Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

PATZ1 Restrains Basal-like Breast Cancer Aggressiveness while Preserving FOXA1-Driven Luminal Identity

Sabrina Battista
CNR: Consiglio Nazionale delle Ricerche
Michela Vitiello
CNR: Consiglio Nazionale delle Ricerche
Maria Pia Albanese
CNR: Consiglio Nazionale delle Ricerche
Cristina Quintavalle
CNR: Consiglio Nazionale delle Ricerche
Tina Di Palma
CNR: Consiglio Nazionale delle Ricerche
Mariastella Zannini
CNR: Consiglio Nazionale delle Ricerche
Gerolama Condorelli
Universita degli Studi di Napoli Federico II
Elvira Crescenzi
CNR: Consiglio Nazionale delle Ricerche
Laura Cerchia
CNR: Consiglio Nazionale delle Ricerche
Monica Fedele
monica.fedele@cnr.it

CNR: Consiglio Nazionale delle Ricerche https://orcid.org/0000-0002-9171-1312

Research Article

Keywords: PATZ1, FOXA1, luminal differentiation, breast cancer plasticity, fibroblast–tumor crosstalk

Posted Date: October 30th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11402423/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

PATZ1 Restrains Basal-like Breast Cancer Aggressiveness while Preserving FOXA1-Driven Luminal Identity

Sabrina Battista[1,†], Michela Vitiello[1,†], Maria Pia Albanese[1], Cristina Quintavalle[1], Tina Di Palma[1], Mariastella Zannini[1], Gerolama Condorelli[1,2], Elvira Crescenzi[1], Laura Cerchia[1], Monica Fedele[1,]*

[1]Institute of Endotypes in Oncology, Metabolism and Immunology "Gaetano Salvatore", National Research Council, 80131, Naples, Italy;

[2]Department of Molecular Medicine and Medical Biotechnology, "Federico II" University of Naples, Italy

† These authors contributed equally to this work and share first authorship

***Corresponding author:** Monica Fedele, Institute of Endotypes in Oncology, Metabolism and Immunology "Gaetano Salvatore", National Research Council. *E-mail address*: monica.fedele@cnr.it

Acknowledgments: The authors gratefully acknowledge Alba Ribot Casasnovas for her contribution to the experimental work during her Bachelor's thesis project on PATZ1 in breast cancer, and Paola Francesca for her contribution to some of the experiments performed during her thesis work.

Abstract

Breast cancer comprises biologically distinct subtypes characterized by differences in lineage identity, tumor aggressiveness, and therapeutic response. However, the transcriptional mechanisms governing subtype specification and cellular plasticity remain incompletely understood. Here, we identify PATZ1 as a key regulator of luminal identity and phenotypic plasticity in breast cancer.

Integrative analyses of The Cancer Genome Atlas and independent transcriptomic datasets revealed that PATZ1 expression is enriched in luminal breast cancers, positively correlates with FOXA1 expression, and associates with lower tumor grade and improved patient outcome. Mechanistically, chromatin immunoprecipitation, promoter-reporter, and loss-of-function assays demonstrated that FOXA1 is a direct transcriptional target of PATZ1 in luminal breast cancer cells.

Functional studies in the Basal B/triple-negative breast cancer cell line MDA-MB-231 showed that PATZ1 overexpression promotes luminal epithelial reprogramming, characterized by increased KRT8 and EpCAM expression and reduced CD44 levels, while significantly suppressing proliferation, migration, invasion, and anchorage-independent growth. In addition, conditioned medium from *Patz1*-deficient fibroblasts induced transcriptional reprogramming in luminal MCF7 cells, downregulating the luminal epithelial markers CDH1 and KRT8 and, notably, PATZ1 itself, while increasing SOX2 and IL6 expression, consistent with loss of luminal identity and enhanced cellular plasticity.

Collectively, our findings identify PATZ1 as a key regulator of breast cancer cell identity and phenotypic plasticity, integrating tumor-intrinsic control of luminal differentiation with microenvironment-dependent signaling and providing a conceptual framework for understanding- and potentially limiting - the plasticity that fuels breast cancer progression.

Keywords: PATZ1; FOXA1; luminal differentiation; breast cancer plasticity; fibroblast–tumor crosstalk.

Introduction

Breast cancer (BC) is one of the most common female cancers, affecting one in nine women during their lifetimes, accounting for approximately 28% of all female cancers, and the second leading cause of cancer-related deaths in women. Progress has been made to the earlier diagnosis and better treatment during the past few decades, leading to the 5-year survival rates of BC patients at approximately 85%. However, distant metastasis and recurrence still occur and result in poor prognosis. Therefore, there is an urgent need for identifying novel biomarkers that can be used to screen high-risk patients and help predict the progression and prognosis of this cancer [1]. Breast cancer is a highly heterogeneous disease encompassing multiple molecular and histological subtypes that differ in clinical outcome, metastatic propensity, and response to therapy [2]. Gene expression profiling, particularly through the PAM50 classification, distinguishes breast tumors into distinct intrinsic molecular subtypes, including luminal A, luminal B, HER2-enriched, basal-like, and normal-like, each characterized by specific transcriptional programs and lineage determinants. Luminal tumors generally retain features of mammary epithelial differentiation and are associated with a more favorable prognosis, whereas basal-like tumors display less differentiated features and a more aggressive clinical behavior [3,4]. Triple-negative breast cancer (TNBC), although substantially overlapping with the basal-like subtype, represents a clinically defined and molecularly heterogeneous group frequently associated with marked phenotypic plasticity, increased invasiveness, and poorer clinical outcome [3,4]. Understanding the molecular mechanisms that maintain luminal identity or drive transition toward more aggressive states remains a central challenge in breast cancer biology.

Increasing evidence indicates that breast cancer cells can dynamically transition between epithelial/luminal and mesenchymal/basal-like states through processes collectively referred to as cellular plasticity [5]. Lineage-tracing studies have further demonstrated that luminal breast cancer cells can acquire basal-like features during tumor evolution, providing direct evidence of lineage plasticity [6]. This phenotypic flexibility contributes to intratumoral heterogeneity, invasion, metastasis, and therapy resistance. In particular, TNBC is enriched for mesenchymal and stem-like programs and lacks actionable molecular targets, highlighting the need to identify transcriptional regulators capable of limiting malignancy or restoring differentiated states.

Cell fate determination and subtype stability are controlled by lineage-restricted transcription factors that shape chromatin accessibility and transcriptional networks. In luminal breast cancer, the Forkhead box protein A1 (FOXA1) is a master pioneer factor that regulates estrogen receptor signaling, epithelial differentiation, and luminal lineage specification [7-10]. FOXA1 expression is strongly associated with hormone receptor-positive disease and favorable prognosis, whereas its loss has been linked to dedifferentiation, endocrine resistance, and acquisition of more aggressive

phenotypes. Thus, factors controlling FOXA1 expression may play critical roles in preserving luminal features and restraining tumor progression.

The POZ/AT-hook/zinc finger protein 1 (PATZ1), also known as ZNF278 or MAZR, is a multifunctional transcription factor belonging to the POK family, characterized by a BTB/POZ domain, AT-hook DNA-binding motifs, and C2H2 zinc fingers [11]. PATZ1 has been implicated in chromatin remodeling, stemness, development, and transcriptional control through context-dependent interactions with diverse cofactors. Importantly, PATZ1 has emerged as a pleiotropic regulator in cancer, where it may function either as an oncogene or tumor suppressor depending on cellular context and tumor type [12]. In both hematopoietic and solid tumors, altered PATZ1 expression has been associated with changes in proliferation, epithelial-to-mesenchymal transition (EMT), migration, and metastatic behavior [13-19]. However, its role in breast cancer subtype specification and progression remains poorly defined.

In the present study, by combining transcriptomic analyses of The Cancer Genome Atlas (TCGA) and other publicly available breast cancer cohorts, mechanistic studies in luminal breast cancer cells, and functional assays in basal-like models, we show that PATZ1 expression positively associates with luminal gene signatures and FOXA1, while inversely correlates with basal/mesenchymal programs, tumor grade, and poor survival. We further demonstrate that PATZ1 directly binds the FOXA1 promoter in luminal cells and positively regulates FOXA1 promoter activity and gene expression, supporting a role in sustaining luminal differentiation. In parallel, enforced PATZ1 expression in basal-like TNBC cells markedly suppresses proliferation, migration, invasion, and tumorigenic potential. Together, our findings identify PATZ1 as a subtype-dependent regulator of breast cancer plasticity that links lineage maintenance with tumor suppression.

Results

PATZ1 is enriched in luminal breast cancer and associated with lower tumor aggressiveness and improved patient survival

To investigate the clinical and molecular relevance of PATZ1 in breast cancer, we first performed an integrative analysis of multiple publicly available transcriptomic datasets, including TCGA and independent breast cancer cohorts through the R2: Genomics Analysis and Visualization Platform (http://r2.amc.nl) and the Gene expression-based Outcome for Breast cancer Online (GOBO) tool [20]. PATZ1 expression was significantly associated with estrogen receptor (ER) status, showing higher levels in ER-positive tumors compared to ER-negative counterparts (Fig.1a). Consistently, stratification by PAM50 molecular subtype revealed a strong enrichment of PATZ1 expression in luminal A and B, HER2-enriched and normal-like subgroups compared to basal-like cancers (Fig.1b).

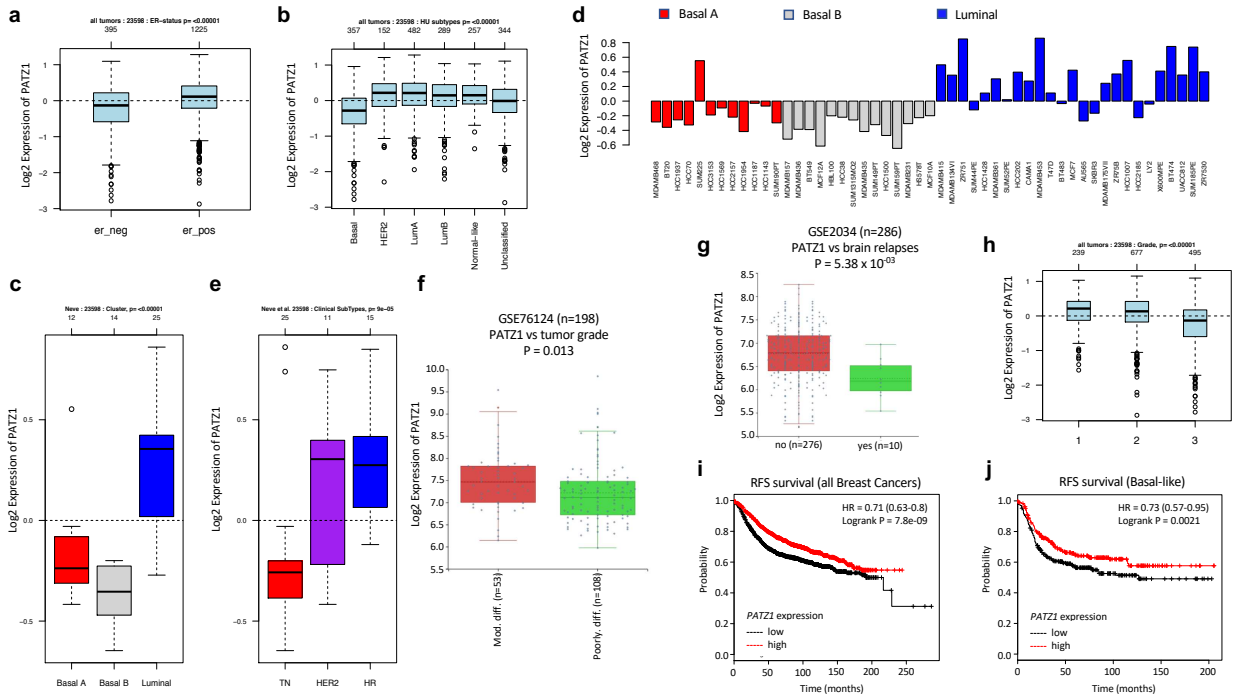


Figure 1

This subtype-specific pattern was further confirmed in independent transcriptomic datasets of BC specimens and cells, where PATZ1 expression was consistently lower in basal-like tumors compared to all other breast cancer subtypes (Supplementary Fig.1a,b). To further determine if PATZ1 correlates with the mammary epithelial/luminal phenotype, also independently from ER status, we looked at PATZ1 expression in breast cancer cell lines previously classified as luminal, basal A or basal B [21]. Basal A cells are more characteristic of human basal-like breast cancers, while basal B cells are mesenchymal and display stem cell features of claudin-low tumors [21]. As shown in Fig. 1c and 1d, PATZ1 expression is significantly higher in luminal compared with basal breast cancer cell lines and is particularly reduced in Basal B/mesenchymal models. Consistently, PATZ1 expression negatively correlates with TNBC, which mainly consists of basal-like tumors, relative to HER2-positive and ER/PR-positive breast cancer subtypes (Fig.1e). However, PATZ1 is also highly expressed in ER-negative luminal cell lines, such as MDA-MB-453 and SUM185PE (Fig.1d), suggesting that, similarly to FOXA1, it correlates more precisely with luminal subtype than ER, and may be independently required for maintaining this phenotype.

Consistent with this association, analysis of single-cell RNA-sequencing data from normal human breast available through the Human Protein Atlas revealed high PATZ1 expression in breast lactating cells and hormone-responsive cells (48 and 27 nCPM, respectively; Supplementary Fig.1c). Thus, the preferential expression of PATZ1 in differentiated and hormone-responsive mammary epithelial populations in normal breast further supports its association with the luminal phenotype observed in breast cancer.

To validate the transcriptomic findings at the protein level, PATZ1 expression was assessed by Western blot analysis in a representative panel of breast cancer cell lines, including two luminal (MCF7 and MDA-MB-453), two Basal A (MDA-MB-468 and BT-20), and two Basal B/mesenchymal models (MDA-MB-231, BT-549). Quantitative densitometric analysis of Western blot signals confirmed a statistically significant downregulation of PATZ1 protein levels from luminal to Basal A/B cell lines (Supplementary Fig.1d,e), further supporting a close association between PATZ1 expression and luminal epithelial differentiation.

Importantly, analysis of tumor grade demonstrated a significant inverse correlation between PATZ1 expression and tumor aggressiveness, with low differentiated, metastatic, and grade 3 tumors displaying the lowest levels of PATZ1 (Fig.1h,i). Finally, survival analysis revealed that elevated PATZ1 expression correlates with improved patient outcome, indicating a potential protective role in breast cancer progression (Fig.1j,k and Supplementary Fig.1f,g). Collectively, these analyses identify PATZ1 as a gene tightly associated with the luminal subtype and inversely correlated to a bad prognosis.

PATZ1 directly regulates FOXA1 expression in luminal breast cancer through promoter binding and transcriptional activation

To further investigate the molecular basis of PATZ1 function in luminal breast cancer, we next explored its relationship with FOXA1, a key lineage-determining transcription factor in this subtype. Integrative analysis of publicly available breast cancer transcriptomic datasets revealed a strong positive correlation between PATZ1 and FOXA1 expression (Fig.2a), suggesting a coordinated regulatory program in luminal tumors.

To determine whether the association between PATZ1 and FOXA1 was supported by direct transcriptional regulation, we first investigated the 5′-flanking genomic region of FOXA1 to identify potential PATZ1-binding sites. *In silico* analysis of the *FOXA1* regulatory region using TraFaC and MatInspector identified a cluster of four closely spaced putative PATZ1/MAZR-binding sites at −176, −162, +110 and +235 bp relative to the TSS (Fig.2b). The two promoter-proximal sites at −176 and −162 bp showed the highest MatInspector matrix similarity scores (0.926 for both) and were independently predicted by both TraFaC and MatInspector, whereas the sites at +110 and +235 bp were identified by MatInspector with scores of 0.901 and 0.888, respectively (Fig.2b).

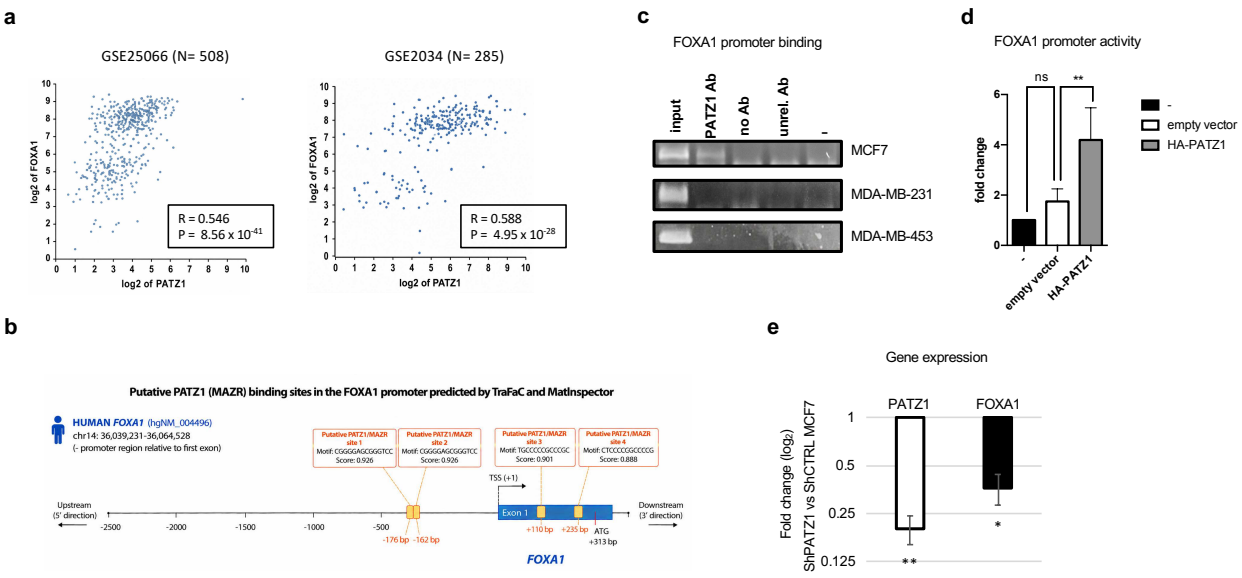


Figure 2

To experimentally validate this prediction, we performed chromatin immunoprecipitation (ChIP) assays in different breast cancer cell lines using a PATZ1 polyclonal antibody. The ChIP results confirmed PATZ1 occupancy of the FOXA1 regulatory region in MCF7 (luminal A/ER+) cells, whereas no enrichment was observed in MDA-MB-231 (Basal B/TNBC) or MDA-MB-453 (Luminal B/ER-) cells (Fig.2c). Because the four predicted sites are located within the same ~500-bp region, corresponding approximately to the size of the sonicated chromatin fragments used for ChIP, these experiments demonstrate PATZ1 recruitment to this regulatory region but do not allow discrimination of binding to individual sites. Nevertheless, the higher prediction scores and independent identification by both algorithms make the −176/−162-bp sites the strongest candidate PATZ1-binding elements within this region.

Notably, PATZ1 and FOXA1 are highly expressed in both MCF7 and MDA-MB-453 cells (Fig.1d and Supplementary Fig.2c), suggesting that PATZ1 occupancy at the FOXA1 promoter is not solely dependent on PATZ1 or FOXA1 abundance, but may require a specific luminal/ER-positive chromatin context.

Consistently, luciferase reporter assays revealed that PATZ1 significantly enhanced FOXA1 promoter activity in MCF7 cells, indicating a direct role in FOXA1 transcriptional regulation (Fig.2d). Furthermore, PATZ1 knockdown in MCF7 cells resulted in a marked reduction of FOXA1 mRNA expression (Fig.2e), demonstrating that endogenous PATZ1 contributes to FOXA1 maintenance in luminal breast cancer cells.

Collectively, these data identify FOXA1 as a direct transcriptional target of PATZ1 and support a model in which PATZ1 contributes to the establishment and maintenance of luminal lineage identity through transcriptional control of a key lineage-specifying factor.

PATZ1 promotes luminal epithelial reprogramming and suppresses malignant traits in TNBC cells

Given the strong association of PATZ1 with luminal breast cancer and the identification of FOXA1 as a direct transcriptional target of PATZ1, we investigated whether PATZ1 could actively influence cell identity in TNBC. To this end, we generated stable HA-tagged PATZ1-overexpressing clones in MDA-MB-231 cells, a highly aggressive Basal B/TNBC model characterized by mesenchymal features and remarkable cellular plasticity (Fig.3a).

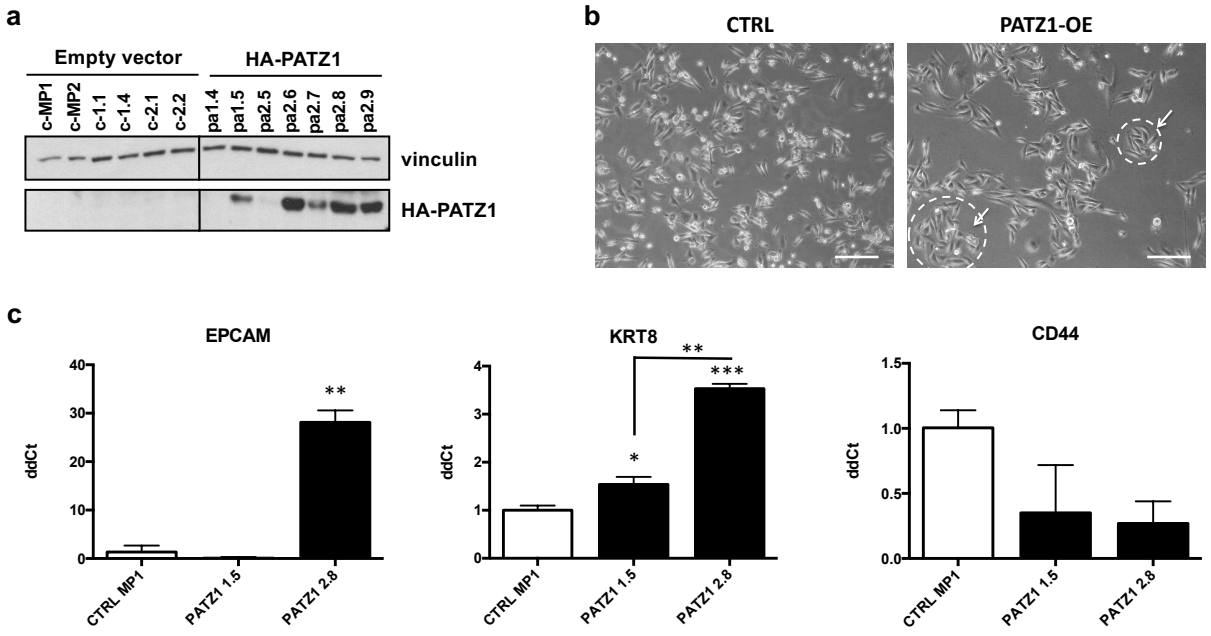


Figure 3

Two PATZ1-overexpressing clones (pa1.5, pa2.8) and a control mass population (c-MP1) were used in the following analyses. Morphological examination revealed that the PATZ1-overexpressing clone pa2.8 displayed increased cell-cell adhesion and a tendency to grow in compact cellular aggregates, in contrast to the scattered growth pattern observed in control cells (Fig.3b). Although the cells retained an overall spindle-shaped appearance, these observations are indicative of a partial transition toward a more epithelial phenotype.

To determine whether PATZ1 could influence lineage commitment in TNBC cells, we examined the expression of markers associated with luminal epithelial differentiation and mesenchymal/stem-like phenotypes. PATZ1 overexpression induced a significant increase in the luminal epithelial markers cytokeratin 8 (KRT8) and EpCAM, while concomitantly reducing the expression of the mesenchymal/stemness associated marker CD44 (Fig.3c). Notably, KRT8 and EpCAM expression increased proportionally to PATZ1 levels across the analyzed clones, indicating a dose-dependent activation of luminal differentiation programs. In contrast, CD44 downregulation was observed in both PATZ1-overexpressing clones irrespective of their relative PATZ1 expression levels. Consistent with these molecular changes, the clone expressing the highest levels of PATZ1 also exhibited the most evident phenotypic alteration (Fig.3b).

Collectively, these findings support a role for PATZ1 in promoting luminal differentiation while restraining mesenchymal/stem-like features, thereby contributing to the regulation of cellular plasticity and lineage specification in TNBC cells.

To assess the biological consequences of this phenotypic reprogramming, we first evaluated cell proliferation (Fig.4a,b). Metabolic viability assays revealed a significant reduction in the growth of PATZ1-overexpressing cells compared with control cells, an effect that became particularly evident 72 hours after plating (Fig.4a).

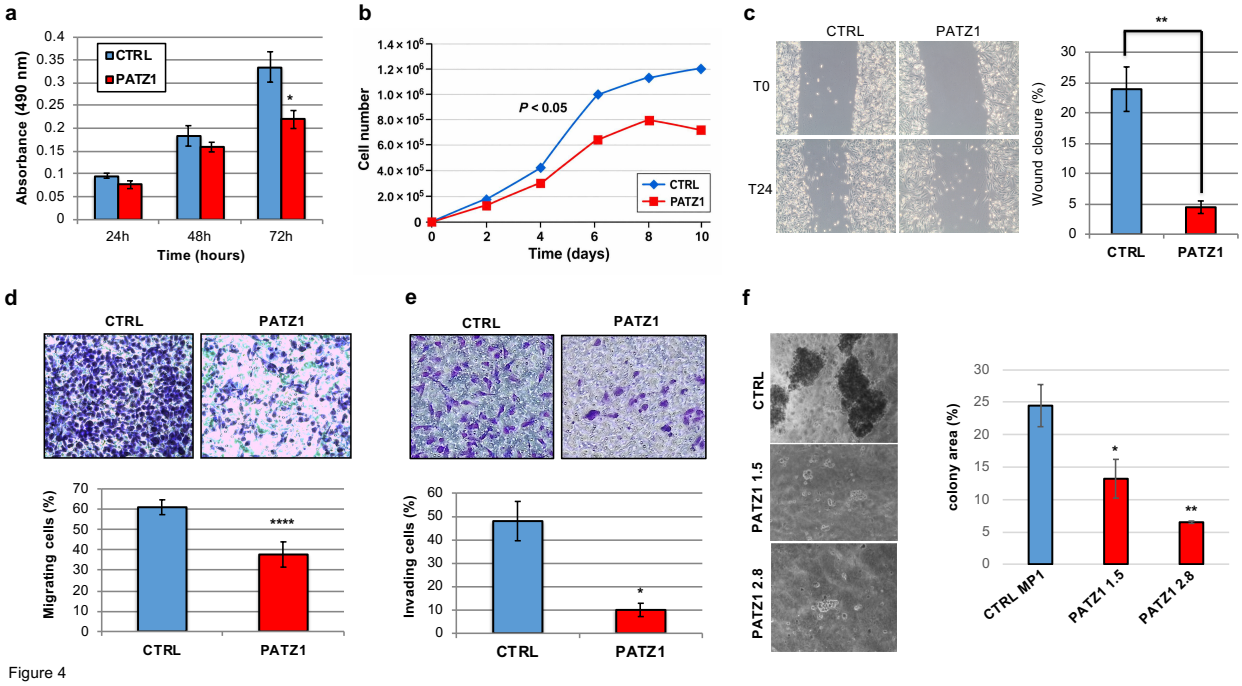


Figure 4

Consistent with these findings, direct cell counting experiments demonstrated a markedly reduced proliferation rate in PATZ1-expressing clones throughout the observation period (Fig.4b). Importantly, Trypan Blue exclusion assays did not reveal significant differences in cell viability between control and PATZ1-overexpressing cells, indicating that the reduced cell numbers primarily reflected impaired proliferative capacity rather than increased cell death.

We next examined whether PATZ1 affected additional hallmarks of TNBC aggressiveness. Both wound-healing and Transwell migration assays demonstrated a marked reduction in the migratory capacity of PATZ1-overexpressing cells compared with controls (Fig.4c,d). Similarly, Matrigel invasion assays revealed a significant impairment of invasive behavior upon PATZ1 expression (Fig.4e). Given the established association of Basal B/claudin-low breast cancer cells with enhanced motility, invasiveness, and metastatic potential, these findings suggest a potential role for PATZ1 in limiting key processes involved in tumor progression.

Finally, we assessed anchorage-independent growth, a hallmark of cellular transformation and tumorigenicity. Whereas control MDA-MB-231 cells efficiently formed large colonies in soft agar, PATZ1-overexpressing cells

exhibited a dramatic reduction in colony formation ability, indicating a profound impairment of tumorigenic potential (Fig.4f).

Collectively, these results demonstrate that PATZ1 exerts potent tumor-suppressive functions in TNBC cells by simultaneously promoting epithelial/luminal differentiation and suppressing multiple malignant traits, including proliferation, migration, invasion, and anchorage-independent growth. These findings support a model in which PATZ1 acts as a key regulator of cellular plasticity in breast cancer, driving TNBC cells away from a mesenchymal/stem-like state toward a more differentiated luminal phenotype.

PATZ1-deficient fibroblasts promote loss of luminal identity and phenotypic plasticity in breast cancer cells

Having demonstrated that PATZ1 promotes luminal differentiation and suppresses malignant traits in TNBC cells, we next investigated whether PATZ1 could also influence breast cancer cell behavior through microenvironment-mediated mechanisms. We recently reported that conditioned medium (CM) derived from *Patz1*-knockout mouse embryonic fibroblasts (MEFs) exerts a pro-tumorigenic effect on MCF7 breast cancer cells, enhancing both cell viability and migratory capacity, thereby suggesting that loss of PATZ1 profoundly alters the fibroblast secretome and generates a tumor-promoting microenvironment [22]. Interestingly, this observation is consistent with our Human Protein Atlas analysis showing that PATZ1 is highly expressed across several glandular and secretory epithelial cell populations, suggesting a previously unrecognized association between PATZ1 and cellular secretory functions (Supplementary Fig.1c).

To gain mechanistic insights into the biological consequences of stromal PATZ1 loss, we analyzed gene expression in MCF7 cells exposed to conditioned medium from *Patz1*-deficient MEFs. RT-qPCR analysis revealed a significant reduction in the luminal/epithelial markers CDH1 (E-cadherin) and KRT8, accompanied by increased expression of SOX2 and IL6 (Fig.5). Consistent with a loss of luminal identity, these changes were also associated with a concomitant downregulation of PATZ1, suggesting that PATZ1-dependent signals released by stromal cells may contribute to maintaining PATZ1 expression and luminal identity in neighboring breast cancer cells. Although not significant induction of analyzed mesenchymal markers, CD44 and FN1 (fibronectin), was observed, the loss of luminal epithelial features, together with the activation of stemness- and inflammation-associated programs, is consistent with the acquisition of a more plastic and potentially aggressive cellular state. These findings suggest that the secretome of *Patz1*-deficient fibroblasts destabilizes luminal identity and promotes early phenotypic reprogramming in breast cancer cells.

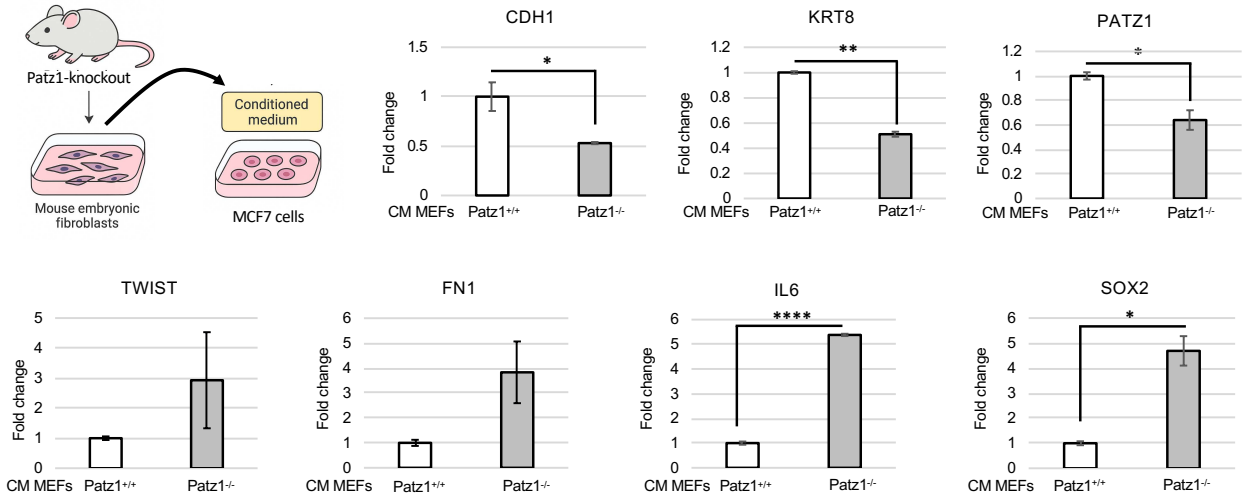


Figure 5

Collectively, these findings demonstrate that PATZ1 regulates breast cancer cell plasticity not only through tumor cell-intrinsic mechanisms but also by shaping the composition of the tumor microenvironment. Loss of PATZ1 in stromal fibroblasts generates a pro-tumorigenic secretome capable of destabilizing luminal identity and promoting phenotypic plasticity, thereby fostering aggressive cellular behaviors in breast cancer cells.

Discussion

In the present study, we identified PATZ1 as a regulator of breast cancer cell identity and phenotypic plasticity. By integrating transcriptomic analyses with functional studies in luminal and basal-like breast cancer models, we show that PATZ1 is preferentially associated with luminal differentiation programs, directly regulates FOXA1 transcription, promotes epithelial lineage features, and suppresses malignant traits in aggressive TNBC cells. Moreover, our findings indicate that PATZ1 may also influence tumor behavior through microenvironment-dependent mechanisms, further supporting its recently recognized role in the regulation of tumor–stroma communication [22].

FOXA1 is widely recognized as a master regulator of luminal lineage specification in breast cancer [9, 23]. As a pioneer transcription factor, FOXA1 establishes chromatin accessibility at luminal regulatory regions and cooperates with ER-dependent and ER-independent transcriptional networks to maintain epithelial differentiation [7]. In this context, the identification of FOXA1 as a direct transcriptional target of PATZ1 provides a potential molecular link between PATZ1 expression and the luminal breast cancer phenotype observed across independent datasets. The positive correlation between the two factors, together with the results of ChIP, promoter-reporter, and silencing experiments, supports a model in which PATZ1 positively regulates FOXA1 transcription and may thereby contribute to the maintenance of luminal epithelial identity.

This finding is consistent with the broader biological functions previously attributed to PATZ1. As a member of the POK (POZ and Krüppel-like zinc finger) family of transcription factors, a group of chromatin-associated regulators known to orchestrate lineage commitment and differentiation programs in multiple tissues [24], PATZ1 has been implicated in embryonic development, stem cell biology, and lineage commitment. Genetic ablation of *Patz1* in mice results in severe developmental defects and impaired tissue differentiation [25, 26], while studies in embryonic stem cells have shown that PATZ1 regulates pluripotency-associated networks and contributes to the balance between self-renewal and differentiation [27, 28]. Together with previous evidence implicating PATZ1 in germ cell development [25], thyroid differentiation and epithelial tumor progression [13, 29, 30], these observations support a unifying view in which PATZ1 acts as a regulator of cell identity and lineage specification. The identification of FOXA1 as a downstream target extends this role to mammary epithelial differentiation and suggests a molecular connection between PATZ1 activity and luminal breast cancer biology.

Within this framework, the phenotypic changes observed following PATZ1 re-expression in MDA-MB-231 are particularly informative. PATZ1 expression in these cells was sufficient to induce a partial shift toward luminal/epithelial features characterized by increased KRT8 and EpCAM expression and reduced CD44 levels. These molecular changes were accompanied by decreased proliferation, migration, invasion, and anchorage-independent growth, indicating that PATZ1 expression is associated with a broad attenuation of the malignant phenotype of mesenchymal TNBC cells. Notably, the clone expressing the highest levels of PATZ1 also displayed the most evident morphological changes, including increased cell–cell adhesion and a more cohesive growth pattern, further supporting the acquisition of epithelial traits. Although these cells did not undergo a complete mesenchymal-to-epithelial transition, the data suggest that PATZ1 promotes a shift toward a more differentiated cellular state.

These observations suggest that, rather than acting exclusively as a conventional tumor suppressor, PATZ1 may influence the cellular state from which aggressive traits emerge, thereby linking lineage specification to the modulation of malignant behavior. Its effects on proliferation, migration and invasion may therefore reflect, at least in part, its broader ability to modulate cellular state and phenotypic plasticity. In support of this concept, Padua et al. demonstrated that UNC5A restricts luminal-to-basal plasticity and metastasis in breast cancer, providing evidence that mechanisms preserving lineage identity can also limit tumor aggressiveness [31].

Our findings are consistent with the emerging concept that maintenance of luminal breast cancer identity requires active transcriptional mechanisms that restrain phenotypic plasticity toward more aggressive basal/mesenchymal states. In this context, Arcuschin et al. recently identified the transcriptional repressor ThPOK/ZBTB7B as a key regulator of luminal breast cancer identity, whose depletion activates ECM-, EMT- and basal-associated transcriptional programs and enhances cell migration, whereas its ectopic expression in TNBC cells promotes a more epithelial, less migratory

phenotype [32]. Similarly, depletion of SNAI1 has been shown to derepress FOXA1 and induce luminal progenitor-like features in invasive TNBC cells [33], while ONECUT2 has been identified as a transcriptional driver of luminal-to-basal plasticity through repression of luminal differentiation programs [34]. Together, these studies support the concept that breast cancer lineage identity is dynamically maintained by interconnected transcriptional networks, whose perturbation can promote phenotypic transitions. Within this framework, our findings identify PATZ1 as an additional regulator of breast cancer identity and, importantly, establish FOXA1 as its direct transcriptional target, providing a distinct molecular mechanism linking PATZ1 activity to the maintenance of luminal differentiation.

Our findings further suggest that PATZ1 regulates breast cancer plasticity through non-cell-autonomous mechanisms as well. We previously demonstrated that conditioned medium from *Patz1*-knockout fibroblasts enhances proliferation and migration of luminal breast cancer cells [22]. Here, we showed that exposure to this conditioned medium destabilizes luminal identity, as evidenced by the downregulation of CDH1, KRT8 and PATZ1 itself. Concomitantly, treated cells exhibited increased expression of SOX2 and IL6, two factors frequently associated with stemness, inflammatory signaling, and cellular plasticity. Although TWIST and FN1 did not reach statistical significance, both genes exhibited a consistent upward trend, suggesting that prolonged exposure to the PATZ1-deficient fibroblast secretome or additional microenvironmental cues may further reinforce mesenchymal-associated programs. In any event, these changes are consistent with the acquisition of a more plastic and potentially aggressive phenotype. Particularly intriguing is the reduction of PATZ1 expression in tumor cells exposed to a PATZ1-deficient stromal secretome. This observation suggests a paracrine regulatory link between stromal and tumor-cell PATZ1 activity and raises the possibility that, under PATZ1-competent conditions, fibroblast-derived signals contribute to maintaining PATZ1 expression and luminal differentiation in neighboring breast cancer cells. Conversely, loss of stromal PATZ1 may disrupt these lineage-supporting signals, thereby reducing tumor-cell PATZ1 expression and further destabilizing the luminal epithelial program. Although the responsible mediators remain to be identified, these findings suggest that stromal PATZ1 loss can influence the transcriptional state of neighboring breast cancer cells, highlighting a potential microenvironment-dependent mechanism of luminal identity destabilization.

Collectively, our findings support a model in which PATZ1 integrates cell-intrinsic and microenvironmental mechanisms to control breast cancer state. At the tumor-cell level, PATZ1 directly promotes FOXA1 transcription and supports luminal epithelial identity, whereas in stromal fibroblasts, PATZ1 appears to shape paracrine signals that favor the maintenance of luminal differentiation in neighboring cells. Loss of PATZ1 at either level may therefore converge on destabilization of the luminal program and increased phenotypic plasticity (Fig.6).

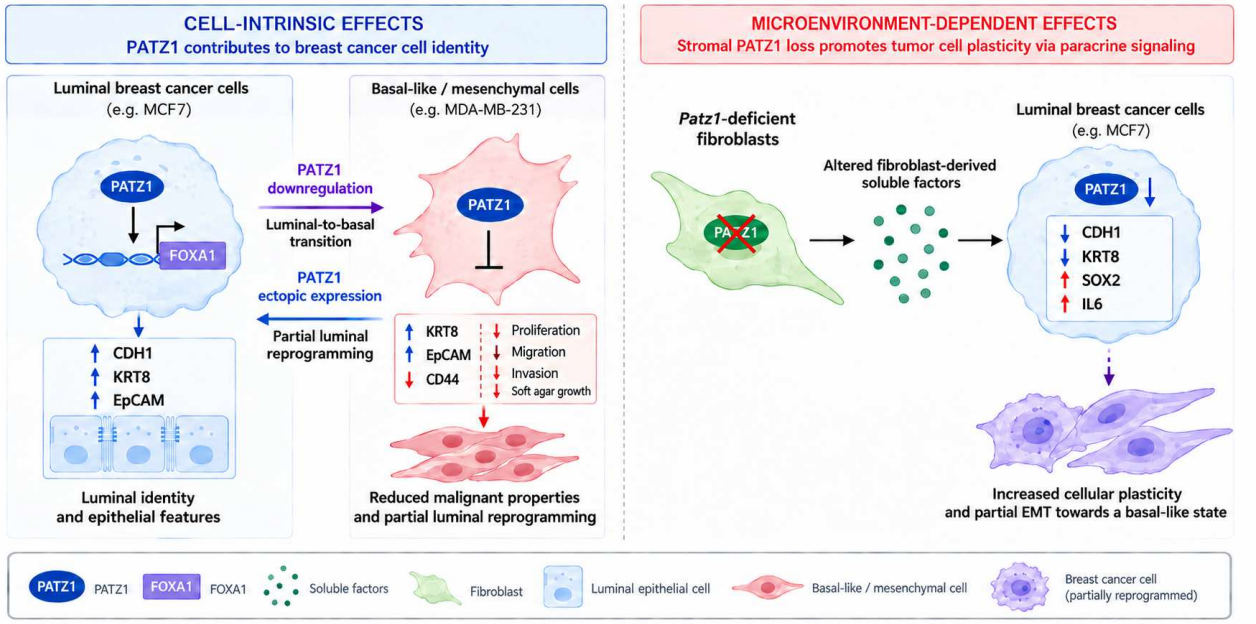


Figure 6

We therefore propose the PATZ1–FOXA1 axis, together with PATZ1-dependent stromal signaling, as previously unrecognized components of the regulatory network governing luminal cell identity in breast cancer. This model provides a conceptual framework for future studies aimed at understanding and potentially limiting tumor plasticity and disease progression.

Materials and methods

Bioinformatic and in silico analyses

Publicly available breast cancer gene expression datasets were interrogated to investigate the association of PATZ1 and FOXA1 expression with breast cancer molecular subtypes, clinicopathological characteristics, and patient outcome. Gene expression analyses were performed using the GOBO (Gene expression-based Outcome for Breast cancer Online) platform (http://co.bmc.lu.se/gobo), version 1.0.3, which integrates gene expression profiles from multiple publicly available breast cancer datasets, including 1881 patients from 11 distinct studies and 51 breast cancer cell lines [21,35]. Additional analyses were carried out using the R2: Genomics Analysis and Visualization Platform (https://r2.amc.nl), for subtype-specific gene expression analyses and Kaplan–Meier survival analyses, using the following datasets from Gene Expression Omnibus (GEO): GSE76124, including 198 TNBC tumors; GSE2034 [36], representing 180 lymph-node negative relapse free patients and 106 lymph-node negate patients that developed a distant metastasis; GSE60031, referring to a cohort of 124 breast cancer tumors from UC San Francisco and the California Pacific Medical Center collected between 1989 and 1997 [37]; GSE1456, representing a cohort of 159 tumors from breast cancer patients receiving surgery at Karolinska Hospital from 1994-1996 [38]; GSE25066, including 508 breast cancer samples from

Prospective multicenter study conducted from June 2000 to March 2010 at the M. D. Anderson Cancer Center [39]; GSE29271, including 210 primary tumors from breast cancer patients with known site of relapse. Event-free survival (EFS) and relapse-free survival (RFS) were analyzed using the Kaplan scan algorithm implemented in the R2 platform. Furthermore, relapse-free survival analyses were independently validated using the Kaplan–Meier Plotter online tool, (http://kmplot.com) that uses more than 25 BC gene expression databases to evaluate BC prognosis [40].

Single-cell RNA expression of PATZ1 in normal human tissues was investigated using the Human Protein Atlas (HPA) Single Cell resource (https://www.proteinatlas.org/ENSG00000100105-PATZ1/single+cell/breast). PATZ1 expression across cell populations of normal human breast tissue was examined using the HPA Single Cell Type dataset, in which publicly available single-cell RNA-sequencing datasets from healthy human tissues are integrated and re-analyzed through a common processing pipeline [41]. Expression levels were reported as normalized counts per million (nCPM), according to the HPA data processing pipeline. PATZ1 expression across cell populations from other normal human tissues was also examined to assess its distribution among glandular and secretory epithelial cell populations.

Prediction of PATZ1-binding sites within the human FOXA1 promoter was performed using the TraFaC web server (https://trafac.cchmc.org), which predicts transcription factor binding sites using TRANSFAC-derived position weight matrices [42] and MatInspector (Genomatix Software GmbH, Munich, Germany - http://www.genomatix.de). MatInspector identifies potential transcription factor-binding sites based on position weight matrices and matrix families, using optimized matrix similarity thresholds to minimize false-positive matches [43]. Candidate binding sites identified in silico were subsequently validated by chromatin immunoprecipitation (ChIP).

Cell culture, generation of stable PATZ1-overexpressing cells, and transient PATZ1-silencing

Human breast cancer cell lines MDA-MB-231, MDA-MB-453, MDA-MB-468, BT-20, and MCF7 were obtained from the American Type Culture Collection (ATCC). MDA-MB-231 cells were cultured in RPMI-1640 medium, whereas MDA-MB-453, MDA-MB-468, BT-20, and MCF7 were maintained in Dulbecco's modified Eagle's medium (DMEM) (Gibco by Invitrogen, Carlsbad, CA). Culture media were supplemented with 10% fetal bovine serum (FBS), except for BT-20, which was maintained in medium containing 20% FBS. All media were additionally supplemented with 1% glutamine and 1% penicillin/streptomycin. Cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. To generate stable PATZ1-overexpressing basal-like cells, MDA-MB-231 were transfected with an expression vector encoding HA-tagged human PATZ1 or the corresponding pCEFL-HA empty vector, as previously described [44]. Stable transfectants were selected with G418 (3 μg/ml). Individual PATZ1-expressing and control clones were isolated and expanded, whereas the remaining resistant cells were maintained as mass populations. Two independent PATZ1-

overexpressing clones displaying different levels of PATZ1 expression (pa1.5 and pa2.8) were selected for subsequent experiments. Corresponding mass population obtained following transfection with the empty vector was used as control. For transient PATZ1-silencing of luminal cells, MCF7 were transfected with human PATZ1-specific shRNA constructs or the corresponding empty vector (KH08765P; Qiagen, Milano, Italy). Cells were collected at 24h after transfection and used for subsequent analysis. PATZ1 overexpression or silencing was verified by RT-PCR and/or Western blot analysis, as detailed below. Cell morphology was examined by phase-contrast microscopy, and representative images were acquired at 10x magnification using a light microscope.

Chromatin immunoprecipitation (ChIP) assay

Chromatin immunoprecipitation (ChIP) was performed as previously described [45]. Briefly, precleared chromatin obtained from breast cancer cell lines was incubated with 1 μg of affinity-purified rabbit polyclonal anti-PATZ1 antibody [44] or with a polyclonal anti-thyroglobulin antibody [46], kindly provided by Prof. Roberto Di Lauro, used as an unrelated antibody control. Samples were rotated at 4 °C for 16 h, and the immunoprecipitated DNA was subsequently analyzed by PCR using primers encompassing the *FOXA1* regulatory region containing the predicted PATZ1/MAZR-binding sites 1 and 2: Forward, 5′-CCCATTTCCCTCTTTCCCTA-3′, and Reverse, 5′-ACCTACAAAGCCCGAGGTG-3′.

Luciferase reporter assay

MCF7 cells (1 x 10^4) were plated in black 96-well (Corning 3603) in triplicate and transiently co-transfected with 50 ng of the pGL3-Basic-hFOXA1 promoter reporter, kindly provided by Dr. Yixiang Xu (Jianming Xu laboratory, Baylor College of Medicine, Houston, TX, USA) [47], 50 ng of the HA-PATZ1 expression vector, and 5 ng of the pRL-TK Renilla luciferase control vector (Promega), using Lipofectamine 3000 (Invitrogen) according to manufacturer's instructions. Control cells were co-transfected with the corresponding pCEFL-HA empty vector in place of HA-PATZ1. At 24 h post-transfection, cells were lysed and Firefly and Renilla luciferase activities were measured using the Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer's instructions. Luminescence was measured using an M PLEX Tecan instrument. Firefly luciferase activity was normalized to Renilla luciferase activity to account for differences in transfection efficiency. Normalized luciferase activity was expressed as fold change relative to cells transfected with the pCEFL-HA empty vector.

Conditioned medium treatment

Primary MEFs were isolated from 12.5-day-old embryos obtained from timed pregnancies of *Patz1*+/- mice, as previously described [26]. Cryopreserved MEFs were thawed and cultured at passage 3 in DMEM (Life Technologies) supplemented with 10% FBS (Hyclone, Erembodegem, Belgium). For conditioned medium (CM) preparation, *Patz1*+/+ and *Patz1*−/−

MEF cultures were washed with serum-free medium and subsequently incubated for 48 h in medium supplemented with 1% FBS. CM was then collected, filtered through sterile 0.2-µm Millipore membranes, and stored at -80°C until use, as previously described [48]. The volume of CM collected from each culture was normalized to the corresponding cell number. For CM treatment, MCF7 cells were seeded in 100-mm culture dishes at a density of 2 x 10^6 cells/dish. After 16 h, the culture medium was replaced with CM derived from either *Patz1*+/+ or *Patz1*−/− MEFs. Following 48 h of treatment, MCF7 cells were harvested for total RNA extraction and subsequent gene expression analysis. Each experimental condition was analyzed in three technical replicates.

RNA extraction and quantitative RT-PCR

Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. One microgram of total RNA was reverse-transcribed into cDNA using the SensiFAST™ cDNA Synthesis Kit (Bioline, London, UK) according to the manufacturer's instructions. Quantitative real-time PCR (RT-qPCR) was performed using SYBR Green Master Mix (Biorad, Segrate, Italy) on a LightCycler 480 system (Roche, Monza, Italy). The amplification protocol consisted of an initial denaturation step at 95°C for 3 min, followed by 45 cycles of denaturation at 95°C for 10 s, annealing at 60°C for 20 s, and extension at 72°C for 60 s. A melting curve analysis was performed at the end of each run to verify the specificity of amplification.

Relative gene expression was calculated using the 2^−ΔΔCt method, with ACTB (ß-actin) as the endogenous reference gene. Primer sequences used for RT-qPCR analyses are listed in Supplementary Table 1.

Western blot analysis

Cells were lysed in buffer containing 1% Nonidet P-40, 1 mmol/liter EDTA, 50 mmol/liter Tris-HCl (pH 7.5), and 150 mmol/liter NaCl supplemented with complete protease and phosphatase inhibitors (Roche). Protein concentration was determined using the Bradford assay, and equal amounts of total protein (30µg) were separated by SDS-PAGE and transferred onto PVDF membranes. Membranes were blocked in 5% non-fat dry milk in TBS containing 0.1% Tween-20 (TBS-T) for 1 h at room temperature and subsequently incubated overnight at 4 °C with the appropriate primary antibodies. After washing with TBS-T, membranes were incubated with the appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies for 1 h at room temperature. Immunoreactive proteins were detected using an enhanced chemiluminescence (ECL) detection system and acquired using autoradiography. PATZ1 expression was detected using our previously characterized anti-PATZ1 polyclonal antibody able to recognize all isoforms of human and mouse protein [44] and/or anti-HA antibody, Santa Cruz, (sc-805, Santa Cruz Biotechnology), as appropriate. Vinculin (sc-7649, Santa Cruz Biotechnology) was used as loading control. Where indicated, band intensities were quantified by densitometric analysis using ImageJ software and normalized to the corresponding loading control.

Cell proliferation and viability assays

Cell proliferation and viability were evaluated by direct cell counting and the CellTiter 96 AQueous One Solution Assay (Promega Italia, Milan, Italy), respectively, according to manufacturer's instructions.

Briefly, control and PATZ1-overexpressing MDA-MB-231 cells were seeded in triplicate into 96-well plates at a density of $5x10^3$ cells/well. At the indicated time points, reagent solution was added and cells were incubated at 37°C for 2h in a humidified, 5% CO_2 atmosphere. Absorbance was measured at 490 nm using a microplate reader. Results were expressed as mean +/- SEM of two mass populations of control cells and two PATZ1-overexpressing clones in three independent experiments.

For growth curves, cells were seeded into 6-well plate at $4x10^4$ cells/well and viable cells were counted at the indicated time points up to 10 days through the Bürker chamber. Cell viability was assessed by Trypan Blue exclusion.

Cell migration assays

Cell migration was evaluated using wound-healing and Transwell assays. For wound-healing assays, control and PATZ1-overexpressing MDA-MB-231 cells were grown to confluence and a linear scratch was generated using a sterile pipette tip. After removal of detached cells, cultures were maintained in medium without serum, and images of the wound area were acquired at 0h and 24h. Wound closure was quantified using ImageJ and expressed as percentage wound closure/migrated area in three independent experiments.

For Transwell migration assays, 3-5 x 10^4 cells were seeded in serum-free medium in the upper chamber of Transwell inserts with 8-μm pores. The lower chamber contained medium supplemented with 10% FBS as chemoattractant. After 24h, non-migrated cells were removed from the upper surface of the membrane, whereas cells that had migrated to the lower surface were fixed and stained with crystal violet. Migrated cells were quantified using ImageJ in 10–11 independent microscopic fields per condition and expressed as the percentage of migrated cells/field of view.

Matrigel invasion assay

Cell invasion was assessed using Transwell inserts coated with Matrigel (BD Bioscience, Heidelberg, Germany) diluted 1:4 in cold serum-free medium. 3-5 x 10^4 control or PATZ1-overexpressing cells were seeded in serum-free medium in the upper chamber, whereas medium containing 10% FBS was added to the lower chamber as chemoattractant. After 48h, cells remaining on the upper surface were removed, and invading cells on the lower surface of the membrane were fixed and stained with 0.1% crystal violet in 20% methanol. Invading cells were quantified in ten microscopic fields using ImageJ and expressed as invaded cells/field of view.

Soft agar colony formation assay

Anchorage-independent growth was evaluated by soft agar colony formation assay. A base layer consisting of 1% agar in complete culture medium was prepared in 100mm dishes and overlaid with 1 x 10^5 control or PATZ1-overexpressing MDA-MB-231 cells suspended in 0.1% agar-containing medium. Cultures were maintained for 4 weeks, with fresh medium added twice a week. Colonies were subsequently stained with 0.01% crystal violet 10% EtOH and imaged. Colony growth was quantified using ImageJ by measuring colony area in 5–10 independent fields of view for each cell population.

Statistical analysis

Data are presented as mean ± SEM of the indicated number of independent experiments or microscopic fields, as specified in the corresponding figure legends. Statistical significance between two groups was assessed using unpaired Student's *t*-test. A P value < 0.05 was considered statistically significant.

Abbreviations

ATCC, American Type Culture Collection;
BC, breast cancer;
ChIP, chromatin immunoprecipitation;
CM, conditioned medium;
DMEM, Dulbecco's modified Eagle's medium;
ECM, extracellular matrix;
EFS, event-free survival;
EMT, epithelial-to-mesenchymal transition;
ER, estrogen receptor;
FBS, fetal bovine serum;
FOXA1, Forkhead box protein A1;
GEO, Gene Expression Omnibus;
GOBO, Gene expression-based Outcome for Breast cancer Online;
HA, hemagglutinin;
HPA, Human Protein Atlas;
MEFs, mouse embryonic fibroblasts;
MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium;
nCPM, normalized counts per million;
PAM50, Prediction Analysis of Microarray 50;
PATZ1, POZ/AT-hook/zinc finger protein 1;
POK, POZ and Krüppel;
PR, progesterone receptor;
PVDF, polyvinylidene difluoride;
RFS, relapse-free survival;
RPMI, Roswell Park Memorial Institute;

RT-qPCR, reverse transcription quantitative polymerase chain reaction;
SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis;
SEM, standard error of the mean;
TCGA, The Cancer Genome Atlas;
TNBC, triple-negative breast cancer;
TSS, transcription start site.

Statements & Declarations

Conflict of Interest Statement

The authors have no relevant financial or non-financial interests to disclose

Ethics approval

No animals were specifically generated or used for the present study. Experiments were performed using previously established and cryopreserved primary MEFs derived from *Patz1*+/+ and *Patz1*−/− embryos. The original animal studies were conducted at the Istituto Nazionale Tumori IRCCS "Fondazione G. Pascale", Naples, Italy, in accordance with Italian regulations on animal experimentation and were approved by the Italian Ministry of Health (protocol no. 576/10, approved on February 3, 2011), as previously reported [49].

Funding

No specific funding was received for this study. The work was supported by research funds available to the participating laboratories through grants awarded for related research activities: CNR project NUTRAGE and European Union–NextGeneration EU PRIN 2022 (202279JKW5) to M.F.; Fondazione AIRC IG 2019 (ID 23052) to L.C; AIRC IG 2025 (ID 32489) and Horizon Europe Project MSCA-DN CanDoIt (ID 101120186) to G.C.; AIRC IG-SIS 2026 (ID 33805) to C.Q.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by S.B., M.V., M.P.A., C.Q. T.D.P., S.Z., and M.F. The first draft of the manuscript was written by M.F., and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability Statement

The data generated and/or analyzed during the current study are included in this published article and its supplementary information files or are available from the corresponding author upon reasonable request. Publicly available datasets analyzed in this study are accessible through the databases and resources indicated in the Materials and Methods section.

References

1. Xu H, Tian Y, Yuan X, Liu Y, Wu H, Liu Q, Wu GS, Wu K (2016) Enrichment of CD44 in basal-type breast cancer correlates with EMT, cancer stem cell gene profile, and prognosis. Onco Targets Ther 9:431-44. https://doi.org/10.2147/OTT.S97192
2. Mardamshina M, Karagach S, Mohan V, et al. (2025) Integrated spatial proteomic analysis of breast cancer heterogeneity unravels cancer cell phenotypic plasticity. Nat Comm 16:10482. https://doi.org/10.1038/s41467-025-65477-6
3. Tsang JYS and Tse GM (2020) Molecular Classification of Breast Cancer. Jan 2020 Adv Anat Pathol 27:27-35. https://doi.org/10.1097/PAP.0000000000000232
4. Lehmann BD, Jovanović B, Chen X, Estrada MV, Johnson KN, Shyr Y, Moses HL, Sanders ME, Pietenpol JA (2016) Refinement of Triple-Negative Breast Cancer Molecular Subtypes: Implications for Neoadjuvant Chemotherapy Selection. PLoS One 11:e0157368. https://doi.org/10.1371/journal.pone.0157368
5. Fedele M, Cerchia L, Battista S (2024) Subtype Transdifferentiation in Human Cancer: The Power of Tissue Plasticity in Tumor Progression. Cells 13:350. https://doi.org/10.3390/cells13040350
6. Mohamed GA, Mahmood S, Ognjenovic NB, Lee MK, Wilkins OM, Christensen BC, Muller KE, Pattabiraman DR (2023) Lineage plasticity enables low-ER luminal tumors to evolve and gain basal-like traits. Breast Cancer Res 25:23. https://doi.org/10.1186/s13058-023-01621-8.
7. Hurtado A, Holmes KA, Ross-Innes CS, Schmidt D, Carroll JS (2010) FOXA1 is a key determinant of estrogen receptor function and endocrine response. Nat Genet 43:27-33. https://doi.org/10.1038/ng.730
8. Bernardo GM, Bebek G, Ginther CL, Sizemore ST, Lozada KL, Miedler JD, Anderson LA, Godwin AK, Abdul-Karim FW, Slamon DJ, Keri RA (2013) FOXA1 represses the molecular phenotype of basal breast cancer cells. Oncogene 32:554-563. https://doi.org/10.1038/onc.2012.62
9. Tachi K, Shiraishi A, Bando H, Yamashita T, Tsuboi I, Kato T, Hara H, Ohneda O (2016) FOXA1 expression affects the proliferation activity of luminal breast cancer stem cell populations. Cancer Sci. 107:281-289. https://doi.org/10.1111/cas.12870
10. Anzai E, Hirata K, Shibazaki M, Yamada C, Morii M, Honda T, Yamaguchi N, Yamaguchi N (2017) FOXA1 Induces E-Cadherin Expression at the Protein Level via Suppression of Slug in Epithelial Breast Cancer Cells. Biol Pharm Bull 40:1483-1489. https://doi.org/10.1248/bpb.b17-00307
11. Fedele M, Benvenuto G, Pero R, et al. (2000) A novel member of the BTB/POZ family, PATZ, associates with the RNF4 RING finger protein and acts as a transcriptional repressor. J Biol Chem 275:7894-7901. https://doi.org/10.1074/jbc.275.11.7894
12. Fedele M, Crescenzi E, Cerchia L (2017) The POZ/BTB and AT-Hook Containing Zinc Finger 1 (PATZ1) Transcription Regulator: Physiological Functions and Disease Involvement. Int J Mol Sci 18:2524. https://doi.org/10.3390/ijms18122524
13. Chiappetta G, Valentino T, Vitiello M, et al. (2015) PATZ1 acts as a tumor suppressor in thyroid cancer via targeting p53-dependent genes involved in EMT and cell migration. Oncotarget 6:5310-5323. https://doi.org/10.18632/oncotarget.2776
14. Franco R, Scognamiglio G, Valentino E, et al. (2016) PATZ1 expression correlates positively with BAX and negatively with BCL6 and survival in human diffuse large B cell lymphomas. Oncotarget 7:59158-59172. https://doi.org/10.18632/oncotarget.10993
15. Guadagno E, Vitiello M, Francesca P, et al. (2017) PATZ1 is a new prognostic marker of glioblastoma associated with the stem-like phenotype and enriched in the proneural subtype. Oncotarget 8:59282-59300. https://doi.org/10.18632/oncotarget.19546
16. Lucà S, Franco R, Napolitano A, et al. (2023) PATZ1 in Non-Small Cell Lung Cancer: A New Biomarker That Negatively Correlates with PD-L1 Expression and Suppresses the Malignant Phenotype. Cancers (Basel) 15:2190. https://doi.org/10.3390/cancers15072190
17. Pan T, Li J, Zhang O, Zhu Y, Zhou H, Ma M, Yu Y, Lyu J, Chen Y, Xu L (2024) Knockdown of ribosome RNA processing protein 15 suppresses migration of hepatocellular carcinoma through inhibiting PATZ1-associated LAMC2/FAK pathway. BMC Cancer 24:334. https://doi.org/10.1186/s12885-024-12065-4
18. Chen L, Tang J, Chang Y, Hang D, Ji J, Chen G (2025) SMURF1 leads to the beta-catenin signaling-mediated progression of esophageal squamous carcinoma by losing PATZ1-induced CCNG2 transcription. Biochem Pharmacol 232:116688. https://doi.org/10.1016/j.bcp.2024.116688
19. Deng C, Cai S, Guo C, Khan S, Liu L, Tian Q, Ma Z, Zhang J, Zhao L (2026) Dysregulation of the PATZ1/CTCF Balance Silences ZBTB20 to Drive Melanoma Progression. Adv Sci (Weinh) 13:e20917. https://doi.org/10.1002/advs.202520917
20. Ringnér M, Fredlund E, Häkkinen J, Borg Å, Staaf J (2011) GOBO: gene expression-based outcome for breast cancer online. PLoS One 6:e17911. https://doi.org/10.1371/journal.pone.0017911
21. Neve RM, Chin K, Fridlyand J, et al. (2006) A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes. Cancer Cell 10:515-27. https://doi.org/10.1016/j.ccr.2006.10.008

22. De Luca G, Petrillo G, Scognamiglio I, et al. (2025) Fibroblasts activated by miRs-185-5p, miR-652-5p, and miR-1246 shape the tumor microenvironment in triple-negative breast cancer via PATZ1 downregulation. Cell Mol Life Sci 82:287. https://doi.org/10.1007/s00018-025-05781-y
23. Yamaguchi N, Nakayama Y, Yamaguchi N (2017) Down-regulation of Forkhead box protein A1 (FOXA1) leads to cancer stem cell-like properties in tamoxifen-resistant breast cancer cells through induction of interleukin-6. J Biol Chem 292:8136-8148. https://doi.org/10.1074/jbc.M116.763276
24. Costoya JA (2007) Functional analysis of the role of POK transcriptional repressors. Brief Funct Genomic Proteomic 6:8-18. https://doi.org/10.1093/bfgp/elm002
25. Fedele M, Franco R, Salvatore G, Paronetto MP, Barbagallo F, Pero R, Chiariotti L, Sette C, Tramontano D, Chieffi G, Fusco A, Chieffi P (2008) PATZ1 gene has a critical role in the spermatogenesis and testicular tumours. J Pathol 215:39-47. https://doi.org/10.1002/path.2323
26. Valentino T, Palmieri D, Vitiello M, Simeone A, Palma G, Arra C, Chieffi P, Chiariotti L, Fusco A, Fedele M (2013) Embryonic defects and growth alteration in mice with homozygous disruption of the Patz1 gene. J Cell Physiol 228:646-653. https://doi.org/10.1002/jcp.24174
27. Ow JR, Ma H, Jean A, Goh Z, Lee YH, Chong YM, Soong R, Fu XY, Yang H, Wu Q (2014) Patz1 regulates embryonic stem cell identity. Stem Cells Dev 23:1062-1073. https://doi.org/10.1089/scd.2013.0430.
28. Ma H, Ow JR, Tan BC, Goh Z, Feng B, Loh YH, Fedele M, Li H, Wu Q (2014) The dosage of Patz1 modulates reprogramming process. Sci Rep 4:7519. https://doi.org/10.1038/srep07519
29. Iesato A, Nakamura T, Izumi H, Uehara T, Ito KI (2017) PATZ1 knockdown enhances malignant phenotype in thyroid epithelial follicular cells and thyroid cancer cells. Oncotarget 8:82754-82772. https://doi.org/10.18632/oncotarget.19787
30. Vitiello M, Palma G, Monaco M, Bello AM, Camorani S, Francesca P, Rea D, Barbieri A, Chiappetta G, Vita G, Cerchia L, Arra C, Fedele M (2019) Dual Oncogenic/Anti-Oncogenic Role of PATZ1 in FRTL5 Rat Thyroid Cells Transformed by the *Ha-Ras*^{V12} Oncogene. Genes (Basel). 10:127. https://doi.org/10.3390/genes10020127
31. Padua MB, Bhat-Nakshatri P, Anjanappa M, et al. (2018) Dependence receptor UNC5A restricts luminal to basal breast cancer plasticity and metastasis. Breast Cancer Res 20:35. https://doi.org/10.1186/s13058-018-0963-5.
32. Arcuschin CD, Kahrizi K, Sayaman RW, et al. (2025) Super-enhancer profiling reveals ThPOK/ZBTB7B, a CD4(+) cell lineage commitment factor, as a master regulator that restricts breast cancer cells to a luminal non-migratory phenotype. Cell Mol Life Sci 82:397. https://doi.org/10.1007/s00018-025-05913-4
33. Tsirigoti C, Ali MM, Maturi V, Heldin CH, Moustakas A (2022) Loss of SNAI1 induces cellular plasticity in invasive triple-negative breast cancer cells. Cell Death Dis 13:832. https://doi.org/10.1038/s41419-022-05280-z.
34. Zamora I, Gutiérrez M, Pascual A, Pajares MJ, Barajas M, Perez LM, You S, Knudsen BS, Freeman MR, Encío IJ, Rotinen M (2025) ONECUT2 is a druggable driver of luminal to basal breast cancer plasticity. Cell Oncol (Dordr) 48:83-99. https://doi.org/10.1007/s13402-024-00957-3.
35. Markus Ringnér, Erik Fredlund, Jari Häkkinen, Åke Borg, Johan Staaf (2011). GOBO: Gene expression-based Outcome for Breast cancer Online. *PLoS One* 6:e17911. https://doi.org/10.1371/journal.pone.0017911
36. Wang Y, Klijn JG, Zhang Y, Sieuwerts AM, Look MP, Yang F, Talantov D, Timmermans M, Meijer-van Gelder ME, Yu J, Jatkoe T, Berns EM, Atkins D, Foekens JA (2005) Gene-expression profiles to predict distant metastasis of lymph-node-negative primary breast cancer. Lancet 365:671-679. https://doi.org/10.1016/S0140-6736(05)17947-1
37. Chin K, DeVries S, Fridlyand J, et al. (2006) Genomic and transcriptional aberrations linked to breast cancer pathophysiologies. Cancer Cell 10:529-541. https://doi.org/10.1016/j.ccr.2006.10.009
38. Pawitan Y, Bjöhle J, Amler L, et al. (2005) Gene expression profiling spares early breast cancer patients from adjuvant therapy: derived and validated in two population-based cohorts. Breast Cancer Res 7:R953-64. https://doi.org/10.1186/bcr1325
39. Hatzis C, Pusztai L, Valero V, et al. (2011) A genomic predictor of response and survival following taxane-anthracycline chemotherapy for invasive breast cancer. JAMA 305:1873-1881. https://doi.org/10.1001/jama.2011.593
40. Gyorffy B (2026) Pharmacologically actionable transcriptomic signatures across immune infiltration subtypes in the KM-plotter breast cancer cohort. Adv Transl Res 1:193-204. https://doi.org/10.1556/1661.2026.00008
41. Karlsson M, Zhang C, Méar L, et al. 2021 A single-cell type transcriptomics map of human tissues. Sci Adv 7:eabh2169. https://doi.org/10.1126/sciadv.abh2169
42. Wingender E, Chen X, Hehl R, Karas H, Liebich I, Matys V, Meinhardt T, Prüss M, Reuter I, Schacherer F (2000) TRANSFAC: an integrated system for gene expression regulation. Nucleic Acids Res 28:316-319. https://doi.org/10.1093/nar/28.1.316
43. Cartharius K, Frech K, Grote K, Klocke B, Haltmeier M, Klingenhoff A, Frisch M, Bayerlein M, Werner T (2005) MatInspector and beyond: promoter analysis based on transcription factor binding sites. Bioinformatics 21:2933–2942. https://doi.org/10.1093/bioinformatics/bti473
44. Valentino T, Palmieri D, Vitiello M, Pierantoni GM, Fusco A, Fedele M (2013) PATZ1 interacts with p53 and regulates expression of p53-target genes enhancing apoptosis or cell survival based on the cellular context. Cell Death Dis 4:e963. https://doi.org/10.1038/cddis.2013.500

45. Di Palma T, Conti A, de Cristofaro T, Scala S, Nitsch L, Zannini M (2011) Identification of novel Pax8 targets in FRTL-5 thyroid cells by gene silencing and expression microarray analysis. PLoS One 6:e25162. https://doi.org/10.1371/journal.pone.0025162
46. D'Andrea B, Iacone R, Di Palma T, Nitsch R, Baratta MG, Nitsch L, Di Lauro R, Zannini M (2006) Functional inactivation of the transcription factor Pax8 through oligomerization chain reaction. Mol Endocrinol 20:1810-1824. https://doi.org/10.1210/me.2005-0463
47. Xu Y, Qin L, Sun T, Wu H, He T, Yang Z, Mo Q, Liao L, Xu J (2017) Twist1 promotes breast cancer invasion and metastasis by silencing Foxa1 expression. Oncogene 36:1157-1166. https://doi.org/10.1038/onc.2016.286
48. Camorani S, Cerchia L, Fedele M, Erba E, D'Incalci M, Crescenzi E (2018) Trabectedin modulates the senescence-associated secretory phenotype and promotes cell death in senescent tumor cells by targeting NF-κB. Oncotarget. 9:19929-19944. https://doi.org/10.18632/oncotarget.24961
49. Mancinelli S, Vitiello M, Donnini M, Mantile F, Palma G, Luciano A, Arra C, Cerchia L, Liguori GL, Fedele M (2021) The Transcription Regulator Patz1 Is Essential for Neural Stem Cell Maintenance and Proliferation. Front Cell Dev Biol 9:657149. https://doi.org/10.3389/fcell.2021.657149

Figure legends

Fig. 1 PATZ1 expression is associated with luminal breast cancer identity and favorable clinical features. Gene expression analyses were performed using the GOBO breast cancer meta-cohort unless otherwise indicated. **(a)** PATZ1 mRNA expression according to estrogen receptor (ER) status in breast tumors (ER-negative, $n = 395$; ER-positive, $n = 1225$), showing significantly higher PATZ1 expression in ER-positive tumors. **(b)** PATZ1 expression across intrinsic molecular subtypes of breast cancer (Basal-like, $n = 357$; HER2-enriched, $n = 152$; Luminal A, $n = 482$; Luminal B, $n = 289$; Normal-like, $n = 257$; Unclassified, $n = 344$), showing preferential expression in luminal tumors. **(c)** PATZ1 expression in the Neve breast cancer cell line panel according to molecular subtype (Basal A, $n = 12$; Basal B, $n = 14$; Luminal, $n = 25$), showing significantly higher PATZ1 expression in luminal cell lines. **(d)** PATZ1 mRNA expression across individual breast cancer cell lines from the Neve dataset, grouped according to molecular subtype (Basal A, Basal B and Luminal). **(e)** PATZ1 expression according to the clinical subtype classification of the Neve breast cancer cell line panel (triple-negative, $n = 25$; HER2-positive, $n = 11$; hormone receptor-positive, $n = 15$). PATZ1 expression is significantly higher in hormone receptor-positive cell lines. **(f)** PATZ1 expression according to histological grade in the GSE76124 dataset ($n = 198$), showing lower PATZ1 levels in poorly differentiated tumors. **(g)** PATZ1 expression in primary breast tumors from the GSE2034 dataset according to the occurrence of brain metastasis (brain relapse: yes, $n = 10$; no, $n = 276$), showing lower PATZ1 expression in tumors from patients who developed brain relapse. (**h)** PATZ1 expression according to histological grade (grade 1, $n = 239$; grade 2, $n = 677$; grade 3, $n = 495$), showing progressive downregulation with increasing tumor grade. **(i, j)** Kaplan–Meier analyses of relapse-free survival (RFS) generated using the Kaplan–Meier Plotter platform, showing that high PATZ1 expression is associated with improved RFS in the overall breast cancer cohort (n = 3557; **i**) and in the basal-like subgroup (n = 581; **j**)

Fig. 2 PATZ1 directly regulates FOXA1 transcription in breast cancer cells. (a) Correlation between PATZ1 and FOXA1 mRNA expression in two independent breast cancer cohorts, showing a significant positive association between the expression of the two genes. **(b)** *In silico* analysis of the human FOXA1 regulatory region identifying four putative PATZ1/MAZR-binding sites predicted by TraFaC and Matinspector. **(c)** Chromatin immunoprecipitation (ChIP)-PCR analysis showing PATZ1 occupancy at the FOXA1 regulatory region in MCF7 cells, whereas no enrichment was detected in MDA-MB-231 or MDA-MB-453 cells. Input DNA and unrelated antibody were used as positive and negative controls, respectively. **(d)** Luciferase reporter assay showing increased FOXA1 promoter activity following ectopic PATZ1 expression in MCF7 cells. Firefly luciferase activity was normalized to Renilla luciferase activity and expressed as fold change relative to empty-vector control cells. Data are presented as mean ± SEM of three independent experiments. **(e)** RT-qPCR analysis of *PATZ1* and *FOXA1* expression following transient PATZ1 silencing in MCF7 cells. Results are expressed as fold change relative to control cells. Statistical significance was determined using an unpaired two-tailed Student's *t*-test. *, $P < 0.05$; **, $P < 0.01$; ns, not significant

Fig. 3 PATZ1 promotes luminal epithelial differentiation in MDA-MB-231 breast cancer cells. (a) Western blot analysis of HA-PATZ1 expression in independent MDA-MB-231 clones stably transfected with either the empty vector or HA-PATZ1. Vinculin was used as a loading control. **(b)** Representative phase-contrast images of control (MP1) and PATZ1-overexpressing (clone 2.8) MDA-MB-231 cells. PATZ1-overexpressing cells exhibit increased cell-cell adhesion and tendency to form cohesive cell clusters (dashed circles), consistent with a shift toward a more epithelial-like morphology. Scale bars, 200 μm. **(c)** RT-qPCR analysis of the luminal epithelial markers *EPCAM* and *KRT8*, and the mesenchymal/stem-associated marker *CD44* in control and PATZ1-overexpressing MDA-MB-231 cells. PATZ1 overexpression increases *EPCAM* and *KRT8* expression and reduces *CD44* expression. Data are presented as mean ±

SEM of two replicate experiments. Statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparisons test. $P < 0.05$; $P < 0.01$; $P < 0.001$

Fig. 4 PATZ1 attenuates the malignant phenotype of MDA-MB-231 breast cancer cells. (a) Metabolic activity of control and PATZ1-overexpressing MDA-MB-231 cells assessed using the MTS assay at the indicated time points. **(b)** Growth curves of control and PATZ1-overexpressing MDA-MB-231 cells determined by direct cell counting, showing reduced proliferative capacity following PATZ1 overexpression. **(c)** Representative images and quantification of wound-healing assays at 0 and 24 h, showing reduced wound closure in PATZ1-overexpressing cells. **(d)** Representative images and quantification of Transwell migration assays, showing reduced migration following PATZ1 overexpression. Migrated cells were quantified in 10 independent microscopic fields per condition and two biological replicates. **(e)** Representative images and quantification of Matrigel invasion assays, showing reduced invasive capacity in PATZ1-overexpressing cells. Invading cells were quantified in 10 independent microscopic fields per condition and two biological replicates. **(f)** Representative images of colony formation in soft agar and quantification of colony area, showing reduced anchorage-independent growth in PATZ1-overexpressing cells. Colony area was quantified in 5-10 microscopic fields per cell population and two biological replicates. Statistical significance was determined using unpaired two-tailed Student's t-test or one-way ANOVA followed by Tukey's multiple-comparisons test, as appropriate. $P < 0.05$; $\mathbf{P < 0.01}$; $\mathbf{P < 0.0001}$

Fig. 5 Loss of PATZ1 in fibroblasts promotes breast cancer cell plasticity through paracrine signaling. Schematic representation of the experimental design and RT-qPCR of MCF7 cells exposed to conditioned media (CM) derived from wild-type ($Patz1^{+/+}$) or Patz1-knockout ($Patz1^{-/-}$) mouse embryonic fibroblasts (MEFs). Expression of *CDH1*, *KRT8*, *PATZ1*, *TWIST*, *FN1*, *IL6*, and *SOX2* was analyzed following CM exposure. Conditioned medium from $Patz1^{-/-}$ MEFs significantly reduced *CDH1*, *KRT8*, and *PATZ1* expression and increased *IL6* and *SOX2* expression. A non-significant trend toward increased *TWIST* and *FN1* expression was also observed. Data are presented as mean ± SEM of three technical replicates from a representative CM treatment experiment. Statistical comparisons between technical replicates were performed using an unpaired two-tailed Student's *t*-test. $P < 0.05$; $\mathbf{P < 0.01}$; $\mathbf{P < 0.0001}$

Fig. 6 Proposed model of PATZ1-mediated regulation of breast cancer cell identity and plasticity. Schematic representation of the dual role of PATZ1 in regulating breast cancer cell identity through cell-intrinsic and microenvironment-dependent mechanisms. **Cell-intrinsic**: in luminal breast cancer cells, PATZ1 directly promotes FOXA1 transcription, thereby supporting luminal epithelial differentiation and cell identity. Conversely, PATZ1 downregulation is associated with destabilization of the luminal program. In basal-like breast cancer cells, ectopic PATZ1 expression induces partial luminal reprogramming, characterized by increased *KRT8*, and *EPCAM* expression, reduced *CD44* levels, and attenuation of malignant properties, including proliferation, migration, invasion, and anchorage-independent growth. **Cell-extrinsic**: loss of PATZ1 in fibroblasts alters paracrine signaling, leading to reduced *PATZ1*, *CDH1*, and *KRT8* expression together with increased *SOX2* and *IL6* expression in neighboring luminal breast cancer cells, thereby promoting loss of luminal identity and increased cellular plasticity

Table 1 | Sequences of oligonucleotide primers used in qPCR

Gene	Primer sequence	Size	GenBank accession number
PATZ1	Forward: 5'-TACATCTGCCAGAGCTGTGG-3' Reverse: 5'-TGCACCTGCTTGATATGTCC-3'	71bp	NM_014323
FOXA1	Forward: 5'-GAAGATGGAAGGGCATGAAA-3' Reverse: 5'-GCCTGAGTTCATGTTGCTGA-3'	97bp	NM_004496
EPCAM	Forward: 5'-CCATGTGCTGGTGTGTGAA-3' Reverse: 5'-TGTGTTTTAGTTCAATGATGATCCA-3'	111bp	NM_002354
KRT8	Forward: 5'-AGGATCTCCGCCTGGTTC-3' Reverse: 5'-TCACCCTGATGGACATGGTA-3'	64bp	NM_001256282
CD44	Forward: 5'-CACAGAGAAAATGCCCTTGA-3' Reverse: 5'-CACAGTCCCCTAAAGGCAGA-3'	110bp	NC_000011
CDH1	Forward: 5'-GCCGAGAGCTACACGTTCA-3' Reverse: 5'-GACCGGTGCAATCTTCAAA-3'	88bp	NM_004360
TWIST	Forward: 5'-GCCAGGTACATCGACTTCCTCT-3' Reverse: 5'-TCCATCCTCCAGACCGAGAAGG-3'	122bp	NM_000474
FN1	Forward: 5'-CTGGCCGAAAATACATTGTAAA-3' Reverse: 5'-CCACAGTCGGGTCAGGAG-3'	114bp	NM_212482
IL6	Forward: 5'-AGACAGCCACTCACCTCTTCAG-3' Reverse: 5'-TTCTGCCAGTGCCTCTTTGCTG-3'	132bp	NM_000600
SOX2	Forward: 5'-GCTACAGCATGATGCAGGACCA-3' Reverse: 5'-TCTGCGAGCTGGTCATGGAGTT-3'	135bp	NM_003106

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryInformation.pdf