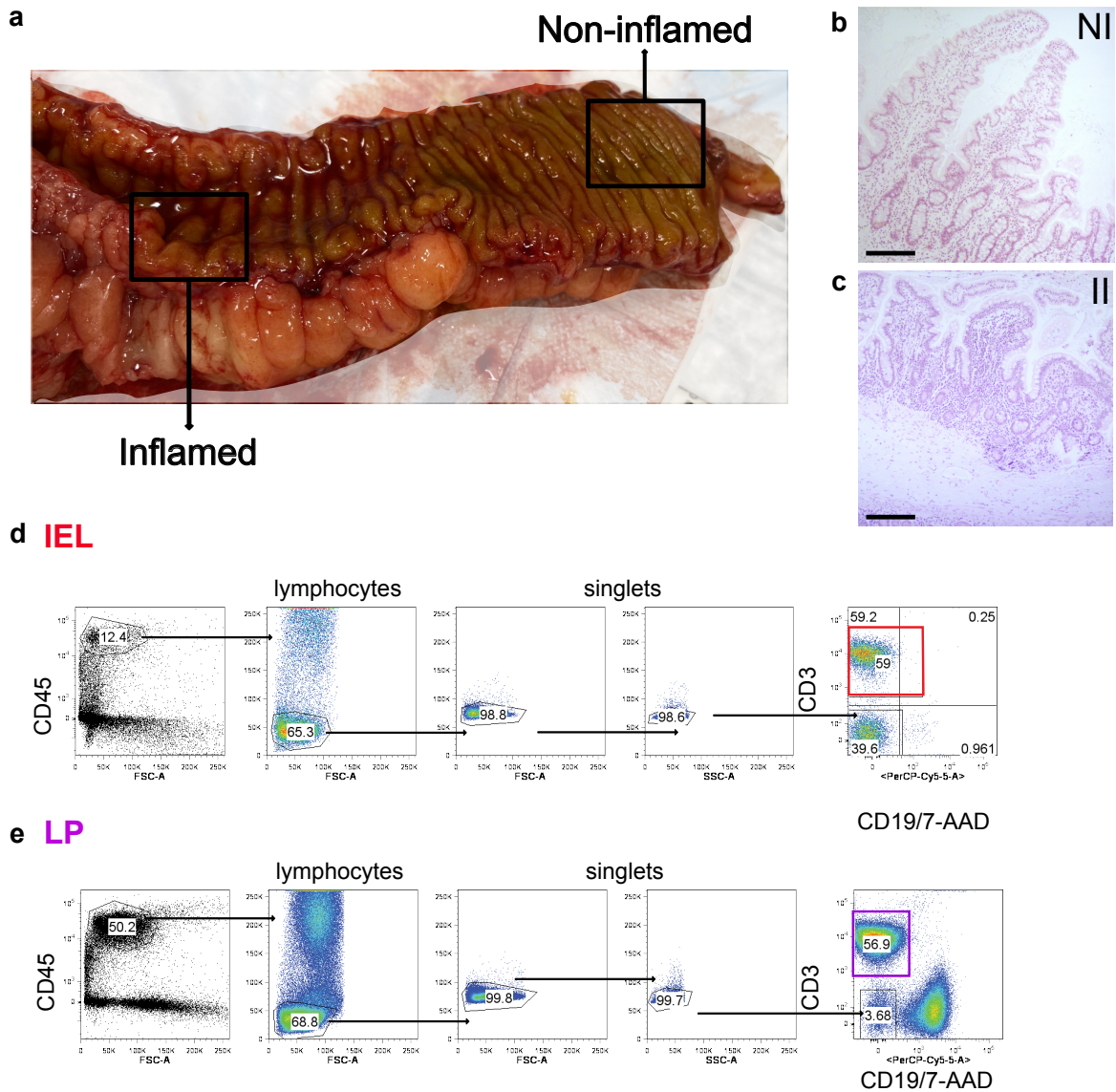


**Single-cell analyses of Crohn's disease tissues reveal intestinal  
intraepithelial T cells heterogeneity and altered subset distributions**

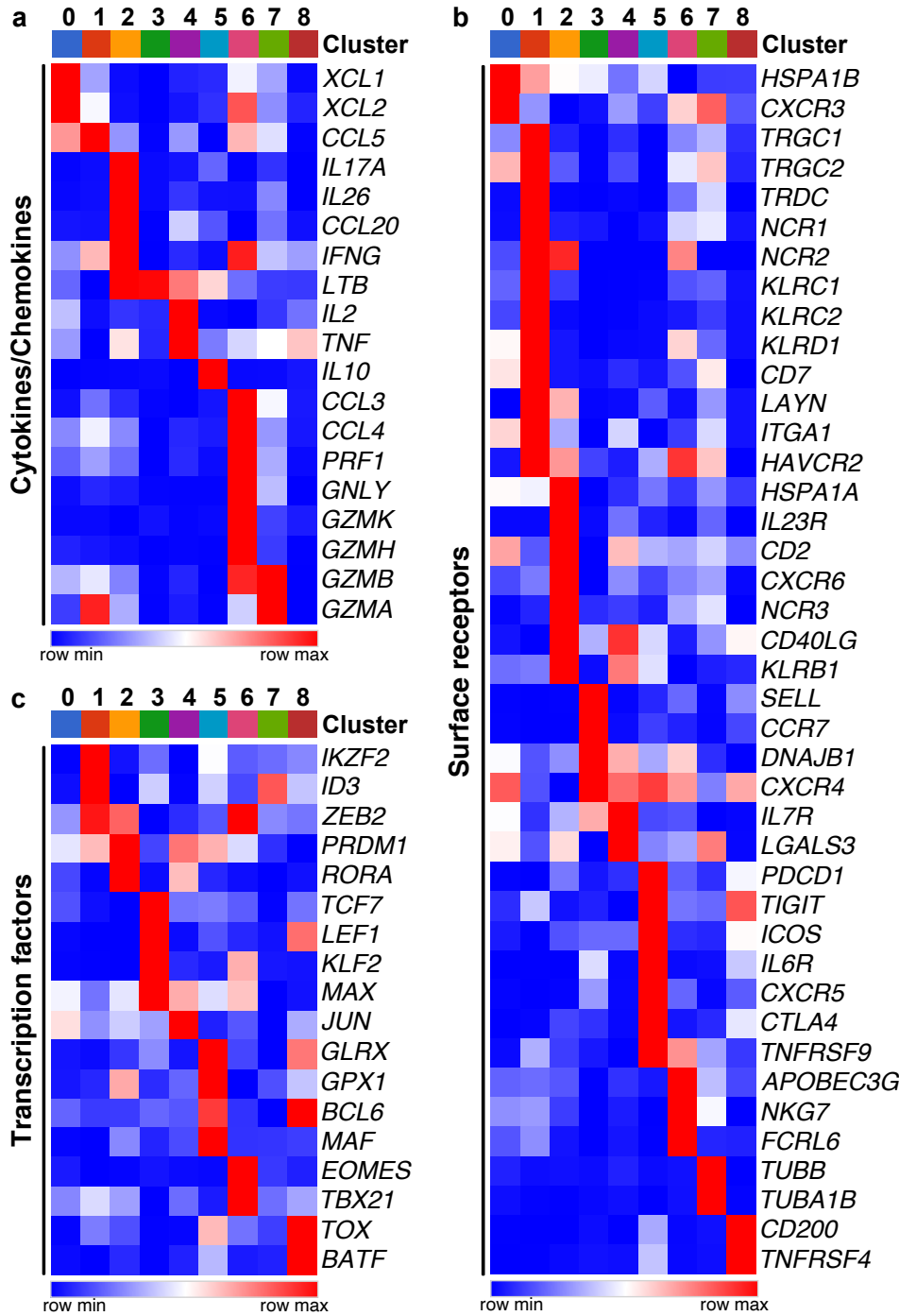
Natalia Jaeger, Marco Colonna

**Supplementary Figure 1**



**Supplementary Figure 1. Tissue collection from paired NI and II areas and gating strategy for T cell sorting from IEL and LPL. a**, Picture showing the paired NI and II areas collected from surgically resected terminal ileum from CD patients. **b-c**, Hematoxylin and eosin staining of terminal ileum samples from paired NI (**b**) and II (**c**) areas. This experiment was performed once. Magnification: 20x, scale bar: 100  $\mu$ m. **d-e**, Gating strategy to sort T cells used on scRNA-seq data shown in Figure 1, 4, 5, Supplementary Figure 2, 4, 5, 6. Gating was applied on alive CD45<sup>+</sup>, lymphocytes, singlets, CD3<sup>+</sup>CD19<sup>-</sup> cells.

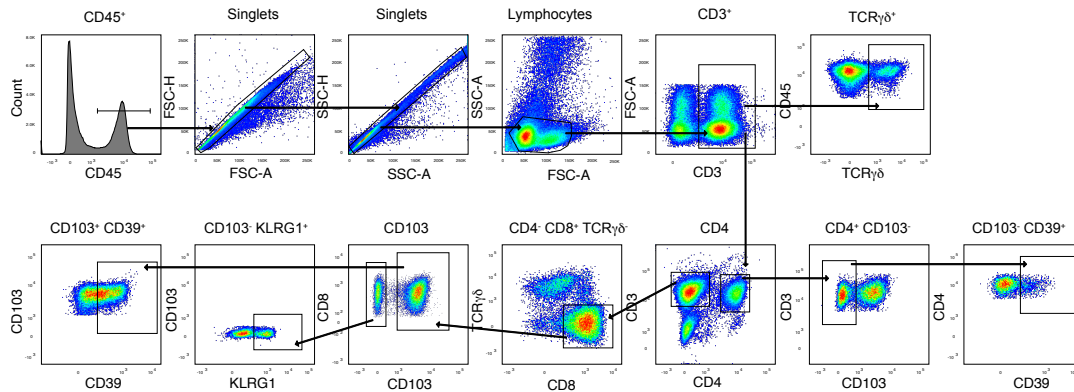
**Supplementary Figure 2**



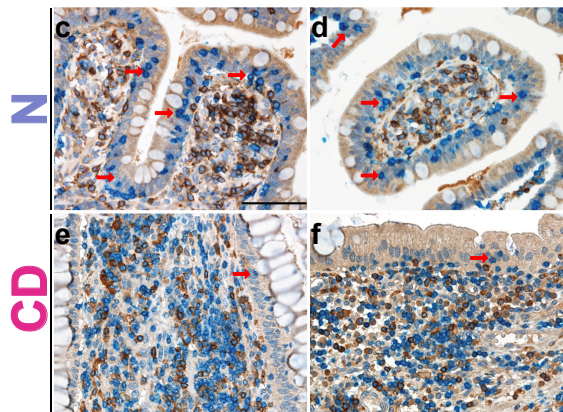
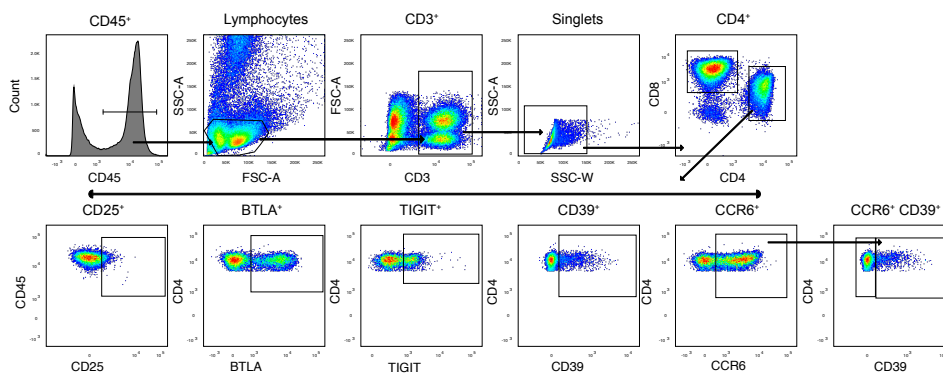
**Supplementary Figure 2. Expression of transcription factors, cell surface receptors and soluble factors in each IEL T cell cluster.** **a**, Heat map depicting the expression level of selected cytokines, chemokines and cytotoxicity mediators in clusters 0 to 8. **b**, Heat map depicting the expression level of selected cell surface receptors in clusters 0 to 8. **c**, Heat map depicting the expression level of selected transcription factors in clusters 0 to 8.

### Supplementary Figure 3

#### a CD8 panel



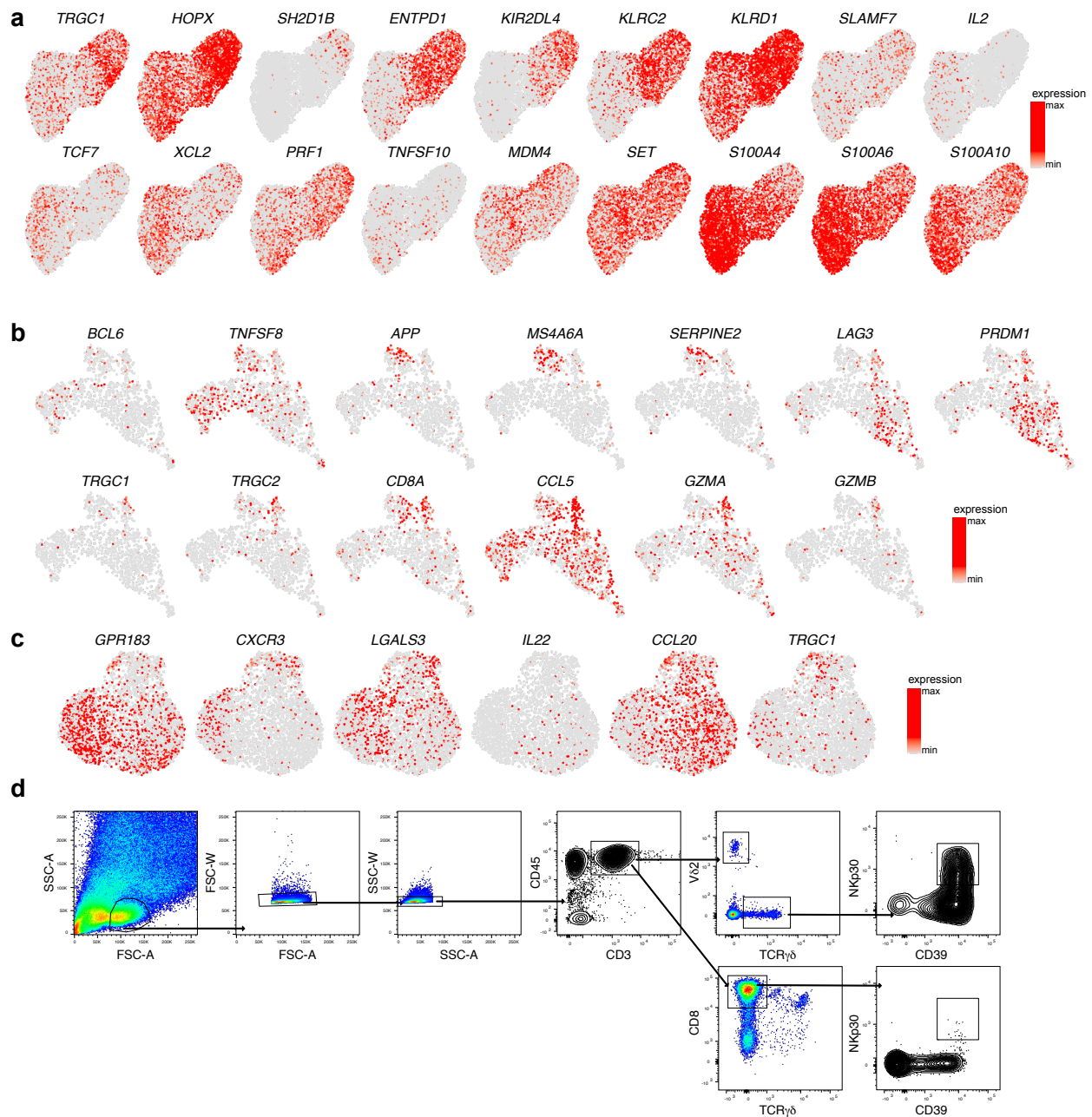
#### b CD4 panel



**Supplementary Figure 3. Gating strategy to quantitate IEL cell subsets by flow cytometry.** Gating strategy used to quantify by flow cytometry the different IEL CD8<sup>+</sup>, TCRγδ<sup>+</sup> and CD4<sup>+</sup> T cell subsets in terminal ileum of controls and CD patients. T cells were gated as singlets, CD45<sup>+</sup>CD3<sup>+</sup>. Gating strategy for the CD4 (a) and CD8 (b) panel presented on Figures 2 and 3. Gating is shown on the top of each panel. **c-f**, IHC of tissue sections stained with anti-CD4 and CD8 from terminal ileum sections of control (**c, d**) and CD (**e, f**) patients. Images are representative of two control (**c, d**) and three CD (**e, f**) patients. Sections are counterstained with hematoxylin. Red arrows indicate CD8<sup>+</sup> T cells in the IEL. Magnification: 200x, scale bar: 100 μm. N= control, CD = Crohn's disease.

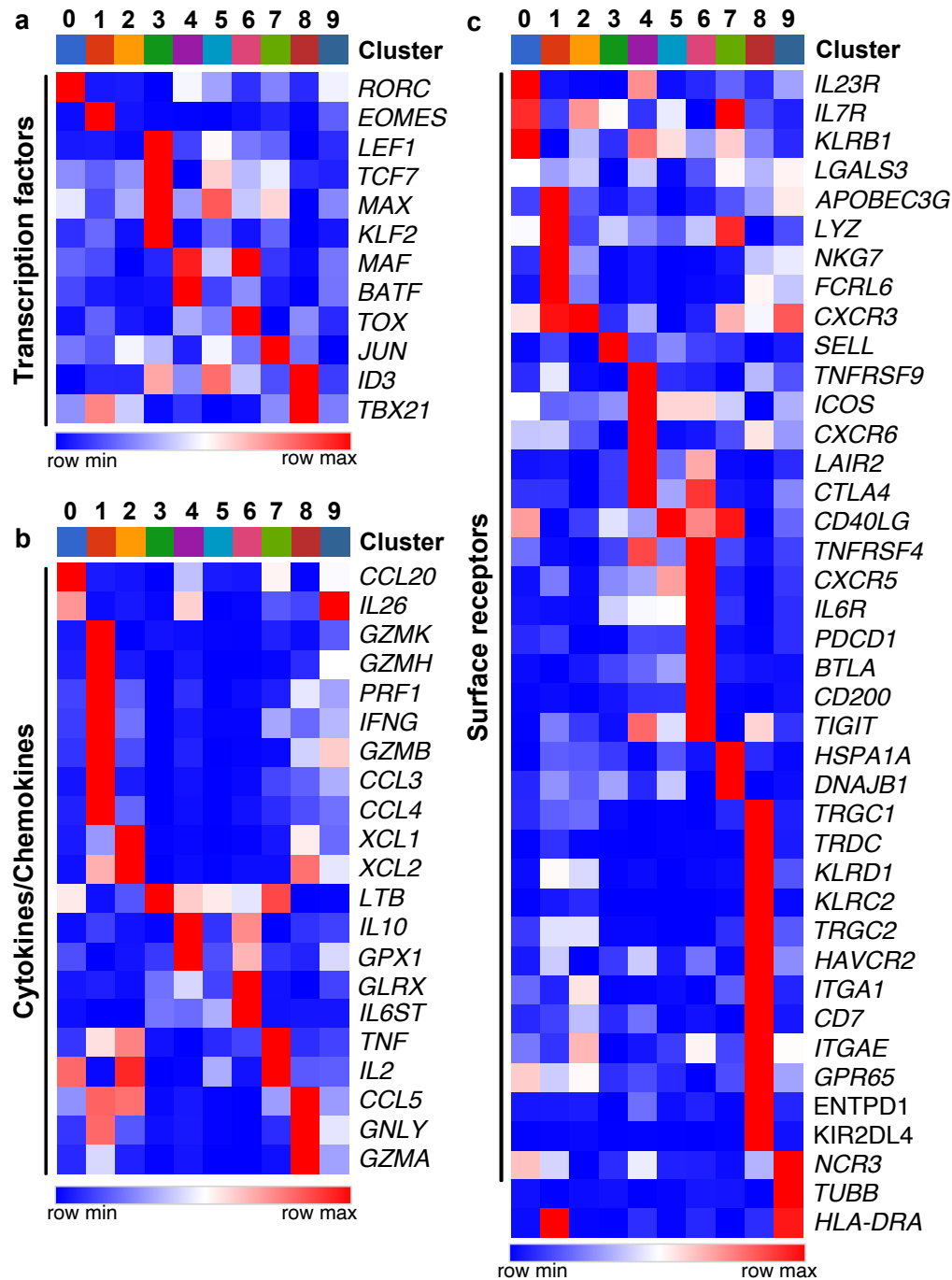


## Supplementary Figure 4



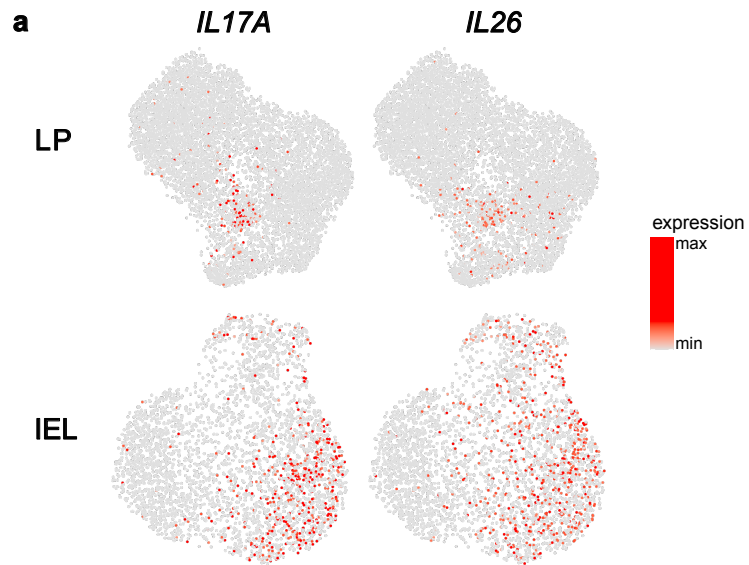
**Supplementary Figure 4. Reclustering of heterogeneous IEL CD8<sup>+</sup> and CD4<sup>+</sup> T cell populations.** **a**, UMAP of representative selected genes associated with the reclustering of clusters 0 and 1 from Fig. 1a. **b**, UMAP of representative selected genes associated with the reclustering of clusters 5 and 8 from Fig. 1a. **c**, UMAP of representative selected genes associated with the reclustering of clusters 2 and 4 from Fig. 1a. **d**, Gating strategy for IEL  $\gamma\delta$  and CD8 T cell expressing NKp30 of the terminal ileum of controls patients. Data presented on Figure 4j-i. Gating was applied on lymphocytes, singlets, CD45<sup>+</sup>, CD3<sup>+</sup> cells and TCR $\gamma\delta$ <sup>+</sup> or V $\delta$ 2<sup>-</sup> for  $\gamma\delta$ <sup>+</sup> T cells or CD8<sup>+</sup> TCR $\gamma\delta$ <sup>+</sup> for CD8 T cells.

Supplementary Figure 5



**Supplementary Figure 5. Expression of transcription factors, cell surface receptors and soluble factors in each LP T cell cluster.** **a**, Heat map depicting the expression level of selected transcription factors in clusters 0 to 9. **b**, Heat map depicting the expression level of selected cytokines, chemokines and cytotoxicity mediators in clusters 0 to 9. **c**, Heat map depicting the expression level of selected cell surface receptors in clusters 0 to 9.

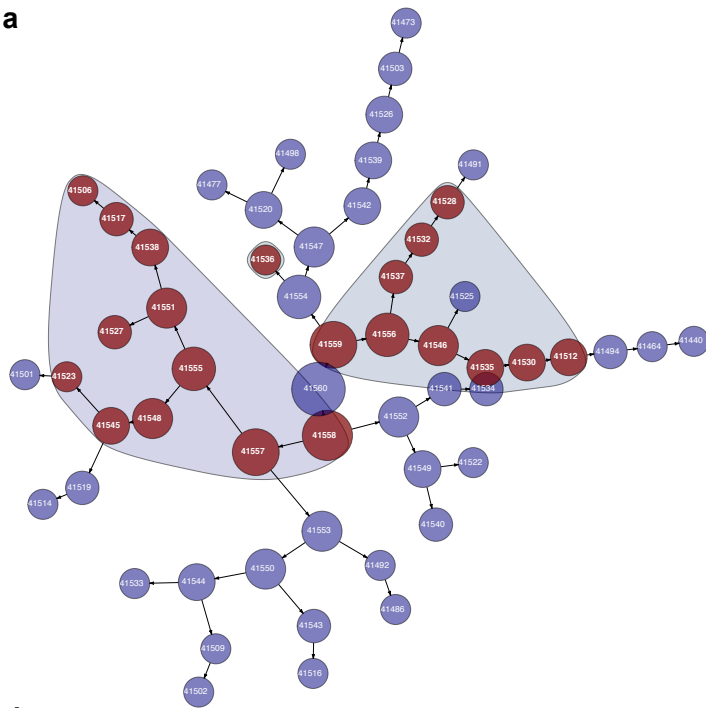
## Supplementary Figure 6



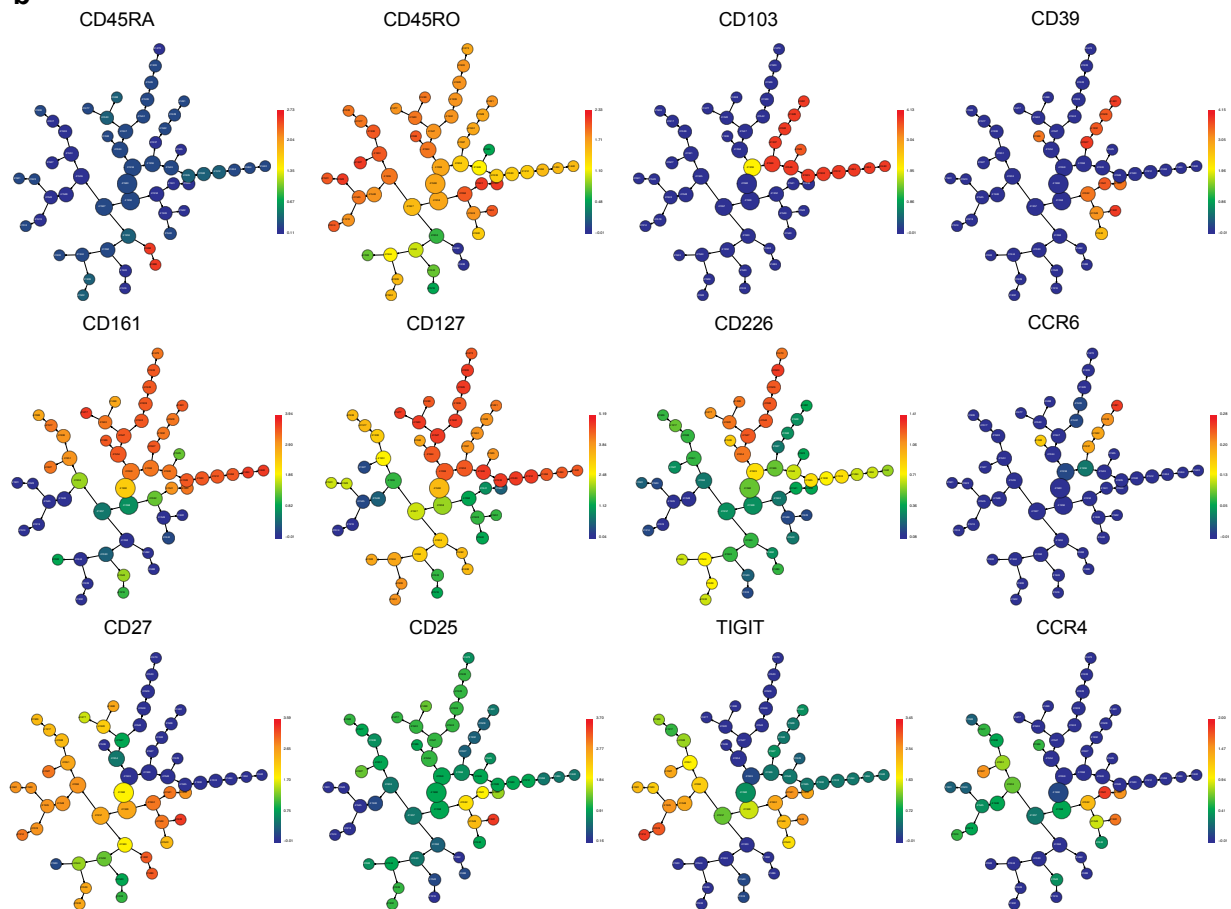
**Supplementary Figure 6. IEL contains more activated Th17 cells. a,** UMAP plots depicting the expression of *IL17A* and *IL26* in the LP and IEL reclustering of clusters 0 (LP, Fig. 5a) and 2 and 4 (IEL, Fig.1a).

# Supplementary Figure 7

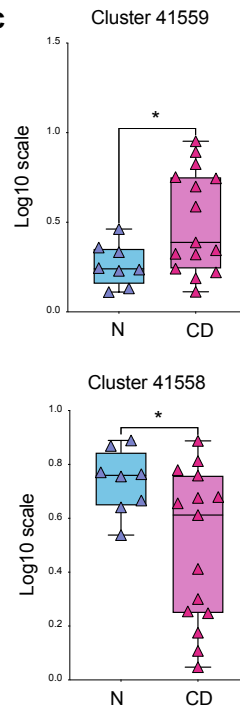
**a**



**b**



**c**



**Supplementary Figure 7. Hierarchical clustering analysis of CD4 T cells subsets by Citrus.** **a**, Schematic CITRUS plot generated to identify and quantify CD4<sup>+</sup> T cells subsets from normal and CD lamina propria samples. **b**, Expression markers that identify the nodes in CD4<sup>+</sup> T cells. **c**, Frequencies of cluster 41559 (Th17) and cluster 41558 (T<sub>FH</sub>) (normal vs CD). Triangles on the boxplots show data collected for each individual donor. Data are median and interquartile range. Significance was calculated using a t-student test with Prism v8 software. **c-d**, \* $P=0.0319$ . **a-d**, Controls (N), n=8; CD (combined inflamed (II) and non-inflamed (NI) samples), n=15 (9 NI, 6 II, total of 10 different patients). Samples were fresh at the time of staining.