SUPPLEMENTARY DATA

Supplementary information

RNA isolation and real-time PCR

Real-time PCR analysis was performed using the primers listed in Supplementary Table 1. Total RNA was extracted using TRIzol reagent (Life Technologies, Eugene, OR, USA), and complementary DNA (cDNA) was prepared using M-MLV reverse transcriptase and oligo-dT primers (Invitrogen, Carlsbad, CA, USA). Quantitative real-time PCR was performed using cDNA, 2× SYBR Green PCR Mix (Applied Biosystems, Foster City, CA, USA), and specific primers. Relative expression was calculated by normalizing against 18s ribosomal RNA using the 7500 Fast Real-Time PCR System (Applied Biosystems). The comparative Ct method was used to quantify the transcripts, expression of which was normalized to that of 18s RNA. Results were analyzed using the ΔΔCt method, and values are expressed as -fold differences.

Immunofluorescence staining

Epididymal adipose tissues were collected from mice injected with vehicle or αGC. The tissues were fixed in 1% paraformaldehyde for 1 h and then washed with tap water. Samples were then incubated for 1 h at room temperature in blocking solution containing 1% BSA in PBST (0.3% Triton X-100 in PBS). Next, samples were incubated for 24 h at 4℃ with anti-IL-13Rα1 (Abcam, Cambridge, MA, USA) diluted 1:500 in PBS with 1% BSA. After washing, samples were incubated with a fluorescently labeled secondary antibody (Alexa594 anti-rabbit; Life Technologies) for 24 h at 4°C. Neutral lipids in adipocytes were stained using 4,4-difluoro-1,3,5,7,8-penta-methyl-4-bora-3a, 4a-diaza-s-indacene (BODIPY, 1 μg/mL in PBS; Molecular Probes). Nuclei were stained using 4, 6-diamidino-2-phenylindole (DAPI; Sigma). After washing, samples were observed under a laser-scanning confocal microscope (FV1000, Olympus Corp., Tokyo, Japan).

Indirect calorimetry

Mice fed either standard chow or a HFD were placed into individual metabolic chambers, with free access to food and water. Oxygen consumption (VO_2), carbon dioxide production (VCO_2), EE (calculated according to the following formula: $1.44 \times VCO_2 \times (3.815 + 1.232 \times \text{respiratory quotient})$), and ambulatory activity were determined by indirect calorimetry using a Physical cage system (Oxylet, Panlab, Cornella, Spain). Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were measured over a 24 h period. Data were analyzed using the METABOLISM® software, version 2.2 (Oxylet, Panlab). Average values during light and dark periods were calculated. *P* values were calculated using Student's *t*-test.

Biochemical measurements

Blood was collected from the mouse heart under general anesthesia. Samples were centrifuged at 16,000 g for 5 min, and the supernatants were collected. Serum insulin (Alpco Diagnostics, Salem, NH, USA), GDF15 (R&D Systems), IL-13 (R&D Systems), IL-4 (eBioscience, San Diego, CA, USA), and IFN-γ (eBioscience) levels were measured by ELISA using kits obtained from the indicated suppliers.

Transfection of 3T3-L1 cells with vectors overexpressing STAT1, 3, or 6

3T3-L1 cells were plated in 12-well culture plates and transfected with the pCMV-STAT6 vector (1 μg), the pRc/CMV-STAT1-flag vector, or the pRC/CMV-STAT3-flag vector using Lipofectamine PLUS (Life Technologies, Eugene, OR, USA). The pRc/CMV-STAT1-flag and pRC/CMV-STAT3-flag vectors were kindly provided by Prof. Jacqueline Bromberg (Weill Cornell Medicine, New York). Next, transfected cells were treated with recombinant mouse IL-13 (100 ng/mL) or IL-4 (100 ng/mL). After 24 h, the amount of GDF15 protein in the supernatant from 3T3-L1 cells exposed to recombinant IL-13 or IL-4 was measured. All assays were performed at least in triplicate.

Measurements of glucose-stimulated insulin secretion from islets

Islets were isolated from 10-week-old wild-type mice fed a normal chow diet. Cultured islets were maintained for 24 hours in complete medium with recombinant IL-13 (10 ng/mL) or saline. On the day of the experiment, batches of 10 islets were hand-picked, starved for 1 h in KRBH/BSA 2.8 mM glucose, and then stimulated for 1 h at 37 °C in KRBH/BSA containing basal glucose (2.8 mM), high glucose (16 mM), or glucose (2.8 mM) with

potassium chloride. At the end of the incubation, the supernatants were collected to measure insulin release, and cellular insulin contents using an insulin ELISA kit (Alpco Diagnostics, Windham, NH, USA)

Growth differentiation factor 15 mediates systemic glucose regulatory action of T helper type 2 cytokines

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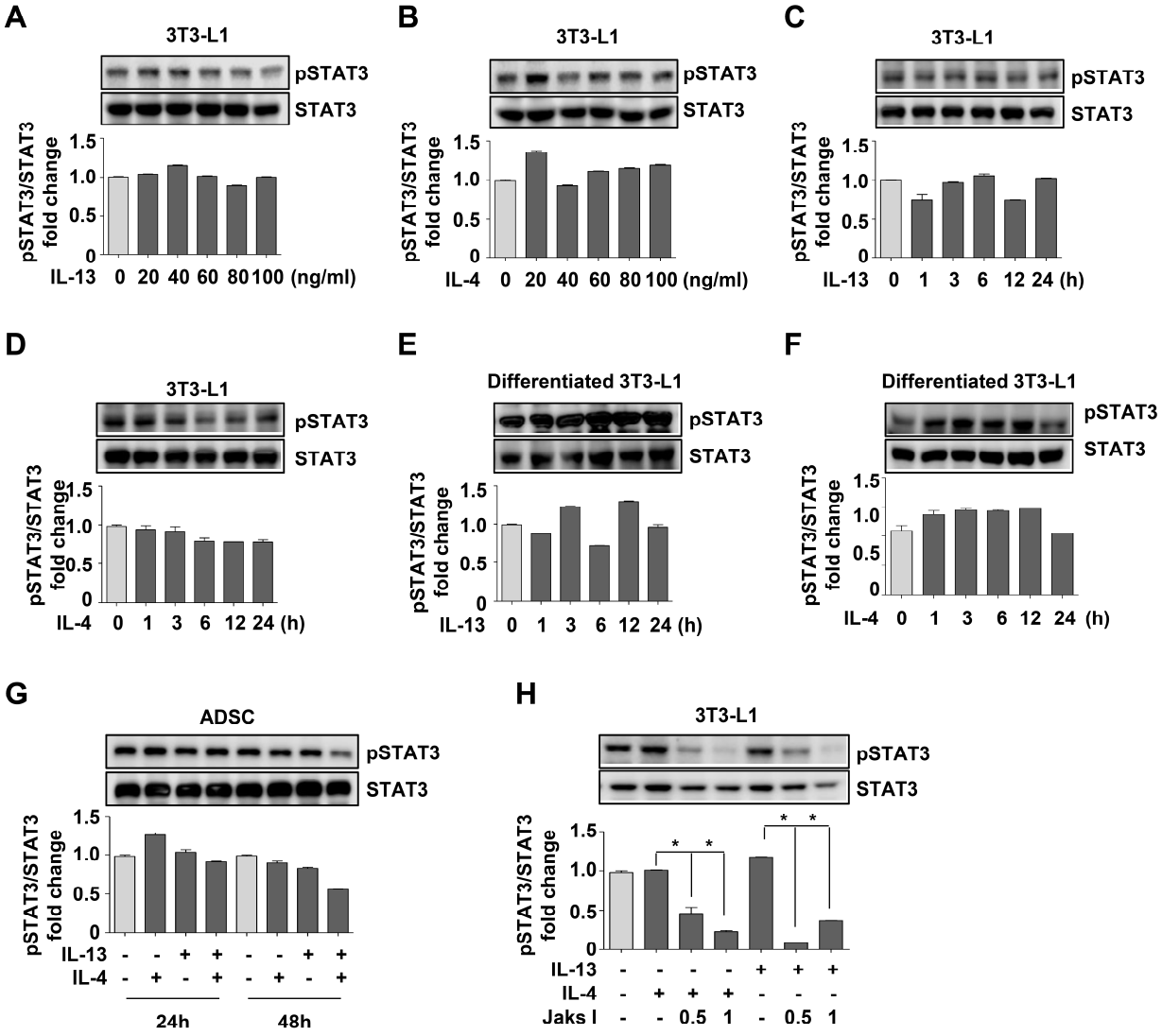
Running head: Th2 cytokines regulate GDF15 in adipose tissue

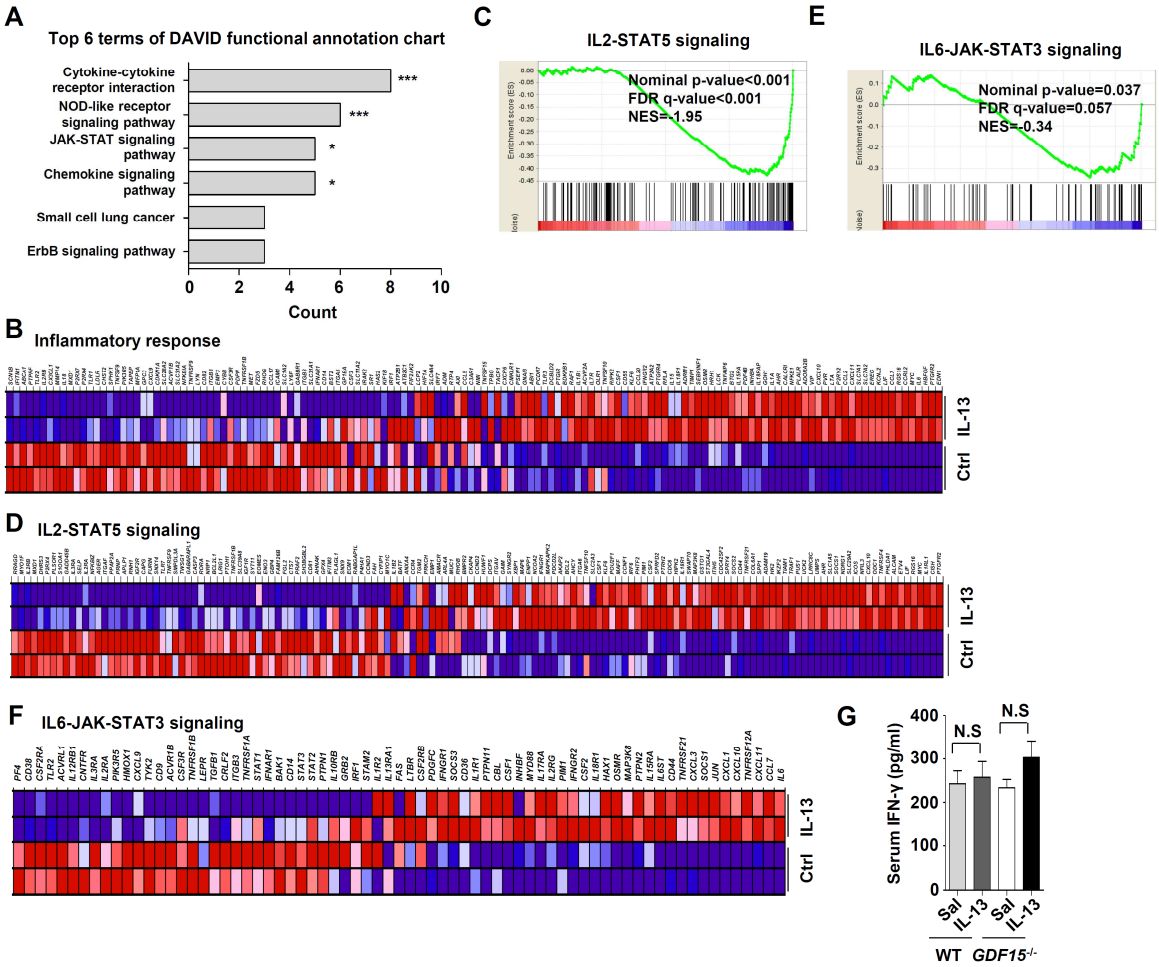
SUPPLEMENTARY DATA

Supplementary Figure 1. Th2 cytokines are not able to phosphorylate STAT3 in cultured adipocytes. (A–D) Western blot analysis of pSTAT3 and STAT3 expression by 3T3-L1 adipocytes treated with recombinant IL-4, IL-13, or vehicle. (E, F) Western blot analysis of pSTAT3 and STAT3 expression by differentiated 3T3-L1 adipocytes treated with recombinant IL-4, IL-13, or vehicle. (G) Western blot analysis of pSTAT3 and STAT3 expression by ADSCs treated with recombinant IL-4, IL-13, or vehicle. (H) Western blot analysis of pSTAT3 and STAT3 expression by 3T3-L1 adipocytes treated with recombinant vehicle, IL-4, IL-13, and/or JAK inhibitors. (A–H) Relative quantification of each protein was performed using densitometry. All data are representative of three independent experiments and are expressed as the mean ± SEM. *P < 0.05, versus the corresponding controls.



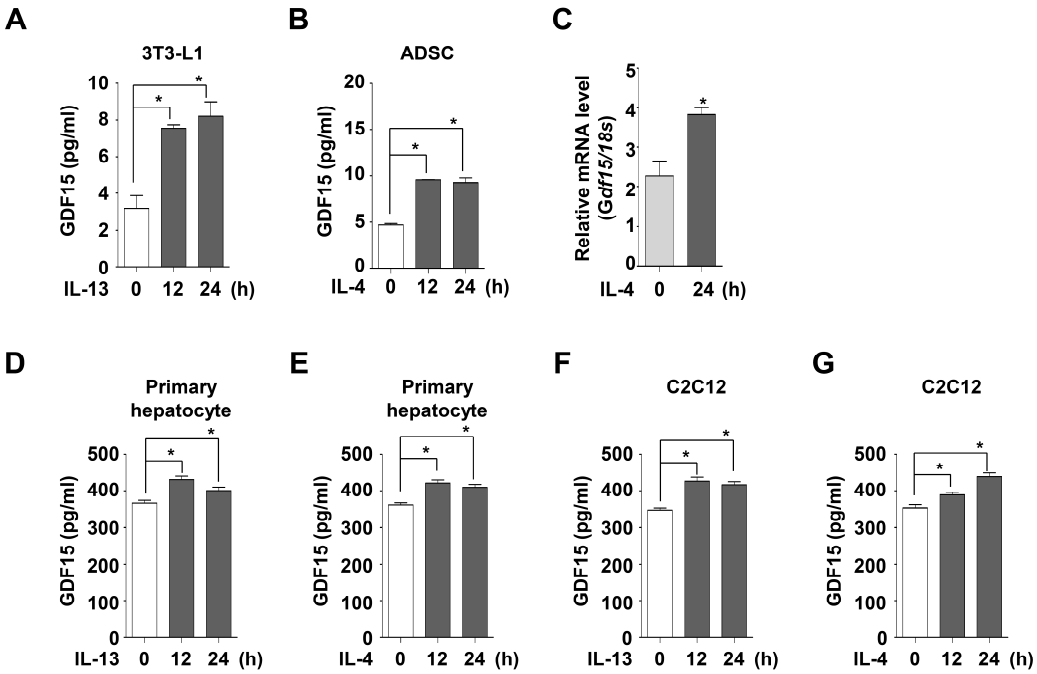
SUPPLEMENTARY DATA

Supplementary Figure 2. RNAseq analysis of cultured adipocytes treated with saline or recombinant IL-13. (A) Representative plot ranking of the top ten Gene Ontology (GO) biological process terms associated with upregulated genes (based on counts). (B) Heatmap of genes related to inflammatory responses. (C) Results of Gene Set Enrichment Analysis of IL-2-STAT5 signaling. (D) Heatmap of genes associated with IL-2-STAT5 signaling. (E) Results of Gene Set Enrichment Analysis of IL6-JAK-STAT3 signaling. (F) Heatmap of genes associated with IL6-JAK-STAT3 signaling. Data are expressed as the mean ± SEM. (G) Serum levels of interferon gamma in wild-type or GDF15 KO mice treated with recombinant IL-13 or saline. *P < 0.05 and ***P < 0.001, compared with the corresponding controls.



SUPPLEMENTARY DATA

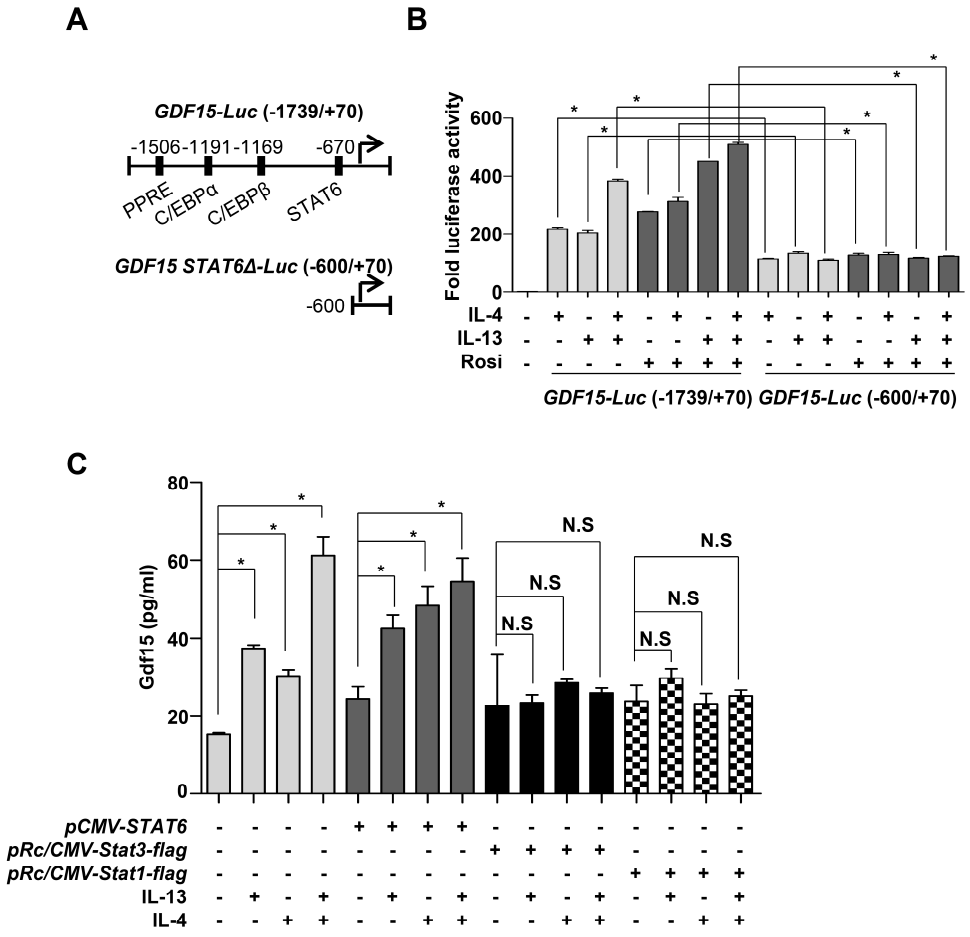
Supplementary Figure 3. Th2 cytokines induce GDF15 production by cultured adipocytes.
(A) GDF15 levels in supernatants from 3T3-L1 adipocytes treated with recombinant IL-13 for 12 or 24 h. (B) GDF15 levels in supernatants from ADSCs treated with recombinant IL-4 for 12 or 24 h. (C) *Gdf15* expression by 3T3-L1 adipocytes treated with recombinant IL-4 for 0 or 24 h. (D, E) GDF15 levels in supernatants from primary hepatocytes treated with recombinant IL-4 or IL-13 for 12 or 24 h. (F, G) GDF15 levels in supernatants from C2C12 myotubes treated with recombinant IL-4 or IL-13 for 12 or 24 h. All data are representative of three independent experiments and are expressed as the mean ± SEM. *P < 0.05, versus the corresponding controls.



SUPPLEMENTARY DATA

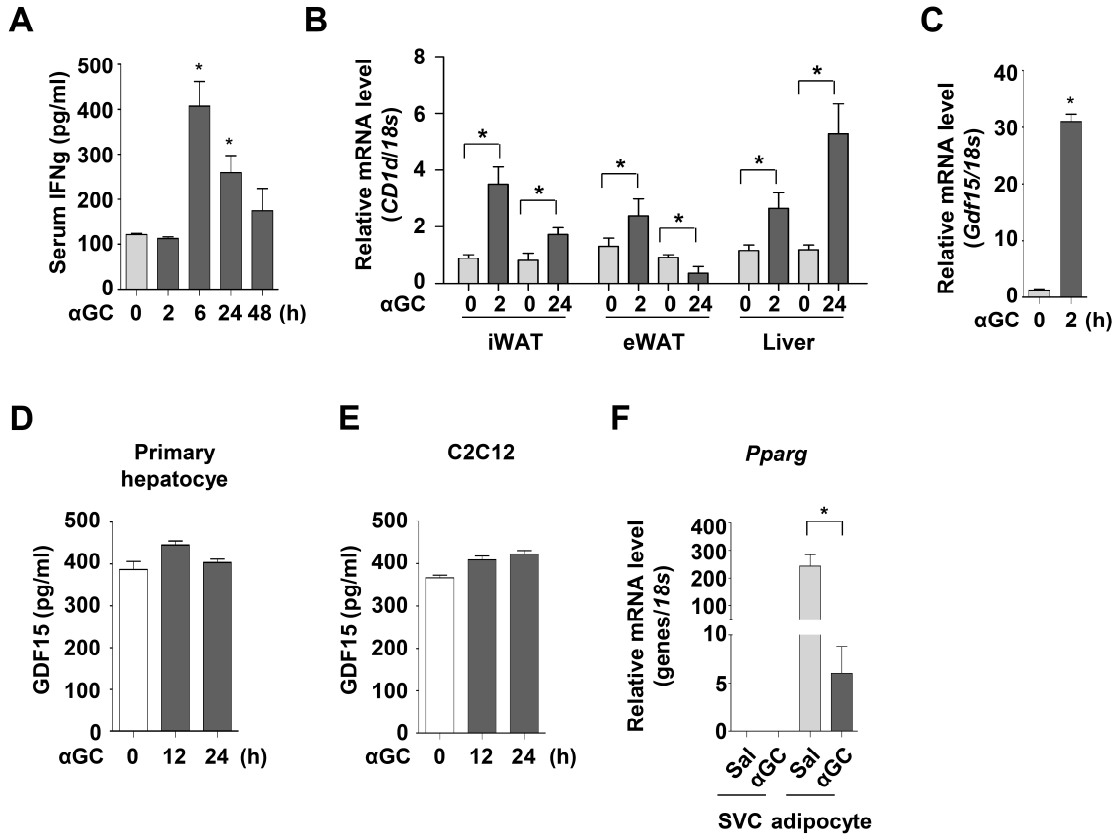
Supplementary Figure 4. STAT6 is critical for GDF15 expression by cultured adipocytes treated with Th2 cytokines.

(A) Response element of the GDF15 luciferase reporter (-1,739/+70) and a deletion mutant (-600/+70) of the human *GDF15* promoter. (B) Luciferase reporter activity of the *GDF15* promoter and the STAT6 deletion mutant of the *GDF15* promoter in response to recombinant IL-13, IL-4, or rosiglitazone. (C) Amount of GDF15 in supernatants from cultured adipocytes transfected with an overexpression vector harboring STAT1, 3, or 6, and exposed to recombinant IL-4 or IL-13. All data are representative of three independent experiments and are expressed as the mean ± SEM. *P < 0.05, versus the corresponding controls.



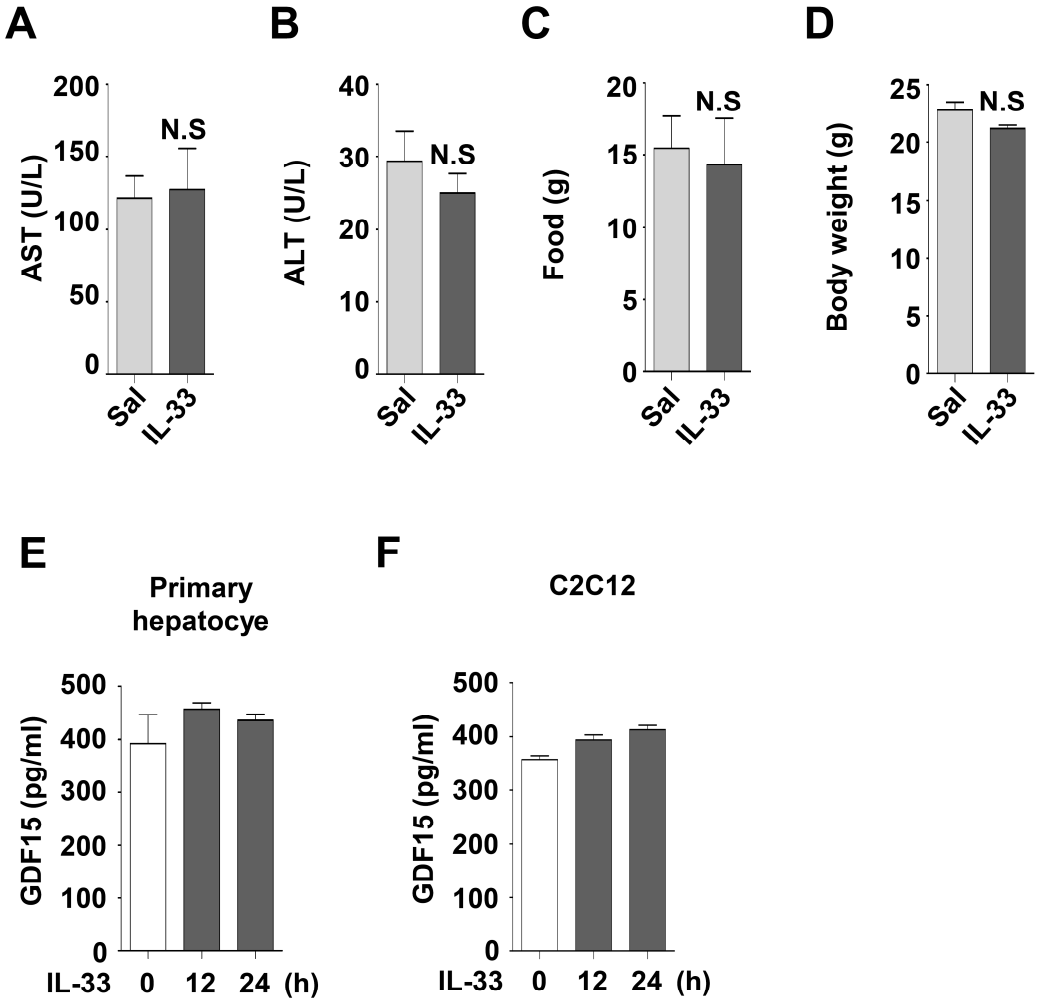
SUPPLEMENTARY DATA

Supplementary Figure 5. αGC increases GDF15 expression in adipose tissue.
(A) Serum levels of interferon gamma in mice treated with αGC. (B) *CD1d* expression in adipose tissues and liver from mice treated with αGC. (C) *Gdf15* expression in adipose tissue from mice treated with αGC for 0 or 2 h. (D, E) GDF15 levels in supernatants from primary hepatocytes and C2C12 myotubes treated with αGC. (F) *Pparg* expression in SVCs and adipocytes from mice treated with saline or αGC. All data are representative of three independent experiments and are expressed as the mean ± SEM. *P < 0.05, versus the corresponding controls.



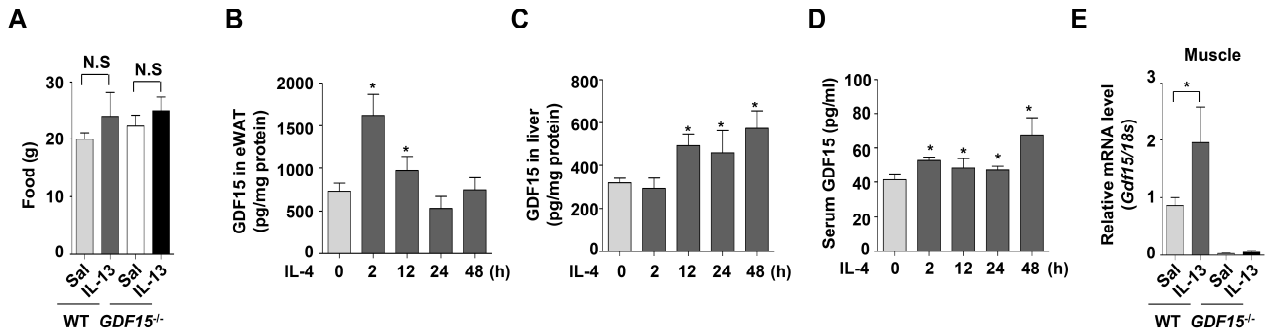
SUPPLEMENTARY DATA

Supplementary Figure 6. IL-33 has no effect on liver damage and body weight.
(A, B) Levels of AST and ALT in serum from mice treated with recombinant IL-33 or saline. (C, D) Differences in food consumption and body weight of mice treated with recombinant IL-33 or saline. (E, F) GDF15 levels in supernatants from primary hepatocytes and C2C12 myotubes treated with recombinant IL-33. All data are representative of three independent experiments and are expressed as the mean ± SEM.



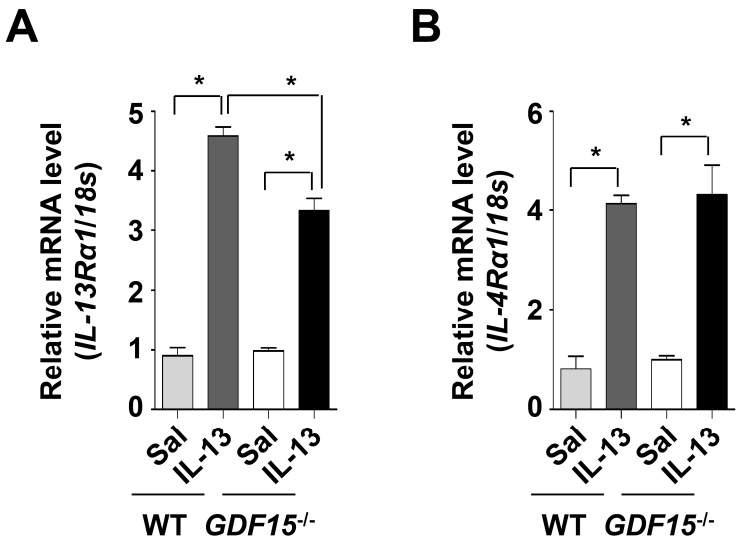
SUPPLEMENTARY DATA

Supplementary Figure 7. IL-4 induces GDF15 expression in adipose tissue and liver.
(A) Daily food intake of wild-type or GDF15 KO mice treated with recombinant IL-13 or saline. (B) Levels of GDF15 protein expression in eWAT or liver from mice treated with recombinant IL-4 for 48 h. (D) Serum levels of GDF15 in mice treated with recombinant IL-4 for 48 h. (E) Real-time PCR analysis of *Gdf15* expression in muscle from WT and GDF15 KO mice treated with saline or recombinant IL-13. All data are representative of three independent experiments and are expressed as the mean ± SEM. *P < 0.05, versus the corresponding controls.



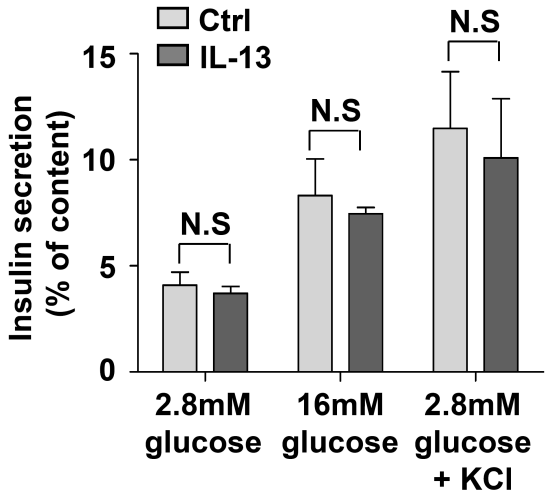
SUPPLEMENTARY DATA

Supplementary Figure 8. GDF15 promotes IL-13-induced receptors expression in adipose tissue.
(A, B) Relative expression of *IL-13Rα1* and *IL-4Rα1* mRNA of adipose tissues from wild-type and GDF15 KO mice treated with saline or recombinant IL-13. All data are representative of three independent experiments and are expressed as the mean ± SEM. *P < 0.05, versus the corresponding controls.



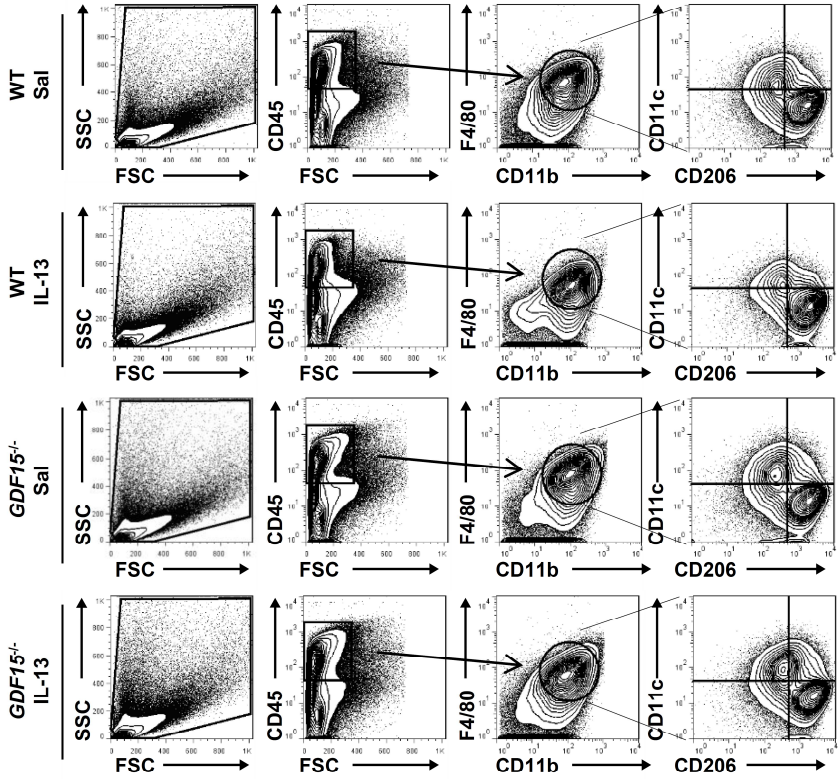
SUPPLEMENTARY DATA

Supplementary Figure 9. Recombinant IL-13 does not alter glucose-stimulated insulin secretion in islet. Glucose-stimulated insulin secretion from islets treated with recombinant IL-13 (10 ng/ml) or saline. All data are representative of three independent experiments and are expressed as the mean ± SEM. *P < 0.05, versus the corresponding controls.



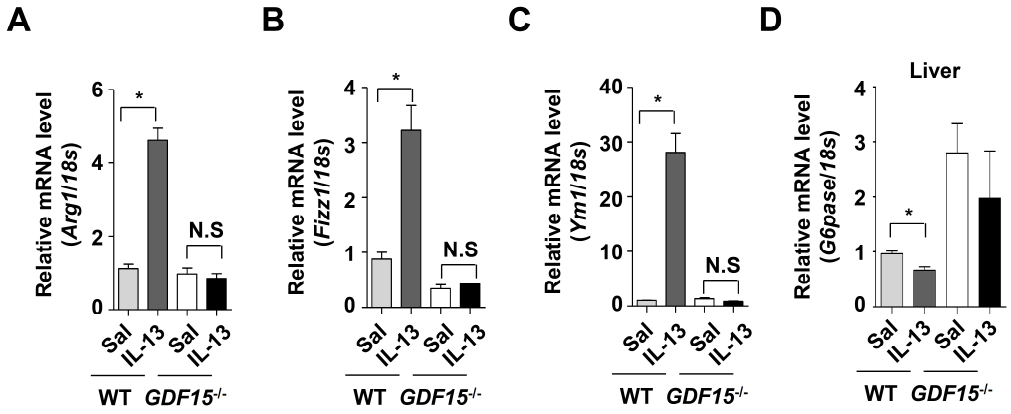
SUPPLEMENTARY DATA

Supplementary Figure 10. Gating strategy for FACS analysis of adipose macrophages.
Adipose SVCs were first gated for single cells (forward scatter vs. side scatter) and isolated $CD45^+$ cells (forward scatter-area vs. CD45). The $CD45^+$ cells were further analyzed using CD11b and F4/80 antibodies. The $CD45^+CD11b^+F4/80^+$ cells were analyzed using CD11c and CD206 antibodies. $CD11c^+CD206^-$ cells are considered to be M1 macrophages, and $CD11c^-CD206^+$ cells are regarded as M2 macrophages.



SUPPLEMENTARY DATA

Supplementary Figure 11. GDF15 promotes IL-13-induced M2 macrophage polarization in adipose tissue. (A–D) Real-time PCR analysis of adipose tissues from wild-type and GDF15 KO mice treated with saline or recombinant IL-13. All data are representative of three independent experiments and are expressed as the mean ± SEM. *P < 0.05, versus the corresponding controls.



SUPPLEMENTARY DATA

Supplementary Table 1. Primers used for real-time PCR

Gene	Forward	Reverse
18s	CTGGTTGATCCTGCCAGTAG	CGACCAAAGGAACCATAACT
IL-13Ra1	TTCCAGTCTTTGTCGCAGTG	CAGGATCAGGAATTGGAGGA
IL-4Ra1	AGGCCCCAGTACAGAATGTG	TCTCAGGTGACATGCTCAGG
Ccl11	GAATCACCAACAACAGAT GCAC	ATCCTGGACCCACTTCTTCTT
Il6	CCGGAGAGGAGACTTCACAG	CAGAATTGCCATTGCACAAC
Ccl7	AAGTGGGTCGAGGAGGCTAT	CTTTGGAGTTGGGGTTTTCA
Ccl2	CCCAATGAGTAGGCTGGAGA	TCTGGACCCATTCCTTCTTG
Ctgf	CACTCTGCCAGTGGAGTTCA	GTAATGGCAGGCACAGGTCT
Gdf15	GAGCTACGGGGTCGCTTC-3	GGGACCCCAATCTCACCT
Lif	GTGGAGCTGTATCGGATGGT	AGTGGGGTTCAGGACCTTCT
Angptl4	TCAAAGACTCCGAGGATAGA	AAAGCCCTTTTCGTAGTTTT
Cxcl1	TGGCTGGGATTCACCTCAAGAACA	TGTGGCTATGACTTCGGTTTGGGT
Cxcl12	GCTCTGCATCAGTGACGGTA	AGATGCTTGACGTTGGCTCT
Tgfbi	TGATAAGAGGGGACGGTTTG	GTCTCCCTTCAGGACATCCA
Perilipin	CTGTGTGCAATGCCTATGAGA	GATCTTTTCCTCCAGGTGGTC
CD1d	ATCAAGGGACAAGTGCAACC	TGACTTCCCTGCCTCTAGGA
Arg1	ATGGAAGAGACCTTCAGCTAC	GCTGTCTTCCCAAGAGTTGGG
Ym1	GGGCATACCTTTATCCTGAG	CCACTGAAGTCATCCATGTC
Fizz1	CCTTCTCATCTGCATCTCCCTG	GCTGGATTGGCAAGAAGTTCC
Tnfa	CCCCAAAGGGATGAGAAGTT	CACTTGGTGGTTTGCTACGA
IL1b	GACCTTCCAGGATGAGGACA	TGTTCATCTCGGAGCCTGTA
iNOS	CACCTTGGAGTTCACCCAGT	ACCACTACTCGTACTTGGGATGC
G6pase	TCTGTCCCGGATCTACCTTG	TGAGAATCCAAGCGCGAAAC