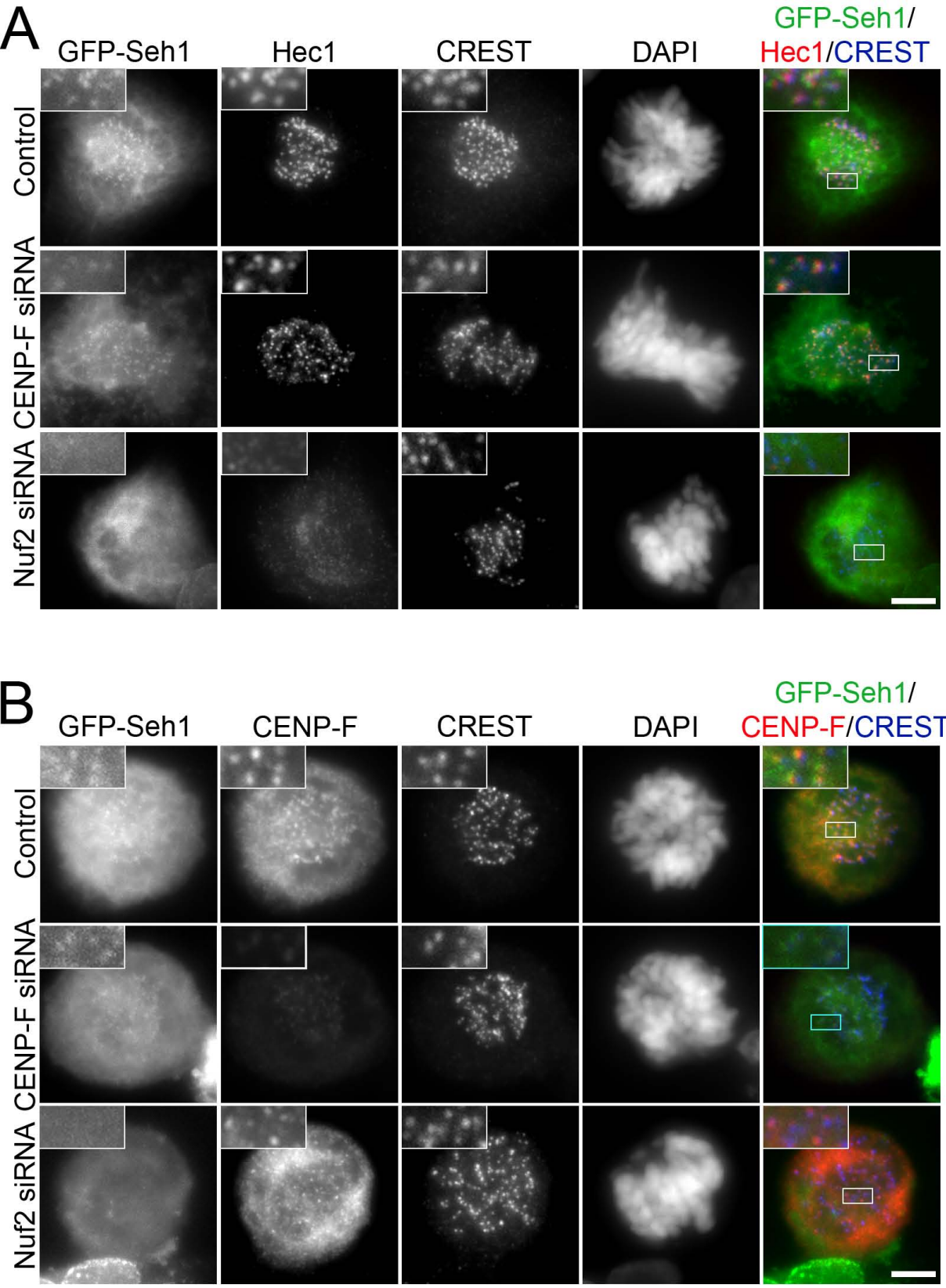
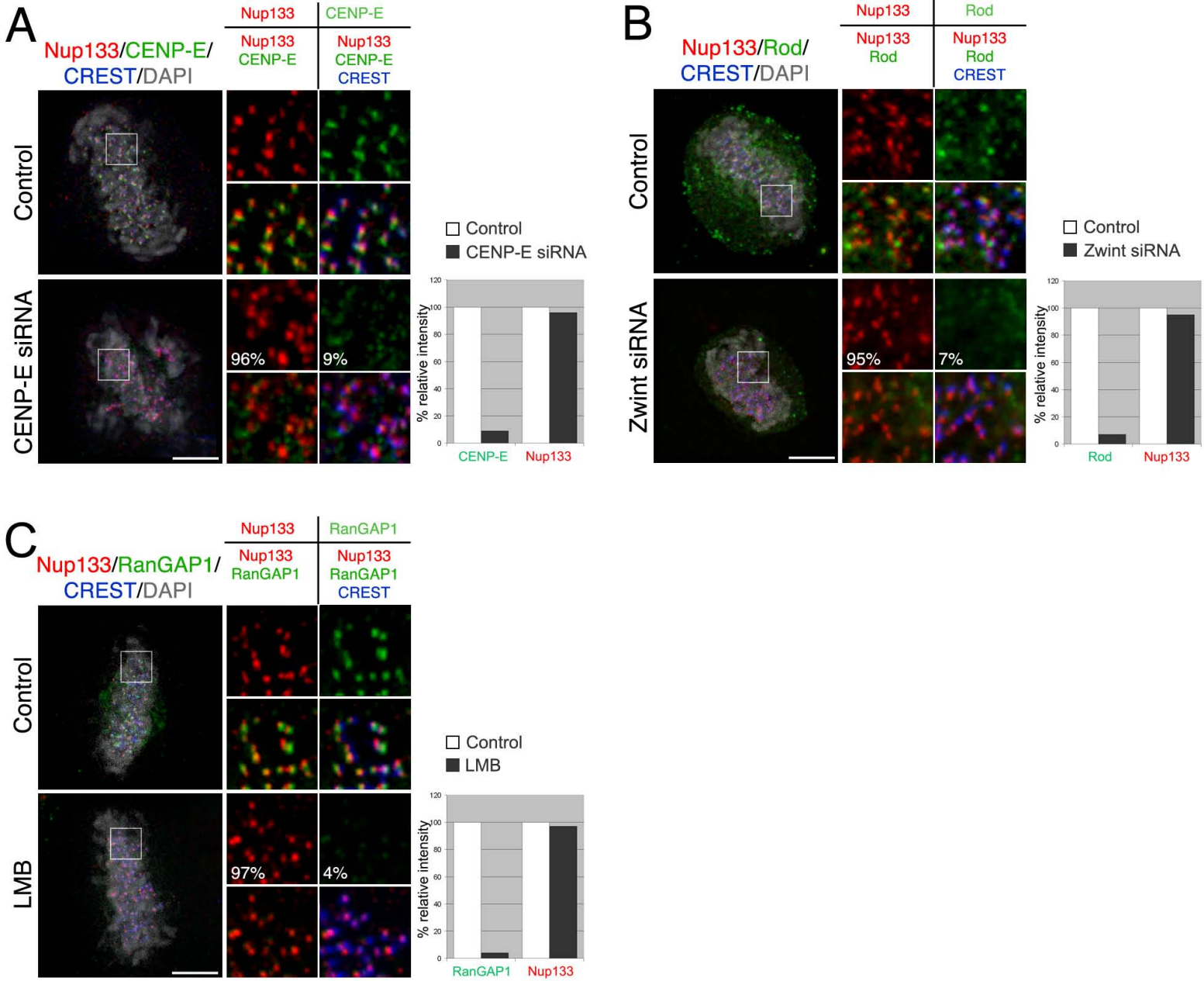


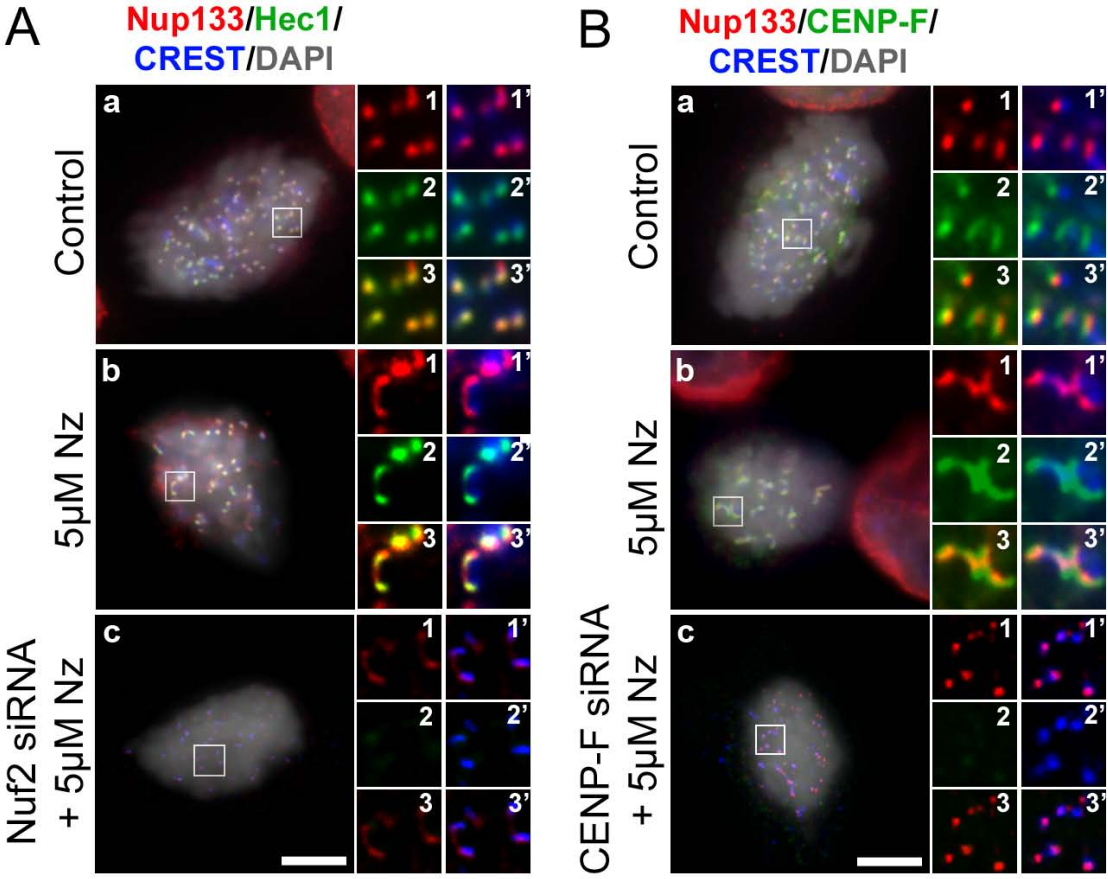
Supplemental Figure 1. The Ndc80 complex and CENP-F contribute to the kinetochore localization of GFP-Seh1.

HeLa cells stably expressing GFP-Seh1 were transfected with control, CENP-F siRNA or Nuf2 siRNA duplexes, fixed 3 days after transfection, and stained with anti Hec1 (A) or anti-CENP-F (B) (red), CREST serum (blue) and DAPI. Maximum projections along the Z-axis of all the images are presented; insets show a 3-fold enlargement of the indicated areas. Bar, 5μm. **C.** Quantification of the fluorescence intensity at kinetochores was performed as described in materials and methods. Note that GFP-Seh1 qualitatively behaves as Nup133 upon depletion of CENP-F or Nuf2, although upon Nuf2 RNAi the kinetochore pool of GFP-Seh1 becomes undistinguishable from the background. The effect of combined Nuf2 + CENP-F siRNA treatment could therefore not be assessed.



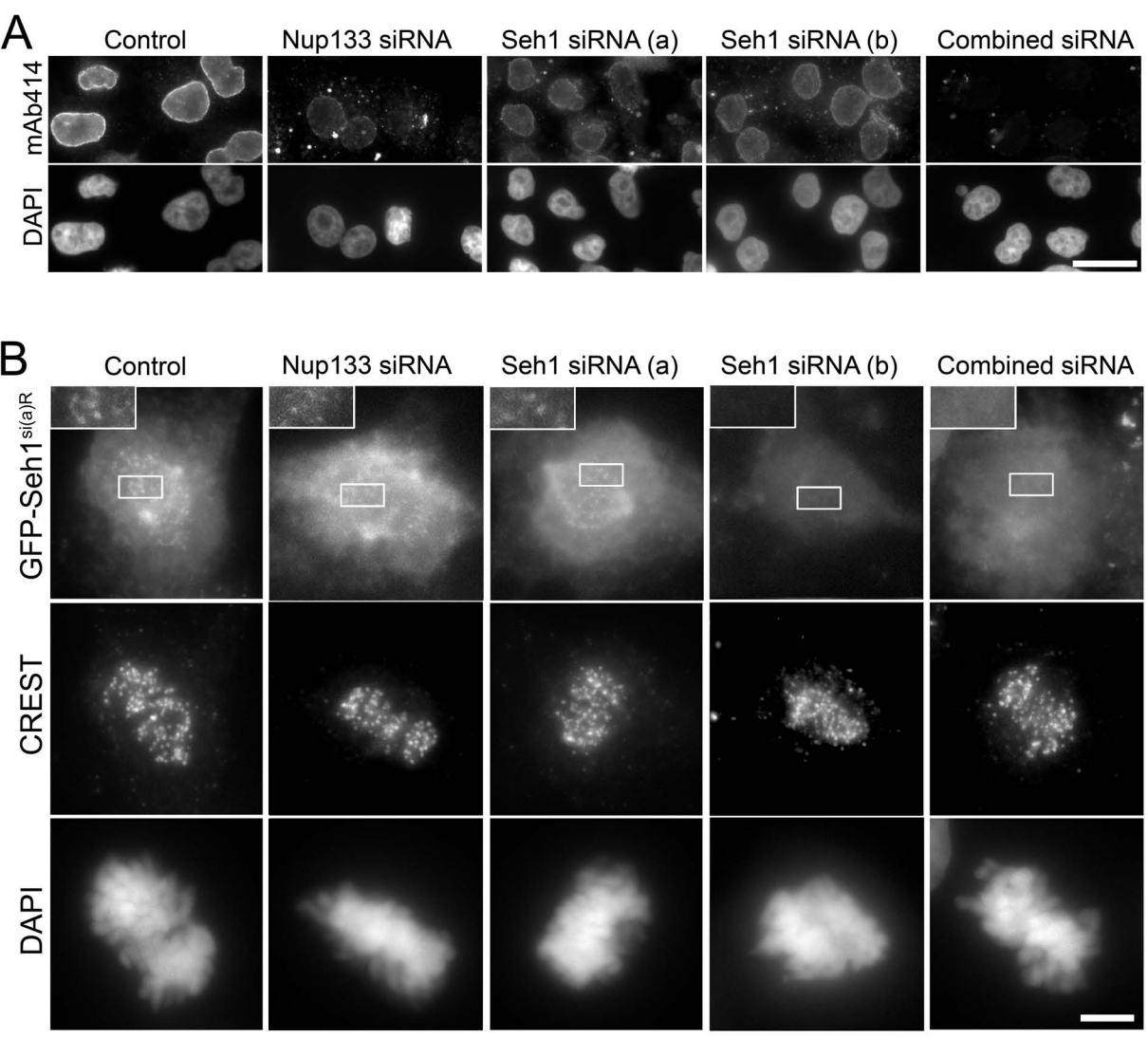
Supplemental Figure 2. Nup133 targeting to kinetochores is not affected upon siRNA-induced depletion of CENP-E or Zwint-1 or upon LMB treatment.

Cells were treated for 72 h with either a control siRNA duplex (upper panels), or CENP-E (**A**) or Zwint (**B**) siRNAs, or for 20 min with 10 ng/ml leptomycin B (**C**) and subsequently pre-extracted, fixed and probed with anti Nup133, the CREST serum, DAPI and respectively anti-CENP-E (**A**), anti-Rod (**B**) or anti-RanGAP1 (**C**) antibodies. Merged maximum projections of deconvolved 3D-stacks images of all the four staining (left) or various combinations of overlay images (3 fold magnification insets, right) are presented. Bar, 5µm. For each marker, the percentage of fluorescence intensity at kinetochores in treated cells as compared to control cells is indicated in the corresponding insets and represented on a histogram.



Supplemental Figure 3. Immunofluorescence colocalization of Nup133 with CENP-F and the Ndc80 complex to the kinetochore outer domain.

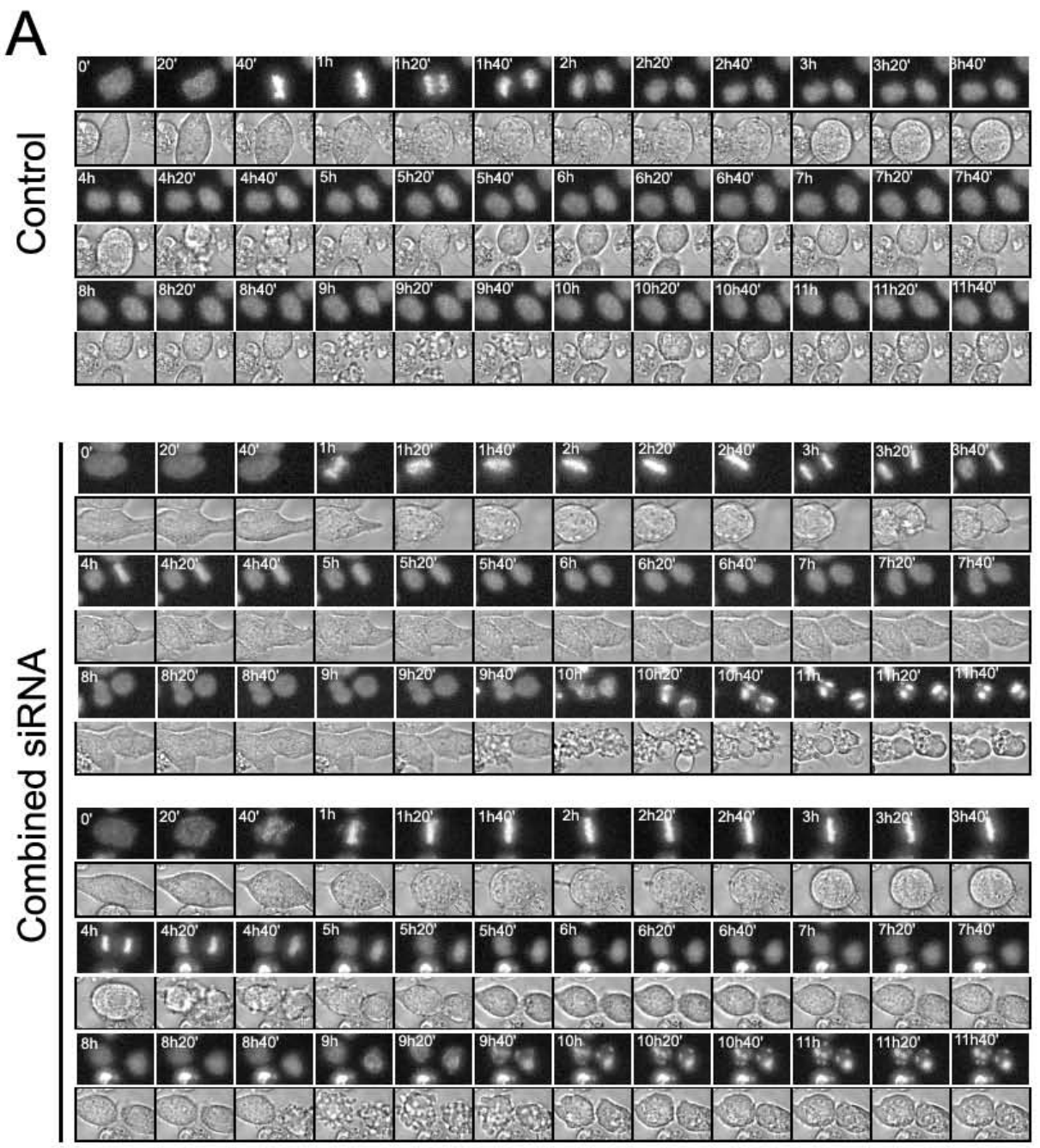
HeLa cells treated for 72 h with either control siRNA duplexes (a and b) or Nuf2 (A-c) or CENP-F (B-c) siRNAs and subsequently incubated for 3 h in 5 μM nocodazole (Nz, b and c) were pre-extracted, fixed, and stained with anti Nup133, the CREST serum, DAPI and either Hec1 (A) or CENP-F (B) antibodies. Merged images of all the four staining and various combinations of overlay images (insets, 3 fold magnification) are presented. Left insets correspond to Nup133 (1, red), Hec1 or CENP-F (2, green), Nup133 + Hec1 or CENP-F (3), and right insets (1', 2' and 3') to the same markers together with the CREST serum (blue). Bar, 5 μm. Note that the crescent shape displayed by Nup133 staining upon Nz treatment is lost in cells depleted for CENP-F (B-c), while it is still visible upon Nuf2 depletion by RNAi (A-c).

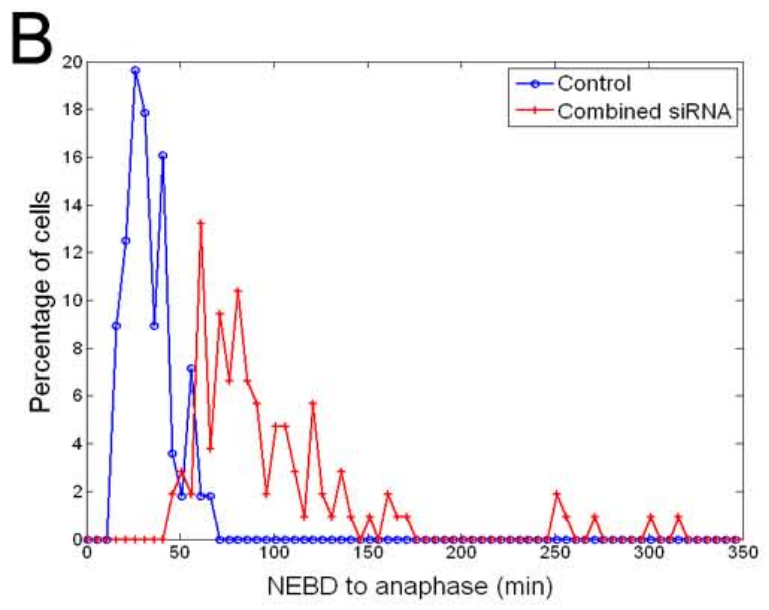


Supplemental Figure 4. Effect of Nup133, Seh1 and 'Combined' siRNAs treatments on NPC assembly and GFP-Seh1 localization.

A. Hela cells treated with siRNAs targeting LaminA/C (control), Nup133, Seh1 (2 different siRNAs, namely 'a' and 'b') or several members of the Nup107-160 complex ("combined" siRNAs) were fixed 3 days after transfection and stained with mAb414 antibody, to assess the overall status of NPCs, and DAPI. While both Seh1 siRNAs have a mild effect on NPC assembly, depletion of Nup133 severely impairs NPC reformation and the "combined" siRNAs have an even stronger effect. Bar, 20μm.

B. Cells stably expressing a mutant version of Seh1 (GFP-Seh1$^{si(a)R}$), which carries a silent mutation that prevent its degradation by the Seh1 siRNA(a) duplex were transfected with the same siRNAs as in A, then fixed and stained with the CREST antigen and DAPI 72h after transfection.

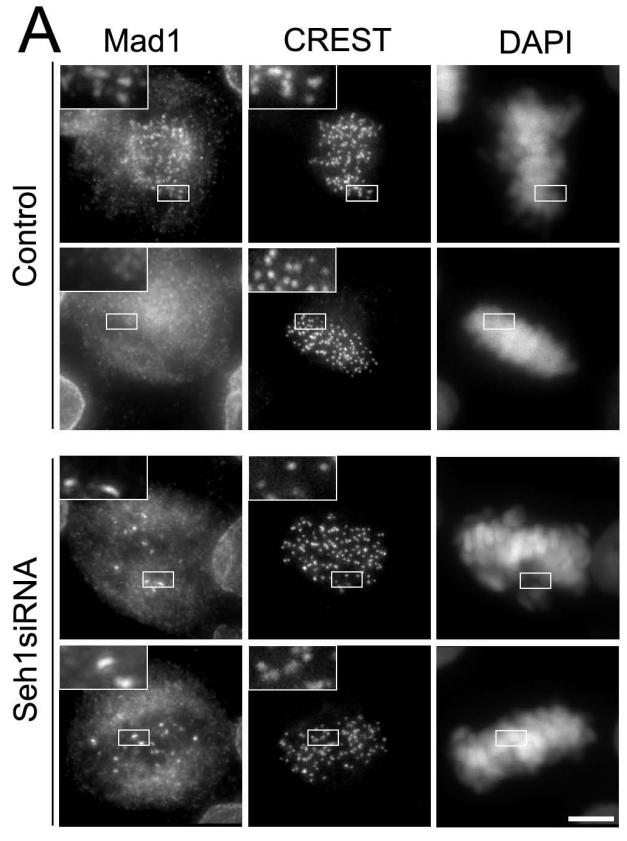


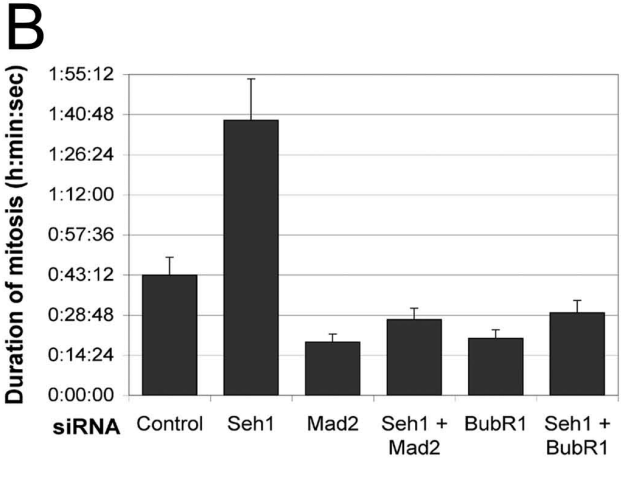


Supplemental Figure 5. Depletion of the Nup107-160 complex upon 'Combined siRNAs' treatment induces a mitotic delay.

A. HeLa cells expressing histone H2B-GFP were transfected whit control (upper panel) or the combined siRNAs (2 lower panels). Cells were recorded by time-lapse videomicroscopy from 36h to 84h post siRNA transfection, acquiring images every 5 min, using a 20x objective on a wide-field microscope. For each time point, maximum projections of 3 Z-stack planes acquired every 3 µm and the corresponding phase contrast images are presented.

B. Duration of mitosis was calculated as the time spent between nuclear envelope breakdown (NEBD) and anaphase onset. The graph shows the percentage of cells displaying a given mitotic duration. At least 100 cells were counted for each condition.

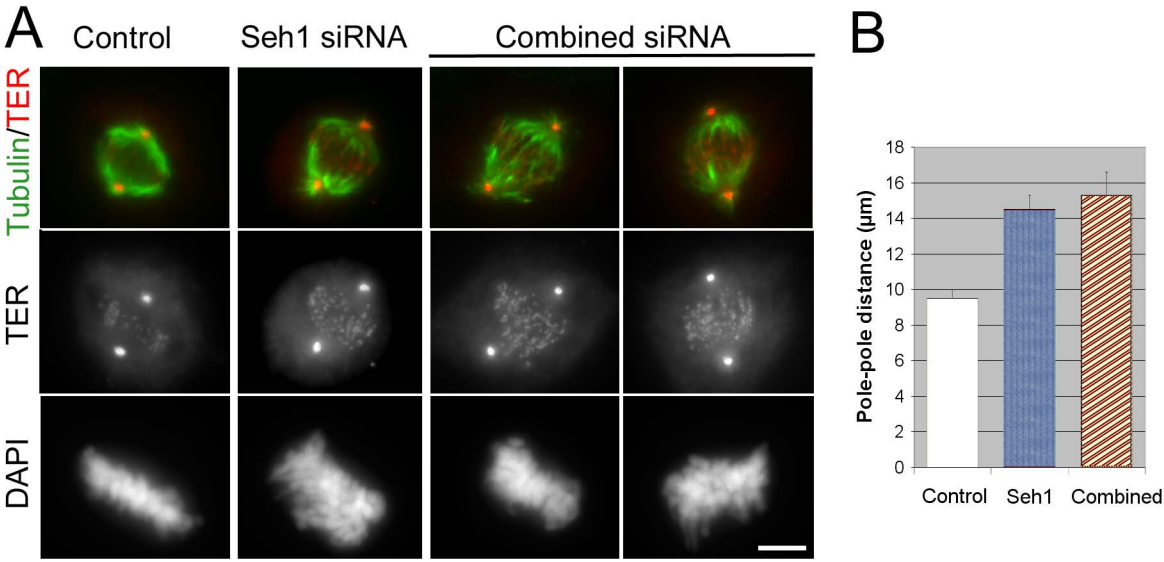




Supplemental Figure 6. Seh1 depletion activates the spindle assembly checkpoint.

A. Immunofluorescence of metaphase or prometaphase HeLa cells upon 72h treatment with control or Seh1 siRNA stained for Mad1, CREST and DAPI. 3-fold magnifications of the marked areas are presented. Bar, 5μm.

B. Duration of mitosis was determined using time lapse videomicroscopy of control cells, and cells either depleted for Seh1, Mad2, and BubR1 or co-depleted for Seh1 and each of these checkpoint proteins. Mean duration of mitosis is presented as an average of at least 50 different cells.



Supplemental Figure 7. Depletion of the Nup107-160 complex upon 'Combined siRNAs' treatment induces an increased pole-pole distance.

A. Cells treated for 72h with control, Seh1 or combined siRNAs were fixed and probed with anti-α-tubulin (green), the TER serum that labels both the centromeres and centrosomes (red), and DAPI. A maximum projection of the deconvolved Z-stack is presented Bar, 5 µm.

B. Pole-pole distance was measured by the distance between the two centrosomes labeled with the TER serum.