

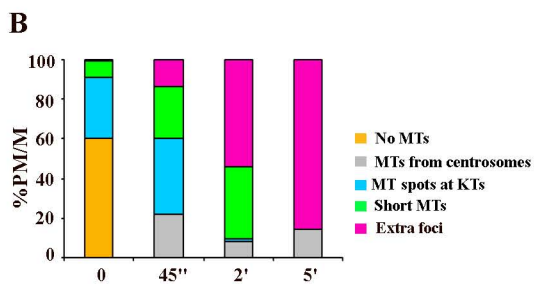
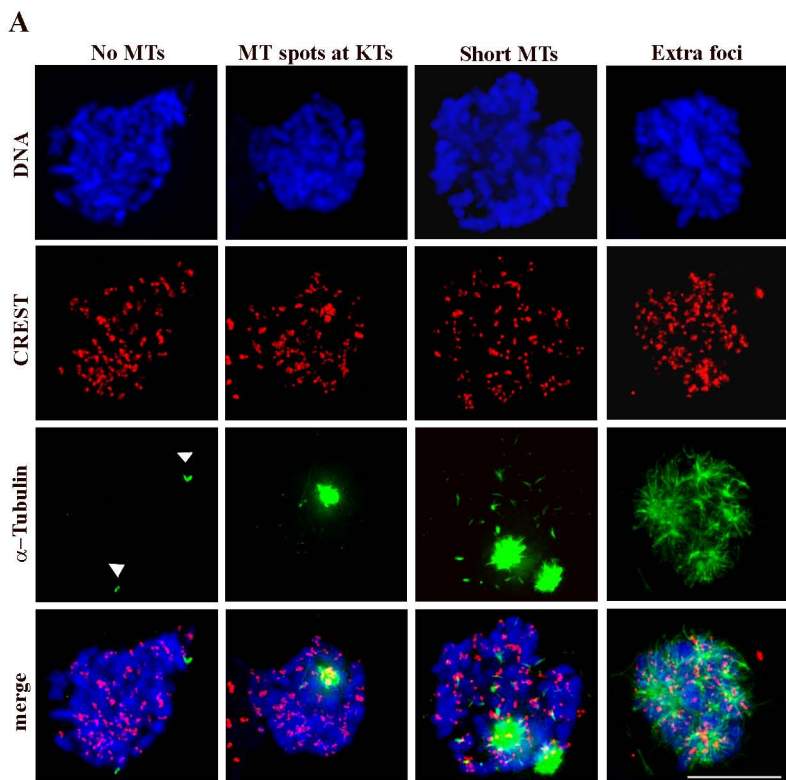
Supplemental Figure 1. Mitotic spindle reassembly after NOC block and release in U2OS cells. (A) Representative images of U2OS prometaphase cells immunostained for CREST and α -tubulin. *No MTs*: No α -tubulin staining at kinetochores, the two α -tubulin spots decorate the centrosomes (arrowhead); *MT spots at KTs*: Kinetochores showing α -tubulin positive spots; *Short MTs*: Short MTs emanating from kinetochores; *Extra foci*: supernumerary α -tubulin foci. Bar, 10 μ m. (B) Relative frequencies of MT nucleation patterns during spindle reformation after NOC washout in U2OS cells. The different categories are described above. More than 200 mitoses were counted for each time point in three experiments

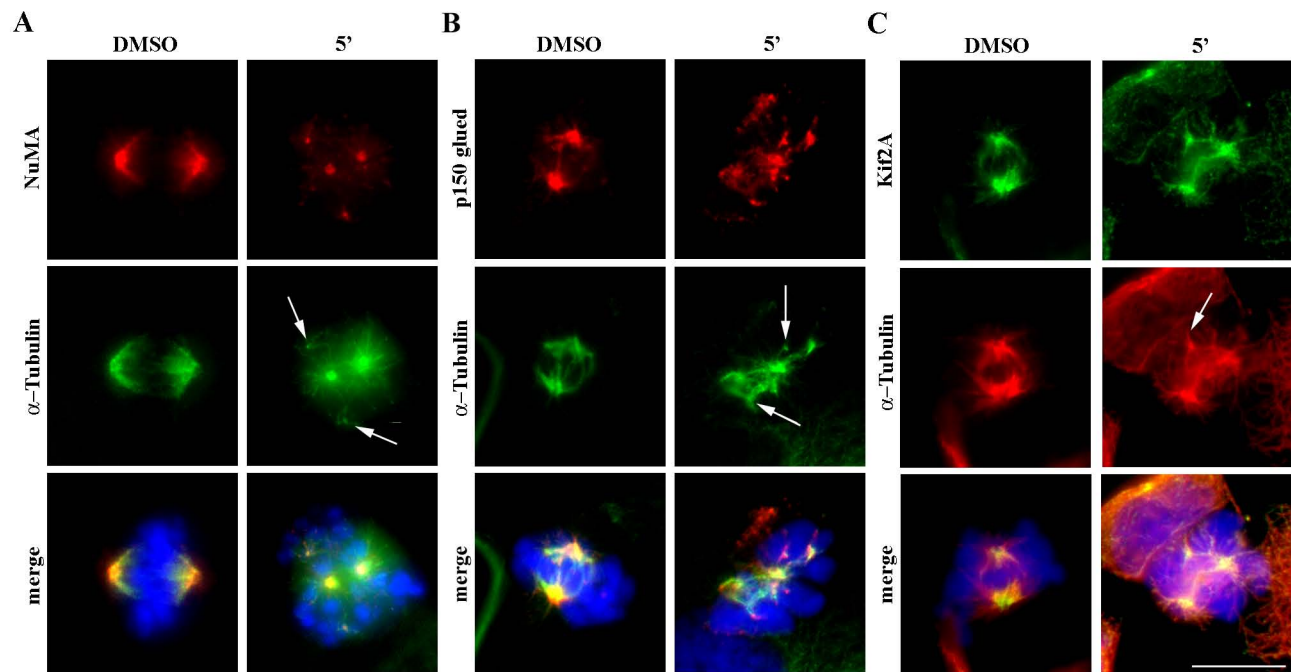
Supplemental Figure 2. Localization of MT minus end-associated proteins to extra-centrosomal foci after NOC treatment. Immunolocalization of NuMA (A), p 150 glued (B) and Kif2A (C) in metaphase cells from exponentially growing cultures (DMSO) and in prometaphase cells released for 5 minutes from a NOC-induced mitotic arrest (5'). Arrows point to α -tubulin extra foci. Bar, 10 μ m.

Supplemental Figure 3. Localization of Aurora-A and Plk1 in NOC-arrested U2OS cells. Aurora-A (A) and Plk1 (B,C) staining of prometaphase cells from a control culture (DMSO) or from a cell culture exposed for 16 h to 1.5 μ M NOC. Kinetochores are visualized by CREST staining. Note that the 14210 anti Plk1 antibody (ABCAM) used in (B) recognizes only the centrosome-associated Plk1 fraction, whereas 14209 antibody (ABCAM) in (C) stains Plk1 both at centrosomes and at kinetochores. Bar, 10 μ m.

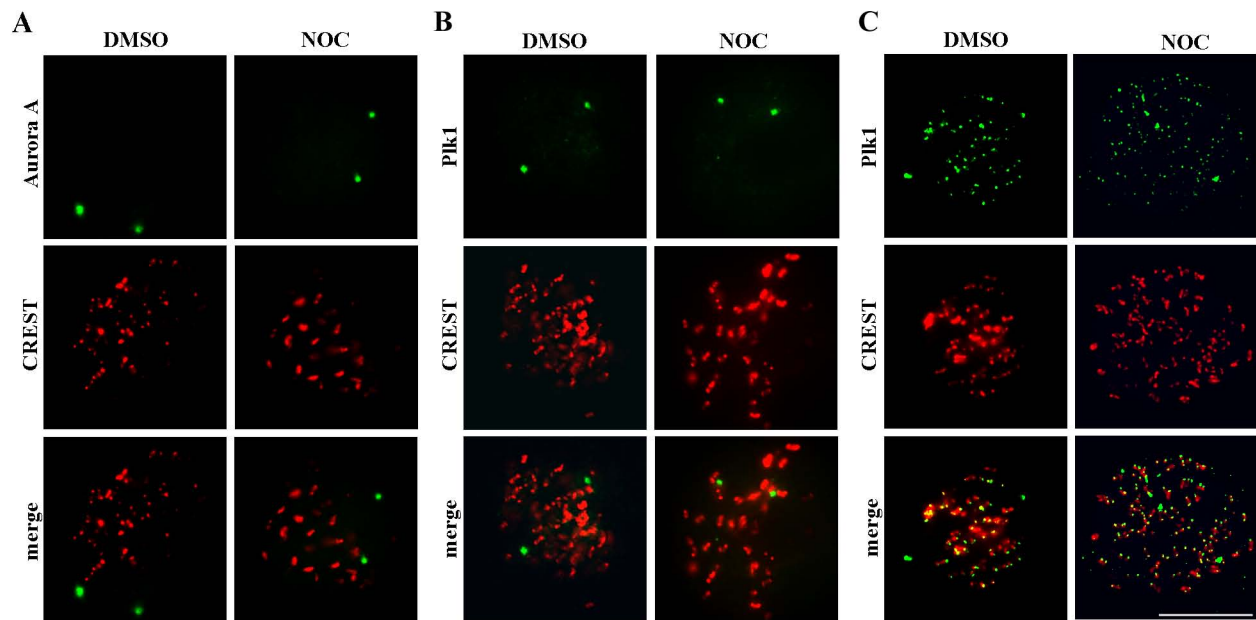
Supplemental Figure 4. RanGTP detection in permissive (32°C) and restrictive (39°C) conditions for RCC1 activity in tsBN2 cells. (A) RanGTP accumulation on microtubules and spindle poles as visualized by AR12 antibody staining at 32°C and 39°C in mitotic tsBN2 cells. (B) Mean \pm SE of the fluorescence intensity (arbitrary units) for AR12 signal at spindle poles at the two temperatures; ($p \leq 0.01$, t test).

Supplemental Figure 5. Western blot analysis of protein extract from U2OS cells transfected with control GL2- or Plk1-specific siRNA. Tubulin immunodetection is used as loading control.

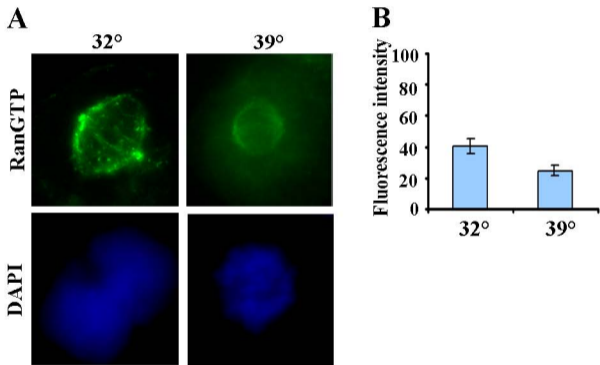




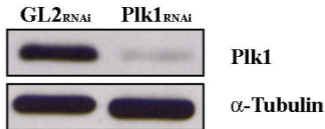
Torosantucci et al. Figure S2



Torosantucci et al. Figure S3



Torosantucci et al. Figure S4



Torosantucci et al. Figure S5