1 **Single-cell tumor-immune microenvironment of BRCA1/2 mutated high-grade serous ovarian cancer**

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38 **Abstract**

39 The majority of high-grade serous ovarian cancers (HGSCs) are deficient in homologous recombination (HR)
40 DNA repair, most commonly due to mutations or hypermethylation of the *BRCA1/2* genes. We aimed to
41 discover how *BRCA1/2* mutations shape the cellular phenotypes and spatial interactions of the tumor
42 microenvironment.

43 Using a highly multiplex immunofluorescence and image analysis on 112 tumor cores we generated single-
44 cell spatial data for 21 markers in 124,623 single cells from 31 tumors with *BRCA1/2* mutation (*BRCA1/2*mut),
45 and 13 tumors without any alterations in HR genes (HRwt). We identified a phenotypically distinct tumor
46 microenvironment in the *BRCA1/2*mut tumors with evidence of increased immunosurveillance. Importantly,
47 we found an opposing prognostic role of a proliferative tumor-cell phenotypic subpopulation in the HR-
48 genotypes, which associated with enhanced spatial interactions in the tumor-immune cellular communities.
49 The single-cell spatial landscapes indicate distinct patterns of spatial immunosurveillance with the premise to
50 improve immunotherapeutic strategies and patient stratification in HGSC.

51

52 **Introduction**

53 Every year almost 240 000 new cases of ovarian cancers are diagnosed, leading to the death of over 150 000
54 women worldwide ¹. Out of all ovarian cancers, high-grade serous ovarian, peritoneal or fallopian-tube cancer
55 (HGSC) is the most common subtype and the cause of 70-80% of all ovarian cancer-related deaths ². The 5-
56 year overall survival of HGSC has remained poor, approximating less than 40%, with DNA-damaging therapy
57 and cytoreductive surgery as the standard of care ^{2,3}. The development of new immunological therapies and
58 patient stratification has been hampered by the lack of a detailed understanding of the tumor-immune
59 microenvironment in HGSC.

60 Characteristically 20-22% of HGSC tumors are deficient in *BRCA1* or *BRCA2* genes, either via germline or
61 somatic mutations, or hypermethylation ⁴. *BRCA1/2* mutations disrupt the high-fidelity homologous
62 recombination (HR) DNA repair pathway, and consequently these cells are dependent on error-prone DNA

63 repair mechanisms and sensitive to both DNA damaging agents and Poly-ADP Ribose Polymerase inhibitors
64 ⁵. In addition to the *BRCA1/2* mutations, HR-deficiency can be caused by mutations or epigenetic modifications
65 in other genes of the HR-pathway ⁵.

66 Notably, preliminary evidence suggests that HR-deficient tumors have a distinct tumor-immune
67 microenvironment ^{6,7}. *BRCA1/2*mut tumors have been predicted to contain more neoantigens than tumors with
68 no alterations in genes of the HR pathway (HRwt)⁶, harbor an increased number of tumor infiltrating
69 lymphocytes ⁶, and have an elevated PD-L1 expression as compared to HR-proficient tumors ^{6 8}. Consistent
70 with these findings, we recently reported enhanced responses to combination of PARP inhibitor plus PD-1
71 inhibitor in patients with HR-deficient tumors⁹. However, a comprehensive view of the tumor
72 microenvironment (TME) and the interplay of tumor, immune and stromal cells of *BRCA1/2* mutated tumors
73 has not yet been described.

74 We performed highly multiplexed imaging utilizing 21 markers in 44 HGSCs and a deep phenotypic profiling
75 of 124,623 single cells to uncover the roles of the tumor- immune- and stromal cell subpopulations in the
76 HGSC TME. We herein report phenotypically and spatially divergent microenvironments in the *BRCA1/2*mut
77 tumors as compared to the HRwt tumors, with distinct cellular subpopulations and their spatial interactions
78 contributing to clinical outcomes in *BRCA1/2* mutated HGSC.

79

80 **Materials and Methods**

81 Cohort description The clinical cohort consisted of 27 patients with *BRCA1* mutated tumors, 4 patients with
82 *BRCA2* mutated tumors and 13 patients with tumors without any alterations in the HR-genes. HR-genes
83 included *ATM*, *ATR*, *BRCA1*, *BRCA2*, *BRIP1*, *CHEK2*, *FANCA*, *FANCC*, *FANCD2*, *FANCE*, *FANCF*,
84 *FANCG*, *NBN*, *PTEN*, and *U2AF1*; they were assessed using OncoPanel Next-generation sequencing assay as
85 previously described ⁶. Tumors harboring mutations in the nucleotide excision repair and mismatch repair
86 pathways were excluded. In addition, immunohistochemistry for *BRCA1* was used to exclude *BRCA1* promoter
87 hypermethylation in tumors without mutations in the HR genes ⁶. The histology of all tumors was confirmed

88 as HGSC by an experienced pathologist. The tumor material consisted of 112 tumor 1.0 mm cores on a single
89 tissue-microarray (TMA); mostly triplicate cores per patient.

90 Highly multiplexed imaging, and image processing. The samples were stained sequentially with the validated
91 antibodies (**Supplementary Table 1**) and scanned with RareCyte CyteFinder scanner following the tCycIF
92 protocol¹⁰. Scanned image files were corrected using the BaSiC tool and stitched and registered using the
93 ASHLAR algorithm (<https://github.com/labsyspharm/ashlar>) to align image tiles and successive images of
94 tiles from all cycles to one another. We detected and cropped the cores from the stitched multi-channel image
95 with Image Processing Toolbox on MATLAB (version 2018b, The MathWorks, Inc., Natick, Massachusetts,
96 United States). This process produced multi-channel .tiff files that were further converted for Ilastik using the
97 Fiji plugin ilastik4ij-1.7.3. Probability maps for segmentation were produced using Ilastik, where all pixel
98 features were used for class discrimination. We trained Ilastik with 10 cores on 3-class manually labeled data
99 (inner nuclei, cell membrane, image background). We segmented the resulting probability maps (exported as
100 unsigned 16-bit tif images with axis order tzyxc=[1, 1, 1400, 1400, 32]) and the simple segmentation results
101 for the label 'nuclei'. The results were processed in Fiji to produce a final set of masks by expanding nuclei
102 masks and removing objects with area < 30 pixels. We quantified log mean fluorescence intensity for each
103 channel as well as default morphological features with histoCAT software. The mean number of cells per core
104 was 1107, median 1030 and range 29 to 3282. Subsequently, the mean number of cells per sample was 2818,
105 median 2524 and range 278 to 5933. The number of cells per patient and the number of TMA-cores per patient
106 is presented in **Supplementary Figure 1A**. We used three channels imaged with secondary-only antibodies
107 (Rabbit 488, Rat 555, Mouse 647) with a background threshold of 97th percentile for quality control to remove
108 unqualified cells. We removed lost cells based on radical changes in the DNA channel across cycles as
109 previously described in Cycif Analysis Suite (https://github.com/yunguan-wang/cycif_analysis_suite). ROIs
110 with artefacts (e.g. bubble, blobs, debris) were identified by visual inspection in the Open Microscopy
111 Environment Omero, and were removed from further analysis. XY-plots of the marker expressions per single
112 cell to were further visualized to confirm data quality.

113 Cell phenotyping and spatial analysis. We utilized our recently published tool CYTO¹¹ to cluster and iteratively
114 assign cell type labels. The quantified cell data were clustered in CYTO, followed by annotation of the clusters

115 based on their median marker expression profiles. The clusters were visualized with heatmaps and minimum
116 spanning trees of marker expressions per cluster in CYTO, and with UMAP using marker expression per cell.
117 The process consisted of five stages: 1) separation of tumor cells from immune and stromal cells, 2) separating
118 immune cells from stromal cells, 3) immune cell subtyping, 4) tumor cell metacluster annotation, and 5)
119 stromal cell metacluster annotation. Cells that did not fit the criteria for any cell types were re-clustered or
120 finally labeled as unknown. Markers used to annotate tumor cells, stromal cells and immune cell subtypes are
121 presented in **Supplementary Figure 1b**.

122 Prior to clustering with FlowSOM (k=20), the data were sample-wise normalized using mean-centering. First,
123 tumor cell clusters were separated, and the remaining cells were further clustered to detect and annotate clusters
124 by their stromal and immune marker expression. The immune-annotated cells were first divided into
125 lymphocytes and myeloid cells with FlowSOM (k=16). Lymphocytes yielded four subtypes (CD8+T-cells,
126 CD4+T-cells, CD4+FOXP3+T-regulatory cells and CD20+B-cells) with PhenoGraph (knn=60, markers
127 CD8a, CD4, FOXP3 and CD20). Myeloid cells we assigned into six subtypes (IBA1+CD163+,
128 IBA1+CD163+CD11c+, IBA1+CD11c+, IBA1+ and CD163+ macrophages, and CD11c+ antigen presenting
129 cells) using FlowSOM (k=16). Separately, tumor cells were clustered using k-means (k=25, markers E-
130 cadherin, Ki67, P21, PDL1, cCasp3, PSTAT1 and Vimentin) and annotated into seven metaclusters: epithelial
131 cells, proliferating epithelial cells, epithelial-mesenchymal-transition (EMT) cells, proliferating EMT cells,
132 mesenchymal cells, apoptotic cells and functional epithelial cells. Stromal cell metaclusters annotation with k-
133 means (knn= 25, markers vimentin, CD31, Ki67, P21, PDL1, cCasp3, pSTAT1 and cell eccentricity – defined
134 as the ratio of the distance between the focus points of an ellipse to its major axis length) produced nine
135 metaclusters: high-proliferative stroma, proliferative stroma, non-proliferative stroma, high-vimentin, low-
136 vimentin, low-eccentricity, functional stroma, high-p21 stroma and endothelia. To confirm the annotations
137 were correct, the identities of the cells were visually confirmed by overlapping the cell class on the original
138 images using Napari image-viewer. The samples were stained for CD3, CD4 and CD8, and scored by a
139 pathologist for the average count of CD3+, CD4+ and CD8+T-cells of three selected CD3+T-cell rich tumor
140 areas per patient ⁶, and these annotations were compared with our image-analysis based annotations. Simpson's

141 diversity index (SDI) was calculated using R-package ([https://cran.r-](https://cran.r-project.org/web/packages/vegan/vignettes/diversity-vegan.pdf)
142 [project.org/web/packages/vegan/vignettes/diversity-vegan.pdf](https://cran.r-project.org/web/packages/vegan/vignettes/diversity-vegan.pdf)).

143 The cell-cell spatial neighborhoods were assessed using a cutoff of 30 pixels from the centroid of each cell to
144 the centroid of neighboring cells using rangesearch function in MATLAB, excluding itself from the resulting
145 list. The mean proportion of each cell type neighboring each specific centering cell type was calculated per
146 tumor, and the log2 fold changes were calculated between *BRCAl/2mut* and HRwt tumors to compare their
147 spatial cell-cell neighborhood frequencies.

148 Statistics

149 Statistical analyses were performed in R version 3.6.1. Unpaired two-tailed Wilcoxon tests were used to
150 calculate two-sided p-values for pairwise comparisons. Spearman correlation coefficients and their p-values
151 were used in correlation analyses. P-values less than 0.05 were considered significant. Kaplan-Meier graphs
152 were plotted according to the method using the survival and survminer packages in R, and patients at risk with
153 no events at the end of follow-up were censored. P-values were calculated with the log-rank test. For immune
154 subtype proportions, the median value was used as a cut-off for high- and low proportion groups. For functional
155 marker expressions, the top 1/3 percentile was annotated as high and the rest as low. The proportional hazards
156 assumption for Cox-proportional hazards were tested using cox.zph function in the survival package in R, and
157 models with values less than 0.05 were considered not suitable for analysis.

158 Results

159 **Multiplexed imaging reveals tumor microenvironment composition at single-cell resolution**

160 We performed highly multiplexed imaging of 21 markers on a TMA with 112 cores (**Supplementary Table**
161 **1**) and quantified the data at single-cell resolution from 44 HGSCs (**Fig. 1a**). We annotated and categorized
162 the single cells to tumor, immune and stroma, and subsequently to functional cell subpopulations. The tumor
163 microenvironment (TME) compositions, cellular diversity and functional states were associated to patient
164 clinical outcomes. The clinical characteristics of the patients are shown in **Table 1**. As expected, the platinum-
165 free interval (PFI), calculated as the time from last platinum-based chemotherapy to tumor relapse, was

166 significantly longer in patients with *BRCA1/2*mut tumors as compared to patients with HRwt tumors in this
167 patient cohort ($p=0.02$, log-rank test, **Fig. S1c**). Overall survival (OS) was not significantly different between
168 the groups ($p=0.3$, log-rank test, **Fig. S1d**).

169 The markers used for annotating the cell types in the TME are shown in **Fig. S1b**. Out of all 124,623 cells,
170 70% were classified as tumor cells, 15% immune cells, 12% stromal cells and 3% remained unannotated
171 (Images, cell segmentation masks, and single-cell data can be found at DOI: [10.7303/syn23747228](https://doi.org/10.7303/syn23747228)). As
172 expected, each tumor was characterized by a unique TME composition, with tumor cells being the dominant
173 cell type in 40 out of 44 patients (**Fig. 1b**). UMAP shows that the tumor cells formed patient-specific clusters,
174 whereas the immune, stromal and endothelial cells formed more common clusters between patients (**Fig. 1c**
175 **and Fig. 1d**). To further characterize the tumor cells according to their functional states, we classified the
176 85,621 tumor cells into seven metaclusters using minimum spanning trees (**Fig. S3a**). The two most abundant
177 metaclusters were proliferating epithelial and epithelial cells, followed by EMT and proliferating EMT
178 phenotypes (**Fig. 1e**), and each sample contained cells belonging to a minimum of five different tumor
179 metaclusters (**Fig. 1f**).

180 We next classified the 19,697 immune cells into distinct immune-cell subtypes. Of the immune cells,
181 IBA1+CD163+ macrophages were among the most abundant, followed by CD8+T-cells (**Fig. 1g**). Each
182 immune cell subtype showed consistent marker expression profiles (**Fig. S1e**) and visual clustering (**Fig. 1h**),
183 underscoring the unique phenotypes of these immune cell populations. We observed significant correlations
184 between the proportions of CD3+ and CD8+T-cells in the imaging-based single-cell data and the pathological
185 scoring from conventional immunohistochemistry evaluated from fields selected for high CD3+T-cell content
186 providing technical validation of the cell type identification (**Fig. S1h-j**).

187 We next classified the 15,402 stromal cells into nine metaclusters using functional markers and morphological
188 features (**Fig. 1j, Fig. S1f**). In contrast to tumor- and immune compositions, the stromal cells showed a
189 heterogeneous composition without a dominant metacluster in any tumor (**Fig. 1j**). However, a subset of the
190 patients formed a cluster characterized by an enriched proportion of proliferative stromal cells (**Fig. S1g**).
191 Interestingly, the high-proliferative stromal cells were projected furthest from high-vimentin stromal cells in
192 UMAP (**Fig. 1i**), underscoring the differential marker profiles of the proliferative activated fibroblast-like

phenotype from the normal activated fibroblast-like phenotype. Furthermore, the *BRCA1/2*mut tumors had a higher overall ratio of tumor cells to stromal cells as compared to the HRwt tumors (**Fig. S1k**).

195

Distinct immune cell subtypes associate with immune diversity and clinical outcomes in HGSC HR-genotypes

Profiling of the immune cell subpopulations revealed highly variable immune compositions across the patients. Using hierarchical clustering of the proportions of different immune cell subtypes out of all immune cells, we observed that a cluster of tumors harboring an IBA1+CD163+ macrophage –dominant immune composition was almost exclusively *BRCA1/2* mutated (**Fig. 2a**). Similarly, the *BRCA1/2*mut tumors were enriched in a cluster with B-cell dominant immune microenvironment (**Fig. 2a**).

Consistently, the *BRCA1/2*mut tumors exhibited a significantly higher infiltration of IBA1+, CD163+ and IBA1+CD163+ macrophages as compared to the HRwt tumors (**Fig. 2b, Fig. S2a**). Additionally, we noted a trend of increased infiltration of CD8+T-cells in the *BRCA1/2*mut tumors when compared to the HRwt (**Fig. S2b**, $p=0.056$). Deeper analysis of the immune compartment of the tumors revealed distinct myeloid cell profiles with the HRwt tumors exhibiting increased proportions of CD11c+ antigen presenting cells (CD11c+ APC), and IBA1+CD163+CD11c+ macrophages out of all immune cells as compared to *BRCA1/2*mut tumors (**Fig. 2c and Fig. 2d**). These findings led us to explore the ratios of CD8+ T-cells to macrophages and antigen-presenting cells in the TME of the different HR-genotypes. Consistently, we observed an increased ratio of CD8+T-cells to both IBA1+CD163+CD11c+ macrophages (**Fig. 2e**) and to IBA1+CD11c+ macrophages in the *BRCA1/2*mut tumors as compared to the HRwt tumors (**Fig. S2c**), indicative of enriched T-cell mediated immunosurveillance over CD11c+ myeloid cell populations in the *BRCA1/2*mut tumors as compared to the HRwt tumors.

215

We next examined whether the proportions of the different immune cells associate with PFI or OS using a median cut-off for high or low proportions of the immune cell infiltration out of all cells. Consistent with previous findings ^{12,13}, higher CD8+T-cell infiltration was associated with longer PFI in all patients (**Fig. 2g**,

219 p=0.015, log-rank test), but not with OS. However, this difference was not significant when the analysis was
220 limited to only patients with *BRCA1/2*mut or HRwt tumors (**Fig. S2d and S2e**). Interestingly, a higher
221 infiltration of CD4+T-cells was associated with longer PFI, also separately in patients with *BRCA1/2*mut
222 tumors (**Fig. 2h**, p=0.0011, log-rank test, HR 0.26, 95% CI 0.10-0.66, **Fig. S2f**). This finding was not
223 significant in patients with HRwt tumors (**Fig. S2g**). Additionally, a high infiltration of FOXP3+CD4+T-
224 regulatory cells was associated with an improved PFI in all patients as well as in patients with *BRCA1/2*mut
225 tumors, and a high infiltration of IBA1+CD163+ macrophages in all patients (**Fig. S2h-2m**). However, in
226 multivariate Cox regression models in patients with *BRCA1/2*mut tumors and in all patients pooled using the
227 significant cell types from Kaplan-Meier analysis as variables, only CD4+T-cells were significant and
228 independently associated with PFI (**Supplementary Table 2**).

229

230 We next calculated a measure of intratumoral diversity for immune cell subtypes using Simpson's diversity
231 index (SDI). Overall, the SDI for immune cells was higher in the HRwt tumors as compared to the
232 *BRCA1/2*mut tumors (**Fig. 2h**), and the SDI did not correlate with the total immune cell infiltration (data not
233 shown). In the *BRCA1/2*mut tumors, the SDI for immune cells correlated positively with the proportions of
234 IBA1+CD163+CD11c+ (**Fig. 2j**) and IBA1+CD11c+ macrophages and CD11c+ APCs (**Fig. S2n and S2o**),
235 suggesting that a more diverse immune microenvironment associates with increased presence of cells with
236 antigen presentation capacity. By contrast, SDI for immune cells correlated negatively with IBA1+CD163+
237 macrophage proportions out of immune cells in both of the tumor HR-genotypes (**Fig. 2i**). Immune diversity
238 was however not associated with PFI in the two clinical groups (**Fig. S2 p-r**).

239

240 Further investigation of the immune suppressive signals in the TME revealed an overall higher expression of
241 PD-L1 in the macrophage and antigen presenting cell subtypes as compared to stromal ($p < 2.2 \times 10^{-16}$) and tumor
242 cells ($p < 2.2 \times 10^{-16}$) (**Fig. S2s**). Importantly, the PD-L1 expression was significantly higher in the CD11c+ APC
243 and IBA+CD11c+ macrophages in the *BRCA1/2*mut, and in IBA1+CD163+ macrophages and
244 IBA1+CD163+CD11c+ macrophages in the HRwt tumors, indicating enhanced immunosuppressive properties

245 of M2-type (defined as CD163+) macrophages in the HRwt tumors. Furthermore, the expression of PD1 was
246 significantly higher in CD8+T-cells, CD4+T-cells, and FOXP3+CD4+T-regulatory cells in *BRCAl/2mut* as
247 compared to HRwt tumors (**Fig. S2t**). Functional marker expressions for the immune cell subtypes are
248 presented in **Supplementary Figure 2u**.

249

250 **Functional states of tumor cell metaclusters associate with distinct outcomes in the HR-genotypes**

251 We next examined the tumor single-cell phenotypes using tumor cell metaclusters annotated by their functional
252 marker expression. In hierarchical clustering of the proportions of tumor cell metaclusters out of all tumor
253 cells, epithelial and proliferating epithelial metaclusters clustered together whereas the EMT and proliferating
254 EMT formed a unique cluster, suggesting that either epithelial or EMT state is favored within each tumor (**Fig.**
255 **3a**). By visual inspection of a minimum spanning tree constructed from the original cell clusters, proliferating
256 epithelial cell clusters and proliferating EMT cell clusters were separated from epithelial and EMT cell clusters
257 into opposite directions (**Fig. 3b**), underscoring the differential proliferative states between these groups (**Fig.S**
258 **3a**). The functional marker expression of each metacluster is presented in **Figure 3c**.

259 Interestingly, the *BRCAl/2mut* tumors contained overall an increased proportion of proliferating epithelial
260 cells out of all cells as compared to the HRwt tumors (**Fig. 3d**). Moreover, the HRwt tumors contained an
261 increased proportion of EMT cells out of all cells as compared to the *BRCAl/2mut* tumors, however the
262 difference in the total EMT proportion out of all cells was not significant when taking into account also
263 proliferating EMT cells. (**Fig. S3b and S3c**). A subset of patients displayed a dominantly mesenchymal or
264 epithelial phenotype as their most abundant tumor metacluster, which associated with low tumor cell
265 metacluster SDI (**Fig. 3a**). The tumor cell metacluster proportions did not associate with clinical outcomes
266 (data not shown). The tumor SDI was similar between the HR genotypes, and did not correlate with the
267 proportion of tumor cells out of all cells (data not shown), or PFI (**Fig. S3d-f**).

268

269 Interestingly, the functional states of the tumor cells were differentially associated with PFI in the HR-
270 genotypes (**Fig. S3g**). Notably, the expression of the proliferation marker Ki67 affected PFI in an opposite

direction in the *BRCA1/2*mut tumors as compared to the HRwt tumors (**Fig. 3e and 3f**). In Kaplan Meier analysis stratified by the highest 1/3rd of median Ki67 expression in the proliferating epithelial cells, a high expression of Ki67 was associated with a prolonged PFI (**Fig. 3g**, $p = 0.029$, log-rank, HR 0.32, 95% CI 0.11-0.94) in patients with *BRCA1/2*mut tumors. By contrast, we observed an opposite trend in patients with HRwt tumors, where a low expression of Ki67 in proliferating epithelial cells was associated with a better PFI (**Fig. S3h**, $p = 0.013$, log-rank, HR 0.10 95% CI 0.10-0.90).

HR genotypes show distinct co-occurrence profiles in the tumor microenvironment

To further investigate the interactions of the tumor metaclusters, immune subtypes and stromal metaclusters in the HR-genotypes, we plotted the correlations between the proportions of cell types separately for *BRCA1/2*mut and the HRwt tumors (**Fig. 4a**). Overall, in the *BRCA1/2*mut tumors, the tumor metaclusters had more significant correlations with immune subtypes than did stromal cell metaclusters. By contrast, in the HRwt tumors, stromal cell metaclusters had more significant correlations with immune subtypes than did tumor cell metaclusters, suggesting distinct shaping of the immune microenvironment in the HR-genotypes.

Overall, the proportions of CD8+T-cells and CD4+T-cells were highly correlated with each other in both of the HR-genotypes, and had similar correlations with other cell types (**Fig. 4a**). In the *BRCA1/2*mut tumors, CD8+T-cells and CD4+T-cells correlated positively with IBA1+CD11c+ macrophages, but CD8+T-cells correlated also positively with FOXP3+CD4+T-regulatory cells, suggestive of simultaneous immune-stimulatory and suppressive environments. Interestingly, in the *BRCA1/2*mut tumors, proliferating epithelial cells correlated negatively with the proportions of CD8+T-cells and CD4+T-cells. Moreover, high-vimentin and proliferative stromal cells correlated negatively with CD8+T-cells, suggesting that higher content of these stromal metaclusters also associates with CD8+T-cell exclusion in the *BRCA1/2*mut tumors. In the HRwt tumors, both CD8+T-cells and CD4+T-cells correlated positively with FOXP3+CD4+T-regulatory cells, as well as with IBA1+CD163+ macrophages indicative of concomitant strong immunosuppression. High-vimentin and proliferative stroma correlated negatively with CD4+T-cells, and these correlations were more significant than the stromal associations to CD8+T-cells in the *BRCA1/2*mut tumors, suggesting that the

stromal compartment may contribute to enhanced immunosuppression via CD4⁺T-cell exclusion in the HRwt tumors.

300

To further explore the relationships between the cell types, we computed Principal Component Analysis (PCA)-plots separately for the *BRCA1/2*mut and the HRwt tumors (**Fig. 4b and 4c**). In both the *BRCA1/2*mut and the HRwt tumors, the first principal components were mainly characterized by either high immune infiltration or high stromal infiltration. In the *BRCA1/2*mut tumors, the second principal component was mainly characterized by proliferative epithelial and mesenchymal tumor metaclusters, with proliferating epithelial cells opposing the mesenchymal metacluster cells and the immune subtypes. In the HRwt tumors, the second principal component was characterized by stromal metaclusters and the mesenchymal tumor metacluster, which oppose the proinflammatory IBA1+CD11c⁺ macrophages. The similar direction of the loading vectors of immune cells in the PCA-plots, especially in the *BRCA1/2*mut tumors, suggests a coordinated immune system with simultaneous recruitment of multiple immune cell subtypes. Consistently, the PCA biplot indicated that patients with a long PFI generally harbored tumors with rich immune infiltration, whereas patients with a short PFI were characterized by tumors rich in stromal metaclusters (**Fig. 4d and 4e**).

313

314 **Spatial cell-cell interactions suggest enhanced immunosurveillance in *BRCA1/2* mutated tumors**

To uncover the physical interactions between the cell types, we explored the TME spatial organization by calculating the fraction of neighboring cell types for each single cell, and computed the mean of these fractions per tumor to compare the cellular communities between the *BRCA1/2*mut and HRwt tumors. We observed marked differences in the cell types that predominantly colocalize in the *BRCA1/2*mut as compared to the HRwt tumors (**Fig. 5a**). Consistent with the overall increased proportion in the *BRCA1/2*mut tumors, the proliferating epithelial cells predominantly neighbored tumor metaclusters, immune subtypes and stromal metaclusters in the *BRCA1/2*mut tumors (**Fig. 5a**). By contrast, although the HRwt tumors contained an overall increased proportion of EMT tumor cells, these cells resided in tumor cell rich neighborhoods in the HRwt tumors, and lacked spatial interaction with other cell types in the TME. Furthermore, in the HRwt tumors, the

stromal metaclusters predominantly neighbored other stromal metaclusters more frequently as compared to the *BRCA1/2*mut tumors, potentially indicative of stromal barriers. Together, with the higher stromal to tumor ratio, these findings suggest a more compartmentalized TME in the HRwt tumors, with a more prominent role of the stromal cells.

328

Interestingly, the proliferating epithelial cells, being the most abundant tumor cell metacluster, were more often surrounded by CD4+T-cells and also endothelial cells in the *BRCA1/2*mut tumors (**Fig. 5a**). Moreover, the proliferating epithelial cells were neighboring multiple immune cell subtypes, including distinct myeloid subpopulations, and CD4+ and CD8+T-cells, significantly more often than in the HRwt tumors. Importantly, the CD4+T-cells resided predominantly in cellular communities rich of proliferating epithelial cells and other CD4+T-cells in the *BRCA1/2*mut tumors, whereas in the HRwt tumors the CD4+T-cells were more often neighbored by FOXP3+CD4+T-regulatory cells and high-proliferative stromal cells. By contrast, in the *BRCA1/2*mut tumors, the FOXP3+CD4+T-regulatory cells were residing in stromal niches and surrounded by high-vimentin stromal cells. Simultaneously, the CD8+T-cell neighborhoods were enriched for proliferating epithelial tumor cells in the *BRCA1/2*mut tumors, and in CD11c+ APCs in the HRwt tumors.

339

Additionally, we observed distinct spatial interactions between the myeloid cells and other cells of TME. In the *BRCA1/2*mut tumors, specific macrophage communities were enriched in proliferating epithelial cells. The IBA1+ macrophages, and the M2-type IBA1+CD163+ macrophages neighbored specific stromal compartments in the *BRCA1/2*mut tumors. By contrast, the antigen-presentation capable CD11c+ APCs, and IBA1+CD163+CD11c+ macrophages had predominantly stromal interactions in the HRwt tumors. Notably, the CD11c+ APCs were dominating the neighborhoods of both FOXP3+CD4+T-regulatory cells and CD8+T-cells in HRwt tumors. Altogether, the distinct spatial cellular communities suggest enhanced immunosurveillance of proliferating epithelial tumor cells by the CD4+ and CD8+T-cells in the *BRCA1/2*mut tumors, and immune-suppressive stromal interactions in the HRwt tumors.

349

350 **Distinct functional states of proliferating epithelial tumor cells associate with immune cell spatial**
351 **interactions in *BRCA1/2* mutated HGSCs**

352 The prognostic roles of CD8⁺ and CD4⁺T-cells, and their observed enhanced spatial interactions with
353 proliferating epithelial cells in the *BRCA1/2*mut tumors prompted us to investigate whether Ki67 expression
354 in proliferating epithelial tumor cells is associated with enhanced cell-to-cell spatial interactions. We compared
355 the Ki67 expression in the proliferating epithelial tumor cells neighbored by CD8⁺ or CD4⁺T-cells as
356 compared to cells without these cells as neighbors (**Fig. 5b**). Interestingly, we observed a higher Ki67
357 expression in the proliferating epithelial cells with one or more CD8⁺T-cells as neighbor as compared to cells
358 without any CD8⁺T-cells as neighbors in the *BRCA1/2*mut tumors, but not in the HRwt tumors (**Fig. 5c, Fig.**
359 **S4a**). A similar phenomenon was observed in proliferating epithelial tumor cells with CD4⁺T-cells as
360 neighbors in patients with *BRCA1/2*mut but not with HRwt tumors (**Fig. 5d, Fig. S4b**). Collectively, these
361 findings suggest that in the *BRCA1/2*mut tumors, high Ki67 expression in proliferating epithelial cells results
362 in improved immunosurveillance, with enhanced cell to cell interaction with CD4⁺T-cells and CD8⁺T-cells,
363 whereas in the HRwt tumors the high-proliferative tumor cells are not spatially interacting with CD4⁺ or
364 CD8⁺T-cells. These distinct spatial tumor-immune interactions provide potential insights into the mechanisms
365 driving the differential prognostic impact of Ki67 expression in proliferative epithelial cells in tumors with
366 different HR-genotypes.

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373 **Discussion**

374 Lack of detailed information on the interplay of tumor, stromal and immune cells in the tumor
375 microenvironment in HGSC has been lacking. Herein, we aimed to shed more light into how *BRCA1/2*
376 mutations and single-cell tumor phenotypes shape the immune microenvironment and prognosis in HGSC.
377 Using imaging-based single-cell data, we captured and annotated 124,623 cells of the tumor, stromal and
378 immune cell populations in the TME of 44 HGSC patients. We discovered distinct immune microenvironments
379 and divergent interactions between the immune subtypes and tumor and stromal metaclusters in tumors with
380 *BRCA1/2*mut as compared to HRwt tumors. Interestingly, we found opposing prognostic roles of proliferative
381 functional states of epithelial tumor cells in patients with *BRCA1/2*mut and HRwt tumors, which also
382 associated with distinct cellular neighborhoods. The graphical summary of the distinct TME phenotypes in the
383 *BRCA1/2*mut and the HRwt tumors is presented in **Figure 6**.

384

385 Single-cell immunophenotyping revealed divergent immune cell compartments in the HR genotypes.
386 Consistent with previous observations from syngeneic mouse models⁷, the *BRCA1/2*mut tumors contained an
387 increased infiltration of IBA1+ and M2-type IBA1+CD163+ and CD163+ macrophages, whereas the HRwt
388 tumors had abundant CD11c+ myeloid cells. The myeloid cells also showed abundant and distinct PD-L1
389 expression profiles between the HR genotypes, with the highest expression in the CD11c+ cells in the
390 *BRCA1/2*mut tumors and M2-type macrophages in the HRwt tumors. Overall, the infiltration of CD8+ and
391 CD4+T-cells correlated positively with the infiltration of various macrophages, as suggested also previously¹⁴.
392 Further, consistent with previous reports^{6,7,15}, we observed a trend towards increased CD8+ T-cell infiltration
393 in the *BRCA1/2*mut tumors, and a balance favoring CD8+T-cell infiltration over infiltration of multiple
394 myeloid cell types in the in the TME of *BRCA1/2*mut tumors, suggesting enhanced immunosurveillance
395 capacity via CD8+ T-cells. Importantly, the CD8+T-cells were characterized by a higher PD-1 expression in
396 the *BRCA1/2*mut tumors implicating potentially enhanced susceptibility to PD-1/PD-L1 targeted immune
397 checkpoint therapies. By contrast, the lower CD8+T-cell infiltration patterns strengthen the notion that T-cell
398 exclusion is more common in the HRwt tumors.

Investigation of the prognostic roles of the immunophenotypes revealed that a higher infiltration of both CD8⁺T-cells and CD4⁺T-cells were associated with prolonged responses to platinum-based chemotherapy, consistent with previous findings^{12,13}. Importantly, in multivariable analysis the CD4⁺T-cell infiltration displayed an independent prognostic role, also in patients with *BRCA1/2*mut tumors. Interestingly, the CD4⁺T-cells had more immune stimulatory correlations with CD11c⁺ myeloid cells and B-cells in the *BRCA1/2*mut tumors. In addition, the proportions of CD11c⁺ antigen presenting cells correlated with increased diversity of other immune cells, and the co-occurrence of tumor infiltrating T-cells, B-cells, and antigen presenting cells suggested a more coordinated immune cell activity in the *BRCA1/2*mut tumors. Further, spatial analysis revealed enhanced immunogenic interactions of the CD4⁺ and CD8⁺T-cells in the *BRCA1/2*mut tumors, whereas in the HRwt tumors the CD4⁺T-cells were more often interacting with immunosuppressive FOXP3⁺CD4⁺T-regulatory cells. Altogether, these findings suggest a coordinated, immune-stimulatory role for CD4⁺T-cells in the *BRCA1/2* mutated HGSCs.

412

Lack of single-cell resolution on large-enough clinical cohorts has hampered the in-depth investigation on the role of tumor cell subpopulations and their functional states on patient prognosis. Previous studies have reported increased proliferative states associated with *BRCA1* mutation in breast^{16,17}, and ovarian tumors¹⁸. However the reports on the potential prognostic role of high Ki67 expression in HGSC have remained conflicting¹⁹⁻²¹. In the present study, the proliferating epithelial cells formed the most abundant tumor cell subpopulation, which was further enriched in the *BRCA1/2*mut tumors. Interestingly, a high Ki67 expression in proliferating epithelial cells conveyed an opposing prognostic effect in the HR-genotypes; high expression of Ki67 in the proliferating epithelial cells associated with a longer PFI in patients with *BRCA1/2*mut tumors, but with a trend for shorter PFI in the HRwt tumors. This difference can be, at least in part, explained by the differential platinum sensitivity due to HR-deficiency: the highly proliferating epithelial cells are likely to be more sensitive to platinum-based chemotherapy in the *BRCA1/2*mut tumors as compared to the HRwt tumors. Thus, using single-cell resolution information in tumors stratified by the predicted HR capacity, we were able

425 to resolve the previously conflicting conclusions, and revealed potentially clinically significant role of tumor
426 cell functional proliferative state with the premise to promote the development of prognostic and predictive
427 biomarkers for HGSC.

428

429 Our unique single-cell dataset enabled further characterization of the associations of the cellular functional
430 states and their spatial interactions. Analysis of the cellular neighborhood frequencies revealed a higher
431 proliferative state of the epithelial cells residing in CD4+ or CD8+T-cell cellular communities in the
432 *BRCA1/2*mut tumors. By contrast, the epithelial cells with a higher proliferative state were residing outside of
433 the CD4+ or CD8+T-cell neighborhoods in the HRwt tumors. Notably, the proportions of proliferating
434 epithelial tumor cells correlated negatively with CD8+ and CD4+T-cell infiltration, as suggested also by a
435 previous report ¹⁴, indicating that the immune surveillance of CD8+ and CD4+T-cells towards the highly
436 proliferating epithelial cells in the *BRCA1/2*mut tumors is regulated by spatial attraction and not just by cellular
437 abundance. Our single-cell spatial TME analysis thus suggests that these tumor-immune spatial communities
438 further contribute to the prognostic roles of T-cell infiltration and tumor cell proliferative states observed in
439 the HR-genotypes.

440

441 The role of the stromal cells in HGSC genotypes has also remained elusive^{14,22}. We observed that the HRwt
442 tumors contained an increased stromal to tumor ratio, and significant intercorrelations of the stromal
443 metaclusters with the immune cell subpopulations, suggesting enhanced stromal contribution to the
444 immunophenotypes and T-cell exclusion. Further, the spatial analysis of the TME revealed significant stromal-
445 to-stromal metacluster spatial interactions in the HRwt tumors, suggesting the presence of physical and
446 biochemical barriers which can further promote therapy resistance^{14,23,24}. Moreover, we found that the HRwt
447 tumors contained an increased proportion of tumor cells with an EMT phenotype without tumor-immune
448 spatial interactions, which also could contribute to chemoresistance²⁵⁻²⁷. Altogether, the stromal dominance,
449 tumor cell EMT functional states, and decreased immune surveillance can contribute to the poor prognosis of
450 the HRwt tumors.

451

452 We acknowledge certain limitations of the study. Our patient cohort consisted of a limited number of HRwt
453 tumors as compared to the *BRCA1/2*mut tumors, rendering the conclusions made on the *BRCA1/2*mut tumors
454 stronger. Although the TME analysis using a tissue microarray minimizes experimental variation, it poses a
455 potential bias on the conclusions regarding tumor heterogeneity as only a maximum of three 1mm cores can
456 be examined, and thus we had to refrain from performing permutation-based spatial analyses and focus on the
457 communities based on neighbor fractions. Further, the experimental design requires the application of a limited
458 set of protein-based markers, thus leaving a small proportion of the cells (average 5% per tumor) unannotated.
459 In addition, the pioneering nature of the work has hampered availability of similar cohorts or datasets, and thus
460 validation in an external cohort, preferably using an unbiased method such as single-cell RNAsequencing,
461 would be needed to strengthen the conclusions of the study.

462

463 In conclusion, single-cell analysis highlights the functional and spatial differences in the tumor
464 microenvironment landscapes of *BRCA1/2*-deficient and HRwt tumors, underscoring the need for differential
465 treatment strategies for these clinical subgroups. Uncovering the effects of tumor genotypes on the TME,
466 cellular phenotypes and spatial communities will enable the development of more effective immunotherapeutic
467 strategies and improved patient stratification in HGSC.

468

469 **Author contributions**

470 I-M.L analyzed the data and wrote the manuscript; N.L, J.C., E.A.A and A.S analyzed the data; C.A.J. and
471 J.R.L performed the experiments and wrote the manuscript; U-M.H, Z.M; S.S; K.A; A.D.A; P.A.K and P.K.S
472 provided materials and resources, supported the data analysis and wrote the manuscript; A.F conceived and
473 supervised the study wrote the manuscript.

474

475 **Competing interests statement**

476 The authors report no competing interests.

477 **Data availability**

478 The source data for Figures 1, 2, 3, 4, and 5, and for Supplementary Figures 1, 2, 3, and 4 are provided in
479 Synapse at DOI: [10.7303/syn23747228](https://doi.org/10.7303/syn23747228), along with highly multiplex 21 channel images and cell segmentation
480 masks.

481 **Code availability**

482 Code used in this study for the multiplexed imaging single-cell data analysis are available at
483 <https://github.com/farkkilab/TMA-project>.

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Figure 1

Fig. 1 Multiplexed imaging reveals tumor microenvironment composition at single-cell resolution

a A schematic representation of the workflow, from tumor tissue microarray (TMA) composition and high-dimensional imaging to cell type calling, population description, spatial and functional analysis, and clinical correlations. Using a TMA, a total of 112 cores from 31 patients with *BRCA1/2*mut tumors and 13 patients with tumors without any mutations in HR genes were imaged with 21 markers. After image processing, labels from cell type calling were used for description of immune subtypes as well as tumor and stromal metaclusters. These profiles were in turn used in diversity analysis. The spatial and functional states of cell types were analyzed and correlations with clinical information were evaluated. **b** Annotated barplot of the tumor, immune, stromal, and endothelial cell composition per patient, showing high tumor content across patients. The barplot is sorted by decreasing tumor proportion. Clinical annotations for barplots include HR status and PFI, with a PFI of <12 months annotated as short and > 12 months long. **c** Semi-supervised UMAP visualizing tumor, immune, stromal, and endothelial cells as distinct populations and **d** colored by their patient of origin. The most abundant subtype were tumor cells, followed by stromal, immune, and endothelial cells. **e** Annotated barplot describing the tumor metacluster composition across different patients and clinical groups, showing heterogeneity across patients. The barplot is sorted by decreasing proliferating epithelial cell proportion. **f** Semi-supervised UMAP visualizing the tumor cell metaclusters as distinct populations. **g** Annotated barplot describing the immune subtype composition across different patients and clinical groups, with a heterogeneous immune composition between patients. The barplot is sorted by decreasing IBA1+CD163+ macrophage proportion. **h** Semi-supervised UMAP visualizing the immune cells as distinct populations, with cells of lymphoid and myeloid lineages grouping to the opposite sides of the UMAP. **i** Annotated barplot describing the stromal metacluster composition across different patients and clinical groups, showing less heterogeneity across patients than tumor metaclusters and immune cell subtypes. The barplot is sorted by decreasing non-proliferative stroma content. **j** Semi-supervised UMAP visualizing the stromal cell metaclusters as distinct populations.

Figure 2

Fig. 2 Distinct immune microenvironments associate with immune diversity and clinical outcomes in HGSC

a A hierarchical clustering heatmap of immune subtype proportions out of all immune cells reveals distinct immune-microenvironments, annotated with immune diversity (immune SDI), and clinical data including HR status and PFI. The barplot annotations for the columns and rows represent the total number of cells per each immune cell subtype and the number of the immune cells in total per patient, respectively. **b** Boxplot showing the combined proportion of IBA1+, CD163+ and IBA1+CD163+ macrophages as a proportion of all cells, stratified by HR status. **c** Boxplot showing the increased proportion of CD11c+ antigen-presenting cells as well as **d** increased proportion of IBA1+CD163+CD11c+ macrophages as a proportion of all immune cells in HRwt as compared to *BRCA1/2*mut tumors **e** Boxplot showing increased ratio of CD8+T-cells to IBA1+CD163+CD11c+ macrophages in *BRCA1/2*mut as compared to HRwt tumors. **f** Boxplot showing higher immune diversity in HRwt as compared to *BRCA1/2*mut tumors. The differences between the boxplot groups were calculated by Wilcoxon signed-rank test. The black horizontal lines represent the sample medians, the boxes extend from first to third quartile and whiskers indicate the values at 1.5 times the interquartile range. Individual dots represent values per each tumor. **g** Kaplan-Meier graphs for PFI show improved PFI for high CD8+T-cell proportion in all tumors pooled as well as **h** for high CD4+T-cell proportion in patients with *BRCA1/2*mut tumors. Proportions were calculated out of all cells, and the median value was used to distinguish high and low groups. Number of patients at risk is shown at the bottom of each Kaplan-Meier graph. The comparisons between the groups were performed using the log-rank test. **i** Scatter plots show negative linear correlation of immune diversity and the proportion of IBA1+CD163+ macrophages in all HR groups and **j** a positive linear correlation of immune diversity and the proportion of IBA1+CD163+CD11c+ macrophages in *BRCA1/2*mut tumors. Proportions were calculated out of immune cells and Spearman correlation coefficients and their p-values are shown. HR groups include *BRCA1/2*mut (blue), HRwt (red) tumors and all tumors pooled (black).

Figure 3

Fig. 3 Tumor metacluster functional states associate with clinical outcomes in *BRCA1/2*mut tumors

a A hierarchical clustering heatmap of tumor metacluster proportions out of all tumor cells shows distinct tumor microenvironments, annotated with tumor SDI representing tumor diversity, and clinical data including HR status and PFI. The barplot annotations for the columns and rows represent the total number of cells per each tumor metacluster and the number of tumor cells in total per patient, respectively. **b** Minimum-spanning tree of tumor cell clusters with circles visualizing the assigned metaclusters based on median marker expression profiles. Smaller metaclusters are marked with letters. **c** Heatmap of tumor metaclusters showing distinct expression levels (Z-score) of the markers in the metaclusters. Rows of the heatmap are sorted by metacluster abundance. **d** Boxplot showing the increased proportion of proliferating epithelial cells as a proportion of all cells in *BRCA1/2*mut as compared to HRwt tumors. The differences between the groups were calculated by Wilcoxon signed-rank test. Boxplots visualize the sample medians, first to third quartile and the values at 1.5 times the interquartile range. Individual dots represent values per each tumor. **e** Dot plot of fold changes ($\log_2$) of functional marker expression in tumor metaclusters in *BRCA1/2*mut tumors, between long and short PFI and **f** in HRwt tumors between long and short PFI. The color of the dots represents the fold change and their size the significance of the p-value. Fold changes with p-values <0.05 are shown. Blue boxes highlight the differential Ki67 expression in proliferating epithelial cells in long and short PFI tumors, in the different HR genotypes. **g** Kaplan-Meier graphs showing high Ki67 expression in proliferating epithelial cells associates with an improved PFI in patients with *BRCA1/2*mut tumors. Patients belonging to the top 1/3 percentile in all tumors in median Ki67 expression were annotated as high and the rest as low. Number of patients at risk is shown at the bottom of each Kaplan-Meier graph. The comparisons between the groups were performed using the log-rank test.

Figure 4

Fig. 4 HR-genotypes show different patterns of cell type co-occurrence with CD8+T-cells and CD4+T-cells

a A Spearman correlation dot plot between tumor metaclusters, immune subtypes and stromal metaclusters as proportions out of all cells, divided by HR status. Dots in white background and light-red background represent correlations in *BRCA1/2*mut and HRwt tumors, respectively. Dots shaded red represent positive correlations while dots shaded blue represent negative correlations. Black lines separate the tumor, immune and stromal cell columns and rows. Blue dashed boxes highlight the interactions that CD4+T-cells and CD8+T-cells have with other cell types. Correlations with a p-value <0.05 are shown. **b** Principal component feature projections for *BRCA1/2*mut tumors and **c** for HRwt tumors. 12 cell types with the highest contributions to PC1 and PC2 are shown for each plot. The more intense the color of the cell type, the higher its contribution is. **d** Plot of patients with *BRCA1/2*mut tumors and **e** plot of patients with HRwt tumors projected onto their first two principal components. Patients are annotated as having a long or short PFI and colored by light blue or dark blue, respectively. Ellipsoids show the 95% confidence intervals for the PFI groups.

Figure 5

Fig. 5 Proliferative state delineates spatial cell-cell interactions of the tumor-immune microenvironment in *BRCA1/2*mut and HRwt tumors

a Dot plot showing the fold change ($\log_2$) between *BRCA1/2*mut and HRwt tumors of the mean proportions of different cell types in the neighborhood (x-axis) of each centering cell type (y-axis). Orange colored dots represent more frequently occurring cellular neighborhoods in *BRCA1/2*mut as compared to HRwt tumors, while blue dots represent more frequently occurring cellular neighborhoods in HRwt as compared to *BRCA1/2*mut tumors. The size of the dot represents the significance of the p-value. P-values were calculated with Wilcoxon signed-rank test. Only fold changes with a p-value of < 0.05 are shown. Black lines separate tumor metaclusters, immune subtypes and stromal metaclusters from each other. Red dashed boxes highlight specific interactions. **b** A schematic illustration of functional state and spatial cell-cell interaction analysis. Proliferating epithelial cells were each characterized by their CD4+T-cell and CD8+T-cell spatial neighborhood status. Next, Ki67 marker expression was compared in proliferating epithelial cells with different cell-cell spatial neighborhoods in *BRCA1/2*mut and HRwt tumors separately. **c** Violin plots showing the probability densities of Ki67 expression in proliferating epithelial cells in the different CD8+T-cell neighborhood and HR-groups. **d** Violin plots showing the probability densities of Ki67 expression in proliferating epithelial cells in the different CD4+T-cell neighborhood and HR-groups. In the box plots inside violin plots the black horizontal lines represent the sample medians, the boxes extend from first to third quartile and whiskers indicate values at 1.5 times the interquartile range. Wilcoxon signed-rank test was used for pairwise comparisons. P-values: $<0.05 = *$, $< 0.005 = **$, $<0.0005 = ***$, $<0.00005 = ****$.

Figure 6

Fig. 6 Graphical summary of the differences in the TME of *BRCA1/2*mut and HRwt tumors

A schematic representation of the differences in cell type infiltration and their spatial interactions in the *BRCA1/2*mut and HRwt tumors, characterized by proliferating epithelial and EMT tumor cells, macrophages and antigen presenting cells, as well as the spatial interactions of CD8+T-cells and CD4+T-cells with differently Ki67 expressing proliferating epithelial cells and other immune cells. Curved arrows represent a spatial interaction.

Tables

Table 1. Summary of patient clinical characteristics

Variable	Category	mean	median	range
Age at diagnosis (years)	All patients	58.1	59.1	42.2-80.0
	<i>BRCA1/2</i> mutated	55.2	53.7	42.2-75.6
	HRwt	65	64.3	49.1-80.0
PFI (months)	All patients	39.4	14.3	0.7-271.7
	<i>BRCA1/2</i> mutated	49.5	24	6.2-271.7
	HRwt	15.3	6.9	0.7-110.4
OS (months)	All patients	66.4	44.6	5.8-276.7
	<i>BRCA1/2</i> mutated	82.1	53.3	7.5-276.7
	HRwt	29	17.9	5.8-116.3