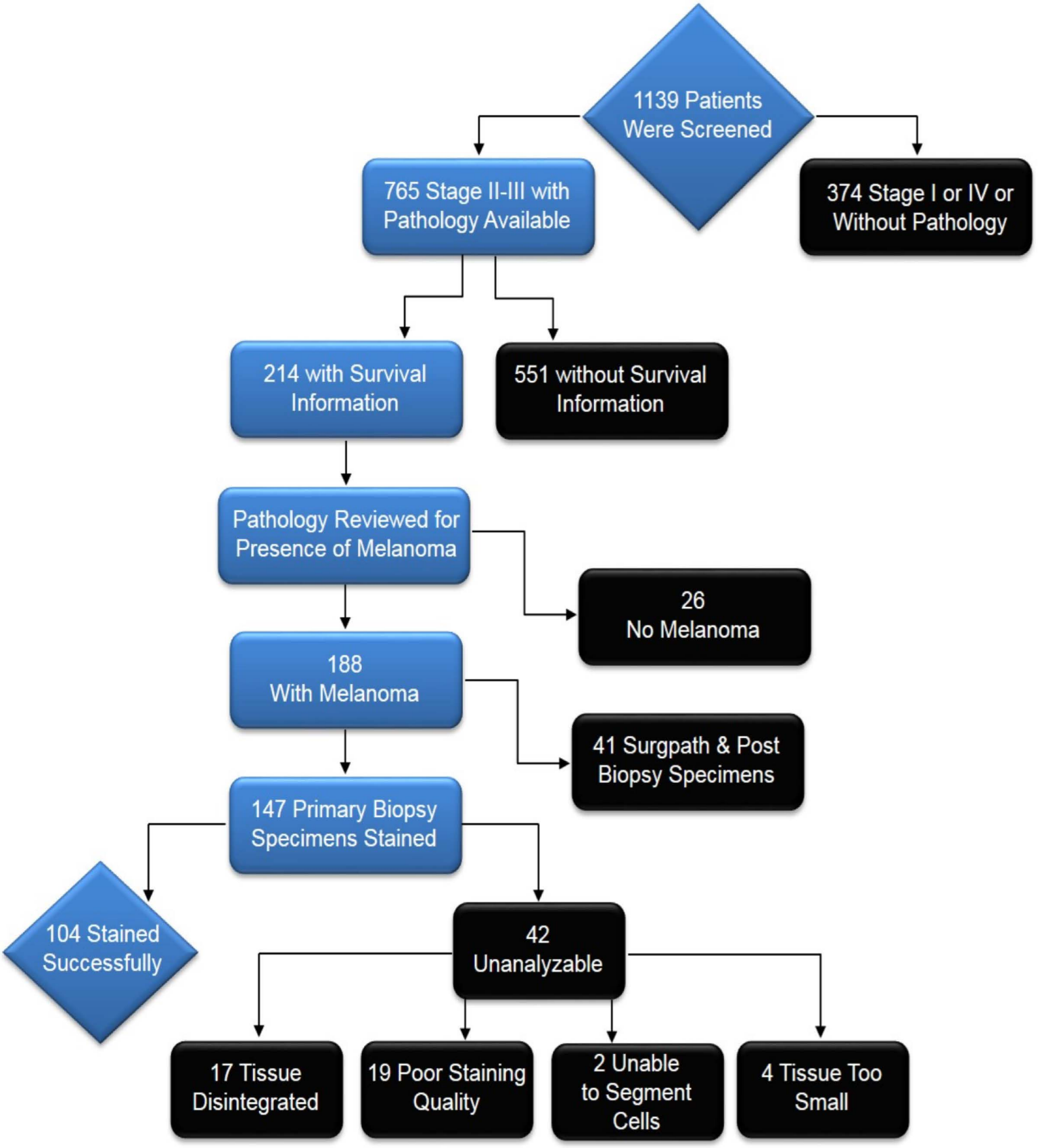
Supplemental Table S1: Melanoma Patient Characteristics for Unanalyzable Patient Cohort (n=42)	
Clinical Characteristics	
Gender	
Male, no. (%)	33 (78.6)
Female, no. (%)	9 (21.4)
Age	
Median, no. (range)	74.5 (34-97)
Location of Tumor	
Trunk, no. (%)	14 (33.3)
Extremity, no. (%)	28 (66.7)
Unknown, no (%)	0 (0)
Stage	
II, no. (%)	37 (88.1)
III, no. (%)	5 (11.9)
Pathological Characteristics	
Depth (mm)	
Median, no. (range)	2.75 (0.32-26)
Ulceration	
Absent, no. (%)	18 (42.8)
Present, no. (%)	22 (52.4)
Unknown, no. (%)	2 (4.8)
Outcome Characteristics	
Patient Follow-Up (months)	
Median, no. (range)	36.5 (3-171)
OS (months)	
Alive (at least 2 years), no. (%)	12 (29.3)
Dead, no. (%)	29 (70.7)
DSS (months)	
Alive or NED at Death, no. (%)	13 (30.9)
Dead with melanoma, no. (%)	6 (14.3)
Unknown, no (%)	23 (54.8)
(OS = Overall Survival, DSS = Disease Specific Survival, NED = No Evidence of Disease)	

Supplemental Table S1: Demographics for Unanalyzable Cohort.

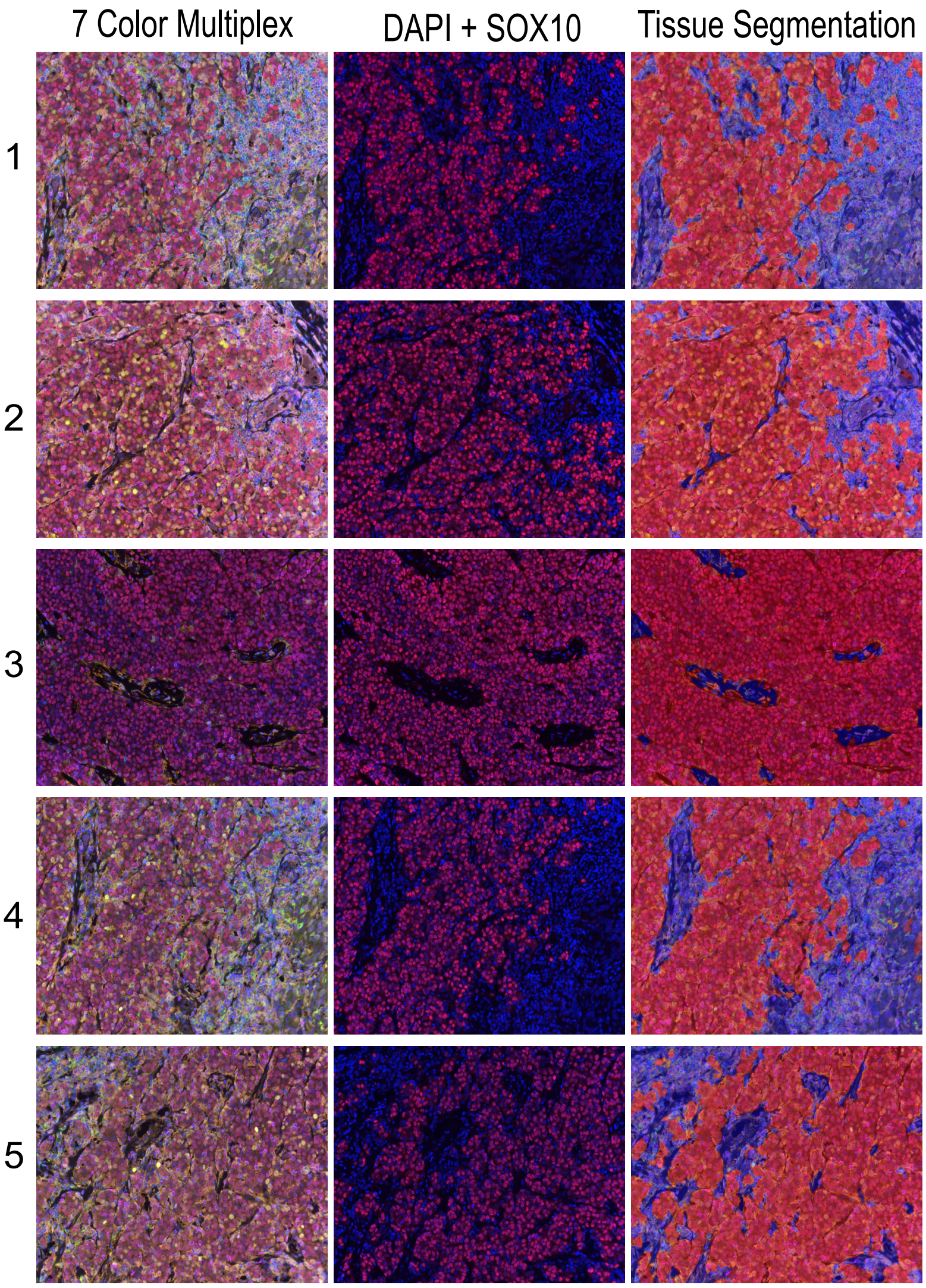
Supplemental Table S2: Melanoma Patient Characteristics for DSS Cohort (n=64)	
Clinical Characteristics	
Gender	
Male, no. (%)	50 (78.1)
Female, no. (%)	14 (21.9)
Age	
Median, no. (range)	68 (22-96)
Location of Tumor	
Trunk, no. (%)	37 (57.8)
Extremity, no. (%)	26 (40.6)
Unknown, no (%)	1 (1.6)
Stage	
II, no. (%)	51 (79.7)
III, no. (%)	13 (20.3)
Pathological Characteristics	
Depth (mm)	
Median, no. (range)	2.6 (0.7-11.4)
Ulceration	
Absent, no. (%)	24 (37.5)
Present, no. (%)	37 (57.8)
Unknown, no. (%)	3 (4.7)
TILs	
Absent, no. (%)	1 (1.6)
Non-Brisk, no. (%)	41 (64.1)
Brisk, no. (%)	18 (28.1)
Unknown, no. (%)	4 (6.2)
Outcome Characteristics	
Patient Follow-Up (months)	
Median, no. (range)	53 (5-173)
OS (months)	
Alive (at least 2 years), no. (%)	31 (48.4)
Dead, no. (%)	33 (51.6)
DSS (months)	
Alive or NED at death, no. (%)	42 (65.6)
Dead with melanoma, no. (%)	22 (34.4)
Unknown, no (%)	0 (0)
(TILs = Tumor Infiltrating Lymphocytes, OS = Overall Survival, DSS = Disease Specific Survival, NED = No Evidence of Disease)	

Supplemental Table S2: Demographics for DSS Cohort.


1139 Patients Were Screened
765 Stage II-III with Pathology Available
374 Stage I or IV or Without Pathology
214 with Survival Information
551 without Survival Information
Pathology Reviewed for Presence of Melanoma
26 No Melanoma
188 With Melanoma
41 Surgpath & Post Biopsy Specimens
147 Primary Biopsy Specimens Stained
104 Stained Successfully
42 Unanalyzable
17 Tissue Disintegrated
19 Poor Staining Quality
2 Unable to Segment Cells
4 Tissue Too Small

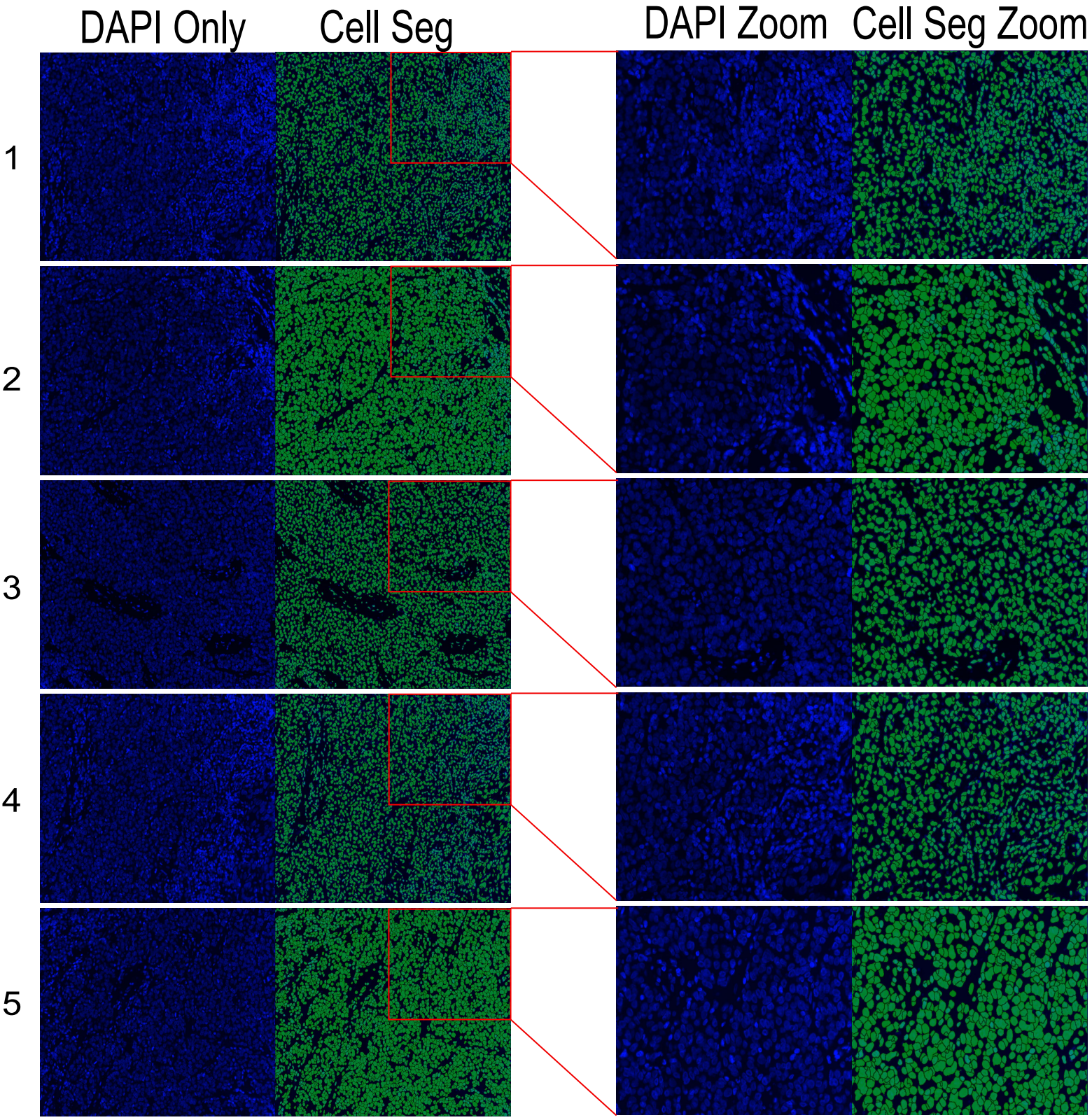
Supplemental Figure S1: Flow chart for database finding 147 patients with clinical follow up and known survival status. Of the 147 patients, we successfully stained and analyzed 104 samples. We were unable to analyze 42 patients (Table S2). Poor sample quality caused loss of tissue during the staining process in 17 patients and led to poor staining in 19 patients for which <6 markers stained successfully. 2 patients stained successfully but could not be analyzed during cell segmentation step due to nuclear hypersegmentation. 4 patients had small tissue which caused overlap of antibodies.

Supplemental Figure S2



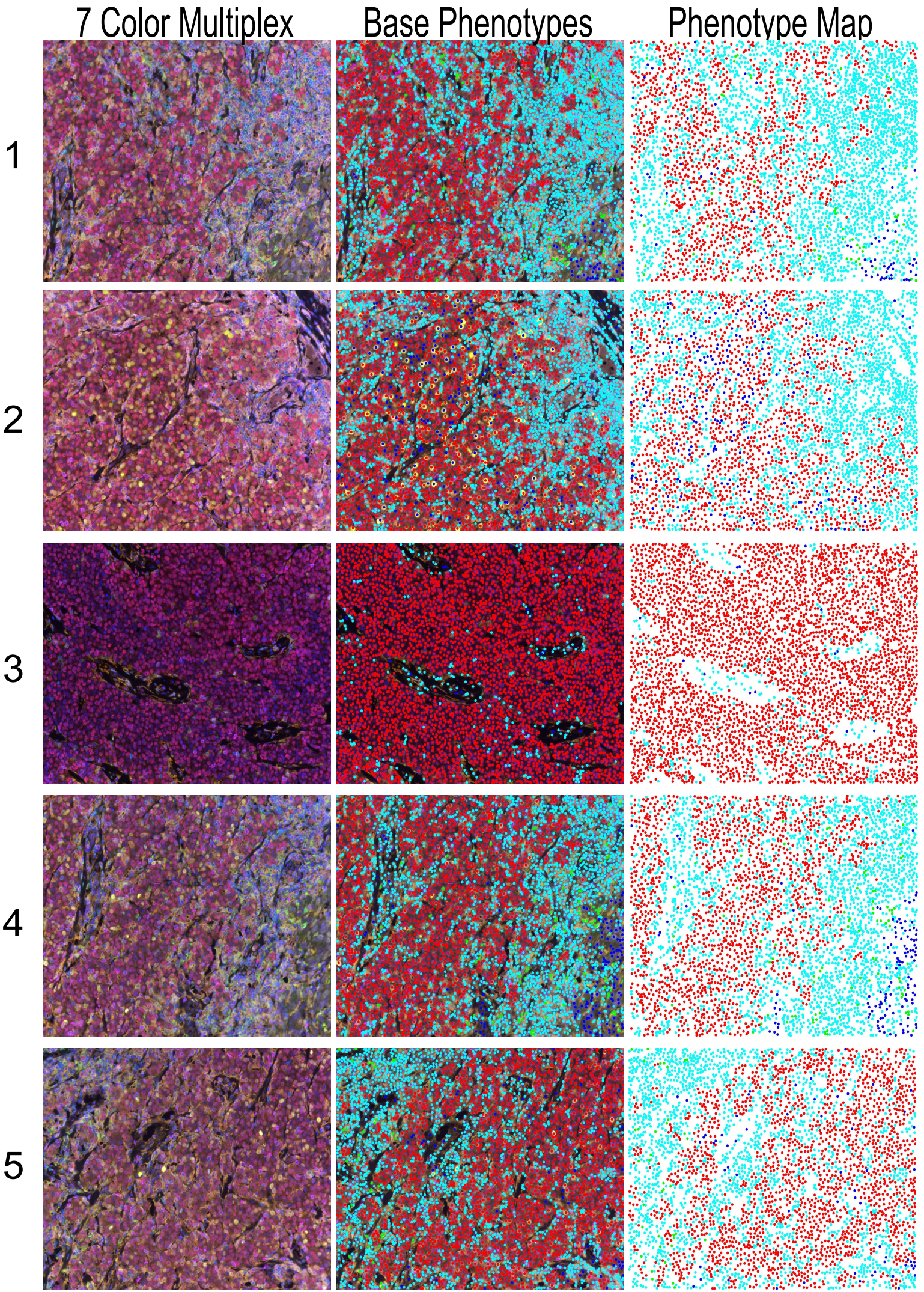
Supplemental Figure S2: Representation of Tissue segmentation for five images from same patient, marked 1 - 5. Tissue segmentation is performed by highlighting examples of tumor (SOX10 positive), and stroma or non-tumor tissue (SOX10 negative). For each image, we show: 7 color multiplex (left), DAPI + SOX10 only (middle) and with respective tissue segmentation (right). Images 1, 4 and 5 are tumor/stroma (up to 50% stroma) and images 2 and 3 are tumor only (at least 90% tumor).

Supplemental Figure S3



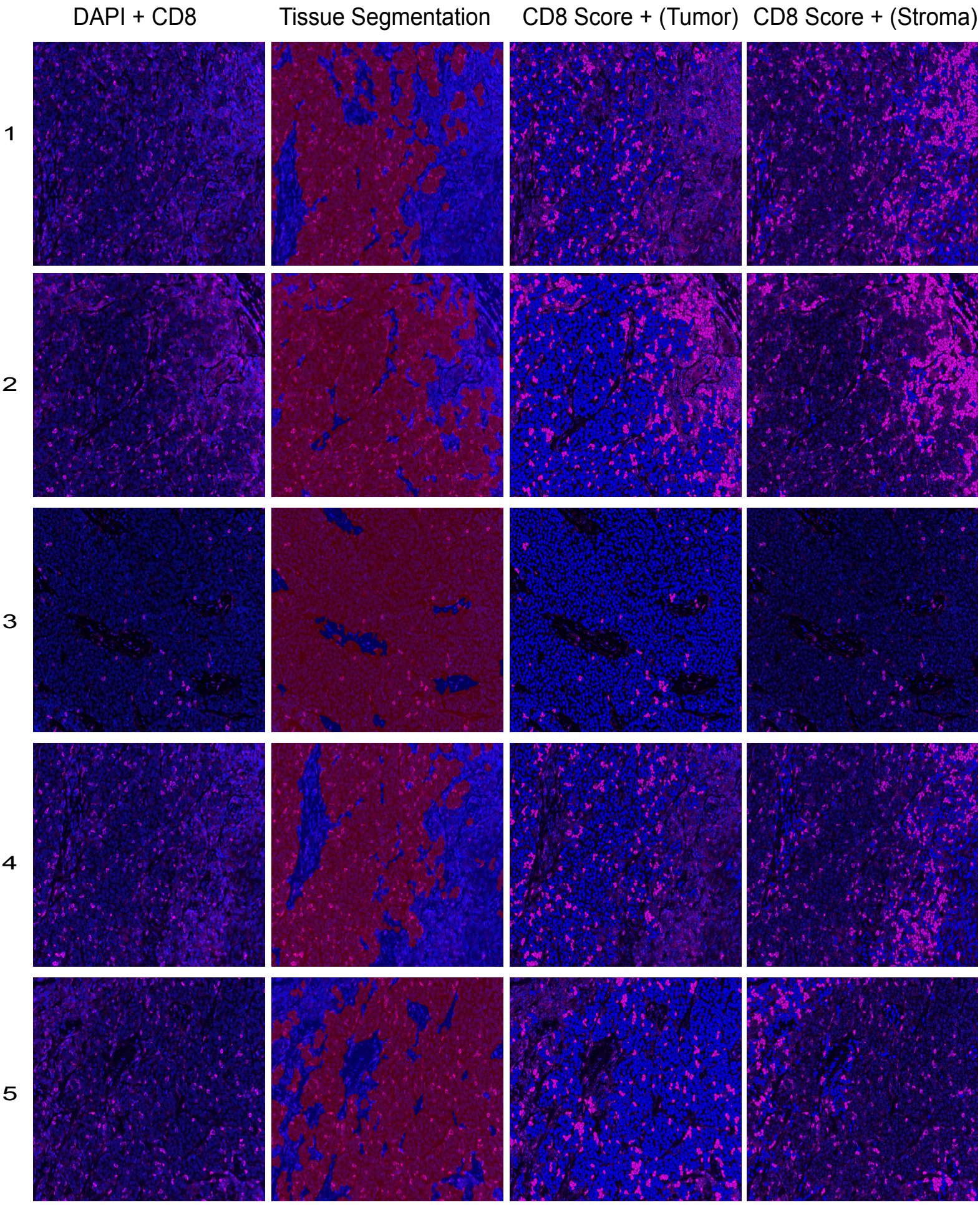
Supplemental Figure S3: Representation of Cell Segmentation for five images from same patient, marked 1 - 5. Cell Segmentation is trained using DAPI as a nuclear counterstain, defining each cell. Left: DAPI only + Cell Segmentation for each 20X image. Right: zoomed in area of the 20X image with DAPI and its respective cell segmentation as well.

Supplemental Figure S4



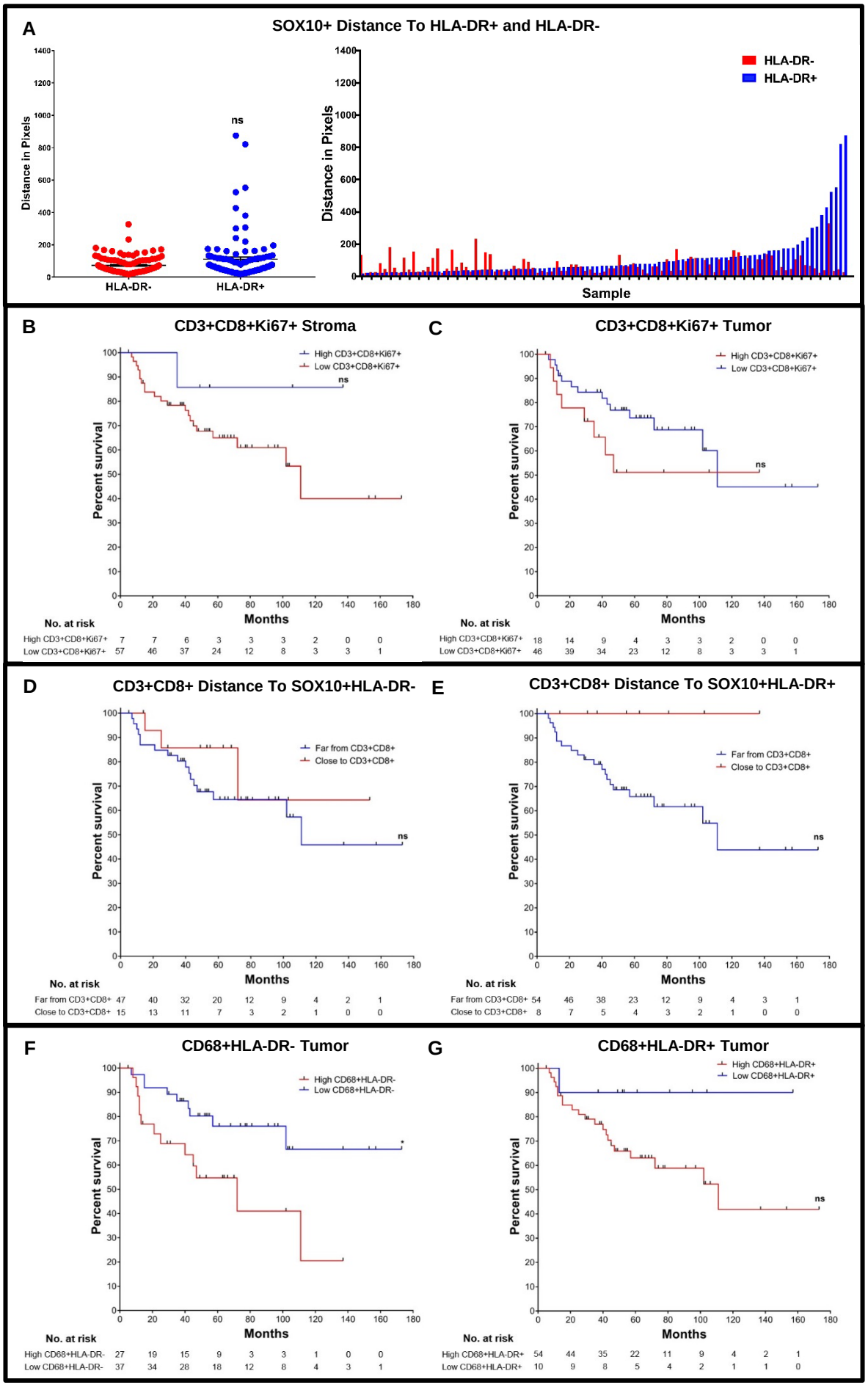
Supplemental Figure S4: Representation of Phenotyping. Phenotyping is trained using 10-15 examples of individual cell types using base variables – Tumor (SOX10+, red dots), CD3+ (cyan dots), CD68+ (green dots) and Other (blue). Left: 7 color multiplex, Middle: phenotyping over multiplex image, Right: phenotype map alone.

Supplemental Figure S5



Supplemental Figure S5: Representation of Scoring. Scoring is done for all concatenated variables including: CD8, Ki67 and HLA-DR. Scoring is evaluated separately in tumor and stroma and a threshold is determined that finds all positive cells in image. As such, we show the representative score for CD8 in tumor and stroma separately. In the figure, the images are a representation of CD8 scoring only. Far Left: DAPI + CD8, Middle Left: Tissue Segmentation over DAPI + CD8 image. This provides the segmentation areas for the two images on the right. Middle Right: CD8 scoring in tumor area only. The stroma area is visible but not scored in this image. Cells highlighted in pink are positive while cells highlighted in blue are negative. Far Right: CD8 scoring in stroma area only. The tumor area is visible but not scored in this image. Cells highlighted in pink are positive while cells highlighted in blue are negative.

Supp Figure S6



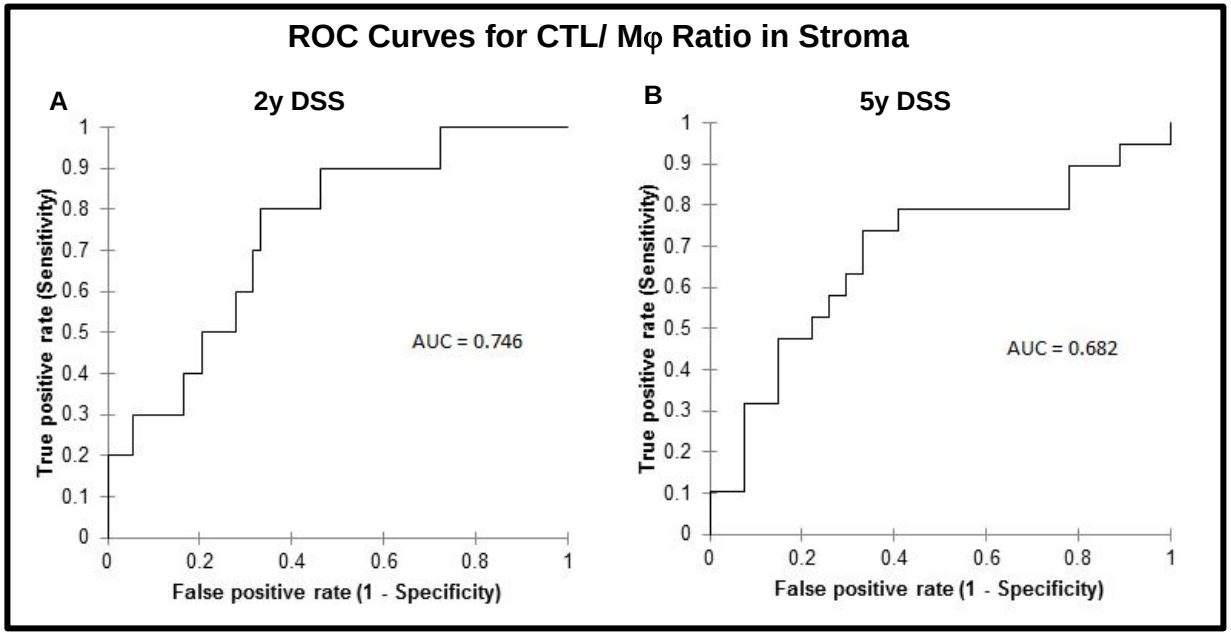
Supplemental Figure S6: A) Median distance of CTLs to SOX10+HLA-DR- (red) or SOX10+HLA-DR+ (blue) grouped (left, p=0.0846), matched median distance to Ki67- and Ki67+ per patient (right). **B)** High (n=7) and Low (n=57) density of CD3+CD8+Ki67 + cells in stroma (p=0.1737). **C)** High (n=18) and Low (n=46) density of CD3+CD8 +Ki67+ cells in tumor (p=0.2161). **D)** Far (n=47) and Close (n=15) distance of CTLs to HLA-DR- tumor (p=0.8436). **E)** Far (n=54) and Close (n=8) distance of CTLs to HLA-DR+ tumor (p=0.0899). **F)** High (n=27) and Low (n=37) density of CD68+HLA-DR- Mφ in tumor (p=0.0163). **G)** High (n=54) and Low (n=10) density of CD68+HLA-DR+ cells in tumor (p=0.1027). Statistical comparison performed using Log-rank (Mantel-Cox) test. ns: not significant (P>0.05), *P≤0.05.

Supplemental Table S3

Receiver Operating Charateristic Curve						
Variable	**Binary**	**AUC**	**Cutoff**	**Sens**	**Spec**	**p**
CD3+CD8+ Stroma	Low vs High	0.668	0.042	0.545	0.762	0.021
CD3+CD8+ Tumor	Low vs High	0.662	0.005	0.636	0.714	0.033
CD3+CD8+ Total	Low vs High	0.640	0.038	0.636	0.548	0.076
CD8 distance to CD68+HLA-DR-	Close vs Far	0.682	165.540	0.550	0.805	0.011
CD68+ Stroma	High vs Low	0.611	0.087	0.409	0.833	0.149
CD68+ Tumor	High vs Low	0.616	0.003	0.909	0.333	0.094
CD68+ Total	High vs Low	0.609	0.047	0.364	0.881	0.148
CD8/CD68 Stroma Ratio	Low vs High	0.724	2.557	0.727	0.667	0.001
CD8/CD68 Total Ratio	Low vs High	0.708	1.521	0.727	0.667	0.004
Univariable Cox Proportional Hazards Model						
Variable	**Binary**	**Value**	**x2**	**p**	**HR**	**95% CI**
CD3+CD8+ Stroma	Low vs High	0.964	5.018	0.025	2.621	1.128 - 6.091
CD3+CD8+ Tumor	Low vs High	1.038	5.440	0.020	2.822	1.180 - 6.749
CD3+CD8+ Total	Low vs High	0.596	1.803	0.179	1.816	0.760 - 4.336
CD8 distance to CD68+HLA-DR-	Close vs Far	1.139	6.386	0.012	3.122	1.291 - 7.551
CD68+ Stroma	High vs Low	1.093	6.306	0.012	2.985	1.271 - 7.007
CD68+ Tumor	High vs Low	1.000	2.580	0.108	2.718	0.802 - 9.209
CD68+ Total	High vs Low	1.052	5.613	0.018	2.863	1.199 - 6.835
CD8/CD68 Stroma Ratio	Low vs High	1.313	7.477	0.006	3.719	1.451 - 9.533
CD8/CD68 Total Ratio	Low vs High	1.458	9.187	0.002	4.297	1.674 - 11.030
TILs	Non-Brisk vs Brisk	1.225	3.840	0.050	3.405	1.000 - 11.597

Supplemental Table S3: ROC Curve and Univariable Cox results (n=64) for density of CTLs in stroma, tumor, and total, distance of CTLs to CD68+HLA-DR- macrophages, density of CD68+ macrophages in stroma, tumor, and total, and CD8/ CD68 Ratio in Stroma and Total. (Abbreviations: Sens = Sensitivity, Spec = Specificity, x2 = Wald Chi-Square, HR = Hazard Ratio, CI = Confidence Interval).

Supplemental Figure S7



Supplemental Figure S7: A) ROC curve for 2y DSS (n=64, AUC = 0.746, p=0.001, cut off = 1.913, Sensitivity = 0.8, Specificity = 0.667) **B)** ROC curve for 5y DSS (n=46, AUC = 0.682, p=0.026, cut off = 2.557, Sensitivity = 0.737, Specificity = 0.667).

Supplemental Table S4

Pearson Correlation Matrix							
Variables	**CD8/ CD68 Stroma**	**CD3+CD8+ Total**	**CD3+CD8+ Tumor**	**CD3+CD8+ Stroma**	**CD68+ Total**	**CD68+ Tumor**	**CD68+ Stroma**
CD8/ CD68 Stroma	**1**	**0.472**	**0.535**	**0.549**	**0.443**	**0.328**	**0.596**
CD3+CD8+ Total	**0.472**	**1**	**0.586**	**0.680**	0.007	-0.100	0.181
CD3+CD8+ Tumor	**0.535**	**0.586**	**1**	**0.607**	0.136	0.022	0.110
CD3+CD8+ Stroma	**0.549**	**0.680**	**0.607**	**1**	0.125	0.014	0.190
CD68+ Total	**0.443**	0.007	0.136	0.125	**1**	**0.316**	**0.695**
CD68+ Tumor	**0.328**	-0.100	0.022	0.014	**0.316**	**1**	**0.361**
CD68+ Stroma	**0.596**	0.181	0.110	0.190	**0.695**	**0.361**	**1**
p Values for Above Correlation							
Variables	**CD8/ CD68 Stroma**	**CD3+CD8+ Total**	**CD3+CD8+ Tumor**	**CD3+CD8+ Stroma**	**CD68+ Total**	**CD68+ Tumor**	**CD68+ Stroma**
CD8/ CD68 Stroma	**0**	**0.000**	**0.000**	**0.000**	**0.000**	**0.008**	**0.000**
CD3+CD8+ Total	**< 0.0001**	**0**	**< 0.0001**	**< 0.0001**	0.954	0.431	0.153
CD3+CD8+ Tumor	**< 0.0001**	**< 0.0001**	**0**	**< 0.0001**	0.284	0.862	0.386
CD3+CD8+ Stroma	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0**	0.324	0.914	0.133
CD68+ Total	**0.000**	0.954	0.284	0.324	**0**	**0.011**	**< 0.0001**
CD68+ Tumor	**0.008**	0.431	0.862	0.914	**0.011**	**0**	**0.003**
CD68+ Stroma	**< 0.0001**	0.153	0.386	0.133	**< 0.0001**	**0.003**	**0**

Supplemental Table S4: Pearson Correlation Matrix showing correlation of CD8/ CD68 (CTL/Mφ) ratio in stroma, with variables of CD8 (Total, Tumor and Stroma) and CD68 (Total, Tumor and Stroma) for all 64 patients with DSS. Values in bold are significant at $p \leq 0.05$.