SUPPLEMENTARY INFORMATION
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γδ T cells producing interleukin-17A regulate adipose regulatory T cell homeostasis and thermogenesis

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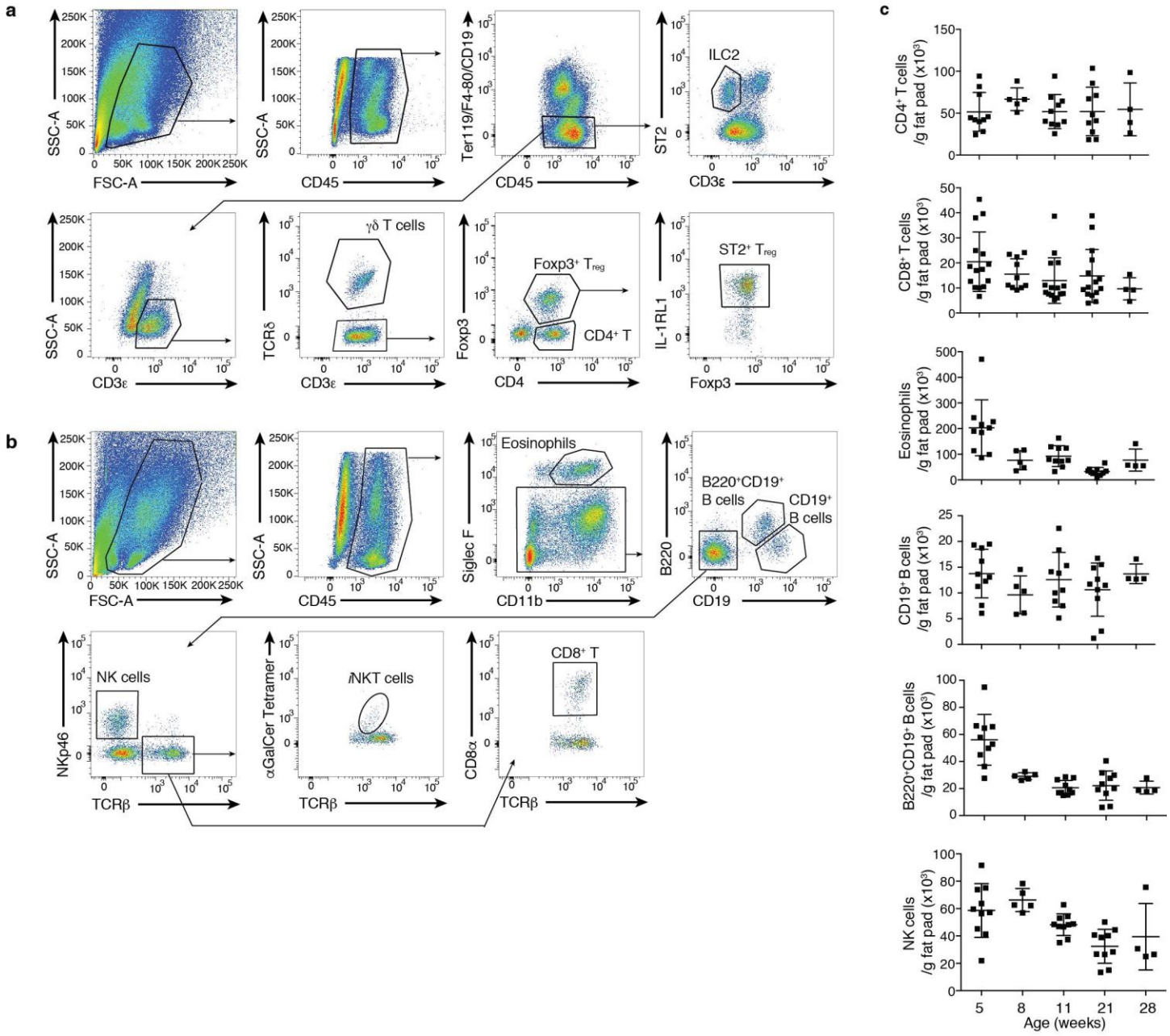
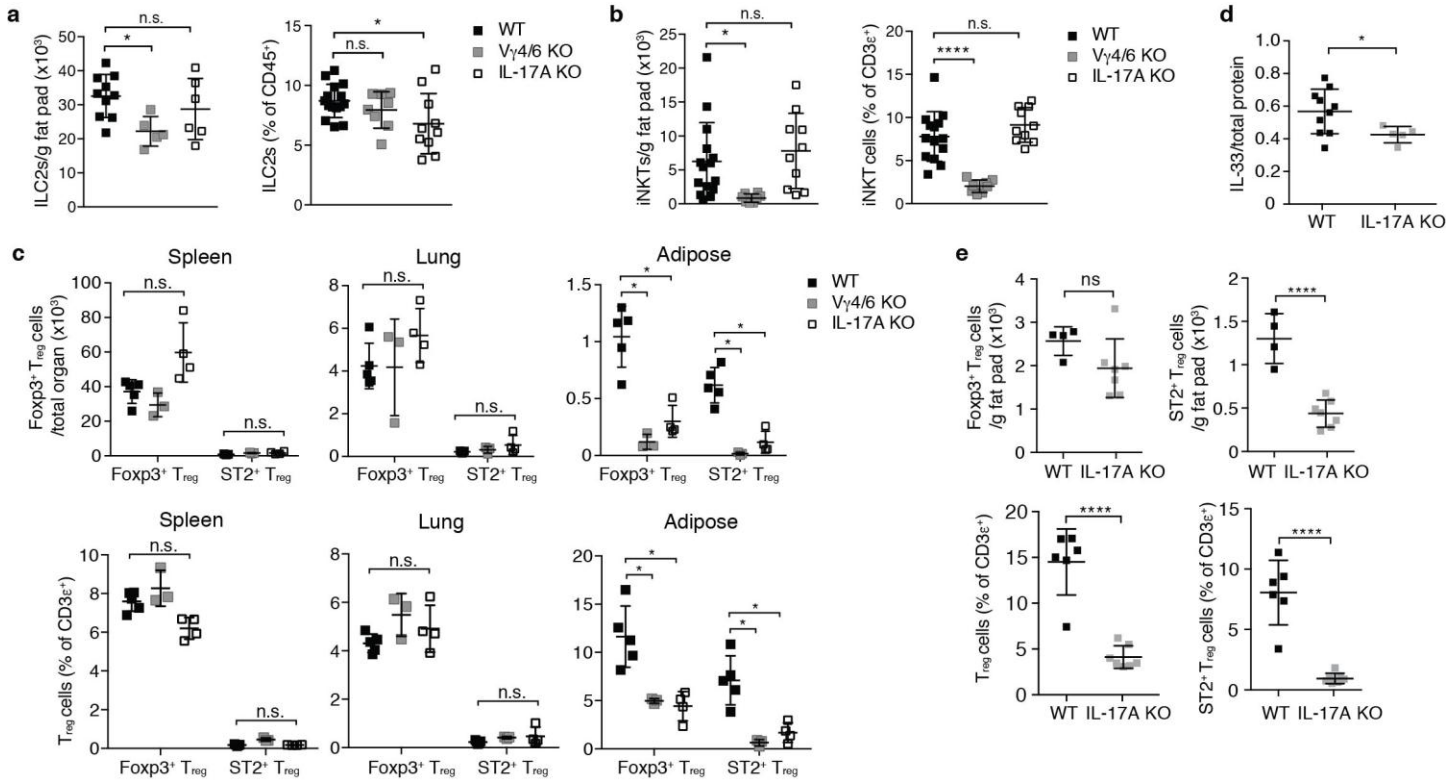


Supplementary Figure 1

Immunophenotyping panels for adipose immune-cell quantification

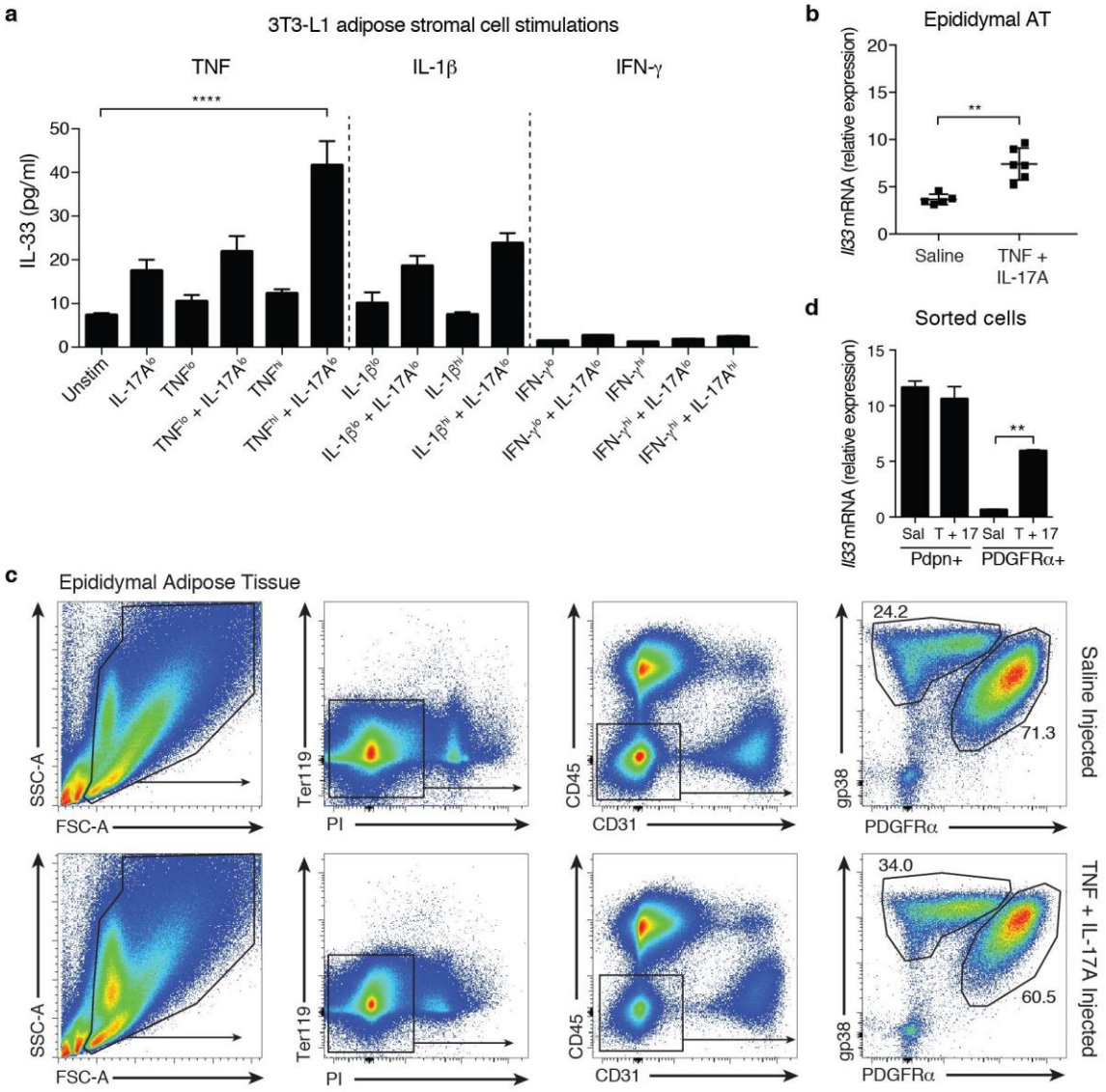
(**a**) Representative flow cytometry plots to identify ILC2s, γδ T, $CD4^+$ T, $Foxp3^+$ T_{reg}, and $ST2^+Foxp3^+$ T_{reg} cells. (**b**) Representative flow cytometry plots to identify eosinophils, $B220^+CD19^+$ B, $CD19^+$ B, NK, *i*NKT, and $CD8^+$ T cells. (**c**) Numbers of $CD4^+$ T, $CD8^+$ 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 ± s.e.m. in **c**).



Supplementary Figure 2

ILC2, iNKT, and T_{reg} numbers in IL-17A-knockout and Vγ4/6-knockout mice

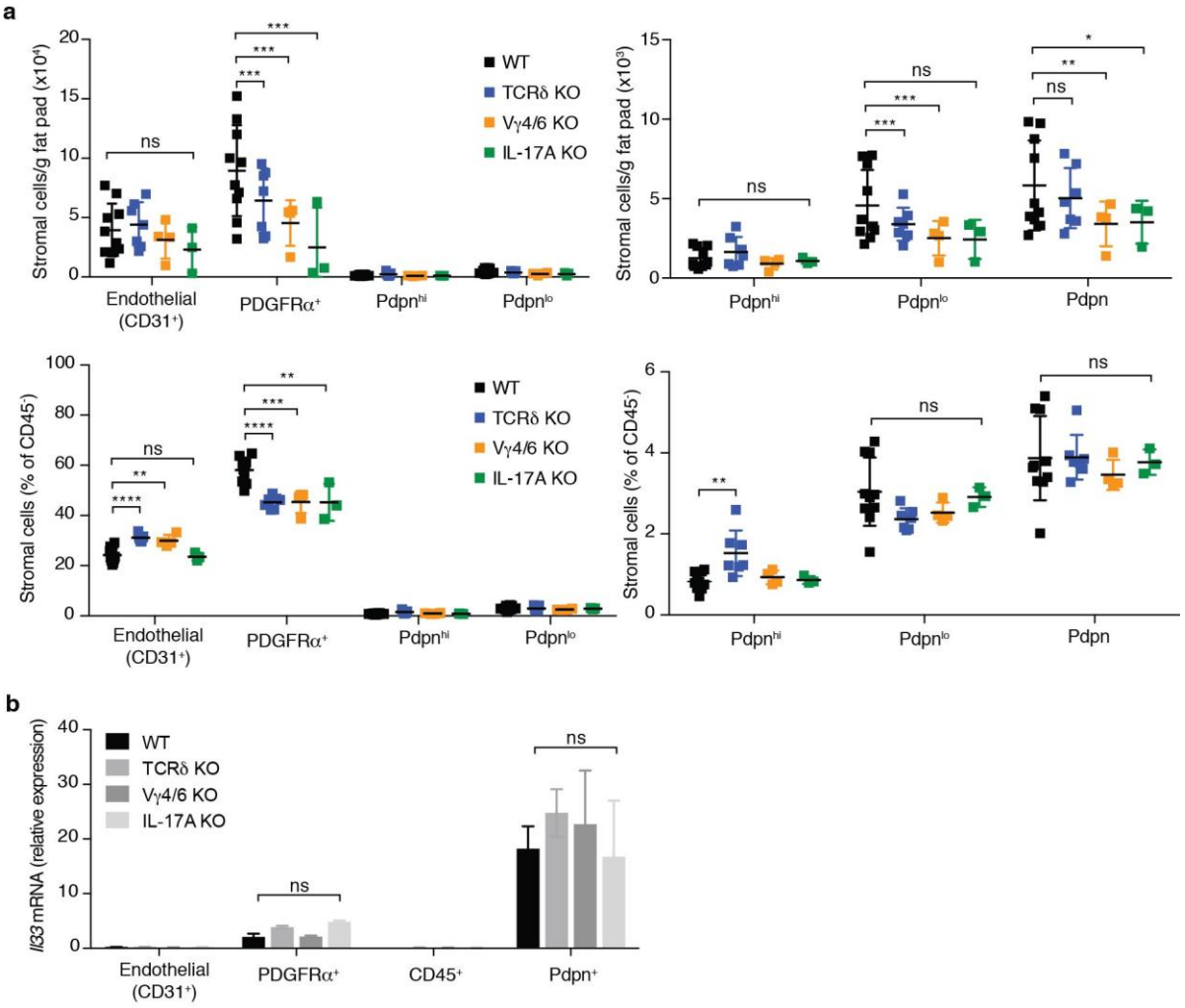
(**a**) Numbers (left) and frequency (right) of ILC2s in eWAT from WT, *Vg4/6*$^{-/-}$ and *Il17a*$^{-/-}$ 16 wk old mice (n = 5, pooled). (**b**) Numbers (left) and frequency (right) of *i*NKTs in eWAT from WT, *Vg4/6*$^{-/-}$ and *Il17a*$^{-/-}$ 16 wk old mice (n = 5, pooled). (**c**) Quantification of numbers (top) and frequencies (bottom) of T_{reg} cells and $ST2^+$ T_{reg} cells from spleen, lung, and adipose tissue from WT, *Vg4/6*$^{-/-}$ 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 ± s.e.m. in **a-e**).



Supplementary Figure 3

In vitro and in vivo cytokine stimulations of epididymal adipose stromal cells

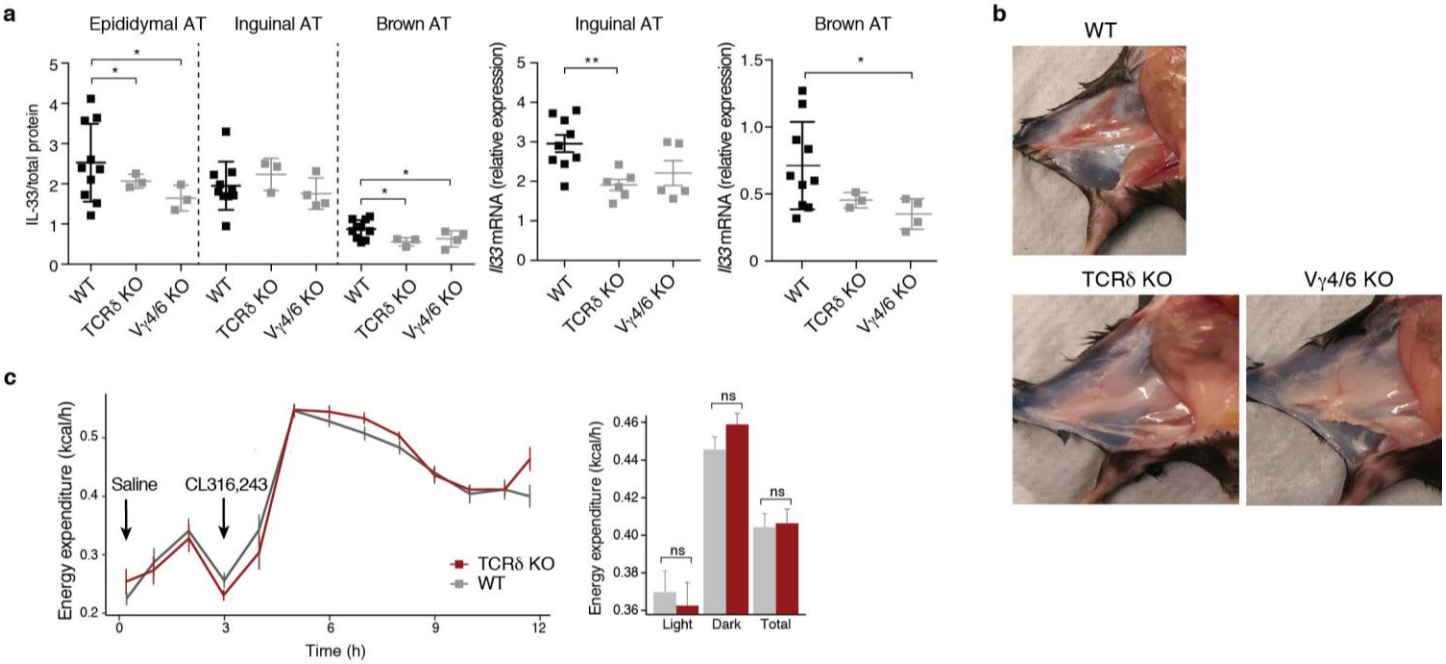
(**a**) 3T3L1 adipose fibroblasts were unstimulated (unstim) or stimulated with TNF^{lo} (0.1ng/mL), TNF^{hi} (1ng/mL), IL-17A^{lo} (0.1ng/mL), IL-17A^{hi} (1ng/mL), IL-1β^{lo} (0.1ng/mL), IL-1β^{hi} (1ng/mL), IFN-γ^{lo} (0.1ng/mL), IFN-γ^{hi} (1ng/mL), or a combination of the cytokines as indicated for 18h. IL-33 protein was measured by ELISA. (**b**) WT mice were injected with saline or TNF (1 μg) and IL-17A (0.5 μg) 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 μg) and IL-17A (0.5 μg) 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 ± s.e.m. in **a**). Data are representative of two experiments (**b-d**; mean ± s.e.m. in **b,d**).



Supplementary Figure 4

Decreased numbers, and not gene expression, probably contribute to lower IL-33 protein

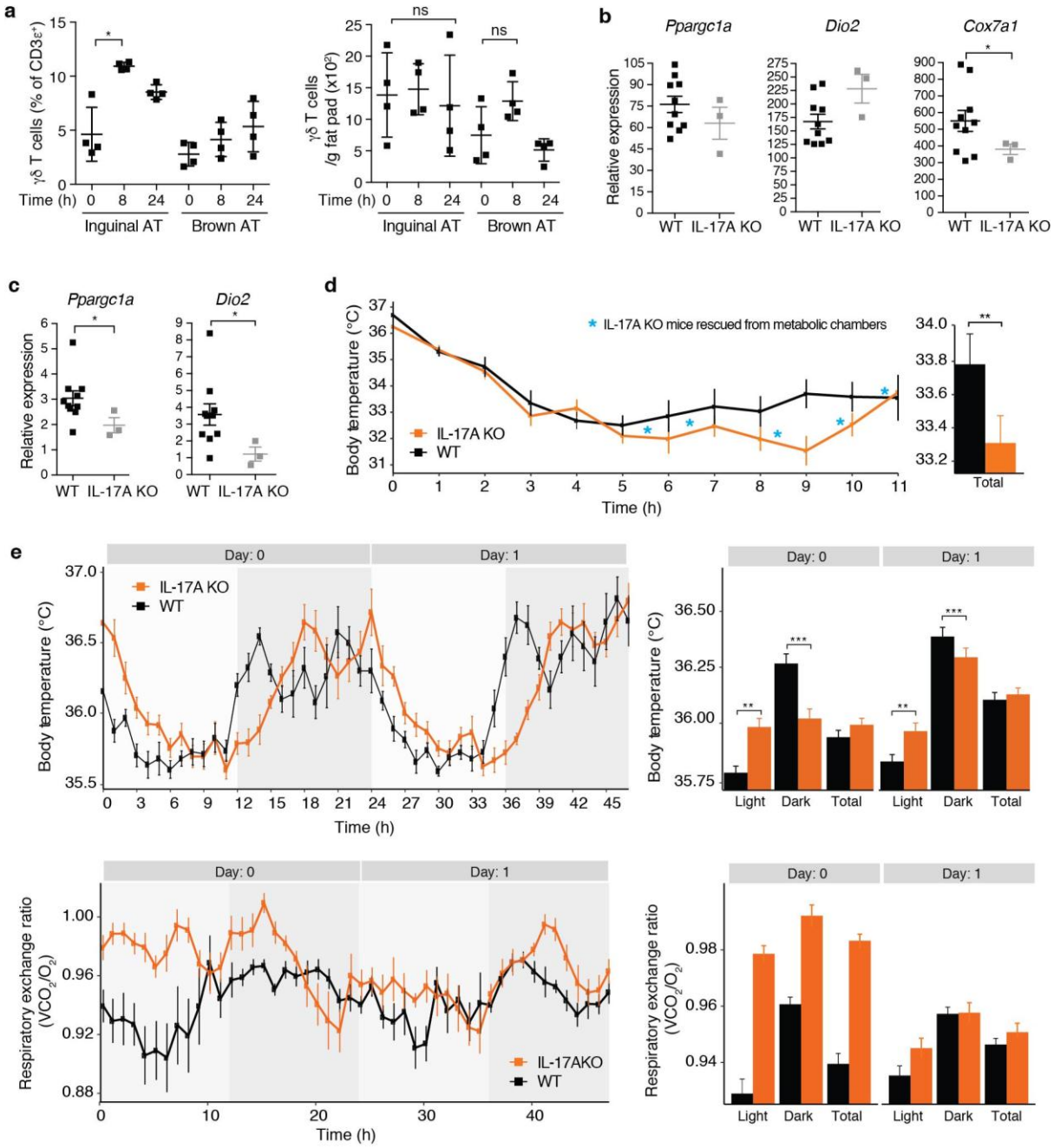
(**a**) Quantification of numbers (top) and frequencies (bottom) of CD31$^+$, PDGFRα$^+$Pdpn$^-$, Pdpn^{hi}, and Pdpn^{lo} eWAT stromal cells from 23 wk old WT, *Tcrd*$^{-/-}$, *Vg4/6*$^{-/-}$, and *Il17a*$^{-/-}$ male mice ($n \geq 3$ mice per genotype). (**b**) Quantitative real-time PCR for *Il33* expression normalized with *Tbp* from sorted Pdpn^{hi}, PDGFRα$^+$, 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 ± s.e.m. in **a-b**).



Supplementary Figure 5

γδ T cells promote temperature regulation and IL-33 homeostasis in BAT and iWAT

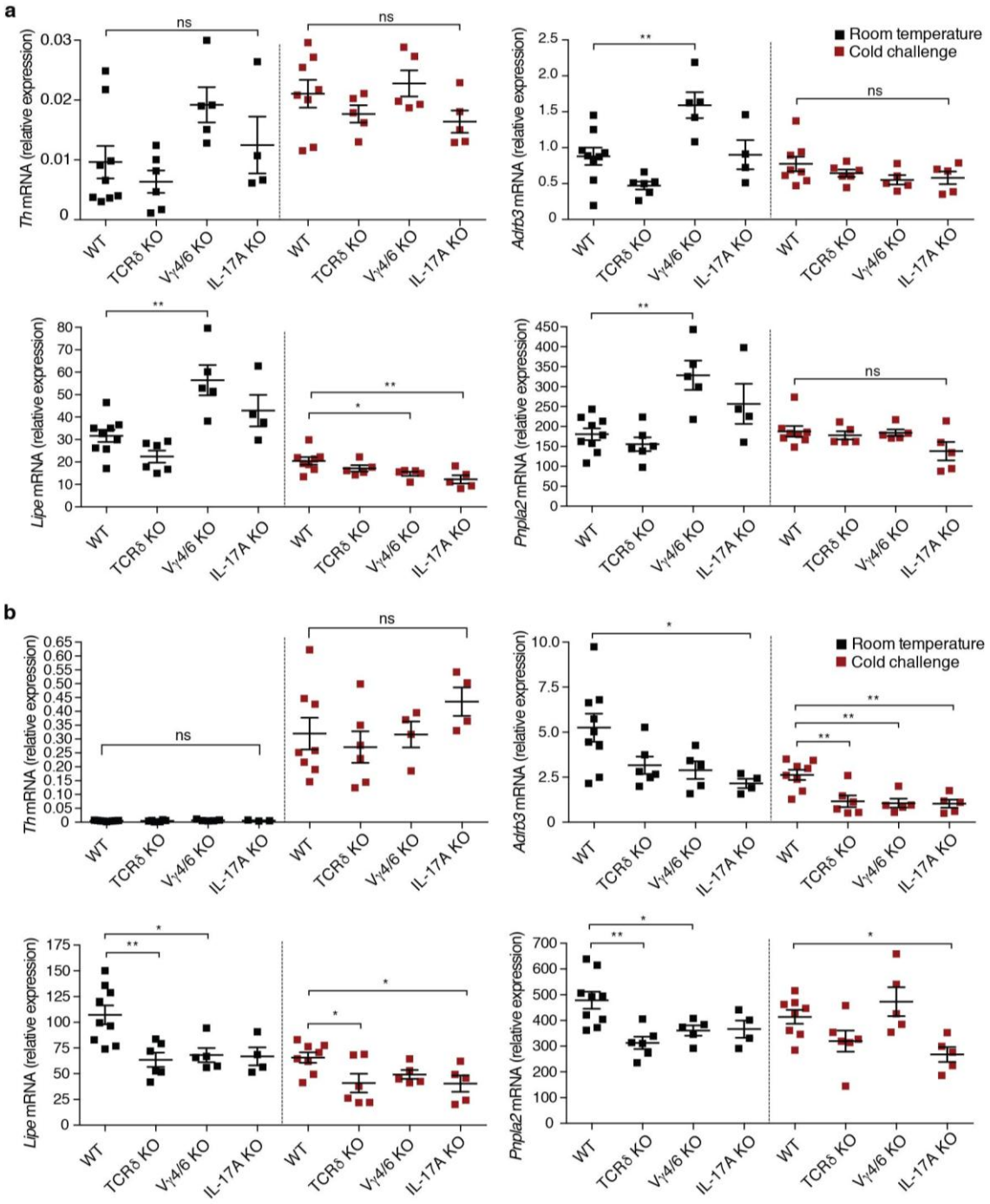
(**a**) IL-33 protein was quantified from cell lysates of eWAT, iWAT, and BAT from WT, *Tcrd*$^{-/-}$ and *Vg4/6*$^{-/-}$ mice using ELISA (left). Quantitative real-time PCR for *Il33* expression normalized with *Tbp* (right) from iWAT and BAT of WT, *Tcrd*$^{-/-}$ and *Vg4/6*$^{-/-}$ mice ($n \geq 4$). (**b**) Representative gross anatomy of iWAT from 22 wk old WT, *Tcrd*$^{-/-}$ and *Vg4/6*$^{-/-}$ mice after 6 h at 4 °C. (**c**) Energy expenditure measured from WT and *Tcrd*$^{-/-}$ mice injected with sterile saline at time 0 h and subsequently injected with selective β3-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 **c**). Data are representative of two experiments (**a,b**; mean ± s.e.m. in **a**) or one experiment (**c**; mean ± s.e.m. in **c**).



Supplementary Figure 6

IL-17A promotes thermogenic responses in BAT and iWAT

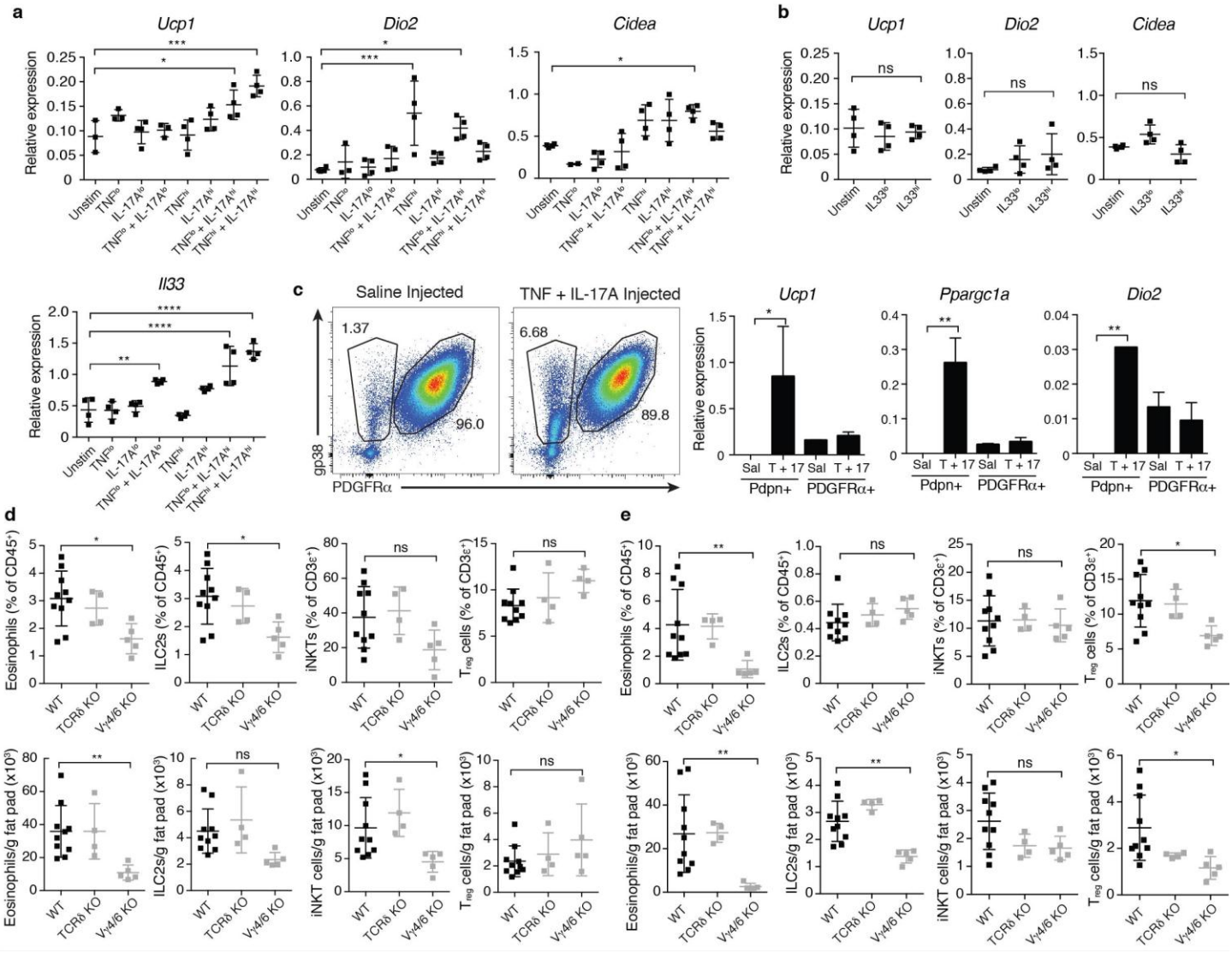
(**a**) Frequency (left) and numbers (right) of γδ T cells at 0, 8, and 24 h at 4 °C in BAT and iWAT ($n \geq 3$ mice per condition). (**b**) Quantitative real-time PCR of *Ppargc1a, Dio2,* and *Cox7a1* normalized to *Tbp* in BAT between WT and *Il17a*$^{-/-}$ mice ($n \geq 3$). (**c**) Quantitative 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 ± s.e.m. in **a-c**).



Supplementary Figure 7

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* ≥ 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 ± s.e.m. in **a,b**).



Supplementary Figure 8

γδ 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), $IL\text{-}17A^{lo}$ (0.1ng/mL), $IL\text{-}17A^{hi}$ (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 $IL\text{-}33^{lo}$ (10ng/mL), $IL\text{-}33^{hi}$ (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 μg) and IL-17A (0.5 μg) every third day for a total of nine days. $Pdpn^{+}PDGFR\alpha^{-}$ and $PDGFR\alpha^{+}$ iWAT stromal cells were sorted and gene expression of *Ucp1, Ppargc1a,* 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 ± 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**).