDT were used. T cells were collected, washed with PBS, and resuspended at 2×10^6 cells in 20 µL P3 nucleofection buffer (Lonza). RNPs were added to the cell suspension, transferred to a 16-well strip (Lonza), and followed by nucleofection using a 4D nucleofection X unit (Lonza) with program EH115. Cells recovered for 24 hours in RPMI GlutaMAX medium (10% FBS), prior to re-activation with anti-CD3/CD28 for an additional 72 hours. Knock-down was validated by Western blot, and IFN γ was measured via ELISA.

Ethics statement

This research complies with all relevant ethics regulations at Swansea University. For healthy controls, informed written consent was obtained from all study participants, and ethical approval was granted by the Swansea University Medical School Research Ethics Committee (SUMSRESC; 2022-0029).

Stable isotope tracing by LC-MS

Stable isotope tracing of U-[$^{13}\text{C}_6$]-glucose (Cambridge Isotope Laboratories) was performed in glucose-free IMDM (Wisent) supplemented with 10% dialyzed FBS (dFBS). CD4⁺ T cells were activated with anti-CD3 (5 $\mu\text{g}\cdot\text{ml}^{-1}$) and anti-CD28 (2 $\mu\text{g}\cdot\text{ml}^{-1}$) for 24h. T cells were counted, washed, and replaced with the 10 mM U-[$^{13}\text{C}_6$]-glucose solution at a concentration of 2×10^6 cells ml^{-1} followed by a 4-hour pulse.

Stable isotope tracing of U-[$^{13}\text{C}_5$]-glutamine and U-[$^{13}\text{C}_3$]-alanine was performed in IMDM (Wisent) supplemented with glucose, 10% FBS, as well as all amino acids, respectively, excluding glutamine and alanine. Cells were processed, and metabolites were extracted as described above.

Following tracer incubation, cells were washed twice with ice-cold PBS and metabolites were extracted with an extraction buffer (50% methanol, 30% acetonitrile and 20% water) for 30 min at 4 °C; 500 μl of extraction buffer was used for 2×10^6 cells. After a short vortex step, the mixture was incubated on ice for 5 minutes. Centrifugation (13,000 rpm, 10 minutes, 4 °C) was used to pellet the protein. The supernatant was transferred to an Eppendorf tube and dried in a rotary vacuum concentrator. Polar metabolites were phase-partitioned from apolar metabolites by addition of 350 μl chloroform:methanol:water (1:3:3 v/v/v, containing 0.375 mol of [^{13}C]valine as an internal standard). The phases were separated by centrifugation (13,000 rpm, 10 min, 4 °C). The polar phase was transferred into a LC–MS vial equipped with an insert and dried in a rotary vacuum concentrator. Finally, 75 μl of a mixture of H₂O:methanol (1:1) were added to the dried extract. The samples were subsequently analysed by LC–MS.

Metabolites were analysed by LC–MS using a Q-EXACTIVE Plus (Orbitrap) mass spectrometer from Thermo Fisher Scientific coupled with a Vanquish UHPLC system from Thermo Fisher Scientific. The chromatographic separation was performed on a SeQuant ZicpHILIC (Merck Millipore) column (5 μm particle size, polymeric, 150 $\times$ 4.6 mm). The injection volume was 5 μl , the oven temperature was maintained at 25 °C, and the autosampler tray temperature was maintained at 4 °C. Chromatographic separation was achieved using a gradient program at a constant flow rate of 300 $\mu\text{l min}^{-1}$ over a total run time of 25 min. The elution gradient was programmed as a decreasing percentage of B from 80% to 5% for 17 min, holding at 5% of B for 3 min and finally re-equilibrating the column at 80% of B for 4 min. Solvent A was 20 mM

ammonium carbonate solution in water supplemented by 1.4 ml l⁻¹ of a solution of ammonium hydroxide at 35% in water and solvent B was acetonitrile. Mass spectrometry was performed in positive/negative ion modes with polarity switching using a Q-EXACTIVE Plus Orbitrap (Thermo Scientific) equipped with an HESI II probe. Mass spectrometry parameters were as follows: spray voltage 3.5 and 3.2 kV for positive and negative modes, respectively; probe temperature 320 °C; sheath and auxiliary gases were 30 and 5 arbitrary units, respectively; and full scan range: 70–1,050 *m/z* with settings of AGC target and resolution as balanced and high (3×10^6 and 70,000), respectively. Data were recorded using Xcalibur 4.2.47 software (Thermo Scientific). Mass calibration was performed for both ESI polarities prior to analysis using the Thermo Scientific Calmix standard solution. To enhance calibration stability, lock-mass correction was also applied to each analytical run using ubiquitous low-mass contaminants. Parallel reaction monitoring (PRM) acquisition parameters were the following: resolution 17,500; collision energies were set individually in HCD (high-energy collisional dissociation) mode. Metabolites were identified and quantified by accurate mass and retention time, and by comparison of retention times, mass spectra, and responses of known amounts of authentic standards using TraceFinder 4.1 EFS software (Thermo Fisher Scientific).

Seahorse analysis

Mitochondrial Stress Test: Mitochondrial respiration was assessed using a Mitochondrial Stress Test with the Agilent Seahorse X Analyzer (2.3.1). 24-hour activated WT-T versus KO-T cells were incubated with Seahorse XF Base Medium, supplemented with 25 mM Glucose, 2 mM glutamine and 25 mM sodium pyruvate (Wisent Multicell) following manufacturer's protocol. 2×10^5 cells CD4⁺ T cells were plated per well. Toxins were injected at the following final concentrations: 1 μM Oligomycin (Cayman Chemicals), 1.5 μM FCCP (Cayman Chemicals), 1 μM Antimycin A (Cayman Chemicals) and 1 μM Rotenone (AdooQ Bioscience).

Glycolysis Stress Test: Glycolytic rate was measured with a Glycolysis stress test with the Agilent Seahorse X Analyzer. 24-hour activated WT-T versus KO-T cells (2×10^5 cells/well) were incubated with Seahorse XF Base Medium, supplemented with 2 mM glutamine and 25 mM sodium pyruvate (Wisent Multicell) following manufacturer's protocol. Toxins were injected at

the following final concentrations: 10mM glucose (Wisent Multicell), 1.5 μ M Oligomycin (Cayman Chemicals), 50 mM 2-deoxy-D-Glucose (Cayman Chemicals).

Fatty Acid Oxidation Assay: Following naive CD4⁺ T cell isolation, TIGAR WT-T and KO-T cells seeded at 2×10^6 cells/well in 24-well anti-CD3/28 plate-bound activated plates in substrate limited growth media (0.5 mM Glucose, 1mM Glutamine, 1%FBS and 0.5mM L-Carnitine). The day of the assay, TIGAR WT-T and KO-T were counted and seeded at 2×10^5 cells/well in poly-D-lysine-coated Seahorse XF96 plates in assay medium containing 2 mM Glucose and 0.5 mM L-carnitine (Sigma Aldrich) and then incubated for 1 h at 37°C without CO₂. Sensor cartridge was hydrated according to manufacturer's recommendations and was calibrated with XF calibrant and incubated for 1 h at 37°C without CO₂. Toxins (Oligomycin, FCCP, and Antimycin A) were used at a final concentration of 2.5 μ M and were loaded in cartridge prior to calibration. BSA-palmitate (50 μ M final concentration) was added immediately before assay initiation. Etomoxir was used at a 4 μ M final concentration.

All bioenergetic metabolic assay data were processed with Agilent Seahorse XFp v2.5.3. program.

GWAS analysis and Mendelian randomization

Mendelian randomization analyses were performed using summary-level data from genome wide association studies (GWASs) to assess whether genetically predicted expression of selected genes influence inflammatory bowel disease (IBD) risk. Cis-eQTLs were obtained from the eQTLGen Consortium blood gene expression meta-analysis ($n = 31,684$). Instrumental SNPs were selected within ± 500 kb of each gene based on genome-wide significance ($P < 5 \times 10^{-8}$) and linkage disequilibrium pruning ($r^2 < 0.001$). After harmonisation with IBD GWAS summary statistics, valid instruments were identified for AKR1B1, RAPGEF2, and MFSD1. Outcome data were obtained from a large IBD GWAS including 42,950 IBD cases and 53,536 controls of predominantly European ancestry, with analyses performed for overall IBD, ulcerative colitis, and Crohn's disease. For genes with a single instrumental SNP, causal effects were estimated using the Wald ratio method^{50,51,53,71}.

Cell Migration tracking

BMDCs were differentiated for 7 days and stimulated with LPS (Millipore SIGMA) one day before live imaging. CD4⁺ T cells (WT-T and KO-T, or supplemented with Sorbitol, or Epalrestat) were activated and stained for VPD450 or CTFR, respectively. Right before imaging 2x10⁵ BMDC and 2x10⁵ T cells are pooled in phenol red-free imaging medium (10% Fetal Bovine Product (HyClone, Cat#SH30109.03), 100 IU Penicillin/Streptomycin) and plated onto 18 well slides (IBIDI, Cat#81816) for imaging. Widefield epifluorescence images of VPD450 (405nm) and CTFR (698nm) are captured every 30 seconds for 3 hours on a Nikon Eclipse Ti2, under a stage top incubator, with mercury lamp illumination.

From raw images, segmentation and tracking of T cells are made by adapting previously published methods^{72,73}. Briefly, in each frame, the centroid of each T cell is localized from the sum of the Indo-1 images using Cellpose. Based on the position of all cells at each timepoint we calculate individual T cell trajectories using particle tracking-based methods, adapted for our particular application.

3D-crystal structure modelisation

3D structural comparison of PFKFB3 (PDB: 3QPV) and TIGAR (PDB: 3DCY) was performed, and substrate interactions with the active sites of each enzyme were visualised by in silico docking of fructose-6-phosphate using PyMOL.

Molecular docking

Molecular docking was performed using AutoDock Vina (version 1.2.7) and Meeko (version 0.7.1). The AlphaFold-predicted structure of human TIGAR was downloaded as a PDB file from UniProt. The receptor structure was prepared by removing non-protein components, adding missing side chains and hydrogen atoms, and correcting protonation states to pH 7.4 using the H++ server from Virginia Tech. The prepared receptor structure was converted to PDBQT format using Meeko. For ligand preparation, files were downloaded as 3D structure PubCSD files from pubchem (links in the supplemental information document). Ligands were protonated at pH 7.4 using Open Babel (version 3.1.0), and Meeko was used to convert SDF files to PDBQT files. Blind (global) docking was performed using AutoDock Vina with a search grid encompassing the entire

receptor, allowing ligand exploration across the full protein surface. The grid box was centred at coordinates $x = -9.534$, $y = 3.415$, and $z = 3.048$, with dimensions of $70.148 \times 58.825 \times 69.314$ Å along the x , y , and z -axes, respectively. Docking calculations were performed using an exhaustiveness value of 32, with 20 binding modes generated per ligand and an energy range of 4 kcal/mol.

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Figure Legends

Figure 1: TIGAR regulates survival in T cells but not proliferation or glycolysis

(a) Immunoblot analysis of TIGAR protein expression in human naïve CD4⁺ T cells and following anti-CD3/CD28 stimulation for 24 h. Actin served as a loading control. **(b)** Immunoblot confirming TIGAR deletion in naïve and activated WT and TIGAR-null mouse CD4⁺ T cells. Vinculin served as a loading control. **(c)** Representative VPD450 histograms of WT and TIGAR-null CD4⁺ T-cell proliferation following stimulation with anti-CD3 alone or anti-CD3/CD28 for 72 h. **(d)** Quantification of early and late apoptotic WT and TIGAR-null CD4⁺ T cells 24 h after activation, determined by Annexin V/propidium iodide (PI) staining. $n = 3$ technical replicates per condition. **(e,f)** Seahorse extracellular flux analysis showing **e**, extracellular acidification rate (ECAR) during glycolytic rate measurements and **f**, glycolytic stress test. Oligo, oligomycin; 2-DG, 2-deoxy-D-glucose. **(g)** Schematic of glycolytic intermediates analysed by stable isotope tracing. Pentose phosphate pathway, PPP; Ribose-5-Phosphate (R5P); UTP, uridine triphosphate; Serine synthesis pathway (SSP). **(h)** LC–MS analysis of ¹³C₆-glucose incorporation into R-5-P, UTP, glycine, pyruvate and lactate. **(i)** schematic of upper glycolysis highlighting TIGAR, fructose-2,6-bisphosphate (F2,6BP) metabolism, and the PFKFB3 inhibitors AZ67 and 3PO. PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3; FBP1, fructose-1,6-bisphosphatase 1; PFK1, phosphofructokinase-1. **(j)** Representative proliferation histograms of CD4⁺ T cells stimulated for 72 h in the presence of AZ67 or 3PO (1 or 2 μ M). **(k)** Structural comparison of the catalytic sites of PFKFB3 and TIGAR, showing interactions with fructose-6-phosphate (F6P). **(l)** Molecular docking analysis of TIGAR with predicted ligands. Table shows the estimated free energy of binding (kcal mol⁻¹) for the highest-scoring docking pose. Data are presented as mean $\pm$ s.d. **(d)**. Statistical significance was determined using an unpaired two-tailed Student's *t*-test **(d)** or two-way ANOVA **(h)**. Immunoblots and proliferation assays are representative of three independent experiments **(a–c, e–j)**; stable isotope tracing is representative of two independent experiments **(h)**.

Figure 2: TIGAR regulates mitochondrial and glutamine metabolism in CD4 T cells, which dictates CD4 T cell fate

(a) Seahorse extracellular flux analysis of WT and TIGAR-null CD4⁺ T cells 24 h after TCR stimulation. Oxygen consumption rate (OCR) versus extracellular acidification rate (ECAR) during the mitochondrial stress test. *n* = 2 biological replicates per condition. (b) Schematic illustrating the contribution of glutamine to the tricarboxylic acid (TCA) cycle. (c,d) LC–MS analysis of ¹³C₅-glutamine tracing in WT and TIGAR-null CD4⁺ T cells 24 h after activation. (c) ¹³C distribution of glutamate, α-ketoglutarate (αKG) and citrate. (d) Isotopomer distribution of aspartate and glutathione (GSH). (e) Representative VPD450 histograms of CD4⁺ T-cell proliferation in the presence of increasing concentrations of the glutaminase inhibitor CB-839 in WT and KO T cells. (f) LC–MS analysis of ¹³C₅-glutamine tracing in activated CD4⁺ T cells from WT and KO-T mice cultured in the presence or absence of CB-839 (5μM), showing isotopomer distribution of glutamate, αKG and citrate. (g) Representative flow cytometry plots of Th1-polarized CD4⁺ T cells showing T-bet and IFNγ expression in WT and kO-T cells. (h) Immunoblot confirming TIGAR depletion in human CD4⁺ T cells transfected with scrambled (SCR) or TIGAR-targeting CRISPR/Cas9 guide RNA. Actin served as a loading control. (i) IFNγ secretion from human CD4⁺ T cells expressing scrambled or TIGAR-targeting guide RNA, measured by ELISA (*n* = 5 donors). (j, k) Representative flow cytometry plots of Th17-polarized WT and KO-T CD4⁺ T cells showing RORγt and IL-17A expression. Data are presented as mean ± s.d or s.e.m. Statistical significance was determined using two-tailed unpaired Student's *t*-tests (c,d) or two-way ANOVA with Šídák multiple comparisons test. (f). Seahorse analysis and stable isotope tracing are representative of two independent experiments (a,c,f). Flow cytometry data are representative of three independent experiments (g,j,k).

Figure 3: TIGAR is a negative regulator of the polyol pathway in CD4⁺ T cells

(a) Volcano plot of quantitative proteomic analysis comparing WT and TIGAR-null CD4⁺ T cells 24 h after anti-CD3/CD28 stimulation. Proteins significantly upregulated (right) and downregulated (left) are shown (*P* < 0.05). Akr1b1, aldo-keto reductase family 1 member B1;

Myh, myosin family proteins. **(b)** Lollipop plot highlighting selected significantly upregulated proteins identified by quantitative proteomics ($P < 0.05$; $n = 4$ biological replicates per condition). **(c)** Schematic of the polyol pathway. AR, aldose reductase; SORD, sorbitol dehydrogenase. **(d,e)** Representative flow cytometry plots of WT CD4⁺ T cells differentiated under **(d)** Th1- or **(e)** Th17-polarizing conditions in the presence of increasing concentrations of sorbitol (1.25, 2.5 or 5 mM), showing T-bet/IFN γ and ROR γ t/IL-17A expression, respectively. **(f,g)** Representative flow cytometry plots of WT CD4⁺ T cells differentiated under **(f)** Th1- or **(g)** Th17-polarizing conditions in the presence of the aldose reductase inhibitor epalrestat (1 or 5 μ M), showing T-bet/IFN γ and ROR γ t/IL-17A expression, respectively. **(h,i)** Uptake of fluorescent fatty acid analogues measured by flow cytometry and quantified as mean fluorescence intensity (MFI) between activated WT and TIGAR-deficient CD4⁺ T cells. **(h)** Medium-chain fatty acid analogue BODIPY FL C12 (lauric acid). **(i)** Long-chain fatty acid analogue BODIPY FL C16 (palmitic acid). **(j)** Seahorse extracellular flux analysis of fatty acid oxidation using octanoate (C8) as substrate in WT and KO-T activated CD4⁺ T cells. OCR, oxygen consumption rate. Eto, etomoxir (5 μ M); oligomycin (1.0 μ M); FCCP (1.5 μ M); rotenone (1 μ M); and antimycin A (1 μ M). Data are presented as mean $\pm$ s.d. **(h,i)**. Statistical significance was determined using two-tailed unpaired Student's *t*-tests **(h,i)**. Flow cytometry and Seahorse data **(d–j)** are representative of three independent experiments.

Figure 4: TIGAR and the aldose reductase modulate T cell movement behaviour

a. Confocal microscopy images of WT-T or KO-T CD4⁺ T cells post anti-CD3/28 stimulation. DAPI is represented in blue and Phalloidin in Red, scale is 2 μ M. **b.** Live imaging diagram of co-culture between activated CD4⁺ T cells (WT-T and KO-T) with anti-CD3/28 for 24 hours and expanded BMDCs (Bone Marrow-Derived Dendritic Cells) for 8 days. WT-T CD4⁺ cells were stained in VPD450 (BD Horizon™ Violet Proliferation Dye 450) whereas KO-T CD4⁺ T cells were stained in CTFR (CellTrace™ Far Red). **c.** Schematic representation of cell trajectory and definitions of migratory parameters. v : speed; d_{net} : net distance; d_{max} : max distance; d_{tot} : total distance. **d.** Formulas of selected migratory parameters. **e.** Comparison between mathematical parameters of migration (average speed, meandering index, outreach ratio and ϕ - phi: relative

turning angle) of WT-T CD4⁺ T cells versus KO-T CD4⁺ T cells. Each point represents the average parameter for all cells in a field of view (FOV). FOV data is normalized to the average of WT-T per experiment. Data are representative of 2 independent experiments. Each point represents the average parameter for all cells in a FOV. Significance was tested using a Mann-Whitney test. **f.** Comparison between migration characteristics (average speed, meandering index, outreach ratio and ϕ - ϕ : relative turning angle) of WT-T CD4⁺ T cells in presence or absence of Epalrestat (1 and 5 μ M). WT-T CD4⁺ cells without sorbitol and with Epalrestat (1 and 5 μ M) were stained in VPD450. Data are normalised to the WT-T average per well. Each point represents the average parameter for all cells in a FOV. Significance was tested with an Ordinary one-way ANOVA. Data are mean $\pm$ s.d. (**e-f**).

Figure 5: TIGAR deficiency promotes IFN γ -dominant responses during *Citrobacter* infection

(a) Experimental design of the *C. rodentium* infection model in WT (n=6) and KO-T (n=5) mice. **(b)** Faecal bacterial burden, expressed as colony-forming units (CFUs) per gram of faeces, on days 7 and 12 post-infection. **(c)** Caecal bacterial burden at day 13 post-infection. **(d)** Representative flow cytometry plots and **(e)** quantification of IL-17A⁺, IFN γ ⁺ and IL-17A⁺IFN γ ⁺ CD4⁺ T cells in the colonic lamina propria following *ex vivo* intracellular cytokine staining. **(f)** Quantification of CD4⁺ T cell frequencies of splenic IL-17A⁺, IFN γ ⁺ and IL-17A⁺IFN γ ⁺ CD4⁺ T cells at day 13 post-infection. **(g)** Quantification of Th17-associated cytokines IL-21, IL-23 and IL-31 in faecal samples collected on day 12 post-infection from WT (*n* = 5) and TIGAR-null (*n* = 4) mice. Data are presented as mean $\pm$ s.e.m. Statistical significance was determined using two-tailed unpaired Student's *t*-tests (**b,c,e-g**).

Figure 6: TIGAR deletion in CD4 T cells exacerbates colitis and identifies aldose reductase as a downstream effector associated with human colonic disease

(a) Experimental design of the adoptive T-cell transfer colitis model. (b) Survival of Rag2^{-/-} recipient mice following adoptive transfer of WT or TIGAR-null CD4⁺CD25⁻ T cells. Statistical significance was determined using a log-rank (Mantel–Cox) test. (c,d) Flow cytometric analysis of recipient mice 21 days after adoptive transfer ($n = 5–6$ biologically independent mice per group). c. Frequencies of IFN γ -producing CD4⁺ T cells in the cLP, spleen and pLN. D. Frequencies of FOXP3-expressing CD4 T cells in the cLP, spleen and mesenteric lymph nodes (mLN). e. Forest plot showing Mendelian randomization estimates of the effect of genetically predicted gene expression on inflammatory bowel disease outcomes (IBD, UC and CD). f. Graphical Abstract. Data are mean $\pm$ s.e.m. (c–d) or odds ratios (OR) with 95% confidence intervals (CI) (e).

Acknowledgements

The authors would like to thank the following: Marie-Laure Durand and Fany DeWilde for the help with animal husbandry; the CR-HMR and the Crick flow cytometry facility for help with sorting and flow cytometry experiments; and the CR-HMR Microscopy Facility. This work was funded by the National Sciences and Engineering Research Council of Canada grants (NSERC-PIN-2023-04494 and NSERC-PIN-2023-00053), the Cole Foundation, start-up funds from the Fondation de l'Hôpital Maisonneuve Rosemont, and supported by the Francis Crick Institute which receives its core funding from Cancer Research UK (FC0010557 & FC001088), the UK Medical Research Council (FC0010557 & FC001088) and the Wellcome Trust (FC0010557 & FC001088). J.B receives salary support from the Fonds de Recherche du Québec Junior 1. E.B.G is funded by the Stavros Niarchos Foundation. Y.R.J. is funded by a Swansea University Research Excellence Scholarship. N.J. was supported by an MRC New Investigator Research Grant (MR/X000095/1). S.T received salary support from the Cancer Research Society. B.D. is supported by a Wellcome Trust studentship (218495/Z/19/Z) at the University of Bristol. B.D and E.E.V. are part of the Medical Research Council Integrative Epidemiology Unit at the University of Bristol, which is supported by the Medical Research Council (MC_UU_00032/03) and the University of Bristol.

Author contributions

J.B, F.Z and K.H.V conceptualised the project. E.B.G, F.Z, and J.B performed most of the experiments and analysed the data. J.B, M.H, and L.M performed metabolomics analysis. F.M.P-G., Y.R.J., and N.J. conceptualised and performed human T-cell experiments. B.D and E.E.V. conceptualised and performed MR analysis. S.T, S.C, and H.M conceptualised and analysed T-cell tracking experiments. M.L performed microscopy. N.L.O and S.G conceptualised and performed the *Citrobacter* model. A.G and B.N performed molecular docking analysis. J.B, F.Z and E.B.G. wrote and prepared the manuscript. All authors critically revised and approved the manuscript.

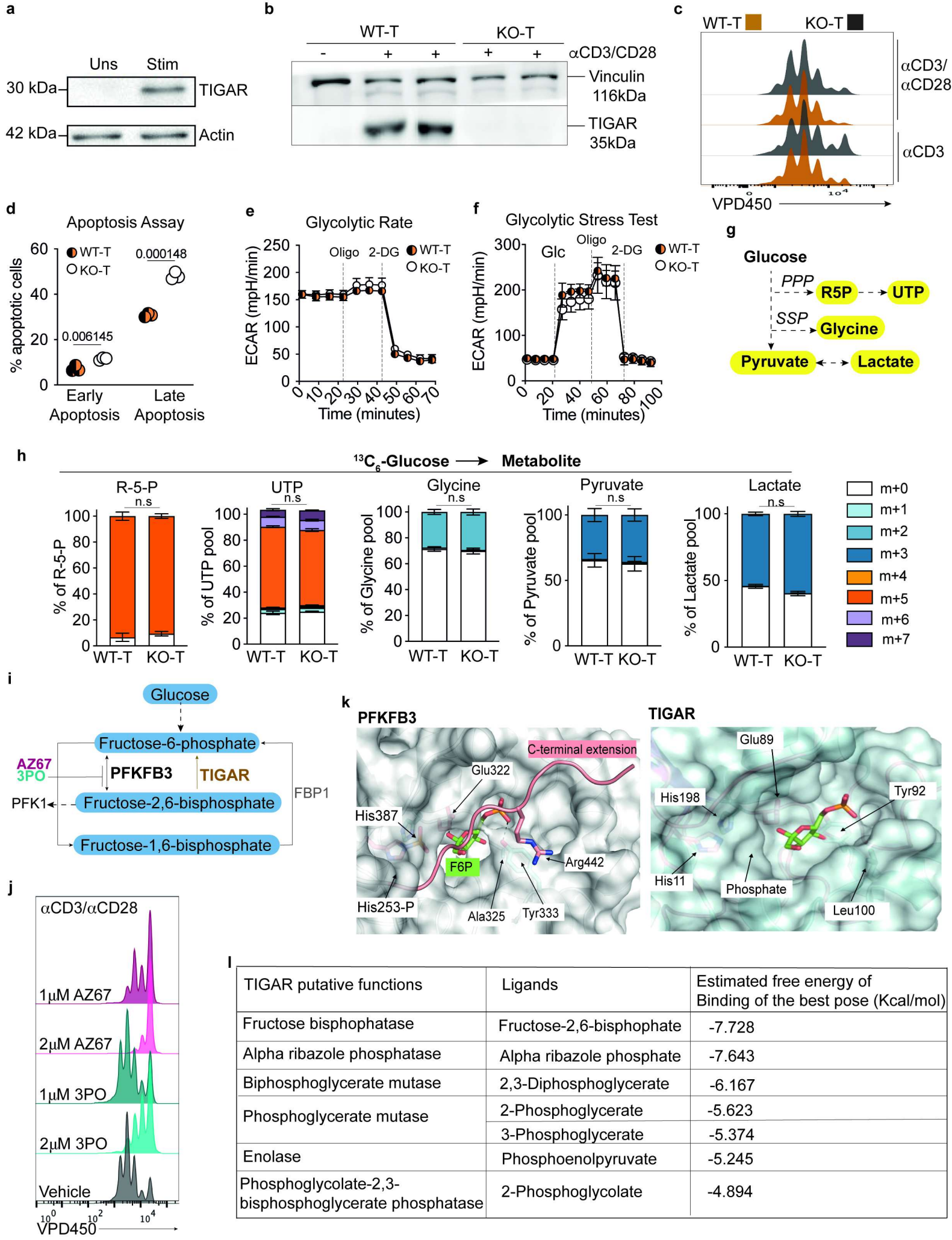
Competing Interest Declaration

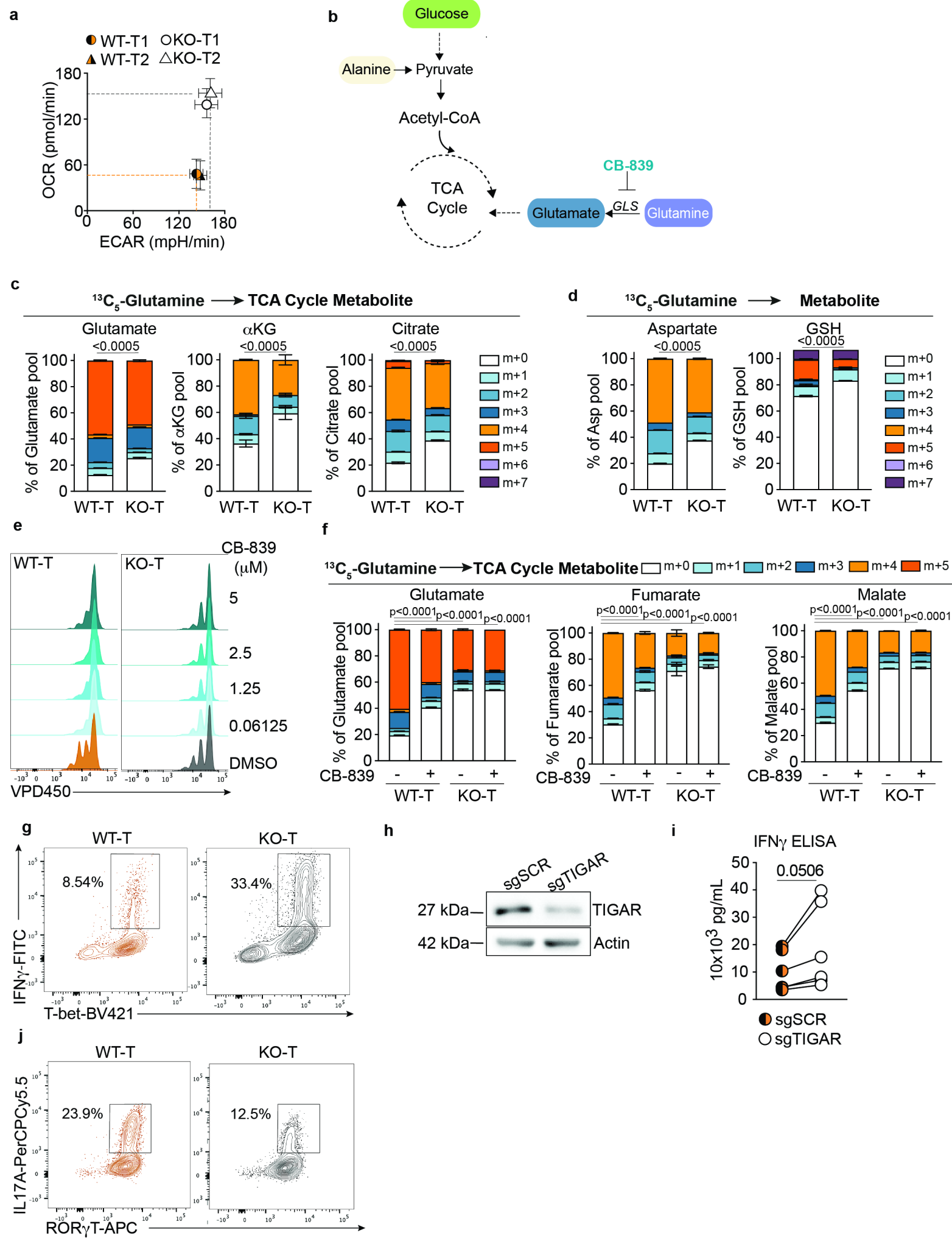
KHV is on the board of directors and shareholder of Bristol Myers Squibb and on the science advisory board (with stock options) of PMV Pharma, RAZE Therapeutics, Volastra Pharmaceuticals and Kovina Therapeutics. She is on the SAB of Ludwig Cancer and a co-founder and consultant of Faeth Therapeutics. She has been in receipt of research funding from Astex Pharmaceuticals and AstraZeneca and contributed to CRUK Cancer Research Technology filing of patent application WO/2017/144877.

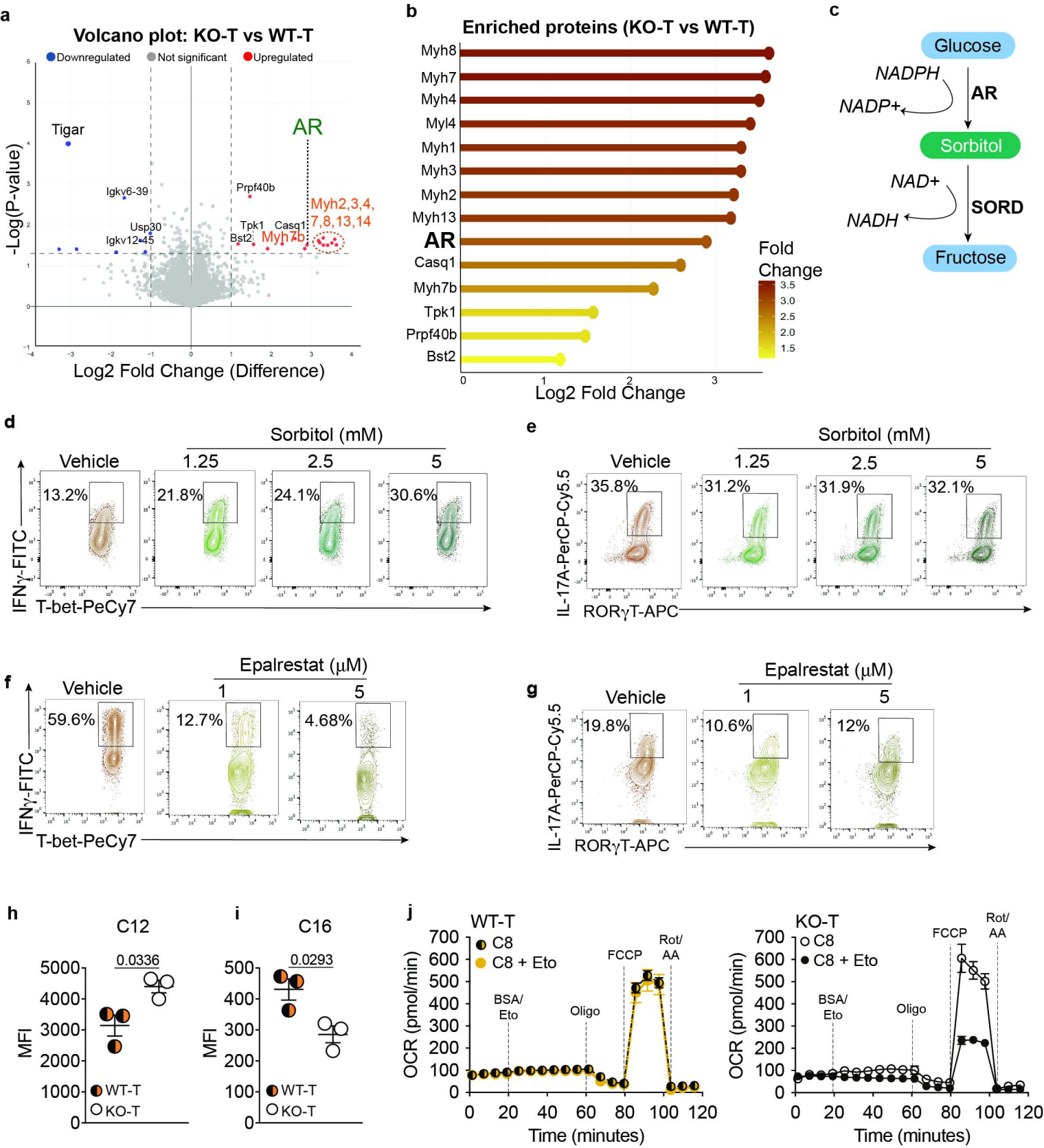
The other authors declare no competing interests.

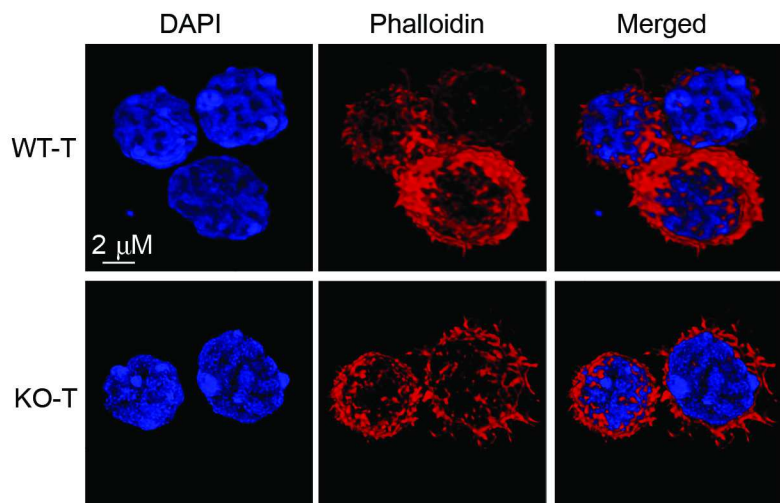
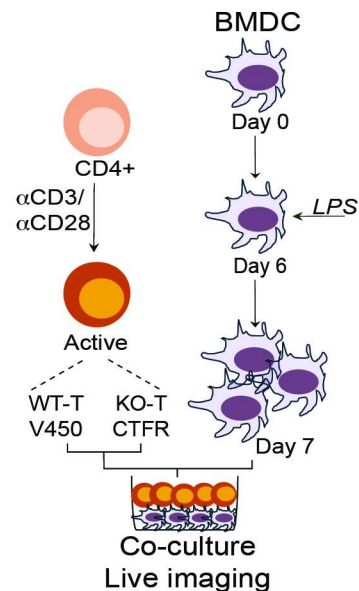
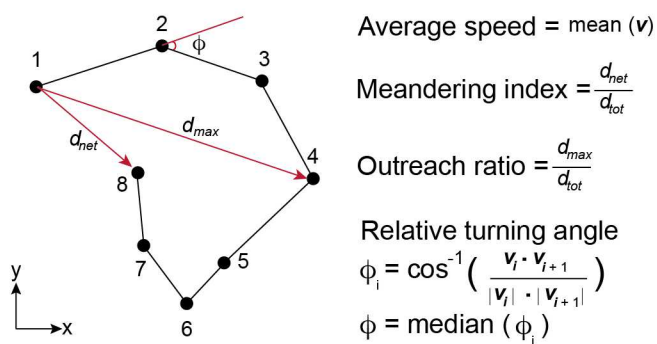
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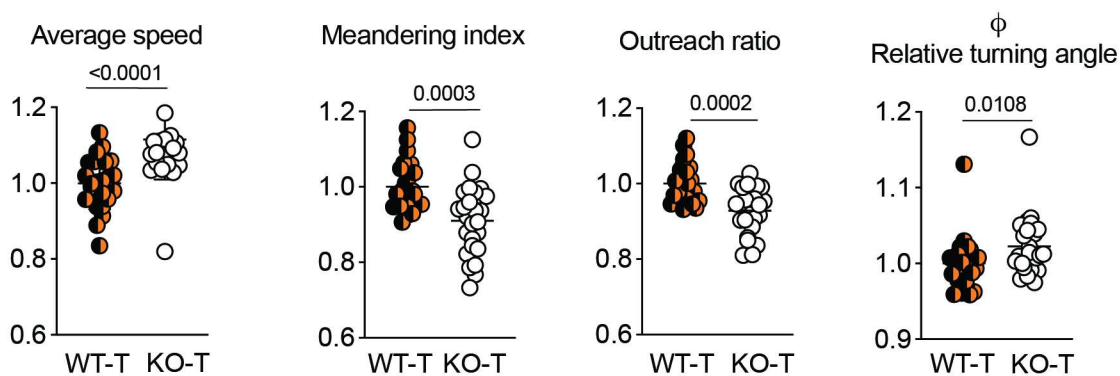
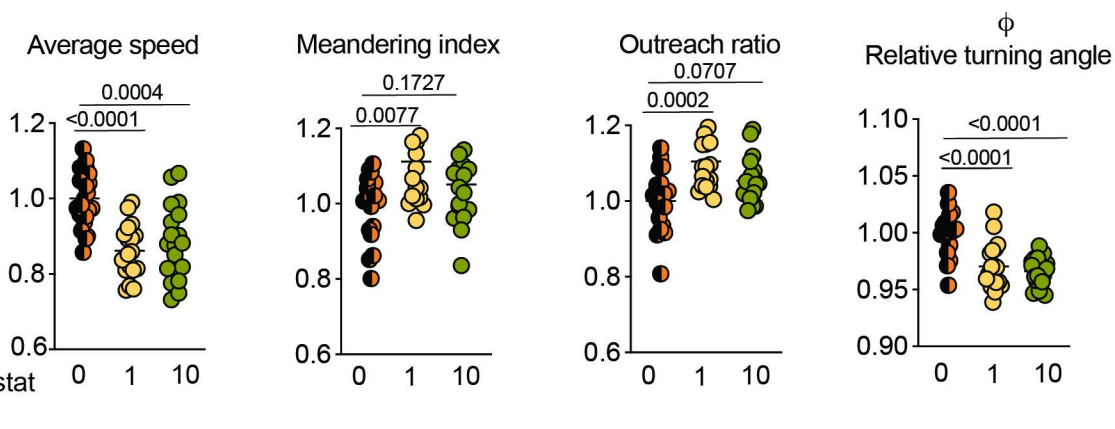


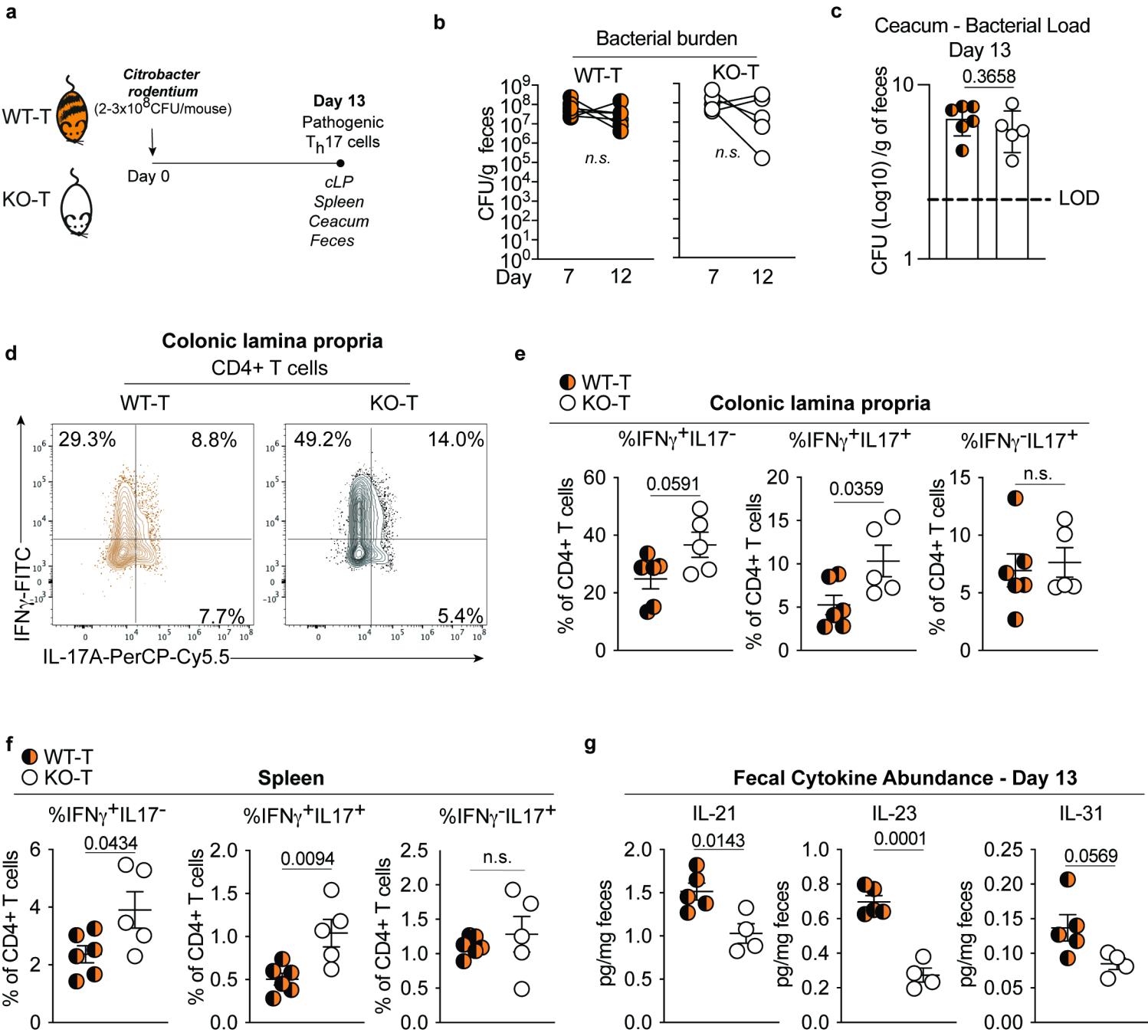


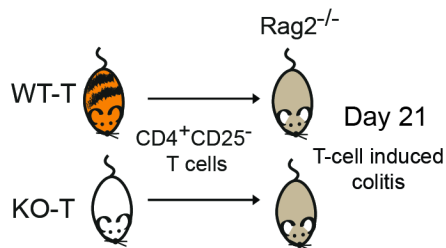
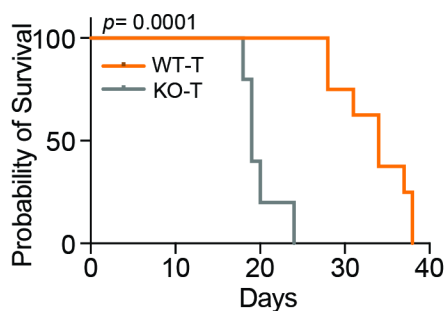
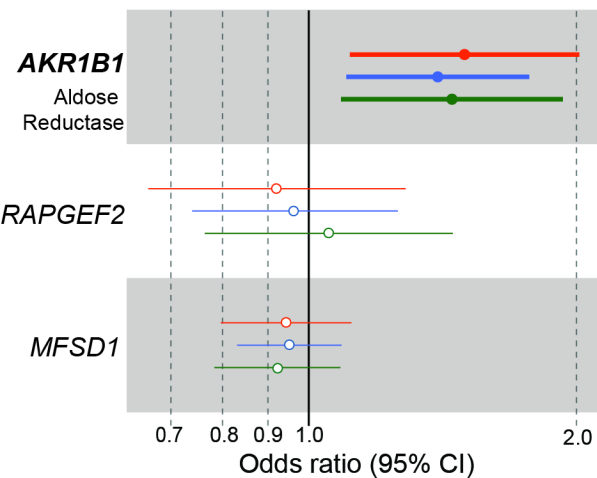
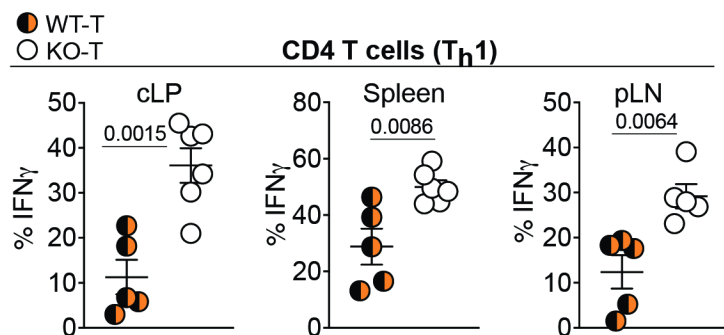
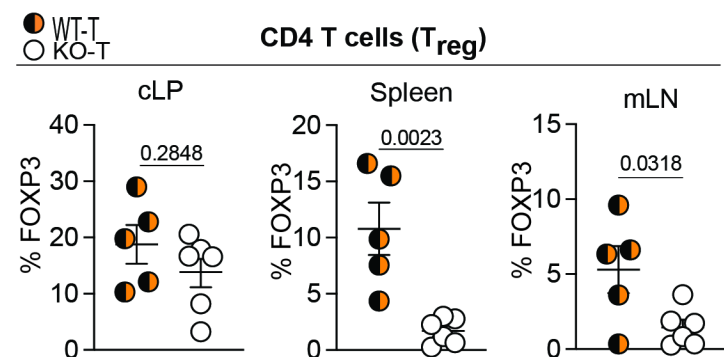
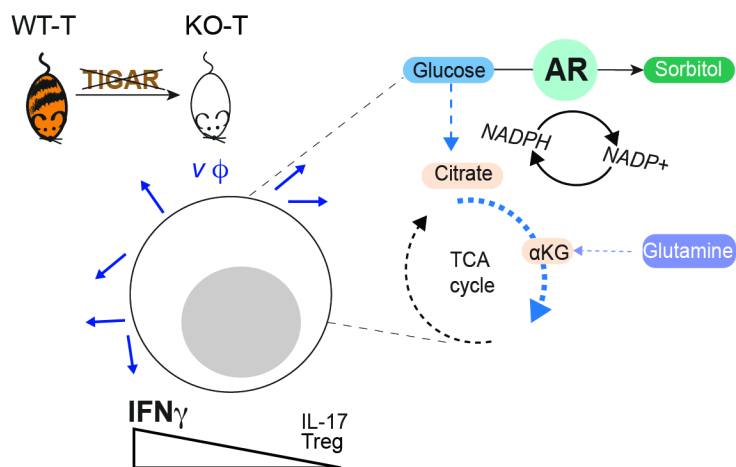
a**b****c****d**

Speed	$\mathbf{v}_t = \frac{d_{t, t+1}}{\Delta_t}$
Distance	$d_{ij} = \sqrt{(x_i - x_j)^2 + (y_i - y_j)^2}$
Net distance	$d_{net} = d_{1,N}$
Max distance	$d_{max} = \max(d_{1,N})$
Total distance	$d_{tot} = \sum_{t=i}^j d_{t, t+1} \cdot \frac{1}{j-i}$

with i, j the first and last time point recorded for the cell

e**f**



a**b****e****c****d****f**

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