Supplemental Materials

Molecular Biology of the Cell

Cavazza et al.

Figure S1. Spindle assembly upon MT regrowth in U2OS and LLC-PK1α cells.

(A) Time required for U2OS γ-tubulin-paGFP/α-tubulin-mCherry cells and for LLC-PK1α α-tubulin-GFP cells to enter anaphase after nocodazole washout. For control cells (MT regrowth before mitosis) the values correspond to the time between NEBD and anaphase entry. For mitotic cells undergoing MT regrowth (MT Regrowth during mitosis) the values correspond to the time between nocodazole washout and anaphase entry. Like in HeLa cells, in these two cell types MT Regrowth cells took significantly longer than Control cells to enter anaphase. U2OS MT regrowth cells took 222±107 min instead of 68±58 min for Control cells, and LLC-PK1α MT Regrowth cells took 94±78 min instead of 77±73 min. The graph is a box and whiskers plot. ** $p<0.01$, *** $p<0.001$. A total of 766 control cells and 136 MT regrowth cells, were analysed in five independent experiments using U2OS cells. A total of 393 control cells and 188 MT regrowth cells were counted in four independent experiments using LLC-PK1α cells.

(B) Spindle organization at anaphase onset. The proportion of bipolar (light blue), multipolar (grey), and disorganized (red) spindles was quantified for the cells analyzed in (A). Like in HeLa cells, upon MT regrowth (MT Regrowth during mitosis) U2OS and LLC-PK1α cells assembled significantly more multipolar and disorganized spindles than control cells (MT regrowth before mitosis): in U2OS MT Regrowth cells 36,9±10,5% of multipolar spindles instead of 3,4±2,1% for Control cells, and in LLC-PK1α MT Regrowth cells 16,5±1,2% of multipolar spindles instead of 2,5±1,2% for Control cells. In both cases the difference is significant with a $p< 0.01$.

Figure S2. MT regrowth after cold MT depolymerization impairs spindle assembly.

(A) Representative immunofluorescence image of a cell supplemented with cold media and incubated on ice for 90 min. The DNA is still along the metaphase plate although not compact and the two centrosomes are juxtaposed on the two sides of the DNA mass. DNA is in blue, Centrin in green, and Tubulin in red. Scale bar = 10 μm.

(B) Spindle organization in mitotic HeLa cells undergoing MT regrowth after cold MT depolymerization. Graph showing the proportion of the different spindle organizations 30 minutes after MT regrowth initiation (Cold MT regrowth) or in control cells. Cold MT regrowth cells have significantly less bipolar spindles properly oriented (55,9±3,0%) than control cells (83,0±1,9%). Moreover, they also had a higher number of tilted (23,4±2,8%) and multipolar spindles (5,0±1,9%) than control cells (11,6±1,9% and 3,2±0,6%

respectively). Representative immunofluorescence images of the different spindle organizations are shown below the graph. Data from 616 controls cells and 617 MT regrowth cells from three independent experiments, counting in each at least 200 cells per condition. DNA is in blue, Centrin in green and Tubulin in red. Scale bar = 10μm. Error bars: SEM. * $p<0.05$; ** $p<0.01$.

Figure S3. Centrosome separation after monastrol and nocodazole treatment and TPX2 silencing control.

(A) Centrosome separation in HeLa cells preincubated in DMSO or Monastrol followed by nocodazole incubation. Graph showing the proportion of separated (distance >3μm) or unseparated (distance <3μm) centrosomes in DMSO cells (grey) and cells incubated with Monastrol (light grey) cells. Representative immunofluorescence images of cells with unseparated and separated centrosomes are shown below the graph. Data from 825 DMSO treated cells and 816 monastrol treated cells from four independent experiments, counting in each at least 200 cells per condition. DNA is in blue and Centrin in green. Scale bar = 5μm. Error bars: SEM. ** $p<0.01$.

(B) Representative western blot analysis of the cells silenced using scrambled siRNA (siCTRL) or an anti TPX2 siRNA (siTPX2). TPX2 is not detectable in siTPX2 cells.

Figure S4. Tubulin availability in control and in MT regrowth treated cells.

(A) Schematic representation of spindle assembly phases in an untreated cell. MTs generated by the centrosomes are in red, by the chromosomal pathway are in green. At the beginning of mitosis many MTs are already formed by the centrosomes, therefore the total polymerized tubulin can increase little and the amount of chromosomal MTs is low.

(B) Schematic representation of spindle assembly phases in a cell undergoing MT regrowth. At the beginning there are no MTs, thus at the initial phases both pathways have much tubulin available to polymerize MTs. Therefore in the initial phases of spindle assembly the chromosomal pathway contributes more than in untreated mitosis (A).

(C) Extension of the limiting component model proposed by (Good *et al.*, 2013). The red line shows the ratio of total cellular tubulin that will be incorporated into the spindle MTs (W_S) for various cell diameters. These MTs are either nucleated by the centrosomes (W_C) or through the chromosomal pathway (W_R). W_C is represented as a blue dashed line and W_R as a blue line. For small cells, the proportion of tubulin that will be incorporated by W_C and W_R is similar, but as the cell diameter increases, the balance shifts towards a larger proportion of

tubulin incorporated by W_R. W_C was estimated considering that a cell entering mitosis has two centrosomes, each having 500 MTs of average length 3µm.

Figure S5. MT regrowth in CSF extract does not impair spindle assembly

(A) Schematic representation of spindle assembly in CSF extracts (Control) and upon MT regrowth after cold treatment (MT Regrowth). Time is in minutes.

(B) Representative images of mitotic structures assembled in CSF extracts in Control conditions and after cold induced MT depolymerization (MT Regrowth) at the indicated times (in min) as shown in (A). At 60 min most of the mitotic structures are monopolar spindles in both conditions. DNA is in blue and tubulin in red. Scale bar = 10µm.

(C) Spindle organization in control CSF extracts (Control, blue) or in cold-treated CSF egg extracts (MT Regrowth, red). Graph showing the proportion of spindle organizations after 60 minutes of incubation. Representative images are shown below the graph. Control and MT Regrowth CSF extracts assemble spindles with similar efficiency. Data from 298 structures in control extracts and 291 structures in MT regrowth treated extracts from three independent experiments, counting in each at least 50 mitotic structures per condition. Error bars: SEM. DNA is in blue, tubulin in red. Scale bar = 10µm. No statistically significant difference was detected between conditions.

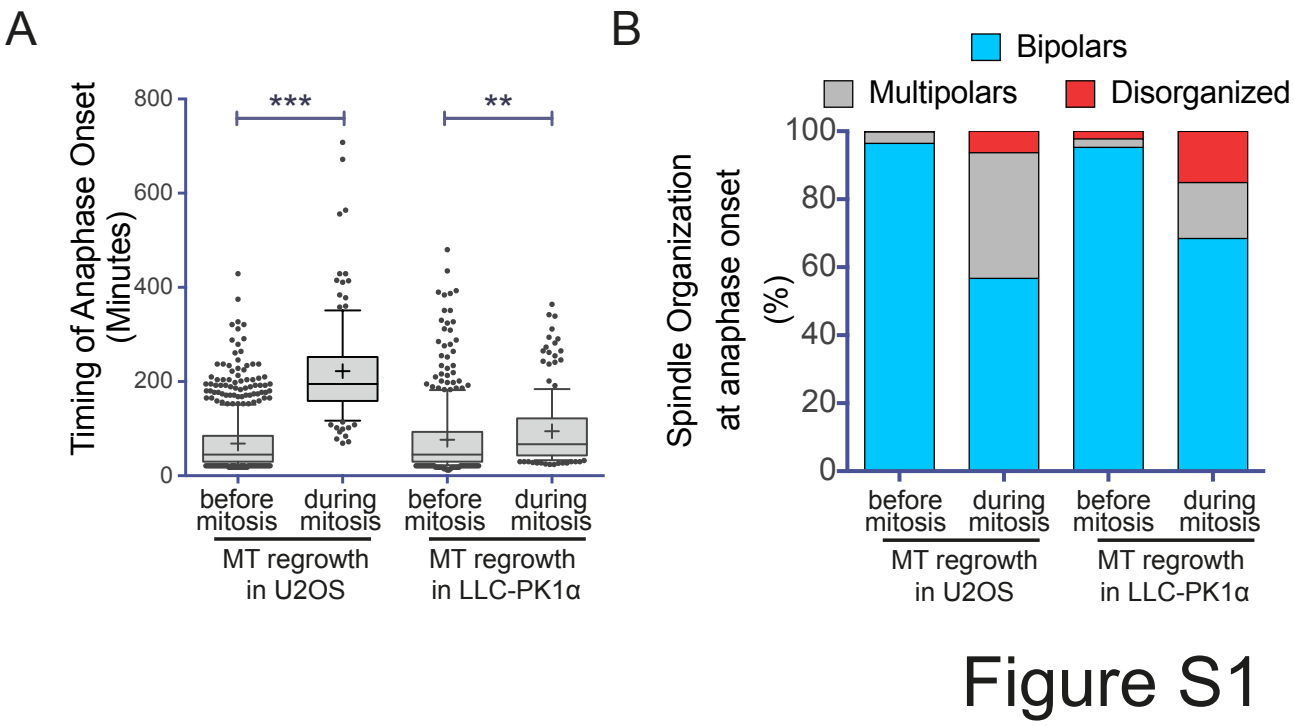
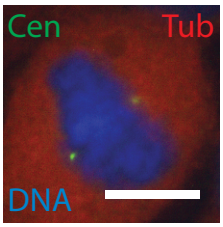

A
Timing of Anaphase Onset (Minutes)
800
600
400
200
0
***
**
before mitosis
during mitosis
before mitosis
during mitosis
MT regrowth in U2OS
MT regrowth in LLC-PK1α
B
Bipolars
Multipolars
Disorganized
Spindle Organization at anaphase onset (%)
100
80
60
40
20
0
before mitosis
during mitosis
before mitosis
during mitosis
MT regrowth in U2OS
MT regrowth in LLC-PK1α

Figure S1

A


Cold
1,5 hours
Cen
Tub
DNA

B

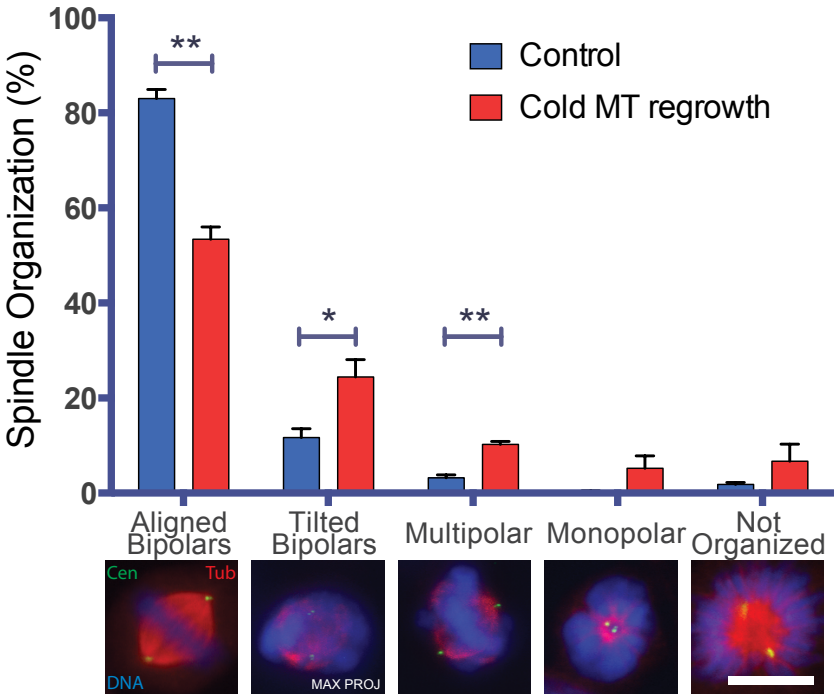

Spindle Organization (%)
100
80
60
40
20
0
**
*
**
Control
Cold MT regrowth
Aligned Bipolars
Tilted Bipolars
Multipolar
Monopolar
Not Organized
Cen
Tub
DNA
MAX PROJ

Figure S2

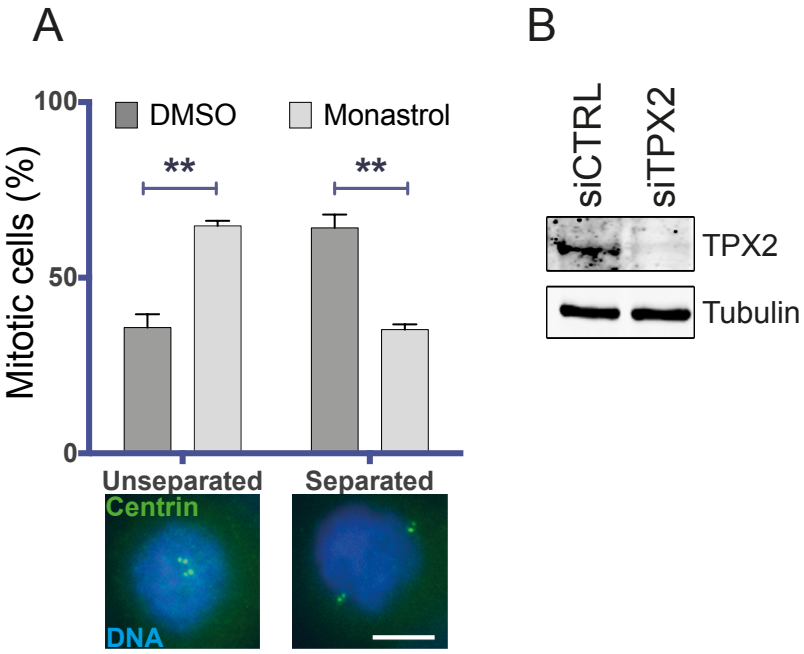
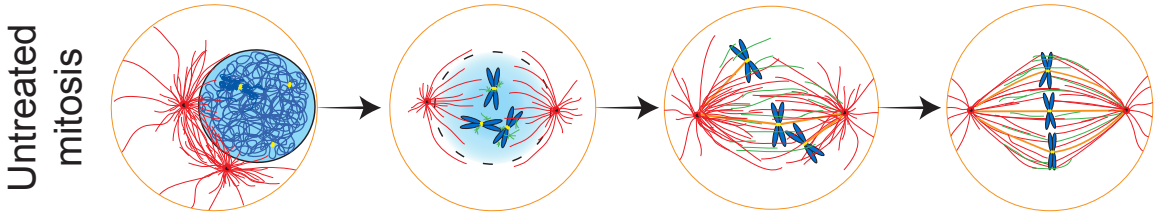
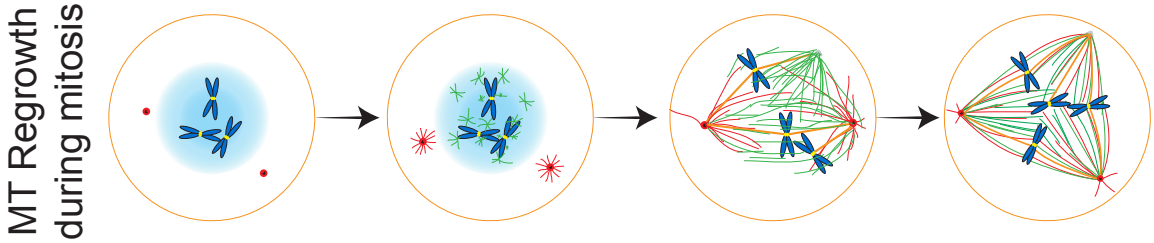

A
B
100
50
0
Mitotic cells (%)
DMSO
Monastrol
**
**
Unseparated
Separated
Centrin
DNA
siCTRL
siTPX2
TPX2
Tubulin

Figure S3

A



B



C

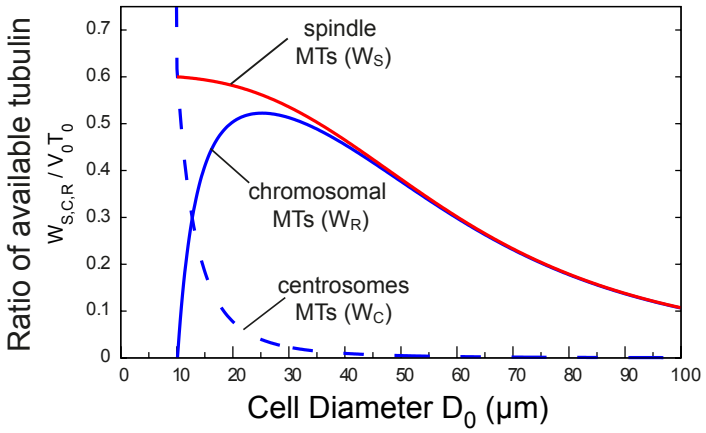


Figure S4

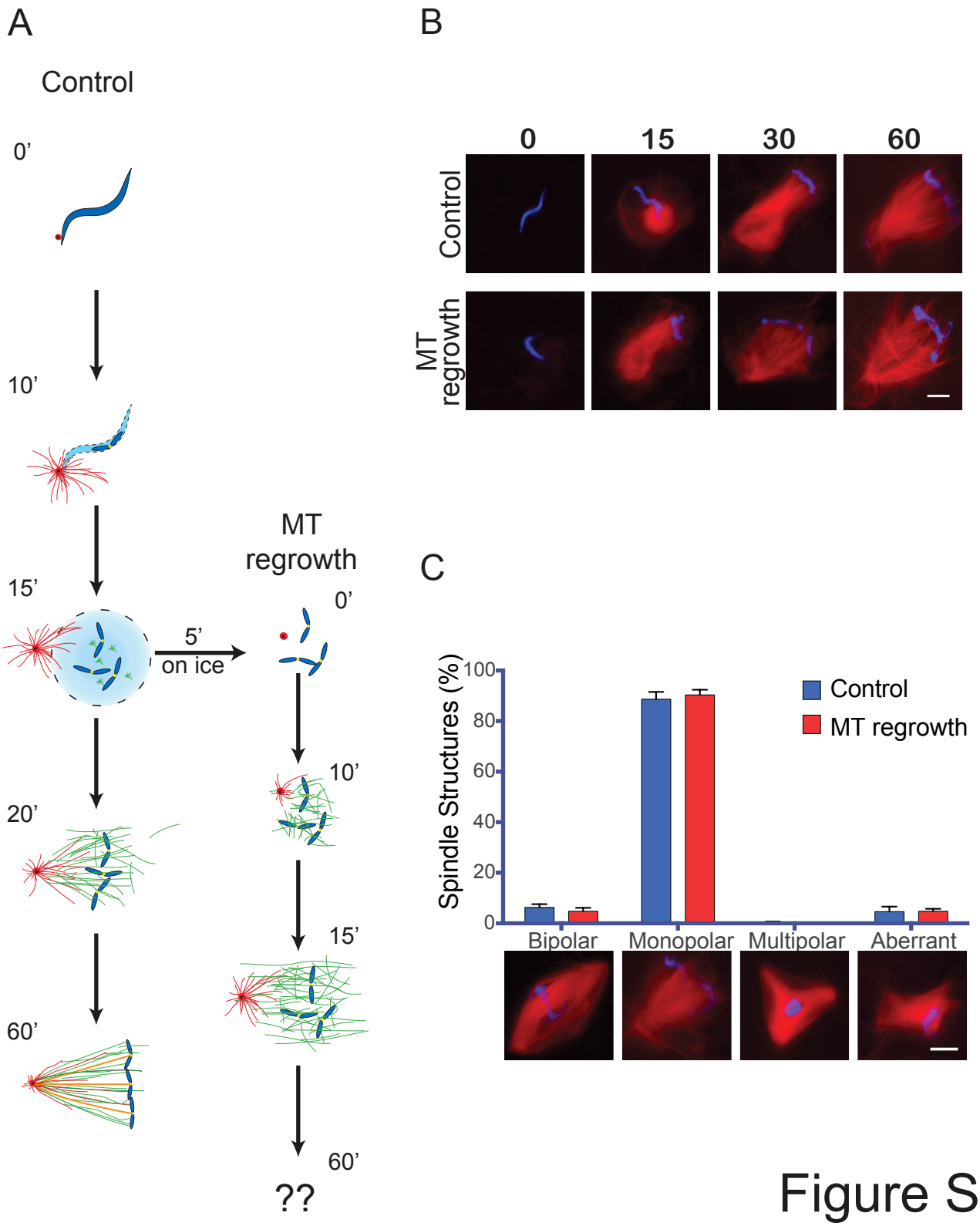

A
Control
0’
10’
15’
20’
60’
5’
on ice
MT
regrowth
0’
10’
15’
60’
??
B
0
15
30
60
Control
MT
regrowth
C
Spindle Structures (%)
100
80
60
40
20
0
Control
MT regrowth
Bipolar
Monopolar
Multipolar
Aberrant

Figure S5