Supporting Information

Fig. S1 Depletion of DDX39 does not affect the levels of TRF2. (A) HT1080 cells stably expressing two different DDX39 shRNAs (shDDX39-1 and shDDX39-2) or the control shRNA (shControl) were analyzed by immunoblotting with anti-DDX39, anti-TRF2, and anti-TRF1 antibodies. (B) Stable clones were analyzed by indirect immunofluorescence. Paraformaldehyde-fixed cells were stained with anti-DDX39 (green) and anti-TRF2 (red) antibodies. DNA was stained by DAPI (blue) in the merged images.

Fig. S2 DDX39 interaction with the TRFH domain of TRF2 is required for DDX39 recruitment to telomeres. (A) HT1080 cells transfected with Myc-DDX39 or Myc-DDX39/2A were analyzed by indirect immunofluorescence for co-localization of DDX39 with telomeres. Immunofluorescence was used to detect Myc-tagged DDX39 proteins (green), and fluorescence *in situ* hybridization (FISH) was used to detect telomeric sites (red). DNA was stained by DAPI (blue) in the merged images. (B) The average percentage of telomere foci co-localized with DDX39 proteins represented in panel A is shown. Statistical analysis was done using a two-tailed Student's *t* test (*, $P < 0.001$).

Fig. S3 DDX39 associates with catalytically competent telomerase. (A) Lysates from 1 x 10^6 H1299 cells were subjected to immunoprecipitation with anti-DDX39, anti-TRF2, anti-p53, and anti-KIP antibodies. Immunoprecipitates were analyzed for telomerase activity by the TRAP assay. Aliquots equivalent to 50,000 cells were loaded for the TRAP assay. (B) HT1080 cells transfected with DDX39-V5 or the empty vector were subjected to immunoprecipitation with anti-V5 antibody, and bound proteins were analyzed for telomerase activity.

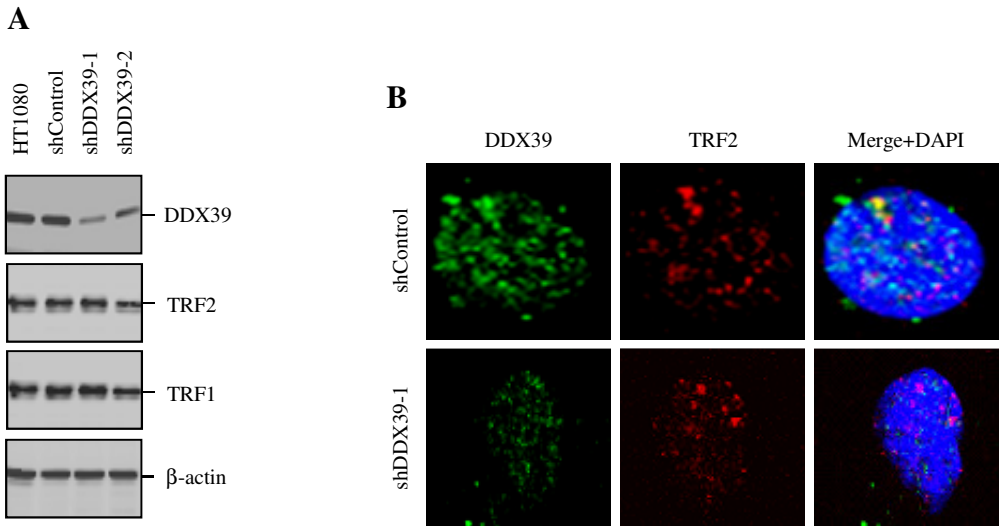
Fig. S4 Telomerase activity is not significantly changed by overexpression or depletion of DDX39. (A) Stable HT1080 cell lines expressing DDX39 (OE-1 and OE-2) or the empty vector were harvested at 46 PD, and telomerase activity was measured by the TRAP assay with different amounts of cell extracts. (B) HT1080 cells stably expressing shDDX39-1, shDDX39-2, or the control shRNA were harvested at 45 PD, and telomerase activity was measured by the TRAP assay with different amounts of cell extracts.

Fig. S5 Depletion of DDX39 leads to progressive telomere shortening. Multiple independent clones stably expressing shDDX39-1, shDDX39-2, or the control shRNA were harvested at various PDs, and genomic DNA was digested with RsaI and HinfI, followed by Southern blot analysis using a telomere repeat probe.

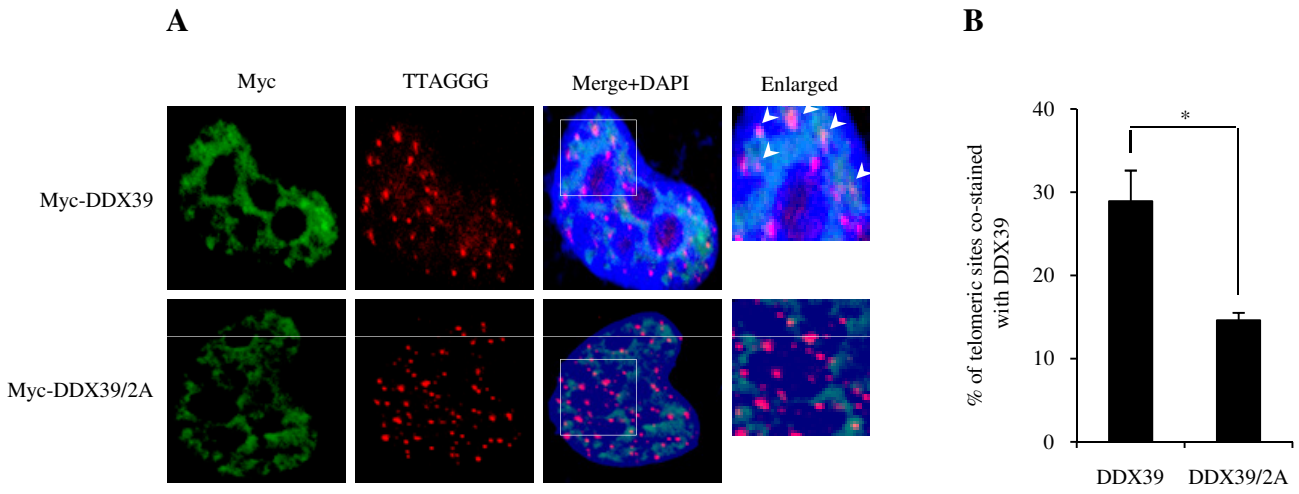
Fig. S6 DDX39-depleted HT1080 cells grow slower than the control cells but with constant rates. (A) Growth curves of HT1080 clones stably expressing shDDX39-1, shDDX39-2, or the control shRNA. Stable clones were replated every 3-4 days to maintain log-phase growth, and cell numbers were measured at the different time points, with day 0 representing the first day after antibiotic selection. (B) Growth curves of U2OS clones stably expressing shDDX39-1, shDDX39-2, or the control shRNA. (C) U2OS clones stably expressing shDDX39-1, shDDX39-2 or the control shRNA were harvested at 42 PD and subjected to immunoblotting with anti-DDX39 antibody.

Fig. S7 Rescue of the DNA-damage phenotypes in DDX39-depleted cells by co-expression of shRNA-resistant DDX39. (A) HT1080 cells stably expressing shDDX39-1 or the control shRNA were transfected with Myc-DDX39 or the version of DDX39 resistant to shDDX39-1 (Myc-RNAi-R-DDX39) and examined for the expression of Myc-DDX39 by immunoblotting with anti-Myc antibody. (B) HT1080 cells stably expressing shDDX39-1 were transfected with DDX39 or RNAi-R-DDX39 and analyzed by indirect immunofluorescence for co-localization of 53BP1 foci (green) with TTAGGG probe (red). DNA was stained by DAPI (blue) in the merged images. (C) The average percentage of cells showing ten or more 53BP1 foci was determined. Statistical analyses were done using a two-tailed Student's *t* test (*, $P = 0.001$).

Fig. S8 Overexpression of DDX39 does not affect the basal levels of the DNA-damage foci. HT1080 cells were transfected with wild-type DDX39 or RNAi-R-DDX39 and analyzed by indirect immunofluorescence for co-localization of 53BP1 foci (green) with TTAGGG probe (red). DNA was stained by DAPI (blue) in the merged images.



Supporting Information Fig. S1



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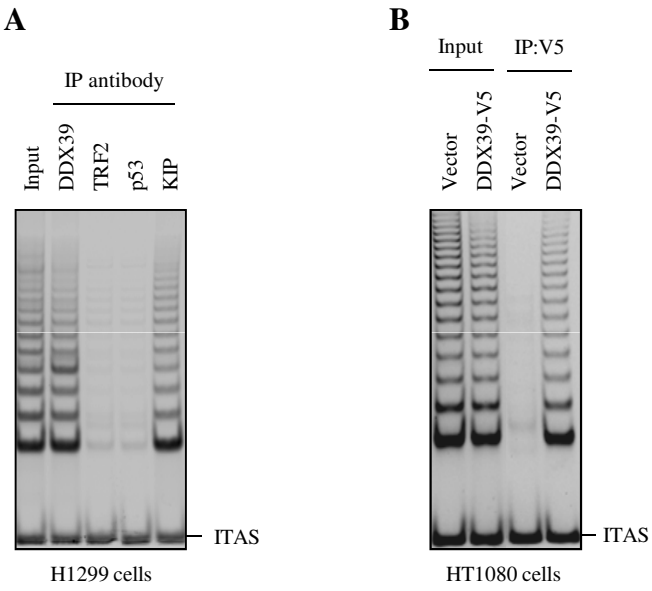

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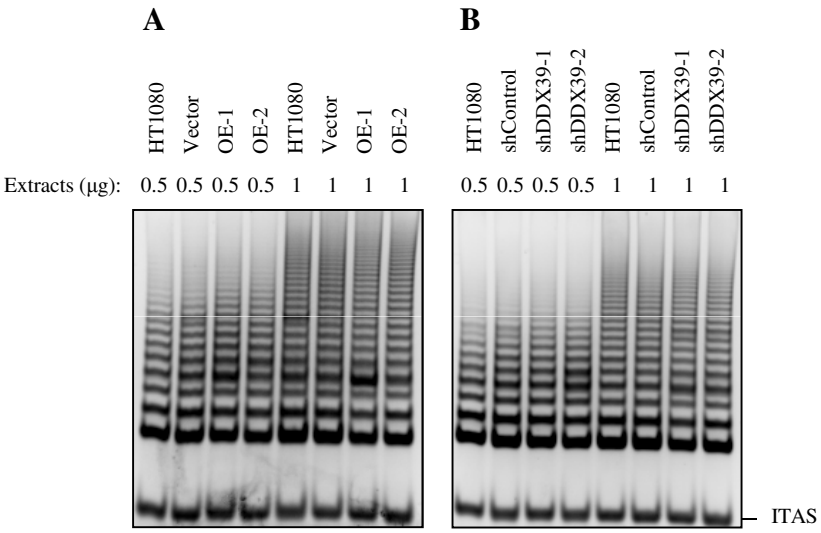

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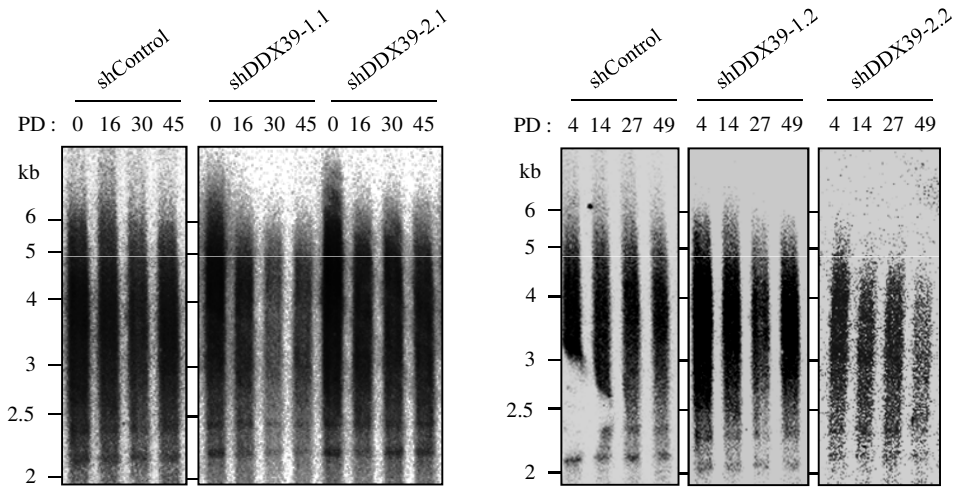

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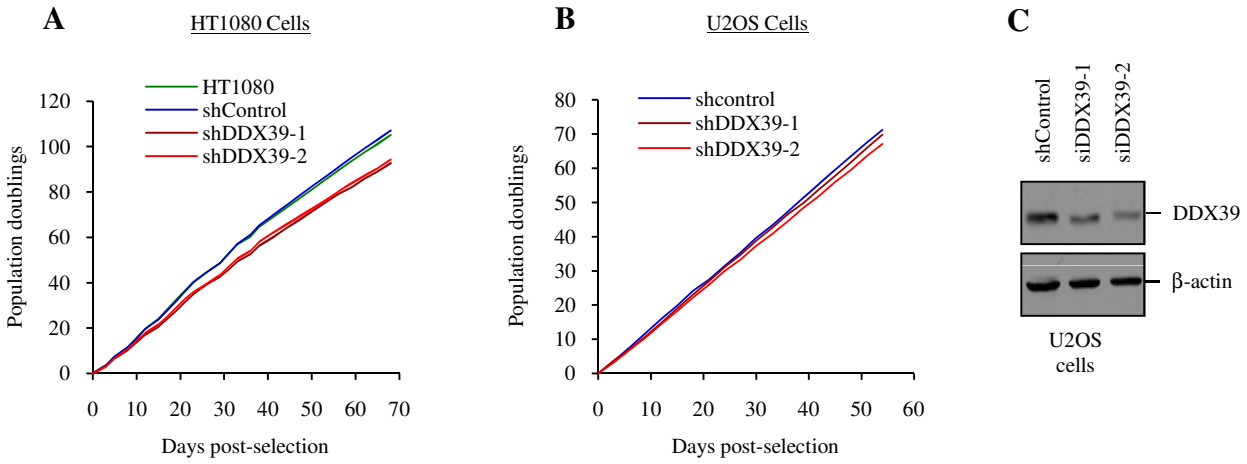

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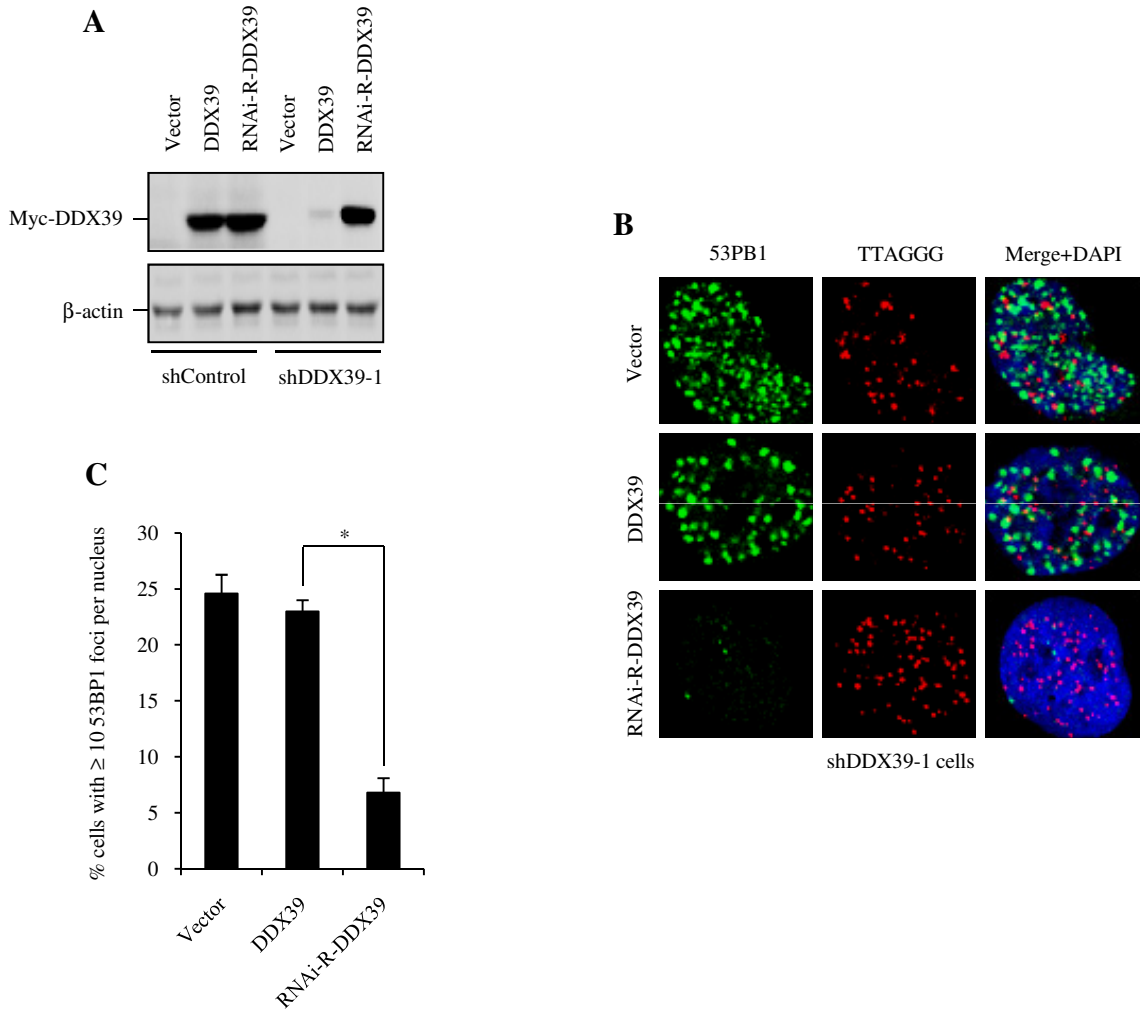

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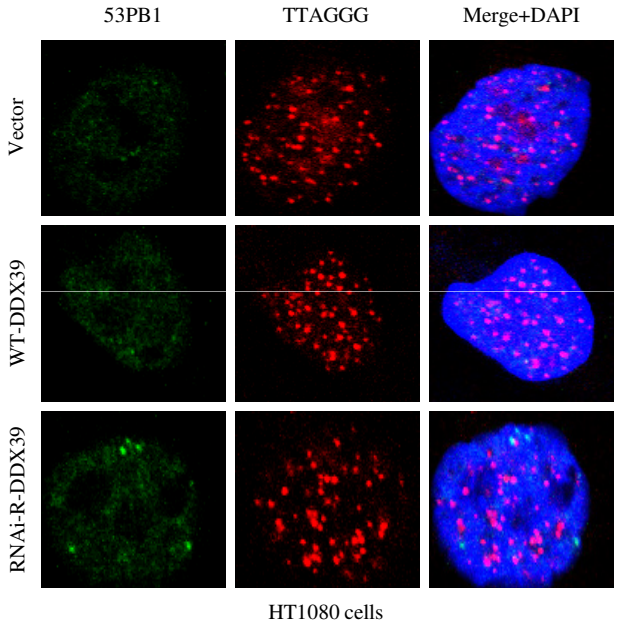

Supporting Information Fig. S8