# Supplementary figure titles and legends

**Figure S1 | Analysis of stromal TILs in residual disease and prognostic implications.** A) Kaplan–Meier curves for i) progression free survival and ii) overall survival in ER+HER2- patients with RCB II and III, stratified according to the median sTIL level (cut-off 3%). B) Kaplan–Meier curves for i) progression free survival and ii) overall survival in TNBC patients with RCB II and III, stratified according to the median sTIL level (cut-off 8.8%).

**Figure S2 | Vectra analysis of residual disease samples.** A) Total immune infiltration per mm2 within pre-treatment (baseline) and residual disease samples (RCB II/III) among i) ER+HER2- and ii) TNBC tumours. B) Stacked bar graph showing the tumoural and stromal PD-L1 expression in TNBC and ER+HER2- RCB II/III disease. C i-ii) Percentage of tumoral and stromal PD-L1 expression within the TME of ER+HER2- RCB II/III disease. C iii-iv) Percentage of PD-1 expression on immune cells within the invasive tumour (iii) and tumour stroma (iv) in ER+HER2- RCB II/III disease (Mann Whitney test).

**Figure S3| Cell family analysis of liquid CyTOF profiling of patient PBMCs.** A) t-SNE analysis of matched PBMCs samples from patients with RD (n=4) and pCR (n=4) at baseline, on-treatment and post-surgery showing expression of 16 immune cell populations. B) Log fold change in naïve and memory B cell clusters at post surgical timepoint, compared to baseline (n=14) LIMMA test.

**Figure S4 | Changes in cancer gene expression after chemotherapy in ER+HER2- and TNBC RCBII/III disease.** A) Heatmap showing expression of pan-cancer profiling gene set in TNBC residual disease samples from patients who received AC-T with or without additional platinum-based chemotherapy. B) Heatmap showing expression of cancer related genes in matched pre-treatment and residual disease ER+HER2- samples. C) Heatmap showing expression of cancer related genes in matched pre-treatment and residual disease TNBC samples.

## Supplementary table 1: Antibodies used for Vectra staining

Antibodies, optimised multiplex conditions and steps used to identify immune cells in human FFPE breast cancer samples

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Panel 01 | Panel 01 | Panel 01 | Panel 01 | Panel 02 | Panel 02 | Panel 02 | Panel 02 | Panel 01 & 02 |
| Antigen | CD8 | CD4 | FoxP3 | PD-1 | CD68 | CD20 | CD56 | PDL-1 | Cytokeratin |
| AR | AR9 (100 degrees) | AR9 (100 degrees) | AR9 (100 degrees) | AR9 (100 degrees) | AR9 (100 degrees) | AR9 (100 degrees) | AR9 (100 degrees) | AR6 (100 degrees) | AR9 (100 degrees) |
| TBST Wash (2m) | x3 | x3 | x3 | x3 | x3 | x3 | x3 | x3 | x3 |
| Block (10m) | x1 | x1 | x1 | x1 | x1 | x1 | x1 | x1 | x1 |
| Primary Antibody | Leica NCL-L-CD8-4B11 | Leica NCL-L-CD4-368 | Abcam 20034 | Abcam 52587 | Dako-M0867 | Dako-M0755 | Leica-NCL-CD56-504 | Cell Signal 13684 | Dako-M3515 |
| Dilution | 1:100 | 1:50 | 1:100 | 1:350 | 1:100 | 1:100 | 1:50 | 1:1000 | 1:100 |
| Incubation Time | 30m | 30m | 30m | 30m | 30m | 30m | 30m | 30m | 30m |
| Incubation Temp | RT | RT | RT | RT | RT | RT | RT | RT | RT |
| TBST Wash (2m) | x3 | x3 | x3 | x3 | x3 | x3 | x3 | x3 | x3 |
| Secondary Antibody | Anti-mouse | Anti-mouse | Anti-mouse | Anti-mouse | Anti-mouse | Anti-mouse | Anti-mouse | Anti-Rabbit | Anti-mouse |
| Incubation Time | 10m | 10m | 10m | 10m | 10m | 10m | 10m | 10m | 10m |
| TBST Wash (2m) | x3 | x3 | x3 | x3 | x3 | x3 | x3 | x3 | x3 |
| Opal Fluorophore | 620 | 520 | 570 | 650 | 570 | 520 | 620 | 520 | 690 |
| Opal Dilution | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 | 1:100 |
| Opal Incubation Time | 10m | 10m | 10m | 10m | 10m | 10m | 10m | 10m | 10m |
| TBST Wash (2m) | x2 | x2 | x2 | x2 | x2 | x2 | x2 | x2 | x2 |

## Supplementary table 2: CyTOF antibodies

Antibodies, metal tags and optimised concentrations used to stain PBMCs for CyTOF analysis

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Antigen | Symbol and Mass | Antibody clone | Volume of antibody (μl) | Source |
| CCR6 | 141Pr | G034E3 | 2 | Fluidigm |
| CD19 | 142Nd | HIB19 | 1 | Fluidigm |
| CD127 | 143Nd | A019D5 | 1 | Fluidigm |
| CD38 | 144Nd | HIT2 | 1 | Fluidigm |
| CD73 | 145Nd | AD2 | 1 | Biolegend |
| IgD | 146Nd | IA6-2 | 1 | Fluidigm |
| CD11c | 147Sm | Bu15 | 1 | Fluidigm |
| CD16 | 148Nd | 3G8 | 1 | Fluidigm |
| CCR4 | 149Sm | L291H4 | 1 | Fluidigm |
| CD294 (CRTH2) | 150Nd | BM16 | 1 | Biolegend |
| CD123 | 151Eu | 6H6 | 1 | Fluidigm |
| TCRgd | 152Sm | 11F2 | 1 | Fluidigm |
| CXCR5 | 153Eu | RF8B2 | 1 | Fluidigm |
| CD3 | 154Sm | UCHT1 | 1 | Fluidigm |
| CD45RA | 155Gd | HI100 | 1 | Fluidigm |
| CD117 (c-Kit) | 156Gd | 104D2 | 2 | Biolegend |
| CD27 | 158Gd | L128 | 1 | Fluidigm |
| CD103 | 159Tb | B-Ly7 | 1 | ThermoFisher Scientific |
| CD28 | 160Gd | CD28.2 | 1 | Fluidigm |
| CD66b | 162Dy | 80H3 | 1 | Fluidigm |
| CXCR3 | 163Dy | G025H7 | 1 | Fluidigm |
| CD161 | 164Dy | HP-3G10 | 1 | Fluidigm |
| CD45RO | 165Ho | UCHL1 | 1 | Fluidigm |
| CD24 | 166Er | ML5 | 1 | Fluidigm |
| CCR7 | 167Er | G043H7 | 1 | Fluidigm |
| CD8 | 168Er | SK1 | 1 | Fluidigm |
| CD25 | 169Tm | 2A3 | 1 | Fluidigm |
| NKp46 | 170Er | 9E2 | 2 | Biolegend |
| CD20 | 171Yb | 2H7 | 1 | Fluidigm |
| CD39 | 172Yb | A1 | 1 | Biolegend |
| HLA-DR | 173Yb | L243 | 1 | Fluidigm |
| CD4 | 174Yb | SK3 | 1 | Fluidigm |
| CD14 | 175Lu | M5E2 | 2 | Fluidigm |
| CD56 | 176Yb | NCAM16.2 | 1 | Fluidigm |
| CD45 | 89Y | HI30 | 1 | Fluidigm |

## Supplementary table 3: Differential immune checkpoint protein abundance analysis based on DSP data between post NAC vs pre-NAC for TNBC RCB II/III disease

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Sample 1 Log2FC | Sample 1  P value | Sample 1  Adjuvant P value (Q) | Sample 2 Log2FC | Sample 2  P value | Sample 2  Adjuvant P value (Q) | Sample 3 Log2FC | Sample 3  P value | Sample 3  Adjuvant P value (Q) | meta.P | meta.Q |
| CD44 | -1.5247921 | 0.00068988 | 0.00406261 | -5.088032 | 4.82E-06 | 4.49E-05 | -1.7702929 | 0.00034879 | 0.00472934 | 4.71E-10 | 2.49E-08 |
| VISTA | -0.7162135 | 0.00105802 | 0.00535274 | -1.8305431 | 3.94E-05 | 0.00011261 | 1.49115118 | 0.00062463 | 0.00472934 | 8.39E-09 | 1.48E-07 |
| PD-1 | -0.6042016 | 0.01599222 | 0.0339035 | -2.6157684 | 1.35E-06 | 4.49E-05 | -0.8255288 | 0.00699038 | 0.02646358 | 4.22E-08 | 5.60E-07 |
| OX40L | -1.2559695 | 0.00111095 | 0.00535274 | -3.7778594 | 0.00020117 | 0.00038161 | -1.0218566 | 0.00508929 | 0.02074866 | 2.66E-07 | 1.76E-06 |
| B7-H3 | -2.2999757 | 0.00019482 | 0.00206504 | -1.9419869 | 0.00251843 | 0.00325553 | -1.4214259 | 0.00454085 | 0.02005543 | 4.89E-07 | 2.88E-06 |
| LAG3 | -0.5987604 | 0.07549329 | 0.09758889 | -2.749193 | 2.87E-06 | 4.49E-05 | -0.6133663 | 0.02147369 | 0.0669474 | 9.52E-07 | 4.25E-06 |
| CTLA4 | -1.2910722 | 0.00064469 | 0.00406261 | -4.1328341 | 4.04E-05 | 0.00011261 | 0.50232183 | 0.18116994 | 0.2909699 | 9.62E-07 | 4.25E-06 |
| 4-1BB | -0.8103672 | 0.02645601 | 0.04835064 | -2.4241144 | 5.85E-06 | 4.49E-05 | -0.4352632 | 0.04874817 | 0.11743878 | 1.47E-06 | 4.86E-06 |
| MART-1/MelanA | -0.6255278 | 0.0498519 | 0.06953028 | -2.6835132 | 6.04E-06 | 4.49E-05 | -0.5850956 | 0.02824238 | 0.07933784 | 1.63E-06 | 5.09E-06 |
| PD-L2 | -0.4482234 | 0.09717356 | 0.11704997 | -2.6702071 | 0.00033871 | 0.00056099 | 2.93491917 | 0.00035124 | 0.00472934 | 2.15E-06 | 6.34E-06 |
| CD40 | -1.0514274 | 0.00485167 | 0.01285693 | -0.671968 | 0.00834879 | 0.01029037 | -0.6993447 | 0.00050044 | 0.00472934 | 3.56E-06 | 8.58E-06 |
| PD-L1 | -0.395734 | 0.14945438 | 0.17219743 | -2.5511522 | 1.00E-05 | 4.49E-05 | -0.4728593 | 0.06162192 | 0.14160259 | 1.37E-05 | 2.80E-05 |
| ICOS | -0.411526 | 0.01858753 | 0.03788997 | -1.1315679 | 0.00026971 | 0.00046112 | -0.4870302 | 0.02844187 | 0.07933784 | 2.01E-05 | 3.95E-05 |
| CD80 | -0.8336816 | 0.03722463 | 0.05802663 | -3.1320245 | 3.79E-05 | 0.00011261 | -0.352866 | 0.32096197 | 0.41490206 | 5.54E-05 | 9.48E-05 |
| CD86 | -1.5203476 | 0.00276427 | 0.00976708 | -2.7752187 | 0.00020161 | 0.00038161 | -0.0304518 | 0.9262279 | 0.9262279 | 6.21E-05 | 0.00010282 |
| CD27 | -0.6668322 | 0.00318391 | 0.00992629 | -1.5016293 | 0.00152718 | 0.00202351 | 0.98946295 | 0.13361287 | 0.23604941 | 7.58E-05 | 0.0001218 |
| GITR | -0.6951216 | 0.0462037 | 0.06618368 | -2.8214514 | 4.57E-05 | 0.0001154 | -0.1999315 | 0.67903358 | 0.76571871 | 0.00015058 | 0.00020463 |
| TIM-3 | -1.104998 | 0.00302914 | 0.00992629 | -2.1267204 | 0.00067496 | 0.00099369 | -0.0693863 | 0.77100821 | 0.79456453 | 0.00016333 | 0.00021641 |
| IDO-1 | 0.68587019 | 0.02976728 | 0.05089245 | -0.2105711 | 0.35150616 | 0.36529071 | 0.54126486 | 0.22031467 | 0.32435216 | 0.05880806 | 0.06111426 |

## Supplementary table 4: LIMMA test of changes in proportions of cell families on treatment and post surgery, compared to baseline, among patients achieving pCR

Results of LIMMA test showing log2 fold change values and p values associated with 4 seeds. Families showing statistical significance in at least 3 out of the 4 seeds tested with a LogFC >1.5 or <-1.5 are highlighted in bold

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **pCR – on treatment - 8 patient analysis** | | | | | | | | | | | |
|  | Seed 1234 | | | Seed 2244 | | | Seed 3324 | | | Seed 8729 | |
| Population of interest | logFC | P.Value | logFC | | P.Value | logFC | | P.Value | logFC | | P.Value |
| **B cell** | **-2.353** | **0.057** | **-2.176** | | **0.009** | **-2.578** | | **0.045** | **-2.622** | | **0.027** |
| CD4+ T cell | -0.380 | 0.377 | -0.169 | | 0.438 | -0.639 | | 0.268 | -0.399 | | 0.361 |
| CD8+ T cell | 0.021 | 0.910 | 0.040 | | 0.880 | 0.248 | | 0.062 | -0.145 | | 0.711 |
| Monocyte & DC | 1.077 | 0.394 | 0.637 | | 0.560 | 0.876 | | 0.448 | 0.786 | | 0.467 |
| NK cell | 0.262 | 0.237 | 0.362 | | 0.506 | 0.502 | | 0.242 | 0.273 | | 0.625 |
| Treg | 0.138 | 0.200 | -0.638 | | 0.446 | 0.285 | | 0.220 | 0.618 | | 0.130 |
| **pCR - post surgical - 8 patient analysis** | | | | | | | | | | | |
|  | Seed 1234 | | | Seed 2244 | | | Seed 3324 | | | Seed 8729 | |
| Population of interest | logFC | P.Value | logFC | | P.Value | logFC | | P.Value | logFC | | P.Value |
| B cell | -1.193 | 0.153 | -1.198 | | 0.120 | -1.278 | | 0.108 | -1.215 | | 0.192 |
| CD4+ T cell | -0.733 | 0.067 | -0.815 | | 0.030 | -0.782 | | 0.042 | -0.972 | | 0.051 |
| CD8+ T cell | 0.404 | 0.105 | 0.383 | | 0.161 | 0.457 | | 0.069 | 0.292 | | 0.440 |
| Monocyte & DC | 1.471 | 0.080 | 1.721 | | 0.030 | 1.817 | | 0.025 | 1.637 | | 0.082 |
| NK cell | 0.043 | 0.951 | 0.024 | | 0.978 | 0.189 | | 0.758 | 0.004 | | 0.995 |
| Treg | 0.445 | 0.012 | 0.178 | | 0.675 | 0.134 | | 0.640 | 0.719 | | 6.30E-07 |
| **pCR - post surgical - 14 patient analysis** | | | | | | | | | | | |
|  | Seed 1234 | | | Seed 2244 | | | Seed 3324 | | | Seed 8729 | |
| Population of interest | logFC | P.Value | logFC | | P.Value | logFC | | P.Value | logFC | | P.Value |
| B cell | -1.055 | 0.072 | -0.809 | | 0.255 | -0.408 | | 0.415 | -0.819 | | 0.229 |
| CD4+ T cell | -0.332 | 0.188 | -0.408 | | 0.067 | -0.562 | | 0.115 | -0.348 | | 0.073 |
| CD8+ T cell | 0.221 | 0.333 | 0.211 | | 0.333 | 0.209 | | 0.251 | 0.338 | | 0.076 |
| **Monocyte & DC** | **1.476** | **0.068** | **1.739** | | **0.012** | **1.537** | | **0.032** | **1.353** | | **0.013** |
| NK cell | -0.479 | 0.442 | -0.849 | | 0.233 | -0.472 | | 0.404 | -0.522 | | 0.406 |
| Treg | -0.270 | 0.520 | -0.116 | | 0.839 | 0.254 | | 0.270 | 0.440 | | 0.243 |

## Supplementary table 5: LIMMA test of changes in proportions of cell families on treatment and post surgery, compared to baseline, among patients with RD

Results of LIMMA test showing log2 fold change values and p values associated with 4 seeds. Families showing statistical significance in at least 3 out of the 4 seeds tested with a LogFC >1.5 or <-1.5 are highlighted in bold

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **RD - on treatment - 8 patient analysis** | | | | | | | | |
|  | Seed 1234 | | Seed 2244 | | Seed 3324 | | Seed 8729 | |
| Population of interest | logFC | P.Value | logFC | P.Value | logFC | P.Value | logFC | P.Value |
| **B cell** | **-3.330** | **0.019** | **-2.662** | **0.023** | **-3.216** | **0.014** | **-2.655** | **0.050** |
| CD4+ T cell | 0.087 | 0.501 | 0.126 | 0.327 | 0.156 | 0.340 | 0.046 | 0.685 |
| CD8+ T cell | 0.542 | 0.188 | 0.504 | 0.323 | 0.431 | 0.242 | 0.303 | 0.512 |
| Monocyte & DC | 0.040 | 0.973 | 0.411 | 0.652 | -0.144 | 0.903 | 0.971 | 0.100 |
| NK cell | 2.443 | 0.045 | 1.407 | 0.200 | 1.684 | 0.139 | 2.176 | 0.028 |
| Treg | 0.664 | 0.123 | -0.166 | 0.781 | 0.594 | 0.043 | 0.535 | 0.035 |
| **RD - post surgical - 8 patient analysis** | | | | | | | | |
|  | Seed 1234 | | Seed 2244 | | Seed 3324 | | Seed 8729 | |
| Population of interest | logFC | P.Value | logFC | P.Value | logFC | P.Value | logFC | P.Value |
| B cell | -0.979 | 0.362 | -1.327 | 0.275 | -0.888 | 0.448 | -1.344 | 0.392 |
| CD4+ T cell | -0.710 | 0.037 | -0.676 | 0.034 | -0.687 | 0.044 | -0.686 | 0.046 |
| CD8+ T cell | 0.539 | 0.228 | 0.513 | 0.202 | 0.557 | 0.279 | 0.386 | 0.299 |
| Monocyte & DC | 1.156 | 0.134 | 2.223 | 0.074 | 1.422 | 0.112 | 1.865 | 0.193 |
| **NK cell** | **3.235** | **2.75E-04** | **1.910** | **0.040** | **2.022** | **0.004** | **2.879** | **1.64E-11** |
| Treg | 1.348 | 0.001 | 0.782 | 0.104 | 0.553 | 0.039 | 0.321 | 0.150 |
| **RD - post surgical - 14 patients** | | | | | | | | |
|  | Seed 1234 | | Seed 2244 | | Seed 3324 | | Seed 8729 | |
| Population of interest | logFC | P.Value | logFC | P.Value | logFC | P.Value | logFC | P.Value |
| B cell | -1.302 | 0.057 | -1.229 | 0.055 | -1.263 | 0.114 | -1.606 | 0.027 |
| CD4+ T cell | -0.338 | 0.063 | -0.356 | 0.118 | -0.502 | 0.028 | -0.380 | 0.056 |
| CD8+ T cell | 0.506 | 0.033 | 0.456 | 0.077 | 0.456 | 0.074 | 0.441 | 0.084 |
| Monocyte & DC | 0.783 | 0.156 | 1.174 | 0.134 | 1.213 | 0.137 | 1.078 | 0.080 |
| **NK cell** | **1.644** | **0.047** | **1.677** | **0.028** | **2.105** | **0.012** | **1.287** | **0.066** |
| Treg | 0.600 | 0.132 | 0.076 | 0.811 | 0.635 | 0.032 | 0.100 | 0.850 |

## Supplementary table 6: Significantly upregulated immune genes in TNBC RCBII/III compared to ER+HER2- RCB II/III disease (FDR-adjusted P < 0.1; log2 FC > 1)

|  |  |  |
| --- | --- | --- |
|  | **log2 Fold Change** | **Adjusted P-value** |
| **PRAME** | 3.09497469 | 0.01748273 |
| **LCN2** | 2.78080874 | 0.00392877 |
| **CCL23** | 2.76711127 | 8.82E-09 |
| **IL12RB2** | 2.42960043 | 0.00105001 |
| **S100A8** | 2.40907818 | 0.01986055 |
| **S100A7** | 2.34933879 | 0.07920329 |
| **MASP2** | 2.2476727 | 4.16E-11 |
| **S100B** | 2.20234488 | 0.0267083 |
| **MARCO** | 2.14725378 | 0.00334457 |
| **TTK** | 2.12835852 | 0.00267514 |
| **BIRC5** | 1.9467763 | 0.02252643 |
| **ROPN1** | 1.84068322 | 0.0447601 |
| **IL8** | 1.79660174 | 0.02252643 |
| **CXCL5** | 1.76901653 | 0.00606892 |
| **MAGEA3** | 1.73972354 | 0.08694986 |
| **PBK** | 1.68440577 | 0.0163169 |
| **SPP1** | 1.67140881 | 0.05340114 |
| **ULBP2** | 1.59689994 | 0.00031499 |
| **CXCL1** | 1.59024189 | 0.01659241 |
| **LAMP3** | 1.56636814 | 0.00328015 |
| **CD24** | 1.52486983 | 0.00606892 |
| **IL22RA2** | 1.50836026 | 0.00135271 |
| **MAGEA4** | 1.47438643 | 0.00192013 |
| **CDK1** | 1.4740285 | 0.06880467 |
| **KIR\_1** | 1.41122411 | 0.01160824 |
| **CCL7** | 1.35682388 | 0.00606892 |
| **IDO1** | 1.3541314 | 0.02252643 |
| **MFGE8** | 1.32738397 | 0.00969749 |
| **GZMB** | 1.31581069 | 0.02339014 |
| **ITGB4** | 1.30300165 | 0.03782807 |
| **CXCL11** | 1.29083294 | 0.06790502 |
| **CD207** | 1.25260579 | 0.04063168 |
| **IL34** | 1.21428482 | 0.03172997 |
| **CXCL10** | 1.19664669 | 0.09332459 |
| **CARD9** | 1.18039927 | 0.00461661 |
| **TNFRSF11A** | 1.09969297 | 0.02339014 |
| **TREM1** | 1.08694323 | 0.07920329 |
| **CXCL3** | 1.01841902 | 0.0571479 |
| **CEBPB** | 1.01559035 | 0.00556273 |
| **RUNX3** | 1.0108552 | 0.03643133 |
| **C8G** | 1.00006443 | 0.00606892 |

## Supplementary table 7: Significantly downregulated immune genes in TNBC RCBII/III compared to ER+HER2- RCB II/III disease (FDR-adjusted P < 0.1; log2 FC < -1)

|  |  |  |
| --- | --- | --- |
|  | **log2 Fold Change** | **Adjusted P-value** |
| **GATA3** | -4.0379021 | 2.67E-09 |
| **TPSAB1** | -3.7373062 | 2.21E-05 |
| **C4B** | -2.8214261 | 2.01 E-03 |
| **MS4A2** | -2.7504664 | 8.82E-09 |
| **CXCL14** | -2.6107236 | 8.45 E-03 |
| **CTSG** | -2.5614254 | 7.87E-05 |
| **MUC1** | -2.3612477 | 3.50 E-03 |
| **CCL14** | -2.2676099 | 2.66 E-03 |
| **C7** | -2.2552449 | 7.42 E-03 |
| **IL1RL1** | -2.1551622 | 1.13E-06 |
| **IL6ST** | -2.1392209 | 6.44E-07 |
| **CMA1** | -1.9655402 | 0.0005755 |
| **DUSP4** | -1.8918982 | 0.0027111 |
| **CFD** | -1.8620983 | 0.00350931 |
| **ALCAM** | -1.8201491 | 0.00268867 |
| **COL3A1** | -1.740867 | 0.00606892 |
| **CD22** | -1.7365663 | 8.20E-05 |
| **FLT3** | -1.7297481 | 0.00022102 |
| **BCL2** | -1.6168336 | 0.00623058 |
| **RORC** | -1.5991993 | 0.05860043 |
| **FOXJ1** | -1.5907413 | 0.04015961 |
| **IGF1R** | -1.5195699 | 0.02755593 |
| **LRRN3** | -1.5059995 | 0.00657639 |
| **SMPD3** | -1.4243115 | 0.00101485 |
| **CX3CR1** | -1.35578 | 0.00350931 |
| **CD36** | -1.3467424 | 0.03857662 |
| **FOS** | -1.3440678 | 0.02252643 |
| **FCER1A** | -1.3332564 | 0.03198965 |
| **BST2** | -1.3331809 | 0.03172997 |
| **SIGIRR** | -1.3312545 | 0.00095367 |
| **CFB** | -1.2825964 | 0.05255611 |
| **BATF** | -1.2787656 | 0.00246985 |
| **CXCL12** | -1.2664067 | 0.01069081 |
| **TNFRSF10C** | -1.252745 | 0.01948862 |
| **F13A1** | -1.2251576 | 0.01400516 |
| **SPA17** | -1.2010757 | 0.0110453 |
| **BMI1** | -1.1723707 | 0.00020704 |
| **SYT17** | -1.1607324 | 0.0267083 |
| **NFATC2** | -1.1568081 | 0.00246985 |
| **IFITM2** | -1.1511091 | 0.00389133 |
| **CDKN1A** | -1.1440004 | 0.00606892 |
| **EGR1** | -1.1375165 | 0.09774278 |
| **LRP1** | -1.1228498 | 0.00405705 |
| **TARP** | -1.1216267 | 0.05201357 |
| **DPP4** | -1.1096515 | 0.01721588 |
| **JAM3** | -1.1088433 | 0.00606892 |
| **IKBKB** | -1.0994051 | 0.00135271 |
| **PDGFRB** | -1.0880165 | 0.00392877 |
| **NEFL** | -1.0627204 | 0.01659241 |
| **MPPED1** | -1.0609079 | 0.023602 |
| **NFATC4** | -1.0415186 | 0.01906239 |
| **TXNIP** | -1.0340124 | 0.00422307 |
| **IL1R1** | -1.0115308 | 0.03172997 |
| **COLEC12** | -1.0090322 | 0.03813559 |

## Supplementary table 8: Significantly upregulated pan-cancer genes in TNBC RCBII/III compared to ER+HER2- RCB II/III disease (FDR-adjusted P < 0.1; log2 FC > 1)

|  |  |  |
| --- | --- | --- |
|  | **log2 Fold Change** | **Adjusted P-value** |
| **MMP7** | 2.81551889 | 0.09230072 |
| **SFRP1** | 2.54625948 | 0.03058373 |
| **IL12RB2** | 2.44478745 | 0.01220459 |
| **CCNE1** | 2.30839596 | 0.00878092 |
| **SHC4** | 2.27308915 | 0.08675487 |
| **CCNA2** | 2.2713914 | 0.01901571 |
| **HMGA1** | 2.25292923 | 0.00399016 |
| **TLX1** | 2.0428526 | 0.09629565 |
| **FZD9** | 2.00710437 | 0.04559027 |
| **RASAL1** | 2.0006358 | 0.0051061 |
| **STMN1** | 1.95287941 | 0.01050243 |
| **LAMC2** | 1.93707739 | 0.04483336 |
| **TCF7L1** | 1.9116742 | 0.00548393 |
| **TTK** | 1.87564163 | 0.0361844 |
| **UBE2T** | 1.80099949 | 0.04765155 |
| **ITGB8** | 1.72853242 | 0.02267941 |
| **PKMYT1** | 1.72655851 | 0.07284936 |
| **SOX9** | 1.69279927 | 0.03346192 |
| **NPM2** | 1.6431394 | 0.07780744 |
| **CHEK1** | 1.63089936 | 0.04765155 |
| **ITGB4** | 1.60955522 | 0.09113943 |
| **CDC25A** | 1.59132845 | 0.08431089 |
| **PRKX** | 1.57698 | 0.01553502 |
| **ETV7** | 1.56531923 | 0.03442183 |
| **EZH2** | 1.54862935 | 0.02552517 |
| **BRIP1** | 1.53212512 | 0.04658094 |
| **SUV39H2** | 1.5076704 | 0.01550666 |
| **CCNB1** | 1.50088535 | 0.08744704 |
| **WNT5B** | 1.48770333 | 0.0679233 |
| **PRKDC** | 1.43220752 | 0.0161489 |
| **DTX4** | 1.41761307 | 0.02031678 |
| **COL27A1** | 1.40667015 | 0.07382312 |
| **PTCRA** | 1.37899952 | 0.00399016 |
| **MCM4** | 1.36921797 | 0.07519675 |
| **FEN1** | 1.31020868 | 0.05418397 |
| **VEGFA** | 1.30934137 | 0.07382312 |
| **MCM5** | 1.30782077 | 0.00406588 |
| **MSH6** | 1.29745617 | 0.00750502 |
| **SKP2** | 1.28898844 | 0.01553502 |
| **E2F5** | 1.28325332 | 0.09771601 |
| **HDAC2** | 1.26836277 | 0.00548393 |
| **PTTG2** | 1.26330799 | 0.09629565 |
| **MCM7** | 1.22924237 | 0.02157688 |
| **HSPA1A** | 1.21609777 | 0.07250389 |
| **H3F3A** | 1.20700651 | 0.00399016 |
| **ITGA6** | 1.19244042 | 0.08756901 |
| **MCM2** | 1.17958301 | 0.08713719 |
| **TFDP1** | 1.17095407 | 0.02247608 |
| **RAD21** | 1.14024569 | 0.0679233 |
| **MSH2** | 1.12915591 | 0.02552517 |
| **EGFR** | 1.11033087 | 0.0951987 |
| **RRAS2** | 1.07805236 | 0.04029891 |
| **DNMT1** | 1.05708939 | 0.01044149 |
| **POLE2** | 1.05517607 | 0.00878092 |
| **FANCE** | 1.03318178 | 0.08284694 |
| **FLNA** | 1.01693344 | 0.01322802 |

## Supplementary table 9: Significantly downregulated pan-cancer genes in TNBC RCBII/III compared to ER+HER2- RCB II/III disease (FDR-adjusted P < 0.1; log2 FC < -1)

|  |  |  |
| --- | --- | --- |
|  | **log2 Fold Change** | **Adjusted P-value** |
| GATA3 | 3.5070205 | 3.29E-05 |
| AR | -3.4351629 | 0.0002162 |
| SFRP4 | -3.1108148 | 0.01739259 |
| GATA2 | -3.0409591 | 0.00026226 |
| CACNA2D2 | -3.0306106 | 0.00019035 |
| MAPT | -2.849994 | 0.0024563 |
| BAIAP3 | -2.7821723 | 8.74E-06 |
| FGF10 | -2.5904979 | 0.00051416 |
| CACNA1H | -2.335507 | 0.00076084 |
| LRP2 | -2.2097149 | 0.05441206 |
| DUSP4 | -2.0957632 | -0.00758861 |
| THBS4 | -2.0407113 | 0.08713719 |
| TNN | -2.0170813 | 0.04834971 |
| PLA2G4F | -2.0155014 | 0.0161489 |
| KITLG | -2.0123505 | 0.00548393 |
| TGFB3 | -2.0068184 | 0.00521628 |
| CCND1 | -1.9972639 | 0.05421642 |
| BMP4 | -1.9776762 | 0.00548393 |
| FGFR3 | -1.9642357 | 0.08372042 |
| SHC2 | -1.9640831 | 0.01220459 |
| HSPA2 | -1.9477736 | 0.00132015 |
| IGF1 | -1.9469202 | 0.03649382 |
| CACNA1G | -1.9435154 | 0.0361844 |
| MYB | -1.9198175 | 0.05418397 |
| NTRK2 | -1.8332002 | 0.0854491 |
| FGF18 | -1.8320371 | 0.015835 |
| ITGA8 | -1.8098126 | 0.00521628 |
| CACNA1D | -1.7885591 | 0.01550666 |
| EPOR | -1.7542255 | 0.0002162 |
| DUSP5 | -1.6810875 | 0.00160041 |
| CHAD | -1.6799872 | 0.09893032 |
| COL4A6 | -1.6622882 | 0.05418397 |
| COL3A1 | -1.6506429 | 0.08709581 |
| NTRK1 | -1.6324194 | 0.04821104 |
| GHR | -1.6055314 | 0.02180975 |
| IRS1 | -1.5712991 | 0.0051061 |
| SOCS2 | -1.565339 | 0.02750839 |
| FUT8 | -1.5381339 | 0.00674055 |
| LEPR | -1.5174172 | 0.02837763 |
| GLI1 | -1.5050614 | 0.02180975 |
| RNF43 | -1.4955011 | 0.01231946 |
| FLNC | -1.4672519 | 0.05418397 |
| FGFR1 | -1.4588551 | 0.00548393 |
| NR4A1 | -1.4342732 | 0.09100549 |
| RUNX1T1 | -1.4278418 | 0.05804827 |
| PRLR | -1.421929 | 0.01050243 |
| RET | -1.4080384 | 0.08675487 |
| AXIN2 | -1.3929689 | 0.07847228 |
| FGF16 | -1.3788115 | 0.03545827 |
| RASGRF2 | -1.3574239 | 0.08284694 |
| HSPB1 | -1.3542525 | 0.06376945 |
| FLT3 | -1.3287892 | 0.05441206 |
| CXXC4 | -1.3162362 | 0.06829974 |
| CREB3L1 | -1.3089821 | 0.09473494 |
| LFNG | -1.2919178 | 0.0334016 |
| GLI3 | -1.2852866 | 0.01550666 |
| BCL2 | -1.2818184 | 0.08713719 |
| FGF12 | -1.2711689 | 0.06693932 |
| GNG7 | -1.2674389 | 0.03474795 |
| PDGFD | -1.2616634 | 0.07391723 |
| KIT | -1.2346213 | 0.08675487 |
| IL5RA | -1.1973788 | 0.03209098 |
| NODAL | -1.1855416 | 0.00750502 |
| CACNA1C | -1.183352 | 0.03075602 |
| DKK2 | -1.1790373 | 0.05418397 |
| CAMK2B | -1.1541391 | 0.08675487 |
| SETBP1 | -1.1351712 | 0.05418397 |
| HGF | -1.1019409 | 0.06693932 |
| FGF23 | -1.0741885 | 0.04347992 |
| SP1 | -1.0647393 | 0.0002162 |
| CDKN1C | -1.0589872 | 0.06693932 |
| PRKAR2B | -1.0400543 | 0.06693932 |
| FGF22 | -1.0200622 | 0.02267941 |
| CACNG1 | -1.0072634 | 0.09100549 |
| CRLF2 | -1.0059322 | 0.05509242 |
| HES1 | -1.0003417 | 0.05418397 |

## Supplementary table 10: Significantly differentially expressed immune genes in TNBC RCBII/III treated with anthracycline, cyclosphoshamide and taxane (AC-T) NAC compared to TNBC RCBII/III treated with AC-T plus carboplatin (FDR-adjusted P < 0.1; |log2 FC| > 1)

|  |  |  |
| --- | --- | --- |
|  | **log2 Fold Change** | **Adjusted P-value** |
| GZMA | 1.36368155 | 0.00611275 |
| KLRC2 | 1.14062163 | 0.02994612 |
| GZMM | 1.08579841 | 0.00611275 |
| CD209 | -1.0850005 | 0.01195353 |
| BTLA | -1.0975274 | 0.04175934 |
| DPP4 | -1.1818392 | 0.04175934 |
| MRC1 | -1.3482563 | 0.00031204 |
| FCER1A | -1.3506659 | 0.08745492 |
| C3 | -1.3817239 | 0.0758893 |
| F13A1 | -1.4055463 | 0.02896047 |
| KIT | -1.4190577 | 0.05286422 |
| SPO11 | -1.4733387 | 4.62E-06 |
| CD36 | -1.4968272 | 0.0758893 |
| MEFV | -2.128531 | 3.61E-11 |
| MPPED1 | -2.1605885 | 2.75E-05 |
| SPACA3 | -2.7431281 | 9.48E-13 |
| USP9Y | -3.4367038 | 2.86E-16 |
| FUT7 | -4.2418942 | 1.82E-23 |

## Supplementary table 11: Differentially expressed immunosuppressive genes in TNBC RCBII/III treated with anthracycline, cyclosphoshamide and taxane (AC-T) NAC compared to baseline like tumours (FDR-adjusted P < 0.1; |log2 FC| > 1)

|  |  |  |
| --- | --- | --- |
|  | **log2 Fold Change** | **Adjusted P-value** |
| MUC1 | -0.9614594 | 0.17376321 |
| MRC1 | -0.6972541 | 0.27089463 |
| IL10RA | -0.4538372 | 0.15518118 |
| CCL5 | 0.10890199 | 0.83687802 |
| CCL22 | 0.16230013 | 0.82076201 |
| CCL18 | 0.21037767 | 0.86320255 |
| IL10 | 0.21462043 | 0.65512215 |
| ARG1 | 0.60795114 | 0.26133535 |
| CCL17 | 0.68994481 | 0.337002 |