

## Supporting Information for

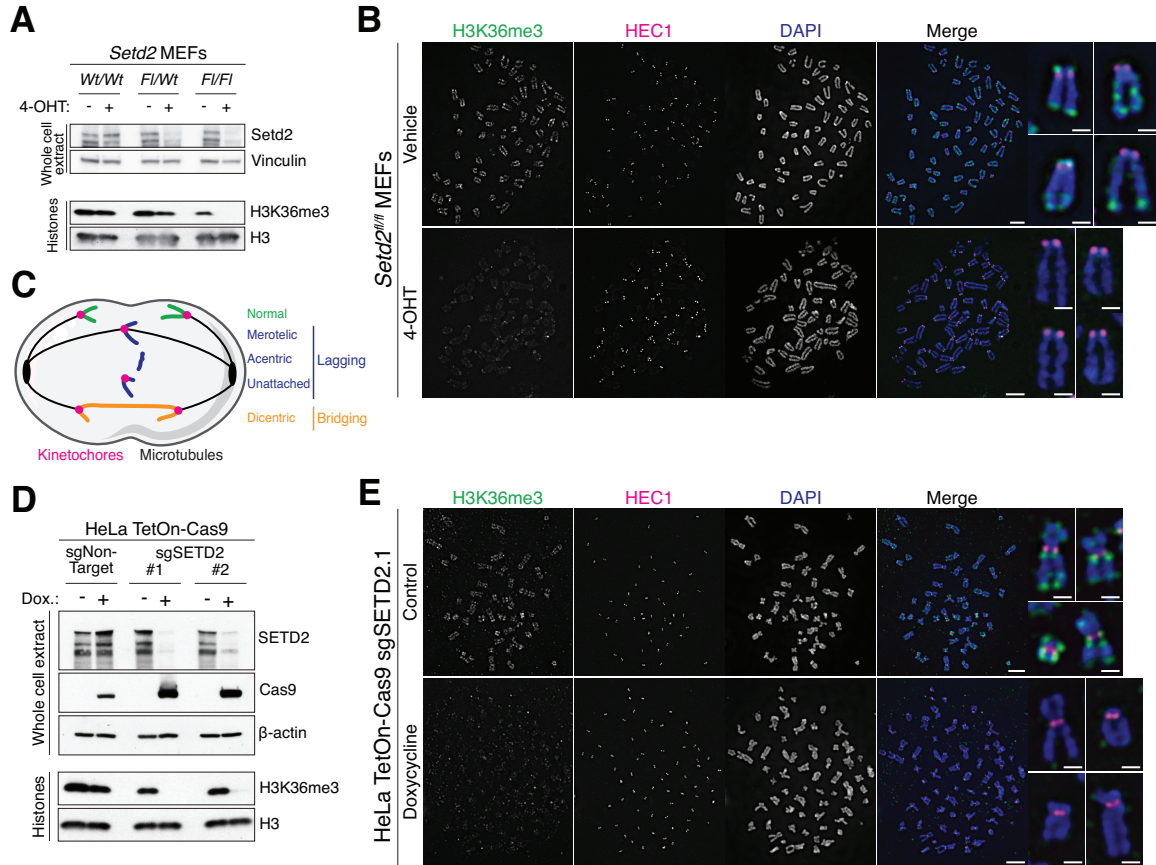
### **SETD2 safeguards the genome against isochromosome formation.**

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**Figure S1: SETD2 is required for H3K36me3, which is present along chromosome arms, sub-telomeric and pericentric regions.**

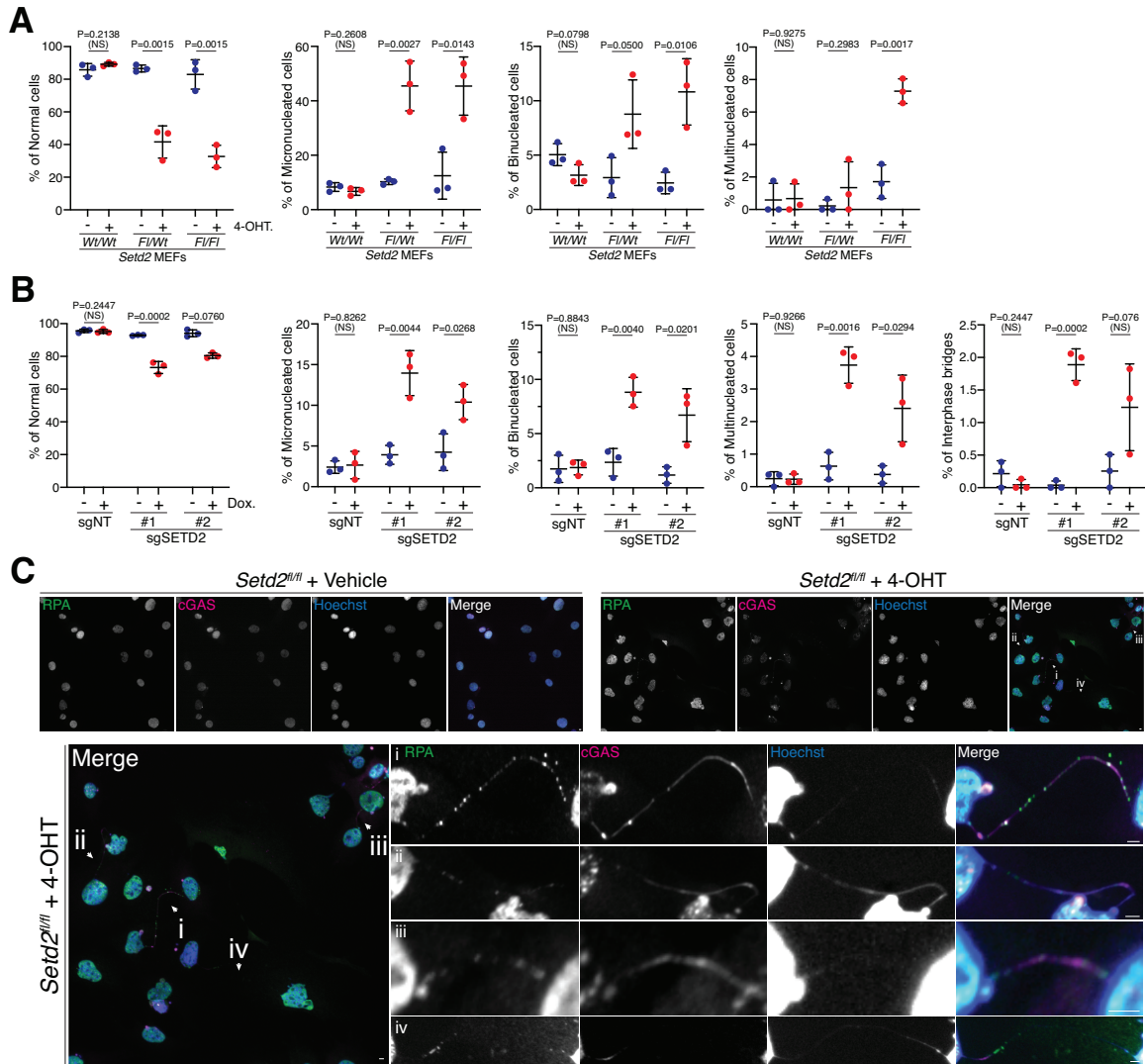
(A) Western blot of MEFs with no, one or two floxed alleles of *Setd2* (Wild-type/*Wt*, *Flox/Wt*, *Flox/Flox*, respectively). 4-OHT leads to deletion of *Setd2* allele(s) and a concurrent decrease in H3K36me3. Top panels are whole cell extract, acid-extracted histones at bottom.

(B) Metaphase spreads from *Setd2*<sup>fl/fl</sup> vehicle (EtOH) or 4-OHT treated MEFs, immunostained for H3K36me3, HEC1 (kinetochore protein) and DAPI (DNA). Scale bars are 5µm at left, and 1µm for individual chromosomes at right.

(C) Model of different types of chromosome mis-segregation.

(D) Western blot of HeLa TetOn-Cas9 cells expressing sgNon-targeting or sgSETD2. Doxycycline is added to activate expression of Cas9, leading to deletion of SETD2 and loss of H3K36me3. Top panels are whole cell extract, acid-extracted histones at bottom.

(E) Metaphase spreads from HeLa TetOn-Cas9 cells expressing sgSETD2, control or doxycycline treated, immunostained for H3K36me3, HEC1 (kinetochore protein) and DAPI (DNA). Scale bars are 5µm at left, and 1µm for individual chromosomes at right.



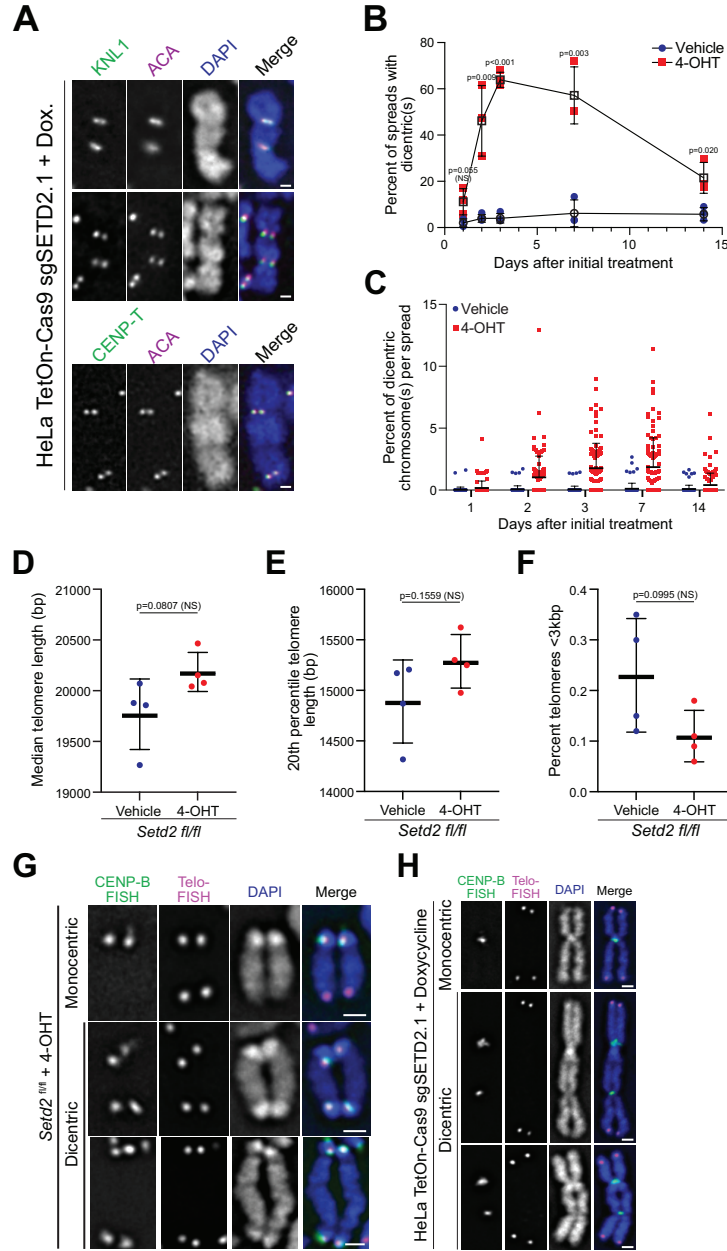
**Figure S2. *Setd2* loss promotes micronucleation, multinucleation, binucleation and bridging.**

(A) Quantification of nuclear phenotypes of MEFs, where *Setd2* deletion leads to less normal nuclei, and more micronucleated, binucleated, and multinucleated cells. N=3 independent experiments.

(B) Quantification of nuclear phenotypes of HeLa cells, where *SETD2* deletion leads to less normal nuclei, and more micro-, bi-, and multi-nucleated cells as well as an increase in cells with interphase bridges. N=3 independent experiments.

For each plot, error bars are s.d. and P-values derived from unpaired t-test between each condition within a genotype.

(C) Image of *Setd2<sup>fl/fl</sup>* MEFs treated with vehicle (control, left) or 4-OHT (*Setd2* knockout, right) demonstrating that there are more interphase bridges in cells lacking *Setd2*. RPA (green) and cGAS (magenta) are markers of extranuclear DNA and bridges. Scale bars are 5 $\mu$ m.



**Figure S3: Telomere attrition or fusion does not occur following acute *Setd2* deletion.**

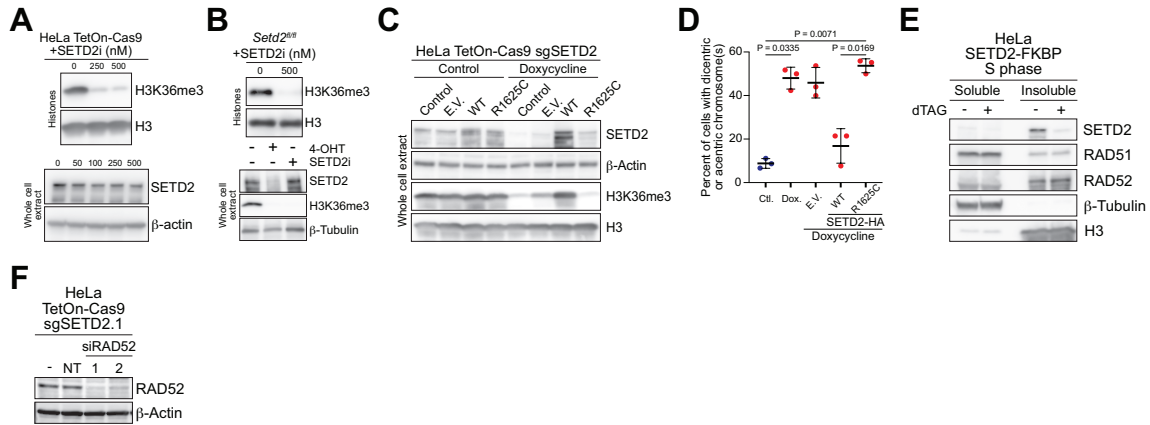
(A) Representative immunofluorescent images of dicentric chromosomes from *SETD2*-knockout HeLa cells, stained for KNL1 or CENP-T with anti-centromere antibody (ACA) and DAPI. Scale bars are 1 $\mu$ m.

(B) Quantification of percent of *Setd2<sup>fl/fl</sup>* cells with dicentric chromosomes, after treatment with vehicle or 4-OHT, n=3 biological replicates. P-values from unpaired t-test between each treatment condition at each time point.

(C) Quantification of percent of dicentric chromosomes per *Setd2<sup>fl/fl</sup>* cell after treatment with vehicle or 4-OHT. N=102 cells (day 1), 101 cells (day 2), 103 cells (day 3), 103 cells (day 7) and 105 cells (day 14) for vehicle and N=102 cells (day 1), 104 cells (day 2), 100 cells (day 3), 99 cells (day 7) and 109 cells (day 14) for 4-OHT treated cells across 3 biological replicates.



- (D) Quantification of median telomere length (base pairs, bp) in vehicle and 4-OHT-treated *Setd2<sup>fl/fl</sup>* MEFs. n=4 technical replicates.
- (E) Quantification of length of 20<sup>th</sup> percentile in vehicle and 4-OHT-treated *Setd2<sup>fl/fl</sup>* MEFs. n=4 technical replicates.
- (F) Quantification of percent of telomeres less than 3 kilobase pairs long (kbp) in vehicle and 4-OHT-treated *Setd2<sup>fl/fl</sup>* MEFs. n=4 technical replicates.
- (G) Representative images of CENP-B box and telomeric FISH on metaphase spreads from 4-OHT-treated *Setd2<sup>fl/fl</sup>* MEFs. Telo-FISH signal is not present between centromeres, suggesting lack of telomeric fusion. Scale bars are 1 $\mu$ m.
- (H) Representative images of CENP-B box and telomeric FISH on metaphase spreads from doxycycline-treated HeLa TetOn-Cas9 sgSETD2.1 cells. Telo-FISH signal is not present between centromeres, suggesting lack of telomeric fusion. Scale bars are 1 $\mu$ m.
- Error bars are s.d. and P-values derived from unpaired t-tests for (D-F).



**Figure S4: SETD2 methyltransferase activity suppresses dicentric chromosome formation.**

- (A) Western blot of histone (top) and whole cell extracts (bottom) from HeLa TetOn-Cas9 sgSETD2.1 cells treated with vehicle (DMSO) or increasing concentrations of SETD2 inhibitor (SETD2i, EPZ-719) for 72 hours, causing a decrease in H3K36me3. SETD2 protein levels are not affected by SETD2i treatment.
- (B) Western blot of histone (top) and whole cell extracts (bottom) from *Setd2<sup>fl/fl</sup>* MEFs treated with vehicle or 500nM SETD2i for 72 hours, causing a 96% decrease in H3K36me3 but not *Setd2*.
- (C) Western blot of whole cell extracts from HeLa TetOn-Cas9 sgSETD2.1 cells with full-length, wild-type (WT) or R1625C catalytically dead SETD2 or empty vector (E.V.) controls, expressed under a tetracycline-inducible promoter. Doxycycline treatment knocks out endogenous *SETD2* and induces expression of rescue constructs.
- (D) Quantification of percentage of cells with dicentric and/or acentric chromosomes from cells described in (C) with n=3 biological replicates per condition. WT SETD2 but not R1625C or empty vector suppresses these chromosomal aberrations. P-values derived from one-way ANOVA with Tukey's multiple comparisons test.
- (E) Western blot of soluble and insoluble, chromatin-bound fractions of control or dTAG-treated HeLa SETD2-FKBP cells during S phase, following double-thymidine block and release. Loading of RAD51 and RAD52 are not affected by SETD2 loss.
- (F) Western blot of HeLa TetOn-Cas9 cells transfected with non-targeting or RAD52-specific siRNA.