Expanded View Figures

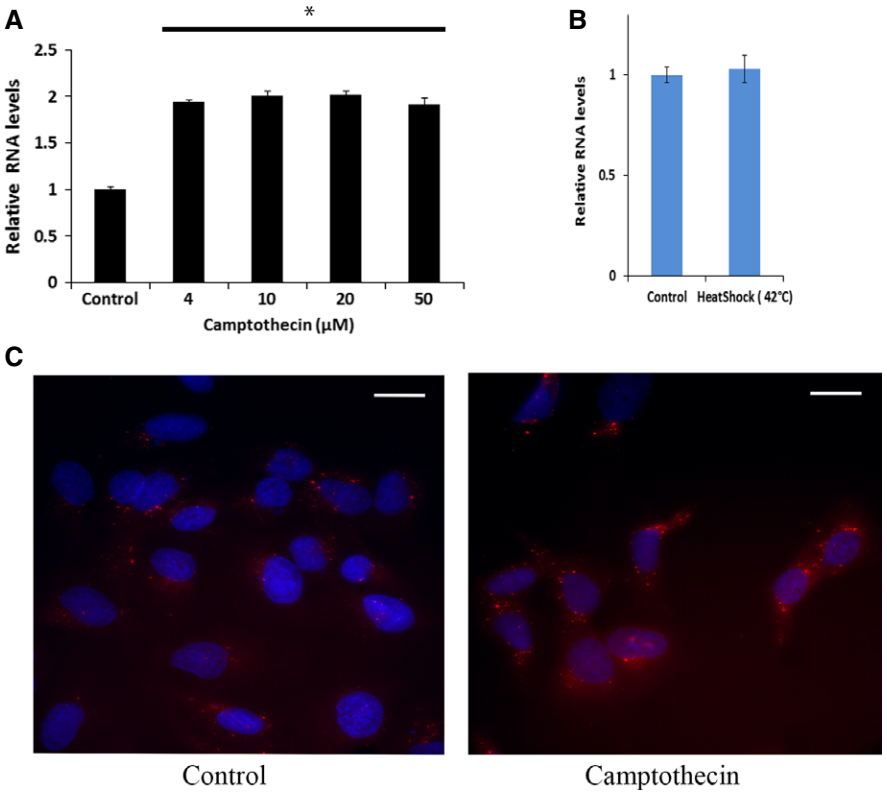

Figure EV1. Further characterization of *DDSR1* levels and subcellular localization.

A *DDSR1* induction is not dose dependent. Human fibroblast cells were treated with the indicated concentration of camptothecin for 3 h and analyzed for *DDSR1* expression by quantitative RT–PCR.

B *DDSR1* does not respond to heat shock. Human fibroblast were kept at 42 or 37°C (control) for 1 h and *DDSR1* expression was determined.

C Visualization of *DDSR1* by RNA fluorescence *in situ* hybridization (FISH) in U2OS cells untreated or treated with camptothecin (10 μM). Scale bar represents 20 μM.

Data information: For (A, B), RNA samples were analyzed by quantitative RT–PCR, and error bars represent the mean ± SEM from three independent experiments. *Significant change compared to control cells ($P < 0.05$). Statistical comparisons were made using Student's *t*-test.

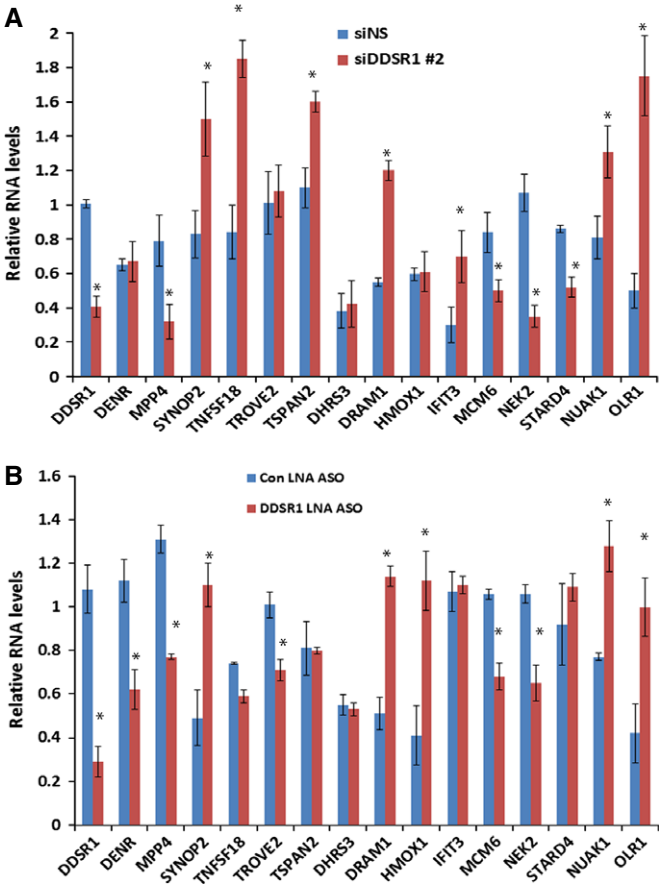

Figure EV2. Validation of *DDSR1*-regulated mRNAs by additional siRNAs.

A Human fibroblasts cells were transfected with siNS or si*DDSR1*#2, and 72 h later, the transcript levels of the genes indicated were measured.

B Human fibroblasts cells were transfected with control locked nucleic acid antisense oligonucleotides or an antisense oligonucleotide against *DDSR1*, and 48 h later, the transcript levels of the genes indicated were measured.

Data information: RNA samples were analyzed by quantitative RT–PCR, and error bars represent the mean ± SEM from three independent experiments. *Significant change compared to control cells ($P < 0.05$). Statistical comparisons were made using Student's *t*-test.

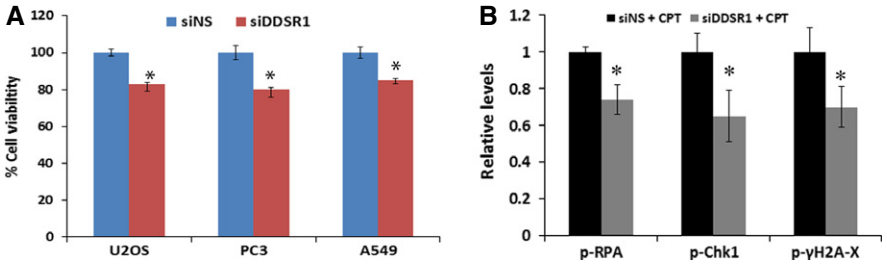

Figure EV3. *DDSR1* knockdown reduces cancer cell proliferation.

A Cell proliferation upon *DDSR1* knockdown. Viability of A549, PC3, and U2OS cells was determined by WST1 assay 72 h after transfection with siNS or si*DDSR1*#1. The graph represents the percentage of viable cells as compared to control cells for each cell type. Viability of cells transfected with siNS for each cell line was taken as a control. Values represent mean ± SD of three independent experiments. *Significant change compared to control cells ($P < 0.05$).

B Densitometry measurements of Western blots shown in Fig 4B for the indicated antibodies. Values represent the means ± SD from three individual experiments. *Significant change compared to siNS cells treated with CPT ($P < 0.05$).

Data information: Statistical comparisons were made using Student's *t*-test.

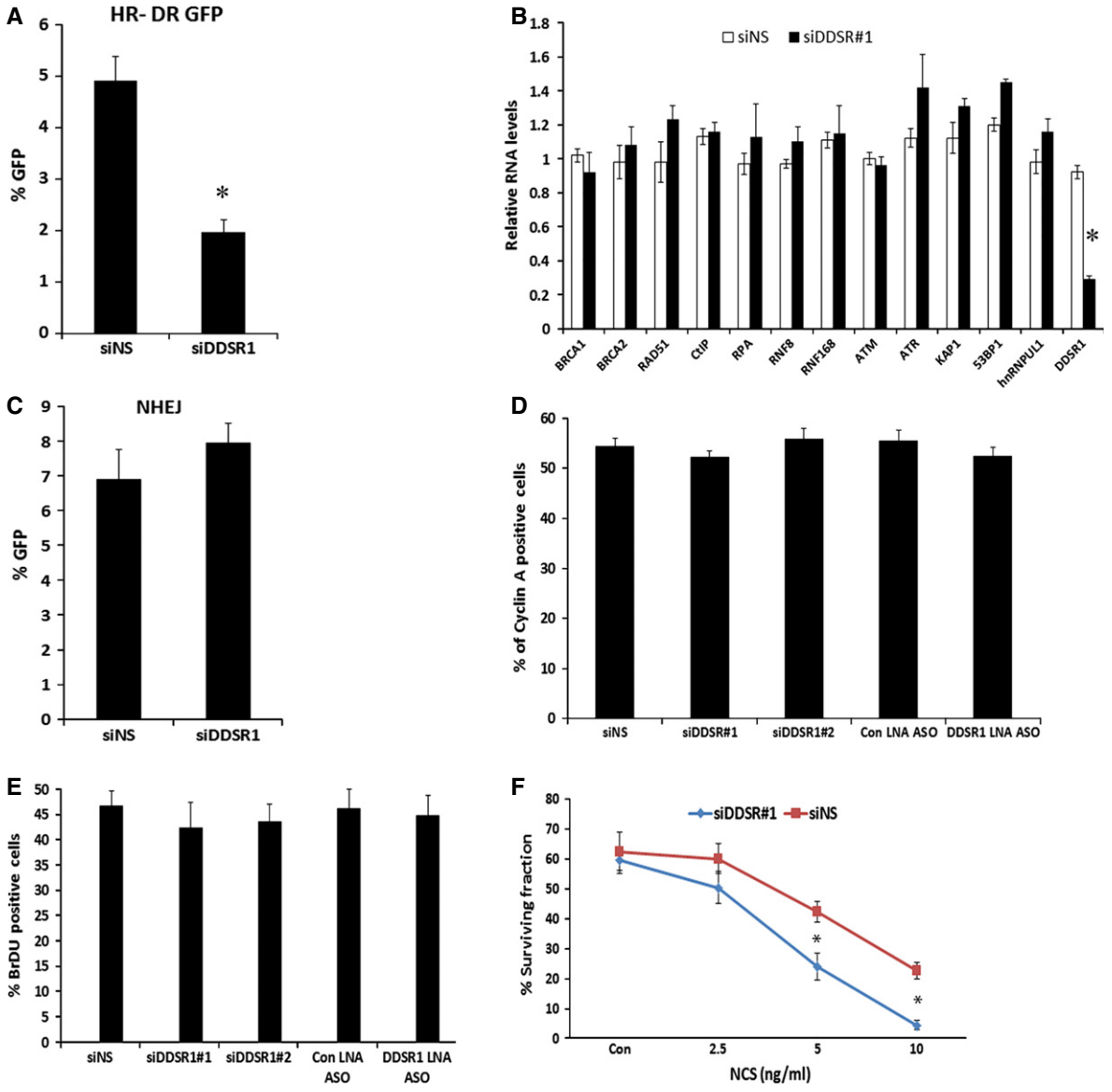

Figure EV4. HR inhibition upon *DDSR1* knockdown is not due to cell proliferation changes.

A HR efficiency in DRGFP-U2OS cells. Cells were transfected with siNS or si*DDSR1*#1 followed by I-SceI expression by transient transfection. HR efficiencies were analyzed by fluorescence-activated cell sorting. Efficiency of repair by HR is shown as % GFP^+ cells. Values represent mean ± SEM from three independent experiments. *Significant change compared to control cells ($P < 0.05$).

B *DDSR1* knockdown does not affect DNA repair gene expression. U2OS cells were transfected with siNS or si*DDSR1*#1, and expression levels of DNA repair genes were determined by qRT–PCR. RNA samples were analyzed by quantitative RT–PCR, and error bars represent the mean ± SEM from three independent experiments. *Significant change compared to cells transfected with siNS ($P < 0.05$).

C DNA repair by NHEJ is not affected by *DDSR1* knockdown. NHEJ efficiencies were analyzed by fluorescence-activated cell sorting after transient transfection of I-SceI in NHEJ-U2OS reporter cells transfected with siNS or si*DDSR1*#1. Efficiency of repair by NHEJ is shown as % GFP^+ cells. Values represent mean ± SEM from three independent experiments.

D *DDSR1* knockdown does not cause cell cycle defects. U2OS cells were transfected with the indicated oligonucleotides for 72 h and probed for cyclin A expression by immunofluorescence. Samples were analyzed in quadruplicates with ≥ 300 cells per sample and values expressed as mean ± SD.

E *DDSR1* knockdown does not perturb DNA replication. Cells transfected with the indicated oligonucleotides were pulsed with BrDU for 2 h and analyzed by fluorescence-activated cell sorting. Values are expressed as the % BrDU-positive cells per 10,000 7-AAD events from triplicates. Bars represent mean values ± SD.

F *DDSR1* regulates cell survival upon genotoxic stress. Clonogenic survival assay in U2OS cells transfected with siNS and si*DDSR1*#1 in response to treatment with the indicated doses of NCS. Samples from three independent experiments were analyzed in triplicates. Values represent mean ± SD. *Significant change compared to siNS cells treated with NCS ($P < 0.05$).

Data information: Statistical comparisons were made using Student's *t*-test.