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Oligoclonal expansion of IgG⁺ B cells along with Tfh cell response is associated with a better outcome in endometrial cancer

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Abstract

B cells play a critical role in tumor immunity, with their presence associated with improved prognosis in various cancers, including endometrial cancer (EC). However, the nature of the B cell response within the tumor microenvironment (TME) remains incompletely understood. In this study, we conducted single-cell analyses of B cells and CD4⁺ T cells in the TME of EC. We found that the TME of EC harbored abundant plasmablasts and plasma cells (PCs), which were rare in normal endometria. PCs primarily expressed either IgG or IgA, and a high abundance of IgG in TME was associated with better overall survival. B cell receptor (BCR) repertoire analysis revealed a clonal expansion of IgG⁺ B cells, coinciding with an increased presence of T follicular helper (Tfh) cells in the TME. Notably, Tfh cells shared T cell receptor clones with cycling CD4⁺ T cells, indicating local proliferation. BCR repertoire analysis also suggested that IgG⁺ PCs differentiate from IFN-responding B cells and double-negative B cells in the TME. Additionally, recombinant oligoclonal IgG antibodies were found to recognize antigens expressed by tumor cells as well as normal endometrial cells. Collectively, our study shows that the clonal expansion of IgG⁺ B cells, along with the Tfh cell response, is associated with a better outcome in EC.

Introduction

B cells are frequently observed in tumor tissues (1). In some cancers, B cells form tertiary lymphoid structures (TLS) in the TME with other immune cells, primarily T cells and myeloid cells (1) (2) (3). Previous studies have suggested that these B cells differentiate into cells producing antibodies, potentially including those recognizing antigens expressed by tumors (4) (5) (6). Accordingly, the presence of B cells in the TME is associated with favorable outcomes in various cancers (2) (3) (7) (8), including bladder cancer (9), breast cancer (10), colorectal cancer (11), esophageal adenocarcinoma (12), non-small cell lung carcinoma (13), melanoma (14), mesothelioma (15), and pancreatic adenocarcinoma (16). However, tumor-infiltrating B cells are correlated with poor prognosis in several cancers, such as renal cell carcinoma (17) and glioblastoma (18). The exact mechanism by which B cells are associated with such divergent prognoses is not fully understood. Given their potential to unlock new therapeutic avenues, B cell responses in the TME, especially those contributing to a favorable prognosis, are now under intense investigation.

Endometrial cancer (EC) is one of the most common cancers affecting women worldwide, particularly in developed countries (19). In EC, the presence of B cells is related to a favorable prognosis in association with the formation of TLS (20) (21). Nevertheless, the exact B cell response linked to a better clinical outcome remains unknown in EC. Furthermore, the heterogeneity of B cells in the TME is not well-characterized in EC partly due to the limited number of B cells analyzed in previous single-cell studies (21) (22). The associated CD4⁺ T cell response in the TME is not fully understood in EC either, even though CD4⁺ T cells have been found within TLS in EC (20). Here, we analyze a total of 47,551 B cells and a total of 13,019 CD4⁺ T cells from 10 EC tumor tissues and a normal endometrial tissue using single-cell multi-omic techniques. Our data demonstrate that B cells, particularly IgG⁺ B cells, undergo clonal expansion within the TME of EC. This clonal expansion appears to be supported by CD4⁺ T cells, especially T follicular helper (Tfh) cells.

Immunohistochemistry and data from The Cancer Genome Atlas (TCGA) further suggest that such B cell response is associated with a better clinical outcome in EC.

Methods

Sample collection and preparation

This study was approved by the ethics committee of Kyoto University Hospital (Study approval number: G1246). All patients provided written informed consent. Tissues were collected from patients undergoing surgery for primary EC and were immediately stored in Dulbecco's PBS without calcium and magnesium (DPBS). Tissue samples were minced into small pieces and digested using Liberase TL (Roche, Ref 5401020001) and DNase I (Roche, Ref 10104159001, from bovine pancreas, grade II) for 20 minutes at 37 °C. The digested tissues were passed through 70 µm cell strainers and washed with DPBS, 2% fetal calf serum (FCS), and 2 mM EDTA. Cell suspensions were then processed using Human CD45 (TIL) MicroBeads (Miltenyi Biotec) to enrich CD45⁺ cells.

Flow cytometry and cell sorting

CD45⁺ cell suspensions isolated from tissues were stained in DPBS containing 2% FCS and 2mM EDTA for 15 minutes on ice with 7-AAD and the following anti-human antibodies: CD45 (BioLegend, HI30), CD19 (BioLegend, HIB19), and CD3 (BioLegend, HIT3a). Samples were sorted using either BD FACSAria Fusion or BD FACSMelody. 7-AAD⁻ CD45⁺ CD19⁺ CD3⁻ cells were sorted as B cells, while 7-AAD⁻ CD45⁺ CD3⁺ CD19⁻ cells were sorted as T cells.

Single-cell library preparation, sequencing, and alignment

Single-cell libraries were generated with 10x Genomics Chromium Single Cell 3' (v3.1) and 5' (v2). After the library quality was confirmed by 2100 BioAnalyzer (Agilent), libraries were sequenced on the Illumina NovaSeq 6000 platform. Single-cell data were processed with Cell Ranger v4 and v5 (10x Genomics). RNA data were aligned and quantified against GRCh38-3.0.0. BCR and TCR data were processed against refdata-cellranger-vdj-GRCh38-alts-ensembl-5.0.0.

Single-cell RNA sequencing (scRNA-seq) clustering analysis

scRNA-seq matrix data were analyzed with Seurat R package (v3.2.2 and v4.0.5) (23) (24). CD4⁺ CD8⁻ T cells were gated and extracted before the clustering analysis using log-normalized TotalSeq-C antibody (anti-CD4, RPA-T4, BioLegend; anti-CD8, SK1, BioLegend) count data. Cells with high mitochondrial percentages and/or low UMI counts were removed as dead cells. The data were then size-scaled and log-normalized using LogNormalize function. Scaling and PCA were performed with ScaleData and RunPCA functions, respectively. Using 30 PC dimensions, clustering and UMAP were performed by FindNeighbors, FindClusters, and RunUMAP functions. Clusters were manually annotated based on the marker gene expression.

Batch correction of scRNA-seq data

In the B cell analysis, batch was corrected across 10 EC tumor tissues using

SelectIntegrationFeatures, FindIntegrationAnchors, and IntegrateData functions of Seurat R package (23) (24). In the CD4⁺ T cell analysis, batch was corrected across 5 EC tumor tissues and 1 normal endometrial tissue using RunHarmony function of harmony R package (25).

Analysis of single-cell BCR sequencing (scBCR-seq) data

The output of Cell Ranger was used to show the top 10 dominant clones. To generate the HoneyComb plot, enclone (v0.5.219) (26) (27) was used. When determining the IgG⁺ PC precursors, TRUST4 (28) was used to reconstruct the BCR repertoire sequence from the scRNA-seq FASTQ data of all patients. Here, BCR clonotypes were defined as clusters with the same V and J gene assignments and similar CDR3 sequences (CDR3 similarity ≥ 0.8), where CDR3 similarity was defined as $1 - \{Levenshtein\ Distance / \max(CDR3\ length)\}$.

Analysis of Human Cell Atlas data

scRNA-seq count matrix data of normal endometria (29) were downloaded. Cells annotated as "immune cells" in the original paper were extracted and analyzed. Clustering analysis was performed using Seurat R package (v4.0.5) (24) as mentioned above.

Immunohistochemistry of CD20, IgG, and IgA and its quantitative evaluation

Immunohistochemistry was performed with anti-CD20 antibody (L26, Roche), anti-IgG antibody (EPR4421, Abcam), and anti-IgA antibody (rabbit polyclonal, Roche) using VENTANA (Roche) and detected with the Roche system. Two trained pathologists independently examined the slides with hematoxylin-eosin (HE) staining and immunohistochemistry. Three lymphoid aggregates per slide were identified to count the number of CD20⁺ cells, IgG⁺ cells, and IgA⁺ cells at 40x resolution. The averaged cell numbers were used for survival analysis.

Immunohistochemistry with recombinant antibodies

Recombinant antibodies of the most expanded clones were generated in EC07 and EC08 at GenScript. The constant regions of the antibodies were replaced with mouse IgG2a. Using the chimeric recombinant antibodies, immunohistochemistry was performed. To detect the chimeric recombinant antibodies, FITC-conjugated goat anti-mouse IgG2a antibody (Abcam, ab6786) was used. The image was acquired using the Keyence BZ-X800.

TCGA-UCEC data analysis

The RNA-seq expression data of The Cancer Genome Atlas Uterine Corpus Endometrial Carcinoma (TCGA-UCEC) and the relevant clinical data (30) (31) (32) were downloaded from NCI Genomic Data Commons (GDC). IgG and IgA expression were calculated by summing the expression levels of IGHG1-4 and IGHA1-2, respectively.

Statistical analyses

All the statistical analyses were performed with R (versions 3.6.3 and 4.0.5) (33). Survival analyses were performed using R package survival.

Graphics

Some graphics were generated using BioRender.

Results

Heterogeneity of B cells in the TME of EC

To investigate B cell heterogeneity in EC, we applied scRNA-seq to FACS-sorted B cells from fresh tumor tissues (Fig. 1A, Supplementary Table 1). We obtained single-cell transcriptomes from 45,425 B cells across 10 EC samples ($4,542 \pm 806$ cells, mean $\pm$ s.d.). UMAP analysis identified two major B cell subpopulations: CD20⁺ (encoded by MS4A1) conventional B cells, and CD20⁻ immunoglobulin gene-expressing plasma cells (PCs) (Fig. 1B, Supplementary Fig. 1A-B). CD20⁺ conventional B cells were further divided into five populations including IGHD⁺ naive B cells and CD27⁺ memory B cells. A population with an interferon-stimulated gene (ISG) signature (e.g., ISG15 and MX1) was noted, likely reflecting the presence of interferons (IFN) in the tumor microenvironment (TME). Double-negative (DN) B cells (IGHD⁻ CD27⁻) were present, which were further subdivided into two subgroups with distinct CR2 (encoding CD21) expression: CD21^{high} and CD21^{low}. CD21^{low} DN B cells expressed ITGAX and TBX21 (encoding CD11c and T-bet, respectively) and thus likely correspond to age-associated B cells (34). Although germinal center (GC)-B cells (BCL6^{high} AICDA^{high} CD38^{high}) have been reported in TME (35), we did not detect such cells in our dataset (Supplementary Fig. 1C). However, a cell population expressing a proliferation marker MKI67, thus in proliferation *in situ*, was detected. Since MKI67⁺ cells in our dataset showed a gene signature of PCs (IGVDJ-sum^{high} CD38^{high} XBP1^{high}), they were *in situ* proliferating plasmablasts. Similar to other types of cancers (1) (2), most PCs expressed either IgG (IGHG1-4) or IgA (IGHA1-2) (Supplementary Fig. 1D). Therefore, B cells in the TME are heterogeneous and include *in situ* proliferating plasmablasts and IgG⁺/IgA⁺ PCs.

B cells comprised $9.9 \pm 8.3\%$ (Mean $\pm$ s.d) of CD45⁺ immune cells in EC, and PCs constituted $24.4 \pm 13.5\%$ of B cells. In contrast, normal postmenopausal endometrium ($n = 1$) contained 3.2% B cells and only 0.7% PCs. (Fig. 1C-D, Supplementary Fig. 2A-B). Analysis of Human Cell Atlas data (29) corroborated this, showing minimal B cells ($1.9 \pm 2.9\%$ of immune cells; total 35 cells) and no PCs in normal endometria ($n = 14$) (Fig. 1D, Supplementary Fig. 3A-C). Furthermore, neither B cells with an IFN-signature nor DN B cells were present in normal tissue (Fig. 1C, Supplementary Fig. 2A). Collectively, these data suggest that the TME in EC promotes B cell responses, including the

differentiation of PCs.

Association between IgG⁺ B cells and a better clinical outcome

Since plasma cells predominantly expressed either IgG or IgA in our scRNA-seq data (Supplementary Fig. 1D), we aimed to investigate the prognostic difference between IgG and IgA expression. To determine whether the gene expression of MS4A1 (encoding CD20), IgG, and IgA in TME is associated with the outcome of EC, we analyzed TCGA data (30) (31) (32). High expression of MS4A1 in EC tissues was correlated with significantly better overall survival ($p = 0.03$) (Supplementary Fig. 4), confirming the favorable prognostic impact of B cells. High expression of IgG genes (sum of IGHG1-4) in EC tissues was also associated with significantly better survival ($p = 0.01$) (Fig. 2A). In contrast, high expression of IgA genes (sum of IGHA1-2) was not associated with better overall survival ($p = 0.17$). These findings suggest that IgG expression, rather than IgA expression, contributes to the better prognosis in EC.

To clarify whether IgG-dominant B cell response is associated with a better clinical outcome, we analyzed EC tissues from 86 patients by immunohistochemistry (IHC) with antibodies for CD20, IgG, and IgA. IgG⁺ B cells and IgA⁺ B cells were identified as cells positive for CD20 and IgG, CD20 and IgA, respectively (Fig. 2B-C, Supplementary Fig. 5A, Supplementary Table 2). The numbers of total B cells, IgG⁺ B cells, and IgA⁺ B cells yielded no difference in the overall survival between the low and high groups ($p = 0.53, 0.61, 0.99$, respectively) (Supplementary Fig. 5B). Likewise, the IgG/CD20 cell number ratio did not show a difference ($p = 0.21$). However, patients with a high IgG/IgA cell number ratio had better overall survival than those with low ratios, albeit not significant in our dataset ($p = 0.057$) (Fig. 2D). These findings suggest that the dominance of IgG⁺ B cells over IgA⁺ B cells is associated with improved clinical outcomes in EC.

Clonal expansion of IgG⁺ B cells in TME

To confirm whether IgG⁺ B cells outnumber IgA⁺ B cells in certain tumor tissues, we analyzed the IgG/IgA cell number ratios among the total B cells in 10 EC tissues using our scRNA-seq data. We determined the immunoglobulin isotype of each B cell based on their expression of immunoglobulin genes. The composition of immunoglobulin isotypes markedly differed among tumor samples (Fig. 3A). However, we noted that IgG/IgA cell number ratios clearly separated tumors into two groups: IgG/IgA high and low groups. Three out of ten tumors belonged to the IgG/IgA high group, showing dominance of IgG⁺ B cells over IgA⁺ B cells (Fig. 3B). Thus, our scRNA-seq data analysis confirmed the dominance of IgG⁺ B cells over IgA⁺ B cells in some tumors.

Two potential mechanisms could explain the dominance of IgG⁺ B cells over IgA⁺ B cells in some tumors. One possibility is a preferential class switching to IgG at an individual B cell level, and the other is a greater clonal expansion of IgG⁺ B cells than IgA⁺ B cells. To explore the clonal diversity of B cells, we performed scBCR-seq with four EC tumor samples (Fig. 3C). Tumors with a high IgG/IgA ratio (EC07 and EC08) contained nine and five clones with an expansion greater than > 1% of the total VDJ-identified B cells, respectively (Fig. 3D). The top clones accounted for 20.3% of total B cells in EC07 and 4.8% in EC08. The top 10 clones in the two tumors dominantly expressed IgG, except four clones in EC07 with IgM dominance. By contrast, tumors with a low IgG/IgA ratio (EC09 and EC10) contained only one clone with an expansion greater than 1% of the total VDJ-identified B cells. Furthermore, eight and six clones among the top 10 clones predominantly expressed IgA in EC09 and EC10, respectively. Some clones in EC09 contained both IgA⁺ and IgG⁺ B cells, suggesting possible co-occurrence of IgA and IgG class switching. The clonality index, calculated using Shannon entropy (36), was higher in the tumors with a high IgG/IgA ratio (Supplementary Fig. 6A), confirming their higher clonality. Taken together, these data suggest that a massive expansion of selective IgG⁺ B cell clones predominantly contributes to the high IgG/IgA in TME, leading to a better prognosis.

To investigate whether affinity maturation could contribute to the improved clinical outcome observed in tumors with a high IgG/IgA ratio, we analyzed somatic hypermutations in these tissues. Using enclone software (26) (27), we quantified mutations outside the junction region. Tumors exhibiting IgG oligoclonal expansion (i.e., high IgG/IgA ratio) showed fewer somatic hypermutations, with IgG⁺ PCs exhibiting lower mutation levels than IgA⁺ PCs (Fig. 3E, Supplementary Fig. 6B). These findings suggest that affinity maturation is unlikely to play a major role in the better prognosis associated with IgG-dominant B cell responses.

Active Tfh responses in high IgG/IgA ratio tumors

Previous studies employing tissue immunostaining techniques on tumor specimens have demonstrated the localization of CD4⁺ T cells, typically with features of T follicular helper (Tfh) cells, in the TLS of TME (20) (37) (38) (39). To explore the contribution of CD4⁺ T cells to B cell responses within the TME, we obtained single-cell transcriptomes of a total of 13,019 CD4⁺ T cells from five EC tumors and a normal endometrium tissue, from which we also analyzed B cells (Supplementary Table 1). Clustering analysis revealed six populations (Fig. 4A-B, Supplementary Fig. 7), including naive (IL7R, CCR7, KLF2), cytotoxic CD4⁺ T (GZMK, NKG7, EOMES), exhausted (LAG3, GZMB, PDCD1), Tfh (CXCL13, PDCD1, CD200, IL21, CXCR5), in proliferation (MKI67), and regulatory T (Treg) cells (FOXP3, IL2RA, IKZF2). Consistent with

recent studies (40) (41), the exhausted cell population shared gene profiles with both Tfh cells and cytotoxic CD4⁺ T cells. Although the Tfh cluster did not express markers for T peripheral helper (Tph) cells, including CCR2, CCR5, and CX3CR1 (42), we cannot exclude the possibility that the Tfh cluster contained Tph cells, given the limited expression of CXCR5.

We then compared the composition of CD4⁺ T cell subsets among three groups: high IgG/IgA ratio tumors ($n = 2$), low IgG/IgA ratio tumors ($n = 3$), and a normal endometrium ($n = 1$). Among the six CD4⁺ T cell populations, three subsets, consisting of Tfh, exhausted, and proliferating, were enriched in the high IgG/IgA ratio tumors (Fig. 4C, Supplementary Fig. 8). TCR clonality analysis with scTCR-seq demonstrated that Tfh cells shared their TCR clones with 31.2% of proliferating CD4⁺ T cells on average (Fig. 4D), highly suggesting their proliferation *in situ*. Collectively, these results suggest that tumors with a high IgG/IgA ratio contain active Tfh cell responses *in situ*. These Tfh cells may contribute to IgG class switching and the clonal expansion of B cells within the TME.

IgG⁺ PC precursors might differentiate from IFN-responding and DN B cells

To describe the precursors of IgG⁺ PCs in TME, we attempted to identify B cells sharing the same BCRs mainly with IgG⁺ PCs. Here, we analyzed all 10 samples by reconstructing the BCR repertoire sequence from the scRNA-seq data using TRUST4 (28). CD20⁺ conventional B cells sharing more BCRs with IgG⁺ PCs than with IgA⁺ PCs were considered as IgG⁺ PC precursors, and *vice versa*. Accordingly, we identified 711 IgG⁺ PC precursors and 220 IgA⁺ PC precursors (Fig. 5A). Differential gene expression analysis revealed that IgG⁺ PC precursors have higher expression of IFN-stimulated genes and genes expressed by DN B cells (Fig. 5B, Supplementary Fig. 9). Consistently, BCR repertoire overlap analysis showed that IgG⁺ PCs shared more BCRs with IFN-responding and DN B cells than IgA⁺ PCs (Fig. 5C). Therefore, IgG⁺ PCs likely differentiate from IFN-responding and DN B cells in the TME.

Reactivities of oligoclonal IgG antibodies towards tumor and normal endometrial cells

To identify the targets of the oligoclonal IgG antibodies, recombinant antibodies were synthesized for the most expanded clones of EC07 and EC08. We found that EC07-IgG recognized the patient's own tumor cells but not the background normal tissues (Fig. 6). By contrast, EC08-IgG recognized some tumor cells and the background normal endometrium. These data suggest that B cell responses in the TME, especially IgG-dominant B cell responses (i.e., high IgG/IgA ratio), can occur against antigens expressed by tumor cells, which may be shared with normal endometrial cells.

Discussion

Here, we analyzed B cell responses in EC through single-cell multi-omics and identified oligoclonal expansion of IgG⁺ B cells in patients with a high IgG/IgA cell number ratio. The oligoclonal expansion of IgG⁺ B cells coincided with an increase in Tfh cells, suggesting active T-B interactions within the TME. The oligoclonal IgG antibodies recognized both tumor and normal endometrial cells.

We found that IgG⁺ B cells clonally expanded in some patients. In these patients, IgG⁺ B cells outnumbered IgA⁺ B cells, resulting in a high IgG/IgA cell number ratio. Since high IgG expression and a high IgG/IgA cell number ratio in the TME are associated with better overall survival, our findings suggest that oligoclonal IgG⁺ B cell expansion in the TME is linked to a favorable prognosis. Although previous studies have shown that the presence of B cells is associated with better clinical outcomes in EC (20) (21), it remained unclear exactly what B cell response improves the prognosis. Our study identifies oligoclonal IgG⁺ B cell expansion as a distinct B cell response that may improve clinical outcomes.

Our simultaneous analysis of CD4⁺ T cells revealed that oligoclonal expansion of IgG⁺ B cells was accompanied by an increase in Tfh cells. In contrast, Tfh cells were rare in tumors without the oligoclonal expansion of IgG⁺ B cells. These findings are consistent with previous reports indicating that Tfh cells are predominantly found in mature TLS (2) (4). Thus, our findings provide additional evidence suggesting that Tfh cells are indispensable for clonal B cell expansion and the formation of mature TLS.

Immunohistochemistry using recombinant antibodies demonstrated that the dominant IgG recognized only tumor cells in one patient, while in another, IgG recognized both tumor and normal endometrial cells. Our analysis of endometrial cancer aligns with a recent study showing that *in situ*-produced antibodies recognized tumor cells in high-grade serous ovarian cancer (6). In addition, many types of solid tumors are coated with endogenous IgG antibodies at varying frequencies (4) (6). Therefore, the production of antibodies recognizing antigens expressed by both tumor cells and normal tissues might be a general phenomenon across cancer types.

In conclusion, our single-cell multi-omic study suggests that B cells interact with Tfh cells and clonally expand in the TME of EC. These clonally expanded B cells differentiate into PCs to secrete IgG antibodies, which recognize antigens expressed by tumor cells and normal endometrial cells. Such B cell responses might contribute to a better prognosis in EC. Therefore, our study deepens the understanding of *in-situ* B cell responses in EC, potentially paving the way for novel immunotherapies.

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Data availability

The single-cell experimental data generated in this study are publicly available through the National Bioscience Database Center (NBDC) Human Database of Japan. Raw data can be accessed via the controlled-access database JGA (accession ID: JGAS000827/JGAD000969) upon application. Processed data are available without application in the GEA database (accession ID: E-GEAD-1121).

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Conflict of interest statement

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Author contributions

Conceptualization: M.F., H.Y. and H.U. Formal analysis: M.F. and S.F. Investigation: M.F., S.F., H.Y., S.M. and H.I. Resources: M.U., H.S., J.H. and Y.T. Data curation: M.F. and S.F. Writing – original draft: M.F., S.F. and H.U. Writing – review and editing: all authors. Visualization: M.F. and S.F. Supervision: M.M. and H.U. Project administration: M.F., M.M. and H.U. Funding acquisition: M.F., H.Y. and H.U.

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Figure 1. Plasma cells are increased in EC

(A) Experimental workflow. CD19⁺ B cells from EC tissues ($n = 10$) and a normal endometrium ($n = 1$) were sorted by FACS and analyzed for their transcriptomes at the single-cell level using scRNA-seq. (B) UMAP (left) and dot plot (right) of 45,425 B cells in 10 EC tissues. DN: double-negative. (C) UMAP plot of 2,126 B cells in a normal endometrium. (D) The frequency of B cells (left) and plasma cells (right) in EC ($n = 10$) and normal endometria. Normal endometrial data were obtained from this study ($n = 1$) and the Human Cell Atlas (HCA, $n = 14$) (29). Frequencies were calculated using FACS data (left two columns) or scRNA-seq data (right four columns). Dots represent each tissue, while bars represent the mean.

Figure 2. IgG⁺ B cells are associated with better overall survival in EC

(A) Survival analysis with TCGA data (30) (31) (32). IgG (left) and IgA (right) expression was analyzed and plotted, respectively. P -values were calculated using log-rank tests. (B) Experimental workflow. Two pathologists independently identified three lymphocyte aggregates per slide and counted the number of CD20⁺, IgG⁺, and IgA⁺ cells in each aggregate for 86 cases. The average number was used for the survival analysis. (C) Representative HE and immunohistochemistry (CD20, IgG, and IgA) images of a lymphocyte aggregate. Arrows indicate IgG⁺ or IgA⁺ cells. Scale bars: 100 μ m. (D) Survival analysis of IgG/IgA cell number ratio based on the immunohistochemistry data. P -values were calculated using log-rank tests.

Figure 3. IgG⁺ B cells expanded clonally in the tumor microenvironment

(A) The composition of immunoglobulin classes in the total B cells from 10 EC tissues. B cells whose constant regions could not be determined are removed. (B) IgG/IgA cell number ratio of the total B cells for each tumor tissue. (C) Honeycomb plot of the total B cells from each tumor sample. Each dot represents a cell, while a cluster of dots represents a clonotype. (D) Top 10 dominant clones in each tumor tissue. The y-axis represents the percentage in the total B cells. (E) The degree of somatic hypermutations (SHM) outside the junction region in each B cell, visualized on the UMAP. $\log_2((\text{number of mutations}) + 1)$ is shown. B cells lacking VDJ information are shown in gray.

Figure 4. IgG⁺ B cell expansion accompanies an active *in situ* Tfh response

(A) UMAP of 10,023 CD4⁺ T cells from EC tissues (left, $n = 5$) and 2,996 CD4⁺ T cells from a normal endometrial tissue (right, $n = 1$). (B) Dot plot showing marker genes of CD4⁺ T cell clusters in EC and normal endometrium. (C) Frequency of Tfh, exhausted CD4⁺ T, and proliferating CD4⁺ T cells among the total CD4⁺ T cells. Data are shown for IgG/IgA high ($n = 2$) and low tumor tissues ($n = 3$) and normal endometrial tissue ($n = 1$). Dots represent each tissue, while bars represent the mean. (D) Frequency of cells sharing TCR clonotypes with Tfh cells. Data are shown for IgG/IgA high ($n = 2$) and low tumor tissues ($n = 3$). Dots represent each tissue, while bars represent the mean.

Figure 5. IgG⁺ PCs likely differentiate from IFN-responding and DN B cells

(A) IgG⁺ PC precursors and IgA⁺ PC precursors, highlighted on the UMAP. IgG⁺ PC precursors were defined as CD20⁺ conventional B cells sharing more BCRs with IgG⁺ PCs than with IgA⁺ PCs, and *vice versa*. (B) Dot plot showing the top 20 differentially expressed genes in IgG⁺ PC precursors compared to IgA⁺ PC precursors. (C) BCR repertoire overlap analysis between IgG⁺/IgA⁺ PCs and CD20⁺ conventional B cell subsets. Overlap coefficients of BCR repertoires were shown.

Figure 6. *In situ* produced IgG antibodies recognized tumor cells as well as normal endometrial cells

Immunohistochemistry using recombinant antibodies derived from the most expanded clones in EC07 (left) and EC08 (right). Tumor tissues and the background normal tissues were stained with recombinant antibodies and DAPI. The same tissues were stained with isotype control and DAPI. rAb: recombinant antibodies. Scale bars: 100 μ m.

Figure 1

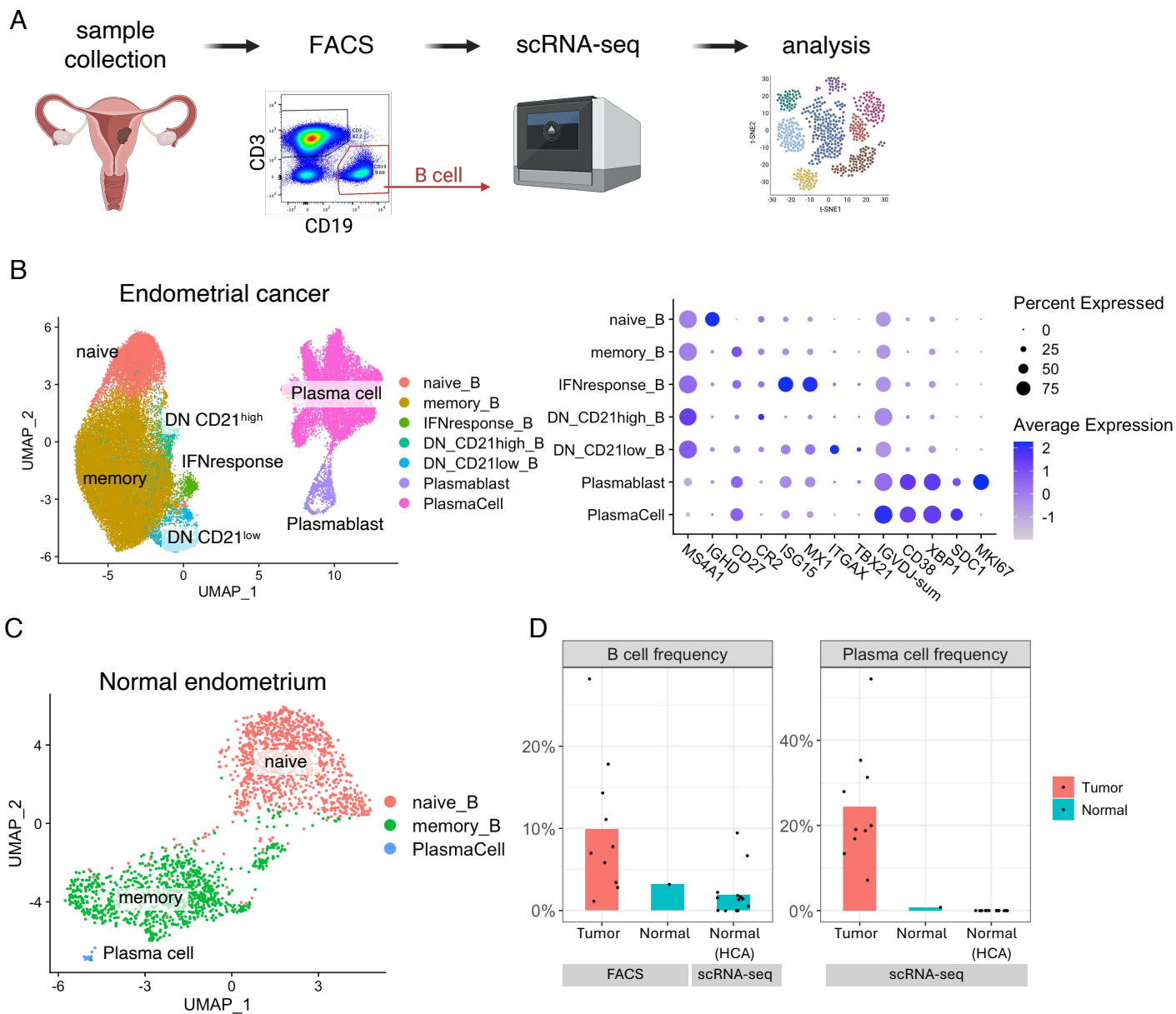
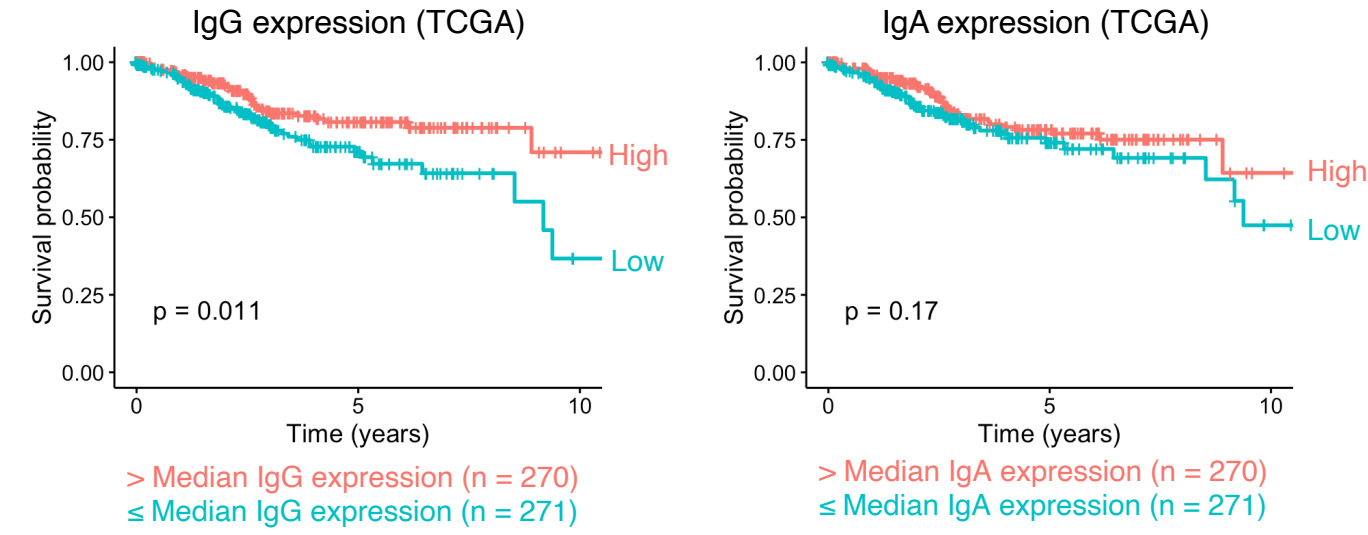
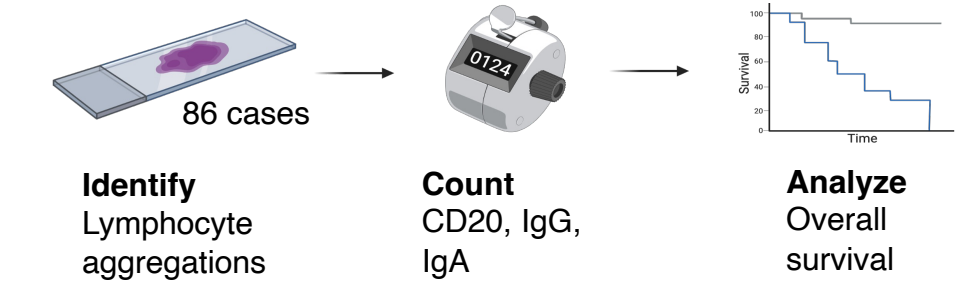


Figure 2

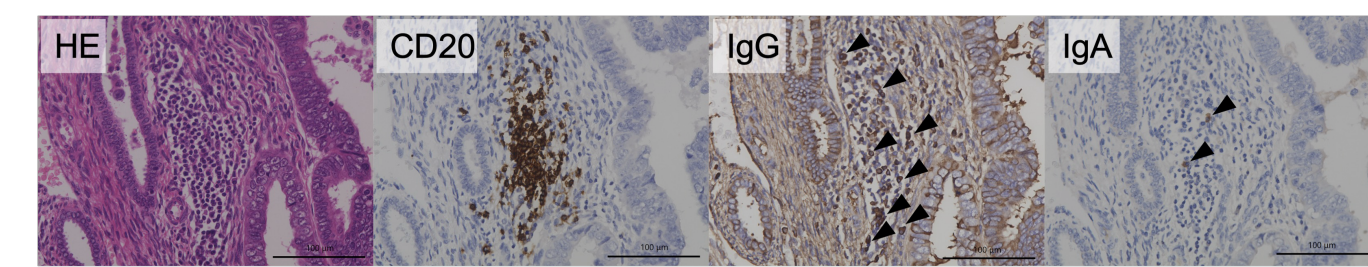
A



B



C



D

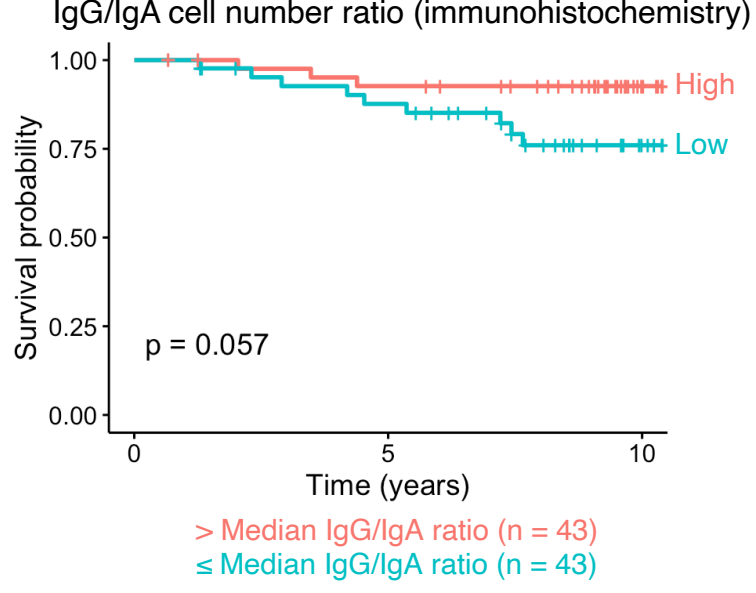


Figure 3

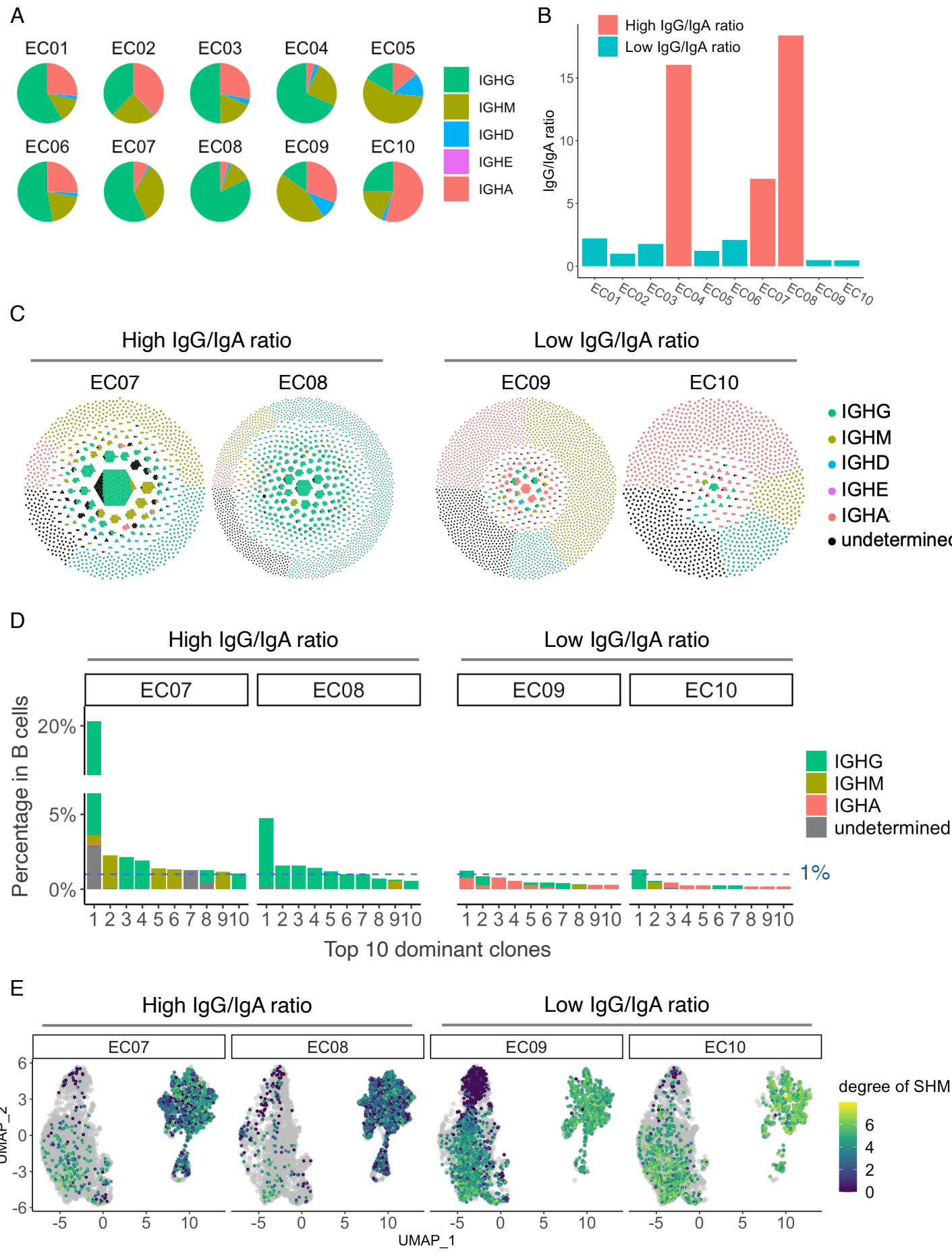


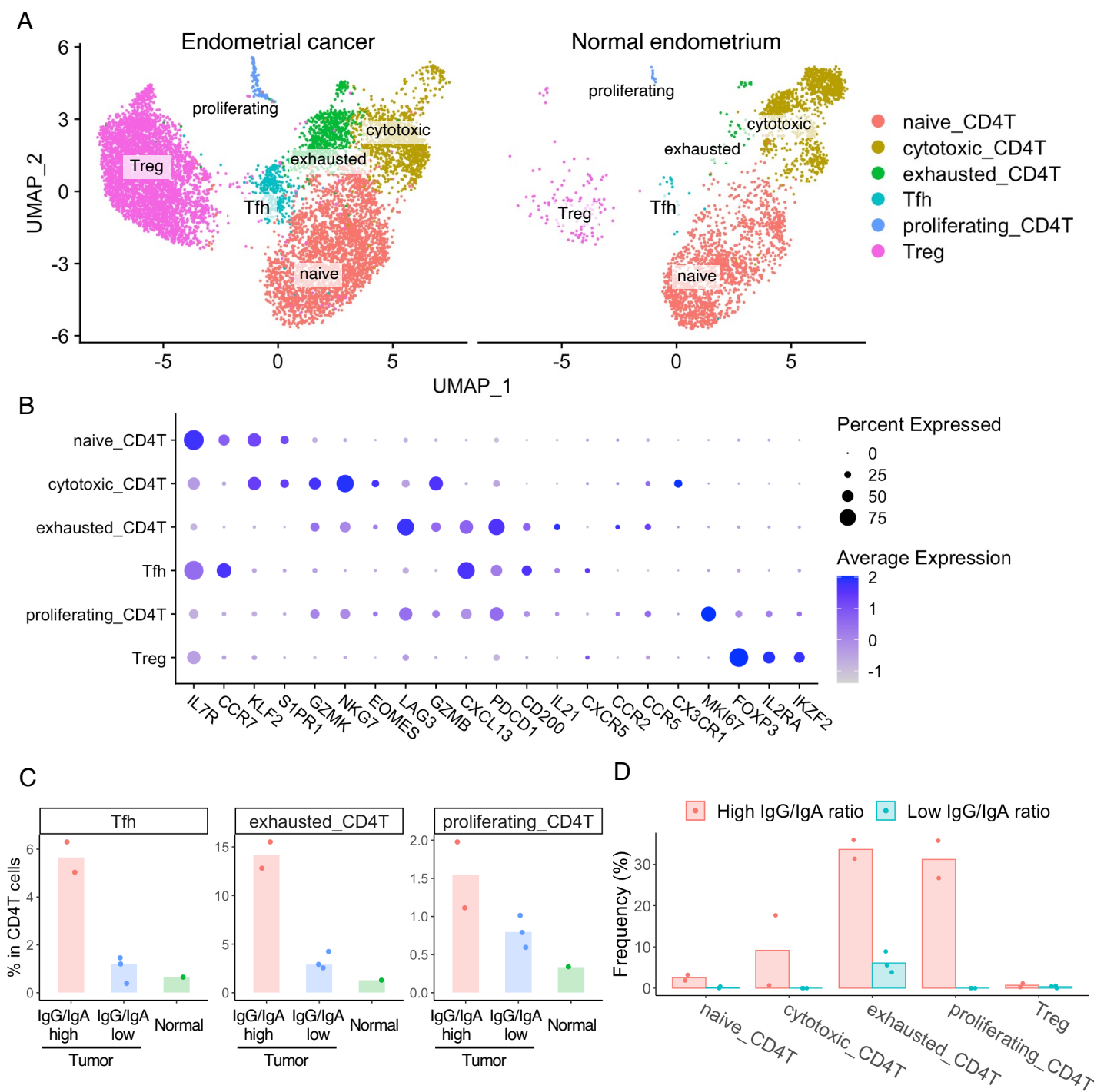
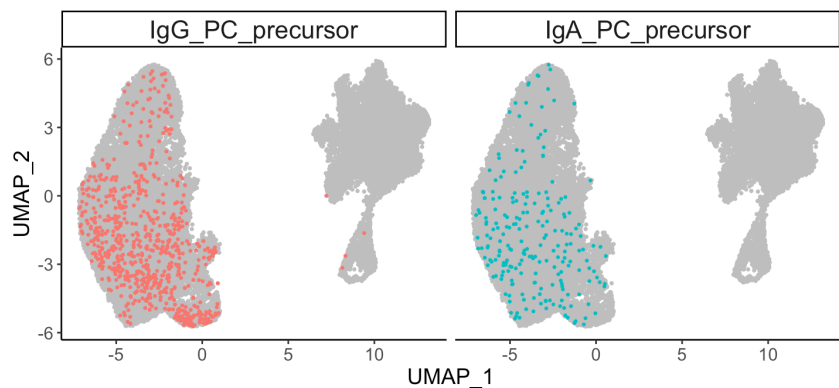
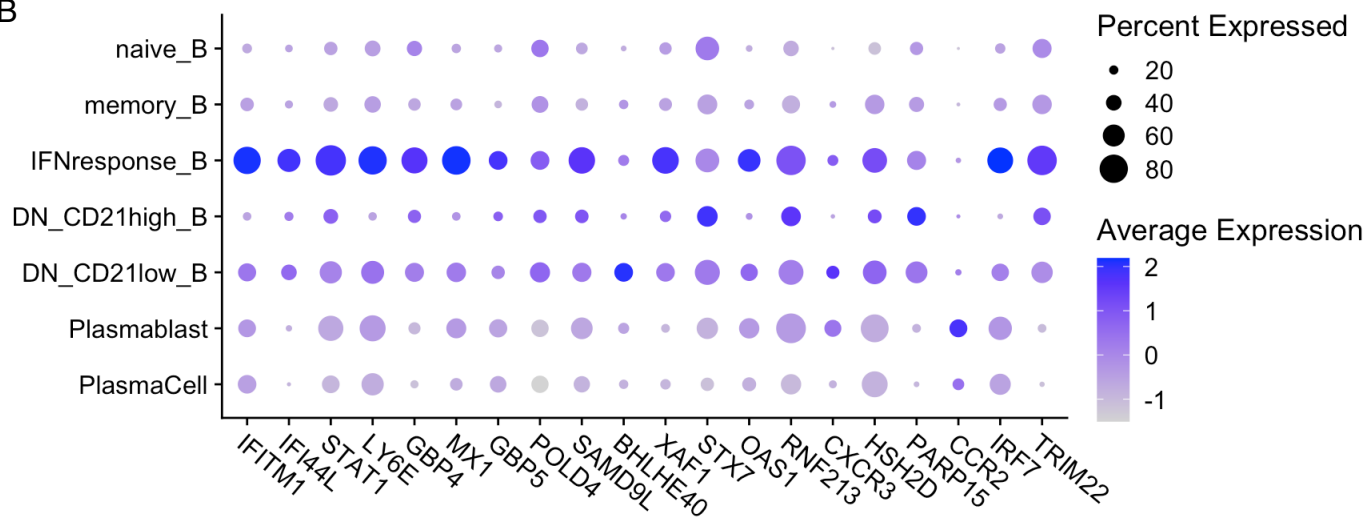
Figure 4

Figure 5

A



B



C

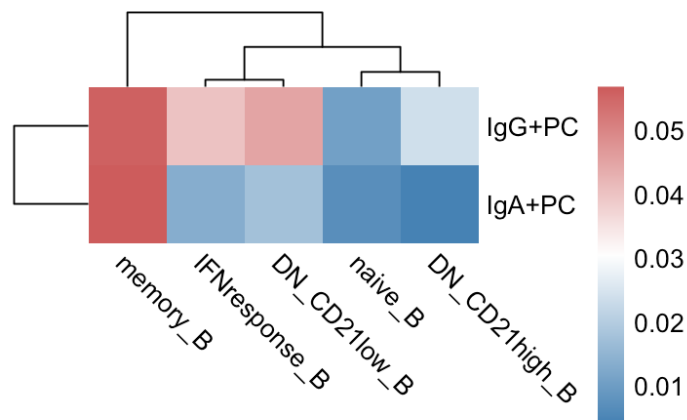
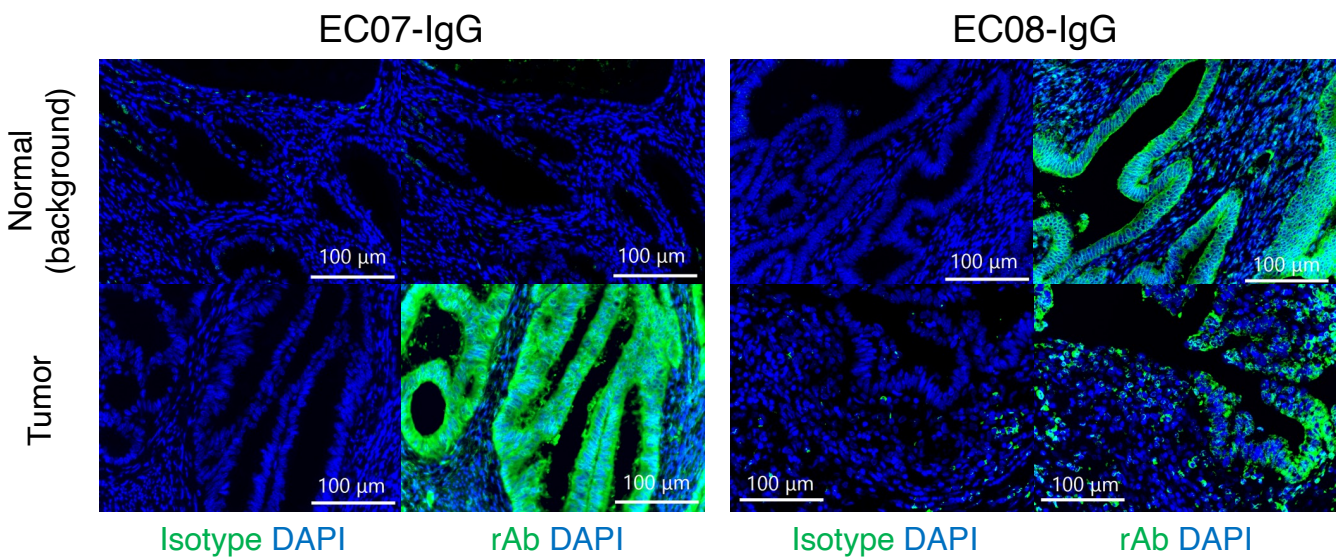
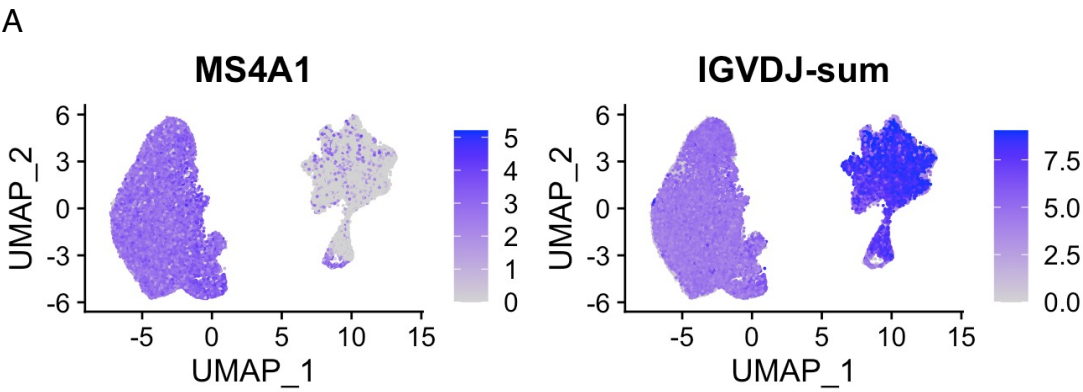


Figure 6



Supplementary Figure 1



Supplementary Figure 1. Phenotype of B cells in endometrial cancer

(A) Feature plot showing marker genes for conventional B cells and plasma cells. (B) Heatmap showing the top 10 differentially expressed genes for each cluster ($p_val_adj < 0.05$). P -values were calculated using a Wilcoxon rank sum test and adjusted by the Bonferroni method. (C) Feature plot showing the expression of GC-B cell marker genes. (D) Feature plot showing the expression of immunoglobulin genes.

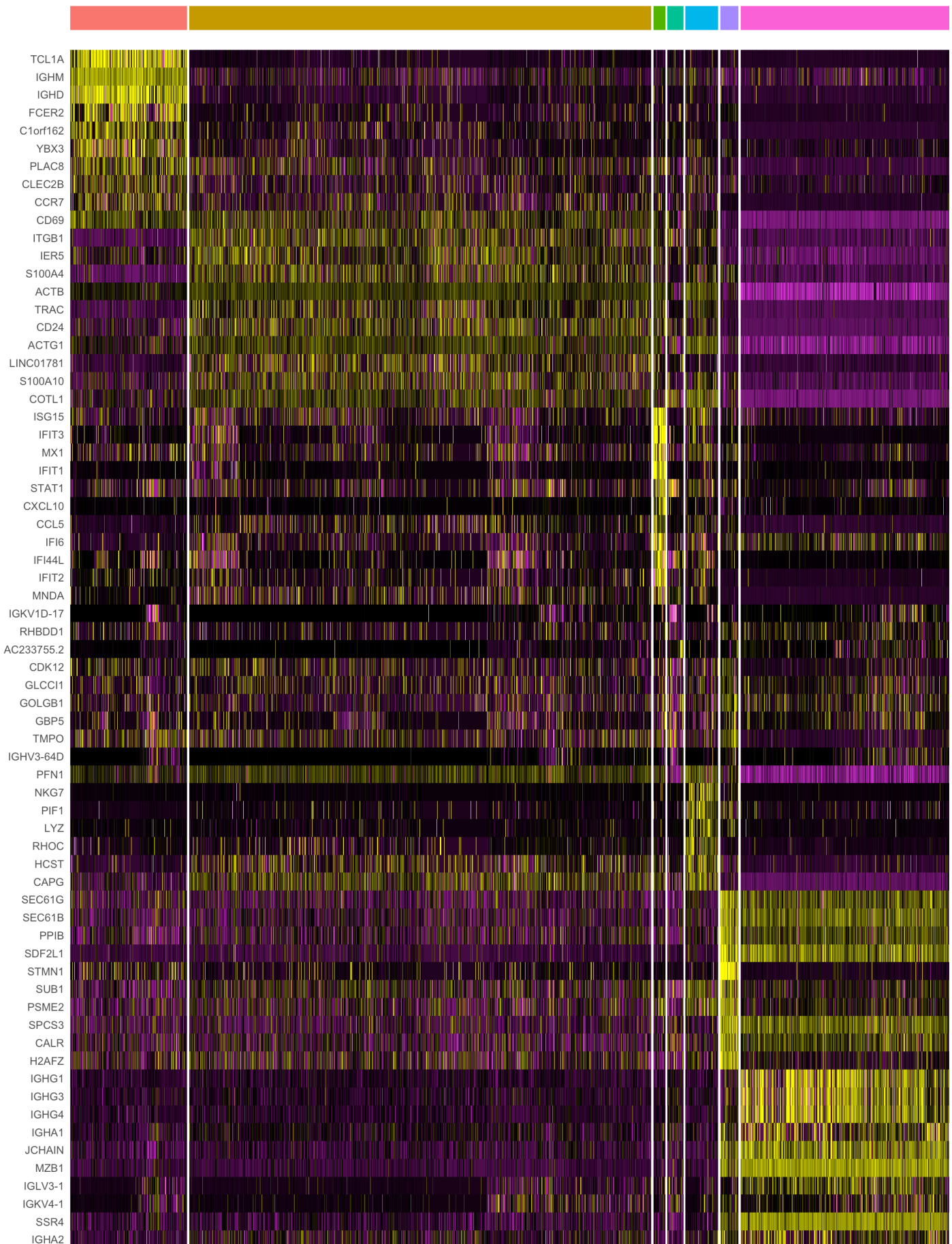
B

naive_B

memory_B

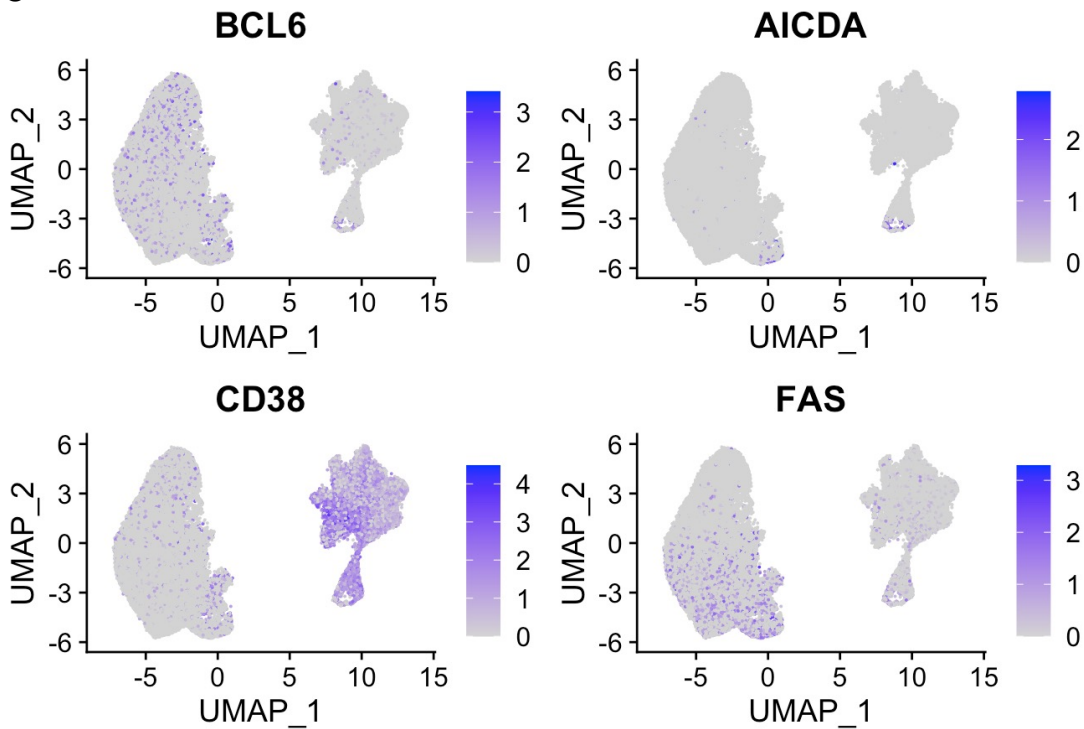
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DN_CD21high_B
DN_CD21low_B
Plasmablast

PlasmaCell

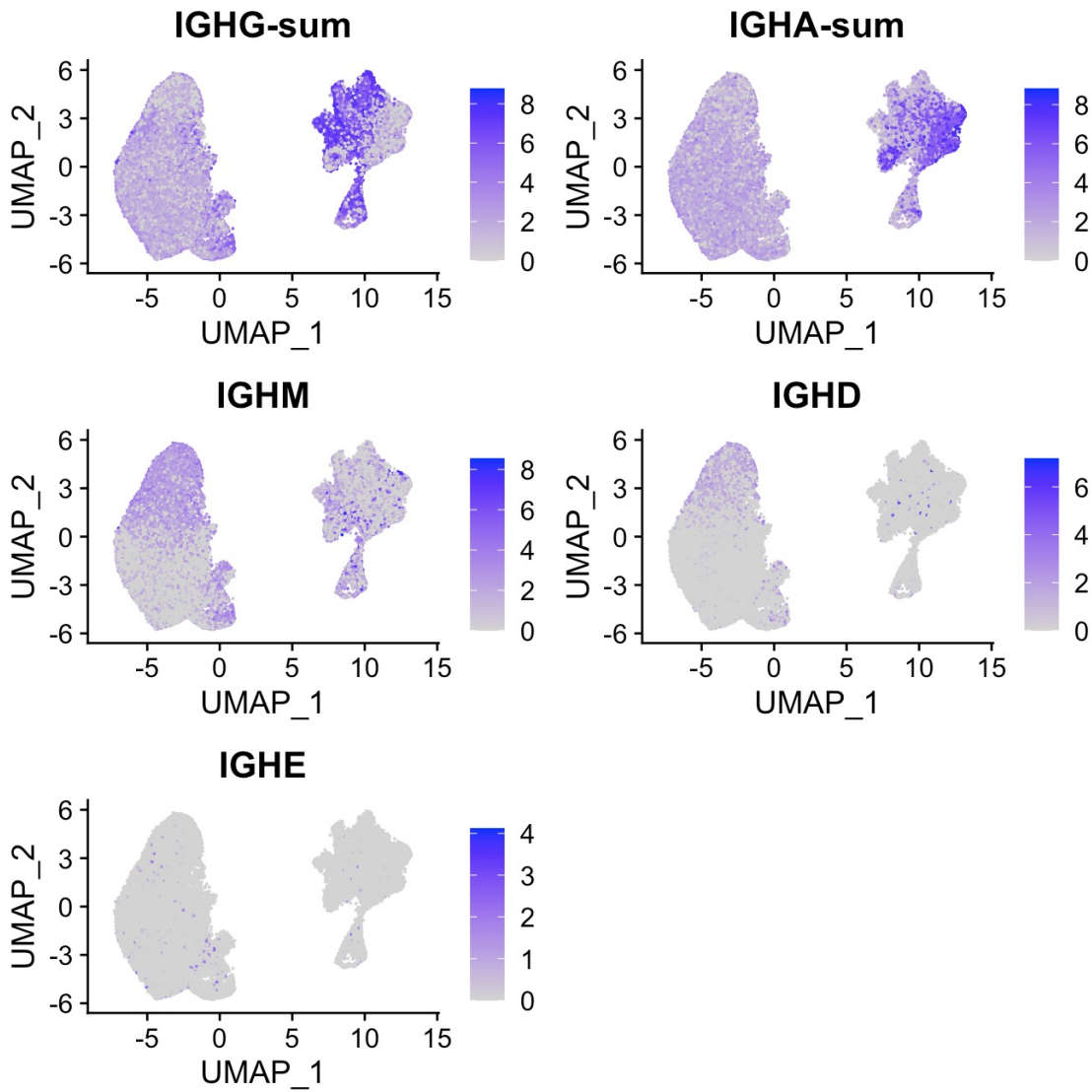


Supplementary Figure 1

C

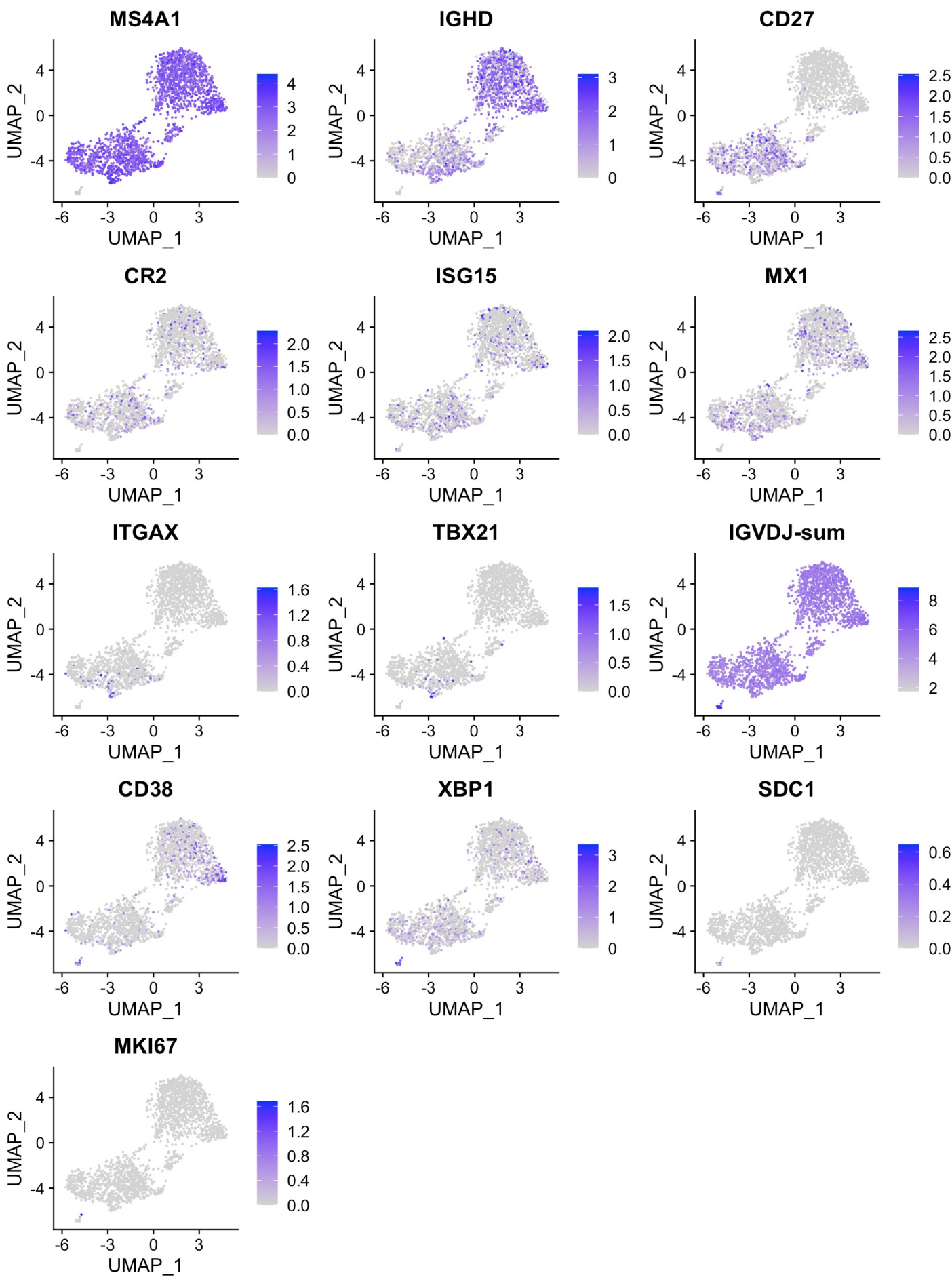


D



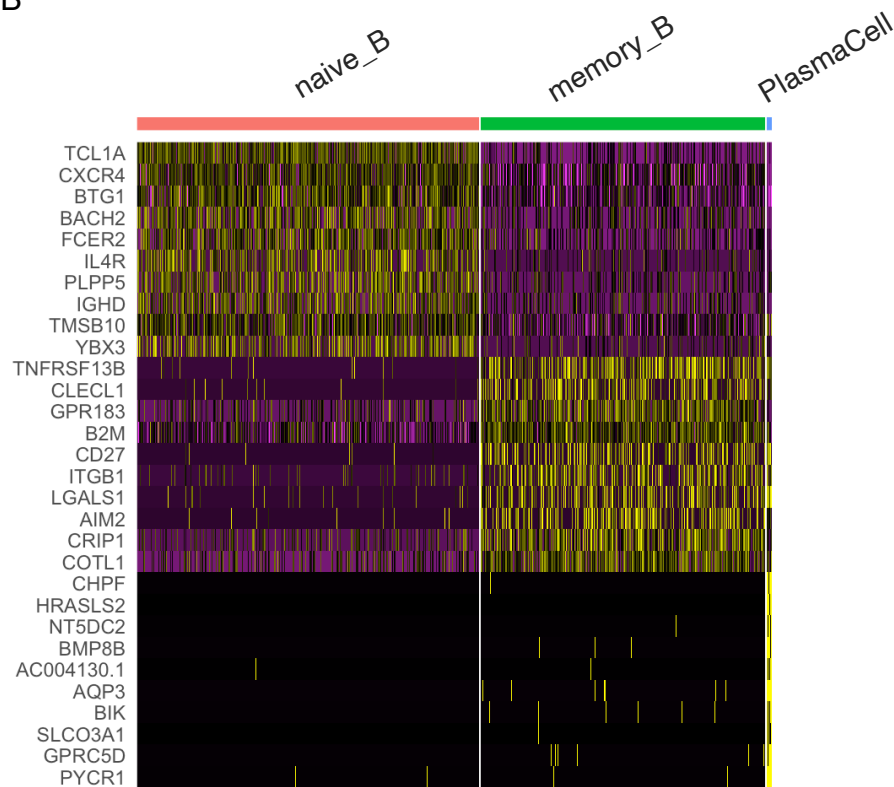
Supplementary Figure 2

A



Supplementary Figure 2

B

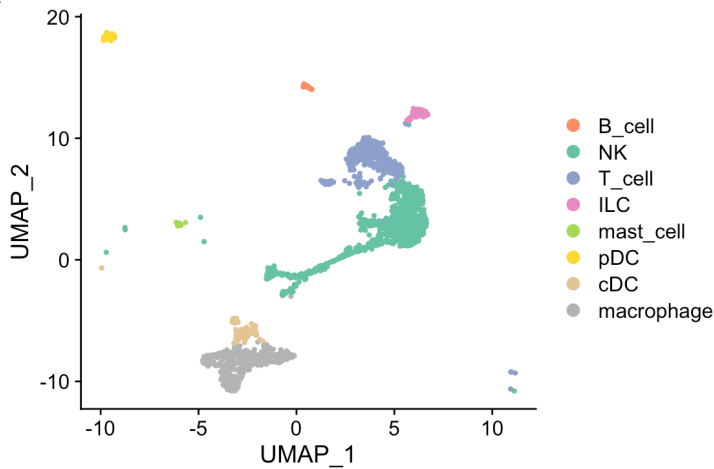


Supplementary Figure 2. Phenotype of B cells in a normal endometrium

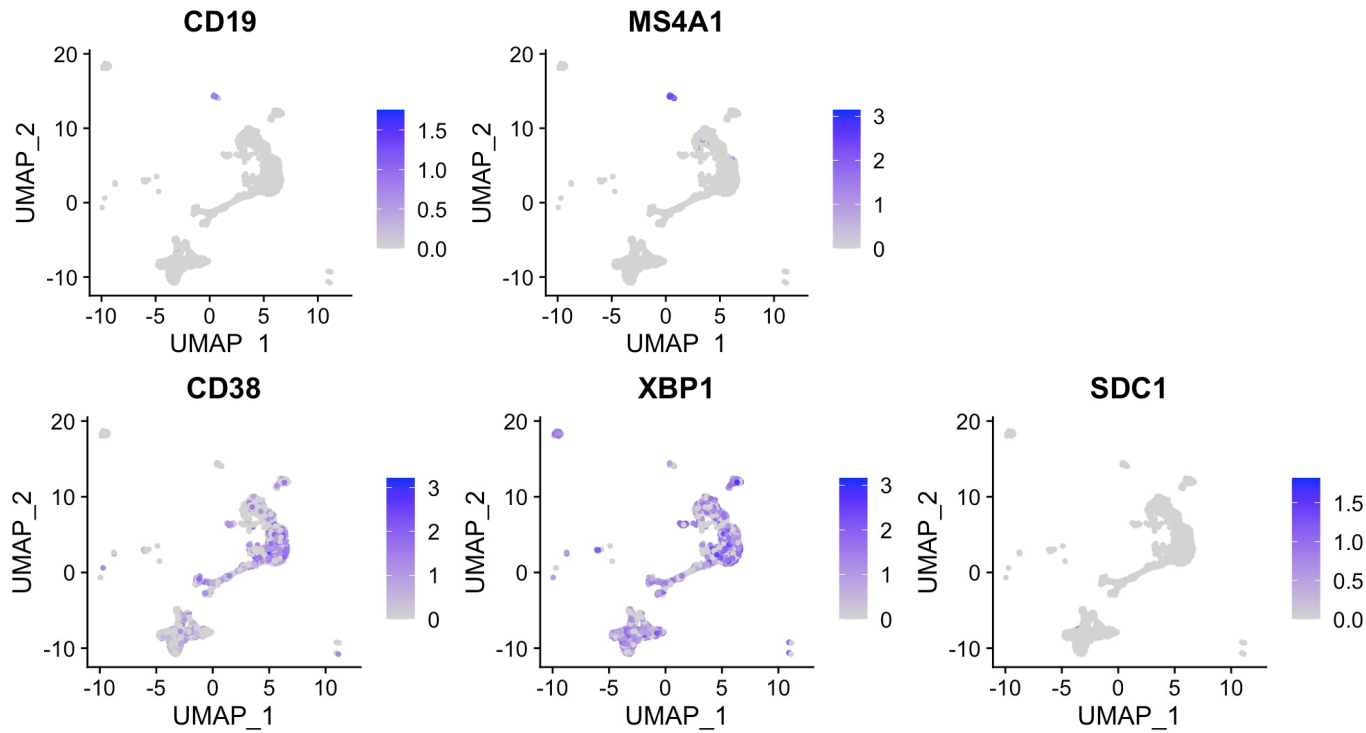
(A) Feature plot showing B cell marker genes, including negatively expressed ones. (B) Heatmap showing the top 10 differentially expressed genes for each cluster ($p_val_adj < 0.05$). P -values were calculated using a Wilcoxon rank sum test and adjusted by the Bonferroni method.

Supplementary Figure 3

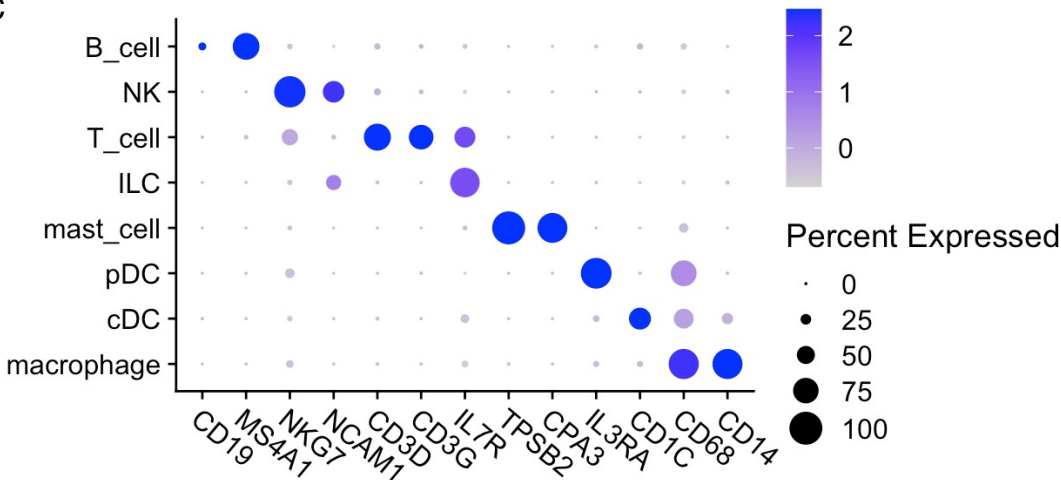
A



B

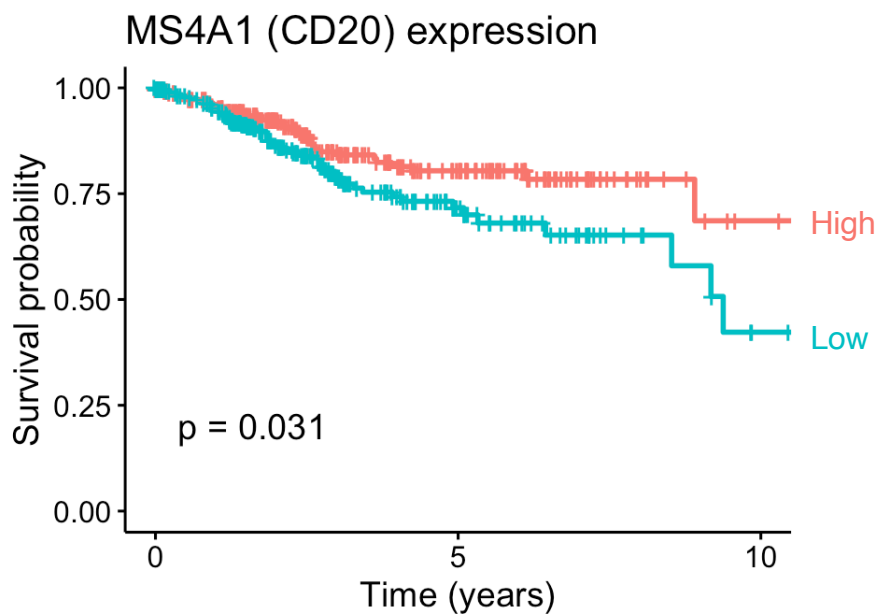


C



Supplementary Figure 3. Phenotype of immune cells in normal endometrial tissues (Human Cell Atlas)
(A) UMAP of immune cells in normal endometria ($n = 14$) (Human Cell Atlas data (29)). (B) Feature plot showing B cell marker genes, including negatively expressed ones. (C) Dot plot showing marker genes for each cluster.

Supplementary Figure 4

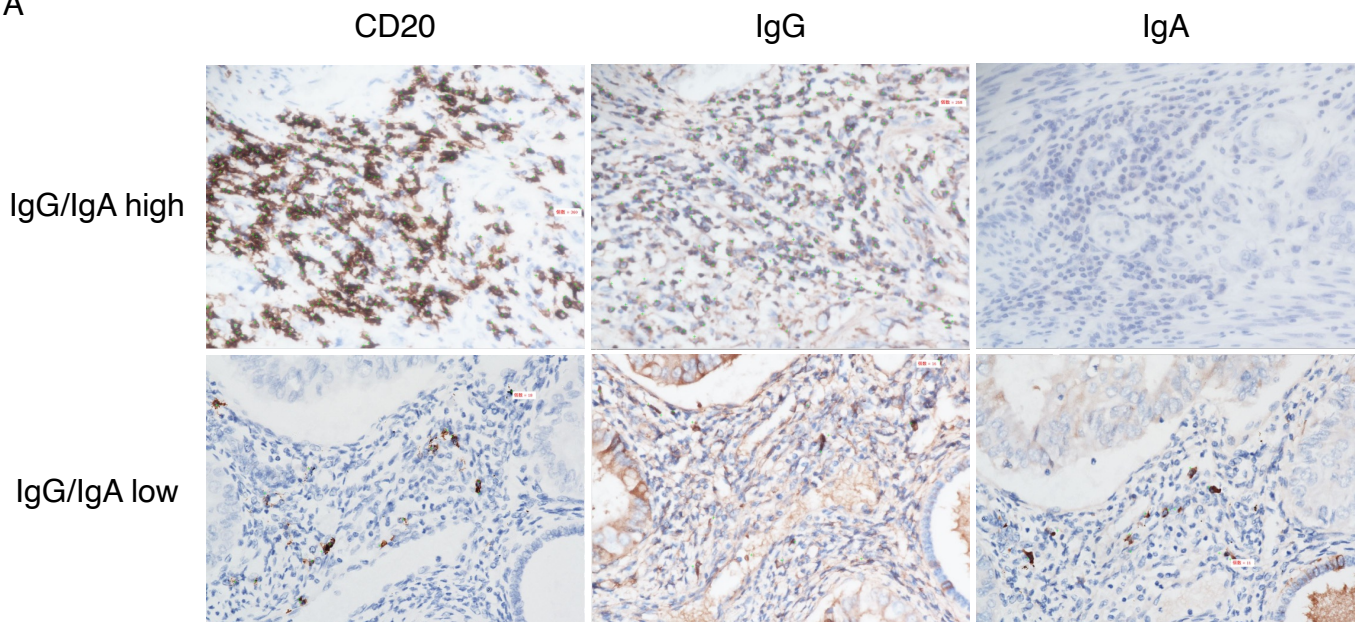


Supplementary Figure 4. Survival analysis using TCGA data

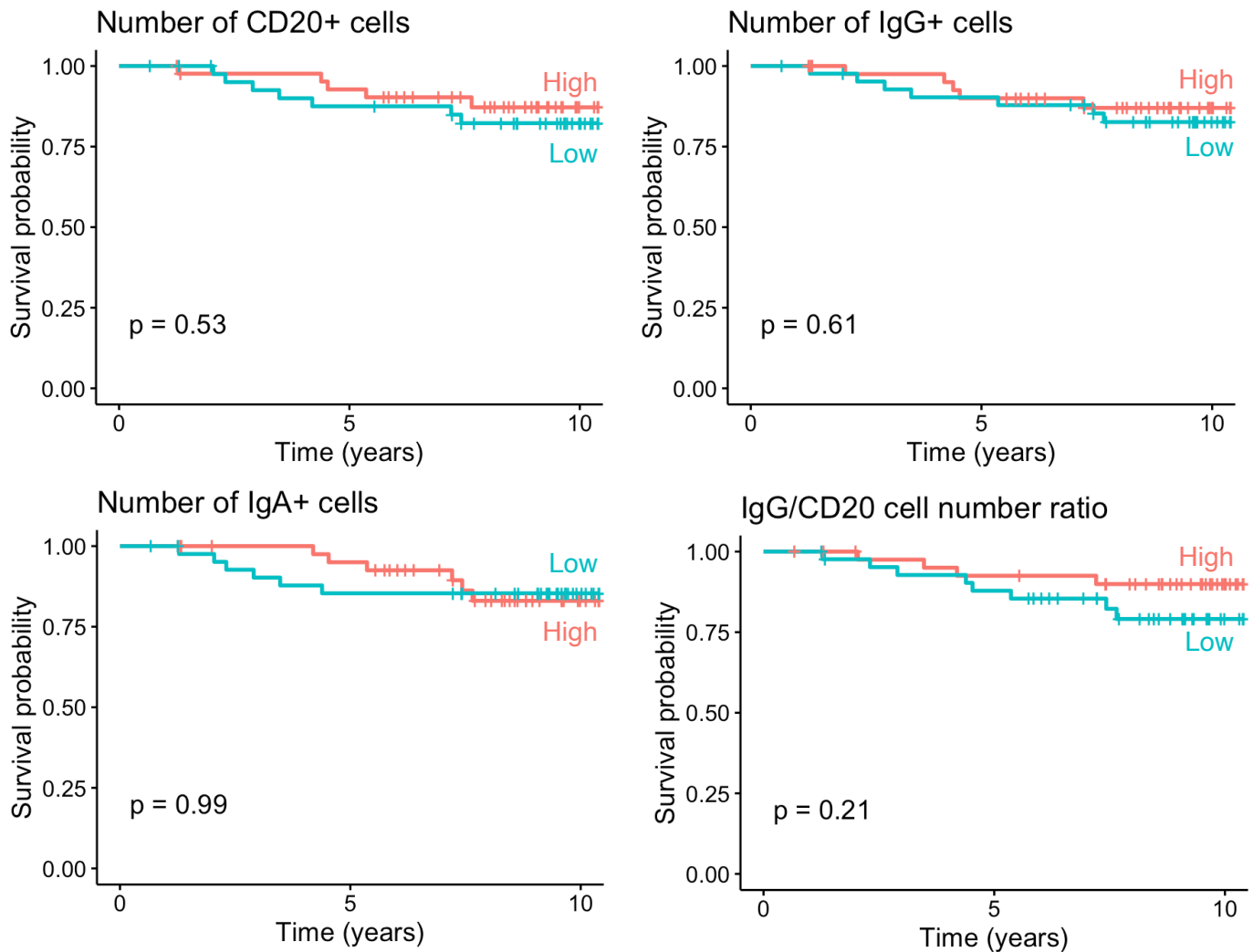
Survival analysis with TCGA data (30) (31) (32). MS4A1 (encoding CD20) expression was analyzed and plotted ($n = 541$). High is defined as larger than or equal to the median, whereas low is defined as smaller than the median. P -values were calculated using log-rank tests.

Supplementary Figure 5

A



B

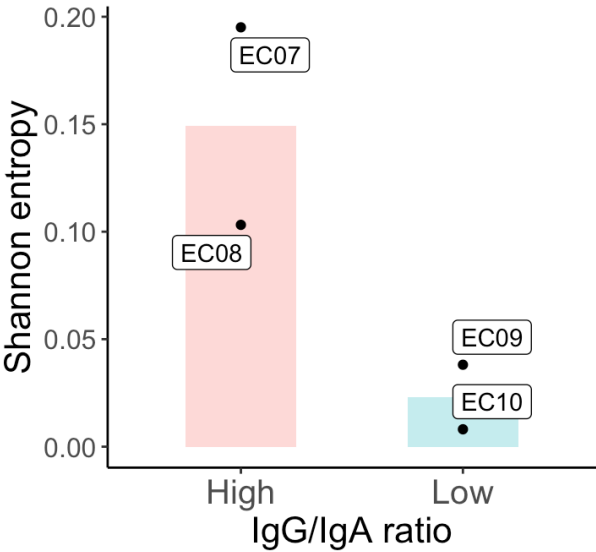


Supplementary Figure 5. Survival analysis using immunohistochemistry

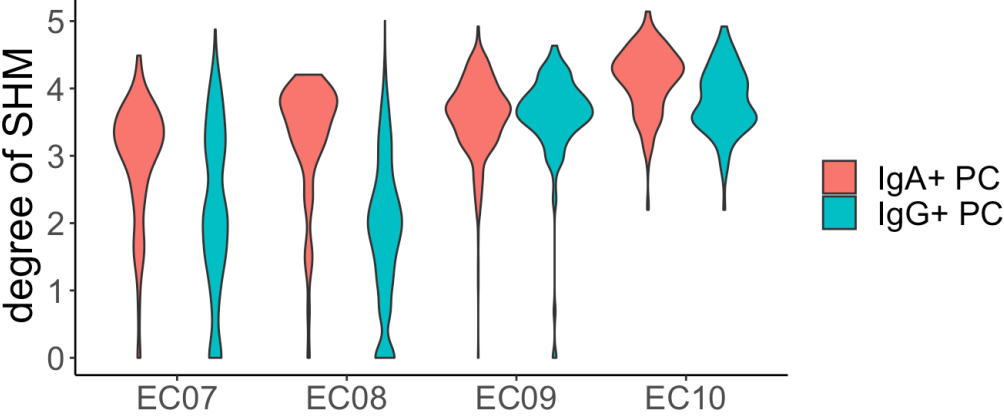
(A) Representative immunohistochemistry images of a lymphocyte aggregate from IgG/IgA ratio high and low tissues. (B) The numbers of CD20⁺, IgG⁺, and IgA⁺ cells, as well as the IgG/CD20 cell number ratio, were analyzed ($n = 86$). High is defined as larger than or equal to the median, whereas low is defined as smaller than the median. P -values were calculated using log-rank tests.

Supplementary Figure 6

A



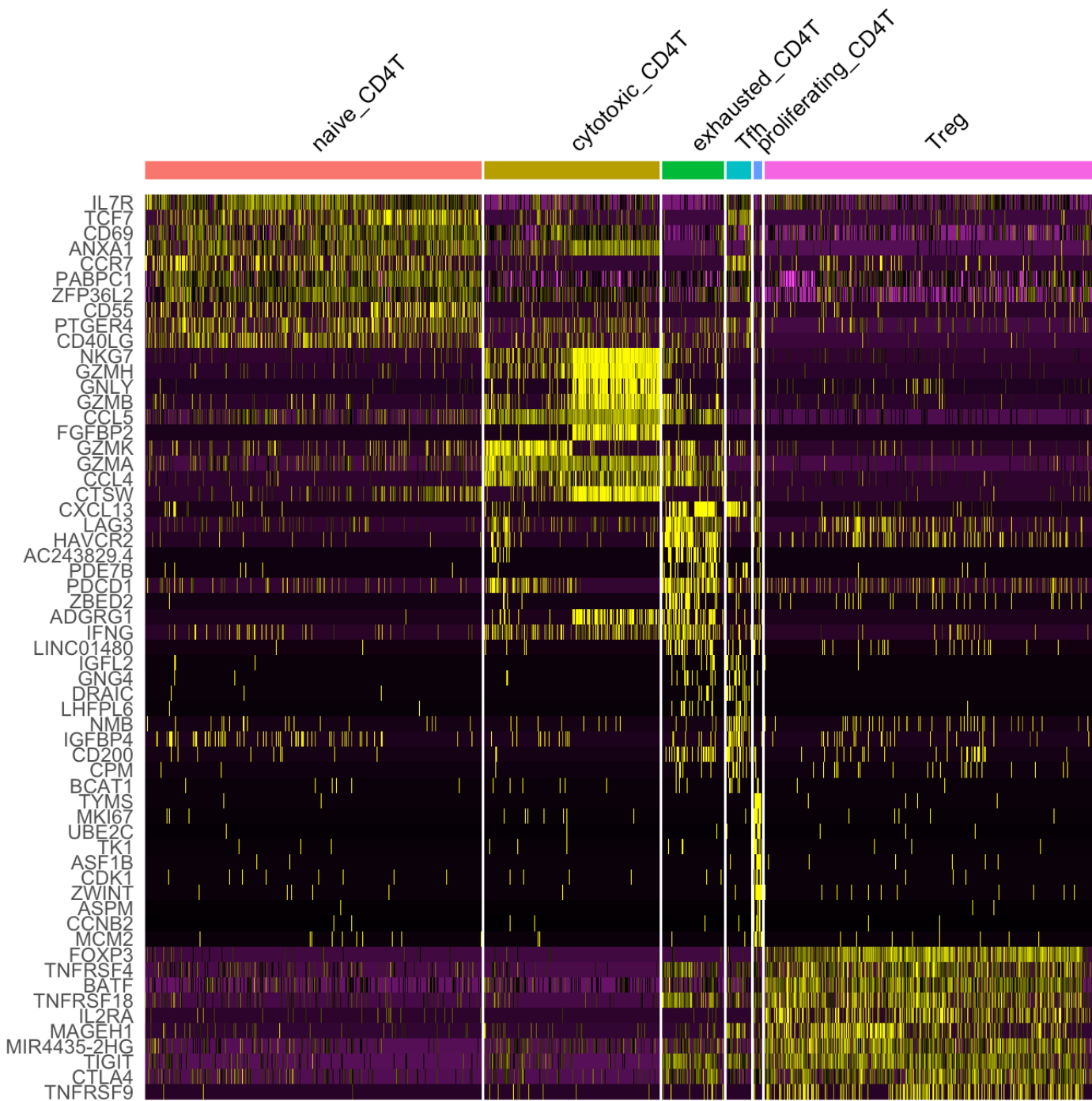
B



Supplementary Figure 6. IgG⁺ B cells clonally expanded with fewer somatic hypermutations

(A) Shannon entropy of the top 100 BCR clonotypes for each patient. (B) Violin plots showing the degree of somatic hypermutations (SHM) outside the junction region of IgA⁺ and IgG⁺ PCs in each tumor tissue. $\log_2((\text{number of mutations}) + 1)$ is shown.

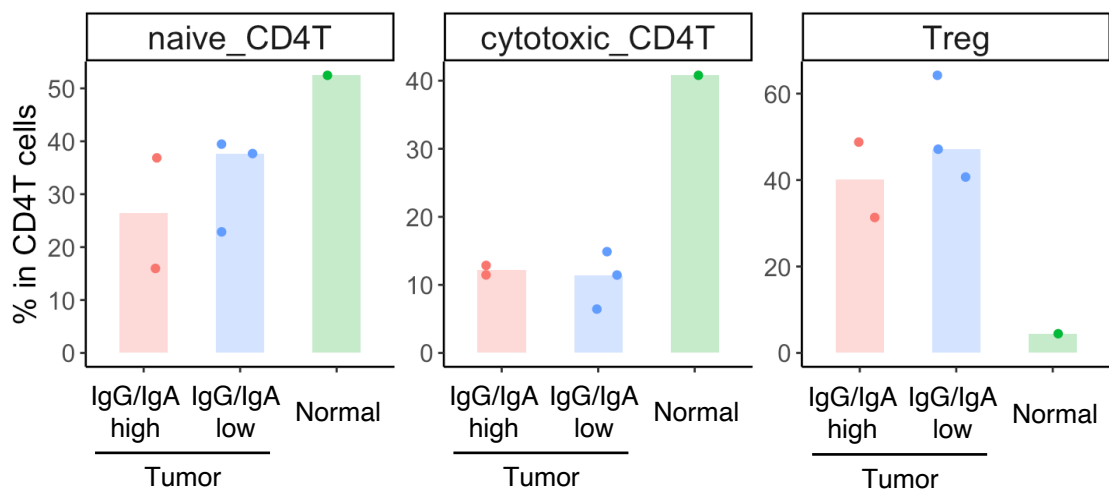
Supplementary Figure 7



Supplementary Figure 7. Phenotype of CD4⁺ T cell subsets in endometrial cancer and normal endometrium

Heatmap showing the top 10 differentially expressed genes of each cluster ($p_val_adj < 0.05$). P -values were calculated using a Wilcoxon rank sum test and adjusted by the Bonferroni method.

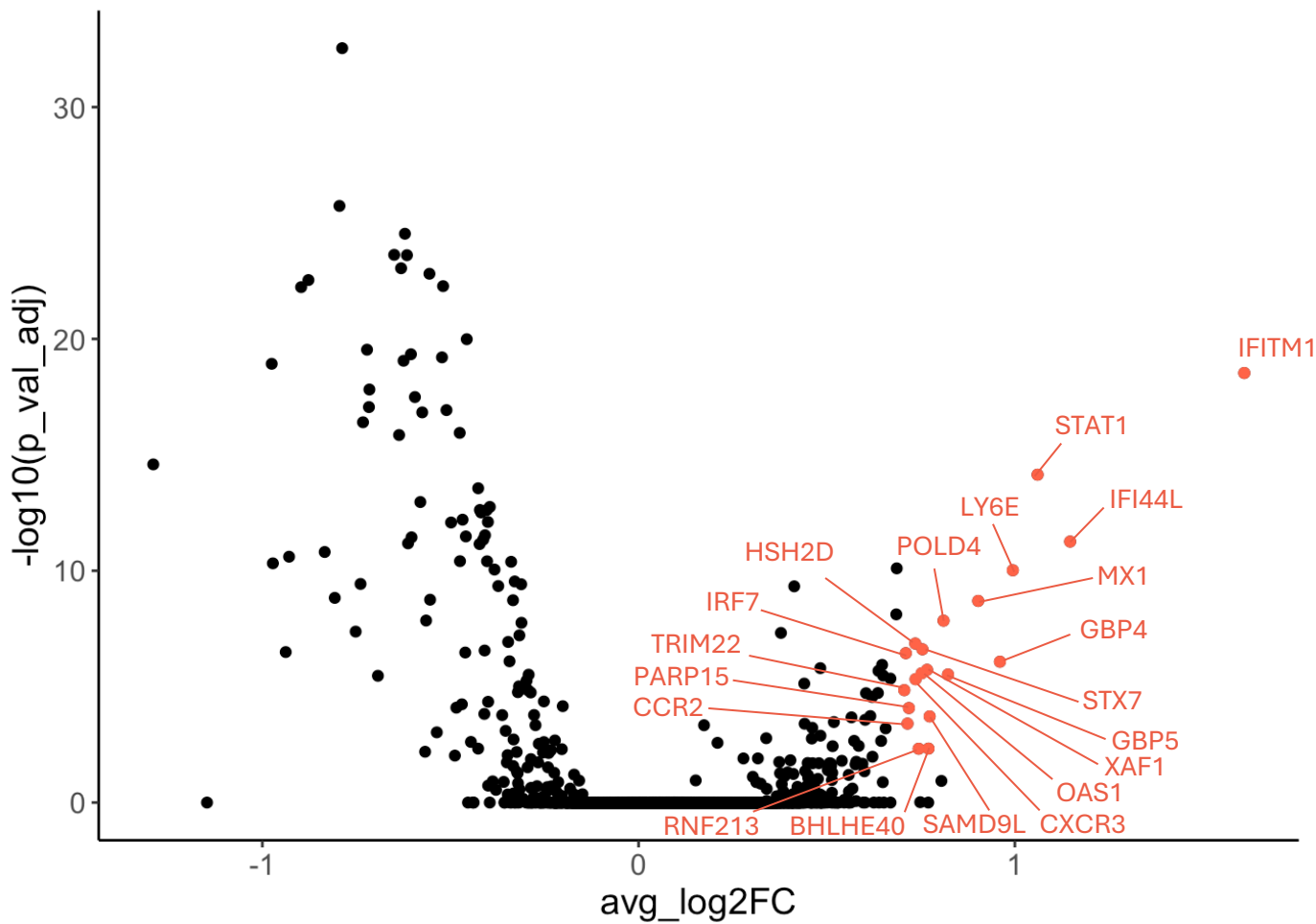
Supplementary Figure 8



Supplementary Figure 8. Frequencies of CD4⁺ T cell subsets in endometrial cancer and normal endometrium

Frequencies of naive CD4⁺ T cells, cytotoxic CD4⁺ T cells, and Treg cells among the total CD4⁺ T cells were compared. Data are shown for IgG/IgA high (*n* = 2) and low tumor tissues (*n* = 3) and normal endometrial tissue (*n* = 1). Dots represent each tissue, while bars represent the mean.

Supplementary Figure 9



Supplementary Figure 9. Differentially expressed genes in IgG⁺ PC precursors
Volcano plot of differential gene expression analysis between IgG⁺ PC precursors and IgA⁺ PC precursors. Highlighted genes are the top 20 significantly ($p_{val_adj} < 0.05$) differentially expressed genes in IgG⁺ PC precursors. P -values were calculated using a Wilcoxon rank sum test and adjusted by the Bonferroni method.

Supplementary Table 1

ID	experiment	age	Gravidity/Parity	BMI	FIGO stage	TNM stage	histology	grade	tumor size (cm)	mutation	CEA (ng/mL)	CA19-9 (U/mL)	CA125 (U/mL)
EC01	RNA_B	48	G0	19.5	IA	pT1aN0	endometrioid adenocarcinoma	G1	4.5	PMS2/MLH1	5.9	1.3	62.2
EC02	RNA_B	70	G2P2	22.55	IIIA	pT3aN0	endometrioid adenocarcinoma	G1	3.5	NA	1.8	47.8	17.5
EC03	RNA_B, RNA_T, TCR	55	G1P1	25.56	IIIC2	pT3N2	endometrioid adenocarcinoma	G1	11	NA	2.6	232.4	858.5
EC04	RNA_B, RNA_T, TCR	58	G2P2	20.04	IIIC	pT1aN1	serous carcinoma	NA	4	NA	1.3	28.5	36.3
EC05	RNA_B	60	G2P1	24.63	IIIC	pT1bN2	endometrioid adenocarcinoma	G2	NA	NA	1.1	32.3	481.8
EC06	RNA_B, RNA_T, TCR	87	G2P2	25.03	IIIC2	pT1bN2	endometrioid adenocarcinoma	G1	5.9	NA	1.8	106.5	48.8
EC07	RNA_B, BCR, RNA_T, TCR	53	G0	29.6	IA	pT1aN0	endometrioid adenocarcinoma	G1	NA	NA	2.4	20.4	16.9
EC08	RNA_B, BCR	50	G0	35	IA	pT1aN0	endometrioid adenocarcinoma	G1	5.8	NA	1.9	95.1	40.4
EC09	RNA_B, BCR	44	G0	41.3	IA	pT1aN0	endometrioid adenocarcinoma	G1	11	NA	NA	327.3	525.9
EC10	RNA_B, BCR, RNA_T, TCR	58	G0	23	IIIC2	pT1bN2	endometrioid adenocarcinoma	G1	7.1	NA	3.9	226.8	40.6

Supplementary Table 1. Endometrial cancer patients analyzed by single-cell multiomics

Endometrial cancer tissues from 10 patients were analyzed with single-cell technologies. RNA_B stands for scRNA-seq of B cells; RNA_T, scRNA-seq of CD4⁺ T cells; BCR, scBCR-seq; TCR, scTCR-seq.

Supplementary Table 2

characteristics		Number of patients	Percentage
Age	<50 year	19	22.1
	≥50 year	67	77.9
Histology	G1	33	38.4
	G2	14	16.3
	G3	21	24.4
	Serous	12	7
	Mixed type	6	14
FIGO stage	I	56	65.1
	II	8	9.3
	III	19	22.1
	IV	3	3.5
Tumor size	<2 cm	14	16.3
	≥2 cm	72	83.7
Survival outcome	recurrence	15	17.4
	death	13	15.1

Supplementary Table 2. Summary statistics of patients analyzed by immunohistochemistry
Endometrial cancer tissues from 86 patients were stained and analyzed.