Supplementary figure legends:

Figure S1, Related to Figure 1. **Mitotic defects caused by I3 depletion.**

(A) Rescue of chromosome alignment by siRNA resistant GFP-I3. Stable inducible GFP-I3 HeLa cells were treated with indicated siRNAs and expression of GFP-I3 (resist) induced by doxycycline. Representative confocal images showing merges of DAPI and GFP channels. Quantification as in Fig. 1 B. Error bars indicate s.d. of 3 independent experiments with 50 cells per condition. Efficiency of siRNA-mediated depletion is shown by Western blot. HSC70 was probed as loading control. The asterisk denotes a non-specific band. Scale bar, 5 µm.

(B) SDS22 or I3 depletion causes delay of anaphase onset. The timing between nuclear envelope breakdown (NEBD) and anaphase onset (AO) was determined in live cells expressing H2B-RFP and IBB-GFP after treatment with indicated siRNAs. NEBD was monitored as a nuclear efflux of IBB-GFP and AO was monitored as the start of separation of the sister chromatids using H2B-RFP. Stills from representative time lapse movies. Scale bar, 10 µm.

Figure S2, Related to Figure 4. **I3 localization during mitosis.**

Cells stably expressing GFP-I3 were fixed and stained with DAPI. Representative images. Scale bar, 10 µm.

Figure S3, Related to Figure 4. **Restoration of SDS22 localization by overexpression of siRNA resistant I3.**

(A) I3- or control-depleted stable SDS22-GFP cells were transiently transfected with mCherry or a mCherry-fusion of I3 resistant to I3 siRNA, fixed, and DAPI stained. Representative confocal images. Scale bar, 10 µm.

(B) Percentage of metaphase cells with detectable SDS22 localization at kinetochores was quantified. Error bars indicate s.d. of 3 independent experiments with 20 cells per condition. P-value ***, $p < 0.001$.

(C) Western blot control of I3 expression levels with specific antibodies. Asterisks denote unspecific bands.

Figure S4, Related to Figure 5. **SDS22 localization to the outer kinetochore.**

SDS22 localizes to the outer kinetochore upon I3 depletion. SDS22-GFP cells were treated with indicated siRNAs, fixed and stained with CREST serum. Representative confocal images. Scale bar, 10 µm.

Figure S5, Related to Figure 5. **PP1γ localization to kinetochores depends on KNL1.**

(A) Stable GFP-PP1γ cells were treated with KNL1 or control siRNA, fixed and DAPI-stained. Representative confocal images. Scale bar, 10 µm.

(B) Percentage of metaphase cells with detectable PP1γ localization at kinetochores was quantified. Error bars indicate s.d. of 3 independent experiments with 25 cells per condition. P-value, ***, $p < 0.001$.

Figure S6, Related to Figure 6. **Partial rescue of the effect of I3 depletion by reduction of SDS22 expression.**

(A) Increased Aurora B autophosphorylation upon I3 depletion is partially reversed by attenuated SDS22 expression. Cells were treated with the first siRNA (siLuc or siI3) for 48h and a second (siLuc or siSDS22) for 24 h as indicated. Cells were fixed and stained with Aurora B-specific (AurB), phospho-T232 (phospho-AurB) antibodies and DAPI. Representative images. Scale bar, 10 µm.

(B) Quantification of the ratio of phospho-AurB versus total AurB signal intensity on chromatin in (A). Box blots show median, lower and upper quartiles (line and box), 10^{th} and 90^{th} percentiles (whiskers) and outliers (●). P-value, ***, $p < 0.001$. Data from 3 independent experiments with a total of 60 cells per condition.

Figure S7, Related to Figure 6. **Effects of I3 overexpression on PP1γ localization and Aurora B pT232 levels.**

(A) I3 does not localize to kinetochores and displaces PP1γ. Stable GFP-PP1γ cells were transiently transfected with mCherry-fusions of I3 wild-type or NIPP1, fixed and DAPI stained. Representative confocal images. Scale bar, 10 µm.

(B) Western blot control of expression levels with anti-dsRED antibodies.

(C) Western blot comparing the expression levels of transiently expressed PP1 subunits with the respective endogenous expression level by staining with anti-I3, anti-NIPP1, and anti-SDS22 antibodies as indicated. Asterisks denote non-specific bands.

(D) Overexpression of I3 increases Aurora B phosphorylation. Cells were transiently transfected with GFP or GFP-fusions of I3 wt, I3-(V41A/W43A), or NIPP1, fixed and stained with DAPI and Aurora B phospho-T232 specific antibodies. Note that overexpression of I3 wt and NIPP1, but not PP1-binding deficient I3-(V41A/W43A) increased Aurora B phosphorylation. Representative confocal images. Scale bar, 5 µm.

(E) Quantification of (D). Box blots show median, lower and upper quartiles (line and box), 10^{th} and 90^{th} percentiles (whiskers) and outliers (●). Data from 3 independent experiments with 20 cells per condition. P-values, ***, $p \leq 0.001$.

(F) Western blot control of expression levels with anti-GFP antibodies.