



Supplementary Figure S4: Rad51 colocalizes with γ H2AX in response to cisplatin treatment

(A) Kuramochi cells were not pretreated (mock) or pretreated with DMSO or 15 μ M ATM inhibitor Ku-55933 for 1 hour and then untreated (U) or treatment with 15 μ M cisplatin for 8 hours (T) (N=3). **(B)** OVCAR5 cells were pretreated with ATM inhibitor as in (A) and then untreated (U) or treated with 6.77 μ M carboplatin for 24 hours. Lysates were analyzed by WB (N=2). **(C)** Representative images of OVCAR5 cells showing RING1A colocalization with γ H2AX with and without ATMi followed by cisplatin treatment. White arrows indicate cells showing colocalization and yellow arrows indicate cells with less or no colocalization. **(D)** OVCAR5 cells were either untreated or treated with 12 μ M cisplatin for 8 hours and then immunofluorescence was performed for γ H2AX (green) and Rad51 (red). White arrows point to examples of γ H2AX and Rad51 foci that co-localize. Graph shows mean percentage of cells with ≥ 4 Rad51 and γ H2AX co-localized foci \pm SEM. Scale bar = 5 μ m. **(E)** OVCAR5 cells were not pretreated (mock) or pretreated with DMSO or 50 μ M Rad51 inhibitor B02 for 2 hours and then untreated (U) or treated with 2 Gy IR. After IR exposure, cells were allowed to recover for 15 minutes at 37°C. Graph shows mean percentage of cells with ≥ 4 γ H2AX and Rad51 co-localized foci \pm SEM (N=3). White arrows show examples of γ H2AX and Rad51 foci that co-localize. Scale bar = 5 μ m. **(F)** EV or XPC KD OVCAR5 cells were either pretreated with DMSO or 50 μ M Rad51 inhibitor (B02) for 2 hours and then (U) or treated (T) with cisplatin for 8 hours. Lysates were analyzed by WB (N=2). Statistical significance was

calculated using Student's t test. For untreated versus cisplatin or 2 Gy IR treated, P-values * < 0.05, ** <0.005, *** < 0.0005. For Mock or DMSO versus Rad51i P – values # < 0.05, ## < 0.005, ### < 0.0005.