Supplementary information

Supplementary materials

Antibodies

The following antibodies were used: monoclonal mouse anti-α-Tubulin (DM1A, Sigma), a polyclonal rabbit anti-AurA generated in the lab and affinity purified, rabbit anti-pT288 AurA (Cell Signalling), rabbit anti pT232 AurB (Rockland), rabbit anti-pS558 TACC3 (Cell Signalling) and rabbit anti-TACC3 (Santa Cruz). For immunofluorescence Alexa 488, Alexa 568 conjugated anti-rabbit or anti-mouse secondary antibodies (Sigma) were used and Hoechst33342 (Invitrogen) was used to visualize the DNA. For Western blots, Alexa 680 or 800 conjugated anti-rabbit or anti-mouse secondary antibodies (Invitrogen) were used.

Supplementary figure legends

Supplementary figure 1.

A- Immunofluorescence images of HeLa cells showing the localization of the active form of AurA (detected with the anti-pT288 AurA antibody) at different cell cycle stages. pT288 AurA is shown in green, α-Tubulin in red and DNA in blue. pT288 Aurora A can be detected in prometaphase, metaphase and anaphase cells but not in telophase.

B- HeLa cells synchronized in prometaphase with STLC were incubated with a range of MLN concentrations as indicated for 30 minutes. Cell lysates were analyzed by Western blot using the anti pT288 AurA and anti-AurA antibodies. The pT288 AurA signal was reduced as the MLN concentration increased whereas the signal for total AurA remained unaltered.

C- Immunofluorescence images of HeLa cells incubated with DMSO (CTRL) or MLN8237 (MLN) showing that active AurB as detected with the phospho specific anti pT232 AurB antibody, is enriched at the spindle midzone in control cells (left) as well as in cells incubated
with MLN (right). In the overlay, AurB pT232 (green), \( \alpha \)-Tubulin (red) and DNA (blue). Scale bar, 5\( \mu \)m. The quantification of the fluorescence intensity signal for pT232 AurB at the spindle midzone is shown at the bottom. The data were obtained from three independent experiments. Bars, SEM.

D- Immunofluorescence on control and AZD treated HeLa cells showing that active AurB as detected with the phospho specific anti pT232 AurB antibody, is absent from the midzone of AZD (100nM) treated cells. In the overlay, AurB pT232 (green), \( \alpha \)-Tubulin (red) and DNA (blue). Scale bar, 5\( \mu \)m. The quantification of the fluorescence intensity signal for pT232 AurB at the midzone is shown at the bottom. The data were obtained from three independent experiments. Bars, SEM.

**Supplementary figure 2.**

A- Quantification of the K-fiber length over time in DMSO (CTRL) (n=15) and MLN (n=6) treated cells obtained from time lapse recordings. Bars, SEM.

B- Quantification of the pole to pole separation over time in DMSO (CTRL) (n=15) and MLN (n=6) treated cells obtained from time lapse recordings. The pole to pole distance is shorter for MLN compared to CTRL treated cells. Bars, SEM.

C- Quantification of the astral MT intensity in CTRL (n=30) and MLN (n=28) treated cells from three independent experiments. The difference was not statistically significant (p= 0.4424). Bars, SEM.

**Supplementary figure 3.**

A- Immunofluorescence images of HeLa cells showing the localization of phospho TACC3 detected with a phosphospecific antibody against S558 TACC3 (pTACC3). The signal for
pS558 TACC3 is reduced in cells incubated with MLN8237 (MLN) and in TACC3 silenced cells (siTACC3). Scale bars, 5μm.

B- Mitotic HeLa cells transfected with control siRNA (siCTRL) and siRNA against TACC3 (siTACC3) were collected by mitotic shake off, lysed and subjected to Western blot analysis with anti TACC3 and anti α-Tubulin antibodies. The efficiency of TACC3 depletion was 58.6% (average of three independent experiments).

C- Mitotic HeLa cells were first transfected with control siRNA (siCTRL) or an siRNA pool against the 3’ and 5’ UTR of TACC3 (siTACC3). After transient expression of Flag alone, Flag-TACC3 WT or Flag-TACC3 S558A, cells were collected by mitotic shake off, lysed and subjected to Western blot analysis with anti TACC3 and anti α-Tubulin antibodies. The efficiency of TACC3 depletion with these siRNAs was 57% (average of three independent experiments). Although the Western blot shows a high degree of overexpression for the exogenous Flag tagged proteins, immunofluorescence analysis showed that high levels of overexpression was only observed in prometaphase like cells. Cells in anaphase showed had low levels of overexpression as detected with the anti-Flag antibody. This suggests that the overexpression of TACC3 prevents cells from entering into anaphase.

Movies legends

Supplementary movie 1

Time lapse analysis of a HeLa cell expressing H2B–eGFP and α-tubulin–mRFP arrested in metaphase with MG132. DMSO containing medium was added at time 0. Images were acquired with an Olympus Andor Revolution XD spinning disc, every 3 minutes for 2,45h. The movie shows maximum intensity projection of 4 stacks every 0.8μm. Image depth is 14bit, pixel size is 0,15μm. Time on top left is in minutes.
**Supplementary movie 2**

Time lapse analysis of a HeLa cell expressing H2B–eGFP and α-tubulin–mRFP arrested in metaphase with MG132. MLN8237 containing medium was added at time 0. Images were acquired with an Olympus Andor Revolution XD spinning disc, every 3 minutes for 2.45h. The movie shows maximum intensity projection of 4 stacks every 0.8µm. Image depth is 14bit, pixel size is 0.15µm. Time on top left is in minutes.

**Supplementary movie 3**

Time lapse analysis of a HeLa cell expressing H2B–eGFP and α-tubulin–mRFP released from STLC arrest. DMSO containing medium was added at time 0. Images were acquired with an Olympus Andor Revolution XD spinning disc, every minute for 19 minutes. The movie shows maximum intensity projection of 4 stacks every 0.8µm. Image depth is 14bit. Pixel size is 0.15µm. Time on top left is in minutes.

**Supplementary movies 4 and 5**

Time lapse analysis of a HeLa cell expressing H2B–eGFP and α-tubulin–mRFP released from STLC arrest. MLN8237 containing medium was added at time 0. Images were acquired with an Olympus Andor Revolution XD spinning disc, every minute for 19 minutes. The movie shows maximum intensity projection of 4 stacks every 0.8µm. Image depth is 14bit. Pixel size is 0.15µm. Time on top left is in minutes.

**Supplementary movie 6**

Time lapse analysis of a control silenced HeLa cells expressing H2B–eGFP and α-tubulin–mRFP released from STLC arrest. Images were acquired with an Olympus Andor Revolution XD spinning disc every 2 minutes for 19 minutes. The movie shows maximum intensity
Supplementary movie 7

Time lapse analysis of a TACC3 silenced HeLa cell expressing H2B–eGFP and α-tubulin–mRFP released from STLC arrest. Images were acquired with an Olympus Andor Revolution XD spinning disc every 2 minutes for 19 minutes. The movie shows maximum intensity projection of 4 stacks every 0.8µm. Image depth is 14bit, pixel size is 0.15µm. Time on top left is in minutes.
Supplementary figure 1

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Supplementary figure 3

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