Eosinophils support adipocyte maturation and promote glucose tolerance in obesity

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Accumulating data have indicated a fundamental role of eosinophils in regulating adipose tissue homeostasis. Here, we performed whole-genome RNA sequencing of the small intestinal tract, which suggested the presence of impaired lipid metabolism in eosinophil-deficient \( \Delta \)dblGATA mice. \( \Delta \)dblGATA mice fed a high-fat diet (HFD) showed reduced body fat mass, impaired enlargement of adipocytes, decreased expression of adipogenic genes, and developed glucose intolerance. HFD induced accumulation of eosinophils in the perigonadal white adipose tissue. Concordantly, adipocyte-differentiated 3T3-L1 cells promoted the migration of eosinophils through the expression of CCL11 (eotaxin-1) and likely promoted their survival through the expression of interleukin (IL)-3, IL-5, and granulocyte-macrophage colony-stimulating factor. HFD-fed \( \Delta \)dblGATA mice showed increased infiltration of macrophages, CD4+ T-cells, and B-cells, increased expression of interferon-\( \gamma \), and decreased expression of IL-4 and IL-13 in white adipose tissue. Interferon-\( \gamma \) treatment significantly decreased lipid deposition in adipocyte-differentiated 3T3-L1 cells, while IL-4 treatment promoted lipid accumulation. Notably, HFD-fed \( \Delta \)dblGATA mice showed increased lipid storage in the liver as compared with wild-type mice. We propose that obesity promotes the infiltration of eosinophils into adipose tissue that subsequently contribute to the metabolic homeostasis by promoting adipocyte maturation.

Eosinophils have been considered as destructive cells involved in T helper cell type (Th) 2 immune responses in parasitic infections or allergic diseases1. However, accumulating evidence has indicated additional roles for eosinophils. For example, eosinophils reside in several organs including the gastrointestinal tract2,3 and adipose tissue and contribute to metabolic homeostasis4,5. Adipose tissue eosinophils secrete interleukin (IL)-4 and induce the polarization of white adipose tissue (WAT) macrophages into alternatively-activated macrophages, which support glucose tolerance by regulating local catecholamine stores in the microenvironment through the import of catecholamines produced by nerve cells4,6,7. Despite recent advances, the roles of eosinophils in the adipose tissue and their effects on adipocyte function remain incompletely understood.

Obesity and its associated metabolic disorders are serious health problems worldwide8. However, obesity is not necessarily an adverse metabolic condition when the excess fat is stored in adipose tissue that responds to insulin9. Adipose tissue regulates energy homeostasis through the storage of excess calories and the secretion of adipocyte-derived secretory proteins such as leptin, adiponectin, and resistin10,11. However, excessive caloric
intake induces the overexpansion of adipocytes, which results in inflammatory responses within adipose tissue.12. Obesity-related metabolic dysfunctions are associated with an excessive infiltration of immune cells and chronic inflammation in adipose tissue.13,14.

Here, we used eosinophil-deficient ΔdblGATA and wild-type (WT) mice to investigate the roles of eosinophils in obesity, adipose tissue maturation, and associated metabolic responses. We performed whole-genome RNA sequencing of the small intestinal tract, which suggested the presence of a defective lipid metabolism in the absence of eosinophils. Furthermore, ΔdblGATA mice fed a high-fat diet (HFD) gained less weight and showed reduced body fat, an impaired enlargement of adipocytes, a decreased expression of adipogenic genes, and a more severe glucose intolerance than the WT group. Thus, we hypothesized that the inability to appropriately expand adipose tissue underlies insulin resistance in ΔdblGATA mice. We found that adipocyte-differentiated 3T3-L1 cells promoted the migration of eosinophils through the expression of CC chemokine ligand (CCL) 11 (eotaxin-1) and promoted their survival through the expression of IL-3, IL-5, and granulocyte-macrophage colony-stimulating factor (GM-CSF). Accordingly, the WAT of HFD-fed WT mice showed an increased infiltration of eosinophils with upregulated CCL11. Compared to WT mice, ΔdblGATA mice showed significantly increased populations of pro-inflammatory immune cells in their WAT. The WAT of HFD-fed ΔdblGATA mice also showed an increased expression of the Th1 cytokine interferon (IFN)-γ and a decreased expression of the Th2 cytokines IL-4 and IL-13. The cytokine expression profile of the WAT of ΔdblGATA mice negatively correlated with adipocyte maturation as demonstrated by attenuated lipid storage in IFN-γ-treated 3T3-L1 cells, which was opposed by IL-4 treatment. Notably, HFD-fed ΔdblGATA mice showed increased lipid storage in the liver as compared with WT mice, suggesting that insulin resistance was induced by an excessive accumulation of lipid in non-adipose tissues. Thus, we propose that cross-talk between adipocytes and eosinophils promotes metabolic homeostasis by supporting the infiltration of eosinophils into adipose tissue and maintaining the microenvironment of adipose tissue to favour adipocyte maturation.

Results

Eosinophil-deficient mice showed impaired glucose tolerance and a decreased expression of genes involved in energy metabolism. Under physiological conditions, eosinophils primarily reside in the small intestine, where they account for a substantial fraction (e.g., 20–30%) of the cellular population.13,14. Therefore, the significant downregulation of genes in the small intestine of ΔdblGATA mice may indicate a role

![Figure 1](https://www.nature.com/scientificreports/)
for eosinophils in regulating certain biological responses. Through the whole-genome RNA sequencing of the small intestine, we observed 379 downregulated and 52 upregulated genes in the small intestine of ΔdblGATA mice (Fig. 1a, Tables S1 and S2) and the expression of the top 10 downregulated genes was validated by real-time PCR (Fig. S1). Of note, genes associated with lipid metabolism, such as Retnlg, Alox15, and Drd215–17 were included in the top 10 downregulated genes (Table S1). Additionally, a GO analysis of the downregulated genes and visualization of a functionally grouped network using the ClueGO plugin18 suggested a defect in lipase activity (Table S3 and Fig. S2) and a significant decrease in the expression of Lpl was observed (Fig. 1b). A decreased expression of Lpl and other lipogenic genes (Slc2a4 and Adipoq) was also observed in the perigonadal WAT of ΔdblGATA mice (Fig. 1c). Although the body weight of 8–10-week-old ΔdblGATA mice was not different from that of WT mice (Fig. 1d), ΔdblGATA mice showed a significantly higher glucose level at the early time point of GTT (Fig. 1e). Our observations suggested that eosinophils regulate lipid metabolism and energy homeostasis.

Figure 2. ΔdblGATA mice gained less weight and body fat and displayed impaired glucose tolerance on a high-fat diet (HFD). Wild-type (WT) and ΔdblGATA male mice were fed a HFD or chow diet for 12 weeks. (a) Weight change during feeding with a HFD or chow diet (n = 20–27 mice/group). *p < 0.05, **p < 0.01, ***p < 0.001 (two-way ANOVA), WT + HFD vs. ΔdblGATA + HFD. *p < 0.05, **p < 0.01, ***p < 0.001 (two-way ANOVA), ΔdblGATA + Chow vs. ΔdblGATA + HFD. (b) Body weight gain, perigonadal fat/body mass, and liver/body mass on a HFD or chow diet. *p < 0.01, ***p < 0.001 (one-way ANOVA). (c) Glucose tolerance test of the indicated mice fasted for 16 h (n = 13–15 mice/group). *p < 0.05, ***p < 0.001 (two-way ANOVA), WT + HFD vs. ΔdblGATA + HFD. (d) Western blots of insulin-stimulated phosphorylation of insulin receptor (IR) and protein kinase B (Akt) in the liver, perigonadal fat, and skeletal muscle. The data are representative of two independent experiments. Graphs show the mean ± standard error of the mean.

Eosinophil-deficient mice gained less weight and perigonadal fat mass and displayed impaired glucose tolerance on a HFD. Excess caloric intake induces expansion of fat mass and accelerates development of insulin resistance15. To further examine the role of eosinophils in adipose tissue expansion and energy homeostasis, we fed 8–10-week-old male WT and ΔdblGATA mice with a HFD for 12 weeks and monitored their body weights weekly. The HFD induced a significant weight gain of ΔdblGATA mice compared to chow diet-fed ΔdblGATA mice (Fig. 2a). However, ΔdblGATA mice showed an approximately 20% lower body weight than WT mice after 12 weeks of a HFD (Fig. 2a,b). Consistent with their lesser weight gain, ΔdblGATA mice fed a HFD had significantly less perigonadal fat mass than WT mice (Figs 2b and S3a). Differences in liver mass were not apparent among the analysed groups of mice (Figs 2b and S3a). Although ΔdblGATA mice showed less weight gain under a HFD than their WT counterparts, their ability to normalize blood glucose in the GTT was significantly impaired (Fig. 2c). HFD-fed ΔdblGATA mice showed significantly higher blood glucose levels until 30 min after insulin administration (ITT, Fig. S3b); however, the slopes of the ITT curves between WT and ΔdblGATA appear to overlap and blood insulin levels did not differ between WT and ΔdblGATA mice (Fig. S3c). To assess the molecular basis of insulin resistance observed in HFD-ΔdblGATA mice, we investigated insulin
receptor signalling after infusion of insulin through the portal vein. Insulin receptor (IR) tyrosine phosphorylation and protein kinase B (Akt) phosphorylation were reduced in the livers, but not in WAT or skeletal muscle from HFD-fed ΔdblGATA mice compared to in their WT counterparts (Fig. 2d). Phosphorylation of either IR or Akt was comparable between WT and ΔdblGATA mice fed a chow diet (Fig. 2d). Taken together, our observations indicate that hepatic insulin resistance was induced in ΔdblGATA mice by HFD feeding.

Although ΔdblGATA mice fed with HFD showed increased level of serum free fatty acid than WT mice (Fig. S4a), it is unlikely that ΔdblGATA mice have defect in energy utilization considering the comparable serum triglyceride and stool fat content of the HFD-fed WT and ΔdblGATA mice (Fig. S4a,b). Energy intake and expenditure were also comparable between HFD-fed WT and ΔdblGATA mice (Fig. S4c).

Eosinophil-deficient mice showed an impaired maturation of perigonadal adipocytes on a HFD. The inability of adipose tissue to expand to accommodate excess calories causes systemic insulin resistance and hyperglycaemia. As we observed an impaired WAT development in HFD-fed ΔdblGATA mice compared to in their WT counterparts (Fig. 2d). Phosphorylation of either IR or Akt was comparable between WT and ΔdblGATA mice fed a chow diet (Fig. 2d). Taken together, our observations indicate that hepatic insulin resistance was induced in ΔdblGATA mice by HFD feeding.

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Adipocytes support the migration and survival of eosinophils. Since eosinophils are resident cells in the adipose tissue and ΔdblGATA mice showed an impairment of adipocyte maturation, we questioned whether adipocytes support the migration or survival of eosinophils. Eosinophils migrate in a manner dependent on CCL11 and its receptor, CC chemokine receptor (CCR) 3-4. We determined that adipocyte-differentiated
mouse embryonic fibroblast 3T3-L1 cells expressed Ccl11 (Figs 4a and S6) and secreted CCL11 (Fig. 4b), which attracted eosinophils, since the application of anti-mouse CCL11 significantly inhibited eosinophil trafficking toward 3T3-L1 cells (Fig. 4c). Additionally, adipocyte-differentiated 3T3-L1 cells expressed cytokines supporting eosinophil survival, such as Il3, Il5, and Csf2 (Figs 4d and S6). Accordingly, the viability of eosinophils was markedly increased by co-culture with adipocyte-differentiated 3T3-L1 cells (Fig. 4e). Next, we assessed eosinophil infiltration in the WAT of HFD-fed WT mice and found a significant increase in the expression of Ccr3 and Ccl11 in the perigonadal WAT (Fig. 4f,g). An increased frequency and number of eosinophils in the WAT was supported by a flow cytometry analysis (Figs 4h and S7a). The frequency of eosinophils in the bone marrow was unaffected by the HFD (Fig. 4h). These results imply that eosinophils preferentially migrate to the perigonadal adipose tissue in diet-induced obesity because of the production of molecules that support their migration and survival by adipocytes.

**The perigonadal adipose tissue of eosinophil-deficient mice showed an increased inflammatory response.** Excess calorie intake results in chronic inflammation in adipose tissue involving an infiltration of
various immune cells. In obesity, the infiltrated immune cells promote the production of pro-inflammatory cytokines that inhibit adipogenesis and insulin signalling. The total numbers of cells isolated from the perigonadal WAT of HFD-fed ΔdblGATA and WT mice were not significantly different ($p = 0.1263$, data not shown).

However, significantly increased abundances of macrophages, CD4$^+$ T cells, and B cells were observed in the perigonadal WAT of HFD-fed ΔdblGATA mice (Figs 5a and S7b). Although HFD-fed ΔdblGATA mice showed an increased abundance of macrophages in W AT as compared with WT mice, the ratio between the classical (M1) and alternative (M2) immune phenotypes of the macrophages was comparable between ΔdblGATA and WT mice (Fig. S8a). This finding was supported by the lack of a significant difference in the expression of Arg1, Nos2, and the ratio of Arg1 and Nos2 between WT and ΔdblGATA mice (Fig. S8b,c). We next measured the mRNA levels of cytokines in the perigonadal WAT and observed a marked increase in the expression of the Th1 cytokine Ifng in HFD-fed ΔdblGATA mice (Fig. 5b). In contrast, the expression levels of the Th2 cytokines Il4 and Il13 were significantly decreased in HFD-fed ΔdblGATA mice (Fig. 5b).

**Figure 5.** ΔdblGATA mice showed increased inflammatory responses in the perigonadal adipose tissue on a high fat diet (HFD). (a) Flow cytometric analysis of SiglecF$^-$$F4/80^+$ macrophages, CD3$^-$$CD4^+$ T cells, CD3$^-$$CD8^+$ T cells, and CD3$^-$$B220^+$ B cells in the perigonadal fat of wild-type (WT) and ΔdblGATA mice on a HFD. Representative dot plots are shown. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (Student’s $t$-test for macrophage, Mann-Whitney test for CD3$^+$ T cells, and B cells). (b) mRNA expression of innate (Il1b, Il6, and Tnf) and adaptive (Ifng, Il4, Il5, and Il13) immune cytokines in the perigonadal fat of mice on a HFD. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ (Student’s $t$-test for Il1b, Il6, Tnf, and Il4, Mann-Whitney test for Ifng, Il5, and Il13). Graphs show the mean ± standard error of the mean.

The cytokine expression profile in the perigonadal adipose tissue of eosinophil-deficient mice reflected defective adipogenic maturation. To determine whether the cytokine profile in the perigonadal WAT of ΔdblGATA mice affects adipocyte maturation, we induced the adipogenesis of 3T3-L1 cells in the presence of IFN-γ or IL-4. As shown in Fig. 6, adipocyte-differentiated 3T3-L1 cells treated with IFN-γ showed a significant decrease in lipid deposition as measured by Oil Red O staining. In contrast, adipocyte-differentiated 3T3-L1 cells treated with IL-4 showed an increase in lipid accumulation (Fig. 6) with significant upregulation of key adipogenic and lipogenic genes including Cebpa, Acaca, Fasn, and Scd (Fig. S9). These findings indicated that eosinophils infiltrated into the perigonadal WAT of HFD-fed mice modulate the immune microenvironment to favour adipocyte maturation.

**Eosinophil-deficient mice showed lipid accumulation in the liver.** Excessive lipid accumulation in non-adipose tissues such as the liver, muscle, and pancreas is closely associated with insulin resistance. Given that lipids tend to be stored ectopically in the absence of functional adipocytes, we hypothesized that eosinophil deficiency would influence the development of ectopic fat accumulation. The livers of HFD-fed ΔdblGATA mice exhibited a paler colour and more prominent lipid deposits than those of WT mice (Fig. 7a,b). The measurement of total liver triglycerides supported the visual observation of lipid storage in the liver of HFD-fed ΔdblGATA
mice (Fig. 7c). An analysis of adipogenic gene expression using real-time PCR revealed a significant increase in the expression of Pparg in the liver of HFD-fed ΔdblGATA mice, although the expression of Lpl and Cav2 was lower in these mice (Fig. 7d). Although the lipid contents in the skeletal muscle of HFD-fed ΔdblGATA mice was comparable to in their WT counterparts, the small intestine of HFD-fed ΔdblGATA showed increased accumulation of triglycerides (Figs S10 and S11).

Figure 6. Effect of interferon (IFN)-γ and interleukin (IL)-4 treatment on 3T3-L1 adipogenesis. (a) 3T3-L1 cells were treated with insulin to differentiate them into adipocytes in the presence of either IFN-γ or IL-4 and stained with Oil Red O. Original magnification × 20. (b) Oil Red O in the adipocyte-differentiated 3T3-L1 cells was eluted using isopropanol and the optical density (OD) of the eluate was analysed. *p < 0.05, **p < 0.01, ***p < 0.001 (one-way ANOVA). Graphs show the mean ± standard error of the mean.

Figure 7. Fatty liver changes induced by high fat diet (HFD) were enhanced in ΔdblGATA mice. (a) Haematoxylin and eosin staining of the liver of wild-type (WT) and ΔdblGATA mice fed a HFD or chow diet. Images are representative of two independent experiments. Original magnification × 20. The scale bar represents 50 μm. (b) Representative image of the liver from a mouse on a HFD. (c) Triglyceride levels in the livers of mice fed a HFD. *p < 0.05 (Student’s t-test). (d) mRNA expression levels of adipogenic genes in the livers of mice fed a HFD. *p < 0.05 (Student’s t-test). Graphs show the mean ± standard error of the mean.
Discussion
Although eosinophils have been characterized as destructive effector cells that mediate tissue damage during helminth infections and allergic diseases, they are found in various tissues under resting conditions without having obvious pro-inflammatory activities. Accumulating evidence has indicated that tissue-resident eosinophils regulate biological processes that are not restricted to Th2 immune responses. Eosinophils in adipose tissue promote the accumulation of alternatively activated macrophages, which protect against insulin resistance induced by obesity-associated changes such as the activation of innate immune activity, alterations in fatty acid uptake, lipogenesis, and energy expenditure that can impact ectopic lipid deposition. Obesity itself does not necessarily induce insulin resistance and primarily results from adipocytes expanding to buffer against excess nutrient uptake. Despite recent advances in understanding the functions of eosinophils in maintaining metabolic homeostasis, their roles in adipocyte development are not fully understood. Nevertheless, multiple lines of our observation have indicated that eosinophils counteract obesity-associated metabolic dysfunctions by promoting adipocyte maturation. A significant decrease of Ccr3 in the small intestine, lung, fat, liver, and muscle of 8–10-week-old chow diet-fed ΔdblGATA mice imply distribution of eosinophils in the metabolic organs under resting conditions (Fig. S12). In addition, a decreased expression of genes associated with lipid metabolism was observed in the small intestine (Figs 1a and S1) and perigonadal WAT (Fig. 1c) of 8–10-week-old ΔdblGATA mice, further suggesting that eosinophils regulate energy homeostasis. However, the expression of Ccr3 significantly increased in the perigonadal WAT on a HFD (Fig. 4f), as well as the frequency and number of eosinophils (Figs 4h and S7a), implying the preferential infiltration of eosinophils into adipose tissue with obesity. Therefore, we propose that mature adipocytes release factors that support the infiltration of eosinophils into adipose tissue. Indeed, adipocyte-differentiated 3T3-L1 cells expressed CCL11, which has an eosinophil-selective chemotactant activity, and the CCL11 produced by 3T3-L1 cells induced functional migration of eosinophils into adipocyte-differentiated 3T3-L1 cells (Fig. 4a–c). Adipocyte-differentiated 3T3-L1 cells also promoted the viability of eosinophils and we observed the expression of IL3, IL5, and CSF2 (the gene encoding GM-CSF) in differentiated 3T3-L1 cells (Fig. 4d,e). These cytokines provide signals that promote the permissive proliferation and differentiation of eosinophils. These findings contrast with previous observations that suggested an inverse correlation between the abundance of eosinophils in adipose tissue and adiposity, implying that the expression of genes associated with lipid metabolism is closely associated with insulin resistance, and we propose that ectopic fat accumulation in HFD-fed ΔdblGATA mice might account for their glucose intolerance. Although increased visceral adiposity has been implicated in hepatic insulin resistance, patients with severe lipodystrophy, as well as a mouse model of lipatrophy, manifest insulin resistance associated with lipid deposition in the liver. The significant decrease in the expression of Pparg, which encodes a transcription factor involved in adipocyte differentiation, and genes associated with lipid droplet formation (Cav1, Cav2, Cdtb, and Cidec) in the perigonadal WAT of HFD-fed ΔdblGATA mice (Fig. 3) suggests insufficient lipid storage in adipose tissue in the absence of eosinophils. Concordantly, the expression of Lpl, Slec2a4, and Adipoq, which are regulated by Pparg and involved in glucose homeostasis, significantly decreased in the perigonadal adipose tissue of HFD-fed ΔdblGATA mice (Fig. 3). We also observed significant decreases in Ptf1 (involved in biogenesis of caveolae), Akt2 (involved in adipocyte differentiation and insulin signalling), and Psmb8 (involved in expression of immunogenic epitopes), which are associated with lipodystrophies, in the WAT of HFD-fed ΔdblGATA mice (Fig. S14). We suggest that an altered immune environment in the perigonadal fat of HFD-fed ΔdblGATA accounts for the decreased expression of adipogenic genes. The exposure of adipocytes to pro-inflammatory cytokines inhibits adipogenesis by reducing the expression of Pparg and inhibiting the adipogenic action of insulin. Th1 cytokines, including IFN-γ, can inhibit insulin signalling and lipid droplet formation, while Th2 cytokines, including IL-4 and IL-13, can suppress inflammatory responses in adipose tissue. The increased expression of Ifng and decreased expression of Il4 and Il13 in the perigonadal fat of HFD-fed ΔdblGATA mice imply that the microenvironments of the WAT of these mice are unfavourable for adipocyte maturation. We supported this idea by demonstrating that IFN-γ attenuated lipid storage while IL-4 promoted lipid deposition in adipocyte-differentiated 3T3-L1 cells (Figs 6 and S9). These changes in cytokine expression were diet- and site-specific, since the expression of Il4, Il13, and Ifng was not different between WT and ΔdblGATA mice either in the perigonadal adipose tissue of chow-fed mice or in the liver of HFD-fed mice (data not shown).

Obesity is associated with an increased infiltration of macrophages, preferentially the pro-inflammatory M1 phenotype, into adipose tissue, and IL-4 produced by adipose tissue eosinophils supports the polarization of anti-inflammatory M2 macrophages. In agreement with this idea, conditioned media (CM) collected from eosinophilic cell line EoL-1 cells or CM from palmitic-acid stimulated EoL-1 cells promoted M2-polarization of monotypic THP-1 cells (Fig. S15). However, the ratio between M1 and M2 macrophages was comparable between WT and ΔdblGATA mice (Fig. S8), although HFD-fed ΔdblGATA mice showed a significant decrease in the expression of Il4 in the perigonadal WAT (Fig. 5). Given that hypoxia induces macrophage proliferation...
and polarization towards the M2 phenotype, it is plausible that hypoxia determined the composition of macrophages in the adipose tissue of HFD-fed ΔdblGATA mice. The significantly elevated concentration of lactate in the perigonadal WAT of HFD-fed ΔdblGATA mice also supports this idea (Fig. S16).

Although adipose tissue is rich in stem cells that can differentiate into fat cells to contain excess energy, the pro-inflammatory microenvironment of adipose tissue induced by obesity is associated with the inhibition of adipocyte maturation and increased adipocyte death. Our data demonstrate that adipocytes provide signals to promote eosinophil migration and survival, and that eosinophils support adipocyte maturation and protect adipose tissue against inflammatory changes. Based on our findings, we propose that eosinophils and adipocytes bidirectionally cooperate to promote metabolic homeostasis in diet-induced obesity.

**Methods**

**Mice.** BALB/c WT mice (Orientbio, Gyeonggi, Korea) and ΔdblGATA mice (C.129S1(B6)-Gata1tm6Sho/J, Jackson Laboratory, Bar Harbor, ME, USA) were housed under standard temperature and humidity in the specific pathogen-free facilities of Gachon University. Cluster of differentiation (CD) 3-IL-5 transgenic mice (NJ.1638, I5575) were kindly provided by Dr. Jamie Lee (Mayo Clinic, Scottsdale, AZ, USA) and housed in the specific pathogen-free facilities of Tel Aviv University. Animal procedures were reviewed and approved by the Center of Animal Care and Use of Lee Gil Ya Cancer and Diabetes Institute, Gachon University (Number: LCDI-2016-0060) or by the Animal Care Committee of Tel-Aviv University (Number: M-13-029, M-13–30), and were performed in accordance with its regulations and guidelines regarding the care and use of animals for experimental procedures.

**RNA sequencing and bioinformatics analysis.** RNA isolated from the small intestine of 8–10-week-old WT (n = 4) and ΔdblGATA mice (n = 4) was subjected to RNA sequencing at the Cincinnati Children’s Hospital Medical Center sequencing core. Sequencing data were demultiplexed and reads were mapped to the mm10 mouse genome reference using TopHat. The total number of mapped reads per transcript was determined and the data were normalized to detect the number of fragments per kilobase of exon per million fragments mapped (FPKM) using Cufflinks. An FPKM > 0.1 in at least 1 of the analysed samples was used to filter for potentially significant gene expression. Transcripts with fold-change values ≥2 with a false discovery rate-corrected p-value < 0.05 were included as differentially expressed genes. Functional groups and pathways encompassing the differentially expressed genes were identified based on a Gene Ontology (GO) analysis using the Database for Annotation, Visualization, and Integrated Discovery. GO terms and pathways having an enrichment score > 1.3, p-value < 0.05, and number of genes ≥ 3 were defined as significantly enriched.

**HFD feeding and metabolic studies.** Eight-to-ten-week-old male mice were fed a HFD (60% fat, D12492, Research Diets, New Brunswick, NJ, USA) or a chow diet (5.0% fat, 5033, LabDiet, St. Louis, MO, USA) for 12 weeks. Body weight was measured weekly. Fasting insulin concentrations were measured by enzyme-linked immunosorbent assay (ELISA, Shibayagi, Gunma, Japan). For glucose tolerance tests (GTTs), glucose (1.5 g/kg) was injected intraperitoneally after starvation for 16 h and blood glucose was measured using a glucometer (Allmedicus, Kyunggi, Korea). For in vivo insulin signalling analysis, mice were anesthetized after overnight fasting. Insulin (0.75 U/kg) or saline was infused into the liver via the portal vein. Five minutes after infusion, liver, perigonadal fat, and skeletal muscle were quickly excised and snap-frozen in liquid nitrogen. At sacrifice, all mice were weighed and the livers and perigonadal fat were removed and weighed.

**Total protein extraction and western blot analysis.** The tissue lysates were prepared in ice-cold tissue lysis buffer (50 mM Tris-HCl, pH 7.5; 150 mM NaCl; 1% Nonidet P-40; 0.5% sodium deoxycholate; 0.1% SDS) containing 50 mM NaF, 2 mM Na3VO4, protease inhibitors (Sigma-Aldrich, St. Louis, MO, USA) and phosphatase inhibitors (Sigma-Aldrich) and total protein was extracted as previously described. Samples from tissue lysates were resolved by SDS-PAGE and then transferred to a nitrocellulose membrane. After 1 h blocking at 4 °C using 5% BSA in phosphate-buffered saline containing 0.1% Tween-20 (PBST), the membrane was incubated overnight with antibodies against phospho-IR (sc-25103, Santa Cruz Biotechnology, Santa Cruz, CA, USA), phospho-Akt (SAB5600064, Sigma-Aldrich), and α-tubulin (T9026, Sigma-Aldrich) in PBST at 4 °C. After 3 PBST washes, membranes were incubated with secondary antibody for 1 h at room temperature. Chemiluminescence was performed using a SuperSignal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific, Waltham, MA, USA).

**PCR analysis.** Total RNA was extracted using QIAzol® lysis reagent (Qiagen, Hilden, Germany) and subsequently column-purified with an RNeasy® Mini Kit (Qiagen). RNA (500 ng) was treated with DNase I (New England Biolabs, Ipswich, MA, USA) and cDNA was synthesized using an iScript™ cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA, USA). Real-time PCR was performed using iQ SYBR® Green Supermix (Bio-Rad Laboratories) on a CFX Connect™ real-time PCR detection system (Bio-Rad Laboratories). Reverse-transcription PCR was performed using Bio-ReadyMix (Bio-Lab, Jerusalem, Israel) and the amplified DNA products were electrophoresed on 2% agarose gels. The primers are detailed in Supplementary Tables S4 and S5.

**Histology.** The perigonadal fat and liver specimens were fixed in 10% buffered formalin and embedded in paraffin. Multiple 4-μm sections were stained with haematoxylin and eosin and visualized using a CX41 microscope (Olympus, Tokyo, Japan).

**Preparation of cell suspension.** Perigonadal adipose tissues were incubated with 0.2% collagenase II (Sigma-Aldrich) in Roswell Park Memorial Institute (RPMI) 1640 medium/0.5% BSA with continuous stirring at 37 °C for 30 min. Bone marrow cells were collected by flushing the femurs with RPMI 1640 medium/5% FBS. Isolated cells were filtered through a 100-μm cell strainer and red blood cells were lysed. For the enrichment of
leukocytes, the cells were subjected to density-gradient centrifugation in 40%/75% Percoll® (Sigma-Aldrich). The cells harvested from the interface were used in subsequent assays.

**Flow cytometry.** To characterize the cell surface phenotype, isolated cells were resuspended in PBS containing 10% FCS, 20 mM HEPES, and 10 mM EDTA. After blocking Fc receptors with anti-mouse CD16/CD32 (2.4G2, BD Biosciences, San Diego, CA, USA) for 15 min at 4 °C, the cells were stained for 30 min at 4 °C with the following antibodies: mAb against CCR3 (83101) from R&D Systems (Minneapolis, MN, USA); anti-SiglecF (E50-2440) from BD Biosciences; anti-CD45 (30-F11), F4/80 (BM8), CD3 (145-2C11), and B220 (RA3-6B2) from BioLegend (San Diego, CA, USA); and anti-CD4 (RM4-5) and CD8 (53–6.7) from eBioscience (San Diego, CA, USA). Each sample was analysed with a FACSCalibur™ flow cytometer (BD Biosciences) and the data were processed using FlowJo software (Tree Star, Ashland, OR, USA).

**Isolation of eosinophils.** Eosinophils were isolated from the peritoneal cavity of CD3-IL-5 transgenic mice as described and used for chemotaxis assay and co-culture with adipocyte-differentiated 3T3-L1 cells. Total cells were extracted and subjected to lymphocyte depletion using a MACS® system with antibodies against CD90.2 and CD45R (Miltenyi Biotec, Auburn, CA, USA). The purity and viability of the isolated cells were determined by flow cytometry and were consistently >95%.

**Adipogenesis of 3T3-L1 cells.** 3T3-L1 preadipocytes were cultured in Dulbecco’s Modified Eagle’s medium (DMEM) containing 10% FBS, 10 mg/mL streptomycin, and 100 U/mL penicillin at 37 °C in 5% CO₂. To induce adipocyte differentiation, at 2 days post-confluence, the cells were treated with 0.5 mM isobutylmethylxanthine (Sigma-Aldrich), 10 μM dexamethasone (Sigma-Aldrich), and 1 μg/mL insulin (Sigma-Aldrich) for 72 h, followed by maintenance in DMEM containing 10% FBS and 1 μg/mL insulin. To examine the effects of cytokines on adipogenesis, the 3T3-L1 cells were treated with IFN-γ (Peprotech, Rocky Hill, NJ, USA) or IL-4 (Peprotech) during their differentiation into adipocytes.

**ELISA.** The concentrations of CCL11 (eotaxin-1) and CCL24 (eotaxin-2) secreted from adipocyte-differentiated 3T3-L1 cells were measured by ELISA according to the manufacturer’s instructions (R&D Systems).

**Chemotaxis assay.** Chemotaxis was assayed using Transwell® inserts (Corning, Corning, NY, USA) with a 3-μm pore diameter. Differentiated 3T3-L1 cells were grown in DMEM containing 10% FBS at 450 μL per well until they achieved confluence. Eosinophils were loaded into each upper insert (200,000 cells in 450 μL medium). To examine the effect of chemokine blockade, anti-mouse CCL11 (Peprotech, Rehovot, Israel) was added (50 μg/mL) to the lower wells. After 24 h, the inserts were removed and the migrated cells were stained and enumerated by flow cytometry.

**Cell viability assay.** Eosinophils were cultured for 24 h with or without adipocyte-differentiated 3T3-L1 cells. Non-viability was determined by staining cells with 4′,6-diamidino-2-phenylindole dihydrochloride (Sigma-Aldrich) and analysing them by flow cytometry.

**Oil Red O staining.** To evaluate lipid accumulation, adipocyte-differentiated 3T3-L1 cells were stained with Oil Red O (Sigma-Aldrich) and analysing them by flow cytometry.

**Liver triglyceride quantification.** Liver triglycerides were determined with an assay kit according to the manufacturer’s instructions (Biovision, Palo Alto, CA, USA).

**Statistical analysis.** Two-group comparisons were performed by Student’s t-test or Mann-Whitney test. Data differences between groups were examined for statistical significance using two-way ANOVA with the Tukey post hoc test. A p-value < 0.05 was considered significant. The data are presented as the mean ± standard error of the mean. GraphPad Prism 5 (GraphPad, San Diego, CA, USA) was used for data analysis.

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Author Contributions
E.H.L. and M.I. researched data and wrote the manuscript. J.J., H.J.G., P.R., M.K.M., T.W., J.Y. and S.Y. researched data. J.Y.R. and C.S.C. analysed data and reviewed the manuscript. W.J.P. and A.M. analysed data, wrote and reviewed the manuscript, and contributed to discussion. Y.J. designed and supervised the studies, analysed data, and wrote and reviewed the manuscript.

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