Expanded View Figures

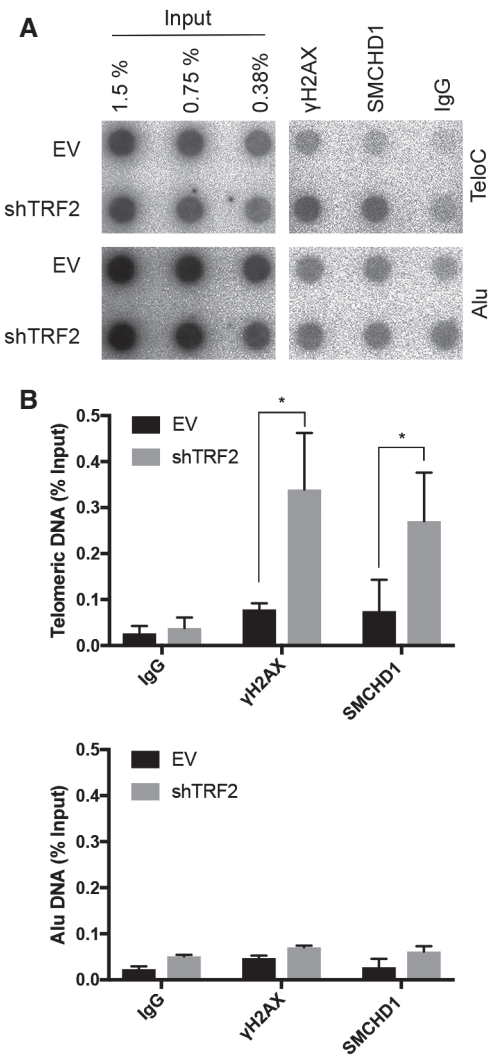
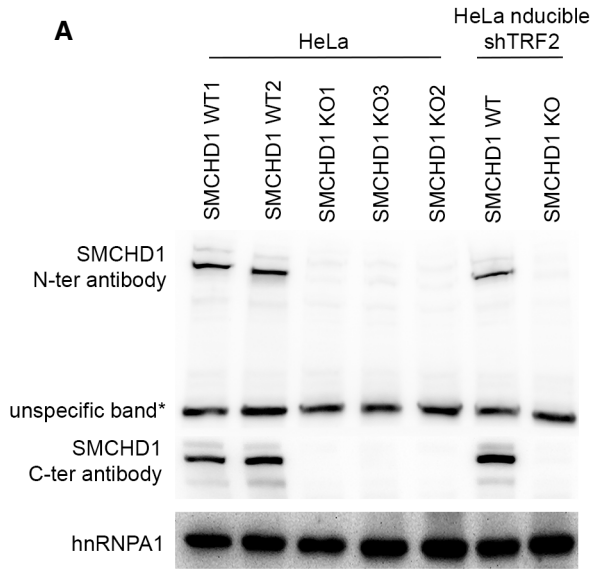


Figure EV1. SMCHD1 association with telomeres is increased upon TRF2 removal.

A Telomeric DNA ChIP with antibodies against γH2AX, SMCHD1, and rabbit IgG. Representative dot blot images of precipitated DNA detected with a (CCCTAA)n or Alu probe. ChIPs were performed in HeLa cells transfected with shTRF2 or empty vector (EV) control.

B Bar graph for quantification of γH2AX, SMCHD1, and rabbit IgG binding to telomeric or Alu DNA as assessed by ChIP. The bars represent average values from three independent experiments for telomeric DNA and two independent experiments for Alu DNA. Error bars represent the standard deviation. *P*-values were calculated by unpaired two-tailed Student's *t*-test $*P < 0.05$.



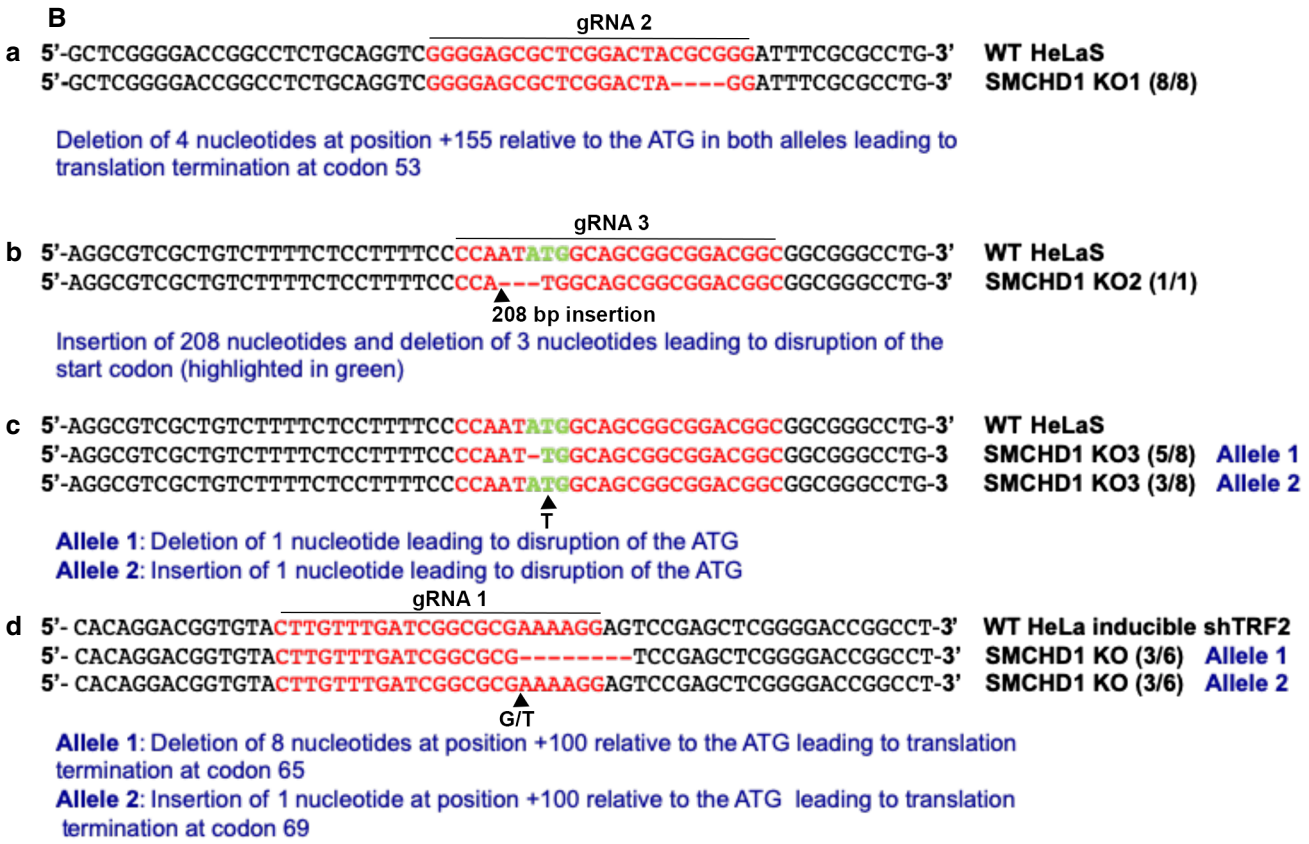


Figure EV2. Generation of SMCHD1 knockout cells using CRISPR-Cas9 technology.

A Western Blot detection of SMCHD1 with an antibody raised against the N-terminus (aa213–aa300) and the C-terminus of the protein (aa1955–2005) in SMCHD1 knockout single-cell clones of HeLa and HeLa inducible shTRF2 cell lines.

B Sequence analysis of edited alleles in SMCHD1 knockout single-cell clones.

Source data are available online for this figure.

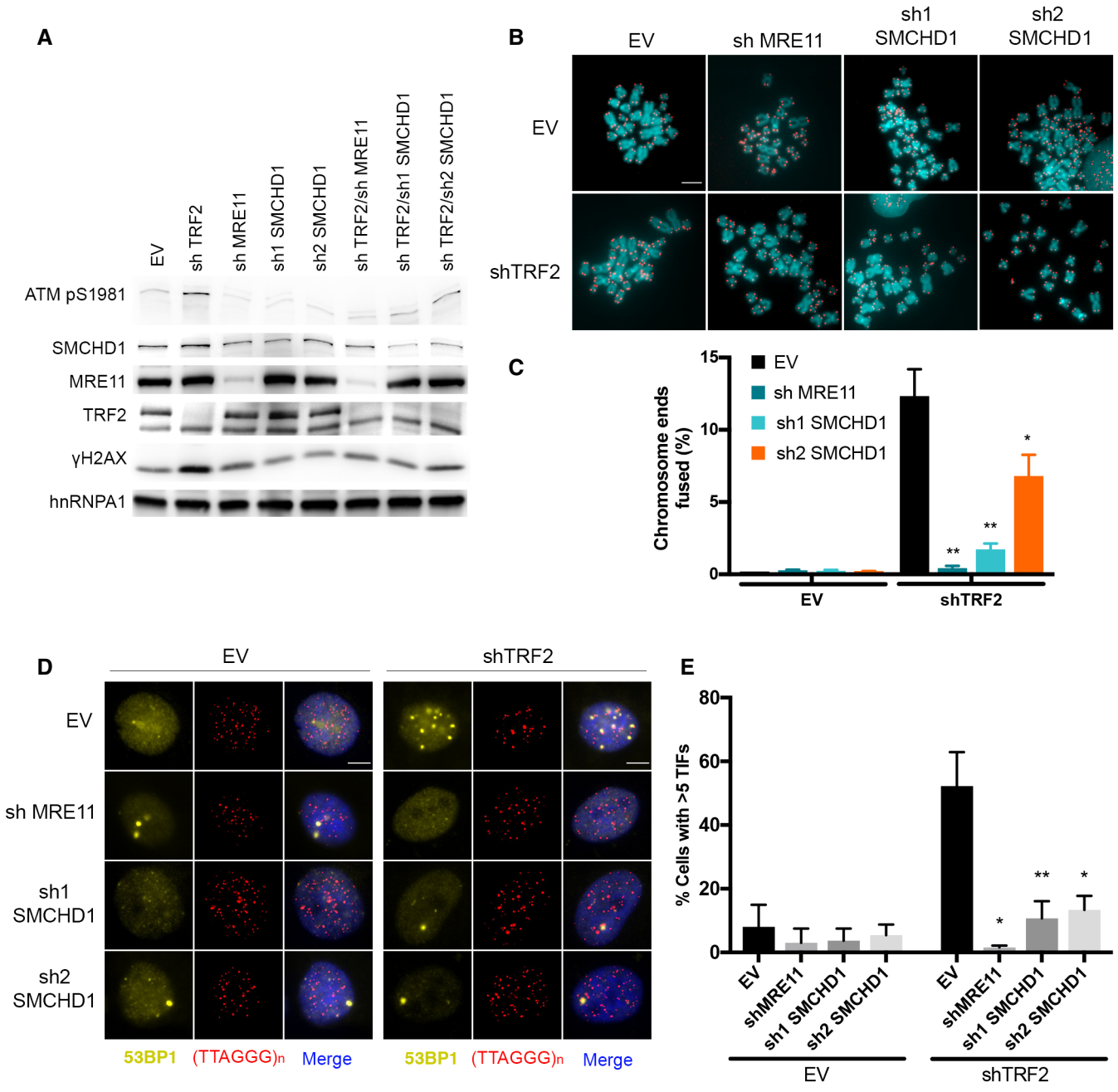


Figure EV3. SMCHD1 stimulates c-NHEJ and promotes DNA damage signaling at TRF2-depleted telomeres.

A Western Blot detection of ATM pS1981, SMCHD1, MRE11, TRF2, and γH2AX, in HeLa cells transfected with the indicated shRNA plasmids (shTRF2, shMRE11, sh1 SMCHD1, sh2 SMCHD1, shTRF2/shMRE11, shTRF2/sh1 SMCHD1, shTRF2/sh2 SMCHD1) and empty vector (EV) control.

B Metaphase spreads from HeLa cells transfected with the indicated shRNA plasmids (shTRF2, shMRE11, sh1 SMCHD1, sh2 SMCHD1, shTRF2/shMRE11, shTRF2/sh1 SMCHD1, and shTRF2/sh2 SMCHD1) and empty vector (EV) control. Telomeric signals were detected with Cy3-(CCCTAA)3 and are false-colored in red. Scale bar: 5 μm.

C Quantification of telomere fusions in HeLa cells transfected with the indicated shRNAs and EV control. Bars represent average numbers of chromosome ends fused in three independent experiments with SDs. $^{**}P < 0.01$; $^{*}P < 0.05$ unpaired two-tailed Student's *t*-test.

D Representative images for detection of 53BP1 at telomeres in HeLa cells transfected with the indicated shRNA plasmids. Immunofluorescence (IF) for 53BP1 (yellow) was combined with telomeric (CCCTAA)3-FISH (red), and the DNA was stained with DAPI. Scale bars: 5 μm.

E Quantification of the number of cells containing > 5 telomere dysfunction-induced foci (TIFs) detected as in (A). Data represent mean of three independent experiments ± SD (> 100 cells/condition/experiment). $^{*}P < 0.05$, $^{**}P < 0.01$, unpaired two-tailed Student's *t*-test.

Source data are available online for this figure.

Figure EV4. Analysis of epistatic relationship between TERRA and MDC1 with SMCHD1.

A Western Blot detection of TRF2 and vinculin in WT and SMCHD1 KO HeLa cells transfected with shTRF2 plasmid or EV control.

B TERRA quantification by RT–qPCR using primers specific for the indicated subtelomeric sequences (10q, 13q, 15q, 1 q, 9p, XpYp, XqYq, 2q, 20q, 12q, 18p). Bars represent average expression levels normalized to EV in each indicated cell line. SDs were obtained from three biological and two technical replicates.

C Western Blot detection of MDC1, TRF2, and hnRNPA1 in WT or SMCHD1 KO HeLa cells transfected with the indicated shRNA plasmids (shTRF2, shTRF2/sh2 MDC1, and shTRF2/sh3 MDC1) and EV control.

D Representative images for detection of ATM pS9181 at telomeres in wild-type (WT) and SMCHD1 KO HeLa cells transfected with indicated shRNA plasmids and empty vector (EV) control. Immunofluorescence (IF) for ATM pS1981 (green) was combined with telomeric (CCCTAA)3-FISH (red), and the DNA was stained with DAPI.

E Quantification of the number of cells containing > 5 telomere dysfunction-induced foci (TIFs) detected as in (B). Data represent mean of two independent experiments ± SD (> 50 cells/condition/experiment).

Source data are available online for this figure.

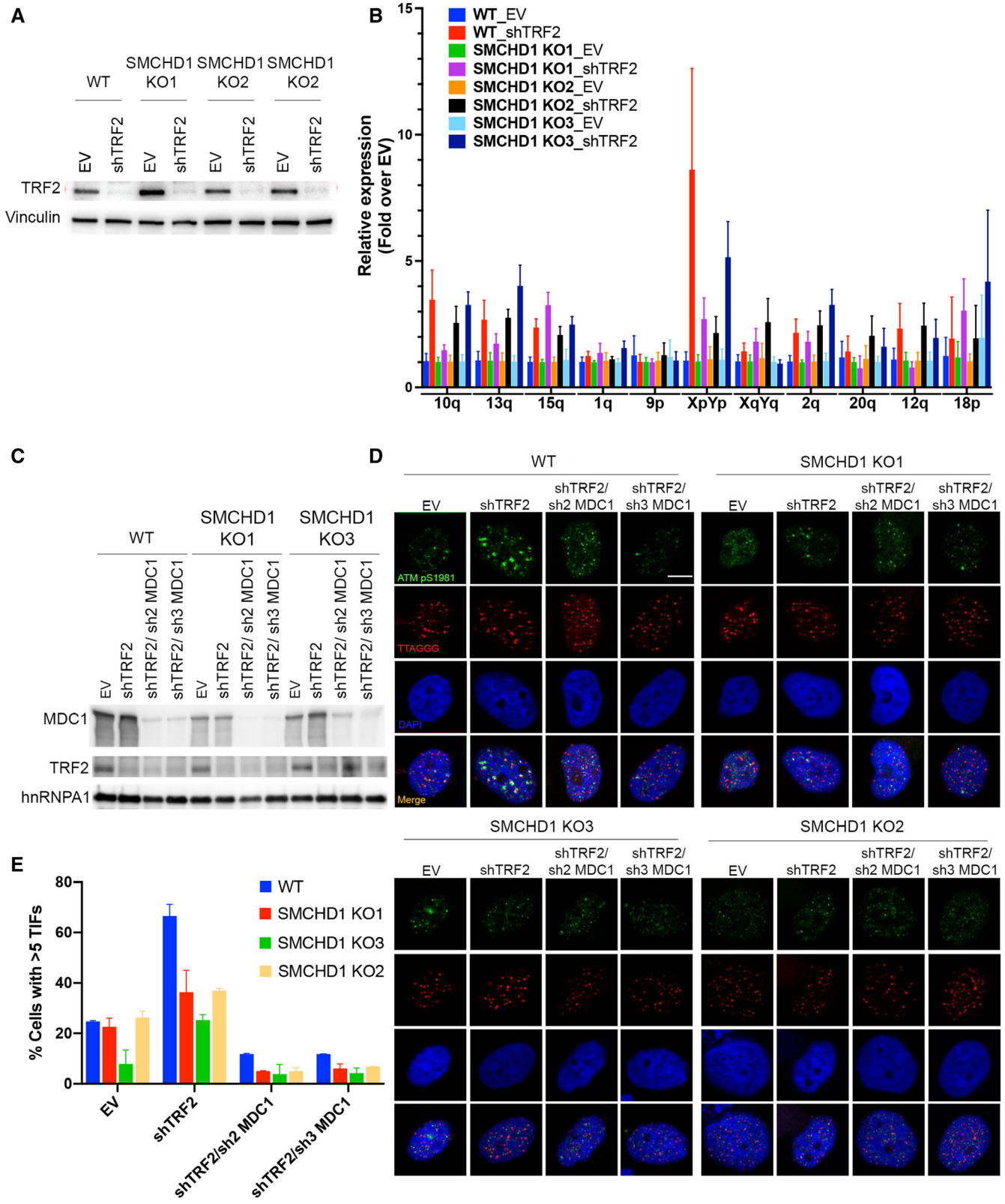


Figure EV4.

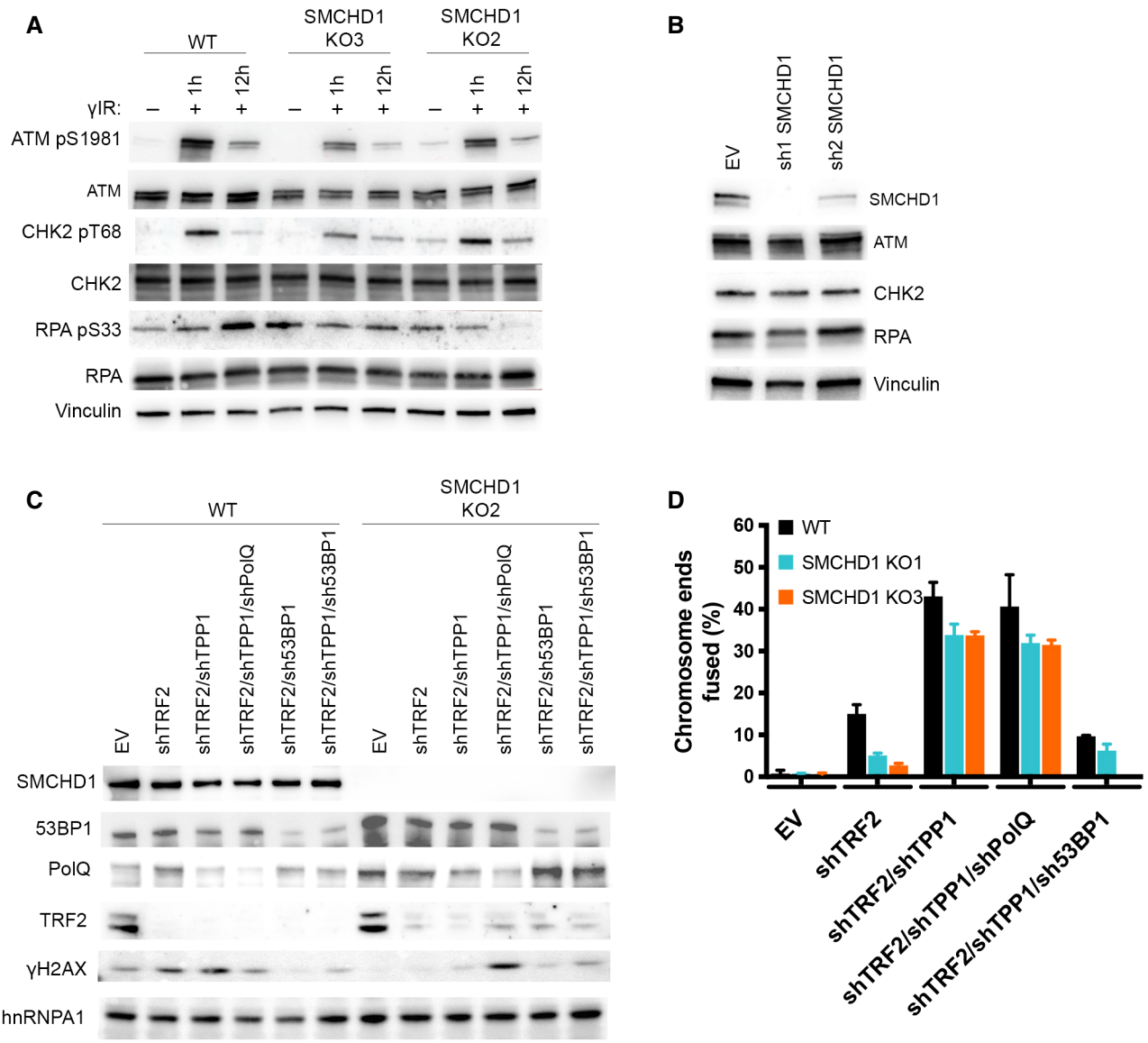


Figure EV5. SMCHD1 loss does not affect genome-wide DNA damage signaling upon γ-irradiation, and depletion of alt-NHEJ repair factor POLθ does not affect repair efficiency after removal of TRF2 and TPP1.

A Western blot detection of ATMpS1981, total ATM, CHK2 pT68, total CHK2, RPA pS33, total RPA, and vinculin in wild-type or *SMCHD1* KO HeLa cells y-irradiated with a dose of 3Gy and harvested at the indicated times (1, 12 h) after the treatment.

B Western blot detection of SMCHD1, total ATM, total CHK2, total RPA, and vinculin in HeLa cells transfected with the indicated shRNA plasmids (sh1 SMCHD1 and sh2 SMCHD1) and empty vector (EV) control.

C Western blot detection of SMCHD1, 53BP1, POLQ, TRF2, γH2AX, and hnRNPA1 in HeLa cells transfected with the indicated shRNA plasmids (shTRF2, shTRF2, shTRF2/shTPP1, shTRF2/shTPP1/shPOLQ, shTRF2/sh53BP1, and shTRF2/shTPP1/sh53BP1) and empty vector (EV) control.

D Quantification of telomere fusions from experiment shown in (A). Bars represent average number of fused chromosome ends. SDs were obtained from two independent experiments (> 2,000 telomeres counted/condition/experiment).

Source data are available online for this figure.