SUPPLEMENTARY FIGURE LEGEND

Supplementary Figure 1. Ska1 antibody specificity. Equal amount of asynchronous Hela S3 extracts were separated on a SDS-PAGE gel and probed after Western blotting with affinity-purified anti-Ska1 antibody and the corresponding preimmune serum.

Supplementary Figure 2. Ska1 KT localisation requires the presence of MTs. A) Hela S3 cells were arrested with noscapine for 14 hours and either directly fixed with PTEMF (top row) or released for 15 min and then fixed (middle row) or treated with nocodazole for another hour (lower row). Cells were stained with anti-Ska1 (red), anti-α-tubulin (green) and CREST serum (blue). B) HeLa S3 cells were treated for 14 hours with nocodazole and subsequently released for the indicated times from this nocodazole block. As a control an untreated cell is shown at the top row. After PTEMF fixation cells were stained with anti-Ska1 antibody (red), with anti-α-tubulin (green) and DAPI (DNA, blue). Scale bars indicate 10 μm.

Supplementary Figure 3. Ska1 but not RanGAP1 KT localisation is rescued by cold treatment in the absence of MTs. A) HeLa S3 cells were either left untreated (control) or arrested in mitosis with nocodazole for 14 hours followed by 2 hours incubation in ice-cold medium (in the continued presence of nocodazole). Cells were fixed with PTEMF and stained with anti-Ska1 antibody (red), DAPI (DNA, blue) and anti-α-tubulin (green) in order to check for complete MT depolymerisation in these cells. B) HeLa S3 cells were either left untreated (control) or arrested in mitosis with nocodazole for 14 hours. The latter cells were then either directly fixed directly with PTEMF or incubated in ice-cold medium for another 2 hours. Cells were stained with anti-RanGAP1 antibody (red), CREST serum (green) or DAPI (DNA, blue). Scale bars indicate 10 μm.

Supplementary Figure 4. Investigation of potential Ska1 interacting proteins by co-immunoprecipitations. Mitotic HeLa S3 cell lysates were used for immunoprecipitations (IPs) with anti-Ska1 antibody and rabbit IgGs, respectively. The isolated protein complexes were separated by SDS-PAGE, followed by transfer onto nitrocellulose membranes. Membranes were subsequently probed with the indicated antibodies to reveal interactions with Ska1.

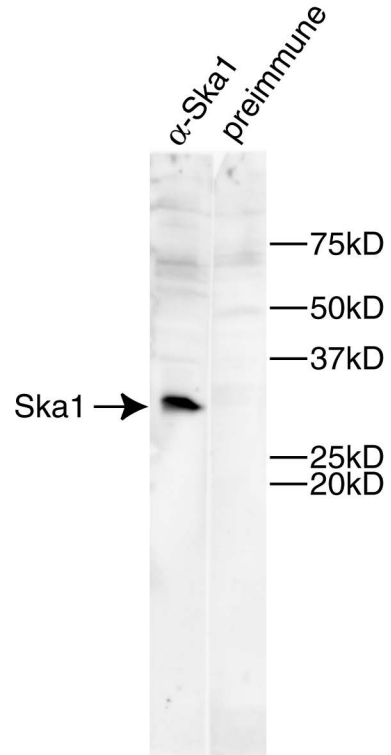
Supplementary Figure 5. Constant expression of Ska1 and Ska2 during the cell cycle and identification of a mitotic Ska2 variant. A) Lysates were prepared from asynchronously growing, aphidicolin arrested (G1/S phase), nocodazole arrested shake off (M phase) and taxol arrested shake off (M phase) HeLa S3 cells, respectively. Equal amounts of cell lysates were separated by SDS-PAGE and probed by Western blotting with the indicated antibodies. B) HeLa S3 lysates from aphidicolin and nocodazole arrested cells, respectively, were either incubated with alkaline phosphatase (AP) or with phosphatase inhibitors (P-inhibitors) for 1 hour at 30°C. Equal amounts of cell lysates were separated by SDS-PAGE and probed after Western blotting with the indicated antibodies. C) HeLa S3 cells were synchronised by a sequential aphidicolin/nocodazole block release protocol. After nocodazole release samples were taken every 20 min. Equal amounts of cell extracts were separated by SDS-PAGE and probed by Western blotting with the indicated antibodies.

Supplementary Movie 1. Movie corresponds to the stills in Figure 5C, upper panel.
Live-cell imaging of H2B-GFP expressing HeLa S3 cells treated with siRNAs against GL2 as a control.

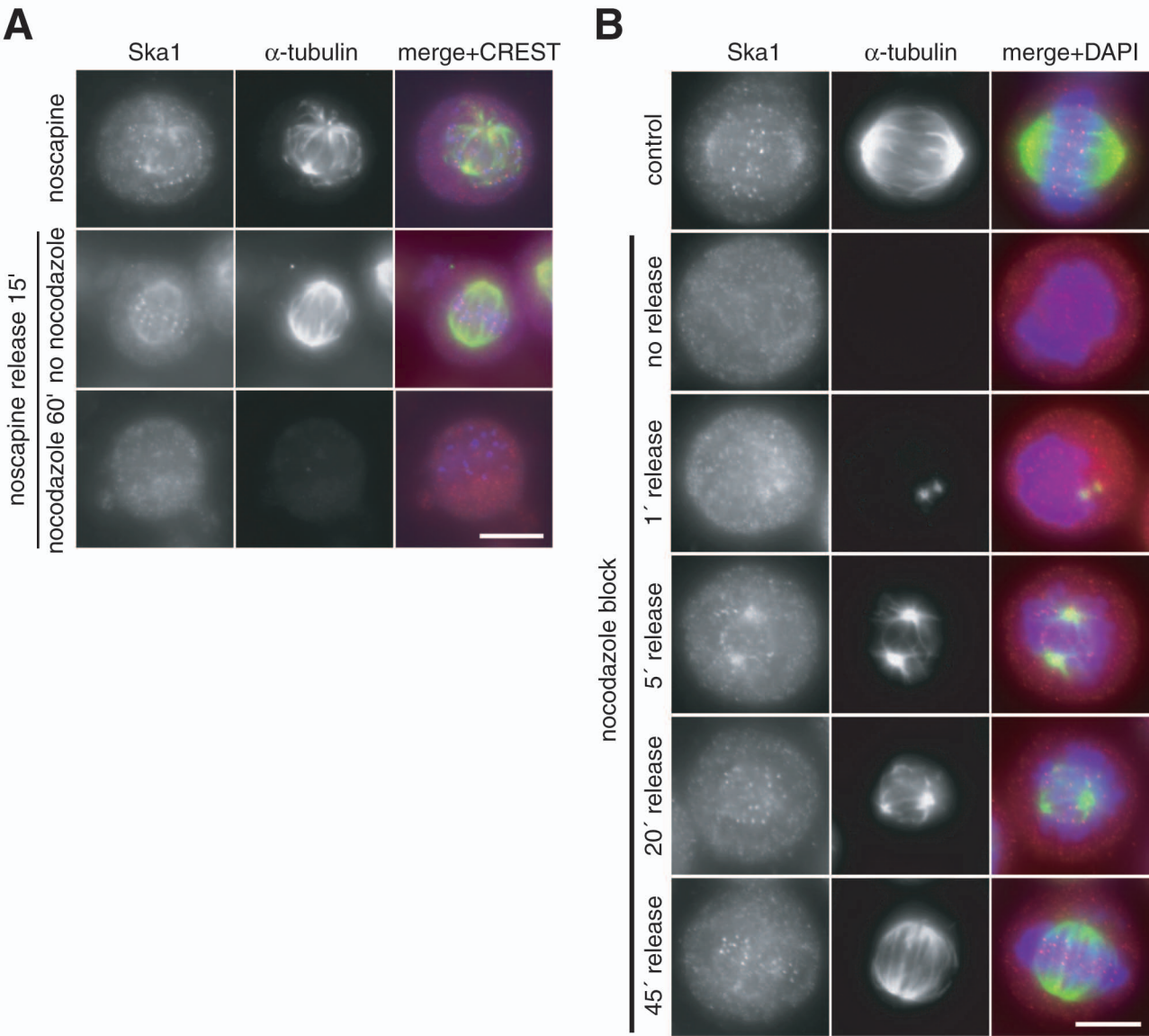
Supplementary Movie 2. Movie corresponds to the stills in Figure 5C, middle panel.
Live-cell imaging of H2B-GFP expressing HeLa S3 cells treated with siRNAs against Ska1.

Supplementary Movie 3. Movie corresponds to the stills in Figure 5C, lower panel.
Live-cell imaging of H2B-GFP expressing HeLa S3 cells treated with siRNAs against Ska2.

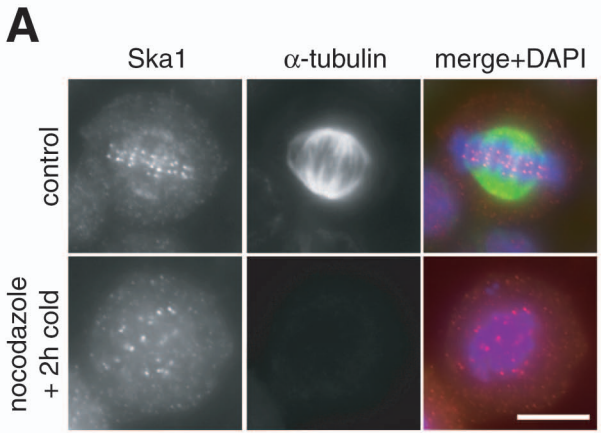
Supplementary Figure 1, Hanisch et al.

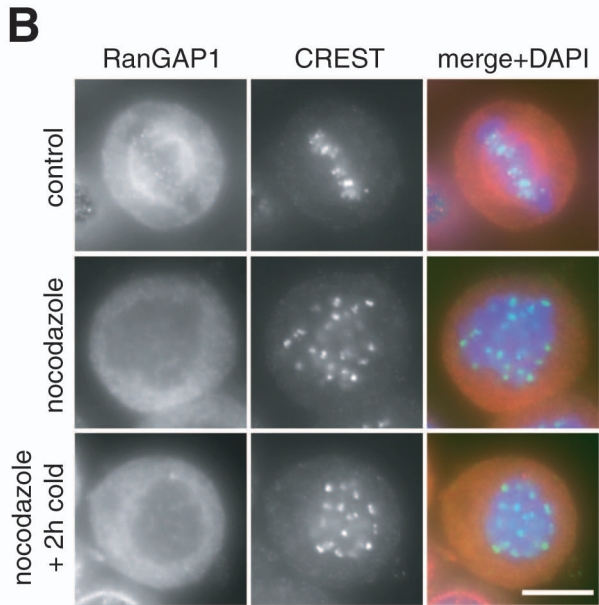


Supplementary Figure 2, Hanisch et al.

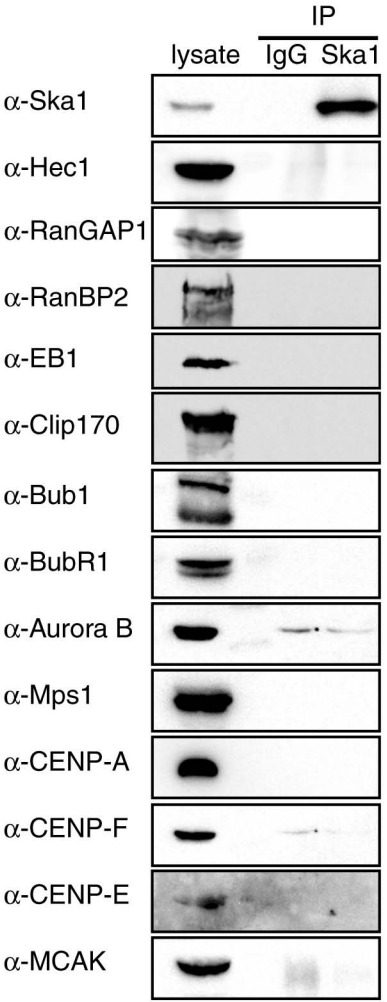


Supplementary Figure 3, Hanisch et al.





Supplementary Figure 4, Hanisch et al.



Supplementary Figure 5, Hanisch et al.