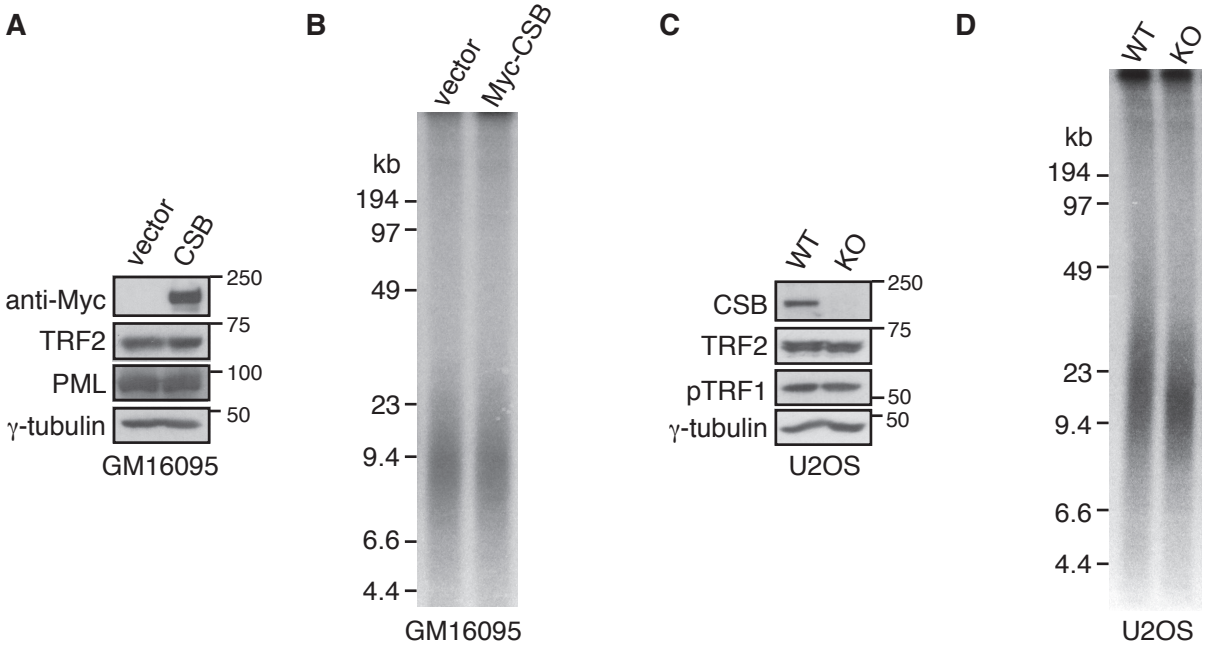

Figure S1. Loss of CSB does not affect telomere length heterogeneity in ALT cells. (**A**) Western analysis of vector- and Myc-CSB-expressing GM16095 cells. Immunoblotting was performed with anti-Myc, anti-TRF2, anti-PML and anti-γ-tubulin antibodies. The γ-tubulin blot was used as a loading control in this and subsequent figures. (**B**) Genomic blot of telomeric restriction fragments. RsaI/HinfI-digested genomic DNA (3 μg) from both vector- and Myc-CSB-expressing GM16095 cells was separated on a CHEF gel. The DNA molecular size markers are shown on the left of the blot. (**C**) Western analysis of U2OS WT and CSB-KO cells. Immunoblotting was performed with anti-CSB, anti-TRF2, anti-(pT371)TRF1 and anti-γ-tubulin antibodies. (**D**) Genomic blot of telomeric restriction fragments. RsaI/HinfI-digested genomic DNA (3 μg) from both U2OS WT and CSB-KO cells was separated on a CHEF gel. The DNA molecular size markers are shown on the left of the blot.

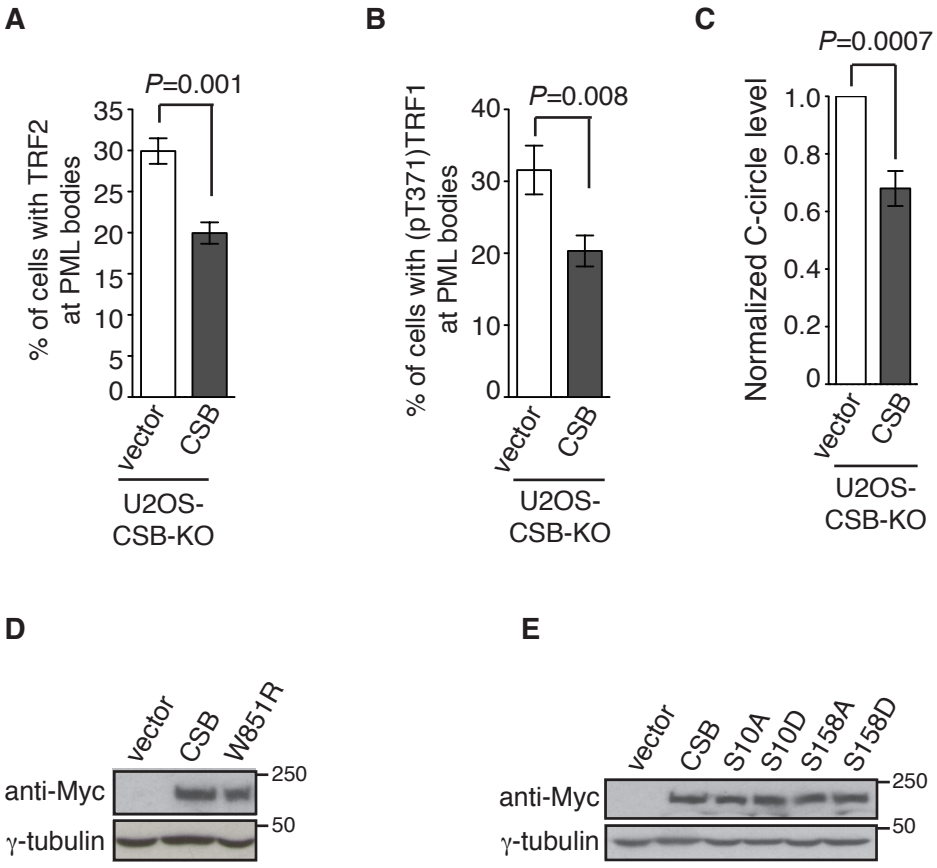

Figure S2. CSB regulates APB formation and C-circle production. (**A**) Quantification of the percentage of cells exhibiting colocalization of telomeric DNA with PML bodies. Vector- and Myc-CSB expressing U2OS CSB-KO cells were costained with a FITC-conjugated-(CCCTAA)3 PNA probe and an anti-PML antibody. At least 1000 cells per experimental condition were scored in blind. Standard deviations from three independent experiments are indicated in this and subsequent panels. (**B**) Quantification of the percentage of vector- and Myc-CSB expressing U2OS CSB-KO cells exhibiting colocalization of TRF2 with PML bodies. Scoring was done as in S2A. (**C**) Quantification of the percentage of vector- and Myc-CSB expressing U2OS CSB-KO cells exhibiting colocalization of (pT371)TRF1 with PML bodies. Scoring was done as in S2A. (**D**) Western analysis of U2OS CSB-KO cells expressing the vector alone, Myc-CSB WT or Myc-CSB-W851R. Immunoblotting was performed with anti-Myc and anti-γ-tubulin antibodies. (**E**) Western analysis of U2OS CSB-KO cells expressing the vector alone or various Myc-tagged CSB alleles as indicated. Immunoblotting was performed with anti-Myc and anti-γ-tubulin antibodies.

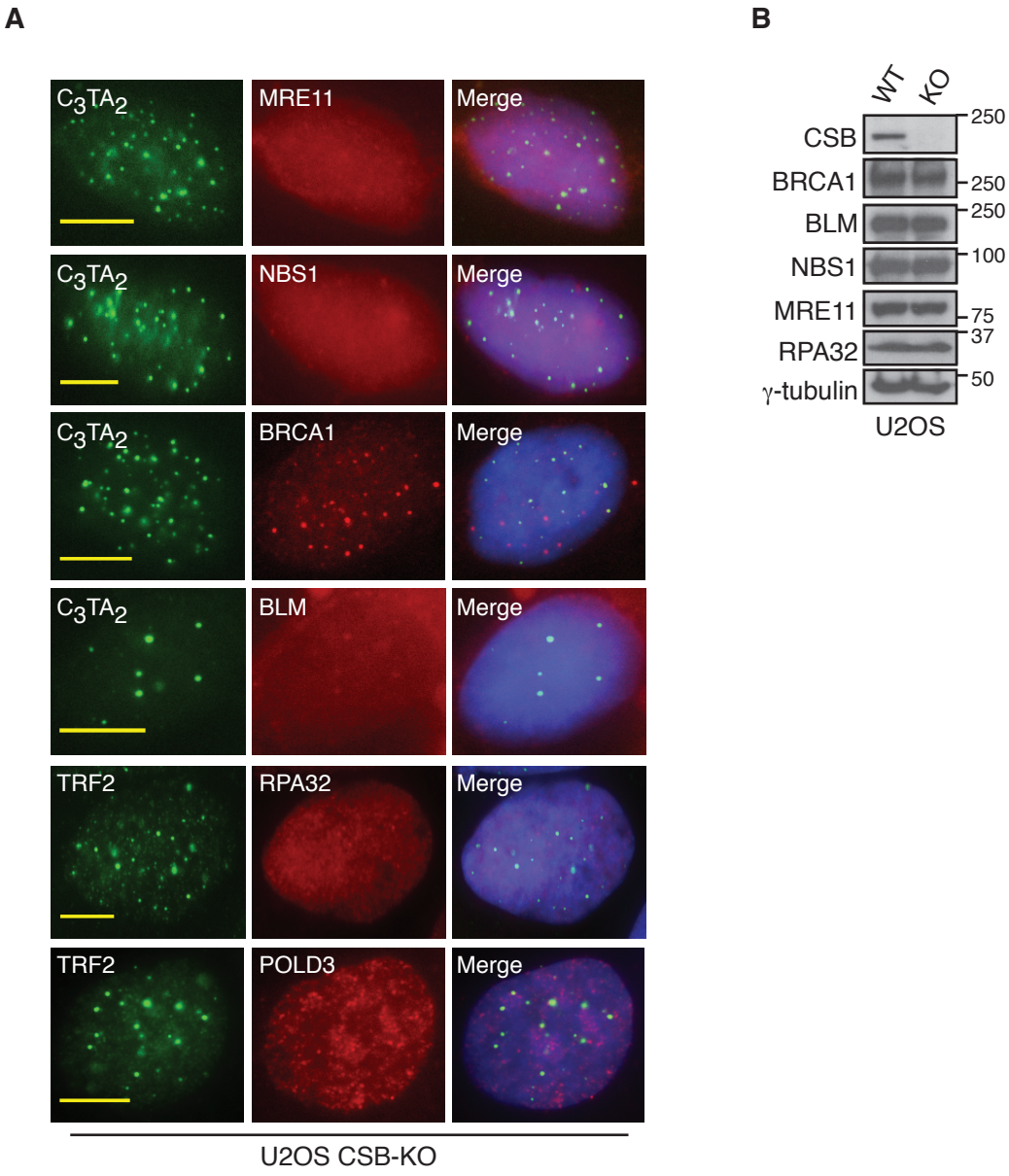

Figure S3. CSB promotes recruitment of HR repair proteins and POLD3 to ALT telomeres. (**A**) Representative images of IF and IF-FISH. For IF, U2OS CSB-KO cells were coimmunostained with an anti-TRF2 antibody in conjunction with either an anti-RPA32 or an anti-POLD3 antibody. For IF-FISH, U2OS CSB-KO cells were immunstained with a FITC-conjugated-(CCCTAA)3 PNA probe (green) in conjunction with an anti-MRE11, an anti-NBS1, an anti-BRCA1 or an anti-BLM antibody. (**B**) Western analysis of U2OS WT and CSB-KO cells. Immunoblotting was done with anti-CSB, anti-BRCA1, anti-BLM, anti-NBS1, anti-MRE11, anti-RPA32 and anti-γ-tubulin antibodies.

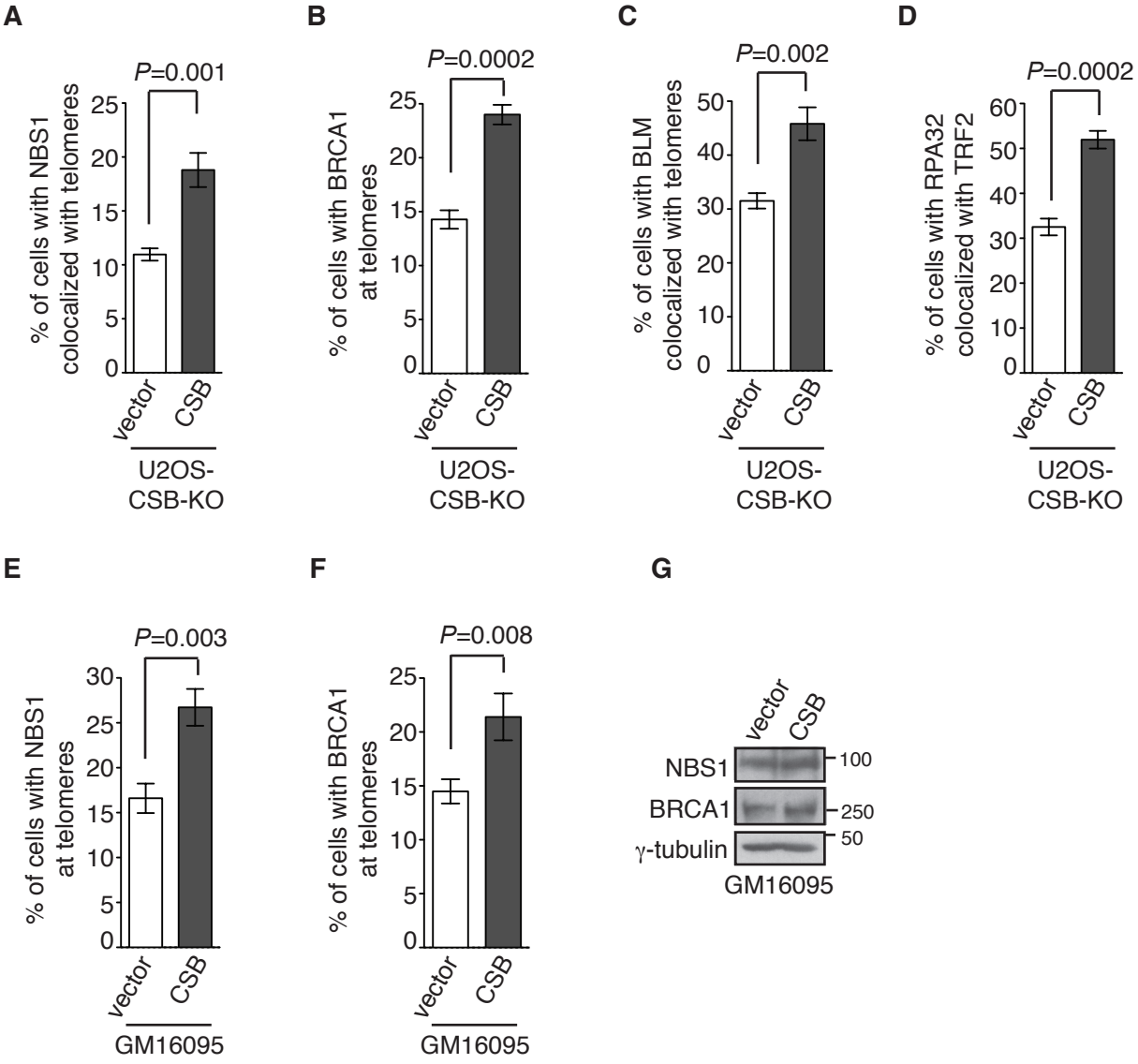

Figure S4. CSB promotes recruitment of HR repair proteins to ALT telomeres. (**A**) Quantification of the percentage of vector- and Myc-CSB-expressing U2OS CSB-KO cells exhibiting NBS1 colocalization with telomeres. At least 1000 cells per experimental condition were scored in blind in this and subsequent panels. Standard deviations from three independent experiments are indicated in this and subsequent panels. (**B**) Quantification of the percentage of vector- and Myc-CSB-expressing U2OS CSB-KO cells exhibiting BRCA1 colocalization with telomeres. (**C**) Quantification of the percentage of vector- and Myc-CSB-expressing U2OS CSB-KO cells exhibiting BLM colocalization with telomeres. (**D**) Quantification of the percentage of vector- and Myc-CSB-expressing U2OS CSB-KO cells exhibiting RPA32 colocalization with TRF2. (**E**) Quantification of the percentage of vector- and Myc-CSB-expressing GM16095 cells exhibiting NBS1 colocalization with telomeres. (**F**) Quantification of the percentage of vector- and Myc-CSB-expressing GM16095 cells exhibiting BRCA1 colocalization with telomeres. (**G**) Western analysis of vector- and Myc-CSB-expressing GM16095 cells. Immunoblotting was done with anti-NBS1, anti-BRCA1 and anti-γ-tubulin antibodies.

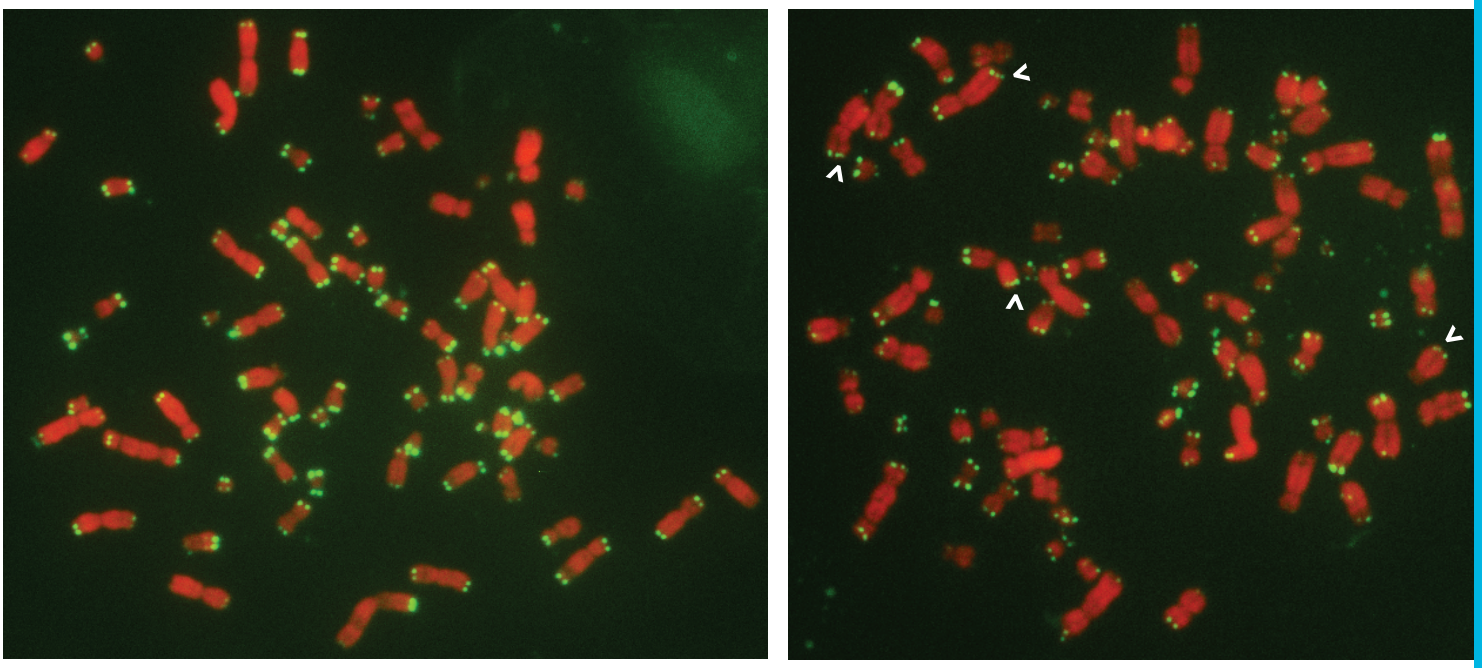

Figure S5. CSB suppresses telomere fragility. Representative images of metaphase chromosome spreads of U2OS WT and CSB-KO cells. Chromosomes were stained with DAPI and false colored in red. Telomeric DNA was detected by FISH using a FITC-conjugated (CCCTAA)3-containing PNA probe (green). Arrowheads indicate fragile telomeres.

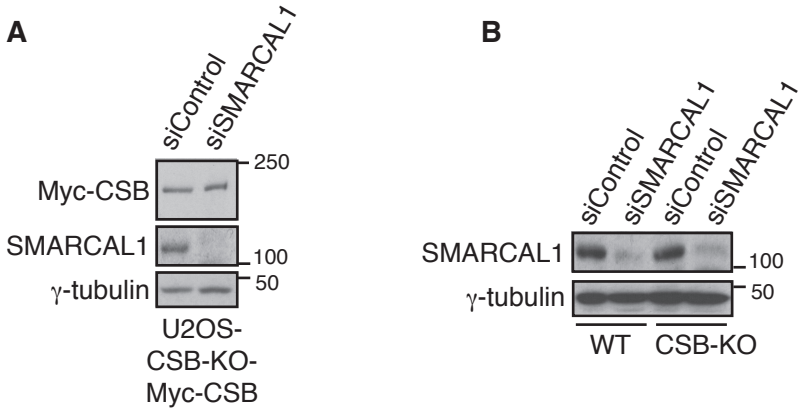

Figure S6. (**A**) Western analysis of Myc-CSB-expressing U2OS CSB-KO depleted for siControl or siSMARCAL1. Immunoblotting was done with anti-Myc, anti-SMARCAL1 and anti-γ-tubulin antibodies. (**B**) Western analysis of U2OS WT and CSB-KO cells transfected with siControl or siSMARCAL1. Immunoblotting was performed with anti-SMARCAL1 and anti-γ-tubulin antibodies.