

Faustrop et al., <http://www.jcb.org/cgi/content/full/jcb.200807137/DC1>

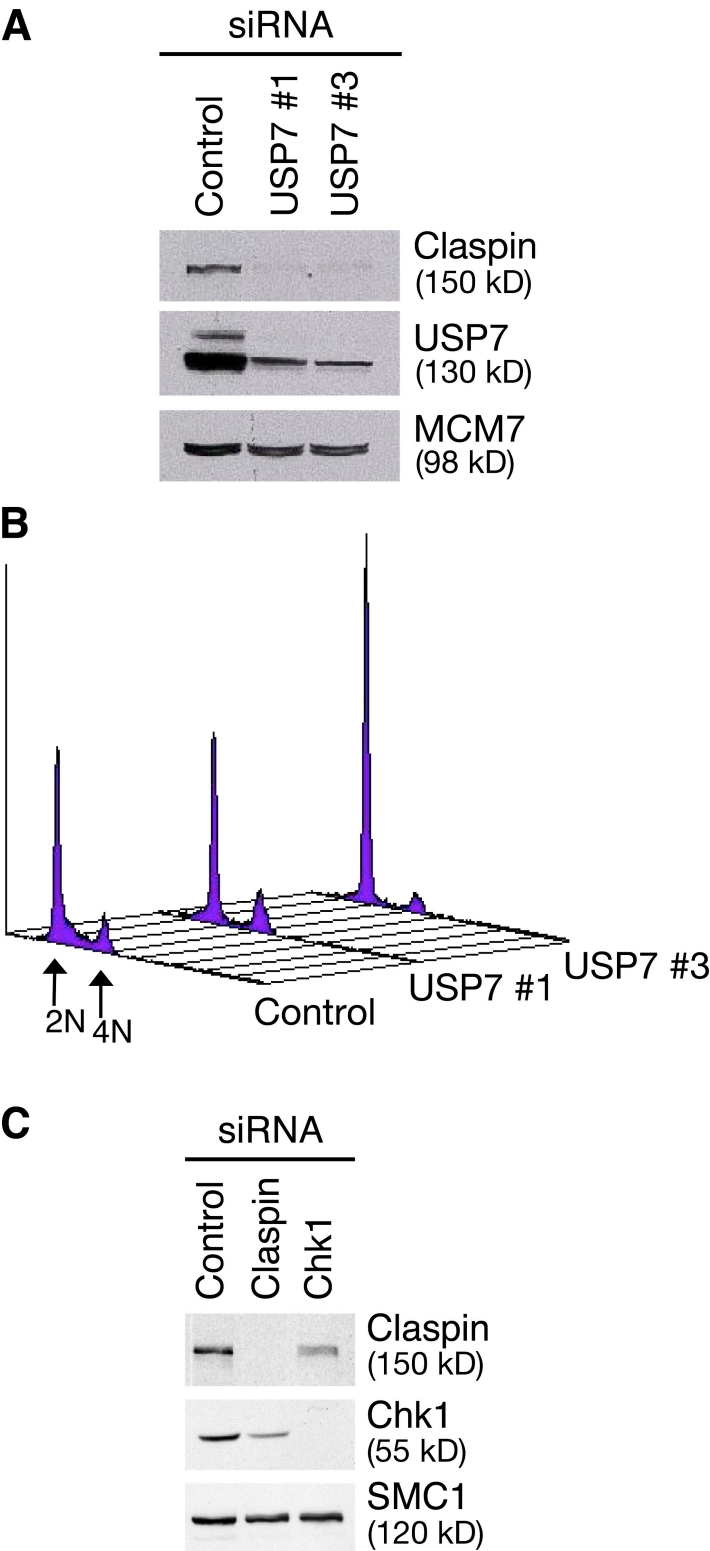


Figure S1. **Effects of USP7, Claspin, and Chk1 siRNAs.** (A and B) siRNA-mediated depletion of USP7 down-regulates Claspin levels. U2OS cells were transfected with control or USP7 siRNAs for 72 h, and total cell extracts were processed for IB of indicated proteins (A) or for flow cytometric analysis of cell cycle distribution (B). (C) Claspin and Chk1 promote the stability of each other. U2OS cells were transfected with indicated siRNAs for 72 h, and the expression levels of Chk1 and Claspin were monitored by IB. SMC1 served as a loading control. siRNA sequences used were: USP7 No. 1 (5'-GGCAACCUUUCAGUUCACUTT-3'), USP7 No. 3 (5'-GGCGAAGUUUUAAAUGUAUTT-3'), and control (Hsp70, 5'-GGGAGGACAAGACGUUCUATT-3'). The Chk1 siRNA has been described previously [Syljuasen, R.G., C.S. Sorensen, L.T. Hansen, K. Fugger, C. Lundin, F. Johansson, T. Helleday, M. Sehested, J. Lukas, and J. Bartek. 2005. *Mol. Cell. Biol.* 25:3553-3562].

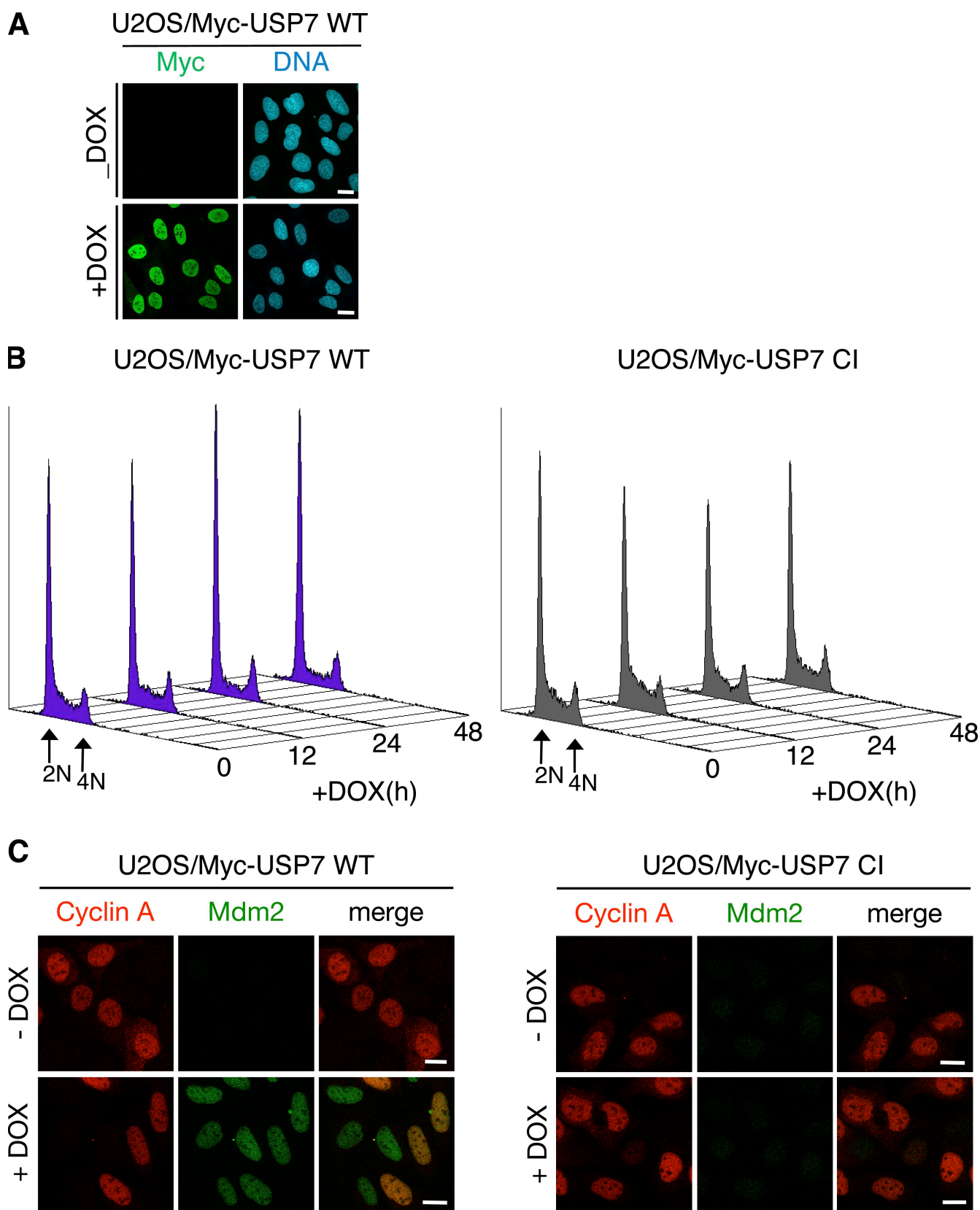
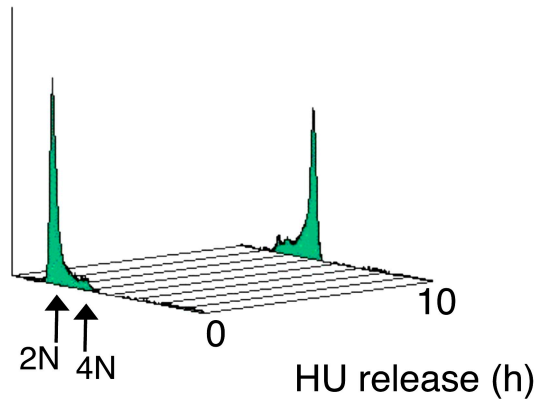


Figure S2. **Characterization of U2OS/Myc-USP7 cell lines.** (A) Conditional U2OS/Myc-USP7 WT cells were left untreated or induced to express the transgene by addition of DOX for 24 h. The cells were then fixed and immunostained with Myc antibody. Bars, 10 μ m. (B) U2OS/Myc-USP7 cell lines were induced with DOX for the indicated times, stained with propidium iodide, and subjected to flow cytometry analysis as described previously (Maidland, N., J. Falck, C. Lukas, R.G. Syljuasen, M. Welcker, J. Bartek, and J. Lukas. 2000. *Science*. 288:1425–1429). (C) Stabilization of Mdm2 in U2OS/Myc-USP7 cells is dependent on the catalytic activity of USP7 but independent of cell cycle stage. U2OS/Myc-USP7 cell lines were left untreated or induced to express the transgenes by the addition of DOX for 24 h. The cells were then fixed and immunostained with Mdm2 and Cyclin A antibodies. Bars, 10 μ m.

U2OS/Myc-USP7 WT

—DOX



U2OS/Myc-USP7 WT

+DOX

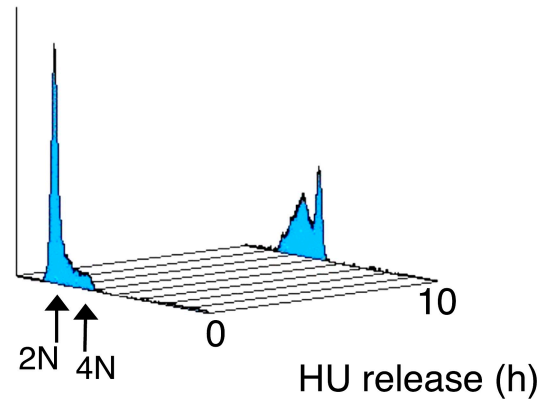


Figure S3. **Representative DNA profiles of the HU release experiment in Fig. 4 B.** U2OS/Myc-USP7 WT cells treated as in Fig. 4 B were processed for flow cytometric analysis of DNA content.