Supplemental Table 1: ROI measurements

Specimen	Avg ROI Diameter (μm)	Avg Nuclei Count
HGG03	490	1214/ROI
HGG05	414.05	1380/ROI
HGG06	539.38	1375/ROI

Supplemental Table 2: Neuropathological assessment of individual ROIs

Sample ID	ROI	Tumor Cellularity (high, medium, low)	Necrosis	Pseudopalisading cells	MVP	Blood vessels - No MVP
HGG_03	ROI 1	>95% tumor (medium)	0%	0%	0%	<5%
HGG_03	ROI 2	95% tumor (low -medium)	0%	0%	0%	5%
HGG_03	ROI 3	100% tumor (medium)	0%	0%	0%	0%
HGG_03	ROI 4	100% tumor (low-medium)	0%	0%	0%	0%
HGG_03	ROI 5	100% tumor (low -medium)	0%	0%	0%	<5%
HGG_03	ROI 6	100% tumor (medium)	0%	0%	0%	<5%
HGG_03	ROI 7	100% tumor (medium)	0%	0%	0%	<5%
HGG_03	ROI 8	90% (Medium-high)	<5%	0%	10%	<5%
HGG_03	ROI 9	100% tumor (low-medium)	0%	0%	0%	0%
HGG_03	ROI 10	5%	0%	0%	0%	95%
HGG_03	ROI 11	95% tumor (low -medium)	0%	0%	0%	5%
HGG_03	ROI 12	30-40% tumor (low - medium)	<5%	0%	0%	60%
HGG_03	ROI 13	100% tumor (medium)	0%	0%	0%	0%
HGG_03	ROI 14	100% tumor (medium)	0%	0%	0%	0%
HGG_03	ROI 15	100% tumor (medium)	0%	0%	0%	0%
HGG_03	ROI 16	10% tumor (medium)	90%	0%	0%	0%
HGG_03	ROI 17	0%	100%	0%	0%	0%
HGG_03	ROI 18	0%	100%	0%	0%	0%
HGG_03	ROI 19	0%	0%	0%	0%	100%
HGG_03	ROI 20	45%	50%	<5%	0%	5%
HGG_03	ROI 21	50%	<5%	0%	0%	50%
HGG_03	ROI 22	80%	5%	0%	<5%	5-10%
HGG_03	ROI 23	See other	30%	0%	0%	10%
HGG_03	ROI 24	See other	75%	0%	0%	0%
HGG_03	ROI 25	80-90% tumor (medium)	0%	0%	0%	0%
HGG_03	ROI 26	75% tumor (medium)	25%	0%	0%	0%
HGG_03	ROI 27	80-90% tumor (medium)	0%	0%	0%	<5%
HGG_03	ROI 28	90-95% tumor (medium)	0%	0%	0%	5-10%
HGG_03	ROI 29	See other	75%	0%	0%	0%
HGG_03	ROI 30	See other	30%	0%	0%	30%
HGG_03	ROI 31	0%	100%	0%	0%	0%
HGG_03	ROI 32	0%	100%	0%	0%	0%
HGG_05	ROI 1	100% tumor (high)	0%	0%	0%	0%
HGG_05	ROI 2	100% tumor (high)	0%	0%	0%	0%
HGG_05	ROI 3	100% tumor (high)	0%	0%	0%	0%
HGG_05	ROI 4	100% tumor (high)	0%	0%	0%	0%
HGG_05	ROI 5	100% tumor (high)	0%	0%	0%	0%
HGG_05	ROI 6	90% tumor (low-medium)	0%	0%	5%	5%
HGG_05	ROI 7	90% tumor (high)	0%	0%	5%	5%
HGG_05	ROI 8	50% tumor (high)	0%	0%	50%	0%
HGG_05	ROI 9	90% tumor (low-medium)	0%	10%	0%	0%
HGG_05	ROI 10	95% tumor (high)	0%	0%	0%	<5%

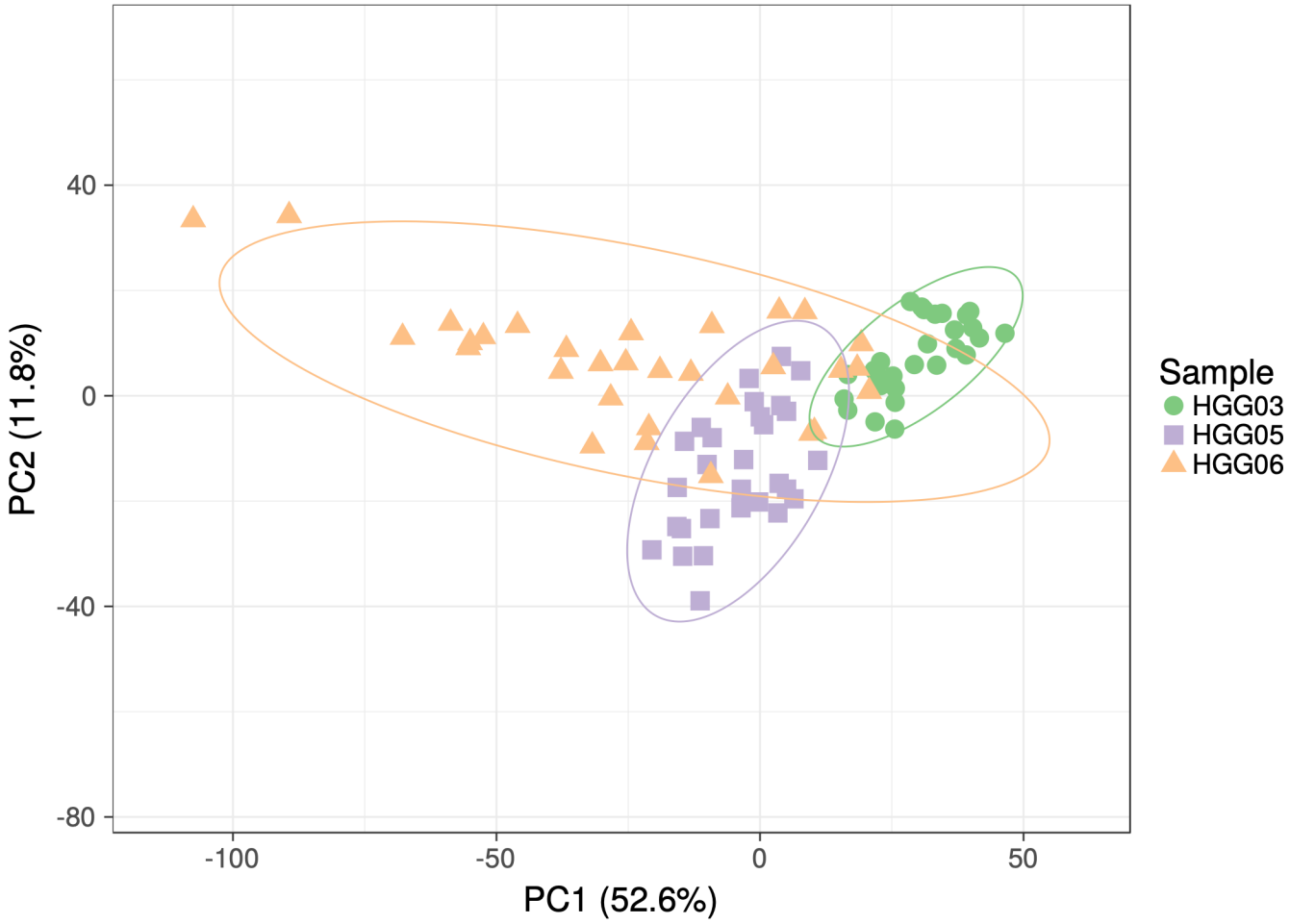
HGG_05	ROI 11	95% tumor (high)	0%	0%	0%	5%
HGG_05	ROI 12	95% tumor (high)	0%	0%	0%	5%
HGG_05	ROI 13	60% tumor (high)	0%	0%	40%	0%
HGG_05	ROI 14	90% tumor (high)	0%	0%	10%	0%
HGG_05	ROI 15	95% tumor (medium-high)	0%	0%	5%	0%
HGG_05	ROI 16	40% tumor (high)	0%	0%	60%	0%
HGG_05	ROI 17	20% tumor (medium)	0%	0%	80%	0%
HGG_05	ROI 18	75% (low-high)	0%	0%	15%	10%
HGG_05	ROI 19	75% (low-high)	0%	0%	15%	10%
HGG_05	ROI 20	See other	See other	0%	0%	5%
HGG_05	ROI 21	See other	See other	0%	0%	75%
HGG_05	ROI 22	95% tumor (medium-high)	0%	0%	0%	<5%
HGG_05	ROI 23	100% tumor (medium-high)	0%	0%	0%	<5%
HGG_05	ROI 24	95% tumor (high)	0%	0%	0%	5%
HGG_05	ROI 25	40% tumor (high)	0%	0%	60%	0%
HGG_05	ROI 26	10% tumor (high)	45%	35%	10%	0%
HGG_05	ROI 27	30% tumor (high)	0%	0%	0%	10%
HGG_05	ROI 28	50% tumor (high)	0%	0%	0%	0%
HGG_05	ROI 29	60% tumor (high)	0%	0%	40%	0%
HGG_05	ROI 30	33% tumor (high)	0%	0%	33%	0%
HGG_05	ROI 31	50% tumor (high)	0%	0%	50%	0%
HGG_05	ROI 32	30% tumor (medium-high)	0%	0%	70%	0%
HGG_06	ROI 1	100% tumor (high)	0%	0%	0%	0%
HGG_06	ROI 2	100% tumor (high)	0%	0%	0%	0%
HGG_06	ROI 3	90% tumor (high)	5%	5%	0%	0%
HGG_06	ROI 4	85% tumor (high)	5%	5%	0%	5%
HGG_06	ROI 5	85% tumor (high)	5%	5%	0%	5%
HGG_06	ROI 6	95% tumor (medium)	0%	0%	0%	5%
HGG_06	ROI 7	70% tumor (high)	5%	5%	30%	0%
HGG_06	ROI 8	70% tumor (high)	5%	5%	30%	0%
HGG_06	ROI 9	>95% tumor (high)	0%	0%	0%	<5%
HGG_06	ROI 10	95% tumor (medium)	0%	0%	0%	5%
HGG_06	ROI 11	>95% tumor (medium-high)	0%	0%	0%	<5%
HGG_06	ROI 12	20% tumor (low)	0%	0%	0%	80%
HGG_06	ROI 13	100% tumor (medium)	0%	0%	0%	0%
HGG_06	ROI 14	90% tumor (low)	0%	0%	0%	10%
HGG_06	ROI 15	90% tumor (medium)	0%	0%	5%	5%
HGG_06	ROI 16	>95% tumor (low)	0%	0%	0%	<5%
HGG_06	ROI 17	>95% tumor (low)	0%	0%	0%	<5%
HGG_06	ROI 18	>95% tumor (low-medium)	0%	0%	0%	<5%
HGG_06	ROI 19	95% tumor (medium)	0%	0%	0%	5%
HGG_06	ROI 20	>95% tumor (medium)	0%	0%	0%	<5%
HGG_06	ROI 21	10-20% tumor (low)	80-90%	0%	0%	0%
HGG_06	ROI 22	50% tumor (medium)	0%	0%	50%	0%
HGG_06	ROI 23	70% tumor (low-medium)	0%	0%	30%	0%
HGG_06	ROI 24	50% tumor (medium)	0%	0%	0%	50%

HGG_06	ROI 25	95% tumor (medium-high)	0%	0%	0%	5%
HGG_06	ROI 26	95% tumor (medium)	0%	0%	0%	5%
HGG_06	ROI 27	80% tumor (medium)	0%	0%	20%	0%
HGG_06	ROI 28	95% tumor (low-medium)	0%	0%	0%	5%
HGG_06	ROI 29	95% tumor (medium)	0%	0%	0%	5%
HGG_06	ROI 30	>95% tumor (medium)	0%	0%	0%	<5%
HGG_06	ROI 31	>95% tumor (medium)	0%	0%	0%	<5%
HGG_06	ROI 32	>95% tumor (medium)	0%	0%	0%	<5%

Supplemental Table 3: Correlation between neuropathology assessment and SpatialDecon cell type scores

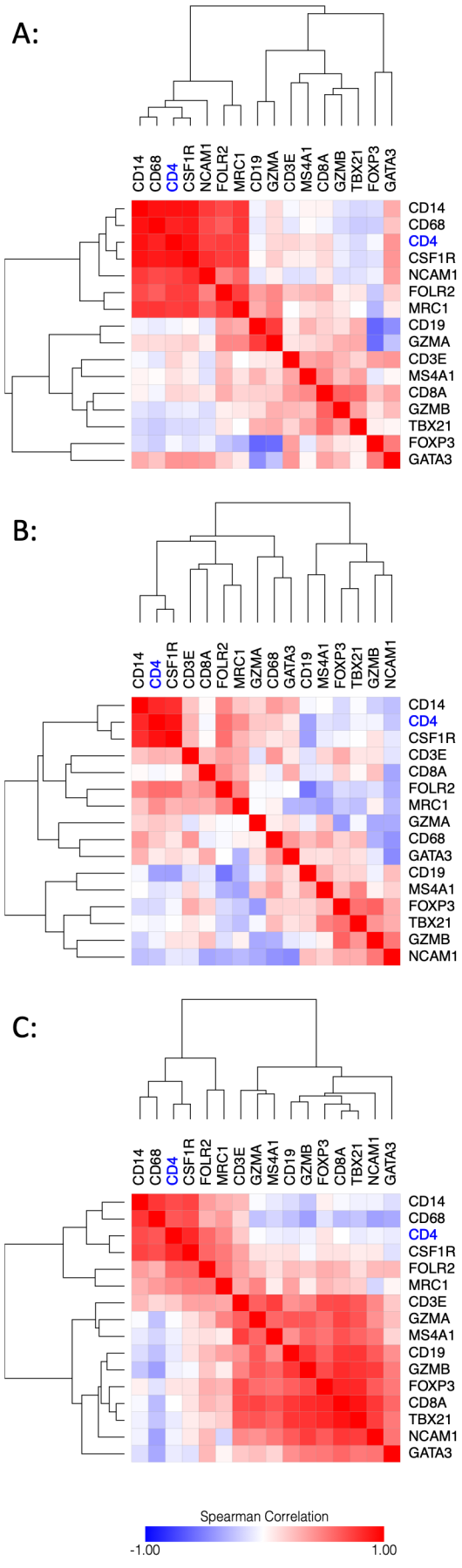
Neuropath. Tumor Cellularity vs SpatialDecon Neoplastic Cell Score	**Spearman**	**p-value**
HGG03	***0.659***	***0.0009***
HGG05	***0.6642***	***0.0002***
HGG06	0.3735	0.055
Neuropath. Microvascular Proliferation vs SpatialDecon Vascular Cell Score	**Spearman**	**p-value**
HGG03	0.07881	0.7208
HGG05	***0.5641***	***0.0014***
HGG06	***0.5852***	***0.0013***
Neuropath. Vessels without Microvascular Proliferation vs SpatialDecon Vascular Cell Score	**Spearman**	**p-value**
HGG03	***0.7053***	***0.0002***
HGG05	0.1218	0.537
HGG06	-0.2914	0.1403

Supplemental Figure 1:



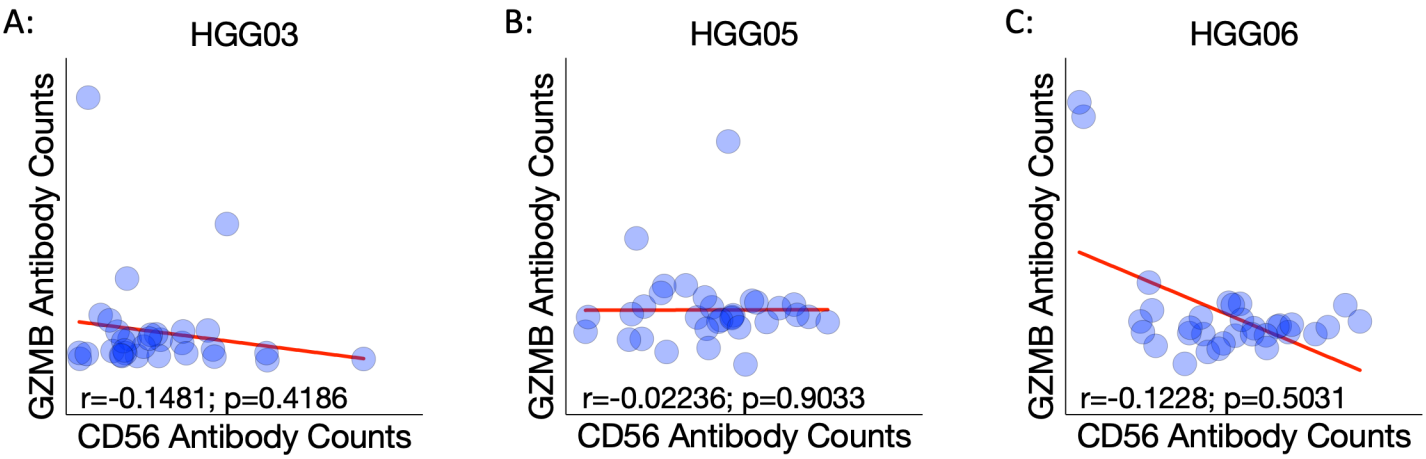
Supplemental Figure 1: Intertumoral heterogeneity in glioblastoma specimens. PCA of the ROIs from specimens HGG03 (green circles), HGG05 (purple squares), and HGG06 (orange triangles). Ellipses represent the 95% confidence interval.

Supplemental Figure 2:



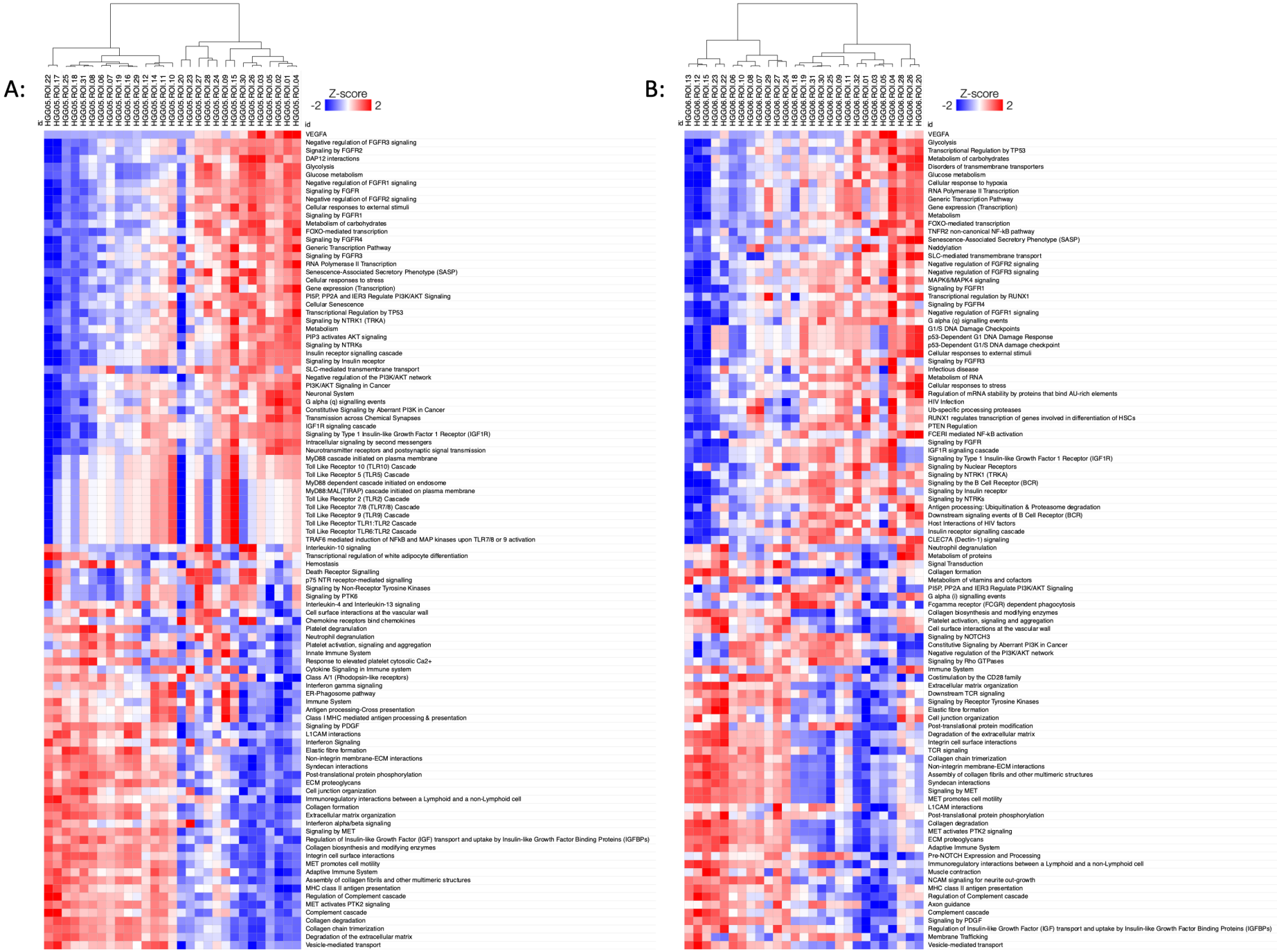
Supplemental Figure 2: Correlation analysis of immune cell markers. Spearman correlation of lymphocyte and myeloid cell marker gene expression in specimen (A) HGG03, (B) HGG05, and (C) HGG06. Color bar indicates Spearman's rho.

Supplemental Figure 3:



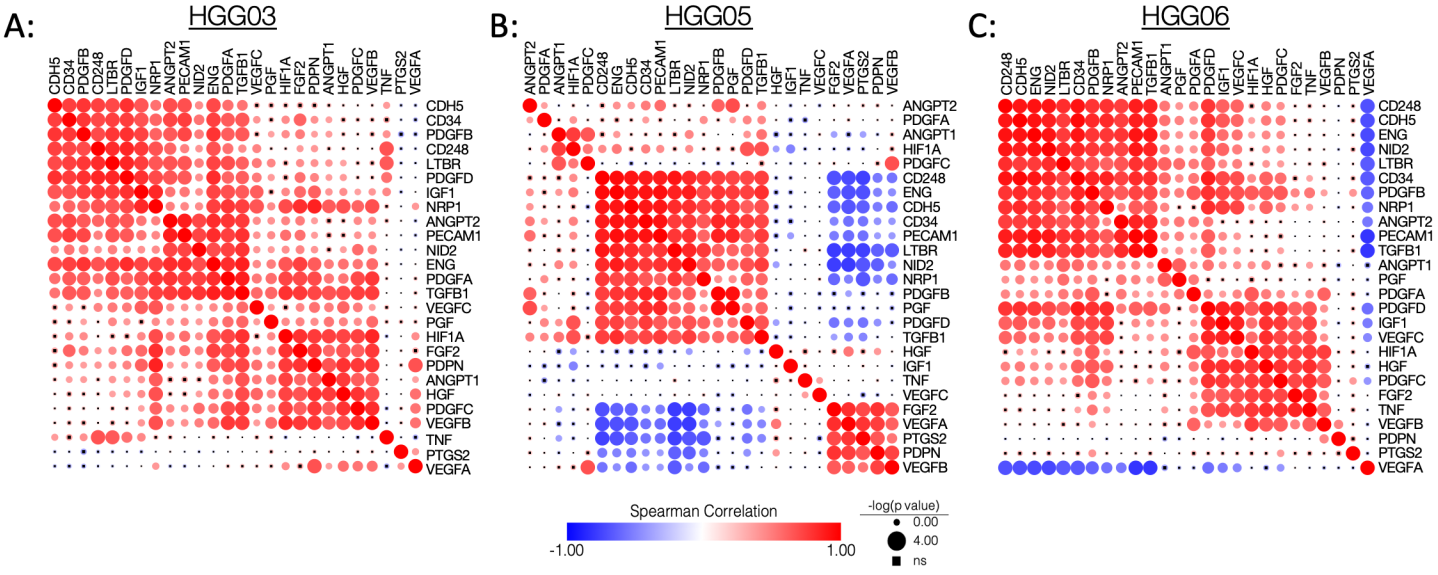
Supplemental Figure 3: Correlation between granzyme B and CD56 protein expression. Spearman correlations between granzyme B and CD56 antibody counts in specimens (A), HGG03, (B) HGG05 and (C) HGG06. Correlation and significance values are listed along the X-axis of each scatter plot. $p < 0.05$ is considered statistically significant.

Supplemental Figure 4:



Supplemental Figure 4: Biological pathways associated with VEGFA expression. Heatmaps showing the results of nearest neighbors analysis using the biological processes from the Reactome database for specimens (A) HGG05 and (B) HGG06. Color bar indicates z-score.

Supplemental Figure 5:



Supplemental Figure 5: Correlation between vascular markers and angiogenic mediators. Spearman correlation matrices showing the relationships between the normalized gene counts of known pro-angiogenic genes and vascular marker genes in tumor specimens (A) HGG03, (B) HGG05, and (C) HGG06. Circle size indicates p value where circles with inlaid black squares indicating failure to reach statistical significance ($p<0.05$). Color bar indicates Spearman's rho.