Supplemental Materials

Molecular Biology of the Cell

Yang et al.
Figure S1. SET protein levels in Log and mitotic phases in cancer cells

A. Log-phase and nocodazole-arrested mitotic cancer cells were collected and subjected to
Western blotting analysis with the indicated antibodies. B. Lysates from the indicated cell lines were resolved with SDS-PAGE and blotted with the indicated antibodies. Pseudo colors were used to mark distinct protein bands: SET in green and actin in red. C. Quantification of SET protein levels for cancer cells in (B). Relative SET protein levels in cancer cells normalized to the ones in RPE-1 cells are shown here.

|        | RPE-1 | A549 | BT-549 | PC3 | 22Rv1 | COS-1 | MDA-MB-213 | U97 | T98G | C4-2B | H1299 | DLD-1 | HT-29 | HCT116 | MCF7 |
|--------|-------|------|--------|-----|-------|-------|------------|-----|------|-------|-------|-------|-------|--------|------|
| Set    | 5%    | 27%  | 24%    | 5%  | 29%   | 23%   | 7%         | 18% | 36%  | 19%   | 10%   | 8%    | 20%   | 19%    | 8%   |
| 3%     | 24%   | 17%  | 10%    | 22% | 34%   | 20%   | 24%        | 13% | 23%  | 18%   | 8%    | 34%   | 15%   | 3%     |
| 7%     | 21%   | 15%  | 7%     | 32% | 35%   | 19%   | 28%        | 17% | 5%   | 14%   | 18%   | 27%   | 33%   | 5%     |

![Table summarizing centromeric cohesion defects for each individual repeat in Figure 2A. The...](image)
number denotes the percentage of cells exhibiting centromeric cohesion defects. B. The non-transformed cells were treated with MG132 for 6 hr and mitotic cells were subjected to chromosome spread. Unseparated and separated sister chromatids were scored according to Figure 2. The mean and standard deviation were calculated from at least three independent experiments. At least 20 mitotic cells were evaluated for each condition in every individual experiment. C. Detailed breakdowns of chromosome morphology in the cancer cells with severe cohesion defects (>30%) in Figure 2A. The mean from three independent experiments is shown here and the error bar is demonstrated in Figure 2A.

Figure S3. Treatment of FTY720 or OP449 does not affect overall PP2A activity and the Sgo1-SET interaction

A. HeLa Tet-On cells stably expressing Myc-Sgo1 were mock-treated or treated with SET siRNAs
and cell lysates were included with anti-Myc antibodies. Immunoprecipitated proteins were subjected to Western blotting analysis with the indicated antibodies. **B.** Pharmacological increase in PP2A activity by FTY720 or OP449 in cancer cells. Lysates of HeLa Tet-On cells treated with FTY720 or OP449 at different times were resolved with SDS-PAGE and blotted with the indicated antibodies. H460 cells were treated with FTY720 for 6 hr before harvest for Western blots. **C.** Pharmacological increase in PP2A activity does not strengthen centromeric cohesion in cancer cells. HeLa Tet-On and H460 cells in (B) were treated with MG132 for 4 hr and then subject to chromosome spread. Separated and unseparated sister chromatids were scored according to Figure 2A. Mean and standard deviation calculated based on at least three independent experiments are shown here. At least 25 mitotic cells were evaluated for each condition. n.s., no significance. **D.** HeLa Tet-On cells stably expressing Myc-Sgo1 were treated with DMSO, FTY720 or OP229 and cell lysates were included with anti-Myc antibodies. Immunoprecipitated proteins were subjected to Western blotting analysis with the indicated antibodies.

![Figure S4](image)

**Figure S4. Analysis of Sgo1 protein levels and Sgo1/SET ratios in cancer cells**

**A.** Lysates of RPE-1 and cancer cells were resolved with SDS-PAGE and blotted with the
indicated antibodies. The relative Sgo1/SET ratios were calculated and are shown in the bottom panels. The asterisks indicate non-specific protein bands. **B.** Pearson’s correlation analysis on relative Sgo1/SET ratios (**A**) and centromeric cohesion defects among all the tested cancer cells in **Figure 2A.** Scatter plot with linear regression is shown in here.
Figure S5. SET overexpression does not affect the phosphorylation of Akt

A, B, C, and D. Cell lysate in Figures C, D, E, and F was resolved with SDS-PAGE and blotted with the indicated antibodies. Fold changes in pAkt/Akt ratios among these cancer cells are shown.
in the top panels. pAkt/Akt ratios were obtained based on the results of Western blots (bottom panels). Mean and standard deviation were calculated from at least three independent experiments. n.s., no significance.

Materials and Methods

Mammalian cell culture, chemicals, siRNAs, and transfection

All the cell lines were cultured in the following media supplemented with 10% fetal bovine serum 10 mM L-glutamine and 10% Penicillin/Streptomycin. DMEM-F12, RPE-1; DMEM, HeLa Tet-On, COS-1, Phoenix, HEK 293T, T47-D, MCF7, BT-549, MDA-MB-231, H460, A549, H1299, Hep G2, Huh-7, C4-2B, HCT116, DLD-1, HT29, RKO, SW620, SW480, T98G, and U-87 MG, BJ-Fibroblasts; RPMI1640, LNCaP, PC-3, 22Rv1, and C4-2. AG09266 and AG08433 were cultured with DMEM supplemented with 15% fetal bovine serum and 1 mM L-glutamine. HeLa Tet-On and RPE-1 cells were authenticated through STR profiling by ATCC. All the other cell lines are gifts from the investigators listed in the Acknowledgement.

Nocodazole (M1404), MG132 (474790) and FTY720 (SML0700) were purchased from Sigma Aldrich. OP449 was a gift from from Oncotide Pharmaceuticals, Inc. The times for MG132, nocodazole, FTY720, and OP449 treatments were specified in each experiment.

To construct the stable cell lines, RPE-1 cells were infected with the lentiviral particles containing GFP-SET WT, 3K, or ΔSET and selected with 1 μg ml⁻¹ puromycin (Sigma).

To overexpress Sgo1 and SET, Sgo1 and SET (WT and mutants) cDNAs were inserted into PCS2-GFP vectors.

For RNAi experiments, the siRNA oligonucleotides were purchased from Thermo Scientific. Cells
were transfected using Lipofectamine RNAiMax (Invitrogen) and analyzed at 36–48 h after transfection. The sequences of the siRNAs used in this study are: SET siRNA #2, GGAUGAAGGUGAAGAAGAU (Thermo Scientific, D-019586-02); SET siRNA #4, CGAGUCAAACGCAGAAUAA (Thermo Scientific, D-019586-04).

**Construction of Lentiviral particles**

Lentiviral particles were generated using the pLVX-Puro system (Clontech) in HEK 293T cells. PEI:DNA complexes containing pRSV-Rev, pMDLg-pRRE, pMD2.G and pLVX inserted of genes of interests (SET WT, 3K or ΔC) were first prepared (Qu et al., 2019). Then the assembled complexes were mixed into cultured HEK 293T cells. After 48 hr, cell culture containing viruses was collected, filtered and stored at -80°C for later use.

**Antibodies and Immunoblotting**

The following antibodies were used in this study: anti-centromere antibody (ACA or CREST-ImmunoVision, HCT-0100), anti-PP2A-Aα (Santa Cruz, Sc-6112), anti-Histone H3-pS10 (Cell signaling, 9706), anti-Smc1 (Bethyl, A300-055A), anti-SET (Bethyl, A302-261A), AKT (Cell signaling, 4691S), pAKT (S473) (Cell signaling, 4060S), and anti-actin (Thermo Scientific, MA5-11869), anti-pHec1 (phospho Ser55, GTX70017, GeneTex), anti-Myc (Millipore, 11667149001). Anti-Sororin is a gift from Dr. Susannah Rankin. Anti-Sgo1 and anti-GFP antibodies were made in-house as described previously (Kim and Yu, 2015; Liu et al., 2013b).

Antibody dilution for immunoblotting was often 1:1000 unless specified.

The secondary antibodies were purchased from *Li-COR*: IRDye® 680RD Goat anti-Mouse IgG
Secondary Antibody (926-68070) and Goat anti-Rabbit IgG Secondary Antibody (926-32211).

Harvested cells were collected and lysed with SDS sample buffer. After being 5-min boiled, lysates were resolved by SDS–PAGE and blotted with indicated antibodies.

For immunoprecipitation, anti-myc or anti-GFP antibodies were coupled to Affi-Prep Protein A beads (Bio-Rad) at a concentration of 1 mg ml⁻¹.

**Immunoprecipitation**

Immunoprecipitation in **Figures S3A and S3D** was performed as described before (Liu et al., 2013b). HeLa Tet-On cells were dissolved in lysis buffer (25 mM Tris–HCl at PH 7.5, 50 mM NaCl, 5 mM MgCl₂, 0.1% NP-40, 1 mM DTT, 0.5 μM okadaic acid, 5 mM NaF, 0.3 mM Na₃VO₄) containing 100 units ml⁻¹ Turbo-nuclease (Accelagen). After a 1-hr incubation on ice followed by a 15-min incubation at room temperature, the lysate was cleared by centrifugation for 20 min at 4°C at 20,817g. The supernatant was then incubated with the beads pre-coupled with anti-Myc antibody (ThermoFisher, 20168) overnight at 4°C. After the beads were washed four times with wash buffer (25 mM Tris–HCl at PH 7.5, 100 mM NaCl, 5 mM MgCl₂, 0.1% NP-40, 1 mM DTT, 0.5 μM okadaic acid, 5 mM NaF, 0.3 mM Na₃VO₄), the proteins bound to the beads were finally dissolved in SDS sample buffer, separated by SDS–PAGE and blotted with the appropriate antibodies.

**Immunofluorescence and chromosome spread**

For chromosome spreads and immunostaining in **Figures 2A, 3B, 4B, 4C, 4F, 5A, 5B, 5C, 6C, 6D, 6E, 6F, and S3C**, collected MG132-treated mitotic cells were swelled in hypotonic solution containing 50 mM KCl for 15 min at room temperature and then spun onto slides with a Shandon Cytospin centrifuge. Cells were first extracted with ice-cold PBS containing 0.2% Triton X-100 for
2 min and then fixed in 4% ice-cold paraformaldehyde for 4 min. After washed with PBS 0.1% Triton X-100, cells were sequentially incubated with primary antibodies (1:1000 dilution) overnight at 4°C and with the appropriate secondary antibodies conjugated to fluorophores (Invitrogen, A11008, A21090 and A31571, 1:1000 dilution) at room temperature for 1 hr. Finally, cells were washed with PBS containing 0.1% Triton X-100, stained with 1 μg ml⁻¹ DAPI and mounted with Vectashield.

The images were taken by a Nikon confocal microscope with a ×60 objective. Image processing was carried out with ImageJ and Adobe Photoshop. Quantification was carried out with ImageJ.

**Quantification and Statistical Analysis**

For quantification of SET protein levels in cells in Figure 1A, masks were generated to mark SET protein bands on WB membranes and each single lane on Gelcode Blue-stained SDS PAGE using the software of Image Studio Lite. After background subtraction, the intensities of SET proteins and total proteins within the masks were obtained in numeric values. The relative SET protein level in each cancer cell line was derived from the intensity of SET proteins normalized to the one of total proteins. Fold changes in the relative SET protein levels were compared between in RPE1 cells and in cancer cells and are shown in Figures 1A and 1B.

For quantification of SET protein levels in cells in Figures S1B, masks were generated to mark the desired protein bands on WB membranes using the software of Image Studio Lite. After background subtraction, the intensities of SET and actin protein bands within the masks were obtained in number. Relative SET protein levels in each cell were derived from the intensity of SET protein bands normalized to the one of actin protein bands. Fold changes in the relative SET protein levels were compared between in RPE1 cells and in cancer cells and are shown in Figures S1B and S1C.
For quantification of Sgo1/SET ratios in Figure S4A, masks were generated to mark the desired protein bands on WB membranes using the software of Image Studio Lite. After background subtraction, the intensities of Sgo1 and SET protein bands within the masks were obtained in number. Sgo1/SET ratios in each cell were obtained from the normalization of the intensity of Sgo1 to the one of SET. Fold changes in Sgo1/SET ratios between in RPE1 cells and in cancer cells and are shown in Figure S4A.

Measurement of sister-centromere distance in Figures 5A, 5B and 5C, was performed using Image J. A straight line was drawn between a pair of sister centromeres, as indicated by ACA signals. Numeric values were automatically generated by Image J.

Pearson correlation analysis was applied in Figures 2B and 4SB. Pearson’s correlation coefficient (Pearson’s r) and the p-value (Schober et al., 2018) were computed by using R software (R version 3.6.1, https://www.r-project.org/).

All the experiments were repeated at least three times. Quantification was usually performed based on the results from all the repeated experiments unless specified. Differences were assessed using one-way ANOVA followed by pairwise comparisons using Tukey’s test for the data in Figures, 5A, 5B, 5C, 6C, 6D, 6E, 6F, S2B, S3C, S5A, S5B, S5C, and S5D. In Figure 2A, differences between RPE-1 and each type of cancer cells were assessed using T-tests. In Figures 3B, 4B and 4C, differences between mock and siSET for each single cancer cell line were also assessed using T-tests. All the samples analyzed were included in quantification. Sample size was recorded in figures and their corresponding legends. No specific statistical methods were used to estimate sample size. No methods were used to determine whether the data met assumptions of the statistical approach.