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Supplemental information

Self-reversal facilitates the resolution of HMCES DNA-protein crosslinks in cells

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SUPPLEMENTAL INFORMATION

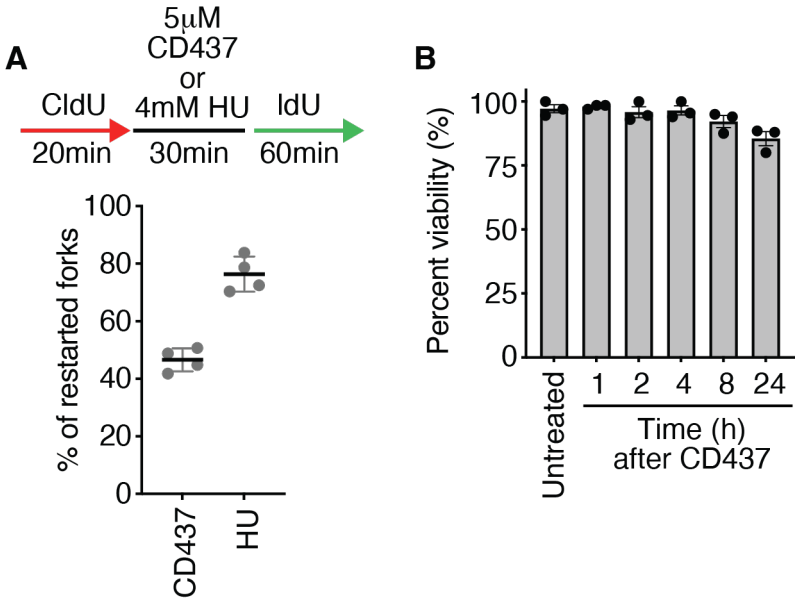


Figure S1. Characterization of CD437 effects on replication and cell viability. (Related to Figure 1)

(A) U2OS cells were labeled and treated as indicated. DNA combing was performed, and the percentage of restarted forks (red + green DNA tracks) compared to total (red and green only) was measured. Mean and SEM from n=4 replicates is shown.

(B) Cells were treated with 5μM CD437 for 30 minutes and then incubated in fresh media. Cell viability was measured at the indicated times using trypan blue staining.

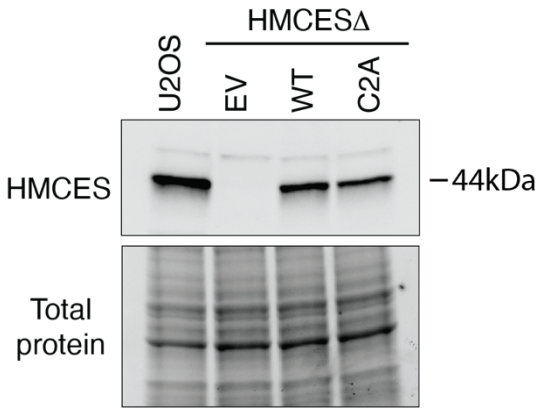
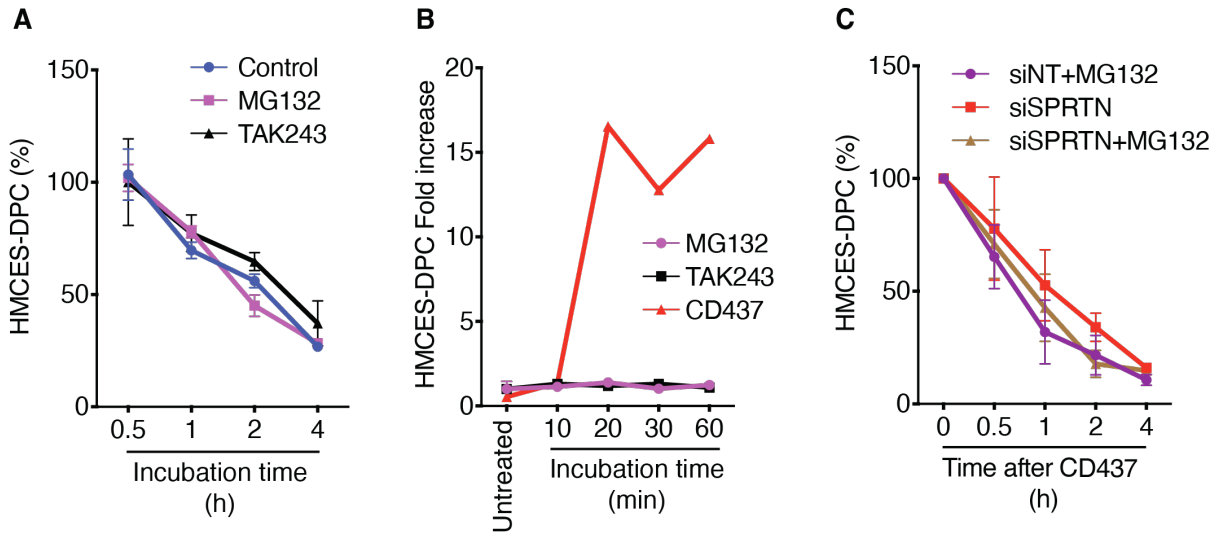


Figure S2. Immunoblot of HMCES expression. (Related to Figure 1)

Immunoblot analysis of total HMCES protein in U2OS cells and HMCESΔ cells complemented with empty vector (EV), WT, or C2A HMCES. Below are total protein levels, using a stain-free image as a loading control.



Figures S3. Analysis of Proteasome, E1 ubiquitin-activating enzyme, and SPRTN contributions to HMCES-DPC resolution. (Related to Figure 2)

(A) Percentages remaining of HMCES-DPC with HMCES-DPC levels at 0.5h set to 100%. RADAR assay was performed as in Figure 1E. Mean ± SEM, n=3.

(B) Quantification of HMCES-DPC formation during incubation with CD437, MG132, or TAK243 for the indicated times.

(C) Quantification of HMCES-DPC removal in cells transfected with non-targeting (siNT) or SPRTN siRNAs and treated with MG132. MG132 was added during and after CD437 treatment.

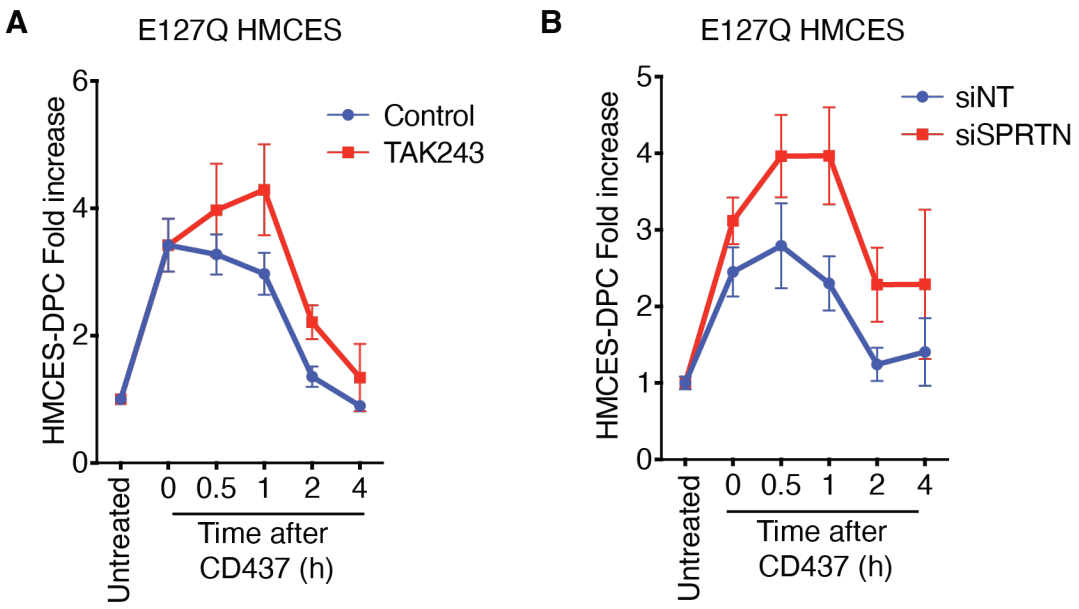
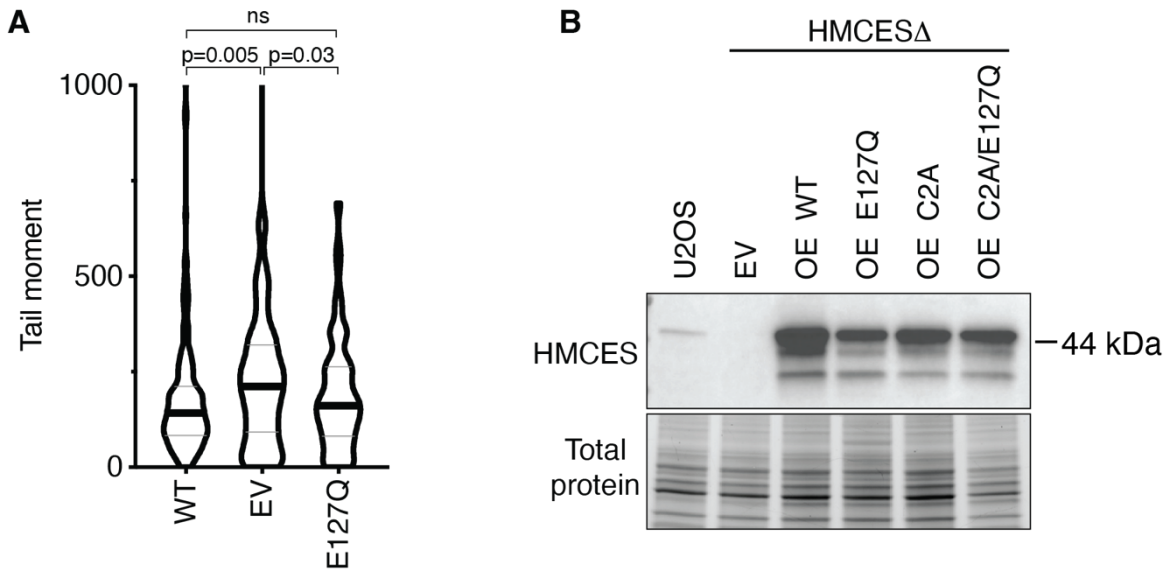


Figure S4. Effects of TAK243 and SPRTN inactivation on stability of the E127Q HMCES-DPC. (Related to Figure 4)

(A) RADAR assay of E127Q HMCES-DPC levels in cells either that were mock treated or treated with TAK243. TAK243 was added immediately after CD437 treatment. Mean ± SEM, n=3.

(B) RADAR assay of E127Q HMCES-DPC levels in cells transfected with non-targeting (siNT) or SPRTN (siSPRTN) siRNAs. Mean ± SEM, n=4.



Figures S5. Comet assay and immunoblot of HMCES overexpression. (Related to Figure 5)

(A) Neutral comet assay was used to measure DSBs in HMCESD cells containing an empty vector (EV) or expressing near endogenous levels of WT or E127Q HMCES. P values were calculated by a one-way ANOVA with a Dunnett posttest.

(B) Immunoblot of U2OS or HMCESΔ cells containing an empty vector (EV) or overexpressing WT, E127Q, C2A, or C2A/E127Q HMCES proteins.