## **Spatial distribution of FoxP3+ and CD8+ tumour infiltrating** T cells reflects their functional activity

## SUPPLEMENTARY FIGURES AND TABLES



**Supplementary Figure S1: Correlation of cell densities in the stromal and epithelial compartment.** Cell densities in pre-RCT biopsies: Epithelial FoxP3+ cells compared to stromal FoxP3+ cells **A**.; epithelial CD8+ cells compared to stromal CD8+ cells **B**.; epithelial FoxP3+ cells compared to epithelial CD8+ cells **C**.; stromal FoxP3+ cells compared to stromal CD8+ cells **D**. Cell densities in post-RCT tumour resections: epithelial CD8+ cells compared to stromal CD8+ cells in the central tumour region **E**. and in the invasive front **F**.



**Supplementary Figure S2: Kaplan-Meier plots for the densities of FoxP3+ and CD8+ cells in the stromal and epithelial compartment of pre RCT biopsies and in post RCT central tumour (no evidence of disease survival).** FoxP3+ density epithelial pre RCT biopsies **A.** CD8+ density epithelial pre RCT biopsies **B.** FoxP3+ density stromal pre RCT biopsies **C.** CD8+ density epithelial post RCT central tumour **E.** CD8+ density epithelial post RCT central tumour **F.** FoxP3+ density stromal post RCT central tumour **G.** CD8+ density stromal post RCT central tumour **H.** 



Supplementary Figure S3: Kaplan-Meier plots for the densities of CD8+ cells in the stromal and epithelial compartment of pre RCT biopsies and in post RCT central tumour (overall survival). CD8+ density epithelial pre RCT biopsies A., CD8+ density stromal pre RCT biopsies B., CD8+ density epithelial post RCT central tumour C., CD8+ density stromal post RCT central tumour D.



**Supplementary Figure S4: Kaplan-Meier plots for the cell-cell distances of FoxP3+-CD8+ and CD8+-FoxP3+ cells in the stromal and epithelial compartment pre and post RCT in the central tumour (no evidence of disease survival).** FoxP3+-CD8+ epithelial pre RCT biopsies **A.**, CD8+-FoxP3+ epithelial pre RCT biopsies **B.** FoxP3+-CD8+ stromal pre RCT biopsies **C.** CD8+-FoxP3+ stromal pre RCT biopsies **D.** FoxP3+-CD8+ epithelial post RCT central tumour **E.** CD8+-FoxP3+ epithelial post RCT central tumour **F.** FoxP3+-CD8+ stromal post RCT central tumour **G.** CD8+-FoxP3+ stromal post RCT central tumour **H.** 



Supplementary Figure S5: Kaplan-Meier plots for the cell-cell distances of CD8+-FoxP3+ cells in the stromal and epithelial compartment pre and post RCT in the central tumour (overall survival). CD8+-FoxP3+ epithelial pre RCT biopsies A., CD8+-FoxP3+ stromal pre RCT biopsies B., CD8+-FoxP3+ epithelial post RCT central tumour C., CD8+-FoxP3+ stromal post RCT central tumour D.

Supplementary Table S1: Correlations between CD8+ or FoxP3+ cells in different compartments, prior RCT (biopsy) or post RCT (central tumour, invasive front), respectively.

| tissue         | compartment     | TIC   |   | tissue         | compartment     | TIC | patients | r     | р       |
|----------------|-----------------|-------|---|----------------|-----------------|-----|----------|-------|---------|
| biopsy         | intraepithelial | FoxP3 | - | biopsy         | intraepithelial | CD8 | 103      | 0.582 | < 0.001 |
| biopsy         | stromal         | FoxP3 | - | biopsy         | stromal         | CD8 | 103      | 0.529 | < 0.001 |
| biopsy         | intraepithelial | CD8   | - | biopsy         | stromal         | CD8 | 105      | 0.400 | < 0.001 |
| central tumour | intraepithelial | FoxP3 | - | central tumour | intraepithelial | CD8 | 153      | 0.480 | < 0.001 |
| central tumour | intraepithelial | FoxP3 | - | central tumour | stromal         | CD8 | 152      | 0.481 | < 0.001 |
| central tumour | stromal         | FoxP3 | - | central tumour | stromal         | CD8 | 152      | 0.281 | < 0.001 |
| central tumour | intraepithelial | CD8   | - | central tumour | stromal         | CD8 | 152      | 0.317 | < 0.001 |
| central tumour | stromal         | CD8   | - | invasive front | stromal         | CD8 | 112      | 0.383 | < 0.001 |
| invasive front | intraepithelial | CD8   | - | invasive front | stromal         | CD8 | 128      | 0.776 | < 0.001 |