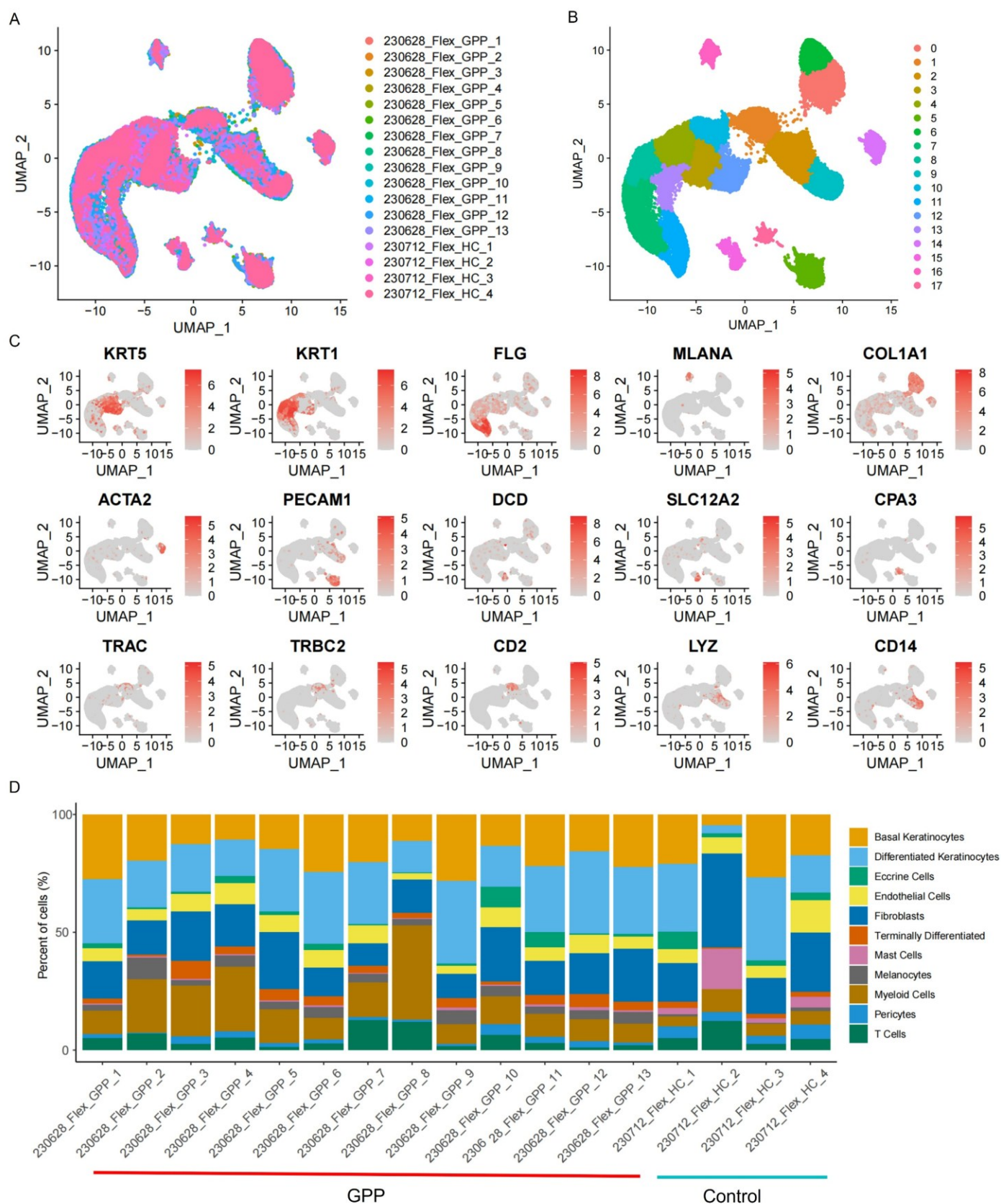


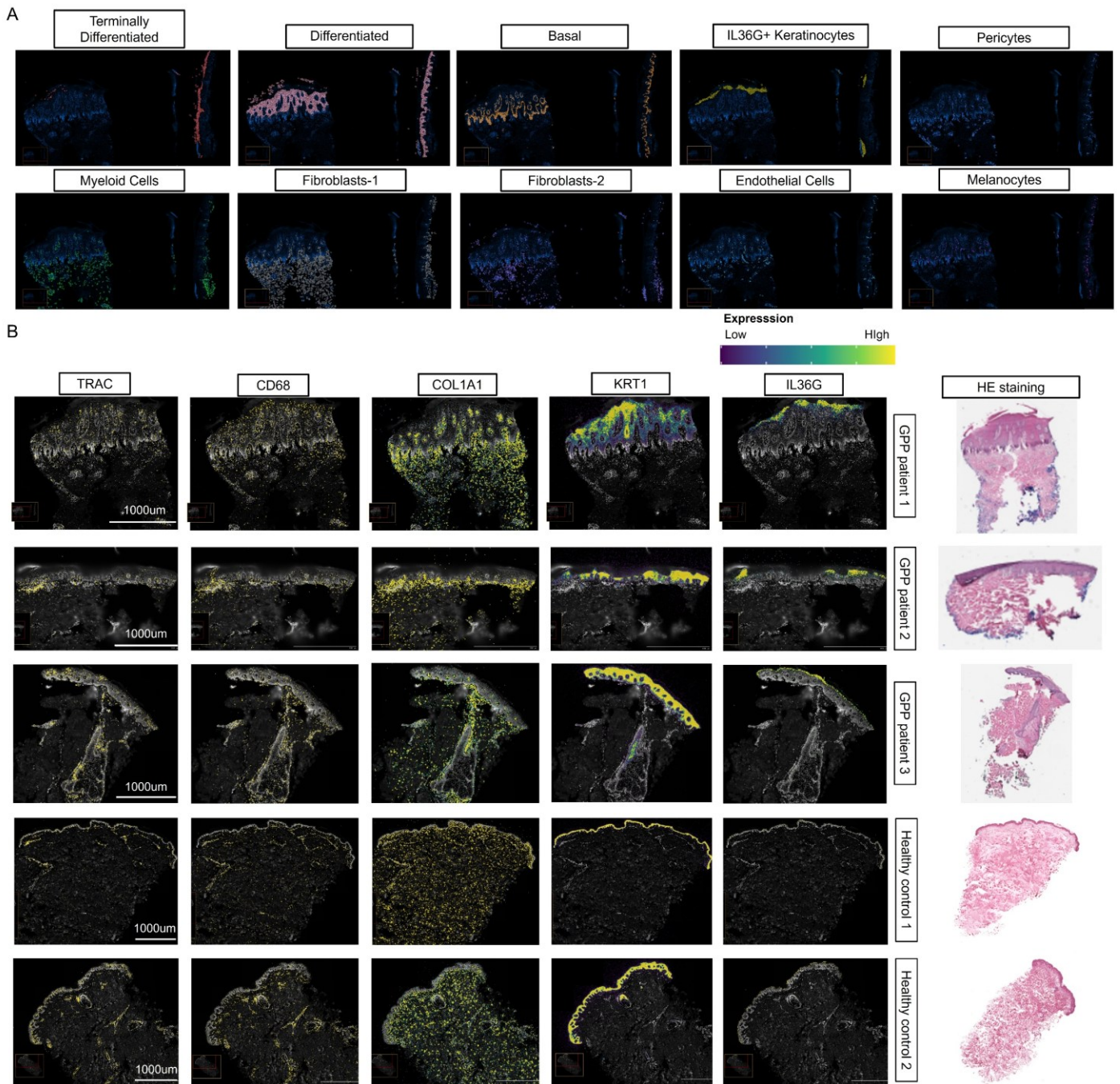
Dynamic neutrophil-keratinocyte communication network centered on IL-36/TNFSF15 responses characterizes inflammatory responses in generalized pustular psoriasis.

SUPPLEMENTARY FIGURES



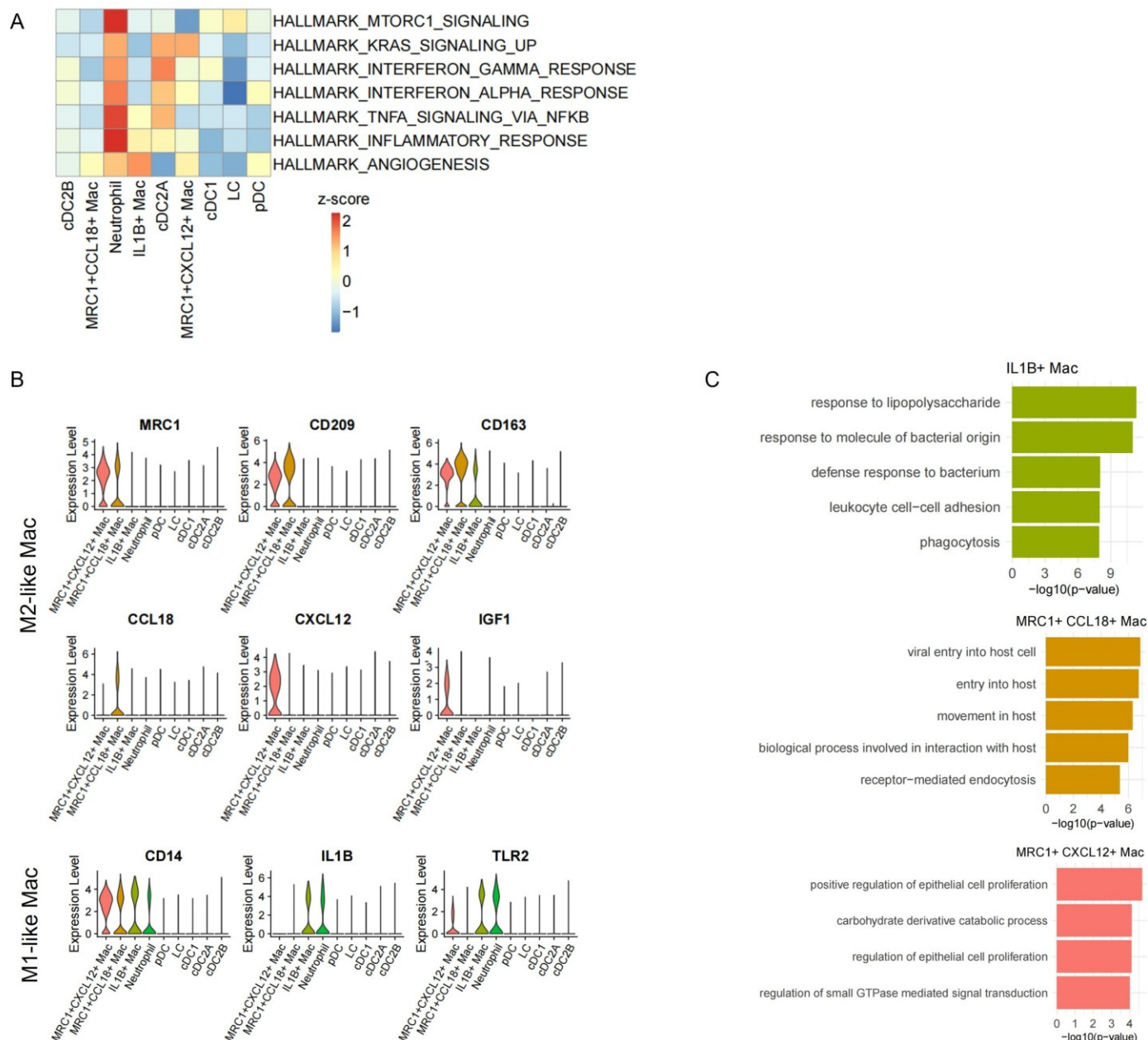
Supplementary Fig.1. Abundance of main cell populations detected in control and GPP skin.

(A) UMAP plot showing cells colored by sequencing libraries. (B) UMAP plot showing cells colored by sub-clusters. (C) Feature plots showing the expression level of markers in various cell types. (D) Bar plot showing the percentage of various cell types in individual skin sample.



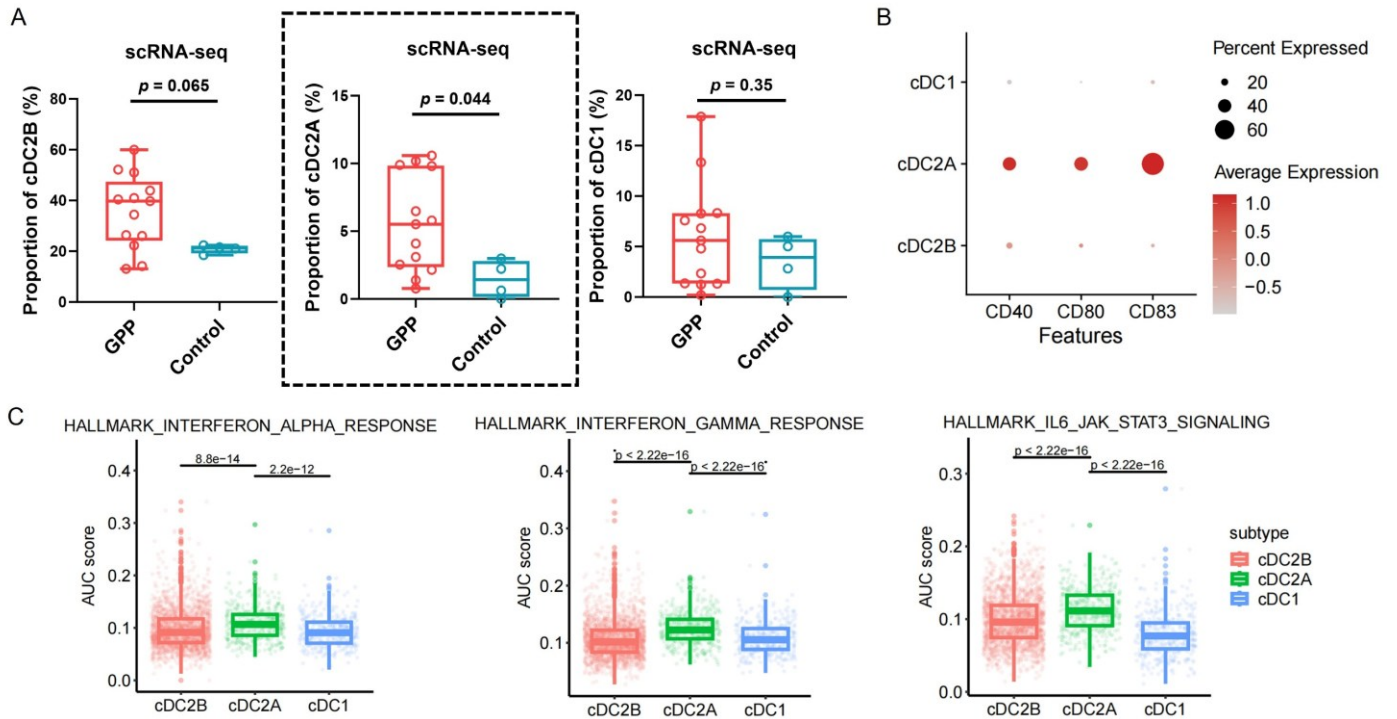
Supplementary Fig.2. Spatial sequencing localizes main cell types in three GPP and two healthy skin samples

(A) Spatial plot showing distribution of Terminally Differentiated keratinocytes, Differentiated keratinocytes, Basal keratinocytes, *IL36G*+ keratinocytes, Myeloid cells, Fibroblasts, Endothelial cells, Pericytes, and Melanocytes within GPP lesional skin. (B) Spatial plot showing detection of *TRAC* (T cell marker), *CD68* (Myeloid cell marker), *COL1A1* (Fibroblast marker), *KRT1* (Keratinocyte marker), and *IL36G* within GPP lesional and healthy skin. H&E staining was listed on the right.



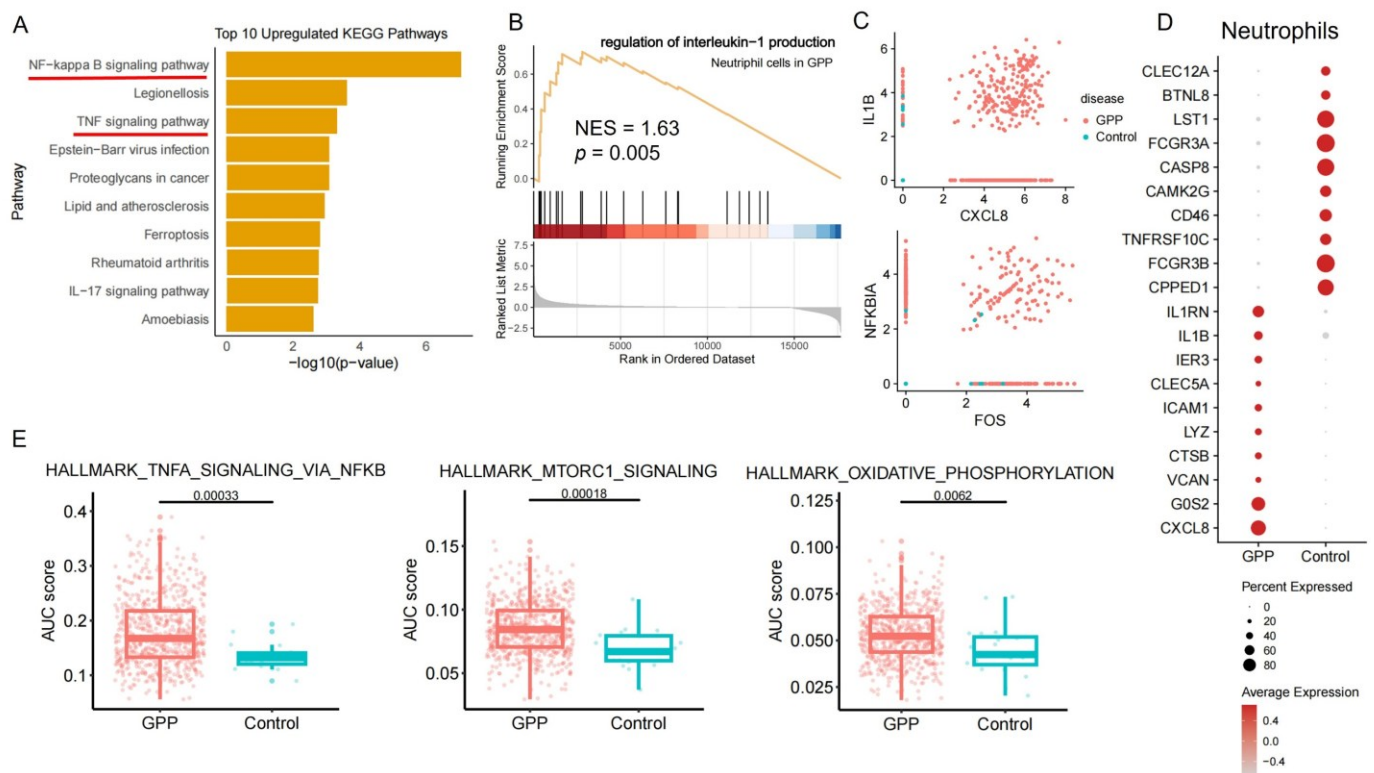
Supplementary Fig.3. Identification of macrophage subtypes

(A) Heatmap of pathway enrichment score in each myeloid cell subtype. (B) Violin plot showing the expression of macrophage marker genes split by myeloid cell subtype. (C) Bar plot showing the top five Gene Ontology pathways enriched for the up-regulated genes in *IL1B*+ Mac, *MRC1*+ *CCL18*+ Mac, and *MRC1*+ *CXCL12*+ Mac.



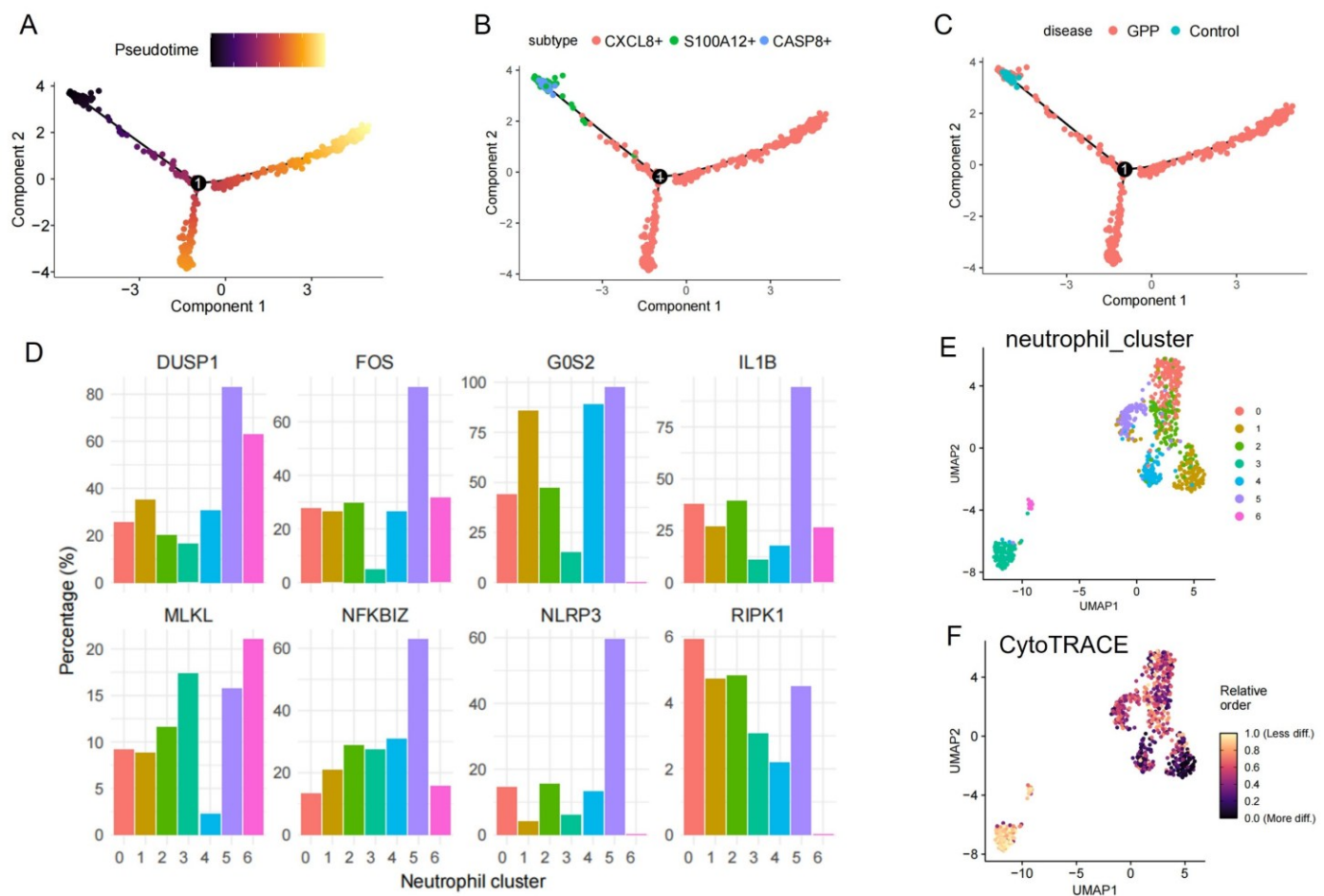
Supplementary Fig. 4. Identification of dendritic cell subtypes

(A) Cell percentage of cDC2B (left), cDC2A (middle), and cDC1 (right) in disease conditions. Every dot represents a sample. Control ($n = 4$) and GPP ($n = 13$). Box plots show the median (center line), interquartile range (box), and $1.5 \times$ interquartile range (whiskers). (B) Dot plot showing *CD40*, *CD80*, and *CD83* expression among cDC2B, cDC2A, and cDC1 cells. (C) Individual cell AUC score overlay for INTERFERON_ALPHA_RESPONSE, INTERFERON_GAMMA_RESPONSE and IL6_STAT3_SIGNALING activity in dendritic cell subtypes. Each dot represents the module score in a single cell. In the box plot, the centerlines denote median values (50th percentile); the bounds of the boxes represent the first and third quartiles (25th and 75th percentiles); the whiskers indicate the smallest and largest values within $1.5 \times$ the interquartile range. The statistical significance was determined by two-tailed unpaired Student's *t* test (A) and Wilcoxon signed-rank test (C).



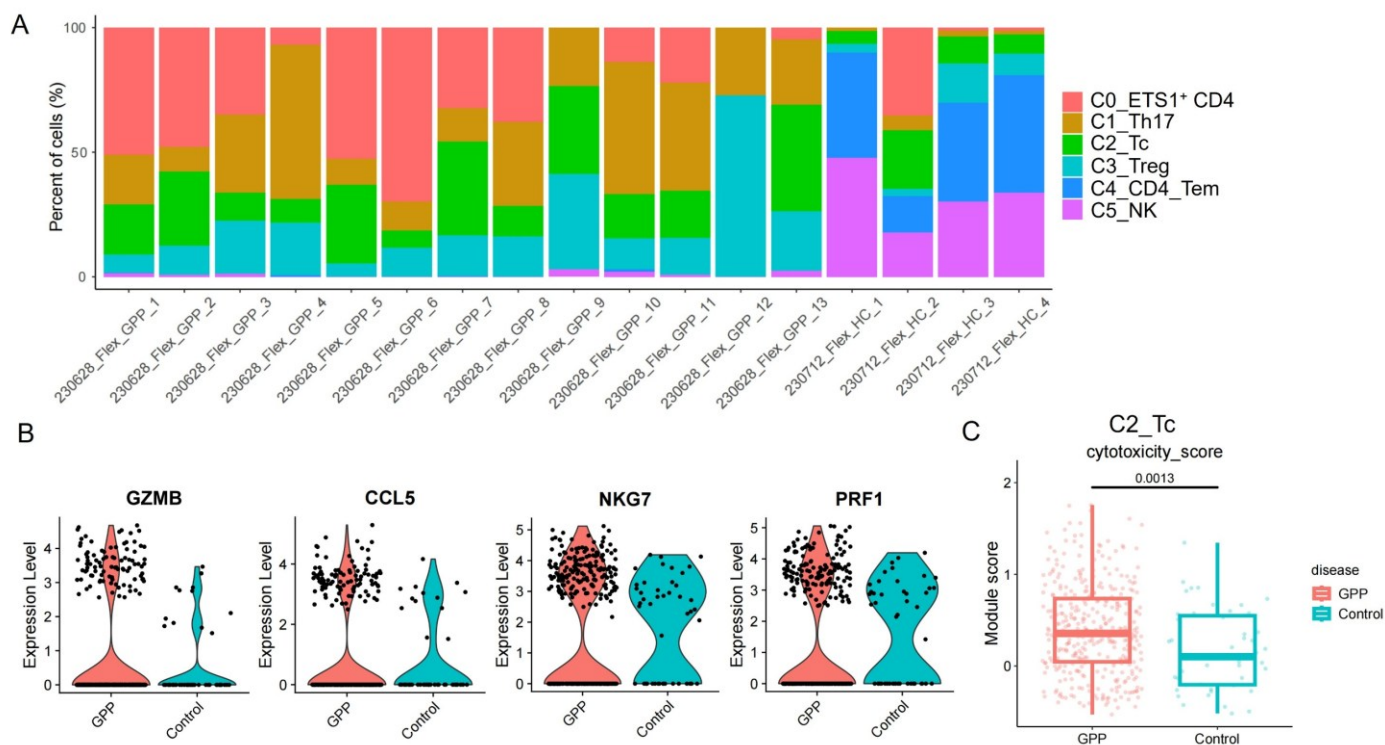
Supplementary Fig. 5. Differential expression analysis in neutrophils

(A) Bar plot showing top ten canonical pathways enriched using the differentially expressed genes comparing GPP to control in neutrophils. (B) GSEA showing the enrichment of Gene Ontology pathways from GPP neutrophils compared with control neutrophils. (C) Scatter plot showing the co-expression of pairs of genes. The color represents the disease identity of the cells. (D) Dot plot showing the top 10 differentially expressed genes comparing GPP to control in neutrophils. (E) Individual cell AUC score overlay for TNFA_SIGNALING_VIA_NFKB, MTORC1_SIGNALING and OXIDATIVE_PHOSPHORYLATION activity in neutrophils, split by the disease conditions. Each dot represents the module score in a single cell. In the box plot, the centerlines denote median values (50th percentile); the bounds of the boxes represent the first and third quartiles (25th and 75th percentiles); the whiskers indicate the smallest and largest values within $1.5 \times$ the interquartile range. The statistical significance was determined by Wilcoxon signed-rank test (E).



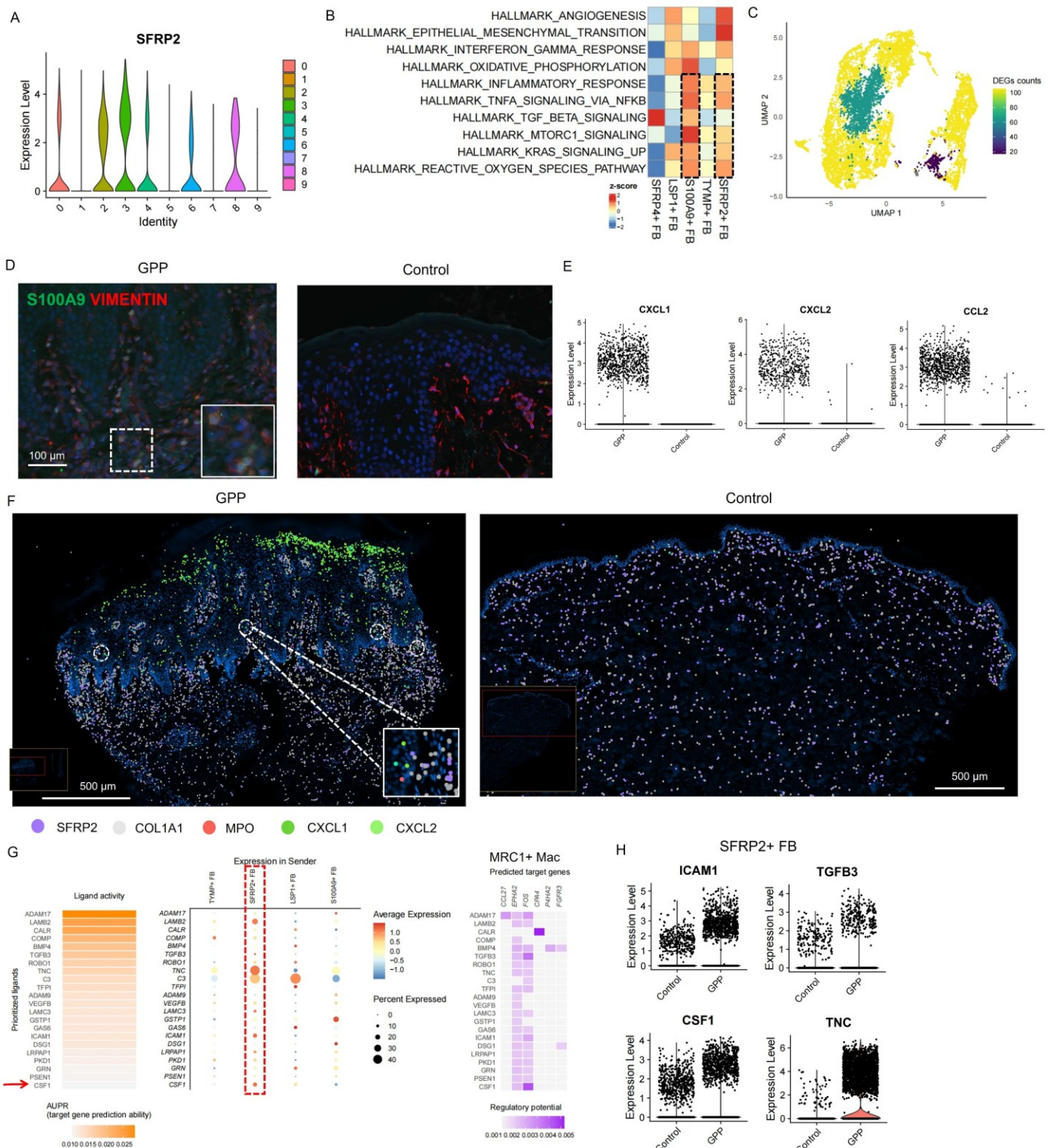
Supplementary Fig.6. Identification of neutrophil subtypes

(A) Pseudotime trajectory colored by the pseudotime of neutrophils. (B) Pseudotime trajectory colored by the subtype identity of neutrophils. (C) Pseudotime trajectory colored by the disease identity of neutrophils. (D) Bar plots showing the percentage of cells expressing the gene in the neutrophil sub-cluster. (E) UMAP plot showing neutrophils colored by sub-clusters conducted by CytoTRACE. (F) UMAP plot showing the CytoTRACE score in the neutrophil sub-clusters. A higher CytoTRACE score suggests the cell being less differentiated.



Supplementary Fig.7. Identification of T cell subtypes

(A) Bar plot showing the percentage of T cell subtypes in respective skin sample. (B) Violin plot showing the expression of genes in Tc cells, split by disease condition. (C) Box plot showing the cytotoxicity module scores in the Tc cells, split by the disease conditions. Each dot represents the module score in a single cell. In the box plot, the centerlines denote median values (50th percentile); the bounds of the boxes represent the first and third quartiles (25th and 75th percentiles); the whiskers indicate the smallest and largest values within $1.5 \times$ the interquartile range. The statistical significance was determined by Wilcoxon signed-rank test (C).



Supplementary Fig.8. Identification of fibroblast subtypes

(A) Violin plots showing the SFRP2 expression in each fibroblast sub-cluster. (B) Heatmap of pathway enrichment score in each fibroblast subtype. (C) UMAP plot showing the number of differentially expressed genes observed in each fibroblast subtype. (D) Immunofluorescence showing the colocalization of S100A9 with VIMENTIN in the dermis. (E) Violin plot showing the expression of genes in *SFRP2*⁺ fibroblasts, split by disease condition. (F) Spatial distributions of *SFRP2*⁺ *CXCL1*⁺ *CXCL2*⁺ fibroblasts and *MPO*⁺ neutrophils in GPP and healthy control skin. White loops point out the colocalization. The size bar represents 500 μm. (G) NicheNet ligand activity prediction for the communication between several fibroblast subtypes and *MRC1*⁺ macrophage. (H) Violin plot showing the expression of genes in *SFRP2*⁺ fibroblasts, split by disease condition.

A

B

Heatmap showing gene expression profiles across various cell lines and conditions. The y-axis lists 15 conditions, and the x-axis lists 100 genes. The heatmap is color-coded by expression level, with a color scale from 0 (blue) to 1 (red).

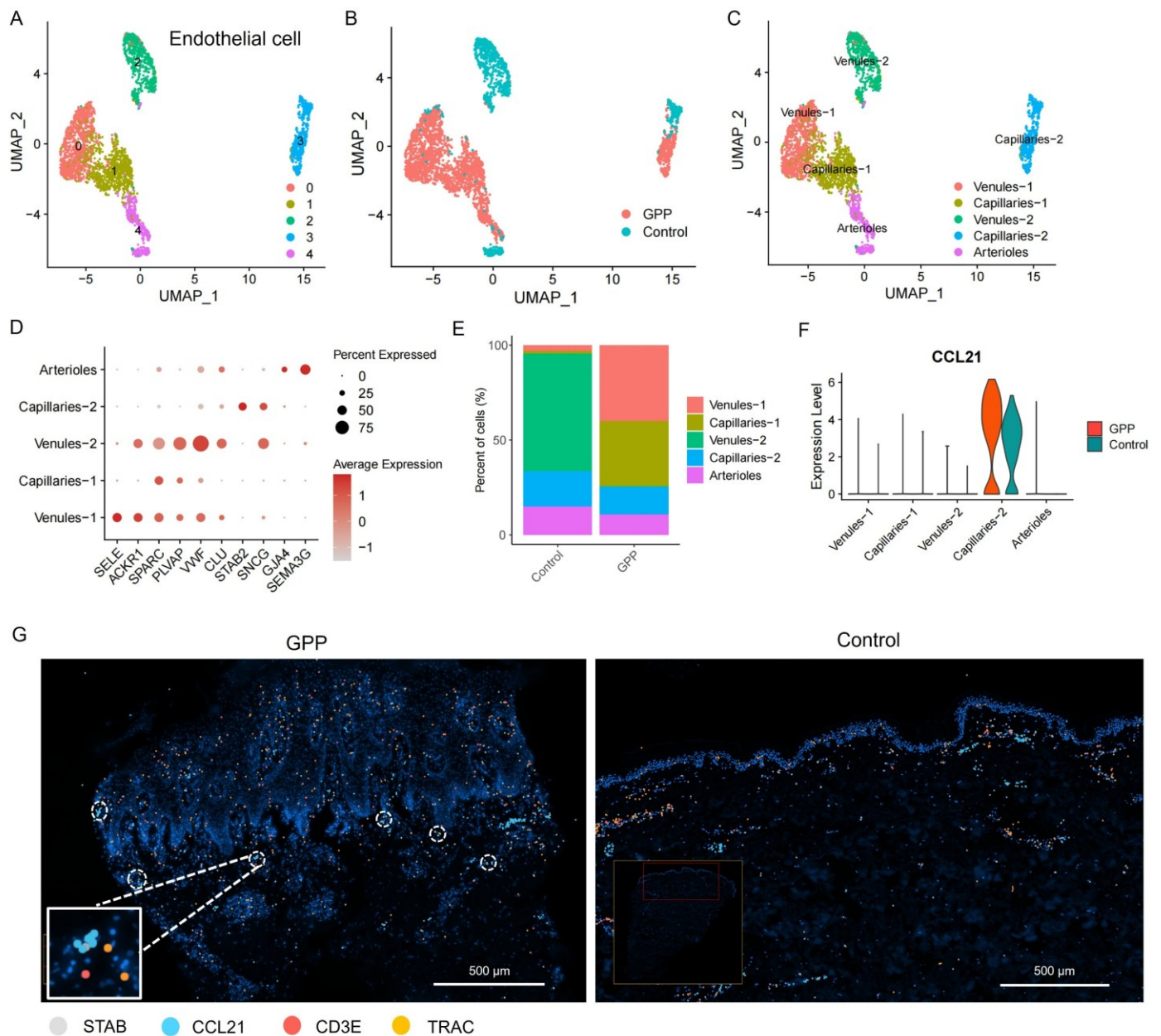
Conditions (Y-axis):

- S100A9+ FB -> cDC1 (Control)
- S100A9+ FB -> cDC1 (GPP)
- S100A9+ FB -> cDC2A (Control)
- S100A9+ FB -> cDC2A (GPP)
- S100A9+ FB -> IL18+ Mac (GPP)
- S100A9+ FB -> LC (Control)
- S100A9+ FB -> LC (GPP)
- S100A9+ FB -> cDC2B (Control)
- S100A9+ FB -> cDC2B (GPP)
- S100A9+ FB -> MRC1+ CCL18+ (Control)
- S100A9+ FB -> MRC1+ CCL18+ (GPP)
- S100A9+ FB -> MRC1+ CXCL12+ (Control)
- S100A9+ FB -> MRC1+ CXCL12+ (GPP)
- S100A9+ FB -> Neutrophil (Control)
- S100A9+ FB -> Neutrophil (GPP)

Genes (X-axis):

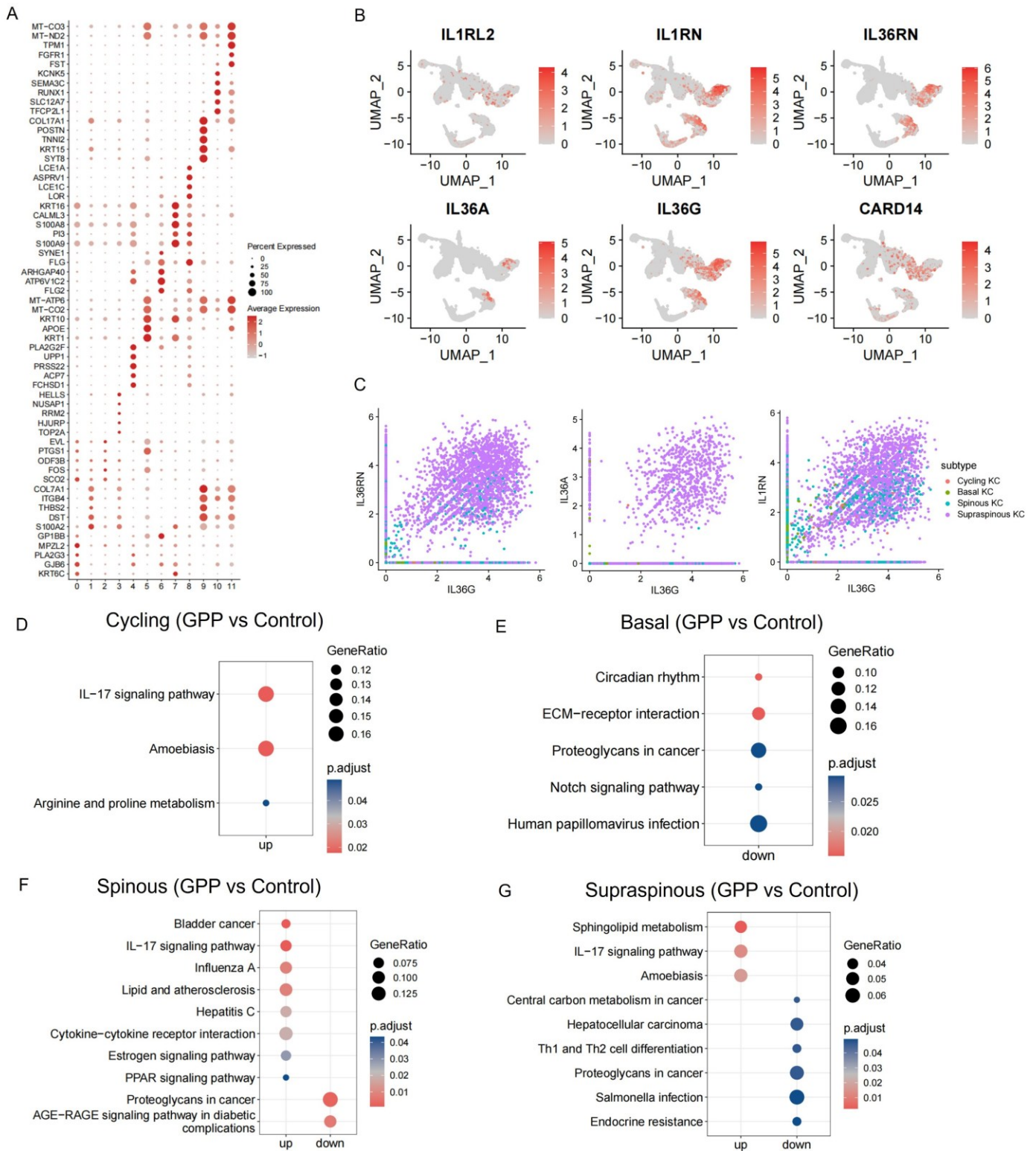
- IL18, IL13, IL12A, IL12B, IL12C, IL12D, IL12E, IL12F, IL12G, IL12H, IL12I, IL12J, IL12K, IL12L, IL12M, IL12N, IL12O, IL12P, IL12Q, IL12R, IL12S, IL12T, IL12U, IL12V, IL12W, IL12X, IL12Y, IL12Z, IL12AA, IL12AB, IL12AC, IL12AD, IL12AE, IL12AF, IL12AG, IL12AH, IL12AI, IL12AJ, IL12AK, IL12AL, IL12AM, IL12AN, IL12AO, IL12AP, IL12AQ, IL12AR, IL12AS, IL12AT, IL12AU, IL12AV, IL12AW, IL12AX, IL12AY, IL12AZ, IL12BA, IL12BB, IL12BC, IL12BD, IL12BE, IL12BF, IL12BG, IL12BH, IL12BI, IL12BJ, IL12BK, IL12BL, IL12BM, IL12BN, IL12BO, IL12BP, IL12BQ, IL12BR, IL12BS, IL12BT, IL12BU, IL12BV, IL12BW, IL12BX, IL12BY, IL12BZ, IL12CA, IL12CB, IL12CC, IL12CD, IL12CE, IL12CF, IL12CG, IL12CH, IL12CI, IL12CJ, IL12CK, IL12CL, IL12CM, IL12CN, IL12CO, IL12CP, IL12CQ, IL12CR, IL12CS, IL12CT, IL12CU, IL12CV, IL12CW, IL12CX, IL12CY, IL12CZ, IL12DA, IL12DB, IL12DC, IL12DD, IL12DE, IL12DF, IL12DG, IL12DH, IL12DI, IL12DJ, IL12DK, IL12DL, IL12DM, IL12DN, IL12DO, IL12DP, IL12DQ, IL12DR, IL12DS, IL12DT, IL12DU, IL12DV, IL12DW, IL12DX, IL12DY, IL12DZ, IL12EA, IL12EB, IL12EC, IL12ED, IL12EE, IL12EF, IL12EG, IL12EH, IL12EI, IL12EJ, IL12EK, IL12EL, IL12EM, IL12EN, IL12EO, IL12EP, IL12EQ, IL12ER, IL12ES, IL12ET, IL12EU, IL12EV, IL12EW, IL12EX, IL12EY, IL12EZ, IL12FA, IL12FB, IL12FC, IL12FD, IL12FE, IL12FF, IL12FG, IL12FH, IL12FI, IL12FJ, IL12FK, IL12FL, IL12FM, IL12FN, IL12FO, IL12FP, IL12FQ, IL12FR, IL12FS, IL12FT, IL12FU, IL12FV, IL12FW, IL12FX, IL12FY, IL12FZ, IL12GA, IL12GB, IL12GC, IL12GD, IL12GE, IL12GF, IL12GG, IL12GH, IL12GI, IL12GJ, IL12GK, IL12GL, IL12GM, IL12GN, IL12GO, IL12GP, IL12GQ, IL12GR, IL12GS, IL12GT, IL12GU, IL12GV, IL12GW, IL12GX, IL12GY, IL12GZ, IL12HA, IL12HB, IL12HC, IL12HD, IL12HE, IL12HF, IL12HG, IL12HH, IL12HI, IL12HJ, IL12HK, IL12HL, IL12HM, IL12HN, IL12HO, IL12HP, IL12HQ, IL12HR, IL12HS, IL12HT, IL12HU, IL12HV, IL12HW, IL12HX, IL12HY, IL12HZ, IL12IA, IL12IB, IL12IC, IL12ID, IL12IE, IL12IF, IL12IG, IL12IH, IL12II, IL12IJ, IL12IK, IL12IL, IL12IM, IL12IN, IL12IO, IL12IP, IL12IQ, 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(A) Ligand-receptor interaction probabilities among *S100A9*+ FB and keratinocyte subtypes. (B) Ligand-receptor interaction probabilities among *S100A9*+ FB and myeloid cell subtypes.



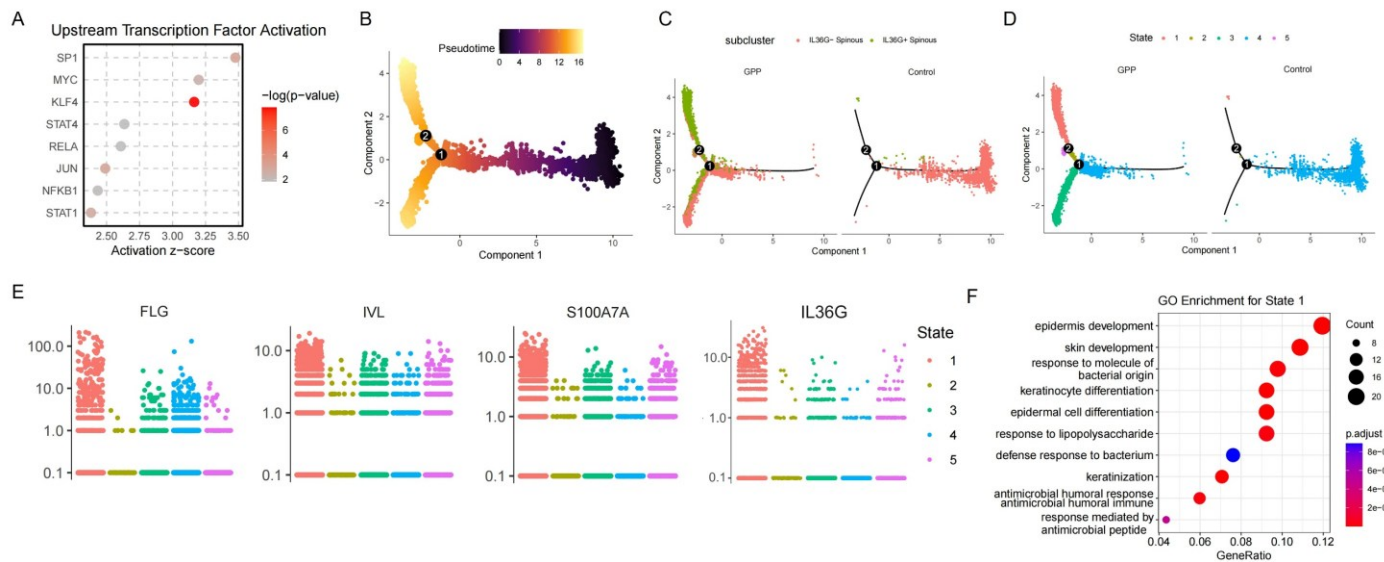
Supplementary Fig.10. Identification of endothelial cell subtypes

(A) UMAP plot showing endothelial cells colored by sub-clusters. (B) UMAP plot showing endothelial cells colored by disease conditions. (C) UMAP plot showing endothelial cells colored by subtypes. (D) Dot plot showing the top marker genes for each endothelial cell subtype. The color scale represents the scaled expression of each gene. (E) Bar charts showing proportion of endothelial cell subtypes in different disease conditions. (F) Violin plot showing the *CCL21* expression in endothelial cell subtypes, and each subtype is split by the disease conditions. (G) Spatial distributions of *CCL21*⁺ capillaries and *CD3E*⁺ or *TRAC*⁺ T cells in GPP and healthy control skin. White loops point out the colocalization. The size bar represents 500 μ m.



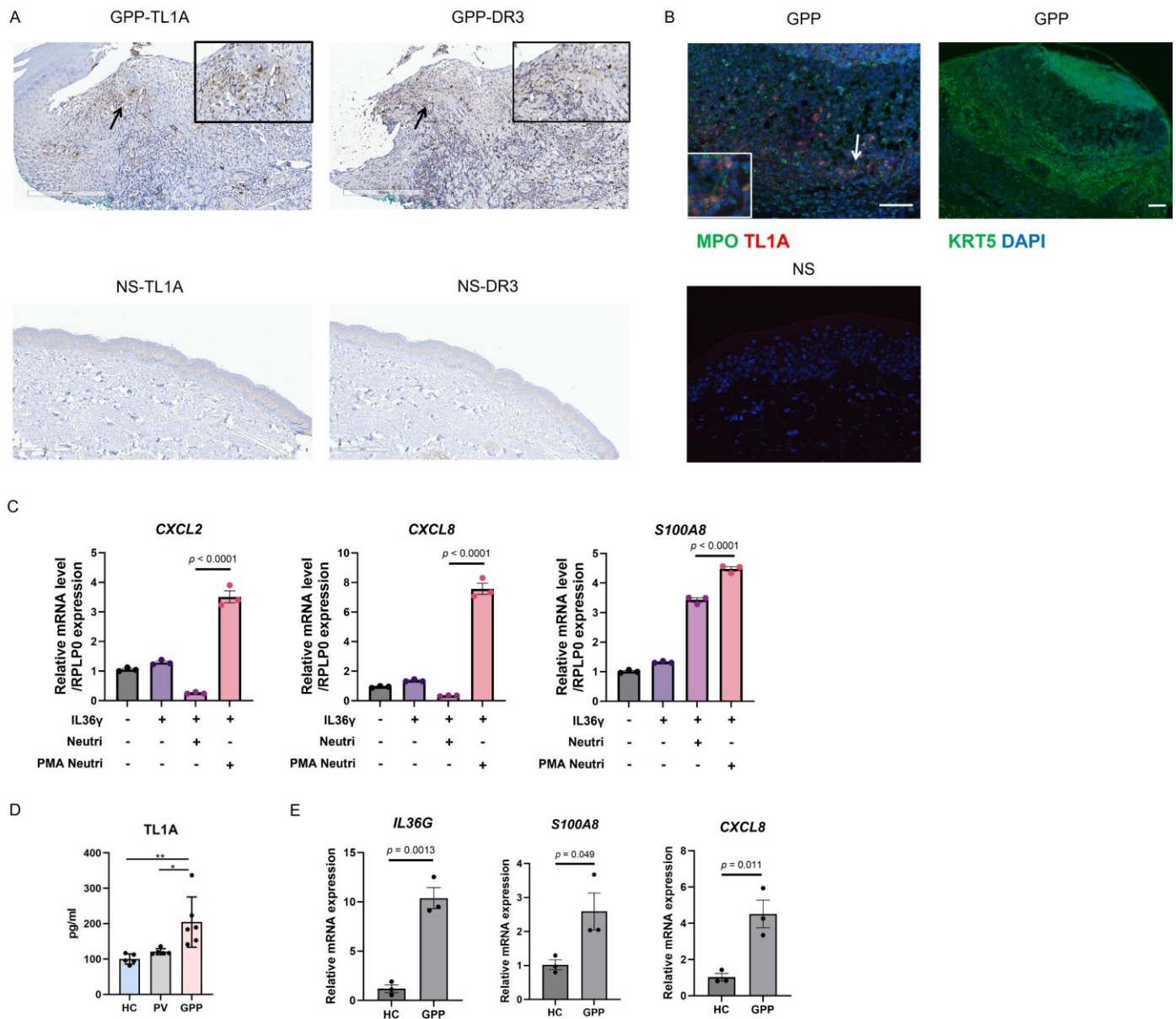
Supplementary Fig.11. Identification of keratinocyte subtypes

(A) Dot plot showing the top marker genes for each keratinocyte sub-cluster. (B) Feature plots showing the expression level of GPP risk genes in keratinocytes. (C) Scatter plot showing the co-expression of pairs of genes. The color represents the subtype identity of keratinocytes. (D) Dot plot showing top canonical pathways enrichment using the differentially expressed genes comparing GPP to control in cycling keratinocytes. (E) Dot plot showing top canonical pathways enrichment using the differentially expressed genes comparing GPP to control in basal keratinocytes. (F) Dot plot showing top canonical pathways enrichment using the differentially expressed genes comparing GPP to control in spinous keratinocytes. (G) Dot plot showing top canonical pathways enrichment using the differentially expressed genes comparing GPP to control in supraspinous keratinocytes.



Supplementary Fig.12. Identification of IL36G+ spinous KCs development

(A) Dot plot showing the activated transcriptional regulators detected in *IL36G*+ Spinous KCs compared with *IL36G*- Spinous KCs through IPA analysis. (B) Pseudotime trajectory colored by the pseudotime of *IL36G*+ Spinous and *IL36G*- Spinous KCs. (C) Pseudotime trajectory colored by the spinous KC subtypes in control and GPP lesions. (D) Pseudotime trajectory colored by the pseudo-state in control and GPP lesions. (E) Jitter plots showing top variable genes along the pseudotime of Spinous KCs. (F) Dot plot showing top GO biological process enrichment for state 1 KCs.



Supplementary Fig.13. Spatial location of the TL1A+ neutrophils and DR3+ keratinocytes.

(A) Immunohistochemistry staining for TL1A and DR3 expression in GPP and control samples. The size bar represents 300 μ m. The black arrow points to the zoom-in location of GPP sample. (B) Immunofluorescence staining on left showing the colocalization of MPO and TL1A in epidermis. The white arrow points to the zoom-in location of GPP sample. Immunofluorescence staining on right showing the expression of Keratin 5 in GPP lesion. The size bar represents 100 μ m. (C) qRT-PCR of inflammatory genes in primary keratinocytes after co-culture with neutrophils, with or without PMA stimulation ($n = 3$ biologically independent samples; one-way ANOVA; mean $\pm$ SEM). (D) ELISA results of TL1A expression in serum of healthy donors, psoriasis vulgaris (PV), and GPP patients. ($n = 5-9$, one-way ANOVA; * $P < 0.05$; ** $P < 0.01$; mean $\pm$ SD). (E) qRT-PCR of inflammatory genes in primary keratinocytes after co-culture with neutrophils from healthy controls (HC) and GPP patients ($n = 3$ biologically independent samples; two-tailed unpaired Student's t test; mean $\pm$ SEM).

SUPPLEMENTARY TABLES

Supplementary Table 1. GPP patient characteristics

Age	Sex	Biopsy site	Medications when biopsied	Background diseases
59	Male	Flank	N/A	N/A
40	Male	Right arm	Betamethasone, clotrimazole	N/A
51	Female	Left shoulder	Cefalexin, diphenhydramine, fluocinolone acetonide, hydrocortisone, hydromorphone, levothyroxine, triamcinolone	Hypothyroidism, psoriasis
38	Male	Left upper back	Clobetasol, hydrocodone-acetaminophen, lisinopril, prednisone, triamcinolone	Hypertension, psoriasis
47	Female	Left arm	N/A	N/A
78	Female	Foot	N/A	N/A
77	Female	N/A	Methotrexate, topical steroids	Psoriasis
80	Female	Back	Asprin, atorvastatin, celecoxib, ciclopirox shampoo, diflorasone, diphenhydramine, griseofulvin, hydrocodone-acetaminophen, hydroxyzine, lansoprazole, levothyroxine, metoprolol, nystatin, triamcinolone, prednisone	Arthritis, basal cell carcinoma, candidal intertrigo, coronary artery disease, gastric reflux, hypercholesterolemia, hypothyroidism, psoriasis, psoriasiform dermatitis
75	Female	Right chest	Alfuzosin, aspirin, ezetimibe/simvastatin, felodipine, fish oil, ibuprofen, insulin, metformin, warfarin	Atrial fibrillation, basal cell carcinoma, coronary artery disease, hyperparathyroidism, hypertension, multiple thromboses, myelofibrosis, osteoarthritis
34	Female	Central upper back	Desonide, triamcinolone	Discoid lupus erythematosus
61	Male	Left big toe	Atorvastatin, glyburide, HIV medication (unspecified)	Diabetes, HIV

69	Male	Right upper arm	Allopurinol, aspirin, clonidine, ezetimibe/simvastatin, furosimide, glimepiride, hydrocortisone, insulin, lisinopril, losartan, metformin, metoprolol, pioglitazone, spironolactone, triamcinolone	Consumptive thrombocytopenia, diabetes mellitus, history of melanoma, hyperlipidemia, hypertension
70	Female	Lower back (external biopsy only)	Cyclosporine, diphenhydramine, furosimide, sucralfate, triamcinolone	non-melanoma skin cancer, psoriasis, psoriasiform dermatitis