

Expanded View Figures

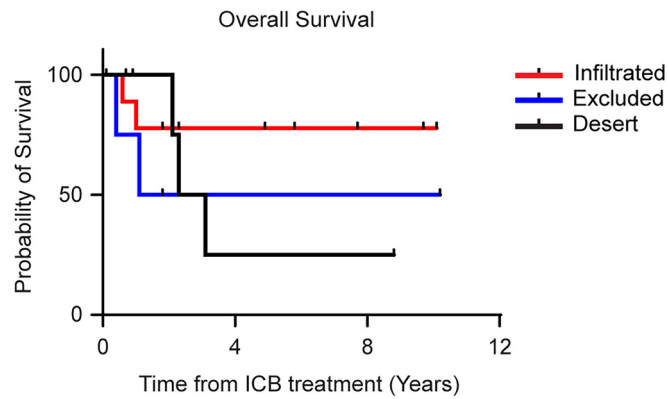


Figure EV1. Overall survival in melanoma patients after ICI treatment.

Kaplan-Meier curves showing overall survival (OS) for patients with three different CD8⁺ T-cell spatial phenotypes: Infiltrated, Excluded, and Desert. The Desert group showed the worst prognosis, followed by the Excluded and Infiltrated groups.

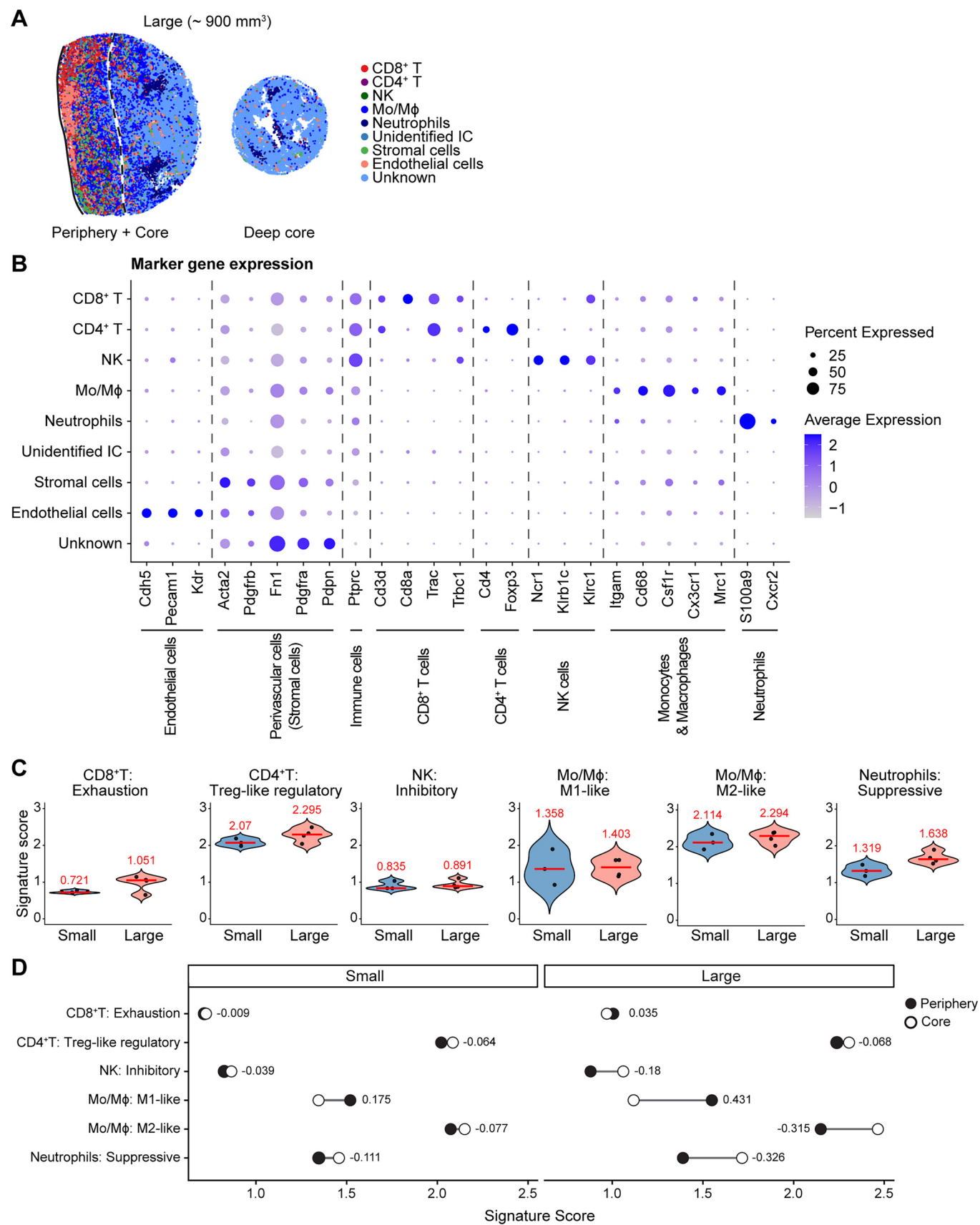


Figure EV2. Xenium-based cell annotation and immunosuppressive signature in small and large YUMMER1.7 tumors.

(A) Representative Xenium in situ map showing spatially resolved cluster distribution in large YUMMER1.7 tumors (~900 mm³), including the tumor periphery and core and deep-core regions used for downstream analysis. Distinct immune clusters are enriched at the tumor periphery and excluded from the tumor core and deep-core regions. (B) Dot plot showing marker gene expression used to annotate major immune and stromal cell types. Dot size indicates the proportion of cells expressing each gene, and the color intensity reflects the average expression level. Mo/MΦ, monocytes/macrophages; Unidentified IC, Unidentified immune cells. (C) Immunoregulatory signature scores by annotated immune cell subset in small and large tumors ($n = 3-4$ per group). (D) Differences in immunoregulatory signature scores between tumor periphery and core in small and large tumors. Signature scores were calculated within the corresponding immune cell subsets using selected phenotype-associated genes detected in the Xenium panel: a T-cell exhaustion score in CD8⁺ T cells, a Treg-like regulatory score in CD4⁺ T cells, an NK inhibitory score in NK cells, an M1-like or M2-like macrophage score in Mo/MΦ, and a neutrophil-associated suppressive score in neutrophils. Gene sets used for each signature are listed in Table EV2.

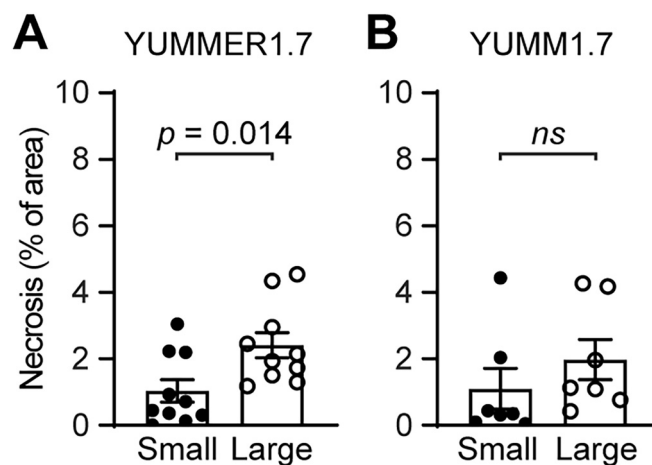


Figure EV3. Necrotic area in mouse melanomas of different sizes.

(A, B) Quantification of necrotic regions, defined by low DAPI signal intensity, across entire tumor sections in small ($\sim 100 \text{ mm}^3$) and large ($\sim 900 \text{ mm}^3$) YUMMER1.7 (A, $n = 10$ per group) and YUMM1.7 (B, $n = 7$ per group) tumors. Each point represents one mouse; bars show mean $\pm$ SEM. Statistical analysis was performed using an unpaired two-tailed Student's t test. P values are shown; ns, not significant.

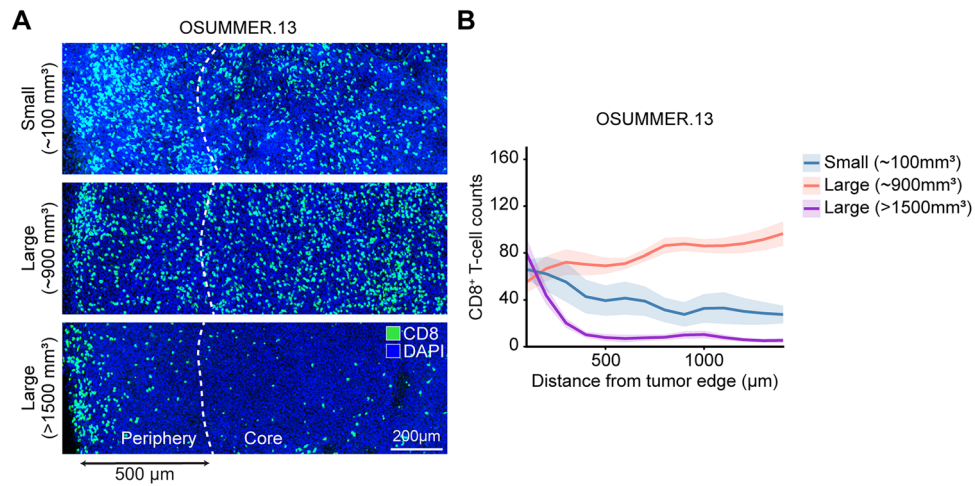


Figure EV4. Spatial distribution of CD8⁺ T cells in OSUMMER.13 tumors.

In total, 1×10^6 OSUMMER.13 cells were subcutaneously inoculated in C57BL/6NHsd mice. Tumors were harvested at ~100 mm³ (small), ~900 mm³ (large), and >1500 mm³ (advanced large). (A) Representative CD8 immunofluorescence staining of small, large, and advanced large tumors. Dashed lines indicate the periphery–core boundary. Scale bar, 200 μm. (B) Corresponding quantification of CD8⁺ T cells (cells per 0.05 mm²) as a function of distance from the tumor margin is shown for tumors ($n = 4$ –5 mice per group).

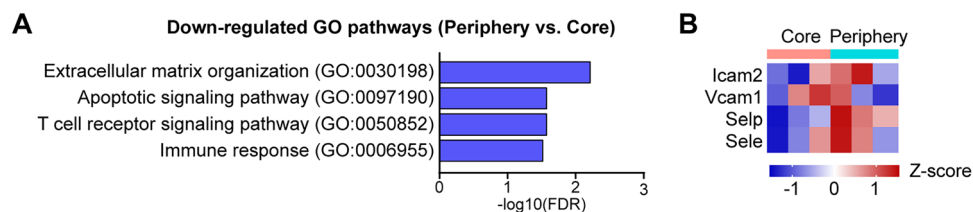


Figure EV5. GO pathways downregulated in peripheral endothelial cells and regional expression of endothelial adhesion molecule genes in YUMMER1.7 tumors.

These analyses were performed using the same bulk RNA-sequencing dataset from sorted core and peripheral endothelial cells of large YUMMER1.7 tumors shown in Fig. 2. **(A)** Gene Ontology enrichment for Biological Process analysis of genes downregulated in peripheral endothelial cells relative to core endothelial cells. Bars show the top terms ranked by $-\log_{10}$ false discovery rate (FDR). **(B)** Heatmap showing the expression of selected endothelial adhesion molecule genes in the core and periphery. Columns represent biological replicates from core and peripheral endothelial cells; values are z-scored expression.

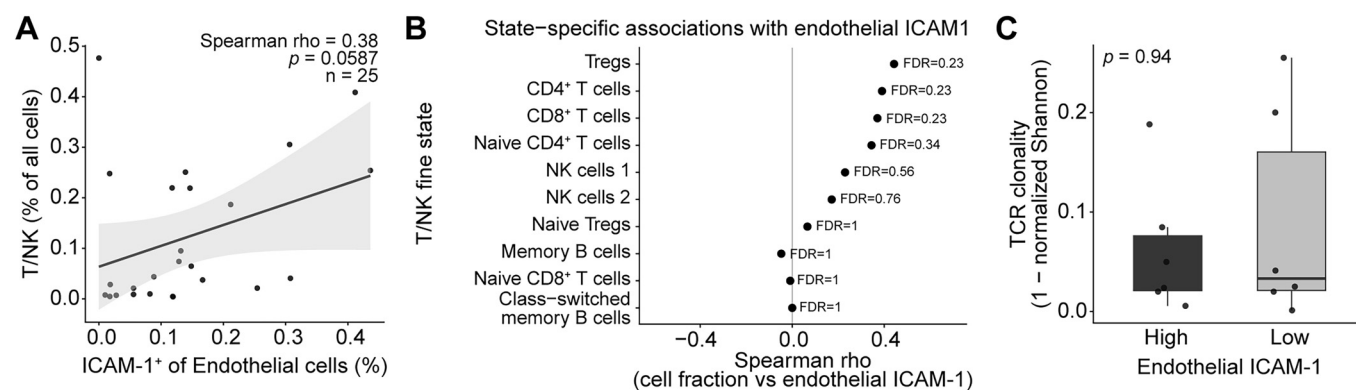


Figure EV6. Association of endothelial ICAM-1 with T/NK abundance, T/NK states, and TCR clonality in human melanoma.

(A) Scatter plot showing the Spearman correlation between the fraction of ICAM-1⁺ endothelial cells and total T/NK abundance (fraction of all cells) at the tumor level. Shaded bands represent 95% confidence intervals from linear regression. Each point represents one tumor ($n = 25$); Spearman's ρ (p) and two-sided P value are shown. (B) Spearman correlations between tumor-level endothelial ICAM-1 expression (mean normalized expression across endothelial cells) and the abundance of annotated T/NK cell states (e.g., CD4⁺ T, CD8⁺ T, Treg, NK, and additional subsets). Points represent individual immune states; effect sizes are shown as Spearman ρ ; P values were adjusted for multiple testing using the Benjamini-Hochberg false discovery rate (FDR). (C) Tumor-level TCR clonality in ICAM-1-high ($n = 6$) versus ICAM-1-low tumors ($n = 6$); upper and lower quartiles of endothelial ICAM-1 expression. Points represent individual tumors; box plots show the median and interquartile range. Statistical comparison was performed using a two-sided Wilcoxon rank-sum test.

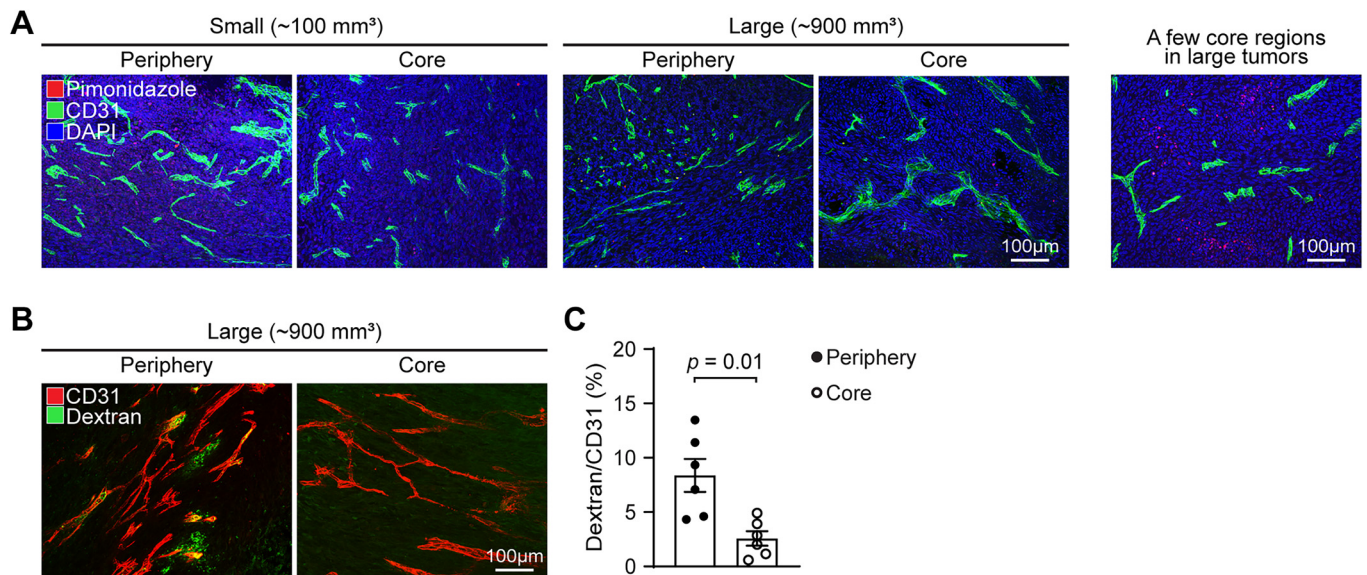


Figure EV7. Hypoxia and vascular permeability in YUMMER1.7 tumors of different sizes.

(A) Representative immunofluorescence image showing pimonidazole (red), CD31⁺ vessels (green), and DAPI-stained nuclei (blue) in small (~100 mm³) and large (~900 mm³) tumors at the periphery and core. A robust pimonidazole signal was not observed in viable tumor regions, although focal pimonidazole-positive areas were detected in a subset of core regions in large tumors (right). (B, C) Representative immunofluorescence showing 40-kDa fluorescein isothiocyanate-dextran (green) and CD31⁺ vessels (red) in large tumors (B) and corresponding quantification of the extravasated dextran⁺/CD31⁺ area (%) (C, $n = 5$). Each point represents one mouse; bars show mean $\pm$ SEM. Statistical analysis was performed using a paired two-tailed Student's t test. P value is shown. Scale bars in (A, B), 100 μ m.

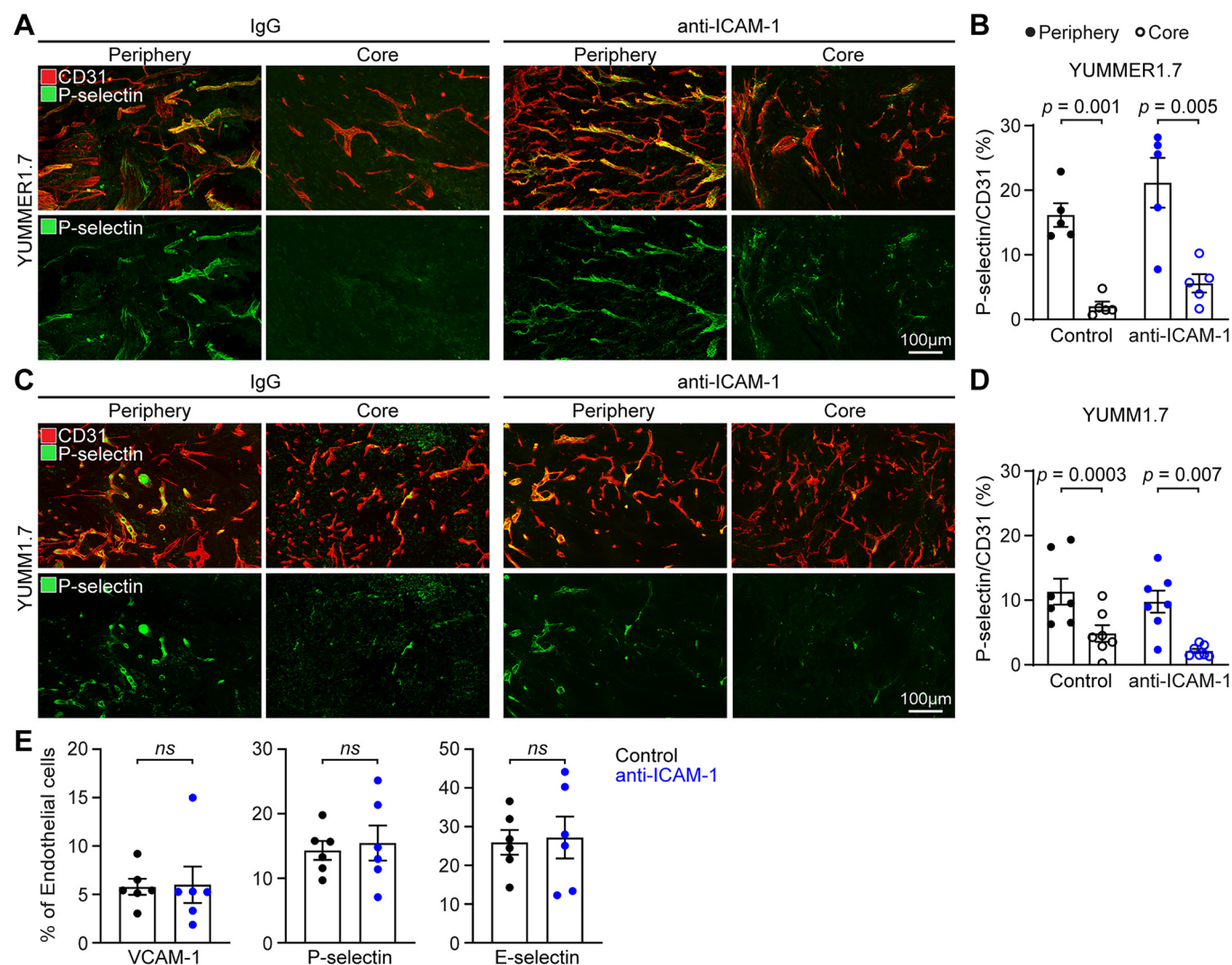


Figure EV8. Expression of adhesion molecules in mouse melanomas following ICAM-1 blockade.

(A–D) Representative immunofluorescence showing P-selectin (green) on CD31⁺ vessels (red) at the tumor periphery and core in IgG-treated (left) and anti-ICAM-1-treated (right) YUMMER1.7 (A) and YUMM1.7 (C) tumors. Corresponding quantification of P-selectin⁺/CD31⁺ area (%) for YUMMER1.7 (B, $n = 5$ per group) and YUMM1.7 (D, $n = 7$ per group) tumors. No significant treatment effect was detected between control and anti-ICAM-1-treated tumors. (E) Flow cytometry analysis showing frequencies of CD31⁺CD45⁺ endothelial cells expressing VCAM-1, P-selectin, and E-selectin in IgG-treated and anti-ICAM-1-treated YUMM1.7 tumors ($n = 6$ per group). Each point represents one mouse; bars show mean $\pm$ SEM. Statistical analysis was performed using a paired or unpaired two-tailed Student's *t* test. *P* values are shown; ns, not significant. Scale bars in (A, C), 100 μ m.

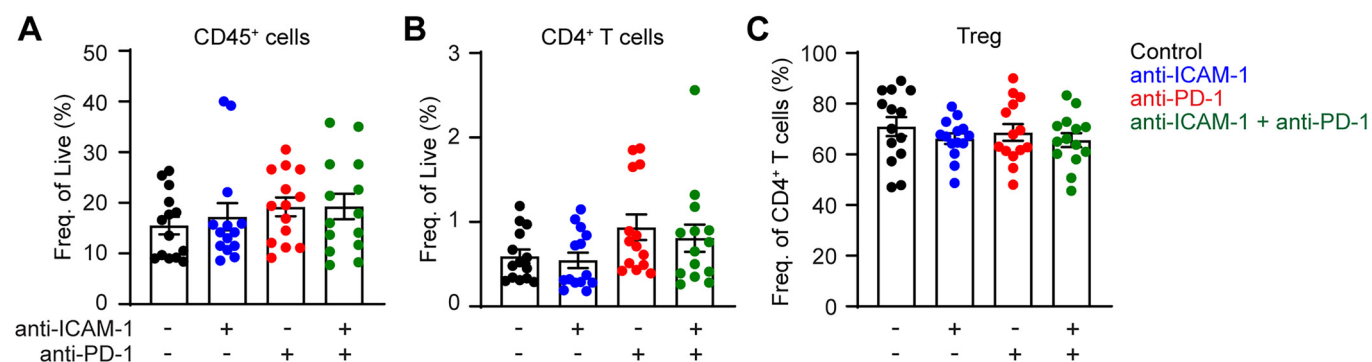


Figure EV9. Immune cell infiltration in YUMM1.7 tumors following anti-ICAM-1 and/or anti-PD-1 treatment.

Flow cytometry data were obtained from the same experiment as in Fig. 5; the experimental schematic diagram is shown in Fig. 5A. (A–C) Flow cytometry analysis of whole tumors showing the frequency of CD45⁺ immune cells (A) and CD4⁺ T cells (B) among live cells, and regulatory T cells (Treg; Foxp3⁺) among CD4⁺ T cells (C) in IgG, anti-ICAM-1, anti-PD-1, and anti-ICAM-1 plus anti-PD-1 treatment groups ($n = 14$ per group). Each point represents one mouse; bars show mean $\pm$ SEM. Statistical analysis was performed using one-way ANOVA.