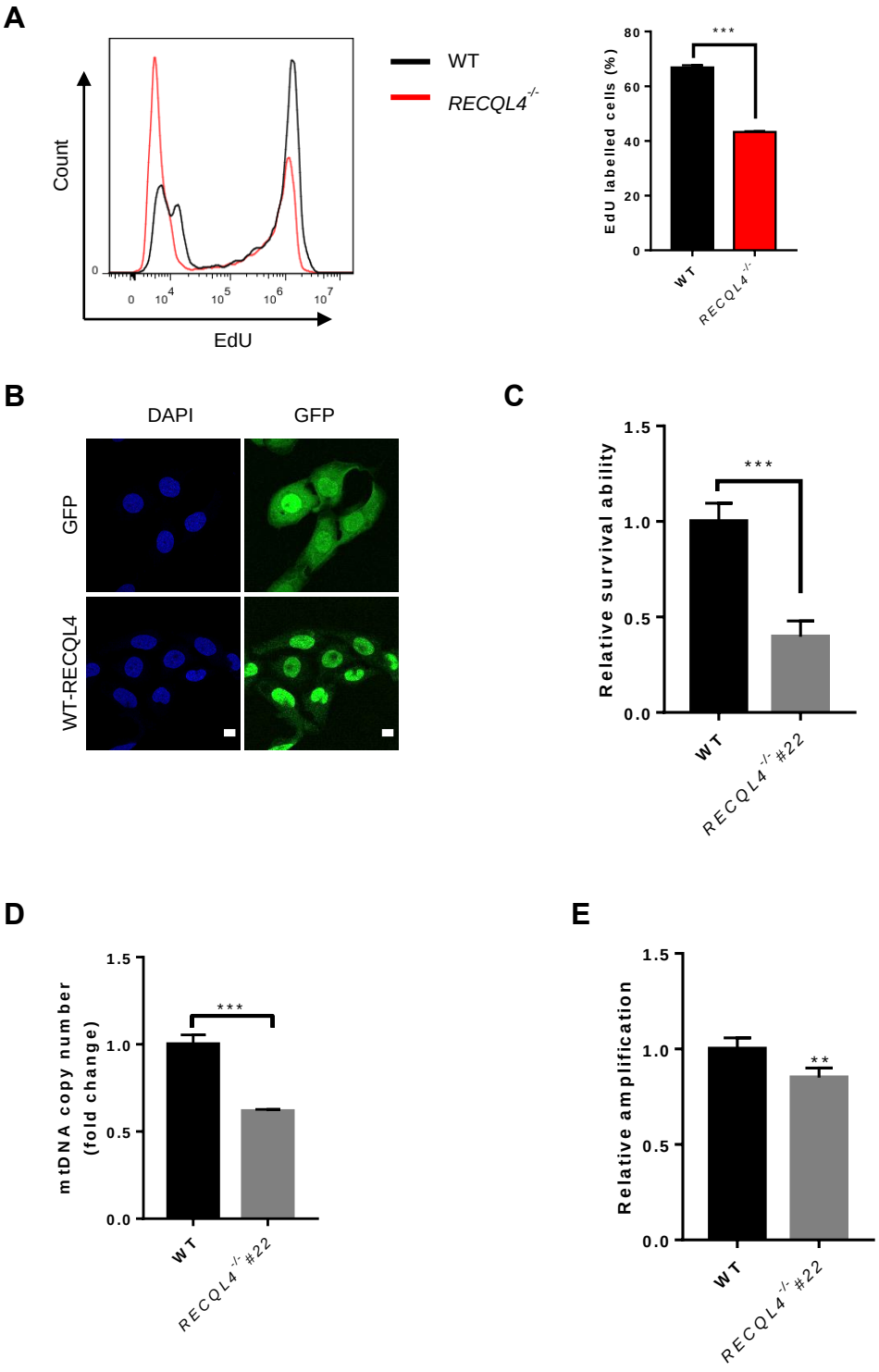
Supplementary data

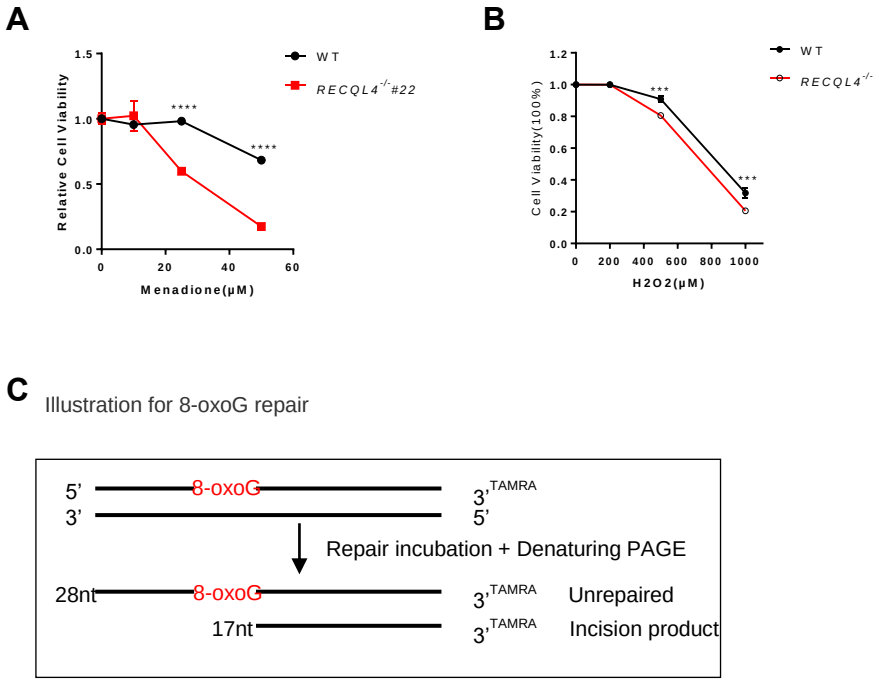
Supplementary Figure S1



Supplementary Figure S1 | Characterization of RECQL4 knockout cells

(A) Detection of EdU incorporation in WT and *RECQL4*$^{-/-}$ cells by flow cytometry. WT and *RECQL4*$^{-/-}$ cells were incubated with 10 μM EdU for 1 h. A representative histogram plot of EdU-labeled cells is shown on the left. The graph on the right shows quantification of the ratio of EdU-positive cells in WT and *RECQL4*$^{-/-}$ cells. Data are shown as mean ± SD from two independent experiments. ***$P < 0.001$, using unpaired two-tailed Student's t test. **(B)** Immunofluorescence images confirm the overexpression of GFP vector or GFP-tagged WT-RECQL4 in RECQL4-/- cells. Scale bar, 10 μm. **(C)** Colony formation assay showing another RECQL4-deficient cell line (*RECQL4*$^{-/-}$ #22) has similar clonogenic survival defects. Data are shown as mean ± SEM, n = 3. ***P < 0.001 (t-test). **(D)** mtDNA copy number in WT and RECQL4-deficient cells (*RECQL4*$^{-/-}$ *#22*). Data are shown as mean ± SEM, n = 3. ***$P < 0.001$ (t-test). **(E)** mtDNA damage analysis in RECQL4-deficient cells (*RECQL4*$^{-/-}$ *#22*). Quantifications are shown as mean ± SEM, n = 3. **$P < 0.01$.

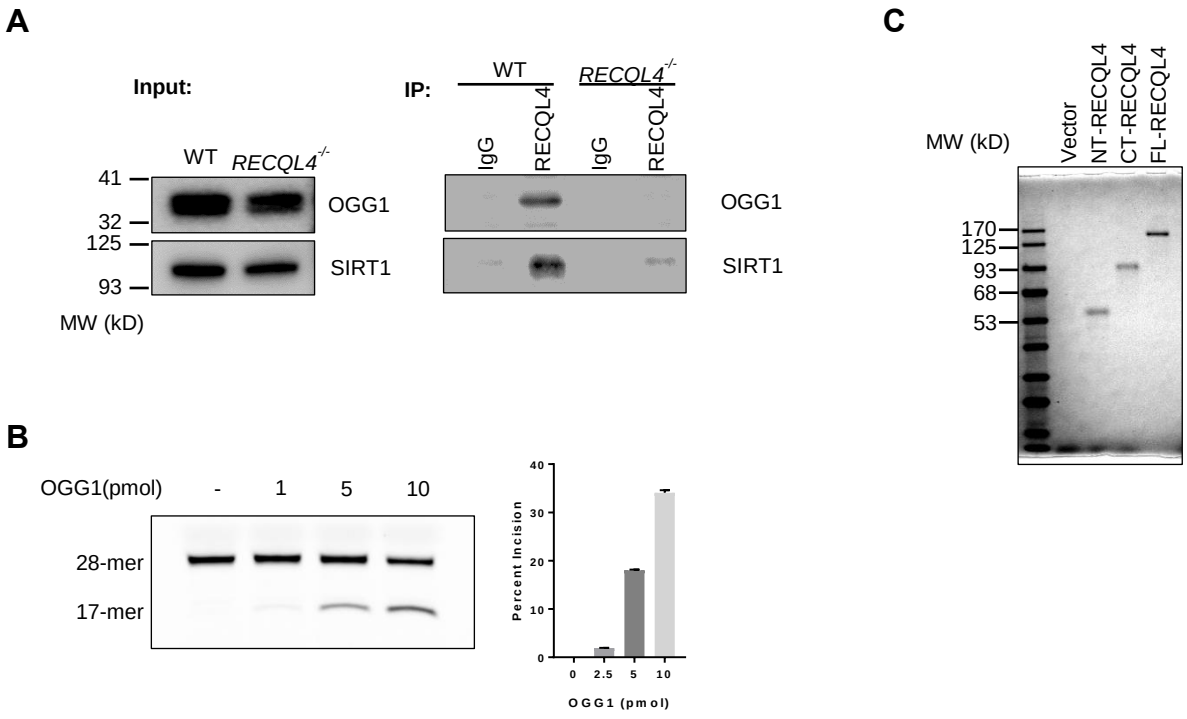
Supplementary Figure S2



Supplementary Figure S2 | RECQL4 is required for BER of 8-oxoG

(A) MTS assay measuring cell viability of WT and RECQL4-deficient cells (*RECQL4*$^{-/-}$ #22) treated with menadione. Data are shown as mean ± SEM, n = 3. ****P < 0.0001 (t-test). **(B)** MTS assay showing cell viability of WT and RECQL4 knockout cells treated with H_2O_2. Data are shown as mean ± SEM, n = 3. ***P < 0.001 (t-test). **(C)** Schematic rationale of BER analysis of 8-oxoG repair *in vitro*. DNA substrates were labelled with Carboxytetramethylrhodamine (TAMRA) at the 3' terminus.

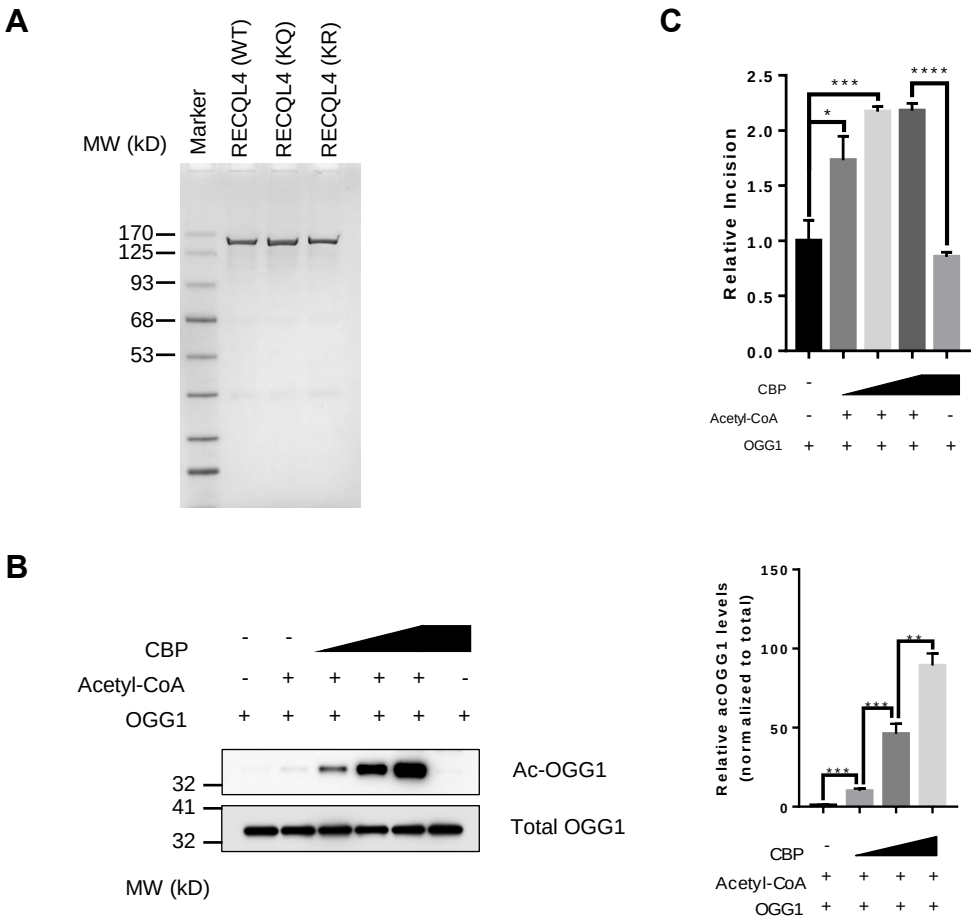
Supplementary Figure S3



Supplementary Figure S3 | RECQL4 selectively interacts with OGG1 and promotes 8-oxoG repair

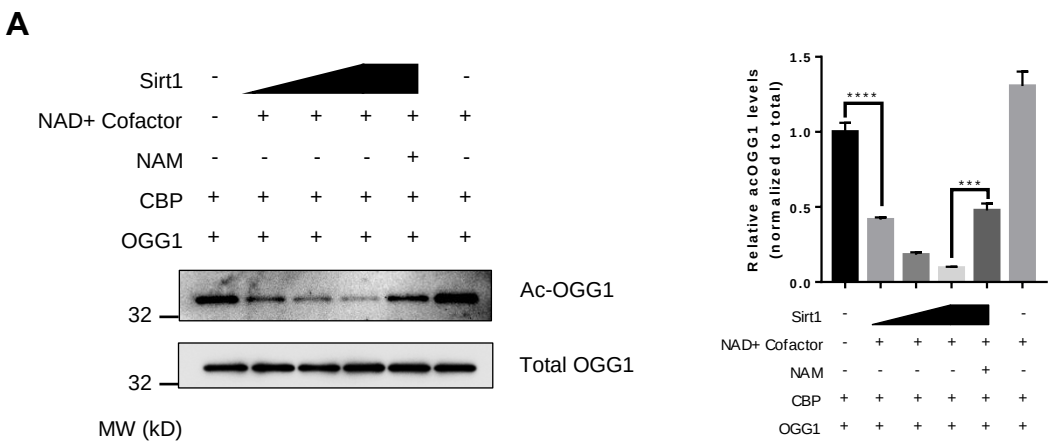
(A) Co-immunoprecipitation of endogenous OGG1 and SIRT1 by RECQL4 antibody from U2OS cells extracts in the presence of benzonase nuclease. **(B)** Representative gel (left) and quantification (right) of the 8-oxoG incision activity of OGG1 alone. **(C)** Coomassie blue staining gels with purified 3xFLAG-NT-RECQL4, 3xFLAG-CT-RECQL4, and 3xFLAG-FL-RECQL4 used for *in vitro* 8-oxoG incision assay, related to Figure 3F.

Supplementary Figure S4



Supplementary Figure S4 | RECQL4 is an acetylated protein and its acetylation is stimulated by oxidative stress (A) Coomassie blue staining gels with purified RECQL4 (WT), RECQL4 (KQ), and RECQL4(KR) used for *in vitro* acetylation and deacetylation assays, related to Figure 4C and Figure 5D. **(B)** *In vitro* acetylation of OGG1 by CBP. Recombinant OGG1(1μg), Acetyl-CoA (2 mM), and different amounts of recombinant CBP (0.1μg, 0.2μg, 0.5μg) were incubated at 30°C for 1 h. Acetylated and total OGG1 proteins were assessed with an anti-acetylated OGG1 antibody (K338 + K341) and an anti-OGG1 antibody, respectively. A representative gel is shown on the left. The graph on the right shows quantification of relative acetylated OGG1 levels normalized to total OGG1. Data are shown as mean ± SD from two independent experiments. $^{**}P < 0.01$, $^{***}P < 0.001$, using unpaired two-tailed Student's t test. **(C)** BER assay measuring 8-oxoG incision activity showing that acetylation of OGG1 enhances its catalytic activity in vitro. Data are shown as mean ± SD from two independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, using unpaired two-tailed Student's t test.

Supplementary Figure S5

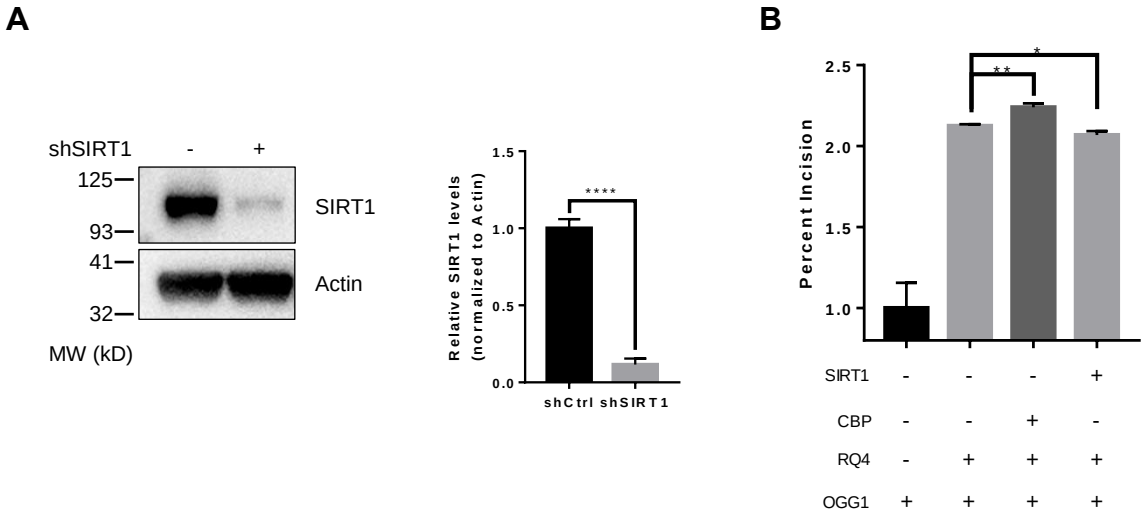


Supplementary Figure S5 | SIRT1 interacts with and deacetylates RECQL4

(A) Deacetylation of OGG1 by SIRT1 *in vitro*. Recombinant OGG1 (1 μg) was first acetylated by CBP (0.1 μg) for the first 1 h, then acetylated OGG1 was incubated with NAD^+ (50 μM), and different amounts of recombinant SIRT1 (0.5 U, 1 U, 2 U) at 30°C for an additional 1 h. Acetylated and total

OGG1 proteins were assessed with an anti-acetylated OGG1 antibody and an anti-OGG1 antibody, respectively. A representative gel is shown on the left. The graph on the right shows quantification of relative acetylated OGG1 levels normalized to total OGG1. Data are shown as mean ± SD from two independent experiments. ***$P < 0.001$, ****$P < 0.0001$, using unpaired two-tailed Student's t test.

Supplementary Figure S6



Supplementary Figure S6 | SIRT1 controls the interaction between OGG1 and RECQL4 following oxidative stress and maintains RECQL4 in a hypoacetylated state (A) Western blot showing reduced SIRT1 protein levels in shRNA-mediated SIRT1-knockdown cells. A representative gel is shown on the left. The graph on the right shows quantification of relative acetylated OGG1 levels normalized to total OGG1. Data are shown as mean ± SD from four independent experiments. ****$P < 0.0001$, using unpaired two-tailed Student's t test. **(B)** BER assay measuring 8-oxoG incision activity shows that RECQL4 stimulates the catalytic activity of OGG1 in the presence of CBP, but not in the presence of SIRT1. Acetyl CoA and NAD+ co-substrate were used, respectively. Data are shown as mean ± SD from two independent experiments. *$P < 0.05$, **$P < 0.01$, using unpaired two-tailed Student's t test.