Supplemental Materials for:

The Mcm2-7-interacting domain of human mini-chromosome maintenance 10 (Mcm10) protein is important for stable chromatin association and origin firing

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Experimental Procedure

siRNA transfection—Stealth small interfering RNAs (Thermo Fisher) were synthesized against Mcm2 and Mcm6. The target sequence for Mcm2 was described previously (1), and the target sequence for Mcm6 was as follows: 5′-AAT AGA CAC GAT CAA TTG ATT CCT C-3′. Stealth RNAi Negative Control Low GC Duplex (Thermo Fisher) was used as a control. HeLa cells plated at 10^5 cells per 6 cm dishes were grown for 24 h, and two rounds of transfection, separated by 24 h, were performed with 100 nM Stealth RNAi and RNAiMax (Thermo Fisher) according to the manufacture's protocol. Then, cells were synchronized at the G1/S boundary by a sequential thymidine-aphidicolin block at 48 h after the second transfection.

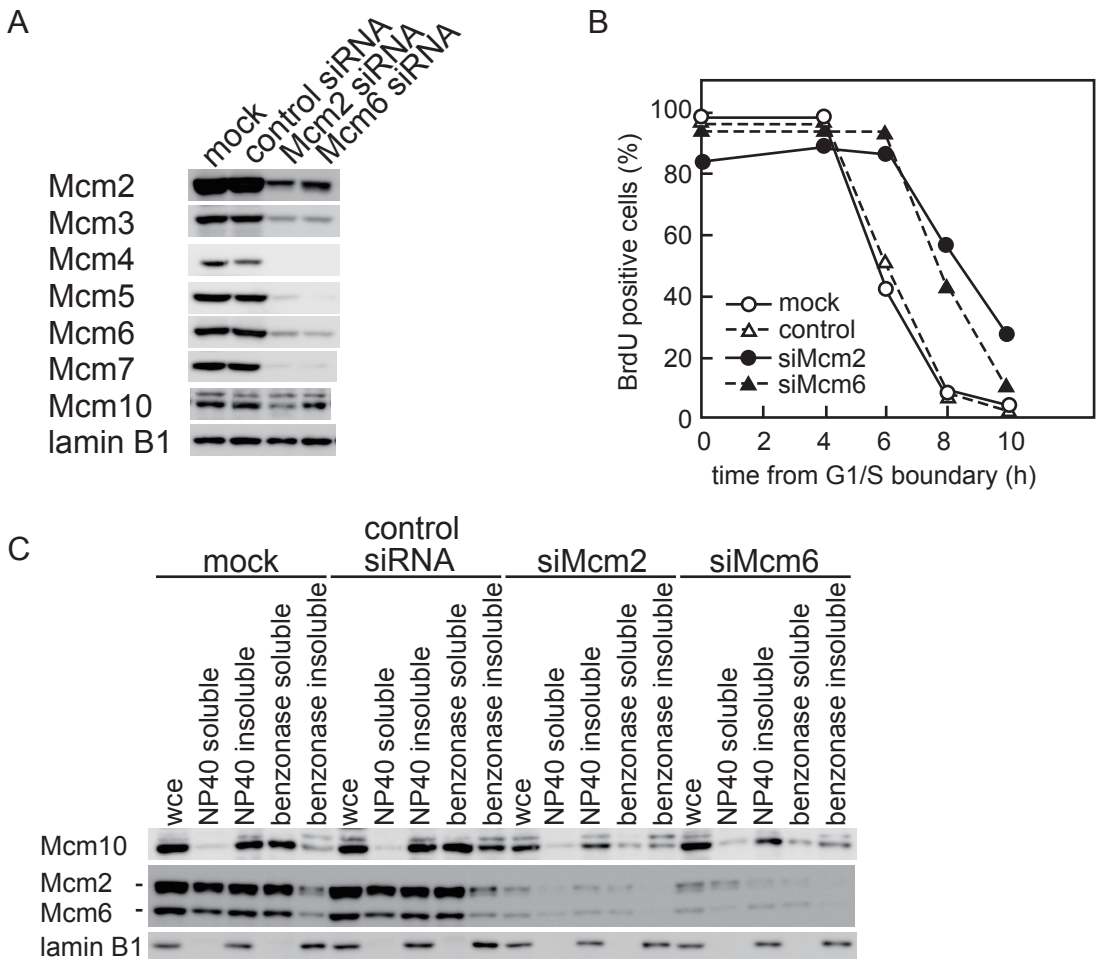

Figure S1. Subcellular distribution of Mcm10 in Mcm2-7-depleted cells. ***A,*** Whole cell extracts were prepared 72 h after the transfection of nonspecific control, Mcm2 or Mcm6 siRNAs and subjected to immunoblotting for the expression of Mcm2-7, Mcm10, and lamin B1 as a loading control. The protein level of Mcm2 and Mcm6 was reduced to 10~12% and 8~14%, respectively. Depletion of Mcm2 or Mcm6 also reduced the levels of other subunits as reported previously (1), probably because the Mcm2-7 complex was destabilized when one of the subunits is missing. ***B,*** To ascertain that the cells were synchronized at the G1/S boundary, HeLa cells transfected with the indicated siRNAs were released from the G1/S boundary, and DNA synthesis was estimated by BrdU incorporation. More than 95% of control cells or Mcm6-depleted cells entered the S-phase, whereas only 83% of Mcm2-depleted cells entered the S-phase. ***C,*** HeLa cells transfected with the indicated siRNAs were synchronized at the G1/S boundary, fractionated as described in Fig. 1A, and subjected to immunoblotting. Mcm10 was mainly localized in the soluble fraction after benzonase digestion in mock- or control siRNA-transfected cells, whereas more than half of Mcm10 remained in the benzonase-insoluble fraction in Mcm2- or Mcm6-depleted cells.

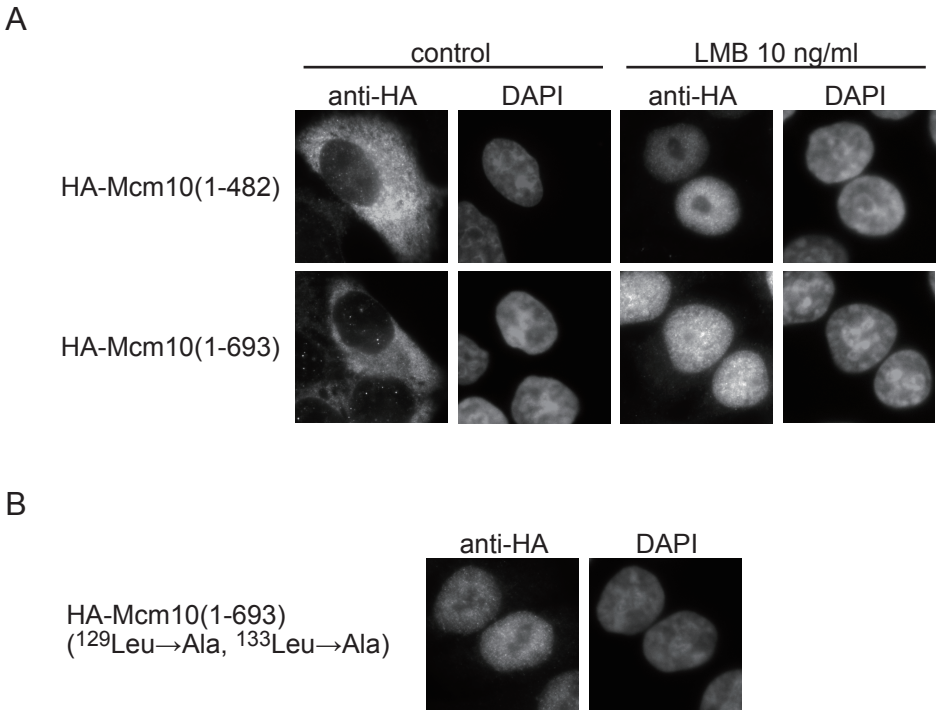

Figure S2. Human Mcm10 has the leucine-rich nuclear export signal in the N-terminal region. ***A,*** Stably transfected HeLa cell lines expressing HA-tagged Mcm10 (1-482) and Mcm10 (1-693) were treated with or without 10 ng/ml of leptomycin B (LMB) for 30 min and fixed with 4% paraformaldehyde. HA-tag was detected by indirect immunofluorescence. DNA was stained with DAPI, and the cells were observed by fluorescence microscopy. ***B,*** The substitution of two amino acids in the nuclear export signal (^{126}MKALQEQLKV) resulted in the nuclear transport of HA-Mcm10 (1-693) .

Reference

1. Ibarra, A., Schwob, E., and Mendez, J. (2008) Excess MCM proteins protect human cells from replicative stress by licensing backup origins of replication. *Proc. Natl. Acad. Sci. U.S.A.* **105,** 8956-8961