Inventory of Supplemental Information

Supplemental Material

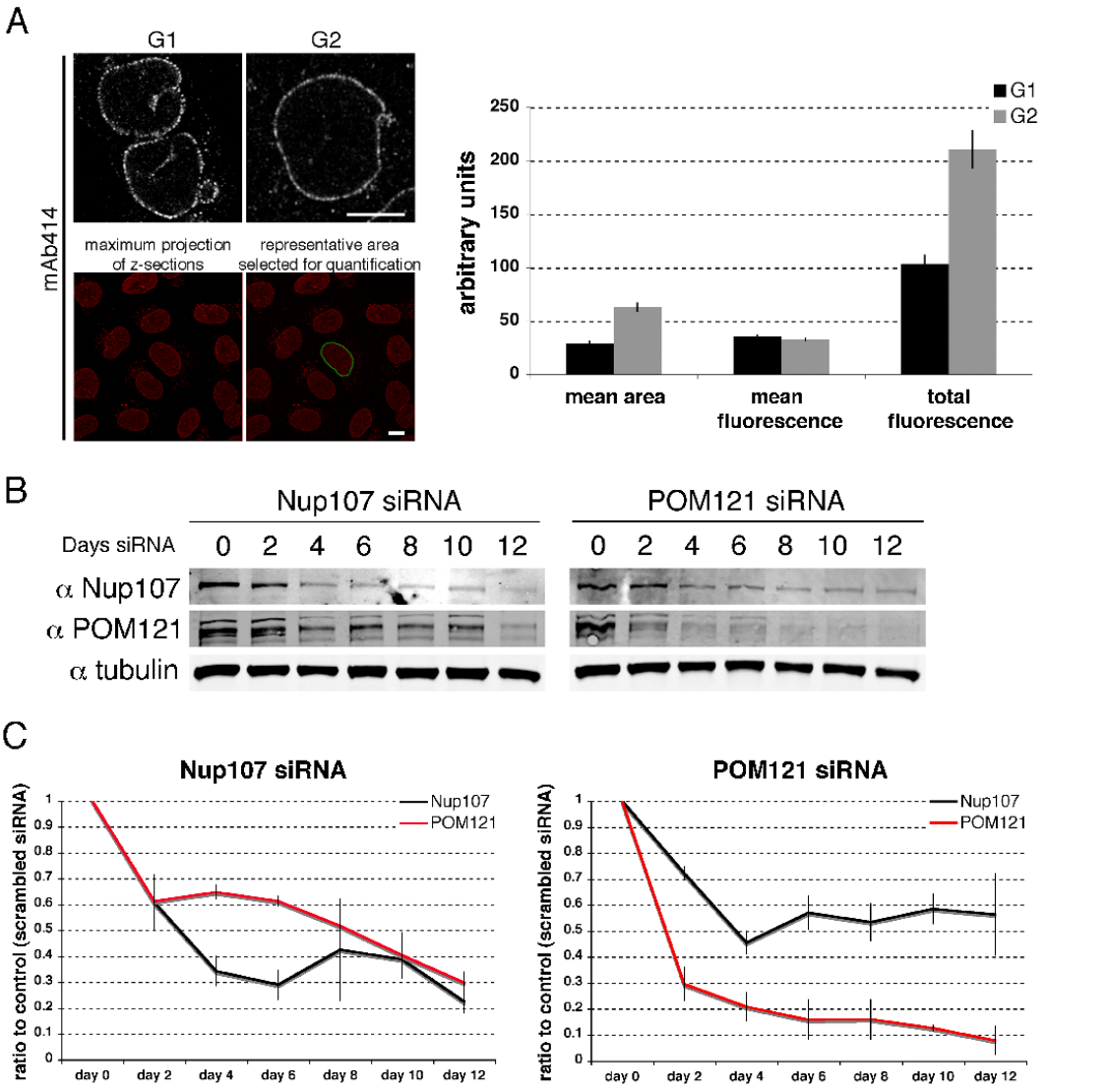


Figure S1 (A) Example and validation of measuring total fluorescence signal by measuring pixel intensity and area of maximum projections of z-stacks taken through nuclei in G1 and G2, N>28 nuclei per condition. Scale bars 10μm. (B) Western blot analysis of U2OS cell lysates harvested every 48 hrs during a 12 day time course of POM121 or Nup107 siRNA treatment. (C) Quantification of protein levels in western blots against Nup107 and POM121 graphed as a ratio to control levels. N=3 independent Western blots. Error bars are standard error. Data is supplemental to Figure 1.

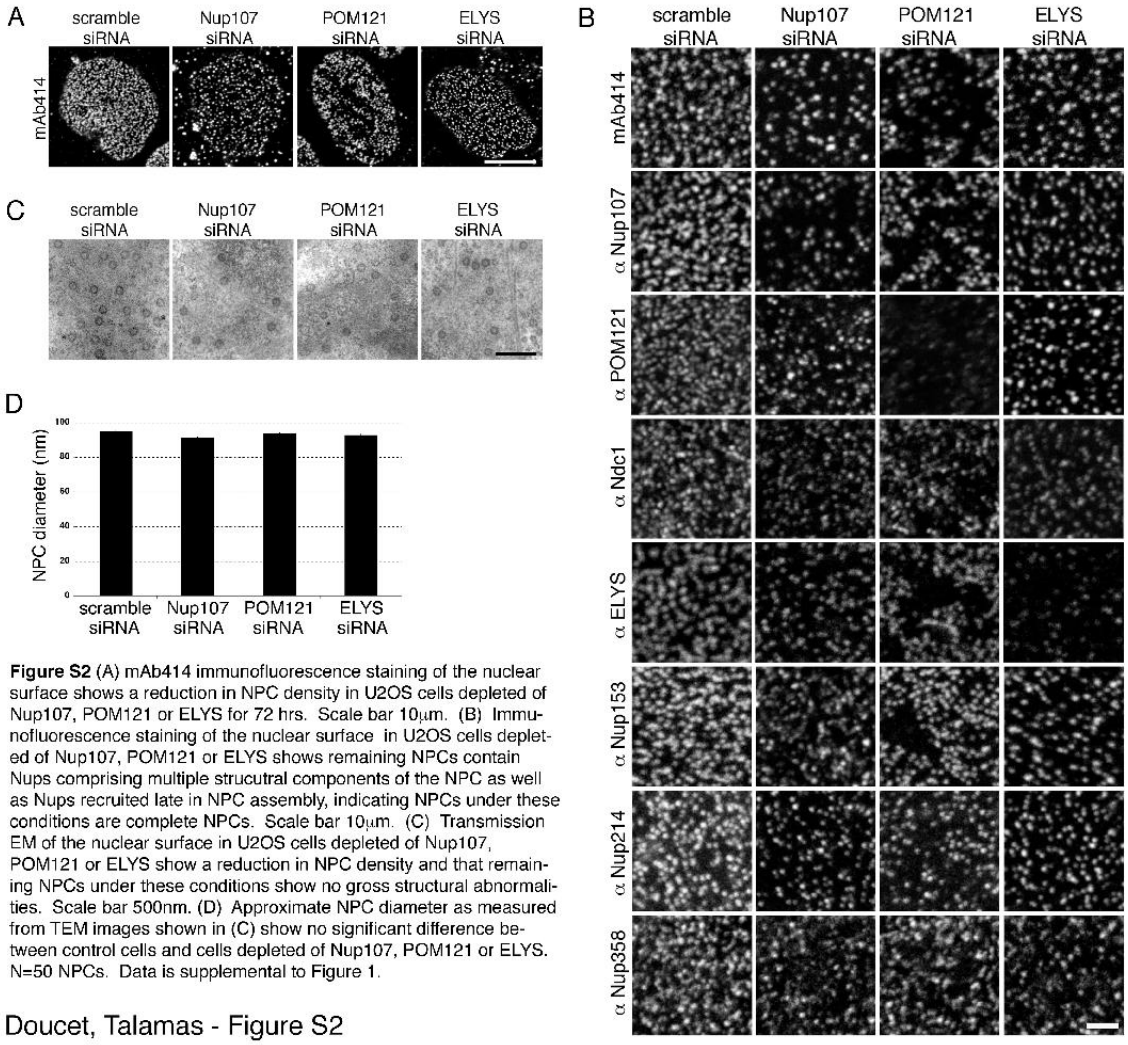


Figure S2 (A) mAb414 immunofluorescence staining of the nuclear surface shows a reduction in NPC density in U2OS cells depleted of Nup107, POM121 or ELYS for 72 hrs. Scale bar 10μm. (B) Immunofluorescence staining of the nuclear surface in U2OS cells depleted of Nup107, POM121 or ELYS shows remaining NPCs contain Nups comprising multiple strucutral components of the NPC as well as Nups recruited late in NPC assembly, indicating NPCs under these conditions are complete NPCs. Scale bar 10μm. (C) Transmission EM of the nuclear surface in U2OS cells depleted of Nup107, POM121 or ELYS show a reduction in NPC density and that remaining NPCs under these conditions show no gross structural abnormalities. Scale bar 500nm. (D) Approximate NPC diameter as measured from TEM images shown in (C) show no significant difference between control cells and cells depleted of Nup107, POM121 or ELYS. N=50 NPCs. Data is supplemental to Figure 1.

Doucet, Talamas - Figure S2

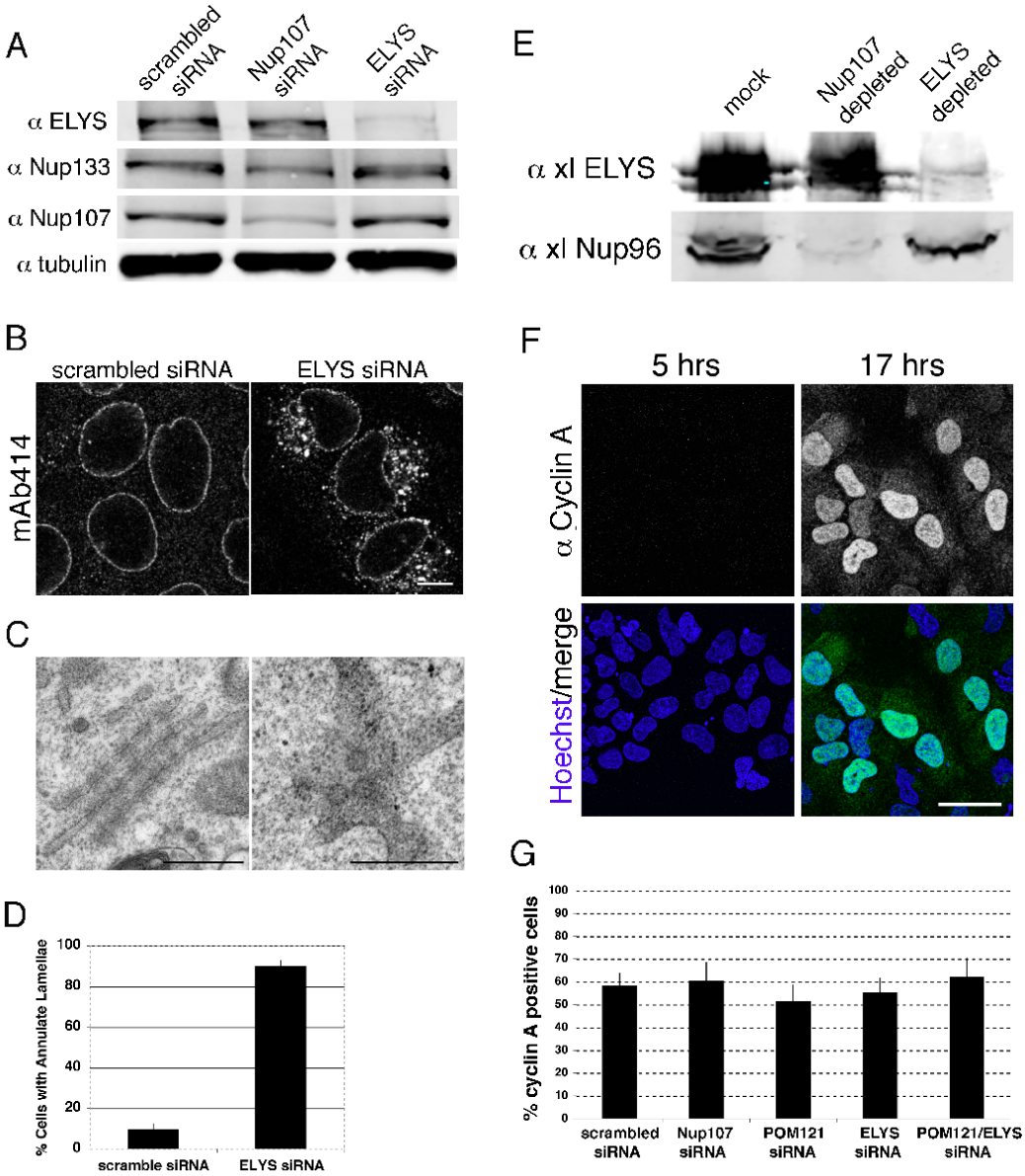
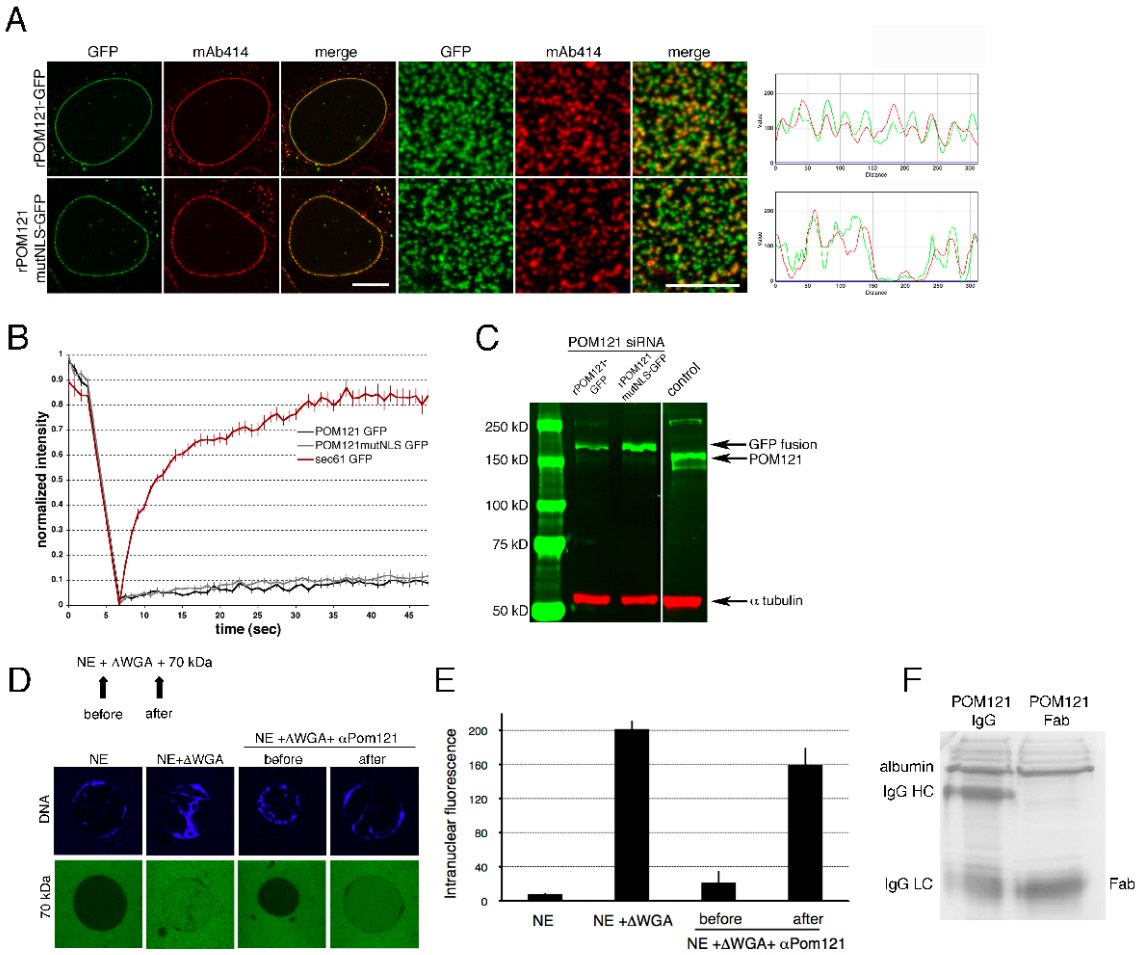


Figure S3 (A) Western blot analysis of lysates from cells treated with control, Nup107 or ELYS specific siRNA oligos. (B) Immunostaining with mAb414 shows a mislocalization of Nups in U2OS cells treated with ELYS specific siRNA oligos. Scale bar 5μm (C) Transmission electron microscopy shows annulate lamellae in U2OS cells treated with ELYS specific siRNA oligos. Scale bars 500 nm. (D) Quantification of cells containing cytoplasmic mAb414 signal in cells treated with control or ELYS specific siRNA oligos. N>160 cells (E) Western blot analysis of immunodepleted xenopus cytosol shows specific depletion of either the Nup107 complex or ELYS (F) U2OS cells immunostained with an α-cyclin A antibody 5 and 17 hours post nocodazole release indicates cells are progressing from phase G1 to G2 of the cell cycle. Scale bar 20 μm. (G) Quantification of cells staining positive for cyclin A under indicated siRNA treatments. N=5 fields. Error bars are standard error. Data is supplemental to Figure 2.



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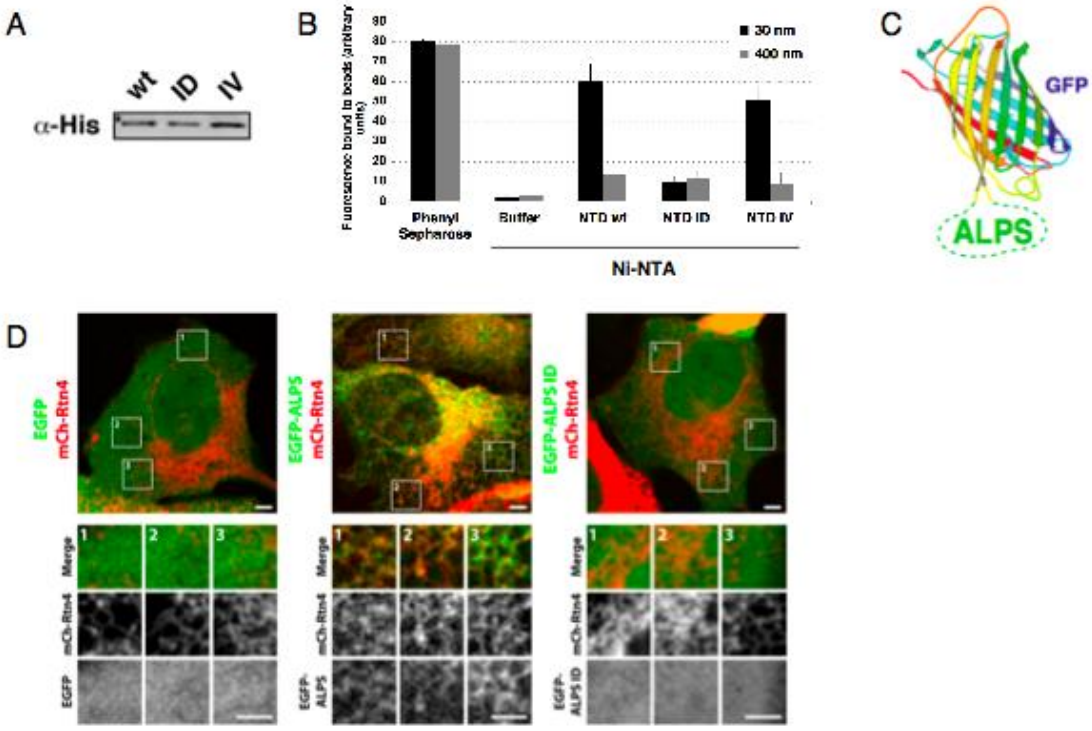

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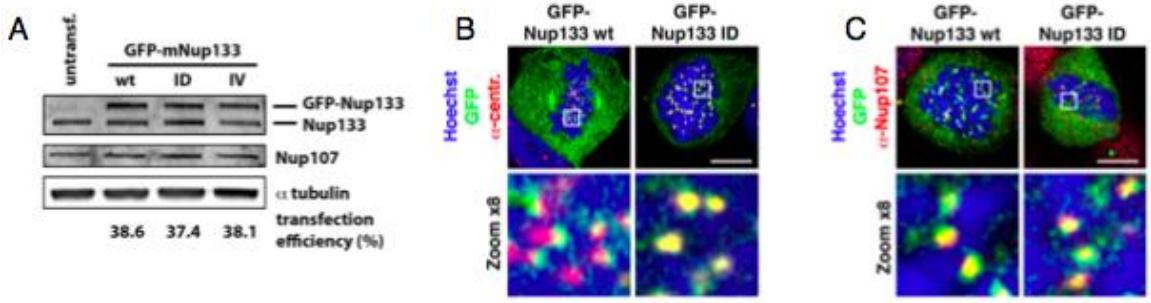


Figure S6 (A) Western blot analysis of U2OS cells transfected with GFP-mNup133 wt, GFP-mNup133 ID or GFP-mNup133 IV for 48h. Untransfected cells were used as a control. The transfection efficiency of each construct is indicated below corresponding lanes.
(B-C) Immunofluorescence staining using Nup107 or centromere specific antibodies in mitotic U2OS cells transfected with either GFP-mNup133 wt or GFP-mNup133 ID. Cells were fixed and stained with Hoechst (blue) 48 hrs post transfection. Scale bars 10 μm.
This data is supplemental to Figure 6.

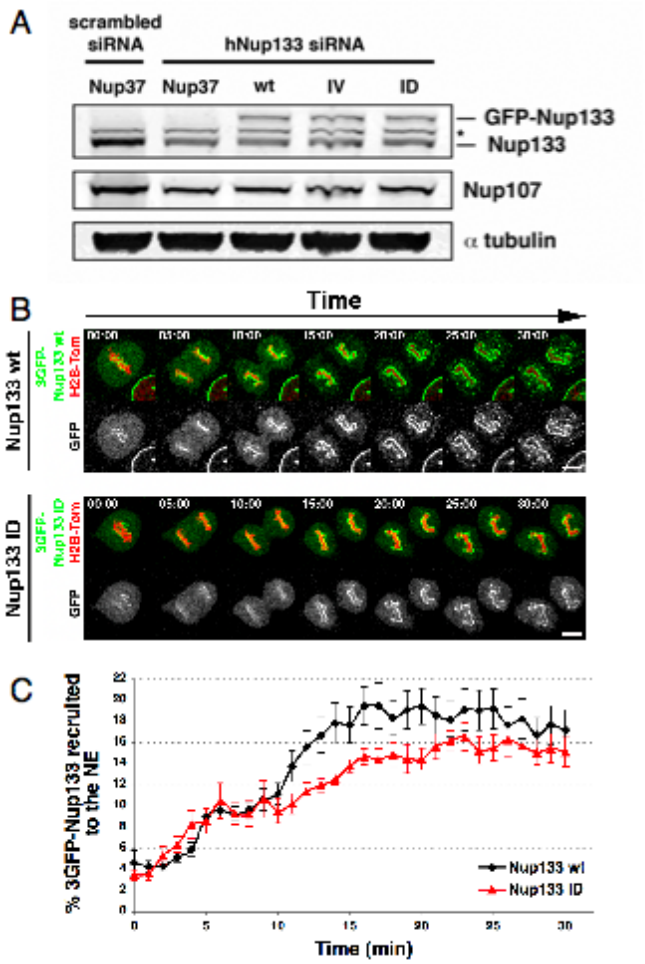


Figure S7 (A) U2OS cells were co-transfected with either control or hNup133 specific siRNA oligos and GFP-hNup37; or the hNup133 specific siRNA oligo and either GFP-mNup133 wt, GFP-mNup133 IV or GFP-mNup133 ID. Cell lysates were analyzed by Western blotting using antibodies against Nup133, Nup107 and α tubulin. * indicates a non-specific band recognized by the Nup133 antibody.
(B) U2OS cells were transfected with a hNup133 specific siRNA oligo and H2B-tdTomato together with either 3GFP-mNup133 wt or 3GFP-mNup133 ID. Cells were imaged from metaphase (condensed and aligned chromatin) for 2 hours at the rate of 1 frame per minute; the onset of chromosome segregation marks t=0. Scale bar 5 μm.
(C) Quantification of GFP signal around chromatin over time, representing the Nup107/160 complex, to the reforming NE; n > 12 cells per condition. Error bars represent standard error.
This data is supplemental to Figure 7.

Table S1 : DNA constructs

Name	Source	Entry vector	Destination vector
rPOM121-GFP	rPOM121,cDNA library	pDONR207	pcDNA6.2 DEST47
rPOM121NLS-3GFP (aa 292-317)	rPOM121	pDONR207 2GFP (modified)	pcDNA6.2 DEST53
rPOM121 (aa 60-1199) - GFP	rPOM121	pDONR207	pcDNA6.2 DEST47
NES-tdTomato-NLS			
Sec61-GFP	hSec61β (aa 1-65)	pDONR207	pcDNA6.2 DEST47
mCherry-Rtn4	hRtn4	pDONR207	pcDNA6.2 DEST53 where GFP has been replaced by mCherry (gift from Clodagh O'Shea Lab)
GFP-mNup133	mNup133, Open Biosystems image clone #4235408	pDONR207	pcDNA6.2 DEST53
NTD mNup133 (aa 67-477)	mNup133	pDONR207	p9His-GW
GFP-Nup37	hNup37	pDONR207	pcDNA6.2 DEST53
3GFP-mNup133	mNup133	pDONR207 2GFP (modified)	pcDNA6.2 DEST53

Table S2: Antibodies

Antibodies generated by Hetzer lab						
Protein	**Antigen used**	**Host**	**Vector**	**Protein purification method**	**Dilution for WB**	**Dilution for IHC**
xPOM121	aa 254-618	Rabbit	pDEST15	Glutathione agarose (Sigma-Aldrich)	1:1000	1:500
hNdc1	aa 296-485	Guinea Pig	pDEST15	Glutathione agarose (Sigma-Aldrich)	N/A	1:200
mNup153	aa 1-198	Rabbit	pDEST15	Glutathione agarose (Sigma-Aldrich)	N/A	1:200
xNup107	aa 76-171	Rabbit	pDEST15	Glutathione agarose (Sigma-Aldrich)	immunodepletion	
xNup96	aa 1-150	Rabbit	pDEST15	Glutathione agarose (Sigma-Aldrich)	1:1000	N/A
mNup107	aa 600-926	Rabbit	pHis9-GW	Ni-NTA (Qiagen)	Affinity purified 1:1000	Affinity purified 1:1000
hNup133	aa 67-478	Rabbit	pHis9-GW	Ni-NTA (Qiagen)	1:200	N/A
Gifts and commercially available antibodies						
Protein	**Host**	**Lab / Vendor**			**Dilution for WB**	**Dilution for IHC**
Cyclin A	Rabbit	Tony Hunter Lab			N/A	1:200
hELYS	Rabbit	Iain Mattaj Lab			1:2000	1:1000
xELYS	Rabbit	Iain Mattaj Lab			1:2000	1:1000
xPOM121	Rabbit	Iain Mattaj Lab (Antonin et al, 2005)			Inhibitory antibody	
Control IgG	Rabbit	Santa Cruz			Immunodepletion / inhibition	
Lamin A	Rabbit	Sigma-Aldrich			1:750	
α-tubulin	Mouse	Sigma-Aldrich			1:5000	1:1000
6His epitope	Mouse	Sigma-Aldrich			1:5000	N/A
Centromere protein	Human	Antibodies Inc			N/A	1:2000
NPC (mAb414)	Mouse	Covance			1:5000	1:1000

Table S3: siRNA oligo sequences

Target	Sequence
unspecific (scramble)	UAGAUACCAUGCACAAUCCdTdT
hELYS	UCGUGGAAAGUUUGCUGCAGGGAAAdTdT
hPOM121	CAGUGGCAGUGGACAUUCAdTdT
hNup107	CUGCGAAUACACUUUCUUCdTdT
hNup96	GCACAAAUUGUGAAGCACUdTdT
hNup133	GAGUGCCGACUUAGUGGCUCUUUCUdTdT

Table S4: buffers

Buffer	Composition
Hypotonic buffer	10 mM KOAc 20 mM Tris Acetate, pH7.5 0.5 mM DTT 1.5 mM $MgCl_2$
Denaturing buffer	60 mM Tris HCl, pH7.5 2% SDS 10% glycerol
S250	250 mM Sucrose 50 mM KCl 2.5 mM $MgCl_2$ 10 mM HEPES pH7.5

SUPPLEMENTAL MATERIAL AND METHODS

Electron Microscopy

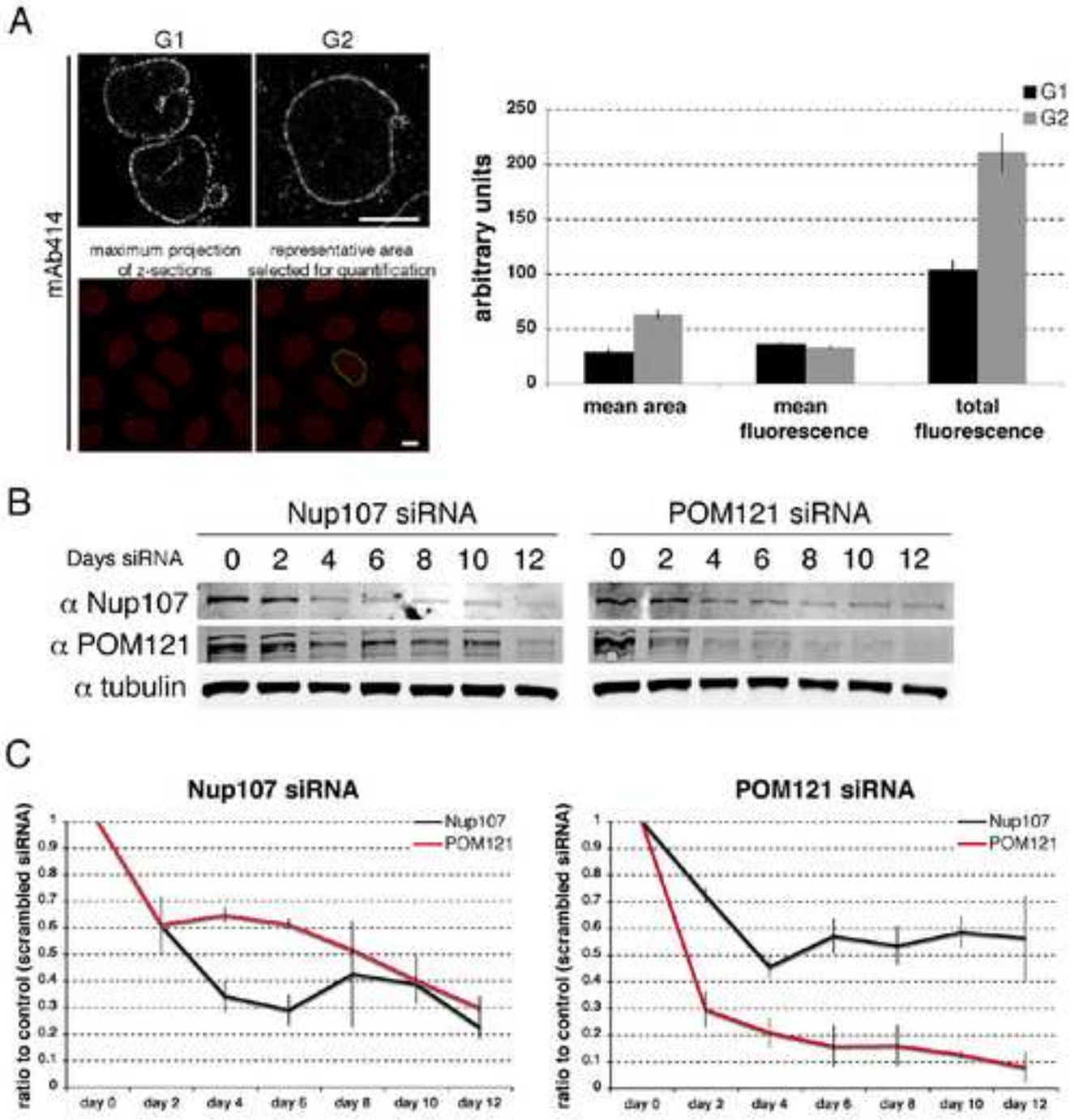
Cells grown in 35mm plastic culture dishes were fixed in 2.5% glutaraldehyde in 0.1M Na cacodylate buffer (pH7.3), washed and fixed in 1% osmium tetroxide in 0.1M Na cacodylate buffer. They were subsequently treated with 0.5% tannic acid followed by 1% sodium sulfate in cacodylate buffer and then dehydrated in graded ethanol series. The cells were cleared in HPMA (2-hydroxypropyl methacrylate: Ladd Research, Williston VT) and embedded in LX112 resin. Following overnight polymerization at 60˚C, small pieces of resin were attached to blank blocks using SuperGlue. Thin sections (70nm) were cut on a Reichert Ultracut E (Leica, Deerfield, IL) using a diamond knife (Diatome, Electron Microscopy Sciences, Hatfield PA), mounted on parlodion coated, copper, slot grids and stained in uranyl acetate and lead citrate. Sections were examined on a Philips CM100 TEM (FEI, Hillsbrough, OR) and data documented on Kodak SO-163 film for later analysis. Alternatively the samples were documented on a SIS Megaview III CCD camera (SIS, Lakewood CO). Negatives were scanned at 600 lpi using a Fuji FineScan 2750xl (Hemel Hempstead, Herts., UK) and converted to tif format for subsequent handling in Adobe Photoshop.

FRAP

Fluorescence recovery after photobleaching (FRAP) was performed on sections of the nuclear rim in U2OS cells transiently expressing GFP fusion proteins using a scanning confocal microscope (DMRIE2; Leica) described above.

FRAP parameters used were: 4 pre-bleach scans, 4 bleaching scans followed by 50 post-bleach scans taken at 0.834 fps. Images and quantitative data were acquired using the Leica Confocal Software (LCS) v1.5. The data were analyzed and presented graphically in Microsoft Excel.

Supplemental Figure1



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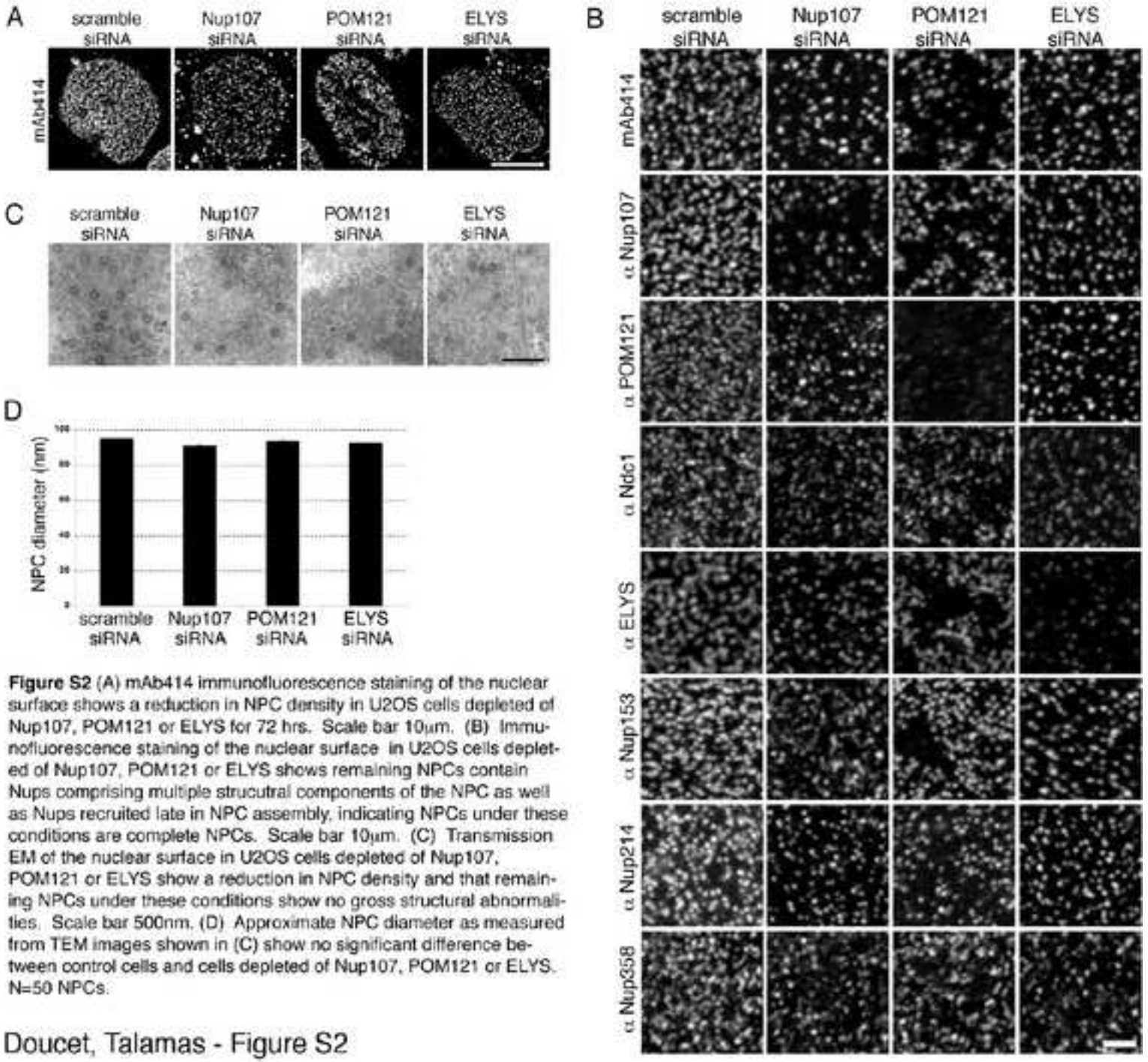

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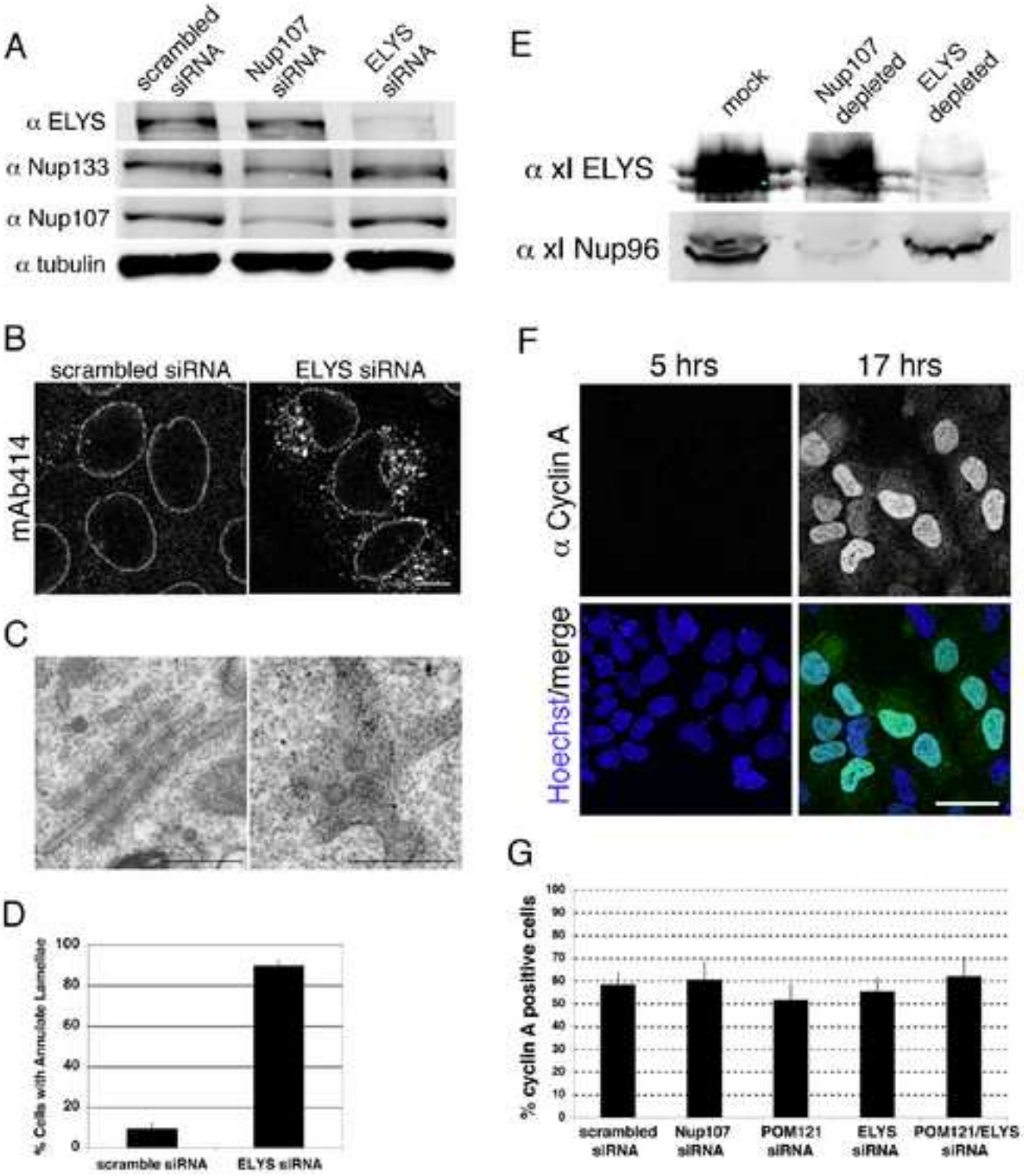
Doucet, Talamas - Figure S2

Supplemental Figure3



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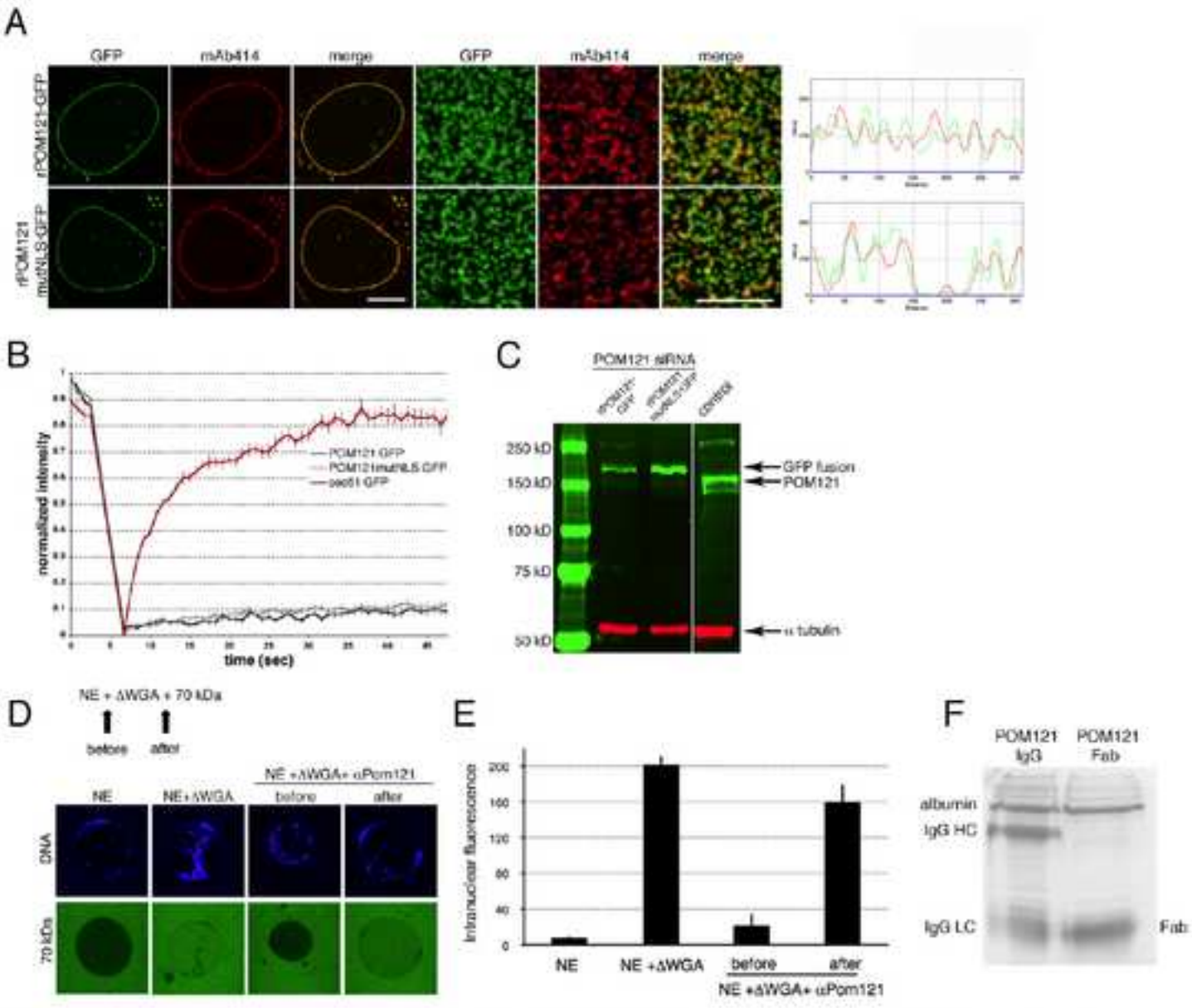

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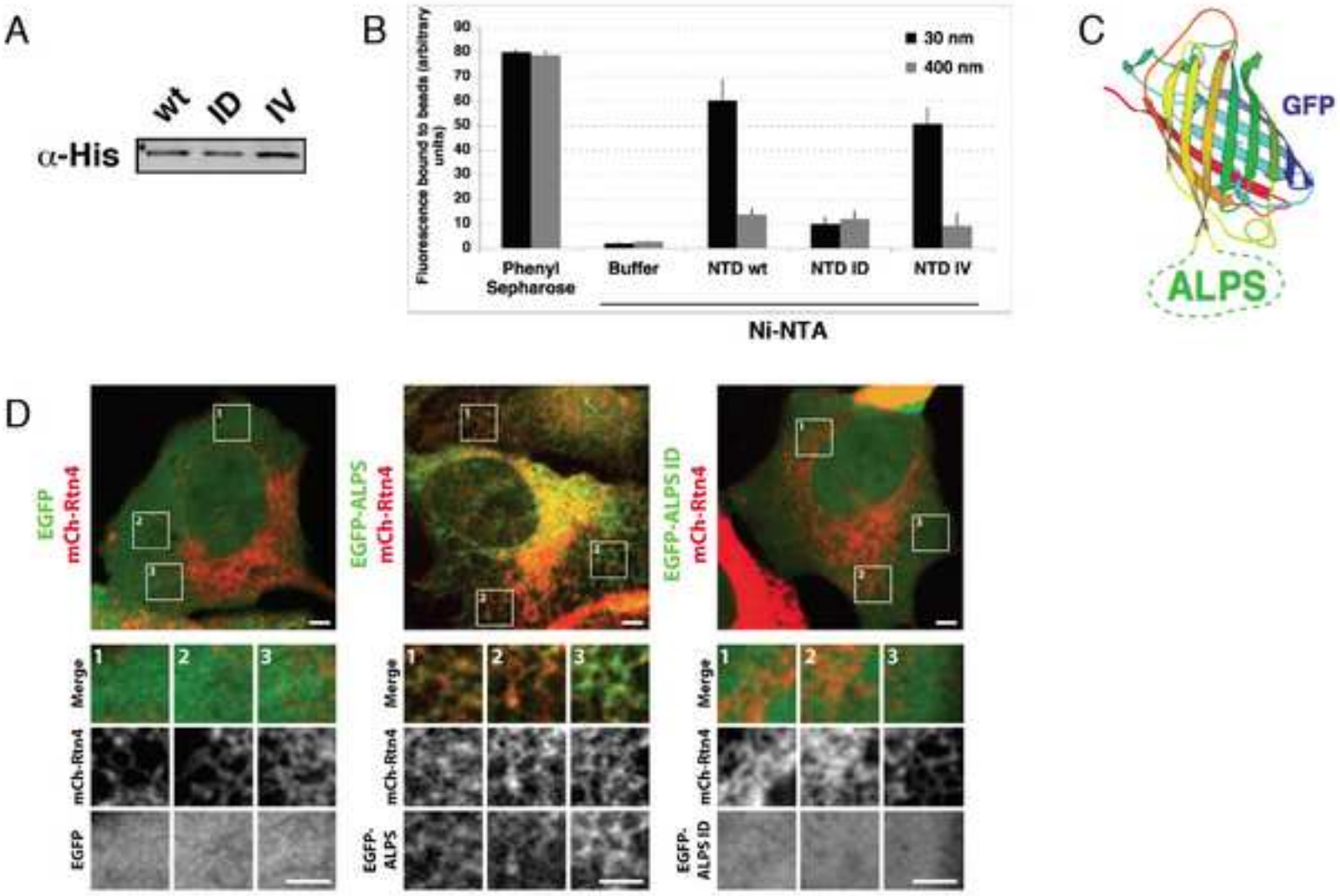
Supplemental Figure5



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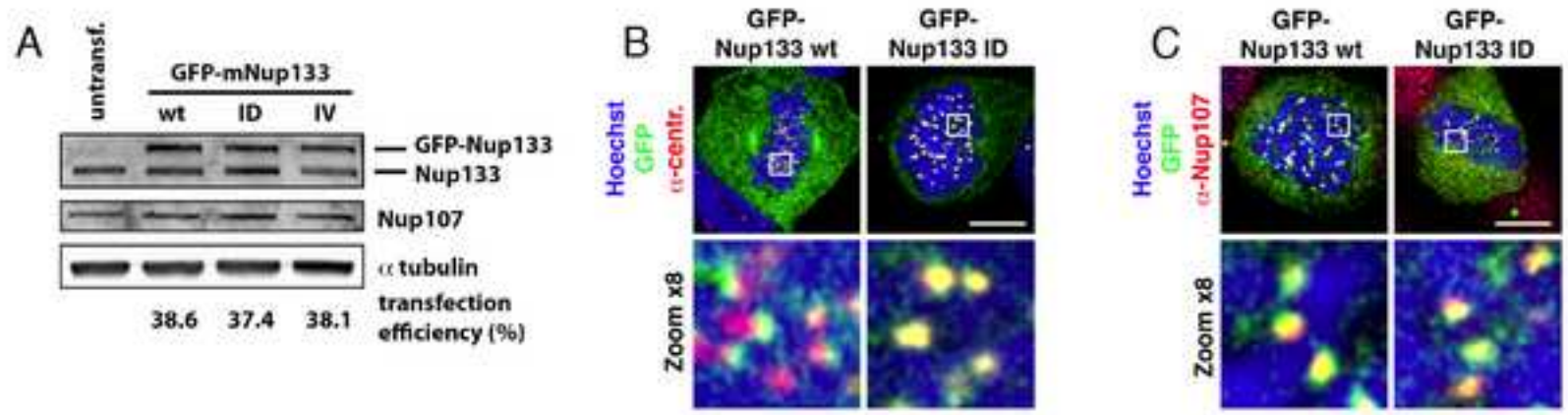
Supplemental Figure6



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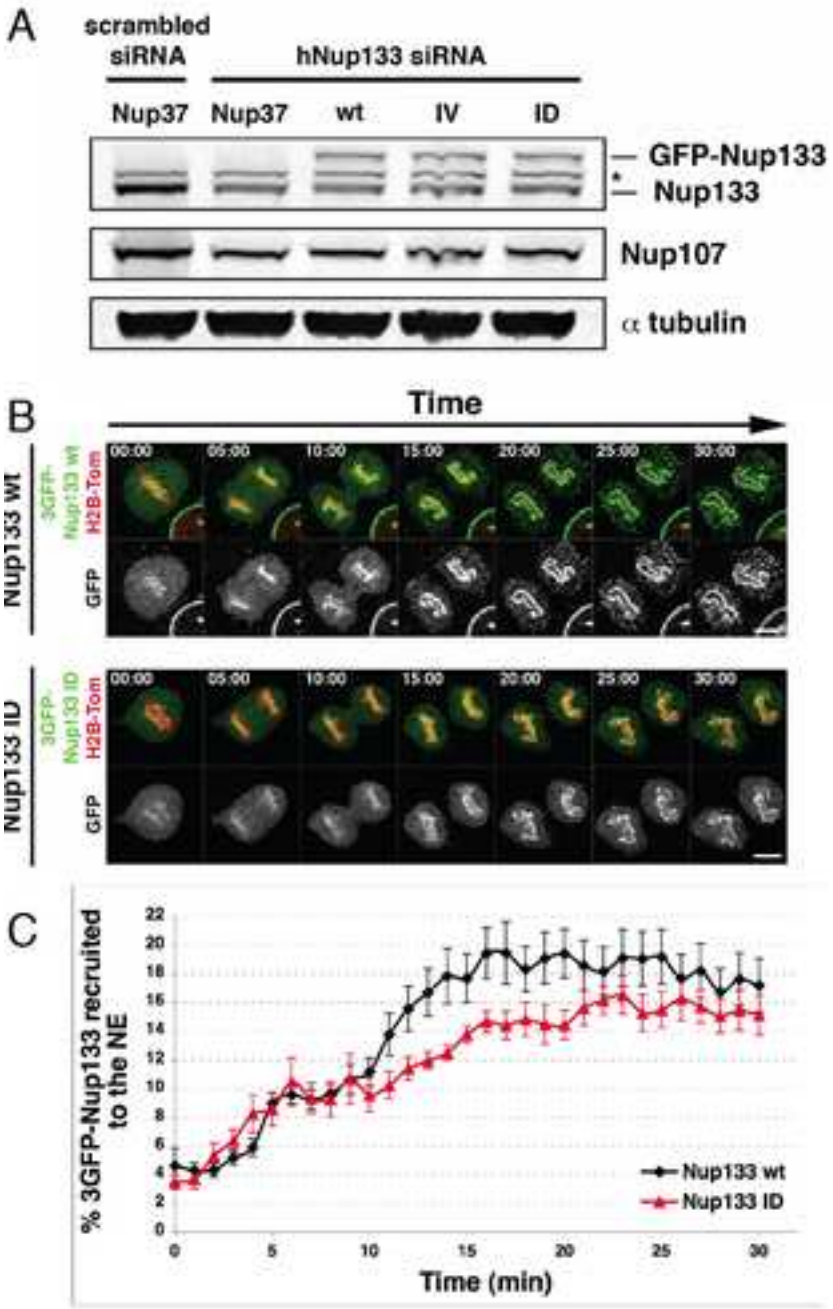
Doucet, Talamas - Figure S6



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(C) Quantification of GFP signal around chromatin over time, representing the Nup107/160 complex, to the reforming NE; n > 12 cells per condition. Error bars represent standard error.

Doucet, Talamas - Figure S7