SUPPLEMENTARY FILE

Chromatin Remodeler Sucrose Nonfermenting 2 Homolog (SNF2H) Is Recruited onto DNA Replication Origins through Interaction with Cdc10-dependent Transcript 1 (Cdt1) and Promotes Pre-replication Complex Formation

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Running title: SNF2H promotes pre-RC formation via Cdt1

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SUPPLEMENTARY FIGURE LEGENDS

Supplementary Table S1. Sequences and annealing temperatures of primers used for quantitative real-time PCR. Primer labels correspond to the amplified region and are followed by F or R to designate forward or reverse primers, respectively. The $T_{annealing}$ is the annealing temperature used in the cycling conditions for the iCycler iQ (Bio-Rad).

Supplementary Figure S1. SNF2H binding to origins is inhibited by Cdt1 silencing. A different set of the experiments shown in Fig. 3C is presented. HeLa cells transfected with control (DS scrambled Neg) or Cdt1 siRNAs for 48hr were subjected to ChIP assay. For ChIP data, the means and standard deviations are shown (n=5). *P<0.05.

Supplementary Figure S2. Simultaneous overexpression of Cdt1+SNF2H or Cdt1+HBO1 does not induce rereplication in HEK293T cells. Cells were transiently transfected with the indicated expression vectors along with a plasmid expressing GFP. Forty-eight hours after transfection, cells were analyzed. *A*. DNA contents were analyzed using a flow cytometer. In these diagrams, the *x*-axis corresponds to FL2-A (area) and represents the PI signals and thus DNA contents. The *y*-axis corresponds to FL2-W (width) and represents the duration of the PI signals. Dots with higher FL2-W signals, which result from aggregated cells or cell debris, were excluded from the measurement of rereplicated cells. *B*. Whole cell lysates were subjected to immunoblotting with the indicated antibodies. GFP served as the control to monitor transfection efficiency.

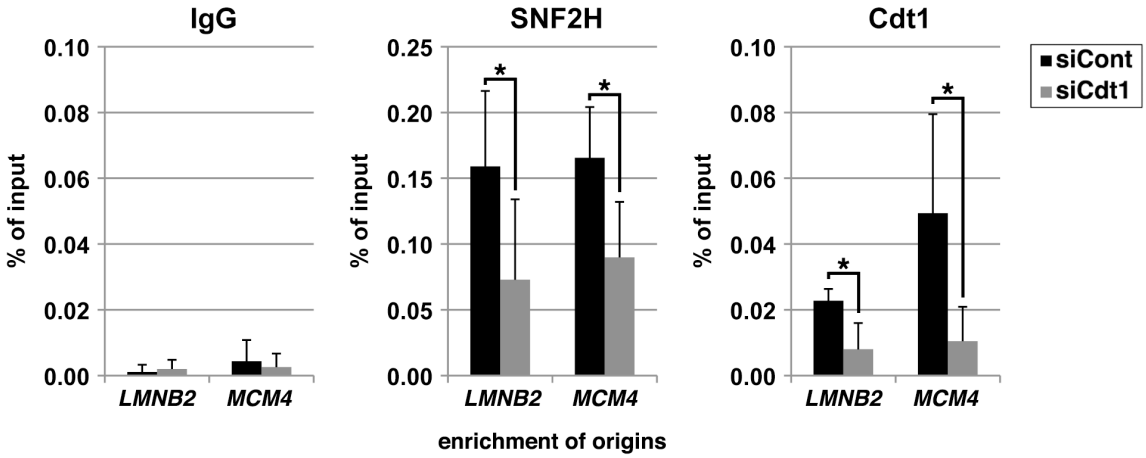
Supplementary Figure S3. Cdt1-induced rereplication is not affected by SNF2H or HBO1 silencing. HEK293T cells were transiently transfected with the indicated expression vector and siRNAs using with Lipofectamine2000, along with a plasmid expressing GFP. Forty-eight hours after transfection, cells were analyzed by flow cytometry or immunoblotting. The means and SDs of the percentage of rereplicated cells (the DNA content higher than 4N) are shown (n=3). *A*. Data for SNF2H silencing experiments. The immunoblotting data that correspond to these results are presented in Figure 5. *B*. Data for HBO1 silencing experiments. DS scrambldNeg siRNAs were used as the control. *C*.

Data for ORC1 or CDC6 silencing experiments. For control siRNAs, a 1:1 mixture of DS scrambldNeg and GL2 was used. For silencing of CDC6, a 1:1 mixture of siCDC6-1 and siCDC6-3 was used. *P<0.05.

Supplementary Figure S4. Simultaneous overexpression of Cdt1 plus SNF2H does not enhance Cdt1 overexpression-induced ATM/Chk2 checkpoint activation. HEK293T cells were transiently transfected with the wild type T7-Cdt1 (T7-Cdt1 WT) with or without SNF2H, along with a plasmid expressing GFP. Forty-eight hours after transfection, whole cell lysates were subjected to immunoblotting with the indicated antibodies. GFP serves as the control to monitor transfection efficiency.

Supplementary Table S1.

Primers	Sequence (5'-3')	$T_{annealing}$ (°C)
Lamin B2 distal F	GTTAACAGTCAGGCGCATGGGCC	67.1
Lamin B2 distal R	CCATCAGGGTCACCTCTGGTTCC	
LaminB2 origin F	GGCTGGCATGGACTTTCATTTCAG	62.9
Lamin B2 origin R	GTGGAGGGATCTTTCTTAGACATC	
MCM4 distal F	TACCTGTGGGTAAGAGATGAGTTG	65.5
MCM4 distal R	CTCTATACATGCAACGACTTGGG	
MCM4 origin F	GGACATTACAGATGCATTTCTC	55.1
MCM4 origin R	AAGAGTTCCAAGTTTGTTCCTC	



Supplementary Figure S1.
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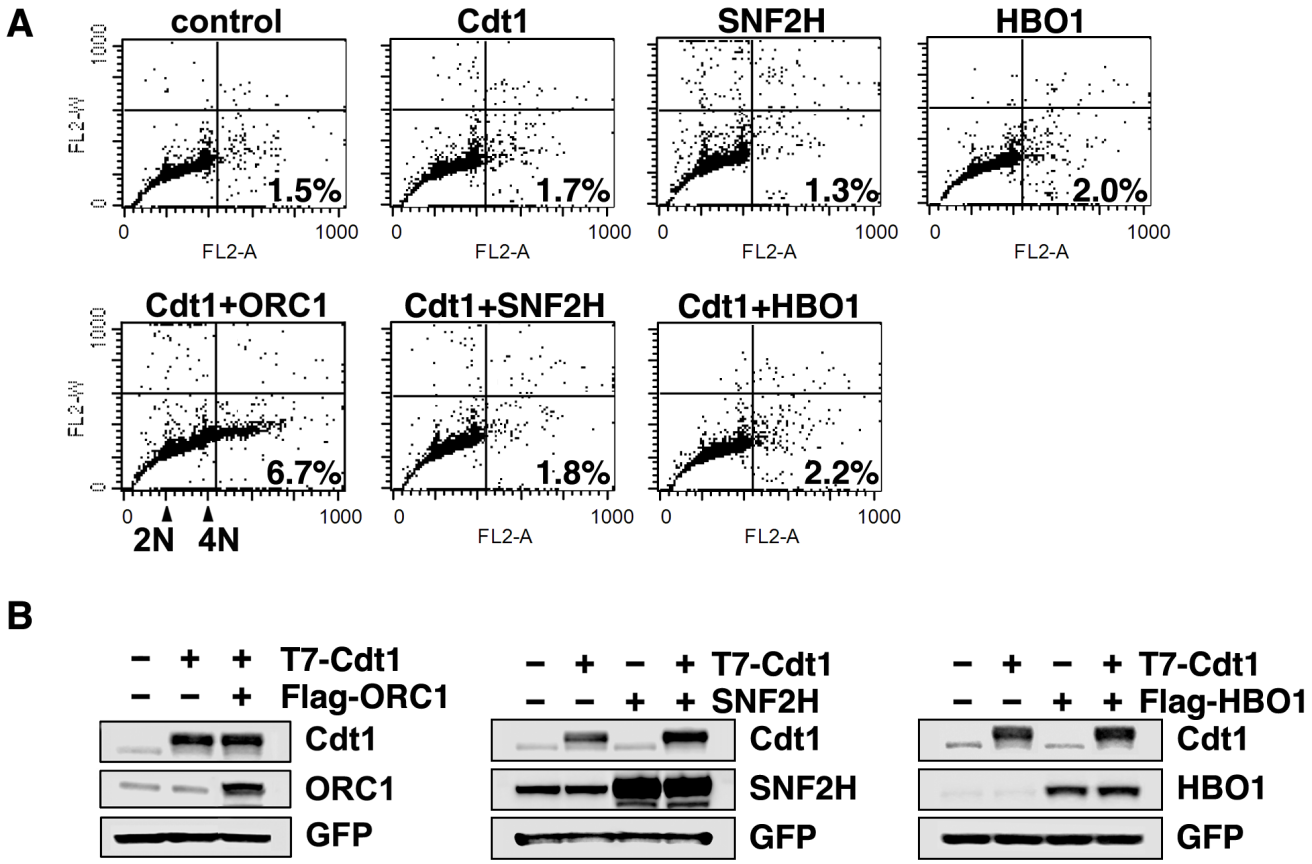

Supplementary Figure S2.
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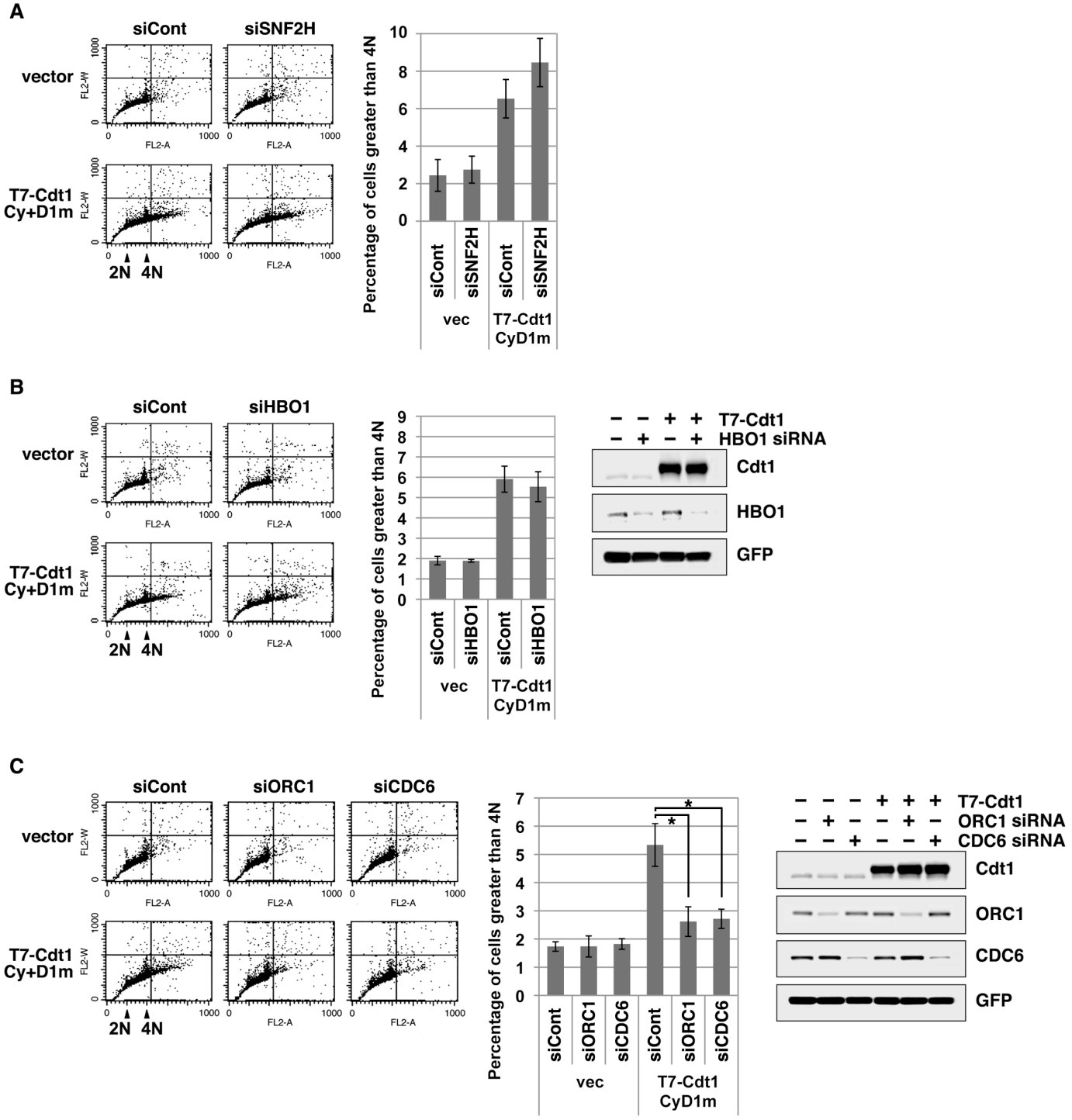

Supplementary Figure S3.
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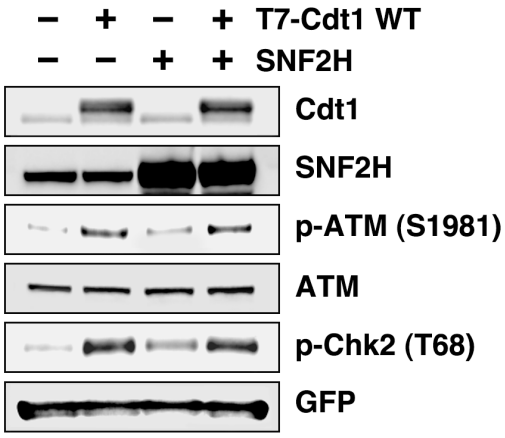

Supplementary Figure S4.
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