SUPPLEMENTARY INFORMATION

Suppl. Fig. 1-4 and Suppl. movies are available.

MATERIALS AND METHODS

Immunoprecipitation. For immunoprecipitation, cells or cells transfected with MCAK expression plasmids were synchronized to prometaphase with 30 ng/ml nocodazole for 14 h. 1-2 µg of cyclin B1, Cdk1 or Flag monoclonal antibodies were bound to Dynabeads® Protein G (Invitrogen). 600-800 µg cellular lysates were added to Dynabeads-IgG complex and incubated for 1 h at 4°C. Beads were then washed and boiled for SDS-PAGE.

RESULTS

MCAK expression is cell cycle regulated. To explore whether Cdk1/cyclin B1 and MCAK cooperate to regulate mitotic events, we studied their expression and possible association throughout the cell cycle. HeLa cells were synchronized and harvested at various time points for Western blot analyses with antibodies against MCAK, cyclin B1 and Cdk1. MCAK began to accumulate in S phase (Suppl. Fig. 1A, upper panel, lane 4), increased in G2 phase (Suppl. Fig. 1A, upper panel, lanes 5 and 6), and an additional slower migrating band of MCAK appeared during mitosis (Suppl. Fig. 1A, upper panel, lane 7). Concomitantly with MCAK, expression of cyclin B1 varied cell cycle dependently and reached its peak in mitosis (Suppl. Fig. 1A, second panel, lane 7). At 12 h, when the cyclin B1 level decreased (Suppl. Fig. 1A, second panel, lane 8), not only the slower migrating MCAK band disappeared but also overall MCAK levels became reduced (Suppl. Fig. 1A, first panel, lane 8), which is in line with a previous report that MCAK is degraded during mitosis (1). These observations indicate that both molecules exhibit similar expression/turnover kinetics and the appearance of the slower migrating MCAK band might relate to the activity of Cdk1.

To examine the spatial relationship between Cdk1 and MCAK in mitosis, HeLa cells were transfected with EGFP-tagged wild-type MCAK. Mitotic cells were immunostained with specific antibodies against Cdk1. MCAK and Cdk1 were seen to colocalize at centrosomes in prometaphase, while MCAK was also detectable at regions occupied by chromatin and chromosomes and in the cytoplasm (Suppl. Fig. 1B, upper panel). In metaphase, MCAK and Cdk1 colocalized mainly to centrosomes, but were also observable along mitotic spindles and at the equatorial plate, where chromosomes aligned (Suppl. Fig. 1B, second panel). In anaphase, while colocalization of MCAK and Cdk1 was weakly detectable at centrosomes, MCAK was more prominent at the midzone (Suppl. Fig. 1B, third panel). In telophase, weak staining of both MCAK and Cdk1 was retained at centrosomes, whereas MCAK was also observed at the midbody, where no Cdk1 immunofluorescence was observed (Suppl. Fig. 1B, lower panel). These data indicate that MCAK and Cdk1 colocalize at centrosomes throughout mitosis, and at mitotic spindles and the equatorial plate during metaphase, supporting the hypothesis that Cdk1 might interact with MCAK.

To test whether MCAK is regulated in a cell cycle dependent manner in human tumor cell lines other than HeLa cells, breast cancer cell line MCF-7, colon cancer cell line SW-480 and osteosarcoma cell line Saos-2 were synchronized to the G1/S boundary or prometaphase for Western blot analysis. Again, MCAK levels clearly correlated with cyclin B1 expression and the active form of Cdk1 (pT161) in all cell lines tested (Suppl. Fig. 1C). The levels of MCAK, pCdk1 (T161) and cyclin B1 increased at the G1/S boundary (Suppl. Fig. 1C, lanes 2, 5, 8 and 11) and peaked in prometaphase (Suppl. Fig. 1C, lanes 3, 6, 9 and 12).

FIGURE LEGENDS

Suppl. FIG. 1. Cell cycle regulated MCAK is colocalized and interacts with Cdk1. (A) Western blot analysis. HeLa cells were synchronized with double thymidine block and released into fresh medium. At indicated time points cells were harvested and cellular extracts

were prepared for Western blot analysis with antibodies against MCAK (upper panel), cyclin B1 (second panel), Cdk1 (third panel) and ß-actin (loading control, lower panel). Cellular extracts from thymidine/nocodazole-treated cells (Noc) or non-treated cells (con) were taken as controls. (B) Immunofluorescence staining. HeLa cells transfected with EGFP-MCAK were synchronized and released for 10 h. Cells were stained for Cdk1 and DNA. Bar: 5 µm. (C) Western blot analysis with extracts from MCF-7, HeLa, SW-480 and Saos-2 cells synchronized either by thymidine block (T) or thymidine/nocodazole treatment (N). Cellular extracts from unsynchronized cells (C) served as control. β-actin served as loading control. (D) Immunoprecipitation was performed using Flag antibodies with mitotic extracts from HeLa cells transfected with Flag-MCAK. The precipitates were subjected to Western blot analysis with antibodies as indicated. (E) Immunoprecipitation was carried out using antibodies against cyclin B1 or Cdk1 with mitotic extracts from HeLa cells without transfection. The precipitates were separated and stained with corresponding antibodies as indicated. IgG was taken as control.

Suppl. FIG. 2. Phosphorylation of MCAK by Cdk1 is specific in a time- and dose-dependent manner. (A) GST-tagged full-length MCAK proteins were subjected to kinase assays with different incubation time as indicated (upper panel). The same gel was stained with Coomassie as input control (lower panel). (B) Kinase assays were carried out with increasing amounts of GST-MCAK protein as indicated (upper panel). Cdk1 or MCAK alone served as control. The same gels were stained with Coomassie as input controls (lower panel).

Suppl. FIG. 3. T537 phosphorylation takes place *in vivo* and is associated with Cdk1 activity. (A) GST-tagged full-length MCAK or its core domain was used for kinase assays in the presence or absence of specific Cdk1 inhibitor RO-3306. DMSO served as control. (B) The T537 phosphorylation signal was reduced in HeLa 776-6 cells. HeLa cells or HeLa 776-6 cells, where cyclin B1 is stably downregulated, were synchronized to the G1/S boundary (T) or prometaphase (N). Cellular lysates were prepared for Western blot analyses with specific

p-MCAK antibodies (upper panels) and MCAK antibodies (middle panels). β-actin served as loading control (lower panels). Cellular extracts from non-treated cells served as controls (C). (C) and (D) Representatives of mitotic HeLa cells, stained for Plk1 (C) or cyclin B1 (D), p-MCAK and DAPI, were illustrated. Bar: 5 µm.

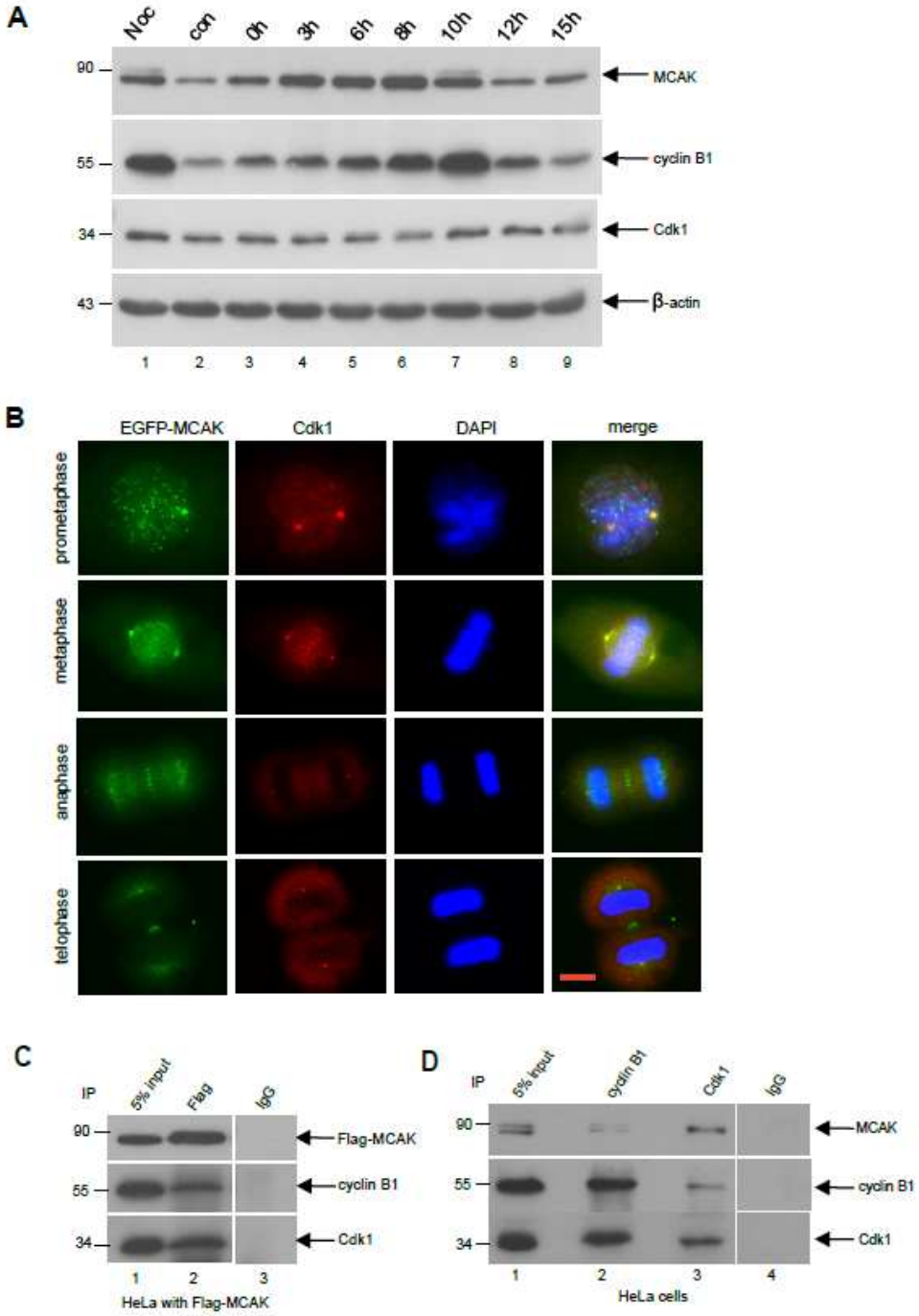
Suppl. FIG. 4. (A) Two siRNAs targeting MCAK are efficient. HeLa cells were transfected with siRNA MCAK1 or siRNA MCAK2 and synchronized to prometaphase. Cellular extracts were prepared for Western blot analyses with antibodies as indicated. (B) To compare the expression levels of EGFP-tagged MCAK and its mutants, HeLa cells were transfected with EGFP-tagged MCAK WT or its mutants. Cells were then harvested and cellular extracts were prepared for Western blot analysis with corresponding antibodies. (C) MCAK T537A localized predominantly to centrosomes. HeLa cells were transfected with EGFP-tagged MCAK T537A, MCAK WT or EGFP vector and synchronized to the G1/S boundary. 10 h after release cells were stained for pericentrin and DAPI. Representative mitotic HeLa cells transfected with MCAK T537A are depicted. Bar: 5 µm.

Suppl. MOVIES. *In vitro* depolymerisation of microtubules by wild-type MCAK and its variants MCAK T537A and MCAK T537E. Epifluorescence images of immobilized rhodamine-labelled, GMPCPP-stabilized microtubules were recorded at 5s intervals. Prior to the first frame of each movie 500 nM wild-type MCAK, MCAK T537A or MCAK T537E was added. Playback is 50x real time.

Reference List

1. **Ganguly, A., R. Bhattacharya, and F. Cabral**. 2008. Cell cycle dependent degradation of MCAK: evidence against a role in anaphase chromosome movement. Cell Cycle **7**:3187-3193.

Suppl. Fig. 1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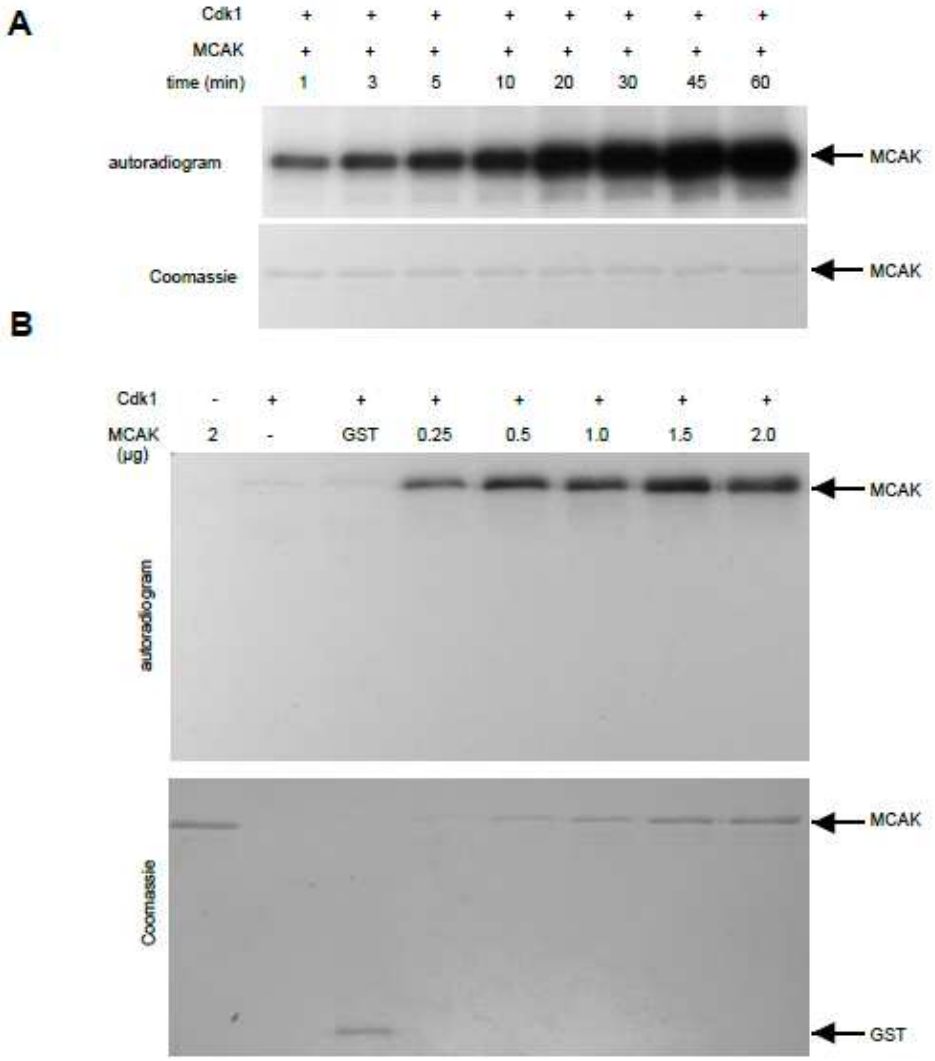
HeLa cells

Suppl. Fig. 2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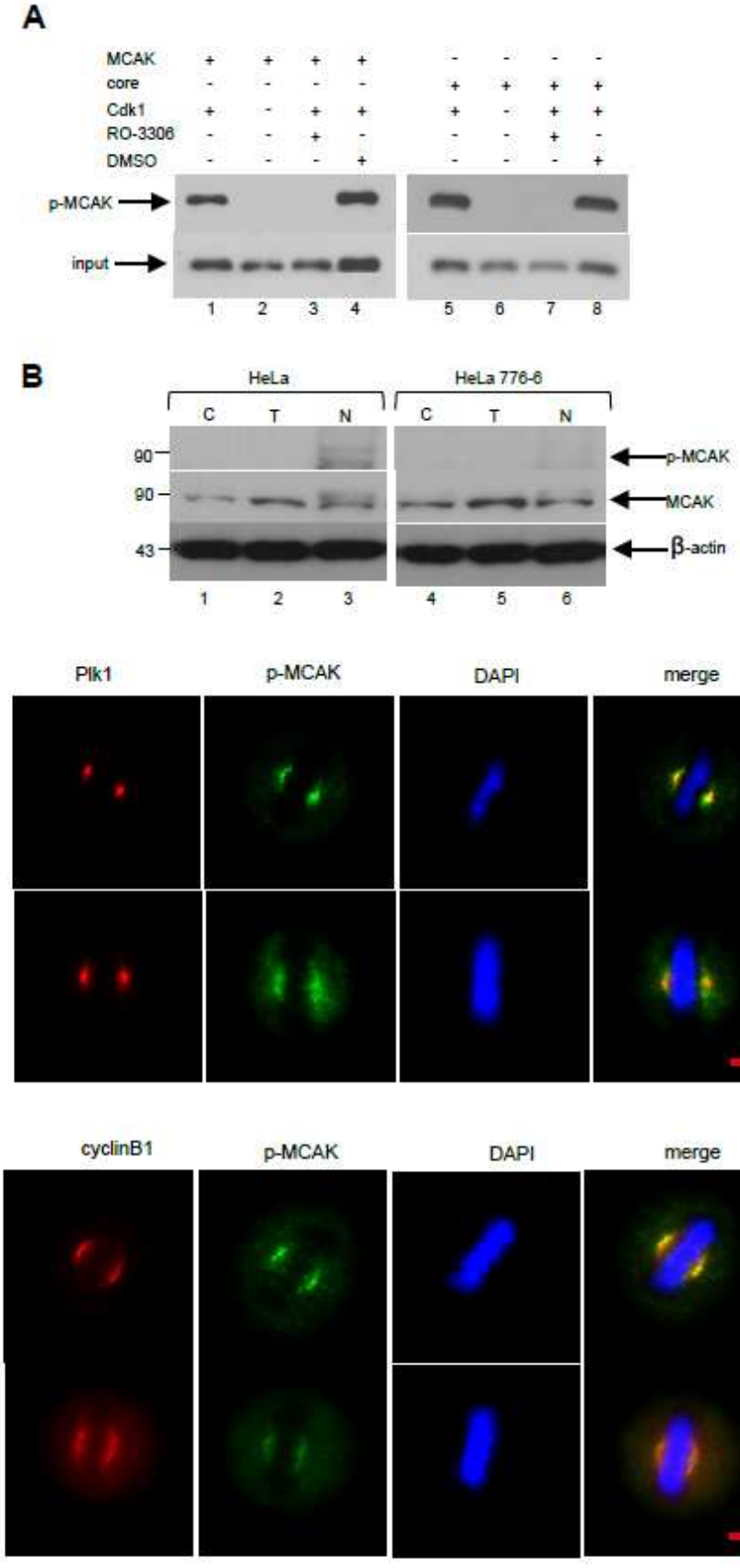
Suppl. Fig. 3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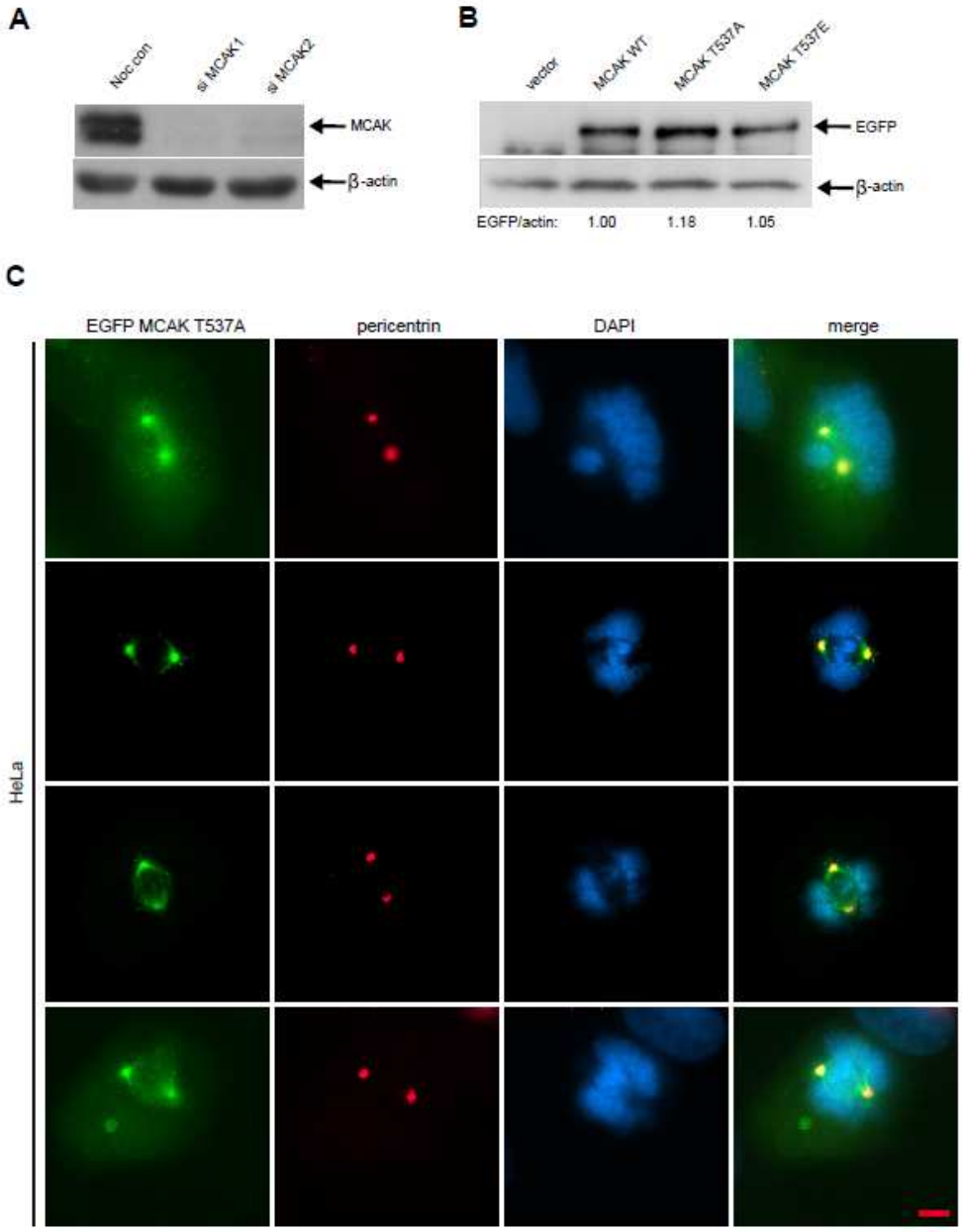
Suppl. Fig. 4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