Clinical impact of tumor DNA repair expression and T cell infiltration in breast cancers

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ABSTRACT

Impaired DNA repair drives mutagenicity, which increases neoantigen load and immunogenicity. We investigated the expression of proteins involved in the DNA damage response (ATM, Chk2), double-strand break repair (BRCA1, BLM, WRN, RECQL4, RECQL5, TOPO2A, DNA-PKcs, Ku70/Ku80), nucleotide excision repair (ERCC1), base excision repair (XRCC1, pol β, FEN1, PARP1), and immune responses (CD8, PD-1, PD-L1, FOXP3) in 1269 breast cancers and validated our findings in an independent estrogen receptor $(ER)^-$ cohort ($n = 279$). Patients with tumors that expressed low XRCC1, low ATM, and low BRCA1 were not only associated with high numbers of $CD8^+$ tumor-infiltrating lymphocyte (TILs), but were also linked to higher grades, high proliferation indexes, presence of dedifferentiated cells, ER^- cells and poor survival (all $P \leq 0.01$). PD-1^+ or PD-$L1^+$ breast cancers with low XRCC1 were also linked to an aggressive phenotype that was high grade, had high proliferation indexes, contained dedifferentiated cells and ER^- (all with P values ≤ 0.01) and poor survival ($P = 0.00021$ and $P = 0.00022$, for PD-1^+ and PD-$L1^+$ cancers, respectively) including in an independent ER^- validation cohort ($P = 0.007$ and $P = 0.047$, respectively). We conclude that the interplay between DNA repair, CD8, PD-L1, and PD-1 can promote aggressive tumor phenotypes. XRCC1-directed personalization of immune checkpoint inhibitor therapy may be feasible and warrants further investigation in breast cancer.

INTRODUCTION

The breast cancer tumor microenvironment includes infiltrating inflammatory cells such as lymphocytes and macrophages. $CD8^+$ T-lymphocytes are critical for tumor-specific adaptive immunity (1). We have previously investigated the clinicopathological and prognostic significance of tumor-infiltrating $CD8^+$ T lymphocytes (TILs) in a large cohort of breast cancers (2). $CD8^+$ TILs were correlated to high tumor grade, hormone receptor negative, and basal-like phenotype. High total $CD8^+$ counts were independently associated with favorable clinical outcome (2). A recent large study in 12,439 breast cancers has provided confirmatory evidence that $CD8^+$ TILs are associated with significant reduction in the relative risk of death in estrogen receptor $(ER)^-$ as well as in $ER^+/HER\text{-}2^+$ breast cancers (3).

Breast cancers with enhanced immunogenicity will be prone to attack by $CD8^+$ T lymphocytes. Impaired DNA repair and the associated genomic instability not only leads to increased mutagenicity/carcinogenicity but can also increase neoantigen load on tumor cell surface resulting in increased immunogenicity. This concept of DNA repair deficiency and enhanced immunogenicity was shown in mismatch repair (MMR)-deficient colorectal cancers that had a good response to PD-1 blockade (pembrolizumab) therapy compared to tumors that are MMR proficient (4, 5). Whether a similar mechanism can also operate in breast cancers is currently unknown. In the current study we profiled proteins involved in the DNA damage response (ATM, Chk2), double strand break repair (BRCA1, BLM, WRN, RECQL4, RECQL5, TOPO2A, DNA-PKcs, Ku70/Ku80), nucleotide excision repair (ERCC1), base excision repair (XRCC1, pol β, FEN1, PARP1) and immune response (CD8, PD-1, PD-L1, FOXP3]) in 1269 breast cancers and validated in an independent ER-cohort (n = 279).

PATIENTS AND METHODS

The study was performed in a consecutive series of 1650 patients with primary invasive breast carcinomas who were diagnosed between 1986 and 1998 and enrolled into the Nottingham Tenovus Primary Breast Carcinoma series. Patient demographics are summarized in **Supplementary Table S1**. This is a well-characterized series of patients with long-term follow-up that have been investigated in a wide range of biomarker studies (6-14). All patients were treated in a uniform way in a single institution with standard surgery (mastectomy or wide local excision), followed by radiotherapy. Prior to 1989, patients did not receive systemic adjuvant treatment (AT). After 1989, AT was scheduled based on prognostic and predictive factor status, including Nottingham prognostic index (NPI), estrogen receptor-α (ER-α) status, and menopausal status. Patients with NPI scores of < 3.4 (low risk) did not receive AT. In premenopausal patients with NPI scores of ≥ 3.4 (high risk), classical cyclophosphamide, methotrexate, and 5-flurouracil (CMF) chemotherapy was given; patients with ER-α positive tumors were also offered endocrine therapy. Postmenopausal patients with NPI scores of ≥ 3.4 and ER positivity were offered endocrine therapy, whereas ER negative patients received classical CMF chemotherapy. Median follow up time was 111 months (range 1 to 233 months). Breast cancer–specific survival (BCSS) data was maintained on a prospective basis and was defined as the number of months from diagnosis to the occurrence of BC-related death. Survival was censored if the patient was still alive at the time of analysis, lost to follow-up, or died from other causes.

We also evaluated an independent series of 279 ER-α negative invasive BCs diagnosed and managed at the Nottingham University Hospitals between 1999 and 2007. All patients were primarily treated with surgery, followed by radiotherapy and anthracycline/CMF chemotherapy. The characteristics of this cohort are summarized in **Supplementary Table S2**.

Tissue Microarrays (TMAs) and immunohistochemistry (IHC): Tumors were arrayed in tissue microarrays (TMAs) constructed with 0.6mm cores. The TMAs were immunohistochemically profiled for ATM, Chk2, BRCA1, BLM, WRN, RECQL4, RECQL5, TOPO2A, DNA-PKcs, Ku70/Ku80, ERCC XRCC1, pol β, FEN1, PARP1, CD8, and FOXP3 expression as previously described (2, 6-15).

Supplementary Table S3 summarizes antigens, primary antibodies, clone, source, optimal dilution, scoring system and cut-offs used for each DNA repair marker, ER, PR and HER-2 expression. The specificity of the antibodies used is described in our recent publications (2, 6-15).

IHC protocol: Detailed IHC protocol and evaluation of immune staining is summarized in **supplementary Table S3**. Immunohistochemical staining was performed using the Thermo Scientific Shandon Sequenza chamber system (REF: 72110017), in combination with the Novolink Max Polymer Detection System (RE7280-K: 1250 tests), and the Leica Bond Primary Antibody Diluent (AR9352), each used according to the manufacturer's instructions (Leica Microsystems). Leica Autostainer XL machine was used to dewax and rehydrate the slides. Pre-treatment antigen retrieval was performed on the TMA sections using sodium citrate buffer (pH 6.0) and heated for 20 minutes at 95^0C in a microwave (Whirlpool JT359 Jet Chef 1000W). Negative and positive (by omission of the primary antibody and IgG-matched serum) controls were included for each marker in each run. The negative control ensured that all the staining was produced from the specific interaction between antibody and antigen. HER2 expression was assessed according to the new ASCO/CAP guidelines using IHC and fluorescence *in situ* hybridization (FISH) (16).

Evaluation of immune staining: Whole field inspection of the core was scored and intensities of nuclear staining for DNA repair markers were grouped as follows: 0 = no staining, 1 = weak staining, 2 = moderate staining, 3 = strong staining. The percentage of each category was estimated (0-100%). H-score (range 0-300) was calculated by multiplying intensity of staining and percentage staining. The number of $CD8^+$ T lymphocytes was counted in each tumor core by using a Nikon Eclipse 80i microscope (Nikon, Tokyo, Japan) and an eyepiece graticule. $CD8^+$and $FOXP3^+$ T cells were counted in three locations in each tumor: intratumoral compartment (within the tumor cell nests), within the distant stroma (defined as more than one tumor cell diameter away from the tumor), and within the adjacent stroma (defined as $CD8^+$ cells within one tumor cell diameter of the tumor). The total number of $CD8^+$ T cells was determined by combining the counts for these three compartments.

Not all cores within the TMA were suitable for IHC assessments as some cores were missing or containing inadequate invasive tumor (< 15% of whole core surface area). Tumor marker prognostic studies (REMARK) criteria, recommended by McShane et al., (17), were followed throughout this study. Ethical approval was obtained from the Nottingham Research Ethics Committee (C202313).

Statistical analysis: Data analysis was performed using SPSS (SPSS, version 21 Chicago, IL). Where appropriate, Pearson's χ^2, Fisher's exact, the student *t* and one-way ANOVA tests were used. Cumulative survival probabilities were estimated using the Kaplan–Meier method, and differences between survival rates were tested for significance using the log-rank test. Multivariate analysis for survival was performed using the Cox proportional hazard model. The proportional hazards assumption was tested using standard log-log plots. Hazard ratios (HR) and 95% confidence intervals (95% CI) were estimated for each variable. All tests were two-sided with a 95% CI and $P < 0.05$ considered significant. For multiple comparisons, *P* values were adjusted according to the Benjamini-Hochberg method (18).

RESULTS

Significance of ATM, BRCA1, and XRCC1 in CD8$^+$ TIL-positive breast cancers

CD8$^+$ T-lymphocytes are critical for tumor-specific adaptive immunity (1). A total of 1269 invasive breast carcinomas [ER$^+$ (*n* = 928), ER$^-$ (*n* = 341), triple negative (*n* = 219), HER2$^+$/ER$^-$ (*n* = 92), and HER2$^+$/ER$^+$ (*n* = 89)] were suitable for CD8$^+$ TIL assessments; 1032 were positive for CD8$^+$ TILs and 237 cases were negative for CD8$^+$ TILs (**Fig. 1A1–1A4**).

Low ATM, BRCA1, and XRCC1 expression was associated with poor BCSS in tumors with CD8$^+$ TILs (*P* = 0.006, 0.001, and 0.000011, respectively; **Fig. 1A9-A12, B, C, and D**), but not in tumors negative for CD8$^+$ TILs (*P* = 0.217, 0.723, and 0.249, respectively; **Supplementary Fig. S1A, B, and C**). Expression of pol β, ERCC1, RECQL4, RECQL5, BLM, PARP1, FEN1, TOPO2A, Ku70/Ku80, and Chk2 was not significantly associated with survival in CD8$^+$ TIL–positive or –negative breast cancers (**Supplementary Fig. S2A–2T)**. In tumors positive for CD8$^+$ TILs, WRN did not influence survival (*P* = 0.332, **Supplementary Fig. S2U)** but in tumors negative for CD8$^+$ TILs, low WRN influenced survival (*P* = 0.026, **Supplementary Fig. S2V**). Similarly, in tumors positive for CD8$^+$ TILs, DNA-PKcs did not influence survival (*P* = 0.996, **Supplementary Fig. S2W)** but in tumors negative for CD8$^+$ TILs, low DNA-PKcs influenced poor survival (*P* = 0.044, **Supplementary Fig. S2X**).

To investigate whether low tumor ATM, BRCA1 or XRCC1 expression increased CD8$^+$ TILs counts and resulted in an aggressive phenotype, we proceeded to investigate clinicopathological associations. The mean CD8$^+$ TIL counts were significantly higher in tumors with low ATM (*P* = 0.004), low BRCA1 (*P* = 2.4 x 10 $^{-9}$) and low XRCC1 (*P* = 0.007; **Supplementary Fig. S3**). Tumors with low ATM, low BRCA1, or low XRCC1 and that contained CD8$^+$ TILs were significantly more likely to manifest aggressive features, including high grade, high mitotic index, de-differentiation, ER negativity, and PR negativity (all adjusted *P* values ≤ 0.05; **Supplementary Tables S4, S5 and S6**).

Significance of ATM, BRCA1 and XRCC1 in FOXP3$^+$ breast cancers

T regulatory cells (Tregs) can inhibit antitumor responses. FOXP3, a member of the forkhead family of transcription factors, is restricted to specific population of Tregs (19). In FOXP3$^+$ breast cancers (**Fig. 1A5**), low BRCA1 (P = 0.016) and low XRCC1 (P = 0.000002) expression influenced survival but ATM did not (P = 0.536) (**Supplementary Fig. S4A-C)**. On the other hand, in FOXP3 negative breast cancers, low ATM influenced poor BCSS (P = 0.001) (**Supplementary Fig. S4D)** but BRCA1 and XRCC1 amounts did not (P = 0.556 and 0.084, respectively) (**Supplementary Fig. S4E and F)**. Low ATM, low BRCA1 or low XRCC1 and FOXP3+ breast cancers were highly significantly associated with high grade, high risk NPI, high mitotic index, pleomorphism, HER-2$^+$, ER$^-$ and PR$^-$ phenotypes (all adjusted p values <0.0001) (**Supplementary Tables S7, S8, and S9**). The data provides compelling evidence that Tregs infiltration along with tumor DNA repair expression can influence breast cancer pathology and outcomes.

Significance of ATM, BRCA1, and XRCC1 in PD-L1$^+$/PD-1$^+$ breast cancers

Programmed death ligand-1 (PD-L1) and programmed death-1 (PD-1) are key members of the PD pathway involved in immune regulation. The interaction of PD-L1 with PD-1 induces T cell suppression. Accordingly the PD-L1/PD-1 pathway has emerged as a key target for immune checkpoint inhibitor therapy (20). We investigated ATM, BRCA1, and XRCC1 expression in PD-L1$^+$/PD-1$^+$ or PD-L1$^-$/PD-1$^-$ breast cancers (**Figures 1A6-A8**).

Low XRCC1 expression was associated with poor BCSS in PD-L1$^+$ (tumor cells), PD-L1$^+$ (TILs), and PD-1$^+$ (TILs) breast cancers (***P*** = 0.00021, 0.007, and 0.00022, respectively; **Fig. 1E, F, and G**). ATM and BRCA1 amounts did not influence survival in PD-L1$^+$/ or PD-1$^+$ breast cancers (**Supplementary Fig. S5A–F)**. In PD-L1$^-$ breast cancers, ATM, BRCA1 and XRCC1 did not influence survival (**Supplementary Fig. S6A–F**). In PD-1$^-$ breast cancers, low ATM, low BRCA1, and low XRCC1 were associated with poor BCSS (**Supplementary Fig. S6G–I**).

In PD-L1$^+$ (tumor cells) (**Table 1)**, PD-L1$^+$ (TILs) (**Table 2)**, or PD-1$^+$ (TILs) (**Table 3)** breast cancers, low XRCC1 amounts were significantly associated with aggressive features including high grade, high risk NPI, high mitotic index, pleomorphism, ER negativity and PR negativity (all adjusted P values < 0.001). Taken

together, the data provides evidence that low XRCC1 expression was associated with aggressive phenotype and poor outcomes in PD-L1$^+$ and PD-1$^+$ breast cancers. We then proceeded to investigate in breast cancers subgroups.

Prognostic significance of ATM, BRCA1, and XRCC1 in ER$^+$ or HER-2$^+$ breast cancers

In ER$^+$ tumors, although CD8$^+$ TILs alone did not influence survival (**Supplementary Fig. S7A– C**), low BRCA1 (P = 0.002) and low XRCC1 (P <0.0001) **were** linked to poor BCSS (**Supplementary Fig. S8A and B**). Low ATM expression was not significant (P = 0.080; **Supplementary Fig. S8C**).

In PD-1$^+$/PD-L1$^+$ (TILs) or PD-L1$^+$ (tumor cells) ER$^+$ breast cancers that received endocrine therapy, ATM, BRCA1, or XRCC1 did not influence survival (unpublished observations). However, XRCC1 expression influenced survival in CD8$^+$, PD-1$^+$, or FOXP3$^+$ ER$^+$ breast cancers that received no endocrine therapy (P = 0.02, 0.038, and 0.026 respectively; **Supplementary Fig. S9A–C**) but was not significant to PD-L1$^+$ (tumor cells) breast cancers (**Supplementary Fig. S9D**, P = 0.078).

XRCC1 amounts influenced survival in PD-1$^+$ HER-2$^+$ breast cancers (P = 0.011; **Supplementary Fig. S10A**). ATM and BRCA1 did not associate with survival in CD8$^+$, FOXP3$^+$, or PD-1$^+$ HER-2$^+$ breast cancers (unpublished observations). BRCA1 expression was borderline associated with survival in FOXP3$^+$ HER-2$^+$ breast cancers (P = 0.05; **Supplementary Fig. S10B**). ATM and XRCC1 did not associate with survival in FOXP3$^+$ HER-2$^+$ breast cancers (unpublished observations). ATM, BRCA1, or XRCC1 did not influence survival in PD-L1$^+$ (TILs) or PD-L1$^+$ (tumor cells) HER2$^+$ breast cancers (unpublished observations).

Prognostic significance of ATM, BRCA1, and XRCC1 in ER$^–$ breast cancers

As expected, CD8$^+$ TILs alone was associated with longer survival in ER$^–$ tumors (P = 0.013) (**Supplementary Fig. S11A**) including in patients who received no chemotherapy (P = 0.029) (**Supplementary Fig. S11B**), but was not significant to patients who received CMF based chemotherapy (P = 0.081) (**Supplementary Fig. S11C**). In ER$^–$ tumors with CD8$^+$ TILs that received CMF chemotherapy, low ATM, low BRCA1, or low XRCC1 did not influence survival (unpublished observations).

In PD-L1$^+$ (TILs) ER$^-$ breast cancers that received no chemotherapy, BRCA1 amounts influenced survival (**Supplementary Fig. S12**), but ATM and XRCC1 did not (unpublished observations). ATM, BRCA1, or XRCC1 did not influence survival in FOXP3$^+$, PD-1$^+$, or PD-L1$^+$ (tumor cells) ER$^-$ breast cancers that received no chemotherapy (unpublished observations). Similarly, ATM, BRCA1, or XRCC1 did not influence survival in FOXP3$^+$, PD-1$^+$, PD-L1$^+$ (TILs) or PD-L1$^+$ (tumor cells) ER$^-$ breast cancers that received CMF chemotherapy (unpublished observations).

We then proceeded to investigate an independent ER$^-$ cohort that received modern anthracycline based chemotherapy. Low XRCC1 expression was associated with poor survival in PD-L1$^+$ (TILs) and PD-1$^+$ (TILs) ER$^-$ breast cancers ($P = 0.047$ and $P = 0.007$ respectively; **Fig. 1I, 1J**). Low XRCC1 expression was not significant ($P = 0.089$) to PD-L1$^+$ (tumor cells) ER$^-$ breast cancers (**Fig. 1H**). ATM or BRCA1 did not influence survival in this cohort (unpublished observations).

DISCUSSION

The presence of TILs is a marker for a good prognosis (1-3, 21, 22) and predicts a favorable response to neoadjuvant chemotherapy in breast cancer (23). Although the biological mechanisms are poorly understood, immune effector cells, their cytokine secretions, or cancer cell immunogenicity may influence biology and antitumor response in breast cancer. In addition, chemotherapy-induced Treg depletion, could also allow pre-existing immune-effector cells to operate effectively and induce antitumor responses (1). $CD8^+$ TILs can be a good marker of response to neoadjuvant chemotherapy, providing evidence that specific immune-effector cells could be essential (23). Another possibility is that the immunogenicity of tumor cells themselves could influence immune-effector cell anticancer activity. Tumor cells with abundant surface neoantigens will be prone to immune-attack compared to tumor cells with low neoantigen load. Emerging data provide evidence that tumors with many somatic mutations accumulate neoantigens and are highly immunogenic (5). A key determinant of mutation load is the DNA repair capacity in cancer cells. DNA repair–deficient cancers have increased genomic instability, leading to a 'mutator phenotype' characterized by the accumulation of mutations. For example, MMR-deficient colorectal cancers not only have 10 to 100 times more somatic mutations compared MMR-proficient colorectal tumors, but also have prominent lymphocytic infiltration (5). A pivotal phase II study of PD-1 blockade by pembrolizumab provided the first compelling evidence that MMR-deficient colorectal cancer are more responsive to immune checkpoint inhibitor therapy compared to MMR-proficient tumors (5). In breast cancers, however, MMR deficiency is rare (24), suggesting that impairment of other DNA repair factors may influence prognosis in tumors with immune cell infiltration.

In the current study, a key initial observation was that low amounts of ATM, BRCA1, and XRCC1 increased $CD8^+$ TIL infiltration, and was associated with aggressive pathology, leading to poor patient survival. Germ-line mutations in ATM or BRCA1 predispose to hereditary breast cancers. In sporadic breast cancers, epigenetic silencing of the BRCA1 promoter has been reported in up to 11%–14% of tumors. About 25% of breast cancers may have a dysfunctional BRCA pathway in which they do not harbor germ-line BRCA mutations, but display similar phenotypes, including HR deficiency. XRCC1 deficiency delays SSB

rejoining, induces mutations, and results in elevated numbers of sister chromatid exchanges, a hallmark of genomic instability. Polymorphism in XRCC1 gene may increase the risk of cancer. We have previously shown that having little ATM or XRCC1 protein in somatic tumors is associated with aggressive breast cancers and poor survival (6, 14). We therefore speculate that reduced protein expression of ATM, BRCA1, or XRCC1 could lead to a 'mutator phenotype', increase immunogenicity, promote $CD8^+$ TILs, and influence tumor biology and outcome. However, a limitation to the current study is that we have not directly shown an increased mutational load in ATM/BRCA1/XRCC1-deficient tumors compared to ATM/BRCA1/XRCC1-proficient tumors. This will be an important area for future investigation. Given the essential role of Tregs in attenuating immune response in the tumor microenvironment (19), we also investigated the expression of ATM, BRCA1, and XRCC1 in $FOXP3^+$ breast cancers. We observed highly significant associations with aggressive phenotypes and outcome implying that ATM-, BRCA1-, and XRCC1-deficient breast cancers elicit complex immune responses including cytotoxic T cell, as well as Treg, infiltration.

The PD-1 pathway is critical for immune regulation. PD-L1/PD-1 interaction induces T-cell repression. PD-L1/PD-1–targeted immune checkpoint inhibitor therapy is an exciting approach in cancers (20). Although durable responses have been seen in PD-$L1^+$ non-small cell lung cancer (25), not all patients respond to pembrolizumab (a humanized monoclonal antibody to PD-1). Therefore, evaluation of potential biomarkers that could allow personalization of PD-$L1^+$ solid tumors is a high priority. We provide clinical evidence that XRCC1 expression can stratify patients into distinct prognostic groups in PD-$L1^+$ and PD-1^+ breast cancers, including in ER negative breast cancers. In addition, low XRCC1 expression also promoted aggressive PD-$L1^+$ and PD-1^+ breast cancer phenotypes, implying potential roles in breast cancer biology. Although none of the patients investigated in the current study received anti PD-1 therapy, our data taken together, would suggest that XRCC1 could aid in the personalization of anti PD-1 therapy that are currently under investigation in PD-$L1^+$ breast cancers. Prospective evaluation of this possibility is warranted in the context of clinical trials.

In conclusion, we provide clinical evidence that the interplay between DNA repair, CD8, PD-L1, and PD-1 can promote aggressive tumor phenotypes. XRCC1-directed personalization of immune checkpoint inhibitor therapy may be feasible in breast cancer.

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FIGURE LEGENDS

Figure 1: **A**. Immunohistochemical expression of CD8, FOXP3, PD-1, PD-L1, ATM, BRCA1, and XRCC1 in breast cancers (all images are at 20x magnification). A1) Invasive carcinoma, infiltrate with minimal lymphocytic infiltrate. A2) Invasive carcinoma with extensive CD8 negative lymphocytic infiltrate. A3) Invasive carcinoma showing CD8 positive intra-tumoral lymphocytic infiltrate. A4) Invasive carcinoma with extensive CD8 positive peri-tumoral lymphocytic infiltrate. A5) FOXP3 positive (TILs staining) invasive carcinoma. A6) PD-1 positive (TILs staining) invasive carcinoma. A7) PD-L1 positive (Tumor cell staining) invasive carcinoma. A8) PD-L1 positive (TILs staining) invasive carcinoma. A9) ATM negative invasive carcinoma. A10) ATM positive invasive carcinoma. A11) BRCA1 positive invasive carcinoma. A12) XRCC1 positive invasive carcinoma. **B**. Prognostic significance of ATM expression in CD8+ TILs positive breast cancers (Kaplan-Meier survival curves is shown here). **C**. Prognostic significance of BRCA1 expression in CD8+ TILs positive breast cancers. **D**. Prognostic significance of XRCC1 expression in CD8+ TILs positive breast cancers. **E**. Prognostic significance of XRCC1 expression in PD-L1 positive (tumor cells) breast cancers. **F**. Prognostic significance of XRCC1 expression in PD-L1 positive (TILs) breast cancers. **G**. Prognostic significance of XRCC1 expression in PD-1 positive (TILs) breast cancers. **H**. Prognostic significance of XRCC1 expression in PD-L1 positive (tumor cells) ER negative breast cancers. **I**. Prognostic significance of XRCC1 expression in PD-L1 positive (TILs) ER negative breast cancers. **J**. Prognostic significance of XRCC1 expression in PD-1 positive (TILs) ER negative breast cancers.

Table 1: Clinicopathological significance of XRCC1 expression in PD-L1 positive (tumor cells) and PDLI negative breast cancers.

	PD-L1 (tumor cells) and XRCC1 expression				*P* value	*P* value (adjusted)
	$XRCC1^{+}$ /$PD\text{-}L1^{+}$	$XRCC1^{+}$ /$PD\text{-}L1^{-}$	$XRCC1^{-}$ /$PD\text{-}L1^{+}$	$XRCC1^{-}$ /$PD\text{-}L1^{-}$		
A) Pathological Parameters						
Tumor Size						
<1cm	39 (69.6%)	7 (12.5%)	9 (16.1%)	1 (1.8%)		
>1-2cm	258 (66.7%)	76 (19.6%)	37 (9.6%)	16 (4.1%)	0.018	**0.022**
>2-5cm	208 (67.1%)	35 (11.3%)	57 (18.4%)	10 (3.2%)		
>5cm	8 (72.7%)	1 (9.1%)	2 (18.2%)	0 (0.0%)		
Tumor Stage						
1	297 (65.4%0	87 (19.2%)	50 (11.0%)	20 (4.4%)		
2	167 (69.0%)	23 (9.5%)	47 (19.4%)	5 (2.1%)	0.000966	**0.00015**
3	52 (74.3%)	9 (12.9%)	8 (11.4%)	1 (1.4%)		
Tumor Grade						
G1	80 (71.4%)	21 (18.8%)	9 (8.0%)	2 (1.8%)		
G2	183 (71.5%)	50 (19.5%)	14 (5.5%)	9 (3.5%)	7.45×10^{-7}	**<0.00001**
G3	251 (63.2%0	48 (12.1%)	82 (20.7%)	16 (4.0%)		
NPI						
≤ 3.4	145 (70.0%)	46 (22.2%)	10 (4.8%)	6 (2.9%)	0.000017	**<0.00001**
>3.4	338 (65.3%)	70 (13.5%)	90 (17.4%)	20 (3.9%)		
Mitotic Index						
M1 (low; mitoses < 10)	172 (72.6%)	45 (19.0%)	16 (6.8%)	4 (1.7%)		
M2 (medium; mitoses 10-18)	102 (67.5%)	23 (15.2%)	17 (11.3%)	9 (6.0%)	0.000029	**0.0001**
M3 (high; mitosis >18)	231 (64.9%)	40 (11.2%)	71 (19.9%)	14 (3.9%)		
Tubule Formation						
1 (>75% definite tubule)	31 (79.5%)	4 (10.3%)	4 (10.3%)	0 (0.0%)	0.175	1.925
2 (10%-75% definite tubule)	175 (72.9%)	31 (12.9%)	26 (10.8%)	8 (3.3%)		
3 (<10% definite tubule)	299 (64.3%)	73 (15.7%)	74 (15.9%)	19 (4.1%)		
Pleomorphism						
1 (small-regular uniform)	7 (63.6%)	3 (27.3%)	1 (9.1%)	0 (0.0%)		
2 (Moderate variation)	203 (70.7%)	54 (18.8%)	19 (6.6%)	11 (3.8%)	0.000108	**0.0002**
3 (Marked variation)	294 (66.1%)	51 (11.5%)	84 (18.9%)	16 (3.6%)		
Tumor Type						
IDC-NST	313 (66.5%)	65 (13.8%)	76 (16.1%)	17 (3.6%)		
Tubular	103 (74.1%)	18 (12.9%)	14 (10.0%)	4 (2.9%)		
Medullary	9 (45.0%)	2 (10.0%)	7 (35.0%)	2 (10.0%)	0.002	**0.0028**
ILC	48 (67.6%)	19 (26.8%)	3 (4.2%)	1 (1.4%)		
Others	4 (44.4%)	3 (33.3%)	2 (22.2%)	0 (0.0%)		
Mixed NST & Lobular/special type	26 (61.9%)	10 (23.8%)	3 (7.1%)	3 (7.1%)		
Her2 overexpression						
No	449 (68.1%)	100 (15.2%)	86 (13.1%)	24 (3.6%)		
Yes	64 (64.0%)	17 (17.0%)	16 (16.0%)	3 (3.0%)	0.022	**0.0242**
ER status						
Negative	117 (58.8%)	23 (11.6%)	46 (23.1%)	13 (6.5%)	6.9×10^{-7}	**<0.00001**
Positive	391 (71.2%)	90 (16.4%)	55 (10.0%)	13 (2.4%)		
PR						
Negative	185 (60.3%)	41 (13.4%)	64 (20.8%)	17 (5.5%)		

Positive	316 (73.1%)	69 (16.0%)	39 (9.0%)	8 (1.9%)	0.000001	**<0.00001**

Table 2: Clinicopathological significance of XRCC1 expression in PD-L1 positive (TILs) and PD-L1 negative breast cancers.

	PD-L1 (TILs) and XRCC1 expression				*P* value	*P* value (adjusted)
	XRCC1^{-} /PD-L1^{-}	XRCC1^{+} /PD-L1^{-}	XRCC1^{-} /PD-L1^{+}	XRCC1^{+} /PD-L1^{+}		
A) Pathological Parameters						
Tumor Size						
<1cm	4(7.4%)	21 (38.9%)	6 (11.1%)	23 (42.6%)		
>1-2cm	25(7.3%)	160(46.5%)	23(6.7%)	136(39.5%)	0.044	0.0484
>2-5cm	23(8.4%)	89 (32.5%)	37(13.5%)	125(45.6%)		
>5cm	1 (9.1%)	3 (27.3%)	1 (9.1%)	6 (54.5%)		
Tumor Stage						
1	31(7.8%)	172(43.1%)	32(8.0%)	164(41.1%)	0.075	0.825
2	20(9.1%)	75(34.1%)	29 (13.2%)	96 (43.6%)		
3	1 (1.5%)	27(40.9%)	6 (9.1%)	32 (48.5%)		
Tumor Grade						
G1	7 (7.0%)	51 (51%)	4 (4%)	38 (38%)	1.57×10^{-12}	**<0.00001**
G2	12(5.3%)	125(55.6%)	6 (2.7%)	82 (36.4%)		
G3	34 (9.5%)	98 (27.3%)	57(15.9%)	170(47.4%)		
NPI						
≤ 3.4	10 (5.4%)	106(57.6%)	4 (2.2%)	64 (34.8%)	3.8×10^{-8}	**<0.00001**
>3.4	41 (8.8%)	159(34.3%)	59 (12.7%)	205(44.2%)		
Mitotic Index						
M1 (low; mitoses < 10)	12 (5.5%)	112(51.6%)	8 (3.7%)	85 (39.2%)	4.1×10^{-8}	**<0.00001**
M2 (medium; mitoses 10-18)	13 (10.1%)	57 (44.2%)	7 (5.4%)	52 (40.3%)		
M3 (high; mitosis >18)	28 (8.7%)	94 (29.1%)	52(16.1%)	149(46.1%)		
Tubule Formation						
1 (>75% definite tubule)	3 (8.6%)	17 (48.6%)	1 (2.9%)	14 (40%)	0.021	**0.0257**
2 (10%-75% definite tubule)	13(6.1%)	102(47.9%)	16(7.5%)	82 (38.5%)		
3 (<10% definite tubule)	37 (8.8%)	144(34.2%)	50 (11.9%)	190(45.1%)		
Pleomorphism						
1 (small-regular uniform)	1 (10.0%)	4 (40.0%)	0 (0%)	5 (50.0%)	8.5×10^{-9}	**<0.00001**
2 (Moderate variation)	18 (7.2%)	136(54.2%)	8 (3.2%)	89 (35.5%)		
3 (Marked variation)	34 (8.4%)	123(30.2%)	59(14.5%)	191(46.9%)		
Tumor Type						
IDC-NST	33 (7.8%)	132(31.4%)	51 (12.1%)	205(48.7%)	1.9×10^{-8}	**<0.00001**
Tubular	9 (7.4%)	69 (56.6%)	7 (5.7%)	37 (30.3%)		
Medullary	3 (15.8%)	2 (10.5%)	6 (31.6%)	8 (42.1%)		
ILC	3 (4.7%)	39 (60.9%)	1 (1.6%)	21 (32.8%)		
Others	1 (20.0%)	2 (40.0%)	0 (0%)	2 (40.0%)		
Mixed NST & Lobular/special type	4 (10.0%)	24 (60.0%)	2 (5.0%)	10 (25.0%)		
Her2 overexpression						
No	44 (7.5%)	248(42.2%)	55 (9.4%)	241(41.0%)	0.01	**0.0138**
Yes	10 (11.0%)	22 (24.2%)	9 (9.9%)	50 (54.9%)		
ER status						
ER –	19 (10.5%)	34 (18.8%)	35(19.3%)	93 (51.4%)	2.4×10^{-12}	**<0.00001**
ER +	31 (6.4%)	232(47.5%)	31 (6.4%)	194(39.8%)		
PR						
Negative	29 (10.7%)	78 (28.7%)	43 (15.8%)	122(44.9%)	4.8×10^{-8}	**<0.00001**
Positive	22 (5.7%)	185(47.7%)	22 (5.7%)	159(41.0%)		

Table 3: Clinicopathological significance of XRCC1 expression in PD-1 positive (TILs) and PD1 negative breast cancers.

	PD-1 and XRCC1 expression				*P* value	*P* value (Adjusted)
	$XRCC1^{-}$ /$PD\text{-}1^{-}$	$XRCC1^{+}$ /$PD\text{-}1^{-}$	$XRCC1^{-}$ /$PD\text{-}1^{+}$	$XRCC1^{+}$ /$PD\text{-}1^{+}$		
A) Pathological Parameters						
Tumor Size						
<1cm	4(6.2%)	32(49.2%)	7 (10.8%)	22(33.8%)	0.111	0.137
>1-2cm	19(4.3%)	210(47.9%)	41(9.4%)	168(38.4%)		
>2-5cm	25(7.1%)	129(36.9%)	48(13.7%)	148(42.3%)		
>5cm	1(7.7%)	4 (30.8%)	2 (15.4%)	6 (46.2%)		
Tumor Stage						
1	26(5.2%)	232(46.2%)	49(9.8%)	195(38.8%)	0.239	2.64
2	19(6.6%)	118(40.8%)	40(13.8%)	112(38.8%)		
3	4 (5.2%)	27(35.1%)	8 (10.4%)	38 (49.4%)		
Tumor Grade						
G1	5 (4.0%)	83(66.9%)	6(4.8%)	30(24.2%)	$1X10^{-13}$	**<0.00001**
G2	11(4.1%)	152(56.1%)	13(4.8%)	95(35.1%)		
G3	33(7.0%)	141(29.9%)	79(16.7%)	219(46.4%)		
NPI						
≤ 3.4	8(3.6%)	139(62.6%)	9 (4.1%)	66(29.7%)	$2.63X10^{-10}$	**<0.00001**
>3.4	39(6.4%)	224(37.0%)	85(14%)	257(42.5%)		
Mitotic Index						
M1 (low; mitoses < 10)	7(2.7%)	155(59.6%)	14(5.4%)	84(32.3%)	$3.7X10^{-12}$	**<0.00001**
M2 (medium; mitoses 10-18)	15(9.1%)	76 (46.1%)	13(7.9%)	61(37.0%)		
M3 (high; mitosis >18)	27(6.4%)	132(31.4%)	70(16.7%)	191(45.5)		
Tubule Formation						
1 (>75% definite tubule)	2(4.7%)	30(69.8%)	3 (7.0%)	8 (18.6%)	0.000001	**<0.00001**
2 (10%-75% definite tubule)	20(7.4%)	139(51.5%)	17 (6.3%)	94 (34.8%)		
3 (<10% definite tubule)	27(5.1%)	194(36.5%)	77 (14.5%)	234 (44%)		
Pleomorphism						
1 (small-regular uniform)	1(8.3%)	7 (58.3%)	0 (0%)	4 (33.3%)	$1\ X10^{-13}$	**<0.00001**
2 (Moderate variation)	15(4.9%)	195 (63.5%)	15 (4.9%)	82 (26.7%)		
3 (Marked variation)	33(6.3%)	160 (30.5%)	82 (15.6%)	250 (47.6)		
Tumor Type						
IDC-NST	35(6.4%)	200(36.5%)	71(13.0%)	242(44.2)	$3.3X10^{-8}$	**<0.00001**
Tubular	8 (5.2%)	93 (60.8%)	9 (5.9%)	43(28.1%)		
Medullary	1(4.3%)	2 (8.7%)	8 (34.8%)	12(52.2%)		
ILC	2(2.6%)	49 (62.8%)	3 (3.8%)	24(30.8%)		
Others	0 (0%)	6 (66.7%)	1 (11.1%)	2 (22.2%)		
Mixed NST & Lobular/special type	3 (6.5%)	19 (41.3%)	6 (13.0%)	18(39.1%)		
Her2 overexpression						
No	40(5.4%)	328(44.5%)	80(10.9%)	289(39.2)	0.123	0.1353
Yes	9 (7.1%)	42 (33.3%)	18 (14.3%)	57(45.2%)		
ER status						
Negative	18(7.6%)	60(25.4%)	49(20.8%)	109(46.2)	$2.87X10^{-12}$	**<0.00001**
Positive	28(4.6%)	308(50.1%)	46(7.5%)	233(37.9)		
PR						
Negative	26(7.1%)	124(33.8%)	67(18.3%)	150(40.9)	$8.1X10^{-9}$	**<0.00001**
Positive	19(4%)	236(50.2%)	31(6.6%)	184(39.1)		

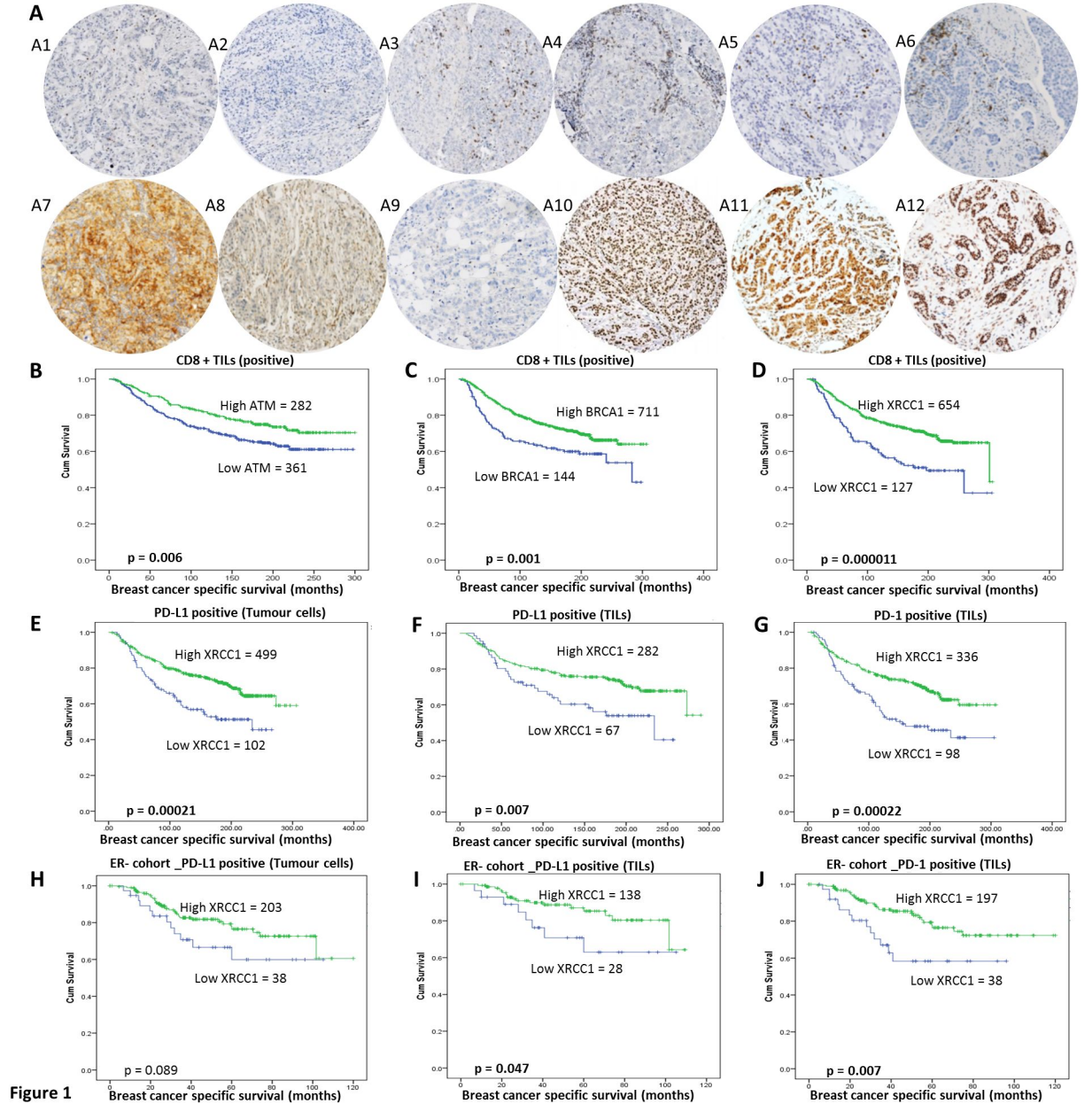

A
A1
A2
A3
A4
A5
A6
A7
A8
A9
A10
A11
A12
B
CD8 + TILs (positive)
High ATM = 282
Low ATM = 361
p = 0.006
Cum Survival
Breast cancer specific survival (months)
C
CD8 + TILs (positive)
High BRCA1 = 711
Low BRCA1 = 144
p = 0.001
Cum Survival
Breast cancer specific survival (months)
D
CD8 + TILs (positive)
High XRCC1 = 654
Low XRCC1 = 127
p = 0.000011
Cum Survival
Breast cancer specific survival (months)
E
PD-L1 positive (Tumour cells)
High XRCC1 = 499
Low XRCC1 = 102
p = 0.00021
Cum Survival
Breast cancer specific survival (months)
F
PD-L1 positive (TILs)
High XRCC1 = 282
Low XRCC1 = 67
p = 0.007
Cum Survival
Breast cancer specific survival (months)
G
PD-1 positive (TILs)
High XRCC1 = 336
Low XRCC1 = 98
p = 0.00022
Cum Survival
Breast cancer specific survival (months)
H
ER- cohort _PD-L1 positive (Tumour cells)
High XRCC1 = 203
Low XRCC1 = 38
p = 0.089
Cum Survival
Breast cancer specific survival (months)
I
ER- cohort _PD-L1 positive (TILs)
High XRCC1 = 138
Low XRCC1 = 28
p = 0.047
Cum Survival
Breast cancer specific survival (months)
J
ER- cohort _PD-1 positive (TILs)
High XRCC1 = 197
Low XRCC1 = 38
p = 0.007
Cum Survival
Breast cancer specific survival (months)

Figure 1

Supplementary Table S1: Clinicopathological characteristics of Nottingham Tenovus series

Variable	n*	Cases	(%)
Menopausal status	1650		
Pre-menopausal		612	(37.0)
postmenopausal		1038	(63.0)
Tumour Grade (NGS)	1650		
G1		306	(18.5)
G2		531	(32.2)
G3		813	(49.3)
Lymph node stage	1650		
Negative		1056	(64.0)
Positive (1-3 nodes)		486	(29.5)
Positive (>3 nodes)		108	(6.5)
Tumour size (cm)	1650		
T1 a + b (≤1.0)		187	(11.0)
T1 c (>1.0 -2.0)		868	(53.0)
T2 (>2.0-5)		579	(35.0)
T3 (>5)		16	(1.0)
Tumour type	1650		
IDC-NST		941	(57)
Tubular		349	(21)
ILC		160	(10)
Medullary (typical/atypical)		41	(2.5)
Others		159	(9.5)
NPI subgroups	1650		
Excellent PG(2.08-2.40)	Low risk	207	(12.5)
Good PG(2.42-3.40)		331	(20.1)
Moderate I PG(3.42 to 4.4)	High risk	488	(29.6)
Moderate II PG(4.42 to 5.4)		395	(23.9)
Poor PG(5.42 to 6.4)		170	(10.3)

Very poor PG(6.5–6.8)		59	(3.6)
Survival at 20 years	1650		
Alive and well		1055	(64.0)
Dead from disease		468	(28.4)
Dead from other causes		127	(7.6)
Adjuvant systemic therapy (AT)			
No AT		665	(42.0)
Hormone therapy (HT)		642	(41.0)
Chemotherapy (CMF)		307	(20.0)
Hormone + chemotherapy		46	(3.0)

* Number of cases for which data were available.

NPI; Nottingham prognostic index, PG; prognostic group

Supplemental Table S2: Clinicopathological characteristics of ER- cohort

Variable		**Cases (%)**
Menopausal status	279	
Pre-menopausal		119 (43.6)
postmenopausal		154 (56.4)
Tumour Grade (NGS)	279	
G1		1 (0.4)
G2		26 (9.5)
G3		248 (90.2)
Tumour size (cm)	279	
≤2.0		140 (52.6)
>2.0		126 (47.4)
Mitotic index	279	
M1		21 (7.5)
M2		47(16.8)
M3		274 (98.9)
Tubule formation	279	
1		1 (0.4)
2		36 (15.0)
3		240 (86.6)
Pleomorphism	279	
1		0 (0)
2		3 (1.1)
3		274(98.9)
Her-2 status	279	
Positive		28 (10)
Negative		251 (90)
NPI	279	
Good (≤ 3.4)		15 (5.5)

Moderate (3.41-5.4)		194 (71.1)
Poor (>5.4)		64 (23.4)

NPI; Nottingham prognostic index, PG; prognostic group

Supplementary Table S3: Antigens, primary antibodies, clone, source, optimal dilution and scoring system used for each immunohistochemical marker

Antigen	Antibody	Clone	Source	Antigen Retrieval	Dilution / Incubation Time	Distribution	Scoring system	Cut-offs
BRCA1	BRCA1	MS110	Calbiochem	Citrate pH6	1:100 60 min	Nuclear	% of positive cells	<25% (negative)
ATM	Rabbit MAb anti-ATM	Y170	Abcam	Citrate pH6	1:100 18 hours	Nuclear	% of positive cells	<25% (negative)
XRCC1	Mouse MAb Anti-XRCC1	33-2-5	Thermo-scientific	Citrate pH6	1:200 20 min	Nuclear	% of positive cells	≥10% (positive)
Pol β	Rabbit anti-polβ	Ab26343	Abcam	Citrate pH6	1:200 60 min	Nuclear	H- Score	≥100 (Median H-score, positive)
BLM	Rabbit anti BLM	Polyclonal	Novus-Biologicals	Citrate pH6	1:100 18 Hours	Nuclear	H- Score	≥50 (Median H-score, positive)
WRN	Rabbit Anti-WRN	Polyclonal	Novus Biologicals	Citrate pH6	1:100 Overnight (18h)	Nuclear	H-score	Nuclear ≥116(Median H-score High)
RECQL4	Rabbit Anti RECQL4	Polyclonal	Novus Biologicals	Citrate pH6	1:1000 60 min	Nuclear	H-score	Nuclear ≥215 (Median H-score High)
Ku70/ Ku80	Mouse Anti-Ku70/Ku80	Monoclonal	Abcam	Citrate pH6	1:2500 60 min	Nuclear	H- Score	>90 (X-tile cut-off, positive)
CHK2	Rabbit Anti CHK2	Polyclonal	Abcam	Citrate pH6	1:100 60 min	Nuclear	H- Score	≥100 (Median H-score, positive)
PARP1	Mouse MAb Anti-PARP1	7D3-6	BD pharmingen	Citrate pH6	1:1000	Nuclear	% of positive cells	≥10% (positive)
TOP2A	Mouse MAb	KiS1	Dako-Cytomation	Citrate pH6	1:150 60 min	Nuclear	% of positive cells	>25% (positive)
FEN1	Rabbit anti-FEN1	polyclonal	Novus Biologicals	Citrate pH6	1:200 60 min	Nuclear	H-score	>100 (positive)
DNA-PKcs	Mouse MAb Anti-	3H6	Abcam	Citrate pH6	1:1000 20 min	Nuclear	H-score	>260 ((Mean H-score, positive)
ER	Mouse MAb anti-ER-α	SP1	Dako-Cytomation	Citrate pH6	1:150 30 min	Nuclear	Allred score	≥3 (positive)

ER	Mouse MAb anti-ER-α	EP1	Dako-Cytomation	Citrate pH6	1:80 30 min	Nuclear	% positive cells	≥1% positive
PR	Mouse MAb anti-PR	PgR636	Dako-Cytomation	Citrate pH6	1:125 30 min	Nuclear	% positive cells	≥1% positive
HER2	Rabbit antihuman c-erbB2	polyclonal	Dako-Cytomation	None	1:400 60 min	Membrane	See text	See text
CD8	Mouse MAb Anti-CD8	1A5	Vector Laboratories	Citrate pH6	1:50 20 min	Membrane	See text	See text
FOXP3	Mouse MAb Anti-FOXP3	236A/E7	Abcam	Citrate pH6	1:100 60 min	Stroma	positive cell counts	≥3 positive
PD-1	Mouse MAb Anti-PD-1	EH33	Cell Signalling Technology	Citrate pH6	1:75 24 hours	Stroma	% positive cells	≥5% positive
PD-L1	Rabbit MAb Anti-PD-L1	E1L3N	Cell Signalling Technology	Epitope retrieval solution 2, pH9, 95°C, 45 min	1:25 24 hours	Membrane Cytoplasm Stroma	% positive in tumour % positive cells in stroma	≥1% positive

All sections were pre-treated with microwave antigen retrieval using 0.1% citrate buffer (pH 6) except for HER2 (no pre-treatment).

Supplementary Table S4: Clinicopathological significance of ATM expression in CD8+ TILs positive and CD8+ TILs negative breast cancer

	CD8 and ATM expression				P- value	*P -Value (Adjusted)
	ATM+ /CD8+	ATM+ /CD8-	ATM- /CD8+	ATM- /CD8-		
A) Pathological Parameters						
Tumour Size						
<1cm	30 (34.9%)	5 (5.8%)	41 (47.7%)	10 (11.6)	0.268	0.2924
>1-2cm	165(38.3%)	51(11.8%)	176(40.8)	39(9.0%)		
>2-5cm	91(32.6%)	27(9.7%)	141(50.5)	20 (1.2%)		
>5cm	3 (21.4%)	2(14.3%)	8 (57.1%)	1 (7.1%)		
Tumour Stage						
1	191(37.3%)	59(11.5%)	214(41.8%)	48 (9.4%)	0.054	0.0810
2	77 (32.8%)	25(10.6%)	114(48.5%)	19(8.1%)		
3	21(33.3%)	2 (3.2%)	38 (60.3%)	2 (3.2%)		
Tumour Grade						
G1	57 (46.0%)	20(16.1%)	40 (32.3)	7 (5.6%)	0.002	**0.0080**
G2	100(38.3%)	26(10.0%)	110(42.1)	25(9.6%)		
G3	132(31.0%)	40(9.4%)	216(50.7)	38(8.9%)		
NPI						
≤ 3.4	100(41.7%)	30(12.5%)	90(37.5%)	20(8.3%)	0.034	0.0680
>3.4	178(33.1%)	53(9.9%)	258(48.0%)	49(9.1%)		
Mitotic Index						
M1 (low; mitoses < 10)	116(43.8%)	28(10.6%)	104(39.2%)	17(6.4%)	0.014	**0.0336**
M2 (medium; mitoses 10-18)	54 (36.5%)	16(10.8%)	62 (41.9%)	16(10.8%)		
M3 (high; mitosis >18)	116(30.2%)	37(9.6%)	195(50.8%)	36(9.4%)		
Tubule Formation						
1 (>75% definite tubule)	12(27.3%)	8(18.2%)	19(43.2%)	5(11.4%)	0.001	**0.0120**
2 (10%-75% definite tubule)	111(43.5%)	31(12.2%)	88(34.5%)	25(9.8%)		
3 (<10% definite tubule)	163(32.7%)	42(8.4%)	254(51.0%)	39(7.8%)		
Pleomorphism						
1 (small-regular uniform)	6 (35.3%)	3 (17.6%)	6 (35.3%)	2(11.8%)	0.077	0.1027
2 (Moderate variation)	112(38.2%)	39(13.3%)	116(39.6%)	26(8.9%)		
3 (Marked variation)	167(34.5%)	38(7.9%)	238(49.2%)	41(8.5%)		
Tumour Type						
IDC-NST	165 (33.8%)	48 (9.8%)	236 (48.4%)	39 (8.0%)		
Tubular	70 (42.9%)	24 (14.7%)	56 (34.4%)	13 (8.0)	0.045	0.0836
Medullary	10 (34.5%)	1 (3.4%)	17 (58.6%)	1 (3.4%)		
ILC	23 (33.8%)	4 (5.9%)	35 (51.5%)	6 (8.8%)		
Others	2 (22.2%)	1 (11.1%)	5 (55.6%)	1 (11.1%)		
Mixed NST &lobular/ special type	16 (37.2%)	6 (14.0%)	13 (30.2%)	8 (18.6%)		
HER-2 overexpression						
No	243(35.8%)	76 (11.2%)	300(44.2%)	60(8.8%)	0.459	0.9670
Yes	41 (34.2%)	9 (7.5%)	61 (50.8%)	9 (7.5%)		
ER						
Negative	6.6 (29.6%)	15 (6.7%)	121(54.3%)	21(9.4%)	0.003	**0.0098**
Positive	220 (38.0%)	71 (12.3%)	242(41.8%)	46(7.9%)		
PR						
Negative	104(31.4%)	23(6.9%)	172(52.0%)	32(9.7%)	0.001	**0.0130**
Positive	175(38.5%)	62(13.6%)	180(39.6%)	38(8.4%)		

Supplementary Table S5: Clinicopathological significance of BRCA1 expression in CD8 positive and CD8 negative breast cancer

					P- value	*P -Value (Adjusted)
	BRCA1+ /CD8+	BRCA1+ /CD8-	BRCA1- /CD8+	BRCA1- /CD8-		
A) Pathological Parameters						
Tumour Size						
<1cm	79 (77.5%)	15 (14.7%)	6 (5.9%)	2 (2.0%)	0.26	0.624
>1-2cm	363(68.6%)	86(16.3%)	64 (12.1%)	16(3.0%)		
>2-5cm	278(68.3%)	51 (12.5%)	70(17.2%)	8 (2.0%)		
>5cm	10 (50%)	3 (15%)	6 (30%)	1 (5.0%)		
Tumour Stage						
1	418(65.3%)	107(16.7%)	95(14.8%)	20(3.1%)	0.40	0.8
2	245(74.7%)	41 (12.5%)	36(11.0%)	6 (1.85%)		
3	67 (73.6%)	8 (8.8%)	15(16.5%)	1 (1.1%)		
Tumour Grade						
G1	112(68.7%)	33 (20.2%)	10 (6.1%)	8 (4.9%)	$3.5x10^{-12}$	**p<0.0001**
G2	263(78.0%)	53 (15.7%)	18 (5.3%)	3 (0.9%)		
G3	355(63.5%)	70 (12.5%)	118(21.1%)	16(2.9%)		
NPI						
≤ 3.4	218(72.7%)	58 (18.7%)	18 (6.0%)	8 (2.7%)	0.000026	**0.0003**
>3.4	474(66.9%)	94 (13.3%)	121 (17.1%)	19 (2.7%)		
Mitotic Index						
M1 (low; mitoses < 10)	260 (76.9%)	56 (16.6%)	17 (5.0%)	5 (1.5%)	$2.4x10^{-9}$	**p<0.0001**
M2 (medium; mitoses 10-18)	142 (73.2%)	29 (14.9%)	19 (9.8%)	4 (2.1%)		
M3 (high; mitosis >18)	317 (63.4%)	62 (12.4%)	106(21.2%)	15(3.0%)		
Tubule Formation						
1 (>75% definite tubule)	36 (64.3%)	14(25.0%)	2 (3.6%)	56 (100%)	0.000207	**0.0012**
2 (10%-75% definite tubule)	231(70.6%)	56(17.1%)	33 (10.1%)	327(100%)		
3 (<10% definite tubule)	452(69.2%)	77(11.9%)	107(16.5%)	649(100%)		
Pleomorphism						
1 (small-regular uniform)	13 (86.7%)	0 (0%)	0 (0%)	2 (13.3%)	$3.5x10^{-10}$	**p<0.0001**
2 (Moderate variation)	282(73.2%)	74(19.2%)	22 (5.7%)	7 (1.8%)		
3 (Marked variation)	421(67.0%)	72(11.5%)	120(19.1%)	15 (2.4%)		
Tumour Type						
IDC-NST	418 (66.5%)	82 (13%)	113 (18%)	16 (2.5%)	$6.4x10^{-10}$	**p <0.0001**
Tubular	144(70.6%)	40 (19.6%)	11 (5.4%)	9 (4.4%)		
Medullary	18 (56.3%)	1 (3.1%)	12 (37.5%)	1 (3.1%)		
ILC	96 (85.7%)	13 (11.6%)	3 (2.7%)	0 (0 %)		
Others	9 (75%)	3 (25%)	0 (0%)	1 (1.7%)		
Mixed NST& Lobular special type	38 (65.5%)	16 (27.6%)	3 (5.2%)	27 (2.6%)		
HER-2						
Negative	626(69.0)	140 (15.4)	116 (12.8)	25 (2.8)	0.037	0.1418
Positive	95 (66.4)	16 (11.2)	30 (21.0)	2 (1.4)		
ER						
Negative	150 (55.8%)	28 (10.4%)	76 (28.3%)	15 (5.6%)	$2.6x10^{-18}$	**p<0.0001**
Positive	574 (74.1%)	124 (16.0%)	66 (8.5%)	11 (1.4%)		
PR						
Negative	260 (61.2%)	47 (11.1%)	101 (23.8%)	17 (4.0%)	$4.6x10^{-15}$	**p<0.0001**
Positive	464 (74.1%)	108(17.3%)	44 (7.0%)	10 (1.6%)		

Supplementary Table S6: Clinicopathological significance of XRCC1 expression in CD8 positive and CD8 negative breast cancers

	CD8 and XRCC1 expression				P- value	*P -Value (Adjusted)
	XRCC1+ /CD8+	XRCC1+ /CD8-	XRCC1- /CD8+	XRCC1- /CD8-		
A) Pathological Parameters						
Tumour Size						
<1cm	61 (71.8%)	11(12.9%)	11(12.9%)	2 (2.4%)		
>1-2cm	346 (76.8%)	90(17.6%)	52(10.2%)	22(4.3%)	0.161	0.1756
>2-5cm	254(67.6%)	52(13.8%)	62(16.5%)	8 (2.1%)		
>5cm	12 (63.2%)	3 (15.8%)	3 (15.8%)	1 (5.3%)		
Tumour Stage						
1	393(66.3%)	106(17.9%)	67(11.3%)	27(4.6%)	0.013	**0.0173**
2	209(67.9%)	45 (14.6%)	49(15.9%)	5 (1.6%)		
3	71 (78.0%)	7 (7.7%)	11(12.1%)	2 (2.2%)		
Tumour Grade						
G1	97 (66%)	33(22.4%)	12(8.2%)	5 (3.4%)	$3.5x10^{-7}$	**p<0.0001**
G2	238(75.8%)	51 (16.2%)	17(5.4%)	8 (2.5%)		
G3	71 (78%)	73 (13.8%)	99(18.7%)	20(3.8%)		
NPI						
≤ 3.4	196(71.3%)	54(19.6%)	16(5.8%)	9 (3.3%)	0.000214	**0.0004**
>3.4	442(65.7%)	99(14.7%)	108(16%)	24(3.6%)		
Mitotic Index						
M1 (low; mitoses < 10)	231(74.3%)	53 (17%)	20 (6.4%)	7(2.3%)	0.000036	**0.0001**
M2 (medium; mitoses 10-18)	128(70.3%)	28(15.4%)	18 (9.9%)	8(4.4%)		
M3 (high; mitosis >18)	301 (64%)	64(13.6%)	89 (18.9%)	16(3.4%)		
Tubule Formation						
1 (>75% definite tubule)	26(56.5%)	16 (34.8%)	3 (6.5%)	1(2.2%)		
2 (10%-75% definite tubule)	211(68.7%)	53(17.3%)	31(10.1%)	12(3.9%)	0.001	**0.0015**
3 (<10% definite tubule)	423(69.3%)	76 (12.5%)	93 (15.2%)	18(3.0%)		
Pleomorphism						
1 (small-regular uniform)	14 (87.5%)	2 (12.5%)	0 (0)	0 (0)		
2 (Moderate variation)	254(71.1%)	70 (19.6%)	23(6.4%)	10(2.8%)	0.000005	**p <0.0001**
3 (Marked variation)	389(66.5%)	143(14.9%)	104(17.8)	21(3.6%)		
Tumour Type						
IDC-NST	408(67.4%)	86(14.2%)	93(15.4%)	18(3.0%)		
Tubular	120(64.5%)	42(22.6%)	17(9.1%)	7 (3.8%)	0.000485	**0.0008**
Medullary	18(64.3%)	0 (0)	8 (28.6%)	2 (7.1%)		
ILC	81(82.7%)	10(10.2%)	5 (5.1%)	2 (2.0%)		
Others	6 (54.5%)	3 (27.3%)	1 (9.1%)	1 (9.1%)		
Mixed NST & Lobular/special type	31(62%)	12(24%)	4 (8%)	3 (6%)		
Her2 overexpression						
No	571(67.6%)	139(16.4%)	107(12.7)	28(3.3%)	0.663	7.9560
Yes	92 (67.2%)	19 (13.9%)	22 (16.1%)	4 (2.9%)		
ER						
Negative	148(59.0%)	29(11.6%)	60(23.9%)	14(5.6%)	$9.3x10^{-7}$	**p <0.0001**
Positive	512(71.2%)	124(17.2%)	66(9.2%)	17(2.4%)		
PR						
Negative	240(60.8%)	56(14.2%)	80(20.3%)	19(4.8%)	$5.4x10^{-8}$	**p <0.0001**
Positive	401(71.7%)	100(17.9%)	45(8.1%)	13(2.3%)		

Supplementary Table S7: Clinicopathological significance of ATM expression in FOXP3 positive and FOXP3negative breast cancer

	FOXP3 and ATM expression				P- value	*P -Value (Adjusted)
	ATM+ / FOXP3+	ATM+ / FOXP3-	ATM- / FOXP3+	ATM- / FOXP3-		
A) Pathological Parameters						
Tumour Size						
<1cm	19 (20%)	25 (26.3%)	31 (32.6	20 (21.1		
>1-2cm	145 (30.7%)	103 (21.8%)	154(32.6	71 (15	0.036	0.3960
>2-5cm	81 (27.7%)	41 (14%)	113 (38.7	57(19.5		
>5cm	2 (15.4%)	2 (15.4%)	6 (46.2	3 (23.1		
Tumour Stage						
1	157 (28.4%)	122 (22.1%)	185 (33.5%)	89 (16.1%)		
2	71 (28.2%)	42 (16.7%)	87 (34.5%)	52 (20.6%)	0.092	0.5060
3	19 (27.9%)	8 (11.8%)	32 (47.1%)	9 (13.2%)		
Tumour Grade						
G1	40 (28%)	56 (39.2%)	26 (18.2%)	21 (14.7%)	$1.7X\ 10^{-21}$	**<0.0001**
G2	66 (23.1%)	73 (25.5%)	74 (25.9%)	73 (25.5%)		
G3	141(31.7%)	43 (9.7%)	204 (45.8%)	57 (12.8%)		
NPI						
≤ 3.4	69 (25.9%)	84 (31.6%)	64 (24.1%)	49 (18.4%)	$6.5\ X10^{-9}$	**<0.0001**
>3.4	170(29.6%)	82 (14.3%)	226 (39.3%)	97 (16.9%)		
Mitotic Index						
M1 (low; mitoses < 10)	72 (24.2%)	99 (33.3%)	66 (22.2%)	60 (20.2%)	$4.1x10^{-16}$	**<0.0001**
M2 (medium; mitoses 10-18)	44 (27.2%)	32 (19.8%)	53 (32.7%)	33 (20.4%)		
M3 (high; mitosis >18)	126 (31.4%)	39 (9.7%)	183(45.6%)	53 (13.2%)		
Tubule Formation						
1 (>75% definite tubule)	10 (20.4%)	16 (32.7%)	14 (28.6%)	9 (18.4%)	$5.5x10^{-7}$	**<0.0001**
2 (10%-75% definite tubule)	82 (29.3%)	80 (28.6%)	70 (25%)	48(17.1%)		
3 (<10% definite tubule)	150 (28.2%)	74 (13.9%)	218(41.1%)	89(16.8%)		
Pleomorphism						
1 (small-regular uniform)	6 (30%)	7 (35%)	6 (30%)	1 (5%)	$7.8x10^{-3}$	**<0.0001**
2 (Moderate variation)	83 (25.2%)	97(29.5%)	74 (22.5%)	75 (22.8%)		
3 (Marked variation)	151(29.7%)	66 (13%)	222 (43.7%)	69 (13.6%)		
Tumour Type						
IDC-NST	158 (30.6%)	72 (13.9%)	212 (41%)	75(14.5%)	$4.4x10^{-13}$	**<0.0001**
Tubular	51 (28%)	59 (32.4%)	41 (22.5%)	31(17%)		
Medullary	11 (36.7%)	1 (3.3%)	18 (60%)	0 (0%)		
ILC	10 (13%)	24 (31.4%)	17 (22.1%)	26(33.8%)		
Others	1 (12.5%)	2 (25%)	2 (25%)	3 (37.5%)		
Mixed NST &lobular/ special type	12 (24.5%)	14 (28.6%)	11 (22.4%)	12 (24.5%)		
HER-2 overexpression						
No	198 (26.8%)	165(22.2%)	244 (32.9%)	135(18.2%)	$1.6x10^{-7}$	**<0.0001**
Yes	48 (39.8%)	4 (3.3%)	57 (46.7%)	13 (10.7%)		
ER						
Negative	76 (32.2%)	18 (7.6%)	117 (49.6%)	25 (10.6%)	$4.7x10^{-12}$	**<0.0001**
Positive	168(26.8%)	153(24.4%)	181 (28.9%)	124(19.8%)		
PR						
Negative	99 (28.7%)	41 (11.9%)	156 (45.2%)	49 (14.2%)	$4.4x10^{-8}$	**<0.0001**
Positive	140(27.9%)	123(24.6%)	141 (28.1%)	97 (19.4%)		

Supplementary Table S8: Clinicopathological significance of BRCA1 expression in FOXP3 positive and FOXP3negative breast cancer

	FOXP3 and ATM expression				P- value	*P -Value (Adjusted)
	BRCA1+ / FOXP3+	BRCA1+ / FOXP3-	BRCA1- / FOXP3+	BRCA1- / FOXP3-		
A) Pathological Parameters						
Tumour Size						
<1cm	60 (51.3%)	48 (41%)	5 (4.3%)	4 (3.4%)		
>1-2cm	308(54.4%)	177(31.3%)	58(10.2%)	23(4.1%)	0.015	**0.0165**
>2-5cm	230(54.1%)	118 (27.8%)	60(14.1%)	17(4%)		
>5cm	8 (38.1%)	6 (28.6%)	6 (28.6%)	1 (4.8%)		
Tumour Stage						
1	354 (51.5%)	217 (31.6%)	86 (12.5%)	30 (4.4%)		
2	196 (56.5%)	109 (109%)	31 (8.9%)	11 (3.2%)	0.397	4.3670
3	56 (58.3%)	24 (25%)	12 (12.5%)	4 (4.2%)		
Tumour Grade						
G1	81 (44.3%)	83 (45.4%)	5 (2.7%)	14 (7.7%)	$2.0X10^{-30}$	**<0.0001**
G2	176 (47.4%)	170(45.8%)	15 (4%)	10 (2.7%)		
G3	349 (60.6%)	97 (16.8%)	109 (18.9%)	21 (3.6%)		
NPI						
≤ 3.4	161 (48.3%)	143 (42.9%)	13 (3.9%)	16 (4.8%)	$9.3\ X10^{-11}$	**<0.0001**
>3.4	416 (55.7%)	192 (25.7%)	112 (15%)	27 (3.6%)		
Mitotic Index						
M1 (low; mitoses < 10)	171 (45.1%)	183(48.3%)	11 (2.9%)	14 (3.7%)	$9.5X10^{-27}$	**<0.0001**
M2 (medium; mitoses 10-18)	108 (51.9%)	76 (36.5%)	17 (8.2%)	7 (3.4%)		
M3 (high; mitosis >18)	312 (60.7%)	84 (16.3%)	97 (18.9%)	21 (4.1%)		
Tubule Formation						
1 (>75% definite tubule)	26 (44.1%)	26 (44.1%)	0 (0%)	7 (11.9%)	$4.0x10^{-9}$	
2 (10%-75% definite tubule)	178(49.7%)	139(38.8%)	25 (7%)	16(4.5%)		**<0.0001**
3 (<10% definite tubule)	387(56.6%)	178(26%)	100(14.6%)	19(2.8%)		
Pleomorphism						
1 (small-regular uniform)	8 (42.1%)	9 (47.4%)	1 (5.3%)	1 (5.3%)	$3.3x10^{-19}$	
2 (Moderate variation)	204(47.8%)	191(44.7%)	12 (2.8%)	20(4.7%)		**<0.0001**
3 (Marked variation)	376(57.8%)	142(21.8%)	112(17.2%)	21(3.2%)		
Tumour Type						
IDC-NST	383(58.4%)	145 (22.1%0	102 (15.5%)	26 (4%)	$1.5x10^{-23}$	
Tubular	107(47.6%)	96 (42.7%)	9 (4%)	13 (5.8%)		**<0.0001**
Medullary	19 (59.4%)	0 (0%)	13 (40.6%)	0 (0%)		
ILC	56 (44.4%)	68 (54%)	1 (0.8%)	1 (0.8%)		
Others	6 (54.5%)	5 (45.5%)	0 (0%)	0 (0%)		
Mixed NST &lobular/ special type	29 (43.3%)	33 (49.3%)	1 (1.5%)	4 (6%)		
HER-2 overexpression						
No	511(52.3%)	323(33.1%)	101(10.3%)	42 (4.3%)	0.000004	
Yes	93 (64.1%)	21 (14.5%)	28 (19.3%)	3 (2.1%)		**<0.0001**
ER						
Negative	163 (58%)	29 (10.3%)	72 (25.6%)	17 (6%)	$2.1x10^{-27}$	
Positive	431 (52%)	318(38.4%)	53 (6.4%)	27 (3.3%)		**<0.0001**
PR						
Negative	239 (54.7%)	85 (19.5%)	90 (20.6%)	23 (5.3%)	$2.2x10^{-18}$	
Positive	363 (53.5%)	256(37.8%)	37 (5.5%)	22 (3.2%)		**<0.0001**

Supplementary Table S9: Clinicopathological significance of XRCC1 expression in FOXP3 positive and FOXP3negative breast cancer.

	FOXP3 and ATM expression				P- value	*P -Value (Adjusted)
	XRCC1+ / FOXP3+	XRCC1+ / FOXP3-	XRCC1- / FOXP3+	XRCC1- / FOXP3-		
A) Pathological Parameters						
Tumour Size						
<1cm	45 (45.5%)	39 (39.4%)	9 (9.1%)	6 (6.1%)	0.219	2.4090
>1-2cm	293(53.5%)	176(32.1%)	52 (9.5%)	28(5.1%)		
>2-5cm	206(52.3%)	111(28.2%)	55 (14%)	22 (5.6%)		
>5cm	10 (52.6%)	5 (26.3%)	4 (21.1%)	0 (0%)		
Tumour Stage						
1	340 (52.5%)	204 (31.5%)	63 (9.7%)	41 (6.3%)	0.044	0.0484
2	158 (49.1%)	107 (33.2%)	43 (13.4%)	14 (4.3%)		
3	57 (60.6%)	22 (23.4%)	14 (14.9%)	1 (1.1%)		
Tumour Grade						
G1	74 (41.8%)	81 (45.8%)	8 (4.5%)	14 (7.9%)	$1.1 X10^{-28}$	**<0.0001**
G2	157(45.8%)	158(46.1%)	9 (2.6%)	19 (5.5%)		
G3	323 (59.6%)	93 (17.2%)	103 (19%)	23 (4.2%)		
NPI						
≤ 3.4	150 (47.8%)	134 (42.7%)	10 (3.2%)	20 (6.4%)	$5.3X10^{-10}$	**<0.0001**
>3.4	379 (53.7%)	187 (26.5%)	104(14.7%)	36 (5.1%)		
Mitotic Index						
M1 (low; mitoses < 10)	154 (43.1%)	170(47.6%)	14 (3.9%)	19 (5.3%)	$1.2x10^{-22}$	**0.0001**
M2 (medium; mitoses 10-18)	101(52.3%)	63 (32.6%)	14 (7.3%)	15 (7.8%)		
M3 (high; mitosis >18)	282(58.8%)	87 (18.1%)	92 (19.2%)	19 (4%)		
Tubule Formation						
1 (>75% definite tubule)	22 (43.1%)	22 (43.1%)	2 (3.9%)	5 (9.8%)	$7.8x10^{-9}$	
2 (10%-75% definite tubule)	168(48.3%)	136(39.1%)	19(5.5%)	25(7.2%)		**<0.0001**
3 (<10% definite tubule)	347(55%)	162(25.7%)	99(15.7%)	23(3.6%)		
Pleomorphism						
1 (small-regular uniform)	10 (41.7%)	11 (45.8%)	1 (4.2%)	2 (8.3%)	$5.2x10^{-19}$	
2 (Moderate variation)	177(44.5%)	183(46%)	15(3.8%)	23(5.8%)		**<0.0001**
3 (Marked variation)	346(57.4%)	125(20.7%)	104(17.2%)	28(4.6%)		
Tumour Type						
IDC-NST	370 (59.2%)	137 (21.9%)	92 (14.7%)	26 (4.2%)	$5.1x10^{-21}$	
Tubular	92 (43.2%)	92 (43.2%)	11 (5.2%)	17 (8%)		**<0.0001**
Medullary	17 (60.7%)	1 (3.6%)	10 (35.7%)	0 (0%)		
ILC	38 (34.2%)	64 (57.7%)	4 (3.6%)	5 (4.5%)		
Others	3 (23.1%)	8 (61.5%)	0 (0%)	2 (15.4%)		
Mixed NST &lobular/ special type	26 (42.6%)	26 (42.6%)	3 (4.9%)	6 (9.8%)		
HER-2 overexpression						
No	458 (50.2%)	308 (33.7%)	100 (11%)	47 (5.1%)	0.000015	
Yes	93 (66.9%)	18 (12.9%)	21 (15.1%)	7 (5%)		**<0.0001**
ER						
Negative	151 (58.8%)	27 (10.5%)	61 (23.7%)	18 (7%)	$1.19x10^{-21}$	
Positive	390 (50.1%)	298 (38.3%)	55 (7.1%)	35 (4.5%)		**<0.0001**
PR						
Negative	216 (53.5%)	83 (20.5%)	80 (19.8%)	25 (6.2%)	$2.4x10^{-14}$	
Positive	316 (52%)	229(37.7%)	35 (5.8%)	28 (4.6%)		**<0.0001**

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1: Prognostic significance of ATM, BRCA1 and XRCC1 in breast cancers negative for CD8+ TILs. Kaplan-Meier survival curves are shown here.

Supplementary Figure S2: Prognostic significance of polβ (A &B), ERCC1 (C&D), RECQL4 (E&F), RECQL5 (G &H), BLM (I & J), PARP1 (K & L), FEN1 (M & N), TOPO2A (O & P), Ku70/Ku80 (Q & R), Chk2 (S & T), WRN (U & V) and DNA-PKcs (W & X) in CD8+ TILs positive and CD8+ TILs negative breast cancers respectively. Kaplan-Meier survival curves are shown here.

Supplementary Figure S3: Correlation between CD8 counts and ATM (A), BRCA1 (B) and XRCC1 (C).

Supplementary Figure S4: Prognostic significance of ATM, BRCA1 and XRCC1 in FOXP3+ or FOXP3 negative breast cancers. Kaplan-Meier survival curves are shown here.

Supplementary Figure S5: Prognostic significance of ATM, BRCA1 in PD-L1+ (tumour cells), PD-L1+ (TILs) or PD-1+ (TILs) is shown here. Kaplan-Meier survival curves are shown here.

Supplementary Figure S6: Prognostic significance of ATM, BRCA1 and XRCC1 in PD-L1 negative (tumour cells), PD-L1 negative (TILs) and PD-1 negative (TILs) breast cancers. Kaplan-Meier survival curves are shown here.

Supplementary Figure S7: Prognostic significance of CD8+ TILs in ER+ breast cancer [whole cohort (A), received no endocrine therapy (B) and received endocrine therapy (C)] is shown here.

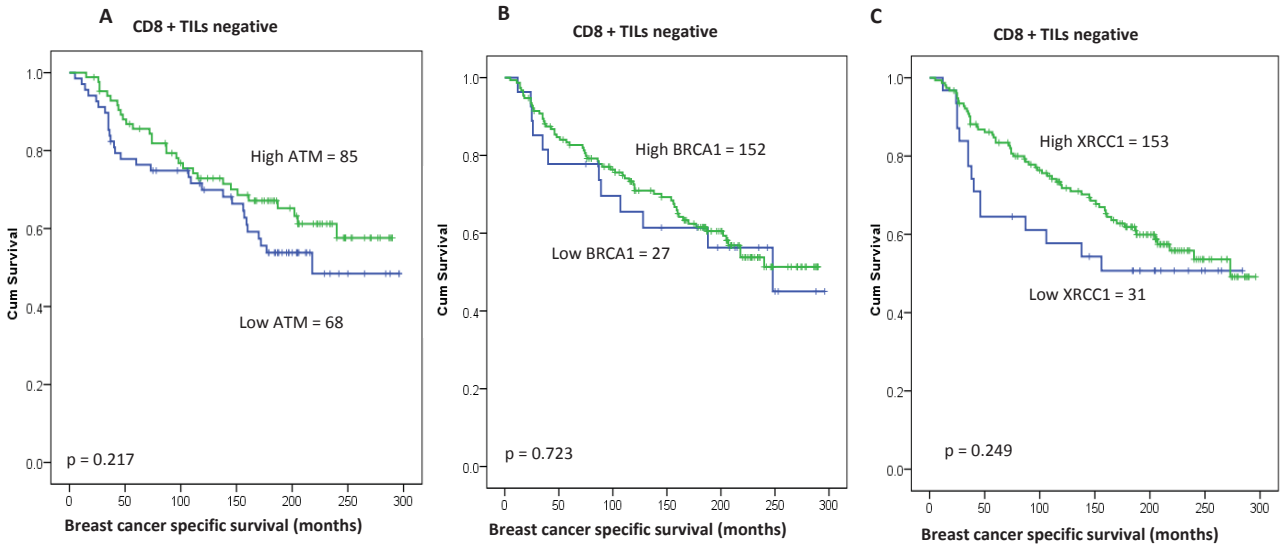
Supplementary Figure S8: Prognostic significance of BRCA1 (A), XRCC1 (B) or ATM (C) in CD8+ TILs positive ER+ breast cancer. Kaplan-Meier survival curves are shown here.

Supplementary Figure S9: Prognostic significance of XRCC1 in CD8+/CD8- (A), PD-1+/PD-1- (B), FOXP3+/FOXP3- (C) and PD-L1+/PD-L1- (D) ER+ breast cancer that received no endocrine therapy.

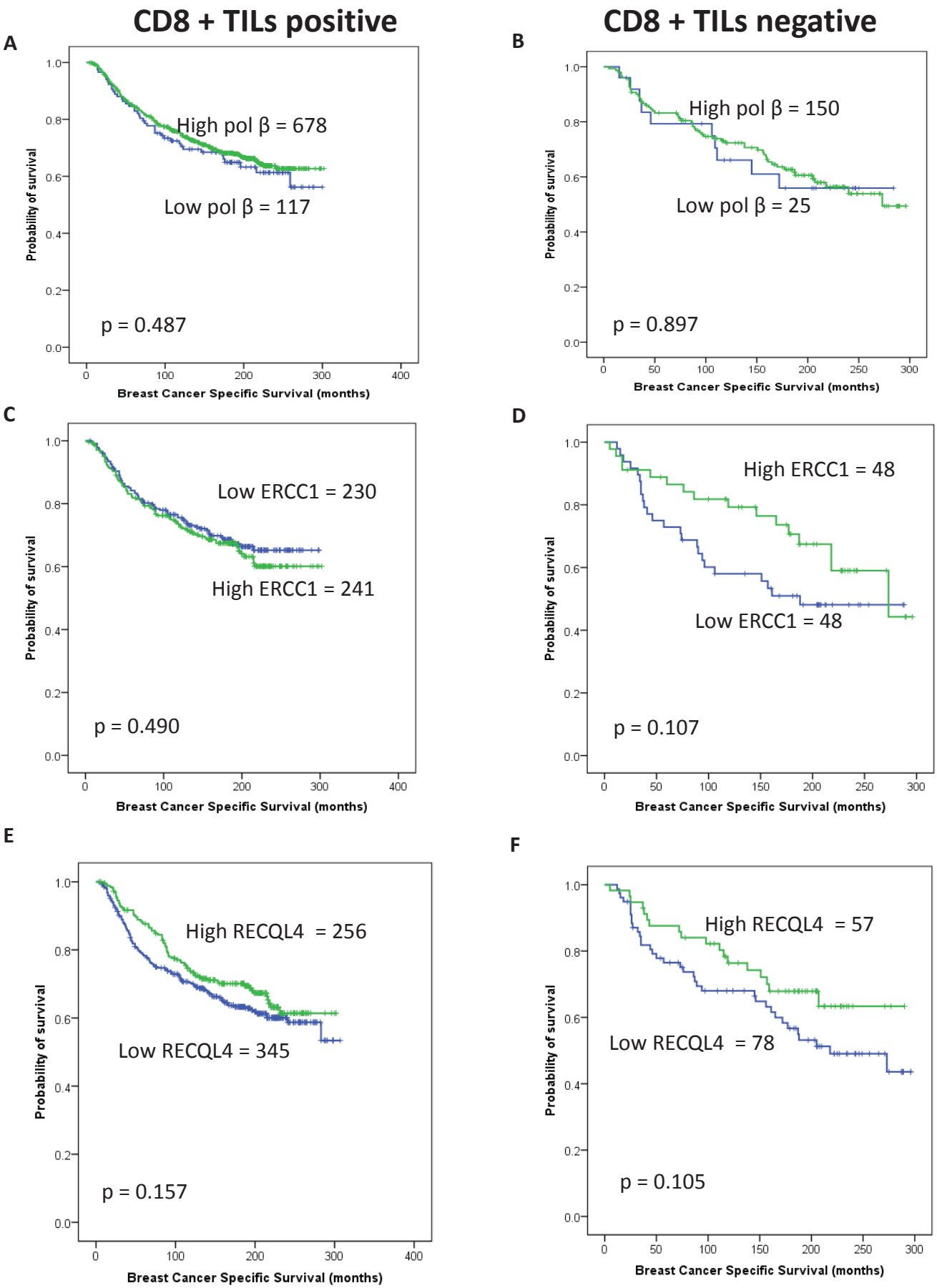
Supplementary Figure S10: A. Prognostic significance of XRCC1 in PD-1+/PD-1- HER2 + breast cancers. **B.** Prognostic significance of BRCA1 in FOXP3+/FOXP3- HER2 + breast cancers. Kaplan-Meier survival curves are shown here.

Supplementary Figure S11: Prognostic significance of CD8+ TILs in ER- breast cancer [whole cohort (A), received no chemotherapy (B) and received chemotherapy (C)] is shown here. Kaplan-Meier survival curves are shown here.

Supplementary Figure S12:.Prognostic significance of BRCA1 in PD-L1+/PD-L1- ER- breast cancers that received no chemotherapy. Kaplan-Meier survival curves are shown here.



Supplementary Figure S1

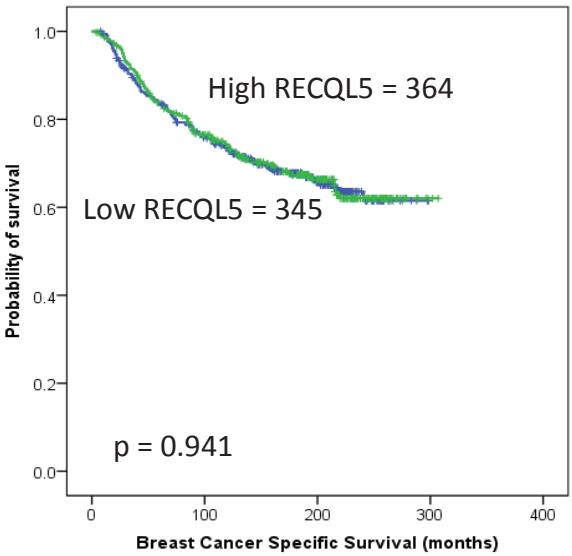


Supplementary Figure S2

CD8 + TILs positive

CD8 + TILs negative

G



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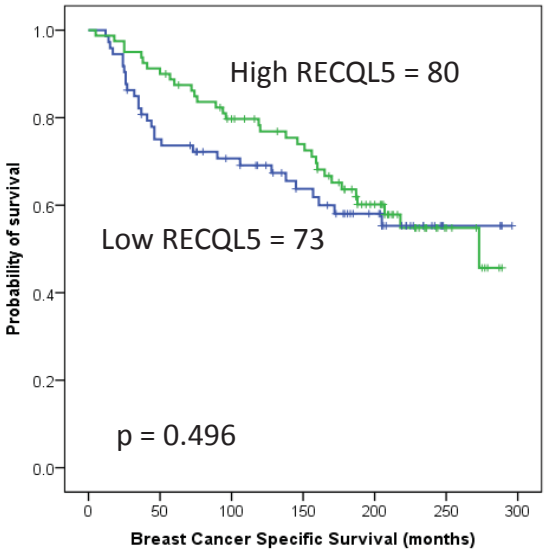

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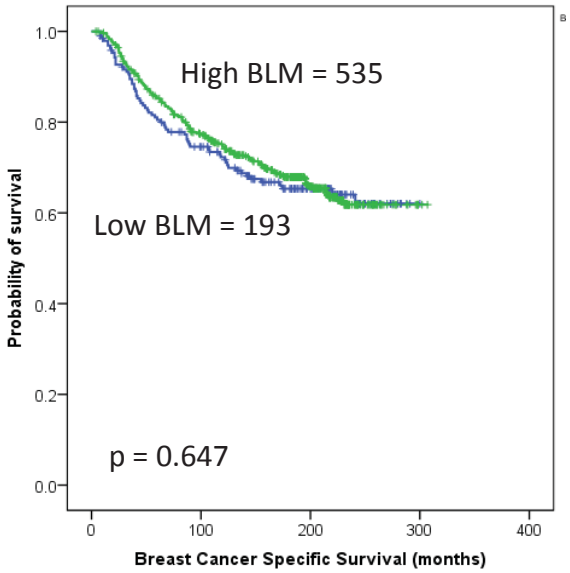

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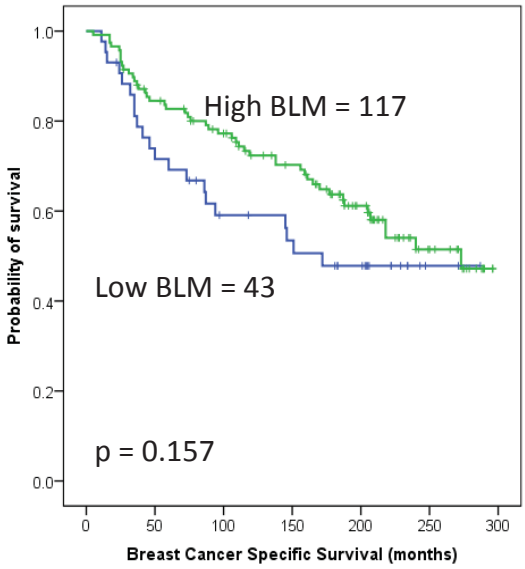

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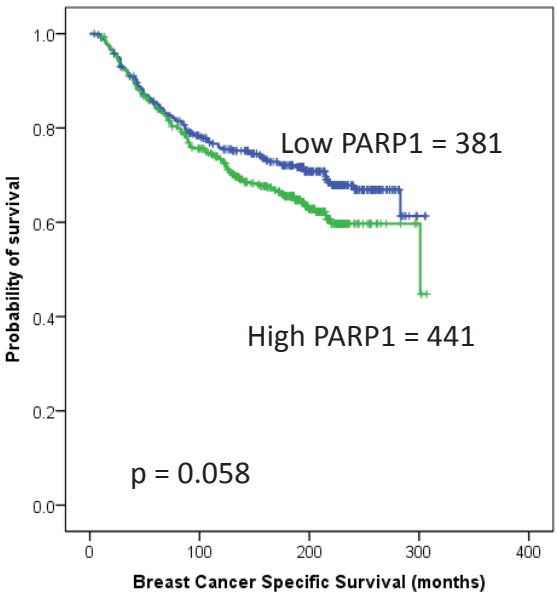

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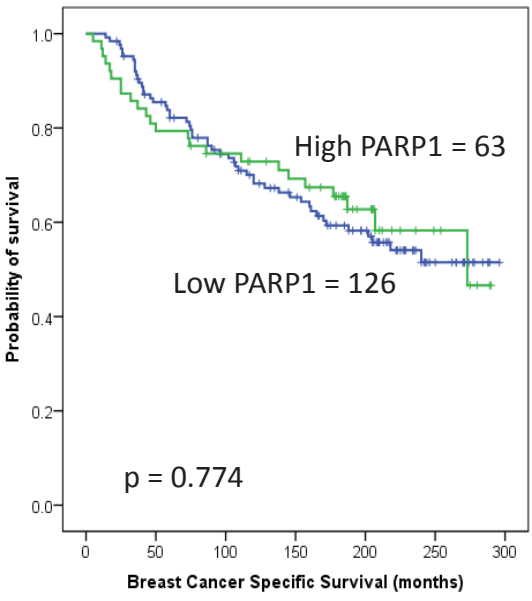
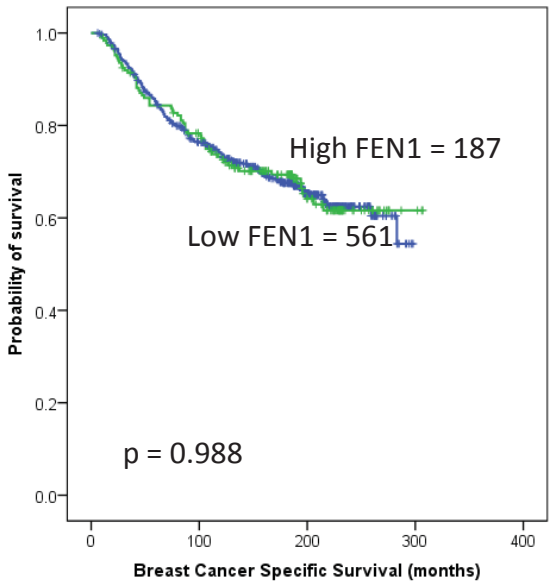


Supplementary Figure S2

CD8 + TILs positive

CD8 + TILs negative

M



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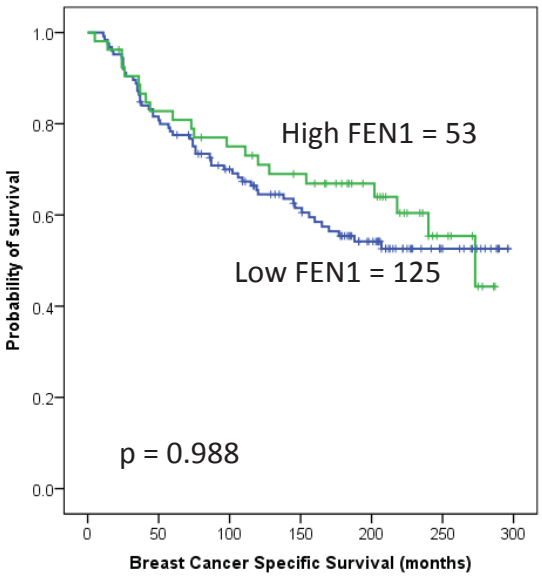

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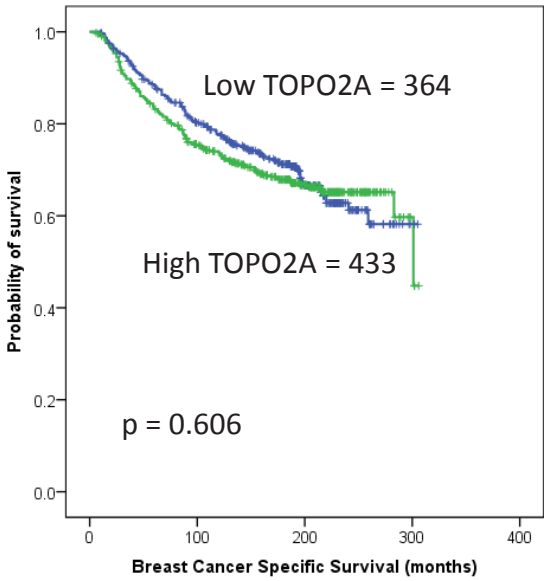

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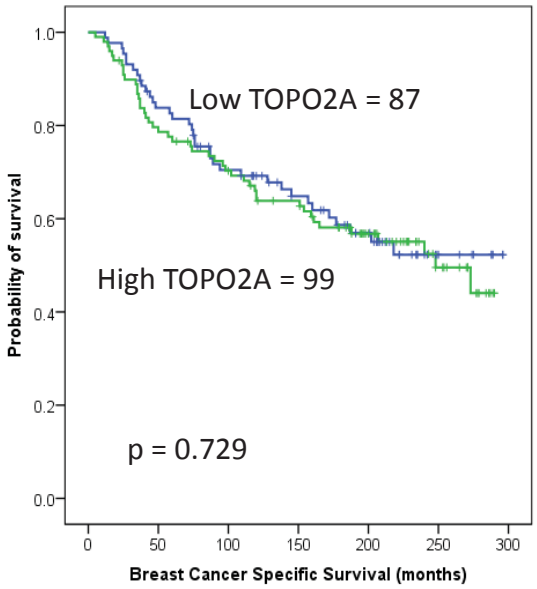

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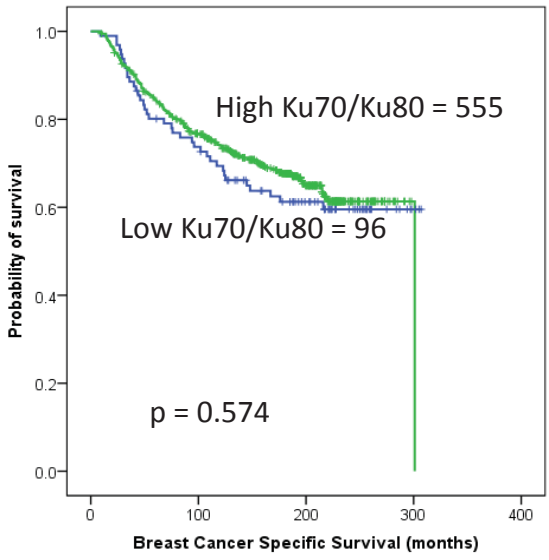

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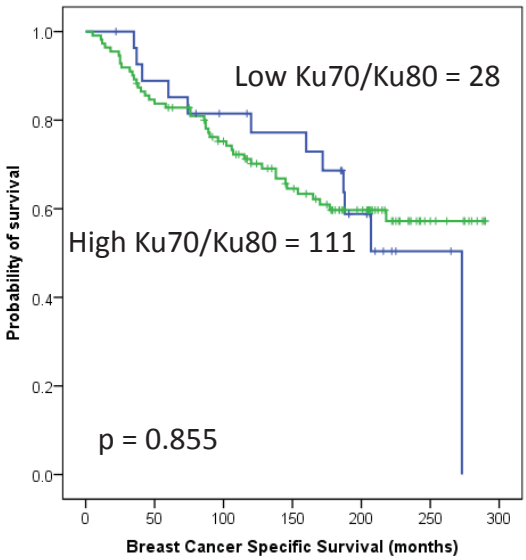
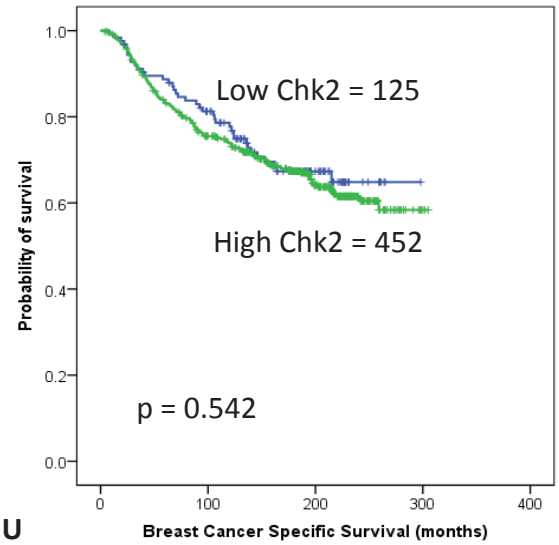


Supplementary Figure S2

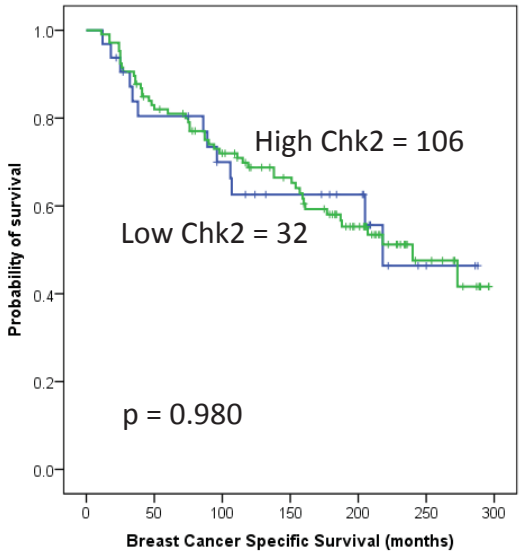
CD8 + TILs positive

S



CD8 + TILs negative

T



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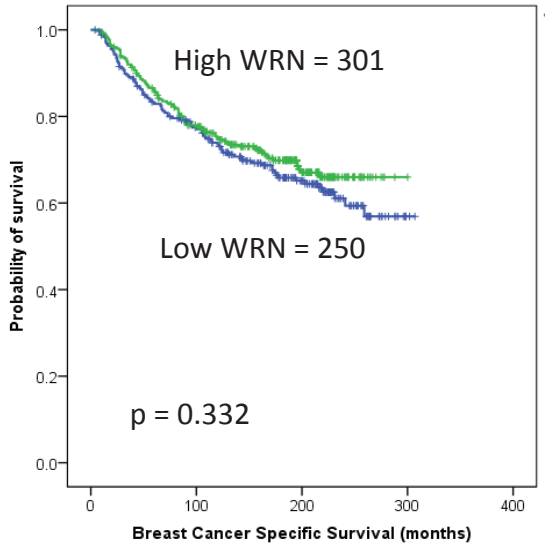

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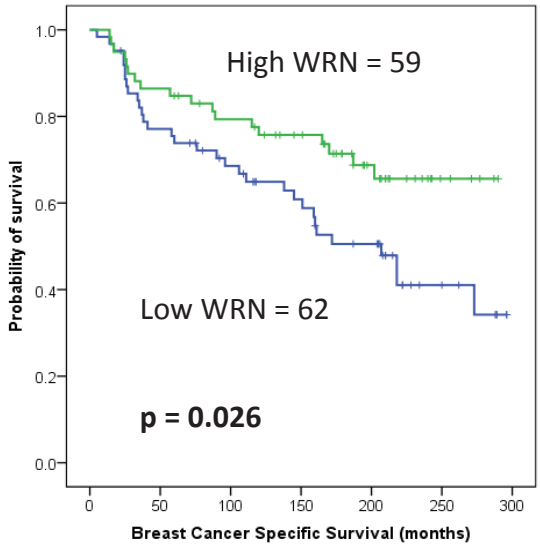

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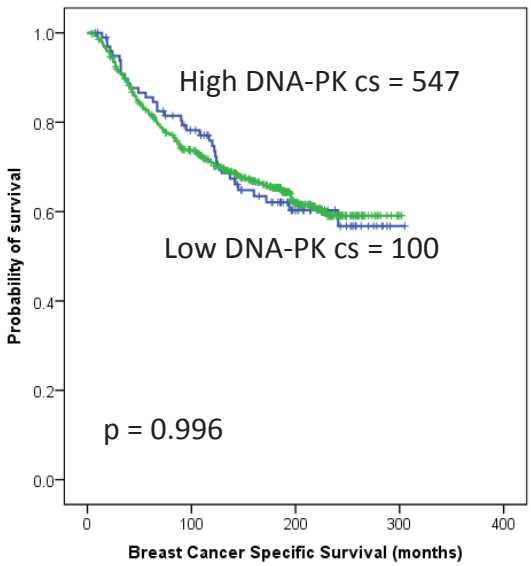

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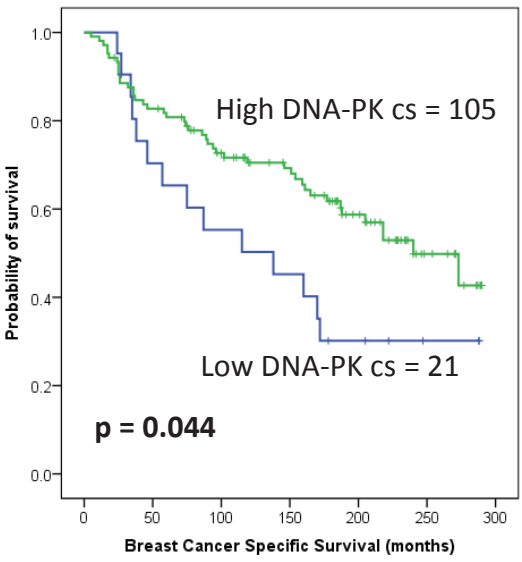
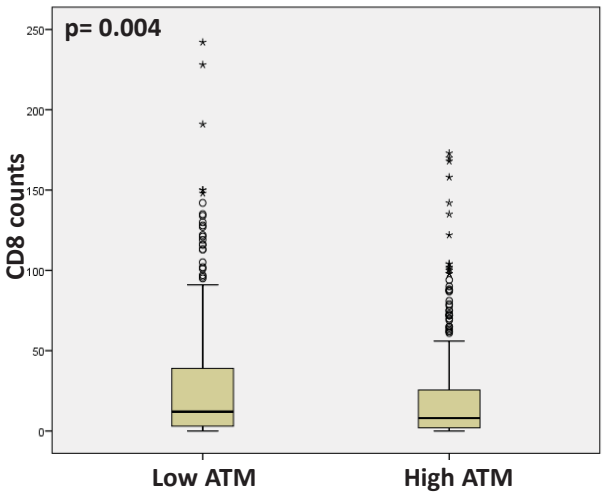


Supplementary Figure S2

A



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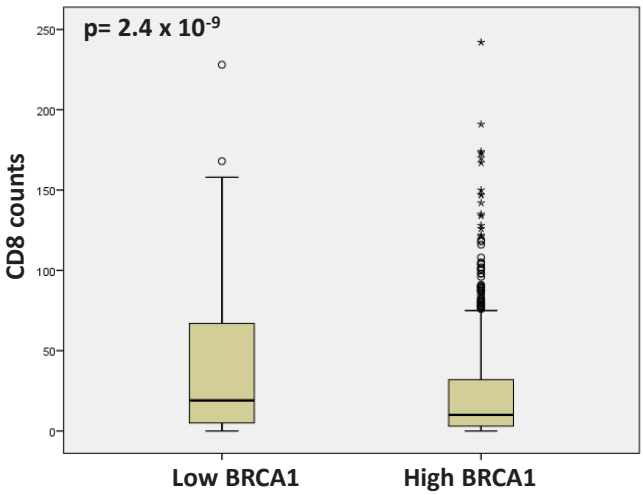

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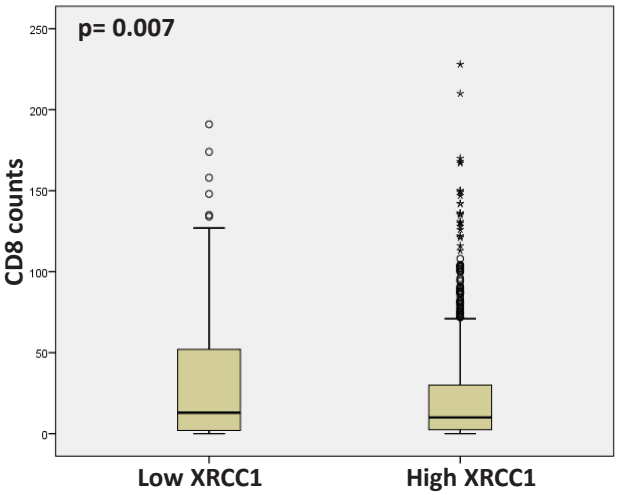
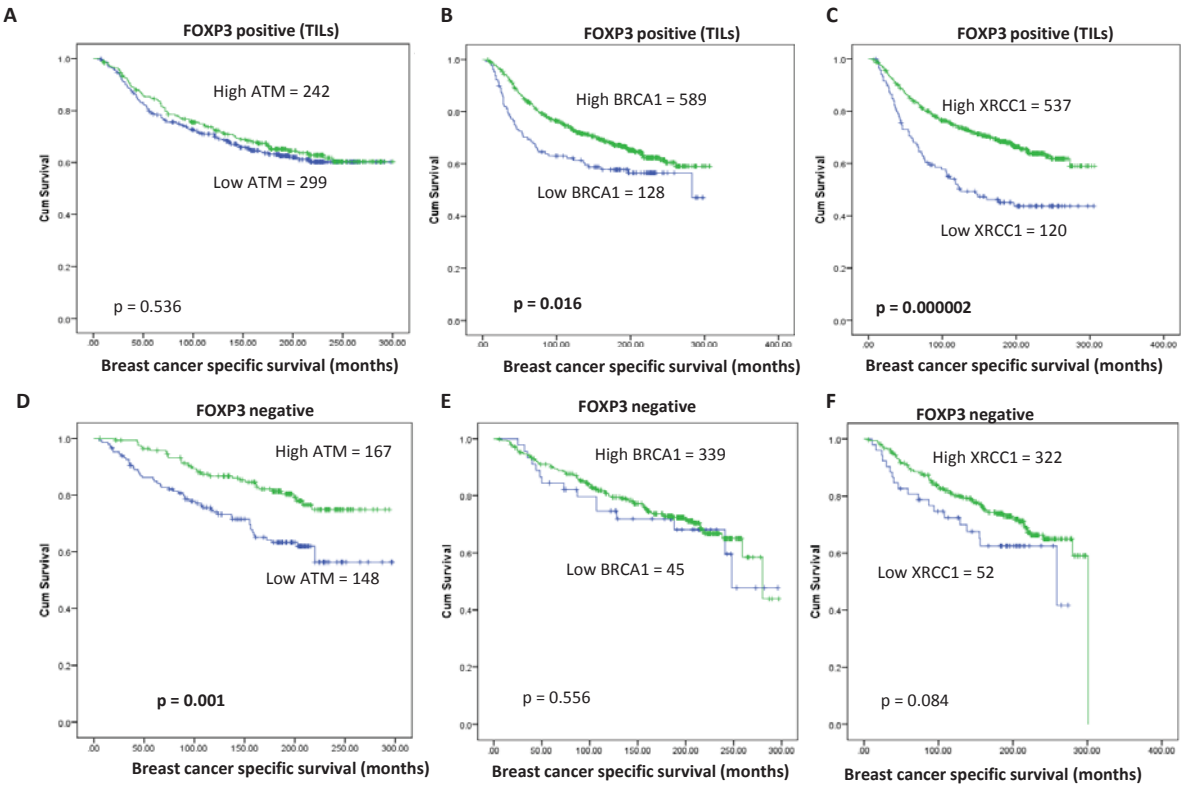
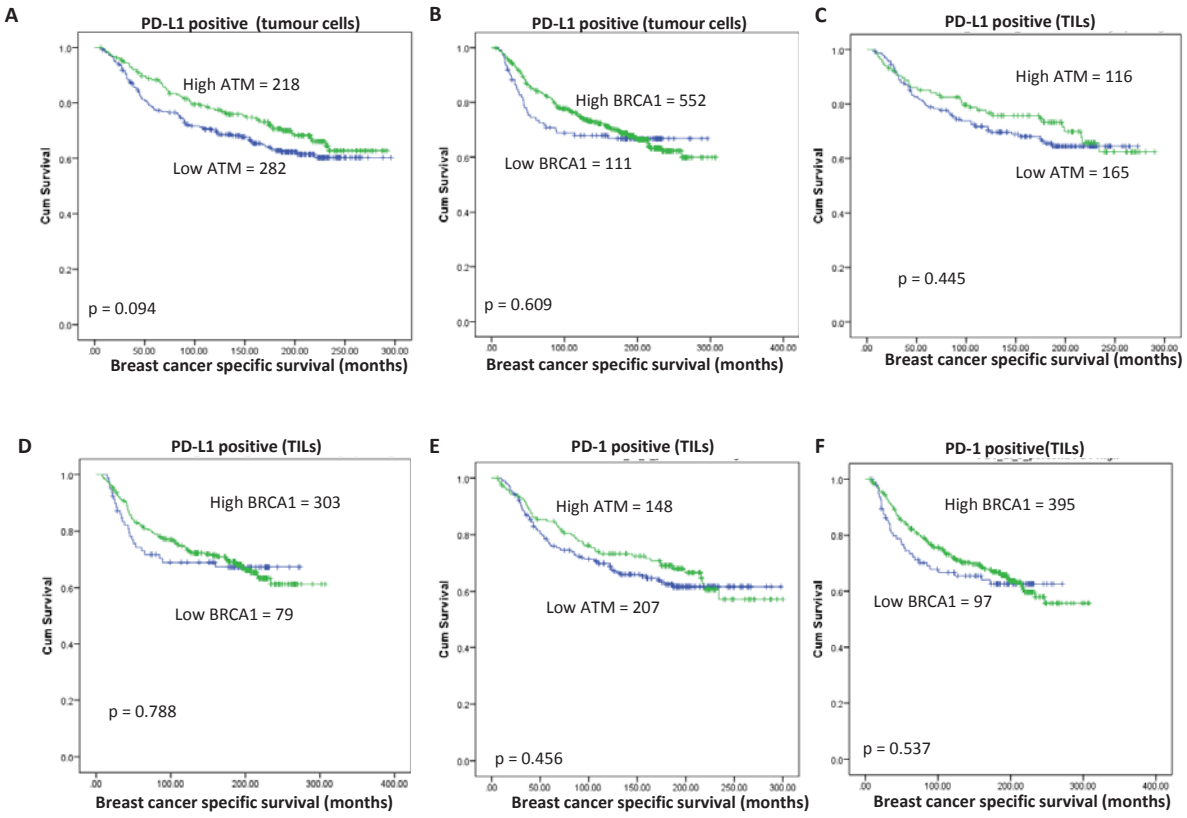


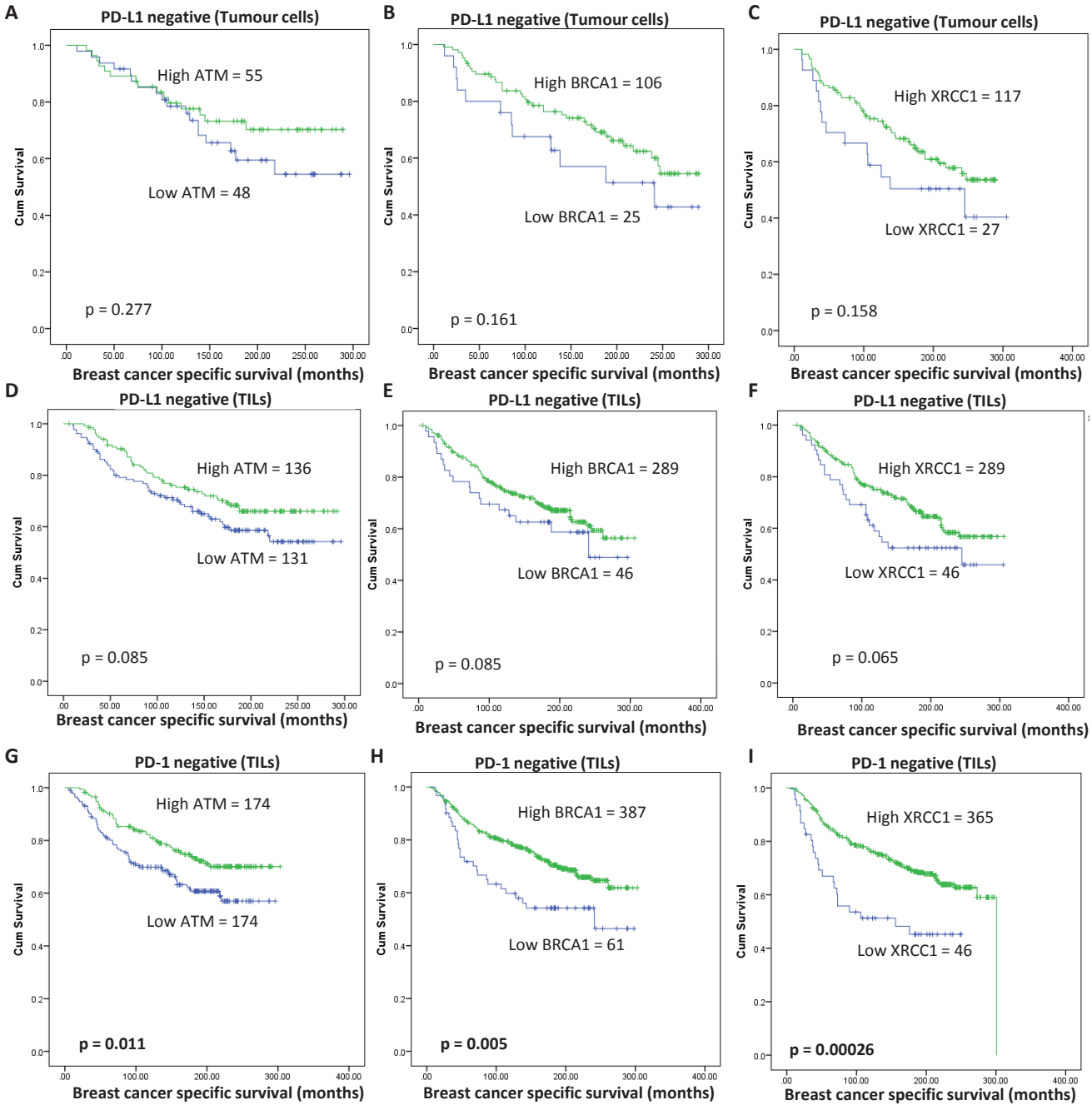
Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure S5



Supplementary Figure S6

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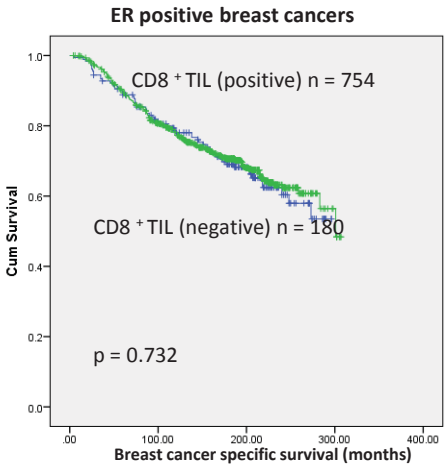

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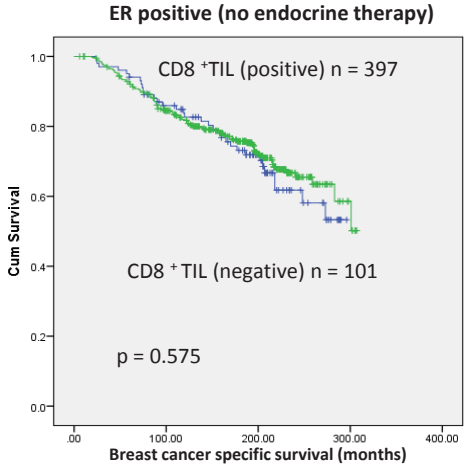

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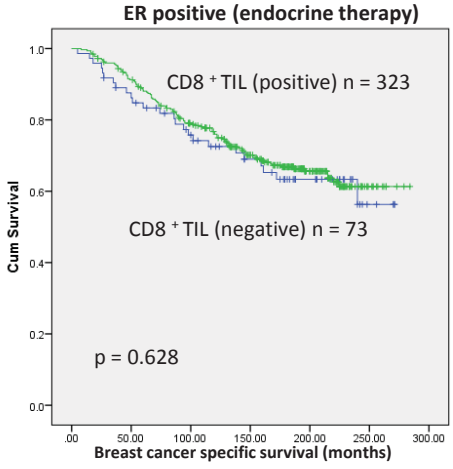
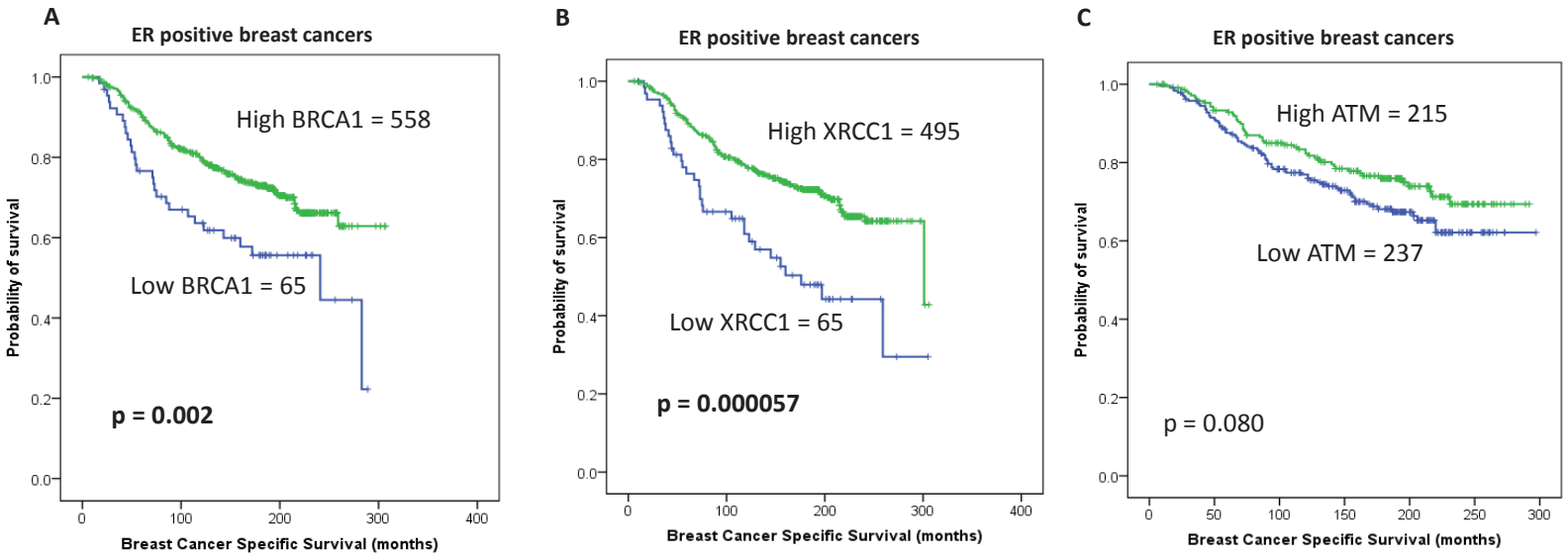


Supplementary Figure S7



Supplementary Figure S8

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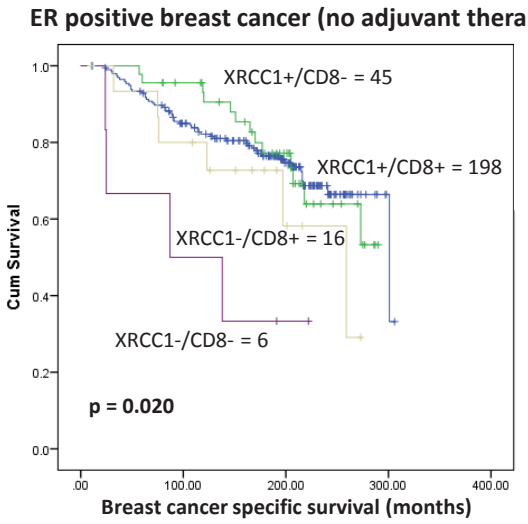

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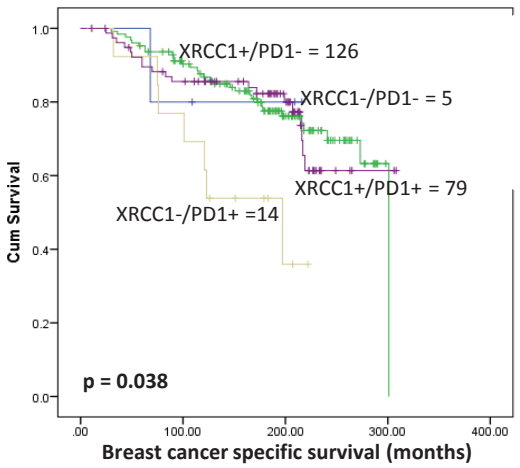

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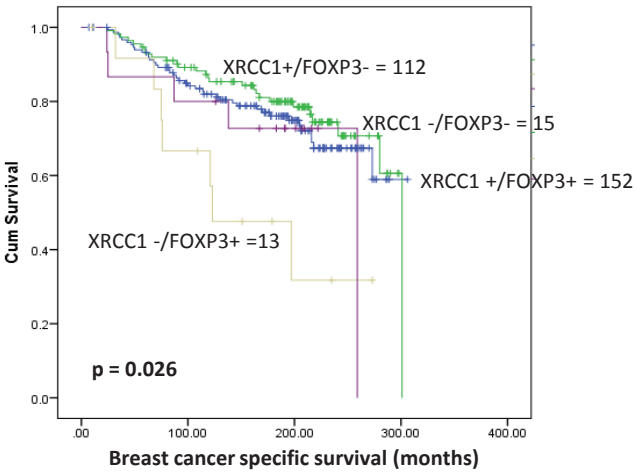

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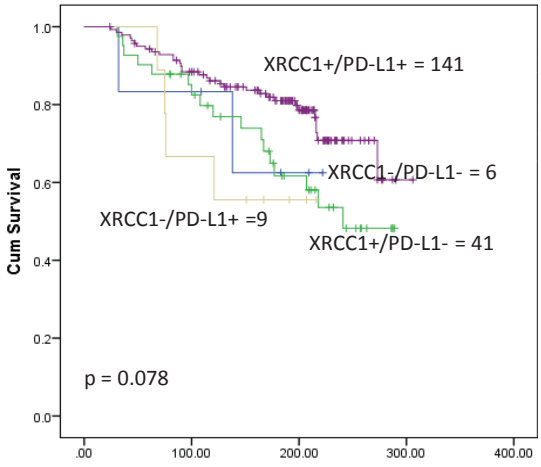

Supplementary Figure S9

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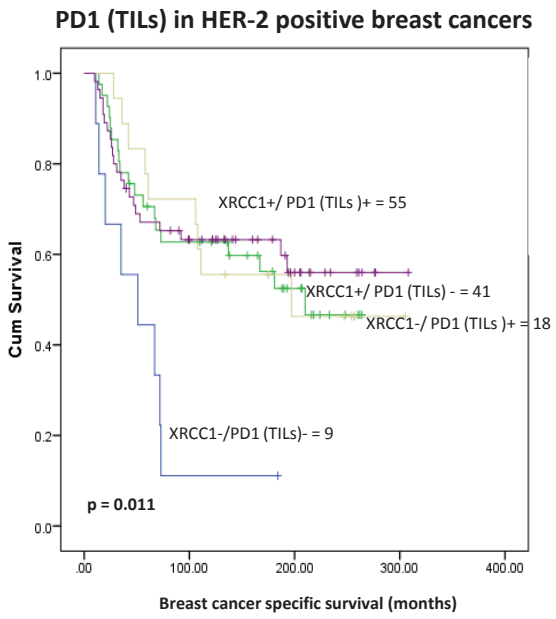

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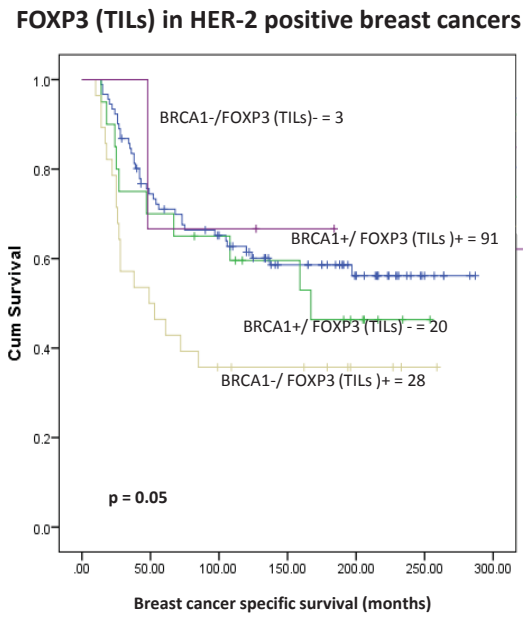

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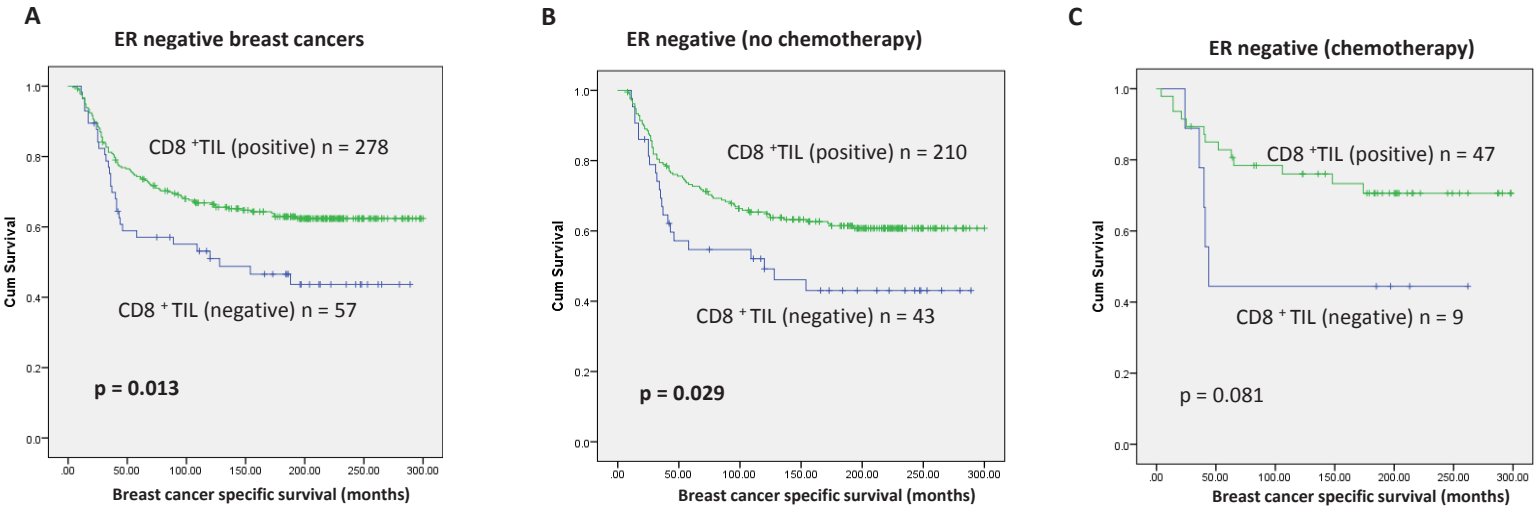

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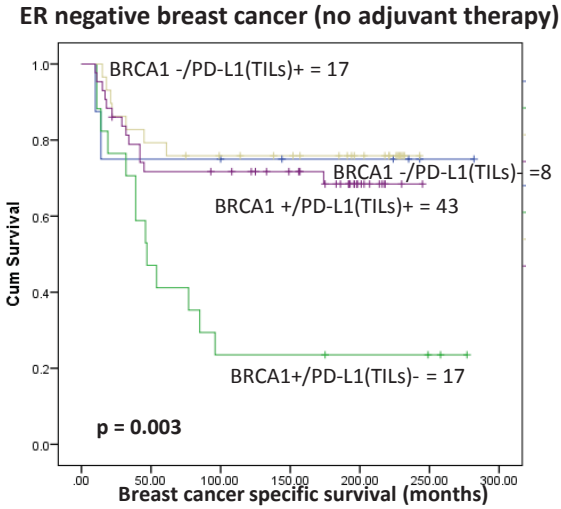

Supplementary Figure S12