B56-PP2A regulates motor dynamics for mitotic chromosome alignment

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Running title: Regulation of chromosome congression by B56-PP2A
SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. Depletion of B56 subunits causes more severe chromosome misalignment than disruption of K-fibers by depletion of the KMN network components.

Using the indicated siRNAs, asynchronously growing HeLa cells were transfected twice with a 24 h interval. 48 h after the initial transfection, cells were harvested or fixed for further analysis.

(A) Immunofluorescence analysis of HeLa cells. Cells were treated with MG132 for 3 h before fixation and representative images are shown (top panels). White bars represent 2 μm. Quantification result of the severity of chromosome misalignment using immunofluorescence analysis (bottom graph) as described in Fig. 1C (n > 19 cells per each condition). NS: not statistically significant. White bars 2 μm.

(B) Real-time quantitative PCR (RT-qPCR) analysis to measure mRNA abundance of Spc24 and Spc25 (top graph) and immunoblot analysis to verify siRNA-mediated knockdown of the KMN network components (bottom panel).

(C) Immunofluorescence analysis of monopolar HeLa cells fixed after treating with Eg5 inhibitor S-trityl-L-cysteine (STLC, 2 μM) for 12 h (left panels). Schematic models of chromosome localization in each indicated condition are shown (right panel). White bars represents 2 μm.

(D) Quantification of kinetochore distance to the center of monopolar spindle (arrow) (n > 20 cells per each condition) from C.

(E) Quantification of aligned chromosome in HeLa cells (n > 100) after transfection with NUF2 siRNA (80 nM) together with control or HSET-3’UTR siRNA (20 nM) (bottom graph). Immunoblot analysis confirming siRNA-mediated depletion of NUF2 and HSET-3’UTR (top panel).

*p < 0.05; **p < 0.01; Data show the mean ± S.D.

Fig. S2. Depletion of dynein does not rescue chromosome misalignment caused in B56-depleted cells.

(A) Immunoblot analysis confirming expression of the indicated GFP-tagged HSET compared to endogenous HSET. The expression levels of GFP-HSET-WT and GFP-HSET-N593K-MD were ~30% of endogenous HSET. As ~50% of transfection efficiency was achieved in HeLa cells, the expression levels of GFP-HSET-WT and GFP-HSET-N593K-MD in single cells were estimated to ~60% of endogenous HSET.
(B, C) Using the indicated siRNAs, asynchronously growing HeLa cells were transfected twice with a 24 h interval. 48 h after the initial transfection, cells were harvested or fixed for further analysis.

(B) Real-time quantitative PCR (RT-qPCR) analysis to measure mRNA abundance of DYNC1H1 after 48 h of initial siRNA (20 nM) transfection (left and middle graphs). Data show the mean ± S.D. Immunoblot analysis confirming siRNA-mediated depletion (20 nM) of dynein heavy chain (HC) (sc-9115, Santa Cruz) and intermediate chain (IC) (sc-13524, Santa Cruz) (right panel).

(C) Quantification of chromosome alignment in HeLa cells (left graph, n > 100 cells per each condition) as described in Fig. 1E.

Fig. S3. HSET binds Hec1 through its stalk domain

For immunoprecipitation (A-C), cells were lysed in 0.5% NP-40 cell lysis buffer [50 mM HEPES (pH 7.5), 150 mM NaCl, 5 mM EDTA, 0.5% NP-40] containing 1 mM DTT, complete Mini (Roche) and phosphatase inhibitor cocktails (Sigma), and subjected to immunoprecipitation with antibodies against HA (Y-11, Santa Cruz) at 4 °C overnight. After incubation, protein A/G-plus agarose beads (Santa Cruz) were added for 2 h at 4 °C. The beads were washed with 0.5% NP-40 cell lysis buffer and subjected to immunoblot analysis.

(A) Mitotic lysates from nocodazole-arrested HEK293 cells transiently expressing GFP-HSET together with either control or Myc-Hec1 (a.a. 1-409) were subjected to immunoprecipitation with antibodies against the Myc epitope. Immunoprecipitated proteins were analyzed by immunoblotting using the indicated antibodies. Whole cell lysate (WCL) was used as the loading control.

(B) Schematic diagram of HSET truncation mutants used to assess the Hec1 interaction.

(C) Mitotic lysates from nocodazole-arrested HeLa cells transiently expressing Myc-Hec1 (a.a. 1-409) together with either control or HA-HSET fragment (a.a. 140-350) were subjected to immunoprecipitation with antibodies against the HA epitope. Immunoprecipitated proteins were analyzed by immunoblotting using the indicated antibodies.

(D) HeLa cells were treated with nocodazole overnight after double transfection of either control or B56 siRNAs. Cells were then washed and released into media containing MG132 for 3 h. Lysates were collected for immunoblot analysis probing with the indicated antibodies including pS55-Hec1 (gift from Jennifer DeLuca). B56δ protein level indicates B56 (αγδε) knockdown efficiency.
SUPPLEMENTARY MOVIE LEGENDS

Movie S1. Mitotic progression of HeLa transfected with control siRNA, Related to Fig. 3. Time-lapse live-cell images. For Movies S1-3, HeLa cells stably expressing GFP-Histone H2B were transfected with the indicated siRNAs twice with a 24 h interval. 12 h after the second transfection, time-lapse live-cell imaging analysis was performed for 14 h with 10 min intervals. Time scale (h:min:sec). White bars 20 µm.

Movie S2. Mitotic progression of B56-depleted HeLa cells, Related to Fig. 3.

Movie S3. Mitotic progression of B56- and HSET-co-depleted HeLa, Related to Fig. 3.
**Movie 1.**
Movie 2.

Movie 3.