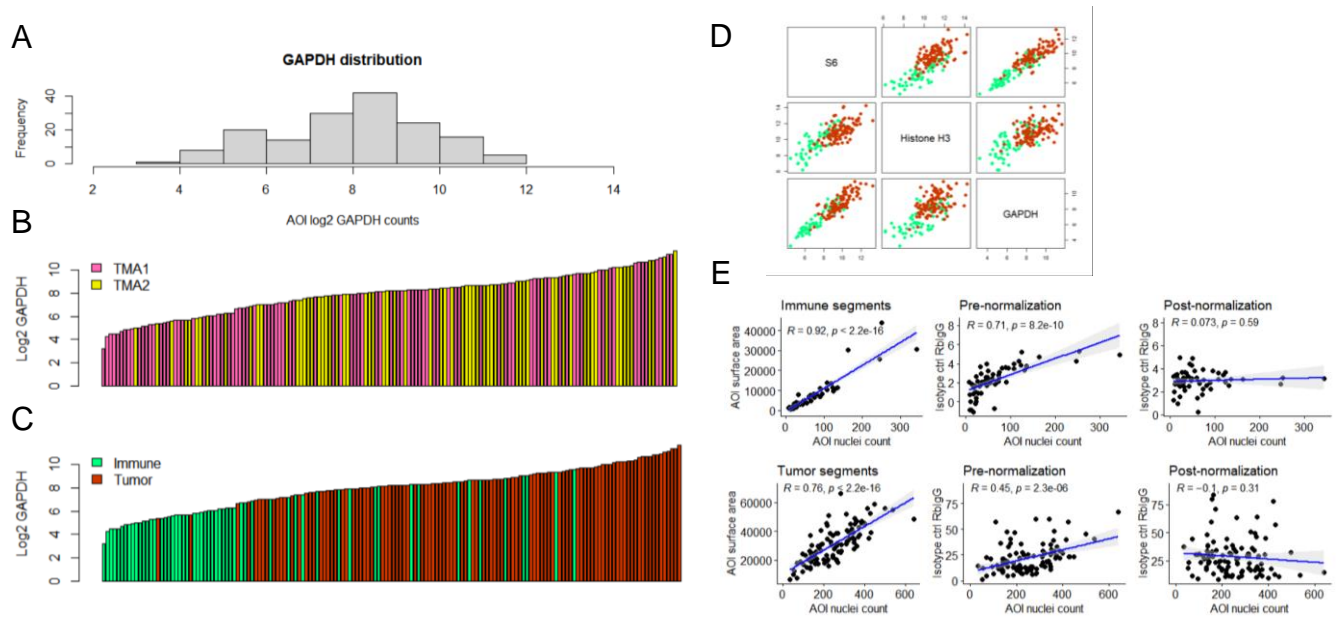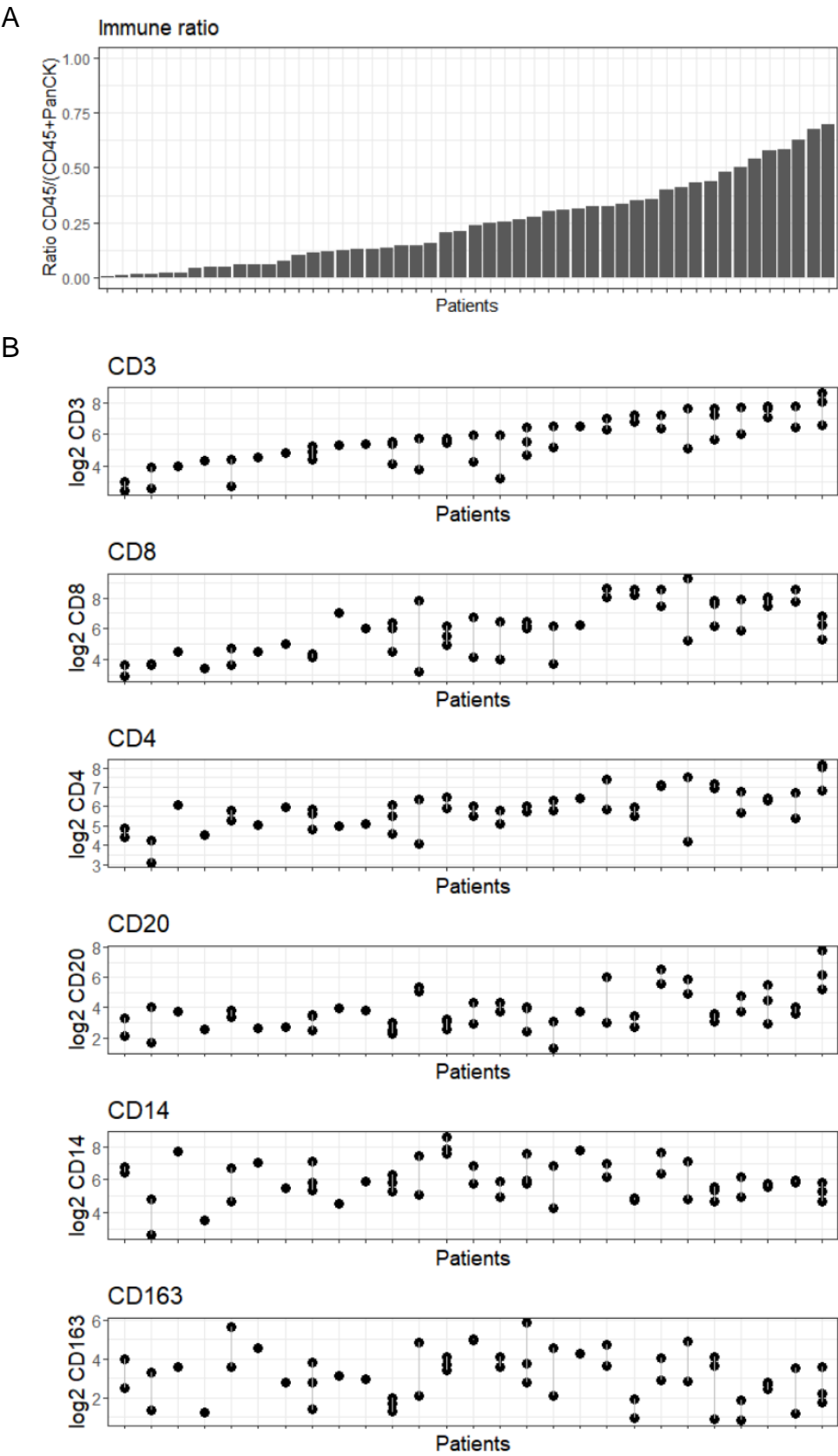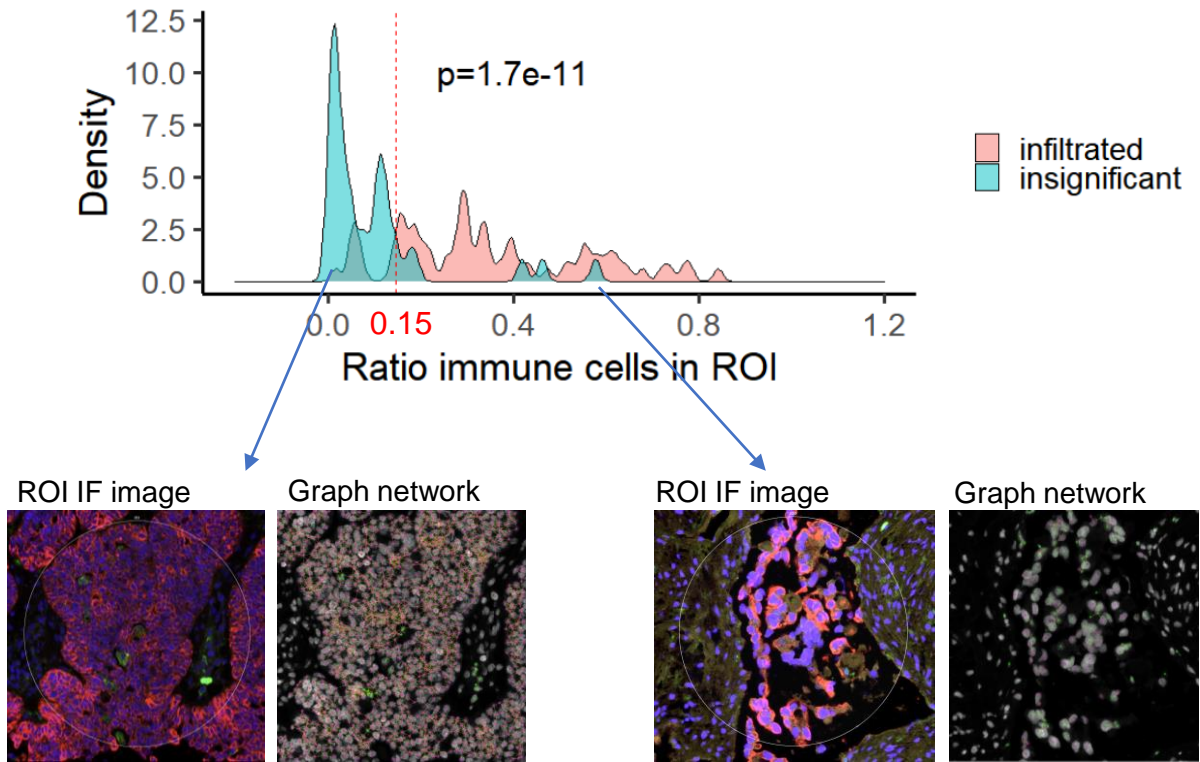# Supplementary Figure 1

Supplementary Figure 1. Data preprocessing. A) Biomarkers generally demonstrated normal distribution, as exemplified by housekeeper protein GAPDH. B) No significant difference between TMA1 and TMA2 was observed, as demonstrated by GAPDH counts in a barplot sorted from lowest to highest AOI. C) Biomarker counts were systematically lower in immune segments compared to tumor segments due to general lower nuclei counts and AOI areas of immune segments compared to tumor segments, as demonstrated by GAPDH counts. D) Correlation between housekeeper proteins S6, GAPDH, and Histone H3. Immune AOIs in green and tumor AOIs in red. E) Top panels: immune AOI data, bottom panels: tumor AOI data. Left: Correlation between AOI surface area and nuclei count. Correlation between isotype control (Rabbit IgG) and AOI nuclei count for raw (quality control adjusted) data (middle panels) and post-normalization (right panels).
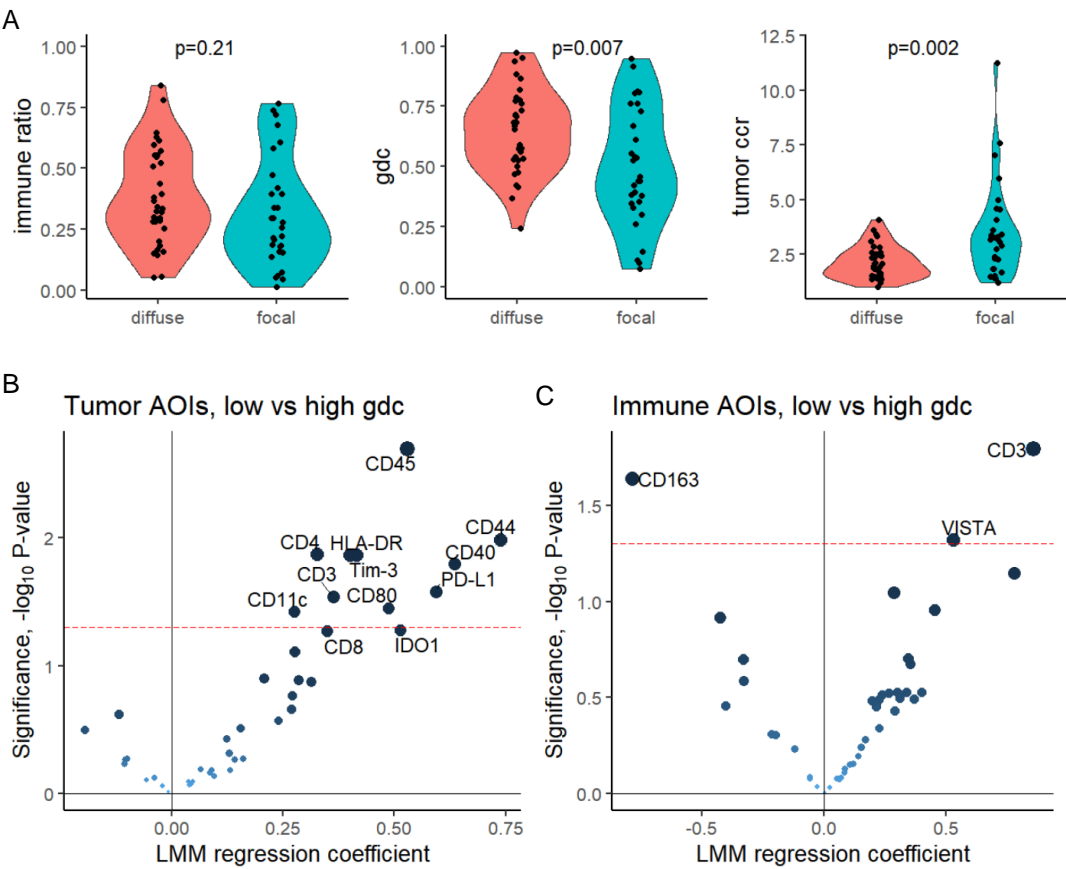
Supplementary Figure 2

Supplementary Figure 2. Immune infiltration heterogeneity in OC. A) Proportion of immune cells per tumor as quantified by immunofluorescence image analysis of sampled areas. Average ratio of CD45+ cells over total count of CD45+ and PanCk+ cells were plotted per patient. B) Variation in DSP-quantified, normalized counts of selected lymphocyte and myeloid lineage markers in sampled CD45+ segments, all plotted in order of increasing CD3 value (highest AOI) for comparison across tumors.
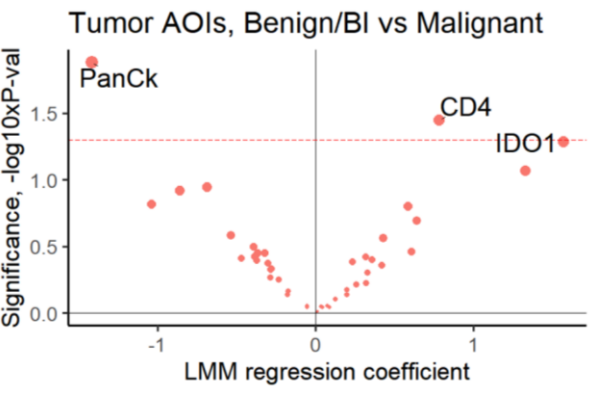
Supplementary Figure 3

Supplementary Figure 3. Ratio immune cells over all cells as determined by image segmentation and classification of images exported from the GeoMx software. Each square image included the ROI in the center, and the area immediately surrounding the circular ROI. Thus, more cells were counted from the image analysis than were sampled from the corresponding ROI. An approximate immune cell ratio of 0.15 were found to best discriminate tumors classified as infiltrated vs insignificant, based on visual scoring. Outliers are exemplified: Left: ROI 012 on TMA1 classified as infiltrated despite a low immune cell ratio determined by image analysis, due to highly dense tumor area and weak CD45 staining. Right: ROI 022 on TMA2 classified as immune-insignificant. Here, the high CD45 staining background resulted in false positive scoring of immune cells in the image analysis.
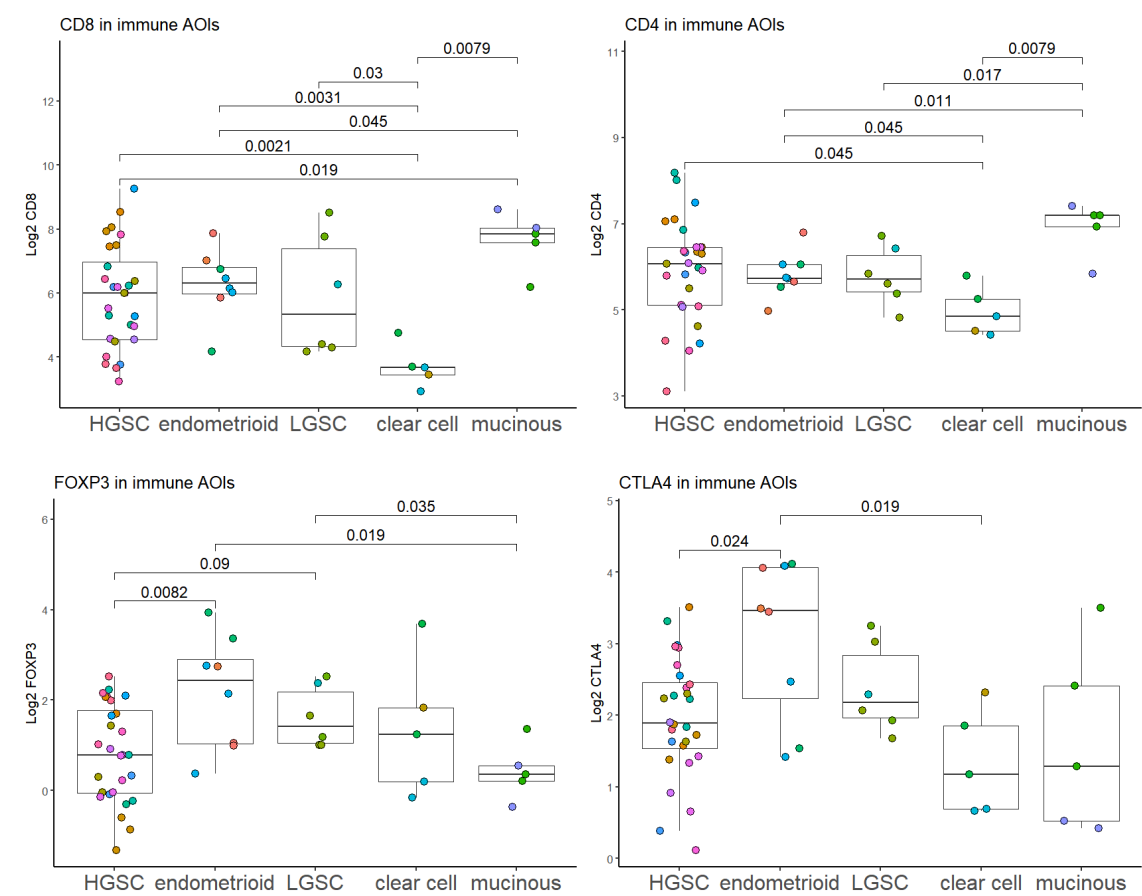
Supplementary Figure 4
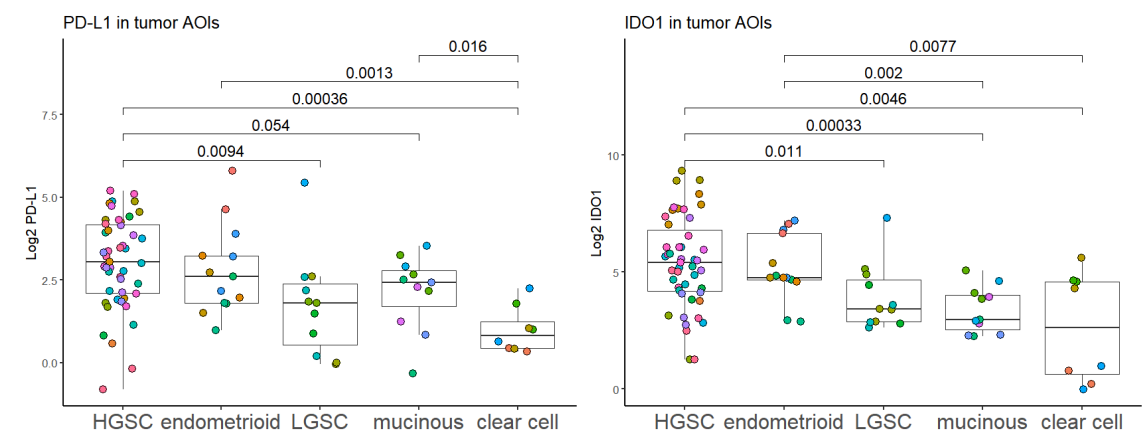
Supplementary Figure 5

Supplementary Figure 5. Tumor-immune microenvironment phenotype in benign and borderline vs malignant tumors. LMM regression with patient as random effect, and benign/borderline/malignant as fixed effect. The number of patients and ROIs were for benign: 1 patient, 2 ROIs; borderline: 6 patients, 9 ROIs; malignant: 43 patients, 91 ROIs. Immune AOIs in malignant and benign/borderline could not be compared as only one immune AOI was collected from benign/borderline tumors.
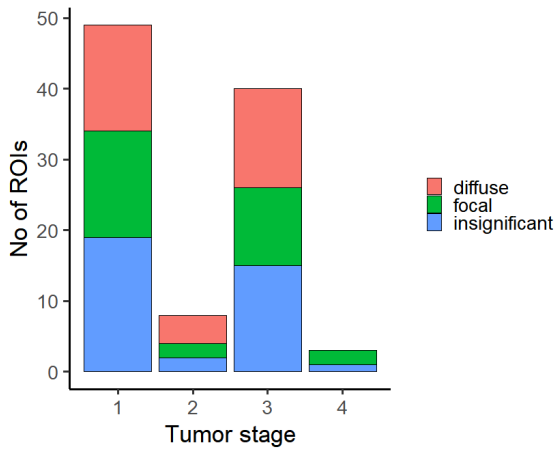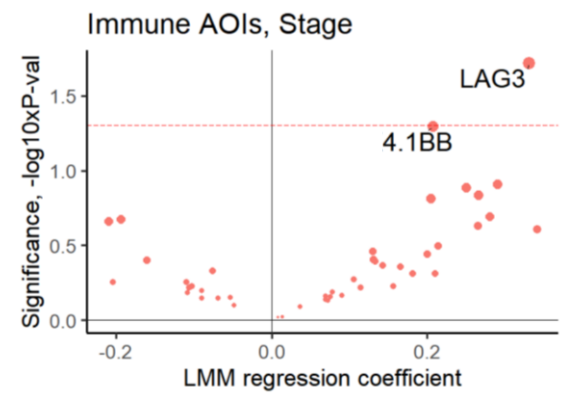
Supplementary Figure 6

A

B

Supplementary Figure 6. Biomarker distribution across OC histotypes. A) immune AOIs: CD8, CD4, FOXP3 and CTLA4; and B) tumor AOIs: PD-L1 and IDO1. Wilcoxon p-values are shown for significant (p<0.05) different groups. Note that p-values were here calculated using all AOI data, including 1-3 AOIs per patient. Overlayed data points are colored by patient.
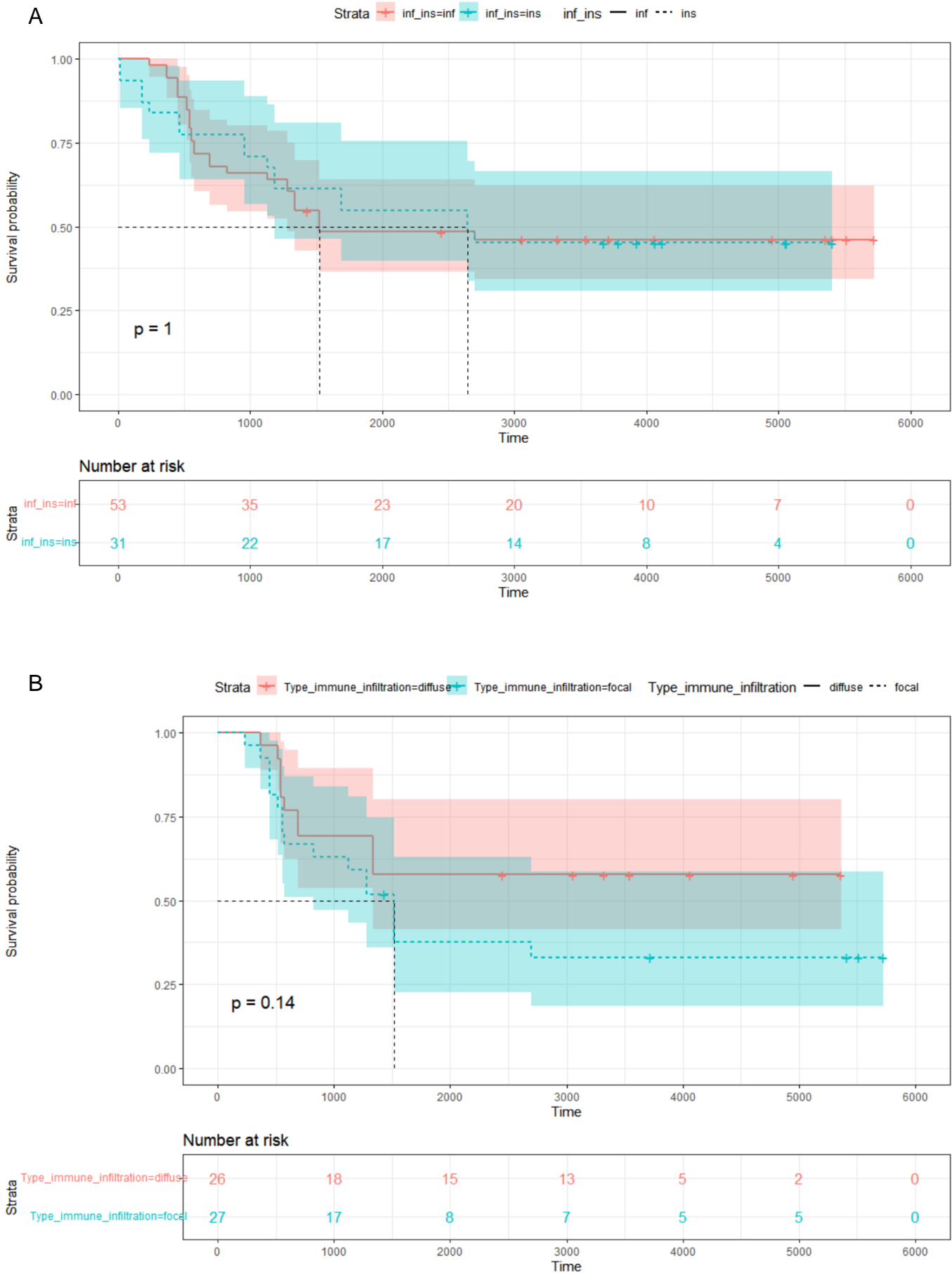
Supplementary Figure 7

A

B

Supplementary Figure 7. Immune infiltration association to tumor stage. A) Number of AOIs per tumor stage. B) LMM regression with patient as random effect, and stage as fixed effect. Stage was annotated as stage 1, 2, 3, 4 (i.e. stage IA, IB, IC were all grouped into stage 1).

Supplementary Figure 8

Supplementary Figure 8. Survival analysis by Kaplan-meier for ROIs annotated as A) immune-infiltrated (inf) versus ignored (insignificant, ins); B) immune infiltrated ROIs annotated as having either diffuse or focal immune infiltration

Supplementary Table 1

Supplementary Table 1. Spatial biomarkers significantly associated with PFS

Biomarker	Segment	Max rank threshold (percentile)	Log rank p-value	PFS effect
OX40L	Tumor	0.74	0.00013	Shorter
PanCK	Tumor	0.77	0.0021	Longer
IDO1	Tumor	0.36	0.0031	Shorter
CD56	Tumor	0.66	0.0047	Longer
CD68	Tumor	0.67	0.014	Longer
Ki67	Tumor	0.60	0.05	Shorter
GZMB	Immune	0.53	0.00053	Longer
CD68	Immune	0.48	0.0014	Longer
OX40L	Immune	0.82	0.014	Shorter
Ki67	Immune	0.59	0.017	Longer