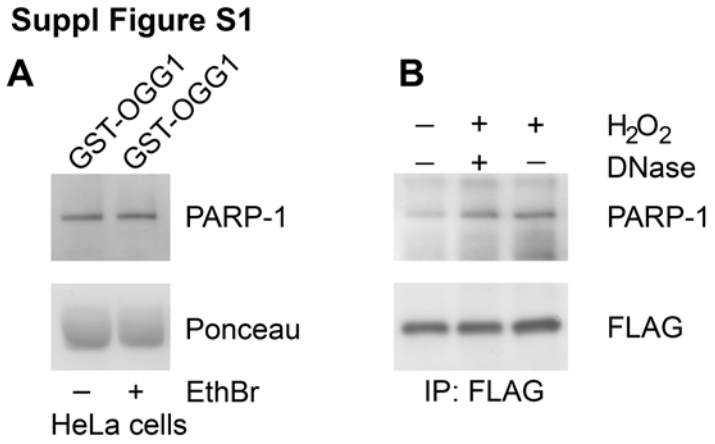
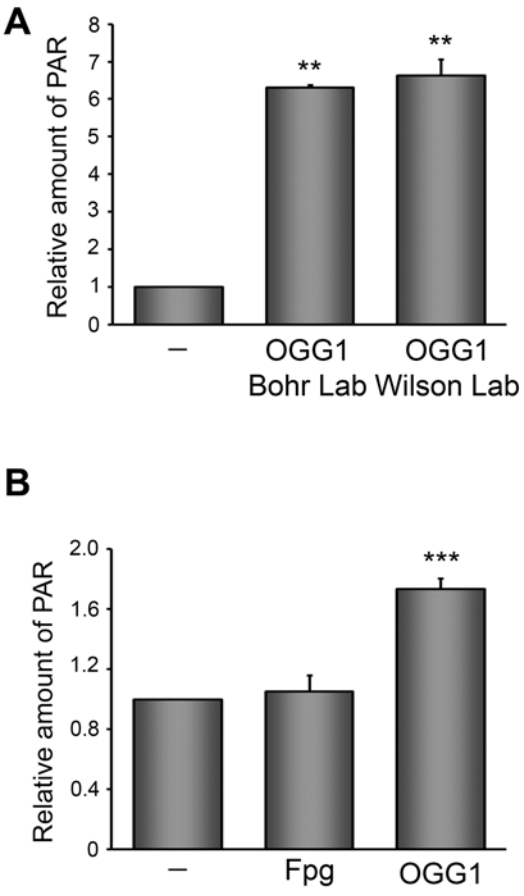
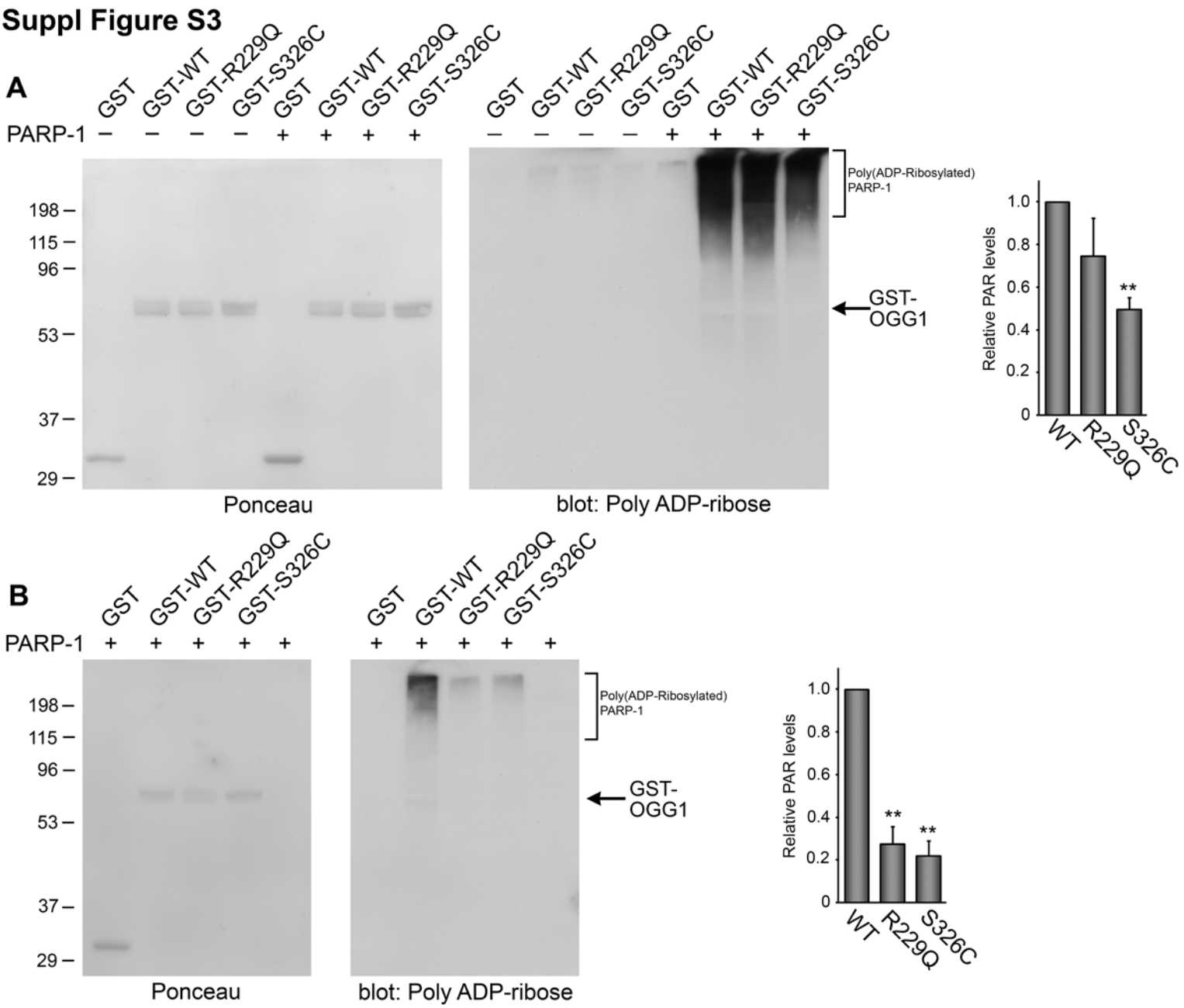


SUPPLEMENTARY FIGURE S1. **OGG1 binds to PARP-1 even in the presence of ethidium bromide and DNase.** *A*, HeLa cell lysates were incubated with immobilized GST-OGG1 (20 μg) in the absence (-) or presence (+) of 10 μg/ml ethidium bromide (EthBr) for 1 hour at 4°C. The proteins remaining associated with GST-OGG1 were probed with anti-PARP-1 antibodies. Ponceau S staining revealed the amount of GST-OGG1 in the precipitations. A represent immunoblot is shown of three independent experiments. *B*, Hela cells transfected with FLAG-OGG1 were untreated or treated with 5 mM H_2O_2 for 30 min, washed with lysis buffer and then treated with 5 units DNase for 15 min at 30°C. FLAG immunoprecipitates were then washed, separated by SDS-PAGE and immunoblotted with anti-PARP-1 and anti-FLAG antibodies. A represent immunoblot is shown of three independent experiments.

Suppl Figure S2

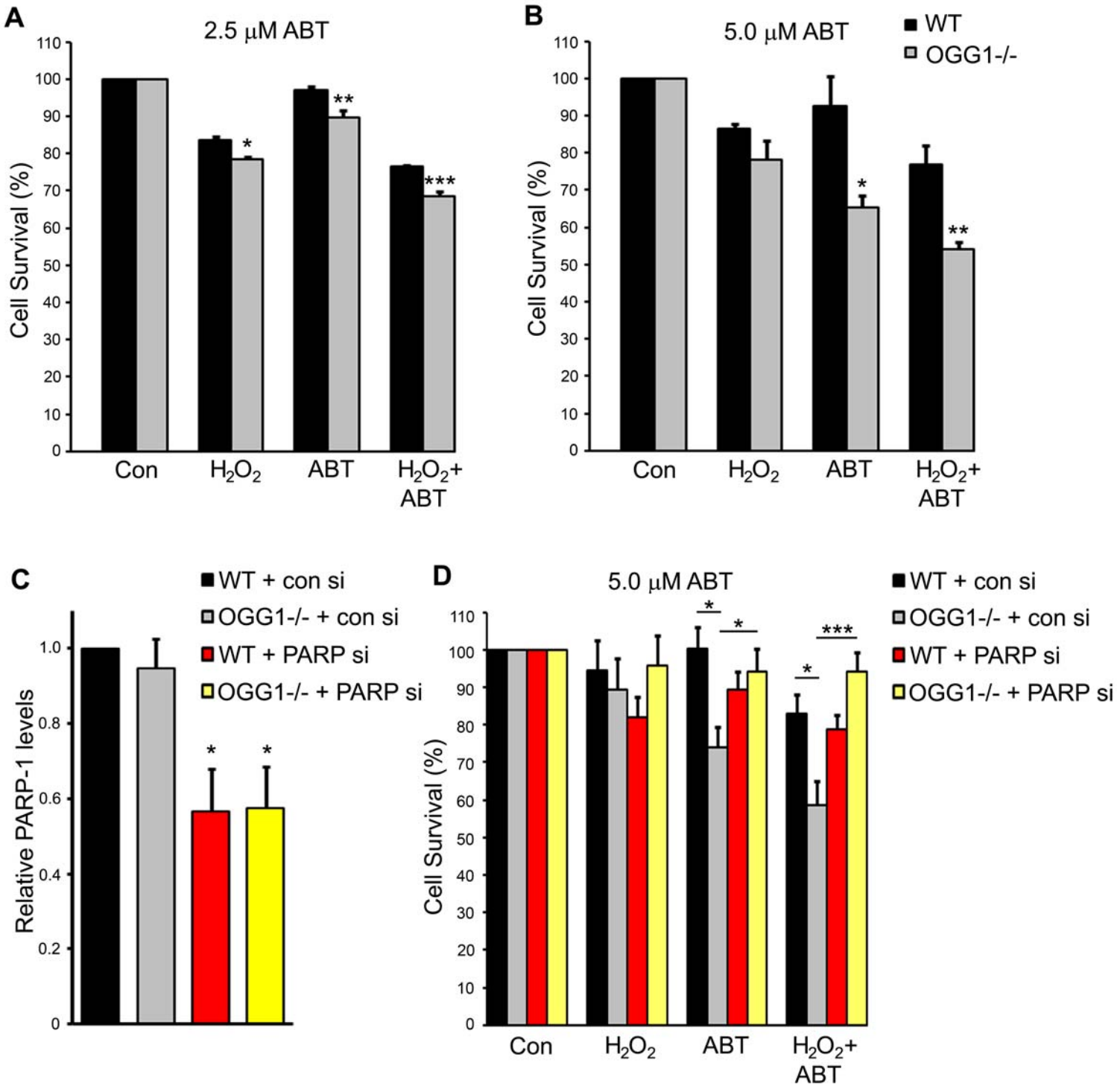


SUPPLEMENTARY FIGURE S2. **OGG1 stimulates the poly (ADP-ribosyl)ation activity of PARP-1.** PARP-1 activity was measured by determining the amount of PAR deposited on immobilized histones. The addition of OGG1 increased the amount of PAR synthesis by PARP-1. *A,* Two different OGG1 protein preparations were used from two different labs. In the indicated histogram, mouse recombinant OGG1 was obtained from Vilhelm Bohr's laboratory from the National Institute on Aging (NIA) and human recombinant OGG1 was obtained from Samuel Wilson's laboratory at the National Institute for Environmental Health Sciences (NIEHS). The ELISA assays were performed as described in the Experimental Procedures and 0.5 μg OGG1 was added to the indicated samples. (-) PARP-1 alone. The histogram shows averages ± SEM from duplicate experiments. *B*, PARP-1 activity is stimulated by OGG1 (0.5 μg; NEB) but not Fpg (0.5 μg; NEB). The histogram shows averages ± SEM from quadruplicate experiments. ** P <0.01, ***P<0.001 compared to PARP-1 alone by one-way ANOVA and Tukey's post-hoc test.



SUPPLEMENTARY FIGURE S3. **The OGG1 polymorphic variants are defective in activating PARP-1**. The indicated fusion proteins were incubated with (+) or without (-) PARP-1. In *A*, PARP-1 buffer containing activated DNA and NAD+ were added to all the lanes to activate PARP-1. In *B*, samples were incubated without activated DNA, but with NAD+. Samples were incubated for 30 min, washed to remove unbound PARP-1 and then terminated by the addition of sample buffer. Boiled samples were separated by SDS-PAGE and immunoblotted with anti-PAR antibodies. Ponceau staining was used to examine loading of the fusion proteins. The arrow indicates GST-OGG1. A representative experiment is shown from four independent experiments for both *A* and *B*. The histograms represent the amount of PAR normalized to the amount of fusion protein from the Ponceau staining from four independent experiments. ** P <0.01 compared to wild-type OGG1 by Student's t-test.

Suppl. Figure S4



SUPPLEMENTARY FIGURE S4. **Inhibition of PARP-1 impairs cell survival in response to DNA damage.** MEFs were treated for 8 hours with 300 μM H_2O_2, 2.5 μM (*A*) or 5 μM ABT-888 (*B,D*) or with both H_2O_2 and ABT-888. Cells were pretreated with ABT-888 for 30 min prior to addition of H_2O_2. Cell survival was measured using a MTT assay (Sigma-Aldrich). In *D*, WT or OGG1-/- cells were transfected with control siRNA (con si) or PARP-1 siRNA and MTT assays were performed 72 hrs after transfection. Histograms show the normalized averages ± SEM from three (*A*) or four (*B,D*) independent experiments. *P<0.05, **P<0.01 and ***P < 0.001 comparing OGG1-/- to WT or the indicated comparisons using one-way ANOVA and Tukey's post-hoc test. *C*, Lysates from WT and OGG1-/- cells transfected with either control siRNA or PARP-1 siRNA were separated by SDS-PAGE and immunoblotted with anti-PARP-1 and anti-GAPDH antibodies as a protein loading control. Relative PARP-1 protein levels were quantified and normalized to the amount of GAPDH. The histogram represents the relative level of PARP-1 quantified from five independent experiments ± SEM. *P<0.05 by Student's t-test.

SUPPLEMENTAL METHODS

PAR assays- To test whether OGG1 polymorphic variants affect PARP-1 activity, we used the HT Universal Colorimetric PARP Assay Kit from Trevigen with some modifications. Equal amounts (10 μg) of GST control, wild-type OGG1 and polymorphic variants of OGG1 were aliquoted into tubes containing PBS with 0.1% Triton X-100. After washing two times with buffer, a ribosylation reaction was added which included 1 ng PARP-1-HSA enzyme, PARP buffer, activated DNA and PARP cocktail (which contains NAD+). As a control in Suppl. Fig. 3A, samples were incubated with all the components of the ribosylation reaction except PARP-1. In Suppl. Fig 3B, the samples were incubated without activated DNA. The reactions were incubated for 30 min at room temperature and washed 3X with PBS, 0.1% Triton X-100. Samples were then separated by SDS-PAGE and immunblotted with anti-PAR antibodies (Clone-10HA; Trevigen). Membranes were stained with Ponceau to visualize loading of the GST-fusion proteins.

siRNA transfections-To knockdown PARP-1, we tested several Stealth™ small interfering RNAs against mouse PARP-1 (Invitrogen Life Technologies; data not shown). The siRNA that most effectively decreased PARP-1 protein expression in WT and OGG1-/- cells was used for the assays (sense sequence AAAGCAAGUUGGAUGGUACCAGCGG and antisense sequence CCGCUGGUACCAUCCAACUUGCUUU). Stealth™ RNAi negative control duplexes (Cat. No. 12935-113) were used as a control. The amount of siRNA and transfection protocol were optimized for the MEF cells. WT and OGG1-/- cells were transfected in 6 cm dishes with 0.2 nanomoles siRNA (58 nM final concentration) using Lipofectamine™ 2000 (Invitrogen Life Technologies) according to the manufacturer's instructions. Cells were used for cell survival MTT assays 72 hrs after transfection.