E07-10-1069 Rogers

SUPPLEMENTARY DATA

Supplemental Figure 1. Interphase day-7 control and γ-Tubulin23C RNAi-treated S2 cells stained for MTs (green), γ-tubulin (red), and DNA (blue). Scale, 5μm.

Supplemental Figure 2. *Drosophila* D16 cells lack a γ-tubulin-containing MTOC during interphase. Interphase (A) and mitotic (B) D16 cells were plated on concanavalin A and processed for immunofluorescence to reveal MTs (red), γ-tubulin (green) and DNA (blue). Scale, 5μm.

Supplemental Figure 3. (A) *Drosophila* SAS-6 co-localizes with the centriolar protein D-PLP. An interphase GFP-SAS-6 (green) expressing stable S2 cell stained for D-PLP (red) and DNA (blue). Magnified views of the centrioles are shown in insets.
(B) Centrioles in *Drosophila* D16 cells co-localize with γ-tubulin in mitosis but not during interphase. D16 cells were plated on concanavalin A and processed for immunofluorescence with antibodies against γ-tubulin (red), D-PLP (green) and stained for DNA (blue). The panels illustrate typical cells in interphase and mitosis. Magnified views of the centrioles are shown in insets. Scale 5μm.

Supplemental Figure 4. The centrosomal proteins Centrosomin (Cnn), CP190, and CP60 localize to the PCM during mitosis but do not localize to centrioles during interphase. Interphase and mitotic stable S2 cells expressing GFP-SAS-6 (green) were fixed and stained for DNA (blue) and Cnn (red, top row), CP190 (red, middle row), or CP60 (red, bottom row). *Top panel,* the mitotic cell shown is in prophase. Magnified views of the centrioles and Cnn (white arrowheads) are shown below the micrographs. *Middle and bottom panels,* both CP190 and CP69 are restricted to the nucleus during interphase (DNA not shown), but are released into the cytoplasm during mitosis and concentrate on centrosomes at spindle poles (white arrowheads) (GFP-SAS-6 centrioles not shown for CP60). Magnified views of CP190-labeled mitotic centrosomes are shown in insets. Scale 5μm.

Supplemental Figure 5. *Drosophila* SAS-6 RNAi eliminates centrioles without altering interphase cell cycle progression. (A) Cells were fixed and stained for SAS-6 (green), γ-tubulin (red), and DNA (blue). Anti-SAS-6 antibody labels spots within γ-tubulin-stained rings at the spindle pole. Note a cluster of centrosomes at the right spindle pole (left pole is in a different focal plane). (B) S2 cell lysates were prepared prior to and 7 days after SAS-6 RNAi-treatment and probed by Western blot using anti-SAS-6 antibody (α-tubulin was used as a loading control). (C) Day 7 control and SAS-6 RNAi-treated S2 cells stained for D-PLP (red) and DNA (blue). White traces outline the cells. (D) Histogram of centrioles per interphase cell counted in control or SAS-6 RNAi-treated S2 cells. (E) SAS-6 RNAi reduces mitotic centrosome number. Control and SAS-6 RNAi cells were stained for γ-tubulin (red), α-tubulin (green) and DNA (blue). Percentages of spindles types are indicated. Scale, 2.5μm. (F) Cell cycle progression is unaffected by SAS-6 RNAi. Histograms of DNA fluorescence intensity (x-axis) and cell number (y-axis) of 7,000 cells with 2C, 4C and 8C populations denoted.

Supplemental Figure 6. Early periods of MT re-growth occur independently of centrioles and Golgi but both organelles can associate with MTs. S2 cells were stained for MTs (green) and either (A) D-PLP centrioles (red) or (B) Golgi (red) at specific early time-points in a MT re-growth assay. MTs were depolymerized by cold-treatment (0 min.) and brought to room temperature to allow polymerization. Cells were fixed at 0, 2.5 and 5 minutes. (A) Centrioles are numbered and shown at higher magnifications (insets). At 2.5 minutes, MTs assemble but most are not associated with centrioles. At 5 minutes, MT foci form, most of which do not contain centrioles (1), although some MT foci do co-localize with centrioles (2). (B) Golgi are numbered and shown at higher magnifications (insets). At 2.5 and 5 minutes, MTs assemble as small (2.5 min.) and larger (5 min.) foci, most of which are not associated with Golgi (1). However, some MT foci do associate with Golgi at these time-points (2). Scale, 5μm.

Supplemental Figure 7. Interphase day-7 control, MAST, and Mini-spindles (Msps) RNAi-treated S2 cells stained for MTs (the cell margin for the MAST depleted cell is traced in white). Scale, 5μm.

Supplemental Figure 8. The kinetics of MT re-growth is not altered in either Ncd, Klp10A, Shortstop (Shot), or Kinesin heavy chain (KHC)-depleted S2 cells. Time-points show representative day 6 RNAi-treated S2 cells stained for MTs during MT re-growth. Scale, 5μm.

MOVIE LEGENDS

Movie 01. Interphase microtubule nucleation is independent of centrioles.

Expression of EB1-mRFP (green) allows the visualization of microtubule nucleation and growth relative to a GFP-SAS-6-labeled centriole (red) in a live interphase S2 cell.

Movie 02. Mitotic microtubule nucleation is associated with centrioles.

Expression of EB1-mRFP (green) allows the visualization of microtubule nucleation and growth relative to GFP-SAS-6-labeled centrioles (red) in a live prophase S2 cell.

Movie 03. Interphase microtubule nucleation is independent of centrioles in live embryonic amnioserosal cells.

Expression of EB1-GFP (green) allows the visualization of microtubule nucleation and growth relative to mCherry-SAS-6-labeled centrioles (red) in live embryonic amnioserosal cells. These differentiated cells are terminally-arrested in interphase (G2 phase). The central cell in this movie has an elongated-diamond shape. Centrioles display wide oscillations in their movements throughout the cell.

Movie 04. Interphase microtubule nucleation is independent of centrioles in live embryonic leading edge cells.

Expression of EB1-GFP (green) allows the visualization of microtubule nucleation and growth relative to mCherry-SAS-6-labeled centrioles (red) in live embryonic leading edge cells. These differentiated cells are terminally-arrested in interphase (G1 phase), and were observed during dorsal closure. The

direction of leading edge cell migration occurs toward the top of the image. Several leading edge cells are seen in this field of view and each has an elongated morphology.

Movie 05. Mitotic microtubule nucleation is associated with centrioles in live embryonic dividing epithelial cells.

Expression of EB1-GFP (green) allows the visualization of microtubule nucleation and growth relative to mCherry-SAS-6-labeled centrioles (red) in live embryonic dividing epithelial cells. Several metaphase-stage spindles are seen within this region of a stage 9 embryo with centrioles at the spindle poles associated with the brightest EB1 signal.

Movie 06. Interphase microtubule nucleation is independent of both centrioles and γTub23C.

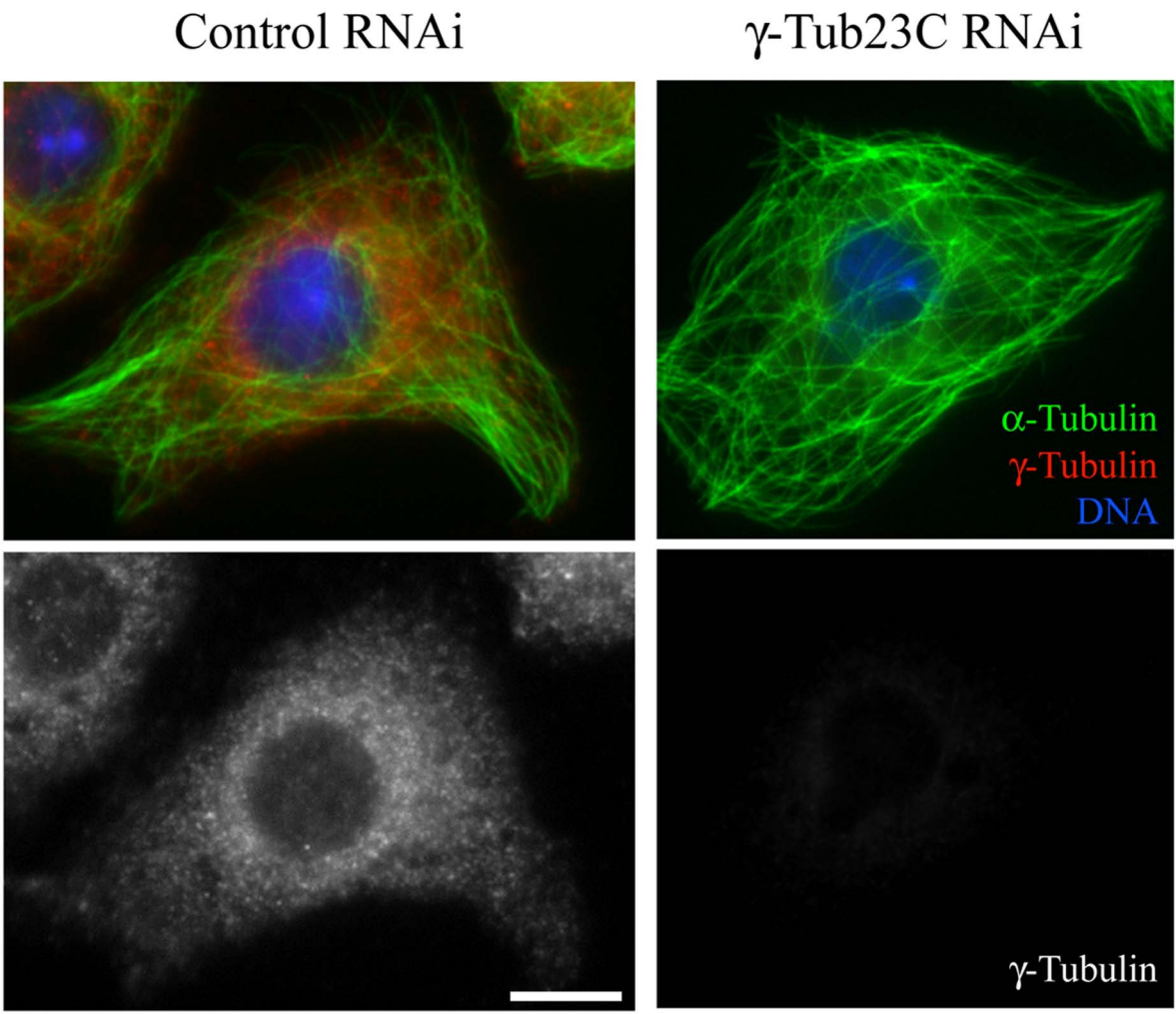
Expression of EB1-mRFP (green) allows the visualization of microtubule nucleation and growth relative to GFP-SAS-6-labeled centrioles (red) in a live 7-day RNAi-treated interphase S2 cell depleted of the major γTub23C isotype. Two centrioles move throughout this cell and are not associated with the microtubule nucleation that appears indistinguishable relative to control cells (see Movie 01). Centrioles are sometimes seen moving in fast linear trajectories which presumably occurs along MT tracks.

Supplemental Table 1. Primer sequences used to generate dsRNA

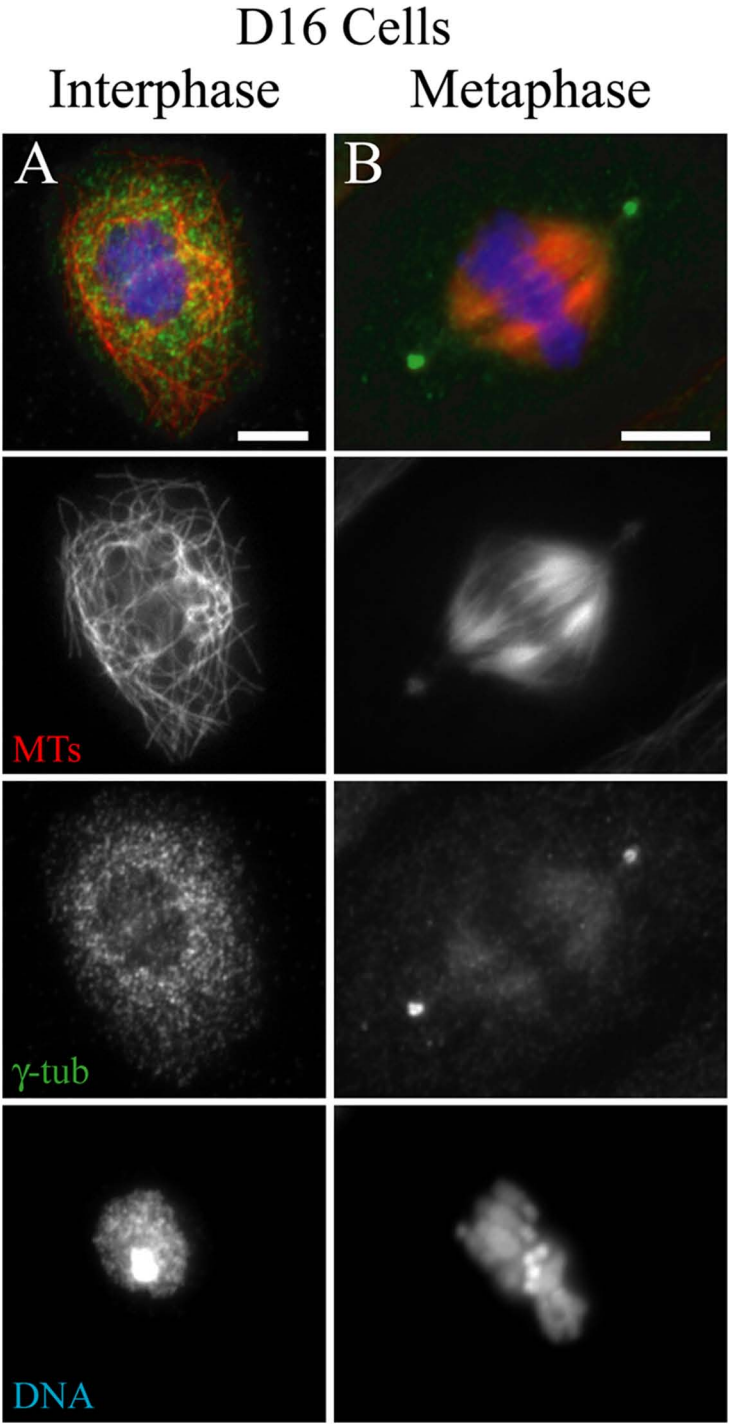
Gene Name	CG Number	Sense Primer [a]	Antisense Primer [a]	Expected Size (bp)[b]
Control (pBluescript SK)		5'-ATGGATAAGTTGTCGATCG	5'-ACCAGGTTCACATGCTTGCG	900
SAS-6	15524	5'-ATGTGGCCTCCAGGGAGC	5'-TGATGTTGGCCACATCCCC	931
γTub23C	3157	5'-TTCGCTTGTGCCAGAAGAAATGCG	5'-ATGTGTCACATATTAAACATGATCGGG	235[c]
γTub37C	17566	5'-AACCCTTCAATTGGGCC	5'-GGGCCGCATGCACATAGGG	1096
DHC	7507	5'-GTGGGAGATGAAGATCTTTTGG	5'-GAACACCCAGATACTCAAAGCC	709
MAST/Orbit	32435	5'-CAGTGATAAAAACGCAGACTGG	5'-GGAACATTAGACATAGCTGCCC	614
CLIP190	5020	5'-AGGAATACGCGGAATCCCGAGCGGAG	5'-TTCTCCTTGGAAGTCTGAGACTCCTGTTGC	800
Mini-spindles	5000	5'-TTACGAGGAGGCCGCAAAGATCTTTCG	5'-TCCATTTCTTTTCCTCCAGCTTATCG	800
EB1	3265	5'-AGAGATGTTAATGGGCACAAATTTTCT	5'-AGATTTGTATAGTTATCCATGCCATG	650

a - all primers began with the T7 promoter sequence 5'-TAATACGACTCACTATAGGG immediately followed by gene specific sequence.
b - in most cases, a cDNA template was used to genereate dsRNA, otherwise a large single exon was PCR amplified from genomic DNA .
c - this dsRNA targets specifically the 3'UTR of 23C-γ-tubulin

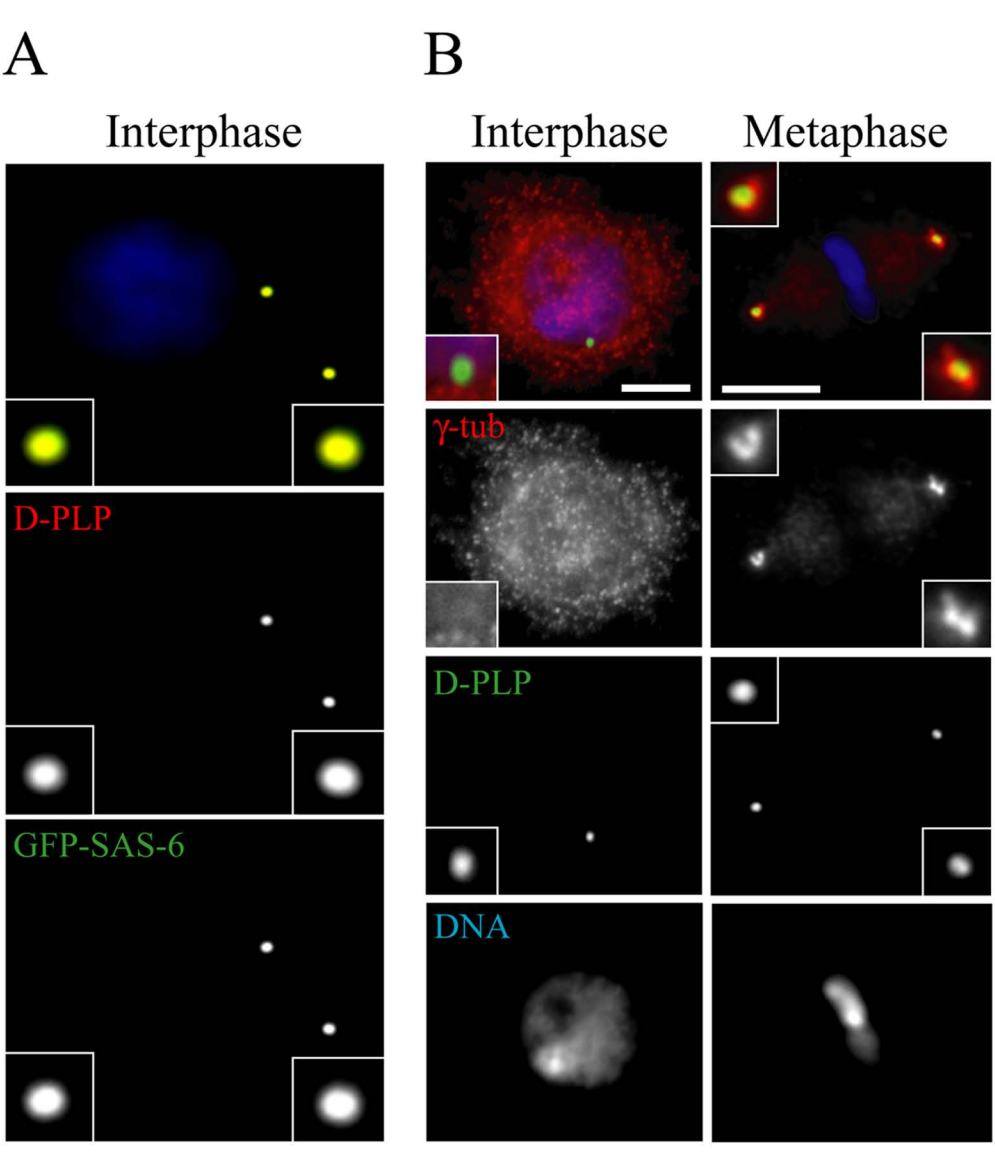
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



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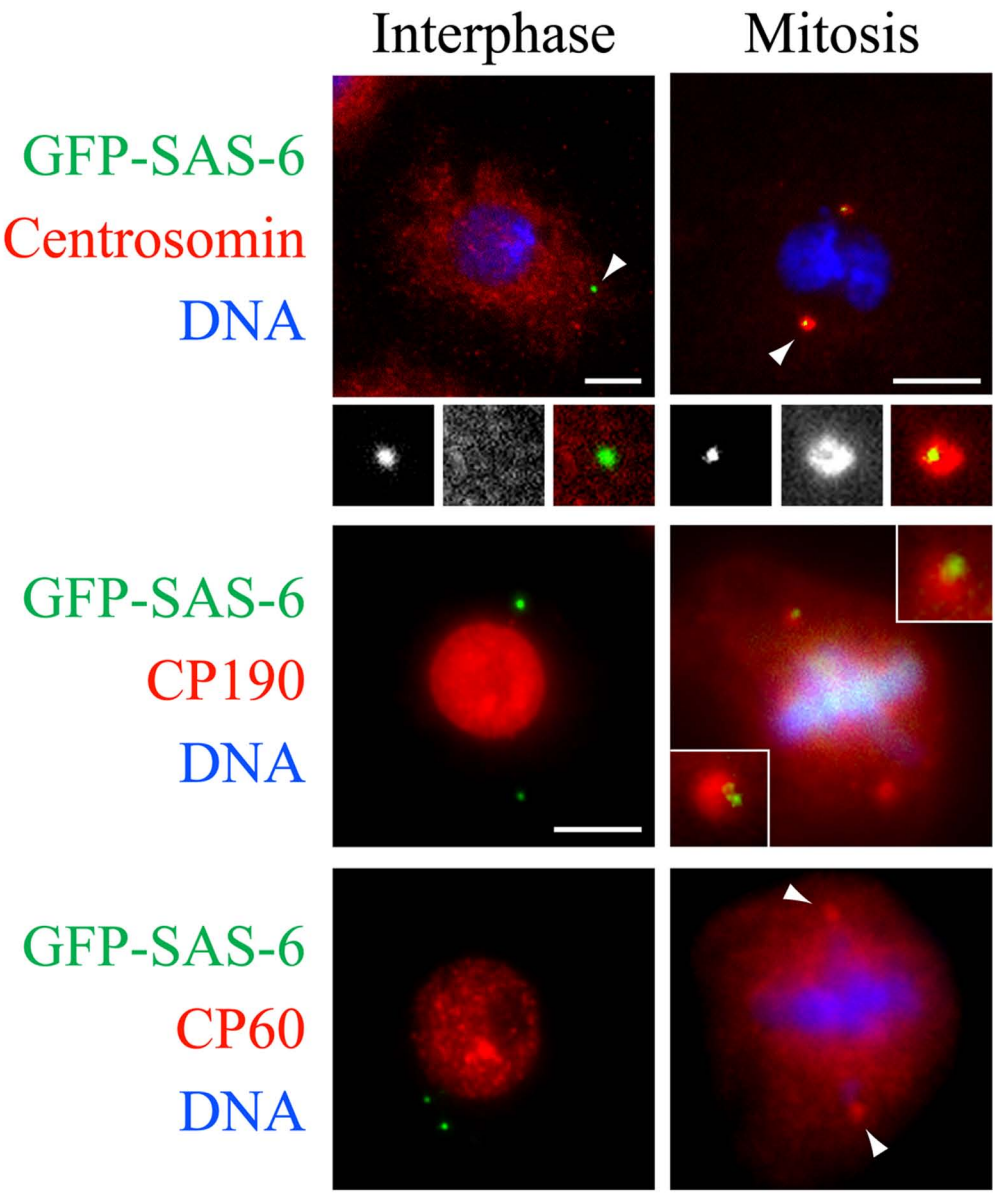
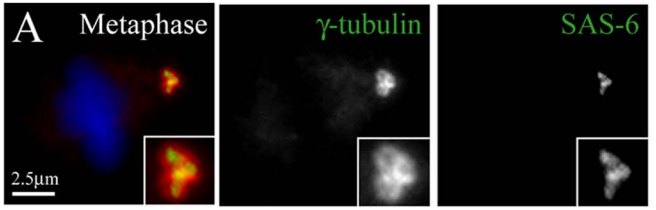
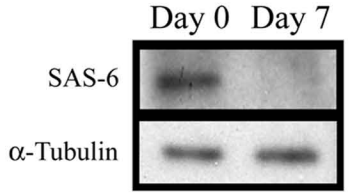
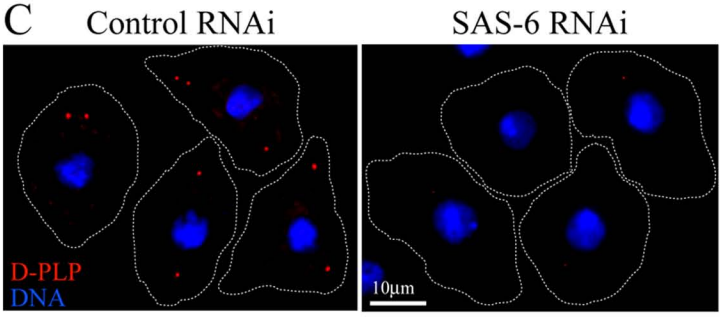
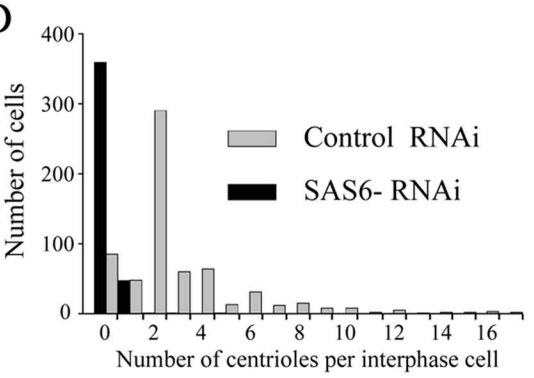


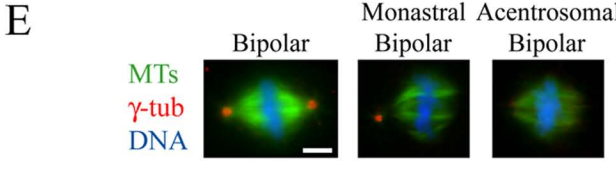
Supplemental Figure 5



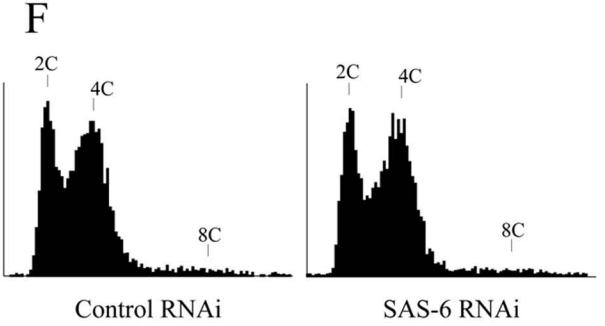




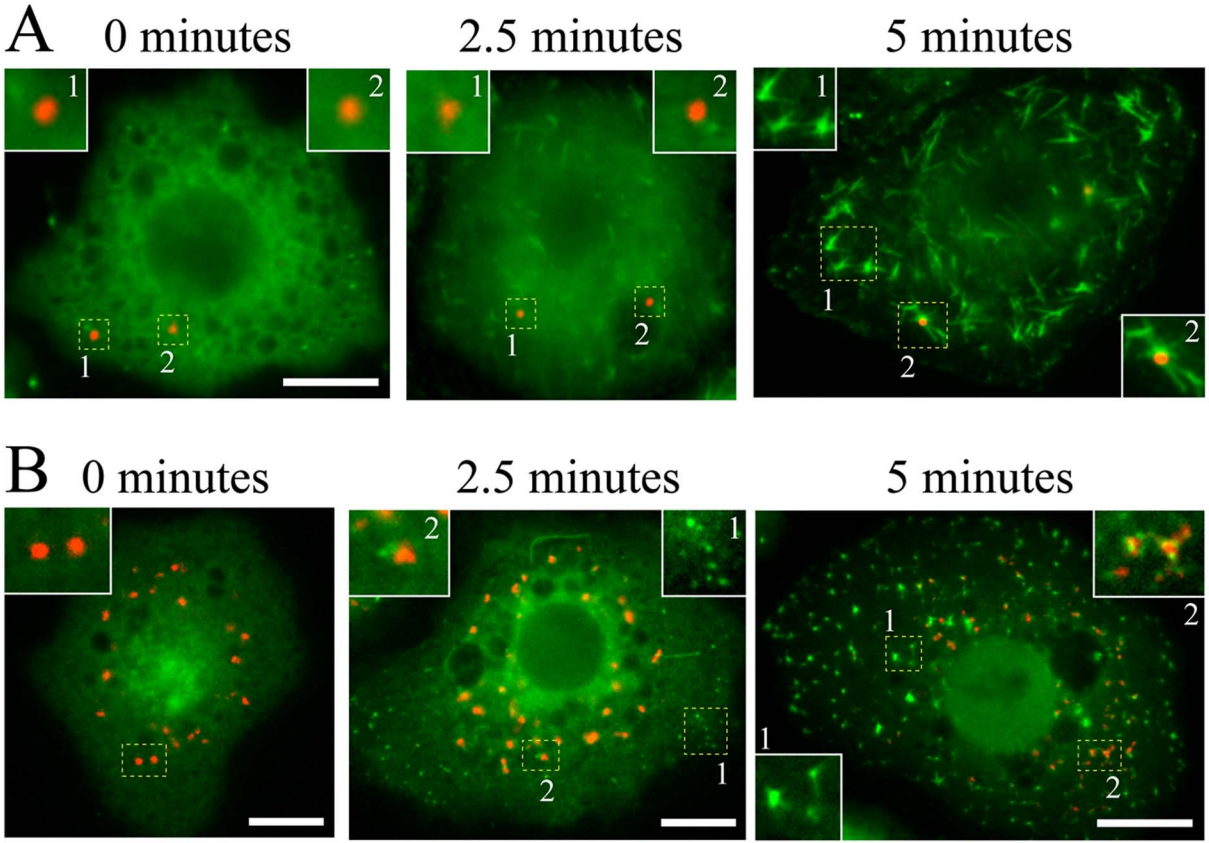




	Bipolar	Monastral Bipolar	Acentrosomal Bipolar
Control RNAi	73.9% 85 of 115	25.2% 29 of 115	0.9% 1 of 115
SAS-6 RNAi	2.0% 2 of 100	12.0% 12 of 100	86.0% 86 of 100

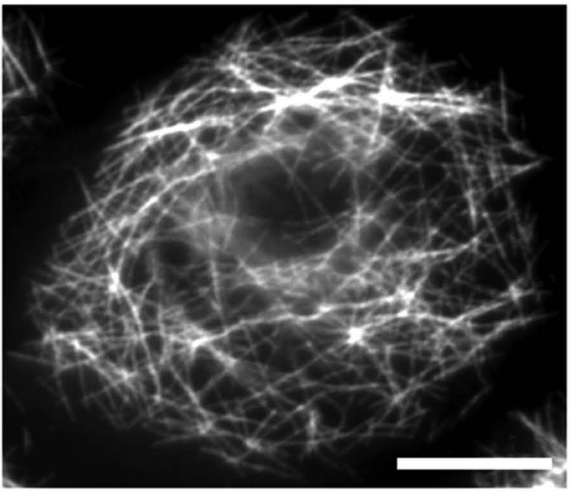


Supplemental Figure 6

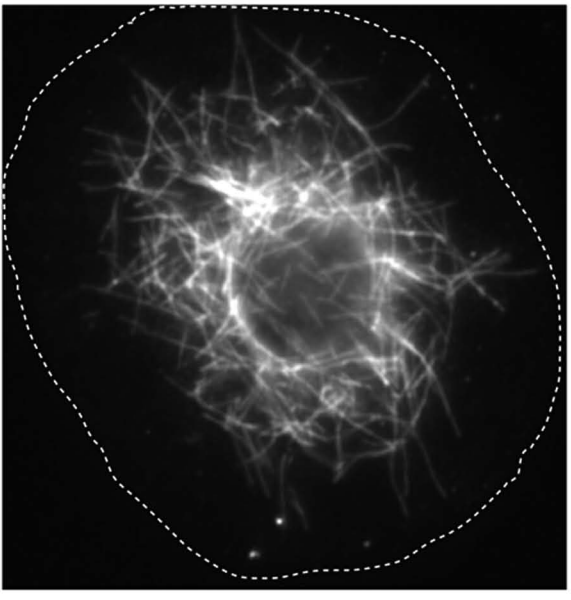


Supplemental Figure 7

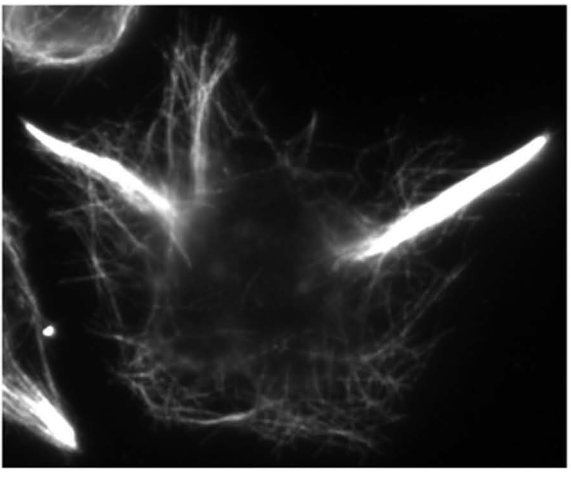
Control RNAi

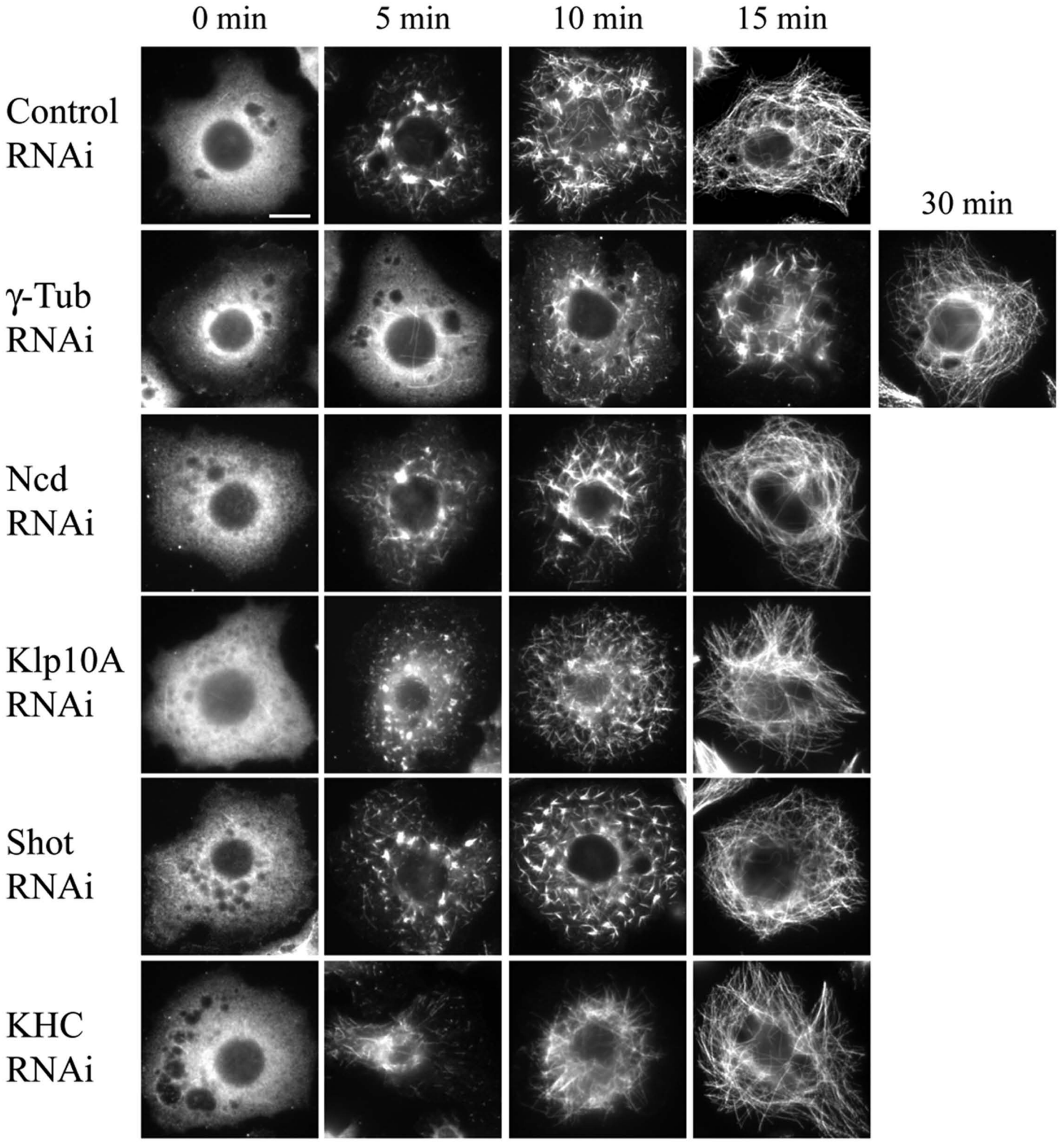


MAST RNAi



Msps RNAi




0 min
5 min
10 min
15 min
30 min
Control RNAi
γ-Tub RNAi
Ncd RNAi
Klp10A RNAi
Shot RNAi
KHC RNAi