The activation of EP300 by F11R leads to EMT and acts as a prognostic factor in triple-negative breast cancers

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Abstract

A role for the JAM family in cancer progression has been demonstrated. Nevertheless, there has been no systematic study of the JAM family in specific cancers through clinical specimens, and the mechanism of the molecule is unclear, particularly in subtypes of breast cancer (BRCA). Based on an extensive analysis of the relative expression of the JAM family in cancer by pan-cancer, we found that the increase of F11R (JAM-A) is the opposite of that of JAM-2, or JAM-3, which is unique to BRCA. BRCA prognoses are related to F11R expression, including survival and recurrence-free survival. It is also noteworthy that F11 has different expression characteristics in subtypes, especially in triple-negative breast cancer (TNBC). Using immunohistochemistry (IHC) analysis as an analytical validation, this study provides consistent results with in-silico. As a result of artificial manipulations of F11R combined with microarray-based analyses, we identified that F11R plays an important role in several aspects of cell motility signaling. An upstream regulators and correlation analysis found that EP300 transcription factors may be involved in TNBC metastasis by modulating RHOA, GSK3B, and TGFBR1 expression through F11R-mediated epithelial-mesenchymal transition (EMT). Accordingly, the findings of this study provide a comprehensive interpretation of the relationship between the clinical value of F11R in BRCA subtype progression and the mechanisms that induce metastasis. These results provide a possible target for the treatment of BRCA.

Introduction

The BRCA gene is associated with one of the most common cancers in women, accounting for 25% of all cancer cases in women [1]. There were 1.68 million new cases of BRCA in 2021 and 522,000 deaths associated with this disease [2]. The onset of the disease is influenced by an individual's age [3]. BRCA is reported to occur at a rate of approximately 1.88% in women older than 50 years old, but increases to 66.66% in those over 70 years of age [4]. There are several subtypes of BRCA, which exhibit substantial differences in their pathology and clinical outcome. According to molecular profiling, there are four main subtypes of BRCA in accordance with their expression levels of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) [5, 6]. Both luminal A and luminal B tumors are ER-positive and PR-positive, with low- and high-Ki67 expressions, respectively. The HER2-enriched subtype is usually ER-/PR-negative, but HER2-overexpressing, while triple-negative breast cancer (TNBC) represents low/none levels of all three [5, 6]. HER2-enriched and TNBC are considered more aggressive than luminal cancers [7]. Luminal BRCA represents 70%-80% of all BRCA, followed by HER2-overexpressing cancers (10%-20%) and TNBC (10%-15%) [8, 9]. As a result of the advent of targeted therapies for BRCA with specific receptor expression (ER, PR, or HER2), patients' disease-free survival (DFS) rates have significantly improved [10]. In contrast, TNBCs are more difficult to treat since hormone therapies that target one of the three receptors do not work on TNBCs.

The F11 receptor (F11R), also known as junctional adhesion molecules-A (JAM-A), is one of the members of the immunoglobulin superfamily of cell adhesion receptors. Many cellular processes depend upon JAMs, including junction assembly and cell polarity [11], cell morphology [12], platelet activation [13], and

leukocyte migration [14]. A number of different cell types, including leukocytes, epithelial and endothelial cells, hematopoietic stem cells, and neurons, express F11R, which was originally characterized as the F11 platelet receptor for a stimulatory anti-platelet antibody [15–17]. An important component of tight junction molecules, F11R is involved in a variety of physiological processes. Dysregulation of F11R has been linked to inflammatory disorders and cancer [18–22]. It was noted that an association existed between F11R upregulation and poor outcome in lung, head, and neck squamous, and renal cell carcinoma [20–22], F11R downregulation negatively affects gastric and pancreatic cancer outcomes [23, 24]. In various pathological tissues, F11R seems to exert diverse functions. Previous studies have documented the presence of F11R in BRCA. However, its relationship to clinical outcome remains unclear. Initially, F11R was found to be expressed in normal mammary epithelium but downregulated in metastatic BRCA [25]. In later studies, higher F11R expression was associated with poorer outcomes in women with BRCA [26, 27]. Furthermore, high F11R expression has been linked to HER2 expression in BRCA tissues, although there were fewer cases of HER2 overexpression in that study [28]. Due to the discrepancy between previous studies regarding F11R expression and its correlation with prognosis, it may be worthwhile to examine the role of F11R in BRCA development further.

In total, 542 cases from two cohorts of patients were examined in this study, with the goal of confirming the association between F11R expression and the clinical outcome of BRCA. In addition, F11R expression was examined in relation to other molecular markers of BRCA subtypes. In addition to the observation that F11R has a unique function in BRCA compared to other family members (JAM-2/JAM-3), we also investigated the prognostic value of F11R in breast cancer, its limitations, and the application of therapeutic strategies in various breast subtypes.

Materials And Methods

Evaluation of the JAM family and clinical correlation

In order to analyze the correlation between different members of the JAM family or genes and various cancer types, several databases were used to analyze the related clinical values, including TIMER 2.0 (http://timer.cistrome.org/), Kaplan-Meier plotter (https://kmplot.com/analysis/index.php?p=background), GEPIA2.0 (http://gepia2.cancer-pku.cn/#index), Prognoscan (http://dna00.bio.kyutech.ac.jp/PrognoScan/) and UCSC XENA (https://xenabrowser.net/). The TIMER 2.0 is used to determine the distribution of intermolecular genes in different cancer types. JAM family expression in BRCA is based on XENA and GEIPA2.0. Based on Kaplan-Meier plotter and Prognoscan, the relationship between genes and prognosis was analyzed. A gene correlation analysis was conducted using GEPIA2.0.

Clinical samples of BRCA patients and tissues

The study collected 542 clinical specimens from two different cohorts of women with primary BRCA. This initial cohort consisted of 302 women, diagnosed with breast ductal carcinoma between 1991 and 1999 through the archives of the Department of Pathology, Kaohsiung Veterans General Hospital (KVGH). From

1998 to 2008, there have been 250 resections of BRCA in total at Wan Fang Hospital of Taipei Medical University (TMU). All data were analyzed anonymously, and the identity of the participants was not disclosed. We constructed tissue microarrays (TMA) using BRCA specimens as described previously [29]. Clinical records and tissue samples were collected in accordance with institutional review board protocols at Taipei Medical University (reference number WFH-IRB-99049, TMU cohort) and the Institutional Review Board at the KVGH (reference number VGHKS12-CT2-07). Using tissue microarray blocks, sections of 2μm thickness were cut out. Clinical records were used to obtain information on clinical pathology and follow-up, including hormone receptor status, tumor recurrence, and survival. Traditionally, the as a measure of patient survival, the overall survival (OS) time was calculated from the time the treatment started to the time of his or her last follow-up or death. An individual's DFS time is defined as the period of time between his or her first symptoms and the date of treatment. Each study participant was monitored for at least 60 months (5 years, and sometimes for as long as 250 months) or until their death.

An IHC staining of the F11R protein in human tissues

As described previously [28, 29], IHC staining of samples was carried out according to the described procedures [29, 30]. Following deparaffinization, rehydration, and blocking with 3% hydrogen peroxide, tissue sections were stripped of their waxy compounds. Tris-EDTA buffer (pH 9.0) was used to heat-induced antigen retrieval. After incubation with first antibody overnight, sections were washed and mounted. Incubation was conducted with anti-mouse probe (MACH 1 Universal HRP Detection System; Biocare Medical) allow to sit for 30 minutes at room temperature. Afterward, HRP-polymer antibody was incubated on the sections for another 30 minutes. When 3,3'-diaminobenzidine was added, immunoreactivity was detected. Hematoxylin counterstained sections were then dehydrated and mounted. F11R antibody (Cat. H00050848-M01, 1:1000 dilution, Abnova, Taiwan) are the first antibodies used in this study.

Evaluation of IHC staining scores

Pathologists who were unaware of the clinical parameters reviewed and scored separately the tissue microarray sections stained with F11R. The intensity and percentage of cells with positive staining were used to score the F11R staining semi-quantitatively. According to the staining intensity, we have 0 no staining, 1 weak staining, 2 moderate staining, and 3 strong staining. An expression score of 0–1 and a score of 2–3 was deemed low and high, respectively. The two pathologists did not observe any significant differences in the interpretation of the IHC results.

DNA Microarray based molecular interaction network

Cells were overexpressed with F11R cDNA (OHu24113, Genscript, USA) and empty vector. The related gene expression level was analysis conducted by Human Genome U133 Plus 2.0 Array. The raw data is export from Greenspring software, and the molecular network were analysis by IPA software (https://analysis.ingenuity.com/pa/installer/select).

The simulation of potential drugs

L1000CDs website (https://maayanlab.cloud/L1000CDS2/#/index/6194cd57d99ec600506d5c0c) were employed to analyze the potential drugs against the F11R-genetic profile. The microarray analysis results (Supplementary Table 4) were submitted to the L1000CDs website for reversed mimic analysis.

An analysis of the statistical data.

A chi-square test for categorical data and a Student *t-test* for continuous data was utilized to analyze the relationships between clinicopathological characteristics and F11R expression. Calculating the DFS and OS curves with the Kaplan-Meier method and evaluating the differences with the log-rank test between the expression groups. Using a Cox proportional-hazards model, univariate and multivariate analyses were conducted to identify significant prognostic factors of OS and DFS. It was considered statistically significant if a value of $p < 0.05$ was obtained.

Results

BRCA survival is correlated with the expression of F11R

To examine the expression of F11R in diverse cancers comprehensively, we conducted a pan-cancer analysis to determine if gene expression correlates with tumor characteristics in the TCGA database. The relative expression of F11R is illustrated in Figure.1A in cancers of different types. Multiple cancers, including BRCA, show significant increases in F11R. Currently, JAM family is composed of F11R, JAM-2, and JAM-3. We also compared the expression of JAM-2 and JAM-3 in other cancers. (Figure.S1A). It is noteworthy that an increase in F11R was associated with BLCA, BRCA, CESC, LUSC, and UCEC, as well as a decrease in JAM-2/JAM-3. To determine whether these trends in tumor and normal cases are relevant to OS. In a correlation analysis of OS and F11R (Figure.S1B), the results revealed that poor survival in BRCA was only positively correlated with high F11R expression. A heatmap analysis of F11R expression in individual cases revealed that F11R is generally lower in normal tissue than tumor, and JAM-2/JAM-3 is higher in normal tissue (Figure.1B) (Supplementary Table.1). A review of different database sources also indicated that F11R is indeed highly expressed in BRCA, in contrast to JAM-2/JAM-3 (Figure.1C). These results indicate that the expression of F11R in BRCA patients is unique to JAM-2/JAM-3, and that patients with higher F11R gene expression in BRCA patients have a correlation between OS and poor prognosis.

F11R expression is specific to BRCA subtypes

Relapse is one of the reasons why cancer treatment is challenging. To understand the relationship between JAM and BRCA, a correlation was also conducted and found between JAM family expression and recurrence-free survival (Figure.2A). Intriguingly, the prognosis analysis indicated that patients with high F11R expression would have a poor chance of surviving cancer free of recurrence, while high JAM-2/JAM-3 implies a good prognosis. In agreement with other clinically relevant data currently available, the

F11R and BRCA have higher hazard ratios for relapse-free survival (RFS), disease-specific survival, and DFS than JAM-2/JAM-3 (Figure.2B).

Nowadays, there are many specific markers ER, PR, and human HER2 that assist in further subdividing BRCA into luminal A (ER+, PR+/-, HER2-), luminal B (ER+, PR+/-, HER2+/-), HER2+ (ER-, PR-, HER2+) and TNBC (Basal-like) (ER-, PR-, HER2-). Therefore, we also examined the correlation between the JAM family and BRCA subtyping. The expression level of F11R in the TCGA_BRCA datasets was analyzed in accordance with the subtype defined by the Prediction Analysis of Microarray (PAM) 50-gene (Figure.2C) (Supplementary Table.2). F11R was found to have a significantly higher expression in patients with HER2-enriched and Basal-like subtypes, whereas JAM-2/JAM-3 is the opposite of F11R. Further analysis revealed no correlation between expression levels of F11R and JAM-2/JAM-3, while expression levels of JAM2 and JAM3 were highly correlated in BRCA (Figure.S2). These results indicate that F11R is unique and has clinical prognostic, subtype classification value in the diagnosis of breast cancer.

BRCA tissue with high F11R expression is significantly associated with TNBC subtype, and tumor recurrence is associated with high expression levels of F11R

For the 240 patients in the TMU cohort, 222 cases had complete records of their ER/PR/HER2 status. In total, 130 luminal (58%), 55 HER2-enriched (25%), and 37 TNBC (17%) cases were observed. 72% of patients (164 out of 227) had early stage (I-II) tumors, while 27% (63 out of 227) had late stage (III-IV) tumors. 51% of patients (113 out of 222) had lymph node involvement, and 18% (44 out of 245) developed tumor recurrences or distant metastases following treatment. There were 302 patients in the KVGH cohort, 282 of whom had ER/PR/ HER2 records. There were 181 luminal type cases (65%), 52 HER2-enriched cases (18%), and 49 TNBC cases (17%). On average, 67% of patients (201 out of 302) had early-stage tumors (I–II), while 33% (101 out of 302) had late-stage tumors (III–IV). 57% of patients (174 out of 302) had lymph node involvement, and 41% (125 out of 302) developed tumor recurrences or distant metastases after treatment.

Using IHC staining, the expression of F11R in TMA samples containing primary BRCA samples from two cohorts was determined. A low level of expression of F11R was observed in normal mammary epithelium (Figure.3A). Cancer tissues varied in their expression of F11R, from no staining to strong membranous staining (Figure.3A). The intensity and percentage of positive-stained cells were used to divide the expression of F11R into low and high groups (see Methods). Table.1 provides an indication of the association between F11R expression and clinicopathological features. According to the TMU cohort, 73 (30%) cases of high expression of F11R were identified, and 114 (38%) out of 302 cases of high expression of F11R were identified in the KVGH cohort. Regardless of age, tumor stage, or lymph node status, F11R levels did not differ significantly. In the TMU cohort, elevated levels of F11R were related to increased tumor grades ($p = 0.001$), but not in the KVGH cohort ($p = 0.078$), while high levels of F11R expression were associated with increased tumor sizes (T1 + T2 v.s. T3 + T4; $p = 0.035$) in the KVGH cohort, but not in the TMU cohort. Interestingly, the expression of F11R was found to be correlated inversely with ER and PR expression ($p < = 0.002$) in both cohorts. The percentage of high F11R

expression was 26% in TMU patients with 0 to 2 + HER2 expression, but it increased to 42% in patients with 3 + HER2 expression (Table.1), although no statistical significance was noted ($p = 0.060$). In the 209 cases of the TMU cohort, the F11R status was determined. In both cohorts, the percentage of high F11R expression was low in luminal cancer (18% in TMU and 31% in KVGH, respectively) and increased to around 40% in HER2-enriched tumors (44% in TMU and 39% in KVGH), while high expression of F11R predominated in TNBC (64% in TMU and 67% in KVGH, $p < 0.001$). In conclusion, tumor recurrence was significantly correlated with high expression of F11R ($p = 0.001$) in the TMU cohort but did not reach significance in the KVGH cohort ($p = 0.01$).

F11R in BRCA reflects poor prognosis, tumor recurrence, and is independently prognostic

The prognostic value of F11R expression was evaluated through a Kaplan-Meier analysis and a log-rank test in BRCA. In both the TMU and KVGH cohorts, patients who had high expression of F11R had a shorter OS ($p < 0.001$) and a shorter DFS ($p < 0.001$, $p = 0.034$) than those who had less expression (Figure.3B). Since the expression of F11R is associated with BRCA subtypes (Table.1), further analysis by stratifying the patients by BRCA subtypes (luminal, HER2-enriched, and TNBC) revealed that high expression of F11R is an adverse prognostic indicator for all three subtypes (Figure.3C; $p < 0.05$), with the exception of the TNBC group of the KVGH cohort where the difference is marginal ($p = 0.065$). Even though the number of cases of high F11R expression in luminal-type BRCA is much smaller than that of low F11R expression, elevated expression of F11R was associated with a shorter OS (Figure.3C). In the TNBC group of the TMU cohort, patients with low F11R expression did not die during the follow-up period. Overall, F11R expression is useful as a prognostic factor for individual subtypes of breast cancer in the multiple cohorts.

To determine the prognostic factors associated with BRCA, Cox proportional-hazards regression analysis was performed. When univariate analyses of both cohorts were performed on OS, age, tumor T status, status of lymph nodes, tumor stage, Status of HER2, as well as the expression of F11R was significantly associated with poor outcomes ($p < 0.05$; Table.2 and 3). The expression of F11R is a significant prognostic marker for multivariate analysis, in addition to age (for both cohorts) and stage of tumor (for the KVGH cohort, Tables.2 and 3). In the multivariate analysis of the TMU cohort, patients with the risk of mortality was increased by 3.32-fold for those with high F11R expression [95% confidence interval (CI) = 1.90–5.80; $p < 0.001$]. As regards the DFS analysis, T status, status of lymph nodes, tumor stage, HER2 status, and analysis of univariate data showed that F11R expression was associated with a short DFS ($p < 0.05$), According to multivariate analyses of the TMU cohort, lymph node metastasis and F11R expression were associated with short DFS ($p < 0.05$). According to our univariate DFS analysis, an there is a 2.45-fold increase in cancer recurrence when F11R is overexpressed (95% CI = 1.50–3.99; $p < 0.001$) as measured by the multivariate analysis in the TMU cohort, and based on the multivariate analysis, the number of recurrences increased by 2.69-fold (95% CI = 1.43–5.05; $p = 0.002$) in the TMU cohort. This study suggests that F11R expression is one of the most crucial biomarkers for poor outcomes (endpoint OS) in patients with BRCA, as well as for poor recurrence-free survival.

Involvement of F11R in the molecular mechanisms of TNBC cells

In light of the fact that pathological features suggest that F11R plays a major role in metastasis (Table.1 and 2), and it is still unclear how F11R is able to function in TNBC. Thus, by using microarrays, we attempted to dissect the possible mechanism of F11R-mediated BRCA progression. Using the CCLE database (Figure.S3) (Supplementary Table.3), we first compared F11R's expression level in BRCA-related cell lines. Using cell lines that express low levels of F11R, stable clones overexpressing F11R were established in breast carcinomas and Breast Ductal Carcinoma, respectively, which are cell lines we have, such as CAL-120_F11R (134.35-fold) and HDQ-P1_F11R (127.61-fold) (Supplementary Table.4). Notably, CAL-120 and HDQ-P1 are classified as TNBC subtypes, HDQ-P1 being Basal-like and CAL-120 being Mesenchymal-like [31]. Microarray and Venn diagram results showed that approximately 24,673 probes were consistently regulated by F11R in CAL-120 and HDQ-P1 cells (Figure.4A) (Supplementary Table.4). Furthermore, by incorporating these co-regulated genes into IPA analyses, we were able to profile the molecular interaction network (Figure.4B) and signaling pathways (Figure.4C) (Supplementary Table.5) involved in F11R regulation. Noteworthy is the fact that these gene ontologies include cell growth and movement related molecular networks. In particular, F11R may mediate cell migration by regulating epithelial adhesion junction signaling, and the EMT through growth factors (Figure.4C). The results showed that F11R was also associated with HER2-signaling in BRCA, which is consistent with previous observations [28, 32, 33]. In accordance with clinical findings, the results of the above microarray analysis indicate that F11R is indeed implicated in cellular EMT changes to contribute to migration, and metastasis may be influenced by these signaling pathways.

The relationship between F11R and EP300 axis in the development of TNBC metastasis

In order to further identify the molecules that F11R regulates primarily to influence cell movement during EMT, Figure.5A highlights key molecules involved in regulation of the epithelial mesenchymal transition by growth factors pathway that are impacted by F11R. A number of these molecules showed positive correlations with F11R in BRCA by greater than ± 0.3 Spearman's correlation, including GSK3B, MAPK1, SMAD4, PTPN11, OCLN, CDC42, MTOR, TGFBR1, RHOA, and IL6R (Figure.5B). CAL-120 and HDQ-P1 show elevated levels of these molecules when F11R is overexpressed. The 10 molecules that were chosen as signature molecules also exhibited a positive correlation with F11R (Figure.5C). An analysis of the downstream regulators showed that EP300 was the most regulated molecule among all transcription factors, with a correlation of over ± 0.3 Spearman's correlation (Supplementary Table.6). In spite of SMAD4, KLF11 and YAP1 having high Spearman's correlation, EP300 has a higher *p*-value than the others. Among those regulated by EP300 are EMT-related molecules such as GSK3B, RHOA and TGFBR1 (Figure.5D). The correlation analysis revealed that EP300 was highly correlated with F11R, GSK3B, RHOA and TGFBR1 in BRACA (Figure.5E). Additionally, analysis of CCLE datasets indicated that F11R and EP300 were also highly correlated in Breast carcinoma or Breast ductal carcinoma cells (Figure.S4)

(Supplementary Table.7). In BRCA, a clinical correlation indicated that patients with F11R and downstream effectors of EP300 have a worse prognosis, particularly with downstream effectors such as GSK3B and TGFB1 (Figure.S5).

It has been reported that EP300 plays an oncogenic role in BRCA malignancy and may also contribute to resistance to drugs [34], which may be related to F11R regulation. For the purpose of identifying potential treatments based on F11R genetic profile, a drug repurposing analysis was performed (Fig. 6) (Supplementary Table 8). Among these candidates, Tyrphostin AG 1478, a tyrosine kinase inhibitor of epidermal growth factor receptor (EGFR), may act as a downstream effector of TGFB1 when inhibiting F11R. Therefore, targeting F11R-related genes by using Tyrphostin AG 1478 may result in improved treatments for TNBC. Accordingly, F11R may play a role in the regulation of the epithelial mesenchymal transition by growth factor pathway via EP300, including GSK3B, RHOA, and TGFBR1, consequently contributing to the metastasis of BRCA.

Discussion

The fact that many biomarkers are used to categorize specific cancers has proven beneficial for people's understanding of cancer and for developing clinical diagnostic methods that allow for more effective treatment. Based on the use of markers such as ER, PR, and HER2, BRCA is currently classified as luminal, HER2-enriched, and TNBC. These subtypes, however, are not yet fully understood in terms of molecular regulation. Identifying specific markers to aid in diagnosis and treatment is therefore extremely important. In this study, we investigate the JAM family of tumors from a pan-cancer perspective through the use of open big data. Several types of cancer express the F11R gene in combination with the JAM family (Figure.1A), which has previously been reported as well [26, 27]. Notably, F11R was confirmed in this study to be elevated in BRCA expression as well as unique among other members of the JAM family in terms of its associated prognostic characteristics. In support of these findings, according to previous studies, F11R is related to many clinical prognoses associated with the BRCA gene [26, 27, 35]. Intriguingly, the results indicate that JAM-2 and JAM-3 act as inverse indicators of F11R. Comparing the pan-cancer analysis, BLCA, BRCA, CESC, LUSC, and UCEC, similar trends were seen (Figure.S1A). In particular, such a relationship is positively correlated with OS and a poor prognosis is associated with F11R gene deficiencies in BRCA patients (Figure.S1B). TCGA-BRCA data also indicated that in the normal case, F11R exhibited a lower distribution than JAM-2 or JAM-3 (Figure.1B). Comparing the differences between normal and tumor populations also revealed that in contrast to the results of JAM-2 or JAM-3, F11R was significantly higher in the tumor group than in the normal group. The clinical correlation between the JAM family and Recurrence Free Survival, in addition to OS, was similar to that seen in BRCA. A review of different clinical data also indicated a similar situation, suggesting that having the F11R results in a high chance of a RFS, disease specific survival or DFS of patients (Figure.2A,B), while JAM-2/JAM-3 is associated with low risk. However, it should be noted that the relationship between JAM family members and BRCA subtypes has not been systematically evaluated. In this study, a series of comparisons are made between different BRCA subtypes based on the expression level of the JAM genes. Both in-silico and IHC results showed that F11R was significantly increased in all subtypes, except

in HER2-enriched and Basal-like subtypes (Figure.2C, 3B), we demonstrate that F11R is associated with OS with Luminal, HER2 and TNBC (Figure.3C). In contrast to F11R, patients with any BRCA subtype will have lower levels of JAM-2/JAM-3 expression. The same trends are also observed in patients with HER2-enriched or Basal-like tumors. However, according to the correlation, there is no correlation between the distributions of F11R and JAM-2/JAM-3 among the inverse indicators (Figure.S2). It is nonetheless noteworthy that there is a strong correlation between JAM-2/JAM-3 (R = 0.8). Despite this, no studies have demonstrated a possible relationship or biological function between JAM-2 or JAM-3.

Currently, TNBC is currently considered to be one of the most difficult subtypes to treat, and metastasis is currently considered to be one of the main causes Considering that there was no detailed analysis to profiling how F11R functions in TNBC cells, we therefore simulated the possible molecular mechanism between F11R and TNBC cells through microarray profiling analysis. Cells overexpressing F11R were shown to activate gene ontology related to cell growth and motility (Figure.4B). Analysis of Canonical pathways also revealed the major signaling mechanisms involved in F11R (Figure.4C). Based on the findings of this study, it is evident that F11R plays a role in several processes related to cell motility, including tight junction signaling, regulation of the EMT by growth factors, HER2 signaling in BRCA, and ER signaling, both of which are associated with BRCA tumorigenesis. It is consistent with recent studies suggesting that HER2-induced resistance to treatment is mediated by HER3 and is mediated by F11R in luminal and basal-like BRCA cell lines [35]. Overexpression of F11R can also be observed in similar cells, regulating Integrin Signaling and influencing cell motility [36]. Our observations are consistent with the results of this study (Figure.4C). Here, we demonstrate that F11R can exhibit consistent signaling regulation in both basal-like and mesenchymal-like TNBC cells. In light of these results, F11R appears to be a therapeutic target that may be cross-subtype applicable, in accordance with the IHC results.

In fact, F11R is involved in EMT-related signaling in cells, which is consistent with the features we have observed in pathological studies. On the molecular level, the EMT is regulated by growth factors via the F11R signaling pathway (Figure.5A), and molecules with high correlation with FSTL3 include GSK3B, MAPK1, SMAD4, PTPN11, OCLN, CDC42, MTQR, TGFBR1, RHOA, and IL6R (Figure.5B). Several of these molecules are indeed highly correlated with F11R in BRCA (Figure.5C) and have previously been implicated in BRCA progression [37–40]. It should be noted that these molecules may serve as cluster signatures and are highly correlated with F11R in BRCA. With a more detailed analysis of Upstream regulators, we identified a novel factor involved in F11R regulation, the EP300 (Figure.5D). EP300 expression is consistently linked to a wide range of biological processes, including cell growth, metastasis, and stemness properties of TNBC [41]. An IPA analysis indicated that EP300 regulates RHOA, GSK3B, and TGFBR1. An analysis of correlations revealed a strong correlation between EP300 and F11R, RHOA, GSK3B and TGFBR1 (Figure.5E). Similar correlations were also observed among different subtypes of BRCA cell lines (Figure.S4). There have been a number of studies that have demonstrated the connection between RHOA, GSK3B, and TGFBR1 and the development of metastatic disease in BRCA [37–40]. This suggests that the crosstalk between F11R and TGFRB1 signaling may also be mediated by EP300. However, the precise mechanism needs to be determined through additional research.

As a result of our study, we suggest that the expression level of biomarker F11R is useful for the diagnosis and evaluation of the disease, especially in subtypes of BRCA. Moreover, as the interpretation between the molecular networks demonstrates, F11R participates in BRCA progression through molecular mechanisms, and can be used as a potential target for future drug design in BRCA therapy.

Declarations

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Author's Contributions

Conception and design: Li CH, Fang CY, Chan MH, Chang YC; Development of methodology: Li CH , Fang CY; Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): Chen CL, Lu PJ, Ger LP, Hsiao M; Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): Li CH, Fang CY, Chang YC; Drafting of the manuscript: Li CH, Fang CY; Critical revision of the manuscript for important intellectual content: all authors.; Study supervision: Chang YC and Hsiao M; All authors read and approved the final manuscript.

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Availability of data and materials

Detailed data are included in the manuscript and its supplementary information files.

Ethics declarations

Ethical Approval and Consent to participate

In the present study, tissue samples were collected and reviewed by the Institutional Review Boards at Kaohsiung Veterans General Hospital (Kaohsiung, Taiwan) and Wan Fang Hospital of Taipei Medical University (Taipei, Taiwan) (VGHKS12-CT9-07, and WFH-IRB-99049). Before any tissue samples were collected during the planned treatment, all patients gave their informed consent. In this study, all procedures were performed in accordance with the ethical standards of the institutional research committee and/or the national research committee and with the 1964 Helsinki Declaration, and later amendments, or comparable ethical standards.

Consent for publication

Not applicable.

Competing interests

There are no competing interests declared by the authors.

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Tables

Table 1. Breast cancer patients' clinical and pathological characteristics and F11R expression.

Cohort	TMU				KVGH			
		F11R				F11R		
Variables	N	Low (n=167)	High (n=73)	p-value	N	Low (n=188)	High (n=114)	p-value
Age	240			0.565	302			0.683
<50yr		73 (71.6)	29 (28.4)			87 (63.5)	50 (36.5)	
>50yr		94 (68.1)	44 (31.9)			101 (61.2)	64 (38.8)	
Stage	217			0.069	302			0.593
I+II		112 (71.8)	44 (28.2)			123 (61.2)	78 (38.8)	
III+IV		36 (59.0)	25 (41.0)			65 (64.4)	36 (35.6)	
Grade	214			0.001	302			0.078
I		24 (88.9)	3 (11.1)			8 (80.0)	2 (20.0)	
II		86 (74.8)	29 (25.2)			146 (64.6)	80 (35.4)	
III		39 (54.2)	33 (54.8)			34 (51.5)	32 (48.5)	
Tumor size	219			0.787	302			0.035
T1+T2		124 (68.9)	56 (31.1)			173 (64.3)	96 (35.7)	
T3+T4		26 (66.7)	13 (33.3)			15 (45.5)	18 (54.5)	
Lymph node status	213			0.184	302			0.752
N0		76 (72.4)	29 (27.6)			81 (63.3)	47 (36.7)	
N1-N3		69 (63.9)	39 (36.1)			107 (61.5)	67 (38.5)	
ER status	233			<0.001	278			<0.001
Negative (0-10%)		47 (51.1)	45 (48.9)			51 (47.7)	56 (52.3)	
Positive (>10%)		115 (81.6)	26 (18.4)			120 (70.2)	51 (29.8)	
PR status	233			<0.001	286			0.002

Negative (0-10%)		80 (58.4)	57 (41.6)			67 (51.5)	63 (48.5)	
Positive (>10%)		82 (85.4)	14 (14.6)			108 (69.2)	48 (30.8)	
HER2 status (I)	222			0.060	293			0.497
0-2+		125 (74%)	51 (26%)			137 (62.6)	82 (37.4)	
3+		26 (58%)	20 (42%)			43 (58.1)	31 (41.9)	
Subtype	222			<0.001	282			<0.001
Luminal A+B		107 (82%)	23 (18%)			125 (69.1)	56 (30.9)	
Her2		31 (56%)	24 (44%)			32 (61.5)	20 (38.5)	
Triple-negative		13 (36%)	24 (64%)			16 (32.7)	33 (67.3)	
Recurrence	237			0.001	302			0.101
No		130 (75.6)	42 (24.4)			117 (66.1)	60 (33.9)	
Yes		35 (53.8)	30 (46.2)			71 (56.8)	54 (43.2)	

Table 2. An analysis of the overall survival and disease-free survival of BRCA patients of the TMU cohort using univariate and multivariate methods.

Variables	Univariate analysis				Multivariate analysis			
	Overall survival		Disease-free survival		Overall survival		Disease-free survival	
	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Age								
<50 Years	1		1		1		1	
≥50 Years	2.23 (1.36-3.66)	0.001	1.23 (0.75-2.02)	0.417	1.76 (1.01-3.07)	0.047	1.10 (0.62-1.95)	0.757
Tumor T status								
T1+T2	1		1		1		1	
T3+T4	2.02 (1.22-3.37)	0.007	2.51 (1.47-4.30)	0.001	1.17 (0.55-2.48)	0.691	1.01 (0.47-2.17)	0.980
Lymph node metastasis								
No	1		1		1		1	
Yes	2.22 (1.39-3.55)	0.001	3.77 (2.10-6.76)	<0.001	1.87 (0.94-3.73)	0.077	2.89 (1.27-6.54)	0.011
AJCC stage								
I + II	1		1		1		1	
III + IV	1.67 (1.33-2.10)	<0.001	2.00 (1.56-2.56)	<.0.001	1.41 (0.98-2.03)	0.068	1.43 (0.96-2.12)	0.078
ER status								
Negative (0-10%)	1		1		1		1	
Positive (>10%)	0.81 (0.51-1.27)	0.350	1.00 (0.60-1.64)	0.938	1.09 (0.54-2.20)	0.803	1.20 (0.55-2.65)	0.648
PR status								
Negative (0-10%)	1		1		1		1	
Positive	0.70	0.137	0.76	0.293	0.80	0.537	0.83	0.627

(>10%)	(0.44-1.12)		(0.46-1.26)		(0.40-1.62)		(0.38-1.79)	
Her2 status								
0-2+	1		1		1		1	
3+	1.85 (1.09-3.12)	0.022	2.07 (1.19-3.60)	0.010	1.06 (0.55-2.03)	0.860	1.25 (0.61-2.55)	0.538
F11R								
Low	1		1		1		1	
High	2.85 (1.14-3.82)	<0.001	2.45 (1.50-3.99)	<0.001	3.32 (1.90-5.80)	<0.001	2.69 (1.43-5.05)	0.002

HR,Hazard ratio; CI, confidence interval; AJCC 8th, American Joint Committee on Cancer.

Table 3. Analysis of univariate and multivariate survival for BRCA patients in the KVGH cohort.

Variables	Univariate analysis				Multivariate analysis			
	Overall survival		Disease-free survival		Overall survival		Disease-free survival	
	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value	HR (95% CI)	*p*-Value
Age								
<50 Years	1		1		1		1	
≥50 Years	1.60 (1.15-2.22)	0.005	1.31 (0.75-2.78)	0.349	1.70 (1.20-2.41)	0.003	1.26 (0.87-1.83)	0.226
Tumor T status								
T1+T2	1		1		1		1	
T3+T4	2.58 (1.67-4.00)	<0.001	2.03 (1.14-3.60)	0.015	1.40 (0.86-2.29)	0.172	0.99 (0.56-1.74)	0.974
Lymph node metastasis								
No	1		1		1		1	
Yes	2.42 (1.70-3.45)	<0.001	1.93 (1.10-3.38)	0.023	1.40 (0.88-2.24)	0.195	2.02 (1.18-3.45)	0.010
AJCC stage								
I + II	1		1		1		1	
III + IV	2.65 (1.92-3.66)	<0.001	1.55 (0.89-2.71)	0.121	2.09 (1.33-3.29)	0.001	2.30 (1.43-3.70)	0.001
ER status								
Negative (0-10%)	1		1		1		1	
Positive (>10%)	0.64 (0.46-0.89)	0.007	1.68 (0.95-2.99)	0.077	0.64 (0.37-1.10)	0.103	0.56 (0.29-1.08)	0.083
PR status								
Negative (0-10%)	1		1		1		1	
Positive	0.75	0.081	1.31	0.362	1.32	0.308	1.50	0.219

(>10%)	(0.54-1.04)		(0.73-2.34)		(0.77-2.27)		(0.79-2.87)	
Her2 status								
0-2+	1		1		1		1	
3+	1.53 (1.07-2.18)	0.019	2.66 (1.39-5.10)	0.003	1.12 (0.75-1.66)	0.579	1.05 (0.68-1.63)	0.824
F11R								
Low	1		1		1		1	
High	1.96 (1.42-2.70)	<0.001	2.66 (1.39-5.10)	0.003	1.92 (1.37-2.68)	<0.001	1.39 (0.95-2.02)	0.089

HR,Hazard ratio; CI, confidence interval; AJCC 8th, American Joint Committee on Cancer.

Figures

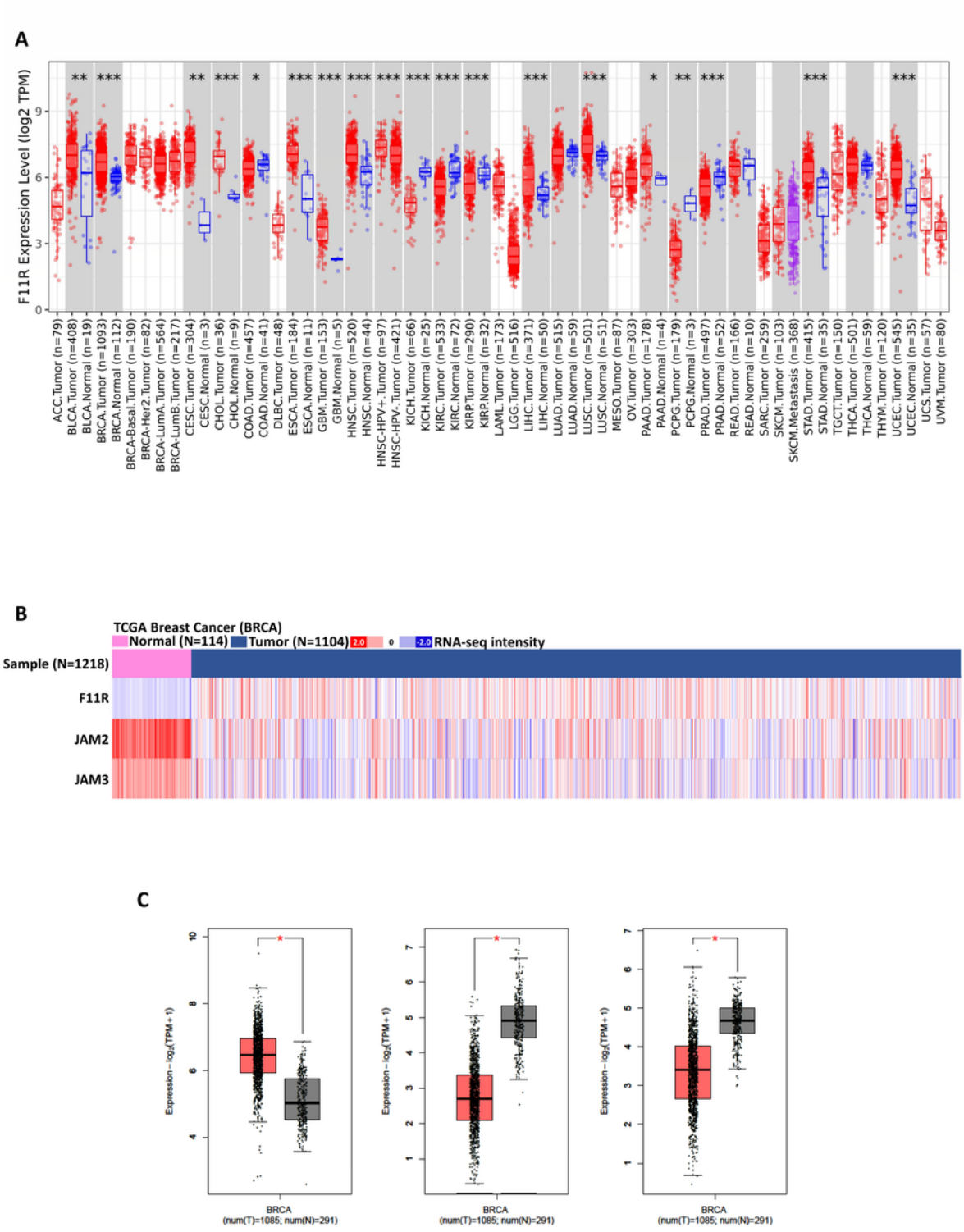


Figure 1

The distribution of JAM members within BRCA. (A) Expression of F11R in cancer types. Analyzed data from TIMER 2.0. (B) This heatmap illustrates the amount of JAM members expressed in BRCA. The data was obtained from XENA. (C) Boxplots illustrating the expression of the JAM gene family in the BRCA normal and tumor groups. The data was analyzed using GEPIA 2.0. (*, $p < 0.05$; $p < 0.01$; ***, $p < 0.001$.)

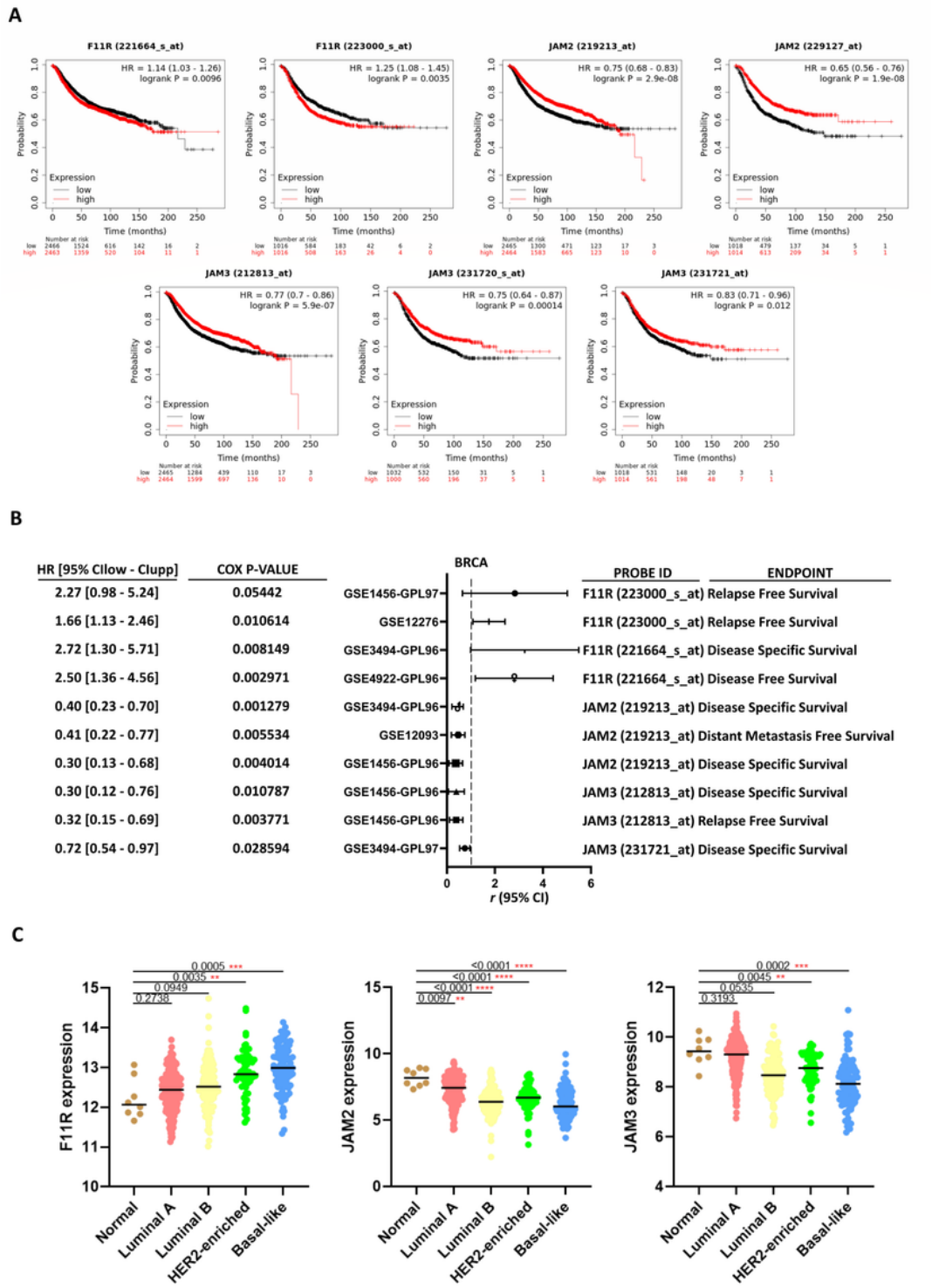


Figure 2

F11R and JAM-2/JAM-3 are inversely related to BRCA prognosis.

(A) Kaplan-Meier survival curves for members of JAM in BRCA with recurrence-free survival. (B) The Hazard Ratio for JAM members with different BRCA prognosis features under different clinical scenarios. (C) The distribution of JAM members within different types of BRCA. (*, $p < 0.05$; $p < 0.01$; ***, $p < 0.001$.)

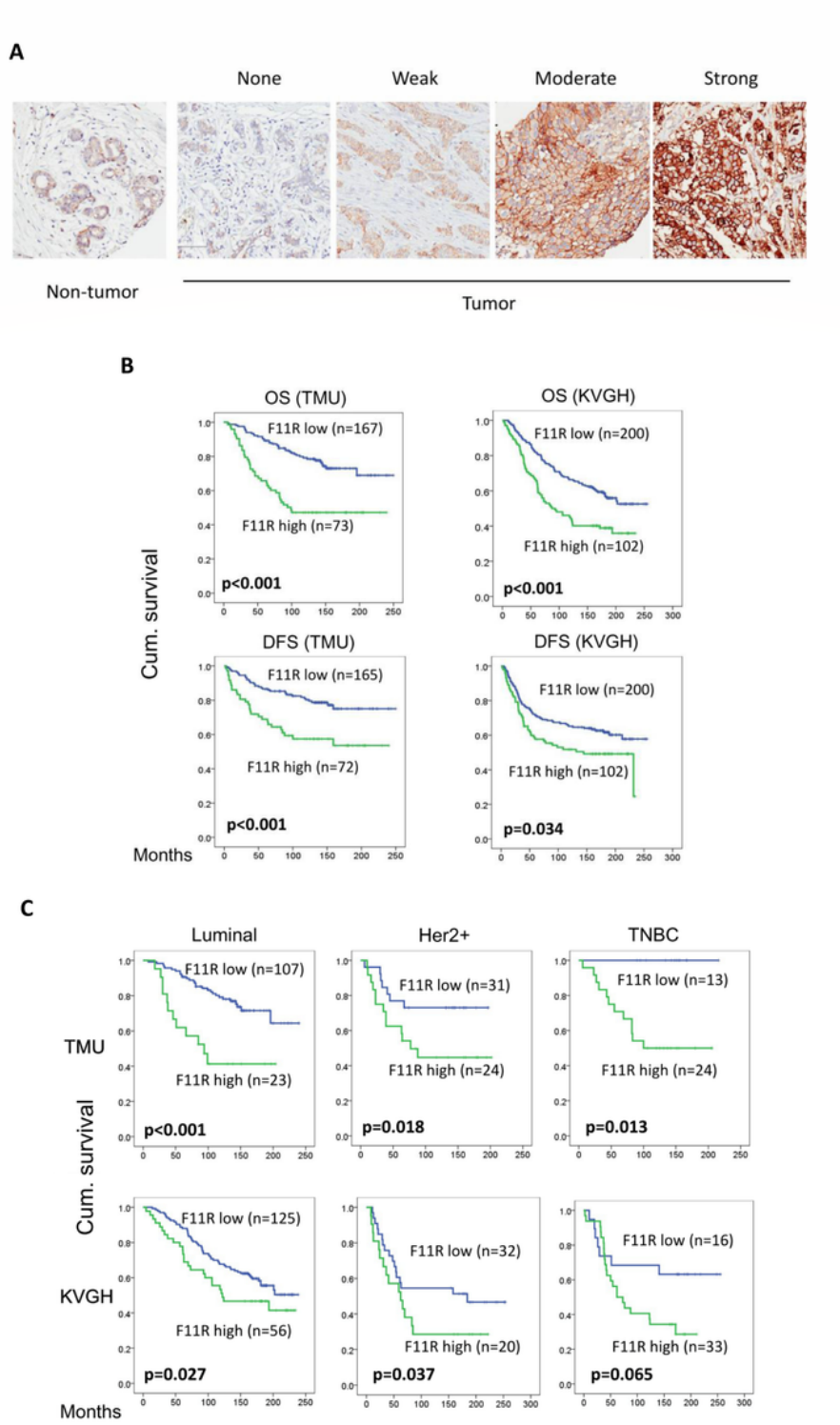


Figure 3

Relationship between F11R protein levels and prognosis in BRCA subtype classification.

(A) Grading of immunohistochemical staining for F11R between tumors and non-tumors of the BRCA. (B) Correlation between expression of F11R and overall survival or disease-free survival in different BRCA tissues. (C) Overall survival according to F11R and different BRCA subtype classifications.

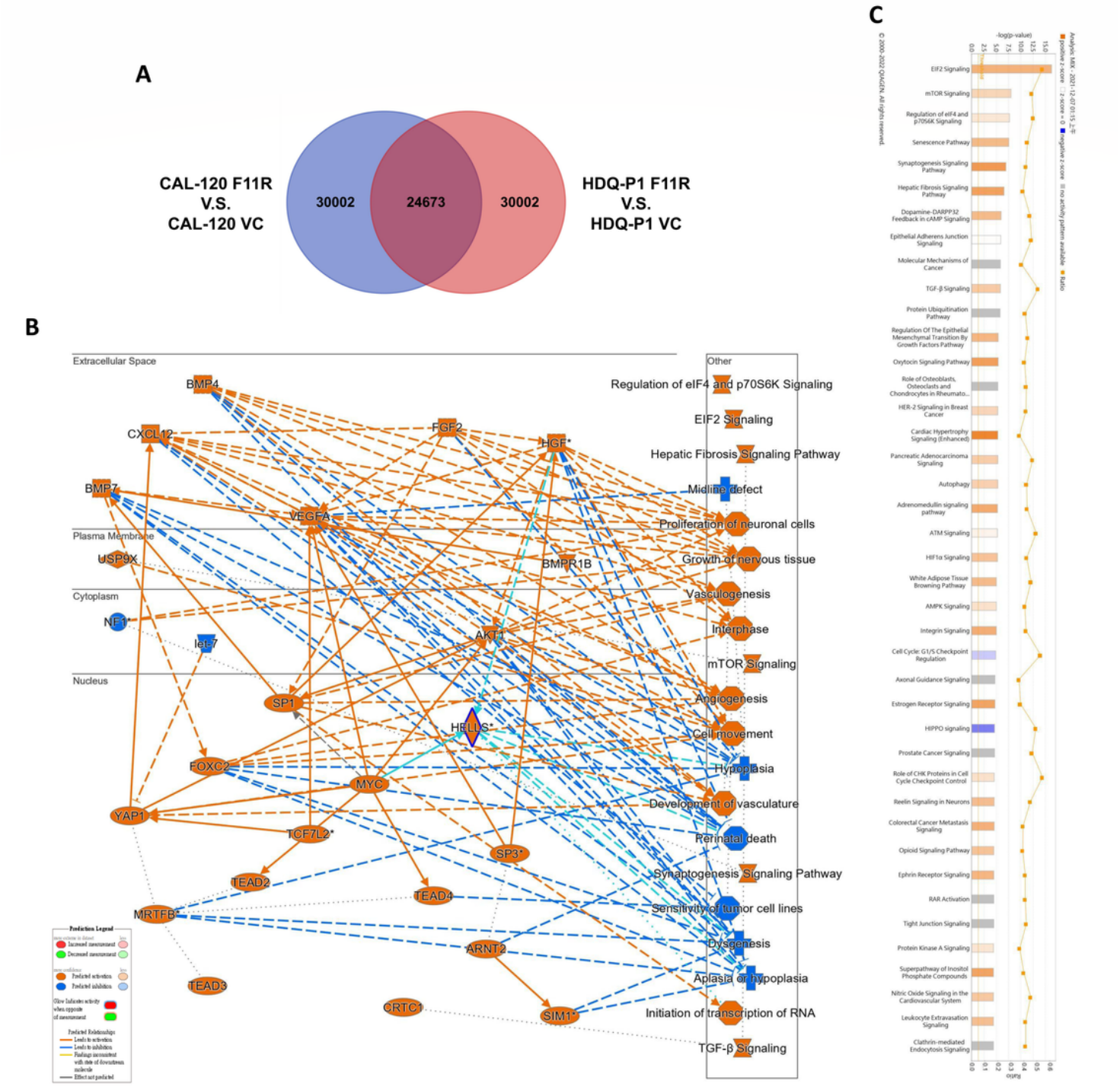
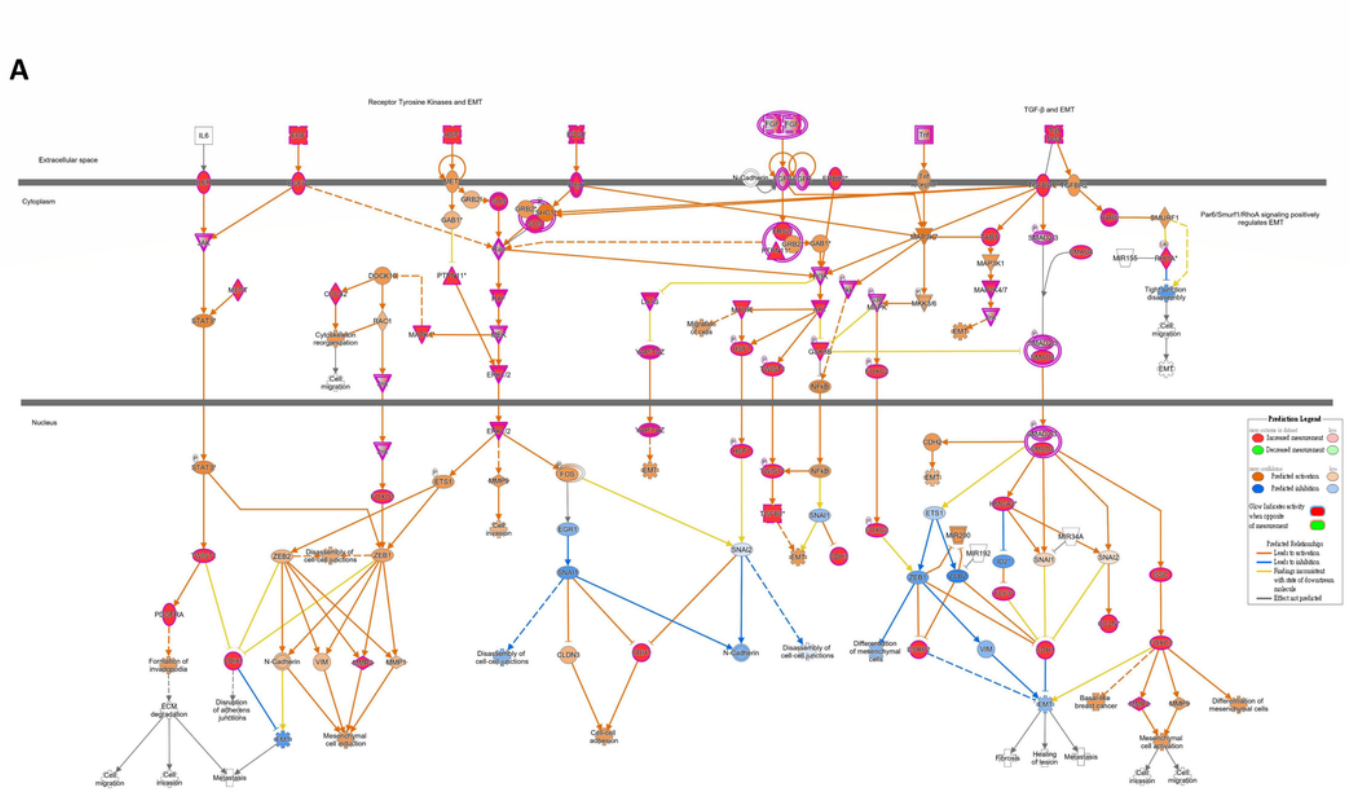


Figure 4

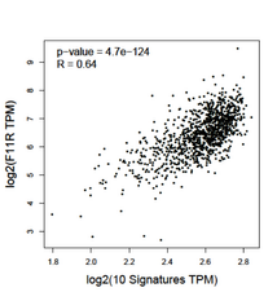
The prediction of molecular biological events using F11R-based transcriptomics in TNBC cells.

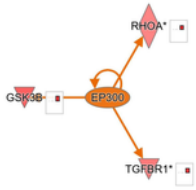
(A) A Venn diagram reveals significant changes in targets following microarray analysis of the F11R overexpression model in CAL120 and HDQ-P1 cells. (B) IPA analysis of F11R-overexpressing cell models of breast cancer cells identified these networks. (C) Potential signaling pathways predicted using IPA that may be affected by F11R overexpression.



B

	CAL120_F11R	HDQP1_F11R	Regulation	F11R	
				Spearman's rank *rho*	*p-value*
GSK3B	1.308	1.036	up	0.61	5.20E-112
MAPK1	1.039	1.314	up	0.58	1.60E+97
SMAD4	1.532	1.005	up	0.54	6.40E-82
PTPN11	1.651	1.076	up	0.49	4.60E-67
OCLN	1.146	1.124	up	0.49	2.00E-66
CDC42	1.125	1.015	up	0.42	3.20E-47
MTOR	1.064	1.089	up	0.42	1.20E-48
TGFBR1	1.094	1.049	up	0.4	4.50E-43
RHOA	1.022	1.171	up	0.38	5.70E-38
IL6R	1.083	1.030	up	0.36	4.00E-34





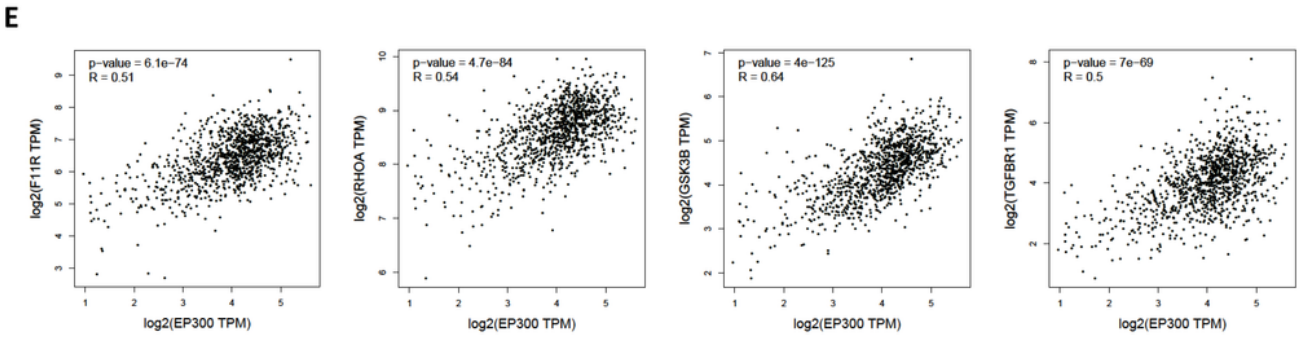


Figure 5

The F11R/EP300 axis is involved in the regulation of cell motility in TNBC.

(A) The molecules activated by overexpression of F11R were found to participate in the regulation of epithelial-mesenchymal transition by growth factors. (B) Correlation of selected EMT-related molecules with F11R in BRCA. (C) An EMT-selected molecule and the F11R gene as a signature of BRCA. (D)

Upstream effectors of EP300 after the overexpression of F11R. (E) A relationship between EP300 and downstream effectors in BRCA.

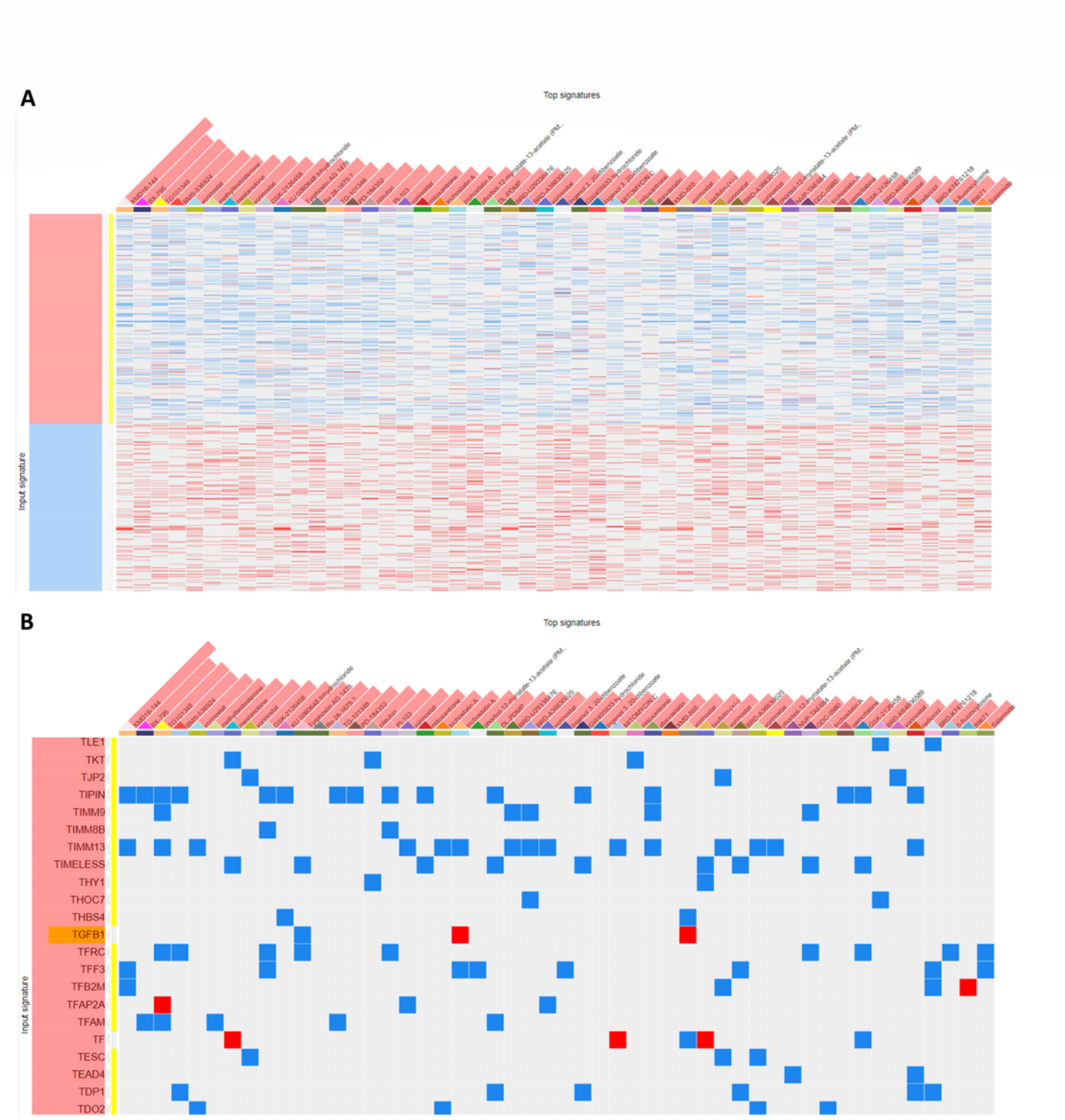


Figure 6

Simulation of drug repurposing based on F11R genetic profiles.

(A) An illustration of the correlation between gene expression and drug candidates can be found in a heatmap. (B) Tyrphostin AG 1478 may act as a candidate for inhibiting TGFB1.

Supplementary Files

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