Supplemental material

Chen et al., https://doi.org/10.1083/jcb.201808134

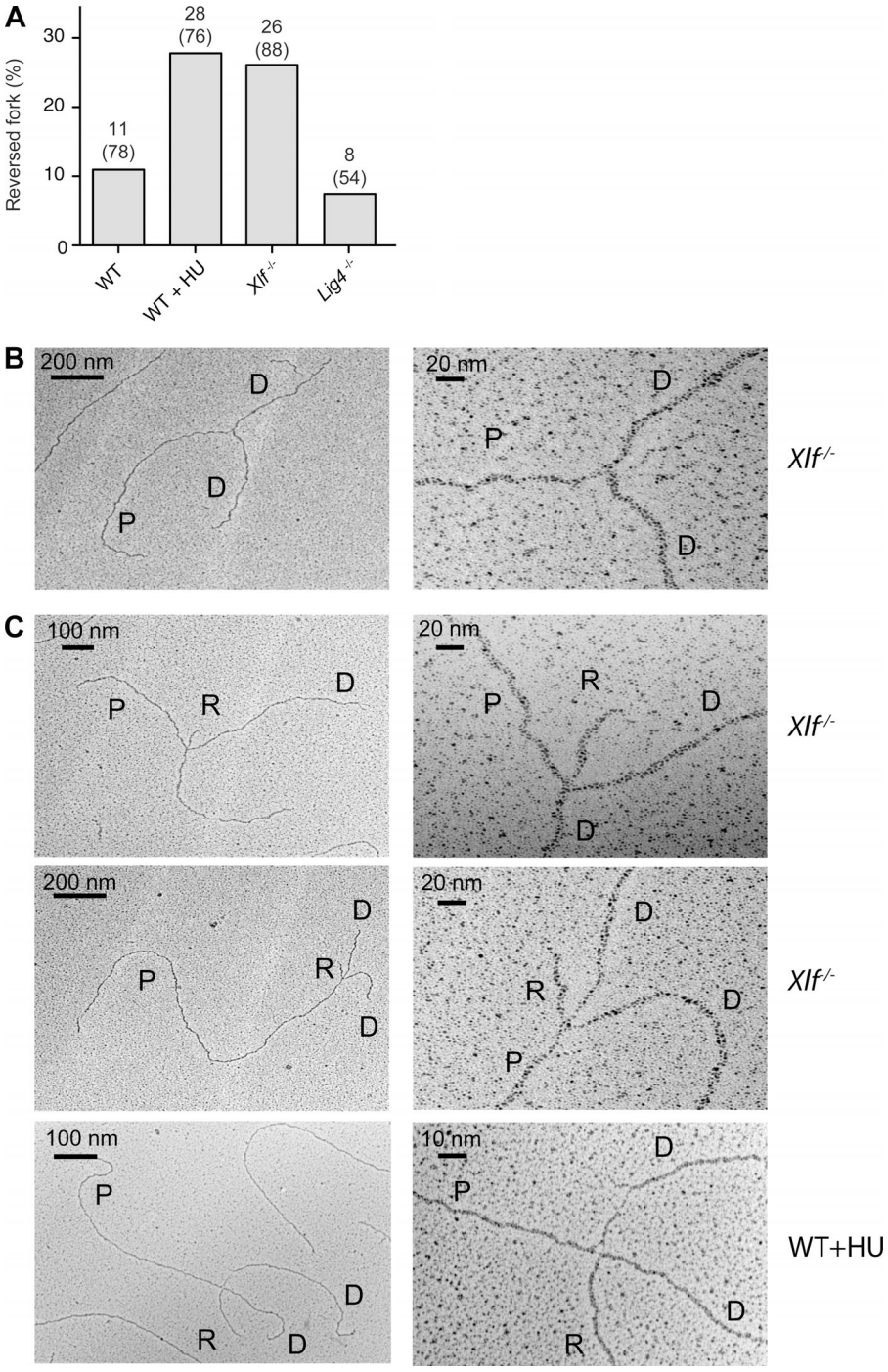

Figure S1. **XLF deficiency promotes replication fork reversal.** Related to Fig. 2. **(A)** Frequency of fork reversal in WT, *Xlf*$^{-/-}$, and *Lig4*$^{-/-}$ MEFs. Cells were treated with 2 mM HU (1 h) where indicated. The percentage values of reversed forks and total number of replication intermediates analyzed (in parentheses) are indicated on the top of the bar. **(B and C)** Electron micrographs of a replication fork (B) and reversed replication forks (C) observed on enriched genomic DNA in *Xlf*$^{-/-}$ MEFs or WT MEFs treated with HU. Right: Magnified three-way junction (B) or four-way junction at the reversed replication fork (C). D, Daughter strand; P, Parental strand; R, Reversed arm. For the criteria used for the assignment of reversed forks, see Vindigni and Lopes (2017) and Neelsen et al. (2014).

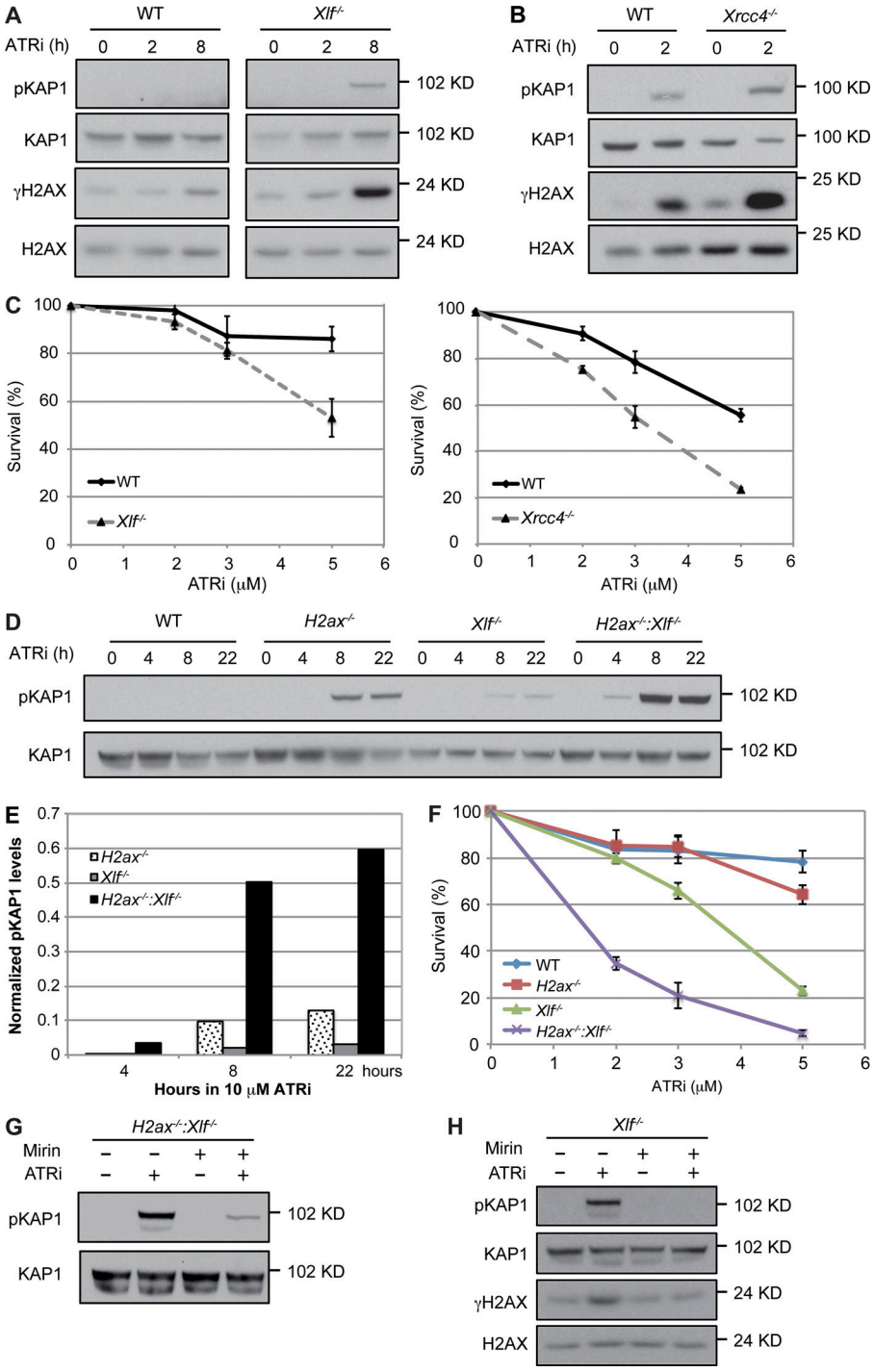

Figure S2. **Biological replicates of key Western blots and survival assays in independently generated set of MEFs.** Related to Figs. 3, 4, and 5. **(A and B)** The indicated MEFs were treated with 10 μM ATRi for indicated times. Whole cell lysate was collected and analyzed with antibodies specified by Western blot. **(C)** WT, *Xlf*$^{-/-}$, and *Xrcc4*$^{-/-}$ MEFs were treated with ATRi at indicated concentrations for 4 d. Cell survival was determined with PrestoBlue Cell Viability Reagent. Error bars indicate SD of three technical repeats in the experiment. **(D)** Whole cell lysate from WT, *H2ax*$^{-/-}$, *Xlf*$^{-/-}$, or *H2ax*$^{-/-}$:*Xlf*$^{-/-}$ MEFs untreated or treated with 10 μM of ATR inhibitor for 4, 8, or 22 h was analyzed by Western blot with indicated antibodies. **(E)** Quantification of D. **(F)** WT, *H2ax*$^{-/-}$, *Xlf*$^{-/-}$, or *H2ax*$^{-/-}$:*Xlf*$^{-/-}$ MEFs were treated with ATR inhibitor at indicated concentrations for 4 d and analyzed as in C. **(G and H)** *H2ax*$^{-/-}$:*Xlf*$^{-/-}$ (G) or *Xlf*$^{-/-}$ (H) MEFs were treated with 10 μM ATRi, 50 μM mirin, or both. Whole cell lysate was prepared and analyzed with indicated antibodies. Western blots in A, B, D, E, G, and H and the survival assays in C and F are representative data on a second set of independently generated cells (data on the other set of cell lines are shown in main figures).

References

Neelsen, K.J., A.R. Chaudhuri, C. Follonier, R. Herrador, and M. Lopes. 2014. Visualization and interpretation of eukaryotic DNA replication intermediates in vivo by electron microscopy. *Methods Mol. Biol.* 1094:177–208. https://doi.org/10.1007/978-1-62703-706-8_15

Vindigni, A., and M. Lopes. 2017. Combining electron microscopy with single molecule DNA fiber approaches to study DNA replication dynamics. *Biophys. Chem.* 225:3–9. https://doi.org/10.1016/j.bpc.2016.11.014

Provided online is one table. Table S1 is related to Fig. 1 and shows proteins and peptides identified in mass spectrometry following HA antibody immunoprecipitation in control (XLF-deficient) and FLAG-HA-XLF–expressing cell lysates.