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Proteomic-based informatics highlights FNBP4 as a prognostically relevant cytoskeletal protein in Breast Cancer

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Abstract

Formin Binding Protein 4 (FNBP4) is a cytoskeletal regulator implicated in actin dynamics, cell migration, and metastasis; however, its prognostic relevance in breast cancer remains poorly defined. This study employed a proteomics-based informatics approach to evaluate the clinical significance of FNBP4 at the protein level and to assess its potential as a prognostic biomarker and therapeutic target. Protein expression patterns of FNBP4 across breast cancer subtypes were analysed using CPTAC, UALCAN, and the Human Protein Atlas (HPA), while survival associations linked to differential protein expression were examined using KM Plotter. Protein–protein interaction networks were constructed using the IntAct database to identify functional partners and elucidate potential mechanistic pathways. FNBP4 was found to exhibit a structurally stable protein conformation with dual cytoplasmic and nuclear localization and moderate nuclear expression in breast cancer tissues. Elevated FNBP4 protein expression was significantly associated with poor overall survival and was particularly enriched in invasive ductal carcinoma and triple-negative breast cancer subtypes. Increased FNBP4 levels correlated with somatic alterations across major oncogenic signalling pathways. Interaction network analysis revealed strong predicted associations with KHDRBS1, SYNJ1, and CSNK2A1, suggesting involvement in RNA regulation, endocytic signalling, and kinase-mediated cellular control. Collectively, these findings identify FNBP4 as a prognostically relevant cytoskeletal protein in breast cancer and provide a strong rationale for its further experimental validation and exploration as a therapeutic target.

INTRODUCTION

Formins are a family of proteins that are crucial for controlling actin filaments formation and organisation within cells[1]. Dysregulation of formins may contribute to the increased motility and invasive properties of cancer cells, facilitating their spread to surrounding tissues[2]. Formin-binding proteins (FNBPs) are a group of proteins that interact with formins to regulate, fine-tune or modulate their activity or functions[3]. The gene for FNBP4(also known as FBP30) is located on chromosome 11p14. This protein contains two tryptophan-rich WW domains, which specifically interact with the proline-rich formin homology 1(FH1) domains found in members of the formin protein family. Despite being the shortest known domains with highly conserved structures, WW domains are known to participate in a diverse array of cellular processes, such as transcription, splicing, organ development, tumorigenesis, ubiquitylation, and much more[4]. Sequence variation confers characteristic ligand specificity to these domains, thus making them capable of binding to different partners and providing the platform for protein-protein interactions. A few of the WW domain-containing formin binding proteins are known to be components of the eukaryotic transcription and splicing machinery as well[5,6,7].

FNBP4 had initially gained attention from a study describing its regulation by p53[8]. Over time, researchers further explored the roles of formin binding proteins across various cancer types [9,10,11] and studied them in relation to their structural characteristics and function,[12,13,14,15].However, the expression of FNBP4 in breast cancer and its potential link to patient prognosis remains unclear.

Breast cancer is among the most common types of cancer diagnosed in women and remains a leading cause of cancer-related deaths globally[16]. The high death rate is mainly due to the spread of tumor cells to distant organs through metastasis. Metastasis involves a complex sequence of events, including detachment from the primary tumor, invasion through the extracellular matrix, intravasation, survival in the circulation, extravasation, and colonization of distant tissues[17]. Regulating the process of actin remodelling is one of the key steps in the

progression of cancer; hence, studying the key components of Epithelial-Mesenchymal Transition (EMT) and Mesenchymal-Epithelial Transition (MET), as well as examining their role in tumor progression[18], can have therapeutic implications, particularly in devising interventions that prevent metastasis[19].

In the current study, we focused specifically on the protein-level expression of FNBP4 to evaluate its potential role in breast cancer. Protein-level analysis provides functionally relevant insights, as proteins are the principal executors of cellular functions. To achieve a comprehensive understanding, multiple publicly available proteomic and interaction databases were utilized. Protein expression data retrieved from the Human Protein Atlas (HPA)[20] and the Clinical Proteomic Tumor Analysis Consortium (CPTAC), accessed via the UALCAN portal[21], provides quantitative protein profiles across diverse tumor subtypes and clinical conditions. To explore FNBP4's functional landscape, the IntAct Molecular Interaction Database[22] was used to identify its potential protein–protein interaction partners, shedding light on its involvement in key cellular pathways. Furthermore, the Kaplan–Meier Plotter (KM Plotter) tool[23] was employed to assess the correlation between FNBP4 protein expression and patient survival outcomes, thereby evaluating its prognostic significance.

METHODOLOGY

1. Human Protein Atlas (HPA)

HPA was utilized to gather comprehensive information on FNBP4, including its protein expression levels, subcellular localization, and predicted functional roles.

2. The University of ALabama at Birmingham CANcer data analysis Portal (UALCAN-CPTAC)

To investigate the differential protein expression of Formin-binding protein 4 (FNBP4) in breast cancer, the UALCAN portal[24], an interactive web resource for analysing cancer omics data, was utilized. Protein expression data were accessed specifically from the Clinical Proteomic Tumor Analysis Consortium (CPTAC) module integrated into UALCAN. The analysis was restricted to the “Breast Invasive Carcinoma” dataset available in CPTAC. FNBP4 protein expression (measured by mass spectrometry-based proteomics) was assessed across normal breast tissue and primary tumor samples. Expression was further stratified based on molecular subtypes (luminal A, luminal B, HER2-enriched, and basal-like) and proteomic subtypes (K1-K10 & S1-S-11), allowing for insights into expression heterogeneity and the probable role in oncogenic pathways.

3. Kaplan-Meier Plotter:

To evaluate the prognostic relevance of FNBP4 expression in breast cancer, Kaplan-Meier survival analysis using the Kaplan-Meier Plotter tool[25], a publicly available resource that integrates gene expression and survival data were performed.

4. IntAct Molecular Interaction Database (IntAct)

To investigate the potential interactome of FNBP4, IntAct Database (https://www.ebi.ac.uk/intact/) was employed —it is a manually curated repository of experimentally validated protein–protein interactions derived from peer-reviewed scientific literature. “FNBP4” was used as the query term, and the search was restricted to interactions specific to Homo sapiens, except for one isoform where data was available only for Mus musculus. Interactions with a MIscore greater than 0.35 were selected, prioritizing those categorized as direct interactions or physical

associations. The MIscore reflects the confidence level of each interaction, which increases proportionally with the number and quality of supporting experimental evidence. This analysis aimed to reveal functionally relevant partners that may contribute to FNBP4's role in breast cancer pathophysiology.

Results

FNBP4 Exhibits Stable Structure, Dual Subcellular Localization, and Moderate Nuclear Expression in Breast Cancer Tissues

HPA provided valuable information regarding FNBP4. Alpha Fold-based structural prediction (A) of FNBP4 revealed a high degree of confidence in most regions of the protein, indicated by the prevalence of blue (pLDDT > 90) and light blue (pLDDT > 70) segments. Western blot analysis (B) confirmed recombinant expression of FNBP4, overexpressed in HEK293T cells co-transfected with the expression vector, showing a protein band with minimal non-specific bands. The higher-than-expected molecular weight (~ 110.3 kDa) in Lane 3 may represent a post-translationally modified protein; however, the presence of a clear band supports the specificity of the antibody and successful overexpression of the tagged FNBP4 protein. Immunofluorescence analysis (C)using independent antibodies in U2OS cells demonstrated consistent localization of FNBP4 to the nucleoplasm. Immunohistochemical (IHC) (D - E) staining in breast cancer tissue microarrays provided further evidence of FNBP4's subcellular localization. Image D showed moderate nuclear staining in tumor cells, while Image E indicated cytoplasmic membranous localization with low staining intensity. Comparative analysis of FNBP4 protein expression across various cancers (F) indicated weak to moderate nuclear positivity with additional cytoplasmic staining in breast cancer tissues. These findings suggest a dual distribution of FNBP4, potentially indicating a shuttling mechanism between the cytoplasm and nucleus or context-dependent functional shifts. Among the twenty cancer types analysed, breast cancer exhibited a notable level of FNBP4 positivity, supporting its relevance in tumor progression.

Poor overall survival at high expression of FNBP4 protein

KM survival plot shows the relationship between the expression levels of the FNBP4 protein (with UniProt ID: Q8N3X1) and patient survival over time, measured in months. The survival rate was assessed using the Tang (2018)[23] dataset (65 samples). The hazard ratio found was 2.86 with a P value of 0.0092 (Fig. 2). Patients with low FNBP4 expression showed a higher probability of survival over time compared to those with high expression levels.

High protein expression in Invasive Ductal Carcinoma and TNBC cancer subclass of breast cancer

FNBP4 protein expression analysis was performed using CPTAC data available on the UALCAN platform, evaluating its differential expression across various clinical parameters including sample types, cancer stages, molecular subtypes, and histological categories (Fig. 3; A-D). The analysis revealed notably higher FNBP4 expression in Invasive Ductal Carcinoma (IDC) and Triple Negative Breast Cancer (TNBC) subtypes, indicating its potential association with aggressive clinical features in breast cancer.

Elevated FNBP4 Protein Expression Correlates with Somatic Alterations in Key Oncogenic Signalling Pathways

FNBP4 expression has been systematically assessed in the context of somatic pathway alterations by integrating proteomic profiles with whole-exome sequencing and copy number alteration (CNA) data. This integrative multi-omics analysis enabled the identification of pathway-level perturbations and gene-specific alterations associated with FNBP4, providing insights into its potential role in key oncogenic signalling events within breast cancer. Figure 4 presents the Z-score normalized expression pattern of FNBP4 protein across various cancer-associated signalling pathways in CPTAC breast cancer samples, comparing normal tissues, pathway-altered tumors, and other tumors.

FNBP4 expression for proteome-based molecular subtypes (K1-K11, S1-S11) and associated mRNA class, notable features for each class, were checked to gain more insights into probable pathways they might be associated with. (Fig. 5 and Table 1)

Table 1
Integrated Proteogenomic Subtypes (K1-K10/S1-S11/associated mRNA class c1-c10) for interpreting FNBP4 Expression in Breast Cancer.

K Subtype	S Subtype	n (K)	n (S)	Associated mRNA Class	Molecular Features	FNBP4 Z-Value (K)	FNBP4 Z-Value (S)	Interpretation
K1	S1	2	1	c1	Proteasome, glycolysis, PPP	~+0.5	~+0.8	Mildly elevated expression in both, likely reflecting proteasome and glycolytic activity.
K2	S2	12	6	c3, c10	Adaptive immune, MHC proteins	~+1.2	~−0.5	Discordant expression; likely reflects microenvironmental diversity (immune-driven K2 vs suppressed S2).
K3	–	5	–	c3, c10	Innate immune, hypoxia, complement system	~−1.5	–	Strong downregulation; may represent suppressed immune or hypoxic subtypes not well captured in S-type.
K4	S3	29	24	c5	Basal-like, MYC/YAP1 targets	~+2.0	~+0.9	Consistently high expression suggests a strong MYC/YAP1-driven basal-like phenotype.
K5	S4	18	25	c6	Epithelial, normoxia, TCA/OXPHOS	~+1.0	~+0.5	Moderate concordance; reflects epithelial phenotype with metabolic activity.
–	S5	–	–	–	B cells, MMPs, eosinophils, complement	–	Not available	Likely heterogeneous, may overlap with immune-stromal mixed types (K3, K6).
K6	–	14	–	c7	Stromal, MMPs, Wnt/Notch, hypoxia	~−1.0	–	Consistently downregulated; reflects desmoplastic or hypoxic stromal context.
K7	S6	31	30	c8	Stromal, collagen VI, Wnt/Notch	~+0.3	~−0.5	Slight disagreement; may indicate functional subclusters in stromal response.

K Subtype	S Subtype	n (K)	n (S)	Associated mRNA Class	Molecular Features	FNBP4 Z-Value (K)	FNBP4 Z-Value (S)	Interpretation
–	S7	–	4	–	Axon guidance, frizzled binding	–	~+0.6	Moderately upregulated; potential neural/migratory component.
–	S8	–	5	–	RCC only, haemoglobin proteins	–	~−1.0	Not relevant to breast cancer; downregulated in RCC cases.
K10	S9	14	17	–	ER-related, steroid biosynthesis	~−0.5	~−0.3	Mild downregulation; similar hormonal profile suggests functional similarity.
–	S10	–	12	–	DNA repair, MYC, Wnt, Hippo pathway	–	~+1.5 to + 2.5	High expression; resembles K4 biologically—potentially an aggressive subtype.
–	S11	–	–	–	Synapse, metabolism (TCA, glycolysis, FA)	–	Not shown	Predicted high expression due to metabolic activity; parallels K5 or K4.

Direct molecular interactions with KHDRBS1 (KH domain-containing, RNA-binding, signal transduction-associated protein 1) and SYNJ1 (Synaptojanin 1) with the highest probability

The IntAct molecular interaction database (https://www.ebi.ac.uk/intact) is a curated resource of molecular interactions, derived from the scientific literature and direct data depositions. IntAct curates' molecular interaction data derived from experimental studies published in scientific literature. The interacting partners of FNBP4 were determined to integrate it into a broader protein interaction network, thereby elucidating its functional context and the biological pathways it may be involved in. These are identified using methods involving purified components, excluding cell extracts or complex mixtures, to ensure no hidden intermediaries are present. Enzymatic reactions (e.g., phosphorylation) were also included in this category, provided purified participants were used, though they are depicted as specific reaction events. A higher MiS score indicates the level of confidence in predicting the specific interaction based on the standardised dataset with a higher number of experimental evidences in support of the claim (Table 2).

Table 2
Experimentally Determined Interaction Partners of FNBP4 and Its Isoforms

S. No	Interactive partner (uniProt)	Host Organism	Detection Method	Interaction type	Mi Score	Publication Id
A. Q8N3X1 (homo sapiens species)						
1	KHDRBS1 Q07666	In vitro	filter binding	Direct interaction	0.54	10748127
2	PRPF40A O75400	Saccharomyces cerevisiae (Baker's yeast)	2 hybrid	Physical association	0.55	15231748
3	H2BC21 Q16778	Homo sapiens U2OS osteosarcoma cell	crosslink	Physical association	0.4	30021884
4	HTT P42858	Saccharomyces cerevisiae (Baker's yeast)	2 hybrid	Physical association	0.37	17500595
5	HSCB Q8IWL3	Saccharomyces cerevisiae (Baker's yeast)	2 hybrid	Physical association	0.37	24606901
6	PLXNA2 O75051	Saccharomyces cerevisiae (Baker's yeast)	2 hybrid	Physical association	0.37	12421765
B. Q8N3X1-1 (Isoform) (homo sapiens species)						
1	CSNK2A1 P68400	In vitro	protein kinase assay	Physical association	0.44	22113938
C. Q8N3X1-2 (Isoform) (homo sapiens species)						
1	DDX17 Q92841-4	Homo sapiens HEK293T embryonic kidney cell	anti tag co ip	association	0.35	28514442
2	ZC3H18 Q86VM9	Homo sapiens HEK293T embryonic kidney cell	anti tag co ip	association	0.35	28514442
D. Q6ZQ03 (Isoform) (with Mus musculus species)						

S. No	Interactive partner (uniProt)	Host Organism	Detection Method	Interaction type	Mi Score	Publication Id
1	SYNJ1 O43426	In vitro	phage display	direct interaction	0.56	11292345
2	Cdk9 Q99J95	Mus musculus	pull down	association	0.35	20593818

DISCUSSION

Cytoskeletal regulators are central drivers of cancer metastasis. Their dysregulation enhances cellular motility, invasiveness, and metastatic potential[26]. Among these regulators, formins and their binding partners play essential roles in actin remodelling. Although direct evidence on the role of FNBP4 in breast cancer remains limited, its Increasing interest stems from emerging proteomic data suggesting its involvement in both clinical aggressiveness and oncogenic signalling pathways.

Proteomic analysis using CPTAC datasets demonstrates a consistent upregulation of FNBP4 in breast tumors compared with normal breast tissue. Stage-wise comparison reveals rising levels from stage II onward, indicating a link with tumor progression. Subtype-specific analysis highlights the highest FNBP4 expression in triple-negative breast cancer (TNBC), followed by luminal and HER2-positive subtypes, suggesting a stronger association with aggressive and therapy-refractory disease. Histological assessment shows substantial upregulation in infiltrating ductal carcinoma, while levels remain comparatively lower in infiltrating lobular carcinoma and mucinous variants.

Survival analysis using Kaplan–Meier Plotter reveals that high FNBP4 protein expression correlates with significantly poorer patient outcomes (HR = 2.86), positioning FNBP4 as a potential marker of increased mortality risk. Complementary data from the Human Protein Atlas indicate that FNBP4 predominantly localizes to the nucleoplasm and cytoplasm, implicating it in processes such as RNA metabolism, chromatin organization, and transcriptional regulation—mechanisms frequently altered in cancer. Its dual localization suggests dynamic shuttling or context-dependent functions, which may be particularly relevant in rapidly evolving tumor environments such as TNBC, where cytoskeletal flexibility and transcriptional adaptability are essential for tumor plasticity and metastasis.

Beyond clinicopathological features, FNBP4 expression shows strong associations with oncogenic pathway alterations. Tumors with somatic changes in pathways such as RTK, WNT, PI3K/AKT/mTOR, MYC/MYCN, p53/Rb, and Hippo exhibit significantly elevated FNBP4 levels. This enrichment suggests that FNBP4 may act as a downstream effector or functional contributor within these pathways, propagating cancer-driving signals that promote cell proliferation, survival, and migration. The highest increases occur in tumors altered in NRF2, MYC/MYCN, RTK, and p53/Rb pathways, emphasising its integration into multiple cancer-relevant networks. Additionally, high copy number alteration (CNA) burden correlates with increased FNBP4 levels, particularly in contexts involving hormone receptor signalling, histone modification, and DNA repair, reinforcing its potential role in the cellular response to genomic instability.

Unsupervised clustering studies further support the variable involvement of FNBP4 across molecular tumor subtypes. Large-scale proteome-based analyses have defined multiple subtypes (K1–K10 and S1–S11)[27,28], each

representing distinct biological programs. FNBP4 expression peaks in subtype K4, characterized by basal-like features and activation of MYC and YAP1 pathways—both powerful regulators of transcriptional amplification, cytoskeletal remodelling, and epithelial-mesenchymal transition (EMT)[29]. Moderately elevated levels appear in K2 and K5 subtypes associated with immune and metabolic signatures, respectively. Conversely, subtypes defined by innate immune activation or hypoxia show reduced expression, indicating subtype-specific functional relevance.

To elucidate FNBP4's mechanistic roles, protein–protein interaction (PPI) mapping was performed using IntAct. The canonical FNBP4 isoform in humans shows eight experimentally validated interactions detected through methods such as two-hybrid assays, crosslinking, and filter binding. Several interactions exhibit moderate to high confidence with MI scores ranging from 0.35 to 0.55. Among these, KHDRBS1 (Sam68) emerges as a strong interactor (MI score 0.54), implicating FNBP4 in RNA splicing and transcriptional regulation. KHDRBS1 regulates alternative splicing events that influence cell adhesion, migration, and apoptosis, often promoting tumor progression[30,31,32] Isoform-specific interactions include CSNK2A1 (CK2α) for isoform Q8N3X1-1, identified through kinase assays, suggesting post-translational modulation of FNBP4 by phosphorylation. CSNK2 (CK2) is a well-known oncogenic kinase that drives survival signalling, cytoskeletal reorganization, and adaptation to stress[33,34,35] Isoform Q8N3X1-2 interacts with DDX17and ZC3H18, both involved in RNA helicase activity and transcriptional regulation[36,37]. In murine models, the Q6ZQ03 isoform interacts strongly with SYNJ1 and moderately with CDK9, linking FNBP4 to vesicle trafficking and cell-cycle control. SYNJ1 contributes to altered membrane trafficking and receptor recycling, facilitating enhanced motility and invasive capacity [38,39,40,41.] Evidently, all these interactors themselves play established roles in breast cancer. Through these interactions, FNBP4 may serve as a scaffold that integrates pathways involving RNA metabolism, cytoskeletal restructuring, and vesicular dynamics-functions that collectively support tumor aggressiveness. Cross-species conservation and high-confidence interactions further underscore the biological significance of FNBP4 in breast cancer progression

CONCLUDING REMARKS

The use of large-scale proteomic datasets offers a valuable, systems-level perspective, facilitating the identification of clinically relevant targets. The availability of global cancer databases has significantly enhanced the understanding of tumorigenesis. This study leverages multiple proteomic and clinical datasets to comprehensively examine FNBP4's role in breast cancer, revealing its potential impact on disease progression and clinical outcomes. Protein-level database analysis is crucial for biomarker discovery and validating targets because it reflects dynamic cellular states and biological function Moving forward, rigorous experimental validation through *in-vitro* and *in-vivo* models is essential to confirm these associations, decipher the underlying molecular mechanisms, and translate these bioinformatic insights into clinical applications for breast cancer management.

Declarations

Author Contribution

RS: Substantial contributions to the conception, design of work; acquisition, analysis, interpretation of data, drafting KK: review, formattingKD: review, formattingMK: critical reviewing, formattingST: Drafting the work, critical

reviewing, formattingSM: intellectual inputs, interpretation of dataHC: conception, design of the work, interpretation of data, Supervision, Final approval of the version to be published

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Figures

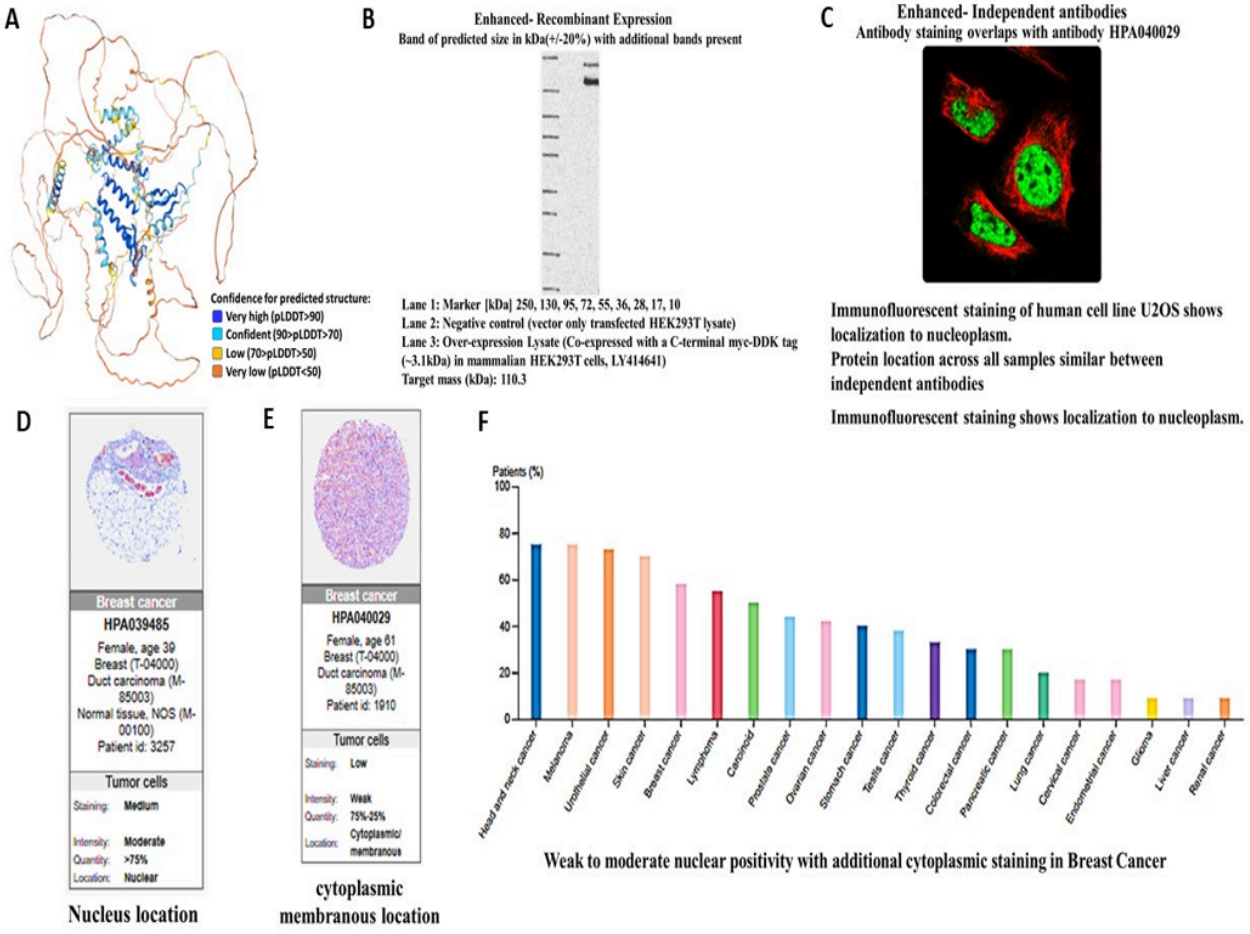


Figure 1

A- Predicted structure; B- Enhanced recombinant expression based on predicted size; C, D, E- Antibody staining showing nuclear and cytoplasmic membranous location; F- weak to moderate nuclear positivity with additional cytoplasmic staining across different cancers[21].

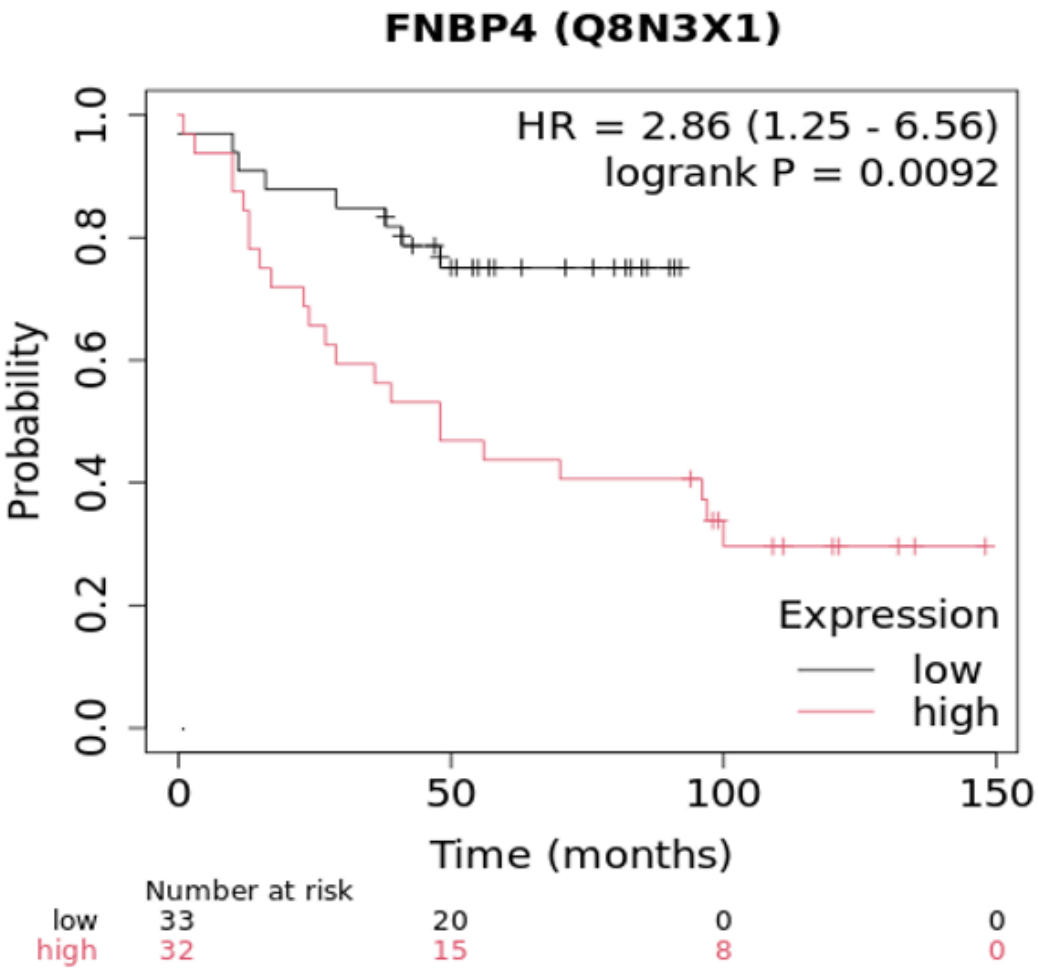


Figure 2

Survival plots. The KM survival plot shows the relationship between the expression levels of the FNBP4 protein (UniProt ID: Q8N3X1) and patient survival over time, measured in months (Hazard ratio= 2.86, P value= 0.0092).

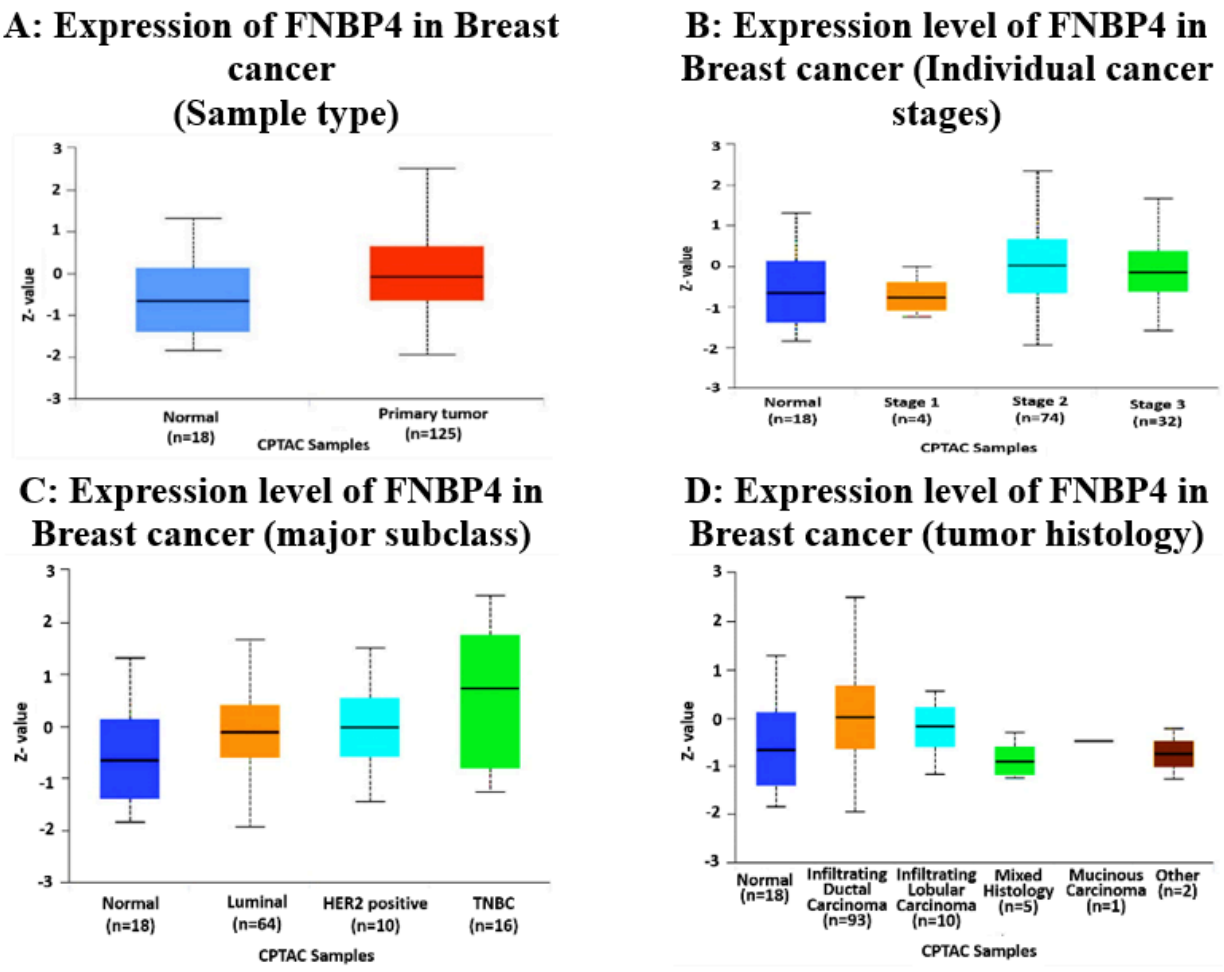


Figure 3

UALCAN-CPTAC data. (A) Expression of protein levels of FNBP4 in normal tissue and primary breast cancer. (B) Expression of FNBP4 protein levels in distinct stages of breast cancer. (C)) Expression of protein levels of FNBP4 in different breast cancer subclasses. (D) Expression of FNBP4 protein levels in breast cancer based on histological subtypes.

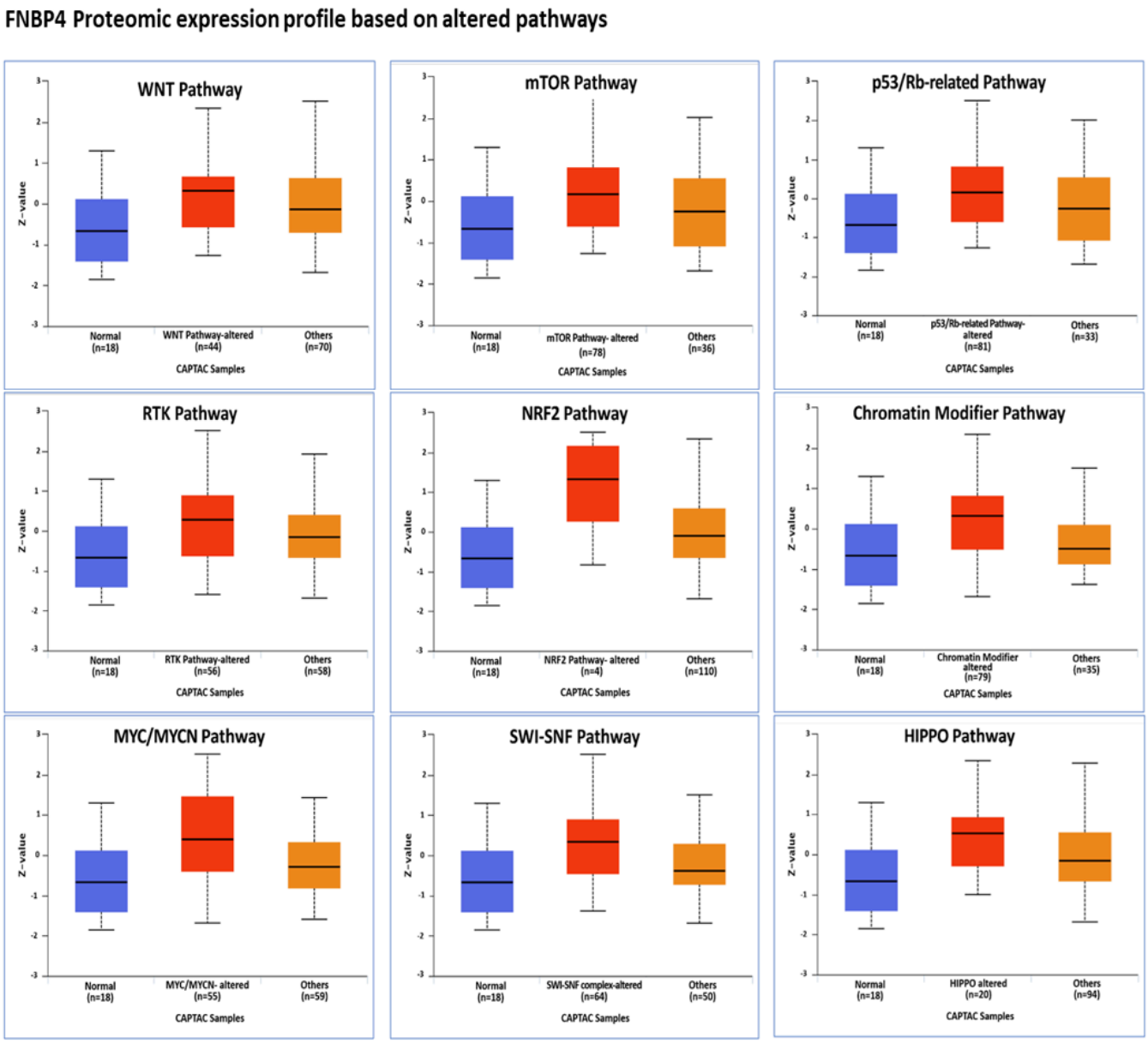


Figure 4

The expression pattern of FNBP4 protein (Z-score normalized) across various cancer-altered signalling pathways in CPTAC breast cancer samples, comparing normal tissue, pathway-altered tumors, and other tumors.

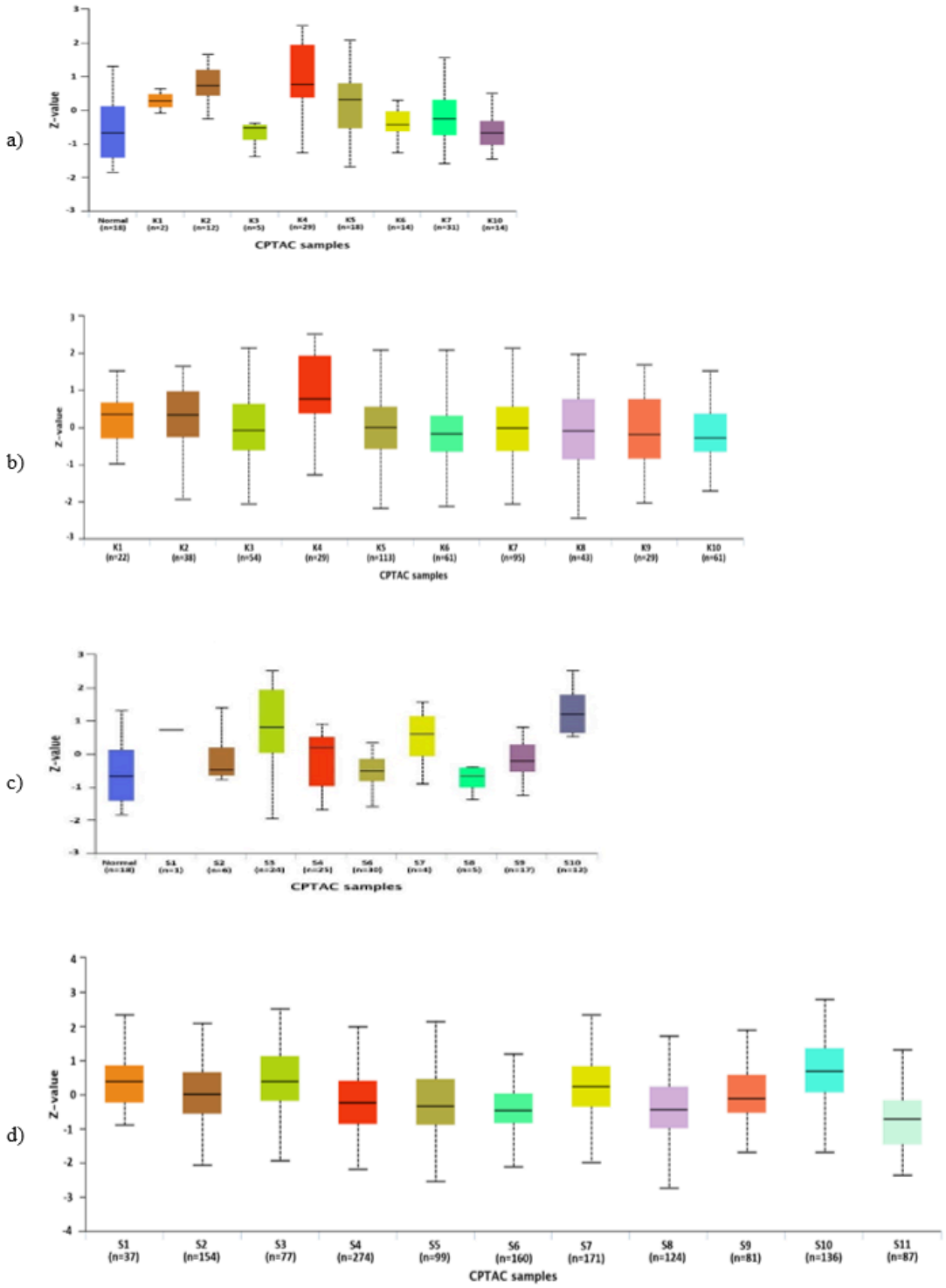


Figure 5

FNBP4 protein expression based on Proteome-based Molecular subtype a) (K1-K10) in breast cancer; b) K1-K10 in Pan cancer; c) Molecular subtype 2 (S1-S10) in breast cancer; d) S1-S11 in Pan cancer.