***eLife’s* transparent reporting form**

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**Sample-size estimation**

* You should state whether an appropriate sample size was computed when the study was being designed
* You should state the statistical method of sample size computation and any required assumptions
* If no explicit power analysis was used, you should describe how you decided what sample (replicate) size (number) to use

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn’t apply to your submission:

**Replicates**

Required sample sizes for the number of mice needed to generate single cell RNA-Seq (scRNA-Seq) and flow cytometry data for splenic and adipose iNKT cells were determined empirically, using data previously published in:

LaMarche, N.M., Kane, H., Kohlgruber, A.C., Dong, H., Lynch, L. and Brenner, M.B., 2020. Distinct iNKT cell populations use IFNγ or ER stress-induced IL-10 to control adipose tissue homeostasis. *Cell Metabolism*, *32*(2), pp.243-258.

Lynch, L., Michelet, X., Zhang, S., Brennan, P.J., Moseman, A., Lester, C., Besra, G., Vomhof-Dekrey, E.E., Tighe, M., Koay, H.F. and Godfrey, D.I., 2015. Regulatory i NKT cells lack expression of the transcription factor PLZF and control the homeostasis of T reg cells and macrophages in adipose tissue. *Nature immunology*, *16*(1), pp.85-95.

* You should report how often each experiment was performed
* You should include a definition of biological versus technical replication
* The data obtained should be provided and sufficient information should be provided to indicate the number of independent biological and/or technical replicates
* If you encountered any outliers, you should describe how these were handled
* Criteria for exclusion/inclusion of data should be clearly stated
* High-throughput sequence data should be uploaded before submission, with a private link for reviewers provided (these are available from both GEO and ArrayExpress)

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn’t apply to your submission:

**Statistical reporting**

Biological and technical replicate information for flow cytometry experiments is provided in the associated figure legends and the Methods section. A single replicate of hepatic iNKT cells at 4 weeks post-αGalCer was excluded due to poor staining and inability to properly identify iNKT cells. This is described in the appropriate figure legend (Figure 6). Gating was performed as described in the figure legends and Methods section.

Biological replicate information for scRNA-Seq experiments is provided in the Methods section. scRNA-Seq experiments were performed once, using multiple biological replicates in the same experiment. Data was processed as described in the Methods section. scRNA-Seq data batch information is provided in Supplementary File 9. Raw and processed scRNA-Seq data has been deposited in GSE190201, the following reviewer token grants access: wlwpkycuxdshbkb.

Other High -Throughput data analyzed in the manuscript is publically available on GEO, and reanalysis of this data is described in the Methods section.

* Statistical analysis methods should be described and justified
* Raw data should be presented in figures whenever informative to do so (typically when N per group is less than 10)
* For each experiment, you should identify the statistical tests used, exact values of N, definitions of center, methods of multiple test correction, and dispersion and precision measures (e.g., mean, median, SD, SEM, confidence intervals; and, for the major substantive results, a measure of effect size (e.g., Pearson's r, Cohen's d)
* Report exact p-values wherever possible alongside the summary statistics and 95% confidence intervals. These should be reported for all key questions and not only when the p-value is less than 0.05.

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn’t apply to your submission:

Statistical methods for flow cytometry data are described in the figure legends and the Methods section.

Statistical methods for scRNA-Seq data are described in the Methods section.

Where referenced in the Results section, Supplementary Files are provided with fold change, percentage expression and adjusted p-values for gene expression analysis.

(For large datasets, or papers with a very large number of statistical tests, you may upload a single table file with tests, Ns, etc., with reference to sections in the manuscript.)

**Group allocation**

* Indicate how samples were allocated into experimental groups (in the case of clinical studies, please specify allocation to treatment method); if randomization was used, please also state if restricted randomization was applied
* Indicate if masking was used during group allocation, data collection and/or data analysis

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn’t apply to your submission:

**Additional data files (“source data”)**

No data masking was used in this study. scRNA-Seq batch information and the availability of samples on GEO is detailed in Supplementary File 9.

We encourage you to upload relevant additional data files, such as numerical data that are represented as a graph in a figure, or as a summary table

* Where provided, these should be in the most useful format, and they can be uploaded as “Source data” files linked to a main figure or table
* Include model definition files including the full list of parameters used
* Include code used for data analysis (e.g., R, MatLab)
* Avoid stating that data files are “available upon request”

Please indicate the figures or tables for which source data files have been provided:

Source data files have been provided for Figure 2, Figure 3, Figure 5 and Figure 7.

Supplementary files have been provided for Figure 1, Figure 2, Figure 3, Figure 4, Figure 7, Supplemental Figures, and the Methods section. A legend for these files has been included in the manuscript.

The R packages, software and parameters used for data analysis are exaplained in the Methods section.