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Supplemental Information

Identification of an Adipogenic Niche for Adipose Tissue Remodeling and Restoration

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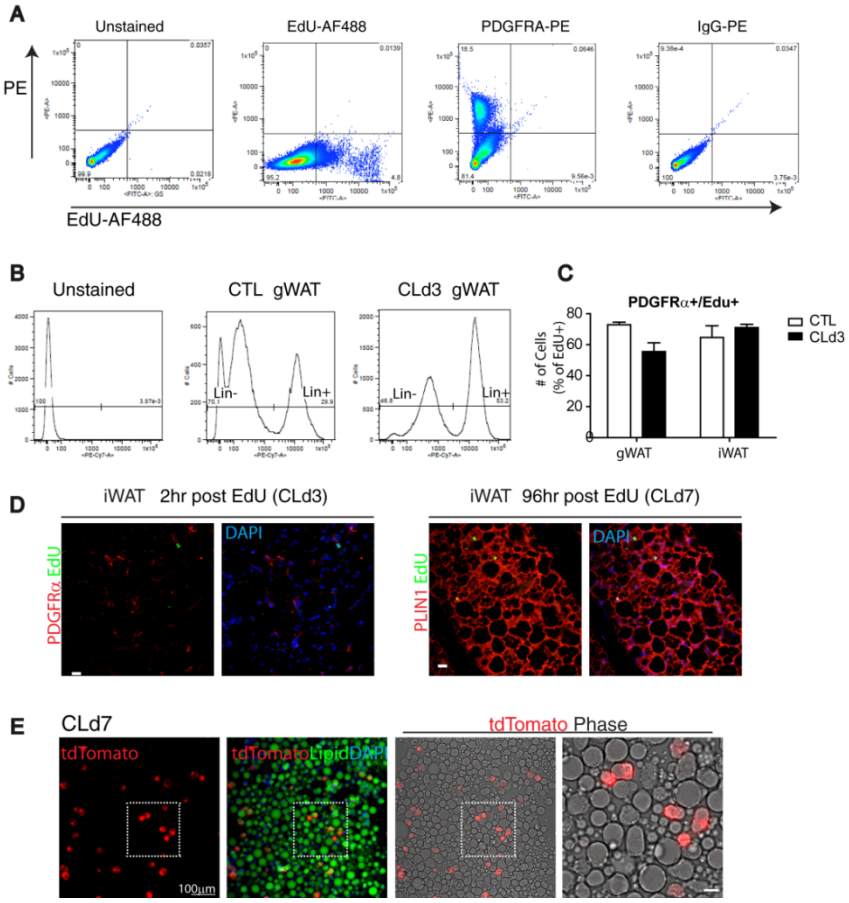

Figure S1, Related to Figure 1. ADRB3 stimulation induces proliferation and differentiation of PDGFRα+ cells in gWAT, (A) Unstained control and single color controls for flow cytometric analysis of EdU incorporation and PDGFRα expression (Figure 1A). The quadrant markers are identical with those placed in Figure 1A. (B) Histogram of lineage marker staining used to exclude hematopoietic lineage from SVC. (C) Quantification of EdU+PDGFRα+ cells in gWAT and iWAT from control mice and mice treated with CL for 3 days. Data are represented as mean ± SEM. (D) Low magnification of paraffin sections stained for EdU and PDGFRα or PLIN1 of iWAT from mice treated with CL up to 7 days and injected with EdU on D3 of CL treatment. (E) Representative images of dissociated adipocytes stained for lipidtox from gWAT of tamoxifen induced *Pdgfra*-Cre/R26-tdTomato mice 7 days after CL treatment. The right panel is a magnified view of a boxed region from low magnification field. Nuclei were counterstained with DAPI (blue). Size bars = 20 μm, except low magnification field in panel E, where bar = 100μm.

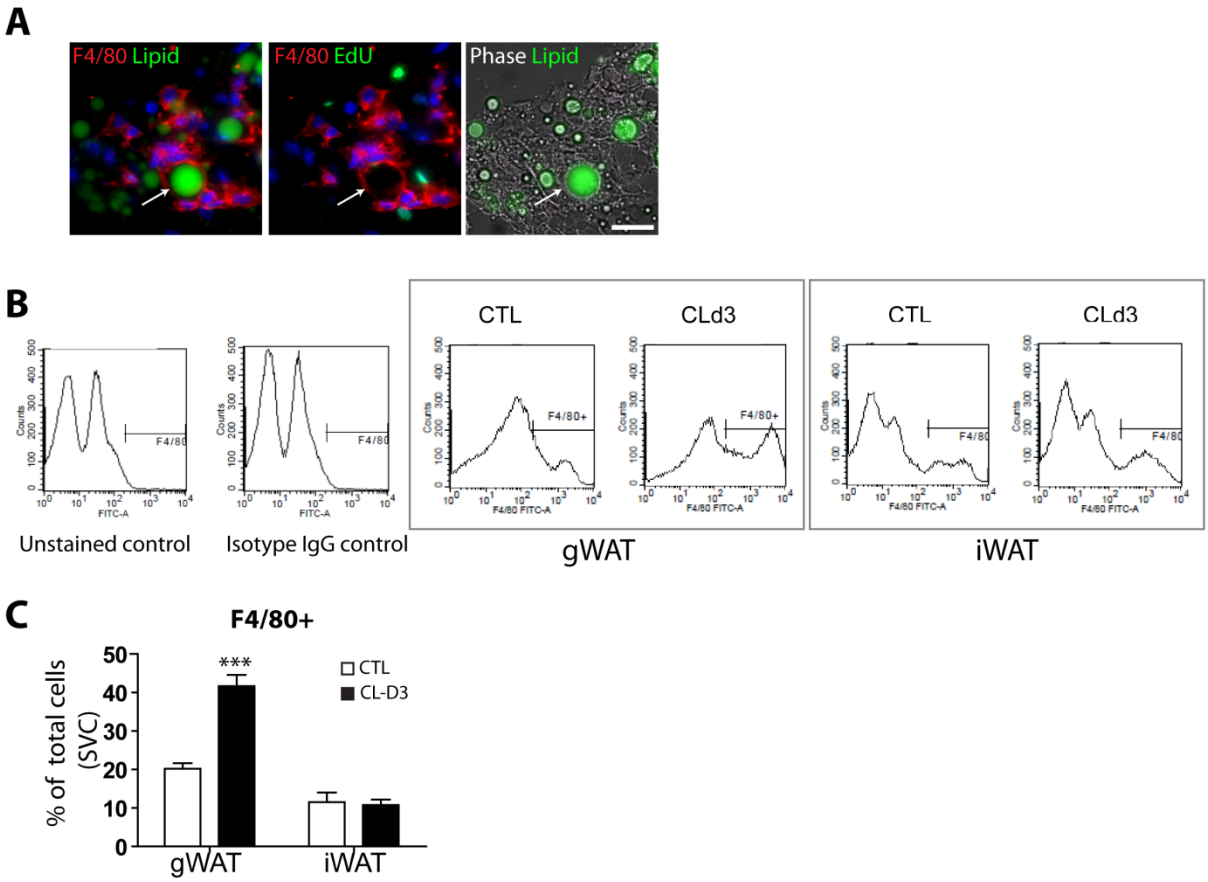

Figure S2, Related to Figure 2. Depot-specific ADRB3-mediated proliferation in WAT correlates with CLS formation and macrophage recruitment. (A) CLS detection in cryosection of eWAT from mice treated with CL for 3d (CLd3). Arrows indicate lipid core surrounded by F4/80^{+} cells. (B-C) FACS analysis of F4/80^{+} cells in SVC obtained from gWAT and iWAT of control and mice treated with CL. Representative histograms (B) and quantification (C) of 7 independent experiments (mean ± S.E.M.).

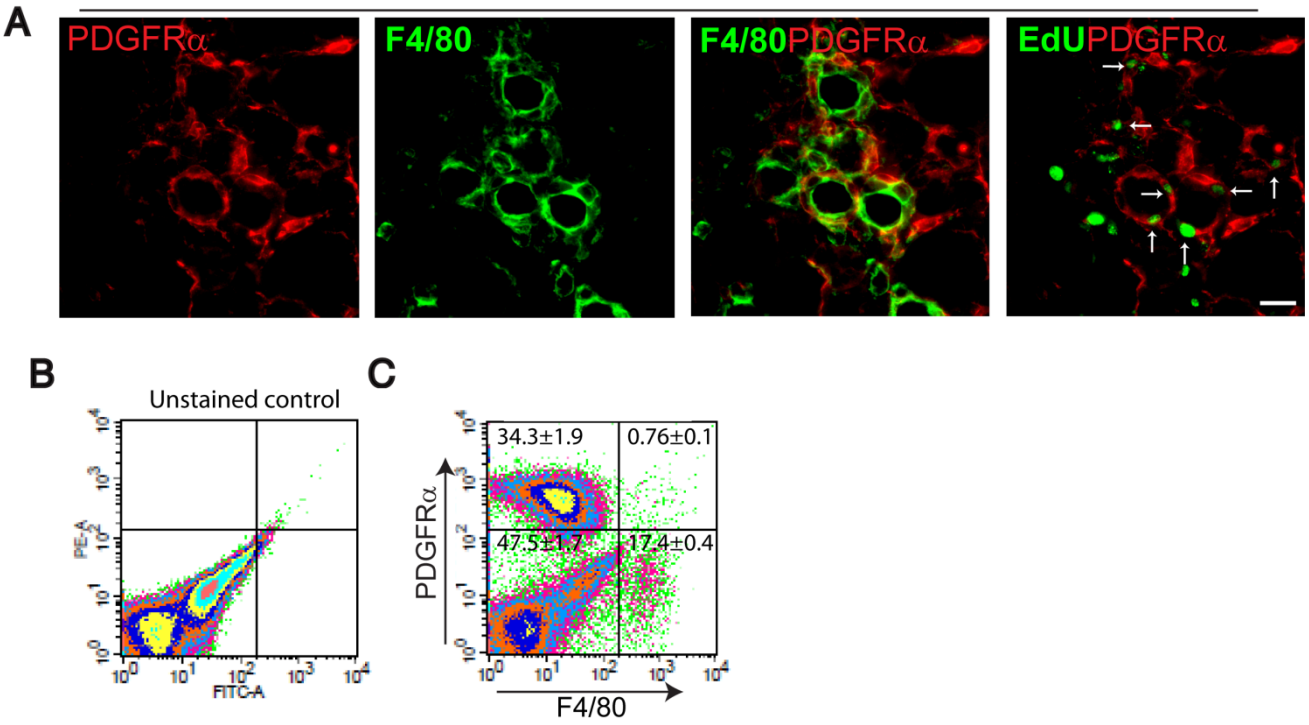

Figure S3, Related to Figure 3. PDGFRα^{+} progenitors are recruited to CLS, where they form a zone of proliferation. (A) Confocal projection images of F4/80, PDGFRα and EdU staining in paraffin sections of gWAT from mice treated with CL for 3 days. (B) Unstained negative control used for flow cytometric analysis of PDGFRα and F4/80 expression (Figure S3C). (C) Flow cytometric analysis of receptor expression status, showing absence of F4/80 expression in PDGFRα^{+} cells. The percentage of cells in each quadrant are indicated on the flow profile (mean ± S.E.M.; n = 3).

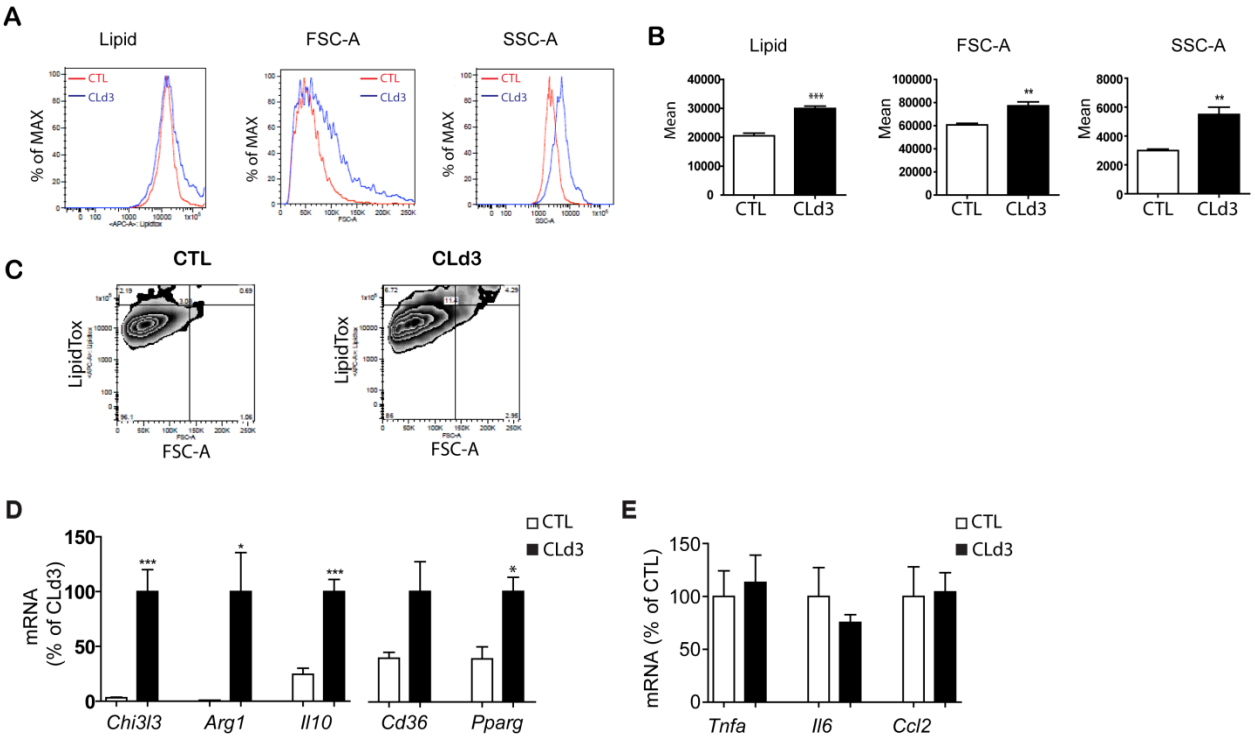

Figure S4, Related to Figure 4. CLS induced by ADRB3 stimulation contain M2-polarized macrophages. **(A-C)** FACS analysis of MGL1^{+}cells in SVC obtained from gWAT of control mice and mice treated with CL for 3d. (A) MGL1^{+} fractions were analyzed for lipid content (LipidTox), size (forward scatter area, FSC-A) and complexity (side scatter area, SSC-A). (B) Quantification of lipid content, size, and complexity of MGL1^{+}cells. (n=4, mean ± S.E.M., ***, p<0.001, **p<0.01) (C) Quadrant analysis of FSC-A/LipidTox zebra plots indicating appearance of a new population (lipid^{hi} and FCS-A^{hi}) in CL-treated group. (D-E) Quantitative PCR analysis of anti- and pro-inflammatory gene expression of isolated F4/80^{+} cells in gWAT from control mice or mice treated with CL for 3 days (n = 4-6, mean ± S.E.M., *p<0.05, ***p<0.001).

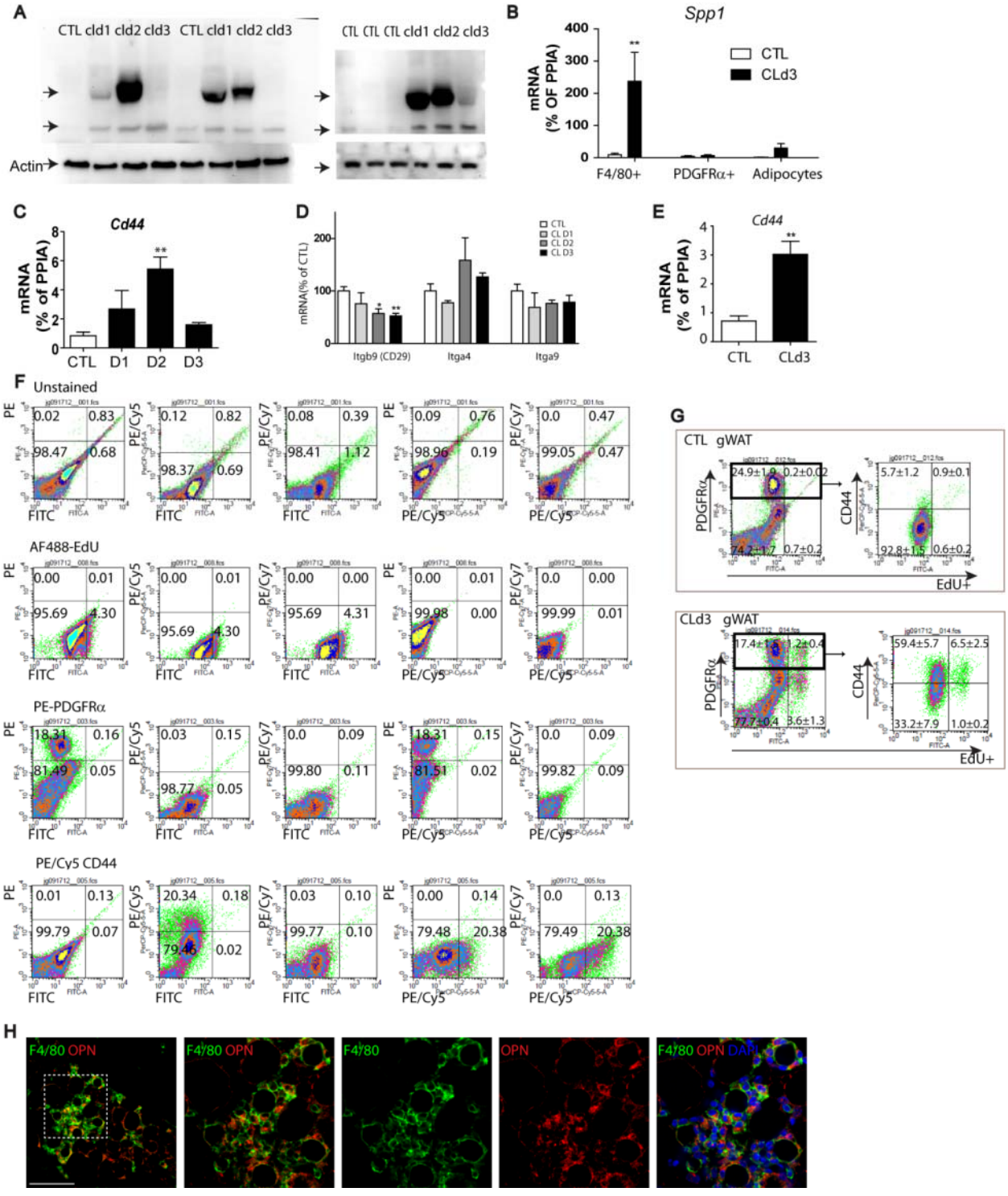

Figure S5, Related to Figure 5. ADRB3 treatment upregulates OPN expression in CLS-associated macrophages and CD44 expression in a subpopulation of PDGFRα expressing cells. (A) Immunoblot analysis of gWAT from control mice and mice treated with CL at indicated

time points. (B) Quantitative PCR analysis of *Spp1* expression in isolated cell fractions of gWAT from control mice and mice treated with CL for 3 days (n = 3-6, mean ± S.E.M., **p<0.01). (C) Quantitative PCR analysis of *Cd44* expression in gWAT control and CL-treated mice (n = 4, mean ± S.E.M., **p<0.01). (D) Quantitative PCR analysis of integrin gene expression in gWAT from control and CL-treated mice (n=4, mean ± S.E.M.). (E) Quantitative PCR analysis of *Cd44* expression in isolated F4/80+ cells of gWAT from control mice and mice treated with CL for 3 days (n = 3, mean ± S.E.M., **p<0.01). (F) Unstained and single color controls used for (G). (G) FACS analysis of EdU^+ cells in SVC of gWAT from mice treated with CL for 3 days, injected with EdU 12 hours before sacrifice. $PDGFR\alpha^+$ fraction was analyzed for CD44 expression and EdU incorporation level. Data show that 86.7% of $EdU^+PDGFR\alpha^+$ cells from CL-treated mice were CD44 positive. The percentage of cells in each quadrant are indicated on the flow profile (n = 3, mean ± S.E.M.). (H) Representative images of paraffin sections stained for OPN and F4/80 of gWAT from mice treated with CL for 3 days. Bar = 100μm.

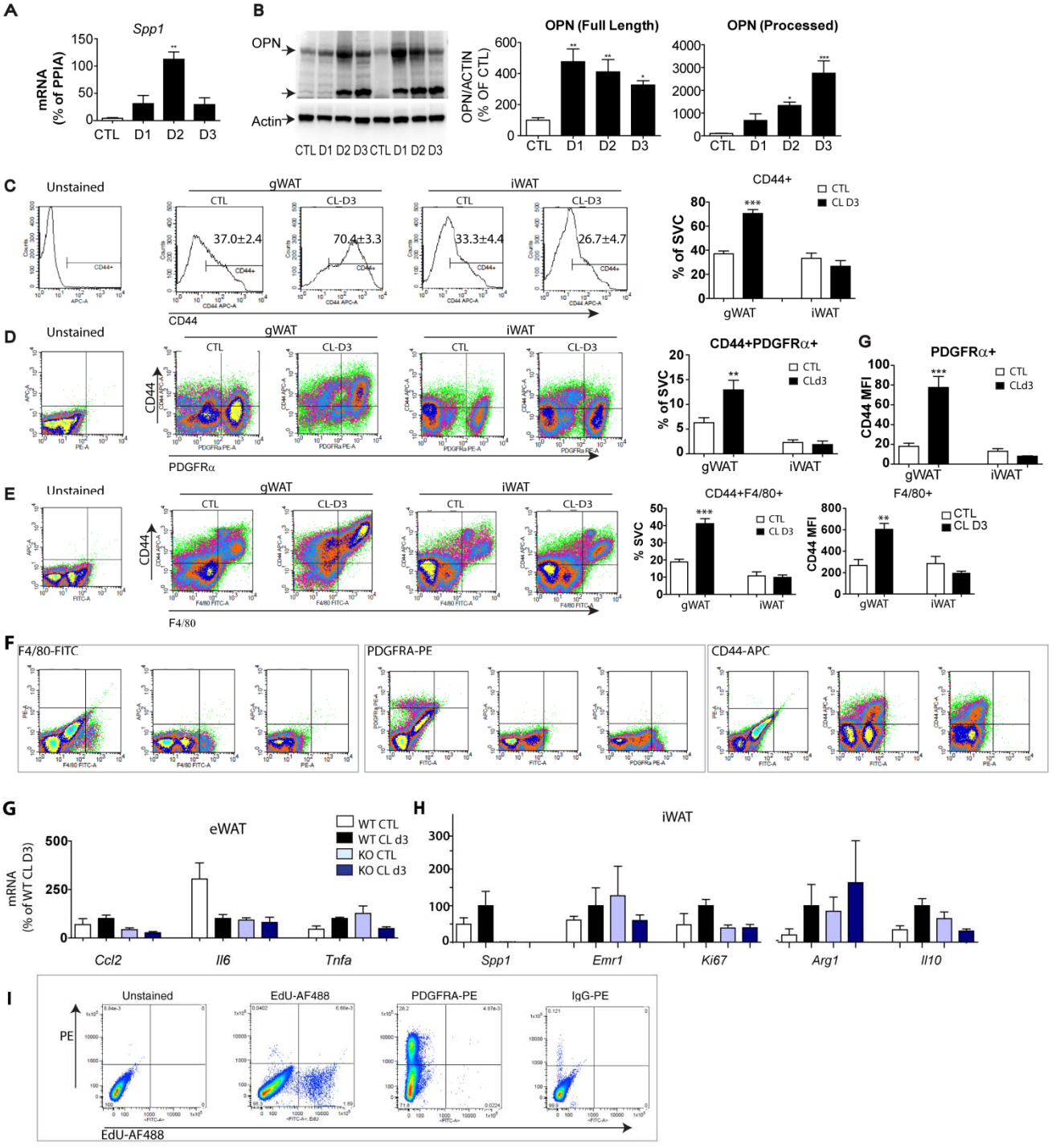

Figure S6, Related to Figure 6. OPN is required for CLS formation and ADRB3 mediated adipogenesis from PDGFRα^{+} cells.

(A) qPCR analysis of Spp1 expression in gWAT from control mice or mice treated with CL (n = 4-6, mean ± S.E.M., **p<0.01). (B) Immunoblot analysis of gWAT from control mice and mice treated with CL at indicated time points (n = 4, mean ± S.E.M., *p<0.05, **p<0.01, ***p<0.001). (C-E) FACS analysis of CD44, F4/80, and PDGFRα expression in SVC obtained from gWAT and iWAT of control and CL-treated mice (n=7, mean ± S.E.M., **p<0.01, ***p<0.001). (F) Single color controls used for (C-E). (G) Quantitative PCR analysis of M1 macrophage marker expression in gWAT from WT and Spp1KO mice (n = 3-5, mean ± S.E.M.). (H) Expression of macrophage and proliferation markers in iWAT from WT and Spp1KO mice (n = 3-5, mean ± S.E.M.). (I) Unstained control and single color controls for flow cytometric analysis of EdU incorporation and PDGFRα expression (Figure 6E). The quadrant markers are identical with those placed in Figure 6E.

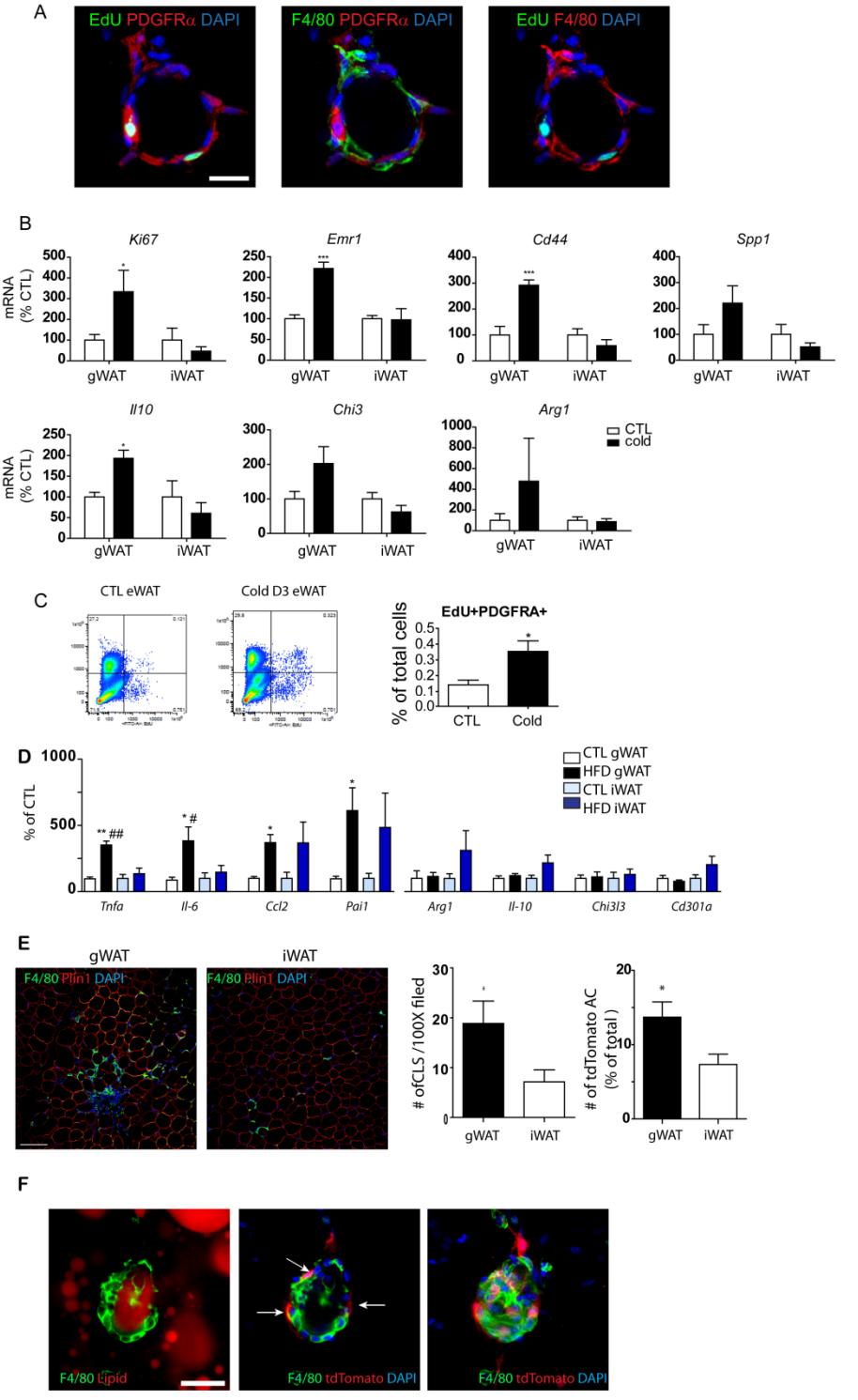

Figure S7, Related to Figure 7. PDGFRα^{+} cells interact with macrophages in adult WAT and contribute to adipogenesis during tissue repair, tissue neogenesis and nutritional hyperplasia. (A) A representative images of CLS in paraffin sections of gWAT from mice exposed

to cold temperature (4°C) for 3 days. Bar = 20 μm. (B) Quantitative PCR analysis of gene expression in gWAT and iWAT from control mice and mice exposed to cold temperature for 3 days (n = 5, mean ± S.E.M., *p<0.05, ***p<0.001). (C) FACS analysis of proliferation of PDGFRα^{+} cells in SVC obtained from gWAT of control mice and mice exposed to cold temperature for 3 days. Quantification of frequency of PDGFRα^{+}EdU^{+} cells in SVC fraction (n = 3, mean ± S.E.M., *p<0.05). (D) Quantitative PCR analysis of pro- and anti-inflammatory gene expression in gWAT and iWAT from control mice and mice fed HFD for 8 weeks (n = 5-12, mean ± S.E.M., *p<0.05). (E) Detection of CLS in gWAT and iWAT paraffin sections. Quantification of CLS in low magnification fields. Data are mean ± S.E.M. (n = 10, *p<0.05). Bar = 100 μm. (F) Representative images of CLS in iWAT whole mount from *Pdgfra*-CreER^{T2}/tdTomato mice. Macrophages and tdTomato^{+} progenitors form a clearing network that surrounds the remnant lipid core undergoing efferocytosis. Bars = 20 μm.

Table S1. Related to gene expression analysis described in extended experimental procedures. Primer sequences used for quantitative PCR

Gene	Symbol	Accession #	Forward (5'-3')	Reverse (5'-3')	Size
arginase, liver	*Arg1*	NM_007482.3	GGCTGGTGTGGTGGCAGAGG	CCTGGCGTGGCCAGAGATGC	100
CD44 antigen	*Cd44*	NM_001039150	CCAGGCTTTCAACAGTACCTTACC	CTGAGGCATTGAAGCAATATGTGTC	212
CD68 antigen	*Cd68*	NM_009853.1	AAGCTTCTGCTGTGGAAATG	ATGATGAGAGGCAGCAAGAG	159
chitinase 3-like 3	*Chi3l3*	NM_009892	TCTGGGTACAAGATCCCTGAA	TTTCTCCAGTGTAGCCATCCTT	97
C-type lectin domain family 10, member A	*Clec10a*	NM_001204252	GCCCCAGAGGCCTCGTGTTTG	GGGAAGGAGCTGCTTTACCAGGC	107
interleukin 10	*Il10*	NM_010548.2	GGCAGAGAAGCATGGCCCAGAA	TCACCTGCTCCACTGCCTTGC	137
interleukin 1 beta	*Il1b*	NM_008361.3	CTGGTGTGTGACGTTCCCATTA	CCGACAGCACGAGGCTTT	76
interleukin 1 receptor antagonist	*Il1rn*	NM_001159562.1	TCCGCTTCTGACAGTGGAAC	TCCCAGATTCTGAAGGCTTGC	103
interleukin 4	*Il4*	NM_021283.2	CATGGGAAAACTCCATGCTT	TGGACTCATTCATGGTGCAG	115
interleukin 6	*Il6*	NM_031168.1	AGTGGCTAAGGACCAAGACC	TCTGACCACAGTGAGGAATG	140
integrin alpha 4	*Itga4*	NM_010576.3	CTAGCTGAGCCACAAGCCAG	TTCACAGAGGCACTAGCGGA	111
integrin alpha 9	*Itga9*	NM_001113514.1	TGCTGCCGACCTGCAGCTTC	GCCCCCAAAGCCAGGTGTGG	78
integrin alpha X	*Itgax*	NM_021334.2	CCTACTTTGGGGCATCTCTTTG	GCACCTCTGTTCTCCTCCTCTC	289
integrin beta 1	*Itgb1*	NM_010578.2	GGACCTTTTGGGTTGAGCTTATT	AAAAAGTCTAACCCCCATATTGCA	106
lymphatic vessel endothelial hyaluronan receptor 1	*Lyve1*	NM_053247.4	CCACAACTCATCCGACACCT	TCTGTTGCGGGTGTTTGAGT	93
mannose receptor, C type 2	*Mrc2*	NM_008626.3	TACAGCTCCACGCTATGGATT	CACTCTCCCAGTTGAGGTACT	100
nitric oxide synthase 2, inducible	*Nos2*	NM_010927.3	CTGCTTTGTGCGAAGTGTCA	GCCAGAAACTTCGGAAGGGA	107
platelet derived growth factor, alpha	*Pdgfa*	NM_008808.3	GTGCGACCTCCAAC	GGCTCATCTCACCTCACATCT	64
platelet derived growth factor, B polypeptide	*Pdgfb*	NM_011057.3	GCCTGTGACTAGAAGTCCTG	GTCATGGGTGTGCTTAAACT	133
platelet-derived growth factor, C polypeptide	*Pdgfc*	NM_019971.2	GCTGGAAGATCCAGAAGACG	CACACCAGCGTCCTAAAACA	89
platelet-derived growth factor, D polypeptide	*Pdgfd*	NM_027924.2	CAGGGAAGACAGTGAAGAAG	GAGCTGCAGATACAGTCACA	139
resistin like alpha	*Retnla*	NM_020509.3	CCTGCTGGGATGACTGCTAC	CAGTGGTCCAGTCAACGAGT	112
serine (or cysteine) peptidase inhibitor, clade E, member 1	*Serpine1*	NM_008871.2	CCTCTTCATGGGCCAAGT	GGTAAGGAGGAGTTGCCTTC	150
secreted phosphoprotein 1	*Spp1*	NM_001204201.1	TCAGAGCCACAAGTTTCACA	AGGAACTGTGTTTTTGCCTCT	151
transforming growth factor beta-1	*Tgfb*	NM_011577.1	CTGCTGACCCCCACTGATAC	GCCCTGTATTCCGTCTCCTT	93

tumor necrosis factor	*TNF*	NM_013693.2	GGGCAGGTCTACTTTGGAGT	CTGAGCCATAATCCCCTTTC	162

Information regarding genes and primer sequences used for quantitative expression profiling by qRT/PCR.

Supplemental Experimental Procedures

Migration analysis

Migration of PDGFRα$^+$ cells was evaluated by Transwell assay. SVCs of gWAT from Pdgfra-H2BeGFP mice were suspended at 2X10^5 cells/ml in DMEM with 0.1% BSA, and 0.2 ml of the suspensions were placed on the upper surfaces of the 8 um pore-size Transwell inserts (4X10^4/ well of 24-well plate). The medium (0.6 ml, 0.1% BSA DMEM) containing PDGF-AA (Invitrogen, 50 ng/ml) or OPN (R&D system, 200 ng/ml) was added in the lower well. The cells were allowed to migrate to the lower surface of the membrane for 16 h at 37°C. The unmigrated cells on the upper surfaces were wiped off using cotton swabs, and the inserts were washed with PBS, fixed with 4% paraformaldehyde for 15min at RT, permeabilized with 0.5% TritonX in PBS and stained with DAPI for 10 min. Membranes were subsequently mounted onto a microscope slide using mounting medium (Dako). Migratory activity was calculated as the average number of migrated GFP$^+$ cells in 5 random 100X fields from each well. Experiments were performed in triplicate, and independently replicated three times.

Antibodies

Antibodies used for immunohistochemistry in this study were: PDGFRα (R&D system, goat, 2μg/ml; Cell Signaling, rabbit, 1:200), F4/80 (ADserotec, rat, 1:400), tdTomato (Clontech, rabbit, 1:100), PLIN1 (rabbit (Moore et al., 2005), 1:500), MGL1 (Abcam, rat, 1:100), UCP1 (rabbit, Alpha Diagnostic International, 0.5ug/ml) and OPN (goat (Liaw et al., 1994)). Following secondary antibodies were used: Alexa Fluor 488– or 594–conjugated donkey antibody to goat IgG, goat or donkey antibody to rabbit IgG, goat antibody to rat IgG (Invitrogen, 1:1,000), and Cy5-conjugated goat antibody to rabbit IgG (Jackson Labs, 1:200). Antibodies used for western blot were OPN (goat (Liaw et al., 1994)) and Actin (goat, Santa Cruz, 1:500). Antibodies used for FACS analysis were:

anti-PDGFRα (CD140a)-PE (Biolegend, rat, 1:200), F4/80-FITC or PE/Cy7 (Biolegend, rat, 1:200), MGL1-FITC (Abserotec, rat, 1:100), CD44-APC or PE/Cy5 (Biolegend, rat, 1:100), CD45-PE/Cy7, and CD11b-PE/Cy7 (Biolegend, rat, 1:300). Species-matched IgG were used as nonspecific controls.

Western blot and gene expression analysis

Protein was extracted using RIPA lysis buffer (25 mM Tris, pH 7.5, 150 mM NaCl, 1% Triton X- 100, 0.5% Na deoxycholate, 1% NP-40, 0.5% sodium dodecyl sulfate, and 1 mM EDTA) containing protease inhibitors (Roche). Western blot was performed as described (Lee et al., 2012). Total RNA was extracted using Trizol (Invitrogen) and mRNA was reverse-transcribed using Superscript III (Invitrogen) and oligo dT primers (Fermentas). 50 ng of cDNA was analyzed in a 20 µl quantitative PCR reaction (ABsolute Blue QPCR SYBR, ThermoScientific) with 70 nM of primers. Expression data were normalized to the house keeping gene peptidyl-prolyl cis-trans isomerase A (PPIA) using the delta-delta CT method (2-ΔΔCT), as described (Li et al., 2005). Specific primer sequences used for PCR are listed in Table S1, and all other primers have been described previously (Lee et al., 2012)

Microscopy

Fluorescence and bright-field microscopy were performed using an Olympus IX-81 microscope equipped with a spinning disc confocal unit and 40X (0.9NA) and 60X (1.2NA) water immersion objectives, using standard excitation and emission filters (Semrock) for visualizing DAPI, FITC (Alexa Fluor 488), Cy3 (Alexa Fluor 594, Nile Red), and Cy5 (Alexa Fluor 647, LipidTox) as described (Granneman et al., 2009). Confocal z stacks were captured for whole-mount tissue by

imaging up to 51 optical sections at 0.4–1micron increments. Raw data were processed using IPlabs software (Scanalytics, BD Biosciences) and Adobe Photoshop.

Supplemental References

Liaw, L., Almeida, M., Hart, C.E., Schwartz, S.M., and Giachelli, C.M. (1994). Osteopontin promotes vascular cell adhesion and spreading and is chemotactic for smooth muscle cells in vitro. Circulation Research 74, 214-224.

Moore, H.-P.H., Silver, R.B., Mottillo, E.P., Bernlohr, D.A., and Granneman, J.G. (2005). Perilipin Targets a Novel Pool of Lipid Droplets for Lipolytic Attack by Hormone-sensitive Lipase. J Biol Chem 280, 43109-43120.