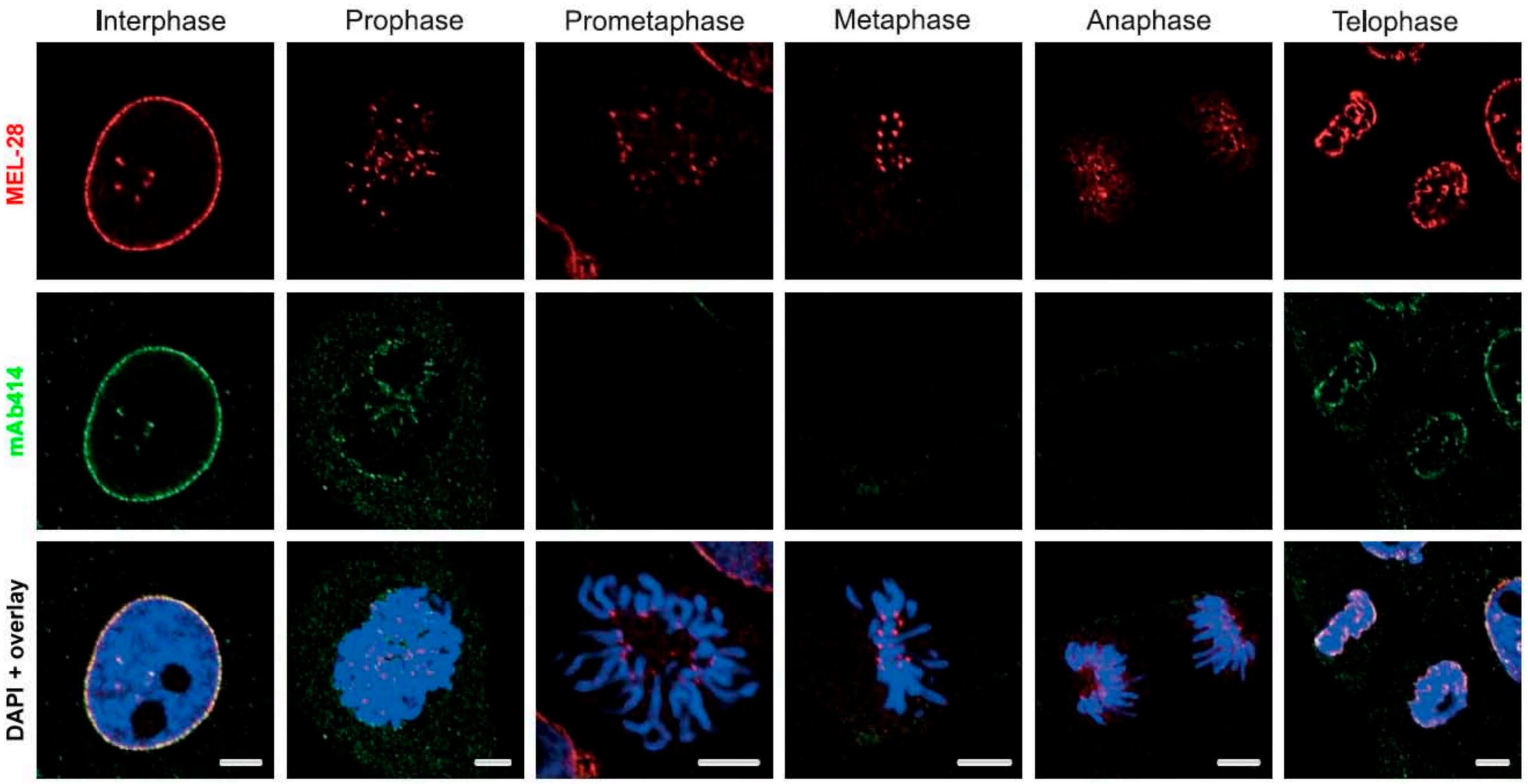
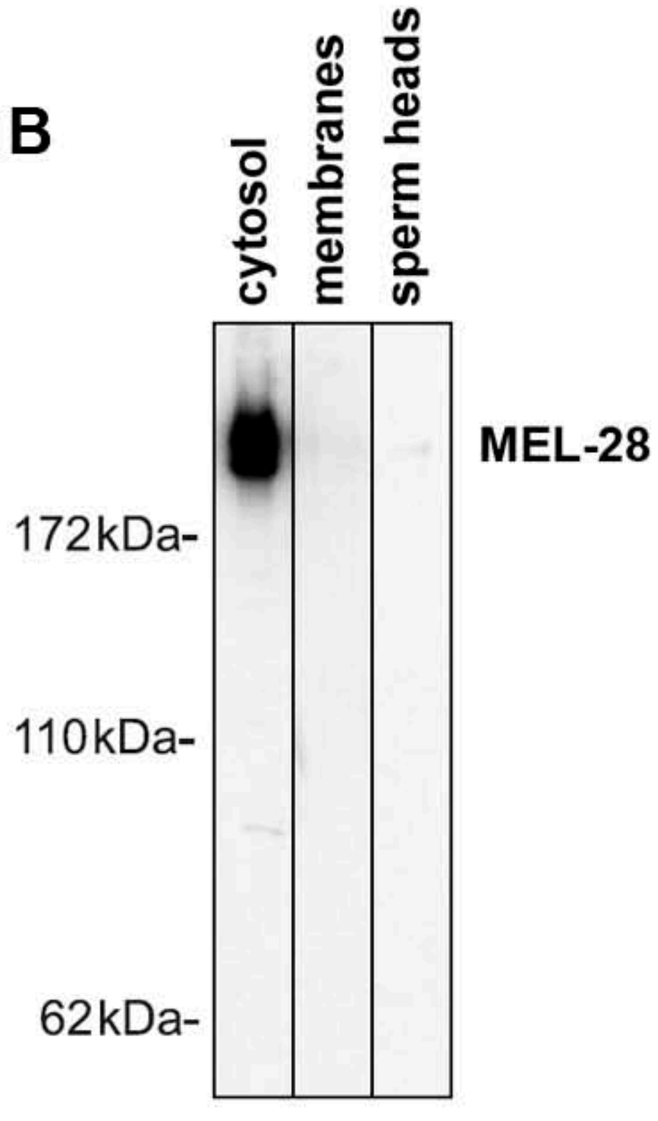
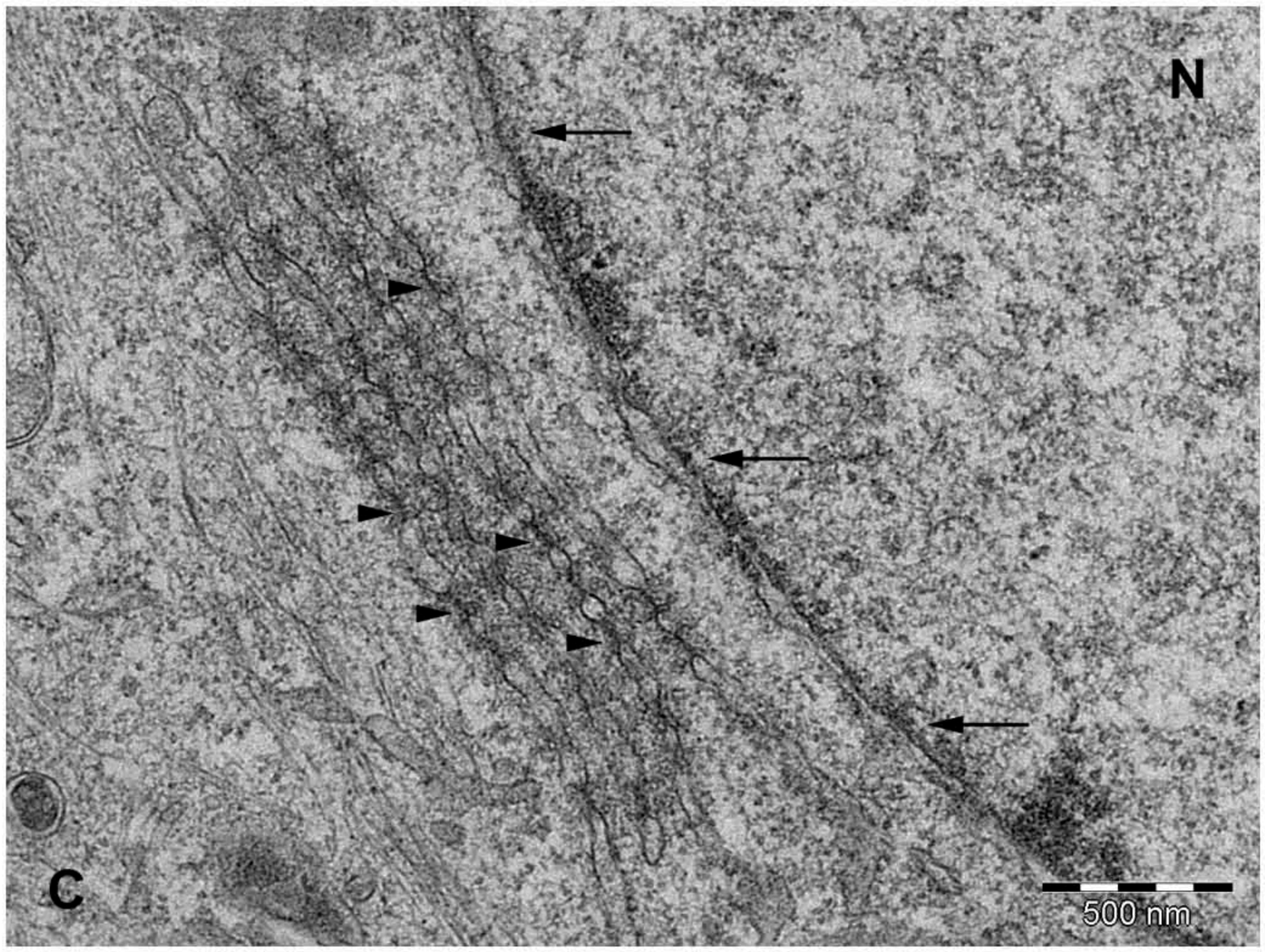
Suppl. Fig. 1A



Suppl. Fig. 1B



Suppl. Fig. 2A



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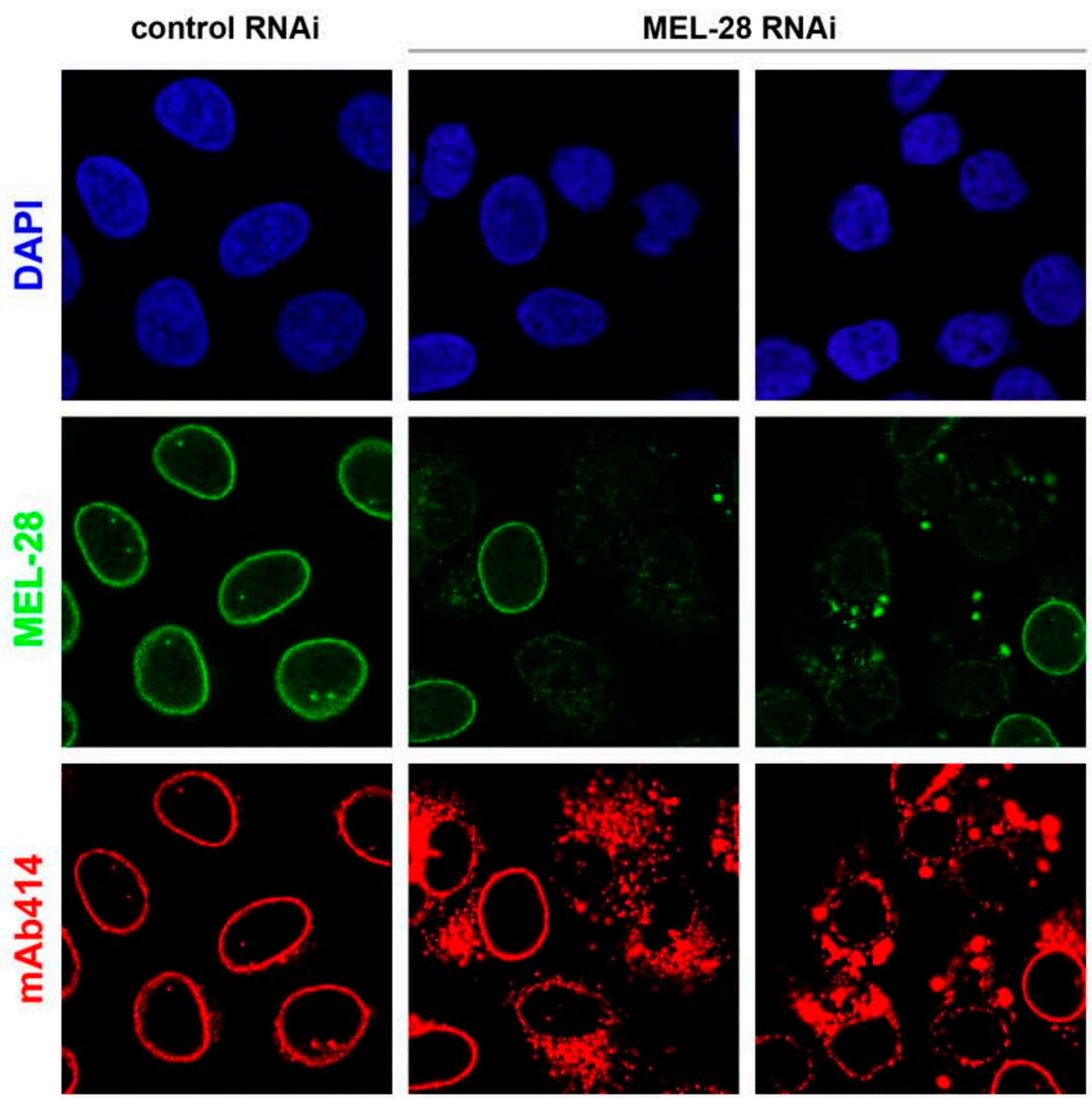
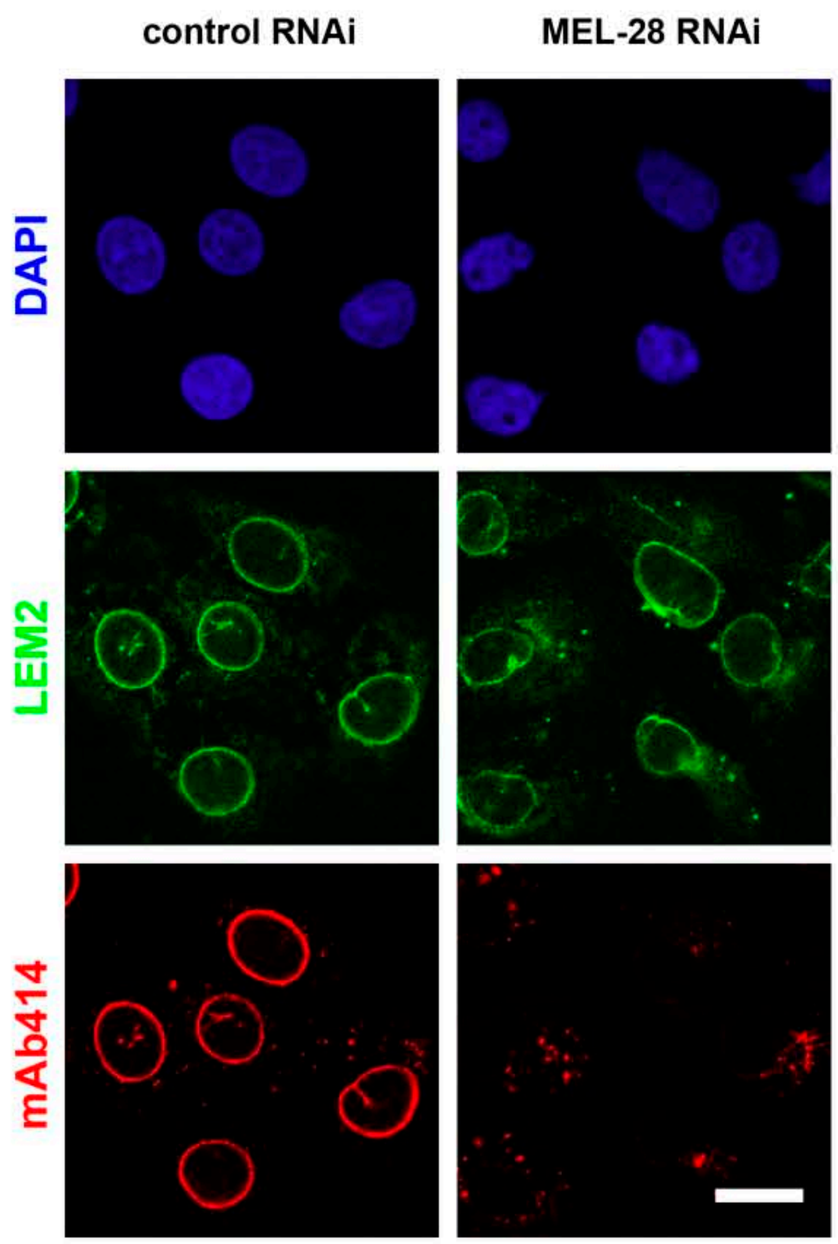
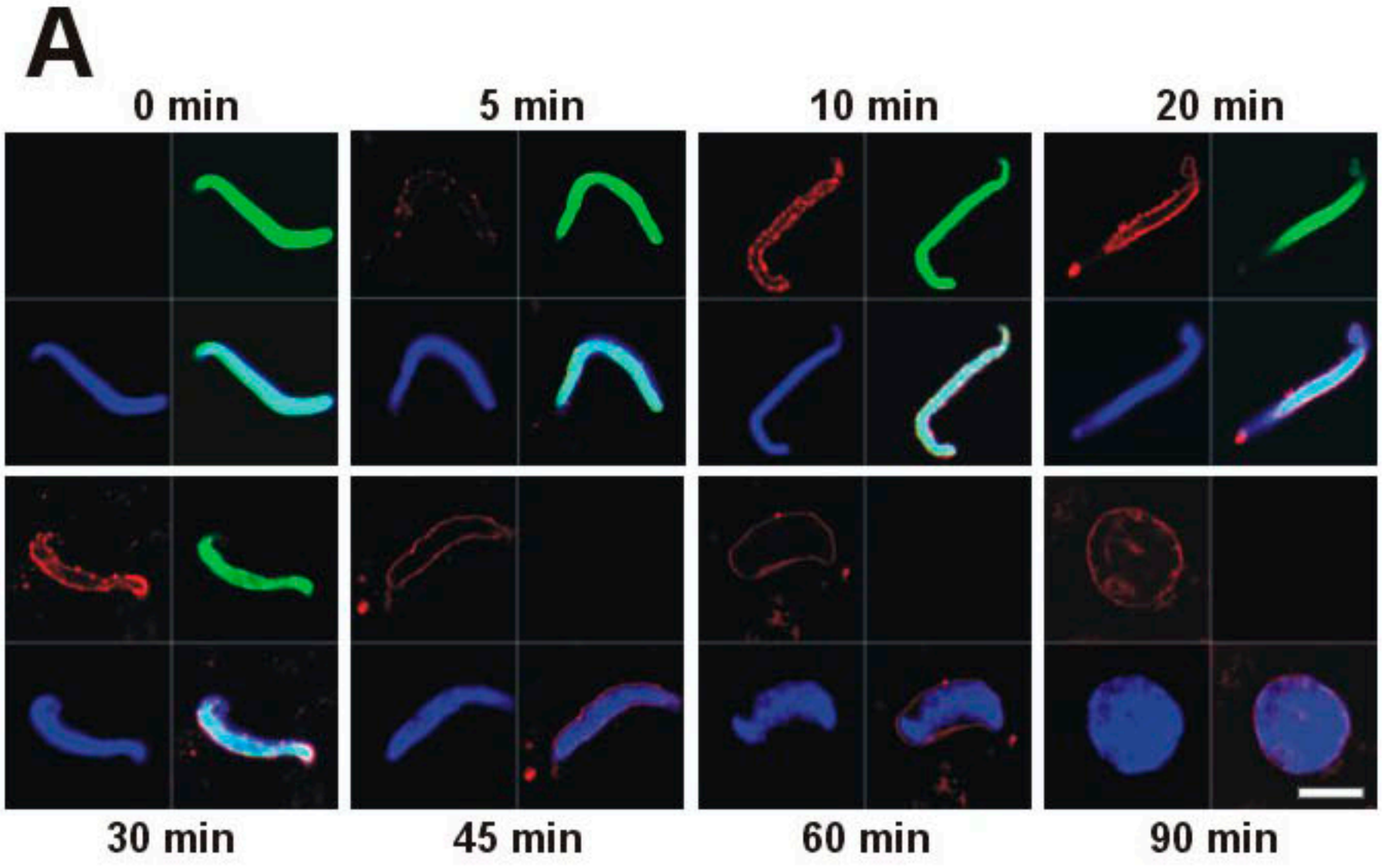




Suppl. Fig. 3


A
0 min
5 min
10 min
20 min
30 min
45 min
60 min
90 min
B
streptavidin-Oregon-geen
added after 30min
streptavidin-Oregon-green
added after 90min
C
mock
Δpom121
ΔNup107-160

Supplementary Figure 1:

(A) Localization of *Xenopus* MEL-28 was followed during the cell cycle in XL-177 cells: MEL-28 is shown in red and NPCs, visualized with mAb414, in green. Chromatin is stained with DAPI (blue in overlays). Bars 5µm.

(B) Western blot analysis of cytosol, membranes and sperm heads using anti *Xenopus* MEL-28 antibodies.

Supplementary Figure 2:

(A) Annulate Lamellae form upon RNAi depletion of MEL-28

Transmission electron micrograph showing Annulate Lamellae (AL) in Hela cells depleted of MEL-28. The number of these cytoplasmic membrane stacks containing nuclear pore complexes is significantly increased by RNAi treatment against MEL-28. Arrows mark the nuclear envelope, arrowheads pore complexes within the AL. N = nucleus, C = cytoplasm.

(B) RNAi depletion of MEL-28 in U2OS cells:

Cells were transfected with either control or MEL-28 siRNA-duplexes and fixed after 72 h. After immunostaining with α-MEL-28, α-LEM2 (green) antibodies or mAb414 they were analysed by confocal microscopy. DAPI staining is shown in blue. Bar, 15µm.

Supplementary Figure 3:

(A) Time course of a nuclear assembly followed with the exclusion assay: Chromatin was preincubated as described in the methods section and the assembly reaction initiated by addition of cytosol. At the indicated time points, Oregon green-streptavidin (green) was added to the reaction for 10 min. Samples were then quenched and processed as described. Membranes were labeled with DiIC18 (red), chromatin with DAPI (blue). Bar 20 µm.

(B) The exclusion assay can monitor the assembly state of the NE during the course of the assembly reaction: Assembly reactions were performed as described in the methods section, but Oregon green-streptavidin (green) was added to the reaction after 30 min (left panel) or 90 min (right panel). After 120 min samples were then quenched and processed as described. Membranes were labeled with DiIC18 (red), chromatin with DAPI (blue). Bar 20 µm.

(C) The exclusion assay can monitor the assembly state of the NE: Assembly reactions were performed using mock, pom121 depleted or Nup107-160 complex depleted extracts. After 90 min, Oregon green-streptavidin (green) was added to the reaction for 10 min. Samples were then quenched and processed as described. Membranes were labeled with DiIC18 (red), chromatin with DAPI (blue). Bar 20 μm.