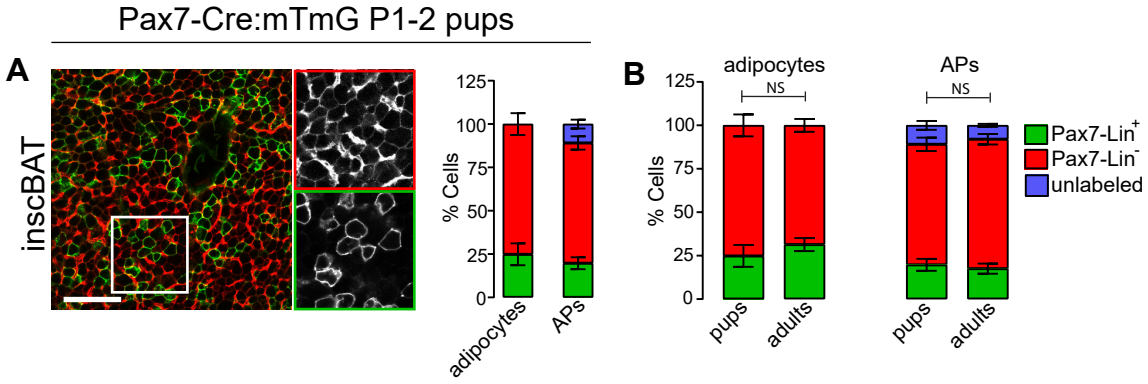
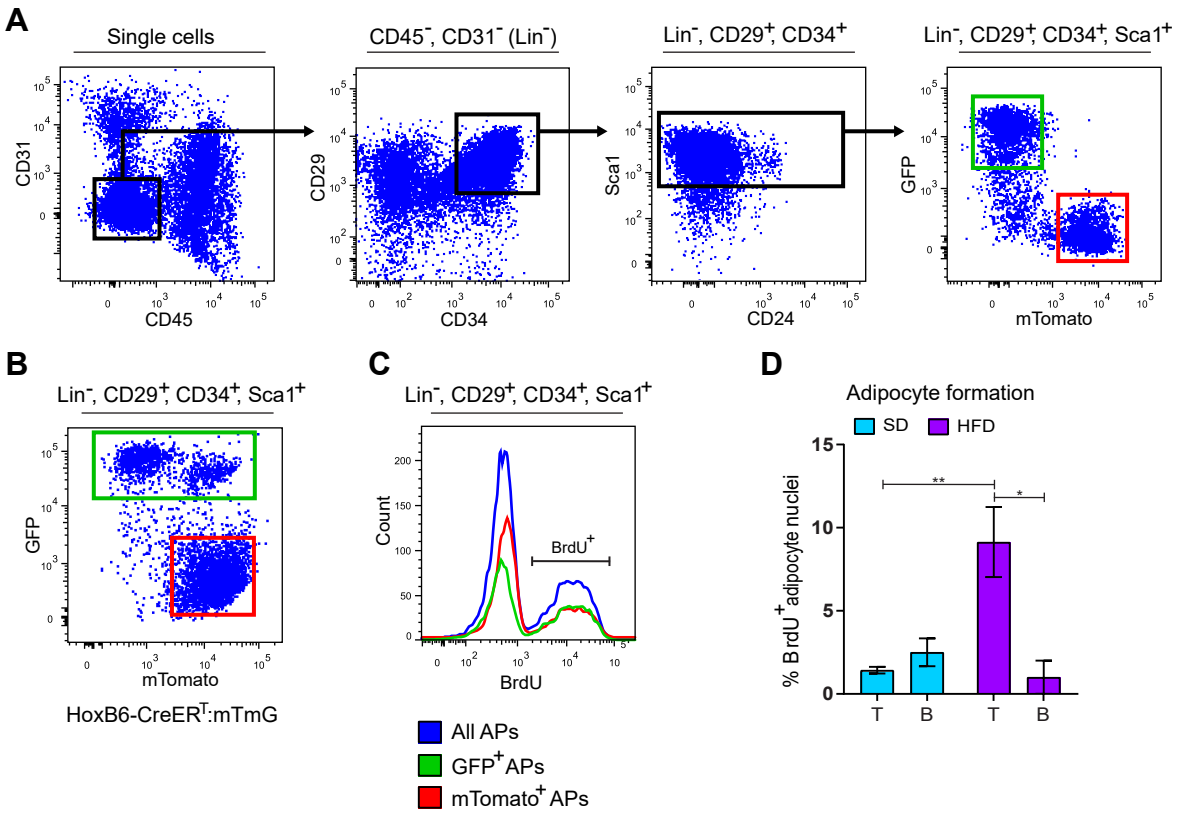
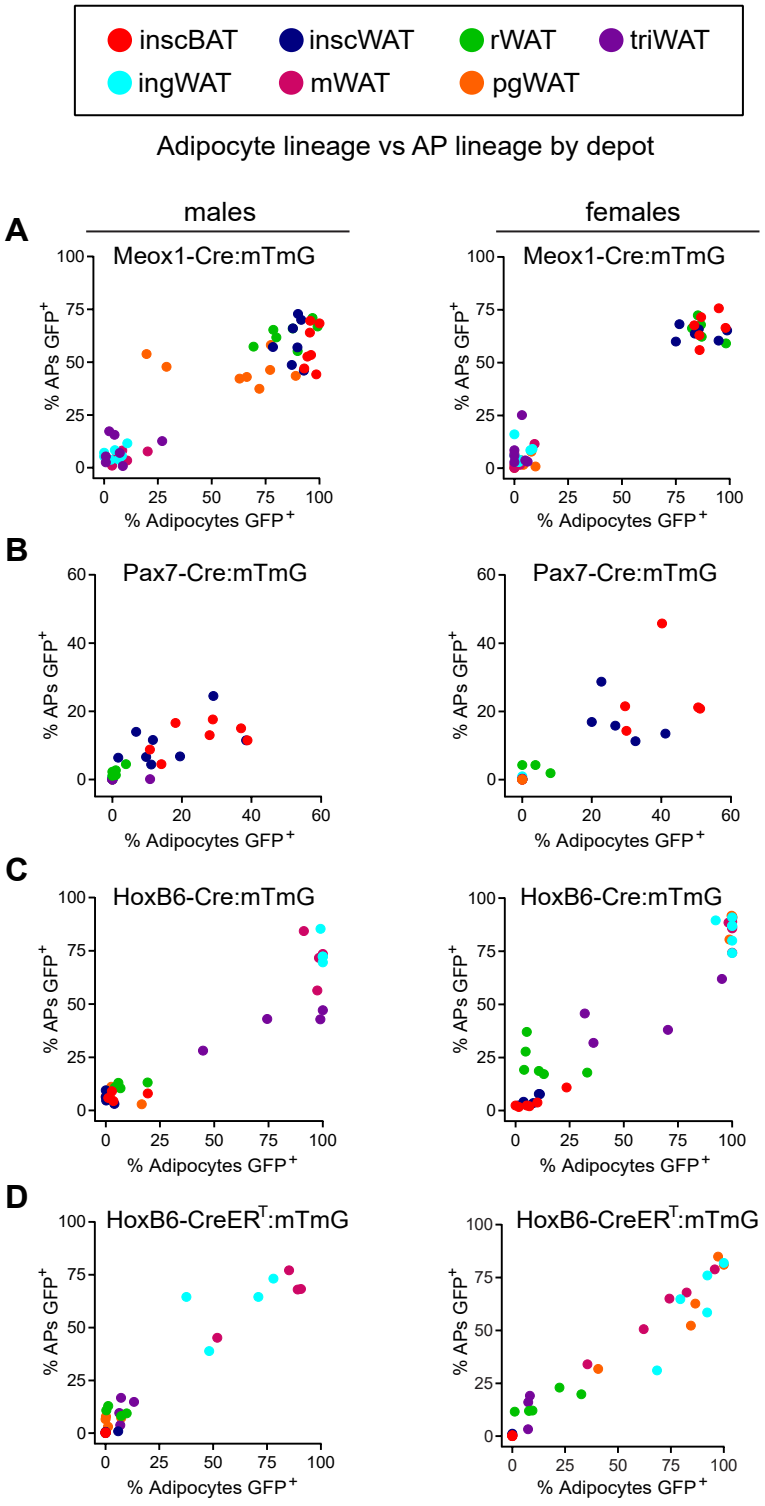


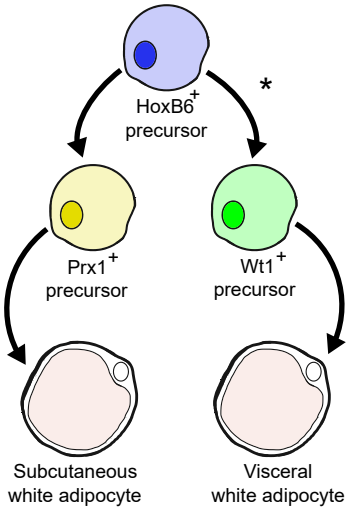
Supplementary Figure 1. Pax7^{Lin+} cells contribute equally to neonatal and adult brown adipocyte lineages. (A) Representative confocal image of brown fat from a 2 day old Pax7-Cre:mTmG pup with quantification of adipocyte and AP labeling. (B) Comparison of adipocyte and AP labeling between 1-2 day old pups and adult Pax7-Cre:mTmG animals from Figure 3. Data from males and females was pooled for pups and adults. The black and white subset images indicate red (top) or green (bottom) channels. n=4 pups, n=12 adults. Error bars indicate mean ± SEM. NS = not significant. Scale bar is 100 um.



Supplementary Figure 2. Representative flow cytometry analysis of APs and adipogenesis in male perigonadal fat. (A) Representative flow plot for the identification of mGFP+ and mTomato+ APs in constitutive Cre strains. (B) Representative flow plot of mGFP+, mTomato+ and mGFP/mTomato double-positive APs in the inducible HoxB6-CreERT:mTmG strain. (C) Method for quantifying BrdU incorporation into APs of distinct lineages. (D) Percent adipocytes arising from proliferative APs in the tip and base of male perigonadal fat (n = 4-5). Adipose tissue was imbedded in paraffin, sectioned and immunostained for BrdU following 1 week of BrdU treatment (pulse) and seven additional weeks of the indicated diet (chase). Sections were imaged via a Leica TCS SP5 confocal microscope and BrdU+ adipocyte nuclei were manually quantified in each condition. SD = standard diet, HFD = high fat diet. *(P<0.05), **(P<0.01). Statistical significance was determined using an unpaired Student's t test for indicated groups.



Supplementary Figure 3. Depot-specific adipocyte and AP labeling are largely concordant across tracing paradigms. Each scatter plot shows percent mGFP+ adipocytes on the x-axis and percent mGFP+ APs on the y-axis. Each adipose depot is denoted by a single color (red = inscBAT, blue = inscWAT, green = rWAT, purple = triWAT, teal = ingWAT, magenta = mWAT, orange = pgWAT). Each dot represents a single biological replicate (i.e. one animal). Given that each adipose depot exists in a pair, one depot was used for quantifying adipocyte labeling and the other was used to quantify AP tracing per animal. Adipocyte tracing is presented alone in figures 2 through 5. AP tracing is presented alone in figure 6. Data from males and females are shown for each tracing paradigm. (A) Meox1-Cre:mTmG. (B) Pax7-Cre:mTmG. (C) HoxB6-Cre:mTmG. (D) HoxB6-CreERT:mTmG. inscBAT = interscapular brown adipose tissue, inscWAT = interscapular white adipose tissue, rWAT = retroperitoneal white adipose tissue, triWAT = triceps white adipose tissue, ingWAT = inguinal white adipose tissue, mWAT = mesenteric white adipose tissue, pgWAT = perigonadal white adipose tissue.



Supplementary Figure 4. Model cell lineage hierarchy for subcutaneous and visceral adipocytes. Based on HoxB6-Cre:mTmG and HoxB6-CreERT:mTmG tracing in this study as well as Prx1-Cre:mTmG and WT1-Cre:mTmG tracing from Sanchez-Gurmaches et al. (Sanchez-Gurmaches et al., 2015) and Chau et al. (Chau et al., 2014), respectively. The asterisk denotes the exception of male perigonadal adipocytes which predominately arise from Pax3 $^{Lin+}$/Meox1 $^{Lin+}$ progenitors in the somites.

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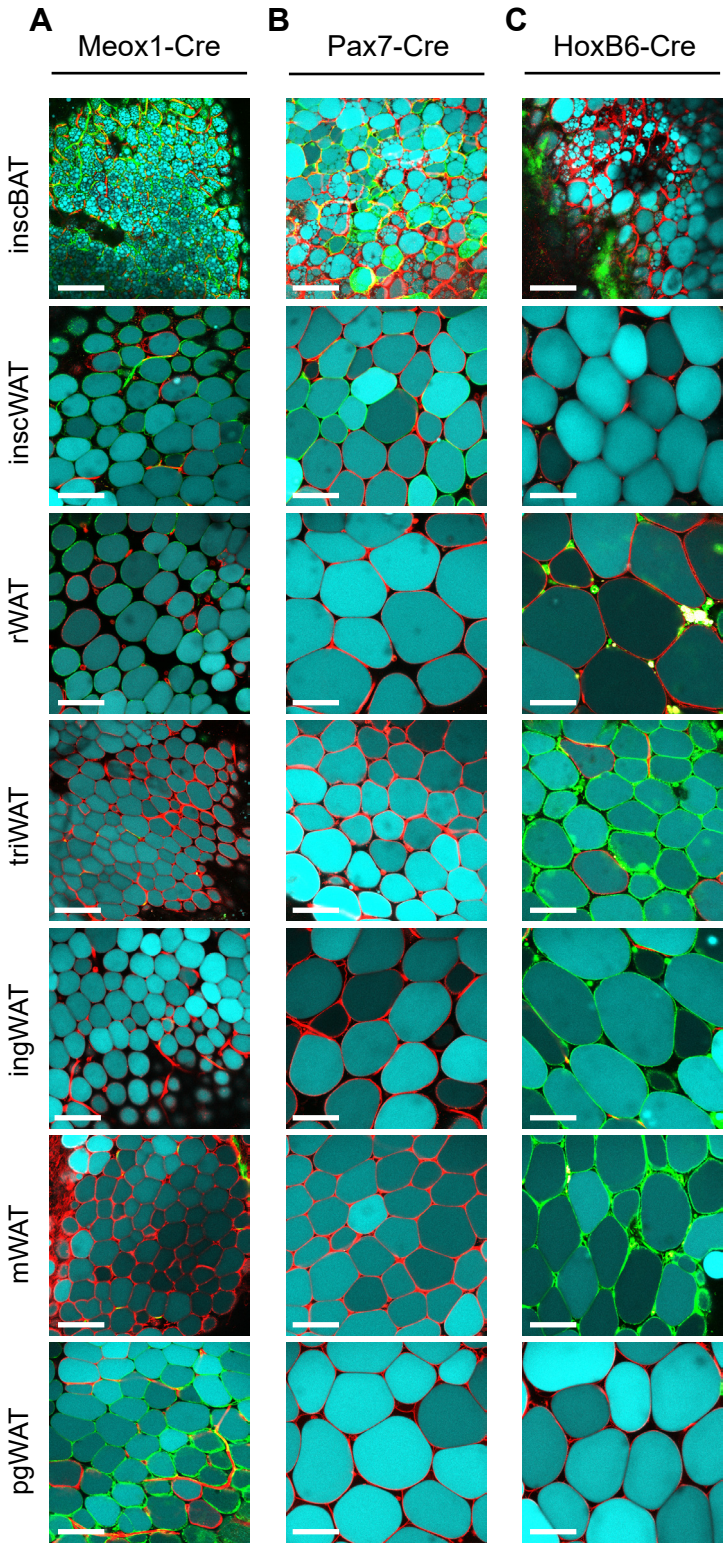
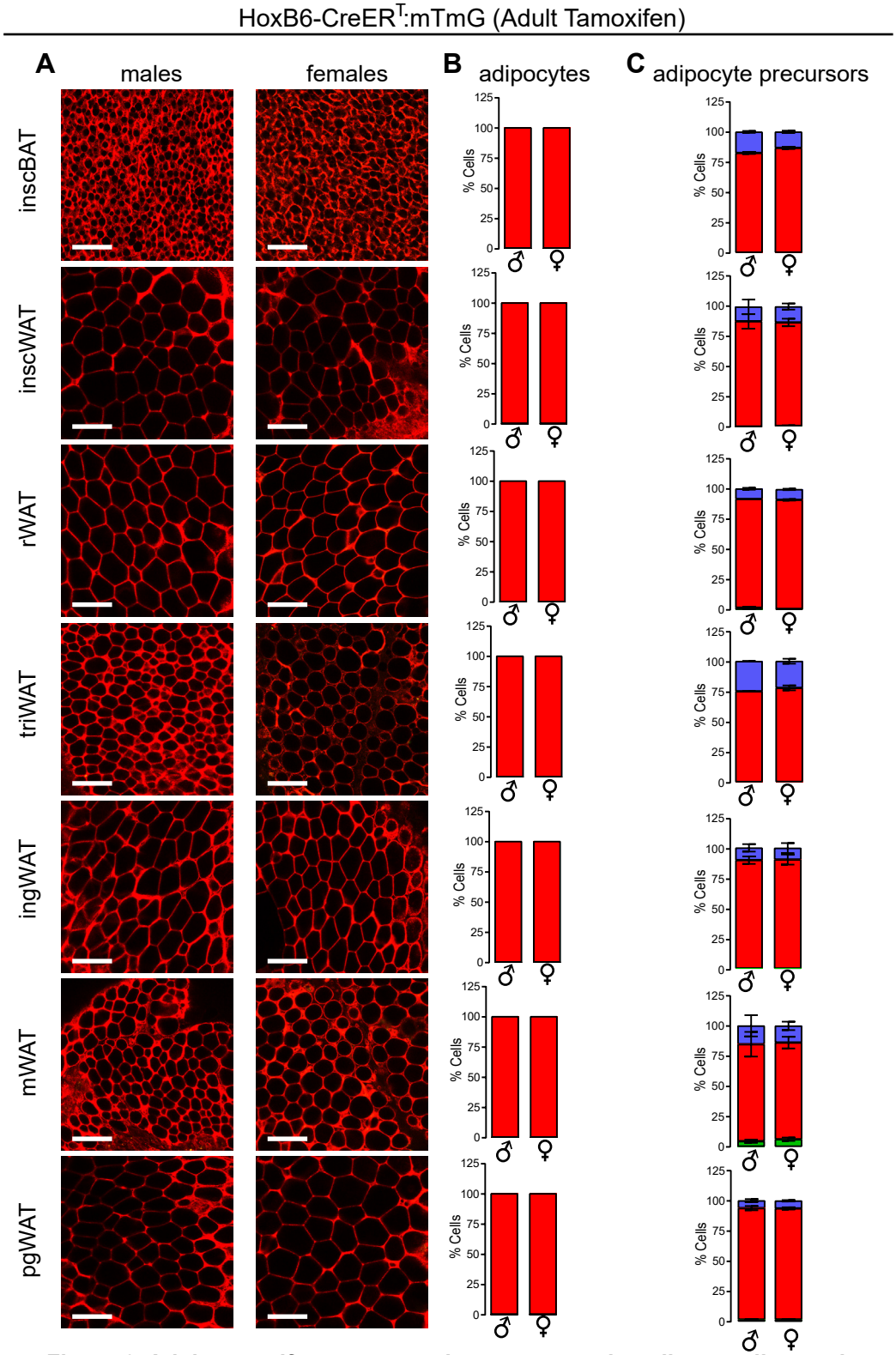
Sanchez-Gurmaches, J., Hsiao, W.-Y. and Guertin, D. A. (2015). Highly selective in vivo labeling of subcutaneous white adipocyte precursors with Prx1-Cre. Stem cell reports 4, 541-550.



Supplementary Figure 5. Lipid staining of adipocytes in lineage tracing systems. Approximately 1.5x1.5 cm chunks of each adipose depot were dissected and stained with LipidTOX Deep Red (Invitrogen H34477) in PBS for 1 hr at room temperature. Following this incubation, tissue was washed once in PBS and mounted for confocal imaging as described in the "Whole Mount and Confocal Imaging" section. (A) Meox1-Cre:mTmG adipose tissue from an ~7 month old male. (B) ~10 month old Pax7-Cre:mTmG male. (C) ~10 month old HoxB6-Cre:mTmG male. Scale bar = 100 um.



Supplementary Figure 6. Adult tamoxifen treatment does not trace the adipocyte lineage in HoxB6-CreER^{T}:mTmG animals. Mice were intraperitoneally injected with 50mg/kg tamoxifen in vegetable oil once a day for five days; 72 hours later, animals were sacrificed and adipose tissue harvested. (A) representative images of indicated adipose depots. (B) quantification of mGFP+ and mTomato+ adipocytes. (C) quantification of mGFP+ and mTomato+ adipocyte precursors. Data are from males (n = 2) and females (n = 2) of ~7.5 months of age. Adipocyte tracing was analyzed by confocal microscopy and adipocyte precursor tracing was analyzed by flow cytometry. Error bars represent mean ± SEM. Scale bar = 100 um.