

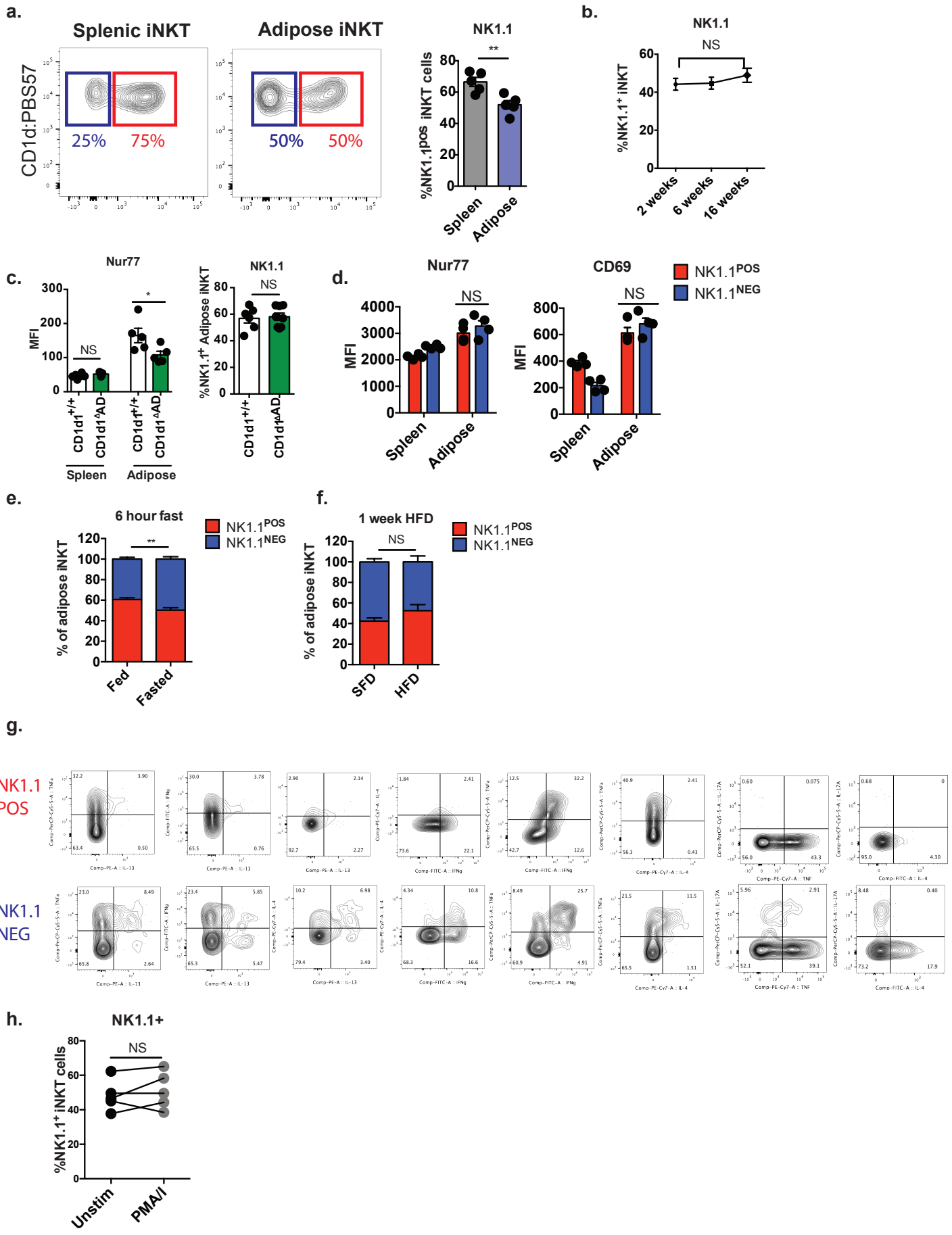
**Figure S1, related to Figure 2. Supplemental figures for scRNAseq analysis.**

(a) UMAP of scRNAseq data from iNKT cells from spleen (grey) and adipose tissue (purple) of 8 week old male C57BL/6 mice.

(b) Normalized expression of genes characteristic of adipose iNKT cells on UMAP shown in (a).

(c) Percentage of BrdU+ iNKT cells in indicated organs of mice after six days of BrdU injections ( $n = 5$  mice).

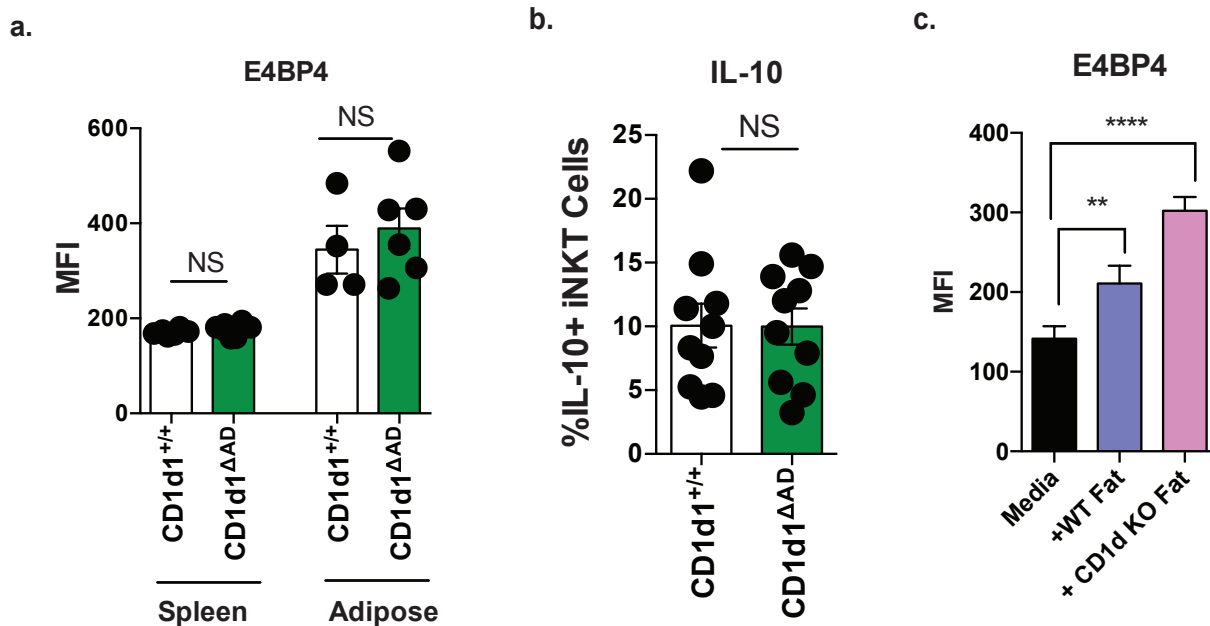
Error bars indicate mean ( $\pm$  S.E.M.). NS, not significant ( $P > 0.05$ ); \*\*\*\* $P < 0.0001$ . ANOVA with *post hoc* Tukey test (c).



**Figure S2, related to Figure 2. Characterization of adipose iNKT cells by NK1.1 expression.**

- (a) Protein expression of NK1.1 in splenic and adipose iNKT cells ( $n = 5$  mice).
- (b) Percentage of NK1.1<sup>POS</sup> iNKT cells in adipose tissue of mice at the indicated ages ( $n = 5$  mice per group).
- (c) Expression of Nur77 and NK1.1 in iNKT cells from *CD1d1*<sup>+/+</sup> and *CD1d1*<sup>ΔΔ</sup> mice ( $n = 5$  mice per group).
- (d) Expression of CD69 and Nur77 in NK1.1<sup>POS</sup> and NK1.1<sup>NEG</sup> iNKT cells from spleen and adipose tissue.
- (e) Percentage of NK1.1<sup>POS</sup> and NK1.1<sup>NEG</sup> adipose iNKT cells at steady state and after six hours of fasting ( $n = 9$  mice per group).
- (f) Percentage of NK1.1<sup>POS</sup> and NK1.1<sup>NEG</sup> adipose iNKT cells after 1 week SFD or HFD ( $n = 5$  mice per group).
- (g) Biaxial flow cytometry plots of sorted iNKT cell populations stimulated with PMA/I.
- (h) Expression of NK1.1 in iNKT cells from paired samples of SVF left unstimulated or stimulated with PMA/I for 4 hours ( $n = 5$  paired samples).

Error bars indicate mean ( $\pm$  S.E.M.). NS, not significant ( $P > 0.05$ ); \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; \*\*\*\* $P < 0.0001$ . Two-tailed unpaired (a-f) or paired (h) Student's T test. Data representative of two or more independent experiments.



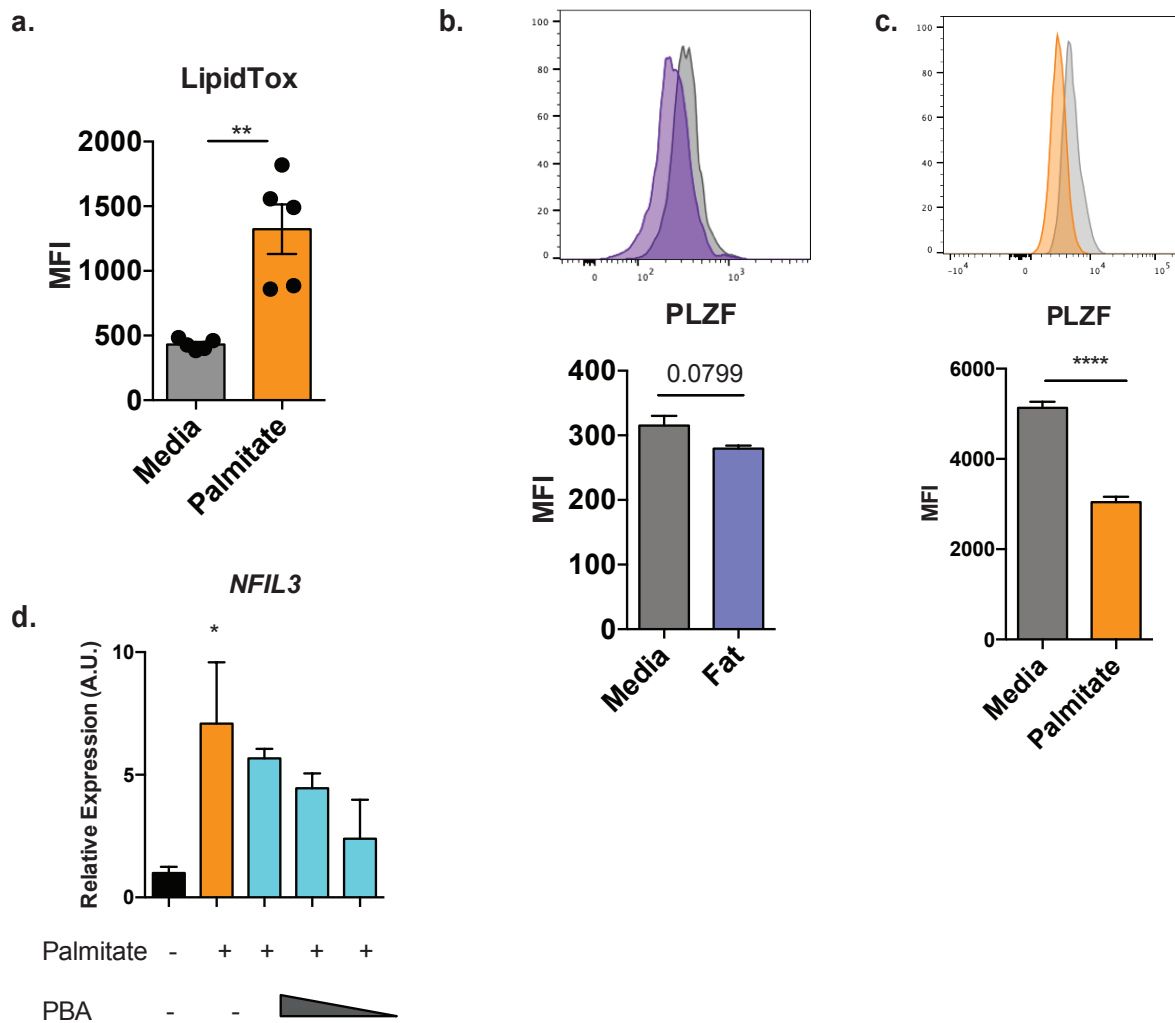
**Figure S3, related to Figure 3. E4BP4 expression and IL-10 production in adipose iNKT cells do not rely on CD1d expression.**

(a) Expression of E4BP4 in iNKT cells from indicated organs of CD1d<sup>+/+</sup> and CD1d<sup>ΔAD</sup> mice. ( $n = 5$  mice/group)

(b) Percentage of IL-10<sup>+</sup> adipose iNKT cells from CD1d<sup>+/+</sup> and CD1d<sup>ΔAD</sup> mice stimulated with PMA/I ( $n = 8-10$  mice per group).

(c) Expression of E4BP4 in splenic iNKT cells cultured overnight in media or in the presence of WT or CD1d KO fat ( $n = 3-4$  technical replicates per group).

Error bars indicate mean ( $\pm$  S.E.M.). NS, not significant ( $P > 0.05$ ); \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; \*\*\*\* $P < 0.0001$ . Two tailed Student's T test (a,b) or ANOVA with *post hoc* Tukey's test (c). Data representative of (a, c) or combined from (b) two independent experiments.



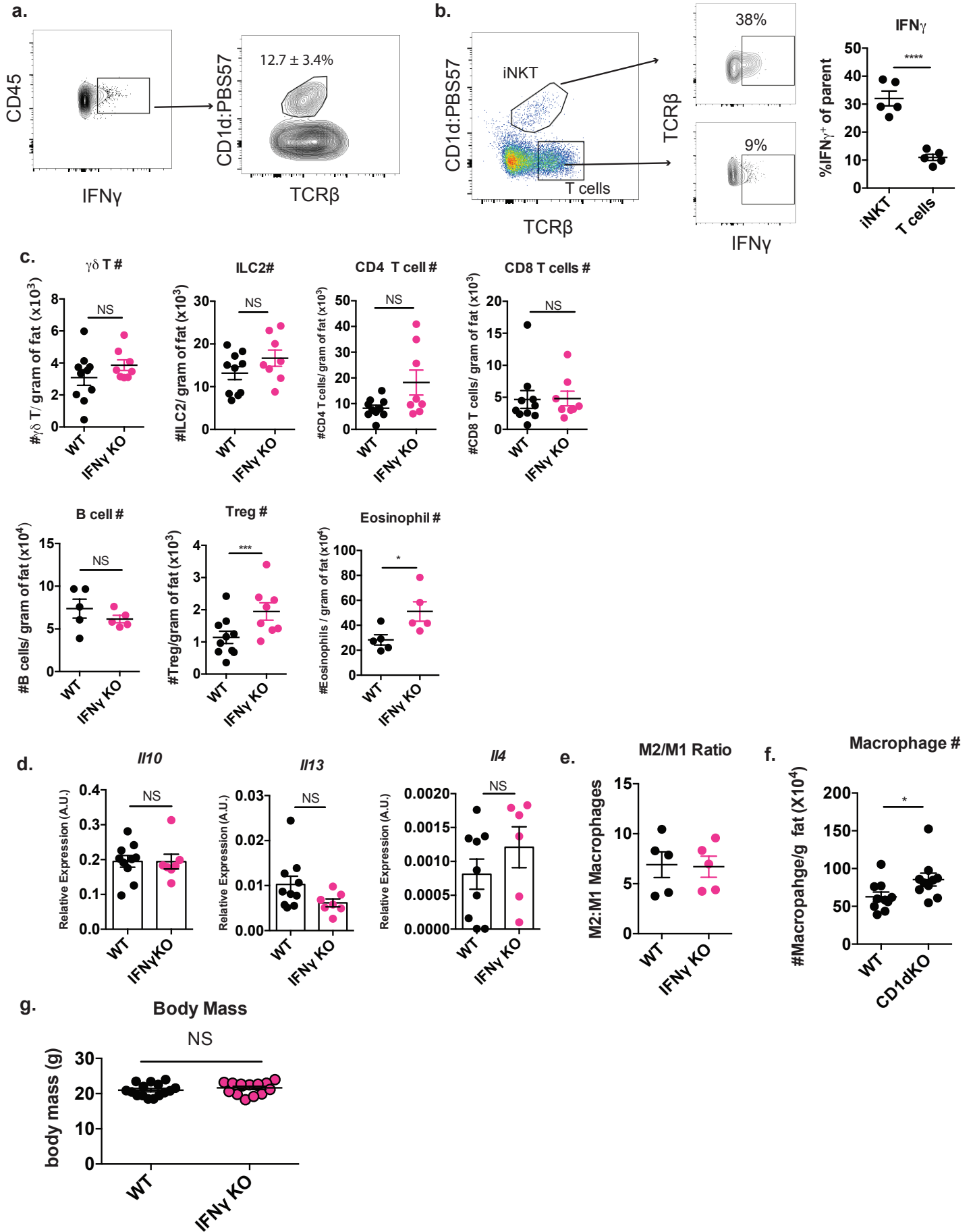
**Figure S4, related to Figure 4. Palmitate drives E4BP4 upregulation and PLZF downregulation in splenic iNKT cells.**

(a) Flow cytometry histograms of Lipidtox staining in splenic versus adipose iNKT cells ( $n = 5$  mice).

(b-c) Expression of PLZF in splenic iNKT cells cultured in control media or in the presence of whole adipose tissue (b) or palmitate (c) ( $n = 4$  technical replicates per group).

(d). *Nfil3* expression in iNKT cells cultured in media, palmitate, or palmitate with increasing concentrations of PBA ( $n = 4$  technical replicates per group).

Error bars indicate mean ( $\pm$  S.E.M.). All data representative of two independent experiments. NS, not significant ( $P > 0.05$ );  $*P < 0.05$ ;  $**P < 0.01$ . Two-tailed Student's t test (a-c) or one-way ANOVA with *post-hoc* Tukey's test (d).



**Figure S5, related to Figure 6. Immunophenotyping of IFN $\gamma$  KO mice.**

(a) Proportion of iNKT cells of IFN $\gamma$ -positive cells after PMA/I stimulation ( $n = 5$  mice).

(b) IFN $\gamma$  production by iNKT cells and tetramer negative T cells from SVF stimulated with PMA/I ( $n = 5$  mice per group).

(c) Quantifications of indicated immune cells in adipose tissue of WT versus IFN $\gamma$  KO mice ( $n = 5$ -10 mice per group).

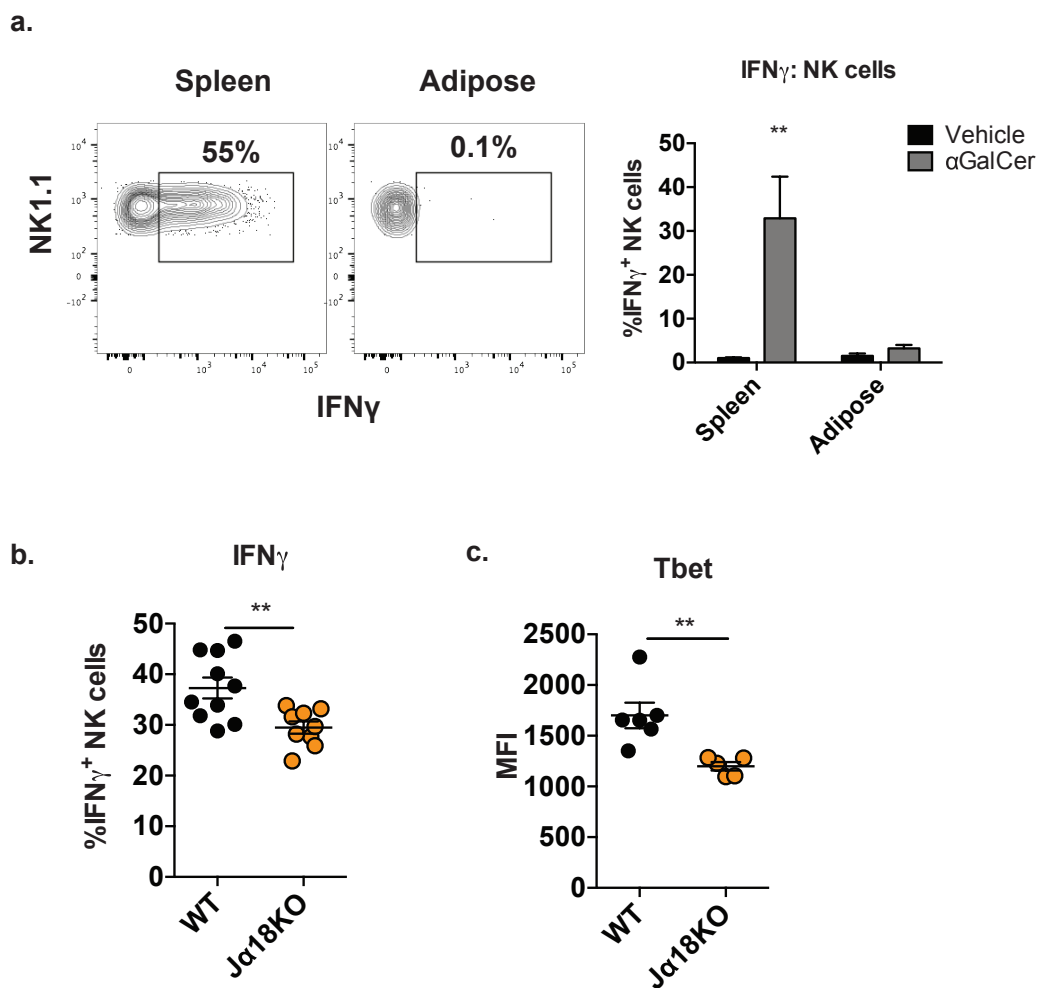
(d) Relative mRNA expression of indicated cytokines in adipose tissue of WT versus IFN $\gamma$  KO mice ( $n = 6 - 10$  mice per group).

(e) Ratio of M2 (CD301<sup>+</sup>) to M1 (CD11c<sup>+</sup>) adipose tissue macrophages in lean WT and IFN $\gamma$ KO mice ( $n = 5$  mice per group).

(f) Number of adipose tissue macrophages in WT versus CD1dKO mice ( $n = 10$  mice per group).

(g) Body mass of lean WT and IFN $\gamma$  KO mice ( $n = 15$  mice per group).

Error bars indicate mean ( $\pm$  S.E.M.). Data representative of (a-e) or combined from (f), two independent experiments or combined from three experiments (g). NS, not significant ( $P > 0.05$ ); \* $P < 0.05$ ; \*\* $P < 0.01$ . Two-tailed Student's t test.



**Figure S6, related to Figure 7. iNKT cells regulate adipose NK cell phenotype.**

(a) Percentage of IFN $\gamma$ <sup>+</sup> NK cells in indicated organs from mice I.P. injected with  $\alpha$ GalCer four hours prior ( $n = 5$  mice per group).

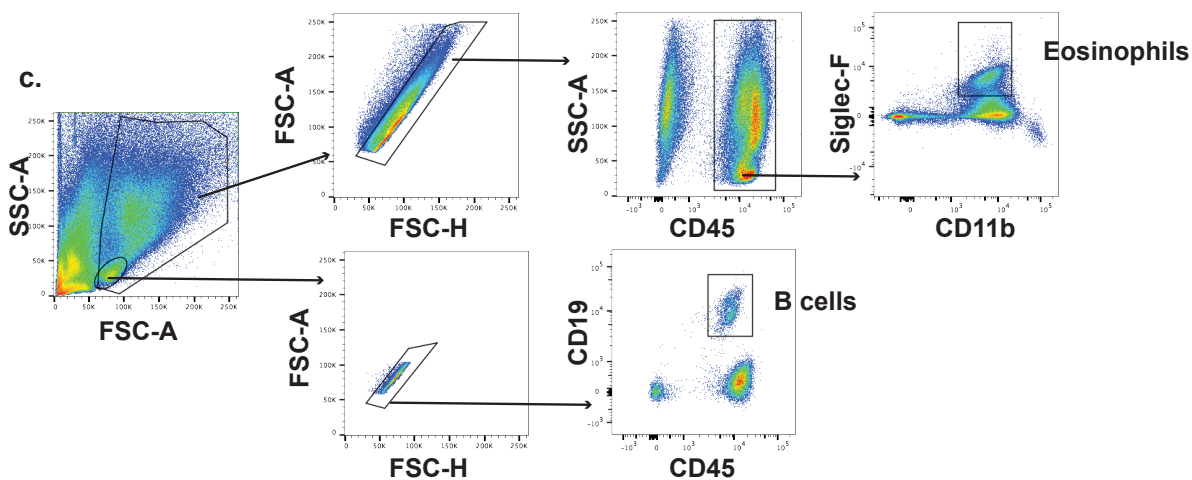
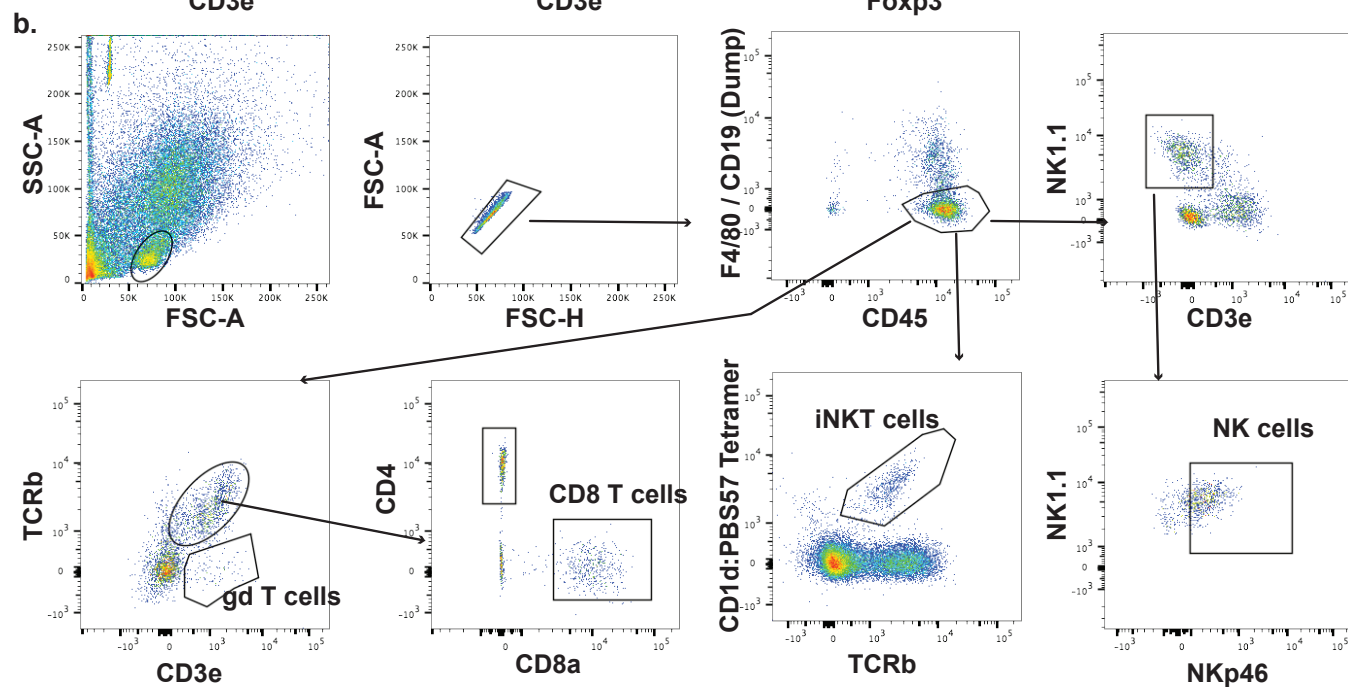
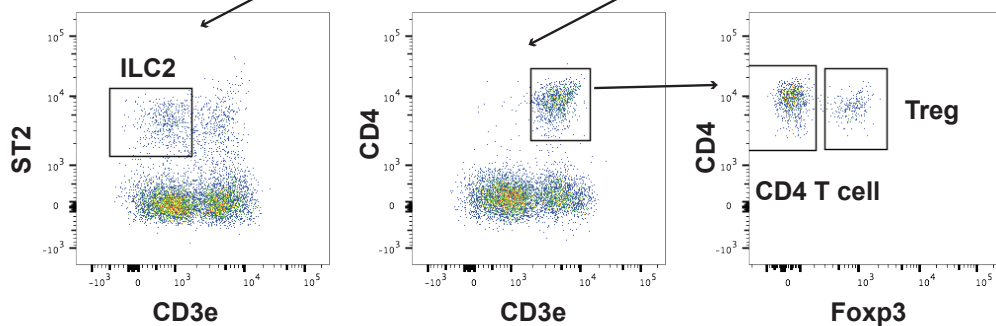
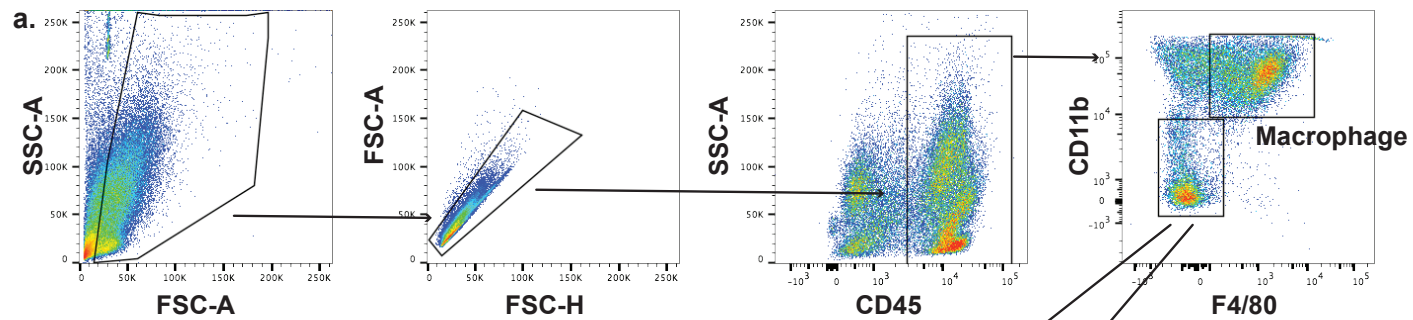
(b) Percentage of IFN $\gamma$ <sup>+</sup> adipose NK cells from WT and Ja18KO mice after PMA/I stimulation ( $n = 9-10$  mice per group).

(c) Expression of Tbet in adipose NK cells from WT and Ja18KO mice ( $n = 5-6$  mice per group).

Error bars indicate mean ( $\pm$  S.E.M.). Representative of (a) or combined from (b) two independent experiments or one experiment (c).

\*\* $P < 0.01$ . Two-tailed Student's  $t$  test.





**Figure S7, related to STAR Methods. Gating Strategies.**

- (a) Representative flow cytometry plots to identify macrophages, ILC2s, CD4<sup>+</sup> T cells, and Tregs.
- (b) Representative flow cytometry plots to identify  $\gamma\delta$  T cells, iNKT cells, NK cells, and CD8<sup>+</sup> T cells.
- (c) Representative flow cytometry plots to identify eosinophils and B cells.

**Table S3, related to STAR Methods. qPCR Primers.**

<b>Gene</b>	<b>Forward Primer (5'-3')</b>	<b>Reverse Primer (5'-3')</b>
<i>18s</i>	GTAACCCGTTGAACCCATT	CCATCCAATCGGTAGTAGCG
<i>Nfil3</i>	CAGTGCAGGTGACGAACATT	TTCCACCACACCTGTTTTGA
<i>Il10</i>	AATAAGCTCCAAGACCAAGG	CAGACTCAATACACACTG
<i>XPB1 total</i>	GACAGAGAGTCAAACCTAACGTGG	GTCCAGCAGGCAAGAAGGT
<i>XPB1s</i>	AAGAACACGCTTGGGAATGG	CTGCACCTGCTGCGGAC
<i>Hprt</i>	CTGGTGAAAAGGACCTCTCGAA	CCAGTTTCACTAATGACACAAA
<i>Nos2</i>	GAGACAGGGAAGTCTGAAGCAC	CCAGCAGTAGTTGCTCCTCTTC
<i>Il6</i>	TACCACTTCACAAGTCGGAGGC	CTGCAAGTGCATCATCGTTGTT
<i>Arg1</i>	CATTGGCTTGCGAGACGTAGAC	GCTGAAGGTCTCTTCCATCACC
<i>Il13</i>	AGCATGGTATGGAGTGTGGAC	CAATTGGAGATGTTGGTCAGGG
<i>Il4</i>	ATCATCGGCATTTTGAACGAGGTC	ACCTTGGAAGCCCTACAGACGA