**Supplementary materials:**

**Table S1.** Antibodies used in IHC and mIF samples.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  |  | Antibody | Clone | AntibodyDilution | Vendor | Flourescence |
| Panel 1 |  | **Pancytokeratin** | AE1/AE3 | 1:100 | Dako, Carpinteria, CA | Opal Polaris 650  |
|  | **CD3** | D7A6E | 1:100 | Cell Signaling Technology, Danvers, MA | Opal Polaris 780 |
|  | **CD8** | C8/144B | 1:25 | Thermo Fisher Scientific, Waltham, MA | Opal Polaris 520 |
|  | **FOXP3** | D2W8E | 1:50 | Cell Signaling Technology, Danvers, MA | Opal Polaris 570 |
|  | **PD-1** | EPR4877-2 | 1:250 | Abcam, Cambridge, MA | Opal Polaris 620  |
|  | **PD-L1** | E1L3N | 1:1500 | Cell Signaling Technology, Danvers, MA | Opal Polaris 690  |
|  | **Ki67** | MIB-1 | 1:100 | Dako, Carpinteria, CA | Opal Polaris 480 |
|  | **CD68** | PG-M1 | 1:50 | Dako | Opal Polaris 540 |
| Panel 2 |  | **TTF-1** | 8G7G3/1 | 1:200 | Agilent Technologies | Opal Polaris 540  |
|  | **WT-1** | 6F-H2 | 1:40 | Sigma-Aldrich | Opal Polaris 780 |
|  | **CD3** | D7A6E | 1:100 | Agilent Technologies | Opal Polaris 4800 |
|  | **CD8** | C8/144B | 1:25 | Thermo Fisher Scientific, Waltham, MA | Opal Polaris 670  |
|  | **FOXP3** | 206D | 1:50 | BioLegend, San Diego, CA | Opal Polaris 570 |
|  | **PD-1** | EPR4877-2 | 1:100 | Abcam, Cambridge, MA | Opal Polaris 620 |
|  | **PD-L1** | E1L3N | 1:300 | Cell Signaling Technology, Danvers, MA | Opal Poliris 650  |
|  | **Ki67** | MIB-1 | 1:100 | Dako, Carpinteria, CA | Opal Polaris 690 |
|  | **CD68** | PG-M1 | 1:25 | Agilent Technologies | Opal Polaris 520 |
| Panel 3 |  | **GATA-3** | EPR16651 | 1:200 | Leica; Abcam | Opal Polaris 540 |
|  | **WT-1** | 6F-H2 | 1:40 | Sigma-Aldrich | Opal Polaris 780 |
|  | **CD3** | D7A6E | 1:200 | Agilent Technologies | Opal Polaris 480 |
|  | **CD8** | C8/144B | 1:25 | Thermo Fisher Scientific, Waltham, MA | Opal Polaris 670 |
|  | **FOXP3** | 206D | 1:50 | BioLegend, San Diego, CA | Opal Polaris 570 |
|  | **PD-1** | EPR4877-2 | 1:100 | Abcam, Cambridge, MA | Opal Polaris 620 |
|  | **PD-L1** | E1L3N | 1:250 | Cell Signaling Technology, Danvers, MA | Opal polaris 650 |
|  | **Ki67** | MIB-1 | 1:100 | Dako, Carpinteria, CA | Opal Polaris 690 |
|  | **CD68** | PG-M1 | 1:25 | Agilent Technologies | Opal Polaris 520 |

CD, cluster of differentiation; FOXP3, forkhead box P3; IHC, immunohistochemistry; mIF, multiplex immunofluorescence; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1;TTF-1, thyroid transcription factor-1; WT-1, Wilms tumor 1.

**Table S2.** Immunophenotyping by mIF using 3 Opal panels.

|  |  |
| --- | --- |
| Phenotype | Marker expression |
| Panel 1 |  |
| Malignant cells | Total PanCK+ |
| PD-L1+ malignant cells | PanCK+PD-L1+ |
| Total T cells  | CD3+ |
| Cytotoxic T cells | CD3+CD8+ |
| Antigen-experienced T cells | CD3+PD-1+ |
| Total macrophages | CD68+ |
| PD-L1+ macrophages | CD68+PD-L1+ |
| Regulatory T cells | CD3+ FOXP3+CD8neg |
| Ki67 expression | Total Ki67+ |
| Panel 2 |  |
| Malignnat cells | Total TTF-1+ |
| Mesothelial cells | WT-1 |
| PD-L1+ malignant cells | TTF-1+PD-L1+ |
| Total T cells | CD3+ |
| Cytotoxic T cells | CD3+CD8+ |
| Antigen-experienced T cells | CD3+PD-1+ |
| Total macrophages | CD68+ |
| PD-L1+ macrophages | CD68+PD-L1+ |
| Regulatory T cells | CD3+FOXP3+CD8neg |
| Ki67 expression | TTF-1+Ki67+ and CD3+Ki67+ |
| Panel 3 |  |
| Malignant cells | Total GATA3+ |
| Mesothelial cells | WT-1 |
| PD-L1+ malignant cells | GATA3+PD-L1+ |
| Total T cells | CD3+ |
| Cytotoxic T cells | CD3+CD8+ |
| Antigen-experienced T cells | CD3+PD-1+ |
| Total macrophages | CD68+ |
| PD-L1+ macrophages | CD68+PD-L1+ |
| Regulatory T cells | CD3+FOXP3+CD8neg |
| Ki67 expression | GATA+Ki67+ and CD3+Ki67+ |

CD, cluster of differentiation; FOXP3, forkhead box P3; mIF, multiplex immunofluorescence; PanCK, pancytokeratin; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; WT-1, Wilms tumor 1.

**Table S3.** Cellular composition ofLADC MPEs in absolute numbers.

|  |  |
| --- | --- |
|  | Samples |
| Cell Phenotype | **S1** | **S2** | **S3** | **S4** | **S5** |
| TTF-1+ | 3200 | 7330 | 4372 | 408 | 6435 |
| WT-1+ | 137 | 259 | 24 | 738 | 67 |
| TTF-1+ PD-L1+ | 1 | 0 | 0 | 0 | 2 |
| TTF-1+ Ki67+ | 473 | 980 | 241 | 10 | 950 |
| CD3+ | 5636 | 338 | 3528 | 5941 | 5885 |
| CD3+ CD8+ | 292 | 36 | 949 | 613 | 901 |
| CD3+ PD-1+ | 85 | 6 | 17 | 55 | 96 |
| CD3+ PD-L1+ | 0 | 0 | 0 | 16 | 33 |
| CD3+Ki67+ | 71 | 5 | 17 | 111 | 130 |
| CD3+ CD8+ Ki67+ | 3 | 1 | 13 | 12 | 44 |
| CD3+ PD-1+ PD-L1+ | 0 | 0 | 0 | 0 | 0 |
| CD3+ CD8+ PD-L1+ | 0 | 0 | 0 | 16 | 29 |
| CD3+CD8+ PD-1+ | 5 | 2 | 2 | 5 | 14 |
| CD3+ FOXP3+ CD8neg  | 287 | 59 | 88 | 517 | 583 |
| CD68+ | 2607 | 2226 | 7972 | 5750 | 686 |
| CD68+ PD-L1+ | 0 | 0 | 0 | 1 | 1 |
| Total cells | 17,771 | 16,331 | 25,674 | 48532 | 25393 |

CD, cluster of differentiation; FOXP3, forkhead box P3; LADC, lung adenocarcinoma; MPE, malignant pleural effusion; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; S,sample; WT-1, Wilms tumor 1.

**Table S4.** Cellular composition ofBC MPEs in absolute numbers.

|  |  |
| --- | --- |
|  | Samples |
| Cell phenotype | **S1** | **S2** | **S3** | **S4** | **S5** | **S6** |
| GATA3+  | 87 | 169 | 9749 | 3155 | 9106 | 1102 |
|  WT-1+  | 480 | 168 | 318 | 2869 | 202 | 1095 |
| GATA3+ PD-L1+ | 0 | 0 | 29 | 11 | 2 | 0 |
| GATA3+ Ki67+ | 20 | 4 | 710 | 153 | 353 | 388 |
| CD3+  | 436 | 3330 | 2274 | 1377 | 170 | 1214 |
| CD3+ CD8+  | 81 | 348 | 306 | 426 | 15 | 48 |
| CD3+ PD-1+  | 7 | 0 | 0 | 128 | 9 | 30 |
| CD3+ PD-L1+  | 0 | 0 | 0 | 31 | 1 | 11 |
| CD3+ Ki67+  | 31 | 75 | 26 | 98 | 2 | 15 |
| CD3+ CD8+ Ki67+ | 4 | 34 | 1 | 51 | 0 | 0 |
| CD3+ PD-1+ PD-L1+  | 0 | 0 | 0 | 6 | 0 | 0 |
| CD3+ CD8+ PD-L1+  | 0 | 0 | 0 | 25 | 1 | 11 |
| CD3+ CD8+ PD-1+  | 1 | 0 | 0 | 61 | 0 | 1 |
| CD3+ FOXP3+CD8neg  | 8 | 137 | 156 | 129 | 21 | 17 |
| CD68+  | 2604 | 47 | 2099 | 8979 | 1325 | 809 |
| CD68+PD-L1+  | 2 | 0 | 2 | 23 | 3 | 0 |
| Total cells  | 31278 | 8695 | 23849 | 32472 | 12960 | 14086 |

BC, breast carcinoma ; CD, cluster of differentiation; FOXP3, forkhead box P3; MPE, malignant pleural effusion; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; S,sample; WT-1, Wilms tumor 1.

**Table S5.** Phenotype spatial distributions in LADC PTs.

|  |
| --- |
| Nearest-neighbor median distances for phenotype pairs (microns) |
| Samples |
| From PanCK+ to | **S1** | **S2** | **S3** | **S4** | **S5** |
| CD3+ | 30.2 | 78.3 | 32.8 | 68.9 | 60.2 |
| CD3+CD8+ | 92.0 | 228.6 | 53.8 | 257.2 | 109.0 |
| CD68+ | 51.3 | 167.5 | 122.2 | 197.6 | 72.5 |

CD, cluster of differentiation; LADC, lung adenocarcinoma; PanCK, pancytokeratin; PT, primary tumor; S,sample.

**Table S6.** Phenotype spatial distributions in BC PTs.

|  |
| --- |
| Nearest-neighbor median distances for phenotype pairs (microns) |
| Samples |
| From PanCK+ to | **S1** | **S2** | **S3** | **S4** | **S5** | **S6** |
| CD3+ | 127.4 | 108.8 | 121.7 | 158.1 | 272.1 | 111.7 |
| CD3+CD8+ | 231.3 | 260.8 | 157.7 | 217.0 | \* | 329.2 |
| CD3+PD-1+ | 265.5 | 333.7 | 369.8 | 267.7 | 573.9 | 342.7 |
| CD68+ | 37.6 | 41.3 | 83.6 | 93.9 | 204.4 | 124.0 |

\* No evaluable cells.

BC, breast carcinoma; CD, cluster of differentiation; PanCK, pancytokeratin; PD-1, programmed cell death protein 1; PT, primary tumor; S,sample.

**Figure S1.** Phenotypes in PTs ,BC MPE panel and LADC MPE panel.



BC, breast carcinoma; CD, cluster of differentiation; FOXP3, forkhead box P3; LADC, lung adenocarcinoma; MPE, malignant pleural effusion; PD-1, programmed cell death protein 1; PD-L1, programmed death-ligand 1; PT, primary tumor; TTF-1, thyroid transcription factor-1.