

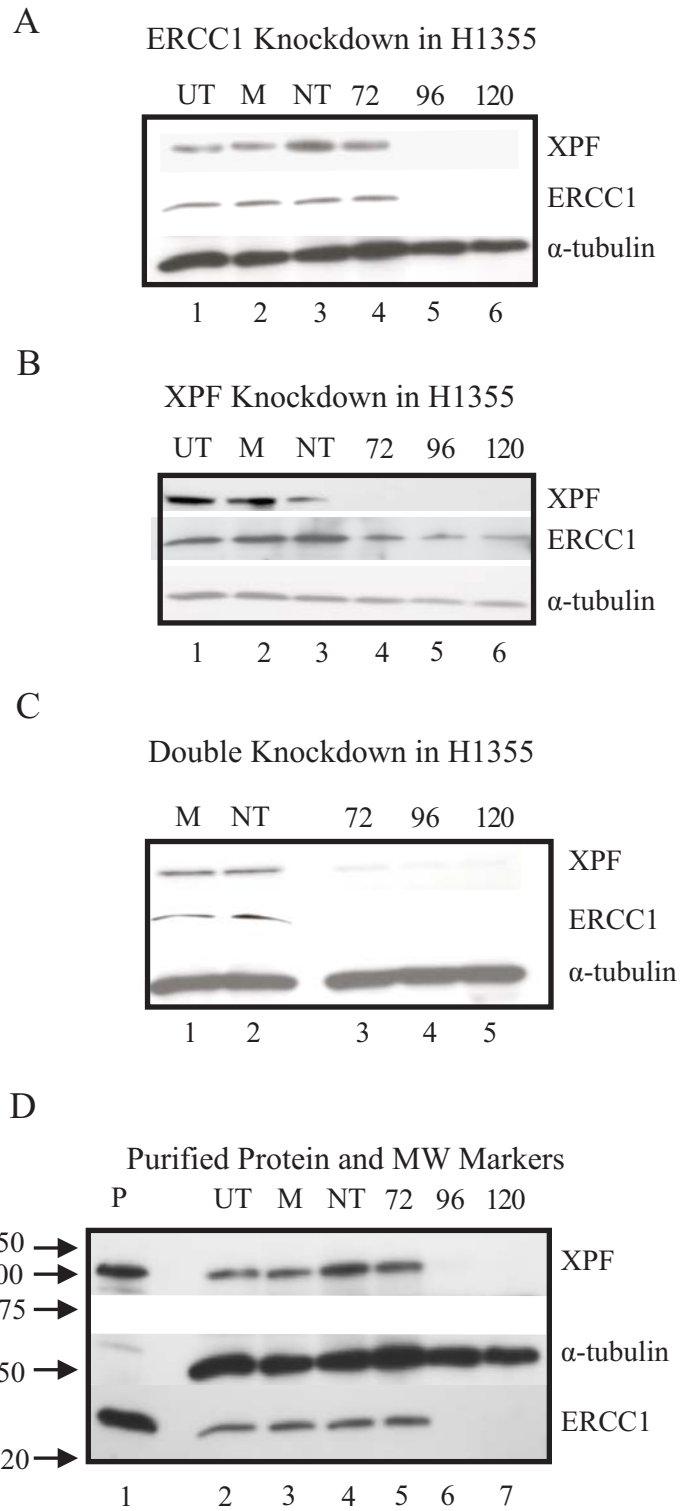
SUPPLEMENTAL FIGURE LEGENDS

Figure 1. Timecourse showing siRNA mediated downregulation of ERCC1 and XPF protein in H1355 cells. Cells were transiently transfected twice at 24 h intervals, each time with smartpool siRNAs (100 nM) directed against ERCC1 (A), XPF (B) and both proteins (C - double knockdown). Control cells were left untransfected (UT), or mock (M) - and Non-targeting (NT) siRNA (100 nM) - transfected. Proteins were extracted at the indicated time points of 72, 96 and 120 h post-transfection, and probed with XPF, ERCC1 and α -tubulin as a loading control. The percent of knockdown was quantified using the Alpha Innotech HD2 and is represented for all cell lines tested in Table 1. Blots from (A) and (B) were also probed for XPF or ERCC1 on individual knockdown of ERCC1 or XPF, respectively. (D) shows XPF and ERCC1 as they run on the gel with purified XPF and ERCC1 protein. The position of the molecular weight markers are shown with arrows. XPF runs at ~110 kD and ERCC1 at ~ at 38 kD. α -Tubulin is shown as a loading control. (P) refers to purified protein.

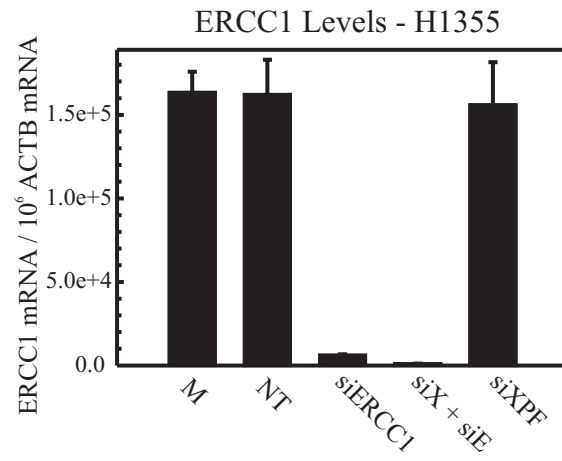
Figure 2. Transcript levels on siRNA mediated downregulation. (A) represents ERCC1 transcript levels. H1355 cells were mock (M) treated or Non-targeting (NT) - , XPF-, ERCC1- , and XPF-ERCC1 (denoted as siX+siE) - siRNA transfected, twice at 24 h intervals each and harvested at 48 h post-transfection. Total RNA was extracted from cells and analyzed using StaRT PCR, as described in the Methods section. Each PCR was run in triplicate. The transcript levels are represented as ERCC1 mRNA / 10^6 ACTB mRNA. The values are represented as mean \pm S.E.M from triplicate PCRs. (B)

represents XPF transcript levels. H1355 cells were mock (M) treated or Non-targeting (NT) - , XPF-, ERCC1-, and XPF-ERCC1 (denoted as siX+siE) - siRNA transfected, twice at 24 h intervals each and harvested at 48 h post-transfection. Total RNA was extracted from cells and analyzed using StaRT PCR, as described. Each PCR was run in triplicate. The transcript levels are represented as XPF mRNA /10⁶ ACTB mRNA. The values are represented as mean ± S.E.M from triplicate PCRs.

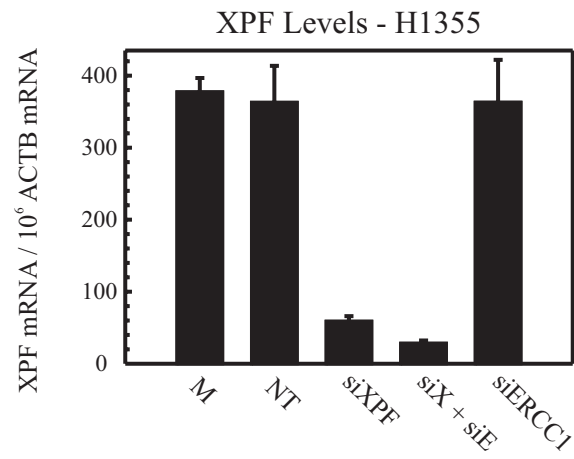
Figure 3. Repair kinetics of γ -H2AX foci post cisplatin treatment in (A and B) 2008 (C and D) MDA-MB-231 show quantitation of γ -H2AX focus formation at various time points post cisplatin treatment in untransfected (3 A and C) and XPF+ERCC1 (3 B and D) double knockdown cells respectively. The cells were seeded onto glass coverslips at 25% confluency. The next day they were treated with cisplatin for 2h and then fresh complete medium was added. The cells were fixed and immunostained for γ -H2AX at the indicated time points post cisplatin treatment. For each data point foci were counted in 250 cells at each time point per condition in each cell line. The foci have been categorized as zero to 5 (2008) or zero (MDA-MB-231), six to ten (2008) or one to ten (MDA-MB-231), and greater than ten foci per nucleus. The results are expressed as % γ -H2AX foci per nuclei, and the data was collected from two individual experiments.



A



B



Supplemental Figure 3

