



Supplementary Materials

A Novel Three-Gene Score as a Predictive Biomarker for Pathologically Complete Response after Neoadjuvant Chemotherapy in Triple-Negative Breast Cancer

Masanori Oshi, Fernando A. Angarita, Yoshihisa Tokumaru, Li Yan, Ryusei Matsuyama, Itaru Endo and Kazuaki Takabe

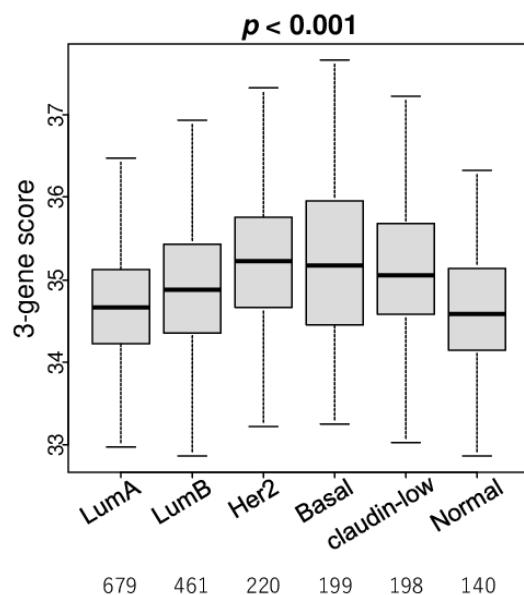


Figure S1. Association of 3-gene with PAM50 subtype in the METABRIC cohort. Boxplots of 3-gene by PAM50 subtype (LumA, LumB, Her2, Basal, claudin-low, and Normal).

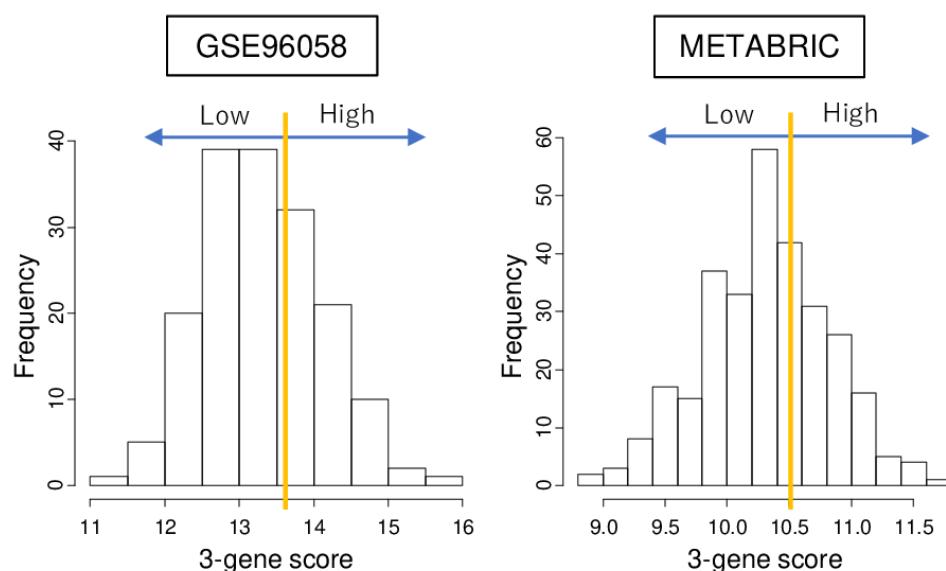


Figure S2. Histogram of the 3-gene score with cut-off line (top one-third) in the GSE96058 and METABRIC cohort.

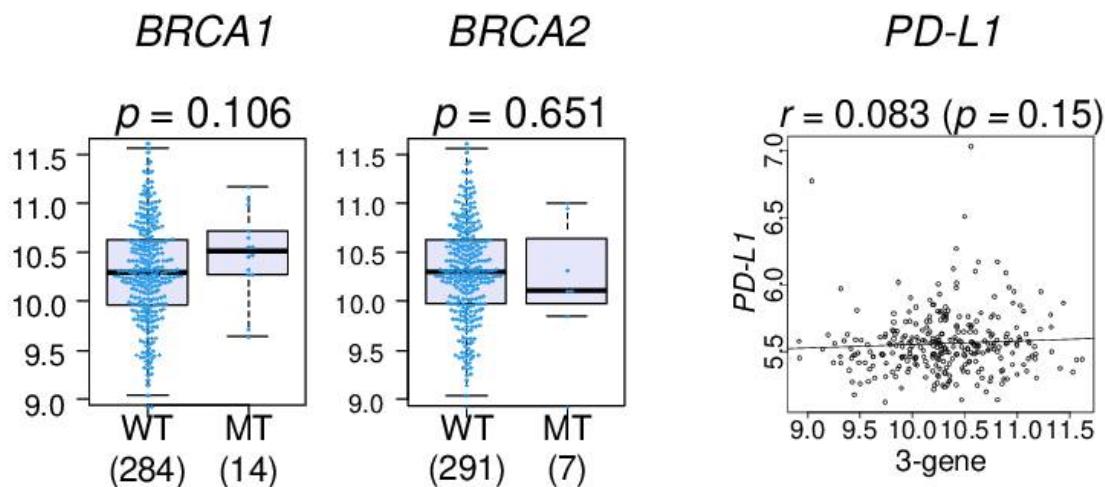


Figure S3. The association of the 3-gene score with *BRCA1* and *BRCA2* mutation, and *PD-L1* expression in TNBC in the METABRIC cohort. Boxplots with Mann-Whitney U *p*-value of the 3-gene score by *BRCA1* and *BRCA2* mutation (MT) and non-mutation (WT) group, and correlation plot with spearman rank correlation value between the 3-gene score and *PD-L1* expression in TNBC.

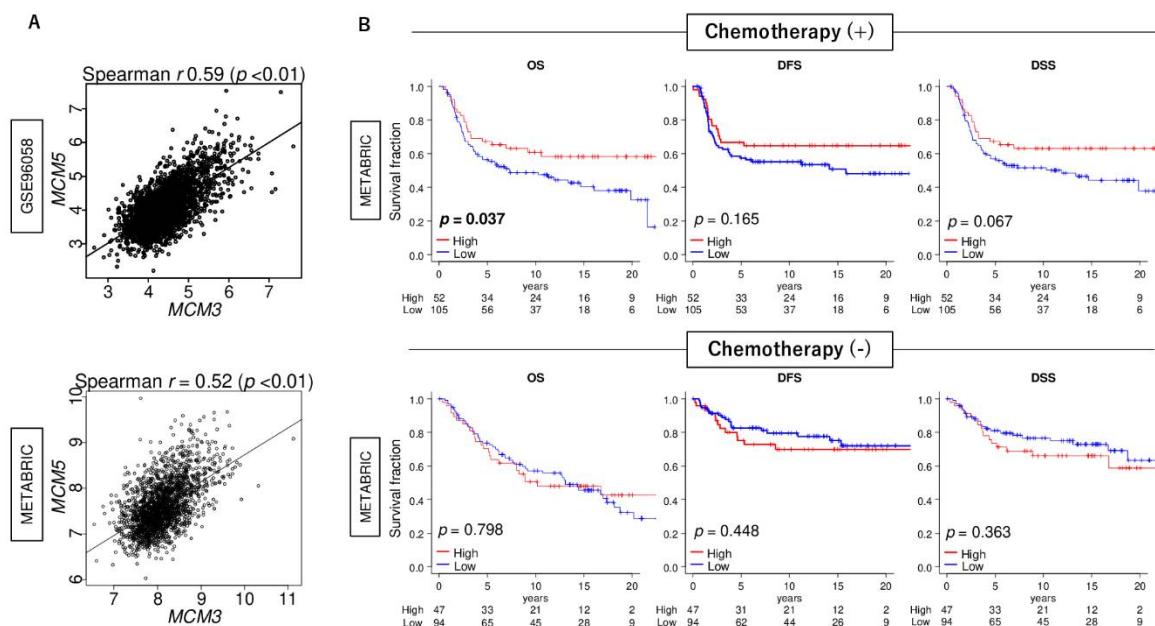


Figure S4. Association between the 3-gene score using *MCM5* and the survival of patients with TNBC with or without chemotherapy. (A) Correlation plots between *MCM3* and *MCM5* gene expression in the GSE96058 and METABRIC cohorts. Spearman rank correlation was used to the analysis. (B) Kaplan-Meier plots of comparison between low and high 3-gene (using *MCM5*) score groups for Overall Survival (OS), disease-free survival (DFS) and Disease Specific Survival (DSS) in treatment group and non-treatment group in the METABRIC cohort. The top one third was defined as the high score group within cohort. Log rank test was used to calculated the *p* value.

Table S1. Area under the curve (AUC) of each gene in E2F target gene sets in TNBC in the GSE25066 cohorts.

| Gene | AUC |
|---------------|-------|
| <i>CDKN2C</i> | 0.687 |
| <i>DEK</i> | 0.671 |
| <i>MCM5</i> | 0.666 |
| <i>TRA2B</i> | 0.659 |
| <i>MCM3</i> | 0.656 |

| | |
|-----------------|-------|
| <i>USP1</i> | 0.653 |
| <i>RPA1</i> | 0.645 |
| <i>STAG1</i> | 0.644 |
| <i>PRIM2</i> | 0.643 |
| <i>MSH2</i> | 0.642 |
| <i>RFC2</i> | 0.641 |
| <i>DEPDC1</i> | 0.640 |
| <i>WEE1</i> | 0.637 |
| <i>MCM6</i> | 0.633 |
| <i>PCNA</i> | 0.633 |
| <i>MCM2</i> | 0.632 |
| <i>PRDX4</i> | 0.628 |
| <i>PLK4</i> | 0.626 |
| <i>PAICS</i> | 0.623 |
| <i>H2AFZ</i> | 0.622 |
| <i>MELK</i> | 0.622 |
| <i>NASP</i> | 0.621 |
| <i>RBBP7</i> | 0.619 |
| <i>RAD51AP1</i> | 0.617 |
| <i>CCP110</i> | 0.617 |
| <i>RAD51C</i> | 0.615 |
| <i>ANP32E</i> | 0.615 |
| <i>CHEK1</i> | 0.614 |
| <i>BARD1</i> | 0.613 |
| <i>NUP153</i> | 0.610 |
| <i>ASF1A</i> | 0.610 |
| <i>TMPO</i> | 0.610 |
| <i>HMGB2</i> | 0.610 |
| <i>RAN</i> | 0.607 |
| <i>CKS1B</i> | 0.606 |
| <i>CDKN1B</i> | 0.603 |
| <i>CDKN2A</i> | 0.601 |
| <i>RPA3</i> | 0.601 |
| <i>KPNA2</i> | 0.601 |
| <i>SMC4</i> | 0.599 |
| <i>GINS1</i> | 0.598 |
| <i>TUBB</i> | 0.595 |
| <i>PSIP1</i> | 0.595 |
| <i>RAD50</i> | 0.595 |
| <i>RACGAP1</i> | 0.594 |
| <i>KIF4A</i> | 0.594 |
| <i>TOP2A</i> | 0.593 |
| <i>CENPM</i> | 0.593 |
| <i>RANBP1</i> | 0.592 |
| <i>UNG</i> | 0.590 |
| <i>DONSON</i> | 0.588 |
| <i>EZH2</i> | 0.588 |
| <i>KIF18B</i> | 0.585 |
| <i>MAD2L1</i> | 0.584 |
| <i>CDCA3</i> | 0.583 |
| <i>AURKB</i> | 0.583 |
| <i>HELLS</i> | 0.582 |
| <i>NOLC1</i> | 0.580 |
| <i>DNMT1</i> | 0.580 |
| <i>TP53</i> | 0.579 |
| <i>CBX5</i> | 0.578 |
| <i>TIPIN</i> | 0.578 |

| | |
|----------------|-------|
| <i>GINS3</i> | 0.578 |
| <i>RRM2</i> | 0.578 |
| <i>PSMC3IP</i> | 0.577 |
| <i>ESPL1</i> | 0.577 |
| <i>CCNE1</i> | 0.574 |
| <i>SMC3</i> | 0.574 |
| <i>CENPE</i> | 0.574 |
| <i>GINS4</i> | 0.573 |
| <i>MLH1</i> | 0.573 |
| <i>SNRPB</i> | 0.572 |
| <i>BRCA1</i> | 0.571 |
| <i>POLD3</i> | 0.569 |
| <i>DIAPH3</i> | 0.568 |
| <i>CHEK2</i> | 0.568 |
| <i>CDK1</i> | 0.567 |
| <i>CDCA8</i> | 0.567 |
| <i>TUBG1</i> | 0.567 |
| <i>SRSF1</i> | 0.567 |
| <i>MXD3</i> | 0.567 |
| <i>UBE2S</i> | 0.566 |
| <i>RFC3</i> | 0.566 |
| <i>ATAD2</i> | 0.565 |
| <i>ORC2</i> | 0.565 |
| <i>CTPS1</i> | 0.565 |
| <i>CDC20</i> | 0.564 |
| <i>CDC25A</i> | 0.564 |
| <i>MKI67</i> | 0.564 |
| <i>RFC1</i> | 0.564 |
| <i>TK1</i> | 0.563 |
| <i>SYNCRIP</i> | 0.563 |
| <i>CKS2</i> | 0.563 |
| <i>DCK</i> | 0.563 |
| <i>CCNB2</i> | 0.563 |
| <i>XPO1</i> | 0.563 |
| <i>E2F8</i> | 0.562 |
| <i>SPAG5</i> | 0.562 |
| <i>LIG1</i> | 0.561 |
| <i>TBRG4</i> | 0.560 |
| <i>PPP1R8</i> | 0.560 |
| <i>PPM1D</i> | 0.560 |
| <i>ILF3</i> | 0.560 |
| <i>POLE</i> | 0.559 |
| <i>CDK4</i> | 0.557 |
| <i>LMNB1</i> | 0.557 |
| <i>PNN</i> | 0.557 |
| <i>GSPT1</i> | 0.557 |
| <i>BIRC5</i> | 0.556 |
| <i>PLK1</i> | 0.555 |
| <i>KIF2C</i> | 0.554 |
| <i>POLA2</i> | 0.554 |
| <i>MCM7</i> | 0.553 |
| <i>XRCC6</i> | 0.551 |
| <i>NBN</i> | 0.551 |
| <i>STMN1</i> | 0.551 |
| <i>HMMR</i> | 0.550 |
| <i>SHMT1</i> | 0.549 |
| <i>MYBL2</i> | 0.548 |

| | |
|-----------------|-------|
| <i>NUDT21</i> | 0.546 |
| <i>LUC7L3</i> | 0.545 |
| <i>DDX39A</i> | 0.545 |
| <i>EXOSC8</i> | 0.543 |
| <i>DLGAP5</i> | 0.542 |
| <i>CDC25B</i> | 0.542 |
| <i>SPC25</i> | 0.542 |
| <i>PA2G4</i> | 0.539 |
| <i>RAD21</i> | 0.538 |
| <i>NUP205</i> | 0.537 |
| <i>MYC</i> | 0.537 |
| <i>PRKDC</i> | 0.536 |
| <i>HMGB3</i> | 0.536 |
| <i>TRIP13</i> | 0.533 |
| <i>POLD1</i> | 0.532 |
| <i>DUT</i> | 0.531 |
| <i>HMGA1</i> | 0.530 |
| <i>LBR</i> | 0.530 |
| <i>CDKN1A</i> | 0.528 |
| <i>PTTG1</i> | 0.528 |
| <i>AK2</i> | 0.527 |
| <i>ING3</i> | 0.527 |
| <i>MCM4</i> | 0.526 |
| <i>PDS5B</i> | 0.525 |
| <i>BUB1B</i> | 0.524 |
| <i>CTCF</i> | 0.522 |
| <i>EED</i> | 0.521 |
| <i>KIF22</i> | 0.521 |
| <i>BRCA2</i> | 0.521 |
| <i>ZW10</i> | 0.520 |
| <i>SSRP1</i> | 0.519 |
| <i>DCLRE1B</i> | 0.519 |
| <i>SMC1A</i> | 0.519 |
| <i>POP7</i> | 0.518 |
| <i>NUP107</i> | 0.518 |
| <i>NAP1L1</i> | 0.518 |
| <i>CDKN3</i> | 0.518 |
| <i>NCAPD2</i> | 0.517 |
| <i>TIMELESS</i> | 0.517 |
| <i>DSCC1</i> | 0.516 |
| <i>IPO7</i> | 0.516 |
| <i>TFRC</i> | 0.516 |
| <i>ORC6</i> | 0.515 |
| <i>PAN2</i> | 0.515 |
| <i>CSE1L</i> | 0.514 |
| <i>MTHFD2</i> | 0.513 |
| <i>NME1</i> | 0.511 |
| <i>POLD2</i> | 0.511 |
| <i>H2AFX</i> | 0.511 |
| <i>SPC24</i> | 0.511 |
| <i>DCTPP1</i> | 0.510 |
| <i>TACC3</i> | 0.510 |
| <i>RAD1</i> | 0.510 |
| <i>SMC6</i> | 0.509 |
| <i>SUV39H1</i> | 0.509 |
| <i>HUS1</i> | 0.509 |
| <i>RNASEH2A</i> | 0.508 |

| | |
|---------------|-------|
| <i>EIF2S1</i> | 0.508 |
| <i>MRE11A</i> | 0.507 |
| <i>SLBP</i> | 0.507 |
| <i>ASF1B</i> | 0.506 |
| <i>RPA2</i> | 0.506 |
| <i>UBR7</i> | 0.505 |
| <i>HNRNPD</i> | 0.501 |
| <i>PRPS1</i> | 0.500 |

Table S2. Triple-negative breast cancer cell lines.

| <u>Cell Line Name</u> |
|-----------------------|
| BT20 |
| BT549 |
| CAL120 |
| CAL148 |
| CAL51 |
| CAL851 |
| DU4475 |
| HCC1143 |
| HCC1187 |
| HCC1395 |
| HCC1599 |
| HCC1806 |
| HCC1937 |
| HCC2157 |
| HCC38 |
| HCC70 |
| HDQP1 |
| HMC18 |
| HS578T |
| MDAMB157 |
| MDAMB231 |
| MDAMB436 |
| MDAMB453 |
| MDAMB468 |
| MFM223 |
| SUM102PT |
| SUM1315MO2 |
| SUM149PT |
| SUM159PT |
| SUM185PE |
| SUM229PE |