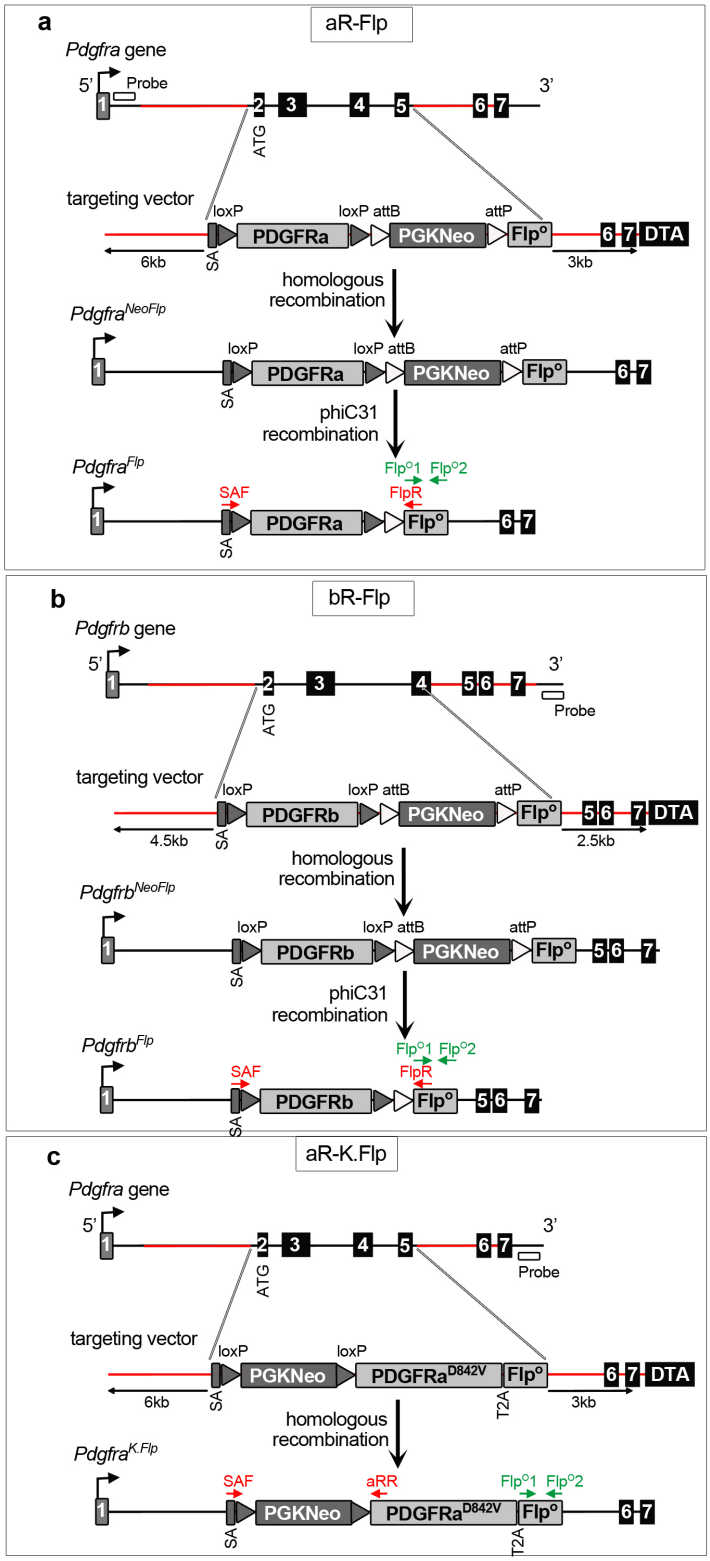
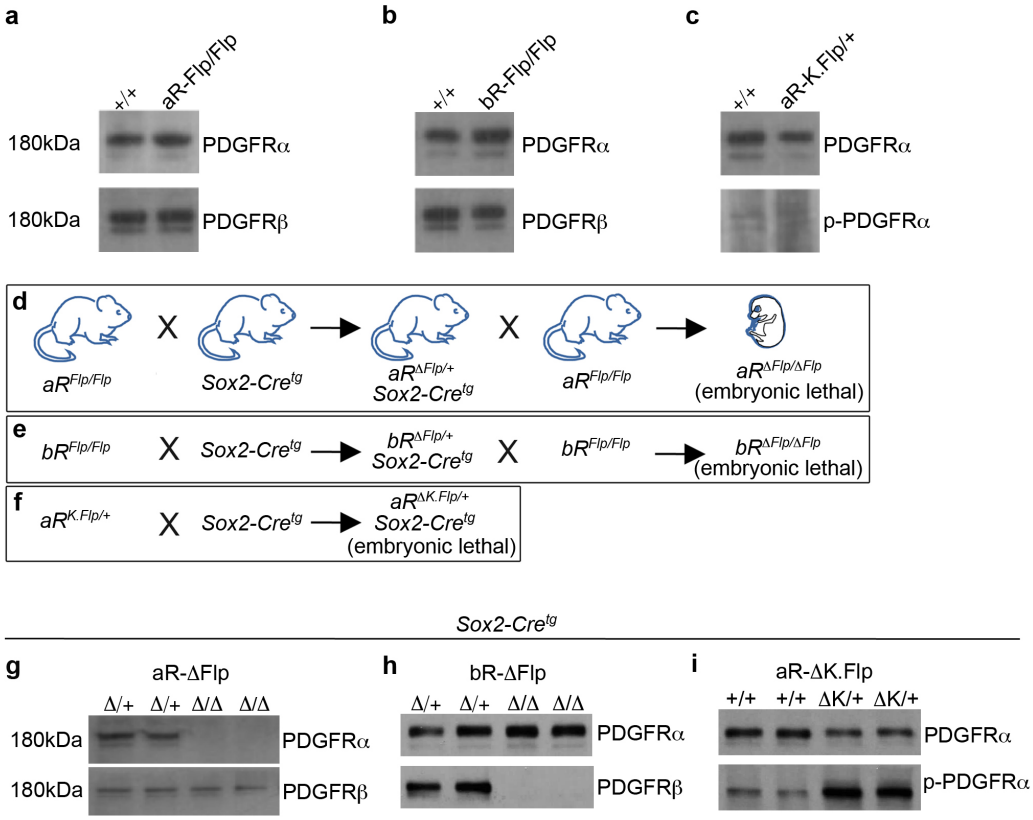
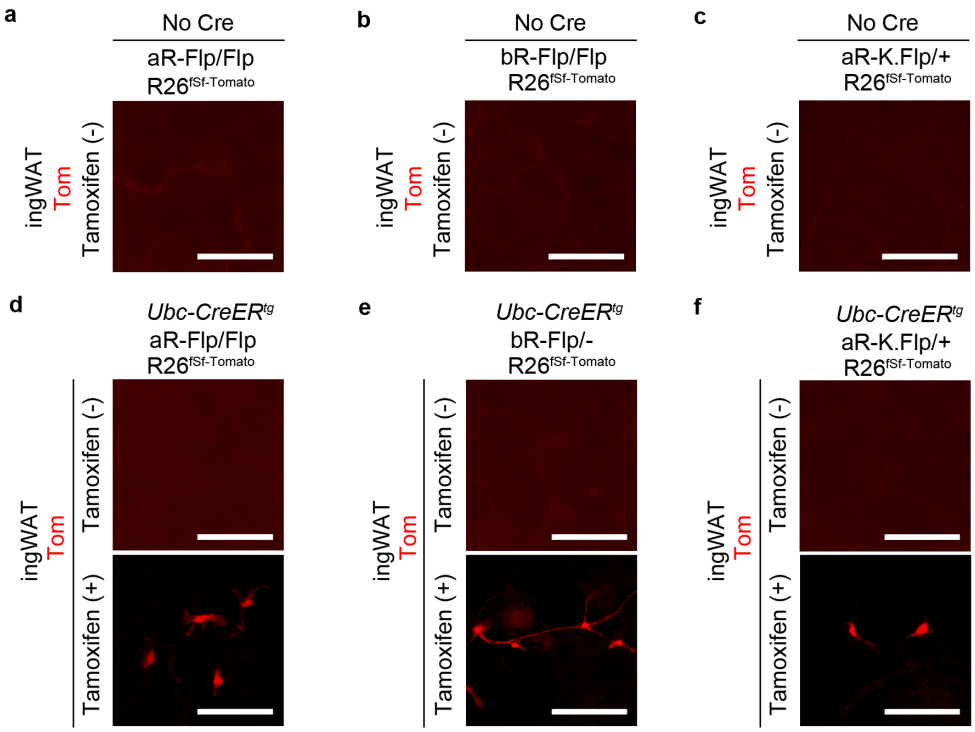
SUPPLEMENTARY INFORMATION



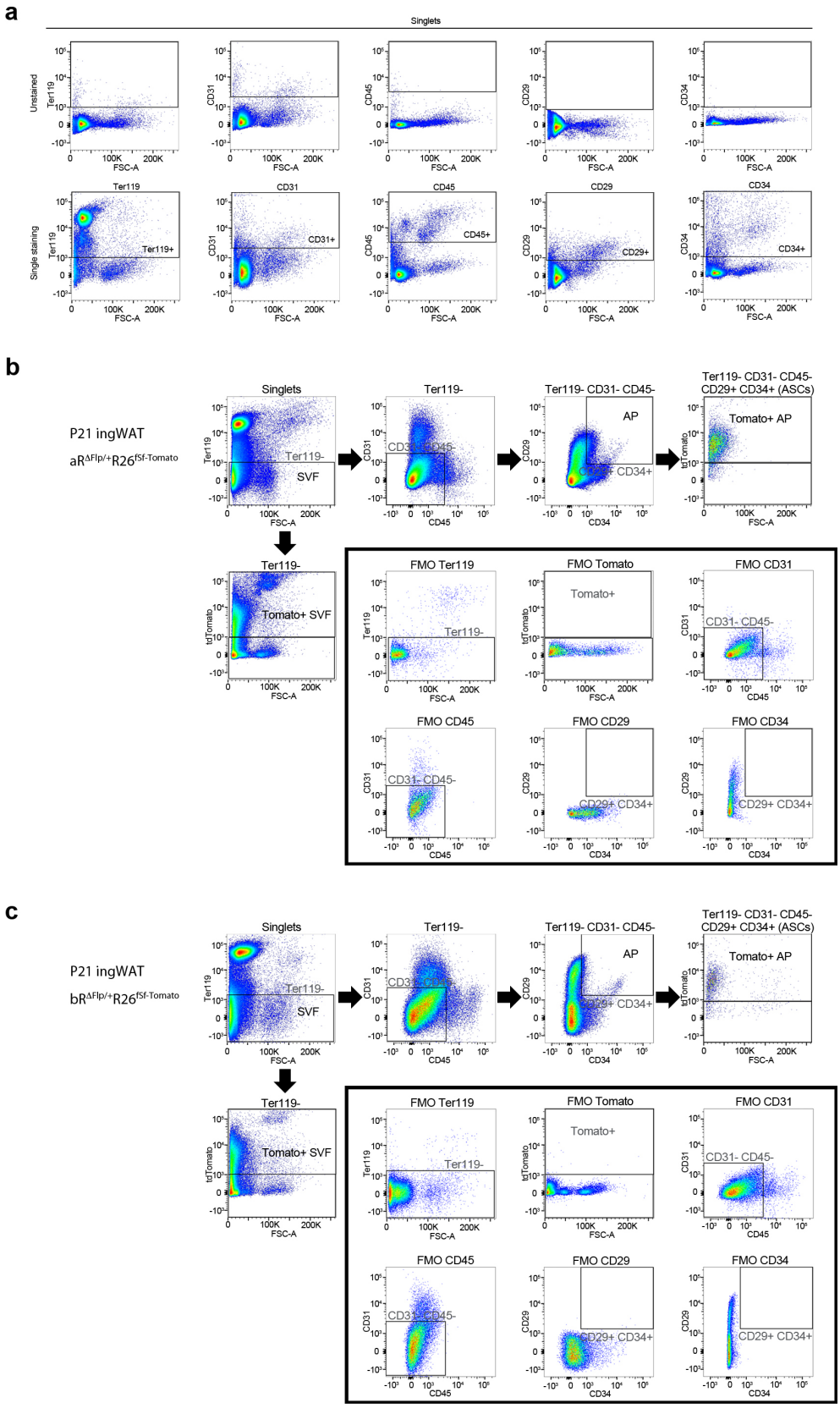
Supplementary Figure 1. Design of Pdgfr-Flp knockin alleles, related to Figure 1. Diagrams representing: (**a**) *Pdgfra* knockin allele aR-Flp, which is designed for conditional deletion of PDGFRα and expression of Flp^{o} recombinase. The location of splice acceptor (SA) sequence and loxP sites are indicated. Red arrows indicate the location of PCR primers used to quantify removal of the lox-PDGFRα-lox cassette, and green arrows indicate primers to quantify the Flp cDNA for normalization. (**b**) *Pdgfrb* knockin allele bR-Flp, which is designed for conditional deletion of PDGFRβ and expression of Flp^{o} recombinase. The location of splice acceptor (SA) sequence and loxP sites are indicated. Red arrows indicate the location of PCR primers used to quantify removal of the lox-PDGFRβ-lox cassette, and green arrows indicate primers to quantify the Flp cDNA for normalization. (**c**) *Pdgfra* knockin allele aR-K.Flp, which is designed for conditional expression of constitutively active PDGFRα^{K} and co-expression of Flp^{o} recombinase. The location of splice acceptor (SA) sequence, loxP sites, and T2A sequence are indicated. Red arrows indicate the location of PCR primers used to quantify removal of the lox-NeoStop-lox cassette, and green arrows indicate primers to quantify the Flp cDNA for normalization.



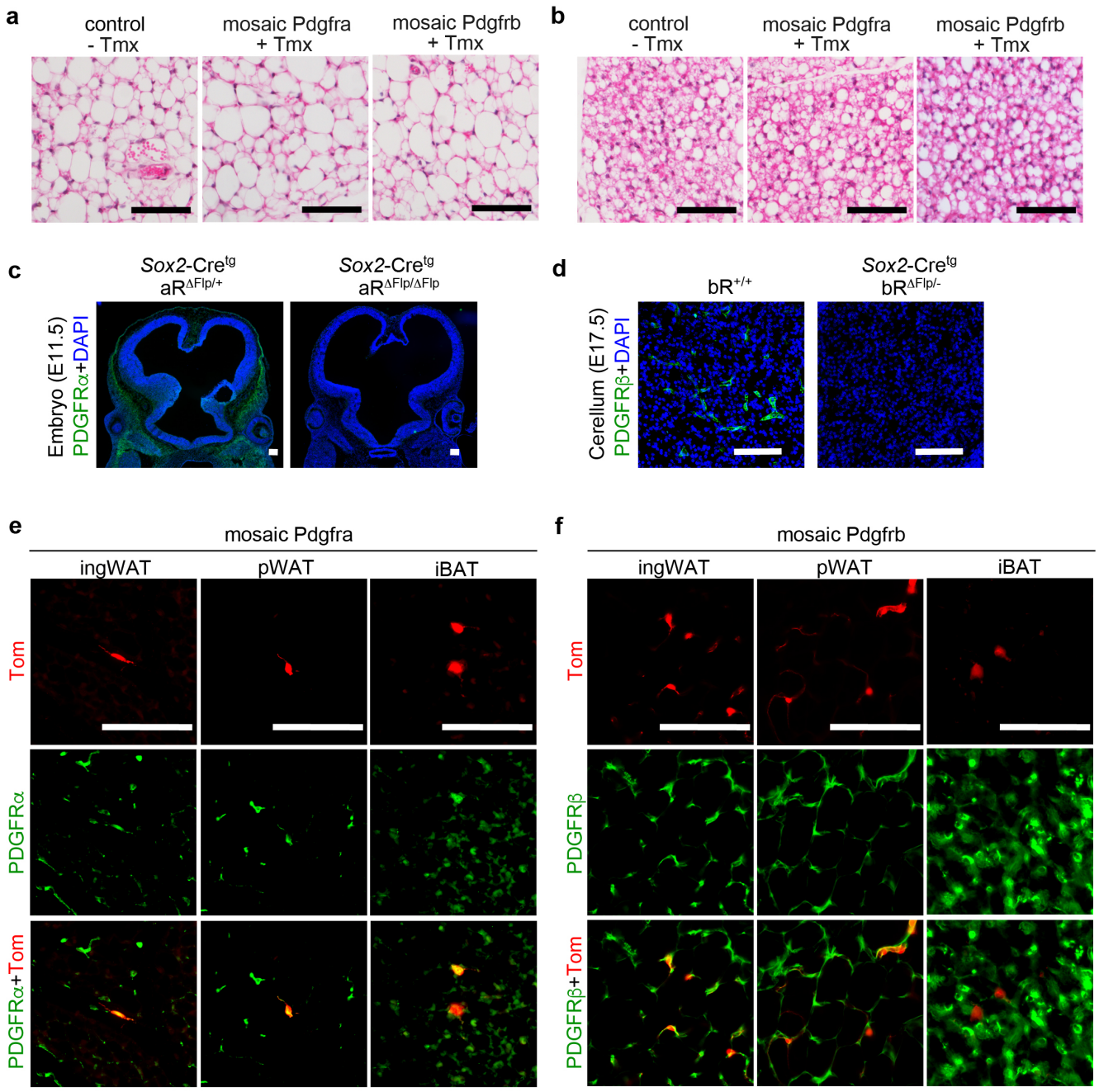
Supplementary Figure 2. PDGFR-Flp constructs are Cre-dependent, related to Figure 1.
(**a-c**) Lung tissue from Cre-naïve mice of the indicated genotypes was used for Western blot for total PDGFRα, total PDGFRβ, or phospho-PDGFRα (pY762) as indicated: (**a**) Tissue lysate from $aR^{+/+}$ or $aR^{Flp/Flp}$ tissue showing equivalent expression of PDGFRα, (**b**) Tissue lysate from $bR^{+/+}$ or $bR^{Flp/Flp}$ tissue showing equivalent expression of PDGFRβ, (**c**) Tissue lysate from $aR^{+/+}$ or $aR^{K.Flp/+}$ tissue showing reduced expression of PDGFRα but no change in phosphorylation.
(**d-f**) *Sox2-Cre*^{tg} breeding strategy to generate global ΔFlp mice. *Sox2-Cre*^{tg} is active in the epiblast and targets the entire embryo proper including the germline. (**d** and **f**) $aR^{\Delta Flp/+}$ and $bR^{\Delta Flp/+}$ mice are produced in the first generation of breeding with *Sox2-Cre*^{tg}, and these mice are used for lineage tracing in Fig. 1e-h. Non-viable $aR^{\Delta Flp/\Delta Flp}$ and $bR^{\Delta Flp/\Delta Flp}$ embryos are produced in the second generation of breeding, and these embryos are used for Western blots in panels g-h of this figure. (**f**) Non-viable $aR^{\Delta K.Flp/+}$ embryos are produced in the first generation of breeding with *Sox2-Cre*^{tg}, and these embryos are used for lineage tracing in Fig. 1i,j and for Western blots in panel i of this figure.
(**g-i**) *Sox2-Cre*^{tg} was used to generate aR-ΔFlp, bR-ΔFlp, and aR-ΔK.Flp embryos for Western blot for total PDGFRα, total PDGFRβ, or phospho-PDGFRα (pY762) as indicated: **(g)** Lysate from $aR^{\Delta Flp/+}$ or $aR^{\Delta Flp/\Delta Flp}$ whole embryos at E11.5, showing deletion of PDGFRα protein. **(h)** Lysate from $bR^{\Delta Flp/+}$ or $bR^{\Delta Flp/\Delta Flp}$ heads at E17.5, with deletion of PDGFRβ protein. **(i)** Lysate from $aR^{+/+}$ or $aR^{\Delta K.Flp/+}$ whole embryos at E13.5, showing increased phosphorylation of PDGFRα.



Supplementary Figure 3. The PDGFR-Flp/R26-fSF-Tomato reporter system is not active without Cre, related to Figures 1 and 2.
(**a-c**) Whole mount fluorescent images of ingWAT from Cre-naïve mice of the indicated genotype, showing only autofluorescence even after long exposure: (**a**) $aR^{Flp/Flp}$ (**b**) $bR^{Flp/Flp}$ (**c**) $aR^{K.Flp/+}$.
(**d-f**) Whole mount fluorescent images of ingWAT from *Ubc-CreER*^{tg} mice of the indicated genotypes, without Tmx treatment or 7 days after 1x Tmx treatment, showing Tomato expression only after Tmx: (**d**) $aR^{Flp/Flp}$ (**e**) $bR^{Flp/-}$ (**f**) $aR^{K.Flp/+}$. Scale bars: 100µm.



Supplementary Figure 4. Flow cytometry of stromal vascular cells and adipocyte progenitors, Related to Figure 1.
(**a**) Single staining controls for each antibody. (**b** and **c**) Representative flow cytometry strategy for Tomato$^+$ cells in P21 ingWAT from mice with constitutive lineage tracing: (**b**) regulated by Pdgfra-Flp, or (**c**) regulated by Pdgfrb-Flp. For Tomato$^+$ stromal vascular fraction (SVF), live Ter119-negative singlets were gated for Tomato. For Tomato$^+$ endothelial cells and hematopoietic cells, live Ter119-negative singlets were gated for CD31 or CD45, respectively. For Tomato$^+$ adipocyte progenitors (APs), live Ter119-negative singlets were gated for lack of CD31 and CD45, then for the CD29$^+$ CD34$^+$ population representing APs, and then for Tomato expression. The same strategy was used throughout the study. Fluorescence minus one (FMO) controls are shown in black boxes.

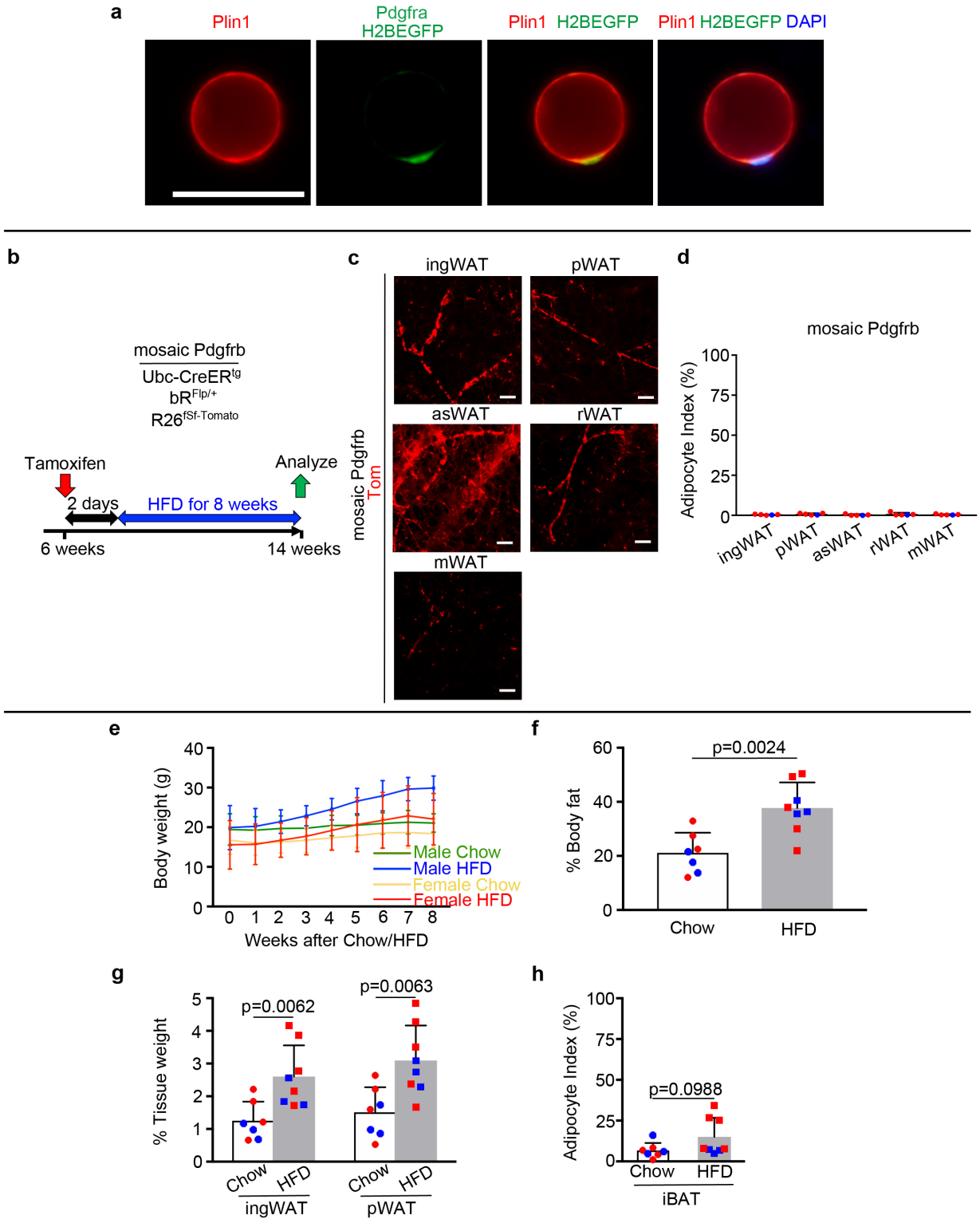


Supplementary Figure 5. Normal morphology of mosaic *Pdgfr* WAT/BAT and *Pdgfra/Pdgfrb* lineage labels PDGFRα+/PDGFRβ+ cells, related to Figure 2.

(**a** and **b**) Hematoxylin/Eosin-stained histological images of white and brown adipose tissue from 7 week old mosaic mice that received Tmx at 6 weeks old. (**a**) ingWAT. (**b**) iBAT. Scale bars: 100µm.

(**c** and **d**) Validation of antibody specificity: (**c**) Heads from E11.5 embryos of the indicated genotypes showing mesenchymal immunofluorescence for PDGFRα in *Pdgfra* heterozygote but no labeling in the homozygous knockout. (**d**) Cerebellum from E17.5 embryos of the indicated genotypes showing pericyte immunofluorescence for PDGFRβ in *Pdgfrb* heterozygote but no labeling in the homozygous knockout.

(**e** and **f**) Validation of lineage labeling specificity: (**e**) adult mosaic $aR^{Flp/+}$ adipose tissues at 7 days after Tmx with PDGFRα immunofluorescence on Tomato^{+} cells. (**f**) adult mosaic $bR^{Flp/+}$ adipose tissues at 7 days after Tmx with PDGFRβ immunofluorescence on Tomato^{+} cells. Scale bar: 50µm.



Supplementary Figure 6. Adult lineage labeling with high fat diet, related to Figure 3

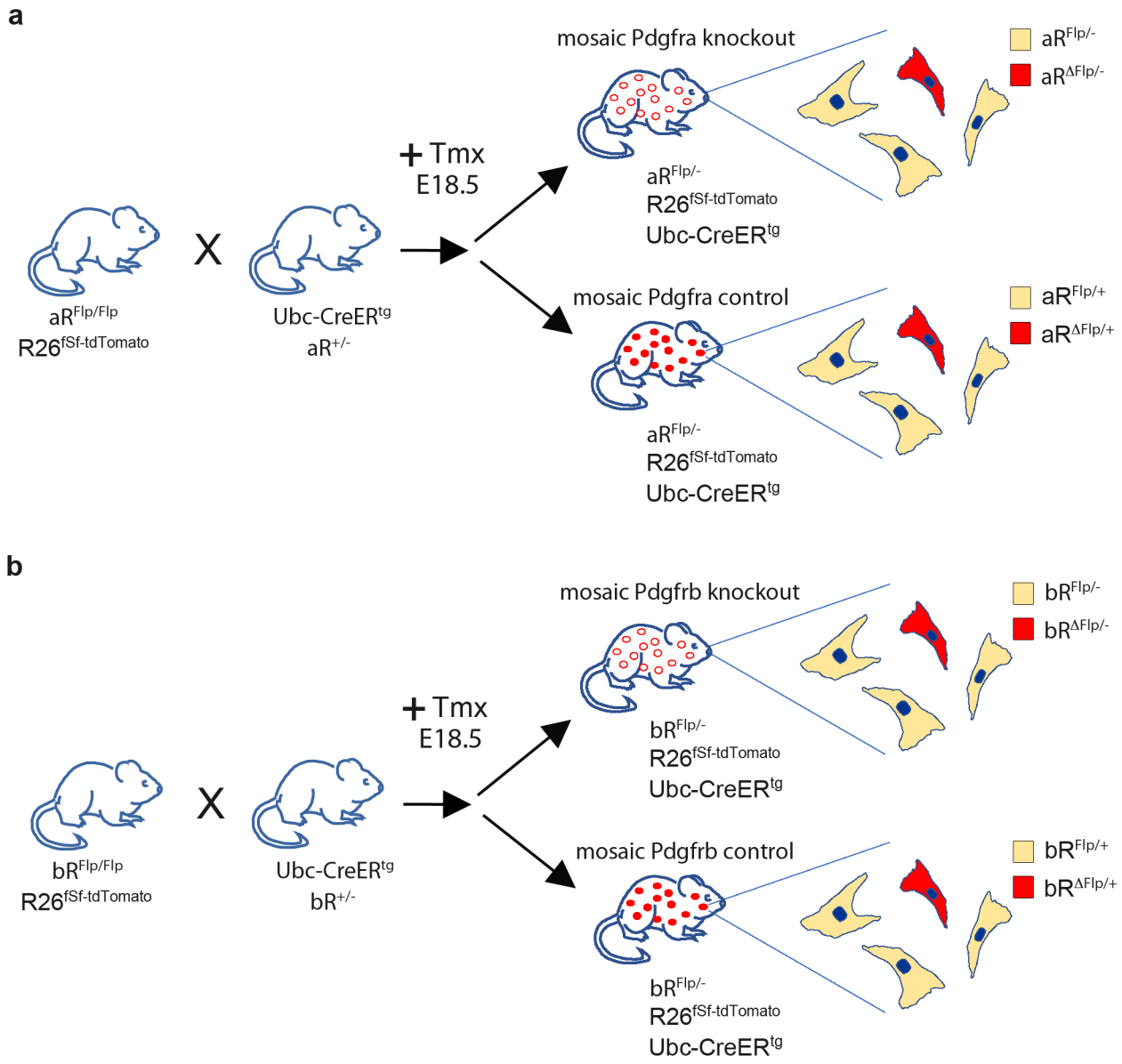
(**a**) Whole mount fluorescent image of an isolated mature adipocyte from mice generated by the experimental scheme shown in Fig. 3d. This adipocyte shows dim nuclear EGFP (green), DAPI stain for DNA (blue) and Plin1 immunofluorescence (red). Scale bar: 50μm

(**b**) Mosaic *Pdgfrb* genotypes with experimental scheme whereby mosaic *Pdgfrb* lineage labeling was induced at 6-weeks old by administering 1x Tmx (100mg/kg) with analysis after 8 weeks of HFD feeding.

(**c**) Whole mount fluorescent images of WAT with mosaic *Pdgfrb* lineage labeling in mural cells. Scale bars: 100μm

(**d**) Near zero adipocyte index for mosaic *Pdgfrb*-lineage WAT after 8 weeks HFD (*n* = 5 mice per tissue).

(**e-h**) Diet effects on body fat and BA adipogenesis in mice with non-mosaic *Pdgfrb* lineage labeling after 5x Tmx by the experimental scheme shown in Fig. 3g (*n* = 7-8 mice per condition). (**e**) Average weight + SD during 8 weeks of normal chow diet (NCD) or high fat diet (HFD). (**f**) Percent body fat + SD. (**g**) Tissue weight + SD. (**h**) Adipocyte index + SD for Tomato^{+} BA.



Supplementary Figure 7. Graphical description of experimental schemes to generate mosaic *Pdgfr* knockouts and controls at birth, related to Figures 4 and 5. (**a** and **b**) Based on timed breeding, Tmx was administered to pregnant dams at E18.5 (one day before birth), resulting in offspring with mosaic *Pdgfr* knockout or control genotypes in the same litter. (**a**) In mosaic *Pdgfrb* knockouts, Tomato-labeled cells have the genotype $bR^{\Delta Flp/-}$ and unlabeled cells have the genotype $bR^{Flp/-}$ (which still expresses PDGFRβ protein). In mosaic *Pdgfrb* controls, Tomato-labeled cells have the genotype $bR^{\Delta Flp/+}$ and unlabeled cells have the genotype $bR^{Flp/+}$. (**b**) In mosaic *Pdgfra* knockouts, Tomato-labeled cells have the genotype $aR^{\Delta Flp/-}$ and unlabeled cells have the genotype $aR^{Flp/-}$ (which still expresses PDGFRα protein). In mosaic *Pdgfra* controls, Tomato-labeled cells have the genotype $aR^{\Delta Flp/+}$ and unlabeled cells have the genotype $aR^{Flp/+}$.