Science Advances
AAAS

advances.sciencemag.org/cgi/content/full/5/8/eaav0318/DC1

Supplementary Materials for

JLP-centrosome is essential for the microtubule-mediated nucleocytoplasmic transport induced by extracellular stimuli

Clement M. Lee*, Ken Aizawa, Joshua Jiang, Sam K.P. Kung, Rinku Jain

*Corresponding author. Email: clement.lee@mssm.edu

Published 28 August 2019, *Sci. Adv.* **5**, eaav0318 (2019)
DOI: 10.1126/sciadv.aav0318

This PDF file includes:

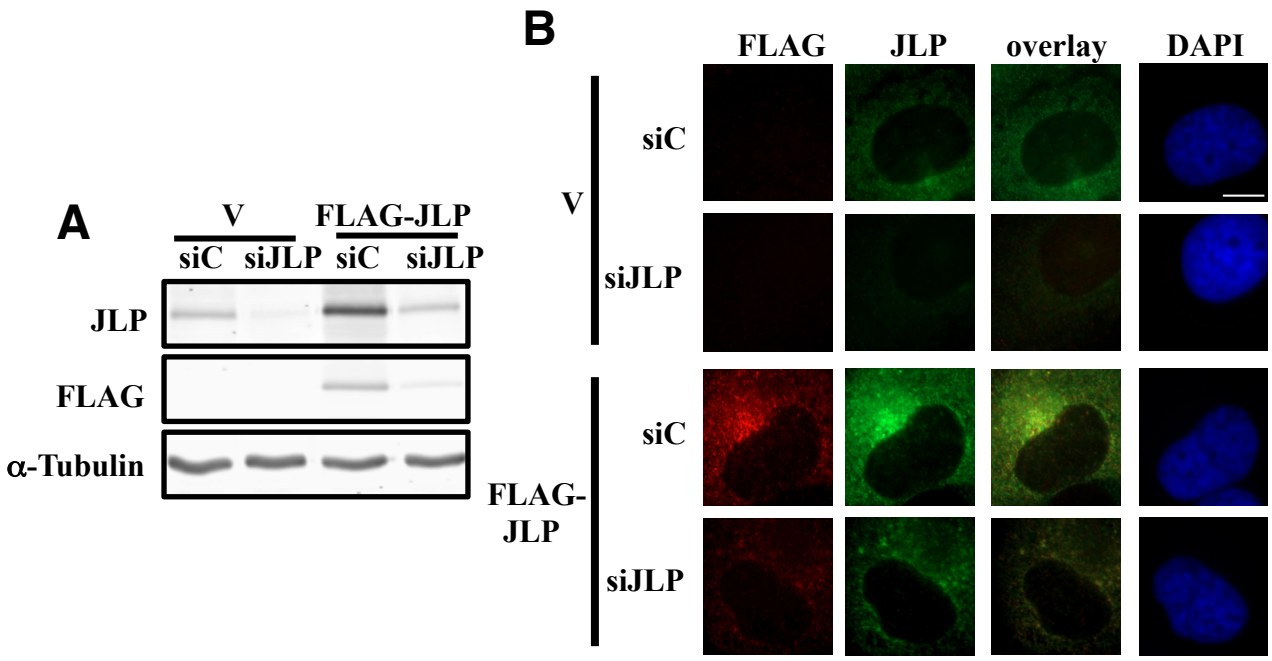

Fig. S1. Specificity of the JLP antibody. H1299 cells expressing the FLAG-tagged JLP and the vector control cells (V) were transfected with a specific siRNA against JLP (siJLP) or a control siRNA (shC). The cells were subjected to western blotting (**A**) and immunofluorescence analysis (**B**). Bar, 10 μm.

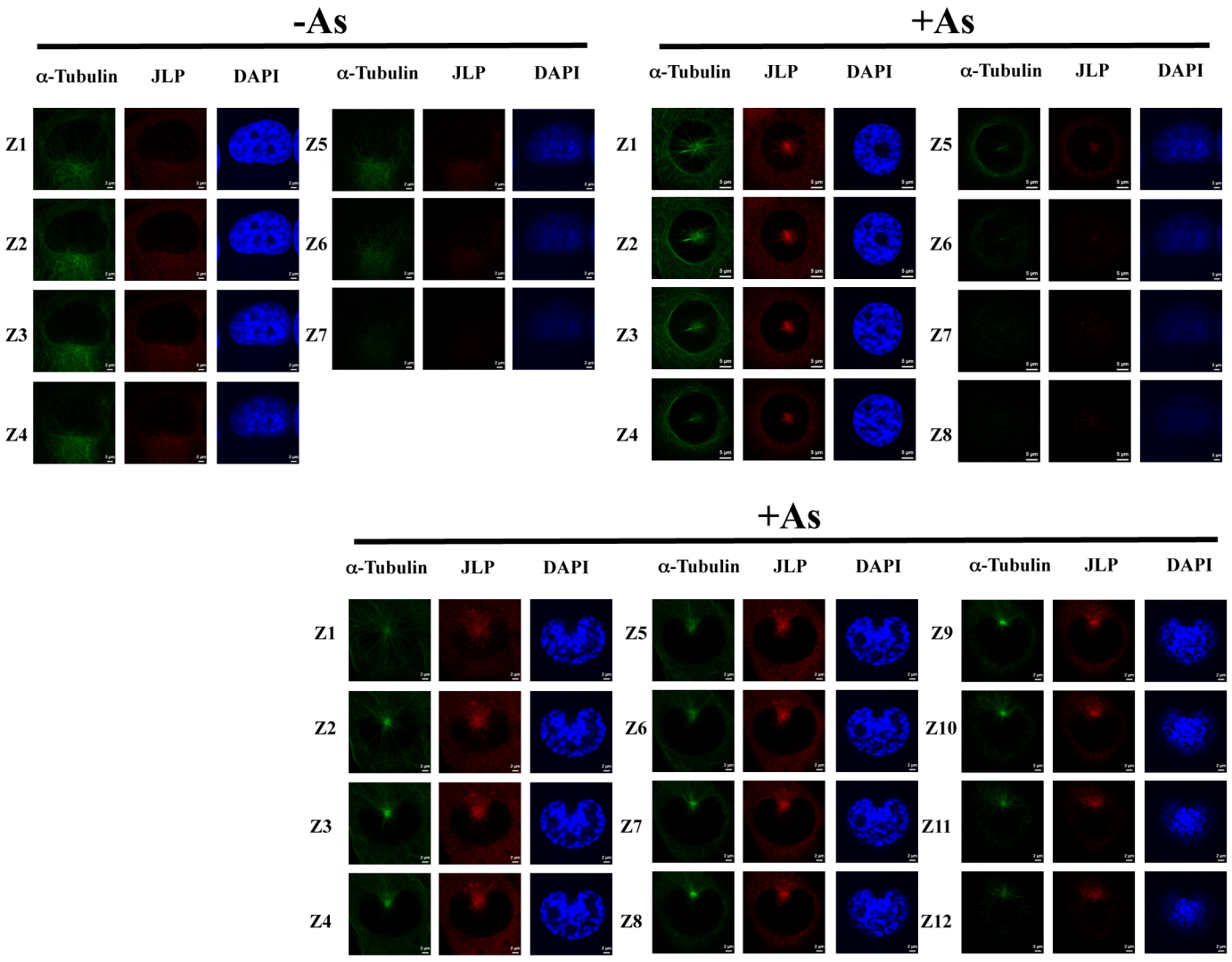

Fig. S2. Co-localiztion of JLP focus and the MTOC. H1299 cells were treated with or without arsenite (0.5 mM) for 30 min. They were then fixed and probed with the antibodies against JLP or α-tubulin. The nuclei were stained with the DNA-intercalating dye DAPI. They were examined by Z-stack imaging using confocal fluorescent microscopy. Bar, 2 μm. The interval between sections is 0.41 μm.

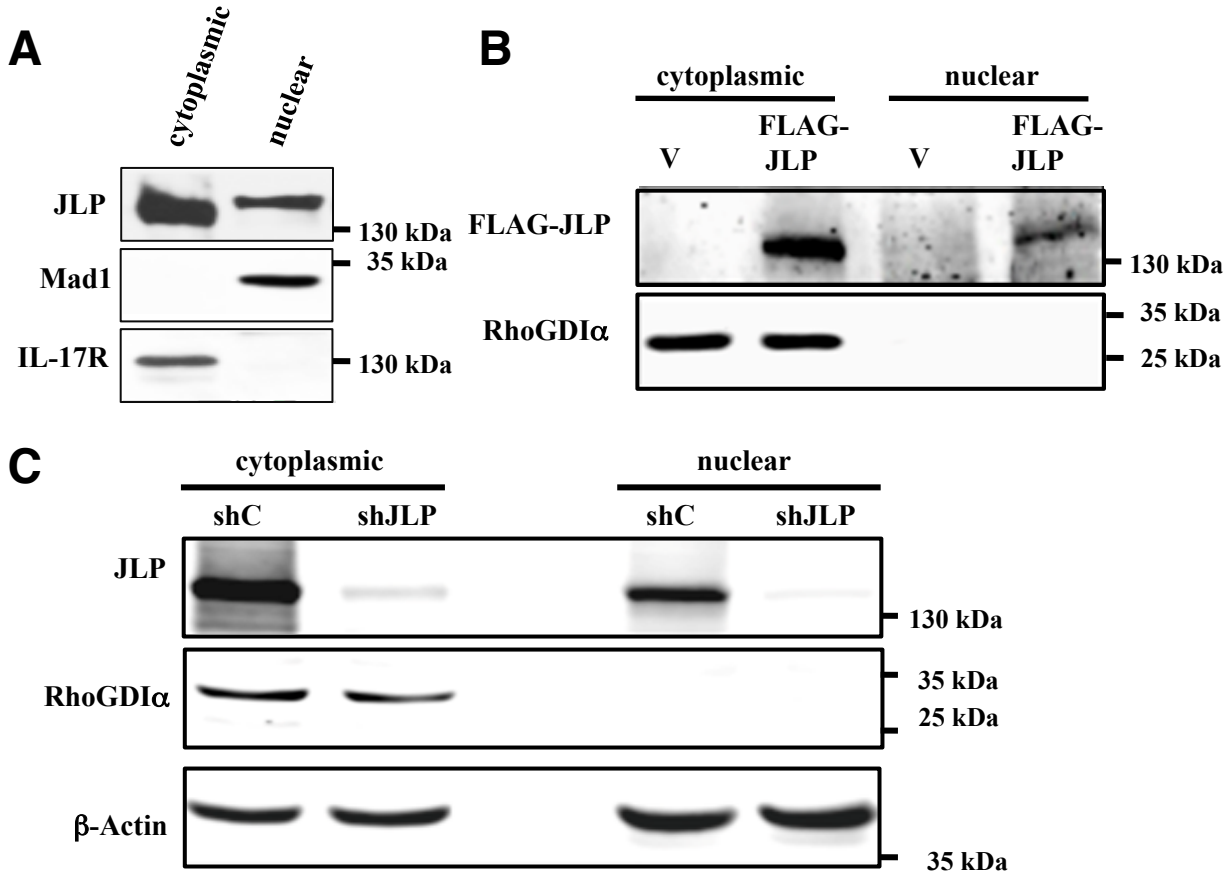

Fig. S3. Nuclear JLP. (**A**) Presence of JLP in the cytoplasmic and nuclear fractions of H1299 cells. The purity of the nuclear and cytoplasmic fractions is shown by the nuclear protein Mad1 and membrane receptor for interleukin-17 (IL-17R). (**B**) Appearance of FLAG-tagged JLP in the cytoplasmic and nuclear fractions. (**C**) Ablation of JLP reduces the nuclear JLP level. shC and shJLP denote the control and JLP-ablated cells, respectively. RhoGDIα is a marker for the cytoplasmic fraction.

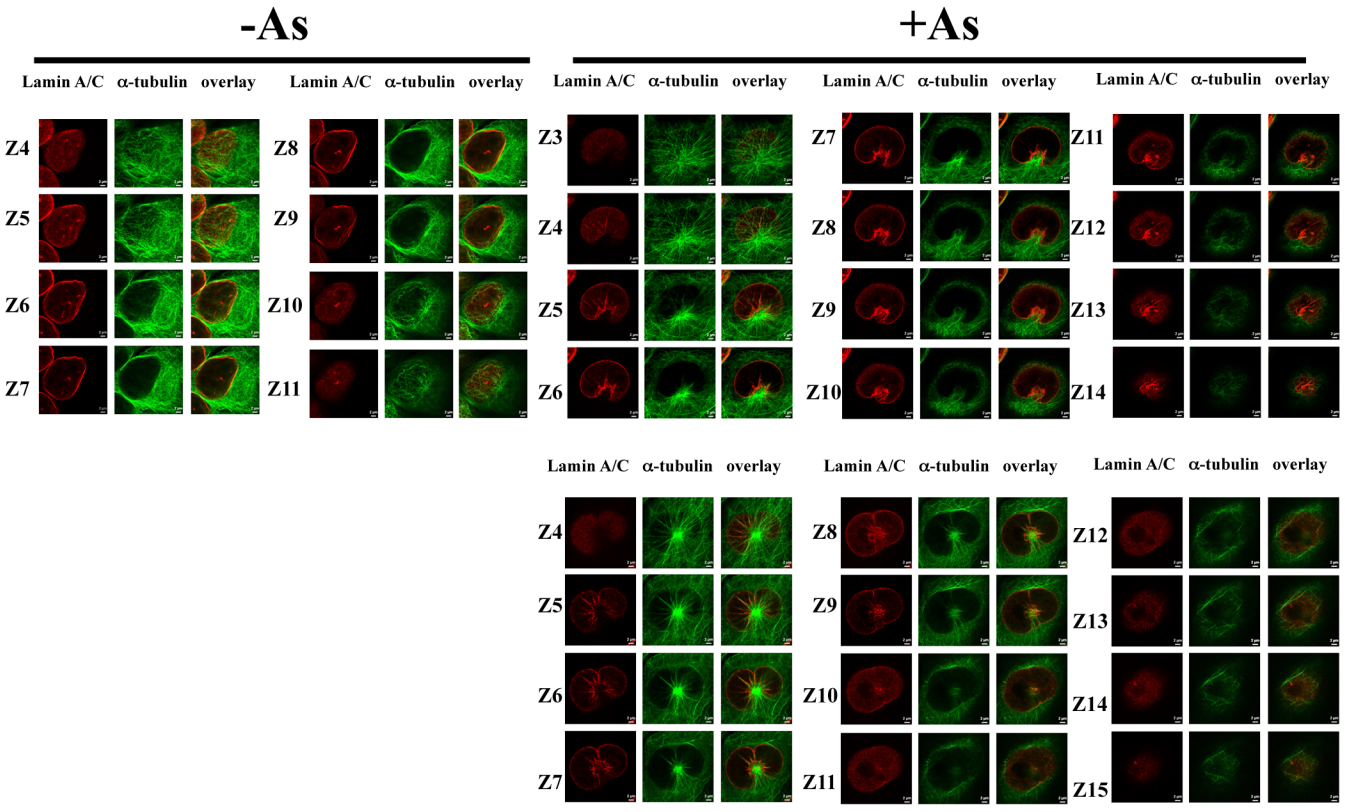

Fig. S4. The MTOC and nuclear membrane. H1299 cells were treated with or without arsenite (0.5 mM) for 30 min. They were then fixed and probed with the antibodies against JLP and lamin A/C. They were examined by Z-stack imaging using confocal fluorescent microscopy. Bar, 2 μm. The interval between sections is 0.41 μm.

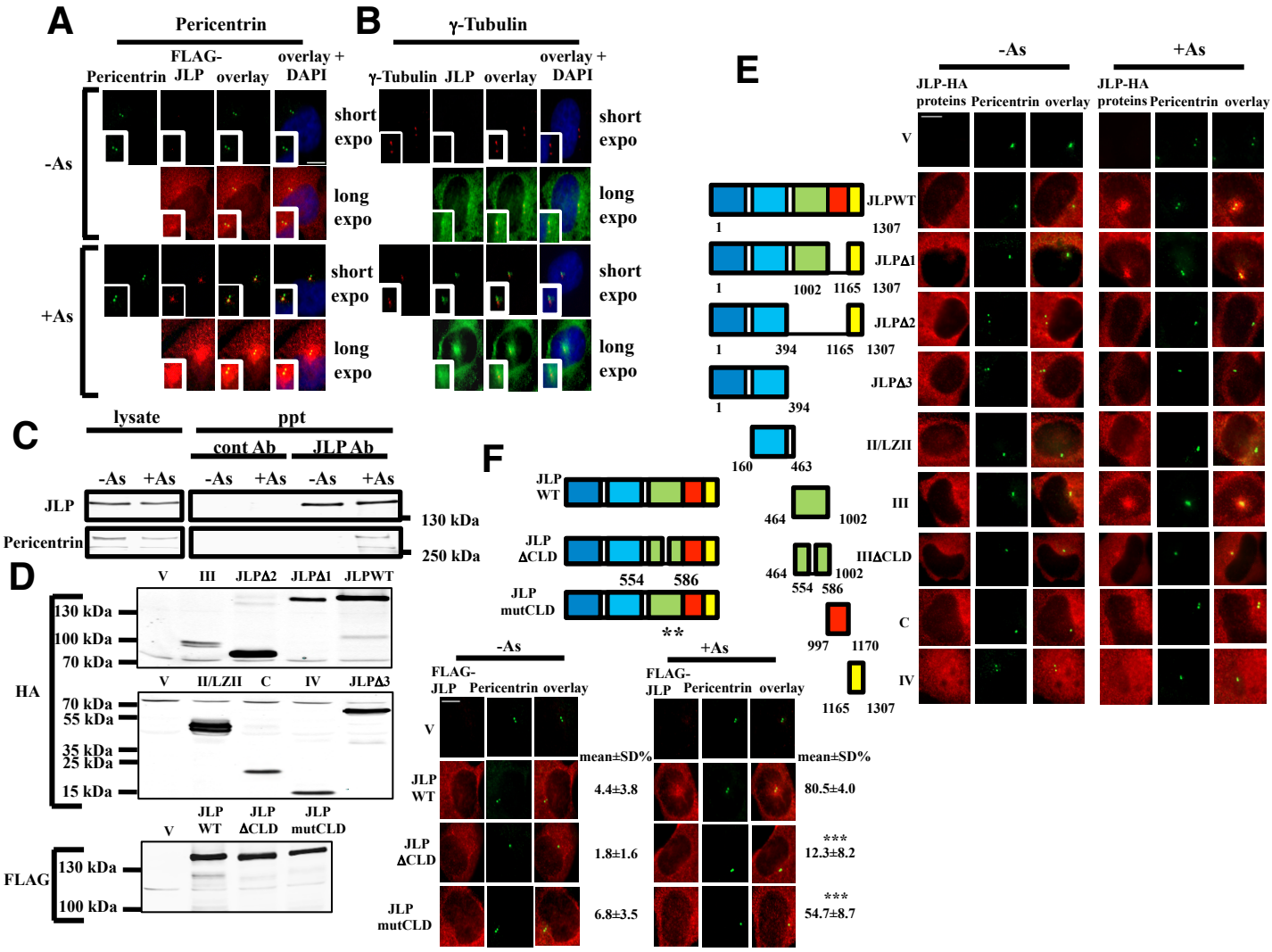

Fig. S5. The role of the CLD in the association of JLP focus with centrosome. H1299 cells with or without arsenite stimulation were probed for pericentrin and the FLAG-tagged JLP (**A**) or γ-tubulin and endogenous JLP (**B**). Short and long exposures of JLP are included in each case. Bar, 10 μm. (**C**) Physical interaction between JLP and pericentrin. (**D**) Expression of various deletion (JLPΔ1-3 and ΔCLD), point (JLPmutCLD) and domain (II/LZII, III, C and IV) mutants of JLP together with the wild-type (WT). (**E**) Cells were transfected with the above JLP proteins and were treated with or without arsenite (0.5 mM) for 30 min. The cells were fixed, stained with the HA-tag antibody and the pericentrin antibody, and then examined under fluorescence microscopy. (**F**) Immunofluorescence studies were preformed as in (**E**) with the FLAG-tagged JLP WT, ΔCLD and mutCLD. The percentages of cells showing co-localization of pericentrin with the JLP foci were scored and presented as mean±SD % (n=3). ***, $P < 0.05$ (as compared with arsenite-treated WT cells). Bar, 10 μm. The point mutant JLPmutCLD contains mutations K580N and R582Q. The schematic diagrams show the structures of the mutants and the numbers denote the amino acids of JLP. The interaction between the JLP mutants and centrosome is indicated by the co-localization of their foci with the centrosomes.

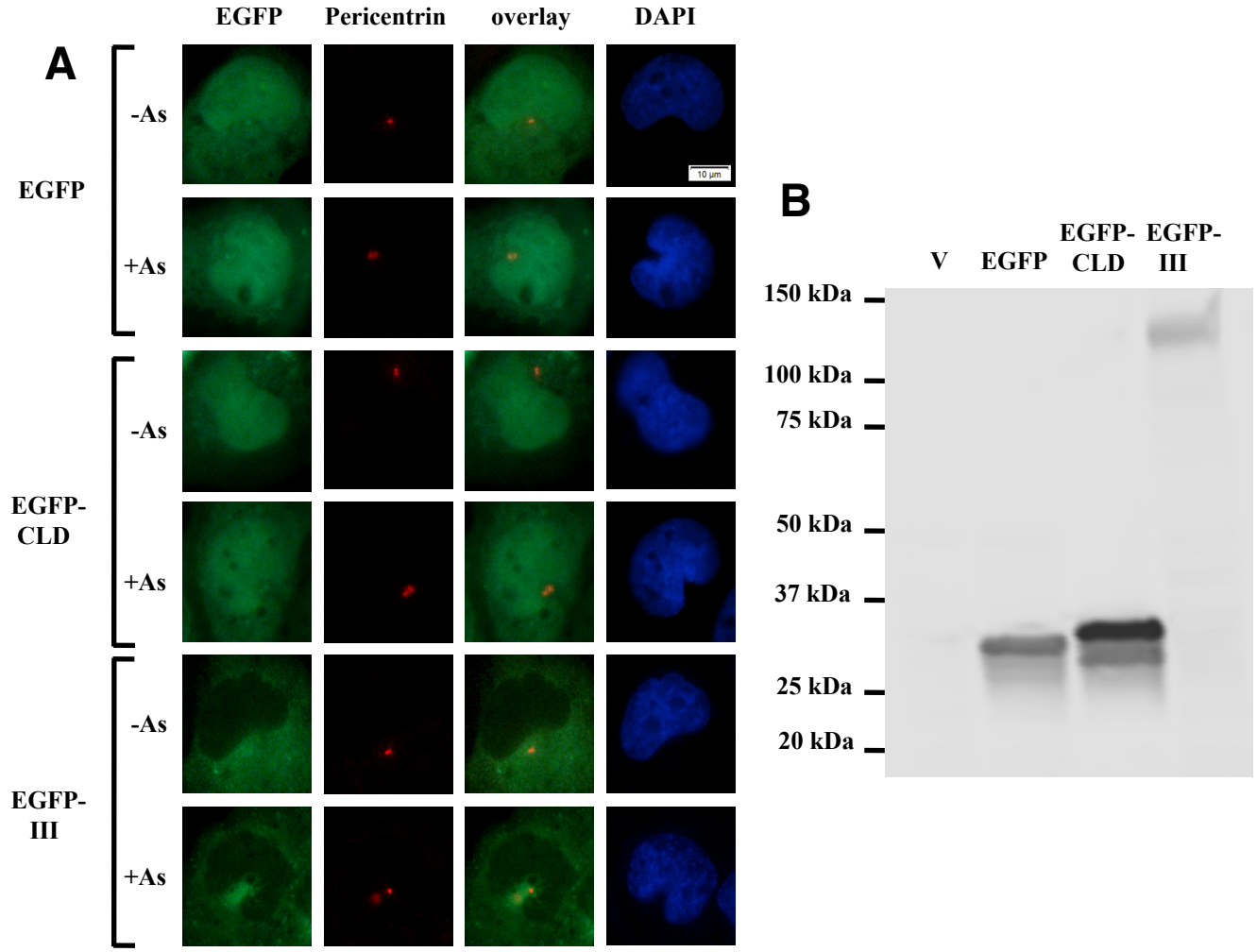

Fig. S6. The CLD does not bind centrosome. The EGFP-tagged CLD and Domain III along with EGFP were expressed in H1299 cells. The cells were treated with or without arsenite (0.5 mM, 30 min). They were stained for pericentrin and examined under fluorescence microscopy (A). Bar, 10 μm. The expression of the proteins was shown in (B).

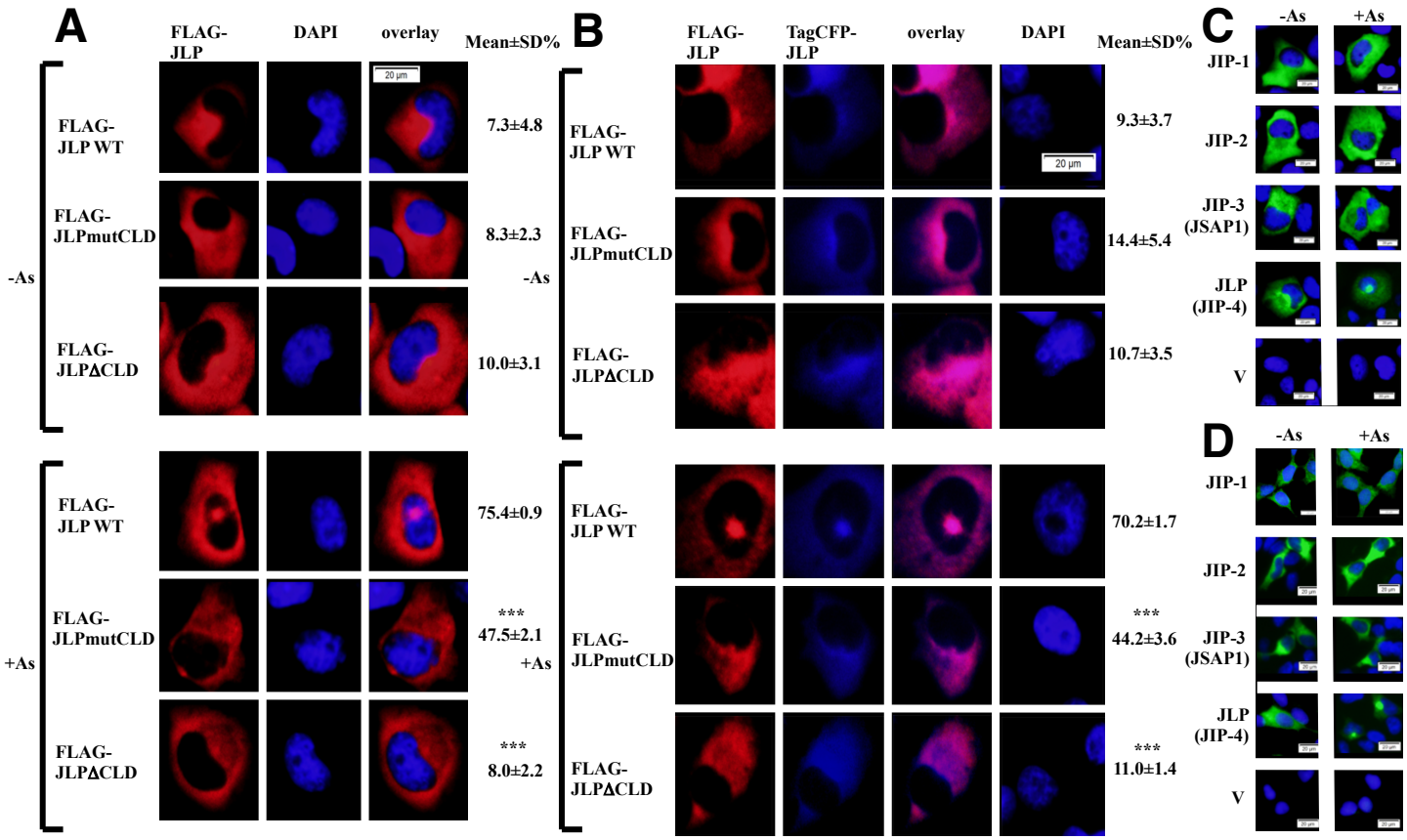

Fig. S7. The role of the CLD in the formation of JLP focus. (**A**) ΔCLD and mutCLD mutants of JLP have reduced ability to form JLP focus in H1299 cells. The percentages of cells showing the JLP foci were scored and presented as mean±SD % (n=3). ***, $P < 0.0001$ (as compared with arsenite-treated FLAG-JLP WT cells). (**B**) ΔCLD and mutCLD mutants of JLP inhibit the focus formation by TagCFP-JLP (wild-type) in H1299 cells. They were treated and examined as in (**A**). The percentages of cells showing the foci of TagCFP-JLP were scored and presented as mean±SD % (n=3). ***, $P < 0.005$ (as compared with arsenite-treated FLAG-JLP WT cells). Arsenite-induced formation of foci mediated by JLP in contrast to other JIP family members in the lung H1299 cells (**C**) or neuronal SHSY-5Y cells (**D**). Bar, 20 μm.

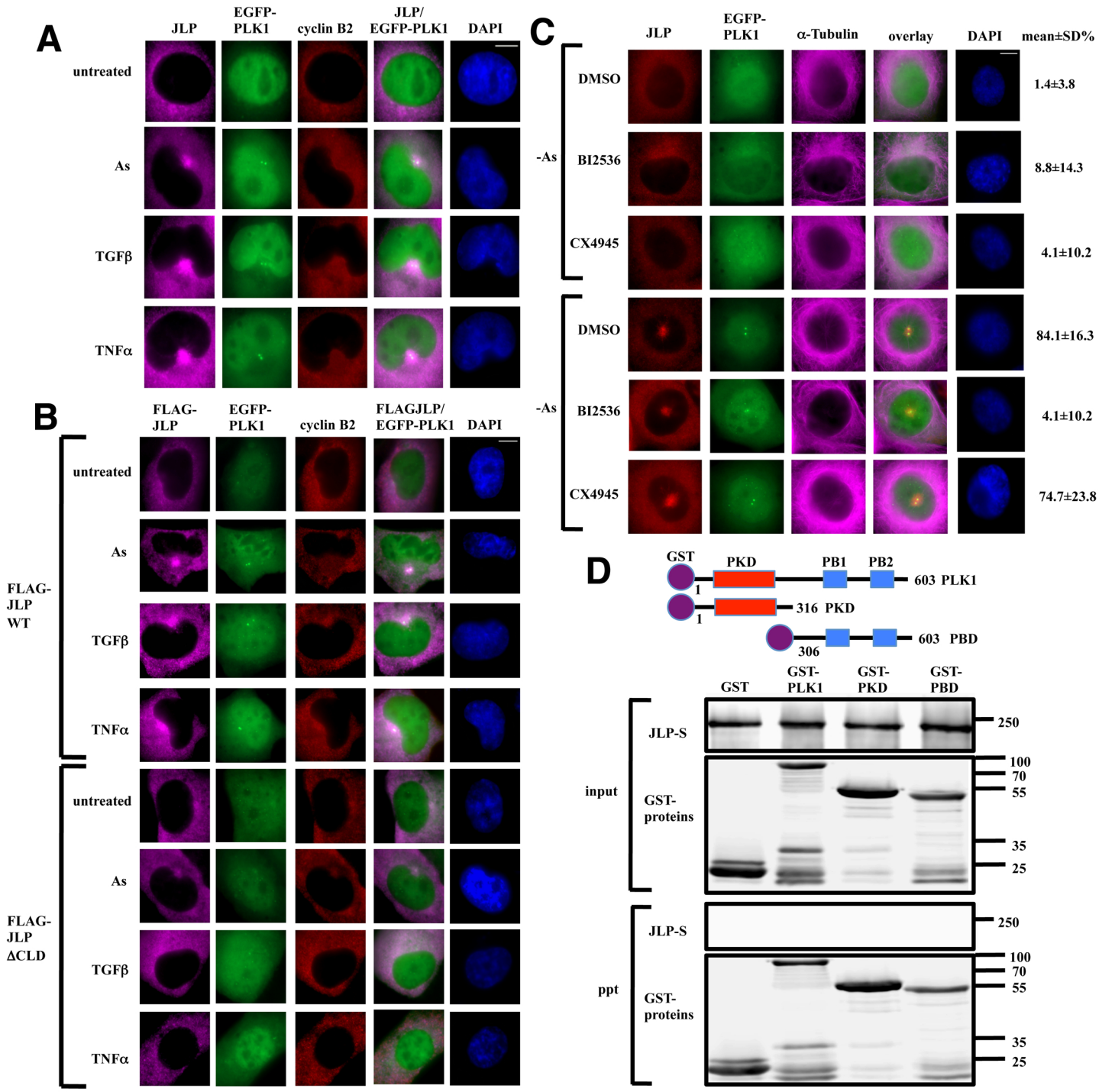

Fig. S8. PLK1 is a part of JLP focus. (**A**) Co-localization of endogenous PLK1 with JLP focus in H1299 cells after stimulation with arsenite (0.5 mM), TGFβ (7.5 ng/ ml) or TNFα (10 ng/ ml) for 25 min. (**B**) JLP tethers PLK1 to the focus after arsenite, TGFβ or TNFα treatment with exogenous FLAG-tagged wild-type (WT) or ΔCLD mutant of JLP together with EGFP-PLK1. Cells were synchronized by double thymidine block, transfected for the exogenous proteins and released from the block for 6 h. They were then examined by fluorescence microscopy. The cells in the G2 phase with the expression of cyclin B2 and intact nuclei were selected for examination in A and B. (**C**) The cells were treated similarly as in (**B**). They were released for 3 h and incubated with DMSO (vehicle), BI2536 (PLK1 inhibitor; 4 mM) or CX4945 (casein kinase II inhibitor as a negative control; 4 mM) for 3 h before they were treated with or without arsenite (0.5 mM) for 30 min. The percentages of cells showing the JLP foci and the MT arrays were scored and presented as mean±SD % (n=3). ***, $P < 0.005$ (compared with arsenite-treated control cells). (**D**) *In vitro* GST pull-down assays for the bacterial recombinant S-tagged JLP and GST-PLK1 proteins. Bar, 10 μm.

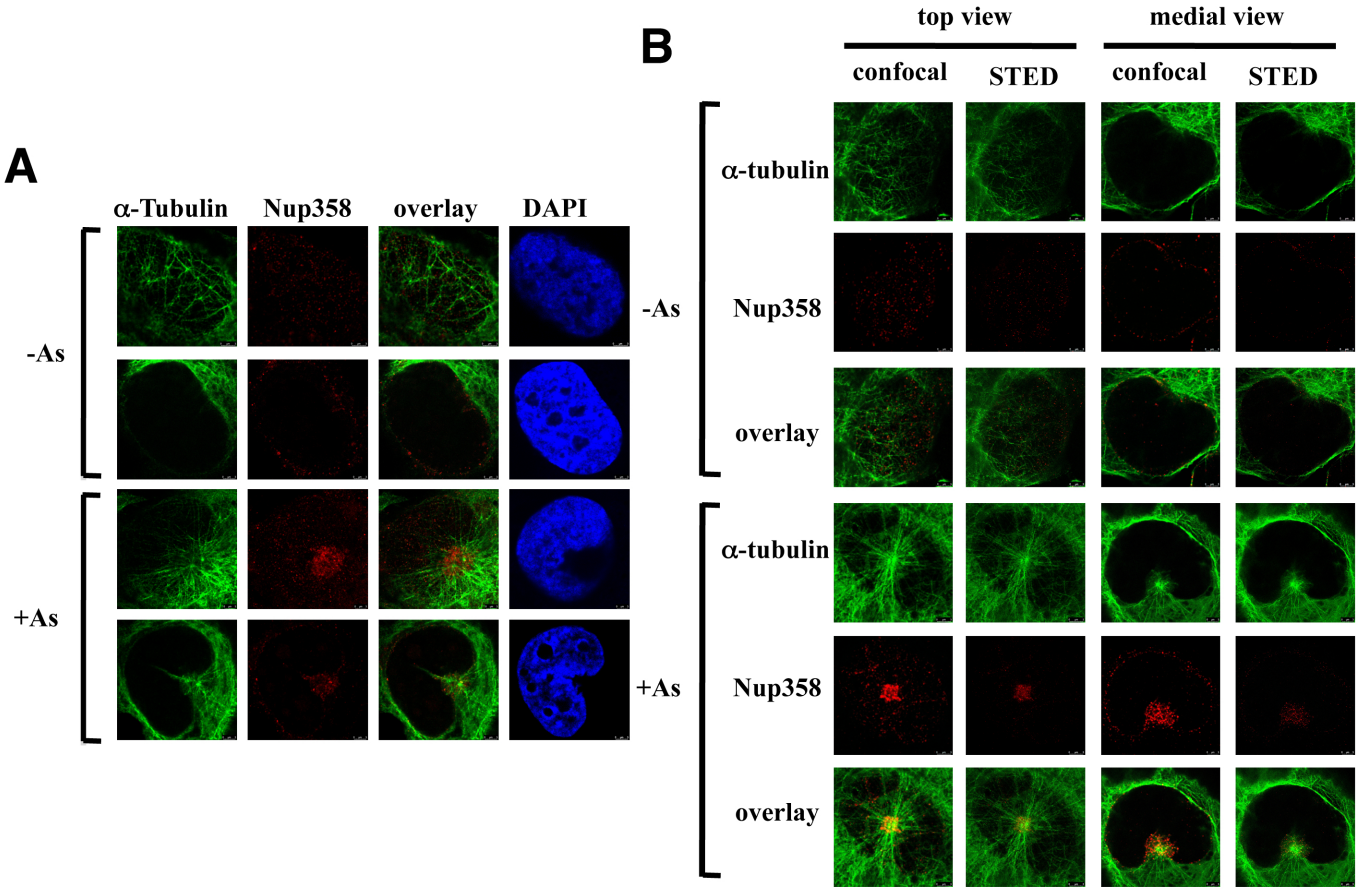

Fig. S9. JLP focus concentrates the nuclear pore protein Nup358. The top surface and medial views of the MT and nuclear pore protein Nup358 on the nuclear membrane were examined by confocal microscopy (**A**) or by high-resolution STED microscopy (**B**) in H1299 cells treated with or without arsenite (0.5 mM, 30 min). Bar; 3 μm.

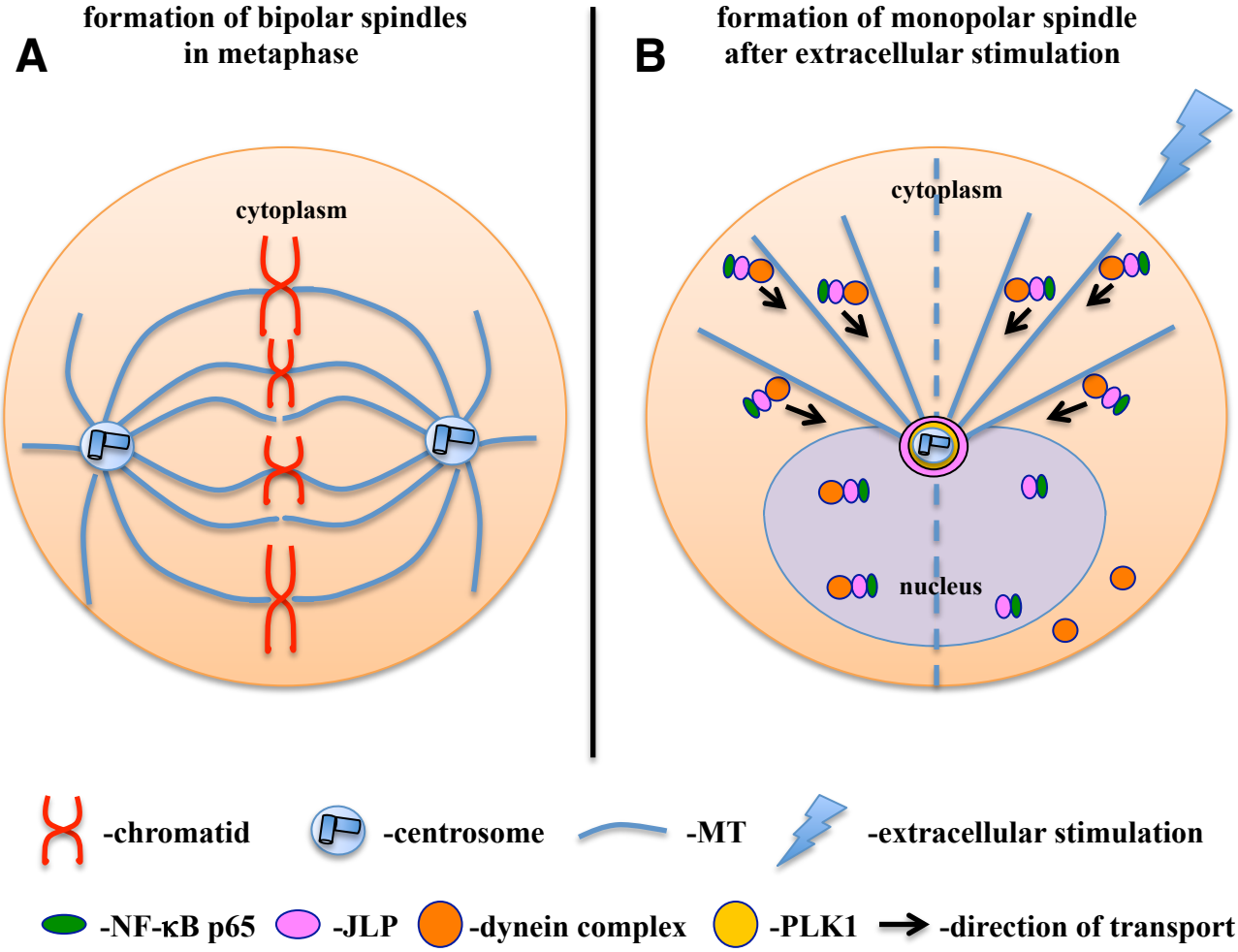

Fig. S10. Models for the formation of JLP focus and its role in nuclear translocation of NF-κB p65. (**A**) Formation of bipolar splindles in mitosis to separate chromatids. The role of JLP in the mitotic asters is undetermined. (**B**) Formation of JLP foci after extracellular stimulation. p38MAPK is activated to induce the formation of JLP focus. JLP tethers PLK1 to the centrosome leading to its maturation and formation of MT array. Here, JLP serves as a structural component of the mature centrosome. Only a monopolar spindle is formed in each cell. The NF-κB p65-JLP-dynein complexes, in which JLP serves as a scaffolding protein to tether NF-κB p65 and dynein, translocate from the cytoplasm to the nucleus membrane in the retrograde direction. The dynein-JLP-NF-κB p65 or JLP-NF-κB p65 complex passes through the nuclear pores to unload NF-κB in the nucleus and JLP (and dynein) is shuttled back to the cytoplasm. The concentration of the nuclear pores at the JLP focus contact surface may enhance the protein transport.