



Published in final edited form as:

Nat Metab. 2025 May ; 7(5): 875–894. doi:10.1038/s42255-025-01296-9.

Towards a Consensus Atlas of Human and Mouse Adipose Tissue at Single-Cell Resolution

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Abstract

Adipose tissue (AT) is a complex connective tissue with a high relative proportion of adipocytes, which are specialized cells with the ability to store lipids in large droplets. AT is found in multiple discrete depots throughout the body, where it serves as the primary repository for excess calories. In addition, AT plays an important role in functions as diverse as insulation, immunity, and regulation of metabolic homeostasis. The Human Cell Atlas (HCA) Adipose Bionetwork was established to support the generation of single-cell atlases of human ATs as well as the development of unified approaches and consensus for cell annotation. Here, we provide a first roadmap from this bionetwork, including our suggested cell annotations for humans and mice, with the aim of describing the state of the field and providing guidelines for the production, analysis, interpretation, and presentation of AT single-cell data.

HCA is a global consortium of researchers working to develop a catalog of all human cell types throughout development (<https://www.humancellatlas.org/>). The HCA comprises a series of bionetworks focused on specific tissues, organs or developmental pathways. These bionetworks are responsible for identifying and addressing critical issues that face researchers in that area, and for helping the HCA generate reference atlases of the particular tissue, organ or pathway. Towards that end, the HCA Adipose Bionetwork will be involved in generating the Adipose Atlas 1.0, which will include (a) the integration of multiple single-nucleus RNA-seq (snRNA-seq) datasets from different healthy human adipose tissue (AT) depots; and (b) the development of unified approaches and consensus on cell annotation. To support this work the bionetwork held a series of virtual meetings open to all researchers in the field aiming to agree on guidelines in four areas of importance for single-cell analyses

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Conflicts of Interest:

MB received honoraria as a consultant and speaker from Amgen, AstraZeneca, Bayer, Boehringer Ingelheim, Daiichi-Sankyo, Lilly, Novo Nordisk, Novartis, and Sanofi.

of ATs, i.e., nomenclature for the anatomical localization of adipose depots, nomenclature for cell annotation, and experimental and computational best practices. In this review, we provide a roadmap of AT at the single-cell resolution based on these meetings.

Adipose Tissue: A Multifocal, Heterogenous, and Dynamic Organ

Once thought to be an inert, cellularly bland storage reservoir for energy in the form of triacylglycerol, AT is now recognized to be a multifocal, complex, heterogenous, and dynamic organ that controls metabolic homeostasis via both endocrine and paracrine mechanisms. Unlike most organs, AT is *multifocal*, found both in discrete depots as well as scattered throughout other organs and tissues. AT is *complex* because it contains a multitude of cell types and subtypes, including adipocytes, fibroblastic stromal cells (many of which represent adipocyte progenitor cells), immune cells, Schwann cells, and vascular cells. AT is *heterogeneous* because its cellular composition varies significantly between and within depots. AT is *dynamic* because its composition changes during development^{1–3} and with perturbation, such as after changes in nutrition, physical activity, or ambient temperature^{4–6}. The transcriptional plasticity of AT has been studied using various methods, including bulk RNA-sequencing and quantitative PCR of whole tissue. More recently, single-cell transcriptome profiling has enabled the cellular and molecular plasticity of AT to be resolved at a single-cell level⁷. Defining “normal AT” in terms of its component cellular subtypes and linking them to function is a critical unmet milestone toward a complete understanding of metabolic homeostasis and disease.

Dysfunctional AT is associated with a variety of cardiometabolic diseases. A clear example is obesity, a condition in which excess adiposity significantly increases the risk of type 2 diabetes (T2D), metabolic dysfunction-associated steatotic liver disease (MASLD), and cardiovascular disease. But AT is not only associated with disease when in excess; lack of AT, either generally or limited to specific regions, also confers risk for severe T2D, MASLD, and atherosclerosis. Individuals with either inherited or acquired forms of lipodystrophy store excess lipids in non-adipose organs such as liver, muscle, and pancreatic islets, contributing to insulin resistance and reduced insulin secretion⁸. Many gene expression changes in whole AT have been associated with cardiometabolic disease, but a true understanding of this relationship will depend on the ability to determine molecular and cellular changes at the level of single cells. For example, some subpopulations of macrophages are more likely to promote insulin resistance than others⁹ and certain adipocyte subtypes are more tightly associated with T2D than others^{5,10}.

Anatomical Location of Adipose Depots

Most AT is found in discrete depots in stereotypical locations throughout the body, though scattered adipocytes can be found in various tissues and organs. Since the cellular composition, molecular attributes, and associations with disease of AT vary depending on the depot, it is critically important that researchers share a common language for describing where their samples originate. Broadly speaking, AT is divided into two major groups: subcutaneous and visceral, which have different associations with cardiometabolic diseases^{11,12}. Excess visceral fat (stereotypically seen in men, thus the older term ‘android’ obesity)

is associated with a relatively high risk of T2D, insulin resistance, and atherosclerosis ¹³. Conversely, excess subcutaneous fat, especially in the gluteofemoral region (stereotypically seen in women, thus the older term ‘gynoid’ obesity), is associated with a relatively lower risk of metabolic dysfunction ^{11,14}. This simple dichotomy obscures several critical issues: (1) most obese patients exhibit a mixed pattern of visceral and subcutaneous adiposity; (2) there are many depots within the broad category of visceral or subcutaneous AT, each possessing distinct compositions and functions, and (3) there are many other depots of biological interest that become enlarged in obesity and that cannot be categorized within the duality of visceral vs. subcutaneous AT.

Another distinction can be made between white AT (WAT) and brown AT (BAT). The latter contains brown adipocytes that express high levels of genes for mitochondrial biogenesis, oxidative phosphorylation, and heat production (thermogenesis) via futile cycling. The best studied thermogenic pathway involves uncoupling protein 1 (UCP1), futile calcium and creatine cycling can also generate heat ^{15,16}. Thermogenic adipocytes can also be found in white fat depots and are variously referred to as beige ^{17,18} or BRITE ¹⁹ adipocytes; these cells are also rich in mitochondria and express *UCP1*. In rodents and other small mammals, brown adipocytes are found in a cluster of small, dedicated BAT depots in the interscapular area, while beige adipocytes emerge in white AT depots in response to specific stimuli, such as cold exposure. Brown and beige adipocytes have different developmental origins and express overlapping but distinct sets of marker genes ^{20,21}. In humans, BAT was thought to exist only in the newborn period, but recent studies show that it can also be found in adults in the deep cervical, supraclavicular, paravertebral and perirenal regions ^{22–26}.

There have been few systematic efforts to catalog and characterize the different adipose depots in any species ^{27,28}. In part, the diffuse nature of some adipocytes in different tissues and organs may make a truly comprehensive list of depots, difficult to compile. This challenge is compounded by the fact that there is generally little correlation between depots across species. For example, the depot most often used by adipose researchers to represent visceral fat in mice is the perigonadal depot (typically the epididymal fat of the male mouse), which has no precise analog in humans ²⁹. Conversely, one of the most important visceral depots in humans, the omental fat, is rudimentary in mice. This issue goes beyond mice and men. For example, the hump of the camel is mainly composed of AT and represents one of the largest depots in that animal, yet it is absent in nearly all other mammalian species. Despite these constraints, here, we present a compendium of generally accepted adipose depots in humans and mice (Figure 1 and Table 1).

Human Adipose Depots

Subcutaneous Adipose Depots

Most studies have not drawn major distinctions between subcutaneous AT (SAT) at different locations; however, it is becoming increasingly clear that there are substantial regional differences between SAT depots. For example, abdominal SAT has different attributes and confers a different metabolic risk profile than gluteofemoral SAT ³⁰. Such differences may be explained by larger, yet more insulin sensitive adipocytes in the thigh ³¹, higher retention of lipids in gluteofemoral adipocytes ¹⁴, differences in cell composition between

abdominal and gluteofemoral depots³², and/or variations in the differentiation capacity of their progenitors³³.

Furthermore, both truncal and gluteofemoral SAT are separated into two planes, called deep and superficial, by a discrete subcutaneous fascia (termed Scarpa's fascia in the abdominal region)³⁴. This fascial plane is discontinuous, and in some parts of the body, the deep SAT can dominate. Deep and superficial truncal SAT exhibit different microanatomy and may have distinct metabolic functions and disease associations^{35–37}.

Craniofacial fat provides another example of the differences found within the broad category of SAT. Not only does craniofacial fat exhibit different metabolic characteristics than SAT found elsewhere in the body, but there are actually several compartments within the category of craniofacial AT (*e.g.*, buccal AT), each of which may have its own molecular and functional characteristics³⁸. We recommend that all studies (single-cell or otherwise) using SAT precisely define the site and depth from which it was collected.

Dermal fat has also been proposed as a separate depot. In mice, a thin muscular layer called the panniculus carnosus separates the dermal AT from SAT, but humans have only vestigial remnants of this layer in scattered areas³⁹. It is therefore unclear whether the subcutaneous and dermal AT represent distinct depots in humans. However, small islands of adipocytes can be found in the human dermis, especially around hair follicles^{40,41}.

Visceral Adipose Depots

Mesenteric and Omental. The mesenteric and omental depots together comprise most of the human visceral fat. The mesentery is a membranous fold that extends from the posterior peritoneum and suspends the loops of the large and small bowel within the peritoneal cavity. The fat that accumulates within the mesentery (mesenteric AT; MAT), also contains lymphoid tissue in the form of fat-associated lymphoid clusters (FALCs), which are milky, spot-like structures that grossly resemble fat. In inflammatory bowel disease, MAT can infiltrate the intestinal wall, a phenomenon called “creeping fat”, which is closely associated with disease pathogenesis⁴². MAT is sometimes named by the specific intestinal segment that it connects (*e.g.*, mesocolic, mesosigmoid, and mesorectal fat), and including this information in the reporting metadata would be of significant value given the recognized heterogeneity of MAT adjacent to specific intestinal segments⁴³. The omentum is a large, apron-like fold from the visceral peritoneum that extends downward from the stomach. Similar to MAT, omental AT (OAT) contains milky spots, which are lymphoid aggregates analogous to FALCs⁴⁴ and a rich vasculature. As the dominant sites of fat storage in obesity, MAT and OAT mass are highly correlated with adverse cardiometabolic outcomes.

Perirenal and Retroperitoneal. Perirenal AT (PRAT), which covers the kidneys and adrenal glands, is in the retroperitoneal space; as such, PRAT is not technically “visceral”, although it is commonly viewed as VAT. PRAT lies between the renal capsule and renal sinus, while the AT within the renal sinus itself is considered perivascular (see below), given its proximity to the renal vasculature. Like other VAT depots, PRAT is associated with obesity and cardiovascular disease. Interestingly, perirenal fat contains numerous

thermogenic brown or beige adipocytes^{45,46}. Retroperitoneal fat describes AT in the retroperitoneal space that is not within the anatomical limits described for perirenal fat.

Epiploic. Epiploic fat, also known as the appendices epiploicae, consists of two rows of 50–100 lobules attached to the outside of the colon. This rarely studied depot has recently been shown to have a unique mRNA and protein expression profile relative to other visceral depots⁴⁷.

Periprostatic. As the name implies, periprostatic AT surrounds the prostate gland, and is a critical part of the tumor microenvironment in prostate cancer⁴⁸.

Pericardial and epicardial. There has been some confusion regarding the depots around the heart. The AT inside the pericardial sac and adherent to the myocardium should be called epicardial AT, while the term pericardial AT should be reserved for fat that is exterior to the pericardium (Figure 1A). One sometimes finds the term “paracardial” AT in the literature – this term has no clear meaning and should be abandoned. Epicardial fat, which can make up 20% of gross cardiac weight, increases with obesity, and is associated with coronary artery disease and atrial fibrillation in humans and non-human primates^{49,50}.

Mediastinal. Roughly 70% of intrathoracic fat in humans is not found in the pericardial or epicardial depots – this fat is called mediastinal. Mediastinal fat has some brown-like characteristics⁵¹. Some mediastinal fat is associated with the thymus, especially as it undergoes involution with aging; adipocytes become a dominant component of the thymus by middle age⁵². Although the origin and function of these adipocytes remain incompletely understood, it has been suggested that thymic fat accumulation may contribute to age-related decline in adaptive immune function.

Other Adipose Depots

There are also adipose tissue depots located outside the primary subcutaneous and visceral regions, each with distinct anatomical locations and metabolic roles.

Bone marrow. AT occupies up to 70% of the marrow cavity, mostly in the so-called “yellow marrow”, although there are adipocytes in the red marrow as well. Bone marrow AT (BMAT) has several unique features, not least of which is its paradoxical expansion in the face of severe calorie restriction and weight loss^{53,54}.

Epineural. Some of the larger nerves have fat depots that adhere to the outer nerve sheath, or epineurium. Epineural fat mass can increase after nerve injury, and leptin from adipocytes enhances neuronal regeneration and recovery⁵⁵.

Mammary. AT makes up approximately 90% of the mass of the breast in nonlactating women. Adipocytes in the breast play an important role in development of the ductal epithelium and contribute to tumorigenesis and metastasis of breast cancer⁵⁶.

Perithyroidal. There is AT adherent to the surface of the thyroid gland, which can be a site of local invasion in some cases of thyroid carcinoma⁵⁷.

Perivascular. As the name implies, perivascular AT (PVAT) encases many blood vessels, especially arteries. By consensus, vascular biologists have agreed that PVAT extends to a distance of one luminal width at the site under examination, or within 2 cm of any vessel >2 cm in diameter⁵⁸. PVAT has been shown to have robust interactions with the encased vessel and may be involved in such conditions as hypertension and atherosclerosis⁵⁸.

Retroorbital. AT provides a cushion for the eye in its socket. In some people, the adipose stromal and progenitor cells (ASPCs) within this retroorbital (sometimes called periorbital) fat express antigens that make the depot expand in the setting of Graves' disease, causing ophthalmopathy⁵⁹.

Intraarticular. Fat also provides an important cushioning function within joints. A recent mixed single-cell/single-nucleus RNA-seq (sc/sn RNA-seq) study of the intraarticular fat of the knee (i.e., the infrapatellar fat pad) demonstrated a developmental relationship between adipocytes here and the synovial lining, and suggested paracrine interactions that may promote osteoarthritis⁶⁰.

Inter/intra-muscular. AT that is located under the deep fascia and between major muscle bundles is called intermuscular AT, or IMAT⁶¹. Some investigators make a distinction between IMAT and fat located within or between muscle fibers (sometimes called intramuscular AT, or intraMAT)⁶², although a functional, molecular, or developmental basis for this distinction is not entirely clear. As one might expect, most of the literature on IMAT emerges from the livestock industry, as a measure of meat quality⁶³. Nonetheless, in humans there are clear associations between IMAT mass, obesity, and insulin resistance⁶¹. Importantly, lipid deposition *within* myocytes (intramyocellular triacylglycerol, or IMTG) is distinct from AT.

Structural fat of heel/fingers. Some parts of the body use adipocytes as a mechanical cushion, such as the heel and the tips of the fingers and toes. This fat is encased in tough septated connective tissue and is relatively inert metabolically⁶⁴.

Brown (and Beige) Adipose Depots

Unlike rodents, most adult humans at room temperature have little constitutively active thermogenic AT. In general, women have more thermogenic AT than men, and the amount diminishes with age⁶⁵. Many people respond to cold exposure by increasing the amount of ¹⁸F-FDG-PET positive thermogenic AT^{22–25}. There are also substantial interindividual differences in BAT volume that may in part be genetic in origin. Interestingly, there is a clear association between the amount of brown and beige fat (as determined by scanning) and metabolic health, with reduced rates of T2D, dyslipidemia, coronary artery disease, cerebrovascular disease, congestive heart failure and hypertension observed in subjects with greater ¹⁸F-FDG uptake⁶⁵. Depots with the highest ¹⁸F-FDG uptake are found in the cervical, supraclavicular, axillary, intercostal, mediastinal, ventral spinal, and perirenal areas^(27; see Table 1). In addition to the depots defined in adults, infants have classical brown AT in the interscapular depot and around the heart and great vessels⁶⁶, but much of this is lost during childhood. There is ongoing debate about whether the other human thermogenic

depots represent brown, beige, some other type of adipocyte, or a mixed population. Marker studies have been contradictory and/or inconclusive, highlighting the complexity of human thermogenic fat and the need for clarity and consensus around nomenclature, cellular annotation, and experimental approaches ^{20,21,67–69}.

Mouse Adipose Depots

There are many similarities between mouse and human AT, but also profound differences. Some of the most obvious differences relate to the specific depots, as highlighted below, but there are also important molecular and functional differences between mouse and human adipocytes. Table 1 lists the murine adipose depots that have been described to date.

Subcutaneous Adipose Depots

Mouse SAT exists in more anatomically defined locations than human SAT. In mice, the major accessible subcutaneous depot is the inguinal fat pad, and virtually all studies that look at mouse SAT focus on this depot. This inguinal fat pad contains a large lymph node which demarcates two distinct lobes, denoted the dorsolumbar and inguinal lobes ⁷⁰. In addition to its distinct structure, the inguinal fat has two features that mark it as significantly different from human SAT. First, the inguinal depot is a major site of thermogenesis in cold-exposed mice, as it houses a large number of inducible beige adipocytes. Importantly, these beige cells are not distributed evenly throughout the depot ⁷⁰. Second, the inguinal depot also contains a significant portion of the mammary ducts, which cannot be easily separated physically from the rest of the AT. Other white subcutaneous depots in the mouse are found in the anterior axillary region, the triceps area, the popliteal fossa, and encasing and infiltrating the interscapular brown depot (see below) ^{28,71}.

Mice have a thin muscular layer below the dermis called the panniculus carnosus; adipocytes located between the panniculus carnosus, and the dermis are called dermal adipocytes (Figure 1B). By contrast, subcutaneous fat lies below the panniculus carnosus. Murine dermal adipocytes have a different developmental origin than subcutaneous fat, and have been implicated in hair growth and cycling, wound healing and antibacterial defense ^{40,72}.

Visceral Adipose Depots

Murine visceral depots also differ significantly from their human counterparts. Mice have a rudimentary omentum and store relatively little fat in their mesenteric depot. Instead, the perigonadal depots (epididymal in males and periovarian in females) are the major sites of intraperitoneal fat storage. Recently, new fat depots affiliated with the perigonadal fat were described around in mice, which was referred to as lymph node-cervical adipose tissue (LNCAT) in female mice and lymph node-prostatic adipose tissue (LNPAT) in male mice ⁷³. Finally, mice have only a small amount of epicardial fat ⁷⁴, which becomes more evident in obese animals.

Other Adipose Depots

Similar to humans, mice also have BMAT, perirenal, retroperitoneal, perivascular, intraarticular, and intra/inter-muscular AT (Figure 1B).

Brown (and Beige) Adipose Depots

Mice have distinct brown adipose depots that maintain their identity regardless of ambient temperature⁷⁵; the best studied of these is the interscapular brown fat, but there are small cervical and axillary depots that share this trait. In addition, almost all “white” depots of the mouse can undergo browning to some degree in the face of a cold challenge. This is most often studied in the inguinal SAT, but most other depots have at least some abilities to remodel to a more thermogenic state²⁸. Accordingly, it is imperative that studies looking at AT composition in mice be rigorous about reporting ambient temperature.

Definition and Annotation of Adipose Cell Types

The rise of single-cell sequencing technologies has revealed a much greater cellular heterogeneity of most tissues, including ATs, than previously appreciated. This has led to confusion regarding the precise definition and annotation of distinct cell types, subtypes and cell states. The concept of ‘*cell type*’ refers to a distinct category of cells that share fundamental characteristics, functions, and lineage. In single-cell data, a cell type is recognized as a distinct cluster of cells sharing overall marker gene expression and transcriptional networks. A cell type may be composed of many different cellular ‘*subtypes*’, which are specific populations within a cell type having specific characteristics and known or inferred functions. In single-cell data, cellular subtypes can be identified as distinct subclusters of a cell type. Finally, a *cell state* is the specific physiological or functional condition of a cell at a given moment. The condition can be dynamic and change in response to environmental cues, developmental signals, or metabolic states. In single-cell data, cell states will often appear as less distinct subclusters of a cell (sub)type. The context-dependent and dynamic nature of cell states makes them more difficult to classify than cell types. Moreover, as they often occur as a continuum of states, their classification is highly sensitive to the choice of computational strategy and the resolution imposed. For now, we propose focusing on the annotation of adipose cell types and subtypes in human and mouse tissues based on transcriptomic markers (Tables 2 and 3), acknowledging the inherent limitations of this approach. For example, since marker genes are rarely unique to one cellular subtype, accurate annotation of closely related cellular subtypes, often requires a combination of multiple marker genes. Moreover, while subtype markers are useful for distinguishing between subtypes within a specific cell type, they may not be effective in differentiating these subtypes from other cell types present in the tissue. Therefore, the use of subtype markers is often limited to identifying subtypes within one or a few specific cell types, rather than across the entire tissue. Importantly, proper annotation of cellular subtypes and states will require inclusion of other modalities such as proteomics, metabolomics, and eventually isolation and functional characterization. Moreover, for cell (sub)types that are also abundant in other tissues, such as vascular cells and immune cells, nomenclature will eventually have to be aligned between tissues, as clarity with respect to functional characteristics is reached.

Here we provide the nomenclature and characteristics of the adipose cell types and subtypes agreed upon in the HCA Adipose Bionetwork.

Adipocytes

Adipocytes are the parenchymal cells of the AT. They are large, lipid-filled cells with the primary function of storing excess calories in the form of triacylglycerol. Furthermore, adipocytes act as endocrine cells that communicate systemically to inform other tissues about the state of their lipid stores, and regulate a large number of physiological functions, including appetite, glucose homeostasis, coagulation, immune activity, and blood pressure ⁷⁶. The large size and fragile nature of adipocytes mean that they are not amenable to conventional flow-sorting and microfluidic devices. Thus, single-cell analyses of AT rely largely on single-nucleus RNA-seq (snRNA-seq) techniques ⁷ (Figure 2).

Adipocytes are easily identifiable in snRNA-seq data by their expression of adipocyte-specific markers like *ADIPOQ* and *PLIN1*. Brown and beige adipocyte subtypes can be distinguished from white adipocytes by their high and moderate expression of *UCP1*, *CIDEA*, *PPARGC1A*, *PRDM16*, and *CKMT2*, respectively ^{77–79}. Several additional markers of brown and beige adipocytes have been reported; however, some of these have turned out to be positional markers that do not distinguish between white, beige and brown ⁸⁰. In addition to the white, brown, and beige subtypes, several lines of evidence indicate that adipocytes from different depots, in addition to their positional markers, also have slightly different properties and transcriptional profiles, which may justify classification as different subtypes. Moreover, different adipocyte cell states, potentially depending on nutritional status, have been reported in both mice and humans ^{3,5,77,78,81–83}. However, the exact classification and characteristics of these cell states remains obscure and controversial.

Adipose Stromal and Progenitor Cells (ASPC)

The stromal cells of the AT are a heterogeneous group of cells generally distinguished by high expression of *PDGFRA* and *DCN* ^{84,85}. These cells have been called adipose stromal cells (ASCs), adipose-derived stromal (or stem) cells (ADSCs), adipose progenitor cells (APCs), and fibro-adipogenic progenitors (FAPs), among dozens of other designations ^{7,86}. We recommend the term adipose stromal and progenitor cells (ASPCs), as this term captures both the supportive (stromal) and differentiative (progenitor) roles played by these cells.

Several subpopulations of ASPCs have been identified, and though the exact taxonomy of these cells is still being worked out, some consensus has been reached. *DPP4*, for example, marks ASPCs that have the capacity to differentiate into adipocytes, but which are the farthest removed from the mature cell ^{87,88}. ASPCs that express mature adipocyte marker genes, such as *PPARG* and *CD36*, represent late preadipocytes ³. Interestingly, subpopulations of ASPCs with the ability to inhibit *in vitro* adipogenesis, called ‘Aregs’, have been highlighted in some studies ^{89–91}. The relative proportions of different ASPCs may depend on depot, age, body weight, metabolic health, sex and ethnicity. The developmental trajectories of different progenitor cells can be inferred computationally by trajectory analyses ^{3,88}; however, detailed insight into the plasticity and lineage relationships between ASPC subtypes will ultimately require the development of new *in vitro* and *in vivo* models.

Mesothelial Cells

The mesothelium consists of a layer of epithelial cells that lines the visceral cavities and organs. From the perspective of AT, mesothelial cells are only found on intraperitoneal (e.g., omental and mesenteric fat) and intrathoracic (e.g., epicardial) depots. These cells are not *per se* adipogenic^{92,93} and can be distinguished from ASCs by the expression of markers like *MSLN* and *KRT19*⁵. There is conflicting evidence about the role that mesothelial cells play in AT. While the data indicate that murine mesothelial cells cannot become adipocytes, single-cell studies have continued to identify a subset of mesothelial cells that share markers with ASCs and exhibit the potential to transition between the mesothelial and mesenchymal state^{93,94}, suggesting that there is a relationship between these cells that is not yet fully understood.

Immune Cells

The AT immune compartment identified by expression of CD45 (*PTPRC*) has representation from all major immune cell types with markers summarized in Table 2 and 3^{95–99}. Single-cell analyses corroborate experimental studies showing that macrophages, dendritic cells, monocytes, and T lymphocytes are the most abundant immune cells in AT, while B cells, natural killer (NK) cells, mast cells, and innate lymphoid cells (ILCs) are comparatively less represented within the immune cell population. There is a disconnect between experimental evidence of adipose neutrophil and eosinophil populations in AT and single-cell studies that show a paucity of these cell types^{100,101}. This disconnect may be due to known limitations in RNA yields from neutrophils in single-cell experiments^{102,102}. Furthermore, blood contamination in highly vascularized AT biopsies may amplify the proportions of cells such as lymphocytes and monocytes^{103,104}. Distinguishing blood and resident tissue immune cells in AT may be important, since resident AT cells have distinct molecular profiles compared to circulating blood immune cells (e.g., Tregs)^{105,106} although monocytes have been reported to have altered profiles as they transit through AT¹⁰⁷.

The proportions and features of immune cells in AT differ based on body weight, age, sex, and anatomical location. The diversity of AT immune cells and their role in obesity and its complications has been extensively reviewed elsewhere^{9,108–110}. Multiple molecular and functional subtypes exist within each broad immune cell type, and a full catalog of all immune types may require enrichment of certain populations prior to single-cell profiling⁹³. For example, AT macrophages can be categorized into various subpopulations, such as perivascular macrophages (PVMs), characterized by *LYVE1* expression^{107,111}; lipid-associated macrophages (LAMs), marked by *CD9* and *TREM2* expression^{112,113}; and non-perivascular macrophages (non-PVMs), which include sympathetic nerve-associated macrophages (SAMs or NAMs) expressing *MAOA*^{104,114–116}. Importantly, the molecular profiles of LAM and PVM are seen in other tissue/disease contexts,^{117,118} suggesting convergence of signals leading to specific macrophage activation profiles.

Vascular Cells

Vascular cells can be classified into three main types: endothelial cells (ECs), which line the interior of blood vessels; smooth muscle cells (SMCs), which regulate vessel diameter and blood flow; and pericytes, which support and stabilize capillaries and venules.

Blood endothelium: Adipose ECs, like those in other tissues, comprise several functionally and transcriptionally distinct subtypes, which express common markers such as *PECAM1* and *CDH5* in humans and mice^{119,120}. The subtypes include arterial ECs, expressing markers like *GJA5* and *NEBL*; venous ECs, expressing *ACKR1*; and capillary ECs, expressing *RBP7*, *BTNL9*, and *CA4*¹²¹. ECs exhibit remarkable plasticity, responding dynamically to changes in their surrounding environment. Integrative data analysis has revealed several additional potential subpopulations of ECs. Some of these subpopulations exhibit hybrid transcriptional profiles, with gene signatures resembling those of adipose monocytes or macrophages, suggesting a possible immunomodulatory role¹²².

Lymphatic endothelium: Lymphatic vessels, lined by lymphatic endothelial cells (LECs), are integral to maintaining fluid balance and conducting immune surveillance within ATs. LECs are particularly prevalent in VAT compared to SAT or BAT¹²³. These cells are identifiable by their expression of *PROX1* and *LYVE1*. However, it is important to note that *LYVE1* also serves as a marker for specific subsets of macrophages and therefore should not be used by itself to identify LECs.

Pericytes and smooth muscle cells: Pericytes and SMCs, both classified as mural cells, play key roles in forming the structure of blood vessel walls. Pericytes are mainly located around small blood vessels, including capillaries and microvessels, while SMCs are the predominant cells in the walls of arterioles and arteries. While both cell types express *PDGFRB*, SMCs in both humans and mice can be further distinguished by their expression of *MYH11* and *MYOCD*, whereas pericytes selectively express *STEAP4*^{5,122}.

Schwann Cells

Schwann cells are glial cells that coat the axon and essential components of the peripheral nerves that innervate AT. They have been captured in some integrated single-cell atlases of AT in humans and mice and are characterized by markers such as *XKR4* and *MPZ*¹²⁴.

Challenges in Adipose Tissue Collection and Processing

Proper collection and handling of AT samples are critical to preserve cellular or nuclear integrity and minimize experimental artifacts in single-cell analyses. Factors such as exact anatomical location, collection technique, and processing protocol need to be thoroughly considered and accurately documented to ensure the generation of reliable and consistent single-cell data (see Figure 2 and Box 1).

Adipose Tissue Sampling

Several methods exist for the collection of AT, each potentially influencing downstream analyses. Here we briefly summarize our collective recommendation for AT sampling and storage. While these recommendations are informed by current scientific understanding and expert insights of the Adipose Biological Network, some aspects may require further investigation and stronger experimental evidence to achieve optimal sampling and storage practices.

The choice of collection method is usually guided by the location of the depots and should be properly documented (see Box 1), since it can help account for potential confounding effects, when analyzing the data. In humans, SAT biopsies can be acquired using minimally invasive techniques like needle aspiration, while surgical procedures are typically employed to obtain VAT, due to its deep location. Most elective intra-abdominal operations are now performed laparoscopically, which limits the amount of tissue that can be collected (approximately 50 mg). However, larger amounts of PVAT can be collected during open heart surgery. An often-overlooked aspect is the impact of surgical procedures on AT ¹²⁵, where factors like prolonged preoperative fasting, exposure to lower ambient temperatures in the operating room, and the choice of tissue collection methods (e.g., aspiration needle, electrocautery, or scalpel) may influence gene expression in AT. Furthermore, the administration of oral or topical anesthetics may also affect gene expression signatures in AT biopsies. For isolation of mouse AT, the mouse is typically euthanized using inhalational anesthetics (e.g., isoflurane) in combination with cervical dislocation or a lethal dose of injectable anesthetics (e.g., ketamine). Perfusion can be performed to flush out blood from the AT before harvest, which could be particularly useful when studying tissue-resident immune compartments ^{103,104}.

After isolation of AT, it is often necessary to remove excess blood or moisture. However, rinsing the tissue with water or PBS may cause damage during subsequent freezing, as formation of ice crystals can disrupt cell membranes and intracellular structures ¹²⁶. To avoid this, the tissue can be cleaned using clean laboratory wipes. Prompt handling of biopsy material is critical to minimize RNA degradation, and when immediate processing is not possible, the tissue should be frozen down as soon as possible after collection ¹²⁷. The most used method is snap-freezing in liquid nitrogen (LN2); however, for some downstream assays this method can be problematic, since LN2 boils in contact with warm tissues and forms a vapor barrier acting as an insulator. As a result, the inner core of the tissue sample may freeze slowly and unevenly. To improve preservation of cellular structures for downstream assays, one can immerse the tissue in pre-cooled isopentane or freeze down tissue pieces in slow freezing media containing DMSO ¹²⁸. Additionally, sample integrity may be affected by the storage condition and duration. While storage at -80°C may be sufficient for most downstream applications, cryotanks with controlled temperature stability may offer advantages for preservation of nuclear integrity.

Preparation of Adipose Tissue for Single-Cell/Nuclei Sequencing Analyses

AT presents unique challenges for single-cell sequencing technologies, particularly due to the fragile nature of adipocytes. scRNA-seq approaches have been widely used to profile cells originating from the stromal vascular fraction (SVF), such as ASCs, endothelial cells, and immune cells ^{1,32,81,83,88,90,97,98,113,129–133}. However, capturing all cell types in AT requires snRNA-seq ^{3,5,82,132,134–136} (Figure 2). The pros and cons of scRNA-seq versus snRNA-seq for AT studies have been discussed in detail elsewhere ^{7,132,134}, and the method of choice depends heavily on the specific research question. For example, snRNA-seq is necessary to map the transition from preadipocytes to fully mature adipocytes during adipogenesis ^{3,132}.

Single cell analyses: Various methods have been employed for dissociating AT for single-cell analyses, including enzymatic, mechanical, and automated methods^{81,97,127,137–139}. Careful consideration is needed regarding differences in the types and proportions of subpopulations that are recovered across these methods, particularly when integrating and comparing various datasets. Gentle dissociation methods are preferred for maintenance of cellular integrity, whereas harsher techniques may be required for enriching certain cell populations, such as vascular cells, thereby potentially compromising the integrity of other cell types. Several single-cell studies of populations from AT employ fluorescence-activated cell sorting (FACS) to enrich specific subpopulations or rare cell types^{93,98,113,129,130,133,140}. In these studies, it is important to consider the potential introduction of bias in the cellular fractions being analyzed as well as potential effects of FACS on cell integrity and features. In all instances, meticulous quality control measures are required to guarantee optimal cell quality during sample preparation.

Single-nucleus analyses: Nuclei are fragile structures that can be easily damaged during isolation, necessitating careful handling and the use of appropriate methods and buffers to maintain their integrity¹³⁷. Furthermore, the use of low-protein-binding tubes and pipette tips during the nuclei extraction process is strongly recommended to minimize contamination from oil droplets and residual tissue fragments. Importantly, contamination with cytoplasmic components, organelles, and debris from ruptured cells can compromise the purity of the isolated nuclei and impact subsequent applications. Pulverization of the tissue is occasionally preferred to achieve a more homogeneous nuclei preparation by effectively releasing cells embedded in the extracellular matrix (ECM).

However, in recent years, automated systems, such as the gentle MACS Dissociator, have gained popularity for isolating high-quality nuclei from AT¹²⁷. These systems combine mechanical agitation and enzymatic digestion, offering standardized and reproducible dissociation that enhances experimental consistency. Regardless of the approach, customizing dissociation protocols to accommodate structural variations in AT, such as those seen in obesity, might be needed to ensure sufficient yield of high quality and representative nuclei for downstream analyses¹³⁷. A major challenge with snRNA-seq is that the enclosed RNA becomes increasingly susceptible to RNases, resulting in rapid RNA degradation. A recent study, however, showed that using a combination of vanadyl ribonucleoside complex and recombinant RNase inhibitors during nuclei isolation from AT preserves RNA integrity and nuclear structure, resulting in high-quality snRNA-seq data with high UMI counts and minimal ambient RNA contamination¹⁴¹. Another strategy that has been shown to increase the quality of nuclei and reduce ambient RNA load for snRNA-seq analyses is fluorescence-activated nuclear sorting (FANS)¹²⁷. However, sorting leads to a loss of nuclei, which could introduce biases in the analysis of biopsies with limited material.

Computational Issues Related to the Analysis of Adipose Tissue Single-Cell Data

Comprehensive guides on best practices for the computational processing of single-cell data in general are available ^{142,143}. Here we describe the major issues, with emphasis on how they relate to the analysis of AT data.^{142,143}.

As described above, most AT studies use snRNA-seq because of its ability to simultaneously capture information all cells, including mature adipocytes. The use of nuclear data requires stricter QC cutoffs for mitochondrial reads than are commonly used in scRNA-seq, as these should not be present in nuclear preparation ¹⁴⁴. Fairly strict cutoffs for ribosomal reads should also be employed. Additionally, it is important not to discard reads that map to intronic regions, as these can comprise up to 50% of the reads in a snRNA-seq dataset, compared to ~10% in a scRNA-seq experiment ¹⁴⁵. Because the cell membrane is destroyed to isolate nuclei, snRNA-seq data tends to be more susceptible to artifacts from ambient RNA more than scRNA-seq. While this should be addressed experimentally by using extra washes and/or FACS sorting, computational methods can also be employed to clean and adjust ambient reads from datasets ¹⁴².

Doublet removal is a major challenge in analysing AT datasets. Several algorithms that detect ‘within-sample’ doublets are available in both Python and R ¹⁴⁶, and at least one of these should be applied to all datasets. Additionally, if samples are multiplexed, demultiplexing using genetic or hashtag identifiers can also detect sample-to-sample doublets. After an initial round of doublet removal, we recommend annotating cells with the “doublet score” calculated by the detection software and plotting this along with other QC metrics like nUMI and mitochondrial percent; cells with higher doublet scores will often cluster together and can be removed. Users should be mindful that overly stringent doublet removal might eliminate transition states or other bona fide cell types. If a cluster is suspected to represent a novel cell type or state, this can be supported by the presence of unique markers that are not present in either of the cell types that make up the putative ‘doublet’, as well as by staining for markers in tissue sections.

Benchmarking analysis comparing ASPC gene expression in whole cell and single nuclear datasets has shown that similar populations are retrieved using both methods, although there is less RNA diversity in nuclear data ¹⁴⁵. This can affect the accuracy of quantifying transcript abundances and the ability to detect low abundance transcripts. Because there is a higher ratio of unspliced to spliced transcripts in the nucleus compared to the cytoplasm, there is increased length-bias in transcript detection for snRNA-seq leading to e.g. enrichment of long noncoding RNAs in snRNA-seq ¹⁴⁵. Additionally, because of the differences in spliced RNA content in the nucleus versus cytoplasm, certain analytical approaches, such as trajectory and velocity analyses, which rely on the ratio of spliced versus unspliced RNA, may require adaptation.

Most single-cell AT datasets have been obtained using the droplet-based 10X Genomics platform ^{3,5,60,82,97,98,133,136,147} and some initial integration efforts have been made ^{124,134,148}. More recently, however, datasets acquired using SMART-seq technology have

been published, offering full-length and more comprehensive transcript coverage that can serve as a complementary resource for characterizing transcriptional variants of interest associated with AT ¹⁴⁹. However, this comes at the expense of reduced capacity to detect rare cellular subpopulations due to the ~10-fold reduction in numbers of profiled cells compared to 10X technology ^{32,132,135}. It is important to note that the platforms used may limit the computational tools available to clean the data. For example, SMART-seq based methods do not automatically sequence “empty” wells, which are typically required to estimate and remove ambient RNA.

Accurate classification and annotation of cell types in AT are crucial for quality control and downstream analysis. Several automated correlation-based annotation tools, such as Azimuth ¹⁵⁰ and CellTypist ¹⁵¹ are effective for annotating datasets that are not expected to contain novel cell types. These methods are built on existing reference datasets ^{5,134}, which limits their use for benchmarking previously uncharacterized tissues and annotating rare cell types. Similarly, supervised classification-based methods such as CellAssign ¹⁵² and Garnett ¹⁵³ use deep learning to annotate cells, which also requires good quality reference single-cell datasets containing all expected cell types.

However, these tools can be useful for datasets where background noise or batch effects make other classification methods problematic. Most other methods rely on marker gene expression to annotate each cell, which has the advantage of not needing a reference dataset and therefore more easily allowing the discovery of novel cell types. However, the expression of many genes can vary considerably due to the inherent heterogeneity across different samples, potentially leading to variable annotations of the same cell type. This inconsistency in cell type labelling across different datasets can hinder comparative analyses and data integration efforts, underscoring the need for the standardized naming conventions that we are working towards as a network.

One of the goals of single-cell and single nuclear RNA-sequencing experiments is the identification of new cell types or subtypes, and any new cell type identified in a dataset should be rigorously validated, both computationally and experimentally. A common pitfall is the annotation of a doublet population as a novel cell population. Groups should therefore ensure that any observed novel population expresses marker genes that are specific to that population and should validate these marker genes using complementary techniques such as immunohistochemistry, immunocytochemistry, and/or flow cytometry.

Integration of data is an important step in creating a usable dataset and is especially important in the development of an atlas based on data from multiple labs using different protocols. Multiple variables must be taken into consideration when integrating, most notably the platform and method (e.g., nuclear vs. whole cell). Differences in cohort composition, batches, tissue lysis and single-nucleus/cell isolation method, library preparation, sequencing platform, sequencing depth, and RNA diversity in differing methodologies can lead to difficulties in harmonizing data. Various integration methods can be explored to assess their impact on the results ¹⁵⁴; however, when comparing two highly distinct datasets, it may be essential to employ techniques like reference mapping ^{150,155},

which enables the comparison of cells across datasets while preserving the unique analysis of the component datasets.

Because a fully integrated adipose atlas will contain data from multiple depots, there is concern that data integration may inadvertently overcorrect true biological differences between depots. To preserve depot-specific features in an individual study, when multiple depot samples (e.g., SAT and VAT) are available from the same individual, they should be aggregated at the individual level before integrating across different individuals. Efforts should be made to collect data for every depot in at least three labs to help identify the differences between datasets that could be lab- versus depot-driven.

Similarly, data integration for comparisons of variables across individuals can lead to overcorrection of biological differences. One way to address this issue is by combining multiple samples into the same run and computationally demultiplexing using genotype information^{156,157}, or by multiplexing through the use of barcoded antibodies¹²⁷ and using run information as the integrating variable. Pseudobulking, i.e., averaging/aggregating expression profiles across biological replicates^{142,158}, effectively reduces overintegration artifacts but sacrifices information that might be gleaned from intra-sample variation. Another approach is to extend deep learning algorithms often used in the analysis of single-cell transcriptomes¹⁵⁹ to explicitly model donor identity and intra-donor variation¹⁶⁰.

Incorporation of Bulk RNA Sequencing Data

Single-cell/nucleus analyses are still performed on relatively small numbers of samples, while translating the knowledge gained by these approaches to the clinic requires validation in large(r) cohorts. snRNA-seq data can help identify novel putative molecular markers that can then be tested individually using dedicated approaches for validation (PCR of specific cell-type markers, as an example). Complementarily, deconvolution algorithms can be used to deduce cell-type proportions from bulk RNA-seq¹⁶¹, some of which are designed to use gene expression derived from snRNA-seq data to provide reliable estimates of cell-type composition of human AT¹⁶². As with annotation, the success of these techniques relies on a robust adipose reference atlas and a consensus definition of cell types.

Adipose Atlas 1.0

Choosing Datasets

The primary objective of Adipose Atlas 1.0 is to create a comprehensive and inclusive representation of the major AT depots of the human body. Based on availability, we will include high quality data sets from abdominal visceral, abdominal subcutaneous, and superficial and deep neck AT, ensuring representation of both white and brown AT and independent replicates from different laboratories. The atlas will ideally include samples from both sexes across a wide range of age. In addition, we will include diverse ethnic and racial backgrounds, when possible, although currently available datasets are somewhat limited in this regard. This information will be included in the metadata, allowing users to easily split and analyze the dataset based on these key variables. We will further strive

to include individuals across a broad spectrum of BMI categories to ensure that the atlas captures the heterogeneity of AT in different metabolic states. Significant differences have been observed between male and female AT ¹⁶³, which imposes an important analytical challenge to seamlessly integrate datasets across both sexes. Whenever possible, data on lifestyle and social determinants of health, such as diet, activity, occupation, socioeconomic status, income, education, and domestic status ¹⁶⁴, will also be included for human subjects.

To ensure comprehensive representation of all cell types in the tissue, we will include both snRNA-seq and scRNA-seq data, despite the integration challenges this may pose. Such challenges arise from variations in the mRNA profiles between cells and nuclei, spliced versus unspliced RNA content, differences in isolation techniques for snRNA- and scRNA-seq, and disparities in the capture efficiency and sensitivity of the two approaches. We will only include studies analysing a meaningful number of cells or nuclei, as a robust sample size is essential to mitigate potential batch effects. The initial focus will be on datasets that do not involve enrichment of specific cellular subpopulations. Given that most data have been generated using the 10X Genomics platform, we will begin by integrating these datasets, with plans to incorporate technologies offering full transcript coverage at a later stage. While we recognize the growing interest in spatial and multiomic approaches, the initial atlas will concentrate solely on RNA-seq data.

Metadata, Required and Suggested

An important task of the HCA Adipose Bionetwork is to ensure that the data are accessible and useful for groups with a wide range of interests. In this regard, we will collect comprehensive metadata for AT samples involved in the HCA integration efforts. It is therefore important to develop clear guidelines for reporting these metadata. This includes the exact anatomical location of the biopsy (Box 1). Currently, there is no consensus on the optimal biopsy site within a depot, such as the omentum, which exhibits considerable regional structural variability. Providing more detailed information on the biopsy site can significantly aid data integration and comparison and offer a more comprehensive overview of subpopulations within the different adipose depots. In addition, specifics should be provided on sample acquisition, including the method of sample collection, storage and handling.

In addition to providing detailed information on the sample itself, individual level metadata focused on physiological and metabolic parameters relevant to AT will ideally also be collected for these studies. This metadata would include anthropometric data, such as age, sex, weight, height, BMI, waist, waist-hip ratio and body fat percentage (with method specified), as well as information on disease states (e.g., T2D, PCOS, and cardiovascular disease), a comprehensive medication list, and relevant blood work such as fasting glucose and insulin as well as lipid panels.

Information on the conditions at the time of sample collection such as donor's feeding status (duration of fast) at the time of biopsy acquisition, ambient temperature at the time of collection, and time of day and season should also be collected. If the study allows for collection of peripheral blood that can be banked along with the samples, it will allow for queries of factors such as circulating leptin and adiponectin levels as hypotheses arise. While

it is unlikely that all recommended metadata will be available for all studies, efforts should be made to collect whatever is possible during study design. When Adipose Atlas 1.0 is created we will include the metadata that is available and make a note of which studies the metadata comes from to assist with interpretation of the datasets.

Data Sharing

As groups generate ever larger sequencing datasets, one question becomes how best to share data with the community. While sharing raw FASTQ files complies with the data sharing policies of many journals, these are often too large and computationally onerous for less bioinformatically inclined groups to query for genes and cell types of interest. Data should therefore ideally be shared both as raw FASTQ files and as processed data matrices and/or data objects such as RDS files. The HCA Data Repository (<https://data.humancellatlas.org/contribute>) offers a platform for sharing FASTQ files and associated metadata for HCA datasets. Additionally, the HCA operates the Cell Annotation Platform (<https://celltype.info/>), which stores matrices and cell annotation metadata in a specialized portal that enables collaborative data annotation within the HCA community. Other similar platforms for querying single-cell datasets online include CZI's CellxGene (<https://cellxgene.cziscience.com/>) and the Broad Institute's Single Cell Portal (https://singlecell.broadinstitute.org/single_cell). The Adipose Biological Network will actively be using these platforms for our atlases to ensure comprehensive data sharing and accessibility within the scientific community.

Ethical and Societal Considerations

As spatial and single-cell technologies advance, consideration must be given to data privacy and ethical use of the detailed molecular data. Ensuring patient privacy and consent is paramount. Raw FASTQ level data can be used to identify a patient¹⁶⁵, and some of the metadata described above may also be specific enough to identify an individual patient. In some regions, rules are already in place that restrict the sharing of raw data and metadata reporting with specific demographic information. The HCA data ingestion process is designed to comply with these laws, and groups that prefer to share their raw data through controlled-access databases can do so via the HCA Data Repository or dbGaP, which is operated by the National Center for Biotechnology Information (NCBI). Groups that cannot share specific demographic information due to concerns about patient privacy should bin these values and report the appropriate range for each individual (e.g., a 49-year-old individual could be reported as being aged 45–50).

To avoid bias and ensure that findings are broadly applicable, it is important to promote equity in research by ensuring access to advanced technologies and inclusion of diverse populations in AT research. When possible, researchers should recruit subjects from a wide demographic range. One of the missions of the HCA is to collect data from researchers around the world, ensuring diversity in both subjects and the researchers themselves.

Atlas 2.0 and Beyond

While the HCA Adipose Atlas 1.0 is focused on currently available snRNA-seq datasets from healthy human subjects, we anticipate that future versions of the Adipose Atlas will include more diverse datasets, comprising different adipose depots, genetically diverse cohorts, and a variety of physiological and pathological conditions. Other modalities, such as spatial transcriptomics, and single-cell epigenomics, proteomics, and metabolomics will also feature in later atlases. This should also include cellular trajectories, including modelling of adipogenesis^{166–170} and activation of immune cells.

Moreover, the human atlas will become more functional and interpretable when comparable atlases are developed for other species, especially for emerging model organisms¹⁷¹ and species of agricultural importance¹⁷², which will also help to evaluate cross-species similarities and differences. Furthermore, adipocytes are not necessarily confined to specific adipose depots but can also be found in a variety of other tissues. As snRNA-seq datasets emerge for more tissues like breast¹⁷³ and skin^{174,175}, we will learn more about adipocytes residing in these tissues. Finally, as mentioned previously, all of these huge datasets need to be accessible widely across the data sharing options suggested above to allow for exploring and querying single-cell datasets.

Metabolic and Disease States

Most studies to date have employed tissue biopsy samples obtained in the fasting state. However, AT is highly responsive to the dynamic hormonal and metabolic signals in the postprandial state, and future studies should also probe the impact of metabolic state and nutrition on the adipose transcriptome and cellular composition. Recent longitudinal snRNA-seq studies have addressed the plasticity of human AT in response to short- and long-term weight loss^{176,177}, revealing an obesogenic memory that may be driven by stable epigenetic changes in certain AT cell types¹⁷⁷. These findings warrant further investigation at the single-cell level which could be incorporated into future versions of the atlases. AT also needs to be studied across the entire life course, as some studies have suggested that AT may be a sentinel tissue for changes associated with aging^{135,178}. Furthermore, AT dysfunction is one of the most significant factors contributing to variability in metabolic disease risk factors, including T2D, cardiovascular disease, polycystic ovary syndrome (PCOS), and other endocrine and reproductive disorders. While the initial version of the adipose atlas will focus on tissue from healthy individuals across a wide range of BMI, one of the intended uses of this atlas is to enable comparison with AT during disease progression. To this end, future expansions of the atlas will incorporate recent single-cell studies that have uncovered associations between specific AT cell types and the severity of certain metabolic disease⁹⁴. In addition to metabolic diseases, future atlases might encompass data from liposarcoma samples, which are malignancies of mesenchymal origin with elements of adipocytic differentiation and unique gene signatures compared to normal AT¹⁷⁹.

Emerging Technologies

Many new technologies are being utilized to query tissues and organs at single-cell resolution, including proteomics, metabolomics, and epigenomics coupled with spatial mapping and profiling of cell types. Advances in single-cell epigenomics have enabled the mapping of accessible genomic regions at single-nucleus resolution using the assay for transposase-accessible chromatin with sequencing (ATAC-seq)^{1,180–182}, despite persistent technical challenges with frozen human AT. DNA methylation can also be assessed at a single-cell level, allowing exploration of how environmental factors like diet or obesity influence epigenetic states in cellular subpopulations¹⁸³. Furthermore, future advances in single-cell long-read sequencing are expected to enhance chromatin accessibility and DNA methylation profiling, enabling simultaneous profiling of single-nucleotide polymorphisms, large-scale genetic alterations, and epigenetic modifications¹⁸⁴.

Some studies have already explored the transcriptional profile of AT using spatial approaches^{10,134,185,186}, though most of these studies lack single-cell resolution. New sequencing- and bioimaging-based technologies now allow the study of spatial cell organization at single-cell and subcellular levels, enhancing our understanding of how spatial relationships between cell types in the adipose niche affect tissue function¹⁸⁷.

Integrating various single-cell data modalities holds the potential to enhance our understanding of how different molecular layers interact in AT biology. For example, combining snRNA-seq and snATAC-seq to detect both the transcriptome and epigenome within the same cell is a powerful approach for inferring gene regulatory networks and causal transcription factors controlling AT function¹. Additionally, scRNA-seq has also recently been combined with cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) to interrogate immunometabolic adaptations in mouse AT during weight loss¹⁸⁸.

Efforts to integrate various multiomics data types from the same cell still face significant technological and computational challenges, including issues related to data alignment, synchronization, and batch effects. Future efforts by members of the HCA and the Adipose Bionetwork will therefore also focus on developing improved techniques for simultaneous or sequential multiomics measurements, as well as sophisticated computational tools for data integration and analysis.

Conclusion

Over the past few decades, AT has emerged as a central player in mammalian metabolism, with important roles in normal physiology and in disease. As a complex, heterogeneous, multifocal, and dynamic tissue, AT needs to be understood at single-cell resolution. Despite the technical challenges associated with this fatty tissue, the field has made significant progress by generating comprehensive cellular atlases of AT, identifying novel cell types and states, and determining the physical and functional relationships between cell types. The adipose research community has begun to establish standards for depot and cellular nomenclature, and for experimental and computational best practices. Going forward, there will be significant efforts throughout the HCA Bionetwork system and within the Adipose Bionetwork to harmonize data and achieve consensus on cell annotation. As a bionetwork,

we hope to establish resources that will enable data mining by experts and non-experts alike to usher in a new paradigm for AT research.

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Acknowledgements

As an initial initiative, members of the HCA Adipose Bionetwork formed subcommittees focused on ‘Anatomical Location,’ ‘Cell Annotation,’ ‘Experimental Best Practices,’ and ‘Computational Best Practices’ to develop recommendations for the entire adipose research community. All listed authors are members of the HCA Adipose Bionetwork and contributed actively, through participation in these subcommittees and/or during the manuscript editing process. Further, a special thanks goes to the following members of the HCA Adipose Bionetwork: Aino R. Peltonen, Aaron Streets, A. Louise Hunter, Antje Körner, Assaf Rudich, Chaitra Sarathy, Dalin Li, Ellen Gammelmark Klinggaard, Erica Pimenta, Feihu Zhao, Halina Dobrzynski, Jacqueline Stephens, Jan Lukas Rinker, Jia Nie, Katia Delgado, Kelly Warmink, Laurine van Gijn, Ling Qin, Mohammad Nadeem Khan, Qiuming Yao, Rasmus Rydbirk, Roberta Gualtierotti, Tong Tong, Yuyan Zhu, and Zinger Yang Loureiro. This work was supported by The Danish National Research Foundation to the Center for Functional Genomics and Tissue Plasticity (ATLAS) (Project grant: 141) to SM and AL, the Lundbeck Foundation (R413–2022-471) to AL, NIH K01 DK134806 to MPE, and NIH RC2 DK116691 to EDR. This publication is part of the HCA (www.humancellatlas.org/publications/).

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Box 1:**Recommended Metadata Reporting**

This is meant to be a wish list of metadata for samples. Not all studies will have all information, but effort should be made during study design and sample collection to collect as much of this information as possible.

Individual information**Demographics:**

Age

Sex

BMI

Disease state

Relevant medications

Relevant bloodwork

Fat distribution information

Collection conditions:

Alive or deceased

Time of day

Time of year

Ambient temperature

Sample information**Depot (see Table 1)**

Sample collection: Surgery, fine needle biopsy, etc

Type of anaesthesia if applicable

Time from tissue isolation to freezing

Storage and handling: Fresh or frozen samples

If frozen, length of freezing, temperature

Cell/nuclei isolation method

Other sample handling such as flow sorting

Any QC measures such as cell/nuclei count per sample

Single cell method

Computational information

Genome build used for alignment

Data processing programs (including version number)

Cell QC information: nUMI

Mitochondrial percent

Ribosomal percent

Score from doublet detection program

Estimated ambient RNA fraction

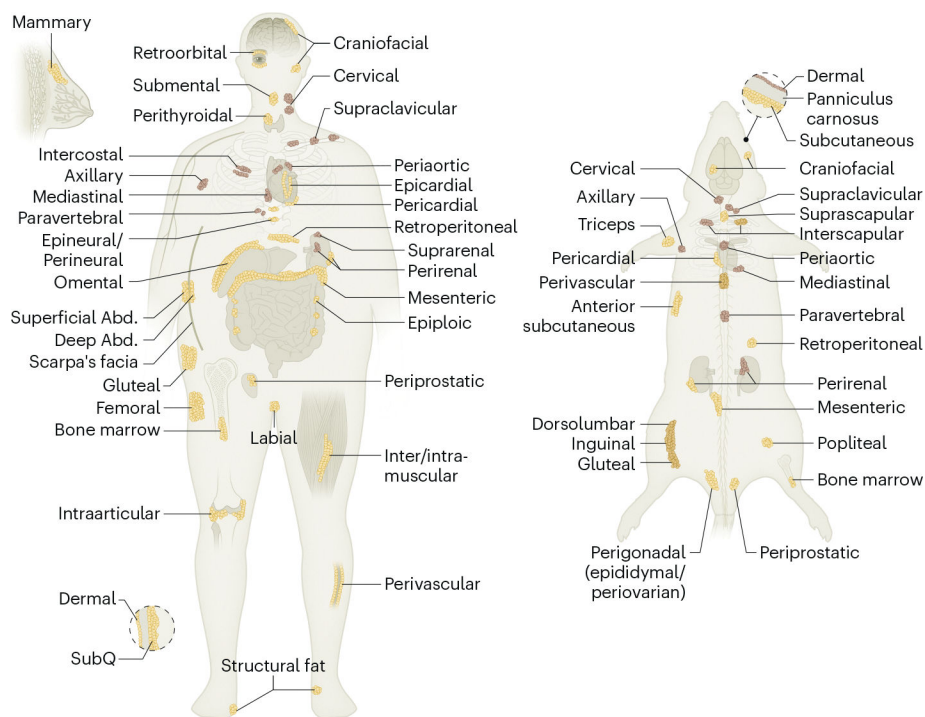


Figure 1. Overview of main adipose depots in human and mouse.
The figure illustrates the approximate anatomical locations of key adipose depots in humans [A] and mice [B]. The colour of the adipose depot links to its classification as white, beige, or brown fat.

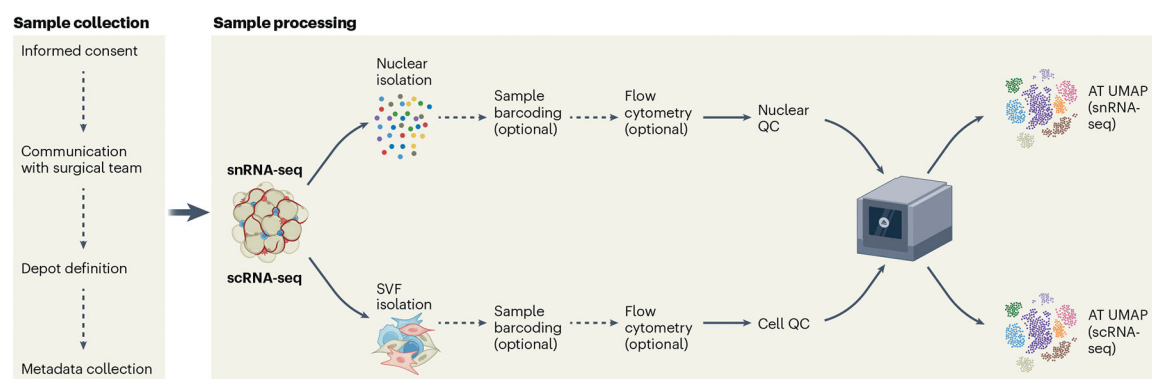


Figure 2. Workflow for single-cell and single-nucleus analysis on human AT using the 10X Genomics platform.

Adipose tissue samples are collected from patients with informed consent, and metadata, including subject demographics and clinical data, are recorded. For snRNA-seq, adipose tissue can be snap-frozen, allowing nuclei to be extracted from frozen samples. For scRNA-seq, fresh tissue is preferred, but fresh-frozen tissue can also be used if dissociated into single-cell suspensions before freezing. The samples are then processed to isolate cells or nuclei through enzymatic digestion or mechanical dissociation. Sample barcoding for multiplexing can be employed to pool multiple samples in a single sequencing run, reducing costs and increasing throughput. Flow cytometry can be used to further sort and enrich specific cell populations before sequencing, or to remove low-quality nuclei or cells. Finally, the prepared samples are loaded onto the 10x Chromium Controller, which uses advanced microfluidics to partition individual cells or nuclei into droplets, each containing a unique barcode for downstream sequencing and analysis.

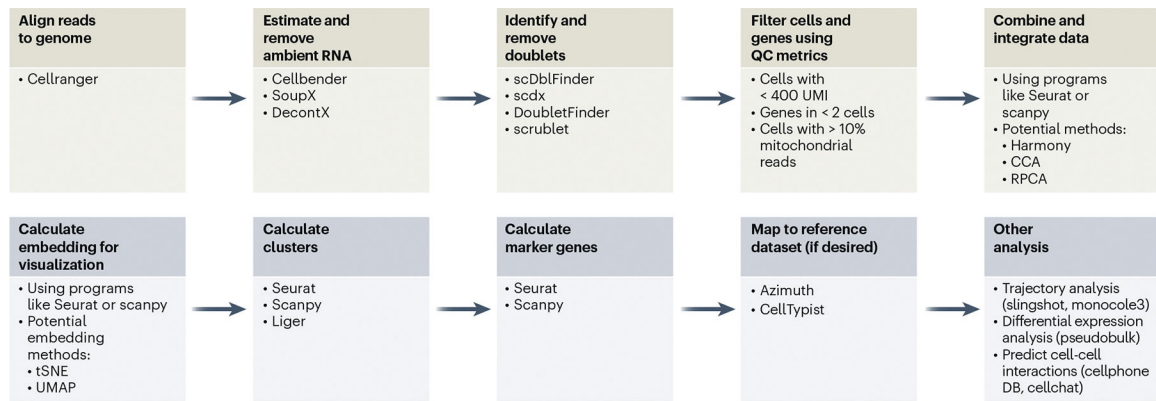


Figure 3. Overview of computational workflow for single-cell analyses on human AT. The workflow begins with aligning reads to the genome using Cellranger. Ambient RNA is removed using tools like Cellbender, SoupX, or DecontX and doublets are identified and removed using scDblFinder, scdx, DoubletFinder, or scrublet. Cells and genes are filtered based on quality control metrics. Data is then combined and integrated using Seurat or scanpy, with integration methods such as Harmony, CCA, and RPCA. Clusters and marker genes are identified using Seurat, Scanpy, or Liger. Data can be mapped to reference datasets using Azimuth or CellTypist. Additional analyses include trajectory analysis with slingshot or monocle3, differential expression analysis using pseudobulk, and prediction of cell-cell interactions with CellphoneDB or Cellchat. Listed programs are suggestions and not exhaustive.

Table 1 - An overview of human and mouse adipose depots.

Adipose tissues can broadly be categorized into subcutaneous (beneath the skin) and visceral (within the peritoneal cavity) depots as well as some other depots that cannot be classified into either of these categories. The table further specifies the predominant AT classifications (i.e., white and/or brown AT), if a human depot is primarily found in infants (§) or if a mouse depot is only observed in severely obese mice (*).

	Subcutaneous	Visceral	Others
Human	<i>Superficial subcutaneous</i> Abdominal Superficial Layer (white) Superficial Gluteofemoral Submental (white) Craniofacial (white) Dermal (white) Labial (white) <i>Deeper subcutaneous</i> Abdominal Deep Layer (white) Deep Gluteofemoral (white) Axillary (white/brown) Cervical (white/brown) Supraclavicular (white/brown)	Mesenteric (white) Omental (white) Retroperitoneal (white) Epiploic (white) Periprostatic (white) Perirenal (white/brown) Suprarenal (white/brown) Intercostal (white/brown) Paravertebral (white/brown) Mediastinal (white/brown§) Periaortic (white/brown§) Pericardial (white/brown§) Epicardial (white/brown§) § primarily in infants	Bone marrow (white/brown potential?) Epineural/Perineural (white) Mammary (white) Perithyroidal (white/brown potential?) Perivascular (white/brown) Retroorbital (white) Intraarticular (e.g., popliteal and infrapatellar) (white) Inter/intra-muscular (white/brown potential?) Structural fat of heel, fingers, etc. (white)
Mouse	<i>Superficial subcutaneous</i> Anterior subcutaneous (white/limited brown) Inguinal (white/brown) Gluteal (white/brown) Interscapular (white/brown) Triceps (white/brown) Craniofacial (white) Dermal (white) Suprascapular (white/brown) Axillary (brown) <i>Deeper subcutaneous</i> Dorsolumbar# (white/brown) Cervical (brown)# Supraclavicular (brown)#	Mesenteric (white/limited brown) Perigonadal (epididymal♂/ periovarian♀) (white/limited brown) Pericardial (white/limited brown) Periprostatic (white/limited brown) Retroperitoneal (white/brown) Perirenal (white/brown) Periaortic (brown) Paravertebral (brown) Omental (white)* Pericardial (white)* Epicardial (white/brown)* *seen only in severely obese mice	Bone marrow (white/brown potential?) Epineural/Perineural (white) Mammary (white) Perithyroidal (white/brown potential?) Perivascular (white/brown) Retroorbital (white) Intraarticular (e.g., popliteal and infrascapular) (white) Inter/intra-muscular (white/brown)

Table 2 - Adipose cell type annotations and proposed marker genes in human single-cell data.

To distinguish closely related cellular subtypes, a combination of marker genes is often required. Additionally, subtype markers may not differentiate subtypes from other cell types in the tissue and therefore may only be useful for distinguishing subtypes within one or a few specific cell types.

Cell types	Human cell type markers	Cell subtypes	Human cell subtype markers
Adipocytes	<i>ADIPOQ</i> ^{5,82,83,94,124,133,176,189,190} , <i>PLIN1</i> ^{124,176}	White	
		Brown/Beige	<i>COBL</i> ⁷⁹ , <i>CKMT2</i> ⁷⁹ , <i>PPARGC1A</i> ⁸² , <i>ATP5K</i> ¹³⁶ , <i>COX7A</i> ¹³⁶
Adipose stromal and progenitor cells (ASPCs)	<i>PDGFRA</i> ^{5,83,94,98,124,134,190} , <i>DCN</i> ^{10,82,190}	Stem cells/early preadipocytes	<i>DPP4</i> ^{88,93,124,133} , <i>CD55</i> ^{88,93,124,133} , <i>PI16</i> ^{93,124,133,176}
		Committed preadipocytes	<i>PPARG</i> ^{88,93,124,176}
Pericytes	<i>STEAP4</i> ^{5,94,124,176,177} , <i>COL25A1</i> ^{124,176}		
Smooth muscle cells	<i>MYOCD</i> ^{5,122,124,176} , <i>MYH11</i> ^{10,82,122,124,133,134,176,177,190}		
Endothelial cells (ECs)	<i>PECAM1</i> ^{5,98,122,124,190} , <i>MECOM</i> ^{82,124,176,177} , <i>CDH5</i> ¹²² , <i>ENPEP</i> ⁵	Lymphatic ECs	<i>PROX1</i> ^{94,124,134,176,189,191} , <i>LYVE1</i> ^{97,124,134,176,191}
		Arterial ECs	<i>PCSK5</i> ^{176,191} , <i>GJA5</i> ^{122,124,191} , <i>NEBL</i> ^{122,176,177,191}
		Venous ECs	<i>ACKR1</i> ^{122,124,134,176,191}
		Capillary ECs	<i>BTNL9</i> ^{124,134,176,190,191} , <i>RBP7</i> ¹²² , <i>CA4</i> ^{122,191}
Mesothelial cells	<i>MSLN</i> ^{5,83,94,124} , <i>KRT19</i> ⁵ , <i>BNC1</i> ¹²⁴		
B cells	<i>MS4A1</i> ^{5,83,94,124,134} , <i>CD79A</i> ^{97,98} , <i>IGKC</i> ⁹⁸		
Plasmablast	<i>JCHAIN</i> ¹²⁴		
T cells	<i>CD3D</i> ^{82,134} , <i>CD3E</i> ⁸²	Tregs	<i>FOXP3</i> ^{98,124} , <i>TIGIT</i> ^{98,124} , <i>CTLA4</i> ^{5,98,124}
		(Naive) CD4 T cells	<i>CD2</i> ⁹⁸ , <i>CD4</i> ¹³⁴ , <i>CD40LG</i> ^{98,124}
		(Naive) CD8 T cells	<i>CD8A</i> ^{98,134} , <i>GZMK</i> ^{98,124}
		Cytotoxic CD8 T cells	<i>CD8A</i> ^{98,134} , <i>GZMB</i> ^{98,134} , <i>GZML</i> ⁹⁸
		CD8 $\gamma\delta$ T cells	<i>CD8A</i> ⁹⁸ , <i>LEF1</i> ⁹⁸ , <i>KLRC2</i> ⁹⁸ , <i>ZNF683</i> ⁹⁸
		Mucosal-associated invariant T (MAIT) cells	<i>CD8A</i> ⁹⁸ , <i>KLRB1</i> ⁹⁸ , <i>GZMK</i> ^{98,124} , <i>SLC4A10</i> ¹²⁴
		Mature NK cells	<i>FCGR3A</i> ^{98,124} , <i>FGFBP2</i> ⁹⁸ , <i>PRF1</i> ⁹⁸ , <i>KLRK1</i> ⁹⁸
		Tissue resident (trNK) cells (NK-like)	<i>XCL2</i> ⁹⁸ , <i>GZMK</i> ⁹⁸ , <i>KLRC1</i> ⁹⁸
Natural killer (NK) cells	<i>GNLY</i> ^{10,97,98,124} , <i>KLRD1</i> ⁵ , <i>PRF1</i> ⁹⁸	Immature (iNK) cells (NK-like)	<i>XCL2</i> ⁹⁸ , <i>GZMK</i> ⁹⁸ , <i>KLRC1</i> ⁹⁸ , <i>NCAM1</i> ⁹⁸ , <i>CD2</i> ⁹⁸ , <i>IL7R</i> ⁹⁸ , <i>SELL</i> ⁹⁸
Innate lymphoid cells (ILC)	<i>IL7R</i> ⁹⁸ , <i>CD200R1</i> ⁹⁸	ILC1	<i>ZNF683</i> ⁹⁸ , <i>TBX21</i> ⁹⁸ , <i>CD200R1</i> ⁹⁸ , <i>IL7R</i> ⁹⁸
		ILC2	<i>GATA3</i> ⁹⁸ , <i>IL1RL1</i> ⁹⁸ , <i>CD200R1</i> ⁹⁸ , <i>IL7R</i> ⁹⁸
		ILC3	<i>CCR6</i> , <i>IL1R1</i> ⁹⁸ , <i>IL23R</i> ⁹⁸ , <i>CD200R1</i> ⁹⁸ , <i>IL7R</i> ⁹⁸
		ILCP	<i>SELL</i> ⁹⁸ , <i>IL1R1</i> ⁹⁸ , <i>CD200R1</i> ⁹⁸ , <i>IL7R</i> ⁹⁸
Dendritic cells (DCs)	<i>FLT3</i> ^{5,82,98}	Conventional DC1 (cDC1)	<i>XCRI</i> ^{98,192} , <i>CLEC9A</i> ^{98,124,192} , <i>CADMT</i> ^{98,124,192} , <i>DPP4</i> ^{98,192}
		Activated DCs	<i>CCR7</i> ^{93,98,124} , <i>FSCN1</i> ⁹⁸ , <i>CCL22</i> ⁹⁸ , <i>LAMP3</i>

Cell types	Human cell type markers	Cell subtypes	Human cell subtype markers
		cDC2B	<i>FCER1A</i> ^{98,124,134,192} , <i>CD1C</i> ^{98,124,134,192} , <i>CLEC10A</i> ^{98,124,176,192} , <i>IRF4</i> ^{98,192}
Neutrophils	<i>S100A12</i> ⁹⁸ , <i>S100A9</i> ⁹⁸ , <i>CSF3R</i> ^{5,124}		
Monocytes	<i>FCN1</i> ^{124,192}	Classical monocytes 1	<i>FCN1</i> ^{98,124} , <i>FCER1A</i> ⁹⁸ , <i>TXNIP</i> ⁹⁸
		Classical monocytes 2	<i>FCN1</i> ^{98,124} , <i>FCAR</i> ⁹⁸
		Non classical monocytes	<i>FCGR3A</i> ^{98,124,134,192} , <i>HES4</i> ^{98,124,192} , <i>LST1</i> ^{98,134} , <i>DGKG5</i>
Macrophages	<i>MRC1</i> ^{134,136,176,177,192} , <i>F13A1</i> ^{124,136,176}	Perivascular macrophages (PVM)	<i>LYVE1</i> ^{98,124,134,176} , <i>SELENOP</i> ^{98,176,192} , <i>CIQA</i> ^{10,98}
		Lipid-associated macrophages (LAMs)	<i>CD9</i> ^{97,98,124,134} , <i>TREM2</i> ^{98,124,134,176} , <i>LPL</i> ^{97,98,124,176}
		Inflammatory macrophages	<i>CCL3L1</i> ⁹⁸ , <i>TNF</i> ⁹⁸ , <i>CXCL3</i> ^{97,98}
Mast cells	<i>KIT</i> ^{83,124,176,190} , <i>CPA3</i> ^{5,94,124,134,176,177}		
Schwann cells	<i>XKR4</i> ¹²⁴		

Table 3 - Adipose cell type annotations and proposed marker genes in mouse single-cell data. Distinguishing closely related cellular subtypes often requires a combination of marker genes. Moreover, subtype markers may not effectively separate subtypes from other cell types in the tissue and are typically only useful for identifying subtypes within one or a few specific cell types.

Cell types	Mouse cell type markers	Cell subtypes	Mouse cell subtype markers
Adipocytes	<i>Adipoq</i> ^{5,133,136} , <i>Plin4</i> ³	White	<i>Retn</i> ¹⁹⁴
		Brown/Beige	<i>Ucp1</i> ^{81,82,136,147,194} , <i>Ppargc1a</i> ^{81,82} , <i>Cidea</i> ^{81,82,194} , <i>Adrb3</i> ⁸¹ , <i>Clstn3</i> ⁸¹ , <i>Atp5k</i> ¹³⁶ , <i>Cox7a1</i> ^{136,194}
Adipose stromal and progenitor cells (ASPCs)	<i>Pdgfra</i> ^{3,5,84,147,177,195} , <i>Dcn</i> ^{3,81,147,196}	Early preadipocytes	<i>Dpp4</i> ^{84,88,89,131,176,196} , <i>Cd55</i> ^{88,89,195} , <i>Pi16</i> ^{84,88,90,133,195-197} , <i>Aldh1a3</i> ³ , <i>Ebf2</i> ^{3,90}
		Committed preadipocytes	<i>Pparg</i> ^{3,88,90,129,196,198} , <i>Icanf</i> ⁸⁸ , <i>Cd36</i> ^{3,129}
		Adipogenesis-regulatory cells (Aregs) in iWAT	<i>Cd142</i> ^{89-91,195} , <i>Clec11a</i> ^{84,89,91,195} , <i>Fmo2</i> ^{89-91,195} , <i>Meox2</i> ^{90,91} , <i>Epha3</i> ³
Pericytes	<i>Steap4</i> ⁵ , <i>Enpep</i> ⁵		
Smooth muscle cells	<i>Myocd</i> ⁵ , <i>Myh11</i> ^{120,133,147,177,195} , <i>Acta2</i> ^{8,120,133,147,195,197,199}		
Endothelial cells (ECs)	<i>Pecam1</i> ^{5,120,147,195} , <i>Cdh5</i> ^{84,120,147,195}	Lymphatic ECs	<i>Prox1</i> ^{5,120,177} , <i>Lyve1</i> ^{5,120,147}
		Arterial ECs	<i>Hey1</i> ¹²⁰ , <i>Gkn3</i> ¹²⁰
		Venous ECs	<i>Vcam1</i> ¹²⁰ , <i>Vwf</i> ¹²⁰ , <i>Ackr1</i> ¹²⁰
		Capillary ECs	<i>Car4</i> ¹²⁰ , <i>Rgcc</i> ¹²⁰
Mesothelial cells	<i>Msln</i> ^{3,5,133,195} , <i>Krt19</i> ^{5,195}		
B cells	<i>Ms4a1</i> ^{3,5,188} , <i>Cd79a</i> ^{3,103,188,193} , <i>Cd79b</i> ^{3,113}	Naive B Cells	<i>Ighd</i> ¹⁸⁸
		Memory B Cells	<i>Zbtb32</i> ¹⁸⁸
Plasmablast	<i>Jchain</i> ¹⁸⁸		
T cells	<i>Cd3d</i> ^{103,113,188,193} , <i>Cd3e</i> ^{103,188}	Tregs	<i>Cd4</i> ^{103,186,188} , <i>Foxp3</i> ^{103,113,186,188}
		CD4 cells	<i>Cd4</i> ¹⁸⁸ , <i>Cxcr3</i> ¹⁸⁸
		CD8 effector memory cells	<i>Cd8b1</i> ¹⁸⁸ , <i>Cxcr3</i> ¹⁸⁸
		CD8 central memory cells	<i>Cd8b1</i> ¹⁸⁸ , <i>Ccr7</i> ¹⁸⁸ , <i>Sell</i> ¹⁸⁸
		Cycling CD8 T cells	<i>Cd8b1</i> ¹⁸⁸ , <i>Cxcr3</i> ¹⁸⁸ , <i>Stmn1</i> ¹⁸⁸ , <i>Pclaf</i> ¹⁸⁸
		CD8 $\gamma\delta$ T cells	<i>Il7r</i> ¹⁸⁸ , <i>Trdv4</i> ¹⁸⁸ , <i>Cd163l1</i> ^{103,188} , <i>Cxcr6</i> ¹⁸⁸ , <i>Trdc</i> ¹⁸⁸
Natural killer (NK) cells	<i>Klrf5</i> ⁵ , <i>Xcl1</i> ¹⁸⁸	Mature NK cells	<i>Ncr1</i> ¹⁸⁸ , <i>Klrb1b</i> ¹⁸⁸ , <i>Klrb1c</i> ¹⁸⁸
		NK-like cells	<i>Ly6c2</i> ¹⁸⁸ , <i>Klrb1c</i> ¹⁸⁸ , <i>Cd3d</i> ¹⁸⁸ , <i>Cd3e</i> ¹⁸⁸
		Invariant NK T (iNKT) cells	<i>Tbx21</i> ²⁰⁰
Innate lymphoid cells (ILC)	<i>Il7r</i> ¹⁸⁸ , <i>Gata3</i> ^{188,193} , <i>Arg1</i> ¹⁰³	ILC2	<i>Klrg1</i> ¹⁸⁸ , <i>Ccdc184</i> ¹⁸⁸
Dendritic cells (DCs)	<i>Flt3</i> ^{3,5,103}	Conventional DC1 (cDC1)	<i>Xcr1</i> ^{103,186,188} , <i>Clec9a</i> ^{5,186,188}
		Activated cDC1s	<i>Ccr7</i> ¹⁸⁸ , <i>Mreg</i> ¹⁸⁸ , <i>Il12b</i> ¹⁸⁸
		Cycling cDC1	<i>Xcr1</i> ¹⁸⁸ , <i>Clec9a</i> ¹⁸⁸ , <i>Stmn1</i> ¹⁸⁸ , <i>Pclaf</i> ¹⁸⁸
		cDC2	<i>Sirpa</i> ^{103,188} , <i>Cd209a</i> ^{104,113,188}

Cell types	Mouse cell type markers	Cell subtypes	Mouse cell subtype markers
		Activated cDC2	<i>Sirpa</i> ¹⁸⁸ , <i>Ccr7</i> ¹⁸⁸ , <i>Mreg</i> ¹⁸⁸
		Cycling cDC2	<i>Sirpa</i> ¹⁸⁸ , <i>Cd209a</i> ^{188,193} , <i>Ear2</i> ¹⁸⁸ , <i>Stmn1</i> ¹⁸⁸ , <i>Pclaf</i> ¹⁸⁸
		Plasmacytoid DCs	<i>Siglech</i> ^{113,188} , <i>Cd209a</i> ¹⁸⁸ , <i>Cox6a2</i> ¹⁸⁸ , <i>Clec9a</i> ¹⁸⁸
		Migratory DCs	<i>Ccr7</i> ¹⁰³ , <i>Ccl22</i> ¹⁰³ , <i>Nudt1</i> ¹⁰³
Neutrophils	<i>S100a8</i> ¹⁸⁸ , <i>Csf3r</i> ⁵ , <i>S100a9</i> ^{113,188}		
Monocytes	<i>Plac8</i> ^{188,193} , <i>Lyz1</i> ¹¹³ , <i>Lyz2</i> ¹⁸⁸	Classical monocytes	<i>Socs6</i> ⁵ , <i>Fcgr1</i> ¹⁸⁸ , <i>Ly6c2</i> ¹⁸⁸ , <i>Ccr2</i> ¹⁸⁸
		Non classical monocytes	<i>Ace</i> ¹⁸⁸ , <i>Dgkg</i> ⁵
Macrophages	<i>Mrc1</i> ^{103,113,188} , <i>Adgre1</i> ^{3,103,177,188}	PVMs	<i>Lyve1</i> ^{3,104,113,177,186} , <i>Cd163</i> ^{3,104,113,177} , <i>Cd209</i> ^{113,186}
		LAMs	<i>Cd9</i> ^{3,113,186} , <i>Trem2</i> ^{3,5,104,113,177,186} , <i>Lp</i> ^{3,104,113}
		Nerve-associated macrophages (NAMs)	<i>Maoa</i> ¹⁰⁴ , <i>St3gal6</i> ¹⁰⁴ , <i>Lilra5</i> ¹⁰⁴
Mast cells	<i>Kit</i> ¹⁸⁸ , <i>Cpa3</i> ^{5,177,188} , <i>Mcpt4</i> ¹⁸⁸		
Schwann cells	<i>Mpz</i> ^{133,147,197}		