Summary of supplemental materials.

We have provided 5 supplemental figures.

Fig. S1. Cell cycle-dependent localization of UNC119a in various cell types.

Fig. S2A. Membrane vesicle-associated p230 was well preserved in paraformaldehyde-fixed cells.

S2B. Localization of GFP-UNC119a in U2OS cells.

Fig. S3. Different regions of UNC119a are required for the regulated localization of the protein.

Fig. S4A. Cell cycle-dependent interaction of UNC119a with Rab11 and Yes.

S4B. Knockdown of both Rab11 isoforms has a similar inhibitory effect on the completion of cytokinesis.

Fig. S5A. Co-localization of Rab11a with Fyn in dividing cells.

S5B. UNC-Ab specifically recognizes endogenous UNC119a from mouse and human cells.

Supplemental Figures.

Fig. S1. Cell cycle-dependent localization of UNC119a in various cell types.

A, B. Cell cycle-dependent localization of UNC119a in U2OS cells (A) and in NIH3T3 cells (B). Cells were double immunostained with UNC-Ab (red) and monoclonal α-tubulin Ab (or monoclonal γ-tubulin Ab, green). Bars, 10 μm.

C. Midbody localization of UNC119a in various cell types. Cells were fixed with cold methanol and double immunostained with monoclonal α-tubulin Ab (green) and UNC-Ab (red). Bar, 10 μm.

Fig. S2A. Membrane vesicle-associated p230 was well preserved in paraformaldehyde-fixed cells. HeLa cells were fixed in either cold methanol or 4% paraformaldehyde. Paraformaldehyde-fixed cells were permeabilized as described in Materials and Methods. Fixed cells were double immunostained with monoclonal p230-Ab (green) and UNC-Ab (red). In cold methanol-fixed cells (Cold-MeOH), UNC119a was concentrated in a spot, at the centrosome, and p230 was present around the UNC119a spot. In paraformaldehyde-fixed cells, both UNC119a and p230 were present as numerous dots. Arrows in cold methanol fixed cells indicate the concentrated UNC119a spots. Bar, 10 μm.

Fig. S2B. Localization of GFP-UNC119a in U2OS cells. U2OS cells were transfected with GFP-UNC119a. After 48 h, the cells were fixed with paraformaldehyde and immunostained with monoclonal γ-tubulin or α-tubulin Ab (red). Bars: 5 μm, in interphase; 10 μm, in other images.

Fig. S3. Different regions are required for the regulated localization of UNC119a.

A. Construction and localization of GFP-UNC119a deletion mutants. Human UNC119a was divided into three regions: N terminal (N, blue), amino acids 1-59; mid-region (M, yellow), amino acids 60-120; and C-terminal (C, pink), amino acids 121-240. Different regions of UNC119a were expressed in U2OS cells as a GFP fusion to examine the cellular localization of the different mutant proteins. The centrosomal localization was examined by fixing the transfected cells with cold methanol at 12 h after transfection and immunostaining the cells with monoclonal γ-tubulin-Ab and Alexa 546-conjugated goat anti-mouse IgG (red). To examine the midbody localization, cells were fixed with paraformaldehyde at 48 h after transfection and permeabilized with Triton X-100. Fixed cells were immunostained with monoclonal α-tubulin-Ab and Alexa 546-conjugated goat anti-mouse IgG (red). Cells that overexpressed and contained aggregated GFP-proteins were not included.

B. Centrosomal concentration of GFP-C and midbody concentration of GFP-N and GFP-M in U2OS cells. GFP-C: Concentration of GFP-C at the centrosome. GFP-N, GFP-M, GFP-NM: Concentration of GFP-N, -M, and -NM at the midbody. GFP: control cells transfected with GFP-expression vector. The nucleus and chromosomes were stained with DAPI. White arrows indicate GFP fusion proteins localized to the midbody. White arrows indicate the midbody. Bars, 20 μm.

Fig. S4A. Mitosis-specific interaction of UNC119a with Rab11 and Yes.

Immunoprecipitations (IP) were carried out using mitotic cell extracts and Rab11-Ab (**Left**) and Yes-Ab (**Right**). Western blotting experiments were performed with indicated Abs.

Fig. S4B. Knockdown of both Rab11 isoforms has a similar inhibitory effect on the completion of cytokinesis. HeLa cells were transfected simultaneously with Rab11a siRNA (siRab11a), Rab11b siRNA (siRab11b), or a mixture of Rab11a siRNA and Rab11b siRNA (siRab11a+b).

Top-left. Depletion of Rab11a and Rab11b. After 72 h, cell lysates were prepared, and Western blotting was performed with monoclonal Rab11a-Ab, polyclonal Rab11b-Ab, and monoclonal α-tubulin-Ab.

Top-right. Inhibition of cytokinesis by Rab11a+b siRNA. The cells treated with a mixture of Rab11a siRNA and Rab11b siRNA were fixed with cold methanol and immunostained with monoclonal α-tubulin-Ab and DAPI. The graphs represent the mean ± S.D. from three independent experiments. siN.C (control siRNA): bi-nucleated cells (5 ± 0.84%), cytokinetic cells (2.22 ± 0.22%) (n=1,000: experiment 1, 300; experiment 2, 300; experiment 3, 400). siRab11a+b: bi-nucleated cells (12.22 ± 0.4%), cytokinetic cells (6.56 ± 0.73%) (n=1,000: experiment 1, 300; experiment 2, 300; experiment 3, 400). $^{*}P < 0.05$.

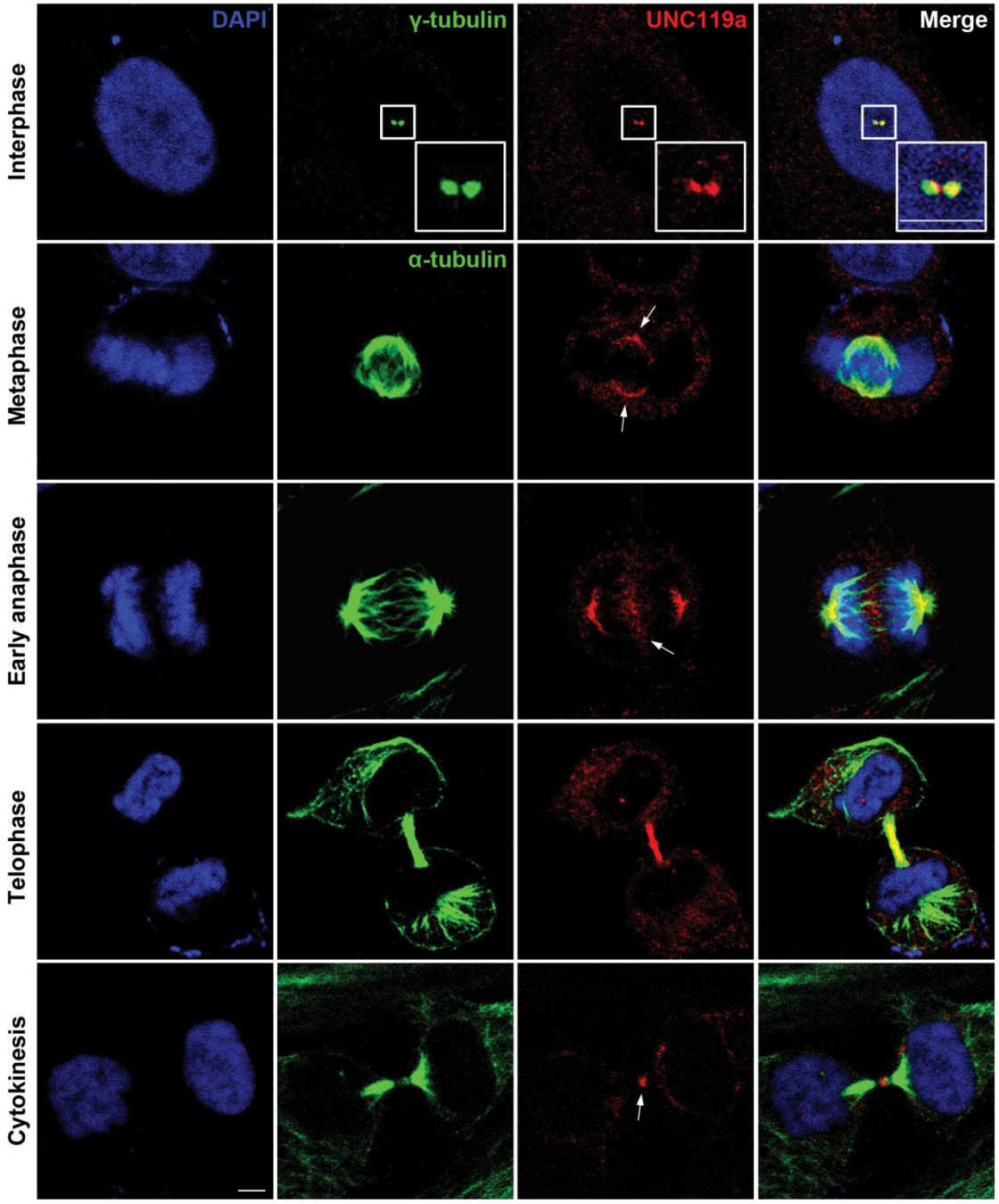
Bottom. The double siRNA treatment inhibited the localization of UNC119a to the intercellular bridge but had no effect on the centrosome/spindle pole localization of the protein. The siRNA-treated HeLa cells were fixed with cold methanol and double immunostained as described in **Fig. 3A**. Nuclei and chromosomes were stained with DAPI. Bar, 20 μm.

Fig. S5A. Co-localization of Rab11a with Fyn in dividing cells. HeLa cells were transfected with GFP tagged Rab11a. 48 h after the transfection, the cells were fixed and

immunostained with Fyn-Ab. Bar, 20 μm.

Fig. S5B. UNC-Ab specifically recognizes endogenous UNC119a from mouse and human cells. Cellular lysates were obtained from various cells and blotted with the indicated antibodies. Lane 1, HEK293 cell lysates; lane 2, HeLa cell lysates; lane 3, hTERT-RPE1 cell lysates; lane 4, U2OS cell lysates; lane 5, MN9D cell lysates; lane 6, MEF cell lysates; lane 7, NIH3T3 cell lysates.

A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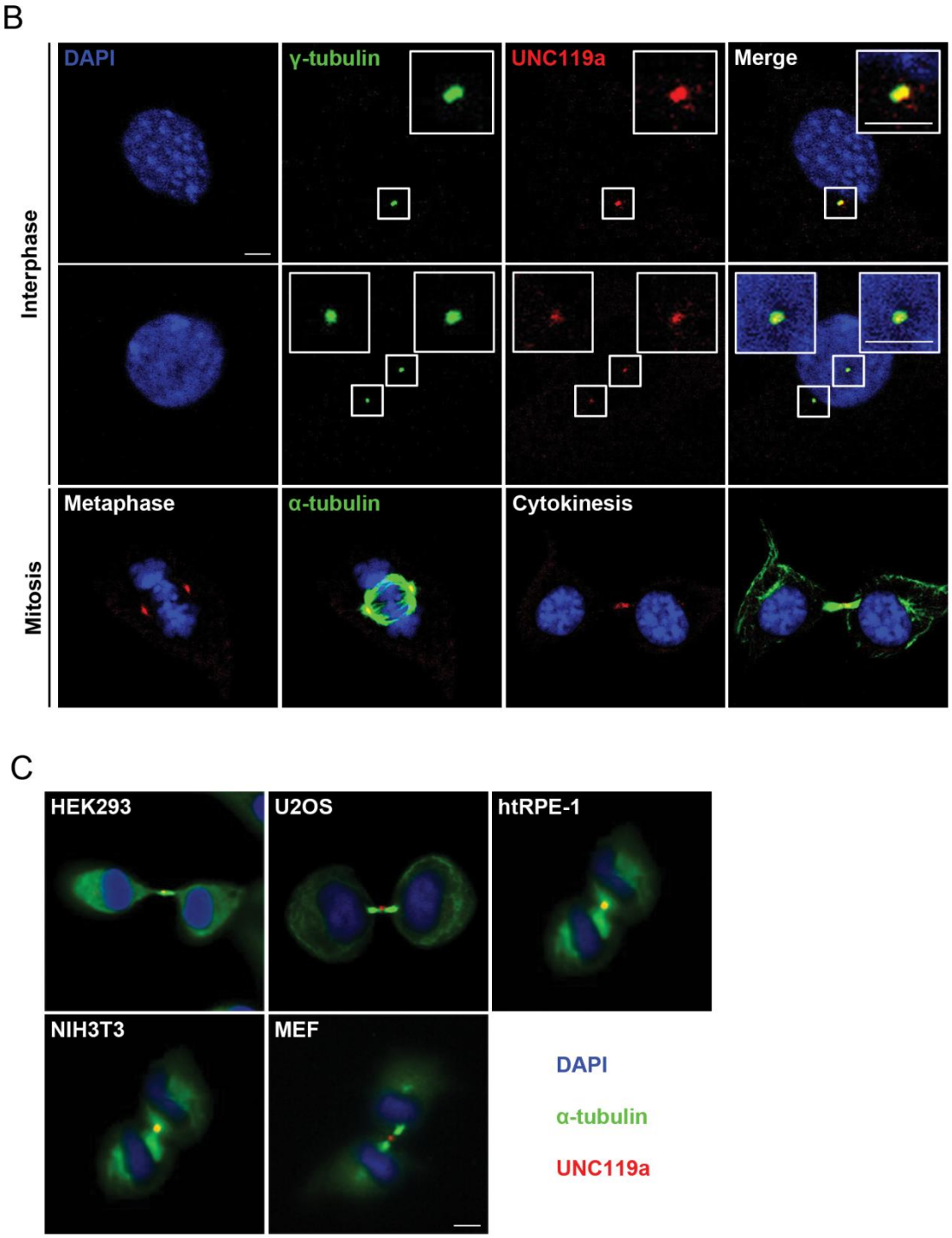
UNC119a
Merge
Interphase
α-tubulin
Metaphase
Early anaphase
Telophase
Cytokinesis



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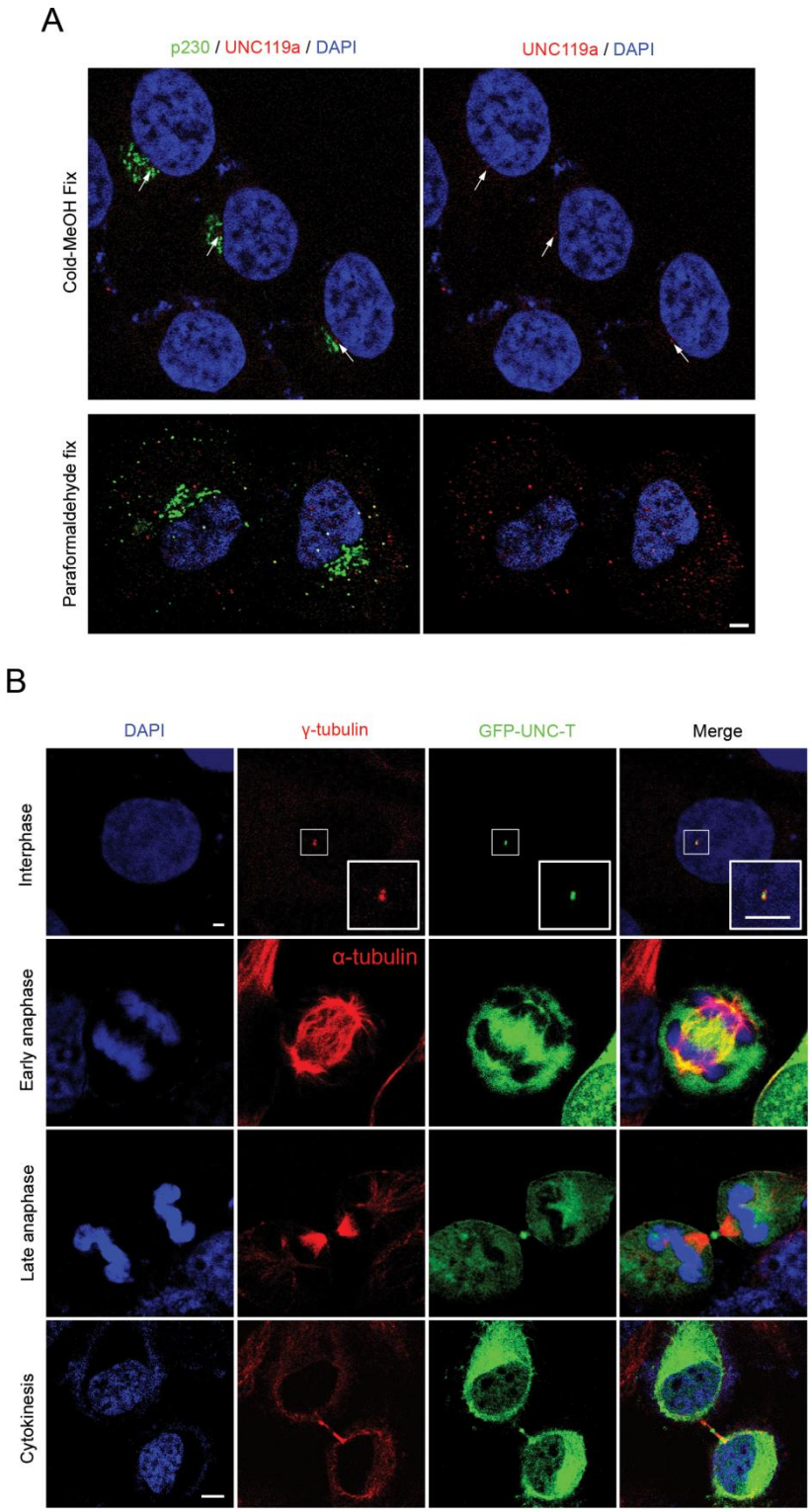

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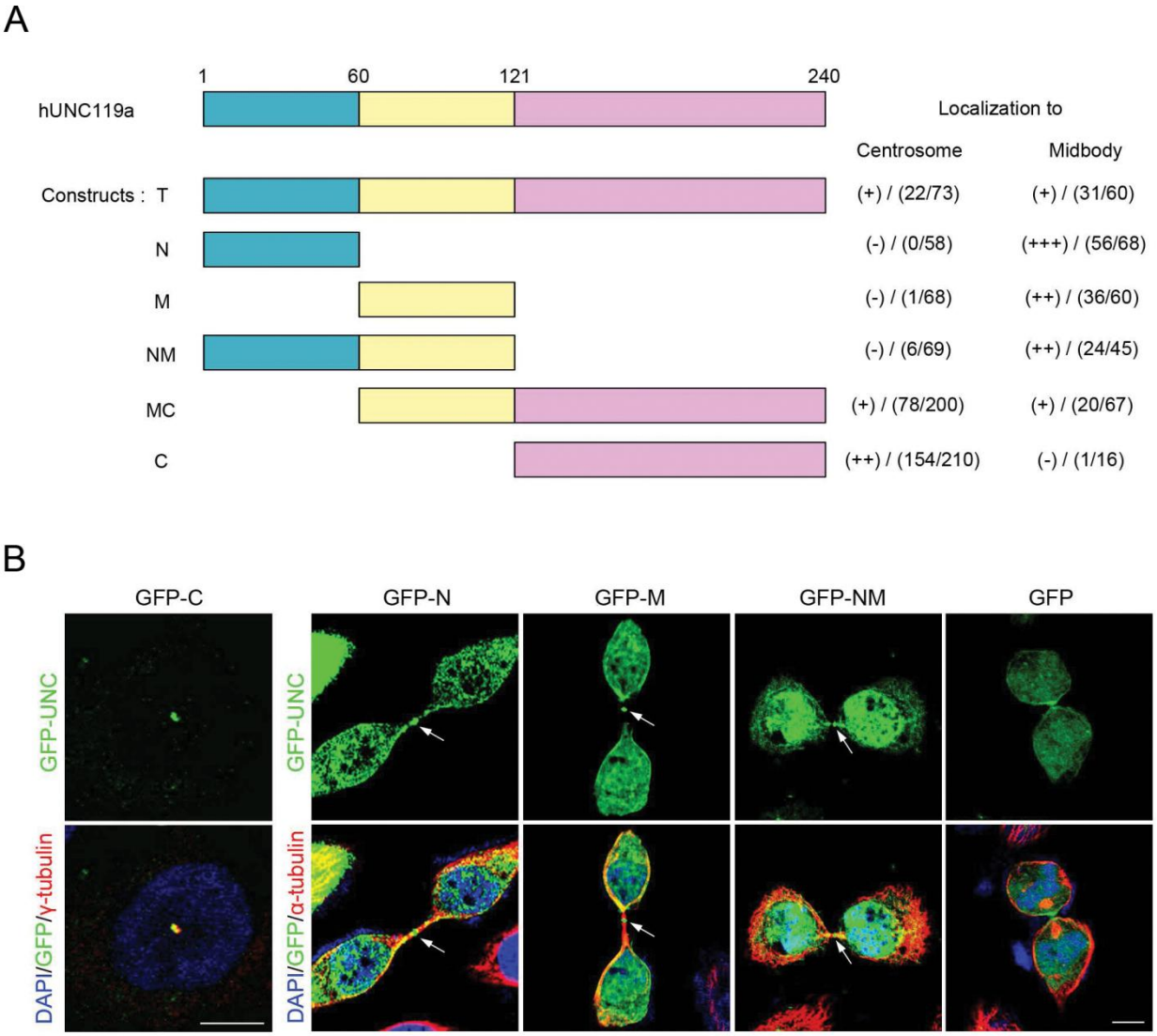

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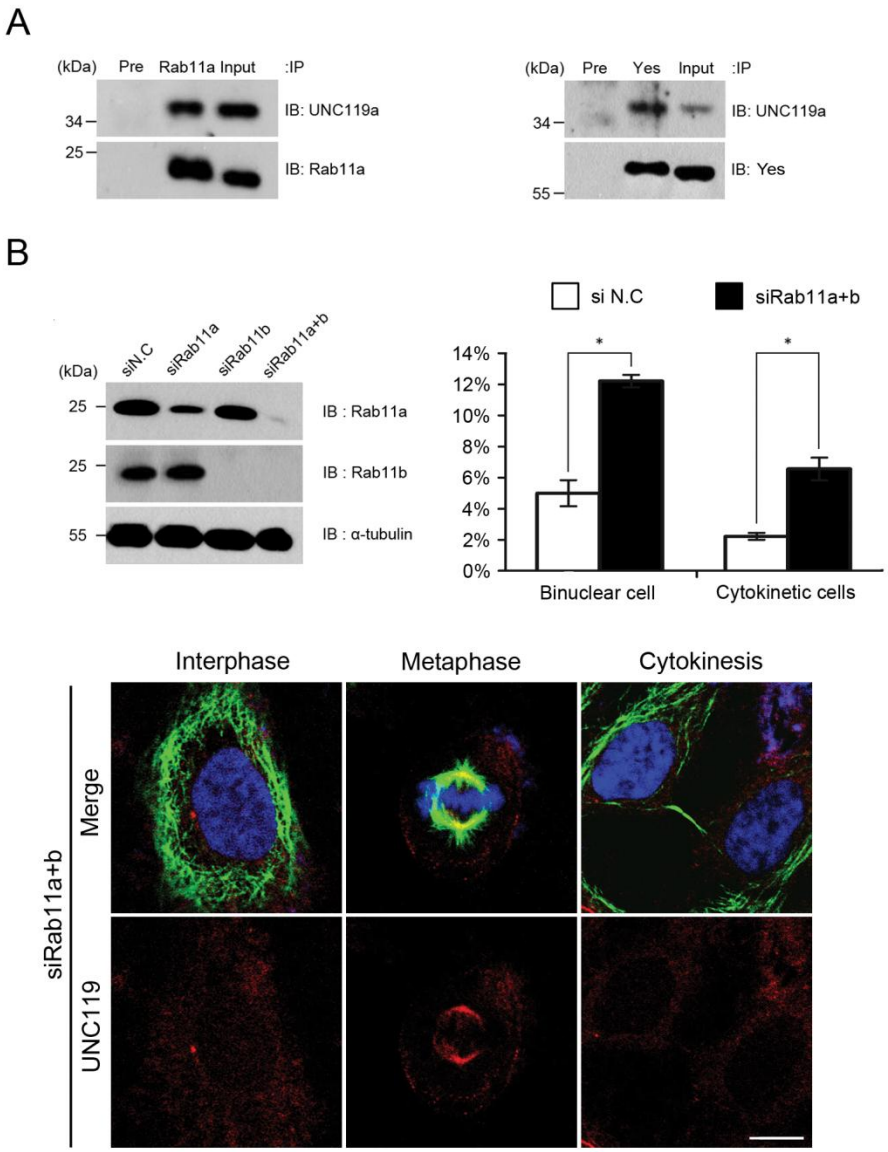

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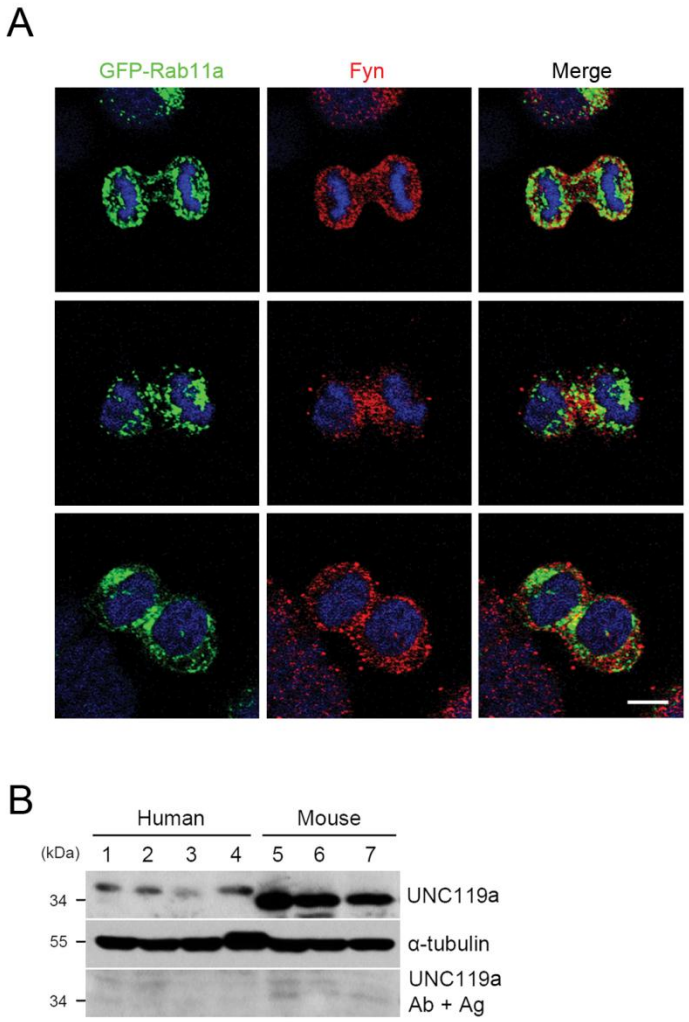

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