Published in final edited form as:

Cell Metab. 2023 March 07; 35(3): 386–413. doi:10.1016/j.cmet.2023.02.002.

Adipose Tissue at Single Cell Resolution

Babukrishna Maniyadath[1,2], **Qianbin Zhang**[2,3,4], **Rana K. Gupta**[3,5,*], **Susanne Mandrup**[1,6,*]

[1]Center for Functional Genomics and Tissue Plasticity, Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense M, Denmark.

[2]These authors contributed equally to this work

[3]Department of Internal Medicine, Touchstone Diabetes Center, UT Southwestern Medical Center, Dallas, TX, USA

[4]Present address: Broad Institute of MIT and Harvard, Cambridge, MA, USA.

[5]Present address: Department of Medicine, Division of Endocrinology, Duke Molecular Physiology Institute, Duke University, Durham, NC, USA

[6]Lead Contact

SUMMARY

Adipose tissue exhibits remarkable plasticity with capacity to change in size and cellular composition under physiological and pathophysiological conditions. The emergence of single cell transcriptomics has rapidly transformed our understanding of the diverse array of cell types and cell states residing in adipose tissues and has provided insight into how transcriptional changes in individual cell types contribute to tissue plasticity. Here, we present a comprehensive overview of the cellular atlas of adipose tissues focusing on the biological insight gained from single cell and single nuclei transcriptomics of murine and human adipose tissues. We also offer our perspective on the exciting opportunities for mapping cellular transitions and crosstalk, which have been made possible by single cell technologies.

Keywords

Single-cell sequencing; single-nuclei sequencing; cellular heterogeneity; adipose tissue plasticity; adipocytes; macrophages; preadipocytes; fibro-adipogenic progenitors; adipogenesis; inflammation

*Correspondence: s.mandrup@bmb.sdu.dk (S.M.) or rana.gupta@duke.edu (R.K.G.).

DECLARATION OF INTERESTS

All the authors declare that they have no competing financial interests.

INTRODUCTION

Adipose tissues are loose connective tissues with a high number of adipocytes. These specialized cells store metabolic energy in the form of large cytoplasmic lipid droplets and at the same time regulate several aspects of organismal physiology through their endocrine functions. Mammals harbor two main classes of adipose tissues, white and brown, that can be classified based on their primary function [1]. White adipose tissue (WAT) serves as the principal site for long-term energy storage and is characterized by the presence of large unilocular white adipocytes, which store surplus metabolic energy as triacylglycerides (TAGs) under anabolic conditions and release that energy as free fatty acids (FFAs) to fuel peripheral tissues under catabolic conditions. WAT is found at many discrete anatomical locations throughout the body of mammals with the major depots broadly sub-characterized as subcutaneous (i.e., located under the skin) or intra-abdominal (within the peritoneum; associated with internal organs). In addition, multiple smaller depots are found at other locations, including the bone marrow, subdermal, perivascular, epicardial, peri- and inter-muscular regions [2–4]. In addition, to its energy storing function, WAT serves numerous nonmetabolic functions, including thermal insulation, and support and cushioning of organs [4].

The second major class of adipose tissue in eutherian mammals is brown adipose tissue (BAT), which plays a major role in thermogenesis and are found in smaller depots at several distinct anatomical locations, including anterior cervical, supraclavicular, intrascapular, perirenal and perivascular in mice and humans [5,6]. Brown adipocytes are characterized by multi-locular lipid droplets, abundance of mitochondria, and expression of uncoupling protein 1 (UCP1), which acts as a proton carrier in the inner mitochondrial membrane [1,7,8]. The presence of UCP1 along with other programs of futile energy cycling allow brown adipocytes to burn excess energy to produce heat and act as a metabolic sink [9]. In adult humans, BAT depots account for maximally 1–2% of total adipose tissue [10]; however, the abundance of BAT in small hibernating mammals or human neonates can constitute up to 5% of total body mass [11].

What separates adipose tissue from most other tissues is its remarkable capacity to change in size and cellular composition under physiological and pathophysiological conditions [12]. The adaptation to environmental and physiological challenges not only involves changes in adipocyte size, number, and function, but also long-lasting quantitative and qualitative changes in the cellular composition of the tissue. The “stromal-vascular” compartment is the term used to describe the non-adipocytes in the adipose tissue, and this consist of highly heterogeneous populations of immune cells, mesenchymal stromal cells, and endothelial cells as well as small populations of other cell types including neurons [12,13]. The development of single cell transcriptomics has rapidly transformed our understanding of the diverse array of cell types and cell states residing in adipose tissue and have shown how they influence the ability of WAT and BAT to exert its multiple functions. In this review, we provide a brief introduction to adipose tissue plasticity and the importance for human health, followed by a comprehensive review of the rapidly growing body of literature describing the cellular heterogeneity of murine and human adipose tissue. We highlight the biological insights gained specifically from single cell transcriptomics and discuss the

new opportunities offered by single cell approaches to acquire mechanistic insight into the regulation of adipose tissue plasticity.

SHORT-TERM METABOLIC PLASTICITY OF ADIPOSE TISSUES

Adipocytes can sense and quickly adapt to acute changes in the metabolic needs of the body. The classical example of a short-term change in adipocyte function is the adaptation that occurs during cycles of feeding and fasting. In the postabsorptive state, insulin acts as the major anabolic hormone stimulating both glucose and fatty acid uptake as well as *de novo* lipogenesis and triglyceride synthesis. Furthermore, insulin inhibits adipocyte lipolysis by stimulating phosphodiesterase 3B (PDE-3B), thereby decreasing cAMP levels [12,14]. As such, insulin sensitivity is essential for adipocyte storage capacity and function. Mouse models with deletion of the insulin receptor specifically in mature adipocytes display WAT atrophy and ectopic lipid deposition [15]. In the fasting state, when circulating insulin levels decline, inhibition of lipolysis is alleviated. Furthermore, release of noradrenaline from the sympathetic nervous system activates β-adrenergic receptors, thereby increasing PKA activity and stimulating lipolysis in adipocytes [16]. This balance between lipogenic and lipolytic signals is critical for the ability of adipose tissue to buffer the daily flux of nutrients and for maintenance of whole body metabolic homeostasis [17].

Another classical example of short-term metabolic plasticity is the activation of brown adipose tissue in response to acute cold exposure. Here norepinephrine released from the sympathetic nervous systems innervating the tissue leads to activation of β-adrenergic receptors and stimulation of the cAMP/PKA axis and induction of lipolysis in brown adipocytes. The fatty acids released by lipolysis simultaneously fuels mitochondrial β-oxidation and activates UCP1-mediated thermogenesis. Furthermore, PKA drives transcriptional activation of gene programs that support a sustained catabolic activity and mitochondrial uncoupling in brown adipocytes [12,18]. Interestingly, it was recently shown that the transcriptional effects are highly dependent on lipolysis which signals through peroxisome proliferator-activated receptor γ (PPARγ) as well as several other transcription factors [19]. Thus, metabolism and transcription are closely linked during thermogenic activation of brown adipocytes.

LONG-TERM CELLULAR AND COMPOSITIONAL PLASTICITY OF ADIPOSE TISSUES

“Browning” and “Whitening” of Adipose Depots

Chronic exposure to environmental and physiological stressors can elicit lasting changes of the cellular composition of adipose tissue and/or modulate the programming of cells within the tissue. One of the most striking examples of the long-term plasticity of adipose tissue is the adaptation to changes in environmental temperatures. In response to cold temperatures, WAT in many mammalian species can adopt a BAT-like phenotype with the emergence of thermogenic brown-like adipocytes commonly referred to as “Beige” or “BRITE adipocytes” [20–22]. This “browning” of WAT can occur upon reactivation of existing dormant (i.e., unilocular) beige adipocytes, by trans-differentiation of white adipocytes, as

well as through *de novo* differentiation of beige adipocytes from progenitor cells [23–26]. In rodents, cold exposure leads to marked browning primarily of subcutaneous inguinal WAT; however, prolonged cold exposure can also drive beige adipocyte accrual in other WAT depots [27]. Moreover, WAT browning can be observed in rodents following calorie restriction, intermittent fasting, gastric bypass surgery, and cachexia [28–32]. The human relevance of WAT browning is not clear; however, beige adipocyte accumulation has been observed in human WAT in response to conditions with increased sympathetic activity, such as severe burns and catecholamine-producing tumors [33–35] as well as in response to cachexia [30]. Cold-induced browning of WAT is driven predominantly by norepinephrine released by sympathetic neurons; however, several other inducers have been reported [36,37]. Catecholamines can act directly on adipocytes to activate a thermogenic gene program [27], or on adipocyte progenitors to drive beige adipogenesis [23]. However, this response is heavily augmented by local stromal cells and cells of the adaptive and innate immune systems [6,38–44].

Importantly, brown and beige adipocytes depend on a continued β-adrenergic tone to maintain their function. Denervation of BAT leads to a loss of thermogenic and oxidative capacity, along with a morphological “whitening” of the depot characterized by the presence of white-like unilocular adipocytes [45]. This “whitening” of BAT occurs naturally in association with aging, obesity, and shifts to warm environmental temperature, facilitated by the presence of inhibitory signals that interfere with β-adrenergic signaling [46–48]. The browning of WAT can also be reversed upon cessation of cold exposure or other thermogenic stimuli. As mice transition from cold temperatures to a warm environment, beige adipocytes slowly revert to a unilocular white adipocyte state with the gross molecular features of white adipocytes. However, these “dormant” beige adipocytes retain an epigenetic memory of their prior cold exposure, allowing them to rapidly reactivate their beige phenotype upon subsequent cold exposure [49].

Adipose Tissue to Mammary Gland Transition during Pregnancy and Lactation

The response of mammary adipose tissue to pregnancy represents another striking example of adipose tissue plasticity [50,51]. In rodents, the mammary gland is contained in the inguinal WAT, and during pregnancy this tissue undergoes a major transformation in preparation for lactation. Lipid-filled adipocytes essentially disappear coincident with a rapid expansion of the mammary epithelium. Adipocytes become delipidated during this time and lineage tracing indicates that cells undergo de-differentiation to a preadipocyte-like state [52,53]. As mammary ducts involute at the end of lactation the small adipocytes undergo hypertrophy as adipocytes take up the milk lipids, and adipocyte-derived preadipocytes re-differentiate [50,53].

Obesity Associated Change in Adipose Tissue Mass

The capacity of WAT to expand and contract in size is arguably unparalleled in adulthood. A chronic state of positive energy balance leads to a significant enlargement of most adipose tissues. WAT expansion is driven by a combination of adipocyte hypertrophy and adipocyte hyperplasia. Mature adipocytes do not undergo cell division; therefore, adipocyte hyperplasia is mediated by *de novo* adipocyte differentiation, or “adipogenesis,” from resident adipocyte precursor cells (APCs). In humans with normal range of body mass

index (BMI), WAT constitute roughly 20% and 30% of the body weight in men and women, respectively. In individuals with obesity, body fat percentage can rise to 50%, primarily due to expansion of the subcutaneous and intraabdominal visceral WAT [54–57]. The expansion of WAT during development of obesity is associated with substantial changes in the cellular composition, referred to as “tissue remodeling”. This includes vascular expansion, extracellular matrix (ECM) remodeling, recruitment of monocytes and activation to pro-inflammatory macrophages, and changes in the mesenchymal stromal cell phenotypes [58].

Conversely, during a negative energy balance, some adipose tissues contract in mass due to adipocyte size reduction and elimination. The remodeling of adipose tissue during weight loss is less well understood; however, increased immune cell infiltration during the early stages of acute weight loss has been observed in both human and rodent WAT[59–61]. On the other hand, decreased adiposity by very low-calorie consumption in rodents and bariatric surgery in humans has been reported to be associated with reduced crown-like structures (CLSs) and improved systemic metabolic parameters [62,63]. Interestingly however, persistent inflammation and metabolic defects of WAT has been reported during prolonged weight loss in some studies in mice and humans, indicating the existence of an obesity memory in the tissue despite improved systemic metabolism [64]. The mechanisms and functional consequences of a obesity memory and how it might affect weight re-gain are currently unknown.

ADIPOSE TISSUE DYSFUNCTION IN HUMAN DISEASES

The importance of adipose tissue in human health is highlighted by the condition of lipodystrophy, a diverse group of metabolic disorders commonly characterized by insufficient capacity to form functional adipose tissue. Adipose deficiency can either be the result of heritable mutations in genes driving adipocyte differentiation and lipid storage or acquired secondary to other conditions or medication [65,66]. The absence of functional adipose tissue leads to deleterious lipid accumulation in peripheral organs, including liver, skeletal muscle, heart, and the endocrine pancreas, resulting in insulin resistance and cardiovascular diseases.

On the other end of the spectrum, elevated BMI significantly confers increased risk of developing several life-threatening comorbidities including type 2 diabetes [67], cardiovascular diseases, fatty liver diseases [68,69], as well as increased risk of certain cancers [69,70]. Importantly however, some individuals with obesity avoid many features of metabolic syndrome, at least for a period. The identification and study of this so-called “metabolically healthy” obesity underscores the notion that factors beyond elevated BMI *per se* lead to the metabolic syndrome [71]. Comparisons to those individuals with obesity and insulin resistance (i.e., “pathologic obesity”) reveals that body fat distribution plays an important role in disease susceptibility. The incidence and severity of cardiometabolic comorbidities are primarily associated with expansion of visceral and abdominal subcutaneous WAT, whereas femoral and gluteal adipose tissues appear to be protective when corrected for fat mass [72]. Genome-wide association studies (GWAS) discovered that genes associated with body fat distribution and healthy obesity are highly enriched for adipocyte genes [73],

indicating that intrinsic properties of adipocytes, and their ability to remain functional, play an important role in determining body fat distribution and cardiometabolic diseases.

Furthermore, the way individual WAT depots expand and remodel in the setting of overnutrition impacts overall metabolic health [74]. Pathologic obesity is often characterized by the presence of hypertrophic adipocytes, excess ECM accumulation (fibrosis), the accumulation of pro-inflammatory immune cells, and decreased expression of protective adipokines (e.g., Adiponectin). Macrophage infiltration and proinflammatory macrophage polarization contribute to adipose tissue dysfunction and may enhance cardiometabolic disorders [75–77]. Healthy adipose tissue expansion is characterized by adipocyte hyperplasia, indicative of increased *de novo* adipogenesis, as well as a relatively lower degree of inflammation and fibrosis. In fact, fibrosis has emerged as a key factor that distinguishes metabolically healthy vs. metabolically unhealthy obesity of humans [78]. As such, lipodystrophy and obesity manifest itself with many of the same cardiometabolic comorbidities, underscoring the notion that limited adipocyte function and storage capacity play a major role in the development of these diseases [79].

The realization that maintaining healthy and functional adipocytes with a high storage capacity is critical for human health emphasizes the need to understand the regulation of adipocyte function in different human depots. Although intrinsic properties of adipocytes have been shown to be major determinants of overall adipose tissue function [73], cells of the tissue microenvironment, including immune cells [80], endothelial cells [81], and neurons [82,83], all play a critical role in controlling adipose tissue plasticity and adipocyte function. Thus, the understanding of human diseases relating to adipose tissue storage depends on in-depth investigations of the molecular properties of adipocytes in their tissue context and understanding how they interact with the array of other cell types in the tissue microenvironment.

CLASSICAL METHODS FOR STUDYING ADIPOSE STRUCTURE AND FUNCTION

The study of adipose tissue has historically been facilitated by classical techniques in bioimaging, cell culture, flow cytometry, and mouse genetics (Figure 1). For nearly a century, critical insights into the morphology and cellularity of adipose tissues have been gained through microscopic examination of tissue sections [84,85]. Some of the earliest observations include hypertrophy of adipocytes and the perivascular localization of pre-adipocytes [86], as well as cold-induced lipid droplet and mitochondrial remodeling in brown adipocytes [87]. These pioneering histological findings have laid the foundation for our current understanding of adipocyte differentiation and adipose tissue plasticity [87–91]. The development of the adipose tissue fractionation approach by Rodbell in the 1960’s facilitated the study of adipocyte metabolism in isolation as well as the ability to study cells of the “stromal vascular fraction” [92]. The derivation of clonal adipogenic fibroblast cells lines (e.g., 3T3-L1) in the 1970’s [93,94] was the key to gaining rapid insight into the functional properties of adipocytes and the molecular basis of adipocyte differentiation. These and other immortalized cell lines have enabled the discovery of adipocyte metabolic pathways

and lineage selective genes [95–99] as well as the transcriptional regulators of adipocyte differentiation and function including the master regulator peroxisome proliferator-activated receptor γ (PPARγ) [100] and CCATT-enhancer binding protein α(C/EBPα)[101]. Furthermore, with the advent of next generation sequencing, immortalized cells have been instrumental for mapping the transcriptional network and epigenomic mechanisms driving lineage determination and differentiation of [102–104] as well as browning of adipocytes [105]. Importantly, despite the artificial conditions of cell culture adipogenesis, many of the basic mechanisms and transcription factors identified also play a role for *in vivo* adipogenesis [104].

At the tissue level high throughput profiling of e.g., the transcriptome, proteome and metabolome have provided unbiased insight into the molecular composition of adipose tissue [106–109]. Biochemical approaches have been used to localize (e.g., immunohistochemistry) and isolate (e.g., flow cytometry) different cell types [7,110,111], whereas genetic approaches (Cre-LoxP) have been used to manipulate or label selective cell types [25,112,113]. Each approach has unique strengths and collectively they continue to be essential for studying aspects of adipose tissue biology. Nevertheless, as detailed below, the growing appreciation of cellular heterogeneity has also highlighted the limitations of these approaches (Figure 1). For instance, immunohistochemistry and flow cytometry rely on knowledge of antigens that discriminate cell populations and the availability of specific antibodies. Major cell types in the adipose tissue (e.g., hematopoietic cells, endothelial cells, mesenchymal stromal cells) can easily be distinguished from one another using a handful of markers; however, further subclassification of these major cell types relies on prior knowledge of these putative subpopulations and the identification of appropriate imaging/sorting strategies. Likewise, current gene targeting approaches in mice leverage the Cre-loxP recombination system, with Cre-recombinase expression ultimately directed by enhancers and promoters that are selectively activated in mature adipocytes (e.g., *Adiponectin*-enhancer and promoter) [114], thermogenic brown/beige adipocytes (*Ucp1*-enhancer and promoter) [115], or a broad pool of mesenchymal cells containing adipose progenitors (*Pdgfra* or *Pdgfrb* enhancer promoter) [113,116]. Current Cre-drivers are limited by the fact that they target broad pools of adipocytes or stromal cells. As such, the classical toolbox for the study of adipose tissue and nearly all other tissues have significant limitations for studies of tissue/cellular heterogeneity and plasticity.

SINGLE-CELL TECHNOLOGIES: PROMISING PATH TO RESOLVING ADIPOSE TISSUE FUNCTION AND HETEROGENEITY

The advent of next-generation sequencing (NGS)-based single-cell technologies has allowed for genomic and transcriptomic profiling of tissues at single cell resolution [117], thus transforming the toolbox for adipose tissue biologists. The resolving power of these methods carry the potential to uncover novel biological insights that may otherwise be hidden when analyzing cells in bulk. In particular, single-cell RNA sequencing (scRNA-seq) methods have proven extremely powerful. Transcriptomics at single-cell resolution can reveal the existence of distinct cellular states in populations of cells that might otherwise appear homogenous. Elucidating these cellular states as distinct snapshots of a dynamic process allows for the reconstruction of biological function at the single cell level. This has

led to discoveries of novel lineage hierarchies driving developmental processes, such as differentiation events [118,119] (Figure 2). Thus, single cell technologies have challenged the concept of cellular identity and plasticity.

With respect to adipose tissue, the use of single-cell technologies is complicated by the buoyant and fragile nature of isolated adipocytes. Many adipocytes are relatively large (up to 300 microns in humans [120]) and therefore incompatible with conventional droplet-based single-cell platforms, where the cell size is restricted by the width of the microfluidic channels (e.g., 50–60 microns for the Chromium platform from 10x Genomics [121]). Furthermore, due to the high lipid content, adipocytes float and cannot be pelleted with other cells in the tissue. Finally, even for strategies that do not involve droplet-based sequencing, the high lipid content interferes with enzymatic reactions. These challenges initially restricted adipose tissue single-cell analyses to non-parenchymal cell types. More recently, development of single-nucleus sequencing-based methods have allowed for inclusion of adipocytes in single-cell studies [122–124]. Single-nucleus RNA-seq (snRNA-seq) is based on isolation of nuclei from tissues/cell populations, followed by sequencing of the RNA from individual nuclei [125–127] (Figure 2). The technique poses a fundamental challenge of low amounts of mature RNA transcripts and comes with an inherent bias for changes in transcript levels that are imposed by differential transcription over those imposed by differential RNA processing. However, recent work comparing snRNA-seq and scRNA-seq for the adipocyte lineage based on cell culture studies found an overall good alignment [128]. A major advantage of snRNA-seq over scRNA-seq is that it does not require tissue dissociation to release individual cells from their native tissue environment, a process which can lead to cell type biases and to dissociation-induced artifacts at the transcriptomic level [129,130]. Furthermore, high quality nuclei can be isolated from frozen tissue, thereby avoiding the need for processing of freshly isolated tissues [131]. Nevertheless, a major challenge for snRNA-seq is that it is highly sensitive to transcript contamination (termed ambient RNA) from broken/damaged nuclei or the cytoplasm [129,132]. Therefore, snRNA-seq studies require high quality nuclei preparations and careful computational analyses to ensure that only high-quality droplets are included and that high abundance ambient transcripts are computationally removed prior to clustering [124,132].

In addition to technologies for high throughput single-cell transcriptomics, a large number of technologies for epigenomic profiling of chromatin accessibility, histone marks, and DNA methylation at single cell resolution have been developed [133]. The integration of single cell transcriptomics and epigenomics data provides a powerful strategy for inferring transcriptional mechanisms at single cell level [134,135] (Figure 2). Furthermore, integration with single cell proteomics allows for in-depth phenotypic characterization of cell states [136–138]. Future results from such integrated analyses are expected to lead to many new mechanistic discoveries in the field of adipose tissue function.

An important development in the single-cell omics field is the introduction of spatial transcriptomics which provides gene expression matrices that can be integrated with information about tissue architecture, cellular features, and relative position of cells [139,140]. These technologies can be categorized into, 1) next generation sequencing-based approaches that are extensions of the scRNA-seq combined with unique positional barcodes; and 2)

bioimaging-based approaches that rely on in situ sequencing or hybridization of probes to transcripts. Given the structure of the adipose tissue with many different types of small stromal vascular cells interspersed between large lipid-filled adipocytes, where most of the volume is devoid of transcripts, techniques based on positional barcoding based on a fixed array slide could be challenging. Here techniques providing true single cell resolution, such as those based on bioimaging, and hybridization are likely to be required. Promising techniques include multiplexed error-robust fluorescence *in situ* hybridization (MERFISH), which uses massively multiplexed single-molecule FISH measurements and provides true single-cell resolution and the ability to quantify the spatial distribution of hundreds to tens of thousands of RNA species at single-cell resolution [139,141,142]. Implementation of such high-resolution bioimaging-based technologies will be important for understanding adipose plasticity and the importance of the cellular microenvironment (Figure 2).

Single-cell omics technologies create a “still picture” of the state of all captured cells in the sample. However, due to the large number of cells, it is also possible to infer relationships between cells. For example, the developmental trajectory or state transitions of cells can be mapped from single cell RNA-seq using a variety of tools that order the cells according to a similarity-based trajectory [143,144] recently combined with directional information [145]. Moreover, cellular crosstalk can be inferred using tools such as CellPhoneDB, NicheNet etc., which predicts crosstalk based on a public repository of ligands, receptors and their interactions [146,147]. Such tools allow researchers to obtain pseudo-time and mechanistic insight into cellular differentiation and tissue plasticity. The combination with the emerging spatial technologies with single cell technologies will provide unique insight into the cellular crosstalk and tissue microenvironments. Finally, single cell transcriptome and/or epigenome data from tissues can be integrated with genetic data to map eQTLs (expression quantitative trait loci) across different cell types and cell states [148] and to infer disease mechanisms [149,150].

Single-cell and single-nuclei transcriptomics analyses have greatly expanded our knowledge of the cellular composition and heterogeneity of adipose tissues. In the sections that follow, we summarize the current knowledge of the cellular atlas of adipose tissue, focusing specifically on insight gained through the employment of these new technologies.

ADIPOCYTE HETEROGENEITY

White Adipocyte Heterogeneity

The importance of adipocytes for whole body physiology and the finding that adipose depots differ in their metabolic functions begs the question how adipocyte functions are regulated by the tissue microenvironment. Interestingly, recent studies mapping the single cell-resolved transcriptome of adipose tissue have revealed that there are multiple adipocyte subpopulations within individual WAT depots in humans as well as in mice [122,124,151] (Table 1). These subpopulations are characterized by expression of subpopulation-specific markers in addition to the canonical adipocyte-identity markers, such as *Adiponectin* and *Perilipin1*. First, snRNA-seq of epididymal WAT from mice, identified three distinct subpopulations of mature white adipocytes differing in their expression of metabolic and inflammatory genes [124]. These subpopulations were denoted 1) lipogenic adipocytes (LGAs) expressing

high levels of genes relating to *de novo* fatty acid synthesis, e.g., *Acaca* and *Acly*; 2) lipid-scavenging adipocytes (LSAs) expressing high levels of genes relating to lipid uptake and transport, e.g., *Cd36* and *Apoe;* and 3) stressed LSAs (SLSAs) which in addition express genes relating to hypoxia and autophagy, e.g., *Hif1a* and *Rab7*. Interestingly, the relative proportions of LSA and SLSA subpopulations are increased in high-fat diet (HFD)-induced obesity at the expense of LGAs. Comparison of cell sizes showed that the SLSAs are the largest, whereas LGAs display the smallest average cell size, consistent with the notion that small adipocytes are more insulin sensitive [152–154]. The cellular and molecular mechanisms underlying changes between the relative abundance of subpopulations are unclear; however, it is likely that adipocytes can switch between "cell states" in response to extrinsic and intrinsic signals. The reduction in LGA in response to HFD-induced obesity may explain the decrease in *de novo* lipogenesis and insulin sensitivity that has previously been reported in obese human and mouse WAT [155–157]. More recently, snRNA-seq analyses of murine inguinal and perigonadal WAT depots, including both epididymal and periovarian WAT, identified six subpopulations of mature adipocytes with no notable enrichment across depots or sex [122]. Some subpopulations could be associated with a lipogenic signature (mAd1, mAd4 and mAd5) similar to the LGAs reported previously; however, the abundance of these subpopulations increase rather than decrease in the obese condition. The discrepancy may relate to the different age and sex of the mice as well as the duration of the HFD-feeding in these studies. More careful time course studies are required to determine and resolve the temporal change in adipocyte plasticity in different depots in response to HFD.

snRNA-seq of WAT from human visceral and subcutaneous adipose tissue depots identified seven subpopulations of mature adipocytes [122], several of which are associated with a lipogenic gene signature, including expression of *FASN*, *ACACA* and *DGAT1*/*2*. Generally, the overlap between human and mouse subpopulations in this study is limited; however, similar to the decrease in LGA abundance in obese mouse epididymal WAT, the abundance of one of the human lipogenic subpopulations correlates negatively with increased BMI in the subcutaneous WAT depot. A smaller, yet significant, population of adipocytes seem to be associated with functions related to smooth muscle cells, such as calcium ion handling/signaling along with focal adhesion and cell substrate junctions.

Spatial transcriptomics of human subcutaneous abdominal WAT using Visium spatial arrays showed that despite the limited resolution of the arrays, three subpopulations of white mature adipocytes displaying distinct transcriptomic signatures (Table 1) and spatial organization could be detected [151]. Interestingly, the Adipo^{PLIN} subpopulation, (high expression of *PLIN1*/*4*), has a transcriptomic signature similar to the LGAs and is correlated with higher *in vivo* insulin sensitivity. Another subpopulation, Adipo^{SAA}, are enriched for genes involved in lipid uptake and handling, thereby resembling LSAs in mice, and the Adipo^{LEP} subpopulation and SLSAs seemed to share common enriched pathways related to leptin synthesis/signaling and ECM remodeling (Table 1). Similar to what was observed in mice, the relative abundance of the Adipo^{PLIN} adipocytes were reduced in obese subjects. Taken together, all studies agree that mice as well as human adipose tissue contain several white adipocyte subpopulations with one or more being more lipogenic and others being more involved in lipid uptake (Figure 3). Going forward, it will be important to gain insight into the developmental and transitional trajectory of these subpopulations to determine for

example whether some subpopulations of adipogenic progenitors are more likely to give rise to specific adipocyte subpopulations and to determine how the tissue microenvironment influence adipocyte cell states. Furthermore, it will be important to determine the role of different adipocyte subpopulations in defining short-term and long-term plasticity of WAT.

Thermogenic Adipocyte Heterogeneity

Investigation of mouse interscapular BAT have identified 10 subpopulations of brown adipocytes many of which are selectively enriched under specific temperature settings [123]. One subpopulation (P4) marked by *Cyp2e1* and *Aldh1a1* increased in abundance in thermoneutrality and appears to be involved in inhibition of thermogenic capacity by a mechanism that involves a paracrine effect of acetate synthesized by aldehyde dehydrogenase 1 A1. Adipocytes with a similar transcriptomic signature were also found in mouse inguinal adipose tissue. In snRNA-seq of human BAT, several of the 8 adipocyte subpopulations display a P4-like transcriptomic signature but the functional relevance of this remains to be determined.

Previous histological and lineage tracing studies in mouse intrascapular BAT have indicated the existence of two distinct subpopulations of brown adipocytes with high (H) and low (L) expression of *Ucp1* and *adiponectin* (termed “BA-H” and “BA-L”). These subpopulations display differential functional parameters like mitochondrial numbers and lipid droplet sizes [158,159]. Multidimensional reconstruction of published snRNA-seq of mouse iWAT adipocytes [40], identified a unique thermogenic subpopulation (Ad1-*Ucp1*^{High}) induced in response to cold or β-adrenergic activation [160]. This population is similar to the mAd1 mouse iWAT adipocyte subpopulation and to the human hAd6 adipocyte subpopulation characterized by others as by the higher expression of browning markers such as *EBF2, ESRRG and PPARGC1A* [122]. These subpopulations may represent beige adipocytes, which, as noted above, are remarkable in their plasticity to revert between a dormant and thermogenic state [49,158]. Future studies employing combined transcriptomics with chromatin accessibility profiling (e.g., snATAC-seq) may aid in predicting and understanding the major transcriptional drivers for such change in cell state.

MESENCHYMAL STROMAL CELL HETEROGENEITY

Preadipocytes and Fibro-adipogenic Progenitors

The subcutaneous inguinal WAT (iWAT) and intra-abdominal perigonadal WAT (gWAT) depots of mice were the focus of several of the first published adipose scRNA-seq analyses designed to interrogate stromal cell heterogeneity. The consensus from these studies is that there are at least two molecularly and functionally distinct subclusters of adipocyte progenitors reside within the iWAT and gWAT depots of adult mice [122,124,161–163]. One subcluster represents cells with a high degree of adipogenic potential *in vitro* and upon transplantation *in vivo* and with expression of adipocyte marker genes (e.g., *Pparg*, *LpL*, *Cd36*) along with the common mesenchymal markers used for lineage tracing such as both *Pdgfra* and *Pdgfrb,* and thus can be considered, “preadipocytes”. These cells have been referred to by many names across both depots (Table 2), including “APCs” (adipocyte precursor cells) [164], “(ASC 1)” (adipocyte stem cell 1) [165], “VmSC4/5” (VAT

mesenchymal stromal cell 4/5) [166], "FAP1/2" (fibro-adipogenic progenitor 1/2)[124], "ES2/3" [167], and "mASPC1/5" (mouse adipose stem and progenitor cell 1/5) [122]. Furthermore, some studies observed a highly committed preadipocyte subpopulation that expresses *Adipoq* and *Lep*, two lineage-defining transcripts associated with terminal differentiation of adipocytes [124,167]. Bioimaging studies indicated that the committed preadipocyte subpopulation appear in proximity to the adipose vasculature, consistent with the long-standing hypothesis that preadipocytes are a subset of adipose perivascular mesenchymal cells [161,163].

The second major cluster of mesenchymal stromal cells lacks *Pparg* expression and instead is enriched in gene signatures associated with extracellular matrix remodeling and inflammation. This cell cluster has also been referred to by many names across both the iWAT and gWAT depots (Table 2), including "fibro-inflammatory precursors" or "FIPs" [164], Group 1/"Interstitial progenitor cells" (IPCs), "adipocyte stem cell 2" (ASC 2)" [165], "VmSC1/2" [166], "FAP3/4" [124], "CD34^{High}" cells [168], "ES1" [167], "collagen-rich (C09) progenitors" [151], and "mASPC2/3" [122]. The proposed functions of these cells in iWAT and/or gWAT include the regulation of inflammation, collagen deposition, and adipogenesis. As such, this distinct subcluster of stromal cells exhibits many properties of interstitial skeletal muscle "fibro-adipogenic progenitors," or "FAPs," and could thus be considered the corresponding "FAPs" of adipose tissue [169]. In iWAT and gWAT, FAPs can be identified based on DPP4 expression (DPP4+). FAPs have been observed in proximity of the adipose vasculature [163]; however, they are readily apparent within the interstitial region on the outer edge of the depots [161]. As such, adipose progenitor cells reside in multiple anatomical progenitor niches. Importantly, human subcutaneous adipose tissue harbors FAPs (DPP4+) and preadipocyes (DPP4-) populations that closely resemble the murine populations, suggesting that distinct progenitor subpopulations also exist in adult humans and can be selected based on DPP4 expression [161,170].

Depot, age, and sex-dependent Properties of Adipocyte Progenitor Cells.

Transcriptomic analyses based on scRNA-seq suggest that subcutaneous iWAT preadipocytes and FAPs are highly similar to their counterparts in intra-abdominal gWAT; however, functional analyses of these distinct subpopulations upon isolation reveal striking depot dependent properties. One notable depot difference lies in the adipogenic potential of isolated FAPs. Both preadipocytes and FAPs isolated from iWAT exhibit a strong capacity to differentiate into adipocytes *in vitro* and upon transplantation into mice. Here, iWAT FAPs give rise to committed preadipocytes in the process of forming adipocytes [161]. This lineage relationship was predicted by cell trajectory analyses of single-cell transcriptomic profiles and supports the existence of a progenitor cell hierarchy [111,171]. Cell trajectory analysis also suggests a hierarchical developmental relationship between gWAT preadipocytes and FAPs [124,167]; however, multiple studies have demonstrated that adult gWAT FAPs lack significant adipogenic potential *in vitro*, even in the presence of strong pro-adipogenic stimuli such as PPARγ agonists. Moreover, gWAT FAPs, unlike iWAT FAPs, do not differentiate into adipocytes upon transplantation into lipodystrophic mice [164,166]. Importantly, these *in vitro* assays and *in vivo* transplantation studies do not preclude the possibility that gWAT FAPs undergo adipocyte differentiation *in vivo* under some conditions. In fact, genetic lineage tracing indicates that FAPs expressing DPP4 in eWAT do undergo *de novo* adipocyte

differentiation at a low frequency in association with HFD feeding [172]. Additional studies will be needed to fully understand the developmental relationship between FAPs and preadipocytes in each depot and the relative contribution of each subpopulation to the maintenance and expansion of the adipocyte pool *in vivo*.

One of the surprising findings from scRNA-seq studies and the functional analyses of isolated subpopulations is the age-dependent changes in function of both preadipocytes and FAPs. In iWAT, a small subpopulation of stromal cells resembling preadipocytes emerges early in the postnatal period. After four weeks of age, this subpopulation, referred to as Aregs, lacks intrinsic adipogenic capacity and can exert an anti-adipogenic effect on adipose stromal cells *in vitro* and upon transplantation *in vivo* [173]. Cells bearing the markers of Aregs can also be found in human adipose tissue; however, the functional properties of these are unknown. Another aging-associated stromal cell subpopulation, termed “aging-dependent regulatory cells (ARCs),” was also described [174]. ARCs arise during aging in mice only in the iWAT depot. ARCs secrete CCL6 and other cytokines to inhibit the differentiation and proliferation of neighboring adipocyte progenitors. The emergence of these cells may contribute to the well-known reduction of subcutaneous adipose tissue mass and plasticity observed in human aging. Interestingly, isolated gWAT FAPs from adult mice have the capacity to inhibit preadipocyte differentiation through the production of secreted factors. This anti-adipogenic property of FAPs is not apparent at the perinatal stage but rather develops with age [175]. Moreover, perinatal gWAT FAPs have a greater potential to undergo adipogenesis when *Pparg* is overexpressed or when exposed to PPARγ agonist compared to their adult counterparts [175].

Sex differences in adipose tissue remodeling and function are widely appreciated; however, less consideration has been given to the intrinsic sex differences in preadipocytes or FAP function. Bulk proteomics analyses of isolated stromal subclusters also highlight considerable sex differences in the expression of genes related to mitochondrial function and lipid metabolism [176]. These findings should serve as a caution to investigators that the functional and molecular properties of adipose progenitors are depot, age, and sex dependent. Age and depot differences in the function of adipose stromal subpopulations may not be readily discerned by single-cell transcriptomics alone [176]. As such, careful consideration should be made to these relevant biological variables in both the design, integration, and interpretation, of studies related to stromal cells.

Beige Adipocyte Progenitors

scRNA-seq has also been utilized to identify putative beige/BRITE adipocyte progenitors in murine iWAT. Fluidigm C1 system-based scRNA-seq identified a subset of PDGFRa+ Sca1+ cells marked by high levels of CD81 gene expression ($CD81^{High}$) [177]. $CD81^{High}$ stromal cells exhibit a vascular smooth muscle cell (VSMC) expression profile (enriched in *Acta2* and *Sm22* transcripts), congruent with prior lineage tracing studies, suggesting a contribution of the smooth muscle cell lineage to beige adipogenesis. In comparison to $CD81^{Low}$ cells, $CD81^{High}$ cells are more adipogenic *in vitro* and differentiate into beige adipocytes upon transplantation and cold stimulation *in vivo*. Genetic lineage tracing (CD81-CreERT2) of CD81+ cells provide clear evidence supporting the proliferation and

differentiation of native CD81 expressing cells into iWAT beige adipocytes upon cold exposure. Notably, CD81 represents a functional marker of beige progenitor cells. CD81 forms a complex with integrins that mediate the integrin-FAK signaling cascade induced by irisin [177]. Importantly, CRISPR-mediated inactivation of *Cd81* attenuates cold-induced beige adipogenesis. Moreover, in line with the rodent data, reduced frequency of CD81+ cells in human subcutaneous WAT is an indicator of metabolic risk, indicating that beige adipocyte biogenesis influences systemic metabolic health. CD81 expression is present within a high proportion of iWAT PDGFRα+ cells, enriched within the aforementioned DPP4-preadipocyte pool. As such, whether CD81+ progenitors also give rise to white adipocytes under permissive conditions remains unclear.

Classical brown adipocyte progenitors

In adult rodents, the adaptation to cold environmental temperatures involves activation of preexisting brown adipocytes within BAT as well as *de novo* differentiation of brown fat cells from resident progenitor cells. Single-cell RNA-seq of BAT stromal vascular cells from mice exposed to cold temperatures (5°C) for either 2 or 7 days followed by cell lineage trajectory analysis pointed to vascular smooth muscle cells (VSMCs) expressing TRPV1 as possible progenitors to cold-induced brown adipocytes [178]. Lineage tracing using the *Trpv1*-Cre line, supported this hypothesis and showed that genetically labeled *Trpv1*-lineage cells proliferate upon cold exposure and differentiate into labeled thermogenic adipocytes. *Trpv1* expression in BAT appears quite specific to VMSCs and these data therefore support the notion of a mural cell origin of adipocytes [179].

Independent single-cell studies of thermogenic adipose depots raise the likelihood that multiple pools of distinct stromal subpopulations give rise to thermogenic adipocytes. scRNA-seq analysis of BAT stromal vascular cells following cold exposure identified multiple fibroblastic *Pdgfra*-expressing subpopulations with distinct tissue localization [180]. One subpopulation, termed “ASC1,” proliferates upon cold exposure and appear poised for differentiation. Genetic pulse-chase lineage tracing confirms that *Pdgfra*-expressing cells undergo brown adipogenesis upon cold exposure. Importantly, *Pdgfra*-expressing fibroblasts are distinct from *Trpv1*+ VSMCs, indicating that multiple progenitor cells populations contribute to cold-induced brown adipogenesis in iBAT.

Aortic perivascular adipose tissue (PVAT) shares several characteristics with iBAT and harbors distinct progenitor cells committed to the brown adipocyte lineage [181]. scRNA-seq analysis of perinatal mouse PVAT supported the notion of brown adipocyte progenitor heterogeneity and indicated that VSMCs as well as multiple *Pdgfra*-expressing fibroblastic subpopulations, including committed preadipocytes (*Pparg*+) and stem cell-like mesenchymal progenitors bearing resemblance to the multipotent cells identified in iWAT (*Pi16*+) [182]. At this stage of development, the preadipocytes and FAPs, but not the VSMCs, are able to differentiate to brown adipocytes *in vitro*. The same progenitor subpopulations are present in adult mouse aortic PVAT; however, here *Trpv1*+ VSMCs can also undergo brown adipocyte differentiation. Lineage tracing using the *Myh11*-Cre confirmed a contribution of VSMCs to the maintenance of brown adipocyte number in adult PVAT [182]. Collectively, these data support a model in which multiple progenitor populations

are utilized within a tissue, with adipogenic PDGFRα+ stromal cells driving tissue genesis, and adipogenic VSMCs serving to help maintain tissue homeostasis in adults. Moreover, these data further highlight how the adipogenic potential of cell populations can change over time.

Non-classical adipose depots: Dermal and skeletal adipose stromal cells

Some of the most plastic WAT depots are relatively understudied and one such depot is the dermal white adipose depot (dWAT). dWAT plays an important role in a wide range of processes including local infection, hair cycling, and wound healing [183–186], and dWAT mass expands and contracts in association with hair cycling and wound healing [187]. scRNA-seq studies have fueled the discovery of an unexpected plasticity of adipocytes within dWAT. Lineage tracing of mature adipocytes and scRNA-seq analysis revealed that mature adipocytes can "de-differentiate" under pathophysiological and physiological conditions into FAP-like cells that can subsequently re-differentiate into mature adipocytes [187,188]. This phenomenon of mature adipocytes reverting to a more primitive lineage state was also unveiled through single cell transcriptomics of mammary gland remodeling during lactation and involution as well as during adipose tissue remodeling in response to a tumor microenvironment [52,189]. As such, scRNA-seq has been instrumental in revealing previously unrecognized lineage relationships.

Bone marrow adipose tissue (BMAT) represents another depot with tremendous plasticity and heterogeneity [190–192]. Here, scRNA-seq has been utilized to explore the heterogeneity of skeletal mesenchymal stromal cells [193]. Cell trajectory analysis identified multiple progenitor subpopulations representing different stages of lineage commitment, and the study revealed the presence of a unique population of committed preadipocytes expressing *Adipoq*, *Pparg*, *Cebpa*, and *Lpl*, but devoid of *Plin1* and lipid droplets. These cells, termed "marrow adipogenic lineage precursors" (MALPs) closely resemble pericytes. MALPs are readily found within bone marrow capillaries where they express *Pdgfrb* and share a basement membrane with endothelial cells. MALPs express notable angiogenic factors, including *Vegfa*, and genetic ablation of these cells resulted in a loss of overall blood vessel density. The identification of MALPs reinforces the notion that pericyte-like cells contribute to the adipogenic lineage and further highlights the multifaceted roles of adipose progenitors in the regulation of tissue homeostasis.

IMMUNE CELL HETEROGENEITY

Obesity-associated adipose tissue macrophages

Adipose tissue macrophages (ATMs) play an important role in adipose tissue homeostasis and adaptive remodeling [76,194]. Obesity is associated with a major increase in the recruitment of bone marrow-derived monocytes into adipose tissues, contributing to a dramatic accumulation of ATMs promoting metabolic inflammation and insulin resistance [195]. ATMs were initially thought to undergo polarization towards proinflammatory M1 macrophages under obese conditions [196,197]; however, in-depth analyses of adipose immune cell populations from mice and humans challenged this view and indicated that obesity rather programs ATMs towards lipid uptake, storage, and catabolism [198–200]. Recently,

single cell technologies have made it possible to resolve the full heterogeneity and plasticity of the adipose immune cell compartment (Figure 4), and these studies all support the notion that ATMs are not strongly polarized towards the classical M1 and M2 macrophage states. Instead, scRNA-seq of human and mouse adipose immune cells or snRNA-seq of whole adipose tissues indicate that macrophage subpopulations constitute a continuum of cell states, where some are more proinflammatory, and others are more metabolically active [124,165,201–204]. Importantly, careful computational analyses using the MacSpectrum tool to classify all macrophages according to their polarization and differentiation indexes support the absence of classical M1 and M2 states [205].

Interestingly, recent results from high-resolution scRNA-seq of Lin^+ cells in murine and human obesogenic conditions [203,206], CITE-seq analyses of mouse eWAT [61], and whole tissue snRNA-seq of mouse eWAT [122,124] and human WAT [122] showed that obesity induces a unique ATM subpopulation marked by *Cd9* and *Trem2*. This subpopulation expresses high levels of lipid handling genes including *Cd36*, *Lpl* and *Lipa*, and is now consensually termed lipid-associated macrophages (LAMs) (Table 3). LAMs are enriched at crown-like structures, where they appear to play an important and Trem2-dependent role in adipose tissue remodeling [203]. Intriguingly, some studies report a smaller subpopulation of LAMs that expresses proliferative genes, hence termed “proliferative-LAMs” (P-LAMs) [124] or “cell-cycling macrophages” (CMs) [61], indicating that the pool of LAMs can expand by local proliferation and not just by increased recruitment and activation of monocytes. In addition to LAMs, “inflammatory macrophage” (IM) subpopulations with high expression of inflammatory gene programs have been shown to be positively associated with obesity [206,207].

Single cell studies have also investigated macrophage subpopulations during obesity regression. Analysis of mouse eWAT by CITE-seq during weight loss demonstrated a decrease in LAMs with weight loss; however, despite the complete normalization of body and tissue weight, the abundance of LAMs in the tissue was higher than that of the lean controls [61]. In addition, the weight regain group in the study showed a rapid increase in LAMs, over and above the obese cohort, suggesting that these cells may encode an obesogenic anticipation or memory.

Despite the clear depot and species differences between obesity-associated ATMs, the metabolic and inflammatory programming is relatively conserved across WAT depots and species (Table 3). A recent direct comparison of mouse and human WAT depots [122] reported differences in the relative abundance of myeloid and lymphoid cell populations across species and depots (Table 3); however, the transcriptomic profiles of the individual macrophage populations were notably similar. Surprisingly, however, this study did not seem to detect the lipid handling gene program in their $Trem2^+$ LAMs.

Vascular associated/perivascular macrophages

Various studies across depots and species have detected subpopulations of macrophages that express genes relevant for vascular development and/or matrix remodeling and maintenance [124,201,208–210]. The consensus across studies suggests that these “vascular-associated macrophages” or “perivascular macrophages” (VAMs or PVMs), marked by *Lyve1,* are

likely the most abundant tissue resident macrophages in lean WAT. The relative abundance of VAMs and PVMs have been shown to decrease with obesity; however it is unclear whether this is due to an increased conversion of PVMs to IMs, as suggested by RNA velocity analyses [206], or primarily due to the large increase in LAM and IM populations. Interestingly, a reduction in the abundance of VAMs in eWAT was observed in response to acute fasting [210]. Notably, the VAMs and PVMs co-express M2-like markers (such as *Mrc1*, *Clec10a,* and *Cd163*) and genes involved in the regulation of complement function and angiogenesis (Table 3). Finally, a minor subpopulation of ATMs with high levels of collagens, especially *Col3a1* and *Col5a2*, have been identified as collagen-expressing macrophages (CEMs) in mouse eWAT[124].

Monocyte heterogeneity and link to ATM subpopulations

Single cell studies have also identified two subpopulations of monocytes in mouse eWAT, termed “Mon 1” and “Mon 2” [203], or “classical” and “nonclassical” [61], whereas three subpopulations have been reported in human sWAT (“Mo-1”, “Mo-2” and “non-canonical”) [206] and mouse iBAT (Ly6c^{low}, Ly6c^{int}, Ly6c^{high}) [211] (Table 4). Interestingly, RNA velocity projections indicate that Mo-1 give rise to PVM and IMs in human sWAT, whereas Mo-2s represent an additional transition state between Mo-1s and IMs in obesity. During obesity, Mo-1 appears to preferentially transition to IMs, whereas PVMs transition to LAMs [206].

ATM subpopulations in BAT

Single cell sorting and transcriptome analyses have also enabled an extensive characterization of the monocyte and macrophage subpopulations of BAT [211], many of which seem to be concordant with those identified in WAT. For example, BAT also contains lipid-associated *Lpl*^{hi} and *Plin2*^{hi} subpopulations, as well as M2-like and matrix macrophages analogous to the LAMs, M2-like, and CMs, of eWAT [211](Table 3). However, further analyses are required to determine the functional similarities between these subpopulations across depots. Trajectory analysis of monocyte subpopulations in BAT indicates that Ly6c^{high} monocytes give rise to LAMs, which further bifurcates into terminally differentiated M2-like or matrix macrophages [211].

Dendritic cells

Adipose tissue dendritic cells (ATDCs) have been previously associated with obesity [212], and single cell/nuclei analyses have now identified immuno-regulatory subpopulations in mouse and human WAT [61,206]. Two major subpopulations of conventional DCs (cDC1 and cDC2) have been found across mouse eWAT and human sWAT. While in humans two further subclusters of cDC2 (cDC2A and cDC2B) were defined based on specific gene expression modules (Table 4), mouse ATDCs were further assigned to subclusters based on their activation state (*Ccr7* expression) and proliferation markers. In addition, monocyte-derived DCs (moDCs) were found to be enriched in lean mouse WAT. Interestingly, activated cDCs were elevated in obesity and failed to regress upon weight loss in mice [61].

Lymphoid cells

The heterogeneity and plasticity of the lymphoid immune compartment of adipose tissue is much less understood. Recent studies have indicated that the adipose tissue lymphoid cells also form subpopulations with some being more responsive to thermogenic cues and others being more responsive to obesogenic cues. Multiple subpopulations of tissue resident T (Naïve, memory and regulatory) and B lymphocytes were reported in mouse iWAT [40], eWAT [61], and human sWAT [206]. In addition, subpopulations of effector memory T cells in the human subcutaneous and omental adipose tissue ($CD8^+$, CCL5 expressing cells), as well as in mouse eWAT ($CD8^+$ TEM) were shown to increase with obesity [61,206,207]. Furthermore, single-cell analyses of human sWAT-resident immune cells demonstrated subpopulations of adipose-resident Natural Killer (NK) cells and innate lymphoid cells (ILCs) [206]. Investigation of the developmental trajectories and inflammatory interactome of the different ILCs identified the ILC3 subpopulation to be positively linked to obesity. Interestingly, iWAT-resident immune cell subpopulations have been shown to undergo cold-induced myeloid-to-lymphoid transition [41].

MESOTHELIAL AND ENDOTHEIAL CELL HETEROGENEITY

Intra-abdominal WAT depots in both humans and rodents contain a defined mesothelium composed of a cobblestone-like monolayer of cells with a mixed mesenchymal/epithelial expression profile. The primary function of this layer is to provide a protective, non-adhesive surface within the abdominal cavity [213]; however, several additional functions of the adipose-associated mesothelium have been hypothesized [214]. Studies of human WAT suggest that these cells adopt a pro-inflammatory phenotype in obesity [215].

Lineage tracing studies in mice have suggested a mesothelial cell origin of intra-abdominal adipocytes [216]; however, recent studies utilizing a more specific Cre-driver targeting epithelial cells (*Krt19*-Cre) did not find evidence of a mesothelial origin of fat cells. Single cell/nuclei transcriptomics have offered an unprecedented view of these cells and have identified distinct mesothelial subpopulations which are largely conserved between mice and humans and appears to be differentially regulated in obesity [122,207].

Endothelial cells represent another important epithelial cell type within adipose tissues. The importance of endothelial cells in the regulation of nutrient transport, hormonal signals, and inflammation is clear; however, the potential heterogeneity of these cells within adipose tissue is still largely unexplored. Single-cell transcriptomic studies have highlighted the presence of multiple endothelial subpopulations in mouse and human WAT [122,207,217], likely representing arteriolar, venular, and lymphatic endothelial cells. One subpopulation expresses high levels of *CD36* and other genes involved in lipid metabolism, possibly representing “lipid handling” endothelial cells [207]. Endothelial cell diversity has also been studied through scRNA analyses of several other tissues [218,219]. Integrating these data with single cell transcriptomics data from adipose tissues may help unveil WAT-specific properties of endothelial cells.

MECHANISTIC INSIGHTS FROM SINGLE CELL OMICS

Intercellular Communication

Intercellular communication between adipocytes and various cellular constituents of the adipose microenvironment is critical for adipose tissue homeostasis and the adaptation to environmental and physiological challenges. The ability to profile adipose tissue transcriptomes at single cell resolution, combined with emerging computational tools, allows for prediction of previously unrecognized intercellular signals. Such predictions can be made based on the expression of known ligand-receptor pairs across different cell types [146,147]. This approach was used to predict potential pro-adipogenic ligands acting on preadipocyte receptors in eWAT [124], and to predict interactions between adipocytes and adipose progenitors and endothelial cells in both murine and human obesity [122]. Similarly, ligand-receptor expression was used to infer cellular crosstalk within adipose tissue as well as potential muscle-adipose crosstalk during exercise training [220]. These computational approaches have huge hypothesis-generating potential; however, experimental studies are required to further investigate and carefully validate predictions.

Recently, several more concrete examples of intercellular communication have emerged involving newly identified stromal cell subpopulations in adipose tissue (Figure 5). For instance, R-spondin2 (RSPO2), an enhancer of WNT signaling, was identified as a secretory protein enriched in the aforementioned Areg subpopulation. RSPO2 targets the Leucine Rich Repeat Containing G Protein-Coupled Receptor 4 (LGR4) in FAPs to block their transition to a more committed preadipocyte state [221]. Likewise, scRNA-seq and follow-up multi-omics analyses of adipose progenitors identified a hypoxia-inducible factor (HIF)1α-dependent inhibitory signaling mechanism underlying sex and depot differences in preadipocyte activity in mouse iWAT and gWAT following the onset of HFD feeding [163]. Activated HIF1α signaling drives the production and secretion of PDGFs, which act in an autocrine/paracrine manner to drive a PDGFR-ERK signaling cascade culminating in inhibitory phosphorylation of PPARγ at serine 112 [163].

Another emerging theme is the importance of intercellular communication between select stromal subpopulations and distinct immune cell types. By applying NicheNet to single-cell transcriptomic data it was proposed that senescent CD9+ macrophages accumulating in obesity block the adipogenic potential of stromal cells via secreted factors (e.g., Osteopontin) [222]. The communication between immune cells and adipose progenitors appears bi-directional. Within the first few days of HFD feeding, eWAT FAPs activate the expression of notable pro-inflammatory cytokines (e.g., *Il6*, *Ccl2*) and extracellular matrix components to a much greater degree than preadipocytes [223]. Genetic mouse models support a critical role for activated FAPs in the development of metabolic adipose tissue inflammation associated with diet-induced obesity [223]. Other scRNA-seq studies of stromal cells from eWAT have revealed the selective expression of IL-33 in FAPs of lean adult mice [166,224]. IL-33 promotes the activity of Tregs, immune cells that are critical to maintain an anti-inflammatory phenotype in adipose tissue. Inactivation of *Il33* in *Pdgfra*-expressing cells (which include FAPs) attenuates Treg accumulation in gonadal adipose tissue [166,224].

These results indicate that, eWAT FAPs play a role in regulating adipose immune cell composition under homeostatic conditions.

Within iWAT, FAPs represent the principal source of IL-33 [225]. Following acute cold exposure, *Il33* is induced in these cells through activation of β-adrenergic receptor/CREB signaling [225]. In the context of thermogenic remodeling, IL-33 drives the recruitment and activation of ILC2s to enhance beige adipocyte accrual [226]. Contemporaneous studies support and extend these observations and show that IL-33 expression is enriched within the *Bst2*^{High} subset of FAPs, which are suggested to reside in proximity to the lymph node within iWAT [167]. This is a region known to be enriched for beige adipocytes following cold exposure. Surgical dissection of the iWAT lymph node attenuated beige adipocyte accumulation, suggesting that the lymph node might provide key signals that modulate thermogenic tissue remodeling [167]. Taken together, scRNA-seq studies have highlighted the important role of crosstalk between adipose progenitors and immune cells in regulating tissue homeostasis and the adaptation to environmental challenges.

Differentiation Trajectories and Transcriptional Drivers

One of the exciting outcomes of snRNA-seq analyses of adipose tissue is the ability to apply pseudotime trajectory analysis tools to map the different stages of *in vivo* adipogenesis. Using this approach, the adipogenic trajectory from early preadipocytes, late preadipocytes and transitioning cells to mature adipocytes could be mapped in lean and obese eWAT [124]. The finding that very few transitioning cells can be detected and that late preadipocytes appear to accumulate at the state just prior to the transition to mature adipocytes suggested the existence of a metastable state highly sensitive to differentiation cues. Furthermore, these findings indicate a switch-like mechanism for induction of differentiation. One of the interesting questions is which are the cues and the drivers that control this switch. Based on more than two decades of analyses of *in vitro* adipogenesis, it seems likely that a main driver of this switch is activation of the master regulator PPARγ above a certain threshold level. Projection of differential gene expression on the pseudo time scale revealed the sequential expression of gene programs during *in vivo* adipogenesis, including the temporal expression of transcriptional regulators that are likely to drive activation of adipocyte genes [124]. This type of analyses combined with single cell epigenomic studies will be instrumental for gaining insight into the transcriptional mechanisms driving cellular transitions, including adipogenesis, *in vivo*.

PERSPECTIVES, CHALLENGES, AND OPPORTUNITIES

The rapid development of single-cell transcriptomics and associated analytical tools has completely reshaped our understanding of the distinct subpopulations of adipocytes, stromal cells, and immune cells, that reside in adipose tissues. The discovery of these new cellular subpopulations has provided new insight into the complex mechanisms by which adipose tissues adapt and remodel under various conditions depending on age, anatomical location, and sex. Moving forward it will be important to determine the role of different cellular subpopulations in defining sex, age, and depot-specific functions of WAT and in regulating short-term and long-term plasticity including metabolic functions of WAT.

The identification of subpopulation selective markers has enabled refinements of the classical strategies to study adipose tissue biology. In particular, the identification of new cell surface markers of stromal cells has fueled the development of new strategies for the isolation and functional analysis of distinct subpopulations by FACS. This now allows investigators to complement classical cell culture studies of primary cells and immortalized preadipocyte cell lines.

Nevertheless, the growing appreciation for intra-and inter-depot heterogeneity now presents numerous challenges to investigators in the field and raises important questions. For instance, do the distinct subpopulations of adipocytes and adipocyte progenitors cells identified within a given depot represent distinct cellular subtypes with distinct cellular origins and stable intrinsic properties (e.g., brown adipocytes vs. white adipocytes), or do these distinct cell subpopulations represent interconvertible “cell states”? Is there even more heterogeneity than current approaches can delineate? The depth of sequencing for individual genes within a single cell can limit the sensitivity of defining subtle differences within cell populations. Furthermore, what is the functional significance of the individual cellular subpopulations? Addressing the functional importance of cell populations and their signaling properties *in vivo* is an enormous challenge given the lack of subpopulation specific strategies for targeting adipocytes, adipose progenitors, or immune cells. The development of intersectional-CRE strategies to target cell subpopulations based on the expression of two defining genes may help gain specificity [227]. Moreover, cell type specific targeting based on cell surface receptors may also constitute a valuable avenue [228].

A major challenge moving forward will be to understand to intercellular crosstalk and the role of this in shaping cellular subpopulations. The emergence of high-resolution spatial omics including transcriptomics approaches constitutes an important next step in understanding cellular subpopulations with their microenvironment. The integration of spatial omics data with single nucleus transcriptomics and epigenomics data constitutes a powerful future approach for obtaining detailed insight into the role of intercellular signaling mechanisms in adipose tissue plasticity.

Finally, as the field moves forward, the ability to integrate datasets gathered from independent studies will rely on unified nomenclature and accurate metadata that enables an accounting for these biological variables. The challenges associated with complexity of adipose tissue can undoubtedly be daunting; however, embracing these challenges may provide opportunities to unravel the natural cellular and molecular mechanisms that allow adipose tissue to maintain its plasticity and function, and identify those mechanisms which are disrupted in obesity. Such efforts may lead to the development of new therapeutic strategies to shape the health and function of adipose tissue as part of an effort to prevent and treat metabolic diseases.

ACKNOWLEDGEMENTS

B.M. and S.M. are supported by the grants from the Danish National Research Foundation (DNRF grant no. 141 to the Center for Functional Genomics and Tissue Plasticity [ATLAS]) and grants from the Novo Nordisk Foundation (NNF18OC0033444 Challenge Grant to the Center for Adipocyte Signaling). Furthermore, B.M. is supported by the EMBO post-doctoral fellowship (ALTF 146-2021). Q.Z. and R.K.G. are supported by National Institutes of Health NIDDK awards R01 DK104789, RC2 DK118620, and R01 DK119163 to R.K.G. B.M and S.M. would like

to acknowledge Elvira Laila van Hauwaert as well as other member of Mandrup Group for the discussions and inputs.

REFERENCES

1. Rosen ED, and Spiegelman BM (2014). What we talk about when we talk about fat. Cell 156, 20–44. 10.1016/j.cell.2013.12.012. [PubMed: 24439368]
2. Tchkonia T, Thomou T, Zhu Y, Karagiannides I, Pothoulakis C, Jensen MD, and Kirkland JL (2013). Mechanisms and metabolic implications of regional differences among fat depots. Cell Metab 17, 644–656. 10.1016/j.cmet.2013.03.008. [PubMed: 23583168]
3. Bagchi DP, Forss I, Mandrup S, and MacDougald OA (2018). SnapShot: Niche Determines Adipocyte Character I. Cell Metab 27, 264–264.e261. 10.1016/j.cmet.2017.11.012. [PubMed: 29320707]
4. Zwick RK, Guerrero-Juarez CF, Horsley V, and Plikus MV (2018). Anatomical, Physiological, and Functional Diversity of Adipose Tissue. Cell Metabolism 27, 68–83. 10.1016/j.cmet.2017.12.002. [PubMed: 29320711]
5. Zhang F, Hao G, Shao M, Nham K, An Y, Wang Q, Zhu Y, Kusminski CM, Hassan G, Gupta RK, et al. (2018). An Adipose Tissue Atlas: An Image-Guided Identification of Human-like BAT and Beige Depots in Rodents. Cell Metab 27, 252–262.e253. 10.1016/j.cmet.2017.12.004. [PubMed: 29320705]
6. Cohen P, and Kajimura S (2021). The cellular and functional complexity of thermogenic fat. Nat Rev Mol Cell Biol 10.1038/s41580-021-00350-0.
7. Cinti S (2005). The adipose organ. Prostaglandins Leukot Essent Fatty Acids 73, 9–15. 10.1016/j.plefa.2005.04.010. [PubMed: 15936182]
8. Nedergaard J, and Cannon B (2018). Brown adipose tissue as a heat-producing thermoeffector. Handb Clin Neurol 156, 137–152. 10.1016/B978-0-444-63912-7.00009-6. [PubMed: 30454587]
9. Chouchani ET, Kazak L, and Spiegelman BM (2019). New Advances in Adaptive Thermogenesis: UCP1 and Beyond. Cell Metab 29, 27–37. 10.1016/j.cmet.2018.11.002. [PubMed: 30503034]
10. Chait A, and den Hartigh LJ (2020). Adipose Tissue Distribution, Inflammation and Its Metabolic Consequences, Including Diabetes and Cardiovascular Disease. Front Cardiovasc Med 7, 22. 10.3389/fcvm.2020.00022. [PubMed: 32158768]
11. Ballinger MA, and Andrews MT (2018). Nature's fat-burning machine: brown adipose tissue in a hibernating mammal. J Exp Biol 221. 10.1242/jeb.162586.
12. Sakers A, De Siqueira MK, Seale P, and Villanueva CJ (2022). Adipose-tissue plasticity in health and disease. Cell 185, 419–446. 10.1016/j.cell.2021.12.016. [PubMed: 35120662]
13. Guilherme A, Pedersen DJ, Henchey E, Henriques FS, Danai LV, Shen Y, Yenilmez B, Jung D, Kim JK, Lodhi IJ, et al. (2017). Adipocyte lipid synthesis coupled to neuronal control of thermogenic programming. Molecular Metabolism 6, 781–796. 10.1016/j.molmet.2017.05.012. [PubMed: 28752043]
14. Petersen MC, and Shulman GI (2018). Mechanisms of Insulin Action and Insulin Resistance. Physiol Rev 98, 2133–2223. 10.1152/physrev.00063.2017. [PubMed: 30067154]
15. Sakaguchi M, Fujisaka S, Cai W, Winnay JN, Konishi M, O'Neill BT, Li M, García-Martín R, Takahashi H, Hu J, et al. (2017). Adipocyte Dynamics and Reversible Metabolic Syndrome in Mice with an Inducible Adipocyte-Specific Deletion of the Insulin Receptor. Cell Metab 25, 448–462. 10.1016/j.cmet.2016.12.008. [PubMed: 28065828]
16. Duncan RE, Ahmadian M, Jaworski K, Sarkadi-Nagy E, and Sul HS (2007). Regulation of lipolysis in adipocytes. Annu Rev Nutr 27, 79–101. 10.1146/annurev.nutr.27.061406.093734. [PubMed: 17313320]
17. Frayn KN (2002). Adipose tissue as a buffer for daily lipid flux. Diabetologia 45, 1201–1210. 10.1007/s00125-002-0873-y. [PubMed: 12242452]
18. Cannon B, and Nedergaard J (2004). Brown adipose tissue: function and physiological significance. Physiol Rev 84, 277–359. 10.1152/physrev.00015.2003. [PubMed: 14715917]

19. Markussen LK, Rondini EA, Johansen OS, Madsen JGS, Sustarsic EG, Marcher A-B, Hansen JB, Gerhart-Hines Z, Granneman JG, and Mandrup S (2022). Lipolysis regulates major transcriptional programs in brown adipocytes. Nature Communications 13, 3956. 10.1038/s41467-022-31525-8.
20. Wu J, Bostrom P, Sparks LM, Ye L, Choi JH, Giang AH, Khandekar M, Virtanen KA, Nuutila P, Schaart G, et al. (2012). Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. Cell 150, 366–376. 10.1016/j.cell.2012.05.016. [PubMed: 22796012]
21. Bonet ML, Oliver P, and Palou A (2013). Pharmacological and nutritional agents promoting browning of white adipose tissue. Biochim Biophys Acta 1831, 969–985. 10.1016/j.bbalip.2012.12.002. [PubMed: 23246573]
22. Loncar D (1991). Convertible adipose tissue in mice. Cell and tissue research 266, 149–161. [PubMed: 1747909]
23. Shao M, Wang QA, Song A, Vishvanath L, Busbuso NC, Scherer PE, and Gupta RK (2019). Cellular Origins of Beige Fat Cells Revisited. Diabetes 68, 1874–1885. 10.2337/db19-0308. [PubMed: 31540940]
24. Lee YH, Petkova AP, Konkar AA, and Granneman JG (2015). Cellular origins of cold-induced brown adipocytes in adult mice. FASEB J 29, 286–299. 10.1096/fj.14-263038. [PubMed: 25392270]
25. Wang QA, Tao C, Gupta RK, and Scherer PE (2013). Tracking adipogenesis during white adipose tissue development, expansion and regeneration. Nat Med 19, 1338–1344. 10.1038/nm.3324. [PubMed: 23995282]
26. Rosenwald M, Perdikari A, Rülicke T, and Wolfrum C (2013). Bi-directional interconversion of brite and white adipocytes. Nat Cell Biol 15, 659–667. 10.1038/ncb2740. [PubMed: 23624403]
27. Barbatelli G, Murano I, Madsen L, Hao Q, Jimenez M, Kristiansen K, Giacobino JP, De Matteis R, and Cinti S (2010). The emergence of cold-induced brown adipocytes in mouse white fat depots is determined predominantly by white to brown adipocyte transdifferentiation. Am J Physiol Endocrinol Metab 298, E1244–1253. 10.1152/ajpendo.00600.2009. [PubMed: 20354155]
28. Fabbiano S, Suárez-Zamorano N, Rigo D, Veyrat-Durebex C, Stevanovic Dokic A, Colin DJ, and Trajkovski M (2016). Caloric Restriction Leads to Browning of White Adipose Tissue through Type 2 Immune Signaling. Cell Metab 24, 434–446. 10.1016/j.cmet.2016.07.023. [PubMed: 27568549]
29. Neinast MD, Frank AP, Zechner JF, Li Q, Vishvanath L, Palmer BF, Aguirre V, Gupta RK, and Clegg DJ (2015). Activation of natriuretic peptides and the sympathetic nervous system following Roux-en-Y gastric bypass is associated with gonadal adipose tissues browning. Mol Metab 4, 427–436. 10.1016/j.molmet.2015.02.006. [PubMed: 25973390]
30. Petruzzelli M, Schweiger M, Schreiber R, Campos-Olivas R, Tsoli M, Allen J, Swarbrick M, Rose-John S, Rincon M, Robertson G, et al. (2014). A switch from white to brown fat increases energy expenditure in cancer-associated cachexia. Cell Metab 20, 433–447. 10.1016/j.cmet.2014.06.011. [PubMed: 25043816]
31. Li G, Xie C, Lu S, Nichols RG, Tian Y, Li L, Patel D, Ma Y, Brocker CN, Yan T, et al. (2017). Intermittent Fasting Promotes White Adipose Browning and Decreases Obesity by Shaping the Gut Microbiota. Cell Metab 26, 801. 10.1016/j.cmet.2017.10.007. [PubMed: 29117546]
32. Kim KH, Kim YH, Son JE, Lee JH, Kim S, Choe MS, Moon JH, Zhong J, Fu K, Lenglin F, et al. (2017). Intermittent fasting promotes adipose thermogenesis and metabolic homeostasis via VEGF-mediated alternative activation of macrophage. Cell Res 27, 1309–1326. 10.1038/cr.2017.126. [PubMed: 29039412]
33. Betz MJ, Slawik M, Lidell ME, Osswald A, Heglind M, Nilsson D, Lichtenauer UD, Mauracher B, Mussack T, Beuschlein F, and Enerback S (2013). Presence of brown adipocytes in retroperitoneal fat from patients with benign adrenal tumors: relationship with outdoor temperature. J Clin Endocrinol Metab 98, 4097–4104. 10.1210/jc.2012-3535. [PubMed: 23744406]
34. Frontini A, Vitali A, Perugini J, Murano I, Romiti C, Ricquier D, Guerrieri M, and Cinti S (2013). White-to-brown transdifferentiation of omental adipocytes in patients affected by pheochromocytoma. Biochim Biophys Acta 1831, 950–959. 10.1016/j.bbalip.2013.02.005. [PubMed: 23454374]

35. Patsouris D, Qi P, Abdullahi A, Stanojcic M, Chen P, Parousis A, Amini-Nik S, and Jeschke Marc G. (2015). Burn Induces Browning of the Subcutaneous White Adipose Tissue in Mice and Humans. Cell Reports 13, 1538–1544. 10.1016/j.celrep.2015.10.028. [PubMed: 26586436]
36. Bartelt A, and Heeren J (2014). Adipose tissue browning and metabolic health. Nature reviews. Endocrinology 10, 24–36. 10.1038/nrendo.2013.204.
37. Wang W, and Seale P (2016). Control of brown and beige fat development. Nat Rev Mol Cell Biol 17, 691–702. 10.1038/nrm.2016.96. [PubMed: 27552974]
38. Jun H, Yu H, Gong J, Jiang J, Qiao X, Perkey E, Kim DI, Emont MP, Zestos AG, Cho JS, et al. (2018). An immune-beige adipocyte communication via nicotinic acetylcholine receptor signaling. Nat Med 24, 814–822. 10.1038/s41591-018-0032-8. [PubMed: 29785025]
39. Rajbhandari P, Thomas BJ, Feng AC, Hong C, Wang J, Vergnes L, Sallam T, Wang B, Sandhu J, Seldin MM, et al. (2018). IL-10 Signaling Remodels Adipose Chromatin Architecture to Limit Thermogenesis and Energy Expenditure. Cell 172, 218–233 e217. 10.1016/j.cell.2017.11.019. [PubMed: 29249357]
40. Rajbhandari P, Arneson D, Hart SK, Ahn IS, Diamante G, Santos LC, Zaghari N, Feng AC, Thomas BJ, Vergnes L, et al. (2019). Single cell analysis reveals immune cell-adipocyte crosstalk regulating the transcription of thermogenic adipocytes. Elife 8. 10.7554/eLife.49501.
41. Rabhi N, Belkina AC, Desevin K, Cortez BN, and Farmer SR (2020). Shifts of Immune Cell Populations Differ in Response to Different Effectors of Beige Remodeling of Adipose Tissue. iScience 23, 101765. 10.1016/j.isci.2020.101765. [PubMed: 33294778]
42. Shamsi F, Tseng YH, and Kahn CR (2021). Adipocyte Microenvironment: Everybody in the Neighborhood Talks about the Temperature. Cell Metab 33, 4–6. 10.1016/j.cmet.2020.12.012. [PubMed: 33406404]
43. Hu B, Jin C, Zeng X, Resch JM, Jedrychowski MP, Yang Z, Desai BN, Banks AS, Lowell BB, Mathis D, and Spiegelman BM (2020). gammadelta T cells and adipocyte IL-17RC control fat innervation and thermogenesis. Nature 578, 610–614. 10.1038/s41586-020-2028-z. [PubMed: 32076265]
44. Henriques F, Bedard AH, Guilherme A, Kelly M, Chi J, Zhang P, Lifshitz LM, Bellve K, Rowland LA, Yenilmez B, et al. (2020). Single-Cell RNA Profiling Reveals Adipocyte to Macrophage Signaling Sufficient to Enhance Thermogenesis. Cell Rep 32, 107998. 10.1016/j.celrep.2020.107998. [PubMed: 32755590]
45. Bartness TJ, Vaughan CH, and Song CK (2010). Sympathetic and sensory innervation of brown adipose tissue. Int J Obes (Lond) 34 Suppl 1, S36–42. 10.1038/ijo.2010.182. [PubMed: 20935665]
46. Kotzbeck P, Giordano A, Mondini E, Murano I, Severi I, Venema W, Cecchini MP, Kershaw EE, Barbatelli G, Haemmerle G, et al. (2018). Brown adipose tissue whitening leads to brown adipocyte death and adipose tissue inflammation. J Lipid Res 59, 784–794. 10.1194/jlr.M079665. [PubMed: 29599420]
47. Shimizu I, and Walsh K (2015). The Whitening of Brown Fat and Its Implications for Weight Management in Obesity. Curr Obes Rep 4, 224–229. 10.1007/s13679-015-0157-8. [PubMed: 26627217]
48. Shimizu I, Aprahamian T, Kikuchi R, Shimizu A, Papanicolaou KN, MacLauchlan S, Maruyama S, and Walsh K (2014). Vascular rarefaction mediates whitening of brown fat in obesity. The Journal of Clinical Investigation 124, 2099–2112. 10.1172/JCI71643. [PubMed: 24713652]
49. Roh HC, Tsai LTY, Shao M, Tenen D, Shen Y, Kumari M, Lyubetskaya A, Jacobs C, Dawes B, Gupta RK, and Rosen ED (2018). Warming Induces Significant Reprogramming of Beige, but Not Brown, Adipocyte Cellular Identity. Cell Metab 27, 1121–1137.e1125. 10.1016/j.cmet.2018.03.005. [PubMed: 29657031]
50. Cinti S (2018). Pink Adipocytes. Trends Endocrinol Metab 29, 651–666. 10.1016/j.tem.2018.05.007. [PubMed: 30017740]
51. Wang QA, and Scherer PE (2019). Remodeling of Murine Mammary Adipose Tissue during Pregnancy, Lactation, and Involution. J Mammary Gland Biol Neoplasia 24, 207–212. 10.1007/s10911-019-09434-2. [PubMed: 31512027]
52. Wang QA, Song A, Chen W, Schwalie PC, Zhang F, Vishvanath L, Jiang L, Ye R, Shao M, Tao C, et al. (2018). Reversible De-differentiation of Mature White Adipocytes into Preadipocyte-

like Precursors during Lactation. Cell Metab 28, 282–288.e283. 10.1016/j.cmet.2018.05.022. [PubMed: 29909970]

53. Zwick RK, Rudolph MC, Shook BA, Holtrup B, Roth E, Lei V, Van Keymeulen A, Seewaldt V, Kwei S, Wysolmerski J, et al. (2018). Adipocyte hypertrophy and lipid dynamics underlie mammary gland remodeling after lactation. Nat Commun 9, 3592. 10.1038/s41467-018-05911-0. [PubMed: 30181538]
54. Karastergiou K, Smith SR, Greenberg AS, and Fried SK (2012). Sex differences in human adipose tissues - the biology of pear shape. Biol Sex Differ 3, 13. 10.1186/2042-6410-3-13. [PubMed: 22651247]
55. Huffman DM, and Barzilai N (2010). Contribution of adipose tissue to health span and longevity. Interdiscip Top Gerontol 37, 1–19. 10.1159/000319991. [PubMed: 20703052]
56. Thomas EL, Saeed N, Hajnal JV, Brynes A, Goldstone AP, Frost G, and Bell JD (1998). Magnetic resonance imaging of total body fat. J Appl Physiol (1985) 85, 1778–1785. 10.1152/jappl.1998.85.5.1778. [PubMed: 9804581]
57. Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, Blomqvist L, Hoffstedt J, Naslund E, Britton T, et al. (2008). Dynamics of fat cell turnover in humans. Nature 453, 783–787. 10.1038/nature06902. [PubMed: 18454136]
58. Sun K, Kusminski CM, and Scherer PE (2011). Adipose tissue remodeling and obesity. J Clin Invest 121, 2094–2101. 10.1172/JCI45887. [PubMed: 21633177]
59. Kosteli A, Sugaru E, Haemmerle G, Martin JF, Lei J, Zechner R, and Ferrante AW Jr. (2010). Weight loss and lipolysis promote a dynamic immune response in murine adipose tissue. J Clin Invest 120, 3466–3479. 10.1172/jci42845. [PubMed: 20877011]
60. Alemán JO, Iyengar NM, Walker JM, Milne GL, Da Rosa JC, Liang Y, Giri DD, Zhou XK, Pollak MN, Hudis CA, et al. (2017). Effects of Rapid Weight Loss on Systemic and Adipose Tissue Inflammation and Metabolism in Obese Postmenopausal Women. J Endocr Soc 1, 625–637. 10.1210/js.2017-00020. [PubMed: 29264516]
61. Cottam MA, Caslin HL, Winn NC, and Hasty AH (2022). Multiomics reveals persistence of obesity-associated immune cell phenotypes in adipose tissue during weight loss and weight regain in mice. Nat Commun 13, 2950. 10.1038/s41467-022-30646-4. [PubMed: 35618862]
62. Cancello R, Henegar C, Viguerie N, Taleb S, Poitou C, Rouault C, Coupaye M, Pelloux V, Hugol D, Bouillot JL, et al. (2005). Reduction of macrophage infiltration and chemoattractant gene expression changes in white adipose tissue of morbidly obese subjects after surgery-induced weight loss. Diabetes 54, 2277–2286. 10.2337/diabetes.54.8.2277. [PubMed: 16046292]
63. Clément K, Viguerie N, Poitou C, Carette C, Pelloux V, Curat CA, Sicard A, Rome S, Benis A, Zucker JD, et al. (2004). Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects. Faseb j 18, 1657–1669. 10.1096/fj.04-2204com. [PubMed: 15522911]
64. Schmitz J, Evers N, Awazawa M, Nicholls HT, Brönneke HS, Dietrich A, Mauer J, Blüher M, and Brüning JC (2016). Obesogenic memory can confer long-term increases in adipose tissue but not liver inflammation and insulin resistance after weight loss. Mol Metab 5, 328–339. 10.1016/j.molmet.2015.12.001. [PubMed: 27110485]
65. Broekema MF, Savage DB, Monajemi H, and Kalkhoven E (2019). Gene-gene and gene-environment interactions in lipodystrophy: Lessons learned from natural PPARγ mutants. Biochim Biophys Acta Mol Cell Biol Lipids 1864, 715–732. 10.1016/j.bbalip.2019.02.002. [PubMed: 30742913]
66. Fiorenza CG, Chou SH, and Mantzoros CS (2011). Lipodystrophy: pathophysiology and advances in treatment. Nature Reviews Endocrinology 7, 137–150. 10.1038/nrendo.2010.199.
67. Despres JP (2006). Is visceral obesity the cause of the metabolic syndrome? Ann Med 38, 52–63. 10.1080/07853890500383895. [PubMed: 16448989]
68. Khaodhiar L, McCowen KC, and Blackburn GL (1999). Obesity and its comorbid conditions. Clin Cornerstone 2, 17–31. 10.1016/s1098-3597(99)90002-9. [PubMed: 10696282]
69. Quail DF, and Dannenberg AJ (2019). The obese adipose tissue microenvironment in cancer development and progression. Nat Rev Endocrinol 15, 139–154. 10.1038/s41574-018-0126-x. [PubMed: 30459447]

70. González-Muniesa P, Mártinez-González MA, Hu FB, Després JP, Matsuzawa Y, Loos RJF, Moreno LA, Bray GA, and Martinez JA (2017). Obesity. Nat Rev Dis Primers 3, 17034. 10.1038/nrdp.2017.34. [PubMed: 28617414]
71. Smith GI, Mittendorfer B, and Klein S (2019). Metabolically healthy obesity: facts and fantasies. J Clin Invest 129, 3978–3989. 10.1172/jci129186. [PubMed: 31524630]
72. Karpe F, and Pinnick KE (2015). Biology of upper-body and lower-body adipose tissue—link to whole-body phenotypes. Nature Reviews Endocrinology 11, 90–100. 10.1038/nrendo.2014.185.
73. Huang LO, Rauch A, Mazzaferro E, Preuss M, Carobbio S, Bayrak CS, Chami N, Wang Z, Schick UM, Yang N, et al. (2021). Genome-wide discovery of genetic loci that uncouple excess adiposity from its comorbidities. Nat Metab 3, 228–243. 10.1038/s42255-021-00346-2. [PubMed: 33619380]
74. Hepler C, and Gupta RK (2017). The expanding problem of adipose depot remodeling and postnatal adipocyte progenitor recruitment. Mol Cell Endocrinol 445, 95–108. 10.1016/j.mce.2016.10.011. [PubMed: 27743993]
75. Reilly SM, and Saltiel AR (2017). Adapting to obesity with adipose tissue inflammation. Nat Rev Endocrinol 13, 633–643. 10.1038/nrendo.2017.90. [PubMed: 28799554]
76. Chakarov S, Blériot C, and Ginhoux F (2022). Role of adipose tissue macrophages in obesity-related disorders. J Exp Med 219. 10.1084/jem.20211948.
77. van Baak MA, and Mariman ECM (2019). Mechanisms of weight regain after weight loss - the role of adipose tissue. Nat Rev Endocrinol 15, 274–287. 10.1038/s41574-018-0148-4. [PubMed: 30655624]
78. Cifarelli V, Beeman SC, Smith GI, Yoshino J, Morozov D, Beals JW, Kayser BD, Watrous JD, Jain M, Patterson BW, and Klein S (2020). Decreased adipose tissue oxygenation associates with insulin resistance in individuals with obesity. J Clin Invest 130, 6688–6699. 10.1172/jci141828. [PubMed: 33164985]
79. Lim K, Haider A, Adams C, Sleigh A, and Savage DB (2021). Lipodistrophy: a paradigm for understanding the consequences of “overloading” adipose tissue. Physiological Reviews 101, 907–993. 10.1152/physrev.00032.2020. [PubMed: 33356916]
80. Kane H, and Lynch L (2019). Innate Immune Control of Adipose Tissue Homeostasis. Trends in Immunology 40, 857–872. 10.1016/j.it.2019.07.006. [PubMed: 31399336]
81. Graupera M, and Claret M (2018). Endothelial Cells: New Players in Obesity and Related Metabolic Disorders. Trends Endocrinol Metab 29, 781–794. 10.1016/j.tem.2018.09.003. [PubMed: 30266200]
82. Larabee CM, Neely OC, and Domingos AI (2020). Obesity: a neuroimmunometabolic perspective. Nature Reviews Endocrinology 16, 30–43. 10.1038/s41574-019-0283-6.
83. Guilherme A, Pedersen DJ, Henchey E, Henriques FS, Danai LV, Shen Y, Yenilmez B, Jung D, Kim JK, Lodhi IJ, et al. (2017). Adipocyte lipid synthesis coupled to neuronal control of thermogenic programming. Mol Metab 6, 781–796. 10.1016/j.molmet.2017.05.012. [PubMed: 28752043]
84. Wasserman F (1926). The fat organs of man: development, structure and systematic place of the so-called adipose tissue. A. Zellforsch. Microskop. Anat. Abt. Histochem 3, 325–329.
85. Wasserman F (1960). Electron microscopic investigation of the surface structures of the fat cell and of their changes during depletion of cell. Z. Zellforsch. Microskop. Anat. Abt. Histochem 52, 778–787.
86. Hausman GJ, and Richardson RL (1983). Cellular and vascular development in immature rat adipose tissue. J Lipid Res 24, 522–532. [PubMed: 6875377]
87. Suter ER (1969). The fine structure of brown adipose tissue. I. Cold-induced changes in the rat. J Ultrastruct Res 26, 216–241. 10.1016/s0022-5320(69)80003-1. [PubMed: 5776618]
88. Napolitano L (1963). THE DIFFERENTIATION OF WHITE ADIPOSE CELLS. AN ELECTRON MICROSCOPE STUDY. J Cell Biol 18, 663–679. 10.1083/jcb.18.3.663. [PubMed: 14064115]
89. Napolitano LM (1965). Observations on the fine structure of adipose cells. Ann N Y Acad Sci 131, 34–42. 10.1111/j.1749-6632.1965.tb34777.x. [PubMed: 5216974]
90. Slavin BG, and Ballard KW (1978). Morphological studies on the adrenergic innervation of white adipose tissue. Anat Rec 191, 377–389. 10.1002/ar.1091910310. [PubMed: 677491]

91. Cinti S (1999). Adipose tissues and obesity. Ital J Anat Embryol 104, 37–51. [PubMed: 10450668]
92. Rodbell M (1964). Metabolism of Isolated Fat Cells. I. Effects of Hormones on Glucose Metabolism and Lipolysis. J Biol Chem 239, 375–380. [PubMed: 14169133]
93. Green H, and Meuth M (1974). An established pre-adipose cell line and its differentiation in culture. Cell 3, 127–133. [PubMed: 4426090]
94. Green Ha.K., O (1974). Sublines of mouse 3T3 cells that accumulate lipid. Cell 1, 113–116.
95. Egan JJ, Greenberg AS, Chang MK, Wek SA, Moos-MC J, and Londos C (1992). Mechanism of hormone-stimulated lipolysis in adipocytes: translocation of hormone-sensitive lipase to the lipid storage droplet. Proc.Natl.Acad.Sci.U.S.A. 89, 8537–8541. [PubMed: 1528859]
96. Student AK, Hsu RY, and Lane MD (1980). Induction of fatty acid synthetase synthesis in differentiating 3T3-L1 preadipocytes. Journal of Biological Chemistry 255, 4745–4750. [PubMed: 7372608]
97. Hu E, Liang P, and Spiegelman BM (1996). AdipoQ is a novel adipose-specific gene dysregulated in obesity. J.Biol.Chem 271, 10697–10703. [PubMed: 8631877]
98. Bernlohr DA, Doering TL, Kelly TJ Jr, and Lane MD (1985). Tissue specific expression of p422 protein, a putative lipid carrier, in mouse adipocytes. Biochem Biophys Res Commun 132, 850–855. [PubMed: 2415129]
99. Cook KS, Groves DL, Min HY, and Spiegelman BM (1985). A developmentally regulated mRNA from 3T3 adipocytes encodes a novel serine protease homologue. Proc.Natl.Acad.Sci.U.S.A. 82, 6480–6484. [PubMed: 3901003]
100. Tontonoz P, Hu E, Graves RA, Budavari AI, and Spiegelman BM (1994). mPPAR gamma 2: tissue-specific regulator of an adipocyte enhancer. Genes Dev 8, 1224–1234. [PubMed: 7926726]
101. Lin FT, and Lane MD (1992). Antisense CCAAT/enhancer-binding protein RNA suppresses coordinate gene expression and triglyceride accumulation during differentiation of 3T3-L1 preadipocytes. Genes Dev. 6, 533–544. [PubMed: 1373117]
102. Lefterova MI, Haakonsson AK, Lazar MA, and Mandrup S (2014). PPARγ and the global map of adipogenesis and beyond. Trends in endocrinology and metabolism: TEM 25, 293–302. 10.1016/j.tem.2014.04.001. [PubMed: 24793638]
103. Siersbaek R, and Mandrup S (2011). Transcriptional Networks Controlling Adipocyte Differentiation. Cold Spring Harbor Symposia on Quantitative Biology, sqb
104. Rauch A, and Mandrup S (2021). Transcriptional networks controlling stromal cell differentiation. Nature Reviews Molecular Cell Biology 22, 465–482. 10.1038/s41580-021-00357-7. [PubMed: 33837369]
105. Loft A, Forss I, and Mandrup S (2017). Genome-Wide Insights into the Development and Function of Thermogenic Adipocytes. Trends Endocrinol Metab 28, 104–120. 10.1016/j.tem.2016.11.005. [PubMed: 27979331]
106. Marcher A-B, Loft A, Nielsen R, Vihervaara T, Madsen Jesper Grud S., Sysi-Aho M, Ekroos K, and Mandrup S (2015). RNA-Seq and Mass-Spectrometry-Based Lipidomics Reveal Extensive Changes of Glycerolipid Pathways in Brown Adipose Tissue in Response to Cold. Cell Reports 13, 2000–2013. 10.1016/j.celrep.2015.10.069. [PubMed: 26628366]
107. Mardinoglu A, Kampf C, Asplund A, Fagerberg L, Hallström BM, Edlund K, Blüher M, Pontén F, Uhlen M, and Nielsen J (2014). Defining the human adipose tissue proteome to reveal metabolic alterations in obesity. J Proteome Res 13, 5106–5119. 10.1021/pr500586e. [PubMed: 25219818]
108. Honecker J, Ruschke S, Seeliger C, Laber S, Strobel S, Pröll P, Nellaker C, Lindgren CM, Kulozik U, Ecker J, et al. (2022). Transcriptome and fatty-acid signatures of adipocyte hypertrophy and its non-invasive MR-based characterization in human adipose tissue. EBioMedicine 79, 104020. 10.1016/j.ebiom.2022.104020. [PubMed: 35490555]
109. Forner F, Kumar C, Luber CA, Fromme T, Klingenspor M, and Mann M (2009). Proteome differences between brown and white fat mitochondria reveal specialized metabolic functions. Cell Metab 10, 324–335. 10.1016/j.cmet.2009.08.014. [PubMed: 19808025]
110. Chi J, Wu Z, Choi CHJ, Nguyen L, Tegegne S, Ackerman SE, Crane A, Marchildon F, Tessier-Lavigne M, and Cohen P (2018). Three-Dimensional Adipose Tissue Imaging Reveals Regional

Variation in Beige Fat Biogenesis and PRDM16-Dependent Sympathetic Neurite Density. Cell Metab 27, 226–236.e223. 10.1016/j.cmet.2017.12.011. [PubMed: 29320703]

111. Rodeheffer MS, Birsoy K, and Friedman JM (2008). Identification of white adipocyte progenitor cells in vivo. Cell 135, 240–249. 10.1016/j.cell.2008.09.036. [PubMed: 18835024]
112. Wang F, Mullican SE, DiSpirito JR, Peed LC, and Lazar MA (2013). Lipoatrophy and severe metabolic disturbance in mice with fat-specific deletion of PPARγ. Proceedings of the National Academy of Sciences 110, 18656–18661. doi:10.1073/pnas.1314863110.
113. Vishvanath L, MacPherson KA, Hepler C, Wang QA, Shao M, Spurgin SB, Wang MY, Kusminski CM, Morley TS, and Gupta RK (2016). Pdgfrbeta+ Mural Preadipocytes Contribute to Adipocyte Hyperplasia Induced by High-Fat-Diet Feeding and Prolonged Cold Exposure in Adult Mice. Cell Metab 23, 350–359. 10.1016/j.cmet.2015.10.018. [PubMed: 26626462]
114. Eguchi J, Wang X, Yu S, Kershaw EE, Chiu PC, Dushay J, Estall JL, Klein U, Maratos-Flier E, and Rosen ED (2011). Transcriptional control of adipose lipid handling by IRF4. Cell Metab 13, 249–259. 10.1016/j.cmet.2011.02.005. [PubMed: 21356515]
115. Kong X, Banks A, Liu T, Kazak L, Rao RR, Cohen P, Wang X, Yu S, Lo JC, Tseng YH, et al. (2014). IRF4 is a key thermogenic transcriptional partner of PGC-1alpha. Cell 158, 69–83. 10.1016/j.cell.2014.04.049. [PubMed: 24995979]
116. Li R, Bernau K, Sandbo N, Gu J, Preissl S, and Sun X (2018). Pdgfra marks a cellular lineage with distinct contributions to myofibroblasts in lung maturation and injury response. Elife 7. 10.7554/eLife.36865.
117. Hwang B, Lee JH, and Bang D (2018). Single-cell RNA sequencing technologies and bioinformatics pipelines. Exp Mol Med 50, 1–14. 10.1038/s12276-018-0071-8.
118. Treutlein B, Brownfield DG, Wu AR, Neff NF, Mantalas GL, Espinoza FH, Desai TJ, Krasnow MA, and Quake SR (2014). Reconstructing lineage hierarchies of the distal lung epithelium using single-cell RNA-seq. Nature 509, 371–375. 10.1038/nature13173. [PubMed: 24739965]
119. Hu WY, Hu DP, Xie L, Nonn L, Lu R, Abern M, Shioda T, and Prins GS (2021). Keratin Profiling by Single-Cell RNA-Sequencing Identifies Human Prostate Stem Cell Lineage Hierarchy and Cancer Stem-Like Cells. Int J Mol Sci 22. 10.3390/ijms22158109.
120. Stenkula KG, and Erlanson-Albertsson C (2018). Adipose cell size: importance in health and disease. Am J Physiol Regul Integr Comp Physiol 315, R284–R295. 10.1152/ajpregu.00257.2017. [PubMed: 29641234]
121. University of Utah Health. Single Cell Sequencing - 10X Genomics 3' Gene Expression https://uofuhealth.utah.edu/huntsman/shared-resources/gba/htg/single-cell/genomics-10x.php.
122. Emont MP, Jacobs C, Essene AL, Pant D, Tenen D, Colleluori G, Di Vincenzo A, Jørgensen AM, Dashti H, Stefek A, et al. (2022). A single-cell atlas of human and mouse white adipose tissue. Nature 603, 926–933. 10.1038/s41586-022-04518-2. [PubMed: 35296864]
123. Sun W, Dong H, Balaz M, Slyper M, Drokhlyansky E, Colleluori G, Giordano A, Kovanicova Z, Stefanicka P, Balazova L, et al. (2020). snRNA-seq reveals a subpopulation of adipocytes that regulates thermogenesis. Nature 587, 98–102. 10.1038/s41586-020-2856-x. [PubMed: 33116305]
124. Sárvári AK, Van Hauwaert EL, Markussen LK, Gammelmark E, Marcher AB, Ebbesen MF, Nielsen R, Brewer JR, Madsen JGS, and Mandrup S (2021). Plasticity of Epididymal Adipose Tissue in Response to Diet-Induced Obesity at Single-Nucleus Resolution. Cell Metab 33, 437–453.e435. 10.1016/j.cmet.2020.12.004. [PubMed: 33378646]
125. Ding J, Adiconis X, Simmons SK, Kowalczyk MS, Hession CC, Marjanovic ND, Hughes TK, Wadsworth MH, Burks T, Nguyen LT, et al. (2020). Systematic comparison of single-cell and single-nucleus RNA-sequencing methods. Nat Biotechnol 38, 737–746. 10.1038/s41587-020-0465-8. [PubMed: 32341560]
126. Bakken TE, Hodge RD, Miller JA, Yao Z, Nguyen TN, Aevermann B, Barkan E, Bertagnolli D, Casper T, Dee N, et al. (2018). Single-nucleus and single-cell transcriptomes compared in matched cortical cell types. PLoS One 13, e0209648. 10.1371/journal.pone.0209648. [PubMed: 30586455]
127. Lake BB, Codeluppi S, Yung YC, Gao D, Chun J, Kharchenko PV, Linnarsson S, and Zhang K (2017). A comparative strategy for single-nucleus and single-cell transcriptomes confirms

accuracy in predicted cell-type expression from nuclear RNA. Sci Rep 7, 6031. 10.1038/s41598-017-04426-w. [PubMed: 28729663]

128. Gupta A, Shamsi F, Altemose N, Dorlhiac GF, Cypess AM, White AP, Yosef N, Patti ME, Tseng Y-H, and Streets A (2022). Characterization of transcript enrichment and detection bias in single-nucleus RNA-seq for mapping of distinct human adipocyte lineages. Genome Research. 10.1101/gr.275509.121.
129. Denisenko E, Guo BB, Jones M, Hou R, de Kock L, Lassmann T, Poppe D, Clement O, Simmons RK, Lister R, and Forrest ARR (2020). Systematic assessment of tissue dissociation and storage biases in single-cell and single-nucleus RNA-seq workflows. Genome Biol 21, 130. 10.1186/s13059-020-02048-6. [PubMed: 32487174]
130. van den Brink SC, Sage F, Vertesy A, Spanjaard B, Peterson-Maduro J, Baron CS, Robin C, and van Oudenaarden A (2017). Single-cell sequencing reveals dissociation-induced gene expression in tissue subpopulations. Nat Methods 14, 935–936. 10.1038/nmeth.4437. [PubMed: 28960196]
131. Van Hauwaert EL, Gammelmark E, Sárvári AK, Larsen L, Nielsen R, Madsen JGS, and Mandrup S (2021). Isolation of nuclei from mouse white adipose tissues for single-nucleus genomics. STAR Protoc 2, 100612. 10.1016/j.xpro.2021.100612. [PubMed: 34189477]
132. Alvarez M, Rahmani E, Jew B, Garske KM, Miao Z, Benhammou JN, Ye CJ, Pisegna JR, Pietilainen KH, Halperin E, and Pajukanta P (2020). Enhancing droplet-based single-nucleus RNA-seq resolution using the semi-supervised machine learning classifier DIEM. Sci Rep 10, 11019. 10.1038/s41598-020-67513-5. [PubMed: 32620816]
133. Stuart T, and Satija R (2019). Integrative single-cell analysis. Nature Reviews Genetics 20, 257–272. 10.1038/s41576-019-0093-7.
134. Avsec Ž, Weilert M, Shrikumar A, Krueger S, Alexandari A, Dalal K, Fropf R, McAnany C, Gagneur J, Kundaje A, and Zeitlinger J (2021). Base-resolution models of transcription-factor binding reveal soft motif syntax. Nature Genetics 53, 354–366. 10.1038/s41588-021-00782-6. [PubMed: 33603233]
135. Cao J, Cusanovich DA, Ramani V, Aghamirzaie D, Pliner HA, Hill AJ, Daza RM, McFaline-Figueroa JL, Packer JS, Christiansen L, et al. (2018). Joint profiling of chromatin accessibility and gene expression in thousands of single cells. Science 361, 1380–1385. doi:10.1126/science.aau0730. [PubMed: 30166440]
136. Schoof EM, Furtwängler B, Üresin N, Rapin N, Savickas S, Gentil C, Lechman E, Keller Ua.d., Dick, J.E., and Porse, B.T. (2021). Quantitative single-cell proteomics as a tool to characterize cellular hierarchies. Nature Communications 12, 3341. 10.1038/s41467-021-23667-y.
137. Labib M, and Kelley SO (2020). Single-cell analysis targeting the proteome. Nature Reviews Chemistry 4, 143–158. 10.1038/s41570-020-0162-7.
138. Brunner A-D, Thielert M, Vasilopoulou C, Ammar C, Coscia F, Mund A, Hoerning OB, Bache N, Apalategui A, Lubeck M, et al. (2022). Ultra-high sensitivity mass spectrometry quantifies single-cell proteome changes upon perturbation. Molecular Systems Biology 18, e10798. 10.15252/msb.202110798. [PubMed: 35226415]
139. Marx V (2021). Method of the Year: spatially resolved transcriptomics. Nat Methods 18, 9–14. 10.1038/s41592-020-01033-y. [PubMed: 33408395]
140. Rao A, Barkley D, França GS, and Yanai I (2021). Exploring tissue architecture using spatial transcriptomics. Nature 596, 211–220. 10.1038/s41586-021-03634-9. [PubMed: 34381231]
141. Xia C, Babcock HP, Moffitt JR, and Zhuang X (2019). Multiplexed detection of RNA using MERFISH and branched DNA amplification. Sci Rep 9, 7721. 10.1038/s41598-019-43943-8. [PubMed: 31118500]
142. Chen KH, Boettiger AN, Moffitt JR, Wang S, and Zhuang X (2015). Spatially resolved, highly multiplexed RNA profiling in single cells. Science 348, aaa6090. doi:10.1126/science.aaa6090. [PubMed: 25858977]
143. Zhang Z, and Zhang X (2021). Inference of high-resolution trajectories in single-cell RNA-seq data by using RNA velocity. Cell Rep Methods 1, 100095. 10.1016/j.crmeth.2021.100095. [PubMed: 35474895]

144. DeTomaso D, Jones MG, Subramaniam M, Ashuach T, Ye CJ, and Yosef N (2019). Functional interpretation of single cell similarity maps. Nat Commun 10, 4376. 10.1038/s41467-019-12235-0. [PubMed: 31558714]

145. Lange M, Bergen V, Klein M, Setty M, Reuter B, Bakhti M, Lickert H, Ansari M, Schniering J, Schiller HB, et al. (2022). CellRank for directed single-cell fate mapping. Nature Methods 19, 159–170. 10.1038/s41592-021-01346-6. [PubMed: 35027767]

146. Efremova M, Vento-Tormo M, Teichmann SA, and Vento-Tormo R (2020). CellPhoneDB: inferring cell–cell communication from combined expression of multi-subunit ligand–receptor complexes. Nature Protocols 15, 1484–1506. 10.1038/s41596-020-0292-x. [PubMed: 32103204]

147. Browaeys R, Saelens W, and Saeys Y (2020). NicheNet: modeling intercellular communication by linking ligands to target genes. Nat Methods 17, 159–162. 10.1038/s41592-019-0667-5. [PubMed: 31819264]

148. van der Wijst MGP, de Vries DH, Groot HE, Trynka G, Hon CC, Bonder MJ, Stegle O, Nawijn MC, Idaghdour Y, van der Harst P, et al. (2020). The single-cell eQTLGen consortium. eLife 9, e52155. 10.7554/eLife.52155. [PubMed: 32149610]

149. Bryois J, Calini D, Macnair W, Foo L, Urich E, Ortmann W, Iglesias VA, Selvaraj S, Nutma E, Marzin M, et al. (2022). Cell-type-specific cis-eQTLs in eight human brain cell types identify novel risk genes for psychiatric and neurological disorders. Nat Neurosci 25, 1104–1112. 10.1038/s41593-022-01128-z. [PubMed: 35915177]

150. Yazar S, Alquicira-Hernandez J, Wing K, Senabouth A, Gordon MG, Andersen S, Lu Q, Rowson A, Taylor TRP, Clarke L, et al. (2022). Single-cell eQTL mapping identifies cell type-specific genetic control of autoimmune disease. Science 376, eabf3041. 10.1126/science.abf3041. [PubMed: 35389779]

151. Backdahl J, Franzen L, Massier L, Li Q, Jalkanen J, Gao H, Andersson A, Bhalla N, Thorell A, Ryden M, et al. (2021). Spatial mapping reveals human adipocyte subpopulations with distinct sensitivities to insulin. Cell Metab 33, 1869–1882 e1866. 10.1016/j.cmet.2021.07.018. [PubMed: 34380013]

152. Klöting N, Fasshauer M, Dietrich A, Kovacs P, Schön MR, Kern M, Stumvoll M, and Blüher M (2010). Insulin-sensitive obesity. Am J Physiol Endocrinol Metab 299, E506–515. 10.1152/ajpendo.00586.2009. [PubMed: 20570822]

153. Gustafson B, Hedjazifar S, Gogg S, Hammarstedt A, and Smith U (2015). Insulin resistance and impaired adipogenesis. Trends Endocrinol Metab 26, 193–200. 10.1016/j.tem.2015.01.006. [PubMed: 25703677]

154. McLaughlin T, Sherman A, Tsao P, Gonzalez O, Yee G, Lamendola C, Reaven GM, and Cushman SW (2007). Enhanced proportion of small adipose cells in insulin-resistant vs insulin-sensitive obese individuals implicates impaired adipogenesis. Diabetologia 50, 1707–1715. 10.1007/s00125-007-0708-y. [PubMed: 17549449]

155. Sanchez-Gurmaches J, Tang Y, Jespersen NZ, Wallace M, Martinez Calejman C, Gujja S, Li H, Edwards YJK, Wolfrum C, Metallo CM, et al. (2018). Brown Fat AKT2 Is a Cold-Induced Kinase that Stimulates ChREBP-Mediated De Novo Lipogenesis to Optimize Fuel Storage and Thermogenesis. Cell Metab 27, 195–209 e196. 10.1016/j.cmet.2017.10.008. [PubMed: 29153407]

156. Tang Y, Wallace M, Sanchez-Gurmaches J, Hsiao WY, Li H, Lee PL, Vernia S, Metallo CM, and Guertin DA (2016). Adipose tissue mTORC2 regulates ChREBP-driven de novo lipogenesis and hepatic glucose metabolism. Nat Commun 7, 11365. 10.1038/ncomms11365. [PubMed: 27098609]

157. Eissing L, Scherer T, Todter K, Knippschild U, Greve JW, Buurman WA, Pinnschmidt HO, Rensen SS, Wolf AM, Bartelt A, et al. (2013). De novo lipogenesis in human fat and liver is linked to ChREBP-beta and metabolic health. Nat Commun 4, 1528. 10.1038/ncomms2537. [PubMed: 23443556]

158. Cinti S, Cancello R, Zingaretti MC, Ceresi E, De Matteis R, Giordano A, Himms-Hagen J, and Ricquier D (2002). CL316,243 and cold stress induce heterogeneous expression of UCP1 mRNA and protein in rodent brown adipocytes. J Histochem Cytochem 50, 21–31. 10.1177/002215540205000103. [PubMed: 11748291]

159. Spaethling JM, Sanchez-Alavez M, Lee J, Xia FC, Dueck H, Wang W, Fisher SA, Sul JY, Seale P, Kim J, et al. (2016). Single-cell transcriptomics and functional target validation of brown adipocytes show their complex roles in metabolic homeostasis. Faseb j 30, 81–92. 10.1096/fj.15-273797. [PubMed: 26304220]

160. Biagi CAO Jr., Cury SS, Alves CP, Rabhi N, Silva WA Jr., Farmer SR, Carvalho RF, and Batista ML Jr. (2021). Multidimensional Single-Nuclei RNA-Seq Reconstruction of Adipose Tissue Reveals Adipocyte Plasticity Underlying Thermogenic Response. Cells 10. 10.3390/cells10113073.

161. Merrick D, Sakers A, Irgebay Z, Okada C, Calvert C, Morley MP, Percec I, and Seale P (2019). Identification of a mesenchymal progenitor cell hierarchy in adipose tissue. Science 364. 10.1126/science.aav2501.

162. Rondini EA, and Granneman JG (2020). Single cell approaches to address adipose tissue stromal cell heterogeneity. Biochem J 477, 583–600. 10.1042/bcj20190467. [PubMed: 32026949]

163. Shao M, Hepler C, Zhang Q, Shan B, Vishvanath L, Henry GH, Zhao S, An YA, Wu Y, Strand DW, and Gupta RK (2021). Pathologic HIF1alpha signaling drives adipose progenitor dysfunction in obesity. Cell Stem Cell 28, 685–701 e687. 10.1016/j.stem.2020.12.008. [PubMed: 33539723]

164. Hepler C, Shan B, Zhang Q, Henry GH, Shao M, Vishvanath L, Ghaben AL, Mobley AB, Strand D, Hon GC, and Gupta RK (2018). Identification of functionally distinct fibro-inflammatory and adipogenic stromal subpopulations in visceral adipose tissue of adult mice. Elife 7. 10.7554/eLife.39636.

165. Burl RB, Ramseyer VD, Rondini EA, Pique-Regi R, Lee YH, and Granneman JG (2018). Deconstructing Adipogenesis Induced by beta3-Adrenergic Receptor Activation with Single-Cell Expression Profiling. Cell Metab 28, 300–309 e304. 10.1016/j.cmet.2018.05.025. [PubMed: 29937373]

166. Spallanzani RG, Zemmour D, Xiao T, Jayewickreme T, Li C, Bryce PJ, Benoist C, and Mathis D (2019). Distinct immunocyte-promoting and adipocyte-generating stromal components coordinate adipose tissue immune and metabolic tenors. Sci Immunol 4. 10.1126/sciimmunol.aaw3658.

167. Nahmgoong H, Jeon YG, Park ES, Choi YH, Han SM, Park J, Ji Y, Sohn JH, Han JS, Kim YY, et al. (2022). Distinct properties of adipose stem cell subpopulations determine fat depot-specific characteristics. Cell Metab 34, 458–472 e456. 10.1016/j.cmet.2021.11.014. [PubMed: 35021043]

168. Buffolo M, Pires KM, Ferhat M, Ilkun O, Makaju A, Achenbach A, Bowman F, Atkinson DL, Holland WL, Amri EZ, et al. (2019). Identification of a Paracrine Signaling Mechanism Linking CD34(high) Progenitors to the Regulation of Visceral Fat Expansion and Remodeling. Cell Rep 29, 270–282 e275. 10.1016/j.celrep.2019.08.092. [PubMed: 31597091]

169. Giuliani G, Rosina M, and Reggio A (2022). Signaling pathways regulating the fate of fibro/adipogenic progenitors (FAPs) in skeletal muscle regeneration and disease. Febs j 289, 6484–6517. 10.1111/febs.16080. [PubMed: 34143565]

170. Hatzmann FM, Grossmann S, Waldegger P, Wiegers GJ, Mandl M, Rauchenwald T, Pierer G, and Zwerschke W (2022). Dipeptidyl peptidase-4 cell surface expression marks an abundant adipose stem/progenitor cell population with high stemness in human white adipose tissue. Adipocyte 11, 601–615. 10.1080/21623945.2022.2129060. [PubMed: 36168895]

171. Berry R, and Rodeheffer MS (2013). Characterization of the adipocyte cellular lineage in vivo. Nat Cell Biol 15, 302–308. 10.1038/ncb2696. [PubMed: 23434825]

172. Stefkovich M, Traynor S, Cheng L, Merrick D, and Seale P (2021). Dpp4+ interstitial progenitor cells contribute to basal and high fat diet-induced adipogenesis. Mol Metab 54, 101357. 10.1016/j.molmet.2021.101357. [PubMed: 34662714]

173. Schwalie PC, Dong H, Zachara M, Russeil J, Alpern D, Akchiche N, Caprara C, Sun W, Schlaudraff KU, Soldati G, et al. (2018). A stromal cell population that inhibits adipogenesis in mammalian fat depots. Nature 559, 103–108. 10.1038/s41586-018-0226-8. [PubMed: 29925944]

174. Nguyen HP, Lin F, Yi D, Xie Y, Dinh J, Xue P, and Sul HS (2021). Aging-dependent regulatory cells emerge in subcutaneous fat to inhibit adipogenesis. Dev Cell 56, 1437–1451 e1433. 10.1016/j.devcel.2021.03.026. [PubMed: 33878347]

175. Zhang Q, Shan B, Guo L, Shao M, Vishvanath L, Elmquist G, Xu L, and Gupta RK (2022). Distinct functional properties of murine perinatal and adult adipose progenitor subpopulations. Nat Metab 4, 1055–1070. 10.1038/s42255-022-00613-w. [PubMed: 35982290]
176. Shan B, Barker CS, Shao M, Zhang Q, Gupta RK, and Wu Y (2022). Multilayered omics reveal sex- and depot-dependent adipose progenitor cell heterogeneity. Cell Metab 34, 783–799 e787. 10.1016/j.cmet.2022.03.012. [PubMed: 35447091]
177. Oguri Y, Shinoda K, Kim H, Alba DL, Bolus WR, Wang Q, Brown Z, Pradhan RN, Tajima K, Yoneshiro T, et al. (2020). CD81 Controls Beige Fat Progenitor Cell Growth and Energy Balance via FAK Signaling. Cell 182, 563–577 e520. 10.1016/j.cell.2020.06.021. [PubMed: 32615086]
178. Shamsi F, Piper M, Ho LL, Huang TL, Gupta A, Streets A, Lynes MD, and Tseng YH (2021). Vascular smooth muscle-derived Trpv1(+) progenitors are a source of cold-induced thermogenic adipocytes. Nat Metab 3, 485–495. 10.1038/s42255-021-00373-z. [PubMed: 33846638]
179. Tang W, Zeve D, Suh JM, Bosnakovski D, Kyba M, Hammer RE, Tallquist MD, and Graff JM (2008). White fat progenitor cells reside in the adipose vasculature. Science 322, 583–586. 10.1126/science.1156232. [PubMed: 18801968]
180. Burl RB, Rondini EA, Wei H, Pique-Regi R, and Granneman JG (2022). Deconstructing cold-induced brown adipocyte neogenesis in mice. Elife 11. 10.7554/eLife.80167.
181. Tran KV, Fitzgibbons T, Min SY, DeSouza T, and Corvera S (2018). Distinct adipocyte progenitor cells are associated with regional phenotypes of perivascular aortic fat in mice. Mol Metab 9, 199–206. 10.1016/j.molmet.2017.12.014. [PubMed: 29396370]
182. Angueira AR, Sakers AP, Holman CD, Cheng L, Arbocco MN, Shamsi F, Lynes MD, Shrestha R, Okada C, Batmanov K, et al. (2021). Defining the lineage of thermogenic perivascular adipose tissue. Nat Metab 3, 469–484. 10.1038/s42255-021-00380-0. [PubMed: 33846639]
183. Festa E, Fretz J, Berry R, Schmidt B, Rodeheffer M, Horowitz M, and Horsley V (2011). Adipocyte lineage cells contribute to the skin stem cell niche to drive hair cycling. Cell 146, 761–771. 10.1016/j.cell.2011.07.019. [PubMed: 21884937]
184. Guerrero-Juarez CF, and Plikus MV (2018). Emerging nonmetabolic functions of skin fat. Nat Rev Endocrinol 14, 163–173. 10.1038/nrendo.2017.162. [PubMed: 29327704]
185. Plikus MV, Guerrero-Juarez CF, Ito M, Li YR, Dedhia PH, Zheng Y, Shao M, Gay DL, Ramos R, Hsi TC, et al. (2017). Regeneration of fat cells from myofibroblasts during wound healing. Science 355, 748–752. 10.1126/science.aai8792. [PubMed: 28059714]
186. Zhang LJ, Guerrero-Juarez CF, Hata T, Bapat SP, Ramos R, Plikus MV, and Gallo RL (2015). Innate immunity. Dermal adipocytes protect against invasive Staphylococcus aureus skin infection. Science 347, 67–71. 10.1126/science.1260972. [PubMed: 25554785]
187. Zhang Z, Shao M, Hepler C, Zi Z, Zhao S, An YA, Zhu Y, Ghaben AL, Wang MY, Li N, et al. (2019). Dermal adipose tissue has high plasticity and undergoes reversible dedifferentiation in mice. J Clin Invest 129, 5327–5342. 10.1172/JCI130239. [PubMed: 31503545]
188. Shook BA, Wasko RR, Mano O, Rutenberg-Schoenberg M, Rudolph MC, Zirak B, Rivera-Gonzalez GC, Lopez-Giraldez F, Zarini S, Rezza A, et al. (2020). Dermal Adipocyte Lipolysis and Myofibroblast Conversion Are Required for Efficient Skin Repair. Cell Stem Cell 26, 880–895 e886. 10.1016/j.stem.2020.03.013. [PubMed: 32302523]
189. Zhu Q, Zhu Y, Hepler C, Zhang Q, Park J, Gliniak C, Henry GH, Crewe C, Bu D, Zhang Z, et al. (2022). Adipocyte mesenchymal transition contributes to mammary tumor progression. Cell Rep 40, 111362. 10.1016/j.celrep.2022.111362. [PubMed: 36103820]
190. Li Z, Hardij J, Bagchi DP, Scheller EL, and MacDougald OA (2018). Development, regulation, metabolism and function of bone marrow adipose tissues. Bone 110, 134–140. 10.1016/j.bone.2018.01.008. [PubMed: 29343445]
191. Scheller EL, Cawthorn WP, Burr AA, Horowitz MC, and MacDougald OA (2016). Marrow Adipose Tissue: Trimming the Fat. Trends Endocrinol Metab 27, 392–403. 10.1016/j.tem.2016.03.016. [PubMed: 27094502]
192. Suchacki KJ, Tavares AAS, Mattiucci D, Scheller EL, Papanastasiou G, Gray C, Sinton MC, Ramage LE, McDougald WA, Lovdel A, et al. (2020). Bone marrow adipose tissue is a unique adipose subtype with distinct roles in glucose homeostasis. Nat Commun 11, 3097. 10.1038/s41467-020-16878-2. [PubMed: 32555194]

193. Zhong L, Yao L, Tower RJ, Wei Y, Miao Z, Park J, Shrestha R, Wang L, Yu W, Holdreith N, et al. (2020). Single cell transcriptomics identifies a unique adipose lineage cell population that regulates bone marrow environment. Elife 9. 10.7554/eLife.54695.

194. Grosjean A, Venteclef N, and Dalmas E (2021). Understanding the heterogeneity and functions of metabolic tissue macrophages. Seminars in Cell & Developmental Biology 119, 130–139. 10.1016/j.semcdb.2021.09.002. [PubMed: 34561168]

195. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, and Ferrante AW Jr. (2003). Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 112, 1796–1808. 10.1172/jci19246. [PubMed: 14679176]

196. Li P, Lu M, Nguyen MTA, Bae EJ, Chapman J, Feng D, Hawkins M, Pessin JE, Sears DD, Nguyen AK, et al. (2010). Functional heterogeneity of CD11c-positive adipose tissue macrophages in diet-induced obese mice. J Biol Chem 285, 15333–15345. 10.1074/jbc.M110.100263. [PubMed: 20308074]

197. Lumeng CN, Bodzin JL, and Saltiel AR (2007). Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest 117, 175–184. 10.1172/jci29881. [PubMed: 17200717]

198. Xu X, Grijalva A, Skowronski A, van Eijk M, Serlie MJ, and Ferrante AW Jr. (2013). Obesity activates a program of lysosomal-dependent lipid metabolism in adipose tissue macrophages independently of classic activation. Cell Metab 18, 816–830. 10.1016/j.cmet.2013.11.001. [PubMed: 24315368]

199. Kratz M, Coats BR, Hisert KB, Hagman D, Mutskov V, Peris E, Schoenfelt KQ, Kuzma JN, Larson I, Billing PS, et al. (2014). Metabolic dysfunction drives a mechanistically distinct proinflammatory phenotype in adipose tissue macrophages. Cell Metab 20, 614–625. 10.1016/j.cmet.2014.08.010. [PubMed: 25242226]

200. Xue J, Schmidt SV, Sander J, Draffehn A, Krebs W, Quester I, De Nardo D, Gohel TD, Emde M, Schmidleithner L, et al. (2014). Transcriptome-based network analysis reveals a spectrum model of human macrophage activation. Immunity 40, 274–288. 10.1016/j.immuni.2014.01.006. [PubMed: 24530056]

201. Hill DA, Lim HW, Kim YH, Ho WY, Foong YH, Nelson VL, Nguyen HCB, Chegireddy K, Kim J, Habertheuer A, et al. (2018). Distinct macrophage populations direct inflammatory versus physiological changes in adipose tissue. Proc Natl Acad Sci U S A 115, E5096–e5105. 10.1073/pnas.1802611115. [PubMed: 29760084]

202. Sharma M, Schlegel M, Brown EJ, Sansbury BE, Weinstock A, Afonso MS, Corr EM, van Solingen C, Shanley LC, Peled D, et al. (2019). Netrin-1 Alters Adipose Tissue Macrophage Fate and Function in Obesity. Immunometabolism 1. 10.20900/immunometab20190010.

203. Jaitin DA, Adlung L, Thaiss CA, Weiner A, Li B, Descamps H, Lundgren P, Bleriot C, Liu Z, Deczkowska A, et al. (2019). Lipid-Associated Macrophages Control Metabolic Homeostasis in a Trem2-Dependent Manner. Cell 178, 686–698.e614. 10.1016/j.cell.2019.05.054. [PubMed: 31257031]

204. Weinstock A, Brown EJ, Garabedian ML, Pena S, Sharma M, Lafaille J, Moore KJ, and Fisher EA (2019). Single-Cell RNA Sequencing of Visceral Adipose Tissue Leukocytes Reveals that Caloric Restriction Following Obesity Promotes the Accumulation of a Distinct Macrophage Population with Features of Phagocytic Cells. Immunometabolism 1. 10.20900/immunometab20190008.

205. Li C, Menoret A, Farragher C, Ouyang Z, Bonin C, Holvoet P, Vella AT, and Zhou B (2019). Single cell transcriptomics based-MacSpectrum reveals novel macrophage activation signatures in diseases. JCI Insight 5. 10.1172/jci.insight.126453.

206. Hildreth AD, Ma F, Wong YY, Sun R, Pellegrini M, and O'Sullivan TE (2021). Single-cell sequencing of human white adipose tissue identifies new cell states in health and obesity. Nat Immunol 22, 639–653. 10.1038/s41590-021-00922-4. [PubMed: 33907320]

207. Vijay J, Gauthier MF, Biswell RL, Louiselle DA, Johnston JJ, Cheung WA, Belden B, Pramatarova A, Biertho L, Gibson M, et al. (2020). Single-cell analysis of human adipose tissue identifies depot and disease specific cell types. Nat Metab 2, 97–109. 10.1038/s42255-019-0152-6. [PubMed: 32066997]

208. Félix I, Jokela H, Karhula J, Kotaja N, Savontaus E, Salmi M, and Rantakari P (2021). Single-Cell Proteomics Reveals the Defined Heterogeneity of Resident Macrophages in White Adipose Tissue. Front Immunol 12, 719979. 10.3389/fimmu.2021.719979. [PubMed: 34381461]

209. Chakarov S, Lim HY, Tan L, Lim SY, See P, Lum J, Zhang XM, Foo S, Nakamizo S, Duan K, et al. (2019). Two distinct interstitial macrophage populations coexist across tissues in specific subtissular niches. Science 363. 10.1126/science.aau0964.

210. Silva HM, Báfica A, Rodrigues-Luiz GF, Chi J, Santos PDA, Reis BS, Hoytema van Konijnenburg DP, Crane A, Arifa RDN, Martin P, et al. (2019). Vasculature-associated fat macrophages readily adapt to inflammatory and metabolic challenges. J Exp Med 216, 786–806. 10.1084/jem.20181049. [PubMed: 30862706]

211. Gallerand A, Stunault MI, Merlin J, Luehmann HP, Sultan DH, Firulyova MM, Magnone V, Khedher N, Jalil A, Dolfi B, et al. (2021). Brown adipose tissue monocytes support tissue expansion. Nat Commun 12, 5255. 10.1038/s41467-021-25616-1. [PubMed: 34489438]

212. Cho KW, Zamarron BF, Muir LA, Singer K, Porsche CE, DelProposto JB, Geletka L, Meyer KA, O'Rourke RW, and Lumeng CN (2016). Adipose Tissue Dendritic Cells Are Independent Contributors to Obesity-Induced Inflammation and Insulin Resistance. J Immunol 197, 3650–3661. 10.4049/jimmunol.1600820. [PubMed: 27683748]

213. Ksiazek K (2013). Mesothelial cell: a multifaceted model of aging. Ageing Res Rev 12, 595–604. 10.1016/j.arr.2013.01.008. [PubMed: 23415666]

214. Gupta OT, and Gupta RK (2015). Visceral Adipose Tissue Mesothelial Cells: Living on the Edge or Just Taking Up Space? Trends Endocrinol Metab 26, 515–523. 10.1016/j.tem.2015.07.003. [PubMed: 26412153]

215. Darimont C, Avanti O, Blancher F, Wagniere S, Mansourian R, Zbinden I, Leone-Vautravers P, Fuerholz A, Giusti V, and Mace K (2008). Contribution of mesothelial cells in the expression of inflammatory-related factors in omental adipose tissue of obese subjects. Int J Obes (Lond) 32, 112–120. 10.1038/sj.ijo.0803688. [PubMed: 17637700]

216. Chau YY, Bandiera R, Serrels A, Martinez-Estrada OM, Qing W, Lee M, Slight J, Thornburn A, Berry R, McHaffie S, et al. (2014). Visceral and subcutaneous fat have different origins and evidence supports a mesothelial source. Nat Cell Biol 16, 367–375. 10.1038/ncb2922. [PubMed: 24609269]

217. Whytock KL, Sun Y, Divoux A, Yu G, Smith SR, Walsh MJ, and Sparks LM (2022). Single cell full-length transcriptome of human subcutaneous adipose tissue reveals unique and heterogeneous cell populations. iScience 25, 104772. 10.1016/j.isci.2022.104772. [PubMed: 35992069]

218. Kalucka J, de Rooij L, Goveia J, Rohlenova K, Dumas SJ, Meta E, Conchinha NV, Taverna F, Teuwen LA, Veys K, et al. (2020). Single-Cell Transcriptome Atlas of Murine Endothelial Cells. Cell 180, 764–779 e720. 10.1016/j.cell.2020.01.015. [PubMed: 32059779]

219. Trimm E, and Red-Horse K (2022). Vascular endothelial cell development and diversity. Nat Rev Cardiol 10.1038/s41569-022-00770-1.

220. Yang J, Vamvini M, Nigro P, Ho LL, Galani K, Alvarez M, Tanigawa Y, Renfro A, Carbone NP, Laakso M, et al. (2022). Single-cell dissection of the obesity-exercise axis in adipose-muscle tissues implies a critical role for mesenchymal stem cells. Cell Metab 34, 1578–1593 e1576. 10.1016/j.cmet.2022.09.004. [PubMed: 36198295]

221. Dong H, Sun W, Shen Y, Baláz M, Balázová L, Ding L, Löffler M, Hamilton B, Klöting N, Blüher M, et al. (2022). Identification of a regulatory pathway inhibiting adipogenesis via RSPO2. Nat Metab 4, 90–105. 10.1038/s42255-021-00509-1. [PubMed: 35027768]

222. Rabhi N, Desevin K, Belkina AC, Tilston-Lunel A, Varelas X, Layne MD, and Farmer SR (2022). Obesity-induced senescent macrophages activate a fibrotic transcriptional program in adipocyte progenitors. Life Sci Alliance 5. 10.26508/lsa.202101286.

223. Shan B, Shao M, Zhang Q, Hepler C, Paschoal VA, Barnes SD, Vishvanath L, An YA, Jia L, Malladi VS, et al. (2020). Perivascular mesenchymal cells control adipose-tissue macrophage accrual in obesity. Nat Metab 2, 1332–1349. 10.1038/s42255-020-00301-7. [PubMed: 33139957]

224. Mahlakoiv T, Flamar AL, Johnston LK, Moriyama S, Putzel GG, Bryce PJ, and Artis D (2019). Stromal cells maintain immune cell homeostasis in adipose tissue via production of interleukin-33. Sci Immunol 4. 10.1126/sciimmunol.aax0416.

225. Shan B, Shao M, Zhang Q, An YA, Vishvanath L, and Gupta RK (2021). Cold-responsive adipocyte progenitors couple adrenergic signaling to immune cell activation to promote beige adipocyte accrual. Genes Dev 35, 1333–1338. 10.1101/gad.348762.121. [PubMed: 34531316]

226. Brestoff JR, Kim BS, Saenz SA, Stine RR, Monticelli LA, Sonnenberg GF, Thome JJ, Farber DL, Lutfy K, Seale P, and Artis D (2015). Group 2 innate lymphoid cells promote beiging of white adipose tissue and limit obesity. Nature 519, 242–246. 10.1038/nature14115. [PubMed: 25533952]

227. Han X, Zhang Z, He L, Zhu H, Li Y, Pu W, Han M, Zhao H, Liu K, Li Y, et al. (2021). A suite of new Dre recombinase drivers markedly expands the ability to perform intersectional genetic targeting. Cell Stem Cell 28, 1160–1176 e1167. 10.1016/j.stem.2021.01.007. [PubMed: 33567267]

228. Zhao Z, Ukidve A, Kim J, and Mitragotri S (2020). Targeting Strategies for Tissue-Specific Drug Delivery. Cell 181, 151–167. 10.1016/j.cell.2020.02.001. [PubMed: 32243788]

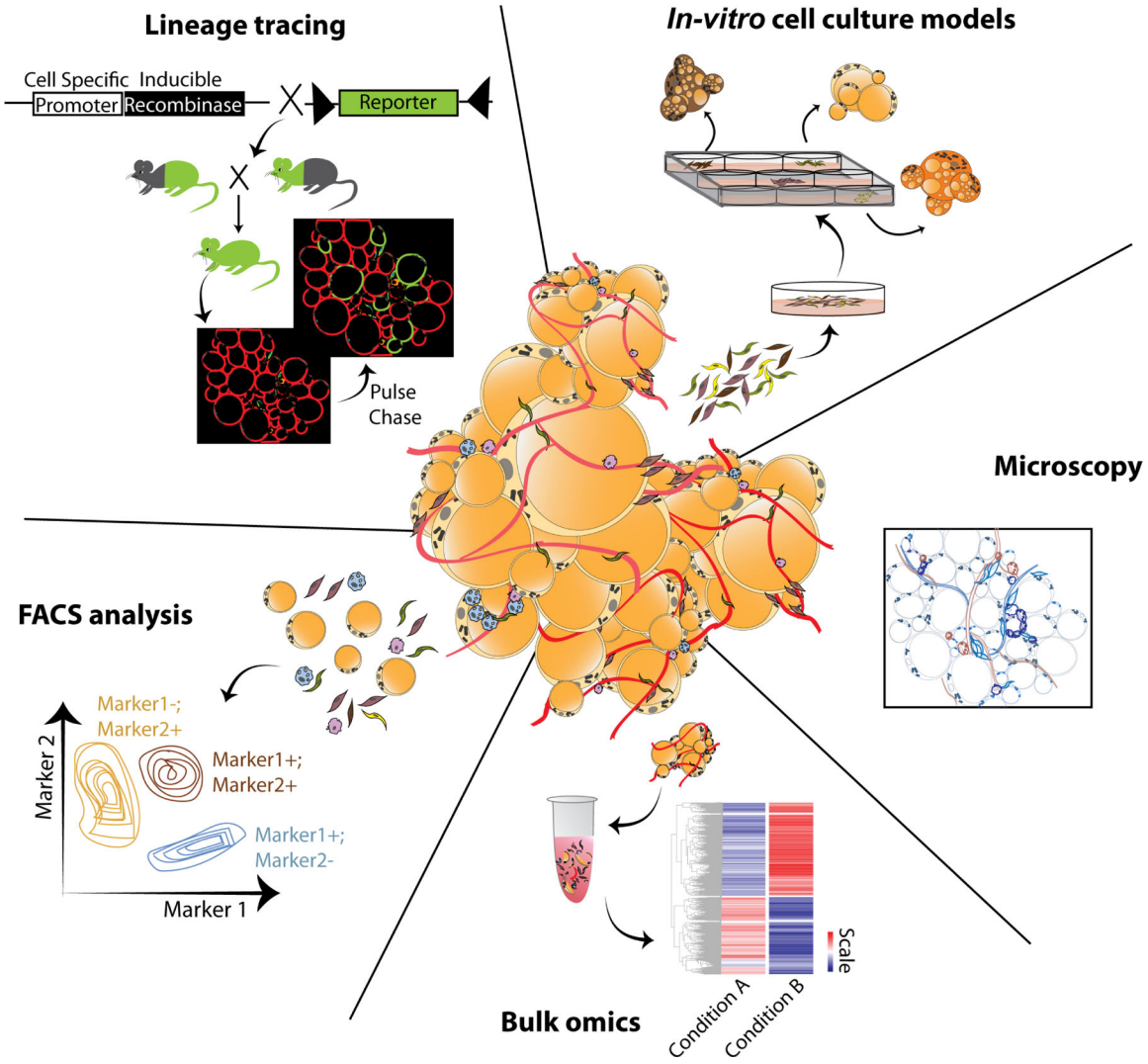

Figure 1: Classical techniques for studying adipose tissue

Conventionally applied microscopy, FACS analyses, genetic lineage tracing, *in vitro* cell culture models, and whole tissue (bulk) omics (genomics/proteomics/metabolomics/lipidomics) represent classical tools for adipose tissue biologists and have been instrumental for our current understanding of the morphological, compositional, functional, and physiological aspects of adipose tissue.

Conventional technologies for studies of adipose tissue		
Whole tissue technologies	**Pros**	**Cons**
Microscopy (immunostaining/RNA FISH)	• Preservation of 2D/3D structure of tissue • Cellular structure and position linked to protein/RNA expression	• Detection of few proteins/RNA • Hypothesis-biased

Conventional technologies for studies of adipose tissue		
Whole tissue technologies	**Pros**	**Cons**
Bulk tissue omics	• Genome-wide quantification of transcriptome/epigenome/ lipidome/metabol ome • Unbiased detection • High dynamic range	• No cell-type or spatial resolution
Studies of cell types		
FACS	• Sorting of primary cell types • /subpopulations • Retrieval of live cells for further studies	• Cell sorting based on few markers • Hypothesis-biased • Stressful sorting conditions • Challenging for adipocyte (size and fragility)
Genetic lineage tracing	• *In vivo* cell type specific tracing	• Limitation of single CRE drivers for labelling of specific cell types • Unwanted physiological effect from CRE-inducing agent • Lack of quantitative output
Cell culture models	• Well-defined culture conditions • Immortalization enables high sample yield • Ease of mechanistic studies	• Lack of signals from tissue microenvironme nt • Culture conditions masking biology

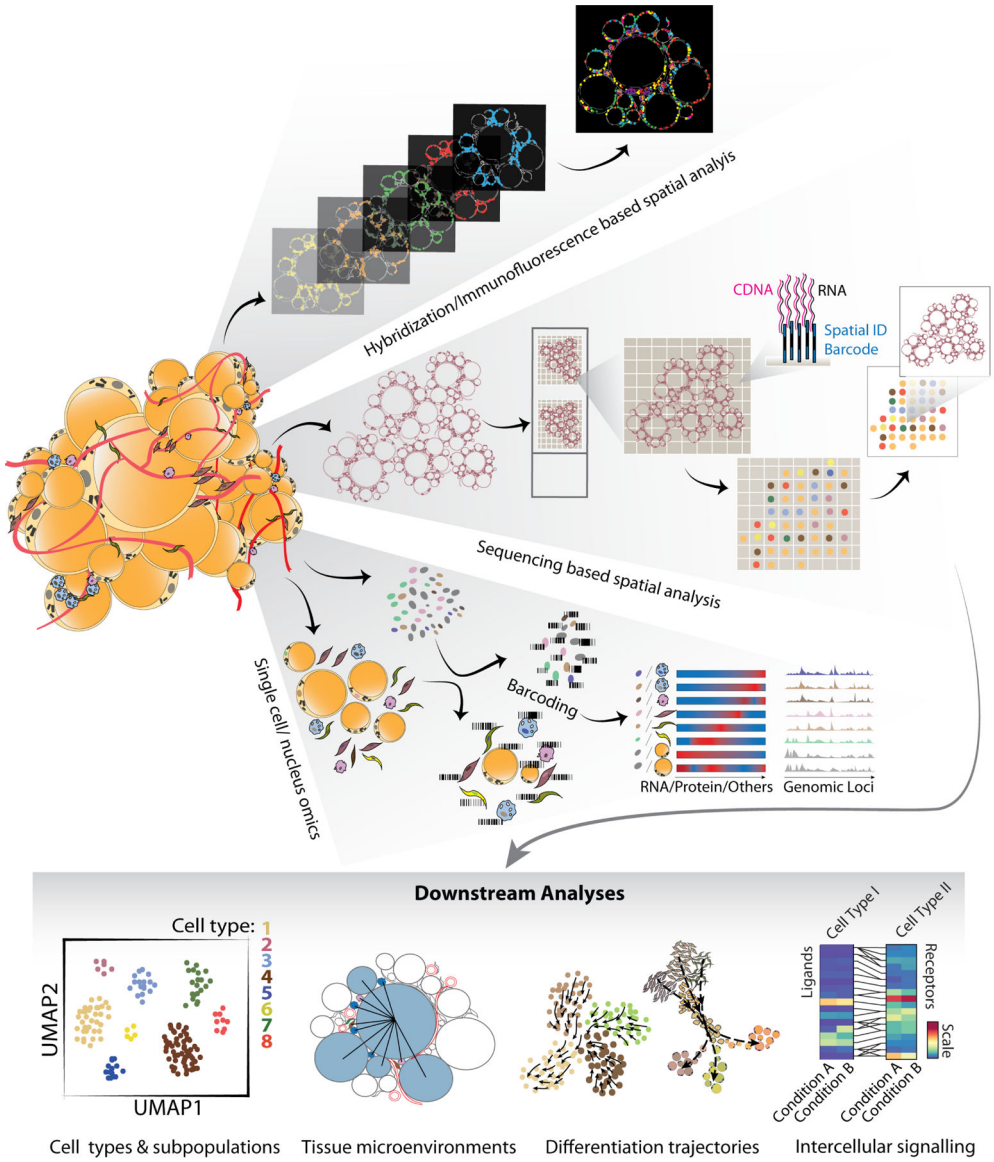

Figure 2: Deconvoluting adipose tissue through single cell/nucleus technologies

Summary of the current available single cell/nucleus technologies that are already in use, or can be implemented, to study adipose tissue functional and compositional heterogeneity. Single cell/nucleus omics based on various cell/nucleus barcoding have been widely applied for whole adipose tissue as well as the stromal vascular fraction of the tissue. Emerging spatial transcriptomics technologies include next generation sequencing-based relying on unique positional barcodes and bioimaging-based approaches rely either on in situ sequencing or hybridization of fluorescent probes to transcripts.

Single cell/nuclei analyses of adipose tissue		
Whole tissue technologies	**Pros**	**Cons**
Single cell/nuclei sequencing (e.g., 10x Genomics and other droplet-based methods)	• Unbiased profiling of all cells in tissue • Genome-wide quantification of transcriptome/epigenome • High throughput for detection of cellular heterogeneity and rare cell types • Detection of cell fate trajectories	• Stressful and biased isolation of cells/nuclei • Ambient RNA contamination (esp. snRNA-seq) • Limited sequencing depth • Lack of spatial information
Sequencing-based spatial transcriptomics (e.g., GeoMx® Digital Spatial Profiler, Illumina Visium)	• Spatial resolution of transcriptome • Detection of tissue microregions • Captures all cell types in tissue section • Limited cross-contamination between cell types	• Lack of full cellular resolution • Adipocyte size and lipid content complicates transcriptome-to-cell assignment • Limited sequencing depth • Limited cell sampling per tissue sections
Hybridization/antibody-based spatial analyses (e.g., MERFISH, CosMx™, 10x Genomics Xenium)	• Subcellular resolution of transcriptome/proteome in tissue • Detection of tissue microregions • Capture of all cell types in tissue section • Limited cross-contamination between cell types	• Lack of unbiased detection • Limited detection depth • Limited cell sampling per tissue sections

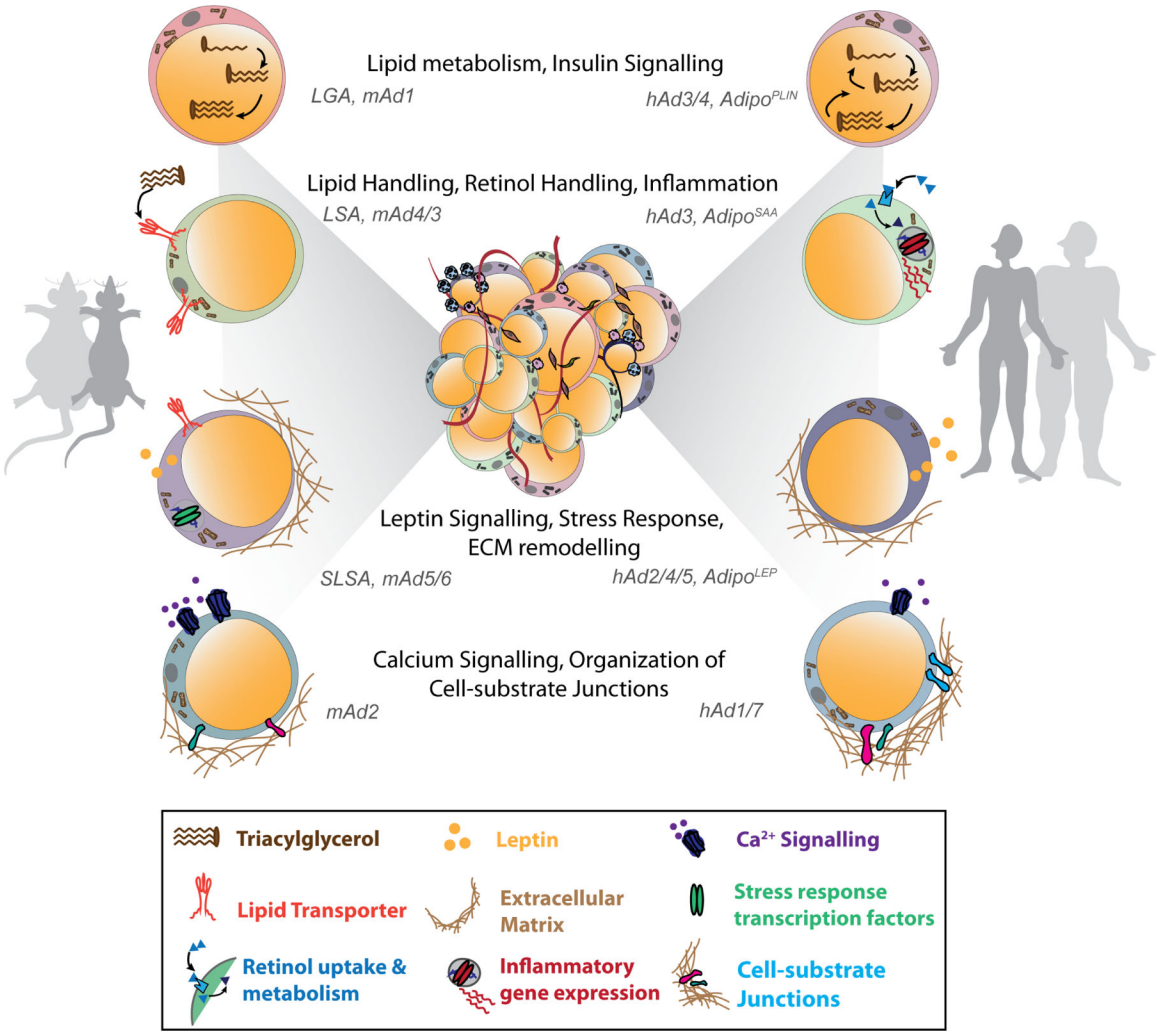

Figure 3: White adipocyte heterogeneity

Subpopulations of white adipocytes identified across different studies in mice and humans, classified based on their gene expression and enriched pathways. The four different subpopulations identified across different studies in mouse and human WAT are shown. Abbreviations: LGA, lipogenic adipocyte; LSA, lipid-scavenging adipocyte; SLSA, stressed lipid-scavenging adipocyte [124]. mAd1–6 are the mouse adipocyte subpopulations and hAd1–7 are the human adipocyte subpopulations defined in [122]. Adipo^{LEP}, Adipo^{PLIN} and Adipo^{SAA} subpopulations were defined by expressing high levels of *LEP, PLIN1/4* and SAA1/2, respectively, in [151].

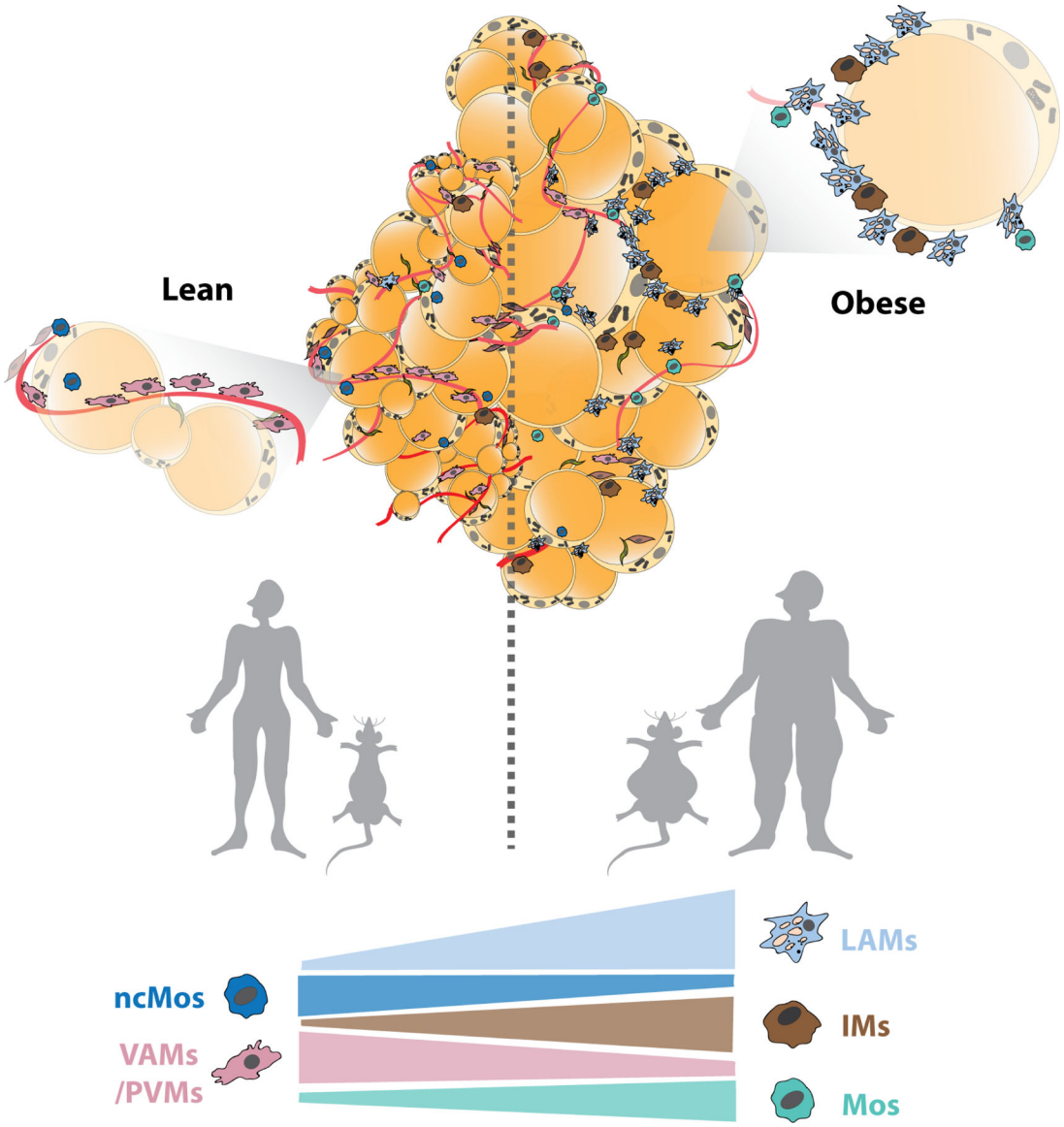

Figure 4: Immune cell (myeloid lineage) composition of adipose tissue.

Obesity-associated remodeling of the myeloid-cell immune compartment of adipose tissue in mice and humans. VAMs/PVMs and ncMos are abundant in the lean adipose tissue, whereas the balance tips towards an enrichment of LAMs, IMs and Mos during obesity progression in mice as well as humans. Abbreviations: LAM, lipid associated macrophage; PVM, perivascular macrophage[124]; IM, inflammatory macrophage [206]; Mos, Monocyte subpopulations Mo1 and Mo2; ncMos, non-canonical monocytes [203].

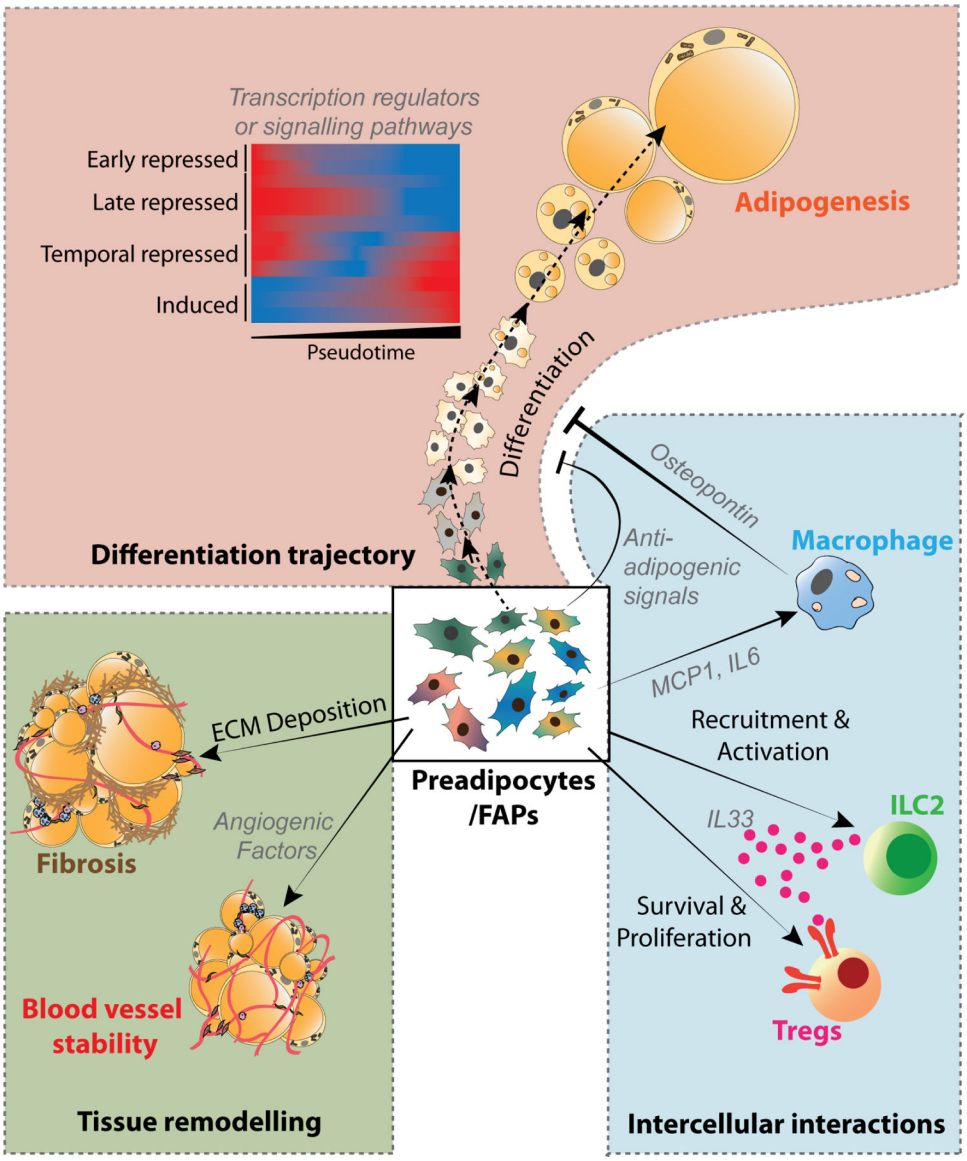

Figure 5: Critical insights from single-cell omics analyses.

Single-cell omics has revealed novel insight into *in vivo* adipogenesis, stromal cell-immune cell crosstalk, and intrinsic regulators of tissue remodeling. Pseudo time analysis of gene programs revealed distinct gene modules that are early/late repressed, induced or transiently repressed during the progression of adipogenesis [124]. Mesenchymal stromal cells, including preadipocytes and FAPs, secrete numerous collagens and modifiers of the extracellular matrix (ECM), thereby contributing to the pathologic tissue fibrosis observed in association with obesity [163,222]. These cells can also secrete angiogenic factors that promote blood vessel growth/stability [193]. Moreover, adipose stromal cells secrete proteins that act in an autocrine or paracrine manner to stimulate or suppress adipocyte differentiation [163,221], and they also modulate the local immune cell composition through the production of pro- (e.g. MCP1 and IL6) [223] and anti-inflammatory factors (e.g. IL-33) [166,224,225]. Importantly,

macrophages can influence the adipogenic and fibrogenic activity of adipose progenitors through the production of secreted factors (e.g., Osteopontin) [222].

Table 1.

Adipocyte subpopulations in white adipose tissue

Species	Gender	Depot	Subpopulation[a]	Markers	Pathways	Reference
Mouse (C57BL/6J)	males	WAT (epididymal)	lipogenic adipocyte (LGA)	*Acaca*, *Acly*, *Fasn*, *Dgat1/2*	triglyceride biosynthesis	Sárvári et al.[123]
			lipid-scavenging adipocyte (LSA)	*Car3*, *Apoe*, *Cd36*	triglyceride handling	
			stressed lipid-scavenging adipocyte (SLSA)	*Apoe*, *Cd36*, *Hif1a*, Ddr2	leptin synthesis + triglyceride handling + stress responses	
	male + female	WAT (inguinal + perigonadal)	mAd1	*Ebf2*, *Pck1*	insulin signaling + lipid handling + thermogenesis	Emont et al.[121]
			mAd2	*Sorbs2*, *Crim1*	Ca^{2+} signaling + organization of cell-substrate junction	
			mAd3	*Apoe*, *campk1d*	OxPhos + lipid handling	
			mAd4	*Cacna1a*, *Fgf1*, *Lep*	PPAR and leptin signaling + ECM remodeling + stress response signaling	
			mAd5	*Acss2*, *Prune2*, *Acly*	lipogenesis + triglyceride biosynthesis	
			mAd6	*Mt2*, *Casp4*, *Bcl3*	stress response + autophagy	
Human	men + women	WAT (subcutaneous + abdominal)	$Adipo^{LEP}$	*LEP*, *DDR2*, *TNS1*	leptin signaling + ECM remodeling	Bäckdahl et al.[150]
			$Adipo^{PLIN}$	*PLIN1/4*, *LIPE*, *DGAT1/2*	lipid biosynthesis and lipolysis	
			$Adipo^{SAA}$	*SAA1/2*, *MGLL*, *AGPAT2*	retinol metabolism + FA handling	
	men + women	WAT (subcutaneous + abdominal)	hAd1	*AFF3*, *WDPCP*	organization of cell-substrate junction + MAPK/Rap1/EGFR signaling	Emont et al.[121]
			hAd2	*NAV2*, *FABP4*	immune response to infection + autophagy	
			hAd3	*SAA1*, *CAV2*	triglyceride biosynthesis + retinol metabolism	
			hAd4	*GRIA4*, *ANKRD30A*	leptin signaling + lipid handling + synaptic control	
			hAd5	*ATP1B3*, *PGAP1*	insulin signaling + lipolysis regulation + HIF1a signaling	
			hAd6	*EBF2*, *ZNF804A*	lipolysis + thermogenic signaling + axon guidance	
			hAd7	*THSD7B*, *AGMO*	hormonal secretion + Ca^{2+} ion handling	

Distinguishing marker genes and pathways enriched in adipocyte subpopulations that have been identified in different depots of white adipose tissue using sc/snRNA-seq studies across mice and humans. LGA, lipogenic adipocyte; LSA, lipid-scavenging adipocyte; SLSA, stressed lipid-scavenging adipocyte.[123] mAd1–6 are the mouse adipocyte subpopulations and hAd1–7 are the human adipocyte subpopulations defined in Emont

et al.[121] Adipo^{LEP}, Adipo^{PLIN}, and Adipo^{SAA} subpopulations were defined by their expression of high levels of *LEP*, *PLIN1/4*, and *SAA1/2*, respectively.[150]

[a]The subpopulations that seem to have similar markers and gene programs identified across different studies can be grouped as LGA, mAd1, Adipo^{PLIN}, hAD4; LSA, mAd3/4, Adipo^{SAA}, hAd3; SLSA, mAd5/6, hAd2/5; mAd2, hAd1/7.

Table 2.

Adipose stromal subpopulations identified by single-cell transcriptomics

Subtypes	Depot	Published nomenclature	Signature transcripts	Functional properties	Human correlate (blank if unknown)	Published isolation strategies (CD45− CD31− = Lin−)	Tissue localization
Preadipocytes (proposed nomenclature)	iWAT	• P2[167] • Group2/committed preadipocytes/ICAM1+ cells[160] • ASC1[164] • DPP4− PDGFRβ+ cells[23] • CD81^{High} APCs[168] • IS3[166]	*Pparg* *Icam1* *Pref1* *Col4a2*	committed preadipocytes	yes	Lin− ICAM1+ CD142− (Merrick et al.[160]) Lin− PDGFRβ+ DPP4−(Shao et al.[162])	perivascular
	eWAT	• APCs[74] • ASC1[161] • VmSC4/5[165] • FAP1/2[123] • ES2/3[166] • mASPC1/5[121]	*Pparg* *Cd36* *Fabp4* *Cd34^{Lo}*	• committed preadipocytes	yes	Lin− PDGFRβ+ LY6C− CD9− (Hepler et al.[163])	perivascular
FAPs (proposed nomenclature)	iWAT	• P1[167] • Group1/interstitial progenitor cells/DPP4+ cells[160] • ASC2[164] • DPP4+ PDGFRβ+ cells[23] • CD81^{Low} cell[168] • IS1[166]	*Dpp4* *Wnt2* *Anxa3* *Pi16* *Cd55*	• multi-mesenchymal lineage potential *in vitro* • adipogenic *in vivo*; give rise to preadipocytes • cold responsive; secrete IL-33	yes	Lin− DPP4+ CD142− (Merrick et al.[160]) Lin− PDGFRβ+ DPP4+ (Shao et al.[162]) Lin− SCA1+ CD55+ (Schwalie et al.[167])	reticular interstitium proximity to blood vessels
	eWAT	• FIPs[74] • ASC2[161] • VmSC1/2/3[165] • FAP3/4[123] • CD34^{High} cells[169] • ES1[166] • mASPC2/3[121]	*Dpp4* *Ly6c* *Fn1* *Pi16* *Il33* *CD55* *Cd34^{High}*	• resistant to adipogenesis (adulthood) • anti-adipogenic (adulthood) • robust proinflammatory response; promote macrophage accrual • robust collagen deposition • secrete IL-33; promote Treg accrual	yes	Lin− PDGFRβ+ LY6C+ (Hepler et al.[163])	within tissue mesothelium proximity to blood vessels
Additional stromal subpopulations	iWAT	• Aregs[160] • Group3[160]	*Cd142, Clec11a* *Fmo2*	• secrete RSPO2, inhibit preadipocyte commitment (adulthood)	yes	Aregs: Lin− SCA1+ CD142++ (Dong et al.[170])	–
		ARCs[171]	*CD36, Lgals3* *Ccl6*	• secrete CCL6; inhibit AP differentiation and proliferation	yes	Lin− CD36+ Lgals3+	–
Brown adipocyte precursors	Interscapular BAT	ASC1[172]	*Col5a3* *Cxcl14* *Bmper*	• ASC1: upregulation of proliferation and adipogenesis gene profiles upon cold exposure	–		mixed location: tissue fascia; interstitial; surrounding vessels;

Subtypes	Depot	Published nomenclature	Signature transcripts	Functional properties	Human correlate (blank if unknown)	Published isolation strategies (CD45− CD31− = Lin−)	Tissue localization
							contact F4/80+ immune cells
		ASC2[172]	*DPP4* *Pi16*				
		ASC3[172]	*Clec11a* *Gdf10*				
		Trpv1+ VSM-APCs[173]	*Trpv1* *Sca1−* *Cd81−*	• proliferate upon cold exposure • give rise to brown and white adipocytes	yes	Trpv1^{Cre} reporter mice	vascular smooth muscle cells
	perivascular BAT (PVAT)	intermediate cells[174]	*Vipr2* *Gli1*	• resistant to adipogenesis	–	PDGFRa+ CD200+	in between the vascular SMCs and adventitial progenitors
		SMC-2 (adult)[174]	*Pparg* *Trpv1* *Myh11*	• adipogenesis	yes	MCAM+ CD200−	aortic adventitia/ vascular smooth muscle cells
Skeletal bone marrow APCs	bone marrow adipose tissue	MALPs[175]	*Adipoq* *Pparg* *Vegfa*	• committed preadipocytes lacking *Plin1* expression and lipid droplets • expressed RANKL and promotes osteoclastogenesis	–	–	bone marrow pericytes
		EMPs[175]	*Ly6a* *Cd34* *Thy1* *Mfap5* *Clec3b*	• number reduces with age • prefer adipogenesis to osteogenesis with age	–	–	–

Single-cell/nuclei RNA sequencing identified adipose stromal cell populations in WAT and BAT depots. Proposed nomenclature, published nomenclature, defining transcripts, proposed presence in human adipose tissue, experimentally validated isolation strategy, and tissue localization are indicated.

Table 3.

Macrophage subpopulations in adipose tissue

Species	Gender	Depot	Subpopulation[a]	Markers	Pathways/polarization	Obesity correlation (−negative/ +positive)	Reference
Mouse (C57BL/6J)	males	WAT (epididymal)	CD11b+ Ly6c+	*Ly6c, Cd74, Dab2, Mgl2*	vascular development and organization	++	Xue et al.[200]
			Ly6c− CD9− CD206+	*CD206*	perivascular-like	−	
			Ly6c− CD9+	*Cd9, Lpl, Plin2, Lamp2*	proinflammatory pathway + lysosomal pathways + lipid handling	++	
			Mac1	*Cd163, Lyve1, Cd209f*	perivascular	−	Jaitin et al.[201]
			Mac2	*Cd9, Nceh1*	not reported	+	
			Mac3	*Cd9, Spp1, Trem2, Lipa, Lpl*	lipid-associated	++	
			LAM	*Trem2, Cd9, Lpl*	phagocytosis + lipid handling+ chemokine signaling	++	Sárvári et al.[123]
			PVM	*Lyve1, Cd163*	statin pathway + EGFR signaling + oxidative stress and redox	−	
			NPVM	*Cd74, Fcrls, Ear5*	phagocytosis + chemokine signaling + PPAR signaling	−	
			CEM	*Col5a2, Tgfbr3, Col3a1*	focal adhesion + PI3K-Akt-mTOR signaling	+ trend	
			P-LAM	*Pola1, Kif11, Kif15*	proliferative pathways + statin pathway	+	
			RM	*Prg4, Tgfb2, Ltbp1*	eicosanoid synthesis + chemokine signaling	no correlation	
			TRM	*Klf4, Cbr2*, and *Stab1, Selenop*	perivascular-like	−	Cottam et al.[61]
			LAM	*Trem2, Cd9, Lpl*	lipid handling	+	
			CM	*Stmn1, Pclaf*	cell cycling	+	
		BAT (intrascapular)	matrix macrophages	*Ecm1, MMP12, MMP19, Fn1*	tissue remodeling	not reported	Silva et al.[208]
			macrophages M2-like	*Mrc1, Clec10a, C1qa, C1qb*	M2-polarized		
			macrophages Lpl^{hi}	*CD36, Lpl, Lipa*	lipid-associated		
			macrophages $Plin2^{hi}$	Fabp4, Trem2, Plin2	lipid-associated		

Species	Gender	Depot	Subpopulation[a]	Markers	Pathways/polarization	Obesity correlation (−negative/ +positive)	Reference
			IS2	*Cd9, Cd68, Lpl, Lipa, Cd36*	lipid metabolism	+	Li et al.[207]
		WAT (inguinal + perigonadal)	IS3	*Cxcl2, Cxcl3, CCL3*	proinflammatory macrophages	not reported	
			IS9	*FOLR2, Klf4*	M2-like	not reported	
	males + females		mMac1	*Fgf13*	not reported	++ in epididymal	Emont et al.[121]
			mMac2	*Plekhg5*		− in epididymal	
			mMac3	*Trem2*		+ in epididymal	
			mMac4	*Prg4*		− in epididymal and periovarian	
Human	men + women	WAT (subcutaneous + abdominal)	Macrophage (M1-like)	*CD68, TREM2*	not reported	not reported	Bäckdahl et al.[150]
			Macrophage (M2-like)	*FOLR2, CD163*			
			hMac1	*PLEKHG5*	not reported	no correlation	Emont et al.[121]
			hMac2	*TREM2*		no correlation	
			hMac3	*PROS1, CLEC10A*		+	
	women	WAT (subcutaneous)	LAM	*TREM2, CD9, LPL*	lipid-associated	+	Weinstock et al.[204]
			PVM	*LYVE1, SELENOP, C1Q*	perivascular	−	
			IM	*CCL3L1, TNF, CXCL3*	inflammatory	++	

Adipose tissue macrophage (ATM) subpopulations in mouse and human WAT and BAT depots. Proposed nomenclature and expression of specific marker genes, enriched pathways, and/or polarization are indicated. Association with obesity, wherever reported, is depicted as + (positive correlation), ++ (strong positive correlation), – (negative correlation), or ‘‘no correlation.’’ Mac1–3, mMac1–4 (mouse), and hMac1–3 (human) are macrophage subpopulations identified in Emont et al.[121] IS2/3/9 are immune subpopulations defined as macrophages in Vijay et al.[207] LAM, lipid-associated macrophage; PVM, perivascular macrophage[123]; NPVM, non-perivascular macrophage; CM, collagen-expressing macrophage; P-LAM, proliferating LAM; RM, regulatory macrophage[123]; TRM, tissue resident macrophage; CM, cycling macrophage[61]; IM, inflammatory macrophage.[206]

[a]The subpopulations that seem to have similar markers and gene programs identified across different studies can be grouped as CD11b+ Ly6c+, Ly6c-CD9-CD206+, Mac1, PVM, TRM, macrophages M2-like, macrophage(M2-like), hMac3; Ly6c− CD9+, mac3, LAM, P-LAM, macrophages Lpl^{hi}, macrophages $Plin2^{hi}$, IS2, mMac3, macrophage(M1-like), hMac2; CEM, matrix macrophages; IS3, IM.

Table 4.

Other myeloid cell subpopulations in adipose tissue

Myeloid cell type	Species	Gender	Depot	Subpopulation	Markers	Obesity correlation (−negative/ +posotive)	Reference
Monocytes	mouse (C57BL/6J)	males	WAT (epididymal)	Mon1	*Fn1, Retnla*	–	Jaitin et al.[201]
				Mon2	*Plac8, Clec4e*	no correlation	
				classical	*Ccr2, Ly6c2*	no correlation	Cottam et al.[61]
				non-classical	*Ear2, Cx3cr1*	no correlation	
			BAT (intrascapular)	monocytes Ly6c^{low}	*Treml4*	not reported	Silva et al.[208]
				monocytes Ly6c^{int}	*Plac8*	not reported	
				monocytes Ly6c^{hi}	*Ly6c2*	not reported	
	human	women	WAT (subcutaneous)	ncMos	*FCGR3A, HES4*	–	Weinstock et al.[204]
				Mo-1	*FCER1A*	+	
				Mo-2	*CSF3R, FCAR, SELL*	+	
Dendritic cells	mouse (C57BL/6J)	males	WAT (epididymal)	cDC1	*Clec9a, Xcr1*	+	Cottam et al.[61]
				activated cDC1	*Clec9a, Xcr2, Ccr7*	+	
				cycling cDC1	*Clec9a, Xcr1, Pclaf, Stmn 1*	no correlation	
				cDC2	*Sirpa, Cd209*	−	
				activated cDC2	*Sirpa, Cd210, Ccr7*	+	
				cycling cDC2	*Sirpa, Cd209, Pclaf, Stmn 1*	no correlation	
				moDCs	*Ear2*	−	
	human	women	WAT (subcutaneous)	cDC1	*IRF8, DPP4, CADM1, XCR1*	+	Weinstock et al.[204]
				cDC2A	*CD1C, IRF4, IL7R, LAMP3*	+	
				cDC2B	*CD1C, IRF4, FCER1A, CLEC10A*	+	

Subpopulations of adipose tissue monocytes and dendritic cells across white and brown adipose depots in mice and humans, defined by the expression of specific marker genes. Their association with obesity, wherever reported, is depicted as + (positive correlation), − (negative correlation), −− (strong negative correlation), or ‘‘no correlation.’’ Mon1/2 and Mo1/2, monocyte subpopulations; ncMos, non-canonical monocytes; cDCs, conventional dendritic cells.[201]