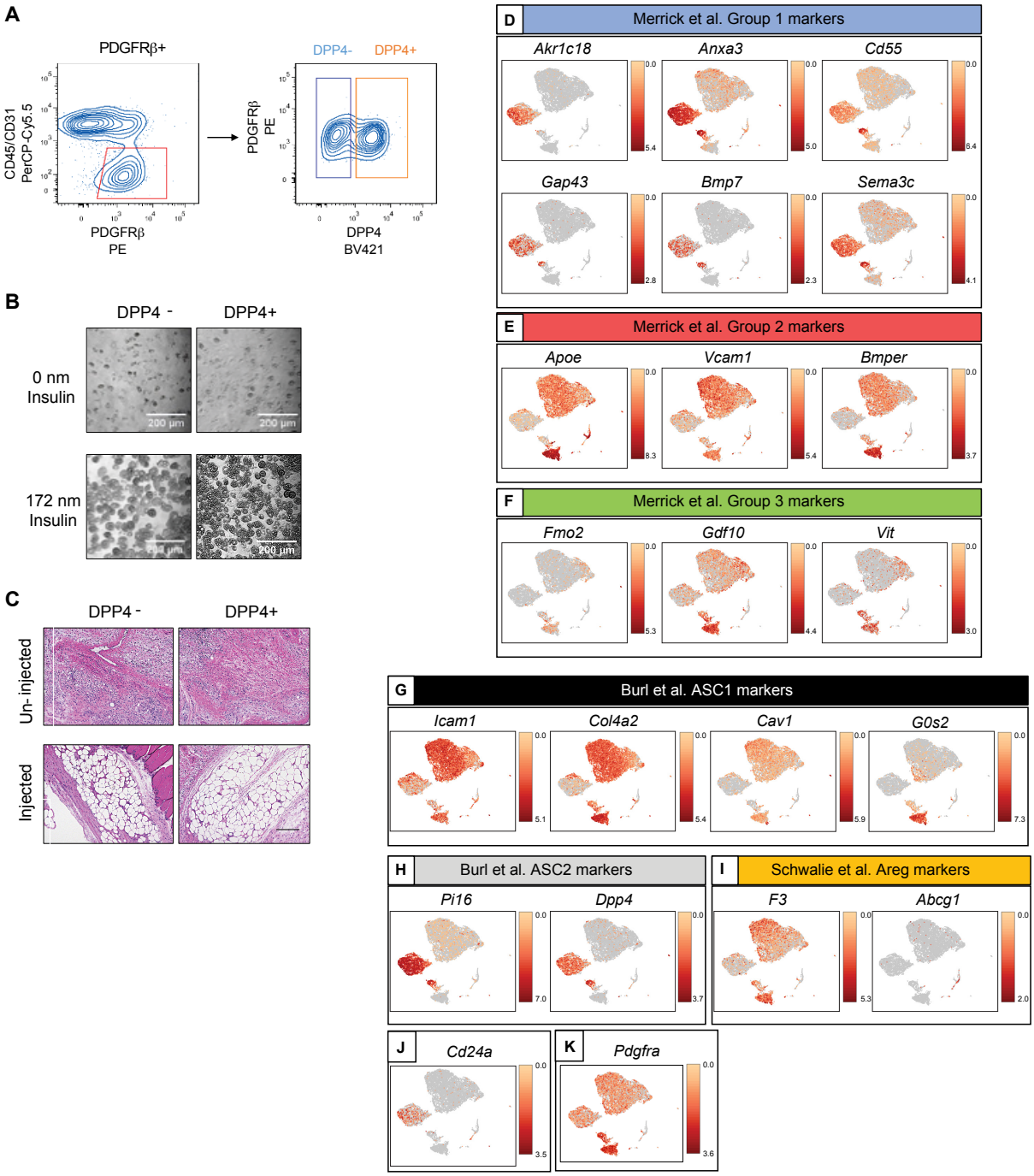

Figure S1: Related to Figure 1. Adipogenic capacity of identified iWAT PDGFRβ+ cell subpopulations and their expression of reported adipocyte precursor markers.

(A) Representative FACS collection gates for the isolation of inguinal WAT PDGFRβ+ mural cell subpopulations.

(B) Representative bright-field images of isolated inguinal DPP4- PDGFRβ+ and DPP4+ PDGFRβ+ cells after 8 days of culture in growth media with or without insulin supplementation.

(C) Representative bright-field images of H&E stained inguinal WAT from lipodystrophic mice (*Adiponectin-Cre*; *Pparg*$^{loxp/loxp}$) transplanted with 100,000 DPP4- PDGFRβ+ cells or 100,000

DPP4+ PDGFRβ+ cells isolated from iWAT of wildtype C57BL/6 mice. The depots were harvested 3 weeks after transplantation. Scale bar denotes 200 μm.

(D) Heat map depicting expression of genes defining “Group 1” adipocyte progenitors (Merrick *et al*.) within iWAT and gWAT PDGFRβ+ subpopulations.

(E) Heat map depicting expression of genes defining “Group 2” adipocyte progenitors (Merrick *et al*.) within iWAT and gWAT PDGFRβ+ subpopulations.

(F) Heat map depicting expression of genes defining “Group 3” adipocyte progenitors (Merrick *et al*.) within iWAT and gWAT PDGFRβ+ subpopulations.

(G) Heat map depicting expression of genes defining “ASC1” (Adipocyte Stem Cell 1) (Burl *et al*.) within iWAT and gWAT PDGFRβ+ subpopulations.

(H) Heat map depicting expression of genes defining “ASC2” (Adipocyte Stem Cell 2) (Burl *et al*.) within iWAT and gWAT PDGFRβ+ subpopulations.

(I) Heat map depicting expression of genes defining “Aregs” (Schwalie *et al*.) within iWAT and gWAT PDGFRβ+ subpopulations.

(J) Heat map depicting expression of *CD24a* within iWAT and gWAT PDGFRβ+ subpopulations.

(K) Heat map depicting expression of *Pdgfra* within iWAT and gWAT PDGFRβ+ subpopulations.

Transcript counts represent Log2 of gene expression.

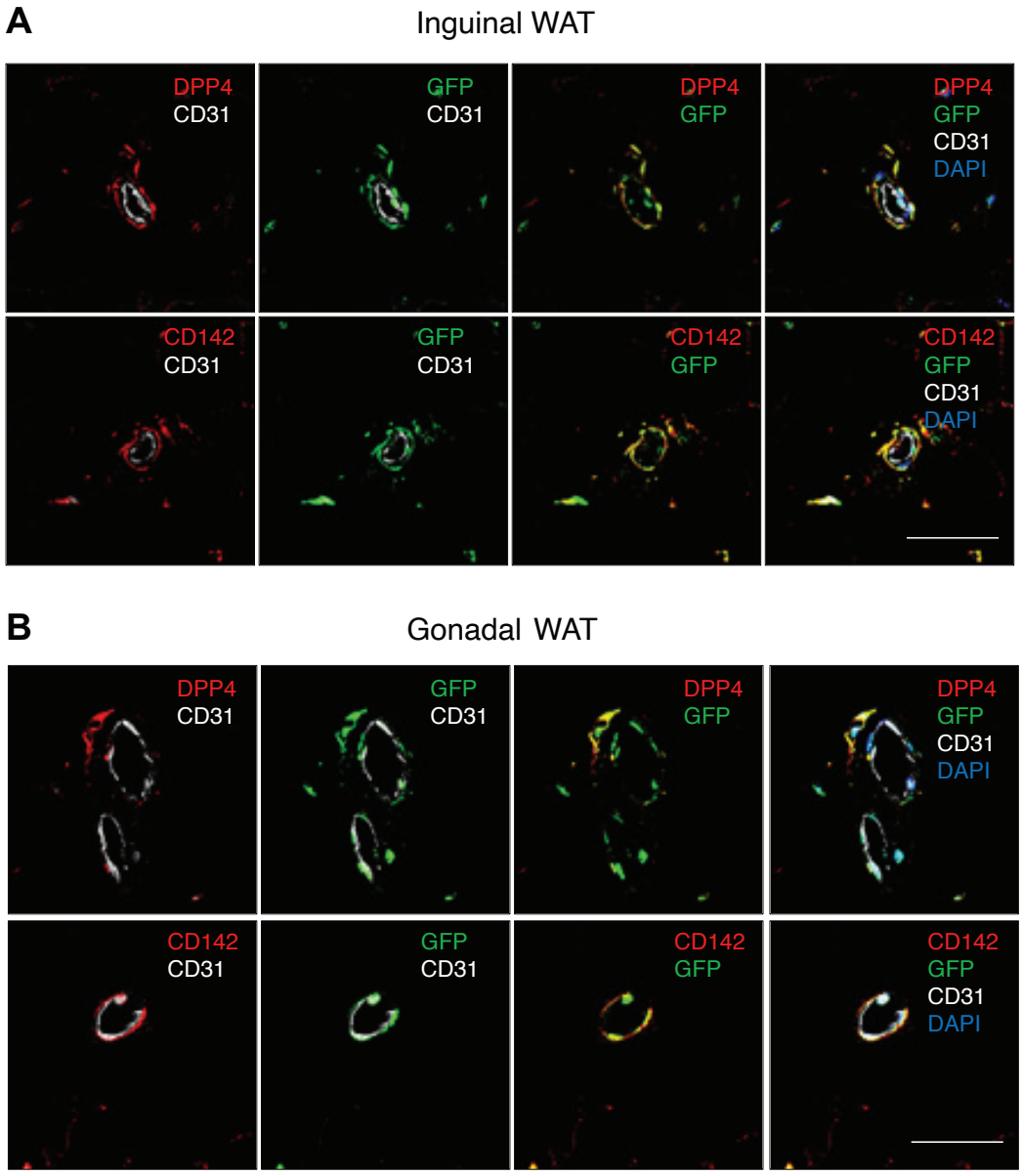

Figure S2: Related to Figure 1. Perivascular localization of adipose tissue PDGFRβ+ cell subpopulations.

(A) Representative confocal immunofluorescence image of paraffin-embedded inguinal WAT sections from 8 week-old MuralChaser mice after 7 days of Dox-chow feeding.

(B) Representative confocal immunofluorescence image of paraffin-embedded gonadal WAT sections from 8 week-old MuralChaser mice after 7 days of Dox-chow feeding.

For panels A and B, sections were stained with antibodies recognizing GFP (identifying PDGFRβ+ cells), DPP4 (identifying iWAT DPP4+ cells and gWAT FIPs), CD142 (identifying iWAT DPP4- cells and gWAT APCs), and CD31 (identifying endothelial cells).

Scale bar denotes 50 μm.

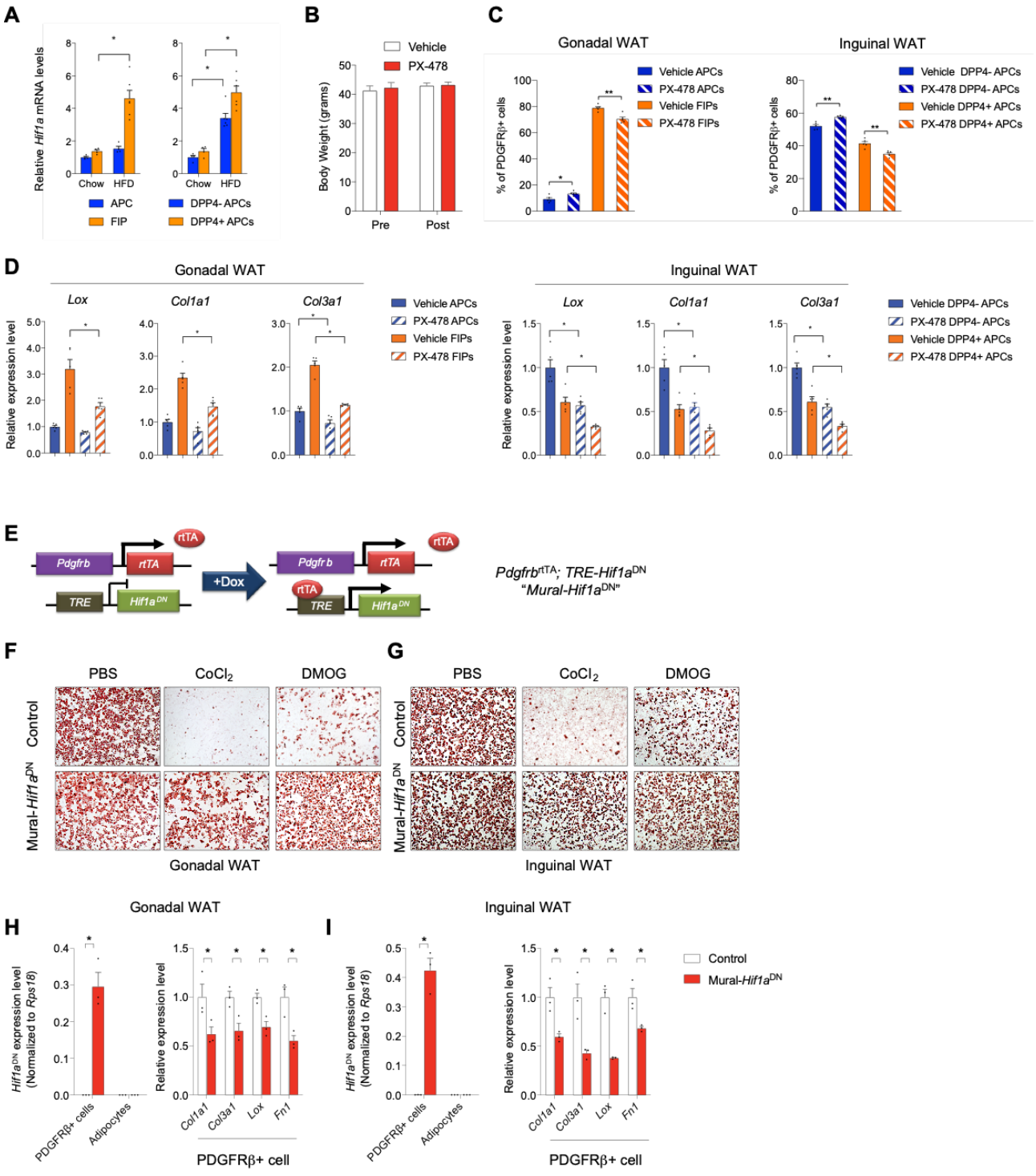

Figure S3: Related to Figure 3. HIFα regulates fibrogenic gene expression in WAT PDGFRβ+ progenitor subpopulations.

(A) mRNA levels of *Hif1a* within freshly isolated gonadal WAT APCs and FIPs, inguinal WAT DPP4- PDGFRβ+ and DPP4+ PDGFRβ+ cells from chow-fed mice or aged-matched animals

maintained on HFD for 10 weeks. Bars represent mean + s.e.m. * denotes $p < 0.05$ by two-way ANOVA. Each sample (n) represents 10,000 freshly isolated cells from 2-3 individual depots. n=6 for HFD FIPs. n=4 for all other subpopulations analyzed.

(B) C57BL/6 wildtype mice were fed a standard chow diet until 8 weeks of age before being switched to HFD for 10 weeks. Mice were then maintained on HFD for an additional 7 days, during which vehicle or PX-478 (5mg/kg) was administered daily by i.p. injection. Body weights were measured before (Pre) and after (Post) the treatment.

(C) Relative frequency of PDGFRβ+ subpopulations within gWAT (left) and iWAT (right) of mice after vehicle or PX-478 treatment. n= 5 per group. Bars represent mean + s.e.m. * denotes $p < 0.05$ by two-way ANOVA.

(D) mRNA levels of indicated fibrosis-related genes within gWAT APCs and FIPs, iWAT DPP4- and DPP4+ PDGFRβ+ cells from mice after vehicle or PX-478 treatment. n= 5 per group. Bars represent mean + s.e.m. * denotes $p < 0.05$ by two-way ANOVA.

(E) *Pdgfrb*^{rtTA}; *TRE-Hif1a*^{DN} (Mural-*Hif1a*^{DN}) mice are generated by breeding the *Pdgfrb*^{rtTA} transgenic mice to animals expressing dominant negative *Hif1a* under the control of the tet-response element (*TRE-Hif1a*^{DN}). The addition of doxycycline activates transgene expression. Littermates carrying only *Pdgfrb*^{rtTA} or *TRE-Hif1a*^{DN} alleles represent Control animals.

(F) Representative bright-field images of Oil-red O stained cultures of adipocytes differentiated from gonadal WAT APCs isolated from Control or Mural-*Hif1a*^{DN} mice.

(G) Representative bright-field images of Oil-red O stained cultures of adipocytes differentiated from inguinal WAT PDGFRβ+ cells isolated from Control or Mural-*Hif1a*^{DN} mice.

For (F) and (G), cells were induced to differentiate in the presence of doxycycline (2 μM) and either PBS (vehicle), $CoCl_2$ (50 μM), or DMOG (50 μM). Oil-red O staining was performed 7 days after the induction of adipogenic differentiation. Scale bar denotes 50 μm.

(H) mRNA levels of *dnHif1a* transgene and fibrosis-associated genes in freshly isolated PDGFRβ+ stromal cells or fractionated adipocytes from gonadal WAT of 8-week old Control or Mural-*Hif1a*^{DN} mice.

(I) mRNA levels of *dnHif1a* transgene and fibrosis-associated genes in freshly isolated PDGFRβ+ stromal cells or fractionated adipocytes from inguinal WAT of 8-week old Control or Mural-*Hif1a*^{DN} mice.

For panels H and I, Control or Mural-*Hif1a*^{DN} mice were fed with Dox-containing chow diet for 7 days prior to harvest. n= 3. Bars represent mean + s.e.m. * denotes $p < 0.05$ by Student's t-test.

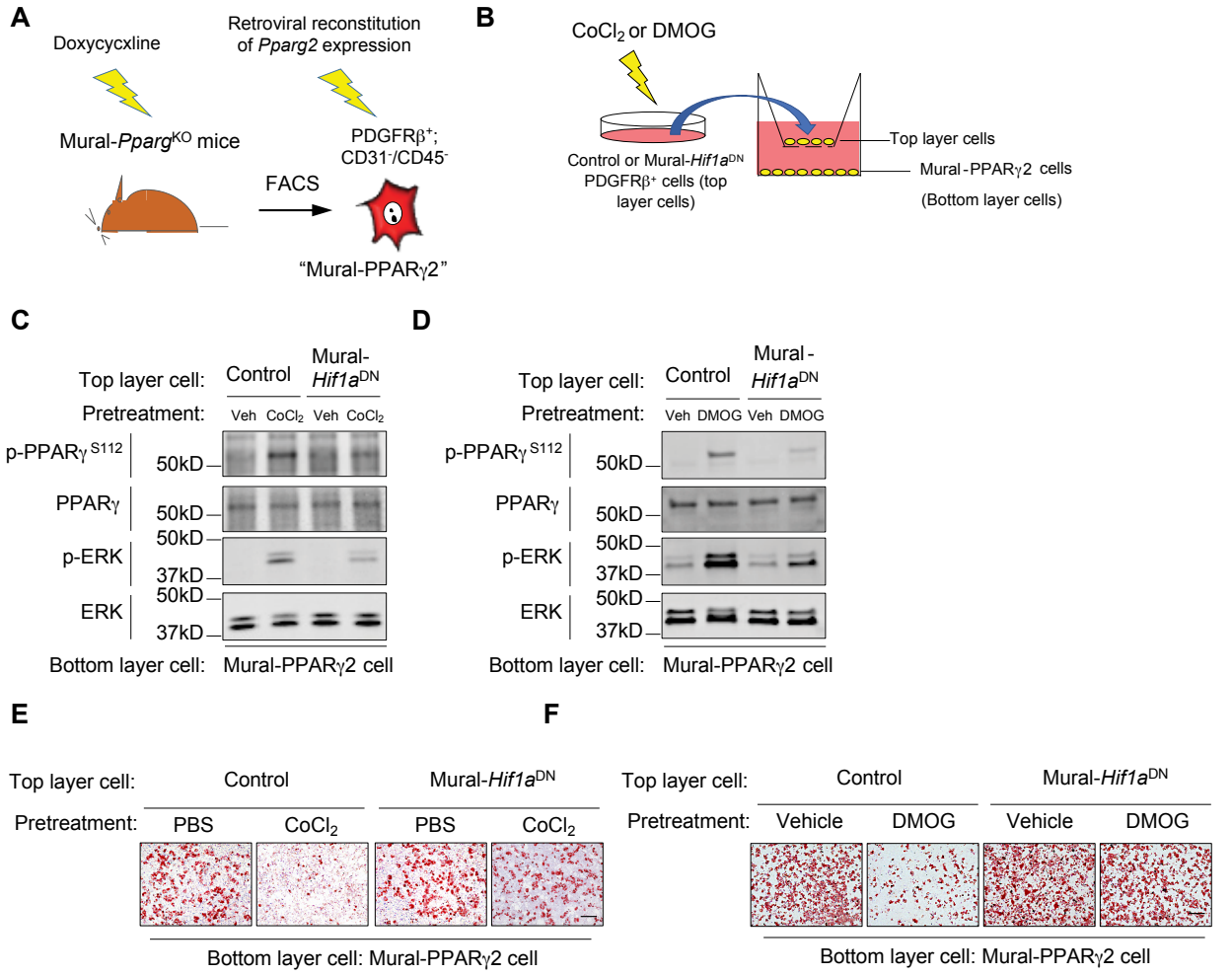

Figure S4: Related to Figure 6. HIFα activation induces PPARγ S112 phosphorylation through autocrine/paracrine signaling mechanisms.

(A) Derivation of stable Mural-PPARγ2 cells: *Pparg*-deficient iWAT PDGFRβ+ cells were isolated by FACS from Doxycyline-treated Mural-*Pparg*^{KO} mice (Dox-chow diet for 7 days) and then transduced in vitro with retrovirus expressing FLAG-PPARγ2.

(B) Design of co-culture experiments: iWAT PDGFRβ+ cells from Control or Mural-*Hif1a*^{DN} mice were treated with 50μM $CoCl_2$ or 50μM DMOG, for 48 hours. After washing, cells were transferred to transwell membranes (Top layer cells) overlaying stable Mural-PPARγ2 cells (without $CoCl_2$ or DMOG) (Bottom later cells). Co-culture of top and bottom layers of cells were performed in serum free media for 6 hours before the bottom layer cells were harvested for western blot analysis of PPARγ phosphorylation or top and bottom layers of cells were co-cultured in adipogenic induction media for 48 hours before the top layer cells were removed. The bottom layer cells were then kept in maintenance media until oil-red O staining was performed 7 days after the adipogenic induction.

(C) Western blot analysis of levels of phosphorylated PPARγ, total PPARγ, phosphorylated ERK, and total ERK, in bottom layer Mural-PPARγ2 cells co-cultured with top layer $CoCl_2$-treated Control or Mural-*Hif1a*^{DN} cells.

(D) Oil-red O staining of adipocytes differentiated from bottom layer Mural-PPARγ2 cells co-cultured with top layer $CoCl_2$-treated Control or Mural-*Hif1a*^{DN} cells 7 days after adipogenic induction.

(E) Western blot analysis of levels of phosphorylated PPARγ, total PPARγ, phosphorylated ERK, and total ERK, in bottom layer Mural-PPARγ2 cells co-cultured with top layer DMOG-treated Control or Mural-*Hif1a*^{DN} cells.

(F) Oil-red O staining of adipocytes differentiated from bottom layer Mural-PPARγ2 cells co-cultured with top layer DMOG-treated Control or Mural-*Hif1a*^{DN} cells 7 days after adipogenic induction.

Scale bar in D, F denotes 200 μm.

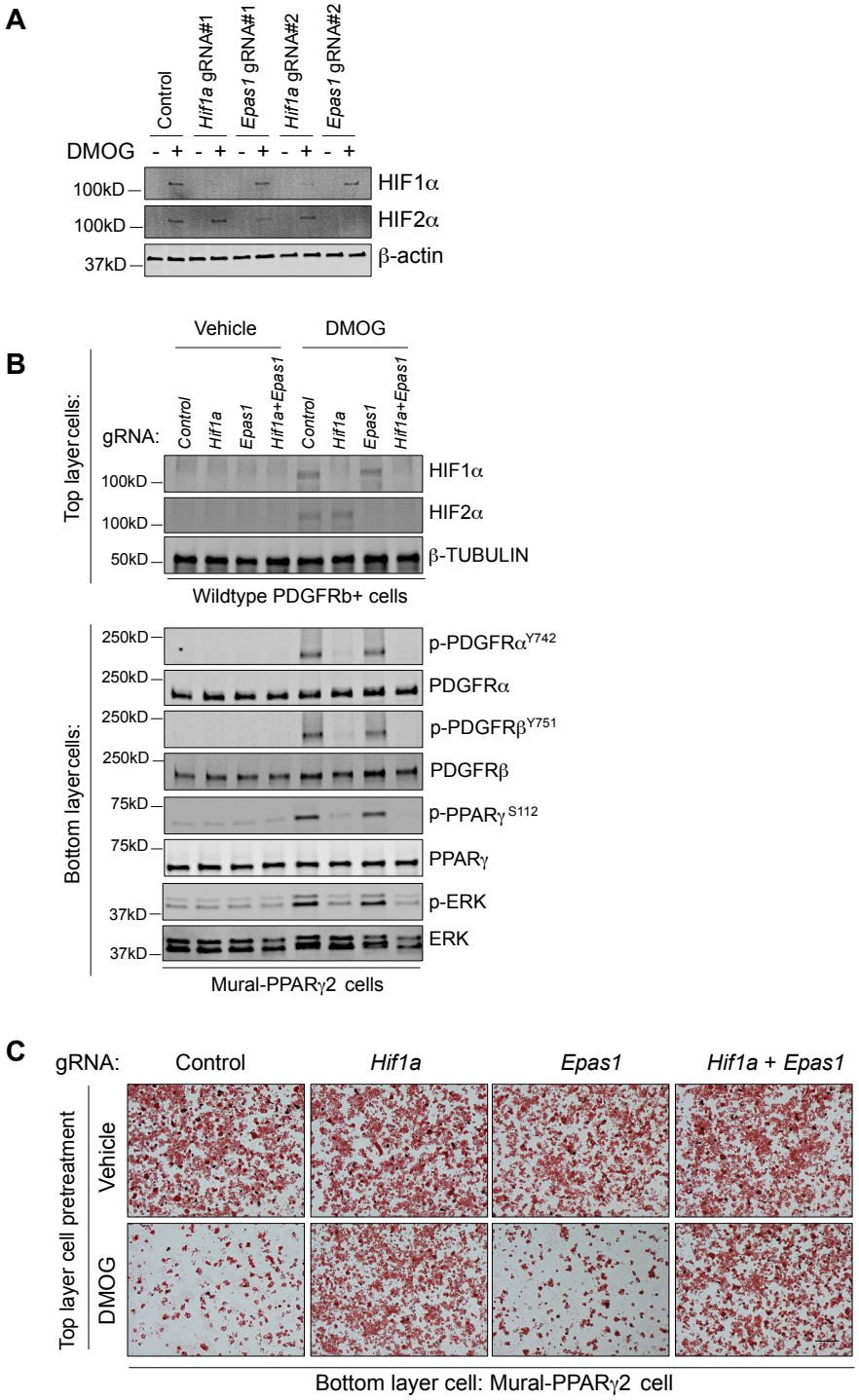

Figure S5: Related to Figure 6. HIF1α, but not HIF2α, mediates the inhibitory effects of DMOG on PPARγ activation in PDGFRβ+ cells.

(A) Western blot analysis for HIF1α, HIF2α, and β-actin in iWAT PDGFRβ+ cells transduced with the indicated CRISPR lentivirus.

(B) iWAT PDGFRβ+ cells from C57BL/6 wildtype mice were transduced with the indicated CRISPR lentivirus and treated with vehicle or 50 μM DMOG for 48 hours. After washing, cells were transferred to transwell membranes (Top layer cells) overlaying stable Mural-PPARγ2 cells (without DMOG) (Bottom later cells). Co-cultures were maintained in serum free media for 6 hours before bottom layer cells were harvested for western blot analysis of HIF1α, HIF2α, phosphorylated/total PDGFRα, phosphorylated/total PDGFRβ, phosphorylated/total PPARγ, and phosphorylated/total ERK.

(C) Parallel co-cultures as described in (B) were established to assay for adipogenesis of Mural-PPARγ2 cells. Bright-field image of lipid accumulation (Red) were captured 7 days after inducing adipogenesis of Mural-PPARγ2 cells exposed to the indicated cells/treatments. Scale bar denotes 200 μm.

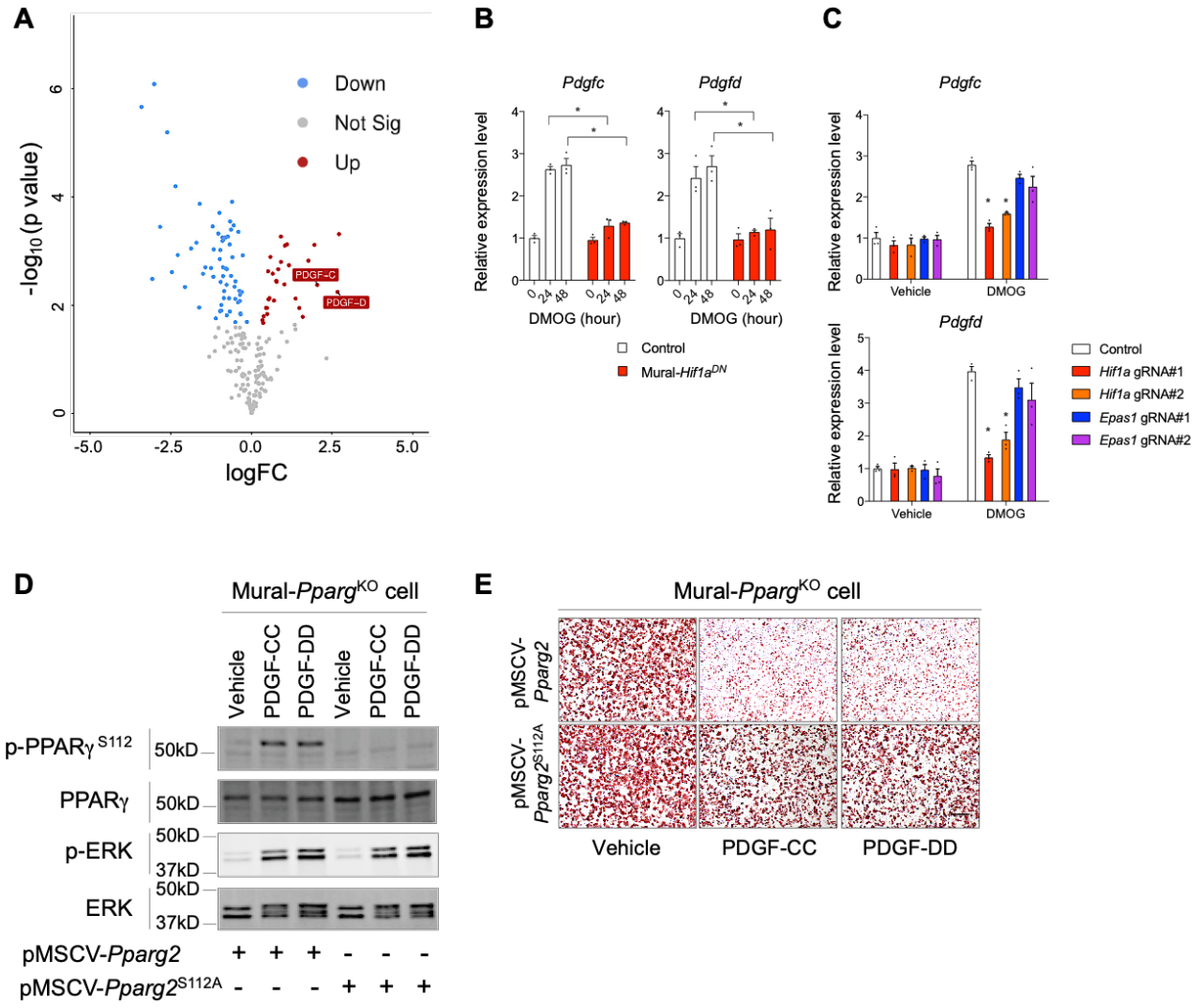

Figure S6: Related to Figure 6. HIFα-activation drives production of anti-adipogenic PDGF-CC and PDGF-DD in adipose PDGFRβ+ cells.

(A) Volcano plot depicting proteins differentially secreted from cultured PDGFRβ+ cells following exposure to the HIF1 stabilizer, DMOG. Secreted proteins annotated in the Uniprot database are shown. DMOG treatment results in increased production of PDGF-C and PDGF-D (See Table S3 for full list of identified proteins).

(B) mRNA levels of *Pdgfc* and *Pdgfd* within cultured inguinal PDGFRβ+ cells at indicated time points following 50 μM DMOG treatment. n= 3. Bars represent mean + s.e.m. * denotes p< 0.05 by Student's t-test.

(C) mRNA levels of *Pdgfc* and *Pdgfd* within cultured inguinal PDGFRβ+ cells transduced with the indicated CRISPR lentivirus following vehicle or 50 μM DMOG treatment for 24 hours. n= 3. Bars represent mean + s.e.m. * denotes p< 0.05 by one-way ANOVA.

(D) *Pparg*-deficient Mural-*Pparg*^{KO} cells transduced with virus expressing PPARγ2, or the PPARγ2 S112A variant were treated with 10 μM recombinant PDGF-CC or PDGF-DD for 30 min. Levels

of phosphorylated PPARγ, total PPARγ, phosphorylated ERK, and total ERK, were determined by western blot analysis.

(E) Oil-red O staining of differentiated cultures of *Pparg*-deficient Mural-*Pparg*^{KO} cells transduced with virus expressing PPARγ2 or the PPARγ2 S112A variant and treated with PDGF-CC or PDGF-DD. Cells were induced to differentiate with adipogenic induction media in the presence of 10 μM recombinant PDGF-CC or PDGF-DD for 48 hours. The treated cells were then cultured in maintenance media for an additional five days before fixation and staining. Scale bar denotes 200 μm.

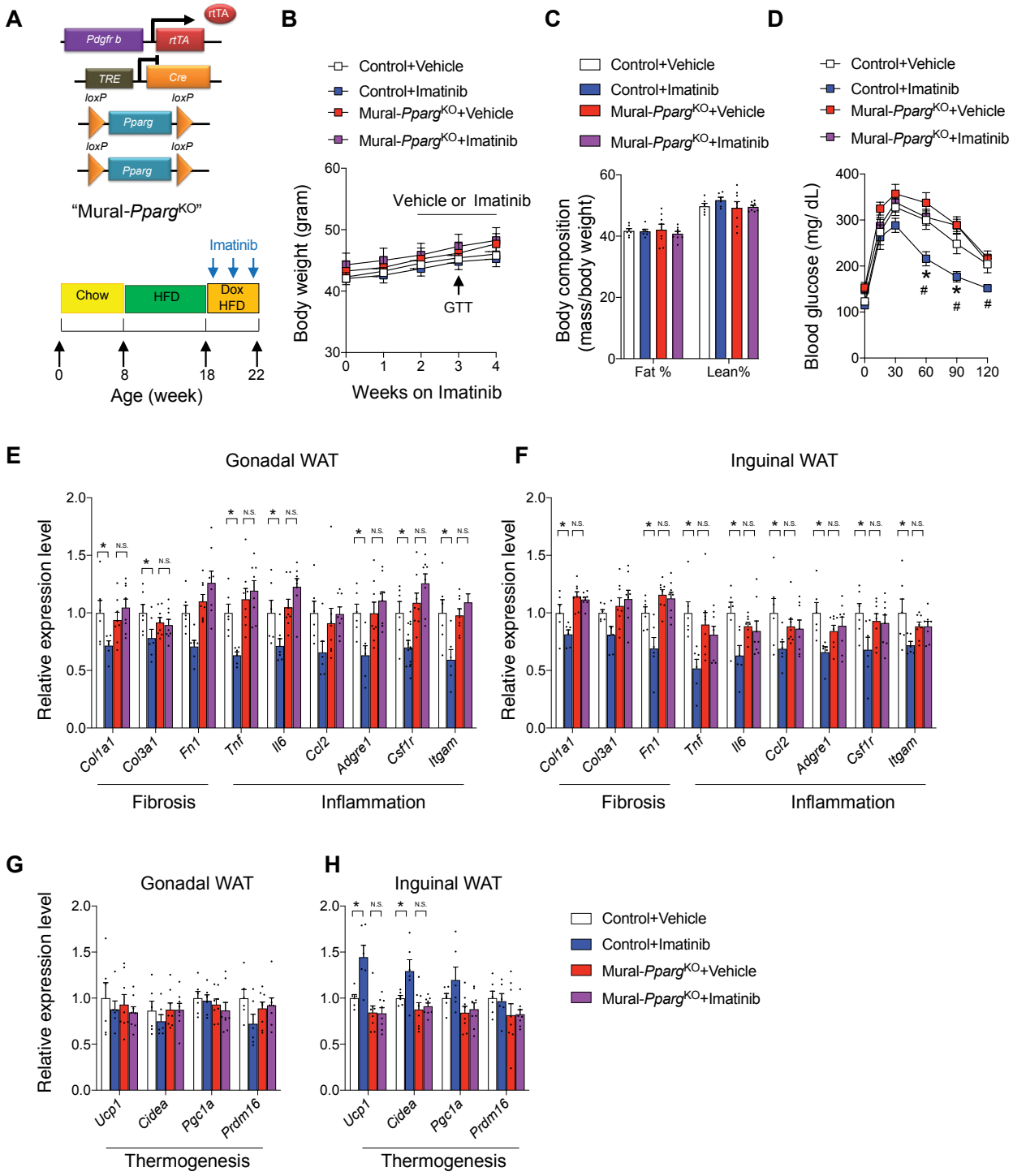

Figure S7: Related to Figure 7. The beneficial effects of Imatinib on WAT and glucose tolerance depend on mural cell PPARγ.

(A) Experimental design: Control and Mural-*Pparg*^{KO} mice were kept on a standard chow diet (Chow) until 8 weeks of age before being switched to HFD for another 10 weeks. To inactivate

mural cell PPARγ, the mice were then administered doxycycline-containing HFD (Dox-HFD) for an additional 4 weeks, during which vehicle or Imatinib was administered four times weekly (25mg/kg) by i.p. injection.

(B) Average weekly body weights during Imatinib administration.

(C) Average fat mass and lean mass (normalized to body weight) of Control and Mural-*Pparg*^{KO} mice after 4 weeks of Imatinib administration.

(D) Glucose tolerance tests of Control or Mural-*Pparg*^{KO} mice after 3 weeks of Imatinib administration.

For panels B-D, n= 6 for Control mice +/- Imatinib. n=8 for Mural-*Pparg*^{KO} mice +/- Imatinib. Data points represent mean + s.e.m. * denotes $p< 0.05$ between Control + Vehicle and Control + Imatinib by two-way ANOVA; $^{\#}$ denotes $p< 0.05$ between Control + Imatinib and Mural-*Pparg*^{KO} + Imatinib by two-way ANOVA.

(E) mRNA levels of fibrosis- and inflammation-related genes in whole gonadal WAT of Control and Mural-*Pparg*^{KO} mice after 4 weeks of Imatinib administration.

(F) mRNA levels of fibrosis- and inflammation-related genes in whole inguinal WAT of Control and Mural-*Pparg*^{KO} mice after 4 weeks of Imatinib administration.

(G) mRNA levels of thermogenesis-related genes in whole gonadal WAT of Control and Mural-*Pparg*^{KO} mice after 4 weeks of Imatinib administration.

(H) mRNA levels of thermogenesis-related genes in whole inguinal WAT of Control and Mural-*Pparg*^{KO} mice after 4 weeks of Imatinib administration.

For panels E-H, n= 6 mice per genotype. Bars represent mean + s.e.m. * denotes $p< 0.05$ by two-way ANOVA.

Table S4: Related to STAR Methods- Gene Expression Analysis. Primer sequences for qPCR

Gene	Forward (5' -3')	Reverse (5' -3')
Acta2	TGACGCTGAAGTATCCGATAGA	GTACGTCCAGAGGCATAGAGG
Adgre1	ATGGACAAACCAACTTTCAAGGC	GCAGACTGAGTTAGGACCACAA
Adipoq	AGATGGCACTCCTGGAGAGAA	TTCTCCAGGCTCTCCTTTCCT
Ccl2	CCACAACCACCTCAAGCACTTC	AAGGCATCACAGTCCGAGTCAC
Cidea	TCCTATGCTGCACAGATGACG	TGCTCTTCTGTATCGCCCAGT
Col1a1	AGATGATGGGGAAGCTGGCAA	AAGCCTCGGTGTCCCTTCATT
Col1a2	GTAACTTCGTGCCTAGCAACA	CCTTTGTCAGAATACTGAGCAGC
Col3a1	ATTCTGCCACCCCGAACTCAA	ACAGTCATGGGGCTGGCATTT
Col5a1	TGTCATGTTTGGCTCCCGGAT	AGTCATAGGCAGCTCGGTTGT
Csf1r	TGTCATCGAGCCTAGTGGC	CGGGAGATTCAGGGTCCAAG
Fn1	GAGAGCACACCCGTTTTCATC	GGGTCCACATGATGGTGACTT
Il6	AAGCCAGAGTCCTTCAGAGAGA	ACTCCTTCTGTGACTCCAGCTT
Itgam	GGCTCCGGTAGCATCAACAA	ATCTTGGGCTAGGGTTTCTCT
Lox	TCGCTACACAGGACATCATGC	ATGTCCAAACACCAGGTACGG
Pgc1a	GGTGCTTTAGAAATGCGGGGT	AGGTTCCCTCTCTGCTGCTTT
Pparg2	GCATGGTGCCTTCGCTGA	TGGCATCTCTGTGTCAACCATG
Prdm16	ACACGCCAGTTCTCCAACCTGT	TGCTTGTTGAGGGAGGAGGTA
Rps18	CATGCAAACCCACGACAGTA	CCTCACGCAGCTTGTTGTCTA
Tgfbi	ATTTCCTGACTGTGCCAAGGG	GCCGGACTGCTTGATTCACAT
Timp1	CTTGGTTCCCTGGCGTACTC	ACCTGATCCGTCCACAAACAG
Tnfa	GAAAGGGGATTATGGCTCAGG	TCACTGTCCCAGCATCTTGTG
*TRE-*dn*Hif1a*	CAGATATGAAGATGACTCAGCTGTTC	CAAGGGACATCTTCCCATTCTAAAC
Ucp1	TCTCAGCCGGCTTAATGACTG	GGCTTGCATTCTGACCTTCAC