Supplementary information

And-1 is required for homologous recombination repair by regulating DNA end resection

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Supplementary figures
Figure S1. (A) And-1 is required for HR repair following DNA damage. U2OS+DR-GFP cells treated with indicated siRNAs were transfected with I-SceI expression vector (pCBASce). The percentage of GFP positive cells was determined by flow cytometry analysis. See method for detail. (B) Cells with And-1 depletion are hypersensitive to PARP1 inhibition by Olaparib. U2OS cells were treated with indicated siRNAs and 48 hr after siRNA transfection, cells were treated with olaparib or vehicle for 24 hr, followed by clonogenic survival assays as described in method. Data represent means ± SD from three independent experiments. **, p ≤ 0.01. (C) Depletion of And-1 increases the chromosomal abnormalities. U2OS cells were treated with 0.1µg/ml colcemid for 2 hr and then released in drug-free medium for 36 hr. Cells were then trypsinized, incubated in 0.8 % Sodium Citrate solution for 20 min, and subsequently fixed in methanol and acetic acid (3:1 ratio) solution for 30 min. Cells were placed onto 50% ethanol-wetted slides and dried, followed by staining in Giemsa solution (Sigma, catalog #GS-500, diluted in water 1:20) for 20 min. For each experiment, more than 100 metaphase spreads were analyzed. Cells with chromosomal breakage, rings and abnormal chromosome structure (indicated by arrows) were regarded as chromosomal aberration. Data represent means ± SD from three independent experiments. *, p≤0.05; **, p≤0.01.
**Figure S2.** (A) And-1 is recruited to DSB sites in response to ionizing radiation (IR). U2OS cells were untreated (upper) or treated (lower) with 10 Gy ionizing radiation and then co-immunostained for γ-H2AX and And-1 three hours post irradiation. For each experiment, >50 cells were counted and the percentage of γ-H2AX cells exhibiting And-1 foci was determined. Data represent means ± SD from three independent experiments. (B) Live imaging of accumulation of And-1 and NBS-1 at damage sites. Laser irradiation was performed in U2OS cells co-transfected with GFP-And-1 and NBS1-TagRFP-T, followed by live cell imaging to assess the kinetics of damage recruitment of And-1 and NBS1 to a damage line.
Figure S3. (A) CtIP is not required for And-1 recruitment to laser-induced DSB sites. U2OS cells treated with siGl2 or siCtIP were micro-irradiated by laser and then co-immunostained for γ-H2AX and And-1 fifteen minutes post irradiation. For each experiment, >50 cells were counted and the percentage of γ-H2AX cells exhibiting And-1 strips was determined. Data represent means ± SD from three independent experiments. (B) Depletion of CtIP impairs Chk1 and RPA phosphorylation after camptothecin exposure. U2OS cells transfected with the indicated siRNAs were treated with camptothecin (1µM) for 1 hr. Cells were then harvested and immunoblotted for indicated proteins. Asterisk indicates the hyper-phosphorylated RPA32. (C) And-1 depletion impairs Rad51 recruitment to DSB sites. U2OS cells transfected with siGl2 or siA-1 were treated with 10 Gy ionizing radiation and co-immunostained for γ-H2AX and Rad51 two hours post radiation.
Figure S4. (A) And-1 interacts with BRCA1 and MDC1. Co-immunoprecipitation (co-IP) assays were performed using U2OS cells. Cell lysates were immunoprecipitated with control IgG, anti-And-1 antibodies and the IPs were then resolved on SDS-PAGE and immunoblotted for the indicated proteins. (B) U2OS cells transfected with Flag-And-1 were immunoprecipitated with anti-FLAG antibody. FLAG-IPs were then immunoblotted for indicated proteins.
Figure S5. (A) And-1 is not required for NBS1 recruitment to laser-induced DSB sites. U2OS cells treated with siGl2 or siAnd-1 were micro-irradiated by laser and then co-immunostained for γ-H2AX and NBS1 ten minutes post irradiation. For each experiment, >50 cells were counted and the percentage of γ-H2AX cells exhibiting NBS1 foci was determined. Data represent means ± SD from three independent experiments. (B) Depletion of And-1 does not affect ATR phosphorylation at T1989 after camptothecin exposure. U2OS cells transfected with the indicated siRNAs were treated with camptothecin (1 μM) for 1 hr. Cells were then harvested and immunoblotted for indicated proteins.
Figure S6. (A) ATR inhibition reduces the interaction between And-1 and CtIP. U2OS cells transfected with plasmid expressing Flag-And-1 were treated with or without VE-821 (10 µM) for 6 hr, followed by CPT (5 µM) treatment for 1 hr. Cells were then lysed and Flag-And-1 IPs were immunoblotted for indicated proteins. (B) And-1 is the substrate of ATM/ATR. U2OS cells expressing FLAG-And-1 were treated with CPT (5µM) for 1 hr. Cells were then harvested, lysed and immunoprecipitated with anti-FLAG antibody and FLAG IPs were then immunoblotted with ATR/ATM phospho-substrate specific antibody (Cell Signaling, #2851).