Shifts of Immune Cell Populations Differ in Response to Different Effectors of Beige Remodeling of Adipose Tissue

Nabil Rabhi, Anna C. Belkina, Kathleen Desevin, Briana Noel Cortez, Stephen R. Farmer

HIGHLIGHTS
ScSeq reveals an extensive remodeling during browning of white adipose tissue
Cold and β3Adr agonist treatment results in distinct brown/beige remodeling
Cold induces a myeloid to lymphoid shift of the immune compartment
β3Adr agonism leads to an interferon/Stat response and infiltration of myeloid cells
Shifts of Immune Cell Populations Differ in Response to Different Effectors of Beige Remodeling of Adipose Tissue

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SUMMARY

White adipose tissue (WAT) is a dynamic tissue, which responds to environmental stimuli and dietary cues by changing its morphology and metabolic capacity. The ability of WAT to undergo a beige remodeling has become an appealing strategy to combat obesity and its comorbidities. Here, by using single-cell RNA sequencing, we provide a comprehensive atlas of the cellular dynamics during beige remodeling. We reveal drastic changes both in the overall cellular composition and transcriptional states of individual cell subtypes between Adrb3- and cold-induced beiging. Moreover, we demonstrate that cold induces a myeloid to lymphoid shift of the immune compartment compared to Adrb3 activation. Further analysis showed that, Adrb3 stimulation leads to activation of the interferon/Stat1 pathways favoring infiltration of myeloid immune cells, while repression of this pathway by cold promotes lymphoid immune cell recruitment. These findings highlight that pharmacological mimetics may not provide the same beneficial effects as physiological stimuli.

INTRODUCTION

Adipose tissue is a central metabolic organ for whole-body energy homeostasis. An imbalance between energy intake and energy expenditure increases adiposity and can lead to severe metabolic disease (Sun et al., 2011). White adipose tissue (WAT) plays a key role as a reservoir for triglyceride storage, whereas brown adipose tissue (BAT) dissipates energy as heat through mitochondrial uncoupling. Under appropriate stimulation, such as cold exposure or beta-3-adrenergic receptor (ADRB3) stimulation, WAT can adopt a thermogenic phenotype, sustained by emergence of uncoupling protein 1 (UCP1) expressing cells (Kajimura et al., 2015). These cells, called beige or brite fat cells, share the same energy-burning capacity as BAT through substrate oxidation but present a distinct molecular and developmental origin. Increasing whole-body thermogenic capacity by activating BAT and promoting beige cells emergence may represent a promising strategy to counteract the development of obesity and diabetes (Cannon and Nedergaard, 2004; Bartelt et al., 2011). Indeed, activation of thermogenesis plays a critical role in promoting a shift in energy expenditure in obese and type 2 diabetes (T2D) individuals through a potent glucose and lipid clearance to fuel thermogenesis. Essentially, all studies to date show that beiging of WAT prevents high-fat, diet-induced insulin resistance and weight gain, resulting in positive metabolic indicators, such as insulin sensitivity and euglycemia (Berbée et al., 2015). Additionally, adult human BAT has been identified with more of a brite/beige character than classic rodent brown fat (Sharp et al., 2012; Cypess et al., 2009). Therefore, a better understanding of mechanisms controlling beige adipogenesis could lead to the development of new therapies for metabolic diseases.

WAT is a complex organ consisting of a mixture of mature adipocytes and stromal vascular cells (SVCs). SVCs, comprising 80% of WAT cells, are a dynamic and complex assortment of resident immune cells, vascular cells, mesenchymal stem cells (MSCs), and pre-adipocytes that can change with development and WAT remodeling (Eto et al., 2009). Furthermore, these changes in cell population can play an important role in the capacity of the tissue to respond to the metabolic needs of the body (Kahn et al., 2019; Choe et al., 2016). While a lot of effort has been devoted to defining the cellular plasticity during obesity, little is known about the landscape of these changes during early beige adipogenesis. Although some studies have attempted to define beige adipogenesis, the focus has been on characterizing progenitor cell origin and fate decisions using either mouse lineage tracing models or cell sorting (Rajbandari et al., 2019;...
Vishvanath et al., 2016; Sanchez-Gurmaches and Guertin, 2014). Moreover, previous investigations have used an ADRB3 activator (CL316,243) as the tool to model WAT response to cold (Lee et al., 2017; Burl et al., 2018; Rajbhandari et al., 2019). Indeed, ADRB3 activation in vivo by CL 316,243 (CL) provides a means to rapidly induce WAT beiging; however, whether CL induces the same adipose tissue remodeling as cold exposure remains to be determined. Herein, we provide a comprehensive atlas of WAT SVC cellular subtypes and address the change in complexity of the tissue during early response to cold and CL using single-cell RNA sequencing (scRNAseq) technology. Our results identify critical cell subpopulations and their dynamic changes that occur following cold or CL treatment. In combination with flow cytometry, we demonstrate that immune cells with a myeloid origin expand in response to CL treatment while cold exposure leads to expansion of lymphoid cells, mainly B cells, CD4, and CD8 T cells. Mechanistically, this immune shift is likely controlled by activation of the interferon pathway and Stat-1 phosphorylation.

RESULTS

Cold and β3Adr Agonist Treatment Lead to Distinct Beige Remodeling

Histological analysis of C57BL/6J mice treated with Aдр3 agonist (CL316,243; CL) or cold for either 3 days or 10 days led to a comparable level of beiging within the subcutaneous inguinal WAT (iWAT) (Figure S1A). However, gene expression analysis of immune markers within the SVC fraction showed a decrease of Ccl2; the monocytes/macrophages chemoattract cytokine in cold conditions while no changes were observed in response to CL at both 3 and 10 days (Figure 1A). In agreement with these results, we found that Adrge1, a macrophage marker, was increased in CL-treated mice while decreased by cold exposure at both monitored time points (Figure 1A). Moreover, extracellular matrix (ECM) markers such as Col1a1 and Col3a1 were increased in response to CL and decreased following cold exposure. Interestingly, adipogenesis markers Fabp4 and adiponectin were only increased by cold exposure at 3 days (Figure 2A), and thermogenic markers were more responsive to CL treatment than cold. To gain more insight into the differences between cold- and Adrb3-induced immune response, we stained for CD45, a common immune cell marker (Figure 1B). We found that CL increases immune cell infiltration at both 3 and 10 days, whereas no changes were observed with cold exposure (Figures 1B and 1C). Surprisingly, we found that more T cells were observed following cold exposure than CL treatment at both monitored times (Figures 1D and 1E).

Single Cell Sequencing Reveals an Extensive Remodeling of Stroma Vascular Compartment during Browning/Beiging of WAT

To understand the full extent of the divergent cellular response to CL and cold, we performed single cell RNA sequencing (scRNA-seq) analysis of the SVC fraction isolated from iWAT of control, CL-, or cold-treated mice for 3 days to capture early changes. Data from all three treatments were pooled together to identify subpopulations within the captured cells. Unsupervised clustering using gene markers singled out 20 distinct cell clusters (Figure 2A). We assigned putative biological identities to each cluster by manual annotation using established gene expression patterns, as well as by interrogating a gene expression atlas (Su et al., 2004; Ravasi et al., 2010). The annotation resulted in several groups of cells including pre-adipocytes, mesenchymal stem cells, immune cells, and neuronal cells (Figure 2B). Previous reports identified two to three cellular subpopulations with an adipogenic potential that were defined as mesenchymal progenitor cells (MSCs) (Merrick et al., 2019; Hepler et al., 2018; Burl et al., 2018). However, these data were either generated from Pdgfrα+ sorted cells or assuming that canonical MSC markers Cd34, Pdgfra, Lys6a (Sca1) are co-expressed within the same cell. Because Cd34 and Sca1 are the major markers of stemness for most of mice progenitors, we plotted them across our single-cell data to discriminate between possible MSCs and other cells subtype. We found that four clusters which we named MSC1, MSC2, MSC3, and pre-adipocytes express both markers (Figures 2B and 2C). Pdgfraf was expressed in most of the cells in the three MSC subpopulations and was highly expressed in MSC2 cluster. Dpp4 and Pit16, markers previously proposed as interstitial progenitors, were exclusively expressed in MSC1. Fabp4 was expressed in both MSC2 and pre-adipocytes, and Ppary was exclusively expressed by the pre-adipocyte cluster suggesting that the MSC2 state may precede the pre-adipocytes state (Figure 2C). Genes encoding ECM components were expressed at different levels within the four clusters, with MSC2 cluster expressing the largest number of ECM-related genes (Figure 2A and Table S1). Interestingly, collagen-type expression was found to be different between populations. Indeed, Col14a was identified as a marker for MSC1 cluster, Col15a as a marker for MSC2 cluster, while both marked MSC3 cluster (Table S1).
Figure 1. CL and Cold Treatment Lead to Distinct Beige Remodeling

All experiments were carried out following 3 days of in vivo CL316,234 or cold exposure.

(A) Real-time PCR analysis of relevant immune (top left), ECM (top right), adipogenesis (bottom left), and thermogenesis markers (bottom right) in the SVF from iWAT.

(B) Representative image of CD45 immunofluorescent staining.

(C) Quantification of CD45+ cells (n = 5).

(D) Representative image of CD3 immunofluorescent staining.

(E) Quantification of CD453+ cells (n = 5).

Data are presented as mean ± SEM. p values. n = 5–6 animals in each group; *p < 0.05, **p < 0.01, ***p < 0.001.
Our merged data from the 3 conditions allowed for the identification of 13 distinct immune clusters (Figure 2B and Table S1). We used unsupervised annotation and cell-type-specific markers to interpret and identify the resulting 13 immune clusters based on literature searches and the Immunological Genome Project database (Figure 2D) (Jojic et al., 2013; Yoshida et al., 2019). We identified 3 clusters that express macrophage markers such as Adgre1 (Figure 2D). Proinflammatory cytokines such as Ccl2, Ccl6, Ccl9, Ccl12,
Cells of lymphoid origin (Figure 3C). We verified the results obtained by scRNAseq using flow cytometry that are increased with CL are mostly from a myeloid origin, whereas cold promotes an increase of immune cell populations. These results suggest a dissimilar immune cell response to CL and cold. Interestingly, the cell populations were reduced by cold compared to vehicle- or CL-treated mice (Figure 3D). More importantly, cold induced lymphoid origin immune cells including B cells (CD19+), CD4, and CD8 T cells (Figures S3B and S3C). In concordance with previous results, immune cells with a myeloid origin were reduced by cold and CL. However, the reduction was more prominent with CL than cold (Figure 3B). Normalized macrophages and monocyte populations were increased by CL and reduced by cold. In contrast, CD4T cells, NKs, and all B cells were increased by cold and reduced by CL treatment (Figure 3B). These results suggest a dissimilar immune cell response to CL and cold. Interestingly, the cell populations that are increased with CL are mostly from a myeloid origin, whereas cold promotes an increase of immune cells of lymphoid origin (Figure 3C). We verified the results obtained by scRNAseq using flow cytometry of the iWat stromal vascular fraction (SVF) from mice treated with a vehicle, CL, or cold for 3 days. We used a panel of antibodies that allowed detailed assessment of multiple immune subsets previously identified by the scRNAseq including B cells, CD4+ and CD8+ T cells, NK cells, DC cells, granulocytes, M1 and M2 macrophages, and monocytes (Figures S3B and S3C). In concordance with previous results, immune cells with a myeloid origin were reduced by cold compared to vehicle- or CL-treated mice (Figure 3D). More importantly, cold induced lymphoid origin immune cells including B cells (CD19+), CD4, and CD8 T cells compared to the other treatments. All together, these data suggest that cold- and CL-induced beiging involves a different immune remodeling leading to the same level of UCP1+ adipocytes (Figure S1A). Indeed, activation of Adrb3 induces a specific activation of cells with a myeloid origin such as macrophages while cold leads to increased recruitment of immune cells with a lymphoid origin, suggesting that CL treatment is not able to activate the full immune system to mimic the cold.

Cold and b3Adr Agonist Differentially Alter the Adipose Resident Immune Compartment

To commence such an examination, we performed a side-by-side comparison of the control, CL-, and cold-treated data sets. While the data showed that the twenty clusters are represented within the three conditions, our analysis revealed drastic changes both in the overall cellular composition and transcriptional states of individual cell subtypes (Figure 3A and 3B). Initial analysis of Uniform Manifold Approximation and Projection (UMAP) maps of the data showed that the pre-adipocyte population increased in CL condition compared to cold and control treatment (Figure 3A). However, normalization of the data to total number of cells sequenced per condition revealed that only cold increased the pre-adipocyte population (Figures 3B and S2A). MSC3 cluster showed the same pattern while both MSC1 and MSC2 clusters were reduced by both cold and CL. However, the reduction was more prominent with CL than cold (Figure 3B). Normalized macrophages and monocyte populations were increased by CL and reduced by cold. In contrast, CD4T cells, NKs, and all B cells were increased by cold and reduced by CL treatment (Figure 3B). These results suggest a dissimilar immune cell response to CL and cold. Interestingly, the cell populations that are increased with CL are mostly from a myeloid origin, whereas cold promotes an increase of immune cells of lymphoid origin (Figure 3C). We verified the results obtained by scRNAseq using flow cytometry of the iWat stromal vascular fraction (SVF) from mice treated with a vehicle, CL, or cold for 3 days. We used a panel of antibodies that allowed detailed assessment of multiple immune subsets previously identified by the scRNAseq including B cells, CD4+ and CD8+ T cells, NK cells, DC cells, granulocytes, M1 and M2 macrophages, and monocytes (Figures S3B and S3C). In concordance with previous results, immune cells with a myeloid origin were reduced by cold compared to vehicle- or CL-treated mice (Figure 3D). More importantly, cold induced lymphoid origin immune cells including B cells (CD19+), CD4, and CD8 T cells compared to the other treatments. All together, these data suggest that cold- and CL-induced beiging involves a different immune remodeling leading to the same level of UCP1+ adipocytes (Figure S1A). Indeed, activation of Adrb3 induces a specific activation of cells with a myeloid origin such as macrophages while cold leads to increased recruitment of immune cells with a lymphoid origin, suggesting that CL treatment is not able to activate the full immune system to mimic the cold.

b3Adr Agonist-Induced Browning/Beiging Lead to an Interferon/Stat1 Response

To gain more insight into the mechanisms controlling the shift from myeloid to lymphoid immune cell recruitment upon cold exposure compared to CL, we performed differential gene expression analyses between the same clusters using the treatments as a variable. The results showed that both immune cells with a myeloid origin or a lymphoid origin activate a different set of genes in response to either CL or cold (Figures 5A and 5B). Because the activation of the monocytes and macrophages precedes the activation of lymphoid cells such as T cells and B cells, we focused on macrophages. Differential analysis revealed that cold induced the upregulation of 140 genes while CL upregulated 23 genes, only 9 of those were overlapping with cold. We also identified 57 genes downregulated by cold while CL only decreased 2 genes (Figure 4A and 4B). Gene ontology analysis of genes downregulated by cold showed an enrichment of genes associated with biological processes including cytokine-mediated signaling, cellular response to type 1 interferon, and type 1 interferon signaling pathway (Figure 4D). We next examined the expression of genes induced by interferons such as Ifit7, Isg15, Ifit1, and Saa3 across all the identified clusters. Surprisingly, we found that interferon target genes are induced in most immune population regardless of their origin. To further confirm these results, we stained tissue from vehicle-, CL-, or cold-treated mice with antibody against pStat1, an interferon-induced signaling component. Our data showed that CL treatment induced a considerable phosphorylation of Stat1 in SVF cells. Collectively, these results strongly suggest that repression of the interferon/pStat1 pathway controls the shift from myeloid to lymphoid immune cell recruitment during cold exposure compared to CL.
DISCUSSION

In the present study, we reveal a complete atlas of cellular complexity of the SVF during beige remodeling. All cell populations were present within iWAT from control mice, suggesting that cells are at a paused-like state to maintain tissue homeostasis. Cold and CL treatments lead to a large modulation of the cellular composition to achieve a beige phenotype. Previous studies have identified two distinct MSC populations within the epidermal WAT (eWAT) and three MSCs within the iWAT (Merrick et al., 2019; Buri et al., 2018;
Our current work reveals the existence of four distinct MSC populations harboring an adipogenic potential which express classical stemness markers and can be distinguished by specific markers including different collagen subtypes. The co-existence of the four populations could explain the high adipogenic potential of the iWAT compared to the eWAT. While all MSCs exist within the control mice iWAT, cold and CL treatments lead to major changes both in the cell number and signaling pathways of individual MSC subtypes. Interestingly, pre-adipocytes and MSC3 cluster were increased in response to cold compared to CL, suggesting that cold leads to significantly more expansion of those populations. However, further studies will be needed to determine if there are any differences between cold and CL stimulation in the recruitment potential to adipocyte progenitors.

Large changes within the immune fraction composition were also observed. In agreement with previous reports from both whole tissue RNA sequencing and scRNAseq, we found that Adrb3-induced beiging...
leads to an increase of macrophage recruitment (Lee et al., 2016; Nguyen et al., 2011; Burl et al., 2018). At a more global scale, immune cells derived from myeloid origin were increased in response to CL compared to cold. In contrast, cold promoted the recruitment of lymphoid originated immune cells including B cells, CD4, and CD8 T cells. This suggests that a shift from myeloid to lymphoid immune cells is an important step to promote the high level of beiging attained in response to cold. Furthermore, it assumes a functional interaction of lymphoid cells with activated MCSs to induce complete beige remodeling. The differences in the origin of the immune cell populations involved in cold- versus Aдрb3-induced beiging will be important to address in future studies looking into the immune implications in thermogenesis.

Our results further showed that the interferon/Stat1 signaling pathway is activated by CL suggesting an importance of these pathways in myeloid activation during CL-induced beiging. Previous work on human peripheral blood mononuclear cells showed that interferon synthesis was suppressed by catecholamines and favors a type 2 cytokine through Aдрb2 stimulation (Wahle et al., 2005). Furthermore, neural inputs have been shown to increase lymphocyte numbers in vitro and in vivo (Agarwal and Marshall, 2000; Araujo et al., 2019). Other studies have documented a need to suppress Interferon/Stat1 signal transduction pathways and transcription factors downstream of cytokines to drive differentiation of Th subtypes (Naka et al., 2001; Yu et al., 2004). Interestingly, catecholamines have been shown to promote an anti-inflammatory effect by inhibiting Stat1 phosphorylation and favor Th2 cytokine type secretion such as IL13 and IL4 (Ishii et al., 2015). Our single-cell data show that Aдрb3 activation alone leads to recruitment of myeloid immune cells and an increase of Stat1 phosphorylation, suggesting that CL is not sufficient to create the cytokine/cellular microenvironment for lymphoid immune recruitment to achieve a cold-like beige remodeling. These studies support a model in which catecholamines released during cold exposure lead to lymphoid immune recruitment through the suppression of the interferon response activated by Aдрb3 stimulation alone. Moreover, our results suggest the involvement of different coordinated signaling pathways to induce beige remodeling during cold and open the possibility that different signaling can lead to distinct adipocytes with an equivalent beige phenotype. Those observations revealed that Aдрb3-induced beiging mimics a hypermetabolic-like response found in the context of thermal injury or cachexia (Petruzelli et al., 2014; Sidossis et al., 2015; Patsouris et al., 2015). Indeed, the hypermetabolic response is characterized by a profound increase in free fatty acids and glycerol release from fat and increase in myeloid immune cell infiltration ultimately resulting in significant elevations in resting energy expenditure (Jeschke et al., 2014). Moreover, Adrb3 activation has been implicated in burn-associated beiging (Kulp et al., 2010; Jeschke et al., 2011). Our data suggest that, although both cold and CL lead to an initial metabolic benefit, prolonged CL exposure is potentially futile and could become devastating, specifically in an already inflamed context such as obesity. More attention should be focused on the immune microenvironments when developing new pharmacological approaches to induce thermogenesis.

In conclusion, these data provide a comprehensive atlas of the cellular dynamics during beige remodeling within WAT. We shed light on the complexity of the immune microenvironment and highlight the differences in the immune cell populations infiltrated in response of Aдрb3- and cold-induced beiging. A better understanding of the signaling pathways and the cellular intra-organ communication influencing beige remodeling during Aдрb3 and cold stimulation could ultimately lead to novel strategies to increase energy expenditure and protect against obesity.

Limitations of the Study

Our study reveals that cold induces a myeloid to lymphoid shift of the immune compartment compared to Aдрb3 activation. We showed that Aдрb3 stimulation leads to activation of the interferon/Stat1 signaling pathway suggesting an importance of these pathways in myeloid activation during CL-induced beiging. However, further investigations of myeloid cells are needed to demonstrate the exact mechanism by which interferon/Stat1 signaling is activated by Aдрb3 stimulation. Myeloid cells do not express Aдрb3, so it is likely that Aдрb3 activation leads to secretion of cytokines by adipocytes that in turn activate the interferon/Stat1 pathway. Blocking secretion of these cytokines could be important to induce the recruitment of lymphoid cells. We also do not know the specific cytokines involved in lymphoid cell recruitment and the exact role of these lymphoid cells. We suggest that Aдрb2 stimulation by catecholamines released upon cold exposure suppresses interferon/Stat1 signaling and favors the recruitment of lymphoid cells. More in vivo experiments including myeloid cell-specific knockout mice models and/or Aдрb2 agonists are needed to demonstrate that activation of Aдрb2 is involved in the myeloid to lymphoid shift upon cold exposure.
Resource Availability

Lead Contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Stephen R Farmer, Boston University School of Medicine (sfarmer@bu.edu).

Materials Availability
This study did not generate new unique reagents.

Data and Code Availability
The data sets/code generated during this study are available at GEO accession GSE159966: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE159966.

METHODS
All methods can be found in the accompanying Transparent Methods supplemental file.

SUPPLEMENTAL INFORMATION
Supplemental Information can be found online at https://doi.org/10.1016/j.isci.2020.101765.

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AUTHOR CONTRIBUTIONS
Conceptualization, N.R. and S.R.F.; Methodology, N.R. and S.R.F.; Investigation, N.R.; Flow cytometry analysis, N.R and A.C.B; Formal Analysis, N.R.; Mouse experiments, N.R, K.D, and B.N.C; Writing – Review & Editing, N.R. and S.R.F.

DECLARATION OF INTERESTS
The authors declare there are no conflicts of interest.

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Supplemental Information

Shifts of Immune Cell Populations Differ
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Supplemental File for:

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TRANSPARENT METHODS

Animals
C57Bl6 mice were purchased from The Jackson Laboratory at 6-week of age and acclimated for 2-week. Mice were housed in a temperature-controlled environment with a 12 hr light-dark cycle and ad libitum water and standard chow diet. For both single cells and flow cytometry experiments, 8-week-old mice were daily injected intraperitoneally (i.p.) with either vehicle (saline) or CL-316,243 (1 mg/kg) for 3 days before euthanasia. For cold exposure experiment, mice were maintained in 4°C room for 3 days. All animal studies were approved by the Boston University School of Medicine Institutional Animal Care and Use Committee.

Histology
Tissue was fixed with paraformaldehyde, paraffin embedded, and sectioned (5 mm) prior to H&E staining or immunohistochemistry for Phospho-Stat1 (Tyr701) (58D6; cell signaling; 1:800).

Immunofluorescence
5 µm slices of paraffin-embedded inguinal adipose tissue were mounted onto slides, deparaffinized and rehydrated before performing antigen retrieval. Tissue sections were stained with rabbit anti-CD3 (D7A6E; cell signaling, 1:200), rabbit anti-CD45 (D3F8Q; Cell Signaling, 1:100) overnight at 4C. After washing with 0.1% tween-20 TBS, sections were incubated for 1 hr at room temperature with fluorophore conjugated secondary antibody (donkey anti-rabbit Alexa 647 (Invitrogen). Slides were then washed three times with 0.1% tween-20 TBS at room temperature in the dark. Coverslips were mounted using Prolong gold antifade (Thermofisher). Fluorescent images for all stained adipose tissue sections were captured with an Axio scan Z1 imager (Zeiss) at 20x magnification.

Real-Time PCR
Total RNA was extracted from frozen tissues and cells using TRIzol reagent according to the manufacturer’s instructions. RNA concentrations were determined on NanaDrop spectrophotometer. Total RNA (100 ng to 1 mg) was transcribed to cDNA using Maxima cDNA synthesis (Thermo Fisher Scientific). Quantitative real-time PCR was performed on ABI Via detection system, and relative mRNA levels were calculated using comparative threshold cycle (CT) method. SYBR green primers are listed in Table S1.

Flow cytometry analysis and data processing
Freshly isolated iWAT stromal vascular cell (SVC) were resuspended in FACS buffer (PBS/1% BSA). Samples were blocked with mouse Fc block (Biolegend; 1:50) for 5 min then incubated with antibody mix supplemented brilliant stain buffer (BD Biosciences) and monocyte blocker (Biolegend) for 20 min at 4C protected from light. SVF cells suspension was rinsed 3 times before flow cytometry analysis with Aurora spectral cytometry analyzer (Cytek Biosciences). Antibodies are listed in Table S2. All data analyses were performed in Omig.ai cloud cytometry data analysis platform. Single live CD45+ cells were clustered with Phenograph (Levine et al., 2015) and visualized with opt-SNE dimensionality reduction algorithm (Belkina et al., 2019). Groupings of clusters based on hierarchical clustering of median fluorescence intensities across multiple surface protein markers were annotated and color-overlaid on the opt-SNE projection of multidimensional data. Frequencies of each cell type were calculated from corresponding clusters and data were plotted and compared using Prism 6.0 (Graphpad).

Isolation of Stromal Vascular Cells from Mouse iWAT:

Inguinal white adipose tissues (WAT) from control and CL- and cold-treated mice were collected after CL treatment and processed for SVC isolation using mouse adipose tissue dissociation kit (Miltenyi biotec) according to manufactures.

Single Cell RNA Sequencing

Cells were prepared for single-cell sequencing according to the 10x Genomics protocols. Sequencing was performed on Illumina NextSeq500. The Cell Ranger Single-Cell Software Suite (v.3.1.0) (available at https://support.10xgenomics.com/single-cell-gene-expression/software/pipelines/latest/what-is-cell-ranger) was used to perform sample demultiplexing, barcode processing, single-cell 3’ counting, and counts alignment to mm10 mouse reference genome. For further analysis, the R (v.3.1) package Seurat was used (adapted workflow available at https://satijalab.org/seurat/v3.1/immune_alignment.html) (Stuart et al., 2019). Briefly, Cells with feature counts over 2500 or less than 200 or have over 5% of mitochondrial genes were filtered out. All the samples were integrated and top 45 dimensions were used to generate the final clusters. Cells are represented with Uniform Manifold Approximation and Projection
(UMAP) plots. The Seurat function “FindNeighbors” followed by the function “FindClusters” were used for clustering using resolution of 0.5. FindAllMarkers function was used to identify specific gene markers for each cluster. Violin plots were used to compare selected gene expression. Differential expression between clusters was obtained using MAST. Specific genes for each cluster were used for functional annotation and Go terms using Enrichr (Chen et al., 2013, Kuleshov et al., 2016).

**Statistical analysis**

Data were analyzed using GraphPad Prism 6.0 software (GraphPad) and are presented as mean ± standard error of mean (SEM). Group comparisons were analyzed using either two-tailed unpaired student t test or a two-way ANOVA followed by multiple comparisons correction method stated in Figure legend. Differences were deemed statistically significant with p < 0.05.

**Code availability**

Codes are publicly available in the relevant citations and custom script is available on request.

**References**

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Figure S1 related to Figure 1: CL and cold treatment lead to distinct beige remodeling. (A) Representative H&E staining sections of from * weeks mice treated with vehicle, CL or cold for 3 and 10 days (n = 5–6 per group).
Figure S2 related to Figure 3: CL and cold differently alter the adipose resident immune compartment. (A) bar charts showing cells populations percentage per treatment from mice treated 3 days with either vehicle, CL or cold. (B) Heatmap of identified Phenograph clusters in flow cytometry data and their assignment to immune cells population of iWAT from mice treated 3 days with vehicle, CL or cold (each row represents a surface marker and Phenograph cell clusters and their assignment to immune cell types are shown in rows). (C) Flow cytometry opt-SNE plot of immune SVC in iWAT from mice treated 3 days with vehicle, CL or cold (All color overlays on t-SNE plots correspond to cell type classes; same color is assigned to Phenograph cluster groupings shown in panel B; grey color indicates debris clusters).
Figure S related to Figure 4: CL-induced beiging leads to an interferon/Stat1 response. (A) Heatmaps of differentially expressed genes in each myeloid cell type identified by single cell from mice treated 3 days with either vehicle, CL or cold. (B) Heatmaps of differentially expressed genes in each lymphoid cell type identified by single cell from mice treated 3 days with either vehicle, CL or cold.
### Table S1: SYBR green primers list related to Figure 1

| gene | forward primer | reverse primer |
|------|----------------|----------------|
| Ccl2 F | TTAAAAACCTGGATCGGAACCAA | GCATTAGCTTCAGATTTACGGGT |
| Ccl2 R | | |
| Adgre1 F | CGTGGTGTGTTGGTGACTGTGA | CCACATCAGTTTCCAGGAGAC |
| Adgre1 R | | |
| Col1a1 F | TAAGGGTCCCCAATGGTGAGA | GGGTCCCTCGACTCCTACAT |
| Col1a1 R | | |
| Col3a1 F | CTGTAAACATGGAAAATCGGGGAAA | CCATAGCTGAACTGAAAACCACC |
| Col3a1 R | | |
| Fabp4 F | TGGTGACAAGCTGGTGTTGGAATG | TCCAGGCTCTTCTTCTTGCTCA |
| Fabp4 R | | |
| AdipoQ F | GTTCCCAATGTCACCATTCG | GTTCCCAATGTACCCATTCGC |
| AdipoQ R | TGTTGCACTAGAAGCTTGCAG | TGTTGCAGTAGAACTTGCCAG |
| UCP1 F | TCCTAGGGACCACCCACCC | TCCAGGCTCTTCTTCTTGCTCA |
| UCP1 R | | |
| Cox8b F | GAA CCA TGA AGC CAA CGA CT | GCG AAG TTC ACA GTG GTT CC |
| Cox8b R | | |
## Table S2: Flow cytometry antibodies list related to Figure 3

| Antibody Name                                      | Biolegend catalog number |
|----------------------------------------------------|--------------------------|
| Anti-CD11c Brilliant Violet 421                    | 117329                   |
| Anti-CD19 Brilliant Violet 605                     | 115539                   |
| Anti-CD4 Brilliant Violet 785                      | 100453                   |
| Anti-Ly-6G/Ly-6C (Gr-1) Alexa Fluor 488           | 108419                   |
| Anti- Ly-6C PE/Dazzle 594                          | 128043                   |
| Anti-CD8a PerCP/Cyanine5.5                         | 100733                   |
| Anti-CD3 PE/Cy7                                    | 100220                   |
| Anti-CD11b APC                                     | 101211                   |
| Anti-NK-1.1 Alexa Fluor 700                        | 108729                   |
| Anti-CD45 APC/Fire 750                             | 103153                   |