



**Figure S3.** In vitro replication assay using plasmids containing single RFB site. (A) The experiment shown in Fig. 1D was repeated using plasmids containing single RFB site. Plasmids constructed as shown in Supplemental Fig. S2 but only containing single RFB were used. RFB was inserted to face nonpermissive (lanes 1–4: 1xRFB<sup>FW</sup>-plasmid) or permissive (lanes 5–8: 1xRFB<sup>RV</sup>-plasmid) side. In vitro replication assay was performed in the presence of Fob1 (5.6 pmol), Tof1–Csm3 (2.0 pmol), and/or Lacl (6.4 pmol), as indicated above the panel. (B) Using 1xRFB<sup>FW</sup>-plasmid, 1xRFB<sup>RV</sup>-plasmid, or 7xRFB<sup>FW</sup>-plasmid (pHZM88 shown in Supplemental Fig. S2A), in vitro replication assay was performed in the presence of Fob1 (5.6 pmol), Tof1–Csm3 (2.0 pmol), and/or Lacl (6.4 pmol), as indicated above the panel. Single RFB<sup>FW</sup> blocked forks similarly to 7 tandem repeats of RFB<sup>FW</sup>.