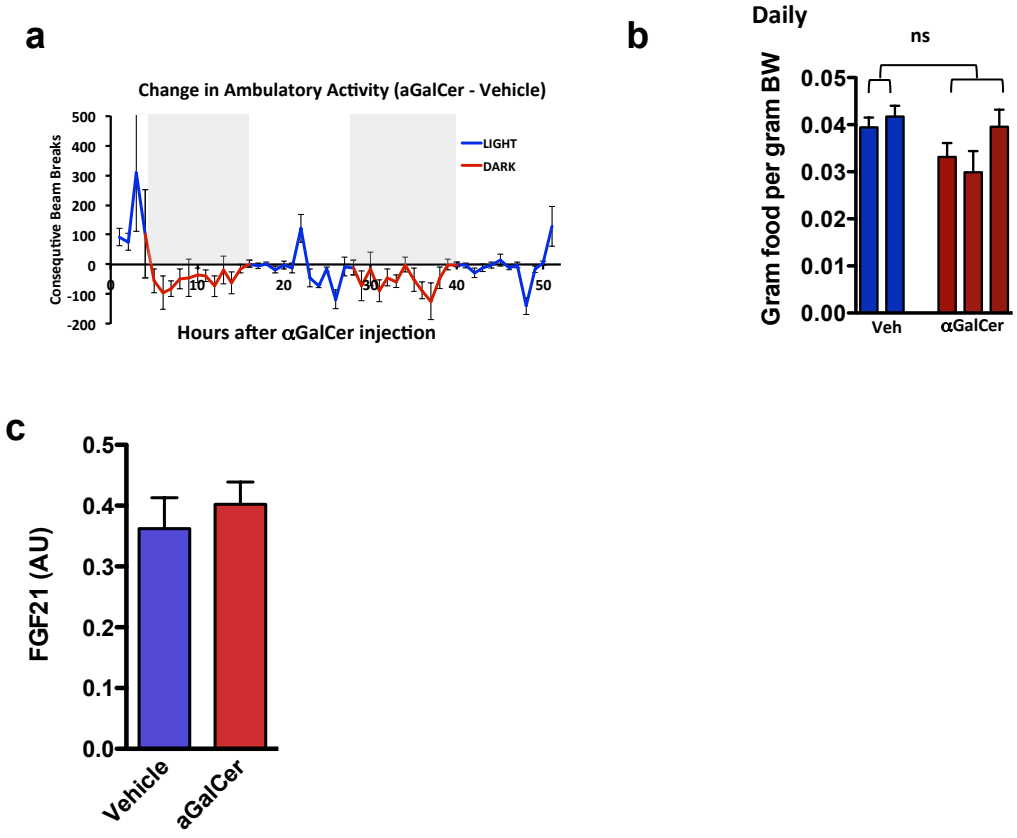


**Figure S1, related to Figure 1: CLAMS analysis measuring movement and food intake of obese WT mice following aGalCer injection**

A) Change in ambulatory movement obese WT mice after receiving αGalCer compared to vehicle i.p. Grey bars indicate dark periods B) Daily food intake in WT obese after receiving αGalCer or vehicle i.p. C) qPCR of FGF21 transcript in liver of obese WT mice treated with one injection of aGalCer or vehicle control.

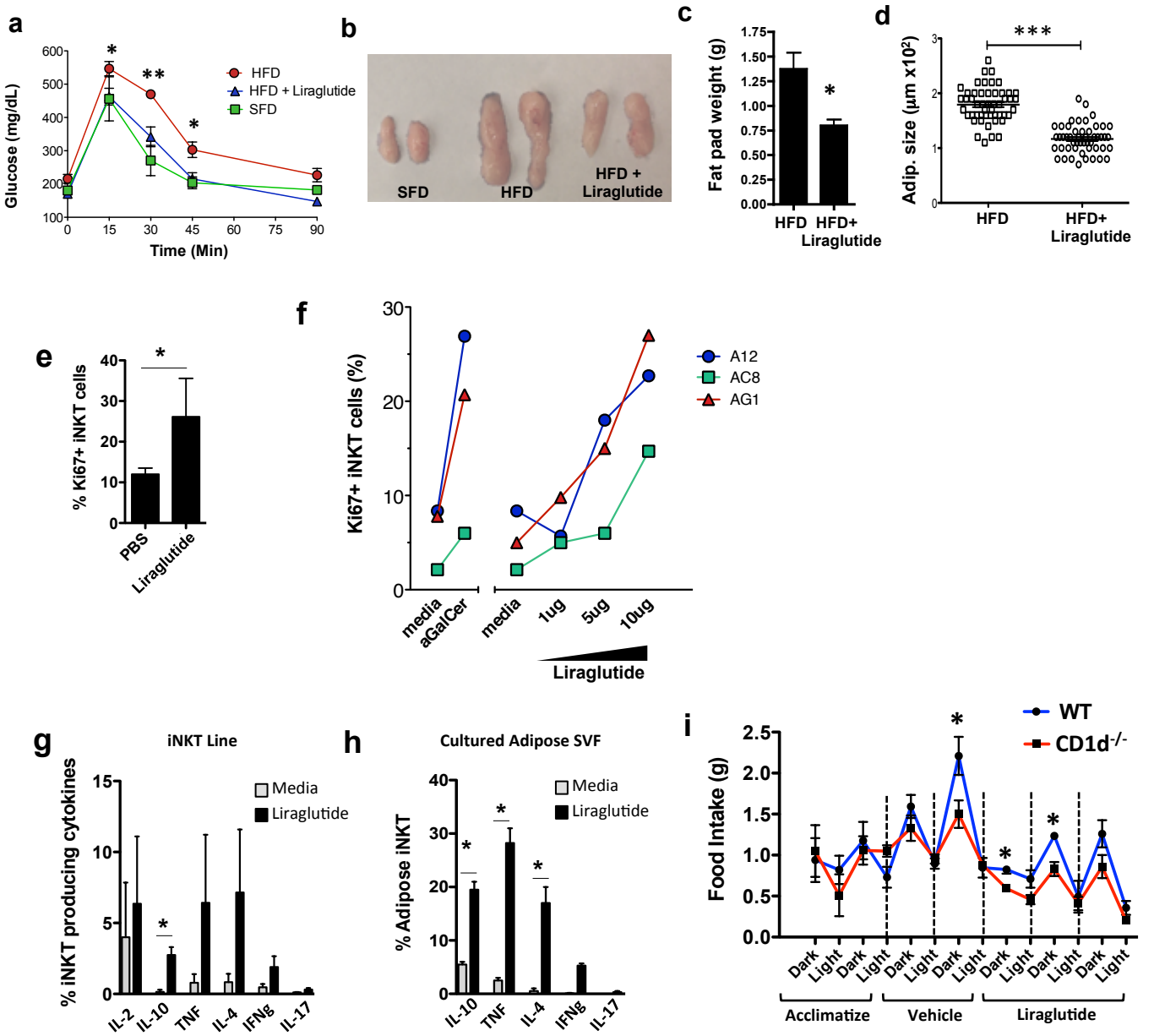


**Figure S2, related to Figure 3: Liraglutide induces metabolic improvements and activates iNKT cells**

A) Glucose tolerance test in WT mice on SFD or HFD receiving a daily injection of Liraglutide. Epididymal fat pad image (B) and weight (C) and adipocyte size (D) of WT mice on SFD or HFD 5 days after receiving a daily injection of Liraglutide or PBS. E) Liraglutide injection induces proliferation of iNKT cells in vivo as measured by Ki67 gated on CD1d-tetramer positive cells. F) Liraglutide activated iNKT cells directly in vitro. 3 primary cultured iNKT cell lines (A12, AC8 and AG1) were incubated *in vitro* with several doses of liraglutide for 18 hrs, or with aGalCer as a positive control for iNKT cell activation and proliferation. Intranuclear Ki67 staining was performed to measure iNKT cell proliferation. G) Intracellular staining for cytokines in iNKT cell lines (G) or adipose iNKT cells (H) cultured with or without liraglutide. I) Liraglutide induces significant decreases in food intake in both WT and CD1d-/- mice. Food intake in obese WT (blue) and CD1d$^{-/-}$ (red) mice during periods of acclimatization, control PBS injection and liraglutide injection. Grey bars indicate dark periods. Graphs show the mean (± s.e.m). Statistics have been calculated using a Student unpaired t-test or ANOVA with Tukey post-hoc test for groups of 3 or more. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

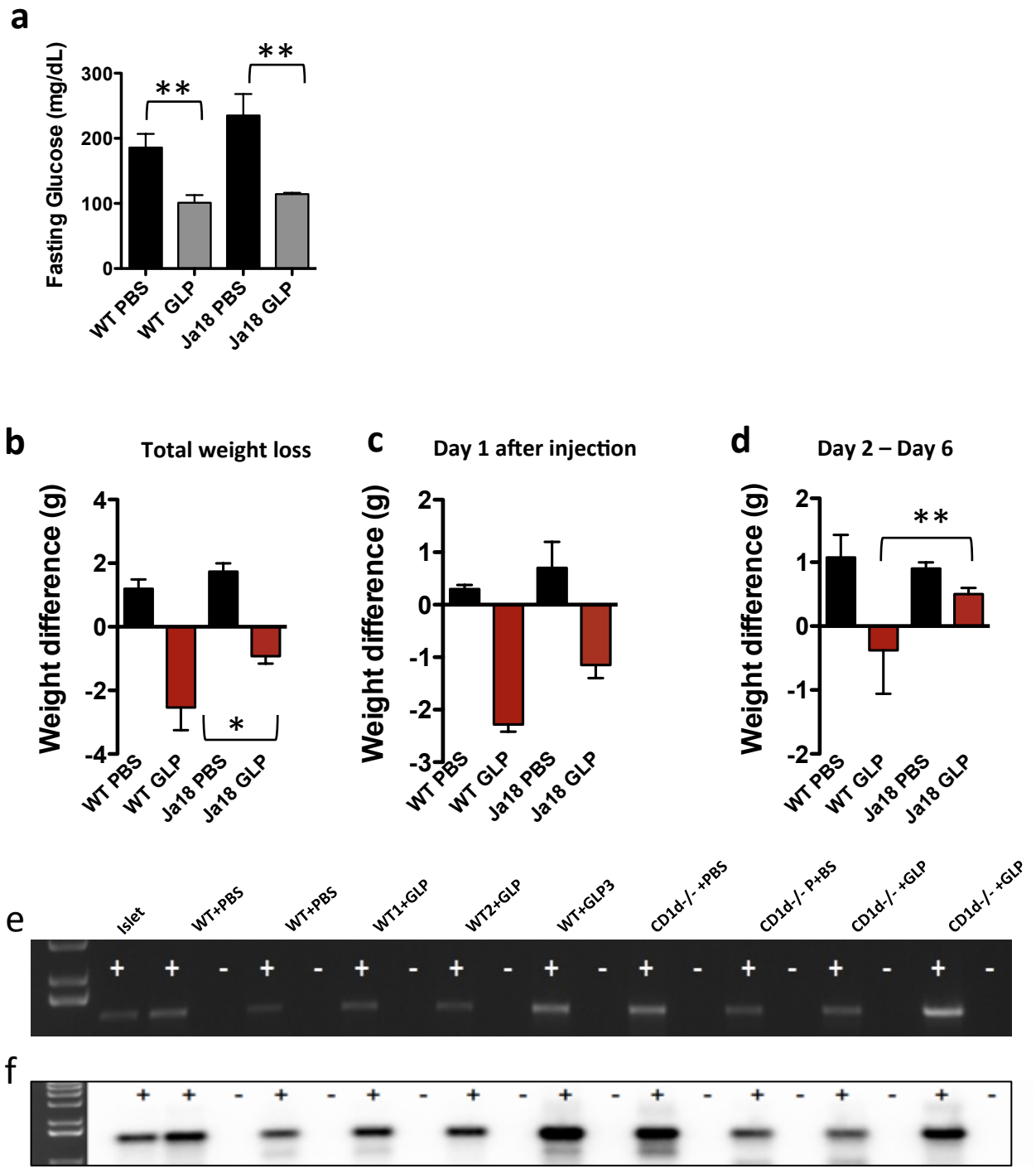


**Figure S3, related to Figure 3: Differential effect of liraglutide on WT versus Ja18$^{-/-}$ mice**
A) Fasting glucose levels in obese WT and Ja18$^{-/-}$ mice after treatment with liraglutide or PBS control for 5 days. B) Total weight loss in grams (g) after 5 days of Liraglutide treatment compared to PBS. C) Weight loss after 24hrs, and D) between 24 hours and 120hrs of Liraglutide treatment. E) PCR products using mGLP1R (1.338kb) primers on islets as a positive control, and hypothalmii from wt and CD1d$^{-/-}$ mice after i.p. injection with PBS or Liraglutide. RT+ = reverse transcriptase positive, RT- = reverse transcriptase minus. F) Southern blot of PCR products.

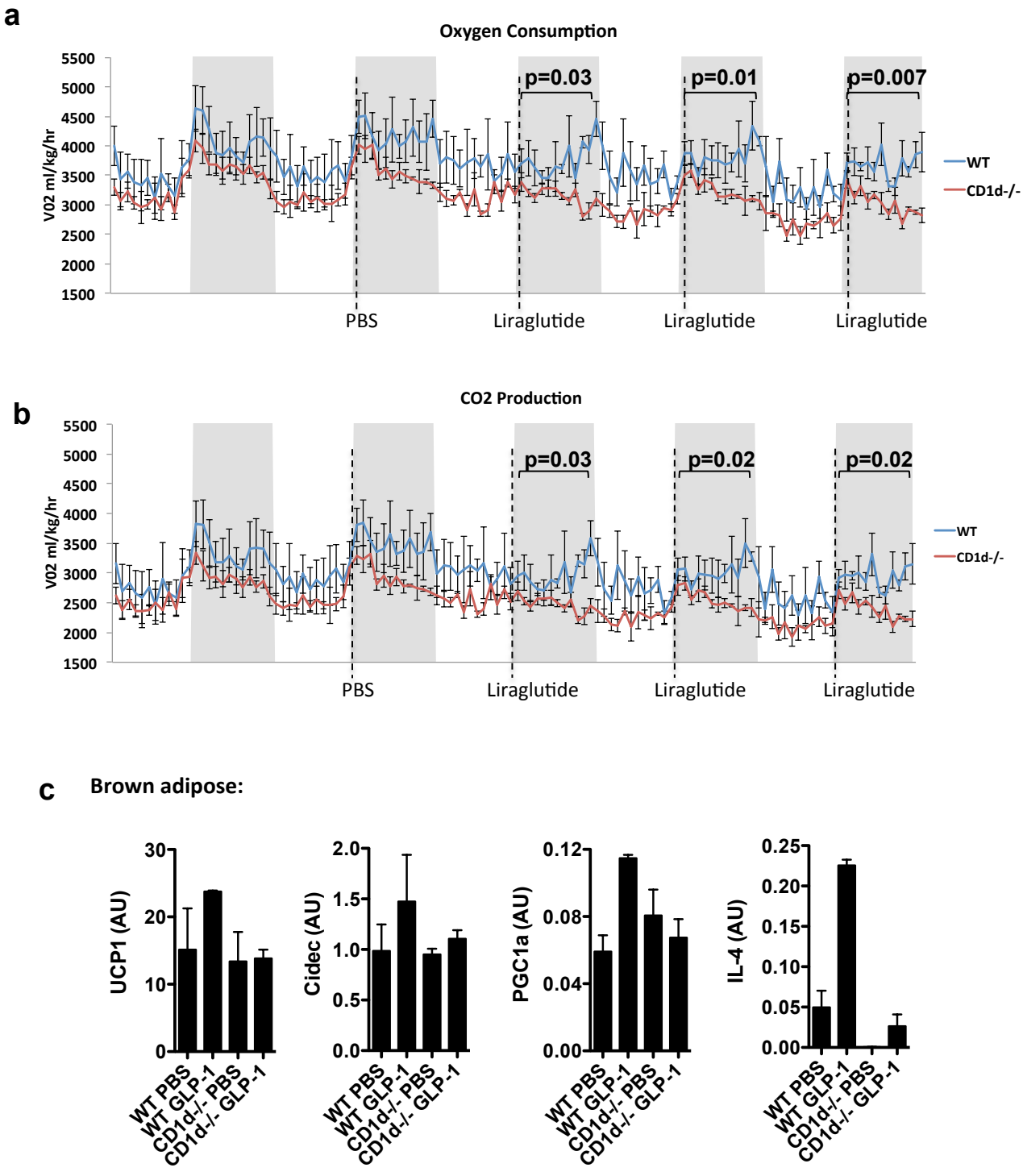


**Figure S4, related to Figure 3: CLAMS analysis and BAT qPCR of obese WT and CD1d$^{-/-}$ mice on liraglutide treatment** A) $VO_2$ and B) $CO_2$ in obese WT and CD1d$^{-/-}$ mice after PBS or liraglutide injection. C) RNA transcript levels (arbitrary units) of UCP1, Cidea, PGC1a and IL-4 in brown adipose tissue (BAT) from obese WT and CD1d-/- mice after treatment with PBS control or liraglutide for 5 days.

## Supplemental Materials and Methods

### GLP1 analog treatment

**Patients:** Ten participants with type 2 diabetes who were starting therapy the GLP-1 analogue in line with good clinical practice were enrolled. Each self-administered liraglutide, by subcutaneous injection, at a dose of 0.6mg once daily for two weeks and then 1.2mg once daily. Before and after 8 weeks of therapy the research participants attended for a visit in the early morning after an overnight fast. At this visit reported adherence to treatment was assessed, weight recorded and sample of blood was taken. The sampled blood was used to determine metabolic status and to assess peripheral iNKT levels. Informed consent was obtained from all participants and approval to conduct this study was obtained from the St Vincent's Healthcare Group Ethics and Medical Research Committee.

**Mice:** C57BL/6, mice were purchased from Jackson Laboratory. $CD1d^{-/-}$ and $Ja18^{-/-}$ were provided by Mark Exley (Harvard Medical School). FGF21-null mice were kindly provided by Eli Lily. FGF21-null mice were generated as previously reported (Badman et al., 2009). For targeted disruption of the Fgf21 locus, a 6.5-kb NheI genomic fragment containing all three exons of the mouse FGF21 gene was subcloned and used as a gene targeting vector by replacing part of exon 1 (30 bp downstream of the ATG), all of exon 2, and the 5′ region of exon 3 with a neomycin resistance gene (pGTN29; New England Biolabs, Ipswich, MA) thus deleting approximately 1200 bp of the genomic FGF21 sequence and deleting the 3′ part of exon 1, all of exon 2, and the 5′ region of exon 3. Founder mice were subsequently backcrossed onto the C57/BL6 line at least 10 times before investigation. In almost all experiments, male mice were used, with the exception of liraglutide treatments in WT and $CD1d^{-/-}$ where male and female mice were used and compared to male and female WT mice. Mice were bred in our specific-pathogen-free facilities at Harvard Medical School. All animal work was approved by and in compliance with the Institutional Animal Care and Use Committee guidelines of The Dana Farber Cancer Institute and Harvard Medical School.

**Tissue processing**

The visceral (epididymal), subcutaneous, peri-renal and brown adipose depots were isolated from the mice and digested with 20 mL collagenase solution (1mg/mL collagenase in PBS) at 37°C for 25 min with shaking. After digestion, adipocytes were isolated by filtering through a 40um nylon mesh and centrifuged for 5 min at 300g to pellet the stromovascular fraction (SVF). The supernatant was discarded and the SVF was washed with FACS buffer (1%FBS and 0.02% NaN3 in PBS), then resuspended in FC block at 4°C for 15 min and then stained with desired antibodies.

**Flow cytometry**

Single-cell suspensions were incubated with Fc receptor–blocking antibody before being stained on ice with specific antibodies and tetramer. Dead cells were excluded with live/dead gate using 7-AAD, or Zombie Aqua (BioLegend) for fixed and permeabilized cells. αGalCer analog PBS-57-loaded or empty CD1d tetramers were provided by the NIH tetramer facility (Emory Vaccine Center, Atlanta). For analysis of iNKT cells, a dump gate with CD19 was used to eliminate non-specific staining, and staining of the same cell preparations with 'empty' tetramer (no αGalCer) confirmed specificity. Antibodies conjugated to fluorescein isothiocyanate, phycoerythrin, phycoerythrin-indotricarbocyanine, peridinine chlorophyll protein complex 5.5, allphycocyanin or allophycocyanin-indotricarbocyanine were as follows: anti-IL-4 (11B11), -IFN-γ (XMG1,2), -IL-10 (JES5-16E3), -TCRβ (G572597). For staining for cytokines, cells were fixed and permeabilized with the Fix/Perm buffer set (eBioscience). Cell doublets were excluded by comparison of side-scatter width to forward-scatter area. For intracellular cytokine staining, single-cell suspension of adipose tissue SVFs were obtained as before, but with the inclusion of Brefeldin A in all media or *in vivo*. First, adipose SVF cells were stained with cell-surface-labelling CD3 mAb and αGalCer-loaded CD1d tetramer. Cells were then fixed, permeabilized, and stained intracellularly for Ki-67, IL-4, IL-10, and IFN-γ using the Cytofix/Cytoperm

kit (BD Biosciences), according to the manufacturer's instructions. For human iNKT cell identification, anti-human 6b11 antibody was used against anti-CD3, after gating on CD45+ cells that were negative for CD19 and CD14 (dump gate).

**In Vivo treatment of mice, including stimulation of iNKT Cells**

Mice were injected i.p. with a single injection of αGalCer or vehicle in 150 ul volume. Mice weighing between 25-42g received 1ug of αGalCer, and mice weighing more than 42g received 1ug of αGalCer. For liraglutide treatment, mice were fed a high fat diet and where randomly assigned to control or GLP-1 treated groups. Mice received daily injection of GLP-1 analogue (50ug/kg I.P) for 5 days. As a positive control for activation of brown adipose tissue through b-adrenergic stimulation, CL316,243 (Sigma-Aldrich) at 1 mg/kg was injected i.p.

**CLAMS analysis**

Wild type C57Bl/6 mice were maintained on a high fat diet before entering the comprehensive laboratory animal monitoring system (CLAMS). Each mouse served as its own control. The mice were singly housed and acclimated to the system for 48 hours then administered a vehicle control (150 ul PBS) by i.p. injection. These mice were further monitored for 70 hours before i.p. injection administration of 1μg αGalCer in 150 μl volume. These mice were monitored for 70 hours. Body weights were measured at the start and end of acclimation, vehicle injection and αGalCer injection. The CLAMS measures each parameter (oxygen consumption, $CO_2$ production, respiratory quotient, locomotor activity, food intake and body temperature) approximately every 10 minutes. For the data analysis, the measurements for each parameter were averaged over every hour for each mouse. For the αGalCer experiment, the data are presented based on the change in response of each mouse to the vehicle versus αGalCer treatment. These changes were averaged. To calculate change from vehicle by αGalCer injection the averages per hour of each mouse post vehicle injection were

subtracted from the averages per hour post αGalCer injection, so that the diurnal variation in measurements was maintained.

**Thermal imaging of body temperature**

Measurement of body temperature was performed using a thermal imaging camera (T300 InfraRed Camera; FLIR Systems). Tail temperature of obese WT mice 48 hours after αGalCer or vehicle treatment was analysed using FLIR Reporter 8.5 software (FLIR Systems). Images were acquired by anesthesizing mice and taking 2–3 images of each mouse from different angles were acquired to minimize temperature variations due to different postures of the animal. Software tools were used to point the region of interest at 1 cm and 2 cm down on the tail and average body temperature was calculated using area in each mouse.

**Statistics**

The continuous outcomes were expressed as the mean ± S.E.M. The changes of outcomes between baseline and the follow-up were evaluated using paired t tests. The difference between treatment groups were tested using unpaired two sample *t* tests with Welsh's correction for unequal variances and one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. $P \leq 0.05$ was considered to denote significance. No exclusion of data points or mice was used. Pilot studies were used for estimation of the sample size required to ensure adequate power. Mice that received treatment were randomized per cage so that each cage contained mice with test treatment versus control treatment (eg. αGalCer vs vehicle, or liraglutide vs. PBS).