Supplemental Information

Lateral to End-on Conversion of Chromosome-Microtubule Attachment Requires Kinesins CENP-E and MCAK

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Figure S1. Lateral-kinetochores are not detached kinetochores that non-specifically overlap with microtubules, Related to Figure 1
A) Images showing levels of kinetochore bound Mis12 in cells treated with siRNA as indicated and exposed to monastrol for 3h before immunostaining. Cells were immunostained with antibodies against Mis12, β-tubulin, CREST antisera and stained with DAPI for DNA. B) Graph of kinetochore-microtubule attachment status showing percentage of laterally, syntelically or monotelically attached or detached kinetochores or kinetochores with unascertainable KT-MT attachment status (unknown) in cells treated as in (A). Scale bar: 5 μm. Error bars represent SEM values across three experiments. Dashed red line marks the degree of uncertainty in determining lateral-kinetochores, in the presence of a large majority of detached kinetochores. p-values representing significance levels were obtained using Proportion test. * and # indicate significant and insignificant difference, respectively. C) Frequency graph shows percentage of lateral-kinetochores with varying intensity of kinetochore-bound Astrin, measured relative to CREST signals. Intensity values were obtained from immunofluorescence images of monastrol treated cells that were fixed and immunostained using antibodies against Astrin, CREST sera and Tubulin. Astrin and CREST intensities show subtracted background noise values before calculating intensity ratio (as described in experimental procedures). Max refers to the maximum possible value, from observations on end-on attached kinetochores. Pink section on left refers to kinetochores where Astrin signals are not visualisable (scored as negative/OFF) and Blue section on right refers to kinetochores were Astrin signals are low yet visualisable (scored as positive/ON).  (n: cells = 9; KTs = 56). Error bars represent SEM values across cells.
Figure S2. MCAK, CENP-E and Kif2B are required for normal congression of chromosomes, Related to Figure 3
A) Representative time-lapse images of HeLa (His2B-GFP; Cherry-Tubulin) cells treated with siRNA as indicated showing congression defects in cells treated with MCAK, CENP-E, or Kif2B as indicated. Scale bar: 10 μm. B) Immunoblots showing extent of protein depletion in lysates of cells treated with siRNA as indicated and probed with antibodies against MCAK, Kif2B or CENP-E, as indicated. Antibody against γ-tubulin was used as a loading control. C) Graph of percentage of mitotic cells displaying congression defects. Cells were siRNA transfected and MG132 treated for 90 min prior to fixing and immunostaining with antibodies against β-tubulin and HEC1Ndc80, CREST anti-sera and DAPI. Congression defect was assigned when one or more chromosomes were unaligned on the metaphase plate. Error bar represents SEM values across three experimental repeats.
Figure S3. Motor activities of MCAK and CENP-E are required for end-on conversion, Related to Figure 3
A) Images showing plus-end localization of MCAK wild-type (WT) and MCAK hypir mutant in cells transfected with plasmids encoding GFP fused to MCAK wild-type or dead mutant ‘hypir’ as indicated and treated with monastrol prior to immunostaining with antibodies against β-tubulin and GFP (to detect MCAK-GFP), CREST antisera and stained with DAPI for DNA. B-D) Graphs of kinetochore-microtubule attachment status showing percentage of laterally, syntelically, monotelically attached or unattached kinetochores or kinetochores with unascertainable KT-MT attachment status in monastrol-treated cells expressing GFP-tagged MCAK wild-type (WT) or dead mutant ‘hypir’ (B) immunostained as in Figures S4A, cells treated with Kif2B siRNA or Control siRNA (C) immunostained as in Figure 4D or cells treated with monastrol in the presence or absence of CENP-E inhibitor (inh.), GSK923295 for an hour (D) immunostained as in Figure 4E. Dashed green line marks the degree of uncertainty in determining lateral-kinetochores, as deduced for monopolar spindles in Figure S1. Error bars represent SEM values across three experimental repeats. p-values were obtained using Mann Whitney U test. * and # indicates significant and insignificant difference respectively.
Figure S4. Lateral-kinetochores in MCAK depleted cells display partial end-on status, Related to Figure 5

A and B) Distribution graph of instantaneous velocity (A) and comet life-time (B) of EB1 comets in HeLa[E1-B1-YFP] cells treated with control or MCAK siRNA as indicated. Cells were treated with monastrol for 3 h prior to filming once every second for a period of 1 min. Error bars represent SEM values across cells. C) Representative immunofluorescence images of Control or MCAK siRNA transfected cells treated with monastrol for 1 h prior to fixation and immunostained with antibodies against Mad2 and Tubulin, CREST antisera and stained with DAPI for DNA. Cropped images are 3x magnified. Bar: 5 μm (in insets: 2 μm). D and E) Graph of percentage of lateral-kinetochore pairs with both, one or neither (none) of the pair displaying Mad2 (D) and Mad1 (E). Cells were treated with siRNA and monastrol as in C and immunostained with antibodies against Tubulin and either Mad2 (D) or Mad1 (E) and CREST antisera. p-values were obtained using Proportion test. * and # indicates significant and insignificant difference respectively. F and G) Image (left panels) of a MCAK depleted HeLa[YFP-Tub; Cen-Red] cell (F) and a HEC1[Ndc80]-depleted cell expressing Tet-inducible siRNA resistant HEC1[Ndc80]-Δloop-YFP and mKate-Tubulin (G) treated as described in Figure 6A and Figure 2E respectively. Graphs (right panels) of total intensity of tubulin and CENP-B signals along the length of the rectangular segment (dashed white) of respective images (left panels). Values in purple indicate ratio of tubulin intensity measured at positions before (blue circle) and after (grey circle) the site of kinetochore interaction with microtubule.
Figure S5. CENP-E is specifically required for wall tethering independent of lateral gliding of kinetochores, Related to Figure 6
A) Graph of percentage of lateral-kinetochore pairs with both, one or neither (none) of the pair displaying Mad1. Cells were treated with siRNA as indicated and treated with monastrol for 3 h prior to fixation. Fixed cells were immunostained with antibodies against β-tubulin and Mad1 and CREST antisera. p-values were obtained using Proportion test. # indicates insignificant difference. B and C) Graph of percentage of detachment episodes observed in lateral-kinetochores during the 5-minute duration of time-lapse movies. HeLa cells co-expressing YFP fused to HEC1<sup>Ndc80</sup>-ΔLoop and mKate-Tubulin were depleted of endogenous HEC1<sup>Ndc80</sup> and treated with Control or CENP-E siRNA for 36 h (B) or Control or MCAK siRNA for 48 h (C). Detachment episodes that lasted at least for 3 consecutive time-frames alone were considered. Error bars represent SEM values across three experiments repeats. p-values were obtained using Proportion test. * and # indicates significant and insignificant difference, respectively.
Table S1. Criteria for defining lateral, end-on and detached kinetochores, Related to Figure 1

| KT-MT attachment states | Kinetochore vs plus-end movements (live-cells) | Geometric configuration of CENP-B and tubulin signals (live-cells) | Tubulin intensity before and after site of attachment | Signal Overlap between width of HEC1<sup>Ndc80</sup> and tubulin wall (fixed-cells) | Recruitment of plus-end protein, Astrin, to the kinetochore (fixed-cells) |
|------------------------|-----------------------------------------------|---------------------------------------------------------------|------------------------------------------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| 1. Lateral             | asynchronous                                   | Tubulin signal extends beyond CENP-B signal.                 | Similar                                              | Overlap along the entire width of KT present; Tubulin signal extends beyond HEC1<sup>Ndc80</sup> signal. | Astrin predominantly absent                                                  |
|                        |                                               | KT-MTend distance: >0.3μ                                      |                                                      |                                                                                   |                                                                                  |
| 2. End-on              | synchronous                                    | Tubulin signal terminates near CENP-B signal.                 | Dissimilar                                           | Minimal overlap only with a portion of outer kinetochore; Tubulin signal terminates at HEC1<sup>Ndc80</sup> signal. | Astrin always present                                                              |
|                        |                                               | KT-MTend distance: <0.2μ                                      |                                                      |                                                                                   |                                                                                  |
| 3. Detached            | asynchronous                                   | CENP-B particles move randomly                                | Irrelevant                                           | Overlap completely absent.                                                        | Astrin always absent                                                              |

Table briefly summarises the five criteria used to distinguish the three different kinetochore-microtubule attachment states as indicated. Conditions used in live- or fixed-cell assays are marked separately.
Supplemental Experimental Procedures

Image analysis: 3D rotation, Deconvolution, volume rendering
Deconvolution of live and fixed-cell images were performed using SoftWorx. Z-slices of 0.1 μm apart were acquired. Rotation angles (α, β, γ) of inter-kinetochore axes for measuring i) change in kinetochore axis > 30 (tumble; T) ii) 3D positions of kinetochores for ΔKT and iii) inter-kinetochore distances were obtained using SoftWorx. For quantifying ΔKT and KT-MT end distances, measurements were made in 3dimensions along the length of the microtubule fibre. Traces of tubulin signal were obtained using line tracing option in Photoshop or plot profile option in ImageJ. 3D Volume rendering was performed in SoftWorx. Pixel intensity of tubulin, CENP-B or HEC1Ndc80 for a defined rectangular segment were obtained using Image J and total intensity along the length of the segment was computed and plotted using Excel.

Statistical Analysis
To confirm that sampling is sufficient, we use two statistical tests: First, for ordinal variables we performed a Mann-Whitney U test to confirm, using p-values, that we have sampled sufficient number of cells for concluding on differences we report. Second, we measure standard error over mean (s.e.m) across experimental repeats and confirm that the differences we report are not only based on differences in mean values, but also the spread of the mean values between experiments. SD values are SEM values obtained across experiments, cells or kinetochores as indicated in legend. p-values representing significance were obtained using Mann-Whitney U test, Proportion test and Paired sample t-test, as appropriate.

PSF measurements
We deduced the lateral (x,y) and axial (z) resolution to be 0.21μm and 0.39μm respectively for Coolsnap camera conditions, and 0.30 μm and 0.43 μm respectively for Cascade2 camera conditions, using 0.1 μm multi-colour beads (TetraSpeck microspheres, Life Technologies) for 405 nm excitation (an average of 3 measurements). For Cascade2 camera, in green (GFP/YFP) channels the lateral and axial resolutions were 0.35 μm and 0.38 μm respectively and in red (mcherry/DsRed) channels the lateral and axial resolutions were 0.32 μm and 0.35 μm respectively.