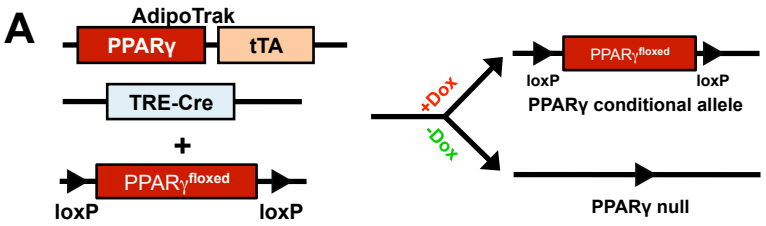
Figure S1, related to Figure 1



	AdipoTrak	Reporter	PPARγ deletion
No Dox	ON	ON	Null
Dox	OFF	OFF	WT

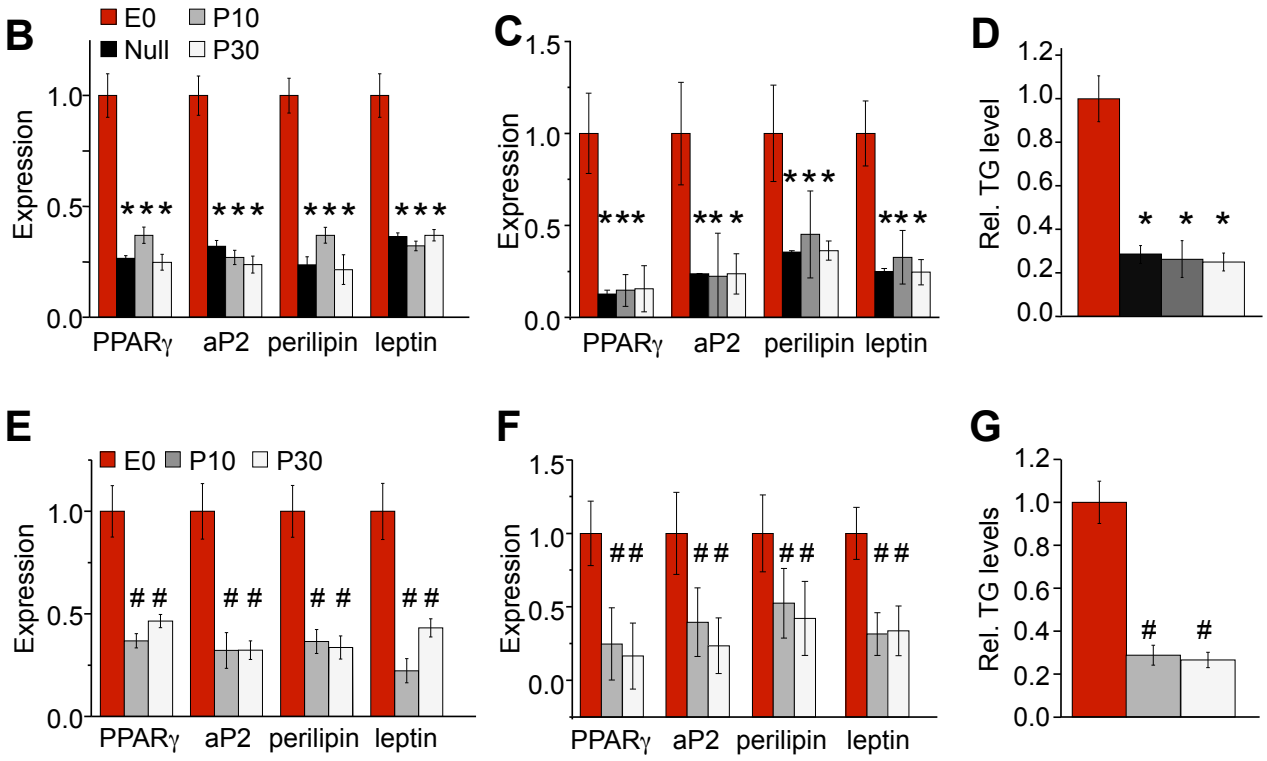
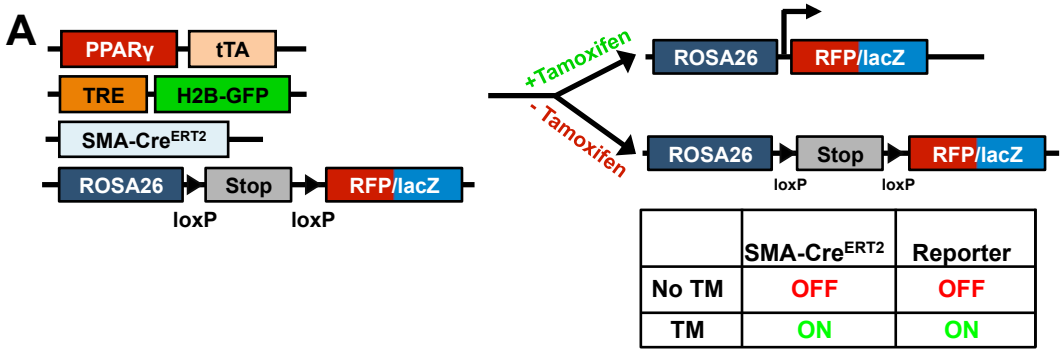
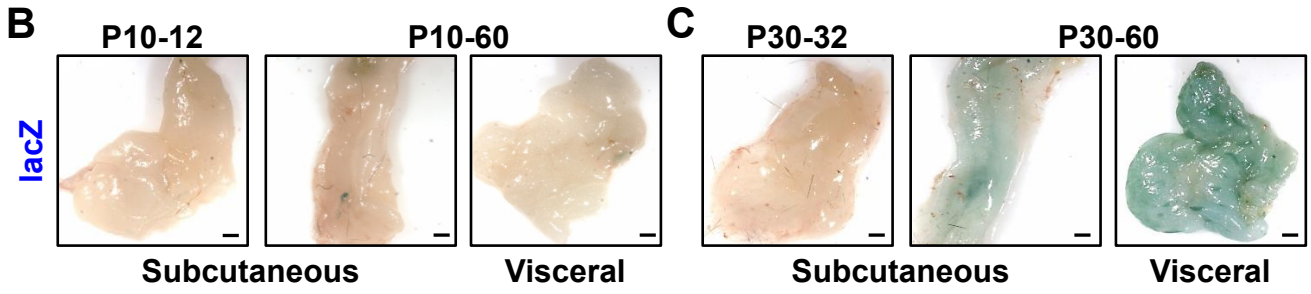
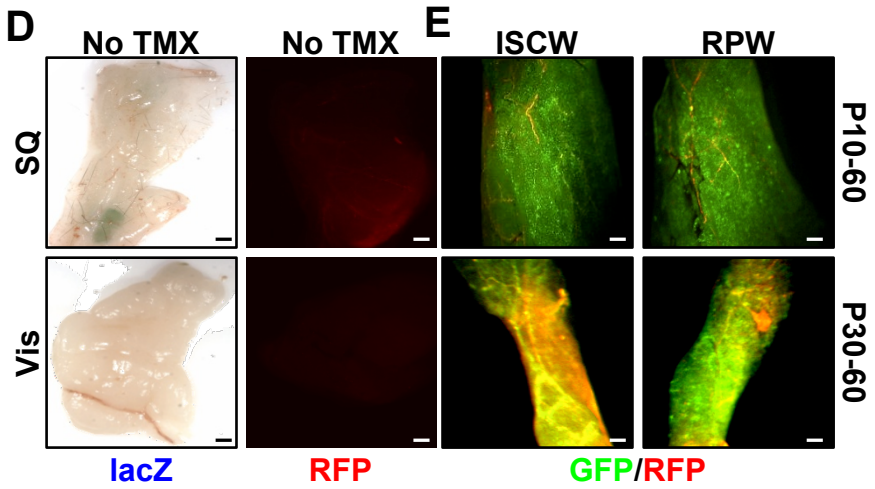


Figure S2, related to Figure 2



	SMA-CreERT2	Reporter
No TM	OFF	OFF
TM	ON	ON





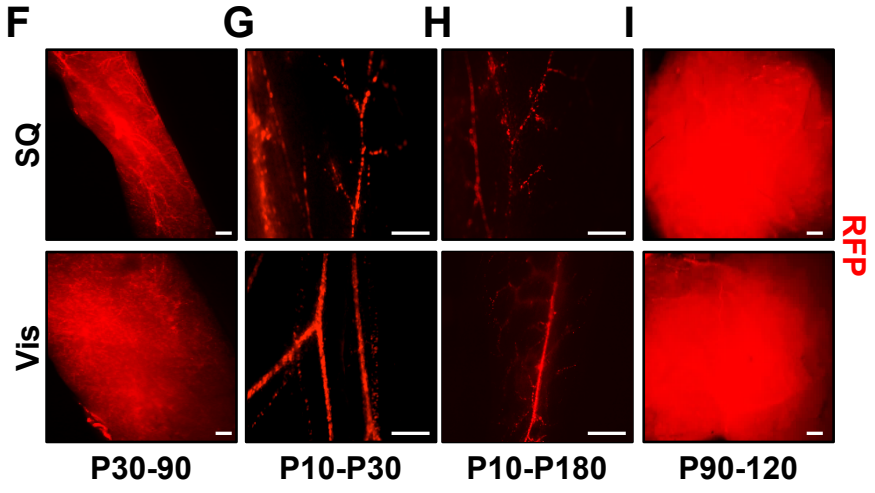


Figure S3, related to Figure 3

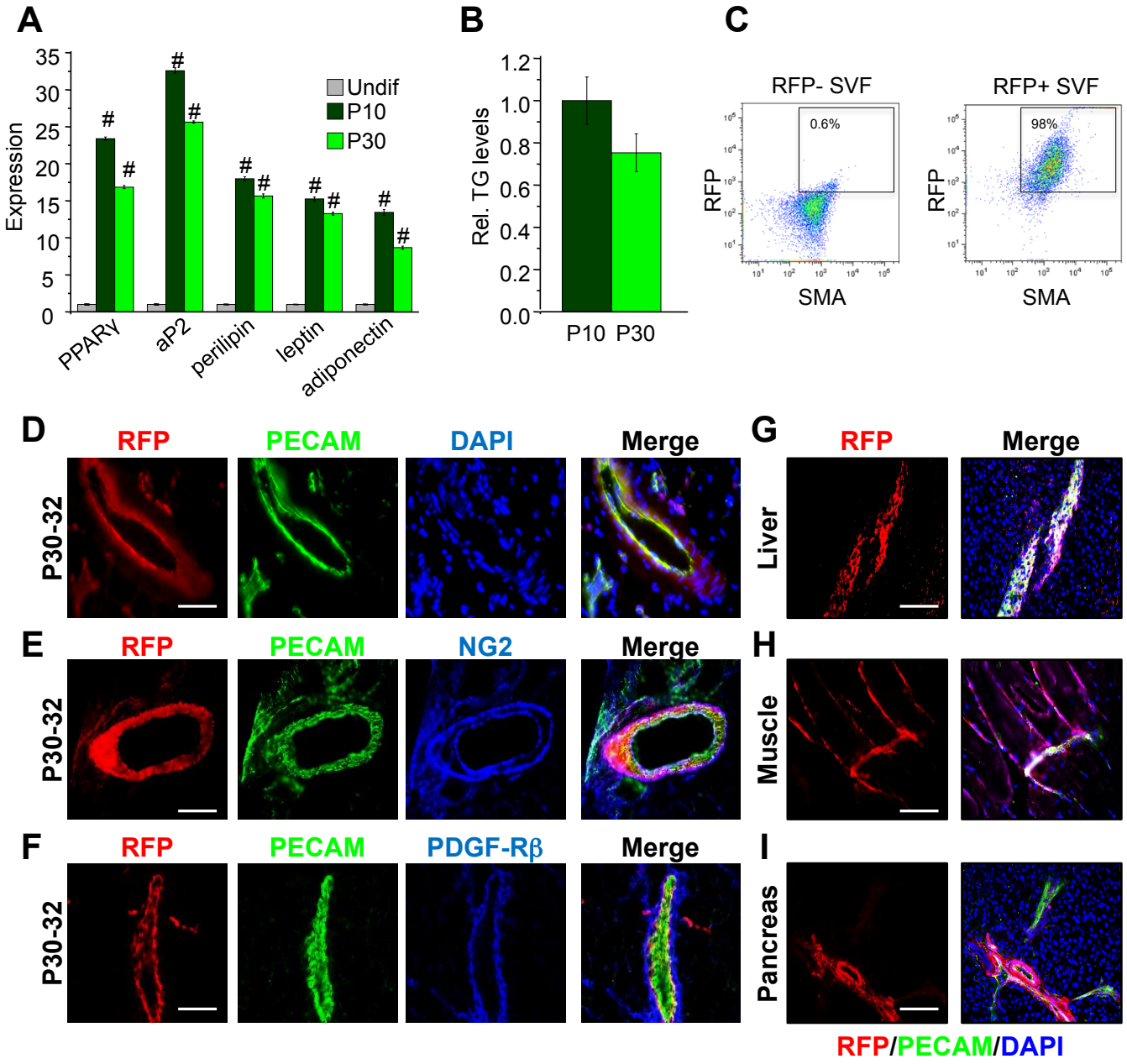


Figure S4, related to Figure 4

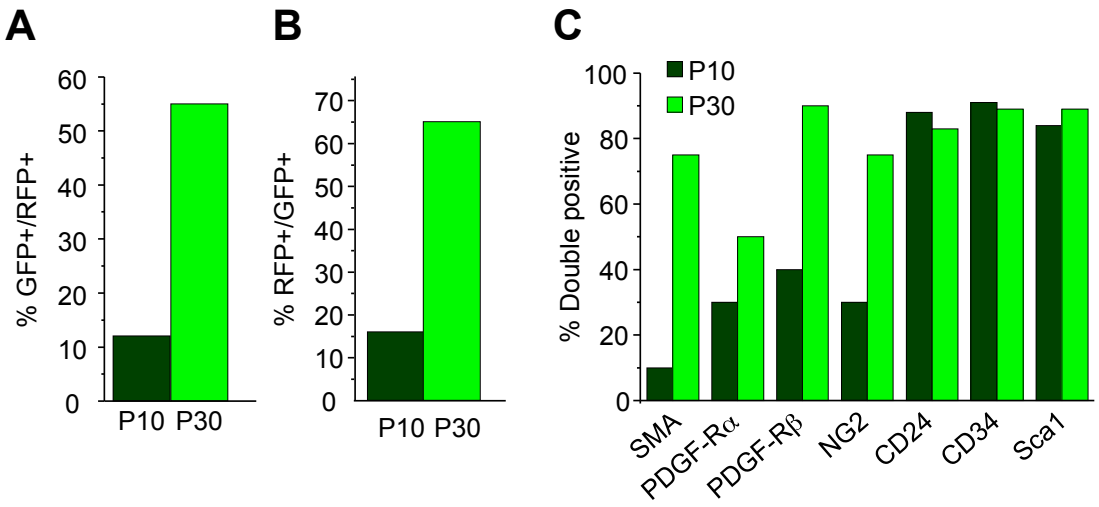


Figure S5, related to Figure 5

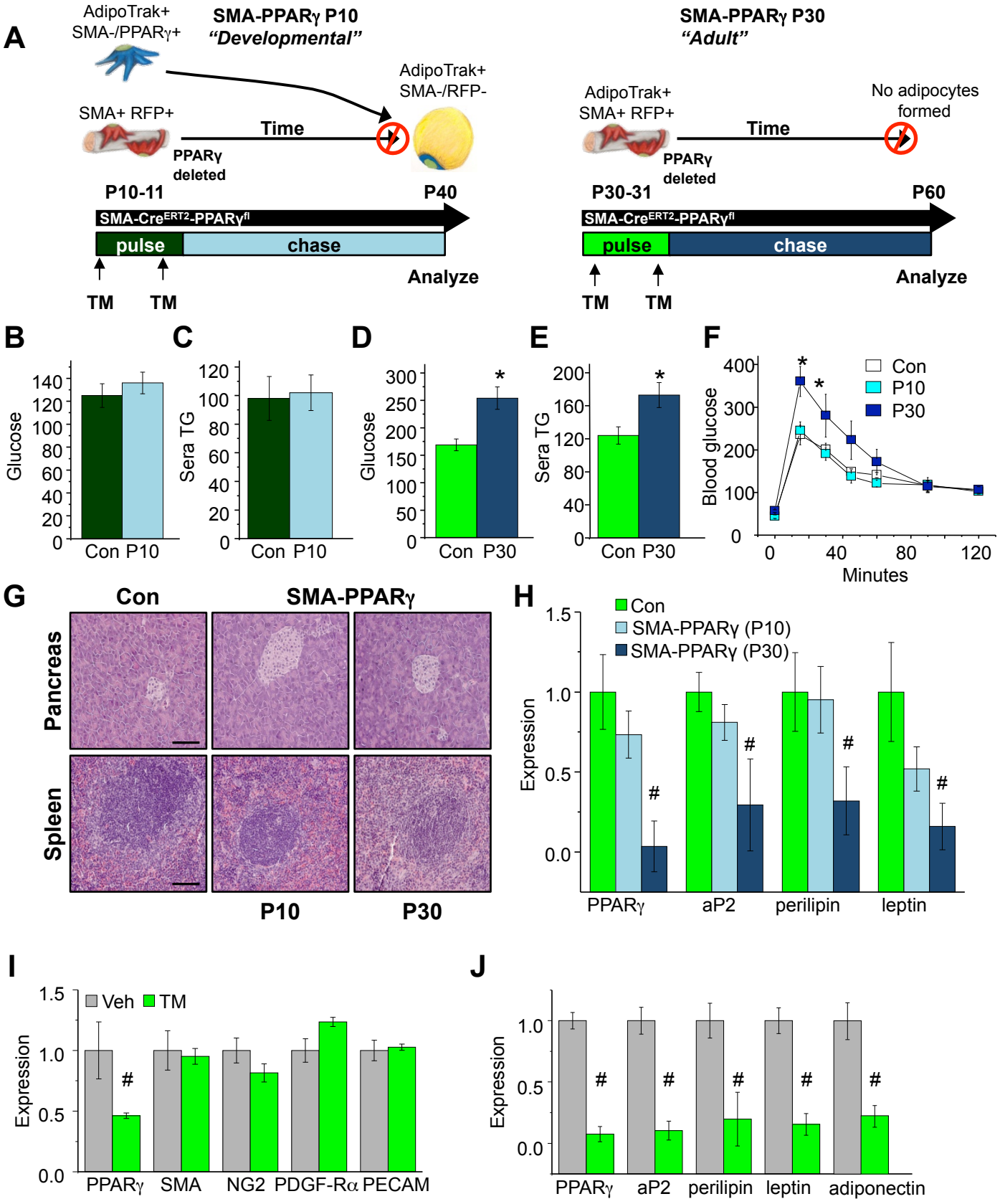


Figure S6, related to Figure 6

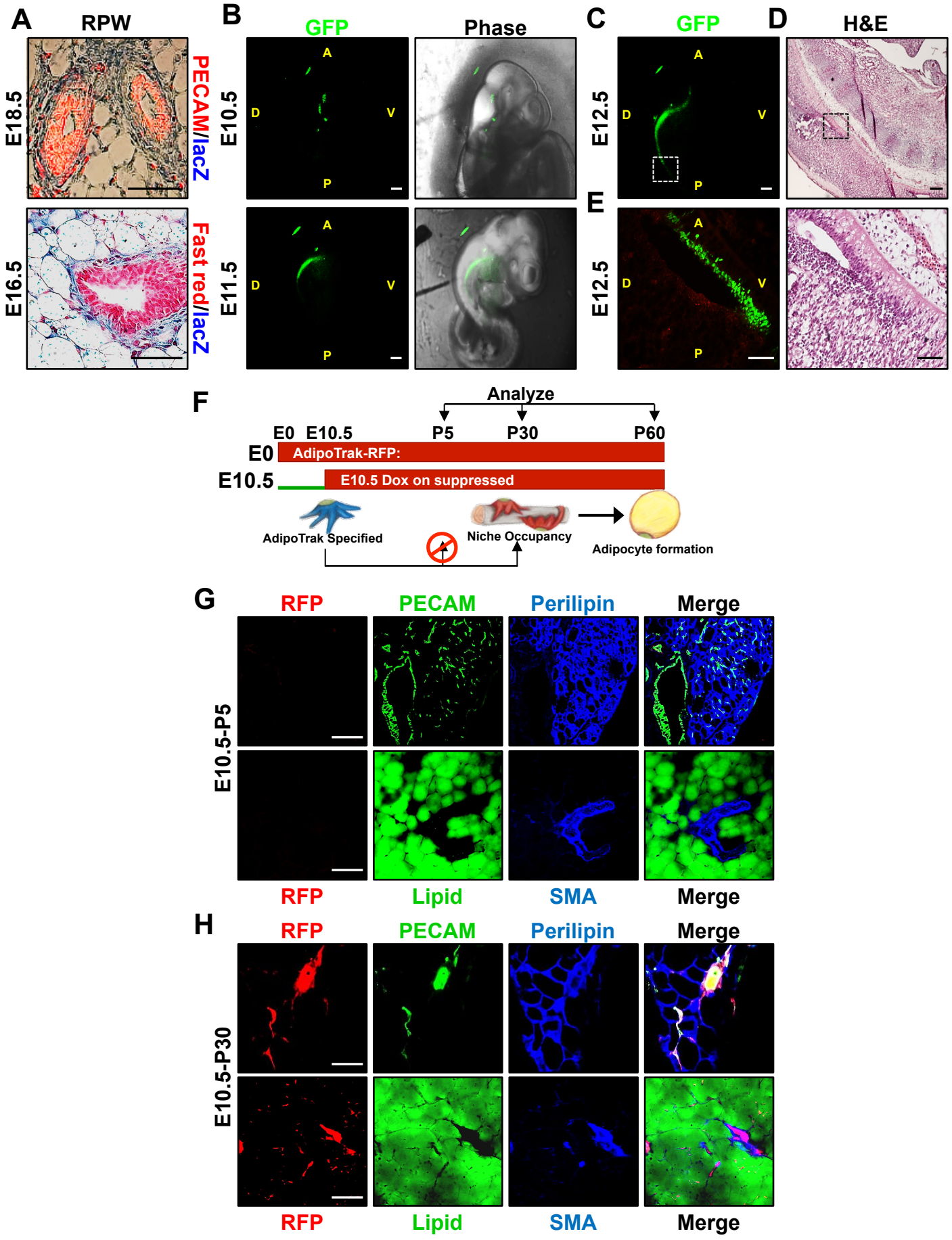
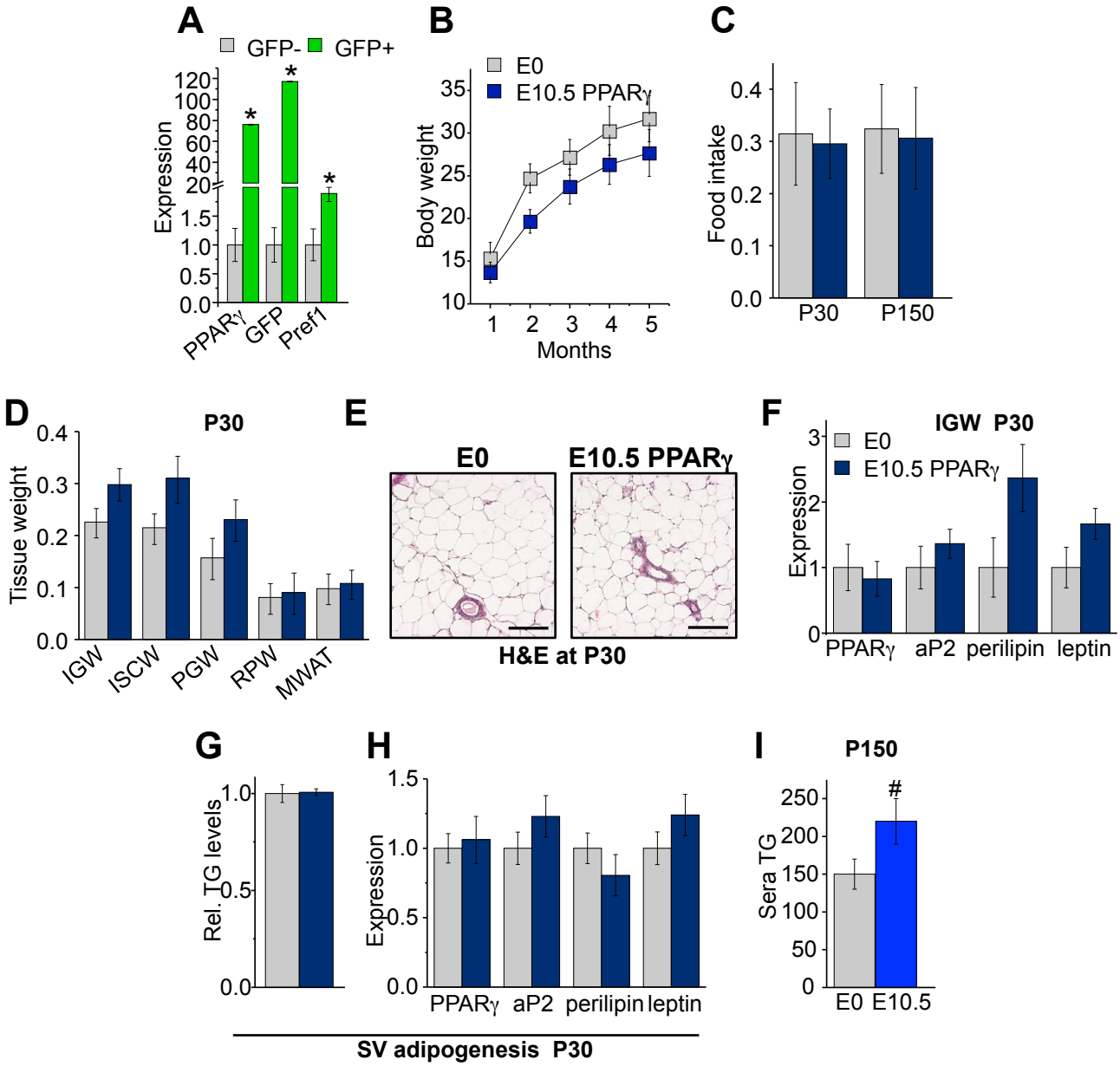


Figure S7, related to Figure 7



Supplemental Figure Legends

Figure S1, related to Figure 1. AdipoTrak lineage marking and conditional PPARγ deletion studies

A) Generation of AdipoTrak-PPARγ mutant mice: the tet-transactivator (tTA, Dox off) was recombined into the PPARγ locus creating a PPARγ-tTA knock-in mouse strain driven from the endogenous PPARγ promoter. AdipoTrak mice, harboring a tTA null PPARγ allele and TRE-Cre, were crossed with a PPARγ floxed allele, floxed sites surround exon 2. When Cre is expressed, the second exon is excised, inactivating this allele; thereby generating PPARγ nullizgosity in this cell and all descendants. Cre expression is repressed by administering Dox. **B)** mRNA expression of denoted genes from subcutaneous depots from AdipoTrak-PPARγ$^{fl/tTA}$ Dox suppressed control mice (E0, always on Dox), constitutive PPARγ null (Null, never on Dox), P10 or P30 Dox off mice . **C-D)** SV cells were isolated from the above mice, cultured and induced to undergo adipocyte differentiation, mRNA of adipocytes genes (C) and triglyceride levels (**D**) were assessed at the end of 7 days. *P<0.05 versus control always on Dox; n=8 mice/group. **E)** AdipoTrak-PPARγ$^{fl/tTA}$ mice described in Figure 1E, AdipoTrak-PPARγ$^{fl/tTA}$ constitutively Dox suppressed (E0), or Dox added at P10 or P30 and mRNA expression of denoted adipocytes genes from the subcutaneous adipose depots. **F-G)** SV cells were isolated from these mice and cultured in adipogenic media for seven days and then mRNA expression (**F**) and triglyceride levels (**G**) were assessed. #P<0.05 versus control always on Dox: Data are ±SEM; n = 6 mice/group unless otherwise noted.

Figure S2, related to Figure 2. Characterization of the AdipoTrak and SMA-Cre^{ERT2} lineage marking system

A) For mural cell SMA fate mapping, we combined the *PPARγ^{tTA}* allele with *SMA-Cre^{ERT2}* and either the ROSA26-flox-stop-flox-RFP or ROSA26-flox-stop-flox-lacZ alleles. The ROSA26 reporters are expressed in a Tamoxifen, and hence Cre, dependent manner. Upon tamoxifen administration Cre is activated, the transcriptional stop is excised and RFP or lacZ is irreversibly expressed, thereby marking SMA-expressing cells and all descendants regardless of whether Cre remains active. In the absence of tamoxifen, SMA-Cre^{ERT2} is inactive and the RFP or lacZ alleles remain silent. *PPARγ^{TA}* transcriptionally regulates TRE-H2B-GFP, inducing nuclear GFP marking. In the presence of Dox, tTA is inactive, blocking new TRE-H2B-GFP expression. GFP is stable in post-mitotic cells. **B-C)** AdipoTrak-GFP (*PPARγ^{tTA}; TRE-H2B-GFP*); *SMA-Cre^{ERT2}; R26R^{lacZ}* mice were administered tamoxifen at P10 or P30 for two consecutive days and subcutaneous (inguinal) and visceral (perigonadal) adipose depots were examined for β-galactosidase (mural lineage) activity either at pulse (P10-12 or P30-32) or chase (P10-P60 or P30-P60). **D)** β−galactosidase activity and RFP fluorescence in vehicle treated *SMA-Cre^{ERT2}; R26R^{RFP}* or *R26R^{LacZ}* mice. **E)** AdipoTrak (*PPARγ^{TA}; TRE-H2B-GFP*); *SMA-Cre^{ERT2}; R26R^{RFP}* mice were injected with tamoxifen at P10 and P11 or P30 and P31 and chased until P60. Interscapular (ISCW) and retroperitoneal (RPW) whole adipose depots were imaged for RFP (mural lineage) and GFP (adipose lineage) signals. The yellow signal indicates that *SMA-Cre^{ERT2}* fate maps into the adipose lineage when induced at P30 but not when induced at P10. **F-I)** *SMA-Cre^{ERT2}; R26R^{RFP}* mice were injected with tamoxifen for two consecutive days (pulse) and chased for various periods,

P30-P90 (**F**), P10-P30 (**G**), P10-180 (**H**) and P90-P120 (**I**) and whole depots were analyzed for RFP fluorescence.

Figure S3, related to Figure 3. The SMA-Cre^{ERT2}; R26R^{RFP} fate mapping model faithfully marks mural cells

A-B) P10 or P30 AdipoTrak SV cells were isolated, cultured, and adipogenically induced. mRNA expression of the indicated adipose markers were analyzed (**A**) and triglycerides were measured (**B**). **C)** Flow cytometry plots indicate that the vast majority of RFP+ cells co-express SMA. Cells were analyzed based on RFP expression and SMA antibodies immunostaining. **D-F)** *SMA-Cre^{ERT2} R26R^{RFP}* mice were given tamoxifen for two consecutive days P30 and P31, and at P32 expression of RFP (red), PECAM (green), NG2 (green, **E**) and PDGF-Rβ (blue, **F**) were assessed. **G-I)** SMA-Cre^{ERT2} *R26R^{RFP}* mice were provided tamoxifen on P30 and P31, and at P32 reporter expression (RFP), PECAM immunostaining and DAPI staining were examined in liver (**G**), skeletal muscle (**H**) and pancreas (**I**). #P<0.05 versus undifferentiated SV cells. *P<0.01 versus RFP positive cells. Data are ± SEM; n= 5 mice/group unless otherwise noted.

Figure S4, related to Figure 4. Functional and molecular characteristics of *Developmental* and *Adult* adipose stem cells

A-B) SV cells isolated from P10 and P30 AdipoTrak-GFP; *SMA-Cre^{ERT2} R26R^{RFP}* mice were flow cytometrically analyzed by for co-expression of GFP and RFP fluorescence (**A**) or RFP and GFP fluorescence (**B**). Plots indicate the percentage of cells that co-

expressed the two markers. **C)** Flow cytometry analyses of denoted mural and stem cell markers in P10 and P30 AdipoTrak GFP positive cells from mice.

Figure S5, related to Figure 5. Characterization of the SMA-Cre^{ERT2} inducible PPARγ deletion.

A) Experimental design for P10 and P30 SMA-Cre^{ERT2} induced PPARγ deletion. Mice were administered Tamoxifen at P10 or P30 to temporally delete PPARγ in SMA+ mural cells. Under the P10 condition, fat cells are allowed to form normally because SMA+ mural cells are not the contributing adipose progenitor cell source (left panel). In contrast, deleting PPARγ in SMA+ mural cells at P30 renders them non-function and unable to form functional fat cells demonstrating that SMA+ mural cells are adipose progenitor cells and essential for adipose tissue homeostasis (right panel). Bottom: experimental protocol. **B-C)** *SMA-Cre^{ERT2}*, *PPARγ$^{fl/tTA}$* mice were randomized to vehicle or tamoxifen at P10, P11. 30 days later we quantified random glucose levels (**B**) and sera triglycerides (**C**). **D-E)** *SMA-Cre^{ERT2}*, *PPARγ$^{fl/tTA}$* mice were administered vehicle or tamoxifen at P30, P31 (pulsed); 30 days later we analyzed random glucose levels (**D**) and sera triglycerides (**E**). **F)** *SMA-Cre^{ERT2}*, *PPARγ$^{fl/tTA}$* mice were randomized to vehicle or tamoxifen at P10/P11 or P30/31 and 30 days later glucose tolerance was analyzed. *P <0.05 versus control; n=10/group. **G)** Hematoxylin and eosin stains of pancreas and spleen 30 days after *SMA-Cre^{ERT2}*-dependent PPARγ deletion. **H)** SV cells were isolated from un-induced P10 and P30 SMA-Cre^{ERT2}; PPARγ$^{f/f}$ mice, cells were plated and cultured with vehicle or tamoxifen (100nM/ml) for 48 hrs. Cells were induced to differentiate, after 7 days mRNA expression of indicated adipose genes was assessed. *P<0.05 versus control.

#P<0.05 versus P60 control. **I-J)** SV cells were isolated from un-induced P30 *SMA-Cre^{ERT2} PPARγ* mice (*SMA-Cre^{ERT2}*, *PPARγ$^{fl/tTA}$*) and cultured in vehicle (Veh) or 100 nM tamoxifen (TAM), to trigger cell culture-induced *PPARγ* deletion, for 48 hours. mRNA was isolated at the 48 hour time point (**I**) or after 7 days culture in adipogenic media (**J**) and analyzed for expression of the indicated genes. #P<0.05 versus *SMA-Cre^{ERT2}* un-induced vehicle treated.

Figure S6, related to Figure 6. Developmental specification of adult adipose stem cells

A) Histological sections of β-galatosidase stained P30 retroperitoneal adipose depot (RPW) from AdipoTrak Dox suppressed mice at E16.5 (left) and E18.5 (right). The E16.5 Dox suppressed section was co-stained with PECAM antibody, and the E18.5 Dox suppressed section was co-stained with Nuclear Fast Red. **B)** AdipoTrak embryos were analyzed at E10.5 and E11.5 days for whole embryo GFP fluorescence. **C)** AdipoTrak embryos were analyzed at E12.5 for whole embryo GFP fluorescence. **D)** H&E section from the white box in (**C**). **E)** GFP fluorescence image (left) and H&E section from the black box in (**D**) (right). A= anterior, P= positerior, D= dorsal and V= ventral. **F)** Diagram depicting the experimental design of AdipoTrak lineage fate mapping at E10.5. AdipoTrak-R26R^{RFP} or R26R^{lacZ} where Dox suppressed from conception (E0=control) or from E10.5. Mice were analyzed at P5, P30 or P120 for RFP fluorescence or β galatosidase activity. Below: cartoon of experimental prediction, illustrating that Adult adipose progenitor cells are specified at E10.5 but are not present in the adipose depots at P5 but become niche residing (SMA+) at P30 and then differentiate in mature white

adipocytes. **G-H)** AdipoTrak *Rosa26R*^{RFP} mice were Dox suppressed from E10.5 to P5 (**G**) or P30 (**H**), sectioned and visualized for either PECAM or LipidTox (green) or Perilipin or SMA (blue).

Figure S7, related to Figure 7. *Adult* adipose progenitor cells are essential for *Adult* adipose tissue homeostasis but not adipose depot formation

A) AdipoTrak GFP+ E10.5 embryos were isolated, GFP+ cells were FACs isolated and mRNA expression of PPARγ and GFP were profiled. **B-F)** Body weight (**B**), food intake (**C**), tissue weight (**D**), histology (**E**) and mRNA expression of mature adipocyte markers (**F**) were analyzed from control and E10.5 PPARγ adult progenitor deleted mice at P30. **G-H)** SV cells were isolated at P30 from control or E10.5 Dox suppressed AdipoTrak PPARγ^{floxed} mice and cultured in Dox and adipogenic media, triglyceride accumulation (**G**) and mRNA expression of mature adipocyte markers (**H**) were assessed. *P<0.05 GFP+ versus GFP- cells. **I**) Sera triglycerides from Dox suppressed E0 or E10.5 PPARγ deleted mice at P150 of age. #P<0.05 Con (Dox suppressed E=0) versus E10.5 Dox suppressed AdipoTrak PPARγ^{floxed} mice.

Supplemental Experimental Procedures

SV, Adipocyte and SVP Fractionation

The fractionation of the stromal-vascular (SV) cells, adipocytes and SVP was as described (Tang et al., 2008). Briefly, we pooled subcutaneous (inguinal, interscapular) or visceral (gonadal and retroperitoneal) white adipose tissues for fractionation, unless indicated otherwise. After 2 hours of slow shaking at 37°C, the digest was pipetted up and down a few times for better dissociation. The suspension was then spun at 800g for 10 minutes; the resultant floating layer was the adipocyte layer and the pellet was a crude SV fraction. The floating adipocyte layer was washed in 1X PBS, spun at 800g for 5 minutes, and the solution was removed from below. The pellet was then resuspended in erythrocyte lysis buffer (0.83% NH_4Cl in H_2O) for 8 minutes, spun at 800g for 5 minutes. The pellet was washed once in 1X PBS, resuspended and passed through 30μm mesh. The SVP tubes that remained on the 30μm mesh were washed off and collected in DMEM+10%FBS.

Cell Culture

Isolated SV cells were cultured; adipogenesis was induced with insulin and analyzed as described (Tang et al., 2008). Cells were maintained in DMEM supplemented with 10% FBS, and with or without Dox at a concentration of 50ng/ml. Oil Red O staining and triglyceride accumulation was performed as previously described (Berry and Noy, 2009; Berry et al., 2010; Tang et al., 2008).

Flow Cytometry and Sorting

SV cells were isolated as above and washed, centrifuged at 800g for 5min, and analyzed with a FACScans analyzer or sorted with a BD FACS Aria operated by the UT Southwestern Flow Cytometry Core. Data analysis was performed using BD FACS Diva software. Sorting of GFP+ and GFP- cells was performed on 1) live SV cells from P10 and P30 AdipoTrak mice and 2) E10.5 embryos stained with propidium iodide (PI, 1mg/ml) to exclude dead cells. For GFP+ and SMA+ flow analysis, SV cells from AdipoTrak mice were stained with rabbit anti-α−smooth muscle actin (1:200, Sigma) on ice for 30 minutes. Cells were then washed twice with the staining buffer and incubated with cy5 donkey anti-rabbit secondary antibody for another 30 minutes on ice before flow cytometric analysis. Other flow cytometry reagents: rabbit anti-NG2 (Chemicon), Sca1-PE (BD Biosciences), PECAM-PE (BD Biosciences), CD45-APC (BD Biosciences), CD106-biotin (eBiosciences), CD24-biotin (eBiosciences), CD34-efluor660 (eBiosciences), PDGFRβ-biotin (eBioscience), PDGFRα-biotin (BD Biosciences), and streptavidin-Alexa Fluor 647 (Invitrogen).

X-gal Staining

Adipose tissues were stained as previously described (Tang et al., 2008). Briefly, adipose tissues were fixed in 4% PFA for 2 hours with gentle shaking then rinsed twice with 1X PBS. Samples were stained overnight in X-gal staining solution at 37°C in the dark followed by washing three times with 1X PBS and post-fixed 4% PFA. The stained tissues were photographed or embedded in paraffin for histology.

Histology

Hematoxylin and eosin (H&E) staining and immunocytochemistry were performed as described (Tang et al., 2008). Antibodies: rat anti-PECAM (1:200, BD Biosciences), rabbit anti-α–smooth muscle actin (1:200, Sigma), rabbit anti-Perilipin (1:400, Sigma). Secondary antibodies including cy3 donkey anti-rabbit, cy3 donkey anti-rat, cy5 donkey anti-rat, cy5 donkey anti-rabbit were from Jackson ImmunoResearch. All secondary antibodies were used at a 1:500 dilution. To stain lipid, cryostat sections were formaldehyde-fixed and incubated in LipidTox Green (Invitrogen, H34475) at 1:200 in PBS for 30 min before washing in PBS and mounting for imaging. Immunostaining images were collected on a Zeiss LSM500 confocal microscope, an Olympus IX70 inverted microscope or an Olympus upright BX40 microscope. Direct GFP and RFP fluorescence for whole adipose depots was observed with a Zeiss Stemi SV11 microscope. Paraffin-embedded tissues were sectioned with a Microm HM 325 microtome. Cryostat sectioning was performed with a Microm HM505 E cryostat. For quantification of images two independent observers assessed 3 random fields in 10 random sections from at least 3 mice per cohort.

Metabolic Phenotyping Experiments

Total body fat mass of mice was measured by NMR spectroscopy on the Minispec mq spectrometer (Bruker). For GTTs, overnight fasted mice received a 1.5 g glucose/kg body weight intraperitoneal injection of 75mg/ml glucose-PBS solution. Tail blood was drawn at the indicated intervals and blood glucose levels were measured with a TrueTrack glucometer.

Quantitative real-time PCR (qPCR)

Total RNA was extracted using TRIzol (Invitrogen) from either mouse tissues or cells. cDNA synthesis was performed using Superscript III reverse transcriptase (Invitrogen) as previously described (Tang et al., 2008). Gene expression was analyzed using Power SYBR Green PCR Master Mix with ABI 7500 Real-Time PCR System. qPCR values were normalized by 18s rRNA expression. Primer sequences are available upon request.

Supplemental references

Berry, D.C., and Noy, N. (2009). All-trans-retinoic acid represses obesity and insulin resistance by activating both peroxisome proliferation-activated receptor beta/delta and retinoic acid receptor. Mol Cell Biol *29*, 3286-3296.

Berry, D.C., Soltanian, H., and Noy, N. (2010). Repression of cellular retinoic acid-binding protein II during adipocyte differentiation. J Biol Chem *285*, 15324-15332.