# Promoter Hypermethylation-Induced Silencing of FXYD1 Drives Breast Cancer Metastasis via DDX5-Mediated Wnt/β-Catenin Pathway Activation

Ping Wen, Guanwen Wang, Fanli Qu, Qing Shao, Long Wang, Ningning Zhang, and Xiaohua Zeng
Department of Breast Cancer Center, Chongqing University Cancer Hospital, Chongqing, China

## Abstract

Epigenetic silencing of tumor suppressor genes is a hallmark of cancer progression. The FXYD family, classically known for ion transport regulation, has recently been implicated in oncogenesis; however, the role of its founding member, FXYD1, in breast cancer remains unclear. Here, we identified FXYD1 as a significantly downregulated gene in breast cancer tissues, with low expression correlating with poor patient prognosis. Integrative transcriptomic, clinical, and epigenetic analyses revealed that promoter hypermethylation drives FXYD1 silencing. Functional restoration of FXYD1 suppressed cell proliferation, migration, and lung metastasis both *in vitro and in vivo*. Mechanistically, FXYD1 acts as a nuclear scaffold that recruits the E3 ubiquitin ligase MAEA to the RNA helicase DDX5, a coactivator of β-catenin, promoting K63-linked ubiquitination and proteasomal degradation of DDX5. This process reduces β-catenin stability, impairs its nuclear translocation, and attenuates Wnt target gene expression. Collectively, our findings uncover a previously unrecognized FXYD1–MAEA–DDX5 axis that inhibits Wnt/β-catenin signaling through a non-canonical ubiquitin–proteasome pathway, establishing FXYD1 as a tumor suppressor and potential prognostic biomarker and therapeutic target in breast cancer.

## Introduction

Breast cancer remains the most prevalent malignancy and a major cause of cancer-related mortality among women. Although early detection and targeted therapies have improved outcomes, recurrence and metastasis, particularly in triple-negative breast cancer, remain critical challenges. Epigenetic dysregulation is increasingly recognized as a central driver of tumorigenesis, often leading to silencing of tumor suppressor genes that sustain malignant progression and therapeutic resistance. The full repertoire and functions of such epigenetically repressed genes in breast cancer, however, remain unclear.

The FXYD family encodes ion transport regulators interacting with $Na^+/K^+$-ATPase, but several members exhibit context-dependent roles in cancer. Here, we identify FXYD1 as a tumor suppressor consistently downregulated in breast cancer through promoter hypermethylation. Unexpectedly, FXYD1 localizes predominantly in the nucleus, where it attenuates oncogenic signaling by acting as a scaffold that recruits the E3 ubiquitin ligase MAEA to the RNA helicase DDX5, a coactivator of β-catenin. This facilitates K63-linked polyubiquitination and proteasomal degradation of DDX5, thereby destabilizing β-catenin and suppressing Wnt target gene expression. Functionally, this axis inhibits tumor proliferation and metastasis.

## Methods and Materials

FXYD1 expression was evaluated using public datasets (TCGA, GEO), breast cancer cell lines, and patient-derived tissues. Functional analyses, including proliferation assays, Transwell invasion assays, and murine xenograft metastasis models, were conducted following FXYD1 overexpression or knockdown. Mechanistic studies involved RNA sequencing, co-immunoprecipitation, DNA methylation analysis, ubiquitination assays, and genetic rescue experiments.

## Results

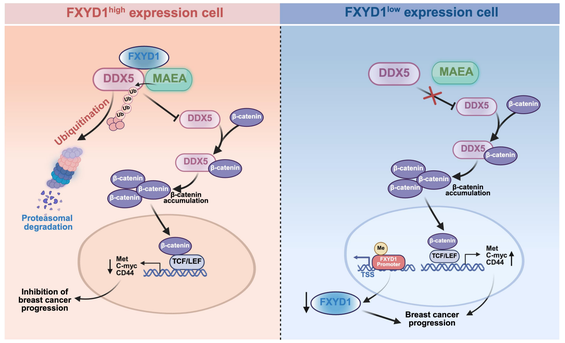



**Figure 1.** Schematic illustration.

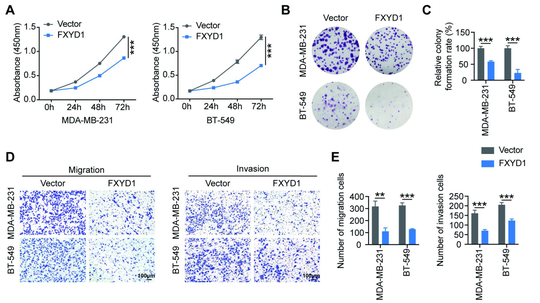



**Figure 3.** FXYD1 suppresses breast cancer cell proliferation and metastasis *in vitro*.

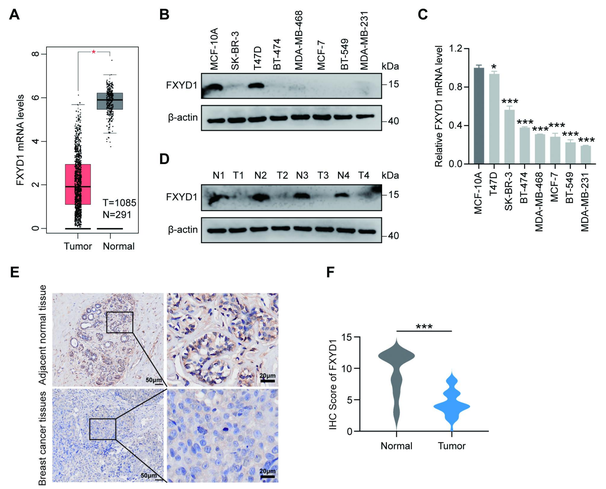



**Figure 2.** FXYD1 is downregulated in breast cancer.

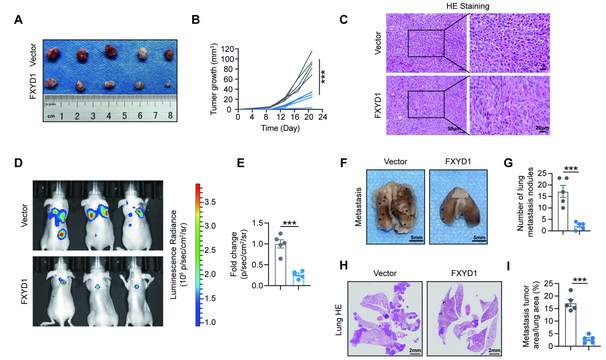



**Figure 4.** FXYD1 suppresses breast cancer cell proliferation and metastasis *in vivo*.

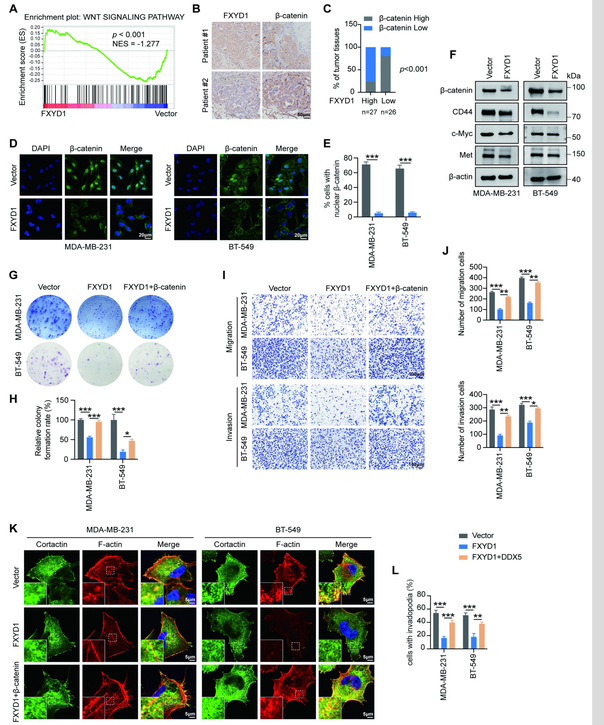



**Figure 5.** FXYD1 inhibits tumor progression by attenuating Wnt/β-catenin signaling.

## Conclusions

Our findings establish FXYD1 as a previously unrecognized tumor suppressor that inhibits breast cancer metastasis via the DDX5-β-catenin axis. This study not only clarifies a novel ubiquitination regulatory mechanism but also highlights FXYD1 as a potential therapeutic target for metastasis intervention.

## Contact

Ping Wen
Chongqing University Cancer Hospital
Email: wenping2529@163.com

## References

1. Bray, F., et al., Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin, 2024. 74(3): p. 229-263.
2. Xiong, X., et al., Breast cancer: pathogenesis and treatments. Signal Transduct Target Ther, 2025. 10(1): p. 49.
3. Fleischer, T., et al., DNA methylation at enhancers identifies distinct breast cancer lineages. Nat Commun, 2017. 8(1): p. 1379.
4. Pasculi, B., R. Barbano, and P. Parrella, Epigenetics of breast cancer: Biology and clinical implication in the era of precision medicine. Semin Cancer Biol, 2018. 51: p. 22-35.
5. Lee, A.V., K.A. Nestler, and K.B. Chiappinelli, Therapeutic targeting of DNA methylation alterations in cancer. Pharmacol Ther, 2024. 258: p. 108640.

© POSTER TEMPLATE BY GENGRAPHICS® 1.800.790.4001 WWW.GENGRAPHICS.COM