

Rhythmic IL-17 production by $\gamma\delta$ T cells maintains adipose de novo lipogenesis

<https://doi.org/10.1038/s41586-024-08131-3>

Received: 26 May 2022

Accepted: 27 September 2024

Published online: 30 October 2024

Open access

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Aaron Douglas¹, Brenneth Stevens¹, Miguel Rendas², Harry Kane^{1,3}, Evan Lynch¹, Britta Kunkemoeller³, Karl Wessendorf-Rodriguez⁴, Emily A. Day¹, Caroline Sutton¹, Martin Brennan¹, Katie O'Brien¹, Ayano C. Kohlgruber³, Hannah Prendeville¹, Amanda E. Garza³, Luke A. J. O'Neill¹, Kingston H. G. Mills¹, Christian M. Metallo⁴, Henrique Veiga-Fernandes² & Lydia Lynch^{1,3,5,6}✉

The circadian rhythm of the immune system helps to protect against pathogens^{1–3}; however, the role of circadian rhythms in immune homeostasis is less well understood. Innate T cells are tissue-resident lymphocytes with key roles in tissue homeostasis^{4–7}. Here we use single-cell RNA sequencing, a molecular-clock reporter and genetic manipulations to show that innate IL-17-producing T cells—including $\gamma\delta$ T cells, invariant natural killer T cells and mucosal-associated invariant T cells—are enriched for molecular-clock genes compared with their IFN γ -producing counterparts. We reveal that IL-17-producing $\gamma\delta$ ($\gamma\delta 17$) T cells, in particular, rely on the molecular clock to maintain adipose tissue homeostasis, and exhibit a robust circadian rhythm for ROR γ t and IL-17A across adipose depots, which peaks at night. In mice, loss of the molecular clock in the CD45 compartment (*Bmal1* ^{Δ^{lav1}}) affects the production of IL-17 by adipose $\gamma\delta 17$ T cells, but not cytokine production by $\alpha\beta$ or IFN γ -producing $\gamma\delta$ ($\gamma\delta^{IFN\gamma}$) T cells. Circadian IL-17 is essential for de novo lipogenesis in adipose tissue, and mice with an adipocyte-specific deficiency in IL-17 receptor C (IL-17RC) have defects in de novo lipogenesis. Whole-body metabolic analysis in vivo shows that *Il17a*^{−/−}*Il17f*^{−/−} mice (which lack expression of IL-17A and IL-17F) have defects in their circadian rhythm for de novo lipogenesis, which results in disruptions to their whole-body metabolic rhythm and core-body-temperature rhythm. This study identifies a crucial role for IL-17 in whole-body metabolic homeostasis and shows that de novo lipogenesis is a major target of IL-17.

Circadian rhythms allow organisms to anticipate and adjust to predictable changes in the external environment, such as light⁸, temperature⁹ and feeding behaviour¹⁰. At the cellular level, circadian rhythms are dictated by the molecular clock, an auto-regulatory system of transcriptional activators (BMAL1 and CLOCK) and repressors (PER1–PER3, CRY1 and CRY2 and REV-ERB α and REV-ERB β), expressed by all mammalian cells¹¹. Cellular rhythms in a tissue are kept in synchrony by a combination of neuronal, hormonal and metabolic cues. These, in turn, give rise to circadian rhythms in physiological processes such as endocrine signalling¹², immune cell function¹ and systemic metabolism¹³.

Maintenance of core body temperature is one such physiological process, which oscillates daily over 24 h and is governed by metabolic rate. In mice, body temperature is highest at night while active and lowest during the day while inactive, and is important for optimal whole-body homeostasis¹⁴. White adipose tissue (WAT) and brown adipose tissue (BAT) are crucial for whole-body metabolic homeostasis, and are influenced by daily fluctuations in energy demands such as feeding, fasting and activity¹⁵. Light and feeding govern the circadian rhythm of adipose lipid metabolism, including lipolysis, lipogenesis and thermogenesis.

Lipolysis peaks during the inactive phase to maintain body temperature, whereas lipogenesis peaks during feeding for lipid uptake and storage from the diet^{16,17}. The molecular clock tightly regulates lipid metabolism in adipose tissue, and circadian disruption can lead to impaired lipid homeostasis and subsequent metabolic disorder. For example, circadian disruption, often observed in shift workers, can impair lipid homeostasis and increase the risk of metabolic disease¹⁸. Thus, it is important to understand how circadian rhythms interact, especially in the setting of metabolic disease.

Adipose tissue has a unique immune environment that is enriched for innate T cells, including $\gamma\delta$ T cells, mucosal-associated invariant T (MAIT) cells and invariant natural killer T (iNKT) cells^{4,5}. The adipose tissue immune system is crucial for maintaining homeostasis, and holds the potential for treating metabolic disease^{6,7}. Cytokines, including TNF¹⁹, IL-6 (ref. 20), IL-17A²¹ and IL-17F²², from adipose immune cells regulate local homeostasis and systemic metabolic processes. We have previously found that clonal IL-17-producing V $\gamma 6^+$ $\gamma\delta$ T cells reside in adipose tissue, and that mice lacking $\gamma\delta$ T cells or IL-17A are susceptible to hypothermia²¹. A subsequent study also showed that

¹School of Biochemistry and Immunology, Trinity Biomedical Science Institute, Trinity College Dublin, Dublin, Ireland. ²Champalimaud Research, Champalimaud Centre for the Unknown, Lisbon, Portugal. ³Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA. ⁴Molecular and Cell Biology Laboratory, Salk Institute for Biological Studies, La Jolla, CA, USA.

⁵Molecular Biology, Princeton University, Princeton, NJ, USA. ⁶Ludwig Cancer Research Institute, Princeton Branch, Princeton University, Princeton, NJ, USA. ✉e-mail: lydiaylynch@princeton.edu

IL-17RC signalling in adipocytes was important for thermogenesis²². These studies highlight members of the IL-17 family as key mediators of adipose thermogenesis.

The molecular clock regulates both T helper 17 (T_H17) cells and type-3 innate lymphoid cells (ILC3s) in the gut, which produce IL-17 and IL-22 (refs. 23,24). Here we investigated whether type-17 innate T cells are also regulated by the molecular clock. We show that type-17 innate T cells (γδ17, MAIT17 and NKT17) are enriched for molecular-clock genes, compared with type-1 innate T cells (γδ^{IFNγ}, MAIT1 and NKT1), particularly in adipose tissue, and that this mediates the circadian fluctuation of RORγt and IL-17A, which peaks at night. We identify adipose de novo lipogenesis (DNL) as a major target of diurnal IL-17 production in adipose tissue. Furthermore, loss of IL-17 signalling in adipocytes disrupts circadian DNL, ultimately impairing systemic metabolic rhythm and circadian body-temperature regulation. We identify a crucial role for adipose IL-17, which is controlled by the molecular clock. In turn, IL-17 regulates circadian physiological processes to support whole-body metabolism. This pathway could provide a therapeutic target for metabolic disease.

Molecular-clock enrichment in innate IL-17⁺ T cells

Adaptive CD4⁺ T_H17 cells and innate gut ILC3s, which produce IL-17, express a rhythmic molecular clock^{23–26}. Whether this is a specific trait of type-17 cells, or a trait of innate T cells, remains unknown. We performed single-cell RNA sequencing (scRNA-seq) in innate T cells from epididymal adipose tissue (epiWAT), and compiled publicly available scRNA-seq data on innate T cells from the lungs, spleen, thymus, skin and lymph nodes of wild-type mice, including iNKT, MAIT and γδ T cells (Fig. 1a). Notably, uniform manifold approximation and projection (UMAP) analysis first separated innate T cells into IL-17-producing (type-17) or IFNγ-producing (type-1) cells, and then separated on the basis of tissue origin and cell subtype (Fig. 1a,b). This highlights the distinct transcriptional program of T_H17-like cells, regardless of tissue or cell type. We then generated a ‘molecular-clock score’ using the average expression of the molecular-clock genes *Arntl* (also known as *Bmal1*), *Clock*, *Nr1d1*, *Nr1d2*, *Cry1*, *Cry2*, *Per1*, *Per2*, *Per3* and *Rora* (Fig. 1c and Extended Data Fig. 1a), and found enriched expression in type-17 innate T cells compared with their type-1 counterparts (Fig. 1d). In addition, tissue-resident innate T cells (adipose and lung) had higher expression of molecular-clock genes than innate T cells in lymphoid organs (spleen, lymph nodes and thymus; Fig. 1d and Extended Data Fig. 1b).

To account for time-of-day variation in the scRNA-seq datasets, we analysed each dataset separately for in-run comparisons, comparing innate T cells collected from the same mice at the same time of day only (Fig. 1e). Regardless of the time at which the sequencing was run, type-17 innate T cells had higher expression of molecular-clock genes (Fig. 1e and Extended Data Fig. 1c–g), with adipose γδ T cells showing the highest expression overall (Fig. 1f). Therefore, to investigate the role of the molecular clock in innate type-17 cells, we focused on adipose γδ17 T cells. To confirm that γδ T cells have rhythmic expression of circadian clock genes, we isolated adipose γδ17 T cells across 24 h, and found robust circadian expression of *Nr1d1*, which peaked before the active phase (Fig. 1g). Next, we used Venus reporter mice to observe *Per1* oscillations (*Per1*^{Venus}; Fig. 1h) in adipose T cells over 24 h. Adipose γδ T cells have rhythmic expression of *Per1*, with peak expression as the mouse dark cycle is entered (Fig. 1i–k), which was not observed in adipose αβ T cells (Fig. 1i–k). In addition, adipose γδ17 T cells had higher expression of *Per1* than adipose αβ T cells in all depots throughout the entire 24-h period, providing further evidence for higher levels of molecular-clock expression in innate type-17 adipose T cells (Fig. 1i–k).

The molecular clock regulates γδ IL-17A

Having established molecular-clock enrichment in γδ T cells, we investigated whether the production of IL-17A and its transcriptional

regulator RORγt exhibit a circadian rhythm over 24 h. We first measured the circadian production of IFNγ and IL-17A ex vivo by collecting lymph-node γδ T cells at zeitgeber time (ZT) 0, then splitting them into eight groups for stimulation at 3-h intervals over 24 h (Extended Data Fig. 2a). Ex vivo stimulated γδ17 T cells had a robust circadian rhythm for IL-17A production, which peaked during the night (Extended Data Fig. 2b–e). By contrast, γδ^{IFNγ} T cells had an opposing diurnal rhythm that peaked during the day (Extended Data Fig. 2f–i). Previous studies on CD4⁺ T cells showed that NFIL3 antagonizes IL-17 circadian rhythm²³. NFIL3 was expressed almost exclusively in γδ17 T cells, not in γδ^{IFNγ} T cells, and exhibited a circadian rhythm in γδ17 T cells (Extended Data Fig. 2j–l). Although lymph-node γδ17 T cells express higher levels of molecular-clock genes than γδ^{IFNγ} T cells, adipose tissue γδ17 T cells have the highest expression of these genes (Fig. 1d,f). Therefore, we investigated whether adipose γδ17 T cells expressed a circadian rhythm for IL-17A. There was no change in the total number of γδ17 T cells over a 24-h period (Extended Data Fig. 3a,b). Although adipose γδ17 T cells exhibit a circadian rhythm to their proportions, no rhythm was observed in absolute cell number (Extended Data Fig. 3a,b), suggesting that the fluctuations in proportions are due to changes in circulating γδ^{IFNγ} T cells, which are higher in the blood. We found a robust circadian rhythm for IL-17A production, and RORγt expression by adipose γδ17 T cells (Fig. 2a–c). Together, these data show that γδ17 T cells have a circadian rhythm for IL-17A production, which can be maintained intrinsically for at least 24 h ex vivo.

To determine the influence of the molecular clock on IL-17A expression by adipose γδ17 T cells, we used SR9009, a REV-ERBα agonist that inhibits RORγt-mediated transcription by competing for DNA-binding sites²⁷. We observed that SR9009 has no effect on RORγt expression by adipose or lymph-node γδ17 T cells (Fig. 2d,e and Extended Data Fig. 3e–g). However, SR9009 completely inhibited IL-17A and IL-17F expression by adipose γδ17 T cells (Fig. 2f–i). SR9009 did not affect lymph-node lymphocyte viability or γδ17 T cell proportions (Extended Data Fig. 3c,d), but it caused a dose-dependent decrease in the production of IL-17A and IL-17F by lymph-node γδ17 T cells (Extended Data Fig. 3h–m). These data demonstrate the molecular-clock modulation of IL-17A expression by γδ17 T cells, particularly in adipose tissue.

To further investigate the role of the molecular clock in IL-17A expression, mice were generated with a specific deletion of *Arntl* in CD45-expressing cells (*Arntl*^{ΔVav1} mice). We found that deletion of *Arntl* decreased the percentage and number of γδ T cells in mesenteric adipose tissue (mesWAT) and epiWAT (Fig. 2j,k), but did not have this effect in γδ intraepithelial lymphocytes (IELs) (Extended Data Fig. 3n). Deletion of *Arntl* in CD45⁺ cells also did not affect the proportion or number of adipose αβ T cells, or IELs (Fig. 2j,l and Extended Data Fig. 3o). Notably, loss of *Arntl* increased both the proportion and the cell number of adipose IL-17A⁺ γδ T cells, compared with *Arntl*^{fl} controls (Fig. 2m–o), but had no effect on IL-17A or IFNγ expression in adipose αβ T cells (Fig. 2p,q). γδ17 T cells have the highest expression of IL-7R in adipose tissue (Extended Data Fig. 3p), so we specifically deleted *Arntl* in IL-7R-expressing cells (*Arntl*^{ΔIL7R} mice). Similarly to the *Arntl*^{ΔVav1} mice, there were decreased levels of adipose γδ T cells in the *Arntl*^{ΔIL7R} mice, but no difference in the lungs and spleen (Extended Data Fig. 3q). Again, adipose γδ T cells lacking *Arntl* had increased IL-17A expression (Extended Data Fig. 3r). These data suggest that the molecular clock is a major regulator of IL-17A and IL-17F, whereby activation of *Rev-erba* (also known as *Nr1d1*) decreases IL-17 expression, whereas loss of *Bmal1* (*Arntl*) increases it. Together, these data show that the molecular clock regulates the homeostatic function of adipose γδ17 T cells.

Environmental factors regulate γδ IL-17A

Light is a major entrainment signal for the circadian regulation of whole-body metabolic rhythm. Therefore, we investigated whether

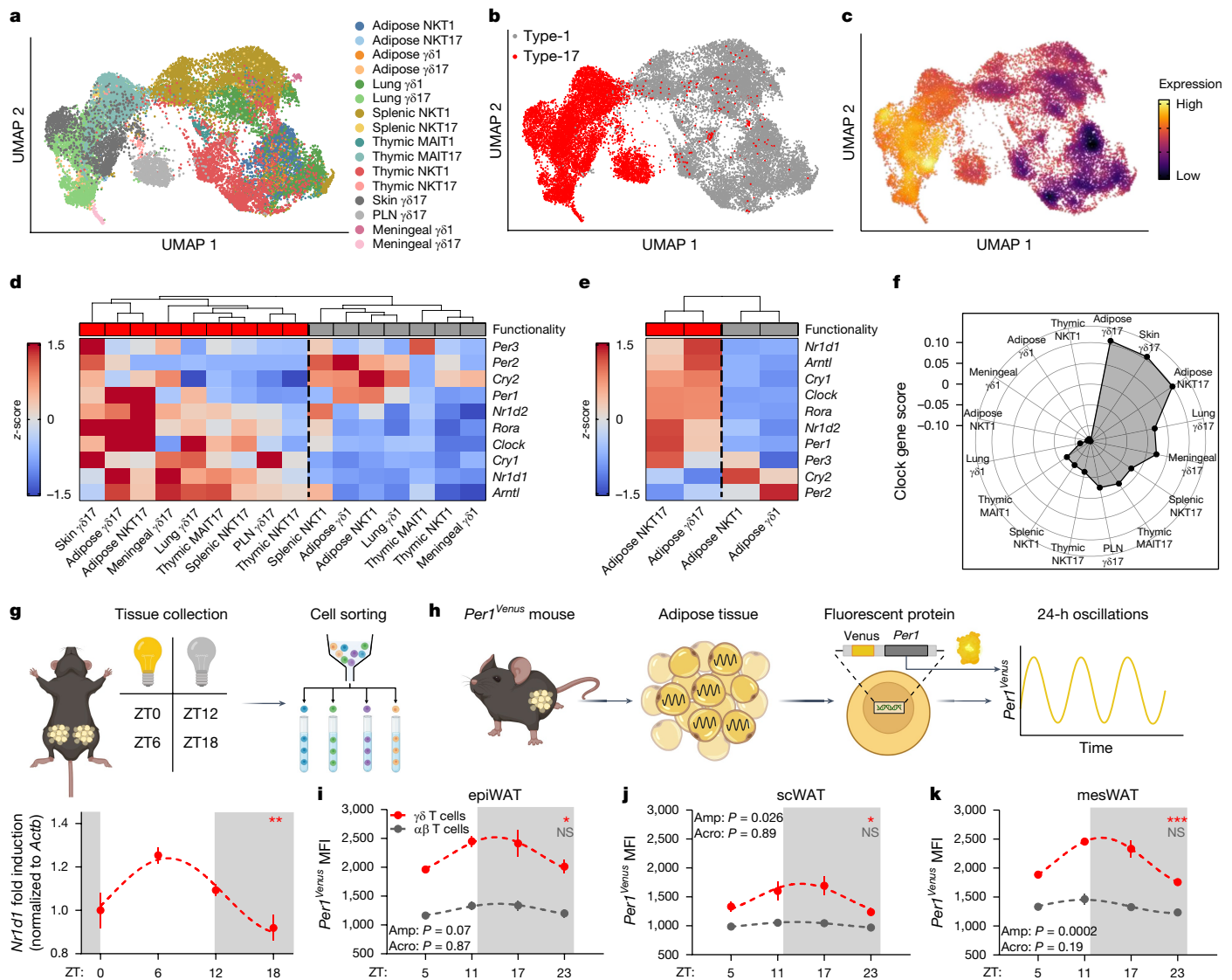


Fig. 1 | Innate IL-17⁺ T cells are enriched for molecular-clock genes. **a**, UMAP of mouse innate T cells across several organs, reanalysed from GSE142845, GSE141895, E-MTAB-7704, E-MTAB-8732, GSE123400 and GSE147262 (all publicly available scRNA-seq datasets). PLN, peripheral lymph node. **b**, UMAP of mouse innate T cells subclustered on the basis of functionality (type 1 (T_H1 -like) or type 17 (T_H17 -like)). **c**, Density plot of the cumulative expression of all molecular-clock genes across innate T cell subsets, designated the molecular-clock score. **d**, Heat map of the averaged gene expression of molecular-clock genes with hierarchical clustering of data from **a**. **e**, Heat map of the averaged gene expression of molecular-clock genes from adipose tissue innate lymphocytes with hierarchical clustering of data generated in this laboratory. **f**, Spider plot showing that adipose $\gamma\delta 17$ T cells have the highest cumulative expression of

molecular-clock genes. **g**, Top, diagram of the isolation and PCR analysis of adipose $\gamma\delta$ T cells. Bottom, relative expression of *Rev-erba* (also known as *Nr1d1*) in epiWAT $\gamma\delta$ T cells across 24 h. **h**, Diagram of *Per1*^{Venus} oscillations in cells isolated from adipose tissue. **i–k**, Circadian time plots of *Per1*^{Venus} expression by $\alpha\beta$ (grey) and $\gamma\delta$ (red) T cells across 24 h in epiWAT (**i**), scWAT (**j**) and mesWAT (**k**). MFI, mean fluorescence intensity. **g, i–k**, White and grey panels represent light and dark periods, respectively. **i–k**, Data are representative of two independent experiments. **g, i–k**, Data are mean $\pm$ s.e.m., $n = 4$ –5 mice per group. **g, i–k**, Significance was calculated using cosinor analysis, with cosine fitted curves; amplitude (amp) and acrophase (acro) were extracted from the cosinor model. NS, not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

light is an entrainment signal for circadian IL-17A production by adipose $\gamma\delta$ T cells. Using metabolic cages, mice were housed under normal light conditions (lights on 07:00–19:00; control) or on a reversed light–dark cycle (lights on 19:00–07:00 for three weeks; Fig. 3a). After three weeks of inverted light, energy expenditure and metabolic rate rhythms were completely reversed (Fig. 3b,d and Extended Data Fig. 4a). Adipose tissue was then collected from both groups at ZT0, ZT6, ZT12 and ZT18 (Fig. 3a). IL-17A production by adipose $\gamma\delta 17$ T cells had a circadian rhythm, which was reversed in the light-inverted group (Fig. 3g). Thus, light is a major entrainment signal for IL-17A expression by $\gamma\delta 17$ T cells.

Previous studies have shown an increase in circulating IL-17A in both mice and humans after eating²⁸. Adipose tissue is sensitive to

feeding behaviour; it expands and contracts during feeding (active) and fasting (inactive) periods, respectively²⁹. A caveat of reversing the light cycle of mice is that the feeding cycle is also reversed (Fig. 3e,f); thus, we investigated whether the adipose IL-17A rhythm was entrained by light as a result of reverse feeding. Mice were placed into metabolic cages under normal light conditions but with reversed feeding schedules, with access to food from 07:00–19:00 (reverse feeding), or from 19:00–07:00 (control) for three weeks (Fig. 3h). Of note, energy expenditure and metabolic rate rhythms did not completely reverse, but rather, were disrupted in reverse-fed mice compared with controls (Fig. 3i–k and Extended Data Fig. 4b). Furthermore, reverse-fed mice had disrupted circadian expression of

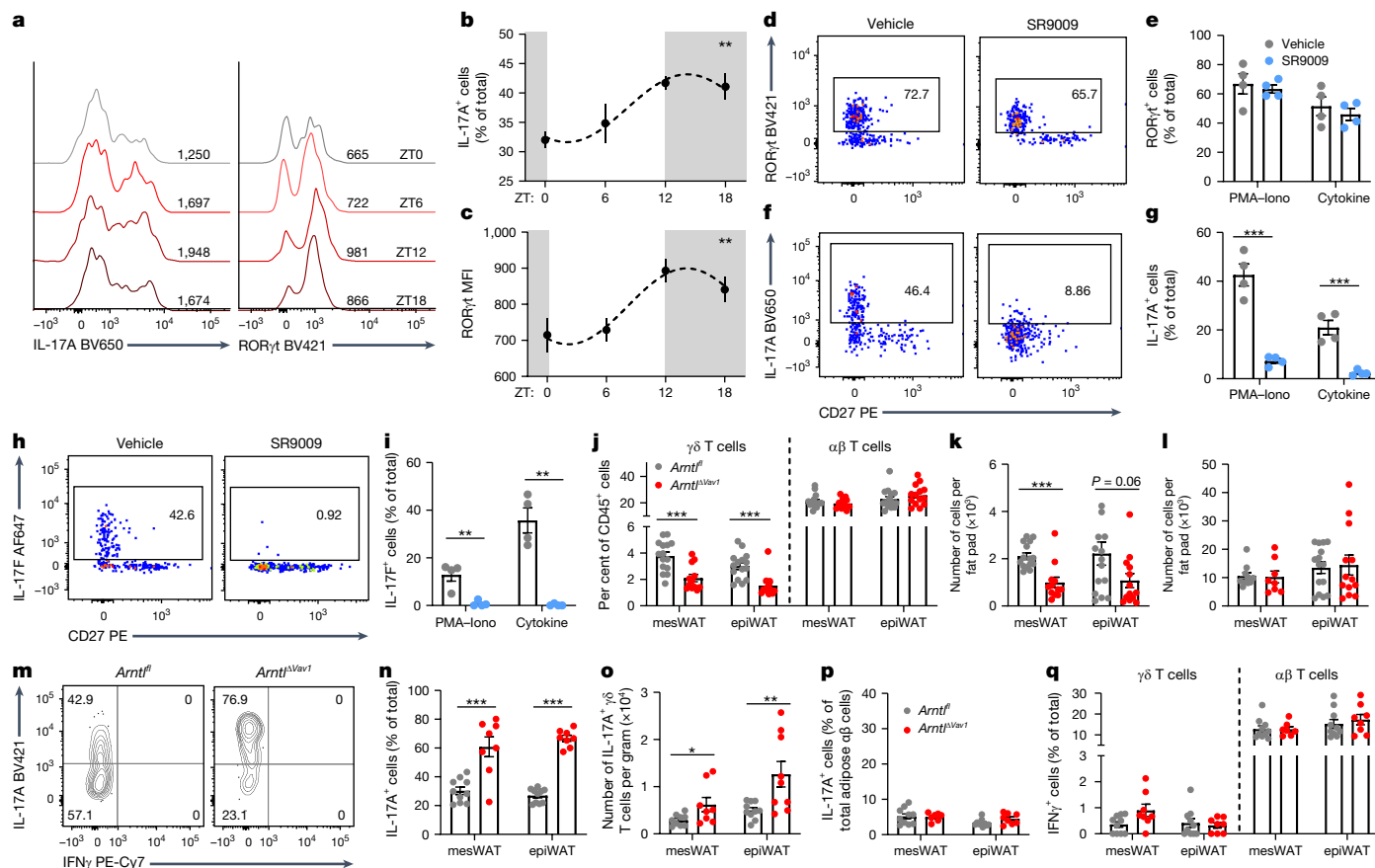


Fig. 2 | IL-17A expression by adipose $\gamma\delta$ T cells is regulated by the molecular clock. **a**, IL-17A and ROR γ t histograms from adipose (epiWAT) $\gamma\delta$ T cells across 24 h. **b**, Percentage of IL-17A $^{+}$ cells. **c**, ROR γ t MFI. **d**, ROR γ t flow plots from adipose (epiWAT) $\gamma\delta$ T cells stimulated with PMA–ionomycin and 40 μ M SR9009. **e**, Percentage of ROR γ t $^{+}$ cells. **f**, IL-17A flow plots from adipose (epiWAT) $\gamma\delta$ T cells stimulated with PMA–ionomycin and 40 μ M SR9009. **g**, Percentage of IL-17A $^{+}$ cells. **h**, IL-17F flow plots from adipose (epiWAT) $\gamma\delta$ T cells stimulated with IL-1 β , IL-23 and 40 μ M SR9009. **i**, Percentage of IL-17F $^{+}$ cells. **j**, Percentage of $\gamma\delta$ T cells and $\alpha\beta$ T cells among total lymphocytes in *Arntl*^{fl/fl} (grey) and *Arntl* ^{Δ Vav1} (red) mice. **k**, Absolute cell numbers of $\gamma\delta$ (k) and $\alpha\beta$ (l) T cells in adipose tissue of *Arntl*^{fl/fl} (grey) and *Arntl* ^{Δ Vav1} (red) mice. **m**, IL-17A flow plots from

Arntl^{fl/fl} and *Arntl* ^{Δ Vav1} adipose $\gamma\delta$ T cells. **n**, Percentage of IL-17A $^{+}$ cells. **o**, IL-17A $^{+}$ $\gamma\delta$ T cell numbers per gram of adipose tissue in *Arntl*^{fl/fl} (grey) and *Arntl* ^{Δ Vav1} (red) mice. **p**, IL-17A $^{+}$ cells as a percentage of the total adipose $\alpha\beta$ T cells. **q**, IFN γ expression by adipose $\alpha\beta$ and $\gamma\delta$ T cells from *Arntl*^{fl/fl} (grey) and *Arntl* ^{Δ Vav1} (red) mice. **b, c**, White and grey panels represent light and dark periods, respectively. Data are representative of three (**a–c, j–q**) or two (**d–i**) independent experiments. **b, c, e, g, i–l, n–q**, Data are mean $\pm$ s.e.m., $n = 4–15$ mice per group. **b, c**, Significance was calculated using cosinor analysis, with cosine fitted curves, **e, g, i–l, n–q**, Two-tailed unpaired student's *t*-test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

IL-17A by adipose $\gamma\delta$ T cells, with a decreased amplitude and an altered acrophase compared with controls (Fig. 3n), suggesting that adipose IL-17 is linked with feeding. However, reverse-fed mice consumed approximately 50% fewer calories than the control mice did (Fig. 3l,m), which could have affected adipose IL-17 production. To investigate whether the disruption to IL-17A expression was due to decreased food intake or the time of feeding, mice were fed ad libitum or fed a 50% calorie-restricted diet (50% CR), reducing the amount of food consumed to match the amount of food eaten by the reverse-fed mice (Fig. 3o). Although the calorie-restricted diet decreased energy expenditure and metabolic rate at all times of day, compared with the ab-libitum-fed mice, the calorie-restricted group maintained the rhythm (amplitude) of energy expenditure and metabolic rate (Fig. 3p,q and Extended Data Fig. 4c,d). Likewise, calorie restriction reduced IL-17 expression (Fig. 3r,s), but did not affect the rhythm of IL-17 production (Fig. 3r,s). Comparing the circadian rhythm of energy expenditure in calorie-restricted and reverse-fed mice, both consuming the same amount of food, revealed differences in amplitude and acrophase between the feeding regimens (Fig. 3t). Furthermore, this was associated with a loss of IL-17 rhythm by adipose $\gamma\delta$ T cells only in the reverse-fed group (Fig. 3u). Together, these data suggest that light entrains the production of IL-17A by adipose $\gamma\delta$ T cells, and that this

effect occurs mostly through feeding behaviour, because disrupted feeding behaviour disturbs IL-17A expression in adipose $\gamma\delta$ T cells.

A high-fat diet disrupts circadian IL-17A expression

Mice exhibit a circadian rhythm in fuel use, switching between carbohydrate and lipid burning across dark and light cycles. This can be measured in metabolic cages by the respiratory exchange ratio (RER), in which 0.7 indicates lipid burning and 1 indicates carbohydrate use. Switching to a 60% high-fat diet (HFD) for 3 weeks disrupts the RER rhythm, causing mice to use lipids throughout both light and dark cycles, as previously described³⁰ (Fig. 3v–x). In addition, a one-week HFD was sufficient to disrupt the normal feeding rhythm of mice; it caused them to feed more during the day, indicating increased grazing, as previously reported^{31,32} (Fig. 3y and Extended Data Fig. 4e). We found that a three-week HFD increased the expression of IL-17A and ROR γ t by adipose $\gamma\delta$ T cells throughout 24 h, disrupting the IL-17A rhythm (Fig. 3z and Extended Data Fig. 4f). Inhibition of the IL-17A signalling axis in adipose tissue has been shown to prevent diet-induced obesity³³. We also found that *Il17a*^{fl/fl} mice were resistant to weight gain after 16 weeks on a HFD (Extended Data Fig. 4g,h). Together, these results suggest that an increase in caloric intake throughout 24 h by HFD feeding

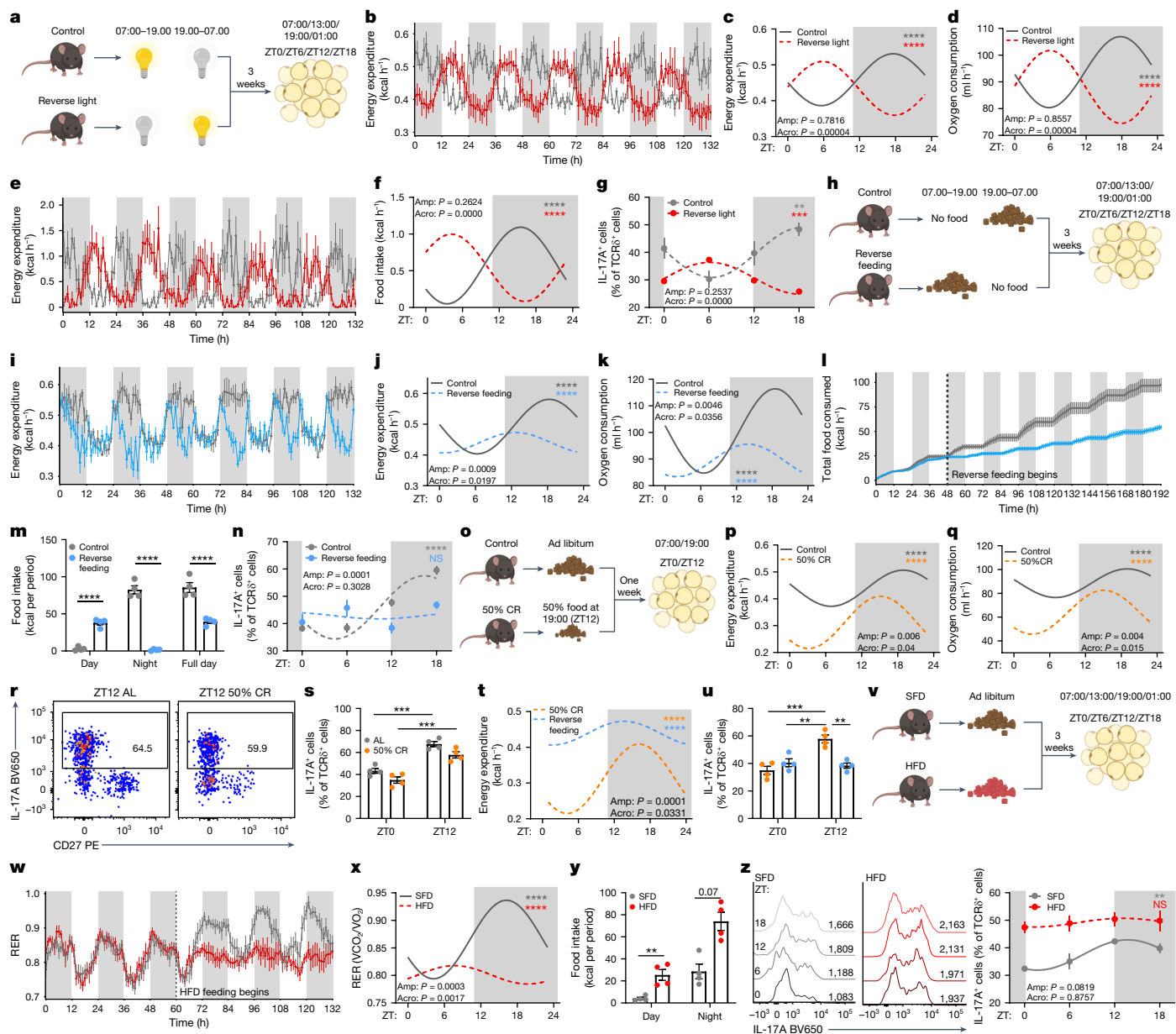


Fig. 3 | Light entrains whole-body metabolism and IL-17A production by adipose $\gamma\delta$ T cells. **a**, Reverse-light-cycle experimental set-up. **b**, Energy expenditure of regular (grey) and inverted-light-cycle (red) mice after three weeks. **c**, **d**, Circadian plots of energy expenditure (**c**) and oxygen consumption (**d**). **e**, Food consumption of regular (grey) and inverted-light-cycle (red) mice after three weeks. **f**, Circadian plot of food consumption. **g**, Percentage of IL-17A⁺ cells among adipose $\gamma\delta$ T cells of normal (grey) and reverse-light-cycle (red) mice over 24 h. **h**, Reverse-feeding experimental set-up. **i**, Energy expenditure of regular (grey) and reverse-fed (blue) mice after three weeks. **j**, **k**, Circadian plots of energy expenditure (**j**) and oxygen consumption (**k**). **l**, Cumulative food consumption of regular (grey) and reverse-fed (blue) mice after three weeks. **m**, Averaged food consumption. **n**, Percentage of IL-17A⁺ cells among adipose $\gamma\delta$ T cells of regular (grey) and reverse-fed (blue) mice over 24 h. **o**, Experimental set-up for 50% CR feeding. **p**, **q**, Energy expenditure (**p**) and oxygen consumption (**q**) of ad-libitum-fed (grey) and 50%-CR-fed (orange) mice over 24 h. **r**, IL-17A flow plots of adipose $\gamma\delta$

T cells from mice fed ad libitum (AL) and mice fed a 50% CR diet. **s**, Percentage of IL-17A⁺ cells. **t**, Energy expenditure from 50%-CR-fed (orange) and reverse-fed (blue) mice over 24 h. **u**, Percentage of IL-17A⁺ cells among adipose $\gamma\delta$ T cells. **v**, HFD experimental set-up. **w**, RER SFD-fed (grey) and HFD-fed (red) mice after three weeks. **x**, Circadian plot of RER. **y**, Bar plot of food intake. **z**, Left, IL-17A histograms of adipose $\gamma\delta$ T cells from SFD-fed and HFD-fed mice. Right, percentage of IL-17A⁺ cells. White and grey panels represent light and dark periods, respectively. **a–u**, Data are representative of three independent experiments; **w–z**, two independent experiments. **c**, **d**, **f**, **j**, **k**, **p**, **q**, **t**, **x**, Data are mean; **b**, **e**, **g**, **i**, **l**, **n**, **s**, **u**, **w**, **y**, **z**, Data are mean $\pm$ s.e.m.; $n = 4–6$ mice per group. **b–g**, **i**, **l**, **n**, **p**, **q**, **t**, **x**, **z**, Significance was calculated using cosinor analysis, with cosine fitted curves; amplitude (amp) and acrophase (acro) were extracted from the cosinor model. **m**, Two-group analysis with ANCOVA. **s**, **u**, Two-way ANOVA. **y**, Two-tailed unpaired student's *t*-test. NS, not significant, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

increases the expression of IL-17A by adipose $\gamma\delta$ T cells, and that loss of IL-17 signalling prevents diet-induced weight gain.

Previous studies have shown that IL-17A signalling has a pathogenic role in neurodegenerative disease, including multiple sclerosis³⁴. In the mouse model of experimental autoimmune encephalomyelitis (EAE),

IL-17A produced by $\gamma\delta$ T cells has a pathological role in the development and onset of EAE³⁴. We examined the pathological role of disrupted IL-17A from a HFD feeding regimen using the EAE mouse model. HFD-fed mice had faster disease progression, compared with controls that were fed a standard-fat diet (SFD) (Extended Data Fig. 5a–d).

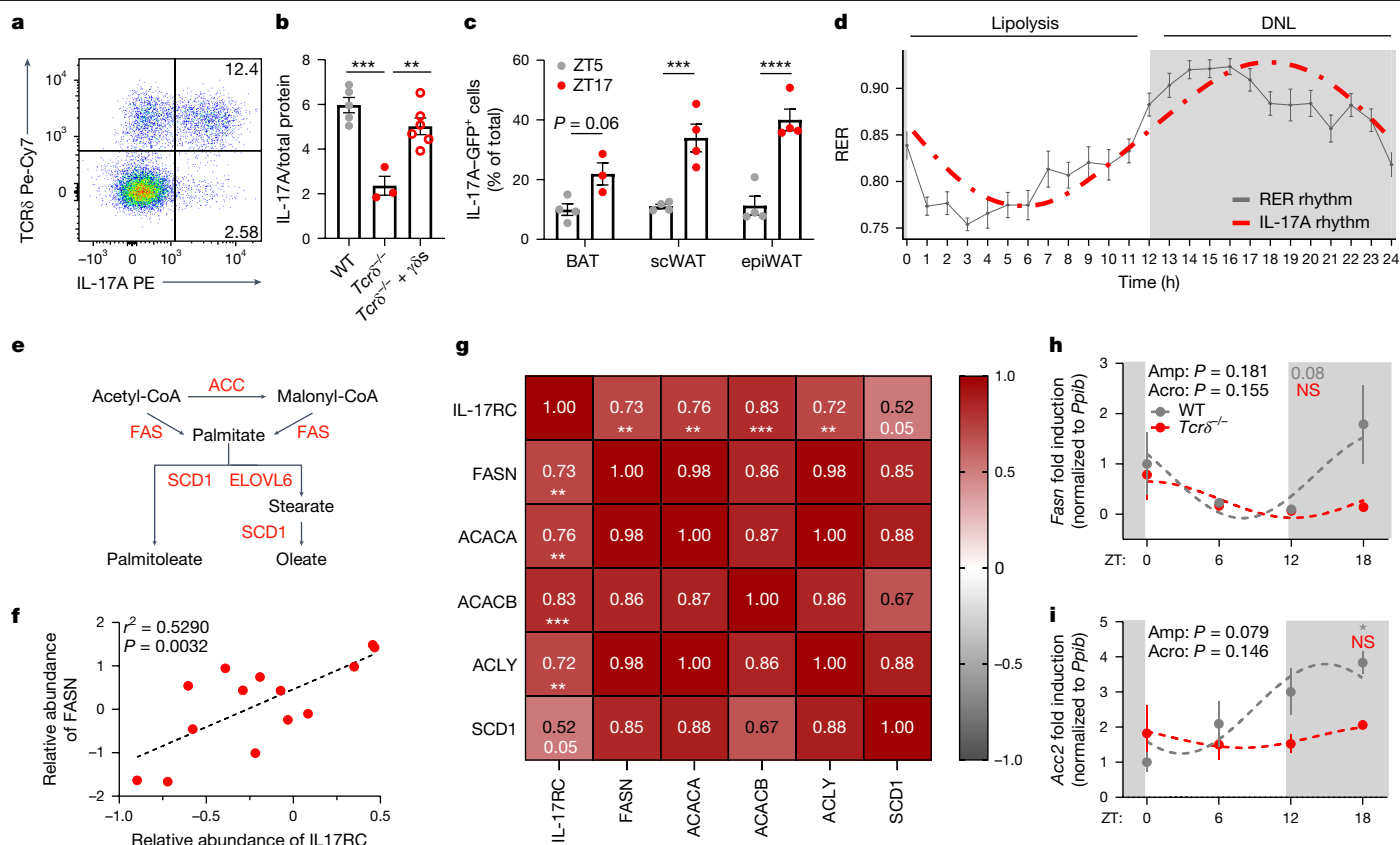


Fig. 4 | $\gamma\delta$ T cells are the main source of adipose tissue IL-17A, and maintain adipose tissue DNL. **a**, Representative flow plot showing that $\gamma\delta$ T cells are the main producer of IL-17A in adipose tissue. **b**, Adipose IL-17A protein levels by enzyme-linked immunosorbent assay (ELISA) from wild-type (WT) mice (grey), *Tcrδ*^{-/-} mice (red) and *Tcrδ*^{-/-} mice reconstituted with wild-type $\gamma\delta$ T cells ($\gamma\delta$ s; open circles). **c**, Diurnal expression of IL-17A-GFP by $\gamma\delta$ 17 T cells across the adipose tissue depots BAT, scWAT and epiWAT. **d**, Diagrammatic representation of circadian whole-body metabolism by RER, showing the most active metabolic processes during the day and night, with the IL-17A circadian rhythm overlaid. **e**, Representation of the DNL pathway. **f**, Correlation analysis of BAT IL-17RC protein levels with FASN abundance from OPABAT. **g**, Correlation

heat map of IL-17RC protein levels with DNL protein abundance from OPABAT. **h, i**, Circadian time plots showing the relative expression of the DNL genes *Fasn* (**h**) and *Acc2* (**i**) from BAT of wild-type (grey) and *Tcrδ*^{-/-} (red) mice. **d, h, i**, White and grey panels represent light and dark periods, respectively. **a–d, h, i**, Data are representative of two independent experiments. **b–d, h, i**, Data are mean $\pm$ s.e.m., $n = 4–12$ mice per group. Significance was calculated using a one-way ANOVA (**b**), two-tailed unpaired student's *t*-test (**c**), simple linear regression (**f, g**) or cosinor analysis, with cosine fitted curves (**h, i**); amplitude (amp) and acrophase (acro) were extracted from the cosinor model. NS, not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

$\gamma\delta$ IL-17A regulates DNL

Adipose $\gamma\delta$ T cells are the main source of IL-17A in adipose tissue, contributing 75% of the IL-17A in adipose tissue (Fig. 4a and Extended Data Fig. 6a). Loss of adipose $\gamma\delta$ T cells decreased the levels of IL-17A protein, and the levels recovered after readministration of $\gamma\delta$ T cells (Fig. 4b). Using IL-17A-GFP mice, we found diurnal expression of IL-17A across all adipose depots including BAT, epiWAT and subcutaneous (scWAT) adipose tissue, which peaked at night during the active phase (Fig. 4c). Studies from our laboratory and others have shown that $\gamma\delta$ 17 T cells have a key role in maintaining body temperature and thermogenesis^{21,22}. We next wondered why fluctuations in IL-17A expression might be important in adipose tissue. Adipose lipolysis and lipogenesis are under strict circadian control, with lipolysis occurring during the day in mice, and lipogenesis happening at night. Overlaying adipose IL-17A expression, we found that IL-17 peaks when lipogenesis peaks¹⁵ (Fig. 4d). Because adipose IL-17 was influenced by food intake, we hypothesized that adipose IL-17A might have a role in DNL (Fig. 4e). Using the public BAT proteome database, OPABAT, we found that the levels of IL-17RC protein directly correlated with the abundance of the DNL proteins FASN, ACACA, ACACB, ACLY and SCD1 (Fig. 4f, g and Extended Data Fig. 6b), but not with proteins involved in lipolysis³⁵ (Extended Data Fig. 7a). In addition, DNL protein abundance did not

correlate with other cytokine receptor levels in BAT (Extended Data Fig. 7b–f). We isolated adipose tissue across 24 h from mice lacking $\gamma\delta$ T cells (*Tcrδ*^{-/-} mice), and found that the circadian expression of the DNL genes *Fasn*, *Acc2* (also known as *Acacb*) and *Scd1* was disrupted in *Tcrδ*^{-/-} mice compared with wild-type controls (Fig. 4h, i and Extended Data Fig. 6c). We next investigated the direct effects of IL-17 on DNL, by treating primary brown adipocytes with recombinant (r)IL-17A or vehicle, and found increased expression of a transcriptional regulator of DNL genes, *Mlxipl*, and fatty acid synthase *Fasn*, by rIL-17A (Fig. 5a, b). Next, we administered IL-17A in vivo over three days, which increased the expression of all BAT DNL genes (*Mlxipl*, *Acaca*, *Fasn*, *Scd1*, *Scd2* and *Elovl6*; Fig. 5c). These experiments support the conclusion that $\gamma\delta$ -T-cell-derived IL-17 regulates adipose DNL.

Because we found an increase in IL-17A expression in adipose $\gamma\delta$ T cells from *Arntl* ^{Δ 17r} mice (Extended Data Fig. 3r and Fig. 5d), we investigated whether there was also a corresponding increase in the expression of DNL genes. Indeed, expression of *Acly*, *Acaca* and *Scd1* was increased in the BAT of *Arntl* ^{Δ 17r} mice, compared with *Arntl*^{*fl*} controls, specifically at ZT18, but not at ZT6 (Fig. 5e and Extended Data Fig. 8a). Previous studies have shown that *Il17a*^{-/-} and *Il17f*^{-/-} mice have impaired thermogenesis^{21,22}. Because adipose $\gamma\delta$ T cells produce both IL-17A and IL-17F, which can compensate for one another, we used IL-17A and IL-17F double-knockout mice (*Il17a/f*^{-/-}). BAT taken from *Il17a/f*^{-/-} mice

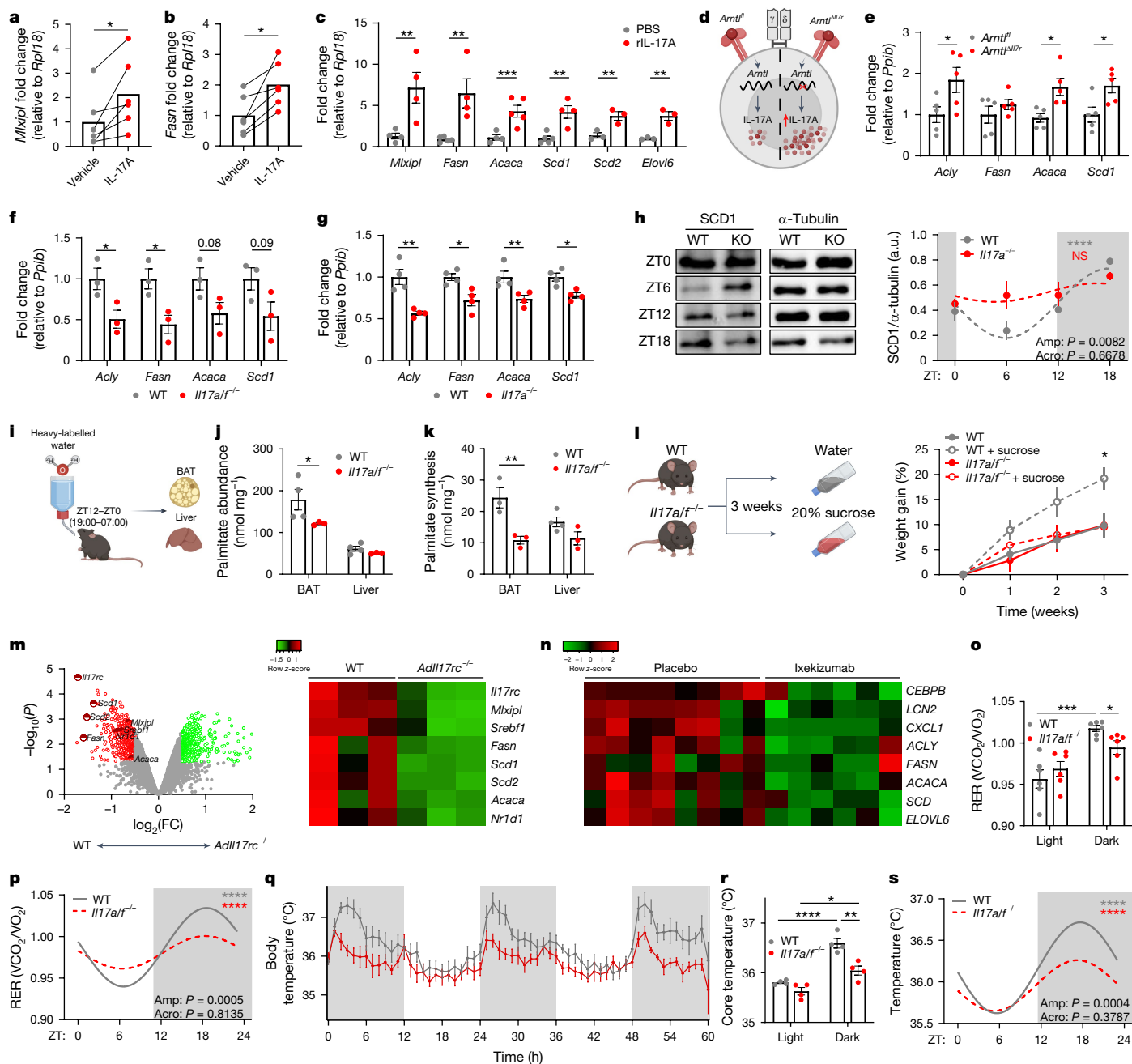


Fig. 5 | IL-17A signalling is necessary for adipocyte DNL, and for whole-body metabolism. **a, b**, Expression of *Mxip1* (**a**) and *Fasn* (**b**) in brown adipocytes treated with IL-17A (2 µg). **c**, BAT DNL gene expression from PBS- or IL-17A-treated mice. **d**, Diagram showing the increased expression of IL-17A in *Arnt1^{Δ17r}* mice. **e**, *Acly*, *Fasn*, *Acaca* and *Scd1* gene expression from wild-type or *Arnt1^{Δ17r}* BAT. **f, g**, *Acly*, *Fasn*, *Acaca* and *Scd1* gene expression from wild-type or *Il17a/f^{-/-}* BAT (**f**), and from wild-type or *Il17a/f^{-/-}* BAT (**g**). **h**, Left, western blots for the circadian expression of SCD1 from wild-type (grey) and *Il17a/f^{-/-}* (knockout; KO) BAT. Right, circadian plot. a.u., arbitrary units. **i**, Heavy-labelled-water protocol for palmitate labelling. **j, k**, Palmitate abundance (**j**) and palmitate synthesis (**k**) in wild-type (grey) and *Il17a/f^{-/-}* (red) BAT and liver. **l**, Left, experimental set-up for high-sucrose feeding. Right, weight gain after three weeks. **m**, Left, volcano plot of DNL-associated gene expression in BAT from wild-type and *Adil17rc^{-/-}* mice, from GSE144255. $\log_2(FC)$, $\log_2$ -transformed fold change. Right, differential expression heat map of DNL-associated genes. **n**, DNL-associated

genes from skin biopsies of patients with psoriasis who were treated with placebo or ixekizumab (150 mg). **o**, RER averaged by light or dark cycle. **p**, Circadian rhythmicity analysis. **q**, Wild-type (grey) and *Il17a/f^{-/-}* body temperature. **r**, Core-temperature data averaged by light or dark cycle. **s**, Circadian rhythmicity analysis. White and grey panels represent light and dark periods, respectively. Data are representative of one independent experiment (**a–k**), two independent experiments (**a–g**), or four independent experiments, with two experiments pooled (**o–s**). **c**, **e**, **h**, **j**, **o**, **q**, **r** Data are mean $\pm$ s.e.m., $n = 3–6$ mice per group. Significance was calculated using two-tailed paired student's *t*-test (**a**, **b**), two-tailed unpaired student's *t*-test (**c**, **e**–**g**), two-way ANOVA (**l**), repeated measures ANOVA, (**j**, **k**, **o**, **r**) or cosinor analysis, with cosine fitted curves (**h**, **p**, **s**); amplitude (amp) and acrophase (acro) were extracted from the cosinor model. NS, not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

at ZT6 and ZT18, in the middle of the inactive and the active phases, respectively, showed lower expression of DNL genes at ZT18, compared with BAT from wild-type mice, but this was not the case at ZT6

(Fig. 5f and Extended Data Fig. 8b). This effect was also observed in *Il17a/f^{-/-}* single-knockout mice (Fig. 5g). Furthermore, we found that *Il17a/f^{-/-}* mice had decreased expression of thermogenesis and lipolysis

genes at ZT6 and ZT18, compared with wild-type mice (Extended Data Fig. 8c,d). However, wild-type and *Il17a/f^{-/-}* mice upregulated DNL genes when administered rIL-17A or rIL-17F (Extended Data Fig. 8e–h). No differences were observed between wild-type and *Il17a/f^{-/-}* groups that were treated with phosphate-buffered saline (PBS), because injections were given during the day, when DNL and IL-17A expression are low (Extended Data Fig. 8e–h). At the protein level, circadian expression of SCD1 was disrupted in *Il17a/f^{-/-}* mice, compared with wild-type controls (Fig. 5h). Next, heavy-labelled water (²H₂O) was used to determine the ability of wild-type and *Il17a/f^{-/-}* BAT to synthesize palmitate. We found decreased lipid synthesis in *Il17a/f^{-/-}* BAT compared with wild-type, but no significant difference in the liver (Fig. 5i–k). Owing to decreased DNL in the BAT of IL-17-deficient mice, we placed wild-type and *Il17a/f^{-/-}* mice on a three-week high-sucrose regime that promotes DNL, and found that *Il17a/f^{-/-}* mice were resistant to high-sucrose-induced weight gain (Fig. 5l).

We next took advantage of publicly available transcriptomic data of adipocyte-specific deletion of the IL-17 receptor, IL-17RC. We found that the DNL genes *Mlxipl*, *Acaca*, *Fasn*, *Scd1*, *Scd2* and *Srebf1* were significantly downregulated in BAT lacking IL-17RC, compared with wild-type BAT (Fig. 5m). Furthermore, publicly available clinical data from skin biopsies of patients with psoriasis who were receiving either a placebo or anti-IL-17 therapy show that individuals who were treated with brodalumab³⁶ (anti-IL-17A; Extended Data Fig. 8i) or ixekizumab³⁷ (anti-IL-17A; Fig. 5n) exhibited a decrease in the expression of DNL genes. Combined, these data show that IL-17 signalling has a role in regulating DNL in BAT and human skin.

Finally, we performed whole-body metabolic analysis in wild-type and *Il17a/f^{-/-}* mice at thermoneutrality (30 °C). Thermoneutrality was chosen to remove any influence from adaptive thermogenesis, because mice that are housed at room temperature expend approximately 40% of their energy to keep warm at room temperature^{38,39}. A previous study suggested that BAT DNL is crucial for the maintenance of body temperature in mice⁴⁰. We found that *Il17a/f^{-/-}* mice have an altered RER, particularly at night (Fig. 5o), and show a blunted circadian RER rhythm (Fig. 5p). Notably, the body temperature of *Il17a/f^{-/-}* mice was decreased at all times of day, but especially during the active phase (Fig. 5r,s). Circadian analysis found that *Il17a/f^{-/-}* mice had a significantly blunted circadian fluctuation of body temperature over 24 h (Fig. 5s). Combined, our results show that circadian IL-17A from adipose-tissue-resident $\gamma\delta$ T cells is important for physiological whole-body metabolism and body temperature, and that it targets the adipose tissue DNL pathway.

Discussion

Here we show that type-17 innate T cells—particularly adipose $\gamma\delta$ 17 T cells—are enriched for the molecular clock. The molecular clock controls adipose $\gamma\delta$ 17 T cell maintenance and IL-17A production, which is essential for adipose tissue circadian homeostasis, whole-body metabolism and body-temperature regulation, through the circadian regulation of DNL. Tissue-resident innate lymphocytes ($\gamma\delta$ T cells²¹, iNKT cells⁷ and ILCs⁴¹) produce effector cytokines that are important for host defence, but also for the normal maintenance of tissue physiology. We show that the molecular clock is more prominent in type-17 tissue-resident innate T cells than it is in type-1 cells, particularly in adipose tissue. The molecular clock has been shown to regulate IL-17A responses in ILC3s²⁶ and T_H17 cells²³; however, circadian rhythms in innate T cells have not been described. In the small intestine, genetic deletion of *Nfil3* or *Rev-erba* disrupted IL-17A expression, but had no effect on the expression of IFN γ by T_H1 cells²³. Furthermore, IL-17A-producing ILC3s also had higher expression of molecular-clock genes than ILC1s²⁶, ILC2s²⁵ and ILC progenitors²⁴. Our data show that $\gamma\delta$ 17 T cells have a daily rhythm to IL-17A and ROR γ t expression, which is negatively regulated by REV-ERB α activity. We found that all adipose

depots, including BAT, had a diurnal rhythm to IL-17A expression by $\gamma\delta$ 17 T cells. These studies, combined with our findings, suggest that enriched expression of the molecular clock is a conserved feature of IL-17A-producing lymphocytes.

Previous work by our laboratory and others have described a key thermogenic role for IL-17A—and/or IL-17F—signalling in adipose tissue^{21,22}. However, these studies were performed in cold-challenged mice, and here we describe a circadian homeostatic role of IL-17A. Given the cold-challenge results, it was at first puzzling that we found peaks of IL-17A expression by adipose $\gamma\delta$ 17 T cells at night during feeding, rather than during the day when lipolysis is highest in mice. At night, when mice feed, energy is stored as triacylglycerides in adipose tissue through DNL⁴². Another study found that DNL and lipolysis in adipose tissue are intricately connected. The research showed that loss of DNL, through the BAT-specific disruption of SREBP activity, was sufficient to disrupt the circadian body temperature of mice, providing evidence that BAT DNL has an essential role in maintaining body temperature⁴⁰. Therefore, loss of DNL circadian rhythm in *Il17a/f^{-/-}* mice might blunt lipolysis owing to a reduction in the lipid stores that act as fuel for circadian lipolysis and body-temperature regulation. We show that disrupted DNL could be restored by administering IL-17A and IL-17F in vitro and in vivo. A reanalysis of a previously published dataset found that loss of IL-17RC specifically in adipocytes significantly reduced BAT DNL gene expression²². The authors concluded that IL-17F, rather than IL-17A, mediates the pro-thermogenic effect on adipose tissue because of the IL-17RC deletion. However, both IL-17A and IL-17F are known to signal through IL-17RC heterodimers with IL-17RA. Thus, both cytokines might act in synergistic or compensatory mechanisms to promote thermogenesis, and maintain body temperature^{43–45}. Thus, here we analysed *Il17a/f^{-/-}* double-knockout mice, and found a marked defect in the daily fluctuations of body-temperature rhythm.

A previous study found that BMAL1 deficiency specifically in ROR γ t-expressing cells (*Bmal1^{ΔRorc}*) led to an increase in the expression of genes that are involved in lipid metabolism in gut epithelial cells²⁴. This study attributed the phenotype to a loss of IL-22 in ILC3s. A similar study found that IL-17A expression was enhanced in *Bmal1^{ΔRorc}* mice, suggesting that REV-ERB α negatively regulates IL-17 expression, in line with our data. Furthermore, *Bmal1^{ΔRorc}* mice had an increase in the mass of gonadal and subcutaneous adipose tissue²⁴, which supports our data linking IL-17 to lipid metabolism and to enhanced lipid synthesis and storage. This suggests that increased IL-17, rather than decreased IL-22 from gut ILC3s, drives the enhanced expression of lipid-metabolism genes in the gut epithelial cells of *Bmal1^{ΔRorc}* mice, although further work is needed to investigate this. Together, these data highlight that the IL-17 cytokine family is important for lipid metabolism in both adipocytes and gut epithelial cells, where innate T cells and ILCs reside.

IL-17A derived from $\gamma\delta$ T cells, among others, has a pathological role in the initiation of EAE³⁴. In addition, a HFD exacerbates EAE pathology, and increases brain infiltration by T_H17 cells, enhancing the production of IL-17A by T_H17 cells. We found that a three-week HFD, before the onset of obesity, enhanced EAE and disrupted the circadian rhythm of IL-17A, leading to constantly elevated levels over 24 h (ref. 46). Shift workers are reported to have an increased preference for high-fat, high-calorie foods, in addition to irregular meal timings^{47–49}. In modern living, all-day grazing and constant food availability have changed the way many people eat in the Western world. In mice, a HFD disrupts feeding behaviour, and increases grazing during the inactive phase as well as the active phase⁵⁰. Furthermore, SFD-fed mice that were forced to eat during the day (inactive phase) gained weight over time^{51,52}. Shift work has been associated with an increased risk of developing multiple sclerosis and metabolic disorders⁵³. Our data suggest that adipose IL-17 production is regulated by food intake, which would enhance DNL and fat storage with all-day grazing. Given the rising number of shift workers⁵⁴, understanding how feeding disruption and timing affect immunometabolism could help to reduce shift work-related diseases.

Our data reveal that IL-17 has a circadian rhythm, with nightly peaks. This rhythm is controlled by the molecular clock, entrained by light and affected by feeding. A major target of IL-17 in adipose tissue is DNL, and the absence of IL-17A and IL-17F results in defects in DNL and a reduced ability to store fat from food, as is also seen³³ in mice with an adipocyte-specific deletion of *Il17rc*. It is becoming increasingly clear that immune cells have a crucial role in regulating local tissue homeostasis, which affects whole-body circadian metabolism. Given the ever-increasing burden of metabolic disease, a better understanding of the links between immune function, circadian rhythm and whole-body metabolic health is essential to combat conditions such as obesity, cachexia and metabolic syndrome. Here we provide evidence for the IL-17 family as potent metabolic modulators that could be therapeutic targets in metabolic disease.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-024-08131-3>.

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Methods

Mice

In all experiments, male C57BL/6J-OlaHsd mice between 8 and 12 weeks of age were used. Mice were bred in-house under specific-pathogen-free conditions in accordance with Irish and European Union regulations. *Il17a^{f/f}* mice were received from the laboratories of V. K. Kuchroo and Y. Iwakura. *Il17a^{-/-}* mice were received from the laboratory of K.H.G.M. IL-17A-GFP mice were purchased from The Jackson Laboratory. The *Vav1^{Cre}*, *Il7^{Cre}*, *Per1^{Venus}* and *Arntl^{fl}* mouse lines were bred and maintained at the Champalimaud Centre for the Unknown (CCU) animal facility. All mouse work was performed in compliance with the L.L. laboratory project licence, with ethical approval from the Trinity College Dublin ethics committee and the Animal Research Ethics Committee from the Health Products Regulatory Authority (HPRA), and the Brigham and Women's Hospital Institutional Animal Care and Use Committee guidelines. Experiments involving the *Vav1^{Cre}* (ref. 55), *Il7^{Cre}*, *Per1^{Venus}* (ref. 56) and *Arntl^{fl}* (ref. 57) mouse lines were approved by national and local institutional review boards, the Direção Geral de Veterinária and CCU ethical committees. For experiments using rIL-17A, mice were administered 100 µl of rIL-17A (2 µg per mouse) or PBS control by intraperitoneal (i.p.) injection three times over five days.

Environmental modulation

Inversion of light–dark cycles. For experiments manipulating light cycles, mice were placed inside a ventilated, light-tight cabinet at room temperature, and light was adjusted such that control mice had lights on from 07:00–19:00, and test mice had lights on from 19:00–07:00.

Diet models. For HFD feeding experiments, mice were fed a 45% or 60% HFD for 3 weeks or 16 weeks ad libitum, compared with control mice that were fed a SFD, in which 10% of the calories were from fat. For reverse-feeding experiments, mice were fed a SFD ad libitum from 07:00–19:00 during the light cycle, compared with controls that were fed a SFD ad libitum from 19:00 to 07:00 during the dark cycle. For isocaloric feeding experiments, mice were placed into metabolic cages, and food consumption was measured over two days. The total calories per day was calculated, and mice were then fed 50% of the total calories consumed per day, for one week, compared with ad-libitum-fed controls that had 24-h access to food. For high-sucrose feeding experiments, mice were provided a 20% sucrose solution as their drinking water ad libitum for three weeks.

Tissue processing

Mice were euthanized by CO₂ inhalation and adipose tissue was removed, minced with a razor and digested in 1 mg ml⁻¹ collagenase type II (Worthington) dissolved in RPMI, in a shaking incubator for 25–30 min at 37 °C. Digested cells were filtered through a 70-µm nylon mesh and centrifuged at 300g for 5 min to pellet the stromovascular fraction (SVF). Lymph nodes were disrupted through a 70-µm filter and centrifuged for 5 min at 300g to pellet. After processing, the adipose SVF and lymph-node leukocytes were stimulated with phorbol 12-myristate 13-acetate (PMA; 10 ng ml⁻¹; Sigma), ionomycin (500 ng ml⁻¹; Sigma) and brefeldin A (BFA; 5 µg ml⁻¹; Sigma) in cRPMI medium, and incubated at 37 °C for 3 h. For cytokine stimulations, adipose SVF was stimulated with rIL-23 (10 ng ml⁻¹; Miltenyi) and rIL-1β (10 ng ml⁻¹; Immunotools) with BFA (5 µg ml⁻¹) for 3 h.

For the circadian cytokine production of lymph-node γδ T cells, all lymph nodes were collected from mice at 07:00 (ZT0) and plated at 3-h time points over 24 h. Each time point was then stimulated with PMA and ionomycin in BFA for 3 h, leading up to the respective time point, before being collected for fluorescent staining for flow cytometry analysis.

For treatments with the REV-ERBβ agonist SR9009 (Sigma), cells were stimulated with PMA–ionomycin or with the recombinant cytokines

rIL-1β and rIL-23 as described above, in the presence or absence of SR9009. For dose–response graphs, lymph-node cells were stimulated in the presence of 0 µM, 5 µM, 10 µM, 20 µM, 40 µM and 80 µM doses of SR9009. Adipose SVF was stimulated with 40 µM of SR9009 only.

ELISA

γδ T cells were pre-expanded in wild-type mice using IL-7 and IL-15 cytokines and sorted to purity. A total of 50,000 γδ T cells were adoptively transferred into *Tcrδ^{-/-}* mice. After one day, wild-type, *Tcrδ^{-/-}* and *Tcrδ^{-/-}* age-matched mice reconstituted with 50,000 γδ T cells were euthanized and adipose tissue lysates were assayed for IL-17A protein levels by ELISA. Processed adipose SVF lysates were diluted 1:2 in reagent diluent (1% bovine serum albumin in PBS) and IL-17A protein levels were quantified using the Mouse IL-17 Quantikine ELISA kit (M1700, R&D Systems).

Culturing of mouse adipocytes

Interscapular BAT was isolated, minced and incubated in Dulbecco's modified Eagle's medium (DMEM) (glucose-free) supplemented with 10% fetal calf serum (FCS), 2.5 mM L-glutamine and 5 mM glucose. Tissue explants were then treated with or without rIL-17A (50 ng ml⁻¹; R&D Systems) for 18 h at 37 °C. Once the medium was removed, explants were snap-frozen in liquid nitrogen for RNA extraction.

Flow cytometry analysis

For intracellular and intranuclear staining, cells were washed with 1 ml PBS and incubated in ZombieAqua Cell Viability Dye (Biolegend; 1:1,000 in PBS) for 20 min at room temperature. Cells were incubated with an extracellular fluorochrome-labelled antibody cocktail with Fc block (1:200; BD Biosciences) in FACS buffer (PBS + 2% FCS) for 20 min at room temperature. Cells were then washed with 2% FACS buffer resuspended in 100 µl of Foxp3 staining kit (eBioscience) and incubated at room temperature for 20 min. Cells were washed with 1× permeabilization buffer (eBioscience) and then incubated with an intracellular fluorochrome-labelled antibody cocktail in 1× permeabilization buffer (eBioscience) for 20 min at room temperature. Cells were incubated on ice for 30 min and subsequently washed in 2% FACS buffer. Cells were acquired on a FACS Canto or LSR Fortessa cytometer (BD Biosciences). Data were analysed with FlowJo v.10 software. Cell sorting was performed using a FACS Aria (BD Biosciences). Sorted populations were more than 95% pure. For a list of flow cytometry antibodies, see Supplementary Table 1.

Induction and assessment of EAE

Mice were fed either ad libitum or a 60% HFD three weeks before EAE induction. EAE was induced in male mice by subcutaneous immunization with MOG_{35–55} peptide (150 µg per mouse; GenScript) emulsified in complete Freund's adjuvant (Condrex) containing 4 mg ml⁻¹ heat-killed *Mycobacterium tuberculosis* (MTB). Mice were injected i.p. with pertussis toxin (200 ng per mouse) (Kaketsuken) on day 0 to induce EAE development. Disease severity was monitored and assessed by clinical scores as follows: no clinical signs, 0; limp tail, 1; ataxic gait, 2; hind limb weakness, 3; hind limb paralysis, 4; tetra paralysis or moribund, 5. A weight loss of more than 20% constituted an additional humane end-point.

RNA extraction and quantitative PCR analysis

RNA extraction from isolated cells. Adipose γδ17 T cells were isolated and sorted from adipose tissue. RNA and cDNA were generated from isolated cells using the SYBR Green Fast Advanced Cells-to-CT Kit (Invitrogen) following the manufacturer's specific instructions. To quantify the relative mRNA expression of genes of interest, quantitative PCR was performed in a 96- or 384-well plate format (Thermo Fisher Scientific) using PowerUp SYBR Green Master Mix (Invitrogen)-based detection (eBioscience). Relative mRNA levels were calculated using the

$\Delta\Delta$ cycle threshold ($\Delta\Delta C_t$) method and normalized to corresponding endogenous controls (*Actb*).

RNA extraction from tissues. Tissues were snap-frozen in liquid nitrogen, defrosted at room temperature and transferred to a 2-ml tube containing a 5-mm stainless steel bead. Tissues were homogenized in 1 ml trizol reagent (Thermo Fisher Scientific) in a tissue lyser for 2.5 min, 25 pulses per second. Then, 200 μ l chloroform was added to each tube, and they were inverted once and left at room temperature for 2–3 min, before centrifuging at 12,000g for 15 min. The aqueous phase containing RNA was transferred into a new Eppendorf tube and 500 μ l isopropanol was added to precipitate the RNA. Tubes were inverted ten times and left at room temperature for 10 min, and then centrifuged at 12,000g for 10 min. Supernatants were discarded and RNA pellets were washed in 1 ml 75% ethanol, diluted in RNase free dH_2O . Tubes were centrifuged at 12,000g for 5 min and supernatants were discarded by inverting the tube. The RNA pellet was left to dry at room temperature for 20–30 min, until transparent, and the pellets were resuspended in 50 μ l RNase free water. RNA was left on ice for 30 min, then in a heat block set at 55 °C for 15 min. RNA quality and concentration were determined using a Nanodrop 2000 UV spectrophotometer (Thermo Fisher Scientific). Twenty microlitres of cDNA was synthesized from 2 μ g of isolated RNA using the High-Capacity cDNA Reverse Transcription Kit (Biosciences) in a MiniAmp Thermal Cycler (BD Biosciences). To quantify the relative mRNA expression of genes of interest, quantitative PCR was performed in a 96- or 384-well plate format (Thermo Fisher Scientific) using SYBR Green-based detection (eBioscience). Relative mRNA levels were calculated using the $\Delta\Delta$ cycle threshold ($\Delta\Delta C_t$) method and normalized to corresponding endogenous controls (*Ppib* or *Rpl18*). For a list of primers, see Supplementary Table 2.

Protein analysis by western blotting

BAT from wild-type and *Il17a*^{−/−} mice was collected over 24 h and snap-frozen. The BAT was then lysed and centrifuged at 14,000g, and the pellet was discarded. The amount of protein was quantified using a BCA kit. Tissue lysates were subsequently boiled at 95 °C for 5 min to denature the proteins. Proteins were resolved, on the basis of their molecular weight, through SDS–PAGE gels in 1× running buffer. Proteins were electro-transferred onto PVDF membranes (Merck) in 1× transfer buffer containing 10% methanol. Membranes were blocked in 5% milk in 1× Tris-buffered saline with Tween-20 and incubated with SCD1, or α -tubulin primary antibodies (Cell Signaling Technologies) overnight at 4 °C and HRP-conjugated secondary antibodies (Jackson ImmunoResearch). Membranes were incubated in ECL substrate (Bio-Rad), and images were developed on a Biorad Gel Doc imaging system. Images were quantified using ImageJ. For raw uncropped blots, see Supplementary Fig. 1.

Plasma ²H₂O enrichment analysis and DNL calculations

For measurements of DNL in vivo, wild-type or *Il17a*^{−/−} mice were placed overnight on 8% enriched D₂O drinking water, with subsequent collection and snap-freezing of BAT and liver. The ²H labelling of water from samples or standards was determined by deuterium acetone exchange, and using equations as previously described⁵⁸.

Metabolic cage analysis

Indirect calorimetry experiments were performed with the Promethion metabolic cage system, or the comprehensive lab animal monitoring system (CLAMS), essentially as described⁵⁹. Mice were singly housed and allowed at least 12 h of acclimatization to the new environment. O₂ consumption, CO₂ emission, energy expenditure, body weight, food and water intake and locomotor activity were monitored throughout the experiment. For experiments involving the manipulation of light cycles, mice were placed inside the metabolic cages at room temperature, and light was adjusted such that the control mice had lights on

from 07:00–19:00, and test mice had lights on from 19:00–07:00. Analysis was performed using the online indirect calorimetry vignettes CalR (ref. 60); online software is available at <https://calrapp.org/>.

Bulk RNA-seq and scRNA-seq data analysis

The bulk RNA-seq dataset of BAT from wild-type and *Adil17rc*^{−/−} mice was downloaded from the GEO repository GSE144255 as cuff gene counts²². The bulk RNA-seq datasets of skin biopsies from patients with psoriasis who were receiving brodalumab³⁶ and ixekizumab³⁷ were downloaded from the GEO repositories GSE117468 and GSE31652, respectively. Heat maps were generated using the online platform Heatmapper⁶¹ (<http://heatmapper.ca/>).

scRNA-seq was performed on single-cell suspensions of sorted $\gamma\delta$ T cells and iNKT cells from the visceral adipose tissue of C57BL/6J mice using the 10x Genomics platform. A total of five visceral adipose tissue deposits from five independent biological replicates were pooled for sequencing. Single-cell suspensions were loaded onto a 10x Chromium Controller to generate gel beads-in-emulsion (GEMS), and GEMs were processed to generate unique molecular identifier (UMI)-based libraries according to the 10x Genomics Chromium Single Cell 3' protocol. Libraries were sequenced using a NextSeq 500 sequencer. Raw BCL files were demultiplexed using Cell Ranger v.3.0.2 mkfastq to generate fastq files with default parameter. Fastq files were aligned to the mm10 genome (v.1.2.0) and feature reads were quantified simultaneously using the Cell Ranger count for feature barcoding. Filtered feature-barcode UMI count matrices containing quantification of gene expression were used for downstream analysis.

Downstream scRNA-seq data analysis. A total of 22,748 cells mouse $\gamma\delta$ T cells, iNKT cells and MAIT cells expressing a median of 1,423 genes and 3,556 UMIs per cell were loaded from feature-barcode UMI count matrices using the Seurat v.4.1.0 package⁶². Adipose and splenic iNKT cells were reanalysed from GSE142845 (ref. 63), thymic iNKT cells were reanalysed from GSE141895 (ref. 64), thymic MAIT cells were reanalysed from E-MTAB-7704 (ref. 65), pulmonary $\gamma\delta$ T cells were reanalysed from E-MTAB-8732 (ref. 66), peripheral lymph node (PLN) and dermal $\gamma\delta$ T cells were reanalysed from GSE123400 (ref. 67) and meningeal $\gamma\delta$ T cells were reanalysed from GSE147262 (ref. 68). Cells expressing more than 11% of mitochondrial genes as a percentage of total gene counts were considered to represent apoptotic or dead cells and were therefore removed from the analysis. Cells were also filtered on the basis of total UMI counts and total gene counts on a per-sample basis to remove empty droplets, poor quality cells and doublets, with a minimum cut-off of at least 300 genes per cell across all samples. UMI counts were normalized using regularized negative binomial regression using scctransform v.0.3.3 (ref. 69).

Dimensionality reduction was performed using principal component analysis (PCA) with $n = 100$ dimensions and 2,000 or 3,000 variable features, and an elbow plot was used to determine the number of PCA dimensions used as input for UMAP⁷⁰. For collective analysis of cells from different batches and cells sequenced using different scRNA-seq technologies the Harmony v.1.0 package⁷¹ was used with default settings to remove batch effects, and batch-corrected harmony embeddings were used for UMAP. UMAP was performed using a minimum distance of 0.3 and a spreading factor of 1. Shared nearest neighbour graphs were calculated using $k = 20$ nearest neighbours. Graph-based clustering was performed using the Louvain algorithm. In some cases, overclustering was performed and clusters were manually collapsed, and/or the first two dimensions of the UMAP reduction were used as input for graph-based clustering instead of PCA or harmony embeddings. Adipose $\gamma\delta$ T cells were distinguished from adipose iNKT cells by expression of *Trdc* and graph-based clustering, and analysed separately for $\gamma\delta 1$ versus $\gamma\delta 17$ cell identification. Type-1 and type-17 innate T cells were individually identified in each dataset using graph-based clustering and gene-expression analysis, and cycling cells were excluded. For thymic

innate T cells, CD44⁺ progenitor populations were also excluded. Raw counts from type-1 and type-17 cell populations were then merged and normalized together into a single file for comparative analysis.

Gene-expression analysis was performed using the FindMarkers() or FindAllMarkers() Seurat functions and the Wilcoxon rank sum test. Heat maps were generated using the Complex Heatmap v.2.7.11 and circlize v.0.4.14 packages⁷², and module scores were calculated using the AddModuleScore() Seurat function with $n = 10$ control features. Density plots were produced using the Nebulosa v.1.1.1 package⁷³. Log-normalized RNA counts were used for all gene-expression analysis and visualization. All computational analysis was performed using R v.4.1.2 and RStudio Desktop v.1.4.1712 on an Ubuntu 20.04 Linux GNU (64 bit) system.

Statistical analysis

GraphPad Prism 9.3.0 was used for statistical analysis. For all experiments, a 95% confidence interval was used and $P \leq 0.05$ was considered statistically significant. A D'Agostino–Pearson omnibus normality test was first performed to test whether the data were normally distributed (Gaussian distribution). If data were normally distributed, parametric testing was performed. If data were not normally distributed, non-parametric testing was performed. When comparing two groups, an unpaired or paired two-tailed student's t -test was used. When comparing more than two groups, an ordinary one-way ANOVA with Dunnett's test was used. When comparing data with two variables, a two-way ANOVA with Bonferroni test was used. Cosine curves were fitted in GraphPad Prism and circadian rhythmicity was evaluated using the cosinor regression model using the cosinor and cosinor2 R packages in RStudio. The circadian period was assumed to be 24 h for all analysis and the significance of the circadian fit was assessed by a zero-amplitude test with 95% confidence. Amplitude and acrophase were extracted from the cosinor model. In all figures, $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$, $****P \leq 0.0001$.

Graphical representations

All diagrams and graphical representations were created using BioRender.

Reporting summary

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

Data availability

The data supporting the findings of this study are publicly available through the Gene Expression Omnibus (GEO) repository at GSE144255, GSE117468 and GSE31652 for the bulk RNA-seq datasets and GSE142845, GSE141895, GSE123400, GSE147262, or from Array-Express at E-MTAB-7704 and E-MTAB-8732 for the scRNA-seq datasets. Raw data that support the findings of this study are available from the corresponding author upon reasonable request. Source data are provided with this paper.

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Acknowledgements The authors acknowledge the work of B. Moran of the flow cytometry facility, in addition to R. Rakhmatullin and all other staff of the animal facilities at our institutions. We thank the past and present members of the laboratories, who have provided invaluable input over the years, and A. Muñoz Zamora, C. Ortega-de San Luis, E. Stewart and J. O'Leary for proofreading and feedback on the manuscript. This work was funded by the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (StG_679173 to L.L.), Science Foundation Ireland (SFI) (16/FRL/3865 to L.L.) and the NIH (R01 AI134861 and metabolic core grant S10 OD020100 to L.L.). This work was also supported by the Irish Research Council (grant GOIPG/2019/421 to A.D.).

Author contributions L.L. provided funding for the study. L.L., L.A.J.O., K.H.G.M., C.M.M. and H.V.-F. supervised the study. A.D. and L.L. conceived and designed the original study. A.D. and L.L. managed the project administration. A.D., B.S. and M.R. performed most of the experiments. E.A.D., C.S., H.P., A.E.G., B.K., A.C.K. and K.W.-R. performed some experiments. M.B., K.O. and E.L. provided technical assistance in some experiments. H.K. and A.D., performed bioinformatic analysis. A.D. analysed and interpreted most of the data. B.S., C.S., E.A.D., B.K. and M.R. analysed some of the data. A.D. and L.L. wrote and edited the manuscript.

Competing interests The authors declare no competing interests.

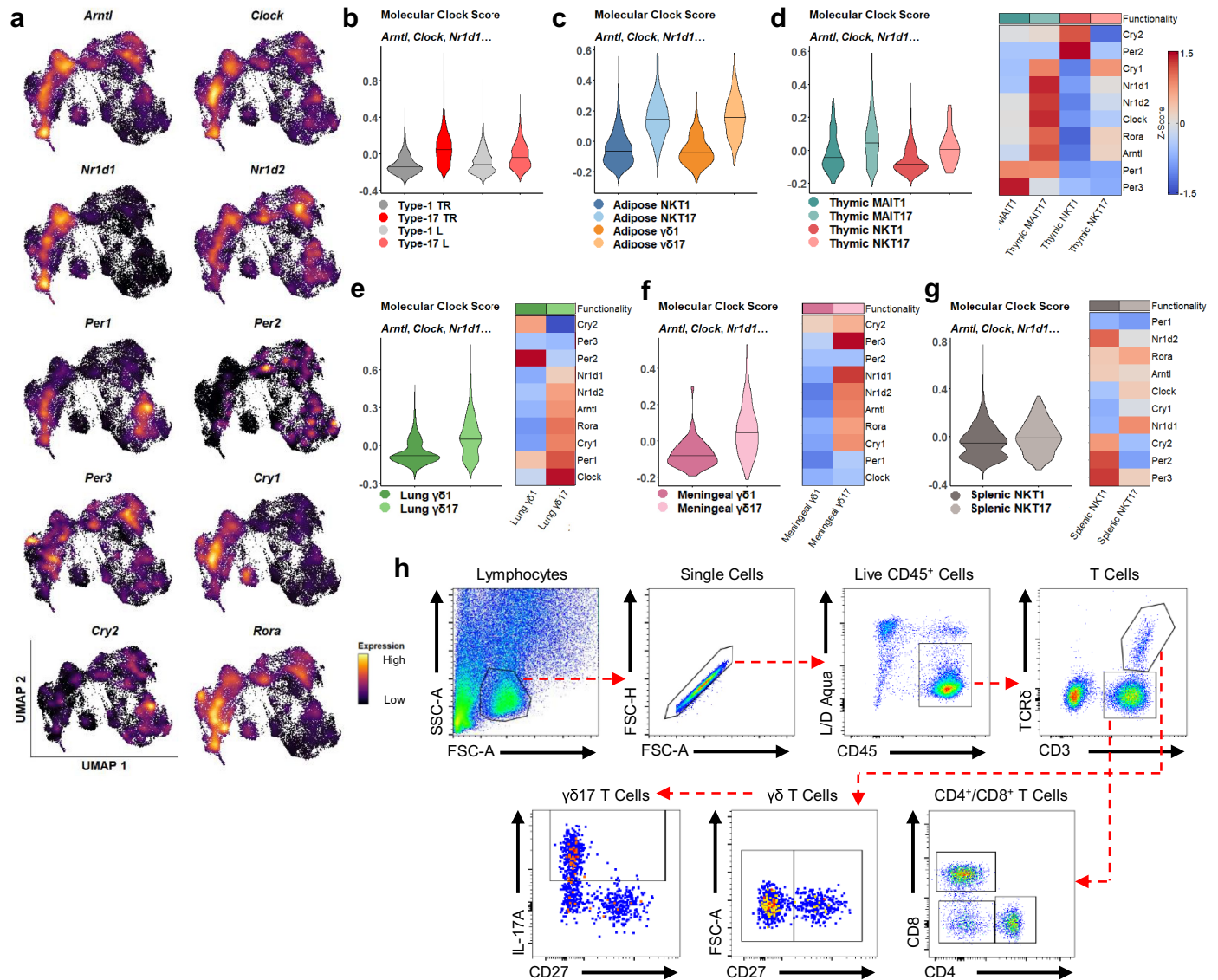
Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41586-024-08131-3>.

Correspondence and requests for materials should be addressed to Lydia Lynch.

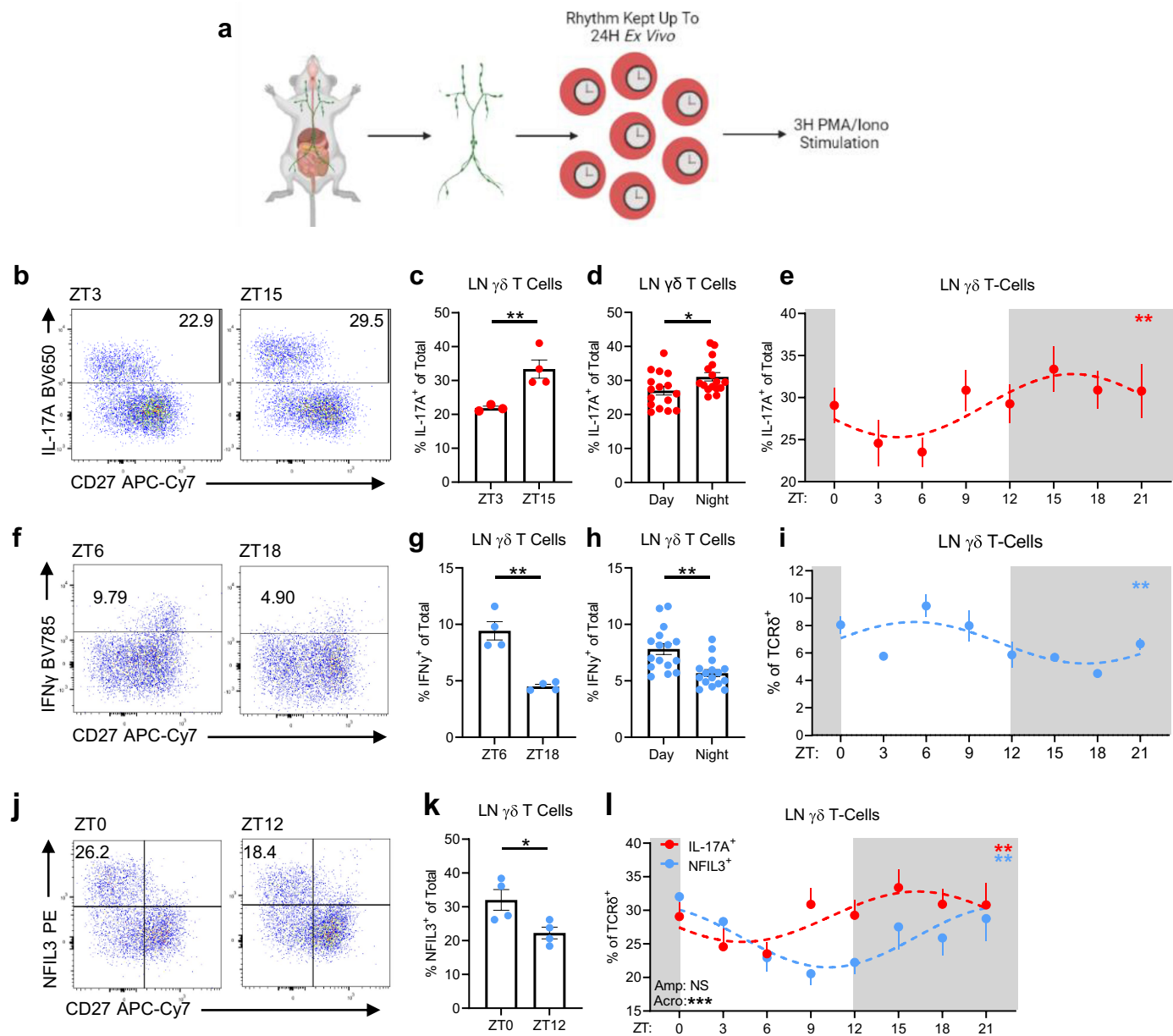
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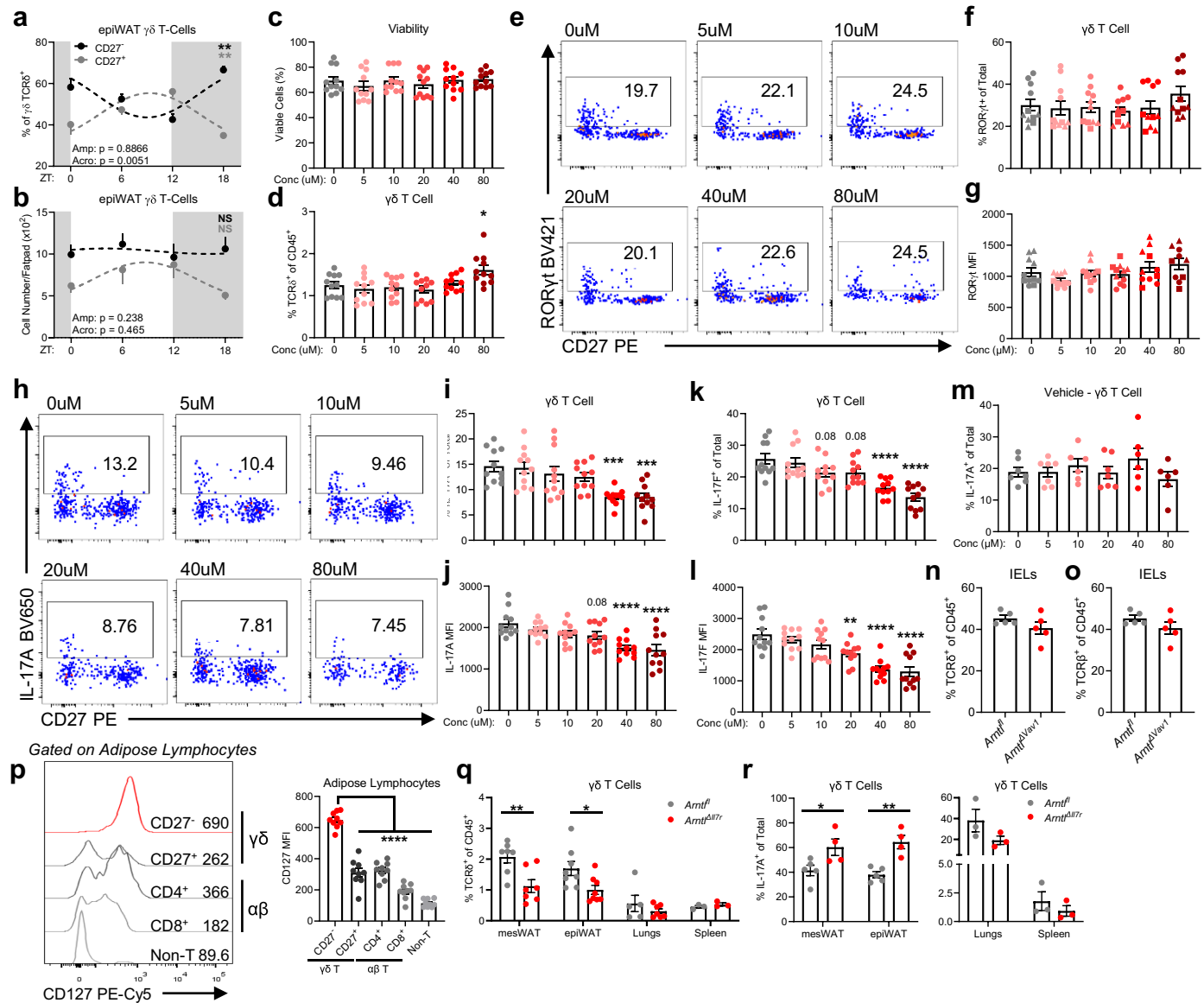
Extended Data Fig. 1 | Innate IL-17⁺ T cells are enriched for molecular-clock genes within tissues. **a**, Density plot expression of individual molecular-clock genes across innate T cell subsets. **b**, Violin plot showing expression of the molecular-clock score of type-17 versus type-1 innate lymphocytes, further divided by tissue-resident or lymphoid. **c**, Violin plot showing expression of the molecular-clock score of adipose innate lymphocytes. **d**, Violin plot showing expression of the molecular-clock score of thymic innate lymphocytes (left), heat map of averaged gene expression of molecular-clock genes with hierarchal clustering of data generated from GSE141895 and E-MTAB-7704 (right). **e**, Violin plot showing expression of the molecular-clock score of lung

innate lymphocytes (left), heat map of averaged gene expression of molecular-clock genes with hierarchal clustering of data generated from E-MTAB-8732 (right). **f**, Violin plot showing expression of the molecular-clock score of meningeal innate lymphocytes (left), heat map of averaged gene expression of molecular-clock genes with hierarchal clustering of data generated from GSE147262 (right). **g**, Violin plot showing expression of the molecular-clock score of splenic innate lymphocytes (left), heat map of averaged gene expression of molecular-clock genes with hierarchal clustering of data generated from GSE142845 (right). **h**, Representative gating strategy for adipose tissue T cells.



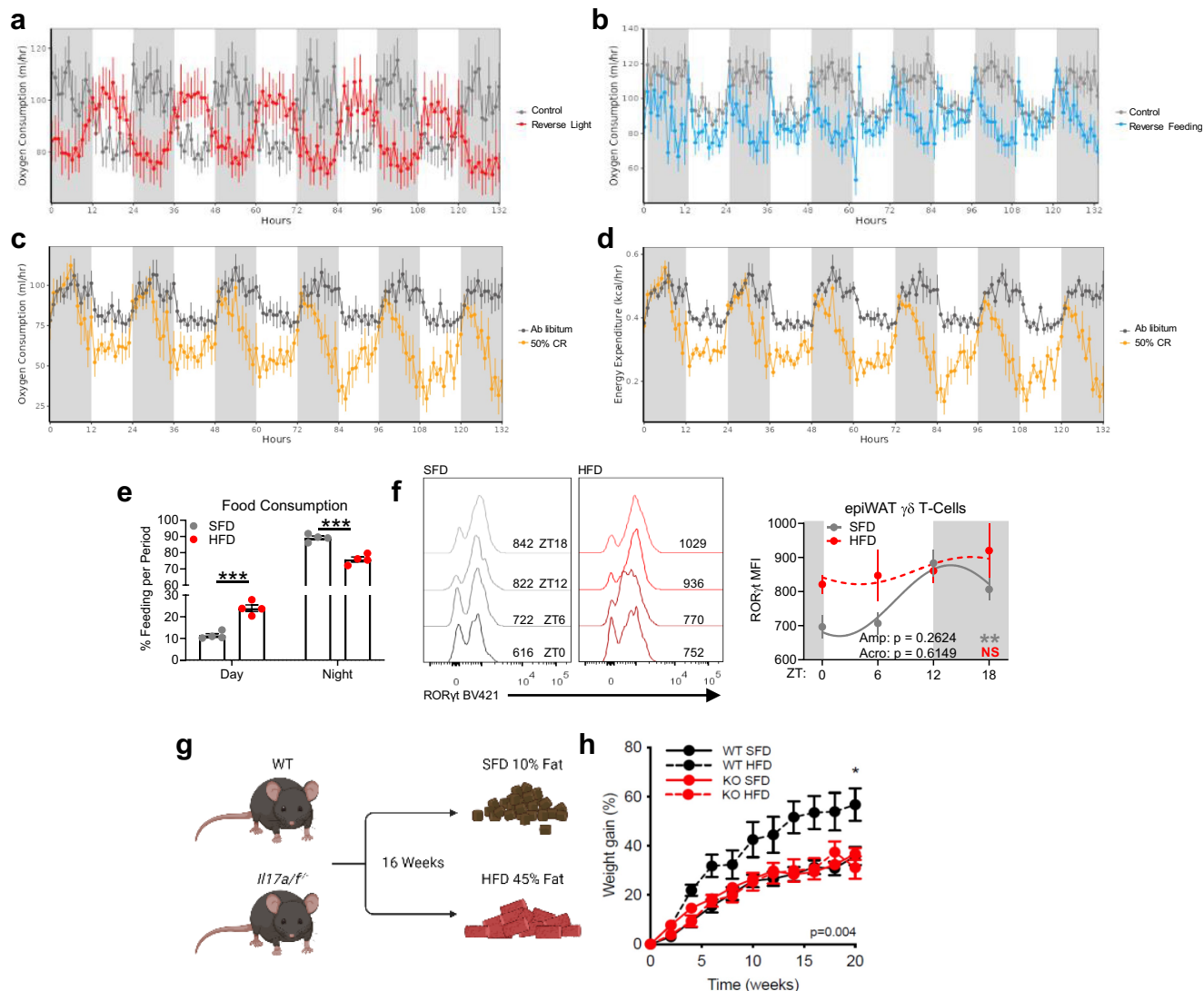
Extended Data Fig. 2 | Lymph-node $\gamma\delta$ T cells express IL-17A and IFN γ rhythmically. **a**, Diagrammatic representation of experimental set-up. **b**, Representative flow plots of IL-17A expression at nadir (ZT5), and peak (ZT15). **c**, IL-17A expression by lymph node (LN) $\gamma\delta$ T cells stimulated ex vivo for 3 h with PMA/Ionomycin, **d**, displaying diurnal rhythm. **e**, Circadian fluctuation of IL-17A production by LN $\gamma\delta$ T cells over 24 h. **f**, Representative flow plots of IFN γ expression at peak (ZT6), and nadir (ZT18). **g**, **h**, IFN γ expression by LN $\gamma\delta$ T cells stimulated ex vivo for 3 h with PMA/Ionomycin, displaying diurnal rhythm (**h**). **i**, Circadian fluctuation of IFN γ production by LN $\gamma\delta$ T cells over 24 h. **j**, Representative flow plots of NFIL3 expression at nadir (ZT12), and

peak (ZT0). **k**, NFIL3 expression by LN $\gamma\delta$ T cells stimulated ex vivo for 3 h with PMA/Ionomycin. **l**, Circadian fluctuation of NFIL3 expression (blue), overlaid with IL-17A production (red), by LN $\gamma\delta$ T cells over 24 h. Data are representative of two independent experiments. White and grey panels represent light and dark periods respectively. **c-e, g-i, k, l**, Data shown as mean $\pm$ SEM, $n = 4$ mice per group. **c, d, g, h, k**, Significance was calculated using two-tailed unpaired students t-test, **e, i, l**, Cosinor analysis, with cosine fitted curves, **l**, amplitude (Amp) and acrophase (Acro) were extracted from the cosinor model. NS = Non-Significant, * $p < 0.05$, ** $p < 0.01$.



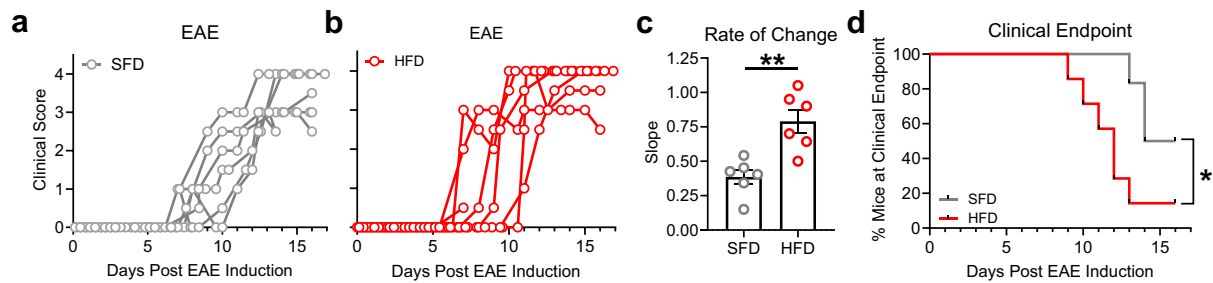
Extended Data Fig. 3 | SR9009 dose-dependently decreases IL-17A and IL-17F expression by lymph-node $\gamma\delta$ T cells. **a**, Circadian time plot of percentage CD27 and CD27 $^+$ $\gamma\delta$ T cell subsets in adipose tissue, with **b**, subset cell numbers of 24 h. **c**, Lymph-node lymphocyte viability with increasing concentration of SR9009. **d**, Percentage of lymph-node $\gamma\delta$ T cell of total lymphocytes with increasing concentrations of SR9009. **e**, Representative flow plots of ROR γ t expression by lymph-node $\gamma\delta$ T cell with increasing concentration of SR9009, with **f**, percentage and **g**, MFI of ROR γ t expressed by lymph-node $\gamma\delta$ T cells. **h**, Representative flow plots of IL-17A expression by lymph-node $\gamma\delta$ T cell with increasing concentration of SR9009, with **i**, percentage and **j**, MFI of IL-17A expressed by lymph-node $\gamma\delta$ T cells. **k**, Percentage and **l**, MFI of IL-17F expressed by lymph-node $\gamma\delta$ T cells with increasing concentration of SR9009. **m**, Percentage of IL-17A expressed by

lymph-node $\gamma\delta$ T cells with increasing concentration of vehicle. **n**, $\gamma\delta$ T cell and $\alpha\beta$ T cell percentage of total lymphocytes in *Arntl* fl (grey) and *Arntl* $^{\Delta Vav1}$ (red) mice. **p**, IL-17R histogram of adipose T cells. **q**, $\gamma\delta$ T cell percentage of total lymphocytes in *Arntl* fl (grey) and *Arntl* $^{\Delta Vav1}$ (red) mice. **r**, Percentage IL-17A of adipose $\gamma\delta$ T cells from *Arntl* fl (grey) and *Arntl* $^{\Delta Vav1}$ (red) mice. **a, b, n-r**, Data shown as mean $\pm$ SEM, $n = 4-8$ mice per group. **c, d, f, g, i-o**, Data shown as mean $\pm$ SEM, $n = 9$ technical replicates from 3 mice per group. **a, b**, Significance was calculated using cosinor analysis, with cosine fitted curves, amplitude (Amp) and acrophase (Acro) were extracted from the cosinor model. **c, d, f, g, i-m, p**, One-way ANOVA, **s, t**, Two-tailed unpaired students t-test. NS = Non-Significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.



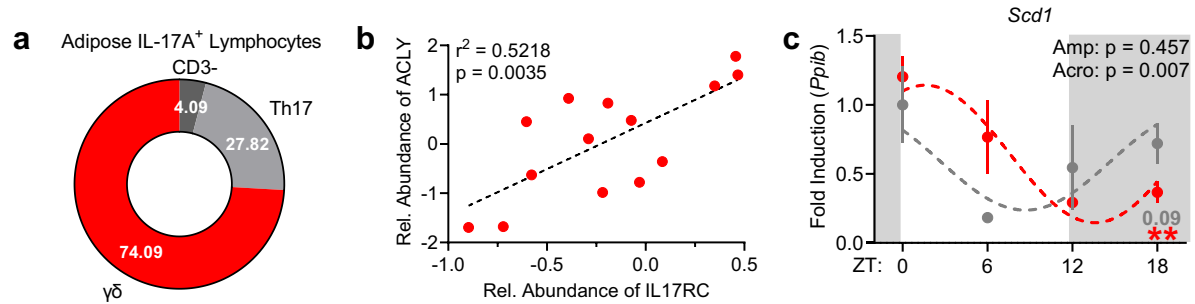
Extended Data Fig. 4 | Whole-body metabolic rate is entrained by light and disrupted by irregular feeding. **a**, Time plot of metabolic rate parameter (oxygen consumption) of mice on a regular light cycle (grey; lights on 7am – 7pm), versus mice on an inverted light cycle (red; lights on 7pm – 7am) after 3 weeks on these light cycles. **b**, Time plot of metabolic rate parameter (oxygen consumption) of mice on a regular feeding cycle (grey; feeding 7pm – 7am), versus mice on a reverse feeding cycle (blue; feeding 7am – 7pm) after 3 weeks on these feeding regimens. **c**, Time plot of metabolic rate parameter (oxygen consumption) of mice on an ad libitum (grey), versus mice on a 50% CR feeding regimen (orange) after 1 week on these feeding regimens. **d**, Time plot of energy expenditure of mice on an ad libitum (grey), versus mice on a 50% CR feeding regimen (orange) after 1 week on these feeding regimens. **e**, Per cent feeding during day or night of SFD and HFD-fed mice. **f**, Representative

histograms (left), of adipose $\gamma\delta$ T cells from SFD (grey) or HFD (red) fed mice with circadian time plots of ROR γ t MFI (right). **g**, Diagrammatic representation of experimental set-up for long-term HFD feeding regimen experiments. **h**, Time plot of weight gain over a 16-week HFD feeding regime. White and grey panels represent light and dark periods respectively. Data are representative of **a–d** three independent experiments, or **e, f**, two independent experiments. **a–h**, data shown as mean $\pm$ SEM, $n = 4–9$ mice per group. **f**, Significance was calculated using cosinor analysis, with cosine fitted curves, amplitude (Amp) and acrophase (Acro) were extracted from the cosinor model, **e**, Two-tailed unpaired students t-test, **h**, two-way repeated measures ANOVA followed by Bonferroni's multiple comparisons test. NS, Non-Significant, ** $p < 0.01$, *** $p < 0.001$.



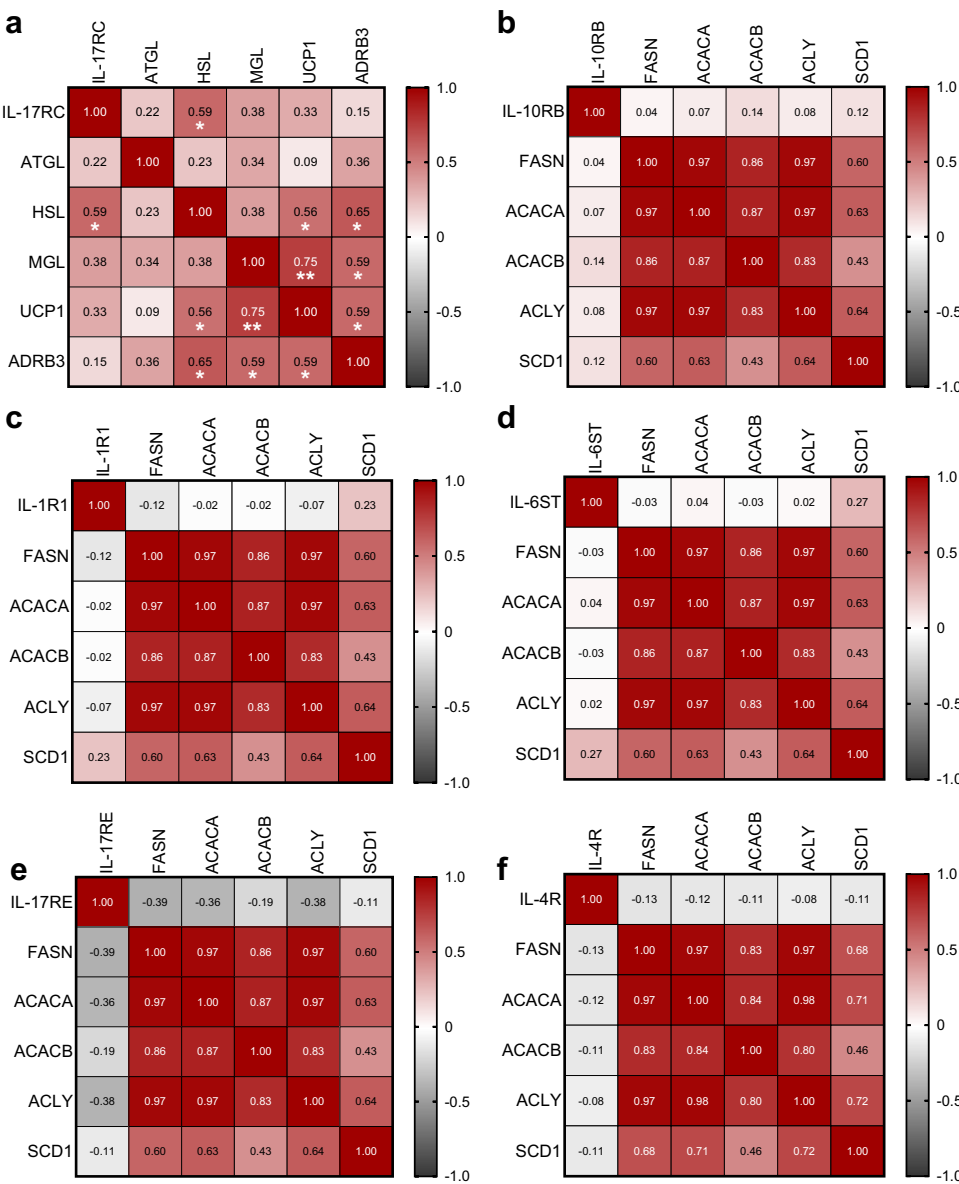
Extended Data Fig. 5 | A three-week HFD feeding regime increases the onset and severity of EAE. a, EAE scores of SFD-fed (grey), and **b,** HFD-fed (red) mice. **c,** Disease progression measured by rate of change (disease onset to disease peak) between feeding regimes. **d,** Time to clinical end-point post EAE induction between feeding regimes. Data are representative of two independent

experiments. **a–d,** data shown as mean $\pm$ SEM, $n = 6$ mice per group. Significance was calculated using **b,** two-way repeated measures ANOVA followed by Bonferroni's multiple comparisons test, **c,** Two-tailed unpaired students t-test, **d,** Simple survival analysis with Gehan-Breslow-Wilcoxon test. * $p < 0.05$, ** $p < 0.01$.



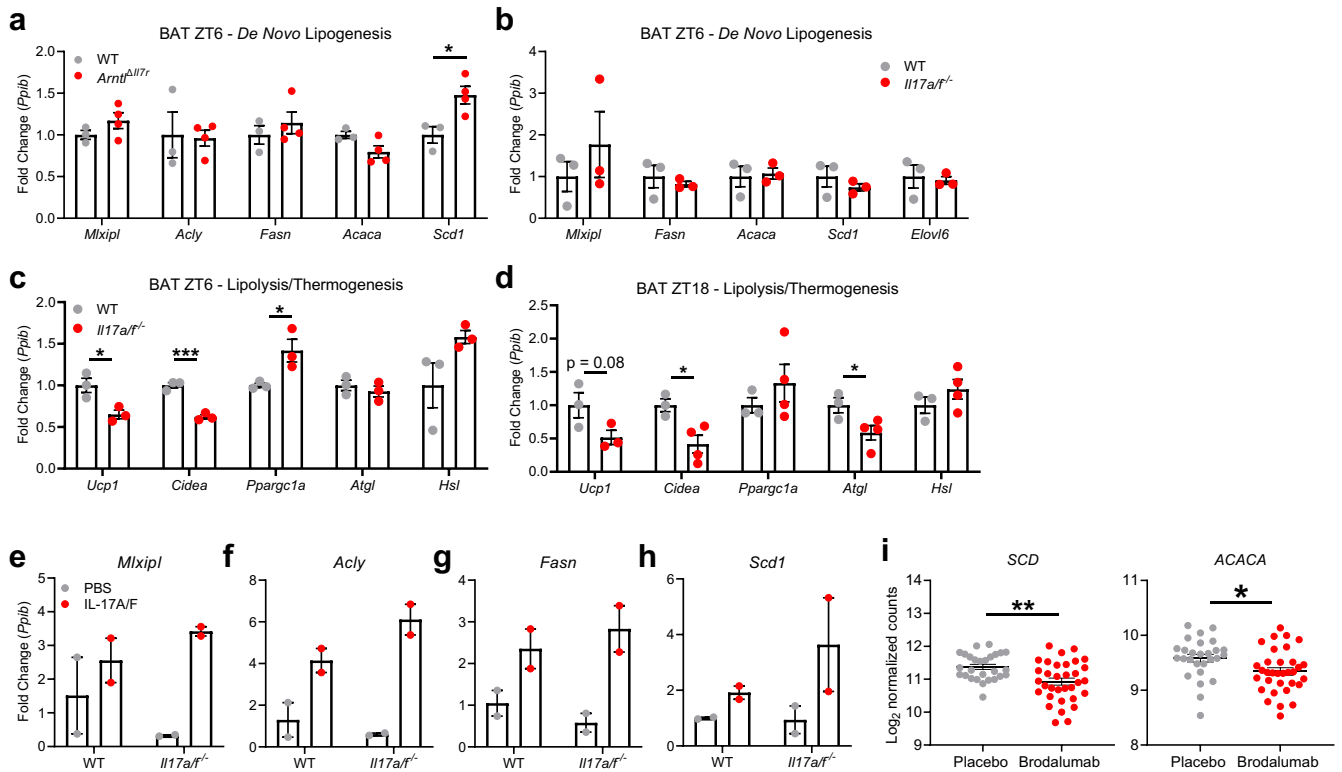
Extended Data Fig. 6 | $\gamma\delta$ T cells are the main source of adipose tissue IL-17A, and maintain adipose tissue DNL. **a**, Pie chart depicting adipose lymphocytes contributing to total IL-17A expression, $\gamma\delta$ T cells (red), non-T lymphocytes (dark grey) and Th17 cells (light grey). **b**, Correlation analysis of BAT IL-17RC protein levels with ACLY abundance from OPABAT. **c**, Circadian time plots showing relative expression of the DNL gene *Scd1* from BAT of wild-type (grey)

and *Tcr δ ^{-/-}* (red) mice. **c**, White and grey panels represent light and dark periods respectively. **c**, Data are representative of two independent experiments. **c**, data shown as mean $\pm$ SEM, $n = 4-14$ mice per group. **b**, Significance was calculated using a simple linear regression, **c**, cosinor analysis, with cosine fitted curves, amplitude (Amp) and acrophase (Acro) were extracted from the cosinor model. ** $p < 0.01$.



Extended Data Fig. 7 | BAT DNL proteins do not correlate with the expression of other cytokine receptors. a, Correlation heat map of IL-17RC protein levels with adipocyte lipolysis-associated protein abundance from OPABAT. **b**, Correlation heat map of IL-10RB protein levels with DNL protein abundance from OPABAT. **c**, Correlation heat map of IL-1R1 protein levels with DNL protein

abundance from OPABAT. **d**, Correlation heat map of IL-6ST protein levels with DNL protein abundance from OPABAT. **e**, Correlation heat map of IL-17RE protein levels with DNL protein abundance from OPABAT. **f**, Correlation heat map of IL-4R protein levels with DNL protein abundance from OPABAT. **a–f**, Significance was calculated using simple linear regression. * $p < 0.05$, ** $p < 0.01$.



Extended Data Fig. 8 | IL-17A regulates DNL and lipolysis gene expression.

a, Relative expression of DNL genes *Mlxipl*, *Fasn*, *Acaca*, *Scd1* and *Elovl6* in the BAT of *Arntl^{fl}* mice compared to BAT of *Arntl^{Δ17r}* mice at ZT6. **b**, Relative expression of DNL genes *Mlxipl*, *Fasn*, *Acaca*, *Scd1* and *Elovl6* in the BAT of wild-type mice compared to BAT of *Il17a/f^{-/-}* mice at ZT6. **c**, Relative expression of lipolysis/thermogenesis genes *Ucp1*, *Cidea*, *Ppargc1a*, *Atgl* and *Hsl* in the BAT of wild-type mice compared to BAT of *Il17a/f^{-/-}* mice at ZT6. **d**, Relative expression of lipolysis/thermogenesis genes *Ucp1*, *Cidea*, *Ppargc1a*, *Atgl* and

Hsl in the BAT of *Arntl^{fl}* mice compared to BAT of *Arntl^{Δ17r}* mice at ZT18. **e-h**, Relative expression of *Mlxipl* (**e**), *Acly* (**f**), *Fasn* (**g**) and *Scd1* (**h**) after administration of IL-17A (1 μg) and IL-17F (1 μg) to wild-type and *Il17a/f^{-/-}* mice compared to PBS administered controls. **i**, Expression of *ACACA* and *SCD* from skin biopsies of patients with psoriasis who were treated with placebo and brodalumab. **a-i**, Data shown as mean ± SEM, n = 2–5 mice per group, or n = 27–33 patients per group. **a-d,i**, Significance was calculated using Two-tailed unpaired student's t-test. *p < 0.05, **p < 0.01, ***p < 0.001.

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	- Flow cytometry data was acquired using FACSDiva software, FACs Canto II, LSR Fortessa and cell sorting on FACSARIA Fusion Sorter (BD Biosciences). - Indirect calorimetry data was recorded using Promethion metabolic cages metascreen (Sable Systems) or by Comprehensive Lab Animal Monitoring System (CLAMS; Columbus Instruments). - PCR data was collected on StepOne and QuantStudio 5 Real-Time PCR systems (Applied Biosystems) . - Single-cell RNA sequencing dataset was generated using the 10x Genomics Chromium Platform and Illumina NextSeq 500 Platform. - Western blot data was collected using Biorad Gel Doce imaging system.
Data analysis	- Flow cytometry data was analyzed using FlowJo v10 Software - Metabolic Cage data was analyzed using the One-Click Macro V2.32.2 (Sable Systems) and CalR Version 1.3 (https://calrapp.org/) - Single-cell RNA sequencing data was analyzed using RStudio and R Version 4.1.2, R package Seurat Version 4.1.0 - PCR data was analyzed using QuantStudio Design and Analysis Software - Circadian analysis was performed using R version 4.2.3 with R packages Cosinor Version 1.2.2 and Cosinor 2 Version 0.2.1 - Prism 9.3.0 - Western blot images were quantified using ImageJ.

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The sequencing data generated for this study will be deposited in a GEO repository in the future.

Research involving human participants, their data, or biological material

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Reporting on sex and gender

N/A

Reporting on race, ethnicity, or other socially relevant groupings

N/A

Population characteristics

N/A

Recruitment

N/A

Ethics oversight

N/A

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

No sample size calculation was performed. The sample size was determined from similar studies in the literature and by performing pilot studies. Experiments were replicated based on the signal-to-noise ratio of the data.

Data exclusions

Outliers were excluded based on outlier detection by Prism 9 software, or data from failed experiments.

Replication

All experiments were replicated to support the conclusions put forward in the manuscript. All experiments were replicated at least twice unless otherwise stated, with multiple animals. We also provide a detailed description of methods for each experiment.

Randomization

Mice were randomly assigned to experimental groups, but ensuring that age and weight were always matched between the groups, particularly for metabolic cage experiments.

Blinding

No blinding was performed during data collection and analysis.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Anti-mouse antibodies used for flow cytometry:

From eBioscience - Anti-CD4 (APCef-780; RM4-5; 1:200) #47-0042-82, Anti-CD27 (PE; LG.7F9; 1:200) #12-0279-42, anti-NFIL3 (PE; S2M-E19; 1:200) #12-5927-82, anti-CD127 (PE-Cy5; A7R34; 1:200) #15-1271-82, anti-TCRd (PE-Cy7; GL-3; 1:200) #25-5711-82,

From BioLegend - Zombie Aqua Viability Dye (1:1000) #423101, anti-CD45 (BV605; 30-F11; 1:200) #103139, anti-CD3 (FITC; 17A2; 1:200) #100203, anti-CD8 (BV650; 53-6.7; 1:200) #100741, anti-CD27 (APC-Cy7; LG.3A10; 1:200) #124225, anti-IL-17A (BV650; TC11-18H10.1; 1:200) #506929, anti-IL-17A (BV421; TC11-18H10.1; 1:200) #506925, anti-IL-17A (PE; TC11-18H10.1; 1:200) #506903, anti-IL-17F (AF647; 8F5.1A9; 1:200) #517104, anti-IFN γ (PE-Cy7; XM61.2; 1:200) #505825, anti-IFN γ (BV785; XM61.2; 1:200) #505837

From BD Biosciences - anti-RORyt (BV421; Q31-378; 1:200) #562894

Validation

All antibodies used in this study are commercially available and used as per the manufacturers instructions. Further validation was performed by using fluorescence minus one (FMO) stained controls.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals

Mice used during this study were bred in-house or were purchased from Jackson Laboratories. Male mice between 8-12 weeks of age were used, and housed under a 12-hour/12-hour light/dark cycle at an ambient temperature of 22 degrees Celsius with 52-55% humidity.

- C57BL/6JOhlsd wild-type mice
 - C57BL/6J wild-type mice (JAX)
 - Tcrd^{-/-} B6.129P2-Tcrdtm1Mom/J (JAX: 002120)
 - Il17a GFP C57BL/6-Il17atm1Bcgen/J (JAX: 018472)
 - Il17a/f⁻ B6.Cg-Il17a/Il17ftm1.1Impr Thy1a/J (JAX: 034140)
 - Vav1Cre, Il7rCre, Per1Venus and Arntlfl mouse lines were bred on a full C57BL/6J background at Champalimaud Centre for the Unknown (CCU)

Wild animals

There were no wild animals used during this study.

Reporting on sex

All mice used during this study were male mice.

Field-collected samples

There were no field-collected samples used during this study.

Ethics oversight

All animal work was performed in compliance with the Lynch laboratory project licence, with ethical approval from the Trinity College Dublin ethics committee and the Animal Research Ethics Committee from the Health Products Regulatory Authority (HPRA), and the Brigham and Women's Hospital Institutional Animal Care and Use Committee guidelines. Experiments involving Vav1Cre 47, Il7rCre, Per1Venus 48 and Arntlfl 49 mouse lines were approved by national and local institutional review boards (IRBs), Direção Geral de Veterinária and CCU ethical committees.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

Adipose tissue was removed, minced with a razor, and digested in a 1mg/ml Collagenase Type II (Worthington) dissolved in RPMI, in a shaking incubator for 25-30 minutes at 37°C. Digested cells were filtered through a 70µM nylon mesh and centrifuged at 300g for 5 minutes to pellet the stromovascular fraction (SVF). Lymph nodes were disrupted through a 70µM filter and centrifuged for 5 minutes at 300g to pellet. Post processing adipose SVF and lymph node leucocytes were stimulated with phorbol 12-myristate 13-acetate (PMA; 10ng/ml; Sigma), ionomycin (500ng/ml; Sigma) and brefeldin A (BFA; 5µg/ml; Sigma) in cRPMI medium, incubated at 37°C for 3 hours. For cytokine stimulations, adipose SVF was stimulated with rIL-23 (10ng/ml; Miltenyi), rIL-1β (10ng/ml; Immunotools) with BFA (5µg/ml) for 3 hours.

Instrument

FACSCanto II, LSR Fortessa, FACSria Fusion Sorter (BD Biosciences).

Software

Flow cytometry data was collected using FACSDiva software (BD Biosciences) and analysed using FlowJo v10

Cell population abundance

Sorted populations were used for further analysis if >95% pure.

Gating strategy

Lymphocytes were discriminated based on FSC-A and SSC-A, then single cells were selected based on FSC-H and FSC-A. Live lymphocytes were then gated on as a ZombieAqua negative, CD45 positive (L/D- vs CD45+) population. From the live CD45+ lymphocyte population, CD4 T cells were defined as CD3+CD4+ cells, and CD8 T cells were defined as a CD3+CD8+ population. Furthermore, the IL-17A-producing population of γδ T cells were defined as CD45+TCRδ+CD27- and IFNγ-producing population defined as CD45+TCRδ+CD27+. IL-17A, IFNγ, RORγt and IL-7R positive and negative populations were gated based on FMO controls.

- ☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.