SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Mass spectrometry identification of C13Orf3

A) Predicted protein sequence of C13Orf3, with peptides detected by MS/MS following Ska2 immunoprecipitation shown in red. Using this method we detected 24% of the total protein after combining two independent experiments.

B) MS/MS spectra of two doubly-charged precursor ions of C13Orf3 at m/z 629.25 (top) and 654.34 (bottom). N- and C-terminal fragments are marked with b and y, respectively. Mox denotes oxidation at methionine and (++) denotes a doubly-charged ion. Both spectra were acquired from a hybrid linear ion trap/Orbitrap tandem mass spectrometer.

Supplementary Figure 2. Phosphoregulation of the Ska complex

A) Primary protein structures of Ska1, Ska2 and Ska3. Lines linking the proteins indicate fragments shown to interact. The potential GLEBS domains on Ska3 are depicted in orange (N-terminus, found by primary sequence alignment, but only partially supported by secondary structure predictions) or yellow (C-terminus, found by secondary structure prediction, not strongly supported by primary sequence alignment). Serine, threonine and tyrosine residues that are known to be phosphorylated are indicated.

B) Table of known phosphorylation sites on Ska proteins. The detected phosphorylated peptide is listed, and the predicted kinase consensus motif shown in red where applicable.

Supplementary Figure 3. Cell cycle localisation of Myc-Ska3

A) HeLa S3 cells were transfected with a Myc-tagged Ska3 construct for 48 hr and fixed with PTEMF. An interphase cell is shown which was stained with 9E10 anti-Myc (red) and anti-α-Tubulin (green) antibodies. DNA was visualized using DAPI (blue). Bar =10 μm.

B) Cells were transfected with a Myc-tagged Ska3 construct for 48 hr and fixed with PTEMF. Cells were stained with 9E10 anti-Myc (red) and anti-Ska1 antibodies (green). DNA was visualized using DAPI (blue). Bar = 10μm.

Supplementary Figure 4. Characterisation of anti-Ska3 antibodies and mitotic-dependent phosphorylation of Ska3

A) Pre-immune and serum from day 73 bleed of an immunized rabbit were tested for reactivity of mitotic HeLa S3 cells by immunofluorescence microscopy. Antibodies from this bleed were then affinity purified and used for all subsequent studies. Bar = 10 μm.

B) Lysates (50 μg) from asynchronously growing HeLa S3 cells and mitotic cells collected by shake-off after control (Gl2 + nocodazole) or Ska3-depletion were resolved by SDS-PAGE and probed with pre-immune serum or affinity-purified antibody. Two bands corresponding to Ska3 are marked (arrows). Asterisks indicate crossreacting bands.

C) Immunoblots were performed on cell lysates from an asynchronously growing population, nocodazole arrested cells and nocodazole arrested cells incubated with either Calf Intestinal Phosphatase (CIP) or λ Phosphatase for 2 hr at 30°C. Equal amounts of cell lysates were separated by SDS-PAGE and probed by Western blotting with anti-Ska3, anti-Securin and anti-α-Tubulin antibodies.

D) Cells were synchronized by a sequential thymidine/nocodazole block release protocol. After nocodazole release, samples were taken every 20 min. Asynchronously growing HeLa S3 cells and cells released for 120 min in the presence of 20 μM MG132 are included in the experiment. Equal amount of cell extracts were separated by SDS-PAGE and probed by Western blotting with the indicated antibodies.

Supplementary Figure 5. Depletion of the Ska Complex

A) HeLa S3 cells were treated for 28 hr with control (Gl2) and Ska1, Ska2 and Ska3, Ska1/Ska2, Ska1/Ska3 and Ska2/Ska3 -specific siRNAs, respectively. Cells were collected and equal amounts of cell extracts were separated by SDS-PAGE and probed by Western blotting with anti-Ska1, anti-Ska2, anti-Ska3 and anti-α-Tubulin antibodies.

B) Corresponding quantification of Western signal intensities from (A) normalised against the signals measured from α-Tubulin.

C) Cells were treated for 48 hr with control (Gl2), Ska1, Ska3 and Ska1/Ska3 specific siRNAs then fixed with PTEMF and stained with anti-Ska3 (red) and anti-α-Tubulin (green) antibodies and CREST serum (far red, depicted in green). The merged image between Ska3 and CREST shows the CREST staining in green. DNA was visualized using DAPI (blue). Bar = 10 μm.

Supplementary Figure 6. KT localisation requirements of the Ska complex

A) HeLa S3 cells were treated with DMSO (control) or nocodazole for 16 hr, then fixed with PTEMF and stained with anti-Ska3 (red), CREST serum (green) and anti-

α-Tubulin (shown in far right column) antibodies. DNA was visualized using DAPI (blue). Bar = 10 µm.

B) Cells were treated for 48 hr with control (Gl2) or Hec1 siRNAs, then fixed with PTEMF and stained with anti-Ska3 (red), CREST serum (green), and anti-Hec1 (shown in far right column) antibodies. DNA was visualized using DAPI (blue). Bar = 10 µm.

Supplementary Figure 7. Ska complex depletion does not strongly affect the KMN

A) HeLa S3 cells were treated for 48 hr with control (Gl2) and Ska1, Ska3, and Ska1/Ska3 -specific siRNAs. To insure that the Ska complex was at the KT, taxol was added to all conditions for 16 hr prior to collection (Hanisch *et al*., 2006). Cells were harvested by shake-off and equal amounts of cell extracts were separated by SDS-PAGE and probed by Western blotting with anti-Ska3, anti-Ska1, anti-Ska2, anti-Hec1, anti-Blinkin and anti-α-Tubulin antibodies.

Immunofluorescent KT signals of anti-Blinkin **(B)** and anti-Hec1 **(C)** were measured and normalised against the corresponding CREST serum signal. A slight decrease was detected between Gl2 and all other conditions for both anti-Blinkin and anti-Hec1 staining. No further decrease was measured from single to double depletion of the Ska components ($p>0.01$, students t-Test). The average intensity of 30 KT per cell was measured, and bars represent mean and SD from 5 cells per condition.

SUPPLEMENTARY MOVIES

Supplementary Movie 1. Control (Gl2) siRNA treated cells

Movie showing segregation of Histone H2B-GFP-labelled chromosomes in Gl2 siRNA treated cells.

Supplementary Movie 2. Ska1 siRNA treated cells

Movie showing segregation of Histone H2B-GFP-labelled chromosomes in Ska1 siRNA treated cells.

Supplementary Movie 3. Ska3 siRNA treated cells

Movie showing segregation of Histone H2B-GFP-labelled chromosomes in Ska3 siRNA treated cells.

Supplementary Movie 4. Control (Gl2) siRNA treated cells

Movie showing mitotic progression of Histone H2B-GFP-labelled chromosomes in Gl2 siRNA treated cells.

Supplementary Movie 5. Ska1/Gl2 siRNA treated cells

Movie showing mitotic progression of Histone H2B-GFP-labelled chromosomes in Ska1 and Gl2 siRNA co-treated cells.

Supplementary Movie 6. Ska3/ Gl2 siRNA treated cells

Movie showing mitotic progression of Histone H2B-GFP-labelled chromosomes in Ska3 and Gl2 siRNA co-treated cells.

Supplementary Movie 7. Ska1/Ska3 siRNA treated cells

Movie showing mitotic progression of Histone H2B-GFP-labelled chromosomes in Ska1 and Ska3 siRNA co-treated cells.

Supplementary Figure 1, Gaitanos et al.

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  1 MDPIRSFCGK LRSLASTLDC ETARLQRALD
 31 GEESDFEDYP MRILYDLHSE VQTLKDDVNI
 61 LLDKARLENQ EGIDFIKATK VLMEKNSMDI
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121 PELSNCENFQ KTDVKDDLSD PPVASSCISE
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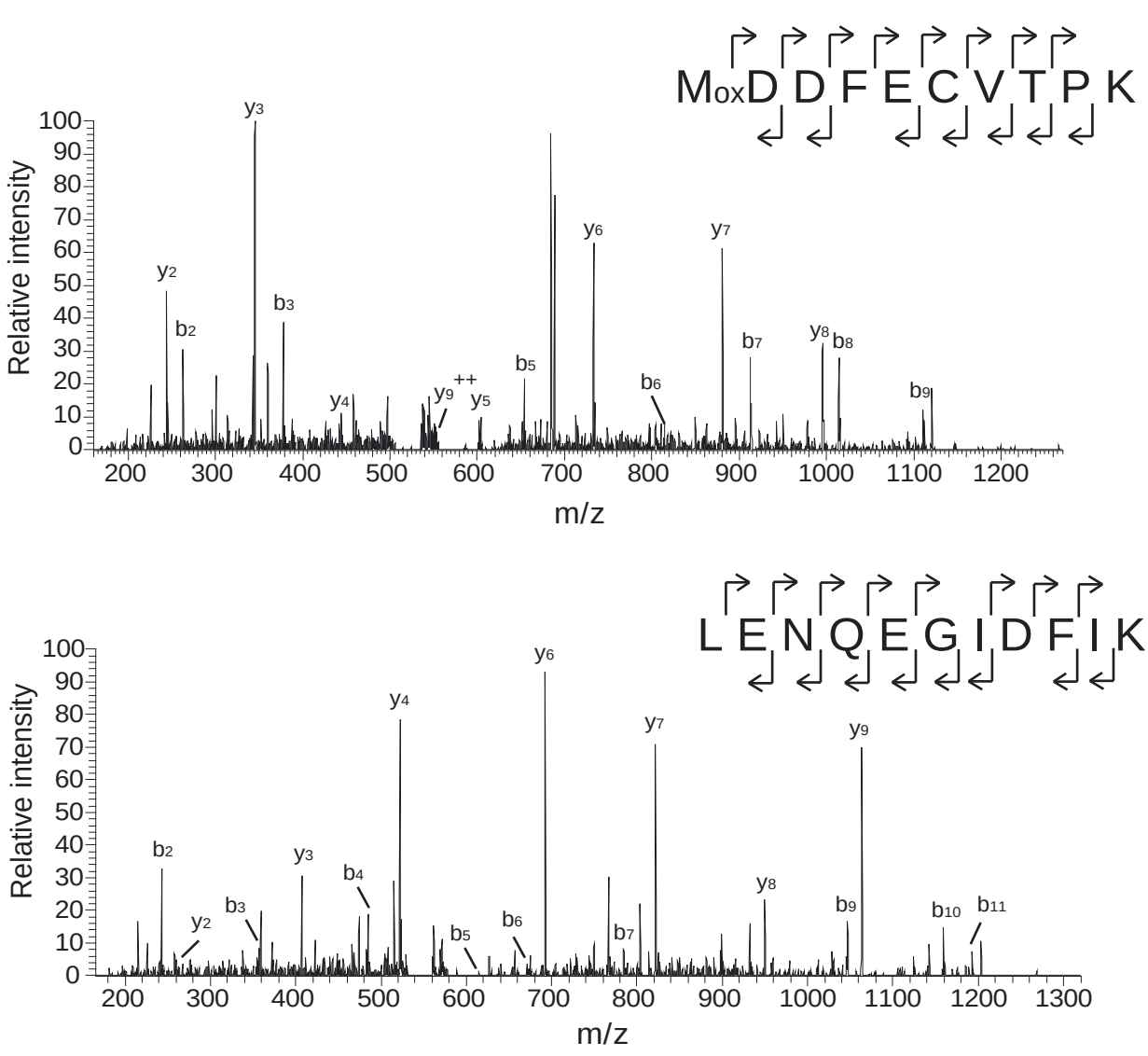

Supplementary Figure 2, Gaitanos et al.

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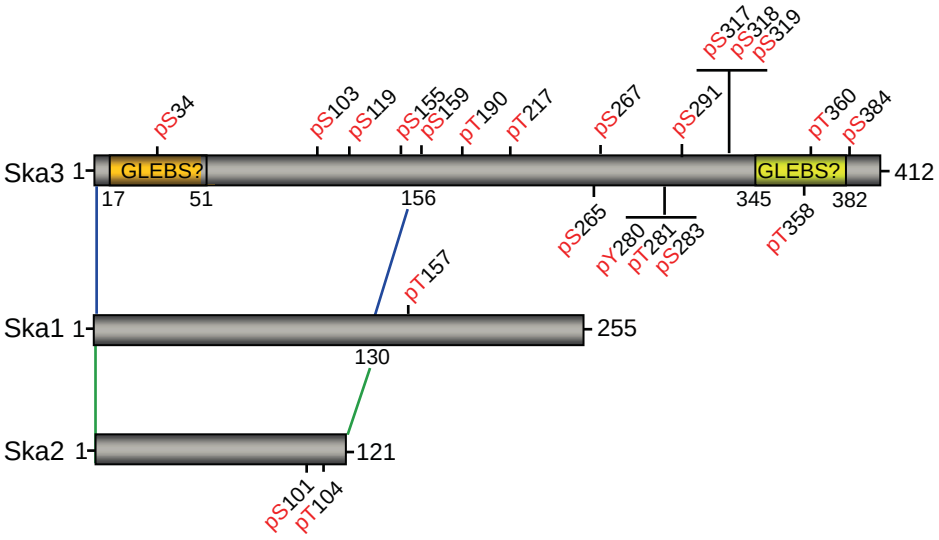

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	Site	Ref.	Phosphorylation peptide	Kinase consensus motif
Ska3 (C13Orf3)	S34	1	DGEES(ph)DFED	Plk1
	S103	2	KYGYS(ph)PRVK	Cdk1
	S119	2	EAINS(ph)DPEL	CK2
	S155	3	KSPRS(ph)PQLS	Cdk1
	S159	1	SPQLS(ph)DFGL	CK2
	T190	5	PVIVT(ph)PPTK	Cdk1
	T217	2	FECVT(ph)PKLE	Cdk1
	T265	1	NVFAT(ph)PSPI	Cdk1
	S267	2	FATPS(ph)PIIQ	Cdk1
	Y280	4	SDAEY(ph)TNSP	
	T281	5	DAEYT(ph)NSPL	Plk1
	S283	5	EYTNS(ph)PLVP	Cdk1
	T291	1	PTFCT(ph)PGLK	Cdk1
	S317	2	SKTNS(ph)SSND	
	S318	6	KTNSS(ph)SNDL	CK2
	S319	5	TNSSS(ph)NDLE	
	T358	1	NLLRT(ph)PTPP	Cdk1
	T360	1	LRTPT(ph)PPEV	Cdk1
	T384	2	SNLAT(ph)PIAI	Cdk1
Ska1 (C18Orf24)	T157	5	KSRLT(ph)YNQI	Aurora
Ska2 (Fam33a)	S101	8	DLELS(ph)PLTK	Cdk1
	T104	6	LSPLT(ph)KEEK	CK2

[1]Nousianen et al., 2006
[2]Dephoure et al., 2008
[3]Brill et al., 2004
[4]Rush et al., 2005
[5]Santamaria et al., unpublished data
[6]Malik et al, manuscript in preparation
[7]Sui et al., 2008
[8]Daub et al., 2008

Supplementary Figure 3, Gaitanos et al.

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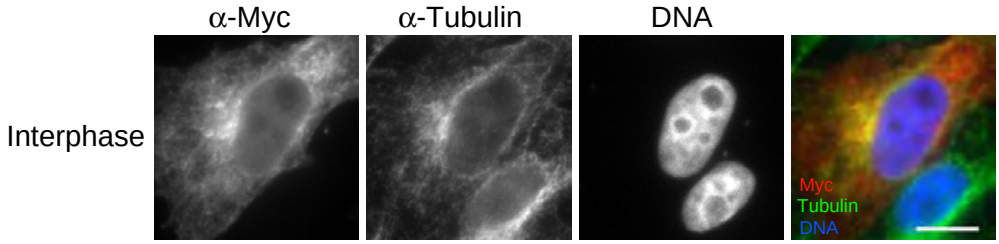

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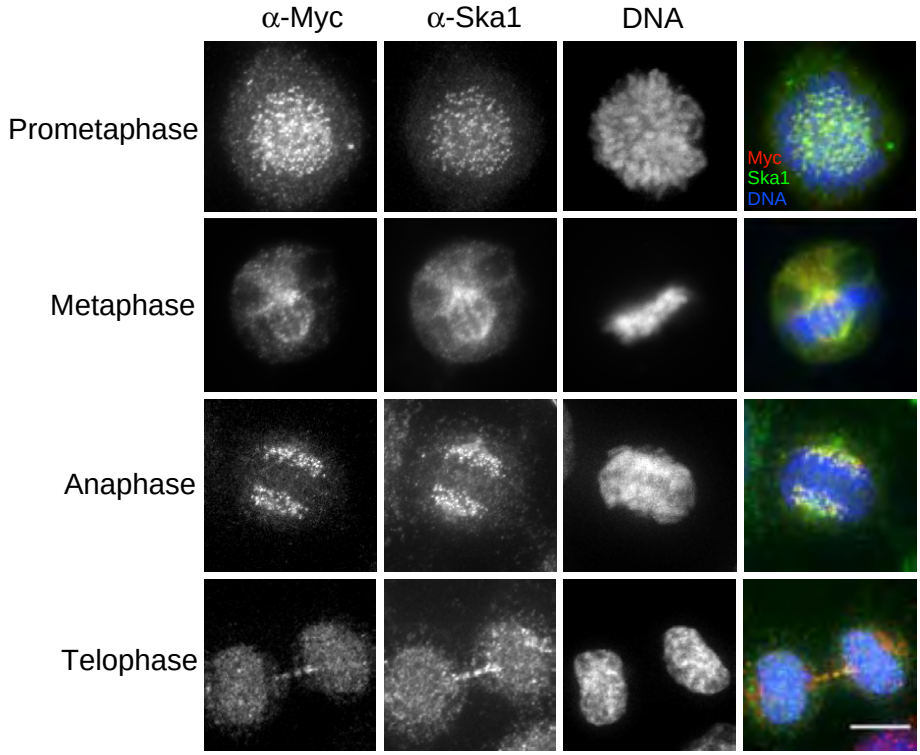
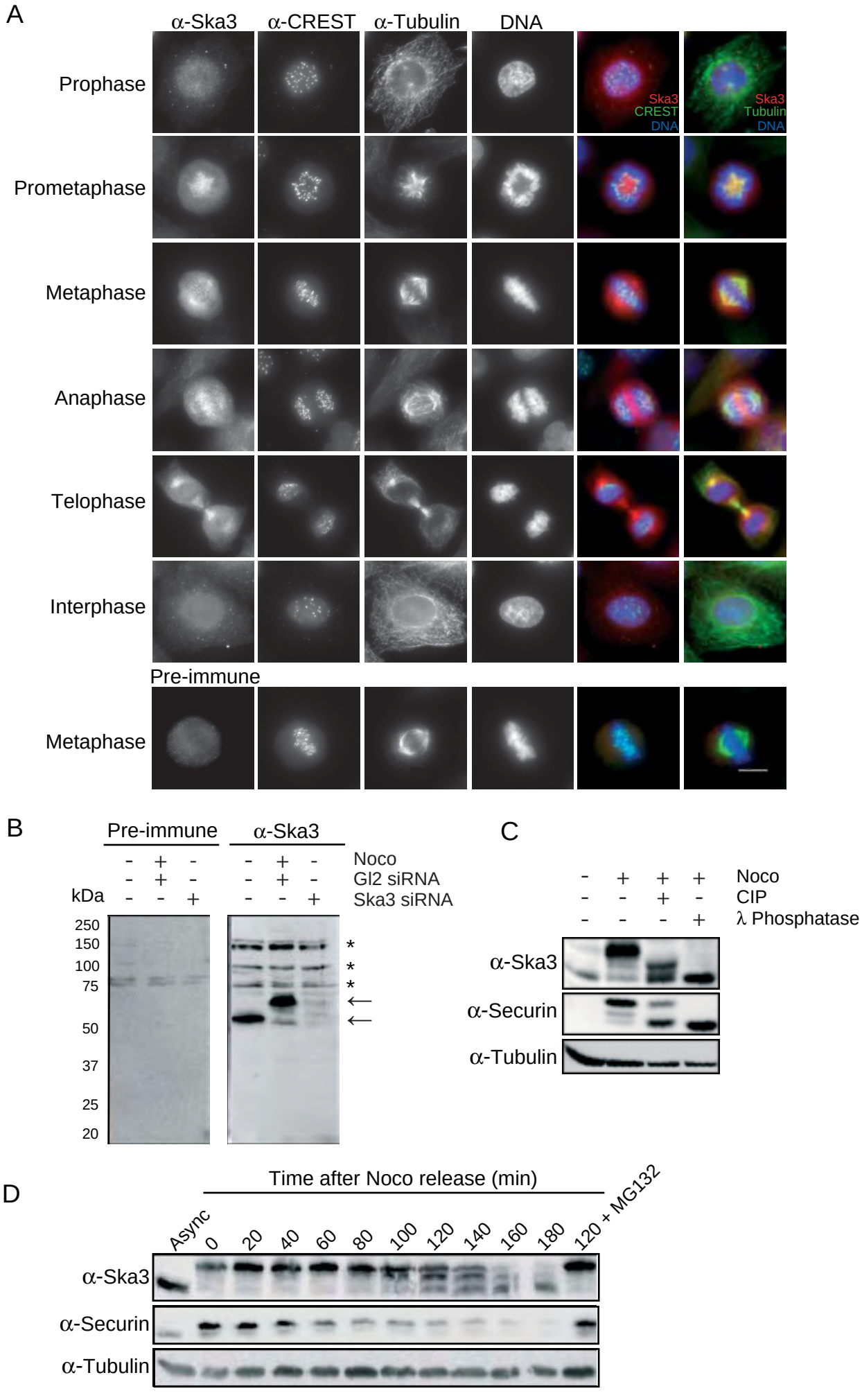
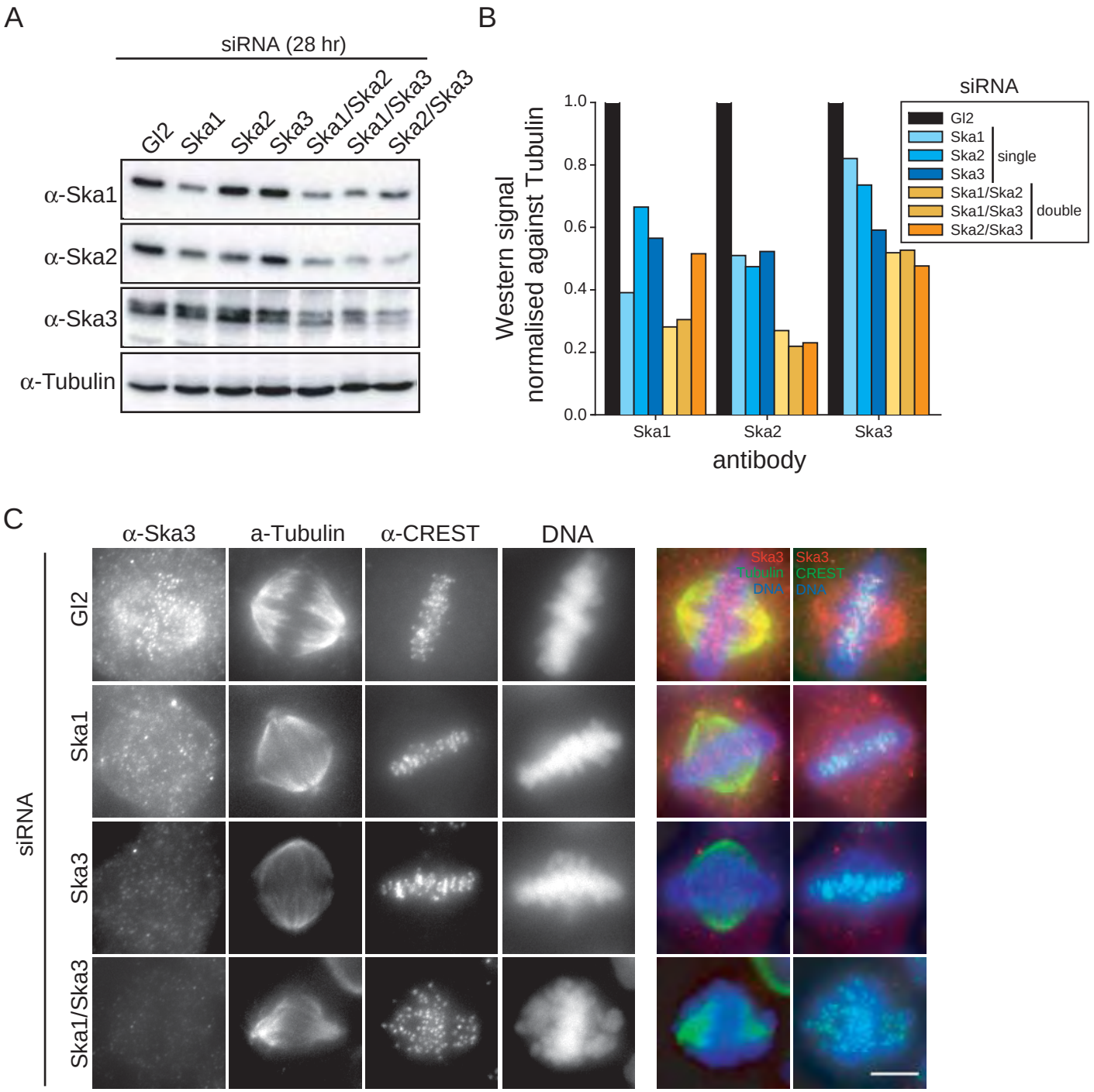


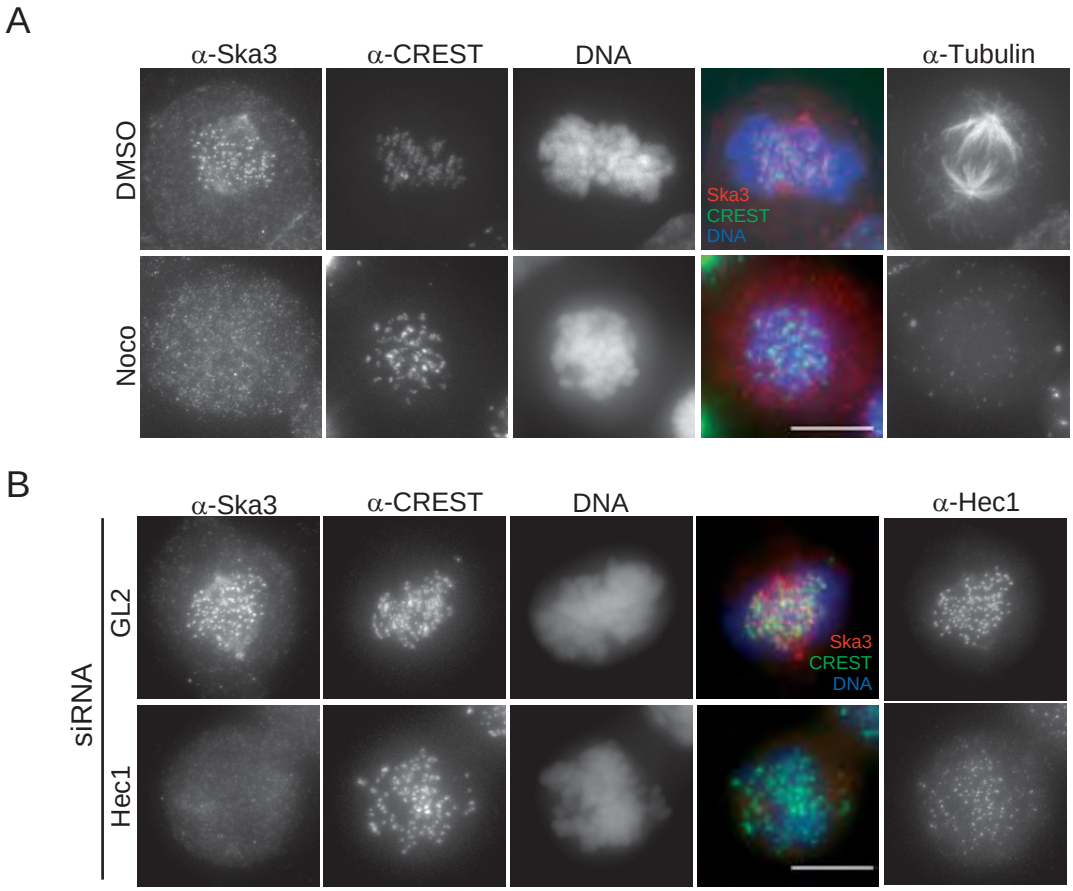
Supplementary Figure 4, Gaitanos et al.



Supplementary Figure 5, Gaitanos et al.



Supplementary Figure 6, Gaitanos et al.



Supplementary Figure 7, Gaitanos et al.

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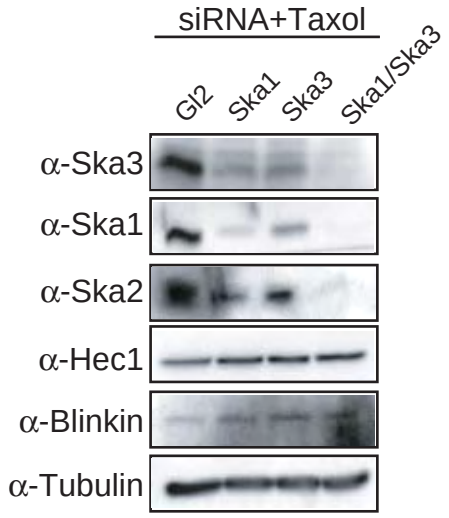

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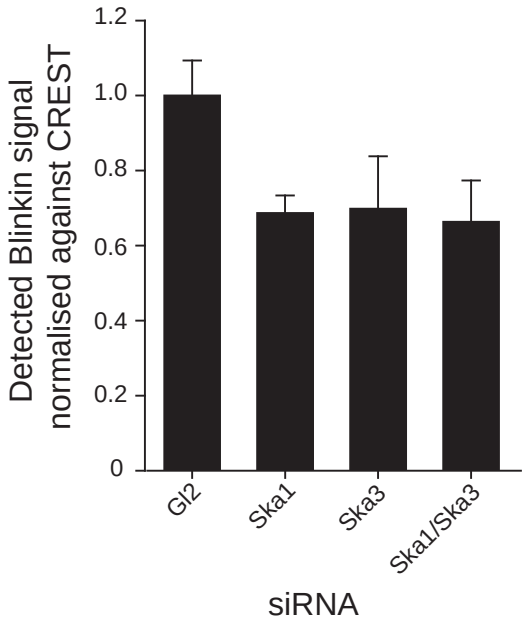

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