

Supplementary Legends

Supplementary Fig. S1 Clusters of immune cell subsets, differentially expressed genes of T cells, and GO analysis of immune cell subsets in ON and YN groups.

- A. Heatmap showing scaled expression of discriminative gene sets for immune cell subsets in CDLNs.
- B. UMAP plots of canonical markers for immune cell subsets.
- C. Representative GO terms and KEGG pathway enriched in downregulated DEGs of total immune cells in ON/YN comparison group.
- D. Volcano plot showing up- and down-regulated DEGs in T cells in the ON/YN comparison group. Red and blue plots indicated up- and down-regulated DEG of T cells in ON group compared to YN group, respectively.
- E. Representative GO terms and KEGG pathway enriched in downregulated DEGs of immune cell subsets in the ON/YN comparison group.

Supplementary Fig. S2 Re-clustering, and pseudotime trajectory analysis of T cells in the ON/YN comparison group.

- A. Heatmap showing scaled expression of discriminative gene sets for T cell subsets in CDLNs.
- B. UMAP plots of canonical markers for T cell subsets.
- C. Pseudotime trajectory analysis corresponding to the differentiation of effector CD4⁺ T cells from naïve CD4⁺ T cells.
- D. Expression transition of differentiation-associated genes along the pseudotime.

Supplementary Fig. S3 Pseudotime trajectory analysis of CD8⁺ T cells, differentially expressed genes and GO analysis of naïve T cells in the YN/ON comparison group.

- A. Pseudotime trajectory analysis corresponding to the differentiation of effector CD8⁺ T cells from naïve CD8⁺ T cells in YN and ON group, respectively. Cells are colored by pseudotime (top) or cell type (bottom).
- B. Pseudotime trajectory analysis corresponding to the differentiation of effector CD8⁺ T cells from naïve CD8⁺ T cells in both YN and ON groups. Cells are colored by pseudotime.
- C. Percentages of CD8⁺ T cells along the pseudotime for YN and ON mice.
- D. Expression transition of differentiation-associated genes along the pseudotime.
- E-F. Volcano plot showing up- and down-regulated DEGs of naïve CD4⁺ T cells (E) or naïve CD8⁺ T cells (F) in the ON/YN comparison group. Red and blue plots indicated up- and down-regulated DEG in ON group compared to YN group, respectively.
- G. Representative GO terms and KEGG pathway enriched in upregulated DEGs of naïve CD8⁺ T cells in the ON/YN comparison group.
- H-I. Violin plots of expression level of *Il17a* and *Il1r1* in T subsets from YN and ON groups.

Supplementary Fig. S4 GO analysis of T cell subsets in the ON/YN comparison group.

- A-B. Representative GO terms and KEGG pathway enriched in upregulated (A) or downregulated (B) DEGs of T cell subsets in the ON/YN comparison group.

Supplementary Fig. S5 Re-clustering, and Pseudotime trajectory analysis of B cells in the ON/YN comparison group.

- A. UMAP plot showing clusters of B cell subsets.

- B.** Heatmap showing scaled expression of discriminative gene sets for B cell subsets.
- C.** UMAP plots of canonical markers for B cell subsets.
- D.** Bar chart of the relative proportion of B cell subsets in the ON/YN comparison group derived from scRNA-seq data.
- E.** Pseudotime trajectory analysis corresponding to the differentiation of effector B cells from naïve B cells in YN and ON groups, respectively. Cells are colored by pseudotime, cell type, as indicated, from the top to the bottom panels.
- F.** Pseudotime trajectory analysis corresponding to the differentiation of effector B cells from naïve B cells in both YN and ON groups. Cells are colored by pseudotime.
- G.** Percentages of B cells along the pseudotime for YN and ON mice.
- H.** Expression transition of differentiation-associated genes along the pseudotime.

Supplementary Fig. S6 GO analysis of B cells subsets and cell-cell interactions of immune cell subsets in the ON/YN comparison group.

- A-B.** Representative GO terms and KEGG pathway enriched in upregulated (A) or downregulated (B) DEGs of B cell subsets in the ON/YN comparison group.
- C.** Heatmap of relative expression of activation-related interaction pairs from T cells and B cells in the ON and YN groups.
- D.** Circle plot showing cell-cell interactions via APRIL signaling in the ON and YN groups.
- E.** Network plots showing the changes in ligand-receptor interaction events between Tregs and other immune cell types in the ON/YN comparison group. Cell-cell communication is indicated by the connected line. The thickness of the lines is positively correlated with the number of ligand-receptor interaction events.

Supplementary Fig. S7 Lymph nodes alteration during EAU, cluster of immune cells subsets, and GO analysis of the YE/YN and OE/ON comparison groups.

- A.** Draining lymph nodes of four groups.
- B.** UMAP plot showing clusters of immune cell subsets across four groups.
- C.** UMAP plots of canonical markers for immune cell subsets.
- D.** Representative GO terms and KEGG pathway enriched in downregulated DEGs of total immune cells in the YE/YN and OE/ON comparison groups.

Supplementary Fig. S8 GO analysis of immune cell subsets in the YE/YN and OE/ON comparison groups.

- A-B.** Representative GO terms and KEGG pathway enriched in upregulated (A) or downregulated (B) DEGs of immune cell subsets in the YE/YN and OE/ON comparison groups.

Supplementary Fig. S9 Canonical markers for T cell subsets, and GO analysis of T cell subsets in the YE/YN and OE/ON comparison groups.

- A.** UMAP plots of canonical markers for T cell subsets.
- B.** Representative GO terms and KEGG pathway enriched in downregulated DEGs of T cell subsets in the YE/YN and OE/ON comparison groups.

Supplementary Fig. S10 Re-clustering, cell ratios and GO analysis of B subsets, proportions of plasma cells and follicular T helper cells across four groups.

A. UMAP plot showing clusters of B cell subsets.

B. UMAP plots of canonical markers for B cell subsets.

C. Relative fold changes (FC) in cell ratios in B cell subsets across the four groups (YN, YE, ON, and OE). The numbers on the right indicate the Log₂FC values of the cell ratios (YE/YN, OE/ON, and OE/YE).

D-E. Representative scatter plots (D) and quantification (E) of the proportion of plasma B cells from the four groups measured by flow cytometry. Each group contains six mice. The values represent the mean $\pm$ SD from three independent experiments. Significance was determined using two-way ANOVA. ****P < 0.0001.

F-G. Representative scatter plots (F) and quantification (G) of the proportion of follicular T helper cells from the four groups were measured by flow cytometry. Each group contains six mice. The values represent the mean $\pm$ SD from three independent experiments. Significance was determined using two-way ANOVA. ****P < 0.0001.

H-I. Representative GO terms and KEGG pathway enriched in upregulated (H) or downregulated (I) DEGs of B cell subsets in the YE/YN and OE/ON comparison groups.

J. Levels of IRBP₁₋₂₀-specific antibodies in the blood serum from the four groups detected by ELISA. Each group contains six mice. The data represent the means $\pm$ SD. Significance was determined using two-way ANOVA. ****P < 0.0001. NS, not significant.

K. Concentration of total IgG in the blood serum from the four groups detected by ELISA. Each group contains six mice. The data represent the means $\pm$ SD. Significance was determined using two-way ANOVA. ****P < 0.0001.

Supplementary Fig. S11 Gating strategy of Th17 cells and schematic of the mechanism of milder EAU in aging mice.

A. Heatmap of relative expression of *Csf2* and *Il23r* from Th17 cells across the four groups.

B. Gating strategy of Th17 cells

C. Schematic illustrating the mechanism of milder EAU during aging.

Supplementary Table Legends

Supplementary Table S1. DEGs in total immune cells (A), eight major immune cell subsets (B-I), ten T cell subsets (J-S) and four B cell subsets (T-W) in the ON/YN comparison group.

Supplementary Table S2. Transcriptional factor activity of Th17 cells in the ON and YN groups.

Supplementary Table S3. DEGs in total immune cells (A), eight major immune cell subsets (B-I), ten T cell subsets (J-S) and four B cell subsets (T-W) in the YE/YN comparison group.

Supplementary Table S4. DEGs in total immune cells (A), eight major immune cell subsets (B-I), ten T cell subsets (J-S) and four B cell subsets (T-W) in the OE/ON comparison group.

Supplementary Table S5. Cell-cell interaction pairs in the YN (A), YE (B), ON (C) and OE (D) groups.

Supplementary Table S6. Clinical and pathological scoring criteria of EAU.

Figure S1

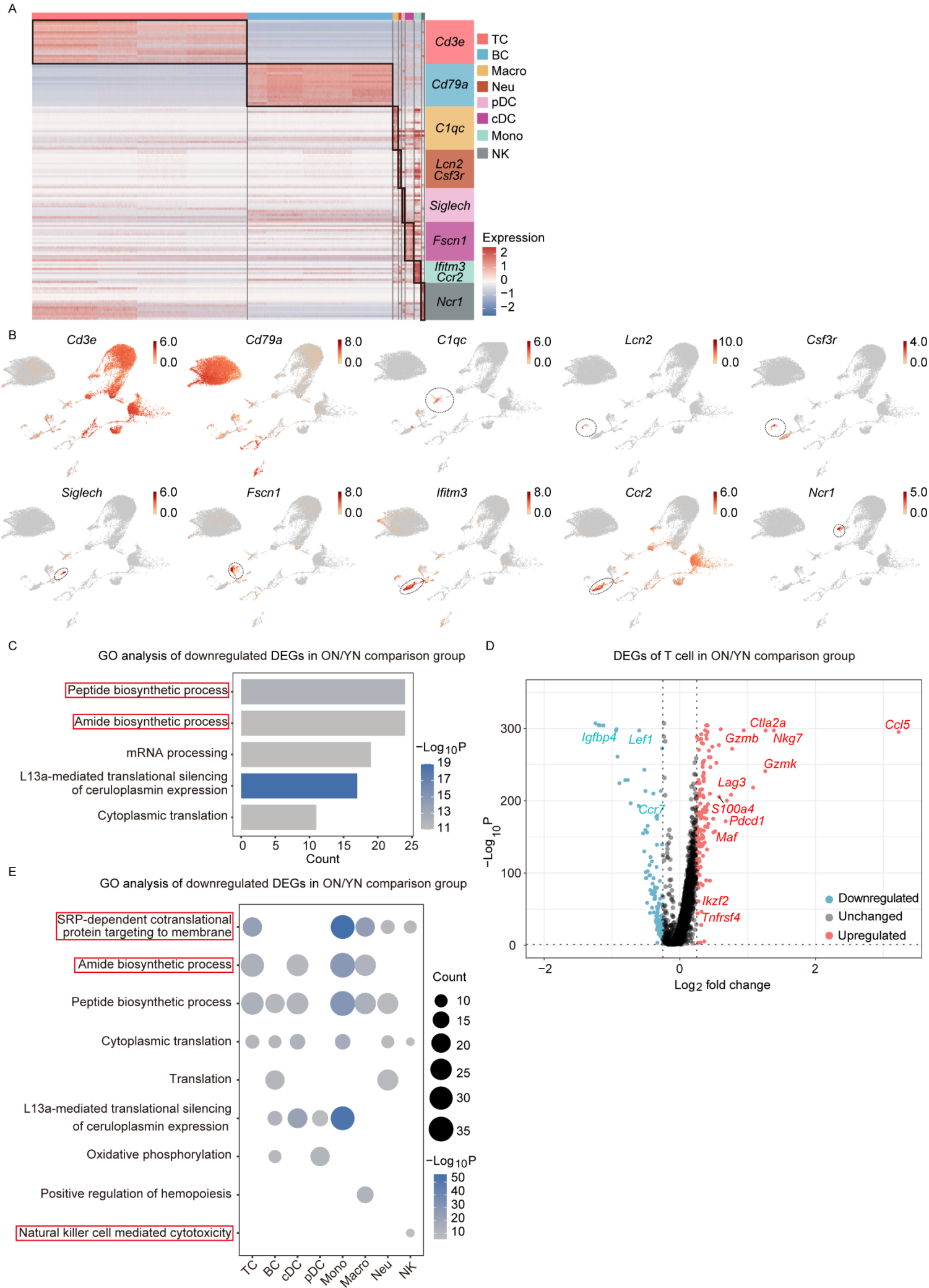
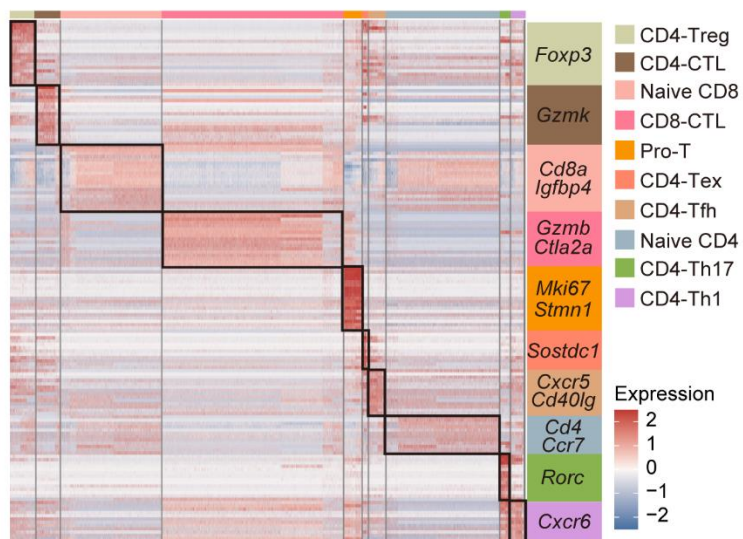
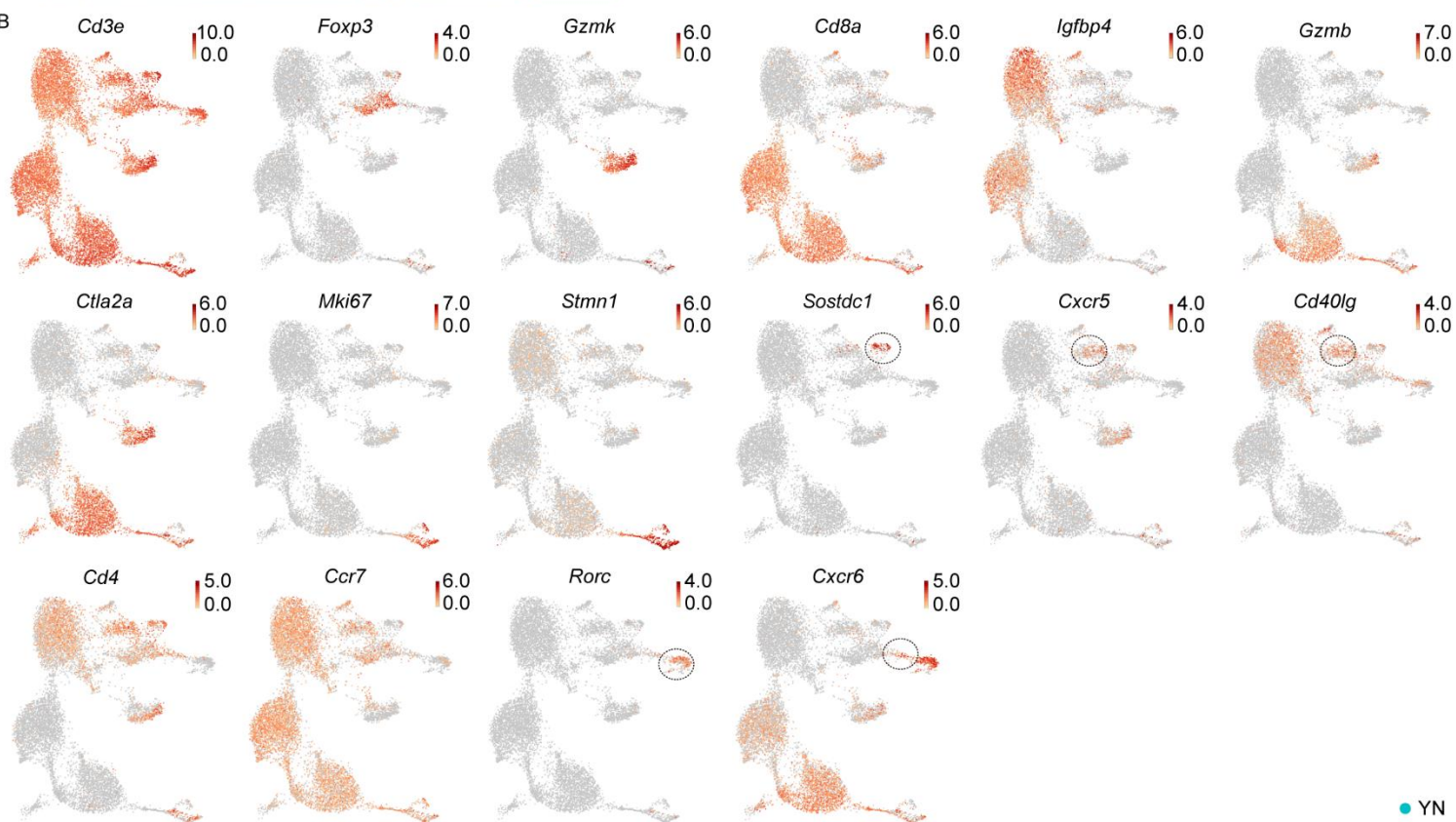


Figure S2

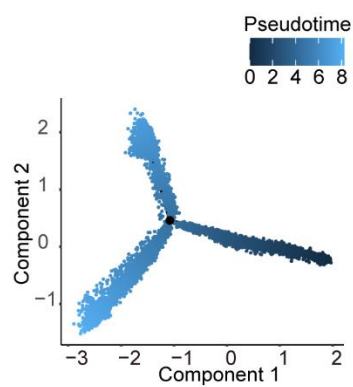
A



B



C



D

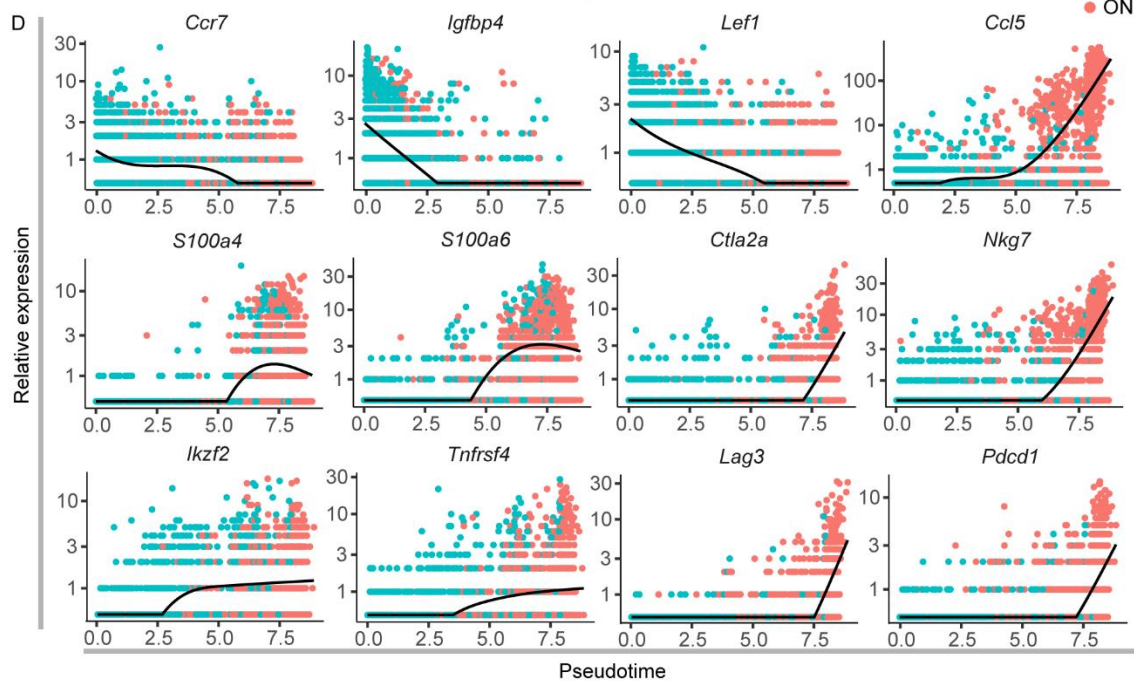


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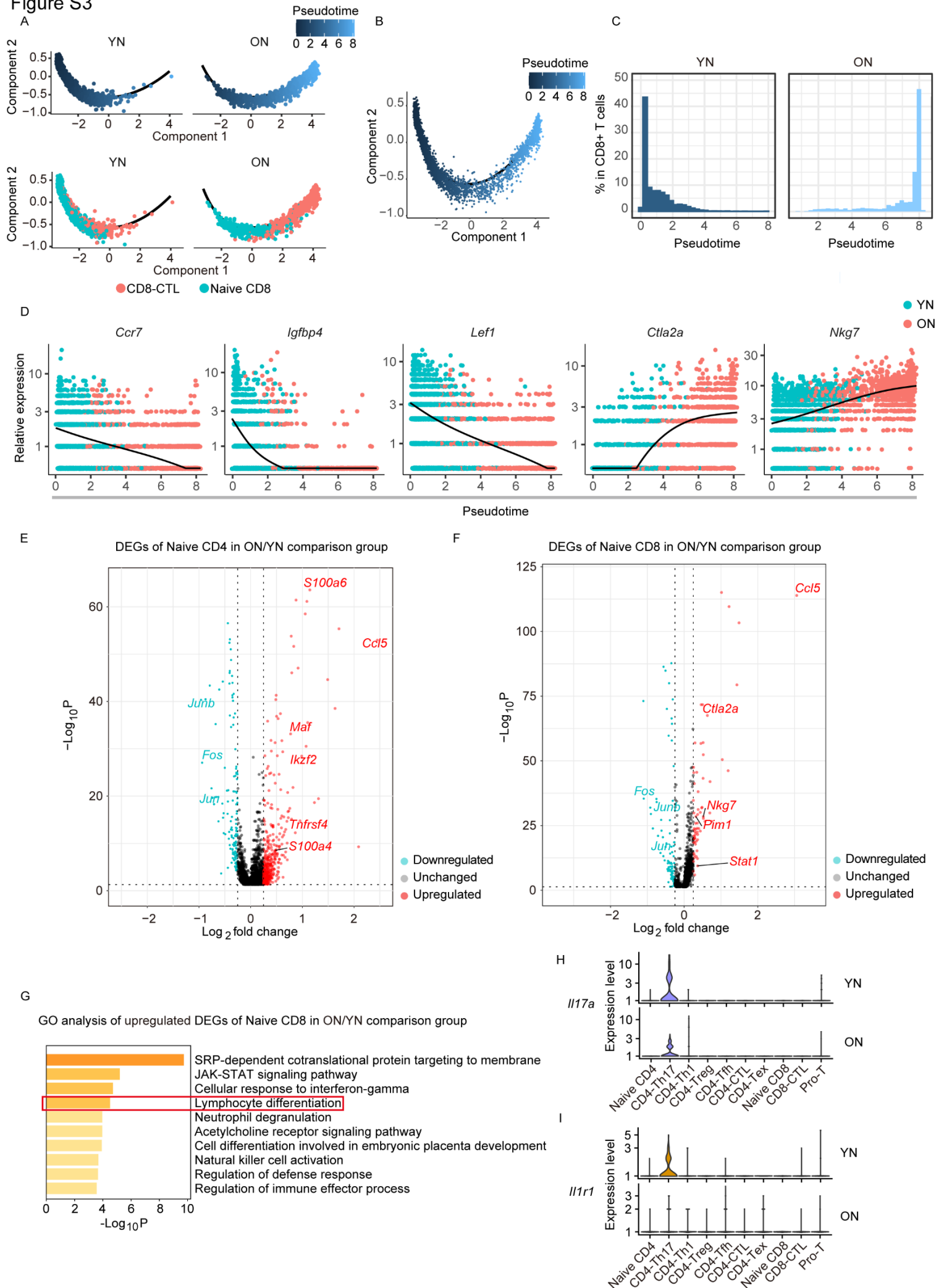
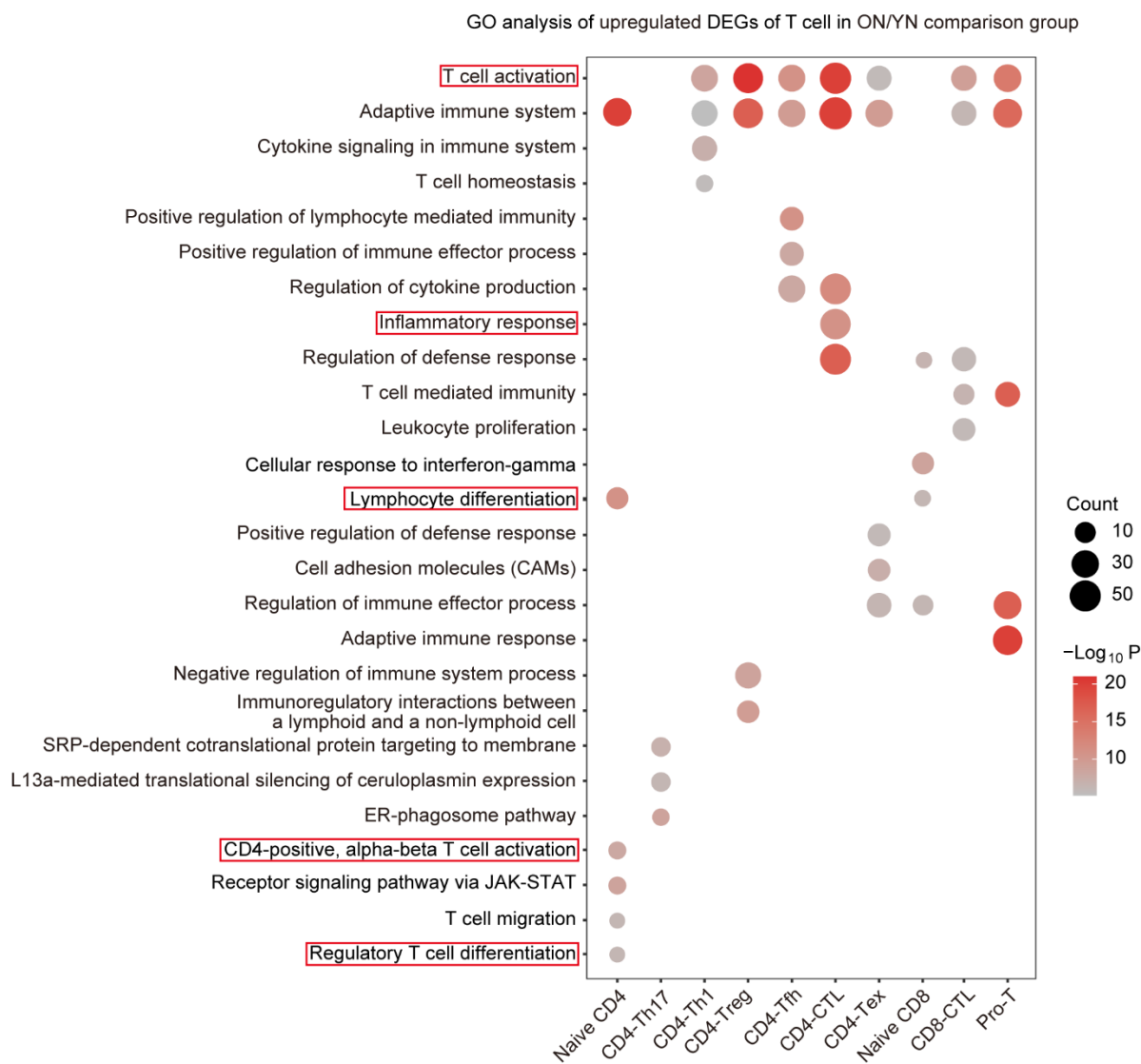


Figure S4

A



B



Figure S5

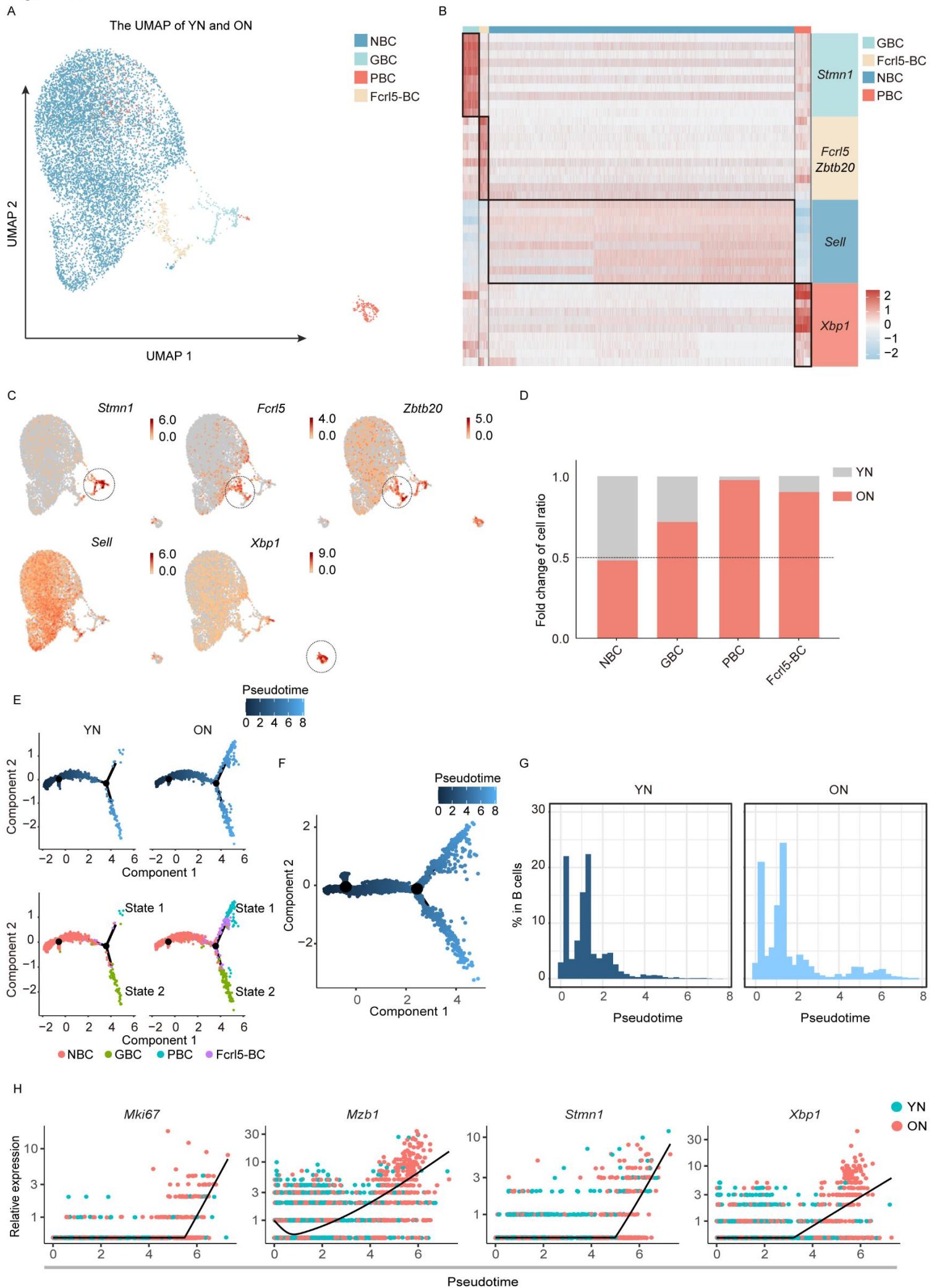


Figure S6

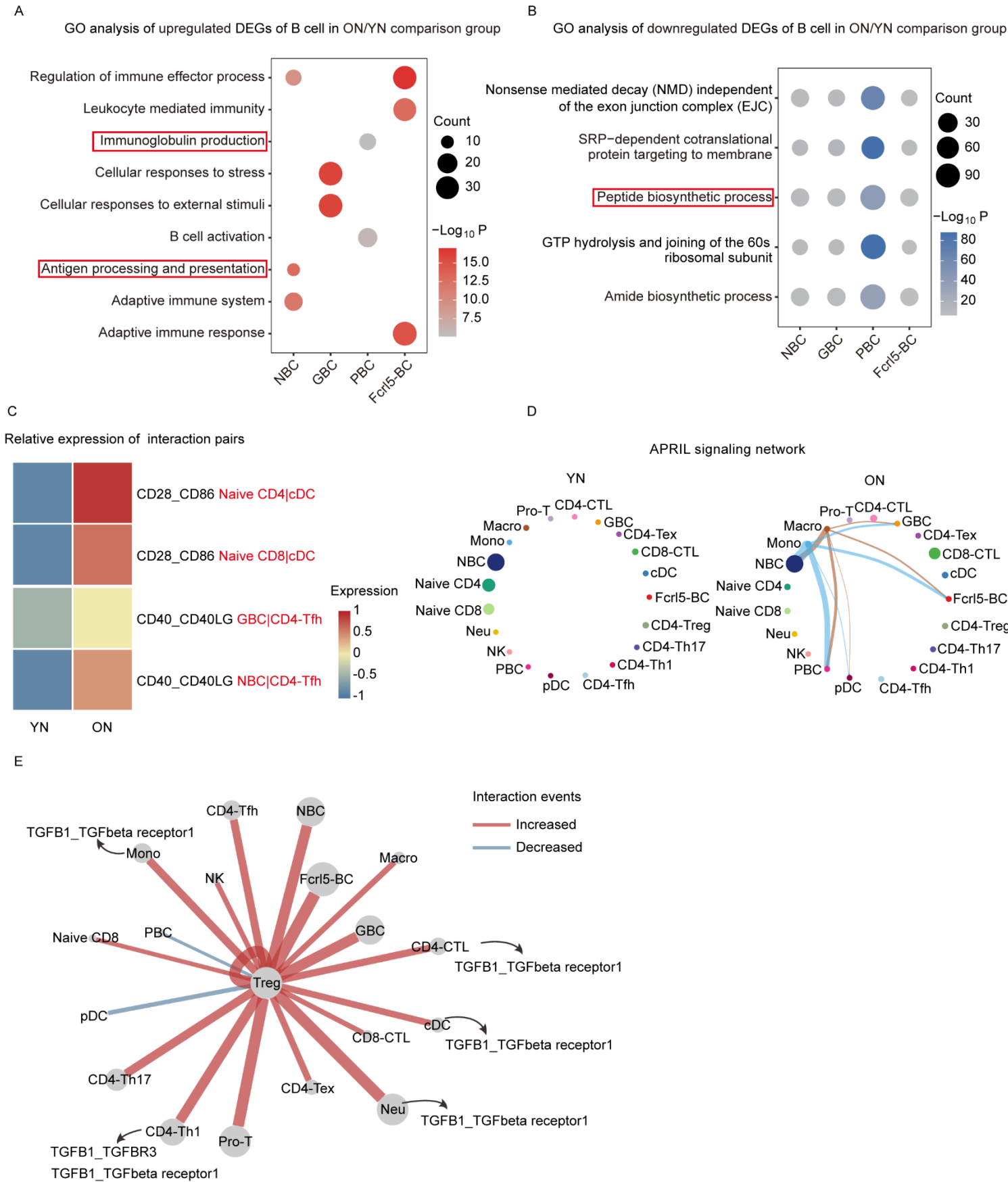
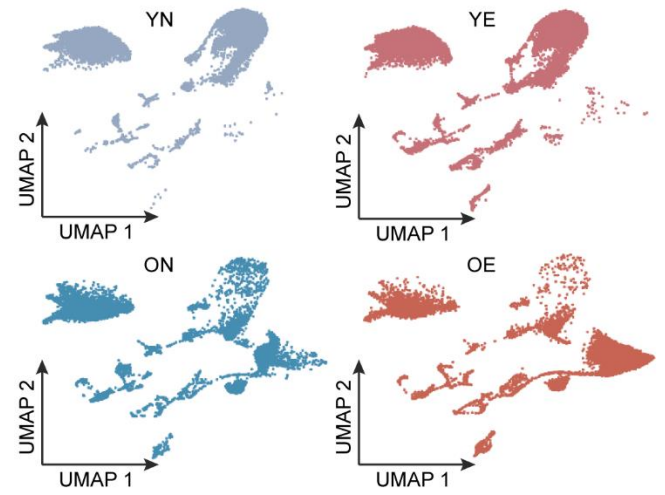


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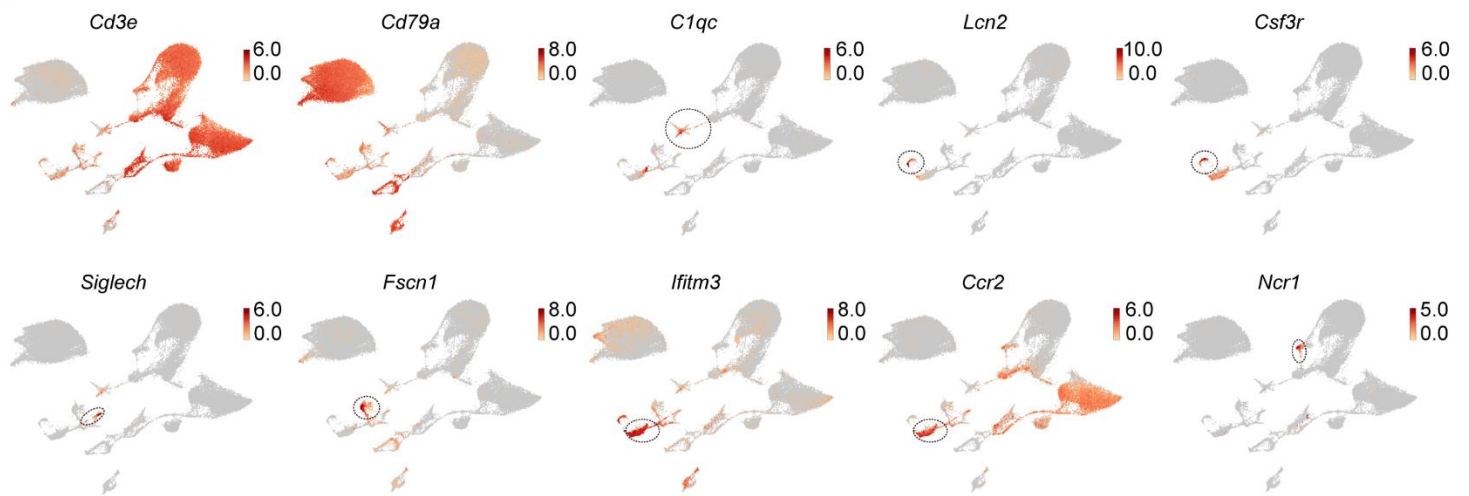
A



B



C



D

GO analysis of downregulated DEGs

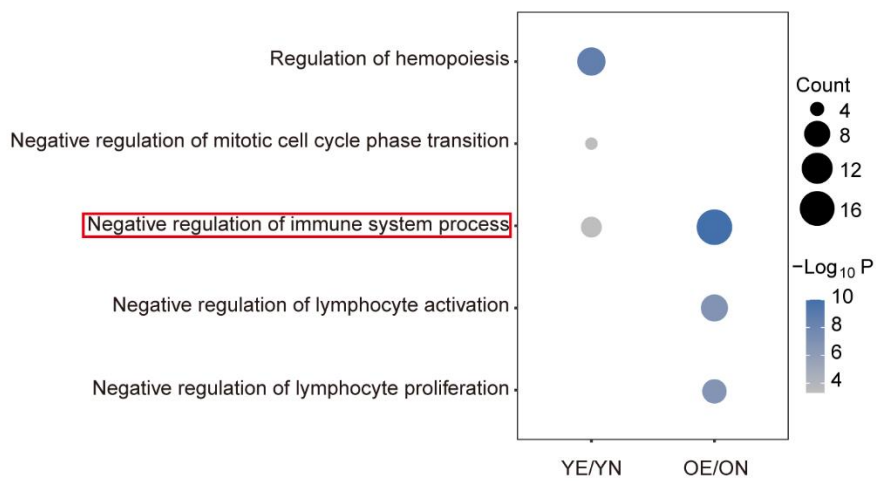


Figure S8

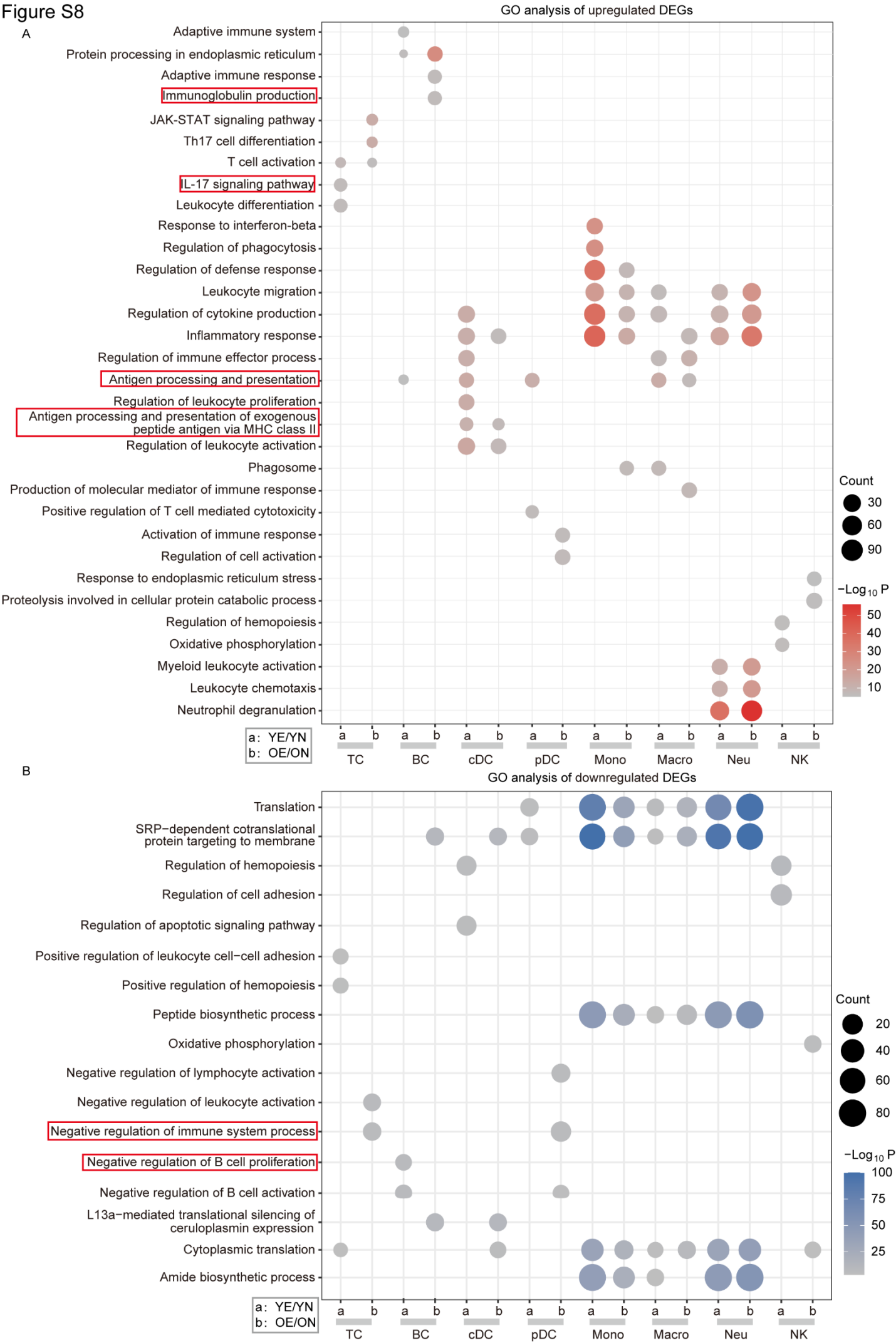
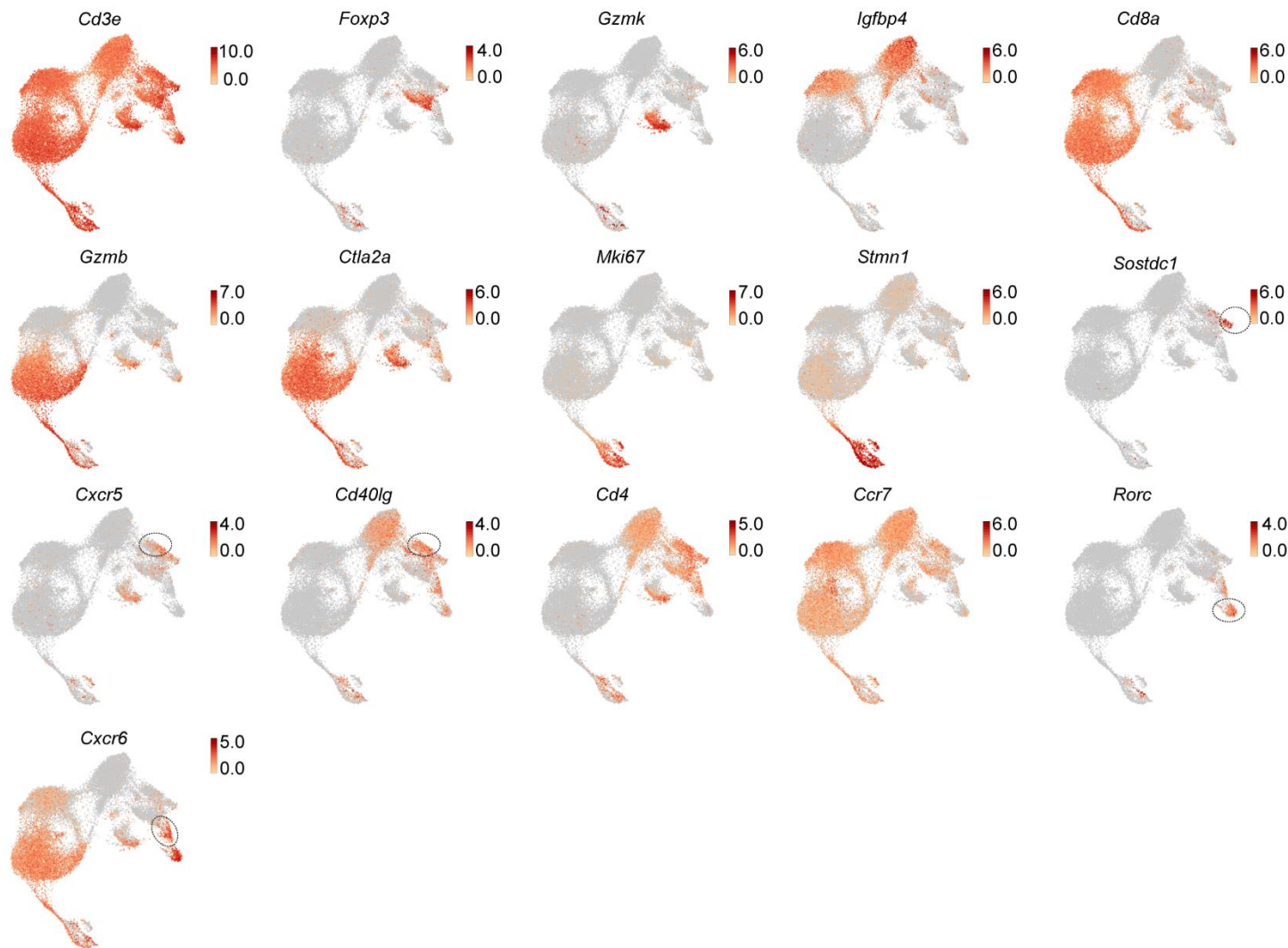


Figure S9

A



B

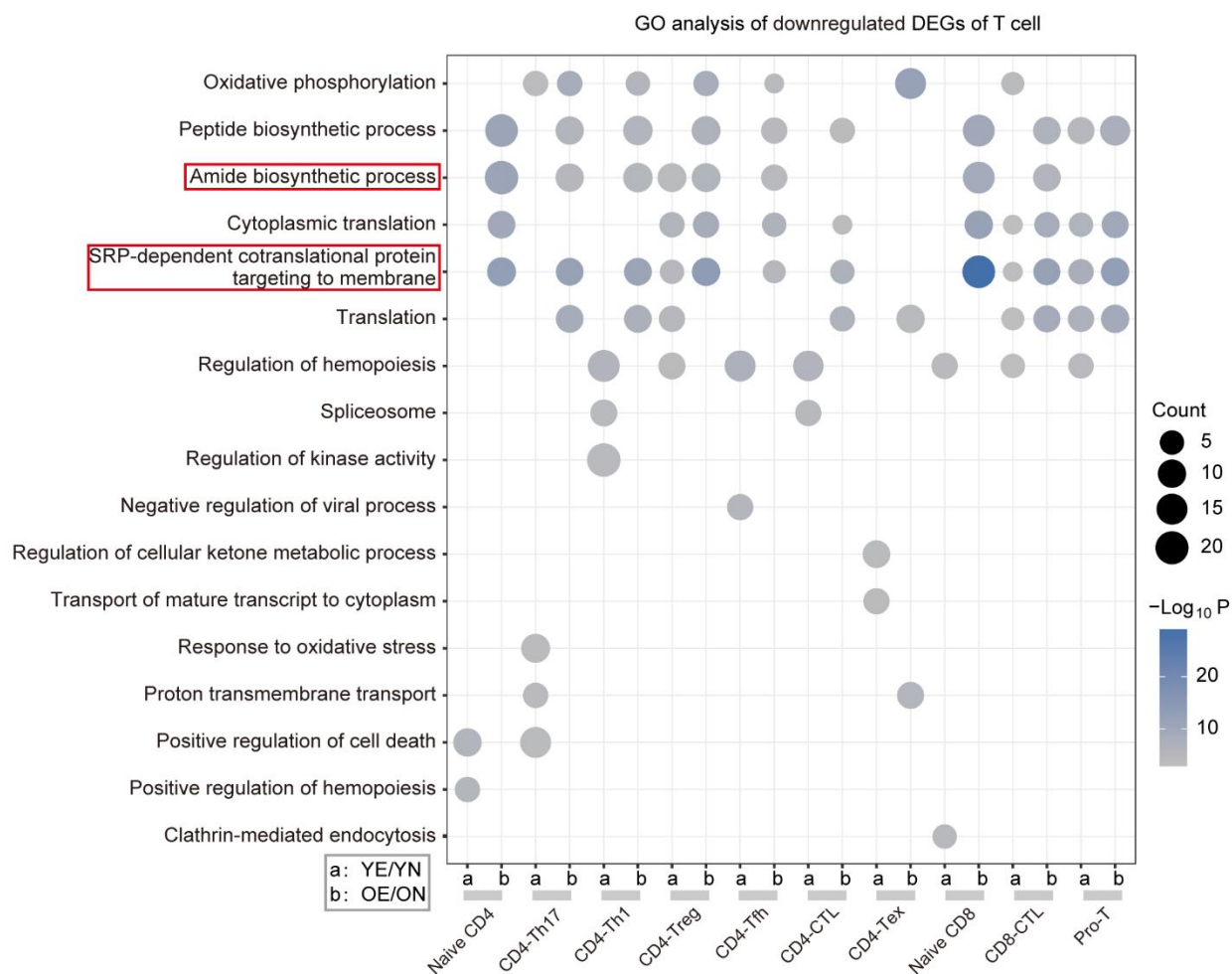


Figure S10

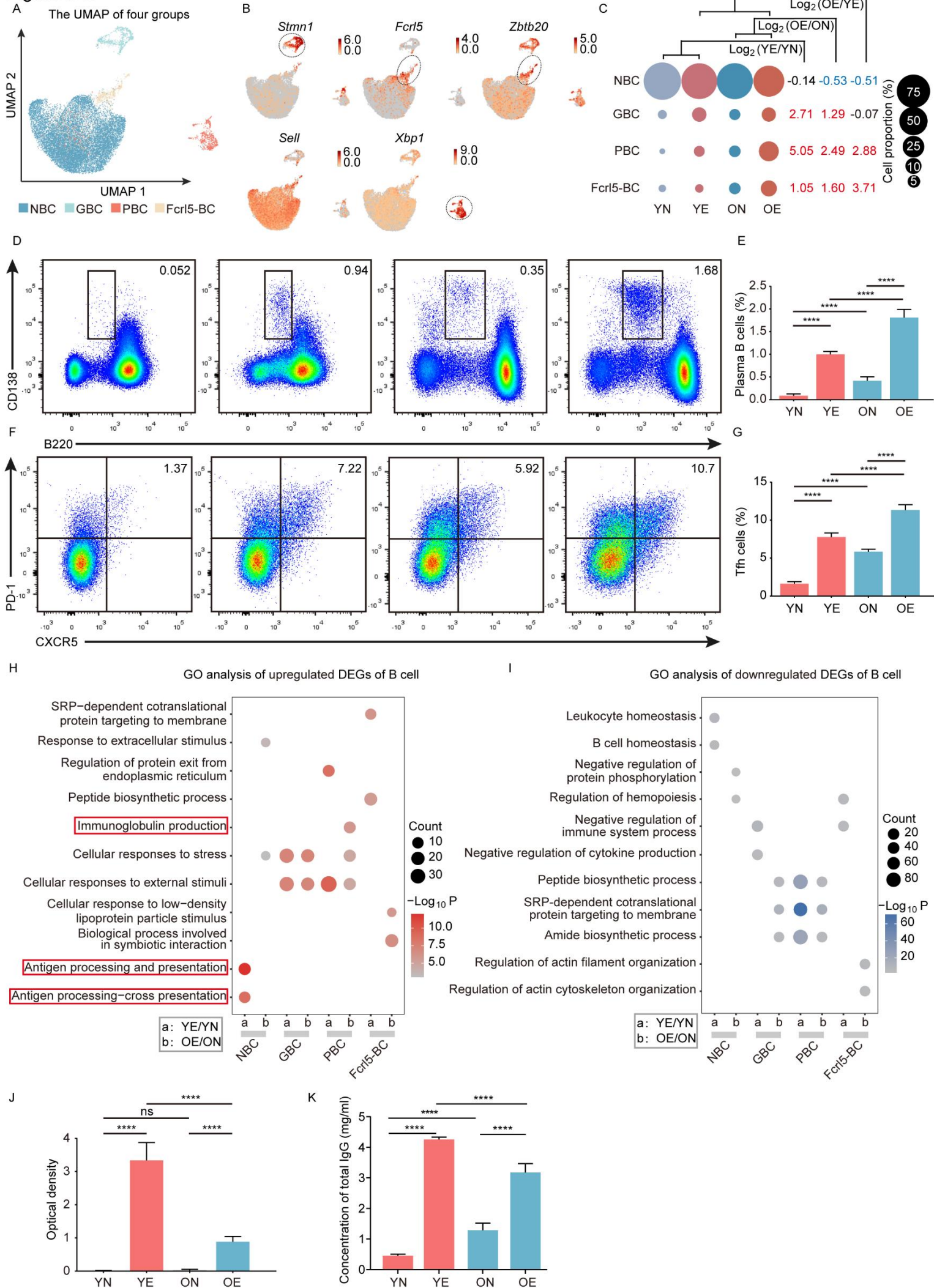


Figure S11

