Figure S1. **RNA-seq analysis of FACS-isolated PDGFRA+ cells from eWAT of control and CL-treated mice.** Related to STAR Methods**.** (A) Upper panel: representative plot of fluorescence-activated cell sorting analysis of stromal vascular cells (SVC) showing PDGFRA+ cells sorted as CD44+ and CD44- in control (CON) and CL-treated mice. Lower panel: Average data from 4 independent experiments. (B) Pearson complete linkage hierarchical clustering analysis of differentially expressed genes among PDGFRA+CD44- (CD44P) and PDGFRA+CD44- (CD44N) cells in control (CON) and CL-treated mice. Colors in the heatmap represent row Z-score calculated from normalized transcript reads (Log2 transformed).
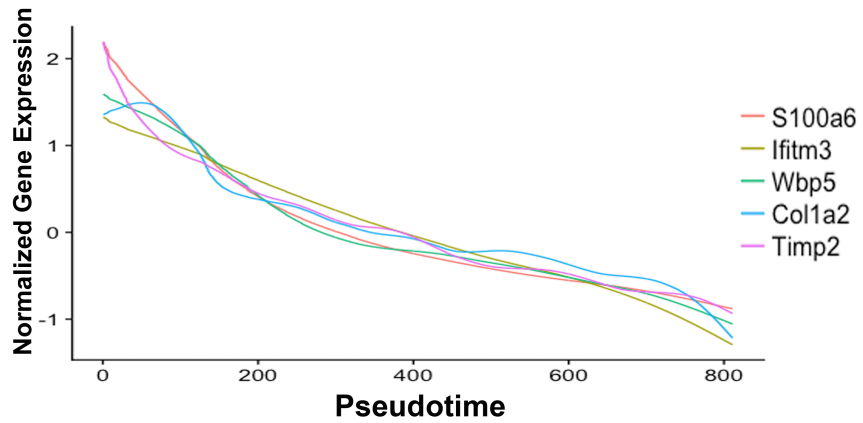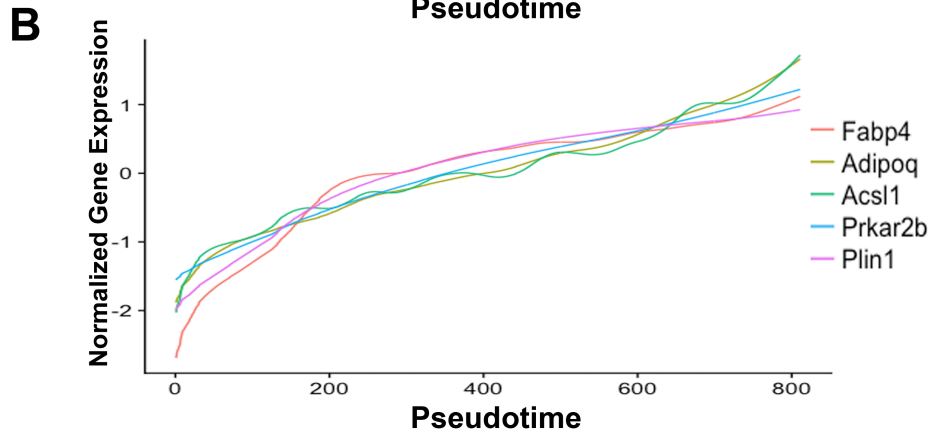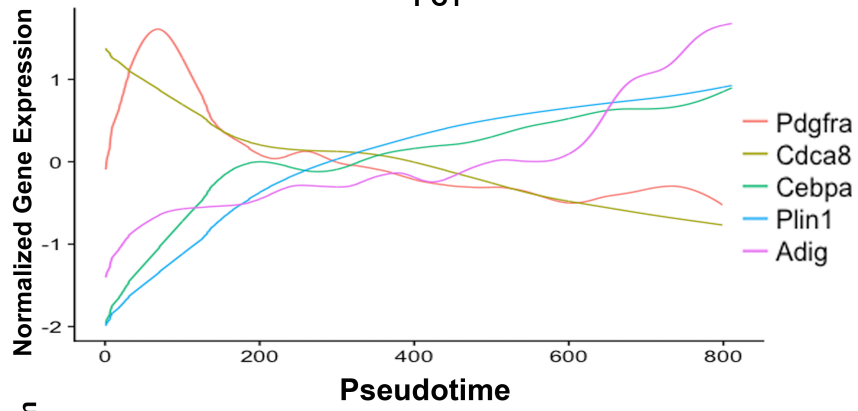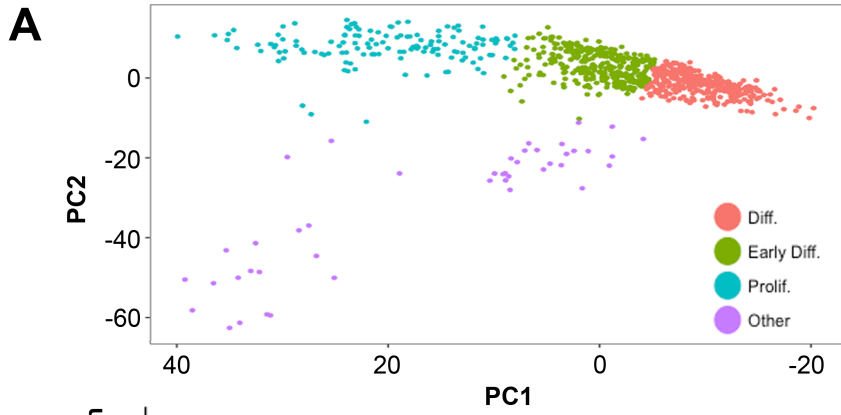
A

B

Figure S2. Pseudotime analysis of proliferating/differentiating cells. Related to Figure 2C.
(A) PCA plot of the first two principal components reveals that principal component 1 (PC1)
orders cells along the differentiation trajectory, and can therefore be used as a surrogate for
ordering cells in pseudotime. Plotting pseudotime versus normalized gene expression for the
genes in Figure 2B follows an expected pattern for adipocyte differentiation. These genes were
significantly correlated with pseudotime analysis: *Pdgfra* (r = -0.46), *Cdca8* (r = -0.53), *Cebpa* (r
= 0.57), *Plin1* (r = 0.74), and *Adig* (r = 0.62). (B) Genes whose expression was correlated with
pseudotime using a Pearson correlation were determined. Shown are the top 5 positively and
negatively correlated genes from this analysis. Positively correlated: *Fabp4* (r = 0.79), *Adipoq* (r
= 0.79), *Acsl1* (r = 0.74), *Prkar2b* (r = 0.74), and *Plin1* (r = 0.74). Negatively correlated: *S100a6*
(r = -0.72), *Ifitm3* (r = -0.72), *Wbp5* (r = -0.68), *Col1a2* (r = -0.66), and *Timp2* (r = -0.66). All
genes significantly correlated with the pseudotime analysis are presented in **Table S2D**.
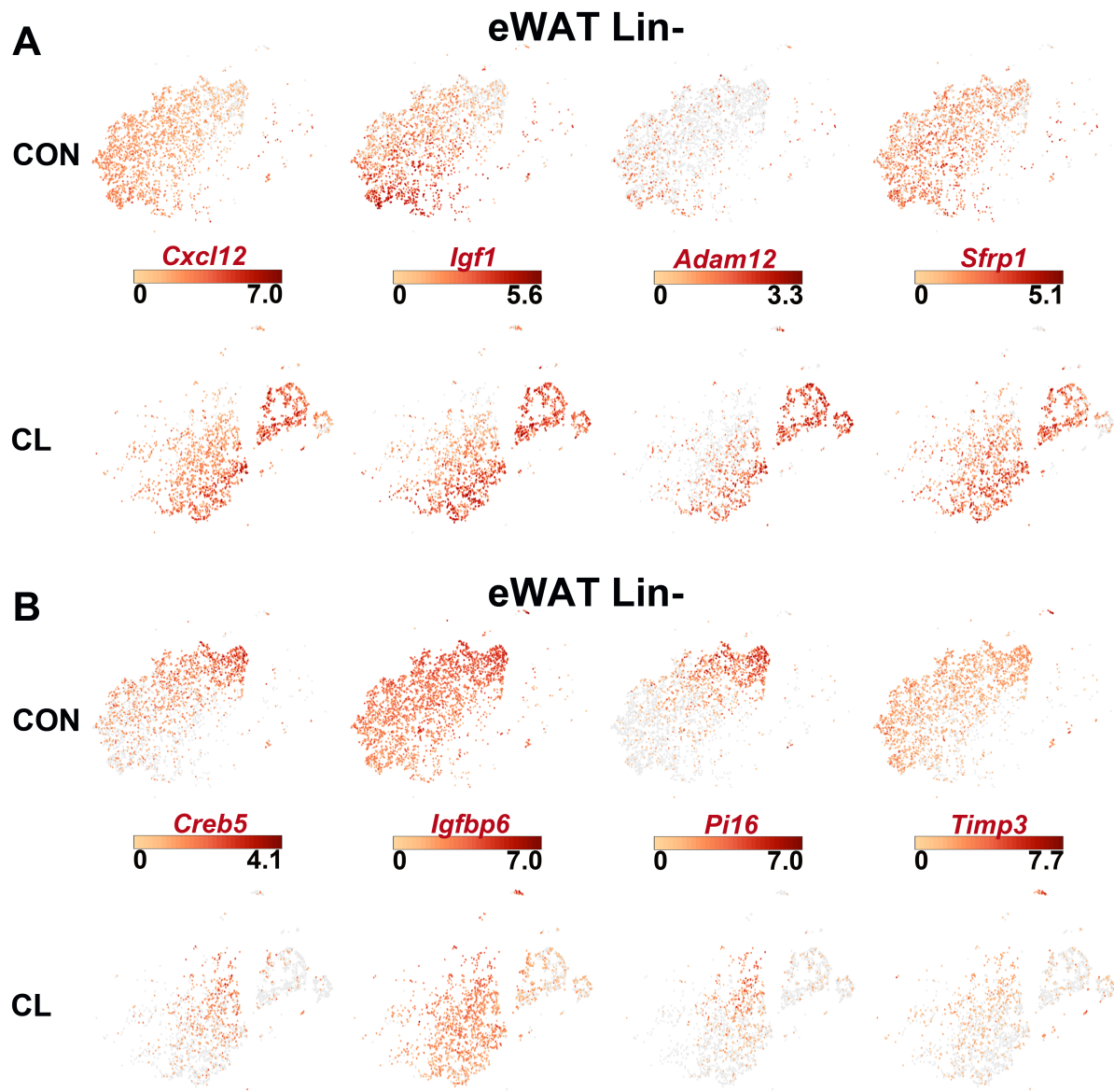
A

eWAT Lin-

Figure S3. CL treatment upregulates expression of genes involved in cell migration, proliferation, and ECM remodeling in eWAT adipocyte stem cells. Related to Figures 1 and 2. t-SNE plots show the ASC populations defined in Figure 1A, segregated by treatment condition. (A) CL upregulates expression of *Cxcl12*, *Igf1*, *Adam12*, and *Sfrp1* and (B) downregulates expression of *Creb5*, *Igfbp6*, *Pi16*, and *Timp3*. Expression scale bars show Log2 values of max gene expression.
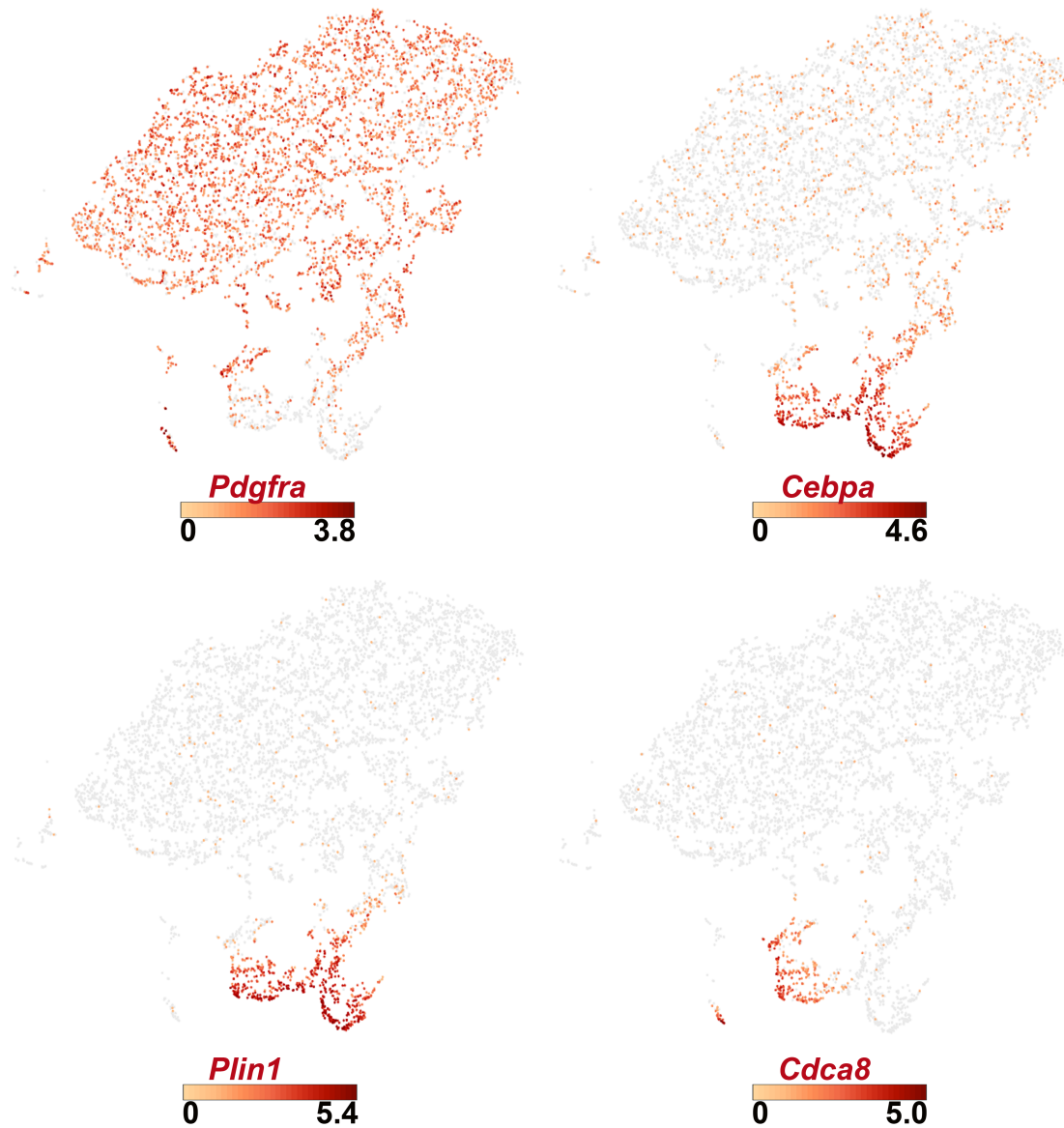
Figure S4. Expression patterns of adipogenic/proliferation genes in ASC from aggregate Lin+ and Lin- fractions identifies proliferating and differentiating ASCs. Related to Figure 3**.** t-SNE plot of clusters ASC and Diff. ASC from Figure 3A. Expression of *Pdgfra* defines ASC. Expression of *Cebpa* and *Plin1* map to population defined in Figure 3A as differentiating ASC. *Cdca8* defines actively proliferating ASC. Expression scale bars represent Log2 values of max gene expression.
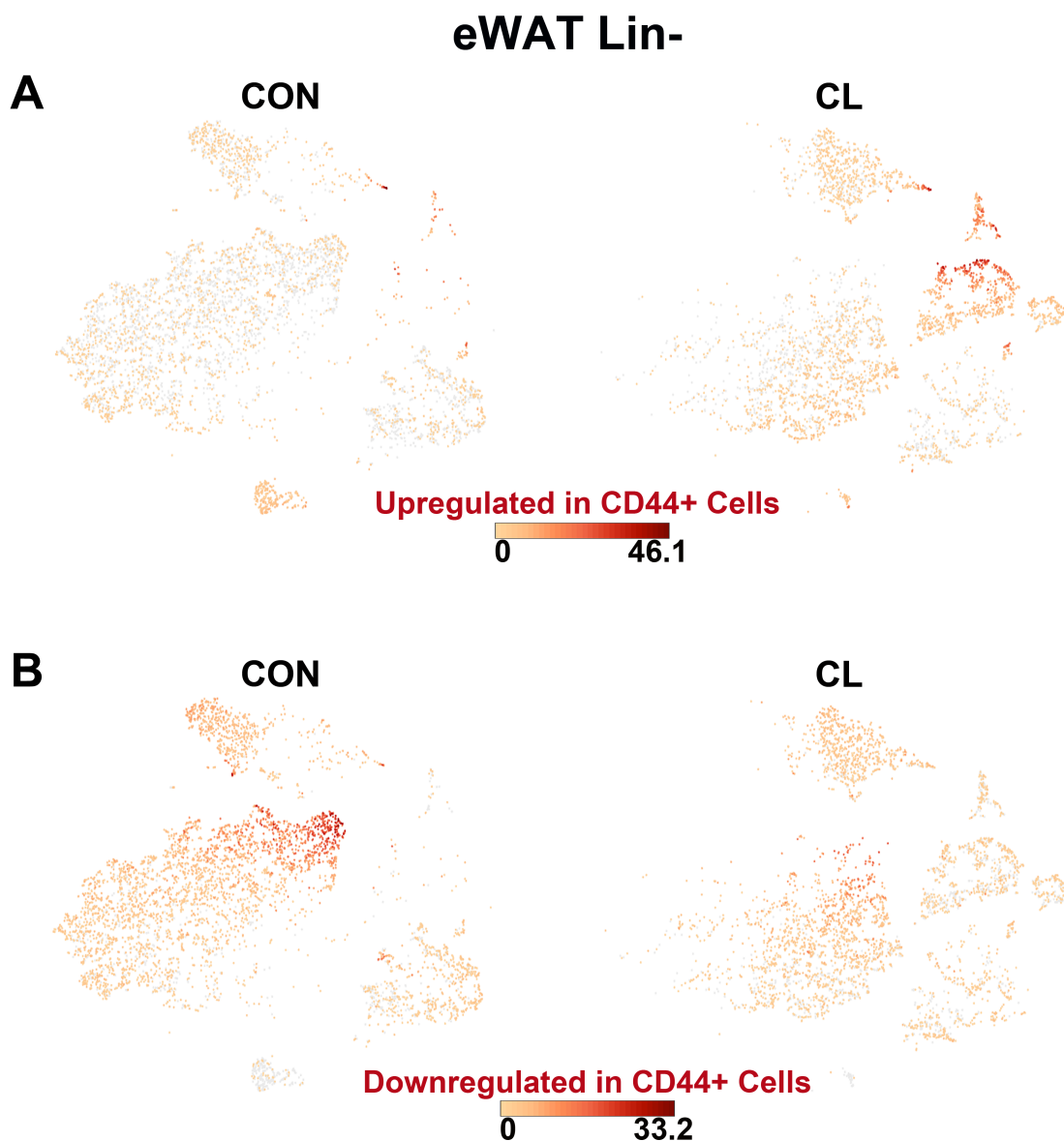
eWAT Lin-

A CON CL

Upregulated in CD44+ Cells

0   46.1

B CON CL

Downregulated in CD44+ Cells

0   33.2

Figure S5. Correspondence of DEGs identified by bulk RNA-sequencing of FACS sorted PDGFRA+CD44+ cells to scRNA-seq. Related to Figures 1 and S1. Lists of the top 25 upregulated or downregulated genes identified from bulk RNA sequencing of PDGFRA+CD44+ cells compared to PDGFRA+CD44- cells were used as input into the 10X Genomics Loupe program. Shown are the t-SNE plots of the eWAT Lin- cell fraction segregated by treatment (CON or CL), also shown in Figure 1B. Color intensities represent the sum of the Log2 expression values for the input gene list. (A) Differentially upregulated genes in PDGFRA+CD44+ cells are enriched with CL treatment and expressed in cells undergoing active adipogenesis. (B) Differentially downregulated genes in PDGFRA+CD44+ cells are reduced in adipogenic clusters that were induced by CL.

eWAT Lin+

| CON | CL |

Arg1
0 — 5.9

Chil3
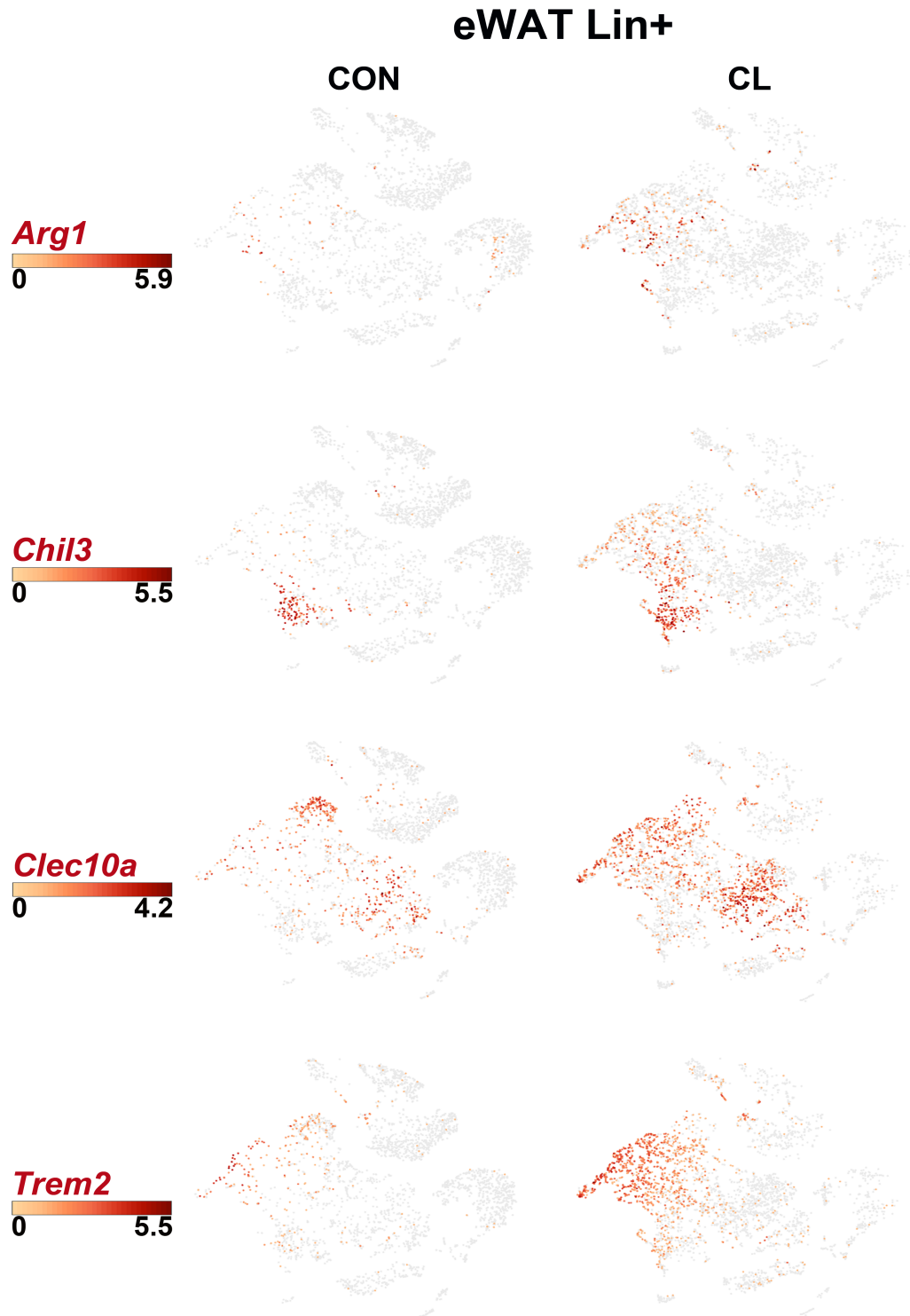0 — 5.5

Clec10a
0 — 4.2

Trem2
0 — 5.5

Figure S6. Expression of M2 macrophage markers in scRNA-seq. Related to Figure 4. Distribution of M2 markers, previously shown to be upregulated by CL, on the t-SNE plot of eWAT Lin+ cells from Figure 4. Scale bars represent Log2 values of maximum gene expression.