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Characterizing excision repair cross-complementing family genes as drug resistance biomarkers in breast cancer

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Abstract

Background Excision repair cross-complementing (ERCC) genes are important regulators of DNA repair processes, the aberrant expression of which may lead to treatment failures of breast cancer. The prognostic significance of the ERCC genes in several cancers has been investigated, except for breast cancer; therefore, we explored the ERCC genes, including *ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, ERCC6,* and *ERCC8* in breast cancer, particularly during drug resistance processes.

Results Using the 2021 provisional study of The Metastatic Breast Cancer Project from cBioPortal, we identified *ERCC* genetic alterations in 8–36% of patients, where most alterations were considered amplifications followed by deep deletions. Pathway enrichment analyses identified Wnt signaling enrichment which contributed to cell proliferation. *ERCC2* had the highest epigenetic alteration levels at 7 DNA methylation sites. Also, the mRNA levels of *ERCC1, ERCC2, ERCC4, ERCC6,* and *ERCC8* were higher in patients with breast cancer when compared to normal breast tissues, with higher *ERCC2* but lower *ERCC8* levels in metastatic breast tissues. Breast cancer patients with low *ERCC6* levels had better overall survival rates than the groups with higher *ERCC6* levels. *ERCC1, ERCC2,* and *ERCC4* were identified as endocrine therapy response predictors. *ERCC1* was specifically an antihuman epidermal growth factor receptor therapy predictor, and *ERCC1, ERCC2, ERCC6*, and *ERCC8* were chemotherapy response predictors.

Conclusion We used bioinformatics to investigate and identify the roles of ERCC genes in breast cancer resistant cells, in particular *ERCC1*, *ERCC2*, and *ERCC6*. We also showed how the Wnt pathway and DNA repair processes had a role in drug resistance in breast cancer cells, but further studies are required to validate those results.

Keywords Excision repair cross-complementing genes, Breast cancer, Drug resistance, Bioinformatics, DNA repair

1 Background

Resistance to therapies, including endocrine, antihuman epidermal growth factor receptor (HER2), and chemo/radiation therapies, is a major hurdle in breast cancer treatment [1]. Therefore, identifying the resistance mechanisms during therapies is the key to successful breast cancer treatment and therapy development [2, 3]. Resistant breast cancer cells may progress to metastatic cells that spread to other tissues and cause patient death; hence, overcoming breast cancer resistance mechanisms can prevent metastasis [4]. Additionally, the discovery of resistance biomarkers can assist in directing treatment

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decisions and improving the outcomes of patients with breast cancer [5, 6].

During therapy, breast cancer cells may develop resistance mechanisms, such as enhanced DNA repair involving upregulated DNA repair genes [1]. Excision repair cross-complementing (ERCC) genes are essential components of the nucleotide excision repair process and important DNA repair regulators [7]. Sophisticated DNA repair processes that remove DNA damage and maintain chromosome stability are generated by the proteins encoded by the ERCC genes, including *ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, ERCC6,* and *ERCC8* [8]. Genomic instability that leads to genetic and epigenetic alterations, and cancer development of are influenced by ERCC pathway dysregulation [9–12].

Importantly, the prognostic significance of the ERCC genes in several cancers has been reported. A previous study indicated that *ERCC1* mRNA and protein overexpression were correlated with lung cancer cell resistance against platinum-based chemotherapy [13, 14]. Bioinformatics analyses of the ERCC genes in ovarian cancer showed that high *ERCC1* and *ERCC8* mRNA levels are associated with poor overall survival (OS) rates in patients with ovarian cancer, whereas patients with increased *ERCC4* mRNA levels had better OS rates than the patients with low *ERCC4* mRNA levels [10]. Another bioinformatic study on the ERCC genes in gastric cancer cells reported that *ERCC4*, *ERCC6*, and *ERCC8* are candidate prognosis biomarkers and could function as potential therapeutic targets [15]. However, the role of ERCC genes, as well as their genetic and epigenetic regulation in breast cancer cells, remains elusive and requires more investigation.

In this review, the ERCC alterations will be carried out using the data of the provisional study of The Metastatic Breast Cancer Project (MBCP, 2021) after previous reports [16–25]. Understanding the role of ERCC genes, including epigenetic and genetic modifications, and other factors in drug resistance in breast cancer may suggest mechanisms and help in identifying potential targets for novel therapies. Therefore, it can be helpful for developing new drugs and therapeutic strategies targeting ERCC genes to overcome drug resistance in breast cancer therapy. In this study, using a bioinformatic approach, we explored the ERCC genes, including *ERCC1*, *ERCC2*, *ERCC3*, *ERCC4*, *ERCC5*, *ERCC6*, and *ERCC8,* in breast cancer, particularly their involvement in drug resistance.

2 Methods

2.1 Genetic alterations

ERCC gene analyses were conducted using cBioPortal (https://www.cbioportal.org/) [26, 27]. Briefly, ERCC gene symbols were entered into cBioPortal, and associated breast cancer studies were selected. Then, studies with the highest number of genetic modifications were selected for additional genetic alteration analyses, including OncoPrint, copy number alterations (CNA), mutations, mutual exclusivity, functional mutant predictions, and pathway enrichment from Pathway Mapper and NDEx. Statistical analyses of CNA were performed using One-Way Analysis of Variance with Tukey's multiple comparison tests. * indicates $p < 0.05$.

2.2 Epigenetic alterations

MethSurv (https://biit.cs.ut.ee/methsurv/) was used to analyze epigenetic changes [28]. Briefly, ERCC gene symbols were entered into MethSurv using several criteria, such as breast invasive carcinoma from the Cancer Genome Atlas (TCGA) study, 2017.

2.3 ERCC mRNA and protein expression

We examined ERCC mRNA expression profiles in normal and tumor breast tissue from The Genotype-Tissue Expression (GTEx) and TCGA studies and analyzed data using GEPIA (http://gepia.cancer-pku.cn/) [29, 30]. Briefly, gene symbols were entered into GEPIA and several parameters were selected, including box plot expression, |Log2FC| cutoff = 1, p-value cutoff < 0.01, using a dataset of BRCA, Jitter Size of 0.4, and match TCGA normal and GTEx data. We analyzed ERCC mRNA expression levels in normal, breast tumor, and metastatic breast tumor tissues from TCGA and GTEx studies using TNM Plot (https://tnmplot.com/analysis/) [31]. Briefly, gene symbols were submitted to TNM Plot using different criteria; RNA-sequencing data, and tumor, normal, and metastatic samples from TCGA and GTEx studies, and statistical analyses were conducted using Kruskal–Wallis tests. ERCC protein expression data were analyzed in normal and breast cancer tissue samples using the Human Protein Atlas (https://www.proteinatlas.org/) [32].

2.4 Prognostic values

OS rates related to ERCC mRNA expression levels were analyzed using the Kaplan–Meier (KM) Plotter (https://kmplot.com/analysis/) using the following criteria: mRNA gene chip data and no subtype and cohort restrictions [33].

2.5 Receiver operating characteristic (ROC) plots

Associations between gene expression levels and sensitivity of breast cancer patients to endocrine, anti-HER2, and chemotherapy were examined in ROC Plotter [34]. Estrogen receptor (ER) and HER2 status, pathological complete response (PCR), relapse-free survival (RFS) for 5 years, and patients receiving endocrine anti-HER2

therapy and chemotherapy were selected. Gene symbols were entered into ROC Plotter and $p < 0.05$ values was selected as statistical significance thresholds.

3 Results

3.1 ERCC genetic alterations

Genetic alteration analyses using cBioPortal showed that *ERCC* alterations mostly occurred in the 2021 provisional study of The Metastatic Breast Cancer Project by Jain et al. (2023), and therefore was used for further analyses [18] (Fig. 1A). Oncoprint analyses identified genetic alterations in *ERCC1* (27%), *ERCC2* (28%), *ERCC3* (16%), *ERCC4* (16%), *ERCC5* (8%), *ERCC6* (36%), and *ERCC8* (13%), where most alterations were amplifications followed by deep deletions, except for *ERCC8* which was dominated by deep deletions (Fig. 1B). CNA analyses (Fig. 1C) showed that *ERCC4* mRNA expression levels were significantly higher in amplification cases when compared to diploid cases, and *ERCC8* mRNA levels were significantly higher in diploid cases when compared to shallow deletion cases. Mutual exclusivity analyses showed that 2 gene pairs exhibited co-occurrence, i.e., *ERCC1-ERCC2* and *ERCC4-ERCC6* (Table 1). We also detected mutations in the ERCC genes (Fig. 1D, Table 2); *ERCC3* (I194M), *ERCC5* (L6H), and *ERCC6* (R670W) mutations were predicted to be highly impactful, deleterious, and probably damaging. Pathway enrichment analyses related to genetic alterations identified enriched Wnt signaling which contributed to cell proliferation (Pathway Mapper, Fig. 1E) and nucleotide excision repair in *Homo sapiens* (NDEx, Fig. 1F).

3.2 Epigenetic alterations

ERCC epigenetic alteration analyses identified 1 alteration in *ERCC1* (cg16629408) (Additional file 1: Fig. S1). *ERCC2* recorded the highest epigenetic alterations with 7 altered methylation sites, including cg01518138, cg17212420, cg03117793, cg01599094, cg18851932, cg02595770, and cg20674128. Epigenetic alterations were also identified in *ERCC3* (cg06373940, cg11957777, and cg26522792), *ERCC4* (cg08296903, cg08387426, cg05348793, and cg26493247), *ERCC5* (cg23044680, cg23957850, cg10258411, cg14904079, cg00691940, and cg00884663), *ERCC6* (cg00025044, cg06437173, cg23926543), and *ERCC8* (cg02408480 and cg25966817).

3.3 ERCC mRNA and protein expression

In GEPIA, *ERCC1, ERCC2, ERCC4, ERCC6*, and *ERCC8* mRNA levels were higher in breast cancer samples when compared to normal breast tissues, whereas *ERCC3* and *ERCC5* mRNA levels were higher in normal breast tissues when compared to breast tumor samples (Fig. 2A). Using TNM Plot, mRNA analyses of normal, tumor, and metastatic breast cancer tissues showed that *ERCC2* mRNA levels were significantly higher in breast tumor tissues when compared to adjacent tissues, but lower when compared to metastatic breast tumor tissues ($p = 1.86 \times 10^{-10}$) (Fig. 2B). Additionally, *ERCC5* mRNA levels were higher in normal breast tissues when compared to breast tumor samples, but breast tumor tissue levels were still lower when compared to metastatic breast tumor levels ($p = 3.93 \times 10^{-7}$). Also, *ERCC8* mRNA levels were higher in normal breast tissue when compared to breast cancer and metastatic breast cancer samples ($p = 1.51 \times 10^{-5}$). From the Human Protein Atlas, ERCC1, ERCC2, ERCC3, ERCC4, and ERCC5 protein levels were increased in breast tumor samples when compared to normal adjacent tissue (Fig. 2C). No data were identified for ERCC6 and ERCC8 protein levels.

3.4 Prognostic values

The prognostic value of the ERCC gene expression across breast cancer samples indicated that only *ERCC6* demonstrated significant results; breast cancer patients with low *ERCC6* levels had better OS rates than the groups with higher *ERCC6* levels Fig. 3). Other gene data were not significant.

3.5 ROC plots

Based on the transcriptome data from patients with breast cancer, we examined relationships between gene expression levels and endocrine/anti-HER2/chemotherapy responses according to PCR and RFS outcomes. Based on the PCR parameters in response to endocrine therapy, 0.689 and 0.743 area under the curve (AUC) values were significantly moderately linked with *ERCC1* ($p = 5.1 \times 10^{-3}$) and *ERCC2* ($p = 1 \times 10^{-4}$) expression levels, respectively (Fig. 4A). Based on the RFS parameters for endocrine therapy responses, 0.578 ($p = 2 \times 10^{-3}$), 0.578 ($p = 1.3 \times 10^{-3}$), and 0.63 ($p = 1.9 \times 10^{-2}$) AUC values were significantly linked with *ERCC1*, *ERCC2*, and *ERCC4* mRNA levels, respectively (Fig. 4B). Based on the ROC on anti-HER2, an AUC value of 0.578 ($p = 2.3 \times 10^{-2}$) was moderately significantly linked to *ERCC1* expression (Fig. 4C). No significant results were identified for RFS values related to anti-HER2 therapy (Fig. 4D). According to the responses to chemotherapy and based on the PCR parameters, an AUC value of 0.613 ($p = 8.3 \times 10^{-6}$) was significantly linked with *ERCC6* levels (Fig. 4E), whereas based on the RFS parameters, 0.598 ($p = 9 \times 10^{-5}$), 0.55 ($p = 3.1 \times 10^{-2}$), 0.618 ($p = 8.7 \times 10^{-3}$), and 0.578 ($p = 1.4 \times 10^{-3}$) AUC values were significantly moderately linked with *ERCC1, ERCC2, ERCC6*, and *ERCC8* expression levels, respectively (Fig. 4F). No data were identified for *ERCC5* across all parameters.

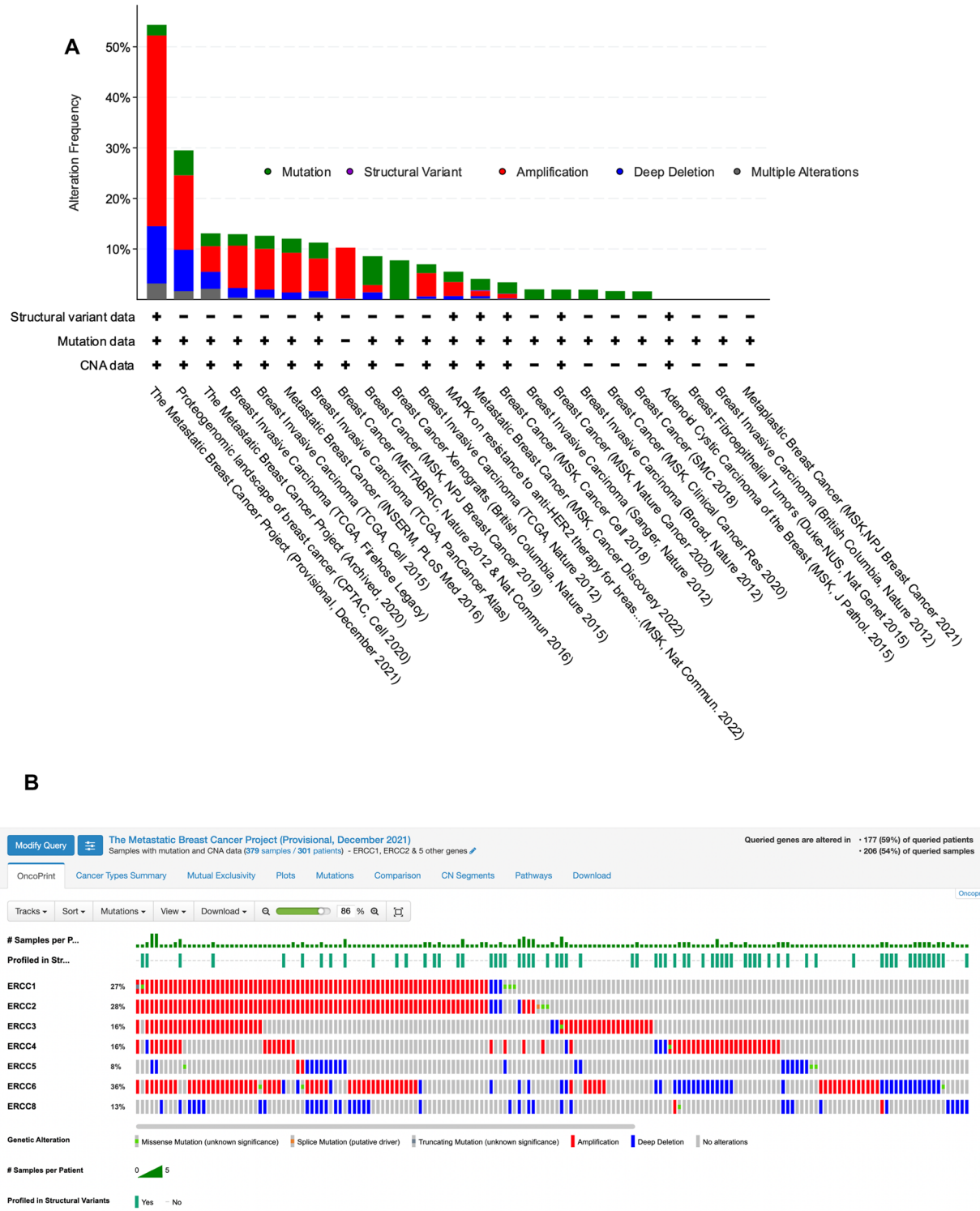
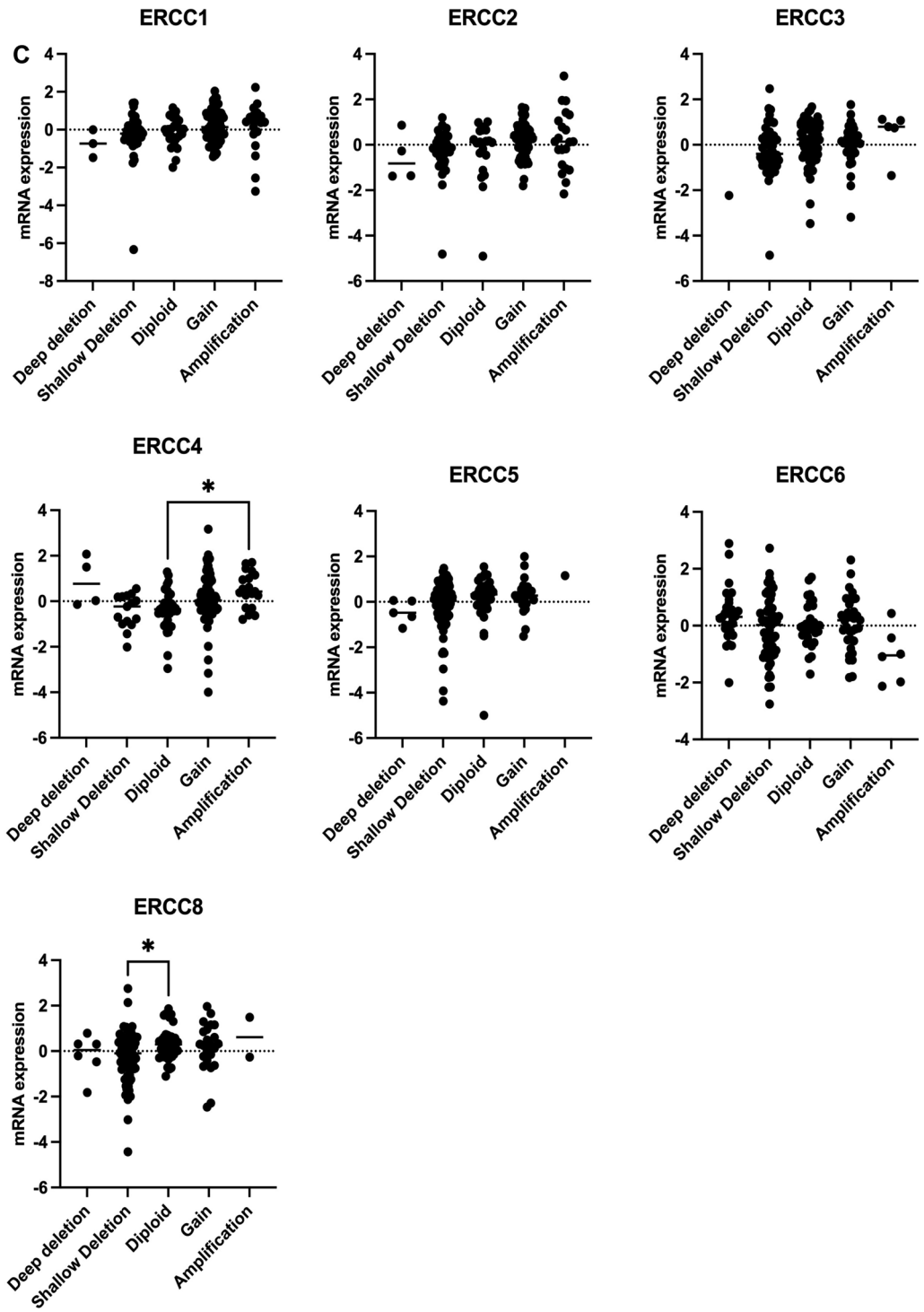


Fig. 1 ERCC genetic alterations: *ERCC1*, *ERCC2*, *ERCC3*, *ERCC4*, *ERCC5*, *ERCC6*, and *ERCC8* analyses using cBioportal. **A** Summary of *ERCC1*, *ERCC2*, *ERCC3*, *ERCC4*, *ERCC5*, *ERCC6*, and *ERCC8* gene alterations in breast cancer studies in cBioportal. **B** Oncoprint *ERCC1*, *ERCC2*, *ERCC3*, *ERCC4*, *ERCC5*, *ERCC6*, and *ERCC8* gene analyses using samples from the 2021 provisional study of The Metastatic Breast Cancer Project. **C** Copy number alterations (CNAs) in *ERCC1*, *ERCC2*, *ERCC3*, *ERCC4*, *ERCC5*, *ERCC6*, and *ERCC8* genes using samples from the 2021 provisional study of The Metastatic Breast Cancer Project. Statistical analyses were performed using One-Way Analysis of Variance with Tukey's multiple comparison tests. * indicates $p < 0.05$. **D** Mutations in *ERCC1*, *ERCC2*, *ERCC3*, *ERCC4*, *ERCC5*, *ERCC6*, and *ERCC8* genes in samples from the 2021 provisional study of The Metastatic Breast Cancer Project. Pathway enrichment analyses of *ERCC1*, *ERCC2*, *ERCC3*, *ERCC4*, *ERCC5*, *ERCC6*, and *ERCC8* gene alterations using (**E**). Pathway Mapper and (**F**). NDEx data



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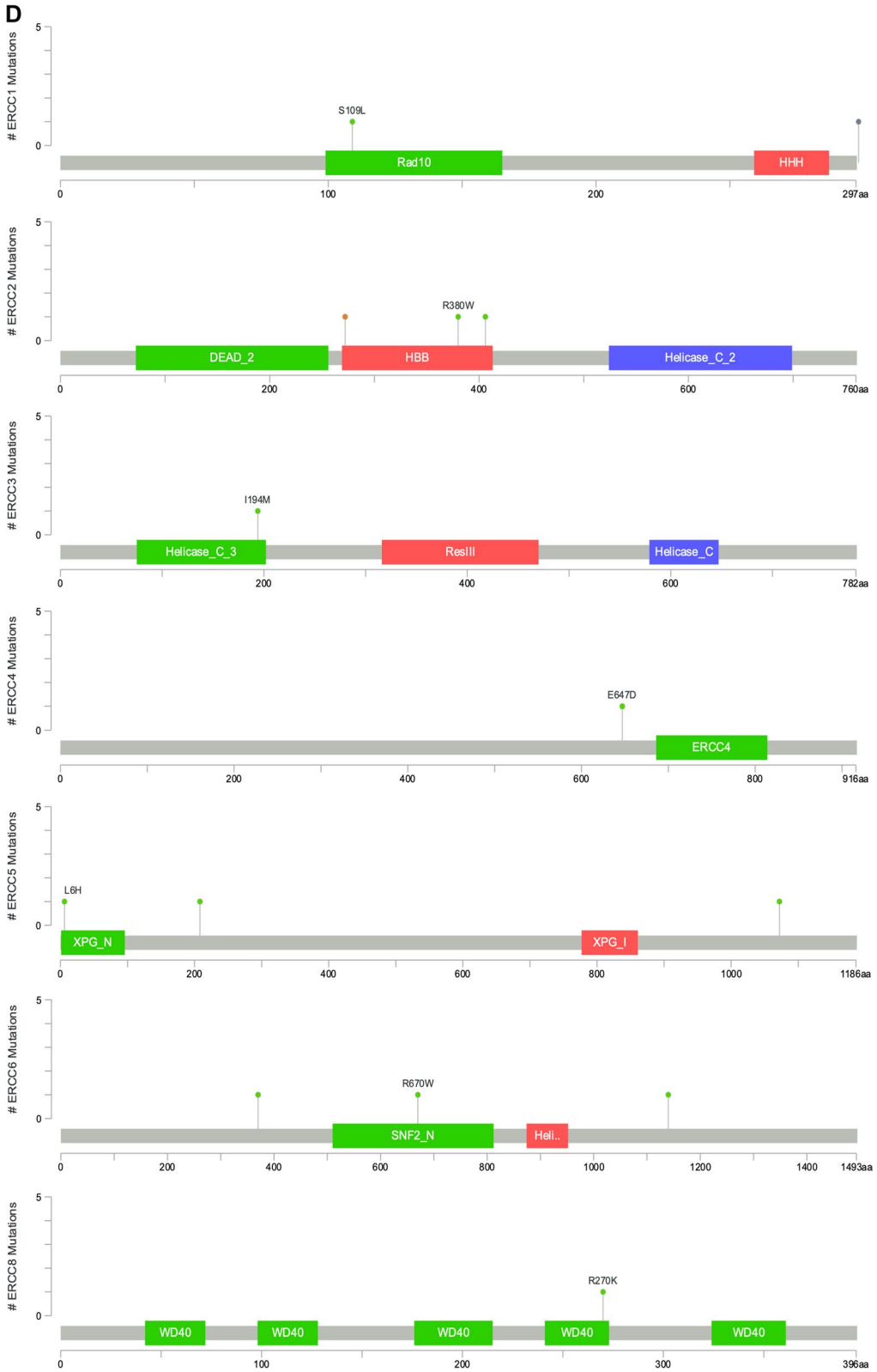

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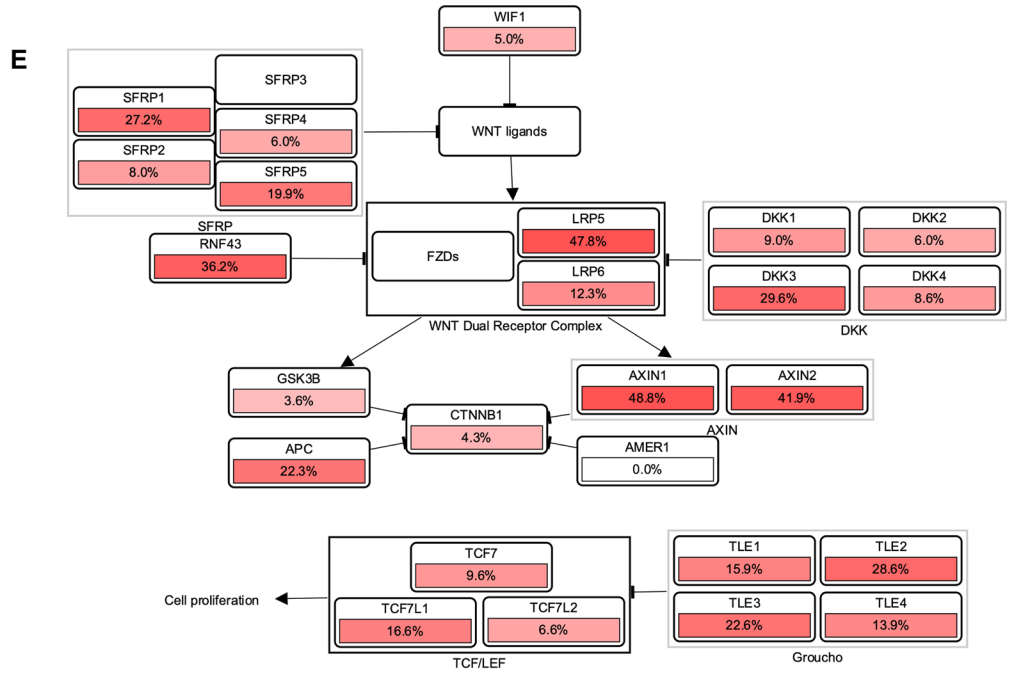

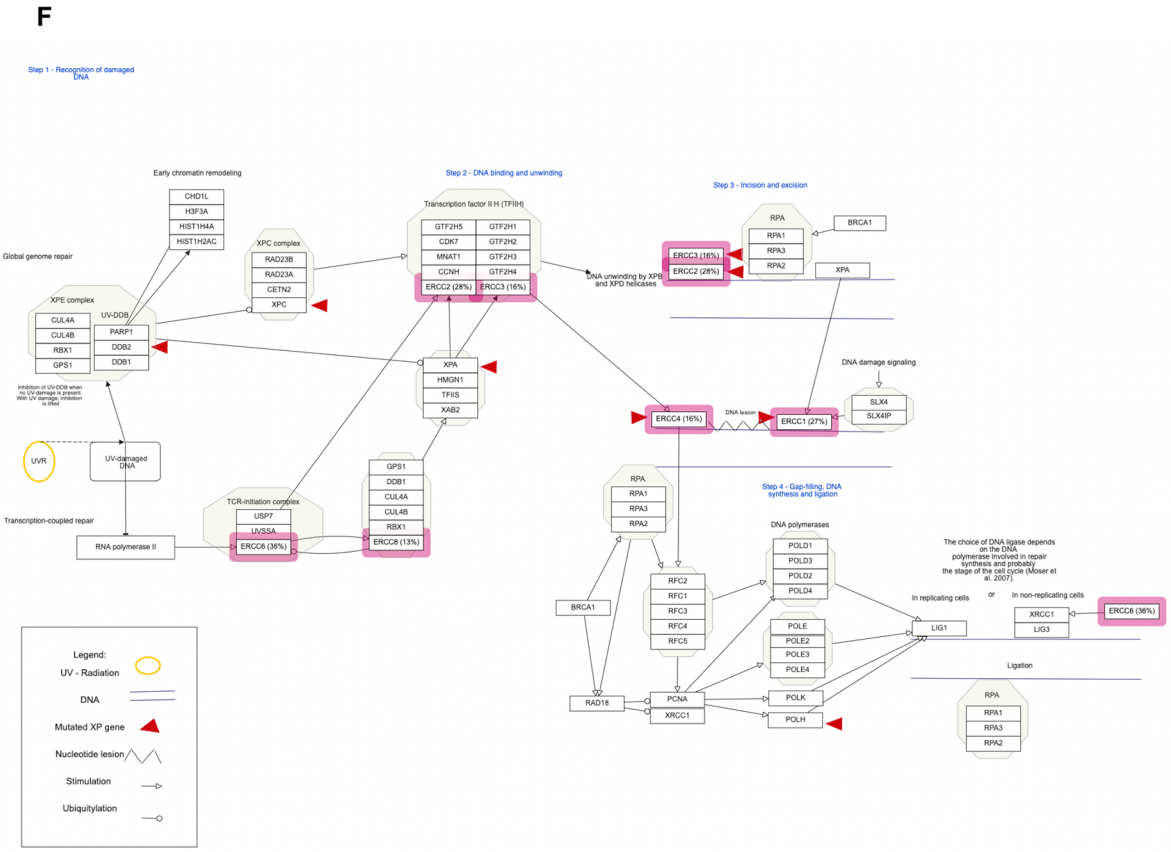

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Table 1 Mutual exclusivity study of ERCC among breast cancer samples from The Metastatic Breast Cancer provisional 2021 study

A	B	*p*-Value	Tendency
ERCC1	*ERCC2*	< 0.001	Co-occurrence
ERCC4	*ERCC6*	< 0.001	Co-occurrence

4 Discussion

We explored ERCC gene roles in breast cancer. Genetic alteration analyses (cBioportal) showed that *ERCC1, ERCC2,* and *ERCC6* had the highest alterations of all the *ERCC* genes. Oncoprint analysis results showed that in the Metastatic Breast Cancer Project Provisional 2021 study, most of genetic alterations are considered as amplification followed by deep deletion. CNA analyses identified significantly altered genes: *ERCC4* and *ERCC8*. Mutual exclusivity analyses showed that 2 gene pairs exhibited co-occurrence traits: *ERCC1-ERCC2* and *ERCC4-ERCC6*. Our findings highlighted the important roles of *ERCC1, ERCC2, ERCC4, ERCC6*, and *ERCC8* in metastatic breast cancer.

Mutations in *ERCC3* (I194M), *ERCC5* (L6H), and *ERCC6* (R670W) genes were predicted to be highly impactful, deleterious, and probably damaging. Previous studies reported that *ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, ERCC6*, and *ERCC8* mutations were associated with the clinical features of some diseases, including xeroderma pigmentosum, and increases the risk of skin cancer, cerebro-oculo-facial-skeletal syndrome, trichothiodystrophy, Cockayne syndrome, and UV-sensitive syndrome [35–38]. Accordingly, *ERCC5* and *ERCC6* mutant functions in breast cancer cells must be clarified in future studies.

Table 2 Functional prediction of the mutant ERCC, as analyzed using cBioportal

Gene	Protein Change	Functional Impact	Copy
ERCC1	E370K	MutationAssessor: impact: neutral, score: -0.345;SIFT: NA;Polyphen-2: NA	Amplification
	E444Q	MutationAssessor: impact: low, score: 1.59;SIFT: NA;Polyphen-2: NA	Gain
	S109L	MutationAssessor: impact: neutral, score: -0.69;SIFT: NA;Polyphen-2: NA	Gain
	P495S	MutationAssessor: impact: low, score: 1.7;SIFT: NA;Polyphen-2: NA	Amplification
	*298Sext*1	MutationAssessor: NA;SIFT: NA;Polyphen-2: NA	Amplification
	R330G	MutationAssessor: impact: low, score: 1.895;SIFT: NA;Polyphen-2: NA	Gain
	R330M	MutationAssessor: impact: low, score: 1.895;SIFT: NA;Polyphen-2: NA	Gain
ERCC2	X272_splice	MutationAssessor: NA;SIFT: NA;Polyphen-2: NA	Gain
	R380W	MutationAssessor: impact: low, score: 1.83;SIFT: NA;Polyphen-2: NA	Shallow Deletion
	L406V	MutationAssessor: impact: medium, score: 3.16;SIFT: NA;Polyphen-2: NA	Diploid
ERCC3	I194M	MutationAssessor: impact: high, score: 3.54;SIFT: impact: deleterious, score: 0;Polyphen-2: impact: probably_damaging, score: 0.983	Amplification
	I194M	MutationAssessor: impact: high, score: 3.54;SIFT: impact: deleterious, score: 0;Polyphen-2: impact: probably_damaging, score: 0.983	Diploid
	I194M	MutationAssessor: impact: high, score: 3.54;SIFT: impact: deleterious, score: 0;Polyphen-2: impact: probably_damaging, score: 0.983	Diploid
ERCC4	E647D	MutationAssessor: impact: low, score: 1.08;SIFT: impact: tolerated, score: 0.63;Polyphen-2: impact: benign, score: 0.015	Amplification
ERCC5	R1072T	MutationAssessor: impact: medium, score: 2.36;SIFT: impact: deleterious, score: 0;Polyphen-2: impact: possibly_damaging, score: 0.617	Gain
	M208T	MutationAssessor: impact: medium, score: 2.535;SIFT: impact: deleterious, score: 0;Polyphen-2: impact: probably_damaging, score: 0.994	Shallow Deletion
	L6H	MutationAssessor: impact: high, score: 3.69;SIFT: NA;Polyphen-2: NA	Shallow Deletion
ERCC6	R670W	MutationAssessor: impact: high, score: 4.78;SIFT: impact: deleterious, score: 0;Polyphen-2: impact: probably_damaging, score: 1	Shallow Deletion
	R670W	MutationAssessor: impact: high, score: 4.78;SIFT: impact: deleterious, score: 0;Polyphen-2: impact: probably_damaging, score: 1	Gain
	M1140I	MutationAssessor: impact: neutral, score: 0;SIFT: impact: tolerated, score: 0.2;Polyphen-2: impact: benign, score: 0	Diploid
	E370K	MutationAssessor: impact: medium, score: 2.425;SIFT: impact: tolerated, score: 0.14;Polyphen-2: impact: benign, score: 0.417	Gain
ERCC8	R270K	MutationAssessor: impact: low, score: 1.225;SIFT: impact: tolerated, score: 0.13;Polyphen-2: impact: possibly_damaging, score: 0.667	Diploid

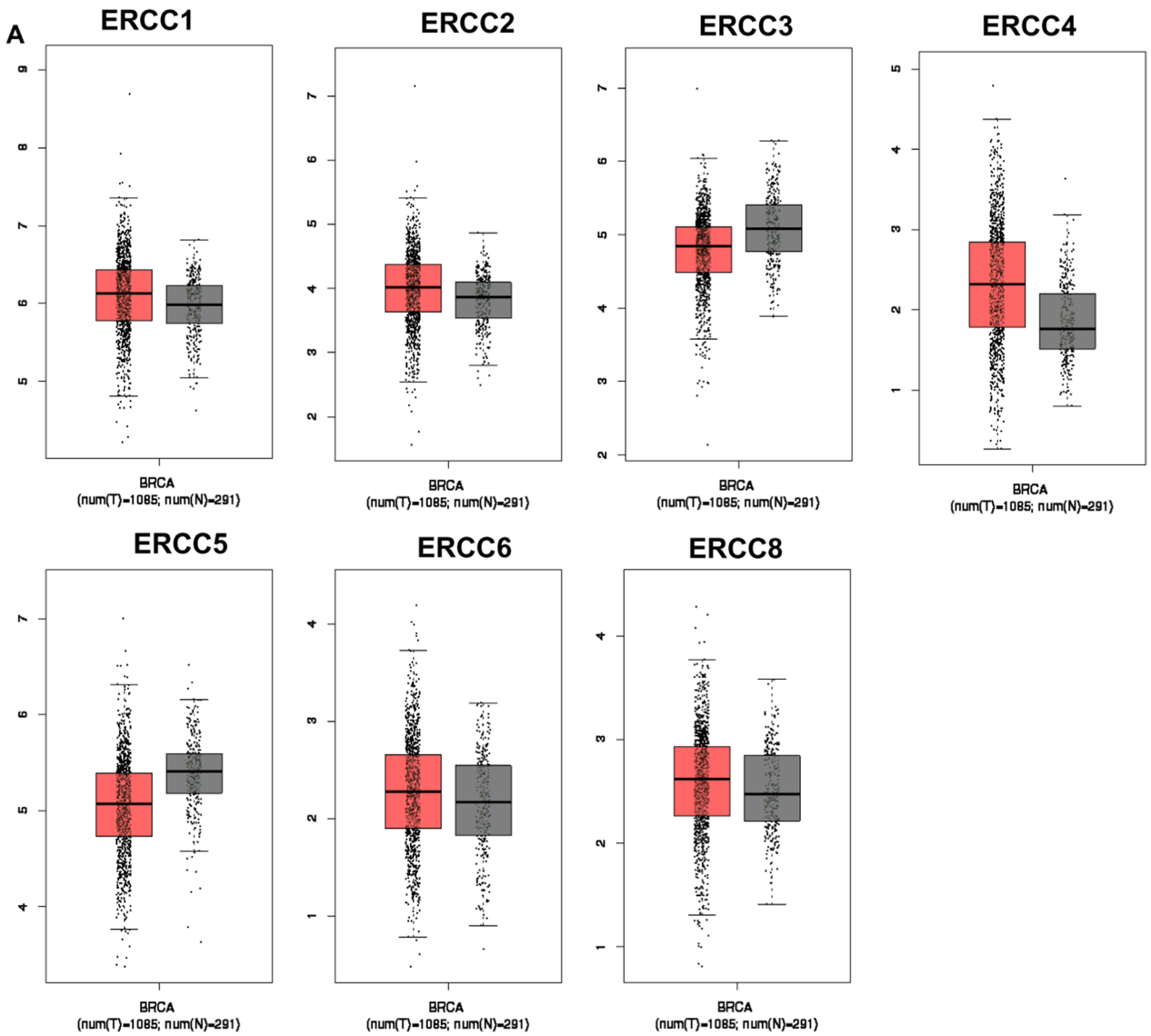
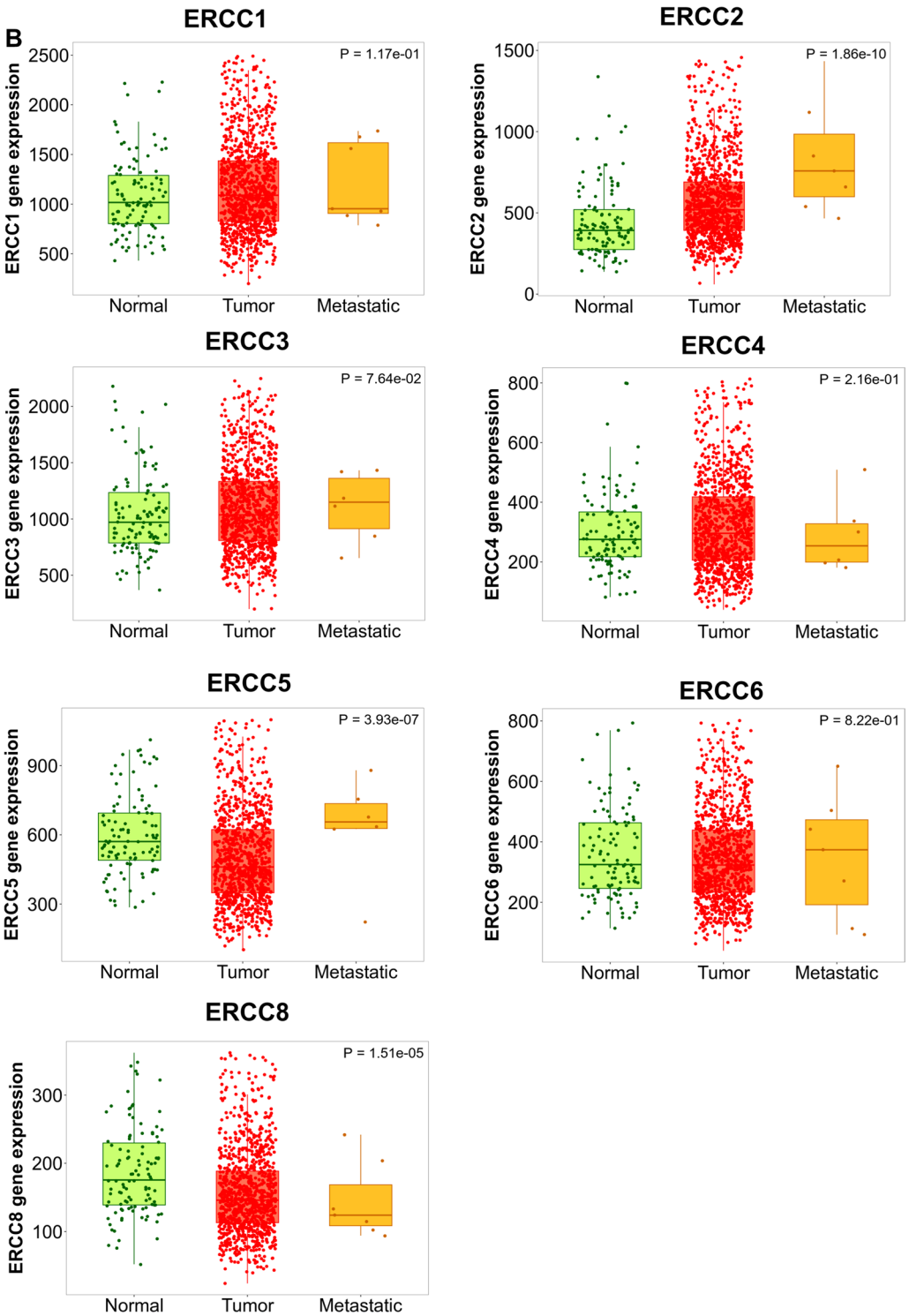


Fig. 2 **A** *ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, ERCC6*, and *ERCC8* mRNA levels in breast cancer (TCGA data) and normal adjacent tissues (GTEx data) using GEPIA. **B** *ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, ERCC6*, and *ERCC8* mRNA levels in normal, breast cancer, and metastatic breast cancer tissues using TNM Plot. Statistical analyses were conducted using Kruskal–Wallis tests. **C** ERCC, ERCC2, ERCC3, ERCC4, ERCC5, ERCC6, and ERCC8 protein levels in normal and breast cancer tissues as determined by The Human Protein Atlas

Our pathway enrichment analyses of ERCC genetic alterations showed enriched Wnt signaling and nucleotide excision repair, although they did not include ERCC genes in the pathway but neighboring genes from ERCC genes that are enriched as genes involved in the Wnt pathway. Previously, Karimaian et al. [39] reported crosstalk between DNA repair and Wnt signaling and highlighted the potential application of Wnt signaling in cancer therapy. Wnt signaling crosstalk with DNA repair pathways plays a role in the genomic stability maintenance due to cisplatin treatment in HeLa, U2OS, and LN229 cells [40]. Additionally, in isogenic triple-negative breast cancer models, Wnt/-catenin inhibition disrupted carboplatin resistance [41]. To date, there has been no study on the relationship between ERCC genes, the Wnt signaling system, and breast cancer resistance. Therefore, we speculate that ERCC might regulate DNA repair and drug resistance in breast cancer by regulating Wnt signaling, but this hypothesis requires experimental confirmation.

Epigenetic *ERCC1* results correlated with Oncoprint results where *ERCC1* was considerably amplified in



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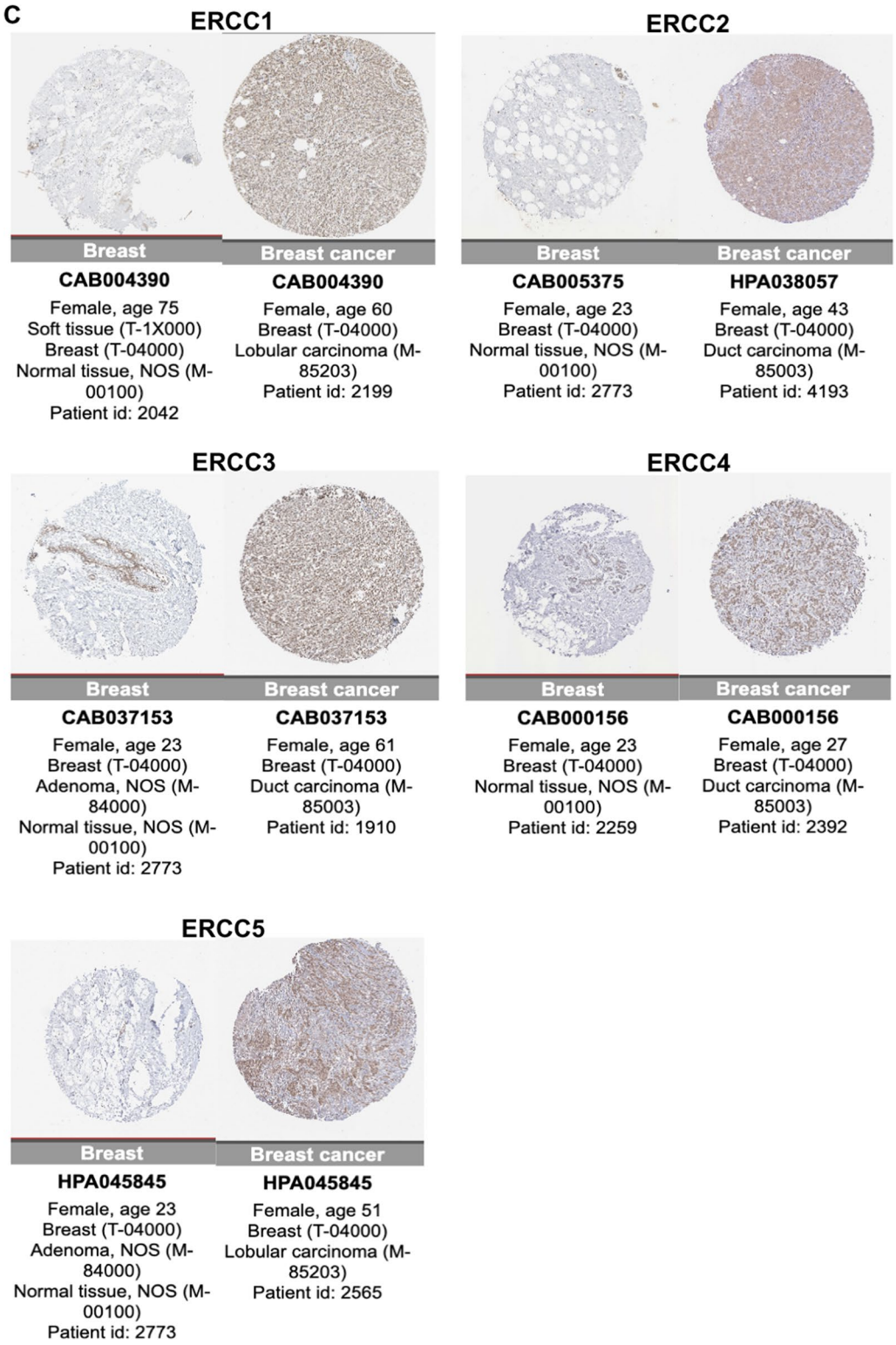

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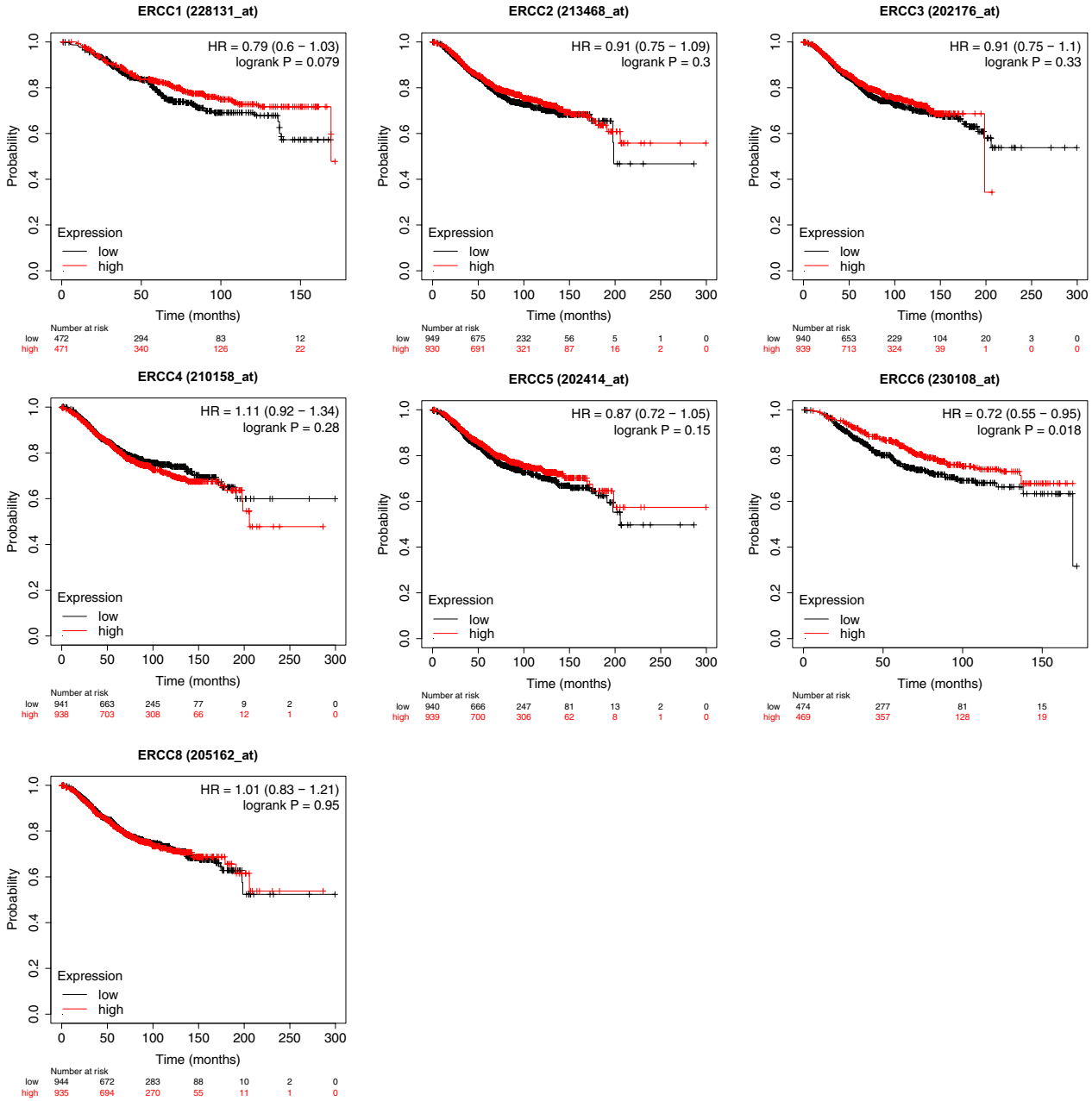


Fig. 3 Prognostic values of *ERCC1*, *ERCC2*, *ERCC3*, *ERCC4*, *ERCC5*, *ERCC6*, and *ERCC8* mRNA expression levels in breast cancer patients using KM Plotter

metastatic breast cancer cell samples, whereas *ERCC2* appeared to have the highest DNA methylation and amplification levels. Epigenetic *ERCC6* alterations indicated 3 DNA methylation profiles, and the number of genetic alterations remains high because the majority of genetic alterations are deep deletions, not amplifications. Previous studies reported *ERCC1* amplifications in metastatic breast cancer cells [42]. Also, patients with non-small cell lung cancer (NSCLC) with adenocarcinoma had significantly varied *ERCC1* mRNA expression levels in main tumors and metastatic sites [43]. Moreover, *ERCC1* overexpression inhibited apoptosis in ovarian cancer cells [44]. However, no studies have yet reported other ERCC gene amplifications in cancer, therefore more studies are warranted.

Few studies have reported ERCC genetic variations and alterations in cancer. Genetic polymorphisms in *ERCC2* (Asp312Asn) and *ERCC4* (Ser835Ser) were

correlated with breast cancer risk in Korean women [45]. Glioma susceptibility was influenced by 2 ERCC2 gene polymorphisms, (rs13181 and rs1799793) and the *ERCC1* polymorphism (rs3212986) [46]. The rs3212986 polymorphism correlated with higher response and PFS rates in patients with advanced NSCLC receiving anti-PD1 nivolumab [9]. *ERCC2* (Lys751Gln) and *ERCC5* (His46His) polymorphisms were correlated with good prognosis rates in osteosarcoma [47]. Thus, deregulated DNA repair pathways may encourage genomic instability and enhance DNA lesion and mutation during carcinogenesis as *ERCC1* was found to be inversely linked with tumor mutation load and neoantigen expression [48].

Using the TNM Plot, mRNA levels in normal, tumor, and metastatic breast cancer tissues showed increased *ERCC2* mRNA expression in breast tumor tissue, *ERCC5* mRNA expression in metastatic breast tumor tissue, and decreased *ERCC8* mRNA expression in breast and metastatic breast tumors. These results were supported by a previous study which showed that high *ERCC*1 expression increased metastasis risks in patients with breast cancer [42], although our *ERCC1*

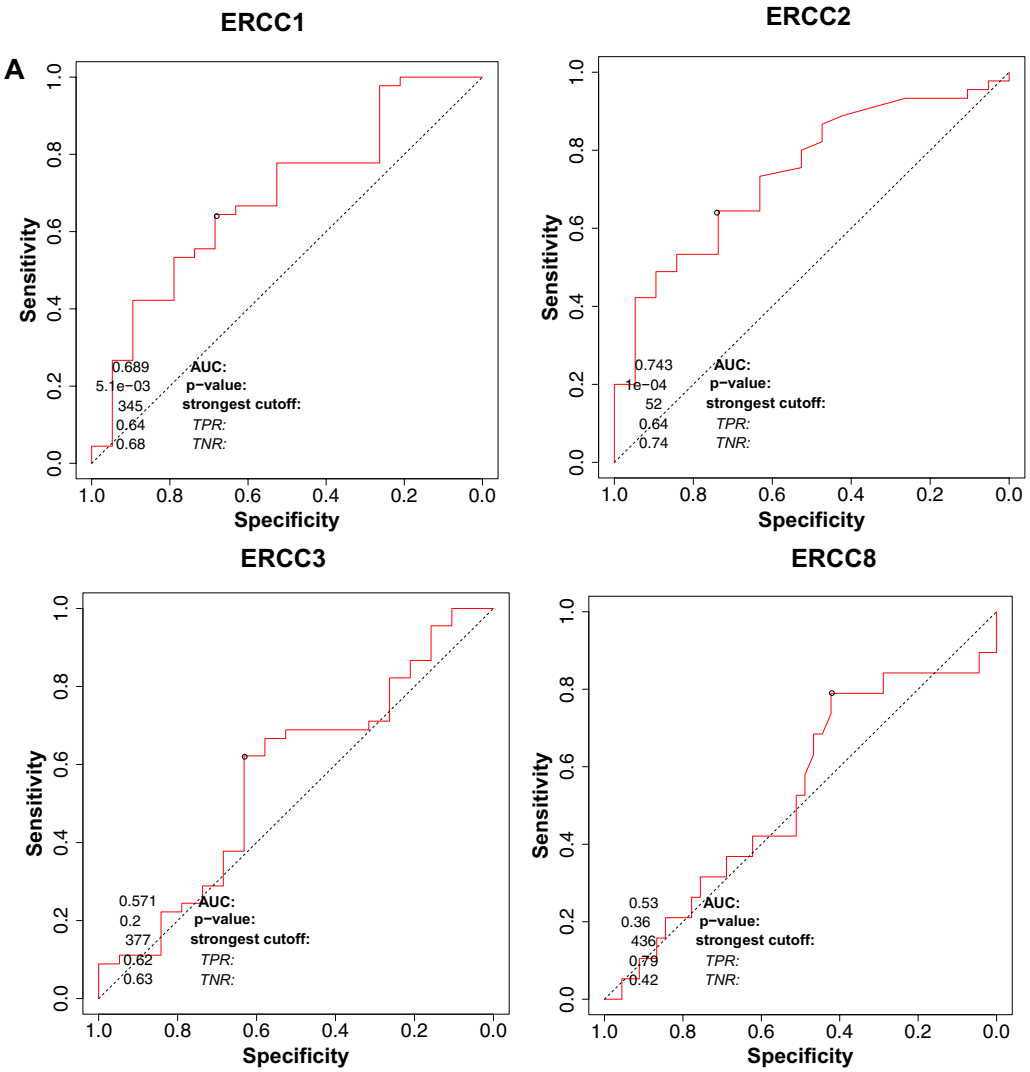
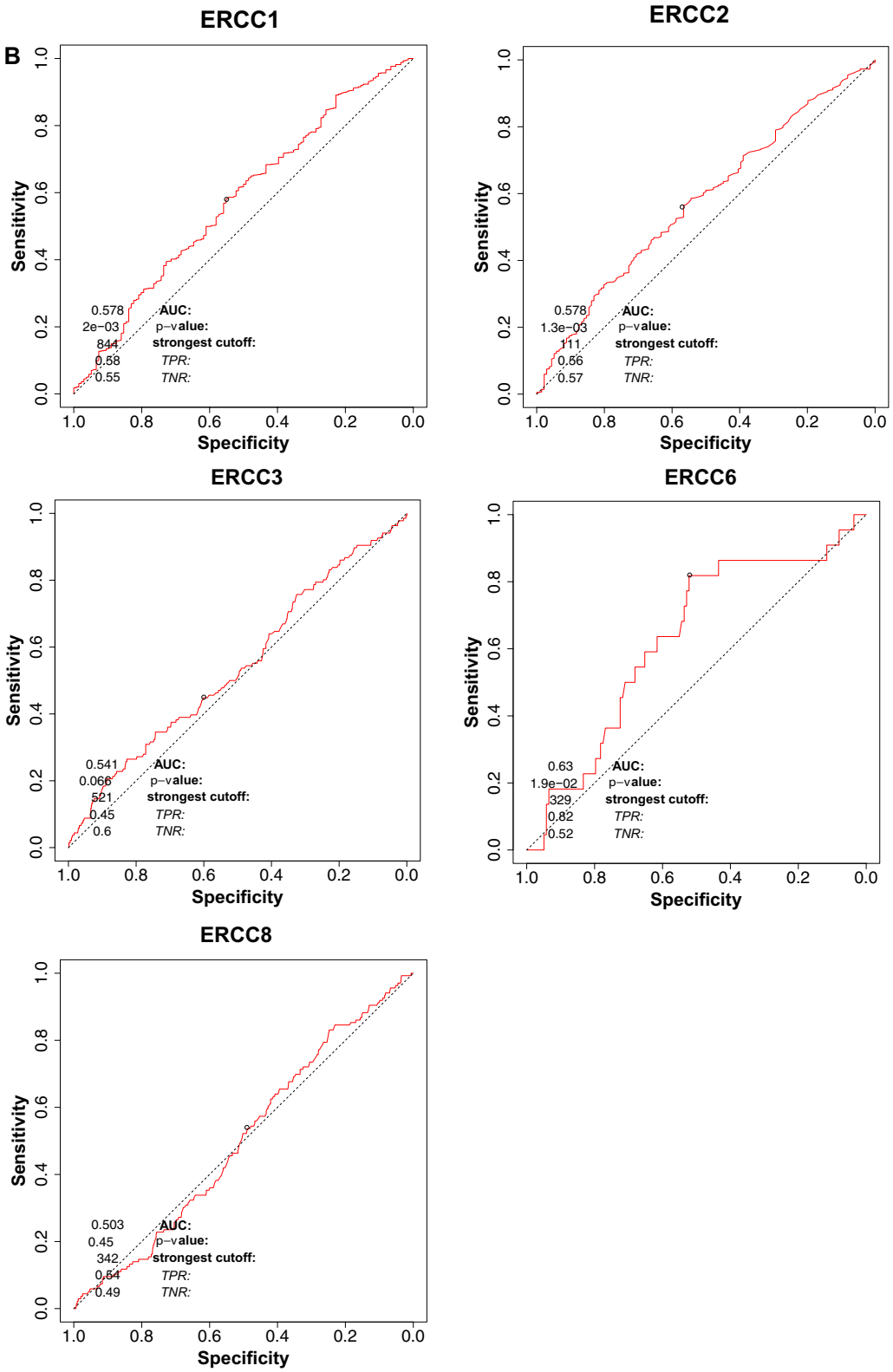


Fig. 4 ROC Plotter showing correlations between *ERCC1*, *ERCC2*, *ERCC3*, *ERCC4*, *ERCC5*, *ERCC6*, and *ERCC8* gene expression levels and endocrine therapy sensitivity using (**A**). pathological complete response (PCR) and (**B**). relapse-free survival (RFS) approaches. ROC Plotter showing correlations between *ERCC1*, *ERCC2*, *ERCC3*, *ERCC4*, *ERCC5*, *ERCC6*, and *ERCC8* gene expression levels and anti-HER2 therapy sensitivity using (**C**). PCR and (**D**). RFS approaches. ROC Plotter showing correlations between *ERCC1*, *ERCC2*, *ERCC3*, *ERCC4*, *ERCC5*, *ERCC6*, and *ERCC8* gene expression levels and chemotherapy sensitivity using (**E**). PCR and (**F**). RFS approaches



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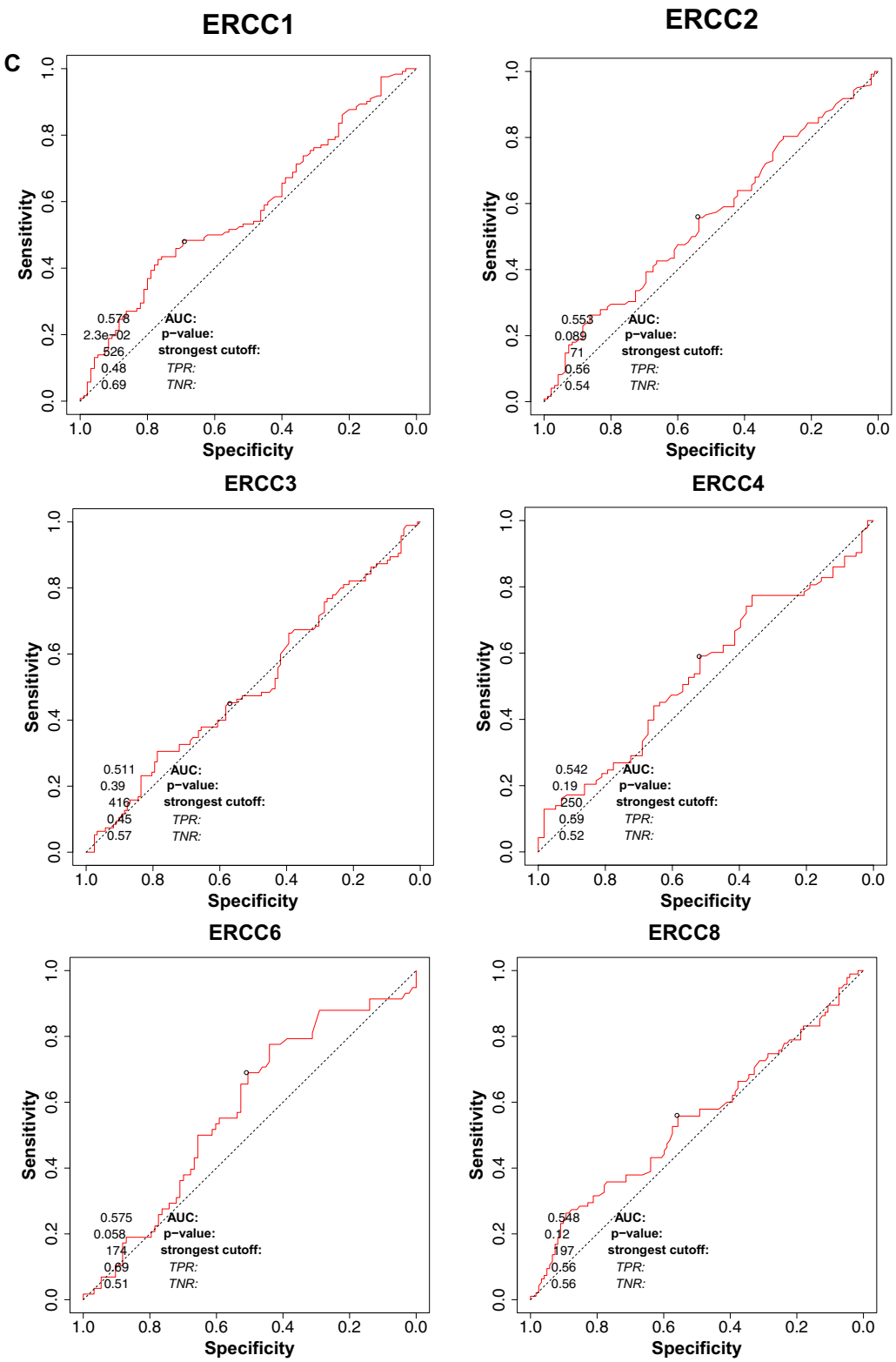

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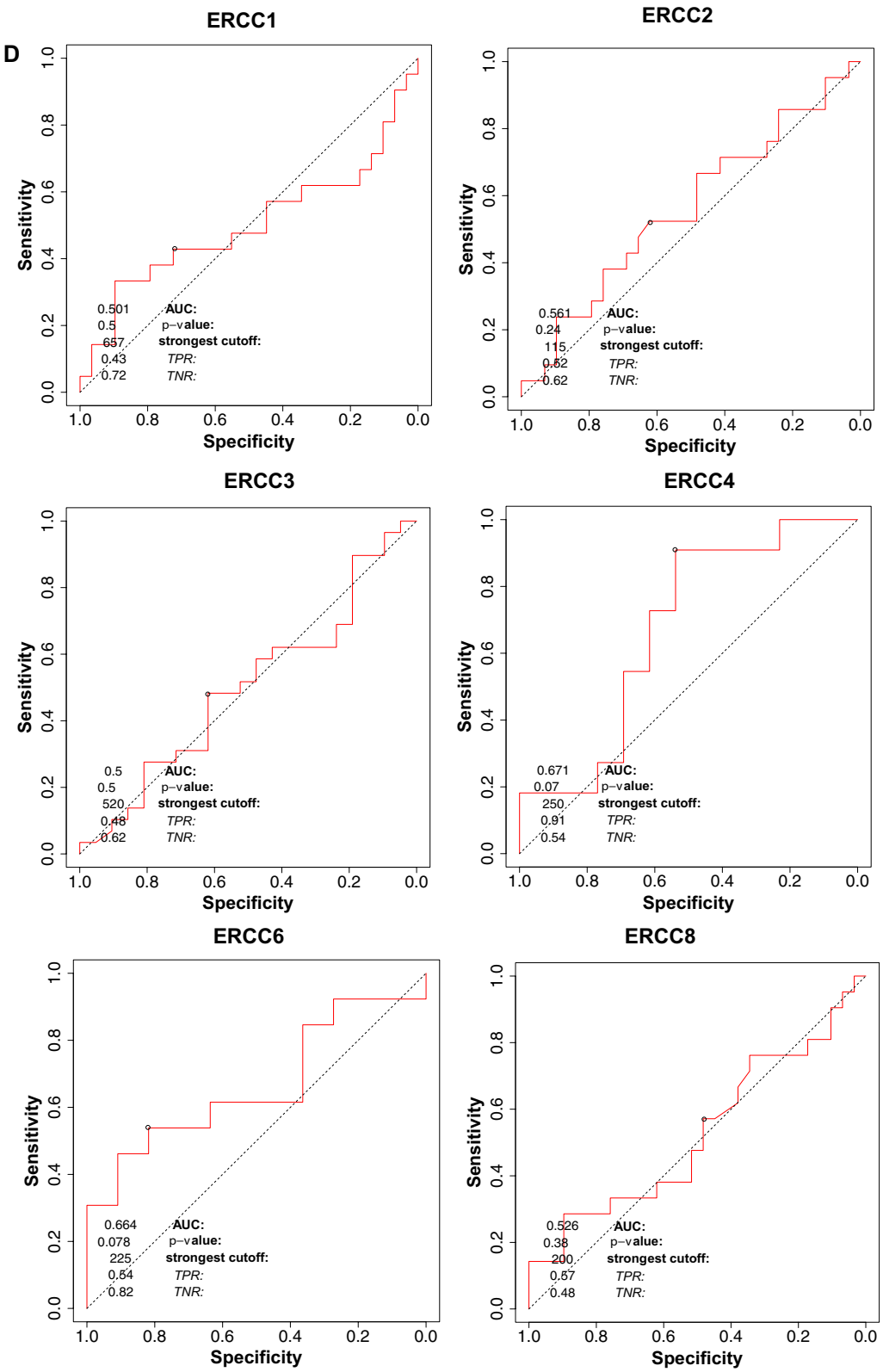

Fig. 4 continued

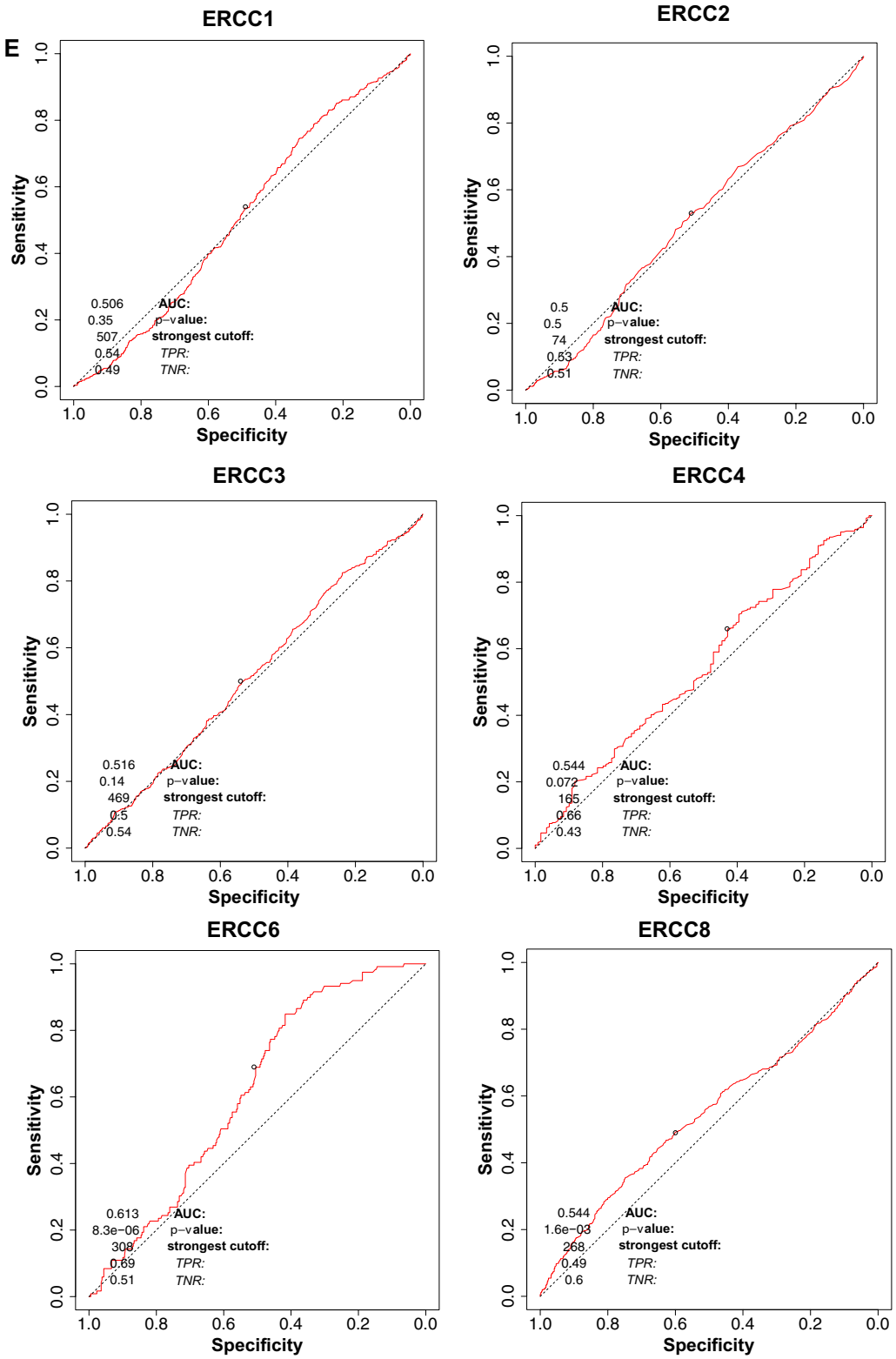

Fig. 4 continued

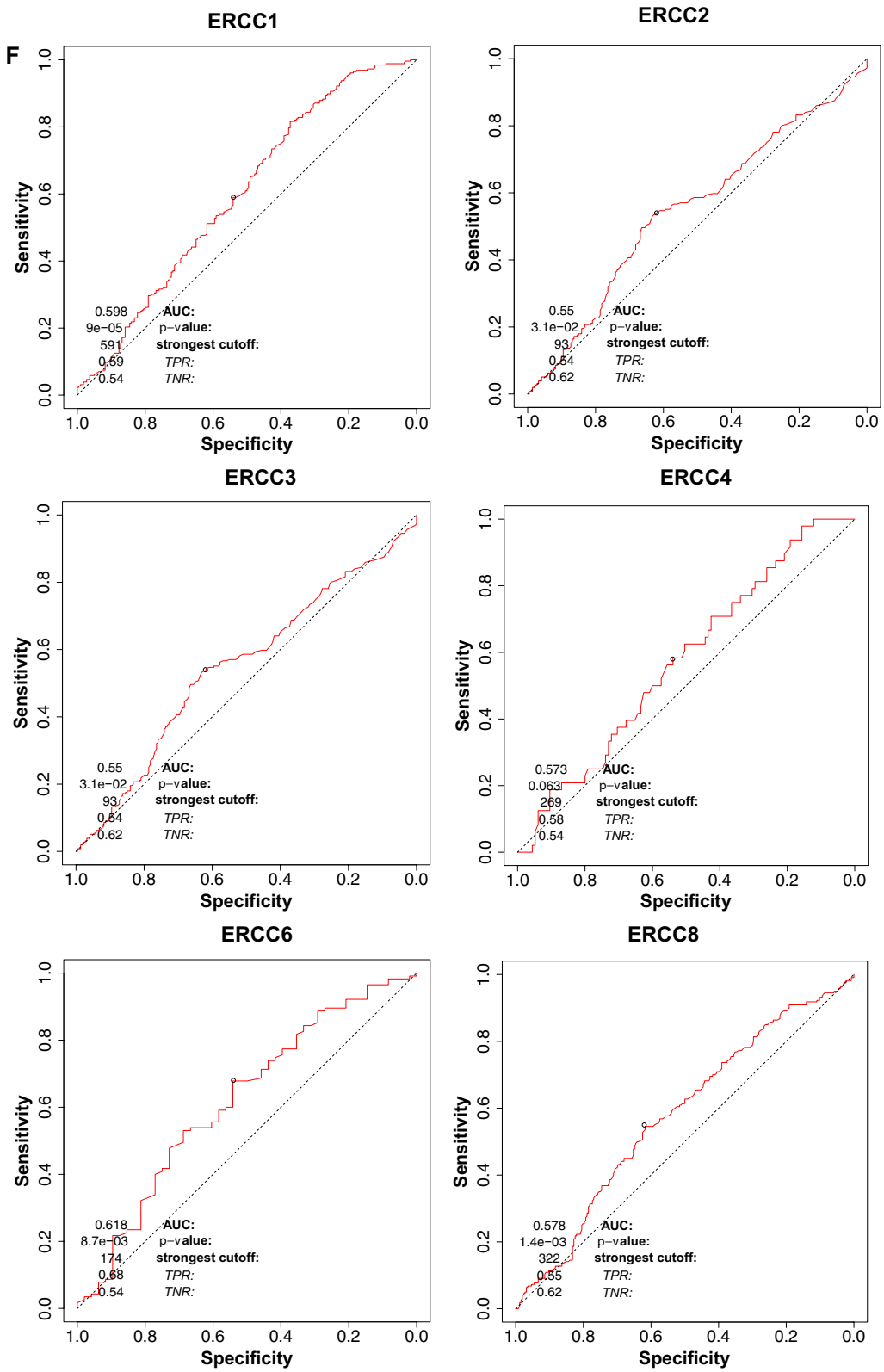

Fig. 4 continued

mRNA overexpression data in metastatic breast cancer were not significant. Using the Human Protein Atlas, ERCC1, ERCC2, ERCC3, ERCC4, and ERCC5 protein levels were increased in breast tumor samples when compared to normal adjacent tissues. No data were identified for ERCC6 and ERCC8 protein levels, therefore future studies must examine the protein levels in patients with breast cancer.

ERCC gene expression analyses across breast cancer samples showed that only *ERCC6* had a significant prognostic value; breast cancer patients with low *ERCC6* levels had better OS when compared to the opposite group. These results were consistent with previous studies which showed that the higher the mRNA ERCC expression, the greater the capability of DNA repair, drug resistance development, and metastasis. Using PCR and RFS parameters, our ROC plots showed that *ERCC1* and *ERCC2* were predictive endocrine therapy markers; *ERCC1* was a predictive marker for anti-HER2 therapy based on RFS. From PCR, *ERCC6* was a predictive marker for chemotherapy, whereas *ERCC1, ERCC2, ERCC6*, and *ERCC8* were predictive markers for chemotherapy (RFS parameters).

Our ROC results were supported by a previous study which reported correlations between high *ERCC1* levels and drug resistance. Upregulated *ERCC1* was identified in cisplatin-resistant A2780 human ovarian carcinoma cells [49]. Low *ERCC1* levels are posited as good prognosis factors for platinum-based chemotherapy at all lung adenocarcinoma stages [50]. In breast cancer, ERCC1 protein levels were correlated with disease resistance to anthracycline therapy, in which samples with high levels of ERCC1 showed poor response to anthracycline therapy, where high elevated ERCC1 levels in samples demonstrated poor responses to anthracycline [51]. The authors concluded that high ERCC1 expression levels were strongly related to poor prognoses in triple-negative breast cancer patients receiving platinum-based chemotherapy [52]. Taken together, ERCC1 is important for predicting chemotherapy responses in breast cancer cells; however, other ERCC gene data are limited and warrant future study.

Our study had some limitations. First, the study was performed using a bioinformatics approach with limited sample numbers; therefore, our data must be validated with other clinical data and in a laboratory setup. Second, ERCC gene functions in breast cancer metastasis, based on subtype, were not examined; therefore, future studies are required. Additionally, protein expression data for several ERCC genes were not identified; therefore, expression data from other databases or protein expression analyses in patients are required. Our study highlighted breast cancer resistance and metastatic mechanisms due to genetic and epigenetic alterations in the ERCC genes, and provided insights on new therapeutic targets, as well as predicted breast cancer patient responses to endocrine, anti-HER2, and chemotherapy.

5 Conclusion

Bioinformatically, we examined and identified roles of *ERCC* in breast cancer resistance cells. Specifically, *ERCC1*, *ERCC2*, and *ERCC6* genes had prominent roles in disease resistance and metastasis. We also demonstrated how Wnt pathway and DNA repair processes contributed to drug resistance in breast cancer cells. However, further research is required to confirm our data so that *ERCC1*, *ERCC2*, and *ERCC6* genes can be used as drug resistance predictors in breast cancer cells.

Abbreviations

ER	Estrogen receptor
ERCC	Excision repair cross-complementing
HER2	Human epidermal growth factor receptor 2
KM	Kaplan–Meier
OS	Overall survival
PCR	Pathological complete response
RFS	Relapse-free survival
ROS	Receiver operator characteristic

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43088-023-00415-3.

Additional file 1: Heatmap of *ERCC1, ERCC2, ERCC3, ERCC4, ERCC5, ERCC6*, and *ERCC8* DNA methylation expression levels in breast cancer cells as determined by the MethSurv database.

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Author contributions
AH was responsible for the design, data curation, formal analysis, original draft writing, review, and editing. HP was responsible for data curation and project administration.

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Availability of data and materials
Study data are available in supplementary files.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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