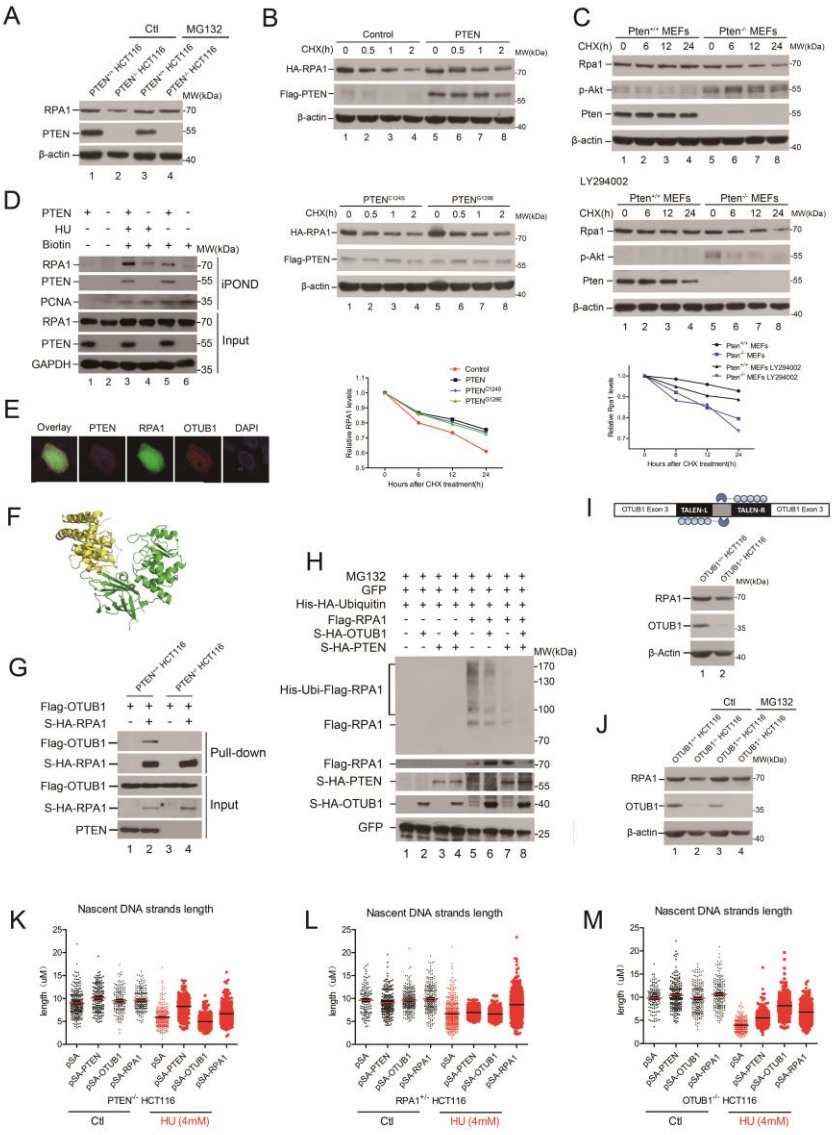
Supplementary Figure 4



Supplementary information, Figure S4. RPA1 is stabilized by PTEN/OTUB1 and acts as a downstream target

(A) Protein expression analysis of RPA1 in PTEN null cells. $PTEN^{+/+}$ and $PTEN^{-/-}$ HCT116 cells were treated with or without MG132 (10 μM) for 12 h before collection. Lysates were subjected to western blotting with antibodies against RPA1, PTEN, and β-actin. **(B)** Half-life analysis of RPA1. $PTEN^{-/-}$ HCT116 cells were co-transfected with HA-RPA1 and Flag-HA-PTEN, Flag-HA-$PTEN^{C124S}$ or Flag-HA-$PTEN^{G129E}$, treated with 100 μg/ml CHX, collected at different time points and immunoblotted with antibodies against HA, Flag and β-actin. Quantification of HA-RPA1 protein levels. **(C)** Half-life analysis of RPA1 in PTEN deleted cells with LY294002 (30 μM) treatment. $Pten^{+/+}$ and $Pten^{-/-}$ MEFs cells were treated with 100 μg/ml CHX, collected at different time points and immunoblotted with antibodies against RPA1, p-Akt, PTEN and β-actin. Quantification of RPA1 protein levels in $Pten^{+/+}$ and $Pten^{-/-}$ MEFs cells. **(D)** $PTEN^{+/+}$ and $PTEN^{-/-}$ HCT116 cells were labeled with EdU (15 min) and treated with or without 4 mM HU for 5h. Proteins isolated on biotin conjugated nascent DNA were detected by western blotting. **(E)** Immunofluorescence analysis of co-localization of ectopic PTEN, RPA1 and OTUB1 in $PTEN^{-/-}$ HCT116. **(F)** In Silico docking analysis of PTEN and OTUB1 complexes within a distance of 3.0 Å . PTEN is represented by green and OTUB1 by yellow. **(G)** *In vivo* binding of exogenous OTUB1 and RPA1 in $PTEN^{+/+}$ and $PTEN^{-/-}$ HCT116 cells. S-tagged-RPA1 and Flag-OTUB1 were co-transfected in $PTEN^{+/+}$ and $PTEN^{-/-}$ HCT116 cells. Cell lysates were pulled down with s-protein beads and immunoblotted with the antibody against Flag and other antibodies as indicated.

(H) *In vivo* deubiquitination of RPA1. PTEN$^{-/-}$ HCT116 cells were co-transfected with S-tagged-HA-PTEN and/or S-tagged-HA-OTUB1 together with His-HA-ubiquitin and Flag-RPA1, then subjected to pull-down assay with Ni-beads and immunoblotted with the antibody against Flag. Cells were treated with MG132 (10 μM) for 12 h prior to collection. **(I)** Experimental design and western blot confirmation of OTUB1 deletion. **(J)** Protein expression analysis of RPA1 in OTUB1 null cells. OTUB1$^{+/+}$ and OTUB1$^{-/-}$ HCT116 cells were treated with or without MG132 (10 μM) for 12 h before collection. Lysates were subjected to western blotting with antibodies against RPA1, OTUB1 and β-actin. **(K-M)** Measurements of IdU tracts in PTEN$^{-/-}$ HCT116 (K), RPA1$^{+/-}$ HCT116 (L) and OTUB 1$^{-/-}$ HCT116 (M) cells transfected with S-tagged-PTEN, S-tagged-RPA1, S-tagged-OTUB1 or S-tagged-mock with or without HU. Data are presented as means ± SEM and analyzed by unpaired t-test.