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Supplemental Information

HMCES Maintains Replication Fork Progression and Prevents Double-Strand Breaks in Response to APOBEC Deamination and Abasic Site Formation

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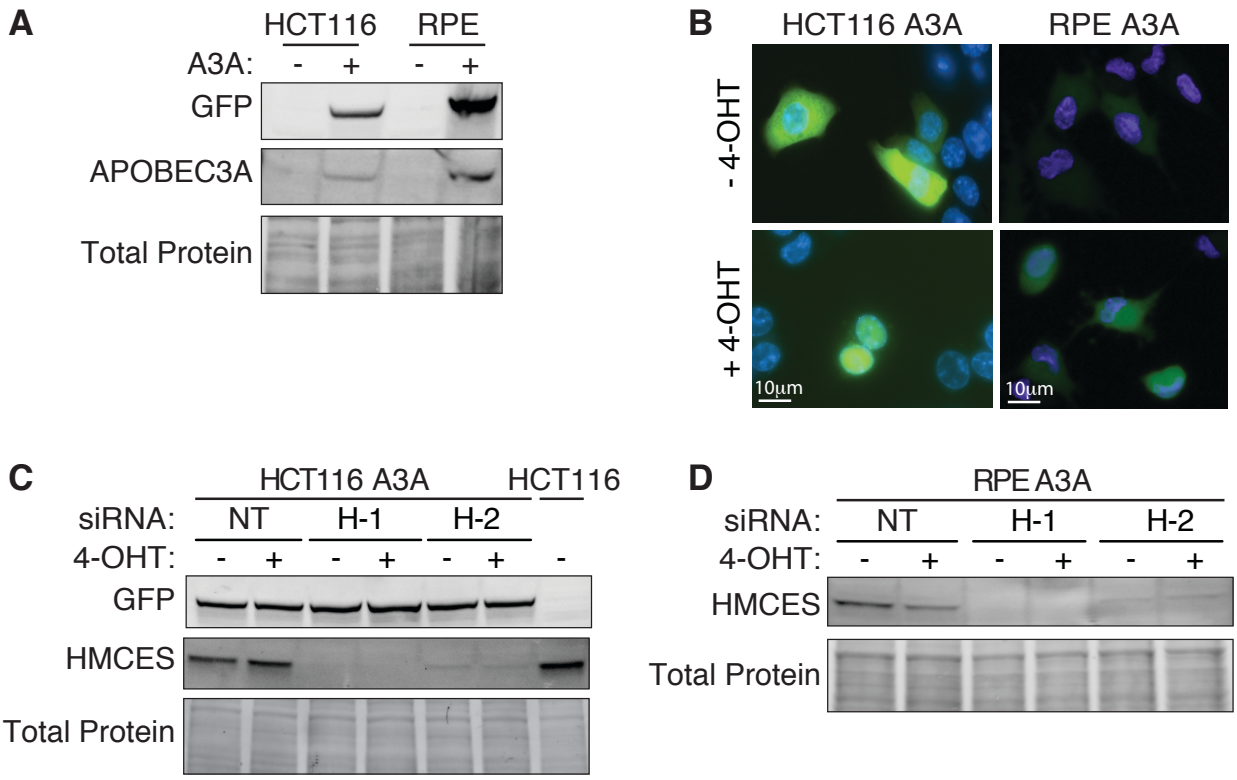

Figure S1. Efficient depletion of HMCES and regulation of APOBEC3A nuclear localization. Related to Figure 1. (A) Immunoblots for GFP and APOBEC3A in HCT116 and hTERT-RPE-1 cell lines with and without stable expression of GFP-APOBEC3A-ERT2. Bio-Rad stain-free total protein was used as a loading control. **(B)** HCT116 GFP-APOBEC3A-ERT2 cells (left) were treated with 10 μM 4-OHT for two hours to induce nuclear localization of APOBEC3A, fixed, and DNA was stained with DAPI (blue). GFP localization was captured by fluorescent imaging. hTERT-RPE-1 GFP-APOBEC3A-ERT2 cells (right) were treated with 10 μM 4-OHT for one hour, DNA was stained with Hoechst 33342 (blue), and live cells were imaged for GFP fluorescence. **(C)** Immunoblots for GFP and HMCES in HCT116 GFP-APOBEC3A-ERT2 cells transfected with two HMCES siRNAs (H-1, H-2) or a non-targeting control siRNA (NT). Cells were treated with 10 μM 4-OHT where indicated. **(D)** Immunoblot for HMCES in hTERT-RPE-1 GFP-APOBEC3A-ERT2 cells transfected with the indicated siRNAs. Cells were treated with 10 μM 4-OHT where indicated.

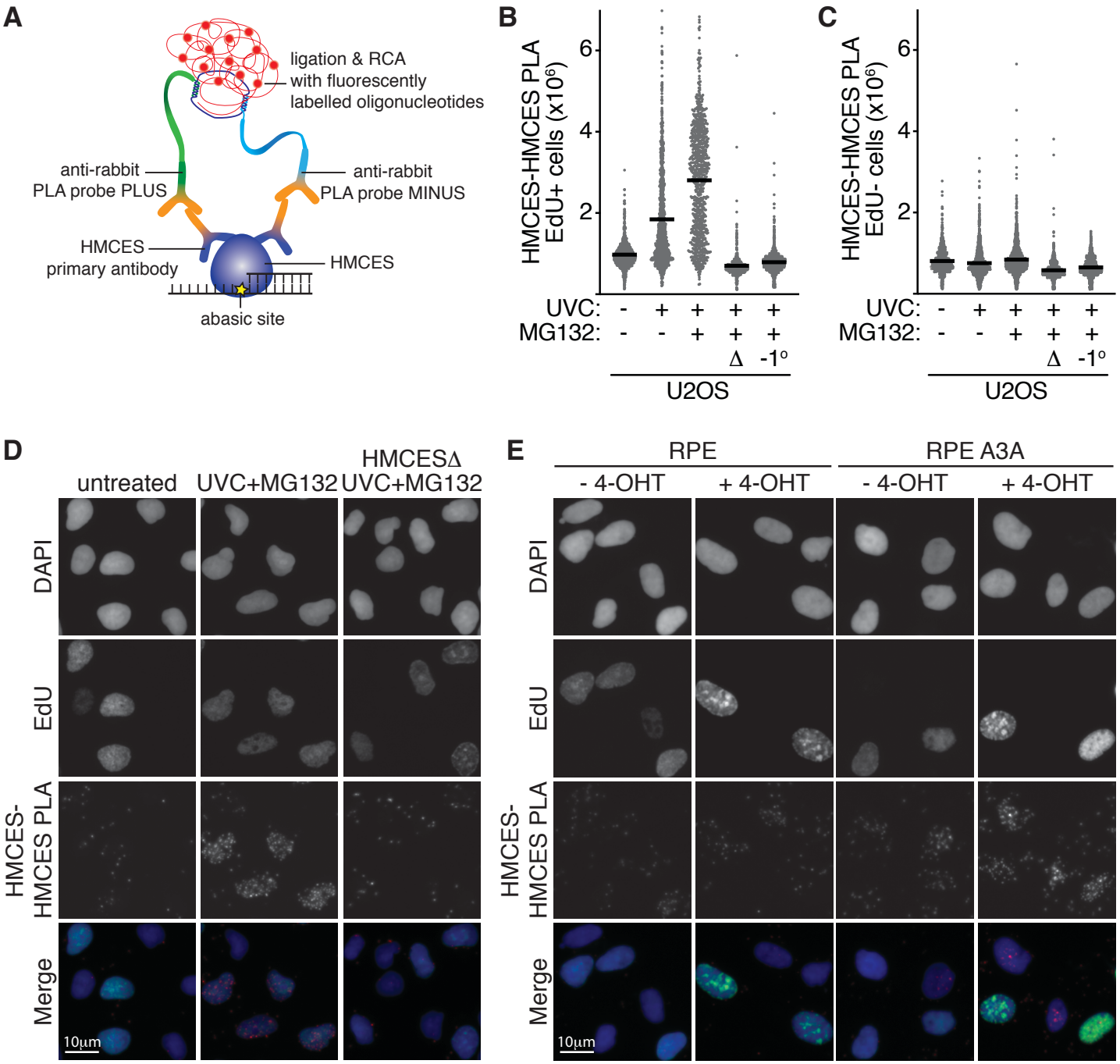

Figure S2. HMCES PLA monitors HMCES localization to the insoluble chromatin fraction in response to UVC and nuclear APOBEC3A. Related to Figure 1. (A) Schematic of the HMCES proximity ligation assay (PLA). Soluble HMCES protein is removed by pre-extraction with Triton X-100 buffer and insoluble HMCES is recognized with a polyclonal HMCES antibody, followed by anti-rabbit secondary antibodies conjugated to PLUS and MINUS PLA probes. Insoluble HMCES is then detected by ligation of the probes and rolling circle amplification (RCA) with fluorescently labeled oligonucleotides. **(B and C)** Quantitation of the insoluble HMCES PLA signal in **(B)** EdU-positive or **(C)** EdU-negative U2OS cells after UVC radiation. U2OS HMCESΔ (Δ) and elimination of the HMCES primary antibody (-1°) were included as negative controls. All bars represent the median. **(D)** Representative images from HMCES PLA in U2OS and U2OS HMCESΔ. Cells were treated with 10 μM EdU for 30 minutes, irradiated with 100 J/m^2 UVC where indicated, and allowed to recover four hours in the presence or absence of a proteasome inhibitor (10 μM MG132). Cells were pre-extracted with 0.5% Triton X-100 buffer and fixed with paraformaldehyde. EdU incorporation was detected by a click reaction using Alexa Fluor 488 azide (green) followed by HMCES PLA (red). DNA was stained with DAPI (blue). **(E)** Representative images from HMCES PLA in hTERT-RPE-1 cells with and without stable expression of GFP-APOBEC3A-ERT2. Cells were treated with EdU +/- 4-OHT, pre-extracted with 0.5% Triton X-100 buffer, and fixed. S-phase cells were identified by EdU staining (green) prior to HMCES PLA (red). DNA was stained with DAPI (blue).

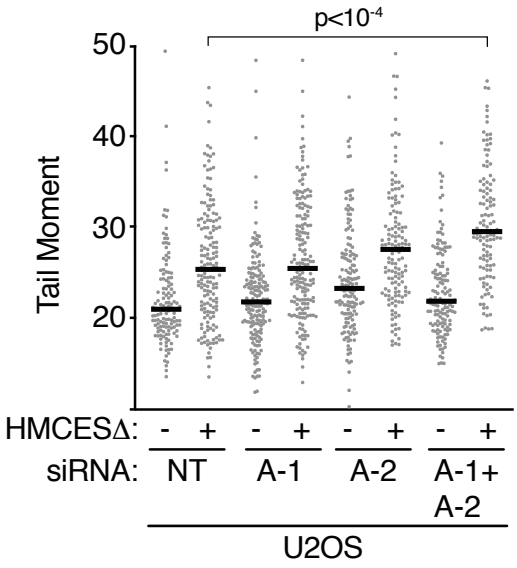

Figure S3. Inactivation of AP endonucleases causes increased DSBs in HMCES-deficient cells. Related to Figure 2. U2OS and U2OS HMCESΔ cells were transfected with APEX-1 (A-1), APEX-2 (A-2), or non-targeting control (NT) siRNAs and DSBs were measured by neutral comet assay. Individual comet tail moments are plotted and bars indicate the median. All p values were derived from an ANOVA with Dunn's multiple comparisons.