

Figure S1

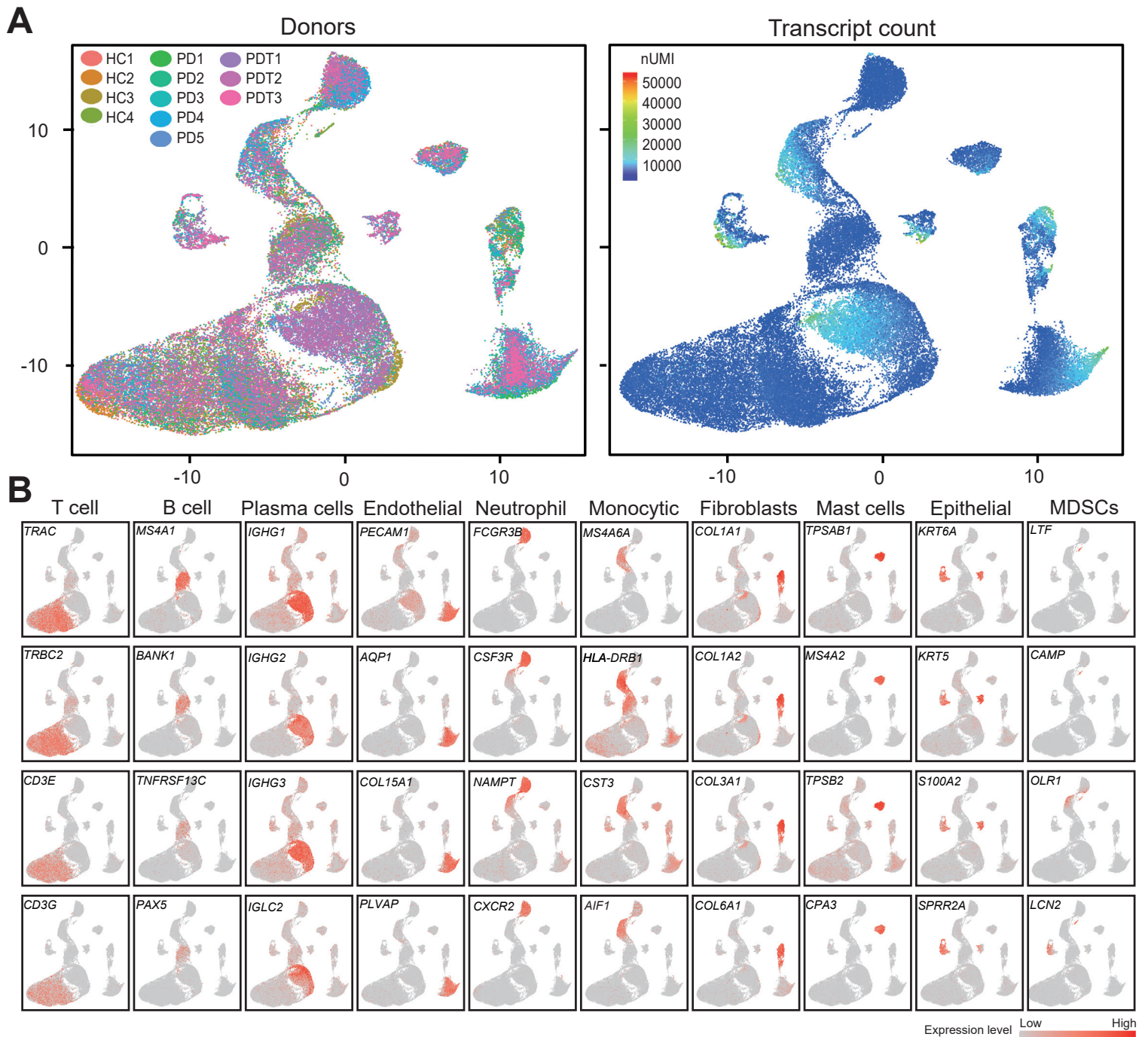


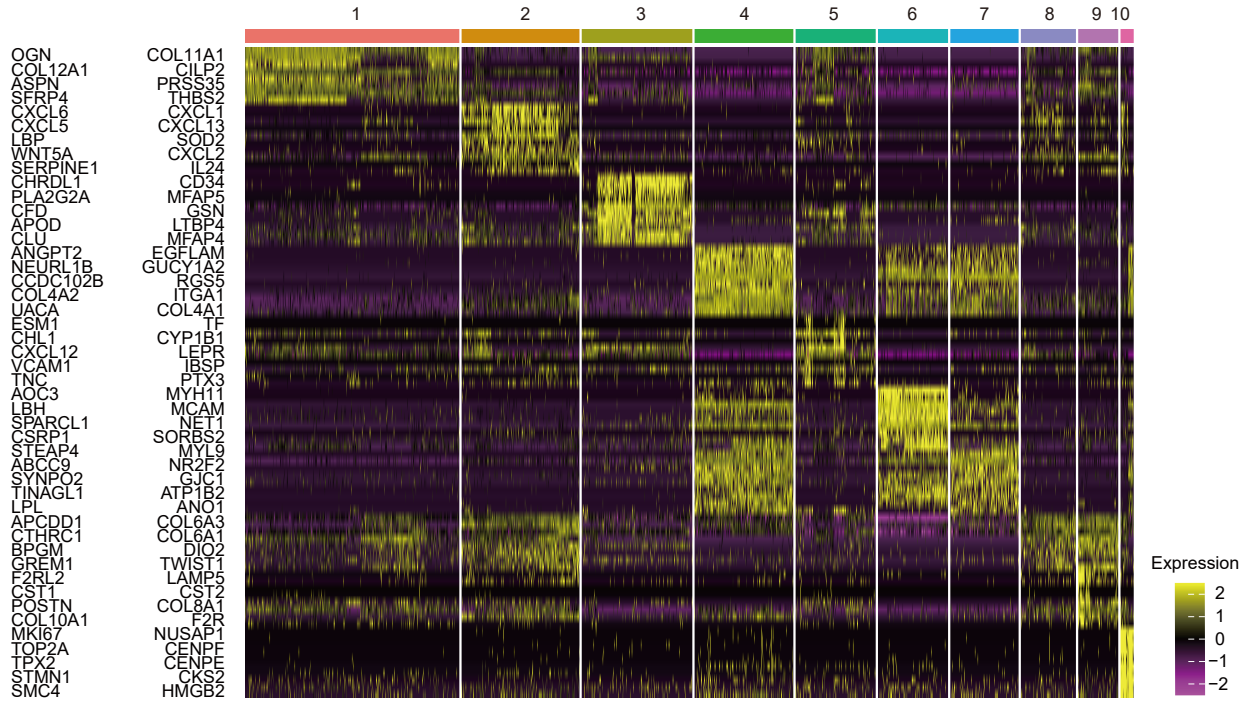
Figure S1. The overview of clustering results related to Figure 1

(A) UMAP of the 51248 cells, annotated and colored by (left to right): the corresponding donors (HCs, PDs, and PDTs) and the number of transcripts (UMIs) detected in that cell (log scale as defined in the inset). MDSCs: Myeloid-derived suppressor cells.

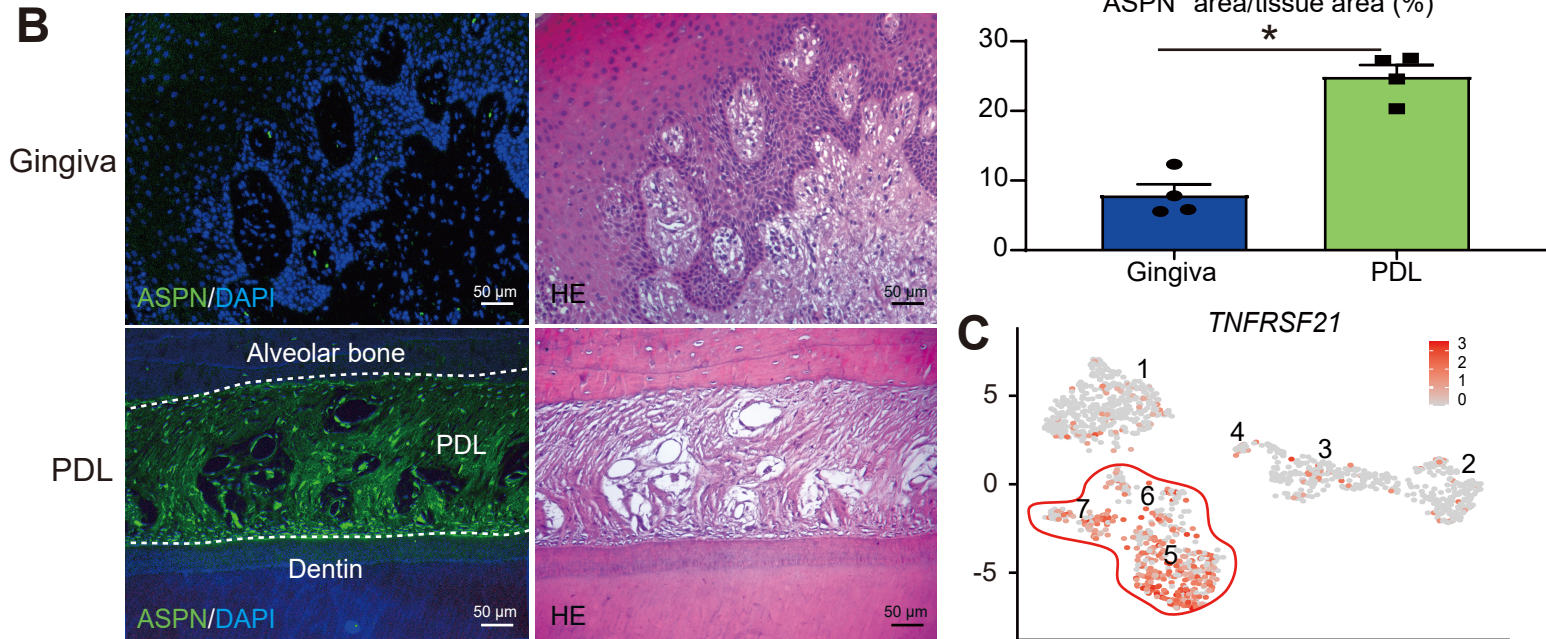
(B) UMAP plots pseudocolored for expression of the marker genes from Fig. 1C (top row) and of 3 additional marker genes (3 bottom rows) for the major cell lineages. Show are, as markers for T cells: genes encoding T-Cell Receptor Alpha Constant (TRAC), T Cell Receptor Beta Constant 2 (TRBC2), CD3e Molecule (CD3E) and CD3g Molecule (CD3G); for B cells: genes encoding Membrane Spanning 4-Domains A1 (MS4A1), B cell scaffold protein with ankyrin repeats 1 (BANK1), TNF Receptor Superfamily Member 13C (TNFRSF13C), Paired Box 5 (PAX5); for Plasma cells: genes encoding Immunoglobulin Heavy Constant Gamma 1 (IGHG1), Immunoglobulin Heavy Constant Gamma 2 (IGHG2), Immunoglobulin Heavy Constant Gamma 3 (IGHG3) and Immunoglobulin Lambda Constant 2 (IGLC2); for endothelial cells: genes encoding Platelet And Endothelial Cell Adhesion Molecule 1 (PECAM1), Aquaporin 1 (AQP1), Collagen type XV alpha 1 chain (COL15A1) and Plasmalemma Vesicle Associated Protein (PLVAP); for neutrophils: genes encoding Fc fragment of IgG receptor IIIb (FCGR3B), Colony stimulating factor 3 receptor (CSF3R), Nicotinamide Phosphoribosyltransferase (NAMPT) and C-X-C Motif Chemokine Receptor 2 (CXCR2); for monocytic cells: genes encoding Membrane Spanning 4-Domains A6A (MS4A6A), Major Histocompatibility Complex, Class II, DR Beta 1 (HLA-DRB1), Cystatin C (CST3) and Allograft Inflammatory Factor 1 (AIF1); for fibroblasts: genes encoding Collagen type I alpha 1 (COL1A1), Collagen type I alpha 2 (COL1A2), Collagen type III alpha 1 (COL3A1) and Collagen type VI alpha 1 (COL6A1); for mast cells: genes encoding Tryptase alpha/beta 1 (TPSAB1), Membrane spanning 4-domains A2 (MS4A2), Tryptase beta 2 (TPSB2) and Carboxypeptidase A3 (CPA3); for Epithelial cells: genes encoding Keratin 6A (KRT6A), keratin 5 (KRT5), S100 calcium binding protein A2 (S100A2) and Small proline rich protein 2A (SPRR2A); for MDSCs: genes encoding Lactotransferrin (LTF), Cathelicidin antimicrobial peptide (CAMP), oxidized low density lipoprotein receptor 1 (OLR1) and Lipocalin 2 (LCN2).

Figure S2

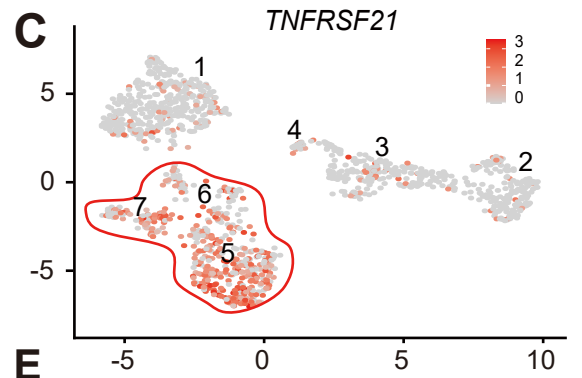
A



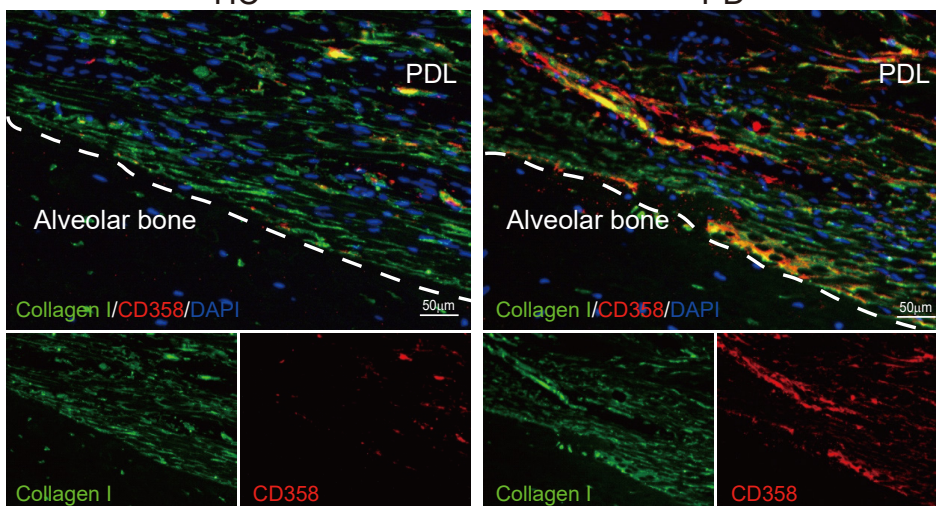
B



C



D



E

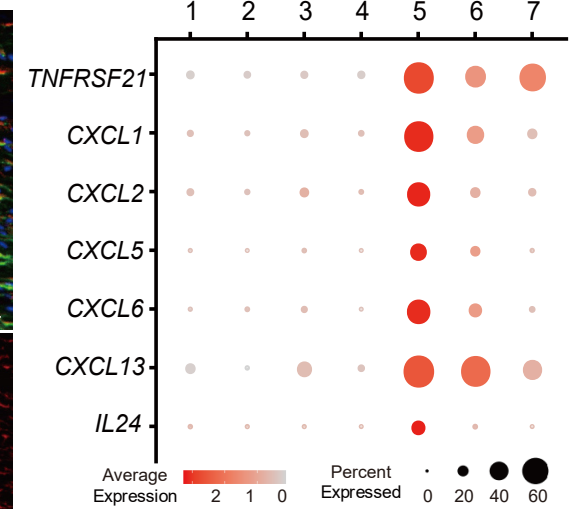


Figure S2. Characterization of Fibroblasts related to Figure 2

(A) Heatmap of the relative expression level of the top 8-10 differentially expressed maker genes identified in the fibroblast clusters in Figure 2A. (B) Representative images of immunofluorescence staining of gingiva (top left panels) and PDL (bottom left panels) from HC sample for showing the location of ASPN (green). Scale bar = 50 μm. Adjacent paraffin sections were stained with H & E staining to show the location. The top right panel shows quantification of ASPN⁺ area/tissue area (%) in gingiva and PDL.

(C) UMAP as in Figure 2B colored by expression of TNFRSF21. Red contour: TNFRSF21⁺ fibroblasts (subcluster 4-6).

(D) Representative images of immunofluorescence staining of periodontal tissues from HC (left panels) and PD samples (right panels) for double fluorescent analysis of Collagen I (green) and CD358 (red) expression. Top panel: Merged; bottom left: Collagen I; bottom right: CD358. Scale bar = 50μm.

(E) Bubble plot showing expressions (dots) of the selected marker genes (columns) in each subcluster (rows, as in Figure 2B). Dot colored by the average expression level, and dot size proportional to the percentage expression.

Figure S3

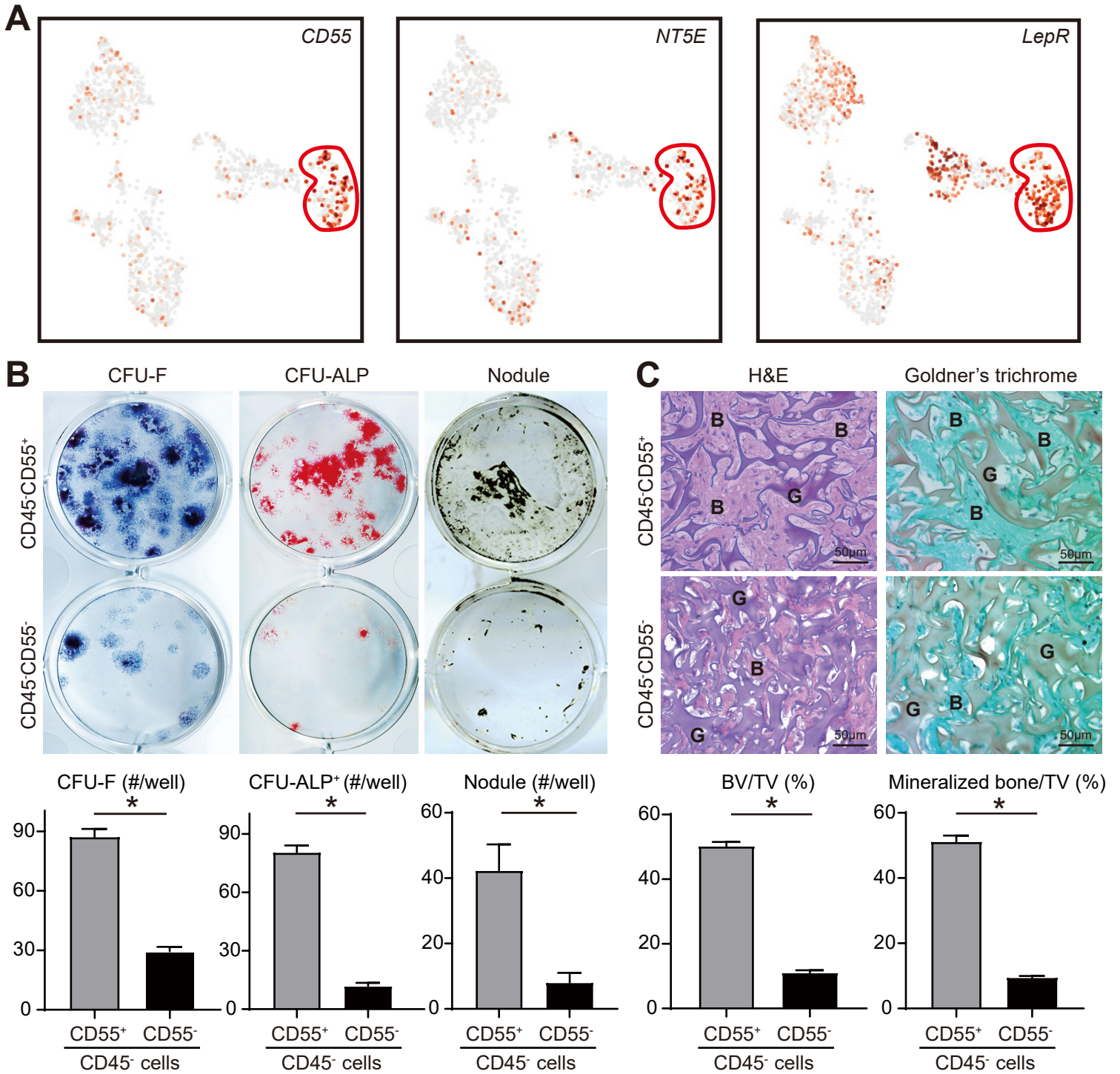


Figure S3. Characterization of CD55⁺ MSCs in periodontal tissue

(A) UMAP as in Figure 2B colored by expression of *CD55*, *NT5E*, and *LepR* (from left to right). Red contour: CD55⁺ MSC (subcluster 2).

(B) Representative images of in vitro assays to determine OB differentiation potential of CD45⁻CD55⁺ cells compared with those of CD45⁻CD55⁻ cells. Periodontal cells of a clinically healthy donor were collected and stained with various antibodies and subjected to flow cytometry sorting. CD45⁻CD55⁺ cells and CD45⁻CD55⁻ cells were collected and subjected to the following assays. For osteogenic induction, colony-forming units (CFU-F, left)/alkaline phosphatase (ALP)-positive colony-forming units (CFU-ALP, middle)/nodules (right) formation were examined.

(C) CD45⁻CD55⁺ cells and CD45⁻CD55⁻ cells were transplanted by subcutaneous surgical implantation into recipient SCID mice. 4 weeks later, the implants were harvested. Upper panels: Representative images of H&E staining (left) and Goldner's Trichrome staining (right) of serial sections of paraffin-embedded ectopic bones. B: bone. G: GelFoam. Bar = 50 μ m. Bottom panels: Histomorphometric analysis of bone volume to tissue volume (%) in H&E-stained sections and mineralized bone to tissue area (%) in Goldner's Trichrome staining.

Figure S4

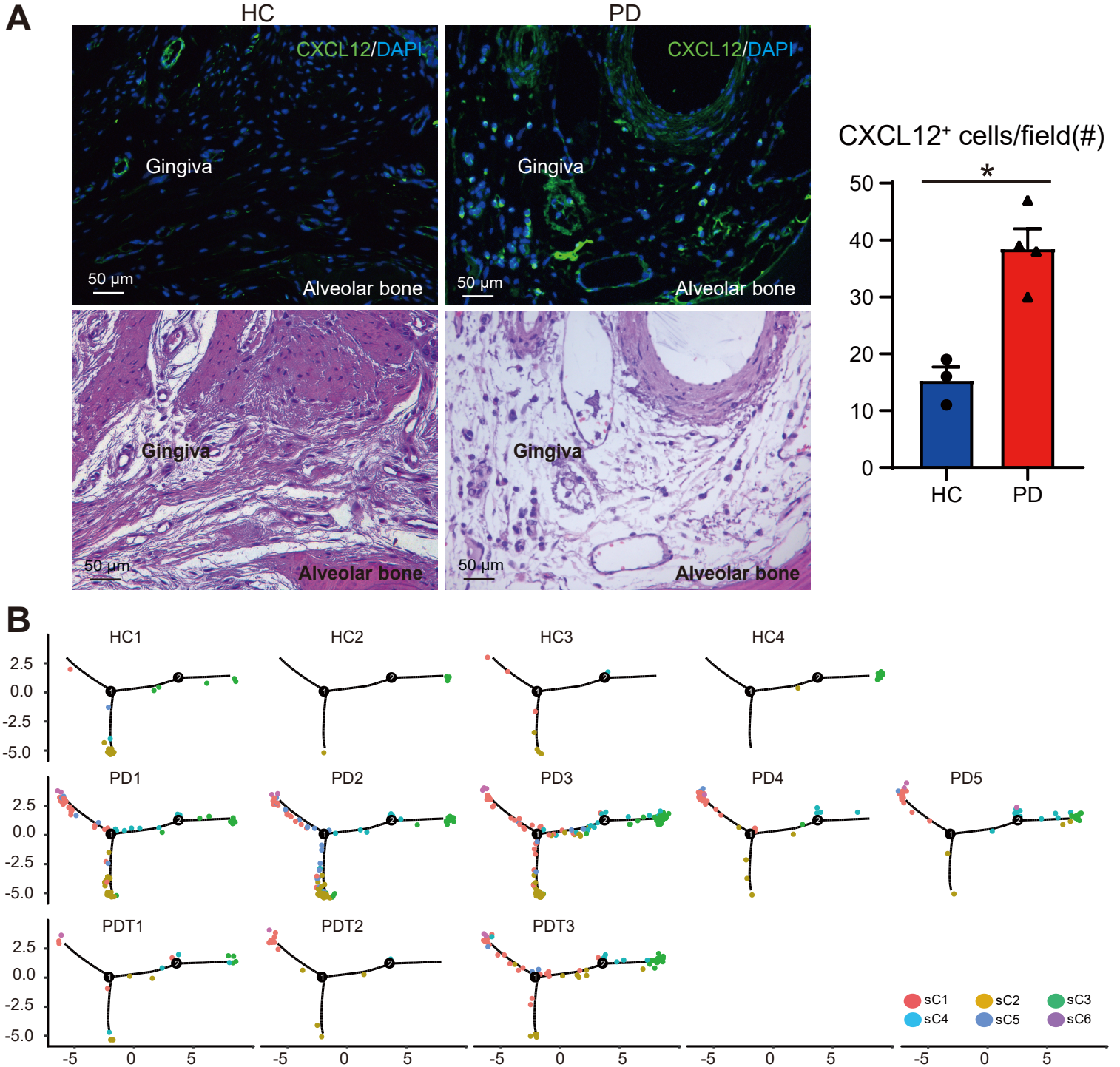


Figure S4. CXCL12 expression in periodontal tissue and the pseudotime trajectory plots from distinct donors related to Figure 3

(A) Representative images of immunofluorescence staining of periodontal tissues from HC (left panel) and PD samples (right panel) for fluorescent analysis of CXCL12 (green). Adjacent paraffin sections were stained with H & E staining to show the location. Scale bar = 50 μ m. The right panel shows the quantification of CXCL12⁺ cells.

(B) The distribution of single cell colored according to subclusters as in Figure 3A from the corresponding donors (HCs, PDs, and PDTs) mapped onto pseudotime trajectory plots as in Figure 3C.

Figure S5

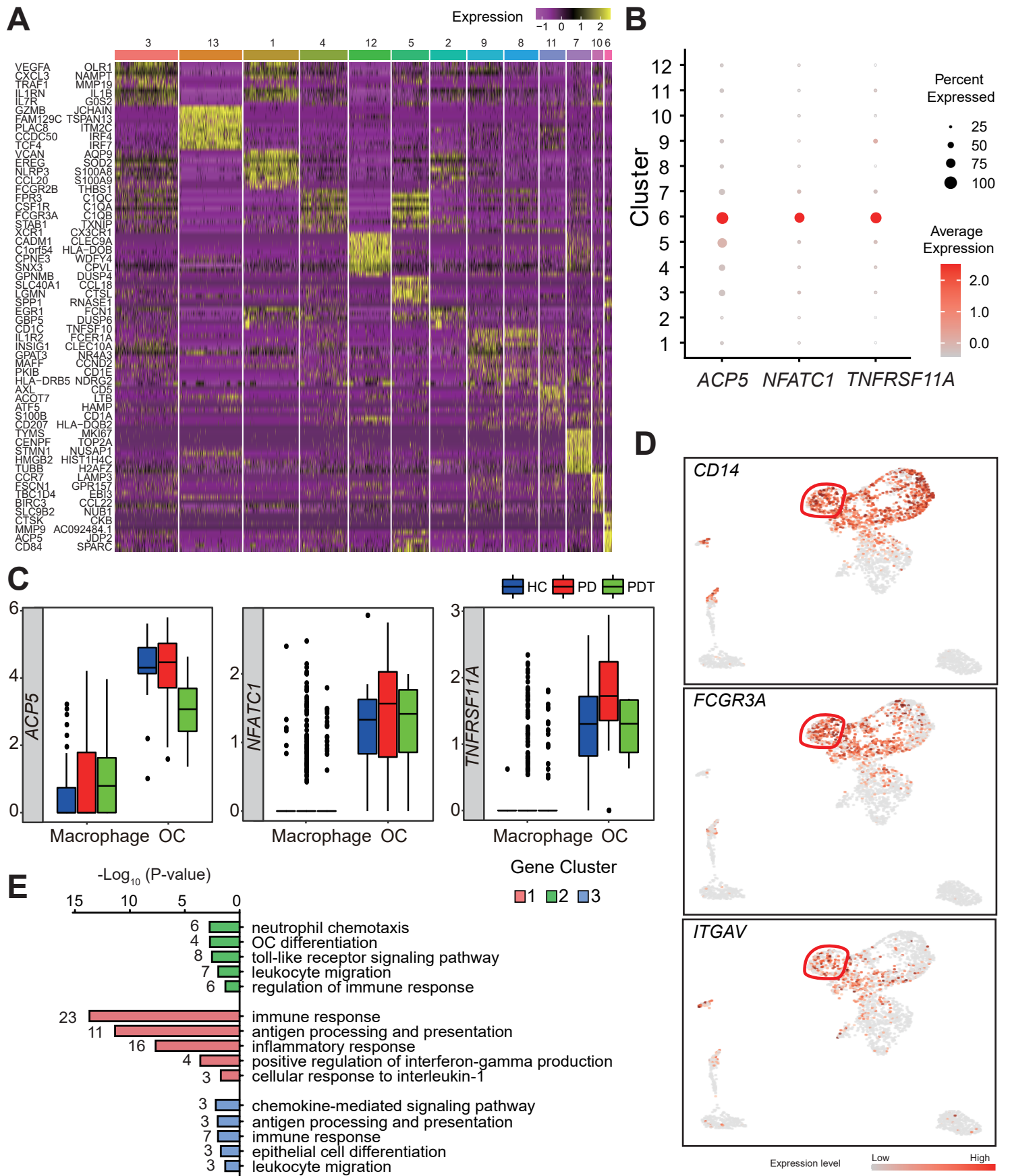
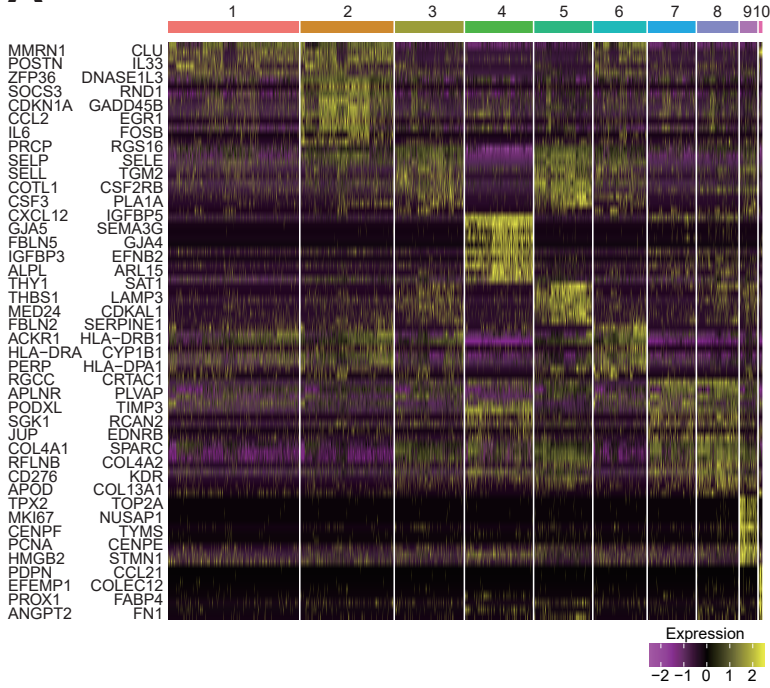


Figure S6

A



B

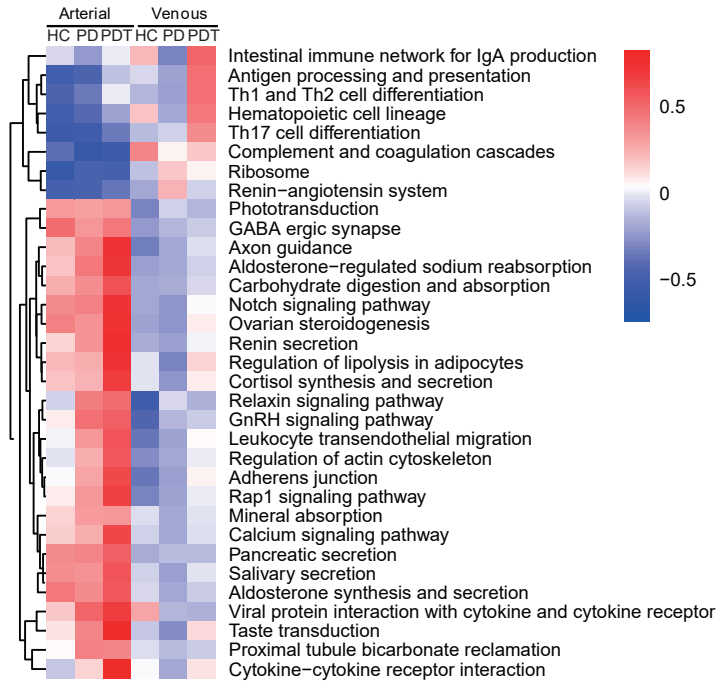


Figure S6. Characterization of ECs related to Figure 5

(A) Heatmap of the relative expression level of the top 8-10 differentially expressed marker genes identified in the endothelial clusters in Figure 5A.

(B) Heatmap of the QuSAGE activity for non-metabolism-related pathways that are associated with differentially expressed genes between the Arterial and Venous ECs clusters from HC, PD, and PDT samples (labels on the top). Red indicating increased average expression of genes in pathways. EC: Endothelial Cell.

Figure S7

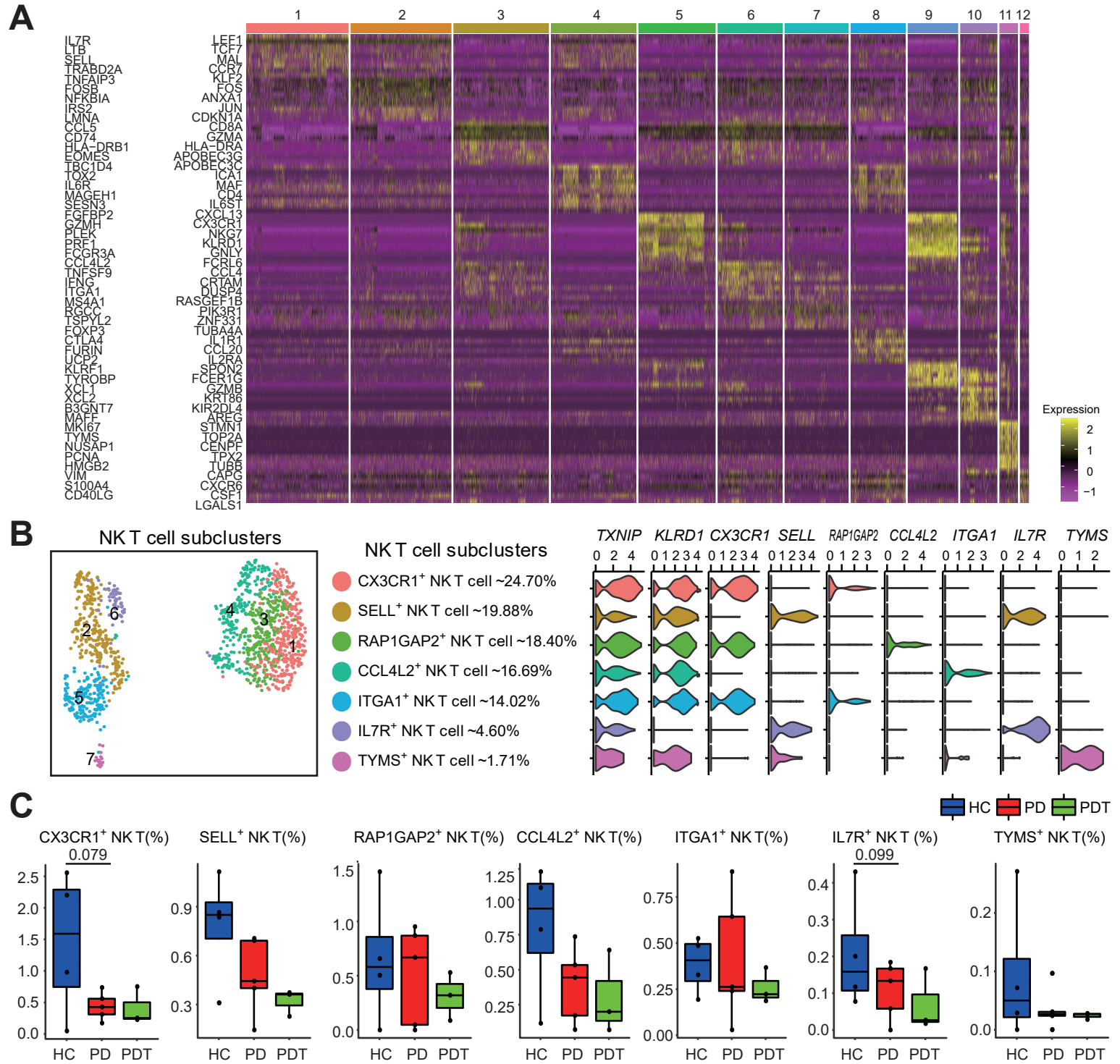


Figure S7. The heterogeneity of T cells and NK T cells

(A) Heatmap of the relative expression level of the top 8-10 differentially expressed maker genes identified in the T cell clusters in Figure 6A.

(B) Left panel: UMAP of 1348 NK T cells, annotated and colored by clustering with the label of each subcluster. Center panel: identified subclusters of NK T cells with the percentages shown. Right panel: Violin plots showing distinct expressions of the selected marker genes (row) in each subcluster of NK T cells as in the center panel.

(C) The box plots showing the percentage of cells for each of seven subclusters of NK T cells as in (A) from HC (blue, n=610), PD (red, n=479), and PDT (green, n=259) samples with plot center, box, whiskers, and points corresponding to median, IQR, $1.5 \times$ IQR and $>1.5 \times$ IQR, respectively. Data were analyzed using t test (two-tailed, two sample, equal variance)

Figure S8

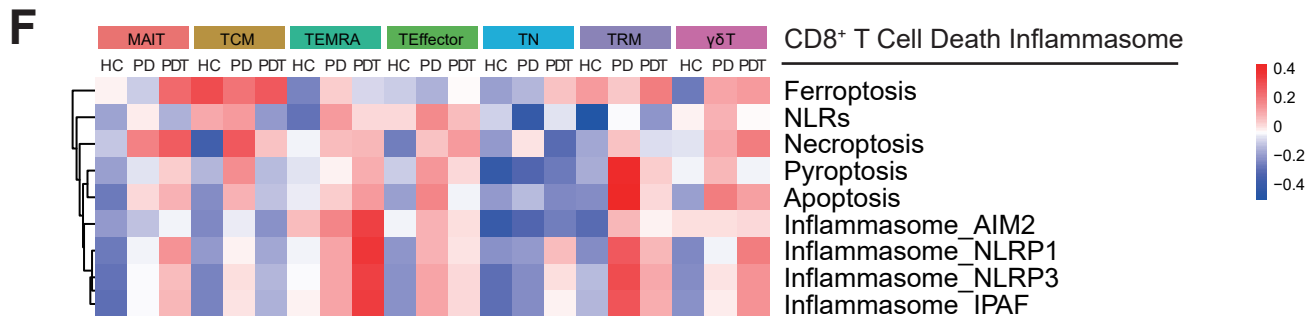
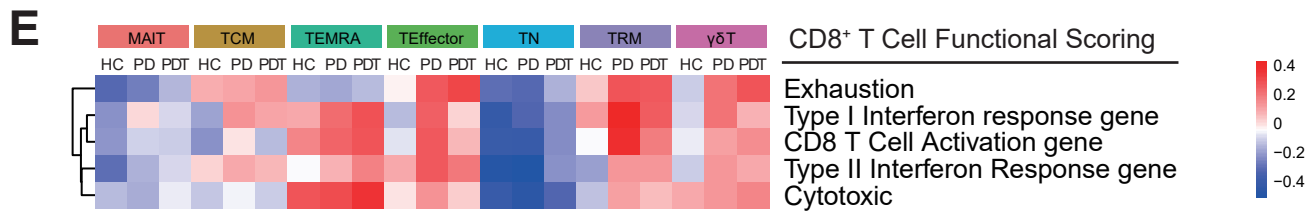
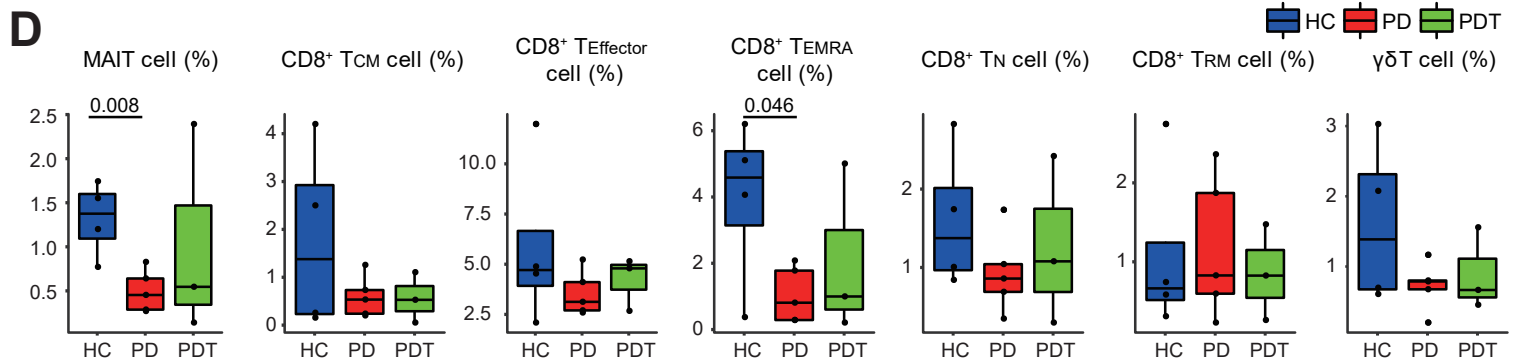
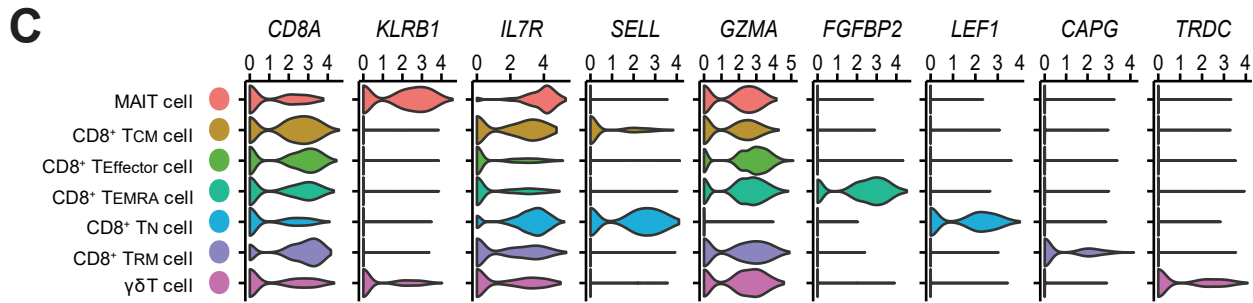
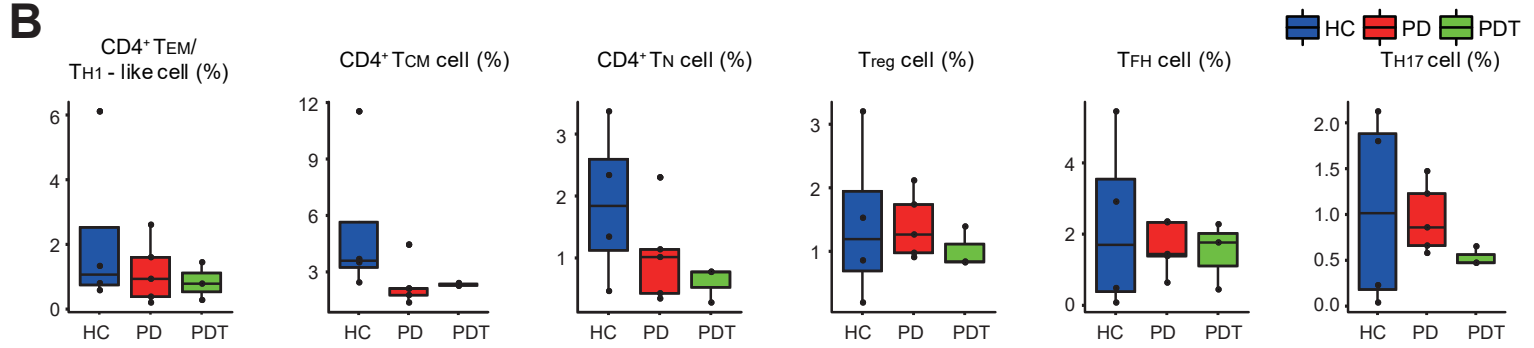
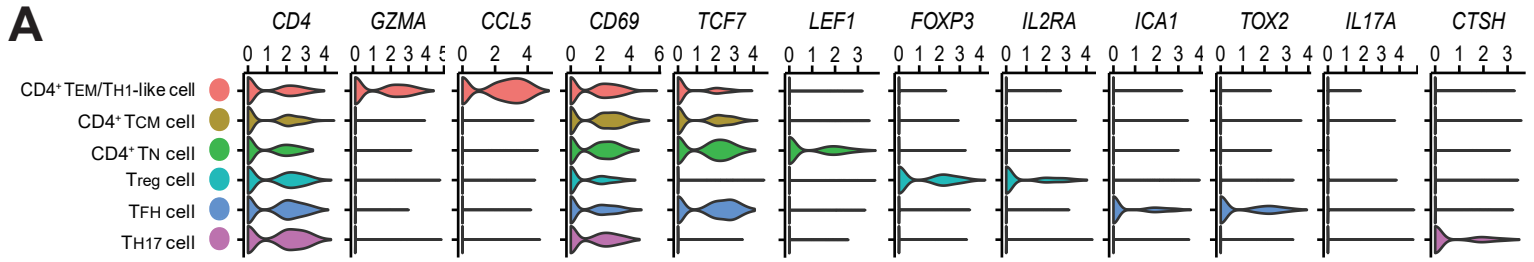


Figure S8. The heterogeneity of CD4⁺ and CD8⁺ T cell, and the characterization of CD8⁺ T cell

(A) Violin plots showing distinct expressions of the selected marker genes (row) in each subcluster (labels on left) of CD4⁺ T cells as in Figure 6C.

(B) The box plots showing the percentage of cells for each subcluster of CD4⁺ T cells from HC (blue, n=2266), PD (red, n=1637), and PDT (green, n=1163) samples with plot center, box, whiskers, and points corresponding to the median, IQR, $1.5 \times \text{IQR}$ and $>1.5 \times \text{IQR}$, respectively. Data were analyzed using t test (two-tailed, two sample, equal variance).

(C) Violin plots showing distinct expressions of the selected marker genes (row) in each subcluster (labels on left) of CD8⁺ T cells as in Figure 6D.

(D) The box plots showing the percentage of cells for each subcluster of CD8⁺ T cells from HC (blue, n=2604), PD (red, n=1695), and PDT (green, n=1652) samples with plot center, box, whiskers, and points corresponding to the median, IQR, $1.5 \times \text{IQR}$ and $>1.5 \times \text{IQR}$, respectively. Data were analyzed using t test (two-tailed, two sample, equal variance).

(E) Heatmap of the QuSAGE activity for the gene modules (labels on the right) that associated with cell functional genes among seven subclusters of CD8⁺ T cells from HC, PD, and PDT samples (labels on the top). Red indicates increased average expression of genes in the modules.

(F) Heatmap of the QuSAGE activity for gene modules (labels on the right) that associated with cell death inflammasome genes among seven subclusters of CD8⁺ T cells from HC, PD, and PDT samples (labels on the top). Red indicates an increased average expression of genes in the modules.

Figure S9

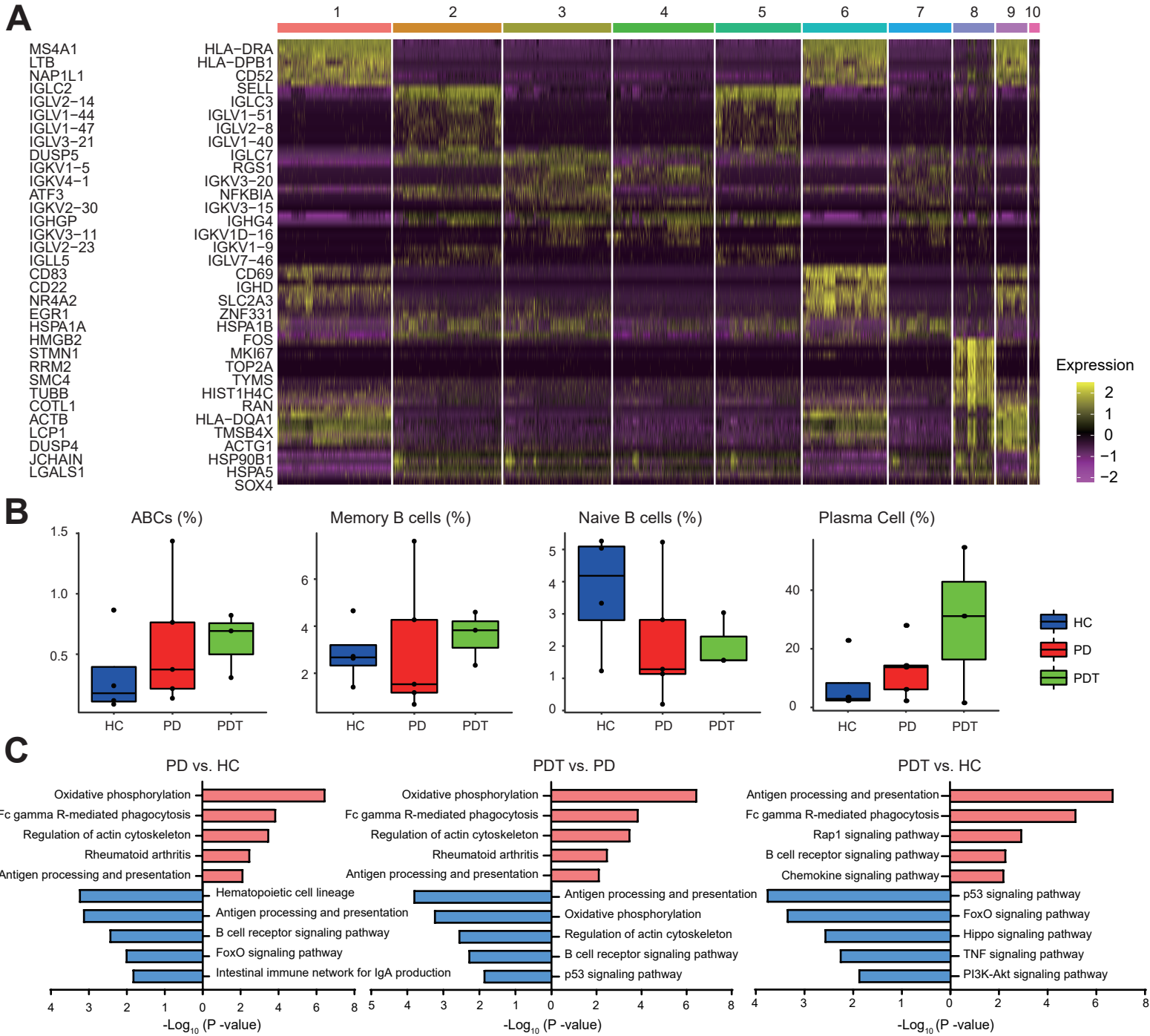


Figure S9. Characterization of B & Plasma cells related to Figure 6

(A) Heatmap of the relative expression level of the top 8-10 differentially expressed marker genes identified in the B & Plasma cells clusters in Figure 6G.

(B) The box plots showing the percentage of cells for each of four subclusters of B & Plasma cells, as in Figure 6G to I, from (blue, n=1039), PD (red, n=1078), and PDT (green, n=1131) samples with plot center, box, whiskers, and points corresponding to the median, IQR, $1.5 \times$ IQR and $>1.5 \times$ IQR, respectively. Data were analyzed using t test (two-tailed, two sample, equal variance).

(C) Pathways enrichment analysis associated with genes upregulated (red) and downregulated (blue) genes in (from left to right) PD vs. HC, PDT vs. PD and PDT vs. HC.

Figure S10

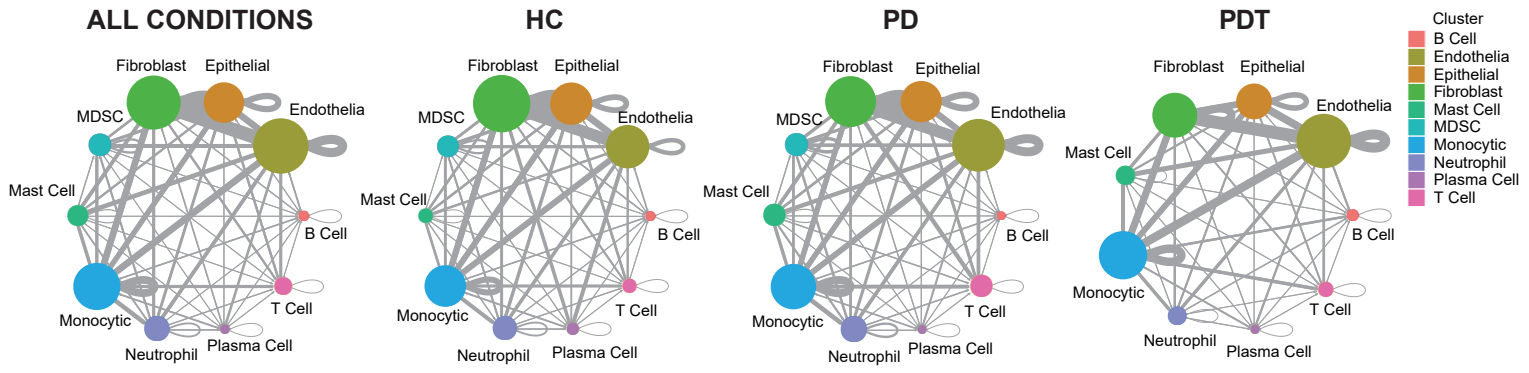


Figure S10. The circo plots under distinct conditions related to Figure 7A

The circo plots showing the potential cell interactions among ten major cell types as in Figure 1B in periodontal tissues predicted by Cellphone DB, from left to right: all conditions, HC, PD and PDT. The node size represents the number of interactions.

Figure S11

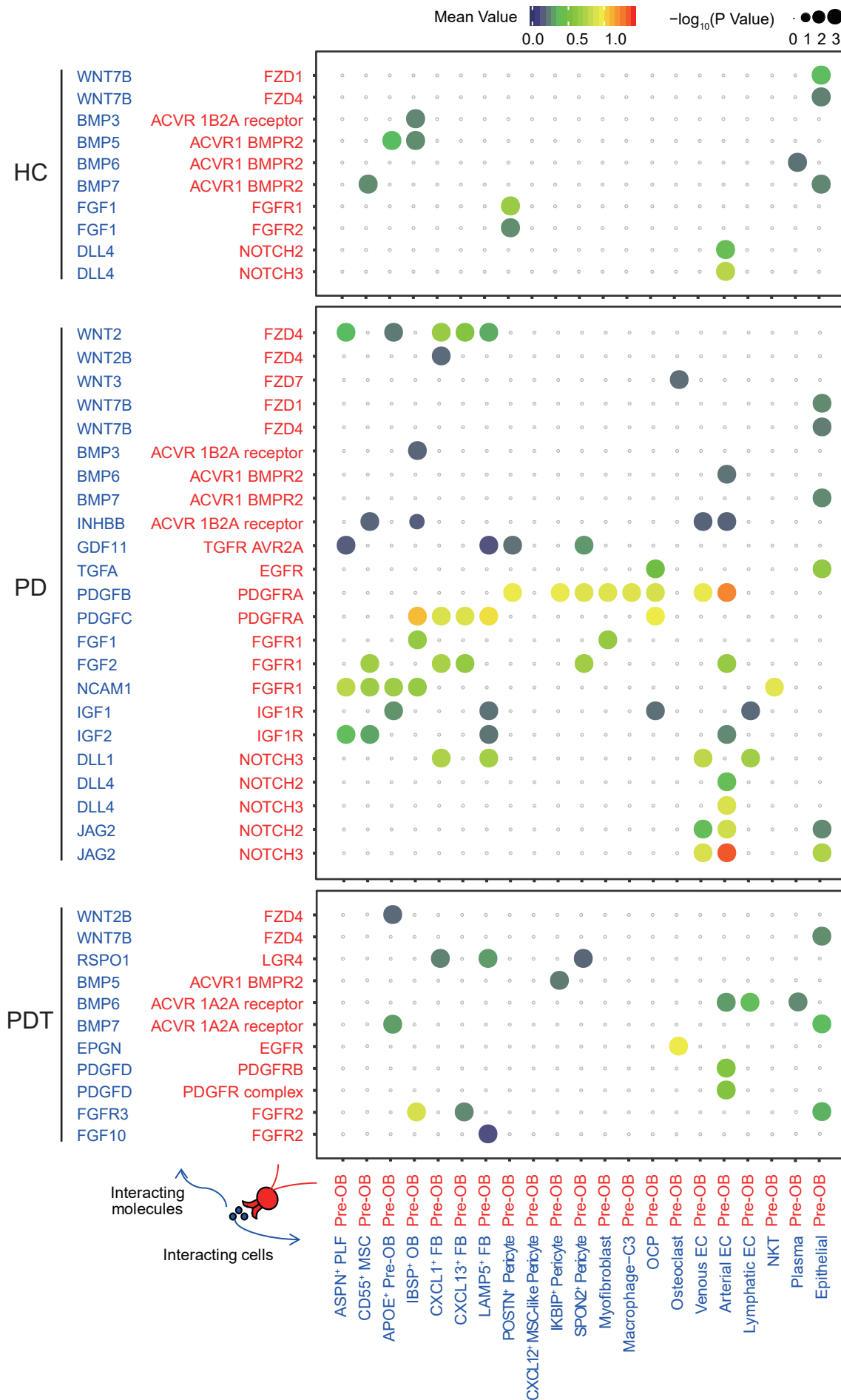


Figure S11. Expression of ligand-receptor pairs between Pre-OB and all cell types in three sample sets, related to Figure 7C

The dot plot generated by CellPhoneDB showing potential ligand-receptor pairs associated with osteogenesis between Pre-OB and all detected cellular types in HC, PD, and PDT groups (from top to bottom). Dots colored by mean expression of ligand-receptor pair between two clusters and dots size proportional to the value of $-\log_{10}(\text{P Value})$.

Figure S12

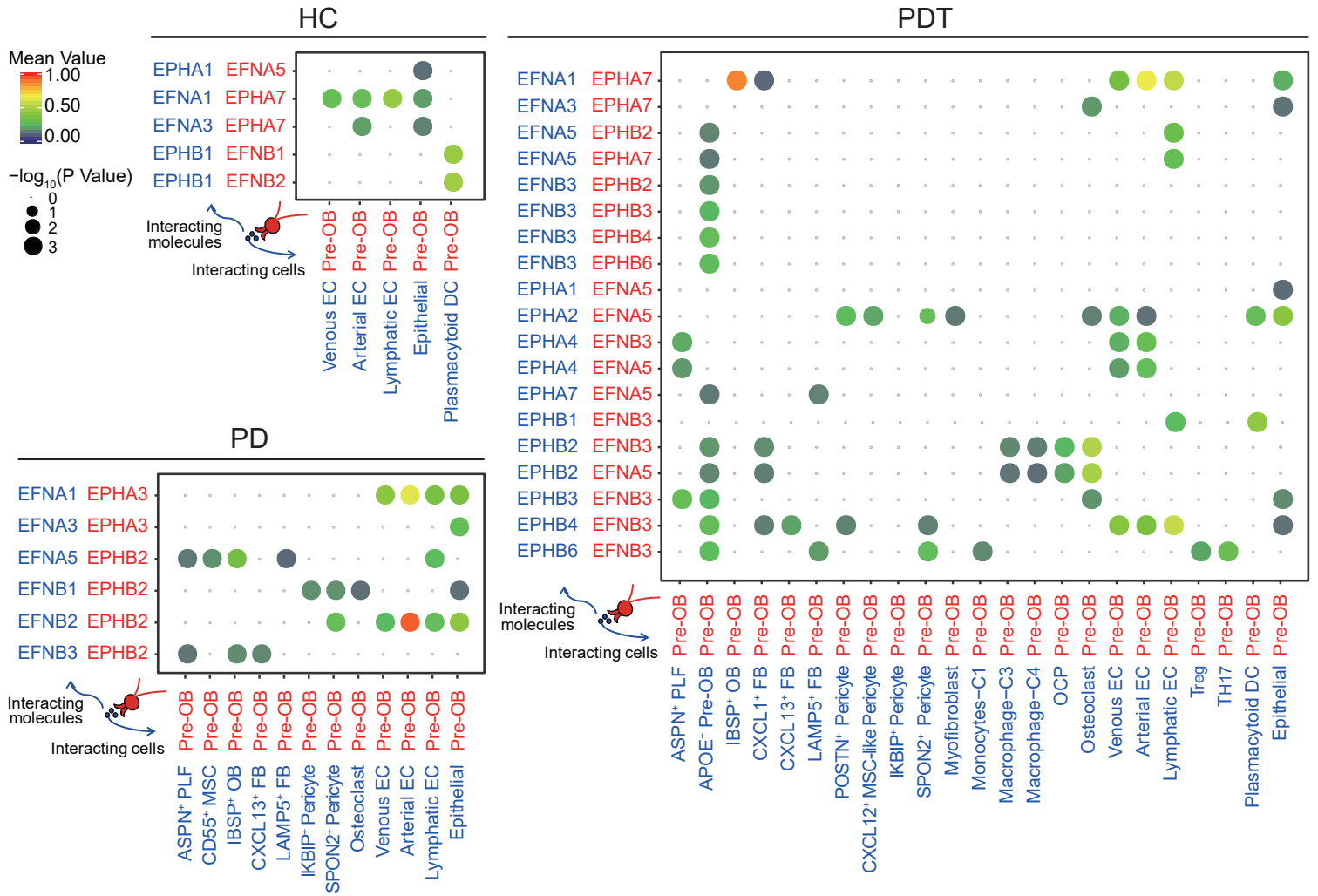


Figure S12. Expression of Ephrin-Ephn interactions between Pre-OB and all cell types in three sample sets, related to Figure 7E

The dot plots generated by CellPhoneDB showing potential ligand-receptor pairs associated with Ephrin-Ephn signaling pathway between Pre-OB and all detected cellular types in HC (top left), PD (bottom left), and PDT (right) groups. Dots colored by mean expression of ligand-receptor pair between two clusters and dots size proportional to the value of $-\log_{10}(P \text{ Value})$.

Figure S13

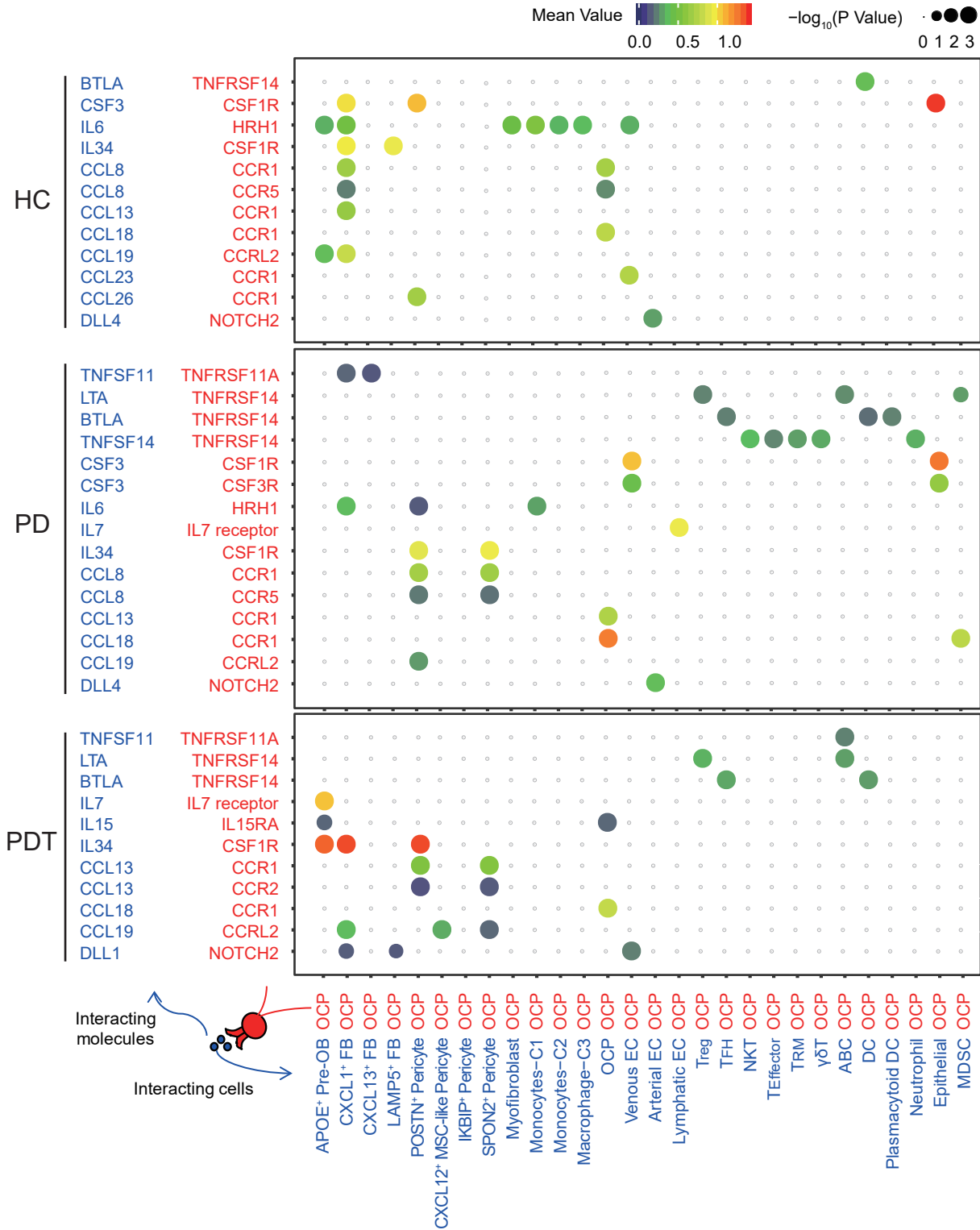
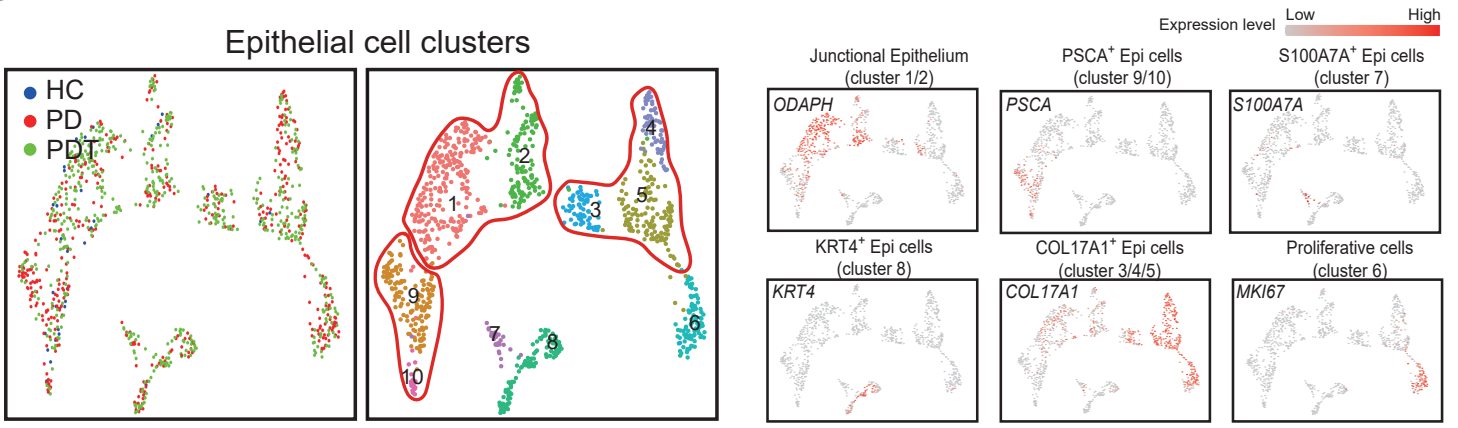


Figure S13. Expression of ligand-receptor pairs associated between OCP and all cell types in three sample sets

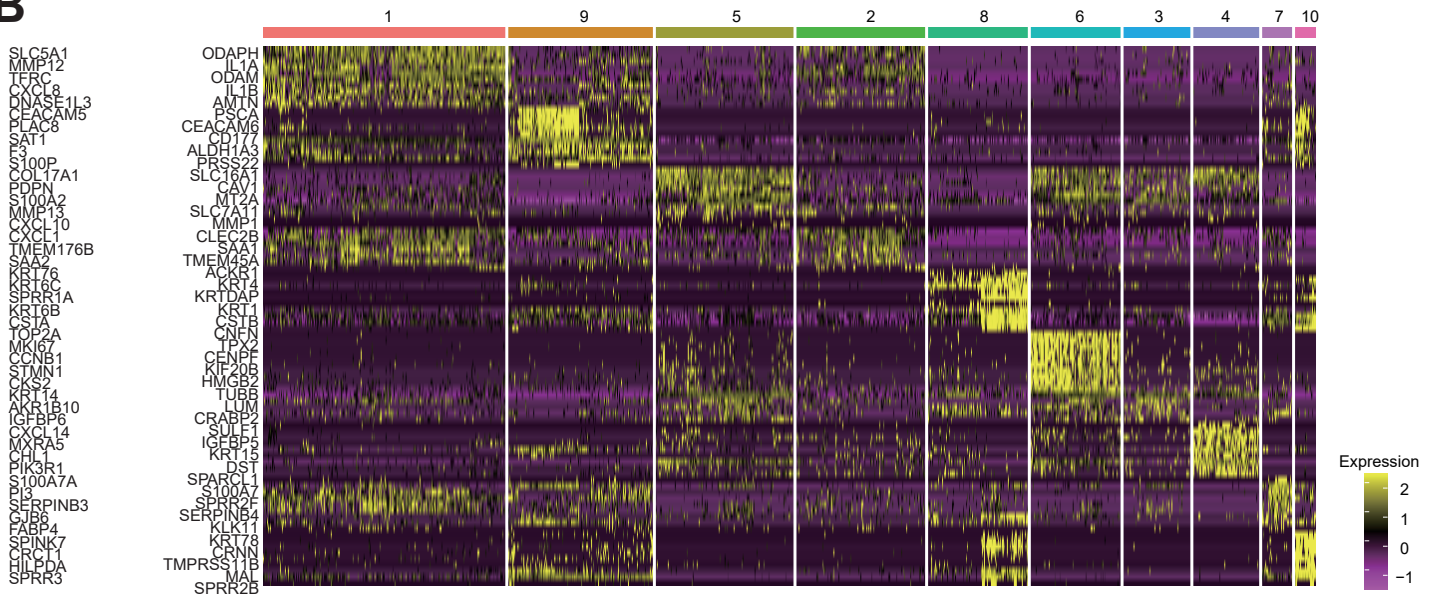
The dot plot generated by CellPhoneDB showing potential ligand-receptor pairs associated with osteoclastogenesis between OCP and all detected cellular types in HC, PD, and PDT groups (from top to bottom). Dots colored by mean expression of ligand-receptor pair between two clusters and dots size proportional to the value of $-\log_{10}(\text{P Value})$.

Figure S14

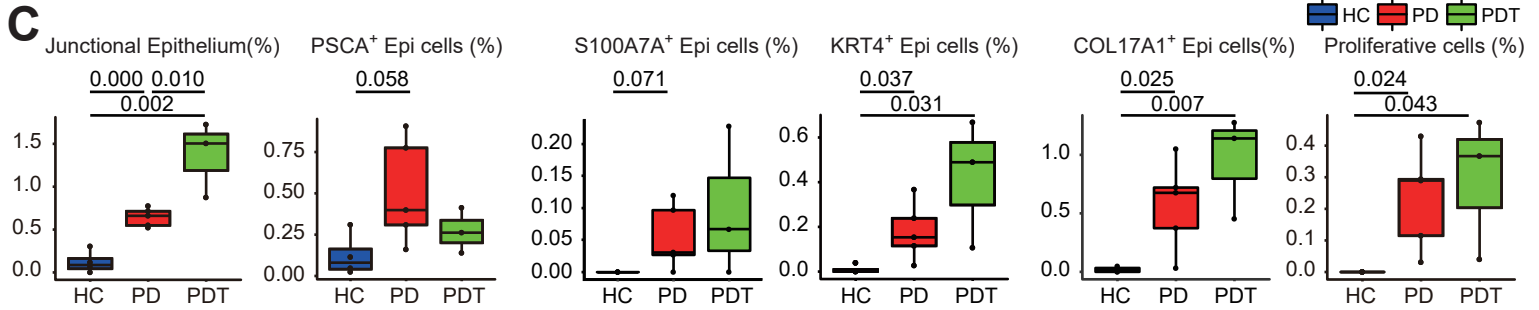
A



B



C



D

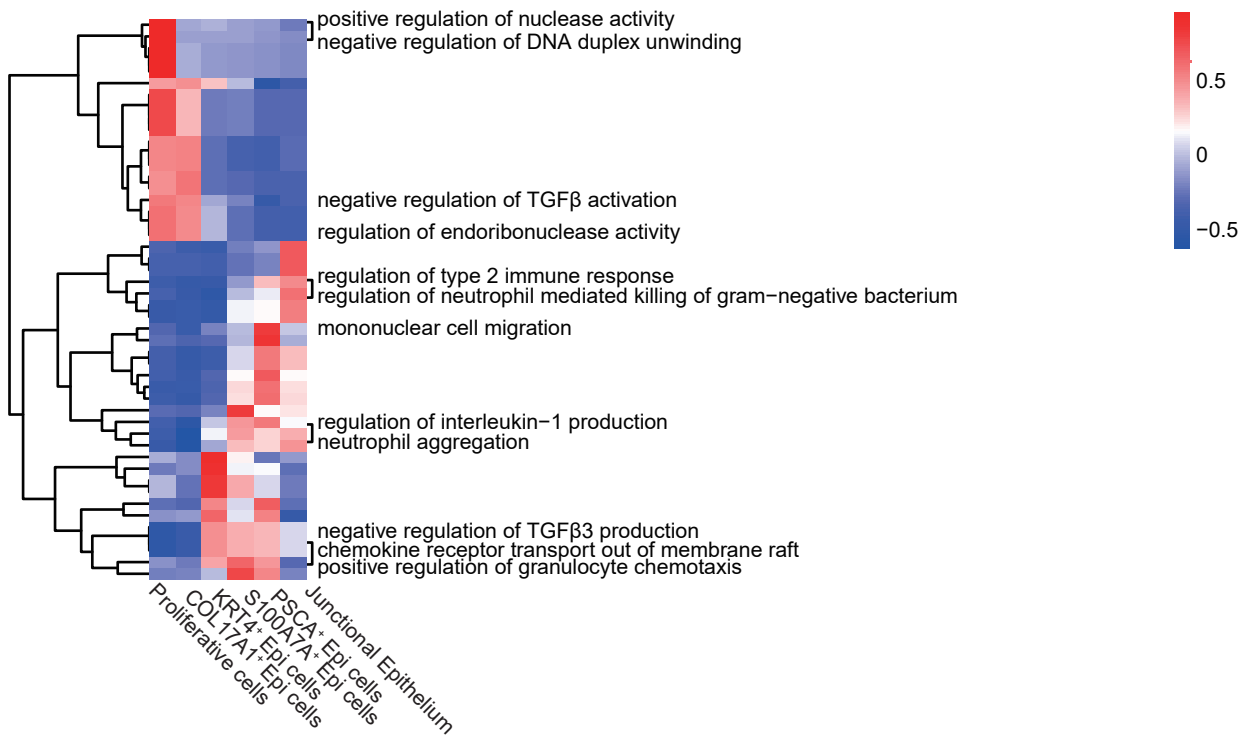


Figure S14. Identification of distinct clusters and functions in epithelial cell population

- (A)** Left panels: Uniform Manifold Approximation and Projection (UMAP) of 1029 epithelial cells (as in Figure 1A), annotated and colored by the sample type of origin (HCs, PDs, and PDTs) and clustering. Right panels: UMAPs color-coded for expression (gray to red) of key cell-type markers to define the clusters. Red contours (from left to right): PSCA⁺ Epi cells (cluster 9/10); Junctional Epithelium (cluster 1/2); COL17A1⁺ Epi cells (cluster 3/4/5). Epi cells: Epithelial cells.
- (B)** Heatmap of the relative expression level of the top 8-10 differentially expressed marker genes identified in the fibroblast clusters in (A).
- (C)** The box plots showing the percentage of cells for each of six clusters as in (A) from HC (blue, n=37), PD (red, n=449), and PDT samples (green, n=543) with plot center, box, whiskers, and points corresponding to the median, IQR, $1.5 \times \text{IQR}$ and $>1.5 \times \text{IQR}$, respectively. Data were analyzed using t-tests (two-tailed, two sample, equal variance).
- (D)** Heatmap of the QuSAGE activity for GO terms that are associated with differentially expressed marker genes among six clusters (labels on the bottom). Red indicating increased average expression of genes in GO term. Epi cells: Epithelial cells.