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

Graphical Abstract

Highlights

- VAT-cDCs acquire a tolerogenic phenotype by upregulating adipocyte-related pathways
- Activation of β-catenin and PPARγ in cDC subsets promotes anti-inflammatory VAT
- Combined, they delay the onset of obesity-induced inflammation and insulin resistance
- Chronic over-nutrition curtails β-catenin and PPARγ pathways, fueling cDC activation

In Brief

Macdougall et al. report key mechanisms that control the immune function of conventional dendritic cells in visceral adipose tissue. The upregulation of adipocyte-related pathways in conventional dendritic cells promotes an anti-inflammatory phenotype in visceral adipose tissue under homeostatic conditions and delays the onset of obesity-induced inflammation and insulin resistance.

Data Resources

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Visceral adipose tissue immune homeostasis is regulated by the crosstalk between adipocytes and dendritic cell subsets

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SUMMARY

Visceral adipose tissue (VAT) has multiple roles in orchestrating whole-body energy homeostasis. In addition, VAT is now considered an immune site harboring an array of innate and adaptive immune cells with a direct role in immune surveillance and host defense. We report that conventional dendritic cells (cDCs) in VAT acquire a tolerogenic phenotype through upregulation of pathways involved in adipocyte differentiation. While activation of the Wnt/β-catenin pathway in cDC1 DCs induces IL-10 production, upregulation of the PPARγ pathway in cDC2 DCs directly suppresses their activation. Combined, they promote an anti-inflammatory milieu in vivo delaying the onset of obesity-induced chronic inflammation and insulin resistance. Under long-term over-nutrition, changes in adipocyte biology curtail β-catenin and PPARγ activation, contributing to VAT inflammation.

INTRODUCTION

Visceral adipose tissue (VAT) is a highly active metabolic and endocrine organ that secretes cytokines and bioactive mediators such as adiponectin, which influences not only body weight homeostasis, but also insulin sensitivity, inflammation, and lipid metabolism. VAT can respond rapidly and dynamically to alterations in nutrient intake through adipocyte expansion (hypertrophy) and adipogenesis (hyperplasia) buffering most of the excess or deficit of energy. In mice, during the early stage of high-fat diet (HFD) exposure, VAT expands at the expense of adipocyte hypertrophy and it is not until after 8 weeks that adipocyte hyperplasia can be observed (Wang et al., 2013b). The expansion and renewal of adipocytes are tightly regulated at the transcriptional level. In mature adipocytes, peroxisome proliferator-activated receptor-γ (PPARγ) regulates lipid accumulation during hypertrophy (Wang et al., 2013a), while the Wnt/β-catenin pathway controls adipocyte hyperplasia (Christodoulides et al., 2009).

VAT contains immune cells that work in cooperation with adipocytes to maintain the overall metabolism and physiology of the organ. Under homeostatic conditions, resident immune cells such as macrophages and regulatory T cells (Tregs) produce anti-inflammatory cytokines that limit inflammation and maintain glucose homeostasis (Cipolletta et al., 2012). In addition, macrophages are present in high numbers in VAT and fulfill housekeeping functions by removing excess lipids and dead adipocytes (Chawla et al., 2011). Under chronic over-nutrition, VAT expansion creates an environmental milieu that promotes low-grade inflammation with local production of IL-6, TNFa, and IL-1, and the influx of pro-inflammatory cells (Schipper et al., 2012). The immune regulatory network in VAT is disrupted with a decrease in numbers of Tregs and eosinophils and increased recruitment of activated T cells, IFNγ-producing natural killer (NK) cells, and inflammatory macrophages (Schipper et al., 2012). This meta-inflammation has been implicated as an important etiological factor in the development of insulin resistance and vascular complications.

The presence of conventional dendritic cells (cDCs) in VAT has been suggested in numerous studies, but their role in VAT inflammation and metabolic homeostasis remains elusive. cDCs are usually defined as CD11c+ MHCII+ expressing cells. However, the surface marker CD11c has been used to identify inflammatory VAT-infiltrated macrophages (Patsouris et al., 2008). This phenotypic overlap has made it difficult to accurately characterize and study cDCs in VAT. Under prolonged over-nutrition, MHCII-CD11c+ cells have been shown to process and present antigens to T cells and to induce Th17 responses. Depletion of all CD11c+ cells (including CD11c+ monocytes/macrophages) resulted in a rapid normalization of insulin sensitivity and decrease of pro-inflammatory cytokines in obese mice (Gertola et al., 2012; Patsouris et al., 2008; Stefanovic-Racic et al., 2012).
Interestingly, following gene set enrichment analysis (GSEA) this “division of labor” could also apply to VAT resident cDCs. and overlapping functions (Merad et al., 2013). We asked if that, unlike in the spleen, approximately 40% of all CD11chi cells control adipocyte expansion. Activation of the Wnt/β-catenin pathway in cDC1 DC subset induces the production of IL-10, while expression of PPARγ in cDC2 subset suppresses the onset of local inflammatory responses. Deletion of β-catenin and PPARγ in cDCs increases VAT-cDC maturation and increases T cell recruitment in lean and obese conditions. The absence of these regulatory pathways accelerates obesity-induced chronic inflammation and insulin resistance. Interestingly, under persistent over-nutrition, adipocyte hyperplasia decreases the availability of β-catenin ligands for cDC1, while expression of PPARγ in cDC2 is reduced, suggesting a possible mechanism for the pro-inflammatory switch of VAT-cDCs in obesity.

RESULTS

**cDC1 and cDC2 DC Subsets Differentially Upregulate the β-Catenin and PPARγ Pathways in VAT**

The promiscuous expression of the markers MHCII and CD11c within phagocytic cell populations in tissues has hampered the study of cDCs in VAT. To understand the distinct contribution of dendritic cells in vivo, new reporter mice have recently been generated that are based on the expression of the highly cDC-specific Zbtb46 promoter (Zbtb46-GFP and Zbtb46-Cre) (Loschko et al., 2016). By using the Zbtb46-GFP mice, which is expressed on cDCs and the MHCII− pre-cDCs, we observed that, unlike in the spleen, approximately 40% of all CD11c+ MHCII+ cells are cDCs (Figures 1A and 1B). The remaining cells are characterized by high expression of CD16/32 and CD11b and negative expression of MerTK and CD64 (Figures S1A and S1B). CD16/32 can be expressed by monocytes and monocyte-derived DCs (mo-DCs) (Menezes et al., 2016). However, they are negative for the mo-DC marker CD206 (Figure S1B), suggesting that they may be monocytes with low CD64 expression. Further future characterization is required, which is beyond the scope of this paper. In concordance with previous reports, Zbtb46-GFP+ cDCs are strategically located close to blood and lymphatic vessels, consistent with their proposed role in antigen sampling (Figure S1C) (Kuan et al., 2015).

CDCs are present in two main subsets, cDC1 (CD103+) and cDC2 (CD11b+), which have been shown to have both distinct and overlapping functions (Merad et al., 2013). We asked if this “division of labor” could also apply to VAT resident cDCs. Interestingly, following gene set enrichment analysis (GSEA) pathway analysis we found that the Wnt and PPARγ pathways, which are important for the regulation of adipocyte differentiation and expansion, were selectively upregulated in cDC1 and cDC2, respectively (Figures 1C and 1D) (Christodoulides et al., 2009; Farmer, 2006). The protein expression of active β-catenin was confirmed in Zbtb46-GFP+ sorted VAT-cDC1 by western blotting, while PPARγ protein expression was observed in Zbtb46-GFP+ VAT-cDC2 (Figure 1E). Interestingly, despite similar gene expression of Tnnt1, Fzd1, and Tcf7l2, stabilization of β-catenin in splenic cDCs could not be detected (Figure S1D). This suggests that these cDCs upregulate adipocyte-relevant pathways in order to “sense” changes in tissue homeostasis.

Wnt/β-catenin signaling in cDCs has been found to limit inflammatory responses in the intestine, to induce Tregs, and to control Th17 responses (Manicassamy et al., 2010; Suryawanshi et al., 2015). Similarly, PPARγ is known to mediate anti-inflammatory effects of several immune cell types (Ahmadian et al., 2013) and to induce CD4 anergy in bone marrow-derived cDCs (Klotz et al., 2007). To confirm the anti-inflammatory effect of both pathways, we stimulated Zbtb46-GFP+ sorted VAT-cDC1 and cDC2 with a TLR4 agonist (GLA) in the presence or absence of the β-catenin pathway activator SB216763 (SB) or the PPARγ agonist Rosiglitazone (RGZ). In line with previous reports, activation of both pathways suppressed inflammatory responses. However, in concordance with our GSEA results, SB preferentially reduced IL-6 production in cDC1 subset, while RGZ had a significant effect on cDC2 (Figure 2A). A reduction of IL-6 production in cDC2 could be observed after SB treatment that could be a result of residual β-catenin expression in those cells or a non-specific effect of the drug as it did not induce IL-10 production. Interestingly, β-catenin activation significantly enhanced the production of IL-10 from cDC1 from VAT, but not spleen (Figure 2B).

Previous research has established Wnt/β-catenin signaling as an important regulator of adipogenesis. Indeed, local production of the β-catenin agonist WNT10B is known to inhibit pre-adipocyte differentiation (Ross et al., 2000). We confirmed that VAT cells produce WNT10B by ELISA (Figure 2C). Furthermore, recombinant WNT10B was sufficient to induce IL-10 and to suppress GLA-induced IL-6 production by VAT-cDC1 at similar levels to SB (Figures 2D–2F). PPARγ can be activated by dietary lipids, resulting in adipocyte-lipid storage (Wang et al., 2013a). Therefore, both pathways are constitutively activated during regular chow-fed diet in adipocytes/pre-adipocytes but also in tissue-resident cDCs. On this basis, we hypothesized that activation of the Wnt/β-catenin and PPARγ pathway in cDC1 and cDC2, respectively, sustains a tolerogenic phenotype in VAT-DCs and contributes to the maintenance of tissue immune-homeostasis.

**Zbtb46-Cre-Driven Loss of β-Catenin Results in Decreased IL-10 Levels in VAT In Vivo**

To directly assess the role of these pathways in vivo, we crossed floxed β-catenin (Ctnnb1fl/fl) or PPARγ (Pparγfl/fl) mice with transgenic Zbtb46-bre (zDC-cre) mice. This abrogates gene expression specifically in cDCs (Loschko et al., 2016). Unexpectedly, β-catenin deletion was lethal due to Zbtb46+ off-target expression in non-hematopoietic cells. To bypass this issue, our...
we generated chimera mice using bone marrows and fetal liver cells from conditional cDC-specific Pparγ and Ctnnb1 knock-down, respectively, to keep experimental settings comparable (chimeric deleted animals are henceforth referred to as Ctnnb1−/− or Pparγ−/−) (Figure S3A). In this model, difference between groups can only be attributed to cDC as zDC-cre is exclusively expressed in cDCs. β-catenin and Pparγ deletion was confirmed by western blotting (Figure S3B). First, we determined if β-catenin and PPARγ signaling pathways are critical for VAT immune homeostasis in steady state. RT-PCR analysis from lean VAT showed significant decrease of IL-10 levels in Ctnnb1−/− compared to wild-type (WT) mice, which reached 10-fold decrease in the stromal vascular fraction (SVF) where immune cells are located (Figures 3A and S3C). IL-17 was significant elevated in the SVF (S3C). This was accompanied by a significant decrease of Tregs and a slightly increase, albeit non-significant, of CD4+ T cell recruitment to VAT (Figures 3B, S2, and S3D). There were no significant differences in IL-10 levels and T cell recruitment between Pparγ−/− and WT mice (Figures 3C, 3D, S3E, and S3F). We hypothesized that this could be the result of two distinct anti-inflammatory mechanisms. While β-catenin prevents the onset of inflammation through IL-10 production, PPARγ exerts its anti-inflammatory effect by interfering with transcriptional regulation of ensuing inflammatory responses, including NF-κB signaling (Pascual et al., 2005; Ricote et al., 1998). Thus, in the absence of inflammation, the effect of PPARγ is not evident.

While immune cells play a key role in the maintenance of a systemic anti-inflammatory state in lean VAT, it has now become evident that they can also participate in local immune responses.
Indeed, phagocytic cells in VAT or associated immune clusters can collect fluids, pathogens, and cells from the peritoneal cavity and present them to T cells, while cDCs can sample antigens traveling in the collecting lymphatic (Cruz-Migoni and Caamaño, 2016; Kuan et al., 2015). To assess the consequences of β-catenin and PPARγ deletion in VAT-cDC antigen presentation capacity, we immunized mice intraperitoneally with ovalbumin (OVA) after OT-II cell transfer. OVA uptake by phagocytic cells was confirmed by fluorescence microscopy in VAT and flow cytometry in VAT and dLN (Figures 3E, 3F, and S3G). As expected due to their high phagocytic capacity, macrophages have increased OVA uptake (high mean fluorescence). However, as confirmed by many recent studies (Schreiber et al., 2013), macrophages have poor antigen presentation function and failed to induce OT-II proliferation (Figure 3F). OVA uptake could also be confirmed after subcutaneous (s.c.) and intravenous (i.v.) immunization (S3H). Consistent with an anti-inflammatory role of β-catenin signaling in the VAT, only Ctnnb1−/− DCs exhibited significantly increased antigen-specific T cell activation, as evidenced by increased OT-II cell division cycles (Figure 3G). This difference was only observed in VAT, but not in LN or spleen (data not shown). Although PPARγ activation in steady-state conditions may not significantly affect VAT immune homeostasis, it may be required to suppress inflammatory responses as observed in vitro (Figure 2A). To test this hypothesis, we immunized mice with OVA in the presence of LPS as adjuvant. As expected, the number of dividing OT-II cells and the amount of divisions were increased in Ctnnb1−/− mice compared to WT mice, but this time the same effect was observed in Pparγ−/− mice in VAT (Figure 3H). Enhanced T cell proliferation was also observed in mesenteric LN (Figure S3I), consistent with a previous report describing increased VAT-DC migration to LN upon inflammation (Kuan et al., 2015). Collectively, these data suggest that, in steady state, β-catenin activation in VAT-cDC1...
is required for IL-10 production, recruitment of Tregs, and the maintenance of an anti-inflammatory state. In addition, IL-10 is known to suppress cDC maturation (Corinti et al., 2001; De Smedt et al., 1997). In contrast, PPARγ stimulation in cDC2 seems to limit VAT-cDC activation under an inflammatory stimulus.
VAT inflammation has been associated with adipocyte dysfunction, increasing the risk of systemic lipid and glucose metabolic alterations. Despite the signs of reduced VAT anti-inflammatory status in lean conditions, this did not translate into changes in metabolic parameters such as serum insulin and adiponectin levels, weight gain (Figures 4A–4D), or increased insulin resistance as measured by glucose (GTT) and insulin (ITT) tolerance tests (Figures 4E and 4F). This was not surprising since in steady state, VAT inflammation is minimal, local, and easily restrained by the resident anti-inflammatory immune network.

Figure 4. Ctnnb1−/− and Pparγ−/− Mice Showed No Statistical Significance in Metabolic Parameters on Chow Diet
Ctnnb1−/− and Pparγ−/− mice were fed a chow diet.

(A) Graph shows representative body weights from three independent experiments.
(B) VAT content was calculated as percentage of body weight. Graphs represent the mean ± SEM and are representative of three independent experiments.
(C and D) Fasting insulin (C) and adiponectin (D) were detected by ELISA. Bars represent the mean ± SEM and are representative of three independent experiments. Statistical analysis was performed with Student’s test.
(E and F) Whole-body glucose homeostasis measured in (E) Ctnnb1−/−, (F) Pparγ−/−, and WT mice on chow diet by i.p. glucose tolerance tests (GTTs; n = 10) and insulin tolerance tests (ITTs; n = 10). Statistical significance at different time points analyzed by two-way ANOVA with Bonferroni’s post-test, *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.
Figure 5. Deletion of β-Catenin and Pparγ on cDCs Stimulates Pro-inflammatory Responses in VAT upon WD

Mice were fed a WD for 12 weeks to induce VAT low-grade chronic inflammation.

(A) qRT-PCR analysis for the gene expression in VAT and SVF from Ctnnb1−/− and WT control mice on WD (12 weeks). Expression levels of all genes were normalized against GAPDH RNA. Bars represent mRNA expression on Ctnnb1−/− compared to WT mice, which was set as 1 as indicated with dotted line. Error bars indicate the geometric mean of ten biological replicates for VAT and five for SVF.

(B) In vivo antigen presentation was assessed after CFSE-labeled OT-II transfer and immunization with 200 μg OVA intraperitoneally. Three days later, cell division and total number of proliferating OT-II T cells were analyzed in VAT from Ctnnb1−/− and WT mice by flow cytometry. In histogram, samples in gray represent undivided/non-immunized cells. CFSE dilution was analyzed on gated CD45−CD3−CD4+TCRvα2+ CFSE+ cells.

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cDC-Specific β-Catenin and PPARγ Deletions Exacerbate Obesity-Induced VAT Inflammation and Insulin Resistance

Our data support a complementary role of cDC1 and cDC2 subsets in the regulation of local VAT homeostasis. A great deal of evidence suggests that obesity is associated with low-grade chronic inflammation and that inflammatory responses in VAT play a causal role in obesity-induced insulin resistance in mice and humans (Donath et al., 2013). It is, therefore, tempting to envisage that the anti-inflammatory properties of VAT-DCs may delay the onset of inflammation during chronic over-nutrition. To ascertain the role of β-catenin and PPARγ in cDCs in the control of obesity-induced VAT inflammation, Ctnnb1<sub>−/−</sub> or Pparγ<sub>−/−</sub> mice were fed a Western diet (WD) for 12 weeks, as well as respective control WT mice. A 10-fold decrease in IL-10 RNA levels in Ctnnb1<sub>−/−</sub> mice was accompanied by significant increase in the T cell chemoattractant CCL17 production and a switch to IL-17 T cell responses in whole VAT as well as the SVF (Figure 5A). In addition, the RNA expression of adiponectin, which is expressed by adipocytes, was reduced, suggesting some degree of adipocyte dysfunction (Figure 5A). Similar to LPS acute inflammation, in vivo OT-II proliferation was enhanced in VAT and dLN from Ctnnb1<sub>−/−</sub> compared to WT mice (Figures 5B and S4A). This effect appears to be mediated by a change in cDC activation status since Ctnnb1<sub>−/−</sub> cDCs isolated from the VAT displayed enhanced activation and stimulatory capacity in a mixed-leukocyte reaction (MLR), demonstrating an induction of Th17 T cell responses and reduction of Tregs (Figures 5D and 5E) compared to WT cDCs. Total numbers of VAT-infiltrating CD4<sup>+</sup> T cells were slightly increased, albeit not significant, while the numbers of Tregs were significantly reduced (Figure 5E). Accordingly, cDC-derived CCL17 has been shown to promote tissue inflammation by restricting Treg recruitment (Webber et al., 2011). We observed no differences in immune cell composition in dLNs and spleen with the exception of increased numbers of migratory cDCs in dLNs (Figure S4C). Interestingly, Pparγ<sub>−/−</sub> mice that failed to show differences during steady-state conditions when fed a chow diet displayed a significant increase in T cell-mediated VAT inflammation as evidenced by elevated CCL17 and IL-17 and reduced IL-10 and IFNγ RNA levels compared to WT mice (Figure 5F). Similar to Ctnnb1<sub>−/−</sub>, Pparγ<sub>−/−</sub> cDCs showed in vivo increased OVA antigen presentation in VAT and dLN (Figures 5G and S4D) as well as ex vivo enhanced stimulatory capacity (MLR) with higher induction of IL-17 CD4<sup>+</sup> T cell responses, reduced Treg recruitment (Figures 5H–5J), and increased migration to dLNs (Figure S4D). In this case, we could also detect a significant increase in VAT-infiltrated neutrophils, also evident on chow diet (Figures S4E and S3E), and a decrease in eosinophils, suggesting a more general pro-inflammatory response in the VAT of Pparγ<sub>−/−</sub> compared to WT mice (Figure S4E).

Based on these observations, we postulated that the increased inflammatory T cell response in VAT of mutant mice could influence whole-body glucose metabolism. β-catenin and PPARγ deletions in cDCs did not affect weight gain, VAT content, or food intake (Figures 6A, S5A, and S5B). No changes were observed in leptin levels, but adiponectin was reduced in Ctnnb1<sub>−/−</sub> compared to WT mice, which confirmed RT-PCR data (Figures 6B and 6C). Liver weight, adipocyte size, and fat deposition in the liver remained unchanged (Figures S5C–S5F). However, cDC-specific Ctnnb1<sub>−/−</sub> and Pparγ<sub>−/−</sub> mice displayed elevated serum insulin levels compared to WT mice (Figure 6D). In addition, GTTs and ITTs revealed that these mice were significantly less glucose tolerant and more insulin resistant than controls (Figures 6E–6H). In addition, insulin-stimulated AKT phosphorylation was markedly reduced in deficient mice compared to controls in VAT and liver (Figures 6I, 6J, S5G, and S5H). Collectively, these results demonstrate that cDC-specific deletion of β-catenin and PPARγ enhances local inflammatory responses and aggravates obesity-induced insulin resistance.

VAT Expansion and Inflammation during Long-Term Over-nutrition Curtail β-Catenin and PPARγ Activation in cDC1 and cDC2, Respectively

During obesity, there is evidence to suggest that cDCs adopt an activated phenotype contributing to VAT inflammation. Indeed, chronic over-nutrition promotes cDC recruitment into VAT and increases cDC activation, resulting in enhanced T cell stimulatory capacity and Th17 responses (Figures S6A–S6D). This can be the consequence of (1) overwhelming inflammation that over-rides suppressive pathways, (2) inhibition of these pathways in obesity, or (3) both. Current literature strongly supports the last hypothesis. WNT10B levels are suppressed in obesity, allowing adipocyte hyperplasia (Ross et al., 2000). In parallel, upregulation of the protein kinase cyclin-dependent kinase 5 in obesity promotes PPARγ post-transcriptional modifications and inactivation in vivo (Choi et al., 2010; Cipolletta et al., 2015). This phenomenon can be replicated with TNFα treatment in vitro, providing evidence for an inflammatory origin. Therefore, we asked if these regulatory pathways remain active under chronic over-nutrition. Significant reduction of WNT10B could be confirmed at the transcriptional and protein level (Figures 7A and 7B). WNT10B expression was only marginally reduced in Ctnnb1<sub>−/−</sub> and Pparγ<sub>−/−</sub> mice, suggesting that it is largely linked to adipocyte differentiation rather than inflammation itself (Figure 7C). As a result, β-catenin activation in vivo was reduced (Figure 7D). However, total expression of β-catenin was unaltered (data not shown) and cells were still able to respond to the β-catenin pathway activator SB<sub>ex vivo</sub>, albeit at lower levels, reflecting the activated state of cDCs in VAT (Figures 7E and 7F). Simultaneously, we observed reduced expression of PPARγ on VAT-cDC2 from obese mice (Figure 7G). Accordingly, cDC2 from obese VAT treated with RZG in vitro failed to upregulate
Figure 6. Deletion of β-Catenin and Pparγ on cDCs Alters Glucose Homeostasis
(A) Body weight of Ctnnb1−/−, Pparγ−/−, and WT mice fed WD for 12 weeks.
(B–D) Serum leptin (B), adiponectin (C), and insulin (D) values from fasted mice representative of two independent experiments (n = 10). Statistical analysis was performed with Student’s test.

(E–H) Systemic glucose homeostasis was measured by ITT (E and G) and GTT (F and H) in Ctnnb1−/− (E), Pparγ−/− (G), and WT control mice fed a WD. Similarly, GTT (F and H) was assessed in Ctnnb1−/− (F) and Pparγ−/− (H) compared to control mice. Statistical significance at different time points was analyzed by two-way ANOVA with Bonferroni’s post-test (n = 10).

(I and J) VAT Akt activation followed acute insulin injection. Western blot analysis of VAT extracts showing phosphor-(Ser473) Akt (p-Akt) levels under control and insulin treatment in Ctnnb1−/− (I) and Pparγ−/− (J) compared to WT mice. Graphs show densitometry of p-Akt/Akt ratios. *p < 0.05, **p < 0.01, and ***p < 0.0005.

See also Figure S5.
Figure 7. Chronic Over-nutrition Reduces Wnt/β-Catenin and Pparγ Pathway Activation in VAT-cDCs
Zbtb46-GFP+ mice were fed a chow or WD for 12 weeks.

(A) mRNA expression of Wnt proteins was analyzed by RT-PCR in mice on WD compared to chow diet (12 weeks). Error bars indicate the geometric mean of five biological replicates.

(B) Wnt10b protein levels were detected in VAT homogenates (n = 3).

(C) mRNA expression of Wnt10b in Ctnnb1−/− and Pparγ−/− mice (n = 10).

(D) Western blot analysis of active β-catenin in purified CD103+ Zbtb46-GFP+ cDCs from chow- and WD-fed mice.

(E and F) cDC purified from spleen and VAT from Zbtb46-GFP mice were incubated overnight with 5 μg GLA in the presence of β-catenin agonist (SB) or DMSO as control. IL-6 (E) and IL-10 (F) production was measured in supernatants. Bars represent mean ± SD and are representative of two independent experiments (n = 6).

(G) Western blot analysis of PPARγ in purified CD11b+ Zbtb46-GFP+ cDCs from chow- and WD-fed mice.

(H and I) VAT-cDCs were purified from chow- and WD-fed Zbtb46-GFP+ mice. Cells were cultured overnight with PPARγ agonist (RGZ), and CD36 expression was evaluated by flow cytometry (H). Histograms are representative of three independent experiments (H) (n = 3) and mRNA expression of CD36 and other downstream genes (I) (n = 5).

(legend continued on next page)
the PPARγ-inducible CD36 and the gene expression of other downstream genes (Figures 7H and 7I), resulting in reduced anti-inflammatory properties as shown by IL-6 and IL-12 production (Figures 7J and 7K). The reduced response to RZG can be solely due to downregulation of PPARγ or to recruitment of newcomer cells. Several reports describe an inhibition of PPARγ signaling in vivo (Choi et al., 2010). However, decreased expression of PPARγ was recently observed in adipocytes exposed to free fatty acids (Nguyen et al., 2012). Overall, our findings suggest that chronic over-nutrition partially abrogates the β-catenin and PPARγ anti-inflammatory pathways fueling cDC activation and VAT T cell-mediated inflammation in vivo.

**DISCUSSION**

cDC function can be dictated by the tissue microenvironment. In this work, we have shown that in order to recognize, integrate, and respond to environmental signals, cDCs must first upregulate pathways relevant for the control of tissue-local homeostasis. In VAT, cDCs are poised to suppress inflammation by activating the β-catenin and PPARγ pathways, which are important regulatory mechanisms for fat expansion. Interestingly, tissue adaptation was cDC subset specific.

The Wnt/β-catenin pathway is evolutionarily conserved and plays a prominent role in cell differentiation, growth, proliferation, survival, and immune cell function (Komiya and Habas, 2008). β-catenin is continuously synthesized but rapidly degraded in the proteasome. The binding of Wnt glycoproteins to Frizzled (Fzd) family receptors in the cell membrane results in the stabilization of β-catenin in the cytoplasm and further translocation into the nucleus, where activation of T cell factor/lymphoid enhancer factor (TCF/LEF) and regulation of gene transcription occur. Several Wnt proteins and Fzd receptors have been described. In VAT, Wnt signaling regulates adipocyte differentiation. Expression of WNT10B is highest in pre-adipocytes and rapidly declines after differentiation. Overexpression of WNT10B stabilizes β-catenin and blocks adipocyte hyperplasia through TCF7/2 activation in vivo and in vitro (Ross et al., 2000). Similar to cDCs, pre-adipocytes are also located close to adipose vessels, suggesting a close interaction and crosstalk (Tang et al., 2008). Interestingly, VAT-cDC1 showed higher expression of one of the WNT10B receptors, Fzd1, and the transcription factor Tcf7l2. Activation of β-catenin in cDC1 by WNT10B in the fat induces a tolerogenic phenotype with high production of the anti-inflammatory cytokine IL-10. Conversely, PPARγ is a member of the nuclear hormone receptor superfamily of transcription factors. PPARγ forms a heterodimer with RXR (retinoic X receptor) and subsequently regulates the transcription of cognate target genes involved in lipid and glucose metabolism. PPARγ is highly expressed in VAT, where it is a master regulator of adipogenesis. In mature adipocytes, PPARγ regulates lipid accumulation and hypertrophy (Wang et al., 2013a). We found that PPARγ expression was limited to cDC2 and that its activation repressed inflammatory responses. Several studies have shown an opposite interplay between PPARγ and Wnt/β-catenin signaling within the same cell (Liu et al., 2006). Activation of β-catenin leads to PPARγ repression. Reciprocally, PPARγ inhibits the transcription of β-catenin (Liu et al., 2006). In this context, this differential pathway expression between subsets allows cDCs to simultaneously recognize and respond to both signals in VAT.

The anti-inflammatory role of the Wnt/β-catenin and the PPARγ pathways has been extensively documented in vitro and in vivo. Indeed, the pathways can protect mice from DSS-induced colitis and obesity-induced insulin resistance, and reduce inflammatory responses during bacterial infections (Gautier et al., 2012; Silva-García et al., 2014). Less is known about their relevance for the control of tissue homeostasis. Here we demonstrate that constitutive activation of β-catenin in VAT-cDC1 promotes an anti-inflammatory milieu under resting conditions. Indeed, the absence of β-catenin in cDCs was sufficient to reduce IL-10 levels and Treg recruitment to VAT rendering the tissue more susceptible to inflammation. By contrast, deletion of PPARγ in Zbtb46-Cre × PPARγflox/flox mice showed minimal changes in VAT inflammation with some evidence of increased T cell and neutrophil infiltrates, albeit not significant. A possible explanation lies in their different mechanism of action. β-catenin activation triggers PI3K/Akt, which in turns induces IL-10 production (Fu et al., 2015; Manoharan et al., 2014). In contrast, PPARγ can negatively regulate inflammatory gene expression through a mechanism termed transrepression. This pathway operates by preventing the clearance of the repressor complex located on the promoter of inflammatory genes such as NF-κB and AP-1 (Glass and Ogawa, 2006). Thus, PPARγ acts as a transcriptional repressor of canonical NF-κB target genes, which are activated upon TLR stimulation, but not in resting conditions (Baratín et al., 2015). Indeed, we observed that IL-6 and T cell stimulatory capacity was elevated in PPARγ−/− cDC2 upon TLR4 stimulation.

Chronic VAT inflammation is a common future of obesity and metabolic syndrome. As individual adipocytes enlarge with excess of lipids, they undergo molecular and cellular alterations, resulting in mitochondrial dysfunction, hypoxia, oxidative stress, and eventually cell death. This creates a first local and then systemic sustained inflammatory response altering whole-body glucose homeostasis in mice and men (Donath and Shoelster, 2011; Okin and Medzhitov, 2016). In this context, activation of the Wnt/β-catenin and PPARγ pathways in cDC1 and cDC2, respectively, restrains VAT-T cell recruitment in an attempt to limit the local inflammatory process. Indeed, mice with cDC-specific deletion of β-catenin or PPARγ show increased VAT inflammation associated with altered T cell responses, as evidenced by increased CCL17 and IL-17 and decreased IL-10, IFNγ, and Treg recruitment in VAT from obese mice. Elevated IL-17 production in knockout mice is most likely a consequence of cDC activation as shown by CD11c+ MHCIId isolated from obese mice. The observed T cell-mediated phenotype, instead of the

(J and K) As described in (E) and (F) but cells were cultured with PPARγ agonist overnight and IL-6 (J) and IL-12 (K) were measured in supernatants. Bars represent mean ± SEM and are representative of two independent experiments. Statistical analysis was performed with Student’s test, *p < 0.05, **p < 0.01, and ***p < 0.0005.

See also Figure S6.
innate pro-inflammatory profile in VAT reported upon monocyte/macrophage manipulation, is likely a consequence of the cDC-specific models used in this study, confirming the relevance of cDCs in the control of T cell responses in vivo (Odegaard et al., 2007). Increased cumulative inflammation in deficient mice resulted in increased systemic insulin resistance. Changes in adipocyte size and ectopic lipid deposition in liver were minimal; however, Ctnnb1−/− mice exhibited reduced VAT and circulating adiponectin, which has anti-inflammatory and cardioprotective functions (den Ruijter et al., 2014). A link between β-catenin signaling and insulin resistance has been previously described. Variants of the TCF7/2 gene are linked to increased susceptibility for type 2 diabetes (Grant et al., 2006), while a SNP in the human WNT10b gene has been associated with early-onset familial obesity (Christodoulides et al., 2006). More compellingly, transgenic mice overexpressing WNT10B in adipocytes resist HFD-induced adipose tissue accumulation (Wright et al., 2007). Paradoxically, despite impaired adipogenesis, WNT10B-overexpressing mice are more glucose tolerant and insulin sensitive, effects attributed partially to reduced VAT inflammation (Wright et al., 2007). Our data suggest an additional mechanism in which activation of β-catenin by WNT10B in cDC1 can contribute to insulin sensitivity in FABP4-WNT10B mice. Similarly, PPARγ agonists, often referred to as an “insulin sensitizer,” are potent oral anti-diabetic drugs. The role of PPARγ in VAT immune function is better known. Macrophage expression of PPARγ is required for their polarization toward an anti-inflammatory phenotype, and mice deficient in PPARγ in their macrophage population are more prone to whole-body insulin resistance (Odegaard et al., 2007). In addition, PPARγ activation in Tregs promotes their accumulation in VAT and protection from obesity-induced insulin resistance (Cipolletta et al., 2012). We are now proposing a third immune cell type that responds to PPARγ ligands to reduce VAT inflammation.

Long-term over-nutrition induces profound changes in adipocyte biology that ultimately affect β-catenin and PPARγ signaling. In mice, adipocyte hyperplasia can be observed after 8 weeks of HFD, which translates into reduced WNT10B production by pre-adipocytes. In parallel, PPARγ signaling is impaired due to post-transcriptional modifications (Choi et al., 2010; Cipolletta et al., 2015). Consistent with this observation, we found that activation of β-catenin in cDC1 was partially reduced while PPARγ expression in cDC2 was decreased. The infiltration of PPARγ low cDC2 cannot be ruled out. In vivo PPARγ post-transcriptional modification in cDC2 from obese mice requires further investigation. Thus, in the long term, reduction of β-catenin and PPARγ signaling in cDC1 and cDC2, respectively, may explain the inflammatory phenotype observed in VAT-cDCs from obese mice and men (Bertola et al., 2012).

The full implications of our findings are yet to be unraveled. In addition to its metabolic role, VAT is believed to support organ and immune cell functions. Nearly all internal organs and large LN in mammals are embedded in firmly attached adipose tissue. This VAT provides protective “padding” against damage but also serves for the paracrine release of fatty acids and immune mediators for proper tissue function (Iacobellis and Bianco, 2011; Pond and Mattacks, 2003). VAT-cDCs are located in close proximity to lymphatic and blood vessels, where they can sample blood and lymph content. An elegant in-depth study demonstrated that the inherent permeability of collective lymphatic vessels allows fat-resident cDCs to sample soluble antigens trafficking from tissue to LNs. Furthermore, cDCs in the fat were also able to enter the lymphatic vessels and emigrate to adjacent LNs during inflammation (Kuan et al., 2015). Thus, the tolerogenic properties of VAT-cDCs may serve as a checkpoint for the control of tissue inflammation. Under pathological conditions in which lymphatic permeability is compromised, e.g., sustained gut inflammation, chronic lymphedema, and obesity, VAT can be highly infiltrated by antigens leaking out from lymphatic vessels (Fonseca et al., 2015) or can act as a reservoir for viruses, bacteria, and parasites (Damouche et al., 2015; Tanowitz et al., 2017). Therefore, activation of the β-catenin and PPARγ pathways in VAT-cDCs may serve not only to restrain obesity-induced inflammation, but also to promote pathogen persistence and tumor growth. Future studies to investigate these possibilities will be of extreme importance.

**Limitations of Study**

During HFD, thorough studies in mice have identified adipocyte hyperplasia in VAT, but not in subcutaneous fat. In addition, the rate of adipocyte hyperplasia is less well understood in human VAT due to technical difficulties. Thus, caution is advised in the extrapolation of these results, in particular the decrease of Wnt10b, to other adipose tissue sites or to human samples.

**STAR METHODS**

Detailed methods are provided in the online version of this paper and include the following:

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**SUPPLEMENTAL INFORMATION**

Supplemental Information includes six figures and one table and can be found with this article online at https://doi.org/10.1016/j.cmet.2018.02.007.

**ACKNOWLEDGMENTS**

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AUTHOR CONTRIBUTIONS

M.P.L. conceived the study; C.E.M., E.G.W., M.C., C.G.-M., and M.P.L. designed experiments; C.E.M., E.G.W., M.-B.V., and M.P.L. performed and analyzed experiments; J.L. generated the Zbtb46-Cre strain; V.S. and F.C.C. provided technical support; M.E.R., L.C., and N.F. performed informatics analysis; F.M.-B. contributed intellectually; C.E.M. and E.G.W. helped with manuscript preparation; and M.P.L. wrote the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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REFERENCES


R Core Team. (2016). R: a language and environment for statistical computing (Vienna, Austria).


# STAR★METHODS

## KEY RESOURCES TABLE

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## Chemicals, Peptides, and Recombinant Proteins

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(Continued on next page)
CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for reagents may be directed to and will be fulfilled by the Lead Contact, M. Paula Longhi (m.longhi@qmul.ac.uk).

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Critical Commercial Assays

- RNeasy MiniElute cleanup kit: QIAGEN Cat#74204
- RNeasy Lipid Tissue mini kit: QIAGEN Cat#74804
- High-Capacity RNA-to-cDNA kit: Applied Biosystems Cat#4387406
- iTaq Universal SYBR Green Supermix: Bio-Rad Cat#1725121
- Mouse Adiponectin ELISA kit: Merck-Millipore RRID: AB_2651034
- Mouse Leptin ELISA kit: Merck-Millipore Cat#EZML-82K
- Mouse Insulin ELISA kit: Merck-Millipore Cat#EZRMI-13K
- Mouse Protein Wnt-10b ELISA kit: Cusabio Cat# CSB-EL026130MO
- FoXP3 / Transcription Factor Staining Buffer Set: eBioscience Cat#00-5523-00
- LIVE/DEAD Fixable Aqua Dead Cell Stain Kit: Invitrogen Cat# L34966
- Dead cell removal kit: MACS Miltenyi Biotec Cat#130-090-101
- Mouse CD11c microbeads (N418): MACS Miltenyi Biotec Cat#130-052-001
- Mouse CD4 microbeads (L3T4): MACS Miltenyi Biotec Cat#130-049-201
- Mouse IL-10 ELISA kit: eBioscience RRID: AB_2574996
- Mouse IL-6 ELISA kit: eBioscience RRID: AB_2574986
- Mouse IL-12p70 ELISA kit: eBioscience RRID: AB_2575018
- Mouse IL-17A ELISA kit: eBioscience RRID: AB_2575100
- VECTASHIELD Mounting Medium with DAPI: VECTOR laboratories Cat# H-1200
- Luminata Forte Western HRP substrate: Millipore Cat#WBLUF0500

Deposited Data

- Raw data files for RNA sequencing: NCBI Gene Expression Omnibus GEO: GSE37448

Experimental Models: Organisms/Strains

- Mouse: C57BL/6J: Charles River Stock No.027
- Mouse: Pparγ²m/dgarv: Jackson Laboratory RRID: IMSR_JAX:004584
- Mouse: Ctnnb1tmKem: Jackson Laboratory RRID: IMSR_JAX:004152
- Mouse: Zbtb46tm1Kmm/J: Jackson Laboratory RRID: IMSR_JAX:018534
- Mouse: BALB/c: Charles River Stock No.028
- Mouse: B6.Cg-Tg(TcraTcrlb)425Cbn/J (OT-II): Jackson Laboratory RRID: IMSR_JAX:004194

Oligonucleotides

See Table S1 for list of primers

Software and Algorithms

- GSEA: Broad Institute http://software.broadinstitute.org/gsea/index.jsp
- IMARIS: Bitplane http://www.bitplane.com
- Axiovision version 4.8: Zeiss https://www.zeiss.com
EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mice
Male Pparγtm2Rev (PparγloxP), Ctnnb1tmKem (Ctnnb1loxP), Zbtb46tm1Kmm/J (Zbtb46GFP) and B6.Cg-Tg(TcraTcrob)425Cbn/J (OT-II) mice were purchased from Jackson Laboratory (US); male C57BL/6 were purchased from Charles river laboratories (UK); male Zbtb46-Cre+ mice were kindly provided by M. Nussenzweig (The Rockefeller University, NY). Mice were housed in temperature- and humidity-controlled rooms (22°C, 55% humidity) with a 12 h light/12 h dark cycle. Mice were fed Chow or Test Diet AIN-76A (Test Diet IPS) and given water ad libitum; animals were rehoused in clean cages weekly. 15 g of Z-NEST (IPS) was used as nesting material to help regulate temperature and light levels (Gaskill et al., 2012). For chimeras, 8–10 week old C57BL/6 male mice were γ-irradiated twice with 500 rad 3 h apart. Three hours later, mice were reconstituted by intravenous (i.v.) injection with marrow cells (3 x 10^8) that had been harvested from the femurs and tibias of conditional PparγCre−/− (PparγG6/6 zDC-cre+) and control WT littersmates or fetal liver cells (1x10^6) from 13 day-old Ctnnb1−/− (Ctnnb1Gfp/ zDC-cre+) or control embryos. Mice were maintained on acidified water during the critical 4-week reconstitution period. All animal work was carried out in accordance with UK government Home Office licensing procedures. Mice fed Western diet were excluded from experiments if they failed to gain more than 20% body weight.

METHOD DETAILS

Real-Time PCR and Expression Profiling
Total RNA was isolated from 10^6 cells using Trizol (Life Technologies) and with RNeasy MinElute cleanup kits (QIAGEN). RNA from 100 mg visceral adipose tissue was isolated using RNeasy Lipid Tissue Mini Kits (QIAGEN) following the manufacturer’s instructions. RNA was quantified with Spectrostar omega (BMG Labtech). Reverse transcription to cDNA was performed using High-Capacity RNA-to-cDNA Kits (Applied Biosystems) and stored at -80°C. Primer sequences can be found in Table S1, purchased from Invitrogen, Paisley, UK. Gene expression was performed using SYBR Green Supermix (Bio-Rad), according to the manufacturer’s instructions, and analyzed using a CFX connect light cycler (Bio-Rad). Gene-relative expression was calculated using the ΔΔCT method and normalized to a reference control (GAPDH) with control sample set as 1. As ΔΔCT is not normally distributed is the geometric mean is a more appropriate representation of the data than the more commonly used arithmetic mean. To note, the propagated error is the product of multiple additive errors, thus it will usually be higher than the error implied by taking the standard error of the ΔΔCT values for each replicate.

Expression profiling was performed using publicly available raw data (ImmGen GEO: GSE37448) in R (R Core Team, 2016). Between-array RNA quantile normalization was performed and unmapped or multi-mapping probes removed prior to differential expression analysis with limma (Ritchie et al., 2015). Fold change rankings were used in GSEA to identify differentially regulated pathways (FDR < 0.25). Processed data are available on ImmGen’s data browsers (http://www.immgen.org/).

Ex Vivo Confocal Microscopy
Zbtb46-GFP mice were injected i.p. with fluorescently conjugated primary antibodies against LYVE-1 (eBioscience) and PECAM-1 (eBioscience) 4 hours prior to surgery. Mice were sacrificed and the mesenteric organs including the associated VAT was exteriorized (Arokiasamy et al., 2017). Acquired confocal images were analyzed using the 3D imaging analysis software IMARIS (bitplane, Switzerland).

Metabolic Assays
Adiponectin and Leptin levels were measured in the serum from fasted lean and obese mice by ELISA (Merck Millipore), as well as by RT-PCR from visceral adipose tissue as described above. Similarly, fasted insulin levels were detected by ELISA (Merck Millipore). Wnt10b was detected from visceral adipose tissue homogenates by ELISA (Cusabio). For Glucose (GTT) and Insulin (ITT) tolerance tests fasted blood glucose levels were measured from initial tail bleeds at room temperature using blood glucose meter and test strips (FreeStyle Optium Neo, Abbott). Mice were administered with 1.5 mg D- Glucose/ g of body weight (Sigma) or 0.5 U Insulin/ kg of body weight (Actrapid) via intraperitoneal (i.p.) injection and blood glucose measurements were taken from the tail bleeds at 15, 30, 60, 90, 120, 180 mins after injection at room temperature.

To assess the insulin signaling, mice were injected i.v. with 5 U Insulin/ kg of body weight at room temperature. After 15 minutes the VAT and liver were harvested and tissue levels of phospho-(Ser473) Akt and total AKT were measured by western blot. Body weight and food intake was recorded weekly. Weight of total visceral adipose tissue harvested was recorded for cell number calculations and to determine the percentage of body weight. Visceral adipose tissue was fixed in 4% PFA and embedded in paraffin. Sectioned slides were stained with hematoxylin and eosin by BCI Pathology core services (QMUL). Adipocytes size were calculated using ImageJ Adiposoft software (Galarraga et al., 2012) by researcher blinded to the tissue genotype. Three fields of view from each image per mouse were analyzed.

Flow Cytometry Staining
Immune cells from spleen, mesenteric lymph nodes and inguinal lymph nodes were isolated after digestion with Collagenase D (Roche). For visceral adipose tissue immune cells, tissue was digested with Collagenase II (Sigma) and DNase (Sigma) for 30 min. Immune cells present in the vascular fraction were obtained after centrifugation and lysed for Red blood cells before staining. All
samples were stained with fixable Aqua Dead cell stain (Invitrogen) to exclude dead cells from analysis. Cells were stained for surface markers using the following antibodies; CD16/32-FITC, F4/80-PerCP, CD206-BV421, CD11c-BV605, CD103-APC, MHCII-AF700, CD11b-AF780, MerK-PE, CD45-PE-CF594, CD64-PE-Cy7, CD8-FITC, Ly6G-BV421, NK1.1-BV605, CD3-AF700, SiglecF-PE-CF594, CD4-PE-Cy7, B220-PerCP, CD36-APC, CD86-BV605, CD80-PE (eBioscience/ BioLegend/ BD Biosciences). Antibodies against non-phospho (active) b-catenin, PPARγ, phospho-(Ser473) Akt and total Akt were purchased from Cell Signaling Technologies; antibodies against Tubulin, GAPDH were purchased from Genetex. Blotted proteins were detected using Luminata Forte Western HRP substrate (Millipore) and exposed on to Hyperfilm photo film (Amersham).

**DC Innate Responses**

Dendritic cells were isolated from the spleen and visceral adipose tissue of 3-5 pooled Zbtb46-GFP mice, by first magnetic depletion of dead cells (MACS Miltenyi Biotec) followed by CD11c+ bead positive selection (MACS Miltenyi Biotec). CD11c+ MHCII+ GFP+ CD11b+ CD2 were purified by cell sorting (FACS Aria; BD Biosciences). Cells were plated at 10^5 cells/well and stimulated with the TLR4 agonist GLA (IDRI) in the presence or absence of Rosiglitazone 2.5 μM (Sigma) or SB216763 20 μM (Abcam Biochemicals). In specific experiments cells were additionally stimulated with 100 ng/mL of recombinant Wnt10b (R&D Systems). After culturing overnight, supernatant was collected and levels of IL-10, IL-6, IL-12p70 were determined by murine ELISA (eBioscience).

**DC Antigen Presentation**

For DC antigen presentation, in vivo, chimera mice were injected intravenously with ovalbumin-specific OT-II cells purified from spleen and lymph nodes by CD4+ bead positive selection (MACS Miltenyi Biotec) and labeled with CFSE 3 μM (Sigma). The next day, mice were immunized with 200 μg of Ovalbumin (Sigma) alone or together with 1 μg of LPS (Sigma). Two (after LPS) or three (after OVA alone) days later, immune cells were isolated from spleen, lymph nodes and visceral adipose tissue and stained, as described above, for analysis by flow cytometry. The dilution of CFSE in the T cell population was used as an indicator of cell division.

To evaluate OVA uptake by visceral adipose tissue phagocytic cells, 10 μg of OVA-AlexaFluor-555 (Invitrogen) was injected i.p. One hour later, visceral adipose tissue was harvested and fixed overnight in 4% PFA containing 30% Sucrose (Sigma) and stained with 0.5 μg/ml of BODIPY as per manufacturer’s instructions (Invitrogen). Tissue was laid onto poly-L-lysine slides with mounting medium containing DAPI (Invitrogen). Tissue was visualized using a Zeiss Z1 fluorescence microscope (Carl Zeiss, Cambridge, UK) equipped with an AxioCam MRm Cooled monochrome digital camera and an Apotome 2 Imaging unit. Images were acquired using a Plan Apochromat 40x or 20x/0.8 NA objective and Axiovision software version 4.8.

To test allostimulatory capacity in vitro, spleen and visceral adipose tissue DCs were isolated, as described above, from lean and obese Zbtb46-GFP or chimera mice. CD4+ T cells were harvested from the spleen of BALB/c mice by CD4+ bead positive selection and labeled with CFSE 3 μM. Cells were mixed at a ratio of 1:5 (5 x 10^5 CD11c+ dendritic cells: 2.5 x 10^6 CD4+ T cells / well) and incubated for 3 - 5 days, after which cells were harvested and CFSE dilution was assessed by flow cytometry and cytokine production in supernatant by ELISA.

**Western Blotting**

Protein lysates were prepared from tissue or purified cDCs using RIPA buffer. Proteins were separated with SDS-PAGE and transferred to Immobilon PVDF membrane (Millipore). Membranes were blocked for 1 h at room temperature in PBST containing 5% (w/v) milk, incubated overnight at 4°C with primary antibodies and subsequently with HRP-conjugated secondary antibody (Amersham Bioscience). Antibodies against non-phospho (active) b-catenin, PPARγ, phospho-(Ser473) Akt and total Akt were purchased from Cell Signaling Technologies; antibodies against Tubulin, GAPDH were purchased from Genetex. Blotted proteins were detected using Luminata Forte Western HRP substrate (Millipore) and exposed on to Hyperfilm photo film (Amersham).

**QUANTIFICATION AND STATISTICAL ANALYSIS**

Data reported in the figures represent the average of at least three independent experiments. Statistical significance was determined by Student’s t test with two-tailed P values of 0.05 or less. For GTT and ITT, statistical significance was evaluated with 2-way ANOVA followed by Bonferroni post-test. Data were analyzed and charts were generated using Prism 5 (GraphPad Software). Normal distribution was assessed with Prism 5 using the Kolmogorov-Smirnov and the Shapiro-Wilk normality test. Differences were considered significant at *p < 0.05, **p < 0.005, ***p < 0.0005, ****p < 0.00005; n.s. non significant.

**DATA AND SOFTWARE AVAILABILITY**

**Data Resources**

Raw data files for the RNA sequencing analysis have been deposited in the NCBI Gene Expression Omnibus under accession number GEO: GSE37448.