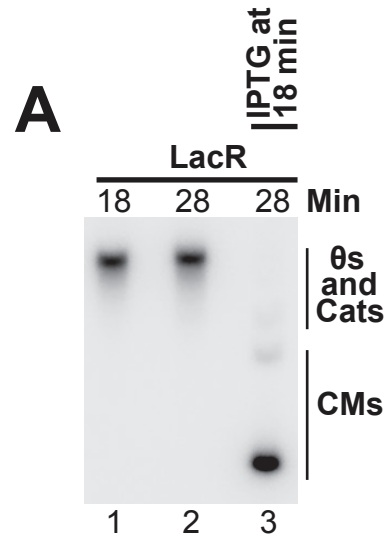
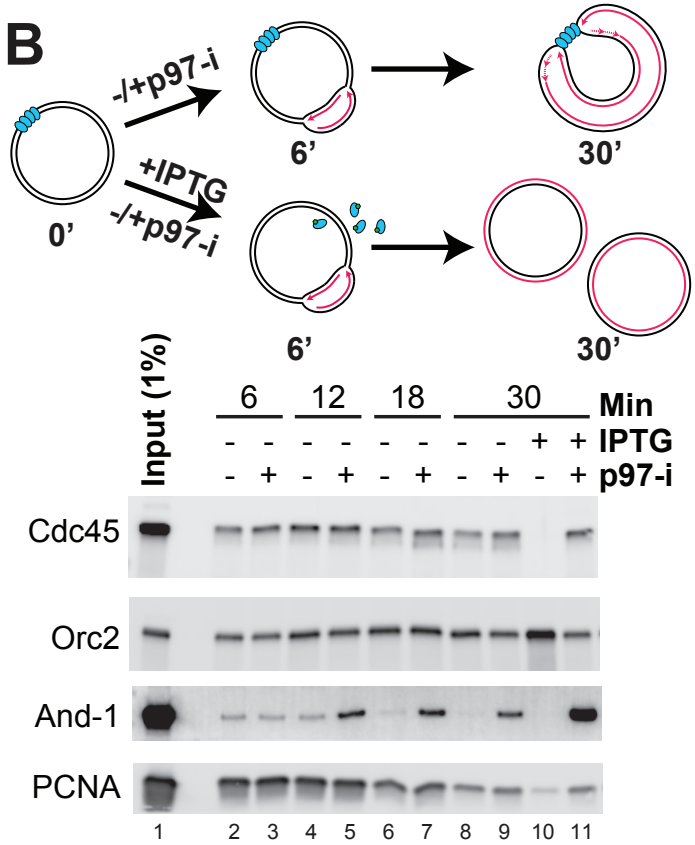
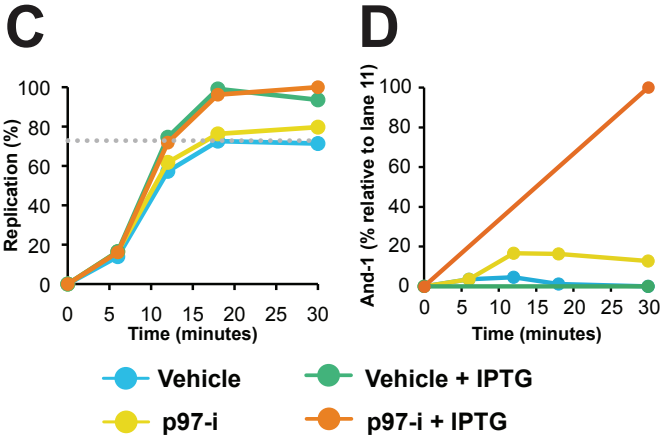
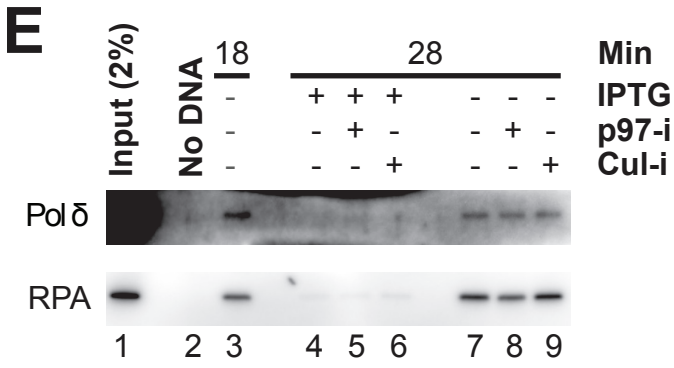
Dewar_FigS7


A
IPTG at 18 min
LacR
18 28 28 Min
θs and Cats
CMs
1 2 3


B
-/+p97-i
+IPTG
-/+p97-i
0'
6'
30'
6'
30'
Input (1%)
6 12 18 30 Min
IPTG
p97-i
Cdc45
Orc2
And-1
PCNA
1 2 3 4 5 6 7 8 9 10 11


C
Replication (%)
Time (minutes)
Vehicle
Vehicle + IPTG
p97-i
p97-i + IPTG
D
And-1 (% relative to lane 11)
Time (minutes)


E
Input (2%)
No DNA
18 28 Min
IPTG
p97-i
Cul-i
Pol δ
RPA
1 2 3 4 5 6 7 8 9

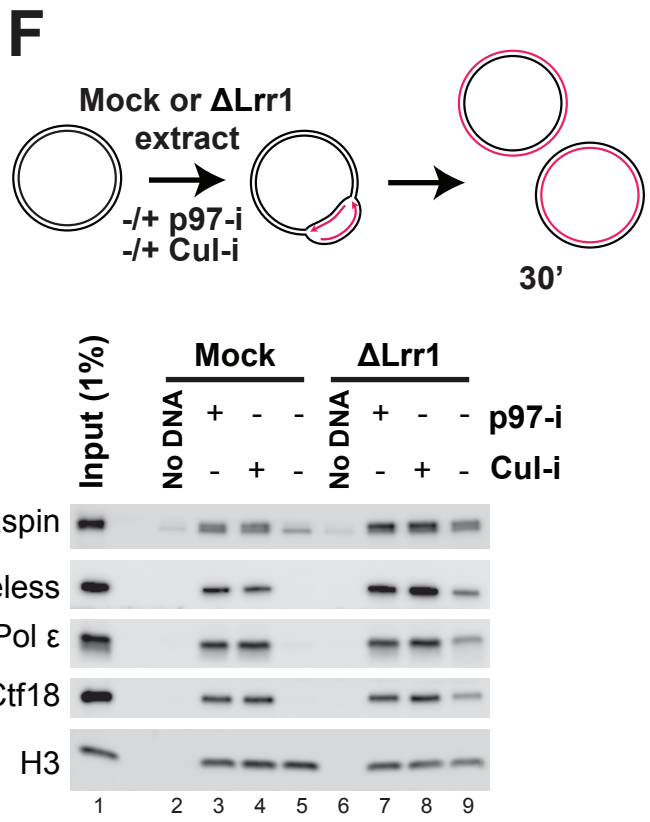

F
Mock or ΔLrr1 extract
-/+ p97-i
-/+ Cul-i
30'
Input (1%)
Mock
ΔLrr1
No DNA
p97-i
Cul-i
Claspin
Timeless
Pol ε
Ctf18
H3
1 2 3 4 5 6 7 8 9

Figure S7: a subassembly of replication proteins is retained on chromatin when CMG unloading is blocked

A. In parallel to Figure 5A-B, reactions including radionucleotides were assembled, and at different time points the DNA structures were analyzed by agarose gel electrophoresis. At 18 and 28 minutes, θ structures were detected (Lanes 1 and 2), which arise from converging forks. Following IPTG addition, only circular monomers were detected at 28 minutes (Lane 3), which are the final products of replication. Therefore, the LacR array robustly arrests forks for 28 minutes, while addition of IPTG at 18 minutes robustly and completely induces termination.

B. Analysis of protein binding during replication of a LacR array. p[*lacO*x32] was incubated with LacR then replicated in the presence or absence of p97-i and/or IPTG, as indicated. Chromatin-bound proteins were recovered by plasmid pull-down at the indicated time points and blotted for Cdc45, Orc2 (loading control), And-1, and PCNA. In the absence of IPTG (lanes 2-9), And-1 binding was low and did not mirror Cdc45 binding, which suggested that And-1 did not stably bind CMG. p97-i dramatically induced And-1 binding, but only when IPTG was added to induce termination (Lane 9 vs. 11). Therefore, And-1 binding in the presence of p97-i is termination-dependent (as indicated in Figure 5B) and not a consequence of And-1 loss from arrested forks. The low level of And-1 binding induced by p97-i in the absence of IPTG (lanes 5, 7, 9) likely reflects termination events on a small number of plasmids where origins fire (as depicted in Figure S2M).

PCNA binding was also measured. PCNA binding was high at early time points, and the majority of PCNA signal was lost by 30 minutes, irrespective of IPTG treatment (Lane 8 and 10 vs. lane 2). In the presence of IPTG, PCNA binding was reduced at 30 minutes (Lane 10 vs. lane 8) but this was blocked by p97-i (Lane 10 vs. lane 11). Therefore, the majority of PCNA

unloading occurs independently of termination but unloading of a small fraction of PCNA is termination- and p97-dependent.

C. In parallel to (B), reactions including radionucleotides were assembled, and the extent of replication was quantified at different time points as in Figure S2N.

D. Quantification of And-1 signal from (B). Quantification is relative to lane 11, which is assigned a value of 100.

E. The same samples in Figure 5A-B were also blotted for DNA Pol δ and RPA.

F. The samples described in Figure 3C were blotted for Timeless, Claspin, Ctf18, Pol ε, and Histone H3 (H3, loading control).