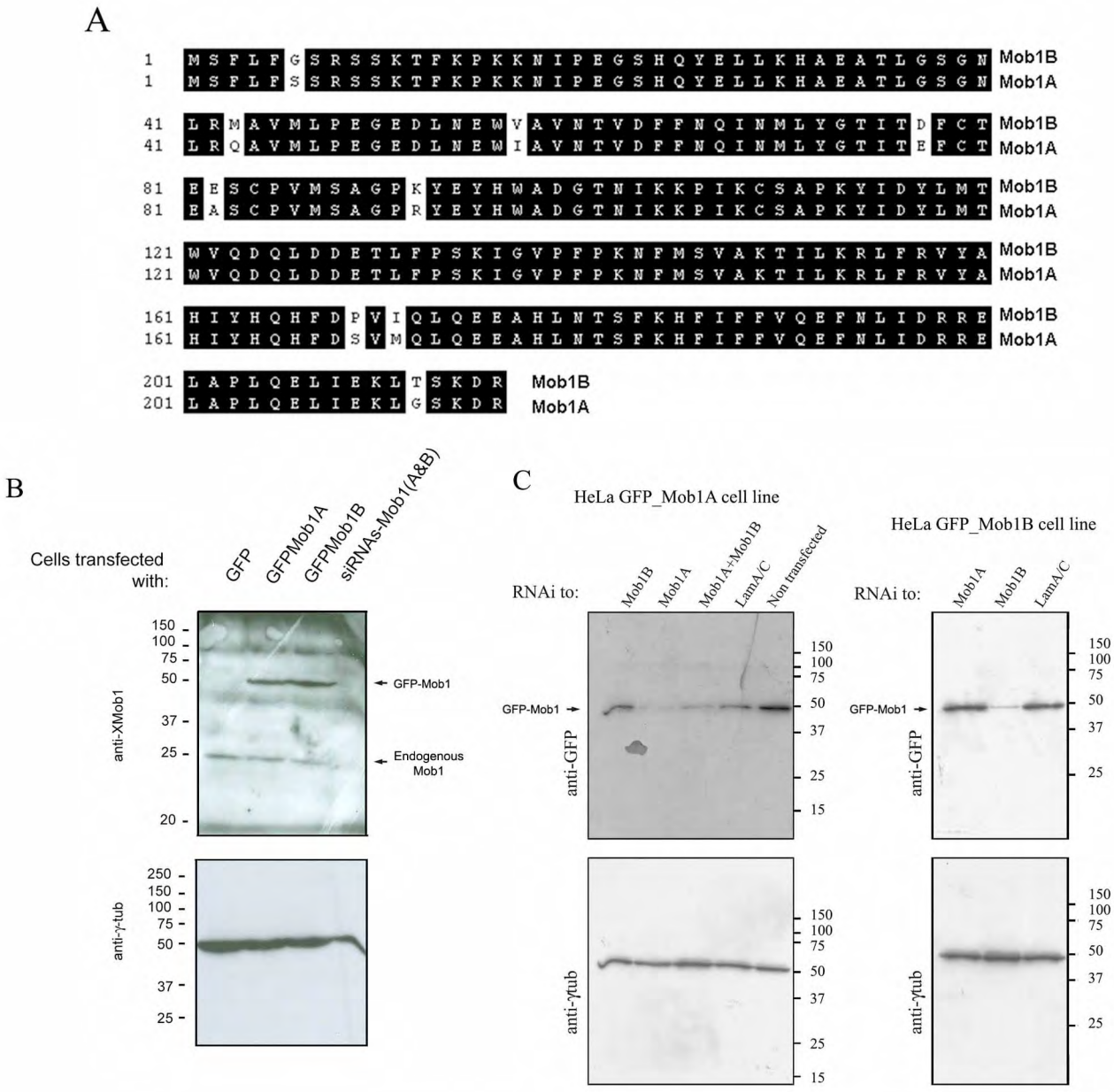
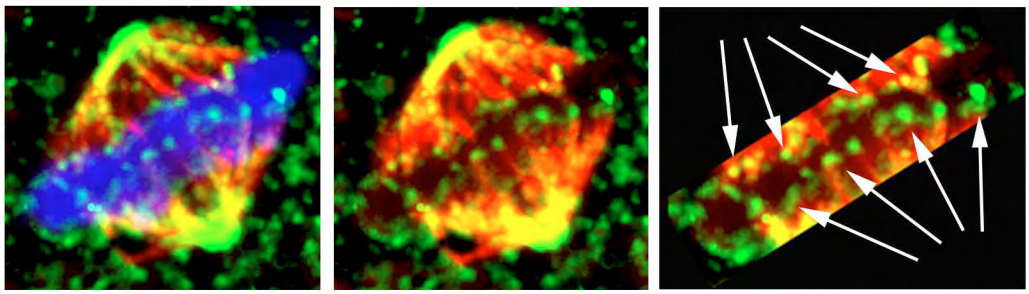
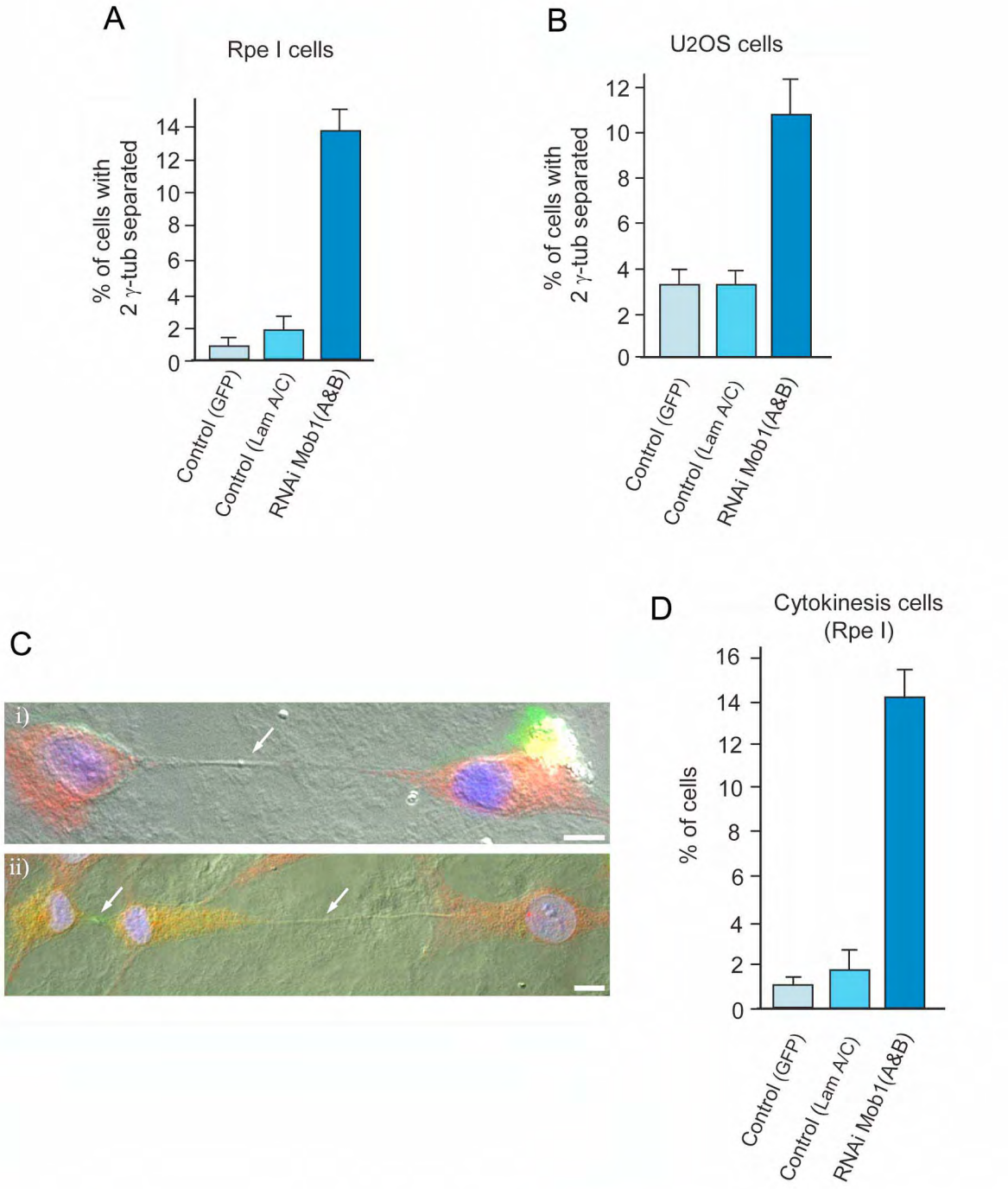


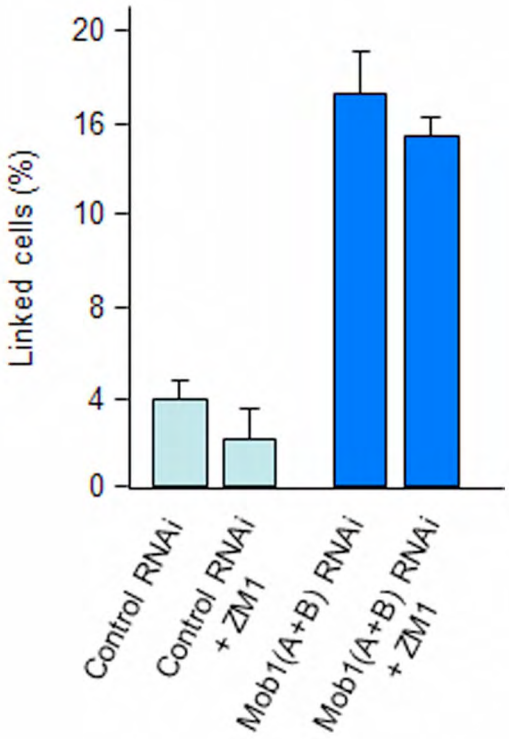
Supplementary Figure 1. *Specificity of Mob1A and Mob1B antibodies and RNAi.* **A)** Amino acid sequence alignment of Mob1A with Mob1B. Identical amino acids are shaded. Human Mob1A and Mob1B are 96% identical at the amino acid level. **B)** Specificity of anti-Mob1 antiserum. Extracts of HeLa cells expressing GFP, GFP-Mob1A or GFP-Mob1B were analyzed by western bolt, 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. γ-tubulin was used as a loading control on B) and C). The numbers indicate the sizes in kilodaltons of the molecular mass markers.



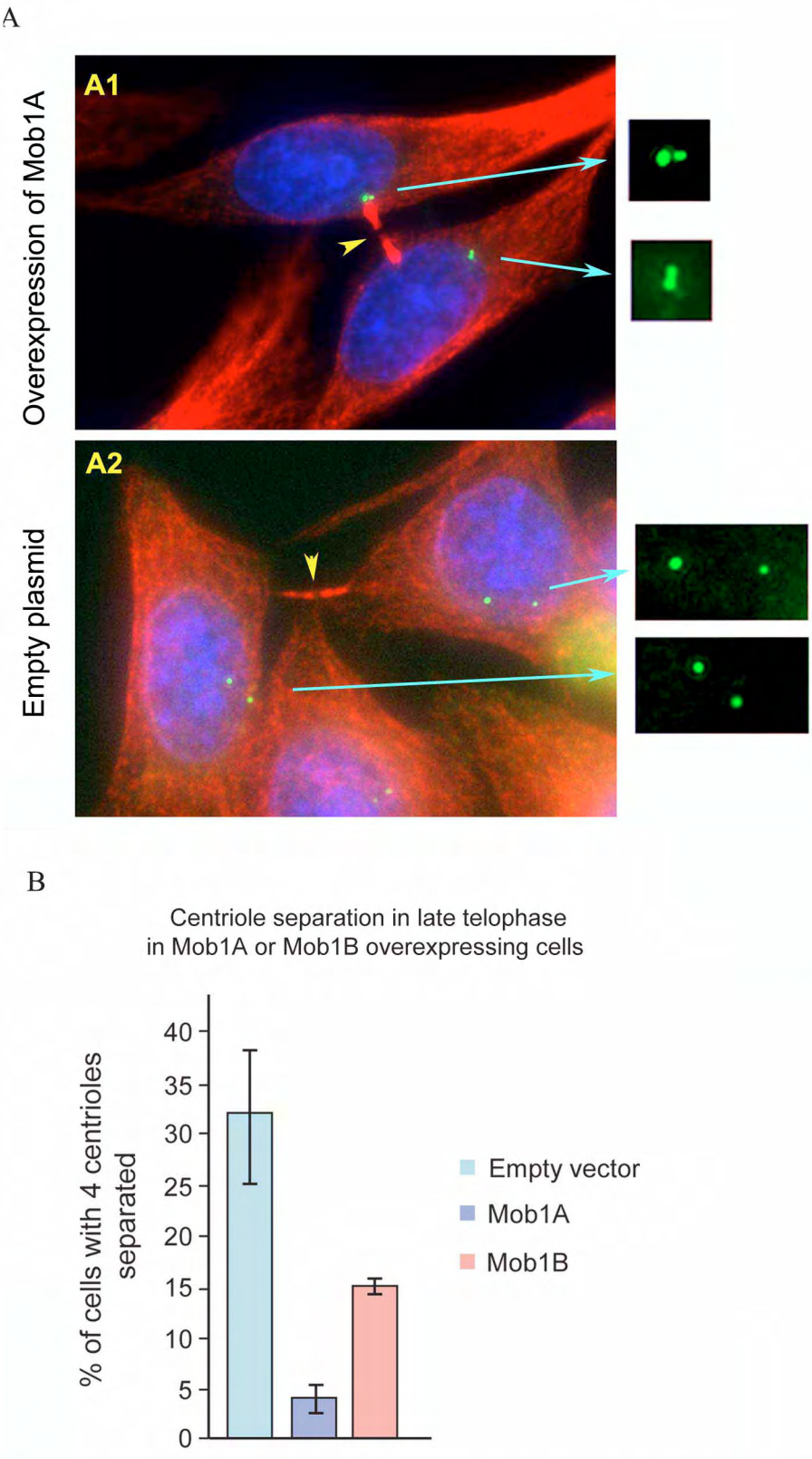
Supplementary Figure 2. *GFP-Mob1 accumulates on the Kinetochores*. Fixed HeLa cells stably expressing GFP-MOB1A were immunostained for α-tubulin (red), and the DNA was stained with DAPI (Blue). GFP-Mob1A is shown in green. The same mitotic figure is shown on three panels. Arrows indicate detection of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



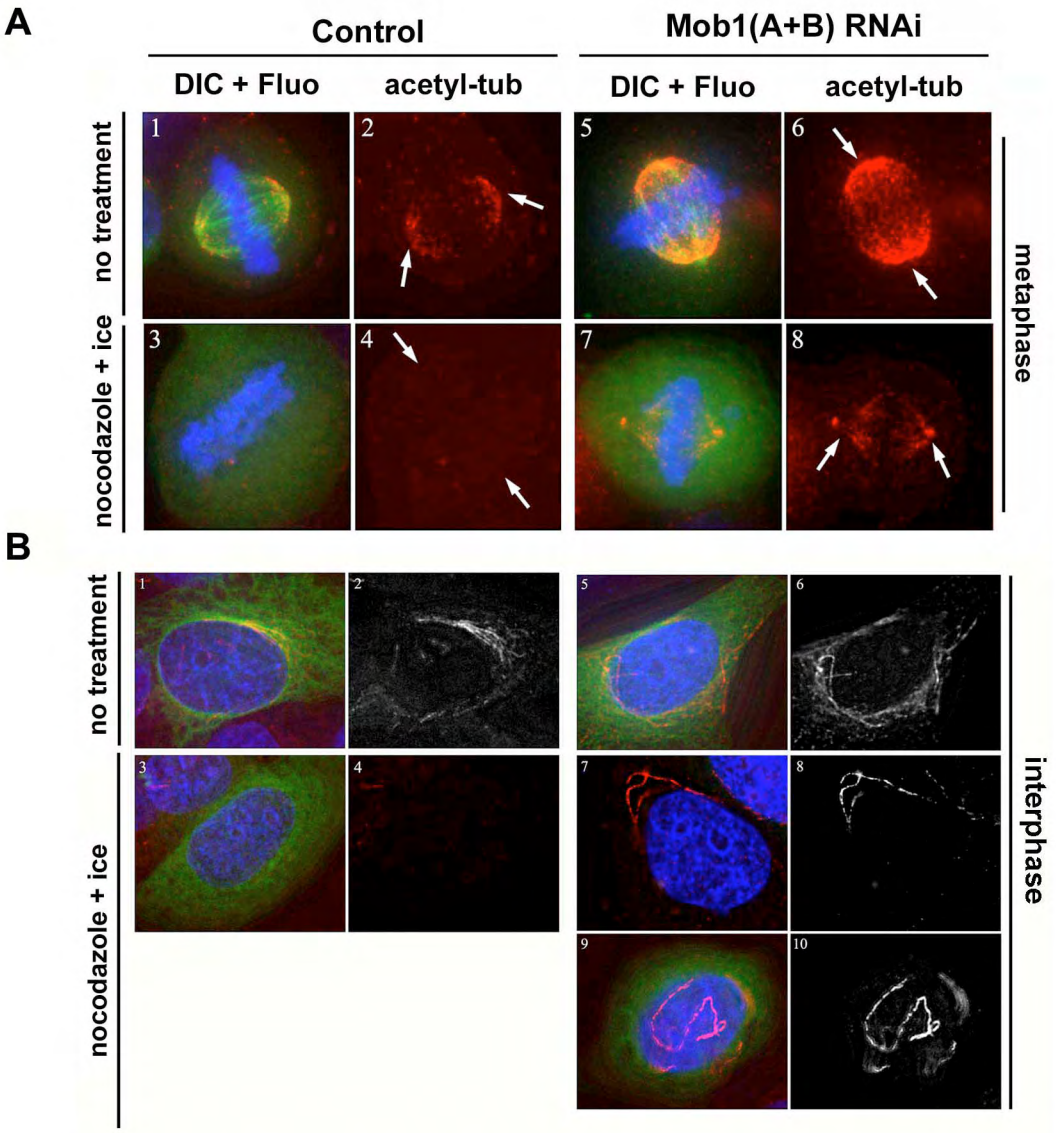
Supplementary Figure 3. *Mob1(A+B) depletion causes centriole separation and cytokinesis defects in RPE I and U2OS cells.* **A, B)** Graphs showing the quantification of centrosome/centriole separation in RPE I (A) and U2OS (B) cells. **C, D)** Mob1 A+B RNAi causes cytokinesis failure in RPE I cells.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



Supplementary Figure 4. *Aurora B inhibition does not significantly decreases 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 for3h and processed for immunofluorescence. The number of linked cells connected by an intercellular bridge was the quantified. (3 experiments, ~450 cells per experiment)



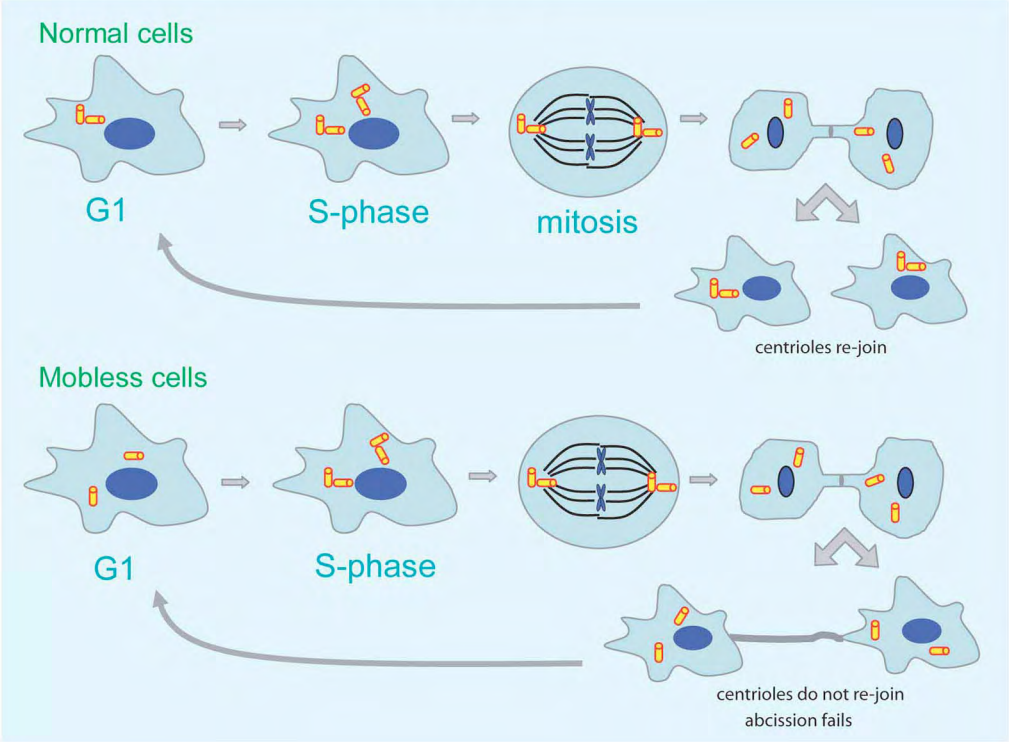
Supplementary Figure 5 *Overexpression of Mob1A or Mob1B impairs centriole separation.* HeLa GFP centrin cells were transfected with plasmids expressing Mob1A or Mob1B or with an empty vector. Two days after transfection cells were fixed and stained to detect GFP, tubulin and DNA. Only cells in late 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 loose 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.

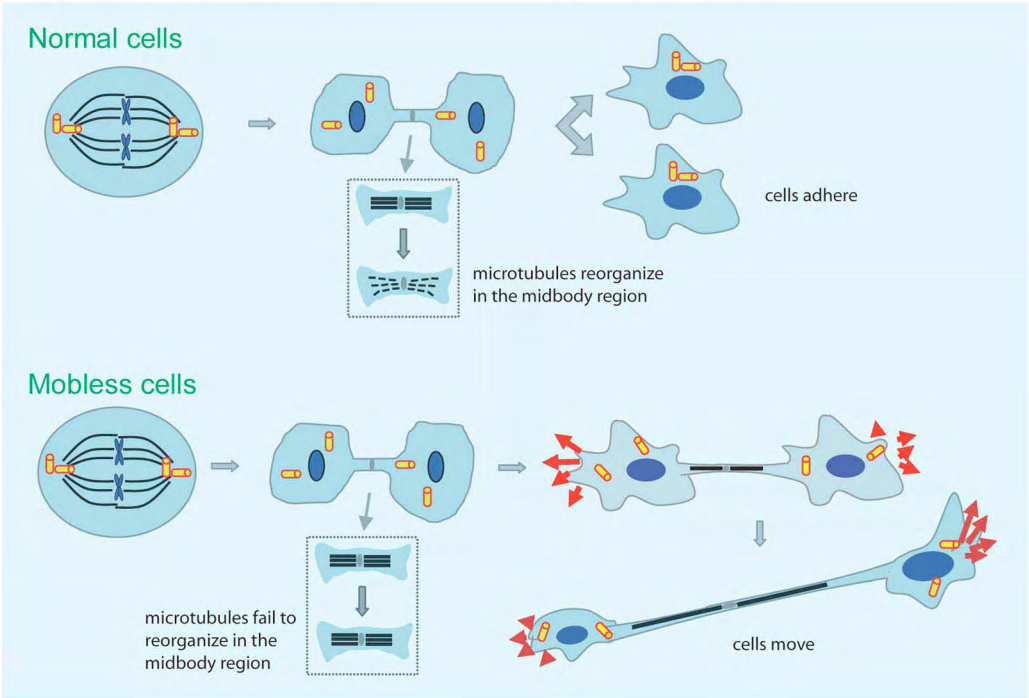
A

Centrosome cycle in control and Mob1-depleted cells



B

Abcission 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 centrioles separate late in telophase and rejoin after abscission; in Mob1-depleted cells, centrioles 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.