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Supplementary Materials for

MRNIP is a replication fork protection factor

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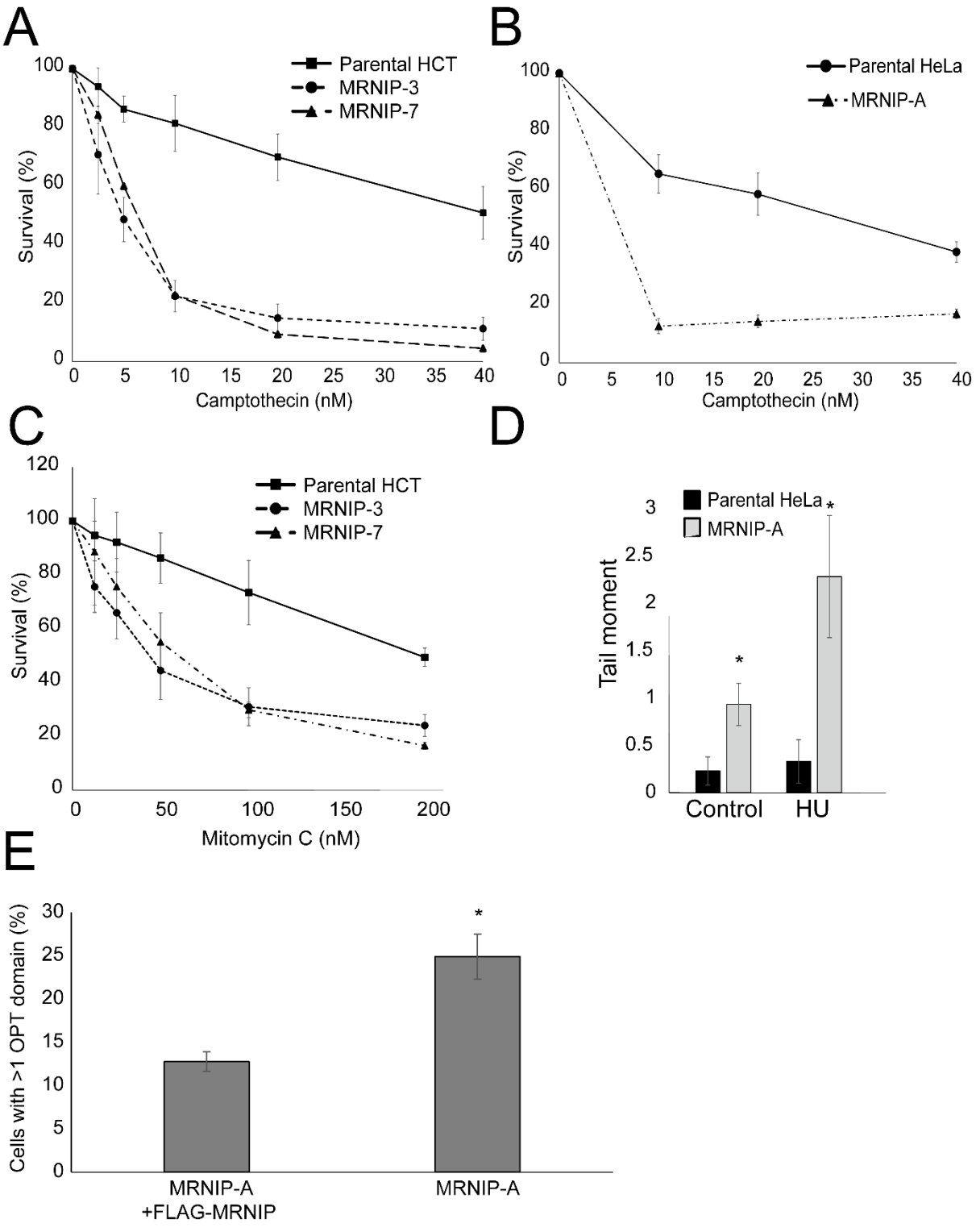
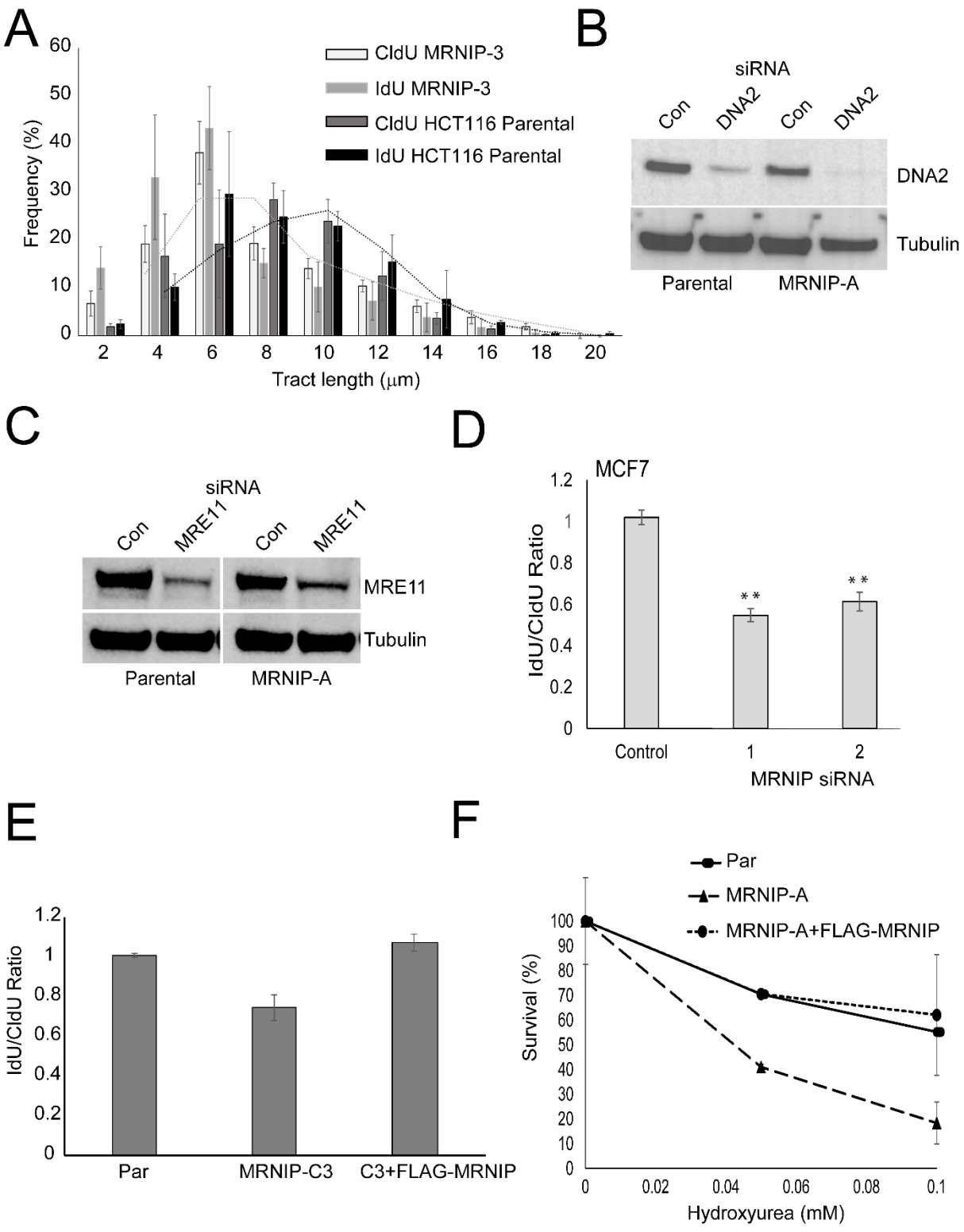
Supplementary Materials

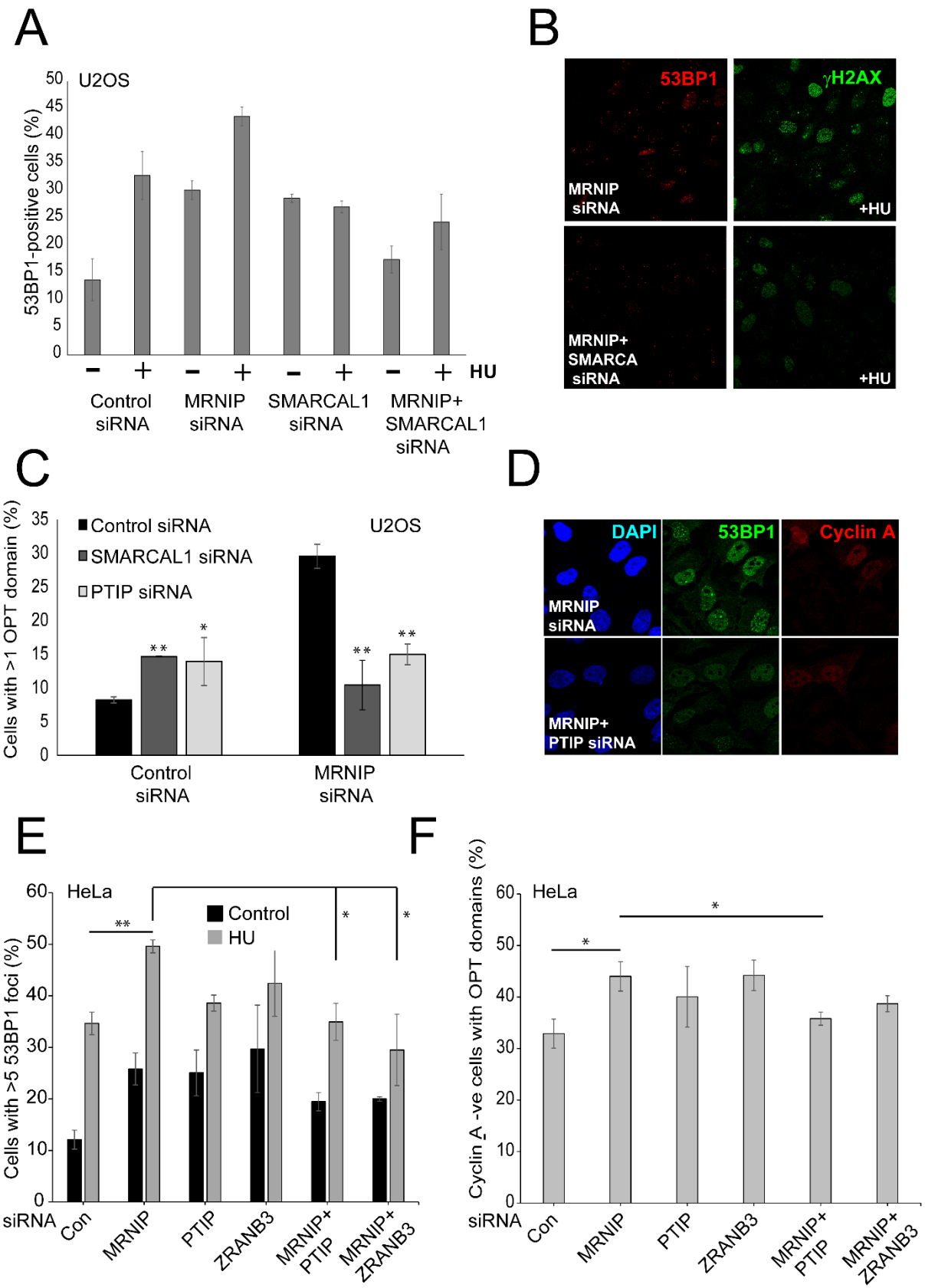
MRNIP is a novel replication fork protection factor

Bennett et al., 2020 Supp Fig 1

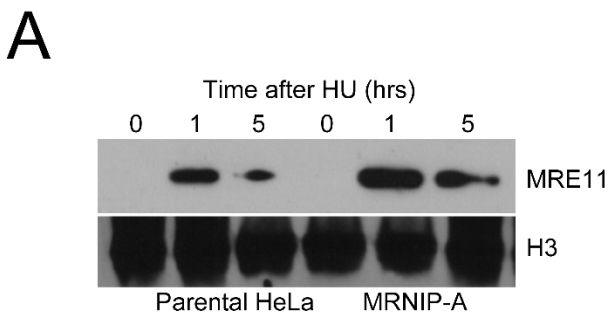


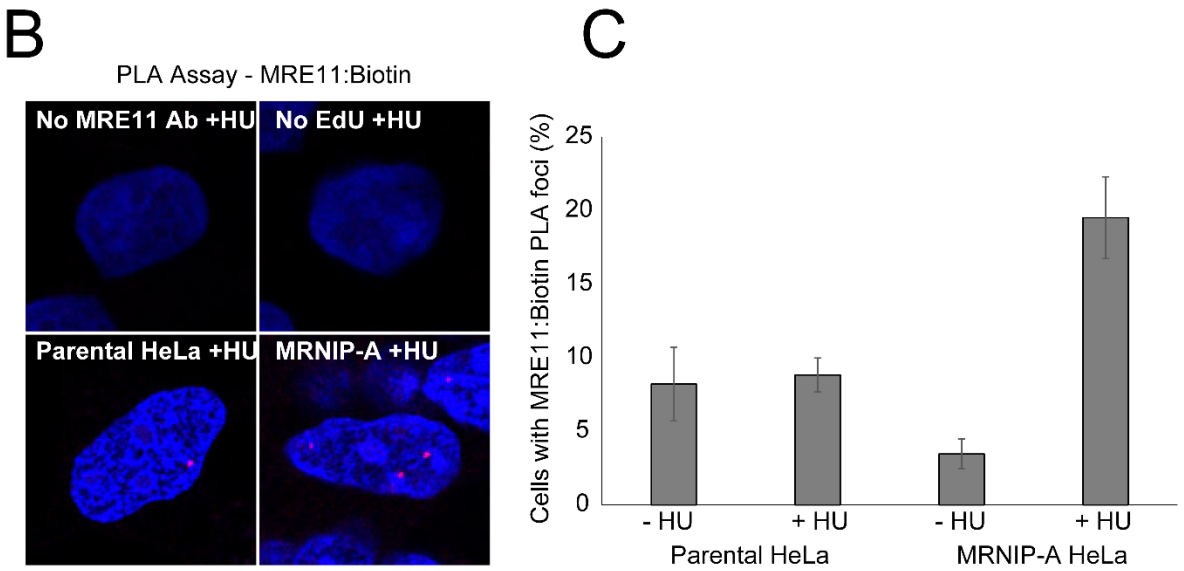


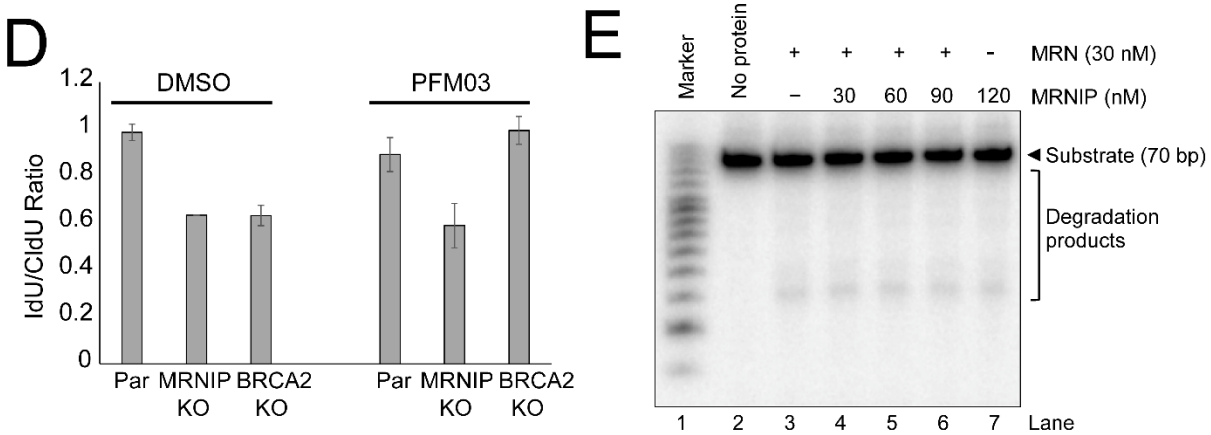
Bennett et al., 2020 Supp Fig 2



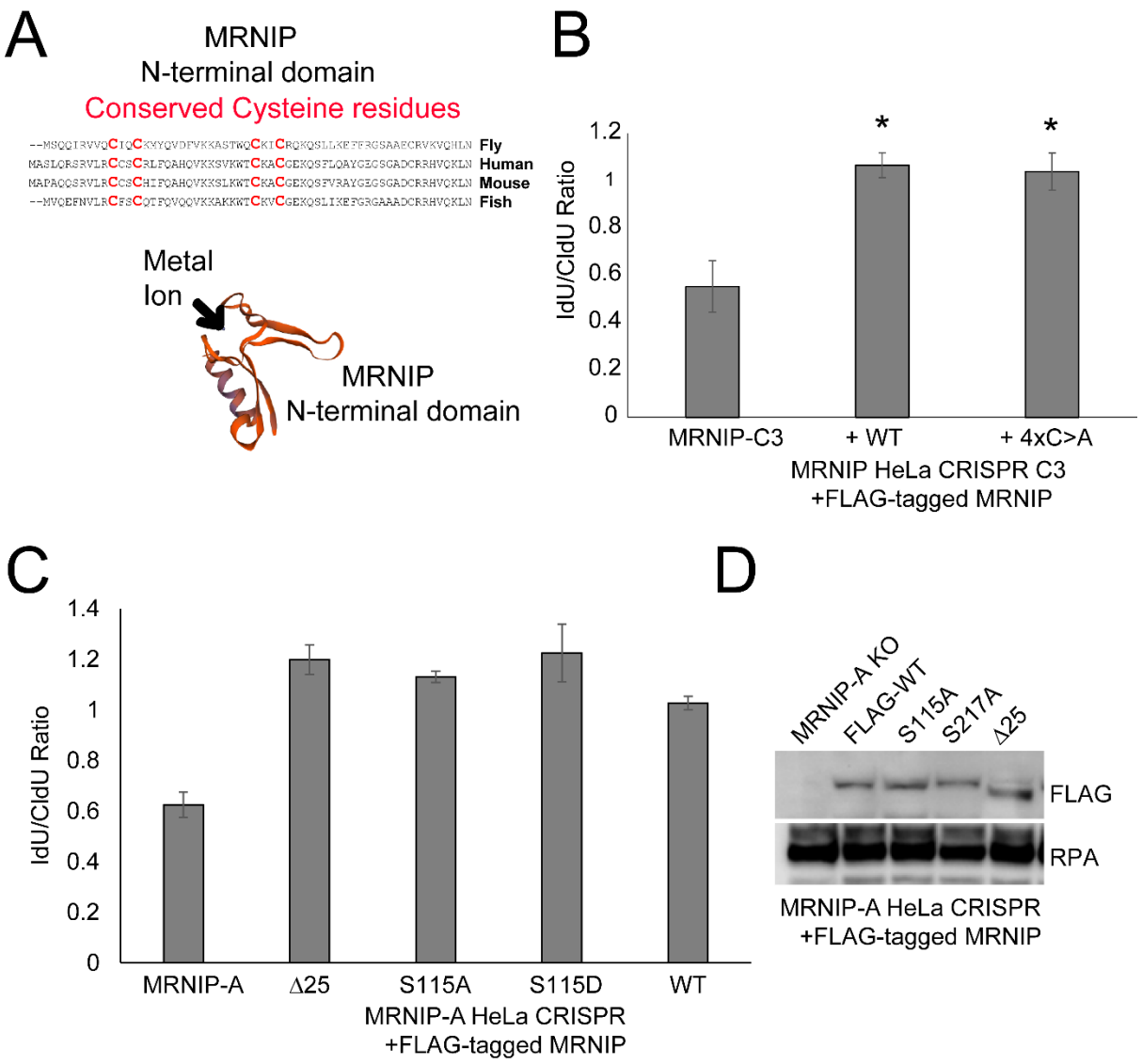
Bennett et al., 2020 Supp Fig 3







Bennett et al., 2020 Supp Fig 4



Bennett et al., 2020 Supp Fig 5

Supplementary Figure 1: MRNIP loss sensitises cells to multiple replication stress agents. A-C: Parental HeLa and HCT cells and MRNIP KO CRISPR derivative lines were treated with the indicated concentrations of MMC or CPT. After 96 hours, an MTT assay was performed, and results normalised to untreated controls. **D:** Parental HeLa cells and a MRNIP KO derivative line were treated with 2 mM HU for 6 hrs, then nuclei were isolated, a neutral COMET assay was performed and the results plotted as tail moment. **E:** MRNIP KO HeLa and KO HeLa cells stably expressing FLAG-MRNIP were fixed and stained with a 53BP1 antibody. The percentage of cells with more than one BP1-containing OPT domain was determined. Data represent the mean from three experimental repeats, and the errors displayed represent standard deviation ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$ where indicated).

Supplementary Figure 2: MRNIP promotes replication fork progression and prevents stalled fork degradation. A: Parental HCT116 and MRNIP KO CRISPR derivative lines were labelled with CldU for 20 min, then IdU for 20 min. Quantification of contiguous tract lengths are displayed as a frequency value for each length. **B** and **C:** Parental HeLa and derivative MRNIP KO cell lines were transfected with a non-targeting control siRNA or siRNAs targeting MRE11 or DNA2. After 48 hrs, whole cell extracts were prepared, resolved by SDS-PAGE and blotted with the indicated antibodies. **D:** MCF7 cells were transfected with a non-targeting control siRNA, or two independent siRNAs targeting MRNIP. After 48 hrs, cells were treated as in (A), and the IdU:CldU tract length ratio for each condition is shown. **E:** Parental HCT116 and derivative MRNIP KO C-3 cells were plated along with stable lines expressing FLAG-MRNIP derived from MRNIP-C3. Cells were labelled with CldU and IdU for 20 min each, then HU was added. Four hours later, cells were fixed and a DNA fibre assay performed. Results are expressed as IdU:CldU ratio. **F:** Parental HeLa, MRNIP-A Hela, or MRNIP-A HeLa cells re-expressing FLAG-MRNIP were treated with the indicated doses of HU and 3 days later survival was assessed by MTT assay. Values were normalized to untreated controls. Data shown represent the mean from three experimental repeats, and the errors displayed represent standard deviation ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$ where indicated).

Supplementary Figure 3: MRNIP deficiency results in increased 53BP1 foci and OPT domains. A and B: U2OS cells were transfected with either control siRNA, MRNIP siRNA, SMARCAL1 siRNA or MRNIP+SMARCAL1 siRNA, and 24 hrs later cells were fixed and stained for 53BP1 and γH2AX, and counterstained with DAPI. Cells with more than 5 foci were counted and represented as a percentage of total cells. **C:** U2OS cells were transfected with either control siRNA, MRNIP siRNA, SMARCAL1 siRNA, PTIP siRNA, MRNIP+PTIP siRNA or MRNIP+SMARCAL1 siRNA, and 24 hrs later cells were fixed and stained for 53BP1, and counterstained with DAPI. The percentage of cells with more than one OPT domain was calculated. **D-F:** HeLa cells were transfected with the indicated siRNAs, and 48 hrs later cells were treated with 3 mM HU for 3 hrs or left untreated. Cells were then fixed and stained for 53BP1 (S3E), or 53BP1 and Cyclin A1 (S3F), counterstained with DAPI and analysed as in A and B, except only cyclin A-negative cells with OPT domains were counted. Data shown represent the mean from three experimental repeats (except E and F, which are from two repeats), and the errors displayed represent standard deviation ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$ where indicated).

Supplementary Figure 4: MRNIP loss results in increased association between MRE11 and nascent DNA. A: Parental HeLa or MRNIP-A KO HeLa cells were treated with either water or 4 mM HU. At the indicated timepoints chromatin was isolated and the extracts resolved by SDS-PAGE prior to blotting with the indicated antibodies. **B and C:** Parental HeLa or MRNIP-A KO HeLa cells were pulsed with 10 mM EdU for 10 min, then treated with either water or 4 mM HU. Two hours later extraction and fixation were performed, and a PLA assay was performed using antibodies raised against MRE11 and EdU. Data represent the mean

number of PLA foci-positive cells from two experimental repeats, and the errors displayed represent standard deviation ($^{*}p \leq 0.05$ where indicated). **D:** Parental HeLa, MRNIP-A KO and BRCA2 KO cell lines were labelled with CldU for 20 min, then IdU for 20 min before the addition of 4 mM HU in the presence of either DMSO or 50 μM PFM03 for 5 hrs. Fork degradation was assessed via IdU:CldU tract length ratio. **E:** Endonuclease assay with MRN (30 nM) and various concentrations of MRNIP (30, 60, 90 and 120 nM) on a 5' end-labeled 70 bp-long dsDNA blocked at both ends with streptavidin. Data shown represent the mean from three experimental repeats, and the errors displayed represent standard deviation ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$ where indicated).

Supplementary Figure 5: Mutant forms of MRNIP are proficient for fork protection. A: Four cysteine residues in the MRNIP N-terminus are conserved between human, mouse, fly and zebrafish. SWISS-MODEL and Phyre2 predict these conserved residues to form a metal ion-binding Zinc finger-like structure. **B:** MRNIP KO HeLa cells, and KO cells stably expressing FLAG-WT MRNIP or FLAG-C4A MRNIP were plated, then labelled with CldU for 20 min, then IdU for 20 min before the addition of 4 mM HU for 5 hrs. **C:** MRNIP KO HeLa cells, and KO cells stably expressing FLAG-WT MRNIP or FLAG-S115A, 115D or Δ25 mutants were plated, then labelled with CldU for 20 min, then IdU for 20 min before the addition of 4 mM HU for 5 hrs. **D:** Cell lines shown in (C) were lysed and whole cell extracts resolved by SDS-PAGE followed by blotting for the indicated antibodies. Data shown represent the mean from three experimental repeats, and the errors displayed represent standard deviation ($^{*}p \leq 0.05$, $^{**}p \leq 0.01$ where indicated).