Supplementary Figure Legends

Sup. Figure 1. γ-tubulin recruitment to spindle poles after DSas-4 RNAi in S2 cells. (A-E and G) Localization of γ-tubulin (red) and DSas-4 (white) in control (A) and DSas-4 depleted (B-E and G) S2 cells for 6 days. Numbered insets correspond to enlarged views of the respective spindle poles. Note that DSas-4 negative poles show different degrees of organization and can be classified according to the γ-tubulin recruited: poles that don't recruit γ-tubulin (C1, E1 and E2), poles that are able to organize aggregates of γ-tubulin (D1 and D2) and poles that are able to organize one well-defined focus of γ-tubulin (B1, B2 and C2). (F) Quantification of γ-tubulin recruitment to DSas-4 negative poles after RNAi. Bars represent the absolute distribution of cells (a total of 100 poles were scored). Note that ~50% of the DSas-4 negative poles are still able to recruit γ-tubulin to different extents. (G) S2 cell showing one pole containing DSas-4 and another pole without DSas-4. Microtubules were stained with anti-α-tubulin antibody (green) and DNA was counterstained with DAPI (blue). Scale bar = 5 μm.

Sup. Figure 2. Western blottings showing γ-tubulin levels in S2 cells stably expressing γ-tubulin-GFP and mCherry-α-tubulin, as well as the depletion efficiency of Ncd, dynein heavy chain, centrosomin, Dgt5, KLP10A, Msps, EB1 and D-CLIP-190 after RNAi. (A) Western blot showing expression levels of γ-tubulin both in parental S2 cells (first lane) and S2 cells stably expressing γ-tubulin-GFP and mCherry-α-tubulin (second lane). The total levels of γ-tubulin in the stable cell line expressing γ-tubulin-GFP correspond to less than 2x the endogenous levels. Also note the down-regulation of endogenous γ-tubulin in the γ-tubulin-GFP line. (B) Western blot showing Ncd expression levels in control (first lane), and after 4 days RNAi (second lane). (C) Western blot showing DHC expression levels in control (first lane), and

after 4 days RNAi (second lane). (D) Western blot showing centrosomin expression levels in control (first lane), and after 4 days RNAi (second lane). (E) Western blot showing Dgt5 expression levels in control (first lane), and after 4 days RNAi (second lane). (F) Western blot showing KLP10A expression levels in control (first lane), and after 4 days RNAi (second lane). (G) Western blot showing Msps expression levels in control (first lane), and after 6 days RNAi (second lane). (H) Western blot showing EB1 expression levels in control (first lane), and after 6 days RNAi (second lane). (I) Western blot showing D-CLIP-190 expression levels in control (first lane), and after 4 days RNAi (second lane). For loading control, unspecific bands recognized by the antibodies were used in A, B, D and G (as indicated), Cenp-C (in C) and α–tubulin (in E, F and H). Molecular weight markers are indicated on the right.

Sup. Figure 3. Cytoplasmic γ-tubulin aggregates are present in control S2 cells from prophase to anaphase and they contain centrosomin, as well as γ-TuRC proteins. (A-C) Immunofluorescence images showing that many cytoplasmic γ-tubulin aggregates (in red) colocalize with Cnn (in green, A), γ-TuRC subunit Dgp71WD (in green, B), and with γ-TuSC subunit Dgrip84 (in green, C) in control S2 cells in prophase. Note that these aggregates can be found in the periphery of centrosomes (arrowheads of the insets 1 and 2 in figures A, B and C) and around the nuclear envelope (A, B and C). (D-F) Immunofluorescence images showing that some γ-tubulin aggregates (in red) colocalize with Cnn (in green, D), γ-TuRC subunit Dgp71WD (in green, E), and with γ-TuSC subunit Dgrip84 (in green, F) in control S2 cells in metaphase (arrowheads of zooms 2x in figures D, E and F). (G-I) Immunofluorescence images showing that some γ-tubulin aggregates (in green) colocalize with Cnn (in red, G), γ-TuRC subunit Dgp71WD (in red, H), and with γ-TuSC subunit Dgrip84 (in red, I) in control S2 cells in anaphase

(arrowheads of zooms in figures G, H and I). Microtubules were stained with anti-α-tubulin antibody (blue). Scale bar = 5 μm.

Sup. Figure 4. Cytoplasmic γ-tubulin aggregates are able to nucleate microtubules prior to anaphase. (A-B) Immunofluorescence images of DSas-4 RNAi (A-A'') and control S2 cells (B-B'') showing microtubule repolymerization at different time points after cold. Note that at 0 min after cold, both DSas-4 depleted cells and control cells show cytoplasmic aggregates of γ-tubulin. After 2 min, microtubules start emanating from these aggregates in both DSas-4 RNAi and control cells (A' and B'). Insets in A' and B' correspond to enlarged views of the yellow-arrowed γ-tubulin aggregates. After 15 min, microtubule formation in the cytoplasm cannot be detected and most microtubules are now associated with the mitotic spindle in both DSas-4 and control cells (A'' and B''). Microtubules are in red, γ-tubulin is in green and DNA was counterstained with DAPI (blue). Scale bar = 5 μm.

Supplementary Video Legends

Sup. Video 1. Live-cell imaging of γ-tubulin and spindle microtubule dynamics in centriolar vs. acentriolar spindle poles of S2 cells after DSas-4 RNAi. S2 cells stably expressing mCherry-α-tubulin (red) and γ-tubulin-GFP (green) were treated with dsRNA for DSas-4 for 6 days (2 pulses). Arrows indicate cytoplasmic γ-tubulin aggregates associated with peripheral microtubule minus-ends, which are subsequently incorporated to the acentriolar pole by a microtubule clustering mechanism. Grayscale sequences show mCherry-α-tubulin and γ-tubulin-GFP alone. Images were acquired every 30 sec by dual-wavelength spinning-disk confocal microscopy.

Sup. Video 2. Live-cell imaging of γ-tubulin recruitment to acentriolar spindle poles in S2 cells after DSas-4 RNAi. S2 cells stably expressing mCherry-α-tubulin (red) and γ-tubulin-GFP (green) were treated with dsRNA for DSas-4 for 16 days (4 pulses). Grayscale sequences show mCherry-α-tubulin and γ-tubulin-GFP alone. Images were acquired every 30 sec by dual-wavelength spinning-disk confocal microscopy.

Sup. Video 3. Live-cell imaging of γ-tubulin at acentriolar spindle poles in S2 cells after DSas-4 and Ncd RNAi. S2 cells stably expressing mCherry-α-tubulin (red) and γ-tubulin-GFP (green) were treated with dsRNA for DSas-4 for 16 days and Ncd dsRNA for the last 4 days. Grayscale sequences show mCherry-α-tubulin and γ-tubulin-GFP alone. Images were acquired every 30 sec by dual-wavelength spinning-disk confocal microscopy.

Sup. Video 4. Live-cell imaging of γ-tubulin at acentriolar spindle poles in S2 cells after DSas-4 and DHC RNAi. S2 cells stably expressing mCherry-α-tubulin (red) and γ-tubulin-GFP (green) were treated with dsRNA for DSas-4 for 16 days and DHC dsRNA for the last 4 days. Grayscale sequences show mCherry-α-tubulin and γ-tubulin-GFP alone. Images were acquired every 30 sec by dual-wavelength spinning-disk confocal microscopy.

Sup. Video 5. *De novo* microtubule nucleation from cytoplasmic aMTOCs after anaphase onset. S2 cell stably expressing mCherry-α-tubulin (red) and γ-tubulin-GFP (green) treated with dsRNA for DSas-4for 16 days. Arrows indicate cytoplasmic aMTOCs (green bright dots) that nucleate microtubules upon anaphase onset. mCherry-α-tubulin channel alone is also shown in grayscale. Time zero was set from the moment of anaphase onset. Images were acquired every 20 sec by dual-wavelength spinning-disk confocal microscopy.

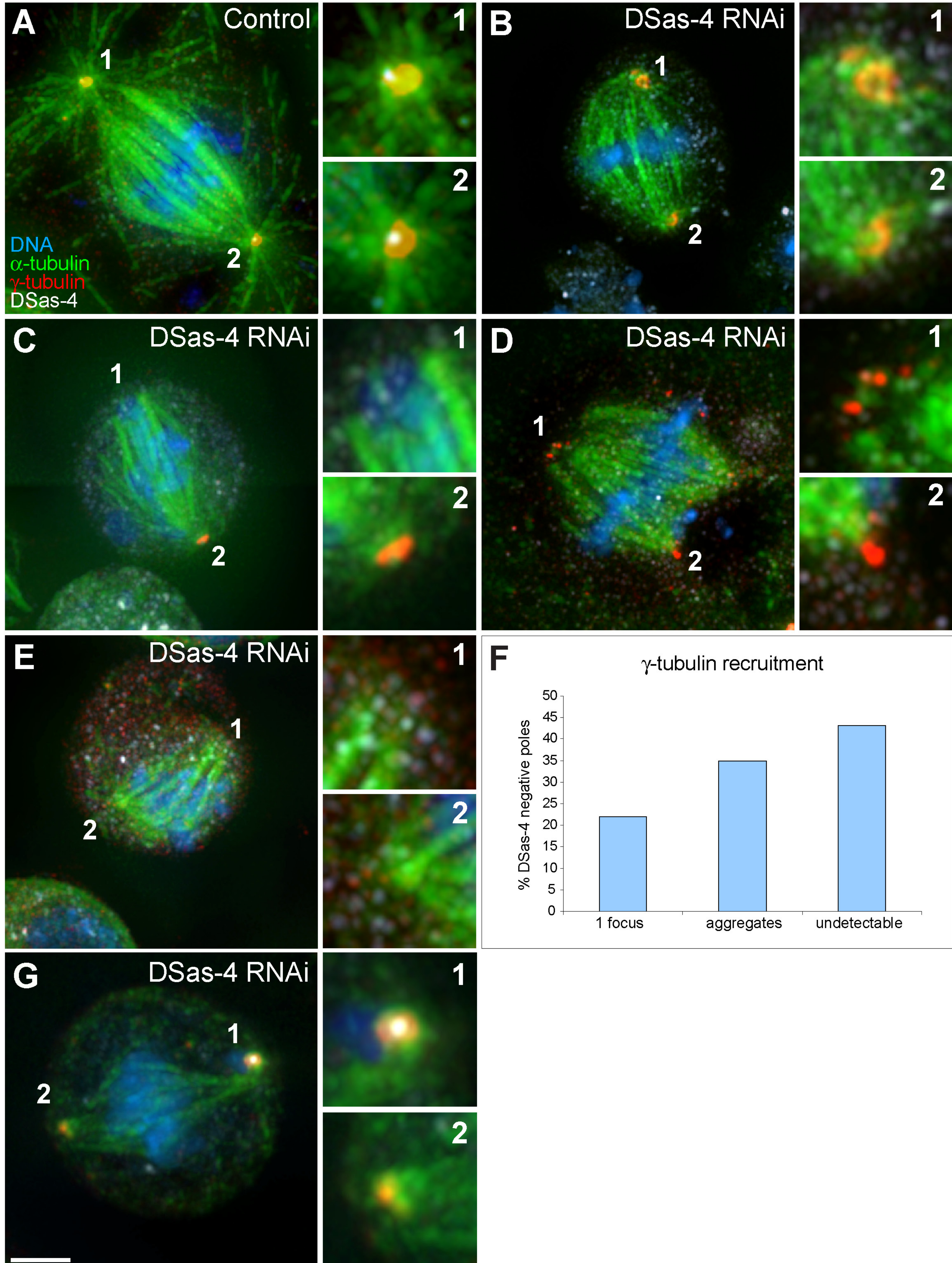
Sup. Video 6. *De novo* microtubule nucleation from cytoplasmic aMTOCs after anaphase onset. Same S2 cell as in Sup. Video 5 showing cytoplasmic γ-tubulin-GFP aggregates forming aMTOCs in grayscale. The sequence on the right panel shows the intensity profile of γ-tubulin-GFP where blue represents high and red represents low. Time zero was set from the moment of anaphase onset. Images were acquired every 20 sec by dual-wavelength spinning-disk confocal microscopy.

Sup. Video 7. *De novo* microtubule nucleation from cytoplasmic aMTOCs after anaphase onset is controlled by CDK1. S2 cells stably expressing mCherry-α-tubulin (red) and γ-tubulin-GFP (green) were treated with dsRNA for DSas-4 for 16 days. Cells were blocked in metaphase

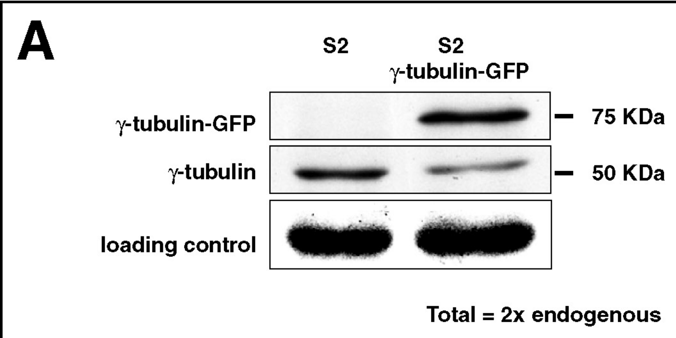
for 2h with MG132 before addition of Roscovitine (time zero). Arrows indicate cytoplasmic aMTOCs that nucleate microtubules immediately upon Roscovitine addition. Grayscale sequences show mCherry-α-tubulin and γ-tubulin-GFP alone. Images were acquired every 20 sec by dual-wavelength spinning-disk confocal microscopy.

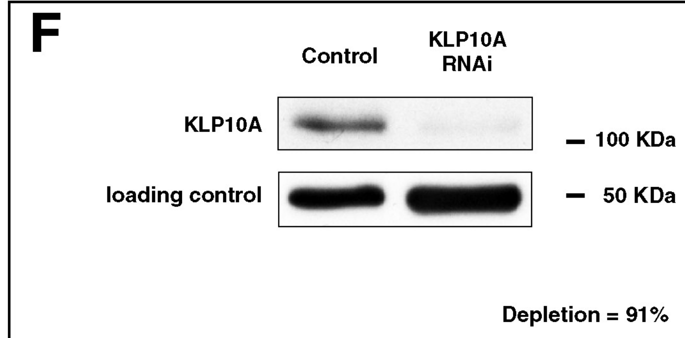
Sup. Table 1 – Distribution of Dsas-4 and γ-tubulin to centriolar vs. acentriolar spindle poles in the presence and absence of microtubules

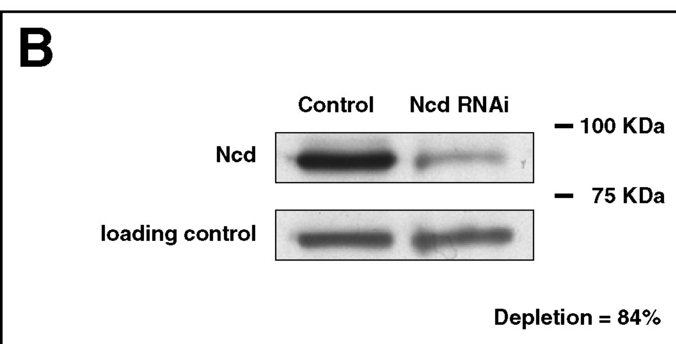
Marker	Cell line	Colchicine	Distribution (% of cells; N=100)				
			2 poles	1 pole	0 poles	Multiple poles	Disperse
DSas-4	S2	-	88.5	8.5	3.0	0	0
		+	2.9	7.7	0	15.4	74
	1182-4	-	5.0	30.5	56.5	0	8.0
		+	4.0	8.0	0	26.0	62.0
γ-tubulin	S2	-	92.5	7.5	0	0	0
		+	79.7	4.9	0	15.4	0
	1182-4	-	31.0	34.5	23.0	0	11.5
		+	11.0	3.0	39.0	47.0	0

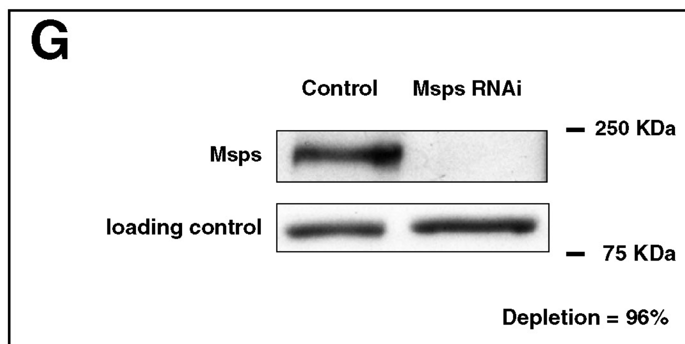


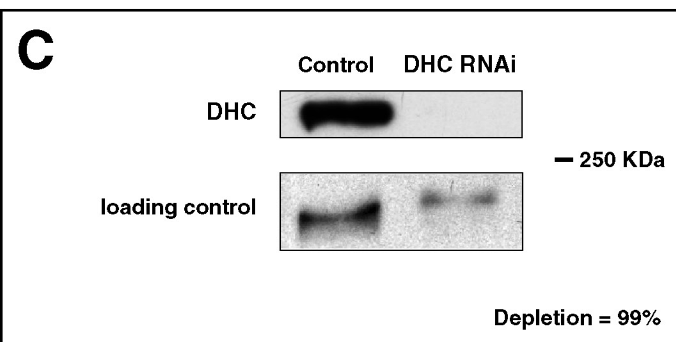
Sup. Figure 1 - Moutinho-Pereira et al. 2009

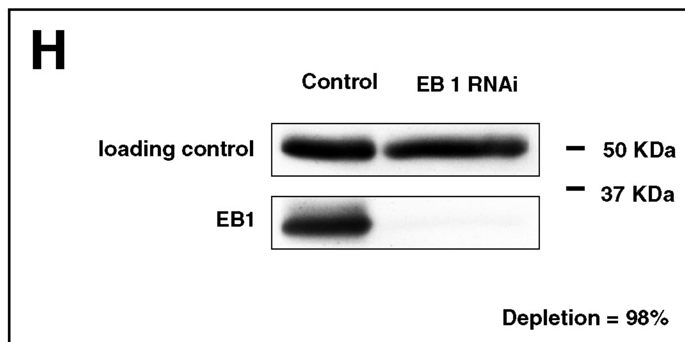


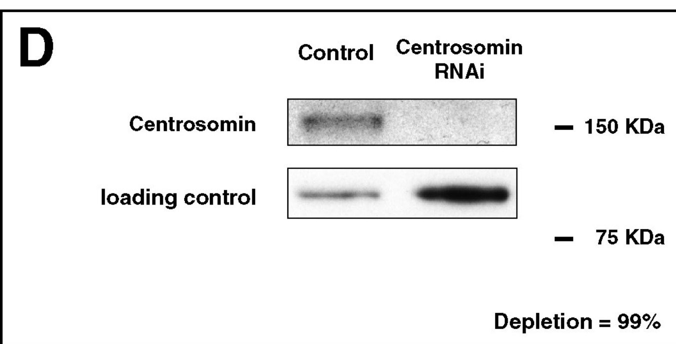


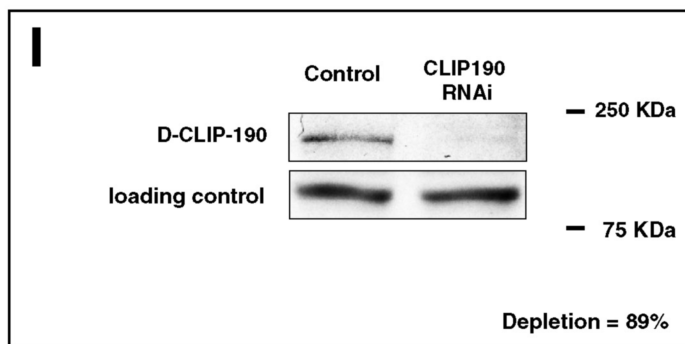


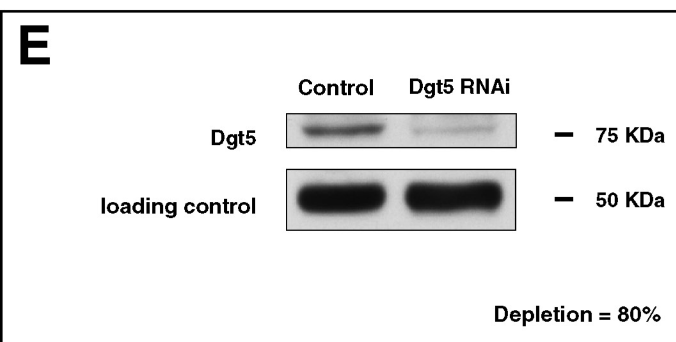




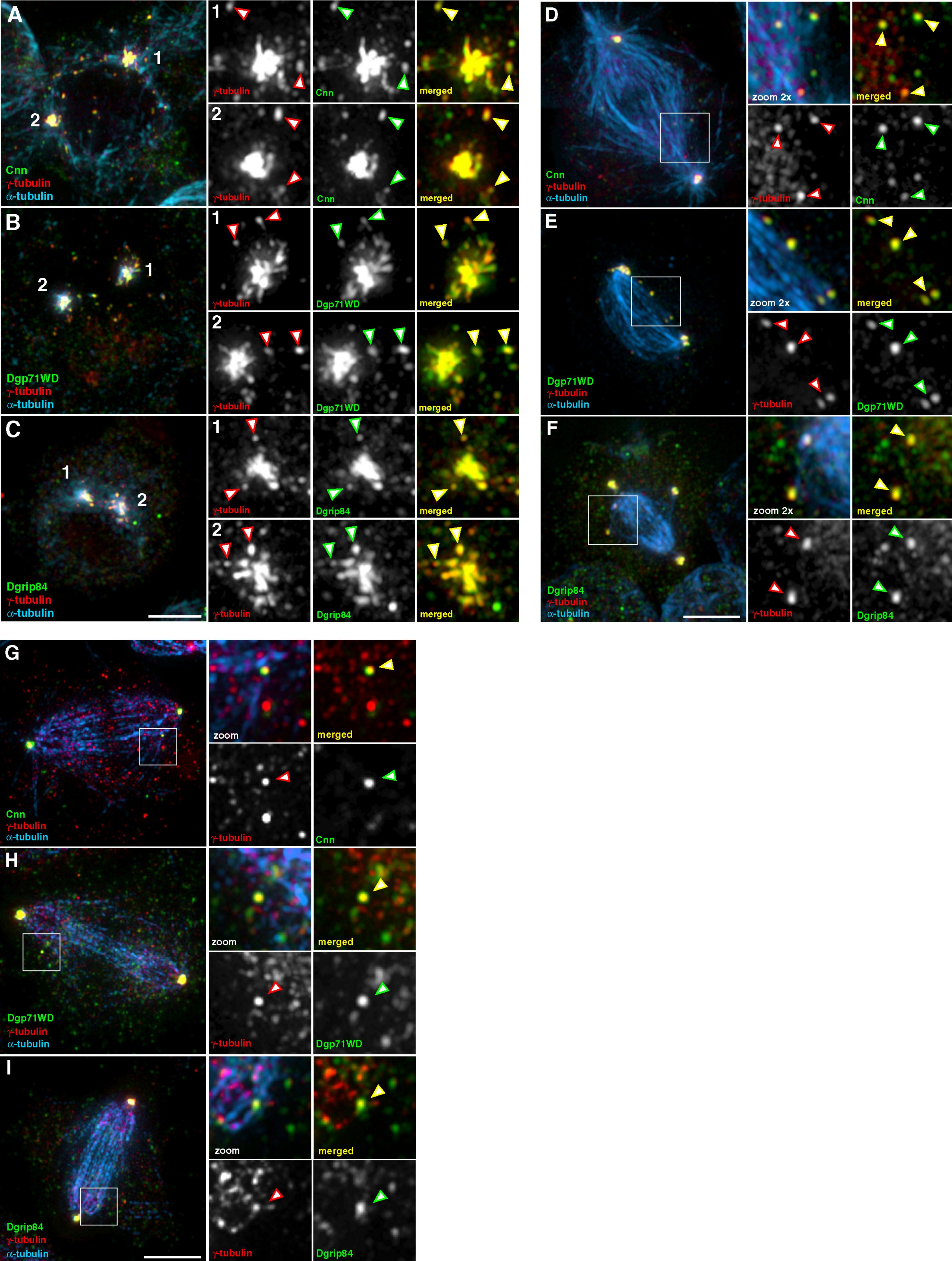




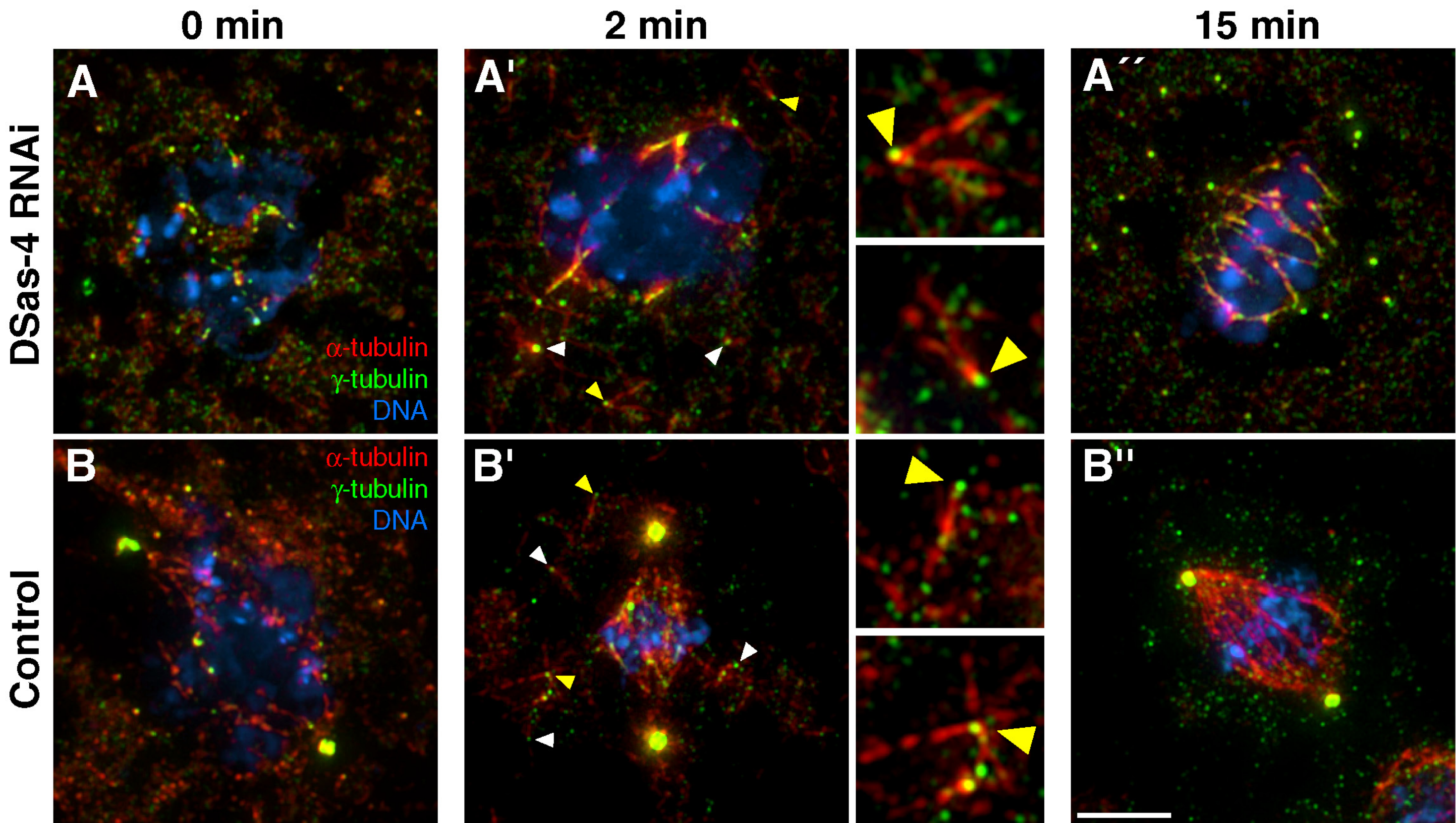




Sup. Figure 2 - Moutinho-Pereira et al., 2009



Sup. Figure 3 - Moutinho-Pereira et al., 2009



Sup. Figure 4 - Moutinho -Pereira et al. 2009