Supplemental material

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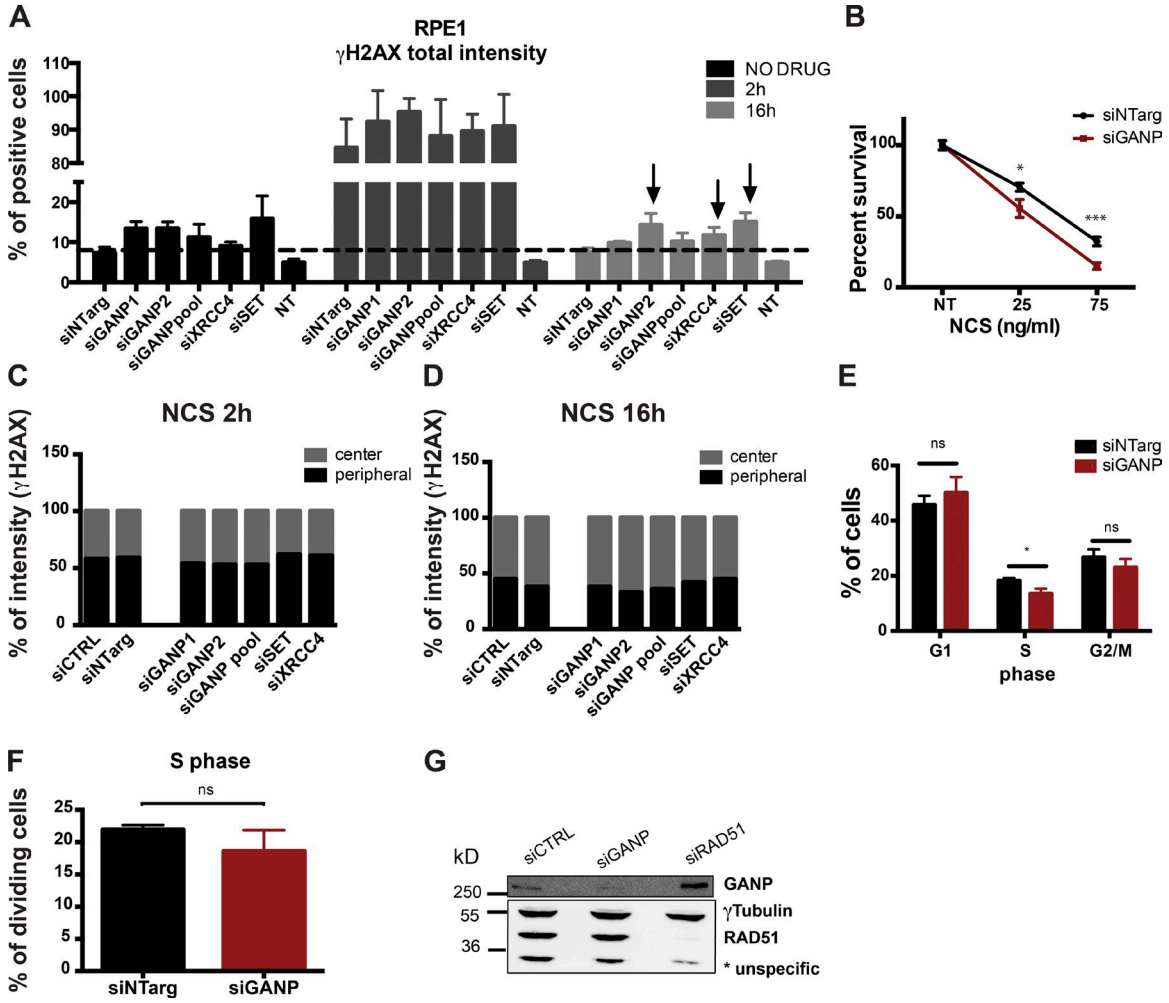

Figure S1. **GANP affects global DNA repair without affecting cell cycle progression and global RAD51 levels. (A)** High-throughput screening was performed to investigate the effect of GANP/TREX-2 KD in hTERT RPE1 cells. The control siRNA for RPE1 cells is represented by siNTarg. Graph represent one experiment with three technical replicates each condition. Error bars represent SD among the three technical replicates. Per each condition and replicate, $n > 1{,}000$ cells (for details, refer to Fig. 1 E). **(B)** Clonogenic survival assay of control HeLa cells and cells depleted of GANP treated with increasing concentrations of NCS. Graph represents the average of three independent experiments with three technical replicates each. Error bars represent SEM. Statistical significance was calculated using the Mann–Whitney test (ns, $P > 0.05$; *, $P \le 0.05$; **, $P \le 0.01$). **(C and D)** Comparison of the percentage of intensity for γH2AX signal between peripheral and inner nuclear environment of the high-throughput screening in Fig. 1 (E and F). $n > 1{,}000$ cells. **(E)** Cell cycle analysis of U2OS cells treated with GANP siRNA and control siRNA for 48 h before propidium iodide staining. Graph represents the percentage of cells in G1, S, and G2/M phase of the cell cycle, based on DNA content as a merge of four independent experiments. Error bars represent the SD. Statistical significance was calculated with multiple *t* test (**, $P \le 0.01$). **(F)** Analysis of HeLa cells in S phase after treatment with GANP siRNA and control siRNA for 48 h. Graph represents the percentage of cells in S-phase using EdU staining as a merge of three independent experiments. Error bars represent SD. Statistical significance was calculated using the *t* test (ns, $P > 0.05$). **(G)** Western blot analyses of total cell lysate obtained by RIPA extraction from cells treated with GANP siRNA, RAD51 siRNA, and control siRNA for 48 h. Membrane was stained with the indicated antibodies. For antibody details, see Table 2. For details about statistical analysis, see Statistical analysis section in Materials and methods. NT, nontreated. siCTRL, control siRNA; siNTarg, nontargeting siRNA (see Table 1).

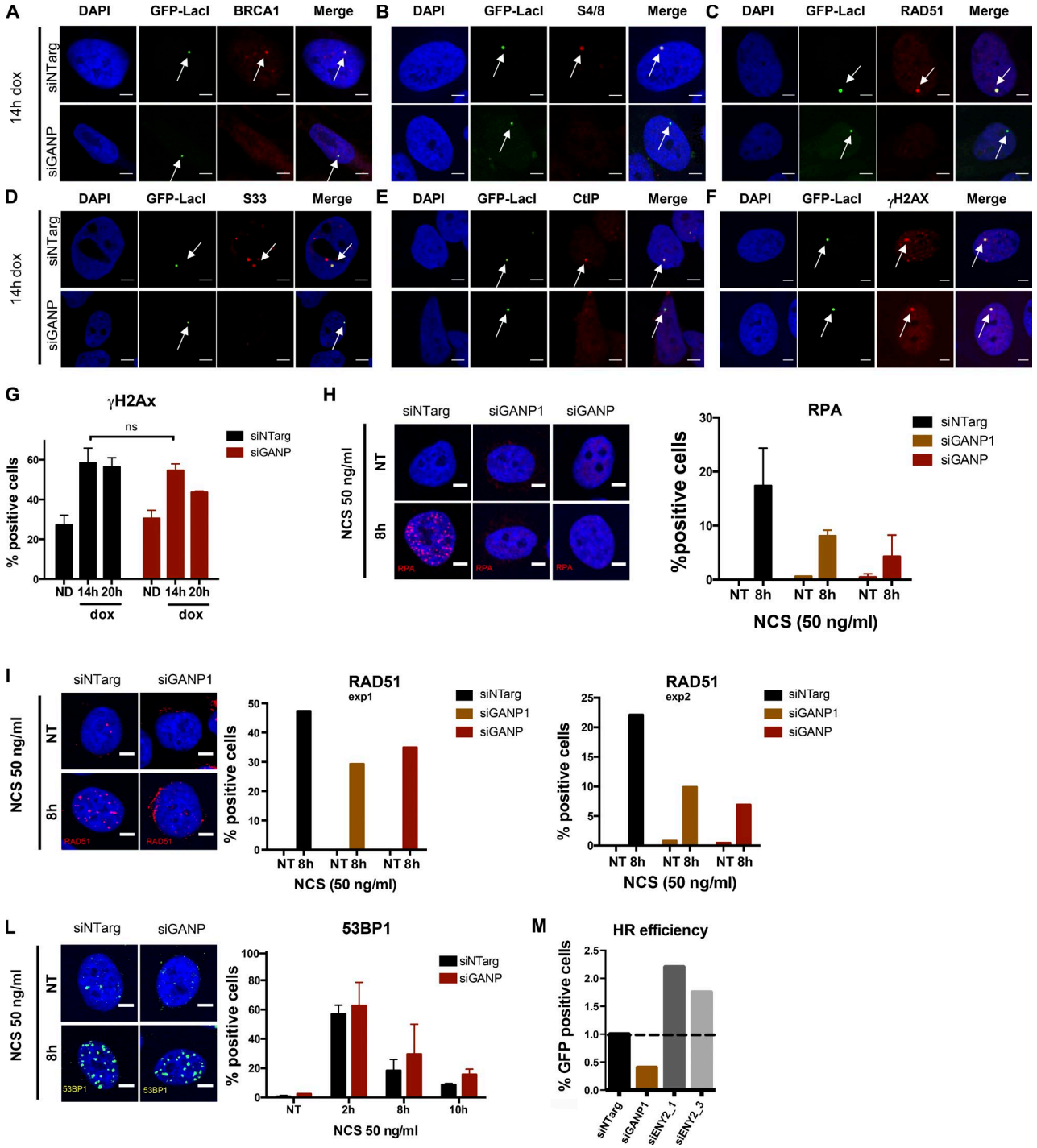

Figure S2. **GANP depletion affects resection. (A–E)** Representative images of the quantified percentages of colocalization shown in Fig. 3 (A–E). Representative images of the LacO array after 14 h dox addition in control cells and cells depleted of GANP. White arrows indicate the GFP-LacI protein (green spots), at the LacO array, and colocalization (in control cells) or no colocalization (in GANP-depleted cells) with BRCA1 (A), phosphoRPA S4/8 (B), RAD51 (C), phosphoRPA S33 (D), and CtIP (E; red spots), respectively. **(F)** Representative images of the efficient H2AX phosphorylation after dox addition in both control and GANP-depleted cells. **(G)** Percentage of colocalization between γH2AX and the LacO array after dox addition. Graph represents the merge of four independent experiments with SD. Statistical analysis was calculated using the *t* test (ns, P > 0.05). **(H–L)** Representative images showing deficient formation of RPA foci (H) and RAD51 foci (I) and efficient formation of 53BP1 foci (L) after NCS treatment at 50 ng/ml in control HeLa cells and cells depleted of GANP with two different siRNAs. Graphs show the percentage of cells showing more than five foci. For each experiment, 10 pictures were acquired using confocal microscope SP5 inverted, with $n > 100$ cells per condition. For details about image acquisition, see the Microscopy section of Materials and methods. Graph in H shows the merge of two independent experiments, graphs in I show two independent experiments separately, and graph in L shows the merge of four independent experiments with SD. **(M)** DR-GFP assay was performed as described in Fig. 2 A and in Fig. 4 (A and D). Different siRNAs for GANP and ENY2 were used. Bars 5 µm. For details about statistical analysis, see Statistical analysis section in Materials and methods. ND, no dox; NT, nontreated; siNTarg, nontargeting siRNA (see Table 1).

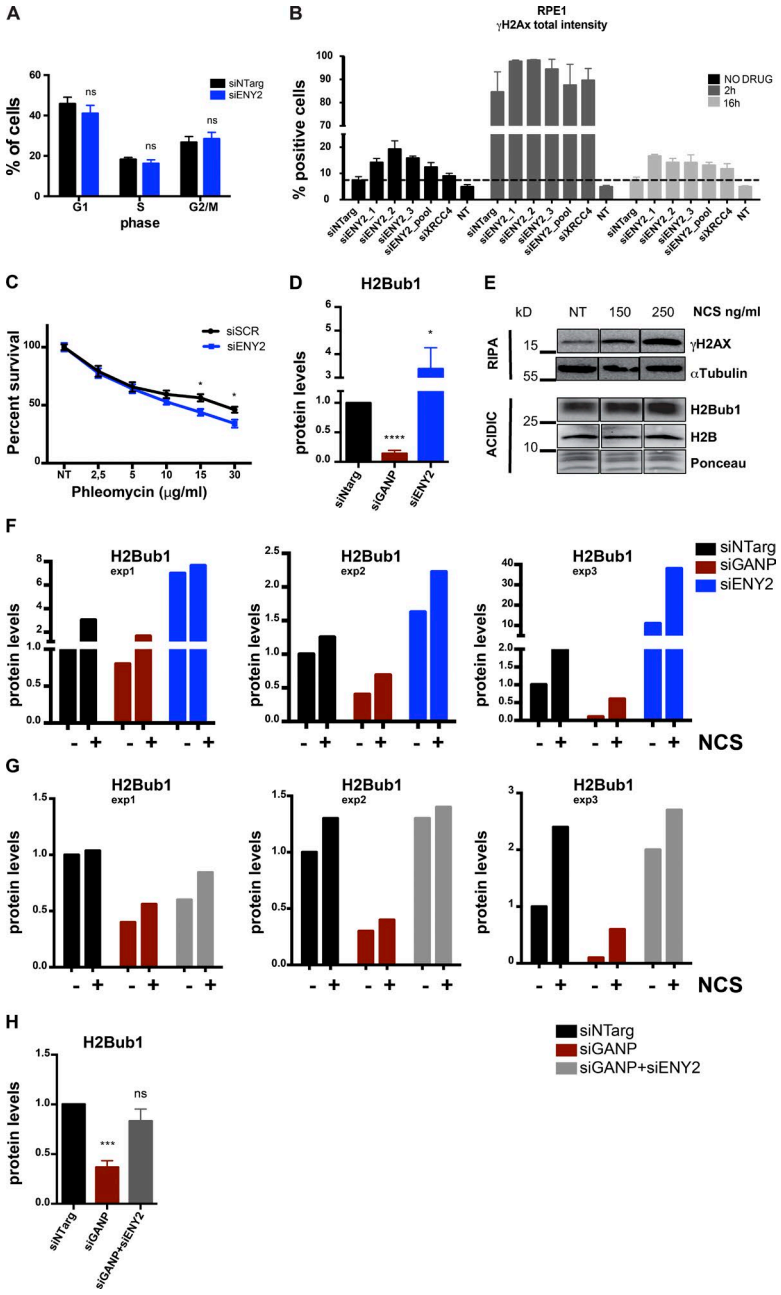

Figure S3. **ENY2 is required to avoid unscheduled HR; TREX-2 and DUB interplay is necessary to maintain balanced h2Bub1 global levels. (A)** Cell cycle analysis of U2OS cells treated with ENY2 siRNA and control siRNA for 48 h before propidium iodide staining. Graph represents the percentage of cells in G1, S, and G2/M phase of the cell cycle based on DNA content as a merge of four independent experiments. Error bars represent SD. Statistical significant was calculated using the multiple *t* test (ns, P > 0.05). **(B)** High-throughput screening was performed to investigate the effect of ENY2 KD in DNA repair in hTERT RPE1 cells. Control siRNA is represented by siNTarg in RPE1 cells. Graph represents one experiment with three technical replicates. Per each condition, *n* > 1,000 cells (for details, refer to Fig. 1 E). **(C)** Clonogenic survival assay of HeLa cells depleted of ENY2 and control cells treated with increasing concentrations of phleomycin. The graph represents the average of three independent experiments with three technical replicates each, where the number of colonies per each concentration was normalized to the respective nontreated (NT) condition. Error bars represent SEM. Statistical significance was calculated using the Mann–Whitney test (ns, P > 0.05; *, P ≤ 0.05). **(D)** H2Bub1 bands intensity from Western blot analyses quantified using Fiji normalized to H2B levels. Graphs represent the merge of four independent experiments with SD included the representative experiment shown in Fig. 5 A. Statistical analysis was calculated using the *t* test (*, P ≤ 0.05; ****, P ≤ 0.001). **(E)** Western blot analysis of total protein lysate (RIPA) and histone proteins (ACIDIC) of HeLa cells upon increasing concentrations of NCS. Membranes were blotted with depicted antibodies. **(F and G)** H2Bub1 bands intensity from Western blot analyses quantified using Fiji normalized to H2B levels of cells depleted of GANP or ENY2 (F) and cells depleted of both (G) with or without damage (NCS treatment). Each panel represents three individual graphs corresponding with three independent experiments and Western blots of which a representative experiment is shown in Fig. 5 (B and D). **(H)** H2Bub1 bands intensity from Western blot analyses quantified using Fiji, normalized to H2B levels. Graphs represent the merge of three independent experiments with SD including the representative experiment shown in Fig. 5 C. Statistical analysis was calculated using the *t* test (ns, P > 0.05; ***, P ≤ 0.001). For details about statistical analysis, see Statistical analysis section in Materials and methods. siNTarg, nontargeting siRNA (see Table 1).

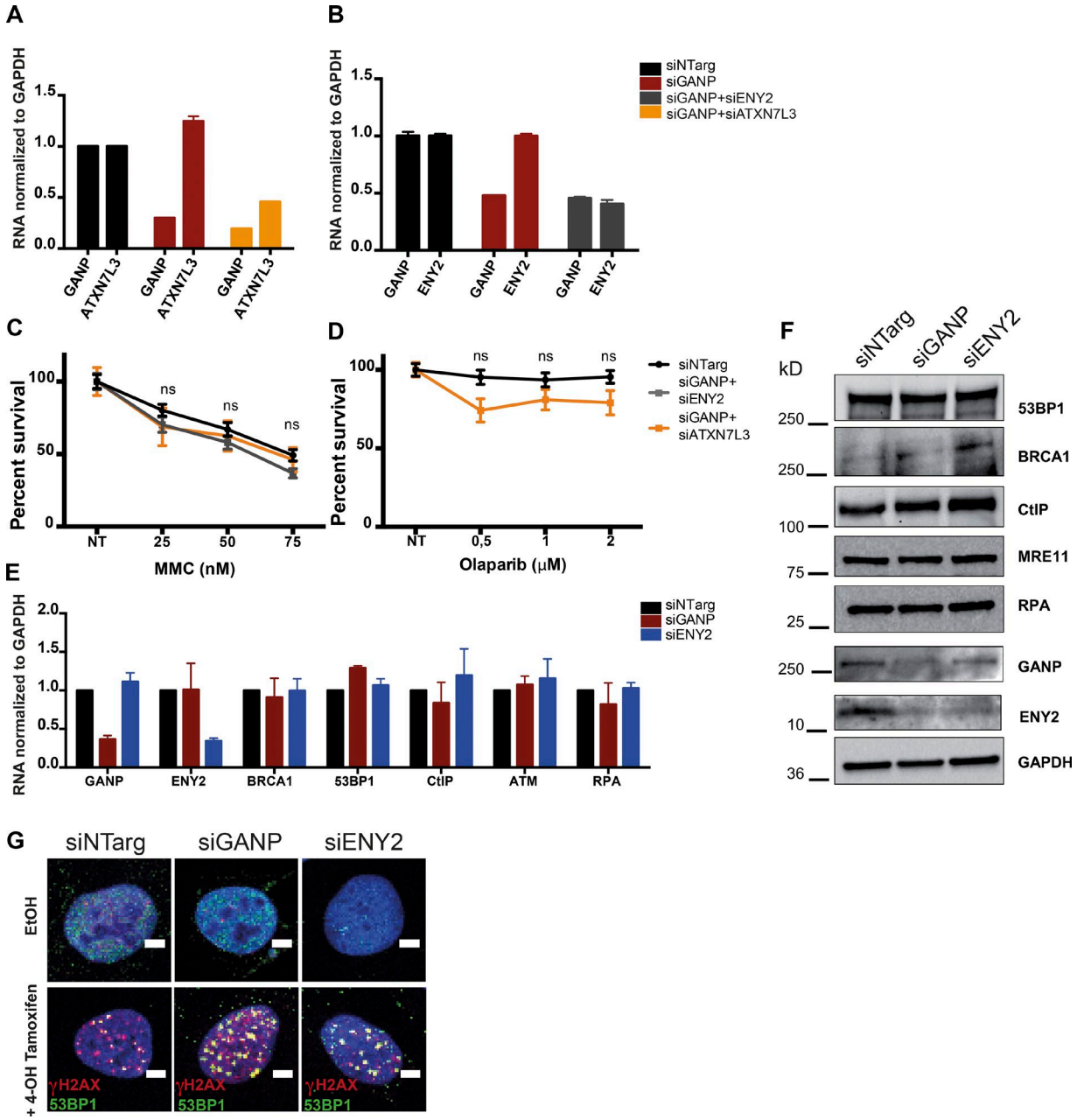

Figure S4. **Double depletion of GANP and the DUB module rescues cell survival to HR-inducing drugs and does not affect the levels of key DNA repair genes. (A and B)** Quantification of GANP, ENY2, and ATXN7L3 RNA levels by qPCR analysis. Graph represents the levels of the RNA normalized to GAPDH RNA levels. In the figure is depicted one representative experiment with qPCR technical duplicates out of three biological replicates. On the x axis is depicted the analyzed RNA with specific primers. Colored boxes represent the different siRNA treatments. **(C and D)** Clonogenic survival assay of HeLa cells depleted of both GANP and ENY2 (gray lines) or GANP and ATXN7L3 (orange lines) and control cells treated with increasing concentration of MMC and Olaparib. Graphs represent the average of three independent experiments with three technical replicates each, where the number of colonies per each concentration was normalized to the respective nontreated (NT) condition. Error bars represent SEM. Statistical significance was calculated using the Mann–Whitney test (ns, $P > 0.05$). **(E)** mRNA levels normalized to GAPDH of key repair genes were analyzed by RT-qPCR as indicated. Graph represents the merge of two independent experiments, each with qPCR technical duplicates. Error bars represent SD. **(F)** Western blot analyses of RIPA extracts of key repair genes after siRNA KD as indicated. **(G)** Representative images of DIvA cells treated with vehicle (ethanol) or 4-OH tamoxifen and the indicated siRNAs. γH2AX (in red) and 53BP1 (in green) were labeled by immunofluorescence. Bars, 5 µm. For details about statistical analysis, see Statistical analysis section in Materials and methods. siNTarg, nontargeting siRNA (see Table 1).