**KIBRA (WWC1) Is a Metastasis Suppressor Gene Affected by Chromosome 5q Loss in Triple-Negative Breast Cancer**

**Graphical Abstract**

**Highlights**
- Reduced KIBRA expression is associated with chr 5q loss in breast cancer
- Restoring Kibra expression inhibits metastatic dissemination in mice
- KIBRA impairs the self-renewal capacity of triple-negative breast cancer cells
- KIBRA blocks mechanotransduction signals required for YAP/TAZ activation

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**In Brief**
Triple-negative breast cancers (TNBCs) frequently lose chromosome 5q. Using a TNBC mouse model with spontaneous loss of a syntenic region, Knight et al. identify KIBRA as a metastasis suppressor. Mechanistically, KIBRA suppresses RHOA activation, impairing nuclear translocation of the oncogenes YAP/TAZ, which drive metastatic and cancer stem cell-like behavior.

**Data and Software Availability**
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**KIBRA (WWC1) Is a Metastasis Suppressor Gene Affected by Chromosome 5q Loss in Triple-Negative Breast Cancer**

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**SUMMARY**

Triple-negative breast cancers (TNBCs) display a complex spectrum of mutations and chromosomal aberrations. Chromosome 5q (5q) loss is detected in up to 70% of TNBCs, but little is known regarding the genetic drivers associated with this event. Here, we show somatic deletion of a region syntenic with human 5q33.2–35.3 in a mouse model of TNBC. Mechanistically, we identify KIBRA as a major factor contributing to the effects of 5q loss on tumor growth and metastatic progression. Re-expression of KIBRA impairs metastasis in vivo and inhibits tumorsphere formation by TNBC cells in vitro. KIBRA functions co-operatively with the protein tyrosine phosphatase PTPN14 to trigger mechanotransduction-regulated signals that inhibit the nuclear localization of oncogenic transcriptional co-activators YAP/TAZ. Our results argue that the selective advantage produced by 5q loss involves reduced dosage of KIBRA, promoting oncogenic functioning of YAP/TAZ in TNBC.

**INTRODUCTION**

Approximately 15% of patients with invasive breast cancer are diagnosed with triple-negative breast cancer (TNBC), defined by the absence of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression (Foulkes et al., 2010). Because TNBC lacks an approved targeted therapy, the only systemic treatment is chemotherapy. Although this can induce a complete pathological response, TNBCs are associated with a high risk of early recurrence, and metastatic disease is virtually incurable (Denkert et al., 2017; Foulkes et al., 2010).

A concerted effort has been undertaken to understand the molecular basis of TNBC heterogeneity and discover actionable targets. Molecular subtyping based on gene expression has defined the majority of TNBCs as basal-like (49%–80%) (Denkert et al., 2017; Lehmann and Pietenpol, 2014; Rakha et al., 2009) or claudin-low (up to ~30%) (Prat et al., 2010; Prat and Perou, 2011). Further studies have refined this classification into four subtypes: basal-like 1, basal-like 2, mesenchymal, and luminal androgen receptor (Lehmann et al., 2016). Integrating mutation status, gene expression, and copy number has shown that breast cancers segregate into 10 “integrative clusters” (Curtis et al., 2012). Most TNBCs (60%) fall into integrative cluster 10 (IntClust10), associated with an elevated 5-year risk of recurrence and frequent TP53 mutations. Up to 70% of TNBCs also undergo deletions on the long arm of chromosome 5, spanning 5q11 to 5q35 (Johannsdottir et al., 2006; Natrajan et al., 2009; Turner et al., 2010). However, with few exceptions (Weigman et al., 2012), genes conferring selective pressure for 5q loss are relatively unknown.

Genetically engineered mouse models are powerful tools for deciphering breast cancer complexity (Cardiff et al., 2000; Herschkowitz et al., 2007). We have previously shown that mammary tumors driven by mouse mammary tumor virus (MMTV)-Met reflect human breast cancer subtypes, including basal-like (Ponzo et al., 2009), whereas conditional deletion of Trp53 in this model (MMTV-Met;Trp53fl/+;Cre) induces mesenchymal tumors modeling the TNBC subtype claudin-low (Knight et al., 2013). Here we show that MMTV-Met;Trp53fl/+;Cre mammary tumors spontaneously lose a region on chromosome 11 that is syntenic with human 5q33.2–35.3. Using gene expression and functional analysis, we show that WWC1 (KIBRA), a scaffold
protein and activator of the Hippo pathway located on 5q (Baumgartner et al., 2010; Genevet et al., 2010; Yu et al., 2010), has tumor- and metastasis-suppressive properties. Our data indicate a multifaceted role of KIBRA upstream of both canonical Hippo signaling and cytoskeletal cues that regulate the activity of the transcriptional coactivators YAP/TAZ.

RESULTS

Chromosome 5q Loss, a Frequent Event in Human TNBC, Is Modeled in Mouse Mammary Tumors

A powerful way to discover genes with causal roles in oncogenesis is to identify frequently altered genomic regions. Applying this approach to TNBC mouse models, we used array-comparative genomic hybridization (aCGH) to identify a region on chromosome 11 that is lost in 18 of 19 MMTV-Met;Trp53fl/+;Cre and Trp53fl/+;Cre tumors (Knight et al., 2013; Figures 1A and S1) but not MMTV-Met tumors (Ponzo et al., 2009), with one exception (Figure S1, 5482; Ponzo et al., 2009). Because the size of the affected region varied, we identified a minimal common region (MCR) of loss extending from 18.9 to 49.8 Mb (Figures 1A and S1).

Mouse chromosome 11:31.4–49.8 Mb is syntenic with human 5q33.2–35.3 (Figure 1B), which is frequently lost in TNBC (Table S1). We used The Cancer Genome Atlas breast cancer patient dataset (Cancer Genome Atlas Network, 2012) to explore the extent of 5q loss among basal and claudin-low subtypes, representing the majority of TNBCs. Segmental losses spanning the entire 5q arm were frequent, with 40%–55% of tumors showing loss of 5q33.2–35.3 (Figure S2A). To identify candidate tumor suppressor genes within 5q, we analyzed 88 mouse-human gene homologs from the syntenic region (Table S2). Because gene expression and copy number alteration are not always correlated, we analyzed their expression in our mouse models, finding 13 genes (orthologous to 11 unique human genes) that were significantly decreased in tumors with loss of the MCR (Figure 1C; Table S3). Analysis of copy number and expression data, available for 10 of these genes, confirmed their hemizygous deletion in 40%–50% of human claudin-low and basal breast cancers (Figure 1D), although only 4 of 10 had negative mRNA Z scores, consistent with decreased expression (Figure 1E).

Furthermore, only CCNG1, CLINT1, and WWC1 had significantly decreased expression in basal and claudin-low patients (Figure 1F). To corroborate our findings, we used the Cancer Cell Line Encyclopedia (CCLE) to analyze expression in human cell lines representing breast cancer subtypes. Although CCNG1 mRNA levels were universally low irrespective of subtype, and CLINT1 levels did not vary significantly, basal B (claudin-low) cell lines had significantly lower expression of WWC1 (also known as KIBRA) (Figure S2B). This is consistent with a previous observation associating low WWC1 expression with a claudin-low phenotype (Moleirinho et al., 2013).

Depletion of WWC1/KIBRA, a 5q Gene, Increases the Metastatic Aggressivity of Mouse Breast Cancer Cells

Low KIBRA expression in murine and human basal B cell lines was validated by real-time qPCR and western blotting (Figures S2C and S2D). KIBRA encodes a multi-domain scaffold protein (Kremerskothen et al., 2003) acting upstream of the Hippo tumor suppressor pathway, interacting with MERLIN and LATS1/2 to inhibit the oncogenic transcriptional co-activators YAP/TAZ (Baumgartner et al., 2010; Genevet et al., 2010; Yu et al., 2010). To understand the role of KIBRA loss, we silenced Kibra in cells from an MMTV-Met tumor, 5156, which retain chromosome 11 (Figure 2A). These cells were transduced with a luciferase-expressing lentivirus and orthotopically injected into nude mice. We observed no difference in primary tumor growth between control and Kibra knockdown cohorts (Figure S3). Because breast cancer morbidity and mortality are caused primarily by metastasis, and TNBC is highly metastatic, we resected primary mammary tumors and monitored mice for metastasis using bioluminescence imaging (Figure 2B). Compared with controls, tumors with Kibra silencing had an elevated capacity to metastasize to lungs and lymph nodes (Figures 2B and 2C). To determine whether this was due to increased invasion, we grew cells as 3D cyst-like structures and monitored their ability to invade a surrounding type I collagen matrix. Kibra knockdown significantly increased the percentage of cysts displaying invasion (Figure 2D). Accordingly, Kibra silencing also enhanced cell migration in two dimensions (Figure 2E). These data support a role for KIBRA in suppressing metastatic dissemination.

Kibra Expression in Mouse Breast Cancer Cells Decreases Metastatic Potential

To further understand the role of KIBRA loss in TNBCs, we overexpressed Kibra in MMTV-Met;Trp53fl/+;Cre tumor cells (A1005 and A1034) with spontaneous loss of chromosome 11 (Figure 3Ai). Kibra expression altered cell morphology (Figure 3Aii) and decreased proliferation in vitro (Figure 3B), and tumor cells grown orthotopically had altered pathology and decreased growth (Figure 3C). Interestingly, Kibra-positive tumors also displayed a significant increase in polyploidy (Figure 3C). This may be due to an increased rate of cytokinesis failure, providing an explanation for the reduced growth and smaller size of Kibra-positive tumors compared with controls (Figure 3C).

Because KIBRA knockdown in MCF10A cells induces EMT (epithelial-to-mesenchymal transition) (Moleirinho et al., 2013), we used real-time qPCR to determine whether Kibra expression modulates the expression of EMT regulators. Although Kibra expression significantly decreased the mRNA levels of Twist2 (a transcriptional driver of EMT), it also increased the expression of its homolog Twist1, with no effect on other EMT drivers (Figure S4). Despite this, the mRNA levels of E-cadherin (Cdh1) and Claudin-1 (Cldn1) were elevated upon Kibra expression, linking Kibra to an epithelial phenotype. These observations are reflected in the Cancer Genome Atlas (TCGA) dataset, where KIBRA and CDH1 mRNA levels positively correlate in basal breast tumors, but no anti-correlation between KIBRA and EMT drivers is apparent (Figure S4).

Because Kibra depletion increased metastatic potential, we investigated the effect of Kibra re-expression on metastasis. Because spontaneous metastasis of A1005 cells from the mammary gland is variable, we injected them into the tail vein (Figure 3D). Strikingly, control cells disseminated extensively to sites outside of the lung 2 weeks post-injection. This was strongly suppressed by Kibra expression (Figure 3D), indicating that, although Kibra-positive cells survive and grow in the lung...
parenchyma, they are unable either to re-enter the bloodstream, survive in the circulation, or establish in sites other than the lungs, pre-requisites for further metastatic dissemination. Supporting an anti-metastatic function of *Kibra*, its expression decreased the invasion of a 3D collagen matrix by A1005 cells (Figure 3E). Together, these *in vivo* and *in vitro* assays demonstrate a metastasis-suppressive role for *Kibra*, consistent with its frequent loss in TNBCs.

**Figure 1. Loss of Heterozygosity in Mouse Mammary Tumors Mimics Chromosome 5q Loss, a Frequent Event in Human TNBC**

(A) Example aCGH profiles of chromosome (chr) 11 in MMTV-Met (5156) and MMTV-Met;Trp53fl/+;Cre (A1005) mammary tumors. Black dots indicate individual microarray probes and red lines segmented means for regions deviating from a log copy number change of 0. The blue arrow indicates a minimal common region (MCR) of loss from 18.9–49.8 Mb.

(B) Alignment of the MCR with human chr 5q.

(C) Heatmap showing significant differential expression among mouse model tumors, with decreased expression of 13 genes in tumors with loss of the MCR.

(D) Frequency of hemizygous deletion for 10 of 11 genes across PAM50 and claudin-low (CLow) breast cancer subtypes in TCGA data.

(E) TCGA mRNA Z scores for all 10 genes among basal and claudin-low tumors with hemizygous loss.

(F) TCGA mRNA Z scores for all molecular subtypes. Asterisks indicate statistical significance for differences in mRNA levels between basal/claudin-low tumors with copy number loss and other PAM50 subtypes. n = number of patients.

See also Figures S1 and S2 and Tables S2 and S3.
Figure 2. Kibra Silencing Increases Tumor Cell Aggressivity in Mice

(A) Knockdown of Kibra in the MMTV-Met mammary tumor cell line 5156-luciferase (5156-luc). Two independent shRNAs (SH3 and SH4) are compared with a pLKO-empty vector control.

(Bi) 5156-luc cells were orthotopically injected and resected after 5 weeks. Representative bioluminescence images of metastatic dissemination are shown. Metastases (white circles) were confirmed in histological sections. n = number of mice.

(Bii) Percentages of mice with confirmed lung and lymph node metastases.

(Ci) H&E-stained lung sections from 3 representative mice per condition. Metastatic lesions are outlined in green.

(Cii) Quantification of lung metastatic burden.

(Ciii) Calculation of the lung area containing tumor (mean ± SEM).

(legend continued on next page)
**KIBRA Expression Inhibits Tumorsphere Formation in Human TNBC Cell Lines**

To determine its effect on the biology of human TNBC, we re-expressed KIBRA in 3 TNBC cell lines (Figure 4A). As with murine TNBC, KIBRA expression altered the morphology, decreased proliferation, and decreased the ability to invade a collagen matrix (Figures 4A–4C). To examine how KIBRA influenced tumorigenic capacity, we grew cells under conditions of anoikis, as tumorspheres, to assay their tumor-initiating capacity and stem-like properties (Pecè et al., 2010). KIBRA expression dramatically decreased tumorsphere propagation (Figure 4D; Figures S5A and S5B). Because sphere-forming efficiency (SFE) can indicate both tumorigenic and metastatic potential (Engler et al., 2006; Levental et al., 2009), we exploited the ability of spheres formed on stiff ECM (Figures 5A and 5B), an effect abrogated by deletion of the WW domains (Figure 5A). These data demonstrate that KIBRA prevents mechanotransduction-dependent nuclear accumulation of YAP/TAZ in a manner dependent on interaction(s) with its WW domains.

**Inhibition of Tumorsphere Formation by KIBRA Requires the WW1/2 Domains**

To identify molecular mechanisms by which KIBRA functions as a tumor/metastasis suppressor, we systematically deleted regions of protein-protein interaction and structural regions and determined their role in tumorsphere formation (Figures S5C and S5D). MDA-MB-231 cells expressing wild-type KIBRA or mutants lacking the PSD95/DLG1/ZO-1 (PDZ)/atypical protein kinase C (aPKC) binding, Glu-rich, or C2 regions displayed reduced SFE compared with the empty vector control (Figure 4E). In contrast, KIBRA mutants lacking the WW1/2 domains did not impair tumorsphere formation, implicating proteins binding the KIBRA WW domains in the repression of tumorsphere formation.

Several studies have shown that increased TAZ activation endows mammary gland cells with stem-like properties (Bartucci et al., 2015; Cordenonsi et al., 2011). To examine the role of KIBRA in inhibiting YAP/TAZ, we initially examined the expression of a YAP/TAZ signature (Cordenonsi et al., 2011) in TCGA breast cancer data. Claudin-low and basal tumors with KIBRA copy number loss showed enrichment of this signature compared with those without KIBRA loss or other PAM50 subtypes (Figure 4F1), suggesting that KIBRA loss increases YAP/TAZ activity in tumors with 5q deletion. Accordingly, KIBRA expression induced a significant WW domain-dependent decrease in mRNA levels of YAP/TAZ transcriptional targets (CYR61 and CTGF) in MDA-MB-231 cells (Figure 4Fii). To examine this further, we assayed the effect of KIBRA on nuclear accumulation of YAP/TAZ using immunofluorescence. Using a stiffness-tenable polycrylamide culture platform mimicking the mechanical rigidities of healthy and diseased breast tissue (Engler et al., 2006; Levental et al., 2009), we exploited the ability of YAP/TAZ to translocate to the nucleus in response to increasing extracellular matrix (ECM) stiffness (Dupont et al., 2011). Importantly, this allowed us to assay single cells, alleviating variability induced by changes in cell-cell contact. Compared with controls, KIBRA expression severely diminished nuclear YAP/TAZ localization in MDA-MB-231 and A1005 cells on stiff ECM (Figures 5A and 5B), an effect abrogated by deletion of the WW domains (Figure 5A). These data demonstrate that KIBRA prevents mechanotransduction-dependent nuclear accumulation of YAP/TAZ in a manner dependent on interaction(s) with its WW domains.

Although YAP and TAZ are generally considered to functionally overlap, it is TAZ specifically that is amplified in basal-like breast cancer and is associated with stem-like characteristics and metastatic potential (Chan et al., 2008; Cordenonsi et al., 2011; Skibinski et al., 2014). To determine the effect of KIBRA expression on YAP and TAZ, we used specific antibodies to examine their status in MDA-MB-231 and A1005 cells (Figure 5C). In agreement with previously published work (Xiao et al., 2011), we detected elevated YAP phosphorylation at Ser127 in cells expressing KIBRA, indicating inhibition. However, the increase in MDA-MB-231 cells was slight and, in A1005, correlated with increased YAP protein levels. More significantly, we observed a decrease in TAZ protein levels upon KIBRA expression in both cell lines (Figure 5C), which is consistent with the protesome degradation of TAZ that occurs following either Hippo pathway activation (Liu et al., 2010) or interference with mechanotransduction (Sorrentino et al., 2014).

To investigate the possibility that KIBRA functions through TAZ inhibition, we grew KIBRA-expressing cells as tumorspheres after transfection with constitutively active, serine-to-alanine mutants of YAP and TAZ (Figure 5Dii), for which we confirmed nuclear localization (Figure 5E). Constitutively active TAZ, but not YAP, significantly increased the SFE of KIBRA-expressing cells (Figures 5Dii and 5Diii). In further support of a role for TAZ inhibition downstream of KIBRA, orthotopic A1005 tumors (Figure 3) showed prominent nuclear localization of TAZ that became cytoplasmic in tumors expressing KIBRA. YAP, however, remained largely cytoplasmic under all conditions (Figure 5E). Collectively, these data indicate that, in claudin-low breast cancer cells, loss of KIBRA promotes tumor progression and metastasis primarily by relieving inhibition of TAZ.

**KIBRA and PTPN14 Co-operate to Impair Breast Cancer Tumorsphere Formation**

To clarify the WW domain interactions critical for KIBRA to suppress tumorsphere formation, we used BioID, a proximity-based strategy using biotinylation and mass spectrometry, for analysis of proximity-dependent interactions (Roux et al., 2012). Figure 6A, i, and Table S4 show high-confidence interactors (significance analysis of interactome [SAINT]express < 0.8) enriched in KIBRA BioID compared with negative controls in MDA-MB-231 cells. The only significant association lost by KIBRA BioID compared with negative controls in MDA-MB-231 cells was with PTPN14 (protein tyrosine phosphatase nonreceptor type 14), a WW domain-containing protein of interest because of its association with other breast cancer risk factors (Chen et al., 2011). KIBRA/WW1/2-KIBRA interaction was highly specific, as the only other significant association lost by KIBRA BioID was with the WW1/2 domain-containing protein YAP (Williams et al., 2011). Importantly, KIBRA co-immunoprecipitated with PTPN14 (Figure 6A, ii–v) and with YAP, in a manner dependent on its WW domains (Figure 5F). In further support of a role for TAZ inhibition downstream of KIBRA, we detected elevated nuclear localization of TAZ that became cytoplasmic in tumors expressing KIBRA and PTPN14 (Figure 5E). Collectively, these data indicate that, in claudin-low breast cancer cells, loss of KIBRA promotes tumor progression and metastasis primarily by relieving inhibition of TAZ.

(Di) Representative images of invasion (white arrows) from cysts into the collagen matrix. Scale bars represent 50 μm.

(Dii) Quantification of invasion (3 independent experiments, means ± SEM).

(Diii) Migration velocity of cells on fibronectin-coated plates (3 independent experiments, 30 cells/condition/experiment, mean ± SEM). See also Figure S3.
Figure 3. *Kibra* Re-expression Has an Anti-tumorigenic Effect

(Ai) Western blot showing stable KIBRA re-expression in MMTV-Met;Trp53fl/+;Cre mammary tumor cells (A1034 and A1005).

(Aii) Altered cell morphology in Kibra-expressing cells. EV, empty vector control. Scale bars, 100 μm.

(B) Proliferation of cell lines with or without Kibra. Shown is the mean of the indicated replicates ± SEM.

(Ci) H&E-stained mammary tumor sections from mice orthotopically injected with A1005 cells with or without Kibra. The arrow indicates an example of polyploidy. Scale bars, 100 μm.

(Cii) Quantification of karyomegalic/multi-nucleated (polyploid) cells per section (mean ± SEM).

(Ciii) Growth of tumors from (Ci) (mean ± SEM), showing significant difference in endpoint tumor size.

(Di) Representative bioluminescent images of mice immediately after and 2 weeks after intravenous injection of A1005-luciferase cells with or without Kibra. n = number of mice. White circles highlight metastases outside of the lungs.

(Dii) Number of metastatic sites per mouse (mean ± SEM).

(Diii) Percentage of mice with metastatic sites outside of the lungs (mean ± SEM).

(Ei) Representative images of invasion of DAPI-stained (white) A1005 cells with or without Kibra. Scale bars, 250 μm.

(Eii) Quantification of invasion as distance traveled through collagen from the seeded area (red line in Ei) (n = 3, mean ± SEM).

See also Figure S4.
Figure 4. KIBRA Expression Reduces the Invasiveness and Tumorsphere-Forming Capacity of Breast Cancer Cells and Correlates with a YAP/TAZ Signature in Human Breast Cancers

(Ai) Western blot showing stable KIBRA expression in 3 basal B breast cancer cell lines. (Aii) Images showing KIBRA-induced loss of mesenchymal features. Scale bars, 100 μm.

(B) Proliferation with or without KIBRA. Shown are the mean values of the indicated replicates ± SEM.

(Ci) Representative images of invasion of DAPI-stained (white) MDA-MB-231 cells with or without KIBRA. Scale bars, 250 μm.

(Cii) Quantification of invasion as distance traveled through collagen from the seeded area (red line). n = 3, mean ± SEM.

(Di) Representative images of MDA-MB-231 tumorspheres with or without KIBRA. Scale bars, 400 μm.

(Dii) Sphere-forming efficiency (SFE) calculated at 3 serial passages (T1, T2, and T3) and normalized to EV control at T1 (n = 3, mean ± SEM).

(Ei) SFE for MDA-MB-231 cells expressing a control (pLVX-GFP) compared with cells expressing either wild-type KIBRA (KIBRA-WT) or KIBRA mutants lacking specific regions as indicated (n = 3, mean ± SEM).

(Eii) Representative images of tumorspheres in (Ei). Scale bars, 400 μm.

(Fi) Gene set variation analysis (GSVA) showing enrichment of a YAP/TAZ gene expression signature. Basal and claudin-low subtypes are divided by KIBRA copy number gain or loss or diploid status. Asterisks indicate statistical differences between PAM50 subtypes and claudin-low (blue) or basal (red) tumors affected by KIBRA copy number loss.

(Fii) qRT-PCR for YAP/TAZ targets in MDA-MB-231 cells expressing vector control, KIBRA-WT, or ΔWW1/2-KIBRA (n = 3, mean ± SEM).

See also Figure S5.
phosphatase non-receptor 14) (Poembacher et al., 2012). We validated this interaction by co-immunoprecipitation (Figure 6Alii). Notably, the BioID failed to detect other known KIBRA interactors, including MERLIN and LAT51/2 (which were readily detected in other cell types such as HeLa; data not shown). Although MDA-MB-231 cells express LAT51, LAT52 is barely detectable, and MERLIN is not expressed (Figure S6A). Consistent with previous work (Xiao et al., 2011), KIBRA expression increased the levels of LAT51 and LAT52. However, KIBRA did not induce their auto-phosphorylation, indicating that KIBRA does not activate LAT51/2 in MDA-MB-231 cells (Figure S6A), possibly because of the absence of MERLIN (Baumgartner et al., 2010; Genevet et al., 2010; Yu et al., 2010). Interestingly, copy number loss of KIBRA can co-occur with that of LAT51/2 or NF2 (MERLIN), supporting LAT51/2 and MERLIN-independent functions of KIBRA in TNBCs (Cancer Genome Atlas Network, 2012; Figure S6B).

To investigate the role of the PTPN14-KIBRA interaction, we stably silenced PTPN14 in MDA-MB-231 cells, expressed GFP-KIBRA, and seeded GFP-positive cells in tumour sphere assays (Figure 6Bl). KIBRA expression in control cells reduced SFE by 85%, which was rescued by PTPN14 silencing in a manner correlative with the extent of knockdown (Figure 6B). This supports the hypothesis that PTPN14 co--operates with KIBRA to inhibit tumour sphere formation in MDA-MB-231 cells. To determine the role of the KIBRA/PTPN14 interaction in YAP/TAZ regulation, we evaluated YAP/TAZ subcellular localization in MDA-MB-231 cells, which express high levels of TAZ that decrease in response to KIBRA expression (Figure 5C). Strikingly, PTPN14 silencing elicited a near-complete rescue of YAP/TAZ nuclear localization in KIBRA-expressing cells (Figure 6C), demonstrating co-operativity between KIBRA and PTPN14 in cytoplasmic sequestration of YAP/TAZ. This is supported by a highly significant correlation between KIBRA and PTPN14 mRNA levels in basal and Claudin-low tumors (Figure 6D).

**KIBRA and PTPN14 Promote YAP/TAZ Cytoplasmic Sequestration through Regulation of Actin Cytoskeletal Dynamics**

The regulation of YAP/TAZ localization by matrix tension or cell density involves modulation of the actin cytoskeleton (Aragona et al., 2013; Dupont et al., 2011). Consistent with this, expression of wild-type KIBRA, but not ΔWW1/2-KIBRA, decreased both actin stress fibers and nuclear localization of YAP/TAZ in MDA-MB-231 (Figures 7Al and 7Bl) and A1005 cells (Figure S7) under stiff matrix conditions. These phenotypes were rescued by PTPN14 silencing in wild-type KIBRA-expressing cells (Figures 7Alii, and 7Bl), demonstrating co-operativity between KIBRA and PTPN14 in regulating actin cytoskeletal dynamics to sequester YAP/TAZ in the cytoplasm. Furthermore, Ptpn14 knockdown increased the metastasis of A1005 cells expressing Kiba to sites outside of the lungs (Figure S7), supporting the role of the KIBRA-PTPN14 interaction in suppressing metastasis in vivo.

The formation of actin stress fibers is controlled by RHOA, which activates forms that assemble F-actin and Rho-associated kinase (ROCK), which is required for stress fiber contractility (Narumiya et al., 2009). RHOA activation is therefore strongly implicated in YAP/TAZ nuclear localization caused by ECM stiffness (Dupont et al., 2011). We used Rhotekin-glutathione S-transferase (GST) pull-down assays (Ren et al., 1999) and an ELISA-based assay to detect guanosine triphosphate (GTP)-bound RHOA in cells expressing KIBRA (Figure 7C). Consistent with loss of stress fibers, KIBRA expression in MDA-MB-231 and A1005 cells decreased ROHA activity (Figure 7Ci; Figures S7Bi and S7Biii). This effect was not observed with ΔWW1/2 KIBRA (Figures 7Cii and 7Ciii), suggesting that the KIBRA-PTPN14 interaction represses RHOA activity to impair mechano-transduction-based regulation of TAZ, as shown schematically in Figure 7D.

**DISCUSSION**

The identification of syntenic regions of chromosomal loss in mouse cancer models and the human tumors they represent can aid in the identification of tumor suppressor genes (Liu et al., 2016; Xue et al., 2012). Here we have applied this strategy to show that mammary tumors from the MMTV-Met;Trp53fl/+;Cre mouse model lose a chromosomal region syntenic with human 5q33.2–35.3. Using a multifaceted approach, we identified KIBRA as a suppressor not only of tumor growth but also of metastasis. Selective pressure for loss of metastasis suppressor genes during tumorigenesis has been...
Figure 6. The KIBRA WW1/2 Domain Interactor PTPN14 Is Required for KIBRA–Mediated Inhibition of Tumorsphere Formation

(Ai) High-confidence KIBRA-proximal proteins from BioID mass spectrometry analysis of MDA-MB-231 cells expressing WT or mutated KIBRA.

(Aii) Co-immunoprecipitation of KIBRA and PTPN14 in MDA-MB-231 cells expressing wild-type or mutated KIBRA.

(Bi) Western blot showing PTPN14 levels in MDA-MB-231-KIBRA cells expressing 3 PTPN14 shRNAs (SH2, SH3, and SH4) or empty vector (pLKO).

(Bii) Representative images of MDA-MB-231 tumorspheres expressing pLKO or PTPN14 shRNA with or without KIBRA. Scale bars, 400 μm.

(Biii) SFE of MDA-MB-231 cells expressing pLKO or PTPN14 shRNA with or without KIBRA, normalized to the appropriate shRNA-alone condition (conditions seeded in triplicate, mean of 2 experiments ± SEM).

(Ci) YAP/TAZ localization in MDA-MB-231 cells expressing pLKO or PTPN14 shRNA with or without KIBRA. Scale bars, 40 μm.

(Cii) Quantification of YAP/TAZ nuclear to cytoplasmic ratios in MDA-MB-231 cells expressing pLKO or PTPN14 shRNA with or without KIBRA, cultured on soft (0.3 kPa) or stiff (17 kPa) matrix or collagen-coated glass coverslips (70 GPa) (n = 3 mean ± SD).

(D) Pearson correlation analysis of WWC1 (KIBRA) and PTPN14 mRNA levels (Z-scores) in pooled basal and claudin-low patients (TCGA data, n = 89). See also Figure S6 and Table S4.
Figure 7. KIBRA and PTPN14 Co-operatively Regulate Actin Cytoskeletal Tension to Inhibit the Nuclear Translocation of YAP/TAZ

(Ai) Representative phalloidin staining and YAP/TAZ immunofluorescence of MDA-MB-231 cells expressing empty vector, wild-type KIBRA, or ΔWW1/2 KIBRA seeded on collagen-coated coverslips. White arrows indicate actin stress fibers. Scale bars, 10 μm.
PTPN14 engage canonical Hippo signaling (Wilson et al., 2014), we found that they also co-operate to inhibit TAZ in TNBCs (Cordenonsi et al., 2011). Hypermethylation of the premise for KIBRA as a tumor suppressor comes from its

Although previous studies have shown that KIBRA and involving its WW1/2 domain-mediated interaction with PTPN14. Indeed, it has been suggested that loss of multiple DNA damage response and cell cycle genes upon 5q deletion may promote genomic instability and tumor progression (Curtis et al., 2012; Weigman et al., 2012). This may explain why the re-introduction of KIBRA alone has a modest effect on tumor growth in vivo.

Diminished expression of KIBRA has been detected in claudin-low breast cancers, leukemia, and osteosarcomas (Basu-Roy et al., 2015; Hill et al., 2011; Moleirinho et al., 2013), although the underlying mechanisms have not been fully explored. Much of the premise for KIBRA as a tumor suppressor comes from its role in activating the Hippo pathway, for which loss of function and the concomitant activation of YAP/TAZ are well-documented in TNBCs (Cordenonsi et al., 2011). Hypermethylation of the LATS1 and LATS2 promoters is observed in 50% of breast cancers (Takahashi et al., 2005), whereas genomic loss of LATS1, LATS2, and NF2 also occurs in TNBC (Figure S6B). Amplification of TAZ occurs in ~44% of basal breast cancers, where its expression confers stem-like and metastatic traits (Chan et al., 2008; Cordenonsi et al., 2011) and predicts poor outcome (Skibinski et al., 2014).

Here, we provide evidence that hemizygous deletion of KIBRA increases TAZ activity in TNBC, with KIBRA expression inhibiting both tumorsphere formation (i.e., self-renewal of tumor-initiating cells) and the mechanosensing of a stiff ECM. The role of KIBRA in suppressing mechanical signals activating TAZ may be related to suppression of self-renewal, given that an undifferentiated stem-like state is maintained through contact with stiff ECM (Engler et al., 2006; Lui et al., 2012). Indeed, cells maintaining ECM contact in the basal layer of breast epithelium have nuclear TAZ, which becomes cytoplasmic as cells lose basement membrane contact and differentiate (Skibinski et al., 2014). KIBRA loss may constitutively activate mechanotransduction pathways that positively regulate TAZ, leading to persistent TAZ nuclear localization and maintenance of the poorly differentiated phenotype associated with basal-like tumors.

The mechanism of tumorsphere suppression by KIBRA involves its WW1/2 domain-mediated interaction with PTPN14. Although previous studies have shown that KIBRA and PTPN14 engage canonical Hippo signaling (Wilson et al., 2014), we found that they also co-operate to inhibit TAZ in MDA-MB-231 cells that lack MERLIN and activated LATS1/2 by inactivating RHOA and impairing actin stress fiber assembly. Although the metastasis suppressor phenotype conferred by KIBRA was only partially rescued by Ptpn14 knockdown in A1005 cells, this may be due to residual inhibition of YAP/TAZ by canonical Hippo signaling, which, as we show, remains active in these cells. Hence, KIBRA inhibits YAP/TAZ via Hippo signaling or by activating the mechanotransduction-sensitive pathways that can promote YAP phosphorylation and TAZ degradation even in the absence of LATS1/2 and MERLIN (Sorrentino et al., 2014).

In addition to migration and invasion, cytoskeletal modulation by RHOA is critical for cytokinesis (Chircop, 2014). The accumulation of polyploid cells in A1005 KIBRA tumors may therefore involve decreased RHOA activity, which is known to cause growth arrest in tetraploid cells via activation of LATS2, subsequent YAP inhibition, and TP53 stabilization (Ganem et al., 2014). Although A1005 cells are Trp53-null, both LATS1/2 and YAP are phosphorylated upon KIBRA expression in A1005 cells (Figure S6), providing a partial mechanism by which KIBRA could impair growth.

Loss of heterozygosity (LOH) affecting large genomic regions occurs frequently in many cancers, including breast cancer (Solimini et al., 2012). The identification of genetic drivers for LOH and determination of their biological functions could provide new approaches for therapy. We demonstrate tumor-suppressive properties for the 5q gene KIBRA, which we link to tumor-initiating capacity and metastatic ability. We identify a Hippo pathway-independent function for KIBRA via its interaction with PTPN14, which itself has metastasis suppressor properties (Belle et al., 2015), in regulating YAP/TAZ localization through modulation of RHOA activity and the actin cytoskeleton. This contributes significantly to the understanding of cross-talk between actin cytoskeletal dynamics and YAP/TAZ function. The potential to target YAP/TAZ therapeutically, including through inhibiting mechanotransduction pathways, is currently being explored (Zanconato et al., 2016). Based on our findings, such therapeutic angles could be applied to TNBCs with 5q loss.

**EXPERIMENTAL PROCEDURES**

**Genomic Analyses**

Genomic DNA and mRNA isolation and microarrays were performed as described previously (Knight et al., 2013). Patient gene expression and copy number information were obtained from a TCGA Breast Invasive Carcinoma

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(Aii) Representative immunofluorescence as in (A) for cells expressing pLKO control or shPTPN14 (SH4) with or without KIBRA.

(Bi and Bii) Number of stress fibers per cell for (A) and (Bii) (n = 3, mean ± SEM).

(Ci) Representative Rhotekin-GST pull-down in MDA-MB-231 cells expressing EV or KIBRA. GST alone was used as a control (Ctrl).

(Cii) RHOA activity determined by G-LISAs (n = 3, mean ± SEM).

(Ciii) RHOA protein levels for (Cii).

(D) Schematic diagram showing regulation of TAZ by KIBRA.

(Dii) Association of KIBRA with PTPN14 inhibits RHOA activation required for actin stress fiber assembly, removing the stimulus for nuclear translocation of TAZ and resulting in its proteasomal degradation.

See also Figure S7.
dataset (Cancer Genome Atlas Network, 2012; http://cancergenome.nih.gov). Further details can be found in the Supplemental Experimental Procedures.

**Statistical Analyses**
Statistical differences were calculated using Student’s t tests, where significance is as follows: p > 0.05, not significant (ns); *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001. Statistical significance in Figures 1F and 4F was calculated by one-way ANOVA with post hoc Tukey’s multiple comparisons test. One luminal B patient-TCGA-E2-A155-01 was an outlier and was removed from all analyses.

**Cell Culture**
Mouse tumor cells were isolated and cultured as described previously (Knight et al., 2013). All human cell lines were obtained from the ATCC and cultured in DMEM (Hi578T and MDA-MB-231) or RPMI medium (BT549) with 10% fetal bovine serum (FBS). In vitro assays are described in the Supplemental Experimental Procedures.

**Generation of Stable Cell Lines**
The retroviral pBabe-KIBRA vector was a kind gift from Dr. Paul Reynolds (Addgene 40887). N-terminally GFP-tagged wild-type and mutant KIBRA were expressed from the pLVX lentiviral vector. Short hairpin RNAs (shRNAs) were expressed from pLKO.1 (Sigma-Aldrich). Further details can be found in the Supplemental Experimental Procedures.

**Transient Transfections**
The vectors pCMV-FLAG YAP2 5SA (Kunliang Guan, Addgene 27371) and 3XFLAG pCMV-TOPO TAZ (S89A) (Jeff Wirana, Addgene 24815) were used. The empty pCMV vector was a negative control. Cells were transfected using Lipofectamine 3000 (Invitrogen) according to the manufacturer’s instructions. Further details can be found in the Supplemental Experimental Procedures.

**KIBRA Mutagenesis**
KIBRA mutants were generated using Q5 site-directed mutagenesis (New England Biolabs) on a pENTR11-wild-type KIBRA vector, as detailed in the Supplemental Experimental Procedures.

**In Vivo Studies**
All procedures involving mice were reviewed and approved by the McGill University Facility Animal Care Committee (FACC) and performed in accordance with university and national guidelines. Female 6-week-old friend virus B/N (FVB/N) mice were used for orthotopic mammary tumor growth experiments, and female 6-week-old NCR athymic nude mice (Taconic) were used for metastasis assays (tail vein injection and primary tumor resection assays). Bioluminescence imaging was performed weekly using the Xenogen IVIS 100 (Caliper LifeSciences) as described previously (Knight et al., 2013). Mammary tumor growth was monitored by twice-weekly caliper measurements. Further details can be found in the Supplemental Experimental Procedures.

**Microscopy**
Phase contrast images were taken on an Axiovert 200M for adherent cells and an AxioScope Zoom for tumorspheres (both from Carl Zeiss). Immunofluorescence was imaged on an LSM800 confocal laser-scanning microscope (Carl Zeiss).

**YAP/TAZ Localization Assays**
Polyacrylamide hydrogels, immunofluorescent staining, and analysis are described in the Supplemental Experimental Procedures. Each experiment was conducted in triplicate. An average of 45 cells was scored per replicate per condition.

**PCRs**
Total RNA was isolated using the RNeasy mini kit (QIAGEN) and reverse-transcribed using the Transcripter First Strand cDNA Synthesis Kit (Roche). Real-time PCR was performed as described previously, normalizing to GAPDH and B2M (human) or Gapdh, Hprt, and Rp113a (mouse) (Knight et al., 2013). Primers (listed in the Supplemental Experimental Procedures) were designed using Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/).

**RHO-A Activity Assays and Actin Stress Fiber Scoring**
GST pull-downs and RHOA G-LISA assays (Cytoskeleton) are described in the Supplemental Experimental Procedures. Stress fibers were counted in ImageJ software, assessing a minimum of 12 cells per experiment in 3 experiments.

**BioID and Mass Spectrometry**
KIBRA constructs were cloned into pSTV2-BirA*-FLAG using Gateway LR cloning (Invitrogen). MDA-MB-231 expressing pSTV2-KIBRA constructs or vector alone were analyzed in biological duplicates. Expression and peptide isolation are described in the Supplemental Experimental Procedures.

**DATA AND SOFTWARE AVAILABILITY**
Supplemental Information includes Supplemental Experimental Procedures, seven figures, and four tables and can be found with this article online at https://doi.org/10.1016/j.celrep.2018.02.095.

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**DECLARATION OF INTERESTS**
The authors declare no competing interests.

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REFERENCES

Al-Mulla, F., AlFadhil, S., Al-Hakim, A.H., Going, J.J., and Bitar, M.S. (2006). Metastatic recurrence of early-stage colorectal cancer is linked to loss of heterozygosity on chromosomes 4 and 14. J. Clin. Pathol. 59, 624–630.

Aragona, M., Panciera, T., Manfrin, A., Giulitti, S., Michielin, F., Elvassoire, N., Dupont, S., and Piccolo, S. (2013). A mechanical checkpoint controls multicellular growth through YAP/TAZ regulation by actin-processing factors. Cell 154, 1047–1059.

Bartucci, M., Dattilo, R., Moriconi, C., Pagliuca, A., Mottolese, M., Federici, G., Benedetto, A.D., Todaro, M., Stassi, G., Sperati, F., et al. (2015). TAZ is required for metastatic activity and chemoresistance of breast cancer stem cells. Oncogene 34, 681–690.

Basu-Roy, U., Bayin, N.S., Rattanakorn, K., Han, E., Placantonakis, D.G., Mansukhani, A., and Basilico, C. (2015). Sox2 antagonizes the Hippo pathway to maintain stemness in cancer cells. Nat. Commun. 6, 6411.

Baumgartner, R., Poernbacher, I., Buser, N., Hafen, E., and Stocker, H. (2010). The WW domain protein Kibra acts upstream of Hippo in Drosophila. Dev. Cell 18, 309–316.

Belk, L. Ali, N., Lonc, A., Li, X., Patridge, J.L., Roslan, S., Hermann, D., Conway, J.R., Gehling, F.K., Bert, A.G., et al. (2015). The tyrosine phosphatase PTPN14 (Pez) inhibits metastasis by altering protein trafficking. Sci. Signal. 8, ra18.

Cancer Genome Atlas Network (2012). Comprehensive molecular portraits of human breast tumours. Nature 490, 61–70.

Cardiff, R.D., Anver, M.R., Gusterson, B.A., Hennighausen, L., Jensen, R.A., Merino, M.J., Rehm, S., Russo, J., Tavassoli, F.A., Wakefield, L.M., et al. (2000). The mammary pathology of genetically engineered mice: the consensus report and recommendations from the Annapolis meeting. Oncogene 19, 968–988.

Chan, S.W., Lim, C.J., Guo, K., Ng, C.P., Lee, I., Hunziker, W., Zeng, Q., and Hong, W. (2008). A role for TAZ in migration, invasion, and tumorigenesis of breast cancer cells. Cancer Res. 68, 2592–2598.

Chircop, M. (2014). Rho GTPases as regulators of mitosis and cytokinesis in mammalian cells. Small GTPases 5, e29770.

Cohn, K.H., Wang, F.S., Desoto-LaPAix, F., Solomon, W.B., Patterson, L.G., Arnold, M.R., Weinra, J., Feldman, J.G., Levy, A.T., Leone, A., et al. (1991). Association of nm23-H1 allelic deletions with distant metastases in colorectal carcinoma. Lancet 339, 722–724.

Cordonensii, M., Zanconato, F., Azzolin, L., Forcato, M., Rosato, A., Frasson, C., Inui, M., Montagnier, M., Parenti, A.R., Poletti, A., et al. (2011). The Hippo transducer TAZ confers cancer stem cell-related traits on breast cancer cells. Cell 147, 759–772.

Curtis, C., Shah, S.P., Chin, S.F., Turashvili, G., Rueda, O.M., Dunning, M.J., Speed, D., Lynch, A.G., Samariajiwa, S., Yuan, Y., et al.; METABRIC Group (2012). The genomic and transcriptomic architecture of 2,000 breast tumours reveals novel subgroups. Nature 486, 346–352.

Denkert, C., Liedtke, C., Tutt, A., and von Minckwitz, G. (2017). Molecular alterations in triple-negative breast cancer-the road to new treatment strategies. Lancet 389, 2430–2442.

Dupont, S., Morsut, L., Aragona, M., Enzo, E., Giulitti, S., Cordonensii, M., Zanconato, F., Le Digabel, J., Forcato, M., Bicciato, S., et al. (2011). Role of YAP/TAZ in mechanotransduction. Nature 474, 179–183.

Engler, A.J., Sen, S., Sweeney, H.L., and Discher, D.E. (2006). Matrix elasticity directs stem cell lineage specification. Cell 126, 677–689.

Foukas, W.D., Smith, I.E., and Reis-Filho, J.S. (2010). Triple-negative breast cancer. N. Engl. J. Med. 363, 1938–1948.

Garmin, N.J., Cornelis, H., Chiu, S.Y., O’Rourke, K.P., Arnaud, J., Yilmamadi, D., Thery, M., Camargo, F.D., and Pellman, D. (2014). Cytokinesis failure triggers hippo tumor suppressor pathway activation. Cell 158, 833–848.

Genevet, A., Wehr, M.C., Brain, R., Thompson, B.J., and Tapon, N. (2010). Kibra is a regulator of the Salvador/Warts/Hippo signaling network. Dev. Cell 18, 300–308.

Herschkowitz, J.I., Simin, K., Weigman, V.J., Mikaelian, I., Usary, J., Hu, Z., Rasmussen, K.E., Jones, L.P., Asselah, S., Chandrasekharan, S., et al. (2007). Identification of conserved gene expression features between murine mammary carcinoma models and human breast tumors. Genome Biol. 8, R76.

Hill, V.K., Dunwell, T.L., Catchpole, D., Krex, D., Brini, A.T., Griffiths, M., Craddock, C., Maher, E.R., and Latif, F. (2011). Frequent epigenetic inactivation of KIBRA, an upstream member of the Salvador/Warts/Hippo (SWH) tumor suppressor network, is associated with specific genetic event in B-cell acute lymphocytic leukemia. Epigenetics 6, 326–332.

Johannsdottir, H.K., Jonsson, G., Johannsdottir, G., Agnarsson, B.A., Erela, H., Arason, A., Heikkila, P., Eglisson, V., Olsson, H., Johannsson, O.T., et al. (2006). Chromosome 5 imbalance mapping in breast tumors from BRCA1 and BRCA2 mutation carriers and sporadic breast tumors. Int. J. Cancer 119, 1052–1060.

Knight, J.F., Lesurf, R., Zhao, H., Pinnaduawage, D., Davis, R.R., Saleh, S.M., Zuo, D., Naujokas, M.A., Chughtai, N., Herschkowitz, J.J., et al. (2013). Met synergizes with p53 loss to induce mammary tumors that possess features of claudin-low breast cancer. Proc. Natl. Acad. Sci. USA 110, E1301–E1310.

Kremerskothen, J., Plaa, S., Bütter, K., Finger, I., Veltel, S., Matanis, T., Liedtke, T., and Barneak, A. (2003). Characterization of KIBRA, a novel WW domain-containing protein. Biochem. Biophys. Res. Commun. 300, 862–867.

Lehmann, B.D., and Pietenpol, J.A. (2014). Identification and use of biomarkers in treatment strategies for triple-negative breast cancer subtypes. J. Pathol. 232, 142–150.

Lehmann, B.D., Jovanovic, B., Chen, X., Estrada, M.V., Johnson, K.N., Shyr, Y., Moses, H.L., Sanders, M.E., and Pietenpol, J.A. (2016). Refinement of Triple-Negative Breast Cancer Molecular Subtypes: Implications for Neoadjuvant Chemotherapy Selection. PLoS ONE 11, e0157368.

Levental, K.R., Yu, H., Kass, L., Laksin, J.N., Egeblad, M., Erler, J.T., Feng, S.F., Ciszars, K., Giaccia, A., Weninger, W., et al. (2009). Matrix crosslinking forces tumor progression by enhancing integrin signaling. Cell 139, 891–906.

Liu, C.Y., Zha, Z.Y., Zhou, X., Zhang, H., Huang, W., Zhao, D., Li, T., Chan, S.W., Lim, C.J., Hong, W., et al. (2010). The hippo tumor pathway promotes TAZ degradation by phosphorylating a phosphodegron and recruiting the SCFbeta-TrCP E3 ligase. J. Biol. Chem. 285, 2592–2598.

Moleinirho, S., Chang, N., Sims, A.H., Tilston-Lmean, A., Angus, L., Steele, A., Boswell, V., Barnett, S.C., Ormandy, C., Faratian, D., et al. (2013). KIBRA exhibits MST-independent functional regulation of the Hippo signaling pathway in mammals. Oncogene 32, 1821–1830.

Narumiya, S., Tanji, M., and Ishizaki, T. (2009). Rho signaling, ROCK and mDia1, in transformation, metastasis and invasion. Cancer Metastasis Rev. 28, 65–76.

Norton, R., Lambros, M.B., Rodriguez-Pinilla, S.M., Moreno-Bueno, G., Tan, D.S., Marchio, C., Vatcheva, R., Rayter, S., Mahler-Araujo, B., Fulford, L.G., et al. (2009). Tiling path genomic profiling of grade 3 invasive ductal breast cancers. Clin. Cancer Res. 15, 2711–2722.

Poembacher, I., Baumgartner, R., Marada, S.K., Edwards, K., and Stocker, H. (2012). Drosophila Pez acts in Hippo signaling to restrict intestinal stem cell proliferation. Curr. Biol. 22, 389–396.

Porzio, M.G., Lesurf, R., Petkiewicz, S., O’Malley, F.P., Pinnaduawage, D., Andrulis, I.L., Bull, S.B., Chughtai, N., Zuo, D., Souleimanova, M., et al. (2009).
Met induces mammary tumors with diverse histologies and is associated with poor outcome and human basal breast cancer. Proc. Natl. Acad. Sci. USA 106, 12903–12908.

Prat, A., and Perou, C.M. (2011). Deconstructing the molecular portraits of breast cancer. Mol. Oncol. 5, 5–23.

Prat, A., Parker, J.S., Karginova, O., Fan, C., Livasy, C., Herschkowitz, J.I., He, X., and Perou, C.M. (2010). Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast Cancer Res. 12, R68, Rakha, E.A., Elsheikh, S.E., Aleskandarany, M.A., Habashi, H.O., Green, A.R., Powe, D.G., El-Sayed, M.E., Benhasouna, A., Brunet, J.S., Akslen, L.A., et al. (2009). Triple-negative breast cancer: distinguishing between basal and non-basal subtypes. Clin. Cancer Res. 15, 2302–2310.

Ren, X.D., Kiosses, W.B., and Schwartz, M.A. (1999). Regulation of the small GTP-binding protein Rho by cell adhesion and the cytoskeleton. EMBO J. 18, 578–585.

Roux, K.J., Kim, D.I., Raida, M., and Burke, B. (2012). A promiscuous biotin ligase fusion protein identifies proximal and interacting proteins in mammalian cells. J. Cell Biol. 196, 801–810.

Skibinski, A., Breindel, J.L., Prat, A., Galván, P., Smith, E., Rolfs, A., Gupta, P.B., Labaer, J., and Kuperwasser, C. (2014). The Hippo transducer TAZ interacts with the SWI/SNF complex to regulate breast epithelial lineage commitment. Cell Rep. 6, 1059–1072.

Solinimi, N.L., Xu, Q., Merpel, C.H., Liang, A.C., Schlabez, M.R., Luo, J., Burrows, A.E., Anselmo, A.N., Bredemeyer, A.L., Li, M.Z., et al. (2012). Recurrent hemizygous deletions in cancers may optimize proliferative potential. Science 337, 104–109.

Sorrentino, G., Ruggeri, N., Specchia, V., Cordenonsi, M., Mano, M., Dupont, S., Manfrin, A., Inaglia, E., Sormaggio, R., Piazza, S., et al. (2014). Metabolic control of YAP and TAZ by the mevalonate pathway. Nat. Cell Biol. 16, 357–366.

Takahashi, Y., Miyoshi, Y., Takahata, C., Irahara, N., Taguchi, T., Tamaki, Y., and Noguchi, S. (2005). Down-regulation of LATS1 and LATS2 mRNA expression by promoter hypermethylation and its association with biologically aggressive phenotype in human breast cancers. Clin. Cancer Res. 11, 1380–1385.

Turner, N., Lambros, M.B., Horlings, H.M., Pearson, A., Sharpe, R., Natrajan, R., Geyer, F.C., van Kouwenhove, M., Kreike, B., Mackay, A., et al. (2010). Integrative molecular profiling of triple negative breast cancers identifies amplicon drivers and potential therapeutic targets. Oncogene 29, 2013–2023.

Weigman, V.J., Chao, H.H., Shabalina, A.A., He, X., Parker, J.S., Nordgard, S.H., Grushko, T., Huo, D., Nwachukwu, C., Nobel, A., et al. (2012). Basal-like Breast cancer DNA copy number losses identify genes involved in genomic instability, response to therapy, and patient survival. Breast Cancer Res. Treat. 133, 865–880.

Wilson, K.E., Li, Y.W., Yang, N., Shen, H., Orillion, A.R., and Zhang, J. (2014). PTPN14 forms a complex with Kibra and LAT51 proteins and negatively regulates the YAP oncogenic function. J. Biol. Chem. 289, 23693–23700.

Xiao, L., Chen, Y., Ji, M., and Dong, J. (2011). KIBRA regulates Hippo signaling activity via interactions with large tumor suppressor kinases. J. Biol. Chem. 286, 7788–7796.

Xue, W., Kitzing, T., Roessler, S., Zuber, J., Krasnitz, A., Schultz, N., Revill, K., Weissmueller, S., Rappaport, A.R., Simon, J., et al. (2012). A cluster of cooperating tumor-suppressor gene candidates in chromosomal deletions. Proc. Natl. Acad. Sci. USA 109, 8212–8217.

Yu, J., Zheng, Y., Dong, J., Kluza, S., Deng, W.M., and Pan, D. (2010). Kibra functions as a tumor suppressor protein that regulates Hippo signaling in conjunction with Merlin and Expanded. Dev. Cell 18, 288–299.

Zanconato, F., Battilana, G., Cordenonsi, M., and Piccolo, S. (2016). YAP/TAZ as therapeutic targets in cancer. Curr. Opin. Pharmacol. 29, 26–33.