Expanded View Figures

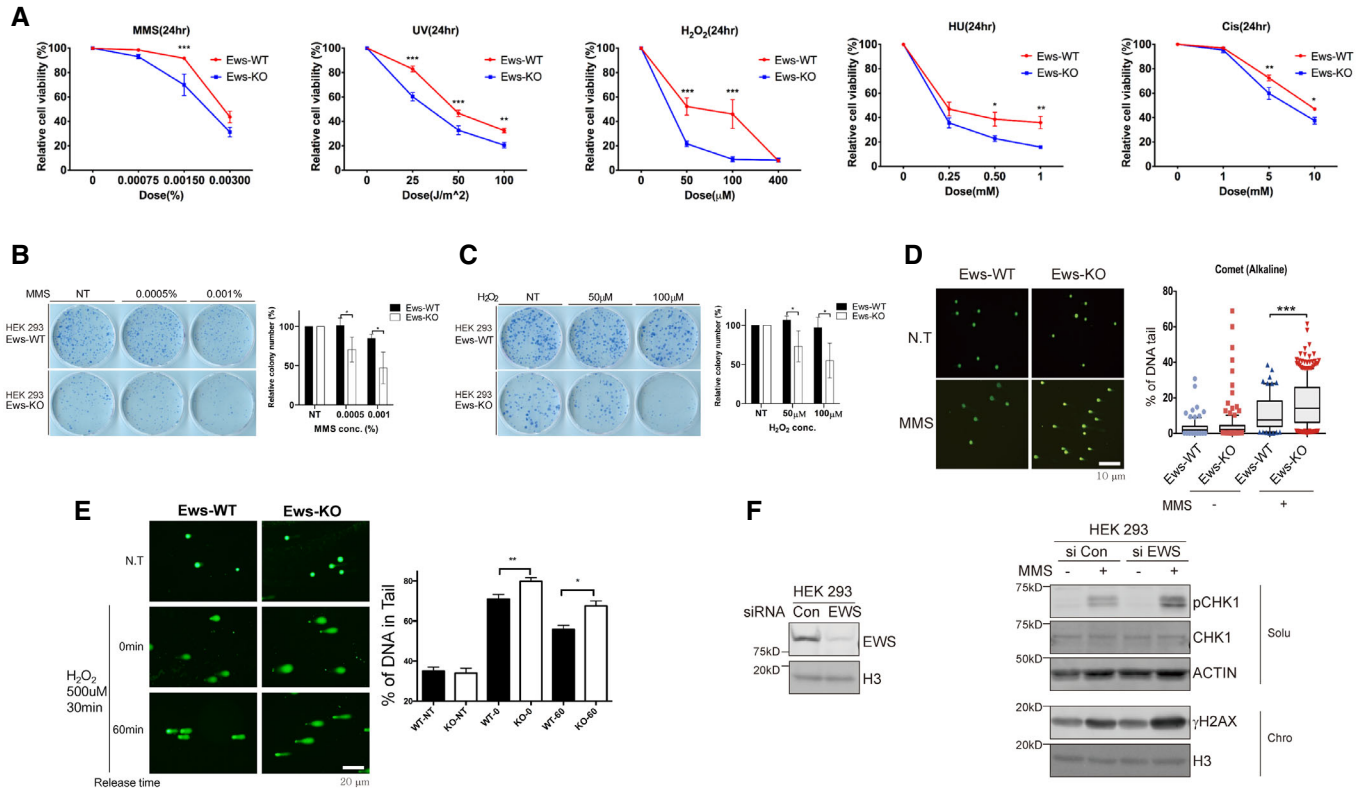

Figure EV1. Loss of *Ews* induces hypersensitivity to DNA-damaging agents.

A Relative cell viability was measured in wild-type (WT) and Ews-KO mBA cells after treatment with various DNA-damaging agents. MMS: Methyl methane sulfonate, H_2O_2: Hydrogen peroxide, Cis: Cisplatin, UV: Ultraviolet, and HU: Hydroxyurea. Error bars represent as mean ± SEMs, and technical repeats (n = 3). Significance determined by two-way ANOVA, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

B, C After 10 days of low dose MMS (B) and H_2O_2 (C) treatment, survived colony were measured using clonogenic assay. Data represented as mean ± SEMs, $n > 3$. Significance determined by Student's t-test, two-tailed, *$P < 0.05$.

D DNA breaks in wild-type (Ews-WT) and Ews-KO mBA cells were measured using Alkaline Comet assay after MMS treatment (0.0015%, 24 h). Error bars represent as mean ± SEMs, $n > 50$. Significance determined by ***$P < 0.001$.

E Alkaline Comet assay were conducted in Ews-WT and Ews-KO mBA cells after treatment and release of H_2O_2. Error bars represent ± SEMs, $n > 50$. Significance determined by two-way ANOVA, *$P < 0.05$, **$P < 0.01$.

F Upon inactivation of EWS in HEK-293 cells, expression of DNA damage markers (pCHK1 and γH2AX) were measured using Western blotting after MMS (0.02%, 1 h) treatment.

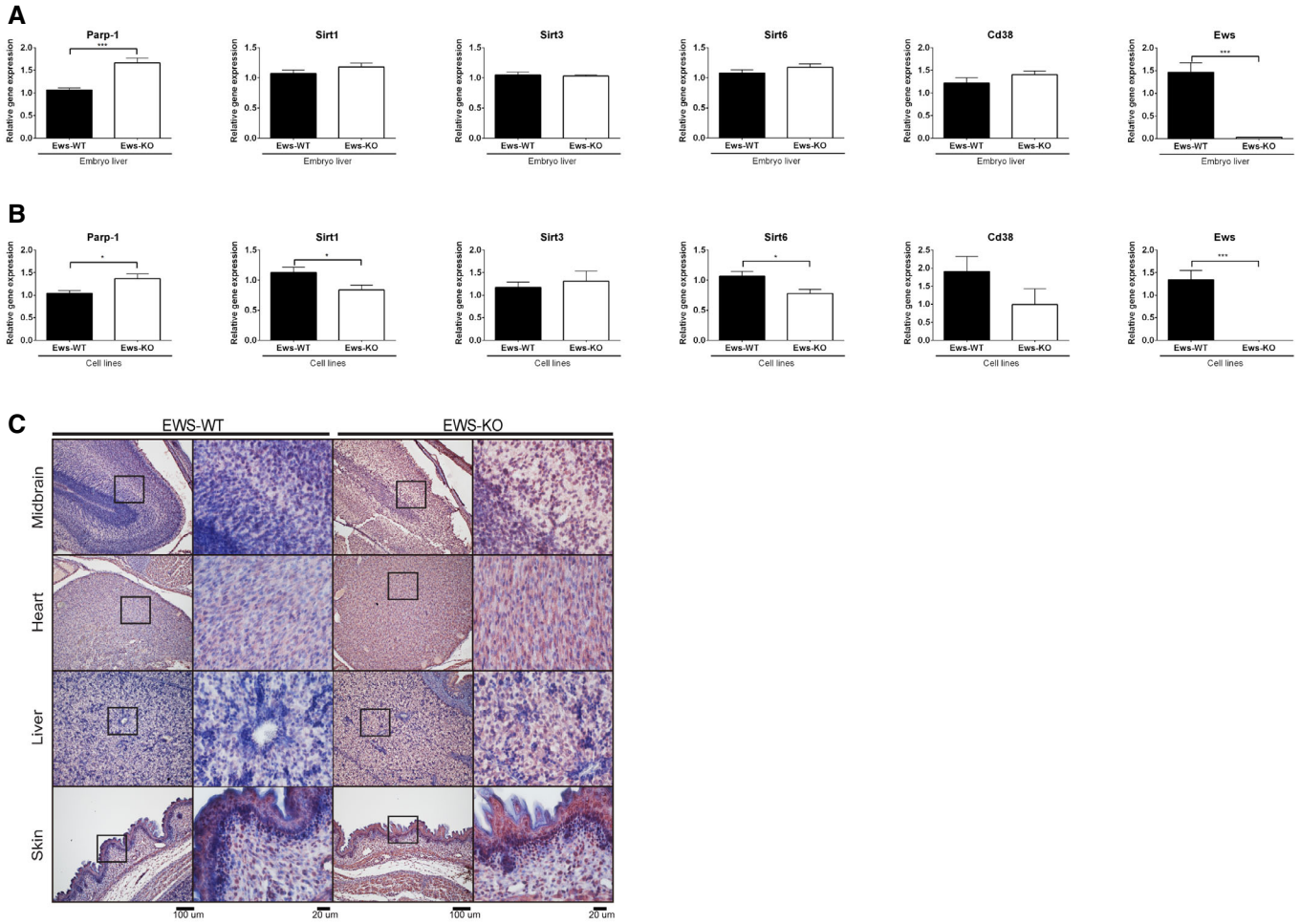

Figure EV2. EWS depletion induces PARP1 expression in mouse embryo and cell lines.

A, B Quantitative real-time PCR was carried out to investigate the expression level of NAD^+ salvage pathway genes using E17.5 day embryos liver (A) and cell lines (B). Data represented as mean ± SEMs, and obtained from three different cell line and embryo liver. Significance determined by Student's *t*-test, two-tailed, $*P < 0.05$, $***P < 0.001$.

C Immunostaining of 17.5 days embryo tissues (mid-brain, heart, liver, and skin) with anti-PARP1-antibody. Insert shows higher magnification. Scale bar indicates 200 μm (left).

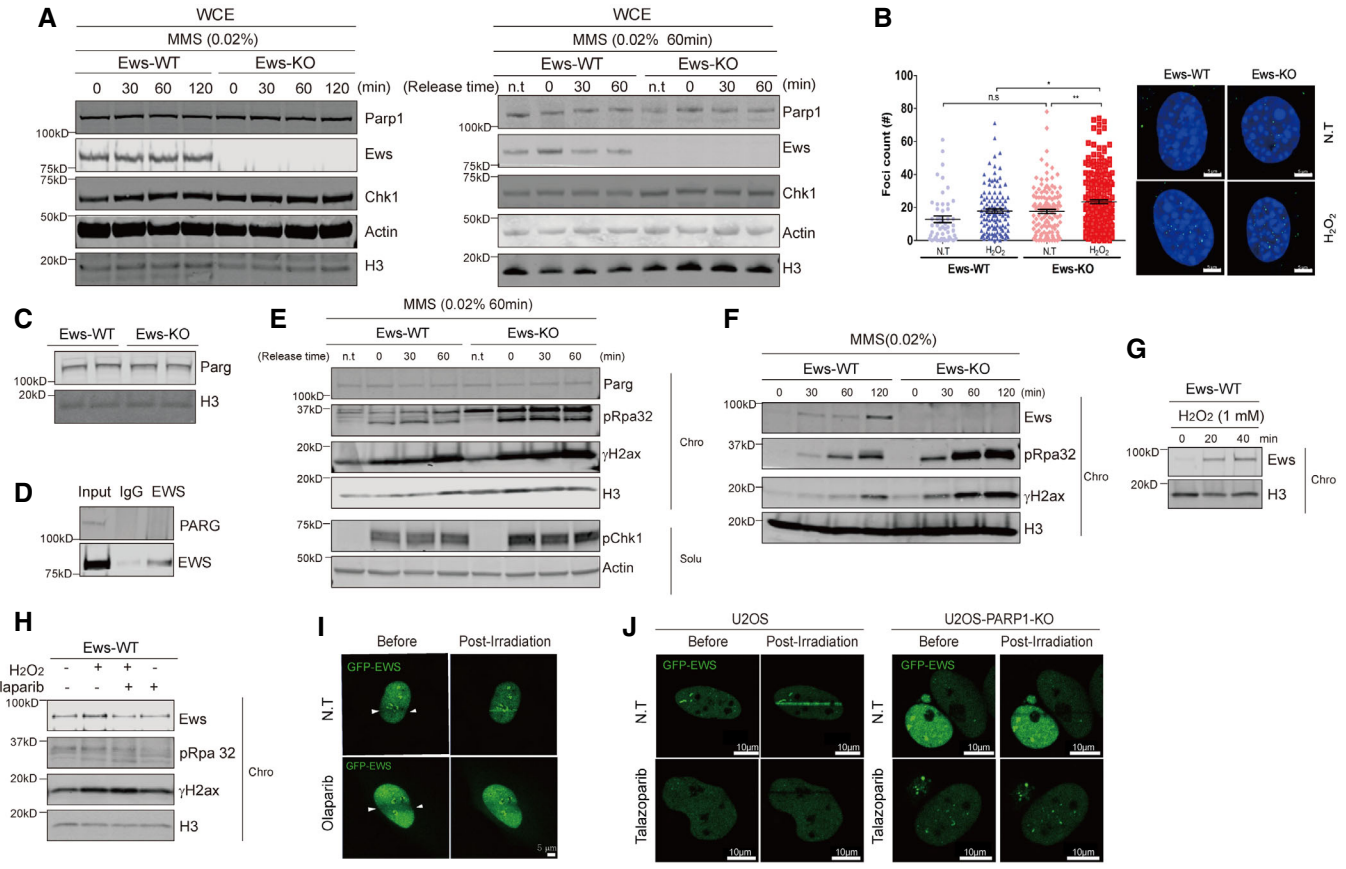

Figure EV3. EWS protein is recruited to DNA damage site in a PARP1 dependent manner and regulates PARP1 dissociation from DNA damage sites.

A Whole cell extract of wild-type and Ews-KO cells following MMS treatment and releasement were subjected to Western blotting.

B, C (B) Immunofluorescence with anti-PARP1 antibody in wild-type and Ews-KO cells following H_2O_2 treatment. Scale bar indicate 5 μm. $n > 150$ (C) PARG expression was measured by Western blot in wild-type and Ews-KO mBA cells.

D Endogenous interaction between EWS and PARG were analyzed by co-immunoprecipitation followed by Western blot analysis with anti-EWS antibody.

E After release from MMS treatment, proteins in cells were fractionated into chromatin-bound and soluble forms to measure PARG kinetics in wild-type and Ews-KO mBA cells.

F, G Chromatin-bound levels of EWS were measured after treatment of MMS (F) or H_2O_2 (G).

H Wild-type cells were treated with 1 mM H_2O_2 for 20 min with or without PARPi (5 μM Olaparib for 7 h). The kinetics of chromatin-bound EWS was analyzed by Western blot.

I Localized specific DNA damage was induced in GFP-EWS cell lines with or without Olaparib using micro-irradiation. Scale bar represents 5 μm.

J DNA damage was induced by micro-irradiation in U2OS and U2OS-PARP1-KO cells with or without Talazoparib (5 μM, 24 h). Scale bar represents 10 μm. $N > 10$.

Source data are available online for this figure.

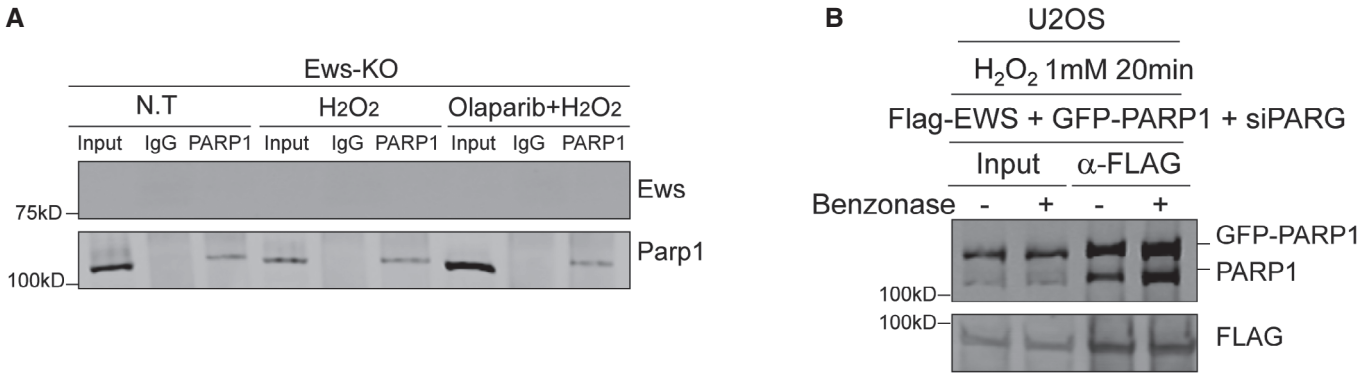

Figure EV4. RGG domain of Ews interact with PARP1.

A Interaction between EWS and PARP1 was analyzed by Immunoprecipitation assay in Ews-KO cells. Cells were treated with H_2O_2 (1 mM, 10 min) with or without Olaparib (5 μM, 7 h).

B With or without Benzonase-treated cells were immune-precipitated by anti-FLAG antibody and immunoblotted by anti-PARP1 and FLAG antibody.

Source data are available online for this figure.

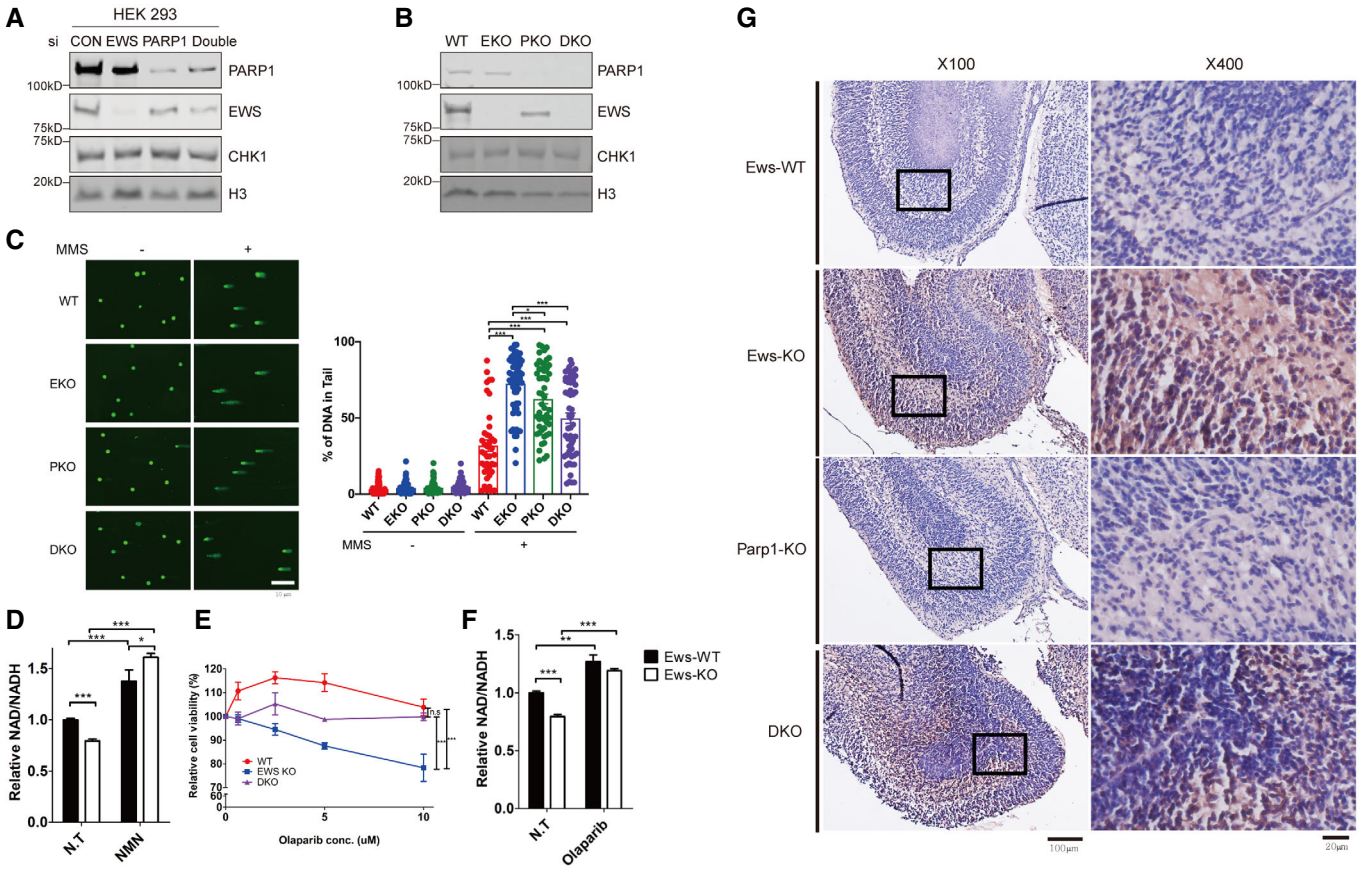

Figure EV5. EWS regulates genomic integrity in PARP1-dependent manner.

A, B Whole cell expressions of PARP1, EWS and CHK1 were measured in Figs 5A (A) and EV5C (B) cells.

C DNA breaks were measured using the Alkaline Comet assay in WT, EKO, PKO, and DKO cells treated with MMS (0.0015%, 24 h). WT: Wild-type, EKO: Ews-KO, PKO: Parp1-KO and DKO: double KO. Error bars represent ± SEMs, $n > 35$. Significance determined by two-way ANOVA, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

D Relative NAD^{+}/NADH ratios were measured in wild-type (Ews-WT) and Ews-KO cells following treatment of NMN (20 μM, 24 h). Error bars represent as mean ± SEMs, and technical repeats ($n = 3$). Significance determined by two-way ANOVA, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

E Relative cell viability was measured in HEK293 WT, EWS-KO and EWS-PARP1 KO (DKO) cell lines upon Olaparib treatment. Error bars represent as mean ± SEMs, and technical repeats ($n = 3$). Significance determined by two-way ANOVA, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

F NAD^{+}/NADH ratios were measured in wild-type (Ews-WT) and Ews-KO cells after treatment of Olaparib (5 μM 24 h). Error bars represent as mean ± SEMs, and technical repeats ($n = 3$). Significance determined by two-way ANOVA, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

G Cell death was observed in wild-type (Ews-WT), Ews-KO, Parp1-KO, and DKO embryos mid-brain at E17.5 days using the TUNEL assay. ×100 and ×400 image scale bar represents 100 μm and 20 μm, respectively.