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Supplemental Material to:

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Human Fidgetin is a microtubule severing the enzyme and minus-end depolymerase that regulates mitosis

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Figure S1

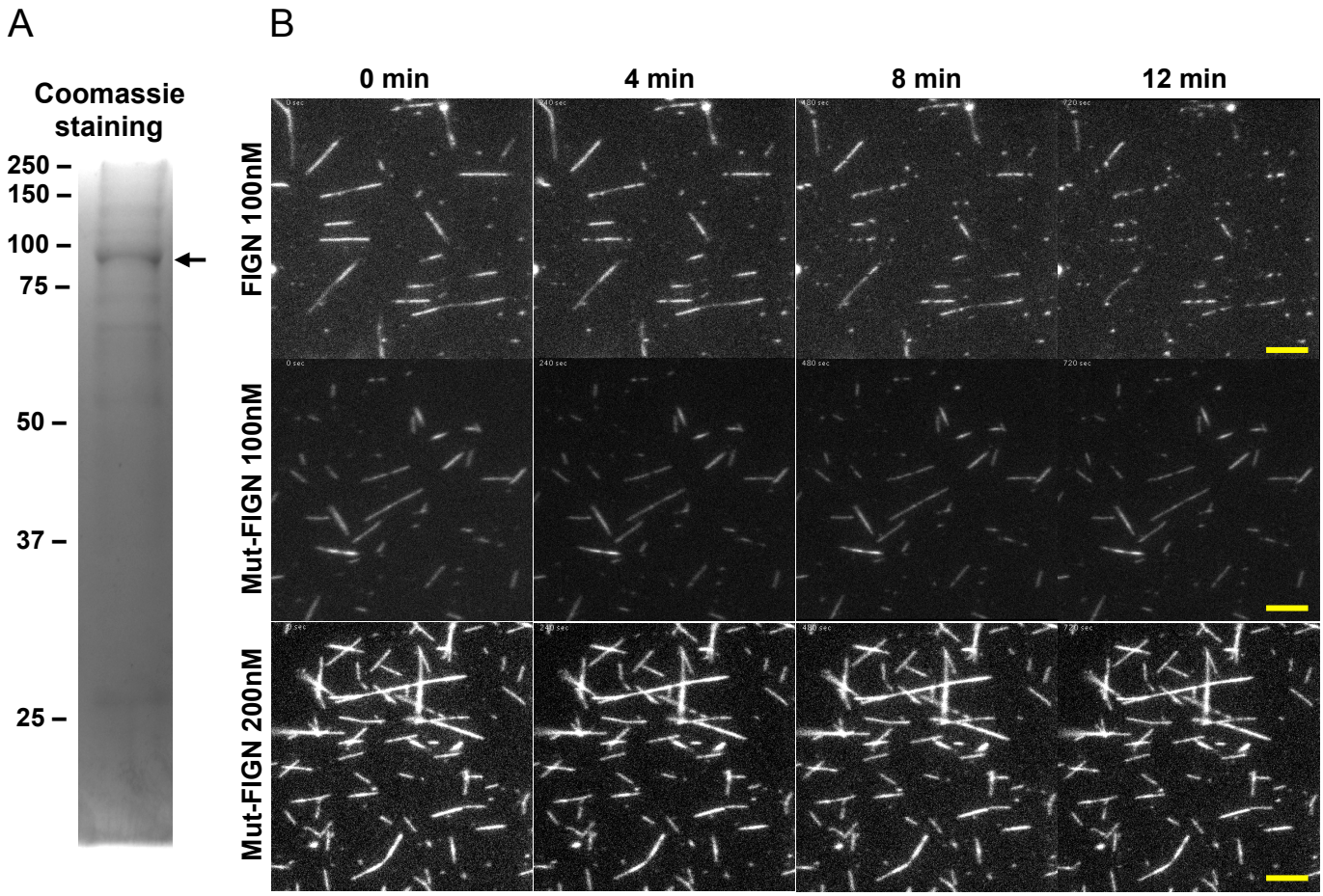

A
Coomassie staining
250 –
150 –
100 –
75 –
50 –
37 –
25 –
B
0 min
4 min
8 min
12 min
FIGN 100nM
Mut-FIGN 100nM
Mut-FIGN 200nM

Figure S2

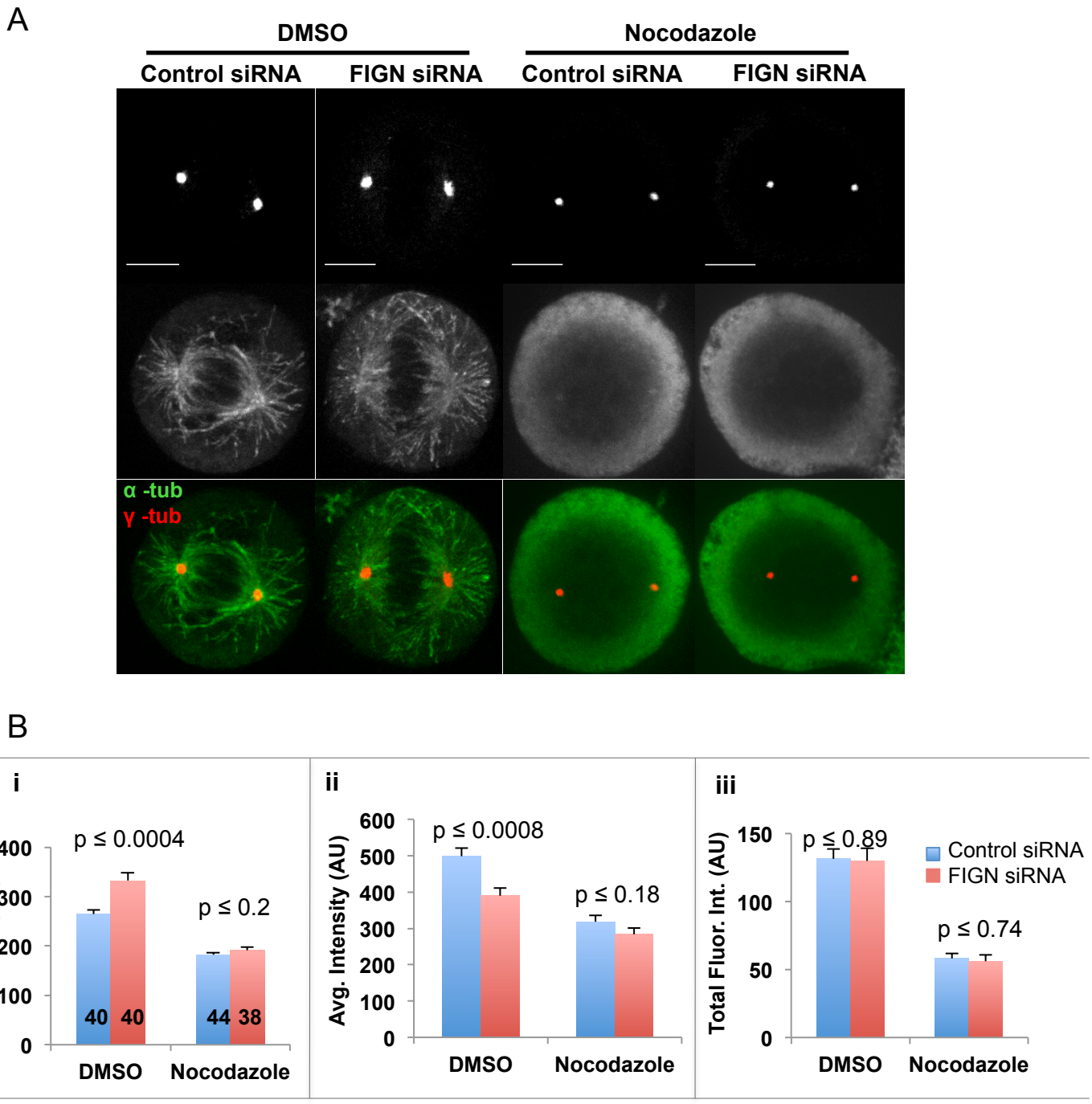
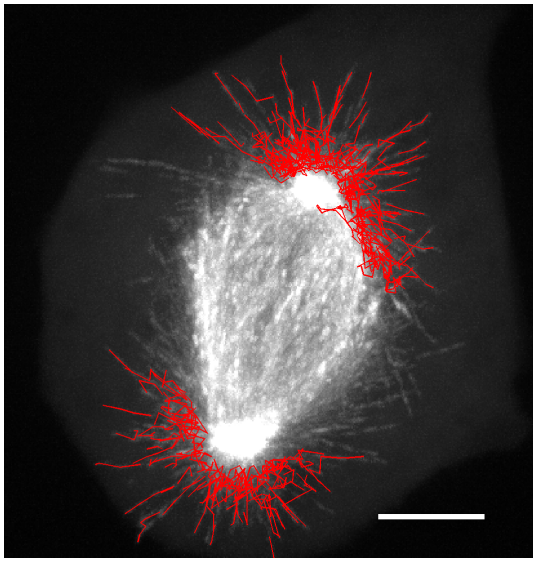

A
DMSO
Nocodazole
Control siRNA
FIGN siRNA
Control siRNA
FIGN siRNA
α -tub
γ -tub
B
i
p ≤ 0.0004
p ≤ 0.2
Area (AU)
400
300
200
100
0
40
40
44
38
DMSO
Nocodazole
ii
p ≤ 0.0008
p ≤ 0.18
Avg. Intensity (AU)
600
500
400
300
200
100
0
DMSO
Nocodazole
iii
p ≤ 0.89
p ≤ 0.74
Total Fluor. Int. (AU)
150
100
50
0
Control siRNA
FIGN siRNA
DMSO
Nocodazole

Figure S3

A



B

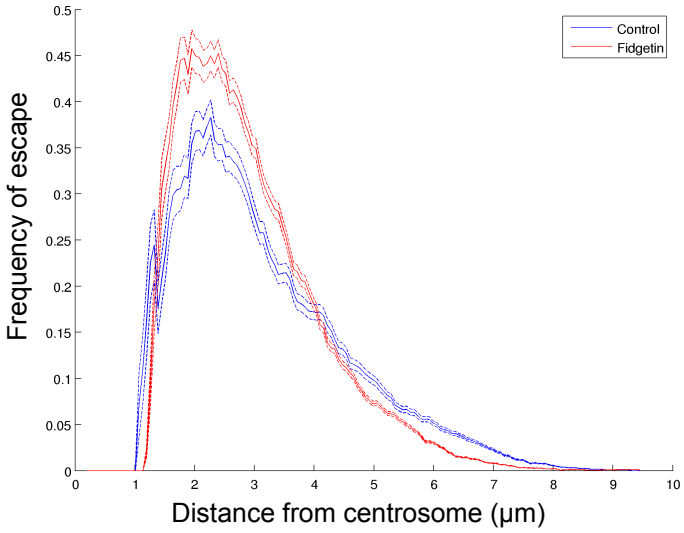

Control
Fidgetin
Frequency of escape
Distance from centrosome (μm)
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5
0
1
2
3
4
5
6
7
8
9
10

Figure S1. A point mutation in the Walker A domain of FIGN inhibits its ability to sever and depolymerize MTs. (A) Coomassie-stained SDS-PAGE gel of the Fidgetin K533A mutant used for the *in vitro* severing assays. (B) Time-series images of the *in vitro* MT severing assays performed in presence of wild type and full-length mutant FIGN. Scale bar 5 μm.

Figure S2. FIGN's impact on centrosome morphology is MT dependent. (A) Confocal images of metaphase U2OS cells double labeled for MTs (green) and γ-tubulin (red) after treatment with DMSO (control) or nocodazole (to depolymerize MTs). Scale bar 5 μm. (B) Measurements reveal that the depolymerization of MTs rescues the effects of FIGN depletion on the area (i) and intensity (ii) of centrosome associated γ-tubulin immunofluorescence. The average total intensity of centrosomal γ-tubulin immunofluorescence remains the same in control vs. FIGN siRNA treated cells (iii). The number of cells analyzed is indicated in the bars shown in (i).

Figure S3. Tracking of EB3-GFP comets within the astral regions of metaphase spindles from control and FIGN siRNA cells. (A) Maximum intensity projection of the frames from a time series movie of a control metaphase spindle labeled with EB3-GFP. The trajectories of tracked EB3-GFP comets within the astral regions of this spindle are shown as red lines. (B) The probability for a MT growth trajectory initiated within a semi-circular region centered at the centrosome to escape that region (units in μm). The dashed lines mark the range of the standard errors.

Mov.S1

Time series movie of the rhodamine-labeled MTs in a glass chamber in the absence of FIGN protein. No changes in MT dynamics were observed. The movie was captured with a TIRF microscope. Images were obtained at 20 second intervals.

Mov.S2

Time series movie showing the severing of rhodamine-labeled MTs incubated with recombinant FIGN and ATP. Images were captured as in Mov.S1.

Mov.S3

Time series movies of the rhodamine-labeled MTs in a glass chamber with 100nM and 200nM mutant FIGN protein respectively. No changes in MT dynamics were observed. The movie was captured in epifluorescence using a Xe-Hg lamp equipped with an electron multiplier CCD Cascade II camera attached to a Nikon Eclipse Ti microscope with a 60X, NA 1.49 objective. Images were obtained at 20 second intervals.

Mov.S4

Anaphase A in a control-treated U2OS cell expressing GFP-γ-tubulin and GFP-CENPB. Time series images were captured using a 4D spinning disk confocal microscope with 100X, 1.4NA objective. Each image is a maximum intensity

projection of 1μm Z sections encompassing the entire spindle.

Mov.S5

Anaphase A in a FIGN siRNA-treated U2OS cells expressing GFP γ-tubulin and GFP-CENPB. Imaging was performed as in Mov.S3.

Mov.S6

Tracking of EB3-GFP labeled astral MTs in a control-treated U2OS cell. In the movie on the left, red circles track the movement of astral EB3-GFP comets while the movie on the right is the same spindle without tracking. Images were captured using a 4D spinning disk confocal microscope with 100X, 1.4NA objective. Time in seconds is indicated in each movie frame.