Supplemental material

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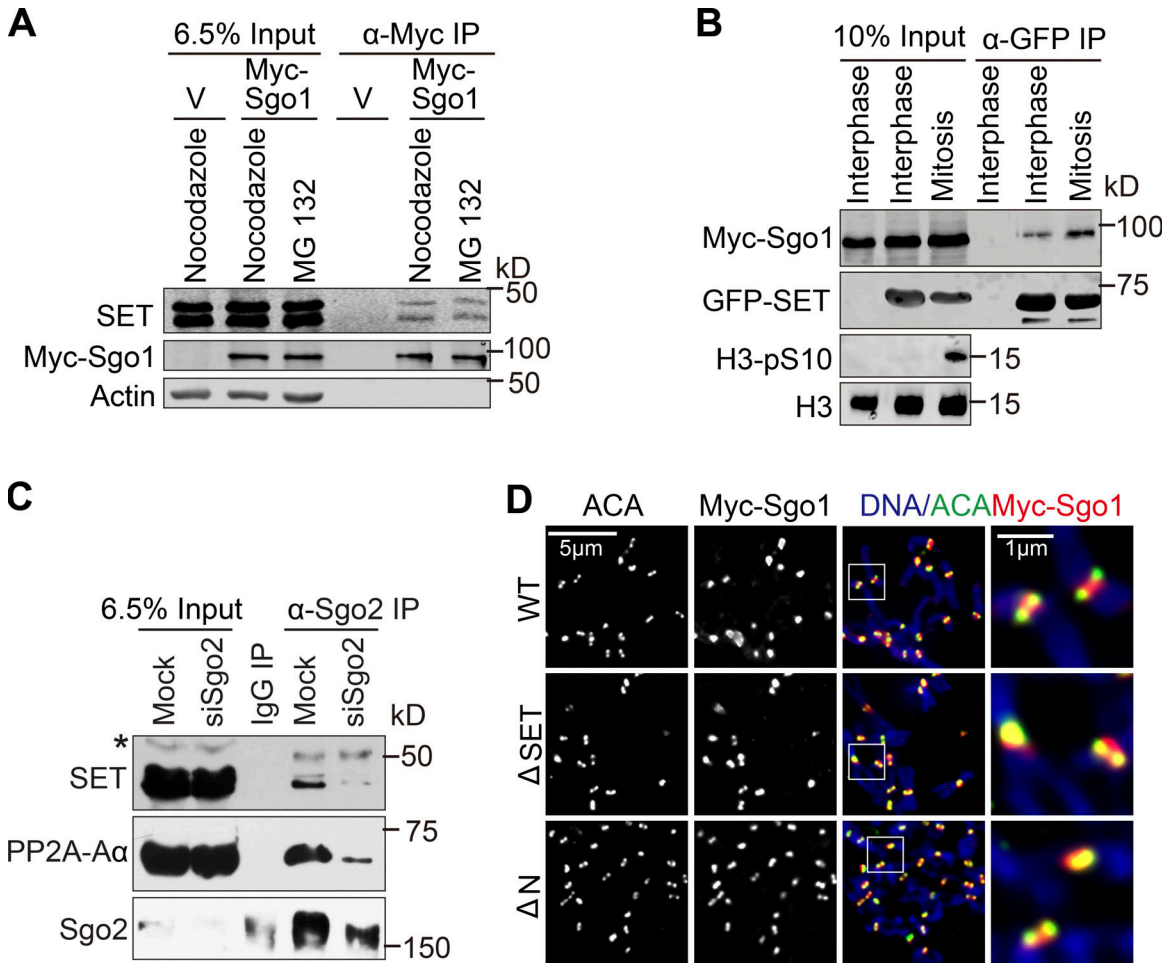

Figure S1. **SET binds Shugoshin in interphase and mitosis. (A)** Lysates of nocodazole-arrested or MG132-arrested HeLa Tet-On cells transfected with vector control or plasmids containing Myc-Sgo1 were incubated with antibody against Myc. Pelleted proteins were resolved on SDS-PAGE and blotted with the indicated antibodies. **(B)** Lysates of interphase or nocodazole-arrested HeLa Tet-On cells transfected with plasmids containing Myc-Sgo1 or GFP-SET were incubated with antibody against GFP. The immunoprecipitated proteins were resolved with SDS-PAGE and blotted with the indicated antibodies. **(C)** Lysates of mitotic HeLa Tet-On cells with mock or siSgo2 treatment were incubated with antibody against Sgo2. The immunoprecipitated proteins were resolved with SDS-PAGE and blotted with the indicated antibodies. The asterisk denotes non-specific bands. **(D)** Representative images of nocodazole-arrested HeLa Tet-On cells expressing Myc-Sgo1 WT, ΔSET, or ΔN. The outlined regions are amplified and shown in the right panel.

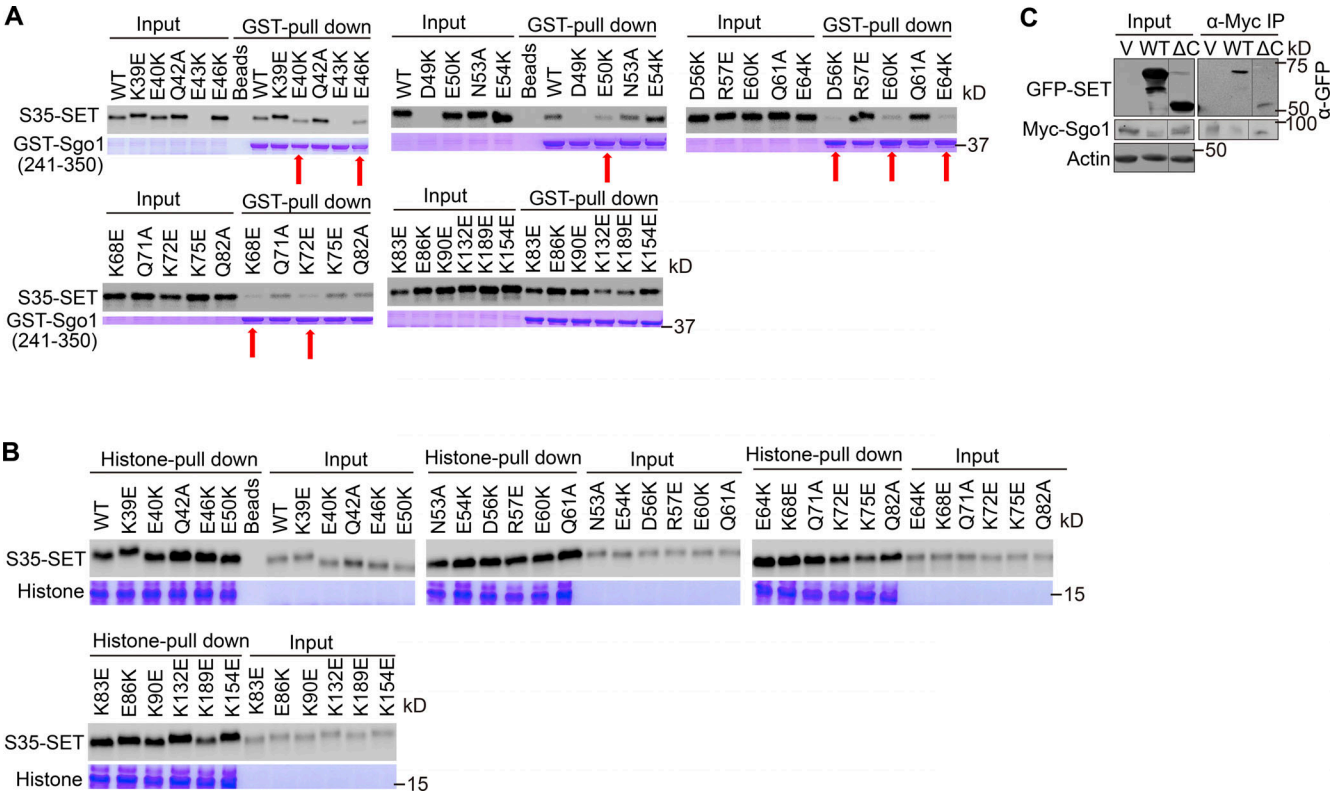

Figure S2. **Identification of the residues in SET important for Sgo1 binding. (A and B)** Mapping the residues of SET responsible for binding Sgo1 (A) and histones (B). In vitro–translated S35-radiolabeled SET proteins were incubated with recombinant GST-Sgo1 (resides 241–350) proteins (A) or bead-coupled bulk histone proteins (B). The pelleted proteins were resolved with SDS-PAGE and stained with Coomassie blue. Dried gels were visualized by a phosphoimager. Various residues in the SET dimerization domain that are not involved in SET dimerization were chosen and residues (K132 and K189) that have been found to cross-link with Sgo1 were also examined. Arrows indicate that single mutations of E40K, E46K, E50K, D56K, E60K, E64K, K68E, and K72E decreased the Sgo1–SET binding. Of note, the mutants E43K and D49K were not translated in vitro. **(C)** Nocodazole-arrested HeLa Tet-On cells stably expressing Myc-Sgo1 (1–472) were transfected with vectors or plasmids containing GFP-SET or ΔC. Cell lysates were then incubated with antibody against Myc. Pelleted proteins were resolved on SDS-PAGE and blotted with the indicated antibodies. The gel was spliced from the same one.

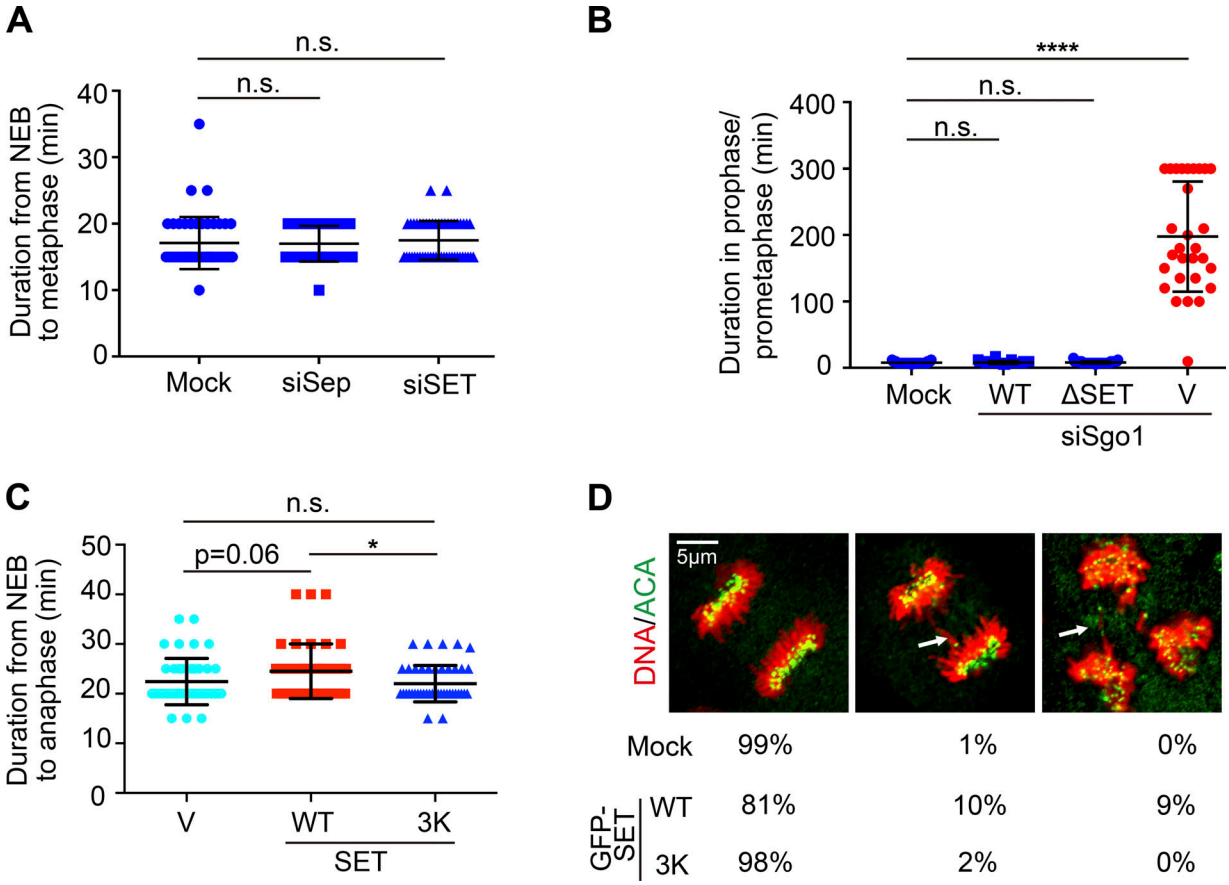

Figure S3. **SET promotes proper chromosome segregation during mitosis. (A and B)** Quantification of the duration from NEB to metaphase in Fig. 4, A and C. "V" in B denotes vector control. The average and standard deviation are shown. One-way ANOVA was performed followed by pairwise comparisons using Tukey's test. n.s., not significant. ****, $P < 0.0001$. **(C)** Time-lapse analysis of H2B-mCherry RPE-1 cells stably expressing Sgo1 WT or ΔSET. Quantification of the duration from NEB to anaphase onset is shown here. The average and standard deviation are shown. At least 40 mitotic cells were analyzed for each condition. *, $P < 0.05$. One-way ANOVA was performed followed by pairwise comparisons using Tukey's test. n.s., not significant**. (D)** Overexpression of SET WT, not 3K, induces chromosome missegregation. Shown are representative images of unperturbed mitotic RPE1 transfected with vectors or plasmids containing GFP-SET WT or 3K. Missegregated chromosomes are marked with arrows. At least 40 anaphase cells were analyzed for each condition. Average from two independent experiment is shown here.

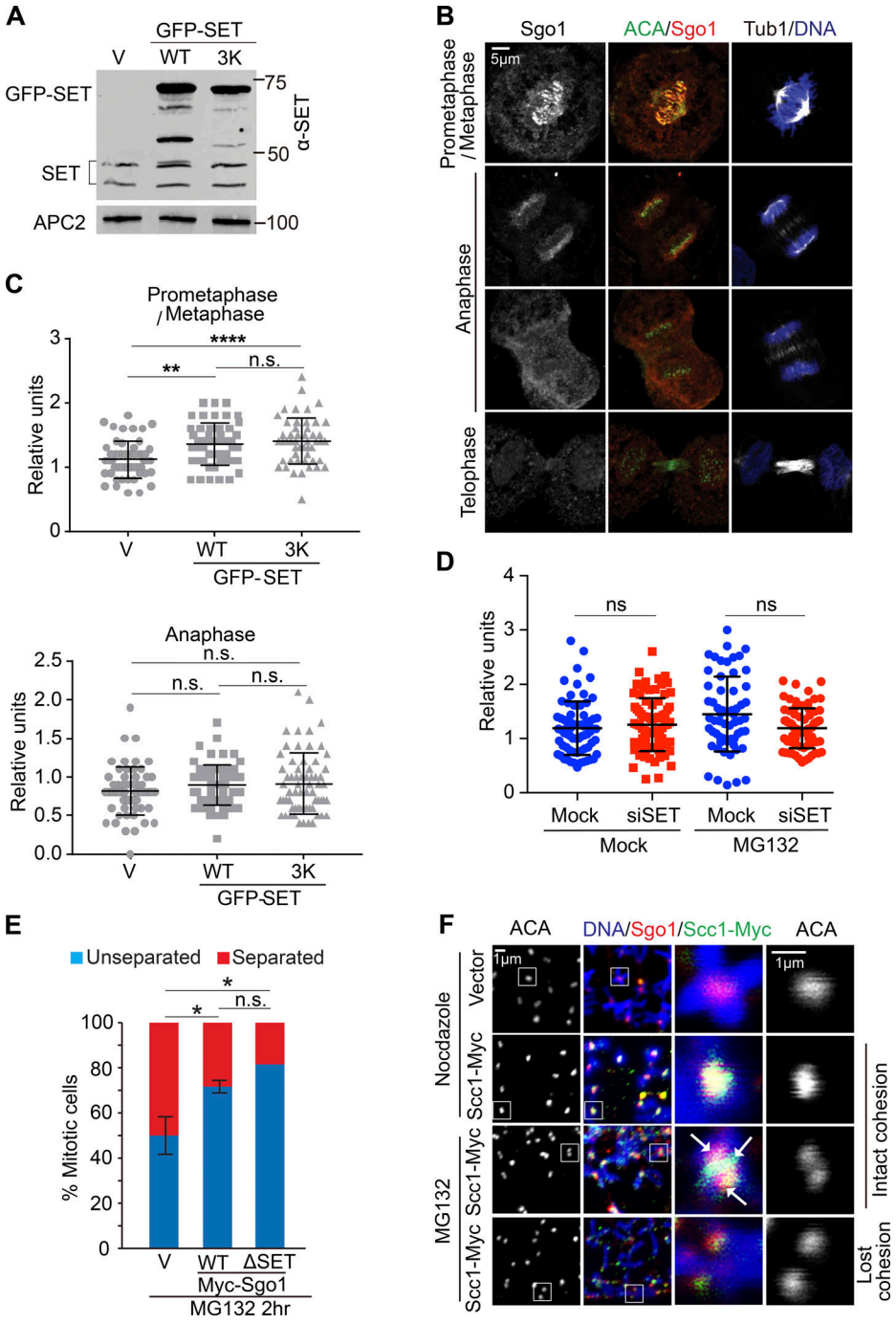

Figure S4. **SET overexpression or depletion does not significantly alter Sgo1 levels on chromosomes. (A)** Lysates of HeLa Tet-On cells transfected with vectors (V) or plasmids containing GFP-SET WT or 3K were resolved with SDS-PAGE and blotted with the indicated antibodies. **(B)** Representative images of unperturbed mitotic HeLa Tet-On cells stained with the indicated antibodies. **(C)** Shown is quantification of the total Sgo1 intensity at kinetochores and inner centromeres in cells transfected with vectors or plasmids containing GFP-SET WT or 3K. The average and standard deviation are shown. At least 60 chromosomes (five per cell) were examined for each condition. The relative intensity was derived from the intensity of Sgo1 signals normalized to the one of ACA signals. **, $P < 0.01$; ****, $P < 0.0001$. One-way ANOVA was performed followed by pairwise comparisons using Tukey's test. n.s., not significant. **(D)** HeLa Tet-On cells with mock or siSET treatment were treated with DMSO (Mock) or MG132 for 1.5 h Mitotic cells were collected for immunostaining with CREST and antibody against Sgo1. Quantification of Sgo1 intensity at kinetochores and inner centromeres is shown here (see the details in Materials and methods). The relative intensity was derived from the intensity of Sgo1 signals normalized to one of the ACA signals. The average and standard deviation are shown. At least 60 kinetochores (five per cell) were examined for each condition. One-way ANOVA was performed followed by pairwise comparisons using Tukey's test. n.s., not significant. **(E)** HeLa Tet-On cells transiently transfected with vectors or plasmids containing Myc-Sgo1 WT or ΔSET were treated with MG132 for 2 h. Quantification of unseparated and separated sister chromatids, described in Fig. 6 B, is shown here. *, $P < 0.05$. The average and standard deviation are shown. One-way ANOVA was performed followed by pairwise comparisons using Tukey's test. n.s., not significant. **(F)** HeLa Tet-On cells transfected with vectors or plasmids containing Scc1-Myc were treated with nocodazole (2 h) or MG132 (2 h). Representatives of chromosome spreads incubated with the indicated antibodies are shown here. Arrows show the separation of Sgo1 and centromeric cohesin.

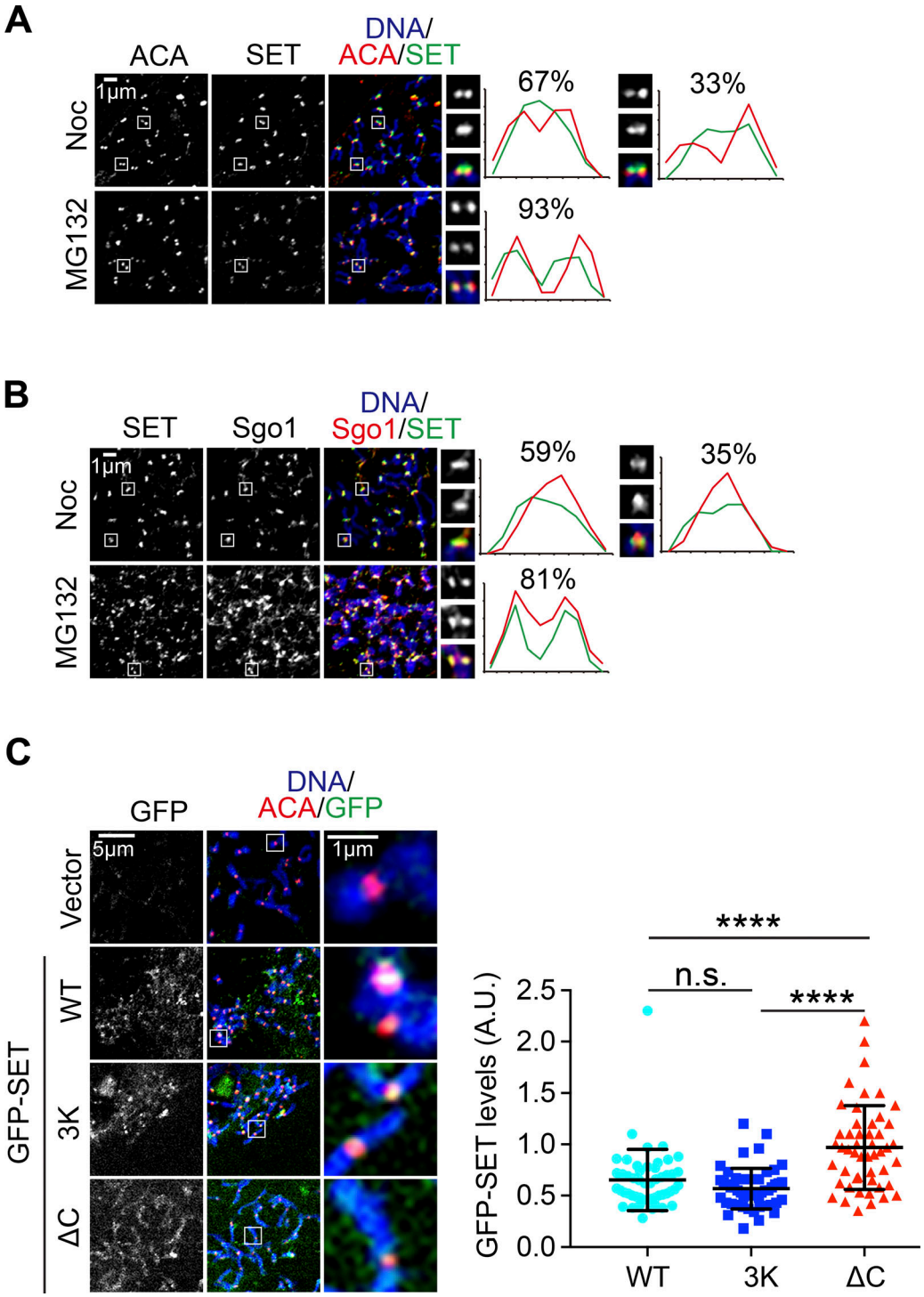

Figure S5. **Localization of SET during mitosis. (A and B)** Representative images of MG132-arrested HeLa Tet-On cells with nocodazole (Noc) or MG132. The outlined regions are amplified and shown in the right panel. The plotted curves define the relative localization of ACA (red) and SET (green) signals (A) and the one of Sgo1 (red) and SET (green) signals (B). At least 90 kinetochores (five per cell) were examined for each condition. **(C)** Representative images of nocodazole-arrested HeLa Tet-On cells overexpressing GFP-SET WT, 3K, or ΔC. The outlined regions are amplified and shown in the right panel. Quantification of GFP-SET levels is also shown. The average and standard deviation are shown. At least 50 kinetochores (five per cell) were examined for each condition. ****, $P < 0.0001$. One-way ANOVA was performed followed by pairwise comparisons using Tukey's test. n.s., not significant.