

The metabolic ER stress sensor IRE1 α suppresses alternative activation of macrophages and impairs energy expenditure in obesity

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Obesity is associated with metabolic inflammation and endoplasmic reticulum (ER) stress, both of which promote metabolic disease progression. Adipose tissue macrophages (ATMs) are key players orchestrating metabolic inflammation, and ER stress enhances macrophage activation. However, whether ER stress pathways underlie ATM regulation of energy homeostasis remains unclear. Here, we identified inositol-requiring enzyme 1 α (IRE1 α) as a critical switch governing M1–M2 macrophage polarization and energy balance. Myeloid-specific IRE1 α abrogation in *Ern1^{fl/fl}*; *Lyz2-Cre* mice largely reversed high-fat diet (HFD)-induced M1–M2 imbalance in white adipose tissue (WAT) and blocked HFD-induced obesity, insulin resistance, hyperlipidemia and hepatic steatosis. Brown adipose tissue (BAT) activity, WAT browning and energy expenditure were significantly higher in *Ern1^{fl/fl}*; *Lyz2-Cre* mice. Furthermore, IRE1 α ablation augmented M2 polarization of macrophages in a cell-autonomous manner. Thus, IRE1 α senses protein unfolding and metabolic and immunological states, and consequently guides ATM polarization. The macrophage IRE1 α pathway drives obesity and metabolic syndrome through impairing BAT activity and WAT browning.

Obesity is associated with a state of chronic low-grade inflammation characterized by infiltration and activation of immune cells in metabolic organs such as adipose tissue^{1,2}. Overnutrition-induced metabolic inflammation in adipose tissue has been proposed to be critical in the pathogenesis of insulin resistance and type 2 diabetes³. Adipose tissue is composed of multiple types of tissue, including white, brown and beige fat, which play pivotal roles in metabolic homeostasis⁴. Various innate and adaptive immune cells communicate with adipocytes and thereby maintain adipose function⁵. In particular, ATMs are critical in orchestrating metabolic inflammation^{6–10}. As the major effector cells mediating both adipose and systemic inflammation, ATMs respond to metabolic cues and are present in a spectrum of functionally distinct activation states, thereby exerting profound regulatory effects on metabolism^{6,8–11}. In obesity, ATMs have been found in the so-called ‘crown-like’ structure (CLS) around dying adipocytes, which display a predominantly proinflammatory classically activated (M1) phenotype¹. ATM M1 polarization is thought to promote insulin resistance and type 2 diabetes^{1,6,9,10}. Whereas in

the lean state, ATMs are uniformly dispersed, primarily exhibiting an anti-inflammatory alternatively activated (M2) phenotype^{1,7,12}. ATM M2 polarization, induced by eosinophil-derived type 2 cytokines (for example, the interleukins IL-4 and IL-13), enhances activation of BAT and browning or beiging of WAT^{13–15}, thereby modulating adaptive thermogenesis and energy expenditure.

The ER is a critical organelle in cellular nutrient sensing and handling². An overload of protein folding can trigger ER stress and consequently activate the unfolded protein response (UPR). Three UPR pathways coordinately restore ER homeostasis and affect many aspects of cellular function^{16–18}. IRE1, the ER-resident transmembrane protein kinase and endoribonuclease, is the most conserved ER stress sensor^{18,19}. Mammalian IRE1 α is activated by ER stress and consequently can catalyze the unconventional splicing of the mRNA encoding the transcription factor X-box binding protein 1 (XBP1) or can degrade select mRNA species in a process termed regulated IRE1-dependent decay (RID^D)^{18,19}. Hyperactivation of the IRE1 α –XBP1 pathway has been documented in the adipose

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tissue of obese humans^{20,21}, but whether this hyperactivation is causally related to energy imbalance remains unknown. Here, we sought to test the possibility that during chronic handling of excess nutrients, ER stress mechanistically converges with metabolic inflammation through ATMs, thereby initiating adipose dysfunction and causing dysregulation of glucose and energy metabolism. We found that the IRE1 α pathway in macrophages regulates a shift in M1–M2 polarization and couples metabolic ER stress to the disruption of energy balance through impairing BAT activity and WAT browning.

RESULTS

Myeloid IRE1 α ablation prevents diet-induced obesity

We first examined whether ER stress in ATMs accompanies overnutrition-induced obesity and metabolic inflammation in adipose tissue, such as in visceral epididymal WAT (epWAT) of obese mice fed an HFD (Supplementary Fig. 1a,b). Not only in adipocytes but also in the stromal vascular fraction (SVF), where resident ATMs are enriched¹, we observed significantly elevated *Xbp1* mRNA splicing and higher expression levels of the UPR marker and proinflammatory genes (Supplementary Fig. 1c–f). Moreover, in cells positive for the integrin CD11b (CD11b⁺), which were isolated from SVFs of obese mice and largely comprised macrophages, we also detected prominently increased *Xbp1* mRNA splicing and decreased mRNA abundance of two known RIDD-target genes²², *Hgsnat* and *Pmp22*, along with elevated expression of other UPR markers and proinflammatory genes (Supplementary Fig. 1g,h). This finding suggested that excessive nutrient handling elicits metabolic ER stress and activation of the IRE1 α branch of the UPR in ATMs in parallel with adipose inflammation. To investigate whether the IRE1 α pathway in macrophages exerts metabolic actions during overnutrition, we intercrossed floxed IRE1 α (*Ern1*^{fl/fl}) mice²³ with the Lysozyme 2-Cre (*Lyz2*-Cre) line¹³ to create myeloid-specific IRE1 α -knockout (*Ern1*^{fl/fl}; *Lyz2*-Cre) mice, in which IRE1 α protein expression was abolished in bone-marrow-derived macrophages (BMDMs) and peritoneal macrophages (pM ϕ) but remained normal in epWAT and subcutaneous WAT (scWAT) (Fig. 1a) as well as in other tissues examined (Supplementary Fig. 2a). RT-PCR analysis with primers spanning the 121-bp exon 2 of *Ern1* that was targeted for deletion revealed that IRE1 α was efficiently ablated in BMDMs and pM ϕ , and was partially ablated in neutrophils but not in T cells positive for the monomorphic co-receptor CD4 (CD4⁺) or B cells (Fig. 1b). In comparison to their *Ern1*^{fl/fl} counterparts, *Ern1*^{fl/fl}; *Lyz2*-Cre mice showed no defective developmental phenotypes (Supplementary Fig. 2b,c) and had insignificant differences in body weight but lower body fat mass when they were fed a normal chow (NC) diet (Fig. 1c,d and Supplementary Fig. 2d). Remarkably, these mice were completely resistant to HFD-induced obesity, exhibiting much lower body weight and adiposity without significant changes in their lean mass (Fig. 1c,d and Supplementary Fig. 2b,e–g). *Ern1*^{fl/fl}; *Lyz2*-Cre mice after HFD feeding also displayed a more beneficial epWAT phenotype with smaller adipocytes (Fig. 1e), along with significantly decreased hyperleptinemia and normal serum adiponectin levels (Fig. 1f), thus indicating improved adipose function. Moreover, HFD-fed *Ern1*^{fl/fl}; *Lyz2*-Cre mice showed greatly decreased hyperinsulinemia (Fig. 1g) and markedly improved insulin sensitivity (Supplementary Fig. 2h) and blood glucose control (Fig. 1h). In addition, *Ern1*^{fl/fl}; *Lyz2*-Cre mice did not exhibit HFD-induced hypertriglyceridemia, hypercholesterolemia or hepatosteatosis (Fig. 1i–k and Supplementary Fig. 2i). These data demonstrated that IRE1 α in myeloid cells couples overnutrition to the development of obesity and obesity-associated metabolic deterioration.

Myeloid IRE1 α abrogation increases energy expenditure

We next determined whether myeloid IRE1 α deficiency affects energy balance. *Ern1*^{fl/fl}; *Lyz2*-Cre mice and their *Ern1*^{fl/fl} counterparts had similar daily food consumption during NC or HFD feeding (Supplementary Fig. 2j) and showed comparable absorption of dietary lipids when they were maintained on an HFD (Supplementary Fig. 2k). However, oxygen consumption, when normalized to the lean mass, was significantly higher in *Ern1*^{fl/fl}; *Lyz2*-Cre mice than in *Ern1*^{fl/fl} littermates fed either NC or an HFD (Fig. 2a,b), although physical activities were similar between these two groups (Supplementary Fig. 2l). In addition, core body temperatures were also higher in HFD-fed *Ern1*^{fl/fl}; *Lyz2*-Cre mice (Fig. 2c). Thus, loss of IRE1 α in myeloid cells resulted in increased energy expenditure.

To determine whether the effect of myeloid IRE1 α deficiency on energy expenditure might be attributable to enhanced heat production, we first examined the activation of BAT, the major organ that dissipates energy through the action of mitochondrial uncoupling protein 1 (UCP1) during adaptive thermogenesis^{4,24}. In the BAT of HFD-fed *Ern1*^{fl/fl}; *Lyz2*-Cre mice, compared with their *Ern1*^{fl/fl} counterparts, much lower lipid-droplet content (Fig. 2d) and higher expression of UCP1 protein were observed (Fig. 2e). *Ern1*^{fl/fl}; *Lyz2*-Cre mice fed either NC or an HFD had significantly elevated expression of key thermogenic genes in BAT, thus further suggesting that these mice had a greater BAT thermogenic capacity (Fig. 2f). In parallel with higher levels of UCP1 protein, BAT from HFD-fed *Ern1*^{fl/fl}; *Lyz2*-Cre mice also had increased levels of phosphorylated STAT3 (p-STAT3) transcription factor and elevated expression of tyrosine hydroxylase (TH) (Fig. 2g), the rate-limiting enzyme in biosynthesis of catecholamines, which activate both brown and beige fat, thereby generating heat^{13,14}. Given the critical function of the inducible beige fat within scWAT in adaptive thermogenesis and maintenance of energy balance^{4,24}, we examined whether beige adipocytes were also involved. Indeed, scWAT from HFD-fed *Ern1*^{fl/fl}; *Lyz2*-Cre animals exhibited significantly elevated *Ucp1* mRNA and UCP1 protein expression levels (Fig. 2h,i), thus indicating enhanced beige-fat activation.

To confirm the effect of myeloid IRE1 α abrogation on adaptive thermogenesis, we subjected mice to cold stress (4 °C). *Ern1*^{fl/fl}; *Lyz2*-Cre mice maintained their body temperatures at higher levels than *Ern1*^{fl/fl} animals after cold exposure for 3 d (Fig. 3a) but showed no significant changes in BAT weight (Fig. 3b). However, cold stress decreased BAT lipid-droplet levels (Fig. 3c) and increased BAT UCP1 and TH expression (Fig. 3d,e) as well as norepinephrine content (Fig. 3f) to a greater extent in *Ern1*^{fl/fl}; *Lyz2*-Cre mice, thus indicating higher BAT activity accompanied by augmented biosynthesis of catecholamines. In addition, scWAT of *Ern1*^{fl/fl}; *Lyz2*-Cre mice subjected to cold exposure also exhibited markedly enhanced remodeling, as evidenced by both increased induction of multilocular UCP1-expressing beige adipocytes (Fig. 3g,h) and robust augmentation of cold-induced UCP1 expression and norepinephrine production (Fig. 3i,j). Together, these results demonstrated that the loss of myeloid IRE1 α promotes energy expenditure through enhancing the thermogenic activity of both brown and beige fat.

To determine whether the effect of myeloid IRE1 α deficiency on adaptive thermogenesis stems primarily from its actions in ATMs, we used liposomal clodronate, which selectively depletes macrophages but not neutrophils through induction of apoptosis²⁵, even in the WAT of obese mice²⁶. Indeed, administration of clodronate-containing liposomes into the scWAT of *Ern1*^{fl/fl} or *Ern1*^{fl/fl}; *Lyz2*-Cre mice resulted in marked decreases in the mRNA expression of the macrophage marker F4/80 (encoded by *Adgre1*) (Fig. 4a), thus indicating efficient ablation of ATMs in scWAT in both groups. Clodronate

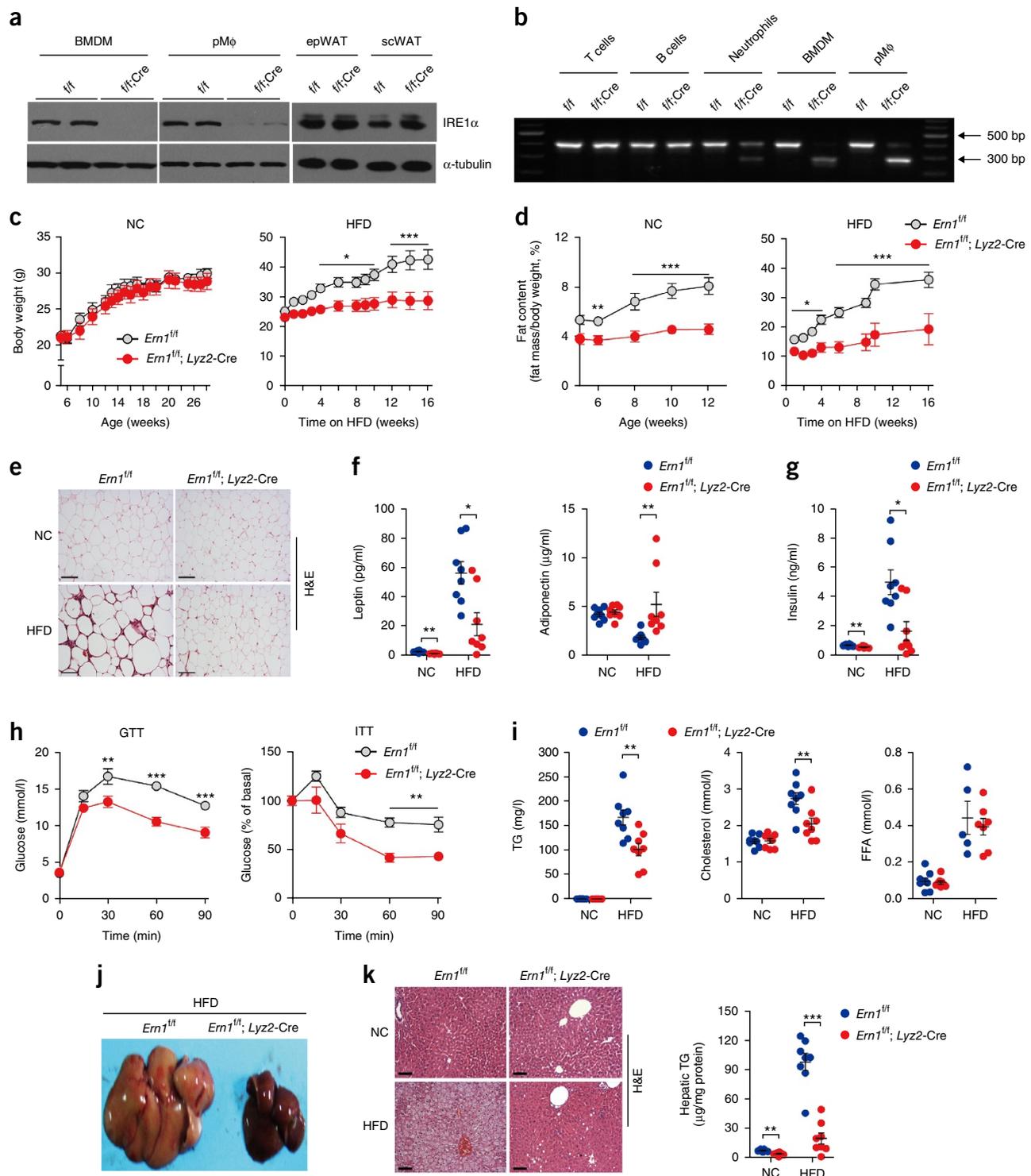


Figure 1 Myeloid IRE1 α abrogation protects mice from diet-induced obesity and metabolic syndrome. **(a)** Immunoblot analyses of IRE1 α protein in bone-marrow-derived macrophages (BMDM), peritoneal macrophages (pM ϕ) and epididymal (ep) and subcutaneous (sc) WAT from male *Ern1^{fl/fl}; Lyz2-Cre* (*f/f;Cre*) mice and *Ern1^{fl/fl}* (*f/f*) littermates. α -tubulin is a loading control. **(b)** RT-PCR analyses of the region spanning the targeted exon 2 within *Ern1* mRNA in CD4⁺ T cells, B cells and neutrophils from the spleen, as well as in BMDMs and pM ϕ . **(c–k)** Male *Ern1^{fl/fl}; Lyz2-Cre* mice and age-matched *Ern1^{fl/fl}* littermates were fed a normal chow diet (NC, 10% fat; $n = 8$ per group) or a high-fat diet (HFD, 60% fat; $n = 10$ per group). HFD feeding started at 8 weeks of age. **(c)** Body weight. **(d)** Body-fat content. **(e–k)** Metabolic measurements in mice after feeding of an NC or HFD for 16 weeks ($n = 8$ per group). **(e)** Hematoxylin and eosin (H&E) staining of epWAT sections (four images per mouse). Scale bars, 10 μ m. **(f)** Serum concentrations of leptin and adiponectin. **(g)** Serum insulin levels. **(h)** Glucose tolerance test (GTT) and insulin tolerance test (ITT). **(i)** Serum levels of triglycerides (TG), cholesterol and free fatty acids (FFA). **(j)** Representative images of livers from HFD-fed mice (3 images per mouse). **(k)** Representative images of H&E staining of liver sections (4 images per mouse). Scale bars, 200 μ m. Hepatic TG content was determined. All data are shown as mean \pm s.e.m., * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ by two-tailed Student's *t* test or two-way ANOVA.

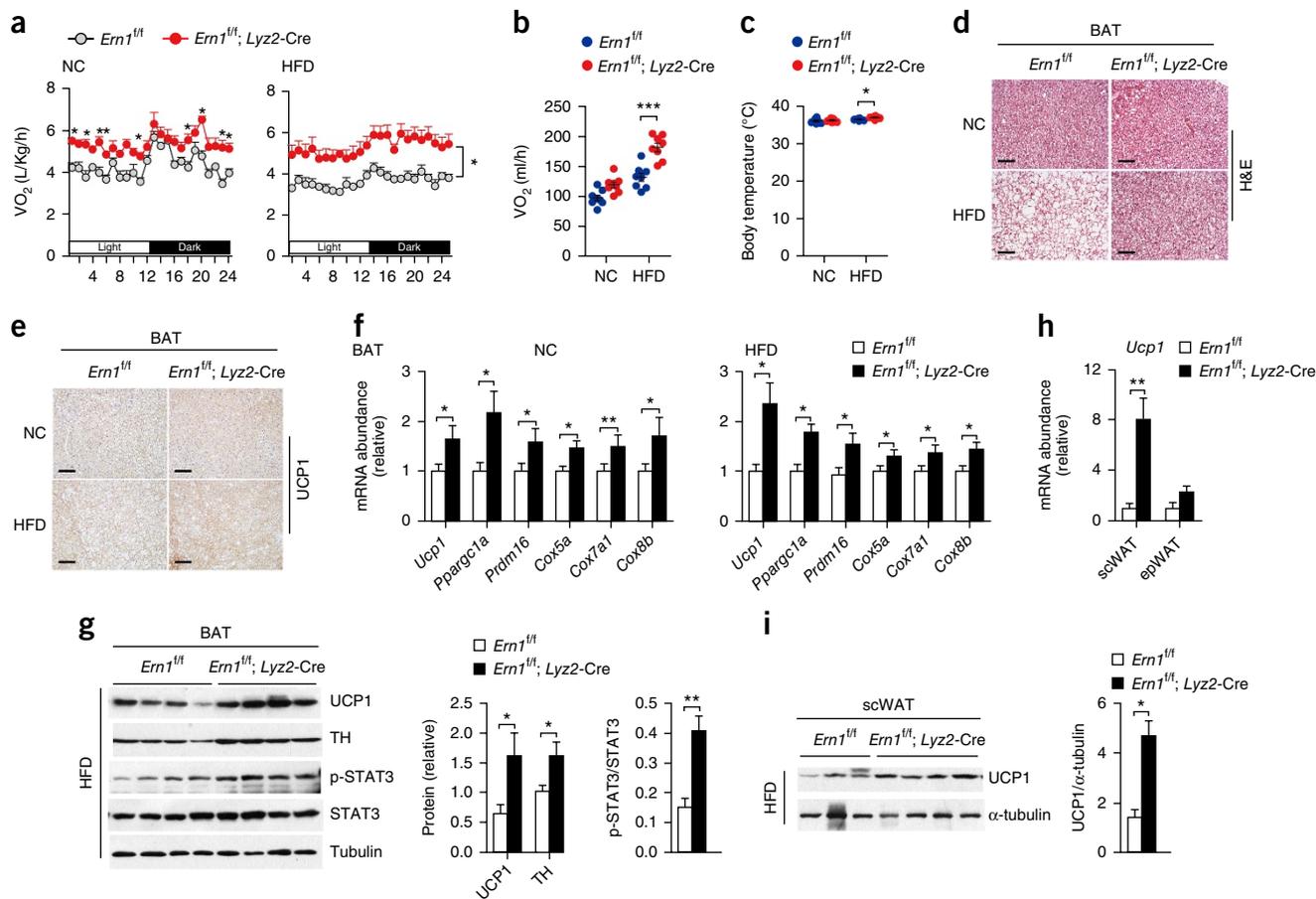


Figure 2 Myeloid IRE1 α ablation increases energy expenditure via enhancing brown- and beige-fat activation. Male *Ern1*^{fl/fl}; *Lyz2-Cre* and *Ern1*^{fl/fl} mice were fed an NC or HFD for 16 weeks ($n = 8$ per group). (a,b) Oxygen consumption (VO_2) monitored over a 24-h period (a) and shown as averaged values (b). (c) Core body temperatures of mice housed at 22 °C. (d,e) Representative images of H&E staining (d) and UCP1 immunohistochemistry (IHC) (e) of BAT sections (5 images per mouse). Scale bars, 200 μ m. (f) Quantitative RT-PCR analysis of the mRNA abundance of the indicated thermogenic genes in BAT. (g) Immunoblot analysis and quantification of UCP1 and tyrosine hydroxylase (TH) protein and STAT3 phosphorylation in BAT from HFD-fed mice. α -tubulin is a loading control. (h) Quantitative RT-PCR analysis of the mRNA abundance of *Ucp1* in scWAT and epWAT of mice after HFD feeding. (i) Immunoblot analysis and quantification of UCP1 protein in scWAT of HFD-fed mice. Results are presented as mean \pm s.e.m., * $P < 0.05$; ** $P < 0.01$ by two-tailed Student's *t* test or two-way ANOVA.

treatment impaired the enhancement of scWAT remodeling (Fig. 4b) and greatly diminished the UCP1 induction in *Ern1*^{fl/fl}; *Lyz2-Cre* mice after cold exposure (Fig. 4c,d). Hence, these results clearly demonstrated the critical role of IRE1 α -deficient ATMs in promoting WAT browning during adaptive thermogenesis.

IRE1 α deficiency reverses the M1–M2 imbalance of ATMs

We then considered whether macrophage IRE1 α might be coupled to metabolic inflammation by mediating a shift in M1–M2 polarization of ATMs. As compared with their *Ern1*^{fl/fl} counterparts, HFD-fed *Ern1*^{fl/fl}; *Lyz2-Cre* mice exhibited significantly decreased serum levels of proinflammatory cytokines such as tumor necrosis factor (TNF), IL-1 β and the chemokine CCL2 (Fig. 5a); however, they produced higher amounts of IL-6 (Fig. 5a), a pleiotropic cytokine that exerts either proinflammatory or anti-inflammatory effects. Immunohistochemistry also revealed lower numbers of epWAT F4/80⁺ CLS in *Ern1*^{fl/fl}; *Lyz2-Cre* than in *Ern1*^{fl/fl} mice after HFD feeding (Fig. 5b). The CD11b⁺F4/80⁺ cell numbers in epWAT and scWAT SVFs, as measured by flow cytometry, were ~58% and ~26% lower, respectively, in HFD-fed *Ern1*^{fl/fl}; *Lyz2-Cre* mice (Fig. 5c,d), thus indicating dramatically decreased macrophage recruitment in WAT. We detected an ~39% decrease in M1-like CD11b⁺CD11c⁺

cells and an ~161% increase in M2-like CD11b⁺CD206⁺ cells in both epWAT (Fig. 5e,f) and scWAT SVFs (Fig. 5g,h). Notably, in the setting of NC feeding, SVFs of epWAT from *Ern1*^{fl/fl}; *Lyz2-Cre* mice, compared with their *Ern1*^{fl/fl} counterparts, had comparable amounts of CD11b⁺F4/80⁺ cells but considerably increased CD11b⁺CD206⁺ cells (Supplementary Fig. 3a,b). These results suggest that in the absence of excess nutrition, IRE1 α ablation does not influence macrophage differentiation or recruitment. Moreover, in SVFs of both epWAT (Fig. 5i) and scWAT (Fig. 5j) of HFD-fed *Ern1*^{fl/fl}; *Lyz2-Cre* mice, gene expression profiling showed robust increases in the expression of signature M2-marker genes (*Arg1*, *Chil3*, *Retnla*, *Mrc1*, *Pcd1lg2* and *Il10*)^{7,12} and significant decreases in the expression of M1 markers (*Nos2*, *Il6* and *Tnf*)¹. Additionally, in SVFs of BAT from *Ern1*^{fl/fl}; *Lyz2-Cre* mice after HFD feeding, M2- but not M1-marker genes were also similarly upregulated (Fig. 5k). In agreement with their serum levels (Fig. 5a), the expression of proinflammatory adipokines *Tnf*, *Ccl2* and *Il1b* markedly decreased, whereas that of *Il6* dramatically increased in adipocytes of both epWAT and scWAT (Fig. 5i,j). Next, we examined whether myeloid IRE1 α deficiency blunted HFD-induced adipose inflammation through altering the UPR pathways. In *Ern1*^{fl/fl}; *Lyz2-Cre* mice subjected to HFD feeding, there were no significant changes in the expression of typical ER stress markers, including *Bip*

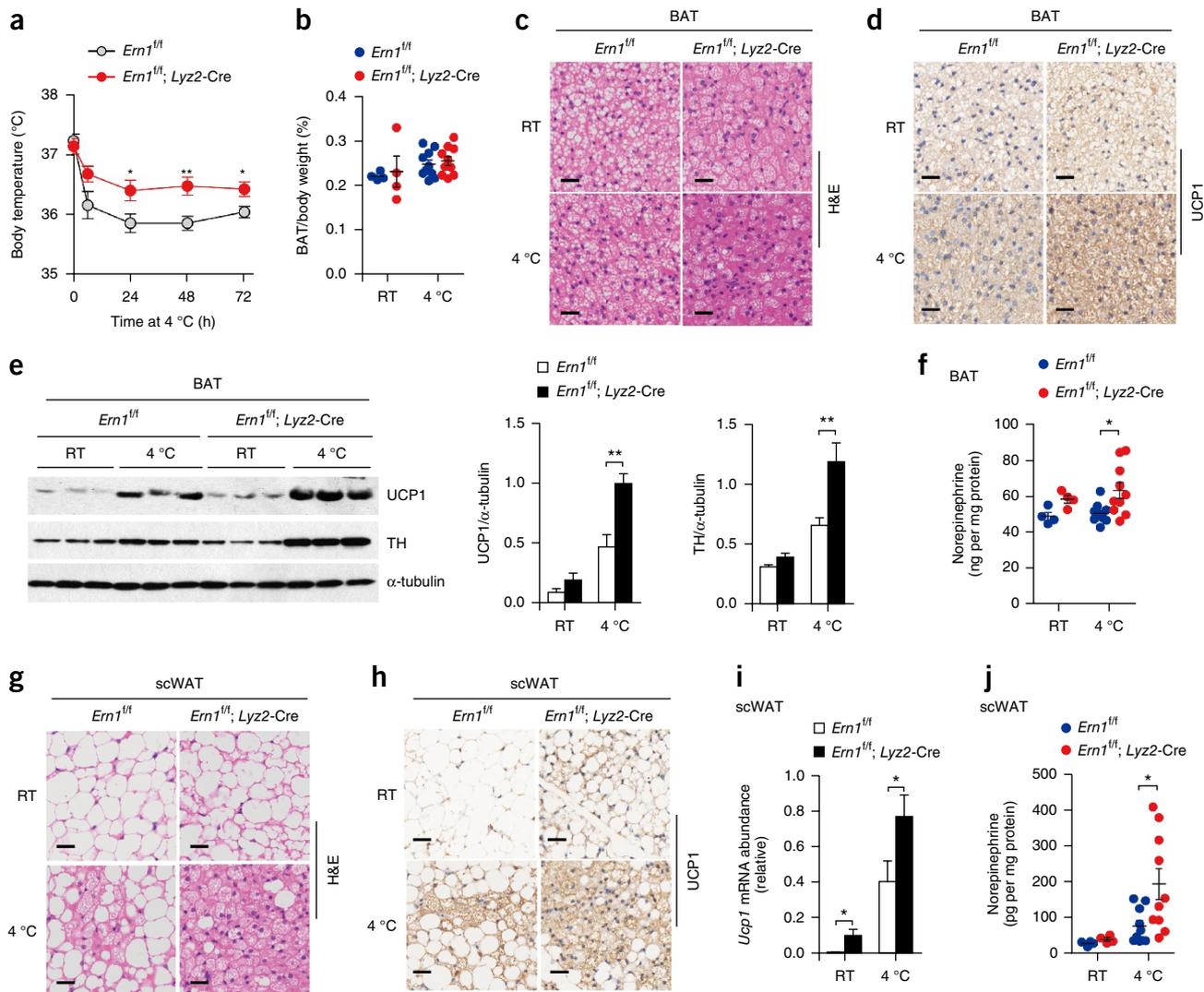


Figure 3 Myeloid IRE1 α ablation augments adaptive thermogenesis. NC-fed *Ern1^{fl/fl}; Lyz2-Cre* and *Ern1^{fl/fl}* mice were maintained at room temperature (RT, ~26 °C; $n = 4$ per group) or at 4 °C for 72 h ($n = 10$ per group). **(a)** Core body temperatures of mice maintained at 4 °C. **(b)** Relative BAT weight after exposure to room temperature or cold stress. **(c,d)** Representative images of H&E staining **(c)** and UCP1 IHC **(d)** of BAT sections (6 images per mouse). Scale bars, 30 μ m. **(e)** Immunoblot analysis and quantification of BAT UCP1 and TH protein. α -tubulin is a loading control. **(f)** BAT norepinephrine content, as measured by enzyme-linked immunosorbent assay. **(g,h)** Representative images of H&E staining **(g)** and UCP1 IHC **(h)** of scWAT sections (6 images per mouse). Scale bars, 30 μ m. **(i)** Relative mRNA abundance of *Ucp1* in scWAT. **(j)** Norepinephrine content in scWAT. Results are presented as mean \pm s.e.m., * $P < 0.05$; ** $P < 0.01$ by two-tailed Student's *t* test or two-way ANOVA.

(official symbol *Hspa5*), *Chop* (official symbol *Ddit3*) and *Atf4*, in the SVFs or adipocytes of epWAT or scWAT (**Supplementary Fig. 3c–f**). Further, in CD11b⁺ cells isolated from SVFs, which predominantly consisted of CD11b⁺F4/80⁺ ATMs (>82% in epWAT, >73% in scWAT; **Supplementary Fig. 4**), elevated levels of the M2-marker protein Ym-1 (encoded by *Chil3*) were detected in HFD-fed *Ern1^{fl/fl}; Lyz2-Cre* mice as compared with their *Ern1^{fl/fl}* counterparts (**Fig. 5l,m**). No apparent alterations in phosphorylation of the initiation factor eIF2 α or levels of the prosurvival ER chaperone BiP and the heat-shock protein Hsp90 were observed in isolated CD11b⁺ cells or adipocytes (**Fig. 5l,m**). Together, these data suggested that macrophage IRE1 α ablation can unlock its suppressive action in M2 polarization of ATMs, thereby reversing HFD-induced M1–M2 imbalance in WAT, decreasing adipose inflammation and enhancing energy expenditure. Without affecting the ER stress state in adipose tissue, loss of IRE1 α in ATMs can resolve overnutrition-induced metabolic inflammation.

IRE1 α promotes M2 polarization in a cell-autonomous manner

To determine whether IRE1 α regulates macrophage polarization in a cell-autonomous fashion, we used lipopolysaccharide (LPS) and IL-4 stimulation of mouse BMDM *in vitro* models to mimic M1 and M2 polarization²⁷, respectively. As assessed with an antibody to IRE1 α phosphorylated at Ser724 within the activation loop of its kinase domain²⁸, LPS treatment increased IRE1 α phosphorylation in BMDMs (**Fig. 6a**); however, phos-tag gel analysis²⁹ indicated that LPS-stimulated IRE1 α phosphorylation was distinct from that induced by the typical ER stressor thapsigargin (**Supplementary Fig. 5a**). In addition, LPS did not affect eIF2 α phosphorylation or BiP protein expression (**Fig. 6a** and **Supplementary Fig. 5b**). This result is consistent with the previously reported finding that Toll-like-receptor ligands such as LPS specifically activate the IRE1 α pathway in macrophages³⁰. In *Ern1^{fl/fl}; Lyz2-Cre* BMDMs, compared with control *Ern1^{fl/fl}* BMDMs, LPS induction of inducible nitric oxide synthase (iNOS)

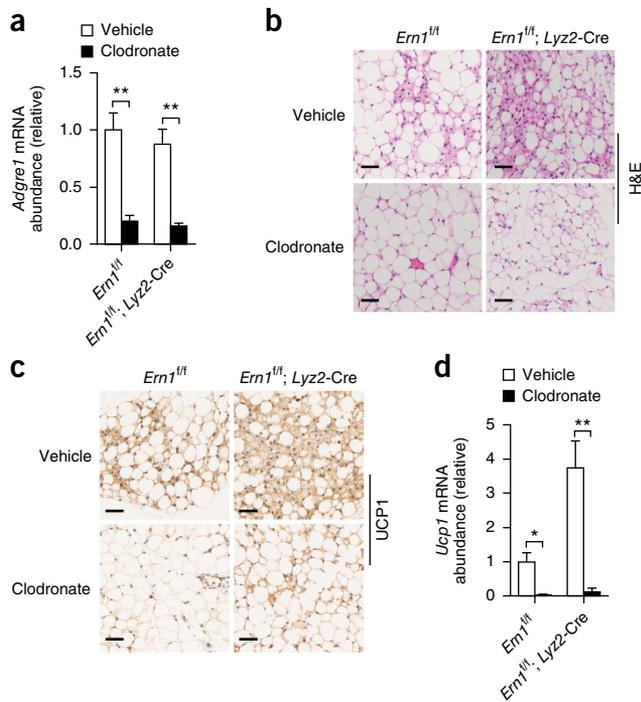


Figure 4 Depletion of ATMs abolishes cold-induced scWAT browning. Clodronate liposomes or control liposomes (vehicle) were subcutaneously delivered into scWAT of NC-fed *Ern1^{fl/fl}*; *Lyz2-Cre* and *Ern1^{fl/fl}* mice. Animals were subsequently maintained at 4 °C for 72 h ($n = 6$ per group). (a) Relative mRNA abundance of *Adgre1* in scWAT. (b, c) Representative images of H&E staining (b) and UCP1 IHC (c) of scWAT sections (6 images per mouse). Scale bars, 30 μ m. (d) Relative mRNA abundance of *Ucp1* in scWAT. Data are shown as mean \pm s.e.m., * $P < 0.05$; ** $P < 0.01$ by two-way ANOVA.

protein expression was significantly lower (Fig. 6a). Flow cytometry analysis showed an ~34% decrease in F4/80⁺CD11c⁺ cell number from LPS-treated *Ern1^{fl/fl}*; *Lyz2-Cre* BMDMs (Fig. 6b). LPS-induced upregulation of *Il6* mRNA was also significantly decreased in *Ern1^{fl/fl}*; *Lyz2-Cre* cells (Fig. 6c), in agreement with results from previous studies showing that IRE1 α increases the expression of proinflammatory cytokines in macrophages^{30,31}. IL-4 also stimulated IRE1 α phosphorylation, as detected by the antibody to phospho-IRE1 α or by phos-tag gel analysis (Fig. 6d and Supplementary Fig. 5c). By contrast, IL-4 treatment had no stimulatory effect on eIF2 α phosphorylation or BiP protein abundance (Fig. 6d and Supplementary Fig. 5d), thus suggesting that IL-4 also selectively activates the IRE1 α branch. In *Ern1^{fl/fl}*; *Lyz2-Cre* BMDMs, compared with *Ern1^{fl/fl}* BMDMs, IL-4 stimulation of Ym-1 protein was significantly elevated, but there were no changes in phosphorylation of the transcription factor STAT6 (Fig. 6d). Furthermore, IL-4-treated *Ern1^{fl/fl}*; *Lyz2-Cre* BMDMs had an ~46% increase in CD11b⁺CD206⁺ cells (Fig. 6e) and showed robustly enhanced expression of signature M2-marker genes (Fig. 6f). These results demonstrated that loss of IRE1 α was sufficient to promote IL-4 induction of M2 polarization while dampening LPS stimulation of M1 polarization.

We then sought to assess metabolic cross-talk between M2-polarized macrophages and beige adipocytes, and to determine whether such cross-talk might be influenced by IRE1 α ablation in macrophages. We used UCP1-expressing beige adipocytes differentiated from mouse preadipocytes³² (Fig. 6g) and cocultured them with IL-4-stimulated *Ern1^{fl/fl}*; *Lyz2-Cre* or *Ern1^{fl/fl}* BMDMs. Coculture with IL-4-treated

BMDMs significantly increased the expression of *Ucp1* and beige-adipocyte markers³³, including *Cd137* (official symbol *Tnfrsf9*), *Ear2* and *Tmem26*, in differentiated beige adipocytes (Fig. 6h); moreover, IRE1 α deficiency robustly enhanced the ability of IL-4-stimulated BMDMs to upregulate these signature genes of beige adipocytes (Fig. 6h). Thus, IRE1 α abrogation augments M2 polarization of ATMs and consequently enables more efficient WAT browning.

Next, to explore the mechanisms by which IRE1 α promotes M2 polarization, we performed global gene expression analysis by RNA-seq on IL-4-stimulated BMDMs. Of the 755 genes significantly upregulated (>1.25-fold, $P < 0.05$ by two-tailed rank product nonparametric method) by IL-4, ~11% were attenuated, and ~10% were further enhanced by IRE1 α ablation in *Ern1^{fl/fl}*; *Lyz2-Cre* BMDMs. In contrast, among the 797 genes significantly downregulated (>1.25-fold, $P < 0.05$), ~15% were attenuated, and ~13% were further suppressed (Supplementary Fig. 6a). Gene set enrichment analysis (GSEA) showed that IRE1 α abrogation resulted in alterations of multiple cellular-function and signaling pathways, including augmented IL-4 stimulation of oxidative phosphorylation, enhanced IL-4 suppression of the Notch pathway, and attenuated cytokine and chemokine signaling (Supplementary Fig. 6b). IRE1 α ablation resulted in changes in the expression of a diversity of IL-4-regulated potential secretory proteins, including cytokines, chemokines, growth factors and proteins implicated in tissue remodeling (Supplementary Fig. 6c). GSEA also revealed enhanced upregulation of most IL-4-induced M2-marker genes as a result of IRE1 α deficiency (Fig. 7a). Moreover, motif analysis identified a number of regulatory transcription factors involved in IL-4-induced gene expression programs, including the interferon regulatory factor (IRF) and Krüppel-like factor (KLF) family members, which were found to be influenced by IRE1 α ablation. Alignment of the expression heat maps indicated that IL-4 upregulated the expression of *Irf1*, *Irf4* and *Klf4*, whose expression was appreciably enhanced in *Ern1^{fl/fl}*; *Lyz2-Cre* cells (Fig. 7b). Given that both IRF4 and KLF4 have been shown to be the critical regulators of M2 polarization^{34–36}, we examined their expression by quantitative RT-PCR. IRE1 α ablation, which abolished IL-4-induced changes in *Xbp1* mRNA splicing and the expression of two RIDD-target genes²², *Pmp22* and *Hgsnat*, significantly augmented IL-4 induction of both *Irf4* and *Klf4* expression in BMDMs (Fig. 7c) but exerted no effect on IL-4 upregulation of *Pparg* or *Ppard*, two other key regulators of M2 activation^{37,38}. Therefore, the effects of IRE1 α deficiency on *Irf4* and *Klf4* expression mirrored the effects on the two RIDD targets examined.

To determine whether IRE1 α downregulated the expression of *Irf4* and *Klf4* through a mechanism dependent on its RNase activity, we treated BMDMs with 4 μ 8C, a pharmacologic inhibitor of IRE1 α 's RNase activity^{39,40}. Although IL-4 stimulated IRE1 α phosphorylation (Fig. 7d) as well as *Xbp1* mRNA splicing (Fig. 7e), treatment with 4 μ 8C further enhanced IRE1 α phosphorylation but effectively blocked *Xbp1* mRNA splicing (Fig. 7d,e) and increased IL-4 induction of Ym-1 protein expression without affecting STAT6 phosphorylation (Fig. 7d). In contrast to its inhibitory effect on IL-4-induced *Xbp1* mRNA splicing, 4 μ 8C significantly enhanced IL-4 induction of *Pmp22* and *Hgsnat* as well as *Irf4*, *Klf4* and other M2-marker genes (Fig. 7e). 4 μ 8C's augmentative effect on IL-4 upregulation of *Irf4*, *Klf4* and *Chil3* was abolished in *Ern1^{fl/fl}*; *Lyz2-Cre* BMDMs, thus indicating its specific action against IRE1 α (Fig. 7f). These data suggested that IRE1 α downregulates *Irf4* and *Klf4* expression and exerts its suppressive effect on M2 polarization, through a mechanism that requires its RNase activity but presumably not its *Xbp1* mRNA splicing activity. To further confirm the involvement of the XBP1 pathway, we tested the effect of ectopic expression of the spliced, transcriptionally active form, XBP1s.

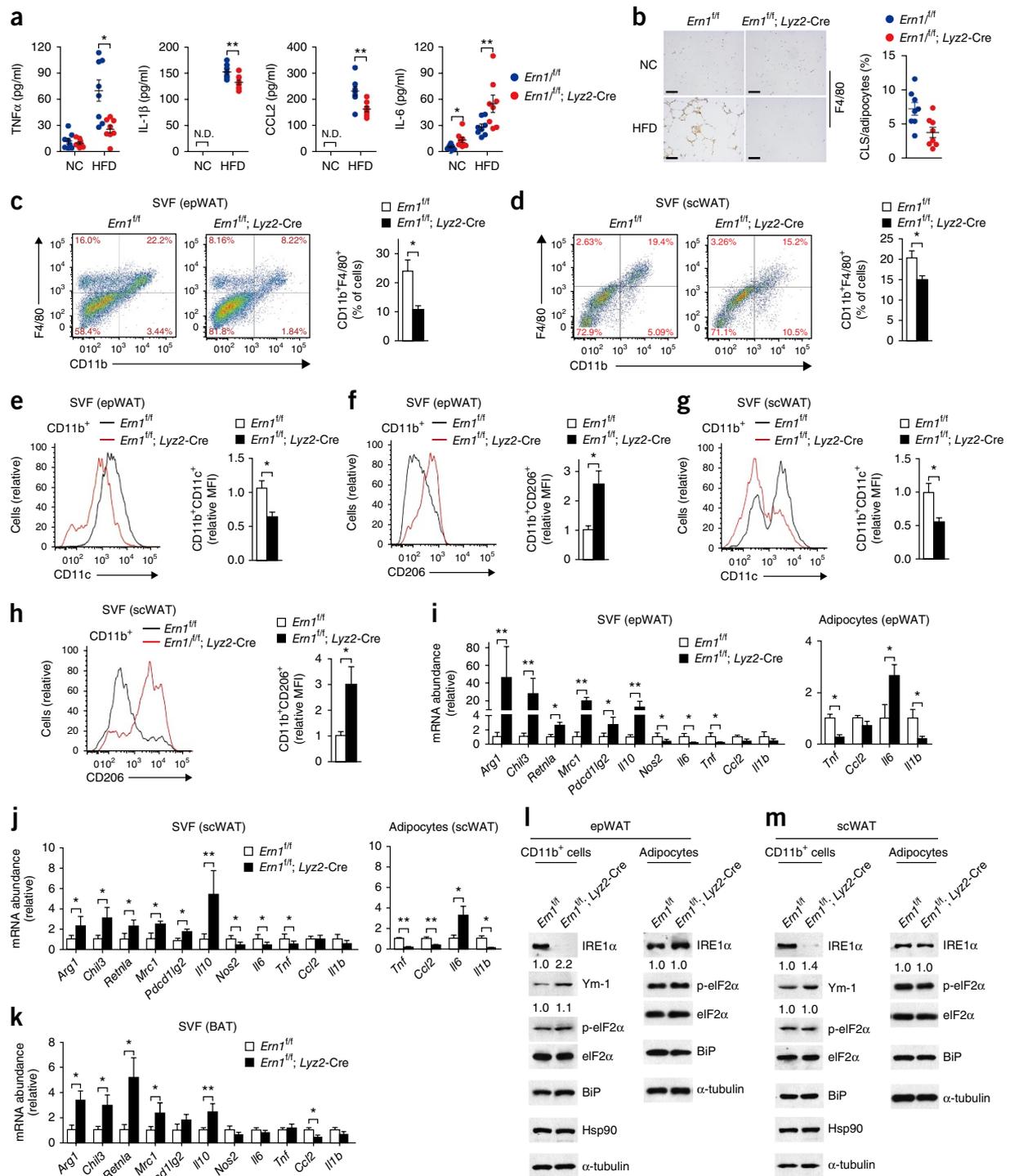


Figure 5 IRE1 α deficiency reverses the M1–M2 imbalance of ATMs. (a,b) *Em1^{fl/fl}*; *Lyz2-Cre* and *Em1^{fl/fl}* mice were fed an NC ($n = 8$ per group) or HFD ($n = 10$ per group) for 16 weeks. (a) Serum concentrations of the indicated cytokines. N.D., not detected. (b) F4/80⁺ crown-like structures (CLS) were quantified. (c–m) *Em1^{fl/fl}*; *Lyz2-Cre* and *Em1^{fl/fl}* mice were fed an HFD for 16 weeks (c–h, $n = 5$ per group; i–k, $n = 8$ per group; l, m, $n = 5$ per group). (c,d) Representative flow cytometry histograms from analysis of CD11b and F4/80 expression in SVFs of epWAT (c) and scWAT (d) ($n = 5$ histograms per group). Percentages of CD11b⁺F4/80⁺ cells are also shown. (e–h) Flow cytometry analyses of the expression of CD11c and CD206 from CD11b⁺ cells in SVFs of epWAT (e,f) and scWAT (g,h), respectively. Amounts of CD11b⁺CD11c⁺ and CD11b⁺CD206⁺ cells are quantified and presented as relative mean fluorescence intensity (MFI). (i–k) Quantitative RT–PCR analyses of the mRNA abundance of the indicated genes in SVFs and adipocytes from epWAT (i) and scWAT (j), and in SVFs from BAT (k). (l,m) Immunoblot analyses of Ym-1 and IRE1 α protein along with the indicated UPR markers in lysates of CD11b⁺ cells or adipocytes isolated from epWAT (l) and scWAT (m). α -tubulin is a loading control. Each lane represents a pooled sample from 5 mice analyzed in duplicate. Numerical values indicate the relative levels of Ym-1 protein and eIF2 α phosphorylation (p-eIF2 α /eIF2 α ratio). Data are shown as mean \pm s.e.m., * $P < 0.05$; ** $P < 0.01$ by two-tailed Student's t test or two-way ANOVA.

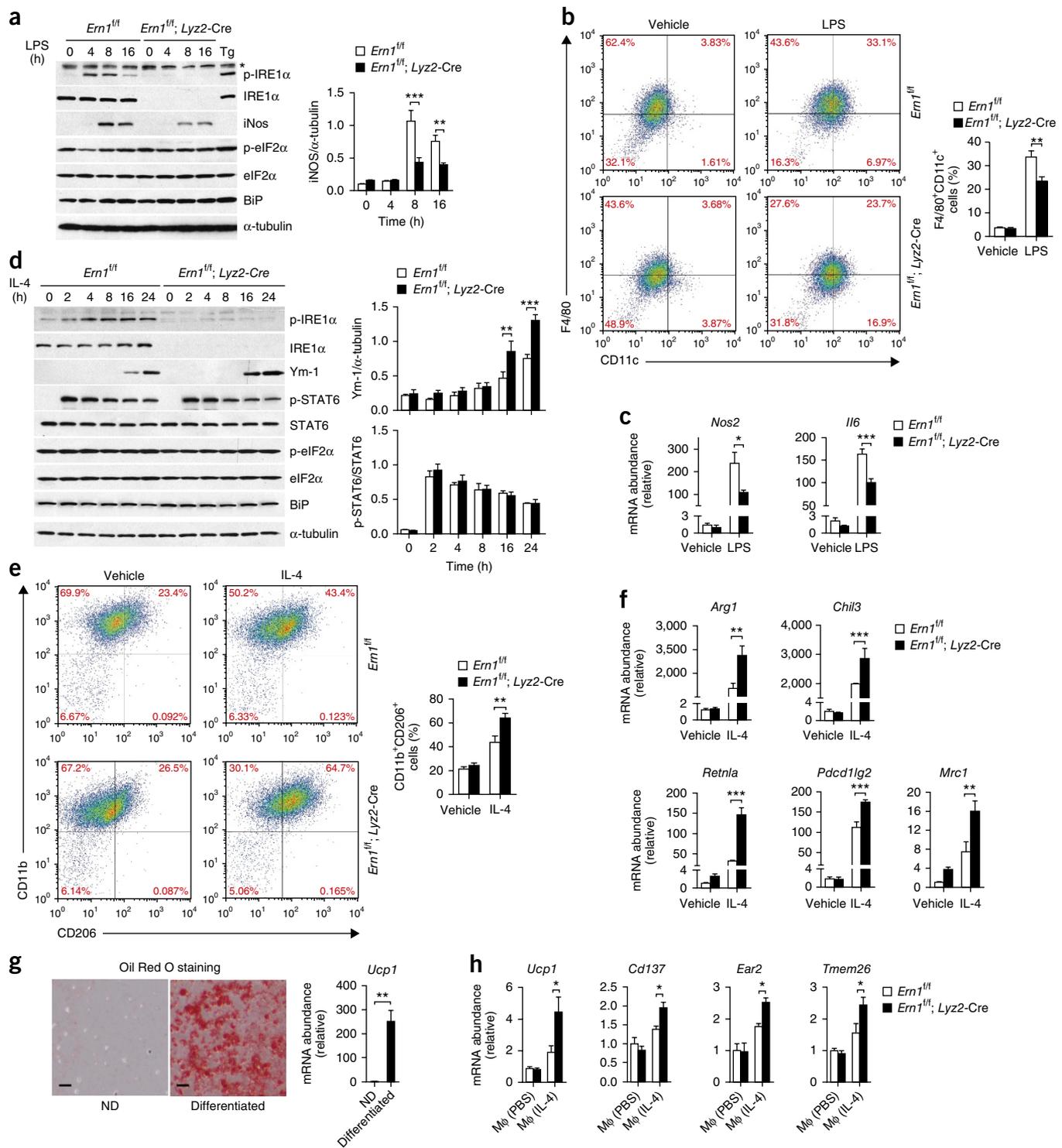


Figure 6 Abrogation of IRE1 α promotes M2 but decreases M1 polarization of macrophages. (a–f) BMDMs from *Ern1^{fl/fl}; Lyz2-Cre* mice and *Ern1^{fl/fl}* littermates were treated with 100 ng/ml LPS (a–c) or 20 ng/ml IL-4 (d–f) or vehicle control (3 and 4 independent experiments, respectively).

(a) Immunoblot analysis of IRE1 α and iNOS protein and the indicated UPR markers in cell lysates of LPS-treated BMDMs. Asterisk indicates a nonspecific band. Quantification of iNOS protein is shown. α -tubulin is a loading control. (b,c) BMDMs were treated with LPS for 24 h. (b) Flow cytometry analysis of the expression of F4/80 and CD11c. Percentages of F4/80⁺CD11c⁺ cells are shown. (c) Relative mRNA abundance of *Nos2* (iNos) and *Il6*. (d) Immunoblot analysis of cell lysates of IL-4-stimulated BMDMs. Quantification of Ym-1 protein and phosphorylated STAT6 is shown. (e,f) BMDMs were treated with IL-4 for 24 h. (e) Flow cytometry analysis of the expression of CD11b and CD206, and percentages of CD11b⁺CD206⁺ cells are shown. (f) Relative mRNA abundance of the indicated M2-marker genes. (g,h) Stimulation of beige adipocytes by IL-4-treated BMDMs *in vitro*. (g) Differentiation of beige adipocytes. Shown are images of cells stained with Oil Red O (Scale bars, 400 μ m) and the relative *Ucp1* mRNA abundance in nondifferentiated (ND) preadipocytes versus differentiated beige cells. (h) Relative mRNA abundance of *Ucp1* and the indicated beige adipocyte markers in differentiated cells after coculture with PBS- or IL-4-treated BMDMs from *Ern1^{fl/fl}; Lyz2-Cre* or *Ern1^{fl/fl}* mice. Results are presented as mean \pm s.e.m., * P < 0.05; ** P < 0.01; *** P < 0.001 by two-tailed Student's *t* test or two-way ANOVA.

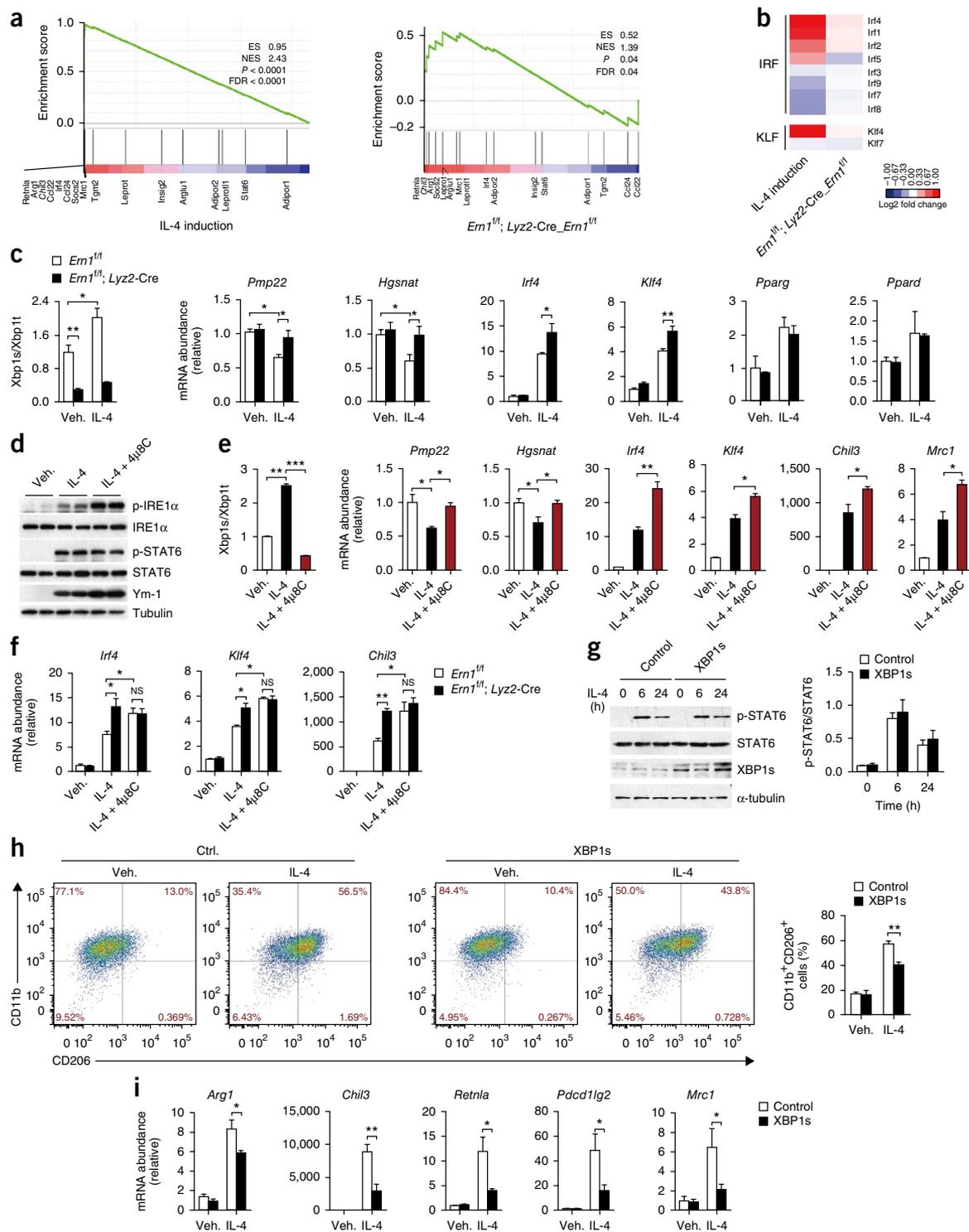


Figure 7 IRE1 α augments M2 polarization via an RNase-dependent mechanism. (a–c) *Ern1^{fl/fl}; Lyz2-Cre* and *Ern1^{fl/fl}* BMDMs were treated with 20 ng/ml IL-4 versus vehicle (veh.) for 24 h. (a,b) RNA-seq analysis (2 independent experiments). (a) Gene set enrichment analysis. Enrichment scores are shown for 16 M2 markers regulated by IL-4 (left) and those affected by IRE1 α deficiency under IL-4 stimulation (right). Genes are ranked according to their expression changes induced by IL-4 or resulting from IRE1 α deficiency. ES, enrichment score; NES, normalized enrichment score; FDR, false discovery rate. (b) Heat maps showing IL-4-induced changes in the expression of the indicated IRF and KLF family members, as aligned with those altered in *Ern1^{fl/fl}; Lyz2-Cre* relative to *Ern1^{fl/fl}* cells under IL-4 stimulation. (c) *Xbp1* mRNA splicing and the mRNA abundance of the indicated genes. (d,e) Mouse BMDMs were treated with vehicle, IL-4 or IL-4 plus 4 μ 8C at 1 μ M for 24 h. (d) Immunoblot analysis of IRE1 α and STAT6 phosphorylation and Ym-1 protein. (e) *Xbp1* mRNA splicing and the mRNA abundance of the indicated genes. (f) *Ern1^{fl/fl}; Lyz2-Cre* and *Ern1^{fl/fl}* BMDMs were stimulated with IL-4 in the absence or presence of 4 μ 8C. Relative mRNA abundance of *Irf4*, *Klf4* and *Chil3*. (g–i) BMDMs were infected by control or XBP1s-expressing lentiviruses and then treated with IL-4. (g) Immunoblot analysis and quantification of STAT6 phosphorylation. (h,i) Infected BMDMs were treated with IL-4 for 24 h. (h) Flow cytometry. Percentages of CD11b⁺CD206⁺ cells. (i) Relative mRNA abundance of the indicated genes. Results are mean \pm s.e.m. from 3 (c–g) or 4 (h–i) independent experiments. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; NS, not significant by one-way or two-way ANOVA.

Lentiviral overexpression of XBP1s in BMDMs did not affect IL-4 stimulation of STAT6 phosphorylation (Fig. 7g) or IL-4 induction of *Irf4* or *Klf4* (Supplementary Fig. 7a,b). However, flow cytometry of IL-4-stimulated, XBP1s-overexpressing BMDMs showed a significant decrease (by ~29%) in CD11b⁺CD206⁺ M2-like cells (Fig. 7h), along with robust decreases in IL-4 induction of signature M2 markers (Fig. 7i). Thus, XBP1s also contributes to IRE1 α -mediated suppression of M2 polarization; however, whether this effect is achieved through its transcriptional activity or other actions remains to be determined. These results suggest that the IRE1 α pathways, which are activated by IL-4 in macrophages, may constitute a negative feedback loop suppressing M2 polarization.

DISCUSSION

ER stress is thought to underlie the pathogenic progression of obesity, insulin resistance and type 2 diabetes^{41,42}, but the precise physiological mechanisms connecting ER stress to metabolic dysfunctions are poorly understood. Here, our studies uncovered a key role of the macrophage IRE1 α pathway in mediating metabolic ER stress-induced malfunction in both brown and beige fat, symptoms increasingly being recognized as a hallmark of the pathogenic development of obesity and metabolic syndrome^{4,24}. Our findings offer a new paradigm with respect to how metabolic ER stress disrupts glucose and energy metabolism: during overnutrition, obesogenic factors (for example, fatty acids and LPS) activate macrophage IRE1 α , thus defining an intracellular context repressing M2 while enhancing M1 polarization; such an M1–M2 imbalance of ATMs in turn severely limits the energy utilization capacity of both brown and beige fat in addition to promoting insulin resistance.

Our results demonstrated that abrogation of myeloid IRE1 α in mice results in a broad range of metabolically beneficial effects, particularly robust augmentation of the thermogenic activity of both brown and beige fat. These metabolic phenotypes are largely ascribable to the functional changes in ATMs, i.e., the enhanced M2 activation of resident macrophages in both BAT and scWAT, which have been reported to secrete catecholamines that drive adaptive thermogenesis^{13,14}. Indeed, we detected higher tissue content of norepinephrine in both BAT and scWAT from *Ern1*^{fl/fl}; *Lyz2*-Cre mice; however, we cannot exclude the possibility that macrophage IRE1 α deficiency might directly affect the biosynthesis and/or secretion of catecholamines within ATMs or indirectly influence catecholamine release by local neurons in adipose tissue. In addition, other macrophage-derived factors affected by IRE1 α ablation may also mediate the intercellular cross-talk between ATMs and adipocytes⁴³, thereby leading to more robust activation of brown and beige fat. For instance, it remains to be defined which IRE1 α -regulated secretory factors from M2-polarized ATMs may modulate the differentiation and thermogenic activation of beige adipocytes. Moreover, the augmentation of BAT activity and WAT browning as a result of the correction of the M1–M2 imbalance of ATMs in *Ern1*^{fl/fl}; *Lyz2*-Cre mice might directly contribute to improvements in insulin sensitivity and systemic glucose homeostasis in a manner independent of fat utilization²³. With regard to the mechanisms by which IRE1 α controls the shift in the M1–M2 polarization programs, it is likely that multiple regulatory modes exist, namely through IRE1 α 's RIDD activity as well as XBP1s-dependent actions.

Notably, IRE1 α was also partially ablated in neutrophils of *Ern1*^{fl/fl}; *Lyz2*-Cre mice. Although the contribution of neutrophil IRE1 α during adipose inflammation has yet to be clarified, our results clearly demonstrated that specific depletion of ATMs abolished the augmentative effects of myeloid IRE1 α deficiency on the browning of scWAT. This finding strongly suggests that IRE1 α ablation in macrophages,

rather than in neutrophils, should account for most of the observed enhancement in energy expenditure and metabolic improvements. This possibility is consistent with findings from a report showing that changes in adipose neutrophils have no effect on adiposity after HFD feeding⁴⁴. We also observed an unusual elevation of circulating IL-6, along with higher *Il6* expression in white adipocytes of *Ern1*^{fl/fl}; *Lyz2*-Cre mice. Given its demonstrated roles in activating brown and beige fat and in promoting M2 polarization of ATMs^{45,46}, IL-6 may also be an important contributor to augmenting adaptive thermogenesis via a feed-forward loop, thereby further enhancing M2 activation of ATMs in *Ern1*^{fl/fl}; *Lyz2*-Cre mice. Currently, how IRE1 α -deficient ATMs communicate with adipocytes in increasing *Il6* expression, while decreasing that of other proinflammatory cytokines, remains enigmatic.

In short, our findings revealed that the macrophage IRE1 α pathway is a crucial driver in metabolic ER stress and the promotion of energy imbalance and obesity. Blocking IRE1 α causes profound alterations in the functional activities of macrophages, which may range beyond the shifting of oversimplified M1–M2 polarization states²⁷. Nonetheless, targeted modulation of IRE1 α activity in ATMs might open new avenues for developing brown- and beige-fat-enhancing therapeutics to treat obesity and metabolic disease.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the [online version of the paper](#).

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

B. Shan, S.-Z.D. and Y.L. conceived and designed the studies. B. Shan, X.W. and Y.W. performed most of the experiments and analyzed the data. C.X. and J.-D.J.H. conducted the bioinformatics analysis. Z.X., J.D., M.S., S.H., F.Z., L.Y. and M.Z. performed some of the animal and cell experiments. F.N., J. Li, Jianmiao Liu, Jianfeng Liu, Y.Q., W.J., B. Song, S.-Z.D. and L.R. provided essential reagents and assisted with experimental design and data analysis. B. Shan and Y.L. wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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