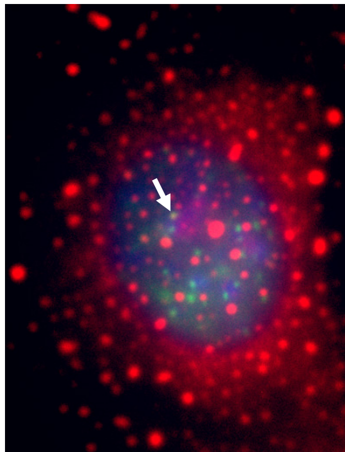
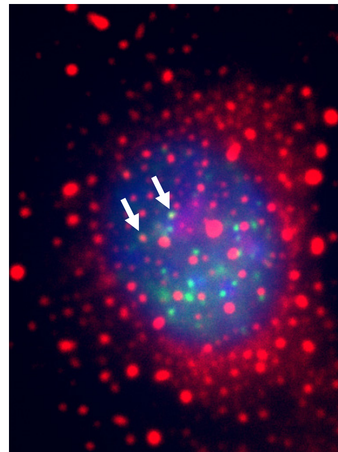


Stack 1



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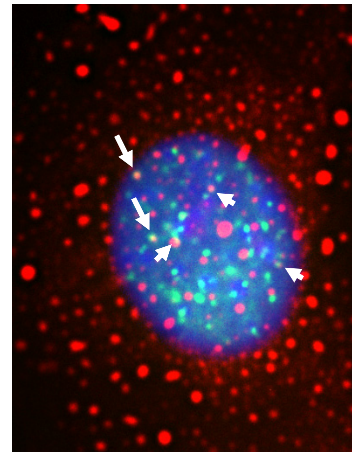

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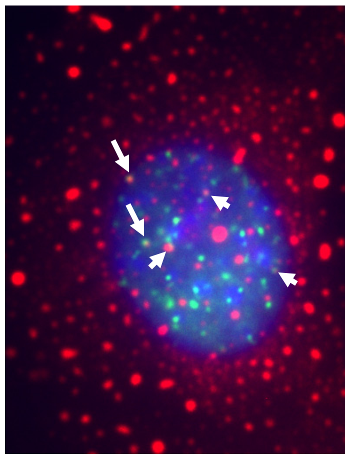
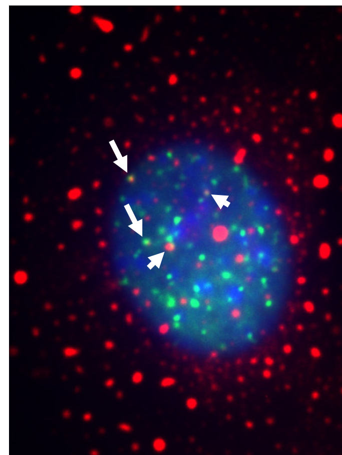
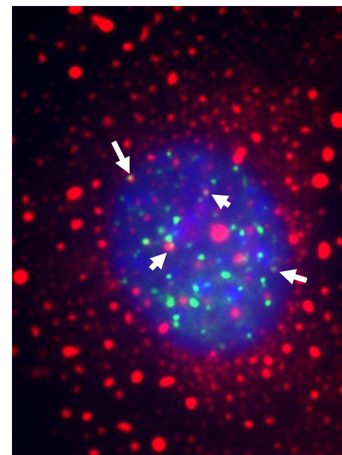


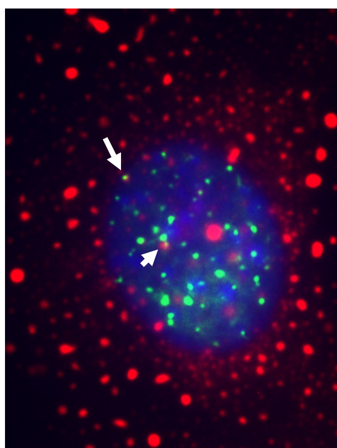
Stack 4



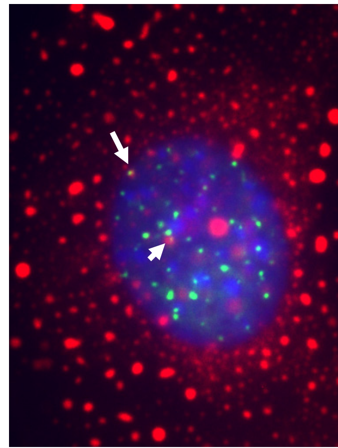
Stack 5



Stack 6



Stack 7



Stack 8

Figure S1. Co-localization of DNA2 with telomeres is not due to random overlap of signals. Various Z-stack images of Fig. 1F are shown. Images were taken at 0.275μM per slice. In each stack, co-localization is indicated by white arrows. The same co-localization signals are observed in various stacks, indicating that it is not due to random overlap of red and green signals.

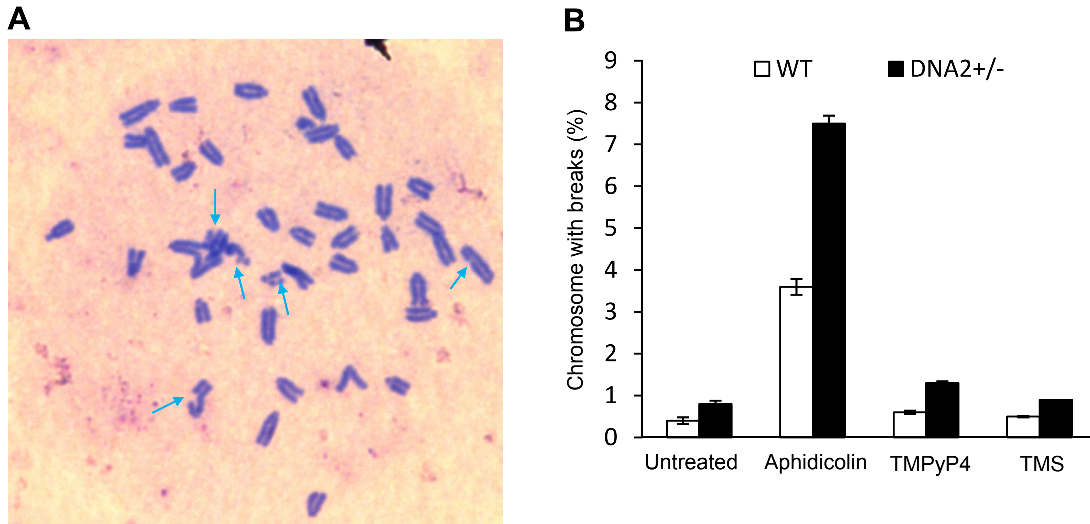


Figure S2. Chromosome breaks in WT and DNA2+/- MEF cells untreated or treated with aphidicolin, TMPyP4, or telomestatin (TMS). A. Representative image showing chromosome breaks in aphidicolin-treated DNA2+/- MEF cells. **B.** Percentage of chromosomes with breaks. Values are means ± s.e.m of three independent assays. In each assay, 3,000 – 4,000 chromosomes are analyzed.

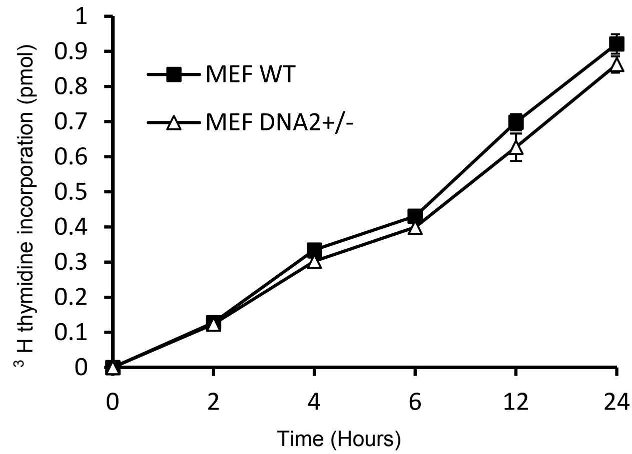


Figure S3. DNA2+/- MEF cells have similar DNA2 replication rate to the WT cells. The DNA replication efficiency in WT and DNA2/+ MEF cells was measured by the ^{3}H incorporation assay. Cells were incubated with ^{3}H-thymidine for 2, 4, 6, 12, or 24 hours. The ^{3}H activity that was incorporated into genomic DNA was determined by liquid scintillation counter. Values are means ± s.d. of five independent assays.

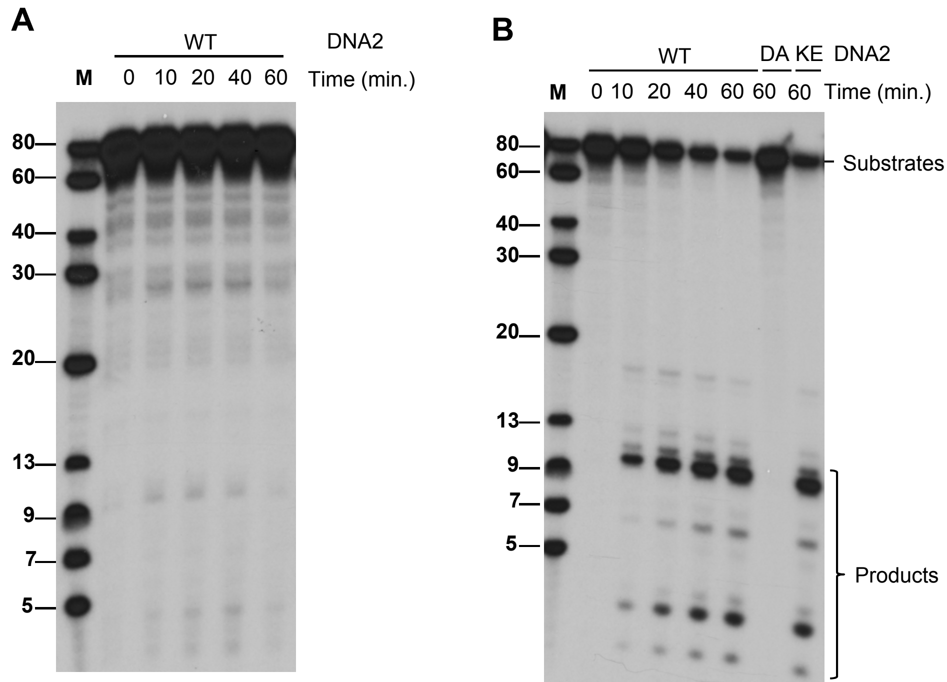


Figure S4. DNA2 nuclease activity on regular bubble or flap DNA substrate. ^{32}P 5' end-labeled regular bubble (A) or flap substrates (B) were incubated with 100 fmol purified human DNA2 at 37 ºC for 0, 5, 10, 20, 40, and 60 min. The reactions were analyzed by 15% denaturing PAGE.

B

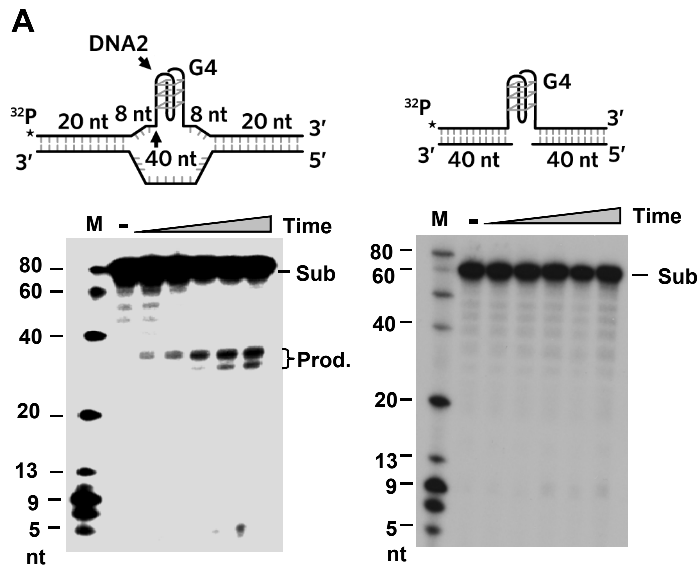


Figure S5. DNA2 specifically cleaves G4 bubble DNA substrate but not G4 gapped substrates. ^{32}P 5’ end-labeled G4 bubble (A) or gap substrates (B) were incubated with 100 fmol purified human DNA2 at 37 ºC for 0, 5, 10, 20, 40, and 60 min. The reactions were analyzed by 15% denaturing PAGE.

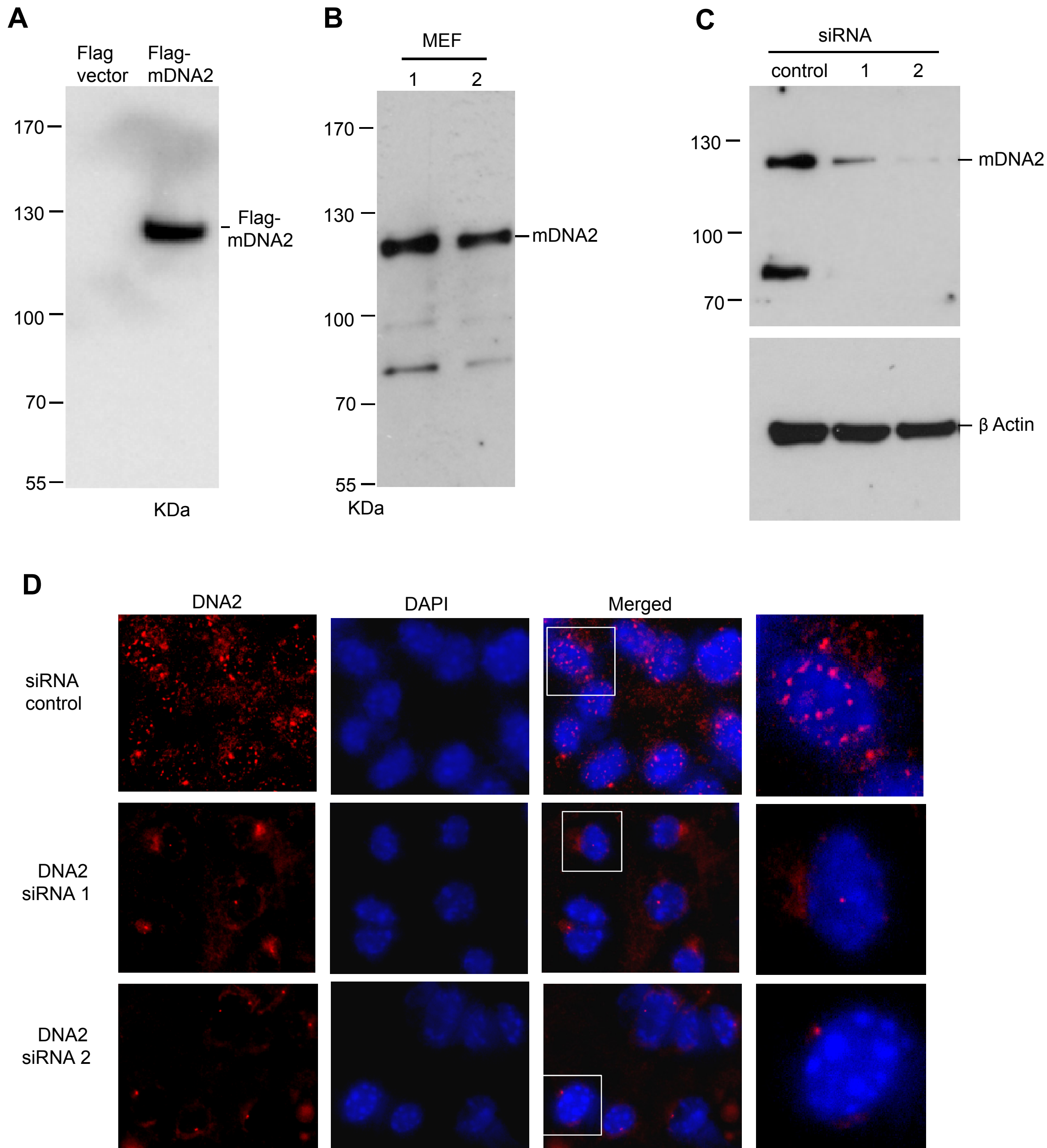


Figure S6. Specificity of rabbit polyclonal antibody against mouse DNA2. A. Western blotting analysis was conducted with mouse recombinant DNA2 protein. Recombinant flag-tagged mouse DNA2 was pulled down with a monoclonal antibody against the flag tag and immunoblotting with the rabbit antiserum against mouse DNA2 (1:000). In the control, 293T cells were transfected with empty vector. Whole cell extracts were incubated with the anti-Flag agarose beads. The proteins pulled down by the beads were used as the negative control. **B.** DNA2 Western blots of nuclear extracts from two different lines of MEF cells. **C.** Knockdown DNA2 in MEF cells. MEF cells were transfected with control siRNA oligos or the siRNA oligo for knocking down mouse DNA2. After 48 hours, the cells were harvested and Western blotting analysis was carried out using the polyclonal antibody against mouse DNA2. **D.** Immunofluorescence staining of mouse DNA2 in MEF pre-treated with the control siRNA oligo or the siRNA oligo for knocking down mouse DNA2. The right panels are the enlarged views of the boxed areas.

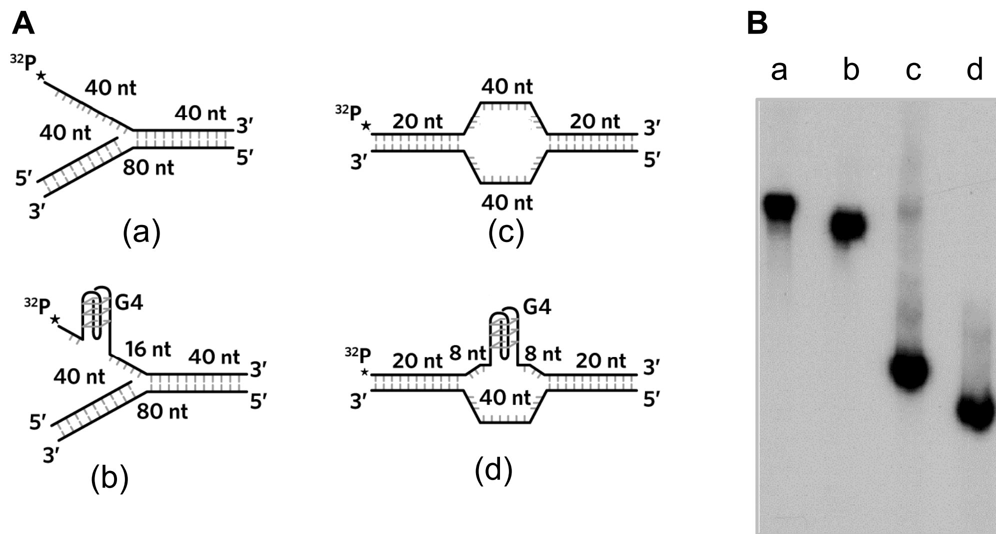


Fig. S7. Confirmation of G4 flap and G4 bubble substrate formation. **A.** Diagram of model substrates with the regular 5′ flap (a), the 5’ flap with telomere G4 (b), the normal bubble, or the telomere G4 bubble (d). **B.** The migration of four different substrates were analyzed with non-denaturing PAGE containing 10 mM KCl. Higher mobility is diagnostic of intramolecular G4 DNA formation by substrates b and d.

Supplementary Table S1. Oligo sequences for preparation of DNA substrates

Name	Sequences
G4 -B	5’-GTTAAGATAGGTCTGCTTGGCATGTCAATTAGGGTTAGGGTTAGGGTTAGGGCTCTGTGGTT GAGGCAGAGTCCTTAAGC-3’
G4 -B-T	5’-GCTTAAGGACTCTGCCTCAAATCGTCAGGGTTTCTAAAGAAGCCGACGGTAGTCAACGTGCC AAGCAGACCTAT CTTAAC-3’
G4-F	5’-TTAGGGTTAGGGTTAGGGTTAGGGATC ATGGCTTGCGATAAGTTAGGACTGCTTGACATCC CAAGCAGACCTATCTTAAC-3
G4 -F-T	5’-GTTAAGATAGGTCTGCTTGGGATGTCAAGCAGTCCTAACTGGAAATCTAGCTCTGTGGAGTT GAGGCAGAGTCCTTAAGC-3’
G4-F-T-C	5’-GCTTAAGGACTCTGCCTCAACTCCACAGAGCTAGATTTCC-3’
G4-F-G-C1	5’-GCCAAGCAGACCTATCTTA-3’
G4-F-G-C12	5’-GCTTAAGGACTCTGCCTCA-3’