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Qiong Wang (✉ qwang@coh.org)

City of Hope/ Beckman Research Institute <https://orcid.org/0000-0003-2224-4287>

Guan Wang

City of Hope/ Beckman Research Institute

Gaoyan Li

UCLA

Anying Song

City of Hope/ Beckman Research Institute

Wenting Dai

City of Hope/ Beckman Research Institute

Nicholas Shamlin

City of Hope/ Beckman Research Institute

Hanjun Qin

Beckman Research Institute, City of Hope

Leonard Medrano

City of Hope/ Beckman Research Institute

Joan Dow

City of Hope/ Beckman Research Institute

Aimin Li

City of Hope/ Beckman Research Institute

Lucy Brown

City of Hope/ Beckman Research Institute

Brian Armstrong

Beckman Research Institute at City of Hope Medical Center

Patrick Fueger

City of Hope

Judith Campisi

Buck Institute

Xiwei Wu

City of Hope

Lei Jiang

City of Hope <https://orcid.org/0000-0002-8596-556X>

Xia Yang

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A unique adipocyte progenitor population promotes age-related adiposity

Guan Wang^{1#}, Gaoyan Li^{2#}, Anying Song¹, Wenting Dai¹, Nicholas Shamlin¹, Hanjun Qin³,
Leonard Medrano⁴, Joan Dow^{1,5}, Aimin Li⁶, Lucy Brown⁷, Brian Armstrong⁸, Patrick T.
Fueger^{1,5,9}, Judith Campisi^{10,11}, Xiwei Wu³, Lei Jiang^{1,9}, Xia Yang^{2,12,13,14*}, Qiong A. Wang^{1,9*}

¹Department of Molecular & Cellular Endocrinology, Arthur Riggs Diabetes and Metabolism
Research Institute, ³The Integrative Genomics Core, ⁴Division of Developmental and Translational
Diabetes and Endocrinology Research, ⁵Comprehensive Metabolic Phenotyping Core, ⁶Pathology
Core of Shared resources, ⁷Analytical Cytometry Core, ⁸Light Microscopy Core, ⁹Comprehensive
Cancer Center, Beckman Research Institute, City of Hope Medical Center, Duarte, California
91010, USA.

¹⁰Buck Institute for Research on Aging, Novato, California.

¹¹Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, California.

²Department of Integrative Biology and Physiology, ¹²Bioinformatics Interdepartmental Program,

¹³Institute for Quantitative and Computational Biosciences, ¹⁴Molecular Biology Institute,
University of California, Los Angeles, Los Angeles, CA 90095 USA

[#]These authors contributed equally.

*Correspondence should be addressed to:

Qiong (Annabel) Wang, Department of Molecular & Cellular Endocrinology, City of Hope.

1500 East Duarte Road, Duarte, CA 91010

E-mail: qwang@coh.org; Phone: 626-218-6419

Xia Yang, Department of Integrative Biology and Physiology, Los Angeles, CA 90095

E-mail: xyang123@ucla.edu; Phone: 310-206-1812

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Abstract

The average fat mass in adults increases dramatically with age, and older people often suffer from visceral obesity and related adverse metabolic disorders. Unfortunately, how aging leads to fat accumulation is poorly understood. It is known that fat cell (adipocyte) turnover is very low in young mice, similar to that in young humans. Here, we find that mice mimic age-related fat expansion in humans. *In vivo* lineage tracing shows that massive adipogenesis (the generation of new adipocytes), especially in the visceral fat, is triggered during aging. Thus, in contrast to most types of adult stem cells that exhibit a reduced ability to proliferate and differentiate, the adipogenic potential of adipocyte progenitor cells (APCs) is unlocked by aging. *In vivo* transplantation and 3D imaging of transplants show that APCs in aged mice cell-autonomously gain high adipogenic capacity. Single-cell RNA sequencing analyses reveal that aging globally remodels APCs. Herein, we identify a novel committed preadipocyte population that is age-specific (CP-A), existing both in mice and humans, with a global activation of proliferation and adipogenesis pathways. CP-A cells display high proliferation and adipogenesis activity, both *in vivo* and *in vitro*. Macrophages may regulate the remodeling of APCs and the generation of CP-A cells during aging. Together, these findings define a new fundamental mechanism involved in fat tissue aging and offer prospects for preventing and treating age-related metabolic disorders.

225 words

Main

At the same BMI, older people, on average, tend to have more body fat (white adipose tissue, WAT) than younger adults, especially in the visceral (abdominal) area^{1,2}. At the same time, skeletal muscle mass declines progressively with age. These aged-related alterations in body composition are defined as sarcopenic obesity^{3,4}. Visceral adiposity accelerates aging by promoting insulin resistance, cardiovascular dysfunction, and many other chronic diseases, significantly shortening both healthspan and lifespan^{5,6}. Thus, preventing or reducing WAT accumulation is critical for healthy aging. A recent study shows that older individuals have reduced energy expenditure, indicating a global remodeling of tissue metabolism during aging⁷. Unfortunately, the cellular mechanism of age-related WAT accumulation is still poorly understood.

WAT regulates numerous hormonal and metabolic processes, and exhibits compositional and phenotypic plasticity⁸. WAT expands via adipocyte hypertrophy (cell enlargement) or adipogenesis (the generation of new adipocytes). We and others generated multiple mouse models that allow *in vivo* tracking of adipogenesis, and showed that the adipogenesis rate is very low in young adult mice⁹⁻¹¹, similar to that in young adult humans¹². During aging, it is known that adipocytes undergo hypertrophy¹³, but it remains unclear if WAT also expands through adipogenesis, which would grant WAT an “unlimited” potential to grow.

Adipocyte progenitor cells (APCs), located within the WAT stroma, are a heterogeneous group of mesenchymal stem cells, including adipocyte stem cells and committed progenitor cells⁸. Adipogenesis involves two steps, the proliferation of APCs and the differentiation of APCs into mature adipocytes. APCs express a set of mesenchymal stem cell surface markers, such as *Pdgfra*,

Pdgfr β , CD29, CD34, and Sca1¹⁴. Recently, single-cell RNA sequencing (scRNA-seq) has provided unprecedented opportunities to define the heterogeneity of murine and human APCs¹⁵⁻²¹, revealing new subtypes and dynamics under various pathophysiological conditions.

In this study, we employed an adipocyte-specific lineage tracing system and found the first cellular evidence that WAT expands with age through massive adipogenesis. Thus, unexpectedly, in contrast to most adult stem cells that exhibit a reduced ability to proliferate and differentiate²²⁻²⁴, adipogenesis of APCs is unlocked by aging. Through a series of *in vivo* transplantations and 3D imaging of transplants, we showed that the APCs in aged mice cell-autonomously gain high adipogenic capacity. scRNA-seq analysis revealed a global transformation of APCs in aged mice and older humans and uncovered a new APC population that is uniquely generated during aging. This new type of APC displays high proliferation and adipogenesis capacity, both *in vivo* and *in vitro*. scRNA-seq analysis also showed that aging transformed the transcriptome of immune cells and generated more communication from macrophages to APCs, which may regulate the remodeling of APCs and the generation of CP-A cells. These data provide new mechanistic insights into the cellular mechanism of age-related adiposity.

Results

Aged-related WAT expansion in mice mimics human sarcopenic obesity

At 12 (aged) vs. 3 (young) months old, male C57BL/6J mice showed a 56% increase in body weight (**Fig. 1a, b**). The age-related body weight gain in male mice was primarily attributed to gain of WAT, as the percentage of fat mass increased >6-fold during aging (**Fig. 1c**), while the percentage of lean mass decreased ~23% (**Fig. 1d**). Total body water content was also decreased

with age (**Extended Data Fig. 1a**). Bodyweight gain with age was much higher in males than females (**Extended Data Fig. 1b, c**). Among the two types of fat depots, gonadal WAT (gWAT), a typical type of visceral WAT, had the most significant weight gain with age (4.6-fold) (**Fig. 1e**), while subcutaneous WAT (sWAT) increased 2.8-fold (**Fig. 1f**). The aged mice exhibited dramatically reduced oxygen consumption (**Fig. 1g**), slightly reduced physical activity (**Fig. 1h**) and total movement (**Extended Data Fig. 1d**), as well as dramatically reduced energy expenditure (**Fig. 1i**). Food intake was also reduced in aged mice (**Extended Data Fig. 1e**). The drop in oxygen consumption and energy expenditure suggested that the aged mice have significantly lower basal metabolism, similar to humans⁷. The decline in basal metabolism causes a positive energy balance (energy intake exceeds expenditure), eventually leads to fat accumulation. Intraperitoneal glucose tolerance tests (GTT) (**Fig. 1j, k**) and insulin tolerance tests (ITT) (**Fig. 1l, m**) showed that aged mice have impaired glucose tolerance and are more insulin resistant. Therefore, mice display a pattern of age-related visceral adiposity, accompanied by positive energy balance and insulin resistance, similar to human sarcopenic obesity.

WAT accumulates with age through massive adipogenesis.

To better understand adipocyte dynamics, we previously developed the AdipoChaser mice for tracking adipogenesis^{16,17,19,31,32}. This mouse model is a doxycycline (dox)-based, tet-responsive labeling system for the inducible, permanent labeling of Adiponectin (Adipoq) expressing cells as LacZ⁺ cells (**Extended Data Fig. 1f**). To determine if aging is accompanied by adipogenesis, we utilized AdipoChaser-LacZ mice to label all adipocytes as LacZ⁺ cells in 3-month-old male mice and tracked adipogenesis during early aging (**Extended Data Fig. 1g**). Consistent with our previous studies, in 6-month-old mice, we observed no new adipocytes (i.e., no lacZ-negative cells)

in the gWAT or sWAT, indicating that the turnover rate of adipocytes is extremely low in young adults (**Fig. 1n**). In contrast, in 9-month-old mice, clusters of newly generated adipocytes started to appear in gWAT. New adipocytes also started to appear in sWAT but did not yet form clusters. As mice continue aging, in 18-month-old mice, gWAT showed numerous new adipocytes, which formed large clusters; sWAT also had clusters of new adipocytes, which are relatively smaller than gWAT. Adipocyte size was also increased in sWAT and gWAT, confirming the age-related adipocyte hypertrophy²¹ (**Fig. 1n**). Female mice were found quite different from the males (**Extended Data Fig. 1h**). In the female gWAT, we observed some adipogenesis in 18-month-old female mice, but the rate is much lower than that in male mice. In the female sWAT, we did not observe any new adipocytes generated during aging. Thus, adipogenesis is the major contributor to age-related gWAT expansion in male mice.

We next tested if aged mice would have a higher adipocyte regeneration rate after inducible adipocyte apoptosis. The FAT-ATTAC mice (fat apoptosis through triggered activation of caspase 8) carry a transgene encoding a caspase 8 protein fused to a dimerization domain under the control of the aP2 adipocyte-specific promoter²⁵. We triggered adipocyte apoptosis through 7 days of daily injection of dimerizer, and checked the adipocyte regeneration post dimerization in young and aged mice (**Extended Data Fig. 2a**). Prior to treatment, the adipocytes in young and aged FAT-ATTAC mice were viable with positive perilipin staining before dimerization (**Extended Data Fig. 2b, c**). After dimerization, apoptosis was initiated in every adipocyte, as adipocytes were uniformly dead, with disrupted morphology and negative perilipin staining (**Extended Data Fig. 2b, c**). One week after dimerization, gWAT volume was markedly reduced in both young and aged mice (**Extended Data Fig. 2b, c**). At two-four weeks after dimerization, the aged mice

demonstrated strong adipocyte regeneration with essentially more adipocytes positive for perilipin. gWAT from young mice, while found to have some perilipin-positive adipocytes, still had an abnormal morphology with many lost cells and vacuoles (**Extended Data Fig. 2b, c**). By four weeks of treatment, the gWAT of aged mice had regained more tissue mass than young mice (**Extended Data Fig. 2d**). Thus, aged mice exhibit higher adipocyte regeneration capacity, through earlier and faster *de novo* adipogenesis.

APCs from aged mice cell-autonomously have a high adipogenic capacity *in vivo*

Do APCs cell-autonomously undergo adipogenesis during aging? Alternatively, is there a systemic stimulus required for age-related adipogenesis? APCs were reported to be less immunogenic and less subject to rejection than other cell types following transplantation into mice³³. We then set out to test the *in vivo* adipogenic ability of APCs from gWAT of young vs. aged male mice through transplantation. An equal number of APCs (Lin-, CD45-CD31-Ter119-) from the stromal vascular fraction (SVF) of gWAT were mixed from 2.5-month-old male CAG-EGFP mice (GFP+, "young" APCs) and from 12-month-old male Rosa26-loxp-mTomato-stop-loxp-GFP (Rosa26-mT/mG) mice (Tomato+, "aged" APCs) (**Fig. 2a**). The cell mixture was transplanted into the sWAT of 2.5-month-old male wide type (WT) mice (**Fig. 2b–f, Extended Data Video 1**). When the Matrigel transplants were dissected one month later, we observed that more "aged" APCs (Tomato+) had differentiated into adipocytes (**Fig. 2b, Extended Data Video 1**), resulting in a 2.5-fold more adipocytes and 2.2-fold greater adipocyte volume compared to "young" APCs (GFP+) (**Fig. 2c, d**). These GFP+ and Tomato+ cells (differentiated from GFP+ or Tomato+ APCs) were indeed mature adipocytes, as they have a unilocular morphology (single lipid droplet) (**Fig. 2e**) and were perilipin positive (**Fig. 2f**). To further confirm our observation,

we also transplanted Tomato+ APCs from the gWAT of 2.5-month-old and 12-month-old male Rosa26-mT/mG mice separately into the different sides of sWATs of the same male WT mice (**Fig. 2g**). Four weeks after transplantation, the "aged" APCs had a significantly higher (3 folds) adipogenesis rate, compared to the "young" APCs (**Fig. 2h–j, Extended Data Video 2, 3**). Thus, the APCs from older mice maintain their high adipogenic potential after transplantation into a “young” environment.

To test if age-related systemic stimulation is sufficient to trigger APC adipogenesis, we then took the reverse approach and transplanted APCs from young mice into aged mice. Equal numbers of “young” Tomato+ APCs from gWAT of 2.5-month-old male Rosa26-mT/mG were transplanted into sWATs of 12-month-old and 2.5-month-old male WT mice (**Extended Data Fig. 3a**). Four weeks after the transplantation, no significant adipogenesis of the “young” APCs was observed in young or old recipient mice (**Extended Data Fig. 3b, Extended Data Video 4, 5**). Similarly, when GFP+ APCs from young CAG-EGFP mice were transplanted into aged WT mice (**Extended Data Fig. 3c**), the age-related systemic stimulation and microenvironment did not trigger significant adipogenesis of the “young” APCs (**Extended Data Fig. 3d, Extended Data Video 6, 7**).

Altogether, these transplantation results indicate that the "aged" APCs cell-autonomously exhibit greater adipogenic rate in the *in vivo* setting, regardless of “young” or “aged” microenvironment.

Aging generates a new APC population with a globally activated adipogenic program

To explore the molecular underpinning of the adipogenic properties of the APCs from aged

189 mice, we performed scRNA-seq of the CD45-CD31-Ter119- stromal vascular cells from the
190 gWAT of aged and young mice (n=3 mice/group) (**Extended Data Fig. 4a**). Among the 19,534
191 cells sequenced, 15,194 cells (78%) were identified as progenitor cells (**Extended Data Fig. 4b**),
192 based on the expression of classic APC markers CD34, CD29, PDGFR α , and Sca-1. While the
193 variation between mice within the same group was minimal (**Extended Data Fig. 4c**), there were
194 dramatic transcriptomic shifts between young and aged cells (**Extended Data Fig. 4d**).

195
196 We combined the "young" and "aged" progenitor cells and used unsupervised clustering of
197 gene expression profiles to identify five cell clusters (**Fig. 3a**). Based on recently published
198 scRNA-seq analysis of WAT APCs^{28,34,35}, we named these clusters: 1) Adipocyte stem cell (ASC);
199 2) intermediate adipocyte progenitor (IAP); 3) committed preadipocyte 1 (CP-1); 4) committed
200 preadipocyte 2 (CP-2); 5) committed preadipocyte, age-specific (CP-A). We found that aging
201 significantly remodeled the progenitor cell population, as "aged" cells had "shifted" away from
202 "young" cells, and cells from the two groups had little overlap (**Fig. 3b, c**). Using Slingshot
203 trajectory analysis, we revealed potential lineage relationships among these APC clusters (**Fig. 3d**,
204 **Extended Data Fig. 4e**). Within the "young" progenitor cells, ASC had two developmental
205 trajectories, both through IAP, and terminated as CP-1 and CP-2 (**Fig. 3d, Extended Data Fig.**
206 **4e**). Interestingly, within the "aged" progenitor cells, ASC had one additional, unique
207 developmental trajectory, which terminated as CP-A (**Fig. 3d, Extended Data Fig. 4e**).
208 Quantifying the percentage of each cell cluster showed that the CP-A population only existed in
209 aged mice, whereas the CP-1 population was dramatically reduced in aged mice (**Fig. 3e**). These
210 results suggest that the aging process generated a new adipogenic lineage from ASC to CP-A.

Next, we used CytoTRACE analysis and sorted the order of these APC clusters based on differentiation status as predicted by transcript abundance (**Fig. 3f, Extended Data Fig. 4f, g**). From stem cells to committed precursor cells, the order of these clusters was: ASC, CP-2, IAP, CP-1, and CP-A. For each type of cluster, the “Young” APCs were always more stem cell-like compared to the “aged” APCs. And among all clusters, the “aged” CP-A cluster was identified as the most committed APC. The mesenchymal progenitor marker *Pdgfra* was universally expressed in all clusters (**Fig. 3g**), but each cluster also showed unique markers (**Extended Data Fig. 4h**). ASC cluster expressed *Cd55*, *Pi16*, and *Dpp4* (**Fig. 3h, Extended Data Fig. 4h, i**); IAP cluster expressed common adipocyte progenitor markers (e.g., *Pdgfra*), and intermediate levels of ASC markers and committed preadipocytes (**Extended Data Fig. 4h**); CP-1 cluster expressed *Apoe*, *Igf1*, and *C7* (**Fig. 3i, Extended Data Fig. 4h**); CP-2 cluster expressed *Mfap4*, *Mgp*, and *Cilp* (**Fig. 3j, Extended Data Fig. 4h**). CP-A cluster were marked by expression of *Thbs1* and *Lifr* (**Fig. 3k, Extended Data Fig. 4h, j**). To determine which cluster(s) within the “aged” APCs undergo active adipogenesis during aging, we assessed the biological pathways enriched in the marker genes of each cluster. Interestingly, many genes involved in the adipogenic pathway is highly upregulated in the CP-A cluster, which is unique to the aged group (**Fig. 3l**). The enrichment of the adipogenic pathway in the CP-A cluster indicates that this population is uniquely generated during aging and may underlie age-related adipogenesis.

We then compared our clusters with the previously published APC clusters in WAT. For each of the published APC clusters in the indicated studies, gene module scores were calculated based on a list of significantly enriched marker genes. Burl et al. identified two major APC clusters (ASC1 and ASC2) in gWAT representing different cell states during adipogenesis. In

comparison¹⁵, the ASC cluster we noted highly overlapped with the Dpp4/Pi16/Cd55+ ASC2 cluster. Our CP-1 cluster highly overlapped with the Icam1/Igf1/Adam12+ ASC1 cluster. Our IAP and CP-2 clusters partially overlapped with both ASC1 and ASC2 clusters, and the CP-A cluster partially overlapped with the ASC1 cluster (**Extended Data Fig. 5a, b**). Sárvári et al. utilized single-nucleus RNA-seq to identify four major APC clusters in gWAT²⁰. Our ASC cluster overlapped with the FAP2, FAP3, and FAP4 clusters. IAP, CP-1, CP-2, and CP-A clusters partially overlapped with the FAP1 and FAP2 clusters (**Extended Data Fig. 5c, d**). Schwalie et al. identified three major APC clusters in sWAT¹⁷. Our ASC cluster overlaps extensively with the P1 cluster. Our IAP, CP-1, CP-2, and CP-A clusters overlap with the P2 cluster, and partially overlap with the adipogenesis-regulatory cells, the P3 cluster (**Extended Data Fig. 5e, f**). Taken together, the five major APC clusters we identified shared similarities with previously defined APC populations both in gWAT and sWAT. Despite the similarities, our study uniquely identified the CP-A cluster as an age- and depot-specific population with unique molecular characteristics.

Human visceral WAT shows similar patterns of age-related APC remodeling

To access the translational relevance of these findings, we set out to verify if humans also have the same age-specific CP-A population with scRNA-seq. CD45-CD31-CD235a- SVFs from human peripancreatic WAT (5 human samples of different sexes and ages) were used for scRNA-seq. Among the 24,794 cells sequenced, 20,431 cells (82%) were identified as progenitor cells (**Extended Data Fig. 6a**). Unsupervised clustering of gene expression profiles divided these APCs into eight cell clusters (Hu0-Hu7) (**Fig. 3m**). To compare the identified APC populations from mice with the human clusters, we calculated the ASC and CP-A gene module score as a sum of mouse marker genes (**Fig. 3n**). Clusters Hu0, 2, 3, 6 were enriched with mouse ASC markers,

while clusters Hu1, 5, 7 were enriched with mouse CP-A markers (**Fig. 3n**). Thus, both mouse ASC and CP-A similar cells can be identified in human visceral WAT. All progenitor cells showed abundant expression of APC marker PDGFRA (**Extended Data Fig. 6b**) and PDGFRB (**Extended Data Fig. 6c**). Human clusters similar to mouse ASC had high expression of the ASC markers CD55 and Pi16+ (**Extended Data Fig. 6d, e**). Human clusters similar to mouse CP-A had high expression of the CP-A marker LIFR (**Extended Data Fig. 6f**), and moderately (or partially) expressed *PPARG*, *FABP4*, and *LPL* (**Extended Data Fig. 6g–i**). We then used flow cytometry to analyze the LIFR+ population in human peripancreatic WAT of different age groups. Among the four samples obtained from male donors, we isolated SVFs and enriched APC (CD31-CD45-CD235a-PDGFR α + cells) (**Extended Data Fig. 6j**), and found a trend for the LIFR+ population to dramatically increase with age (**Fig. 3o**). Thus, APCs in human vWAT may undergo similar remodeling during aging, and the human CP-A population may also contribute to sarcopenic obesity.

CP-A population has a high proliferation and adipogenesis rate both *in vitro* and *in vivo*

Pathway analysis suggested that proliferation-related pathways, such as TGF β ¹⁸ and mitotic signaling, are enriched in the “aged” ASCs compared to the “young” ASCs (**Fig. 4a, Extended Data Table 1**). Moreover, the adipogenesis pathway is highly enriched in the age-specific CP-A population (**Fig. 4b, Extended Data Table 2**). This analysis suggests that the “aged” ASC and CP-A populations may have increased proliferation and inflammation, and the CP-A population also has increased adipogenesis.

We then determined the capacity of total APC to proliferate and differentiate into adipocytes

in 3D culture *in vitro* (**Fig. 4c**). The enriched APCs were first mixed with Matrigel and cultured in the growth medium for 72 hours. An adipogenic cocktail was then added to differentiate these cells into adipocytes. Starting with the same number of cells, “aged” APCs proliferated into more cells compared to the “young” APCs (**Extended Data Fig. 7a**). However, the adipogenesis rate (the percentage of Bodipy⁺ adipocytes in Tomato⁺ cells) for both “young” and “aged” APCs were similarly high, close to 100%. The advantage of the 3D culture system is that we do not need the APCs to grow densely and attach prior to adipogenesis. We then added the adipogenic cocktail 16 hours later after seeding to the enriched APC populations (**Extended Data Fig. 7b**). “Aged” APCs had higher (but not significant) adipogenesis rate, and significantly higher proliferation rate, as the total Tomato⁺ cell number from “aged” APCs was more than 2 folds greater than the “young” APCs (**Extended Data Fig. 7b**).

Flow analysis showed that the LIFR⁺ (CP-A) population did significantly increase with age (**Extended Data Fig. 7c**). In light of results from the pathway analysis, we next inquired whether the CP-A population generated during aging has greater capacity in proliferation and differentiation. We next enriched individual APC populations and tested their proliferation and adipogenic capacity *in vitro*. To minimize stress to cells, a magnetic beads-based negative selection was used to enrich APCs (Lin⁻, CD31-CD45-Ter119⁻) from freshly isolated SVFs from the gWAT of young and aged male Rosa26-mT/mG mice. To enrich the ASC population, we isolated the DPP4⁺ population from APCs. DPP4⁺ and DPP4⁻ populations were validated by flow analysis (**Extended Data Fig. 7d**). To enrich the CP-1 and CP-A population, negative selection, depleting DPP4⁺ ASC, CD9⁺ CP-2, and IAP populations in APCs were employed. The enriched populations were validated and showed a low percentage of DPP4 and CD9 expression and a high percentage

of LIFR expression on flow analysis (**Extended Data Fig. 7e, f**). Interestingly, more DPP4⁺ APCs from the gWAT of aged male mice switched from CD9⁻ cells to CD9⁺ cells, suggesting that a certain number of cells in the aged ASC population may have started the adipogenesis program along the trajectory to IAP (**Extended Data Fig. 7g**). This also validates that aged ASCs are more differentiated than young ASC as suggested by the CytoTRACE analysis (**Fig. 3f**). The “aged” ASC population had an adipogenesis rate similar to the “young” ASCs, but they had a significantly higher proliferation rate (**Fig. 4d, e**). We also compared the age-specific CP-A population with the “young” CP-1 population, which shares the highest molecular similarity with the CP-A population according to the scRNA-seq results. Importantly, the CP-A population not only had a more than four folds higher proliferation rate compared to the CP-1 population, but had the highest adipogenesis rate among all populations tested (**Fig. 4f, g**). Thus, the CP-A population, uniquely generated with age, has the most remarkable capacity for proliferation and differentiation *in vitro*.

We then enriched APC populations and tested their proliferation and adipogenic capacity *in vivo*. A large amount of DPP4⁻ APCs (containing all APC populations other than ASC), CP-1, and CP-A populations were collected from freshly isolated SVFs from gWAT of 2.5-month-old and 12-month-old male Rosa26-mT/mG mice, and equal numbers of these enriched “young” and “aged” populations were transplanted into the different sides of the sWATs of male WT mice (**Fig. 4h**). The “aged” DPP4⁻ APCs grew substantially larger in volume compared to the “young” DPP4⁻ APCs (**Fig. 4i–k, Extended Data Video 8, 9**). More notably, the CP-A population grew six times larger in volume compared to the CP-1 population (**Fig. 4l–n, Extended Data Video 10, 11**), indicating that the CP-A population has a striking ability to proliferate *in vivo*. Not surprisingly, most of the CP-A population differentiated into adipocytes.

Taken together, both the *in vitro* and *in vivo* results indicate that the CP-A population has a greater capacity for proliferation and adipogenesis compared to the other APC populations.

Enhanced interactions from macrophages to “aged” APCs

Aging has been reported to alter the immune system in WAT²⁶⁻²⁸, we next investigated potential immune cell-APC interactions. We performed scRNA-seq of the CD45+ SVFs from the gWAT of aged and young mice (n=3 mice/group: the same group of mice were used for the APC analysis), which identified 10,486 cells (**Extended Data Fig. 8a**). Unsupervised clustering of gene expression profiles identified all the major immune cell clusters, including macrophage and M2-like macrophage, T cell, B cell, and neutrophil (**Extended Data Fig. 8a, b**). The variation between mice within the same age group was minimal (**Extended Data Fig. 8c**), but there was not much mixture of the “aged” cells with the “young” cells in each immune cluster, reflecting a dramatic remodeling of immune cells by aging (**Extended Data Fig. 8d**). Flow cytometry showed no difference in the percentage of total macrophage or M2-like macrophage in total immune cells isolated from the gWAT of young and aged mice (**Extended Data Fig. 8e**).

We inferred the interaction frequency between immune cells and APC populations based on ligand-receptor pairs using the CellphoneDB database and the iTALK database, which indicated that the interactions between macrophages and APC populations are dramatically increased in aged mice (**Extended Data Fig. 8f**). We then analyzed the one-way ligand-receptor communication from immune cells to APC populations. We selected three ligand-receptor groups: adipogenesis (**Extended Data Fig. 8g, h**), TGF β signaling (**Extended Data Fig. 8i, j**), and insulin signaling

(**Extended Data Fig. 8k, l**). Among all the immune cell types, macrophages were found to have increased ligand-receptor pairing with all of the APC populations. We then narrowed down the analysis by selecting one-way ligand-receptor communication only from macrophages to APC populations (**Extended Data Fig. 9**). More ligand-receptor pairing showed up as we narrowed down the cell types, again, signals that promote adipogenesis (**Extended Data Fig. 9a**), TGF β (**Extended Data Fig. 9b**), and insulin signaling (**Extended Data Fig. 9c**), were uniquely increased from the “aged” macrophages to “aged” ASC. Signals sent from the “aged” macrophages to the “aged” CP-A were compared with signaling sent from the “young” macrophages to the “young” CP-1 (**Extended Data Fig. 9d–i**). Indeed, from the “aged” macrophages to “aged” CP-A, signaling that promotes adipogenesis (**Extended Data Fig. 9d, g**), and insulin signaling (**Extended Data Fig. 9f, i**), were increased both in the number of ligand-receptor pairs and the signal intensity. TGF β signaling was not altered in the number of ligand-receptor pairs, but many of them did increase in signal intensity in the aged condition (**Extended Data Fig. 9e, h**). In summary, the increased proliferation and adipogenesis phenotype in the “aged” APC subpopulations is highly likely regulated by local macrophages.

Evidence of macrophage-APC crosstalk suggested possible effects on phenotype. We then enriched APCs and tested if macrophages could promote the phenotype of the “aged” APCs. APCs (Lin⁻, CD31-CD45-Ter119⁻) from freshly isolated SVFs from the gWAT of young and aged male Rosa26-mT/mG mice were mixed with macrophages from young and aged male WT mice. Matrigel containing the cell mixture was seeded in the growth medium for 16 hours, then the adipogenic cocktail was added to drive adipocyte differentiation (**Extended Data Fig. 10a**). Macrophages, regardless of the age of the donor mice, increased the proliferation rate of APCs of

both “young” and “aged” mice. However, co-culture with macrophage did not increase the adipogenesis rate of APCs of both “young” and “aged” mice (**Extended Data Fig. 10b**). We note that the in vitro co-culture experiment may not capture the role of macrophages in the remodeling of APCs during aging, which may take weeks and months.

Discussion

As a highly plastic organ, WAT alters in volume in adaptation to a variety of physiological and pathological metabolic challenges. We and others have shown that without any metabolic challenge, healthy young adult mice or humans have extremely low adipocyte turnover rates. WAT plasticity under many physiological and pathological challenges has been extensively studied, such as cold exposure and high fat diet feeding^{9,10}. However, the expansion of WAT during early aging is surprisingly understudied. In this study, we provide the first cellular and molecular evidence that aging triggers massive adipogenesis of unique APC populations (**Extended Data Fig. 10c**).

Among the two major fat depots, vWAT and sWAT, vWAT has the most adipogenesis during aging. As vWAT and sWAT are fundamentally distinct organs with distinct development timelines, molecular signatures, and metabolic functions^{9,10,29}, it is not surprising that age-related adiposity happens in the vWAT at a much higher rate. Compared to hypertrophy, which gives WAT only limited potential to expand, adipogenesis is alarming because it grants WAT unlimited potential for growth. Our findings on the age-related adipogenesis, therefore, highlights adipogenesis but not hypertrophy, as the key process to intervene for age-related obesity and other associated diseases.

Age-related adiposity accelerates aging and shortens life expectancy. Signaling pathways that are known to extend lifespan, e.g., SIRT, FOXO, and JNK, often inhibit adipogenesis (the making of new adipocytes) and decrease WAT mass³⁰⁻³⁴. Conversely, signaling pathways that are known to shorten lifespan, e.g., insulin/IGF-1 and mTOR, often do the opposite³⁵⁻³⁷. Similarly, strategies that promote longevity and youthfulness often inhibit WAT expansion in aged animals and older humans, such as caloric restriction³⁸⁻⁴⁰ and fasting⁴¹. These critical studies suggest that inhibiting WAT expansion during aging is closely linked to increased healthspan and lifespan. Our study expands on this and suggests that to limit WAT expansion during aging, it is crucial to understand the underlying mechanism of age-related adipogenesis.

Our *in vivo* transplant and *in vitro* differentiation results suggest that “aged” APCs cell-autonomously have a greater adipogenic capacity, even when disassociated with the aging microenvironment. However, the aging microenvironment is potentially crucial for driving the remodeling of APCs, which may have started during early aging. Dr. James Kirkland’s group elegantly showed that APCs could undergo cellular senescence and lose the ability to differentiate into adipocytes⁴². Therefore, their study suggested that cellular senescence contributes to age-related fat loss, which happens in the very aged population. In this study, we focused on WAT expansion during early and middle-aging. Our study indicates that although some APCs may become senescent and stop undergoing adipogenesis during aging, sufficient APCs are generated during early aging and contribute to the massive adipogenesis we observed *in vivo*. Interestingly, senescent cells accumulate specifically in vWAT during early aging⁴³⁻⁴⁵, and we showed that the vWAT has the highest adipogenic rate. It is possible that the senescent microenvironment promotes adipogenesis of APCs in vWAT during early aging. Firstly, cellular senescence

positively correlates with WAT mass. Inducing cellular senescence through genetic manipulation often results in WAT expansion^{46,47}; in addition to accumulating during aging, senescent cells also accumulate in obesity, especially in the vWAT⁴⁸⁻⁵³, which has active adipogenesis. Secondly, senescent cells are known to stimulate adult stem cell proliferation and differentiation or tumor proliferation⁵⁴⁻⁵⁶. Lastly, inflammatory signals are known to promote adipogenesis⁵⁷⁻⁵⁹; senescent cells may promote adipogenesis through the senescence-associated secretory phenotype⁶⁰⁻⁶², which contains numerous pro-inflammatory cytokines. Currently, we are trying to determine if cellular senescence promotes adipogenesis by remodeling the APC niche.

Driven by the state-of-art scRNA-seq technique, several elegant studies provided new insights into APC heterogeneity in a fat depot-specific manner^{63,64}. Around four major APC subpopulations had been identified, including 1) adipose stem cells (ASCs)^{15,16,18}, which is also identified in our study as the ASC population; 2) committed preadipocytes (adipocyte progenitors)^{15,16,18}, which is also identified in our study as the CP-1 and CP-2 populations; 3) fibro-inflammatory progenitors (FIPs)^{16,65,66}; and 4) adipogenesis regulators (Aregs). Aregs inhibit adipogenesis and were found in both adult sWAT¹⁷ and aged sWAT²¹. Our ASC cluster bears a close resemblance to the gWAT PDGFR β + stromal subpopulation¹⁶. The gene signatures of our ASC cells are molecularly similar to those of APCs (adipogenic progenitor cells) and CPs (committed preadipocytes), the two adipogenic clusters defined previously¹⁶. In the same study, another anti-adipogenic subpopulation, or FIPs (fibro-inflammatory progenitors), was also defined in gWAT. In our gWAT scRNA-seq data, the FIP markers were not apparently enriched within the defined clusters, likely because the previous study analyzed pre-selected PDGFR β + cells, which represent a fraction of SVF cells so that FIPs are not resolved by scRNA-seq in our samples. In the present study, we identified a new,

committed preadipocyte population, the CP-A population, which is uniquely generated in the vWAT of aged mice and older humans. Importantly, the CP-A cells displayed superior capacity, compared to all other known APC subpopulations, to proliferate and differentiate into mature adipocytes, both *in vitro* and *in vivo*.

The CP-A markers LIFR and Thbs1 distinguish these cells from other committed preadipocytes. LIFR forms a heterodimer with gp130 and induces JAK-STAT3 signaling pathway⁶⁷, which has been demonstrated to promote adipogenesis from APCs⁶⁸. Pathway Enrichment analysis also indicates upregulated JAK-STAT3 pathway in the CP-A population, suggesting the activation of LIFR signaling. The other CP-A marker, Thbs1 is recognized as an adipokine that is associated with obesity and insulin resistance⁶⁹. Adding exogenous Thbs1 was found to promote the proliferation of 3T3-L1 cells⁷⁰. Thbs1 also activates TGFβ1 signaling⁷¹, which might promote a positive interacting loop between ASC and CP-A populations. Thus, LIFR and Thbs1 may play essential roles in promoting CP-A proliferation and differentiation.

In addition to the discovery of the new age-specific CP-A population, our scRNA-seq data showed that the other APC populations also had little overlap between the aged and young groups, indicating that the aging process significantly transformed all APC sub-populations. Indeed, we found that the “aged” ASC population had a higher proliferation rate, which is also essential for age-related adipogenesis. Interestingly, a large proportion of aged ASCs also expressed CD9, a representative marker of IAP and CP-2 populations. These findings suggest that aged ASCs might have started the differentiation program and have a higher tendency to differentiate into mature adipocytes. CD9 is a marker of the FIP population¹⁶. Thus, CP-2 is likely to be the analogous

population of FIPs. CD9+ progenitors have been reported to control adipose tissue fibrosis⁷². As vWAT fibrosis progresses during aging⁷³, the ASC to CP-2 transition could contribute to age-related adipose fibrosis and metabolic dysfunction. Considering the presence of anti-adipogenic stromal cell subpopulations as identified in multiple fat depots^{16,17,21,65,66}, it is not excluded that the age-dependent gWAT hyperplastic expansion can be ascribed to the dysregulation of suppressive mechanisms governed by anti-adipogenic cells such as FIPs or Aregs, which also requires further investigation in the future.

Besides APCs, various immune cell types have been reported to regulate APC differentiation and adipose homeostasis under different conditions in mice and humans^{26,74-76}. Similar to APCs, there was a significant shift in nearly all the immune cells in aged gWAT. We also showed that adipose tissue macrophages (ATMs) account for the majority cell type among the immune cells. These ATMs are Lyve1+, indicating that they might resemble the previously reported perivascular-like macrophages (PVM)²⁰. They also have a high expression level of Retnla, a specific marker of adipose infiltrating M2 macrophages⁷⁷. This suggests that adipose inflammation in age-related obesity is different from that in high fat diet-induced obesity. In accordance with this, these ATMs did not express Trem2, a marker of previously identified lipid-associated macrophages (LAM) emerging in gWAT after high fat diet feeding⁷⁵. Additionally, CD44 signaling was increased in the aged ASC population, which has been reported to mediate the interaction with macrophage⁷⁸. Co-culture with ATMs did not increase the differentiation of APCs, but did promote cell growth, suggesting that ATMs regulate APCs during aging. Ligand-receptor analysis suggests that the cell-cell communications between macrophages and APCs increase in aged mice compared to other immune cell types. There would be a positive feedback loop within ATMs and APCs in mediating

age-related adipogenesis. Besides, a recent paper demonstrates that adipose-resident macrophages promote adipocyte hypertrophy through PDGFC⁷⁹. Our scRNA-seq analysis indicates that Pdgfc is highly expressed in the MAC2 population, which may indicate that hyperplasia rather than hypertrophy is the dominant factor to control age-related adiposity. Thus, how macrophages remodel APCs during aging needs further investigation.

In conclusion, we found that massive adipogenesis is triggered during aging, and largely contributes to age-related visceral adiposity. We then discovered a new, age-specific APC subpopulation: the CP-A cells, which cell-autonomously mastered high proliferation and adipogenesis capacity. Future studies will need to determine how the CP-A population emerges during aging and whether the immune-regulation of adipose tissue plasticity during aging plays a role in this process. It is worth further investigating the crosstalk between different immune cell types and all the APC subpopulations to better understand the molecular and cellular dynamics in adipose remodeling to pinpoint key regulatory cell types and genes. Other than immune cells, other key regulators discussed above, such as cellular senescence, adipose fibrosis, may also remodel the APC subpopulations and promote the generation of the CP-A population. Most importantly, effective strategies eliminating the CP-A population or inhibiting its proliferation and differentiation will prevent the development of visceral adiposity during aging, improve age-related metabolic disorders, and eventually increase healthspan and lifespan.

Author contributions

QAW and G.W. designed the experiments. QAW, G.W., G.L., and X.Y. wrote the manuscript. G.W., A.S., and Q.A.W. handled all the mouse experiments. G.W. and J.D. performed mouse MRI

and metabolic cage studies. G.W., Q.A.W., A.S., and N.S. performed β -gal staining. G.W. and N.S. performed 2D immunofluorescence staining and imaging. G.W. and W.D. prepared the primary SVFs for scRNA-seq, H.Q. and X.W. conducted the scRNA-seq experiments. G.L. and X.Y. analyzed the scRNA-seq data. A.L. performed tissue embedding and sectioning. G.W. and N.S. performed all the *in vivo* transplantations. G.W. performed all *in vitro* culture and co-culture experiments. G.W. and B.A. performed all the 3D immunofluorescence imaging. G.W. and L.B. performed FACS and analysis. P.F., J.C. and L.J. contributed to experimental design and discussion.

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Method

Mice

Mice were maintained in a 12h dark/light cycle and housed in groups of three to five with unlimited access to water and food (chow diet, number 5058, lab diet; or doxycycline chow diet [600 mg/kg], S4107, Bio-Serv, as described for individual experiments). All mice were on a pure C57BL/6J background. Adn-rtTA and FAT-ATTAC mouse lines were generated in the Scherer laboratory as previously described^{1,2}. TRE-Cre (Jax stock no. 006234), Rosa26-loxP-STOP-loxP-LacZ (Jax stock no. 003309), Rosa26-loxP-STOP-loxP-mT/mG (Jax stock no. 007676), and CAG-EGFP (Jax Stock no. 006567) mouse lines were obtained from the Jackson Laboratories. The Institutional Animal Care and Use Committees of City of Hope, Duarte, have approved all animal experiments.

Metabolic cage studies were performed in the City of Hope Comprehensive Metabolic Phenotyping Core facility. Oxygen consumption and energy expenditure were calculated relative to lean mass. GTT was performed in mice without access to food for 12 h before administration of 2 g/kg body weight glucose by intraperitoneal injection. ITT was performed in mice without access to food for six hours before administering 0.75 U/kg body weight insulin (Eli Lilly) by intraperitoneal injection. To induce adipocyte apoptosis in FAT-ATTAC mice, AP21087 (Ariad Pharmaceuticals) in 4% ethanol, 10% PEG-400, 1.75% Tween-20 in water were administered by intraperitoneal injection at a dose of 0.2 µg/g body weight every day for 7 days.

β-gal staining

Mice were anesthetized and perfused with 0.2% glutaraldehyde in PBS. Adipose tissue was collected and chopped into small pieces. The tissue pieces were fixed in 0.2% glutaraldehyde/PBS overnight and followed by washed with rinse buffer (100 mM sodium phosphate, 2 mM MgCl₂, 0.01% sodium deoxycholate, and 0.02% NP-40) three times. Then the tissue pieces were stained with 1 mg/mL β -gal in staining buffer containing 5 mM potassium ferricyanide and 5 mM potassium ferrocyanide for 48 h at room temperature with mild shaking. After staining, the tissue pieces were moved into 10% neutral buffered formalin overnight for post-fixation. The fixed tissues were processed with a standard paraffin tissue embedding protocol to produce 7 μ m sections by the City of Hope Pathology Core facility. The sections were then counterstained with nuclear fast red and imaged under a microscope (Keyence).

Isolation of mouse adipose SVFs and enrichment of APC subpopulations

gWAT was minced and transferred to a 50 mL Falcon tube containing 10 mL digestion buffer (100mM HEPES pH 7.4; 120mM NaCl; 50mM KCl; 5mM Glucose; 1mM CaCl₂, 1.5% BSA, and 1 mg/mL collagenase D). The tissue was incubated in a 37°C shaking water bath for 30 min. Digestion was stopped by adding 35 mL 2% FBS in PBS. The solution was passed through 100 μ m and then 40 μ m strainers and centrifuged at 400 g for 5 min. The supernatant was aspirated, and the SVF pellet was collected.

Lin⁻ SVF and enriched ASC, CP-A, CP-1 were obtained by MACS (Stemcell Technologies, Cat# 17656). To obtain Lin⁻ SVF, anti-mouse-CD45-PE, anti-mouse-CD31-PE, and anti-mouse-Ter119-PE (1:200, BioLegend) were used. To enrich the ASC subpopulation, additional anti-mouse-DPP4/CD26-PE antibody (1:50, BioLegend) was used. To enrich CP-A and CP-1

subpopulations, the above 4 antibodies plus anti-mouse-CD9-PE (1:200, BioLegend) antibodies were used. To obtain immune cells from gWAT, anti-mouse-CD45-PE was used and isolated by MACS.

Flow cytometry

For flow cytometry analysis, cells were blocked with anti-mouse-CD16/32 (BioLegend, 1:500) for 10 min, and then stained with anti-mouse-CD45-Pacific Blue (BioLegend, 1:400), anti-mouse-CD31-Pacific Blue (BioLegend, 1:400), anti-mouse-Ter119-Pacific Blue (BioLegend, 1:400), anti-mouse-CD140a-APC (BioLegend, 1:200), anti-mouse-DPP4/CD26-PE/Cy7 (BioLegend, 1:100), anti-mouse-CD9-PerCP/Cy5.5 (BioLegend, 1:400), anti-LIFR (Proteintech; 1:200), and anti-rabbit-PE/Cy5.5 (Invitrogen, 1:200) ; anti-human-CD45-PE (BioLegend, 1:400), anti-human-CD31-PE (BioLegend, 1:400), anti-human-CD235a-PE (BioLegend, 1:400), and anti-human-CD140a-APC (BioLegend; 1:100) for individual experiments for 20 min on ice. After antibodies incubation, the cells were washed once with 2% FBS/PBS, and analyzed by BD Fortessa cytometer. The flow plots were analyzed by FlowJo (V10).

For FACS isolation of macrophages from gWAT, total SVF were blocked with anti-mouse-CD16/32 (BioLegend, 1:500), and then stained with anti-mouse-CD45-PE (BioLegend, 1:400), anti-mouse-CD11b-APC (BioLegend, 1:200), and anti-mouse-F4/80-Alexa Fluor 488 (BioLegend, 1:200). Macrophages were obtained using BD Aria Fusion.

Isolation of human adipose SVFs

Peri-pancreatic WAT samples from deceased cadaveric donors were dissected from the pancreas (process time within ~8-12 hours). All of the proposed experiments will be immediately performed using the fresh tissue samples, without freezing or any preservation. The sampling and examination of human tissues have been approved by the Ethics Committee of COH. WAT from five donors (male, 44-year-old; male, 21-year-old; female, 42-year-old; male, 24-year-old; male, 57-year-old) were used for scRNA-seq, and WAT from four donors (all males, 39, 56, 57, and 58-year-old) were used for flow cytometry analysis. Adipose tissue was minced and transferred to a 50 mL Falcon tube containing 10 mL digestion buffer (1 × PBS, and 10 mg/mL collagenase D). The tissue was incubated in a 37°C shaking water bath for 45 min. Digestion was stopped by adding 35 mL 2% FBS in PBS. The solution was passed through a 250 µm mesh (Spectrum), and then 100 µm and 40 µm strainers, and centrifuged at 600 g for 5 min. The supernatant was aspirated, and the SVF pellet was collected. To obtain Lin- SVFs, cells were incubated with anti-human-CD45-PE, anti-human-CD31-PE, and anti-human-CD235a-PE (1:200, BioLegend), and isolated by MACS (Stemcell Technologies, Cat# 17654). To enrich immune cells, anti-human-CD45-PE (1:200, BioLegend) was used and isolated by MACS.

scRNA-seq

Lin- SVF and CD45+ immune cells from 2.5-month-old or 12-month-old C57BL/6J male mice gWAT or Lin- SVFs from human peri-pancreatic adipose tissue (Extended Data Supplementary Table 4) were freshly collected and resuspended in PBS containing 0.04% BSA at a concentration of 800~1000 cells/µl. Cell number and viability were measured by trypan blue using a TC20 Automated Cell Counter (BioRad). Single-cell RNA libraries were prepared by the City of Hope Integrative Genomics Core facility according to the ChromiumTM Single Cell 3' Reagent Kits v3

User Guide (10x Genomics). Approximately 10,000 cells were loaded on a Chromium single cell Controller instrument (10x Genomics) to form single cell gel beads in emulsion (GEMs). After reverse transcriptions, GEMs were harvested and the cDNAs were amplified and cleaned up with the SPRIselect Reagent Kit (Beckman Coulter). The barcoded sequencing libraries were constructed using the Chromium Single-Cell 3' Library Kit (10x Genomics) for enzymatic fragmentation, end-repair, A tailing, adaptor ligation, ligation cleanup, sample index PCR, and PCR cleanup. Libraries were then sequenced with a NovaSeq 6000 instrument (Illumina) with a depth of 50-100 k reads per cell.

scRNA-seq data processing and quality control

The 10X Genomics Cell Ranger version 3.0.2 single-cell software³ was used to perform sample demultiplexing sequencing alignment to mouse genome mm10, filtering, and unique molecular identifier (UMI) counting to generate gene count matrices. Single cells were identified from background noise by filtering on the proportion of mitochondrial reads (threshold: < 10%), the number of UMI (thresholds: 700-22,000), and the number of detected genes (thresholds: 200-6,000).

Identification of cell clusters

The Seurat R package version 3.0⁴ was used to determine cell clusters by the Louvain algorithm based on similarities in the transcriptome patterns and visualized with t-Distributed Stochastic Neighbor Embedding (t-SNE). The optimal number of Principal Components used for t-SNE/UMAP dimensionality reduction and Louvain clustering was determined using the Jackstraw permutation approach and a grid-search of the parameters. The identities of the cell types were

resolved by comparing the cell cluster specific marker genes expressed in each cluster in our own dataset, as identified with a Wilcoxon rank sum test, with known cell-type specific markers curated from literature, single cell atlases, and previous studies in the SVF. To be considered in the cell cluster marker analysis, a gene had to be expressed in at least 10% of the single cells from the cluster of interest and there had to be at least a 0.25 log fold change in the cell cluster of interest than in other cells. A multiple testing was corrected using the Benjamini-Hochberg method to estimate false discovery rate (FDR).

Identification of differentially expressed genes (DEGs) and pathways

To quantitatively determine which genes were affected by aging, we compared the cell transcriptome of each cell type between age groups using a Wilcoxon rank sum test. To be considered in the analysis, a gene had to be expressed in at least 10% of the single cells from at least one of the two groups for that cell type and there had to be at least 1.1-fold change in gene expression between the groups. Multiple testing correction was done using the Benjamini-Hochberg method to estimate FDR.

For ASC, CP-1 and CP-2 populations shared between both the young and aged mice, the DEGs were identified by comparing the two age groups and then subjected to pathway enrichment analysis. Since CP-A is only present in the aged group, the CP-A marker genes in the aged condition were used as DEGs and subject to pathway enrichment analysis. Pathways from HALLMARK⁵ were assessed for overlap with the DEGs using Fisher's exact test and corrected for multiple testing using the Benjamini-Hochberg method to estimate FDR.

Single-cell trajectory analysis

To infer cell lineages of complex communities of heterogeneous cells, pseudo-temporal reconstruction was performed by two methods. The Slingshot R package⁶ was used first to infer multiple branching lineages based on the minimum spanning tree followed by simultaneous principal curves. The Cytotrace R package⁷ was then used to estimate the developmental potential of ten cell sets (five APC subtypes in “aged” and “young” conditions) based on feature selected gene-count measures.

Cell-cell communication analysis

Both the Cellphone DB database⁸ and the iTALK database⁹ were used to curate ligand-receptor pairs that may mediate intercellular cross-talk between the APC subtypes and immune cell types. Pathway enrichment of the ligand-receptor pairs between cell types were further analyzed based on the overlap with selected pathways from KEGG¹⁰, Reactome¹¹, BIOCARTA¹², and HALLMARK⁵. Top ligand-receptors were ranked based on the formula: the expression value of the ligand gene from the ligand-secreting cell type X the expression value of the receptor gene from the receptor cell type.

***In vivo* transplantation of APCs and APC subpopulations**

Lin⁻ SVF from the epididymal white adipose tissue (gWAT) of 12-month-old aged Rosa26-loxP-STOP-loxP-mT/mG and 2.5-month-old young CAG-GFP mice were isolated and mixed together at a 1:1 ratio. The cells were resuspended in Matrigel (Corning). A total volume of 20 μ l Matrigel was injected into the subcutaneous adipose depot of recipient mice at a concentration of 10,000 cells/ μ l. After four weeks, the recipient mice were euthanized, and sWAT was collected.

The Matrigel containing transplant was dissected under a fluorescence microscope (Keyence), and the whole transplant was captured under a confocal microscope (Zeiss, LSM880).

For single Tomato+ SVF transplant, Lin- SVF from the epididymal white adipose tissue (gWAT) of 12-month-old aged and 2.5-month-old young Rosa26-loxP-STOP-loxP-mT/mG mice were isolated. The cells were resuspended in Matrigel (Corning). A total volume of 20 μ l Matrigel was injected to the subcutaneous adipose depot of recipient mice at a concentration of 5,000 cells/ μ l. The comparable aged and young Lin- SVF were injected to either side of the subcutaneous adipose depot of the same recipient. After four weeks, the recipient mice were euthanized, and subcutaneous adipose tissue was collected.

For transplantation of ASC, CP-1, and CP-A, each cell population was isolated from Rosa26-loxP-STOP-loxP-mT/mG and resuspended in Matrigel at a concentration of 1,000 cells/ μ l. Then 20 μ l Matrigel of ASC from 12-month-old aged and 2.5-month-old young mice, or enriched CP-A from aged mice and CP-1 from young mice were injected into either side of sWATs of recipient mice. After two weeks, the recipient mice were euthanized, and subcutaneous adipose tissue was collected.

For 3D imaging and quantification of Matrigel transplants, The Matrigel containing transplant was dissected under a fluorescence microscope (Keyence), and the whole transplant was captured under a confocal microscope (Zeiss, LSM880). Original files were imported to Imaris (Bitplane). All the images and videos were made using Imaris. Quantification of mature adipocyte number

and volume were analyzed by the spot function of Imaris. The function parameters were kept the same between different samples.

***In vitro* 3D differentiation and differentiation of APCs and APC subpopulations**

Lin- SVF or enriched ASC, CP-1, and CP-A populations were isolated from gWAT of Rosa26-loxP-STOP-loxP-mT/mG mice that were 12-month-old or 2.5-month-old. The cells were cultured as described previously¹³. After isolation by MACS, the cells were resuspended in complete SVF culture medium (DMEM/F12, Life technologies 10565018) containing 10% FBS (Sigma, 16H328), 1% penicillin/streptomycin, (Hyclone SV30010), and 0.1% gentamicin (Life technologies 1855724) at a concentration of 2,000 cells/ μ l. The same volume of Matrigel was added to the suspension, and 10 μ l mixture was added to the 12-well plate. The plate was incubated in the 37°C CO₂ incubator for 15 min to induce the formation of the gel. Then complete SVF culture medium was added to the wells, and the cells were cultured in the 37°C CO₂ incubator. After three days or 16 hours, an adipogenic cocktail containing dexamethasone (1 μ M), insulin (5 μ g/ml), isobutylmethylxanthine (0.5 mM), and rosiglitazone (1 μ M) was added to induce adipocyte differentiation. After six days of adipogenic induction, the cells were then stained with Bodipy (Invitrogen) and imaged under a confocal microscope (Zeiss, LSM880). The images were analyzed using Zen blue (Zeiss).

***In vitro* 3D macrophage and APC co-culture**

Lin- SVF were isolated from gWAT of Rosa26-loxP-STOP-loxP-mT/mG mice that were 12-month-old or 2.5-month-old. Macrophages were isolated from gWAT of C57BL/6 mice that were 12-month-old or 2.5 month-old by FACS. “Aged” Lin- SVF and “aged” macrophages, “aged” Lin-

SVF and “young” macrophages, “young” Lin- SVF and “aged” macrophage, and “young” Lin- SVF and “young” macrophages were mixed at 1:1 ratio in the complete SVF medium at a concentration of 4,000 cells/ μ l. The same volume of Matrigel was added to the suspension, and 10 μ l mixture was added to the 12-well plate. The plate was incubated in the 37°C CO₂ incubator for 15 min to induce the formation of the gel. Then complete SVF culture medium was added to the wells, and the cells were cultured in the 37°C CO₂ incubator. After 16 hours, the adipogenic cocktail was added. Adipocytes were stained with Bodipy (Invitrogen) 6 days after adipogenic induction. Images were captured under a confocal microscope (Zeiss, LSM880), and analyzed using Zen blue (Zeiss).

Histological analysis

10% formalin-fixed tissues were processed with a standard paraffin tissue embedding protocol to produce sections for H&E staining by the City of Hope Pathology Core facility. For immunofluorescence staining, the sections were dewaxed in xylene and rehydrated in a graded series of ethanol to ddH₂O. Slides were placed in 10 mM sodium citrate buffer and boiled for 30 min for antigen retrieval, then blocked in PBST with 5% BSA for 30 min. Primary antibodies used were LIFR (1:200, Proteintech), PDGFR α (1:400, R&D), Perilipin (1:500, Novus), F4/80 (1:400, Abcam), GFP (1:400, Abcam), and mCherry (1:100, Abcam) as described for individual experiments, and incubated at 4°C overnight. After three times wash with PBS, secondary antibodies used were Alexa Fluor 647 Donkey-anti-Rabbit IgG H&L (1:200, Invitrogen), Alexa Fluor 488 Donkey-anti-Goat IgG H&L (1:200, Invitrogen), Alexa Fluor 594 Donkey-anti-Rat IgG H&L (1:200, Invitrogen), and Cy3 Donkey-anti-Chicken IgG H&L (1:200, Jackson Immuno, Inc.), and incubated at room temperature for 2 hours. Slides were then counterstained with DAPI and

mounted with prolong Anti-Fade mounting medium (Invitrogen) after being washed with PBS three times. Images were acquired using a fluorescence microscope (Keyence).

Statistical analysis

Graphs are generated with GraphPad Prism. Statistical analysis was also performed within GraphPad Prism. Data values are presented as means $\pm$ s.e.m.. The statistical parameters (i.e. n number, p values, and numbers of biological repeats) can be found in Figure Legends.

Reference for method

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Figure legend

Fig. 1: Aged mice display substantial fat gain and reduced metabolic rate, accompanied by massive adipogenesis.

a, b, Images (a) and body weight (b) of young (2.5-month-old) and aged (12-month-old) male mice (n=8 mice per age group).

c, d, Magnetic resonance imaging (MRI) was used to measure fat mass (c) and lean mass (d) (n=8 mice per age group).

e, f, Tissue weight and whole-tissue pictures of gWAT (e) and sWAT (f). n=9 mice per each age group.

g–i, Metabolic cage determines oxygen consumption (g), physical activity (h), and energy expenditure (i) in young and aged mice. n=8 mice per age group.

j–m, GTT (j, k) and ITT (l, m) were performed on young (2.5-month-old) and aged (12-month-old) mice. For GTT, n=8 per age group; for ITT, n=7, young; n=8, aged.

n, Representative β -gal staining of mouse gWAT and sWAT samples from the male mice at the indicated ages. Red dashed line circles clusters of new, LacZ-negative adipocytes. These images are representative of three independent experiments.

b–f, k, m, **p<0.01; two-tailed Student's t-test. j, l, *p<0.05; **p<0.01; Two-way ANOVA. All data represent the mean $\pm$ s.e.m.

Fig. 2: APCs in aged mice have a high adipogenic rate *in vivo*.

a, APCs (Lin⁻, CD45-CD31-Ter119⁻) were enriched from isolated SVFs from both 2.5-month-old (young) male CAG-EGFP and 12-month-old (aged) Rosa26-mT/mG mice. An equal number of GFP⁺ and Tomato⁺ cells (1×10^5) were mixed together and suspended in Matrigel. The cell mixture

was transplanted into the sWAT of 2.5-month-old male WT mice.

b, Dissected Matrigel plugs were profiled for 3-dimensional Tomato and GFP signals.

c, d, Quantification of Tomato⁺ and GFP⁺ adipocyte number (c) and volume (d). n=3 transplantations.

e, Hematoxylin and eosin (HE) staining in the transplanted Matrigel plug within the sWAT.

f, Immunofluorescence staining of GFP, Tomato, and Perilipin in the transplanted Matrigel plug.

g, APCs (Lin⁻, CD45-CD31-Ter119⁻) were enriched from isolated SVFs from both 2.5-month-old (young) or 12-month-old (aged) Rosa26-mT/mG mice. These Tomato⁺ cells (1×10^5) were suspended in Matrigel. The cell mixture was transplanted into either side of the sWAT of the same 2.5-month-old male WT mice.

h, Dissected Matrigel plugs were profiled for 3-dimensional Tomato signal.

i, j, Quantification of Tomato⁺ adipocyte number (i) and volume (j). n=4 mice (transplants).

c, d, i, j, *p<0.05; **p<0.01; two-tailed Student's t-test. All data represent the mean $\pm$ s.e.m.

Fig. 3: ScRNA-seq analysis identifies a novel, age-specific APC population.

a, Progenitor populations from each group (4,337 "young" and 10,757 "aged" cells) were selected for t-distributed stochastic neighbor embedding (tSNE) plot. K-means clustering (k=25) was applied to the tSNE plot to identify five clusters, ASC, IAP, CP-1, CP-2, and CP-A. This data is from a single experiment. n=3 per group.

b, c, tSNE plot showing differences between young and aged adipocyte APCs, indicating the CP-A cluster uniquely exists in the aged group.

d, Slingshot trajectory analysis of five clusters showing a new, age-specific trajectory from ASC to CP-A.

e, The percentage of each cluster in young and aged APCs. n=3 per group. **p<0.01; Two-way ANOVA. All data represent the mean $\pm$ s.e.m.

f, Box plot showing median and distribution of CytoTRACE values (modified gene count measure) per APC subtypes in young and aged mice. A Higher CytoTRACE value means less differentiated status.

g–k, Individual gene tSNE and violin plots showing the expression and distribution of representative marker genes: general APC marker *Pdgfra* (g), cluster-specific markers *Cd55* for ASC (h), *Apoe* for CP-1 (i), *Mfap4* for CP2 (j), and *Thbs1* for CP-A (k).

l, Expression levels of genes related to adipogenesis in the five APC populations.

m, The progenitor populations from 5 human donors were selected for tSNE plot.

n, ASC and CP-A score of clusters in human APCs. The scores calculate the top 20 ASC and CP-A marker genes for each cell.

o, Flow cytometry analysis shows the percentage of LIFR⁺ populations within the PDGFR α ⁺ population in each peripancreatic WAT sample from men of different ages.

Fig. 4: CP-A cells are highly proliferative and adipogenic *in vitro* and *in vivo*.

a, Top upregulated pathways in “aged” ASCs compared to “young” ASCs.

b, Top pathways in the age-specific CP-A population.

Enrichment score (a and b): Number of Overlapped genes/number of genes in our cell type specific geneset $\times$ 20000/number of genes in the pathway. Pathway source: HALLMARK. Statistic cut off: FDR<0.05

c, Young ASC and CP-1 populations were enriched from the SVFs isolated from the gWAT of 2.5-month-old (young) male Rosa26-mT/mG mice; aged ASC and CP-A populations were

803 enriched from the SVFs isolated from the gWAT of 12-month-old (aged) male Rosa26-mT/mG
804 mice. For each APC population, 1×10^4 cells were suspended in Matrigel and seeded in tissue
805 culture dishes. 16 hours later, the adipogenic cocktail was added to induce APC differentiation.
806 Cells were imaged 6 days later with BODIPY labeling lipid droplets.

807 **d, f**, Representative confocal fluorescent images show Tomato⁺ cells (all cells seeded) and GFP⁺
808 cells (adipocytes are positive for BODIPY staining) from the young and aged ASC populations
809 (d), young CP-1 population and aged CP-A population (f).

810 **e, g**, Quantification of adipogenesis rate, adipocyte number (GFP⁺ cells), and total cell number
811 (Tomato⁺ cells). e, n=3 transplantations; g, n=3 transplantations (young) n=4 transplantations
812 (aged).

813 **h**, Young DPP4⁻ (none ASC APCs) and CP-1 populations were enriched from the SVFs isolated
814 from the gWAT of 2.5-month-old (young) male Rosa26-mT/mG mice. Aged DPP4⁻ (none ASC
815 APCs) and CP-A populations were enriched from the SVFs isolated from the gWAT of 12-month-
816 old (aged) male Rosa26-mT/mG mice. For each APC population, 2×10^4 cells were enriched. Each
817 enriched APC population was suspended in Matrigel and transplanted into the sWAT of 2.5-
818 month-old WT male mice. Young and aged DPP4⁻ populations were transplanted into either side
819 of the sWAT of the same 2.5-month-old male WT mice. Young CP-1 and aged CP-A populations
820 were transplanted into either side of the sWAT of the same 2.5-month-old male WT mice.

821 **i, j, l, m**, Dissected Matrigel plugs were profiled for 3-dimensional Tomato signals.

822 **k**, Quantification of Tomato⁺ adipocyte number and volume generated from young and aged
823 DPP4⁻ cells. n=3 transplantations.

824 **n**, Quantification of Tomato⁺ adipocyte number and volume generated from young CP-1 and Aged
825 CP-A cells. n=4 transplantations.

826 *p<0.05; **p<0.01; two-tailed Student's t-test. All data represent the mean $\pm$ s.e.m.

827

Figure 1

Figure 2

Figure 3

Figure 4

Extended data figure legend

Extended Data Fig. 1: *In vivo* tracking of adipogenesis shows sex difference in age-related adiposity and adipogenesis

a, Magnetic resonance imaging (MRI) was used to measure water content (n=8 mice per age group).

b, c, Bodyweight curve (b) and body weight gain (c) of male and female C57BL/6J mice from 8-week-old to 44-week-old (n=9 male mice; n=8 female mice).

d, e, Metabolic cage studies show total distance (d), and food intake (e) in young and aged mice. n=8 mice per age group.

f, Doxycycline inducible, adipocyte-specific labeling with LacZ in the AdipoChaser-LacZ mouse model. The doxycycline (dox)-based, tet-responsive, Cre-loxP "pulse-chase" labeling system is derived from interbreeding three transgenic strains: 1) mice expressing the "tet-on" transcription factor rtTA under the adiponectin promoter (Adn-rtTA); 2) mice expressing tet-responsive CRE (TRE-Cre) that is activated by rtTA in the presence of doxycycline; and 3) reporter mice expressing a LacZ reporter gene from the Rosa26 locus in a Cre-dependent manner (Rosa26-loxp-stop-loxP-LacZ). This model allows tracking the fate of pre-existing adipocytes (LacZ⁺) and the identification of newly generated adipocytes (LacZ-negative) in mice exposed to doxycycline.

g, 3-month-old male and female AdipoChaser-LacZ mice were fed with a doxycycline diet for 1 week to ensure a uniform and permanent labeling of all mature adipocytes, followed by a standard chow diet for 3 to 15 months.

h, Representative β -gal staining of mouse gWAT and sWAT samples from the female mice at the indicated ages. Arrows indicate new, LacZ-negative adipocytes. These images are representative of three independent experiments.

a, c–e, * $p < 0.05$; ** $p < 0.01$; two-tailed Student's t-test. All data represent the mean $\pm$ s.e.m.

Extended Data Fig. 2: Aged mice have higher WAT regeneration rate.

a, Experimental design: young or aged FAT-ATTAC mice were dimerized daily for seven days, and gWAT samples were collected before, or 0, 1, 2, 4 weeks after dimerization.

b, Hematoxylin and eosin (HE) staining in the gWAT of young (2.5-month-old) and aged (12-month-old) FAT-ATTAC mice before and after dimerization. These images are representative of two independent experiments.

c, Perilipin (green), F4/80 (red), and DAPI (blue) immunofluorescence staining in gWAT of young (2.5-month-old) and aged (12-month-old) FAT-ATTAC mice after dimerization. These images are representative of two independent experiments.

d, Whole-tissue images of gWAT from young (2.5-month-old) and aged (12-month-old) FAT-ATTAC mice after dimerization.

Extended Data Fig. 3: Aged microenvironment does not promote adipogenesis of APCs in young mice.

a, Lin⁻ (CD45-CD31-Ter119⁻) SVFs were isolated from 2.5-month-old (young) male Rosa26-mT/mG mice. 1×10^5 cells were suspended in Matrigel and transplanted into the sWAT of 2.5-month-old (young) or 12-month-old (aged) male WT mice.

b, Dissected Matrigel plugs were profiled for 3-dimensional Tomato signal.

c, Similar experiments were performed using Lin⁻ (CD45-CD31-Ter119⁻) SVFs isolated from 2.5-month-old (young) male CAG-EGFP mice.

d, Dissected Matrigel plugs were profiled for 3-dimensional GFP signal.

Extended Data Fig. 4: scRNA-seq of Lin- SVFs in gWAT of young and aged male mice.

a, Lin- (CD45-CD31-Ter119-) SVFs isolated from gWAT of 2.5-month-old (young) and 12-month-old (age) male WT mice were used for scRNA-seq.

b, Three major cell clusters, progenitor cells, immune cells, and endothelial cells were identified using k-means = 25.

c, Lin- SVFs from each mouse (2,199 cells from "young 1", 2,394 cells from "young 2", 2,539 cells from "young 3", 5,150 cells from "aged 1", 4,109 cells from "aged 2", and 3,072 cells from "aged 3") are illustrated in the tSNE plot.

d, tSNE plot showing differences between young and aged Lin- SVFs.

e, Slingshot trajectory analysis of five APC clusters in each age group, showing a new, age-specific trajectory from ASC to CP-A in the aged group.

f, Barplot shows the top 10 (less differentiated; red) and bottom 10 (most differentiated; blue) genes in this dataset based on their correlation with CytoTRACE values.

g, Box plot showing the median and distribution of CytoTRACE values (modified gene count measure) per APC population in young and aged mice, separately.

h, Dot plot showing the scaled average expression of the top three marker genes in each APC cluster (young and aged combined).

i, j, Individual gene tSNE and violin plots, showing the expression and distribution of the ASC marker *Dpp4* (i), and the CP-A marker *Lifr* (j).

Extended Data Fig. 5: Comparison of ACS, IAP, CP-1, CP-2, and CP-A populations with previously identified APC populations.

a, tSNE plot showing the top 50 markers of ASC1 and ASC2 as reported by Burl et al.¹⁵ overlaid on the scRNA-seq analysis of eWAT from 2.5-month-old (young) or 12-month-old (aged) male WT mice.

b, Group identities with the 50 top cluster markers as reported by Burl et al.¹⁵.

c, tSNE plot showing the top 50 markers of FAP1-4 as reported by Sárvári et al.²⁰ overlaid on the scRNA-seq analysis of eWAT from 2.5-month-old (young) or 12-month-old (aged) male WT mice.

d, Group identities with the 50 top cluster markers as reported by Sárvári et al.²⁰.

e, tSNE plot showing the top 50 markers of P1-3 as reported by Schwalie et al.¹⁷ overlaid on the scRNA-seq analysis of sWAT from 2.5-month-old (young) or 12-month-old (aged) male WT mice.

f, Group identities with the 50 top cluster markers as reported by Schwalie et al.¹⁷.

Extended Data Fig. 6: Single-cell RNA-seq analysis of human APCs

a, Four major cell clusters, progenitor cells, immune cells, mesothelial cells and endothelial cells were identified with k-means clustering (k-means=25) applied to the tSNE plot.

b–i, Individual gene tSNE plots showing the expression levels of the indicated genes: adipocyte stem cell/progenitor markers *Pdgfra* (b) and *Pdgfrb* (c), ASC markers *Cd55* (d), and *Pil6* (e), CP-A marker *Lifr* (f), and committed preadipocyte markers *Pparg* (g), *Fabp4* (h), and *Lpl* (i).

j, Flow cytometry strategy for identifying PDGFR α ⁺ cells. Single cells were selected based on forward and side scatter profiles. Live cells were gated as DAPI⁻. Immune cells, endothelial cells, and erythrocytes were gated out using CD45, CD31, and CD235a antibodies.

Extended Data Fig. 7: Enriching APC populations and *in vitro* 3D culture of APCs.

a, Representative confocal fluorescent images show young and aged Lin⁻ (CD45-CD31-Ter119⁻)

total APCs (all Tomato⁺ cells that seeded) and GFP⁺ cells (BODIPY⁺, adipocytes). The adipogenic cocktail was added 3 days post-seeding. Other experimental conditions are the same as described in Fig. 4c. n=2 per group. All data represent the mean $\pm$ s.e.m.

b, Representative confocal fluorescent images show young and aged Lin⁻ (CD45-CD31-Ter119⁻) total APCs (all Tomato⁺ cells that seeded) and GFP⁺ cells (adipocytes). The adipogenic cocktail was added 16 hours post-seeding. Other experimental conditions are the same as described in Fig. 4c. n=3 per group. *p<0.05; **p<0.01; two-tailed Student's t-test. All data represent the mean $\pm$ s.e.m.

c, SVFs from gWAT of young and aged male WT mice were subjected to flow cytometry analysis. Cells were first enriched for APCs (CD31-CD45-Ter119-PDGFR α ⁺). Flow cytometry analysis shows the percentage of LIFR⁺ populations within the PDGFR α ⁺ population in gWAT of 2.5-month-old (young) or 12-month-old (aged) male WT mice.

d, DPP4⁺ population was enriched by magnetic bead-associated cell sorting (MACS). Flow cytometry analysis validates the isolation of DPP4⁺ and DPP4⁻ populations within the Lin⁻ SVF after MACS in 2.5-month-old (young) or 12-month-old (aged) male WT mice.

e, CP-1 and CP-A populations were enriched through MACS-based negative selection, depleting DPP4⁺ ASC, CD9⁺ CP-2, and IAP populations in Lin⁻ SVFs. Flow cytometry analysis validates depletion of DPP4⁺ and CD9⁺ populations from the enriched CP-A population after MACS from 2.5-month-old (young) or 12-month-old (aged) male WT mice.

f, Flow cytometry analysis validates increased LIFR⁺ cell percentage in enriched CP-A population after MACS.

g, Flow cytometry analysis shows DPP4⁺ and CD9⁺ population percentages in PDGFR α ⁺ eWAT SVFs from 2.5-month-old (young) or 12-month-old (aged) male WT mice.

Extended Data Fig. 8: scRNA-seq of immune cells isolated from the sWAT of young and aged mice.

a, CD45⁺ SVFs from each group (5,528 "young" and 4,986 "aged" cells) were selected for tSNE plot. 15 clusters, including 12 types of immune cell were generated using k-means = 25. This data is from a single experiment. "MAC" indicates macrophages, "MAC2" indicate M2 macrophages. n=3 per group.

b, Dot plot showing the scaled average expression of representative marker genes in each immune cell clusters (young and aged combined).

c, CD45⁺ SVFs from each mouse (2,046 "young 1", 1,686 "young 2", 1,796 "young 3", 1,784 "aged 1", 1,130 "aged 2", and 2,072 "aged 3") are illustrated in tSNE plot.

d, tSNE plot showing differences between young and aged immune cells.

e. Flow cytometry analysis shows the percentage of macrophage (CD11b⁺) within the CD45⁺ population in 2.5-month-old (young) or 12-month-old (aged) male WT mice, and the percentage of M2 macrophages (CD206⁺) within the CD11b⁺ population.

f, Heatmap illustrating the numbers of ligand receptor pairs gained or lost in aged mice comparing to young mice between immune cell types and APC populations. 2D-clustering was applied to order the cell types.

g–l, One-way ligand-receptor communication analysis of the top immune cell-APC interaction events in selected pathways: adipogenesis (g, h), TGFβ (i, j), and insulin (k, l), in young and aged mice. Circos plots showing top 40 highly expressed ligand-receptor pair signaling with ligand from immune cells to receptors in APC populations.

Extended Data Fig. 9: Increased proliferation and adipogenesis signaling from macrophage to aged APC populations.

a–c, One-way ligand-receptor communication analysis for all putative gain or loss of macrophage-ASC interaction events in aging via selected pathways: adipogenesis (a), TGF β (b), and insulin (c). Circos plots showing ligand-receptor pair signaling with ligand from MAC, MAC2 to receptors in ASC.

d–i, One-way ligand-receptor communication analysis the top young macrophage-young CP-1 and aged macrophage-aged CP-A interaction events in selected pathways: adipogenesis (d, g), TGF β (e, h), and insulin (f, i). Circos plots showing top 40 highly expressed ligand-receptor pair signaling with ligand from MAC, MAC2 to receptors in young CP-1 (d–f) and aged CP-A (g–i).

Extended Data Fig. 10: Macrophages co-culture increases proliferation of both “young” and “aged” APCs.

a, Total APCs (Lin⁻, CD45-CD31-Ter119⁻) were enriched from 2.5-month-old (young) or 12-month-old (aged) male Rosa26-mT/mG mice were enriched. CD45⁺ immune cells were enriched from 2.5-month-old (young) or 12-month-old (aged) male WT mice. 1×10^4 APCs and 1×10^4 immune Cells were mixed in Matrigel and seeded in tissue culture dishes. 16 hours later, the adipogenic cocktail was added to induce APC differentiation. Cells were imaged 6 days later with BODIPY labeling lipid droplets.

b, Representative confocal fluorescent images show Tomato⁺ cells (all cells seeded) and GFP⁺ cells (adipocytes) and quantification of adipogenesis rate, adipocyte number (GFP⁺ cells), and total cell number (Tomato⁺ cells). n=2 per group. All data represent the mean $\pm$ s.e.m.

c, Summarization of key findings: APCs in young adult mice are quiescent. These cells do not

undergo adipogenesis unless triggered by metabolic challenges (such as high fat diet feeding or cold exposure). The aging process remodels APCs, generates ASC cells that are highly proliferative and CP-A cells that are both highly proliferative and adipogenic. The proliferation and differentiation of APCs in aged mice are spontaneous, without any external metabolic challenges. Illustration created with BioRender.com.

Extended Data Fig. 1

Extended Data Fig. 2

Extended Data Fig. 3

Extended Data Fig. 4

Extended Data Fig. 5

Extended Data Fig. 6

Extended Data Fig. 7

a Complete cocktail added 3d post seeding

b Adipogenic cocktail added 16h post seeding

c Young

Extended Data Fig. 8

Extended Data Fig. 9

Extended Data Fig. 10

a

b

c

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