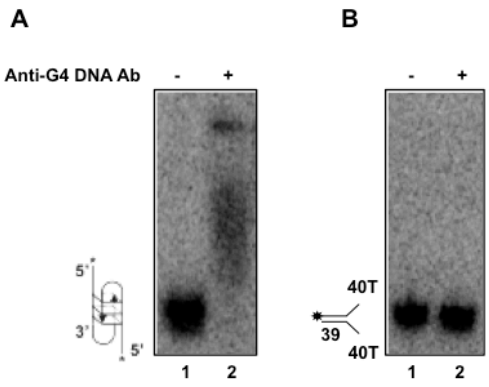
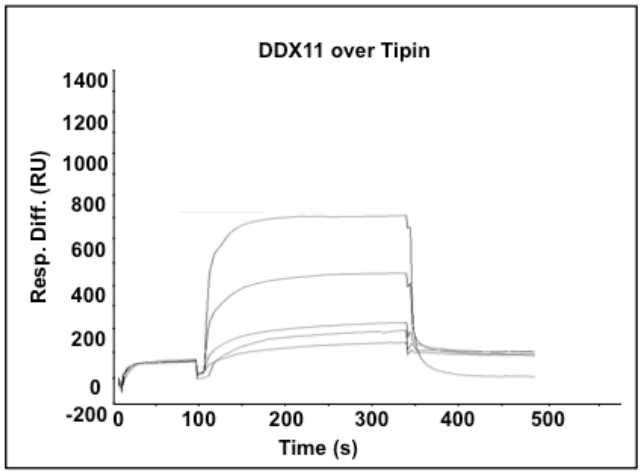
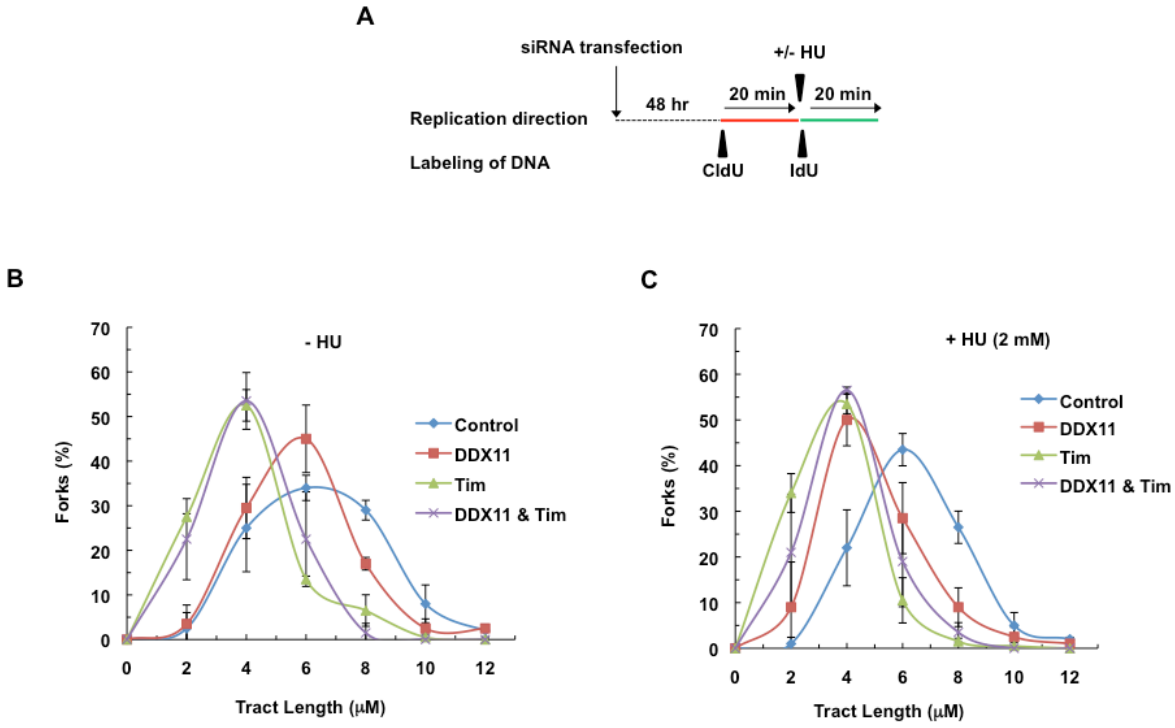


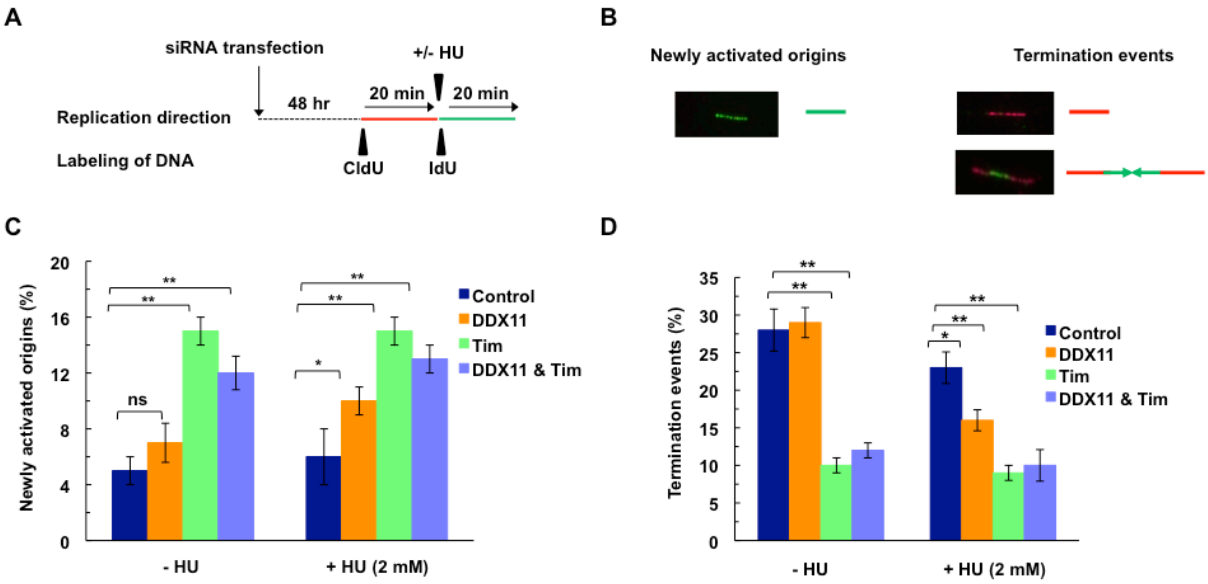
Supplementary Figure 1



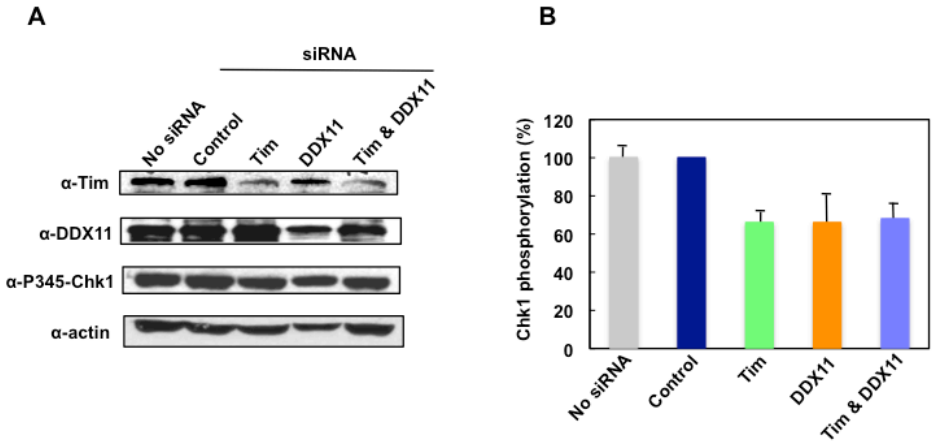
Supplementary Figure 2



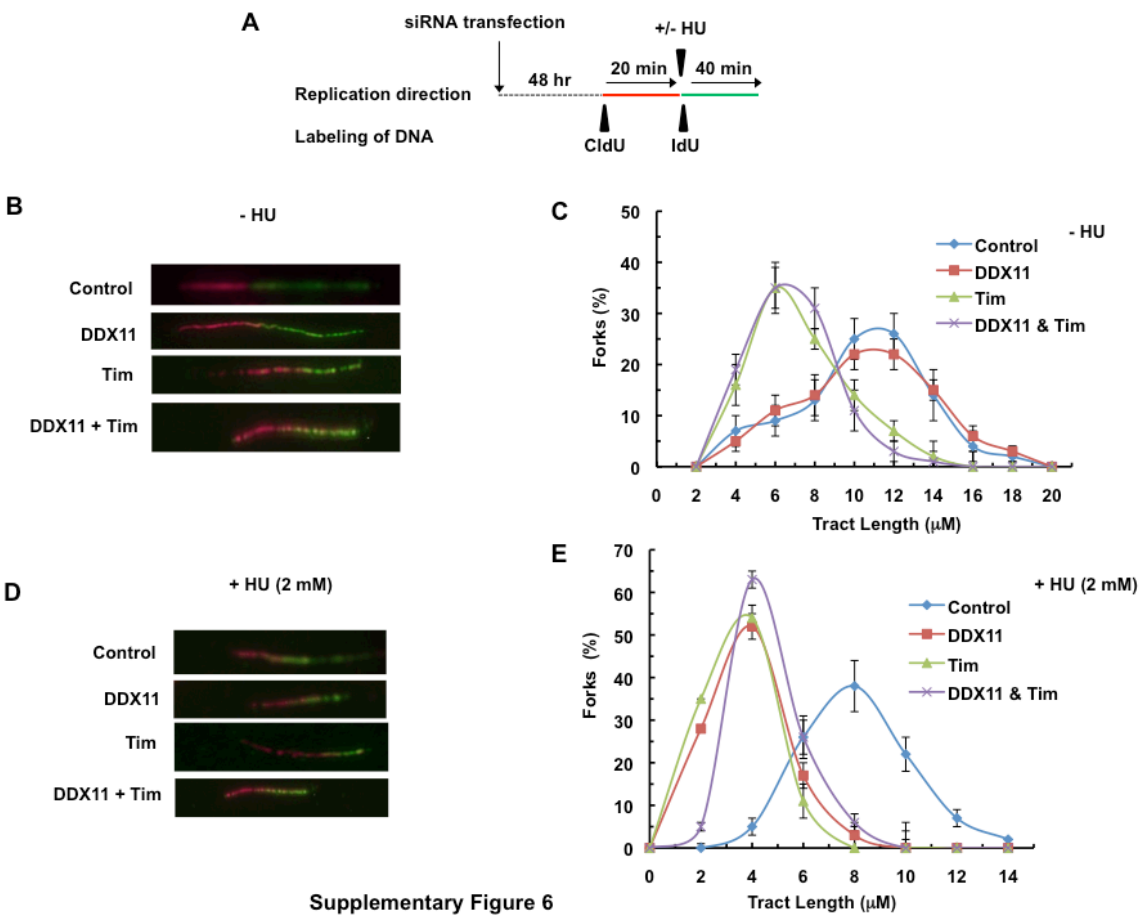
Supplementary Figure 3



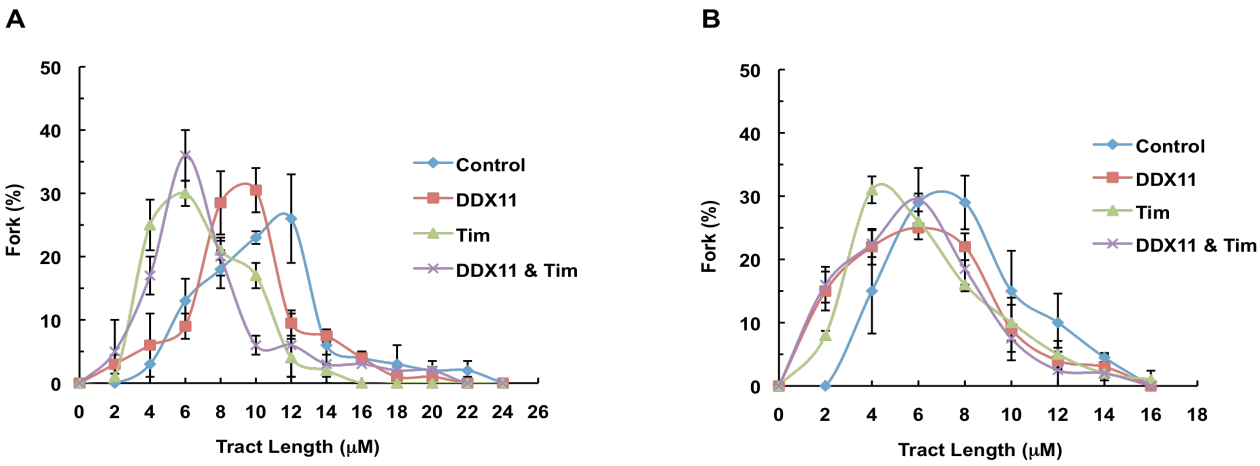
Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7

Supplementary Figure legends

Supplementary Figure 1. OX-1-G2' DNA substrate contains a G-quadruplex DNA structure. Mixtures (volume: 10 µl) were prepared that contained anti-parallel bimolecular G-quadruplex DNA (OX-1-G2'; 87.5 ng, in *Panel A*) or forked DNA substrate (20 fmol, in *Panel B*) and no protein (*lane 1*) or anti-G4 DNA single chain BG4 antibody (20 fmol, *lane 2*), described by Biffi *et al.* (54). Following incubation for 15 min at 25 °C, complexes were separated by electrophoresis through 5% polyacrylamide/bis gels (37.5:1) in 0.5 X TBE containing 10 mM KCl.

Supplementary Figure 2. Dynamic interaction of DDX11 with Tipin. Surface plasmon resonance measurements were carried out to analyze the interaction of DDX11 with Tipin using a Biacore2000 instrument. Tipin (6000 RU) was immobilized on a CM5 sensor chip in 10 mM sodium acetate buffer pH 3.5, according to the manufacturer's instructions. To collect sensorgrams five increasing concentration values of human purified recombinant DDX11 (from 0 to 80 nM) in PBS buffer (50 mM sodium phosphate pH 7.5, 150 mM NaCl) were fluxed over the sensor chip surface at a flow rate of 20 µl/min. Recorded sensorgrams were normalized to a baseline of 0 RU and a dissociation constant (K_D = 6.55 x 10^{-7} M) value was calculated using the BIA Evaluation software.

Supplementary Figure 3. DNA fiber track assays on Tim- and/or DDX11-depleted HeLa cells. *A*) Schematic representation of the protocol used to track DNA fiber replication. Labeling of cells were done 48 hours after transfection of siRNAs (Tim #1 and/or DDX11 #2). First, cells were pulse-chased with CldU (*red label*); then, they were labeled with IdU (*green label*) for the indicated times. *B*) and *C*) Distribution of replication fork tract measured by CldU tract length in a DNA fiber labeled with CldU and IdU in cells transfected with the indicated siRNAs without and with HU treatment, respectively**.** CldU tract length represents mean of at least three experiments with SD and analysis is based on 150-250 DNA fibers from each experiment. CldU mean track length for the cells treated with indicated siRNA in the absence of HU are 6.4 ± 0.15 µM (control), 5.0 ± 0.29 µM (DDX11), 3.0 ± 0.11 µM (Tim), 3.1 ± 0.57 µM (Tim + DDX11); whereas, in the presence of HU, the mean track length are 5.0 ± 0.41 µM (control), 4.2 ± 0.81 µM (DDX11), 2.7 ± 0.22 µM (Tim), 3.1 ± 0.49 µM (Tim + DDX11).

Supplementary Figure 4. Frequency of newly fired origins and termination events in Tim- and/or DDX11-depleted HeLa cells. *A*) Schematic representation of the protocol used to track DNA fiber replication. *B*) Representatives of fluorescently-labeled DNA fibers from HeLa cells accompanied with a schematic of the corresponding replication events. Since no fork pausing is evident under the indicated treatment, fibers showing CldU-only labeling represent termination and not fork stalling events. *C*) Frequency of newly activated replication origins calculated from number of IdU tract (*green labe*l) only and *D*) frequency of termination events based on the CldU (*red*) and CldU-IdU-CldU (*red-green-red*) tract in HeLa cells transfected with siRNAs (Tim #1 and/or DDX11 #2), as described in *Material and Methods* in the main text. Data represent mean of at least three experiments with SD and analysis is based on 150-250 DNA fibers from each experiment. * = P <0.05, ** = P <0.001 (Student's *t*-test), *ns* = not significant.

Supplementary Figure 5. *A*) Western blot analysis of Chk1 phosphorylation at Serine 345 in Tim- and/or DDX11-depleted HeLa cells. HeLa cells were transfected with the indicated siRNAs (Tim #2 and/or DDX11 #5), as described in the main text. After 48 hours, cells were treated with HU at 2 mM for 20 min. Whole cell extract was prepared using lysis buffer (10 mM Hepes-NaOH, pH 7.5, 150 mM NaCl, 1 mM dithiotreitol, 0.1% Triton X-100, 0.5 mM phenylmethylsulfonyl fluoride, protease and phosphatase inhibitors cocktails from Roche). Aliquots (10 μg of total protein/lane) were subjected to electrophoresis on a 10% polyacrylamide/bis gel (19:1) containing SDS. The gel was electro-transferred onto a membrane and analyzed by immuno-blot using the indicated antibodies. *B*) Densities of phosphorylated Chk1 band were quantified using the computer software Image J and data are expressed as percentages of the sample treated with scrambled siRNA (named *Control*). Mean values and standard deviations are calculated from three independent experiments.

Supplementary Figure 6. DNA fiber track assays on Tim- and/or DDX11-depleted HeLa cell after 40 min incubation with IdU. *A*) Schematic representation of the protocol used to track DNA fiber replication. Labeling of cells were done 48 hours after transfection of siRNAs (Tim #2 and/or DDX11 #5). First, cells were pulse-chased with CldU (*red label*); then, they were labeled with IdU (*green label*) for the indicated times. *B*) and *D*) Representatives of fluorescently-labeled DNA fibers from cells treated with the indicated siRNAs, as described in the main text, without and with HU treatment, respectively. *C*) and *E*) Distribution of replication fork tract measured by IdU tract length of cells transfected the indicated siRNAs, as described in the main text, in a DNA fiber labeled with CldU and IdU, without and with HU treatment, respectively**.** IdU tract length represents mean of at least three experiments with SD and analysis is based on 150-250 DNA fibers from each experiment. IdU mean track length for the cells treated with indicated siRNA in the absence of HU are 11.6 ± 0.4 μM (control), 10.6 ± 0.85 μM (DDX11), 6.3 ± 0.28 μM (Tim), 6.1 ± 0.35 μM (Tim + DDX11); whereas, in the presence of HU, the mean track length are 7.9 ± 0.11 μM (control), 4.9 ± 0.97 μM (DDX11), 4.7 ± 0.28 μM (Tim), 3.8 ± 0.36 (Tim + DDX11).

Supplementary Figure 7. IdU track length during replication fork recovery after HU treatment. 48 hours after transfection of siRNAs (Tim #2 and/or DDX11 #5), HeLa cells were labeled for 30 min with CldU (*red label*); then they were treated with (or without) HU at 2 mM for 14 hours and then labeled for 60 min with IdU (*green label*). Distribution of replication fork tract measured by IdU tract length in cells transfected with the indicated siRNAs, as described in the main text, in a DNA fiber labeled with CldU and IdU, without (*A*) and with (*B*) HU treatment, respectively**.** IdU tract length represents mean of at least three experiments with SD and analysis is based on 50-100 DNA fibers from each experiment. IdU mean track length for the cells treated with indicated siRNA in the absence of HU treatment are 11.2 ± 2 μM (control), 8.7 ± 1.2 μM (DDX11), 5.7 ± 0.5 μM (Tim), 5.7 ± 1 μM (Tim + DDX11); whereas, in the presence of HU treatment, the mean track length are 7.6 ± 0.5 μM (control), 5.6 ± 0.4 μM (DDX11), 5.1 ± 0.8 μM (Tim), 5.6 ± 0.2 μM (Tim + DDX11).

SUPPLEMENTARY REFERENCES

54. Biffi, G., Tannahill, D., McCafferty, J. and Balasubramanian, S. (2013) Quantitative visualization of DNA G-quadruplex structures in human cells. Nat. Chem., 5,182-186.