Figures and figure supplements

Single cell analysis reveals immune cell–adipocyte crosstalk regulating the transcription of thermogenic adipocytes

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Figure 1. Ablation of IL10Rα in adipocytes increases energy expenditure and protects mice from diet-induced obesity. (A) Immunoblot analysis of IL10Rα expression in SVF and adipocyte fractions of iWAT from chow-fed 10 week-old mice. (B) Oxygen consumption (VO₂, ml/hr), energy expenditure
Figure 1 continued

(EE, kCal/hr), and carbon dioxide production (VCO2, ml/hr) of chow-fed 10-week-old IL10Rα/F and AdIL10Rα KO mice were analyzed in Columbus Oxymax metabolic chambers (12 hr light/dark cycles, 72 hr total duration, each light/dark bar represents 12 hr duration). N = 9,7. Statistical analysis was performed using ANCOVA. (C) External appearance of representative 9 week HFD-fed mice. (D) Body weight of mice fed chow diet for 10 weeks and then 60% high-fat diet (HFD) for 9 weeks. N = 13, 7. (E) Fat and lean mass of mice in D fed HFD for 6 weeks. (F) Internal and gross appearance of iWAT from representative IL10Rα/F and AdIL10RαKO mice. *, p<0.05.

DOI: https://doi.org/10.7554/eLife.49501.002
Figure 1—figure supplement 1. AdIL10Rα KO mice are protected against diet-induced obesity. (A) Body mass and composition of 10 week-old chow-fed mice. (B) Food intake of chow-fed mice. (C) RER, locomotion and ambulatory activity of chow-fed 10-week-old IL10Rα F/F and AdIL10Rα KO mice.
analyzed by Columbus Oxymax metabolic chambers (12 hr light/dark cycles, 72 hr total duration, each light/dark bar represents 12 hr duration; N = 9,7).

(D) Body weight of mice fed chow diet for 10 weeks and then 60% high-fat diet (HFD) for 9 weeks. N = 7,5.

DOI: https://doi.org/10.7554/eLife.49501.003
Figure 2. IL10R deficiency promotes adipose tissue browning. (A and B) Real-time PCR analysis of gene expression in iWAT from 10 week 24 hr cold-exposed (A) or CL 1 mg/kg/day for 4 days; B) IL10Rα/F/F and AdIL10RαKO mice. N = 5, 5. *, p<0.05; **, p<0.01; ***, p<0.0001. (C) Heatmap Figure 2 continued on next page
Figure 2 continued

representation of genes that changed >1.5 fold (p-value<0.01) as a function of percentile expression by RNA-Seq of iWAT from 10 week-old 24 hr cold-
exposed IL10Rα and AdIL10Rα KO mice. Genes are grouped as upregulated (Red) or downregulated (Blue). 

(D) Average oxygen consumption rate (OCR) in coupling (left) and electron flow (right) assays of mitochondria isolated from iWAT of mice in (A).

DOI: https://doi.org/10.7554/eLife.49501.004
Figure 3. SNAP-seq reveals heterogeneity of tissue adipocytes from iWAT. (A) Workflow showing DAPI-stained nuclei pre- and post-FACS/filtration that underwent microfluidic partitioning and library preparation in the 10X genomics platform followed by sequencing using an Illumina HiSeq 4000. (B) Figure 3 continued on next page.
Figure 3 continued

tSNE-plot showing 14 clusters from ~6000 adipocytes derived from iWAT of mice exposed to cold for 24 hr. Each colored dot is an adipocyte assigned to a cluster based on transcriptomic signature. (C) Normalized expression values of the top two adipocyte subtype-specific cluster genes from (B) plotted as violin plots with clusters as rows and genes as columns. (D) tSNE-plot showing cluster-specific expression of selected marker genes from (C). (E) Normalized expression values of indicated genes in subtype-specific clusters plotted as violin plots with clusters as rows and genes as columns.

Black arrow is pointing toward metabolically active Type nine adipocyte cluster and enriched gene.

DOI: https://doi.org/10.7554/eLife.49501.005
Figure 3—figure supplement 1. Multimodal analysis of SVF and adipocyte single-cell sequencing data. Hierarchical plot showing correlation of single cell/nuclei gene expression between SVF and adipocyte clusters. Cell types from both mature adipocyte nuclei and SVF single cells were used in this analysis.
analysis. They were grouped using hierarchical clustering with tiles colored by unadjusted (top) -Log10 Bonferroni adjusted p-values (bottom). Unadjusted (top) and adjusted (bottom) p-values were thresholded to aid in visualization with values less than $10^{-5}$ set to $10^{-5}$.

DOI: https://doi.org/10.7554/eLife.49501.006
Figure 4. Transcriptomic visualization of thermogenic adipocytes in iWAT. (A) Aggregated tSNE-plot showing 17 clusters from ~54,000 adipocytes derived from iWAT of mice at RT, cold-exposed (24 hr, 48 hr, and 4 days), or treated with CL for 4 days at 1 mg/kg/day. Each colored dot is an

Figure 4 continued on next page
Figure 4 continued

adipocyte that falls into a cluster based on transcriptomic signature. (B) tSNE-plot showing indicated treatment-dependent sub-clustering of aggregated clusters shown in (A). Black arrow indicates the Type nine adipocyte cluster. (C) Normalized expression values of the top two adipocyte subtype-specific cluster genes from (A and B) plotted as violin plots with clusters as rows and genes as columns. (D) tSNE-plot showing distribution of indicated genes from adipocytes from (A). (E) Normalized expression values of indicated genes in the Type nine adipocyte cluster under different treatment condition plotted as violin plots with treatment conditions as rows and genes as columns. (F) RNAscope FISH (see Materials and methods) of indicated probes from iWAT of mice treated with saline or CL for 4 days at 1 mg/kg/day.

DOI: https://doi.org/10.7554/eLife.49501.007
**Figure 4—figure supplement 1.** Enrichment of cells involved in lipid mobilization in Cluster nine adipocytes. (A and B) Gene set pathways obtained from GO Biological Processes and KEGG were used to find the functions of the different cell types. The top five enriched pathways determined by FDR.
from each cell type cluster were selected (from Supplementary file 1) and only unique pathways were kept (some top enriched pathways were shared across cell type clusters). Enriched pathways (rows) were clustered with hierarchical clustering. The size of each dot represents the -log10 FDR of the pathway enrichment and the color of each dot corresponds to the fold enrichment of each pathway (red- higher enrichment, gray- lower enrichment). Dotted blue squares highlight pathways enriched in cluster 9.

DOI: https://doi.org/10.7554/eLife.49501.008
Figure 4—figure supplement 2. Type nine cluster constitute of thermogenic adipocytes. (A) Normalized expression values of top adipocyte subtype-specific major clusters (1, 4, and 9) genes from Figure 4A plotted as violin plots with clusters as rows and expression levels of genes as columns. (B) Figure 4—figure supplement 2 continued on next page
tSNE-plot showing cluster-specific expression of selected marker genes from aggregated tSNE-plot in Figure 4A. Black arrow indicates Type nine cluster. (C) Normalized expression values of indicated genes plotted as violin plots with clusters as rows and expression levels of genes as columns. DOI: https://doi.org/10.7554/eLife.49501.009
Figure 5. Enrichment of the thermogenic adipocyte cluster in IL10Rα-depleted mice. (A) Top: tSNE-plot showing percentage of clusters from aggregated (All) or from indicated treatments. Bottom: Colored dot plot showing percentage of fractions plotted in y-axis and cell types in x-axis under Figure 5 continued on next page.
Figure 5 continued

(B) tSNE-plot showing differences in percentage of clusters between adipocytes from iWAT from control and AdIL10Rα KO mice under RT, 24 hr, 48 hr or 4 days cold exposure, or CL treated conditions. (C) Colored dot plot showing percentage of fractions plotted in y-axis and cell types in x-axis under indicated conditions and genotype. N denotes Adipoq-Cre-Negative IL10RαF/F (IL10RαF/F) and P denotes Adipoq-Cre-Positive (AdIL10Rα KO) mice. (D) Volcano plot showing adipocyte gene expression differences between indicated mice from cluster 9. The Log2Fold Change (FC) ratio of floxed control vs. AdIL10Rα KO was plotted as a function of log10 p-value, with select genes indicated with text. Black arrow indicates the Type nine adipocyte cluster.

DOI: https://doi.org/10.7554/eLife.49501.010
Figure 5—figure supplement 1. Thermogenic pathway is enriched in the Type 9 cluster of IL10Rα-deficient adipocytes. (A) Heatmap showing fold enrichment of pathways abundant in the adipocytes of AdIL10Rα KO mice compared to controls. DEG analysis of N represent Adipq-CRE Negative.

Figure 5—figure supplement 1 continued on next page.

A

- *mitochondrial electron transport, cytochrome c to oxygen (GO:0006123)*
- aerobic electron transport chain (GO:0019646)
- oxidative phosphorylation (GO:0006119)
- *respiratory electron transport chain (GO:0022904)*
- electron transport chain (GO:0022900)
- *cellular response to calcium ion (GO:0071277)*
- positive regulation of mRNA metabolic process (GO:1903313)
- cellular respiration (GO:0045333)
- *positive regulation of cold-induced thermogenesis (GO:0120162)*
- energy derivation by oxidation of organic compounds (GO:0015980)

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*Il10ra<sup>−/−</sup>*, *AdIL10RAKO*
IL10Rα/F/F (IL10Rα/F/F) and P represent Adipoq-Cre Positive (AdIL10Rα KO) mice was performed and fold-change was calculated as Log2FC(N/P) and gene ontology analysis was performed for genes enriched in P over N and plotted as a heatmap as fold-enrichment (0 to >10) and p<0.001. Shades of blue indicate highly enriched pathway in P with fold-enrichment >10. (B) Violin plot showing expression levels of indicated genes in iWAT adipocytes of AdIL10Rα KO mice and controls from SNAP-seq data (Figure 4).

DOI: https://doi.org/10.7554/eLife.49501.011
Figure 6. scRNA-Seq reveals an increase in adaptive immune cells under adrenergic stress. (A) Aggregated tSNE-plot of combined ~10,000 SVF cells isolated from iWAT of mice treated with CL or saline for 4 days. Colored dots are cells assigned to clusters based on similar transcriptomic signatures.
and these clusters correspond to specific cell-types. **(B)** Segregated tSNE-plot from **(A)** showing percentage of cell-types between control (Saline) and β3-agonist CL-treated mice. The tSNE-plot also shows differences in percentage of clusters between control and CL-treated mice. Black arrows indicate major B-cell population. **(C)** Colored dot plot showing percentage of fractions plotted in y-axis and cell types in x-axis under indicated conditions. **(D)** tSNE-plot showing cells expressing Il10 in control and CL-treated mice. Black arrows indicate major cell clusters with Il10 expression. **(E)** Dot plot showing expression levels of Il10 in indicated cells comparing control and CL-treated mice. The Log2Fold Change (FC) ratio of saline vs. CL was plotted as a function of log10 p-value and indicated as different sizes of dots. Fold-change and p-value was compared with saline condition represents as a dotted line.

DOI: https://doi.org/10.7554/eLife.49501.012
Figure 7. Increased adipose thermogenesis and EE in lymphocyte-deficient mice. (A and B) Real-time PCR analysis of gene expression in iWAT from chow-fed 10 week 24 hr cold-exposed (A) or CL (1 mg/kg/day for 4 days (B) mice. (C, D) Bar graphs (C) and scatter plot (D) (Light cycle) showing oxygen consumption, energy expenditure, carbon dioxide production, and RER in CL-treated mice with body mass as covariate. N = 6, 5. *, p<0.05, **, p<0.01.
DOI: https://doi.org/10.7554/eLife.49501.013