Supporting Information

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SI Materials and Methods

Cell Culture. The human colon cancer cell line DLD-1 and derivatives were cultured in McCoy's 5A medium supplemented with 6% FCS and penicillin/streptomycin (Invitrogen). The human lung cancer cell line H1299 was cultured in RPMI medium 1640 supplemented with 10% FCS. 293 cells were cultured in DMEM supplemented with 10% FCS and penicillin/streptomycin.

Expression of Epitope-Tagged Chk1 Proteins. Chk1 proteins containing the V5-His tag (Chk1-V5) were expressed from pcDNA3.1/GS-Chk1-V5-His (Invitrogen). Point mutations in pChk1-S317A-V5 and pChk1-S345A-V5 were introduced by site-directed mutagenesis with the Quickchange kit (Stratagene). Transient transfections were performed with LipofectAMINE (Invitrogen) according to manufacturer's protocols. At 48 h after transfection, cells were treated as indicated and harvested in NuPage lysis buffer (Invitrogen).

Antibodies and Immunoblotting. Whole-cell lysates were denatured and fractionated on NuPAGE gels (Invitrogen). Proteins were transferred to PVDF membranes (Millipore) and then incubated with antibodies directed against Chk1, α-tubulin, Cdc25A, Cdk1, Cyclin B1 (Santa Cruz Biotechnology), V5 (Invitrogen), Chk1S296-P, Chk1S317-P, and Chk1S345-P (Cell Signaling Technologies) under conditions recommended by the manufacturers. Blots were developed using enhanced chemiluminescence (Amersham).

Cell Cycle Analysis. HU treatment and cell cycle analysis was performed as described previously (1). The status of the G_2/M checkpoint was assessed using a nocodazole trap (2).

DNA Combing. Subconfluent cell cultures were preincubated with the Chk1 inhibitor 500 nM CEP-3891 or equal volumes of DMSO (control) for 1 h. Cells were then sequentially pulse labeled with 25 μM CIdU and 250 μM IdU for 20 min. Labeled cells were harvested and DNA fiber spreads prepared as previously described (3). For immunodetection of CIdU-labeled tracts, acid-treated fiber spreads were incubated with a rat anti-BrdU monoclonal antibody that recognizes CIdU but not IdU (Oxford Biotechnology) at 1:1,000 dilution for 1 h at room temperature. Slides were then fixed with 4% paraformaldehyde and incubated with an AlexaFluor 555-conjugated goat anti-rat IgG (Molecular Probes) at 4 μg/ml for 1.5 h at room temperature. To detect IdU-labeled patches, a mouse anti-BrdU monoclonal antibody that recognizes IdU but not CIdU (Becton Dickinson) was used at 17 ng/ml overnight at 4 °C, followed by an AlexaFluor 488-conjugated goat anti-mouse F(ab′)$_2$ fragment (Molecular Probes) at 4 μg/ml for 1.5 h at room temperature. Fibers were examined using a Leica SP2 confocal microscope using a 63× (1.3 NA) glycerol immersion objective. The lengths of red (AF 555)- or green (AF 488)-labeled patches were measured using the ImageJ software (National Institutes of Health), and arbitrary length values were converted into micrometers using the scale bars created by the microscope. At least 125 replication tracks were measured per experiment. For quantification of stalled forks and origins, at least 250 replication structures were counted per experiment.

Immunofluorescence. Subconfluent cell cultures growing on chamber slides (Nunc) were fixed in 3% paraformaldehyde, permeabilized with 0.5% Triton X-100, and incubated with primary antibodies overnight. For Chk1S345-P staining, cells were incubated with a phospho-specific rabbit monoclonal antibody (clone 133D3; Cell Signaling Technologies) at a dilution of 1:300. A secondary biotin-conjugated anti-rabbit IgG antibody (Sigma) was then applied at a concentration of 0.75 ng/ml and visualized by 2 ng/ml fluorescein-conjugated avidin (Vector Laboratories). Centrosomes were detected by direct fluorescence using a CY3-conjugated anti-γ-tubulin antibody (Sigma). Images were captured on a Nikon E800 fluorescence microscope with a 60× oil immersion Plan Apo objective (1.4 aperature) and a 5 MHz interline CCD camera (Princeton Instruments) using Metamorph imaging software (version 6.0).

1. Wilsker D, Bunz F (2007) Loss of ataxia telangiectasia mutated- and Rad3-related function potentiates the effects of chemotherapeutic drugs on cancer cell survival. *Mol Cancer Ther* 6:1406–1413.
2. Bunz F, *et al.* (1998) Requirement for p53 and p21 to sustain G2 arrest after DNA damage. *Science* 282:1497–1501.
3. Henry-Mowatt J, *et al.* (2003) XRCC3 and Rad51 modulate replication fork progression on damaged vertebrate chromosomes. *Mol Cell* 11:1109–1117.

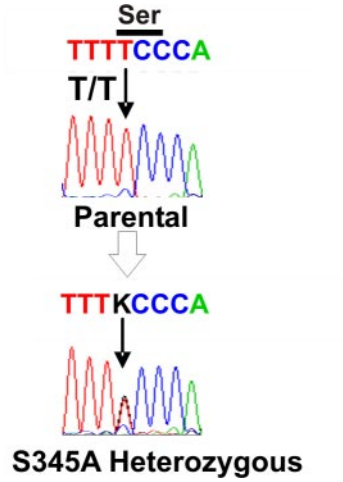

Fig. S1. Derivation of Chk1 S345A heterozygous cells from DLD-1. Homologous integration of knockin vector pAAV-Chk1S345A was assessed by PCR. Heterozygous expression of Chk1 in expanded clones was confirmed by DNA sequence analysis of RT-PCR products at nucleotide position 1212 (NM_001274).

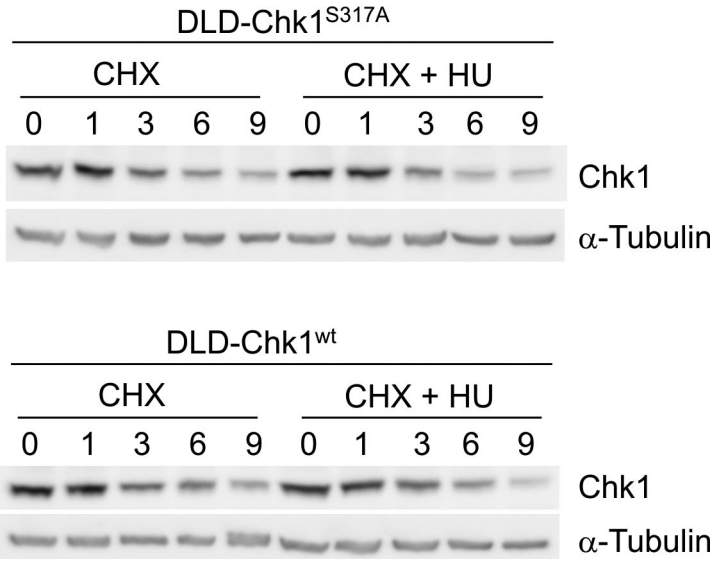

Fig. S2. Stability of the Chk1 S317A mutant protein. DLD-Chk1^{WT} and DLD-Chk1^{S317A} cells were pretreated with the protein synthesis inhibitor cycloheximide (100 μg/ml) for 15 min. Cells were either treated with 0.5 mM hydroxyurea in the presence of cycloheximide, or with cycloheximide only for the times indicated. Cell lysates were harvested and probed with an antibody to total Chk1 protein.

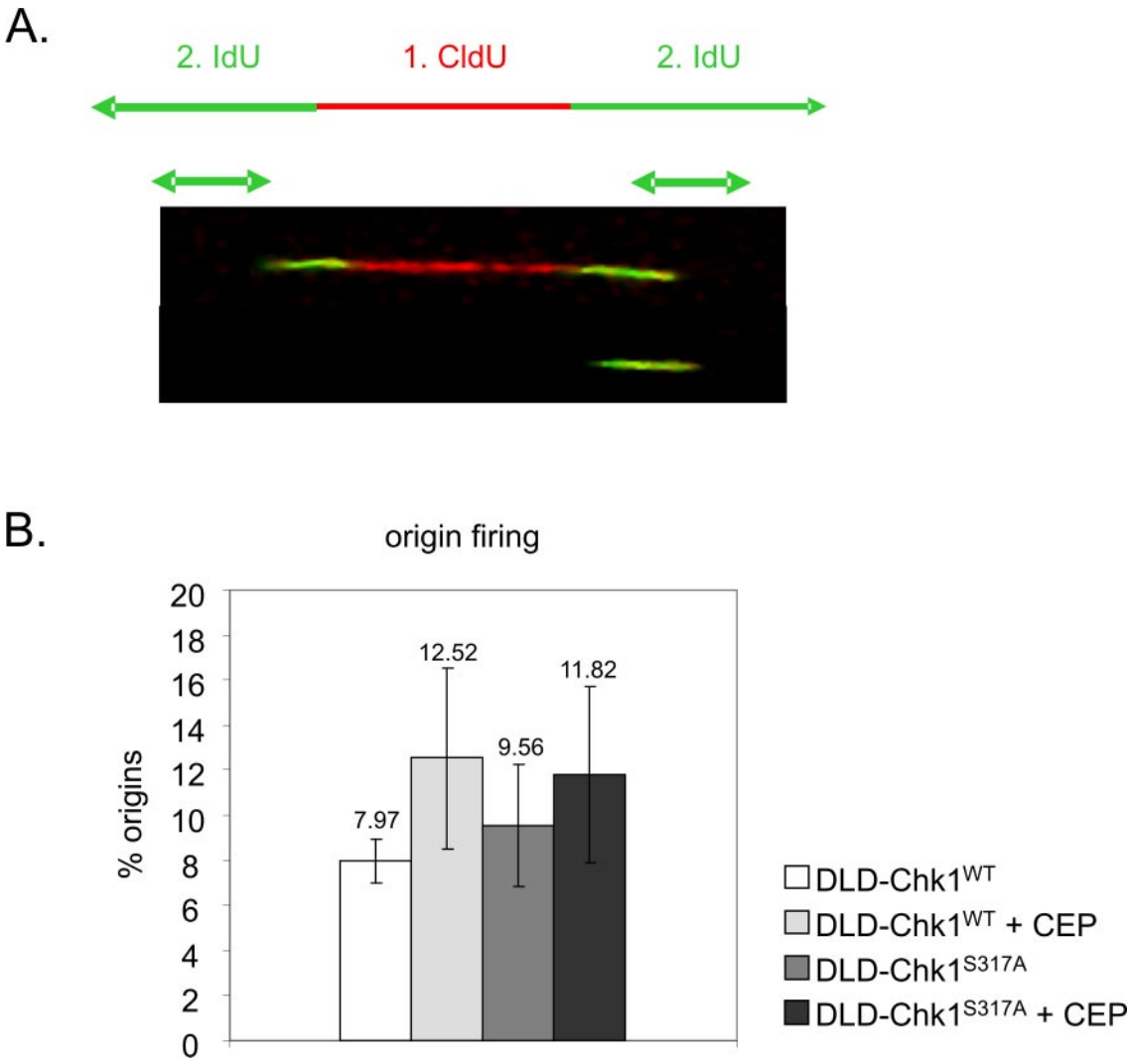

Fig. S3. Origin firing density in DLD-Chk1^{WT} and DLD-Chk1^{S317A} cells. (*A*) The rate of origin firing was determined by quantitation of CldU labeled fibers with flanking IdU signals. (*B*) Average rates of origin firing were determined in the presence and absence of the Chk1 inhibitor CEP-3891 (CEP).

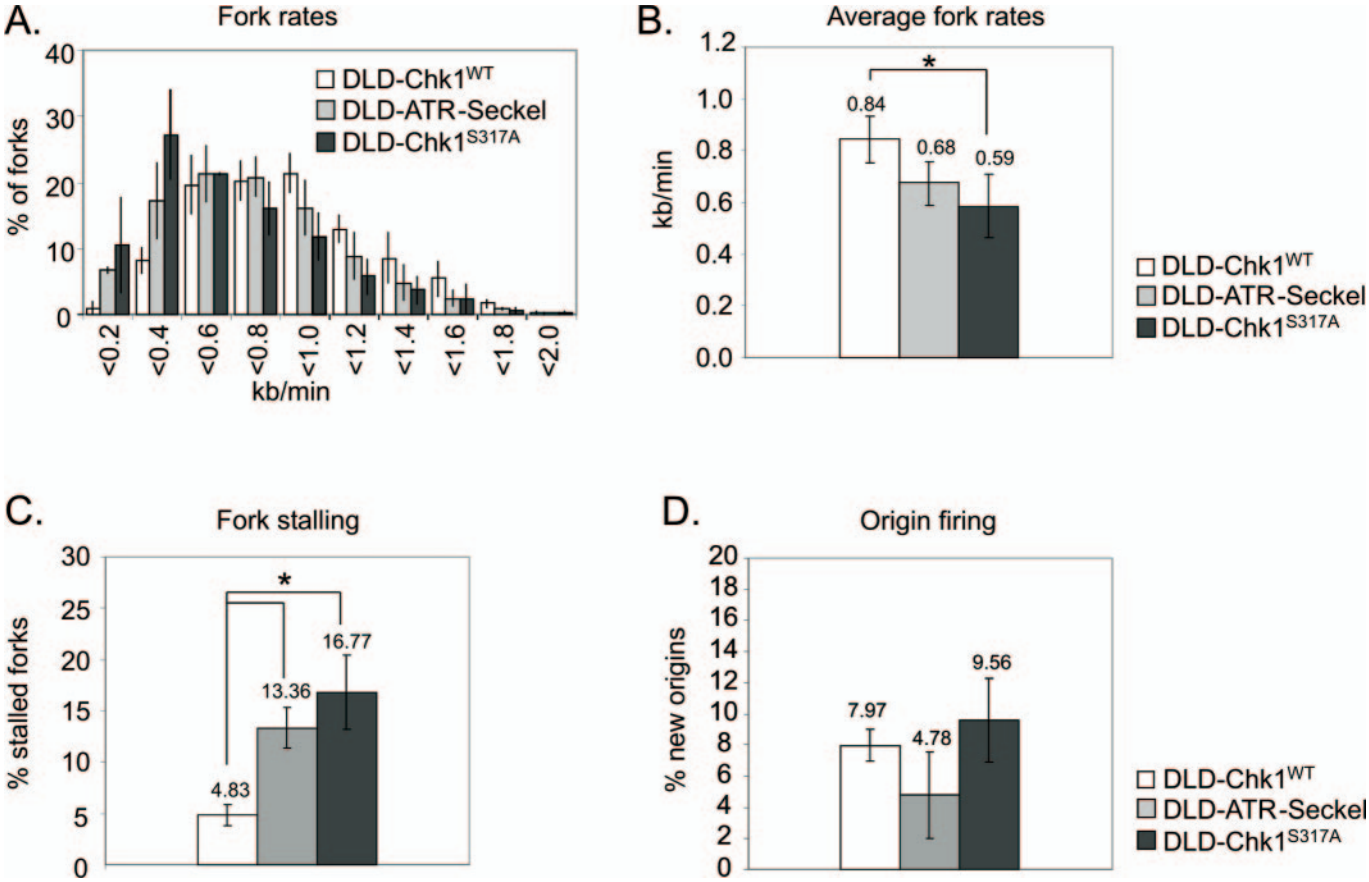

Fig. S4. ATR and Chk1 S317 are required for proper replication fork progression. (*A*) The rate of bidirectional origin firing was determined by quantitation of CldU-labeled fibers with flanking IdU signals. (*B*) Average fork rates in DLD-Chk1^{WT}, DLD-ATR-Seckel, and DLD-Chk1^{S317A} cells. Values connected by lines are significantly different (Student's *t* test, $P < 0.05$). (*C*) Stalled forks as percentage of all CldU-labeled tracks. Values connected by lines are significantly different ($P < 0.05$). The means and standard deviations of three independent repeats are shown. (*D*) The rates of origin firing were determined by quantitation of CldU fibers with flanking IdU signals. The average rates are presented.