Figure S1

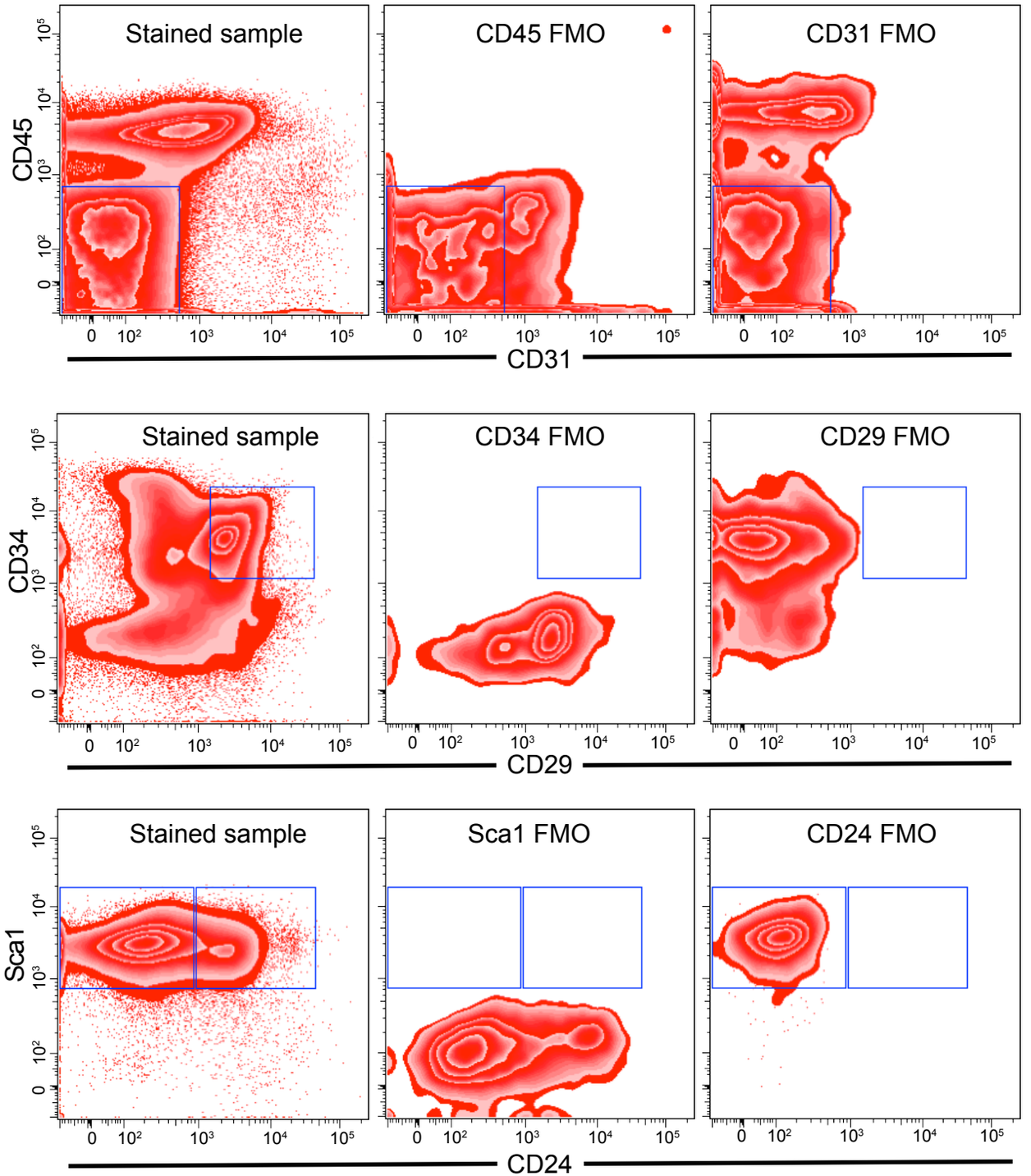
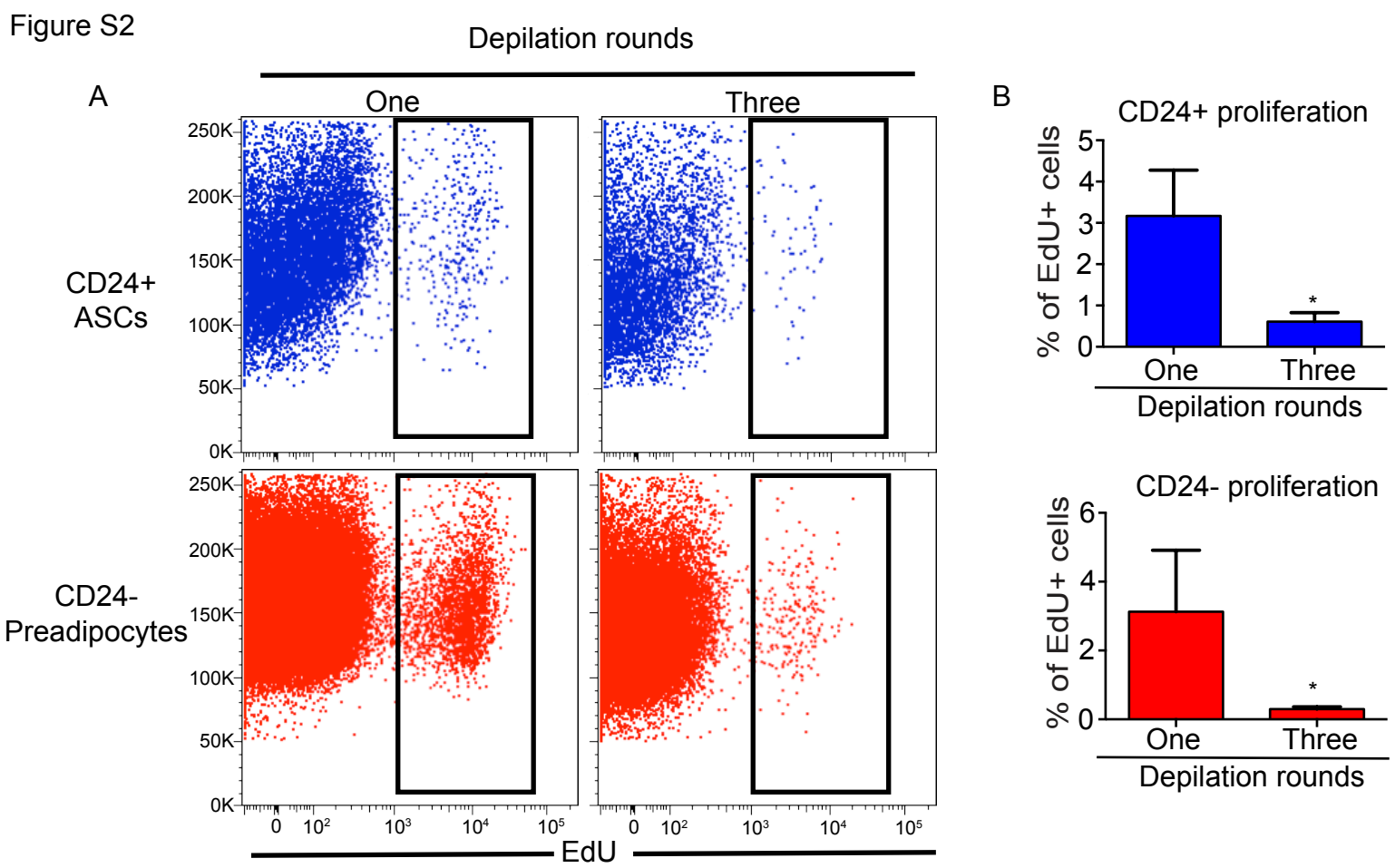

Stained sample
CD45 FMO
CD31 FMO
CD45
CD31
Stained sample
CD34 FMO
CD29 FMO
CD34
CD29
Stained sample
Sca1 FMO
CD24 FMO
Sca1
CD24
0
10^2
10^3
10^4
10^5

Figure S2


Depilation rounds
A
One
Three
CD24+ ASCs
CD24- Preadipocytes
250K
200K
150K
100K
50K
0K
0
10²
10³
10⁴
10⁵
EdU
B
CD24+ proliferation
% of EdU+ cells
CD24- proliferation
One
Three
Depilation rounds
*
n.s.

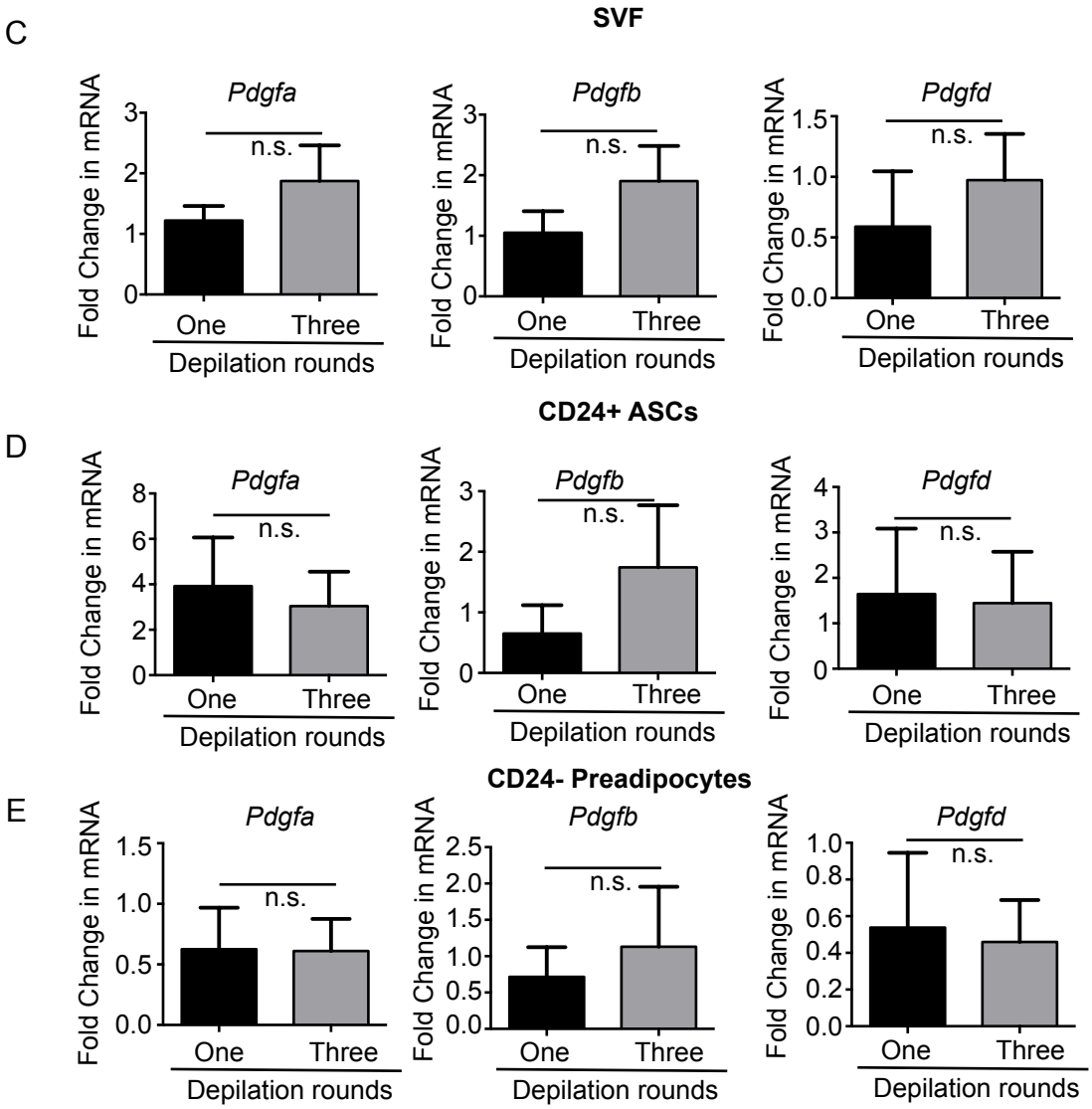

C
SVF
D
CD24+ ASCs
E
CD24- Preadipocytes
Pdgfa
Pdgfb
Pdgfd
Fold Change in mRNA
n.s.
One
Three
Depilation rounds

Figure S3

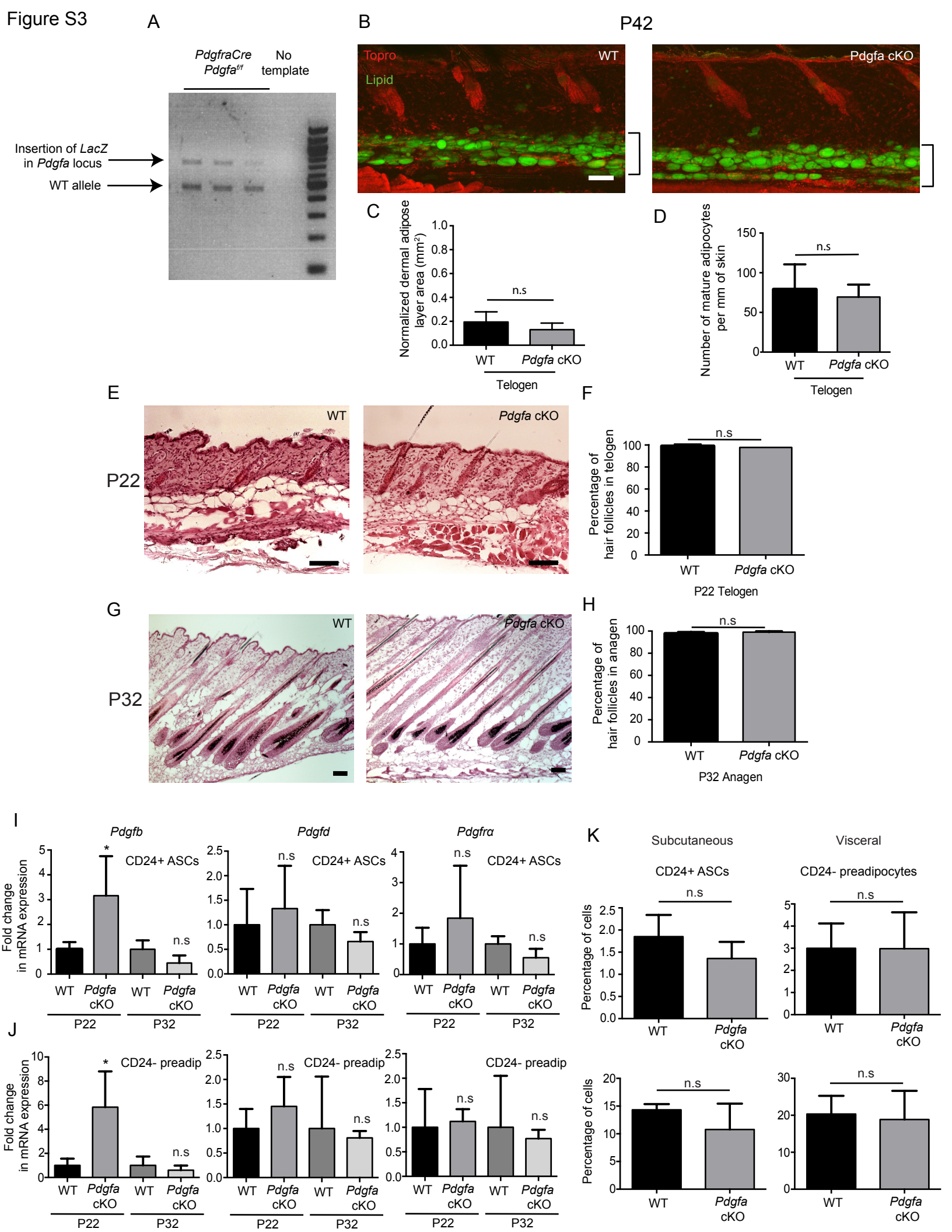
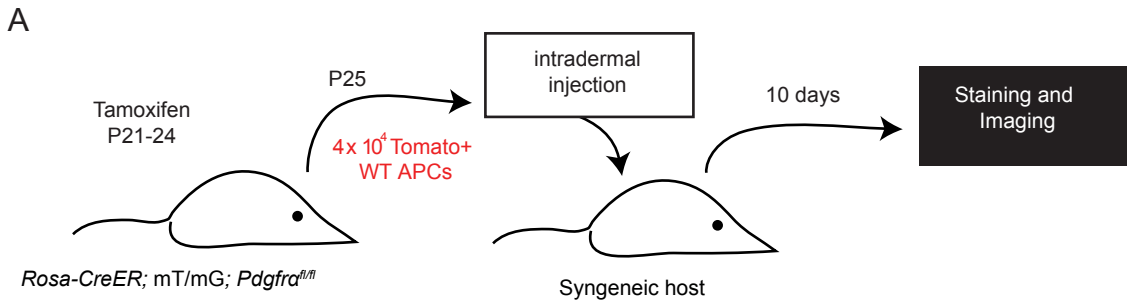

A
PdgfraCre Pdgfa f/f
No template
Insertion of LacZ in Pdgfa locus
WT allele
B
P42
Topro
Lipid
WT
Pdgfa cKO
C
Normalized dermal adipose layer area (mm2)
n.s
WT
Pdgfa cKO
Telogen
D
Number of mature adipocytes per mm of skin
n.s
WT
Pdgfa cKO
Telogen
E
P22
WT
Pdgfa cKO
F
Percentage of hair follicles in telogen
n.s
WT
Pdgfa cKO
P22 Telogen
G
P32
WT
Pdgfa cKO
H
Percentage of hair follicles in anagen
n.s
WT
Pdgfa cKO
P32 Anagen
I
Pdgfb
Pdgfd
Pdgfrα
CD24+ ASCs
Fold change in mRNA expression
*
n.s
WT
Pdgfa cKO
P22
P32
J
CD24- preadip
K
Subcutaneous
Visceral
CD24+ ASCs
CD24- preadipocytes
Percentage of cells
n.s
WT
Pdgfa cKO

Figure S4


A
Tamoxifen
P21-24
P25
4x 10^4 Tomato+
WT APCs
intradermal
injection
10 days
Staining and
Imaging
Rosa-CreER; mT/mG; Pdgfra^fl/fl
Syngeneic host

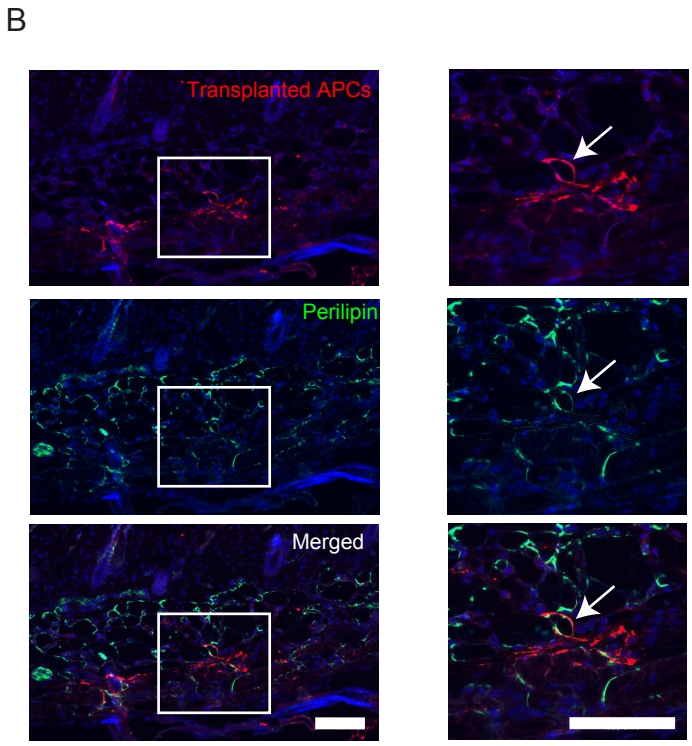

B
Transplanted APCs
Perilipin
Merged

Figure S5

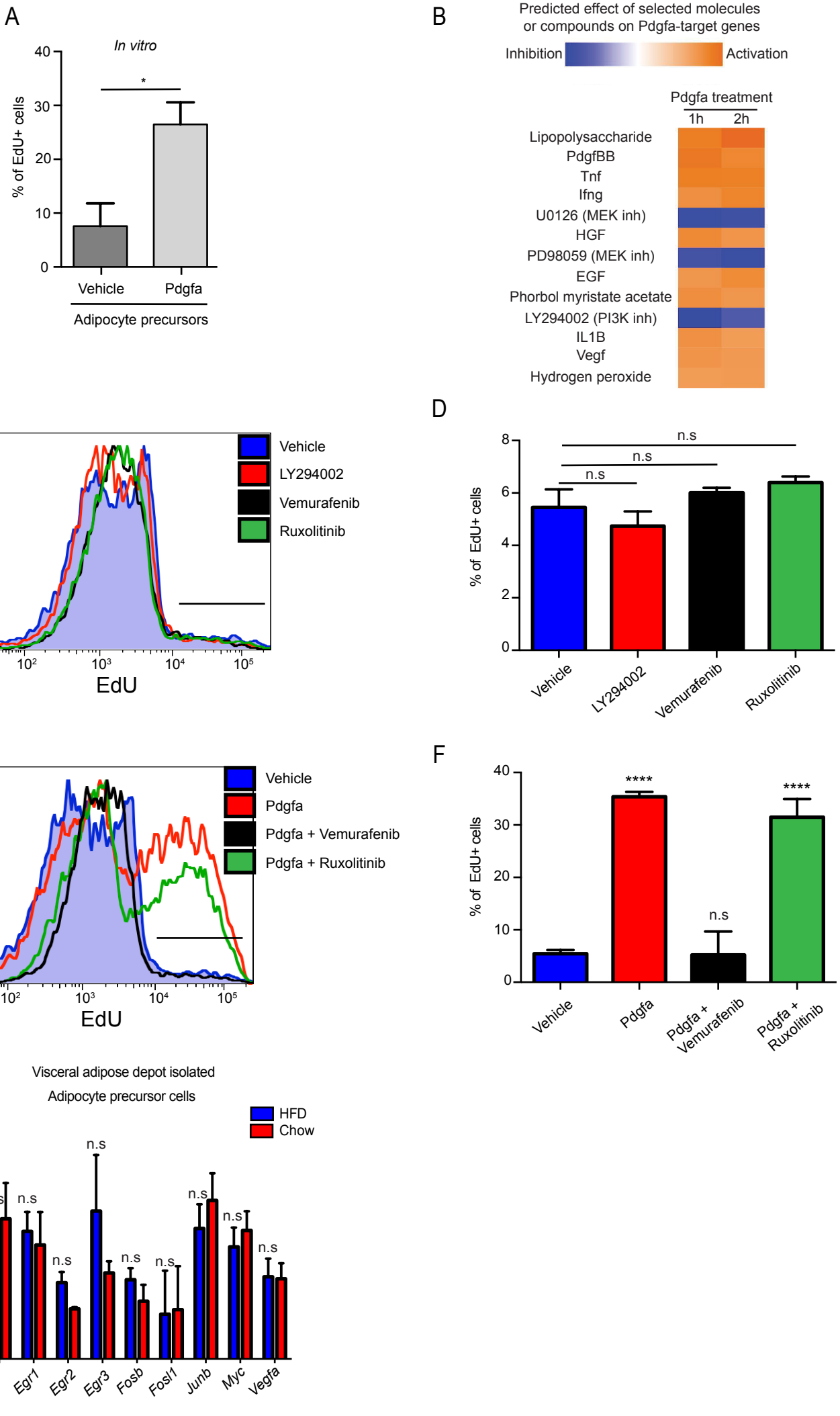

A
In vitro
% of EdU+ cells
Vehicle
Pdgfa
Adipocyte precursors
B
Predicted effect of selected molecules or compounds on Pdgfa-target genes
Inhibition
Activation
Pdgfa treatment
1h
2h
Lipopolysaccharide
PdgfBB
Tnf
Ifng
U0126 (MEK inh)
HGF
PD98059 (MEK inh)
EGF
Phorbol myristate acetate
LY294002 (PI3K inh)
IL1B
Vegf
Hydrogen peroxide
C
% of cells
EdU
Vehicle
LY294002
Vemurafenib
Ruxolitinib
D
% of EdU+ cells
n.s
Vehicle
LY294002
Vemurafenib
Ruxolitinib
E
% of cells
EdU
Vehicle
Pdgfa
Pdgfa + Vemurafenib
Pdgfa + Ruxolitinib
F
% of EdU+ cells
****
n.s
Vehicle
Pdgfa
Pdgfa + Vemurafenib
Pdgfa + Ruxolitinib
G
Visceral adipose depot isolated
Adipocyte precursor cells
HFD
Chow
Fold change in mRNA expression
n.s
Egr1
Egr2
Egr3
Fosb
Fosl1
Junb
Myc
Vegfa

Figure S6

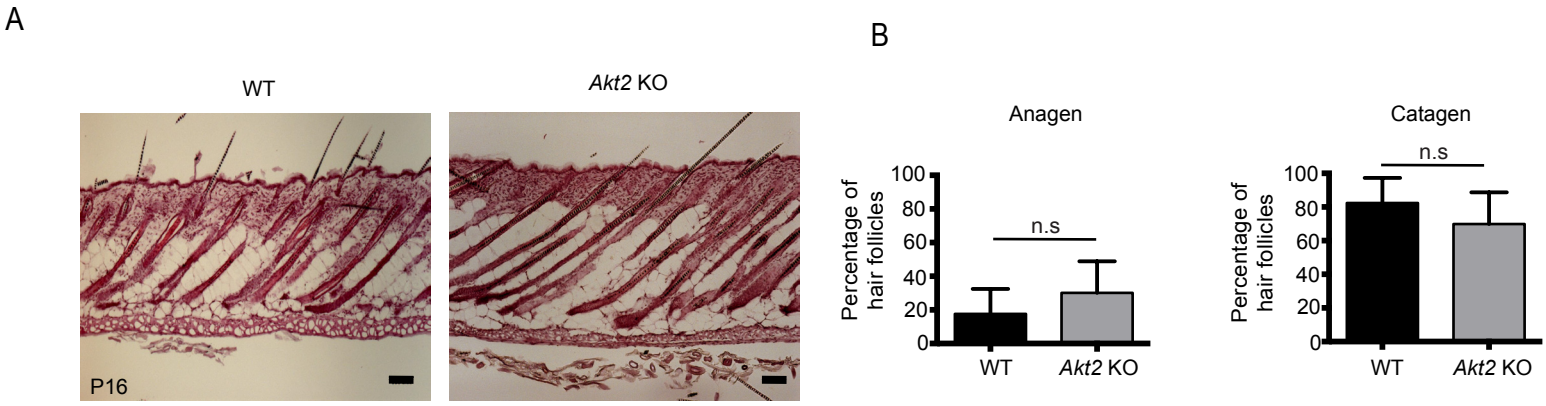

A
WT
Akt2 KO
P16
B
Anagen
Percentage of hair follicles
100
80
60
40
20
0
n.s
WT
Akt2 KO
Catagen
Percentage of hair follicles
100
80
60
40
20
0
n.s
WT
Akt2 KO

Figure Legends

Figure S1. Fluorescent activated cell sorting (FACS) gating strategy. Related to Figure 1. SVF from mouse dermis was stained with the set of antibodies that allow AP identification (antibodies against CD45, CD31, CD34, CD29, Sca1 and CD24) or with the complete set minus one specific antibody (fluorescence minus one control (FMO). To determine where to draw the gates for our FACS experiments, we used the FMO controls for each specific marker as shown in the figure. The settings for these measurements were saved and used for all FACS experiments.

Figure S2. Multiple depilation rounds induce loss of proliferation in APs of the skin. A-B. Related to Figure 2. Flow cytometry analysis (A) and quantification (B) of EdU-positive CD24+ and CD24- intradermal APs depilated one or three times. Mice were injected with EdU 6h prior to each time point. (n = 3 mice). Data are mean ± SD. **C-E**. Gene expression of *Pdgf* family molecules in SVF, CD24+ ASCs and CD24- preadipocytes from single or serially (three rounds) depilated mice. Data are mean ± SD normalized to single depilated mice (n= 3 mice per group). Asterisk indicates significance, *p<0.05, calculated with Student's T test.

Figure S3. Effect of dermal Pdgfa loss on HF cycle and adipose tissue in the skin and other WATs. Related to Figure 3. A. PCR amplification of inserted *LacZ* allele in DNA extracted from *Pdgfrα*Cre*;Pdgfa*^{fl} mice The upper band (680 bp) represents the correctly reversed *LacZ* allele in the Pdgfa gene. The lower band (484 bp) represents unrecombined *Pdgfa*^{fl} DNA. **B**. Whole mount confocal images of P42 skin from WT and *Pdgfa* cKO mice stained with Bodipy to label neutral lipids and TOPRO3 to stain nuclei. Scale bar is 100μm. Bracket indicates dermal white adipose (dWAT) thickness. **C**. Quantification of dermal adipose tissue area per mm of skin in WT and *Pdgfa* cKO mice at age P42. (n=4 mice per genotype). Data are mean ± SD of the area of adipose tissue per mm of skin. **D**. Number of mature adipocytes per mm of skin at P42 in WT and *Pdgfa* cKO mice (n=4 mice per genotype). **E-H**. Analysis of hair follicle cycle stage on WT and Pdgfa cKO mice at P22 and P32. At least fifty follicles per mouse with visible dermal papillae were evaluated and classified (n=3 mice per genotype). Scale bar is 100μm and data are mean ± SD. **I-J.** *Pdgfb*, *Pdgfd* and *Pdgfrα* expression in CD24+ ASCs and CD24- preadipocytes isolated from WT and Pdgfa cKO mice at P22 and P32 (n=3 mice per genotype). Data are mean ± SD. **K.** APs were isolated from vWAT and sWAT of 5-6 week old WT or *Pdgfa* cKO mice and analyzed by flow cytometry. Data are mean ± SD of AP percentage of total isolated cells (n= 3-4 mice per genotype). Asterisk indicates significance, *p<0.05, calculated with One-way ANOVA with Dunnett's post-test for multiple comparisons or Student's T tests for two groups.

Figure S4. Transplanted APs can engraft and form mature adipocytes. Related to Figure 4. A. Schematic of transplantation experiments with Tomato+ (WT) tamoxifen-treated *Rosa*-CreER*;Pdgfrα*$^{fl/fl}$ mice into the skin of P15 syngeneic mice. **B**. Ten days after injection the tissue was isolated and imaged to assay for the presence of dTomato+ and perilipin. The images are representative of three independent experiments. The white arrow indicates a dTomato+ and perilipin+ mature adipocyte. Scale bar is 100μm.

Figure S5. Akt pathway is activated following Pdgfa treatment in APs from the skin. Related to Figure 5. A. Percentage of proliferating cells (EdU+) from APs treated with vehicle or Pdgfa (30ng/ml) *in vitro* from 24h. Data are mean ± SD, three biological replicates per experimental group **B**. Heat map of Ingenuity pathway analysis of molecules that regulate Pdgfa target genes in APs. **C**-**D**. Flow cytometry analysis (C) and quantification (D) of EdU incorporation in cultured APs treated with vehicle indicated pathway inhibitors for 24 h in the presence of EdU. Data are mean ± SD, three biological replicates per experimental group. **E-F**. Flow cytometry analysis (E) and quantification (F) of EdU incorporation in cultured APs treated with vehicle, Pdgfa or Pdgfa and specific pathway inhibitors for 24h. Data are mean ± SD, three biological replicates per experimental group. **G**. Pdgfa-target gene expression in APs from vWAT of WT mice given chow or high fat diet (HFD) for seven days measured by qPCR. Data are mean ± SD, three biological replicates per experimental group. Asterisk indicates significance, *p<0.05 and ****p<0.0001 calculated with One-way ANOVA with Dunnett's post-test for multiple comparisons or Student's T tests for two groups.

Figure S6. Hair follicle cycle of *Akt2* KO mice is not disturbed at P16. Related to Figure 6. Analysis of hair follicle cycle stage on WT and *Akt2* KO mice at P16. At least fifty follicles per mouse with visible dermal papillae were evaluated and classified (n=3 mice per genotype). Scale bar is 100μm and data are mean ± SD. Statistical significance was calculated using Student's T test.

Supplemental Experimental Procedures

Primer sequence information.

Gene	Primer sequence 5'-3'
Adora2b	**For** CGTGGCGCTGGAGCTGGTTATC **Rev** GGCAAAGGGGATGGCGAAGAGTC
Egr1	**For** CCATCCCCTGCCACCACCTCATT **Rev** TGCGGCCATCTCTTCCCTCCTGT
Egr2	**For** TTCGGCAGAAGGAACGGAAGAGCAG **Rev** ATGGGGAGGAACAGGGAAGGGTGGTAG
Egr3	**For** AGCCGCAGCGACCACCTCACCACTC **Rev** GGCGCACCCCCTTTCTCCGACTTCT
Egr4	**For** GGAGCGGCGGCGAAGGCGGAGAGTT **Rev** GGGGAAGGATGCGGCGGGGGATGAG
Fos	**For** ATTCCCCAGCCGACTCCTTCTCCA **Rev** GCTCTGCGCTCTGCCTCCTGACAC
Fosb	**For** ACCCGCAAGGAACAAGGAGGAGGAAGAT **Rev** CGCCGGGGCACAGAAAACCAGAG
Fosl1	**For** GACCCACTGGCTATCCCCGACCTCT **Rev** CGCTGCAGCCCCGATTTCTCAT
Junb	**For** GGCCCCCTTCCAGCGTATTTTGTATG **Rev** AGCCCCCTTCTCCCCTCCCTGTTA
Klf4	**For** CCGGCGGGAAGGGAGAAGACACT **Rev** CGCCGGGGAAGACGAGGATGAA
Lif	**For** TGAGGCCAGGCAAGCAGAGGAAC **Rev** GCAAAACCGCGTGAAGCAGAATC
Myc	**For** ACAACGAAAAGGCCCCCAAGGTAGTGAT **Rev** AAAGCCCCAGCCAAGGTTGTGAGGTTA
Sox9	**For** AGCGAACGCACATCAAGACGGAGCAG **Rev** GAGCGGGTGATGGGCGGGTAGG
Vegfa	**For** CGACACGGGAGACAATGGGATGAA **Rev** AGGGGCGGGGTGCTTTTGTAGACT
Wnt2	**For** GAATCTGGCTCTGGCTCCCTCTGCTCT **Rev** GCGTAAACAAAGGCCGATTCCCGACTA
Pdgfa	**For** CGCTGCACTGGCTGTTGTAA **Rev** GCCGGCTCATCTCACCTCA
Pdgfra	**For** ACGCATGCGGGTGGACTC **Rev** GATACCCGGAGCGTGTCAGTTAC
Pdgfb	**For** GCCGGTCCAGGTGAGAAAGATT **Rev** AGGCCGAGGGGTCACTACTGTC
Pdgfc	**For** TGCCCCCTTCATCTTTGTCATTGG **Rev** TCCGGGGTGTGCAGCTGTAGAGTT
Pdgfd	**For** TCTCCTGTGCGCGAACTTTAG **Rev** CATGGCCATTGCTTGTCACC