# SUPPLEMENTARY DATA

## Supplementary tables

**Table S1:** Patient characteristics among the whole population and by pCR status.

*Missing data:* Family history, n=1; Menopausal status, n=4; BMI classes, n=1; Smoking status, n=71; Comorbidity, n=56; Nuclear ER staining (%), n=20; Nuclear PR staining (%), n=18; SBR grade, n=9; KI67 classes, n=209; Mitotic index class, n=42; Ductal carcinoma in situ, n=43; % stromal lymphocytes, n=57; % intra-tumoral lymphocytes, n=57; Lymphovascular invasion, n=256; NAC regimen, n=1; Lymphovascular invasion postNAC, n=142; RCB index, n=57; RCB classes, n=57; % stromal lymphocytes postNAC, n=57; % intra-tumoral lymphocytes postNAC, n=197.
*Abbreviations*: BMI= body mass index; T= tumor; N= node; ER= estrogen receptor; PR= progesterone receptor; SBR= grade Scarff-Bloom and Richardson; BC= Breast cancer; NAC=neoadjuvant chemotherapy; anthra-taxans= anthracyclines and taxanes; anthra= anthracyclines; RCB= Residual Cancer Burden.
*The "n" denotes the number of patients. In case of categorical variables, percentages are expressed between brackets. In case of continuous variables, mean value is reported. In case of nonnormal continuous variables, median value is reported, with interquartile range between brackets.*

**Table S2:** Association of clinical and pathological pre and post-NAC parameters with PD-L1-TC expressio after univariate and multivariate analysis in the whole population.
*Abbreviations*: BMI=body mass index; ER=oestrogen receptor; PR=progesterone receptor; TNBC= triple negative breast cancer ; str TILs= stromal tumor-infiltrating lymphocytes ; IT TILs= intratumoral-infiltrating lymphocytes ; NAC=neoadjuvant chemotherapy ; AC=anthracyclines ; pCR=Pathologic complete response.

**Table S3:** Studies on PD-L1 expression in BC RD
Abbreviations: breast cancer (BC), triple-negative breast cancer (TNBC), hazard ratio (HR), confidence interval (CI), not available (NA), relative risk (RR)

**Table S1:** Patient characteristics among the whole population and by pCR status.

| Characteristics | Class | No pCR | pCR | *p* |
|---|---|---|---|---|
| **n=** | | 236 | 139 | |
| **Baseline characteristics** | | | | |
| **Age** | | 48.75 (10.41) | 47.67 (10.05) | 0.327 |
| **Family history** | No | 188 (79.7) | 94 (68.1) | 0.017 |
| | Yes | 48 (20.3) | 44 (31.9) | |
| **BRCA screening** | No | 175 (74.2) | 90 (64.7) | 0.070 |
| | Yes | 61 (25.8) | 49 (35.3) | |
| **Menopausal status** | Premenopausal | 140 (59.6) | 89 (65.4) | 0.313 |
| | Postmenopausal | 95 (40.4) | 47 (34.6) | |
| **BMI classes** | 18.5-24.9 | 124 (52.5) | 86 (62.3) | 0.081 |
| | <18.5 | 11 (4.7) | 1 (0.7) | |
| | 25-29.9 | 70 (29.7) | 33 (23.9) | |
| | >=30 | 31 (13.1) | 18 (13.0) | |
| **Smoking status** | Never | 128 (69.6) | 78 (64.5) | 0.280 |
| | Current | 32 (17.4) | 19 (15.7) | |
| | Former | 24 (13.0) | 24 (19.8) | |
| **Comorbidity** | No | 114 (55.9) | 72 (62.1) | 0.337 |
| | Yes | 90 (44.1) | 44 (37.9) | |
| **Clinicar T stage** | T0-T1 | 11 (4.7) | 20 (14.4) | **0.003** |
| | T2 | 155 (65.7) | 87 (62.6) | |
| | T3-T4 | 70 (29.7) | 32 (23.0) | |
| **Clinical N stage** | N0 | 110 (46.6) | 61 (43.9) | 0.686 |
| | N1-N2-N3 | 126 (53.4) | 78 (56.1) | |
| **SBR grade** | Grade I-II | 44 (19.2) | 9 (6.6) | **0.002** |
| | Grade III | 185 (80.8) | 128 (93.4) | |
| **Mitotic index class** | [0,20) | 82 (40.2) | 33 (25.6) | **0.009** |
| | >=20 | 122 (59.8) | 96 (74.4) | |
| **Ductal carcinoma in situ** | No | 170 (82.5) | 112 (88.2) | 0.216 |
| | Yes | 36 (17.5) | 15 (11.8) | |
| **% stromal lymphocytes** | | 20.00 [5.00, 30.00] | 30.00 [15.00, 60.00] | **<0.001** |
| **% intra-tumoral lymphocytes** | | 5.00 [1.00, 5.00] | 5.00 [5.00, 20.00] | **<0.001** |
| **Lymphovascular invasion** | No | 61 (71.8) | 21 (60.0) | 0.297 |
| | Yes | 24 (28.2) | 14 (40.0) | |
| **NAC regimen** | anthra-taxans | 173 (73.3) | 114 (82.0) | 0.227 |
| | anthra | 46 (19.5) | 17 (12.2) | |
| | taxanes | 1 (0.4) | 0 (0.0) | |
| | others | 16 (6.8) | 8 (5.8) | |
| **Post-NAC characteristics** | | | | |
| **N stage** | 0 | 141 (59.7) | 139 (100.0) | **<0.001** |
| | [1-3] | 54 (22.9) | 0 (0.0) | |
| | [4-9] | 33 (14.0) | 0 (0.0) | |
| | 10 and more | 8 (3.4) | 0 (0.0) | |
| **RCB index** | | 2.39 (0.93) | 0.00 (0.00) | **<0.001** |
| **RCB classes** | RCB-0 | 0 (0.0) | 123 (100.0) | **<0.001** |
| | RCB-I | 23 (11.7) | 0 (0.0) | |
| | RCB-II | 131 (66.8) | 0 (0.0) | |
| | RCB-III | 42 (21.4) | 0 (0.0) | |
| **Lymphovascular invasion pos** | No | 125 (66.1) | 45 (100.0) | **<0.001** |
| | Yes | 64 (33.9) | 0 (0.0) | |
| **% stromal lymphocytes post-NAC** | | 15.00 [5.00, 30.00] | 5.00 [5.00, 10.00] | **<0.001** |

*Missing data:* Family history, n=1; Menopausal status, n=4; BMI classes, n=1; Smoking status, n=71; Comorbidity, n=56; Nuclear ER staining (%), n=20; Nuclear PR staining (%), n=18; SBR grade, n=9; KI67 classes, n=209; Mitotic index class, n=42; Ductal carcinoma in situ, n=43; % stromal lymphocytes, n=57; % intra-tumoral lymphocytes, n=57; Lymphovascular invasion, n=256; NAC regimen, n=1; Lymphovascular invasion postNAC, n=142; RCB index, n=57; RCB classes, n=57; % stromal lymphocytes postNAC, n=57; % intra-tumoral lymphocytes postNAC, n=197.

*Abbreviations:* BMI= body mass index; T= tumor; N= node; SBR= grade Scarff-Bloom and Richardson; BC= Breast cancer; NAC=neoadjuvant chemotherapy; anthra-taxans= anthracyclines and taxanes; anthra= anthracyclines; RCB= Residual Cancer Burden.

*The "n" denotes the number of patients. In case of categorical variables, percentages are expressed between brackets. In case of continuous variables, mean value is reported. In case of nonnormal continuous variables, median value is reported, with interquartile range between brackets.*

**Table S2:** Association of clinical and pathological pre and post-NAC parameters with PD-L1-TC expressio after univariate and multivariate analysis in the whole population

| | | | | | Univariate | | | | Multivariate | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Variable** | **Class** | **Nb total** | **Nb in model** | **Events** | **HR** | **CI** | **RCH** | ***p*** | **HR** | **CI** | ***p*** |
| **Pre-NAC parameters** | | | | | | | | | | | |
| **Age** | | | | 31 | 1 | [0.96 - 1.04] | | 0.959 | | | |
| **Family history** | No | 68 | 68 | 24 | 1 | | 35.3 % | | | | |
| | Yes | 21 | 21 | 7 | 0.92 | [0.31 - 2.53] | 33.3 % | 0.869 | | | |
| **BRCA screening** | No | 56 | 56 | 20 | 1 | | 35.7 % | | | | |
| | Yes | 33 | 33 | 11 | 0.9 | [0.36 - 2.21] | 33.3 % | 0.82 | | | |
| **Menopausal status** | Premenopausal | 51 | 51 | 18 | 1 | | 35.3 % | | | | |
| | Postmenopausal | 38 | 38 | 13 | 0.95 | [0.39 - 2.3] | 34.2 % | 0.915 | | | |
| **BMI classes** | 18.5-24.9 | 47 | NA | 13 | | | 27.7 % | | | | |
| | <18.5 | 2 | NA | 0 | | | 0% | | | | |
| | 25-29.9 | 22 | NA | 10 | | | 45.5 % | | | | |
| | >=30 | 18 | NA | 8 | | | 44.4 % | | | | |
| **Smoking status** | Never | 61 | 61 | 19 | 1 | | 31.1 % | | | | |
| | Current | 12 | 12 | 4 | 1.11 | [0.27 - 3.98] | 33.3 % | 0.882 | | | |
| | Former | 12 | 12 | 6 | 2.21 | [0.62 - 7.96] | 50% | 0.215 | | | |
| **Comorbidity** | No | 35 | 35 | 10 | 1 | | 28.6 % | | | | |
| | Yes | 42 | 42 | 16 | 1.54 | [0.59 - 4.12] | 38.1 % | 0.38 | | | |
| **Clinicar T stage** | T0-T1 | 6 | 6 | 4 | 1 | | 66.7 % | | | | |
| | T2 | 62 | 62 | 23 | 0.29 | [0.04 - 1.63] | 37.1 % | 0.177 | 0.41 | [ 0.05 - 2.68 ] | 0.36 |
| | T3-T4 | 21 | 21 | 4 | 0.12 | [0.01 - 0.81] | 19% | **0.038** | 0.16 | [ 0.01 - 1.43 ] | 0.114 |
| **Clinical N stage** | N0 | 38 | 38 | 13 | 1 | | 34.2 % | | | | |
| | N1-N2-N3 | 51 | 51 | 18 | 1.05 | [0.43 - 2.57] | 35.3 % | 0.915 | | | |
| **SBR grade** | Grade I-II | 12 | 12 | 1 | 1 | | 8.3 % | | | | |
| | Grade III | 75 | 75 | 29 | 6.93 | [1.25 - 130.09] | 38.7 % | 0.07 | 3.87 | [ 0.55 - 79.01 ] | 0.24 |
| **Mitotic index** | | | | 31 | 1.01 | [0.99 - 1.03] | | 0.185 | | | |
| **Mitotic index status** | [0,20) | 34 | 34 | 10 | 1 | | 29.4 % | | | | |
| | >=20 | 48 | 48 | 18 | 1.44 | [0.57 - 3.78] | 37.5 % | 0.448 | | | |
| **Ductal carcinoma in situ** | No | 83 | 83 | 30 | 1 | | 36.1 % | | | | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Yes | 6 | 6 | 1 | 0.35 | [0.02 - 2.33] | 16.7 % | 0.353 | | | |
| **% stromal lymphocytes** | | | | 31 | 1.02 | [1 - 1.04] | | 0.071 | 1 | [ 0.96 - 1.05 ] | 0.812 |
| **% intra-tumoral lymphocytes** | | | | 31 | 1.04 | [1 - 1.09] | | 0.086 | 1.02 | [ 0.95 - 1.1 ] | 0.599 |
| **Lymphovascular invasion** | No | 2 | NA | 1 | | | 50% | | | | |
| | Yes | 1 | NA | 1 | | | 100% | | | | |
| **BC surgery** | Lumpectomy | 59 | 59 | 18 | 1 | | 30.5 % | | | | |
| | Mastectomy | 30 | 30 | 13 | 1.74 | [0.7 - 4.35] | 43.3 % | 0.232 | | | |
| **NAC regimen** | anthra-taxans | 85 | NA | 30 | | | 35.3 % | | | | |
| | anthra | 3 | NA | 1 | | | 33.3 % | | | | |
| | taxanes | 1 | NA | 0 | | | 0% | | | | |
| **Chemotherapy setting** | NAC | 59 | 59 | 19 | 1 | | 32.2 % | | | | |
| | NAC and adjuvant | 30 | 30 | 12 | 1.4 | [0.56 - 3.49] | 40% | 0.466 | | | |
| **Post-NAC parameters** | | | | | | | | | | | |
| **N stage post-NAC** | 0 | 53 | NA | 18 | | | 34% | | | | |
| | [1-3] | 22 | NA | 5 | | | 22.7 % | | | | |
| | [4-9] | 13 | NA | 8 | | | 61.5 % | | | | |
| | 10 and more | 1 | NA | 0 | | | 0% | | | | |
| **RCB classes** | RCB-I | 15 | 15 | 3 | 1 | | 20% | | | | |
| | RCB-II | 53 | 53 | 16 | 1.73 | [0.47 - 8.34] | 30.2 % | 0.441 | 1.02 | [ 0.21 - 5.91 ] | 0.978 |
| | RCB-III | 19 | 19 | 11 | 5.5 | [1.26 - 30.51] | 57.9 % | **0.032** | 2.94 | [ 0.48 - 21.7 ] | 0.259 |
| **KI67 status post-NAC** | [0,20) | 51 | 51 | 17 | 1 | | 33.3 % | | | | |
| | >=20 | 38 | 38 | 14 | 1.17 | [0.48 - 2.82] | 36.8 % | 0.731 | | | |
| **Mitotic index post-NAC (%)** | | | | 31 | 1.01 | [1 - 1.03] | | **0.035** | 1.01 | [ 0.99 - 1.02 ] | 0.431 |
| **Mitotic index status post-NAC** | [0,20) | 43 | 43 | 14 | 1 | | 32.6 % | | | | |
| | >=20 | 35 | 35 | 12 | 1.08 | [0.42 - 2.79] | 34.3 % | 0.872 | | | |
| **% stromal lymphocytes post-NAC** | | | | 31 | 1 | [0.98 - 1.03] | | 0.758 | | | |
| **% intra-tumoral lymphocytes post-NAC** | | | | 31 | 0.99 | [0.91 - 1.06] | | 0.718 | | | |
| **Lymphovascular invasion post-NAC** | No | 54 | 54 | 22 | 1 | | 40.7 % | | | | |
| | Yes | 23 | 23 | 6 | 0.51 | [0.16 - 1.45] | 26.1 % | 0.225 | | | |

*Abbreviations:* BMI=body mass index; TNBC= triple negative breast cancer ; str TILs= stromal tumor-infiltrating lymphocytes ; IT TILs= intratumoral-infiltrating lymphocytes ; NAC=neoadjuvant chemotherapy ; AC=anthracyclines ; pCR=Pathologic complete response.

| First author | Journal | Year | Country | n clinical studies | n patients | PDL-1 prevalence | Association with clinicopathological characteristics | PDL1 and pCR | PDL1 and DFS | PDL1 and OS |
|---|---|---|---|---|---|---|---|---|---|---|
| Wang | The Breast Journal | 2017 | China | 9 | 8583 | 25.8% (2141/8288) | Ductal BC (p=0.037)<br>High tumor grade (p=0.0001)<br>ER negative (p=0.0001)<br>PR negative (p=0.0001)<br>Her2+ (p=0.001)<br>Basal like (p=0.0001) | - | HR = 1.122<br>95% CI [0.878; 1.434]<br>p = 0.357 | HR 1.573<br>95% CI [1.01; 2.45]<br>p = 0.045 |
| Kim | BMC Cancer | 2017 | Korea | 6 | 7877 (827 TNBC) | - | Tumor and immune cells:<br>Lymph node metastasis (p=0.06)<br>High tumor grade (p<0.001) | - | HR = 1.36<br>95% CI [1.03; 1.79]<br>p = 0.03 | HR = 1.908<br>95% CI [0.91; 4.00]<br>p = 0.09 |
| Zhang | Oncotarget | 2017 | China | 5 | 2546 | 21.7% to 56.6% | Lymph node metastasis (p=0.02)<br>High tumor grade (p<0.001)<br>ER negative (p=0.008)<br>TNBC (p<0.001) | - | - | HR=1.76<br>95% CI [1.09; 2.82]<br>p=0.02 |
| Huang | Breast Cancer Research and Treatment | 2019 | China | 41 | 14367 | - | Ductal BC (p=0.03)<br>Large tumor size (p=0.05)<br>High tumor grade (p<0.00001)<br>ER negative (p=0.0009)<br>PR negative (p=004)<br>High Ki67 (p=0.01)<br>TNBC (p=0.0001)<br>High TILS (p<0.00001) | Tumor c. : RR = 1.64<br>95% CI [0.99–2.73]<br>p = 0.05<br>(n=1085) | Tumor c. :<br>HR = 1.43<br>95% CI [1.21; 1.70]<br>p < 0.0001 (n=4349)<br>Immune c. :<br>HR = 0.45<br>95% CI [0.28; 0.73]<br>p = 0.001 | Tumor c. : HR = 1.58<br>95% CI [1.14; 2.20]<br>p = 0.006 (n=5890)<br>Immune c. :<br>HR = 0.41<br>95% CI [0.27; 0.63]<br>p < 0.0001 |
| Li | Medicine | 2019 | China | 19 | 12505 | 6.4% to 76.4% | Lymph node metastasis (p<0.001)<br>High tumor grade (p<0.001)<br>Negative HR (p<0.001)<br>High Ki67 (p<0.001)<br>High TILS (p<0.001) | - | HR = 1.31<br>CI95% [1.14; 1.51]<br>p = 0.0002 | HR = 1.52<br>CI95% [1.14;2.03]<br>p=0.004 |
| Matikas | Clinical Cancer Research | 2019 | Sweden | 38 | 9500 | In the TNBC subgroup:<br>tumor cells 41% (n =2740)<br>immune c. 48% (n=1313)<br>tumor and immune c. 46% (n=903) | - | - | Tumor c. overall :<br>HR 1.62<br>95% CI [1.14–2.33]<br>p=0.008<br>Immune c. TNBC :<br>HR =0.61<br>95% CI [0.51–0.73]<br>p < 0.001 (n=969) | Tumor c. overall :<br>HR = 1.93<br>95% CI [1.20; 3.09]<br>p=0.006<br>Immune c. TNBC :<br>HR = 0.53<br>95% CI [0.39; 0.73]<br>p < 0.001 |
| Lotfinejad | Diagnostics | 2020 | Iran | 7 | 1152 | - | No associations | - | HR = 0.65<br>95% CI [0.37; 1.16]<br>p = 0.15 | HR = 0.56<br>95% CI [0.32 to 1.01]<br>p = 0.056 |

**Table S3.** Recent meta-analyses of the prognostic value of PD-L1 (IHC) in BC *Abbreviations:* breast cancer (BC), estrogen receptor (ER), progesterone receptor (PR), hormone receptor (HR), triple-negative breast cancer (TNBC), tumor-infiltrating lymphocytes (TILS), cells (c.), hazard ratio (HR), confidence Interval (CI), relative risk (RR), pathological complete response (pCR), overall survival (OS, disease-free survival (DFS)

## Supplementary Figures

**Fig. S1** Immunohistochemical analysis of PD-L1 in surgical specimens (Cell Signaling #13684S (E1L3N®) XP® rabbit mAb) staining. **A,** Positive control, breast carcinoma cells cytoblock, PD-L1 strong membrane expression, reported at ≥50% (x400); **B,** Positive control, Tonsil, PD-L1 strong expression of epithelial and microenvironment cells, reported at ≥50% (x200); **C,** residual invasive breast cancer obtained after NAC, strong membrane and cytoplasmic PDL1 expression by tumor and microenvironment cells, reported at ≥50% (x200); **D,** residual invasive breast cancer obtained after NAC, strong PDL1 expression by immune cells of the tumor microenvironment, reported at ≥50% (x400); **E,** residual invasive breast cancer obtained after NAC, strong PDL1 by breast carcinoma cells, reported at ≥50% (x400).

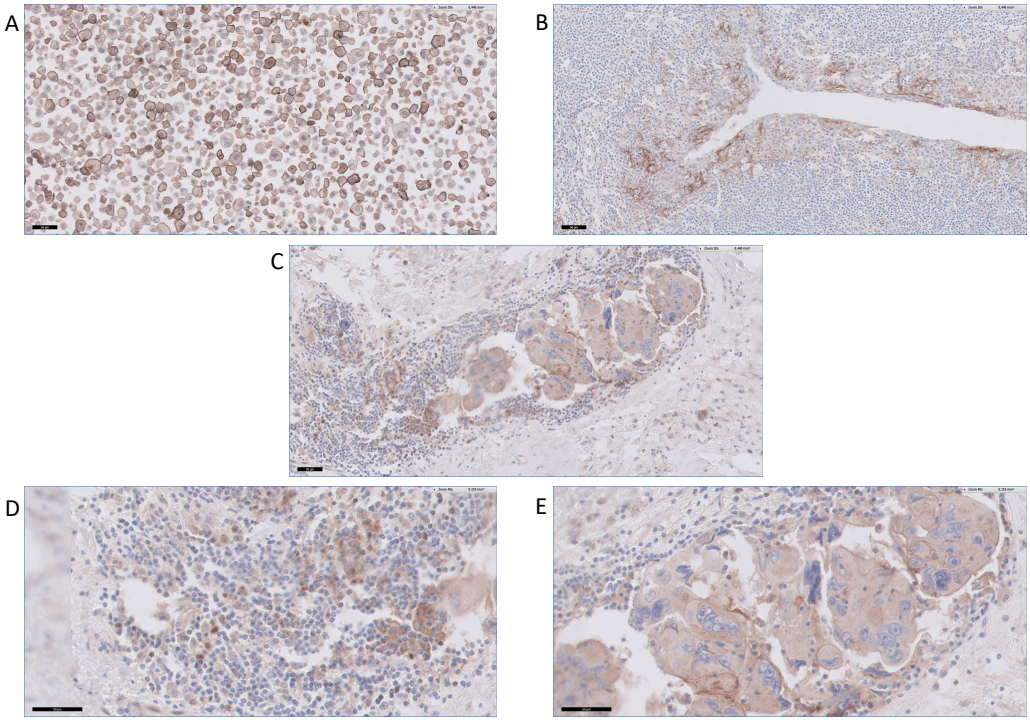
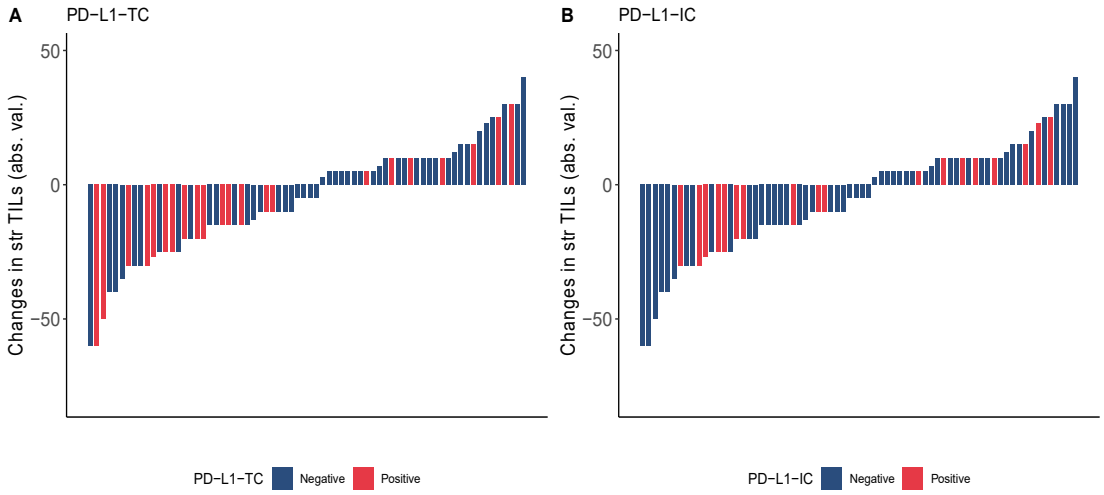



**Fig. S2:** Pre-NAC and post-NAC stromal immune infiltration rates in the whole population and by BRCA status. Waterfall plot representing the variation of TIL levels according to PD-L1-TC and PD-L1-IC; each bar represents one sample, and samples are ranked by increasing order of TIL level change. Paired samples for which no change was observed have been removed from the graph. **A,** by PD-L1_TC. B, by PD-L1-IC.


A
PD−L1−TC
50
0
−50
Changes in str TILs (abs. val.)
PD−L1−TC
Negative
Positive
B
PD−L1−IC
50
0
−50
Changes in str TILs (abs. val.)
PD−L1−IC
Negative
Positive