Early macrophage response to obesity encompasses Interferon Regulatory Factor 5 regulated mitochondrial architecture remodelling

Received: 6 May 2021
Accepted: 16 August 2022
Published online: 30 August 2022

Adipose tissue macrophages (ATM) adapt to changes in their energetic microenvironment. Caloric excess, in a range from transient to diet-induced obesity, could result in the transition of ATMs from highly oxidative and protective to highly inflammatory and metabolically deleterious. Here, we demonstrate that Interferon Regulatory Factor 5 (IRF5) is a key regulator of macrophage oxidative capacity in response to caloric excess. ATMs from mice with genetic deficiency of Irf5 are characterised by increased oxidative respiration and mitochondrial membrane potential. Transient inhibition of IRF5 activity leads to a similar respiratory phenotype as genomic deletion, and is reversible by reconstitution of IRF5 expression. We find that the highly oxidative nature of Irf5-deficient macrophages results from transcriptional derepression of the mitochondrial matrix component Growth Hormone Inducible Transmembrane Protein (GHITM) gene. The Irf5-deficiency-associated high oxygen consumption could be alleviated by experimental suppression of Ghitm expression. ATMs and monocytes from patients with obesity or with type-2 diabetes retain the reciprocal regulatory relationship between Irf5 and Ghitm. Thus, our study provides insights into the mechanism of how the inflammatory transcription factor IRF5 controls physiological adaptation to diet-induced obesity via regulating mitochondrial architecture in macrophages.

Macrophage metabolism is a powerful mitigating or optimising factor influencing function\(^1\). Generally speaking, pro-inflammatory polarisation relies on glycolysis, mediated by hypoxia-inducible factor (HIF) \(\text{HIF}^-\alpha\)^2, with specific interruptions of the tricarboxylic acid (TCA) cycle\(^1\). Conversely, anti-inflammatory polarisation or regulatory function is supported by mitochondria and oxidative respiration\(^1\). Microenvironmental niches also impose energetic specificities on macrophages\(^1\). For example, adipose tissue macrophages (ATM) can range from being...
metabolically quiescent to overall hypermetabolic. In diet-induced obesity (DIO), ATMs are exposed to the same dysmetabolism as all peripheral tissues, that is, glucolipotoxicity, providing a systemic abundance of metabolic substrates. It is in this context that ATMs are hypermetabolic, with highly glycolytic and highly oxidative fluxes. ATMs are, however, a heterogenous population of cells that exhibit a range of beneficial and detrimental phenotypes over the course of obesity. Lipid-associated macrophages (LAM) have been characterised, they are highly oxidative and have a high capacity to clear lipids and dying adipocytes. Also highly responsive to lipids are the phenotypically similar MARCO lipid-buffering ATMs and metabolically activated macrophages (MMe). Expansion of these ATM populations and their functional contribution to maintaining tissue homeostasis or to metabolic decline varies with the duration of caloric excess. Such reports indicate that macrophages are reactive beyond their inflammatory roles, and their successful adaptation early in the course of DIO, may be sufficient to mitigate systemic metabolic decline.

The rise of lipid-buffering ATMs represents initial adaptation in a physiological attempt to maintain homeostasis. Over prolonged caloric excess ATMs become predominantly inflammatory and contribute to insulin resistance in DIO. The early adaptive step is marked by high oxidative capacity, and the later predominance of inflammatory ATMs marks their rise as critical actors in the development of insulin resistance and type-2 diabetes.

The Interferon Regulatory Factor (IRF) is a key molecular switch mediating M1-like polarisation of ATMs. ATMs expression of IRF5 is increased in DIO, promoting pro-inflammatory polarisation and repressing TGFβ-signalling. This favours maladaptive white adipose tissue (WAT) expansion and insulin resistance. IRF5 is physiologically required to respond to bacterial and viral stimuli, evidence also implicates deregulated expression in conditions of chronic inflammation (e.g. auto-immune, metabolic diseases). Gain-of-function Irf5 variants associated with the auto-immune disease have more recently been found to promote macrophage glycolytic programming.

Here, we reveal a non-canonical function for IRF5 in orienting macrophage energetic adaptation to caloric excess. IRF5 transcriptionally represses the gene encoding Growth hormone inducible transmembrane protein (Ghitm), a key mitochondrial component required for oxidative respiration. Through this interaction, IRF5 contributes to failure in maintaining normal mitochondrial cristae structures that support effective oxidative respiration. Ghitm repression and failure to maintain cristae structure restrain ATM oxidative capacity in DIO. The Irf5-Ghitm regulatory axis extends from short- to long-term high-fat feeding and to monocytes and ATMs in patients with obesity and T2D.

**Results**

**IRF5 is associated with ATM metabolic adaptation upon short-term high-fat diet**

We started by analysing the ATM transcriptome from mice with a myeloid-deficiency of Irf5 (IRF5-KO) or wild-type (WT) mice on 4 and 12 weeks of a high-fat diet (HFD). On a 12-week, long-term HFD (LT-HFD), differentially expressed genes were associated with inflammatory response and tissue remodelling (Fig. 1A, S1A). This confirms previously reported phenotypic features. On a 4-week HFD, qualified short term (ST-HFD), differentially expressed genes enriched several GO terms for metabolic process. Interestingly, terms relating to immune function (humoral immune response, phagocytosis/recognisation) were under-represented (Fig. lb, S1B). These results indicate that IRF5 may influence ATM metabolic adaptation, in particular, in response to short-term caloric excess.

To associate ATM metabolism with IRF5, we evaluated ATM metabolic adaptation and IRF5 expression upon ST- and LT-HFD in C57BL/6j mice. On ST-HFD and LT-HFD gained weight, increasing WAT mass and losing glycaemic homeostasis over time (Fig. SIC-E). IRF5 expression also increased in epididymal fat pads (EpiWAT) on ST-HFD and LT-HFD (Fig. 1C). We characterised ATM metabolic adaptation using the fluorescent lipid dye BODIPY and the JC-1 dye, a sensor for mitochondrial mass (Mt Mass) and membrane potential (ΔΨm). On ST- and LT-HFD, ATMs have a higher lipid content and Mt Mass but decreasedΔΨm and mΔΨ-to-mass ratio, relative to mice on a normal chow diet (NCD) (Fig. 1D, S2A). Interestingly, effects on Mt Mass and ΔΨm upon ST-HFD are similar in magnitude to LT-HFD. These data are consistent with previous reports that ATMs become hypermetabolic in DIO; however, metabolic adaptation occurs within short-term caloric excess. This was confirmed to contribute to cellular respiration by extracellular flux analyses on F4/80+ ATMs (Fig. 1E, S2B).

On ST-HFD, correlations revealed that ATM Mt Mass was positively associated with IRF5 expression and mΔΨ-to-mass ratio was negatively associated (Fig. 1F). ATM lipid content was not associated (Fig. S2C). ATM numbers were positively correlated to IRF5 expression, this was observed by FACS and by qPCR analysis of F4/80 and IRF5 expression in EpiWAT (Fig. 1G, S2D). A UMAP showed that IRF5 was highly expressed in cells that also highly express F4/80 (F4/80hi, Fig. S2E), a population reported to be monocyte-derived. We quantified IRF5 expression in F4/80hi and F4/80lo ATMs and found IRF5 to be upregulated in F4/80lo ATMs on ST-HFD (Fig. 1H, S2F). F4/80hi ATMs had markedly increased Mt Mass and decreased ΔΨm (Fig. 1I). These results suggest that IRF5 plays a role in ATM mitochondrial adaptation, in particular in F4/80lo ATMs.

**IRF5 deficiency alters ATM oxidative respiration in response to a short-term high-fat diet**

We applied the same model of ST-HFD to IRF5-KO and WT mice. Weight gain and EpiWAT weight were similar between genotypes (Fig. S3A). ATMs from IRF5-KO mice had increased ΔΨm and mΔΨ-to-mass ratio relative to WT mice, lipid content and Mt Mass were not altered (Fig. 1J; Fig. S3B). Analysis by TSE confirmed JCI red fluorescence, indicating mΔΨ was highest in F4/80hi ATMs and these cells had higher fluorescence in IRF5-KO (Fig. 1K). Under basal conditions, on NCD, IRF5-KO did not affect ATM metabolic phenotype (Fig. S3C), and upon LT-HFD, only a trend to increased intracellular lipid content persisted (Fig. S3D).

To link cytometric analyses to functional respiration, we analysed extracellular flux from magnetically sorted F4/80+ ATMs of IRF5-KO and WT mice under NCD and following ST- and LT-HFD. ATMs from IRF5-KO mice had a higher oxygen consumption rate (OCR) following ST-HFD, but not on NCD nor LT-HFD (Fig. 2A). F4/80hi cells were unaffected by IRF5 deficiency (Fig. S4A). However, higher OCR in IRF5-KO ATMs remains apparent when whole SVF is analysed under conditions testing mitochondrial, or glycolytic, respiration (Fig. 2B, C). OCR reflects a number of oxygen-consuming processes, a major contributor to which is fatty acid oxidation (FAO). To evaluate the contribution of FAO to the IRF5-KO respiratory phenotype, we carried out a palmitate oxidation test on SVF, with or without etomoxir, an inhibitor of carnitine palmitoyltransferase (CPT)−1. OCR was higher in palmitate-loaded SVF from IRF5-KO mice upon ST-HFD. This was normalised to WT levels in the presence of etomoxir (Fig. 2D, S4B), indicating that FAO contributes to higher OCR in cells from IRF5-KO mice.

**ATM adaptation in IRF5 deficiency alters adipose tissue phenotypic response to a short-term high-fat diet**

As a consequence of ATM phenotype, analysing EpiWAT sections revealed that average adipocyte diameter and frequency of large (>100 um) adipocytes were higher in EpiWAT from IRF5-KO mice (Fig. 2E, S4C). The number of crown-like structures (CLS) was higher, and the number of MAC2+ cells had an increasing trend (Fig. 2F, S4D). Despite CLS accumulation in IRF5-KO, we found no difference in the expression of inflammatory markers in EpiWAT, (e.g. IL6, TNF, some...
markers were not reliably detectable). Cytokine and adipokine levels in circulation were also similar between genotypes (Fig. S4E–G). These phenotypic tissue features were concurrent to a functional increase in glucose uptake capacity in fat pads from IRF5-KO mice (Fig. 2g), and this occurs on a background of similar glycaemia and insulin levels to WT mice (Fig. S4H). Increased glucose uptake might reflect improved glucose homeostasis and insulin sensitivity at the tissue level and could also explain increased adipocyte size in IRF5-KO mice. The EpiWAT phenotype of IRF5-KO mice upon ST-HFD presents similarities with the protective EpiWAT phenotype of IRF5-KO mice upon LT-HFD (i.e. increased ATM content, improved insulin sensitivity). Importantly, the IRF5-linked respiratory phenotype of ATMs occurs transiently and orchestrates tissue level adaptation at a stage when systemic metabolism is not yet impacted (Fig. S4I). Such early adaptation,
underpinned by ATM mitochondrial respiration, is a key event that precedes IRF5 deficiency’s protective metabolic phenotype at the stage of systemic insulin resistance (LT-HFD).

IRF5 repression of mitochondrial respiration is cell intrinsic, reversible and inducible in mature macrophages To carry out mechanistic investigations, we moved to bone-marrow-derived macrophages (BMDM). BMDMs from IRF5-KO and WT mice were differentiated and treated for 24 h with bacterial lipopolysaccharides (LPS), a canonical stimulant of the IRF5 signalling pathway, or with palmitate to model lipotoxicity. Testing glycolysis, we found no genotype difference in extracellular acidification rates (ECAR) in control or treated cells (Fig. S5A). Glucose-stimulated OCR was increased in IRF5-KO BMDMs following treatment with LPS or palmitate (Fig. 3a, S5B). Under conditions testing mitochondrial respiration, OCR was increased in IRF5-KO BMDMs following LPS or palmitate treatment, with no difference in untreated cells (Fig. 3b, S5C). The IRF5-linked respiratory phenotype is cell intrinsic and mirrors what we observed in ATMs.

To evaluate whether the respiratory phenotype is the result of genetic deficiency or if it is inducible in mature macrophages, we applied an IRF5 inhibitory decoy peptide (IRF5-DP) to mature BMDMs from WT mice. IRF5-DP binds to IRF5, preventing its nuclear translocation18. LPS-induction of TNF is prevented by IRF5-DP, confirming that it blocks the transcriptional activity of IRF5 (Fig. S5D). When treated with palmitate, metabolic flux analyses showed that IRF5-DP increased OCR relative to the vehicle, replicating the effect of genetic deficiency (Fig. 3c). This result indicates a requirement for IRF5 nuclear translocation and rules out a differentiation effect of genetic deficiency. We next used adenoviral delivery to re-introduce IRF5 expression in BMDMs from IRF5-KO mice, IRF5 adenovirus (adIRF5) resulted in a 1.4-fold increase in IRF5 expression (Fig. S5F). Following palmitate treatment, OCR was decreased in cells treated with adIRF5, but not in cells treated with the control adenovirus (adGFP; Fig. 3d, S5G).

IRF5 deficiency alters concentrations of TCA cycle metabolites and structural components of mitochondria in response to palmitate treatment To understand how IRF5 affects mitochondrial function, we quantified TCA cycle metabolites in IRF5-KO, and WT BMDMs treated with palmitate or with LPS. A PCA score plot revealed a genotype-dependent difference in metabolite profile within 2 h of treatment with palmitate but not with LPS, and most differences were normalised by 24 h (Fig. 4a, S6A, B). This was confirmed by carrying out a PCA only on 2 h Palmit-treated samples (Fig. 4b). Variable ranking revealed lactate was the biggest contributor to the IRF5-dependent response to palmitate, and it had a higher concentration in IRF5-KO BMDMs (Fig. 4c). Lactate is a glycolysis end-product destined for extracellular release20 (Fig. 4d). However, ECAR was lower in IRF5-KO BMDM under these test conditions, indicating lactate is released at a slower rate (Fig. 4e). Thus, lactate accumulation in IRF5-KO BMDM can be explained by its increased retention. Interestingly, intracellular lactate has recently been reported to be subject to oxidation in M2-like macrophages, potentially contributing to oxygen consumption19. Consequently, analysing mitochondrial respiration found increased OCR in IRF5-KO BMDM under these conditions.

We also applied electron microscopy to BMDMs under these same conditions to evaluate potential structural mechanisms. Mitochondrial density, form factor and aspect ratio were not altered between IRF5-KO and WT BMDM (Fig. 4g, S6C), suggesting no adaptation in mitochondrial morphology was observed in response to palmitate or LPS. Mitochondrial translation; Fig. 5b). We also acquired ChIP-seq data that maps IRF5 binding in BMDM 30. Of 526 bound genes, 77 (1%) were represented in all conditions. Intersection with ChIP-seq datasets revealed 6 genes were differentially expressed and were also bound by IRF5 in BMDMs from IRF5-KO mice. Irf5 expression was downregulated in response to palmitate, consistent with an increase in oxidative respiration (Fig. 5e). These data indicate that Ghitm, a mitochondrial respiratory process, may be a mechanistic target of IRF5 that can influence mitochondrial respiration.

To resolve a transcriptional mechanism, we carried out RNA-seq on ATMs (ST- and LT-HFD) and BMDM (0, 2 and 24 h stimulation with LPS or Palmit) from IRF5-KO and WT mice. Coregulated clusters were defined based on genotype effect and on a trajectory over time (Fig. 5a, S7A). A number of terms relating to lipotoxicity and mitochondrial function were enriched across all conditions (e.g. response to cholesterol, mitochondrial translation; Fig. 5b). We also acquired ChIP-seq data that maps IRF5 binding in BMDM. Of 526 bound genes, 77 (14.6%) enriched the metabolic process GO term (Fig. 5c), indicating a level of transcriptional control over metabolism.

To define a list of targets, we carried out differential expression analyses of RNA-seq data between genotypes, per condition and per timepoint (Fig. 5d). Palmitate treatment had the highest number of differentially expressed genes, followed by LPS and HFD; 34 targets (1%) were represented in all conditions. Intersection with ChIP-seq revealed 6 genes were differentially expressed and were also bound by IRF5 at, or upstream of, transcription start sites: Afp3, Sce2, Abcg1, Lrtr27, Fnrp2 and Ghitm. Ghitm has an overt function in maintaining inner membrane cristae structures21 (Fig. 5d), and its expression was negatively correlated with Irf5 expression in ATMs from WT mice (Fig. 5e). These data indicate that Ghitm may be a mechanistic target of IRF5 that can influence mitochondrial respiration.
expression was higher than all other targets identified (Figs. 6a, b, SSA). *Irf5* and *Ghitm* expression were negatively correlated ($R = -0.44$; $p < 0.001$), supporting our own data (Fig. 6c, d). *Irf5* expression increased over time and remained negatively correlated to *Ghitm* expression in monocytes and ATMs (Fig. 6b, d). Data analysed from Jaitin et al.12, Saliba et al.30, together with our work, strongly suggest that the interaction between IRF5 and Ghitm influences macrophage respiratory phenotype.

We chose to pursue a GHITM-mediated mechanism as a contributor to increased oxygen consumption through the maintenance of cristae structures in IRF5-deficient macrophages (Fig. 4h). As for the potential contribution of lactate oxidation, none of the identified targets had a described function in lactate metabolism, and thus IRF5-dependent remodelling of the TCA cycle could be an area for future investigation beyond the scope of current work.

**GHITM knockdown reverses hyperoxidative phenotype of IRF5-deficient macrophages**

With guide RNAs (gRNA) targeting Ghitm (gGHITM), we transduced BMDM expressing the Clustered Regularly Interspaced Short
Palindromic Repeats (CRISPR)-Associated Protein (Cas)–9 linked to EGFP and under control of the Lyz2 promoter (Fig. S8B). Transfection with gGHITM resulted in a 40% decrease in expression (Fig. S8C). We subjected BMDM to palmitate treatment, Ghitm expression decreased in response to palmitate and upon transfection (Fig. 6e, S9D, S8E). Transfection with gGHITM also decreased OCR measures, in particular at maximal respiration, in untreated and palmitate-treated cells (Fig. 6f). We then targeted Irf5 alone (gIRF5) or co-transfected with gIRF5 and gGHITM (Fig. S8F). Extracellular flux analysis after palmitate treatment revealed that gIRF5 increased OCR, reproducing the Irf5-KO phenotype (Fig. 6h, S8G). Co-transfection with gGHITM normalised respiration to control levels (Fig. 6h, S8G). These results indicate that GHTM contributes to increased oxidative respiration in IRF5-deficient macrophages.

IRF5-GHTM regulatory axis is conserved in patients with obesity and type-2 diabetes

RNA-seq on IRF5+ and IRF5− monocytes from patients with T2D revealed 3211 upregulated and 295 downregulated genes in IRF5+ monocytes (Fig. 7a). Terms for mitochondrial organisation and protein localisation to mitochondria were under-represented amongst upregulated genes while downregulated genes enriched lipid catabolism (Fig. 7j). On this same resource, the expression of Ghitm was consistently downregulated in IRF5+ relative to IRF5− cells (Fig. 7c).

ScRNA-seq on SVF from lean and obese humans2 confirmed previous reports that Irf5 expression is increased with obesity and revealed a concurrent decrease in Ghitm expression (Fig. 7d, S9A). Cell-by-cell visualisation indicated that as cells gain expression of Irf5, they lose expression of Ghitm (Fig. 7d, S9B). We next binned cells by increasing levels of Irf5 expression and found that as Irf5 expression increased, the proportion of Ghitm′ cells decreased (Fig. 7e). Correlative analyses revealed a strong negative association between Irf5 and Ghitm mean expression per bin (Fig. 7f). For further analysis, we obtained WAT biopsies from a cohort of patients with obesity and sorted CD14+ ATMs from subcutaneous and visceral fat depots (scATMs, vATMs) for qRT-PCR analysis. Samples were designated as IRF5hi or IRF5lo expressers, in which we found similar counter-regulation of Ghitm in vATMs, but not in scATMs (Fig. 7g, S9C). In functional analyses, we found negative association trends between IRF5 expression and Mt Mass, mΔΨ and mΔΨ-to-mass ratio in vATMs, and mΔΨ-to-mass ratio in monocytes (Fig. 7h, S9D). These results demonstrate that the IRF5-GHITM axis is conserved in humans and may be associated with mitochondrial adaptation of ATMs and monocytes in obesity and T2D.

To evaluate the potential for transcriptional regulation, we stained monocytes from patients with T2D for IRF5 and for oxidative phosphorylation (OXPHOS) enzyme complexes (Fig. 7i). These complexes are typically anchored to the cristae structures maintained by GHTM3,4. Monocytes with nuclear localisation of IRF5 (Nuc) had lower OXPHOS staining density relative to those with cytoplasmic staining (Cyt). Loss of OXPHOS complex density is associated with mitochondrial adaptation of ATMs and monocytes in obesity and T2D.

Discussion

WAT is a key responder to caloric excess. Adaptive responses dictate disease course in metabolic syndrome, and a major determinant of tissue adaptation is the phenotype and function of ATMs. ATMs are a heterogenous population of cells ranging from regulatory to highly polarised (HMDM), and this coincides with a decrease in active transcription histone mark H3K27ac. The above analyses demonstrate that IRF5 can bind to the Ghitm gene in humans, Irf5 and Ghitm are also reciprocally regulated, indicating that IRF5’s transcriptional activity may be targeted to Ghitm upon macrophage polarisation.

regulation of Ghitm in vATMs, but not in scATMs (Fig. 7g, S9C). In functional analyses, we found negative association trends between IRF5 expression and Mt Mass, mΔΨ and mΔΨ-to-mass ratio in vATMs, and mΔΨ-to-mass ratio in monocytes (Fig. 7h, S9D). These results demonstrate that the IRF5-GHITM axis is conserved in humans and may be associated with mitochondrial adaptation of ATMs and monocytes in obesity and T2D.

To evaluate the potential for transcriptional regulation, we stained monocytes from patients with T2D for IRF5 and for oxidative phosphorylation (OXPHOS) enzyme complexes (Fig. 7i). These complexes are typically anchored to the cristae structures maintained by GHTM3,4. Monocytes with nuclear localisation of IRF5 (Nuc) had lower OXPHOS staining density relative to those with cytoplasmic staining (Cyt). Loss of OXPHOS complex density is associated with mitochondrial adaptation of IRF5, indicating a transcriptional mechanism. Lastly, we used the University of California Santa Cruz (UCSC) genome browser to visualise IRF5 binding regions around the Ghitm gene. Several IRF5 binding regions were found on and upstream of Ghitm (Fig. 7j). On this same resource, the expression of Ghitm mRNA is decreased in LPS-treated human monocyte-derived macrophages (HMDM), and this coincides with a decrease in active transcription histone mark H3K27ac. The above analyses demonstrate that IRF5 can bind to the Ghitm gene in humans, Irf5 and Ghitm are also reciprocally regulated, indicating that IRF5’s transcriptional activity may be targeted to Ghitm upon macrophage polarisation.

Discussion

WAT is a key responder to caloric excess. Adaptive responses dictate disease course in metabolic syndrome, and a major determinant of tissue adaptation is the phenotype and function of ATMs. ATMs are a heterogenous population of cells ranging from regulatory to highly inflammatory, the latter contributing to the systemic metabolic decline in obesity and T2D. As sentinel cells with roles in maintaining homeostasis, the molecular mechanisms of ATM adaptation to early caloric excess remain to be fully understood. Here we demonstrate that ATMs undergo extensive IRF5-dependent energetic adaptation upon short-term caloric excess. ATM oxidative capacity is limited by...
IRF5’s transcriptional interaction with *Ghitm*, the gene coding an inner mitochondrial membrane protein that maintains mitochondrial architecture for efficient oxidative respiration. Decreased GHITM expression and loss of cristae organisation occur at an early stage of DIO and represent an IRF5-dependent mechanism that may contribute to loss of microenvironmental homeostasis and development of insulin resistance (Fig. 8). Previous studies show that inflammation arises in WAT and is mediated by ATMs. The key implication of IRF5 in this inflammation and the development of T2D has been demonstrated2. Irf5 gain-of-function risk variants have also been associated with increasing macrophage glycolytic flux3, a cellular process that supports inflammatory effector function. A recent study
also demonstrated that IRF5 regulates airway macrophage metabolic response to viral infection. Here we hypothesised that this transcription factor may have a role to play in adapting ATM metabolism in response to caloric excess. We first found that metabolically relevant genes were disproportionally represented in the IRF5-deficient transcriptome upon short-term but not long-term high-fat feeding. The latter is enriched by inflammation-related genes. In coherence with a study by Lee et al. that demonstrated immunocompromised mice developed insulin resistance upon short-term high-fat feeding, indicating that inflammation is not required for loss of glycaemic homeostasis in short-term caloric excess. A further study by Shimobayashi et al. confirmed this, demonstrating that WAT was disproportionally

Nature Communications | (2022) 13:5089
Fig. 5 | IRF5 binds to and regulates expression of genes that control mitochondrial structure and metabolism in bone-marrow-derived macrophages and adipose tissue macrophages in response to metabolic stress. 

- **a** Clustering analysis on RNA sequencing from bone-marrow-derived macrophages (BMDM) from IRF5-KO and WT mice treated for 2 or 24 h with bacterial lipopolysaccharides (LPS) or palmitate (Palm) and epididymal white adipose tissue (EpiWAT) F4/80 + macrophages (ATMs) from IRF5-KO and WT mice following short-term (ST) or long-term (LT) high-fat diet (HFD). Clustering analyses was applied to genes differentially expressed between genotypes in at least one condition.

- **b** Gene ontology (GO) term enrichment, related to mitochondria and lipotoxicity. Genes from differentially regulated clusters in panel **a** and Fig S7A (Binomial test of gene list compared to species reference genome).

- **c** Publicly available chromatin immunoprecipitation (ChIP) seq of IRF5 in BMDMs treated with LPS for 120 min was procured. Peaks of interest were determined as either at or upstream of transcription start sites (TSS). Annotated genes were subject to gene ontology (GO) enrichment analyses. Differential analysis between genotypes per treatment: 2- or 24-h treatment with LPS or Palm and in EpiWAT ATMs following ST- or LT-HFD (Wald test p-value <0.05, equivalent to \(-\log_{10}(p\text{-value}) > 1.3\), and \(\log_{2}(\text{FC}) > 1.0\)). Venn diagram of differentially expressed genes between genotypes, per treatment condition. Percentage refers to proportion of genes in overlap. Gene track from ChIP-seq in c. of GHTM gene which overlaps all conditions and also bound by IRF5.

- **d** Correlative analyses of IRF5 expression and the expression of previously identified overlapping genes in d; and notably GHTM expression in ATMs from IRF5-competent mice fed a ST- or LT-HFD (Pearson’s correlation, \(r = -0.83\), two-tailed \(p = 0.009\)). Data presented as mean ± SEM. Source Data file provided.

Fig. 6 | IRF5 and GHTM are highly expressed and reciprocally regulated in epididymal white adipose tissue macrophages and monocytes. 

- **a** Single-cell RNA sequencing of the epididymal white adipose tissue (EpiWAT) stromal vascular fraction (SVF) of C57BL/6 J mice following 6 or 12 weeks of high-fat feeding (Jaitin et al., 2019). Macrophages and monocytes were identified and expression of IRF5 and of GHTM were projected onto tSNE plots per cell type and duration of high-fat feeding.

- **b** Heatmap of mean expression values of IRF5, GHTM, ABCG1, SYCE2, FNIP2, ATF5 and LRRC27 over time and by cell type [monocytes (Mono) or macrophages (Mac)]. Correlative analyses between IRF5 expression and expression of GHTM, ABCG1, SYCE2, FNIP2, ATF5 and LRRC27 at the single-cell level (Pearson’s correlation, \(r\); two-tailed ***\(p < 0.0001\) and **\(p = 0.004\)). Heatmap of IRF5 and GHTM expression, each line represents a single cell.

- **c** Correlative analyses between IRF5 expression and GHTM expression, ABCG1, SYCE2, FNIP2, ATF5 and LRRC27 at the single-cell level (Pearson’s correlation, \(r\); two-tailed ***\(p = 0.0003\), left \(p = 0.0423\), right \(p = 0.0167\)). Western blotting against GHTM in the same experimental design, quantification and blot in Fig. S8D, S8E (\(n = 2\) per condition).

- **d** Oxygen consumption rate (OCR) from extracellular flux analysis in BMDMs with or without Palm treatment following transfection with gGHTM or with lipofection agent alone (Ctrl). Oligomycin (Oli), carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP) and Rotenone/Antimycin A (Rot/AA) were administered (\(n = 5\) for Ctrl, Ctrl + Palm and gGHTM; \(n = 8\) for gGHTM + Palm).

- **e** Maximal respiration from extracellular flux analysis on Palm-treated BMDMs following transfection with a gRNA targeting IRF5 (gIRF5), double transfection with gGHTM and gIRF5 or with lipofection agent alone (Ctrl) (\(n = 3\) per condition; one-way ANOVA, *\(p = 0.0428\)). Data presented as mean ± SEM. Source Data file provided.
affected and that early loss of glycaemic homeostasis precedes inflammation. Our findings are supported by these studies, within 4 weeks of high-fat feeding, mice develop altered glucose homeostasis, however, without an overt IRF5-linked inflammatory signature. We did, however, demonstrate that ATMs undergo adaptation and are energetically distinct at this timepoint when compared to mice on a NCD.

ATMs reside in a lipid-rich environment and take on an overall hypermetabolic phenotype in DIO, increasing glycolysis as well as mitochondrial respiration. More recent studies report specific LAM expansion on HFD, with LAMs being metabolically protective. Similarly, CD11c+CD206+ double-positive macrophages were found to expand on short-term HFD and are a highly oxidative population.
intracellular lactate is itself susceptible to oxidation and can be metabolised in mitochondria\[^{29,36,37}\]. We found that decreased lactate secretion leads to its accumulation in IRF5-KO macrophages; however, we do not have direct evidence of its contribution to oxygen consumption. Moreover, the target genes that we identified have not been found to directly interact with pathways for lactate metabolism. This mechanism may contribute to the observed phenotype; however, it may also run in parallel to the structural mechanism we resolved in the IRF5-GHITM interaction. Whilst out of the scope of the current work, future investigations can focus on the mechanism by which IRF5 alters TCA cycle dynamics.

We were data-driven in resolving the current mechanism, in which we combined public datasets with our own RNA-seq to reveal that a transcriptional target of IRF5 impairs macrophage mitochondrial respiration at the early stage of glucose intolerance but prior to the onset of insulin resistance. Interestingly, this mechanism is transient, as ATMs from IRF5-KO and WT mice do not show any difference in respiratory phenotype following 12 weeks of high-fat feeding. This may be due to the function of IRF5 being more inflammatory over time or when supported by other microenvironmental cues (e.g. hypoxia, cytokines, hyperglycaemia). Such functional specificity is clearly...
represented by the IRF5-deficient ATM transcriptome, which is enri-
ched by metabolism-related genes in the short-term, and then enri-
ched by inflammation-related genes in long-term high-fat feeding.

Previous studies found that Ghitm knocks down causes cristae
disorganisation and mitochondrial fragmentation. Mitochondrial
fragmentation has been associated with inflammatory polarisation,
both in response to LPS and to fatty acids. Studies in lymphocyte
lines stimulated with inflammatory cytokines and from virus-exposed
monocytes also report downregulation of Ghitm. These reports are
in line with our current findings that loss of GHTM is associated with
compromised cristae in macrophages and with increased inflamma-
tion under lipotoxic stress. We report this role for GHTM in macro-
phages, and monocytes, in humans and mice in response to metabolic
stress. Decreased expression of Ghitm, and decreased ATM oxidative
capacity is an early and potentially key mechanism of WAT mala-
daptation to caloric excess.

In summary, we deciphered a mechanism by which IRF5, a well-
characterised pro-inflammatory transcription factor, alters cellular
mitochondrial respiration. Having identified this mechanism to control
mitochondrial metabolism, a number of questions remain unanswered. For
example, to elucidate how and through which regulatory elements
IRF5 may be binding to such targets as Ghitm. While it is widely
accepted that IRF5, target interferon-sensitive regulatory elements, it is
unknown whether these response elements populate genes that regu-
late mitochondrial metabolism and structural components, such as
GHTM. Furthermore, the specific functional contribution of GHTM
downregulation to effective inflammation is unknown, for example,
consequent mitochondrial fragmentation may be a source of reactive
oxygen species required for bacterial killing. Lastly, despite several
lines of evidence implicating IRF5 in metabolic decline associated with
weight). Glycaemia was monitored for 120 min after insulin injection.

Organ collection and histology
Mice were sacrificed by cervical dislocation. Upon dissection, tissues
were weighed. Immediately after collection, samples were either
digested with collagenase, snap-frozen for further analysis or drop-
fixed into 10% formalin (Sigma–Aldrich) for 24 h for histological anal-
ysis. For histological analysis, tissues were processed for dehydration,
clearing and paraffin embedding with an automated carousel (Leica).
Sections (6 µm thick) were stained with haematoxylin and eosin
according to standard procedures. Images were acquired with a slide
scanner (Zeiss Axio Scan Z1). Adipocyte diameter was measured
(3 sections per mouse) with ImageJ (Fiji).

Methods

EpicWAT explants were processed to measure glucose uptake with
2-DG. After starvation and 2-DG uptake, explants were lysed in an extraction buffer. Lysates were processed according to the
manufacturer’s protocol (Glucose Uptake Fluorometric Assay Kit,
MAK084, Sigma–Aldrich).

Glucose uptake assay

Experimental animals and in vivo studies

Male C57BL/6j mice (5–7 weeks) were purchased from Charles River.
To generate mice with myeloid-specific deletion of IRF5, IRF5 flox/flox
mice (C57BL/6-IRF5tm1Ppr/J; stock no. 017311) were crossed with LysM-
cre mice (B6.129P2-LyZ2tm1(cre)If/J; stock no. 04781), purchased from The Jackson Laboratory. To generate mice with a restricted
myeloid expression of the Cas9 endonuclease, Rosa26Cas9K1 mice
(Gt(Rosa)26Sortm1.1(CAG-cas9*,-EGFP)Fezh/J; stock no. 024858, The
Jackson Laboratory) were crossed with LysM-Cre mice.

Mice carrying mutated alleles were identified by PCR screening
performed on genomic DNA (DNase Blood & Tissue Kit, Qiagen) with
specific primers (Table S1). Mice were housed at 21 °C and 50%
humidity, on average, on a 12 h light/dark cycle in the “Centre d’Ex-
plorations Fonctionnelles” of Sorbonne University (UMS-28). All mice
used in the study were male and aged between 7 and 10 weeks old at
the time of the experiment’s starting point. The number of mice used
per experiment is detailed in figure legends.

Mice were fed with High Fat Diet (HFD) (60% fat, D12492, Research
Diets) or a normal chow diet for 4 or 12 weeks. Mice had ad libitum
access to food and water. Mice were weighed weekly and glycaemia
measured.

For the oral glucose tolerance test (GTT), mice were fasted over-
night before being gavaged with glucose (2 g/kg of body weight). Tail
vein blood was collected to measure glycaemia with a glucometer
(Verio, One Touch). For the insulin tolerance test (ITT), mice were
fasted for 5 h before being i.p. injected with insulin (0.5 U/kg of body
weight). Glycaemia was monitored for 120 min after insulin injection.

Analysis of circulating plasma parameters

Adiponectin (Mouse Adiponectin/Acrp30 DuoSet ELISA, DY119, R&D
Systems), leptin (Mouse Leptin DuoSet ELISA, DY498-05, R&D Sys-
tems) and insulin (U-PLEX Mouse Insulin Assay, MSD) concentrations
were determined by immunoassay. Plasma cytokines were quantified with LEGENDplex Mouse Inflammation Kit (Biolegend) according to
the manufacturer’s instructions.

Stromal vascular fraction

The stromal vascular fraction (SVF) containing mononuclear cells and
preadipocytes was isolated from the adipose tissue after collagenase
digestion. Briefly, adipose tissue biopsies were minced in collagenase
solution (1 mg/ml collagenase (C6885, Sigma–Aldrich), diluted in
Dulbecco’s Modified Eagle Medium (DMEM) (Gibco) supplemented
with 1% penicillin/streptomycin (P/S), Heps and 2% BSA) for 20 min at
37 °C. The lysate was then passed through a 200 µm filter. After cen-
trifugation, the resulting cell pellet was resuspended in red blood cell
lysis buffer (155 mM NH4Cl, 12 mM NaHCO3, 0.1 mM EDTA) and passed

Human samples and study populations

Participants were consecutively recruited, and blood samples and
adipose tissue biopsies were obtained from different populations
admitted to the Lariboisière and Geoffroy Saint Hilaire hospitals
(Paris, France), respectively. Adipose tissue biopsies were obtained
from obese subjects during bariatric surgery. Sorted and
sequenced monocytes (Fig. 7a–c) were from patients with T2D aged
67–73 years old (4 male/1 female). Sorted ATMs (Fig. 7g) were from
patients with obesity aged 37–54 years old (gender was anonymised
for these patients). Samples analysed by cytometry (Fig. 7h) were
monocytes from patients with T2D aged 45–74 years old (6 male/7
female) and ATMs from patients with obesity aged 41–59 years
old (1 male/8 female). Blood samples prepared for immuno-
fluorescence (Fig. 7i) were from patients with T2D aged 47–81 years
old (9 male/1 female).
through a 70 µm filter. Cells were centrifuged and resuspended in FACS buffer (1× PBS supplemented with 0.5% BSA and 5 mM EDTA) for further analysis.

**Flow cytometry and cell sorting**

SVF cells were prepared as described above. Blood cells were obtained from 1 ml of venous blood after red blood cells lysis and resuspended in FACS buffer.

Cells were incubated with an Fc-blocker (120-000-422, Miltenyi Biotech) for 10 min. For metabolic analysis, cells were incubated with 200 µM JC-1 (T3168, Thermofisher Scientific) for 30 min at 37°C. Finally, cells were stained for surface markers (Table S2) and a Live/Dead viability dye (L34957, Thermofisher Scientific) according to the manufacturer’s protocol. For intracellular lipid staining, BODIPY (D9222, Thermofisher Scientific) was added to the surface markers antibodies mix. For IRF5 staining, cells were fixed with Foxp3-staining kit (00-5523-00, Thermofisher Scientific) and then stained with an anti-IRF5 (10547-1-AP, Proteintech) and OXPHOS (MS604, Abcam) with the appropriate secondary antibodies (goat anti-mouse FITC (A11001) and anti-rabbit-AF555 (A21428), Invitrogen). Nuclei were counterstained with Hoescht 33342 (Thermofisher Scientific). Images were acquired with a confocal microscope (Zeiss LSM 710) and analysed with ImageJ (Fiji).

Adipose tissue sections were stained for Mac2 (CL5942AP, Cedarlanelabs) overnight and then with the appropriate secondary antibody. Nuclei were counterstained with Hoescht 33342 (Thermofisher Scientific). Slides were scanned using Zeiss Axiol Scan Z1, and Mac2 staining was quantified with Visiopharm.

**Quantitative PCR with reverse transcription**

RNA was extracted from cells or tissue using RNeasy Plus Mini or Micro kit (Qiagen). Complementary DNA was synthesised with M-MLV Reverse Transcriptase kit (Promega). SYBR Green qRT-PCR reactions were performed with MESA green MasterMix (Eurogentec) and sequence-specific primers (Table S3), using QuantStudio 3 Real-Time PCR Systems (Thermofisher Scientific). 18S was used for normalisation to quantify relative mRNA expression levels.

**Western blotting**

To extract proteins, cells were lysed in RIPA lysis buffer (Sigma), supplemented with proteases (A29255, Thermofisher Scientific) and phosphatases inhibitors (1862495, Thermofisher Scientific). Proteins were separated on NuPAGE 4–12% polyacrylamide gels (Thermofisher Scientific) and then transferred onto nitrocellulose membranes. Membranes were probed with the appropriate primary (anti-GHITM, 16296-1-AP, Proteintech; anti-Actin, ab8226, Abcam) and secondary antibodies (34130 and 34660, Invitrogen) and visualised with SuperSignal West Pico Substrate (34080, Thermofisher Scientific). Images were analysed with ImageJ (Fiji).

**Extracellular flux measurements**

Real-time extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) were measured using Seahorse XF24 or XF96 extracellular flux analyser (Agilent). Briefly, cells were differentiated in XF24 or XF96 cell culture plate (15,000–30,000 cells per well). Adipose stromal vascular cells (800,000 cells per well) were seeded in an XF96 cell culture plate pre-treated with CellTak (Corning). F4/80- and F4/80- cells were allowed to adhere overnight in RPMI medium supplemented with 10% FBS and 1% P/S. Cells were incubated in Seahorse XF base medium supplemented with either 2 mM L-glutamine, 10 mM glucose and 1 mM sodium pyruvate (pH = 7.4) for mitochondrial stress test or only 2 mM L-glutamine (pH = 7.4) for glycolysis stress test, for 1 h at 37°C in a CO2 incubator. For palmitate oxidation test, cells were placed in substrate limited medium (DMEM supplemented with 0.5 mM glucose, 1 mM GlutaMAX (Life Technologies), 0.5 mM carnitine and 1% FBS) for 24 h. Assay was performed in fatty acid oxidation assay buffer (111 mM NaCl, 4.7 mM KCl, 1.25 mM CaCl2, 2 mM MgSO4, 1.2 mM Na2HPO4, 2.5 mM glucose, 0.5 mM carnitine, 5 mM Heps, pH = 7.4). Cells were pre-treated with etomoxir (40 µM) and then with palmitate (175 µM) before the assay. ECAR and OCR were measured in response to injections of either glucose (10 mM), oleicmycin (1µM) and 2-deoxyglucose (2-DG) (50 mM) for glycolysis stress test or oleicmycin (1µM), carbonyl cyanide 4-(trifluoro)phenylhydrazone (FCCP) (1µM) and rotenone/antimycin A (0.5 µM) for mitochondrial stress and palmitate oxidation tests. All compounds were purchased from Sigma–Aldrich.
Three measurements were made under basal conditions and after each drug injection. Each measurement cycle had the following time parameters: ‘mix’ 3 min, ‘wait’ 2 min, ‘measure’ 3 min.

Electron microscopy and structural analyses

BMDMs were scraped and fixed in 2 % glutaraldehyde for 2 h at 4 °C, postfixed in 1 % Osmium tetroxide for 1 h at 4 °C, dehydrated, and embedded in Epon. Samples were then cut using an RMC/MTX ultra-microtome (Elexience), and ultrathin sections (60–80 nm) were mounted on copper grids, contrasted with 5 % uranyl acetate and lead citrate, and observed with a Jeol 1200 EX transmission electron microscope (Jeol LTD) equipped with a MegaView II high-resolution transmission electron microscopy camera. Pictures of cell sections were taken at 45,000 magnification. Mitochondria number per section was measured to evaluate mitochondria density. For crista analysis, mitochondria and cristae were outlined using Imagem (Fiji) and both the total length and number of cristae in each mitochondrion were calculated, as previously described42. For the analysis of mitochondria dynamics, the long and short axis of each mitochondrion, as well as their perimeter and area, were measured. From these values, aspect ratio (major axis/minor axis) and form factor (perimeter)^2/(4•pi•Area) were calculated. TEM analyses were performed in triplicate, and a minimum of 11 images per sample were taken.

Quantification of TCA metabolites by liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS)

Metabolite extraction. A volume of 170 µL of ultrapure water was added to the frozen cell pellets. At this step, 20 µL of each sample was withdrawn to further determine the total protein concentration (colormetric quantification / Pierce BCA Protein Assay Kit, ThermoFisher Scientific). Then, 10 µL of 11 internal standards at 50 g/mL were added to the remaining 150 µL of cell lysate: 13C5-glutamic acid, 13C2-phosphoenolpyruvic acid, 13C4-fumaric acid, 13C3-pyruvic acid, 13C4-succinic acid (Merck), and D4-citric acid, 13C5-glutamine, D3-malic acid, 13C4,15N-aspartic acid, 13C5-α-ketoglutaric acid and 13C5-glutamic acid (Eurisotop), followed by a volume of 350 µL of cold methanol. The resulting samples were left on ice for 90 min. After a final centrifugation step at 20,000 x g for 15 min at 4 °C, supernatants were recovered and dried under a stream of nitrogen using a TurboVap instrument (ThermoFisher Scientific) and stored at -80 °C until analysis. Prior to LC-HRMS analysis, dried extracts were dissolved in 100 µL of 40 µL of chromatographic mobile phase A + 60 µL of mobile phase B (see below).

Preparation of calibration standards. Working solution (WS) for calibration curves and quality control solutions were prepared from two separate mother solutions (100 µg/mL in water) of each quantified compound: L-glutamic acid, L-aspartic acid, L-glutamine, succinic acid, alpha-ketoglutaric acid, trans-aconitic acid, L(-)-malic acid, D,L-isocitric acid, D-glyceric acid, fumaric acid, citric acid, pyruvic acid, D-α-hydroxyglutaric acid disodium salt, D(-)-lactic acid, D(+)-3-phosphoglyceric acid, phosphoenolpyruvic acid and itaconic acid (all from Sigma). Several diluted solutions of calibration standard solutions (CSS) and quality control solutions (QCS) were prepared by successive two-fold dilutions of WS in ultrapure water. Then, a three-fold dilution in a BSA solution (7200 µg/mL), of each previous diluted solution (CSSI-8 and QCSI-3) was applied to prepare standards for the calibration curve (from 33.33 to 0.26 µg/mL), and quality control (from 53.33 to 1.31 µg/mL). A volume of 350 µL of cold methanol was added to each calibration curve and quality control solution and followed the metabolite extraction process.

LC-HRMS analysis. Targeted LC-HRMS experiments were performed using an U3000 liquid chromatography system coupled to a Q Exactive Plus mass spectrometer (ThermoFisher Scientific). The software interface was Xcalibur (version 2.1) (ThermoFisher Scientific). The mass spectrometer was externally calibrated before each analysis in ES- polarity using the manufacturer’s predefined methods and recommended calibration mixture. The LC separation was performed on a Sequant ZIC-pHILIC 5 µm, 2.1 × 150 mm column (HILIC) maintained at 45 °C (Merck, Darmstadt, Germany). Mobile phase A consisted of an aqueous buffer of 10 mM of ammonium acetate, and mobile phase B of 100% acetonitrile. Chromatographic elution was achieved with a flow rate of 200 µL/min. After injection of 10 µL of sample, elution started with an isocratic step of 2 min at 70% B, followed by a linear gradient from 70 to 40% of phase B from 2 to 7 min. The chromatographic system was then rinsed for 5 min at 0% phase B, and the run was ended with an equilibration step of 9 min. The column effluent was directly introduced into the electrospray source of the mass spectrometer, and analyses were performed in the negative ion mode. The Q Exactive Plus mass spectrometer was operated with capillary voltage set at -2 kV and a capillary temperature set at 350 °C. The sheath gas pressure and the auxiliary gas pressure (nitrogen) were set at 60 and 10 arbitrary units, respectively. The detection was achieved from m/z 50 to 600 in the negative ion mode and at a resolution of 70,000 at m/z 200 (full width at half maximum). All metabolites were detected as their deprotonated [M-H]- species.

Succinic acid, glycine, itaconic acid, and lactic acid were detected at m/z 117.01933 (retention time (rt): 4.50 min); 105.01933 (rt 3.20 min); 129.01933 (rt 3.73 min); 89.02441 (rt 2.40 min), respectively; and quantified using 13C4-succinic acid (m/z 121.03251) as internal standard (ISTD). Malic acid and aconitic acid were monitored at m/z 133.01424 (rt 6.70 min); 173.0091 (rt 7.15 min), respectively; and quantified with D3-malic acid (m/z 136.03276). Citric acid, isocitric acid and 3-phosphoglyceric acid were monitored at m/z 191.01944 (rt 7.70); 191.01952 (rt 8.35); 184.98866 (rt 7.70 min), respectively; and quantified with D4-citric acid (m/z 195.04455). Pyruvic acid (m/z 87.00876, rt 2.25 min), aspartic acid (m/z 132.03023, rt 5.20 min), glutamine (m/z 145.06186, rt 4.75 min), glutamic acid (m/z 146.04388 rt 4.80 min), alpha-ketoglutaric acid (m/z 147.02989, rt 6.20 min), alpha-ketoglutaric acid (m/z 152.04637, rt 6.50 min), fumaric acid (m/z 150.03702, rt 7.05 min) and phosphoenolpyruvic acid (m/z 166.97509, rt 8.20 min) were all quantified with their isotopically labeled homologues (see above).

Metabolomic data processing and quantification. Xcalibur software was used for peak detection and integration. Metabolite quantification was performed using calibration curves established from peak area ratios between metabolites and their respective internal standard. Each metabolite amount was normalised by the protein quantity measured in each sample by BCA assay.

RNA sequencing of BMDMs and F4/80+ ATMs

After extraction, total RNA was analysed using Agilent RNA 6000 Pico Kit on the Agilent 2100 Bioanalyzer System. RNA quality was estimated based on capillary electrophoresis profiles using the RNA Integrity Number (RIN) and DV200 values. RNA-sequencing libraries were prepared using the SMARTer Stranded Total RNA-Seq Kit v2 – Pico Input Mammalian (Clontech/Takara) from 10 ng of total RNA. This protocol includes a first step of RNA fragmentation using a proprietary fragmentation mix at 94 °C. The time of incubation was set up for all samples at 4 min, based on the RNA quality, and according to the manufacturer’s recommendations. After fragmentation, indexed cDNA synthesis and amplification were performed, followed by a ribo-depletion step using probes targeting mammalian rRNAs. PCR amplification was finally achieved on ribodepleted cDNAs, using 12 cycles estimated in accordance with the input quantity of total RNA. Library quantification and quality assessment were performed using Qubit fluorometric assay (Invitrogen) with dsDNA HS (High Sensitivity) Assay Kit and LabChip GX Touch using a High Sensitivity DNA chip (Perkin
Elmer). Libraries were then equimolarly pooled and quantified by qPCR using the KAPA library quantification kit (Roche). Sequencing was carried out using a pair-end 2 × 100 bp mode on the NovaSeq 6000 system (Illumina), targeting between 10 and 15 M clusters per sample.

STAR v2.7.3a (Spliced Transcripts Alignment To a Reference) was used to align reads to the mouse mm10 genome and generate raw counts. We processed normalisation and differential expression gene analysis with DESeq244. Pathway enrichment analyses were performed using clusterProfiler45 with differentially expressed genes (abs(log2-FoldChange) > 1.3 and/or adj p-value < 0.05).

RNA sequencing of IRF5+/− human monocytes
Complementary DNA libraries and RNA-sequencing Library preparation and Illumina sequencing were performed at the École Normale Supérieure genomics core facility (Paris, France). Twenty nanograms of total RNA were amplified and converted to cDNA using SMART-Seq v4 Ultra Low Input RNA kit (Clontech). Afterwards an average of 150 pg of amplified cDNA was used to prepare the library following Nextera XT DNA kit (Illumina). Libraries were multiplexed by 12 on high-output flowcells. A 75 bp read sequencing was performed on a NextSeq 500 device (Illumina). A mean of 38.9 ± 8 million passing Illumina quality filter reads was obtained for each of the 12 samples.

The analyses were performed using the Eoulsan pipeline, including read filtering, mapping, alignment filtering, read quantification, normalisation and differential analysis. Before mapping, poly N read tails were trimmed, reads ≤40 bases were removed, and reads with quality mean ≤30 were discarded. Reads were then aligned against the hg19 genome from Ensembl version 91 using STAR (version 2.5.2b)46. Alignments from reads matching more than once on the reference genome were removed using Java version of samtools47. To compute gene expression, hg19 GTF genome annotation version 91 from Ensembl database was used. All overlapping regions between alignments and referenced exons were counted and aggregated by genes using HTSeq-count 0.3.5.48. The sample counts were normalised using DESeq2 1.8.149. Statistical treatments and differential analyses were also performed using DESeq2 1.8.1.

Statistics
Data analysis was performed using Microsoft Excel for Mac 16.47. Statistical analysis was performed using a two-tailed t-test for two groups, an ordinary one-way ANOVA followed by Tukey’s multiple-comparisons test for multiple groups and a two-way ANOVA followed by Bonferroni’s multiple comparison test on Prism 9 for macOS (GraphPad). Correlative analyses were performed on Prism 9 for macOS, computing Pearson coefficients for normally distributed data or Spearman coefficients for non-normally distributed data (GraphPad). PCA analyses were carried out on Prism 9 for macOS. Trajectory-resolved clustering was carried out on the Orange (v. 3.28.0) Python toolbox47. Statistical approaches per data panel are detailed in figure legends.

Public data
Single-cell sequencing data. Murine single-cell sequencing data from Jaitin et al.12 were downloaded and treated using BioTuring BBrowser (v. 2.7.48)50. Data were filtered in BBrowser and exported in tabular format for subsequent treatment with Microsoft Excel for Mac and Prism 9 for macOS. Human single-cell sequencing data were retrieved from GSE156110 raw data. Clustering was performed according to the authors’ instructions, and IRF5 and GHTM expressions were analysed in all macrophages and monocytes according to their lean/obese status.

UCSC genome browser. Gene tracks in Fig. 6e were visualised with the UCSC genome browser http://genome.ucsc.edu52, using the track hubs53. JASPAR2020 was used to visualise transcription factor binding sites54. The BLUEPRINT track-set was used for RNA expression and H3K27 Ac lines55,56. Sample lines and tracks available through this session link / live link. The Human Dec. 2013 (GRC38/hg38) assembly was used57,58.

Reporting summary
Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
Gene raw counts and raw fastq files for RNA-seq data generated in this study are available on GEO repository (www.ncbi.nlm.nih.gov/geo/). RNA-seq of IRF5+/− human monocytes (Fig. 7a–c) available under accession number: GSE176216 (GSM360191-4 and GSM360167-70 not included in study). RNA-seq of F4/80+ ATMs and BMDM from IRF5-KO and WT mice (Fig. 1a, b; Fig. 5a, b, d, e, Fig. S1A, B; Fig. S7A) available under accession numbers: GSE208648 and GSE208667, respectively. Previously published dataset analysed in this paper are from ref. 12 (Fig. 6a-d; Fig. S8A) (GSE128518) and from ref. 32 (Fig. 7d-f; Fig. S9A, B) (GSE155960). ChIP-seq data from ref. 30 (Fig. 3c; d; Fig. S7B) is available under accession number E-MTAB-2661. The GRC38/hg38 assembly was used is accessible via GenBank/RefSeq assembly accession numbers GCA_000001405.15/GCA_000001405.26. Source data are provided with this paper.

References
1. O’Neill, L. A. J., Kishton, R. J. & Rathmell, J. A guide to immuno-metabolism for immunologists. Nat. Rev. Immunol. 16, 553–565 (2016).
2. Li, C. et al. HIF1α-dependent glycolysis promotes macrophage functional activities in protecting against bacterial and fungal infection. Sci. Rep. 8, 3603 (2018).
3. Mills, E. L. et al. Succinate dehydrogenase supports metabolic repurposing of mitochondria to drive inflammatory macrophages. Cell 167, 457–470.e13 (2016).
4. Van den Bossche, J. et al. Mitochondrial dysfunction prevents repolarization of inflammatory macrophages. Cell Rep. 17, 684–696 (2016).
5. Wculek, S. K., Dunphy, G., Heras-Murillo, I., Mastrangelo, A. & Sancho, D. Metabolism of tissue macrophages in homeostasis and pathology. Cell. Mol. Immunol. https://doi.org/10.1038/s41423-021-00791-9 (2021).
6. Angelin, A. et al. Foxp3 reprograms t cell metabolism to function in low-glucose, high-lactate environments. Cell Metab. 28, 1282–1293.e7 (2017).
7. Serbulea, V. et al. Macrophage phenotype and bioenergetics are controlled by oxidized phospholipids identified in lean and obese adipose tissue. Proc. Natl Acad. Sci. USA 115, E6254–E6263 (2018).
8. Boutens, L. et al. Unique metabolic activation of adipose tissue macrophages in obesity promotes inflammatory responses. Diabetologia 61, 942–953 (2018).
9. Sharma, M. et al. Enhanced glycolysis and HIF-1α activation in adipose tissue macrophages sustains local and systemic interleukin-1β production in obesity. Sci. Rep. 10, 5555 (2020).
10. Hill, D. A. et al. Distinct macrophage populations direct inflammation versus physiological changes in adipose tissue. Proc. Natl Acad. Sci. USA 115, E5096–E5105 (2018).
11. Särväri, A. K. et al. Plasticity of epididymal adipose tissue in response to diet-induced obesity at single-nucleus resolution. Cell Metab. 33, 437–453.e5 (2021).
12. Jaitin, D. A. et al. Lipid-associated macrophages control metabolic homeostasis in a Trem2-dependent manner. Cell 178, 686–698.e14 (2019).
23. Reers, M. et al. Mitochondrial membrane potential monitored by JC-22. Hedl, M., Yan, J. & Abraham, C. IRF5 and IRF5 disease-risk variants 19. Yanaï, H. et al. Role of IFN regulatory factor 5 transcription factor in 17. Dalmas, E. et al. Irf5 de 16. Lee, Y. S. et al. In 32. Hildreth, A. D. et al. Single-cell sequencing of human white adipose 15. Weisberg, S. P. et al. Obesity is associated with macrophage 14. Brunner, J. S. et al. The PI3K pathway preserves metabolic health 33. Seitaj, B. et al. Transmembrane BAX inhibitor-1 motif containing 29. Noe, J. T. et al. Lactate supports a metabolic-epigenetic link in 30. Saliba, D. G. et al. IRF5:RelA interaction targets in 26. Cao, T. et al. Fatty acid oxidation promotes cardiomyocyte pro- 25. Nomura, M. et al. Fatty acid oxidation in macrophage polarization. 24. Cao, T. et al. Fatty acid oxidation promotes cardiomyocyte prolif- 23. Cao, T. et al. Fatty acid oxidation promotes cardiomyocyte prolif- 22. Hedl, M., Yan, J. & Abraham, C. IRF5 is required for bacterial clearance in human M1-polarized macrophages, and IRF5 immune- mediated disease risk variants modulate this outcome. J. Immunol. Baltim. Md 1950 202, 920–930 (2019). 21. Weiss, M. et al. IRF5 controls both acute and chronic inflammation. Proc. Natl Acad. Sci. USA 112, 11001–11006 (2015). 20. Reers, M. et al. Mitochondrial membrane potential potential monitored by JC-1 dye. Methods Enzymol. 260, 406–417 (1995). 19. Bassaganya-Riera, J., Misyak, S., Guri, A. J. & Hontecillas, R. PPAR gamma is highly expressed in F4/80(hi) adipose tissue macrophages and dampens adipose-tissue inflammation. Cell. Immunol. 258, 138–146 (2009). 18. Nomura, M. et al. Fatty acid oxidation in macrophage polarization. Nat. Immunol. 17, 216–217 (2016). 17. Cao, T. et al. Fatty acid oxidation promotes cardiomyocyte prolif- eration rate but does not change cardiomyocyte number in infant mice. Front. Cell Dev. Biol. 7, 42 (2019). 16. Weihrauch, D. et al. An IRF5 decoy peptide reduces myocardial inflammation and fibrosis and improves endothelial cell function in tight-skin mice. PLoS ONE 11, e0151999 (2016). 15. Grist, J. T. et al. Extracellular lactate: a novel measure of T cell proliferation. J. Immunol. Baltim. Md 1950 200, 1220–1226 (2018). 14. Noe, J. T. et al. Lactate supports a metabolic-epigenetic link in macrophage polarization. Sci. Adv. 7, eabj6802 (2021). 13. Saliba, D. O. et al. IRF5:RelA interaction targets inflammatory genes in macrophages. Cell Rep. 8, 1308–1317 (2014). 12. Oka, T. et al. Identification of a novel protein MICS1 that is involved in maintenance of mitochondrial morphology and apoptotic release of cytochrome c. Mol. Biol. Cell 19, 2597–2608 (2008). 11. Hildreth, A. D. et al. Single-cell sequencing of human white adipose tissue identifies new cell states in health and obesity. Nat. Immunol. 22, 639–653 (2021). 10. Seiltaj, B. et al. Transmembrane BAX inhibitor-1 motif containing protein 5 (TMBIM5) sustains mitochondrial structure, shape, and function by impacting the mitochondrial protein synthesis machinery. Cells 9, 2147 (2020). 9. Albers, G. J. et al. IRF5 regulates airway macrophage metabolic responses. Clin. Exp. Immunol. 204, 134–143 (2021). 8. Shimobayashi, M. et al. Insulin resistance causes inflammation in adipose tissue. J. Clin. Invest. 128, 1538–1550 (2018). 7. Young, A., Oldford, C. & Mailoux, R. J. Lactate dehydrogenase supports lactate oxidation in mitochondria isolated from different mouse tissues. Redox Biol. 28, 101339 (2020). 6. Glancy, B. et al. Mitochondrial lactate metabolism: history and implications for exercise and disease. J. Physiol. 599, 863–888 (2021). 5. Zoaña, E. et al. Mitochondrial quantification in human macrophages attenuates palmitate-induced inflammatory responses. Biochem. Biophys. Acta Mol. Cell Biol. Lipids 1863, 433–446 (2018). 4. Kapetanovic, R. et al. Lipopolysaccharide promotes Drp1- dependent mitochondrial fission and associated inflammatory responses in macrophages. Immunol. Cell Biol. 98, 528–539 (2020). 3. Nagel, J. E. et al. Identification of genes differentially expressed in T cells following stimulation with the chemokines CXCL12 and CXCL10. BMC Immunol. 5, 17 (2004). 2. Moni, M. A. & Liò, P. Network-based analysis of comorbidities risk during an infection: SARS and HIV case studies. BMC Bioinforma. 15, 333 (2014). 1. Sood, A. et al. Mitofusin-2-dependent inactivating cleavage of Opal1 links changes in mitochondrial cristae and ER contacts in the postprandial liver. Proc. Natl Acad. Sci. USA 111, 16017–16022 (2014). 0. Dobin, A. et al. STAR: ultrafast universal RNA-seq aligner. Bioin- forma. Oxf. Engl. 29, 15–21 (2013). 11. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 550 (2014). 10. Yu, G., Wang, L.-G., Han, Y. & He, Q.-Y. clusterProfiler: an R package for comparing biological themes among gene clusters. Omics J. Integr. Biol. 16, 284–287 (2012). 9. Jourden, L., Bernard, M., Dillies, M.-A. & Le Crom, S. Eoulson: a cloud computing-based framework facilitating high throughput sequencing analyses. Bioinforma. Oxf. Engl. 28, 1542–1543 (2012). 8. Li, H. et al. The Sequence Alignment/Map Format and SAMtools. Bioinforma. Oxf. Engl. 25, 2078–2079 (2009). 7. Anders, S., Pyl, P. T. & Huber, W. HTSeq—a Python framework to work with high-throughput sequencing data. Bioinforma. Oxf. Engl. 31, 166–169 (2015). 6. Deksi, J. et al. Orange: data mining toolbox in python. J. Machine Learn. Res. 14, 2349–2353 (2013). 5. Le, T. et al. BBBrowser: Making single-cell data easily accessible. bioRxiv https://doi.org/10.1101/2020.12.11.414136 (2020). 4. Kent, W. J. et al. The human genome browser at UCSC. Genome Res. 12, 996–1006 (2002). 3. Navarro Gonzalez, J. et al. The UCSC Genome Browser database: 2021 update. Nucleic Acids Res. 49, D1046–D1057 (2021). 2. Raney, B. J. et al. Track data hubs enable visualization of user- defined genome-wide annotations on the UCSC Genome Browser. Bioinforma. Oxf. Engl. 30, 1003–1005 (2014). 1. Fornes, O. et al. JASPAR 2020: update of the open-access database of transcription factor binding profiles. Nucleic Acids Res. 48, D87–D92 (2020). 0. Fernández, J. M. et al. The BLUEPRINT data analysis portal. Cell Rep. 3, 491–495.e5 (2016). 1548 (2001). 1442 (2020). 11001 (2015). 4302–3407 (2007). 216–217 (2016). 2012). 2001). 1005 (2014). 2012). 2012). 2019). 2001). 2001). 2009). 533 (2014). 15:5089
of the SU Cell Imaging and Flow Cytometry network (LUMIC) and UPD cell imaging networks. Transmission electronic microscopy was performed at CIOLE platform (Centre d’Imagerie Quantitative Lyon-Est, Lyon, France) and we thank Elisabeth Errazuriz and Christel Cassin for their technical help. This work was supported by the France Génomique national infrastructure, funded as part of the “Investissements d’Avenir” program managed by the Agence Nationale de la Recherche (contract ANR-10-INBS-09). High-throughput sequencing has been performed by the ICGex NGS platform of the Institut Curie supported by the grant ANR-10-EQPX-03 (Equipex) from the French National Research Agency (Agence Nationale de la Recherche; ANR; “Investissements d’Avenir” program), by the Canceropole Ile-de-France and by the SiRIC-Curie program - SiRIC Grant “INCa-DGOS-4654”. This research was supported by the French National Research Agency (Agence Nationale de la Recherche; ANR) ANR-JCJC grant for the MitoFLAME Project (ANR-19-CE14-0005) and by the French Society for Diabetes (Société Françophone du Diabète; SFD) Allocation Exceptionnelle to F.A. Collaboration with AstraZeneca provided support to F.A. and N.V. The European Foundation for the Study of Diabetes provided support to F.A. and J.F.G. Support was also provided by the Commissariat à l’Energie Atomique et aux Energies Alternatives and the MetaboHUB infrastructure (ANR-11-INBS-0010) to F.C. and F.F. Grants from the European Union H2020 framework (ERC-EpiFAT 725790), ANR-PUMAS (ANR-19-CE14-0020) and INFLAMEX supported N.V. Support was provided from the Fondation de la Recherche Médicale (FDT202106013230) to L.O.

Author contributions
L.O., N.V. and F.A. conceived and designed the study. L.O., T.E., R.B., J.M., D.C., Jo.C., Ju.C., C.P., A.I.H., A.K.H., F.C. and F.A. performed experiments and collected data. L.O., T.E., A.I.H., M.D., C.P., C.B., Sop.L., Son.L., J.R. and F.A. analysed data. A.H., M.D., J.M., P.I., L.G.B., S.B., Sop.L., Son.L., C.B.; F.F. and F.A.C. contributed data or analysis tools. L.O., D.L., J.B., E.D., J.P.R., J.R. and J.F.G. provided key resources. L.O., D.L., J.B., E.D., J.P.R., J.R. and J.F.G. performed experiments and collected data. L.O., T.E., A.I.H., M.D., C.P., C.B., Sop.L., Son.L., C.B., F.F. and F.A.C. provided data or analysis tools. L.O., D.L., J.B., E.D., J.P.R., J.R., J.F.G., N.V. and F.A. provided intellectual input. L.O., N.V. and F.A. wrote the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-022-32813-z.

Correspondence and requests for materials should be addressed to N. Venteclef or F. Alzaid.

Peer review information Nature Communications thanks Jan Van den Bossche and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Peer reviewer reports are available.

Reprints and permission information is available at http://www.nature.com/reprints

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2022