

Supplementary Materials for
**Cytokinesis involves a nontranscriptional function of the Hippo
pathway effector YAP**

Duyen Amy Bui, Wendy Lee, Anne E. White, J. Wade Harper, Ron C. J. Schackmann,
Michael Overholtzer, Laura M. Selfors, Joan S. Brugge*

*Corresponding author. E-mail: joan_brugge@hms.harvard.edu

Published 1 March 2016, *Sci. Signal.* **9**, ra23 (2016)
DOI: 10.1126/scisignal.aaa9227

This PDF file includes:

Fig. S1. Immunofluorescence analysis of YAP-depleted cells.
Fig. S2. YAP hairpins.
Fig. S3. Spindle misorientation after YAP knockdown.
Fig. S4. YAP phospho-mutant combinations.
Fig. S5. Phosphorylation of MLC is increased by expression of the YAP 3A mutant.
Fig. S6. YAP and PATJ coimmunoprecipitation.
Fig. S7. Immunofluorescence of PATJ-depleted cells.
Legend for table S1
Legends for movies S1 to S10

Other Supplementary Material for this manuscript includes the following:
(available at www.sciencesignaling.org/cgi/content/full/9/417/ra23/DC1)

Table S1 (Microsoft Excel format). HCIPs for YAP wild type, YAP 3D, and YAP 3A.
Movie S1 (.mov format). Mitosis of MCF-10A cells depleted of YAP with five distinct shRNA sequences.
Movie S2 (.mov format). Mitosis of YAP-depleted MCF-10A cells expressing GFP-tubulin.
Movie S3 (.mov format). Mitosis of MCF-10A cells treated with YAP siRNA.
Movie S4 (.mov format). Mitosis of YAP-depleted MCF-10A cells in a monolayer.
Movie S5 (.mov format). Mitosis of YAP-depleted HeLa cells.
Movie S6 (.mov format). Mitosis of YAP-depleted multiple HeLa cells.

Movie S7 (.mov format). RHOA localization in a mitotic YAP-depleted MCF-10A cell.

Movie S8 (.mov format). Mitosis of YAP-depleted MCF-10A cells treated with C3 transferase or Y-27632.

Movie S9 (.mov format). Mitosis of MCF-10A cells expressing wild-type YAP or YAP 3A or YAP 3D mutants.

Movie S10 (.mov format). Mitosis of PATJ-depleted MCF-10A cells.

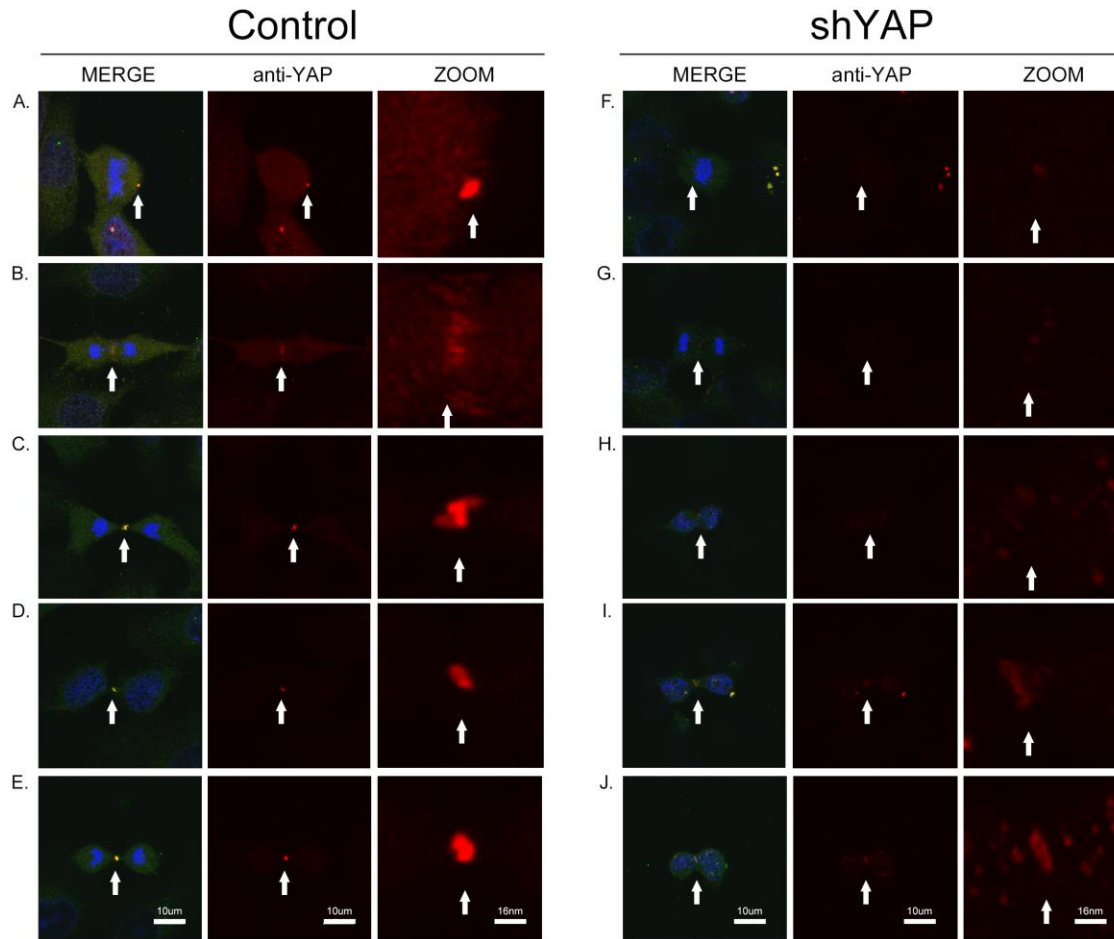


Fig. S1

Immunofluorescence analysis of YAP-depleted cells

Representative images showing localization of YAP (red) and Dapi (blue) for control LKO (**A-E**) or shYAP (**F-J**) cells in early mitosis and cytokinesis. Scale bar = 16 nm in zoomed images and 10 μ m in all others. Representative images from 60 control cells and 50 shYAP cells from two independent experiments.

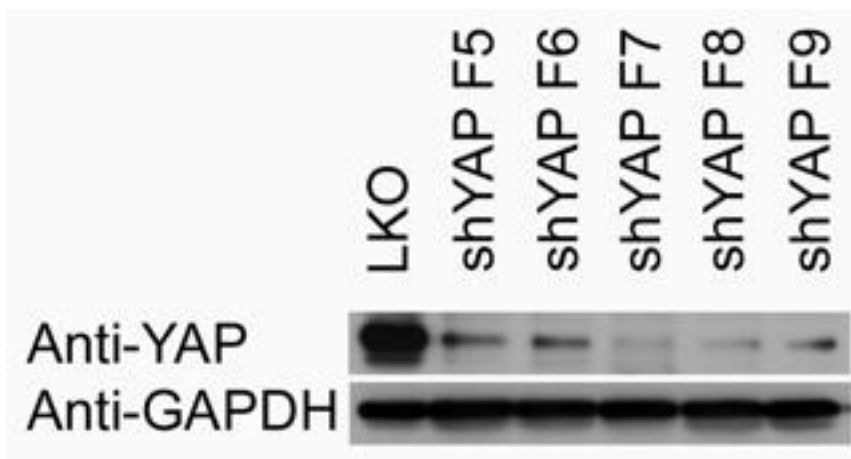


Fig. S2

YAP hairpins

Western blot showing YAP abundance in MCF-10A cells after knockdown with five different shRNA sequences. LKO is empty-vector control and GAPDH was used as loading control. Immunoblots are representative of two independent experiments.

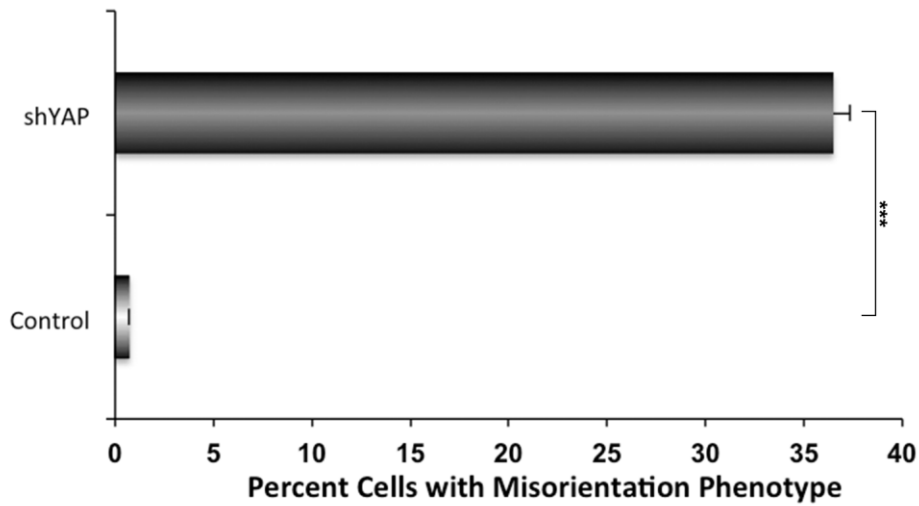


Fig. S3

Spindle misorientation after YAP knockdown

Quantification of spindle misorientation during cell division from time-lapse movies of cells after knockdown of YAP. Quantification was performed on 277 YAP knockdown cells and 2206 control cells from three independent experiments. Bars represent mean+SEM of three experiments each performed in triplicate. Statistical significance (***) $P < 0.001$; ** $P < 0.01$; * $P < 0.05$; NS, $P > 0.05$) was assessed by unpaired Student's *t*-test versus control LKO.

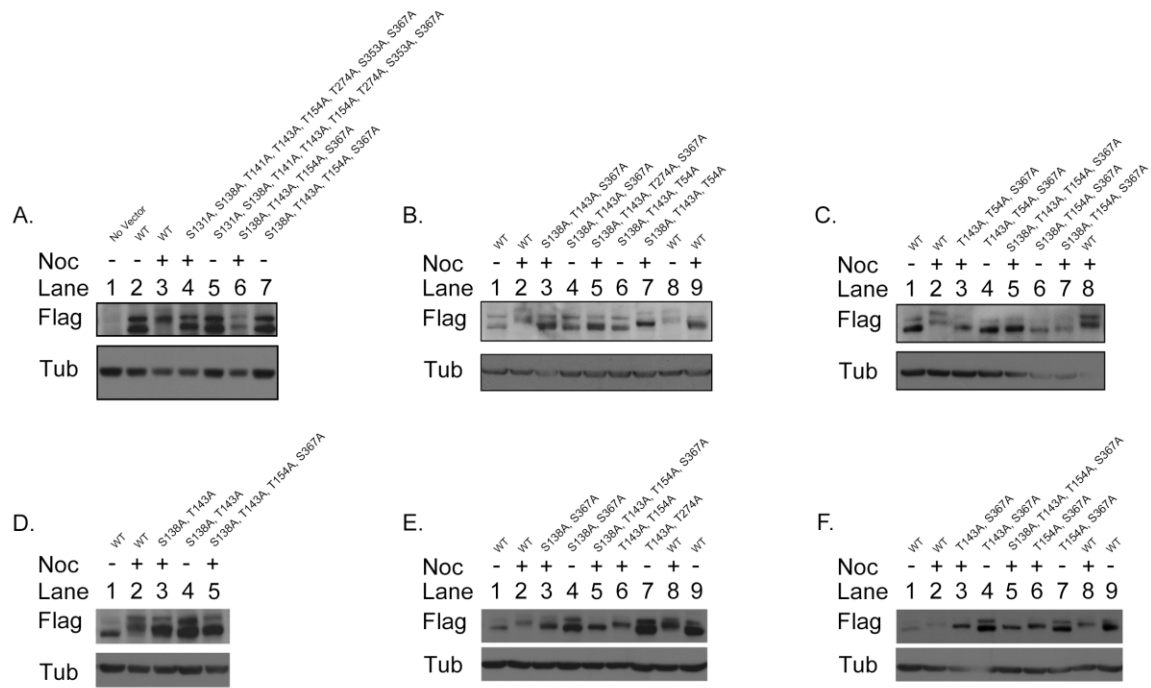


Fig. S4

YAP phospho-mutant combinations

(A) Anti-Flag and anti-Tubulin immunoblot of extracts from HeLa cells expressing different mutant variants of Flag-YAP in which all eight or four serine or threonine sites were mutated to alanine. Alternate lanes of the gels show cells treated with nocodazole. **(B and C)** Same as panel A except three serine or threonine sites on Flag-YAP were mutated into alanine. **(D to F)** Same as A except two serine or threonine sites were mutated to alanine. Immunoblots are representative of two independent experiments.

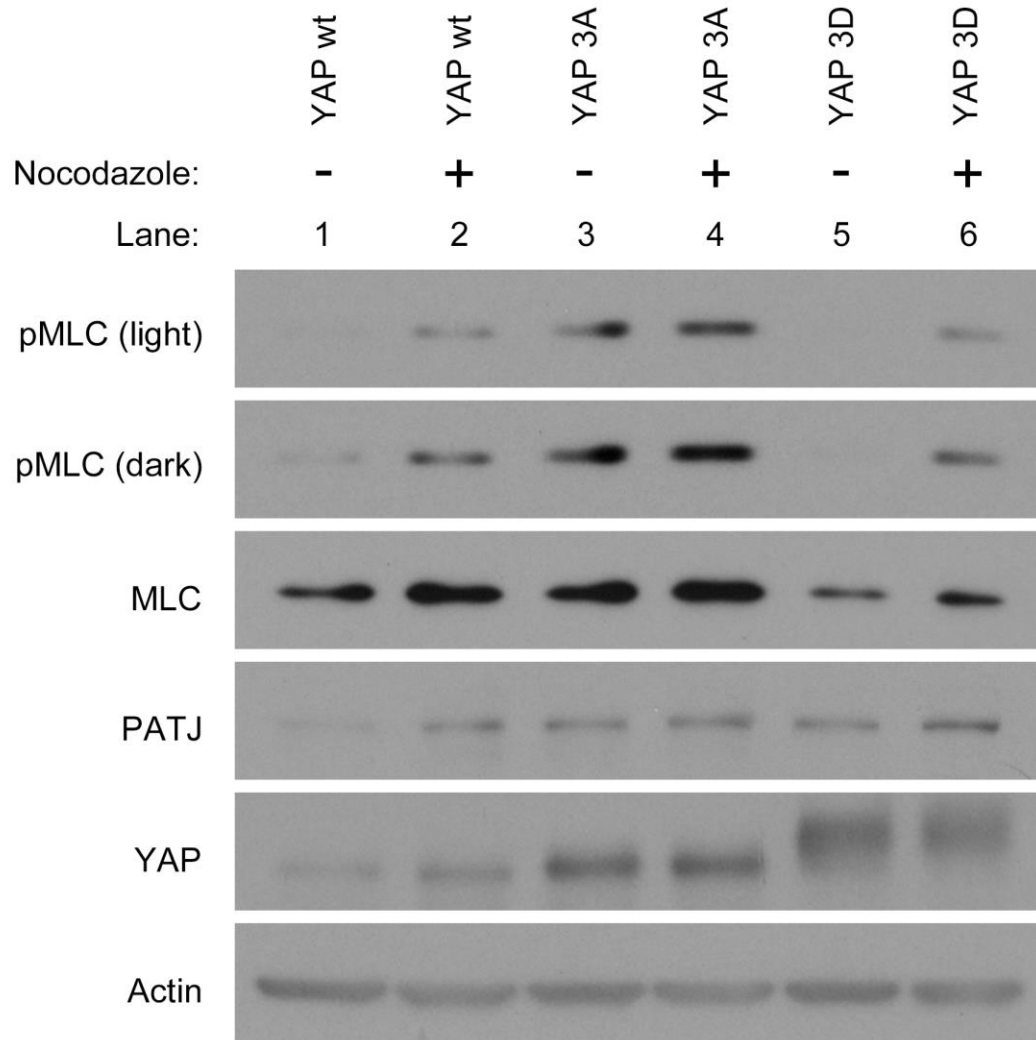


Fig. S5

Phosphorylation of MLC is increased by expression of the YAP 3A mutant

Anti-phosphoMLC immunoblot of HeLa cells expressing YAP WT, YAP 3A or 3D phosphorylation mutants and treated with or without nocodazole. Immunoblots are representative of two independent experiments.

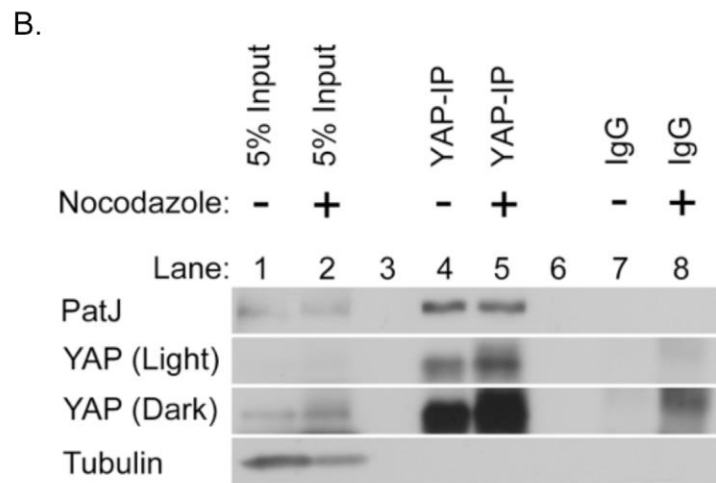
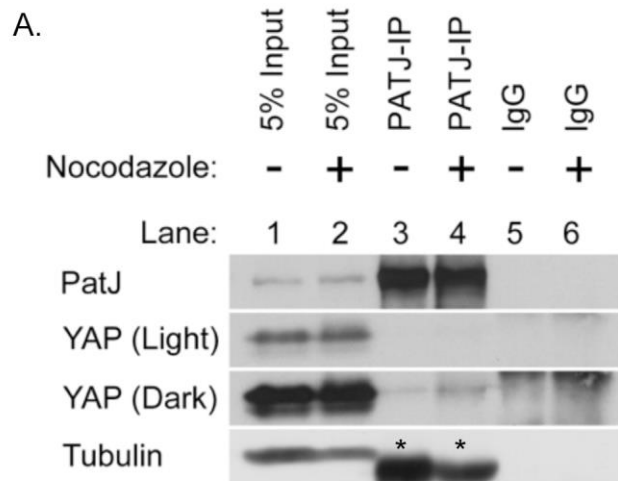


Fig. S6
YAP and PATJ coimmunoprecipitation

(A) MCF-10A cell lysates were immunoprecipitated with anti-PATJ antibody and assessed for the ability of endogenous YAP to coprecipitate. Asterisk indicates IgG band in lane 3 and 4 of the immunoblot. Immunoblots are representative of three independent experiments. **(B)** MCF-10A cell lysates were immunoprecipitated with anti-YAP antibody and assessed for the ability of endogenous PATJ to coprecipitate. Immunoblots are representative of four independent experiments.

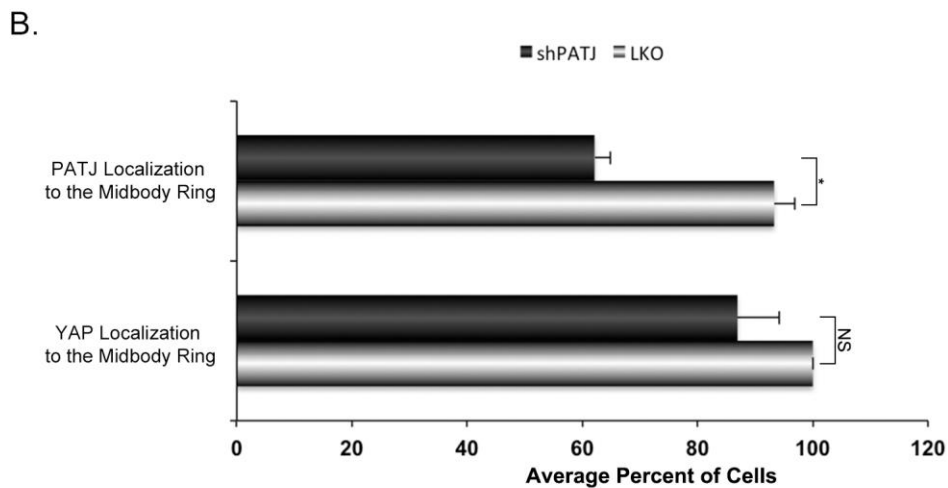
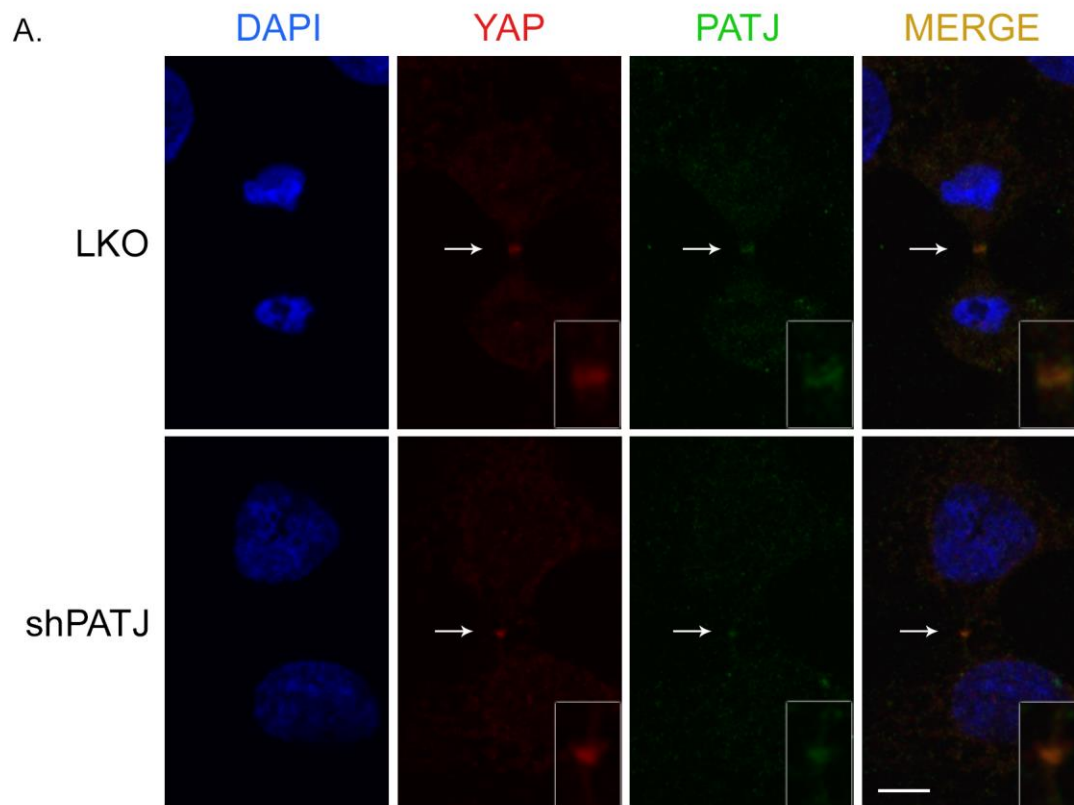


Fig. S7

Immunofluorescence analysis of PATJ-depleted cells

A. Representative immunofluorescence images of PATJ and YAP in control (n=44) and shYAP (n=21) cells using PATJ antibody Santa Cruz ab151257 from three independent experiments. Three different PATJ antibodies were tested: Novus Santa NBP2-19707, Santa Cruz ab151257, and Santa Cruz ab102113.

Note the co-localization of YAP and PATJ in the midbody ring and the localization of YAP to midbody in PATJ-depleted cells. **B.** Quantification of PATJ and YAP localization to the midbody in control (LKO) or shPATJ MCF-10A cells. Note the reduction in PATJ detection at the midbody in cells expressing PATJ shRNA (35% of cytokinetic figures showed dim to lightly speckled to no PATJ staining in the midbody ring area), yet no significant reduction in YAP staining. Scale bar = 2.5 μm . Statistical significance ($***P<0.001$; $**P<0.01$; $*P<0.05$; NS, $P>0.05$) was assessed by unpaired Student's t-test. Bars represent mean+SEM from three independent experiments.

Table S1

HCIPs for YAP wild type, YAP 3D, and YAP 3A

MCF-10A N-TapYAP (Flag & HA-tagged at the N-terminus) wild-type, YAP 3A, or YAP 3D cells were synchronized with nocodazole and released at 0, 30, 60, 90, 120, 180 minutes. IP-MS/MS and *Comparative Proteomics Analysis Software Suite* (CompPASS) Analysis were used to assign confidence values to interacting proteins and the values were collected and normalized.

Movie S1

Mitosis of MCF-10A cells depleted of YAP with five distinct shRNA sequences

Representative time-lapse DIC microscopy movies of MCF-10A cells undergoing mitosis after knockdown of YAP with five distinct shRNA sequences. LKO is empty-vector control.

Movie S2

Mitosis of YAP-depleted MCF-10A cells expressing GFP-tubulin

Representative time-lapse movies of mitotic MCF-10A cells expressing GFP-tubulin. Control LKO vector (top) or shYAP (bottom) cells. Left panels show phase contrast and right panels show corresponding fluorescence microscopy of GFP-tubulin.

Movie S3

Mitosis of MCF-10A cells treated with YAP siRNA

Representative time-lapse DIC movies of MCF-10A cells undergoing mitosis after treatment with a YAP-targeted siRNA SMARTpool. An siRNA SMARTpool against Gapdh (siGapdh) was used as a control.

Movie S4

Mitosis of YAP-depleted MCF-10A cells in a monolayer

Representative time-lapse movies of a confluent monolayer of MCF-10A cells after knockdown of YAP showing multiple mitoses. Phase-contrast images are shown.

Movie S5

Mitosis of YAP-depleted HeLa cells

Representative time-lapse movies of mitotic HeLa cells expressing GFP-tubulin after knockdown of YAP. Left images are phase-contrast; right images are fluorescence microscopy of GFP-tubulin.

Movie S6

Mitosis of YAP-depleted multiple HeLa cells

Representative time-lapse movie of multiple HeLa cells undergoing mitosis after knockdown of YAP. Phase-contrast images are shown.

Movie S7

RHOA localization in a mitotic YAP-depleted MCF-10A cell

Top images: representative time-lapse movie of MCF-10A cells initiating cytokinesis after knockdown of YAP. Left images are phase-contrast, right images are fluorescence microscopy of GFP-Tubulin. Bottom panel: same cells from the time-lapse were fixed and immunostained for RHOA (red) and tubulin

(green). A 3D reconstruction of a confocal z-series is shown. Note mislocalization of Rho (red).

Movie S8

Mitosis of YAP-depleted MCF-10A cells treated with C3 transferase or Y-27632

Representative time-lapse phase microscopy of MCF-10A cells undergoing mitosis after knockdown of YAP treated with the Rho inhibitor TAT-C3 transferase or the ROCK inhibitor Y-27632.

Movie S9

Mitosis of MCF-10A cells expressing wild-type YAP or YAP 3A or YAP 3D mutants

Representative time-lapse phase microscopy of mitosis of control LKO or YAP-depleted MCF-10A cells expressing wild-type YAP, phospho-mutant YAP 3A, or phospho-mimetic mutant YAP 3D.

Movie S10

Mitosis of PATJ-depleted MCF-10A cells

Representative time-lapse DIC microscopy movies of cells undergoing mitosis after knockdown of PATJ with two distinct shRNA sequences. LKO is empty-vector control.