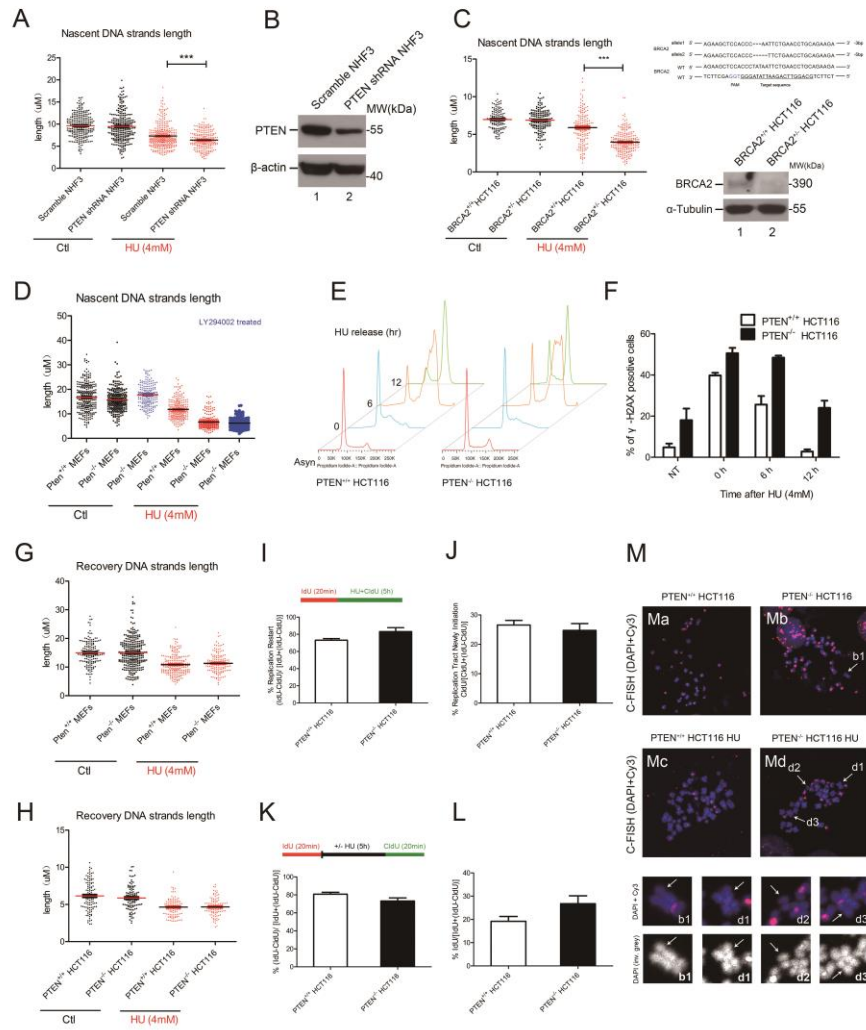


Supplementary Figure 1



Supplementary information, Figure S1. PTEN regulates DNA replication mainly through stalled replication forks protection

(A) IdU tract lengths in NHF3 cells transfected with PTEN shRNA and control. NHF3 cells transfected with PTEN shRNA lentivirus, but not cells transfected with Scramble shRNA lentivirus exhibited shortening with HU treatment. **(B)** Western blot confirmation of PTEN deletions in NHF3 cells. **(C)** Positive control for IdU tracts shortening under HU treatment in BRCA2^{+/-} HCT116 cells. Efficiency of CRISPR targeted BRCA2 was confirmed by DNA sequencing in HCT116 cells. **(D)** IdU tracts in *Pten*^{-/-} MEFs cells treated with 6 h 30 μ M LY294002. **(E)** FACS analysis of PTEN in replication recovery. PTEN^{+/+} HCT116 and PTEN^{-/-} HCT116 cells were arrested in S phase by treatment with 10 mM HU for 16 h, and media was replaced with fresh media containing 0.1 μ g/mL of nocodazole. Cellular DNA content was analyzed with FACS at stated time points. **(F)** γ -H2AX foci formation assay in PTEN^{+/+} and PTEN^{-/-} HCT116 cells after 4 mM HU treatment for 5 h. **(G)** CldU tracts in *Pten*^{+/+} and *Pten*^{-/-} MEFs cells. *Pten*^{-/-} MEFs cells showed no significant differences under HU treatment as compared with *Pten*^{+/+} MEF cells. CldU tracts reflected the restart of DNA replication stalling. **(H)** CldU tracts of replication after exposure to HU or media in PTEN^{+/+} and PTEN^{-/-} HCT116 cells. Frequency of replication restart and new replication initiation **(I and J)** and continuous and non-continuous IdU-CldU tracts **(K and L)** in PTEN^{+/+} and PTEN^{-/-} HCT116 cells with HU. **(M)** Centromere fluorescence *in situ* hybridization (C-FISH) analysis. Representative metaphase spreads from PTEN^{+/+} and PTEN^{-/-} HCT116 cells treated with or without HU (4 mM) hybridized with

a Cy3 centromeric probe are shown. Merged images of DAPI (blue) and Cy3-labeled (red) metaphase spreads are shown in the top panel. Selected regions of metaphase spreads from PTEN^{-/-} HCT116 cells with or without HU treatment are magnified in the lower panel to show chromosomal aberrations indicated by arrows. Metaphase spreads counterstained for DNA with DAPI are illustrated below in inverted gray scale for visual enhancement. All data are presented as means \pm SEM and analyzed by unpaired t-test. *** $P < 0.001$.