Protein tyrosine phosphatase *UBASH3B* is overexpressed in triple-negative breast cancer and promotes invasion and metastasis

Shuet Theng Lee[a,1], Min Feng[a,1], Yong Wei[b,1], Zhimei Li[a], Yuanyuan Qiao[a], Peiyong Guan[c], Xia Jiang[a], Chew Hooi Wong[a], Kelly Huynh[d], Jinhua Wang[d], Juntao Li[c], K. Murthy Karuturi[c], Ern Yu Tan[e], Dave S. B. Hoon[d], Yibin Kang[b], and Qiang Yu[a,f,g,2]

[a]Cancer Biology and Pharmacology, Genome Institute of Singapore, Agency for Science, Technology and Research, Singapore 138672; [b]Department of Molecular Biology, Princeton University, Princeton, NJ 08544; [c]Information and Mathematical Science, Genome Institute of Singapore, Agency for Science, Technology and Research, Singapore 138672; [d]Department Molecular Oncology, John Wayne Cancer Institute, Santa Monica, CA 90404; [e]Department of General Surgery, Tan Tock Seng Hospital, Singapore 308433; [f]Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 117597; and [g]Cancer and Stem Cell Biology, Duke-National University of Singapore Graduate Medical School, Singapore 169857

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Efforts to improve the clinical outcome of highly aggressive triple-negative breast cancer (TNBC) have been hindered by the lack of effective targeted therapies. Thus, it is important to identify the specific gene targets/pathways driving the invasive phenotype to develop more effective therapeutics. Here we show that ubiquitin-associated and SH3 domain-containing B (UBASH3B), a protein tyrosine phosphatase, is overexpressed in TNBC, where it supports malignant growth, invasion, and metastasis largely through modulating epidermal growth factor receptor (EGFR). We also show that *UBASH3B* is a functional target of anti-invasive microRNA*200a* (*miR200a*) that is down-regulated in TNBC. Importantly, the oncogenic potential of *UBASH3B* is dependent on its tyrosine phosphatase activity, which targets CBL ubiquitin ligase for dephosphorylation and inactivation, leading to EGFR up-regulation. Thus, *UBASH3B* may function as a crucial node in bridging multiple invasion-promoting pathways, thereby providing a potential therapeutic target for TNBC.

Triple-negative breast cancer (TNBC)—so named for its absent expression of estrogen receptor (ER), progesterone receptor, and human epidermal growth factor receptor 2 (HER2)—is among the most aggressive of breast cancer subtypes, with a high propensity for metastasis and poor prognosis (1). Current treatment modalities for TNBC are limited to surgery, radiation, and systemic chemotherapy, given the absence of more specific therapeutic targets. However, patients often experience early relapse from distant tumor metastasis, although they may initially respond well to the treatments (2).

Over the past few decades, tremendous effort has been expended in the search for molecular targeted therapy for TNBC, with only limited success. Epidermal growth factor receptor (EGFR)-targeted therapy is an example of these investigational therapies. Although TNBC often harbors EGFR overexpression (3), anti-EGFR treatments have shown only limited favorable response in clinical trials, however (4). Another example is poly-(ADP ribose) polymerase inhibitor, which can induce synthetic lethality in *BRCA1*-deficient TNBC tumors, although its clinical efficacy in a Phase II trial remains to be determined (5). Although the reasons for the ineffectiveness of these molecular targeted therapies are unclear, one hypothesis is that breast tumors, particularly TNBCs, are highly heterogeneous and use multiple mechanisms, including invasion and metastasis, to enable the aggressive phenotypes. These pathways then may become integrated as specific signaling steps that collectively contribute to the disease progression. Pathways and mechanisms involved in invasion and metastasis often involve transcriptional reprogramming induced by deregulated transcriptional and epigenetic regulators, as well as miRNAs such as *miR200* (6–11). Although the breast cancer transcriptome has been investigated extensively, how these pathways are interconnected in disease progression is unclear, and the regulatory elements crucial for network integrity remain to be identified. The availability of improved genomic technologies allows us to readdress these issues.

The goal of the present study was to identify "druggable" targets in TNBC that may inform new treatment options. Using the latest gene array technology, we have identified the overexpression of *Ubiquitin-Associated and SH3 Domain-Containing B* (*UBASH3B*) tyrosine phosphatase as an oncogenic driver in TNBC invasion and metastasis. We show that *UBASH3B* acts as a crucial downstream target of invasive regulator *miR200a*, and that its oncogenic function requires phosphatase activity. Our work thus identifies an oncogenic protein phosphatase as a potential therapeutic target for TNBC.

Results

Functional Genomics Identifies Overexpression of *UBASH3B* in TNBC. To identify specific targets associated with TNBC, we used the Illumina HumanHT-12 V4.0 expression Beadchip to profile the gene expression of a panel of TNBC and non-TNBC human breast cancer cell lines and primary tumor tissues. Toward this end, we identified a set of 103 genes as overexpressed in both TNBC cell lines and patient tissues compared with respective non-TNBC counterparts (Fig. 1*A*, Fig. S1*A*, and Table S1). Among these genes, 19 were annotated as enzymes and thus considered potential "druggable" targets (Fig. 1*A*). Moreover, seven of these genes showed a significant association with disease outcome in at least one examined breast cancer cohort (Fig. S1*B*).

To examine whether the foregoing genes could have roles in TNBC, we knocked down their expression individually in the highly aggressive breast cancer cell line MDA-MB-231 (MB231 hereinafter) by transfecting two independent siRNA oligos. We monitored the efficiency of gene knockdown by quantitative PCR (qPCR) and assessed the phenotypic changes on 3D Matrigel growth as an indicator of malignant growth. Our results showed that five of the seven genes (*UBASH3B*, *AGPAT4*, *ACOT9*, *CDK6*, and *HYAL3*) may be required for TNBC

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[1]S.T.L., M.F., and Y.W. contributed equally to this work.

[2]To whom correspondence should be addressed. E-mail: yuq@gis.a-star.edu.sg.

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progression, given that their knockdown strongly reduced the Matrigel growth of MB231 cells (Fig. 1*B*). Among these genes, *UBASH3B*, also called *Suppressor of T-Cell Receptor Signaling 1* (*STS-1*), is of particular interest for further study, as it was top-ranked in causing phenotypic change upon knockdown and known to regulate EGFR endocytosis and degradation (12). Moreover, *UBASH3B* was recently shown to have tyrosine protein phosphatase activity (13). Nevertheless, a role of *UBASH3B* in promoting tumorigenesis has yet to be described.

Further validation analysis using qPCR and Western blot analysis revealed high levels of both *UBASH3B* mRNA and protein in highly invasive TNBC cell lines compared with the less-invasive luminal lines (Fig. 1*C*). In contrast, *Ubiquitin-Associated and SH3 Domain-Containing A* (*UBASH3A*), a close family member of *UBASH3B,* showed no such difference (Fig. S1*C*). Interestingly, *UBASH3B* was overexpressed in invasive prostate cancer cells as well (Fig. S1*D*). Furthermore, gene expression analysis based on Oncomine datasets also showed the overexpression of *UBASH3B* in TNBC and metastatic prostate cancer specimens (Fig. 1*D* and Fig. S1*E*). The overexpression of *UBASH3B* in TNBC was further verified by immunohistochemistry (IHC) analysis of clinical tumor specimens ($P = 0.0018$) (Fig. 1*E*). Furthermore, IHC analysis of a cohort of primary and invasive breast tumors on a tissue microarray revealed higher *UBASH3B* expression in invasive breast cancer compared with noninvasive counterparts ($P = 0.0456$) (Fig. 1*F*). More strikingly, higher expression of *UBASH3B* was consistently observed in patients undergoing early metastasis events (Fig. 1*G*). These results indicate an association between *UBASH3B* and breast cancer invasion and metastasis in patients with breast cancer.

Finally, we performed a meta-analysis by combining three independent patient cohorts to assess the overall breast cancer patient survival in relation to the expression level of *UBASH3B*. The results indicated that high expression of *UBASH3B* confers poor overall survival (particularly 5-y survival) in patients with ER-negative breast cancer (Fig. 1*H*), but no significant survival disadvantage in patients with ER-positive breast cancer. This observation suggests that high *UBASH3B* expression may predict early relapse in ER-negative patients. Taken together, these findings suggest a potential role of *UBASH3B* in TNBC invasion and metastasis.

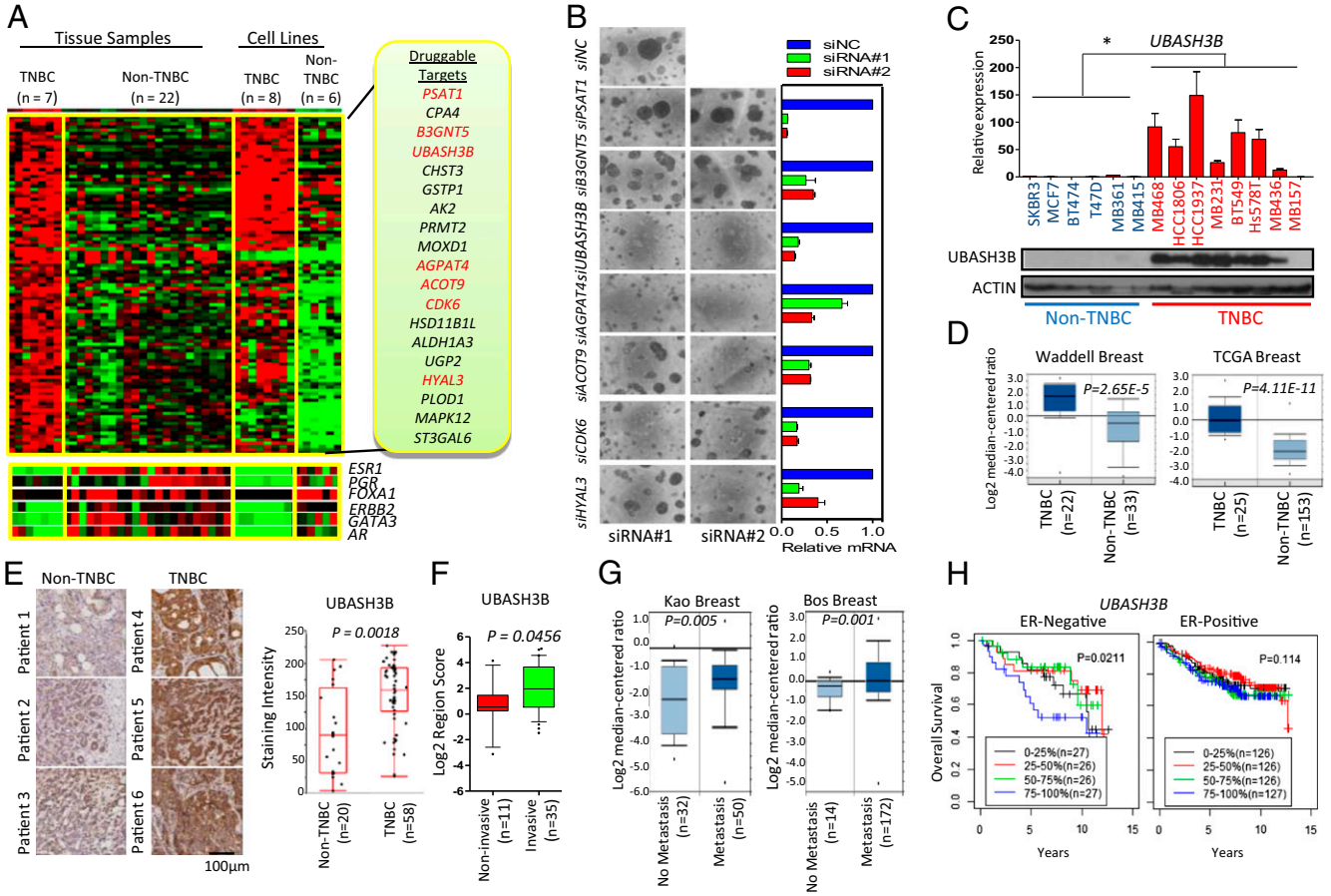

Fig. 1. Functional genomics identifies the overexpression of *UBASH3B* in TNBC. (*A*) Heat map representation of 103 genes expressed more than twofold higher in TNBC compared with non-TNBC breast cancer tissues and cell lines. Expressions of *ER* (*ESR1*) and its related genes *FOXA1* and *GATA3*, as well as *ERBB2*, *AR*, and *PGR*, are also shown. A total of 19 druggable genes were identified (*Right*); the genes in red were associated with poor patient survival. (*B*) Representative images of MB231 cells cultured in 3D Matrigel. (*Left*) Cells were depleted of indicated genes using two independent siRNA sequences. (*Right*) Knockdown efficiency was assessed by qPCR. (*C*) qPCR and Western blot analysis of *UBASH3B* expression in a panel of breast cancer cell lines. $^*P < 0.05$, unpaired two-tailed *t* test. (*D*) Boxplots showing the mRNA expression of *UBASH3B* in two Oncomine datasets, Waddell and TCGA breast. (*E*) Representative images of IHC staining of UBASH3B on paraffin-embedded breast cancer tissues from the John Wayne Cancer Institute patient cohort. Staining was graded based on the intensity, and *P* values were calculated by the Wilcoxon test. (*F*) IHC staining of UBASH3B on a tissue microarray consisting of invasive and noninvasive breast cancer tissues. The proportion of positive-stained cytoplasm was scored and plotted. *P* values were calculated by the unpaired two-tailed *t* test. (*G*) Boxplots showing the mRNA expression of *UBASH3B* in two Oncomine datasets, Kao (metastatic event at 3 y) and Bos (metastatic event at 5 y). (*H*) Kaplan–Meier analyses of overall survival of breast cancer patients in the Oxford/Stockholm/Uppsala datasets. Patients were stratified based on quartile mRNA expression of *UBASH3B*. *P* values were calculated by the Wald test. Error bars represent mean ± SEM.

***UBASH3B* Is a Functional Target of *miR200a*.** To identify the molecular events leading to *UBASH3B* up-regulation associated with invasion, we examined the regulatory regions of *UBASH3B*. We found that several *miR200a* target sequences were present in the 3′ UTR of *UBASH3B* (Fig. 2*A*). *miR200*s are known to be anti-invasive, and their expression is down-regulated in aggressive breast cancer and prostate cancer (14, 15).

We next investigated the possibility of *UBASH3B* serving as a target of *miR200*s. qPCR analysis revealed the down-regulation of *miR200a*/*b*/*c* in mesenchymal TNBC cell lines (Fig. 2*B* and Fig. S2*A*). Consistent with the foregoing analysis indicating that the *UBASH3B* 3′ UTR contains sites for *miR200a*, but not for *miR200b*/*c*, we were able to demonstrate direct and specific targeting and inhibition of *UBASH3B* by *miR200a*, but not by *miR200c*, using *UBASH3B* 3′ UTR reporters containing WT or a variant in which the *miR200a*-binding sequences were mutated (Fig. 2*C*). As expected, enforced expression of *miR200a*, but not of *miR200c*, was able to reduce the *UBASH3B* mRNA and protein expression in invasive breast and prostate cancer cells (Fig. 2*D* and Fig. S2*C*), although both *miR200a* and *miR200c* could repress the expression of ZEB1 and ZEB2 (Fig. 2*D*), two well-known common targets of *miR200a*/*b*/*c* (7, 16). Conversely, *miR200a* antagomir treatment of MCF7 and T47D cells that express high levels of *miR200a* resulted in up-regulation of *UBASH3B* mRNA and protein levels (Fig. 2*E*). We also confirmed the down-regulation of *miR200a* ($P = 0.032$), together with *miR200b*/*c*, in clinical TNBC samples compared with non-TNBC samples (Fig. 2*F* and Fig. S2*B*), in concordance with the up-regulation of *UBASH3B* in TNBC samples ($P = 0.0007$) (Fig. 2*F*).

We performed a rescue invasion assay to investigate the functional association between *UBASH3B* and *miR200a*., and found that treatment with either *miR200a* or *miR200c* could reduce the invasive capacity of MB231 cells (Fig. 2*G*). Ectopic expression of *UBASH3B*, which could not be targeted by *miR200a*, effectively restored the reduced invasion by *miR200a*, but not that by *miR200c*. Thus, *UBASH3B* is a functional target of *miR200a*, supporting a *miR200a–UBASH3B* functional axis in promoting cell invasion in TNBC.

***UBASH3B* Knockdown Reduces Malignancy by Modulating EGFR.** We next assessed the relevance of *UBASH3B* overexpression in tumorigenesis. Because UBASH3B is implicated in antagonizing EGFR degradation during endocytosis on EGF treatment (12), we asked whether *UBASH3B* has a role in EGF-induced invasion, a process of particular relevance in TNBC. *UBASH3B* knockdown by two independent siRNAs resulted in marked inhibition of EGF-induced Transwell invasion in invasive breast and prostate cancer cells (Fig. 3*A*, *Upper* and Fig. S3*A*). Consistently, *UBASH3B* knockdown facilitated EGF-induced EGFR protein degradation and abolished EGF-induced EGFR phosphorylation (Fig. 3*A*, *Lower*), These findings are consistent with the known role of EGFR as a well-established invasive effector in TNBC and other invasive tumors (17–19).

We further confirmed the specificity of knockdown effects on invasion by a rescue experiment using ectopic expression of *UBASH3B* and the third siRNA targeting the 3′ UTR of *UBASH3B* (Fig. S3*B*). Conversely, ectopic *UBASH3B* overexpression was able to increase EGFR protein abundance in a noncancerous human mammary epithelial MCF10A cells, although it could not induce transformation or enhance invasive growth (Fig. S3*C*). This indicates that *UBASH3B* expression is required but is insufficient to induce malignancy. The positive correlation between *UBASH3B* and EGFR in primary breast tumors was further validated by IHC analysis of 73 patient tissues revealing that patients with higher EGFR expression were significantly enriched with higher *UBASH3B* expression ($P = 0.034$) (Fig. 3*B*).

In addition to affecting cell invasion, *UBASH3B* knockdown also reduced the ability of breast and prostate cancer cells to form tumorspheres (Fig. S3 *D* and *E*), a growth feature associated with cancer stem cells. Notably, *UBASH3B* knockdown did not affect cell proliferation when grown on monolayers (Fig. S3*F*). This indicates that *UBASH3B* is not required for cell proliferation in general, and is more involved in regulating cellular phenotypes associated with malignancy. Consistent with these observations, we found that *UBASH3B* expression is further up-regulated in a more aggressive subline of MB231 derived from lymph node metastasis (MB231-LN) (20), which displayed a more disorganized morphology compared with the parental cells under 3D Matrigel growth conditions (Fig. 3*C*). As expected, *UBASH3B* knockdown in MB231-LN cells attenuated the aggressive morphology (Fig. 3*C*).

To examine whether the phenotypic changes after *UBASH3B* knockdown are related to the reduction of EGFR protein, we performed a rescue assay by stably ectopic expression of EGFR in MB231 cells depleted of *UBASH3B*. We found that the ectopic EGFR overexpression largely restored the invasive capacity of *UBASH3B*-depleted cells (Fig. 3*D* and Fig. S3 *G* and *H*), consistent with our hypothesis that *UBASH3B*, at least in large part, drives invasion through EGFR.

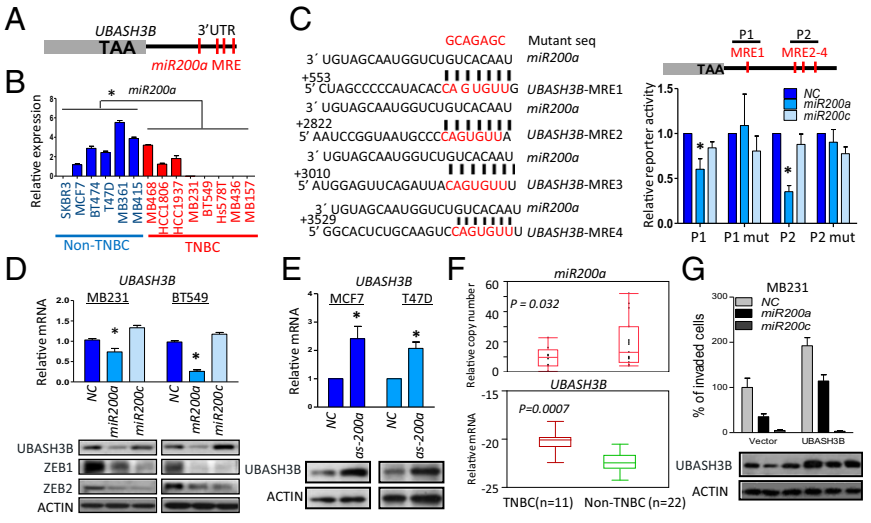

Fig. 2. *UBASH3B* is a target of *miR200a*. (*A*) Schematic diagram of *UBASH3B* gene structure. TAA, stop codon; MRE, miRNA response element. (*B*) qPCR analysis of *miR200a* expression in a panel of breast cancer cell lines. **P* < 0.05, unpaired two-tailed *t* test. (*C*) Schematic diagram showing the four MREs in the 3′ UTR of the *UBASH3B* gene base-paired to an *miR200a* mature sequence. Two regions (P1 and P2) were cloned into a luciferase reporter, pMIR-REPORT. (*Left*) P1 mutation (mut) and P2 mut were constructed by mutating the MRE to the mutant sequence as indicated. (*Right*) Luciferase reporter assay in cells cotransfected with *miR200a* or *miR200c* mimics and pMIR-REPORT containing WT or mutant P1 or P2. **P* < 0.05, paired two-tailed *t* test. (*D*) qPCR and Western blot analysis of *UBASH3B* expression in MB231- and BT549-overexpressing *miR200a* or *miR200c* mimics. Protein expression of ZEB1 and ZEB2 was assessed as well. **P* < 0.05, paired two-tailed *t* test. (*E*) qPCR and Western blot analysis of *UBASH3B* expression in indicated cells transfected with *miR200a* antagomir. **P* < 0.05, paired two-tailed *t* test. (*F*) qPCR analysis of *miR200a* and *UBASH3B* expression in TNBC and non-TNBC tumor samples. (*G*) (*Upper*) Transwell Matrigel invasion assay conducted on MB231 transfected with *miR200a* or *miR200c* mimics in the presence of EGF as a chemoattractant. (*Lower*) UBASH3B expression assessed by Western blot analysis. **P* < 0.05, paired two-tailed *t* test. Error bars indicate mean ± SEM.

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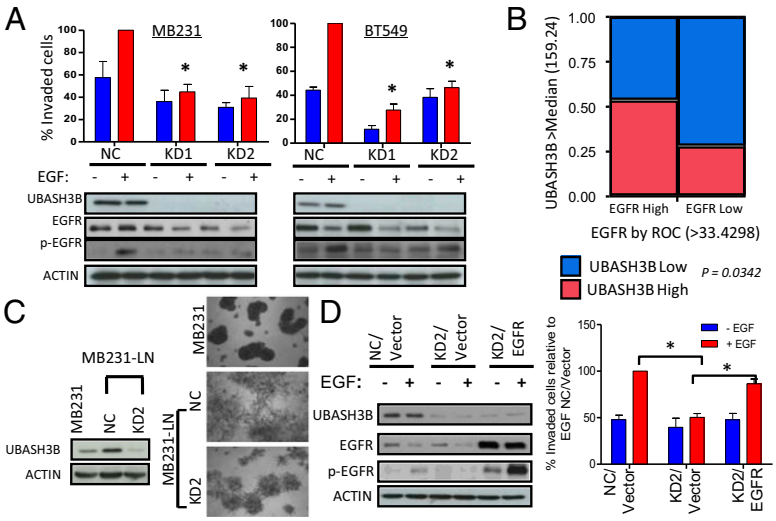

Fig. 3. *UBASH3B* knockdown reduces malignant phenotypes and facilitates EGFR degradation (*A*) (*Upper*) Transwell Matrigel invasion assays were performed on MB231 and BT549 depleted of *UBASH3B* using two independent siRNAs in the presence or absence of EGF as a chemoattractant. (*Lower*) EGFR and UBASH3B expression was assessed by Western blot analysis. $*P < 0.05$, paired two-tailed *t* test. (*B*) Contingency analysis of UBASH3B and EGFR IHC staining on 73 breast cancer tissues from the John Wayne Cancer Institute patient cohort. *P* values were calculated with Fisher's exact test. (*C*) (*Left*) UBASH3B expression as assessed by Western blot analysis. (*Right*) Representative images of indicated cells grown in 3D Matrigel. (*D*) Western blot analysis of EGFR and UBASH3B expression (*Left*) and Transwell invasion assay (*Right*) using MB231-LN cells with UBASH3B depletion and EGFR overexpression. $*P < 0.05$, paired two-tailed *t* test.

***UBASH3B* Knockdown Reduces Breast Cancer Metastasis.** To determine whether *UBASH3B* is required for tumor metastasis, we used a lung metastatic MB231 subline (LM2) (21) that expresses luciferase reporter, and generated short hairpin (sh) RNA knockdown (KD) *shNC*, *shUBASH3B1* (*KD1*), and *shUBASH3B2* (*KD2*) stable cell lines (Fig. 4*A*). Tail vein injection of these cells resulted in lung metastasis in nude mice. Bioluminescent imaging (BLI) measurement revealed significant reduction in lung metastasis compared with controls, with a 50% and 77% reduction of metastasis burden at week 4 after injection of *KD1* and *KD2*, respectively (Fig. 4 *B*–*D*), closely corresponding to the respective knockdown efficiency in these cells. The reduced metastasis provided a significant survival advantage in these mice. Knockdown of *UBASH3B* substantially reduced the risk of mortality in mice, by 3.51-fold (95% CI, 1.10–11.18) with *KD1* and by 6.99-fold (95% CI, 1.46–33.50) with *KD2*, which significantly prolonged survival in these mice ($P = 0.02$ for *KD1*; $P = 0.004$ for *KD2*) (Fig. 4*E*).

To further test the generality of the prometastatic function of *UBASH3B*, we also knocked down *UBASH3B* in the 4T1 mouse mammary tumor cell line and tested the effects in both experimental and orthotopic spontaneous metastasis models by tail vein and mammary gland injection, respectively. In both models, *UBASH3B* knockdown strongly inhibited lung metastasis (Fig. 4 *F*–*J*). Nevertheless, depletion of *UBASH3B* had little effect on primary tumor growth, as shown in the model of 4T1 cell line mammary fat pad injection (Fig. 4*I*). Interestingly, we found that ectopic expression of EGFR partially rescued inhibited metastasis in *UBASH3B*-depleted cells, although this rescue was seen only in the later weeks of metastasis progression (Fig. 4*K*). These data from both in vitro and in vivo studies demonstrate an important role of *UBASH3B*, at least in part through EGFR, in the progression of TNBC invasion and metastasis.

Oncogenic Role of UBASH3B Requires Phosphatase Activity Targeting CBL Phosphorylation and EGFR Degradation. Considering that UBASH3B recently has been identified as a tyrosine phosphatase

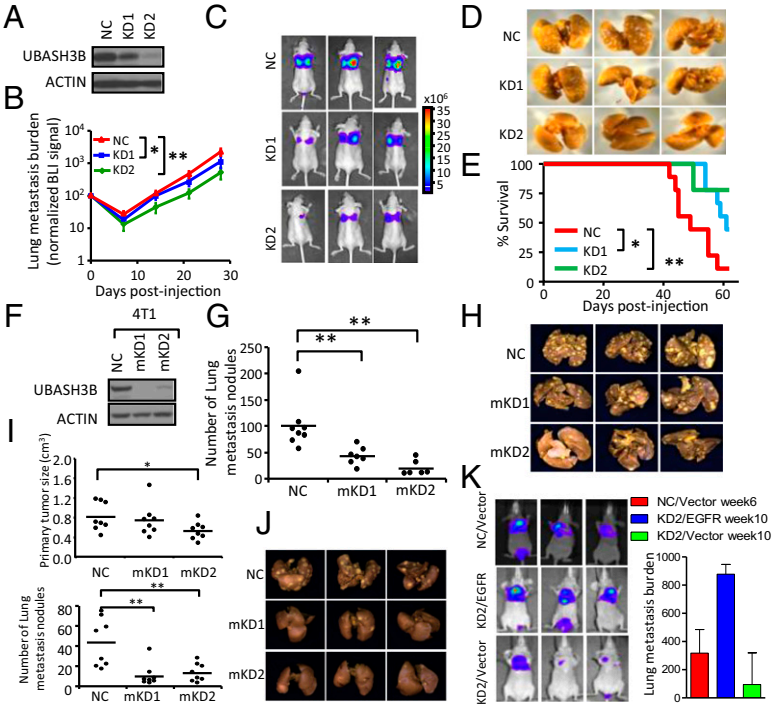

Fig. 4. *UBASH3B* knockdown reduces breast cancer metastasis. (*A*) *UBASH3B* knockdown by shRNAs in LM2 cells as detected by Western blot analysis. (*B*) BLI curves of lung metastasis development in female athymic nude mice injected via the lateral tail vein with control and *UBASH3B* KD cells. Data are mean ± SEM; $n = 9$. $*P < 0.05$; $**P < 0.01$, Mann–Whitney *U* test. (*C*) Representative BLI images of mice in *B* at 4 wk postinjection. (*D*) Whole lung images of mice from a repeat experiment as in *B*, at 4 wk postinjection. (*E*) Kaplan–Meier curves of mice from *B*. $n = 9$. $*P < 0.05$; Cox proportional HR, 3.51 (95% CI, 1.10–11.18). $**P < 0.005$; Cox proportional HR, 6.99 (95% CI, 1.46–33.50). (*F*) *UBASH3B* knockdown by shRNA in 4T1 cells detected by Western blot analysis. (*G*) Boxplot showing the number of lung metastasis nodules from experimental metastases generated by control or *UBASH3B KD* of 4T1 cells at 2 wk after tail vein injection. $n = 7$–8. *P* values were determined by the Student *t* test. (*H*) Representative gross images of lung metastasis from mice as in *G*. (*I*) (*Upper*) Size of primary tumors generated by control or *UBASH3B* KD 4T1 cells at 24 d after mammary fat pad injection. (*Lower*) Boxplot showing the number of lung metastasis nodules from spontaneous metastases generated by control or *UBASH3B* KD 4T1 cells after mammary fat pad injection. $n = 8$. $**P < 0.01$, Student *t* test. (*J*) Representative gross images of spontaneous lung metastasis from mice as in *I*. (*K*) Representative images (*Left*) and BLI signals (*Right*) of lung metastasis of MB231-LN cells of control (NC) and *UBASH3B KD2*, with ectopic expression of vector or EGFR.

(13), we explored whether phosphatase activity is required for its invasion-promoting capability. To do this, we created a phosphatase-dead mutant *UBASH3B* (H391A) (13) and compared it with the WT *UBASH3B* in terms of the ability to promote cell invasion. Although the overexpression of WT *UBASH3B* in BT549 and MB231 cells robustly promoted both basal and EGF-induced invasion, overexpression of *UBASH3B* (*H391A*) did not (Fig. 5*A*). Consistently, EGF-induced EGFR degradation over time was dampened by ectopic overexpression of WT *UBASH3B*, but not of *UBASH3B* (*H391A*) (Fig. 5*B*). We further validated the functionality of this observation using a lung metastasis model. Consistent with in the vitro data, WT *UBASH3B*, but not the mutant *UBASH3B* lacking phosphatase activity, enhanced lung metastasis and concurrently reduced mouse survival (Fig. 5*C*). These in vitro and in vivo results demonstrate that the phosphatase activity of *UBASH3B* is required for its oncogenic activity to promote EGFR protein abundance, invasion, and metastasis in TNBC.

It has been reported previously that *UBASH3B* promotes EGFR protein stability by binding to and inhibiting CBL ubiquitin ligase-mediated EGFR degradation (12); however, whether this *UBASH3B* inhibition of CBL is mediated by its tyrosine phosphatase activity through dephosphorylating CBL is unknown. We found that overexpression of the WT *UBASH3B*, but not of the mutant *UBASH3B* (*H391A*), resulted in a loss of EGF-induced tyrosine phosphorylation of ectopic CBL in HEK293T cells, as assessed by probing the immunoprecipitated CBL with a pan-phospho-Tyr antibody, although interaction was observed between CBL and both WT and mutant *UBASH3B* (Fig. 5*D*). Moreover, in BT549 cells, EGF failed to induce CBL phosphorylation on overexpression of WT *UBASH3B*, but did induce robust CBL phosphorylation in the presence of *UBASH3B* (*H391A*) mutant (Fig. 5*E*). We further showed that *UBASH3B* knockdown-induced EGFR degradation and abolished EGFR phosphorylation could be restored by the concomitant *CBL* knockdown (Fig. 5*F*), indicating an antagonistic role of *CBL* in promoting *UBASH3B*-induced EGFR stabilization.

We further streamlined the functional connection of UBASH3B, CBL, and EGFR by measuring 3D Matrigel invasive growth. We found that the WT *UBASH3B*, but not the *UBASH3B* (*H391A*) mutant, promoted invasive growth, which was rescued by concomitant EGFR knockdown (Fig. S4). In addition, UBASH3B-promoted invasive growth was also rescued by cotransfection of ectopic CBL Y371E mutant, which is known to have a constitutive E3 ligase activity toward EGFR (22) (Fig. S4). Taken together, our biochemical and phenotypical analysis data support the hypothesis that *UBASH3B* promotes invasion and aggressive growth by promoting EGFR stability through dephosphorylation and inhibition of CBL function. Thus, the oncogenic phosphatase activity of *UBASH3B* makes this gene an excellent therapeutic target for cancer invasion and metastasis.

Discussion

Unlike for ER- or HER2-positive breast tumors, there currently are no targeted therapies for TNBC. Given the high genetic heterogeneity of TNBC and the complex gene network driving malignancy, along with the limited benefits of current targeted therapies, continued efforts to identify specific enzymes that represent crucial nodal points of a control network for pharmacologic intervention are of particular relevance. Given our finding that *UBASH3B* overexpression is associated with early relapse of TNBC, along with its fundamental role as a tyrosine phosphatase in invasive progression and disease outcome, we contend that *UBASH3B* overexpression is an important oncogenic event in TNBC. Importantly, *UBASH3B* acts to bridge several key invasive pathways, raising the possibility that therapeutic targeting *UBASH3B* may provide additional benefits for patients with TNBC.

UBASH3B up-regulation in TNBC can be the result of deregulation of *miR200a*, an important member of the miR200 family whose loss is often associated with cancer invasion through promotion of epithelial-mesenchymal transition (EMT). We have shown that *UBASH3B* is a direct and functional target of *miR200a*, and thus up-regulation of *UBASH3B* could be a result of *miR200a* down-regulation, as seen in TNBC. Although *miR200*s can suppress EMT by repressing the expression of *ZEB1/2*, thereby facilitating the spread of cancer cells (7), *UBASH3B* does not seem to affect EMT; its knockdown does not change cell morphology or the expression of EMT markers

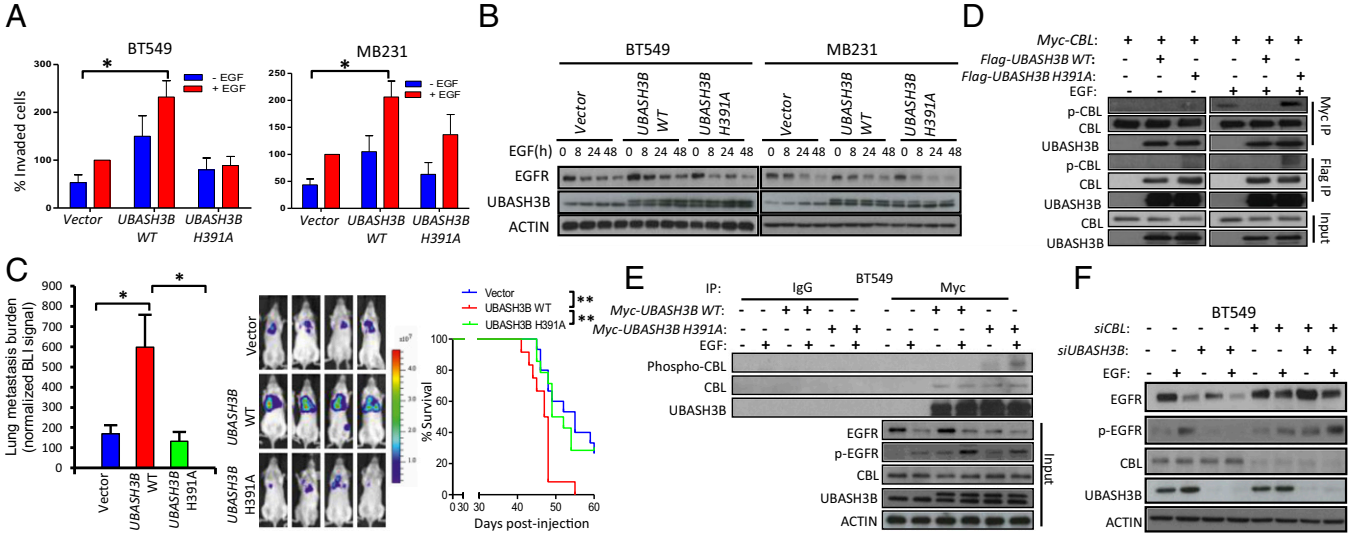

Fig. 5. Oncogenic role of *UBASH3B* requires phosphatase activity targeting CBL phosphorylation and EGFR degradation (*A*) Transwell invasion of cells expressing WT or mutant *UBASH3B* (*H391A*) in the presence or absence of EGF as a chemoattractant. *$P < 0.05$, paired two-tailed *t* test. (*B*) EGFR and UBASH3B expression assessed by Western blot analysis. (*C*) (*Left*) BLI values of lung metastasis development in female NOD/SCID mice at 6 wk after injection with control and WT and mutant *UBASH3B* cells via the lateral tail vein. Data are mean ± SEM; $n = 12$ per category. *$P < 0.05$, unpaired two-sided Student *t* test. (*Center*) Representative BLI images of mice at 6 wk postinjection. (*Right*) Kaplan–Meier analysis. **$P < 0.01$, log-rank test. (*D*) Coimmunoprecipitation assays conducted on HEK293T with transient expression of *CBL* and WT or mutant *UBASH3B* in the presence or absence of EGF. (*E*) Coimmunoprecipitation assays conducted on BT549 stably expressing WT or mutant *UBASH3B* in the presence or absence of EGF. (*F*) Western blot analysis of EGFR, UBASH3B, and CBL protein expression in BT549 depleted with *UBASH3B* and/or *CBL*. Error bars represent mean ± SEM.

such as E-cadherin and vimentin. Thus, in early stages of disease progression, *UBASH3B* may coordinate with other *miR200* targets to promote invasive capacity by enabling extravasation and subsequent migration to distal sites. We have previously shown that when circulating cancer cells colonize a distal site (eg, lung), *miR200* needs to be reexpressed to induce mesenchymal-to-epithelial transition (MET), a rate-limiting step that allows for attachment and colonization of the new niche (23). Thus, *UBASH3B* expression might not be required during the late stages of metastasis progression, and its role in metastasis colonization may require further investigation.

In the present study, we have shown that *UBASH3B* functions as an oncogenic protein tyrosine phosphatase. At first glance, this finding may appear counterintuitive, considering that TNBC is known to harbor an overall induction of protein tyrosine phosphorylation activity owing to activation of a number of receptor tyrosine kinases, such as EGFR and MET, as well as nonreceptor tyrosine kinase Src (24). Moreover, other protein phosphatases, such as PTEN and PTPN12, often function as tumor suppressors that are inactivated in TNBC (25, 26). Thus, it is conceivable that *UBASH3B* as a tyrosine phosphatase likely exerts its oncogenic function by selectively targeting a specific tumor-suppressive tyrosine phosphorylation event. An example of this is tyrosine phosphatase PTP1B, which has been shown exert oncogenic effects by inhibiting growth-inhibitory Src Y530 phosphophorylaton in prostate and colon cancers (27). Of note, a recent study implicated another oncogenic tyrosine phosphatase, SHP2, in breast cancer progression (28), although SHP2's association with TNBC, as well as the direct targets for dephosphorylation, remain to be elucidated.

A possible mechanism by which *UBASH3B* promotes invasive growth and metastasis in TNBC is at least partially through CBL-mediated EGFR regulation. We have shown that *UBASH3B* directly targets CBL phosphorylation to promote EGFR stability. CBL is long known to have dual roles in oncogenesis. In its tumor-suppressive role, CBL acts as an ubiquitin ligase to degrade multiple oncogenic tyrosine kinases, including EGFR (29). CBL's oncogenic role has been linked to its capability to be recruited to activate receptors and to serve as adaptor for the docking of downstream kinases cascade to relay oncogenic signaling (30). Tumor-suppressive ubiquitin ligase activity is associated with phosphorylation at tyrosine residue 371, although other phosphorylation sites also have been linked to its oncogenic activity (31). We have shown that *UBASH3B* is able to bind to and dephosphorylate CBL using a pan-tyrosine phosphorylation antibody, leading to EGFR stabilization. It will be interesting to investigate whether *UBASH3B* specifically targets Y371 or other phosphorylation sites. Although *UBASH3B* may have effects on both tumor-suppressive and oncogenic phosphorylation sites, it may preferentially modulate CBL toward oncogenic activity when it is overexpressed in aggressive cancers like TNBC.

Materials and Methods

Primary breast tumor specimens were obtained from John Wayne Cancer Institute (Santa Monica, CA). All animal procedures were approved by the Institutional Animal Care and Use Committees at the University of Princeton and National University of Singapore.

Statistical Analysis. All statistical analyses were performed using GraphPad Prism. Significance was evaluated by Student's *t* test or one-way ANOVA.

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