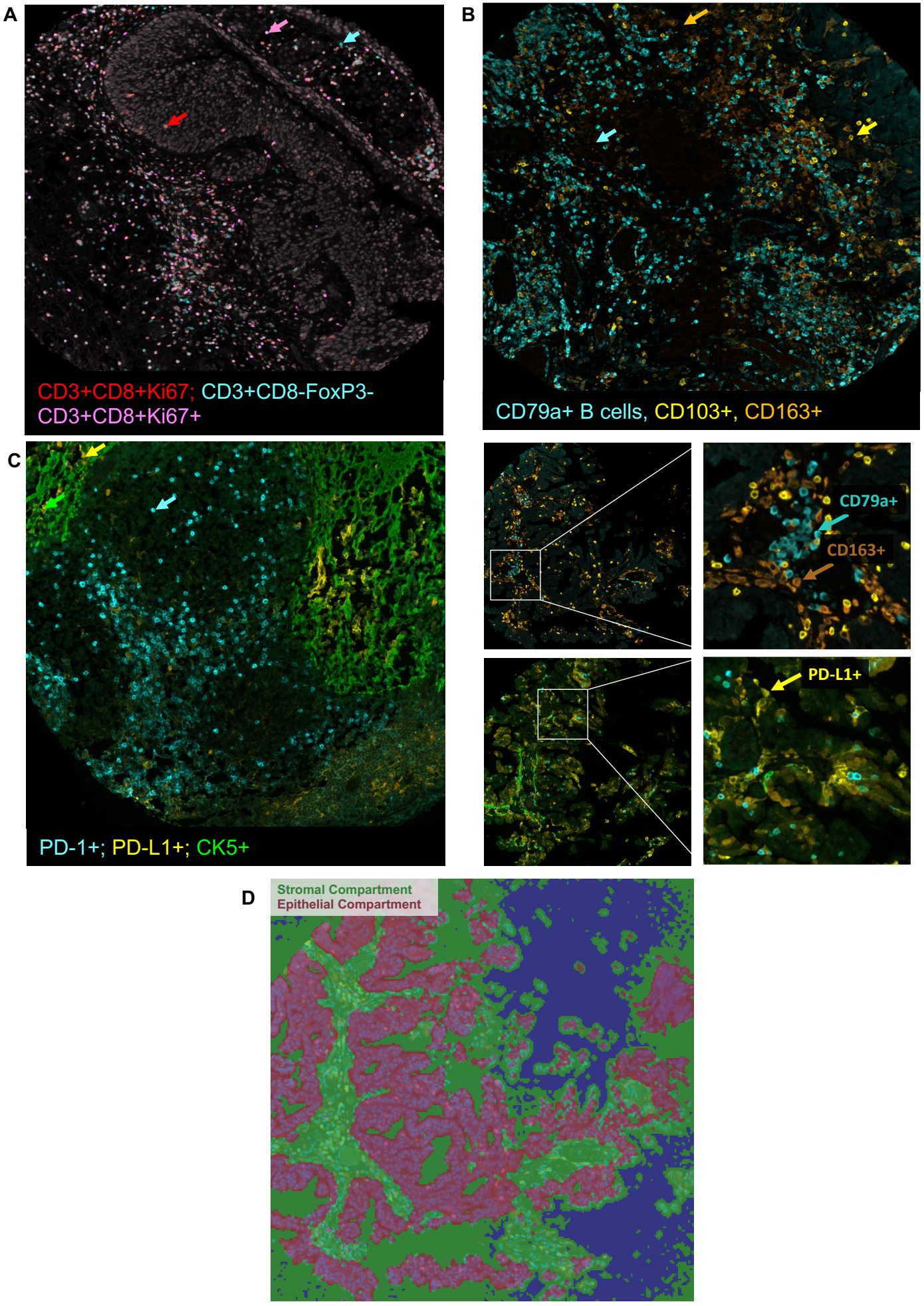
Supplementary Figures

Supplementary Figure 1. Multiplex immunofluorescence staining and automated tissue segmentation of representative tumor core. A composite view of a representative tumor core, highlighting antibody panels distinguishing CD3+CD8+Ki67+/- and CD3+CD8-FoxP3- cells (A); CD79a+ B, CD103+ T resident and CD163+ M2-like TAMs (B); PD-1+, PD-L1+ and CK5+ cells (C). PerkinElmer's Inform software based automated segmentation of tumor core into epithelial (red) and stromal (green) compartments prior to automated scoring of positively stained cells (D).

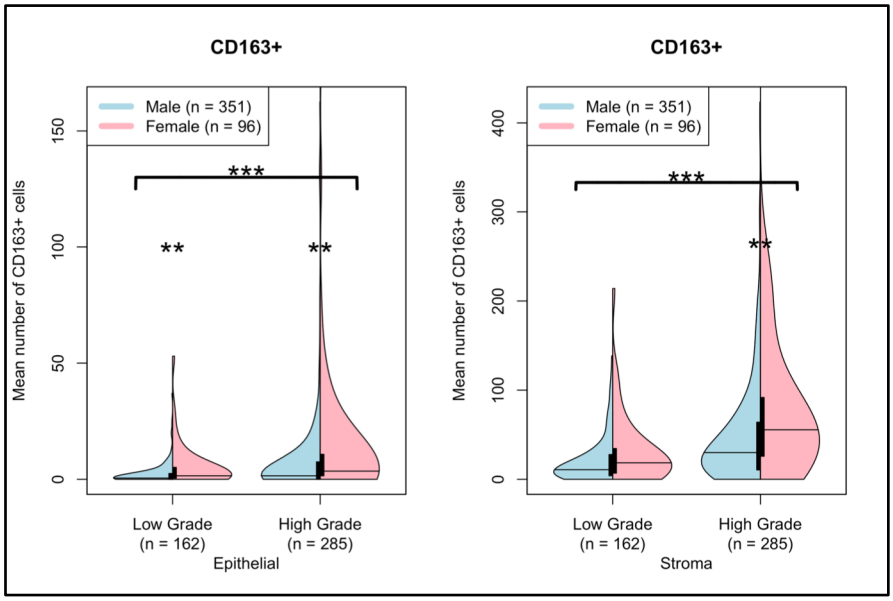


Supplementary Figure 1. Multiplex immunofluorescence staining and automated tissue segmentation of representative tumor core.

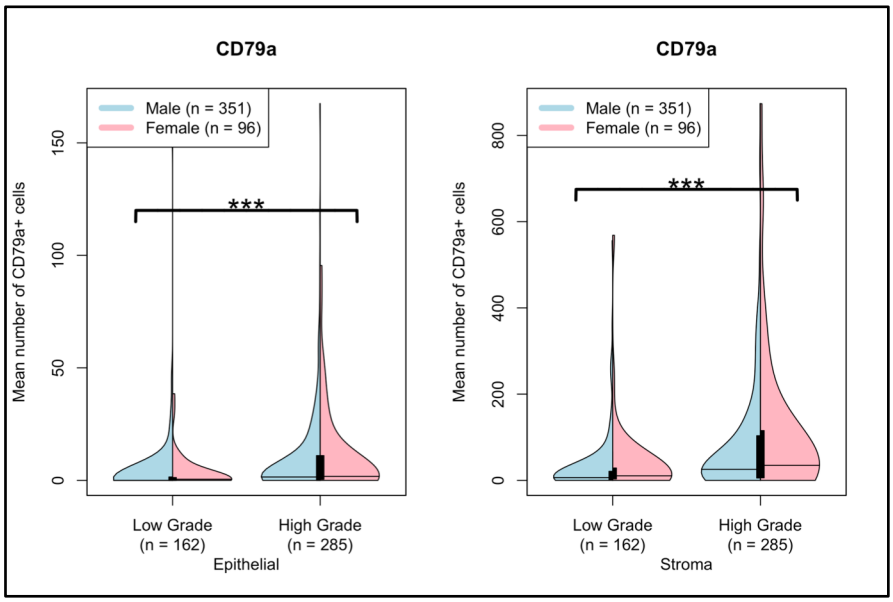
Supplementary Figure 2. Profiles of CD163, CD79a and PD-L1 in tumors from BCG naïve patients

Violin plots of mean cell counts for CD163+ (A), CD79a+ (B) and PD-L1+ cell (C) populations respectively, in the epithelial (left) and stromal (right) compartments of tumors from the KHSC cohort with no evidence of BCG immunotherapy prior to collection of their specimens (BCG naïve). Asterisks indicate level of significance as determined by Mann-Whitney-U statistics: ***p-value < 0.001 **p-value < 0.01 *p-value < 0.05.

2A



2B



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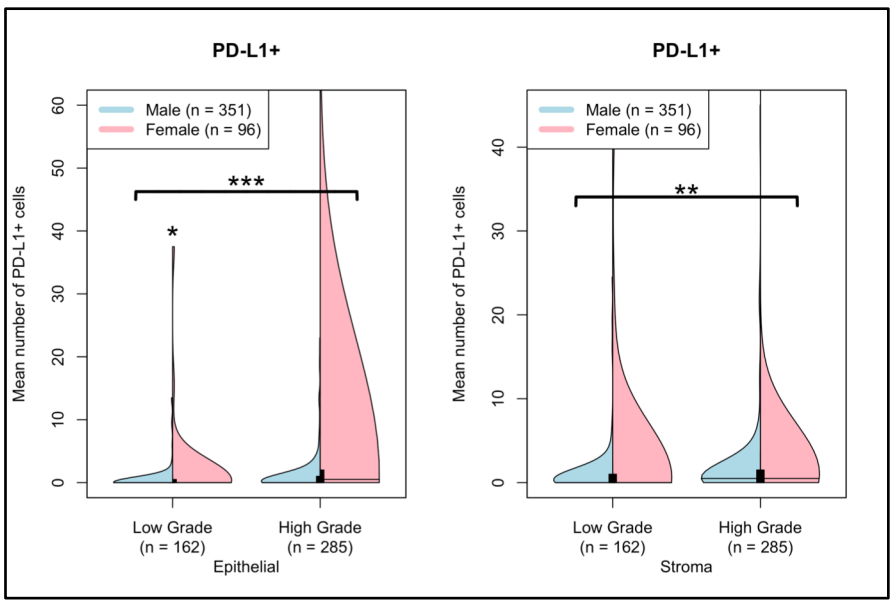
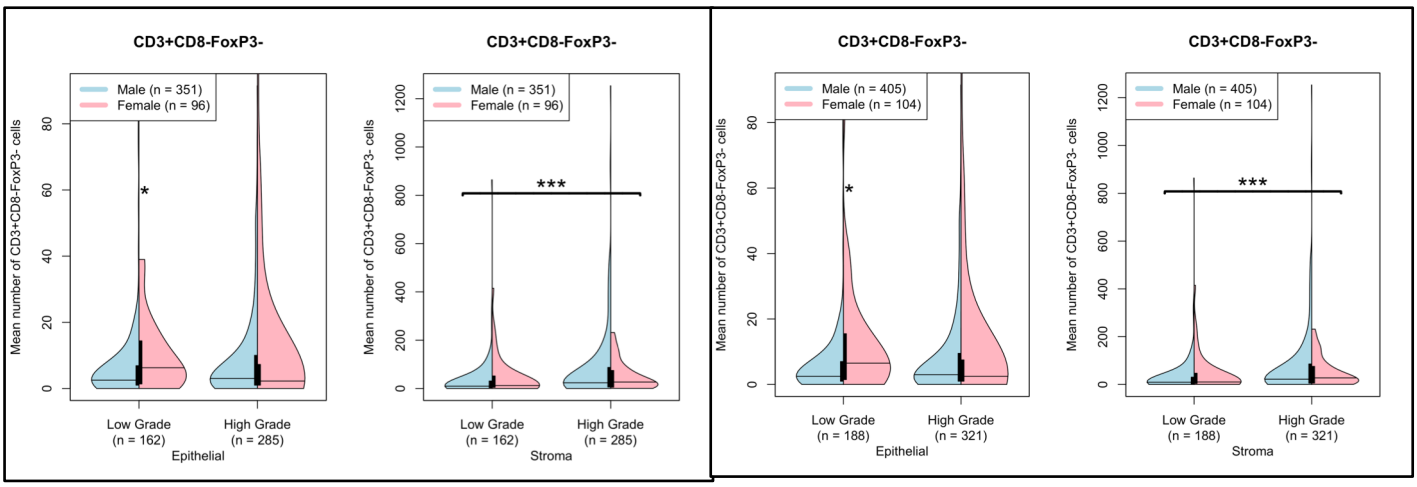


Supplementary Figure 2: Profiles of CD163, CD79a and PD-L1 in tumors from BCG naïve patients

Supplementary Figure 3. Profiles of CD3+CD8-T cells (3A) and regulatory T cells (3B) in tumors from BCG naïve patients and overall cohort

Violin plots of mean cell counts for CD3+CD8- T(A) and CD3+CD8-FoxP3+ T regulatory cells (B) in BCG naïve and overall cohort. Plots stratified by low-grade and high-grade samples for males (blue) versus females (pink). Asterisks indicate level of significance as determined by Mann-Whitney-U statistics: ***p-value < 0.001 **p-value < 0.01 *p-value < 0.05.

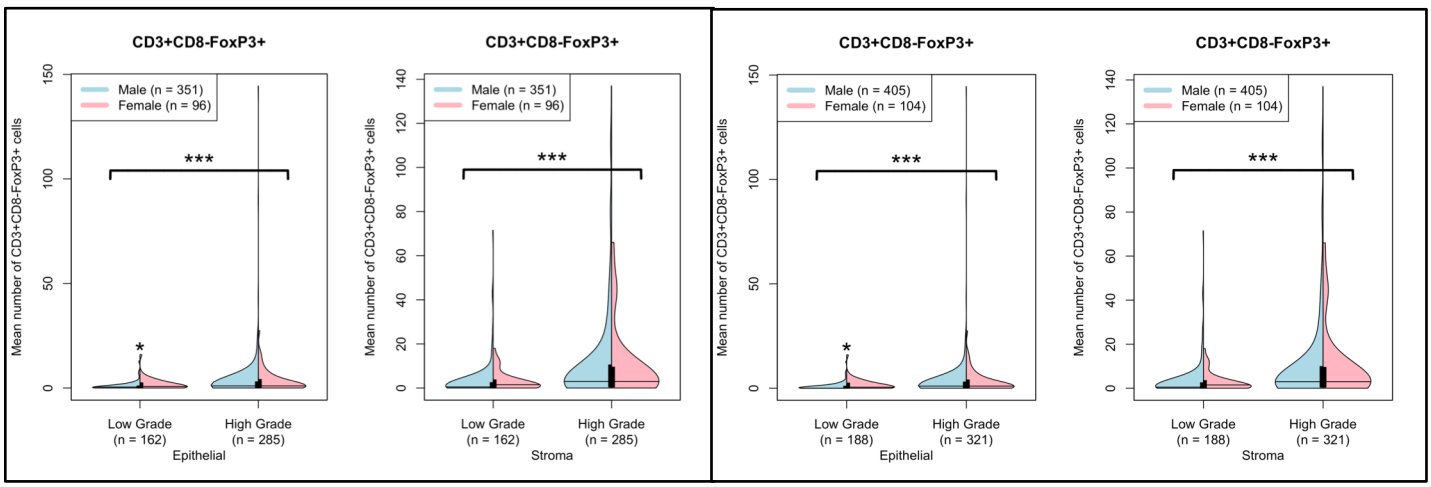
3A



BCG naïve tumors

Overall cohort

3B



BCG naïve tumors

Overall cohort

Supplementary Figure 3: Profiles of CD3+CD8- T (3A) and regulatory T cells (3B) in tumors from BCG naïve patients and overall cohort

Supplementary Figure 4. CD79a+ B cell density is associated with recurrence free survival in patients with high-grade NMIBC and no prior history of BCG before specimen collection (BCG naïve).

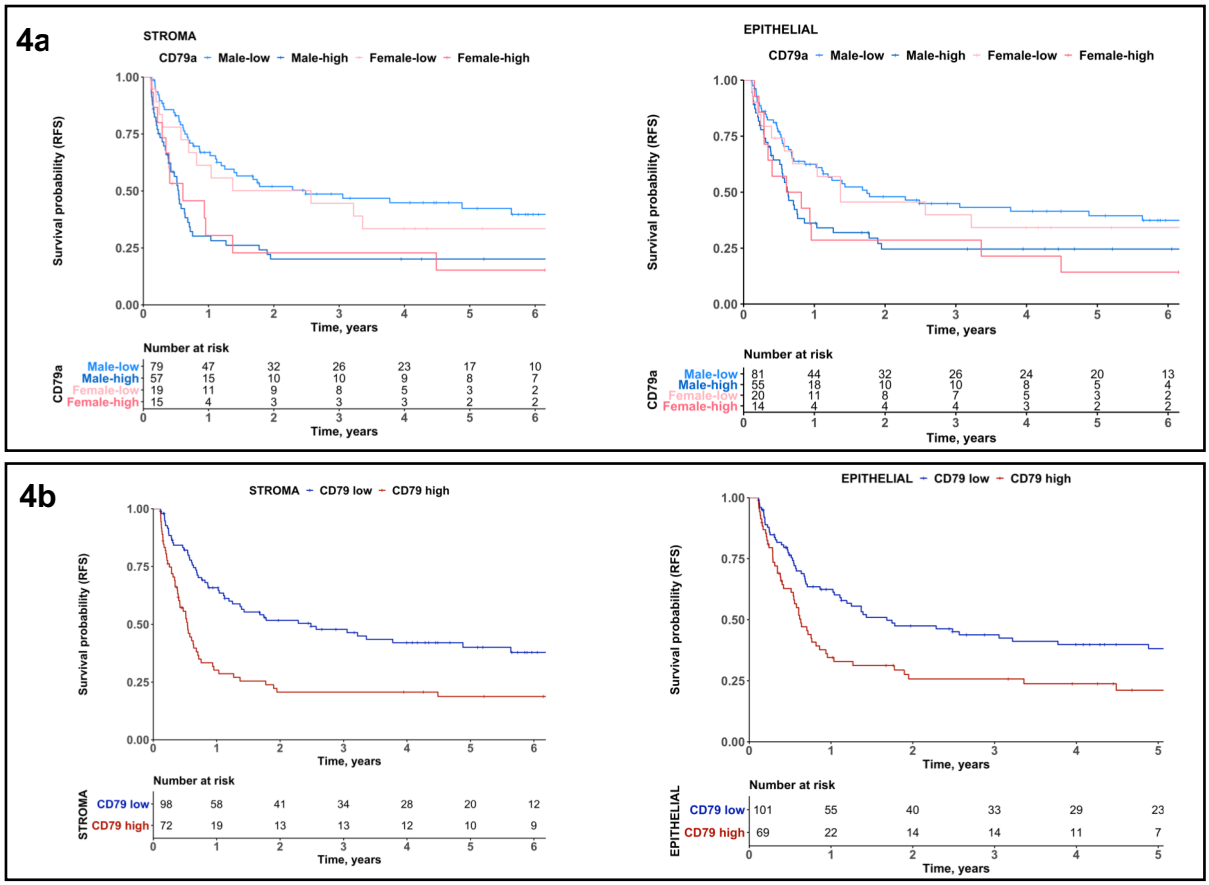
(a) Recurrence-free survival of a subset of patients with high-grade disease and no previous BCG therapy prior to specimen collection (n=170). Within this cohort 74% had evidence of adequate BCG therapy after specimen collection (TURBT). Based on log-rank optimized cut-offs for stromal (left; $p<0.001$) and epithelial (right; $p<0.056$) CD79a+ cells stratified by males (blue) versus females (pink).

(b) Recurrence-free survival based on log-rank optimized cut-offs for stromal (left) and epithelial (right) CD79a+ cells stratified by high CD79a+ cells versus low CD79a+ cells. High versus low stromal CD79a+ cells defined as > 35 or < 35 cells, respectively. High vs low epithelial CD79a+ cells defined as > 3 or < 3 cells, respectively. Associated p-values for the epithelial and stromal compartments is <0.01 and <0.0001, respectively.

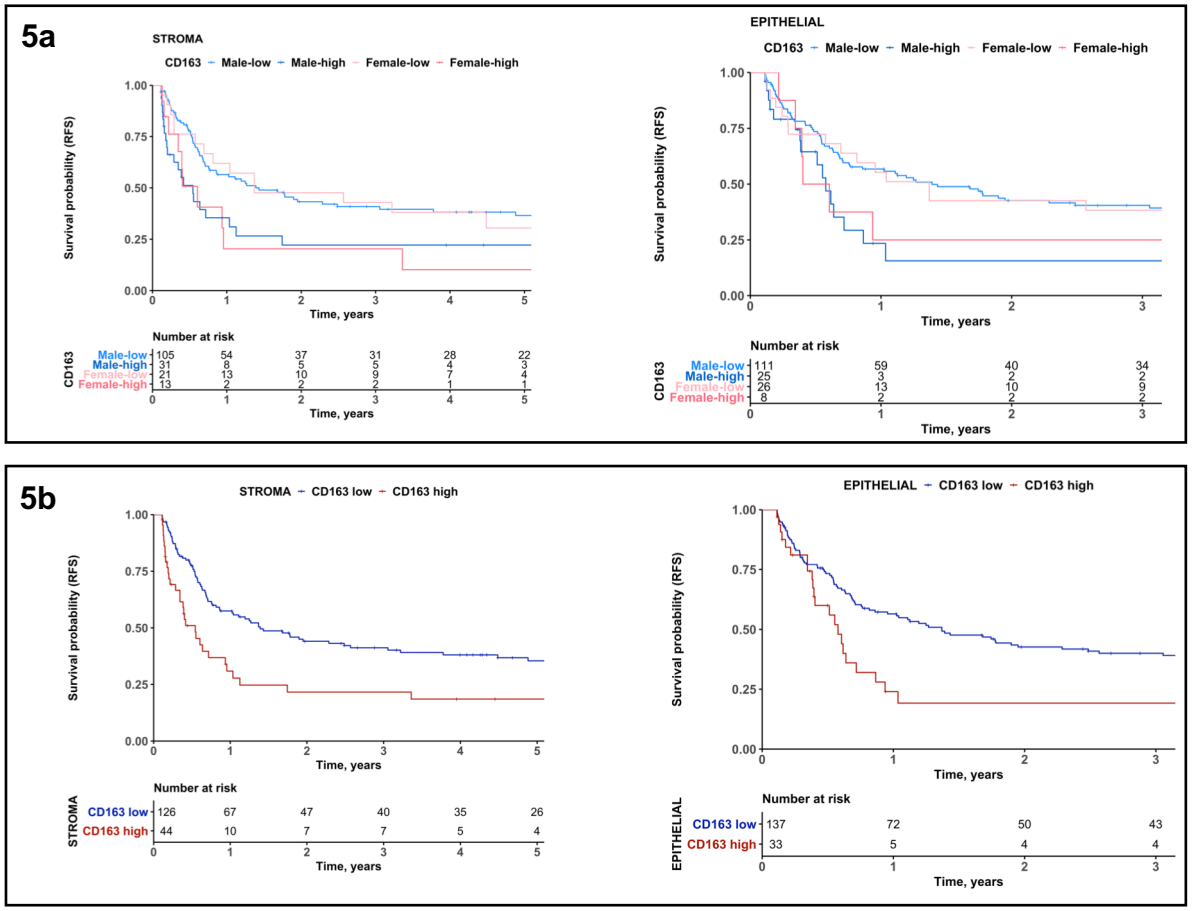
Supplementary Figure 5. Higher CD163+ cell infiltration is associated with shorter recurrence free survival in BCG-naïve patients with high-grade NMIBC.

(a) Recurrence-free survival of high-grade, BCG naïve patients (n=170) based on log-rank optimized cut-offs for stromal (left; P=0.017) and epithelial (right; 0.057) CD7163+ cells stratified by males (blue) versus females (pink).

(b) Recurrence-free survival based on log-rank optimized cut-offs for stromal (left) and epithelial (right) CD163+ cells stratified by high CD163+ cells versus low CD163+ cells. High versus low stromal CD163+ cells defined as > 64 or < 64 cells, respectively. High vs low epithelial CD163+ cells defined as > 12 or < 12 cells, respectively. Associated p-values for the epithelial (right) and stromal (left) compartments are both $p<0.01$.



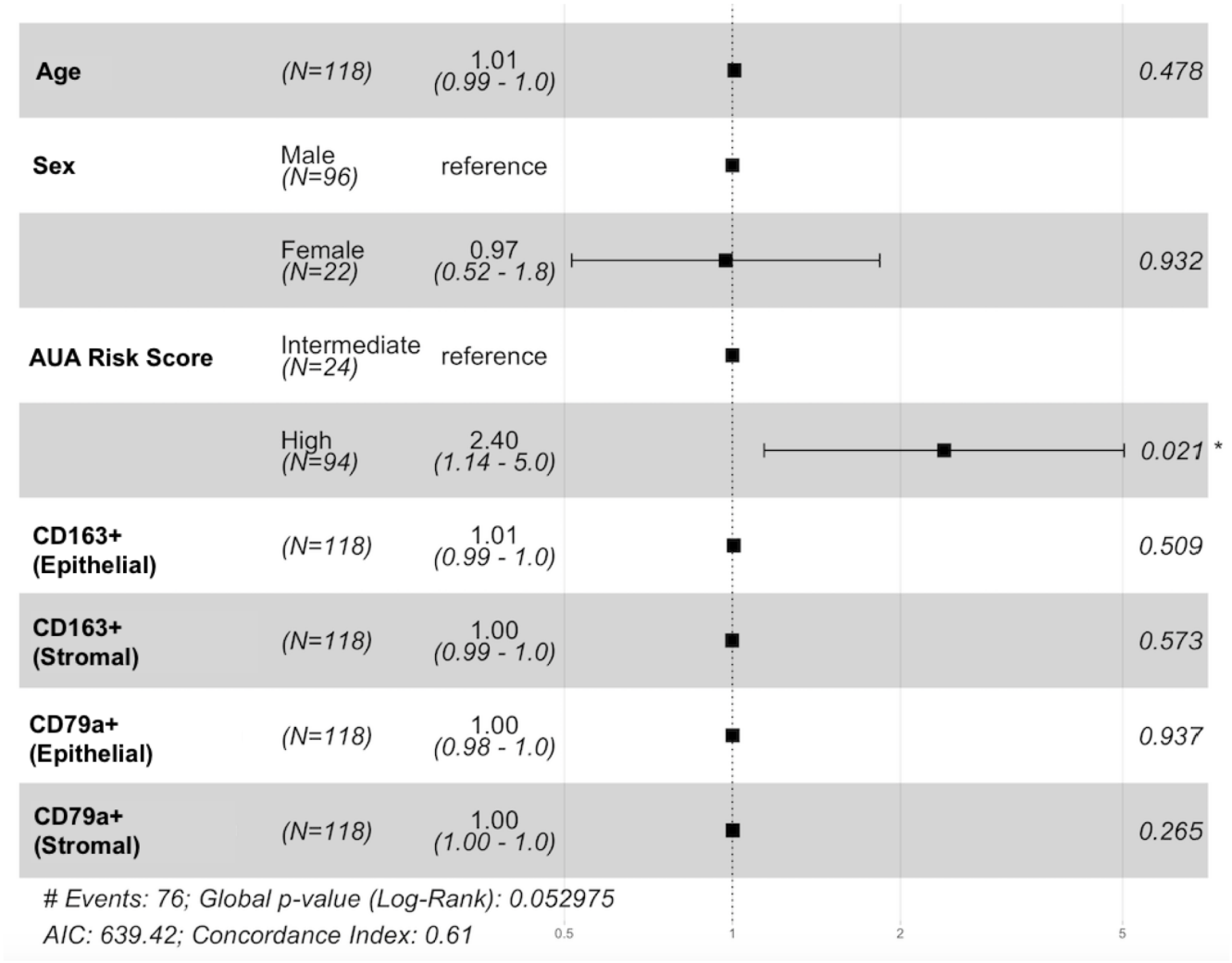
Supplementary Figure 4. CD79a+ B cell density is associated with recurrence free survival in patients with high-grade NMIBC



Supplementary Figure 5. Higher CD163+ cell infiltration is associated with shorter recurrence free survival in patients with high-grade NMIBC

Supplementary Figure 6. AUA Risk Score is associated with increased risk of recurrence for patients with high-grade NMIBC who received adequate induction BCG therapy.

(a) Forest plot of multivariate analysis examining the relationship between potential risk factors (age, sex, previous BCG, induction BCG, CD163, CD79a and AUA risk score) and recurrence-free survival for all patients with high-grade NMIBC who received adequate induction BCG therapy (>5 doses) in the KHSC cohort (n=118). Patients with high AUA risk score were 2.40 times more likely to suffer disease recurrence than patients with intermediate AUA risk score (95% CI, 1.14-5.0; $p<0.021$). No other risk factors were found to be significantly associated with RFS ($p>0.05$).



Supplementary Figure 6. AUA Risk Score is associated with increased risk of recurrence for patients with high-grade NMIBC who received adequate induction BCG therapy.

Supplementary Tables

S1. Clinical characteristics of patients in the KHSC cohort (n=332).

S2. Sample characteristics (grade, stage and sex) for overall KHSC cohort (n = 509)

S3. Optimized log-rank thresholds for recurrence-free survival for individual immune markers