SUPPORTING ONLINE MATERIAL

SUPPLEMENTARY FIGURES

Fig S1 | (**A-C**) Pharmacological inactivation of Crm1 by LMB affects the centromeric localization of (**A**) Survivin-GFP, Aurora-B, (**C**) INCENP and, (**D**) Borealin-GFP. (**B**) LMB treatment also affects the localization of Survivin-GFP to the midbody. Cells were treated with LMB for 6 h. Aurora-B, INCENP and Survivin were detected by immunostaining (red). DNA was stained with Hoechst. Arrows mark midbody. (**E**) The Survivin NES is active *in trans*. Nuclear injected recombinant GST-SurvNES-GFP is efficiently exported, in contrast to export deficient GST-SurvNESmut-GFP. GFP-fusions were detected by fluorescence microscopy. Bars, 10 μm. (**F**) Alignment of mammalian Survivin NESs: *Homo sapiens* (Hs), *Pan trophogloydes* (Pt), *Bos taurus* (Bt), *Sus scrofa* (Ss), *Canis familiarias* (Cf), *Felis canis* (Fc), *Mus musculus* (Mm), *Rattus norvegicus* (Rn). Ω denotes aa M, V, or F. (**G**) Localization of siRNA-resistant $\text{Survivin}_{\text{mNES}}$-GFP in cells transfected with Survivin siRNA or a control siRNA together with a RFP expression plasmid as the transfection control. (**H**) Crm1-siRNA induced ablation of Crm1. Survivin-GFP expressing cells were transfected with Crm1 or a control (ctl) siRNA. 48 h later, the expression of Crm1 and Survivin-GFP was analyzed by immunoblot. Actin served as the loading control. (**J**) Crm1 and Survivin-GFP partially colocalize at the centromere. Survivin-GFP shows a significant colocalization with Crm1 compared to $\text{Survivin}_{\text{mNES}}$-GFP and metaphase chromosomes, which served as the negative control. Maximal colocalization is observed for Survivin-GFP stained with α-Survivin Ab. Cells were pre-extracted with digitonin before fixation and stained using α-Crm1 and α-Survivin Abs. DNA was visualized using TO-PRO®-3 iodide. The correlation coefficient was quantified by 2D scatterplot analysis using the Leica colocalization software. Bars, 10 μm.

Fig S2 | (**A-D**) Time-lapse imaging of GFP-fusion proteins during G2/M transition was followed by confocal microscopy in A431 cells. Bars, 10 μm.

(**E**) The localization of Survivin-GFP, $\text{Survivin}_{\text{mNES}}$-GFP and Borealin-GFP during mitosis was followed in the cells depicted in Fig 2F, S2A,D.

(**F**) IPs from metaphase enriched synchronised HeLa cell extracts was performed using α-Survivin Ab. Cells arrested in mitosis were collected by shake-off after overnight incubation with 50 ng/ml nocodazole and released from the arrest in nocodazole-free medium for 1 h prior to lysate preparation. Precipitated proteins were detected by immunoblot using α-Crm1, -Survivin, and -Aurora-B Ab.

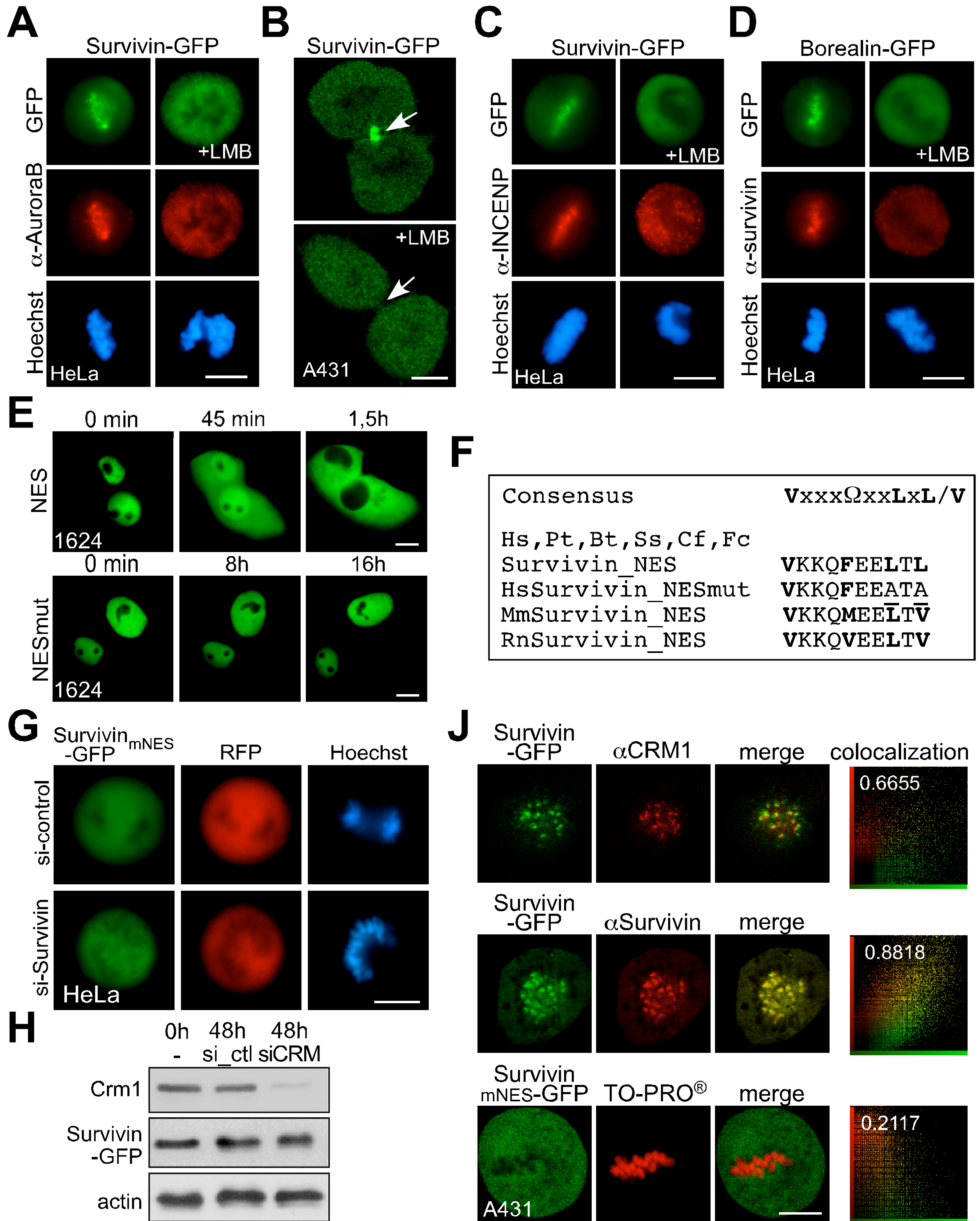
(**G**) IPs from mitotic HeLa cell extracts was performed using α-Borealin Ab. Borealin, INCENP, Aurora-B and Survivin-FLAG or $\text{Survivin}_{\text{mNES}}$-FLAG were detected by immunoblot.

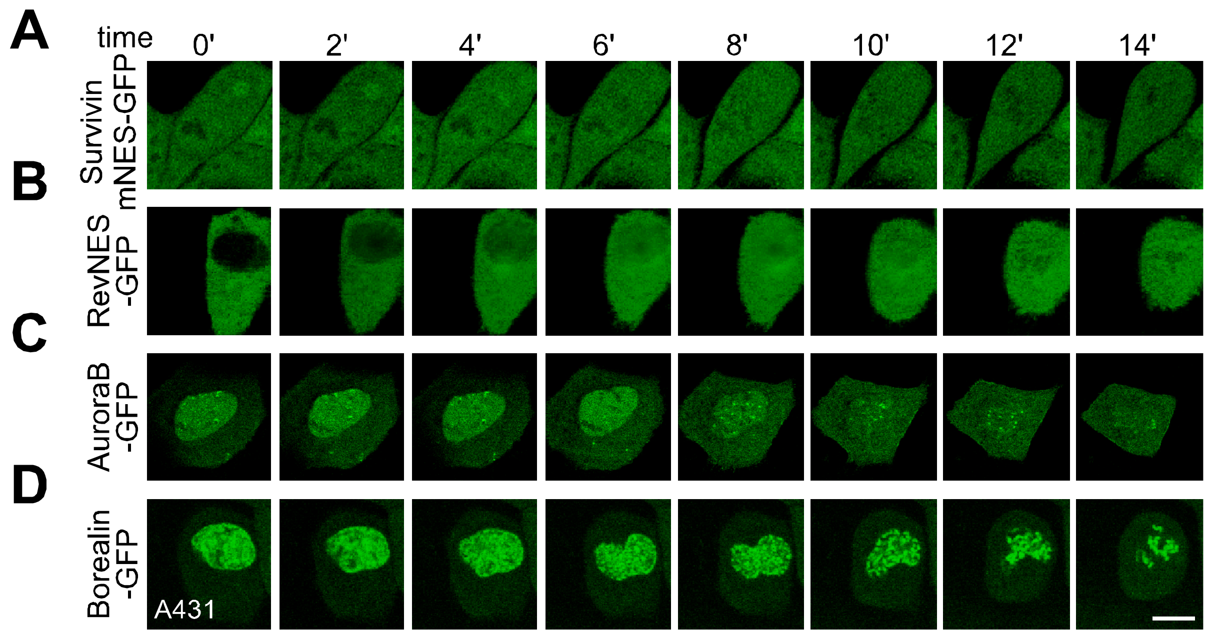
(**H**) HeLa cells stably expressing intermediate (sort1) or low (sort2) amounts of siRNA-resistant Survivin-GFP fusions (Survivin$_{R\text{-}mNES}$-GFP), were transfected with Survivin siRNA or a control siRNA together with a RFP expression plasmid. The number of cells with two or more nuclei was examined in at least 100 GFP- and RFP-double-positive cells, and the percentages of multinucleated cells were determined (mean±SD, n=3).

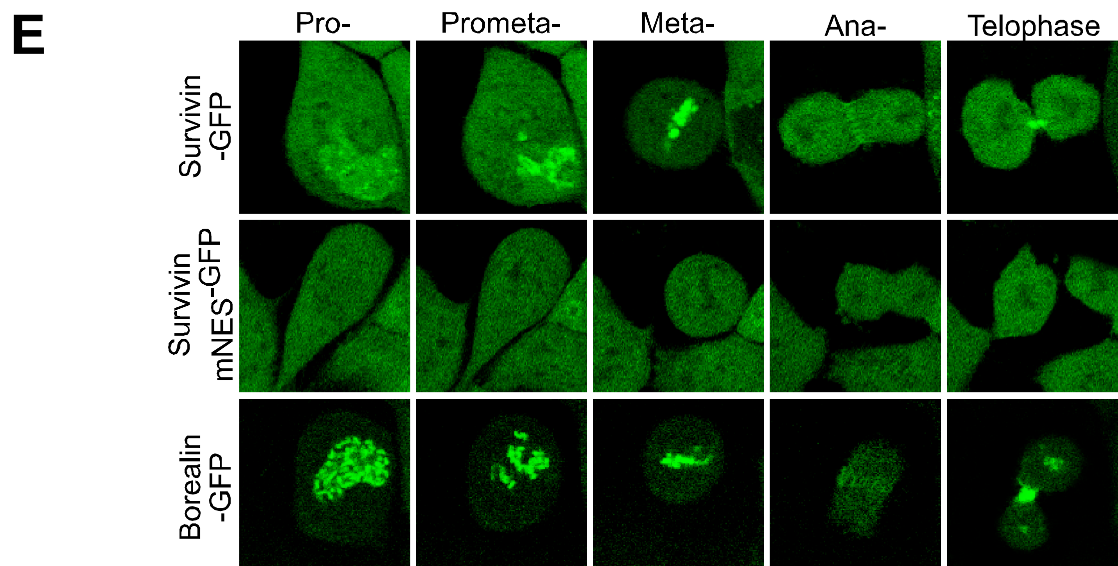
(**J**) Expression levels of Survivin$_{R\text{-}mNES}$-GFP and endogenous Survivin as well as depletion of endogenous Survivin were analyzed by immunoblot using α-Survivin Ab. Actin served as the loading control.

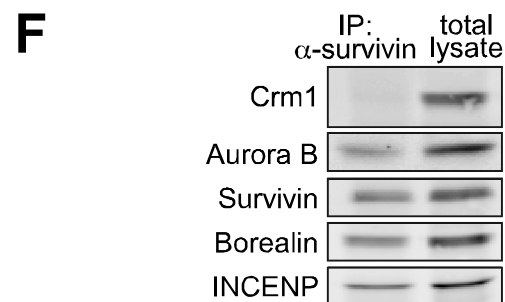
Fig S3 | Model for the role of Crm1 in targeting the CPC to the centromere. Borealin is complexed with Survivin, which can bind to Aurora-B kinase and is incorporated into the CP-holocomplex by interacting with INCENP. The NES in Survivin mediates recruitment of Crm1/RanGTP, which seems to be involved in guiding the CPC to the centromeres in early prophase by a still unknown mechanism. This process might be catalyzed by the activity of the guanine nucleotide-exchange factor RCC1 or TD60. Hydrolysis of RanGTP, by factors like RanBPs/Ran-GAP1, may facilitate the release of Crm1 and deposition of the CPC at the inner centromere.

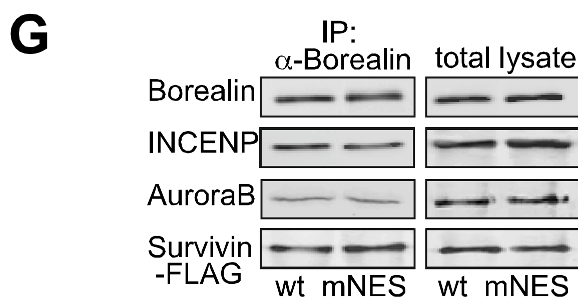
Supplementary Figure 1

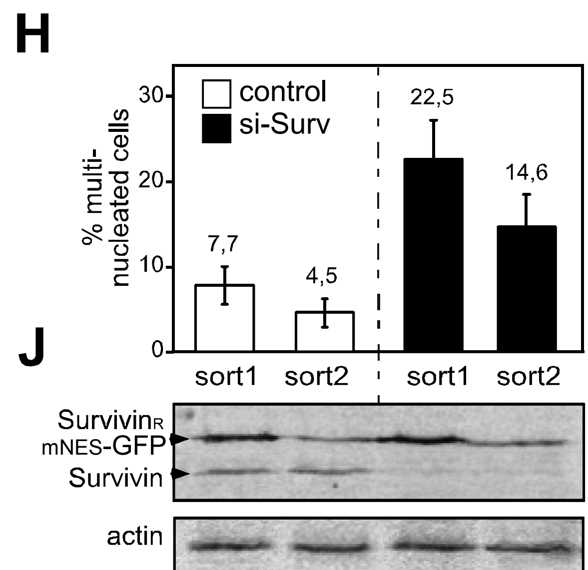


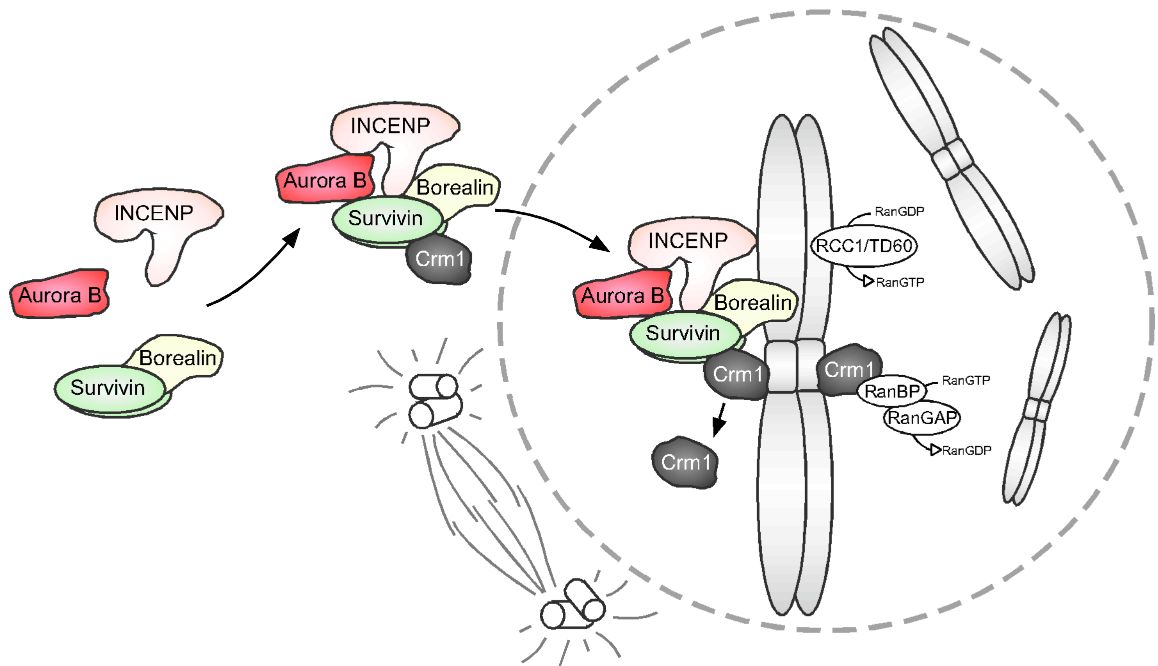

A
B
C
D
time
0'
2'
4'
6'
8'
10'
12'
14'
Survivin
mNES-GFP
RevNES
-GFP
AuroraB
-GFP
Borealin
-GFP
A431


E
Pro-
Prometa-
Meta-
Ana-
Telophase
Survivin
-GFP
Survivin
mNES-GFP
Borealin
-GFP


F
IP:
α-survivin
total
lysate
Crm1
Aurora B
Survivin
Borealin
INCENP


G
IP:
α-Borealin
total lysate
Borealin
INCENP
AuroraB
Survivin
-FLAG
wt
mNES
wt
mNES


H
% multi-
nucleated cells
control
si-Surv
30
20
10
0
7,7
4,5
22,5
14,6
sort1
sort2
sort1
sort2
J
SurvivinR
mNES-GFP
Survivin
actin


INCENP
Aurora B
Borealin
Survivin
INCENP
Aurora B
Borealin
Survivin
Crm1
INCENP
Aurora B
Borealin
Survivin
Crm1
Crm1
Crm1
RanGDP
RCC1/TD60
RanGTP
RanGTP
RanBP
RanGAP
RanGDP

SUPPLEMENTARY METHODS

Plasmids. To generate NES-deficient Survivin, aa ^{89}VKKQFEELTL^{98} were changed to ^{89}VKKQFEEATA^{98} by mutagenesis as described (Knauer et al., 2005a). Likewise, pc3Survivin_T^{97}A-GFP and pc3Survivin_T^{97}D-GFP encode Survivin mutants in which the T^{97} residue was changed to A or D by mutagenesis. Plasmids pc3Survivin$_{R\text{-}WT}$-GFP and pc3Survivin$_{R\text{-}mNES}$-GFP encode siRNA-resistant Survivin mutants, and were generated by introducing silent mutations at positions essential for siRNA-binding (nucleotides ^{1229}C→T and ^{1236}A→C). Plasmids are: pc3Survivin and pc3Survivin$_{mNES}$, encoding WT and NES-deficient Survivin; pc3Survivin-GFP and pc3Survivin$_{mNES}$-GFP, and pc3Survivin-FLAG and pc3Survivin$_{mNES}$-FLAG encode WT and NES-deficient Survivin-FLAG or -GFP fusions. Bacterial expression plasmids are: pGEX-Survivin-GFP and pGEX-Survivin$_{mNES}$-GFP, encoding WT and NES-deficient GST-Survivin-GFP fusions; pGEX-SurvivinNES-GFP and pGEX-SurvivinNESmut-GFP, encoding active and inactive GST-SurvivinNES-GFP fusions.

***In vitro* protein binding assay.** Coupled transcription/translation was performed using the TNT reticulocyte lysate system (Promega) supplemented with [^{35}S] labelled methionine (Amersham Pharmacia Biotech) and the plasmids pc3Crm1-HA, pc3Survivin-GFP, pBSK_hINCENP, pc3-Borealin, and pc3AuroraB-GFP as the templates.

Pull-down assays with the specific recombinant GST-GFP substrates bound to glutathione sepharose beads were carried according to (Knauer et al., 2005a). Briefly, 4 μg of the bound GST-GFP proteins were incubated with equal amounts of the transcription/translation mix. Following several washing steps, complexes were resolved by SDS-PAGE and visualized by fluorography. Crm1 pull-down assays were performed in the presence of 5 mM GTP-RanQ69L. GST-RanQ69L was purified in the presence of 0.1 mM GTP and stored in Ran-Buffer (50 mM HEPES, pH 7.6, 100 mM NaCl, 2 mM $MgCl_2$, 0.5 mM EDTA, 1 mM DTT, 0.1 mM GTP) following proteolytic removal of GST to ensure GTP-loading.