

TOPICAL REVIEW

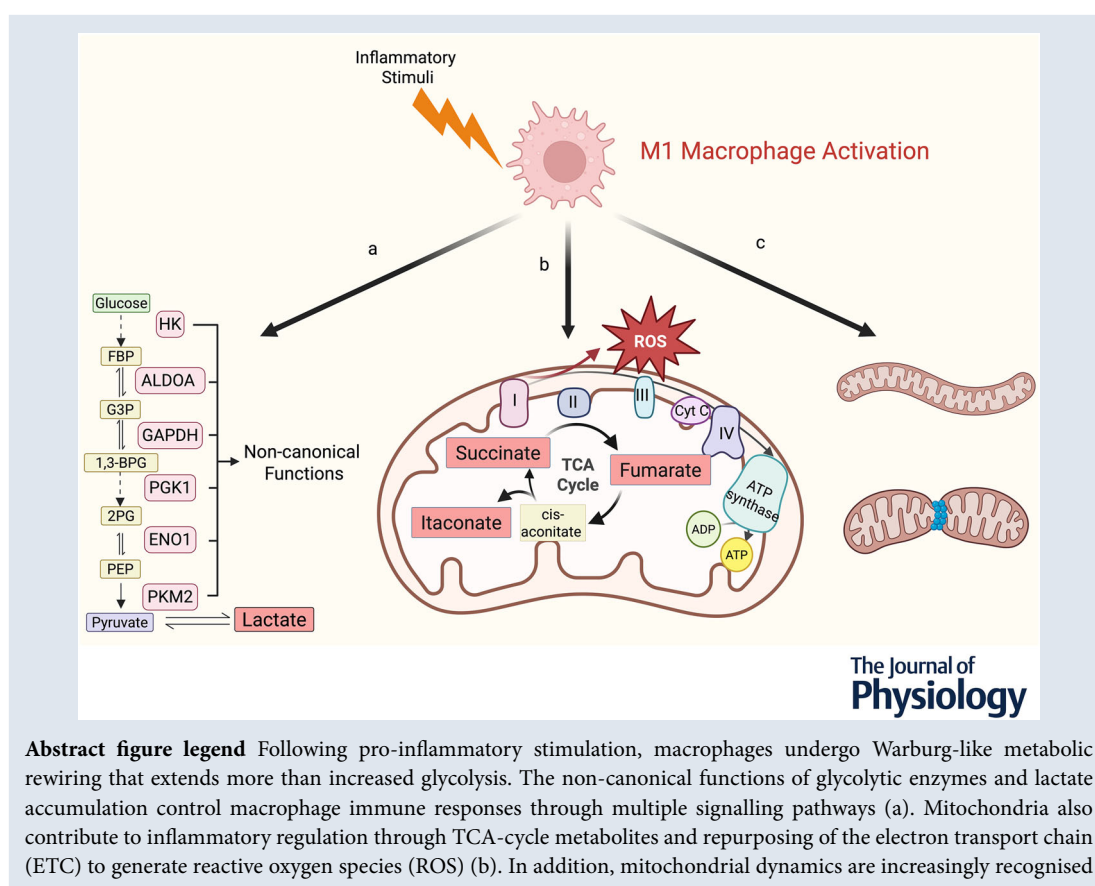
Warburg redefined: Metabolites, mitochondria and macrophages in immune regulation

Yukun Min, Órlaith C. Henry  and Luke A. J. O'Neill 

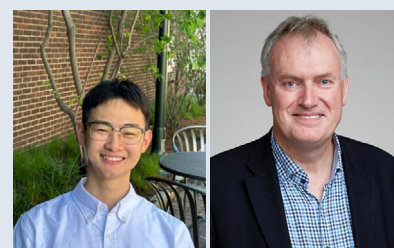
School of Biochemistry & Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland

Handling Editors: Laura Bennet & Cormac Taylor

The peer review history is available in the Supporting Information section of this article (<https://doi.org/10.1113/JP291073#support-information-section>).



Yukun Min is a PhD researcher in the O'Neill laboratory at Trinity College Dublin. His research focuses on immunometabolism, with particular interests in macrophage metabolic reprogramming and innate immune signalling. His work explores how metabolic pathways and metabolites, such as fumarate, influence type I interferon signalling and macrophage function. **Luke O'Neill** is a professor of biochemistry at Trinity College Dublin and a world-leading researcher in innate immunity and immunometabolism. His research focuses on the molecular mechanisms that regulate inflammation, with particular interests in macrophage metabolism and the roles of metabolites in immune responses. His work has contributed substantially to understanding how metabolic pathways shape innate immune function and inflammatory disease.



as active participants in macrophage metabolic and immune responses rather than merely a consequence of the pro-inflammatory activation of macrophages (c).

Abstract Nearly 100 years ago, Otto Warburg observed that cancer cells preferentially use glycolysis even in the presence of oxygen, supporting rapid cell growth. Since then, the concept of the Warburg effect has expanded far beyond tumour bioenergetics and is now recognised as a key feature of immune cell activation. A notable example is in macrophages, which, following pro-inflammatory stimulation, undergo Warburg-like metabolic reprogramming, characterised by increased glycolysis. Recent studies have shown that accumulated metabolites such as lactate have a range of immunoregulatory functions that shape macrophage inflammatory responses. In addition, glycolytic enzymes have been found to exert non-canonical functions that directly regulate macrophage immune signalling. Mitochondria, once considered suppressed during Warburg reprogramming, also undergo profound changes, with the electron transport chain generating reactive oxygen species and metabolites such as succinate, fumarate and itaconate playing complex immunoregulatory roles, and mitochondrial dynamics actively shaping metabolism and immune responses. Together, these findings redefine the Warburg effect in macrophages as a coordinated metabolic and signalling process that integrates glycolysis, mitochondrial function and immunometabolite signalling to optimise context-dependent immune responses.

(Received 17 July 2026; accepted after revision 28 September 2026; first published online 15 October 2026)

Corresponding author L. A. J. O'Neill: School of Biochemistry & Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Dublin, Ireland. Email: LAONEILL@tcd.ie

Introduction

Cellular metabolism comprises a highly coordinated network of biochemical reactions that maintains homeostasis and supports cell survival, growth and proliferation. Among the various metabolic pathways, glucose metabolism plays a central role by fuelling cell activities and synthesising building blocks such as nucleic acids, lipids and amino acids. Under aerobic conditions, glucose metabolism is divided into three interconnected stages: glycolysis, the tricarboxylic acid (TCA) cycle and oxidative phosphorylation (OxPhos). With electrons flowing from the TCA cycle via nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH₂), oxygen serves as the terminal electron acceptor in this process, allowing efficient ATP production via proton pumping through the ATP synthase. On the other hand, under oxygen-limited conditions, mitochondrial respiration is reduced, and cells rely mainly on glycolysis for ATP generation.

However, a century ago, Otto Warburg observed that cancer cells preferentially convert glucose to lactate even with sufficient oxygen (Warburg, 1923, 1925). This phenomenon was termed the Warburg effect and has since become a hallmark of cancer metabolism and a major focus of cancer research. Although aerobic glycolysis is less efficient than OxPhos, it enables rapid generation of ATP and metabolic intermediates required for biosynthesis, facilitating tumour growth and proliferation.

Oncogenic signalling pathways, including those targeted by Ras and c-Myc, promote activation of mammalian target of rapamycin (mTOR) and hypoxia-inducible factor-1 (HIF-1), which then stimulate glycolysis and suppress mitochondrial respiration in cancer cells (De Leon-Oliva et al., 2025; Vaupel & Multhoff, 2021).

Macrophages are innate immune cells that recognise pathogens and danger signals through a wide range of pattern-recognition receptors (PRRs). Following activation, macrophages generally diverge into two broad phenotypes: classically activated M1 macrophages and alternatively activated M2 macrophages (Yunna et al., 2020). They then move from a basal state into either M1 or M2 macrophages. M1 activation is typically induced by microbial infections, inflammatory cytokines or damage-associated molecular patterns (DAMPs). These macrophages produce pro-inflammatory cytokines and reactive oxygen species (ROS), supporting antimicrobial defence and amplifying inflammatory responses (Yunna et al., 2020). In contrast, M2-like macrophages are commonly induced by regulatory cytokines or parasitic infection (Yunna et al., 2020). They generally perform immunoregulatory and tissue-repair functions by producing mediators such as interleukin (IL)-10 and transforming growth factor (TGF)- β .

These distinct macrophage functions are accompanied by extensive metabolic reprogramming. Resting basal state macrophages predominantly rely on mitochondrial OxPhos to meet their basal energetic requirements

(Wculek et al., 2022). M2-like macrophages also generally maintain a relatively intact TCA cycle and mitochondrial respiration (Wculek et al., 2022). Fatty acid oxidation further contributes to metabolic support for OxPhos. In contrast, M1-like macrophages have a metabolic phenotype with features similar to the Warburg effect observed in cancer cells. Glycolysis is markedly enhanced to support rapid ATP production, while mitochondrial respiration and the TCA cycle are extensively remodelled (Wculek et al., 2022). This metabolic reprogramming also contributes to the production of ROS and other inflammatory mediators. These changes suggest that the Warburg effect is not unique to cancer cells but is also a fundamental feature of M1 macrophages.

Despite the historical emphasis on instant energy supply, recent advances have substantially broadened our understanding of Warburg metabolism in macrophages. Glycolytic enzymes perform functions beyond their conventional metabolic activities, and lactate has emerged as a pleiotropic signal in macrophages. Furthermore, rather than representing a complete metabolic switch from OxPhos to aerobic glycolysis, mitochondria are increasingly considered as key participants in macrophage immune responses (Wang et al., 2021). In this review, we will describe these advances with a particular focus on macrophage metabolism. The hope is that these discoveries will help in the development of new therapies for immune and inflammatory diseases.

Rediscovering glycolysis in macrophage Warburg-like metabolism

Non-canonical functions of glycolytic enzymes in macrophages. Following bacterial or inflammatory stimulation, macrophages markedly increase glycolysis while mitochondrial respiration and OxPhos are impaired (Hard, 1970). Subsequent studies demonstrated that activated macrophages divert a substantial proportion of glucose-derived carbon through glycolysis towards lactate production rather than complete mitochondrial oxidation (Newsholme et al., 1986). Mechanistic studies further demonstrate the importance of glycolysis in macrophage inflammatory responses, as deletion of HIF-1 α impaired glycolysis and attenuated inflammatory gene expression (Cramer et al., 2003). However, accumulating evidence suggests that the glycolytic enzymes also regulate immune responses independent of their metabolic function. In this section, non-canonical functions of hexokinase (HK), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), aldolase A (ALDOA), phosphoglycerate kinase 1 (PGK1), enolase 1 (ENO1) and pyruvate kinase M2 (PKM2) in macrophages are discussed in detail.

Hexokinase. HK is the first enzyme of glycolysis and catalyses the conversion of glucose to

glucose-6-phosphate (G6P). This reaction controls whether glucose enters glycolysis, the pentose phosphate pathway (PPP) and other biosynthetic processes. Increased HK activity has long been recognised as a feature of the Warburg effect in tumour cells (Li et al., 2022). A similar increase in HK activity has also been observed in M1 macrophages (Newsholme et al., 1986). HK activity influences macrophage immune activation not only by controlling glycolytic flux but also by shaping downstream metabolic and signalling pathways.

Recent studies have shown that overexpression of HK2, an isomer of HK, increased glycolysis and lactate production, leading to enhanced histone lactylation at the promoters of glycolytic genes (Li, Chen, Song et al., 2025). Blocking HK2 reduced histone lactylation and pro-inflammatory cytokine production. HK spatial location also influences macrophage glucose metabolism and inflammatory responses (De Jesus et al., 2022). Under normal conditions, HK1 and HK2 can bind voltage-dependent anion channel (VDAC) on the outer mitochondrial membrane (OMM). However, cytosolic localisation of HK1 reduced glycolytic flux beyond GAPDH while enhancing PPP activity in response to lipopolysaccharide (LPS) (Fig. 1a). Mechanistically, cytosolic HK1 interacted with the DAMP S100A8/A9 and inhibited GAPDH by inducible nitric oxide synthase (iNOS)-dependent S-nitrosylation. As a result, G6P was redirected towards the PPP, supporting inflammatory cytokine production. These findings indicate that HK activity affects the metabolic rewiring of M1 macrophages.

Beyond its catalytic function, HK can also act as a sensor for microbial and metabolic stimuli. Degradation of Gram-positive bacterial peptidoglycan releases the sugar subunit *N*-acetylglucosamine (NAG), which inhibits HK2 and induces its dissociation from VDAC (Wolf et al., 2016). Experimental induction of HK2 release triggers NLR family pyrin domain containing 3 (NLRP3) inflammasome activation, a key pro-inflammatory signal in macrophages, which promotes caspase-1-mediated processing of pro-IL-1 β and pro-IL-18 and Gasdermin (GSDM) D to drive pyroptosis. A subsequent study further clarified how HK2 dissociation promotes NLRP3 inflammasome assembly (Baik et al., 2023). HK2 dissociation promoted activation of inositol trisphosphate receptors (IP3Rs) and transferred calcium from the endoplasmic reticulum (ER) to mitochondria. This then induced VDAC oligomerisation and mitochondrial DNA (mtDNA) release for NLRP3 inflammasome activation (Fig. 1a). Inhibition of VDAC oligomerisation reduced caspase-1 activation and IL-1 β secretion in response to multiple NLRP3 stimuli.

However, HK-dependent signalling does not always promote macrophage inflammation. In tumour-associated macrophages (TAMs), fructose enhanced the interaction between HK2 and the ER

calcium channel ITPR3 (Yan, Wang et al., 2024). This interaction disrupted the calcium transfer from ER to cytosol and mitochondria. Nuclear factor kappa-light-chain-enhancer of activated b cells (NF- κ B) and NLRP3 inflammasome activation were suppressed by reduced calcium signalling, thereby impairing M1-like macrophage polarisation. Thus, HK2 can either promote or restrain inflammatory activation depending on the stimulus, binding partner and subcellular context. Pathogens may also exploit the HK–VDAC axis to promote their survival (Li, Chen, Wang et al., 2025). During *Mycobacterium bovis* infection, HK2 dissociated from VDAC, leading to mitochondrial stress and damage. This then activated mitophagy and promoted intracellular persistence of the pathogen. These results indicate that HK acts as both a metabolic gatekeeper and an immune signalling sensor that connects Warburg-like metabolic reprogramming to macrophage inflammatory function.

Aldolase A. ALDOA is a glycolytic enzyme that catalyses the reversible conversion of fructose-1,6-bisphosphate (FBP) into glyceraldehyde-3-phosphate (G3P) and dihydroxyacetone phosphate (DHAP). Although it is

classically viewed as a glycolytic enzyme, recent evidence suggests that ALDOA can also regulate macrophage antimicrobial function through a non-canonical role in phagosome maturation.

In macrophages infected with *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*), glycolytic metabolism was initially induced but later suppressed by the pathogen (Gutierrez et al., 2021). This reduction in glycolytic flux was associated with impaired vacuolar H⁺-ATPase (v-ATPase) complex and lysosomal activity. Inhibition of glycolysis impaired recruitment of the cytosolic V1 subunit to phagosomes and reduced formation of the functional complex. Under glucose-replete conditions, ALDOA localised to bacteria-containing or particle-containing phagosomes and interacted with v-ATPase components (Gutierrez et al., 2021) (Fig. 1b). In contrast, glycolysis inhibition reduced ALDOA recruitment to phagosomes and weakened its interaction with the v-ATPase V0 domain. Knockdown of ALDOA directly reduced phagosomal acidification and increased intracellular *S. Typhimurium* survival. Notably, a catalytically inactive ALDOA mutant still interacted with the v-ATPase subunit, suggesting that ALDOA

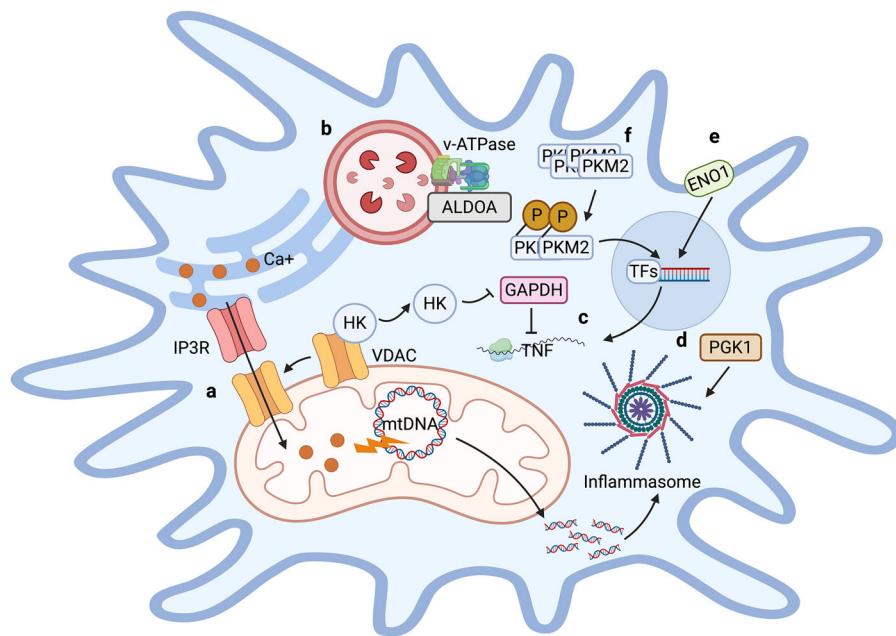


Figure 1. Non-canonical functions of glycolytic enzymes

Glycolytic enzymes can have functions besides their original enzymatic activity. After LPS stimulation, hexokinase (HK) dissociates from the voltage-dependent anion channel (VDAC) and inhibits glyceraldehyde 3-phosphate dehydrogenase (GAPDH), diverting carbon flux toward the pentose phosphate pathway (PPP) and supporting inflammatory cytokine production (a). Disassociated VDAC interacts with IP3R and promotes calcium translocation into the mitochondrial matrix (a). This then disturbs mtDNA and induces its release to the cytosol and subsequently activates inflammasome. Aldolase A (ALDOA) binds to v-ATPase and facilitates bacterial degradation (b). GAPDH binds to *TNF* mRNA to reduce its translation (c). Phosphoglycerate kinase 1 (PGK1) interacts with NLRP3 and boosts inflammasome activation (d). Enolase 1 (ENO1) translocates to the plasma membrane and induces activation of proinflammatory transcription factors (TFs) such as NF- κ B to induce inflammatory responses (e). Phosphorylation of pyruvate kinase M2 (PKM2) forms dimers and translocates into the nucleus (f). It then interacts with multiple TFs to promote the expression of glycolysis enzymes and pro-inflammatory cytokines.

may support phagosome acidification independent of its enzymatic activity.

Glyceraldehyde-3-phosphate dehydrogenase. GAPDH catalyses the conversion of G3P to 1,3-bisphosphoglycerate (1,3-BPG) during glycolysis. In resting cells, GAPDH is not considered as a major rate-limiting step in glucose metabolism. However, when macrophages undergo Warburg-like metabolic reprogramming after pro-inflammatory stimulation, glycolytic flux becomes highly dependent on GAPDH activity. GAPDH therefore represents an important metabolic checkpoint. Several anti-inflammatory drugs and metabolite derivatives can induce post-translational modifications (PTMs) on GAPDH and inhibit its enzymatic function, thereby alleviating macrophage inflammatory responses (Jiang et al., 2026; Kornberg et al., 2018; Liao et al., 2019; Wu et al., 2026).

In contrast to the anti-inflammatory effects of GAPDH inhibition, GAPDH can also promote inflammation through non-glycolytic RNA-binding functions. In resting macrophages, GAPDH binds to AU-rich elements in inflammatory mRNAs, such as tumour necrosis factor alpha (TNF), and suppresses their translation (Fig. 1c) (Galvan-Pena et al., 2019). Following LPS stimulation, citrate-derived malonyl-CoA promotes GAPDH malonylation at lysine residues. Modified GAPDH then dissociates from *Tnfa* mRNA, allowing TNF production. These findings indicate that GAPDH can regulate inflammatory mRNA translation and act as an immune-response regulator in Warburg-reprogrammed macrophages.

Phosphoglycerate kinase 1. PGK1 catalyses the conversion of 1,3-BPG to 3-phosphoglycerate (3PG), generating the first ATP molecule in the glycolytic pathway. A study identified PGK1 as a regulator of cytokine production in LPS-stimulated macrophages (Liao et al., 2022). Inhibition of PGK1 reduced glucose consumption and lactate production in activated macrophages. Functionally, PGK1 inhibition reduced LPS-induced IL-1 β and IL-6 protein production. Mechanistically, nuclear factor erythroid 2-related factor 2 (NRF2) expression and nuclear translocation were increased by PGK1 inhibition and Kelch-like ECH-associated protein 1 (KEAP1) degradation. NRF2 then bound near the *Il1b* and *Il6* promoters and restricted their transcription. This suggests that PGK1 supports inflammatory cytokine production partly by limiting NRF2-dependent transcriptional repression.

Another study identified PGK1 as a potential regulator of NLRP3 activation (Zhu et al., 2024). In LPS-stimulated macrophages, PGK1 interacted with NLRP3, and LPS stimulation enhanced this association (Fig. 1d). Silencing PGK1 reduced NLRP3 phosphorylation and suppressed GSDMD-mediated pyroptosis. Molecular docking

suggested that PGK1 may interact with NLRP3 near residues that could support phosphorylation (Zhu et al., 2024). Inhibition of PGK1 also reduced inflammatory injury in the LPS-induced acute lung injury model. These findings suggest that PGK1 may promote macrophage inflammation through a non-canonical kinase-related mechanism that enhances NLRP3 inflammasome activation.

Enolase 1. ENO1 is a glycolytic enzyme that catalyses the conversion of 2-phosphoglycerate (2PG) to phosphoenolpyruvate (PEP). Although ENO1 is classically considered a cytosolic glycolytic enzyme, recent studies suggest that it can also regulate immune responses through non-canonical functions, particularly after translocation to the cell surface.

In rheumatoid arthritis, surface ENO1 was found to be increased on monocytes and macrophages from peripheral blood and synovial fluid (Bae et al., 2012). ENO1-positive cells were also detected in inflamed synovial tissues from rheumatoid arthritis patients and in arthritic tissues from mice with collagen-induced arthritis. Functionally, stimulation of surface ENO1 with an anti-ENO1 auto-antibody induced robust production of pro-inflammatory mediators, including TNF, IL-1 α/β , IL-18, interferon (IFN)- γ and prostaglandin E2 (PGE2) (Fig. 1e). This response was mediated through p38 mitogen-activated protein kinase (MAPK) and NF- κ B signalling. Surface ENO1 can also function as a plasminogen receptor. In rheumatoid arthritis mononuclear cells, plasminogen binding to surface ENO1 promoted inflammatory cytokine production through plasmin-dependent signalling. These findings indicate that ENO1 subcellular localisation determines its effect on macrophage function.

Pyruvate kinase M2. PKM2 catalyses the final step of glycolysis by converting PEP to pyruvate. LPS-stimulated macrophages induce PKM2, which is required for the increased lactate production during Warburg metabolism (Palsson-McDermott et al., 2015). Similar to HK, PKM2 is an immune-regulatory protein in macrophages. A key feature of PKM2 is its ability to switch between dimeric and tetrameric conformations.

LPS stimulation phosphorylates PKM2 and favours dimeric PKM2 accumulation (Palsson-McDermott et al., 2015). Dimeric PKM2 is enzymatically less active. Instead, it can translocate into the nucleus and regulate transcription. Nuclear PKM2 interacts with HIF-1 α and enhances HIF-1 α -dependent transcription, including the induction of inflammatory and glycolytic genes (Fig. 1f). PKM2 also modulates other inflammatory transcriptional pathways. In atherosclerosis, macrophage JAML promotes PKM2 phosphorylation and the formation of a complex with p65, thereby activating NF- κ B signalling and the NLRP3 inflammasome (Cui et al., 2025). The PKM2-p65

interaction can also be exploited during viral infection. HIV-1-infected macrophages increased dimeric PKM2 expression and nuclear localisation (Sen et al., 2017). PKM2 interacted with the HIV-1 long terminal repeat and associated with p65, acting as a transcriptional co-activator of HIV-1 activity.

PKM2 dimer can also regulate transcription factors through its protein kinase activity. In macrophages from patients with coronary artery disease, increased glycolysis and mitochondrial ROS promoted PKM2 dimerisation and nuclear translocation, where it phosphorylated signal transducer and activator of transcription 3 (STAT3) and induced IL-6 and IL-1 β production (Shirai et al., 2016). In acute liver failure, nuclear PKM2 similarly phosphorylated STAT3 (Yu et al., 2024). This then caused STAT3 recruitment to the *Nlrp3* promoter and enhanced pro-inflammatory pyroptosis.

In contrast, tetrameric PKM2 can promote anti-inflammatory effects by improving mitochondrial fitness. Enforced PKM2 tetramerisation enhanced pyruvate kinase activity and increased ATP production in LPS-activated macrophages (Toller-Kawahisa et al., 2025). Released ATP was subsequently converted by CD39 and CD73 into adenosine, which activated A2A receptor signalling and increased IL-10 production. This response improved mitochondrial respiration and mitochondrial fusion. Another study showed that PKM2 tetramerisation promoted PGC-1 α -dependent mitochondrial biogenesis and macrophage endotoxin tolerance after LPS stimulation (Yi et al., 2020).

Lactate as a regulator of macrophage functions. Lactate, the end product of aerobic glycolysis, accumulates in cancer cells and the tumour microenvironment (TME). It is increasingly recognised as an active metabolic and signalling molecule rather than a simple byproduct (De Leon-Oliva et al., 2025). Lactate promotes tumour-supportive signalling in cancer cells while suppressing the function of surrounding immune cells. Similarly, lactate levels increase following M1 macrophage activation and contribute to the regulation of immune responses. In this section, we discuss the effects of lactate on histone and non-histone protein lactylation, as well as additional lactate-mediated signalling mechanisms.

Histone lactylation. Protein lactylation is a relatively newly discovered PTM, with histone lysine lactylation first identified in macrophages. Prolonged LPS stimulation or bacterial infection enhances histone lactylation (Zhang et al., 2019). Curiously, H3K18 lactylation was enriched at promoters of several genes characteristic of M2-like macrophages, the subtype involved in resolution of inflammation. LPS is best known as a driver of M1-like inflammatory macrophages, although at later time points it promotes expression of anti-inflammatory genes such

as IL-10. Manipulating intracellular lactate availability through LDHA deletion or exogenous lactate treatment altered histone lactylation and Arg1 expression, a classical marker of M2 macrophages (Fig. 2a). p300 was implicated as a potential histone lactyltransferase because p300 depletion substantially reduced histone lactylation levels.

Subsequent studies further linked lactate-driven histone lactylation to macrophage repair programmes. Following LPS or heat-killed bacteria stimulation, B-cell adaptor for PI3K (BCAP) promoted PI3K–AKT activation, glycolysis and lactate production (Irizarry-Caro et al., 2020). BCAP-deficient macrophages showed reduced glycolysis and histone lactylation and failed to efficiently undergo a reparative transition. Exogenous lactate restored both histone lactylation and reparative gene expression, supporting a role for lactate in the transition from inflammatory activation towards tissue repair. Similarly, inhibition of monocarboxylate transporter 4 (MCT4), which can mediate lactate release, increased intracellular lactate levels and promoted p300-dependent histone lactylation and reparative gene expression after oxidised low-density lipoprotein (ox-LDL) exposure (Zhang et al., 2024). Consistent with these findings, macrophage-specific MCT4 deletion alleviated atherosclerosis in Apoe-deficient mice fed a high-fat diet.

During methicillin-resistant *Staphylococcus aureus* (MRSA) infection, macrophages initially displayed enhanced glycolysis, followed by iNOS-dependent disruption of mitochondrial respiration and reduced OxPhos 24 h after infection (Reynolds et al., 2024). Lactate subsequently accumulated and was associated with increased H3K18 lactylation at the iNOS gene nitric oxide synthase 2 (*Nos2*) promoter, reduced *Nos2* expression and limited sustained iNOS activity. However, the precise mechanism by which promoter-associated H3K18-Lac suppresses *Nos2* transcription remains unclear. This late lactate-dependent checkpoint may restrain the nitric oxide (NO)-driven type I IFN-biased inflammatory programme and facilitate inflammatory resolution.

LPS-induced sustained lactate accumulation can suppress the NLRP3 inflammasome. In glucose-6-phosphate transporter (G6PT)-deficient macrophages, increased glycolysis and lactate production enhanced histone lactylation and induced expression of the m6A demethylase AlkB homolog 5 (ALKBH5) (Su et al., 2025). ALKBH5 subsequently reduced m6A modification of *NLRP3* mRNA, decreasing transcript stability and constraining NLRP3 expression, which again would have an anti-inflammatory effect. This is also the case in a model of heart ischaemia–reperfusion injury, where macrophages and monocytes displayed increased histone lactylation (Wang, Wang et al., 2022). LDHA inhibition reduced histone lactylation and reparative

cytokine IL-10 production, while IL-1 β promoted nuclear localisation of general control non-depressible 5 (GCN5) and enhanced p300-associated lactylation which could be anti-inflammatory.

Longer-term lactate accumulation can also shape epigenetic innate immune memory. The tuberculosis (TB) vaccine *Bacillus Calmette-Guérin* (BCG) can be used to 'train' macrophages causing epigenetic alterations that subsequently allow for enhanced stimulation in a process termed innate immune training. BCG-trained macrophages exhibit increased basal lactate levels and further lactate accumulation after LPS stimulation (Ziogas et al., 2025). BCG training increased histone lactylation and enhanced chromatin accessibility at active enhancer regions. Inhibition of LDH or p300 reduced histone lactylation and impaired epigenetic features of trained immunity. Although both BCG and LPS induced lactylation at transcriptional regulatory regions, they were associated with distinct motif enrichments: BCG-induced lactylation was enriched at bZIP motifs, whereas LPS-induced lactylation was enriched at Ets motifs. BCG-trained macrophages also showed increased CCCTC-binding factor (CTCF) binding and long-range chromatin interactions associated with IL-1 β

transcription. In this context, the effect of lactylation was pro-inflammatory. Consistent with these findings, monocytes from BCG-vaccinated individuals displayed persistent increases in histone lactylation at inflammatory genes. These contrasting findings demonstrate that the effects of histone lactylation on macrophage function are context-dependent. In the TME, persistent lactate accumulation favours M2 macrophage differentiation and immunosuppressive responses, thereby leading to tumour immune evasion (Colegio et al., 2014).

Lactylation of other proteins. In addition to histones, lactate can regulate macrophage responses through lactylation of non-histone proteins. These processes are greatly pro-inflammatory. During LPS stimulation, p300/CREB-binding protein (CBP)-dependent lactylation of the classic DAMP protein high mobility group box 1 (HMGB1) promoted its nuclear export and extracellular release (Yang, Fan et al., 2022) (Fig. 2b). Lactate also acted through an autocrine GPR81-dependent pathway involving large tumour suppressor (LATS)-mediated yes-associated protein (YAP) degradation, sirtuin 1 (SIRT1) inhibition, β -arrestin2 activation and recruitment of P300/CBP. Released HMGB1-containing extracellular vesicles

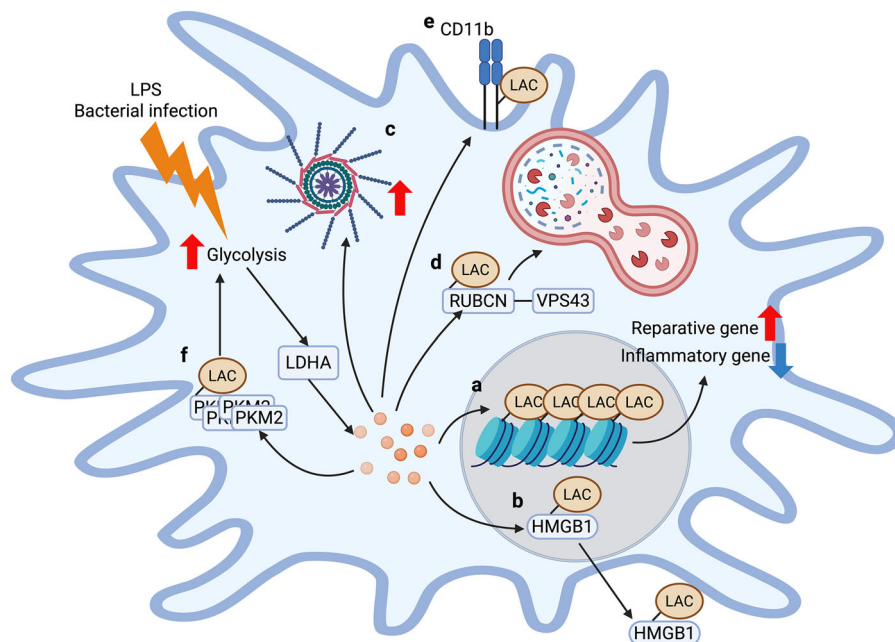


Figure 2. Lactylation targets in macrophages

Following LPS stimulation or bacterial infection, macrophages increase glycolysis and LDHA activity, resulting in lactate accumulation. Lactate can then drive lysine lactylation of histone and non-histone proteins to regulate macrophage immune responses (a). At later stages of LPS stimulation, histone lactylation promotes the expression of reparative genes and contributes to the resolution of inflammation. Lactate can lactylate HMGB1, promoting its release as a DAMP (b). In addition, lactylation of NLRP3 promotes inflammasome assembly (c), and lactylation of RUBCN enhances LC3-associated phagocytosis by inducing RUBCN–VPS4 interaction (d). Lactylation of CD11b, however, impairs apoptotic-cell recognition and clearance (e). It also modifies PKM2 to promote tetramer formation. This boosts glycolysis and limits PKM2-dependent pro-inflammatory signalling (f). Together, these illustrate how lactate accumulation translates metabolic rewiring into diverse macrophage immune functions.

reduced VE-cadherin and claudin-5 expression, increased intercellular adhesion molecule-1 (ICAM1) expression in endothelial cells and promoted vascular permeability (Yang, Fan et al., 2022).

Lactate can also promote inflammasome activation through direct lactylation of NLRP3 (Liu et al., 2026) (Fig. 2c). LPS priming followed by the classic NLRP3 activator nigericin stimulation increased lactate accumulation, whereas LDHA inhibition or silencing reduced NLRP3 activation and IL-1 β secretion. Mechanistically, alanyl-tRNA synthetase (AARS) 2 is reported to lactylate NLRP3 at K24 and K565, which in turn promoted ASC recruitment and inflammasome assembly. Inhibition of lactate production or silencing AARS2 also reduced inflammation in a polymicrobial sepsis model. This pro-inflammatory effect is different from the suppressive effect of lactate observed in G6PT-deficient macrophages described above, probably because acute lactate accumulation enhances the activation of pre-existing NLRP3, whereas sustained lactate accumulation reduces NLRP3 expression through histone lactylation, ALKBH5 induction and m6A-dependent destabilisation of *NLRP3* mRNA.

Lactate-dependent protein lactylation can regulate antibacterial responses (Fig. 2d). In macrophages challenged with intracellular bacteria, LDH is upregulated to induce lactate production. Accumulated lactate then promoted lactylation of the LC3-associated phagocytosis regulator rubicon (RUBCN) (Sun et al., 2024). RUBCN lactylation enhanced its interaction with vacuolar protein sorting (VPS) 34, promoting phosphatidylinositol 3-phosphate (PI3P) production, NADPH oxidase 2 (NOX2)-dependent ROS generation, microtubule-associated protein 1 light chain 3 (LC3) recruitment to bacteria-containing phagosomes and bacterial killing. Inhibition of LDHA or mutation of the RUBCN lactylation site impaired these protective effects. The study further identified programmed cell death protein (PDCD) 6 as a negative regulator of LDHA activity and lactate production; PDCD6 deficiency increased LDHA activation and lactate accumulation, thereby enhancing antibacterial activity in macrophages *in vitro* and *in vivo*.

Lactylation can additionally impair macrophage engulfing of apoptotic cells, termed efferocytosis (Fig. 2e). In synovial macrophages, high-glucose exposure enhanced glycolysis and lactate accumulation, resulting in CBP-mediated lactylation of the efferocytosis receptor CD11b at K575 (Zhou et al., 2025). Molecular-dynamics analysis predicted that this modification altered CD11b conformation and weakened its interaction with phosphatidylserine on apoptotic cells, impairing apoptotic-cell recognition and clearance. CBP silencing reduced CD11b lactylation, restored efferocytosis, and

attenuated synovial inflammation and osteoarthritis progression in hyperglycaemic mice.

Lactylation can also modulate macrophage metabolism directly. Lactate promoted PKM2 lactylation and tetramer formation, which promote glycolysis since the tetrameric form is more enzymatically active than the dimeric form (Fig. 2f) (Wang, Yang et al., 2022). The PKM2 dimer is pro-inflammatory as it translocates to the nucleus, boosting the expression of HIF-1 α -dependent genes including IL-1 β (Palsson-McDermott et al., 2015). Blocking its dimerisation therefore limits inflammation.

Other targets for lactate. Lactate can also affect NLRP3 inflammasome activation independently of protein lactylation. During pyroptotic stimulation, intracellular lactic acid accumulation causes cytoplasmic acidification, which promotes mitochondrial dysfunction, ROS production and phosphorylation of the stress sensor PKR (Lin et al., 2026). Phosphorylated PKR interacts with NLRP3 and promotes inflammasome assembly. Extracellular alkalinisation alleviated intracellular acidification and reduced NLRP3 activation, indicating that the protonated form of lactate and the local pH environment are important determinants of this response.

Lactate accumulation can also impair antimicrobial responses. In iron regulatory protein (IRP) 2-deficient macrophages, elevated HIF-2 α signalling increased LDHA expression and lactate production during acute gram-negative bacteria challenge (Cheng et al., 2024). Increased lactate was associated with impaired transcription factor EB (TFEB), a master regulator of lysosomal biogenesis, nuclear localisation and reduced lysosomal function. Inhibition of LDHA or HIF-2 α restored TFEB activity, and HIF-2 α inhibition reduced liver abscess formation in myeloid-specific *Irf2*-deficient mice. This contrasting study indicates that lactate can enhance antibacterial activity through RUBCN lactylation as above but impair lysosomal antimicrobial capacity through TFEB suppression, depending on the upstream stimuli.

Infection-induced lactate may also be exploited by intracellular pathogens. During infection with gram-negative *S. Typhimurium*, macrophages undergo enhanced glycolysis and accumulate lactate (Jiang et al., 2021; Wang, Yang et al., 2023). *Salmonella* senses host-derived lactate and pyruvate through the CreBC two-component system, which promotes VrpB-dependent expression of SPI-2 virulence genes. Lactate-mediated induction of SPI-2 increases translocation of the bacterial effector SteE into macrophages, promoting STAT3 phosphorylation and M2-like macrophage polarisation. This shift suppresses M1-associated bactericidal responses and creates a permissive intracellular niche that promotes *Salmonella* replication and systemic dissemination.

Table 1. Mechanism of lactate regulating inflammatory responses

Stimulation	Mechanism	Pro/anti-inflammatory	Reference(s)
LPS	Histone lactylation	Anti-inflammatory	(Irizarry-Caro et al., 2020; Zhang et al., 2019)
MRSA		Anti-inflammatory	(Reynolds et al., 2024)
BCG		Pro-inflammatory	(Ziogas et al., 2025)
LPS		Anti-inflammatory	(Su et al., 2025)
LPS	HMGB1 lactylation	Pro-inflammatory	(Yang, Fan et al., 2022)
LPS+Nigericin/ATP	NLRP3 lactylation	Pro-inflammatory	(Liu et al., 2026)
Bacterial infection	RUBCN lactylation	Pro-inflammatory	(Sun et al., 2024)
High glucose	CD11b lactylation	Pro-inflammatory	(Zhou et al., 2025)
LPS	PKM2 lactylation	Anti-inflammatory	(Wang et al., 2025)
Nigericin	Intracellular acidification	Pro-inflammatory	(Lin et al., 2026)
<i>E. coli</i>	TFEB inhibition	Anti-inflammatory	(Cheng et al., 2024)

Lactate can be pro- or anti-inflammatory in different stimulations. This could be time-dependent, with early effects being largely pro-inflammatory, while later effects are anti-inflammatory.

Intracellular lactate trafficking can also shape macrophage polarisation. LPS-induced M1-like polarisation was associated with decreased expression of the lactate transporter MCT1 and increased MCT4 expression, whereas IL-4-induced M2-like polarisation increased MCT1 expression (Chen et al., 2025). MCT1 was reported to localise predominantly to mitochondria, while MCT4 was enriched at the plasma membrane. M1-like macrophages therefore accumulated cytosolic lactate and showed reduced mitochondrial lactate, whereas M2-like macrophages displayed increased lactate flux towards mitochondria. Inhibition of MCT1 reduced mitochondrial lactate and respiratory capacity and enhanced LPS-induced pro-inflammatory cytokine expression. In contrast, MCT4 inhibition suppressed M1-like inflammatory responses. These findings support a model in which MCT4 facilitates lactate export during inflammatory activation, while mitochondrial MCT1 supports intracellular lactate transport into mitochondria and oxidative metabolism during M2-like polarisation.

Macrophage-derived lactate can also regulate tissue remodelling through paracrine metabolic signalling to promote fibrosis. In hypertrophic scar tissue, mechanically stressed macrophages displayed increased glycolysis and lactate production, promoting fibroblast-to-myofibroblast phenotypic remodelling (Yuan et al., 2026). Inhibition of macrophage LDHA or blockade of fibroblast MCT1 attenuated the remodelling. Mechanistically, MCT1-mediated lactate uptake in fibroblasts increased histone H3K23 lactylation of the profibrotic genes hairy/enhancer-of-split related with (YRPW motif 2 (HEY2) and collagen type XI alpha 1 (COL11A1). HEY2 promoted YAP1/SMAD family member 2 (SMAD2) signalling, while COL11A1 stabilised MCT1 and enhanced lactate transport, establishing a positive-feedback loop that sustained fibroblast activation

and collagen deposition. Fibroblast-specific MCT1 inhibition reduced scar formation and collagen deposition in a murine wound-healing model.

Collectively, these diverse effects demonstrate that lactate is a critical product of Warburg metabolism and regulates macrophage inflammatory responses. The pro- and anti-inflammatory effects of lactate are summarised in Table 1.

Mitochondrial remodelling in Warburg metabolism

Although Warburg metabolism was traditionally linked to glycolysis, with scant regard paid to mitochondria, changes in mitochondrial metabolism emerged as important for tumorigenesis (Du et al., 2025). Similar to cancer cells, mitochondria in activated M1 macrophages also undergo various changes and regulate immune responses. Mitochondria participate in Warburg metabolism at three interconnected levels: accumulation of TCA cycle immunometabolites, reprogramming of the electron transport chain (ETC) and dynamic changes in mitochondria.

Mitochondrial immunometabolites

Succinate. Succinate is a TCA cycle intermediate that accumulates in macrophages undergoing Warburg-like metabolic reprogramming (Tannahill et al., 2013). Glutamine-dependent anaplerosis is reported to be a major source of this succinate pool (Tannahill et al., 2013). Endogenously accumulated succinate acts as a pro-inflammatory signal. Succinate stabilises HIF-1α by inhibiting prolyl hydroxylase (PHD) activity, thereby enhancing HIF-1α-dependent transcription. LPS-induced succinate accumulation promotes HIF-1α binding to the *Il1b* promoter, increasing IL-1β expression.

Inhibition of glycolysis with 2-deoxyglucose reduces succinate accumulation, leading to suppressed HIF-1 α activation and IL-1 β expression.

In addition to its intracellular effects, succinate can be released from inflammatory macrophages and act extracellularly through succinate receptor 1 (SUCNR1 or GPR91) (Littlewood-Evans et al., 2016). LPS or IL-1 β stimulation increases *Sucnr1* expression in macrophages and promotes succinate release into the extracellular environment (Littlewood-Evans et al., 2016). In inflammatory macrophages, extracellular succinate enhances LPS-induced HIF-1 α expression and IL-1 β production in a GPR91-dependent manner. GPR91 deficiency reduced macrophage activation and IL-1 β production in a model of arthritis. These findings suggest that macrophage-derived succinate can form an autocrine or paracrine feed-forward loop that amplifies inflammation.

However, the immunological effect of succinate is highly context-dependent. In obesity-associated inflammation, myeloid-specific SUCNR1 deficiency promoted a more pro-inflammatory tissue phenotype, worsened glucose intolerance and impaired metabolic adaptation (Keiran et al., 2019). In this setting, SUCNR1 signalling supported anti-inflammatory macrophage programmes and enhanced responses to type 2 cytokines such as IL-4. Similarly, comparison of different experimental models showed that cell-permeable succinate derivatives or SDH inhibition can suppress IL-6, TNF, NO and IL-1 β expression in LPS-stimulated macrophages, while SUCNR1 deficiency itself may enhance inflammatory responses (Harber et al., 2020). These findings indicate that succinate and SUCNR1 signalling can either promote or restrain inflammation depending on the disease setting, macrophage state, succinate form, receptor availability and timing of stimulation.

Fumarate. Fumarate has recently emerged as another immunoregulatory metabolite that accumulates during macrophage Warburg-like metabolic reprogramming. In LPS-activated macrophages, fumarate is a highly upregulated metabolite (Hooftman et al., 2023). Acute fumarate accumulation is due to increased argininosuccinate synthase (ASS1) expression and aspartate–argininosuccinate shunt, whereas reduced fumarate hydratase (FH) expression accounts for late fumarate accumulation. Macrophages are more pro-inflammatory with FH inhibition. FH inhibition increases mitochondrial stress as it enhances mitochondrial membrane potential and mitochondrial ROS (mtROS). Fumarate reduces IL-10 production by suppressing c-Fos activity, probably through succination-dependent mechanisms. FH inhibition enhances TNF secretion and amplifies inflammatory

macrophage activation as a consequence of IL-10 down-regulation. Fumarate is also reported to succinate GSDMD (Humphries et al., 2020). This modification inhibits caspase-1-induced cleavage and downstream pyroptosis and IL-1 β release.

On the other hand, fumarate accumulation induces type I IFN responses through mitochondrial nucleic acid release (Hooftman et al., 2023). LPS stimulation induces release of mitochondrial RNA (mtRNA) into the cytosol in cells treated with an FH inhibitor. This cytosolic mtRNA is sensed by TLR7, retinoic acid-inducible gene I (RIG-I) and melanoma differentiation-associated protein 5 (MDA5), leading to IFN- β production. Importantly, this response is not reproduced by dimethyl fumarate (DMF), suggesting that FH inhibition promotes IFN- β primarily through mitochondrial disturbance and mtRNA release rather than succination alone.

Itaconate. One particular mitochondrial metabolite, itaconate, which is strongly induced during Warburg-like metabolism, has received a lot of attention in its regulatory role in inflammation. Itaconate was identified in the 1970s as having antibacterial activity. Early studies demonstrated that it inhibits the bacterial enzyme isocitrate lyase (ICL), thereby restricting bacterial growth (Rittenhouse & McFadden, 1974). Later, a gene that was shown to be strongly induced by LPS in macrophages of unknown function, termed immune-responsive gene 1 (IRG1), was subsequently shown to encode aconitate decarboxylase 1 (ACOD1), which catalyses the conversion of TCA cycle intermediate *cis*-aconitate into itaconate (Lee et al., 1995; Michelucci et al., 2013). Deletion of IRG1 significantly reduced intracellular itaconate levels. Further metabolomic analysis demonstrated that itaconate is among the most accumulated metabolites in LPS-activated macrophages (Jha et al., 2015).

Several transcriptional pathways contribute to this induction (Fig. 3a). IRF family members are central regulators. IRF1 was the first transcription factor shown to promote LPS-induced IRG1 expression and itaconate production, while IRF3, IRF2 and IRF9-related pathways have also been implicated following activation by various pathogen-associated molecular patterns (PAMPs) and cytokines (Bourner et al., 2024; Chen, Wu et al., 2022; Ganta et al., 2017; Mills et al., 2018; Qin et al., 2024; Tallam et al., 2016). In addition, TFEB can directly bind the *IRG1* promoter after LPS or bacterial stimulation and promote ACOD1 expression independently of IRF signalling (Schuster et al., 2022). Interestingly, itaconate can also activate TFEB through cysteine alkylation, suggesting a potential positive feedback loop between TFEB and ACOD1 (Zhang et al., 2022). ACOD1 expression is also regulated epigenetically, as SIRT1 promotes IRG1 expression by deacetylating EZH2 and reducing repressive histone trimethylation at the IRG1 locus (Yao et al., 2025).

These observations raised the question of why inflammatory macrophages produce such large quantities of this metabolite. Research from the last 10 years gradually revealed how itaconate could regulate macrophage immune responses. In mitochondria, it directly inhibits complex II, thereby disrupting TCA cycle activity and limiting succinate accumulation induced by reverse electron transport (RET) at mitochondrial Complex I, thereby eliciting an anti-inflammatory effect (Lampropoulou et al., 2016). Deletion of ACOD1 increases mtROS production and promotes pro-inflammatory cytokine expression. Itaconate-induced complex II inhibition also induces mtRNA release, triggering RIG-I and MDA5 activation and IFN- β expression (O'Carroll et al., 2024). Loss of ACOD1 has attenuated IFN- β expression after LPS stimulation.

Once itaconate is released into the cytosol through mitochondrial carriers solute carrier (SLC)25A1, SLC25A10 and SLC25A11 (Fig. 3b), it also functions as a signalling molecule through protein PTM. Itaconate derivatives can modify cysteine residues on KEAP1, leading to activation of the NRF2 antioxidant pathway and suppression of inflammatory responses (Mills et al., 2018). Another derivative, 4-octyl itaconate (4-OI), has also been shown to inhibit inflammasome activation through alkylation of NLRP3, reducing pyroptosis and IL-1 β release (Fig. 4a) (Hooftman et al., 2020). Itaconate

has also been reported to inhibit NLRP3-triggered GSDMD activation by alkylation (Fig. 4a) (Bambouskova et al., 2021). In contrast, a recent study suggested that excessive itaconate accumulation promoted alkylation of absent in melanoma 2 (AIM2) (Ma et al., 2026). This modification increased AIM2 stability and promoted ASC oligomerisation and PANoptosome assembly, and macrophage PANoptosis. Endogenous itaconate suppresses LPS-activated signalling pathway by alkylating IL-1 receptor-associated kinase 4 (IRAK4) (Fig. 4b) (Bourner et al., 2024). Itaconate has been found to alkylate STING, TANK binding kinase 1 (TBK1) and tyrosine kinase 2 (TYK2) at a later time of inflammatory stimulation (Fig. 4b and c) (Chai et al., 2025; Li et al., 2026; Wu et al., 2023). This then causes a reduction of IFN- β expression. Itaconate and its derivatives also limit glycolytic flux by alkylating rate-limiting enzymes such as ALDOA, GAPDH and LDHB (Fig. 3c) (Bourner et al., 2024; Liao et al., 2019; Qin et al., 2019). Itaconate can therefore block the hallmark of Warburg metabolism, decreasing lactate accumulation.

Furthermore, evidence suggests that itaconate can regulate macrophage inflammatory responses through epigenetic control (Chen, Morcelle et al., 2022). Itaconate binds directly to the active site of tet methylcytosine dioxygenase 2 (TET2), thereby competitively inhibiting TET2 catalytic activity (Chen, Morcelle et al., 2022).

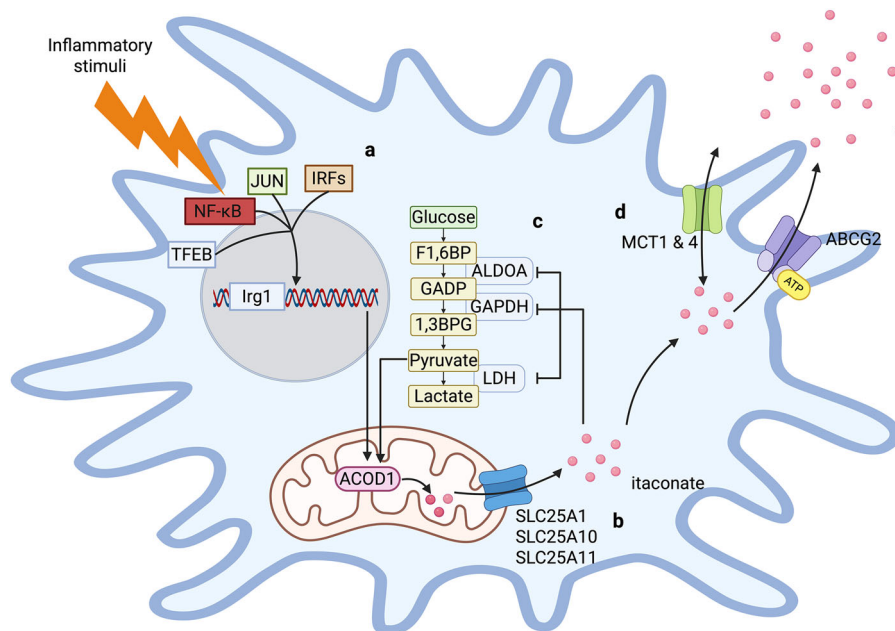


Figure 3. ACOD1 regulation and Warburg effect inhibition by itaconate

Following inflammatory stimulation, transcription factors including TFEB, NF- κ B, JUN and IRFs promote *Irf1*/*Acod1* transcription and subsequent ACOD1 expression (a). Itaconate is exported to the cytosol through the mitochondrial carriers SLC25A1, SLC25A10 and SLC25A11 (b). Cytosolic itaconate alkylates aldolase A (ALDOA), glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and lactate dehydrogenase B (LDHB) to reduce glycolysis and lactate accumulation (c). It can also be released extracellularly through ATP-dependent ABCG2-mediated export or transported through MCT1 and MCT4 (d).

LPS stimulation increases IRG1-derived itaconate levels, which then reduce global 5-hydroxymethylcytosine (5hmC) concentration. However, deletion of *Irg1* maintained higher 5hmC, indicating that endogenously produced itaconate suppresses TET2 activity during inflammatory activation. Transcriptomic analysis further showed that LPS-induced NF- κ B- and STAT-dependent inflammatory genes are suppressed by both itaconate treatment and *TET2*-deficient cells. By inhibiting TET2, itaconate reduces the expression of downstream inflammatory genes such as *Il6*, C-X-C motif chemokine ligand (*Cxcl*)9, *Cxcl*10 and *Cxcl*11 (Chen, Morcelle et al., 2022).

In addition to its intracellular effects, itaconate trafficking also regulates immune responses. The mechanisms controlling its transport across the plasma membrane have only recently been revealed. ABCG2 was the first transporter identified as an ATP-dependent itaconate exporter using a CRISPR–Cas9 screen with the fluorescent itaconate sensor cBioITA in THP-1 cells (Fig. 3d) (Chen, Zhang, et al., 2024). ABCG2 silencing increased intracellular itaconate without

altering ACOD1 expression, and ATP hydrolysis-deficient ABCG2 mutants produced a similar effect. Functionally, ABCG2 deficiency promoted intracellular itaconate accumulation and TFEB activation. This then induced lysosome biogenesis and antibacterial immunity (Chen, Zhang, et al., 2024). The same group subsequently identified SLC13A3 as an itaconate importer (Chen, Liu, et al., 2024). SLC13A3 deletion or inhibition reduced itaconate uptake. In hepatocytes, SLC13A3-mediated itaconate uptake activated TFEB and enhanced antibacterial defence (Chen, Liu, et al., 2024). In addition, MCT1 and MCT4 have been implicated in itaconate transport and intracellular trafficking (Fig. 3d) (Meng et al., 2025). Their inhibition increases intracellular itaconate and restricts *Salmonella* proliferation in macrophages. Together with RAB32, MCT1 and MCT4 may facilitate itaconate trafficking into *Salmonella*-containing vacuoles (Meng et al., 2025).

As a result of these groundbreaking discoveries, itaconate and its derivatives have been widely tested in a wide range of inflammatory disease models, and anti-inflammatory and tissue-protective effects are

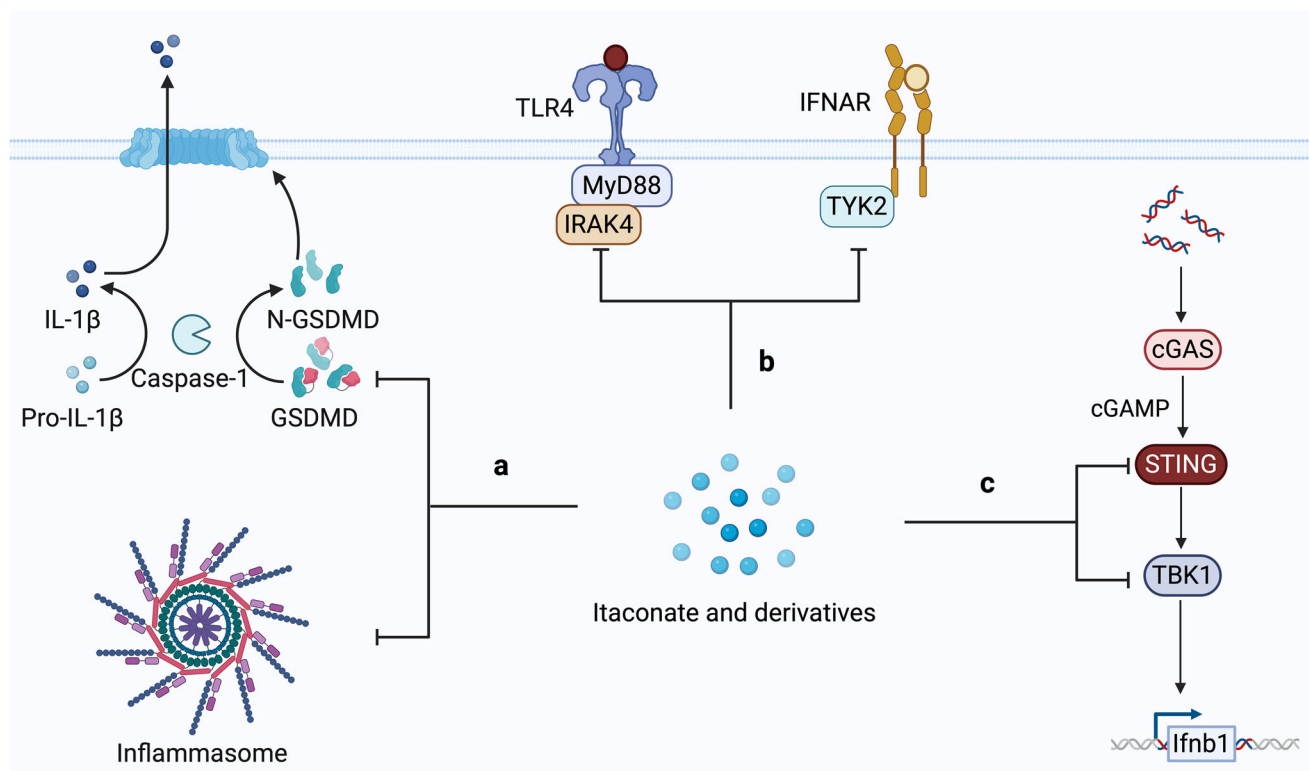


Figure 4. Inhibition of pro-inflammatory signalling pathways by itaconate-mediated alkylation

Itaconate- and itaconate-derivative-induced alkylation can exert anti-inflammatory effects in macrophages. 4-OI modifies NLRP3 and blocks its interaction with NEK7, thereby inhibiting inflammasome activation (a). Downstream of this pathway, itaconate alkylates GSDMD and suppresses pyroptosis (a). Itaconate can also inhibit signal transduction from pattern-recognition receptors and cytokine receptors. Itaconate targets IRAK4 and TYK2, key mediators of TLR4 and IFNAR signalling, thereby reducing downstream pro-inflammatory pathway activation (b). Itaconate and its derivatives also alkylate STING and TBK1, suppressing antiviral signalling and IFN- β expression (c).

observed (Yukun Min, 2023). Collectively, these studies establish itaconate as a central immunometabolite linking mitochondrial metabolism to inflammatory responses and highlight its critical role in the Warburg-like metabolic reprogramming associated with activated M1 macrophages.

ETC reprogramming. Pharmacological inhibition of ETC Complex I with metformin has demonstrated anti-tumour effects in clinical studies (Goodwin et al., 2021; Zannella et al., 2013). Furthermore, recent evidence suggests that ETC complexes II, III and IV also play critical but context-dependent roles in tumour development, as inhibition of these complexes can impair tumour growth in some settings while promoting adaptation in others (Gandhirajan et al., 2018; Jia et al., 2026; Martinez-Reyes et al., 2020; Stewart et al., 2025).

In macrophages, LPS-induced inflammatory activation is commonly associated with inhibition of OxPhos, while restoration of mitochondrial metabolism has been linked to macrophage repolarisation (Van den Bossche et al., 2016).

Mechanistically, ETC function can be shaped by iNOS-derived NO, which inhibits aconitase 2 and pyruvate dehydrogenase, reroutes carbon flux away from mitochondrial oxidation, and reduces ETC complex abundance and respiration (Palmieri et al., 2020). Although Warburg metabolic reprogramming in macrophages is often described as a shift from OxPhos to glycolysis, attention has started shifting toward the role of mitochondrial ETC and OxPhos in macrophage activation. Activated macrophages were found to increase mtROS production, which contributes to antimicrobial defence (Cramer et al., 2003). Rather than becoming completely inactive, mitochondria are repurposed during inflammatory activation to generate immune-regulatory signals.

Complex I. Complex I integrity is important not only for respiration but also for antibacterial signalling and maintenance of a functional mitochondrial pool. Early studies showed that stimulation with pro-inflammatory pathogens can directly link innate immune recognition to ETC-dependent mtROS production. TNF receptor associated factor 6 (TRAF6) ubiquitinates the complex I-associated protein ECSIT, inducing complex I-dependent mtROS generation (West et al., 2011). The subsequent study showed that macrophage-specific deletion of ECSIT disrupts complex I assembly and stability, inducing a compensatory glycolytic shift and impairing mitochondrial ROS responses (Carneiro et al., 2018). Loss of ECSIT also disrupts PTEN induced kinase 1 (PINK1)/parkin-dependent mitophagy, leading to defective mitochondrial quality control.

The ETC can also be remodelled in a stimulus-specific manner. LPS stimulation increases glycolysis and suppresses OxPhos, whereas Pam3CysSK4 treatment increases both glycolysis and oxygen consumption (Lachmandas et al., 2016). In this toll-like receptor (TLR)2-driven state, pharmacological inhibition of complex I reduces cytokine production and phagocytosis. Similarly, mitochondrial respiratory-chain adaptation has been reported to support macrophage bactericidal responses, emphasising that OxPhos inhibition is not a universal requirement for macrophage activation (Garaude et al., 2016). These findings challenge the simple model in which pro-inflammatory macrophages suppress ETC activity.

De novo NAD⁺ synthesis through the kynurenine pathway also appears to support complex I activity (Minhas et al., 2019). Genetic deletion of indoleamine 2,3-dioxygenase 1 (IDO1) or inhibition of quinolinate phosphoribosyltransferase (QPRT) causes NAD⁺ depletion, which increases acetylation of complex I subunits and superoxide dismutase 2 (SOD2), which then reduces OxPhos and enhances mitochondrial ROS accumulation. These findings suggest that NAD⁺ availability is required for electron flow through complex I and for mitochondrial redox control. Blocking *de novo* NAD⁺ synthesis increases pro-inflammatory cytokines such as IL-1 β and IL-18 and impairs phagocytosis. LPS stimulation impairs conversion of kynurenine-pathway intermediates into NAD⁺ and contributes to NAD⁺ depletion, defective complex I function and a more pro-inflammatory macrophage phenotype.

Complex II and reverse electron transport. Complex II, or succinate dehydrogenase (SDH), has been particularly implicated in inflammatory macrophage activation. LPS stimulation leads to succinate accumulation, and oxidation of succinate through complex II can drive mitochondrial hyperpolarisation and mtROS production (Mills et al., 2016). This study proposed that mitochondrial ROS is generated through RET. When mitochondrial membrane potential is excessively high, electrons can flow backwards through complex I and generate mtROS. These ROS promote HIF-1 α -dependent inflammatory signalling and IL-1 β induction, whereas inhibition of complex II or complex I reduces ROS production and suppresses IL-1 β (Fig. 5a). A recent study further refined the mechanism of mtROS generation in LPS-activated macrophages, showing that RET at complex I is a major source of mitochondrial superoxide (Casey et al., 2025). LPS-induced metabolic reprogramming reduces reliance on OxPhos for ATP production because of increased glycolysis, thereby increasing proton motive force and mitochondrial membrane potential. At the same time, succinate oxidation maintains a reduced coenzyme Q pool, allowing electrons to flow backwards

through complex I and generate mitochondrial superoxide. RET-derived mtROS regulates IL-1 β release by inducing NLRP3 inflammasome activation.

In contrast, another study linked ETC function to NLRP3 inflammasome activation independently of RET-derived ROS (Billingham et al., 2022). This study reported that pharmacological inhibition or genetic disruption of ETC components reduced NLRP3 activation and IL-1 β release (Fig. 5a). However, inflammasome activation could be restored by preserving electron flow through ETC bypass systems that did not generate mtROS. Instead of mtROS, phosphocreatine-dependent cytosolic ATP generation was proposed as the key driver of NLRP3 activation (Fig. 5b).

The discrepancy between these studies may be partly explained by differences in experimental

timing and the specific stage of the NLRP3 response being examined. Billingham et al. (2022) focused on relatively acute caspase-1 activation and IL-1 β secretion, whereas Casey et al. (2025) showed that RET-deficient macrophages had comparable ASC complex formation and caspase-1 cleavage but impaired IL-1 β release. Therefore, ETC-dependent ATP production may support inflammasome activation, while RET-derived ROS may act at a later stage, possibly by regulating GSDMD-dependent pore formation and cytokine release.

Complex II itself is dynamically regulated during inflammatory activation. LPS stimulation induces complex II disassembly, which involves sequestration and selective mitophagy-mediated degradation of the SDHB subunit (Reynolds et al., 2023). Mitochondrial fission facilitates SDHB degradation, while the

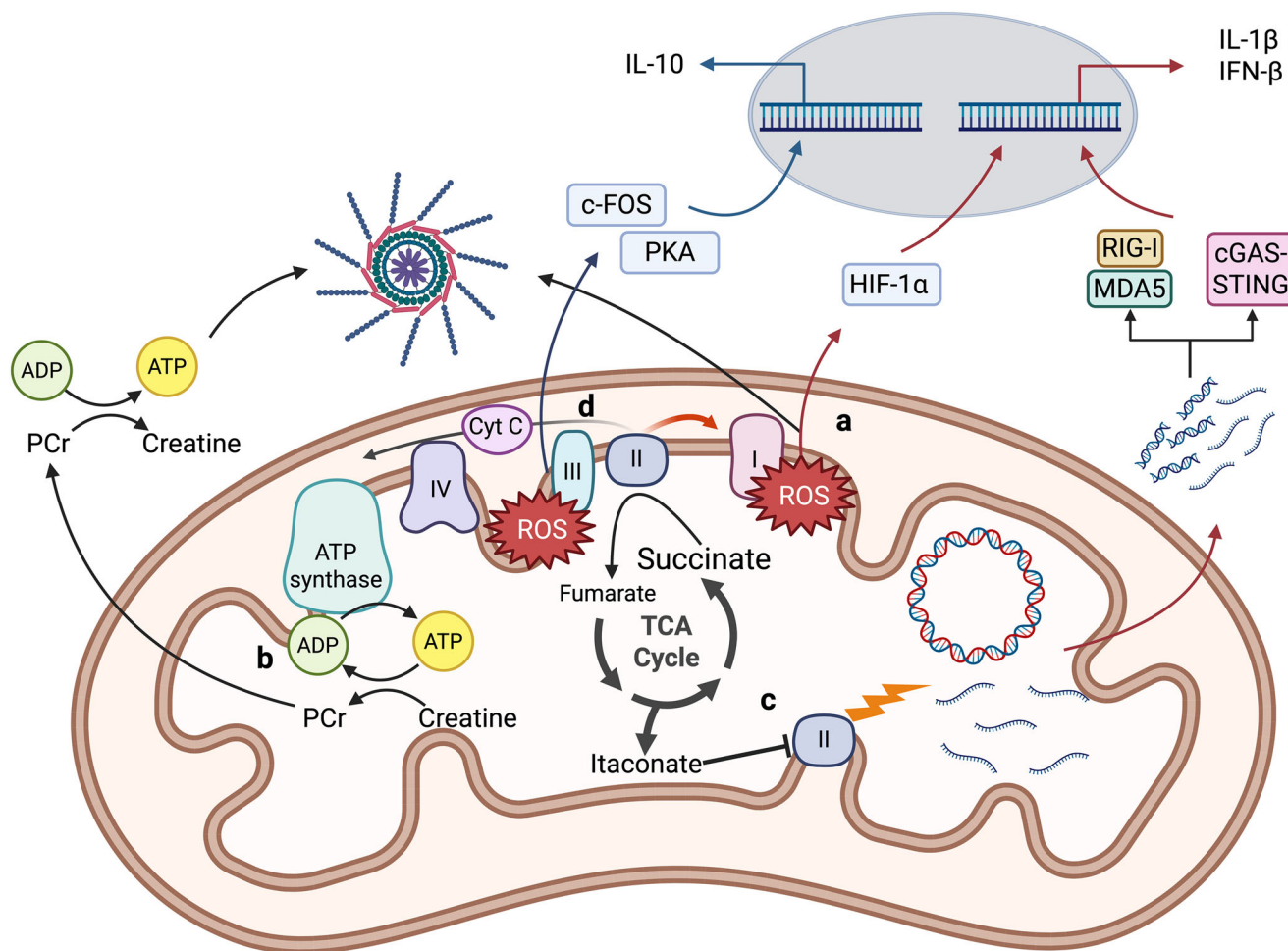


Figure 5. ETC affects macrophage immune responses

ETC is rewired during Warburg metabolic reprogramming of macrophages. Succinate accumulation triggers reverse electron transport backwards through complex II and induces mitochondrial ROS (mtROS) production (a). This then activates HIF-1 α and *Il1b* expression. mtROS from complex I also induces inflammasome activation. ATP production from ETC also boosts NLRP3 activation through phosphocreatine (PCr) (b). The TCA metabolite itaconate or inhibition of complex II also drives mitochondrial nucleic acid release and IFN- β expression (c). mtROS from complex III sustains IL-10 expression through PKA or c-FOS (d).

mitochondrial phospholipid cardiolipin coordinates complex II disassembly and inflammatory metabolic remodelling. Knockdown of cardiolipin synthase prevents LPS-induced complex II disassembly and impairs pro-inflammatory cytokine production. This phenotype can be partially rescued by complex II inhibition, suggesting that regulated complex II remodelling is not just a consequence of inflammation, but actively contributes to the inflammatory metabolic programme.

Studies revealed that endogenous complex II inhibition by the TCA cycle metabolite itaconate (which will be discussed in detail later) is required for optimal IFN β production in macrophages (Bourner et al., 2024; Swain et al., 2020). Exogenous complex II inhibitor treatment boosts IFN β . Mechanistically, complex II inhibition induces IFN β by triggering mtRNA release into the cytosol, where it is detected by the RNA-sensing PRRs RIG-I and MDA5 (O'Carroll et al., 2024) (Fig. 5b). Consistent with this mechanism, silencing RIG-I or MDA5, or inhibiting mtDNA transcription, effectively abolishes itaconate-induced IFN β production. Similarly, complex II has been shown to promote IFN β expression in macrophages stimulated with the TLR3 agonist Poly:IC (Paulenda et al., 2025). Similar to LPS and complex II inhibition, increased mtROS and mtDNA release activate the cyclic GMP-AMP synthase (cGAS)-stimulator of interferon response cGAMP interactor 1 (STING) pathway driving IFN β production. IRG1-deficient macrophages exhibit reduced IFN β production following LPS stimulation.

Complex III. Complex III is another important source of immune-regulatory mtROS. Inflammatory macrophage activation generates complex III-derived mtROS, which disrupts NAD $^{+}$ homeostasis (Cameron et al., 2019). This mtROS induces DNA damage and activates poly(ADP-ribose) polymerases (PARPs), which consume NAD $^{+}$ during DNA repair. As a result, the nicotinamide phosphoribosyltransferase (NAMPT)-dependent NAD $^{+}$ salvage pathway is required to replenish NAD $^{+}$ pools. NAD $^{+}$ salvage is also required to sustain Warburg metabolism and pro-inflammatory macrophage responses. NAMPT inhibition reduces expression of pro-IL-1 β , IL-6 and TNF, whereas supplementation with the NAD $^{+}$ precursor NMN partially restores inflammatory activation. These findings indicate that complex III-derived ROS can indirectly restrain inflammatory metabolism by promoting DNA damage and NAD $^{+}$ consumption, while NAD $^{+}$ salvage allows macrophages to maintain the Warburg programme required for inflammatory activation.

Complex III-derived ROS can also support anti-inflammatory signalling. Recent studies showed that genetic or pharmacological suppression of complex III ROS reduces IL-10 production following bacterial

or viral stimulation (Stoolman et al., 2025; Zotta et al., 2025). Mechanistically, complex III-derived mtROS promotes IL-10 expression through signalling pathways involving protein kinase A (PKA) and c-Fos (Fig. 5c). Therefore, complex III-derived ROS does not just act as a pro-inflammatory signal, but can also promote an anti-inflammatory response depending on the context.

Collectively, these studies redefine the role of the ETC in M1 macrophage Warburg-like metabolism. The ETC is not simply switched off during macrophage inflammatory activation. Instead, individual ETC complexes are dynamically remodelled to generate mtROS, control metabolite signalling and regulate inflammasome activation.

Mitochondrial dynamics and the Warburg effect.

Mitochondrial morphology in cells is highly dynamic and may change according to activation stage, metabolic demand and immune function. A recent study showed that even within the same cell, mitochondria can separate and specialise in either OxPhos or proline synthesis subset (Ryu et al., 2024). This functional separation required continuous mitochondrial fusion and fission. In M1 macrophages, mitochondrial dynamics have also become recognised as important features. They act as modulators of metabolic reprogramming during inflammatory activation, anti-bacterial defence and tissue repair (Li, Fan et al., 2025; Pfefer et al., 2026). A clear connection has also emerged linking mitochondrial change to glycolysis (Fig. 6).

Fission. Mechanistically, mitochondrial fission, meaning the breaking up of mitochondria, supports Warburg metabolism through mtROS production which promotes PHD2 dimerisation and hence HIF-1 α activation (Sun et al., 2025). Several studies indicate that mitochondrial fission contributes to the inflammatory functions associated with Warburg metabolic reprogramming. Aerobic glycolysis is prevented in LPS-activated bone marrow-derived macrophages (BMDMs) when mitochondrial fission is inhibited (Buck et al., 2016). A correlation was observed between this reduction in glycolysis in fission inhibitor-treated BMDMs and decreased *Nos2*. Reduced glycolysis following inhibition of mitochondrial fission repressed macrophage activation. This positions mitochondrial fission as a potential upstream regulator of Warburg metabolism, suggesting that mitochondrial morphology actively shapes rather than simply being a consequence of inflammatory metabolic reprogramming.

LPS stimulation promotes TLR4- and MyD88-dependent recruitment of the fission protein dynamin-related protein 1 (DRP1) to mitochondria, increasing mitochondrial fragmentation (Kapetanovic et al., 2020). STAT2 promoted DRP1 activation and

resulted in increased mitochondrial mass and fragmented morphology. This also disrupted cristae structure and reduced mitochondrial membrane potential, changing mitochondrial activity from ATP generation towards ROS production (Fig. 6a) (Yu et al., 2020). This mtROS promoted NF- κ B-dependent expression of inflammatory cytokines, linking DRP1-dependent remodelling to macrophage inflammatory activation. DRP1 has also been shown to induce IL-1 β and TNF production (Gao et al., 2020). However, DRP1 depletion did not prevent the LPS- or MRSA-induced change from OxPhos towards aerobic glycolysis, indicating fission facilitates how mitochondria function within the glycolytic state by generating ROS and cytokine production.

DRP1 has also been reported to regulate NLRP3 activation in macrophages. ox-LDL stimulation causes

excessive fission by DRP1, which then triggers increased mtROS production (Zhang et al., 2023). In contrast, DRP1 deficiency induced persistent loss of mitochondrial fission, enhancing ATP- or nigericin-induced NLRP3 inflammasome activation through ERK-dependent recruitment of NLRP3 to mitochondria (Park et al., 2015). These studies show that regulated fission supports mitochondrial homeostasis, whereas either excessive fission or chronic fission failure can induce ROS production for NLRP3 activation.

Fusion. Fusion involves the formation of long tubular networks of mitochondria that allow sharing of mitochondrial contents between those in close proximity (Yan, Luo et al., 2024). In agreement with the above findings, mitochondrial fusion has been shown to facilitate OxPhos in various cells (Hu et al., 2025;

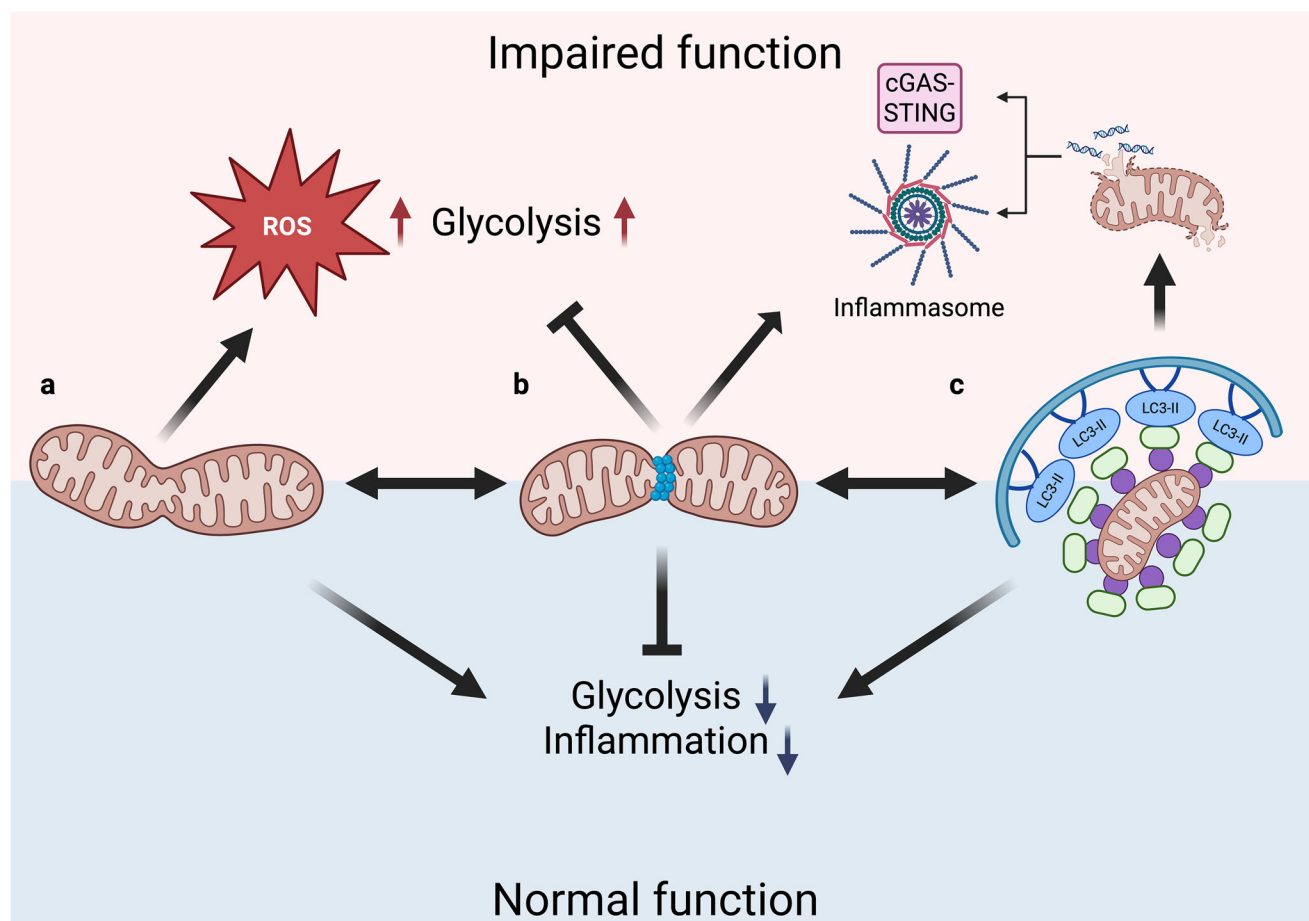


Figure 6. Mitochondrial dynamics and its effect on glycolysis and inflammatory responses

Recent studies have shown that mitochondrial dynamics regulate the macrophage Warburg effect and inflammatory responses. Under homeostatic conditions, mitochondrial fusion helps restrain glycolysis and inflammation (a). However, impaired fusion can promote mitochondrial dysfunction, leading to increased mtROS production and enhanced glycolytic metabolism. In contrast, mitochondrial fission is closely associated with mtROS generation and inflammatory activation (b). Dysregulated fission can further promote NLRP3 inflammasome activation. Mitophagy also contributes to this regulatory network by limiting glycolysis and removing damaged mitochondria (c). When mitophagy is impaired, mitochondrial stress increases, promoting mtDNA release and subsequent activation of cGAS–STING signalling and the NLRP3 inflammasome.

Mourier et al., 2015; Yan, Luo et al., 2024). These results suggest that mitochondrial fusion acts as a shield against sustained Warburg metabolism. By allowing cells to maintain or return to OxPhos, mitochondrial fusion may provide a mechanism for controlling and resolving inflammatory states. This potentially reframes OxPhos as an essential player in preserving the reversibility of Warburg metabolism.

Fusion-associated proteins are also required for specific inflammatory and metabolic functions. In macrophages, MFN2 is required for specific inflammatory and metabolic functions (Tur et al., 2020). MFN2-deficient cells had impaired OxPhos and ROS production, which reduced NF- κ B activation (Fig. 6b). Pro-inflammatory cytokine production was also dampened by MFN2 deletion. MFN2 also regulates phagocytosis and autophagy. This shows the importance of Mfn2 and mitochondrial fusion during inflammatory macrophage activation. Similarly, deletion of the inner mitochondrial membrane fusion protein mitochondrial dynamin like GTPase (OPA1) impaired ETC organisation and altered TCA-cycle flux (Sanchez-Rodriguez et al., 2023). These changes weakened NF- κ B activation. Although OPA1-deficient macrophages showed a greater reliance on glycolytic metabolism, M2-associated and profibrotic markers were increased through suppressing NF- κ B signalling. In a muscle-injury model, these macrophages persisted within damaged tissue and were associated with excess collagen deposition and delayed regeneration. Therefore, mitochondrial fusion and cristae integrity can support appropriate inflammatory activation and the subsequent transition towards tissue repair.

Mitophagy. Beyond fusion and fission, mitophagy provides another layer of metabolic control by selectively removing mitochondria (Wang, Long et al., 2023). Loss of BNIP3 or PINK1 is associated with increased glycolysis and reduced OxPhos (Agnihotri et al., 2016; Chourasia et al., 2015). This also increased ROS production and HIF-1 α stabilisation, suggesting that mitophagy can also restrain the Warburg effect. Mitophagy has also been linked to the 'Reverse Warburg Effect', in which reduced mitochondrial mass in cancer-associated fibroblasts promotes glycolysis and the release of lactate and pyruvate to fuel tumour-cell OxPhos (Jiang et al., 2022; Naik, 2020; Pavlides et al., 2009; Warburg, 1925). In macrophages, programmed mitophagy has been shown to facilitate a shift to a glycolytic metabolic profile, characteristic of M1 macrophages (Esteban-Martinez et al., 2017). This is thought to reflect, at least in part, a decrease in mitochondrial mass, consequently reducing the oxidative capacity of cells. Compared with M2 macrophages, M1 macrophages display decreased mitochondrial mass (Esteban-Martinez et al., 2017). Mitophagy inhibition caused an increase in mitochondrial mass in M1

macrophages but not in M2 macrophages, suggesting that mitophagy is responsible for the observed decrease in mitochondrial mass in M1 macrophages. This mitophagy inhibition also caused a decrease in expression of M1 macrophage markers, such as TNF, iNOS and IL-1 β , as well as glycolytic enzyme expression such as HK2, PFKFB3, GAPDH and PKM (Esteban-Martinez et al., 2017).

After LPS-induced inflammatory activation, transient inhibition of mitophagy can contribute to macrophage activation (Patoli et al., 2020). STAT1-activated caspases 1 and 11 induced inhibition of PINK1-dependent mitophagy in macrophages. This was associated with increased ROS, glycolysis and reduced mitochondrial respiration (Fig. 6c). Inhibition of mitophagy reproduced parts of this metabolic rewiring, but mitochondrial uncouplers that promoted mitophagy reversed LPS-induced macrophage activation. Functionally, inhibition of mitophagy enhanced macrophage anti-bacterial activity and improved survival in the polymicrobial sepsis model, suggesting that early suppression of mitophagy can support host defence by allowing mitochondria to function as inflammatory signalling organelles.

However, persistent impairment of mitophagy can have pathological consequences. Defective removal of damaged mitochondria allows accumulation of mtROS and mtDNA release, which can activate the NLRP3 inflammasome and cGAS-STING signalling (Fig. 6c) (Zhong et al., 2022). In aged macrophages, impaired PINK1/Parkin-dependent mitochondrial ubiquitination and defective mTOR-TFEB-dependent lysosomal function reduced mitophagic flux. This promoted mtDNA release and cGAS-STING activation during sterile liver inflammation, while restoration of mitophagy suppressed this inflammatory signalling. Similarly, prolonged TNF stimulation inhibited PINK1-mediated mitophagy, which then altered mitochondrial function and promoted mtDNA release (Willemsen et al., 2021). This subsequently led to cGAS-STING-dependent type I IFN responses in inflammatory arthritis. These studies indicate that mitophagy normally limits sterile inflammatory signalling by preventing damaged mitochondria from releasing immunostimulatory mtDNA.

Collectively, the macrophage Warburg-like metabolism should not be interpreted as a uniform loss of mitochondrial function, but rather as a dynamic redistribution of mitochondrial activities that supports context-dependent immune and metabolic demands (Fig. 6). These findings challenge the view of changes in mitochondrial dynamics as merely a housekeeping process or a downstream consequence of inflammation-induced mitochondrial damage. Instead, these changes are an active regulator of the Warburg-like reprogramming in M1 macrophages, shaping both

metabolic enzyme activity and inflammatory signalling pathways.

Therapeutic possibilities

Which of the observations on the Warburg effect (which also includes mitochondrial changes) might lead to therapeutic targeting?

Regarding the section 'Non-canonical functions of glycolytic enzymes in macrophages', tetramerisation of PKM2 in macrophages has also been shown to have anti-inflammatory therapeutic potential across several diseases. In LPS-activated macrophages, TEPP-46 enforced tetramerisation prevents nuclear PKM2–HIF-1 α signalling, reducing IL-1 β while enhancing IL-10 responses (Palsson-McDermott et al., 2015). TEPP-46 reduced *S. Typhimurium*-induced IL-1 β production and inflammation *in vivo*. Protective effects have also been shown in ischaemic stroke, where TEPP-46 suppressed microglial PKM2 nuclear translocation and PKM2–HIF-1 α interaction (Xia et al., 2025). This reduced neuroinflammation and decreased infarct volume after cerebral ischaemia–reperfusion injury. Beyond macrophages, TEPP-46-induced PKM2 tetramerisation can limit pathogenic adaptive immune responses in CD4⁺ T cells by suppressing PKM2 nuclear translocation (Angiari et al., 2020). This reduces T-cell proliferation and inhibits Th1 and Th17 polarisation. TEPP46 ameliorates autoimmune encephalomyelitis (EAE) development, suggesting its therapeutic relevance in T-cell-mediated autoimmunity. More recently, PKM2 activation has also been implicated in fibrosis. S-nitrosylation of PKM2 reduced its tetramerisation and pyruvate kinase activity in cardiac fibroblasts (Luo et al., 2026). This drives mitochondrial fission and cardiac fibrosis. TEPP-46 and the clinically approved pyruvate kinase activator Mitapivat alleviated fibroblast activation and cardiac fibrosis. Moreover, a clinical trial for the PKM2 modulator SYX-5219 is currently running in atopic dermatitis (Sitryx, 2026a). A Phase 1a trial has shown that this drug can be well tolerated by healthy volunteers. Also, an *in vivo* model revealed that CCL17/thymus and activation-regulated chemokine (TARC) levels are reduced by SYX-5219 in healthy volunteers, showing its therapeutic potential against atopic dermatitis (Sitryx, 2026b).

Regarding the section 'Lactate as a regulator of macrophage functions', recent studies suggest that targeting lactate signalling may provide therapeutic potential in cancer. Genetic deletion or silencing of the lactate receptor GPR132 can block lactate sensing in macrophages and reshape macrophage polarisation within the TME (Chen et al., 2017). Tumour-derived lactate promotes M2-like macrophage differentiation,

whereas GPR132 silencing drives macrophages towards an M1-like phenotype and suppresses breast cancer metastasis. In addition to targeting lactate receptors, inhibition of lactate transport may also be therapeutically useful. The MCT1 inhibitor AZD3965 has been shown to reduce tumour growth in multiple cancer models by disrupting lactate transport and metabolism (Silva et al., 2021). AZD3965 has completed phase I clinical testing and has entered phase II clinical trials for advanced solid tumours and lymphomas (Halford et al., 2023). However, its direct effects on TAMs and lactate-dependent immune regulation in the TME require further investigation.

Regarding 'Mitochondrial immunometabolites', itaconate mimetics and derivatives, particularly 4-OI, have shown anti-inflammatory effects in multiple models of inflammation (Yukun Min, 2023). 4-OI reduces excessive inflammatory responses induced by both bacterial and viral infections (Hooftman et al., 2020; Li et al., 2026; Sun et al., 2019). Through activation of NRF2, 4-OI also exerts protective effects in several liver and kidney disease models, including acute kidney injury, acute liver failure, liver fibrosis and autoimmune hepatitis (Fan et al., 2022; Li et al., 2021; Xu et al., 2023; Yang, Wang et al., 2022). In addition, 4-OI has been reported to alleviate neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease by inhibiting NLRP3 inflammasome activation and promoting NRF2-dependent antioxidant responses (Jyotirmaya Behera, 2023; Sun et al., 2022). Beyond NRF2 and NLRP3, 4-OI can also suppress MAPK signalling, thereby reducing disease severity in inflammatory conditions such as colitis and type 1 diabetes (He et al., 2023; Yang et al., 2023). Several approaches are being taken to exploit the anti-inflammatory effect of itaconate across multiple disease indicators (Evans & O'Neill, 2026).

Regarding 'ETC reprogramming', locking SDH in macrophages limits RET-induced ROS production and is effective in ischaemia reperfusion injury. Rapid reoxygenation drives the oxidation of this accumulated succinate via SDH during ischaemia–reperfusion. This then promotes RET-mediated mtROS generation, causing oxidative damage, inflammation and tissue injury (Chouchani et al., 2014). Pharmacological inhibition of SDH with malonate or dimethyl malonate reduces succinate oxidation, decreases complex I RET-driven ROS production, and alleviates injury in experimental models of myocardial and cerebral ischaemia–reperfusion. More selective approaches further support this concept. Suppressors of site IQ electron leak (S1QELs) inhibit superoxide production from complex I during RET without impairing OxPhos (Brand et al., 2016). S1QEL1.1 treatment during reperfusion facilitated post-ischaemic recovery of cardiac function and reduced the infarct size of perfused mouse hearts. Similarly, inhibition of complex I with amobarbital (AMO) during early reperfusion

reduced ROS-related injury and improved recovery in transplanted donation-after-circulatory-death rat hearts (Quader et al., 2023).

Regarding 'Mitochondrial dynamics and the Warburg effect', inhibition of mitochondrial fission has shown therapeutic potential in several animal models of inflammatory and cardiovascular disease. For example, Mdivi-1 treatment reduced LPS-induced inflammatory cytokine production *in vivo* (Yu et al., 2020). It also attenuated atherosclerotic plaque formation by suppressing macrophage M1 polarisation and NLRP3 activation (Su et al., 2023). However, because mitochondrial morphology is highly dynamic and context-dependent, inhibition of fission may not always be beneficial and can impair physiological processes such as macrophage efferocytosis. Therefore, further studies are required to define therapeutic strategies that restore mitochondrial dynamics in a disease- and cell-type-specific manner.

Conclusion

The Warburg effect in both tumours and inflammatory macrophages is now understood as more than a simple increase in glycolysis. Rather, it involves extensive metabolic remodelling that generates immuno-metabolites capable of regulating tumour cell and immune cell signalling through diverse mechanisms. Lactate and lactylation are recently recognised components of macrophage immunometabolism. They have already been shown to influence a broad range of macrophage functions, including inflammatory signalling, metabolism, phagocytosis, efferocytosis, antimicrobial activity and tissue repair. Glycolytic enzymes have more non-canonical functions outside their enzyme activity that can affect macrophages' immune response. ETC complexes are critical in producing mtROS and downstream inflammatory responses. The TCA cycle metabolites succinate, fumarate and itaconate have also shown immunoregulatory effects on inflammatory signalling. Mitochondrial dynamics provide an additional layer of regulation within the Warburg effect. Mitochondria remain functionally important during macrophage activation, despite the increased reliance on glycolysis, because they regulate innate immune signalling. Mitochondrial fission, fusion and mitophagy are all required for normal macrophage function, and disruption of their balance can promote mitochondrial stress, mtROS generation and mtDNA release, thereby activating inflammatory pathways such as NLRP3 and cGAS-STING (Park et al., 2015; Willemsen et al., 2021; Zhang et al., 2023; Zheng et al., 2025). However, the consequences of altered mitochondrial morphology are highly context-dependent (Casey et al.,

2025; Kapetanovic et al., 2020). Mitochondrial dynamics can also link metabolic reprogramming to macrophage inflammatory responses. Increased mitochondrial fission or impaired mitochondrial fusion promotes glycolysis and M1 macrophage activation (Gao et al., 2020; Kapetanovic et al., 2020; Sanchez-Rodriguez et al., 2023; Tur et al., 2020; Yu et al., 2020). Mitophagy further contributes to this process by regulating mitochondrial mass and respiratory capacity, thereby regulating glycolytic reprogramming and inflammatory signalling (Esteban-Martinez et al., 2017; Patoli et al., 2020). Further work is required to clarify how mitochondrial fission, fusion and mitophagy are coordinated during macrophage activation. All of these insights may give rise to new therapeutic opportunities to target Warburg metabolism in immune and inflammatory diseases.

References

- Agnihotri, S., Golbourn, B., Huang, X., Remke, M., Younger, S., Cairns, R. A., Chalil, A., Smith, C. A., Krumholtz, S. L., Mackenzie, D., Rakopoulos, P., Ramaswamy, V., Taccone, M. S., Mischel, P. S., Fuller, G. N., Hawkins, C., Stanford, W. L., Taylor, M. D., Zadeh, G., & Rutka, J. T. (2016). PINK1 is a negative regulator of growth and the Warburg effect in glioblastoma. *Cancer Research*, **76**(16), 4708–4719.
- Angiari, S., Runtsch, M. C., Sutton, C. E., Palsson-McDermott, E. M., Kelly, B., Rana, N., Kane, H., Papadopoulou, G., Pearce, E. L., Mills, K. H. G., & O'Neill, L. A. J. (2020). Pharmacological activation of pyruvate kinase M2 inhibits CD4⁺ T cell pathogenicity and suppresses autoimmunity. *Cell Metabolism*, **31**(2), 391–405e398.
- Bae, S., Kim, H., Lee, N., Won, C., Kim, H. R., Hwang, Y. I., Song, Y. W., Kang, J. S., & Lee, W. J. (2012). alpha-enolase expressed on the surfaces of monocytes and macrophages induces robust synovial inflammation in rheumatoid arthritis. *Journal of Immunology*, **189**(1), 365–372.
- Baik, S. H., Ramanujan, V. K., Becker, C., Fett, S., Underhill, D. M., & Wolf, A. J. (2023). Hexokinase dissociation from mitochondria promotes oligomerization of VDAC that facilitates NLRP3 inflammasome assembly and activation. *Science Immunology*, **8**(84), eade7652.
- Bambouskova, M., Potuckova, L., Paulenda, T., Kerndl, M., Mogilenko, D. A., Lizotte, K., Swain, A., Hayes, S., Sheldon, R. D., Kim, H., Kapadnis, U., Ellis, A. E., Isaguirre, C., Burdess, S., Laha, A., Amarasinghe, G. K., Chubukov, V., Roddy, T. P., Diamond, M. S., ... Artyomov, M. N. (2021). Itaconate confers tolerance to late NLRP3 inflammasome activation. *Cell Reports*, **34**(10), 108756.
- Billingham, L. K., Stoolman, J. S., Vasan, K., Rodriguez, A. E., Poor, T. A., Szibor, M., Jacobs, H. T., Reczek, C. R., Rashidi, A., Zhang, P., Miska, J., & Chandel, N. S. (2022). Mitochondrial electron transport chain is necessary for NLRP3 inflammasome activation. *Nature Immunology*, **23**(5), 692–704.

- Bourner, L. A., Chung, L. A., Long, H., McGettrick, A. F., Xiao, J., Roth, K., Bailey, J. D., Strickland, M., Tan, B., Cunningham, J., Lutzke, B., McGee, J., Otero, F. J., Gemperline, D. C., Zhang, L., Wang, Y. C., Chalmers, M. J., Yang, C. W., Gutierrez, J. A., ... Dorsey, F. C. (2024). Endogenously produced itaconate negatively regulates innate-driven cytokine production and drives global ubiquitination in human macrophages. *Cell Reports*, **43**(8), 114570.
- Brand, M. D., Goncalves, R. L., Orr, A. L., Vargas, L., Gerencser, A. A., Borch Jensen, M., Wang, Y. T., Melov, S., Turk, C. N., Matzen, J. T., Dardov, V. J., Petrassi, H. M., Meeusen, S. L., Perevoshchikova, I. V., Jasper, H., Brookes, P. S., & Ainscow, E. K. (2016). Suppressors of superoxide-H(2)O(2) production at site I(Q) of mitochondrial complex I protect against stem cell hyperplasia and ischemia-reperfusion injury. *Cell Metabolism*, **24**(4), 582–592.
- Buck, M. D., O'Sullivan, D., Klein Geltink, R. I., Curtis, J. D., Chang, C. H., Sanin, D. E., Qiu, J., Kretz, O., Braas, D., van der Windt, G. J., Chen, Q., Huang, S. C., O'Neill, C. M., Edelson, B. T., Pearce, E. J., Sesaki, H., Huber, T. B., Rambold, A. S., & Pearce, E. L. (2016). Mitochondrial dynamics controls T cell fate through metabolic programming. *Cell*, **166**(1), 63–76.
- Cameron, A. M., Castoldi, A., Sanin, D. E., Flachsmann, L. J., Field, C. S., Puleston, D. J., Kyle, R. L., Patterson, A. E., Hassler, F., Buescher, J. M., Kelly, B., Pearce, E. L., & Pearce, E. J. (2019). Inflammatory macrophage dependence on NAD⁺ salvage is a consequence of reactive oxygen species-mediated DNA damage. *Nature Immunology*, **20**(4), 420–432.
- Carneiro, F. R. G., Lepelley, A., Seeley, J. J., Hayden, M. S., & Ghosh, S. (2018). An essential role for ECSIT in mitochondrial complex I assembly and mitophagy in macrophages. *Cell Reports*, **22**(10), 2654–2666.
- Casey, A. M., Ryan, D. G., Prag, H. A., Chowdhury, S. R., Marques, E., Turner, K., Gruszczak, A. V., Yang, M., Wolf, D. M., Miljkovic, J. L., Valadares, J., Chinnery, P. F., Hartley, R. C., Frezza, C., Prudent, J., & Murphy, M. P. (2025). Pro-inflammatory macrophages produce mitochondria-derived superoxide by reverse electron transport at complex I that regulates IL-1 β release during NLRP3 inflammasome activation. *Nature Metabolism*, **7**(3), 493–507.
- Chai, L., Li, C., Wang, X., Qin, Y., Sun, H., Du, J., Yang, L., Hu, D., Xiong, J., Zhao, Z., Gong, R., Wu, T., Wu, M., Nie, M., Gao, J., Jia, J., Gao, C., Zhao, W., Zhou, H., ... Jia, M. (2025). IRG1 catalyzed energy metabolite itaconic acid restrains type I interferon-dependent immune responses by alkylation of TBK1. *Cell Reports*, **44**(10), 116336.
- Chen, C., Liu, C., Sun, P., Zhang, Z., Wang, Z., Liu, P., & Li, X. (2024). Itaconate uptake via SLC13A3 improves hepatic antibacterial innate immunity. *Developmental Cell*, **59**(21), 2807–2817e2808.
- Chen, C., Zhang, Z., Liu, C., Sun, P., Liu, P., & Li, X. (2024). ABCG2 is an itaconate exporter that limits antibacterial innate immunity by alleviating TFEB-dependent lysosomal biogenesis. *Cell Metabolism*, **36**(3), 498–510e411.
- Chen, F., Wu, R., Liu, J., Kang, R., Li, J., & Tang, D. (2022). The STING1-MYD88 complex drives ACOD1/IRG1 expression and function in lethal innate immunity. *Science*, **25**(7), 104561.
- Chen, L., Lin, Y., Zhu, X., Zhuo, S., Li, Z., Guo, C., Ye, X., Chen, J., Wang, S., & Chen, Y. (2025). MCT1-mediated lactate shuttle to mitochondria governs macrophage polarization and modulates glucose homeostasis by affecting beta cells. *Advanced Science*, **12**(38), e14760.
- Chen, L. L., Morcelle, C., Cheng, Z. L., Chen, X., Xu, Y., Gao, Y., Song, J., Li, Z., Smith, M. D., Shi, M., Zhu, Y., Zhou, N., Cheng, M., He, C., Liu, K. Y., Lu, G., Zhang, L., Zhang, C., Zhang, J., ... Ye, D. (2022). Itaconate inhibits TET DNA dioxygenases to dampen inflammatory responses. *Nature Cell Biology*, **24**(3), 353–363.
- Chen, P., Zuo, H., Xiong, H., Kolar, M. J., Chu, Q., Saghatelian, A., Siegwart, D. J., & Wan, Y. (2017). Gpr132 sensing of lactate mediates tumor-macrophage interplay to promote breast cancer metastasis. *Proceedings of the National Academy of Sciences*, **114**(3), 580–585.
- Cheng, C., Xing, Z., Zhang, W., Zheng, L., Zhao, H., Zhang, X., Ding, Y., Qiao, T., Li, Y., Meyron-Holtz, E. G., Missirlis, F., Fan, Z., & Li, K. (2024). Iron regulatory protein 2 contributes to antimicrobial immunity by preserving lysosomal function in macrophages. *Proceedings of the National Academy of Sciences of the United States of America*, **121**(31), e2321929121.
- Chouchani, E. T., Pell, V. R., Gaude, E., Aksentijevic, D., Sundier, S. Y., Robb, E. L., Logan, A., Nadtochiy, S. M., Ord, E. N. J., Smith, A. C., Eyassu, F., Shirley, R., Hu, C. H., Dare, A. J., James, A. M., Rogatti, S., Hartley, R. C., Eaton, S., Costa, A. S. H., ... Murphy, M. P. (2014). Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. *Nature*, **515**(7527), 431–435.
- Chourasia, A. H., Tracy, K., Frankenberger, C., Boland, M. L., Sharifi, M. N., Drake, L. E., Sachleben, J. R., Asara, J. M., Locasale, J. W., Karczmar, G. S., & Macleod, K. F. (2015). Mitophagy defects arising from BNip3 loss promote mammary tumor progression to metastasis. *European Molecular Biology Organization Reports*, **16**(9), 1145–1163.
- Colegio, O. R., Chu, N. Q., Szabo, A. L., Chu, T., Rhebergen, A. M., Jairam, V., Cyrus, N., Brokowski, C. E., Eisenbarth, S. C., Phillips, G. M., Cline, G. W., Phillips, A. J., & Medzhitov, R. (2014). Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. *Nature*, **513**(7519), 559–563.
- Cramer, T., Yamanishi, Y., Clausen, B. E., Forster, I., Pawlinski, R., Mackman, N., Haase, V. H., Jaenisch, R., Corr, M., Nizet, V., Firestein, G. S., Gerber, H. P., Ferrara, N., & Johnson, R. S. (2003). HIF-1 α is essential for myeloid cell-mediated inflammation. *Cell*, **112**(5), 645–657.
- Cui, H., Xie, L., Lu, H., Cheng, C., Xue, F., Wu, Z., Liu, L., Qiao, L., Zhang, C., Zhang, W., & Yang, J. (2025). Macrophage junctional adhesion molecule-like (JAML) protein promotes NLRP3 inflammasome activation in the development of atherosclerosis. *Cell Death and Differentiation*, **32**(10), 1763–1776.

- De Jesus, A., Keyhani-Nejad, F., Pusec, C. M., Goodman, L., Geier, J. A., Stoolman, J. S., Stanczyk, P. J., Nguyen, T., Xu, K., Suresh, K. V., Chen, Y., Rodriguez, A. E., Shapiro, J. S., Chang, H. C., Chen, C., Shah, K. P., Ben-Sahra, I., Layden, B. T., Chandel, N. S., ... Ardehali, H. (2022). Hexokinase 1 cellular localization regulates the metabolic fate of glucose. *Molecular Cell*, **82**(7), 1261–1277e1269.
- De Leon-Oliva, D., Gonzalez-Prieto, P., De Castro-Martinez, P., Boaru, D. L., Laguna-Hernandez, P., Fraile-Martinez, O., Garcia-Montero, C., Guijarro, L. G., Lopez-Gonzalez, L., Diaz-Pedrero, R., Alvarez-Mon, M., Saez, M. A., & Ortega, M. A. (2025). Revisiting the biological role of the Warburg effect: Evolving perspectives on cancer metabolism. *Pathology, Research and Practice*, **273**, 156151.
- Du, H., Xu, T., Yu, S., Wu, S., & Zhang, J. (2025). Mitochondrial metabolism and cancer therapeutic innovation. *Signal Transduct Target Ther*, **10**(1), 245.
- Esteban-Martinez, L., Sierra-Filardi, E., McGreal, R. S., Salazar-Roa, M., Marino, G., Seco, E., Durand, S., Enot, D., Grana, O., Malumbres, M., Cvekl, A., Cuervo, A. M., Kroemer, G., & Boya, P. (2017). Programmed mitophagy is essential for the glycolytic switch during cell differentiation. *European Molecular Biology Organization Journal*, **36**(12), 1688–1706.
- Evans, V. A., & O'Neill, L. A. J. (2026). Lessons from glucocorticoids, metformin, and dimethyl fumarate: Could targeting immunometabolism lead to better anti-inflammatory therapies? *Annual Review of Pharmacology and Toxicology*, **66**(1), 419–440.
- Fan, K., Zan, X., Zhi, Y., Yang, Y., Hu, K., Zhang, X., Zhang, X., Zhao, S., Chen, K., Gong, X., Tian, R., & Zhang, L. (2022). Immune response gene 1 deficiency impairs Nrf2 activation and aggravates liver fibrosis in mice. *Biochemical and Biophysical Research Communications*, **607**, 103–109.
- Galvan-Pena, S., Carroll, R. G., Newman, C., Hinchey, E. C., Palsson-McDermott, E., Robinson, E. K., Covarrubias, S., Nadin, A., James, A. M., Haneklaus, M., Carpenter, S., Kelly, V. P., Murphy, M. P., Modis, L. K., & O'Neill, L. A. (2019). Malonylation of GAPDH is an inflammatory signal in macrophages. *Nature Communications*, **10**(1), 338.
- Gandhirajan, R. K., Rodder, K., Bodnar, Y., Pasqual-Melo, G., Emmert, S., Griguer, C. E., Weltmann, K. D., & Bekeschus, S. (2018). Cytochrome C oxidase inhibition and cold plasma-derived oxidants synergize in melanoma cell death induction. *Scientific Reports*, **8**(1), 12734.
- Ganta, V. C., Choi, M. H., Kutateladze, A., Fox, T. E., Farber, C. R., & Annex, B. H. (2017). A MicroRNA93-interferon regulatory factor-9-immunoresponsive gene-1-itaconic acid pathway modulates M2-like macrophage polarization to revascularize ischemic muscle. *Circulation*, **135**(24), 2403–2425.
- Gao, F., Reynolds, M. B., Passalacqua, K. D., Sexton, J. Z., Abuaita, B. H., & O'Riordan, M. X. D. (2020). The mitochondrial fission regulator DRP1 controls post-transcriptional regulation of TNF- α . *Frontiers in Cellular and Infection Microbiology*, **10**, 593805.
- Garaude, J., Acin-Perez, R., Martinez-Cano, S., Enamorado, M., Ugolini, M., Nistal-Villan, E., Hervás-Stubbbs, S., Pelegrin, P., Sander, L. E., Enriquez, J. A., & Sancho, D. (2016). Mitochondrial respiratory-chain adaptations in macrophages contribute to antibacterial host defense. *Nature Immunology*, **17**(9), 1037–1045.
- Goodwin, P. J., Dowling, R. J. O., Ennis, M., Chen, B. E., Parulekar, W. R., Shepherd, L. E., Burnell, M. J., Vander Meer, R., Molckovsky, A., Gurjal, A., Gelmon, K. A., Ligibel, J. A., Hershman, D. L., Mayer, I. A., Whelan, T. J., Hobday, T. J., Rastogi, P., Rabaglio-Poretti, M., Lemieux, J., ... Stambolic, V. (2021). Effect of metformin versus placebo on metabolic factors in the MA.32 randomized breast cancer trial. *Nature Partner Journal Breast Cancer*, **7**(1), 74.
- Gutierrez, S., Fischer, J., Ganesan, R., Hos, N. J., Cildir, G., Wolke, M., Pessia, A., Frommolt, P., Desiderio, V., Velagapudi, V., & Robinson, N. (2021). *Salmonella typhimurium* impairs glycolysis-mediated acidification of phagosomes to evade macrophage defense. *PLoS Pathogens*, **17**(9), e1009943.
- Halford, S., Veal, G. J., Wedge, S. R., Payne, G. S., Bacon, C. M., Sloan, P., Dragoni, I., Heinzmann, K., Potter, S., Salisbury, B. M., Chenard-Poirier, M., Greystoke, A., Howell, E. C., Innes, W. A., Morris, K., Plummer, C., Rata, M., Petrides, G., Keun, H. C., ... Plummer, R. (2023). A Phase I dose-escalation study of AZD3965, an oral monocarboxylate transporter 1 inhibitor, in patients with advanced cancer. *Clinical Cancer Research*, **29**(8), 1429–1439.
- Harber, K. J., de Goede, K. E., Verberk, S. G. S., Meinster, E., de Vries, H. E., van Weeghel, M., de Winther, M. P. J., & Van den Bossche, J. (2020). Succinate is an inflammation-induced immunoregulatory metabolite in macrophages. *Metabolites*, **10**(9), 372.
- Hard, G. C. (1970). Some biochemical aspects of the immune macrophage. *British Journal of Experimental Pathology*, **51**(1), 97–105.
- He, S., Zhao, Y., Wang, G., Ke, Q., Wu, N., Lu, L., Wu, J., Sun, S., Jin, W., Zhang, W., & Zhou, J. (2023). 4-Octyl itaconate attenuates glycaemic deterioration by regulating macrophage polarization in mouse models of type 1 diabetes. *Molecular Medicine*, **29**(1), 31.
- Hooftman, A., Angiari, S., Hester, S., Corcoran, S. E., Runttsch, M. C., Ling, C., Ruzek, M. C., Slivka, P. F., McGettrick, A. F., Banahan, K., Hughes, M. M., Irvine, A. D., Fischer, R., & O'Neill, L. A. J. (2020). The immunomodulatory metabolite itaconate modifies NLRP3 and inhibits inflammasome activation. *Cell Metabolism*, **32**(3), 468–478e467.
- Hooftman, A., Peace, C. G., Ryan, D. G., Day, E. A., Yang, M., McGettrick, A. F., Yin, M., Montano, E. N., Huo, L., Toller-Kawahisa, J. E., Zecchini, V., Ryan, T. A. J., Bolado-Carrancio, A., Casey, A. M., Prag, H. A., Costa, A. S. H., De Los Santos, G., Ishimori, M., Wallace, D. J., ... O'Neill, L. A. J. (2023). Macrophage fumarate hydratase restrains mtRNA-mediated interferon production. *Nature*, **615**(7952), 490–498.

- Hu, D. W., Huang, C. H., He, Y. H., Wei, Y. L., Hu, S. W., Cheng, F. J., Huynh, T. K., Chen, B. R., Wang, B. W., Kuan, L. C., Lee, D. Y., Yeh, M. H., Chang, Y. J., Liu, L. C., Hung, M. C., & Huang, W. C. (2025). SLC6A14 drives mitochondrial fusion and oxidative phosphorylation to promote cancer stemness and early-onset of breast cancer. *Advanced Science*, **12**(45), e10811.
- Humphries, F., Shmuel-Galia, L., Ketelut-Carneiro, N., Li, S., Wang, B., Nemmara, V. V., Wilson, R., Jiang, Z., Khalighinejad, F., Muneeruddin, K., Shaffer, S. A., Dutta, R., Ionete, C., Pesiridis, S., Yang, S., Thompson, P. R., & Fitzgerald, K. A. (2020). Succination inactivates gasdermin D and blocks pyroptosis. *Science*, **369**(6511), 1633–1637.
- Irizarry-Caro, R. A., McDaniel, M. M., Overcast, G. R., Jain, V. G., Troutman, T. D., & Pasare, C. (2020). TLR signaling adapter BCAP regulates inflammatory to reparatory macrophage transition by promoting histone lactylation. *Proceedings of the National Academy of Sciences of the United States of America*, **117**(48), 30628–30638.
- Jha, A. K., Huang, S. C., Sergushichev, A., Lampropoulou, V., Ivanova, Y., Loginicheva, E., Chmielewski, K., Stewart, K. M., Ashall, J., Everts, B., Pearce, E. J., Driggers, E. M., & Artyomov, M. N. (2015). Network integration of parallel metabolic and transcriptional data reveals metabolic modules that regulate macrophage polarization. *Immunity*, **42**(3), 419–430.
- Jia, A., Zhang, X., Zhou, J. H., Ma, Y., Wang, J., Gong, Y., Song, W., Hu, H., Yu, Y., Yang, H., Lu, Y., Huang, L., Du, X., Chang, C., Pei, S., Li, P., Jordan, C. T., Zhao, T. J., & Ye, H. (2026). Dual targeting of SLC25A51 and succinate dehydrogenase selectively depletes mitochondrial NAD⁺ to eradicate KRAS-driven AML. *Cell Metabolism*, **38**(6), 1113–1129.e1112.
- Jiang, L., Wang, P., Song, X., Zhang, H., Ma, S., Wang, J., Li, W., Lv, R., Liu, X., Ma, S., Yan, J., Zhou, H., Huang, D., Cheng, Z., Yang, C., Feng, L., & Wang, L. (2021). *Salmonella typhimurium* reprograms macrophage metabolism via T3SS effector SopE2 to promote intracellular replication and virulence. *Nature Communications*, **12**(1), 879.
- Jiang, M., Yang, Y., Zhang, Y., Zhang, P., Zhang, Q., Yang, T., Zhang, G., Zhou, J., Chu, Z., Li, S., Wang, Y., Zhang, J., Shen, S., Song, P., Wang, J., & Tang, H. (2026). Covalent targeting GAPDH by Pudilan formula and baicalein suppresses macrophage Warburg effect to alleviate acute lung injury. *Phytomedicine*, **151**, 157727.
- Jiang, Y., Krantz, S., Qin, X., Li, S., Gunasekara, H., Kim, Y. M., Zimnicka, A., Bae, M., Ma, K., Toth, P. T., Hu, Y., Shajahan-Haq, A. N., Patel, H. H., Gentile, S., Bonini, M. G., Rehman, J., Liu, Y., & Minshall, R. D. (2022). Caveolin-1 controls mitochondrial damage and ROS production by regulating fission–fusion dynamics and mitophagy. *Redox Biology*, **52**, 102304.
- Behera, J., N, F., Zhang, Y. U.-T., Smolenkova, I., & Tyagi, a.N. (2023). 4-octyl itaconate ameliorate cognitive impairment in 3xTg mouse model of Alzheimer's disease. *Physiology*, **38**(51), 5732886.
- Kapetanovic, R., Afroz, S. F., Ramnath, D., Lawrence, G. M., Okada, T., Curson, J. E., de Bruin, J., Fairlie, D. P., Schroder, K., St John, J. C., Blumenthal, A., & Sweet, M. J. (2020). Lipopolysaccharide promotes Drp1-dependent mitochondrial fission and associated inflammatory responses in macrophages. *Immunology and Cell Biology*, **98**(7), 528–539.
- Keiran, N., Ceperuelo-Mallafre, V., Calvo, E., Hernandez-Alvarez, M. I., Ejarque, M., Nunez-Roa, C., Horrillo, D., Maymo-Masip, E., Rodriguez, M. M., Fradera, R., de la Rosa, J. V., Jorba, R., Megia, A., Zorzano, A., Medina-Gomez, G., Serena, C., Castrillo, A., Vendrell, J., & Fernandez-Veledo, S. (2019). SUCNR1 controls an anti-inflammatory program in macrophages to regulate the metabolic response to obesity. *Nature Immunology*, **20**(5), 581–592.
- Kornberg, M. D., Bhargava, P., Kim, P. M., Putluri, V., Snowman, A. M., Putluri, N., Calabresi, P. A., & Snyder, S. H. (2018). Dimethyl fumarate targets GAPDH and aerobic glycolysis to modulate immunity. *Science*, **360**(6387), 449–453.
- Lachmandas, E., Boutens, L., Ratter, J. M., Hijmans, A., Hooiveld, G. J., Joosten, L. A., Rodenburg, R. J., Franssen, J. A., Houtkooper, R. H., van Crevel, R., Netea, M. G., & Stienstra, R. (2016). Microbial stimulation of different toll-like receptor signalling pathways induces diverse metabolic programmes in human monocytes. *Nature Microbiology*, **2**, 16246.
- Lampropoulou, V., Sergushichev, A., Bambouskova, M., Nair, S., Vincent, E. E., Loginicheva, E., Cervantes-Barragan, L., Ma, X., Huang, S. C., Griss, T., Weinheimer, C. J., Khader, S., Randolph, G. J., Pearce, E. J., Jones, R. G., Diwan, A., Diamond, M. S., & Artyomov, M. N. (2016). Itaconate links inhibition of succinate dehydrogenase with macrophage metabolic remodeling and regulation of inflammation. *Cell Metabolism*, **24**(1), 158–166.
- Lee, C. G., Jenkins, N. A., Gilbert, D. J., Copeland, N. G., & O'Brien, W. E. (1995). Cloning and analysis of gene regulation of a novel LPS-inducible cDNA. *Immunogenetics*, **41**(5), 263–270.
- Li, J., Chen, X., Song, S., Jiang, W., Geng, T., Wang, T., Xu, Y., Zhu, Y., Lu, J., Xia, Y., & Wang, R. (2025). Hexokinase 2-mediated metabolic stress and inflammation burden of liver macrophages via histone lactylation in MASLD. *Cell Reports*, **44**(3), 115350.
- Li, L., Chen, Y., Wang, Y., Dong, Y., Wang, H., Liu, Z., Ge, X., Shen, P., Li, Y., Liu, D., & Zhou, X. (2025). *Mycobacterium bovis* induces macrophage mitochondrial release of hexokinase 2 to enhance intracellular survival. *Cell Reports*, **44**(10), 116421.
- Li, P., Fan, Z., Huang, Y., Luo, L., & Wu, X. (2025). Mitochondrial dynamics at the intersection of macrophage polarization and metabolism. *Frontiers in Immunology*, **16**, 1520814.
- Li, R., Mei, S., Ding, Q., Wang, Q., Yu, L., & Zi, F. (2022). A pan-cancer analysis of the role of hexokinase II (HK2) in human tumors. *Scientific Reports*, **12**(1), 18807.

- Li, R., Yang, W., Yin, Y., Zhang, P., Wang, Y., & Tao, K. (2021). Protective role of 4-octyl itaconate in murine LPS/D-GalN-induced acute liver failure via inhibiting inflammation, oxidative stress, and apoptosis. *Oxidative Medicine and Cellular Longevity*, **2021**, 9932099.
- Li, W., Zhang, Q., Jing, S., Deng, L., Jiang, H., Zhang, J., Hong, Z., Zhang, Z., Hao, S., Wu, L., Zheng, T., Yu, Y., Ma, X., Wang, X., Li, Q., Yu, Z., Liu, M., Yang, J., Liu, B., ... Wu, X. (2026). 4-octyl itaconate inhibits cytokine-mediated inflammation via alkylation of TYK2 and JAK1. *Cell Reports*, **45**(4), 117179.
- Liao, L., Dang, W., Lin, T., Yu, J., Liu, T., Li, W., Xiao, S., Feng, L., Huang, J., Fu, R., Li, J., Liu, L., Wang, M., Tao, H., Jiang, H., Chen, K., Diao, X., Zhou, B., Shen, X., & Luo, C. (2022). A potent PGK1 antagonist reveals PGK1 regulates the production of IL-1 β and IL-6. *Acta Pharmaceutica Sinica B*, **12**(11), 4180–4192.
- Liao, S. T., Han, C., Xu, D. Q., Fu, X. W., Wang, J. S., & Kong, L. Y. (2019). 4-Octyl itaconate inhibits aerobic glycolysis by targeting GAPDH to exert anti-inflammatory effects. *Nature Communications*, **10**(1), 5091.
- Lin, H. A., Lin, H. C., Tsai, M. H., Chen, Y. J., Bao, B. Y., Tsai, K. J., Shih, C. T., Yu, J. S., Chien, K. Y., Huang, K. Y., Ojcius, D. M., & Chen, L. C. (2026). Lactic acid drives NLRP3 inflammasome activation and caspase-1-like cytokine cleavage via intracellular acidification. *Cell Death & Disease*, **17**(1), 450.
- Littlewood-Evans, A., Sarret, S., Apfel, V., Loesle, P., Dawson, J., Zhang, J., Muller, A., Tigani, B., Kneuer, R., Patel, S., Valeaux, S., Gommermann, N., Rubic-Schneider, T., Junt, T., & Carballido, J. M. (2016). GPR91 senses extracellular succinate released from inflammatory macrophages and exacerbates rheumatoid arthritis. *Journal of Experimental Medicine*, **213**(9), 1655–1662.
- Liu, W., Zhang, T., Wang, B., & Yin, H. (2026). Aerobic glycolysis promotes NLRP3 inflammasome activation via NLRP3 lactylation. *Cell Chemical Biology*, **33**(2), 213–226e215.
- Luo, S., Ye, D., Zhang, Y., Wang, Y., Zhou, M., Lv, M., Wang, X., Zhong, K., Zhang, Y., Hu, L., Sun, S., Zhang, Z., Yu, B., Sun, C., Kong, X., Huang, Z., Chen, X., Han, Y., Xie, L., & Ji, Y. (2026). S-nitrosylation of pyruvate kinase isoform 2 drives cardiac fibrosis by promoting mitochondrial fission. *Circulation*, **153**(5), 338–357.
- Ma, J., Chen, Y., Wang, P., Zhang, Y., Li, Y., Tu, Q., Zhao, X., Yao, X., Li, F., Yuan, Y., Wu, C., Wang, L., Chen, Y., Liu, C., Kang, R., Tang, D., Yao, L., Chen, F., & Li, J. (2026). The alkylation of AIM2 by itaconate mediates macrophage PANoptosis during sepsis. *Cellular & Molecular Immunology*, **23**(6), 619–634.
- Martinez-Reyes, I., Cardona, L. R., Kong, H., Vasan, K., McElroy, G. S., Werner, M., Kihshen, H., Reczek, C. R., Weinberg, S. E., Gao, P., Steinert, E. M., Piseaux, R., Budinger, G. R. S., & Chandel, N. S. (2020). Mitochondrial ubiquinol oxidation is necessary for tumour growth. *Nature*, **585**(7824), 288–292.
- Meng, Q., Li, C., Cai, Y., Chen, Y., Chen, X., Wang, X., Zhang, B., Zhang, Y., Liu, F., & Chen, M. (2025). Itaconate transport across the plasma membrane and Salmonella-containing vacuole via MCT1/4 modulates macrophage antibacterial activity. *Nature Communications*, **16**(1), 10551.
- Michelucci, A., Cordes, T., Ghelfi, J., Pailot, A., Reiling, N., Goldmann, O., Binz, T., Wegner, A., Tallam, A., Rausell, A., Buttini, M., Linster, C. L., Medina, E., Balling, R., & Hiller, K. (2013). Immune-responsive gene 1 protein links metabolism to immunity by catalyzing itaconic acid production. *Proceedings of the National Academy of Sciences of the United States of America*, **110**(19), 7820–7825.
- Mills, E. L., Kelly, B., Logan, A., Costa, A. S. H., Varma, M., Bryant, C. E., Tourlomousis, P., Dabritz, J. H. M., Gottlieb, E., Latorre, I., Corr, S. C., McManus, G., Ryan, D., Jacobs, H. T., Szibor, M., Xavier, R. J., Braun, T., Frezza, C., Murphy, M. P., & O'Neill, L. A. (2016). Succinate dehydrogenase supports metabolic repurposing of mitochondria to drive inflammatory macrophages. *Cell*, **167**(2), 457–470 e413.
- Mills, E. L., Ryan, D. G., Prag, H. A., Dikovskaya, D., Menon, D., Zaslona, Z., Jedrychowski, M. P., Costa, A. S. H., Higgins, M., Hams, E., Szpyt, J., Runtsch, M. C., King, M. S., McGouran, J. F., Fischer, R., Kessler, B. M., McGettrick, A. F., Hughes, M. M., Carroll, R. G., ... O'Neill, L. A. (2018). Itaconate is an anti-inflammatory metabolite that activates Nrf2 via alkylation of KEAP1. *Nature*, **556**(7699), 113–117.
- Minhas, P. S., Liu, L., Moon, P. K., Joshi, A. U., Dove, C., Mhatre, S., Contrepolis, K., Wang, Q., Lee, B. A., Coronado, M., Bernstein, D., Snyder, M. P., Migaud, M., Majeti, R., Mochly-Rosen, D., Rabinowitz, J. D., & Andreasson, K. I. (2019). Macrophage de novo NAD⁺ synthesis specifies immune function in aging and inflammation. *Nature Immunology*, **20**(1), 50–63.
- Mourier, A., Motori, E., Brandt, T., Lagouge, M., Atanassov, I., Galinier, A., Rappl, G., Brodesser, S., Hultenby, K., Dieterich, C., & Larsson, N. G. (2015). Mitofusin 2 is required to maintain mitochondrial coenzyme Q levels. *Journal of Cell Biology*, **208**(4), 429–442.
- Naik, P. P. (2020). Mitophagy and reverse Warburg effect: Metabolic compartmentalization of tumor micro-environment. In S. K. Bhutia (Ed.), *Autophagy in tumor and tumor microenvironment* (pp. 117–140). Springer Singapore.
- Newsholme, P., Curi, R., Gordon, S., & Newsholme, E. A. (1986). Metabolism of glucose, glutamine, long-chain fatty acids and ketone bodies by murine macrophages. *Biochemical Journal*, **239**(1), 121–125.
- O'Carroll, S. M., Peace, C. G., Toller-Kawahisa, J. E., Min, Y., Hooftman, A., Charki, S., Kehoe, L., O'Sullivan, M. J., Zoller, A., McGettrick, A. F., Zotta, A., Day, E. A., Simarro, M., Armstrong, N., Annes, J. P., & O'Neill, L. A. J. (2024). Itaconate drives mtRNA-mediated type I interferon production through inhibition of succinate dehydrogenase. *Nature Metabolism*, **6**(11), 2060–2069.

- Palmieri, E. M., Gonzalez-Cotto, M., Baseler, W. A., Davies, L. C., Ghesquiere, B., Maio, N., Rice, C. M., Rouault, T. A., Cassel, T., Higashi, R. M., Lane, A. N., Fan, T. W., Wink, D. A., & McVicar, D. W. (2020). Nitric oxide orchestrates metabolic rewiring in M1 macrophages by targeting aconitase 2 and pyruvate dehydrogenase. *Nature Communications*, **11**(1), 698.
- Palsson-McDermott, E. M., Curtis, A. M., Goel, G., Lauterbach, M. A., Sheedy, F. J., Gleeson, L. E., van den Bosch, M. W., Quinn, S. R., Domingo-Fernandez, R., Johnston, D. G., Jiang, J. K., Israelsen, W. J., Keane, J., Thomas, C., Clish, C., Heiden, V. M., X., R. J., & O'Neill, L. A. (2015). Pyruvate kinase M2 regulates hif-1 α activity and IL-1 β induction and is a critical determinant of the warburg effect in LPS-activated macrophages. *Cell Metabolism*, **21**(1), 65–80.
- Park, S., Won, J. H., Hwang, I., Hong, S., Lee, H. K., & Yu, J. W. (2015). Defective mitochondrial fission augments NLRP3 inflammasome activation. *Scientific Reports*, **5**, 15489.
- Patoli, D., Mignotte, F., Deckert, V., Dusuel, A., Dumont, A., Rieu, A., Jalil, A., Van Dongen, K., Bourgeois, T., Gautier, T., Magnani, C., Le Guern, N., Mandard, S., Bastin, J., Djouadi, F., Schaeffer, C., Guillaumot, N., Narce, M., Nguyen, M., ... Thomas, C. (2020). Inhibition of mitophagy drives macrophage activation and antibacterial defense during sepsis. *Journal of Clinical Investigation*, **130**(11), 5858–5874.
- Paulenda, T., Echalar, B., Potuckova, L., Vachova, V., Kleverov, D. A., Mehringer, J., Potekhina, E., Jacoby, A., Sen, D., Nelson, C., Stegeman, R., Sukhov, V., Kemper, D., Lichti, C. F., Day, N. J., Zhang, T., Husarcikova, K., Bambouskova, M., Fremont, D. H., ... Artyomov, M. N. (2025). Itaconate modulates immune responses via inhibition of peroxiredoxin 5. *Nature Metabolism*, **7**(6), 1183–1203.
- Pavlidis, S., Whitaker-Menezes, D., Castello-Cros, R., Flomenberg, N., Witkiewicz, A. K., Frank, P. G., Casimiro, M. C., Wang, C., Fortina, P., Addya, S., Pestell, R. G., Martinez-Outschoorn, U. E., Sotgia, F., & Lisanti, M. P. (2009). The reverse Warburg effect: Aerobic glycolysis in cancer associated fibroblasts and the tumor stroma. *Cell Cycle*, **8**(23), 3984–4001.
- Pfefer, T., Bessenyei, A. H., & O'Neill, L. A. (2026). Immuno-metabolites and cancer: The role of 2-hydroxyglutarate, succinate, fumarate and itaconate in tumour development and anti-tumour immunity. *Immunology Letters*, **279**, 107153.
- Qin, R. X., Ma, X. Y., Han, Z. Y., Ma, S. Y., Shen, Z. J., Lu, Z. H., Sun, Y., & Yu, W. L. (2024). IRF2 affects LPS- and IFN- γ -induced pro-inflammatory responses, cell viability, migration and apoptosis of macrophages by regulating IRG1. *Journal of Inflammation Research*, **17**, 9651–9664.
- Qin, W., Qin, K., Zhang, Y., Jia, W., Chen, Y., Cheng, B., Peng, L., Chen, N., Liu, Y., Zhou, W., Wang, Y. L., Chen, X., & Wang, C. (2019). S-glycosylation-based cysteine profiling reveals regulation of glycolysis by itaconate. *Nature Chemical Biology*, **15**(10), 983–991.
- Quader, M., Chen, Q., Akande, O., Cholyway, R., Mezzaroma, E., Lesnefsky, E. J., & Toldo, S. (2023). Electron transport chain inhibition to decrease injury in transplanted donation after circulatory death rat hearts. *Journal of Cardiovascular Pharmacology*, **81**(6), 389–391.
- Reynolds, M. B., Hong, H. S., Michmerhuizen, B. C., Lawrence, A. E., Zhang, L., Knight, J. S., Lyssiotis, C. A., Abuaita, B. H., & O'Riordan, M. X. (2023). Cardiolipin coordinates inflammatory metabolic reprogramming through regulation of complex II disassembly and degradation. *Science Advances*, **9**(5), eade8701.
- Reynolds, M. B., Klein, B., McFadden, M. J., Judge, N. K., Navarrete, H. E., Michmerhuizen, B. C., Awad, D., Schultz, T. L., Harms, P. W., Zhang, L., O'Meara, T. R., Sexton, J. Z., Lyssiotis, C. A., Kahlenberg, J. M., & O'Riordan, M. X. (2024). Type I interferon governs immunometabolic checkpoints that coordinate inflammation during staphylococcal infection. *Cell Reports*, **43**(8), 114607.
- Rittenhouse, J. W., & McFadden, B. A. (1974). Inhibition of isocitrate lyase from *Pseudomonas indigofera* by itaconate. *Archives of Biochemistry and Biophysics*, **163**(1), 79–86.
- Ryu, K. W., Fung, T. S., Baker, D. C., Saoi, M., Park, J., Febres-Aldana, C. A., Aly, R. G., Cui, R., Sharma, A., Fu, Y., Jones, O. L., Cai, X., Pasolli, H. A., Cross, J. R., Rudin, C. M., & Thompson, C. B. (2024). Cellular ATP demand creates metabolically distinct subpopulations of mitochondria. *Nature*, **635**(8039), 746–754.
- Sanchez-Rodriguez, R., Tezze, C., Agnellini, A. H. R., Angioni, R., Venegas, F. C., Cioccarelli, C., Munari, F., Bertoldi, N., Canton, M., Desbats, M. A., Salvati, L., Gissi, R., Castegna, A., Soriano, M. E., Sandri, M., Scorrano, L., Viola, A., & Molon, B. (2023). OPA1 drives macrophage metabolism and functional commitment via p65 signaling. *Cell Death and Differentiation*, **30**(3), 742–752.
- Schuster, E. M., Eppele, M. W., Glaser, K. M., Mihlan, M., Lucht, K., Zimmermann, J. A., Bremser, A., Polyzou, A., Obier, N., Cabezas-Wallscheid, N., Trompouki, E., Ballabio, A., Vogel, J., Buescher, J. M., Westermann, A. J., & Rambold, A. S. (2022). TFEB induces mitochondrial itaconate synthesis to suppress bacterial growth in macrophages. *Nature Metabolism*, **4**(7), 856–866.
- Sen, S., Deshmane, S. L., Kaminski, R., Amini, S., & Datta, P. K. (2017). Non-metabolic role of PKM2 in regulation of the HIV-1 LTR. *Journal of Cellular Physiology*, **232**(3), 517–525.
- Shirai, T., Nazarewicz, R. R., Wallis, B. B., Yanes, R. E., Watanabe, R., Hilhorst, M., Tian, L., Harrison, D. G., Giacomini, J. C., Assimes, T. L., Goronzy, J. J., & Weyand, C. M. (2016). The glycolytic enzyme PKM2 bridges metabolic and inflammatory dysfunction in coronary artery disease. *Journal of Experimental Medicine*, **213**(3), 337–354.
- Silva, A., Antunes, B., Batista, A., Pinto-Ribeiro, F., Baltazar, F., & Afonso, J. (2021). In vivo anticancer activity of AZD3965: A systematic review. *Molecules*, **27**(1), 181.
- Sitryx advances development of PKM2 modulator SYX-5219 into atopic dermatitis patients following clinical evidence of immunomodulatory activity (2026). Sytrix. <https://www.sitryx.com/news/sitryx-advances-development-of-pkm2-modulator-syx-5219-into-atopic-dermatitis-patients-following-clinical-evidence-of-immunomodulatory-activity>

- Sitryx completes enrollment in phase 1b trial of first-in-class oral PKM2 modulator SYX-5219 in atopic dermatitis (2026). Sytryx. <https://www.sitryx.com/news/sitryx-completes-enrollment-in-phase-1b-trial-of-first-in-class-oral-pkm2-modulator-syx-5219-in-atopic-dermatitis>
- Stewart, A. E., Zachman, D. K., Castellano-Escuder, P., Kelly, L. M., Zolyomi, B., Aiduk, M. D. I., Delaney, C. D., Lock, I. C., Bosc, C., Bradley, J., Killarney, S. T., Stuart, J. D., Grimsrud, P. A., Ilkayeva, O. R., Newgard, C. B., Chandel, N. S., Puissant, A., Wood, K. C., & Hirschey, M. D. (2025). Pathway coessentiality mapping reveals complex II is required for de novo purine biosynthesis in acute myeloid leukaemia. *Nature Metabolism*, 7(12), 2474–2488.
- Stoolman, J. S., Grant, R. A., Billingham, L. K., Poor, T. A., Weinberg, S. E., Harding, M. C., Lu, Z., Miska, J., Szibor, M., Budinger, G. S., & Chandel, N. S. (2025). Mitochondria complex III-generated superoxide is essential for IL-10 secretion in macrophages. *Science Advances*, 11(4), eadu4369.
- Su, Z., Lan, J., Wang, Y., Ma, N., Yang, J., Liang, D., Zeng, H., & Yang, M. (2025). Lactylation-driven ALKBH5 diminishes macrophage NLRP3 inflammasome activation in patients with G6PT deficiency. *Journal of Allergy and Clinical Immunology*, 155(6), 1783–1799 e1788.
- Su, Z. D., Li, C. Q., Wang, H. W., Zheng, M. M., & Chen, Q. W. (2023). Inhibition of DRP1-dependent mitochondrial fission by Mdivi-1 alleviates atherosclerosis through the modulation of M1 polarization. *Journal of Translational Medicine*, 21(1), 427.
- Sun, G., Zhang, R., Liu, C., Meng, W., & Pang, Q. (2022). Itaconate attenuates neuroinflammation and exerts dopamine neuroprotection in Parkinson's disease through inhibiting NLRP3 inflammasome. *Brain Sciences*, 12(9), 1255.
- Sun, L., Wu, S., Wang, H., Zhang, T., Zhang, M., Bai, X., Zhang, X., Li, B., Zhang, C., Li, Y., Zhou, J., & Li, T. (2024). PDCD6 regulates lactate metabolism to modulate LC3-associated phagocytosis and antibacterial defense. *Nature Communications*, 15(1), 10157.
- Sun, X., Yegambaram, M., Lu, Q., Garcia Flores, A. E., Pokharel, M. D., Soto, J., Aggarwal, S., Wang, T., Fineman, J. R., & Black, S. M. (2025). Mitochondrial fission produces a Warburg effect via the oxidative inhibition of prolyl hydroxylase domain-2. *Redox Biology*, 81, 103529.
- Sun, X., Zhang, B., Pan, X., Huang, H., Xie, Z., Ma, Y., Hu, B., Wang, J., Chen, Z., & Shi, P. (2019). Octyl itaconate inhibits osteoclastogenesis by suppressing Hrd1 and activating Nrf2 signaling. *Federation of American Societies for Experimental Biology Journal*, 33(11), 12929–12940.
- Swain, A., Bambouskova, M., Kim, H., Andhey, P. S., Duncan, D., Auclair, K., Chubukov, V., Simons, D. M., Roddy, T. P., Stewart, K. M., & Artyomov, M. N. (2020). Comparative evaluation of itaconate and its derivatives reveals divergent inflammasome and type I interferon regulation in macrophages. *Nature Metabolism*, 2(7), 594–602.
- Tallam, A., Perumal, T. M., Antony, P. M., Jager, C., Fritz, J. V., Vallar, L., Balling, R., Del Sol, A., & Michelucci, A. (2016). Gene regulatory network inference of immunoresponsive gene 1 (IRG1) identifies interferon regulatory factor 1 (IRF1) as its transcriptional regulator in mammalian macrophages. *PLoS ONE*, 11(2), e0149050.
- Tannahill, G. M., Curtis, A. M., Adamik, J., Palsson-McDermott, E. M., McGettrick, A. F., Goel, G., Frezza, C., Bernard, N. J., Kelly, B., Foley, N. H., Zheng, L., Gardet, A., Tong, Z., Jany, S. S., Corr, S. C., Haneklaus, M., Caffrey, B. E., Pierce, K., Walmsley, S., ... O'Neill, L. A. (2013). Succinate is an inflammatory signal that induces IL-1 β through HIF-1 α . *Nature*, 496(7444), 238–242.
- Toller-Kawahisa, J. E., Viacava, P. R., Palsson-McDermott, E. M., Nascimento, D. C., Cervantes-Silva, M. P., O'Carroll, S. M., Zotta, A., Damasceno, L. E. A., Publio, G. A., Forti, P., Luiz, J. P. M., Silva de Melo, B. M., Martins, T. V., Faca, V. M., Curtis, A., Cunha, T. M., Cunha, F. Q., O'Neill, L. A. J., & Alves-Filho, J. C. (2025). Metabolic reprogramming of macrophages by PKM2 promotes IL-10 production via adenosine. *Cell Reports*, 44(1), 115172.
- Tur, J., Pereira-Lopes, S., Vico, T., Marin, E. A., Munoz, J. P., Hernandez-Alvarez, M., Cardona, P. J., Zorzano, A., Lloberas, J., & Celada, A. (2020). Mitofusin 2 in macrophages links mitochondrial ROS production, cytokine release, phagocytosis, autophagy, and bactericidal activity. *Cell Reports*, 32(8), 108079.
- Van den Bossche, J., Baardman, J., Otto, N. A., van der Velden, S., Neele, A. E., van den Berg, S. M., Luque-Martin, R., Chen, H. J., Boshuizen, M. C., Ahmed, M., Hoeksema, M. A., de Vos, A. F., & de Winther, M. P. (2016). Mitochondrial dysfunction prevents repolarization of inflammatory macrophages. *Cell Reports*, 17(3), 684–696.
- Vaupel, P., & Multhoff, G. (2021). Revisiting the Warburg effect: Historical dogma versus current understanding. *Journal De Physiologie*, 599(6), 1745–1757.
- Wang, J., Yang, P., Yu, T., Gao, M., Liu, D., Zhang, J., Lu, C., Chen, X., Zhang, X., & Liu, Y. (2022). Lactylation of PKM2 suppresses inflammatory metabolic adaptation in pro-inflammatory macrophages. *International Journal of Biological Sciences*, 18(16), 6210–6225.
- Wang, N., Wang, W., Wang, X., Mang, G., Chen, J., Yan, X., Tong, Z., Yang, Q., Wang, M., Chen, L., Sun, P., Yang, Y., Cui, J., Yang, M., Zhang, Y., Wang, D., Wu, J., Zhang, M., & Yu, B. (2022). Histone lactylation boosts reparative gene activation post-myocardial infarction. *Circulation Research*, 131(11), 893–908.
- Wang, S., Long, H., Hou, L., Feng, B., Ma, Z., Wu, Y., Zeng, Y., Cai, J., Zhang, D. W., & Zhao, G. (2023). The mitophagy pathway and its implications in human diseases. *Signal Transduction and Targeted Therapy*, 8(1), 304.
- Wang, T., Ma, X., Wang, Y., Liu, H., Ding, G., Li, Y., Yuan, H., Gao, J., Sun, F., Guo, Y., Ma, J., & Wu, J. (2025). Lactate-driven lactylation of HNRNP1 orchestrates PKM2 splicing and glycolytic reprogramming in bladder cancer. *Journal of Experimental & Clinical Cancer Research*, 44(1), 331.

- Wang, X., Yang, B., Ma, S., Yan, X., Ma, S., Sun, H., Sun, Y., & Jiang, L. (2023). Lactate promotes salmonella intracellular replication and systemic infection via driving macrophage M2 polarization. *Microbiology Spectrum*, **11**(6), e0225323.
- Wang, Y., Li, N., Zhang, X., & Horng, T. (2021). Mitochondrial metabolism regulates macrophage biology. *Journal of Biological Chemistry*, **297**(1), 100904.
- Warburg, O. (1923). Tests on surviving carcinoma cultures. *Biochemische Zeitschrift*, **142**, 317–333.
- Warburg, O. (1925). The metabolism of carcinoma cells. *The Journal of Cancer Research*, **9**, 148–163.
- Wculek, S. K., Dunphy, G., Heras-Murillo, I., Mastrangelo, A., & Sancho, D. (2022). Metabolism of tissue macrophages in homeostasis and pathology. *Cellular & Molecular Immunology*, **19**(3), 384–408.
- West, A. P., Brodsky, I. E., Rahner, C., Woo, D. K., Erdjument-Bromage, H., Tempst, P., Walsh, M. C., Choi, Y., Shadel, G. S., & Ghosh, S. (2011). TLR signalling augments macrophage bactericidal activity through mitochondrial ROS. *Nature*, **472**(7344), 476–480.
- Willemsen, J., Neuhoff, M. T., Hoyler, T., Noir, E., Tessier, C., Sarret, S., Thorsen, T. N., Littlewood-Evans, A., Zhang, J., Hasan, M., Rush, J. S., Guerini, D., & Siegel, R. M. (2021). TNF leads to mtDNA release and cGAS/STING-dependent interferon responses that support inflammatory arthritis. *Cell Reports*, **37**(6), 109977.
- Wolf, A. J., Reyes, C. N., Liang, W., Becker, C., Shimada, K., Wheeler, M. L., Cho, H. C., Popescu, N. I., Coggeshall, K. M., Arditi, M., & Underhill, D. M. (2016). Hexokinase is an innate immune receptor for the detection of bacterial peptidoglycan. *Cell*, **166**(3), 624–636.
- Wu, K., Jia, Y., Lin, Q., Wang, F., Liu, T., Gao, M., Gao, B., Zhu, Y., Xiong, S., Sun, D., Li, L., Lu, H., Zhu, P., & Zhai, Y. (2026). 3-Hydroxypropionic acid converts inflammatory macrophage glycolysis into mitochondrial oxidation through GAPDH carboxyethylation. *Iscience*, **29**(6), 116258.
- Wu, Y. T., Xu, W. T., Zheng, L., Wang, S., Wei, J., Liu, M. Y., Zhou, H. P., Li, Q. F., Shi, X., & Lv, X. (2023). 4-octyl itaconate ameliorates alveolar macrophage pyroptosis against ARDS via rescuing mitochondrial dysfunction and suppressing the cGAS/STING pathway. *International Immunopharmacology*, **118**, 110104.
- Xia, X., Chen, W., Zhou, T., Zhou, F., Lu, C., Yan, Z., Zhao, Q., & Su, Q. (2025). TEPP-46 inhibits glycolysis to promote M2 polarization of microglia after ischemic stroke. *International Immunopharmacology*, **149**, 114148.
- Xu, L., Cai, J., Li, C., Yang, M., Duan, T., Zhao, Q., Xi, Y., Sun, L., He, L., Tang, C., & Sun, L. (2023). 4-Octyl itaconate attenuates LPS-induced acute kidney injury by activating Nrf2 and inhibiting STAT3 signaling. *Molecular Medicine*, **29**(1), 58.
- Yan, H., Wang, Z., Teng, D., Chen, X., Zhu, Z., Chen, H., Wang, W., Wei, Z., Wu, Z., Chai, Q., Zhang, F., Wang, Y., Shu, K., Li, S., Shi, G., Zhu, M., Piao, H. L., Shen, X., & Bu, P. (2024). Hexokinase 2 senses fructose in tumor-associated macrophages to promote colorectal cancer growth. *Cell Metabolism*, **36**(11), 2449–2467e2446.
- Yan, L., Luo, X., Hang, C., YuWang, Z., Z. X. U., S., & Du, L. (2024). Unraveling the Mfn2-Warburg effect nexus: A therapeutic strategy to combat pulmonary arterial hypertension arising from catch-up growth after IUGR. *Respiratory Research*, **25**(1), 328.
- Yang, K., Fan, M., Wang, X., Xu, J., Wang, Y., Tu, F., Gill, P. S., Ha, T., Liu, L., Williams, D. L., & Li, C. (2022). Lactate promotes macrophage HMGB1 lactylation, acetylation, and exosomal release in polymicrobial sepsis. *Cell Death and Differentiation*, **29**(1), 133–146.
- Yang, W., Wang, Y., Wang, T., Li, C., Shi, L., Zhang, P., Yin, Y., Tao, K., & Li, R. (2023). Protective effects of IRG1/itaconate on acute colitis through the inhibition of gasdermins-mediated pyroptosis and inflammation response. *Genes & Diseases*, **10**(4), 1552–1563.
- Yang, W., Wang, Y., Zhang, P., Wang, T., Li, C., Tong, X., Zeng, X., Yin, Y., Tao, K., & Li, R. (2022). Hepatoprotective role of 4-octyl itaconate in concanavalin A-induced autoimmune hepatitis. *Mediators of Inflammation*, **2022**, 5766434.
- Yao, X., Liu, H., Duan, C., Zhang, Y., Wu, X., Li, B., Li, S., Gong, Y., Liu, T., Wang, X., & Xu, H. (2025). Sirtuin1 mitigation of calcium oxalate nephropathy via enhancing itaconate abundance through reduction of histone trimethylation. *Clinical and Translational Medicine*, **15**(8), e70450.
- Yi, Z., Wu, Y., Zhang, W., Wang, T., Gong, J., Cheng, Y., & Miao, C. (2020). Activator-mediated pyruvate kinase M2 activation contributes to endotoxin tolerance by promoting mitochondrial biogenesis. *Frontiers in Immunology*, **11**, 595316.
- Yu, S., Pei, S., Zhang, M., Gao, S., Chen, J., Duan, L., Hu, E., Wang, Y., & Huang, Y. (2024). PKM2-mediated STAT3 phosphorylation promotes acute liver failure via regulating NLRP3-dependent pyroptosis. *Communications Biology*, **7**(1), 1694.
- Yu, W., Wang, X., Zhao, J., Liu, R., Liu, J., Wang, Z., Peng, J., Wu, H., Zhang, X., Long, Z., Kong, D., Li, W., & Hai, C. (2020). Stat2-Drp1 mediated mitochondrial mass increase is necessary for pro-inflammatory differentiation of macrophages. *Redox Biology*, **37**, 101761.
- Yuan, Y., Xiao, Y., Zou, J., Luo, L., Li, M., Shen, K., Wei, L., Zhang, Y., Wang, P., Chen, Y., Zhuo, S., Zhang, H., Song, S., Jia, Y., Wang, K., Jiang, S., Guan, H., & Hu, D. (2026). Lactate derived from macrophages drives skin dermal fibroblasts phenotypic remodeling via MCT1-primed histone H3 lysine 23 lactylation in hypertrophic scar. *Nature Communications*, **17**(1), 2694.
- Yukun Min, L. O. N. (2023). Targeting mitochondrial metabolites and nucleic acids as an anti-inflammatory strategy [Review]. *Frontiers in Drug Discovery*, **3**, 1294454.
- Yunna, C., Mengru, H., Lei, W., & Weidong, C. (2020). Macrophage M1/M2 polarization. *European Journal of Pharmacology*, **877**, 173090.
- Zannella, V. E., Dal Pra, A., Muaddi, H., McKee, T. D., Stapleton, S., Sykes, J., Glicksman, R., Chaib, S., Zamara, P., Milosevic, M., Wouters, B. G., Bristow, R. G., & Koritzinsky, M. (2013). Reprogramming metabolism with metformin improves tumor oxygenation and radiotherapy response. *Clinical Cancer Research*, **19**(24), 6741–6750.

- Zhang, D., Tang, Z., Huang, H., Zhou, G., Cui, C., Weng, Y., Liu, W., Kim, S., Lee, S., Perez-Neut, M., Ding, J., Czyz, D., Hu, R., Ye, Z., He, M., Zheng, Y. G., Shuman, H. A., Dai, L., Ren, B., ... Zhao, Y. (2019). Metabolic regulation of gene expression by histone lactylation. *Nature*, **574**(7779), 575–580.
- Zhang, L., Wang, M., An, R., Dai, J., Liu, S., Chen, M., & Ding, H. (2023). Activation of NLRP3 inflammasome via Drp1 overexpression in Kupffer cells aggravates ischemia-reperfusion injury in hepatic steatosis. *Journal of Clinical and Translational Hepatology*, **11**(5), 1069–1078.
- Zhang, Y., Jiang, H., Dong, M., Min, J., He, X., Tan, Y., Liu, F., Chan, M., Chen, X., Yin, Q., Zheng, L., Shao, Y., Li, X., & Chen, H. (2024). Macrophage MCT4 inhibition activates reparative genes and protects from atherosclerosis by histone H3 lysine 18 lactylation. *Cell Reports*, **43**(5), 114180.
- Zhang, Z., Chen, C., Yang, F., Zeng, Y. X., Sun, P., Liu, P., & Li, X. (2022). Itaconate is a lysosomal inducer that promotes antibacterial innate immunity. *Molecular Cell*, **82**(15), 2844–2857e2810.
- Zheng, Y., Lin, J., Wan, G., Gu, X., & Ma, J. (2025). Macrophage Notch1 drives septic cardiac dysfunction by impairing mitophagy and promoting NLRP3 activation. *Biology Direct*, **20**(1), 65.
- Zhong, W., Rao, Z., Xu, J., Sun, Y., Hu, H., Wang, P., Xia, Y., Pan, X., Tang, W., Chen, Z., Zhou, H., & Wang, X. (2022). Defective mitophagy in aged macrophages promotes mitochondrial DNA cytosolic leakage to activate STING signaling during liver sterile inflammation. *Aging Cell*, **21**(6), e13622.
- Zhou, H., Xiao, Y., Xue, X., Liu, J., Xu, H., Wang, J., Zhou, J., Zuo, Q., Liang, W., Song, H., Chen, Z., Liu, F., Li, X., Shen, K., & Fan, W. (2025). Hyperglycemia exacerbates osteoarthritis by impairing macrophage efferocytosis through modulation of CD11b lactylation. *Nature Communications*, **17**(1), 724.
- Zhu, G., Yu, H., Peng, T., Yang, K., Xu, X., & Gu, W. (2024). Glycolytic enzyme PGK1 promotes M1 macrophage polarization and induces pyroptosis of acute lung injury via regulation of NLRP3. *Respiratory Research*, **25**(1), 291.
- Ziogas, A., Novakovic, B., Ventriglia, L., Galang, N., Tran, K. A., Li, W., Matzaraki, V., van Unen, N., Schluter, T., Ferreira, A. V., Moorlag, S., Koeken, V., Moyo, M., Li, X., Baltissen, M. P. A., Martens, J. H. A., Li, Y., Divangahi, M., Joosten, L. A. B., ..., & Netea, M. G. (2025). Long-term histone lactylation connects metabolic and epigenetic rewiring in innate immune memory. *Cell*, **188**(11), 2992–3012e2916.
- Zotta, A., Toller-Kawahisa, J., Palsson-McDermott, E. M., O'Carroll, S. M., Henry, O. C., Day, E. A., McGettrick, A. F., Ward, R. W., Ryan, D. G., Watson, M. A., Brand, M. D., Runtsch, M. C., Maitz, K., Lueger, A., Kargl, J., Miljkovic, J. L., Lavalle, E. C., & O'Neill, L. A. J. (2025). Mitochondrial respiratory complex III sustains IL-10 production in activated macrophages and promotes tumor-mediated immune evasion. *Science Advances*, **11**(4), eadq7307.

Additional information

Competing interests

The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

Y.M.: Writing – original draft; preparation of illustrative figures and tables. Ó.C.H.: Writing – original draft. L.O': Supervision; writing – review and editing.

Funding

The authors declare that financial support was received for the research, authorship and/or publication of this article. This work was supported by grants from the European Research Council (834370) and Science Foundation Ireland (12/IA/1531).

Acknowledgements

We would like to thank all members of the O'Neill laboratory for helpful discussions.

Generative AI statement

OpenAI ChatGPT (GPT-5.6) was used during the writing of the manuscript to assist with language editing. All scientific content, interpretations, conclusions and references were reviewed and verified by the authors, who take full responsibility for the final content of the paper.

Keywords

ETC, glycolysis, itaconate, lactate, macrophage, mitochondria, TCA cycle, Warburg metabolism

Supporting information

Additional supporting information can be found online in the Supporting Information section at the end of the HTML view of the article. Supporting information files available:

Peer Review History