JCB

Supplemental material

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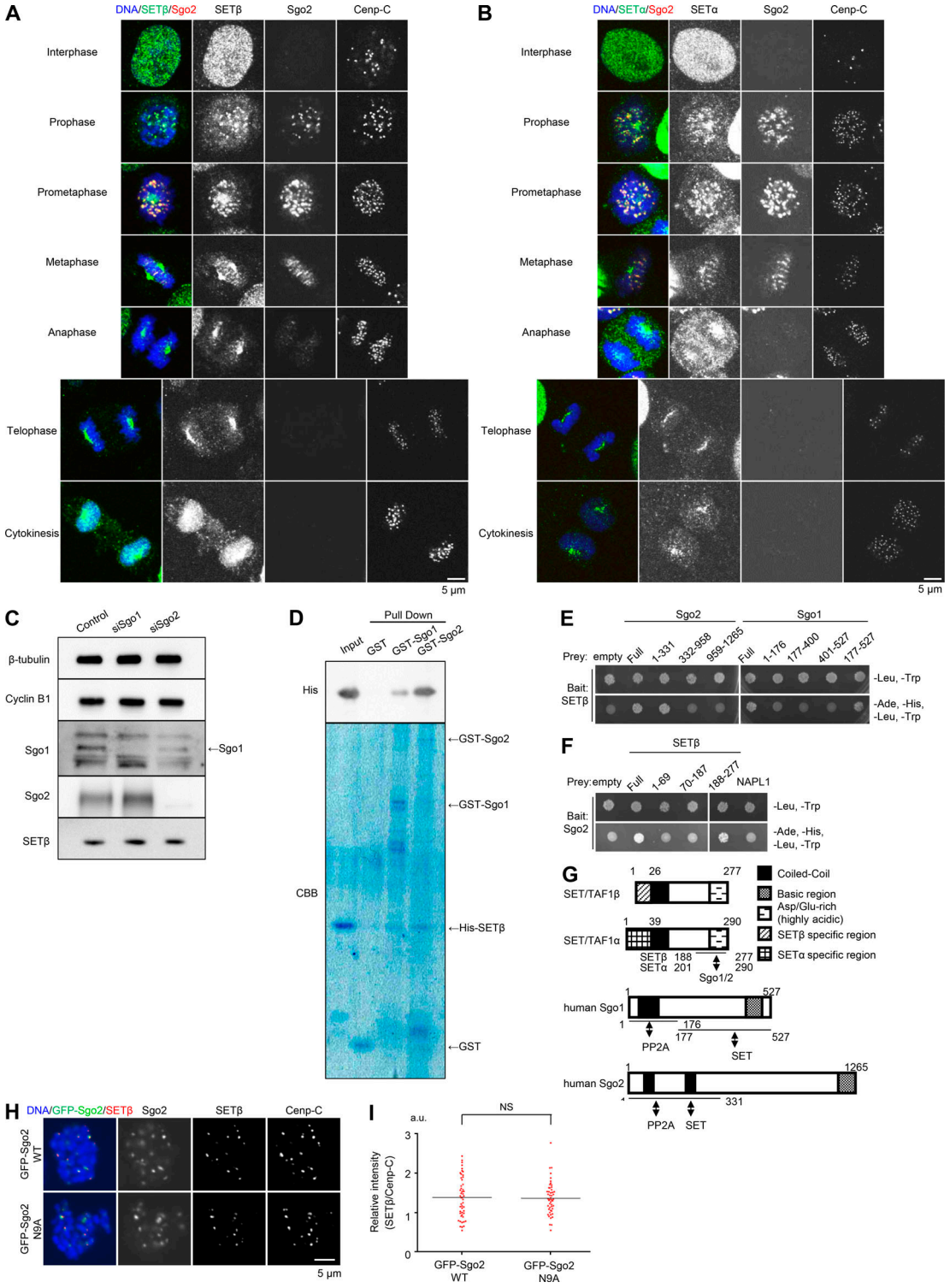

Figure S1. **SET localizes at centromeres through direct interaction with Sgo2 during mitosis. (A and B)** SETβ (A) and SETα (B) localize in the nucleus during interphase and at the centromere during mitosis. **(C)** Sgo1 and Sgo2 were knocked down with siRNA. HeLa cells treated with Sgo1, Sgo2, or control siRNA were analyzed by immunoblot using anti-β-tubulin, anti-SETβ, anti-Sgo1, and anti-Sgo2 antibodies. **(D)** His_6-SETβ protein was pre-incubated with GST, GST-Sgo1, or GST-Sgo2, pulled down with glutathione-sepharose beads, and analyzed by immunoblot using anti-His antibody or Coomassie brilliant blue. **(E and F)** Yeast two-hybrid assay indicates that SETβ interacts with the middle and C-terminus of Sgo1 (177–527 aa) and the N-terminus of Sgo2 (1–331 aa) through its C-terminal domain (188–277 aa). **(G)** Schematic diagrams indicate the interaction domains between human Sgo1, human Sgo2, and human SET/TAF1α/β. Coiled-coil domain (Zaytsev et al., 2016), basic region (gray), and Asp/Glu-rich (highly acidic) region (dotted) of SETα/β. **(H and I)** SETβ localizes at centromeres without direct interaction with PP2A on Sgo2. Signals for GFP-Sgo2 WT or N9A and SETβ were arrested at prometaphase in Sgo2 RNAi cells expressing RNAi-resistant GFP-Sgo2 WT or N9A defective for interaction with PP2A. Each bar represents the mean (n = 50 centromeres from 10 cells, 5 centromeres per cell; Mann-Whitney U test). Scale bars, 5 μm.

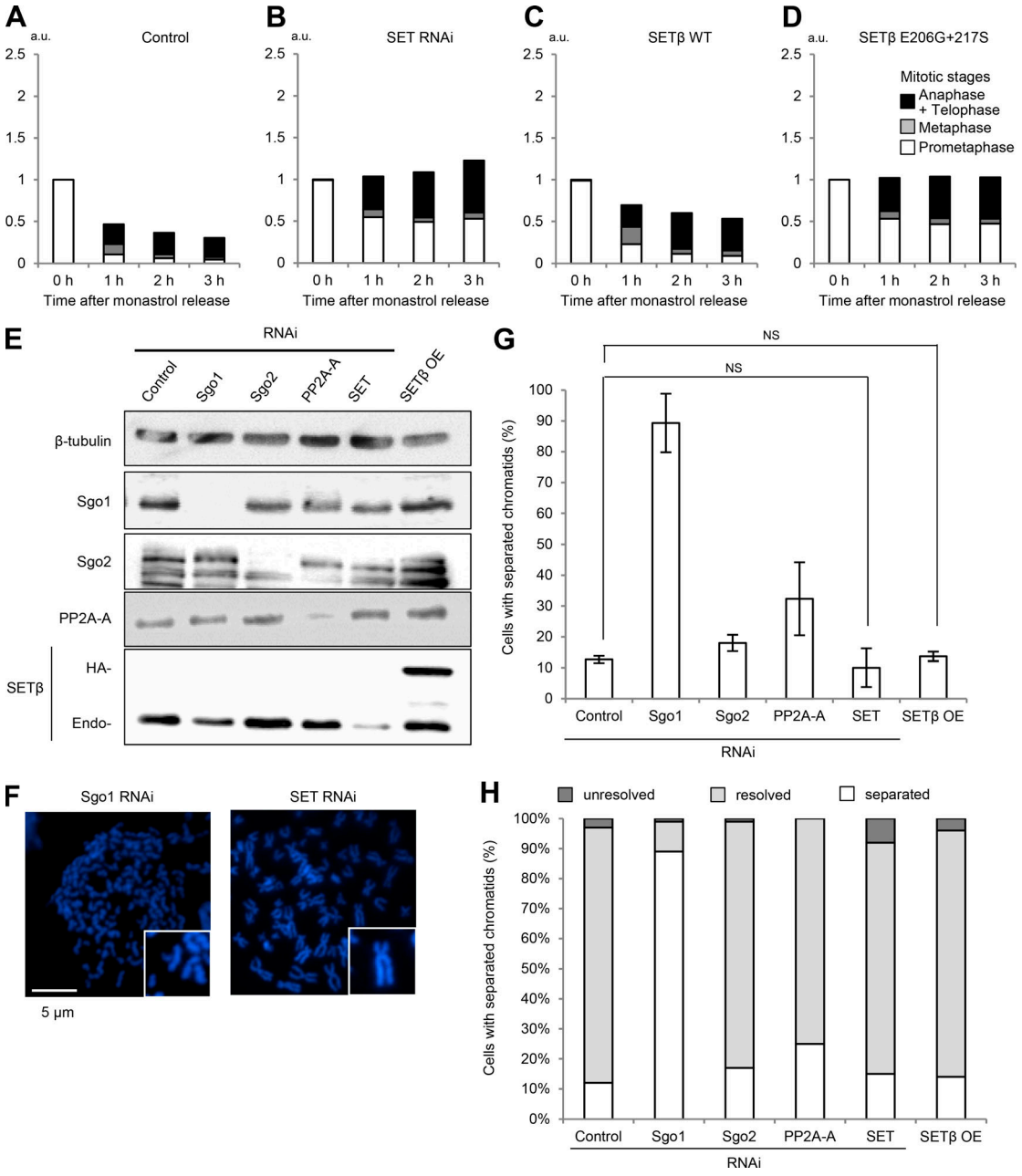

Figure S2. **SET depletion causes mitotic delay following chromosome misalignment. (A–D)** After double-thymidine block and release, cells with or without HA-SET WT or E206G+L217S replacement were arrested with monastrol for 5 h and released. Graph shows the populations of the indicated stages in mitosis at each time point after release from monastrol (n = 3 independent experiments, 100 mitotic cells per experiment). **(E)** Extracts from mitotic HeLa cells after treatment with siRNA or shRNA virus, or transfection with HA-SETβ were immunoblotted with the indicated antibodies. Endo, endogeneous; OE, overexpression. **(F)** Mitotically synchronized HeLa cells after treatment with siRNA or shRNA, or transfection with HA-SETβ, were collected by mitotic shake-off, and the chromosomes were spread and stained with DAPI. Scale bar, 5 μm. **(G and H)** The frequencies (%) of cells showing separated chromatids (G) and unresolved chromatids (H) were determined. Each bar represents SD (n = 3 independent experiments, 100 cells per experiment).

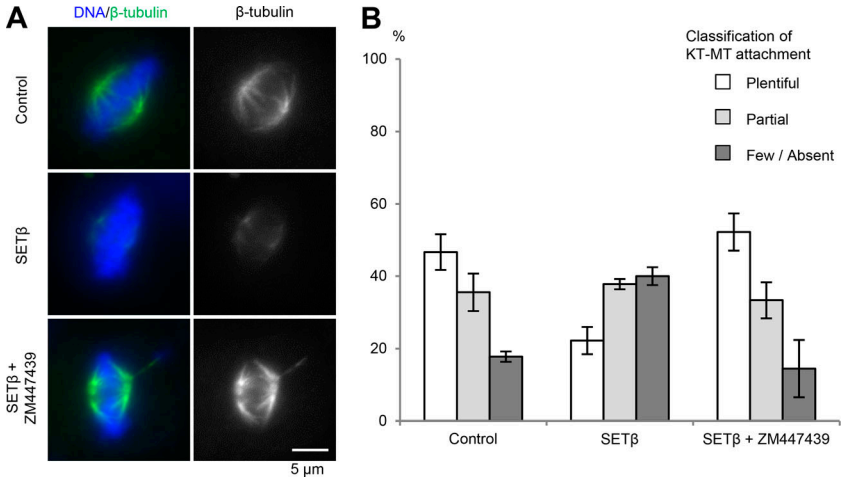

Figure S3. **Aurora B inactivation stabilizes K-fibers, counteracting SETβ overexpression. (A and B)** Cells were transfected with control or HA-SETβ plasmids, arrested in mitosis by nocodazole treatment for 6 h, and released into MG132 for 2 h with or without ZM447439 treatment for 30 min, before cold treatment and fixation as shown in Fig. 3 D. Bars represent SD (*n* = 3 independent experiments, 100 cells per experiment).

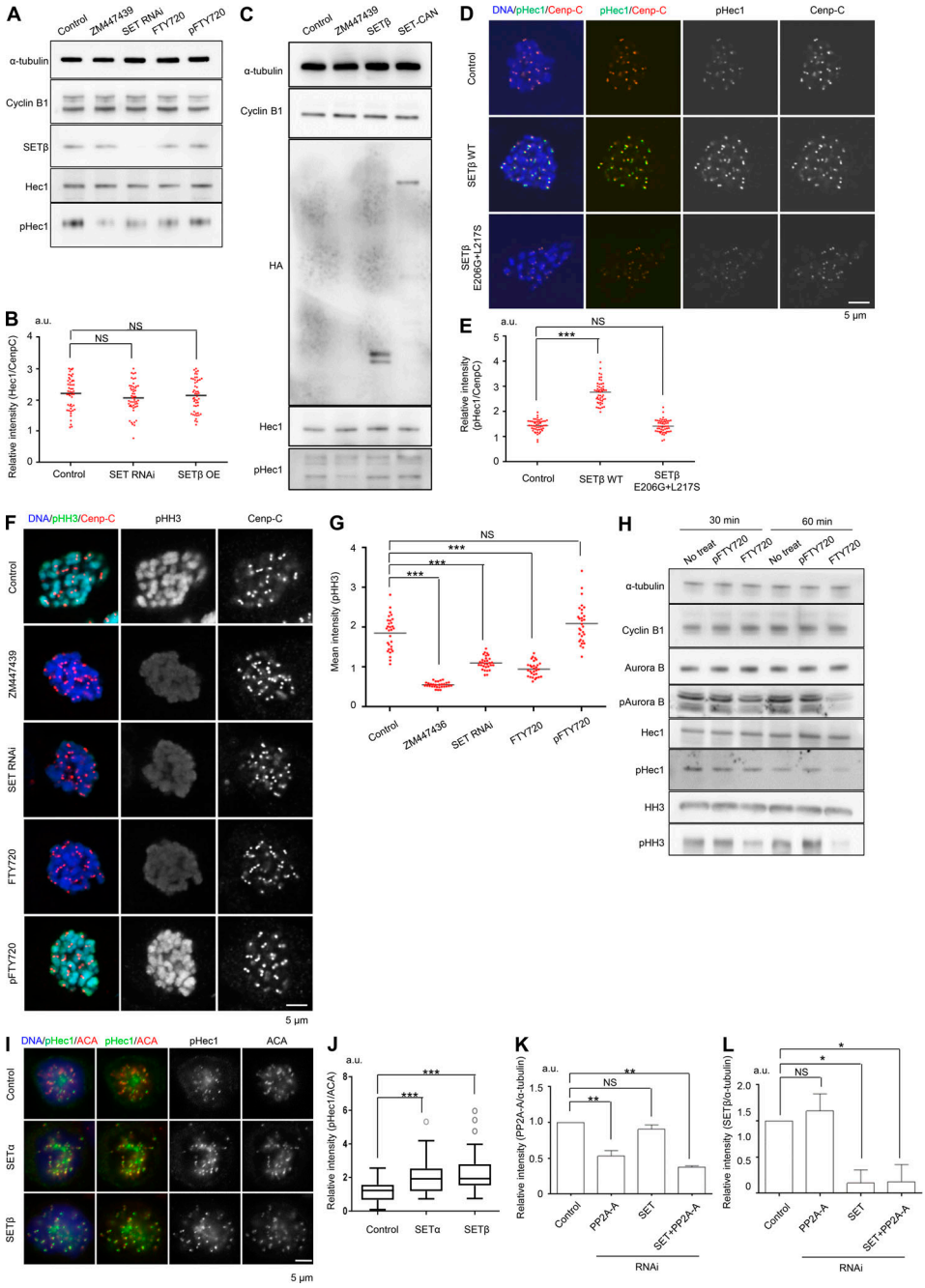

Figure S4. **SETβ is required for the phosphorylation of Aurora B substrates. (A)** HeLa cells infected with control or shSET viruses were synchronized at prometaphase with nocodazole for 3 h, including treatment with or without ZM447439 for 30 min, FTY720 for 1 h, or pFTY720 for 1 h. Mitotic cells were collected by shake-off and analyzed by immunoblotting with the indicated antibodies. **(B)** Neither SET depletion nor SETβ overexpression affected the amount of Hec1 protein at kinetochores. HeLa cells infected with control or shSET viruses and transfected with HA-SETβ or control plasmids were synchronized with nocodazole for 3 h and then immunostained with anti-Hec1 antibody and Cenp-C. Each bar represents the mean (n = 50 kinetochore pairs from 10 cells; Mann-Whitney U test). **(C)** HeLa cells transfected with HA-SETβ or HA-SET-CAN were synchronized at prometaphase with nocodazole for 3 h, including treatment with or without ZM447439 for 30 min. Mitotic cells were collected by shake-off and analyzed by immunoblotting with the indicated antibodies. **(D and E)** HeLa cells transfected with control, HA-SETβ WT, or E206G+L217S plasmids were synchronized at prometaphase with colcemid for 3 h and then immunostained with anti–phospho-Hec1 and anti–Cenp-C antibodies. Each bar represents the mean (n = 50 kinetochore pairs from 10 cells; Mann-Whitney U test; ***, P < 0.001). Scale bar, 5 µm. **(F and G)** HeLa cells infected with control or shSET viruses were synchronized at prometaphase with colcemid for 3 h, including treatment with or without ZM447439 for 30 min, FTY720, or pFTY720 for 60 min. Then, cells were immunostained with anti–phospho-histone H3 (HH3 pS10) and anti–Cenp-C antibodies. Scale bar, 5 µm. Each bar represents the mean (n = 30 cells; Mann-Whitney U test; ***, P < 0.001). **(H)** HeLa cells were synchronized at prometaphase with nocodazole for 3 h, including treatment with or without FTY720 or pFTY720 as a negative control for 30 or 60 min and analyzed by immunoblotting with the indicated antibodies. **(I and J)** Mitotically synchronized HeLa cells transfected with control, HA-SETβ or HA-SETα plasmids were immunostained with anti–phospho-Hec1 antibody and ACA. Bars represent whiskers and median (n = 40 kinetochore pairs from 8 cells; Mann-Whitney U test; ***, P < 0.001). Scale bar, 5 µm. **(K and L)** Relative intensities of PP2A-A/α-tubulin or SETβ/α-tubulin of Fig. 4 F were quantified, respectively. Bars represent SD (n = 3 independent experiments; Welch's t test; *, P < 0.05; **, P < 0.01).

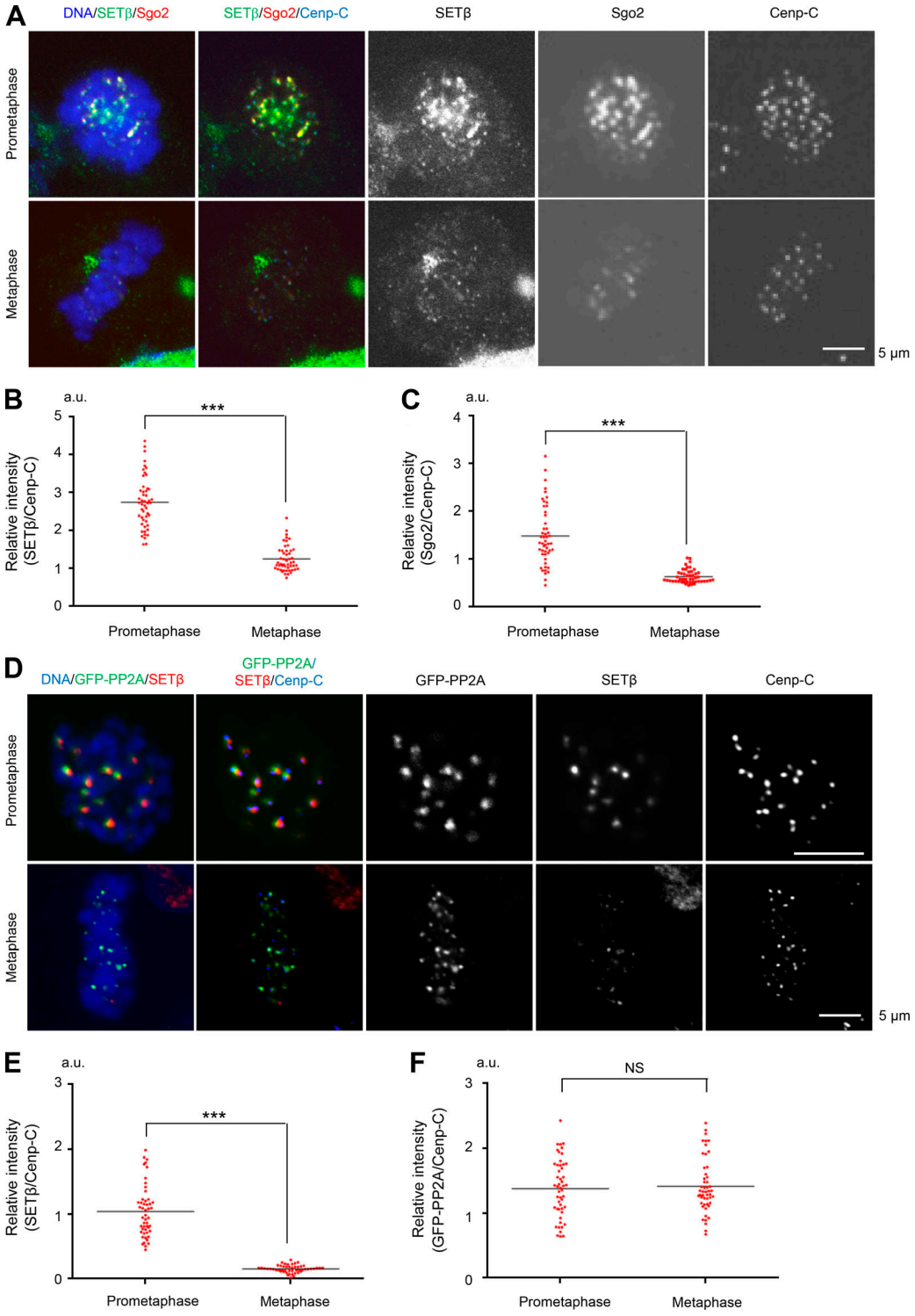

Figure S5. **The SETβ signal at centromere/kinetochore weakens in metaphase. (A–C)** Comparison between centromeric localization at prometaphase and kinetochore localization at metaphase of SETβ and Sgo2 (n = 50 kinetochore pairs from 10 cells; Mann-Whitney U test; ***, P < 0.001). **(D–F)** Comparison of centromere/kinetochore localization of SETβ and GFP-PP2A B56α between prometaphase and metaphase. HeLa cells expressing GFP-PP2A-B56α were synchronized at prometaphase with colcemid or at metaphase with MG132 and then stained with anti-SETβ and Cenp-C antibodies. Relative fluorescence intensities of GFP-PP2A B56α or SETβ toward anti–Cenp-C antibodies were quantified at five centromeres in each cell. Each bar represents the mean (n = 50 kinetochore pairs from 10 cells; Mann-Whitney U test; ***, P < 0.001). Scale bars, 5 μm.