Molecular basis of MKLP2-dependent Aurora B transport from chromatin to the anaphase central spindle

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Supplemental Figure Legends

Figure S1. INCENP and stable cell lines. (A) HeLa cells, transiently expressing different GFP-INCENP constructs (full-length (f) WT, the T59E f point mutant, or truncations 1-58, 1-80, 1-80 RRKKRR (1-80 RRKKRR to hexa-alanine mutant), 58-end and 80-end were stained for the centromere marker CREST. Representative images of thymidine-treated cells in metaphase are shown. For INCENP\textsuperscript{1-58}, 62.5% of cells showed only cytoplasmic targeting, and 16.7% showed weak centromere targeting. For INCENP\textsuperscript{1-80} RRKKRR, 57.1% of cells showed only cytoplasmic targeting, and 21.4% showed weak centromere targeting. The scale bar marks 10\textmu m. (B) HeLa Flp-In T-Rex cells, expressing doxycycline-inducible GFP-INCENP constructs (full-length WT or truncations 1-58, 1-80, RRKKRR were induced and then stained for the centromere marker CREST. Metaphase cells are shown and the scale bar marks 10\textmu m. (C) HeLa Flp-In T-Rex cells, expressing doxycycline-inducible GFP-INCENP constructs (full-length WT, T59E f or T59A f point mutants or truncations 1-58, 1-80, RRKKRR) were depleted of endogenous INCENP using a 3'-UTR siRNA, then western blotted after doxycycline induction. Tubulin was used as a loading control. The INCENP antibody recognises an epitope in the C-terminus and does not detect the N-terminal fragments. The induced cells were stained for MKLP2 and representative images of cells in either (D) anaphase or (E) metaphase are shown. Note that N-terminal INCENP fragments unable to bind Aurora B failed to support full chromosome alignment and exhibited lagging chromatin in anaphase. The scale bar marks 10\textmu m.

Figure S2. Structure of CPI58 and CPI80 peptide complexes. (A) CPI\textsuperscript{NT} (survivin in green, borealin in light blue and INCENP in pink) crystallised as a dimer, inhibiting
H3pT3 peptide binding. INCENP N-terminus region of the symmetry related molecule is interacting with survivin, occupying the same region where the peptide should have bound. Survivin H80 and K62 are shown. (B) Electrostatic potential maps of the CPI58 and (C) CPI80 surfaces shows the negatively charged (red) peptide-binding groove, and the positive charged (blue) cleft where the T3 phosphate sits. The green density map indicates the presence of H3pT3 peptide bound to survivin of both (B) CPI58 and (C) CPI80. Superimposition of (B) CPI58 and (C) CPI80 with PDB 2qfa (yellow) (Jeyaprakash et al., 2007) highlights the different orientations of the side chain of Lys62 of survivin (green) in the presence of H3pT3, confirming its role in phosphate recognition, as previously observed for survivin (Du et al., 2012). (D) ITC data plots of survivinE65A-H80A CPI58 and (E) CPI80 mutants in the presence of H3 or H3pT3. (F) ITC data plots of CPI58 and (G) CPI80 in the presence of H2A or H2ApT121.

**Figure S3. Mapping the CPC-binding domain using an MKLP2 rigor mutant trap assay.** (A) HeLa cells transfected with full-length (amino acids 1-890) GFP-MKLP2 E413A ATPase defective rigor mutant and C-terminal truncations to positions 850, 750 and 650 were fixed, and then stained for Aurora B (red) and DNA (blue). GFP fluorescence for MKLP2 (green) was visualised directly. (B) Comparison of 10 amino acid truncations from MKLP2 position 650 to 690. Cells were stained for DNA, Aurora B, and PRC1 to mark the central spindle. GFP fluorescence for MKLP2 was visualised directly. Representative cells in anaphase are shown. (C) HeLa T-Rex GFP-MKLP2535-718 cells were left uninduced or induced with doxycycline for 18h, then fixed and stained for INCENP and Aurora B. Representative cells in metaphase, anaphase A and B are shown. Scale bars indicate 10µm in all image panels. (D)
HeLa T-Rex GFP-MKLP2<sup>535-718</sup> cells were left uninduced or induced with doxycycline for 18h, then arrested in mitosis with nocodazole for 18h. Cells were forced into anaphase using 5 µM flavopiridol to rapidly inhibit CDK1, and samples collected at the times indicated. MKLP2 complexes were isolated by GFP IP and blotted for the proteins shown in the figure.

**Figure S4. Structure of the MKLP2 CPC-binding region.** (A) A Clustal X alignment of MKLP2 sequences from different species highlighting different conserved features. (B) Crystal structure of dimeric MKLP2<sup>596-668</sup> at 1.43Å shows the disulphide bond occurring between C607 of chain A and B. Electrostatic potential on the two sides of MKLP2 indicates a highly negatively charged region (red). (C) Electrostatic potential on the surface of MKLP2 fragment 596-668 shows the negatively charged (red) 636-652 region, involved in CPC interaction. (D) Western blot of siControl and siMKLP2 cells confirmed depletion of MKLP2 and following rescue with GFP-MKLP2 (WT and mutants). Tubulin was used as a loading control. (E) SEC-MALS calculated weight-average molar masses for MKLP2<sup>557-668</sup> (yellow), CPI80 (orange) and MKLP2:CPI80 (blue) are plotted versus the elution volume. Data were analysed using the ASTRA software package (Wyatt Technology).

**Figure S5. Survivin mediates centromeric localisation of CPC through recognition of Histone H3.** (A) HeLa cells expressing different survivin-GFP constructs (WT, K62A, E65A, H80A, K62A-H80A, and E65A-H80A) were depleted of endogenous survivin using a 3'-UTR siRNA and then western blotted. Tubulin was used as a loading control. (B) HeLa Flp-In T-Rex cells, expressing doxycycline-inducible full-length GFP-INCNENP or the RRKKRR-motif mutant were depleted of
endogenous INCENP using a 3'-UTR siRNA, then western blotted following doxycycline induction. Tubulin was used as a loading control. (C) The induced cells were stained for Aurora B and histone H3 pS10. Representative images of prometaphase cells are shown. The scale bar marks 10µm. (D) GFP-INCENP enrichment (f_{centromere}/f_{cytoplasmic}) at centromeres and (E) the relative level of histone H3 pS10 phosphorylation metaphase chromatin (f_{pS10}/f_{GFP-INCENP}) are plotted in the graphs (WT n = 57, RRKKRR f n = 56), where the mean with individual data points are marked and error bars indicate SEM. An unpaired t test with Welch’s correction and 99% confidence intervals was performed (****, P < 0.0001).
Figure S2

**A**

CPI\textsubscript{INT}

Survivin
INCENP
Borealin

**B**

CPI\textsubscript{58} H3pT3

Survivin H3pT3$^1$-4
Survivin (2qfa)

**C**

CPI\textsubscript{80} H3pT3

Survivin H3pT3$^1$-7
Survivin (2qfa)

**D**

CPI\textsubscript{58} Survivin(E65A-H80A)

**E**

CPI\textsubscript{80} Survivin(E65A-H80A)

**F**

CPI\textsubscript{58} H2A

H2ApT121

**G**

CPI\textsubscript{80} H2A

H2ApT121

**Figure S2**

**A**

CPI\textsubscript{INT}

Survivin
INCENP
Borealin

**B**

CPI\textsubscript{58} H3pT3

Survivin H3pT3$^1$-4
Survivin (2qfa)

**C**

CPI\textsubscript{80} H3pT3

Survivin H3pT3$^1$-7
Survivin (2qfa)

**D**

CPI\textsubscript{58} Survivin(E65A-H80A)

**E**

CPI\textsubscript{80} Survivin(E65A-H80A)

**F**

CPI\textsubscript{58} H2A

H2ApT121

**G**

CPI\textsubscript{80} H2A

H2ApT121
Figure S3

A  

|       | 1-890 | 1-850 | 1-750 | 1-650 |
|-------|-------|-------|-------|-------|
| DNA   |       |       |       |       |
| GFP   |       |       |       |       |
| Aurora B |     |       |       |       |
| GFP AurB |   |       |       |       |

B  

|       | 1-640 | 1-660 | 1-670 | 1-680 | 1-690 |
|-------|-------|-------|-------|-------|-------|
| DNA   |       |       |       |       |       |
| GFP   |       |       |       |       |       |
| Aurora B |     |       |       |       |       |
| PRC1  |       |       |       |       |       |

C  

DNA  GFP  INCENP  Aurora B

GFP-MKLP2 (minus doxycycline)

DNA  GFP  INCENP  Aurora B

GFP-MKLP2 (plus doxycycline)

D  

|       | Inputs  | α-GFP  | Inputs  | α-GFP  |
|-------|---------|--------|---------|--------|
| 0 min |         |        | 0 min   |        |
| 2.5 min |       |        | 2.5 min |        |
| 5.0 min |       |        | 5.0 min |        |

CDK1 inhibition

GFP
MKLP2
INCENP
Aurora B
Cyclin B
PRC1
pT481

KDa: 46 100 135 46 32 58

minus doxycycline  plus doxycycline

HeLa Flip-In T-REx GFP-MKLP2
Figure S4

A

MKLP2

"Rigor" mutant E413A

Kinesin motor domain

Conservation

B

MKLP2

Chain A 596-667

Chain A side

θ - 180°

Chain B 600-663

Chain B side

C

MKLP2 CPI binding region

D

GFP-MKLP2

siControl

WT

Δ636-652

Δ690-705

RRSQR

MKLP2

GFP

Tubulin

E

Molar mass x 10^3 (g/mol)

Volume (ml)

MKLP2

MKLP2: CPI80

CPI80

63.7 kDa

100.3 kDa

25.4 kDa

41.7 kDa

63.7 kDa

100.3 kDa

25.4 kDa

41.7 kDa

63.7 kDa

100.3 kDa

25.4 kDa

41.7 kDa

63.7 kDa

100.3 kDa

25.4 kDa

41.7 kDa

63.7 kDa

100.3 kDa

25.4 kDa

41.7 kDa

63.7 kDa

100.3 kDa

25.4 kDa

41.7 kDa

63.7 kDa

100.3 kDa

25.4 kDa

41.7 kDa
Survivin

Figure S5

A

B

C

D

E

**Figure S5**

A

Survivin-GFP

B

GFP-INCENP

C

siControl - GFP-INCENP

D

INCENP enrichment on centromeres (\(f_{\text{cen}}\))

E

Relative H3pS10 intensity (\(f_{\text{pS10}}/f_{\text{GFP-INCENP}}\))