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Supplemental Material to:

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Nucleoporin Nup62 maintains centrosome homeostasis

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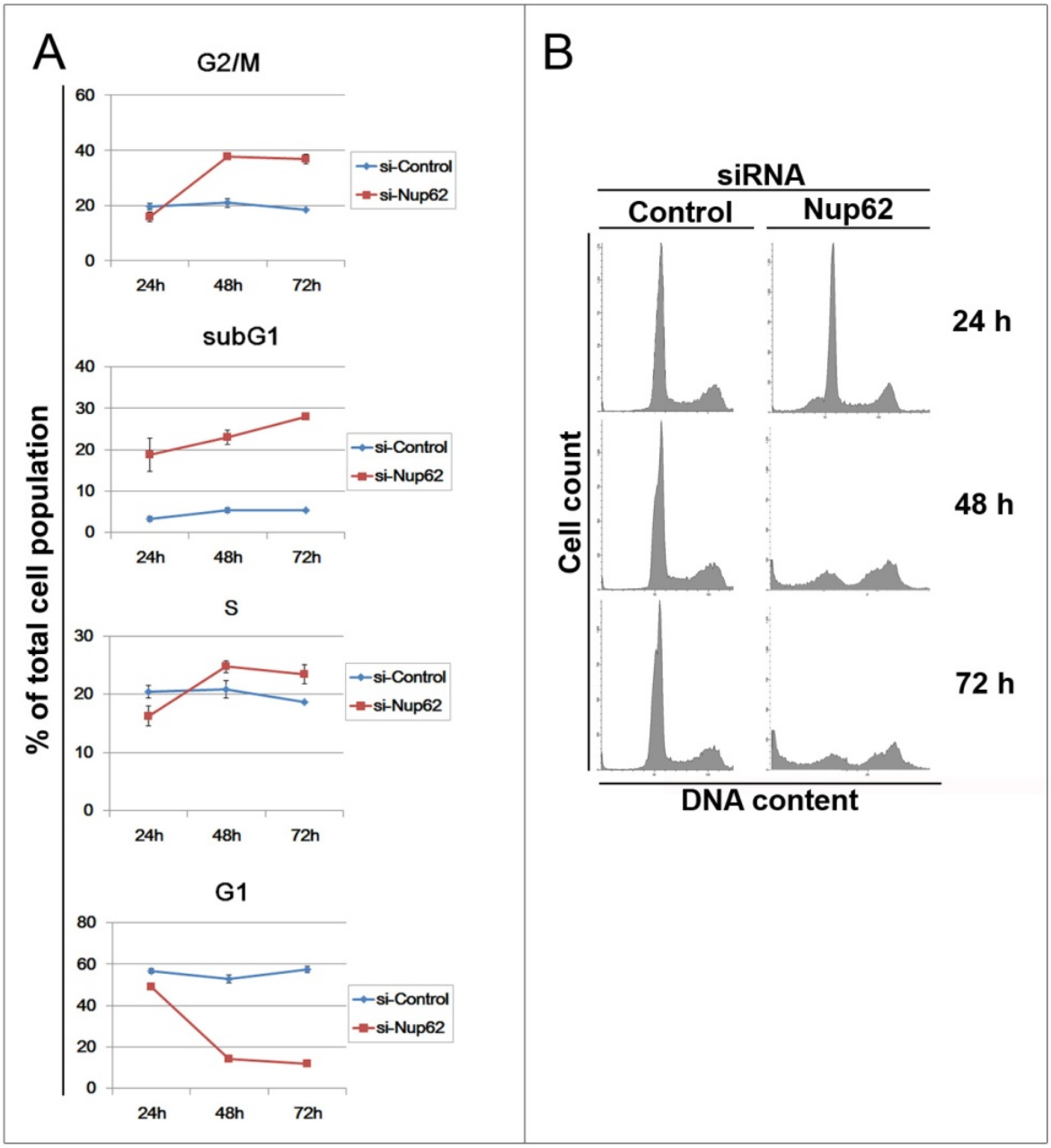
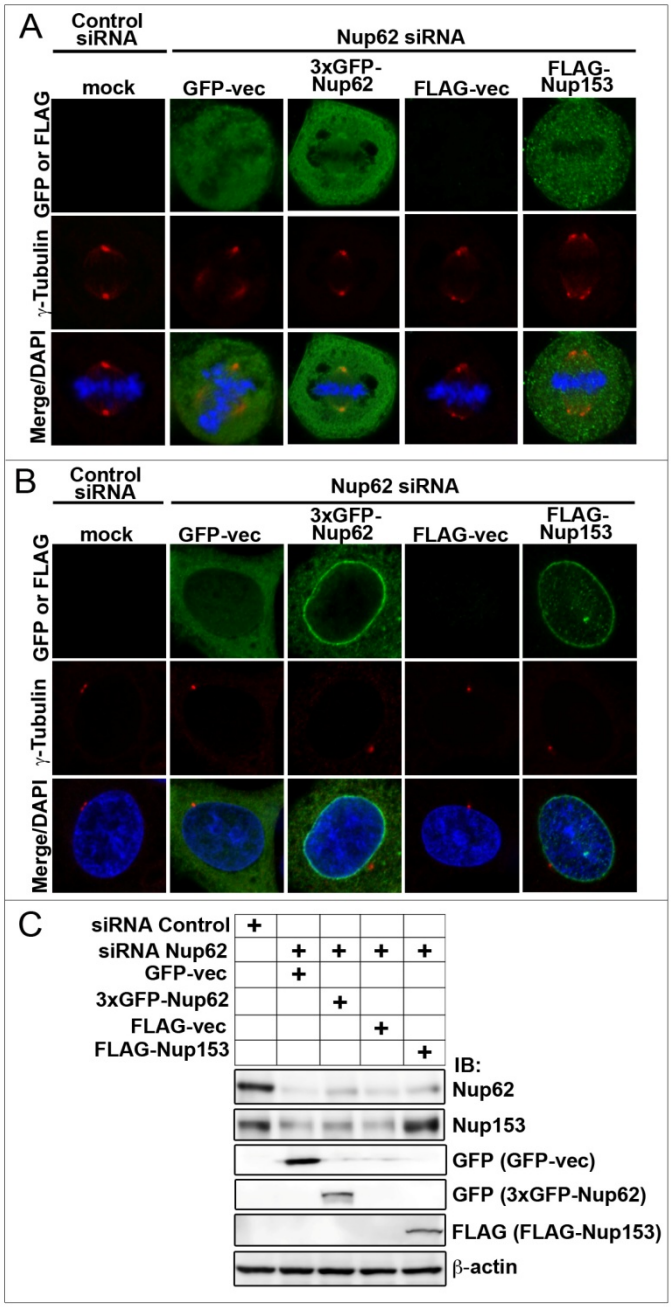


Supplemental Figure S1. Depletion of Nup62 induced G2/M mitotic arrest

A. Percentage of G1, G2/M, S, and Sub-G1 cells were calculated based on the results shown in Figure 1G.

B. Flow cytometric profiles of HeLa cells transfected with control or Nup62 siRNA. DNA was stained quantitatively with propidium iodide.

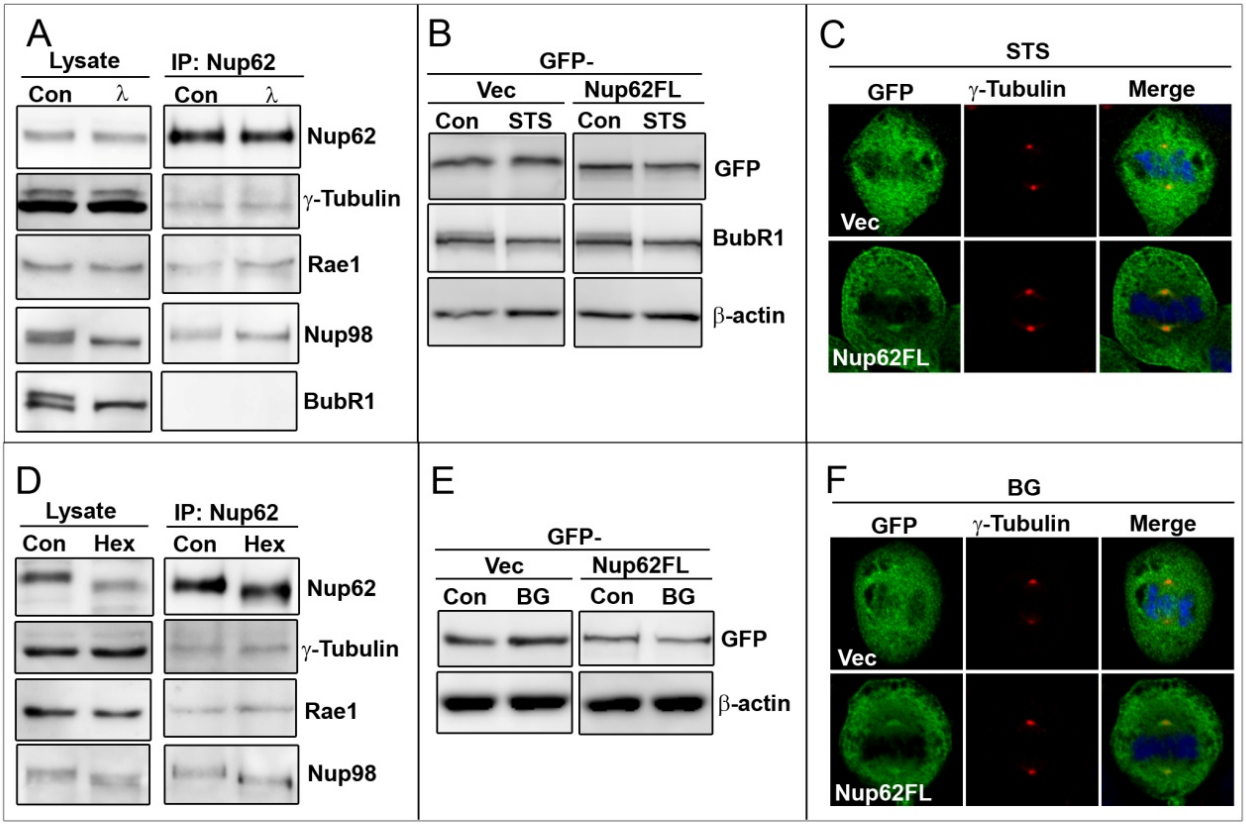


Supplemental Figure S2. Nup153 could not rescue Nup62 mitotic defects.

A. Representative images of mitotic HeLa cells, transfected with indicated siRNA and/or plasmid. Seventy-two hours after transfection, cells were stained with centrosome marker anti-GFP/FLAG (green), anti-γ-tubulin (red). All cells were treated with double thymidine block, stained with DAPI, and visualized by confocal microscopy. Scale bars, 5 μm.

B. Representative images of mitotic HeLa cells, transfected with indicated siRNA and/or plasmid. Seventy-two hours after transfection, cells were stained with centrosome marker anti-GFP/FLAG (green), anti-γ-tubulin (red), and analyzed by confocal laser microscopy. Chromatin was stained with DAPI (blue). Scale bars, 5 μm.

C. Immunoblotting of protein expression for quantification (relative percentage) of centrosomal defect phenotypes for the indicated siRNA and/or plasmid (6G).



Supplemental Figure 3

Neither phosphorylation nor O-glycosylation of Nup62 significantly altered its centrosome localization and expression during mitosis

A and D, Mitotic HeLa cell extract or IP with anti-Nup62 antibody were treated or untreated with λ protein phosphatase (PPase) (a common chemical used to release phosphate groups from phosphorylated serine, threonine and tyrosine residues in proteins) (A), or β-N-Acetyl-Hexosaminidase f (Hex) was used to remove O-GlcNAc (N-Acetylglucosaminidase on serine/threonine) (D). These lysates were analyzed by IB with the indicated antibodies.

B and E, HeLa cells transfected with GFP-vector or GFP-tagged full-length Nup62 plasmid

and treated with DMSO (Con), 10 μM of Staurosporine (STS) (B), 33 nM of Benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside (BG) (E), or 3 μM of MG-132 (MG) were analyzed by IB with the indicated antibodies.

C and F, Confocal microscopy images of HeLa cells transfected with GFP-vector or GFP-tagged full-length Nup62 plasmid were treated with DMSO (Con), 10 μM of STS (C), 33 nM of BG (F), or 3 μM of MG-132 (H) and stained with anti-GFP (green) or anti-γ-tubulin (red) antibodies.

Supplemental Table1. Plasmids construction details in this study.

Protein	Residues (aa)	Vector	Restriction sites 5', 3'	Tags N/C
Subclone constructs				
Nup62 (full length)	1–523	pJET1.2	bluntend	none/none
Nup153 (full length)	1–1476	pCMV6–XL4	from OriGene Technologies	none/none
Immunofluorescence microscopy constructs				
mNup62FL	1–526	p3xEGFP	from Euroscarf	none/3xGFP
Nup62N1	1–150	pEGFP–C3	XhoI, HindIII	GFP/none
Nup62N2	151–327	pEGFP–N1	XhoI, HindIII	none/GFP
Nup62C1	328–458	pEGFP–N1	XhoI, HindIII	none/GFP
Nup62C2	328–523	pEGFP–N1	XhoI, HindIII	none/GFP
Nup153 (full length)	1–1476	p3xFLAG–CMV	NotI, NotI	

Supplemental Table 2. Primer sequences in this study.

	5' Primer	3' Primer
Subcloning		
Nup62FL	GCTCGAGCTCATATGAGCGGGTTTAATTTT	CAAGCTTGTCAAAGGTGATCCGGAA
$Nup62N1_{1-150}$	GCTCGAGCATATGAGCGGGTTTAATTTTGGA	CAAGCTTAGCCACAGAGGTGGTGGA
$Nup62N2_{151-327}$	GCTCGAGCATATGCCAGCTACCACATCTGGA	CAAGCTTGGCGGAGCTGGCAGCCGC
$Nup62C1_{328-458}$	GCTCGAGCATATGACCTACGCGCAGCTGGAG	CAAGCTTCAGGTGCTCGATGATGTC
$Nup62C2_{328-523}$	GCTCGAGCATATGACCTACGCGCAGCTGGAG	CAAGCTTGTCAAAGGTGATCCGGAA