

A cross-tissue single-cell and multiomics atlas of cold induced immunometabolic remodeling

Guoqing Wang

qing@jlu.edu.cn

Key Laboratory of Pathobiology Ministry of Education, China-Japan Union Hospital of Jilin University, Jilin University, Changchun, China <https://orcid.org/0000-0001-9326-3755>

Zecheng Chang

Key Laboratory of Pathobiology Ministry of Education, China-Japan Union Hospital of Jilin University, Jilin University, Changchun, China

Jiahui Pan

Department of Cell Biology, School of Basic Medical Sciences, Harbin Medical University, Harbin, Heilongjiang, China

Qin Zhou Dong

College of Mathematics, Jilin University, Changchun, China

Pengfei Hao

Changchun Veterinary Research Institute, Chinese Academy of Agricultural Sciences

Mingying Liu

State Key Laboratory for Diagnosis and Treatment of Severe Zoonotic Infectious Diseases, College of Basic Medical Sciences, Jilin University

Xinyu Hu

State Key Laboratory for Diagnosis and Treatment of Severe Zoonotic Infectious Diseases, College of Basic Medical Sciences, Jilin University

Tingting Zhao

State Key Laboratory for Diagnosis and Treatment of Severe Zoonotic Infectious Diseases, College of Basic Medical Sciences, Jilin University

Zhuoyuan Xin

State Key Laboratory for Diagnosis and Treatment of Severe Zoonotic Infectious Diseases, College of Basic Medical Sciences, Jilin University

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A cross-tissue single-cell and multiomics atlas of cold induced immunometabolic remodeling

Zecheng Chang^{1,2†}, Jiahui Pan^{4†}, Qinzhou Dong^{2,3}, Pengfei Hao², Mingying Liu², Xinyu Hu², Tingting Zhao², Zhuoyuan Xin^{2*}, Guoqing Wang^{1,2*}

1 Key Laboratory of Pathobiology Ministry of Education, China-Japan Union Hospital of Jilin University, Jilin University, Changchun, China

2 State Key Laboratory for Diagnosis and Treatment of Severe Zoonotic Infectious Diseases, Key Laboratory for Zoonosis Research of the Ministry of Education, College of Basic Medical Sciences, Jilin University, Changchun, China

3 College of Mathematics, Jilin University, Changchun, China

4 Department of Cell Biology, School of Basic Medical Sciences, Harbin Medical University, Harbin, Heilongjiang, China

† Equal contribution.

* Address correspondence to Zhuoyuan Xin, xinzy@jlu.edu.cn and Guoqing Wang, qing@jlu.edu.cn

Abstract

Cold exposure is a defined environmental perturbation that rapidly induces organism-wide metabolic reprogramming and challenges immune homeostasis. To characterize how cold exposure reshapes immune and metabolic states across tissues, we established a mouse model of cold exposure and generated a multidimensional single-cell atlas comprising 501,543 single cells from nine tissues. We then further integrated this atlas with 4D proteomic and succinylomic datasets generated at the tissue level. Cross-tissue comparisons revealed widespread but highly tissue selective immune remodeling. Macrophages showed pronounced responses to cold exposure, with particularly extensive changes in macrophage abundance and transcriptional states in the lung. Within this response, we identified a cold associated macrophage subpopulation characterized by enhanced metabolic programs and reduced immune and inflammatory programs. Integration of the transcriptomic, proteomic and succinylomic datasets identified *Chil3* as a candidate multiomics node linked to this macrophage reprogramming program. Together, this study provides a valuable resource and an integrative framework for understanding how environmental stress reshapes organismal immunometabolic homeostasis.

Keyword

Cold exposure; Single-cell atlas; Multiomics integration; Transcriptomics; Immunometabolism; Macrophage

Introduction

Cold exposure places sustained environmental stress on mammalian physiology by altering energy metabolism and tissue homeostasis¹⁻³. To maintain core body temperature, mammals rapidly engage coordinated adaptive responses, including neuroendocrine regulation, muscle shivering, lipid mobilization, and nonshivering thermogenesis⁴⁻⁸. Thermogenesis during cold exposure therefore depends on metabolic coordination among tissues. Brown adipose tissue (BAT) is a principal effector of cold induced thermogenesis, whereas sustained thermogenic activity also requires glucose and fatty acids supplied by the liver, white adipose tissue (WAT), the circulation and other metabolic tissues⁹. Together, these findings support that cold adaptation is systemically coordinated by multiple organizations.

Studies in individual tissues further indicates that cold exposure can reshape local immune function. In obese mice, cold exposure promotes BAT to produce maresin 2 (MaR2), which contributes to inflammation resolution by targeting macrophages¹⁰. At respiratory barriers, reduced temperature weakens epithelial antiviral defenses and permits greater rhinovirus replication in mouse airway epithelial cells¹¹. Cold exposure also impairs the antiviral activity of extracellular vesicles released through TLR3 signaling in human nasal epithelium¹². Macrophages couple cellular metabolism and lipid handling to inflammatory regulation and antigen presentation, thereby occupying an important position in the adaptive response to cold exposure¹³⁻¹⁶. An early study reported that cold exposure induces IL-4 dependent alternative macrophage activation, promoting thermogenic gene expression in BAT and lipolysis in WAT¹⁷. These studies indicate that the immune system is not merely a passive target of cold stress but an active participant in tissue metabolic adaptation and host-defense remodeling.

However, immune functions, including immune surveillance, inflammatory responses, and immune cell renewal, also depend on continuous energy supply, nutrient availability, and supportive tissue microenvironments^{14,18,19}. From a resource allocation perspective, immune defense is energetically demanding and may compete with other physiological maintenance programs for limited energetic and nutritional resources¹⁹. Consequently, cold induced thermogenesis may alter the allocation of resources

available to immune functions²⁰.

Recent single-cell and multiomics atlases have enabled high resolution dissection of how tissue ecosystems are organized and remodeled during development, aging, sex-specific physiology, and disease^{21–25}. Unlike these gradual processes and those associated with disease, cold exposure represents a systemic environmental perturbation with a defined thermogenic output, capable of eliciting rapid cross-tissue energy redistribution and microenvironmental remodeling within a short timeframe²⁶. Although previous studies have characterized local immunometabolic responses to cold exposure within individual tissues or cell types^{27–29}, such focused analyses may obscure the full landscape of the systemic response. Thus, a cross-tissue atlas is needed to characterize how immune cell states change across tissues and relate to broader immunometabolic remodeling during cold exposure.

Here, we established a mouse model of cold exposure, generated a multidimensional single-cell atlas and integrated this atlas with 4D proteomic and succinylomic datasets generated at the tissue level. This panoramic atlas resolves both shared and tissue selective immune and metabolic responses to cold exposure and identifies macrophages as one of the most responsive immune compartments. We further identified a cold-associated macrophage subpopulation characterized by enhanced metabolic programs and reduced inflammatory, antigen presentation, and adaptive immune signatures. Integration of the transcriptomic, proteomic and succinylomic datasets identified *Chil3* as a candidate molecule associated with this macrophage state across multiple molecular layers. Together, these data provide a systematic resource for understanding cross-tissue immunometabolic remodeling under cold exposure and establish a framework for investigating how environmental temperature reshapes organismal immunometabolic homeostasis and tissue inflammatory states.

RESULT

Cold exposure reshapes physiology and immune homeostasis

To explore the impact of cold exposure on organismal homeostasis, we exposed C57BL/6 mice, following an acclimatization period, to 4°C for 7 consecutive days and subsequently used H3N2 influenza A virus (IAV) infection as an immune challenge (Fig. 1a). Mice exposed to cold rapidly diverged from control mice in their body weight and temperature trajectories. Body weight declined from day 2 onward. Although body weight partially recovered at later time points, it remained below that of control mice for the remainder of the exposure period. Body temperature decreased within 24h of cold exposure and remained lower thereafter (Fig. 1b). These longitudinal changes showed a sustained physiological response to cold exposure. We next examined whether cold exposure was accompanied by changes in individual organs. Cold exposure increased the relative weights of the lung and heart but reduced that of the thymus (Fig. 1c). Gross examination of freshly isolated organs further revealed macroscopic differences in the lung, heart and thymus between Cold exposure group and Control group (Supplementary Fig. 1a). Histological analysis showed that cold exposure alone altered lung architecture, with thickened alveolar septa, increased pulmonary cellularity and focal vascular congestion relative to controls (Fig. 1e). These findings indicate that the physiological response to cold extends beyond thermoregulation and is accompanied by organ selective phenotypic changes. Cold exposure was also associated with compartment specific changes in immune mediator concentrations. In serum, IFN- γ concentrations were reduced, whereas IP-10, IL-1 β and IL-6 concentrations were increased (Fig. 1d). In the spleen, concentrations of IFN- γ , IP-10, IL-1 β and IL-6 were all increased after cold exposure (Supplementary Fig. 1b). Collectively, the serum and splenic measurements showed cold exposure did not produce a uniform state of immune activation or suppression. Instead, cold exposure altered immune homeostasis in a compartment dependent manner.

We next asked whether these cold induced physiological and immune changes altered the outcome of a subsequent infectious challenge. Cold exposure alone caused no mortality during the observation period, but it markedly worsened the outcome of

H3N2 IAV infection. Some mice infected with H3N2 IAV under control housing conditions survived, whereas all mice exposed to cold before H3N2 IAV infection reached the predefined survival endpoint by day 5 after infection (Fig. 1f). This accelerated disease course was accompanied by increased infectious viral titers and greater H3N2 viral RNA abundance in the lung (Fig. 1g, h), demonstrating that cold exposure increased pulmonary viral burden. Consistent with this increased disease severity, Cold exposure & H3N2 IAV group showed higher splenic protein levels of IFN- γ , IP-10, IL-6 and IL-1 β than H3N2 IAV group. Transcript levels of the corresponding inflammatory mediators were also increased in the spleen (Supplementary Fig. 1c, d). Lung histology further showed more pronounced pathological features in Cold exposure & H3N2 IAV group, including increased inflammatory cell accumulation, disruption of alveolar architecture and areas of hemorrhage, relative to mice infected under control housing conditions (Supplementary Fig. 1e). Together, these results show that sustained cold exposure alters physiological and immune homeostasis and is associated with increased susceptibility to subsequent H3N2 IAV infection.

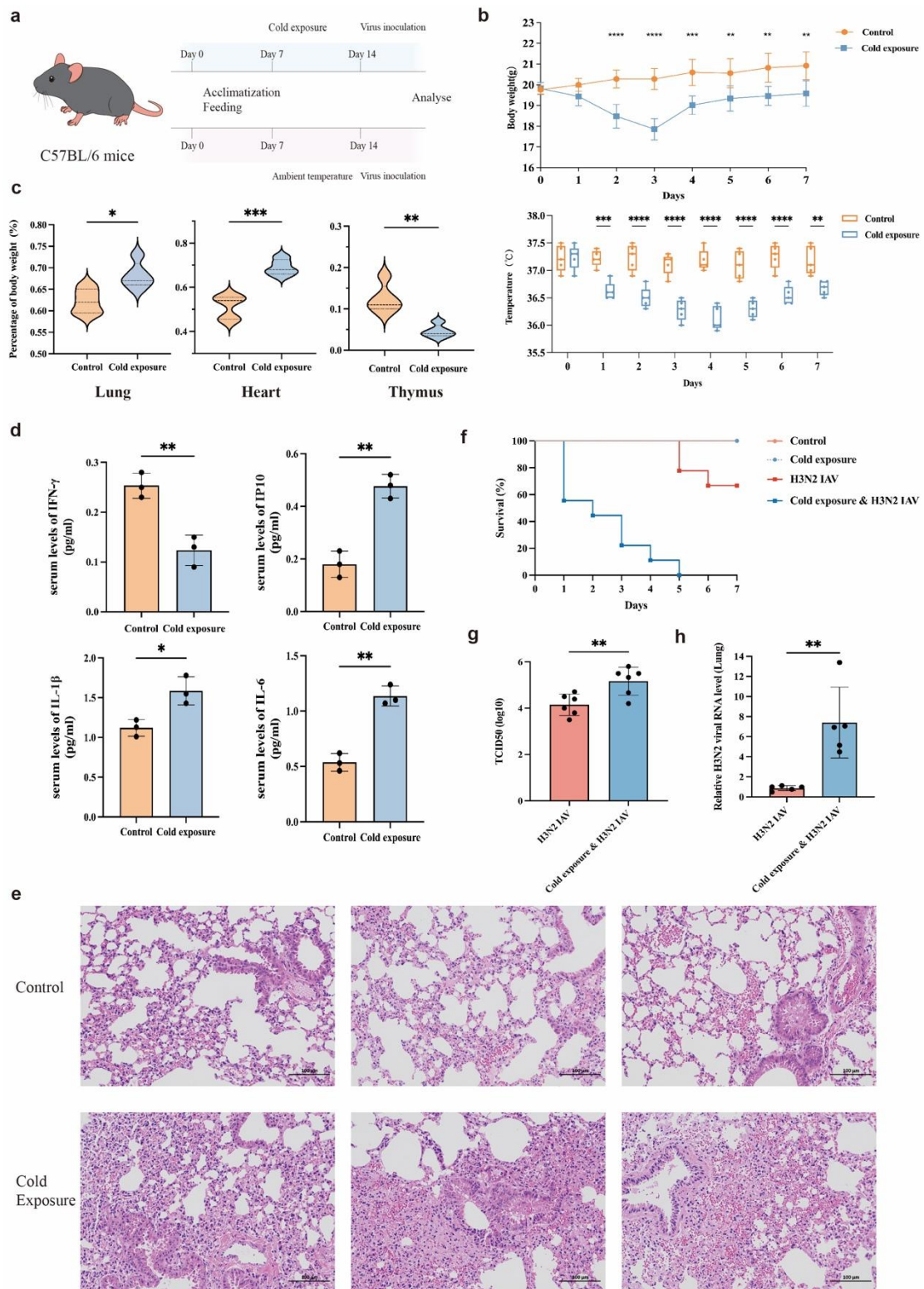


Figure 1 Cold exposure reshapes physiological and immune homeostasis and increases susceptibility to subsequent H3N2 IAV infection. **a**, Schematic of the experimental design. **b**, Body weight and body temperature during the 7 days exposure period ($n = 5$ per group). **c**, Lung, heart and thymus weights normalized to body weight after 7 days of exposure ($n = 5$ per group). **d**, Serum concentrations of IFN- γ , IP-10, IL-1 β and IL-6 after 7 days of exposure, measured by ELISA ($n = 3$ per group). **e**, Representative hematoxylin and eosin (H&E) stained lung sections from the

Control group and Cold exposure group (n = 3 per group, scale bars: 100 μ m). **f**, Kaplan–Meier survival curves for the Control, Cold-exposure, H3N2 IAV and Cold-exposure & H3N2 IAV groups (n=9 per group). **g**, H3N2 IAV titers in lung tissue from the H3N2 IAV and Cold exposure & H3N2 IAV groups, measured by 50% tissue culture infectious dose assay (TCID₅₀, n = 6 per group). **h**, RT–qPCR analysis of H3N2 IAV matrix gene (M) RNA abundance in lung tissue from the H3N2 IAV group and Cold exposure & H3N2 IAV group. Viral RNA abundance was normalized to *Gapdh* and expressed relative to the H3N2 IAV group (n = 5 per group). Each point represents one biologically independent mouse. Shown is mean $\pm$ SD. Significance is represented with * P < 0.05, ** P < 0.01, *** P < 0.001, and **** P < 0.0001.

A cross-tissue single-cell transcriptomic atlas of cold exposure

To systematically characterize how cold exposure reshapes organismal physiology at the cellular and molecular levels, we performed single-cell RNA sequencing (scRNA-seq) of tissues from C57BL/6 mice maintained at ambient temperature or exposed to 4 °C for 7 consecutive days (Fig. 2a). The sampling strategy covered nine tissues and cell compartments representing neuroendocrine, immune, hematopoietic and peripheral systems, including hypothalamus, thymus, peripheral blood mononuclear cells (PBMCs), heart, lung, spleen, liver, kidney and bone marrow (Fig. 2b). In each condition, each tissue contained at least three biological replicates (three mice) (Supplementary Table 1). After stringent quality control, doublet removal and exclusion of low-quality cells, we retained 501,543 high-quality cells, including 253,140 cells from Cold exposure group and 248,403 cells from Control group (Fig. 2c and Supplementary Table 1).

We performed organ-resolved analyses of uniform manifold approximation and projection (UMAP) defined major cell populations, compared the cellular proportions between the Control group and Cold exposure group within each tissue (Supplementary Fig. 2a and Supplementary Table 2). We selected the top three cell types with the largest proportional changes (absolute log₂ fold change in cell proportion, with cell proportion >1% in at least one group) in each tissue between the two groups (Fig. 2d). The top three cell types with the largest proportional differences between control and cold exposure tended to vary across tissues (Fig. 2d). For example, in the heart, dendritic cells, cardiomyocytes and neutrophils were among the populations showing the most pronounced proportional shifts; in the liver, endothelial cells and neutrophils

exhibited a higher proportion in Cold exposure group (Fig. 2d).

We identified differentially expressed genes (DEGs) (P adjusted < 0.05 and $|\log_2$ fold change (FC)| > 0.25) in each cell type across 9 tissues between Control group and Cold exposure group (Supplementary Table 3). The DEGs were minimally confounded by tissue-level compositional shifts and primarily reflected biological differences^{30,31}. We determined the top three cold-responsive cell types for each tissue by ranking the cell types according to the number of DEGs (Fig. 2e). The number of DEGs varied substantially across cellular populations, indicating heterogeneous transcriptional responses to cold exposure. For example, in lung B cells, cold exposure upregulated genes associated with lipid uptake and metabolic adaptation, including *Lpl*, *Cd36* and *Sgkl*, while concomitantly downregulating genes involved in immune signaling and NF- κ B associated responses, including *Rel*, *Nfkb1* and *Fyn* (Supplementary Fig. 2b).

We next performed Gene Ontology enrichment analyses (GO) of DEGs in each tissue and cell type combination, considering the direction of cold-induced changes (biological process, cellular component and molecular function; adjusted $P < 0.05$; Supplementary Table 4). These analyses identified shared and tissue specific functional annotations associated with the transcriptional responses to cold exposure. For example, hepatic CD8⁺ T cells showed induction of protein folding and chaperone-mediated stress response pathways, while genes downregulated by cold exposure were enriched for T cell activation, leukocyte cell–cell adhesion, type II interferon production and immune effector processes, consistent with previous findings^{20,32} (Supplementary Fig. 2c and Supplementary Fig. 2d).

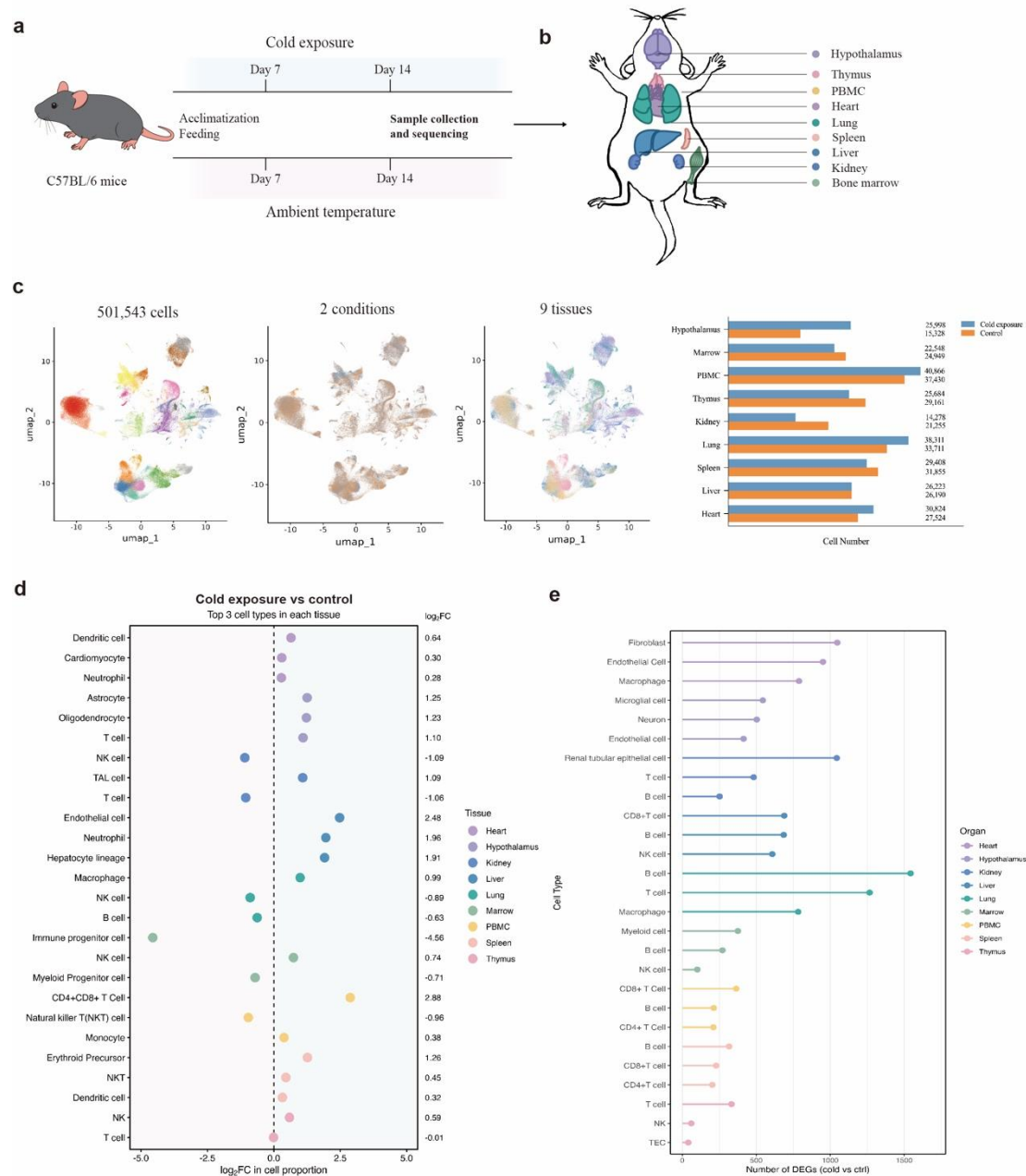


Figure 2 A cross-tissue single-cell transcriptomic atlas of cold exposure. **a**, Schematic overview of the experimental design for profiling cross-tissue responses to cold exposure. **b**, Construction of the single-cell transcriptomic landscape based on 9 tissues. **c**, UMAP plots showing cell clusters (left), experimental condition (middle) and tissue origin (right). The bar plot illustrates the distribution of high-quality single cells retained after quality control for each tissue and experimental group. Each experimental group included at least three biological replicates (three mice). **d**, Lollipop plot showing the top three cell types (cell proportion > 1% in at least one group) from each tissue with the largest absolute log₂ fold changes in cell proportion between two groups. NK, natural killer; TAL, thick ascending limb. **e**, Lollipop plot showing the top three cell types from each tissue with the largest numbers of DEGs based on the comparison of Cold exposure group vs Control group. TEC, thymic epithelial cell.

Cold exposure drives systemic remodeling of immune cell states

To systematically characterize immune remodeling across tissues after cold exposure, we extracted immune cells from the cross-tissue single-cell atlas and reintegrated them for comparative analysis. UMAP embedding demonstrated clear separation among major immune cell types and effective integration across tissues and datasets (Fig. 3a). Following stringent quality control, we obtained 173,182 high-quality immune cells from nine tissues, establishing an integrated cross-tissue immune atlas that captured eight major immune compartments: B cells, T cells, neutrophils, microglia, NK cells, macrophages, monocytes and dendritic cells (Fig. 3a and Supplementary Table 5). This atlas revealed pronounced tissue-specific shifts in immune composition between the Control group and Cold exposure group. For example, cold exposure was associated with a marked increase in the proportion of macrophages in the lung, whereas neutrophils underwent a pronounced proportional expansion in the liver (Fig. 3b and Supplementary Table 5).

We next subclustered each major immune cell types and identified 34 transcriptionally distinct cell states across tissues (Fig. 3c). We assessed the tissue preference of these subsets by calculating the ratio of observed to expected cell numbers ($R_{o/e}$)³³ (Fig. 3d). Developmental lymphoid states were enriched in their expected anatomical niches, including pre-B cells in bone marrow and immature thymocytes in thymus, consistent with previous studies^{34,35}. The lung contained multiple represented lymphoid and myeloid states, including T/NK/ILC, monocyte–macrophage and dendritic/APC subsets, suggesting a central role for this organ in cold induced immune remodeling (Fig. 3d). Consistently, Augur-based prioritization, a machine-learning framework that ranks cell states according to perturbation responsiveness, further identified the lung as a major site of cold-responsive immune remodeling³⁶. Direction-resolved analyses separated this response into two opposing transcriptional axes. Antigen-presenting cells, T cells and NK cells converged on a downregulated immune-response axis. In contrast, the lung monocyte–macrophage continuum was dominated by an upregulated remodeling axis, consistent with cold induced myeloid activation and state repolarization (Fig. 3e). Together, these analyses identified a tissue specific

immune responses to cold exposure.

To characterize the transcriptional basis of these tissue dependent responses, we examined cold associated expression programs across major immune lineages and their constituent states. Differential expression analysis across the eight major immune compartments identified both lineage dependent and recurrent responses to cold exposure. The number and direction of expression changes varied across lineages, whereas increased expression of *Fkbp5*, *Tsc22d3* and *Hspa1a* and decreased expression of *Rel*, *Spag9* and *Cd274* recurred in multiple immune compartments (Supplementary Fig. 3a and Supplementary Table 6). Functional enrichment analysis across the refined immune states identified cell-state dependent differences in cold-associated transcriptional programs. Myeloid populations showed a coherent shift towards metabolic programs and away from immune-activation, antigen-presentation and inflammatory-signaling pathways (Fig. 3f). The macrophage compartment was one of the most extensively remodeled immune compartments. Cold exposure promoted a metabolically rewired state characterized by enhanced fatty acid oxidation, PPAR signaling, lipid and cholesterol metabolism, and mitochondrial carbon metabolism, alongside broad attenuation of leukocyte activation, inflammatory-response, cytokine-signaling, cell-adhesion and interferon-associated programs (Supplementary Fig. 3b, c). Single-cell metabolic pathway analysis further indicated that cold exposure reshaped immune cell function through a coordinated metabolic program. Metabolic pathway scoring using scMetabolism³⁷ showed marked heterogeneity across the 34 immune cell states, with distinct lymphoid and myeloid states displaying differential engagement of glycolytic, mitochondrial, lipid-derived and amino acid catabolic programs (Fig. 3g).

Comparison of the two groups further resolved this response into a structured metabolic remodeling landscape, rather than a uniform shift across the immune compartment (Fig. 3h). Notably, macrophages showed one of the clearest cold induced metabolic shifts. This response was marked by increased scores for the pentose phosphate pathway, pyruvate metabolism, the citrate cycle, oxidative phosphorylation, fatty acid degradation, propanoate metabolism, branched-chain amino acid degradation and glutathione metabolism, together with reduced arachidonic acid and ketone-body

metabolic programs. This pattern suggests that cold exposure redirects macrophages towards a catabolic, oxidative and redox-buffering metabolic transcriptional program, rather than inducing uniform activation of lipid-associated metabolism (Fig. 3h). These findings indicate that cold exposure imposed a structured transcriptional and functional rewiring of the immune system, redirecting macrophages away from immune-effector and inflammatory programs and towards a catabolic, oxidative and redox-adaptive state. Together, these results identify macrophages as a prominent cold responsive immune compartment characterized by coupled changes in immune and metabolic transcriptional programs.

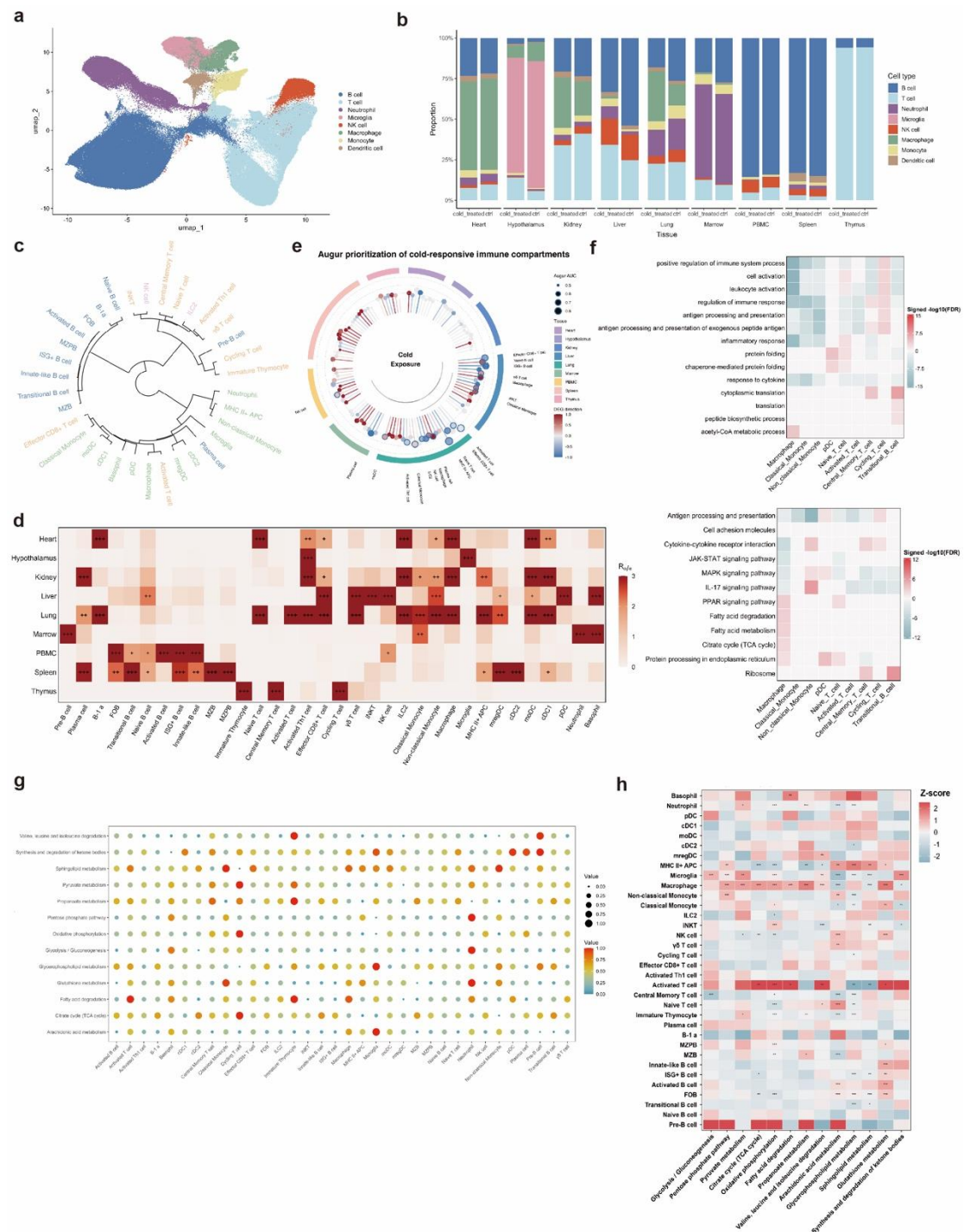


Figure 3 Cold exposure remodels immune cell states and metabolic programs across tissues.

a, UMAP visualization of immune cells integrated across tissues and conditions, colored by major immune lineage. **b**, Stacked bar plots showing the relative proportions of major immune cell types in the Control and Cold exposure groups across the heart, hypothalamus, kidney, liver, lung, bone marrow, PBMC, spleen and thymus. Cell-type colors match those in **a**. **c**, Unsupervised hierarchical clustering of 34 immune cell subsets colored by cell type. B1-a, B1-a B cells; cDC1, conventional type 1 dendritic cells; cDC2, conventional type 2 dendritic cells; mregDC, mature dendritic cells enriched in immunoregulatory molecules; pDC, plasmacytoid dendritic cells; FOB, follicular B cells; ILC2, group 2 innate lymphoid cells; ISG⁺ B cell, interferon-stimulated gene-positive B cells;

MHC II+ APC, MHC class II-positive antigen-presenting cells; MZB, marginal zone B cells; NK cell, natural killer cells; NKT, natural killer T cells; Pre-B cell, precursor B cells. **d**, Tissue distribution preferences of refined immune cell subsets measured by $R_{o/e}$ (the ratio of observed to expected cell numbers) (Methods). Immune cell states are grouped by major immune lineage. **e**, Augur based prioritization of tissue resolved immune cell states according to their transcriptional responsiveness to cold exposure. Each point represents a tissue-resolved immune cell state; point size indicates Augur AUC, the outer-ring color indicates tissue identity, and point color indicates the DEG-based direction score, with positive and negative values indicating predominant upregulation or downregulation in Cold exposure group, respectively. Black inner-ring outlines mark hotspot compartments defined by the top 10% of Augur AUC scores, and outer labels denote the top eight tissue-resolved immune cell states ranked by Augur AUC. **f**, Signed enrichment heat maps of representative GO biological processes (top) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (bottom) across selected cold-responsive immune subclusters. Colors indicate signed $-\log_{10}(\text{FDR})$ values, with red and blue denoting enrichment among genes upregulated and downregulated in cold-treated cells, respectively. **g**, Dot plot showing selected metabolic pathway activities across refined immune cell states. Dot size and color indicate normalized pathway values. **h**, Heat map showing cold associated differences in metabolic pathway scores across refined immune cell states. Metabolic pathway scores were calculated using scMetabolism and compared between the Control group and Cold exposure group within each immune subcluster. Colors denote z-scored changes in median pathway activity, calculated as cold exposure group minus the Control group. Significance is represented with *FDR < 0.05, **FDR < 0.01, ***FDR < 0.001.

Cold exposure rewires macrophage immunometabolism

Having identified macrophages as a prominent cold responsive population, we next examined their transcriptional heterogeneity in greater detail. We further carried out unsupervised clustering and used the expression patterns of subtype-specific markers to define 12 macrophage subsets, including macrophage subsets and monocyte-derived macrophage (MDM) subsets (Fig. 4a and Supplementary Fig. 4a). The 09_Macro_ISG+ subset was defined by the expression of interferon-stimulated genes, and the 10_Macro_Mki67+ subset, characterized by specific expression of *Mki67*, represented macrophages under proliferative states.

We also identified two MDM subsets, 11_MDMs_Plac8+Ccr2+, marked by *Plac8* and *Ccr2* expression, and 12_MDMs_Adgre4+Ace+, marked by *Adgre4* and *Ace* expression^{38–41}. Cold exposure was associated with subset specific differences in macrophage composition (Fig. 4b, c). Subset composition and differential abundance analyses revealed that the 01_Macro_Plet1+Atp6v0d2+ (hereafter C01) underwent the

most pronounced expansion after cold exposure, identifying C01 as the macrophage state most closely associated with cold induced compartmental remodeling (Fig. 4b, c). By contrast, 02_Macro_Tanc2+Lacc1+ and 06_Macro_Hspa1a+Cd4+ were relatively depleted (Fig. 4b, c). To assess whether the higher C01 proportion was associated with altered cell cycle programs, we calculated cell-cycle scoring across macrophage subsets. Notably, C01 showed no concomitant increase in cell cycle activity, suggesting that its increased abundance after cold exposure reflected selective remodeling of macrophage state composition rather than a generalized proliferative response. (Supplementary Fig. 4b).

To characterize gene expression programs across macrophage subsets, we performed gene set variation analysis (GSVA) across the 12 subsets⁴². GSVA distinguished macrophage states by the relative enrichment of metabolic and immune gene sets. C01 was distinguished by strong enrichment of lipid catabolism, fatty acid metabolism and lipid oxidation, together with organic-acid metabolism and PPAR signaling (Fig. 4d). In the group resolved analysis, C01 cells from the Cold exposure group retained high lipid-metabolic and PPAR signaling scores and showed a higher oxidative phosphorylation score than Control group (Supplementary Fig. 4c). By contrast, NF- κ B signaling, inflammatory-response, immune-effector and antigen-presentation programs were comparatively low in C01 (Fig. 4d). Together, these findings indicate that cold exposure selectively expands a metabolically biased C01 macrophage state, enhancing metabolic programs while attenuating immune-inflammatory and antigen-presentation signatures.

To determine the transcriptional relationships among macrophage states, we constructed a pseudotemporal trajectory using Monocle3⁴³. Monocle3 ordered macrophage subsets along a branching pseudotemporal trajectory, with metabolic gene programs associated with cold exposure enriched toward later pseudotime positions (Supplementary Fig. 4d). Module analysis along pseudotime identified opposing expression patterns. Inflammatory and antigen presentation programs were enriched toward earlier pseudotime positions, whereas lipid handling, PPAR related and mitochondrial metabolic programs were enriched toward later positions (Fig. 4e).

Representative gene kinetics supported this program-level switch, with antigen-presentation markers such as *Cd74* and *H2-Aa* declining as cold-remodeled metabolic markers such as *Chil3*, *Cd9* and *Pparg* increased along pseudotime (Supplementary Fig. 4e). These data indicate that cold exposure does not simply expand a preexisting macrophage subset. Instead, cold exposure can reshape macrophage state architecture along an immunometabolism trajectory, culminating in a metabolically adapted state characterized by enhanced lipid-mitochondrial metabolism and reduced immune-effector and antigen-presenting programs.

We next asked whether the cold induced metabolic specialization of macrophages was accompanied by a coordinated remodeling of immune activity. We quantified immune and metabolic gene expression signatures across macrophage states using MSigDB Hallmark immune gene set scoring⁴⁴ and scMetabolism based KEGG pathway scoring³⁷, respectively. Immune gene set scores varied across macrophage states, with C01 showing comparatively low scores for several immune programs (Supplementary Fig. 4f). Metabolically, C01 exhibited enhanced PPAR-linked lipid metabolism, oxidative phosphorylation and thermogenesis, redox metabolism and fatty-acid degradation, whereas canonical inflammatory, interferon and complement programs were reduced (Fig. 4f, g). Notably, the magnitude of metabolic program activity across C01 was significantly negatively correlated with Hallmark immune program activity (Spearman's $\rho = -0.37$, $P = 3.3 \times 10^{-120}$; Fig. 4h). Together, these results identify C01 as a key macrophage subset associated with the cold induced shift toward enhanced metabolic activity and attenuated immune function.

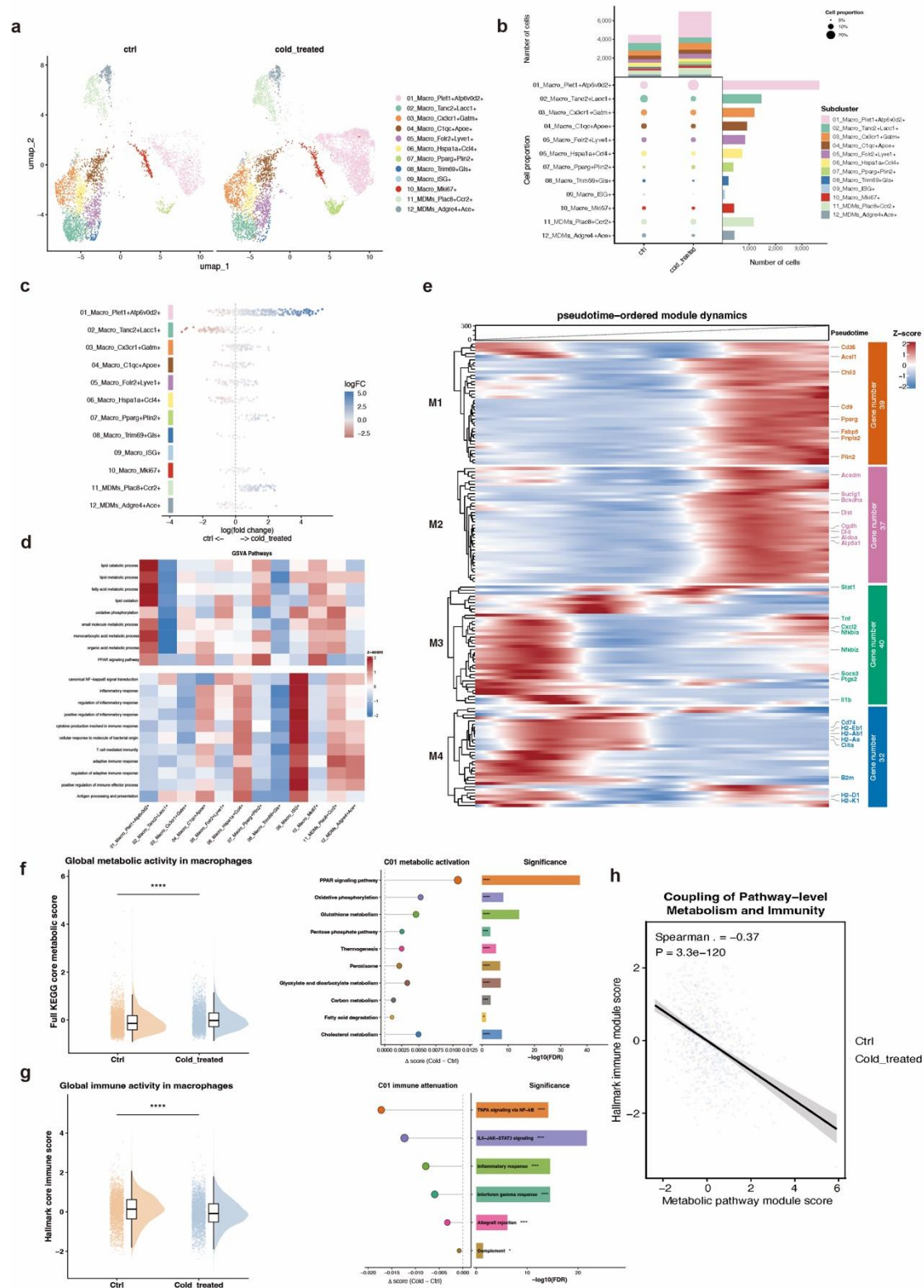


Figure 4 Cold exposure rewires macrophage immunometabolism. **a**, UMAP of macrophages from the Control group and Cold exposure group, colored by macrophage subset. **b**, Macrophage subset compositions in Control group and Cold exposure group inferred from single-cell transcriptomic data. Middle, bubble plot showing the relative proportion of each macrophage subset within control and cold-exposed conditions; circle size denotes the proportion of each subset, and circles are color-coded by macrophage subset as indicated in the legend. The stacked bar graph on

the top shows the cell-number composition of macrophage subsets within each condition. The histogram on the right shows the absolute number of cells in each macrophage subset. **c**, Violin plots show the differential abundance across major cell types. The 01_Macro_Plet1+Atp6v0d2+ (C01) showed the most pronounced expansion after cold exposure. **d**, GSVA heatmap of functional pathway activities across macrophage subsets. **e**, Pseudotime-ordered gene-module dynamics inferred by Monocle3. Heat map shows scaled expression of genes ordered along pseudotime, grouped into modules with distinct temporal dynamics. **f, g**, Metabolic and immune program activity in C01 macrophages. **f**, Metabolic program activity in C01 macrophages assessed by scMetabolism-based metabolic pathway scoring. Violin plots show the global metabolic activity score in control and cold-exposed C01 macrophages (left). Lollipop and bar plots show metabolic pathways differentially activated between cold-exposed and control C01 macrophages (right). **g**, Immune program activity assessed by MSigDB Hallmark immune-signature scoring. Violin plots show the global immune activity score in control and cold-exposed C01 macrophages (left), and lollipop and bar plots show immune programs differentially attenuated between cold-exposed and control C01 macrophages (right). Boxes indicate the median and interquartile range, with whiskers extending to $1.5 \times$ the interquartile range. In the lollipop plots, dot position indicates the mean score difference between cold-exposed and control C01 macrophages, dot size denotes $-\log_{10}(\text{FDR})$, and colors indicate individual metabolic pathways or immune programs. The adjacent bar plots show $-\log_{10}(\text{FDR})$. Significance is represented with * P or $\text{FDR} < 0.05$, ** P or $\text{FDR} < 0.01$, *** P or $\text{FDR} < 0.001$, and **** P or $\text{FDR} < 0.0001$. **h**, Correlation between pathway-level metabolic and immune module activity in C01 macrophages. Each point represents a single C01 macrophage and is colored by condition. Metabolic module scores were derived from KEGG metabolic pathways, and immune module scores were derived from curated MSigDB Hallmark immune signatures. Scores were adjusted for condition before Spearman correlation analysis. The black line indicates the fitted linear regression trend, with the shaded area denoting the 95% confidence interval. Spearman's correlation coefficient and associated P value are shown.

Cold exposure reveals a metabolically activated and immunologically attenuated macrophage state

Cold exposure imposed a lipid-oxidative transcriptional program within C01, marked by enhanced PPAR signaling, lipid handling, fatty-acid oxidation, oxidative phosphorylation and thermogenesis. This shift was accompanied by reduced antigen-presentation and inflammatory signaling modules, including NF- κ B and IL-17 related responses. *Chil3* was robustly upregulated in C01 macrophages following cold exposure, emerging as one of the most prominent cold induced transcripts within this state (Fig. 5a). Pathway analysis further substantiated these transcriptional patterns. Gene set enrichment analysis (GSEA)⁴⁴ showed enrichment of PPAR signaling, thermogenesis and oxidative phosphorylation in the Cold exposure group, whereas

antigen processing and presentation, IL-17 signaling and TNF–NF-κB signaling were enriched toward the Control group (Fig. 5b).

To determine whether these pathway changes formed coordinated co-expression patterns, we next constructed the C01 co-expression architecture using hdWGCNA⁴⁵. This analysis separated the cold response into two opposing network states. One module contained genes associated with PPAR related lipid handling, fatty acid oxidation, electron transport and redox metabolism and showed higher expression after cold exposure (Fig. 5c). Another module contained genes related to NF-κB activity, cytokine induction and innate immune sensing (Fig. 5c). Within this network, *Chil3* emerged as a prominent molecular feature whose expression was associated with increased lipid and mitochondrial metabolic gene programs and decreased inflammatory and antigen presentation programs. We next examined whether the opposing metabolic and immune programs within C01 were organized along an inferred transcriptional trajectory. Pseudotime analysis ordered C01 macrophages along a transcriptional continuum. Early pseudotime was associated with higher NF κB related and antigen presentation program scores. By contrast, later pseudotime was associated with higher *Chil3* expression and enrichment of PPAR signaling, lipid handling, and mitochondrial metabolic programs. (Fig. 5d). SCENIC analysis⁴⁶ identified prominent reductions in the activity of NF-κB family regulons associated with *Rel*, *Nfkb1* and *Relb*, together with reduced activity of the *Fosl2* associated AP-1 regulon in cold exposed C01 cells (Fig. 5e). Together, these analyses defined two coordinated features of the cold response in C01 macrophages: a metabolic program marked by increased *Chil3* expression and an attenuated NF-κB program reflected by reduced *Nfkb1* regulon activity.

To test whether these two components actively contributed to shaping the cold induced C01 state, we performed complementary in silico perturbation analyses across transcription factor regulatory and gene network layers. At the transcription factor level, we used CellOracle⁴⁷ to simulate transcriptional responses to perturbation. Because *Chil3* is not a transcription factor and was not directly modeled by CellOracle, we selected *Sp1* as an upstream proxy for the *Chil3* associated program. Previous studies

have established *Spi1* encoded PU.1 as a core transcription factor governing macrophage lineage determination and enhancer selection, with additional roles in the establishment of alternatively activated macrophage programs and close links to *Chil3* associated expression programs^{48–52}. In parallel, the CellOracle inferred regulatory relationship between *Spi1* and *Chil3*, their baseline coexpression, and the predicted response of *Chil3* to in silico *Spi1* knockout collectively supported the use of *Spi1* as an upstream regulatory proxy for perturbation analyses centered on *Chil3* (Supplementary Table 7). In silico *Spi1* knockout caused the perturbation vectors of cold-exposed C01 cells to shift globally towards the control state and partially restored the immune module suppressed by cold exposure (recovery-to-control = 32.6%; Fig. 5f). These findings suggest that the metabolic activation program marked by increased *Chil3* expression is an active component of the cold response. This program may contribute to the establishment and persistence of the C01 transcriptional state. The result further suggests a relationship between the *Chil3* associated metabolic program and the reduced NF- κ B related immune program.

In contrast, based on the previous SCENIC transcription factor analysis results (Fig. 5e), we modeled *Nfkb1* overexpression to test whether reactivating the immune regulatory could counteract the cold induced metabolic bias. Simulated *Nfkb1* overexpression shifted the predicted metabolic module score toward the Control group (recovery to control = 76.9%), with perturbation vectors directed toward the control enriched region (Fig. 5f). These predictions indicate that NF- κ B attenuation is not merely a downstream correlate of the cold-exposed C01 state, but a regulatory pivot that helps drive and maintain its metabolically biased transcriptional program. Together, the *Spi1* and *Chil3* associated metabolic activation program and the *Nfkb1* and NF- κ B associated immune attenuation program form a coupled regulatory structure underlying the cold induced transition of C01 macrophages.

We next directly assessed the regulatory contribution of *Chil3* to C01 transcriptional state by using scTenifoldKnk⁵³. The perturbation landscape showed that the significantly affected genes covered both NF- κ B and inflammatory response genes and mitochondrial metabolism genes (Fig. 5g). GO biological process enrichment

analysis further showed that the *Chil3* perturbation responsive genes were enriched for terms including response to lipopolysaccharide and immune response activating signaling pathway (Fig. 5h). These results associate the inferred network position of *Chil3* with immune and inflammatory gene programs. Pathway recovery analysis further predicted that *Chil3* knockout restored the NF- κ B and inflammatory module towards the control level (recovery-to-control = 83.8%) while reducing the cold-elevated PPAR and lipid-metabolism module (recovery-to-control = 82.4%; Fig. 5i). These in silico perturbation results suggest that *Chil3* loss is sufficient to both release the cold-suppressed inflammatory program and weaken the associated lipid-metabolic bias. Together, these analyses prioritize *Chil3* as key network node between the enhancement of lipid metabolism and the suppression of immune inflammation.

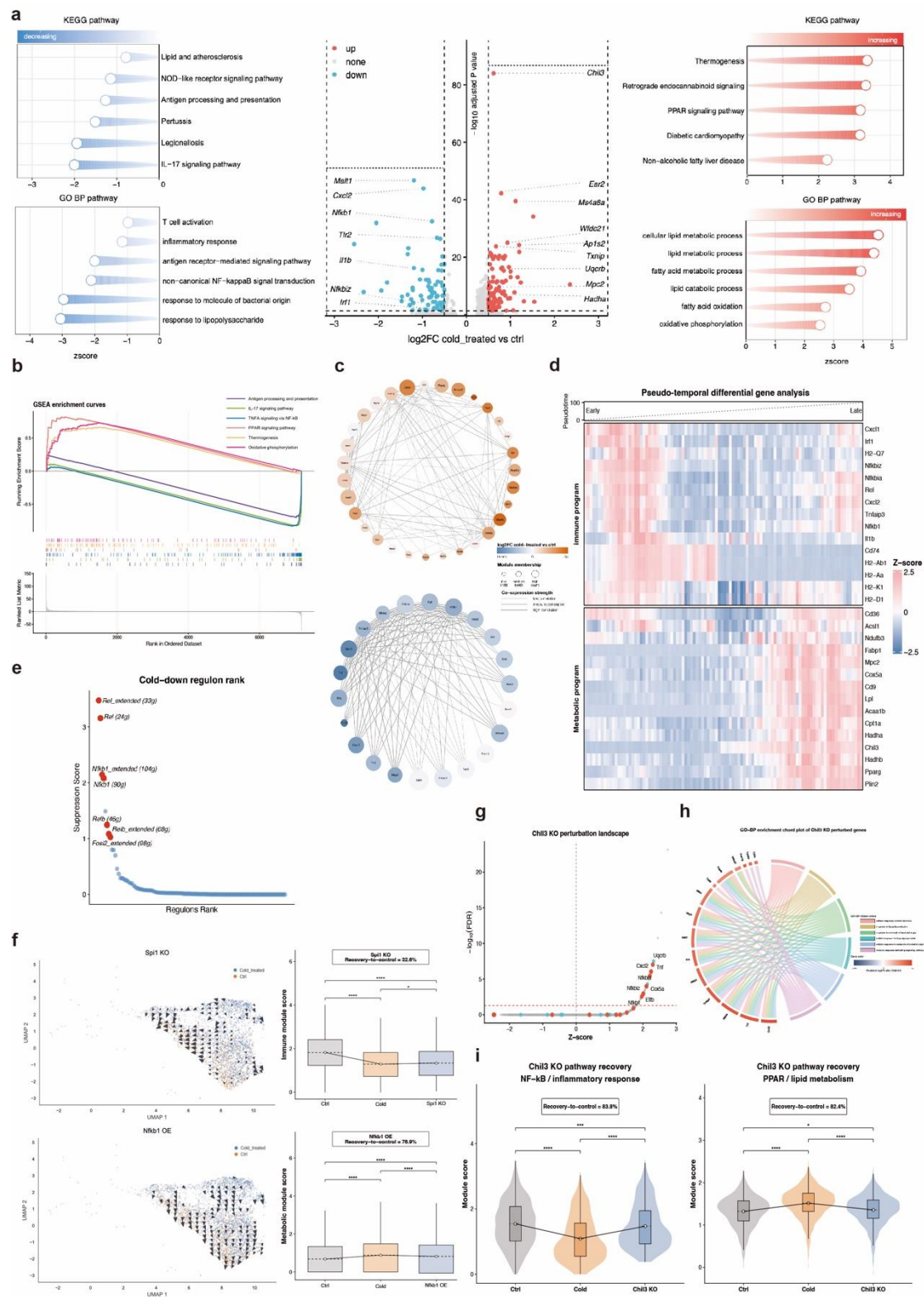


Figure 5 Cold exposure is associated with a *Chl3*-macrophage subset characterized by increased lipid metabolic and reduced NF- κ B related programs. **a**, Differential transcriptional landscape and pathway enrichment in C01 after cold exposure. The center volcano plot shows DEGs ranked by $\log_2$ fold change and statistical significance. Red, cyan and gray points respectively denote upregulated, downregulated and non-significant genes. The flanking panels show KEGG (top) and GO biological process (bottom) pathway. Dot position indicates pathway z score. **b**, GSEA

plots for representative pathways in cold-exposed versus control C01. Genes were ranked according to the differential expression statistic. Enrichment curves are shown for PPAR signaling, thermogenesis, oxidative phosphorylation, antigen processing and presentation, IL-17 signaling and TNF signaling. **c**, hdWGCNA coexpression networks of C01. The upper network shows a metabolic module containing genes associated with PPAR related lipid handling, fatty-acid oxidation and mitochondrial metabolism. The lower network shows an immune related module containing genes associated with NF- κ B signaling, cytokine responses and innate immune sensing. Each node represents a gene. Node color indicates the $\log_2$ fold change between the Cold exposure group and Control group, node size indicates module membership, and edges indicate weighted gene coexpression relationships. **d**, Heat map showing representative immune and metabolic gene expression patterns along the inferred C01 pseudotemporal trajectory. Cells are ordered from early to late pseudotime. Rows represent selected genes, and expression values are scaled by gene and shown as z scores. **e**, SCENIC regulon ranking analysis of transcriptional regulatory programs decreased in C01. Regulons are ranked according to their relative activity change between the Cold exposure group and Control group. Highlighted regulons include NF- κ B family circuits centered on Rel, Nfkb1 and Relb, together with the Fosl2-associated AP-1 regulon. **f**, CellOracle in silico perturbation analysis in C01. UMAP plots show perturbation vectors projected onto the C01 transcriptional space after in silico Spi1 knockout (top) or *Nfkb1* overexpression (bottom). Arrows indicate the predicted direction and magnitude of cell-state displacement. The accompanying module-score plots show the change in the cold-suppressed immune module after Spi1 knockout and the change in the cold-elevated metabolic module after *Nfkb1* overexpression. **g**, scTenifoldKnn based in silico *Chil3* knockout perturbation landscape in C01. Genes are plotted according to perturbation z score on the x axis and $-\log_{10}(\text{FDR})$ on the y axis. Significantly perturbed genes are highlighted. **h**, GO biological process chord plot of genes significantly affected by in silico *Chil3* knockout. The outer sectors show enriched GO biological process terms and perturbation-responsive genes. Links connect individual genes to their assigned GO terms. **i**, Pathway recovery analysis after in silico *Chil3* knockout in C01. Violin plots show cell level distributions of the NF- κ B and inflammatory response module score and the PPAR and lipid-metabolism module score across the indicated states. Box plots show the median and interquartile range. Significance is represented with * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$. Recovery-to-control values quantify the extent to which the perturbation shifts the cold-exposure module score towards the control state.

Integration of transcriptomic, proteomic and succinylomic data identifies *Chil3* as a convergent node of cold induced immunometabolic remodeling

scRNA-seq analysis identified C01 as the macrophage state showing the strongest association with cold exposure. C01 showed a strongly tissue biased distribution, with most cells derived from the lung and a higher C01 proportion among lung macrophages than among macrophages from the other sampled tissues (Supplementary Fig. 5a, b). Because the C01 transcriptional program was characterized by lipid-metabolic and

mitochondrial features, we next asked whether this transcriptional state was accompanied by coordinated changes in protein abundance and posttranslational modification profiles

We first performed 4D quantitative proteomics of lung tissue (Fig. 6a). Proteomic profiling revealed cold-associated remodeling of the tissue proteome, identifying 7,339 proteins and quantifying 125 differentially abundant proteins, including 34 increased and 91 decreased after cold exposure (Supplementary Fig. 5c). The most strongly increased proteins included *Chil3* and several lipid or metabolism associated proteins, such as *Arg1*, *Pdk4*, *Lpl* and *Plin2* (Fig. 6b). Enrichment analysis showed that the up-regulated proteins were involved in lipid-handling and fatty-acid pathways, including glycerolipid metabolism, fatty acid β -oxidation and PPAR signaling pathways, and down-regulated proteins were related to innate and adaptive immune modules, including MyD88/TLR4, TRAF6-NF- κ B -MAPK, IL-17 and T cell receptor pathways (Fig. 6c and Supplementary Table 8).

Protein posttranslational modifications (PTMs) are considered as emerging critical regulators of biological mechanisms⁵⁴⁻⁵⁶. To explore the effect of cold exposure on lung PTMs during cold adaptation, we first examined the overall profiles of six PTM types upon cold exposure, including acetylation, succinylation, crotonylation, ubiquitination, β -hydroxybutyrylation and, lactoylation, respectively (Supplementary Fig. 5d). Interestingly, global protein succinylation showed the most evident cold associated increase among the surveyed PTM types, prompting us to focus on the lung succinylome for subsequent quantitative analysis (Fig. 6a and Supplementary Fig. 5d). Based on succinylome profiling, we identified 7,254 succinylation sites across 1,756 modified proteins, including 128 increased sites (92 proteins) and 66 decreased sites (62 proteins) upon cold exposure (Supplementary Fig. 5e). Notably, the differentially regulated succinylome included reduced succinylation of *Chil3* at K173, indicating that *Chil3* was remodeled not only at the protein abundance level but also at the succinylation level (Fig. 6d). Enrichment analysis showed that proteins with increased succinylation were mainly involved in lipid and mitochondrial metabolic pathways, including fatty acid oxidation, oxidative phosphorylation, respiratory electron transport,

the citrate cycle and PPAR signaling, whereas proteins with decreased succinylation were related to immune effector and antigen presentation programs, including T cell mediated cytotoxicity, leukocyte mediated cytotoxicity and antigen processing and presentation (Fig. 6e and Supplementary Table 9). Given that alterations in protein abundance may affect the interpretation of post translational modification changes⁵⁷, we further compared differentially expressed proteins with differentially succinylated sites after cold exposure. Quadrant analysis identified 123 upregulated and 65 downregulated succinylation sites on proteins whose abundance remained unchanged. In addition, Chil3_K173 was the only event assigned to the protein up and succinylation down quadrant, indicating a discordant succinylation change that could not be explained by reduced *Chil3* protein abundance (Supplementary Fig. 5f). Together, these results indicate that cold exposure reshaped the proteome at both the protein abundance and succinylation levels.

To determine whether molecular features of the cold expanded C01 macrophage state were also detectable at the whole lung protein abundance and succinylation layers, we integrated cold responsive molecules from scRNA-seq, 4D proteomics and succinylomics (Fig. 6f). Within the single cell transcriptomic landscape, *Chil3* emerged as a defining transcriptional feature of the cold expanded C01 macrophage state. 4D proteomics then captured this response at the protein abundance level, identifying *Chil3* as one of the most strongly increased proteins after cold exposure. In parallel, succinylomic profiling extended this signal to the post translational modification layer, detecting Chil3_K173 among cold exposure succinylation events. Of the 348 C01 DEGs, 125 differentially abundant proteins and 194 differentially succinylated sites, *Chil3* was the only feature common to all three datasets (Fig. 6f). Together, these findings prioritize *Chil3* as a cold responsive molecular target candidate, suggesting its role in cold induced immunometabolic remodeling.

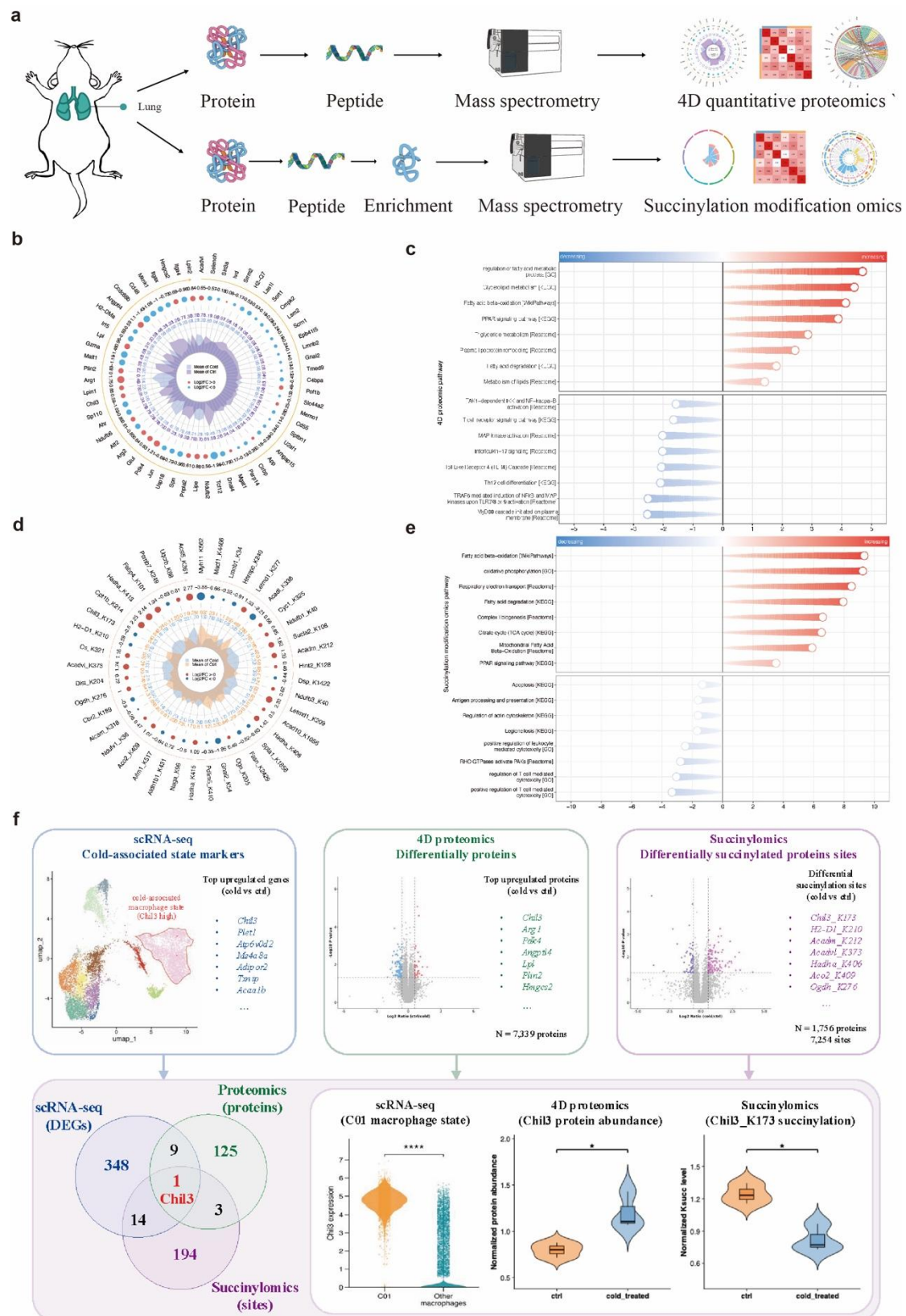


Figure 6 Global proteomics of protein abundance and succinylation upon cold exposure. a. Workflow of lung 4D quantitative proteomics and lysine succinylation proteomics. Each experimental group included at least three biological replicates (three mice). **b.** Global landscape of differentially abundant proteins after cold exposure. The circular plot summarizes proteins with

altered abundance in cold exposed versus control lungs. Red and cyan dots respectively denote increased and decreased proteins, and representative differentially abundant proteins are labelled. Inner radial profiles show mean abundance in each group. Outer symbols indicate the direction of abundance change. **c**, Pathway level organization of differentially abundant proteins. Positive z scores mark pathways associated with increased protein abundance after cold exposure, whereas negative z scores mark pathways associated with decreased abundance. **d**, Global landscape of differentially regulated succinylation sites after cold exposure. The circular plot summarizes differentially regulated succinylation sites in cold exposed versus control lungs. Red and cyan dots respectively denote increased and decreased succinylation sites, and representative sites are labelled. Inner radial profiles show mean succinylation signal in each group. Outer symbols indicate the direction of site-level succinylation change. **e**, Pathway level organization of proteins carrying cold-regulated succinylation sites. Positive z scores mark pathways associated with increased succinylation after cold exposure, whereas negative z scores mark pathways associated with decreased succinylation. **f**, Integrated analysis of scRNA-seq, 4D proteomics and succinyloics identified *Chil3* as a critical target under cold exposure. The upper left panel shows the cold associated macrophage state identified by scRNA-seq, with the *Chil3* high C01 macrophage state highlighted in the UMAP space and representative cold increased C01 genes listed. The upper middle panel shows the protein abundance volcano plot from lung proteomics. The upper right panel shows the succinylation site volcano plot from lung succinyloics. Vertical dashed lines in the volcano plots denote the $\log_2$ fold change thresholds corresponding to a fold change of 1.5 in either direction, and horizontal dashed lines denote the nominal P value threshold of 0.05, and colored points denote features passing these criteria. The Venn diagram integrates cold responsive C01 transcriptional features, differentially abundant proteins and differentially regulated succinylation sites, identifying *Chil3* as the single shared feature across all three datasets. The lower violin plots quantify *Chil3* expression in C01 macrophages relative to other macrophages, *Chil3* protein abundance in control and cold treated lung proteomes, and *Chil3*_K173 succinylation in control and cold treated lung succinyloics. Box plots within violins show the median and interquartile range. For 4D proteomics and succinyloics, differential features were defined by $|\log_2(\text{fold change})| > \log_2(1.5)$ and nominal P value < 0.05 . Significance is represented with $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and $****P < 0.0001$.

DISCUSSION

We generated an integrative cross-tissue multi-omics atlas comprising 501,543 single-cell transcriptomes from nine tissues, together with lung 4D proteomic and lysine-succinylomic profiles. This atlas resolves the tissue distribution and molecular heterogeneity of cold induced responses, revealing coordinated immune and metabolic remodeling across organs. Cross-tissue atlases have previously revealed extensive tissue-specific variation in cellular composition and gene expression^{58,59}. Our study extends this principle to a defined environmental perturbation by integrating transcriptomic, proteomic and succinylomic analyses, revealing that organismal adaptation to cold is achieved through coordinated but tissue and cell type specific remodeling.

These cross-tissue changes support a finite energy-allocation framework for cold exposure. Our findings indicate that, under limited metabolic resources, the thermogenic demand required to maintain body temperature competes with immune responses, thereby establishing a reconfigured immunometabolic balance. This interpretation is consistent with previous studies⁶⁰. The context-dependent consequences of this rebalancing are further illustrated by studies of autoimmune disease. Cold exposure attenuates autoimmune neuroinflammation by reducing MHC-II expression in monocytes and suppressing T cell priming²⁰. These findings indicate that cold exposure reconfigures organismal immunity, providing a physiological basis for an energetic trade-off between thermogenesis and host defense.

Importantly, the physiological basis of this resource-allocation framework is not restricted to rodents. Human studies currently demonstrate thermogenic fuel redistribution directly. In healthy adults, controlled cold exposure activates BAT oxidative metabolism, increases glucose and non-esterified fatty-acid uptake, and raises whole-body energy expenditure⁶¹. When intracellular triglyceride lipolysis is pharmacologically inhibited, BAT oxidative metabolism declines but shivering increases commensurately, preserving total heat production and demonstrating compensatory redistribution of thermogenic work between adipose tissue and skeletal muscle⁶². In a pilot human observation, mild cold exposure activated BAT and reduced

glucose uptake by a tumor, providing proof of principle that cold-driven thermogenesis can alter substrate access in another tissue²⁶. Together, these studies establish the upstream human physiology required by our model: cold induced thermogenesis reorganizes substrate use across tissues, providing the physiological premise for competition with other energy-demanding processes, including immunity. The immune consequences of cold exposure are likewise evident in human tissue. In human nasal epithelial cells and fresh nasal mucosal specimens, cooling reduced TLR3 dependent extracellular vesicle release, antiviral microRNA content and viral neutralization, directly linking lower mucosal temperature to weaker epithelial antiviral defense¹². It parallels our observation that reduced pulmonary antigen presentation and antiviral transcriptional programs preceded a poorer outcome after IAV challenge. Together, these observations support a model in which cold reconfigures immune competence according to tissue context. Our cross-tissue atlas advances this framework by resolving the cell states in which metabolic enhancement and immune attenuation coincide, thereby providing a testable cellular basis for the organ-selective immune costs of cold adaptation.

The lung occupies a distinctive position during cold exposure because the respiratory surface encounters cooled inspired air while maintaining gas exchange and barrier immune defense. It may therefore experience both local cooling at the respiratory surface and systemic metabolic changes associated with thermogenesis^{9,63}. In our study, pulmonary structural and immune related changes were evident after cold exposure. These findings identify the lung as one of the tissues in which physiological and immune changes associated with cold exposure are readily detectable.

Macrophage identity is shaped by developmental origin and local tissue signals, including metabolic features of the tissue environment⁵². Macrophage transcriptional and functional states are influenced by lipid availability, mitochondrial metabolism, inflammatory signals and tissue specific environmental cues⁶⁴. Collectively, these studies indicate that the role of macrophages in cold adaptation cannot be reduced to a single thermogenic mediator or explained solely within the traditional binary M1 and M2 framework. Our cross-tissue atlas showed that macrophages are not only potential

regulators of thermogenic tissues but are themselves prominent targets of cold induced metabolic reprogramming. Single cell analysis identified a distinct macrophage subset, designated 01_Macro_Plet1+Atp6v0d2+. Following cold exposure, this state showed high expression of *Chil3*. Its expansion without increased cell-cycle activity indicates redistribution of macrophage-state architecture rather than generalized proliferation. Moreover, this state exhibited enhanced PPAR signaling, fatty acid catabolism and mitochondrial metabolism, together with coordinated attenuation of antigen presentation, NF- κ B signaling and antiviral programs. Thus, it is not a conventional alternatively activated population, but a cold-adapted macrophage state in which metabolic specialization is coupled to reduced immune reserve.

Previous studies indicate that the biological effects associated with *Chil3* vary across tissues and disease settings¹⁵. During the differentiation of macrophages associated with inflammation resolution, IL33 induces a mitochondrial program that depends on UCP2 and is accompanied by increased *Chil3* expression and sustained oxidative metabolism⁶⁵. In pulmonary helminth infection, Ym1, the protein encoded by *Chil3*, promotes IL17 production by $\gamma\delta$ T cells and neutrophil recruitment, improving parasite control while increasing lung injury⁶⁶. These contrasting outcomes suggest that the immune effects associated with *Chil3* depend on the surrounding tissue and pathological context. Separate evidence links *Chil3* more directly to macrophage lipid metabolism. Macrophage derived *Chil3* can bind and de-N-glycosylate *CD36*, thereby increasing lipid uptake and altering cholesterol metabolism⁶⁷. Although *Chil3* has no direct human ortholog, the same study identified *CHI3L2* as a functional counterpart in human macrophages and reported similar effects on lipid handling through *CD36*⁶⁷. These findings suggest that this lipid handling function may extend to human macrophages at the level of the functional module rather than through direct gene orthology.

Lysine succinylation is one of an identified type of PTMs involved in regulating metabolic processes, immunity, and inflammation⁵⁵. In macrophages, SIRT5 mediated desuccinylation of PKM2 at K311 restores its tetrameric enzymatic state and restrains HIF-1 α -dependent IL-1 β production, directly linking succinylation to

immunometabolic polarization⁶⁸. Conversely, SIRT5-dependent desuccinylation of MAVS suppresses its aggregation and reduces type I interferon and antiviral gene expression⁶⁹. Global succinylome analysis of SARS-CoV-2-infected cells further showed that changes in site-specific succinylation can occur in the opposite direction to total protein abundance, demonstrating that modification state and protein quantity represent distinct regulatory dimensions⁵⁴. In this context, decreased Chil3_K173 succinylation can coexist with increased *Chil3* transcript and total *Chil3* protein abundance. Several mechanisms could potentially account for this pattern. Cold exposure may promote rapid synthesis of new Chil3 that has not yet acquired K173 succinylation, thereby reducing the relative occupancy of this modification within the expanding protein pool. K173 may also undergo selective desuccinylation, while the form with lower K173 succinylation may be preferentially secreted or remain more stable in the extracellular space. These possibilities are not mutually exclusive. Beyond explaining the discordance between *Chil3* protein abundance and K173 succinylation, this site-specific change may also have functional consequences. Because Chil3 is a secreted glycan interacting protein implicated in CD36 dependent lipid uptake, altered charge at K173 could plausibly affect ligand binding, intracellular trafficking, secretion or extracellular persistence⁶⁷. Therefore, cold exposure may regulate Chil3 at two separable levels: increased transcript and protein abundance expands the Chil3 associated macrophage program, whereas reduced K173 succinylation alters the proteoform composition of the resulting protein pool.

Taken together, we constructed a cross-tissue multiomics atlas that reveals how cold exposure reshapes metabolism and immunity across organs and cell types. By integrating single cell transcriptomic, proteomic and succinylomic data, we further revealed organism wide metabolic adaptation and immune remodeling, identified a distinct macrophage subpopulation, and highlighted *Chil3* as a key molecular node regulated at the transcriptional, protein and posttranslational levels. As these findings are extended to humans, future studies should focus on the conserved relationship between enhanced lipid metabolism and attenuated immune activity, rather than seek direct counterparts of the murine C01 population, Chil3 or an individual modification

site. Our atlas converts a broad energetic trade off into a mechanistically tractable framework, providing a valuable resource for investigating how cold adaptation reshapes immune competence across species.

MATERIALS AND METHODS

Mice and experimental design

Male 8 weeks old C57BL/6 mice were obtained from Changchun Yisi Laboratory Animal Technology Co. Ltd (License No. SCXK 2020–0002). Experiments were started following one week of acclimation of the purchased mice. Mice were housed in a Specific Pathogen Free (SPF) facility in 12 h day/night cycles at 25 °C and 40% to 60% humidity, with unrestricted access to standard laboratory chow and water. All protocols involving animal experiments in this experiment were approved by the Ethics Committee for Animal Experiments of the College of Basic Medical Sciences of Jilin University (Approval No.2025–011), and were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Mice were randomly assigned to the indicated experimental groups. For cold stimulation, mice were housed in pre-chilled cages (one mouse per cage) at 4 °C for 7 days without bedding or nesting material. Body weight and body temperature were monitored daily throughout the experimental period. H3N2 influenza A virus (H3N2 IAV, A/Canine/Jilin/Qiaoke01/2017) was provided by the Changchun Veterinary Research Institute, Chinese Academy of Agricultural Sciences. Mice were inoculated with H3N2 IAV at a dose of $10^{5.5}$ TCID₅₀ per mouse via intranasal challenge. For the Cold exposure & H3N2 IAV group, mice were exposed to 4 °C for 7 days before viral inoculation and were subsequently maintained at 4 °C until the experimental endpoint. All viruses were manipulated under BSL-2 conditions. Body weight and body temperature were measured daily in the same mice over 7 consecutive days, whereas all other endpoints were assessed in biologically independent mice.

Organ collection and molecular analysis

At the experimental endpoint, blood samples were collected from the retro orbital sinus, after which the mice were euthanized, and nine tissues or organs were harvested for subsequent analyses. The weights of the lung, heart and thymus were measured, and the organ coefficient was calculated as the percentage of organ weight to total weight. The organs were preserved in 4% paraformaldehyde for histological investigation using hematoxylin and eosin (H&E) staining. Serum levels and spleen levels of inflammatory

factors were determined using an ELISA kit (Bioswamp, Wuhan, China). For mice subjected to H3N2 IAV challenge, we collected lung tissues for the assessment of viral burden. Infectious virus titers in lung homogenates were quantified using a TCID₅₀ assay. For viral RNA quantification, RNA was extracted from lung tissues using the viral RNA extraction kit (Tiangen Biotech, Beijing, China), and H3N2 IAV RNA abundance was determined by the HiScript II U + one step qRT-PCR probe kit (Vazyme Biotech, Nanjing, China), targeting the viral matrix (*M*) gene. Relative *M*-gene RNA levels were normalized to *Gapdh* and expressed relative to those in the H3N2 IAV group, which served as the calibrator. The *M*-gene primers were 5'-GACCAATCCTGTCACCTCTGA-3' (forward) and 5'-GTATATGAGGCCCATRCAACTG-3' (reverse), yielding a 150-bp amplicon, whereas the *Gapdh* primers were 5'-CTACATGGTTTACATGTTCC-3' (forward) and 5'-GGATCTCGCTCCTGGAAGAT-3' (reverse), yielding a 123-bp amplicon. For inflammatory cytokine analysis, RNA isolated from spleen tissues was analyzed by RT-qPCR to determine the relative transcript levels of *Ifng*, *Cxcl10*, *Il1b* and *Il6*, with *Gapdh* serving as the internal reference gene.

Library preparation for scRNA-seq data

scRNA-seq libraries were prepared using the MobiCube High Throughput Single Cell 3' RNA-Seq Kit v2.1 and MobiCube 3' Dual Index Kit (MobiDrop) according to the manufacturer's instructions. The resulting libraries were sequenced on an Illumina NovaSeq X Plus platform with 150-bp paired-end reads.

Data preprocessing and quality control for scRNA-seq data

We used fastp (v0.20.0) to perform basic statistics on the quality of the raw reads. FASTQ files were processed using MobiVision (v3.2, intron-included mode; MobiDrop), which performed cell-barcode correction, STARsolo-based alignment to the mm10_2020A mouse reference genome, and UMI counting to generate raw and filtered gene-by-cell count matrices. The filtered count matrices were imported into R using the Read10X function and used to construct sample-level Seurat objects with CreateSeuratObject (min.cells = 3 and min.features = 200). Low-quality cells were excluded based on the number of detected genes, UMI counts, mitochondrial transcript

proportions and hemoglobin-gene proportions. In addition, DoubletFinder (v2.0.3) was applied to identify and remove doublets based on the expected doublet rate derived from the loading rate.

Clustering and annotation of cell types

Data normalization, dimensionality reduction, clustering and identification of cell types were also conducted using Seurat (v5.5.1) and Harmony (v2.0.5) in R (v4.4.1). (1) Filtered gene–barcode matrices were normalized with the NormalizeData function using the ‘LogNormalize’ method. (2) The FindVariableFeatures function was utilized to identify the top 2,000 variable genes using the default ‘vst’ method. (3) Batch effects across samples were corrected using the IntegrateLayers function with HarmonyIntegration based on the PCA embeddings. (4) Dimensional reduction was performed using the RunPCA and RunUMAP functions. Subsequently, the FindNeighbors and FindClusters functions were used to cluster cells. We used clustree (v0.5.1) to determine the appropriate resolution. (5) Analyses of DEGs for each cluster were performed using the Wilcoxon rank-sum test as implemented in the FindAllMarkers function, retaining positive markers expressed in at least 25% of cells with a $\log_2$ fold change threshold of 0.25. Cell clusters were annotated according to the representative cell markers, with scMayoMap (v0.2.0) and SingleR (v2.8.0) predictions used as complementary references.

Cell proportion analysis

For each tissue and experimental condition, the number of cells assigned to each annotated cell type was determined, and the proportion of each cell type was calculated by dividing this number by the total number of cells pooled across all samples from the corresponding condition. To evaluate differences in the proportion of each cell type between the cold-treated and control conditions, the cell-type proportion calculated from cells pooled within the cold-treated condition was divided by the corresponding proportion in the control condition, and the resulting ratio was transformed by $\log_2$ to generate the $\log_2$ FC value. For each tissue, cell types with a proportion greater than 1% in either condition were ranked according to their absolute $\log_2$ FC values, and the three cell types showing the largest proportional changes were displayed.

Definition of DEGs and pathway enrichment analyses

Cell types with fewer than six cells in total or fewer than three cells in either the cold-treated or control group were excluded from downstream differential expression analysis. Within each annotated cell type, DEGs between the two conditions were identified using the Wilcoxon rank-sum test implemented in the FindMarkers function of Seurat (v5.5.1). The 'logfc.threshold' and 'min.pct' parameters were set to 0.25 and 0.10, respectively. DEGs were defined as genes with $|\log_2FC| > 0.25$ and adjusted $P < 0.05$. P values were adjusted using the Bonferroni correction implemented in Seurat. Positive $\log_2FC$ values indicated higher expression in cold-treated mice, whereas negative values indicated higher expression in control mice.

GO and KEGG enrichment analyses were performed for significant DEGs in each cell type using the enrichGO and enrichKEGG functions in clusterProfiler (v4.14.6) (P value cut-off = 0.05, q value cut-off = 0.20). WikiPathways enrichment analysis was conducted separately for significantly upregulated and downregulated genes using the enricher function and C2 CP gene sets retrieved from MSigDB with msigdb (v26.1.0) (P value cut-off = 0.05, q value cut-off = 0.25). GSEA was performed using the gseKEGG and GSEA functions for KEGG pathways and custom gene sets, respectively. GSVA was conducted using the gsva function in GSVA (v2.0.7) with averaged log normalized expression values and GO Biological Process and KEGG gene sets obtained from MSigDB to calculate pathway activity scores across macrophage subclusters and experimental conditions using a Gaussian kernel. P values were adjusted by the Benjamini–Hochberg method.

Tissue distribution preferences of immune cell subpopulations

We calculated the ratio of observed to expected cell numbers ($R_{o/e}$) for each immune cell subpopulation across tissues to quantify its tissue distribution preference. The expected cell numbers for each combination of immune cell subsets and tissues were calculated using the expected-frequency formulation of the χ^2 test. $R_{o/e}$ values reveal whether a specific immune cell subset is enriched or depleted in a particular tissue. For example, $R_{o/e} > 1$ suggests that the given immune cell subset is more frequently observed than expected by chance, indicating enrichment in that tissue.

Quantifying metabolism activity at the single-cell resolution

The R package scMetabolism (v0.2.1) was used to evaluate metabolism activities at the single-cell resolution. Raw RNA count data were extracted from the Seurat object, mouse genes were converted to human orthologues using the babelgene method in orthogene, and pathway activity scores were calculated using AUCell after retaining pathways with at least 20% gene coverage.

Pseudotime trajectory and gene module analyses

To reconstruct macrophage-state transitions between control and cold-treated conditions, pseudotime analysis was performed using Monocle3 (v1.3.7). The proliferating 10_Macro_Mki67+ subcluster was excluded. We performed monocle3 clustering on the original UMAP representation generated in Seurat and inferred developmental trajectories along pseudotime by choosing the node assigned the highest number of control cells from the 04_Macro_C1qc+Apoe+ subcluster as the root node. We then learned the principal trajectory graph across the selected macrophage subclusters using ‘monocle3::learn_graph’ without partitioning the cells. Finally, we determined genes whose expression changes along pseudotime by using monocle3::graph_test, which leverages a Moran’s *I*-test considering gene expression changes within groups of $k = 25$ neighboring cells on the principal trajectory graph. Four functional gene modules representing PPAR–lipid metabolism, the TCA cycle and acyl-CoA metabolism, inflammatory responses, and antigen presentation and T-cell interactions were manually curated on the basis of previously published studies^{70–73}.

Network inference and in silico gene perturbation

We applied CellOracle (v0.20.0) in Python (v3.10.20) to conduct the in silico perturbation analysis following the tutorial provided by the original authors (<https://morris-lab.github.io/CellOracle.documentation/>)⁴⁷. After removing hemoglobin genes detected in the dataset, raw counts from 3,655 C01 macrophages were restricted to 3,000 genes. Simulated *Spi1* knockout and *Nfkb1* overexpression were propagated for five iterations and projected onto the original UMAP embedding using randomized sampling of 200 neighbors. To assess the network-level effects predicted for *Chil3* loss, we performed virtual knockout analysis using scTenifoldKnn⁵³

(v1.0.3) in R (v4.4.1). For this analysis, raw counts from the same C01 cells were restricted to a 3,083-gene feature set comprising the 3,000 genes with the highest variance after CPM normalization and log_{1p} transformation, together with prespecified module genes and knockout targets. Ten directed networks were inferred from all cells by principal-component regression and denoised using rank-three CANDECOMP/PARAFAC tensor decomposition. The resulting reference and *Chil3* pseudo-knockout networks were compared by two-dimensional nonlinear manifold alignment, and genes with a Benjamini–Hochberg-adjusted $P < 0.05$ were subjected to Gene Ontology Biological Process enrichment.

Immunoblot

For detection of protein PTMs, lung tissues were harvested from Control group and Cold exposure group for immunoblot analysis. Total protein (15 µg) from lung samples was separated by 12% SDS/PAGE using Tris–glycine–SDS running buffer (Yeasen Biotech, Shanghai, China). The proteins were then transferred onto a 0.22-µm nitrocellulose membrane (PALL) at 200 mA for 1 h at 4 °C. The membranes were blocked with 5% skimmed milk in TBST for 1 h at room temperature and incubated at 4 °C overnight with anti-acetyllysine antibody (PTM Biolabs, PTM-105RM; 1:500), anti-succinyllysine antibody (PTM Biolabs, PTM-419; 1:1,000), anti-crotonyllysine antibody (PTM Biolabs, PTM-502; 1:1,000), anti-ubiquitin antibody (PTM Biolabs, PTM-1106RM; 1:200), anti-β-hydroxybutyryllysine antibody (PTM Biolabs, PTM-1201RM; 1:200) or anti-lactyllysine antibody (PTM Biolabs, PTM-1401RM; 1:1,000) as the primary antibodies. After washing three times with TBST, the membranes were incubated with HRP-conjugated goat anti-rabbit or goat anti-mouse IgG (H+L) secondary antibodies (Pierce; 1:10,000) for 1 h at room temperature. Chemiluminescence was detected using an HRP substrate (Millipore), and protein bands were visualized using a Tanon 5200 imaging system.

4D proteomics and lysine succinylome analysis

Lung tissues from the Cold exposure group and Control group were collected and lysed by ultrasonication. Equal amounts of protein samples were precipitated with

trichloroacetic acid, washed with acetone, and subjected to trypsin digestion for MS analysis to determine both total protein abundance and lysine succinylation.

Affinity enrichment of succinylated peptides and liquid-chromatography MS of proteome and succinyl-proteome were conducted by PTM BioLab in Hangzhou, China. Briefly, peptides from each sample were separated using a nanoElute system (Bruker), and all MS spectra were acquired on a timsTOF Pro 2 (Bruker) with parallel accumulation-serial fragmentation (PASEF) acquisition mode.

Raw MS data from proteomics and succinyl-proteomics were processed, and proteins and succinylated sites were identified using Spectronaut (v18) with default settings. All MS data were searched against the UniProt-reviewed mouse protein database (17,191 protein sequences, downloaded on December 20, 2023) for peptide and protein identification. For protein abundance analysis, data were searched using the default Pulsar settings, with methionine oxidation and protein N-terminal acetylation as variable modifications and carbamidomethylation of cysteine as a fixed modification. For succinylpeptide-enriched analysis, the Spectronaut settings were modified to include lysine succinylation as a variable modification.

We used fold change analysis and *t*-tests to identify differentially abundant proteins and succinylated sites between cold-exposed and control lung tissues. Relative quantitative intensity values of proteins or succinylated sites were log₂ transformed before the *t*-tests. Proteins or succinylated sites with $P < 0.05$ and fold changes of >1.5 or $< 1/1.5$ were considered significantly increased or decreased, respectively.

Statistical analysis and plotting

R (v4.4.1) and GraphPad Prism (v10.4.1) were used for statistical analysis. We did not predetermine the sample size using statistical methods. Comparisons between two independent groups were performed using two-tailed unpaired Student's *t*-tests. Multiple-group comparisons were analyzed using ordinary one-way ANOVA followed by Tukey's multiple-comparisons test for the qPCR datasets. Body temperature and body weight were analyzed using two-way ANOVA. $P < 0.05$ was considered statistically significant.

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Author contributions

Z.Z.C. contributed the animal experiments, performed data analysis and contributed to manuscript writing. J.H.P. contributed the literature and contributed to manuscript writing. Q.Z.D. contributed preprocessing on the scRNA-seq data. P.F.H. contributed the animal experiments. M.Y.L. contributed preprocessing on the proteomic data. X.Y.H. contributed preprocessing on the succinylomic data. T.T.Z. contributed the molecular biology experiments. Z.Y.X. designed the overall study. G.Q.W. conceived, designed, and supervised the study and acquired funding. The first author and corresponding authors are ultimately responsible for this work.

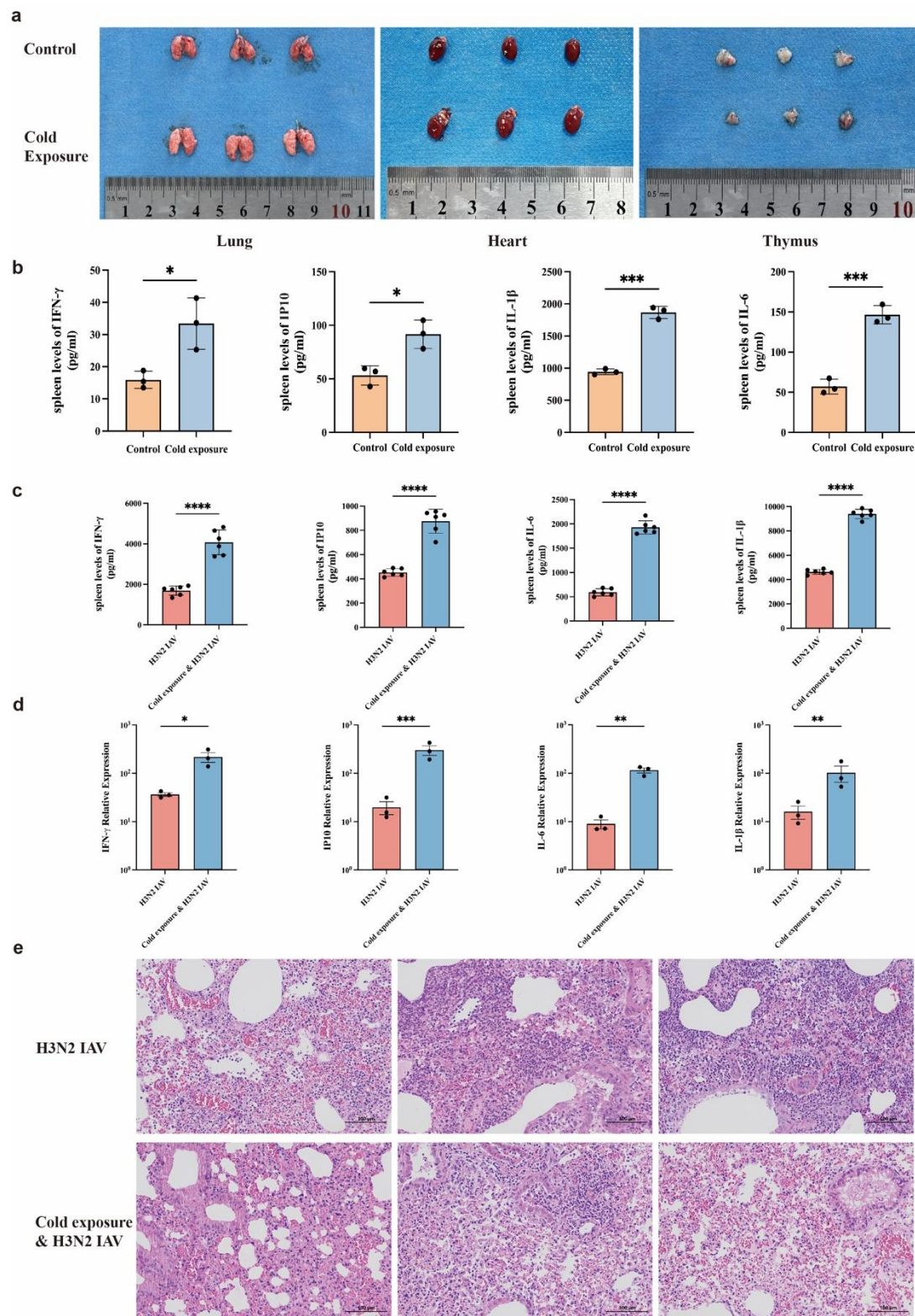
Competing interests

The authors declare that they have no competing interests.

Data availability

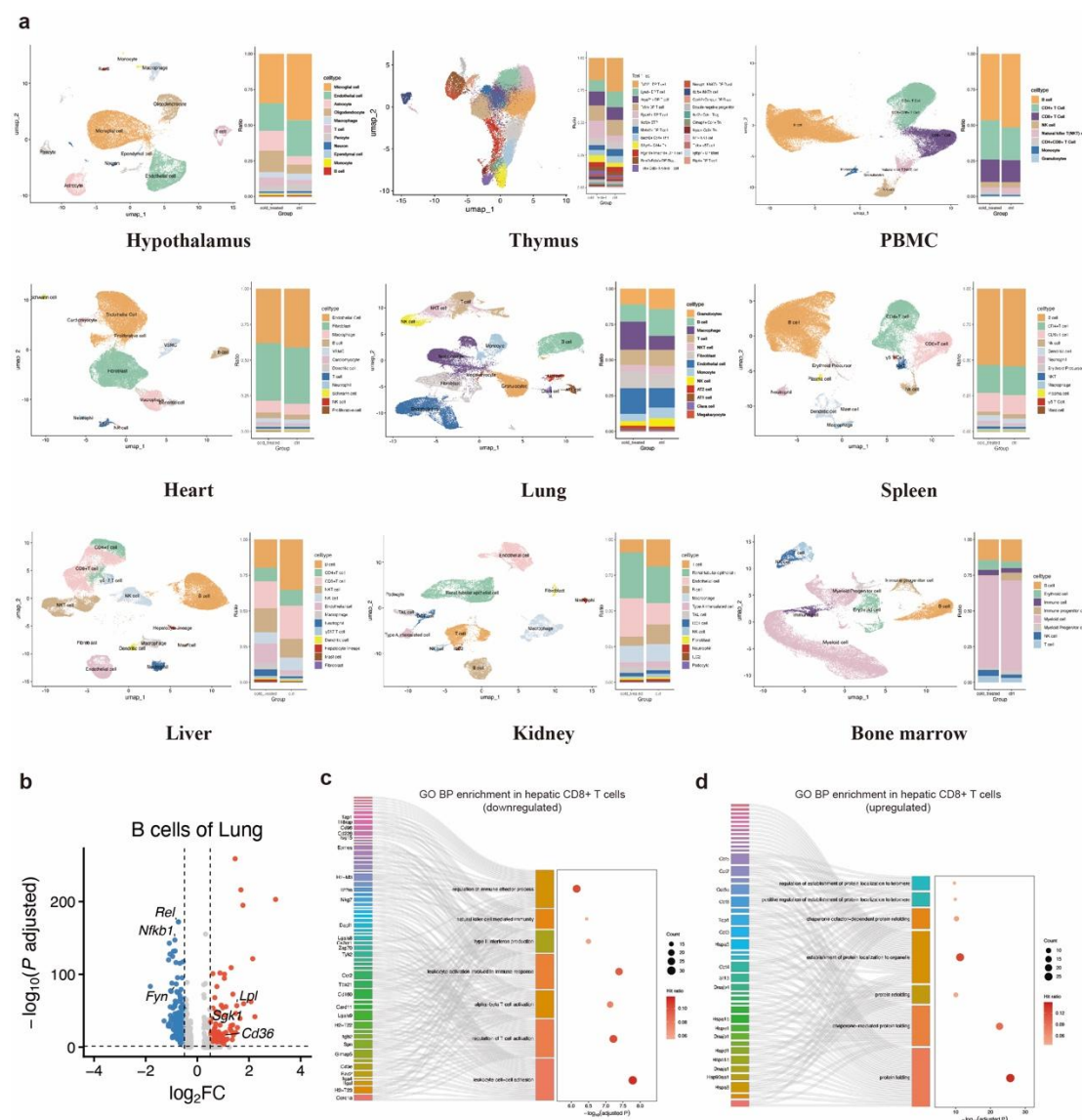
The raw data for scRNA-seq have been deposited in the GSA (<https://ngdc.cncb.ac.cn/gsa/>) under CRA076351. The processed data for scRNA-seq have been deposited in the OMIX under OMIX018812-01. The proteomic and succinylomic data were uploaded to iProX (IPX0018480000). Source data are provided with this paper.

Supplemental information



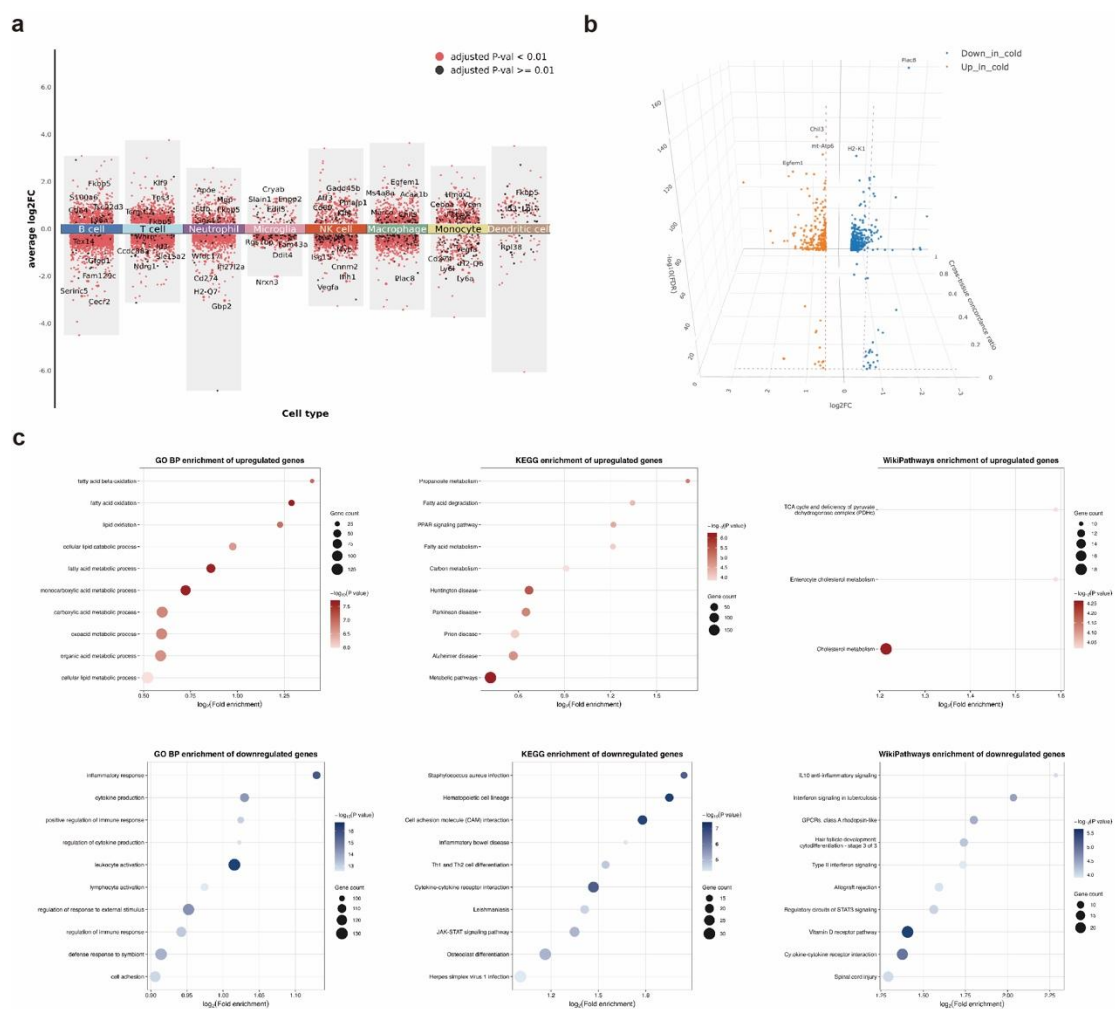
Supplementary Fig. 1 Cold exposure promotes tissue-level remodeling and heightens inflammatory responses to H3N2 IAV infection. **a**, Gross morphology of lungs, hearts and thymuses from mice maintained at ambient temperature (25 °C, control) or exposed to cold (4 °C, cold exposure) for 7 consecutive days (n = 3 per group). **b**, Splenic concentrations of IFN- γ , IP-10, IL-1 β and IL-6 in the Control and Cold exposure groups (n = 3 per group, measured by ELISA,

shown is mean $\pm$ SD). **c**, Splenic concentrations of IFN- γ , IP-10, IL-1 β and IL-6 in the H3N2 IAV and Cold exposure plus H3N2 IAV groups ($n = 6$ per group, measured by ELISA, shown is mean $\pm$ SD). **d**, RT-qPCR analysis of *Ifng*, *Cxcl10*, *Il1 β* and *Il6* mRNA abundance in spleen tissue from the H3N2 IAV group and Cold exposure & H3N2 IAV group. Transcript abundance was normalized to *Gapdh* and expressed relative to the Control group ($n = 3$ per group, shown is mean $\pm$ SEM). **e**, Representative H&E staining lung sections from the H3N2 IAV group and Cold exposure & H3N2 IAV group ($n = 3$ per group, scale bars: 100 μ m). Each point represents one biologically independent mouse. Significance is represented with a * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$.



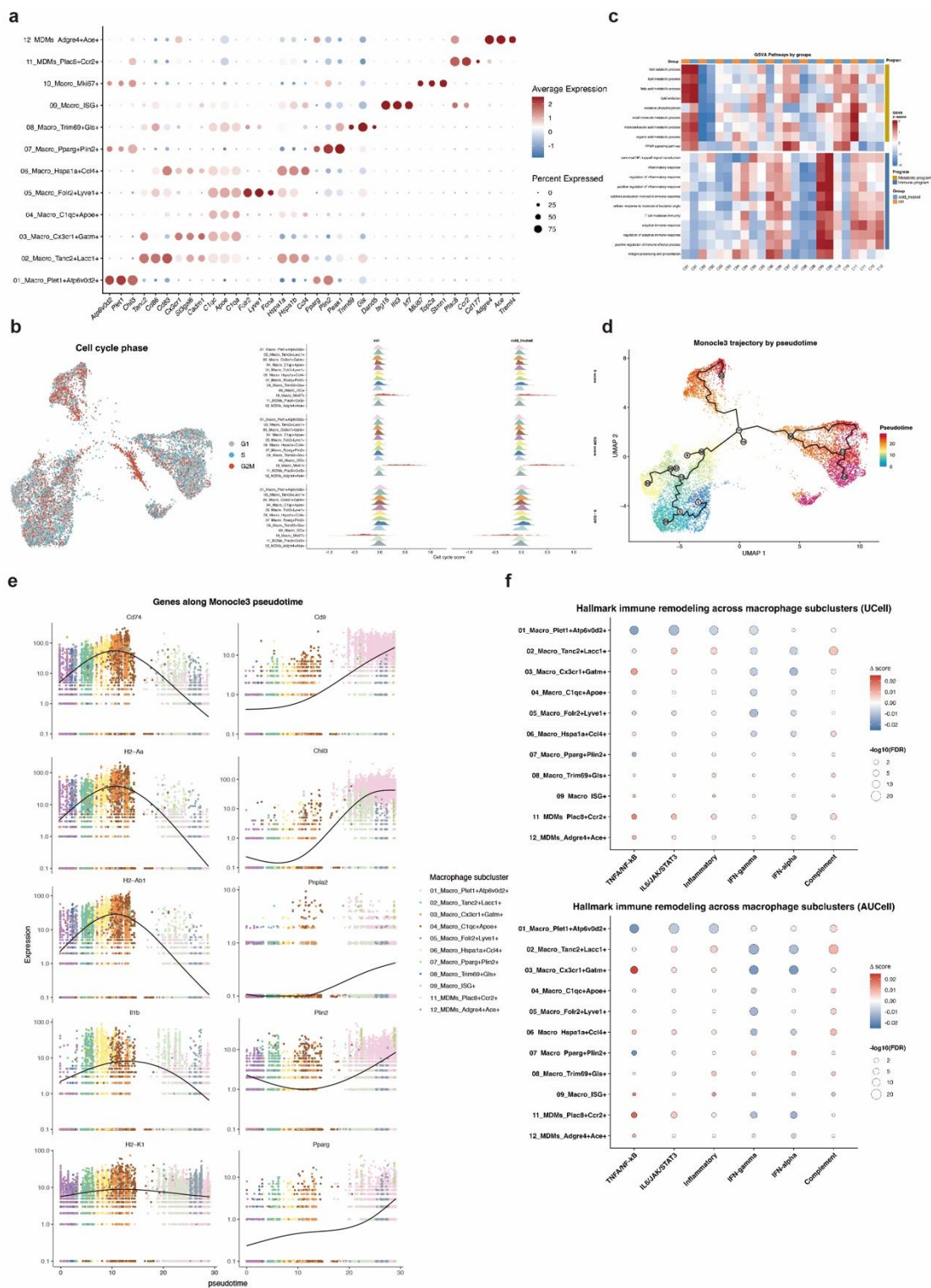
Supplementary Fig. 2 Cold exposure remodels tissue resolved cellular and transcriptional states. **a**, UMAP embeddings of single-cell transcriptomes from nine tissues, colored by annotated cell type. Adjacent stacked bar plots show the relative abundance of major cell populations in Control group and Cold exposed group within each tissue. Cell populations were annotated separately within each tissue. **b**, Volcano plot showing DEGs in lung B cells between two groups. Three representative upregulated and three representative downregulated DEGs are labelled. DEGs were identified using the Wilcoxon rank-sum test, and P values were adjusted by Bonferroni

correction using the FindMarkers function implemented in Seurat. The horizontal dashed line represents adjusted $P = 0.05$, and the vertical dashed lines represent $|\log_2FC| = 0.5$. **c, d**, GO biological process enrichment of genes downregulated (**c**) or upregulated (**d**) by cold exposure in hepatic CD8⁺ T cells. Links connect individual DEGs to selected enriched GO terms shown in the plot. Bubble size denotes gene count, color denotes the hit ratio, and the x axis shows $-\log_{10}(\text{adjusted } P)$. DEGs were defined as adjusted $P < 0.05$ and $|\log_2 \text{fold change}| > 0.25$; GO terms were considered enriched at adjusted $P < 0.05$.



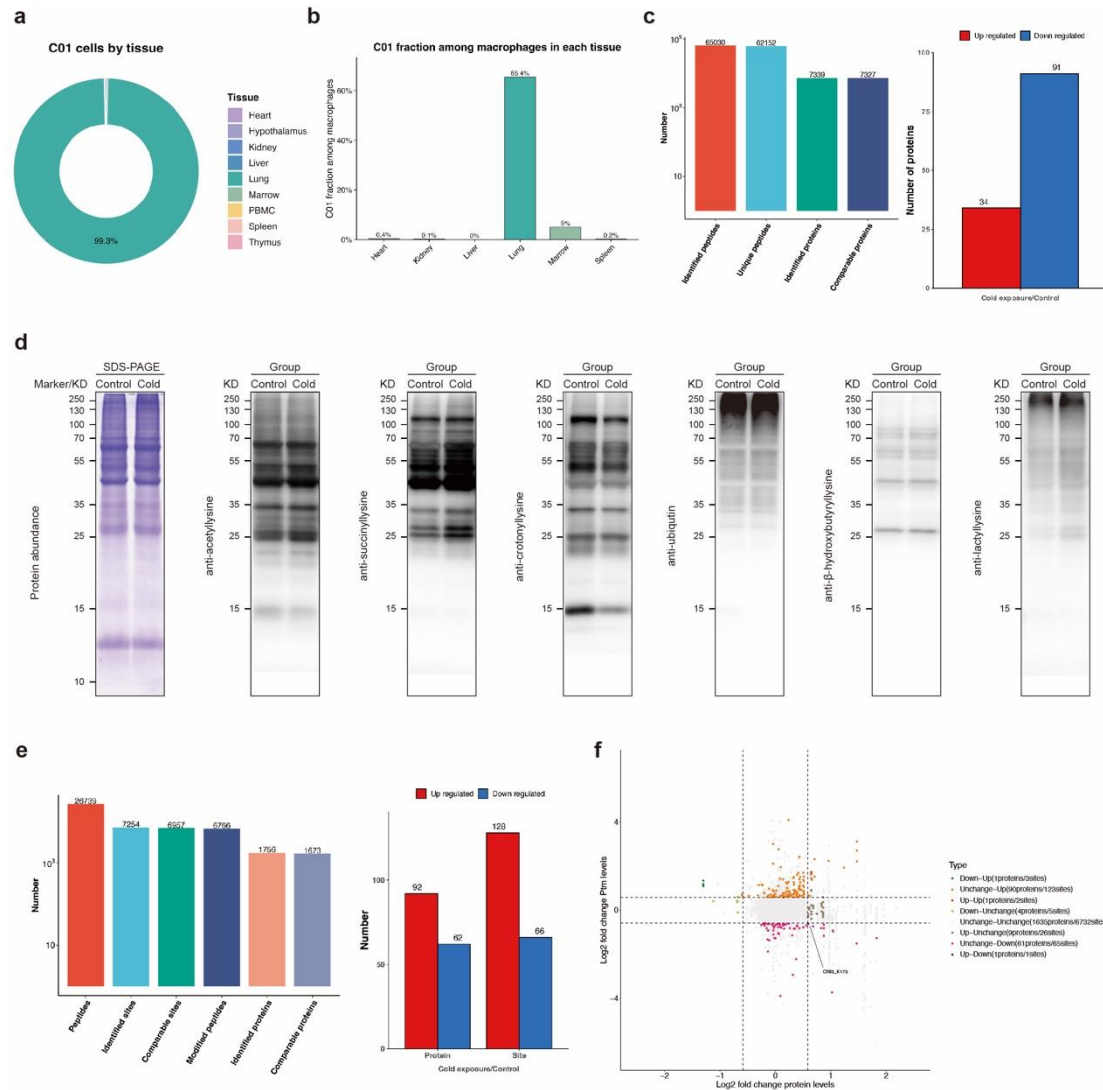
Supplementary Fig. 3 Cold exposure remodels transcriptional and pathway programs across immune compartments. **a**, Differential gene expression analysis showing upregulated and downregulated genes across major immune cell types. An adjusted P value < 0.01 is indicated in red, while an adjusted P value ≥ 0.01 is indicated in black. **b**, Three-dimensional volcano plot of cold-associated DEGs in macrophages. Genes are positioned according to representative $\log_2$ fold change, $-\log_{10}(\text{FDR})$ and cross-tissue concordance ratio. For each gene, the representative $\log_2$ fold change and FDR were taken from the tissue comparison with the largest absolute $\log_2$ fold change. The cross-tissue concordance ratio indicates the proportion of tissues in which the gene changed in the consensus direction. Orange and blue points denote genes upregulated and downregulated in

cold exposed macrophages, respectively; labeled genes indicate the top five genes ranked by $-\log_{10}(\text{FDR})$. c, Enrichment analysis of macrophage genes upregulated or downregulated after cold exposure using GO biological process, KEGG and WikiPathways annotations. Dot position indicates $\log_2$ fold enrichment, dot size indicates gene count and color denotes $-\log_{10}(P \text{ value})$.



Supplementary Fig. 4 Cold exposure drives macrophage state remodeling toward lipid-metabolic adaptation and immune attenuation. a, Dot plot showing the expression of

representative marker genes across the 12 macrophage subsets. Dot size denotes the percentage of cells expressing each gene, and color indicates scaled average expression. **b**, Cell-cycle analysis of macrophage subsets. UMAP visualization shows inferred cell-cycle phases across macrophages (left), and distributions of S-phase and G2/M-phase scores across macrophage subsets and conditions are shown (right). **c**, GSVA heat map showing pathway activity across macrophage subsets from Control group and Cold exposure group. Rows represent related pathway or program names, and columns indicate macrophage subsets stratified by condition. Color denotes scaled GSVA enrichment scores. **d**, Monocle3 pseudotime trajectory of macrophage-state transitions. Cells are embedded in UMAP space and colored by pseudotime, with black lines indicating the inferred trajectory. **e**, Pseudotime dependent expression dynamics of representative genes associated with antigen presentation and inflammatory activity, or with metabolic macrophage states associated with cold exposure. Each point represents a single cell, colored by macrophage subset, and fitted curves show smoothed gene-expression trends along pseudotime. **f**, Remodeling of Hallmark immune programs across macrophage subsets assessed by UCell and AUCell scoring. Dot plots show changes induced by cold exposure in selected MSigDB Hallmark immune signatures across macrophage subclusters, quantified using UCell (top) and AUCell (bottom). Dot color indicates the mean score difference between two groups for each subcluster–signature pair, calculated as cold minus control; blue indicates lower activity and red indicates higher activity after cold exposure. Dot size denotes $-\log_{10}(\text{FDR})$.



Supplementary Fig. 5 Cold exposure remodels global lung protein abundance and lysine succinylation landscape. a, b, Tissue distribution of 01_Macro_Plet1+Atp6v0d2+ (C01) in the integrated macrophage atlas. **a,** Proportion of all C01 cells contributed by each tissue. **b,** Fraction of C01 cells among macrophages in each tissue. **c,** Summary of global 4D quantitative proteomic profiling. The left bar plot shows the numbers of identified peptides, unique peptides, identified proteins and comparable proteins. The right bar plot shows the numbers of proteins with increased or decreased abundance in cold treated lungs relative to control lungs. **d,** Protein post-translational modifications in lungs after cold exposure. Total protein expression was analyzed by SDS-PAGE (first), and posttranslational modifications were detected by Western blot for acetyllysine (second), succinyllysine (third), crotonyllysine (fourth), ubiquitin (fifth), β -hydroxybutyryllysine (sixth), and lactyllysine (seventh). **e,** Summary of lysine succinylome profiling. The left bar plot shows the numbers of detected peptides, identified succinylation sites, comparable succinylation sites, modified peptides, identified succinylated proteins and comparable succinylated proteins. The right bar plot shows the numbers of proteins and sites with increased or decreased succinylation in cold treated lungs relative to control lungs. **f,** The nine-quadrant diagram shows the differences between t changes in total protein abundance and changes in lysine succinylation.

Supplementary Table 1

General information for the control and cold exposure multi tissue single cell atlas.

Supplementary Table 2

Cell proportions across tissues in the control and cold exposure multi tissue single cell atlas.

Supplementary Table 3

Differentially expressed genes (DEGs) across tissues and cell types in the control and cold exposure multi tissue single cell atlas.

Supplementary Table 4

Gene Ontology enrichment analysis of differentially expressed genes (DEGs) across tissues and cell types in the control and cold exposure multi tissue single cell atlas.

Supplementary Table 5

Immune cell type proportions across tissues in the control and cold exposure multi tissue single cell atlas.

Supplementary Table 6

Differentially expressed genes (DEGs) across all immune cell types in the control and cold exposure multi tissue single cell atlas.

Supplementary Table 7

Baseline association between Spi1 and Chil3 and predicted Chil3 response to in silico Spi1 knockout in C01 macrophages.

Supplementary Table 8

Functional enrichment analysis of differentially abundant proteins in lung under cold exposure versus control.

Supplementary Table 9

Functional enrichment analysis of proteins carrying differentially regulated succinylation sites in lung tissue under cold exposure versus control.

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