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Supplemental Material to:

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Automated tracking of mitotic spindle pole positions shows that LGN is required for spindle rotation but not orientation maintenance

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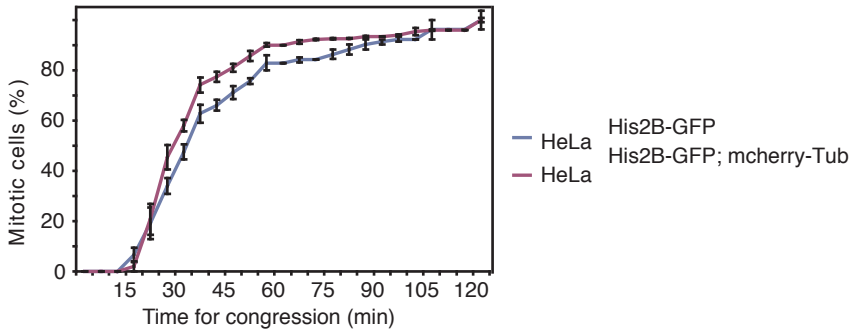
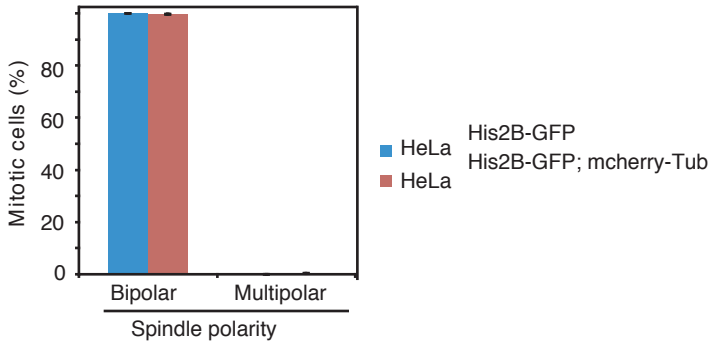
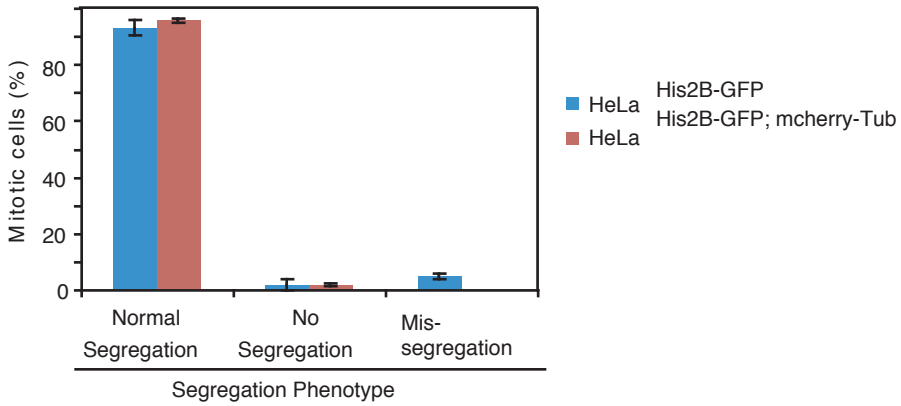
A Cumulative Mitotic Timing



B Spindle architecture

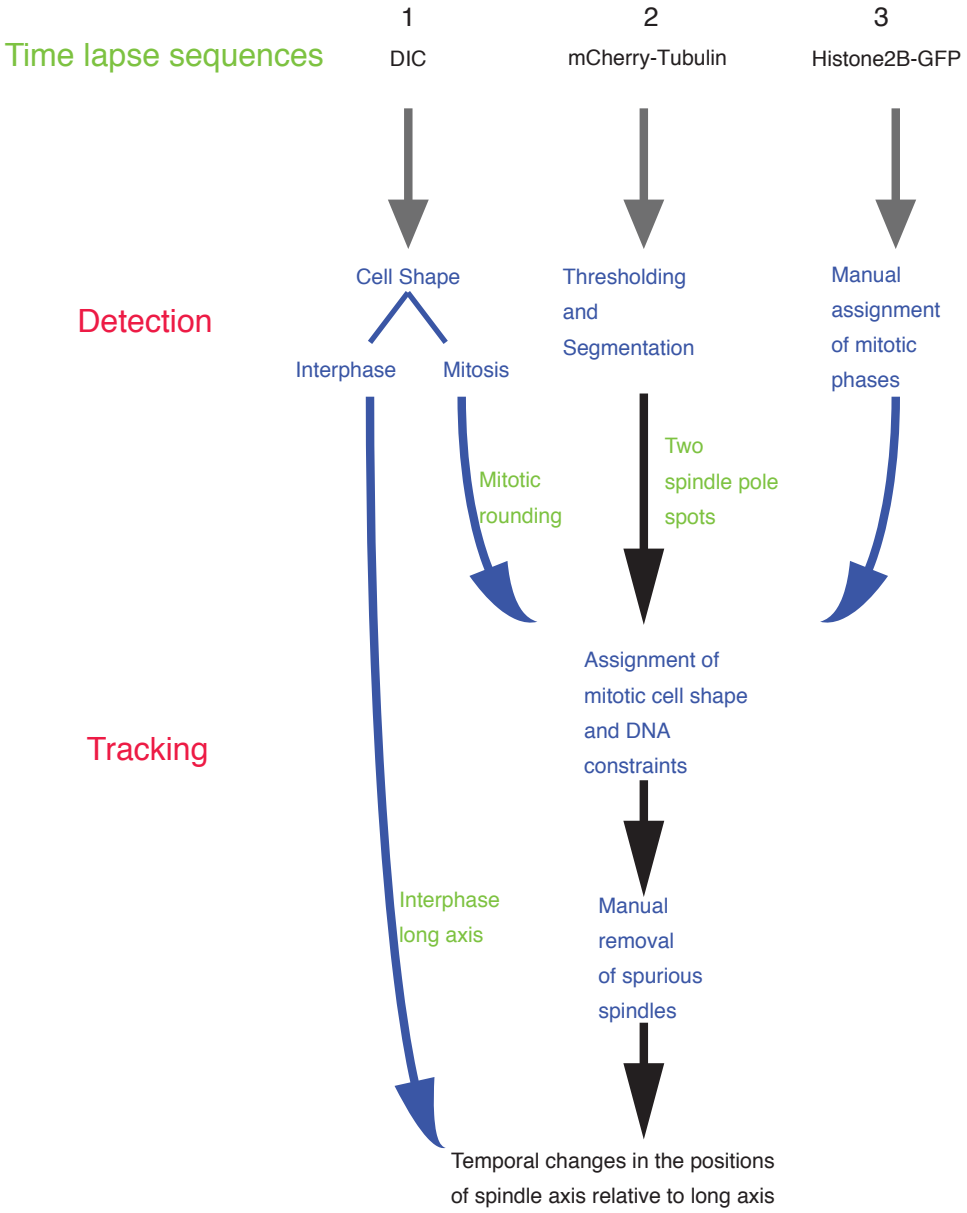


C Chromosome segregation accuracy

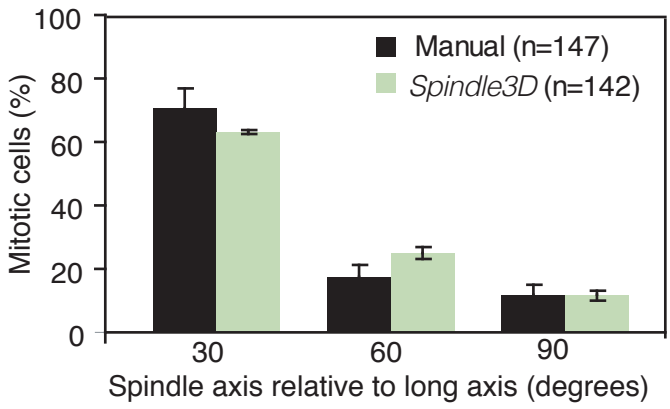


Supplementary Figure-2 Corrigan *et al.*, 2013

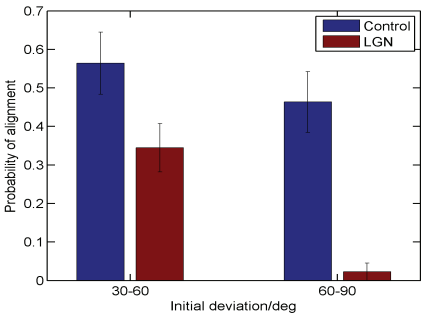
A Flow diagram of Spindle pole detection, tracking and spindle axis determination

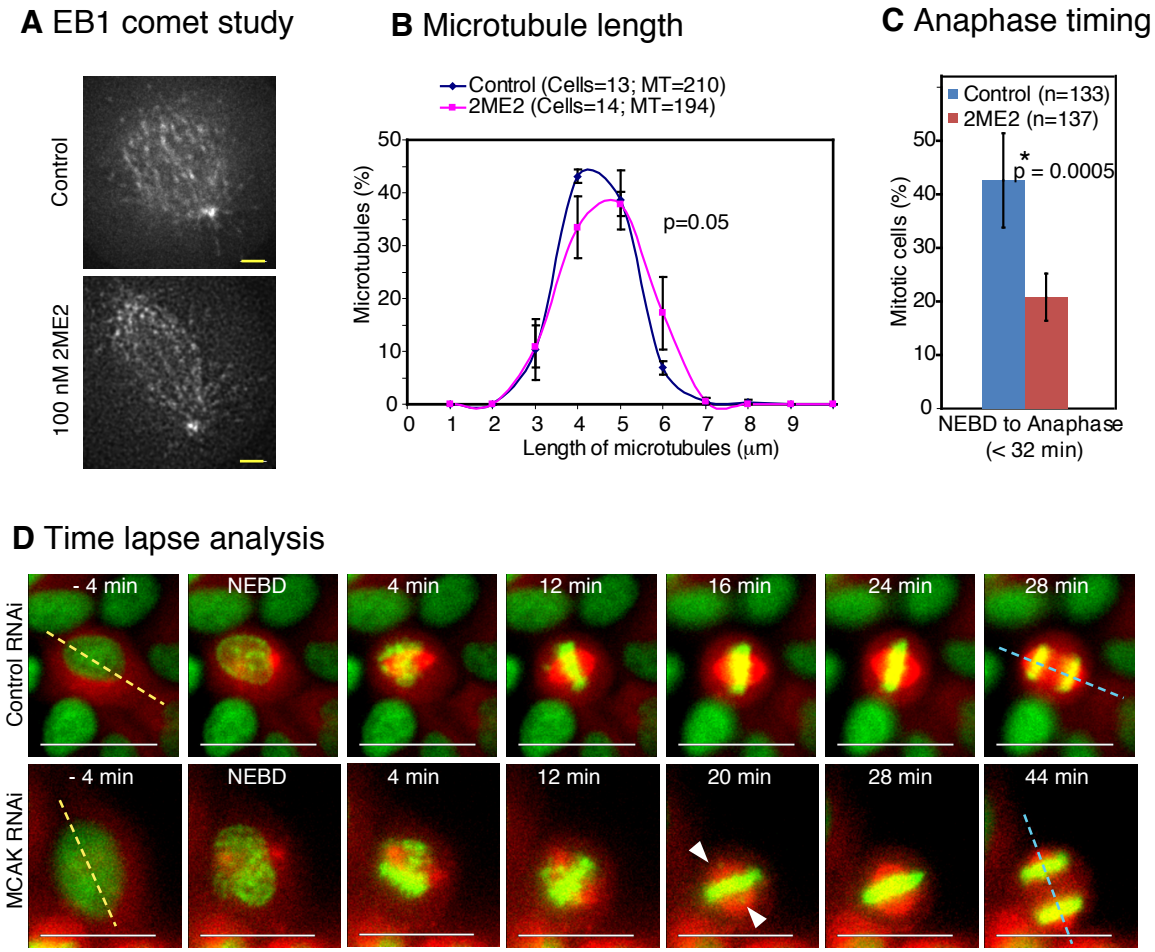


B Validation of *Spindle3D* output



SUPPLEMENTARY FIGURE-3 Corrigan *et al*., 2013




A EB1 comet study
Control
100 nM 2ME2
B Microtubule length
Control (Cells=13; MT=210)
2ME2 (Cells=14; MT=194)
Microtubules (%)
p=0.05
Length of microtubules (μm)
C Anaphase timing
Control (n=133)
2ME2 (n=137)
Mitotic cells (%)
* p = 0.0005
NEBD to Anaphase
(< 32 min)
D Time lapse analysis
Control RNAi
- 4 min
NEBD
4 min
12 min
16 min
24 min
28 min
MCAK RNAi
- 4 min
NEBD
4 min
12 min
20 min
28 min
44 min

MOVIE LEGENDS

Movie 1:

Spindle orientation is predictable in cells displaying a continuum of shapes

Uncropped movie of HeLa$^{His2B\text{-}GFP;\ mCherry\text{-}Tub}$ cells synchronized in S-phase using aphidicolin and filmed 7 hours after aphidicolin wash. Movie was compiled using single Z plane images from 3D image stacks, acquired once every 4 minutes. Scale bar indicates 10 μm. Movie highlights the cell-to-cell variability in rotational and oscillatory movements of the spindle.

Movie 2:

Cells align their spindle axis along the long-axis

Cropped and enlarged (2X) movie of a HeLa$^{His2B\text{-}GFP;\ mCherry\text{-}Tub}$ cell that underwent synchronization, as described in Movie 1. Movie was compiled using single Z plane images from 3D image stacks, acquired once every 4 minutes. Scale bar indicates 10 μm. Yellow line indicates interphase long-axis.

SUPPLEMENTARY INFORMATION

Supplementary Fig 1: Validation of spindle reporter cell line

(A) Cumulative frequency distribution of time consumed from NEBD to anaphase in HeLa cells expressing either His2B-GFP alone ($HeLa^{His2B\text{-}GFP}$) or both His2B-GFP and mCherry Tubulin-DsRed ($HeLa^{His2B\text{-}GFP;\ mCherry\text{-}Tub}$). **(B)** Graph of percentage of bipolar and multipolar spindles as assessed using single or multiple metaphase plates in $HeLa^{His2B\text{-}GFP;\ mCherry\text{-}Tub}$ and $HeLa^{His2B\text{-}GFP}$ cell lines. **(C)** Graph of percentage of cells that underwent normal segregation, no segregation (mitotic arrest), or mis-segregation (lagging chromatids) in $HeLa^{His2B\text{-}GFP;\ mCherry\text{-}Tub}$ and $HeLa^{His2B\text{-}GFP}$ cell lines, as assessed from time-lapse movies. Error bars represent SEM. n indicates the number of cells from three independent experiments.

Supplementary Fig 2: Validation of *Spindle3D*

(A) Flow diagram of *Spindle3D* software outlining the steps of spindle pole detection, tracking and spindle axis determination as detailed in Image Processing Methods (*Spindle3D*) section. **(B)** Graph of frequency distributions of final orientation angles (spindle axis relative to long-axis) in Control RNAi treated $HeLa^{His2B\text{-}GFP;\ mCherry\text{-}Tub}$ cells from two independent experiments that were analysed manually and using *Spindle3D*. Error bars represent SEM values. n indicates the number of cells from two independent experiments.

Supplementary Fig 3: Spindle alignment probability following LGN depletion

Graph shows the alignment probability of unaligned spindles in LGN depleted cells. Spindles were grouped by initial angular deviation from the long-axis into 30-60 and 60-90 degree groups. The number of cells in 30-60 group was 58 (LGN RNAi); 39 (Control RNAi) and 60-90 group was 44 (LGN RNAi); 41 (Control RNAi). Spindles beginning close to the long-axis (<30 degrees) were excluded. From each group, the fraction of spindles achieved alignment within 20 degrees of the long-axis is referred to as the alignment probability. Error bars refer to S.E.M values.

Supplementary Fig 4: Validation of microtubule lesions

(A) Representative still images of a single Z-plane of 3D time-lapse movies of $HeLa^{EB1\text{-}YFP}$ cells treated or untreated with 100 nM 2ME2 as indicated. Scale bar = 2 μm. **(B)** Graph showing distribution of microtubule (MT) lengths (μm) in monopolar spindles of cells treated with monastrol for 3h and exposed to 100 nM 2ME2 or DMSO control as indicated for 1h prior to immunostaining with antibodies against β-tubulin and stained with DAPI (DNA dye) **(C)** Graph of percentage of 2ME2 treated or untreated cells that completed anaphase onset within 32 minutes following mitotic entry, as defined by NEBD. **(D)** Representative time-lapse images showing chromosome movements in $HeLa^{His2B\text{-}GFP;\ mCherry\text{-}Tub}$ cells in Control, MCAK siRNA treated cells. Yellow line indicates long-axis and blue indicates pole-pole spindle axis. White arrows mark uncongressed chromosomes. Scale bar= 40 μm. Error bar refers to SEM from two (B) or three (C) experiments. p values were obtained using proportion test. # and * refer to insignificant and significant differences, respectively.