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Supplemental information

CHAMP1 binds to REV7/FANCV and promotes homologous recombination repair

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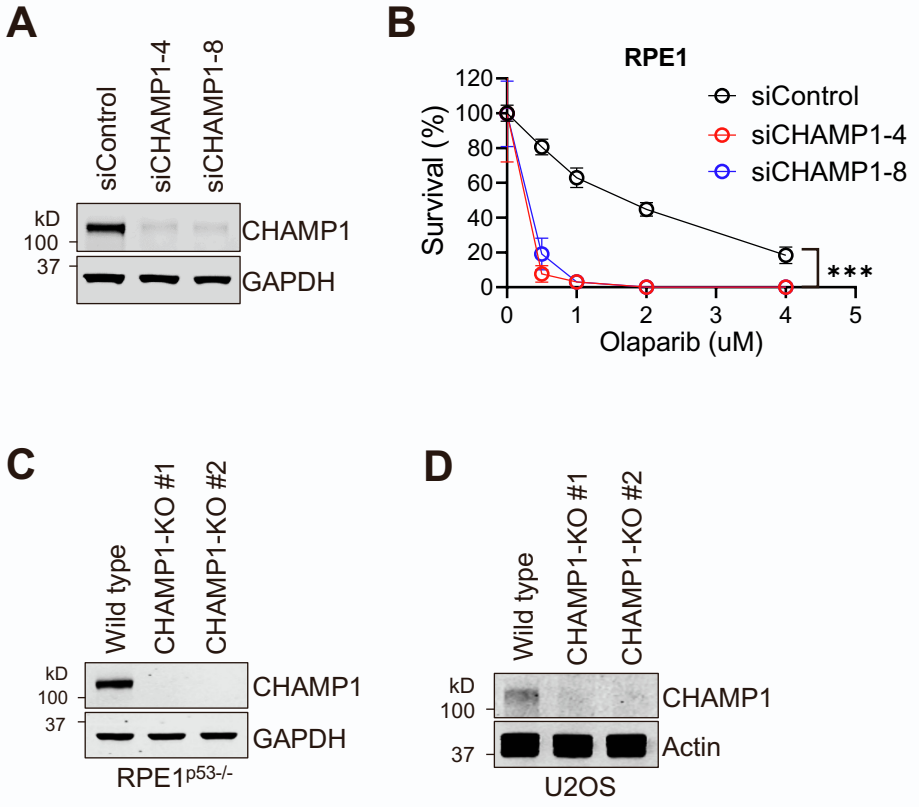
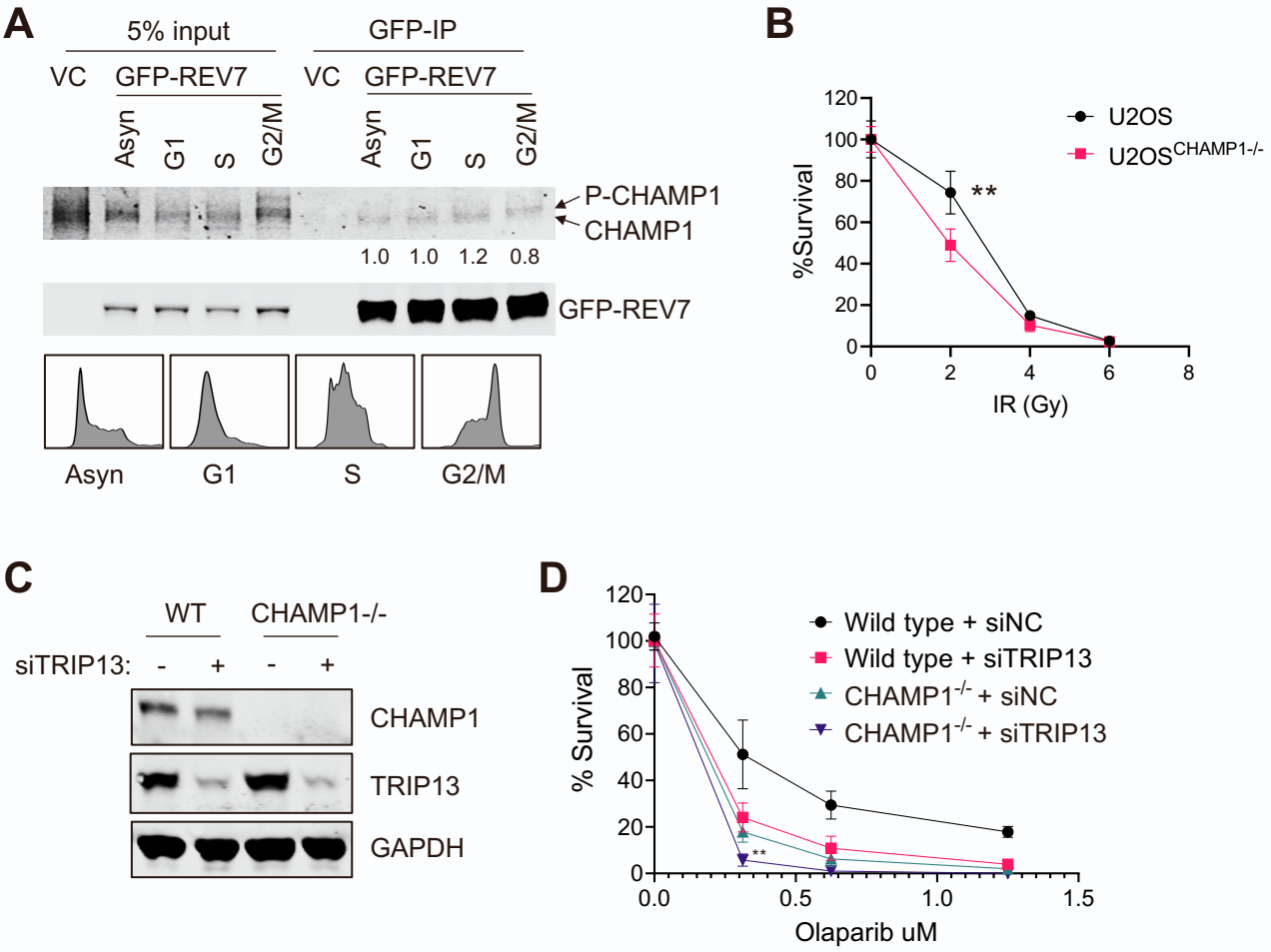
Figure S1, **CHAMP1 depletion sensitizes cells to PARPi,** related to Fig. 1.



Supplemental figure 1, related to Figure 1.

A, Western blot showing knocking down efficiency of siRNA targeting CHAMP1. U2OS cells were treated with siRNA negative control or siCHAMP1 for 48hrs. **B**, A 14 days clonogenic assay of RPE1 cells treated with siRNA control and siCHAMP1, and treated with various doses of Olaparib; n=3 independent experiments, ***P<0.001. Statistical analysis was performed using two-way ANOVA. **C**, Western blot showing the lack of CHAMP1 expression in two CHAMP1 knockout RPE1 cell lines. **D**, Western blot showing the lack of CHAMP1 expression in two CHAMP1 knockout U2OS cell lines.

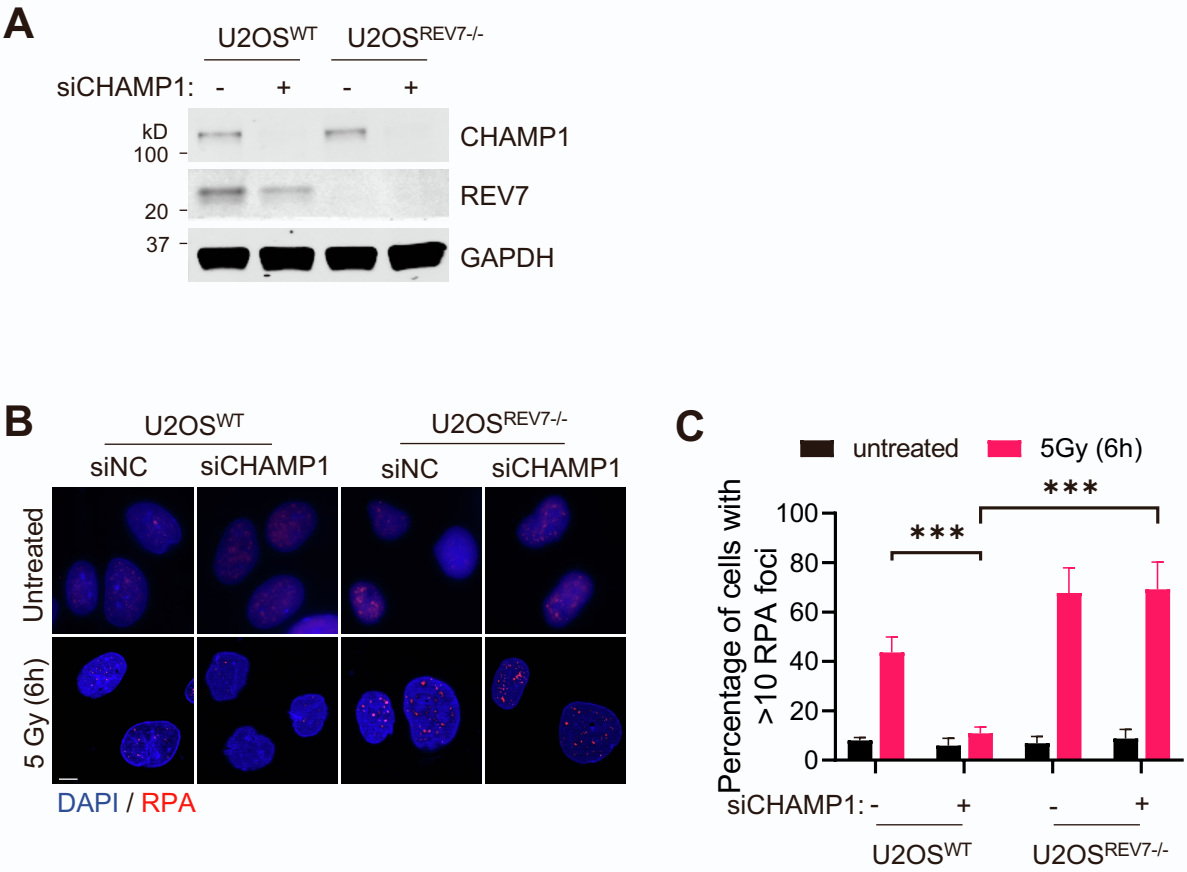
Figure S2, **CHAMP1 and TRIP13 have non-epistatic activity,** related to Fig. 2.



Supplemental figure 2, related to Figure 2.

A, 293T cells were transfected with GFP-tagged REV7, and following treatment with thymidine (2.5mM for 16h, G1) or thymidine (2.5mM for 16h, and release for 2h, S) or nocodazole (0.5ug/ml for 16h, G2/M). (Top) Western blot showing the GFP-REV7 and the co-immunoprecipitation of endogenous CHAMP1. (Bottom) Same cells were analyzed by flow cytometry to detect cell cycle. **B**, A 14 days clonogenic assay of U2OS wild type and CHAMP1-KO U2OS cell line, treated with various doses of IR; n=3 independent experiments. **P<0.01. Statistical analysis was performed using two-way ANOVA. **C**, The U2OS-wild type (WT) and U2OS$^{CHAMP1-/-}$ cells were transfect with siRNA targeting TRIP13 for 48 hours. Western blot shows the expression of endogenous CHAMP1 and TRIP13. **D**. A 14 days clonogenic assay of indicated cell lines treated with various doses of Olaparib; n=3 independent experiments, **P<0.01. Statistical analysis was performed using two-way ANOVA.

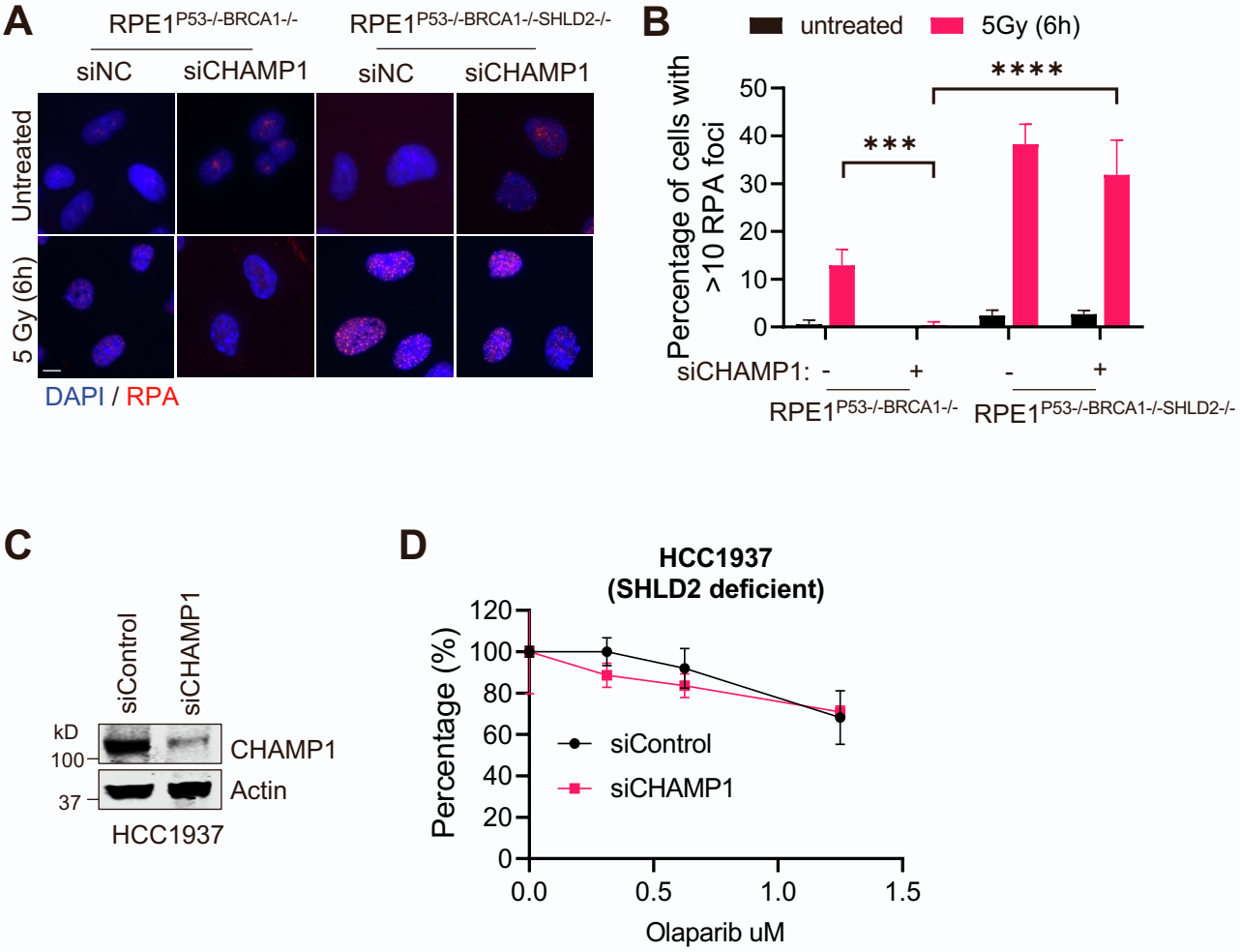
Figure S3, **CHAMP1 regulates HR through REV7,** related to Fig. 3.



Supplemental figure 3, related to Figure 3.

A, The U2OS-wild type (WT) and U2OS$^{REV7-/-}$ cells were transfect with siRNA targeting CHAMP1 for 48 hours. Western blot shows the expression of endogenous CHAMP1 and REV7. **B**, Representative images of p-RPA32(S33) foci formation in wild-type and *REV7*$^{-/-}$ U2OS cells treated with siRNA negative control (siNC) and siCHAMP1, and 6 hours after 5Gy IR treatment. Scale bar, 5 μm. **C**, Quantification of p-RPA32(S33) foci in A. More than 10 p-RPA32(S33) foci were counted. n=3 biologically independent experiments, ***P < 0.001. Statistical analysis was performed using two-tailed student's t-tests.

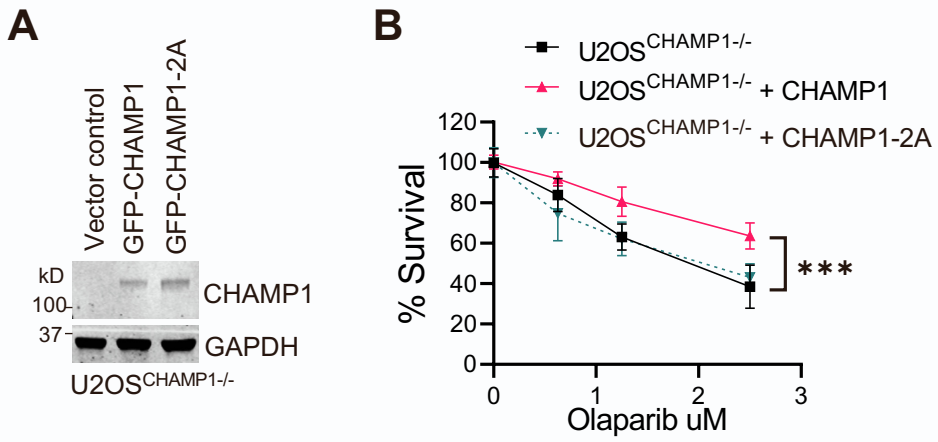
Figure S4, **CHAMP1 regulates HR through Shieldin complex,** related to Fig. 4.



Supplemental figure 4, related to Figure 4.

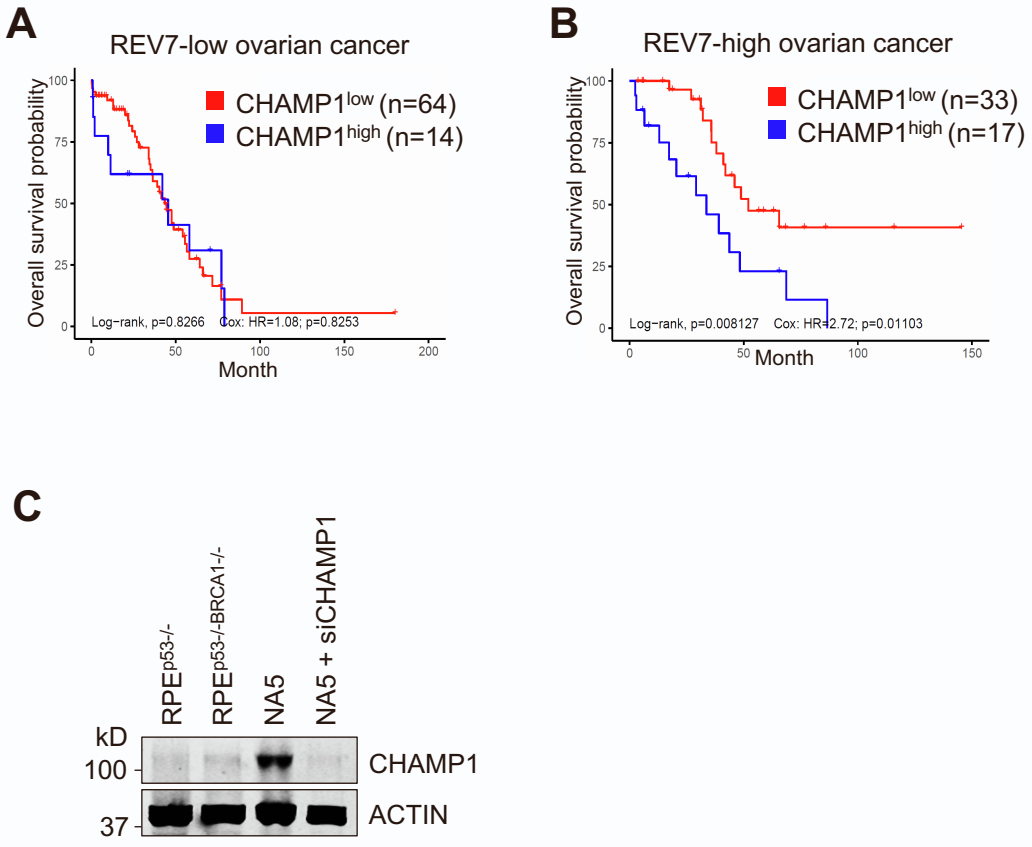
A, Representative images of p-RPA32(S33) foci formation in RPE1$^{P53-/-BRCA1-/-}$ and RPE1$^{P53-/-BRCA1-/-SHLD2-/-}$ cells treated with siRNA negative control (siNC) and siCHAMP1, and 6 hours after 5Gy IR treatment. Scale bar, 5 μm. **B**, Quantification of p-RPA32(S33) foci in C. More than 10 p-RPA32(S33) foci were counted. n=3 biologically independent experiments, ***P < 0.001. Statistical analysis was performed using two-tailed student's t-tests. **C**, HCC1937 cells (SHLD2 deficient) treated with siControl or siCHAMP1 for 48h. Western blot showing the expression of CHAMP1. Actin acts as loading control. **D**, A 14 days clonogenic assay of HCC1937 cells (SHLD2 deficient) treated with siControl or siCHAMP1 with various doses of Olaparib.

Figure S5, **CHAMP1 increases HR activity by binding to REV7,** related to Fig. 5.



Supplemental figure 5, related to Figure 5.
A, U2OS$^{CHAMP1-/-}$ cells were transfected with GFP-Empty Vector, GFP-CHAMP1 wild-type or GFP-CHAMP1-2A mutant. Western blot showing the expression of CHAMP1. GAPDH acts as loading control. **B**. A 14 days clonogenic assay of indicated cell lines treated with various doses of Olaparib; n=3 independent experiments, **P<0.001. Statistical analysis was performed using two-way ANOVA.

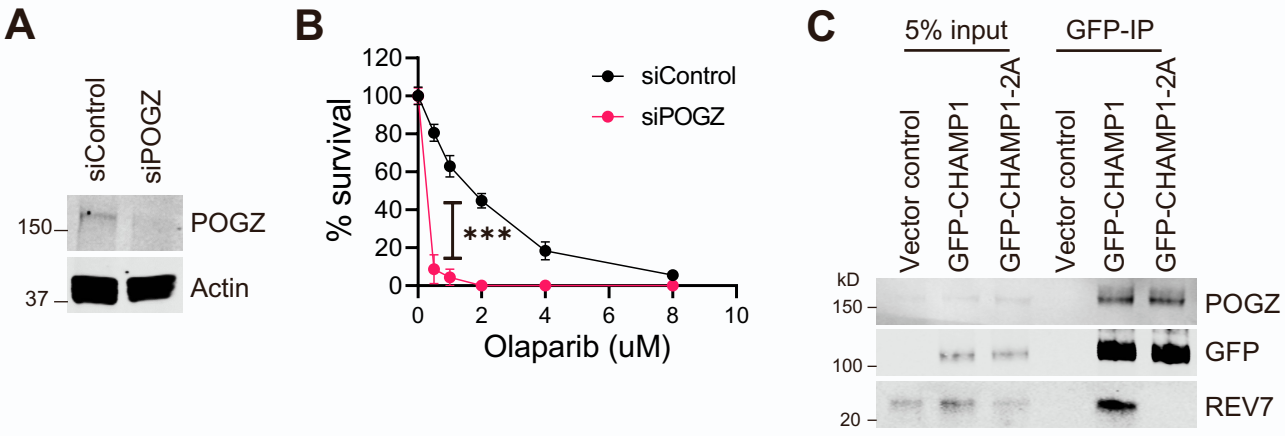
Figure S6, **high CHAMP1 expression correlated with poor patient prognosis in high REV7 expression patients,** related to Fig. 6.

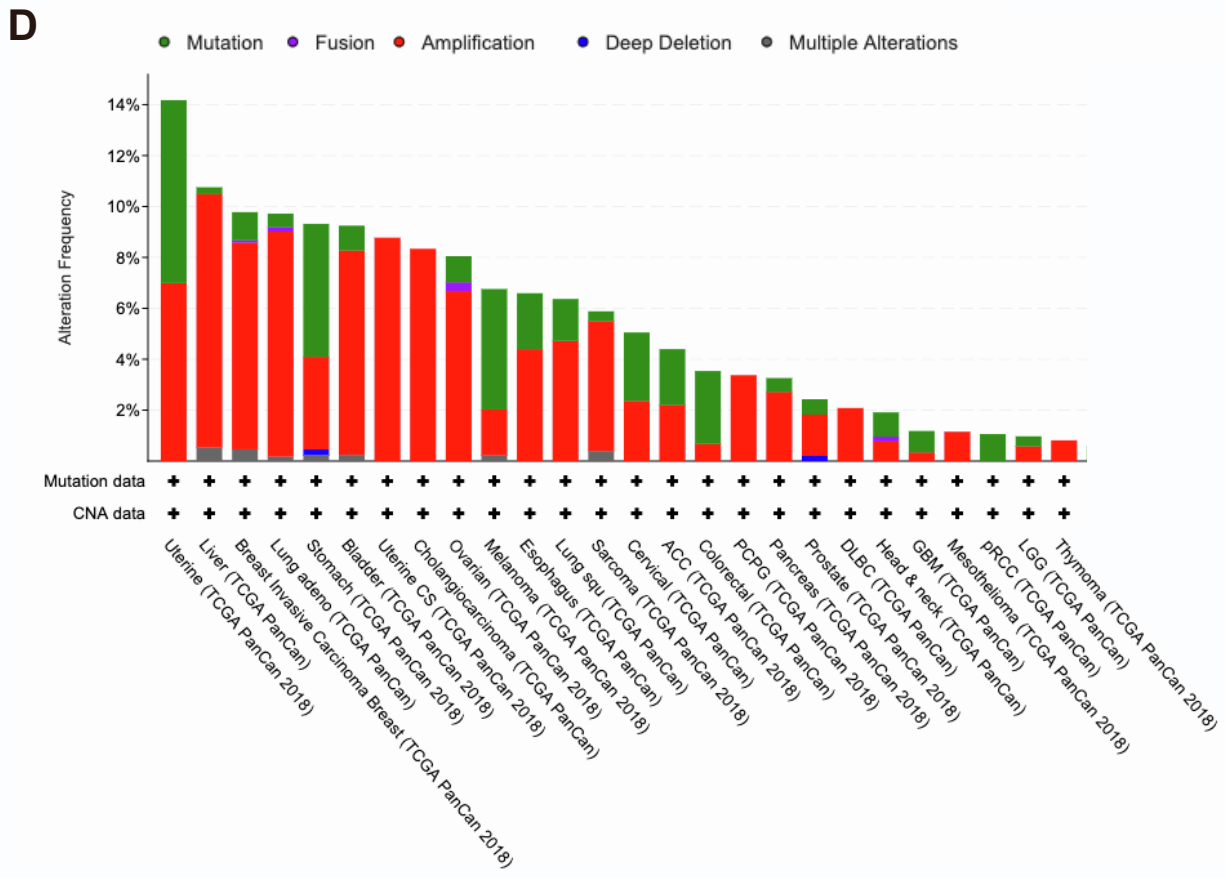


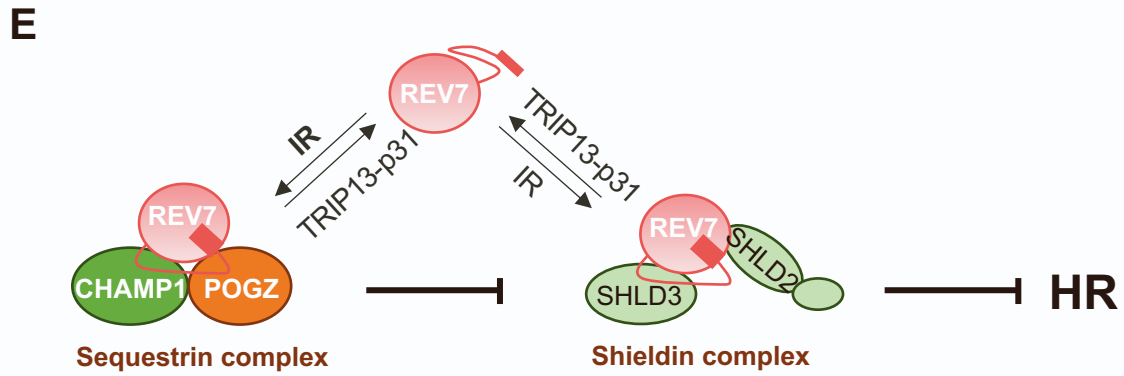
Supplemental figure 6, related to Figure 6.

A-B, Kaplan–Meier curves depicting overall survival of ovarian cancer patients with CHAMP1 expression and REV7 expression. N represents number of patients. **C**, Western blot showing the expression of CHAMP1 in indicated cells.

Figure S7, **POGZ promotes homologous recombination,** related to Fig. 7.







Supplemental figure 7, related to Figure 7.

A, Western blot showing knocking down efficiency of siRNA targeting POGZ. U2OS cells were treated with siRNA negative control or siCHAMP1 for 48hrs. **B**, A 14 days clonogenic assay of RPE1 cells treated with siRNA control or siPOGZ with various doses of Olaparib; n=3 independent experiments, ***P<0.001. Statistical analysis was performed using two-way ANOVA. **C**, Western blot showing GFP-immunoprecipitation of GFP-CHAMP1 or -CHAMP1-2A mutant, and the co-immunoprecipitation of endogenous REV7 and POGZ in HEK293T cells. **D**, Bar chart showing the prevalence of amplifications (red), deletions (blue), and mutations (green) of the POGZ gene across an array of cancer types in TCGA. **E**, Schematic of our proposed model of CHAMP1/POGZ function in HR regulation.