



**Figure S6: Mcm7 is ubiquitylated when CMGs, but not pre-RCs, encircle dsDNA**

**A.** Analysis of Mcm7, Mcm6, and Cdc45 binding during termination. p[*lacOx32*] was incubated with LacR, then replicated until forks converged upon the LacR array (cartoon). IPTG was added at 18 minutes to release the block and induce termination. Chromatin-bound proteins were recovered by plasmid pull-down at 17 minutes, 18.5 minutes, and every 30 seconds thereafter, incubated either with buffer control or Usp21, then blotted for Mcm7, Mcm6, Cdc45, and Histone H3 (H3, loading control). In the mock-treated samples, the band corresponding to unmodified Mcm7 disappeared more rapidly than the Mcm6 and Cdc45 bands (lanes 4-7). In the Usp21-treated samples, the unmodified Mcm7 band disappeared with similar kinetics to the Mcm6 and Cdc45 bands (lanes 16-19). Therefore, disappearance of the unmodified Mcm7 band during termination reflects Mcm7 ubiquitylation.

**B.** Band intensities from (A) and two additional experiments were quantified (see methods). For Mcm7, only the unmodified band was quantified. Means and standard deviations are plotted.

**C.** In parallel to Figure 4E, reactions including radionucleotides were assembled, and the extent of replication was assessed at different time points. In the presence of Cdc7-i, incorporation of radioactivity was abolished (compare lanes 1-3 to lanes 4-6), consistent with Cdc7-i blocking the formation of CMG helicases.