YAP Regulates Actin Dynamics through ARHGAP29 and Promotes Metastasis

Graphical Abstract

Highlights

- YAP transcriptionally upregulates ARHGAP29, a suppressor of RhoA
- YAP reduces the cytoskeleton rigidity by promoting actin depolymerization via ARHGAP29
- YAP regulates the turnover of F- and G-actin to promote cancer metastasis

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In Brief
Qiao et al. discover a role for YAP in regulating actin dynamics. YAP activates the transcription of ARHGAP29 to suppress RhoA activity, resulting in F-actin depolymerization, which reduces cytoskeletal rigidity and promotes a metastatic phenotype.

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YAP Regulates Actin Dynamics through ARHGAP29 and Promotes Metastasis

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SUMMARY
Yes-associated protein (YAP) is regulated by mechanical cues via the interaction of the Hippo pathway with cytoskeleton. Previous studies showed that YAP plays a role in regulating the actomyosin network by suppressing Rho GTPase in medaka fish. Here, we identify Rho GTPase activating protein 29 (ARHGAP29) as a transcriptional target of YAP in a human gastric cancer cell line. YAP promotes the expression of ARHGAP29 to suppress the RhoA-LIMK-cofilin pathway, destabilizing F-actin. The overexpression of YAP causes cytoskeletal rearrangement by altering the dynamics of F-actin/G-actin turnover, thus promoting migration. In a mouse model, circulating tumor cells (CTCs) exhibit an increased ARHGAP29 RNA level compared with cells at primary tumor sites, and the metastatic potential of CTCs is positively correlated with ARHGAP29 expression. Moreover, increased ARHGAP29 expression is correlated with shortened survival of human gastric cancer patients. Our study provides a model to understand YAP’s contribution to cancer metastasis via regulation of actin dynamics.

INTRODUCTION
Yes-associated protein (YAP) is a potent oncprotein, functioning as a transcriptional co-activator that responds to transducer of mechanical cues (Halder et al., 2012). In addition to canonical Hippo pathway signaling, which results in YAP phosphorylation and cytoplasmic retention in response to cell-cell contact, YAP’s subcellular localization can also be regulated by cellular tension, morphology, and the rigidity of the extracellular matrix (ECM) (Low et al., 2014; Wada et al., 2011; Yu and Guan, 2013). In Drosophila, spectrin, an actin-binding protein that localizes to the intracellular side of the plasma membrane, serves as a clustering center for the upstream regulators of the Hippo pathway (Deng et al., 2015). Stretching of spectrin in response to mechanical stimuli increases the spatial distance between Hippo complexes, causing a reduction of Hpo and Wts activation and an increase in nuclear Yki (Deng et al., 2015; Fletcher et al., 2015; Wong et al., 2015). In parallel, the actomyosin network and Rho GTPase signaling are known to contribute to YAP’s translocation upon mechanical stimulation (Dupont et al., 2011).

Nuclear YAP associates with TEAD proteins to promote the transcription of pro-proliferative and pro-migratory genes such as CTGF and Cyr61 (Zhao et al., 2008). Stringently regulated YAP activity is thus crucial for appropriate tissue growth and/or homeostasis, with abnormally accumulated nuclear YAP indicative of poor prognosis in many types of cancer, including liver, breast, and gastric carcinomas (Zanchonato et al., 2016).

Recently, mutation of YAP in the medaka fish mutant, hirame (hir), in which the transcriptional activation domain of YAP had been deleted, was identified as the cause of severe tissue flattening and misalignment in the embryo. This was due to deregulation of the cortical actomyosin network, affecting tissue tension and fibronectin assembly (Porazinski et al., 2015). This finding indicates that YAP is not only a transducer of mechanical cues but also a regulator of cellular mechanical behavior.

We studied YAP’s role in the regulation of F- and G-actin turnover, which is an important process to maintain cytoskeletal homeostasis in mammalian cells. As a transcriptional co-activator, YAP upregulates the expression of ARHGAP29, a Rho GTPase activating protein and known regulator of the RhoA-LIMK-cofilin pathway. Cofillin, an actin depolymerizing factor, is functionally blocked by the activation of this pathway. Therefore, loss of YAP reduces the expression of ARHGAP29, increasing RhoA activity to inhibit cofillin, eventually resulting in the stabilization of actin filaments. Importantly, this YAP-ARHGAP29-actin
depolymerization signaling axis is positively correlated with cancer metastasis.

RESULTS

YAP Negatively Regulates F-Actin Levels

In healthy gastric epithelium, YAP is minimally expressed, whereas YAP is frequently upregulated in cancerous gastric epithelium (Zhang et al., 2012). The MKN28 cell line was previously identified as one of the highest YAP-expressing gastric cancer cell lines (Qiao et al., 2016); thus it was used here as a model of human gastric carcinoma.

Characterization of YAP knockout cells by fluorescent phalloidin staining showed that actin filaments were more prominent. Elongated stress fibers, which were primarily observed in YAP-null cells, were shortened and weakened when exogenous YAP was reintroduced into the YAP-null cells (Figures 1A and 1B). A similar phenotype was observed in a mouse mammary gland epithelial cell line (EPH4) (Figure S1A). In addition, a greater proportion of F-actin was fractionated in the YAP-null cells when F- and G-actin were separated by differential sedimentation (Figure 1C). In wild-type cells, actin polymerization increased with cell density, but this was not observed in YAP-null cells (Figures 1D and 1E), suggesting that canonical Hippo signaling might affect cell density-dependent actin polymerization via YAP. As a major component of the cytoskeleton, actin polymerization is critical for maintaining cell rigidity (Calzado-Martín et al., 2016). Using atomic force microscopy (AFM) (Figure 1F), we showed that YAP-null cells were twice as stiff as wild-type or YAP-rescue cells (Figure 1G).

YAP Transcriptionally Regulates ARHGAP29, a Rho GTPase Activating Protein

Rho GTPase activating proteins (ARHGAPs) are important regulators of actin dynamics that inhibit the Rho-family of small GTPases (Moon and Zheng, 2003). In the hir medaka fish mutant, ARHGAP18 was downregulated by 20% by the loss of YAP transcriptional activity, and reintroduction of ARHGAP18 partially rescued the mutant phenotype (Porazinski et al., 2015). In another study, five ARHGAP transcripts were upregulated when YAP was overexpressed in human MCF10A cells (Figure 2A), among which ARHGAP29 showed the largest increase (Zhao et al., 2008). In MKN28 cells, the expression of ARHGAP29 RNA decreased by 50% in YAP-null cells, and this decrease could be reversed by reintroduction of YAP (Figures 2C and S2).

Two regions in the ARHGAP29 promoter, which contain the consensus binding sequence for the TEAD-YAP transcriptional complex (Figure 2D), were enriched by antibodies targeting TEAD4 and YAP during chromatin immunoprecipitation (ChIP)
experiments (Figures 2E–2G). When this 1,500 bp promoter was inserted upstream of the luciferase open reading frame, co-expression of TEAD4 and YAP increased luciferase activity. In contrast, the mutant promoter, in which both TEAD-consensus sequences were deleted, showed no significant response (Figures 2H and 2I). These experiments indicate that the TEAD-YAP complex physically binds to the ARHGAP29 promoter, and that ARHGAP29 is a bona fide transcriptional target of this complex.

YAP Suppresses F-Actin Formation Partially via ARHGAP29-RhoA-LIMK-Cofilin Signaling

ARHGAP29 suppresses RhoA activity by accelerating the conversion of GTP-bound (active) RhoA to its GDP-bound (inactive) form (Post et al., 2015). Only GTP-bound RhoA can activate Rho-associated kinase (ROCK), which then activates LIM kinase 1 and 2 (LIMK1/2) by phosphorylation of Thr508 and Thr505, respectively. Cofilin, a potent actin depolymerizing factor, is one of LIMK's major substrates. Phosphorylation of cofilin at Ser3 by LIMK blocks its activity, resulting in the stabilization of actin filaments (Maekawa et al., 1999; Sumi et al., 1999).

Compared with wild-type cells, YAP-null cells had remarkably reduced ARHGAP29 protein expression, and exhibited a higher level of active RhoA, activated LIMK and Ser3-phosphorylated cofilin, all of which could be rescued by reintroduction of YAP or inhibition of Rho activity (Figure 3A). Elevated RhoA activity was also observed in YAP-null cells (Figures S1B and S1C). Consistent with previous studies indicating that ARHGAP29 shows specificity for interaction with RhoA compared with other Rho GTPases (Xu et al., 2011), the activity of Rac1 and Cdc42 was not changed in YAP-null cells (Figure S3). Moreover, the increased activity of the RhoA-ROCK-LIMK-cofilin pathway in YAP-null cells was consistent with our previous observation that YAP-null cells exhibited more stress fibers (Figure 1).

In wild-type cells, small interfering RNA (siRNA)-mediated knockdown of ARHGAP29 increased the level of Ser3-phosphorylated cofilin to a similar level as that observed in YAP-null cells (Figure S3B). Interestingly, when ARHGAP29 was reduced to a minimal level in YAP-null cells, there was no additional increase of Ser3-phosphorylated cofilin (Figure 3B). This indicates that loss of YAP is sufficient to reduce ARHGAP29 to a functionally negligible level.

Consistent with previous results indicating that ARHGAP29 regulates RhoA-LIMK-cofilin signaling, overexpression of ARHGAP29 decreased the Ser3 phosphorylation of cofilin in YAP-null cells.
The ratio of F- and G-actin was also significantly reduced by ARHGAP29 overexpression in YAP-null cells (Figures 3D and 3E). Taken together, reintroduction of ARHGAP29 into YAP-null cells partially suppresses the increase of F-actin caused by the loss of YAP.

YAP Promotes Metastasis by Modulating Actin Turnover

Neoplastic cells often use an amoeboid modality to migrate through extracellular matrix, requiring a flexible cytoskeleton (Sanz-Moreno and Marshall, 2010). The more rigid cytoskeleton of YAP-null cells is expected to adversely affect cell migration. Wound healing assays (in 2D), as well as trans-well migration and invasion assays (in 3D) are common means to evaluate the tendency for cell migration in vitro. In trans-well migration and invasion assays, significantly more wild-type cells migrated through the membrane compared with YAP-null cells, and overexpression of ARHGAP29 either knocked down using small interfering RNA (B) or overexpressed (C) in WT and YAP K/O MKN28 cells, and the abundance of total and inactive (phospho-Ser3) cofilin, ARHGAP29, and YAP was analyzed by western blotting.

Intriguingly, no obvious difference was observed between wild-type and YAP-null cells in the wound-healing assays (Figures S4 C and S4D). This could be explained by the elevated RhoA activity in YAP-null cells offsetting some of the adverse effects of a rigid cytoskeleton by increasing cell contractility during migration across a flat surface (Raftopoulou and Hall, 2004).

Circulating tumor cells (CTCs) are a commonly acknowledged source of distal metastases in malignant cancer (Massagué and Obenauf, 2016). Dynamic cytoskeletal rearrangement is critical for a primary tumor cell to become a CTC. In a previously published mouse model of pancreatic cancer (Ting et al., 2014), expression of ARHGAP29 was found to be significantly elevated in CTCs compared with single cells extracted from primary tumors or white blood cells (WBCs) (Figure 4C). This suggests that ARHGAP29 contributes to the cytoskeletal rearrangement during the transition from primary tumor cell to CTCs. On the basis of gene set enrichment analysis (GSEA), CTCs exhibiting increased ARHGAP29 expression had higher YAP activity (Figure 4D), further supporting our hypothesis that regulation of actin dynamics via YAP-ARHGAP29 signaling promotes metastasis. Moreover, in an orthotopic spontaneous hepatocellular carcinoma (HCC) metastasis assay, cells with silenced ARHGAP29 had a significantly impaired ability to metastasize to the lung in vivo (Figures S4 E–S4G). The human relevance of these observations was reinforced by a survival analysis of 876 human gastric cancer patients among whom those with lower ARHGAP29 expression level had significantly improved overall survival (Figure 4F).

Here, we have shown that YAP plays a critical role in modulating actin disassembly in a gastric cancer cell model. YAP activates the transcription of ARHGAP29 to suppress RhoA activity, resulting in F-actin depolymerization. The increased
Figure 4. YAP Promotes Cell Migration via Regulation of ARHGAP29

(A and B) Wild-type (WT) and YAP knockout (YAP K/O) MKN28 cells stably overexpressing empty vector or ARHGAP29 were used for trans-well migration assays (A) and Matrigel invasion assays (B). Migrated cells on the lower membrane surface were stained with crystal violet and counted in three randomly chosen fields, and the average numbers and SD of cells per field are shown. The data were from three independent experiments (a t test was performed, n = 3, ***p < 0.001).

(C) The expression profile of ARHGAP29 was extracted from an RNA sequencing database of a mouse pancreatic cancer model comparing circulating tumor cells (CTC; n = 75), single cells from primary tumor (tumor, n = 20), and white blood cells (WBC; n = 12) (Ting et al., 2014). Mean ± SD was plotted. A t test was performed (**p < 0.01).

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turnover of F- to G-actin softens the cytoskeleton to promote a metastatic phenotype (Figure 4G).

**DISCUSSION**

**The Hippo-YAP Pathway Is a Key Regulator of the Actin Cytoskeleton**

It has been repeatedly shown that increased actin polymerization induces the nuclear accumulation of YAP (Das et al., 2016; Dupont et al., 2011). Our findings suggest that YAP in turn suppresses the polymerization of actin by regulation of ARHGAP29 and the RhoA-LIMK-cofilin pathway. By this negative feedback loop, YAP plays a central role in fine-tuning actin cytoskeleton dynamics, which is consistent with the study of hir medaka mutant (Porazinski et al., 2015), as well as a mouse kidney model in which YAP knockout causes abnormal nephron induction and morphogenesis (Reginensi et al., 2013).

We report here that ARHGAP29 is a downstream target of YAP, which regulates RhoA activity. Important to note is that in the hir medaka mutant, a different ARHGAP (ARHGAP18) was identified as the major downstream effector of YAP (Porazinski et al., 2015). This disparity suggests that YAP may regulate multiple RhoGAPs in various tissues (Figure S2). In addition to RhoGAPs, YAP has been shown to promote the expression of myosin light chain 9 in mouse hepatocytes, indicating that YAP regulates not only actin reorganization but also myosin-dependent contraction (Bai et al., 2016). Thus, YAP is a key regulator of the cytoskeleton in mammalian cells.

Interestingly, in Drosophila, the Hpo kinase cassette was reported to modulate actin organization independently of Yorkie by phosphorylation of Enabled (Ena), an actin assembly and elongation regulator (Lucas et al., 2013; Sakuma et al., 2016). Within ovary border cells, Ena drives actin polymerization and cortical protrusions by competing with F-actin capping proteins. Wts phosphorylates and suppresses Ena, activating F-actin capping proteins on inner membranes where more Hpo kinase complex is present. This signaling event restricts F-actin polymerization to the outer rim of the migrating cluster to regulate motility of cell clusters (Lucas et al., 2013). Furthermore, at neuromuscular synapses, Wts-mediated Ena phosphorylation activates Arp2/3, resulting in the assembly of branched actin to form satellite boutons (Sakuma et al., 2016).

From these studies, we see that in contrast to their opposite effects on cell growth, YAP and Hippo pathway components both appear to regulate actin dynamics but via different mechanisms. Specifically, activation of YAP suppresses RhoA activity and linear F-actin elongation, while activation of Hpo kinase activates F-actin capping proteins, leading to branched actin elongation (Lucas et al., 2013). Moreover, whereas YAP regulates ARHGAPs at the level of transcription, which is temporal, Hpo kinase spatially regulates the actin branching process. This provides an interesting model to explain how cells deform and migrate in response to their micro-environment, highlighting the importance of the Hippo-YAP pathway in regulating actin dynamics, which is essential for development, tissue homeostasis, and tumorigenesis.

**YAP Promotes Metastasis via Multiple Mechanisms**

YAP promotes proliferation, metastasis, and drug resistance, resulting in poor prognosis (Figure 4G) (Zanconato et al., 2016). Among these processes, metastasis is the major cause of cancer-related death, and YAP is known to contribute to metastasis via multiple mechanisms (Massague and Obenauf, 2016). First, YAP interacts with TEAD and FOS in the nucleus to reprogram gene expression to induce epithelial-mesenchymal transition (EMT), characterized by a loss of cell-cell adhesions and apical-basal polarity and acquisition of mesenchymal motility (Shao et al., 2014). In addition, YAP antagonizes E-cadherin junction assembly by regulating actin cytoskeleton organization, which also contributes to EMT (Bai et al., 2016). Second, as shown here, YAP expression decreases the rigidity of cancer cells. Softening of the cytoskeleton is frequently observed during cancer progression, which assists cancer cells to squeeze through extracellular matrix and extravasate through vasculature to achieve distal metastasis via the circulation (Cross et al., 2007). Conversely, YAP activation actually promotes stiffening of the extracellular matrix of cancer-associated fibroblasts (CAFs) to reinforce YAP nuclear localization in cancer cells (Calvo et al., 2013). Such interplay between cancer cells and CAFs might amplify the effects of YAP during tumorigenesis.

Finally, the plasma membrane of YAP-null cells could be detached from the cytoskeleton more easily upon negative pressure compared with wild-type cells (Figure S3). This suggests that YAP enhances membrane-cytoskeletal integrity, which increases cell viability when they travel through narrow spaces such as capillary vessels during metastasis (Arpin et al., 2011; Elliott et al., 2005). However, the mechanism of this regulation is not yet clear, and future studies should investigate this further.

Our study of YAP’s regulatory role of actin cytoskeleton dynamics provides a model to understand of YAP’s function in metastasis. Furthermore, the link identified here between YAP and Rho GTPase activity presents an innovative opportunity for the development of an indirect therapeutic targeting strategy for Rho GTPases in cancer, because previous drug discovery efforts have deemed them “undruggable” because of systemic toxicity (Lin and Zheng, 2015).

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Transfections**

MKN28 cells (a gift from Professor Yoshiaki Ito’s group) were grown in RPMI-1640 medium (Nacalai Tesque), supplemented with 10% fetal bovine serum (FBS; HyClone) and 100 U/mL penicillin/streptomycin (Pen/Strep; Nacalai Tesque). HEK293T and EPH4-EV (purchased from ATCC) and HCCLM3-Luc (a gift from Professor Yoshiaki Ito’s group) were grown in RPMI-1640 medium (Nacalai Tesque), supplemented with 10% fetal bovine serum (FBS; HyClone) and 100 U/mL penicillin/streptomycin (Pen/Strep; Nacalai Tesque).
from Professor Hui Kam Man’s group) cell lines were grown in DMEM (Nacalai Tesque), supplemented with 10% FBS and 100 U/mL Pen/Strep.

For the generation of YAP knockout cells, guide RNA (gRNA) was designed using the online CRISPR design tool (http://crispr.mit.edu), and oligos harboring the targeting gRNA were inserted into the CRISPR/NuclEase Vector with OFP Reporter (Thermo Fisher Scientific). Cells were transected using Lipofectamine2000 (Thermo Fisher Scientific), sorted as single cells into 96-well plates by fluorescence-activated cell sorting, and validated for YAP knockout by western blot. YAP-knockout cells were transduced by retrovirus carrying pRetroX-Tet-on (Clontech), in addition to retrovirus carrying empty vector or pRetroX-tight-puro-Flag-YAP to generate a stable, inducible YAP-rescue cell line. Cells were selected using 1.5 mg/mL Genetecin (Thermo Fisher Scientific) and 1 μg/mL puromycin (Thermo Fisher Scientific) for 2 weeks. Doxycycline hydrochloride 0.1 μg/mL (Sigma-Aldrich) was added to the medium for 24 hr for experiments requiring pRetro-tight-Flag-YAP over-expression. ARHGAP29 was overexpressed using the pBABE-puro retrovirus system.

HCC-LM3-Luc cells were transduced by lentivirus carrying scramble shCTR or shARHGAP29 (#1-TRCN0000426540 and #2-TRCN0000429397, Sigma-Aldrich), and were selected using 2 μg/mL puromycin to generate stable cell lines.

All small interfering RNA was transfected using Lipofectamine iMAX (Thermo Fisher Scientific) according to the manufacturer’s instructions.

The sequence information for all gRNA, cloning primers, and small interfering RNAs used is provided in Supplemental Experimental Procedures.

**Actin Segmentation by Ultracentrifugation**

Cells were lysed directly in the dish using actin stabilization buffer (50 mM PIPES [pH 6.9], 50 mM NaCl, 5 mM MgCl₂, 5 mM EGTA, 2 mM ATP, 5% glycerol, 0.1% Nonidet P-40, 0.1% Triton X-100, 0.1% Tween 20, 0.1% i-mercaptoethanol, 1:100 protease inhibitor mixture [Sigma-Aldrich], and 1:100 phosphatase inhibitor mixture [Sigma-Aldrich]) for 10 min at 37°C before being collected into Eppendorf tubes by scraping, followed by centrifugation at 300 × g at room temperature to remove insoluble particles. The protein concentration was determined using the Bradford method. Cell lysates were diluted with actin stabilization buffer to achieve the same concentration among all samples, and an equal volume of diluted lysates was transferred to fresh tubes for fractionation. An aliquot of the diluted cell lysates (10% of the volume) was kept separately as “total protein inputs,” to which Laemmli buffer was added before being boiled and analyzed by western blot.

The F-actin and G-actin pools of the diluted cell lysates were separated by ultracentrifugation at 100,000 × g for 1 hr at 37°C. After centrifugation, F-actin sediments while G-actin remains in the supernatant, because of their different molecular weights. The supernatant containing the G-actin pool was removed to a fresh tube, while the pellet containing F-actin was resuspended in cold distilled water with 1 μM cytochalasin D (Sigma-Aldrich) and kept on ice for 45 min to dissolve F-actin. Laemmli buffer was added to both fractions before being boiled and analyzed by western blotting.

After boiling with Laemmli buffer, both F-actin and G-actin became β-actin monomers, which can be detected using an antibody against β-actin by western blotting. The band intensity of β-actin was determined using ImageJ for F-actin and G-actin fractions, and their ratio was plotted as column graphs.

**AFM**

A silicon AFM tip with a 4.5 μm diameter polystyrene bead (Novascan) was first mounted onto the JPK NanoWizard II before placing on the stage of an Olympus IX81 microscope. After the system was stabilized at 37 ± 1°C, the tip was then calibrated in situ using thermal fluctuation analysis in JPK SPMC/Control Software version 4. Spring constants ranged from 0.025–0.03 N/m. Force mapping was subsequently conducted on single cells adhered to the cover glass. For each indentation, the following parameters were used: 0.2 nN set point, 4.0 μm Z length, extend speed of 2.0 μm/s with constant duration of 2.0 s, and a sample rate of 2,048 with Z closed loop. Indentations were carried out in a 1.5 × 1.5 μm grid (with 8 × 8 resolution) at a consistent location beside each cell nucleus. Twelve cells were probed for each cell line. JPKSPM Data Processing software was used to process the raw data obtained from the procedure described. Batch processing of each force map was conducted using the Hertz-fit process.

Before fitting each curve, the curve is off-set and tilted to ensure that the baseline is horizontal. Subsequently, Young’s modulus could be obtained by fitting the Hertz model to the extend curve within the range of ~300 nm to 1.0 μm. At least 40 points were used to calculate the average Young’s modulus of each cell. Twelve cells from each cell line were measured to obtain the average stiffness. A two-tailed Student’s t test with unequal variance was finally used to compare among the three cell lines.

**Bioinformatic Analysis**

**Mouse Pancreatic Cancer Dataset**

Data were downloaded from GSE51372 (Ting et al., 2014). The sample sizes of the groups were single cells circulating in mouse blood enriched for tumor cells (CTC, n = 75), single cells from pancreatic tumor (n = 20), and single mouse white blood cells (n = 12). Data analysis was performed using the partek genomic suite. The normalized reads of RNA sequencing samples were imported. The zero values were replaced by 0.3 to enable log₂ transformation. The expression of ARHGAP29 was extracted from the database. Seventy-five CTCs were grouped on the basis of the medium expression of ARHGAP29. Gene set enrichment analysis version 2.0 was performed. The gene sets extracted from broad institute libraries C6: oncogenic signature were as follows: CORDENOSI_YAP_CONSERVED_SIGNATURE and LI400 METASTASIS.

**Human Gastric Cancer Dataset**

Kaplan-Meier plotter (http://kmplot.com/privates) was used to perform survival analysis (Szász et al., 2016). Eight hundred seventy-six gastric cancer patients were grouped at lower quartile according to the expression level of ARHGAP29 (low-ARHGAP29 group, n = 219; high-ARHGAP29 group, n = 657). The Kaplan-Meier survival plot was generated, and the hazard ratio with 95% confidence intervals and log-rank p value were calculated.

**Orthotopic HCC Spontaneous Metastasis Model**

All procedures involving animals were reviewed and approved by the SingHealth Animal Use and Care Committee (AUC/C Reference No. 2014/SHS/0967). Five million human HCCLM3 cells expressing firefly luciferase (HCCLM3-Luc-shCTR or HCCLM3-Luc-shARHGAP29) in 0.05 mL were injected subcutaneously into the rear flank of 10-week-old female BALB/c nude mice (Invivos). When the tumor size reached approximately 1 cm³, the tumor was harvested, cut into 1 mm³ pieces, and surgically implanted into the liver of new BALB/c nude mice (six mice per group).

Tumor growth was monitored weekly by bioluminescent imaging using the IVIS camera system (Xenogen). Tumor-bearing mice were euthanized once the tumor signal reached 1 × 10¹⁰ photons per second (p/s). For ex vivo imaging, 150 mg/kg D-luciferin (Xenogen) was injected into the mice intraperitoneally just before necropsy. Lungs were excised, placed into tissue culture dishes, and imaged for 1 min. Regions of interest in the displayed images were quantified using Living Image software (Xenogen).

**General Statistical Analysis**

Experimental data are presented as the mean ± SD or mean ± SE. Two-tailed Student’s t tests with unequal variance were performed using GraphPad Prism 5 (GraphPad Software). Differences were considered statistically significant when p values were less than 0.05 (*p < 0.05, **p < 0.01, ***p < 0.001).

Please refer to Supplemental Experimental Procedures for a detailed description of other experiments.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures and five figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2017.04.075.

**AUTHOR CONTRIBUTIONS**

Y.Q. and M.S. designed the study. Y.Q. performed most experiments and wrote the manuscript. J.C. and V.P.S. conducted trans-well assays, bioinformatic analysis, and metastasis mice modeling. Y.B.L., C.T.L., and H.S. performed AFM and micropippette aspiration assays. M.L.F.-E. designed and cloned CRISPR-Cas9 constructs, generated EFH4 YAP knockout cells.
and revised the draft. L.Q. helped with the confocal imaging of EPH4 cells. T.J. and B.C.L. helped with RBD and PBD assays.

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