Expanded View Figures

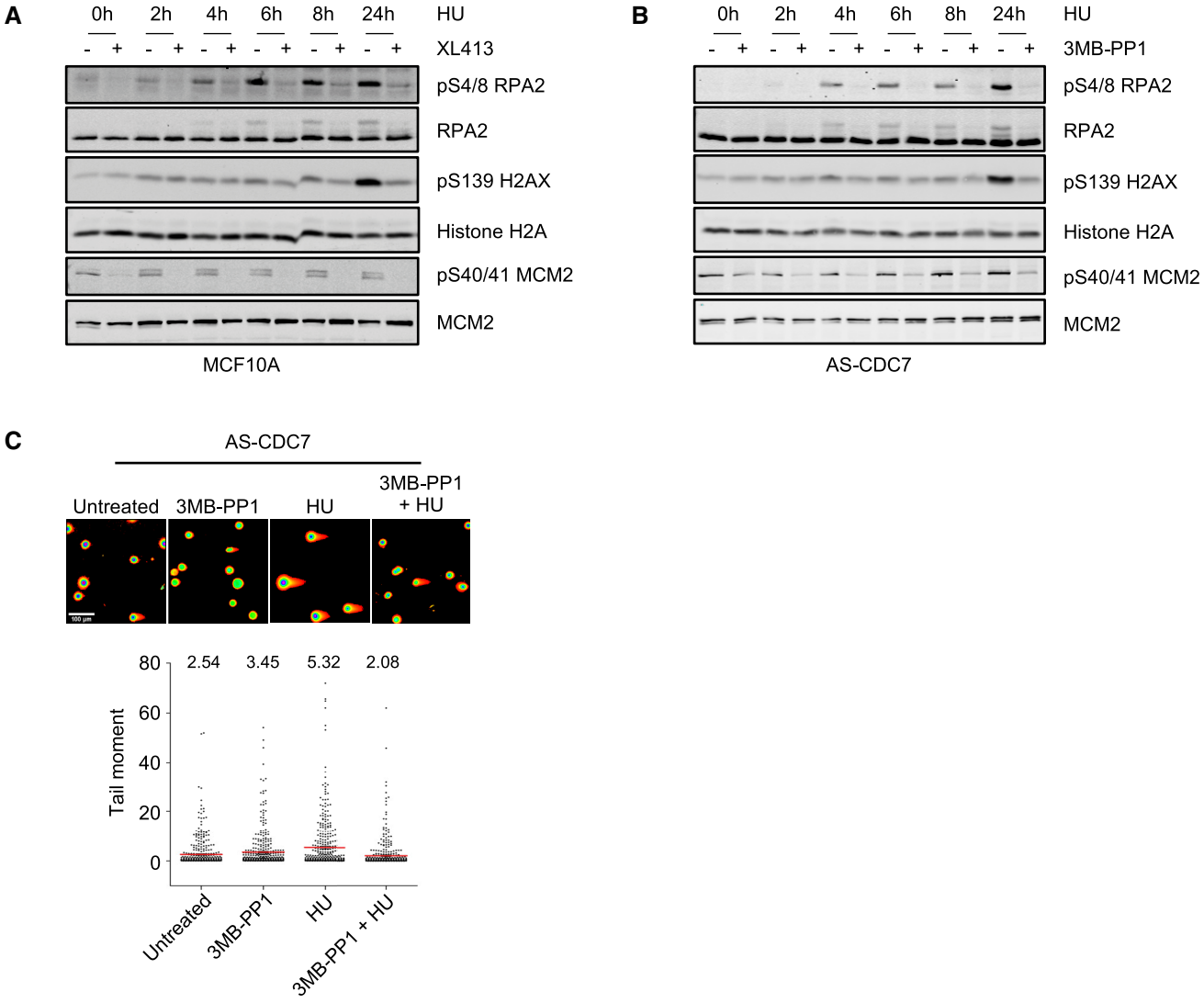

Figure EV1. (related to Fig 1): Inhibition of CDC7 with XL413 in MCF10A cells and with 3MB-PP1 in AS-CDC7 cells suppresses histone H2AX and RPA2 phosphorylation and DNA double-strand break formation in HU.

A, B MCF10A or AS-CDC7 cells were either mock-treated or treated with 10 μM XL413 for 30 min, at which point 4 mM HU was added and cells further incubated for the indicated times. Whole-cell extracts were then analysed by Western blotting with the indicated antibodies. Data are representative of at least two independent experiments.

C AS-CDC7 cells were either mock-treated or treated with 10 μM 3MB-PP1, 4 mM HU or both for 24 h before performing neutral comet assays. Representative images of cells are shown. Scale bar = 100 μm. In the dot plots, ~400 comets per each condition were analysed, means are indicated with red lines, and their values are shown above the plots. Data are from two independent experiments.

Source data are available online for this figure.

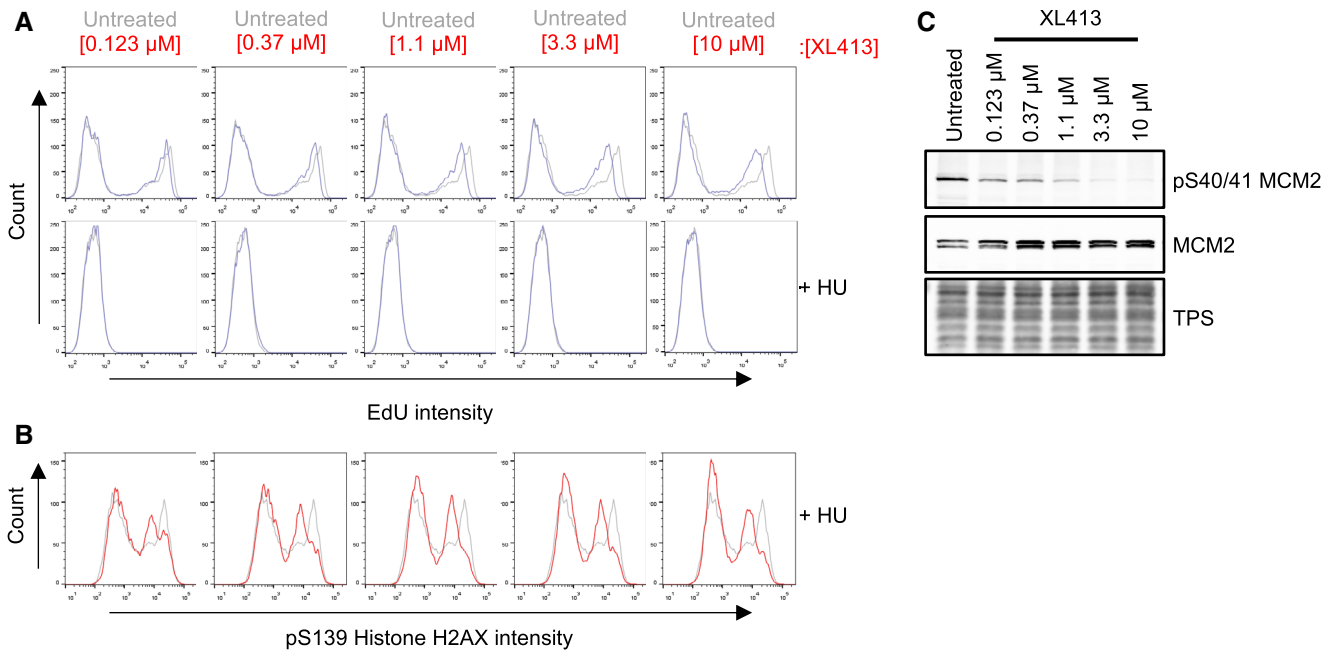

Figure EV2. Partial inhibition of CDC7 has a minor effect on DNA synthesis but markedly affects pS139 H2AX phosphorylation.

U2OS cells were treated with the indicated concentrations of XL413 in the presence or absence of 4 mM HU for 5 h and then labelled with 10 μM EdU 30 min prior to harvest. Samples were processed for flow cytometry analysis.

A Mono-parametric analysis of cell count against EdU intensity. Histograms are overlaid to appreciate changes in EdU intensity upon treatment with XL413 (blue lines) relative to the untreated control (grey lines).

B Mono-parametric analysis of cell count against pS139 histone H2AX intensity in HU-treated cells. Histograms are overlaid to appreciate changes in pS139 histone H2AX intensity upon treatment (red lines) relative to untreated controls (grey lines).

C Whole-cell extracts were analysed by Western blotting with the indicated antibodies, and total protein stain (TPS) is used as a loading control. Reduction of phosphorylation of Ser40/41 on MCM2 is indicative of CDC7 inhibition. Data are representative of two repeat experiments.

Source data are available online for this figure.

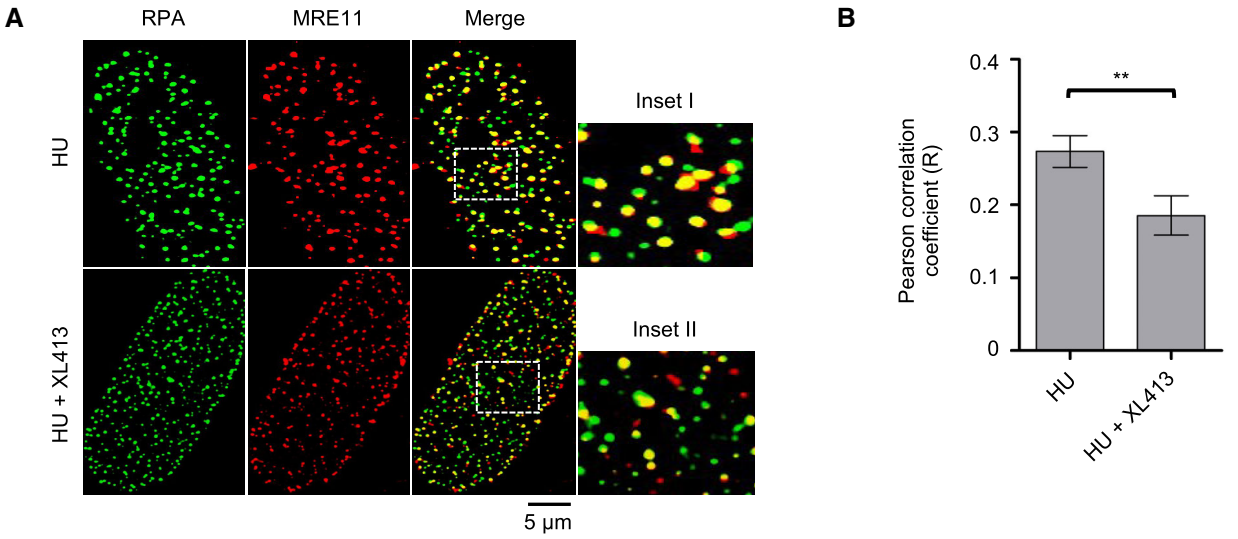

Figure EV3. CDC7 inhibition decreases colocalization of MRE11 with RPA2.

A U2OS cells were treated with 4 mM HU in the presence or absence of 10 μM XL413 for 24 h. RPA2 (green) and MRE11 (red) were detected by immunofluorescence. Insets I–II represent enlargements of selected region of the merged images.

B Quantification of RPA2 and MRE11 colocalization was assessed with ImageJ in ~200 randomly selected cells for each condition from three biological replicates and expressed as Pearson's correlation coefficient. Error bars represent SEM. Statistical significance was assessed by Student's *t*-test (**$P < 0.01$).

Source data are available online for this figure.