Supplementary figure legends

Supplementary Figure S1.

The knockdown efficiency of Claspin between Claspin-single knockdown and Claspin/HERC2-double knockdown. Long exposure of the anti-Claspin immunoblotting demonstrated in Fig. 1C, 3A, 4B and Supplementary Fig. S4B. Note that the Claspin expression in Claspin-single knockdown cells was at the same level as that in Claspin/HERC2-double knockdown cells, or both were undetectable even with the long exposure.

Supplementary Figure S2. Reliability test for the DNA fiber labeling experiments. (A) Schematic representation: HCT116 cells were sequentially treated with BrdU for 10, 20 or 40 min and IdU for 20 min to label the nascent DNAs. (B) Representative images of labeled DNA fibers from cells with indicated BrdU labeling time lengths measured with confocal microscopy. (D) Distributions of length of replication fork labeled with BrdU (left panel) and IdU (right panel) are shown with the mean percentages (bars).

Supplementary Figure S3. Proteasome inhibition does no affect nascent DNA length shortened by Claspin inhibition. HCT116 cells transfected with Claspin-specific siRNA were subjected to immunoblot (A) or sequentially treated with BrdU and IdU for 20 min each in the presence (+) or absence (-) of 5μM of MG132(B). Distributions of replication fork length during the entire labeling period in cells are shown with the mean percentages (bars). Significance was analyzed by Student's t-test.

Supplementary Figure S4. Nuclear RPA foci formation induced by Claspin depletion was not affected by HERC2 status. U2OS cells stably expressing GFP-RPA70 (1) were transfected with indicated siRNA oligonucleotides and either analyzed for nuclear GFP-RPA70 foci with confocal microscopy (**A**), or subjected to immunoblot with indicated antibodies (**B**). (**C**) The percentage of cells containing ten or more GFP-RPA70 foci. Bars represent standard deviation, based on three independent experiments.

Supplementary Figure S5. HERC2-ATRIP interaction detected with immunoprecipitation from cell lysate in the presence of endonuclease. HeLa cells were immunoprecipitated (IP) in the absence or presence of endonuclease, and immunoblotted (IB) with indicated antibodies. Inputs (1.5%) were also loaded. Note that although large amount of ATRIP was immunoprecipitated when compared to the inputs, only small fraction of total ATRIP were additionally eluted by the endonuclease treatment when judged by the amount of ATRIP in both endonuclease-treated and untreated samples. The small fraction coprecipitated MCM2 and HERC2.

Supplementary Figure S6. A model for HERC2 function in DNA replication. ATR-Claspin-Chk1 pathway up regulates DNA elongation while it inhibits excess origin firing. On the other hand, ATR-dependent phosphorylation of Mcm2 recruits Plk1 that enhances origin firing through inhibition of Claspin-Chk1 pathway or through different mechanisms such as DNA replication licensing mediated by Plk1 phosphorylation of Hbo1 (histone acetyltransferase binding to Orc1). Persistent inhibition of replication fork stability mediated by siRNA inhibition of Claspin results in increased origin firing as a

compensatory mechanism for survival of replication blocks. Enhanced MCM2 phosphorylation observed in the Claspin-deficient cells suggests that the increased origin firing could be attributed to activation of MCM2-Plk1-Hbo1 pathway in addition to inhibition of Chk1. HERC2 may play a role in the ATR-dependent phosphorylation of MCM2 and subsequent activation of origin firing. Because replication elongation and origin firing are balanced each other, the inhibition of origin firing mediated by HERC2 inhibition may alleviate the slow replication fork progression in Claspin-deficient cells.

Reference

1. Nakamura K, Kato A, Kobayashi J, Yanagihara H, Sakamoto S, Oliveira DV, et al. Regulation of homologous recombination by RNF20-dependent H2B ubiquitination. Mol Cell. 2011;41:515-28.