Supplementary Figure 1. Example DNA fiber spreads.
Representative images of replication tracks from cells pulse labeled with 25μM BrdU for 20 minutes (green track) followed by 250μM IdU for 20mins (red tracks) then prepared for DNA fiber spreads. Continuous red-green labeling patterns are classed as ongoing replication events that can be further subdivided into progressing forks, bidirectional forks and terminal fusions. Green only tracks represent forks labeled during the first pulse only and are classed as fork stalling events. Red only tracks represent origins that fired during the second label and interspersed patterns representative of closely spaced active origins are also interpreted as origin firing events.

Supplementary Figure 2. Similar increased origin firing and fork stalling phenotypes observed in response to independent siRNAs against Chk1 and Claspin.
(A) HeLa cells were transfected with control siRNA (siCon), or siRNA oligonucleotides designed to target independent sequences in Chk1 (siChk1 and siChk1-2) or Claspin (siClasp and siClasp-2). Cells were transfected twice, 24 hours apart. Forty-eight hours following initial transfection, levels of Chk1 and Claspin protein were monitored using the relevant antibodies. Ku80 was used to verify equal loading. (B) HeLa cells transfected with the indicated siRNA were pulsed with 25μM BrdU for 10 minutes followed by 250μM IdU for 20 minutes before DNA fiber spreads were prepared. Origin firing was calculated as above. (C) Fork stalling was calculated as described above. Graphs represents average fraction of origin firing or fork stalling measured from three independent experiments; at least 100 replication tracks were counted in each experiment. Error bars represent standard error of the mean.

Supplementary Figure 3. Examples of replication events scored in presence and absence of aphidicolin.
(A) Representative images of ongoing replication in HeLa cells transfected with the indicated siRNA labeled with 25μM BrdU for 10 minutes followed by 250μM IdU for 40 minutes. (B) Examples of ongoing replication forks (left panel) and stalled replication forks (right panel) in siRNA-treated cells labeled with 25μM BrdU for 10 minutes followed by aphidicolin and IdU for 40 minutes.

Supplementary Figure 4. Increased origin firing in siChk1, but not Claspin-siRNA, cells upon release from a replication block.
HeLa cells transfected with the indicated siRNA were labeled with 25μM BrdU for 10 minutes followed by HU (A) or aphidicolin (B) for 0, 2 or 6 hours. Aphidicolin or HU was then washed off and cells were labeled with 250μM IdU for 20 minutes before DNA fiber spreads were prepared. The fraction of origin firing was determined as described in Figure 2. Graphs show data measured from three independent experiments; at least 100 replication tracks were counted in each experiment. Error bars represent standard error of the mean.