

# Human Mob1 proteins are required for cytokinesis by controlling microtubule stability

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## Summary

The completion of cytokinesis requires abscission of the midbody, a microtubule-rich cytoplasmic bridge that connects the daughter cells before their final separation. Although it has been established that both the midbody structure and membrane fusion are essential for abscission, the biochemical machinery and the cellular processes of abscission remain ill-defined. Here we report that human Mob1A and Mob1B proteins are involved in the regulation of abscission of the intercellular bridge. The Mob family is a group of highly conserved proteins in eukaryotes, described as binding partners as well as co-activators of protein kinases of the Ndr family, and as members of the Hippo pathway. We show that depletion of Mob1A and Mob1B by RNAi causes abscission failure as a consequence of hyper-stabilization of microtubules in the midbody region. Interestingly, depleting Mob1 also increases cell motility after cytokinesis, and induces prolonged centriole separation in G1 phase. In contrast, centrosomes fail to split when either Mob1A or Mob1B is overexpressed. Our findings indicate that human Mob1 proteins are involved in the regulation of microtubule stability at the midbody. We conclude that Mob1A and Mob1B are needed for cell abscission and centriole re-joining after telophase and cytokinesis.

**Key words:** Cytokinesis, Mob1, Microtubule stability

## Introduction

At the end of cytokinesis cells have to execute abscission, which results in the physical separation of two daughter cells, and return to interphase. It is generally accepted that a particular sequence of events are required for abscission to occur: cortical anchorage of the ingressed furrow, splitting the plasma membrane between the sister cells, and the disassembly of midbody-associated microtubules (Barr and Gruneberg, 2007; Guizetti and Gerlich, 2010). Recent work has demonstrated that endosome fusion and microtubule reorganization are essential for abscission. It was shown that disassembly of microtubules can be mediated by the microtubule-severing protein spastin (Connell et al., 2009; Guizetti et al., 2011; Schiel et al., 2011) and several proteins of the endosomal sorting complex are required for transport (ESCRT)-III localize to the midbody and are required for cytokinesis (Morita et al., 2007; Wollert et al., 2009; Guizetti et al., 2011; Schiel et al., 2011).

Mob1 is a conserved protein required for cytokinesis and for the regulation of mitotic exit (McCollum and Gould, 2001; Hergovich, 2011). Mob1 proteins are binding partners and coactivators, of protein kinases of the Ndr and Lats families (reviewed in Hergovich et al., 2006; Trammell et al., 2008). In fly and human cells, Mob1 participates in the control of cell proliferation and apoptosis as part of the Hippo pathway (Lai et al., 2005; Wei et al., 2007; Praskova et al., 2008). There is also a genetic interaction between the *Drosophila* Mob-like genes and

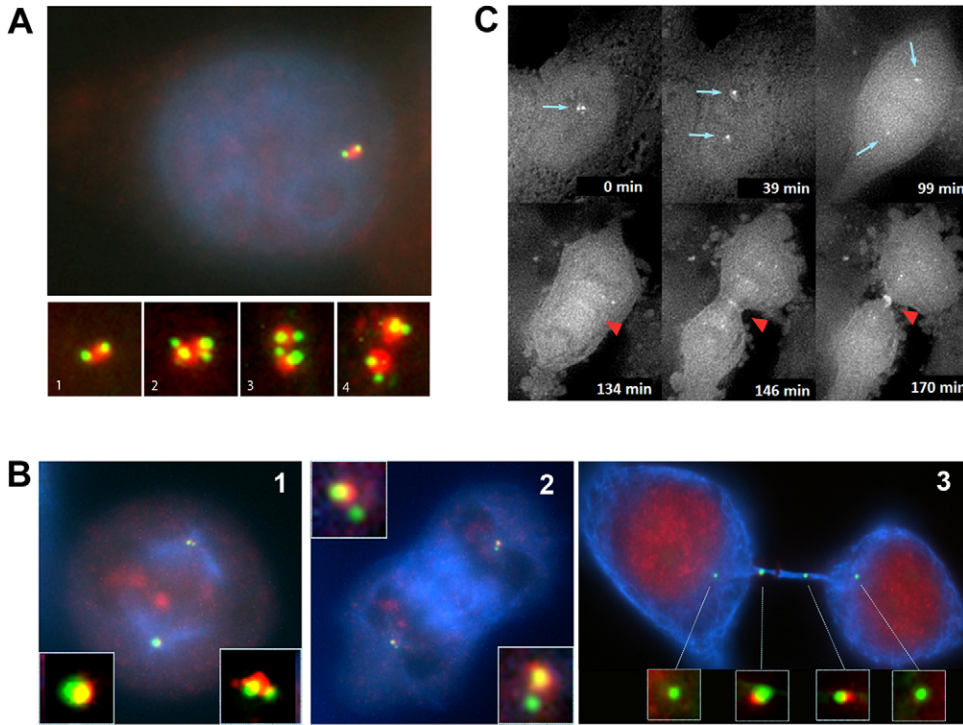
*tricornered* (*trc*), another Ndr protein kinase required for normal morphogenesis of a variety of polarized outgrowths (He et al., 2005). In humans, Lats1 interacts with Mob1 and this complex appears to be functionally required for cytokinesis (Bothos et al., 2005).

Here we have investigated the function of the human Mob1A and Mob1B proteins to further explore their role during cytokinesis. We show that Mob1 proteins are required for abscission. Furthermore, we show that upon Mob1 downregulation, centrioles fail to rejoin at the end of cytokinesis, cells acquire increased motility, and microtubule stability is increased. Finally, we also show that depolymerization of microtubules is a prerequisite for abscission, and that Mob1 proteins are involved in the regulation of this process.

## Results and Discussion

### Human Mob1A and Mob1B proteins are essential for centriole cohesion

We first examined the distribution of human Mob1A and Mob1B proteins. In HeLa cells expressing GFP-centrin, where the two centrioles were clearly distinguishable, we detected a broad single Mob1 dot in the center of the centrosome by indirect immunofluorescence microscopy (Fig. 1A). This staining persisted until the end of mitosis (Fig. 1B). However, in late telophase cells with two separated centrioles, Mob1 was detected just in the stronger GFP-centrin signal, often the one closer to the midbody



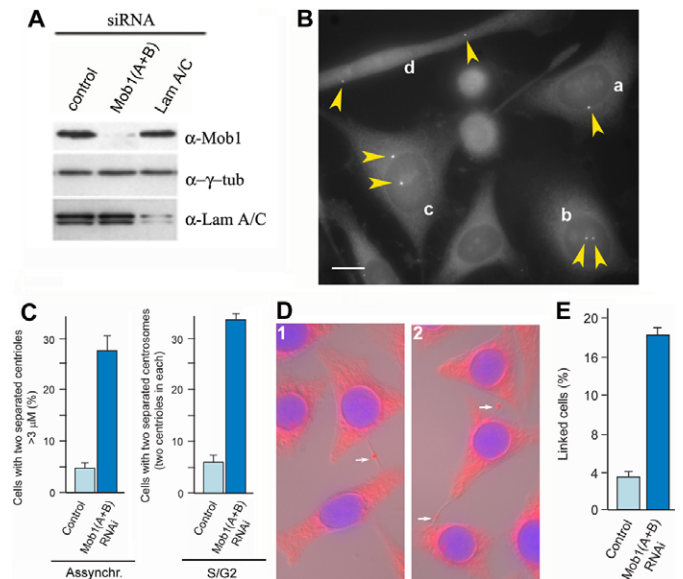
**Fig. 1. Mob1 localizes to the centrosome and to the midbody.** (A) Cells were stained for GFP-centrin (green), DAPI (blue) and anti-Mob1 (red). Mob1 accumulates on the centrosome but does not completely overlap with the centrioles (two centrin dots). This pattern remains while the centrioles duplicate and centrosomes separate (A1 to A4). (B) HeLa GFP-centrin cells immunostained for  $\alpha$ -tubulin (blue) and Mob1 (red). At the end of telophase (3), Mob1 is detected in the midbody and in just one centriole. (C) Time lapse analysis of a stable cell line expressing GFP-Mob1A. GFP-Mob1A is visible on the centrosomes from the start of mitosis until anaphase (arrows), and in the central spindle and midbody (arrowheads) at the end of mitosis. GFP-Mob1B protein has an identical behavior.

(Fig. 1B3). Mob1 was also observed at the kinetochores (supplementary material Fig. S2) and in the central spindle and midbody regions during late anaphase and telophase (Fig. 1). The specificity of the Mob1 centrosomal staining was confirmed by time-lapse video microscopy of HeLa cells expressing either GFP-Mob1A or GFP-Mob1B (Fig. 1C).

The high degree of identity between the two Mob1 proteins indicated a possible functional redundancy (supplementary material Fig. S1) (Vitulo et al., 2007). Therefore, we sought to disrupt the expression of both Mob1s by simultaneous co-transfection of siRNA directed against each Mob1. We used three different siRNA duplexes for each type of Mob1, and found that Mob1 protein levels were substantially repressed (>95%) by 48 h post-transfection (Fig. 2A; supplementary material Fig. S1C). Identical results were obtained using any pair of different siRNAs against either Mob1s, indicating that siRNA treatment effectively and specifically reduced the abundance of Mob1 proteins. We will refer to cells in which neither Mob1A nor Mob1B were detected by immunoblotting as 'Mob1(A+B)-depleted'.

Although Mob1(A+B)-depleted cells formed apparently normal interphase arrays of microtubules and mitotic spindles, downregulation of Mob1 had a striking effect upon centriole behavior. Two distinct, widely separated GFP-centrin positive structures were observed in >28% of interphase Mob1(A+B)-depleted cells, compared with 5% in control cells (Fig. 2B). The distance between separated centrioles varied considerably (between 2 and 30  $\mu$ m, Fig. 2B), compared to control cells (1.4  $\pm$  0.2  $\mu$ m). This effect on centrioles was observed with all Mob1-siRNAs, but not with the control siRNAs, and was also observed in U2OS and RPE1 cell lines (supplementary material Fig. S3A,B), indicating that is not cell type specific, nor linked to the transformed state of HeLa cells.

The abrogation of Mob1 function seemed to cause centrioles to split during G1 phase, firstly because mitotic spindle poles



**Fig. 2. Depletion of Mob1 results in centriole separation and cytokinesis failure.** Unsynchronized HeLa-GFPcentrin cells were analyzed after RNAi for Mob1(A+B) two days post-transfection. (A) Western blots using  $\alpha$ -Mob1,  $\alpha$ -Lamin A/C and anti- $\gamma$ -tubulin antibodies. (B) HeLa-GFPcentrin cells with centriole/centrosome separation induced by Mob1(A+B) depletion. Cell a shows normal centriole separation; cell b has centrosome separation of  $\sim$ 3  $\mu$ m; cell c: this distance between centrosomes is rarely seen in control cells; cell d has centrioles separated more than 30  $\mu$ m, never detected in control cells. Scale bar: 10  $\mu$ m. (C) Percentage of cells with centriole/centrosome separation greater than 3  $\mu$ m in asynchronous cultures, and in synchronized cells analyzed in S/G2 phase. (D) DIC + fluorescence images for Mob1(A+B) depleted cells showing delayed/failed cytokinesis (1) and three connected cells (2) with the midbodies in the intercellular bridges (arrows) (DNA: blue,  $\gamma$ -tub: red). (E) For the quantification of linked cells after Mob1(A+B) depletion only cells with a visible midbody, as in D1, were considered.

always had two centrioles each (even after 5 days of siRNA treatment); secondly, because we never observed cells with four separated centrioles (or with one pair plus two separated centrioles) indicating that no splitting occurred after centriole duplication. To confirm this hypothesis, we analyzed cells 3.5 h after release from a S-phase block and we found that 32% of the Mob1(A+B)-depleted cells had two separated pairs of centrioles compared to ~7% in control cells (Fig. 2C), indicating that a large proportion of Mob1-depleted cells initiated centriole duplication with already separated centrioles.

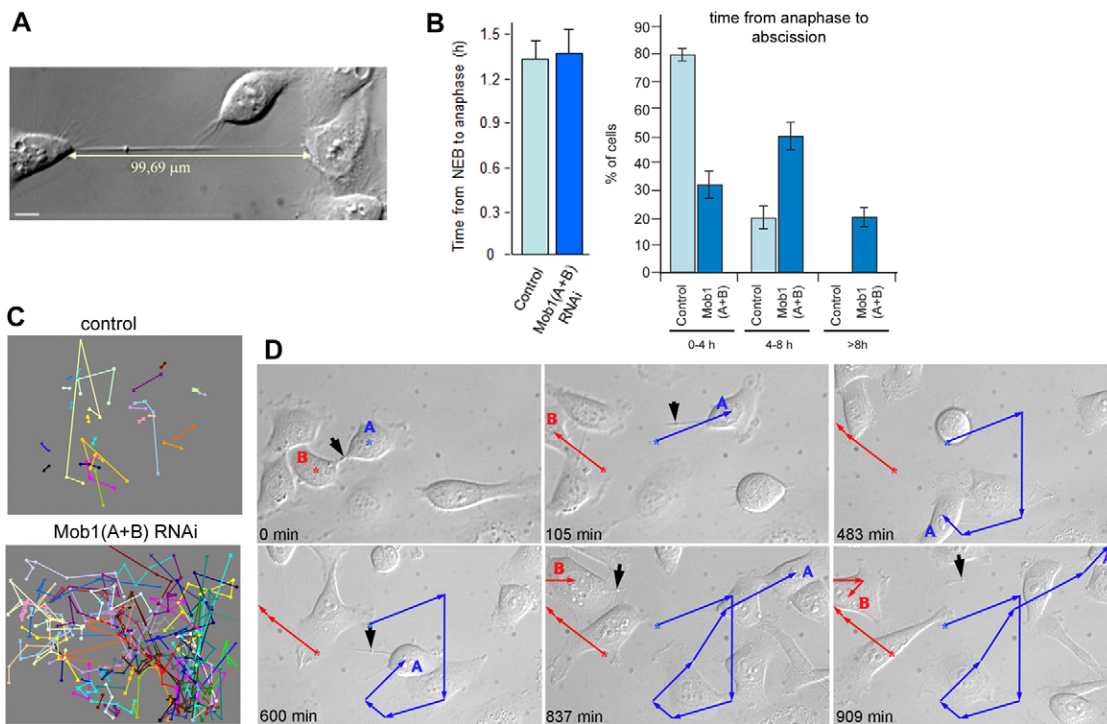
Centrioles often split at the end telophase and re-join after abscission (Chevrier et al., 2002; Meraldi and Nigg, 2002). In contrast to the effect of depletion, overexpression of either Mob1 reduced the percentage of cells with split centrioles. We detected four distinct separated centrioles in 32% of control cells undergoing telophase/cytokinesis (supplementary material Fig. S5). On the other hand, overexpression of Mob1A or Mob1B drastically reduced this number to 15% and 4%, respectively (three independent experiments,  $n \sim 100$  dividing cells per exp.). Therefore, an excess of Mob1 prevents centriole separation at the end of cell division, and Mob1 is important for the re-association of centrioles after mitosis (supplementary material Fig. S7).

### Mob1 downregulation results in abscission failure and increased cell motility

We found that Mob1(A+B)-depleted cells remained connected at the end of cytokinesis by thin cytoplasmic bridges containing the

midbody (Fig. 2D; Fig. 3A; supplementary material Fig. S3C,D). Chains of 3 or 4 connected cells were also frequently observed (Fig. 2D2). Time-lapse imaging revealed that Mob1(A+B)-depleted cells proceeded through mitosis with similar kinetics to control cells but took longer to execute abscission (Fig. 3). Most control cells took up to 4 h (80%,  $n=60$ ) from constriction to complete separation of daughter cells. In contrast, abscission was completed by 4 h in only 30% Mob1(A+B)-depleted cells (Fig. 3B), and 38% took longer than 6 h ( $n=70$ ). No DAPI-staining or anti-lamin staining was detected at the midbody region after Mob1(A+B)-depletion (data not shown), indicating that chromosome bridges or lagging chromosomes were unlikely to be the major cause of abscission failure (Steigemann et al., 2009). In addition, treatment of the cells with ZM1 (a specific Aurora B inhibitor) did not accelerate the timing of abscission, indicating that the delay was not caused by activation of the Aurora B-dependent checkpoint (supplementary material Fig. S4).

Remarkably, while control cells moved slightly apart after cytokinesis before adhering again and flattening onto the dish (supplementary material Movie 1), Mob1-depleted cells became highly mobile immediately after telophase/cytokinesis and remained connected by long intercellular bridges (Fig. 3; supplementary material Movies 2, 3). Cells moved continuously for several hours and with frequent changes of direction (Fig. 3C–D) even after resolution of the intercellular bridge, indicating that movement was not dependent on abscission failure.



**Fig. 3. Depletion of Mob1 increases cell motility.** (A) Mob1(A+B) depleted cells are frequently connected by very long intercellular bridges, indicating that cells have moved away from each other (scale bar: 10  $\mu$ m). (B) The time from nuclear envelope breakdown (NEB) to the start of anaphase and from anaphase until abscission was measured by time-lapse imaging. 65 control and 70 Mob1(A+B)-depleted cells were analyzed. (C) Representation of the movement of all dividing cells in an optical field over 23 h. Each pair of cells is represented in a different color. 30 control and 36 Mob1(A+B)-depleted cells divided in each movie respectively. (D) Time-lapse of Mob1-depleted cells (supplementary material Movie 2). The blue and red arrows indicate the path followed by daughter cells A and B, respectively. The black arrow indicates the position of the midbody, between the two connected cells. After Mob1(A+B) down regulation daughter cells fail abscission, do not adhere strongly to the dish, and continue moving for hours.



### Mob1 depletion reduces dynamic instability of microtubules

We found that Mob1(A+B)-depletion did not affect the midbody localization of Aurora B, MKPL1, PLK1 or Cep55, in agreement with (Wilmeth et al., 2010). Both endobrevin and syntaxin 2 also localized correctly, indicating that abscission failure was not caused by deficient recruitment of exocyst components to the midbody region (not shown).

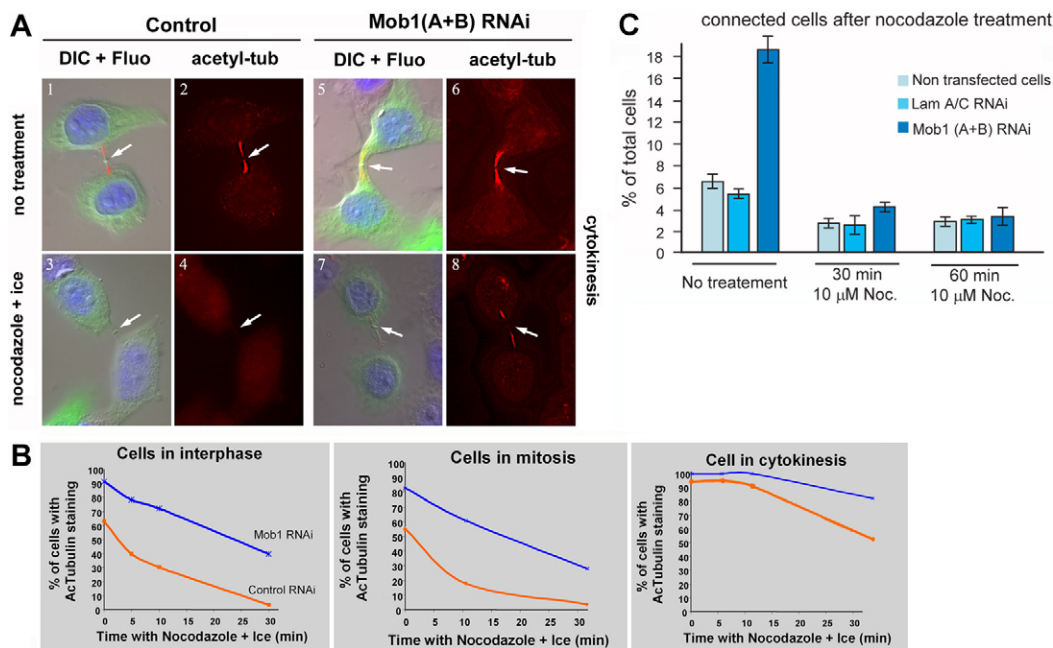
Because over-stabilization of microtubules can prevent or delay cytokinesis (Hong et al., 2007; Manabe et al., 2002), we wondered whether hyper-stabilization of microtubules prevented abscission in Mob1-depleted cells. We found that Mob1-depleted cells had increased levels of acetylated tubulin (a marker of stabilised microtubules) (Piperno et al., 1987), and of microtubules resistant to both nocodazole and cold treatments (Fig. 4; supplementary material Fig. S6). In dividing cells, acetylated microtubules were easily detected in the midbody region (Fig. 4A). But after 30min incubation with nocodazole and cold this staining was lost in 46% of control cells, while the majority of Mob1(A+B)-depleted cells (>80%) retained strong acetylated tubulin staining on the intercellular bridge (Fig. 4). Therefore down regulation of Mob1A+B resulted in increased microtubule stability. If hyper-stabilization of the microtubules did prevent abscission, then treatments that reduced microtubule stability should decrease the number of unsuccessful cell divisions. In agreement with this, we found that Mob1(A+B)-depleted cells could execute abscission if microtubules were artificially destabilized by nocodazole treatment (Fig. 4C).

In summary, we have shown that Mob1(A+B) depletion inhibits the removal of midbody-localized microtubules that is

required to allow membrane fusion and abscission (Guizetti and Gerlich, 2010). Although poorly explored, microtubule dynamics do have an important role in cytokinesis (D'Avino et al., 2005; Glotzer, 2009). Cells lacking Spastin, a microtubule-severing protein, fail abscission because they retain midbody microtubules (Yang et al., 2008; Connell et al., 2009; Guizetti et al., 2011) making spastin a potential candidate for regulation by Mob1. Also, it is tempting to think that there may be a causal connection between the unusual centriole behavior in Mob1-depleted cells and the contribution of the mother centriole to signal abscission observed by (Piel et al., 2001).

Populations of microtubules with different stabilities are involved in several aspects of cytokinesis (Foe and von Dassow, 2008; Shen et al., 2009; Connell et al., 2009). Microtubule dynamics and reorganization are also crucial for cell motility (Gundersen and Bulinski, 1988; Pegtel et al., 2007; Drabek et al., 2006) and, interestingly, altered microtubule dynamics can induce centriole separation (Jean et al., 1999). At present it is unclear whether the centriole splitting, abscission failure and hypermotility of Mob1(A+B)-depleted cells are all consequences of altered microtubule stability or reflect different functions of Mob1A and Mob1B (supplementary material Fig. S7).

The only Mob-like protein in *Tetrahymena* (TtMob1) is a cell polarity marker and is essential for division plane placement and cytokinesis (Tavares et al., 2012), therefore, since directed cell movement is dependent on cell polarization, the effect on cytoskeletal organization may be the ancestral function of Mob-like proteins. The underlying molecular mechanism of Mob1 activity likely involves binding and activating Dbf2-like



**Fig. 4. Depletion of Mob1 results in increased microtubule acetylation and stability.** Two days post-transfection cells were incubated with 10 μM of nocodazole + ice for 30 minutes before fixation. Acetylated tubulin (red), DAPI (blue), GFP-Tubulin (green). (A) After treatment only bridges between Mob1(A+B)-depleted cells retain acetylated tubulin (compare A3-4 with A7-8). All images were taken with the same exposure settings. Arrows indicate midbody. (B) Percentage of cells with acetylated tubulin staining at different cell cycle stages. 350 interphase cells, 75 mitotic cells and 200 cells in cytokinesis were scored in each of three independent experiments. (C) Brief treatment with nocodazole allows abscission in Mob1(A+B)-depleted cells. Two days post-transfection with siRNAs, cells were incubated with 10 μM nocodazole for 30min or 60min before fixation. Cells connected by an intercellular bridge containing a midbody were counted (~1500 cells analyzed in each of three separate experiments).

kinases, since both Lats1/2 and NDR kinases contribute to the regulation of the cytoskeleton (Justice et al., 1995; Yang et al., 2004; Hergovich et al., 2006). Consistent with this idea, components of the Hippo pathway cooperate with the Nek2 kinase to regulate centrosome disjunction (Mardin et al., 2010). Although other centrosome components are required to prevent premature centriole/centrosome splitting (Bahmanyar et al., 2008; Wang et al., 2008; Nakamura et al., 2009), Mob1 is unique in the sense that it affects centriole re-joining.

In conclusion, we have shown that membrane recruitment to the midbody area is not in itself enough, and that microtubules have to be reorganised before membrane vesicles can fuse. Our results open the door to explore the regulation of microtubule dynamics by the Mob1-dependant kinase(s). Moreover, they indicate the exciting possibility that the Hippo pathway, which has a key role in regulating cell proliferation and apoptosis (reviewed in Pan, 2010), may influence microtubule dynamics in response to extracellular and intracellular signals and thereby influence cell polarity.

## Materials and Methods

### Antibodies and siRNAs

The  $\alpha$ -Mob1 serum (Ponchon et al., 2004), recognized both Mob1A and Mob1B in HeLa extracts. The signal could be competed with either Mob1A or Mob1B expressed bacterially, and was suppressed upon Mob1A+Mob1B RNAi depletion (supplementary material Fig. S1). Primary antibodies:  $\gamma$ -tubulin (GTU-88, Sigma),  $\alpha$ -tubulin (DM1 $\alpha$ , Sigma), acetylated tubulin (C3B9). Three different siRNAs were used per gene with identical results: 5'-AAACACGCAGAA-GCCACACUU-3' and Ambion's #s30511 and #s30510 for Mob1A, oligos 5'-AACACCUUUUAAGCACUUU-3', and Ambion's #s40977 and #s40978 for Mob1B. As controls Lamin A/C oligo (Elbashir et al., 2001) and Dharmachon Scramble II oligo were used. GenBank sequences: Human Mob1A, AJ577473; Human Mob1B, AJ577474.

### Cell culture and synchronization

HeLa and U2OS cells were cultured in DMEM supplemented with 10% heat-inactivated fetal calf serum (FCS) and 2 mM glutamine. HeLa-GFP-centrin cells were cultured in RPMI 1640 with GLUTAMAX-I, supplemented with 10% FCS. RPE-I cells were cultured using D-MEM/F12 media supplemented with 10% FCS, 2 mM glutamine and 0.348% sodium bicarbonate. HeLa cells were synchronized in S phase using a double thymidine (2.5 mM) block of 24 h, with a 12 h interval between the blocks. Cells entered mitosis 8–10 h after release of the second block.

### Transfections

(A) fixed cell analysis: Cells were seeded on sterile coverslips at a density of 2–3 $\times$ 10<sup>4</sup> cells per well in a 24 well plate. Next day cells were transfected using 1  $\mu$ l Lipofectamine2000 (Life technologies) with siRNAs at a final concentration of 100 nM. Two days after transfection the cells were analyzed. For siRNA-DNA co-transfections we used LF2000 and 200 ng of DNA per reaction.

(B) live imaging analysis: 2 $\times$ 10<sup>5</sup> HeLa and HeLa GFPcentrin cells were seeded on 0.17  $\Delta$ mm dish (Biotechs). Cells were synchronized in S-phase and transfections were done between the two blocks. Cells were filmed 8–10 hours after released from the second block. Images were acquired every 3 minutes.

### Nocodazole and cold treatment assays

Two days post-transfection, cells were treated with ice cold D-MEM + Nocodazole (10  $\mu$ M) for 5, 10 and 30 minutes, on top of an ice bed. Non-treated cells were used as controls. Cells were fixed with methanol/acetone and processed for immunofluorescence. Only YFP positive cells were scored: 350 interphase cells, 75 mitotic cells and 75 linked cells were scored per experiment in three independent experiments.

For Aurora B inhibition we used an adapted protocol from (Steigemann et al., 2009). Two days after RNAi treatment, ZM1 (an Aurora B inhibitor) (Tocris) was added to a final concentration of 2  $\mu$ M and incubated for 3 h. After this time cells were washed, fixed and processed for immunofluorescence.

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Supplementary material available online at

<http://jcs.biologists.org/lookup/suppl/doi:10.1242/jcs.097147/-/DC1>

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A

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1  MSFLFLFGSRSSKTFKPKKNIPEGSHQYELLKHAEATLGSNGN Mob1B
1  MSFLFLFS SRSSKTFKPKKNIPEGSHQYELLKHAEATLGSNGN Mob1A

41  LRM AVMLPEGEDLNEWV AVNTVDFFNQINMLYGTITD FCT Mob1B
41  LRQ AVMLPEGEDLNEWI AVNTVDFFNQINMLYGTITE FCT Mob1A

81  EESCVPMSAGPKY EYHWADGTNIKKPKIKCSAPKYIDYLMT Mob1B
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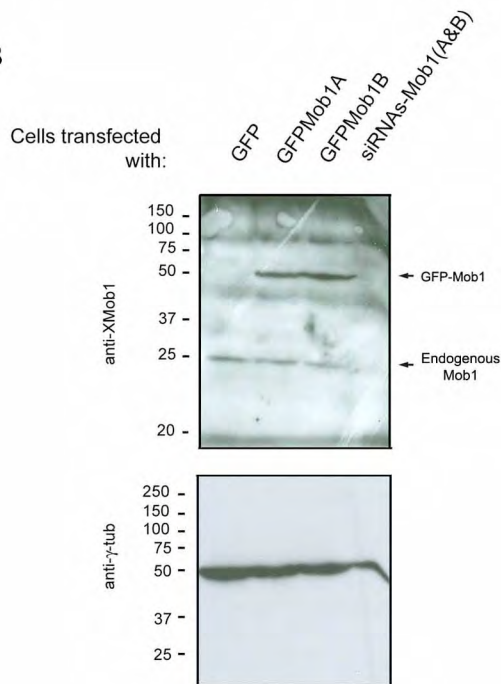
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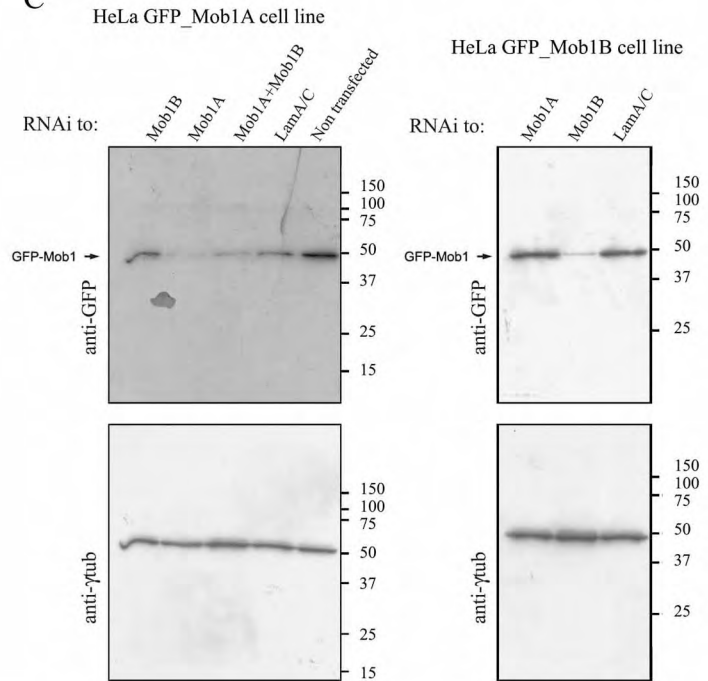
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B



C

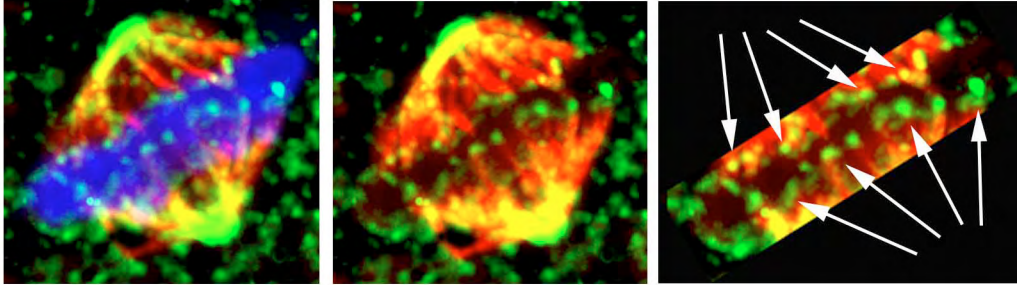


**Supplementary Figure 1. Specificity of Mob1A and Mob1B antibodies and RNAi. A)** Amino acid sequence alignment of Mob1A with Mob1B. Identical amino acids are shaded. Human Mob1A and Mob1B are 96% identical at the amino acid level. **B)** Specificity of anti-Mob1 antiserum. Extracts of HeLa cells expressing GFP, GFP-Mob1A or GFP-Mob1B were analyzed by western blot, together with extracts of cells treated with siRNA oligos for Mob1(A+B). The serum recognizes both Mob1A and Mob1B and the fusion proteins GFP-Mob1A and GFP-Mob1B. **C)** **C1)** HeLa cells stably expressing GFP-Mob1A were transfected with siRNA oligos for Mob1B, Mob1A, Mob1(A+B) or Lamin A/C (control). Non transfected cells were used as another control. Cell extracts were prepared 2 days post transfection and probed by western blot to detect the GFP. The GFP protein levels are identical in cells treated with Mob1B and Lamin A/C oligos, and are significantly reduced in cells treated with Mob1A or Mob1(A+B) oligos, indicating the specificity of Mob1A oligo. **C2)** HeLa Cells stably expressing GFP-Mob1B were transfected with oligos against Mob1B, Mob1A or LaminA/C. Cell extracts were made 2 days post transfection and analyzed by western blot with anti-GFP antibodies. As can be seen, the GFP protein levels are reduced after treatment with Mob1B directed oligos, but are not affected in cells treated with Mob1A or Lamin A/C oligos, confirming the specificity of Mob1B directed oligos.  $\gamma$ -tubulin was used as a loading control on B) and C). The numbers indicate the sizes in kilodaltons of the molecular mass markers.

DNA /  $\alpha$ -tubulin / GFP-Mob1A

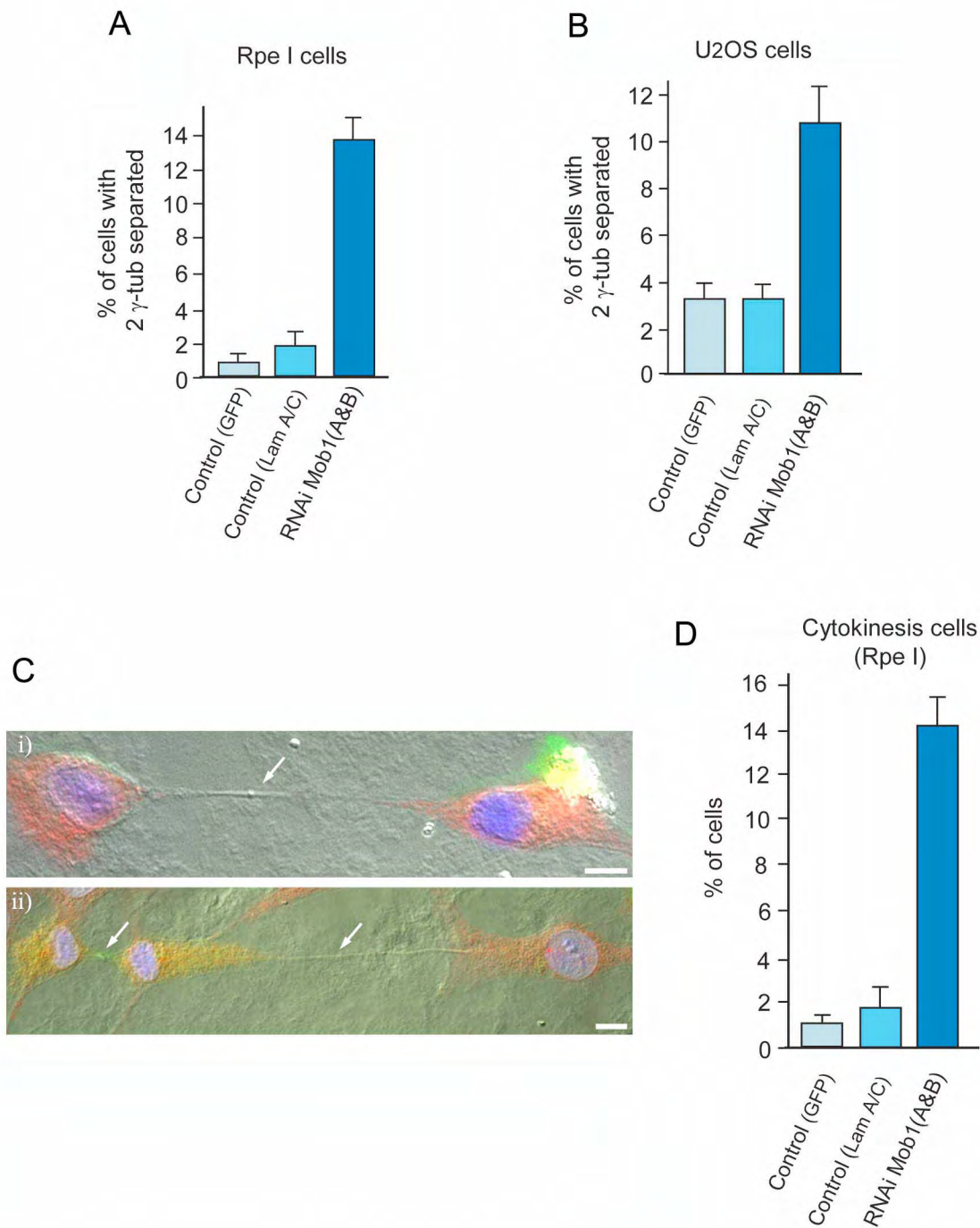
$\alpha$ -tubulin / GFP-Mob1A

$\alpha$ -tubulin / GFP-Mob1A

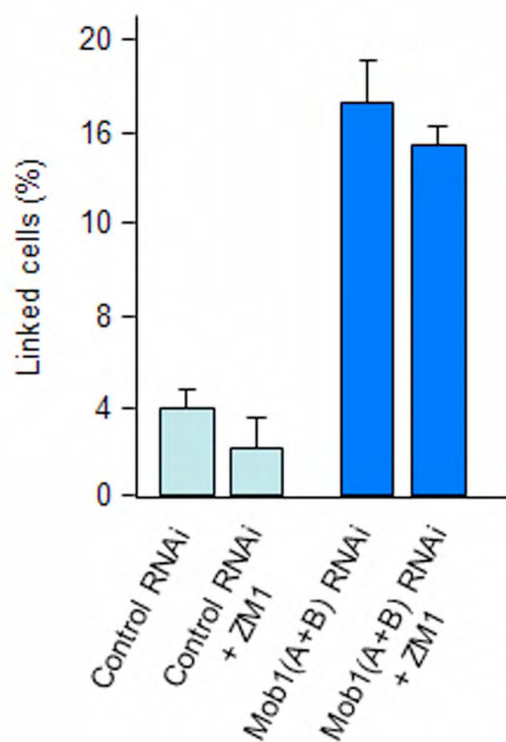


**Supplementary Figure 2.** *GFP-Mob1 accumulates on the Kinetochores.* Fixed HeLa cells stably expressing GFP-MOB1A were immunostained for  $\alpha$ -tubulin (red), and the DNA was stained with DAPI (Blue). GFP-Mob1A is shown in green. The same mitotic figure is shown on three panels. Arrows indicate detection of Mob1 on the end of microtubules in contact with the chromatin (kinetochores).



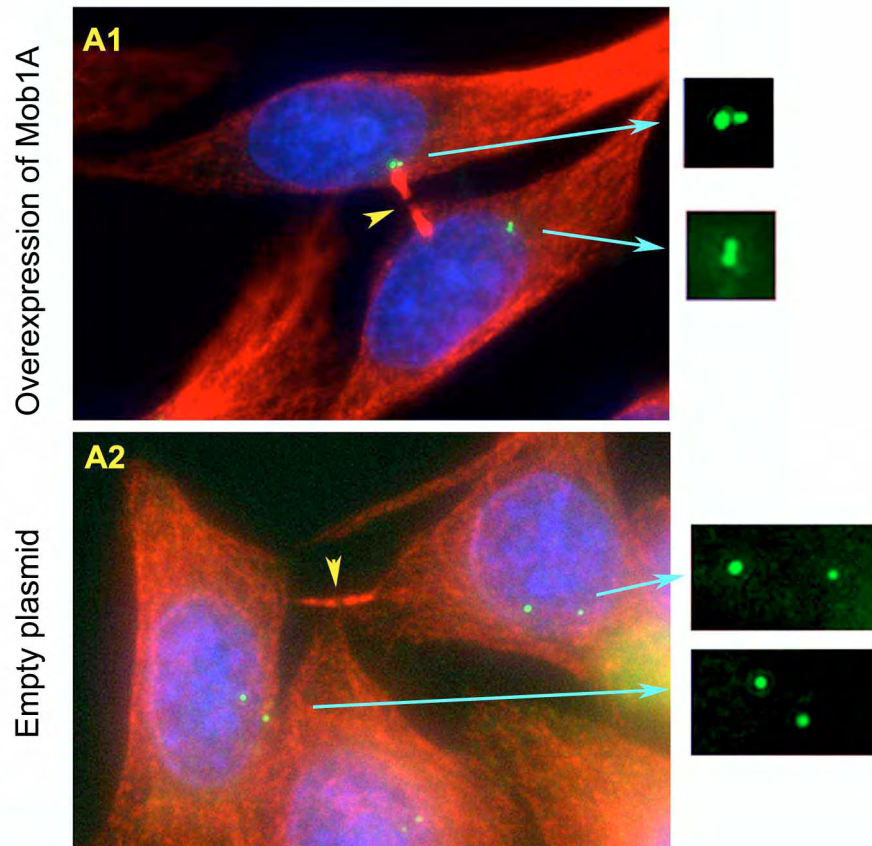


**Supplementary Figure 3.** *Mob1(A+B)* depletion causes centriole separation and cytokinesis defects in RPE I and U2OS cells. **A, B)** Graphs showing the quantification of centrosome/centriole separation in RPE I (A) and U2OS (B) cells. **C, D)** *Mob1* A+B RNAi causes cytokinesis failure in RPE I cells. **C)** (i) Cells connected by very long intercellular bridge, (ii) and a chain of interconnected cells. Arrows indicate the midbody rings (scale bar 10  $\mu$ m). **D)** Percentage of cells connected by a bridge containing the midbody ring.



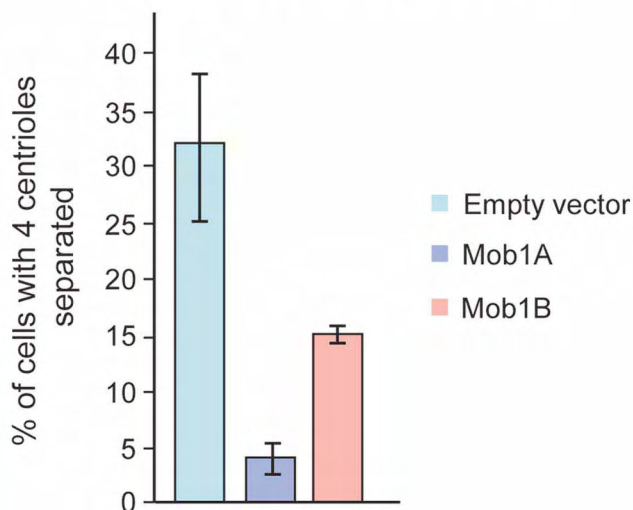
**Supplementary Figure 4.** *Aurora B inhibition does not significantly decrease the number of linked cells by intercellular bridge upon Mob1(A+B)RNAi.* Cells were transfected with oligos against Mob1(A+B) and scramble duplex oligos, incubated with Aurora B inhibitor for 3h and processed for immunofluorescence. The number of linked cells connected by an intercellular bridge was quantified. (3 experiments, ~450 cells per experiment)

A



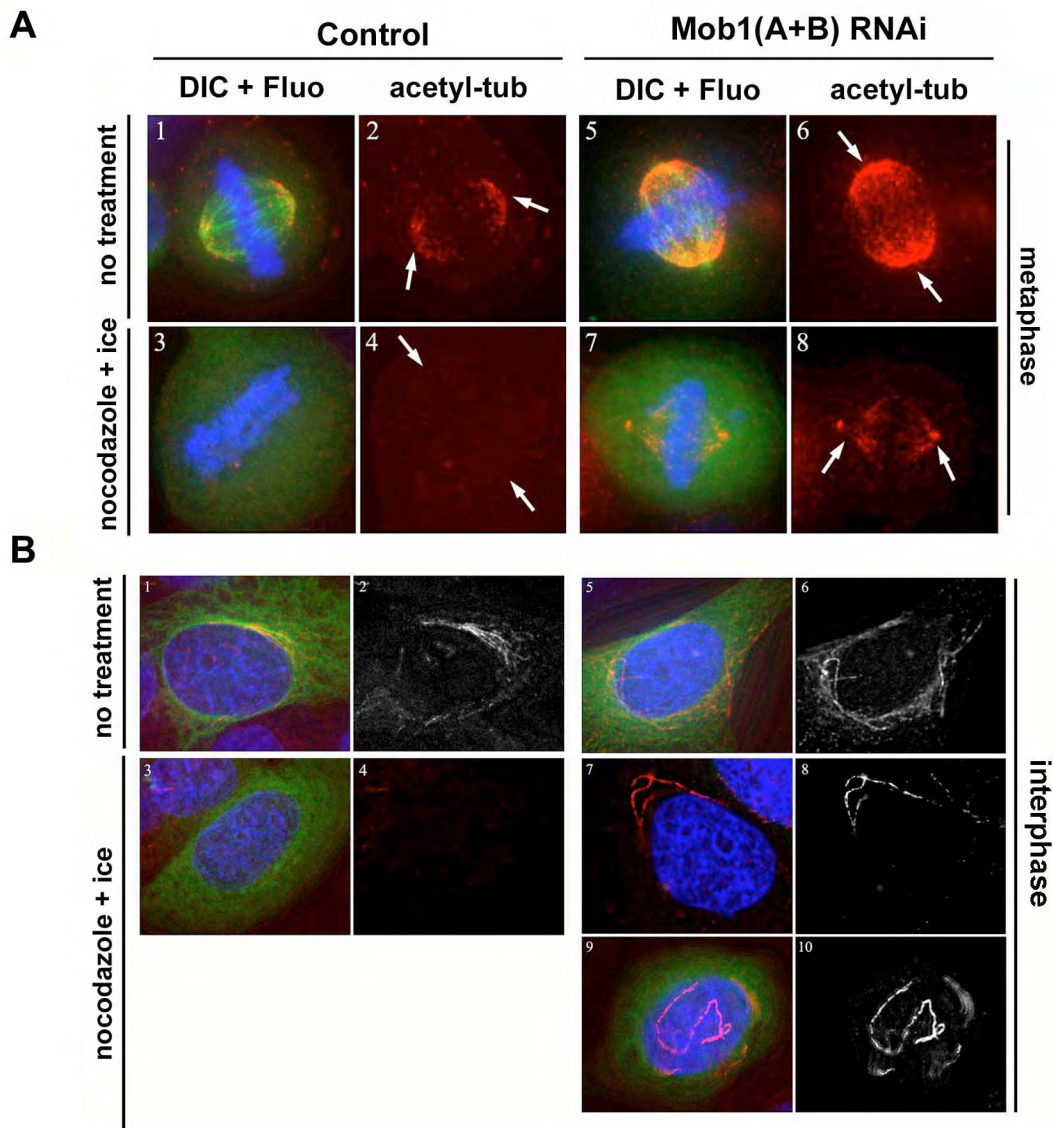
B

Centriole separation in late telophase  
in Mob1A or Mob1B overexpressing cells



**Supplementary Figure 5** *Overexpression of Mob1A or Mob1B impairs centriole separation.* HeLa GFP centrin cells were transfected with plasmids expressing Mob1A or Mob1B or with an empty vector. Two days after transfection cells were fixed and stained to detect GFP, tubulin and DNA. Only cells in late telophase/cytokinesis, with visible intercellular bridge were analyzed (~ 75 cell/experiment, 3 experiments). **A)** Centrioles are frequently separated in control cells (A2), but after overexpression of Mob1A or Mob1B centrioles are not separated (A1). **B)** Percentage of cells overexpressing Mob1A, Mob1B or control vector, in late cytokinesis containing separated centrioles.

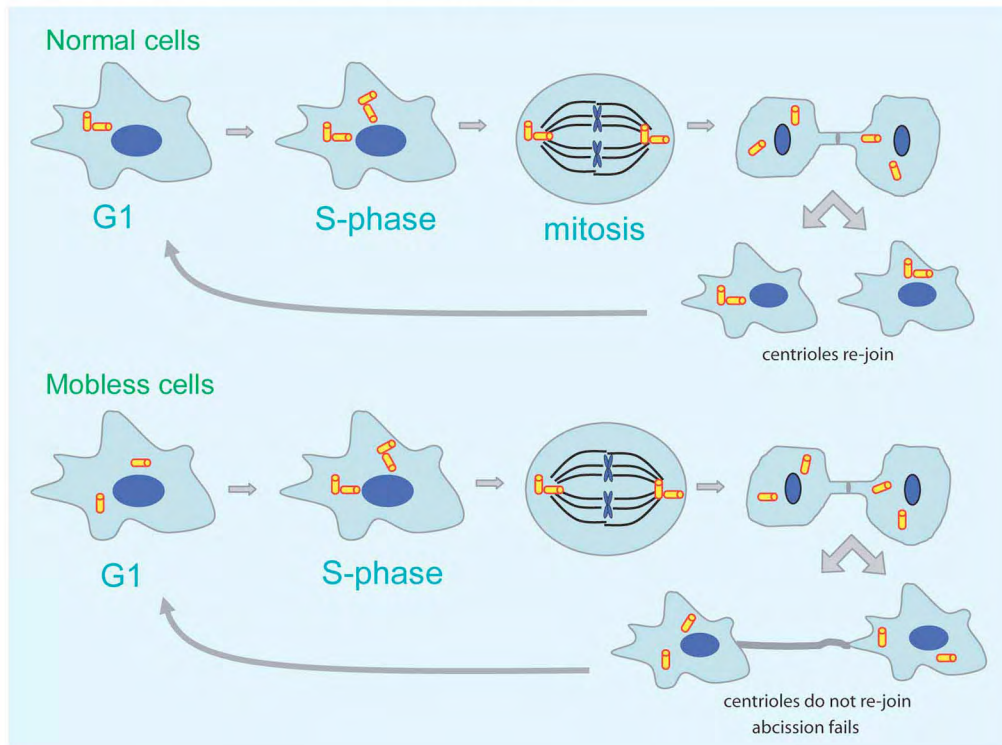




**Supplementary Figure 6.** Depletion of *Mob1* results in increased microtubule acetylation and stability. All images were acquired with the same exposure settings. Acetylated tubulin (red), DAPI (blue), GFP-tubulin (green) are shown. **A)** Acetylated tubulin detected on mitotic spindles is clearly more intense on *Mob1*(A+B) depleted cells than on control cells (compare 1-2 with 5-6). After nocodazole+ice treatment acetylated tubulin is not detected on control cells but is still detected in 24% of *Mob1*(A+B) depleted cells (compare 3-4 with 7-8). Arrows indicate the spindle poles **B)** Cells in interphase lose most acetylated microtubules after 30 minutes of nocodazole+ice treatment: 3% of cells still retain acetylated microtubules, while 40% of *Mob1*-depleted cells do so.

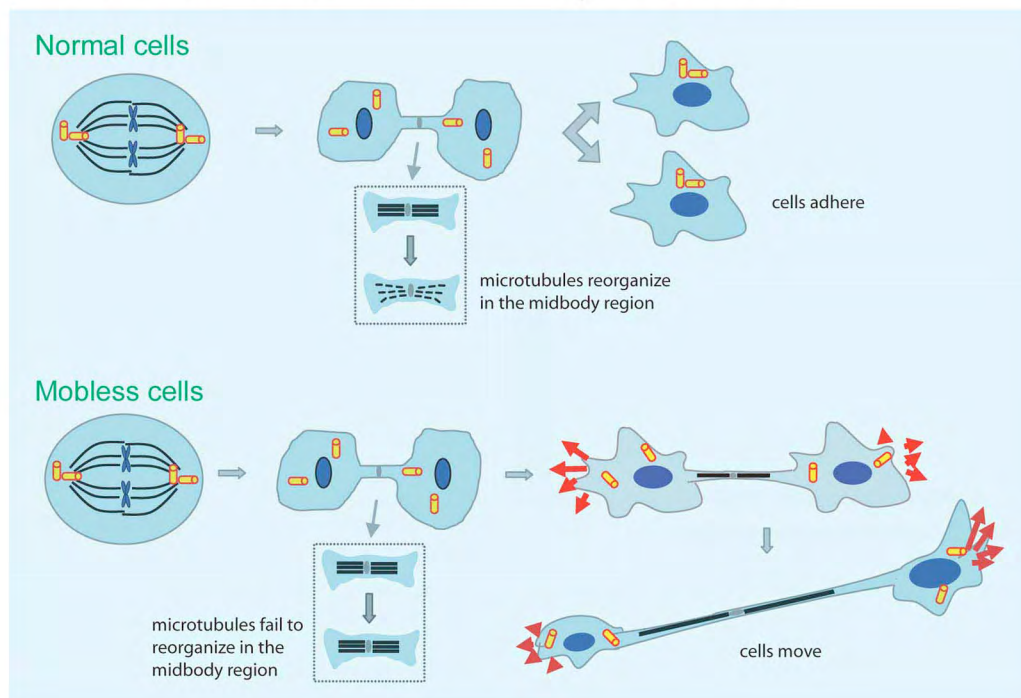
A

Centrosome cycle in control and Mob1-depleted cells



B

Abscission failure and cell movement in control and Mob-1 depleted cells



**Supplementary Figure 7. Proposed model of centrosome cycle, abscission failure and cell movement of Mob1-depleted cells. A)** In control cells centrosomes separate late in telophase and rejoin after abscission; in Mob1-depleted cells, centrosomes do not rejoin and abscission fails. Centrioles are represented by yellow cylinders, and the nucleus by blue circles. **B)** At the end of cytokinesis, control cells move slightly apart to adhere to the dish. Microtubules in the midbody region (black lines) reorganize allowing membrane fusion and cell abscission. In Mob1-depleted cells, microtubules increased stability prevents membrane fusion causing a failure in cell abscission. Simultaneously, cells do not adhere to the dish completely and continue their movement.