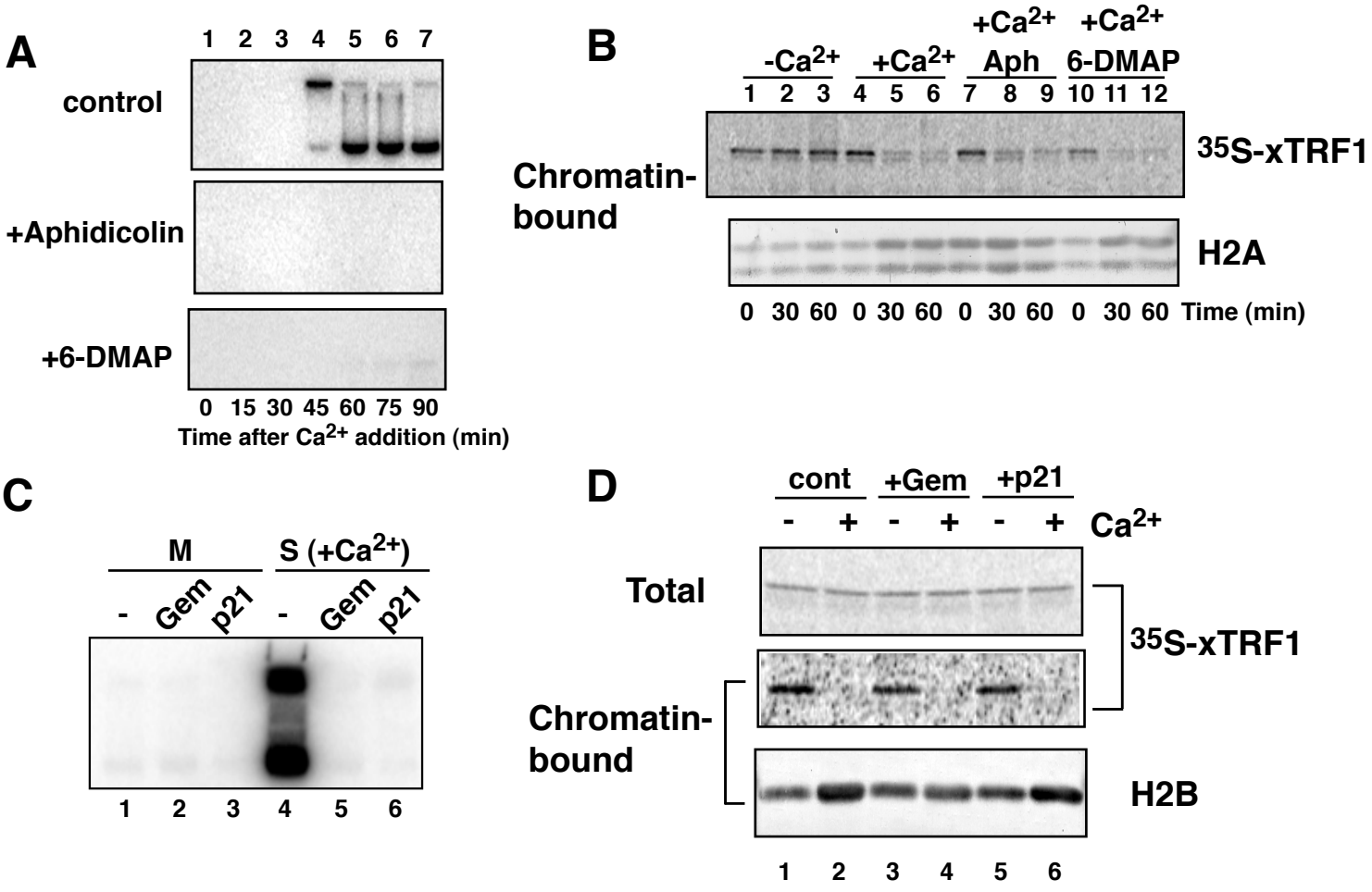

Nishiyama *et al.* Supplementary Figure 3

Supplementary Figure 3

xTRF1 dissociates from chromatin independently of DNA replication

(A) DNA replication in egg extracts was inhibited by aphidicolin or 6-DMAP. Sperm chromatin was added to CSF extracts in the absence (top panel) or presence of aphidicolin (20 μg/ml, middle panel) or 6-dimethylaminopurine (6-DMAP; 2.5 mM, bottom panel) and incubation was carried out at 22˚C for 10 min. After the incubation, Ca^{2+} was added to make a final concentration of 0.6 mM at time 0. Aliquots were removed at the indicated time points and DNA replication in the extracts was analyzed by measuring the incorporation of [α-^{32}P]dATP into sperm DNA. Replication products were subjected to agarose gel electrophoresis, followed by autoradiography.

(B) Aphidicolin or 6-DMAP treatment does not affect the kinetics of xTRF1 dissociation from chromatin in interphase egg extracts. Sperm chromatin was incubated for 10 min with ^{35}S-labeled xTRF1 and CSF extracts with or without 20 μg/ml aphidicolin (Aph) or 2.5 mM 6-DMAP as indicated. Ca^{2+} was added to the extracts to make a final concentration of 0.6 mM at time 0, and chromatin-bound xTRF1 (top panel) and histone H2A (as loading control, bottom panel) were analyzed at the indicated time points (lanes 4-12). As control, chromatin incubated in the absence of Ca^{2+} was also analyzed (lanes 1-3). Note that the amount of xTRF1 bound to chromatin in the 6-DMAP-treated CSF extracts prior to Ca^{2+} addition (lane 10) is smaller than those in mock-treated or aphidicolin-treated CSF extracts (lanes 1, 4 and 7).

(C) Recombinant xGeminin or human p21 inhibits DNA replication of chromatin in interphase egg extracts. CSF extracts were incubated with purified GST-p21 (50 μg/ml, p21) or GST-xGeminin (15 μg/ml, Gem) at 22˚C for 1 hr in the absence (lanes 1-3) or presence (lanes 4-6) of 0.6 mM Ca^{2+}. DNA replication in the extracts was analyzed as described in (A).

(D) xTRF1 dissociates from interphase chromatin in egg extracts treated with xGeminin or human p21. Sperm chromatin was incubated in xGeminin- or human p21-treated CSF extracts containing ^{35}S-labeled xTRF1 with (lanes 2, 4, and 6) or without (lanes 1, 3, and 5) Ca^{2+}. After 1 hr incubation, total extracts (top panel) or chromatin-bound proteins (middle and bottom panels) were analyzed for labeled xTRF1 and histone H2B (as loading control), as indicated.