Characterization of the adipocyte cellular lineage in vivo

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Mature adipocytes are generated through the proliferation and differentiation of precursor cells. Our previous studies identified adipocyte progenitors in white adipose tissue (WAT) as Lin−:CD29+:CD34+:Sca-1+:CD24+:CD24+ (CD24+) cells that are capable of generating functional WAT (ref. 1). Here, we employ several Cre recombinase mouse models to identify the adipocyte cellular lineage in vivo. Although it has been proposed that white adipocytes are derived from endothelial2 and haematopoietic3,4 lineages, we find that neither of these lineages label white adipocytes. However, platelet-derived growth factor receptor α (PdgfRα)–Cre trace labels all white adipocytes. Analysis of WAT from PdgfRα–Cre reporter mice identifies CD24+ and Lin−:CD29+ :CD34+ :Sca-1+:CD24− (CD24−) cells as adipocyte precursors. We show that CD24+ cells generate the CD24+ population in vivo and the CD24− cells express late markers of adipogenesis. From these data we propose a model where the CD24+ adipocyte progenitors become further committed to the adipocyte lineage as CD24 expression is lost, generating CD24− preadipocytes. This characterization of the adipocyte cellular lineage will facilitate the study of the mechanisms that regulate WAT formation in vivo and WAT mass expansion in obesity.

The number of mature adipocytes in WAT of adults is tightly regulated, despite their continual turnover5. As mature adipocytes are post-mitotic5,2, a change in adipocyte number occurs through disruption of the balance between rates of adipogenesis and adipocyte death. Therefore, characterization of the adipocyte cellular lineage is required for a mechanistic understanding of WAT homeostasis and growth.

Various methods have been used to study adipocyte precursors ex vivo and in vivo. One common method is to culture the whole stromal-vascular fraction (SVF) from adipose tissues and select cell populations by their adherence to plastic5,9. The cells derived from this method are referred to as preadipocytes or adipocyte-derived stem cells. However, these cells have not been shown to have de novo adipogenic capacity in vivo and their relationship to adipocyte lineage cells in vivo is not known.

Alternatively, several groups have used fluorescence-activated cell sorting (FACS) in a prospective approach to identify adipogenic cell populations from various tissues1,10–12. Two cell populations derived from WAT, defined by the marker profiles Lin−:CD34+ :CD29+ :Sca-1+:CD24+ (CD24+) and Lin−:CD34− :CD29+:Sca-1+:CD24− (CD24−), are adipogenic in vitro but only the CD24+ population is capable of generating a functional WAT depot following transplantation into a residual WAT depot of lipodystrophic mice1, indicating that the CD24+ population contains adipocyte progenitors. Cells with similar marker profiles have been shown to be adipogenic within the skin10 and skeletal muscle11.

Genetic approaches have also been used to investigate the adipocyte cellular lineage. A previous study showed, through crossing cadherin-5 (Cdhs)–Cre mice into reporter lines that express cytoplasmic β− galactosidase and GFP, that Cdhs–Cre labels mature adipocytes12, suggesting an endothelial origin for white adipocytes as Cdhs labels endothelial lineages14. However, for studies of WAT the cellular specificity of reporters that stain the cytoplasm is difficult to delineate given the paucity of cytoplasm in mature adipocytes and the high vascularity of WAT. To overcome this limitation, we employed a mouse strain harbouring a fluorescent-membrane dTomato/membrane eGFP (mT/mG) Cre reporter construct that marks Cre excision by a heritable switch from membrane-targeted tdTomato expression to membrane-targeted eGFP expression15. We crossed this reporter to several mouse lines expressing Cre recombinase from various promoters to more specifically determine the identity of the adipocyte precursors. Whole-mount confocal microscopy of WAT from adiponectin (Adipoq)–Cre;mT/mG mice demonstrates GFP expression in mature adipocytes of all WAT depots assayed, with no GFP fluorescence signal in the

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Figure 1 Adipocytes are derived from PdgfRα precursor cells in subcutaneous WAT. (a) Confocal images of whole-mounted SWAT from the indicated 4-week-old Cre: mT/mG male mice (red: membrane-targeted dTomato; green: membrane-targeted eGFP, indicating Cre excision of dTomato). (b) Confocal images of membrane-targeted eGFP and isolectin GS-IB4 Alexa Fluor 647 staining endothelial cells of Cdh5-Cre:mT/mG SWAT. (c) Quantification of flow cytometry analysis of SVF populations from the indicated 4-week-old Cre: mT/mG mice (n = 3). (d) Quantification of quantitative PCR analysis of PdgfRα in mature adipocytes and FACS-sorted SVF, Lin−:CD29+;CD34−;Sca-1+;CD24+ (CD24+) and Lin−:CD29+;CD34−;Sca-1+;CD24− (CD24−) cell populations (n = 5 RNA extractions from independently isolated cell samples, **P < 0.001). (e) Quantification of flow cytometry analysis of anti-PdgfRα-PE antibody staining in the indicated cell populations from 6-week-old male C57BL/6 SWAT (n = 3 SWAT SVF preparations). (f) A histogram of the distribution of CD24 staining in PdgfRα−:Lin−:CD29−;CD34−;Sca-1− cells from e. All error bars represent s.e.m. Scale bars, 100 μm.

absence of Cre expression, indicating that the mT/mG reporter model is appropriate for lineage tracing of mature adipocytes (Fig. 1a and Supplementary Fig. S1a). Flow cytometry analysis of the SVF from mT/mG models (Supplementary Fig. S2a) demonstrates that this model is also suitable for the study of potential precursor populations. However, flow cytometry analysis of Adipoq−CremT/mG SWAT shows
Figure 2 CD24+ adipocyte progenitors give rise to CD24− cells in vivo. (a) Flow cytometry plots of SVF from a time course of SWAT development in C57BL/6 mice at the indicated embryonic (e) and postnatal (P) days. Dot plots show Lin−:CD29+:CD34+ cells. Black squares denote the Lin−:CD29+:CD34+:Sca1+:CD24+ adipocyte progenitor population. (b) CD24 staining in Lin−:CD29+:CD34+:Sca1+ cells from e17.5 and P42 SWAT. (c) Oil Red O staining of differentiated FACS-sorted cell populations from e17.5 SWAT. Scale bar, 100 μm. (d) Representative images of flow cytometry plots of SWAT Lin−:CD29+:CD34+ cells from 6-week-old Rag2−/−:IL2Rγ−/−:A-Zip mice at the indicated days post transplantation of 100,000 FACS-isolated dTomato+, Lin−:CD29+:CD34+:Sca1+:CD24+ (CD24+) cells from 6-week-old mT/mG mice. Plots show overlay of dTomato+ transplanted cells (white) on Rag2−/−:IL2Rγ−/−:A-Zip recipient SVF cells (blue). (e) Quantification of flow cytometry analysis of dTomato+CD24+ transplants into Rag2−/−:IL2Rγ−/−:A-Zip mice (n = 4 transplantations, **** P < 0.0001, error bar represent s.e.m.). (f) Representative flow cytometry plot of SWAT Lin−:CD29+:CD34+ cells from 6-week-old Rag2−/−:IL2Rγ−/−:A-Zip mice at 1 day post transplantation of 100,000 FACS-isolated dTomato+, CD24− cells from 6-week-old mT/mG mice. The dot plot shows overlay of dTomato+−transplanted cells (white) on Rag2−/−:IL2Rγ−/−:A-Zip recipient SVF cells (blue). (g) Quantification of flow cytometry analysis of dTomato+CD24− transplants into Rag2−/−:IL2Rγ−/−:A-Zip mice (n = 3 transplants, error bars represent s.e.m.).

We next generated Cdh5−Cre mT/mG mice, using the same mouse line used in the previous study1, to determine whether adipocyte precursors within the SVF are derived from Cdh5−expressing cells. Whereas CD31+ endothelial cells were almost completely labelled...
by Cdh5–Cre, mature adipocytes from WAT depots and brown adipose tissue did not express GFP in this model (Fig. 1a–c and Supplementary Fig. S1). Analysis of another endothelial lineage marker, endothelial-specific receptor tyrosine kinase (Tie2)–Cre, produced similar results (Fig. 1a,c and Supplementary Fig. S1), indicating that adipocytes are not derived from endothelial cells under normal conditions. Owing to the high vacuularity of WAT and the tight association of capillaries with mature adipocytes, some adipocytes are completely surrounded by GFP+ vasculature, giving the appearance that the adipocyte may be GFP+. However, labelling endothelial cells with isoelectin GS-IB4 clearly shows that the GFP signal is derived from endothelial cells in Cdh5–Cre and Tie2–Cre and the mature adipocytes are dTomato+ (Fig. 1b and Supplementary Fig. S1c).

It has also been proposed that adipocytes are derived from haematopoietic lineages16–22, and recent studies of transplant and injury models have shown that at least some adipocytes are derived from circulating cells of haematopoietic origin3,4. However, Tie2–Cre labels nearly all CD45+ cells in WAT, yet does not label mature adipocytes. Similarly, the Vav 1 oncogene (Vav1)–Cre, another marker of haematopoietic lineages4,23, fails to label adipocytes despite the near complete labelling of CD45+ cells in WAT (Fig. 1a,c and Supplementary Fig. S1a,b). These data indicate that adipocytes are not normally derived from haematopoietic lineages.

To determine whether endothelial or haematopoietic lineages contribute mature adipocytes in the context of obesity we placed Cdh5–Cre and Vav1–Cre mT/mG reporter mice on a high-fat diet (HFD). Following 10 weeks of HFD, no GFP+ adipocytes were observed in the Cdh5–Cre WAT, whereas adipocyte-like GFP+ structures are present in Vav1–Cre WAT (Supplementary Fig. S3). However, all of these GFP+ structures are multi-nucleated and stain positive for the macrophage markers F4/80, CD11b and CD45 (Supplementary Fig. S3a–d), indicating that the GFP fluorescence signal is not derived from adipocyte membranes but from macrophages forming crown-like structures24. These data indicate that haematopoietic and endothelial lineage cells do not contribute to mature adipocyte formation in HFD-induced obesity.

Previous studies demonstrated that PdgfRα is expressed in adipogenic cells from skeletal muscle11,12. In addition, PdgfRα is expressed in WAT-resident cells that produce brown-like adipocytes in response to β-adrenergic stimulation and white adipocytes on HFD feeding25. Therefore, we determined whether PdgfRα labels mature adipocytes during normal formation of WAT. PdgfRα–Cre labels all mature adipocytes in all major WAT depots, as indicated by GFP+ adipocyte membranes (Fig. 1a and Supplementary Fig. S1a). As PdgfRα is not expressed in mature adipocytes25 (Fig. 1d), the GFP expression observed in adipocytes of PdgfRα–Cre;mT/mG mice is due to lineage tracing.

We next examined the labelling of SVF cells in PdgfRα–Cre mice to identify potential adipocyte precursors. The SVF of PdgfRα–Cre;mT/mG mice contains a low percentage of GFP+ cells in CD31+ and CD45+ populations. In contrast, both the CD24+ and CD24– cells are nearly completely traced by PdgfRα–Cre (Fig. 1c) and PdgfRα is expressed in these cell populations (Fig. 1d–f). These data suggest that both the CD24+ and CD24– populations are part of the in vivo adipocyte cellular lineage.

Therefore, we next examined the relationship between the CD24+ and CD24– cells in vivo. During development of subcutaneous WAT (SWAT), lipid filling of differentiating adipocytes is not observed until birth26. Flow cytometry analysis of embryonic SWAT (embryonic day 15.5–18.5) shows that there is a large population of cells with the cell surface marker profile of CD24+ adipogenic precursors (Fig. 2a,b). These embryonic CD24+ cells are 98.62 ± 1.08% PdgfRα+ and are adipogenic in cell culture (Fig. 2c), suggesting that they are adipocyte progenitors. Before birth there is no discernible CD24– population (Fig. 2a). Following birth (P0) there is a shift in the population...
Figure 4  CD24+ cells are further committed to an adipogenic fate. (a) Breakdown of PdgfRα+ (α+ ) SVF into subpopulations. Flow cytometry plots and percentages are shown in Supplementary Fig. S2b. α+ : CD24+, α+ : CD24+, and α+ : Sca1+ are Lin− : CD29− : CD34+. α+ : CD34+ are Lin− : CD29+. (b) Luminescence in 12-week-old FVB or A-Zip mice at 6 weeks post subcutaneous sternum transplantation of indicated FACS-isolated populations from 6-week-old leptin–luciferase BAC transgenic mice. Per experiment, 50,000 cells were transplanted. (c) Quantification of luminescence signal from FVB and A-Zip mice before and 6 weeks post subcutaneous sternum transplantation of 50,000 α+ : CD24+ as shown in b,c. (g) A model of in vivo adipogenesis.

profiles, with the appearance of a large population of CD24− cells and a simultaneous reduction in the amount of CD24+ cells to levels similar to those observed in adult WAT (Fig. 2a). These population dynamics during development of WAT suggests that the CD24+ cells may generate the CD24− cell population.

To test this hypothesis we employed an A-Zip transplantation model similar to the assay previously used to demonstrate the capacity of CD24+ cells to reconstitute functional WAT (ref. 1). In these assays, dTomato-labelled CD24+ cells from mT/mG mice were transplanted into the undeveloped WAT of A-Zip mice and injected tissues were analysed before the appearance of mature adipocytes, 1–4 days post-injection. When dTomato− : CD24− cells are transplanted, dTomato+ cells recovered one day post-injection are nearly all CD24− with no dTomato+ cells in the CD24+ gate, demonstrating that the assay accurately identifies the CD24− population (Fig. 2f,g). In contrast, when dTomato+ : CD24+ cells are transplanted, 51.7% of dTomato+ cells are CD24− after one day, and four days post-injection 91.4% of dTomato+ cells are CD24− (Fig. 2d,e). The increasing appearance of CD24+ donor cells over time after transplantation of CD24+ adipocyte progenitors indicates that the CD24+ adipocyte progenitors generate the CD24+ adipogenic cell population.

The finding that the CD24+ cell population is derived from the CD24+ adipocyte progenitors, coupled with the reduced ability of the CD24− population to generate adipose tissue in vivo, suggests that the CD24− cell population contains cells that are further differentiated towards mature adipocytes. Thus, we performed gene expression analysis on sorted cells to investigate the relative state of differentiation in these adipocyte precursor populations. Real-time PCR shows that CD24 messenger RNA is enriched in the CD24− population, validating the sorting of these populations (Fig. 3a,b). CD24 is also
highly expressed in unfractionated SVF as CD24 is expressed in several other cell types in SVF, such as B lymphocytes, and only 0.61% ± 0.12 of SVF cells that express CD24 exhibit the complete cell surface marker profile of adipocyte progenitors (Supplementary Fig. S4a,b). The late adipogenic markers Ppary2 and Cebpa are enriched in the CD24+ population whereas markers of mature adipocytes, Adipoq and Perilipin, are expressed only in isolated mature adipocytes (Fig. 3c).

The enrichment of late adipogenic markers in CD24+ cells suggests that this population may contain cells that are committed to an adipogenic fate. Thus, we examined the adipogenic capacity of the CD24+ and CD24− cells in a previously developed in vivo adipocyte commitment assay (Supplementary Fig. S4a,b). PdgfRα populations (Fig. 4a and Supplementary Fig. S2b) were sorted from leptin–luciferase mice and injected subcutaneously over the sternum of wild-type mice, a region where adipose tissue does not normally form. Whereas the PdgfRα+ : CD24+ adipocyte progenitors and PdgfRα− : Lin+ cells did not produce luciferase signal, the transplanted PdgfRα+CD24+ cells produced a significant luciferase signal after 6 weeks, indicative of the formation of mature adipocytes (Fig. 4b,c).

Despite the inability of the CD24+ adipocyte progenitors to form mature adipocytes outside the WAT microenvironment (Fig. 4b,c), previous work has shown that this cell population is capable of reconstituting a functional WAT depot that rescues hyperglycaemia when transplanted into the residual perigonadal WAT (GWAT) of A-Zip mice. The same study also showed that the CD24− cell population has limited adipogenic capacity in this WAT microenvironment. To more directly assess the capacity of CD24− cells to form adipocytes, we performed sternum injections of PdgfRα− : CD24− cells from Adipoq−Cre:mT/mG mice. These injections resulted in the formation of lipid filled, Adipoq−expressing mature adipocytes (Fig. 4d,e). However, the adipose tissue formed by differentiation of these PdgfRα− : CD24− cells is several fold smaller than the tissue observed in GWAT reconstitution by CD24+ adipocyte progenitors in A-Zip mice (Fig. 4d,e). In addition, the adipose tissue resulting from the injection of PdgfRα− : CD24− cells above the sternum of A-Zip mice does not rescue their hyperglycaemia (Fig. 4c,f). The ability of the PdgfRα− : CD24− population to form small amounts of adipose tissue both within and outside the WAT microenvironment indicates that this population harbours cells that are further committed to adipogenesis yet have limited adipogenic capacity as compared with CD24+ adipocyte progenitors.

Taken together our data characterize a previously unappreciated cellular lineage of adipocytes within WAT. Our recent prospective studies identified the CD24+ population as a rare cell type in WAT that is capable of forming a functional WAT depot in vivo. We show here that these CD24+ adipocyte progenitors are indeed a component of the adipocyte lineage in vivo, becoming further committed to the adipocyte lineage as they lose CD24 expression, forming a CD24− preadipocyte population in the course of differentiating into mature adipocytes in vivo (Fig. 4g).

Our data also indicate that adipocytes are derived from PdgfRα+ expressing cells during the normal establishment of WAT. Previous data have demonstrated that adipocytes formed in response to β-adrenergic stimulation and HFD are also derived from PdgfRα+ cells that share other markers with the preadipocytes described here, including Sca-1 and CD34 (ref. 25), although this previous study did not investigate the role of the CD24+ adipocyte progenitors. Using PdgfRα as a single marker of adipocyte precursors, this previous study found that PdgfRα+ cells are associated with the vasculature. However, we have shown that there are multiple PdgfRα+ populations and some of them are not adipogenic (Fig. 4b,c). Therefore, PdgfRα expression alone cannot definitively identify adipocyte progenitors within WAT. Similarly, CD24 cannot be used as a sole marker of adipocyte progenitors, as only 0.61% of CD24+ cells within WAT are adipocyte progenitors (Supplementary Fig. S4a,b). In addition, only 17.42% of PdgfRα− : CD24− cells within WAT are adipocyte progenitors (Supplementary Fig. S4a,b). These PdgfRα− : CD24− cells are dispersed throughout WAT and are not preferentially localized to vasculature (Supplementary Fig. S4c), indicating that precise localization of adipocyte progenitors requires more exclusive markers.

Although many factors are known to affect adipocyte formation in vitro, the precise role of these factors in adipocyte dynamics in vivo remains uncharacterized. For example, the expression of Pdgfα on adipocyte precursors suggests that PDGF signalling plays a role in WAT mass regulation in vivo. However, previous studies of the role of PDGF in adipogenesis in vitro are conflicting, with PDGF signalling either inducing or inhibiting adipogenesis. The identification of the adipocyte cellular lineage in vivo and the characterization of PdgfRα−Cre mice as a model for genetic manipulation of adipocyte precursors will now permit detailed studies of the cellular and molecular mechanisms that regulate adipogenesis and WAT mass in vivo under various conditions, including the establishment of the adipose tissue set point during development and the expansion of WAT mass in obesity. Determining the mechanisms involved in these different states of WAT mass regulation may lead to the development of new therapeutic strategies for controlling WAT mass.

**METHODS**

Methods and any associated references are available in the online version of the paper.

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**AUTHOR CONTRIBUTIONS**

R.B. and M.S.R. designed and performed experiments, analysed and interpreted data involved in these different states of WAT mass regulation may lead to the development of new therapeutic strategies for controlling WAT mass.

**COMPETING FINANCIAL INTERESTS**

The authors declare no competing financial interests.

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METHODS

Mice. The following mouse lines were obtained from Jackson Laboratory for WAT lineage analysis: Adipoq–Cre (B6;FVB–Tg(Adipoq–cre)1Eshr/J), stock no. 010803; mT/mG (B6.129.Cg-Gt(Rosa)26Sortm4(ActCAG-GFP)1/LongJ), stock no. 006757; Cd31–PE (B6.Cg–Tg(Cd31–PE)15Flk/J), stock no. 002624; Tie2–Cre (B6.Cg–Tg(Tie2–cre)1Ywa/J), stock no. 008863; Pdgfra–Cre (C57BL/6–Tg(Pdgfra– cre)1Cle/J), stock no. 013148; Pdgfra–H2B2GFP (B6.129S4-Pdgfratm11(CAG-GFP)2Orj/J), stock #007664. For indicated experiments, Vav1–cre and Cd53–cre mice were fed a HFD containing 60% of the kilocalories from fat (Research Diets) for 10 weeks before being killed. Male mice were used for all experiments.

Whole-mount confocal microscopy. Approximately 4 mm × 4 mm sections of indicated white adipose depots were dissected from 4-week-old male mice and mounted onto charged slides in Fluoromount-G (Southern Biotech; 0100-01). Images were taken on a Leica TCS SP5 confocal microscope. Gain and offset were set with mT/mG and Adipoq–Cre:mT/mG for mT and GFP, respectively. mT was excited at 488 nm and detected from 505 to 540 nm. To stain vasculature, lipid or cell surface antigens, adipose tissue was incubated in isocyanate GS-IB4 Alexa 647 (Invitrogen, I32450), HCS LipidTox Deep Red (Invitrogen, H34477), CD24 Brilliant Violet 421 (Biolegend; 101235) and CD45 Alexa 647 (Biolegend, 103123) at 1:100 in PBS containing 3% BSA, 1.2 mM calcium chloride, 1.0 mM magnesium chloride and 0.01% sodium azide. Cells were then incubated with nuclei with DAPI. SVF was sequentially filtered through 70- and 40-μm filters and single cells were collected. Positive adipocytes were verified by staining for plasma membrane with Cell Mass Orange and negative adipocytes were verified by staining for dystrophin with Cell Mass Blue. Isolated adipocytes were stained with 10 μg/ml of 15 mg ml⁻¹ dexamethasone and 0.5 mM IBMX) in fresh media. After 48 h, cells were switched to DMEM supplemented with 10% FBS. Cells were grown to confluence and held at confluence for 2 days without changing the media. Cell culture. FACS-isolated cell populations were plated in 24-well plates (BD Biosciences; 354775) in DMEM supplemented with 10% FBS. Cells were grown to confluence and held at confluence for 2 days without changing the media before exposure to the differentiation cocktail (1 mg ml⁻¹ insulin, 0.25 mg ml⁻¹ dexamethasone and 0.5 mM IBMX) in fresh media. After 48 h, cells were switched back to DMEM with 10% FBS and maintained for 6 days before collection. Cells were fixed and stained with oil red O as previously described.

Flow cytometry and cell sorting. Excised white adipose tissue was digested in 0.8 mg ml⁻¹ collagenase type 2 (Worthington Biochemical; LS004174) in HBSS containing 3% BSA, 1.2 mM calcium chloride, 1.0 mM magnesium chloride and 0.01% sodium azide. Floating adipocytes were separated from the SVF by centrifugation at 300g for 5 min. Isolation of intact adipocytes was verified by staining for plasma membrane with Cell Mass Orange and nuclei with DAPL. SVF was sequentially filtered through 70- and 40-μm filters before staining with the following antibodies for 20 min: CD45 APC-eFluor 780 at 1:5,000 (eBioscience; 45-0451-80), CD31 PE-Cy7 at 1:1,200 (eBioscience, 25-0311-82), CD29 Alexa Fluor 700 at 1:400 (BioLegend, 102218), CD34 Alexa Fluor 647 at 1:200 (BioLegend, 119314), Sca-1 Pacific Blue at 1:1,000 (BD Biosciences, 560653), CD24 eBioscience; 47-0451-80), CD31 PE (Biolegend; 101825), F4/80 Alexa 647 (Biolegend, 123121) and CD11b Brilliant Violet 421 (Biolegend, 101235) and CD45 Alexa 647 (Biolegend, 103123) at 1:100 in PBS for at least 30 min before washing in PBS and mounting for imaging.

Statistical analysis. The transplant studies were analysed by Student’s t-test. One-way analysis of variance with Tukey’s post-hoc test was used to determine significant differences in gene expression between sorted cell populations. Statistical analysis was performed using GraphPad Prism. P < 0.05 was determined to be significant for all experiments. Actual P values are listed in each figure legend.
Figure S1 Adipocytes in all major WAT depots are derived from PdgfRα+ precursor cells. (a) Confocal images of whole mounted gonadal (GWAT), retroperitoneal (RWAT), and mesenteric WAT (MWAT) from indicated 4-week old Cre; mT/mG mice (red: membrane-targeted dTomato; green: membrane-targeted eGFP, indicating Cre excision). All images were taken at 20x magnification. (b) Quantification of flow cytometry analysis of SVF populations from indicated 4-week old Cre;mT/mG mice (n=3 WAT SVF preparations, error bars represent S.E.M.). CD24+ and CD24- populations are also Lin-, CD29+, CD34+, Sca-1+. (c) Confocal images of Tie2-Cre;mT/mG GWAT stained with Isolectin GS-IB4 Alexa Fluor 647. Individual channels for eGFP, Isolectin and dTomato are shown along with merged images of eGFP/dTomato and eGFP/A647/dTomato at 20x. (d) Confocal image of 4-week old Cdh5-Cre;mT/mG brown adipose tissue (BAT) taken at 20x. All scale bars represent 100 µm.
**Figure S2** mT/mG labeling of adipose SVF. (a) Representative flow cytometry plots from the indicated promoter driven-Cre:mT/mG mice. Cell populations are identified via flow cytometry by staining of SVF cells with fluorescently labeled antibodies against the indicated cell surface proteins (Left three panels). The percentage of cells in each population that express membrane target eGFP or membrane target dTomato can then be assessed (Right panels show dTomato and eGFP analysis of the Lin-, CD29+, CD34+, Sca-1+, CD24- cell population in each Cre:mT/mG line.). (b) Representative flow cytometry plots showing the identification and quantification of PdgfRα+ subpopulations in 6-week old C57BL/6 SWAT. Percentages shown indicate the portion of PdgfRα+ cells present in each subpopulation.
Figure S3 Endothelial and hematopoietic lineage cells do not produce mature adipocytes in response to high fat diet feeding. (a-c) Representative confocal images of whole mounted SWAT and GWAT from Vav1-Cre:mT/mG mice fed HFD for 10 weeks stained with fluorescent F4/80 (a), CD11b (b), or CD45 (c) antibodies. (d) Confocal image of whole mounted GWAT stained with CD45 and DAPI following 10 weeks of HFD. (e) Representative confocal images of whole mounted adipose depots from Cdh5-Cre:mT/mG mice fed HFD for 10 weeks. All scale bars represent 100 µm.
**Figure S4** Pdgfrα+;CD24+ cells are not preferentially localized to the vasculature. (a) Representative flow cytometry plots showing CD45 and CD31 staining of SWAT CD24+ singlets (left plot) and Pdgfrα+;CD24+ singlets (right plot). Pdgfrα+;Lin-;CD29+;CD34+;Sca-1+;CD24+ APCs (red) are overlayed on top of SVF singlets that are single positive for CD24 (left plot) or double positive for CD24 and Pdgfrα but are not Lin-;CD29+;CD34+;Sca-1+ and are therefore not APCs (white). (b) Quantification of results from (a) showing the percentage of CD24+ singlets in SWAT that are adipocyte progenitors (left bar) or the percentage of Pdgfrα+;CD24+ singlets that are adipocyte progenitors (right bar) (n=5 SWAT SVF preparations, error bars represent S.E.M.). (c) Representative confocal images of whole mounted Pdgfrα-H2B:GFP SWAT stained with fluorescently labeled CD24 antibody and Isolectin GSIB4. White arrows mark Pdgfrα+;CD24+ cells associated with vasculature while yellow arrows mark Pdgfrα+;CD24+ cells not associated with vasculature. All scale bars represent 100 µm.