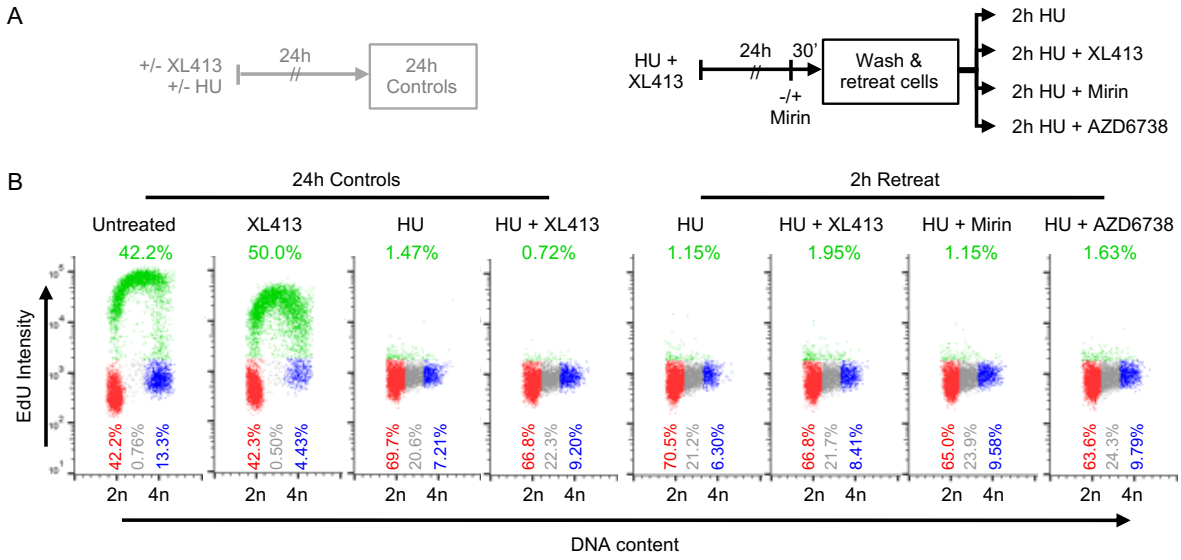
APPENDIX

Appendix Figure S1: related to Figure 2



Analysis of DNA replication in cells analysed in Figure 2.

A. U2OS cells were either untreated, treated with 10 μM XL413 or with 4 mM HU in the presence or absence of 10 μM XL413 for 24 hours. Cells that had been treated with both XL413 and HU for 24 hours, were washed with equilibrated media and retreated as indicated for a further 2 hours. Cells were EdU labelled for 30 minutes before harvesting and analysed by flow cytometry.

B. Biparametric analysis of EdU intensity and DNA content as measured by DAPI. The percentage of cells in each of the cell cycle phase is indicated: G1 (red); S-phase, EdU negative (grey) and EdU positive (green); and G2/M (blue). Data are representative of two independent experiments.

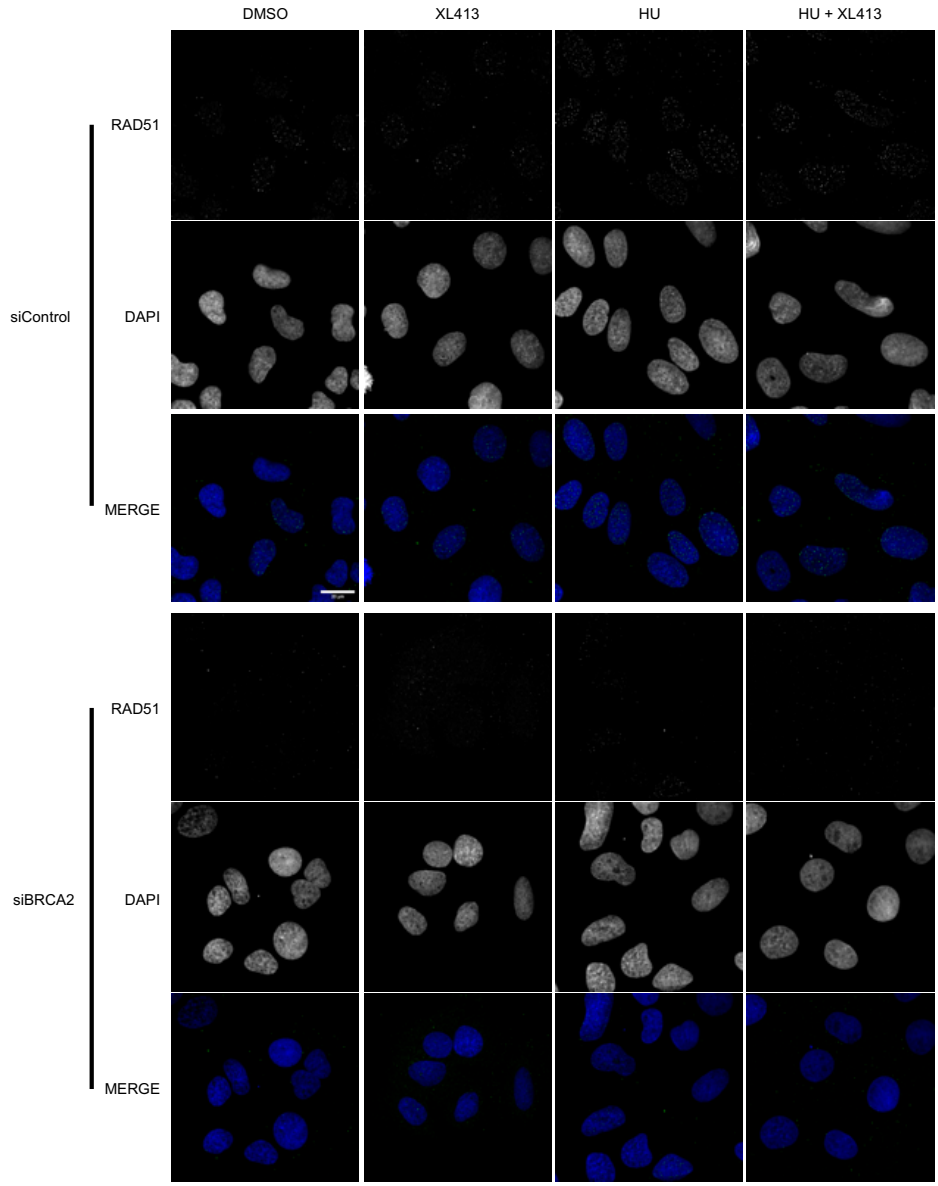
Appendix Figure S2 - related to Figure 3

XL413 does not decrease EXO1 levels.

A. U2OS cells were treated with 4 mM HU in the presence or absence of 10 μM XL413 or 5 μM PHA-767491 for the indicated times. Whole cell extracts were then analysed by western blotting with the indicated antibodies and total protein stain (TPS) as a loading control. Reduction of phosphorylation of Ser40/41 on MCM2 is indicative of CDC7 inhibition.

B. U2OS cells were transfected with non-targeting (siCon) or an siRNA targeting EXO1 (siEXO1) and incubated for the indicated times. Whole cell extracts were analysed by western blotting with anti-EXO1 antibodies. Total protein stain (TPS) as a loading control. Image is representative of two independent experiments.

Appendix Figure 3 – related to figure 6



XL413 treatment does not rescue RAD51 focal recruitment in HU in BRCA2 depleted cells.

U2OS cells were transfected with either non targeting siRNA (siControl) or with BRCA2 targeting siRNAs. After 48 hours cells were treated with 10 μM XL413, 4 mM HU or both and incubated for a further 24 hours. Cells were then analysed by immunofluorescence microscopy with anti-RAD51 antibodies. DNA was stained with DAPI. Scale bar = 20 μM.

Appendix Table S1 - related to Figure 5

U2OS	siLuc	siLuc	siBRCA2	siBRCA2
HU	+	+	+	+
XL413	-	+	-	+
% reversed forks Exp #1 (Figure 5)	**23 (78)**	**23 (92)**	**12 (72)**	**22 (74)**
% reversed forks Exp #2 (Repeat)	**21 (72)**	**22 (75)**	**10 (83)**	**24 (88)**

Inhibition of CDC7 protects reversed forks in BRCA2 deficient cells.

U2OS cells were transfected with control (siLUC) or BRCA2 targeting siRNA (siBRCA2) and after 48 hours were treated with 4 mM HU for 5 hours and 10 μM XL413 where indicated. Replication intermediates were then analysed by electron microscopy. The frequency of reversed replication forks is displayed and the number of replication intermediates analysed is indicated in parentheses for two independent experiments.