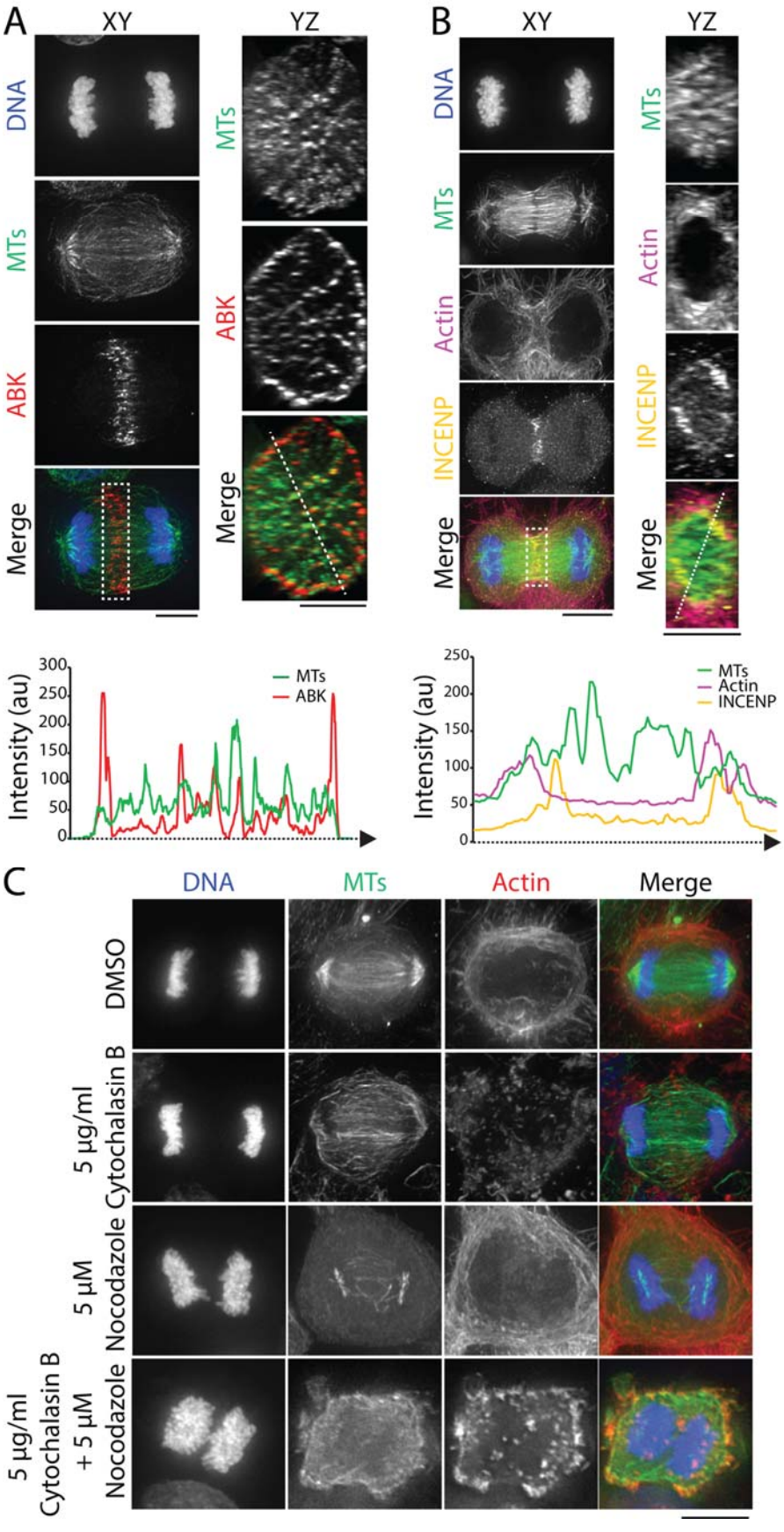
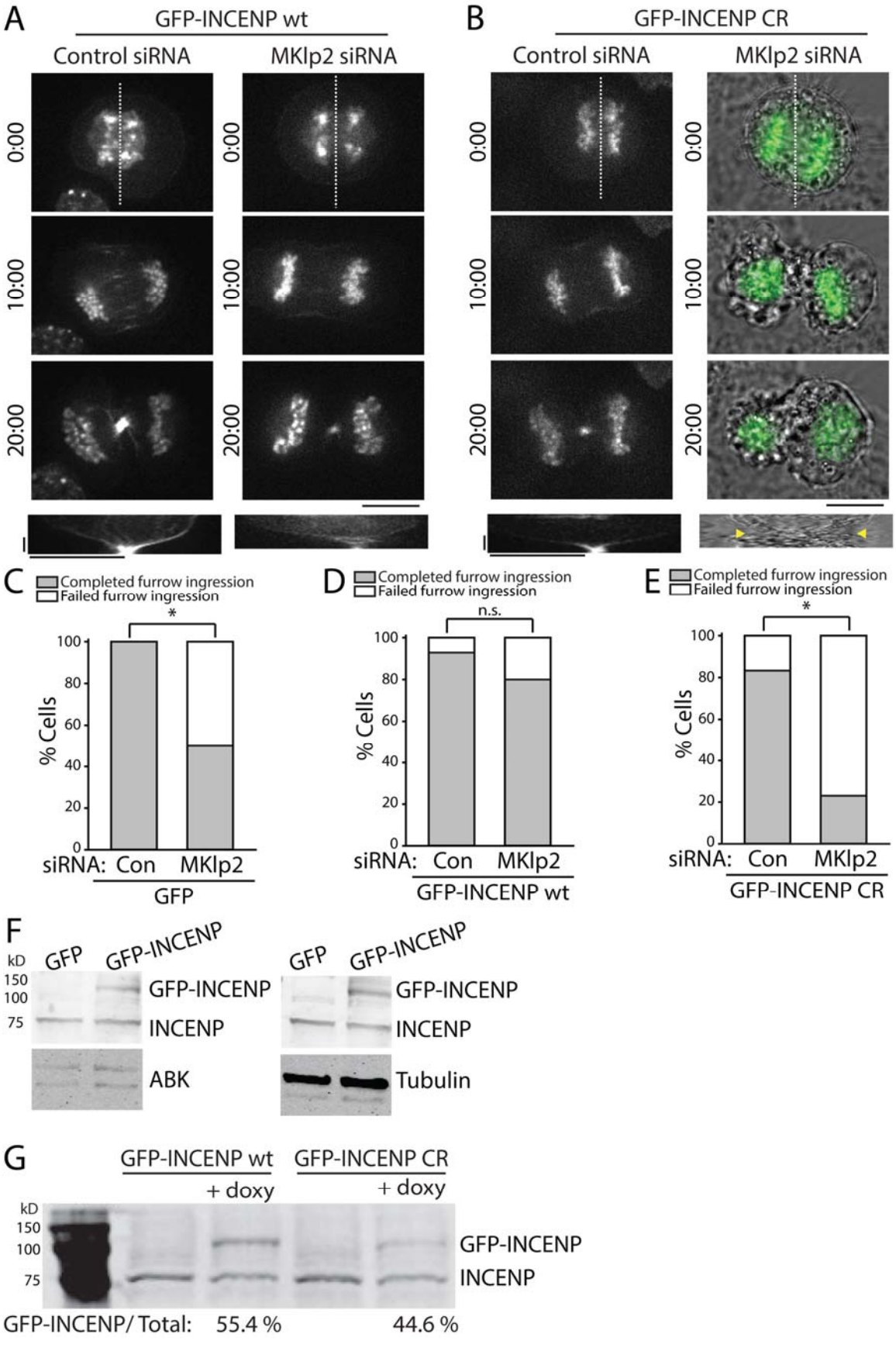
Supplemental Materials

Molecular Biology of the Cell

Landino et al.


A
XY
YZ
DNA
MTs
ABK
Merge
B
Actin
INCENP
Intensity (au)
C
DMSO
5 µg/ml Cytochalasin B
5 µM Nocodazole
5 µg/ml Cytochalasin B + 5 µM Nocodazole

Supplemental Figure 1. (A) Top: Maximum z-projections of the XY dimension of an anaphase HeLa cell (left panels). Dashed box indicates section used to generate volume projection of YZ dimensions (right panels). Dashed line was used to generate line scans. Cells were stained with antibodies to tubulin (green) and ABK (red). DNA was counterstained with Hoechst 33342 (blue). Scale bars, 10 μm. Bottom: line scans of MT and ABK fluorescence intensity across the YZ projection of the division plane. (B) Top: Maximum z-projections of the XY dimension of ananaphase HeLacell (left panels). Dashed box indicates section used to generate volume projection of YZ dimensions (right panels). Dashed line was used to generate line scans. Cells were stained with antibodies to tubulin (green) and INCENP (yellow). Actin was visualized with phalloidin-TRITC (pink, B). DNA was counterstained with Hoechst 33342 (blue). Scale bars, 10 μm. Bottom: line scans of MT, actin and INCENP fluorescence intensity across the YZ projection of the division plane. C) Maximum z-projections of HeLa cells treated with DMSO, 5 μg/ml cytochalasin B, 5 μM nocodazole, or both drugs simultaneously.Cells were stained with antibodies to tubulin (green) and actin was visualized using phalloidin-TRITC (red). DNA (blue) was counterstained with Hoechst 33342. Scale bar, 10 μm.


A
GFP-INCENP wt
Control siRNA
MKlp2 siRNA
0:00
10:00
20:00
B
GFP-INCENP CR
Control siRNA
MKlp2 siRNA
0:00
10:00
20:00
C
Completed furrow ingression
Failed furrow ingression
*
% Cells
siRNA: Con MKlp2
GFP
D
Completed furrow ingression
Failed furrow ingression
n.s.
% Cells
siRNA: Con MKlp2
GFP-INCENP wt
E
Completed furrow ingression
Failed furrow ingression
*
% Cells
siRNA: Con MKlp2
GFP-INCENP CR
F
kD
GFP
GFP-INCENP
150
100
75
GFP-INCENP
INCENP
ABK
Tubulin
G
GFP-INCENP wt
+ doxy
GFP-INCENP CR
+ doxy
kD
150
100
75
GFP-INCENP
INCENP
GFP-INCENP/ Total: 55.4 % 44.6 %

Supplemental Figure 2. (A)Top: Single z-plane micrographs taken from time-lapse movies of cells stably expressing GFP-INCENP, after 24 hours in 2 μg/ml doxycycline, treated with a control or MKlp2-targeing siRNA. Time is indicated in minutes. Dashed lines were used to generate kymographs. Scale bars, 10 μm. Bottom: Kymographs of GFP-INCENP along the division plane. Scale bars, 2.5 minutes (y axis) and 10 μm (x axis). (B) Top: Single z-plane micrographs taken from time-lapse movies of cells stably expressing GFP-INCENP CR, after 24 hours in 2 μg/ml doxycycline, after treatment with a control or MKlp2-targeting siRNA. Due to the dim cortical signal of GFP-INCENP CR (green) after Mkp2 depletion, we tracked furrow ingression by DIC. Time is indicated in minutes. Dashed lines were used to generate kymographs. Scale bars, 10 μm. Bottom: Kymographs of GFP-INCENP CR along the division plane. Yellow arrowheads mark the start of furrow regression. Scale bars, 2.5 minutes (y axis) and 10 μm (x axis). (C) Quantification of the percentage of cells stably expressing GFP successful in cleavage furrow ingression in control or MKlp2-depleted cells, n=8 (control) and 10 (MKlp2 siRNA) cells. * = $p < 0.05$(D) Quantification of the percentage of cells successful in cleavage furrow ingression as described in (A), n=14 (control) and 10 (MKlp2 siRNA) cells. (E) Quantification of the percentage of cells successful in cleavage furrow ingression as described in (B), n=12 (control) and 13 (MKlp2 siRNA) cells. * = $p < 0.05$ (F) Immunoblots of cell extracts prepared from HeLa cells transfected with GFP or GFP-INCENP and probed for INCENP or ABK. Tubulin is shown as a loading control. Molecular weight standards are indicated in kDa. (G) Immunoblots of cell extracts prepared from cells stably expressing GFP-INCENP or GFP-INCENP CR after 24 hours in 2 μg/ml doxycycline

probed for INCENP. Tubulin is shown as a loading control. Molecular weight standards are indicated in kDa. Boxes (bottom) represent the percent of GFP-INCENP present in cells after doxycycline addition relative to the total cellular INCENP.