Supplementary Materials for

Quantitative Spatial Profiling of Immune Populations Reveals Prognostic Significances in Patients with Pancreatic Ductal Adenocarcinoma

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**Figure. S1**

**Diagram

Description automatically generated**

**Gating strategy used for classifying cell types**. **A,** Manual gating was performed in FCS Express Image Gating Cytometry RUO with gated cell selections overlaid on signal to visually validate classification. Text boxes label each cell population, while red boxes indicate backgated populations. Segmented cells were gated through a hierarchy starting with CD45 to identify leukocytes. B cells were identified by CD45+ CD3- CD20+ CD8- marker expression. CD8 T cells were classified by CD45+ CD3+ CD8+ and also backgated from CD3-/CD20+ and CD3-/CD20- cell populations. CD4 T cells were classified by CD3+ CD8- CD20- PanCK- expression. Myelo-monocytic cells were classified as cells with CD45+ CD3- CD20- CD68+ expression. Neoplastic epithelial cells were identified by CD45- PanCK+ and also backgated on the CD3+/CD8- (CD4 T cell) population, as well as the CD45+ CD3- CD20- CD68- population. The gate labeled, CD45+ Other, contained remaining cells that had CD45 expression, but no other markers utilized in this gating strategy. Functional markers (PD1, PDL1, Ki-67, GranzymeB, EOMES, ICOS, IL-10) were classified as positive or negative by thresholding based on the mean intensity of each marker on all cells.

**Figure S2.**

Chart, scatter chart

Description automatically generated

**Correlation analysis between CD4+ T cell density and total immune density as well as macrophage density and total immune density. A and D,** cell densities computed from the entire cohort. **B and C**, cell densities computed from short-term survivors. **G and F,** cell densities computed from long-term survivors.

**Figure S3.**

图表, 散点图

描述已自动生成

Results of **A,** Univariate survival analysis and **B,** multivariate survival analysis on first-order features using Cox regression model. **C,** correlation analysis on discovery cohort between CD8+ T cell density in lymphoid aggregates (LAs) and overall survival (OS) using spearman’s method.

**Figure S4.**

**Diagram, box and whisker chart

Description automatically generated**

**A,** Kaplan-Meier curves revealing overall survival (OS) of patients based on classes defined by treatment strategies. **B,** comparisons between class 1 and 2 patients for each immune population. ns: not significant..

**Figure S5.**

图表, 散点图

描述已自动生成

Correlation analysis on validation cohort (presurgically treated) between CD8+ T cell density in lymphoid aggregates (LAs) and overall survival (OS) using spearman’s method.

**Table S1. Multiplex immunohistochemistry (mIHC) antibody panels**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Primary Antibody** | **Hematoxylin** | **CD68** | **CD45** | **PD-L1** | **CD3/CD20/NKp46** | **Pan-cytokeratin** | **PD-1** | **CD3** |
| RRID | N/A | AB\_306119 | AB\_467274 | AB\_2687655 | See footnotes | AB\_777047 | AB\_881954 | AB\_149922 |
| Clone | N/A | PG-M1 | HI30 | E1L3N | See footnotes | AE1/AE3 | NAT105 | SP7 |
| Vendor | Dako | Abcam | ThermoFisher | Cell Signaling | See footnotes | Abcam | Abcam | ThermoFisher |
| Catalog # | S3301 | ab783 | 14-0459-82 | 13684S | See footnotes | ab27988 | ab52587 | RM-9107-S |
| Concentration | N/A | 1:50 | 1:100 | 1:100 | See footnotes | 1:2000 | 1:50 | 1:150 |
| Incubation | 1 min @ RT | 30 min @ RT | 60 min @ RT | O/N @ 4oC | 30 min @ RT | 30 min @ RT | 30 min @ RT | 30 min @ RT |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Primary Antibody** | **CD8α** | **T-bet** | **CD20** | **CD8α** | **CD138** | **CD38** | **IDO** | **CD4** |
| RRID | AB\_11000353 | AB\_2616022 | AB\_1139386 | AB\_11000353 | AB\_10987019 | AB\_10986743 | AB\_1977098 | AB\_2335982 |
| Clone | C8/144B | D6N8B | Polyclonal | C8/144B | MI15 | 38C03 (SPC32) | IF8.2 | SP35 |
| Vendor | ThermoFisher | Cell Signaling | Abcam | ThermoFisher | ThermoFisher | ThermoFisher | Millipore Sigma | Ventana |
| Catalog # | MA5-13473 | 13232S | ab5694 | MA5-13473 | MA5-12400 | MA5-14413 | MAB10009 | 790-4423 |
| Concentration | 1:100 | 1:500 | 1:200 | 1:100 | 1:20 | 1:100 | 1:100 | 1:4 |
| Incubation | 30 min @ RT | O/N @ 4oC | 60 min @ RT | 30 min @ RT | 30 min @ RT | 30 min @ RT | 30 min @ RT | 30 min @ RT |

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Primary Antibody** | **CD3** | **Granzyme B** | **CD278/ICOS** | **CD27** | **CD5** | **IgD** | **MECA-79** | **EOMES** |
| RRID | AB\_149922 | AB\_304251 | AB\_2827535 | AB\_2827537 | AB\_10985112 | AB\_10974228 | AB\_395099 | AB\_10806889 |
| Clone | SP7 | Polyclonal | SP98 | Polyclonal | 4C7 | EPR6146 | MECA-79 | Polyclonal |
| Vendor | ThermoFisher | Abcam | LifeSpan Bio | Novus Biologicals | ThermoFisher | Abcam | BD Bioscience | Millipore Sigma |
| Catalog # | RM-9107-S | ab4059 | LS-C210350-500 | NBP2-38434 | MA5-13308 | ab124795 | 553863 | AB2283 |
| Concentration | 1:150 | 1:200 | 1:100 | 1:500 | 1:40 | 1:200 | 1:500 | 1:1000 |
| Incubation | 30 min @ RT | 30 min @ RT | 30 min @ RT | 30 min @ RT | O/N @ 4oC | O/N @ 4oC | O/N @ 4oC | 30 min @ RT |
|  |  |  |  |  |  |  |  |  |

|  |  |  |  |
| --- | --- | --- | --- |
| **Primary Antibody** | **IL-10** | **TCRB** | **Ki67** |
| RRID | AB\_1056749 |  | AB\_1158031 |
| Clone | JES3-12G8 |  | SP6 |
| Vendor | LifeSpan Biosciences |  | Millipore Sigma |
| Catalog # | LS-C44484 |  | 275R-14 |
| Concentration | 1:200 |  | 1:500 |
| Incubation | O/N @ 4ºC |  | 30 min @ RT |

**Table S2.** Baseline characteristics of presurgical treatment-naïve cohort.

|  |  |
| --- | --- |
|  | **Neoadjuvant-treated cohort (validation cohort)** |
| **Number of subjects*a*** | 46 |
| **Women, n (%)** | 24 (52%) |
| **Age in years, median (Q1, Q3)** | 63.5 (58.0, 71.0) |
| **Tumor location, n (%)** |  |
| Head/Uncinate | 40 (87%) |
| Body/Tail | 6 (13%) |
| **Tumor size in cm, median (Q1, Q3)*b*** | 3.4 (2.5, 4.5) |
| **AJCC 8th ed. pT stage, n (%)** |  |
| T1 | 7 (15%) |
| T2 | 23 (50%) |
| T3 | 15 (33%) |
| T4 | 1 (2%) |
| Tx (cannot be assessed) | - |
| AJCC 8th ed. pT stage, n (%) |  |
| N0 | 12 (26%) |
| N1 | 15 (33%) |
| N2 | 19 (41%) |
| **Tumor differentiation, n (%)** |  |
| Well/Moderately differentiated | 23 (51%) |
| Poorly differentiated/Undifferentiated | 22 (49%) |
| Unknown | 1 |
| **Resection margin status, n (%)** |  |
| R0 | 20 (44%) |
| R1 | 25 (54%) |
| R2 | 1 (2%) |
| **Lymphovascular invasion, n (%)** |  |
| Negative | 11 (24%) |
| Positive | 34 (76%) |
| Unknown | 1 |
| **Perineural invasion, n (%)** |  |
| Negative | 3 (7%) |
| Positive | 42 (93%) |
| Unknown | 1 |
| **Adjuvant treatment, n (%)** |  |
| None | 5 (11%) |
| Chemotherapy only | 17 (37%) |
| Radiation or chemoradiation only | 4 (9%) |
| Chemoradiation and chemotherapy | 15 (32%) |
| Other/Unknown | 5 (11%) |
| ***SMAD4*, n (%)** |  |
| Lost | 6 (46%) |
| Intact | 5 (38%) |
| ***KRAS*, n (%)** |  |
| Wild-type | 0 |
| Mutant | 22 (100%) |
| Unknown | 24 |
| ***TP53,* n (%)** |  |
| Wild-type | 5 (23%) |
| Altered | 17 (77%) |
| Unknown | 24 |
| ***CDKN2A*, n (%)** |  |
| Lost | 6 (27) |
| Intact | 16 (73%) |
| Unknown | 24 |

*a*Among 45/46 patients with evaluable single-cell data.

*b*Abbreviations: Q1, 25th percentile; Q3, 75th percentile; AJCC, American Joint Committee on Cancer

**Table S3.** Baseline characteristics of presurgically treated cohort.

|  |  |
| --- | --- |
|  | **Neoadjuvant-treated cohort (validation cohort)** |
| **Number of subjects*a*** | 13 |
| **Women, n (%)** | 4 (31%) |
| **Age in years, median (Q1, Q3)*b*** | 61.0 (56.0, 59.0) |
| **Tumor location, n (%)** |  |
| Head/Uncinate | 10 (77%) |
| Body/Tail | 3 (23%) |
| **Tumor size in cm, median (Q1, Q3)** | 3.8 (2.9, 4.4) |
| **AJCC 8th ed. pT stage, n (%)** |  |
| T1 | 3 (23%) |
| T2 | 3 (23%) |
| T3 | 5 (38%) |
| T4 | 2 (15%) |
| AJCC 8th ed. pT stage, n (%) |  |
| N0 | 7 (54%) |
| N1 | 4 (31%) |
| N2 | 2 (15%) |
| **Tumor differentiation, n (%)** |  |
| Well/Moderately differentiated | 4 (31%) |
| Poorly differentiated/Undifferentiated | 6 (46%) |
| Unknown | 3 (23%) |
| **Resection margin status, n (%)** |  |
| R0 | 5 (38%) |
| R1 | 7 (54%) |
| R2 | 1 ( 8%) |
| **Lymphovascular invasion, n (%)** |  |
| Negative | 7 (54%) |
| Positive | 6 (46%) |
| **Perineural invasion, n (%)** |  |
| Negative | 0 (0%) |
| Positive | 13 (100%) |
| **Presurgical treatment, n (%)** |  |
| Chemotherapy only | 6 (46%) |
| Radiation or chemotherapy only | 1 (8%) |
| (Chemo)radiation and chemotherapy | 6 (46%) |
| Unknown | 0 (0%) |
| **Presurgical chemotherapy agent, n (%)** |  |
| FOLFIRINOX | 3 (23%) |
| Gemcitabine/nab-paclitaxel | 7 (54%) |
| Other combination | 3 (23%) |
| Unknown | 0 (0%) |
| **Presurgical chemoradiation agent, n (%)** |  |
| No concurrent chemoradiotherapy | 9 (69%) |
| Gemcitabine/nab-paclitaxel | 1 (8%) |
| 5-FU or capecitabine | 3 (23%) |
| **Histologic response to presurgical/neoadjuvant therapy, n (%)** |  |
| Poor or no response | 3 (23%) |
| Moderate response | 3 (23%) |
| Marked response (minimal residual disease) | 6 (46%) |
| Unknown | 1 (8%) |
| **Adjuvant treatment, n (%)** |  |
| None | 6 (46%) |
| Chemotherapy only | 5 (38%) |
| Radiation or chemoradiation only | 0 (0%) |
| Chemoradiation and chemotherapy | 0 (0%) |
| Other/Unknown | 2 (15%) |

*a*Among 10/13 patients with evaluable single-cell data.

*b*Abbreviations: Q1, 25th percentile; Q3, 75th percentile; AJCC, American Joint Committee on Cancer

**Table S4.** Region of interest (ROI) areas summarized for each patient

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Patient** | **ROI area (mm2)** | | | | | | | | | | | | |
| **ID** | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **mean** |
| **2009** | 1.57 | 0.79 | 1.64 | 11.30 | 20.01 | 14.26 | 18.25 | 29.41 | / | / | / | / | 12.15 |
| **2089** | 29.63 | 40.87 | 34.30 | 14.39 | 14.49 | 10.82 | / | / | / | / | / | / | 24.08 |
| **2117** | 1.00 | 0.98 | 0.70 | 45.20 | 14.74 | 0.43 | 42.20 | 36.01 | 39.08 | / | / | / | 20.04 |
| **2148** | 12.16 | 55.70 | 42.77 | 36.56 | 19.59 | 30.91 | 1.40 | 5.30 | 9.21 | 4.29 | / | / | 21.79 |
| **2170** | 28.06 | 24.55 | 27.07 | 11.73 | / | / | / | / | / | / | / | / | 22.85 |
| **2196** | 0.30 | 0.66 | 0.52 | 37.63 | 37.22 | 42.21 | 31.83 | 23.60 | 14.78 | / | / | / | 20.97 |
| **2238** | 50.39 | 42.78 | 53.03 | 2.29 | 1.99 | 23.48 | 28.95 | 1.63 | / | / | / | / | 25.57 |
| **2243** | 5.49 | 1.73 | 40.45 | 38.21 | 27.93 | 28.44 | 5.29 | 8.79 | 5.21 | 13.05 | 6.55 | / | 16.47 |
| **2250** | / | / | 1.21 | 0.82 | 0.88 | 0.75 | 21.58 | 32.04 | 28.28 | 18.68 | 5.50 | 6.09 | 11.58 |
| **2256** | 16.45 | 6.17 | 9.73 | 8.29 | / | 14.08 | / | / | / | / | / | / | 10.94 |
| **2289** | 38.03 | 36.94 | / | 25.37 | 27.20 | 7.61 | / | / | / | / | / | / | 27.03 |
| **3082** | 4.48 | 1.05 | 6.58 | 3.99 | 39.65 | 42.15 | 24.3 | 13.41 | 9.55 | / | / | / | 16.14 |
| **4066** | 46.65 | 55.41 | 39.35 | 22.44 | 13.84 | / | / | / | / | / | / | / | 35.54 |
| **4086** | 47.96 | 44.04 | 31.93 | 25.89 | 20.28 | 3.04 | / | / | / | / | / | / | 28.86 |
| **4101** | 11.16 | 13.13 | 11.98 | / | / | / | / | / | / | / | / | / | 12.09 |
| **4145** | 4.25 | 4.40 | 3.71 | 2.83 | 4.21 | / | / | / | / | / | / | / | 3.88 |
| **4165** | 3.11 | 2.66 | 5.41 | 1.59 | 6.69 | 6.78 | / | / | / | / | / | / | 4.37 |
| **4183** | 8.56 | 1.69 | 2.90 | 1.00 | 1.63 | 2.80 | 9.10 | / | / | / | / | / | 3.95 |
| **4188** | 40.40 | 34.17 | 27.82 | 33.66 | 21.46 | 12.44 | / | / | / | / | / | / | 28.33 |
| **4304** | 37.47 | 42.97 | 34.43 | 25.20 | 23.54 | 12.09 | / | / | / | / | / | / | 29.28 |
| **4366** | 49.63 | 50.25 | 28.24 | 2.06 | 35.88 | 100.00 | / | / | / | / | / | / | 29.34 |
| **4373** | 2.70 | 5.71 | 4.30 | 3.57 | 43.99 | 46.82 | 42.51 | 37.41 | 33.98 | / | / | / | 24.55 |
| **4380** | 10.89 | 35.22 | 35.16 | 28.05 | 2.47 | 37.54 | 4.53 | 29.62 | 1.43 | 17.78 | / | / | 20.27 |
| **4399** | 6.05 | 6.66 | 32.21 | 29.06 | 42.73 | 29.06 | 19.74 | 3.20 | 1.52 | / | / | / | 19.61 |
| **4409** | 30.87 | 2.40 | / | / | 1.58 | 7.05 | 2.67 | 7.84 | / | / | / | / | 10.18 |
| **4414** | 48.47 | 42.79 | / | 0.99 | 34.20 | 41.77 | / | / | / | / | / | / | 33.64 |
| **4422** | 37.02 | 29.44 | 46.01 | 36.49 | 24.72 | 3.87 | 7.34 | / | / | / | / | / | 26.41 |
| **4423** | 10.99 | 26.09 | 31.60 | 28.24 | 20.02 | 0.70 | / | / | / | / | / | / | 19.61 |
| **4523** | 35.67 | 21.30 | 13.29 | 29.29 | 19.27 | 9.32 | / | / | / | / | / | / | 21.36 |
| **4525** | 23.98 | 15.88 | 5.87 | 1.33 | 2.56 | 10.45 | 6.05 | 10.91 | 12.22 | / | / | / | 9.92 |
| **5202** | 2.32 | 1.12 | 48.33 | 43.39 | 44.03 | 21.04 | 21.89 | 13.68 | / | / | / | / | 24.48 |
| **5203** | 65.94 | 38.51 | 38.11 | 8.10 | 6.72 | 20.06 | 12.87 | 14.03 | / | / | / | / | 25.54 |
| **5204** | 36.14 | 24.21 | 15.25 | 2.03 | 1.95 | 24.38 | 30.45 | / | / | / | / | / | 19.20 |
| **5205** | 1.60 | 11.96 | 6.68 | 2.12 | 32.93 | 45.17 | 39.73 | 19.27 | 19.66 | / | / | / | 19.90 |
| **5206** | 1.19 | 1.14 | 8.23 | 49.61 | 39.63 | 48.90 | / | / | / | / | / | / | 24.78 |
| **5207** | 12.38 | 9.75 | 6.21 | 3.83 | 19.71 | 22.49 | / | / | / | / | / | / | 12.39 |
| **5209** | 44.72 | 50.60 | 47.54 | 0.40 | 2.24 | 12.68 | 7.94 | 4.81 | / | / | / | / | 21.36 |
| **5210** | 39.58 | 19.71 | 37.54 | 38.09 | 17.02 | 8.82 | / | / | / | / | / | / | 26.79 |
| **5211** | 14.28 | 17.99 | 17.40 | 14.79 | 15.01 | / | / | / | / | / | / | / | 15.91 |
| **5212** | 32.63 | 12.39 | 44.83 | / | / | / | / | / | / | / | / | / | 29.95 |
| **5213** | 13.43 | 3.20 | 2.44 | 40.75 | 52.86 | 48.47 | / | / | / | / | / | / | 26.86 |
| **5214** | 27.23 | 22.02 | 41.89 | 0.78 | 3.63 | 10.20 | 28.05 | 29.49 | 4.17 | 4.51 | / | / | 17.20 |
| **5215** | 6.67 | 1.83 | 1.18 | 16.05 | 32.57 | 13.91 | 20.86 | 21.57 | 3.20 | / | / | / | 13.09 |
| **5217** | 1.88 | 1.73 | / | 11.53 | 8.58 | 22.51 | 6.88 | 19.54 | 12.60 | / | / | / | 10.66 |
| **5218** | 0.62 | 1.21 | 27.00 | 40.29 | 21.77 | 9.70 | 9.91 | 3.94 | / | / | / | / | 14.31 |

**Table S5.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Cell pair** | | **Distance interval (m)** | | | | |
| **From** | **To** | **10 - 20** | **20 – 30** | **30 – 40** | **40 - 50** | **Entire span** |
| CD4+ T | CD8+ T | 0.2240 | 0.0683 | 0.2686 | 0.5165 | 0.0264 |
| CD8+ T | CD4+ T | 1 | 1 | 1 | 1 | 1 |
| CD4+ T | B | 0.4622 | 0.0713 | 0.0847 | 0.1638 | 0.0037 |
| B | CD4+ T | 1 | 1 | 1 | 1 | 1 |
| CD4+ T | Macrophage | 2.3548e-5 | 2.3048e-6 | 3.773e-7 | 7.5463e-7 | 4.1068e-11 |
| Macrophage | CD4+ T | 0.0435 | 0.0372 | 0.004 | 0.0080 | 2.1789e-4 |
| CD8+ T | B | 0.0002 | 0.0014 | 0.0017 | 0.0032 | 4.5496e-8 |
| B | CD8+ T | 0.0002 | 0.0087 | 0.1731 | 0.3338 | 2.2273e-5 |
| CD8+ T | Macrophage | 0.0001 | 2.4120e-5 | 3.2874e-5 | 6.5749e-5 | 1.7637e-6 |
| Macrophage | CD8+ T | 1 | 1 | 1 | 1 | 0.8938 |
| B | Macrophage | 1 | 1 | 1 | 1 | 1 |
| Macrophage | B | 1 | 1 | 1 | 1 | 1 |

**Spatial correlation analysis of immune phenotype pairs using spatial G(r) function (Gcross).** Gcross values were evaluated and compared between short- and long-term groups at each distance interval starting from 10m to 50 m and the entire span (Wilcoxon rank-sum test). P values were recorded and FDR-adjusted. Adjusted *P* < 0.05 was considered significant.

**Table S6.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Enrichment score (SEn) – 400 simulations** | | | | | | | |
| **Macrophage** | | | | | | | |
| **Marker** | **PD-L1** | **PD-1** | **IL-10** | **GZMB** | **Ki67** | **EOMES** | **ICOS** |
| **Median SEn** | 0.0425 | 0 | 1 | 0 | 0.0075 | 0.0025 | 0 |
| **Mean SEn** | 0.3808 | 0.0788 | 0.6488 | 0.1280 | 0.2688 | 0.2915 | 0.0310 |
| **CD4+ T cell** | | | | | | | |
| **Marker** | **PD-L1** | **PD-1** | **IL-10** | **GZMB** | **Ki67** | **EOMES** | **ICOS** |
| **Median SEn** | 0.0475 | 1 | 1 | 0 | 0.4725 | 0.185 | 0 |
| **Mean SEn** | 0.3325 | 0.7028 | 0.6587 | 0.1367 | 0.4840 | 0.3784 | 0.0355 |
|  | | | | | | | |
| **Enrichment score (SEn) – 500 simulations** | | | | | | | |
| **Macrophage** | | | | | | | |
| **Marker** | **PD-L1** | **PD-1** | **IL-10** | **GZMB** | **Ki67** | **EOMES** | **ICOS** |
| **Median SEn** | 0.0400 | 0 | 1 | 0 | 0.0060 | 0.0020 | 0 |
| **Mean SEn** | 0.3809 | 0.0788 | 0.6489 | 0.1282 | 0.2692 | 0.2917 | 0.0312 |
| **CD4+ T cell** | | | | | | | |
| **Marker** | **PD-L1** | **PD-1** | **IL-10** | **GZMB** | **Ki67** | **EOMES** | **ICOS** |
| **Median SEn** | 0.0440 | 1 | 1 | 0 | 0.4920 | 0.2140 | 0 |
| **Mean SEn** | 0.3325 | 0.7026 | 0.6584 | 0.1367 | 0.4857 | 0.3786 | 0.0358 |
|  | | | | | | | |
| **Enrichment score (SEn) – 600 simulations** | | | | | | | |
| **Macrophage** | | | | | | | |
| **Marker** | **PD-L1** | **PD-1** | **IL-10** | **GZMB** | **Ki67** | **EOMES** | **ICOS** |
| **Median SEn** | 0.04 | 0 | 1 | 0 | 0.0067 | 0.0017 | 0 |
| **Mean SEn** | 0.3811 | 0.079 | 0.6488 | 0.1282 | 0.2689 | 0.2915 | 0.0311 |
| **CD4+ T cell** | | | | | | | |
| **Marker** | **PD-L1** | **PD-1** | **IL-10** | **GZMB** | **Ki67** | **EOMES** | **ICOS** |
| **Median SEn** | 0.0533 | 1 | 1 | 0 | 0.4767 | 0.2167 | 0 |
| **Mean SEn** | 0.3328 | 0.7036 | 0.6587 | 0.1370 | 0.4843 | 0.3783 | 0.0361 |

**Enrichment score computed for all functional markers on both macrophages and CD4+ T cells at per-ROI basis, denoted as SEn using different simulation times.** Mean and median SEn were derived by summarizing values from all ROIs. Marker with highest mean and median SEn was selected as the primary contributing factor to the spatial clustering

**Table S7.** Exclusion analysis test result.

|  |  |
| --- | --- |
| **Removed Patient** | **P value** |
| **2117** | < 2.2e-16 |
| **2148** | < 2.2e-16 |
| **2243** | < 2.2e-16 |
| **2256** | < 2.2e-16 |
| **2289** | < 2.2e-16 |
| **3082** | < 2.2e-16 |
| **4066** | < 2.2e-16 |
| **4101** | < 2.2e-16 |
| **4165** | < 2.2e-16 |
| **4183** | < 2.2e-16 |
| **4304** | < 2.2e-16 |
| **4373** | < 2.2e-16 |
| **4380** | < 2.2e-16 |
| **4399** | < 2.2e-16 |
| **4409** | < 2.2e-16 |
| **4414** | < 2.2e-16 |
| **4422** | < 2.2e-16 |
| **4423** | < 2.2e-16 |
| **4523** | < 2.2e-16 |
| **4525** | < 2.2e-16 |
| **5202** | < 2.2e-16 |
| **5205** | < 2.2e-16 |
| **5209** | < 2.2e-16 |
| **5210** | < 2.2e-16 |
| **5211** | < 2.2e-16 |
| **5212** | < 2.2e-16 |
| **5213** | < 2.2e-16 |
| **5214** | < 2.2e-16 |
| **5215** | < 2.2e-16 |
| **5217** | < 2.2e-16 |
| **5218** | < 2.2e-16 |