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(54) Title: TARGETING GAMMA-DELTA T CELLS IN OBESITY AND CACHEXIA

(57) Abstract: Methods of promoting or inhibiting weight loss by modulating activity levels of $\gamma\delta$ T cells.

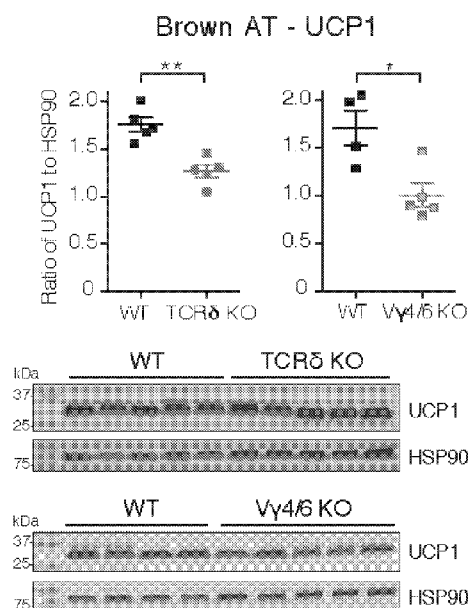


FIG. 7B

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Targeting gamma-delta T Cells in Obesity and Cachexia

CLAIM OF PRIORITY

This application claims priority under 35 USC §119(e) to U.S. Patent Application Serial No. 62/491,753, filed on April 28, 2017. The entire contents of the foregoing are hereby incorporated by reference.

FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with Government support under Grant Nos. AI11304603 and AI113046 awarded by the National Institutes of Health. The Government has certain rights in the invention.

TECHNICAL FIELD

Described herein are methods of promoting or inhibiting weight loss by modulating activity or levels of $\gamma\delta$ T cells.

BACKGROUND

Adipose tissue harbors a unique immune compartment that is important for physiologic responses to fasting and feeding, regulation of body weight, and thermogenesis. Compared to lymphoid organs, approximately 80-90% of the adipose immune system is innate. Much of what we know about the adipose tissue immune system suggests its major roles are not focused on fighting infection. Instead, obesity studies reveal that perturbations in immune cells or signaling molecules can either protect from or contribute to inflammation and insulin sensitivity. Although less understood, the resident innate immune compartment of adipose tissue also likely has important functions in the absence of obesity.

Besides innate myeloid cells, a substantial component of adipose tissue is comprised of innate lymphocytes such as type 2 innate lymphoid cells (ILC2s), invariant natural killer T (*i*NKT) cells, mucosal-associated invariant T (MAIT) cells, natural killer (NK) cells, and $\gamma\delta$ T cells^{1,2}. In the lean state, ILC2s and *i*NKT cells are critical for maintaining an anti-inflammatory environment through secretion of type 2 cytokines that support the function and survival of eosinophils, alternatively activated macrophages (AAM), and regulatory T (T_{reg}) cells³⁻⁶. Moreover, under other

conditions including cold challenge, ILC2s and *i*NKT cells can induce thermogenic programs in adipose tissue^{7,8}. Conversely, in obesity, NK^{8,9} cells and MAIT¹⁰⁻¹² cells secrete pro-inflammatory cytokines that impair glucose handling by adipocytes, hepatocytes and muscle cells and interfere with insulin production and insulin signaling. Despite recent advances in our understanding of adipose innate lymphocytes, the role of $\gamma\delta$ T cells in this dynamic organ remains largely unknown.

SUMMARY

$\gamma\delta$ T cells are situated at barrier sites and guard the body from infection and damage. However, little is known about their role(s) outside of host defense in non-barrier tissues. Here, we characterize a highly enriched, tissue-resident population of $\gamma\delta$ T cells in adipose tissue that regulates age-dependent regulatory T (T_{reg}) cell expansion and controls core body temperature in response to environmental fluctuations. Mechanistically, innate PLZF⁺ $\gamma\delta$ T cells produced tumor necrosis factor (TNF) and interleukin-17A (IL-17A) and determined PDGFR α ⁺ and Pdpn⁺ stromal cell production of IL-33 in adipose tissue. Mice lacking $\gamma\delta$ T cells or IL-17A exhibited reductions in both ST2⁺ T_{reg} cells and IL-33 abundance in visceral adipose tissue. Remarkably, these mice also lack the ability to regulate core body temperature at thermoneutrality and after cold challenge. Together, these findings support the methods of targeting resident $\gamma\delta$ T cells in adipose tissue to alter immune homeostasis and body temperature control, to reduce obesity or to treat cachexia.

Thus, provided herein are methods for promoting weight loss. The methods can include administering to a subject in need thereof therapeutically effective amounts of a combination of agents comprising: (i) Tumor Necrosis Factor alpha (TNF α) or an agonist of the TNF Receptor (TNFR), and (ii) Interleukin 17 (IL-17) or IL-33. Alternatively or in addition, e.g., as an alternative to or in addition to either or both of the previously listed agents, the methods can include administration of an agent that activates $\gamma\delta$ T cells, e.g., an activating monoclonal antibody against a specific TCR, e.g., a monoclonal antibody targeting Vd2, a number of which are available commercially, pyrophosphates or butyrophilins. In some embodiments, the subject has a BMI of 29 or above. In some embodiments, the agents are modified to increase half-life. In some embodiments, the agents are targeted to adipose tissue.

Also provided herein are methods of inhibiting weight loss (or promoting weight gain). The methods include administering to a subject in need thereof therapeutically effective amounts of a combination of agents comprising: (i) an inhibitor of Tumor Necrosis Factor alpha (TNF α) or the TNF Receptor (TNFR), and
5 (ii) an inhibitor of Interleukin 17 (IL-17), IL-17 Receptor, or IL-33.

Further, provided herein are methods for inhibiting weight loss that include administering to a subject in need thereof a therapeutically effective amount of an agent comprising an inhibitory anti-IL-33 antibody.

In some embodiments, the subject has a chronic illness or cancer. In some
10 embodiments, the cancer is not IL-17 dependent, e.g., wherein IL-17 is not present in significant levels in the tumor. In some embodiments, the cancer is pancreatic cancer.

In some embodiments, the subject has COPD, diabetic kidney disease, or heart failure.

In some embodiments, the agents are modified to increase half-life. In some
15 embodiments, the agents are targeted to adipose tissue.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Methods and materials are described herein for use in the present invention; other, suitable methods and materials known in the art can also
20 be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

25 Other features and advantages of the invention will be apparent from the following detailed description and figures, and from the claims.

DESCRIPTION OF DRAWINGS

Figures 1A-1D. $\gamma\delta$ T cells are enriched and resident in adipose tissue. (A)

Representative flow cytometry plots of $\gamma\delta$ T cells from eWAT SVF. (B) Frequency of
30 $\gamma\delta$ T cells across various adipose tissue depots as a percentage of CD3 ϵ^+ T cells in male eWAT ($n \geq 4$ mice per group). (C) Flow cytometry of lymphocytes in blood, liver, spleen, and adipose tissue of CD45.1 $^+$ and CD45.2 $^+$ congenic C57BL/6 parabiotic pairs joined at 6 wks of age and analyzed 2 wks later (left); and frequency

pie charts of CD45.1⁺ and CD45.2⁺ CD8⁺ and $\gamma\delta$ T cells (right). **(D)** Frequency of V δ 1⁺, V δ 2⁺, and V δ 3⁺ $\gamma\delta$ T cells (% of CD45⁺PI⁻ cells) in peripheral blood (PBMCs) or from matched omental fat from patients before bariatric surgery. SVF, stromal vascular fraction. eWAT, epididymal white adipose tissue. Each symbol represents an individual mouse; small horizontal lines indicate the mean. Data are representative of three experiments **(A,B,D)**; mean $\pm$ s.e.m. in **(B)** or one experiment **(C)**.

Figures 2A-2G. PLZF discriminates two $\gamma\delta$ T cell populations. (A)

Representative flow cytometry and frequency quantification of CD3 ϵ ^{hi} and CD3 ϵ ^{lo} $\gamma\delta$ T cells from eWAT SVF across adipose, liver, and spleen ($n = 5$ mice). **(B)** CD27 expression by CD3 ϵ ^{hi} and CD3 ϵ ^{lo} $\gamma\delta$ T cells (left) and subset quantification from adipose, liver, and spleen (right) ($n = 5$ mice). **(C)** Representative histograms of mean fluorescence intensity (MFI) of CD69, CD44, CD127, and CD45RB expression by CD3 ϵ ^{hi} and CD3 ϵ ^{lo} $\gamma\delta$ T cells. **(D)** Representative histogram of PLZF expression by CD3 ϵ ^{hi} and CD3 ϵ ^{lo} $\gamma\delta$ T cells (left) and MFI quantification across adipose, liver, and spleen (right) ($n = 5$ mice). **(E)** Representative flow cytometry of TCR β ⁺ versus TCR δ ⁺ cells of PLZF⁺CD45⁺ cells from eWAT SVF. **(F)** Immunofluorescence microscopy of whole-mount adipose tissue from *Zbtb16*^{GFP} mice injected with dextran (grey line in bottom left quarter). Scale bar, 100 μ m. **(G)** Frequency of $\gamma\delta$ T cells from wild-type (WT) and *Zbtb16*^{-/-} mice ($n = 5$). NS, not significant ($P > 0.05$); * $P < 0.05$ and **** $P < 0.0001$ (One-way ANOVA). Data are representative of three experiments **(A,B,C,D,E)**; mean $\pm$ s.e.m. in **(A,D)** or two experiments **(F,G)**; mean $\pm$ s.e.m. in **(G)**.

Figures 3A-3F. $\gamma\delta$ T cells are important for adipose T_{reg} accumulation.

Numbers per gram eWAT of $\gamma\delta$ T and Foxp3⁺ T_{reg} cells **(A)** and ILC2s and *i*NKT cells **(B)** at 5, 8, 11, 21 and 28 wks of age ($n \geq 4$ mice per timepoint). **(C)** Ratio (log₂ normalized fold change) of CD8⁺ T, NK, CD4⁺ T, ILC2s, and T_{reg} cell numbers from *Tcrd*^{-/-} mice compared to WT mice (left) at 16 wks ($n = 10$ mice). Frequency and numbers of Foxp3⁺ T_{reg} cells in eWAT between WT and *Tcrd*^{-/-} mice (right) at 16wks ($n = 4$ mice per group). **(D)** Quantitative real-time PCR for *Il10* expression normalized to *Tbp* from sorted CD25⁺CD4⁺ T_{reg} cells from WT and *Tcrd*^{-/-} mice at 16 wks ($n = 5$ mice). **(E)** Frequency (left) and MFI (right) of cell surface KLRG1 expression on eWAT Foxp3⁺ T_{reg} cells at 16 wks ($n = 5$ mice). **(F)** Representative flow cytometry of ST2⁺Foxp3⁺ T_{reg} cells from eWAT (left) and cell numbers at 5, 8, 11 and

22 wks of age between WT and *Tcrd*^{-/-} littermates (right) (*n* = 5 mice). Each symbol represents an individual mouse; small horizontal lines indicate the mean. NS, not significant (*P* > 0.05); * *P* < 0.05; ** *P* < 0.01; *** *P* < 0.001. (Student's *t* test in C,D,E; One-way ANOVA in F). Data are representative of three experiments (A,B,F; mean ± s.e.m. in A,B,F) or two experiments (C,D,E; mean ± s.e.m. in C,D,E).

Figures 4A-4D. PLZF⁺ γδ T cells are innate-IL17A producing cells. (A)

Heatmap of top 60 genes differentially expressed (FDR-adjusted *P* value < 0.01) between PLZF⁺ (left) and PLZF⁻ (right) γδ T cells. (B) Scatter plot of gene transcripts differentially expressed by PLZF⁺ and PLZF⁻ γδ T cells from 14 wk old male mice. (C) Flow cytometry of RORγt (top) and T-bet (bottom) expression in PLZF⁺ and PLZF⁻ γδ T cells from eWAT SVF. (D) Representative intracellular cytokine staining (top) and quantification (bottom) on gated γδ T cells from eWAT SVF for TNF, IL-17A and IFN-γ after 4 h stimulation with PMA and ionomycin (*n* = 4 mice). NS, not significant (*P* > 0.05); * *P* < 0.05; ** *P* < 0.01; **** *P* < 0.0001. (One-way ANOVA in D). Data are representative of two experiments (C,D; mean ± s.e.m. in D).

Figures 5A-5F. ST2⁺ T_{reg} numbers depend on PLZF⁺ γδ T cells and IL-

17A. (A) Frequency of PLZF⁺ and PLZF⁻ γδ T cells from eWAT at 5, 8, 11 and 22 wks of age (*n* = 5 mice per timepoint). (B) Numbers of GFP⁺ CD3ε^{hi} and CD3ε^{lo} γδ T cells from eWAT of *Il17a*^{GFP} male mice at 10, 13, 20 and 27 wks of age (*n* = 5 mice per timepoint). (C) Numbers and frequency of ST2⁺ and total T_{reg} cells from eWAT of 16 wk old males from WT and *Il17a*^{-/-} mice (*n* = 5 mice, pooled). (D) Representative flow cytometry of γδ T cells stained with anti-CD27, 17D1, anti-Vγ1 and anti-Vγ4 antibodies to characterize TCR usage. (E) Representative flow cytometry plots of γδ T cells from WT and *Vg4/6*^{-/-} eWAT (left) and quantification of CD3ε^{hi} and CD3ε^{lo} γδ frequencies (right) (*n* = 5 mice). (F) Numbers and frequency of ST2⁺ and total T_{reg} cells from eWAT of 16 wk old WT and *Vg4/6*^{-/-} male mice (*n* ≥ 4 mice). Each symbol represents an individual mouse; small horizontal lines indicate the mean. NS, not significant (*P* > 0.05); * *P* < 0.05 (One-way ANOVA in A,C,F). Data are pooled across two experiments (A,B,C,F; mean ± s.e.m. in A,B,C,F) or representative of two experiments (D,E; mean ± s.e.m. in E).

Figures 6A-6H. TNF and IL-17A induce IL-33 in adipose stromal cells. (a)

IL-33 protein from SVF eWAT lysates of 5-8, 15-16 and 20+ wk WT male mice normalized to total SVF protein by ELISA (*n* = 4 mice per timepoint). (B) IL-33 (left)

and IL-2 (right) protein from SVF eWAT and splenic lysates of 14 wk old WT and *Tcrd*^{-/-} male mice ($n \geq 3$ mice, pooled). (C) IL-33 protein from SVF eWAT lysates of 16 wk old WT, *Vg4/6*^{-/-} and *Il17a*^{-/-} male mice normalized to total SVF protein ($n \geq 4$ mice). (D) Representative flow cytometry plots for stromal cell subsets in eWAT SVF of male WT mice. (E) Cell surface IL-17R, CD26, CD9 and Cdh11 MFI of CD45⁻ Pdpn^{hi}PDGFR α ⁻ and CD45⁻Pdpn^{lo}PDGFR α ⁺ stromal cells. (F) Quantitative real-time PCR for *Il33* mRNA normalized to *Tbp* from sorted Pdpn^{hi}, PDGFR α ⁺, CD31⁺, and CD45⁺ cells from WT mice ($n = 6$). (G) 3T3L1 or primary adipose stromal cells derived from eWAT SVF were unstimulated (unstim) or stimulated with TNF^{lo} (0.1ng/mL), TNF (1ng/mL), IL-17A^{lo} (0.1ng/mL), IL-17A (1ng/mL), or TNF (1ng/mL) + IL-17A (1ng/mL) for 18 h. *Il33* transcript levels were measured by quantitative real-time PCR (left) and protein levels measured by ELISA (right). (H) Primary human stromal cells derived from visceral (Lonza) and subcutaneous (ATCC) adipose tissues were unstimulated (unstim) or stimulated with TNF^{lo} (0.1ng/mL), TNF (1ng/mL), IL-17A^{lo} (0.1ng/mL), IL-17A (1ng/mL), or TNF (1ng/mL) + IL-17A (1ng/mL) for 18 h. Cell lysates were collected and IL-33 protein measured by ELISA. Each symbol represents an individual mouse; small horizontal lines indicate the mean. NS, not significant ($P > 0.05$); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$ (One-way ANOVA in A,B,C,F,G,H). Data are pooled across three experiments (A,B,C; mean $\pm$ s.e.m. in A,B,C) or representative of two experiments (D,E,F,G,H; mean $\pm$ s.e.m. in F,G,H).

Figures 7A-7F. $\gamma\delta$ T cells are important for adaptive thermogenesis after cold. (A) Representative histology of hematoxylin- and eosin-stained BAT and lipid droplet quantification from WT, *Tcrd*^{-/-} and *Vg4/6*^{-/-} mice after 6 h at 4 °C. Scale bars, 500 μ m. (B) Immunoblot analysis and densitometry quantification of UCP1 and HSP90 in BAT of WT, *Tcrd*^{-/-} and *Vg4/6*^{-/-} mice after 6 h at 4 °C ($n = 5$ mice). (C) Quantitative real-time PCR of thermogenesis genes from WT and *Tcrd*^{-/-} BAT (top), and WT and *Vg4/6*^{-/-} BAT (bottom) after 6 h at 4 °C ($n \geq 5$ mice). (D) Immunoblot analysis of pHSL and HSL and quantitative real-time PCR of *Lipe* (*Hsl*) from iWAT of WT, *Tcrd*^{-/-} and *Vg4/6*^{-/-} mice after 6 h at 4 °C ($n \geq 5$ mice). (E) Quantitative real-time PCR of thermogenesis genes from WT, *Tcrd*^{-/-} and *Vg4/6*^{-/-} iWAT after 6 h at 4 °C ($n \geq 5$ mice). (F) Body temperature (left) and energy expenditure (right) measured between WT and *Tcrd*^{-/-} male mice from 30 °C to 4 °C ($n = 5$). Each symbol

represents an individual mouse; small horizontal lines indicate the mean. Gene expression normalized to *Tbp*. Immunoblots have been cropped to show relevant proteins. NS, not significant ($P > 0.05$); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (metabolic variable adjusted for differences in body composition by ANCOVA in **F**;
 5 One-way ANOVA in **A,E,F**; Student's *t* test in **B,C**). Data are representative across two experiments (**A-F**; mean $\pm$ s.e.m. in **A-C,D-F**).

Figures 8A-I. IL-17A promotes thermogenic responses in brown and inguinal adipose tissue. (**A**) Frequency of $\gamma\delta$ T cells of CD45⁺ cells in BAT and iWAT 0, 8, 24 h at 4 °C ($n = 4$ mice). (**B**) Frequency of IL-17A producing $\gamma\delta$ T cells
 10 from BAT and iWAT after 5 h stimulation with PMA and ionomycin ($n = 10$ mice). (**C**) Frequency of immune cells from BAT and iWAT that produce TNF, IL-17A, or TNF + IL-17A after 4 h stimulation with PMA and ionomycin at 4 °C ($n = 5$ mice). (**D**) Representative histology of hematoxylin- and eosin-stained BAT and lipid droplet quantification from WT and *Il17a*^{-/-} mice after 6 h at 4 °C. Scale bars, 500 μ m. (**E**)
 15 Quantitative real-time PCR of *Ucp1* (left) and immunoblot analysis of UCP1 and HSP90 (right) in BAT tissue obtained from WT and *Il17a*^{-/-} mice after 6 h at 4 °C ($n \geq 5$ mice). (**F**) Quantitative real-time PCR of *Lipe* (*Hsl*) from WT and *Il17a*^{-/-} iWAT after 6 h at 4 °C ($n \geq 5$ mice). (**G**) Quantitative real-time PCR of *Ucp1* from WT and *Il17a*^{-/-} iWAT after 6 h at 4 °C ($n \geq 5$ mice). (**H**) WT and *Il17a*^{-/-} mice were gradually
 20 shifted from 30 °C to 4 °C at a continuous rate and monitored for survival. Mice were rescued when body temperature dropped to < 28 °C ($n = 5$ mice). (**I**) Energy expenditure measured between WT and *Il17a*^{-/-} male mice ($n = 5$ mice). Each symbol represents an individual mouse; small horizontal lines indicate the mean. Gene expression normalized to *Tbp*. Immunoblots have been cropped to show relevant
 25 proteins. NS, not significant ($P > 0.05$); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ (metabolic variable adjusted for differences in body composition by ANCOVA in **H,I**; One-way ANOVA in **A**; Student's *t* test in **D-G**; Log-rank Mantel-Cox test in **H**). Data are representative across two experiments (**A-G**; mean $\pm$ s.e.m. in **A-G**).

Figures 9A-9C. Immunophenotyping panels for adipose immune cell quantification. (**A**) Representative flow cytometry plots to identify ILC2s, $\gamma\delta$ T, CD4⁺ T, Foxp3⁺ T_{reg}, and ST2⁺Foxp3⁺ T_{reg} cells. (**B**) Representative flow cytometry
 30 plots to identify eosinophils, B220⁺CD19⁺ B, CD19⁺ B, NK, *i*NKT, and CD8⁺ T cells. (**C**) Numbers of CD4⁺ T, CD8⁺ T, eosinophils, CD19⁺ B, B220⁺CD19⁺ B, and NK

cells per gram of eWAT at 5, 8, 11, 21 and 28 wks of age in male mice ($n = 5$, pooled). Each symbol represents an individual mouse; small horizontal lines indicate the mean. Data are representative across two experiments (A,B,C; mean $\pm$ s.e.m. in c).

Figures 10A-10E. ILC2, iNKT, and T_{reg} numbers in IL-17A KO and V γ 4/6 KO mice. (A) Numbers (left) and frequency (right) of ILC2s in eWAT from WT, V γ 4/6^{-/-} and *Il17a*^{-/-} 16 wk old mice ($n = 5$, pooled). (B) Numbers (left) and frequency (right) of iNKTs in eWAT from WT, V γ 4/6^{-/-} and *Il17a*^{-/-} 16 wk old mice ($n = 5$, pooled). (C) Quantification of numbers (top) and frequencies (bottom) of T_{reg} cells and ST2⁺ T_{reg} cells from spleen, lung, and adipose tissue from WT, V γ 4/6^{-/-} and *Il17a*^{-/-} 16 wk old mice ($n \geq 3$). (D) IL-33 protein from SVF eWAT lysates of 11 wk male WT and *Il17a*^{-/-} mice normalized to total SVF protein by ELISA ($n \geq 3$). (E) Numbers (top) and frequency (bottom) of T_{reg} cells and ST2⁺ T_{reg} cells from WT and *Il17a*^{-/-} eWAT at 11 wks of age ($n \geq 4$). Each symbol represents an individual mouse; small horizontal lines indicate the mean. NS, not significant ($P > 0.05$); * $P < 0.05$; **** $P < 0.0001$ (One-way ANOVA in A-C; Student's *t* test in D-E). Data are pooled across two experiments (A-E; mean $\pm$ s.e.m. in A-E).

Figures 11A-11D. In vitro and in vivo cytokine stimulations of epididymal adipose stromal cells. (A) 3T3L1 adipose fibroblasts were unstimulated (unstim) or stimulated with TNF^{lo} (0.1ng/mL), TNF^{hi} (1ng/mL), IL-17A^{lo} (0.1ng/mL), IL-17A^{hi} (1ng/mL), IL-1 β ^{lo} (0.1ng/mL), IL-1 β ^{hi} (1ng/mL), IFN- γ ^{lo} (0.1ng/mL), IFN- γ ^{hi} (1ng/mL), or a combination of the cytokines as indicated for 18h. IL-33 protein was measured by ELISA. (B) WT mice were injected with saline or TNF (1 μ g) and IL-17A (0.5 μ g) every third day for a total of nine days and eWAT RNA isolated. *Il33* transcript levels were measured by quantitative real-time PCR and normalized to *Tbp* ($n \geq 5$). Representative flow cytometry plots (C) and *Il33* expression from iWAT stromal cells (D) after WT mice were injected with saline or TNF (1 μ g) and IL-17A (0.5 μ g) every third day for a total of nine days. *Il33* normalized with *Tbp* ($n \geq 3$, pooled). Small horizontal lines indicate the mean. ** $P < 0.01$; **** $P < 0.0001$ (One-way ANOVA in A,D; Student's *t* test in B). Data are pooled across two experiments run in triplicates (A; mean $\pm$ s.e.m. in A). Data are representative of two experiments (B-D; mean $\pm$ s.e.m. in B,D).

Figures 12A-B. Decreased numbers and not gene expression likely contributes to lower IL-33 protein. (A) Quantification of numbers (top) and

frequencies (bottom) of CD31⁺, PDGFR α ⁺Pdnp⁻, Pdnp^{hi}, and Pdnp^{lo} eWAT stromal cells from 23 wk old WT, *Tcrd*^{-/-}, *Vg4/6*^{-/-}, and *Il17a*^{-/-} male mice ($n \geq 3$ mice per genotype). (B) Quantitative real-time PCR for *Il33* expression normalized with *Tbp* from sorted Pdnp^{hi}, PDGFR α ⁺, CD31⁺, and CD45⁺ cells from WT, *Tcrd*^{-/-}, *Vg4/6*^{-/-}, and *Il17a*^{-/-} mice ($n \geq 3$ mice per genotype). Each symbol represents an individual mouse; small horizontal lines indicate the mean. NS, not significant ($P > 0.05$); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. (One-way ANOVA in A-B). Data are representative of two experiments (A-B; mean $\pm$ s.e.m. in A-B).

Figures 13A-13B. $\gamma\delta$ T cells promote temperature regulation and IL-33

homeostasis in BAT and iWAT. (A) IL-33 protein was quantified from cell lysates of eWAT, iWAT, and BAT from WT, *Tcrd*^{-/-} and *Vg4/6*^{-/-} mice using ELISA (left). Quantitative real-time PCR for *Il33* expression normalized with *Tbp* (right) from iWAT and BAT of WT, *Tcrd*^{-/-} and *Vg4/6*^{-/-} mice ($n \geq 4$). (B) Energy expenditure measured from WT and *Tcrd*^{-/-} mice injected with sterile saline at time 0 h and subsequently injected with selective β 3-adrenergic receptor, CL-316 243, (1mg/kg) at 3 h ($n = 5$ per genotype). Small horizontal lines indicate the mean. NS, not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. (One-way ANOVA in A; Metabolic variable adjusted for differences in body composition by ANCOVA in B). Data are representative of two experiments (A; mean $\pm$ s.e.m. in A) or one experiment (C; mean $\pm$ s.e.m. in C).

Figures 14A-14E. IL-17A promotes thermogenic responses in BAT and iWAT.

(A) Frequency (left) and numbers (right) of $\gamma\delta$ T cells at 0, 8, and 24 h at 4 °C in BAT and iWAT ($n \geq 3$ mice per condition). (B) Quantitative real-time PCR of *Ppargc1a*, *Dio2*, and *Cox7a1* normalized to *Tbp* in BAT between WT and *Il17a*^{-/-} mice ($n \geq 3$). (C) Quantitative real-time PCR of *Ppargc1a* and *Dio2* normalized to *Tbp* in iWAT between WT and *Il17a*^{-/-} mice ($n \geq 3$). (D) Mice were gradually shifted from 30 °C to 4 °C at a continuous rate and body temperature measured between WT and *Il17a*^{-/-} male mice ($n = 5$ mice per genotype). (E) Body temperature (top) and RER (bottom) measured for 72 h at thermoneutrality after acclimation between WT and *Il17a*^{-/-} male mice ($n = 5$ per genotype). Each symbol represents an individual mouse; small horizontal lines indicate the mean. NS, not significant ($P > 0.05$); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. (Student's *t* test in B-C; One-way ANOVA in A;

Metabolic variable adjusted for differences in body composition by ANCOVA in **D-E**). Data are representative of two experiments (**A-C**; mean $\pm$ s.e.m. in **A-C**).

Figures 15A-15B. Gene expression analysis of BAT and iWAT. Quantitative real-time PCR of *Th*, *Adrb3*, *Lipe* (*Hsl*), and *Pnpla2* (*Atgl*) in brown (**A**) and inguinal (**B**) adipose tissue obtained from WT, *Tcrd*^{-/-}, *Vg4/6*^{-/-} and *Il17a*^{-/-} mice at room temperature (25 °C) and after 6 h cold at 4 °C. Genes normalized to *Tbp* ($n \geq 4$ mice per condition). Each symbol represents an individual mouse; small horizontal lines indicate the mean. NS, not significant ($P > 0.05$); * $P < 0.05$; ** $P < 0.01$. (One-way ANOVA in **A,B**). Data are representative of two experiments (**A,B**; mean $\pm$ s.e.m. in **A,B**).

Figures 16A-16E. $\gamma\delta$ T cells directly and indirectly influence adaptive thermogenesis. (**A**) Differentiated brown adipocytes were stimulated with indicated amounts of TNF^{lo} (0.1ng/mL), TNF^{hi} (1ng/mL), IL-17A^{lo} (0.1ng/mL), IL-17A^{hi} (1ng/mL), for 18 h and *Ucp1*, *Dio2*, *Cidea*, and *Il33* transcript levels were measured by quantitative real-time PCR and normalized with *Tbp*. (**B**) Differentiated brown adipocytes were stimulated with either IL-33^{lo} (10ng/mL), IL-33^{hi} (100ng/mL), and analyzed as in **A**. (**C**) Representative flow cytometry plots (left) of iWAT stromal cells after WT mice were injected with saline (top row) or TNF (1 μ g) and IL-17A (0.5 μ g) every third day for a total of nine days. Pdpn⁺PDGFR α ⁻ and PDGFR α ⁺ iWAT stromal cells were sorted and gene expression of *Ucp1*, *Pparg1a*, and *Dio2* measured by quantitative real-time PCR and normalized with *Tbp* ($n \geq 3$). Frequency (top) and numbers (bottom) of eosinophils, ILC2s, *i*NKT, and T_{reg} cells from WT, *Tcrd*^{-/-} and *Vg4/6*^{-/-} brown (**D**) and inguinal (**E**) adipose tissue from 22 wk male mice ($n \geq 4$ mice per group). Each symbol represents an individual replicate or mouse. Data are representative of two experiments (**A-E**; mean $\pm$ s.e.m. in **A-E**). NS, not significant ($P > 0.05$); * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. (One-way ANOVA in **A-E**).

DETAILED DESCRIPTION

A key adaptive cell type in adipose tissue is the Foxp3⁺ T_{reg} cell. T_{reg} cells are low in numbers in adipose tissue of mice until 20 weeks of age, after which they greatly expand and comprise 40-80% of the CD4⁺ T cell population⁹⁻¹². Adipose T_{reg} cells have enhanced expression of genes such as *Il10*, *Gata3*, *Pparg*, and *Ilrl1* that define their adipose and anti-inflammatory phenotype^{9,10,13,14}. Furthermore, they

express high amounts of the interleukin-33 (IL-33) receptor ST2 (IL-1R4), and IL-33 is critical for their local expansion and transcriptional stability^{11,15}. We previously described a critical role for *i*NKT cell derived IL-2 to maintain T_{reg} cell numbers and to boost their function in adipose tissue³. In addition, ILC2s have been shown to play a role in T_{reg} cell homeostasis via ICOSL-ICOS interactions after IL-33 administration, and T_{reg} cells from ICOSL-deficient mice fail to expand with IL-33 treatment¹⁶. While these studies provide mechanistic insights into regulation of T_{reg} homeostasis, the basis for the marked increase in T_{reg} cell numbers with age is unknown.

IL-33 is an important factor for non-shivering thermogenesis, the metabolic adaptation to cold temperatures^{17,18}. Adaptive thermogenesis is mediated in large part by uncoupling protein 1 (UCP1), which uncouples oxidative phosphorylation from ATP synthesis to generate heat. IL-33 is critical for body temperature regulation in newborns¹⁸, and adult mice deficient in IL-33 cannot induce UCP1 and exhibit defects in thermogenesis^{8,19}. In adipose tissue, the main source of IL-33 is debated, and has been ascribed to a number of different stromal cell types including mesenchymal Cadherin-11⁺ (Cdh11) cells, podoplanin⁺ (Pdpn⁺) fibroblasts, or CD31⁺ endothelial cells^{15,16,20,21}. Importantly, the mechanisms that regulate the endogenous expression of IL-33 in adipose tissue remain elusive.

The role of $\gamma\delta$ T cells as guardians against pathogens at barrier sites has been well documented. However, the physiological role of $\gamma\delta$ T cells at steady state and in non-barrier tissues is less appreciated. We uncover a new biological axis where PLZF⁺ IL-17A-producing $\gamma\delta$ T cells crosstalk with adipose stromal cells to regulate IL-33 abundance with downstream effects on T_{reg} cell accumulation and thermoregulation. Post-translational processing tightly controls the levels of IL-33³⁹, but the upstream mechanisms that control its transcription have been less studied. In addition, the main cell type that produces IL-33 in murine adipose tissue had been a matter of debate. Our studies point to Pdpn⁺ and PDGFR α ⁺ as IL-17A responsive stromal cells in visceral adipose tissue. Moreover, TNF and IL-17A synergize to increase the numbers and *Il33* expression, respectively, of Pdpn⁺ and PDGFR α ⁺ cells to modulate IL-33 amounts *in situ*. These findings highlight an important homeostatic role for IL-17A in adipose tissue, independent from microbial insult.

Such effects mediated by $\gamma\delta$ T cells are also intriguing given the previously defined roles for *i*NKT cells that also regulate T_{reg} cell homeostasis^{3,7}. We suggest that while *i*NKT cells play a key role in regulating T_{reg} numbers and function in young mice and via IL-2, PLZF⁺ $\gamma\delta$ T cells play a key role in adult mice via IL-33 when *i*NKT cell numbers decline. Despite their important roles as regulators of type 2 immunity in young mice, ILC2s and *i*NKT cells decrease with age, while a new wave of immune cells composed of $\gamma\delta$ T cells and T_{reg} cells expand. This temporal regulation of adipose lymphocytes may ensure redundancies in the molecular pathways that maintain healthy adipose tissue, which is critical for local and systemic metabolic homeostasis.

In addition, we have discovered $\gamma\delta$ T cells and the cytokine IL-17A as critical regulators of thermogenesis, a distinctive function of adipose tissue. $\gamma\delta$ T cell- and IL-17A-deficiency dramatically affect the ability of mice to survive upon cold challenge and robustly induce UCP1-dependent thermogenic responses. Remarkably, both *i*NKT cells and PLZF⁺ $\gamma\delta$ T cells in adipose tissue regulate thermogenesis through FGF-21 and IL-17A, respectively^{3,7}. These roles of *i*NKT cells and $\gamma\delta$ T cells to both regulate T_{reg} cells and thermogenesis by complementary mechanisms underscores the remarkable importance of these innate T cell populations in adipose tissue.

Methods of treatment – Obesity

$\gamma\delta$ T cells are canonical, meaning they have an invariant or semi-invariant T cell receptor. They produce IL-17 and TNF and express the transcription factor PLZF, unlike most $\gamma\delta$ T cells at other sites. Their actions in adipose tissue, partly through production of synergistic IL-17 and TNF which induces IL-33 production by adipose stromal cells, enhances Tregulatory cell survival and/or expansion which is beneficial for adipose tissue and systemic health. These actions, again partly through IL-33, also generates body heat through thermogenesis. This can induce weight loss in obesity, and can lower fasting glucose and improve metabolism and insulin resistance. We have also found that this pathway exists in human adipose tissue. The present methods can be used, e.g., in subjects who are obese (BMI of 30 kg/m² or above, calculated as weight in kilograms divided by the square of height in meters). In some embodiments, the subjects do not have diabetes, e.g., does not have type 2 diabetes.

Thus, these methods can include activation of $\gamma\delta$ T cells in adipose tissue, through stimulating their specific T cell receptor. In mice, the relevant $\gamma\delta$ subset are Vd1Vg6. In humans, the methods can include activating Vd2Vg9 or Vg9 negative Vd2 cells; Vd1, or Vd3 cells, which are also present in human adipose tissue, may also produce IL-17 and TNF α and thus can also be activated. As shown in Figure 1D, the Vd1,2,3 in the human adipose tissue, and Figure 6H shows that human IL-17 and TNF induced IL-33 from human adipose stromal cells. In addition, we sequenced the human $\gamma\delta$ population and determined that Vd2 have IL-17 associated genes (IL23R and RORA).

The $\gamma\delta$ cells can be targeted with an activating monoclonal antibody against a specific TCR, e.g., a monoclonal antibody targeting Vd2, a number of which are available commercially, e.g., from Abcam, GeneTex, Invitrogen Antibodies, Miltenyi Biotec, and United States Biological. Activating or inhibitory antibodies can be made and identified using assays known in the art. Other methods of activating $\gamma\delta$ T cells, can be used, e.g., administration of pyrophosphates or butyrophilins (see, e.g., Alexander et al., Clin Cancer Res. 2008 Jul 1; 14(13): 4232–4240; Barros et al., Cell. 2016 Sep 22;167(1):203-218.e17; and Gu et al. PNAS August 29, 2017. 114 (35) E7311-E7320 (butyrophilin 3A1 (BTN3A1)).

Alternatively or in addition, the methods can include administration of the factors produced by adipose $\gamma\delta$ T cells that control inflammation and/or thermogenesis. These factors include, for example, a combination of TNF α (or an agonist of the TNF Receptor (TNFR)) and IL-17 (or an agonist of the IL-17 Receptor) and/or an IL-17 receptor agonist.

Administration of TNF α can include administration of the purified protein. Exemplary sequences of human TNF α are known in the art, e.g., NCBI RefSeq ID. NP_000585.2 (SEQ ID NO:1).

Human TNF α (SEQ ID NO:1)

```
1 mstesmirdv elaealpkk tggpqqsrcc lflslfsfli vagattlfcl lhfgvigpqr
61 eefprdlsls splaqavrss srtpsdkpva hvvanpqaeg qlqwlrran allangvelr
121 dnqlvvpseg lyliysqvlf kgqgcpsthv llthtisria vsyqtkvnll saikspcpre
181 tpegaeakpw yepiylggvf qlekgdrlsa einrpdylf aesgqvfygi ial
```

Agonists of the TNFR are also known in the art. The tumor necrosis factor receptors (TNFRs), include glucocorticoid-induced TNFR (GITR; CD357), CD27, OX40 (CD134), and 4-1BB (CD137). Agonists include Varlilumab, Urelumab,

utomilumab, PRS-343, MEDI0562, MOXRO0916, GSK3174998, PF-04518600, TRX518. See, e.g., Sturgill and Redmond, *AJHO*. 2017;13(11):4-15.

Administration of IL-17 can include administration of the purified protein.

Exemplary sequences of human IL-17 are known in the art, e.g., NCBI RefSeq ID.

NP_002181.1 (SEQ ID NO:2), e.g., a protein comprising amino acids 69-147 of SEQ ID NO:2.

Human IL-17 (SEQ ID NO:2)

```
1 mtpgktslvs lllllsleai vkagitiprn pgcpnsedkn fprrvmvnlh ihnrrntntnp
61 krssdyynrs tspwnlhrne dperypsviw eakcrhlhci nadgnvdyhm nsvpiqqeil
10 121 vlrrepphpc nsfrlekilv svgtctvtpi vhhva
```

Agonists of the IL-17R can be identified using methods known in the art.

Alternatively or in addition (e.g., in combination with TNF α), the methods can include administration of IL-33, e.g., purified IL-33 protein. Exemplary sequences of human IL-33 are known in the art, e.g., NCBI RefSeq ID. NP_254274.1 (SEQ ID NO:3), e.g., a protein comprising amino acids 95-270, 99-270, or 109-270 of SEQ ID NO:3. Other isoforms can also be used.

Human IL-33 (SEQ ID NO:3)

```
1 mkpkmkystn kistakwknt askalcflkg ksqqkakevc pmyfmklrsg lmikkeacyf
61 rrettkrpsl ktgrkhkrhl vlaacqqgst vecfafgisg vqkytralhd ssitgispit
20 121 eylaslstyn dqsitfaled esyeiyvedl kkdekkdkvl lsyyesqhps nesgdgvdgk
181 mlmvtlsptk dfwlhannke hselhkcck plpdqaffvl hnmhsncvsv ecktdpgvfi
241 gvkdnhlali kvdsenlct enilfklset
```

Methods of treatment – Cachexia

We have identified a role for innate lymphocytes that are resident in adipose tissue, and the cytokines they produce, in cachexia. Cachexia is a wasting of the body (e.g., fat and muscle) due to cancers and chronic illnesses including COPD, diabetic kidney disease, heart failure and others, and is responsible for the death of ~30% of cancer patients. The first step in cachexia is the activation of browning in white adipose tissue, which occurs before any significant weight loss is seen in cachectic animal models. Using parabiosis as a tool, we have shown that innate $\gamma\delta$ T cells and iNKT are resident in adipose tissue and produce cytokines including TNF and IL-17 in response to changes in the environment including cold exposure and changes in the diet. In addition to cytokines usually associated with these immune cells, transcriptomics on these subsets in adipose tissue has revealed that these cells also produce other factors that can modulate neurons, which are also involved in thermogenesis. The activation of these innate resident T cells induced browning of

white adipose tissue and thermogenesis and increased systemic energy expenditure, which are the key steps in induction of cachexia.

Thus, these methods can include blocking the activation of $\gamma\delta$ T cells and/or iNKT cells in adipose tissue, or depletion of these cells (e.g., of Vd2Vg9 or Vg9 negative Vd2 $\gamma\delta$ T cells; Vd1 $\gamma\delta$ T cells; or Vd3 $\gamma\delta$ T cells) through selective depleting antibodies against their specific T cell receptor or surface markers. Antibodies specific for the iNKT cell TCR for activation are known in the art, e.g., the 6b11 antibody (Exley et al., Eur J Immunol. 2008 Jun; 38(6): 1756–1766) targets the iNKT TCR).

For example, in some embodiments the methods can include blocking the factors produced by adipose iNKT, $\gamma\delta$ T cells and ILC3, that control inflammation and/or thermogenesis could be new treatments for cachexia or other wasting diseases. For example, the methods can include administration of an inhibitor of IL-17 or the IL-17R, e.g., Secukinumab, ustekinumab, brodalumab or ixekizumab; see, e.g., Rizvi et al., Nature Reviews Drug Discovery 14, 745–746 (2015). These methods can also include administration of an inhibitor of TNF α or the TNFR, e.g., a monoclonal antibody such as infliximab, adalimumab, certolizumab pegol, and golimumab, or with a circulating receptor fusion protein such as etanercept. The inhibitors of IL-17/IL-17R (e.g., Brodalumab and others) and of TNF α /TNFR can be administered together, e.g., in a single composition, or in separate compositions.

Alternatively or in addition, the methods can include administering an IL-33 antibody, e.g., a long-acting IL-33 neutralizing antibody. The sequence of human IL-33 is known in the art (see above). Anti-IL-33 antibodies are known in the art; see, e.g., WO2014164959A2, and are commercially available from Abbexa Ltd; Abcam; ABclonal; Abnova Corporation; antibodies-online; Assay Biotech; AssayPro; Atlas Antibodies; Aviva Systems Biology; BioLegend; Biomatik; Bio-Rad; Biorbyt; Bioss Inc.; BioVision; BosterBio; Cell Sciences; Cloud-Clone; Creative Biolabs; Creative Diagnostics; Elabscience Biotechnology Inc.; Enzo Life Sciences, Inc.; Fitzgerald Industries International; GeneTex; IBL – America Immuno-Biological Laboratories); Invitrogen Antibodies; LifeSpan BioSciences; MBL International; MilliporeSigma; MyBioSource.com; Novus Biologicals; NSJ Bioreagents; OriGene Technologies; PeproTech; ProSci, Inc; Proteintech Group Inc; R&D Systems; Raybiotech, Inc.; Rockland Immunochemicals, Inc.; Santa Cruz Biotechnology, Inc.; Signalway

Antibody LLC; Sino Biological; SouthernBiotech; St John's Laboratory; United States Biological; and Abbexa Ltd.

Pharmaceutical Compositions and Methods of Administration

The methods described herein include the use of pharmaceutical compositions comprising one or more active ingredients as described herein. In some embodiments, no other active ingredients are used or administered.

Pharmaceutical compositions typically include a pharmaceutically acceptable carrier. As used herein the language “pharmaceutically acceptable carrier” includes saline, solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration.

Pharmaceutical compositions are typically formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration.

Methods of formulating suitable pharmaceutical compositions are known in the art, see, e.g., *Remington: The Science and Practice of Pharmacy*, 21st ed., 2005; and the books in the series *Drugs and the Pharmaceutical Sciences: a Series of Textbooks and Monographs* (Dekker, NY). For example, solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use can include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline,

bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of
5 microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of
10 dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the
15 injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization.

20 Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle, which contains a basic dispersion medium and the required other ingredients from those enumerated herein. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying, which yield a powder of the active ingredient plus
25 any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules, e.g., gelatin capsules. Oral compositions can also be prepared using a fluid carrier for use
30 as a mouthwash. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin;

an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

5 In some embodiments, the proteins or antibodies can be modified, e.g. pegylated to extend half-life. Methods for producing recombinant protein and/or purifying native proteins are known in the art. In some embodiments, the methods include administration by injection into adipose tissue, to target adipose-resident cells. In some embodiments, the drug is formulated to target adipose, e.g., using adipo-8
10 aptamers (Liu et al., May 25, 2012; doi.org/10.1371/journal.pone.0037789). In some embodiments, an extended release formulation is used.

In some embodiments, the therapeutic compounds are prepared with carriers that will protect the therapeutic compounds against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated
15 delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Such formulations can be prepared using standard techniques, or obtained commercially, e.g., from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to selected cells with
20 monoclonal antibodies to cellular antigens) can also be used as pharmaceutically acceptable carriers. Nanoparticles (1 to 1,000 nm) and microparticles (1 to 1,000 μ m), e.g., nanospheres and microspheres and nanocapsules and microcapsules, can also be used, e.g., adipose-targeted nanoparticles (see, e.g., Xue et al., PNAS May 17, 2016. 113 (20) 5552-5557).

25 The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for use in a method described herein.

EXAMPLES

The invention is further described in the following examples, which do not limit the scope of the invention described in the claims.

30 Methods

The following materials and methods were used in the Examples below.

Mice. C57BL/6J (WT), *Il17a*^{-/-}, *Tcrd*^{-/-}, and *Il17a*^{GFP} mice were purchased from Jackson Laboratory. Littermates were bred and maintained in specific-pathogen-free animal facilities at Brigham and Women's Center for Comparative Medicine. In almost all experiments, male mice of specified age were used. PLZF reporter mice (*Zbtb16*^{GFP} mice) and *Zbtb16*^{-/-} were generated in the Sant'Angelo laboratory as previously described^{3,40}. *Vgamma4/6*^{-/-} (*Vg4/6*^{-/-}) mice were a kind gift from R. O'Brien³⁰. All studies were executed by following relevant ethical regulations detailed in animal use protocols. All animal work and protocols were approved by and was in compliance with the Institutional Animal Care and Use Committee guidelines of Brigham and Women's Hospital and Harvard Medical School.

Parabiosis. Parabiosis studies were conducted as previously described⁴¹. CD45.1⁺ and CD45.2⁺ mice were first anesthetized with ketamine (100 mg per kg body weight) and xylazine (10 mg per kg body weight). After mice were shaved, a linear incision was made from the scapulae to the lower abdomen on opposing sides of each member of the pair. Mice were placed side by side and skin edges were sewn together. Each pair was housed singly, with food placed on the floor of the cage for the first week during recovery. Parabiotic mice were kept together for 2–3 weeks. Chimerism in the blood and tissues was defined for gated lymphocytes, or lymphocyte subsets as the percentage of CD45.1⁺ cells over the percentage of CD45.1⁺ cells plus CD45.2⁺ cells in CD45.2⁺ mice, and as the percentage of CD45.2⁺ cells over the percentage of CD45.2⁺ cells plus CD45.1⁺ cells in CD45.1⁺ mice.

CLAMS. Indirect calorimetry experiments were performed with a Comprehensive Lab Animal Monitoring System (CLAMS, Columbus Instruments) essentially as described⁴². Mice were surgically implanted with intraperitoneal wireless temperature transmitters. Following recovery, mice were housed in the CLAMS and maintained at thermoneutrality for 3 days. To observe the response to adrenergic agonist versus effects of injection alone, mice housed at thermoneutrality were first injected with a control solution of sterile saline (200 μ l) and monitored for 3 h. The animals were then injected (1 mg/kg) with the selective β 3-adrenergic receptor agonist CL316,243 (Sigma Chemical Co.) and monitored as indicated. For 4 °C cold challenge, mice were gradually shifted from 30 °C to 4 °C at a continuous rate over 3 h. For each experimental condition, metabolic variables were adjusted for differences

in body composition by analysis of covariance (ANCOVA) in the R programming language with a custom package for indirect calorimetry experiments (CalR).

Tissue digestion. Adipose tissue was carefully excised, minced, and digested with 1 mg/ml collagenase type 2 (Worthington #LS004188) in RPMI (Life Technologies) for 25 min at 37 °C, with shaking. Cells were passed through a 70 µm cell strainer, washed, and centrifuged for 5 min at 300×g to pellet the stromal-vascular fraction from floating mature adipocytes. For the preparation of single-cell suspensions of liver, the organ was perfused via the portal vein with 10 ml PBS and mashed through a 70 µm cell strainer. Liver samples underwent enrichment for lymphocytes by centrifugation using a Percoll gradient. Spleens were strained through a 70 µm cell strainer and spun. Pellets from all tissues were subjected to red blood cell lysis and subsequently resuspended in flow cytometry buffer (2% FBS and 0.02% NaN₃ in phenol-free DMEM) for further staining.

Flow cytometry and cell sorting. Single-cell suspensions were incubated with Fc receptor-blocking antibody (14-0161-82; eBioscience) before being stained on ice. Dead cells were excluded with a live/dead Zombie Aqua stain (BioLegend). For intracellular transcription factor staining, cells were fixed and permeabilized using the eBioscience Transcription Factor Fix/Perm Buffer. Mouse antibodies were as follows: anti-CD45 (30-F11), anti-CD45.1 (A20), anti-CD45.2 (104), anti-CD27 (LG.3A10), anti-CD69 (FN50), anti-CD44 (BJ18), anti-CD127 (A019D5), anti-CD4 (RM4-5), anti-CD8 α (53-6.7), anti-TCR β (H57-597), anti-KLRG1 (2F1/KLRG1), anti-pdnp (8.1.1), anti-CD26 (H194-112FC), and anti-CD31 (390) were all purchased from BioLegend. Anti-Ter119 (TER-119), anti-F4/80 (BM8), anti-CD19 (ID3), anti- $\gamma\delta$ TCR (GL3), anti-CD3e (500A2), anti-PLZF (Mags.21F7), anti-Foxp3 (FJK-16s), anti-ST2 (RMST2-2), anti-T-bet (4B10), anti-ROR γ t (B2D), anti-TNF (MP6-XT22), anti-IL-17A (17B7), anti-IFN- γ (XMB1.2), anti-PDGFR α (APA5), anti-IL-17RA (PAJ-17R), and streptavidin-APC were all purchased from eBioscience. To stain for V γ 6⁺ cells, GL3 (anti-TCR $\gamma\delta$) and 17D1 (anti-V γ 5V δ 1) antibodies were used as previously described³⁰. Anti-V γ 4 (UC3-10A6) was purchased from BD Pharmingen. Biotinylated anti-cad11 was generated in house. Mouse PBS-57-loaded CD1d tetramers were from the NIH tetramer facility. Human antibodies were as follows: anti-TCR V δ 1 (REA173) and anti-TCR V δ 2 (123R3) were purchased from Miltenyi Biotec. Anti-CD3 (UCHT1) was purchased from BioLegend. Anti-TCR V δ 3 was

custom made in Labpan (Europe). After staining, cells were passed through a 70 μ m filter, and data acquired on a BD FACS Aria Fusion, BD Fortessa, or BD Canto II analyzer using FACSDiva software. Spherotech AccuCount Fluorescent particles were added for cell quantification prior to analysis on the flow cytometers. Cell doublets were excluded by comparison of side-scatter width to forward-scatter area. For analysis of $\gamma\delta$ T cells, a 'dump gate' with Ter119, CD19, and F4/80 was used for elimination of nonspecific staining.

PLZF⁺ and PLZF⁻ $\gamma\delta$ T cells were sorted directly from freshly digested adipose tissue using PLZF^{GFP} mice. Fibroblast subsets were sorted according to the gating scheme outlined in Fig. 6d. Cell sorting was performed on a BD FACS Aria Fusion sorter using a 70 μ m nozzle. Cell purity was routinely >98%. For RNA analyses, sorted cells were lysed in either Trizol (Qiagen) or RLT lysis buffer (Qiagen) with 1% β -mercaptoethanol (2-ME, Sigma).

Immunofluorescence. For detection of GFP, epididymal adipose tissue was harvested into 0.02% sodium azide and 5% normal mouse serum in PBS (Jackson ImmunoResearch Laboratories). Adipose tissue mounted on glass slides with Aqua Poly/Mount (Polysciences) and a coverslip was imaged by confocal microscopy (Leica TCS SP5).

In vitro stimulations. Adipose tissue was digested as described above and bulk stromal vascular fraction (SVF) was stimulated with phorbol 12-myristate 13-acetate (PMA, 50 ng/ml) and ionomycin (1 μ g/ml) for 6 h in complete RPMI medium (RPMI supplemented with 10% FBS [Gemini], HEPES [Invitrogen], L-glutamine, penicillin/streptomycin, and 2-ME). Brefeldin A (1:1000, eBioscience) was added for the last 5 h. Cells were washed twice in 2% FBS in DMEM, surface stained, and fixed and permeabilized using the eBioscience Transcription Factor Fix/Perm Buffer to assay cytokine production by $\gamma\delta$ T cells.

Primary stromal cells from mouse and human adipose tissue were generated by digesting adipose tissue and expanding bulk cells in 6-well plates in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Gemini), 2 mM L-glutamine, 50 μ M 2-ME, and antibiotics (penicillin and streptomycin). After 3-5 days, non-adherent cells are washed off and stromal cells trypsinized. Stromal cells were plated on day 1 at 5×10^4 cells per well in 24-well plates in 10% FBS-containing media. Human cells were serum starved on day 2 by

changing to 1% FBS-containing media. Cells were left unstimulated or stimulated with the indicated concentrations of TNF (Peprotech), IL-17A (Peprotech), or a combination of the two, for 18 h prior to washing in PBS and harvesting cell lysates for protein analysis.

Immunoblotting. Whole adipose tissues were homogenized in lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 5 mM EDTA, 1% Triton X-100, Protease inhibitor cocktail (Roche), 500 U/ml benzonase nuclease (Novagen), 1 mM PMSF, 1 mM Na₃VO₄, 10 mM NaF). Samples were clarified by centrifugation for 15 min at 16,100×g. Protein concentration was measured with a BCA protein assay kit (Pierce). 20-50 µg of protein was loaded on 4-20% Mini Proteon TGX gradient gels (Bio-Rad). Protein was transferred to 0.2 µm PVDF membranes (Bio-Rad). Membranes were blocked in Tris-buffered saline plus 0.1% Tween 20 (TBS-T) containing 5% BSA or 5% milk for 1 h at 25 °C followed by overnight incubation with primary antibody at 4 °C. Primary antibodies were diluted 1:1000 in 5% BSA or 5% milk in TBS-T. Primary antibodies used were against UCP1 (Abcam, #10983) and HSP90 (Cell signaling, #4877, C45G5). Membranes were washed with TBS-T and incubated with HRP-conjugated secondary antibodies for 1 h at 25 °C. HRP was activated with Bio-Rad Clarity Western ECL Substrate (Bio-Rad) and visualized with a chemiluminescent detection system using Bio-Rad Chemidoc. Densitometry of blots were analyzed using ImageJ.

ELISA. Processed adipose SVF lysates or stromal cell cultures were diluted 1:2 in reagent diluent (1% BSA in PBS) and IL-33 protein concentrations quantified using Mouse/Rat IL-33 Quantikine ELISA kit (M3300, R&D Systems). Adipose SVF lysates were similarly analyzed for IL-2 using Ready-SET-Go!® ELISA kit (eBioscience).

RT-PCR analyses. Tissues were snap frozen in liquid nitrogen and stored at -80°C until use. Inguinal, epididymal, and brown adipose tissue depots were homogenized in TRIzol® Reagent (#15596026, Life Technologies) and mixed with chloroform at a ratio of 5:1. After spinning, the upper aqueous phase was mixed with the same volume of 70% EtOH and RNA was isolated using RNeasy Mini Kits (#74104, Qiagen). cDNA was prepared using Quantitect RT-PCR (#205311 Qiagen) and PCR performed with Brilliant III SYBRGreen (#600882, Agilent Technologies) on a Stratagene Mx3000. Primers used were as follows: *Tbp* (forward: 5'-

CTACCGTGAATCTTGGCTGTAAAC-3' (SEQ ID NO:1); reverse: 5'-
 AATCAACGCAGTTGTCCGTGGC-3' (SEQ ID NO:2)), *Il10* (forward: 5'-
 AATAAGCTCCAAGACCAAGG-3' (SEQ ID NO:3); reverse: 5'-
 CAGACTCAATACACACTG-3' (SEQ ID NO:4)), *Il33* (forward: 5'-
 5 ATGGAAGAAGCTGATGGTG-3' (SEQ ID NO:5); reverse: 5'-
 CCGAGGACTTTTTGTGAAGG-3' (SEQ ID NO:6)), *Ucp1* (forward: 5'-
 GGCTCTACGACTCAGTCCA-3' (SEQ ID NO:7); reverse: 5'-
 TAAGCCGGCTGAGATCTTGT-3' (SEQ ID NO:8)), *Ppargc1a* (forward: 5'-
 AGCCGTGACCACTGACAACGAG-3' (SEQ ID NO:9); reverse: 5'-
 10 GCTGCATGGTTCTGAGTGCTAAG-3' (SEQ ID NO:10)), *Dio2* (forward: 5'-
 TGCCACCTTCTTGACTTTGC-3' (SEQ ID NO:11); reverse: 5'-
 GGTTCGGTGCTTCTTAACC-3' (SEQ ID NO:12)), *Cox7a1* (forward: 5'-
 AAACCGTGTGGCAGAGAAGCAG-3' (SEQ ID NO:13); reverse: 5'-
 CCCAAGCAGTATAAGCAGTAGGC-3' (SEQ ID NO:14)), *Adrb3* (forward: 5'-
 15 AACTGAAACAGCAGACAGGGAC-3' (SEQ ID NO:15); reverse: 5'-
 CCCCCATGTACACCCTAGTT-3' (SEQ ID NO:16)), *Th* (forward: 5'-
 CCAAGGTTTCATTGGACGGC-3' (SEQ ID NO:17); reverse: 5'-
 CTCTCCTCGAATACCACAGC-3' (SEQ ID NO:18)), *Lipe* (forward: 5'-
 GCTCATCTCCTATGACCTACGG-3' (SEQ ID NO:19); reverse: 5'-
 20 TCCGTGGATGTGAACAACCAGG-3' (SEQ ID NO:20)), and *Pnpla2* (forward: 5'-
 GGAACCAAAGGACCTGATGACC-3' (SEQ ID NO:21); reverse: 5'-
 ACATCAGGCAGCCACTCCAACA-3' (SEQ ID NO:22)).

Histology. For whole fat tissue staining, 5 mm² of brown adipose tissue was
 fixed in 4% of paraformaldehyde in PBS overnight, washed in PBS, and stored in
 25 70% ethanol. Samples were processed and embedded in paraffin and stained with
 H&E by the Dana Farber Rodent Histopathology Core. For lipid droplet
 quantification, histological sections of brown adipose tissue were placed under a
 microscope, and images were acquired under 100× magnification. These images in
 TIFF format were analyzed using the automated Fiji-based Open Source software
 30 package Adiposoft. Under 0.485 microns per pixel, a minimum diameter of 20 μm
 and a maximum diameter of 100 μm was set for the calculation of adipocyte area.

RNA sequencing. RNA was isolated from 800–1,000 cells from sorted γδ T
 cell populations using *Zbtb16*^{GFP} mice as described. 5 μl of total RNA were placed in

wells of a 96-well plate and RNA sequencing libraries were prepared at Broad Technology Labs at the Broad Institute of Harvard and MIT using the Illumina SmartSeq2 platform. Samples were sequenced on a NextSeq500 using 75 bp paired-end reads to an average depth of 9M pairs of reads per sample by the Broad Genomics Platform. Reads were mapped to the mouse genome (mm10) using HISAT⁴³ (0.1.6-beta release). Bam files were sorted and indexed by SAMtools⁴⁴ (1.2 release). Assembly, quantification and normalization were performed using CuffLinks⁴⁵ (1.2 release), according to the Tuxedo pipeline⁴⁶. A merged transcriptome constructed from all samples was used as a reference annotation for quantification (by CuffQuant) and normalization (by CuffNorm) stages. Differentially expressed genes between PLZF⁺ and PLZF⁻ $\gamma\delta$ T cell subsets were identified using CuffDiff⁴⁷ (false-discovery rate and adjusted *P*-value < 0.1). Genes with calculated FPKM values (according to CuffDiff's pooled dispersion measure) lower than 2⁶ in both subsets were removed from the analysis to avoid low noisy measurements. For heatmaps, values lower than one were replaced by 1, and then data was log₂ transformed.

Human tissue. Omental adipose tissue was obtained from patients undergoing weight-loss surgery with approval of the Brigham and Women's Hospital Institutional Review Board. Tissue was processed similar to mouse adipose tissue. Matched peripheral blood was also collected for analysis. Informed consent was obtained from all patients and samples collected following BWH ethical regulations. Cultured stromal cell fibroblast lines were generated from visceral preadipocytes (Lonza, Cat# PT-5005) and subcutaneous preadipocytes (ATCC, Cat# PLS-210-010) and grown in T-75 cm² flasks in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Gemini), 2 mM L-glutamine, 50 μ M 2-ME, and antibiotics (penicillin and streptomycin).

Statistics. Independent experiments were repeated at least two to three times, and the data were presented as mean $\pm$ SEM. Statistical significance was determined using the Student's *t* test, one-way ANOVA, or two-way ANOVA as indicated. *P*-value of < 0.05 was considered to be statistically significant and is presented as * *P* < 0.05, ** *P* < 0.01, *** *P* < 0.001, or **** *P* < 0.0001. No exclusion of data points or mice was used. Pilot studies were used for estimation of the sample size required to ensure adequate power. GraphPad Prism 6 was used for all statistical analyses.

Life Sciences Reporting Summary. Further information on experimental design and reagents is available in the Life Sciences Reporting Summary.

Data availability. RNAseq expression data has been deposited in the gene expression omnibus under Series GSE103742. The data that support the findings of this study are additionally available from the corresponding author upon request.

Example 1. $\gamma\delta$ T cells are enriched and resident in adipose tissue

We profiled visceral adipose tissue using flow cytometry and found $\gamma\delta$ T cells to be highly enriched in adipose tissue compared to other organs (**Figs. 1A,B**). $\gamma\delta$ T cells were abundant across several adipose depots compared to peripheral sites including lung, liver, blood, and spleen (**Fig. 1B**). To determine if the enriched population of $\gamma\delta$ T cells in visceral adipose tissue was constantly replenished from the periphery or if they represented a tissue-resident population, parabiosis experiments were performed using congenic CD45.1 and CD45.2 mice (**Fig. 1C**). While CD8⁺ T cells reached near-complete chimerism for blood, spleen, liver, and adipose, $\gamma\delta$ T cells showed full recirculation only in the blood and spleen, while the liver and more strikingly, adipose tissue, showed reduced chimerism (**Fig. 1C**). In adipose tissue, >90% of $\gamma\delta$ T cells were endogenous to the host mice, supporting a resident or long-dwelling phenotype. Importantly, $\gamma\delta$ T cells, identified by their V δ chain usage, were also found in human omental adipose tissue (**Fig. 1D**). When compared to peripheral blood, V δ 1, V δ 2, and V δ 3 subsets were increased in human omentum as a percentage of CD45⁺ lymphocytes. Thus, $\gamma\delta$ T cells are enriched and resident in murine and human adipose tissues.

Example 2. PLZF discriminates two $\gamma\delta$ T cell populations

When profiling $\gamma\delta$ T cells in adipose tissue, we found they could be separated into two distinct populations based on CD3 ϵ intensity (**Fig. 2A**)²². CD3 ϵ^{hi} $\gamma\delta$ T cells were more abundant compared to CD3 ϵ^{lo} cells in adipose tissue, making up to two-thirds of the total $\gamma\delta$ T cell pool, while other organs such as the liver and spleen had few CD3 ϵ^{hi} cells (**Fig. 2A**). CD27, a TNFR superfamily member and costimulatory molecule, demarcates functionally distinct $\gamma\delta$ subsets in mice²³. We found that adipose $\gamma\delta$ T cells were largely CD27⁻, which corresponded to the CD3 ϵ^{hi} subset, whereas in the spleen and liver, $\gamma\delta$ T cells were largely CD3 ϵ^{lo} CD27⁺ (**Fig. 2B**).

Additional phenotyping showed the enriched population of CD3 ϵ^{hi} CD27 $^{-}$ $\gamma\delta$ T cells to be CD69 $^{\text{hi}}$ CD44 $^{\text{hi}}$ CD127 $^{+}$ CD45RB $^{-}$ (**Fig. 2C**).

Promyelocytic leukemia zinc finger protein (PLZF) is a BTB-POZ transcription factor encoded by *Zbtb16* and imparts lymphocytes with innate-like qualities. PLZF is expressed on certain $\gamma\delta$ T cells from other organs^{24,25}, *i*NKT cells²⁶ and human MAIT cells²⁷. We analyzed PLZF expression in adipose $\gamma\delta$ T cells by flow cytometry and found that CD3 ϵ^{hi} $\gamma\delta$ T cells highly expressed PLZF compared to CD3 ϵ^{lo} cells by flow cytometry (**Fig. 2D**). PLZF expression was significantly higher in CD3 ϵ^{hi} $\gamma\delta$ T cells in adipose tissue compared to those in liver and spleen (**Fig. 2D**). Furthermore, using *Zbtb16*^{GFP} mice, we found that almost all the PLZF signal (>92% of PLZF $^{+}$ cells) from adipose CD45 $^{+}$ cells was attributed to $\gamma\delta$ T cells, while in the liver most of the PLZF $^{+}$ CD45 $^{+}$ lymphocytes were TCR β^{+} cells (**Fig. 2E**). Whole mount staining of adipose tissue from *Zbtb16*^{GFP} mice further confirmed the presence of PLZF $^{+}$ cells and found them to be interspersed between adipocytes (**Fig. 2F**). PLZF-deficient (Luxoid) mice showed a two-thirds reduction in the frequency of adipose $\gamma\delta$ T cells, corresponding with the relative frequency of PLZF $^{+}$ CD3 ϵ^{hi} $\gamma\delta$ T cells in adipose tissue (**Fig. 2G**). $\gamma\delta$ T cell frequencies in other organs were unaffected by loss of PLZF, as they harbored fewer PLZF $^{+}$ CD3 ϵ^{hi} $\gamma\delta$ T cells (**Fig. 2G**), highlighting the requirement of PLZF for the CD27 $^{-}$ $\gamma\delta$ T cell population resident in adipose tissue.

Example 3. $\gamma\delta$ T cells are important for adipose T_{reg} accumulation

Next, we quantified the numbers of $\gamma\delta$ T cells to determine changes in their frequency in adipose tissue over time. Interestingly, $\gamma\delta$ T cells displayed similar accumulation kinetics as Foxp3 $^{+}$ T_{reg} cells in adipose tissue (**Fig. 3A**). In contrast, both ILC2s and *i*NKT cells, two populations previously shown to influence adipose T_{reg} numbers, decreased at the time of T_{reg} expansion (**Fig. 3B**). Besides T_{reg} cells and $\gamma\delta$ T cells, no other lymphocyte population quantified increased with age (**Figs. 9A-9C**). It is well described that T_{reg} cells expand in adipose tissue with age, but a unifying mechanism to explain their temporal accumulation remains unknown. Thus, we wondered if $\gamma\delta$ T cells played a role in adipose T_{reg} homeostasis. To test this, we profiled wild-type and TCR δ -deficient (*Tcrd*^{-/-}) mice and found that the frequency of T_{reg} cells was significantly reduced in *Tcrd*^{-/-} mice compared to wild-type counterparts after 20 weeks (**Fig. 3C**). A characteristic feature of adipose T_{reg} cells is

their high expression of IL-10, KLRG1, and ST2¹⁰. T_{reg} cells sorted from *Tcrd*^{-/-} adipose tissue expressed significantly less *Il10* (**Fig. 3D**) and surface KLRG1 compared to T_{reg} cells from wild-type mice (**Fig. 3E**). Lastly, we found a striking defect in ST2⁺ T_{reg} cell accumulation in *Tcrd*^{-/-} compared to wild-type littermates at 22 weeks of age, at the time of physiologic T_{reg} cell expansion (**Fig. 3F**). Together these results reveal a concomitant increase of $\gamma\delta$ T cells and T_{reg} cells with age and the requirement of $\gamma\delta$ T cells for visceral adipose T_{reg} accumulation.

Example 4. PLZF⁺ $\gamma\delta$ T cells are innate IL-17A producing cells

$\gamma\delta$ T cells are generally recognized as innate-like lymphocytes that induce inflammation in response to pathogens and cellular stress. They rapidly secrete inflammatory cytokines such as TNF, interferon- γ (IFN- γ) and IL-17, as well as chemokines that recruit key phagocytes to injured or infected tissues²⁸. To understand how $\gamma\delta$ T cells modulate T_{reg} numbers in adipose tissue, we further characterized their transcriptional phenotype and function. PLZF⁺ and PLZF⁻ $\gamma\delta$ T cells were sorted from adipose tissue of *Zbtb16*^{GFP} mice for RNA sequencing and gene-expression analysis. Differential expression analysis showed 247 and 205 genes to be significantly upregulated in adipose PLZF⁺ and PLZF⁻ $\gamma\delta$ T cells, respectively, of which the top 60 genes are shown (**Fig. 4A**). Consistent with the sorting strategy, *Zbtb16* was among the most differential genes (**Figs. 4A,B**). Notably, transcripts for genes including *Sox13*, *Rorc*, *Il1r1*, and *Il23r* were significantly upregulated in PLZF⁺ $\gamma\delta$ T cells compared to PLZF⁻ counterparts (**Fig. 4A,B**). Moreover, the high transcript levels of *Tcr γ* , *Tcr δ* , *Cd3e*, and *Cd3g* in PLZF⁺ $\gamma\delta$ T cells reaffirmed the flow cytometry data that distinguished CD3 Σ ^{hi} versus CD3 Σ ^{lo} $\gamma\delta$ subsets (**Fig. 4A**). In contrast, PLZF⁻ $\gamma\delta$ T cells showed significant expression of genes characteristic of NK cells including *Ncr*, *Nkg7*, *Klrk1*, *Gzmb*, and *Gzma* (**Fig. 4A,B**). These genes, combined with overexpression of genes encoding T-bet (*Tbx21*) and CD27 (*Cd27*), highlight their T_H1- and NK-like transcriptional phenotype (**Fig. 4B**). Lastly, we validated protein expression of ROR γ t and T-bet in $\gamma\delta$ T cells and found them to be discretely expressed by PLZF⁺ and PLZF⁻ subsets, respectively (**Fig. 4C**).

Transcriptional analysis revealed that PLZF⁺ $\gamma\delta$ T cells expressed *Il17a*, while PLZF⁻ $\gamma\delta$ T cells expressed *Ifng*. As CD27 is useful to demarcate IL-17A-producing (CD27⁻) versus IFN- γ -producing (CD27⁺) $\gamma\delta$ T cells, we used CD27 as a marker for functional analysis. Upon stimulation, PLZF⁺ $\gamma\delta$ T cells exclusively produced IL-17A

and TNF, while PLZF⁻ $\gamma\delta$ T cells exclusively produced IFN- γ , consistent with their gene expression (**Fig. 4D**). Together, our transcriptional, phenotypic, and functional characterization highlight PLZF⁺ $\gamma\delta$ T cells as innate IL-17A-producing cells that exhibit a distinct effector program from the NK-like PLZF⁻ $\gamma\delta$ T cells in adipose tissue.

Example 5. ST2⁺ T_{reg} numbers depend on PLZF⁺ $\gamma\delta$ T cells and IL-17A

To parse the relative contributions of the two $\gamma\delta$ T cell populations to the observed T_{reg} phenotype, we determined the kinetics of accumulation of the two subsets over time (**Fig. 5A**). Flow cytometry analysis of visceral fat pads showed IL-17A-producing PLZF⁺ $\gamma\delta$ T cells, but not PLZF⁻ $\gamma\delta$ T cells, expand with age (**Fig. 5A**). Importantly, profiling $\gamma\delta$ T cells from *Il17a*^{GFP} mice showed that the numbers significantly increased in adipose tissue with age (**Fig. 5B**). We next asked if IL-17A was important for the observed T_{reg} cell accumulation. We found that IL-17A-deficient (*Il17a*^{-/-}) mice had significantly reduced numbers and frequencies of total Foxp3⁺ T_{reg} cells and failed to accumulate ST2⁺ T_{reg} cells in adipose tissue at 20 weeks of age (**Fig. 5C**). *i*NKT and ILC2 numbers were not different between wild-type and *Il17a*^{-/-} mice (**Figs. 10A-10E**). This data suggests that IL-17A is a key factor to the homeostatic expansion of T_{reg} cells in visceral adipose tissue.

$\gamma\delta$ T cells with specific V-gene rearrangements leave the thymus in concerted waves during neonatal development and seed tissues²⁹. The innate-IL17A producing subset is largely dominated by V γ 6⁺ TCRs, although other IL-17A-producing V γ 4⁺ cells can arise later. As some PLZF⁺ $\gamma\delta$ T cells have been reported to harbor the canonical V γ 6⁺ TCR chain, we stained $\gamma\delta$ T cells from adipose tissue with antibodies to determine TCR usage³⁰. At 20 weeks of age, the majority of CD3 ϵ ^{hi}PLZF⁺CD27⁻ $\gamma\delta$ T cells were V γ 6⁺, while CD3 ϵ ^{lo}PLZF⁻CD27⁺ $\gamma\delta$ T cells expressed V γ 1⁺ and V γ 4⁺ TCR chains and comprised a smaller fraction of total adipose $\gamma\delta$ T cells (**Fig. 5D**).

To deplete the majority of PLZF⁺ $\gamma\delta$ T cells, we characterized adipose tissue from V γ 4–V γ 6-deficient (*Vg4/6*^{-/-}) mice. We confirmed that *Vg4/6*^{-/-} mice had severely reduced numbers of PLZF⁺CD3 ϵ ^{hi} $\gamma\delta$ T cells in adipose tissue, while PLZF⁻CD3 ϵ ^{lo} $\gamma\delta$ T cells were still present (**Fig. 5E**). Next, we analyzed 20-week-old wild-type and *Vg4/6*^{-/-} mice and found that, like *Il17a*^{-/-} and *Tcrd*^{-/-} mice, *Vg4/6*^{-/-} mice displayed significant reductions in total adipose Foxp3⁺ and ST2⁺ T_{reg} cells (**Fig. 5F**). When profiling other immune populations, we observed similar frequencies of ILC2s,

but surprisingly, *i*NKT cells were significantly decreased in *Vg4/6^{-/-}* mice compared to wild-type, suggesting there may be crosstalk between PLZF⁺ $\gamma\delta$ T cells and *i*NKT cells in adipose tissue (**Figs. 10A-10E**). Interestingly, the reduced frequencies of ST2⁺ T_{reg} cells were not observed in spleen and lung of *Il17a^{-/-}* and *Vg4/6^{-/-}* mice (**Figs. 10A-10E**). In summary, these data uncover an important role for both IL-17A and PLZF⁺ $\gamma\delta$ T cells in age-dependent increases of adipose tissue T_{reg} cell numbers.

Example 6. TNF and IL-17A induce IL-33 in adipose stromal cells

IL-33, a member of the IL-1 family of cytokines, is an important regulator of adipose ILC2 and T_{reg} cell homeostasis owing to the fact that both cell types express the cognate receptor, ST2^{11,15,31-34}. Adipose T_{reg} cells have high expression of ST2, and engagement of the ST2 receptor by IL-33 results in T_{reg} proliferation^{11,15}. We found that IL-33 protein increased with age in visceral adipose tissue, concomitant with T_{reg} and PLZF⁺ $\gamma\delta$ T cell accumulation (**Fig. 6A**). Because of the significant reduction of T_{reg} numbers in *Tcrd^{-/-}*, *Il17a^{-/-}* and *Vg4/6^{-/-}* mice, we asked if $\gamma\delta$ T cells affected IL-33 levels in adipose tissue. Indeed, *Tcrd^{-/-}* mice showed a significant decrease in IL-33 protein in adipose tissue but not spleen in 20-week-old mice (**Fig. 6B**). IL-2, a critical cytokine for T_{reg} maintenance in other peripheral organs was not different between wild-type and *Tcrd^{-/-}* mice (**Fig. 6B**). There was also a clear reduction in IL-33 protein in adipose tissue of *Il17a^{-/-}* and *Vg4/6^{-/-}* mice compared to wild-type (**Fig. 6C**). Interestingly, *Il17a^{-/-}* mice have significantly lower IL-33 protein and fewer ST2⁺ T_{reg} cell numbers in visceral adipose tissue even at 11 weeks of age, suggesting that this phenotype manifests earlier in *Il17a^{-/-}* mice compared to wild-type (**Figs. 10A-10E**). Together, these results suggest that IL-17A-producing PLZF⁺ $\gamma\delta$ T cells are necessary for age-induced increases IL-33 protein in visceral adipose tissue.

To assess the contribution of IL-17A producing PLZF⁺ $\gamma\delta$ T cells to IL-33 concentrations, we sought to identify the IL-33 expressing cell type and importantly, test whether it would respond to IL-17A. Flow cytometry of the adipose stromal compartment identified a substantial population of CD31⁺ endothelial cells, as well as Pdpn⁺ (gp38⁺) stromal cells, among the non-hematopoietic (CD45⁻) pool (**Fig. 6D**). These Pdpn⁺ cells could further be segregated by their expression of the surface marker platelet-derived growth factor receptor-alpha (PDGFR α)(**Fig. 6D**), as well as CD26, CD9, and Cadherin-11 (Cdh11)(**Fig. 6E**). Staining for interleukin-17 receptor

(IL-17R) to identify the subset that would respond to IL-17A identified Pdpn⁺CD26⁺PDGFR α ⁻Cdh11⁺ stromal cells as the dominant IL-17R expressing population in adipose tissue (**Fig. 6E**). Intriguingly, when we quantified *Il33* mRNA from sorted stromal cells, we found that Pdpn⁺PDGFR α ⁻ cells highly expressed *Il33* compared to other cells in adipose tissue (**Fig. 6E**). Our data highlighted Pdpn⁺CD26⁺PDGFR α ⁻Cdh11⁺ stromal cells as the main IL-33 and IL-17R expressing cell type in visceral adipose tissue.

In addition to amplifying inflammation, IL-17A plays important homeostatic and anti-microbial roles at mucosal sites³⁵. However, its effects on adipose tissue stromal cells (fibroblasts and adipocytes), as well as other resident immune cells, are less appreciated³⁶. Because *Il17a*^{-/-} mice exhibited severe decreases in T_{reg} cell numbers and IL-33 protein, we asked if IL-17A was sufficient to induce IL-33 expression in adipose stromal cells. Using 3T3L1 preadipocytes and fresh primary adipose stromal cells, we found that IL-17A stimulated *Il33* mRNA and IL-33 protein, but it was the presence of both TNF and IL-17A that synergized to induce high expression of IL-33 (**Fig. 6E**). Moreover, this stimulation was specific for the combination of TNF and IL-17A, as IFN- γ or IL-1 β failed to increase IL-33 to the same extent (**Figs. 11A-11D**).

We next injected purified recombinant TNF and IL-17A into mice, and found that, upon injection, *Il33* mRNA increased in adipose tissue (**Figs. 11A-11D**). Mechanistically, TNF and IL-17A expanded IL-33 expressing Pdpn⁺ stromal cells and upregulated *Il33* mRNA within the PDGFR α ⁺ population (**Figs. 11A-11D**). Although Pdpn⁺ stromal cells expressed the highest amounts of IL-33 at steady state, it appears that *in vivo*, both Pdpn⁺ and PDGFR α ⁺ stromal subsets can contribute to endogenous IL-33 in visceral adipose tissue and can both be affected by TNF and IL-17A.

To assess why mice deficient in $\gamma\delta$ T cells or IL-17A exhibited decreases in IL-33 in adipose tissue, we quantified the numbers and IL-33 expression of stromal populations from wild-type, *Tcrd*^{-/-}, *Vg4/6*^{-/-} and *Il17a*^{-/-} mice. We found that both Pdpn⁺ and PDGFR α ⁺ stromal subset numbers were greatly decreased in *Tcrd*^{-/-}, *Vg4/6*^{-/-} and *Il17a*^{-/-} mice, while IL-33 expression within stromal cells showed no significant differences (**Figs. 12A-12B**). Our data suggest that in $\gamma\delta$ -deficient and IL-17A-deficient animals, the decreased IL-33 owes largely to decreased numbers of IL-

33 expressing or producing Pdpn⁺ and PDGFR α ⁺ cells, respectively, and less so on lower *Il33* mRNA within stromal populations.

Recent reports have demonstrated IL-33 expression in human adipocytes and endothelial cells^{37,38} and found that human omental T_{reg} cells express ST2¹¹. We asked whether this newly defined IL-17A–TNF–IL-33 axis existed in human adipose tissue. In human primary preadipocytes from both visceral and subcutaneous adipose depots, stimulation with TNF and IL-17A greatly enhanced expression of IL-33 (**Fig. 6H**). Taken together, TNF and IL-17A produced by PLZF⁺ $\gamma\delta$ T cells promotes IL-33 within the stromal compartment in adipose tissue.

Example 7. $\gamma\delta$ T cells and IL-17A regulate body temperature

In addition to adipose immune regulation, IL-33 is important for thermogenesis, the metabolic adaptation to cold temperatures^{17,18}. When we extended our analysis to brown (BAT) and inguinal adipose tissue (iWAT), depots important for increasing body temperature, we found IL-33 protein to be significantly decreased in BAT of *Tcrd*^{-/-} and *Vg4/6*^{-/-} mice compared to wild-type (**Figs. 13A-13B**). Although protein levels were not different in inguinal adipose tissue, *Il33* mRNA was decreased in *Tcrd*^{-/-} and *Vg4/6*^{-/-} mice compared to wild-type (**Figs. 13A-13B**), raising the possibility that mice lacking $\gamma\delta$ T cells might exhibit defects in body temperature regulation.

In support, histological sections of BAT from cold challenged mice showed *Tcrd*^{-/-} and *Vg4/6*^{-/-} mice to harbor more lipids in brown adipocytes compared to wild-type littermates (**Fig. 7A**). Importantly, *Tcrd*^{-/-} and *Vg4/6*^{-/-} mice had decreased UCP1 protein in BAT, and lower expression of thermogenic genes including *Ppargc1a*, *Dio2*, and *Cox7a1* compared to wild-type after cold challenge (**Fig. 7B,C**). Remarkably, when we evaluated iWAT after cold challenge, *Tcrd*^{-/-} and *Vg4/6*^{-/-} mice were unable to burn lipids evidenced by gross anatomy (**Figs. 13A-13B**), and had decreased expression and activation of hormone sensitive lipase (HSL), a critical lipolysis enzyme (**Fig. 7D**). Like brown adipose tissue, the inguinal depot from *Tcrd*^{-/-} and *Vg4/6*^{-/-} mice after cold challenge showed decreased expression of thermogenic genes (**Fig. 7E**), suggesting mice deficient in $\gamma\delta$ T cells are unable to upregulate adaptive thermogenesis in BAT and iWAT.

To determine whether these local adipose defects had physiological consequences, we measured body temperature and energy expenditure by indirect

calorimetry. *Tcrd*^{-/-} mice dropped body temperature significantly more rapidly than wild-type in the cold and correspondingly were unable to increase energy expenditure (**Fig. 7F**). Of note, when challenged with the β -adrenergic agonist CL-316 243, which maximally activates BAT thermogenesis, *Tcrd*^{-/-} mice did not show a defect in their ability to expend energy as heat, suggesting that β -adrenergic signaling was downstream of $\gamma\delta$ T cell control (**Figs. 13A-13B**). Thus, mice deficient in $\gamma\delta$ T cells were unable to engage non-shivering thermogenesis in response to cold, in part due to an inability to upregulate factors important for turning on the thermogenic program in BAT and iWAT.

To better understand how $\gamma\delta$ T cells promoted thermogenic responses, we quantified the frequency and cytokine profile of $\gamma\delta$ T cells in BAT and iWAT after cold challenge. We found that 8 hours after cold challenge, $\gamma\delta$ T cells significantly increased in frequency in both BAT and iWAT and remained elevated at 24 hours (**Fig. 8A**). Despite their increased frequency, the number of $\gamma\delta$ T cells did not change, suggesting the decline of other immune populations with cold (**Figs. 14A-14D**). When $\gamma\delta$ T cells were stimulated with PMA and ionomycin, greater than 40% produced IL-17A in BAT and iWAT (**Fig. 8B**). Conversely, other immune populations produced little IL-17A, highlighting the role of $\gamma\delta$ T cells as the dominant source of IL-17A in both depots (**Fig. 8C**). $\gamma\delta$ T cells thus become a larger percentage of immune cells after cold challenge and are a major source of IL-17A *in situ*.

As $\gamma\delta$ T cells produce IL-17A in BAT and iWAT, we hypothesized that IL-17A might be an important regulator of the thermogenic phenotype observed. Indeed, like *Tcrd*^{-/-} and *Vg4/6*^{-/-} mice, the BAT of *Il17a*^{-/-} mice contained more lipid droplets (**Fig. 8D**) and failed to upregulate UCP1 (**Fig. 8E**) compared to wild-type after cold. Moreover, defects in lipolysis were observed in the iWAT of *Il17a*^{-/-} mice (**Fig. 8F**), and a similar inability of *Il17a*^{-/-} mice to upregulate *Ucp1* in iWAT was measured (**Fig. 8G**). *Il17a*^{-/-} mice showed decreased expression of other thermogenic genes in both BAT and iWAT (**Figs. 14A-14D**). Strikingly, when WT and *Il17a*^{-/-} mice were placed in metabolic cages for indirect calorimetry assessment, all of the *Il17a*^{-/-} mice had to be rescued from death 5-12 hours after cold challenge because of their inability to increase energy expenditure (**Fig. 8H,L**). This is evidenced by their inability to increase body temperature 5 hours after cold challenge (**Figs. 14A-14D**). Interestingly, *Il17a*^{-/-} mice also showed abnormal circadian control of body

temperature and respiratory exchange ratio (RER) (**Figs. 14A-14D**). Together, these data support a critical role for IL-17A in body temperature regulation.

To understand the mechanism behind thermogenic control by $\gamma\delta$ T cells and IL-17A, we first quantified gene expression for thermogenic enzymes and receptors (**Figs. 15A-15B**). Interestingly, *Adrb3* mRNA was decreased across all genotypes in iWAT at thermoneutrality and after cold. *Lipe* and *Pnpla2*, two key genes of lipolysis, were also significantly decreased, suggesting mice lacking $\gamma\delta$ T cells or IL-17A were less sensitive to catecholamine stimulation for lipolysis induction. Second, stimulation with TNF and IL-17A synergized to upregulate thermogenic genes including *Ucp1*, *Dio2*, *Cidea*, and *Il33* in brown adipocyte cultures (**Figs. 15A-15B**). Stimulation with IL-33 itself did not induce the same increases in gene expression, suggesting that TNF and IL-17A can induce a thermogenic program in BAT independent of IL-33 (**Figs. 16A-16C**). In iWAT, TNF and IL-17A injections *in vivo* were sufficient to induce *Ucp1*, *Ppargc1a*, and *Dio2* in sorted Pdpn⁺ stromal cells, but not PDGFR α ⁺ cells (**Figs. 16A-16C**). Lastly, we quantified BAT and iWAT of wild-type, *Tcrd*^{-/-} and V γ 4/6^{-/-} mice for immune cells known to be important for thermogenic responses (**Figs. 16A-16C**). There were no differences in ILC2, eosinophil, *i*NKT, and Treg frequencies between wild-type and *Tcrd*^{-/-} mice in both depots, suggesting the thermogenic defects seen in $\gamma\delta$ T cell deficient mice were independent of other immune populations. Together, our data suggest $\gamma\delta$ T cells can promote thermogenic responses directly through the cytokines they produce, namely TNF and IL-17A, and indirectly through maintenance of catecholamine sensitivity.

References

1. Brestoff, J. R. & Artis, D. Immune regulation of metabolic homeostasis in health and disease. *Cell* **161**, 146–160 (2015).
2. Kohlgruber, A. & Lynch, L. Adipose tissue inflammation in the pathogenesis of type 2 diabetes. *Curr. Diab. Rep.* **15**, 92;1-11 (2015).
3. Lynch, L. *et al.* Regulatory *i*NKT cells lack expression of the transcription factor PLZF and control the homeostasis of T(reg) cells and macrophages in adipose tissue. *Nat. Immunol.* **16**, 85–95 (2015).
4. Lynch, L. *et al.* Adipose tissue invariant NKT cells protect against diet-induced obesity and metabolic disorder through regulatory cytokine production. *Immunity* **37**, 574–587 (2012).

5. Molofsky, A. B. *et al.* Innate lymphoid type 2 cells sustain visceral adipose tissue eosinophils and alternatively activated macrophages. *Journal of Experimental Medicine* **210**, 535–549 (2013).
6. Nussbaum, J. C. *et al.* Type 2 innate lymphoid cells control eosinophil homeostasis. *Nature* **502**, 245–248 (2013).
7. Lynch, L. *et al.* iNKT Cells Induce FGF21 for Thermogenesis and Are Required for Maximal Weight Loss in GLP1 Therapy. *Cell Metab.* **24**, 510–519 (2016).
8. Brestoff, J. R. *et al.* Group 2 innate lymphoid cells promote beiging of white adipose tissue and limit obesity. *Nature* **519**, 242–246 (2015).
9. Feuerer, M. *et al.* Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. *Nat. Med.* **15**, 930–939 (2009).
10. Cipolletta, D. *et al.* PPAR- γ is a major driver of the accumulation and phenotype of adipose tissue Treg cells. *Nature* **486**, 549–553 (2012).
11. Vasanthakumar, A. *et al.* The transcriptional regulators IRF4, BATF and IL-33 orchestrate development and maintenance of adipose tissue-resident regulatory T cells. *Nat. Immunol.* **16**, 276–285 (2015).
12. Bapat, S. P. *et al.* Depletion of fat-resident Treg cells prevents age-associated insulin resistance. *Nature* **528**, 137–141 (2015).
13. Cipolletta, D. *et al.* Adipose tissue-resident regulatory T cells: phenotypic specialization, functions and therapeutic potential. *Immunology* **142**, 517–525 (2014).
14. Panduro, M. *et al.* Tissue Tregs. *Annu. Rev. Immunol.* **34**, 609–633 (2016).
15. Kolodin, D. *et al.* Antigen- and cytokine-driven accumulation of regulatory T cells in visceral adipose tissue of lean mice. *Cell Metab.* **21**, 543–557 (2015).
16. Molofsky, A. B. *et al.* Interleukin-33 and Interferon- γ Counter-Regulate Group 2 Innate Lymphoid Cell Activation during Immune Perturbation. *Immunity* **43**, 161–174 (2015).
17. Mathis, D. IL-33, Imprimatur of Adipocyte Thermogenesis. *Cell* **166**, 794–795 (2016).

18. Odegaard, J. I. *et al.* Perinatal Licensing of Thermogenesis by IL-33 and ST2. *Cell* **166**, 841–854 (2016).
19. Lee, M.-W. *et al.* Activated type 2 innate lymphoid cells regulate beige fat biogenesis. *Cell* **160**, 74–87 (2015).
- 5 20. Jackson-Jones, L. H. *et al.* Fat-associated lymphoid clusters control local IgM secretion during pleural infection and lung inflammation. *Nat. Commun.* **7**, 12651 (2016).
- 10 21. Pichery, M. *et al.* Endogenous IL-33 is highly expressed in mouse epithelial barrier tissues, lymphoid organs, brain, embryos, and inflamed tissues: in situ analysis using a novel IL-33-LacZ gene trap reporter strain. *The Journal of Immunology* **188**, 3488–3495 (2012).
22. Paget, C. *et al.* CD3bright signals on $\gamma\delta$ T cells identify IL-17A-producing V γ 6V δ 1+ T cells. *Immunol. Cell Biol.* **93**, 198–212 (2015).
- 15 23. Ribot, J. C. *et al.* CD27 is a thymic determinant of the balance between interferon-gamma- and interleukin 17-producing gammadelta T cell subsets. *Nat. Immunol.* **10**, 427–436 (2009).
24. Kreslavsky, T. *et al.* TCR-inducible PLZF transcription factor required for innate phenotype of a subset of gammadelta T cells with restricted TCR diversity. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 12453–12458 (2009).
- 20 25. Lu, Y. *et al.* PLZF Controls the Development of Fetal-Derived IL-17+V γ 6+ $\gamma\delta$ T Cells. *The Journal of Immunology* **195**, 4273–4281 (2015).
26. Savage, A. K. *et al.* The transcription factor PLZF directs the effector program of the NKT cell lineage. *Immunity* **29**, 391–403 (2008).
- 25 27. Alonzo, E. S. & Sant'Angelo, D. B. Development of PLZF-expressing innate T cells. *Current Opinion in Immunology* **23**, 220–227 (2011).
28. Vantourout, P. & Hayday, A. Six-of-the-best: unique contributions of $\gamma\delta$ T cells to immunology. *Nat. Rev. Immunol.* **13**, 88–100 (2013).
29. Wei, Y.-L. *et al.* A Highly Focused Antigen Receptor Repertoire Characterizes $\gamma\delta$ T Cells That are Poised to Make IL-17 Rapidly in Naive Animals. *Front. Immunol.* **6**, 118 (2015).
- 30 30. Roark, C. L. *et al.* Subset-specific, uniform activation among V γ 6/V δ 1+ $\gamma\delta$ T cells elicited by inflammation. *Journal of Leukocyte Biology* **75**, 68–75 (2004).

31. Van Dyken, S. J. *et al.* Chitin activates parallel immune modules that direct distinct inflammatory responses via innate lymphoid type 2 and $\gamma\delta$ T cells. *Immunity* **40**, 414–424 (2014).
32. Vannella, K. M. *et al.* Combinatorial targeting of TSLP, IL-25, and IL-33 in type 2 cytokine-driven inflammation and fibrosis. *Science Translational Medicine* **8**, 337ra65 (2016).
33. Schiering, C. *et al.* The alarmin IL-33 promotes regulatory T-cell function in the intestine. *Nature* **513**, 564–568 (2014).
34. Kuswanto, W. *et al.* Poor Repair of Skeletal Muscle in Aging Mice Reflects a Defect in Local, Interleukin-33-Dependent Accumulation of Regulatory T Cells. *Immunity* **44**, 355–367 (2016).
35. Hepworth, M. R. Innate Lymphoid Cell Regulation: Meeting the Long-Lost Cousin. *Trends Immunol.* **38**, 873–874 (2017).
36. Zúñiga, L. A. *et al.* IL-17 regulates adipogenesis, glucose homeostasis, and obesity. *The Journal of Immunology* **185**, 6947–6959 (2010).
37. Wood, I. S. *et al.* IL-33, a recently identified interleukin-1 gene family member, is expressed in human adipocytes. *Biochem. Biophys. Res. Commun.* **384**, 105–109 (2009).
38. Zeyda, M. *et al.* Severe obesity increases adipose tissue expression of interleukin-33 and its receptor ST2, both predominantly detectable in endothelial cells of human adipose tissue. *Int J Obes (Lond)* **37**, 658–665 (2013).
39. Martin, N. T. & Martin, M. U. Interleukin 33 is a guardian of barriers and a local alarmin. *Nat. Immunol.* **17**, 122–131 (2016).
40. Kovalovsky, D. *et al.* The BTB-zinc finger transcriptional regulator PLZF controls the development of invariant natural killer T cell effector functions. *Nat. Immunol.* **9**, 1055–1064 (2008).
41. Wagers, A. J. *et al.* Little evidence for developmental plasticity of adult hematopoietic stem cells. *Science* **297**, 2256–2259 (2002).
42. Okada, K. *et al.* Thioesterase superfamily member 1 suppresses cold thermogenesis by limiting the oxidation of lipid droplet-derived fatty acids in brown adipose tissue. *Mol Metab* **5**, 340–351 (2016).
43. Kim, D., Langmead, B. & Salzberg, S. L. HISAT: a fast spliced aligner with low memory requirements. *Nature Methods* **12**, 357–360 (2015).

44. Li, H. *et al.* The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**, 2078–2079 (2009).
45. Trapnell, C. *et al.* Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nat. Biotechnol.* **28**, 511–515 (2010).
46. Trapnell, C. *et al.* Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. *Nat Protoc* **7**, 562–578 (2012).
47. Trapnell, C. *et al.* Differential analysis of gene regulation at transcript resolution with RNA-seq. *Nat. Biotechnol.* **31**, 46–53 (2013).

10

OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

15

WHAT IS CLAIMED IS:

1. A method of promoting weight loss, the method comprising administering to a subject in need thereof therapeutically effective amounts of a combination of agents comprising:
 - (i) Tumor Necrosis Factor alpha (TNF α) or an agonist of the TNF Receptor (TNFR), and
 - (ii) Interleukin 17 (IL-17) or IL-33.
2. The method of claim 1, wherein the subject has a BMI of 29 or above.
3. The method of claim 1, wherein the agents are modified to increase half-life.
4. The method of claim 1, wherein the agents are targeted to adipose tissue.
5. The method of claim 1, wherein the TNFR agonist is selected from the group consisting of Varlilumab, Urelumab, utomilumab, PRS-343, MEDI0562, MOXRO0916, GSK3174998, PF-04518600, and TRX518.
6. The method of claim 1, further comprising administering an agent that activates $\gamma\delta$ T cells, preferably wherein the agent that activates $\gamma\delta$ T cells is selected from the group consisting of an activating monoclonal antibody against a $\gamma\delta$ specific TCR, pyrophosphates or butyrophilins.
7. The method of claim 6, wherein the activating monoclonal antibody against a $\gamma\delta$ specific TCR is a monoclonal antibody targeting Vd2.
8. A method of inhibiting weight loss, the method comprising administering to a subject in need thereof therapeutically effective amounts of a combination of agents comprising:
 - (i) an inhibitor of Tumor Necrosis Factor alpha (TNF α) or the TNF Receptor (TNFR), and
 - (ii) an inhibitor of Interleukin 17 (IL-17), IL-17 Receptor, or IL-33.
9. A method of inhibiting weight loss, the method comprising administering to a subject in need thereof a therapeutically effective amount of an inhibitory anti- IL-33 antibody.

10. The method of claim 8 or 9, wherein the subject has a chronic illness or cancer.
11. The method of claim 10, wherein the cancer is not IL-17 dependent.
12. The method of claim 10, wherein the cancer is pancreatic cancer.
13. The method of claim 10, wherein the subject has COPD, diabetic kidney disease, or heart failure.
14. The method of claims 8 or 9, wherein the agents are modified to increase half-life.
15. The method of claims 8 or 9, wherein the agents are targeted to adipose tissue.
16. The method of claim 8, wherein the inhibitor of IL-17 or the IL-17R is selected from the group consisting of secukinumab, ustekinumab, brodalumab and ixekizumab.
17. The method of claim 8, wherein the inhibitor of TNF α or the TNFR is selected from the group consisting of infliximab, adalimumab, certolizumab pegol, golimumab, and etanercept.

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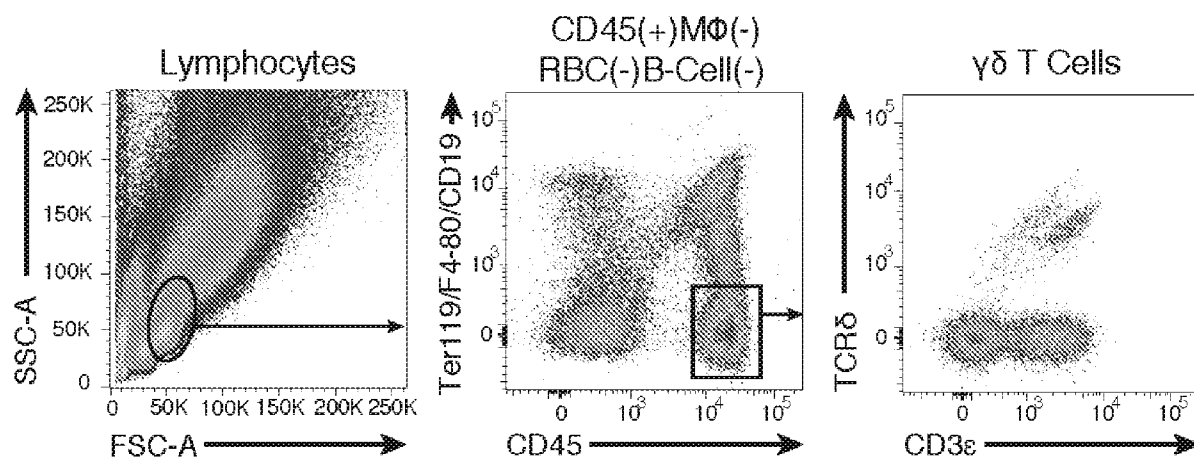


FIG. 1A

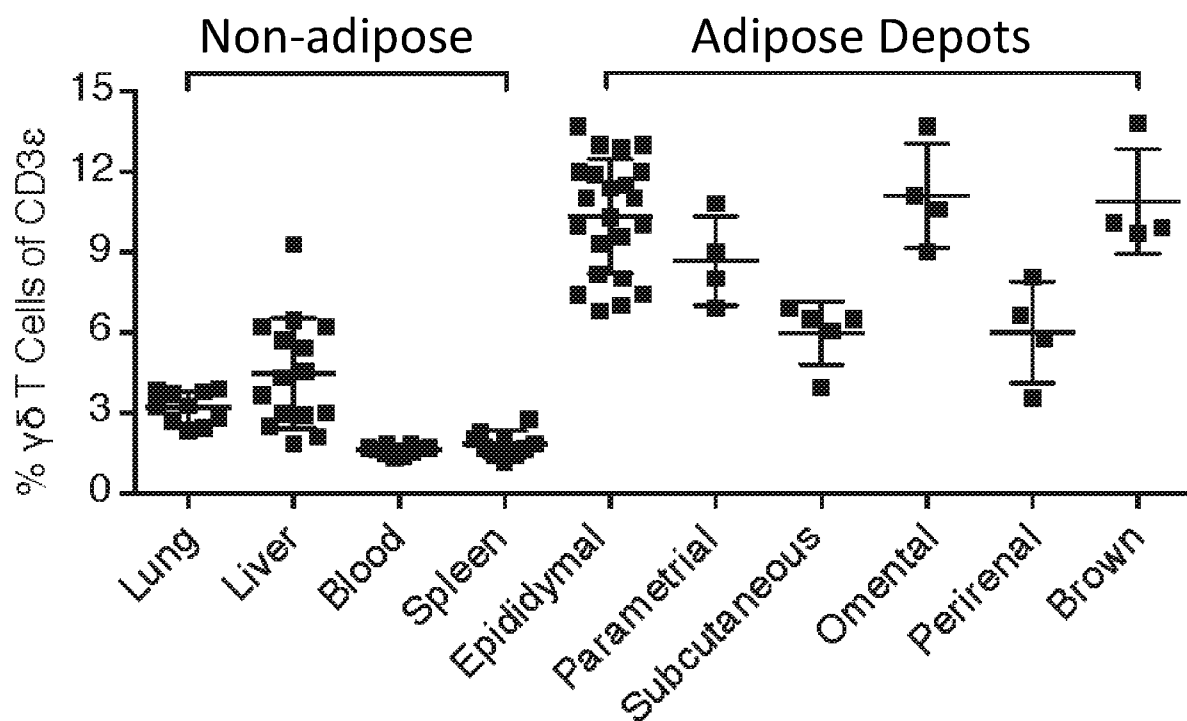


FIG. 1B

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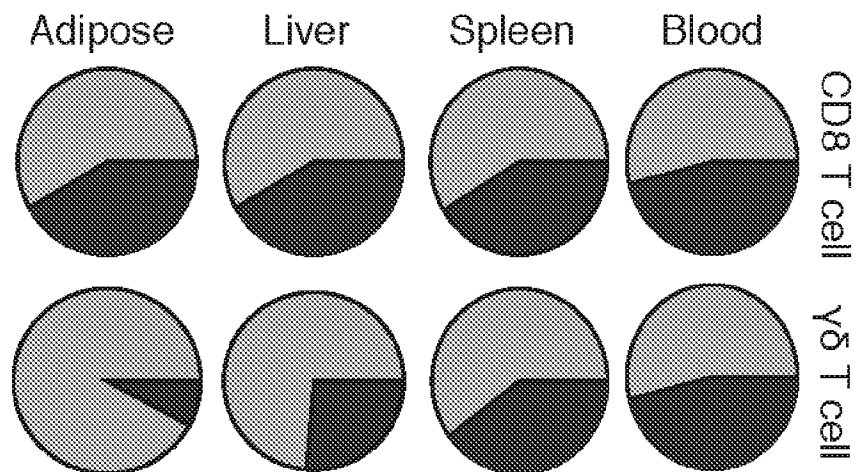
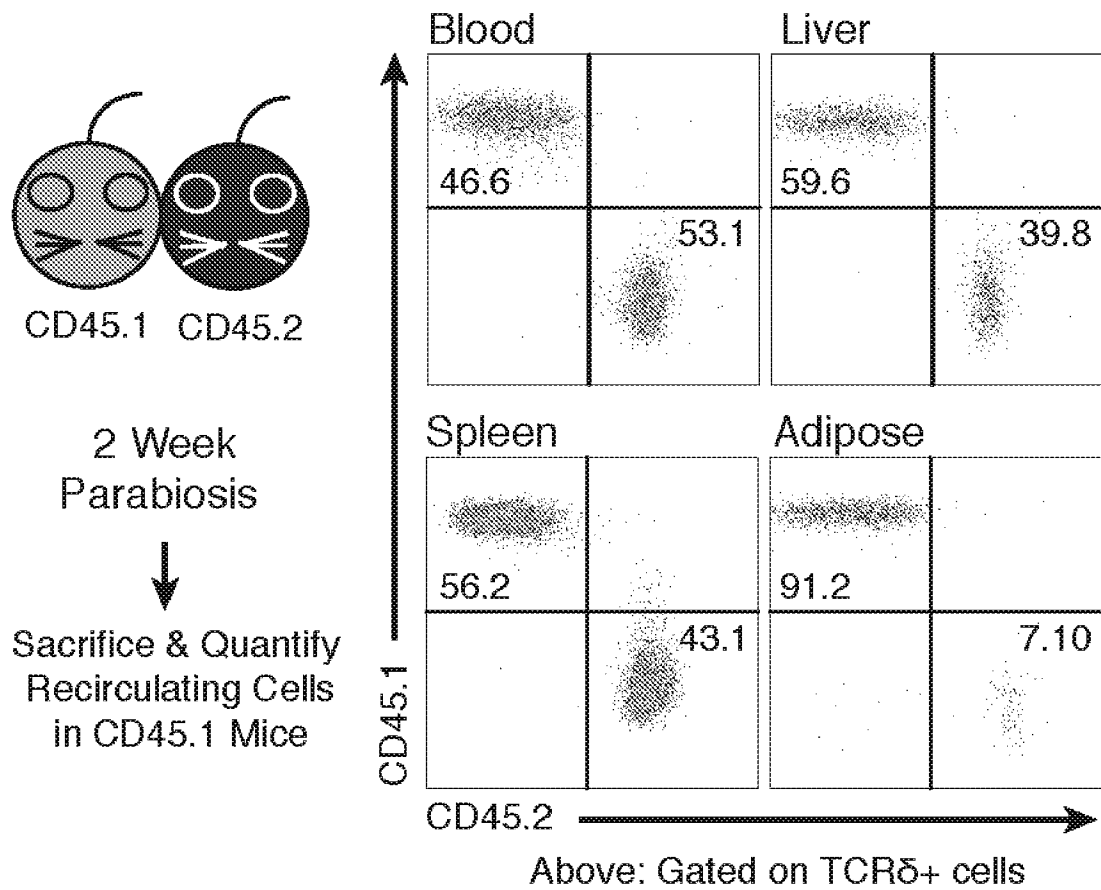


FIG. 1C

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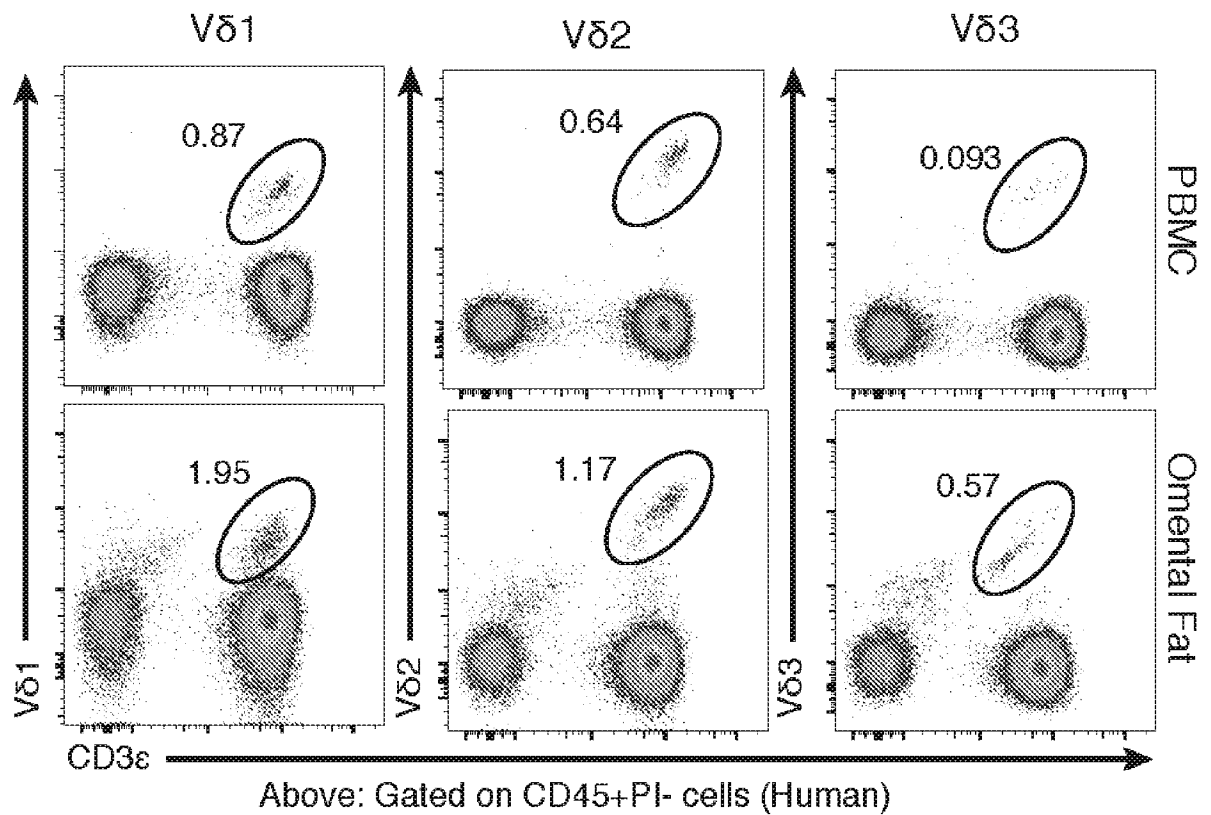


FIG. 1D

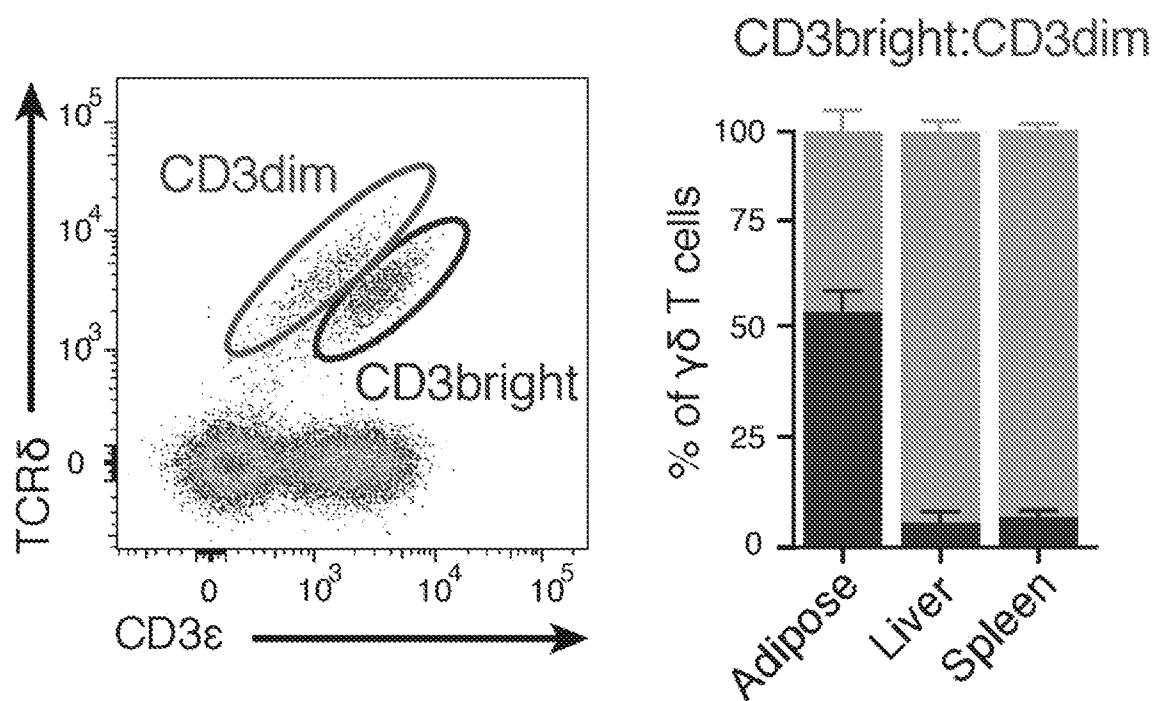


FIG. 2A

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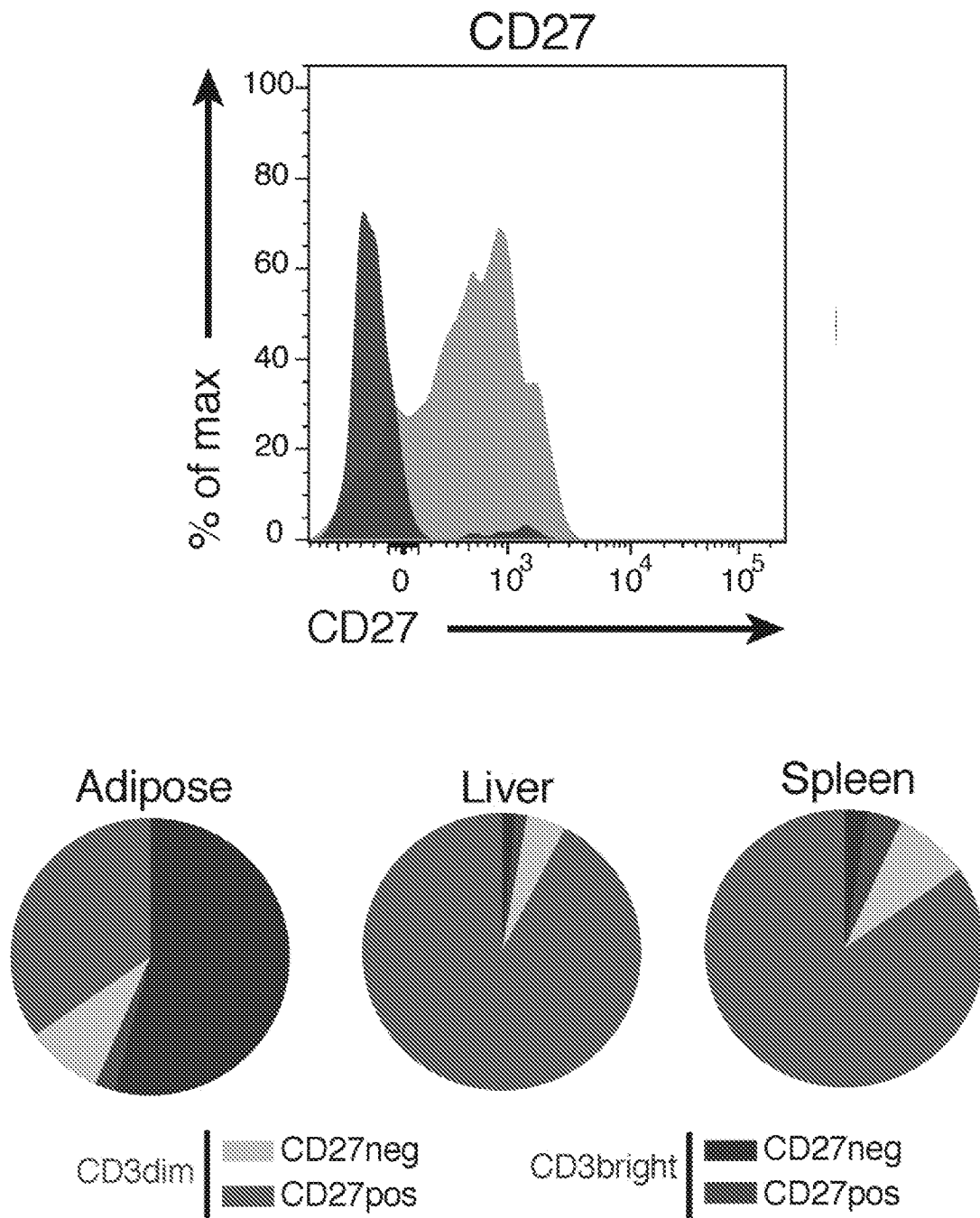


FIG. 2B

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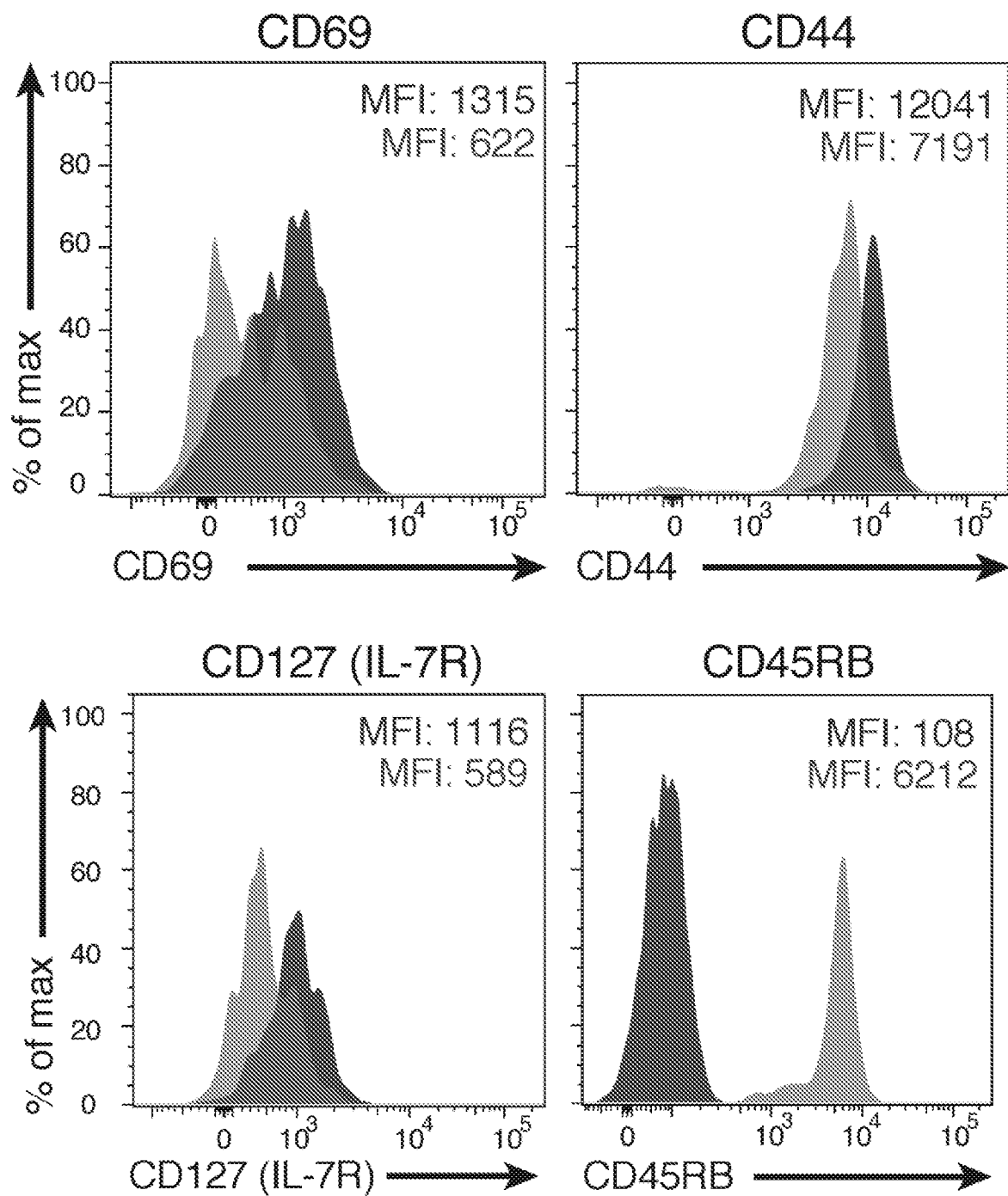


FIG. 2C

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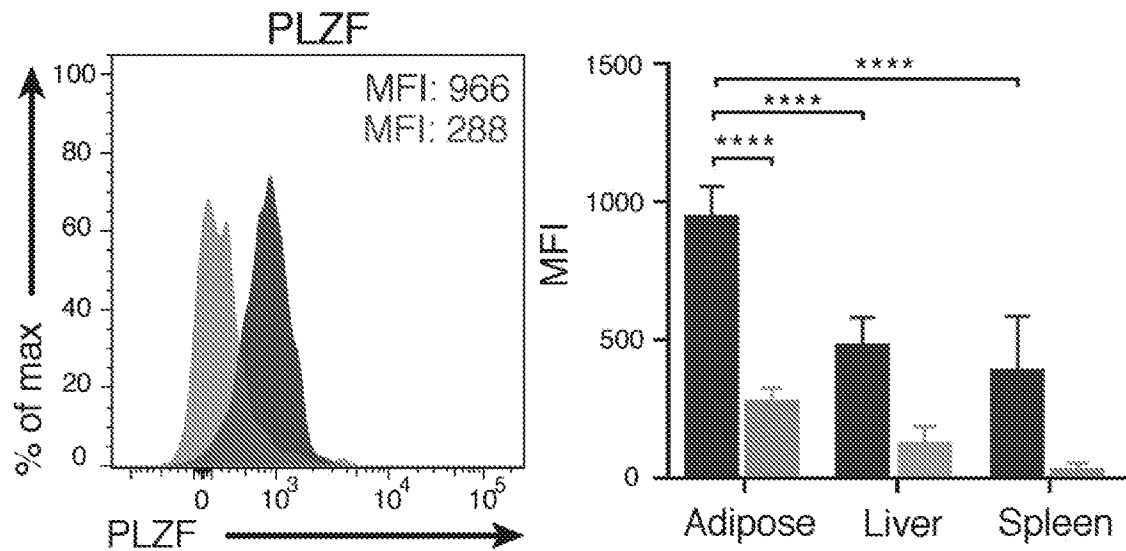


FIG. 2D

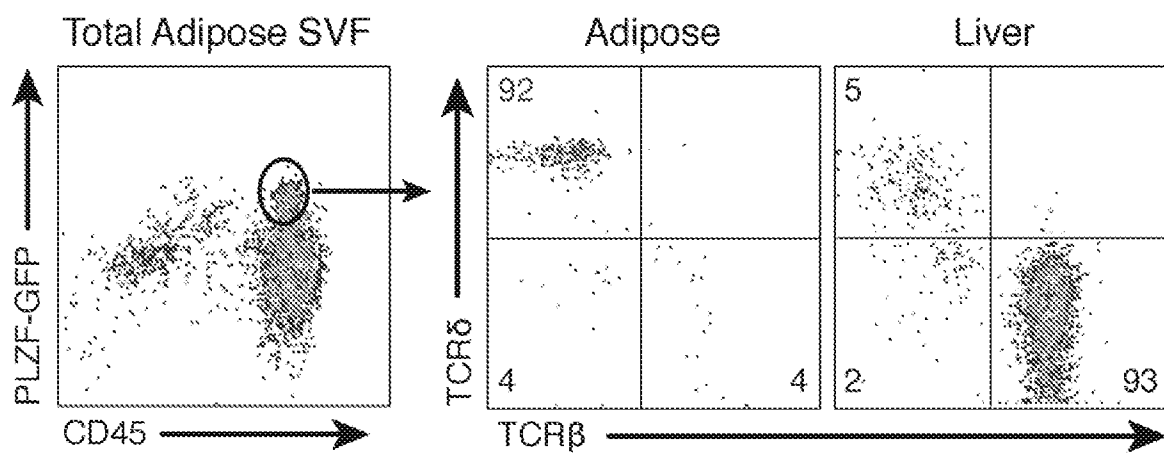


FIG. 2E

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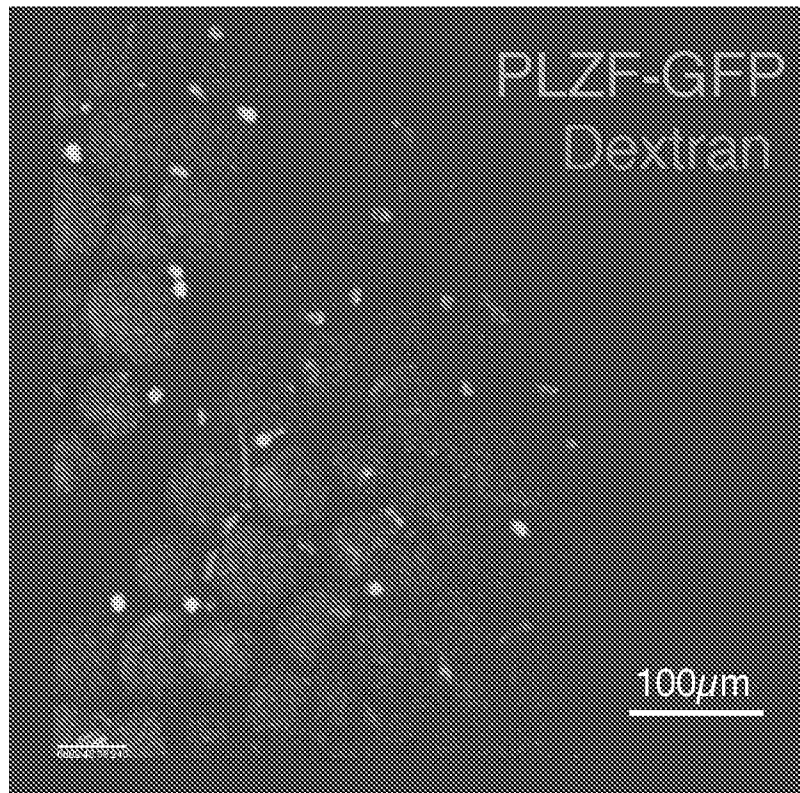


FIG. 2F

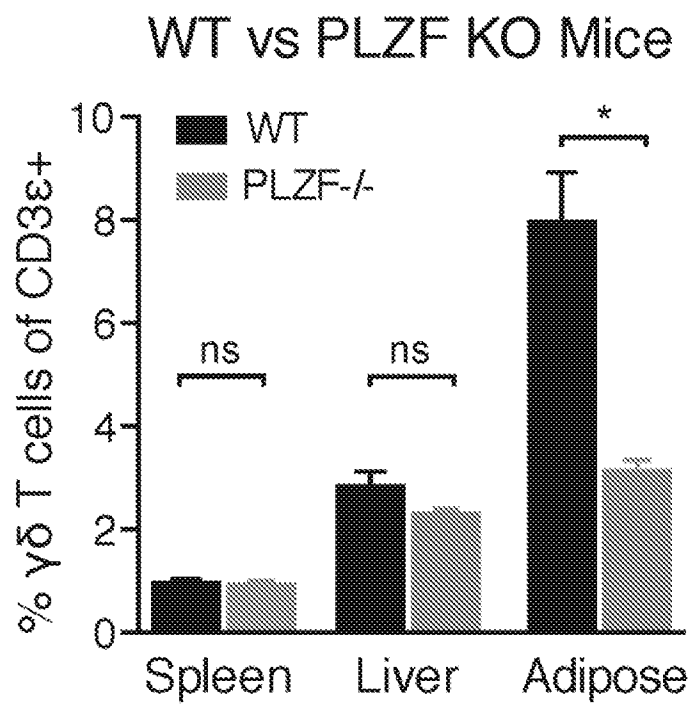


FIG. 2G

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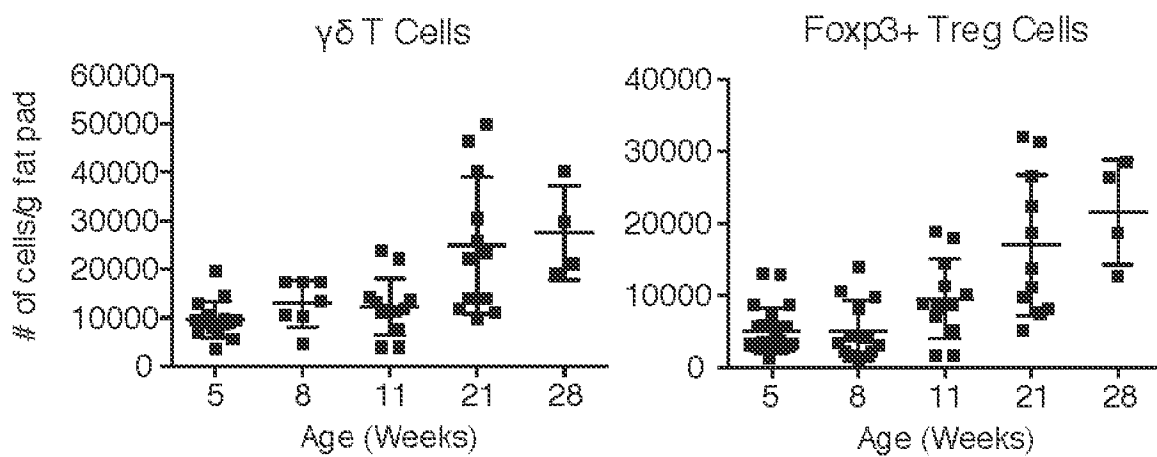


FIG. 3A

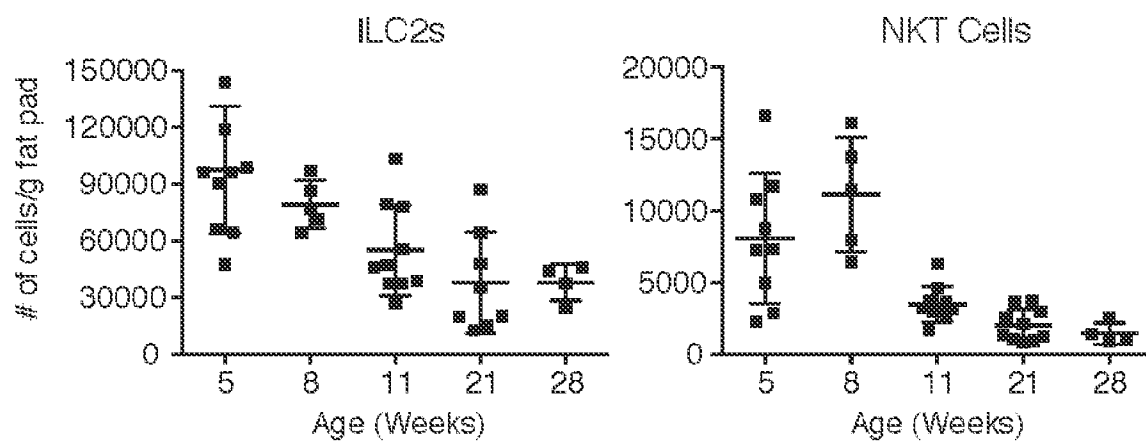


FIG. 3B

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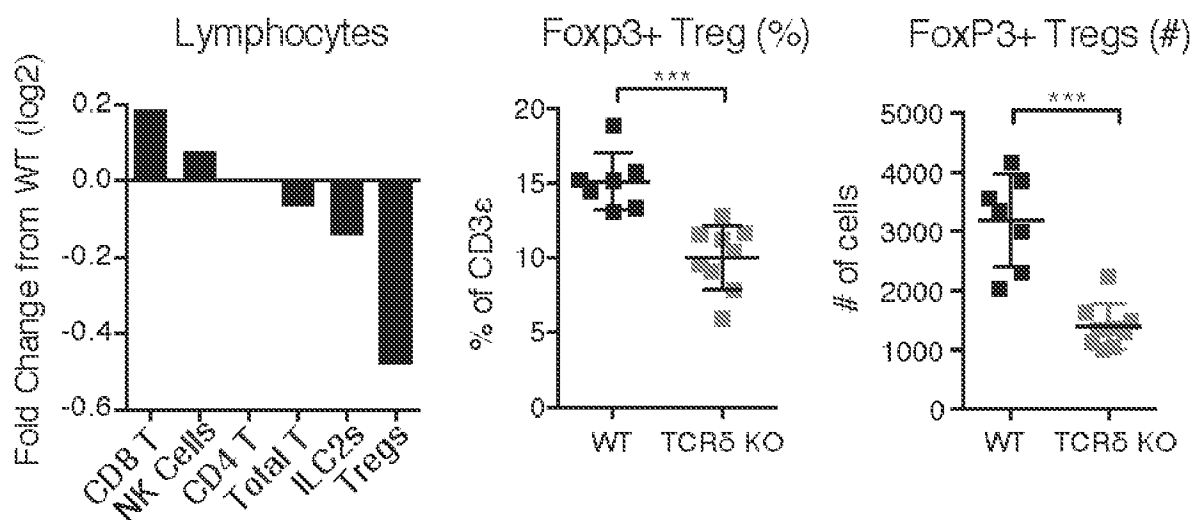


FIG. 3C

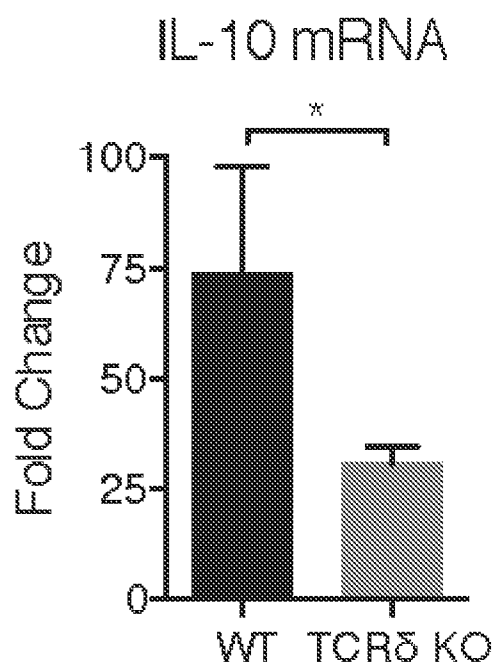


FIG. 3D

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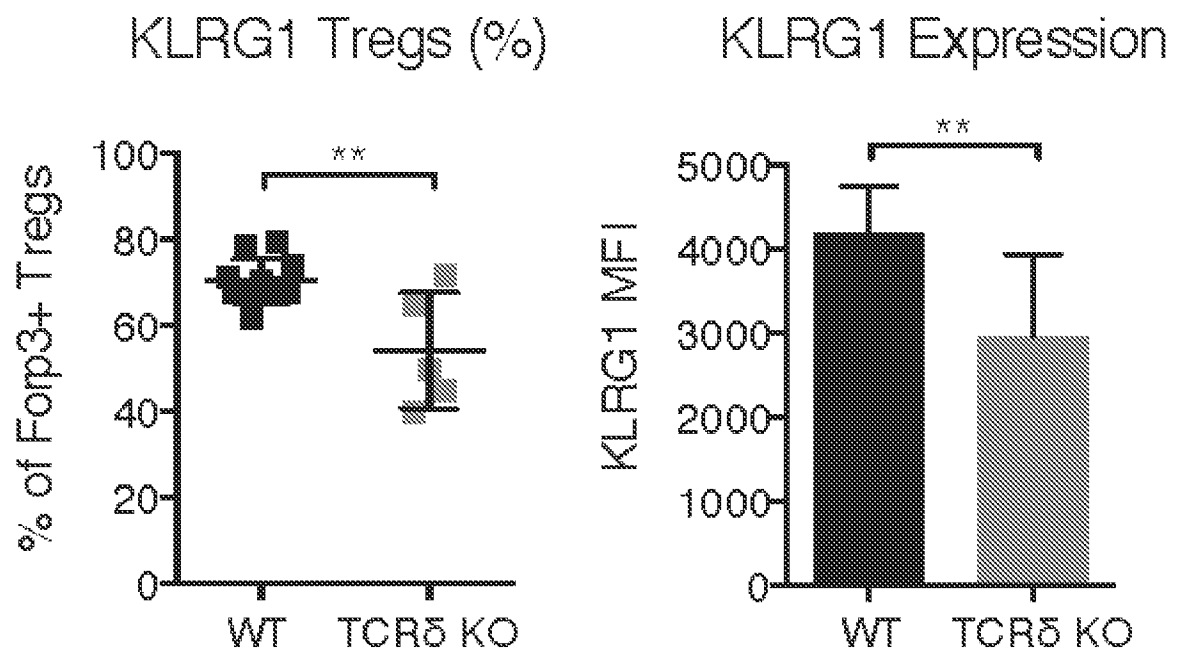


FIG. 3E

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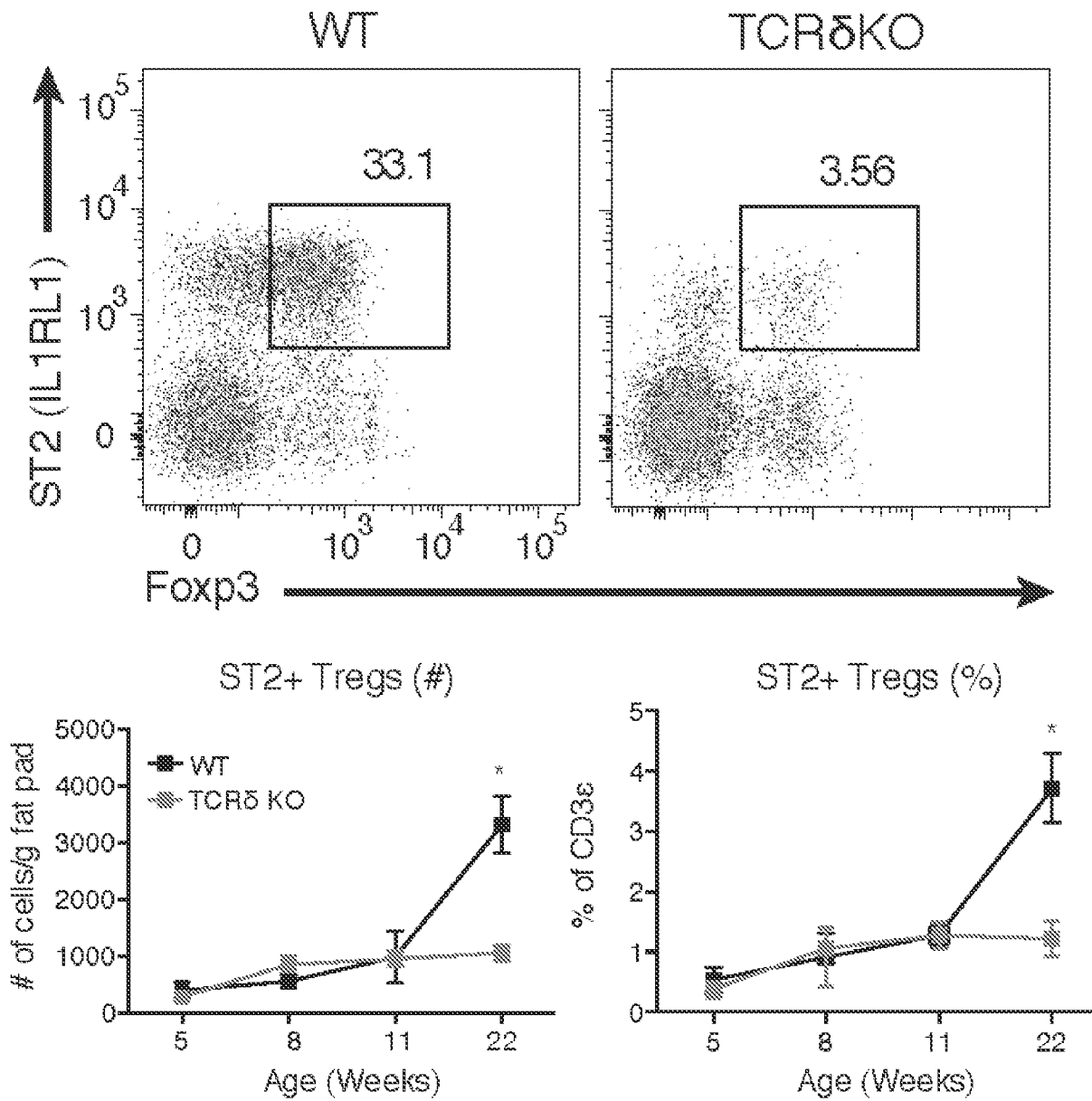


FIG. 3F

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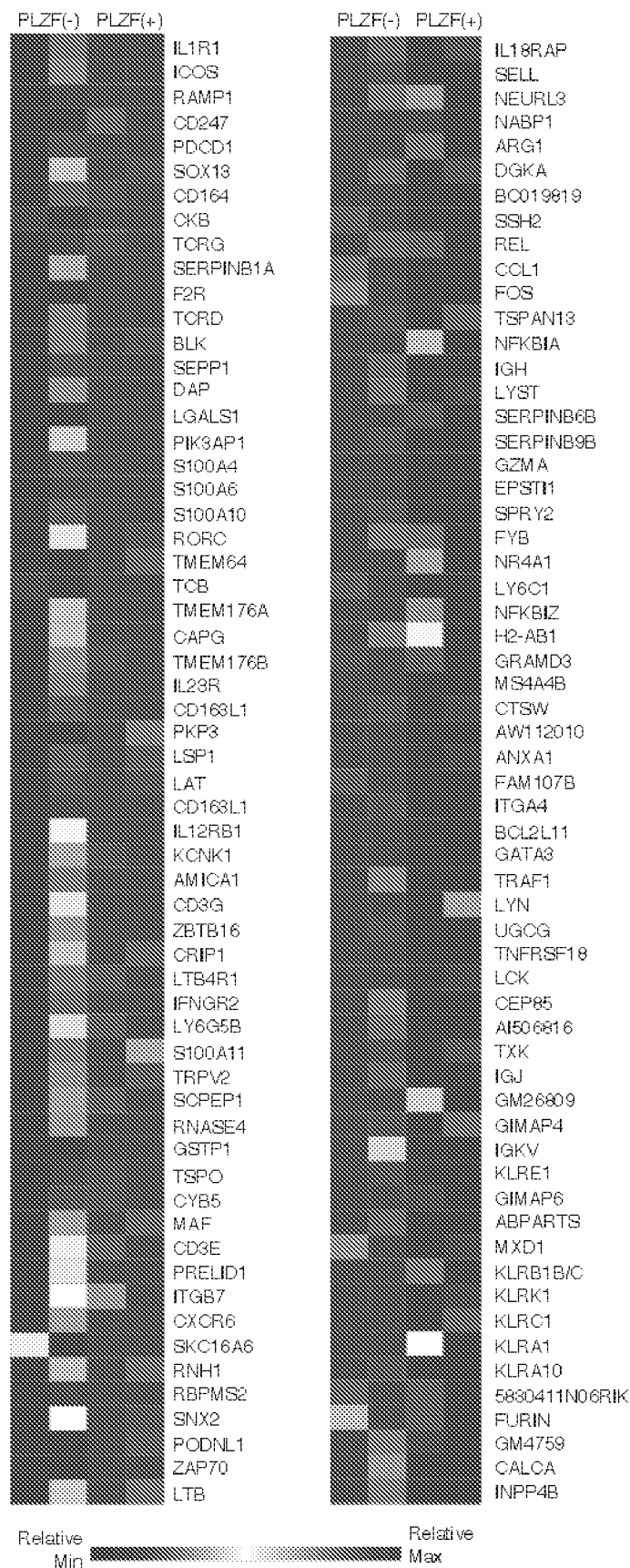
Top 60 genes
PLZF(+) $\gamma\delta$ TTop 60 genes
PLZF(-) $\gamma\delta$ T

FIG. 4A

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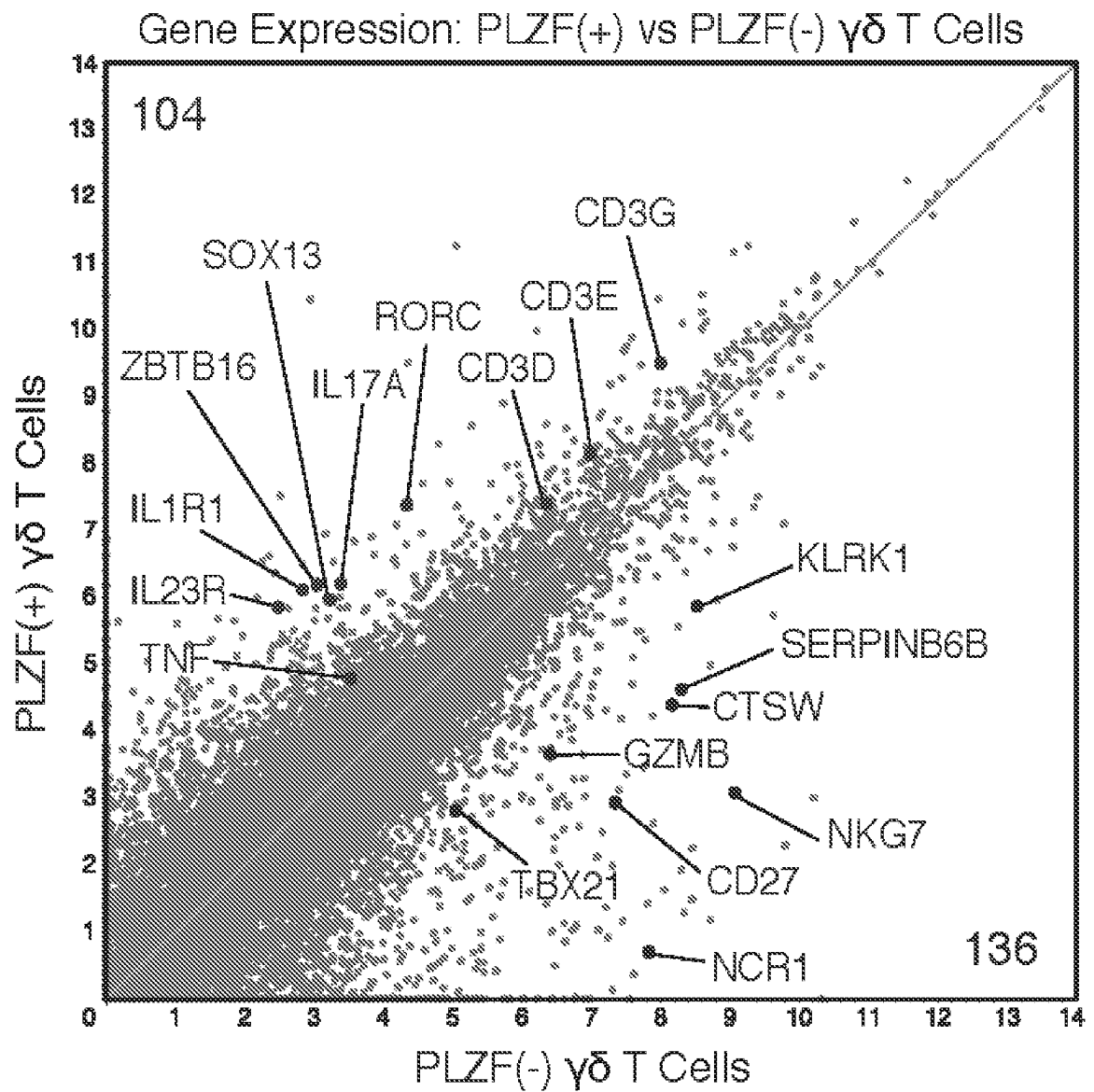
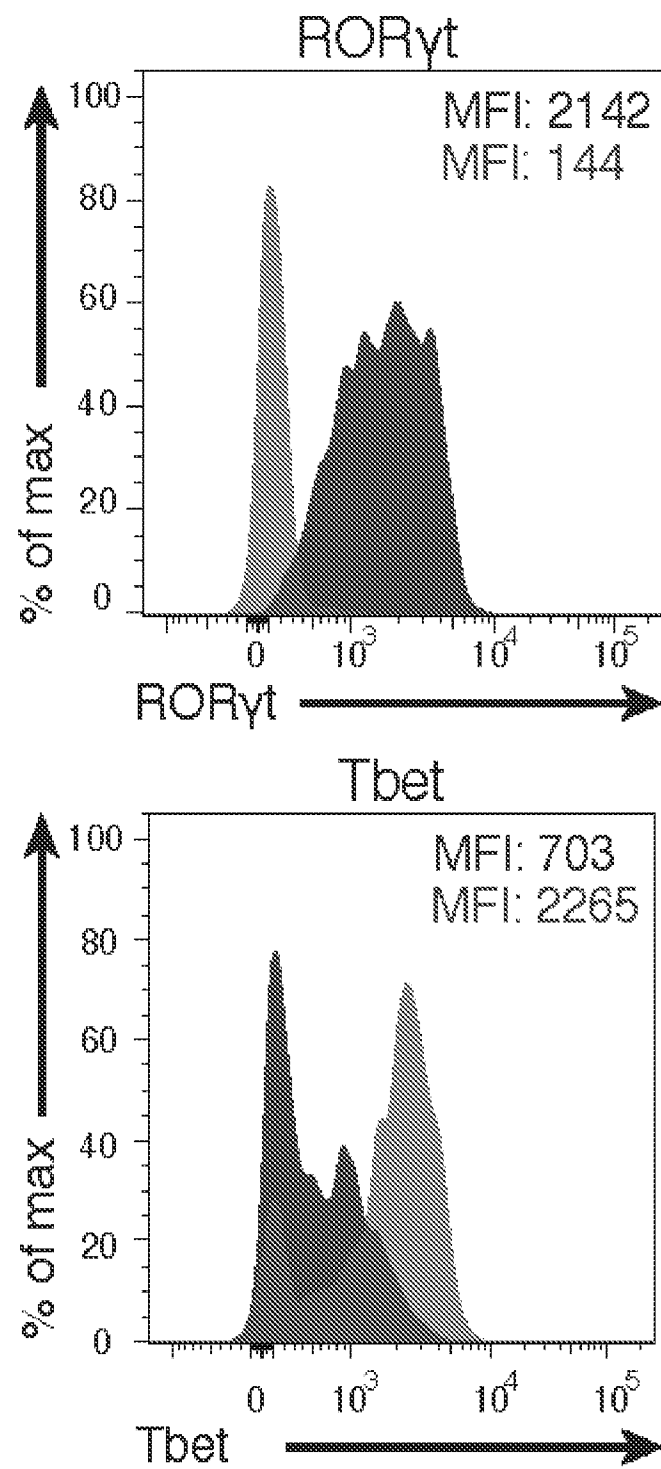


FIG. 4B

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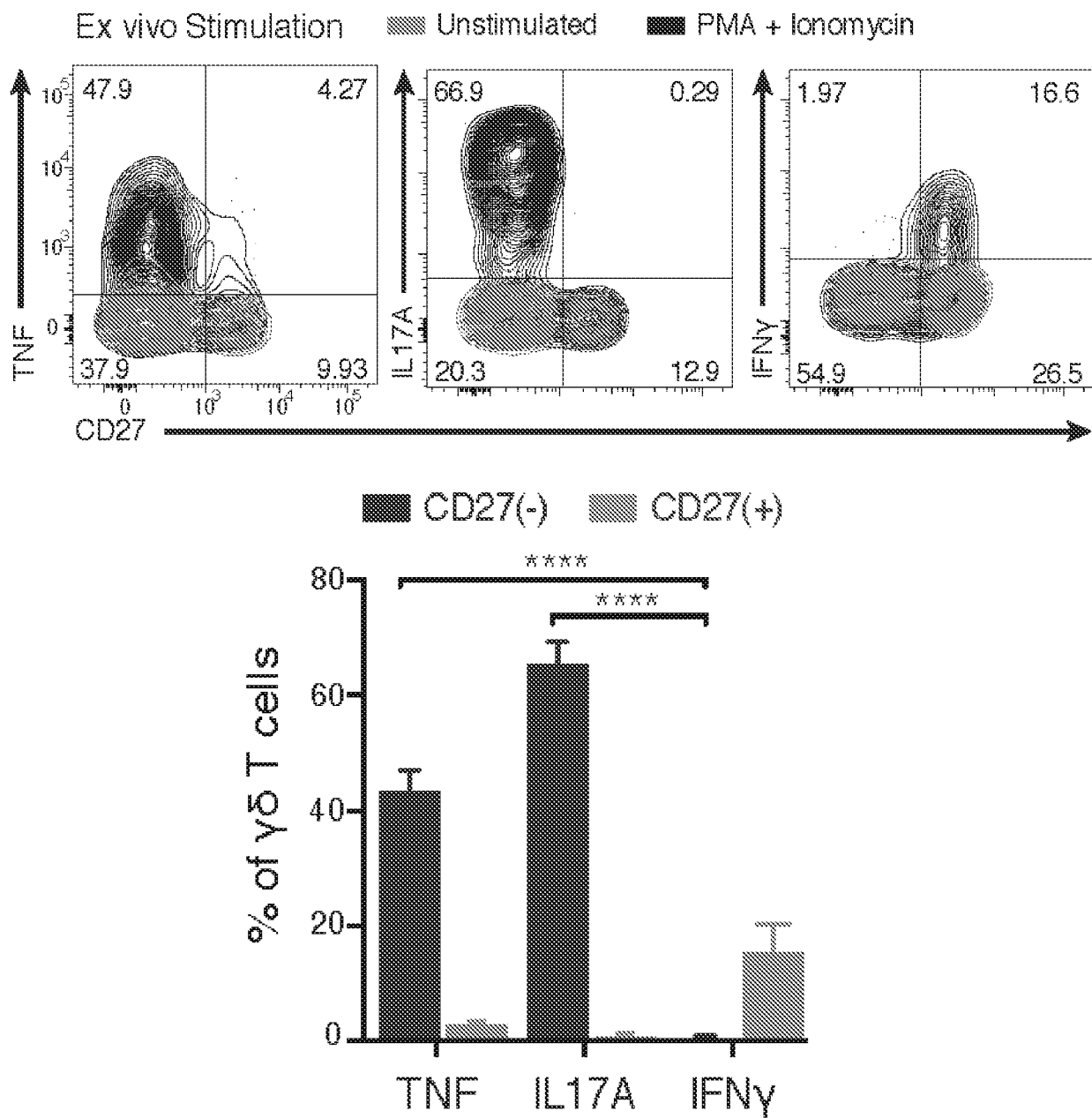


FIG. 4D

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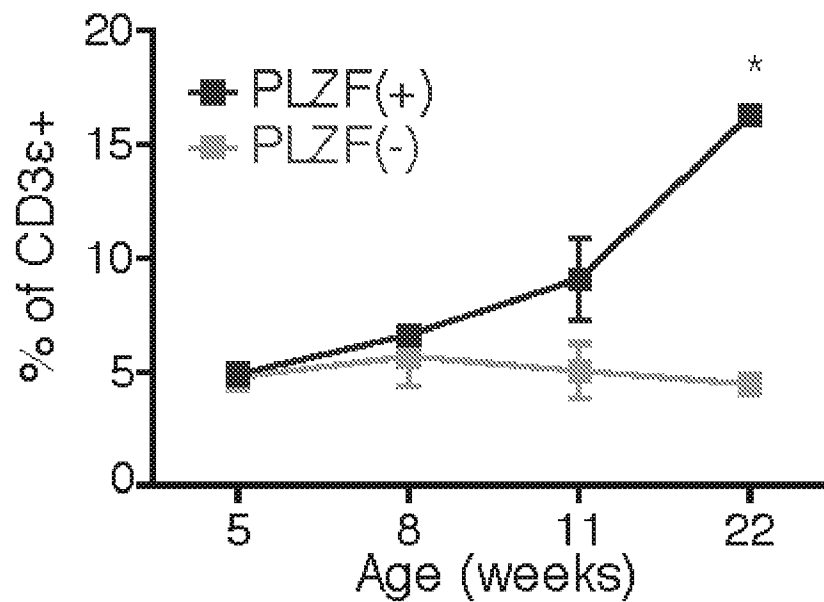
 $\gamma\delta$ T Cells

FIG. 5A

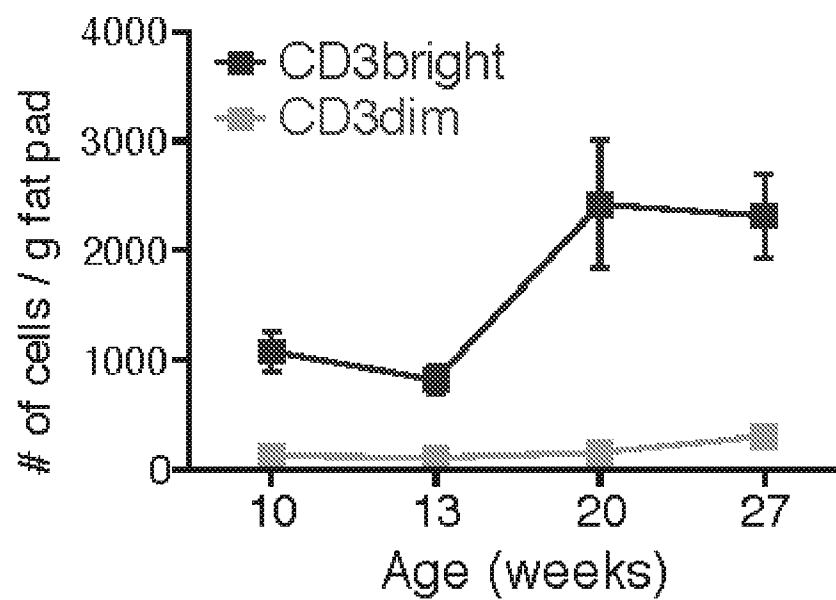
IL17A⁺ $\gamma\delta$ T Cell Subsets (#)

FIG. 5B

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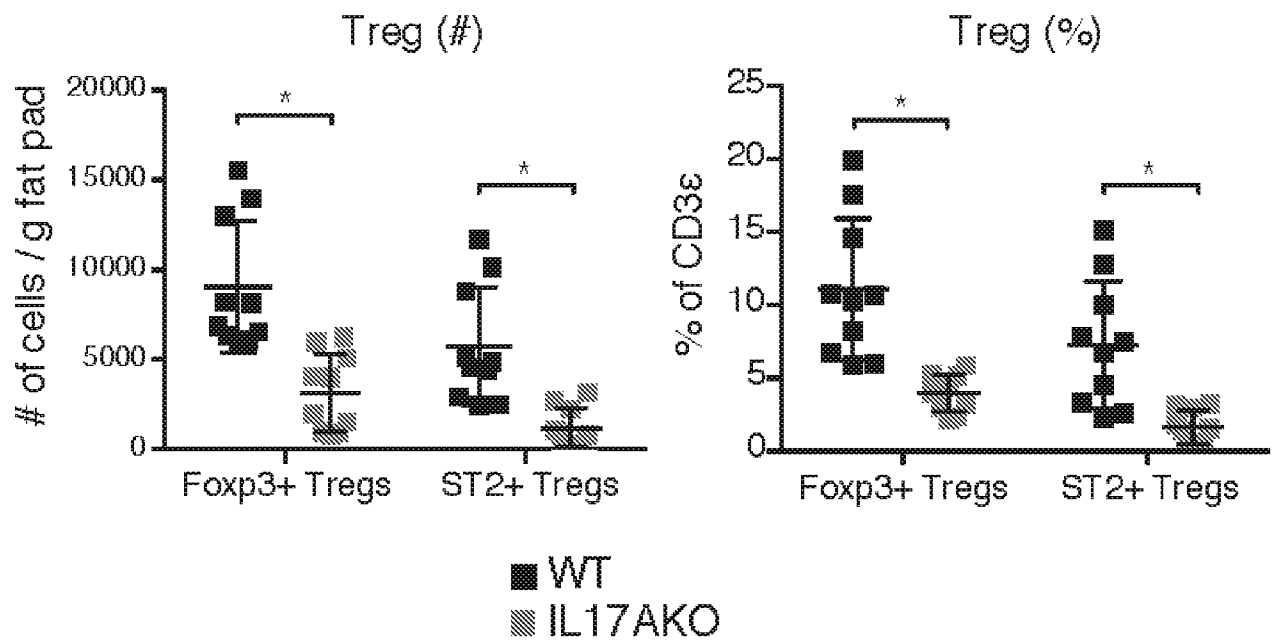


FIG. 5C

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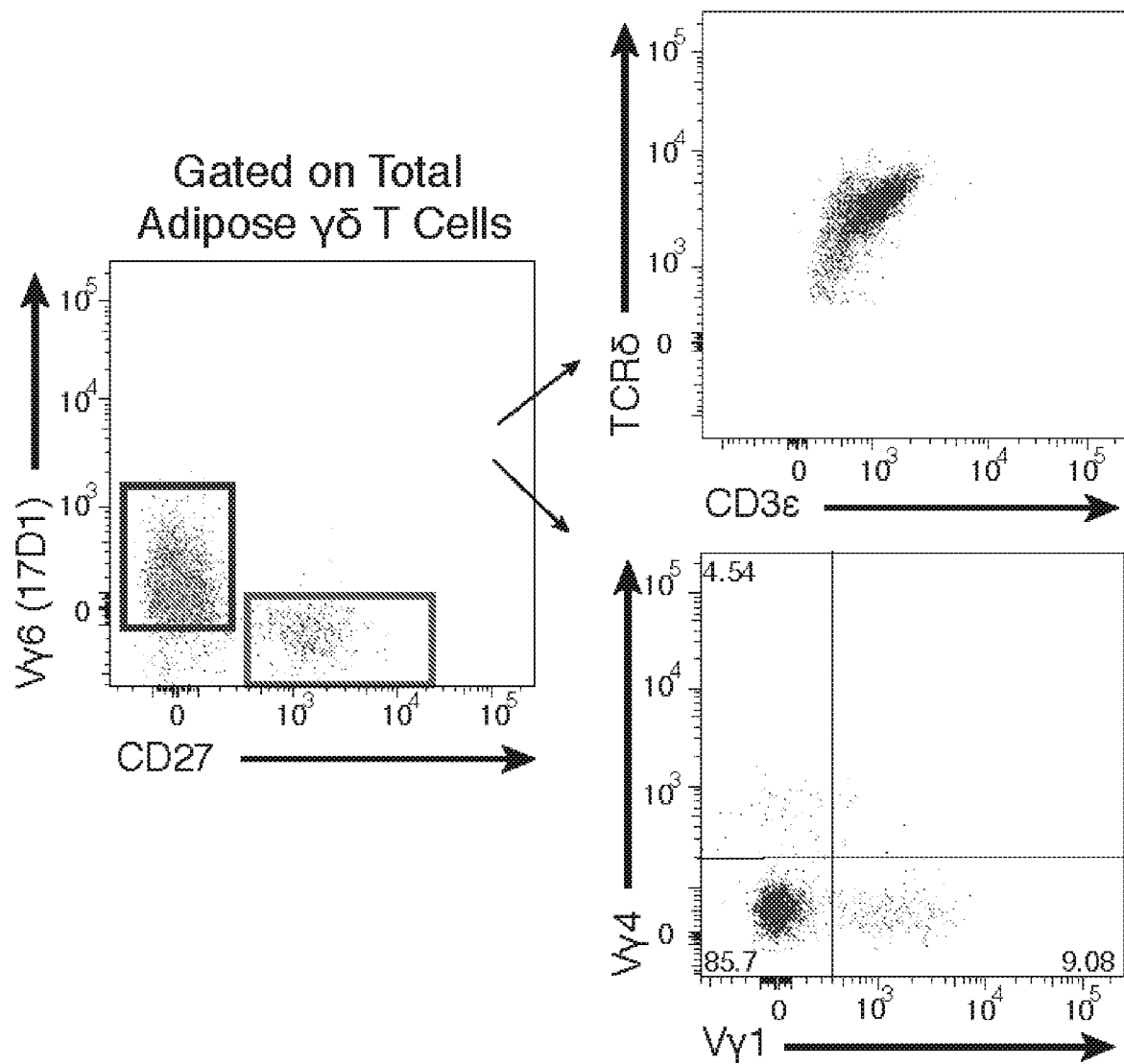


FIG. 5D

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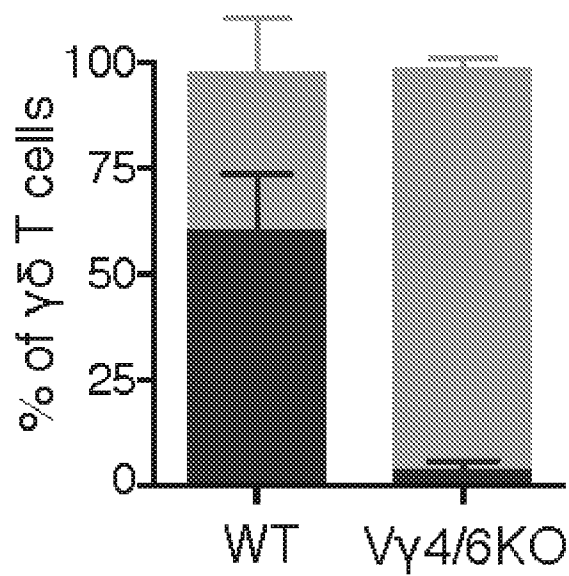
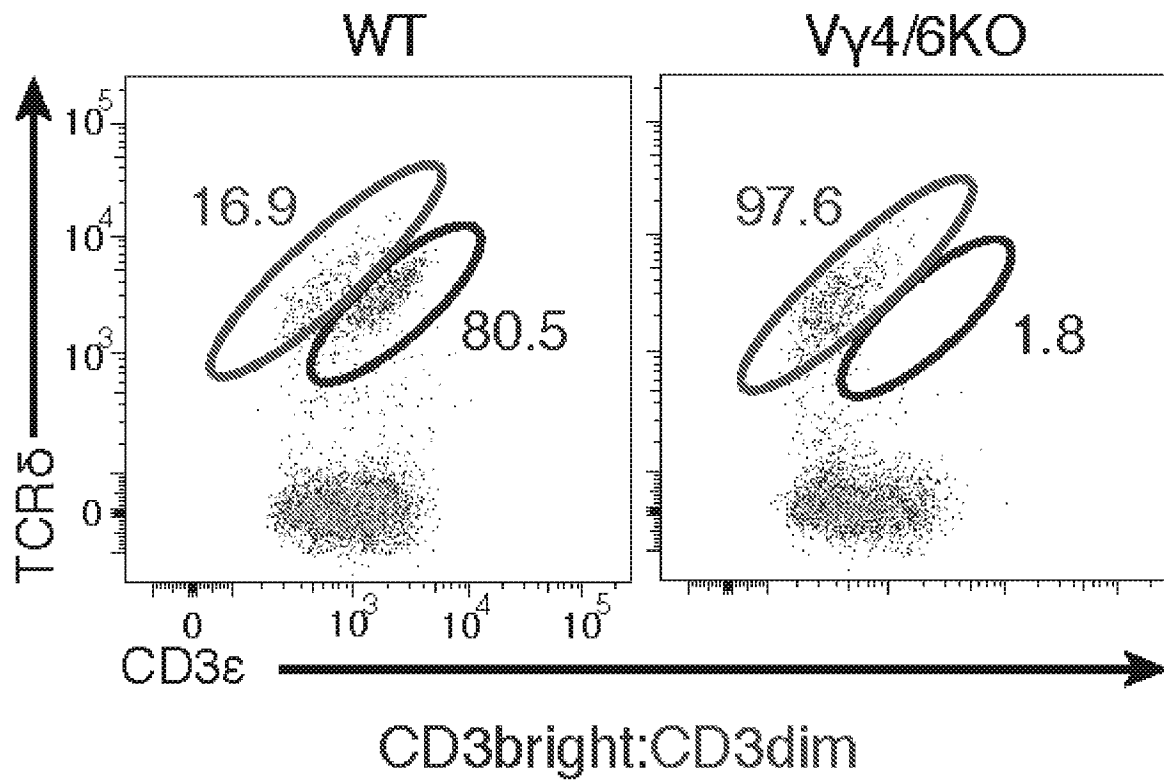


FIG. 5E

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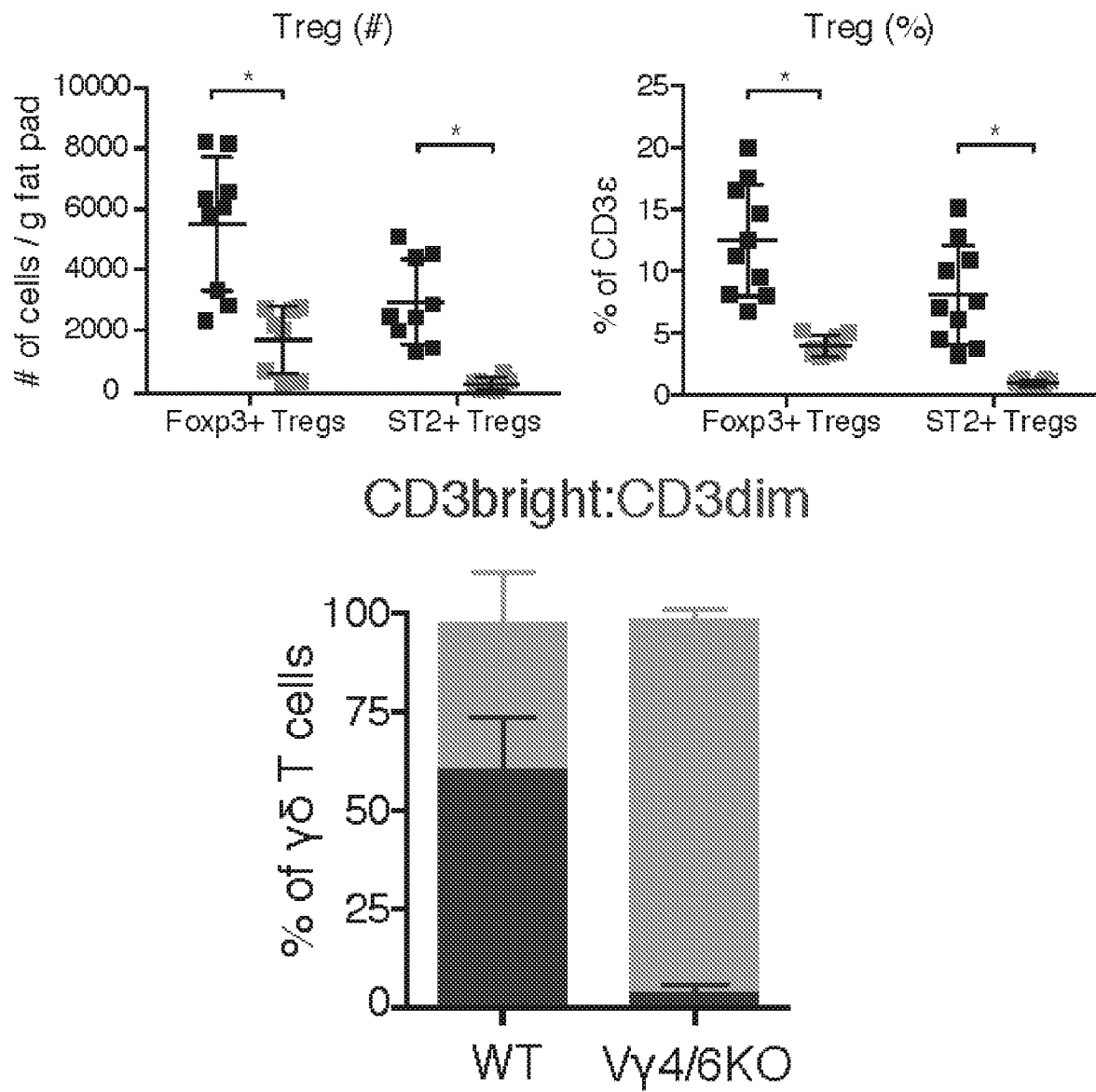


FIG. 5F

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Adipose IL-33 Protein

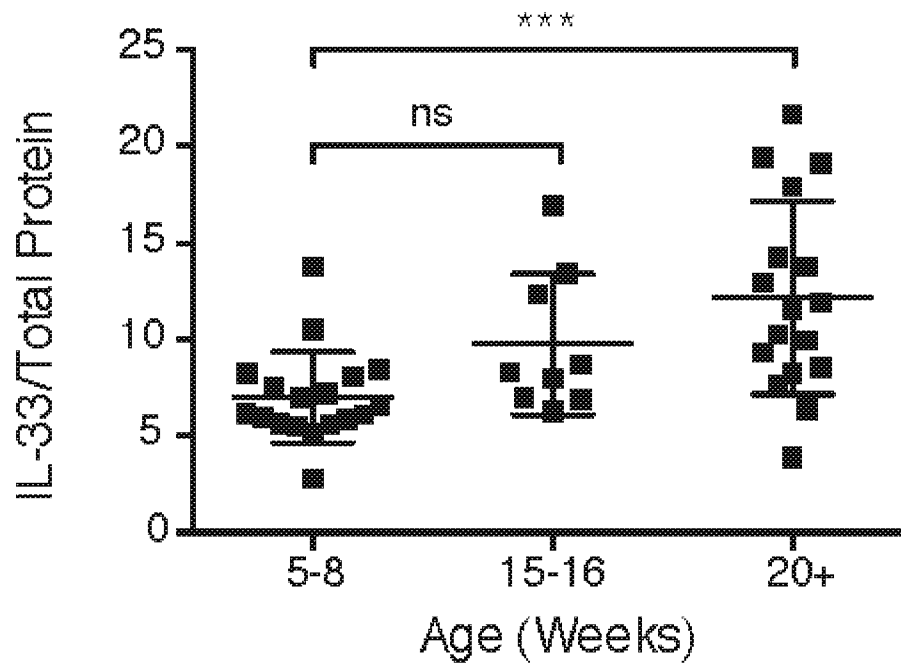


FIG. 6A

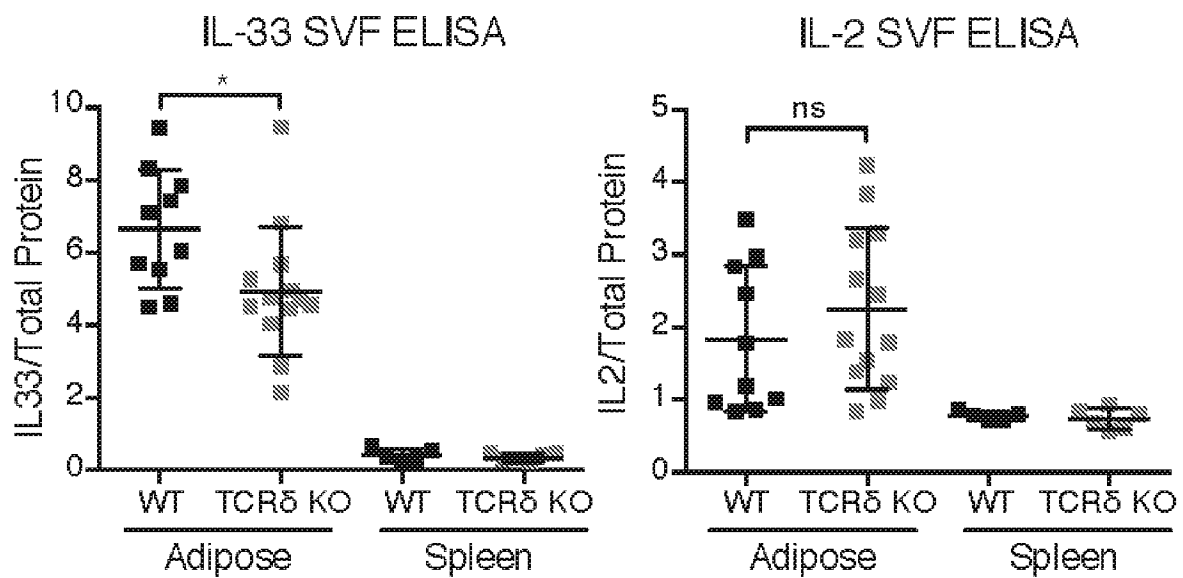
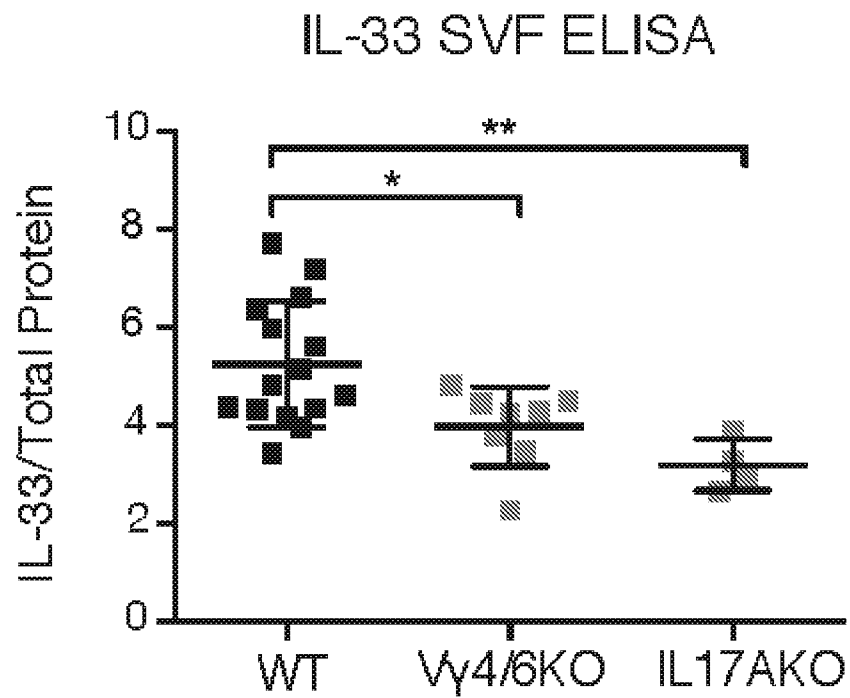


FIG. 6B

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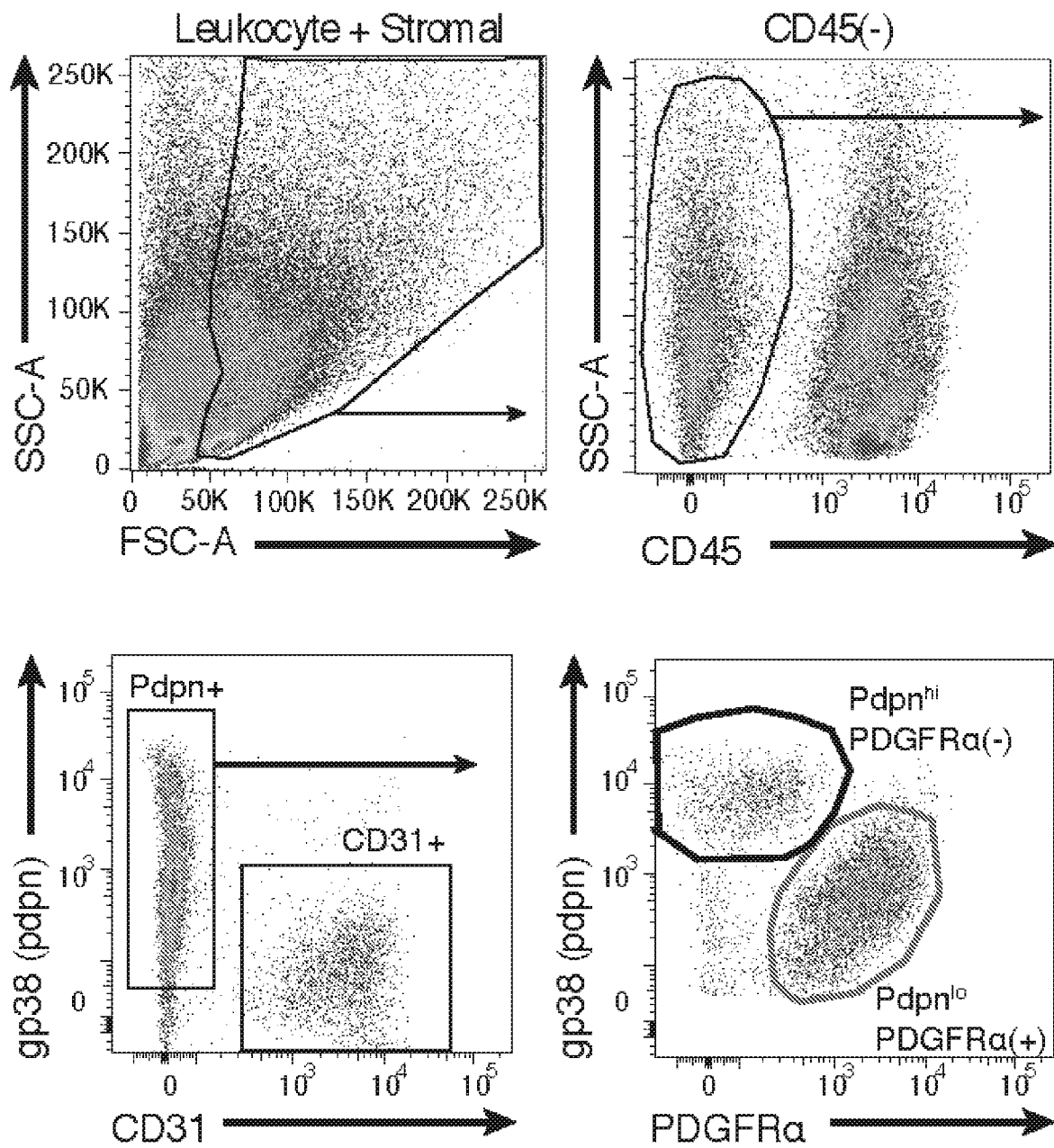


FIG. 6D

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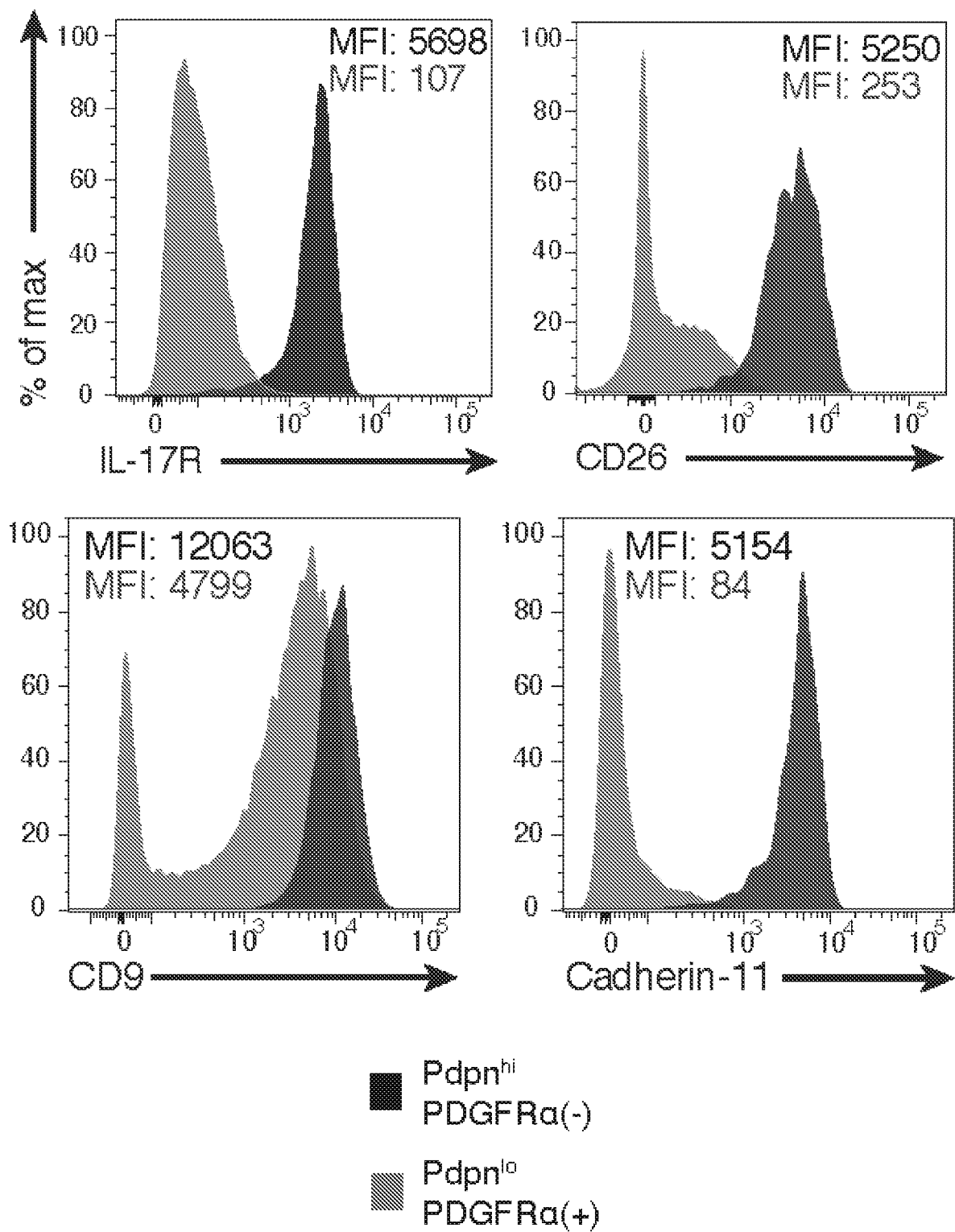


FIG. 6E

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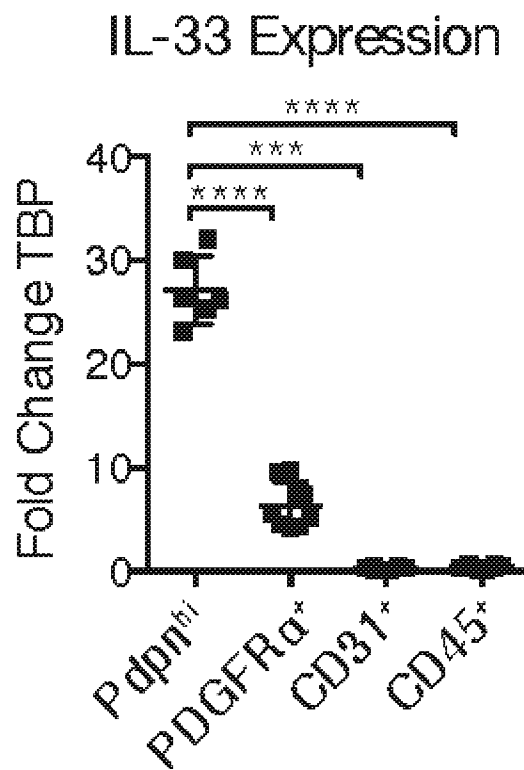


FIG. 6F

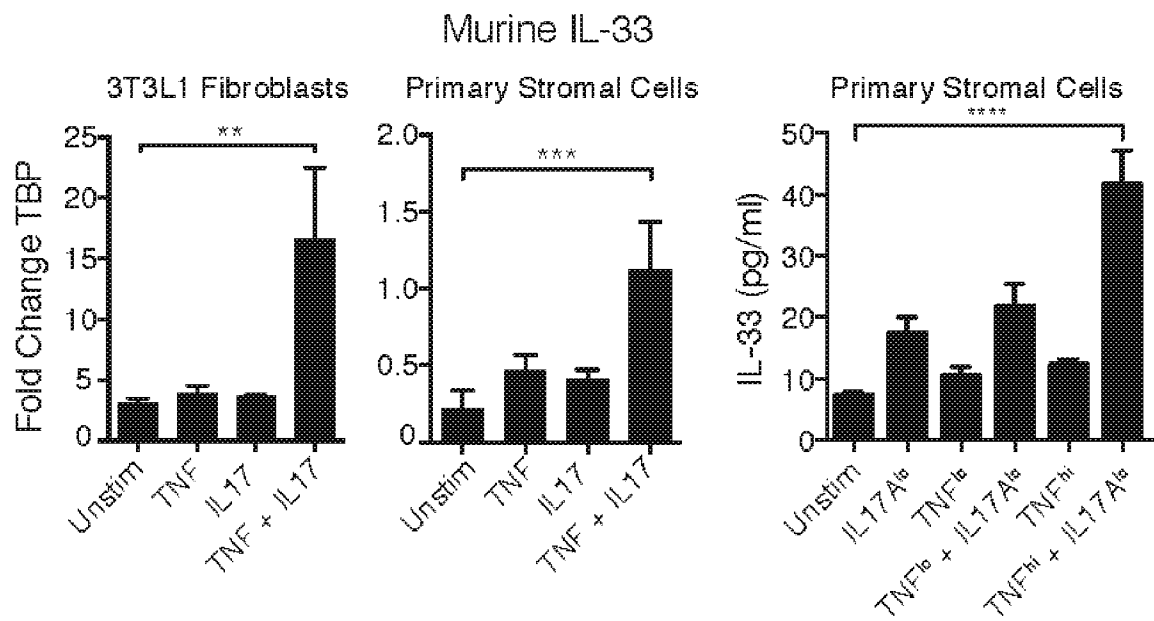


FIG. 6G

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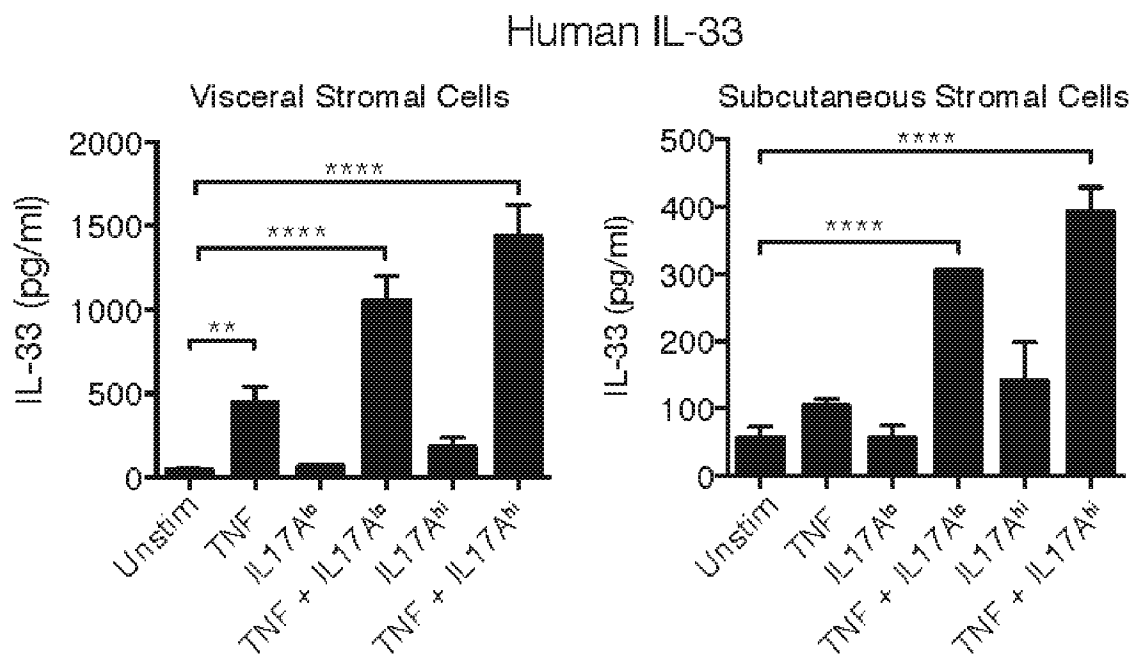


FIG. 6H

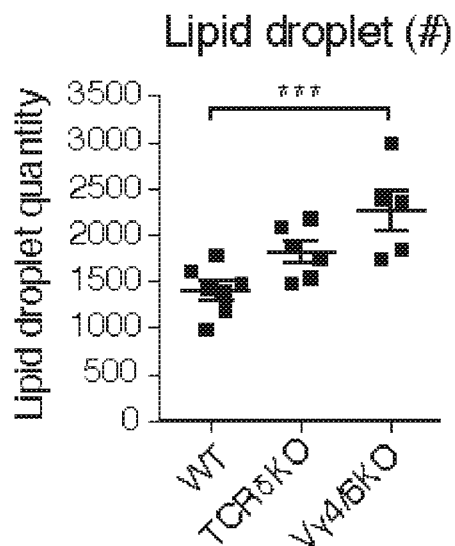
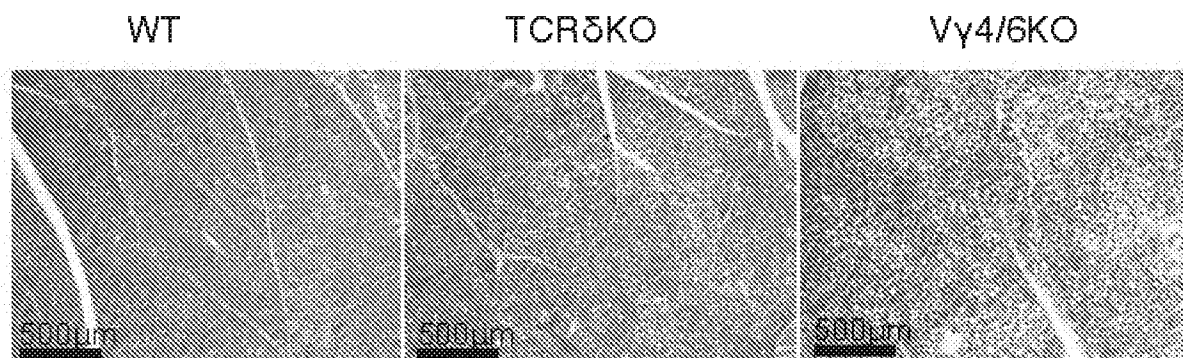


FIG. 7A

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Brown AT - UCP1

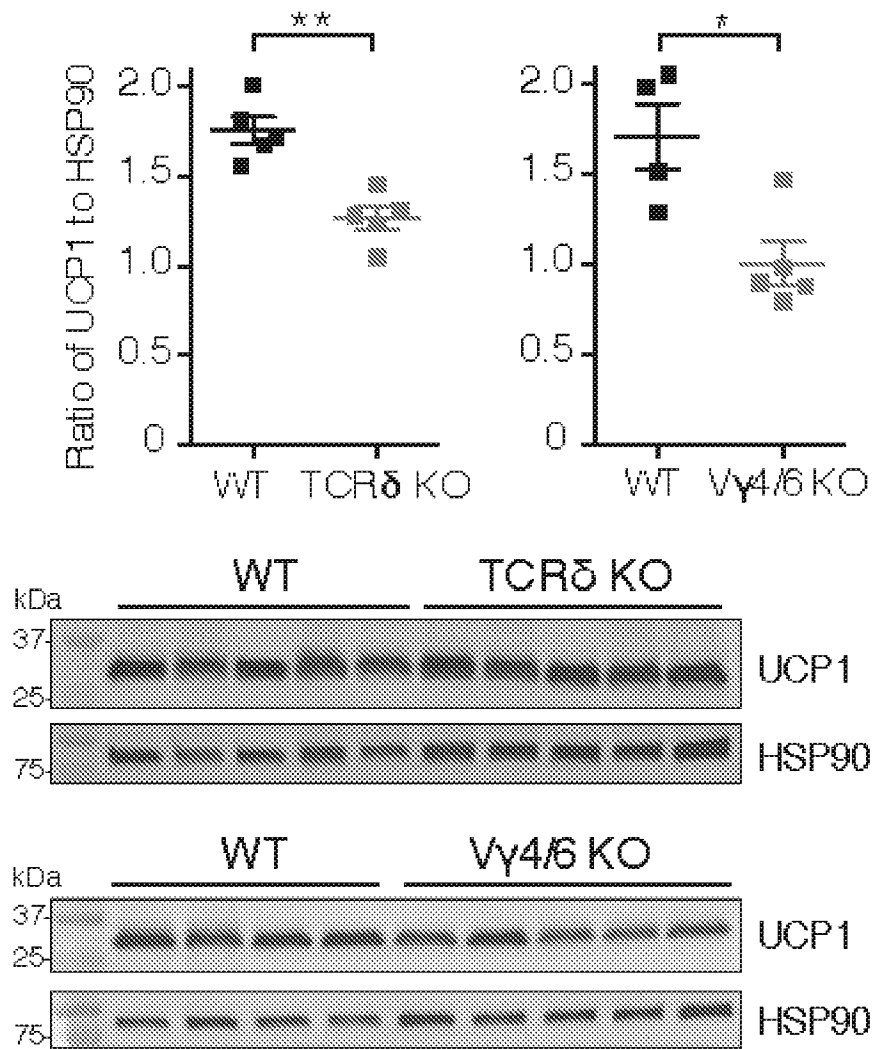


FIG. 7B

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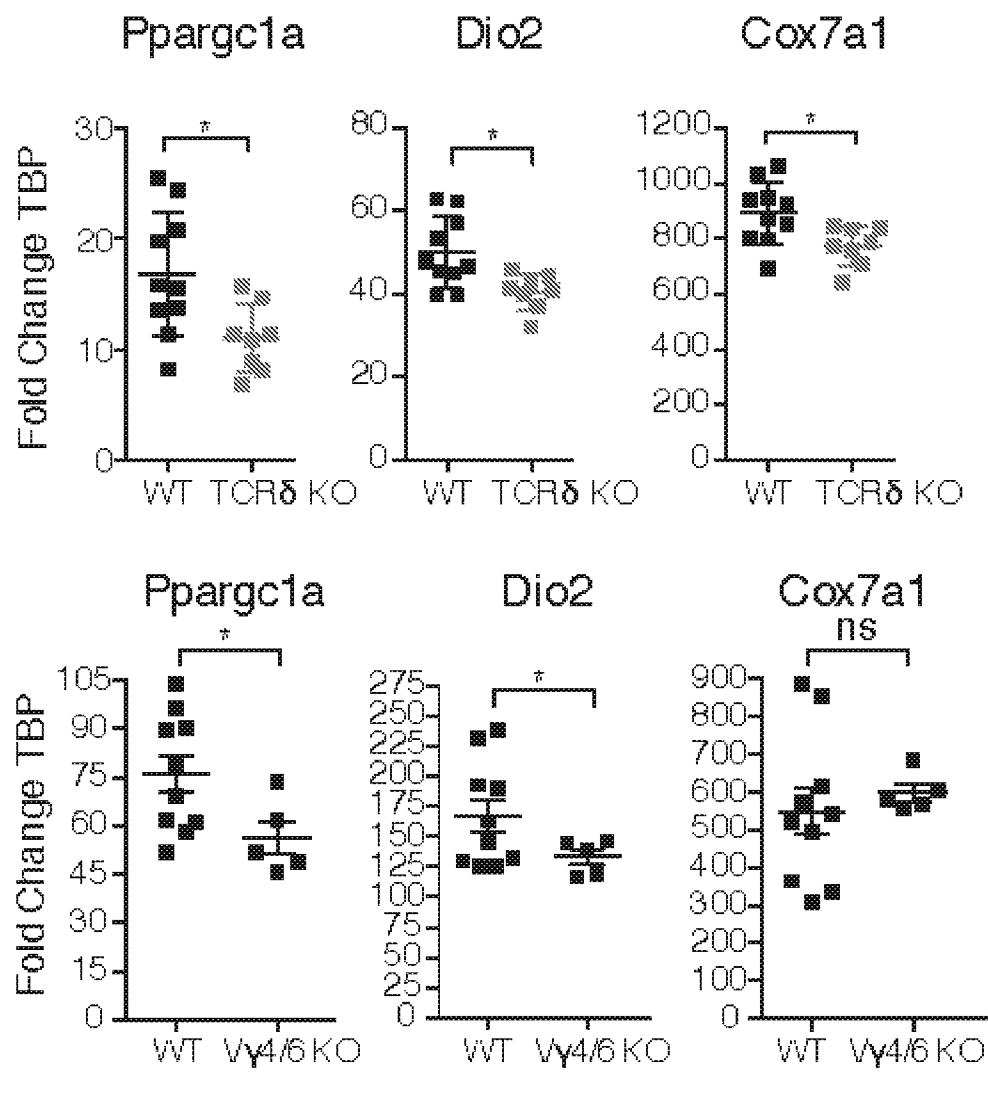


FIG. 7C

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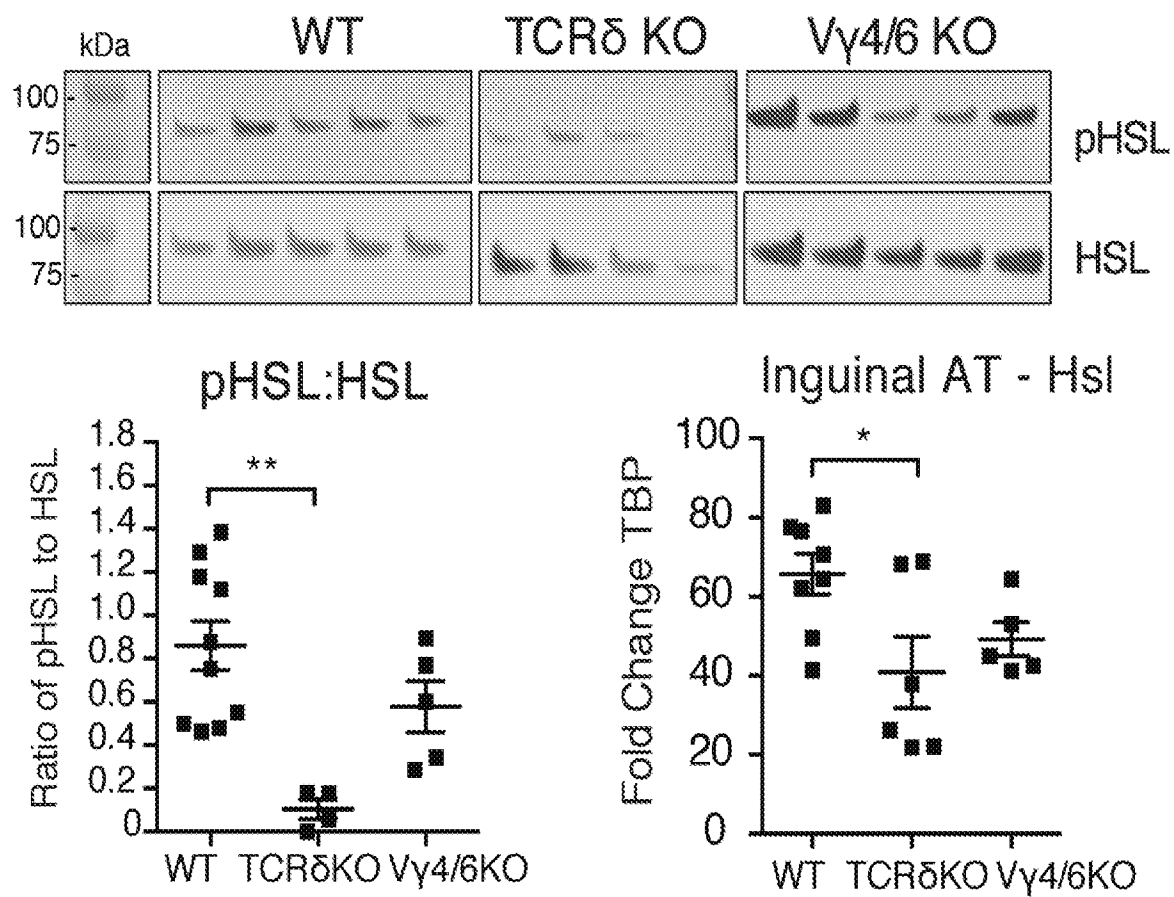


FIG. 7D

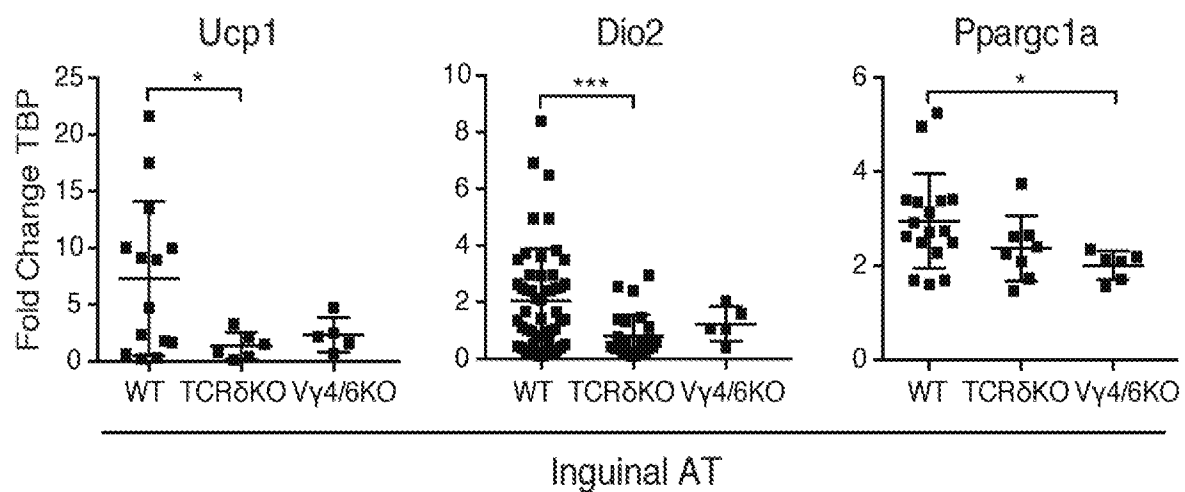


FIG. 7E

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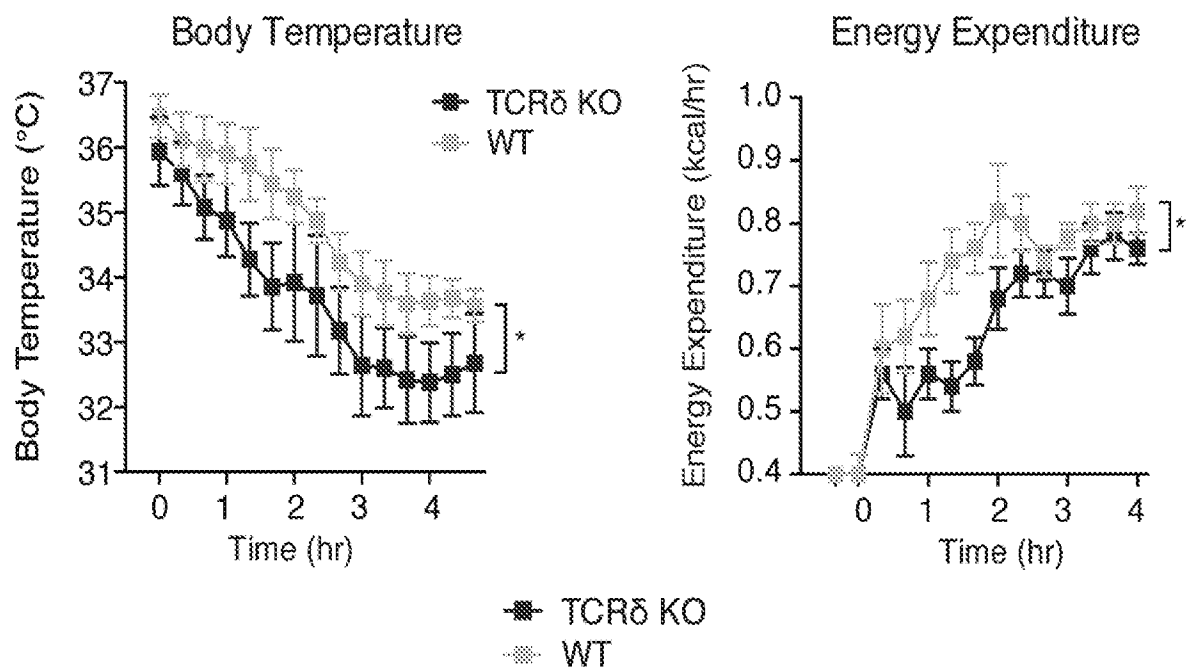


FIG. 7F

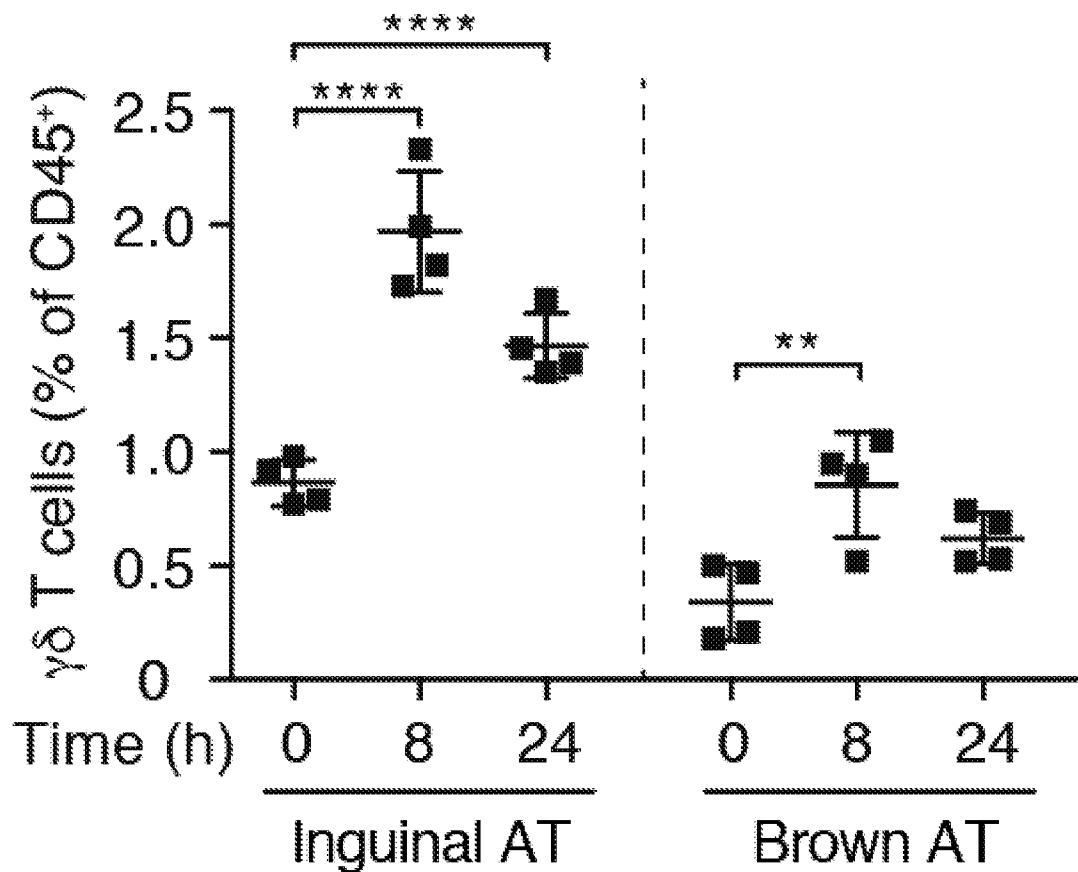


FIG. 8A

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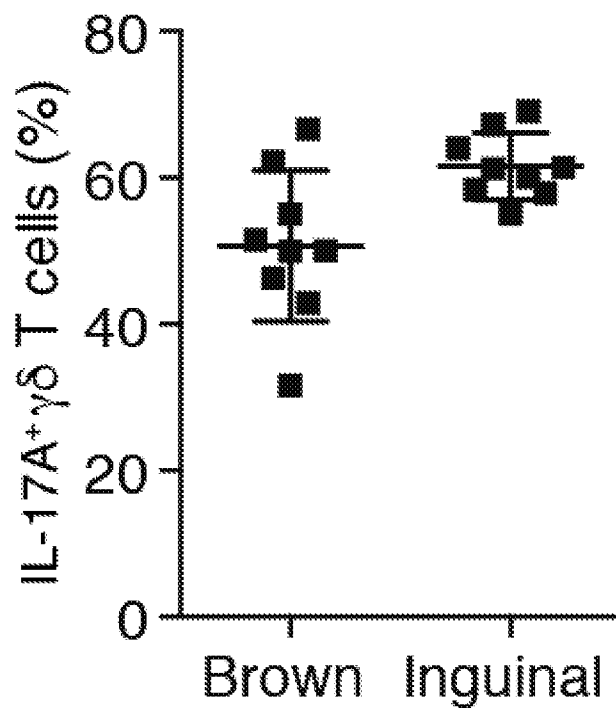


FIG. 8B

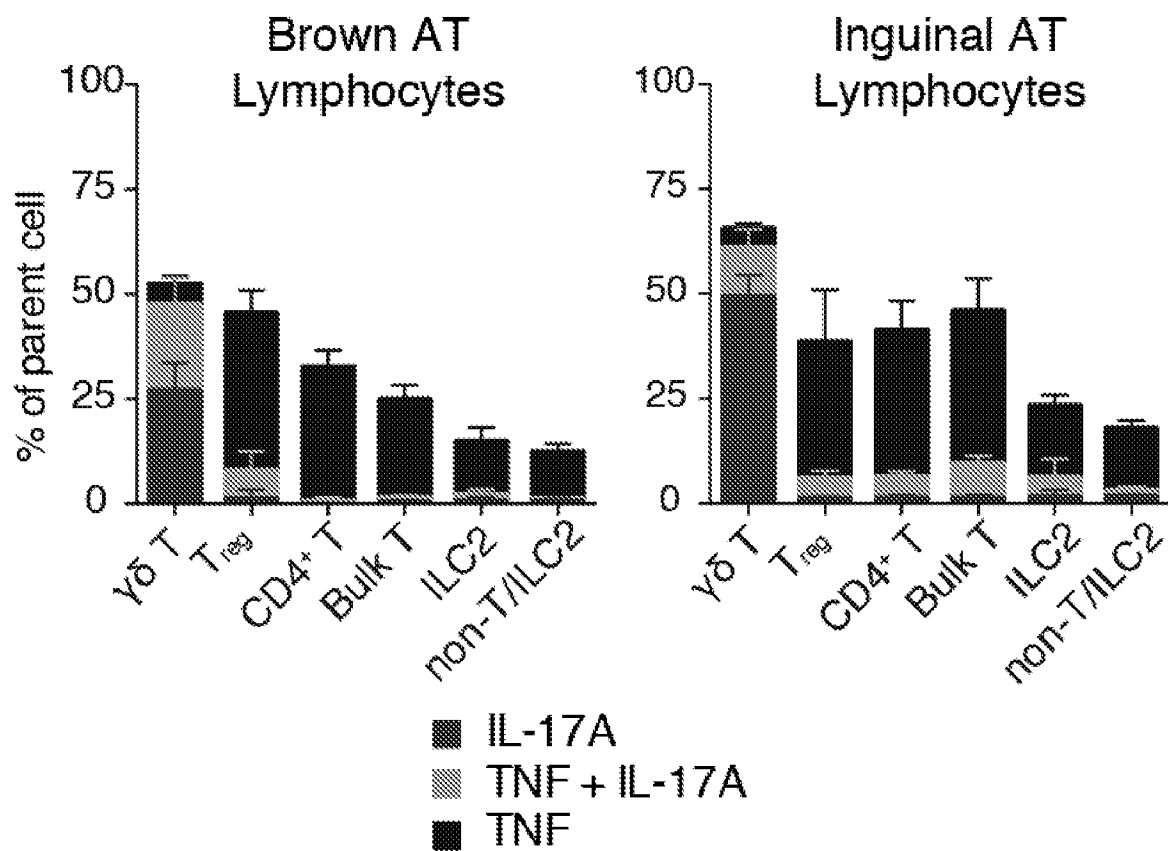


FIG. 8C

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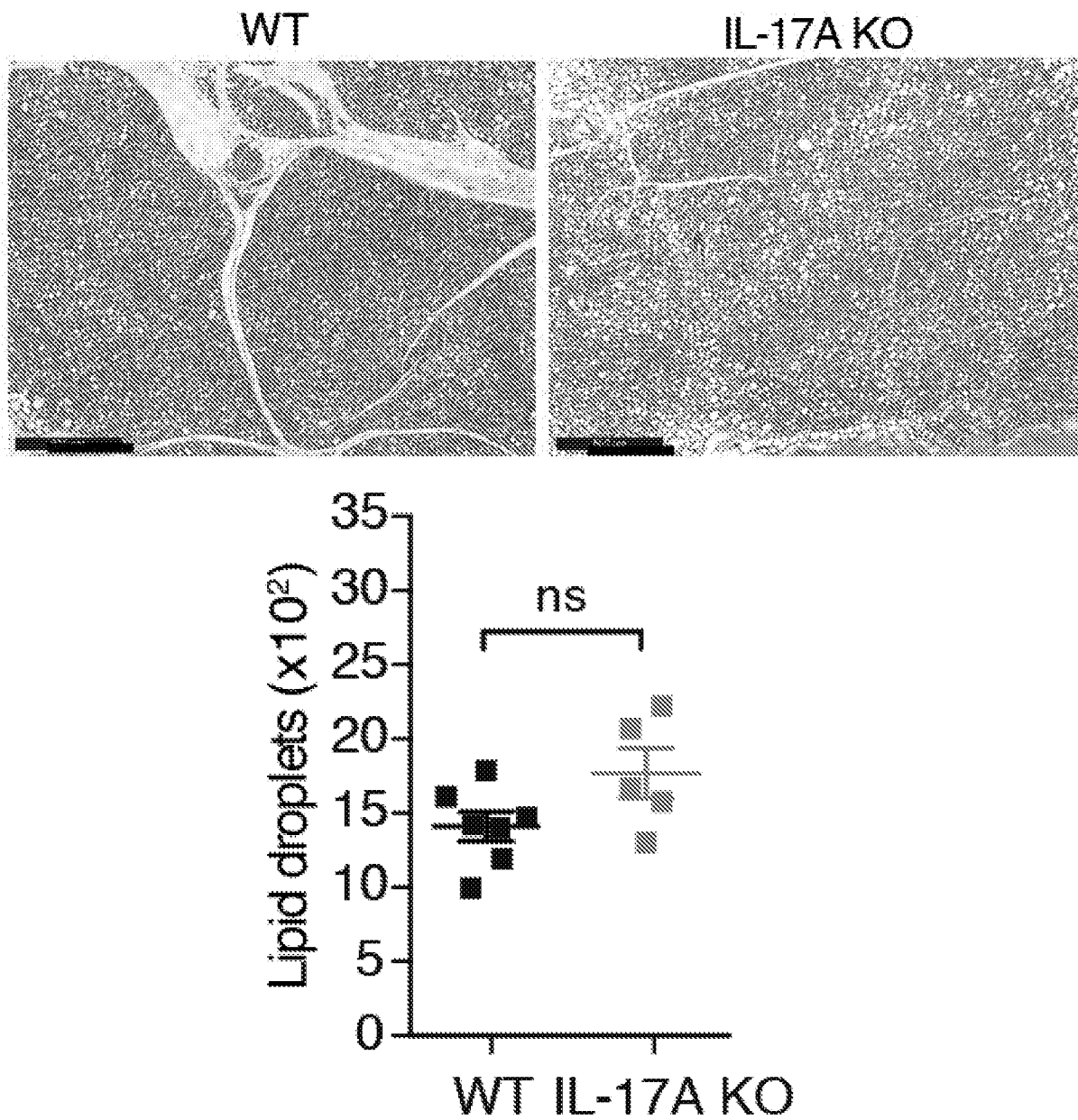


FIG. 8D

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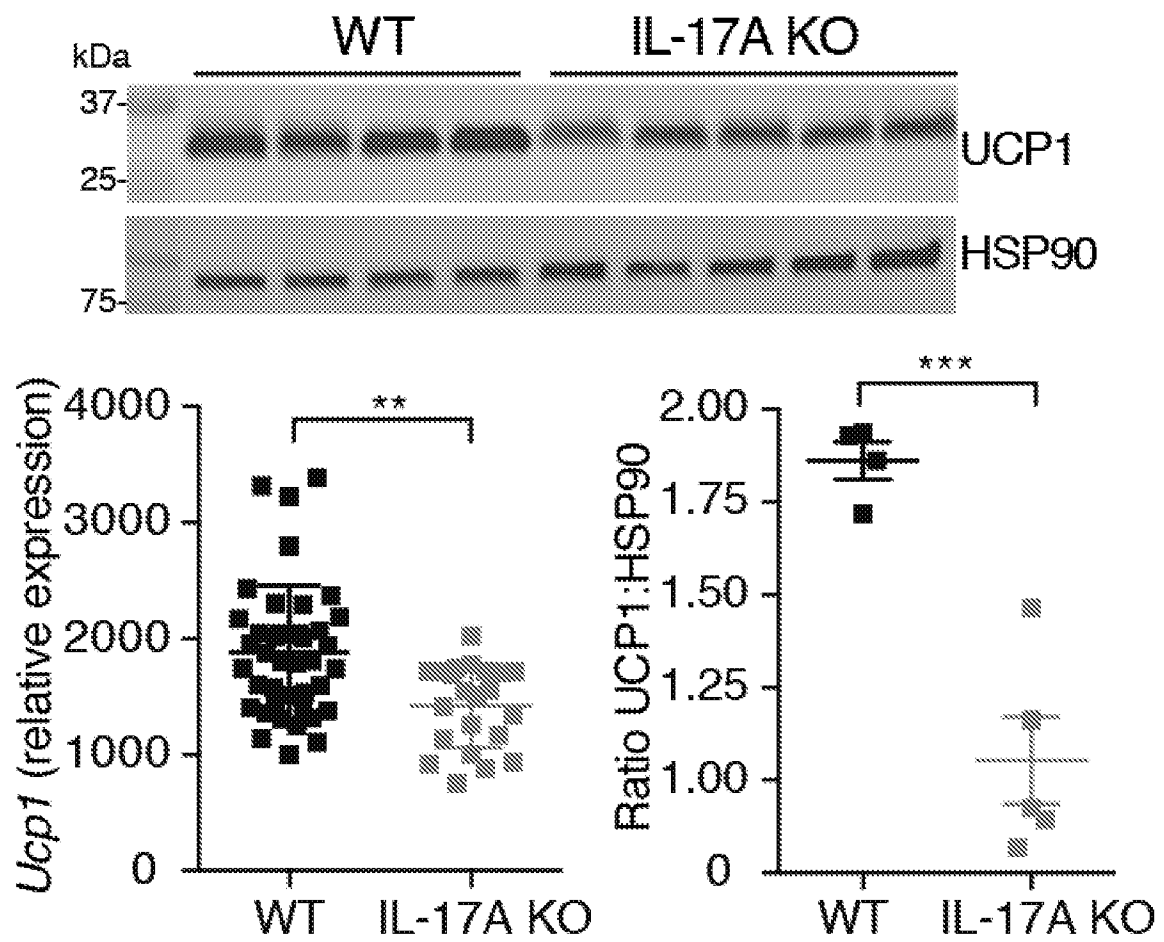


FIG. 8E

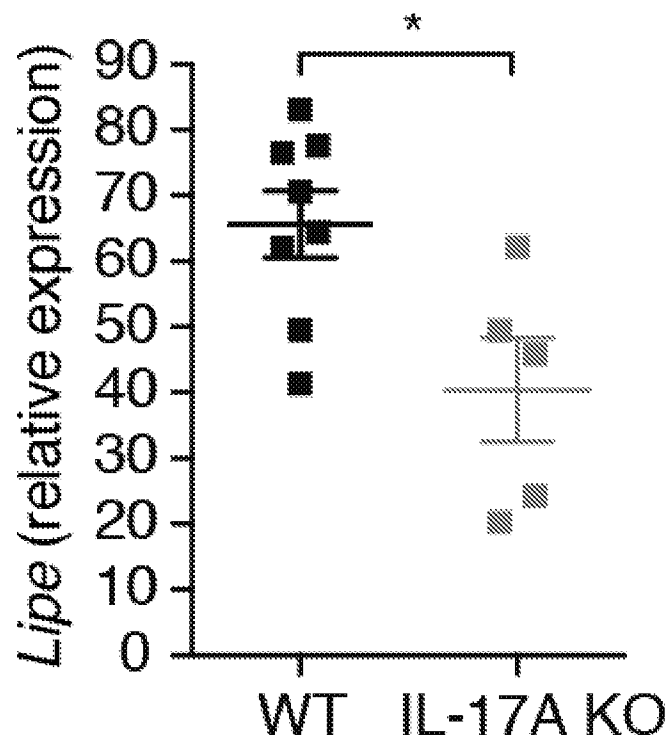


FIG. 8F

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Inguinal AT

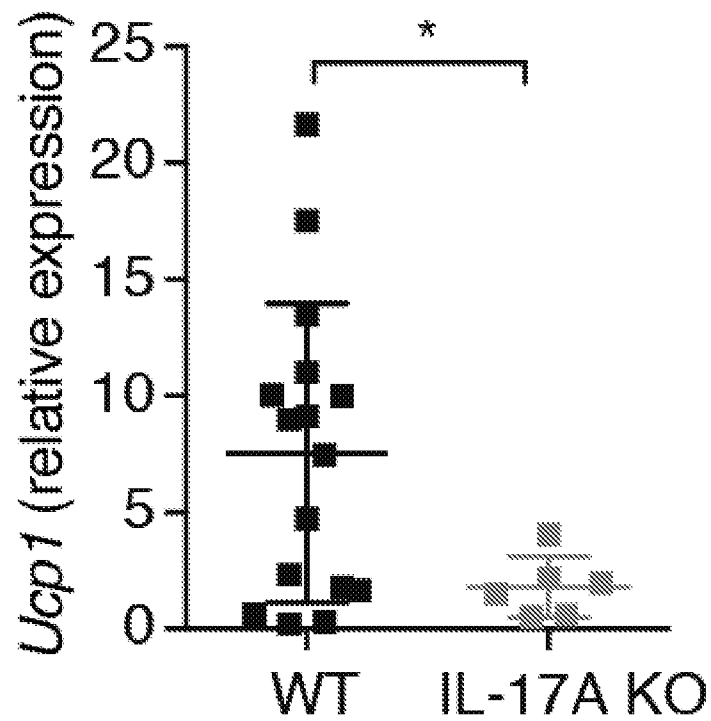


FIG. 8G

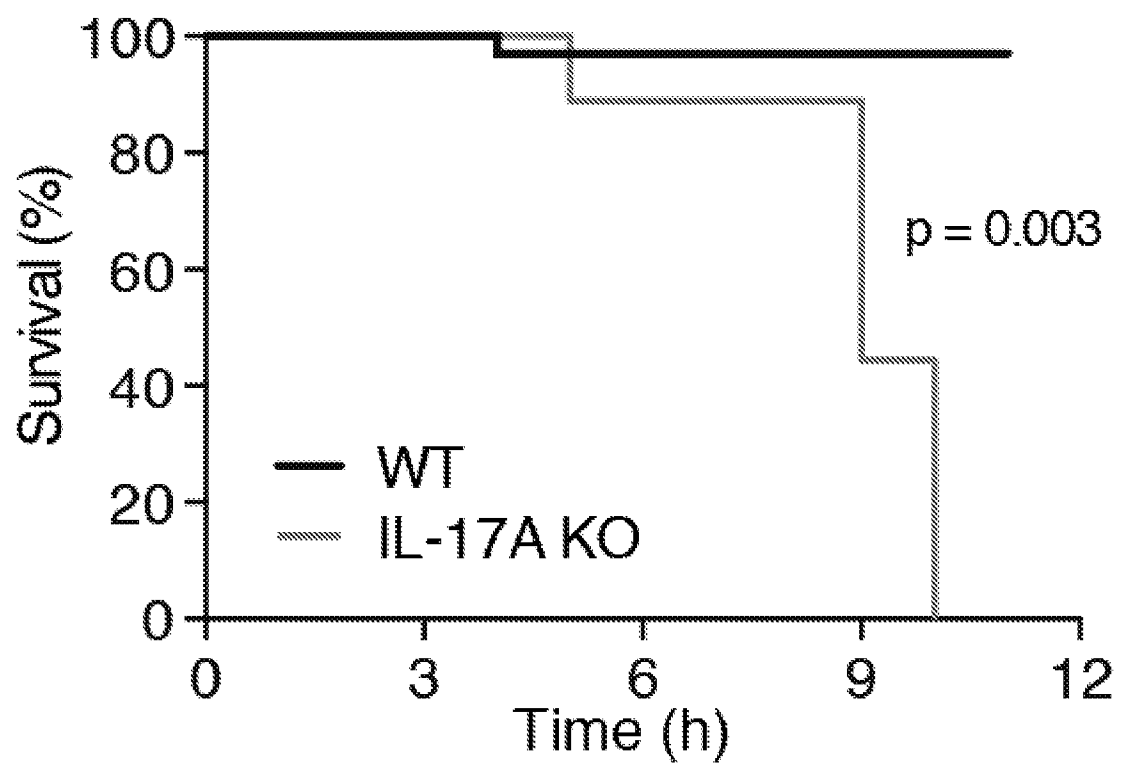
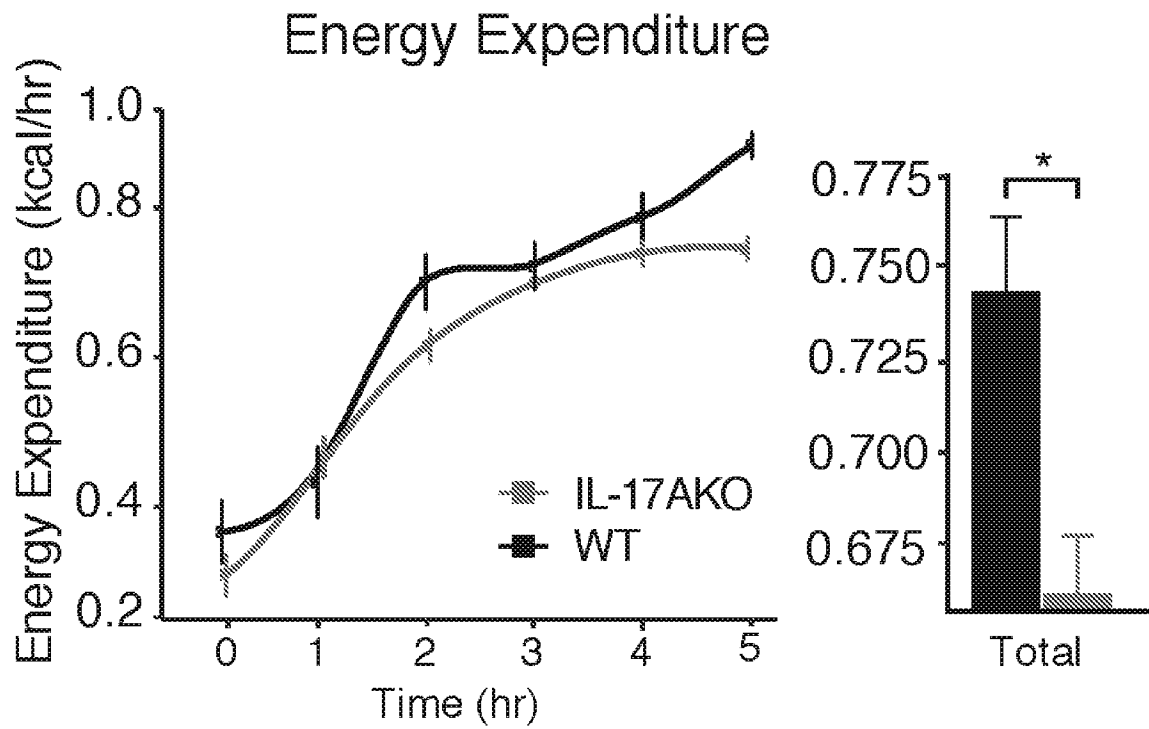


FIG. 8H

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*FIG. 8I*

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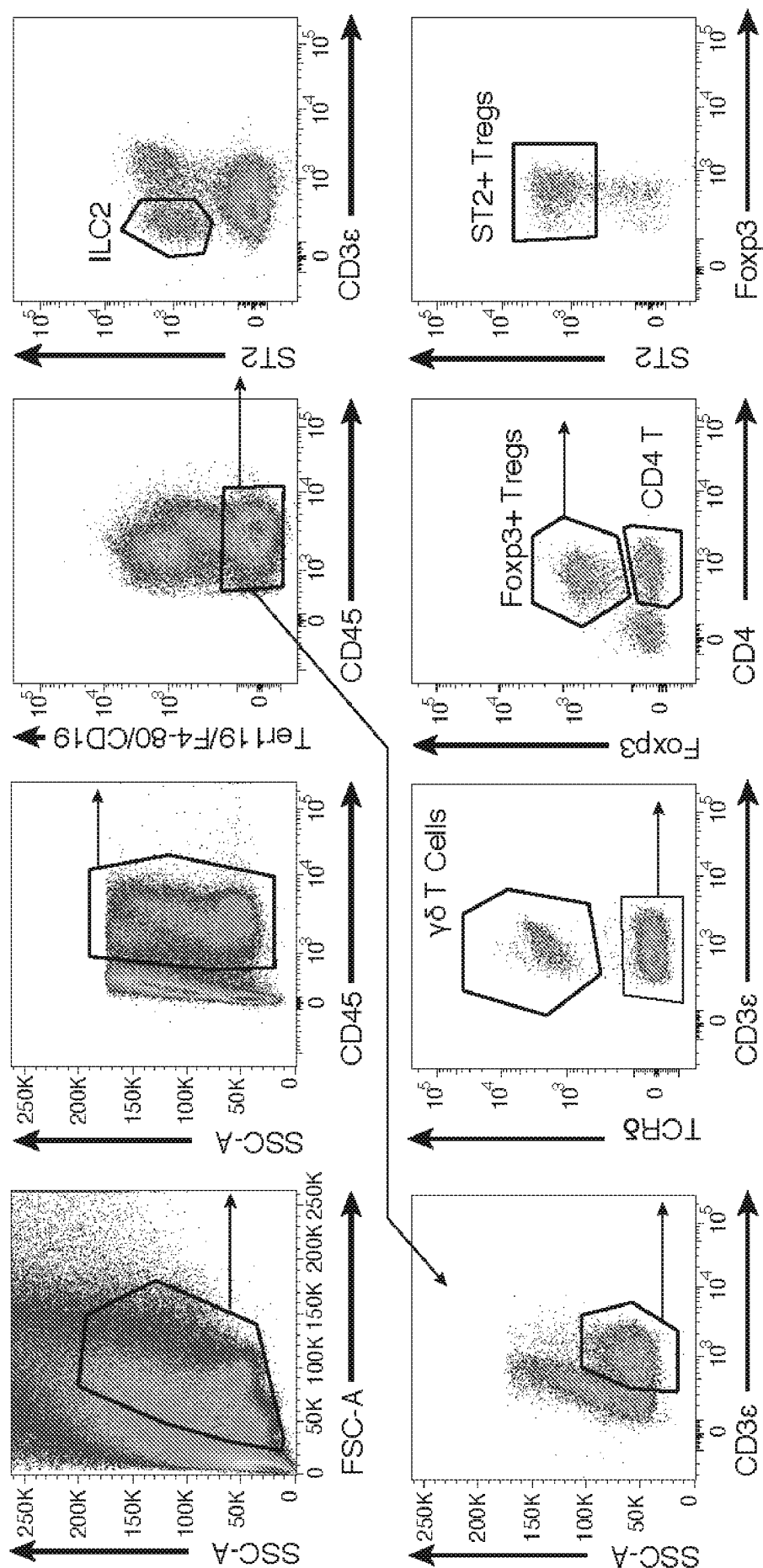


FIG. 9A

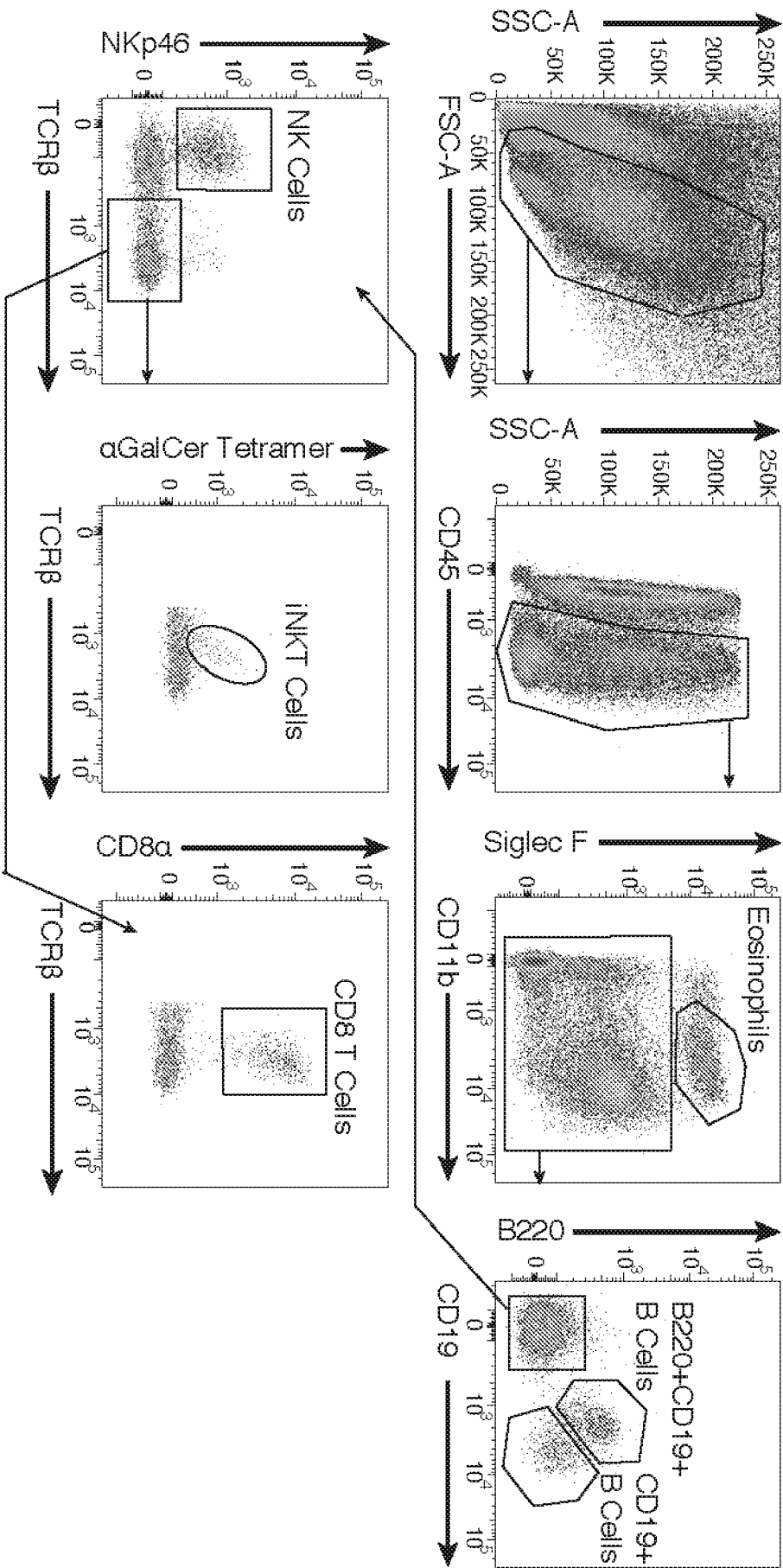


FIG. 9B

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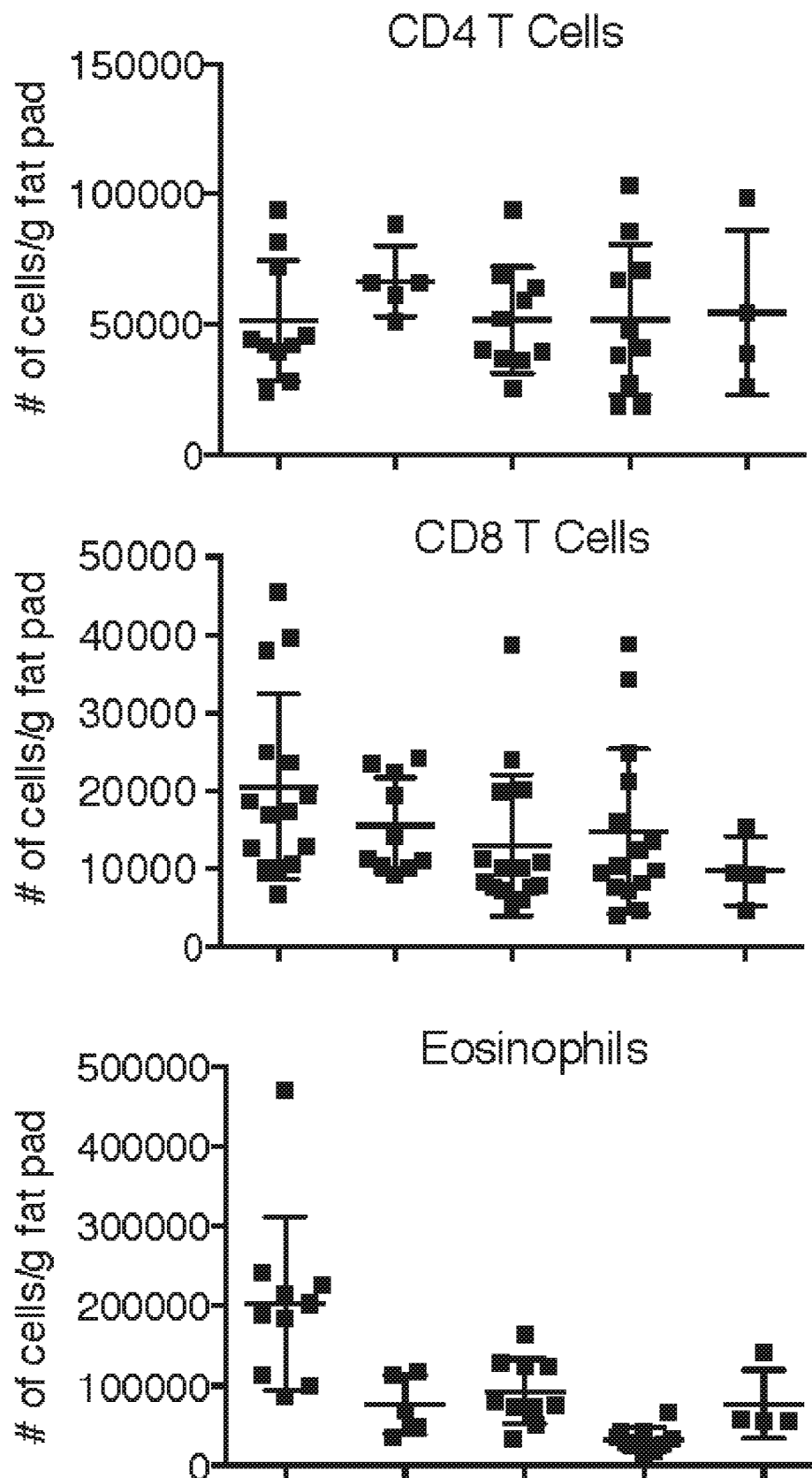


FIG. 9C

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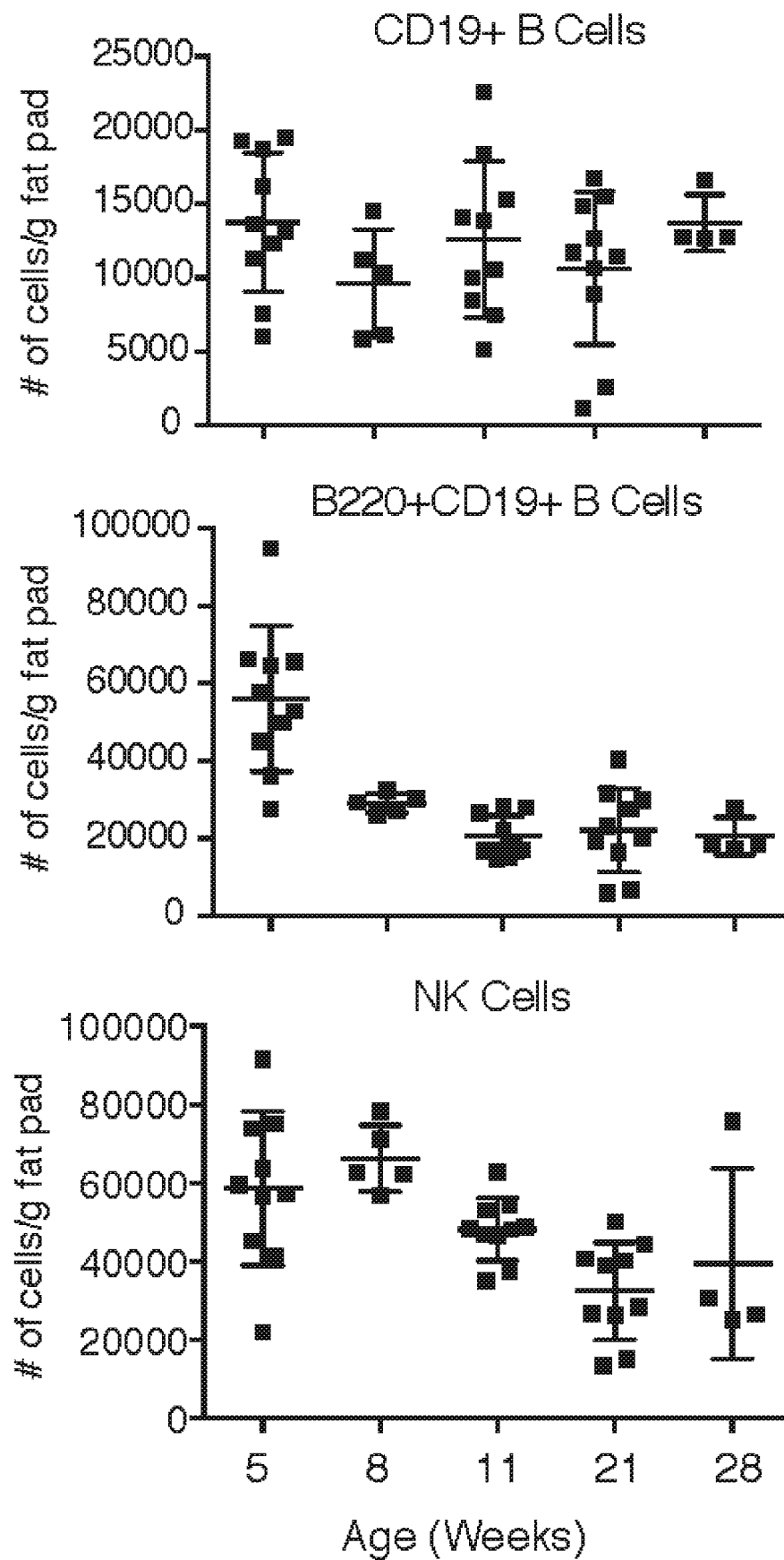


FIG. 9C, Continued

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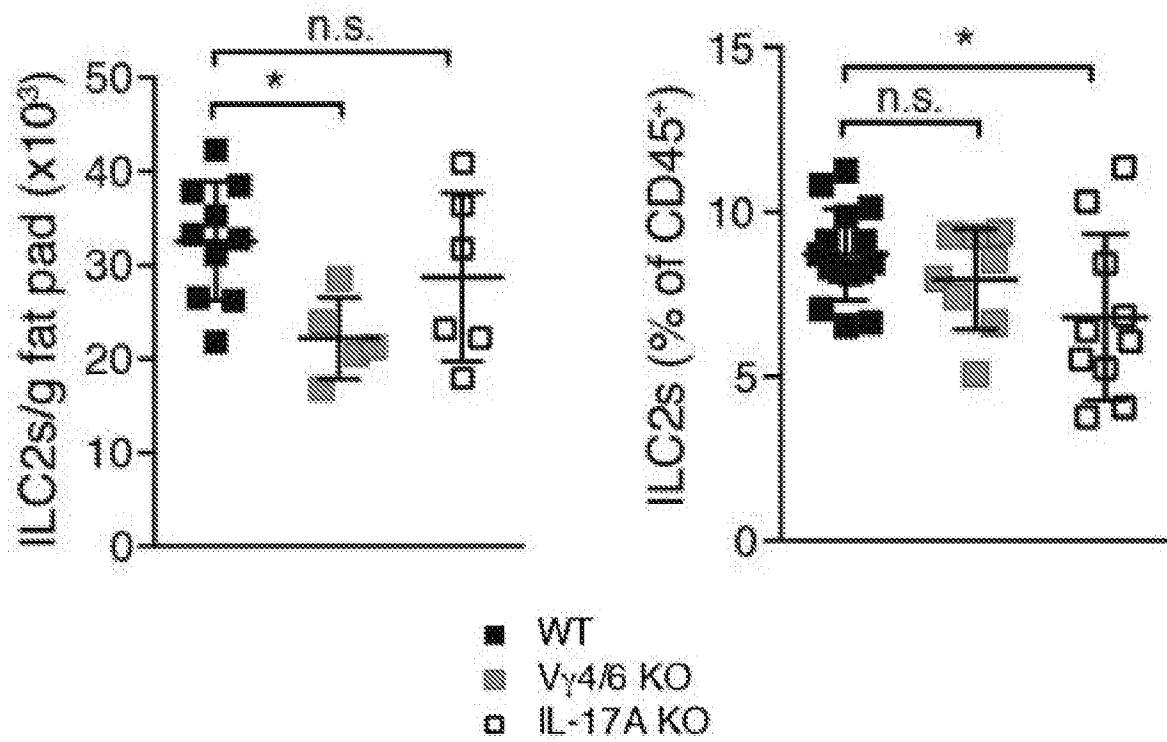


FIG. 10A

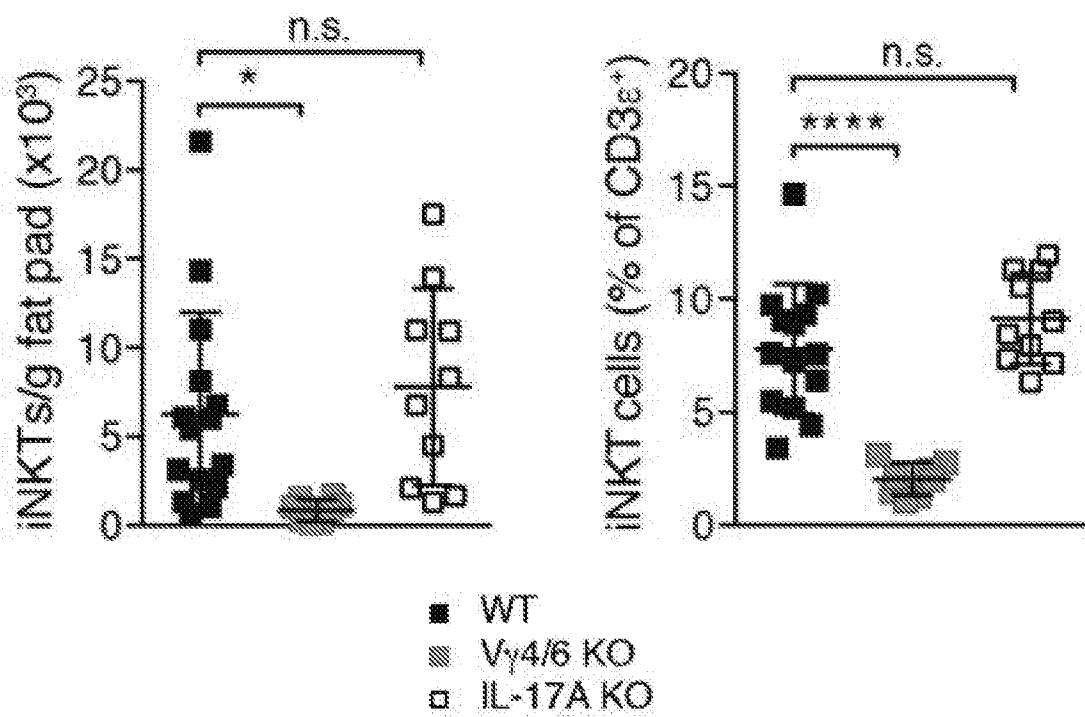


FIG. 10B

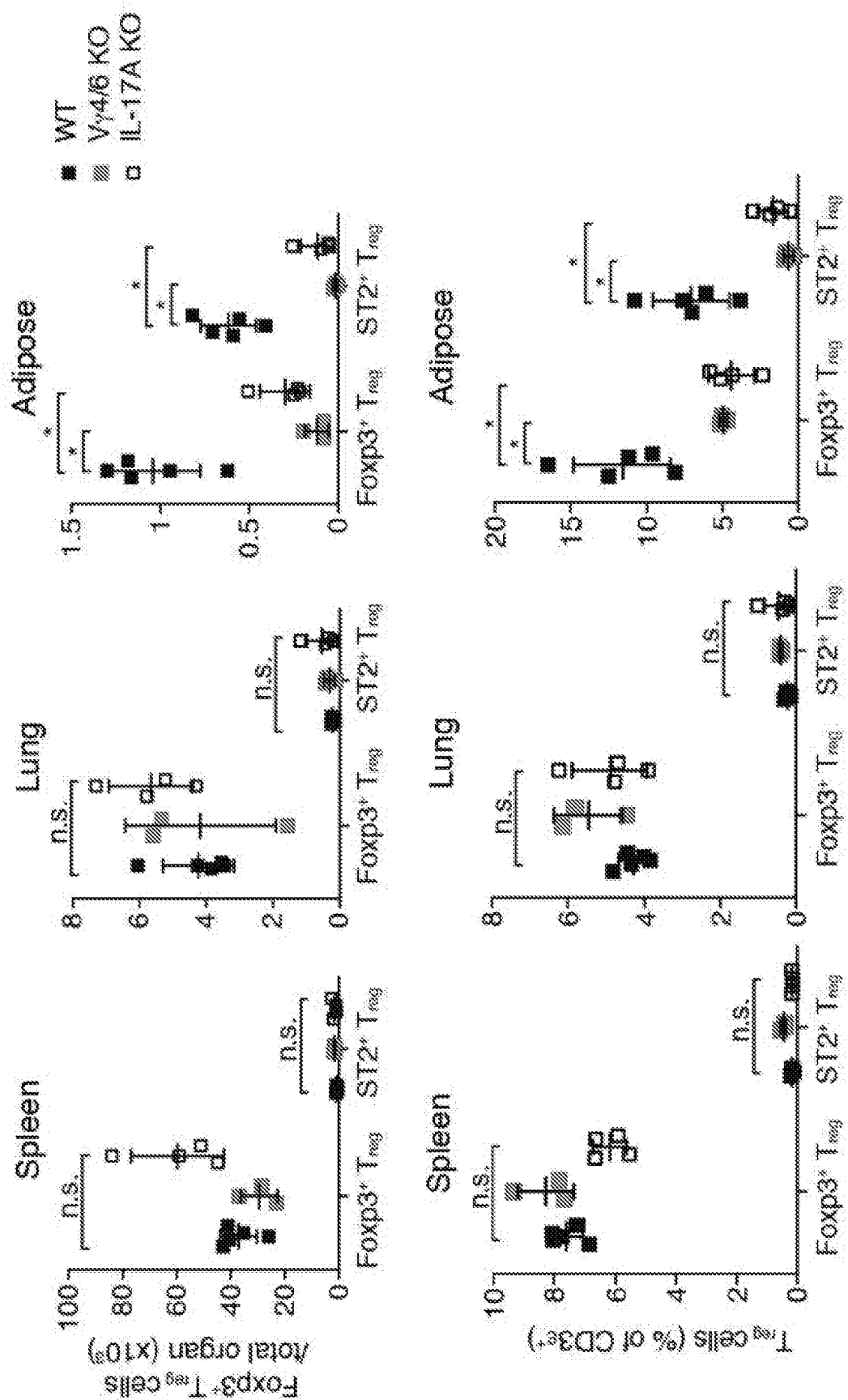


FIG. 10C

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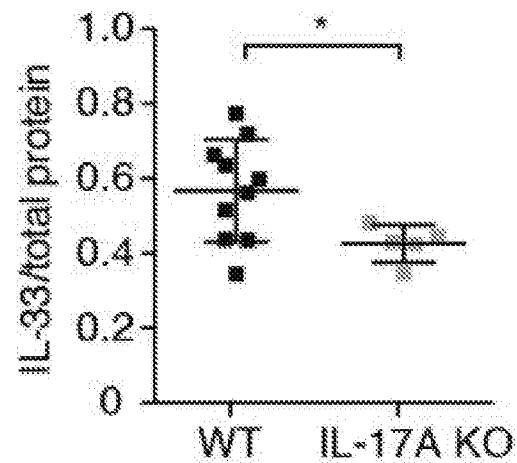


FIG. 10D

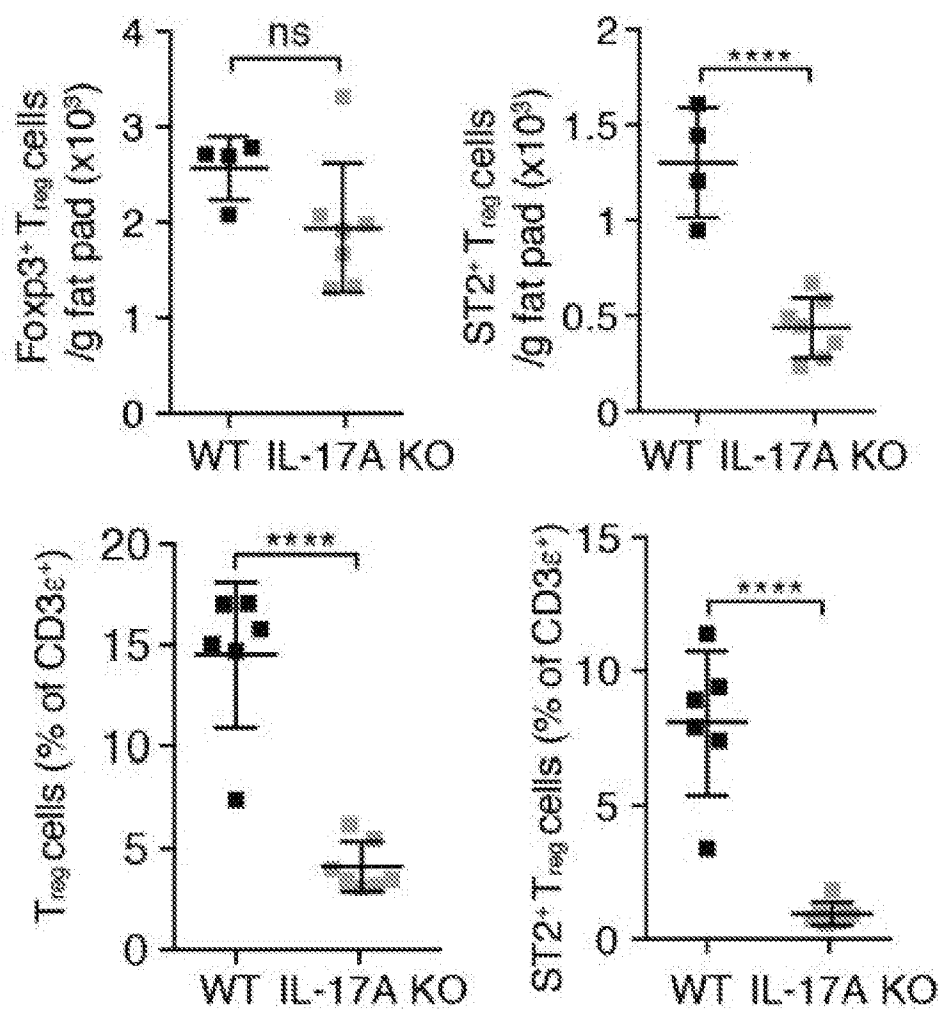


FIG. 10E

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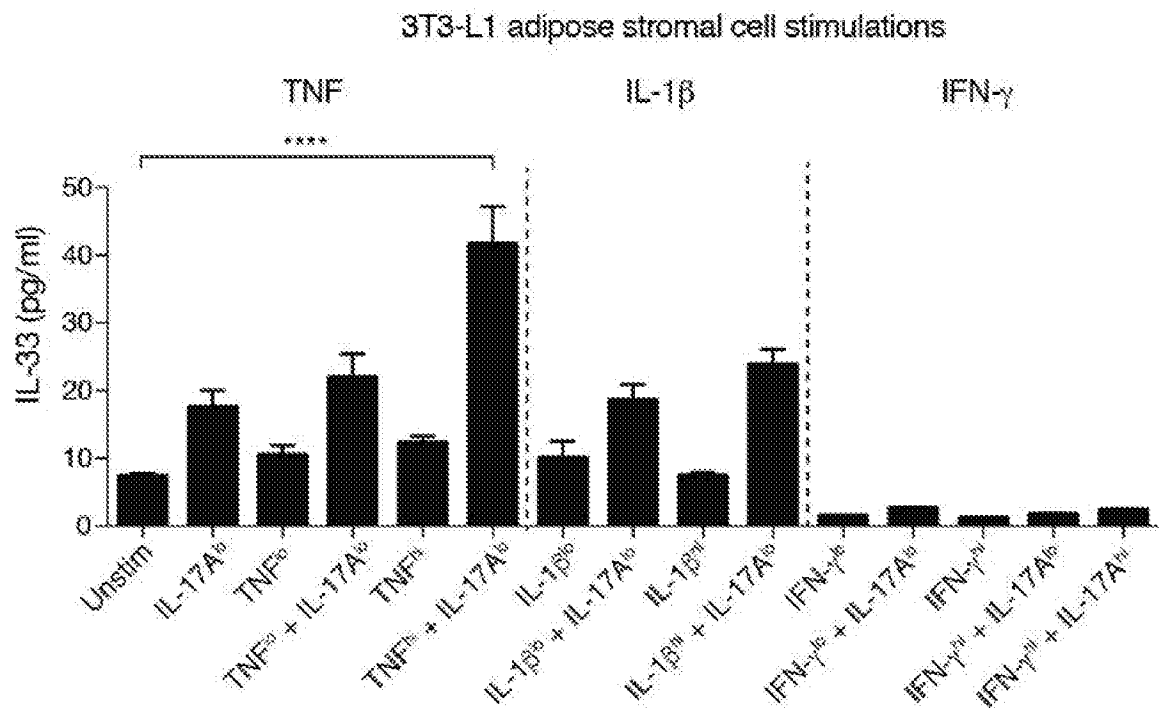


FIG. 11A

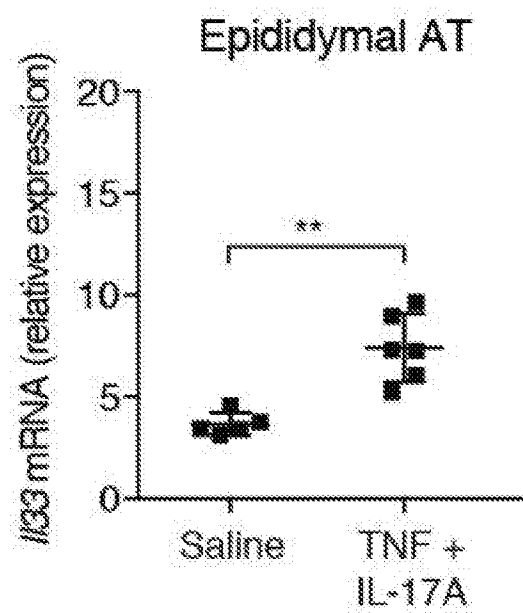


FIG. 11B

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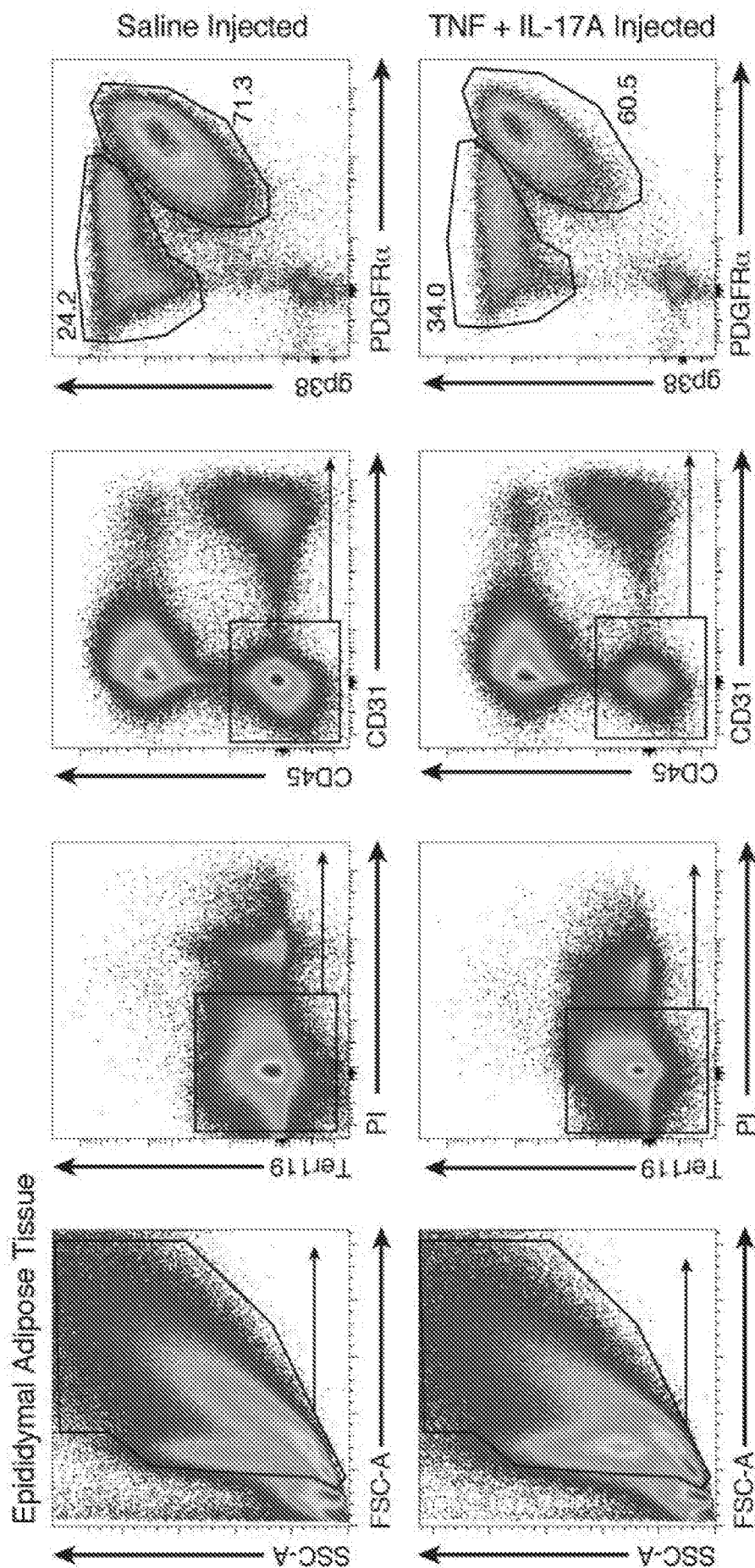
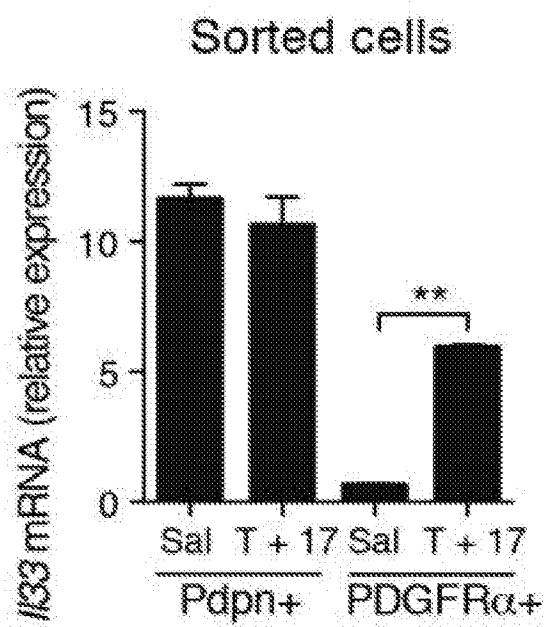


FIG. 11C

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*FIG. 11D*

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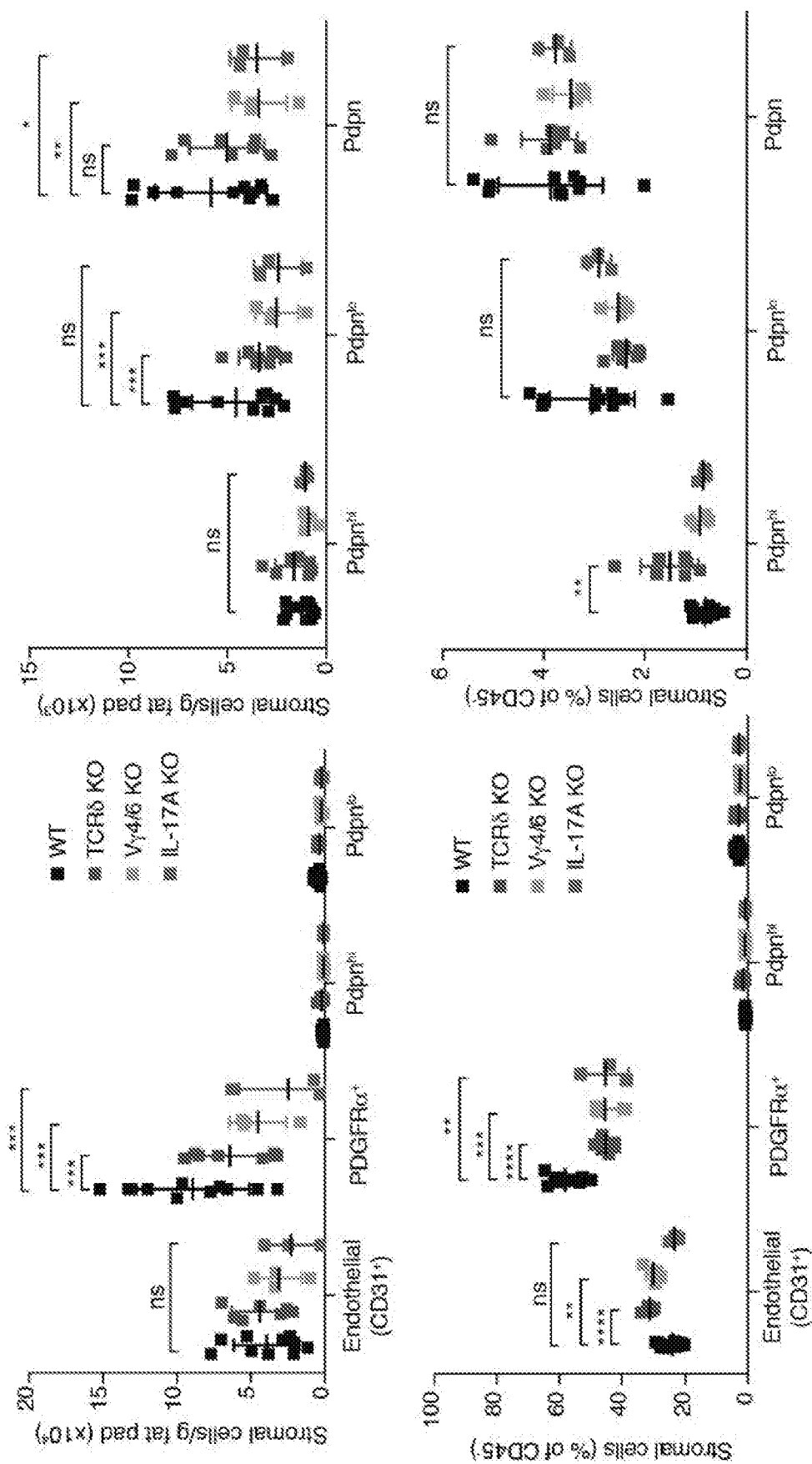


FIG. 12A

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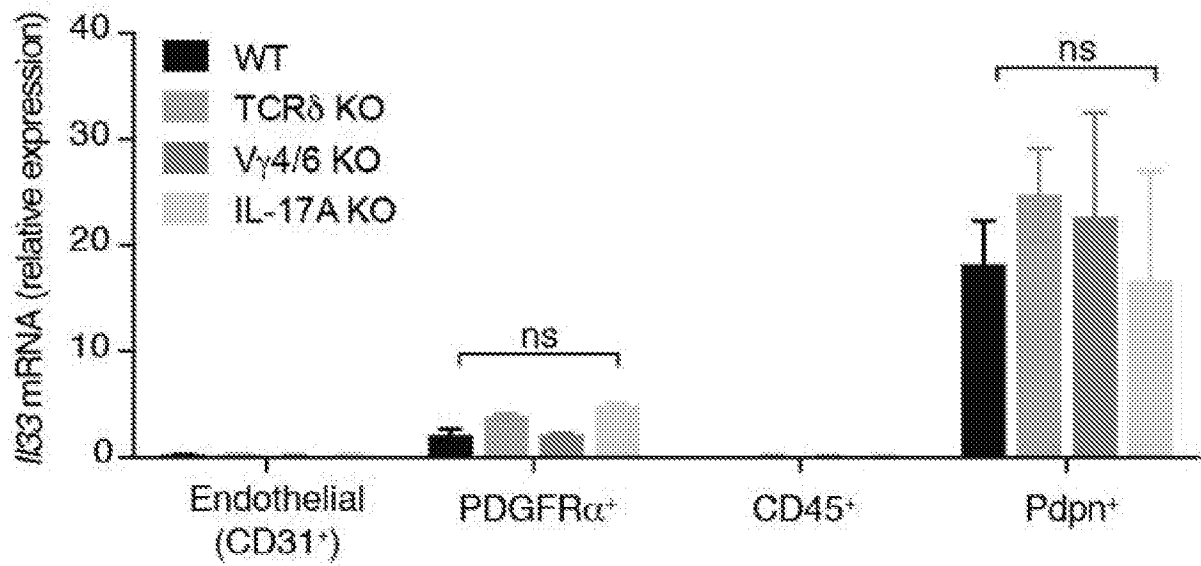


FIG. 12B

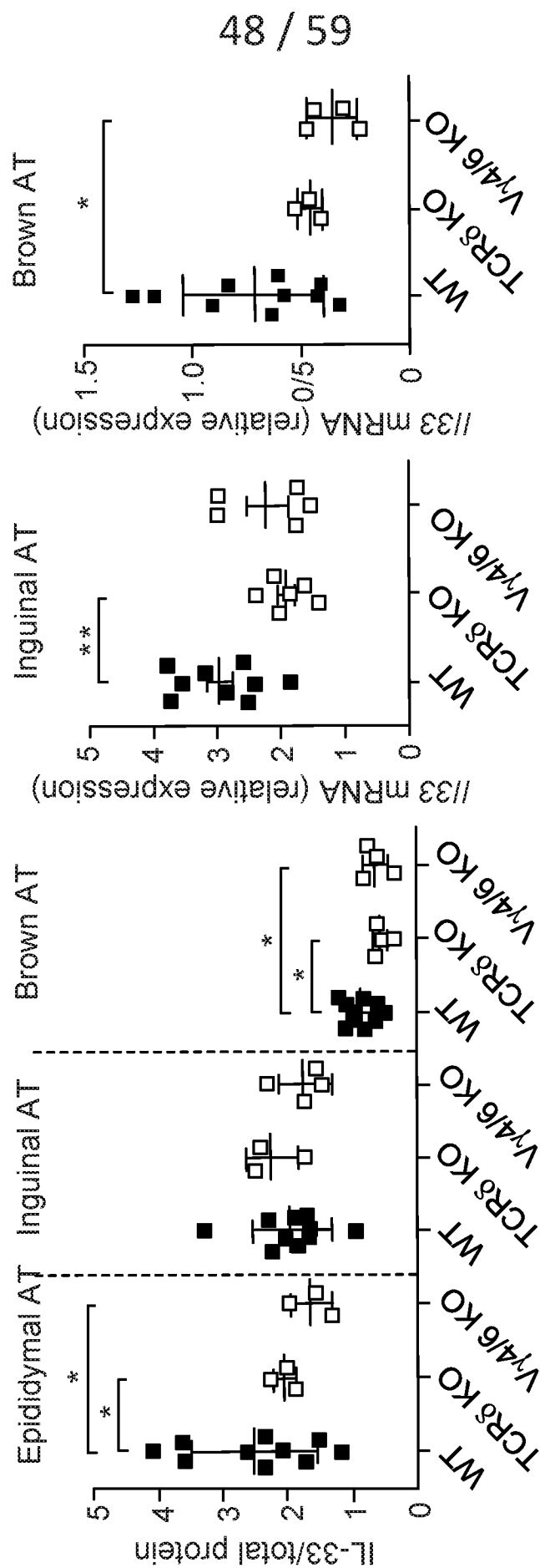
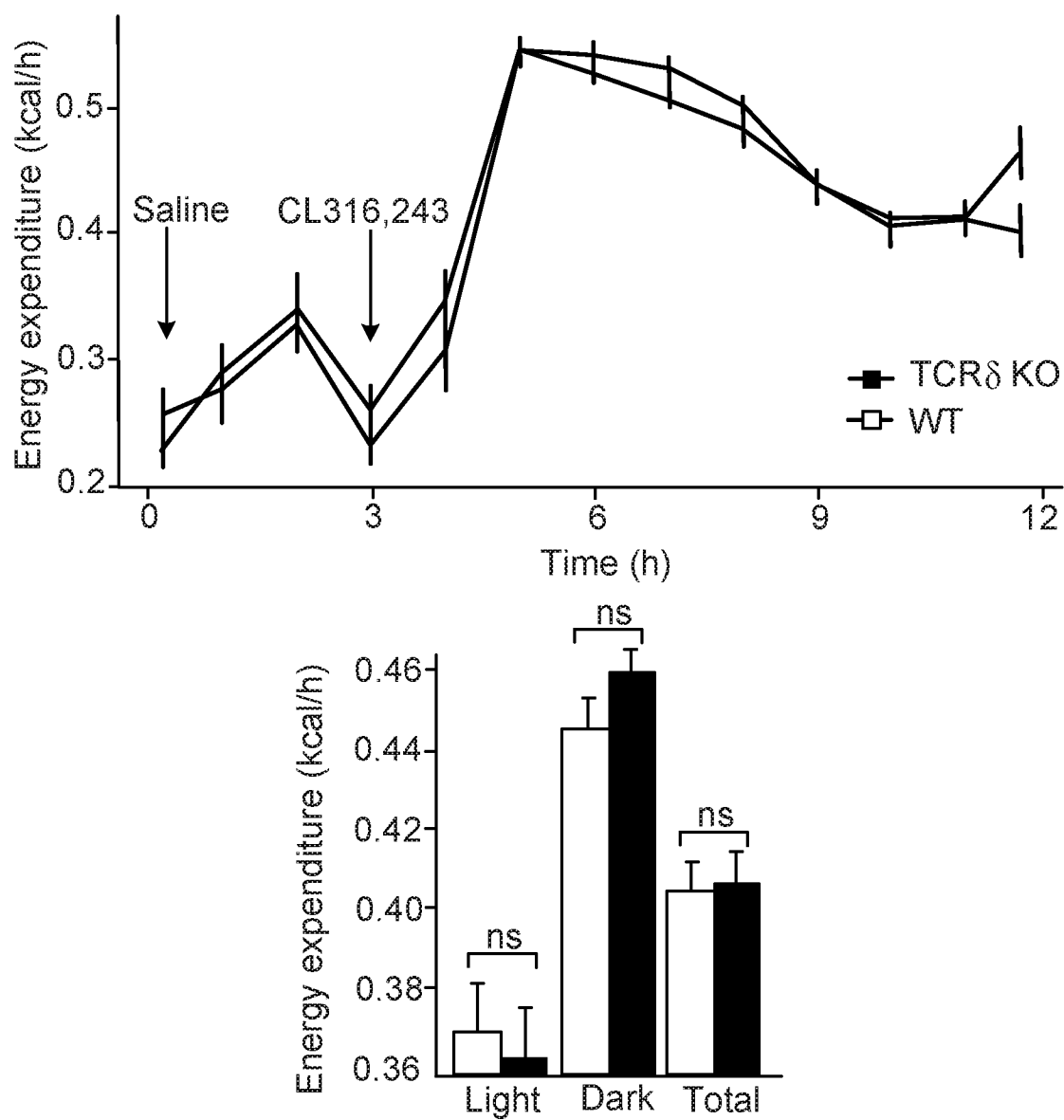


FIG. 13A

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*FIG. 13B*

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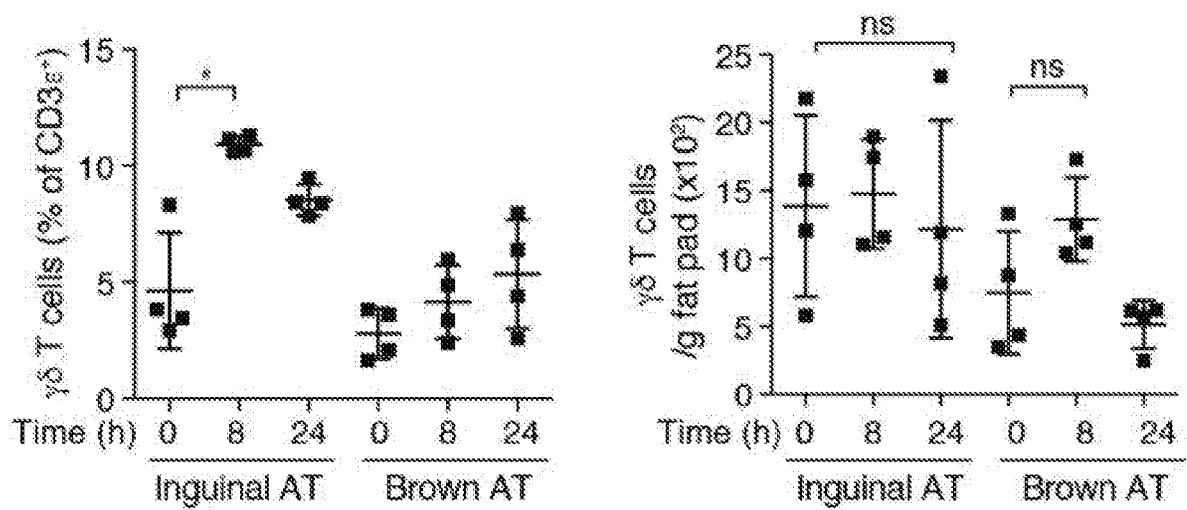


FIG. 14A

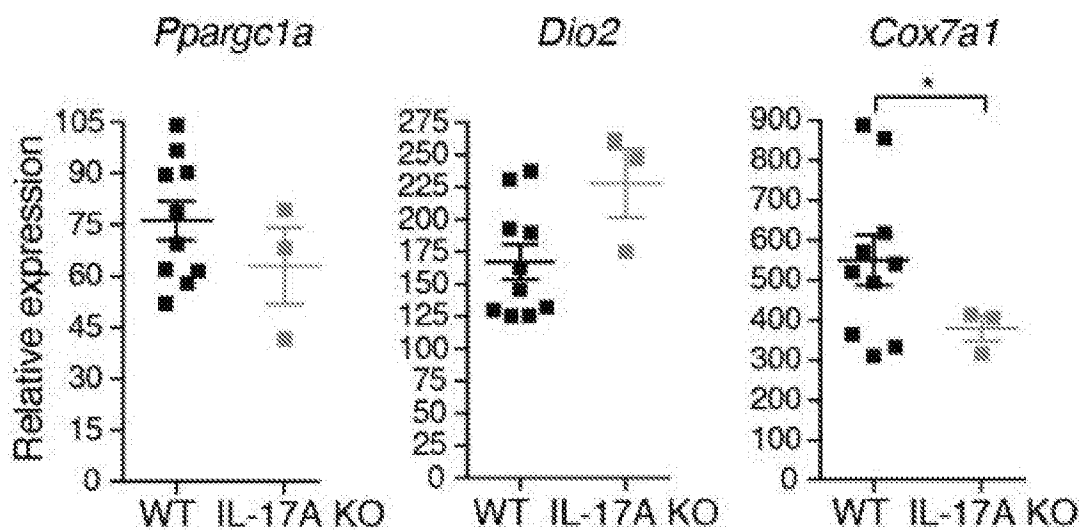


FIG. 14B

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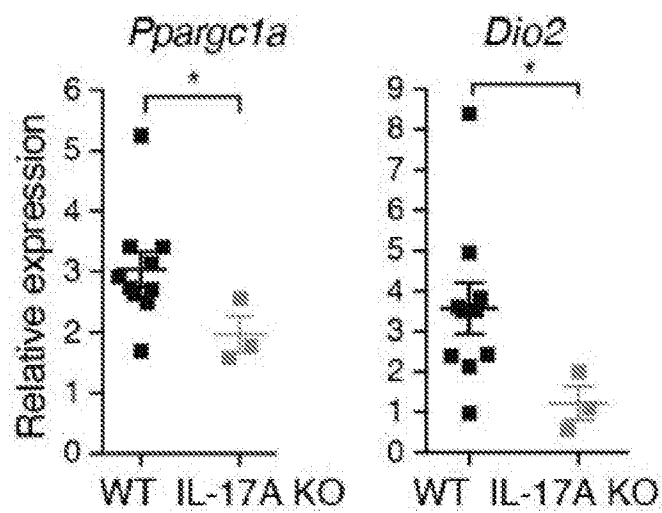


FIG. 14C

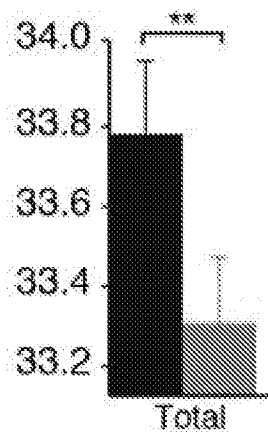
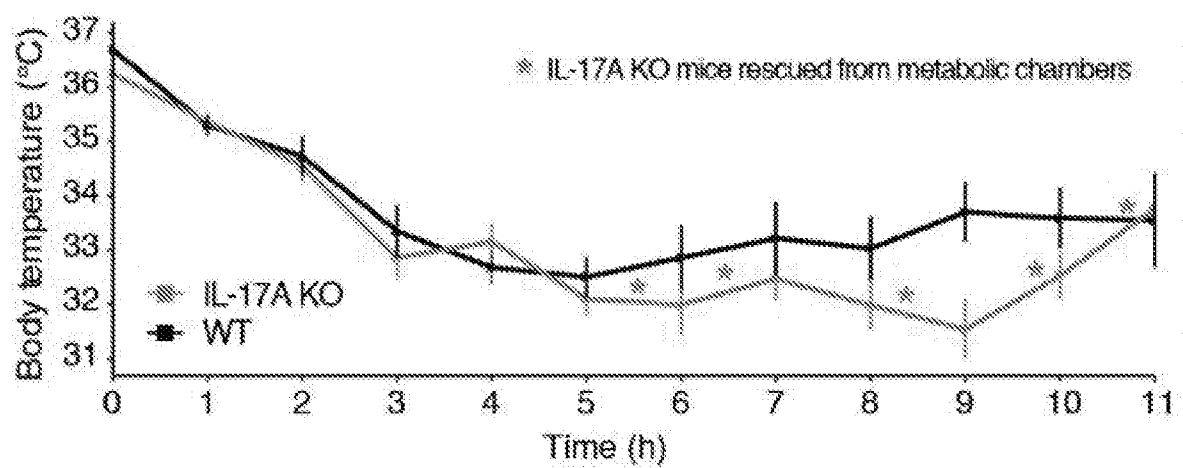


FIG. 14D

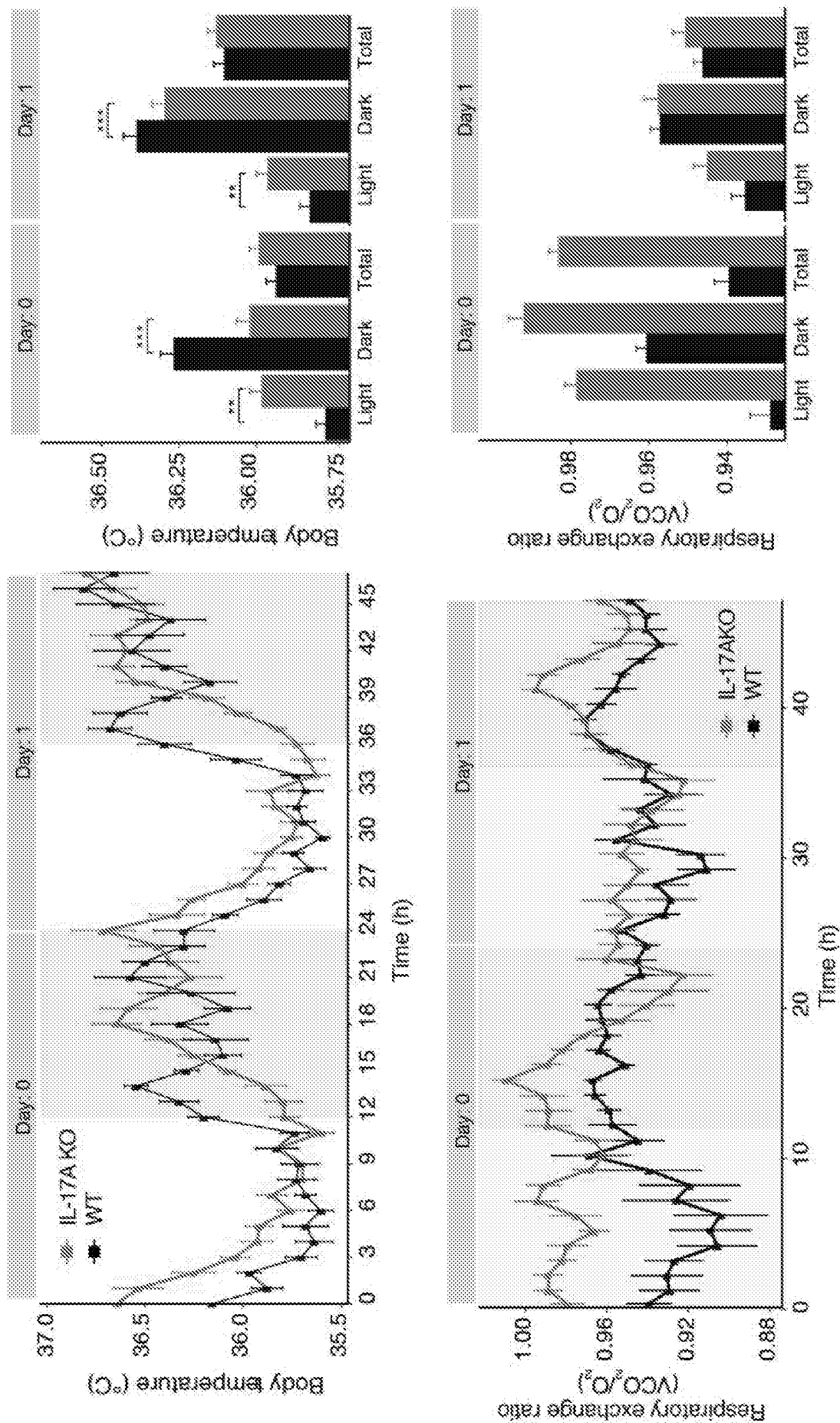


FIG. 14E

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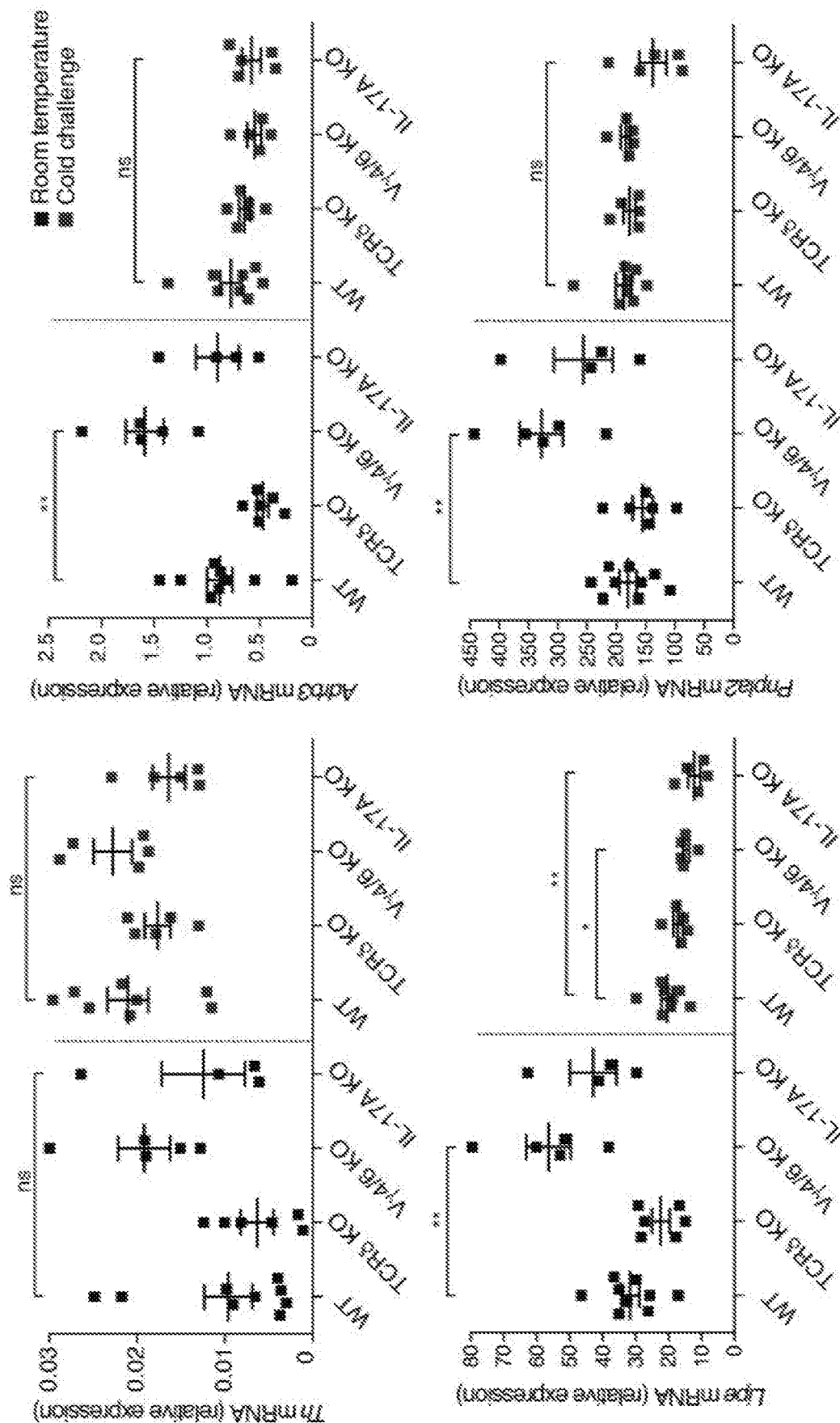


FIG. 15A

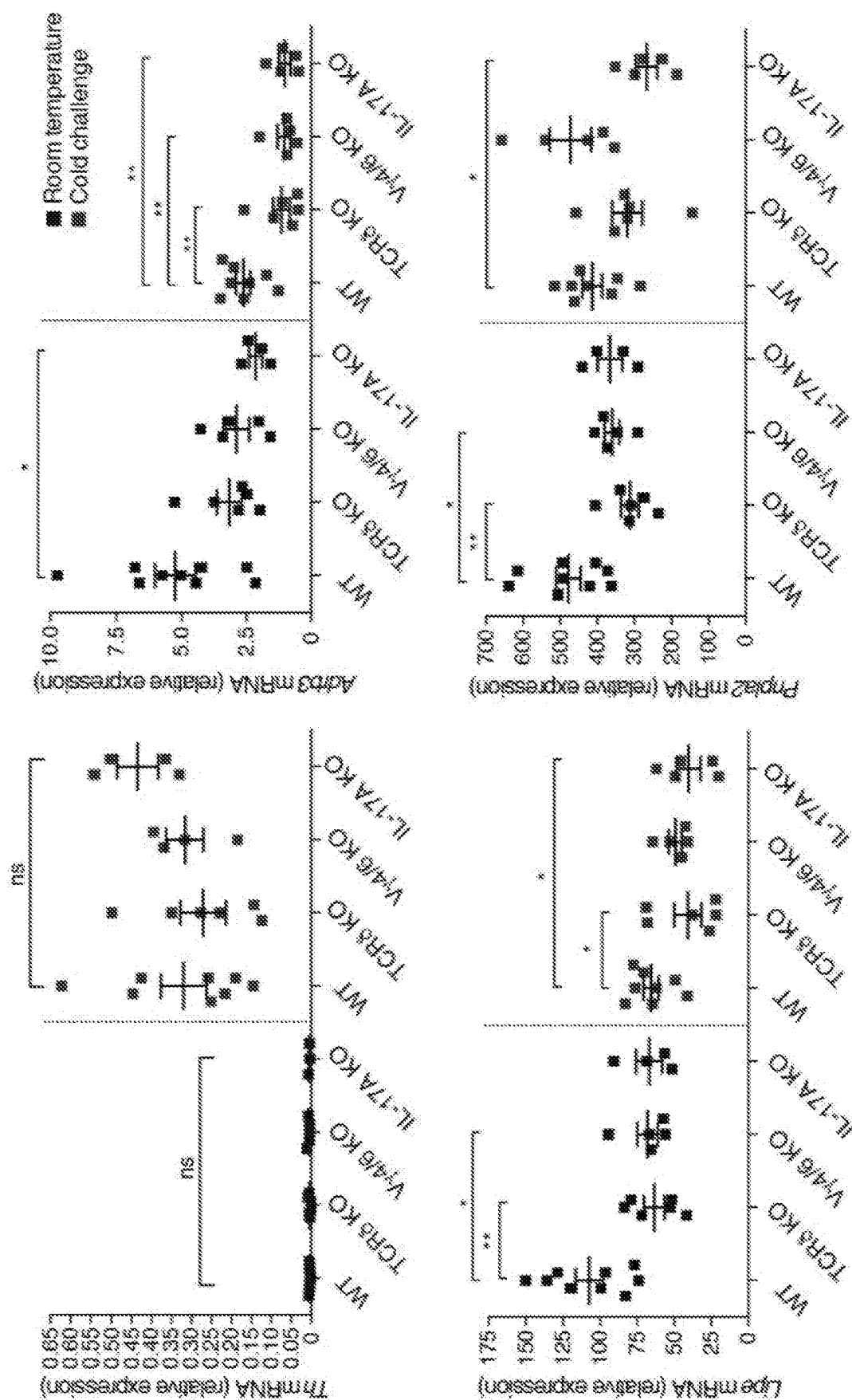


FIG. 15B

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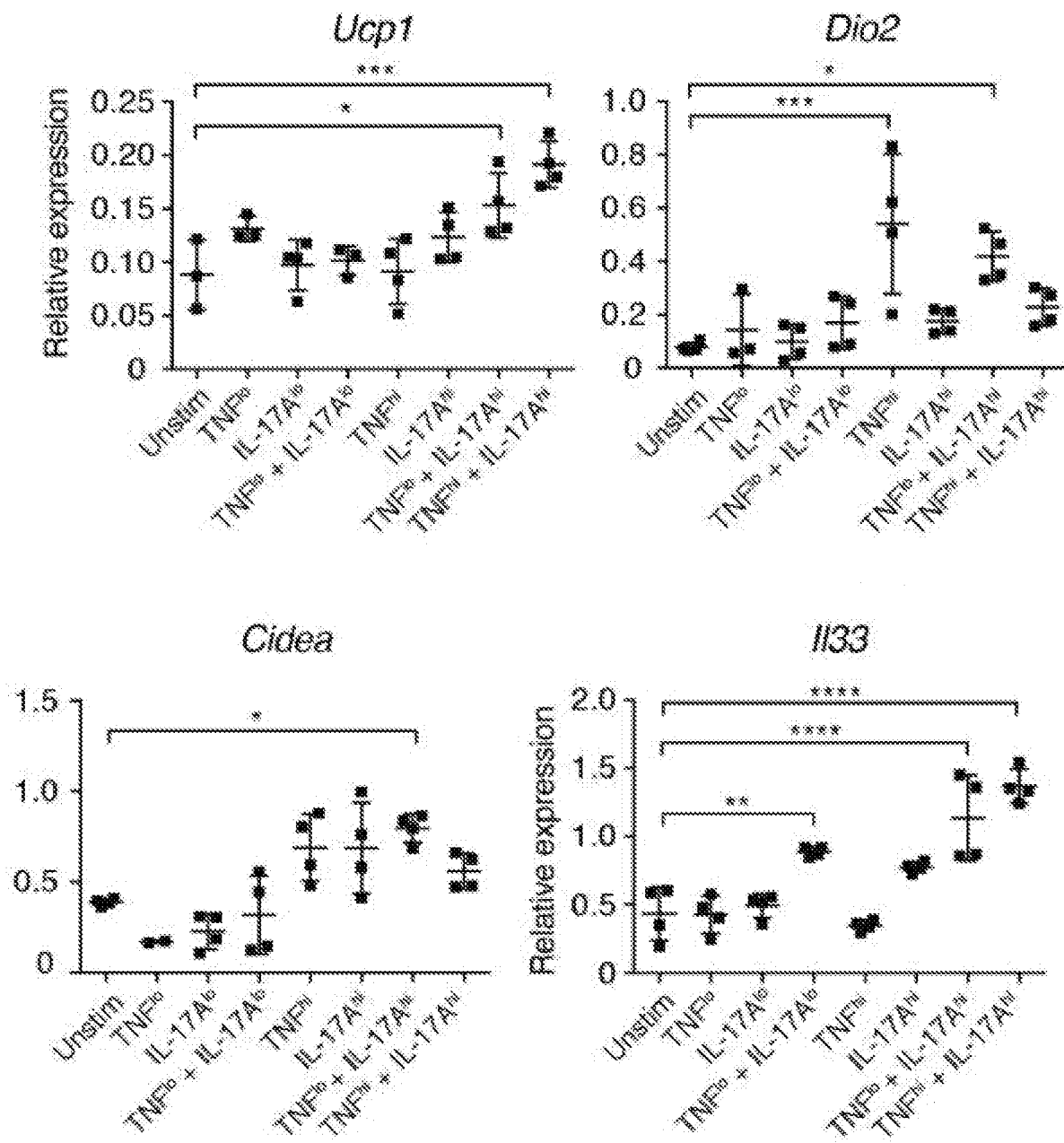


FIG. 16A

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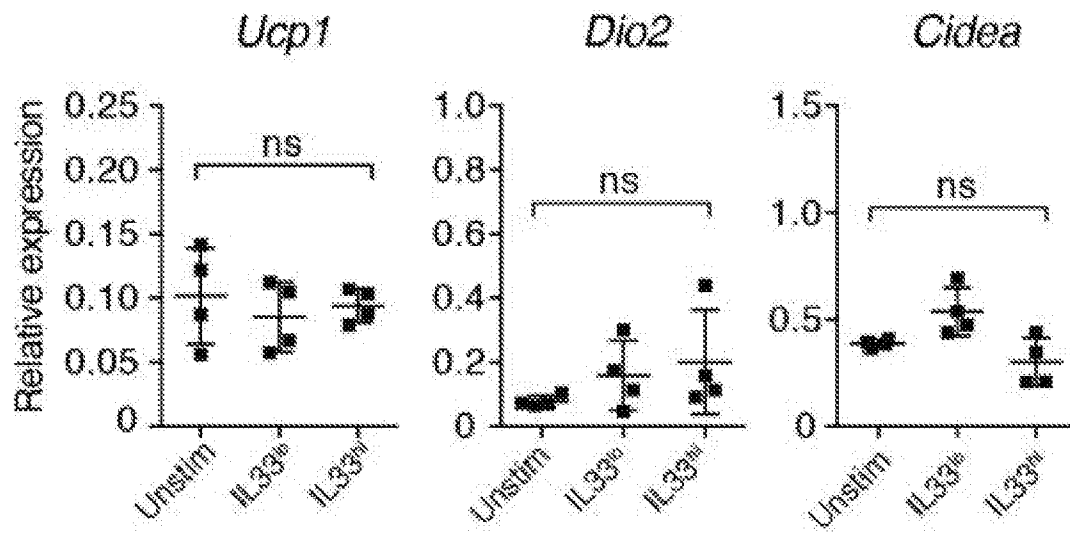


FIG. 16B

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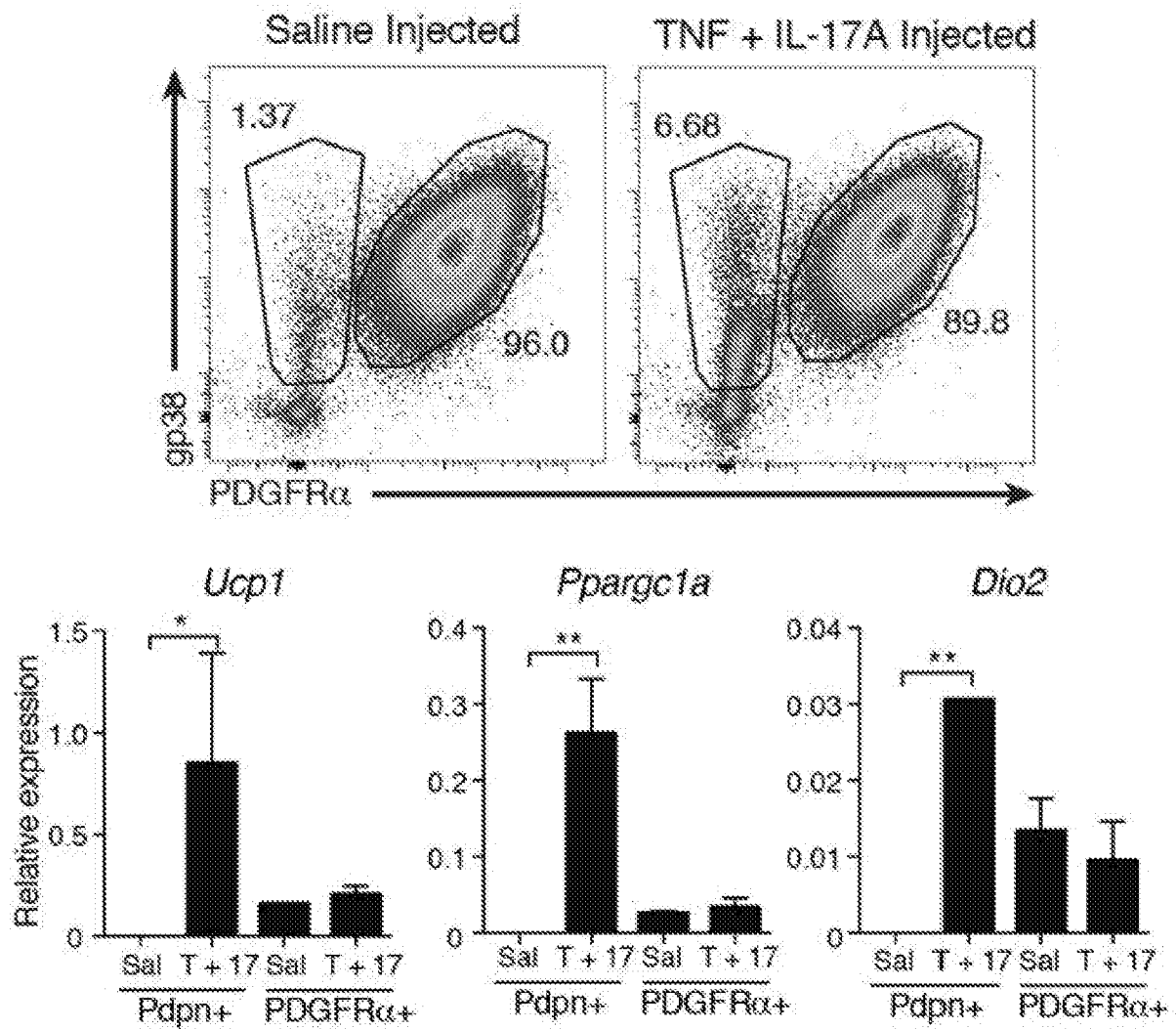


FIG. 16C

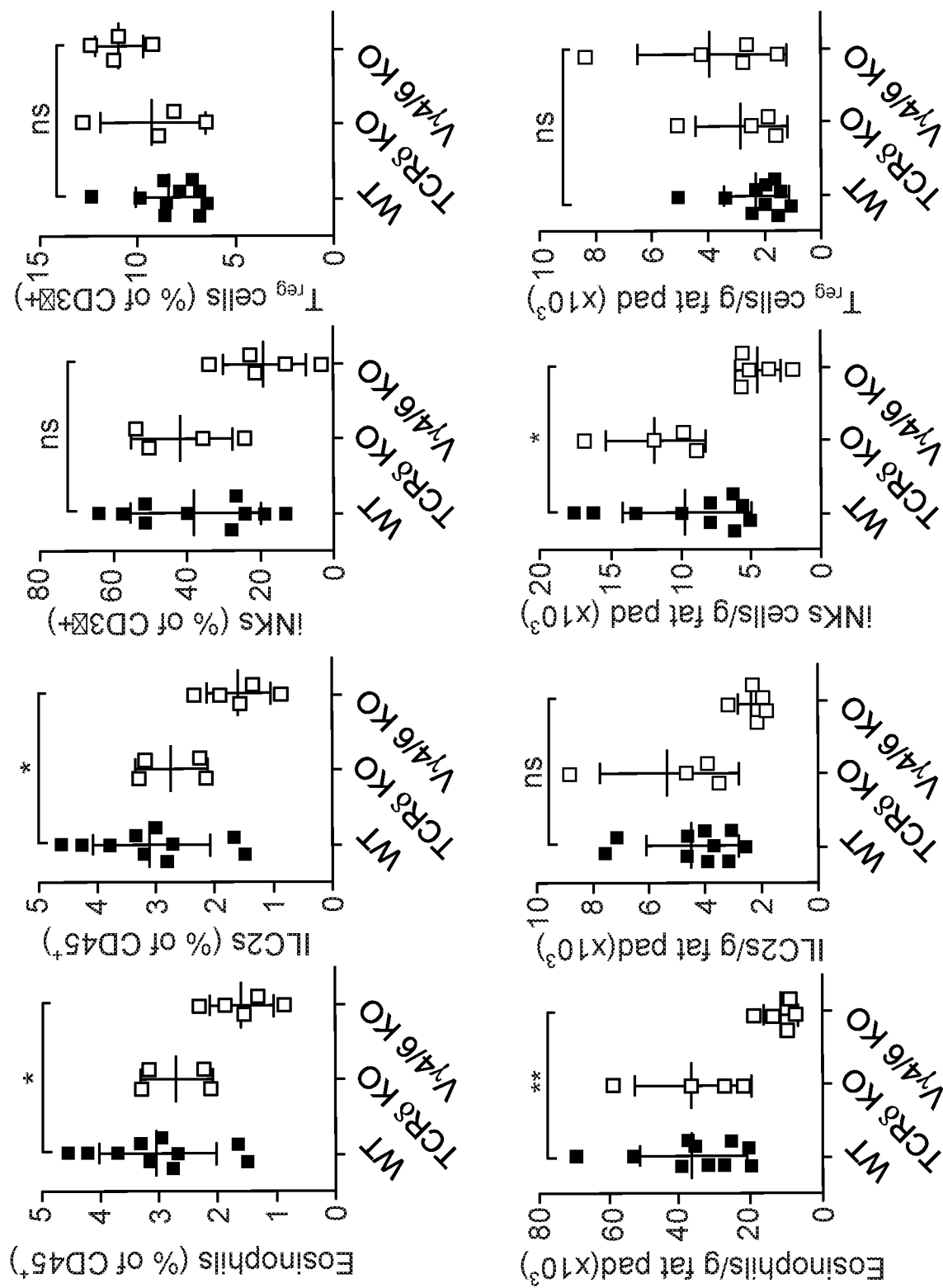


FIG. 16D

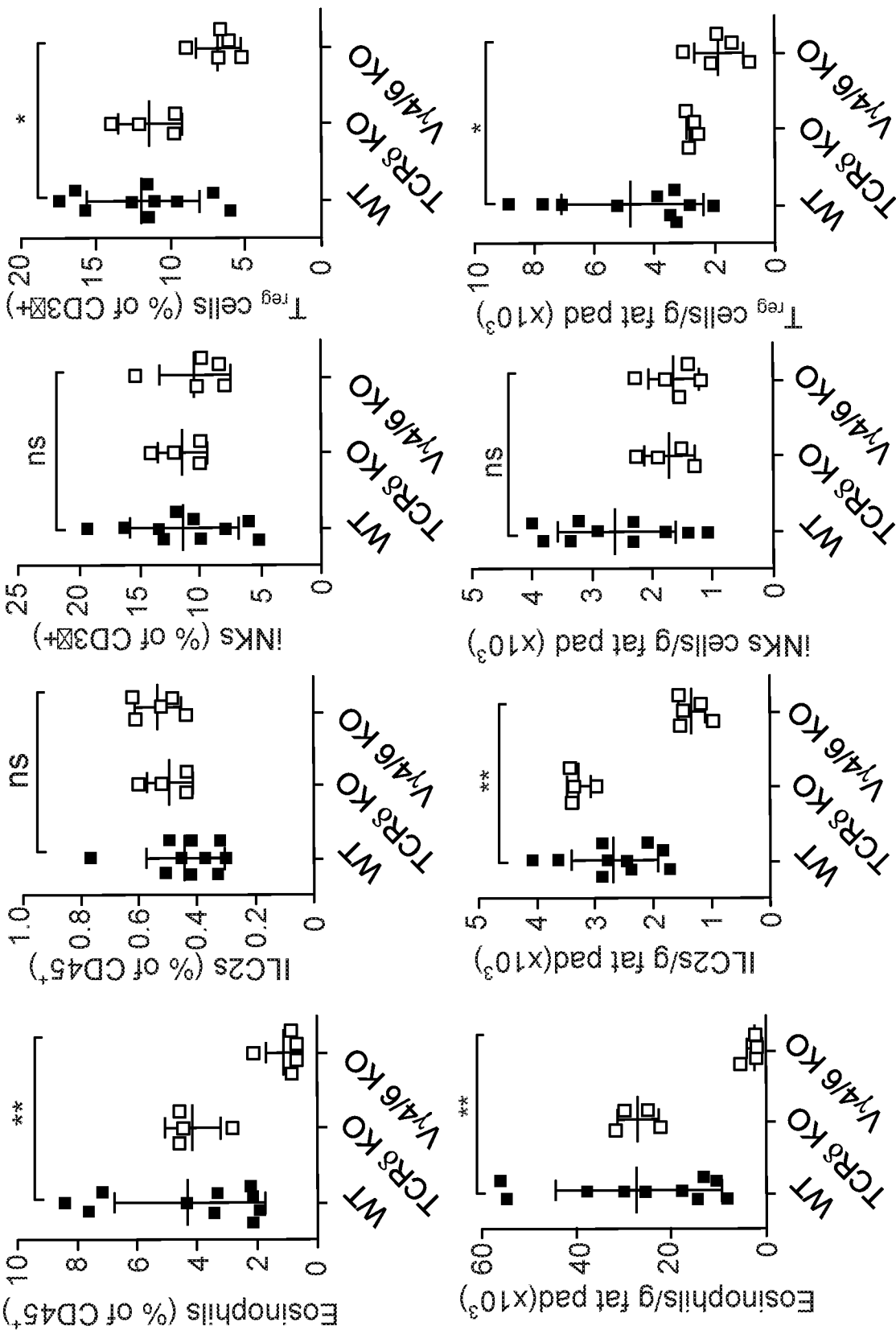


FIG. 16E

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US18/30153

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61K 38/00, 39/395; A61P 17/06, 37/00; C07K 16/24 (2018.01)

CPC - A61K 9/0019, 31/519, 39/3955; C07K 16/241

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2016/0289171 A1 (BRISTOL-MYERS SQUIBB COMPANY) October 6, 2016; abstract; paragraphs [0125], [0182], [0184]	1-7
Y	US 2014/0037578 A1 (ZHAO et al.) February 6, 2014; paragraphs [0018], [0037], [0077]; claims 1, 8	1-7
Y	US 2015/0175690 A1 (ABBVIE BIOTECHNOLOGY LTD) June 25, 2015; paragraphs [0213], [0214], [0224], [0243]	2-3
Y	WO 2012/080769 A1 (INSERM (INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE) June 21, 2012; page 4, lines 32-33; page 5, lines 16-17; page 23, lines 1-3, page 31, lines 14-18, page 44, lines 4-6; claim 5	6-7
Y	(COSTANZO, AE et al.) Obesity Impairs $\gamma\delta$ T Cell Homeostasis and Antiviral Function in Humans. PLoS ONE. 2015, Vol. 10, No. 3, pages 1-16; abstract. doi:10.1371/journal.pone.0120918	6-7

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

29 August 2018 (29.08.2018)

Date of mailing of the international search report

18 SEP 2018

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US18/30153

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

-Please See Supplemental Page-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Group I, Claims 1-7

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/US18/30153

-***-Continued from Box No. III Observations where unity of invention is lacking: -***-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, Claims 1-7 are directed toward a method of promoting weight loss.

Group II, Claims 8-17 are directed toward a method of inhibiting weight loss.

The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: the special technical features of Group I include promoting weight loss, not present in Group II; the special technical features of Group II include an inhibitor of IL-17, IL-17R or IL-33, not present in Group I.

Groups I and II Share the technical features including: a method of modulating weight loss, the method comprising administering to a subject in need thereof therapeutically effective amounts of a combination of agents comprising: an agent related to Tumor Necrosis Factor alpha (TNFa) activity or TNF Receptor (TNFR) activity, and an agent related to Interleukin 17 (IL-17) or IL-33.

However, these shared technical features are previously disclosed by US 7,820,168 B2 to Cua et al. (hereinafter 'Cua').

Cua discloses a method of modulating weight loss (a method of treating a metabolic disease associated with obesity (column 1, lines 32-34; column 2, lines 51-58), the method comprising administering to a subject in need thereof therapeutically effective amounts of a combination of agents (the method comprising administering to a subject in need thereof therapeutically effective amounts of a combination of agents; column 2, lines 51-64; column 14, lines 57-60 Claim 1) comprising: an agent related to Tumor Necrosis Factor alpha (TNFa) activity or TNF Receptor (TNFR) activity (comprising: an agent related to Tumor Necrosis Factor alpha (TNFa) activity or TNF Receptor (TNFR) activity; column 3, lines 8-11; column 14, lines 57-60), and an agent related to Interleukin 17 (IL-17) (and an agent related to Interleukin 17 (IL-17); column 3, lines 8-11; Column 14, lines 57-60; Claim 1).

Since none of the special technical features of the Groups I and II inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Cua reference, unity of invention is lacking.