Supplemental Information

Visceral Adipose Tissue Immune Homeostasis Is Regulated by the Crosstalk between Adipocytes and Dendritic Cell Subsets

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Supplementary Figure 1. Phenotypic characterization of cDCs in VAT. Related to Figure 1. VAT was isolated from Zbtb46-GFP+ mice. (A) cDCs were identified as MHCII+ CD11c+ GFP+ cells and macrophages as MerTK+ CD64+ cells. (B) Phenotypic characterization of MHCII+ CD11c+ GFP+, MHCII+ CD11c+ GFP- cells and macrophages. Dot plots and histograms are representative of 3 independent experiments. (C) Mesenteric VAT and vessels from Zbtb46-GFP+ mice were imaged by ex vivo confocal microscopy. Tissue was visualized viewed using a Leica SP8 confocal microscope. GFP+ cDCs (green) located in close proximity (black arrows) to PECAM-1+ capillary vessels (BV, blue) of the VAT (left picture) but also close to mesenteric afferent lymphatics (LV, middle picture) and
LYVE-1+ initial lymphatic vessels (iLV, red) near the gut (right picture). (D) Expression of active β-catenin and PPARγ in spleen and VAT detected by Western blot representative of 3 independent experiments.
Supplementary Figure 2. Gating strategy. Related to Figure 3 and 5. VAT was isolated from Zbtb46-GFP\(^+\) mice and immune populations were analyzed by flow cytometry. Dot plots show the gating strategy to identify cDCs, monocytes and macrophages (A, stain 1) as well as T cell populations, neutrophils, eosinophils, NK cells, NK T cells (B, stain 2).
Supplementary Figure 3. Characterization of VAT-immune infiltrates in Ctnnb1−/− and Pparγ−/− mice on chow diet. Related to Figure 3. (A) Scheme showing experimental mice model. (B) Western blot showing lack of β-catenin and PPARγ expression in Ctnnb1−/− and Pparγ−/− mice respectively. (C) Quantitative RT PCR analysis for the gene expression in SVF from Ctnnb1−/− and WT mice in resting conditions. Expression levels of all genes were normalized against GAPDH RNA. Bars represent mRNA expression of conditional KO compared to WT mice, which was set at 1 as indicated with a dotted line. Error bars indicate the geometric mean of 5 biological replicates. (D-E) VAT was digested from lean Ctnnb1−/− (D), Pparγ−/− (E) and respective WT control mice. Total numbers of infiltrated immune cells identified as described in Supplementary Figure 2, were quantified by flow cytometry. Bars represent the mean ± SEM (n=6). (F) As in (C) but quantitative RT PCR
analysis for the gene expression in SVF from $Ppar\gamma^{/-}$ and WT mice in resting conditions. 

(G) Dot plots indicate *in vivo* AF555-OVA uptake by resident and migratory cDCs in LN.

(H) Histograms indicate *in vivo* AF555-OVA uptake by VAT-cDCs after i.v. and s.c. immunization. (I) CFSE-labeled OT-II cells were transferred i.v. one day before inoculation of 200 μg OVA plus 1 μg of LPS i.p. Two days later, OT-II cell proliferation was assessed by flow cytometry. Dot plots show CFSE dilution of OT-II cells in LN (left). Bar graphs represent the average frequency of division of proliferating OT-II T cells in LN and spleen from 3 independent experiments (n=6).
Supplementary Figure 4. Characterization of VAT-immune infiltrates in Ctnnb1−/− and Pparγ−/− mice on WD. Related to Figure 5. (A) CFSE-labeled OT-II cells were transferred i.v. one day before inoculation of 200 μg OVA i.p in Ctnnb1−/− and WT control mice. Three days later, OT-II cell proliferation was assessed by flow cytometry. Histogram shows CFSE dilution of OT-II cells in LN. Bar graphs represent the average frequency of division of proliferating OT-II T cells in LN in 2 independent experiments. Ctnnb1−/− (B-C), Pparγ−/− (E-F) and respective control mice were fed a WD for 12 weeks. Immune cells were identified as described in Supplementary Figure 2. Bar graphs represent the total numbers of immune cells per gram of VAT (B) and total cell numbers in LN (C) from Ctnnb1−/− and WT control mice. (D) In vivo OVA presentation as in (A) but in Pparγ−/− and WT control mice. (E-
F) Similar to (B-C) but immune cell numbers were quantified in \textit{Ppar γ}⁻/⁻ and WT control mice. Bars represent the mean ± SEM (n=6).
Supplementary Figure 5. Minor changes in adipocyte function and size in \textit{Ctnnb1}\textsuperscript{−/−} and \textit{Pparγ}\textsuperscript{−/−} mice on WD. Related to Figure 6. \textit{Ctnnb1}\textsuperscript{−/−} and \textit{Pparγ}\textsuperscript{−/−} and WT control.
mice were fed a WD for 12 weeks. (A) Food intake was monitored weekly. Bars represent the mean ± SEM (n=10). (B) VAT content was calculated as percentage of body weight. Graphs represent the mean ± SEM. (C) Liver weight at 12 weeks of WD. Each dot represents an individual mouse. (D) Frequency distribution of adipocyte area isolated from VAT. Frequency of cell area is expressed in number of cells per area bin using ImageJ Adiposoft software. (E-F) Representative H&E staining from VAT and liver from Ctnnb1−/− (E) and Pparγ−/− (F) and WT control mice. (G-H) Western blot analysis of Liver extracts showing phosphor-(Ser473) Akt (p-Akt) levels under control and insulin treatment. Graphs show densitometry of p-Akt/Akt ratios in arbitrary units (A.U.). *p<0.05, **p<0.01 and ***p<0.0005.
Supplementary Figure 6. WD induces activation of VAT-cDCs. Related to Figure 7.

Zbtb46-GFP⁺ mice were fed a chow or WD diet for 12 weeks. (A) Expression of CD80 and CD86 in VAT-cDC isolated from chow or WD fed mice. Histograms are representative of 3 independent experiments. (B) VAT-cDCs were purified and plated with purified allogeneic Balb/c CD4⁺ T cells (1:5) for 5 days. T cell proliferation was assessed by CFSE dilution by flow cytometry. Histograms are representative of 3 independent experiments. (C) IL-17 production by allogeneic T cells was measured in supernatant by ELISA. (D) Numbers of VAT-cDC were calculated by flow cytometry. Bars represent the mean ± SEM (n=10).
Table S1.
Related to STAR Methods Key Resource Table (Oligonucleotides section):
List of primers used in this paper

| Oligonucleotide name | Sequence (5’ to 3’) |
|----------------------|---------------------|
| IL10_forward         | AAA CAA AGG ACC AGC TGG AC |
| IL10_reverse         | TTC CGA TAA GGC TTG GCA AC |
| TNFα_forward         | TCG TAG CAA ACC ACC AAG TG |
| TNFα_reverse         | TTT GAG ATC CAT GCC GTT GG |
| IL6_forward          | TAG TCC TTC CTA CCC CAA TTT CC |
| IL6_reverse          | TTG GTC CTT AGC CAC TCC TTC |
| IL1_forward          | AAC CTG CTG GTG TGT GAC GTT C |
| IL1_reverse          | CAG CAC GAG GCT TTT TTG TTG T |
| INFγ_forward         | CAG CAA CAG CAA GGC GAA A |
| INFγ_reverse         | CTG GAC CTG TGG GTT GTT GAC |
| IL17_forward         | AAA GCT CAG CGT GTG TAC AC |
| IL17_reverse         | TTC TGG AGC CTA CTT TGG CG |
| CCL2_forward         | GCT GGA GCA TCC ACG TGT T |
| CCL2_reverse         | ATC TTG CTG GTG AAT GAG TAG CA |
| CCL17_forward        | GGA TGC CAT CGT GTT TCT GA |
| CCL17_reverse        | GCC TTC TTC ACA TGT TTG TCT TG |
| Apidoq_forward       | GAC GTT ACT ACA ACT GAA GAG C |
| Apidoq_reverse       | CAT TCT TTT CCT GAT ACT GCT C |
| Leptin_forward       | TGA GTT TGT CCA AGA TGG ACC |
| Leptin_reverse       | GCC ATC CAG GCT CTC TGG |
| GAPDH_forward        | GGC TCA TGA CCA CAG TCC A |
| GAPDH_reverse        | CAC ATT GGG GTG AGG AAC AC |
| TGFβ_forward         | CCCGAAGCGGACTACTATGC |
| TGFβ_reverse         | ATAGATGGCGGTGTTGCGGCT |
| WNT1_forward         | ATT TTG CGC TGT GAC CTC TT |
| WNT1_reverse         | AGC AAC CTG CTT TCC CAC TT |
| WNT2_forward         | GGT CAG CTC TTC ATG GTG GT |
| WNT2_reverse         | GGA ACT GTT GTC GGC ACT CT |
| WNT3a_forward        | TCG GAG ATG GTG GTA GAG AAA |
| WNT3a_reverse        | CGC AGA AGT TGG GTG AGG |
| WNT5b_forward        | TCT CCG CCT AAC AAG ATG CT |
| WNT5b_reverse        | CAG CAC CTC TGC CAA GCA |
| WNT6_forward         | GCG GAG AGC ATG TGG ACT TC |
| WNT6_reverse         | ATG CAC GGA TAT CTC CAC GC |
| WNT7a_forward        | GAC AAA TAC AAC GAG GCC GT |
| WNT7a_reverse        | GGC TGT CTT ATT GCA GGC TC |
| WNT8b_forward        | CCA GAG TCC CGG GAG GTA G |
| WNT8b_reverse        | GAG ATG GAG CGG AAG GTG T |
| WNT9a_forward        | GGA CAA CCT CAA GTA CAG CAG |
| WNT9a_reverse        | TCC ACT CCA GCC TTT ATC ACC |
| WNT9b_forward        | ACA GCA CCA AGT TCC TCA GC |
| WNT9b_reverse        | CTT GCA GGT TGT TCT CAG GC |
| WNT10a_forward       | CCA CTC CGA CCT GGT CTA CTT TG |
| Gene       | Primer Direction | Sequence                          |
|------------|------------------|-----------------------------------|
| WNT10a_reverse | Forward          | TGC TGC TCT TAT TGC ACA GGC       |
| WNT10b Forward  | Reverse          | AAT GCG GAT CCA CAA CAA CA       |
| WNT10b_reverse | Forward          | TTC CAT GGC ATT TGC ACT TC       |
| WNT11 Forward   | Reverse          | TGC TTG ACC TGG AGA GAG GT       |
| WNT11_reverse  | Forward          | AGC CCG TAG CTG AGG TTG T        |
| Fabp4 Forward    | Reverse          | ATGTGCAGACCAGTTTGTG               |
| Fabp4_reverse   | Forward          | TTTGCCATCCACCTTCTG                |
| Fabp5 Forward    | Reverse          | TGAAGGAGCTAGGAGTGCGGA            |
| Fabp5_reverse   | Forward          | TGCACCATCTGTAAAGTTGCA            |
| Dgat1 Forward    | Reverse          | GGCCTTCTTCCACGAGTACC             |
| Dgat1_reverse   | Forward          | GCCCTCATAGTGAGCAGCAG             |
| CD36 Forward     | Reverse          | TCTTTCCCTGCAAGCCCAATG            |
| CD36_reverse    | Forward          | AGCCTCTGTCTCAACTGATAGTGA         |