Expanded View Figures

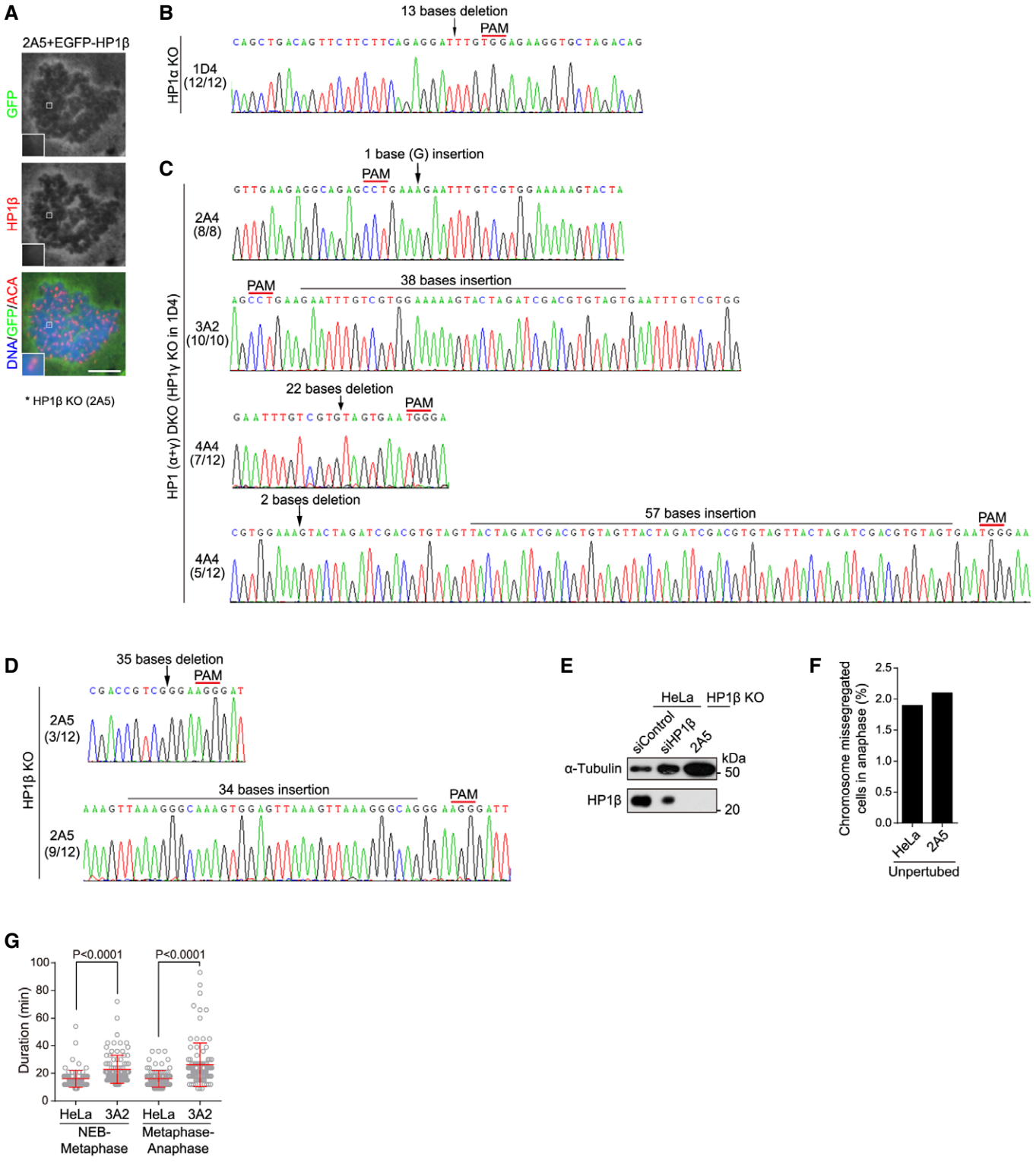

Figure EV1.

Figure EV1. DKO of HP1α and HP1γ causes defective mitosis progression (related to Fig 1).

A EGFP-HP1β was transiently expressed in HeLa cells in which endogenous HP1β was stably knocked out by CRISPR/Cas9. Chromosome spreads prepared from nocodazole-arrested mitotic cells were immunostained. Scale bar, 10 μm.

B, C Genomic DNA sequencing of HeLa-derived clones in which HP1α (B), or HP1α and HP1γ (C), were knocked out. The genomic DNA PCR fragments were subcloned and sequenced. For clone 1D4 cells, all 12 bacterial colonies showed insertion of 13 bases. For clone 2A4 cells, all eight bacterial colonies showed insertion of one base. For clone 3A2 cells, all 10 bacterial colonies showed insertion of 38 bases. For clone 4A4 cells, 7 out of 12 bacterial colonies showed insertion of 22 bases, whereas the rest five bacterial colonies showed insertion of 57 bases and deletion of two bases.

D Genomic DNA sequencing of HeLa-derived clone 2A5 cells in which HP1β was knocked out. The genomic DNA PCR fragments were subcloned and sequenced. Three out of 12 bacterial colonies showed deletion of 35 bases, whereas the rest nine bacterial colonies showed insertion of 34 bases.

E, F Asynchronous HeLa and the indicated HP1β KO clone 2A5 were immunoblotted (E), or were fixed and stained with ACA and DAPI. The percentage of cells with lagging chromosomes was determined in 100 anaphase cells.

G The mitosis progression of HeLa and HP1 DKO clone 3A2 cells stably expressing H2B-GFP were analyzed by time-lapse live imaging (related to Fig 1D). The time from NEB to metaphase chromosome alignment, and from metaphase to anaphase onset, was determined (unpaired *t*-test). Means and SDs are shown.

Source data are available online for this figure.

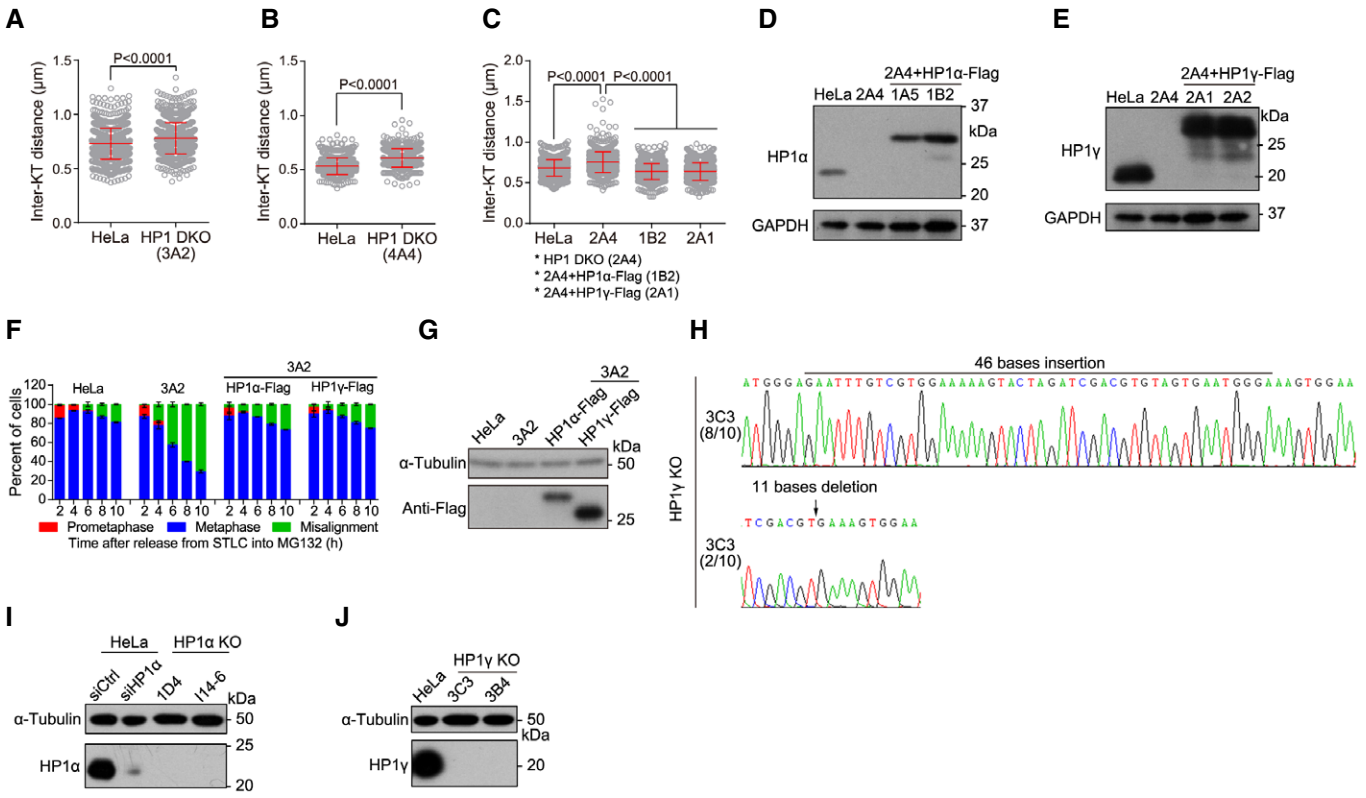

Figure EV2. HP1α and HP1γ are redundantly required to protect mitotic centromere cohesion (related to Fig 2).

A–C The indicated stable cell lines were treated with nocodazole for 3 h. Mitotic chromosome spreads were stained with CENP-C or ACA antibodies and DAPI. The inter-KT distance was measured on over 400 chromosomes in over 20 cells (unpaired *t*-test). Means and SDs are shown.

D, E Lysates of asynchronous HeLa and HP1 DKO clone 2A4 cells with or without stable expression of the indicated exogenous HP1 were immunoblotted.

F, G HeLa and HP1 DKO clone 3A2 cells with or without transient expression of HP1α-Flag or HP1γ-Flag were analyzed for metaphase chromosome alignment in around 200 cells. Means and ranges are shown (F; $n = 2$). Lysates of asynchronous cells were immunoblotted (G).

H Genomic DNA sequencing of HP1γ KO clone 3C3 cells. The genomic DNA PCR fragments were subcloned and sequenced. Eight out of 10 bacterial colonies showed insertion of 46 bases, whereas the rest two bacterial colonies showed deletion of 11 bases.

I, J Lysates of asynchronous HeLa and HP1α or HP1γ KO cells were immunoblotted.

Source data are available online for this figure.

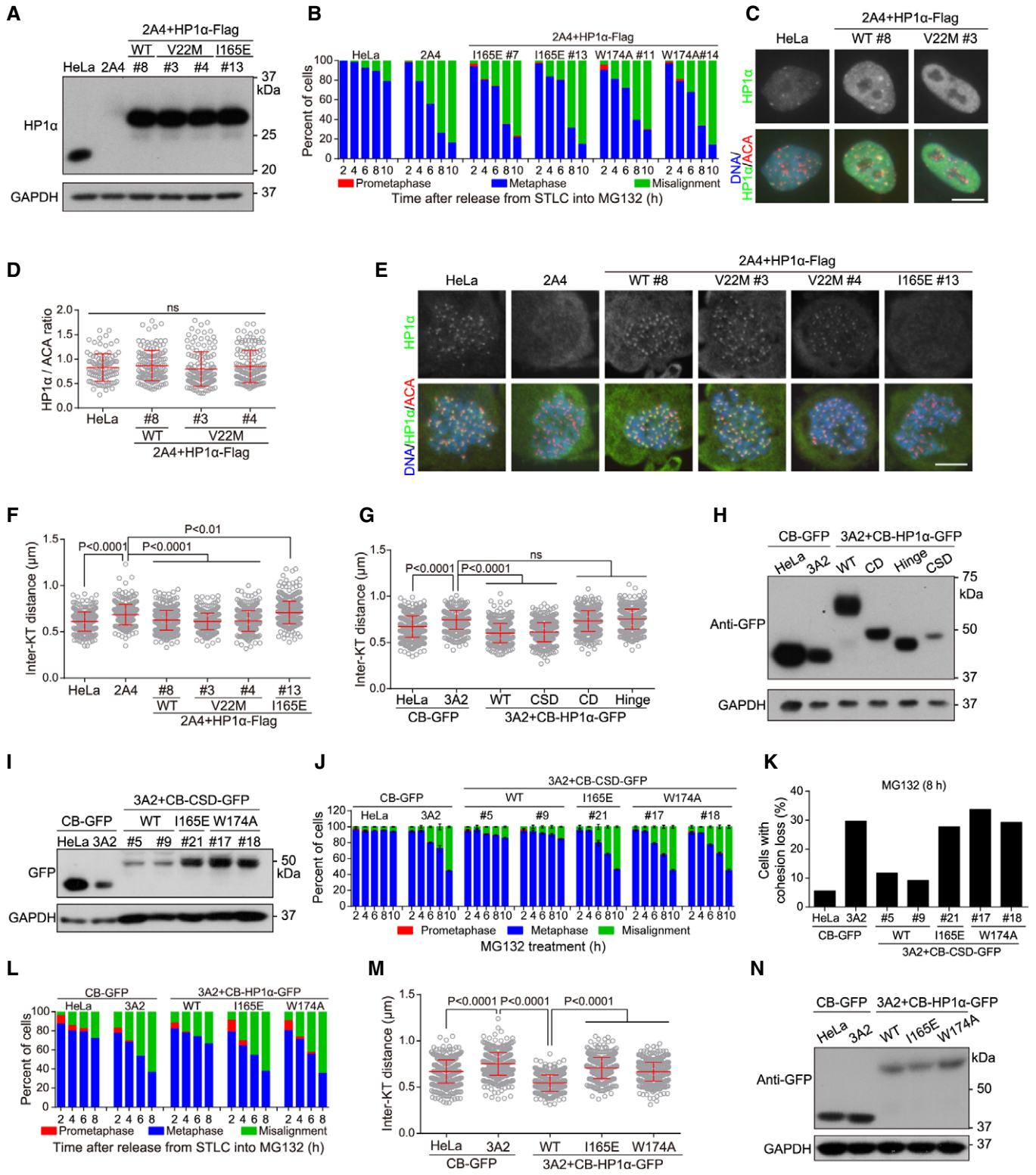

Figure EV3.

Figure EV3. CSD localization at centromeres is necessary and sufficient for HP1 to protect centromeric cohesion (related to Fig 3).

A Lysates of asynchronous HeLa and HP1 DKO clone 2A4 cells with or without stable expression of the indicated exogenous HP1α were immunoblotted.

B HeLa and the indicated stable cell lines were released from 5-h treatment with STLC into MG132-containing medium, fixed at the indicated time points for DNA staining, and then quantified in around 100 cells.

C Asynchronous HeLa cells and the indicated stable cell lines were immunostained.

D, E HeLa and the indicated stable cell lines were treated with nocodazole for 3 h. Mitotic chromosome spreads were immunostained. The centromeric HP1α/ACA immunofluorescence intensity ratio was determined on over 100 chromosomes in 10 cells (D) (unpaired *t*-test). Example images are shown (E).

F HeLa and the indicated stable cell lines were treated with nocodazole for 3 h. Mitotic chromosome spreads were stained with ACA antibodies and DAPI. The inter-KT distance was measured on over 400 chromosomes in over 20 cells (unpaired *t*-test).

G HeLa and HP1 DKO clone 3A2 cells transiently expressing the indicated fusion proteins were treated with nocodazole for 3 h. Mitotic chromosome spreads were stained with CENP-C antibodies and DAPI. The inter-KT distance was measured on over 400 chromosomes in over 20 cells (unpaired *t*-test).

H Lysates of asynchronous HeLa and HP1 DKO clone 3A2 cells transiently expressing the indicated fusion proteins were immunoblotted.

I Lysates of asynchronous HeLa and HP1 DKO clone 3A2 cells stably expressing the indicated fusion proteins were immunoblotted.

J The indicated stable cell lines were analyzed for metaphase chromosome alignment in around 200 cells. Means and ranges are shown (n = 2).

K The indicated stable cell lines were analyzed for cohesion loss in around 100 cells.

L HeLa and HP1 DKO clone 3A2 cells transiently expressing the indicated fusion proteins were released from 5-h treatment with STLC into MG132-containing medium, fixed at the indicated time points for DNA staining, and then quantified in around 100 cells.

M HeLa and HP1 DKO clone 3A2 cells transiently expressing the indicated fusion proteins were treated with nocodazole for 3 h. Mitotic chromosome spreads were stained with CENP-C antibodies and DAPI. The inter-KT distance was measured on over 400 chromosomes in over 20 cells (unpaired *t*-test).

N Lysates of asynchronous HeLa and HP1 DKO clone 3A2 cells transiently expressing the indicated fusion proteins were immunoblotted.

Data information: Means and SDs are shown (D, F, G, J, and M). Scale bars, 10 μm.
Source data are available online for this figure.

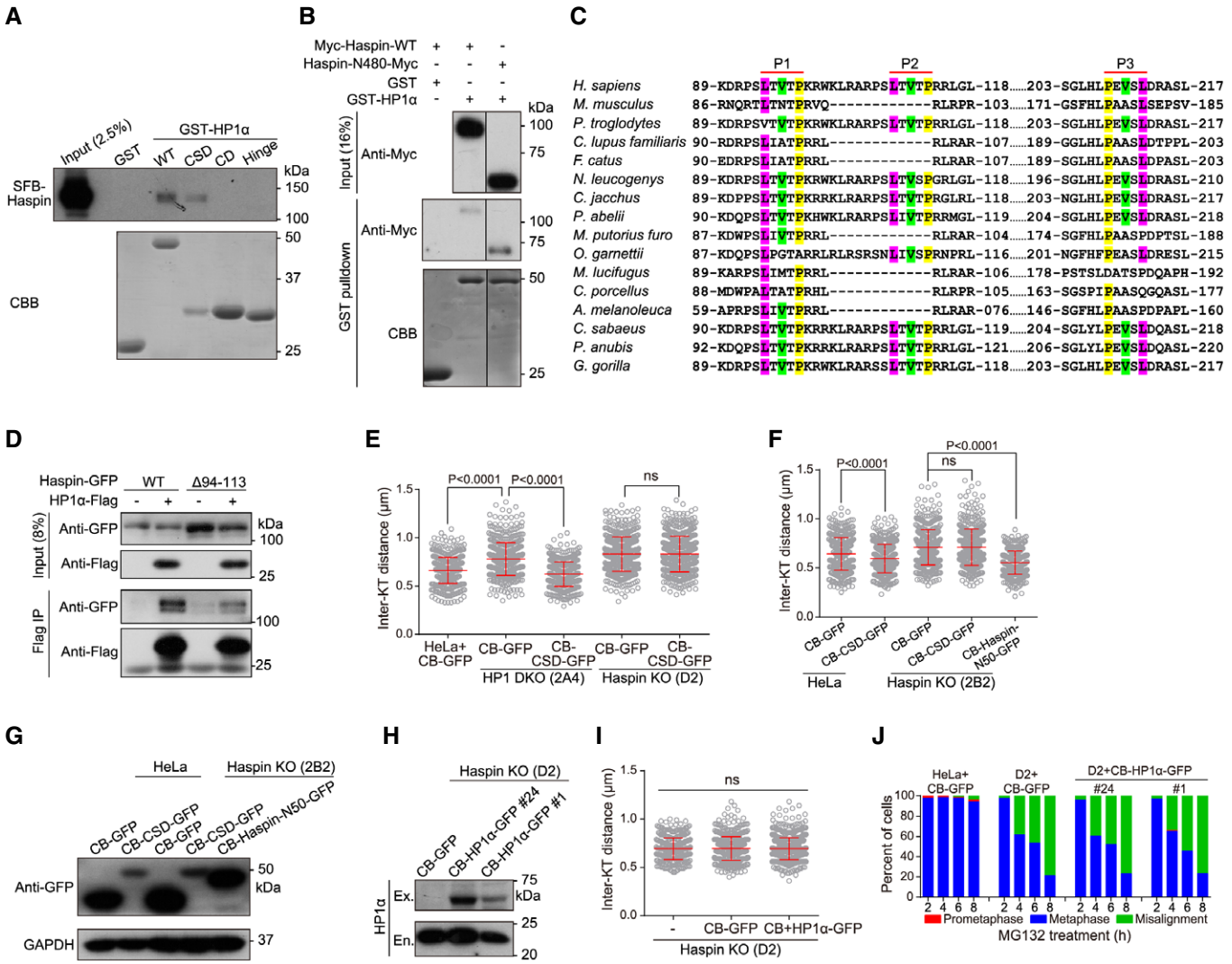

Figure EV4. Haspin binds HP1 CSD and is required for HP1-dependent cohesion protection (related to Fig 4).

A Lysates of nocodazole-arrested mitotic HeLa cells stably expressing SFB-Haspin were subjected to pulldown by GST or GST-fused HP1α (WT and the indicated domains/fragments), followed by anti-Flag immunoblotting and CBB staining for GST proteins.

B Lysates of nocodazole-arrested mitotic HeLa cells transiently expressing Myc-Haspin or Haspin-N480-Myc were subjected to pulldown by GST or GST-fused HP1α.

C Alignment of Haspin N-terminal sequences surrounding three PxVxL motifs.

D Lysates of nocodazole-arrested mitotic HeLa cells transiently expressing HP1α-Flag and Haspin-GFP (WT or Δ94–113) were subjected to immunoprecipitation followed by immunoblotting. Parts of the images are shown in Fig 4A.

E The indicated stable cell lines were treated with nocodazole for 3 h. Mitotic chromosome spreads were stained with CENP-C antibodies and DAPI. The inter-KT distance was measured on over 400 chromosomes in over 20 cells (unpaired *t*-test).

F, G HeLa and Haspin KO clone 2B2 cells transiently expressing the indicated fusion proteins were treated with nocodazole for 3 h. Mitotic chromosome spreads were stained with CENP-C antibodies and DAPI. The inter-KT distance was measured on over 400 chromosomes in over 20 cells (F) (unpaired *t*-test). Asynchronous cell lysates were immunoblotted (G).

H Lysates of asynchronous Haspin KO clone D2 cells stably expressing the indicated fusion proteins were immunoblotted. Ex., exogenous, En., endogenous.

I Haspin KO clone D2 cells stably expressing the indicated fusion proteins were treated with nocodazole for 3 h. Mitotic chromosome spreads were stained with CENP-C antibodies and DAPI. The inter-KT distance was measured on over 400 chromosomes in over 20 cells (unpaired *t*-test).

J HeLa and Haspin KO clone D2 cells stably expressing the indicated fusion proteins were exposed to MG132, then fixed at the indicated time points for DNA staining, and quantified in around 100 cells.

Data information: Means and SDs are shown (E, F and I).
Source data are available online for this figure.

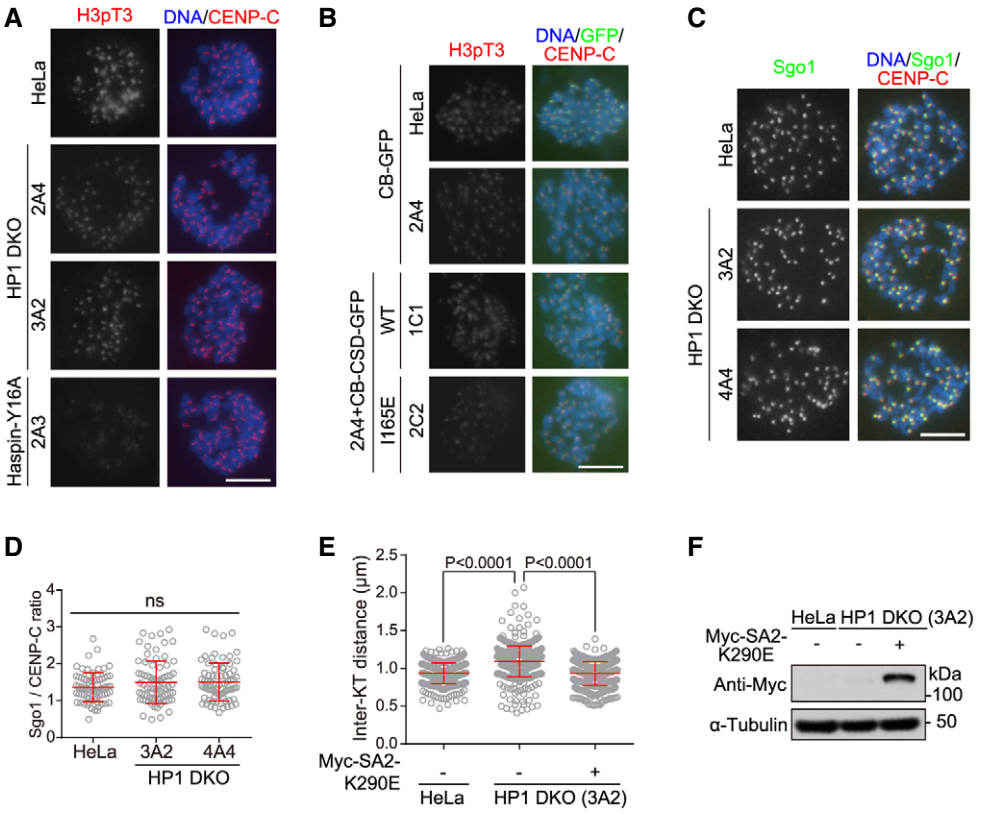

Figure EV5. HP1 promotes centromeric localization of Haspin to antagonize Wapl (related to Fig 5).

A, B Example images of cells described in Fig 5A and C.

C, D HeLa and the indicated HP1 DKO clones were treated with nocodazole for 3 h. Mitotic chromosome spreads were immunostained. Example images are shown (C). The centromeric Sgo1/CENP-C immunofluorescence intensity ratio was determined on around 100 chromosomes in 10 cells (D) (unpaired *t*-test).

E, F Chromosome spreads prepared from nocodazole-arrested mitotic HeLa and HP1 DKO clone 3A2 cells transiently expressing Myc-SA2-K290E were immunostained. Then, the inter-KT distance was measured on over 400 chromosomes in over 20 cells (unpaired *t*-test) (E), and lysates of asynchronous cells were immunoblotted (F).

Data information: Means and SDs are shown (D and E). Scale bars, 10 μm.
Source data are available online for this figure.