

## Supplementary Information

### Telomere recombination requires the MUS81 endonuclease

Sicong Zeng<sup>1</sup>, Tao Xiang<sup>1</sup>, Tej K. Pandita<sup>1</sup>, Ignacio Gonzalez-Suarez<sup>1,2</sup>, Susana Gonzalo<sup>1,2</sup>, Curtis C. Harris<sup>3</sup> and Qin Yang<sup>1\*</sup>

<sup>1</sup>Department of Radiation Oncology, Washington University School of Medicine, 4511 Forest Park, St. Louis, MO 63108, USA. <sup>2</sup>Department of Cell Biology & Physiology, Washington University, St. Louis, MO, USA. <sup>3</sup>Laboratory of Human Carcinogenesis, NCI, NIH, Bethesda, MD, USA.

\* Correspondence should be addressed to QY. Email: [qyang@wustl.edu](mailto:qyang@wustl.edu)

**This PDF file includes:**

**Supplementary Information, Figs. S1 to S9**

## Supplementary figure legends:

**Fig. S1. A.** TRF1 and TRF2 co-localize to APBs in GM847 cells. Cells were processed for immunofluorescence with PML, TRF1 and TRF2 antibodies, and for telomeric DNA-FISH. Bright green staining with the FITC-labeled PNA probe (telomeric C-strand oligo) indicated APBs in less than 5% of GM847 cells. The merged images (yellow foci) showed that TRF1, TRF2, PML or MUS81 co-localized with telomeric DNA in APBs. **B.** Knockdown of MUS81 decreases immunostaining signals of MUS81 in GM847 cells. Cells transduced with MUS81 shRNAs were stained with MUS81 and telomeric DNA. **C.** Cell cycle distribution of GM847 cells. GM847 cells at different time points following release from double thymidine block were fixed and processed for Flow cytometric analysis (FACS) analyses. The asterisk (\*) shows an additional block by Hoechst 33342, in addition to the double thymidine treatment, which enriches G2 cells more efficiently than double thymidine block alone. –Met: methionine restriction for 4 days; HU: 5 mM hydroxyurea treatment for 24 hours. **D.** MUS81 localizes in nucleoli in HT1080 and GM847 cells. Cells were processed for immunofluorescence combined with telomeric DNA-FISH. FITC-labeled PNA probe (green staining) represented telomeric DNA signals in HT1080 (Non-ALT) and GM847 (ALT) cells. Red staining indicated MUS81 protein. The merged image showed that MUS81 only localized in nucleoli in HT1080 cells. In GM847 cells, MUS81 localized in nucleoli and formed foci in APBs. **E.** Localization of MUS81 in nucleoli was confirmed by immunofluorescence assay with MUS81 and Nucleoli antibodies in HT1080 and GM847 cells.

**Fig. S2. A.** Knockdown of MUS81 induces the cell growth arrest in ALT cells. Colony formation assays were performed in three ALT cells (U2OS, GM847 and SAOS-2) and two non-ALT cells (MCF7, HT1080). Cells were equally seeded after infection with control lentiviral vector or shMUS81-A in antibiotics selected medium for two weeks. Viable cell colonies were stained and counted. **B.** Knockdown of MUS81 decreased incorporation of BrdU in GM847 cells. Cells were stained with anti-BrdU antibody and Alexa 499-conjugated secondary antibody after cultured with BrdU for 30 min. Red signal indicated BrdU incorporated cells. Quantitative data were summarized from three independent experiments (mean  $\pm$  S.D.) and showed that incorporation ratio of BrdU was largely reduced after MUS81-A shRNA expression.

**Fig. S3. A.** Effects of MUS81 on cell cycle in ALT cells. The cell cycle distribution of U2OS and HT1080 cells with or without expression MUS81-shRNA-A was determined by FACS of BrdU- and propidium iodide (PI)-stained cells. **B.** Depletion of MUS81 does not induce apoptosis. TUNEL assay was performed in U2OS cells after expression with or without MUS81 shRNAs. Etoposide treatment (5  $\mu$ M) was used as a positive control.

**Fig. S4.** MUS81-deficiency does not induce telomere loss and telomere recombination in non-ALT cells. Telomere-FISH assay and T-SCE assay were performed to evaluate telomere loss and T-SCE rate in telomerase positive cells (MCF7 and HT1080) with or without expression MUS81-shRNAs. Depletion of MUS81 did not induce telomere loss and did not change T-SCE rate in these non-ALT cells. *Mus81*<sup>+/+</sup> and *Mus81*<sup>-/-</sup> MEFs were also used and there were no significant difference in telomere loss and T-SCE rate.

**Fig. S5.** Effects of MUS81 on telomere length. **A.** Quantitative FISH assay indicated that knockdown of MUS81 did not affect telomere length distribution in GM847 cells. Maps illustrated telomere length distribution range in cells after infection with lentiviral vector or MUS81-A shRNA for one and three weeks. Average telomere length and telomere distribution showed in the Table (the lower panel). \* SD. Quantitative data represent three independent experiments (mean  $\pm$  S.D.). **B.** Telomere length analysis by TRF assay in GM847 and MCF7 cells. After one or three weeks stably expressing MUS81-A shRNA or vector control, genomic DNA were digested by Hinf I and separated by pulse-electrophoresis. Southern blot were performed with telomeric DNA probe. Quantitative data represent five independent experiments (mean  $\pm$  S.D.).

**Fig. S6.** Depletion of MUS81 does not induce telomere dysfunctional induced foci in ALT cells. U2OS cells transduced with control shRNA, MUS81-shRNAs or POT1-shRNA were immunostained with anti- $\gamma$ -H2AX mouse monoclonal antibody (*green*) together with anti-TRF1 antibody (*red*). The nuclei were counterstained with DAPI.

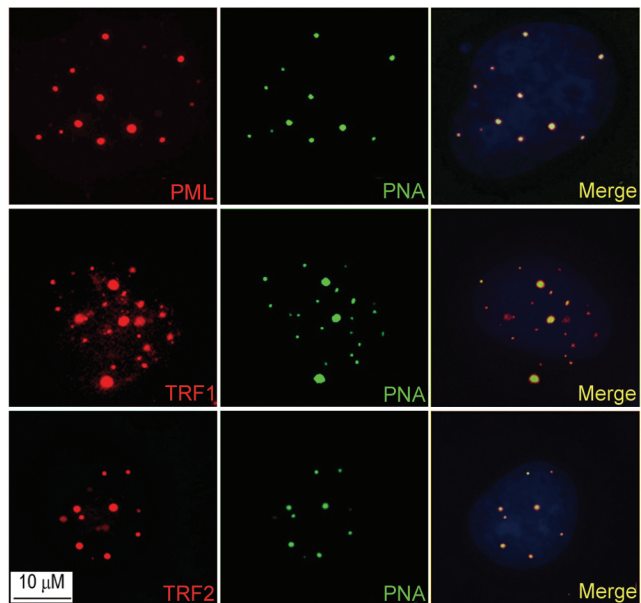
**Fig. S7. A.** Depletion of TRF2 increases the endonuclease activity of MUS81 in ALT cells. HA-tagged MUS81 wild-type (WT) and nuclease dead mutant constructs (338A/339A) (Mu) were expressed in U2OS and HeLa cells. Then, TRF2-shRNA or control shRNA vector was expressed in the cells. The endonuclease activity assay was carried out by incubating the  $^{32}P$  3'-flap telomeric DNA sequence substrate with immunoprecipitates of anti-HA from cell lysates. Quantitative data were summarized from three independent experiments (mean  $\pm$  S.D.). Equivalent amounts of immunoprecipitated HA-MUS81 were used, as determined by WB analysis (data not shown). The decreased expressing levels of TRF2 protein by TRF2-shRNA were confirmed by WB analysis (data not shown). **B.** The endonuclease activity assay was carried out by incubating the  $^{32}P$  3'-flap telomeric DNA sequence substrate (the right panel) and non-telomeric DNA sequence substrate (the left panel) with immunoprecipitates of either anti-MUS81 or IgG from U2OS cell lysates in the presence or absence of TRF2-shRNA expression. Quantitative data represent three independent experiments (mean  $\pm$  S.D.). **C.** MUS81 wild-type, but not the mutant, rescues the ALT cell growth arrest induced by depletion of MUS81. shMUS81-B (in the 3'UTR region) were expressed in U2OS cells for three days and then wild-type or mutant MUS81 were over-expressed in these cells. After two weeks with drug-selection, cell colonies were stained and counted. **D.** Recombinant TRF2 inhibits MUS81 binding to the 3'-flap DNA substrate. The biotin 3'-flap DNA was incubated with *in vitro* translated HA-MUS81 in the absence (*lane 3*) or presence of various amounts of recombinant TRF2 (5 and 20 nmol, *lanes 4* and *5*). The biotinylated DNA substrate and its bound proteins on the beads were analyzed by SDS-PAGE gel. The quantitative data represent three independent experiments (mean  $\pm$  S.D.). Bovine serum albumin did not affect TRF2-inhibited MUS81 binding to the DNA substrate (*lanes 6* and *7*). HA-MUS81 was detected by WB with the anti-HA antibody. Input, 50% of the total HA-MUS81 used in each reaction (*lane 1*). The DNA substrates made by telomere sequence were showed in the upper panel and non-telomere sequence in the lower panel.

**Fig. S8.** Knockdown of MUS81 does not affect t-circle formation. MUS81-A shRNA or vector control was stably expressed in GM847 cells for 2 weeks. 2-D gel analysis was conducted to detect t-circle formation.

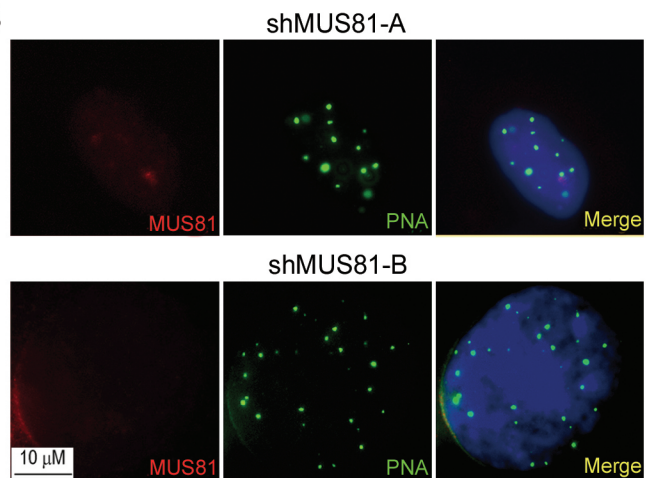
**Fig. S9.** Full scans of Western blot data.



A



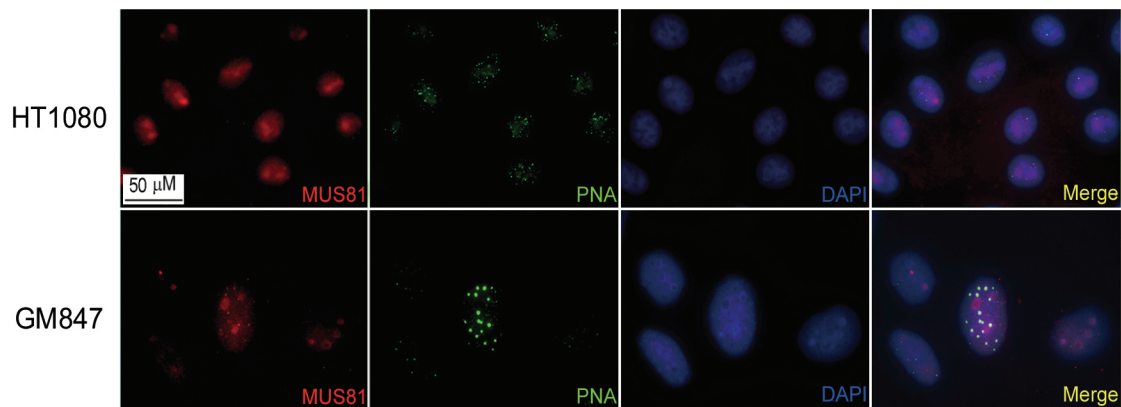
B



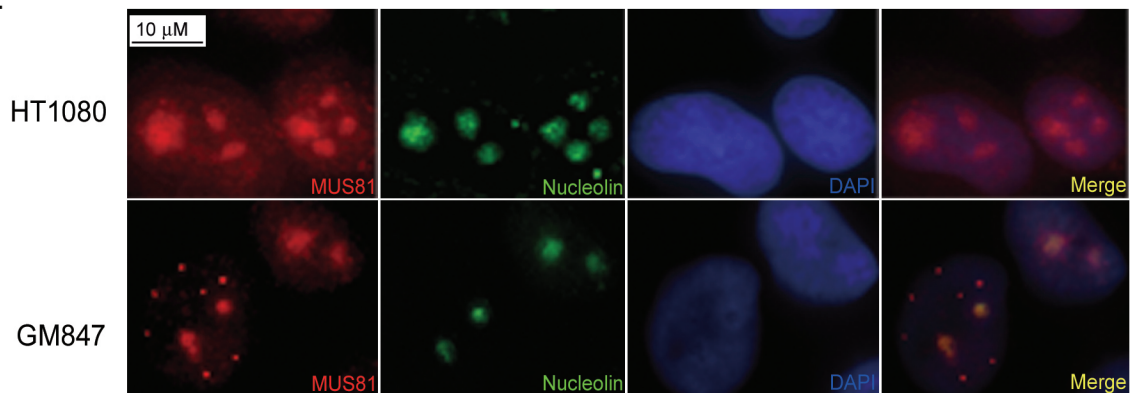
C

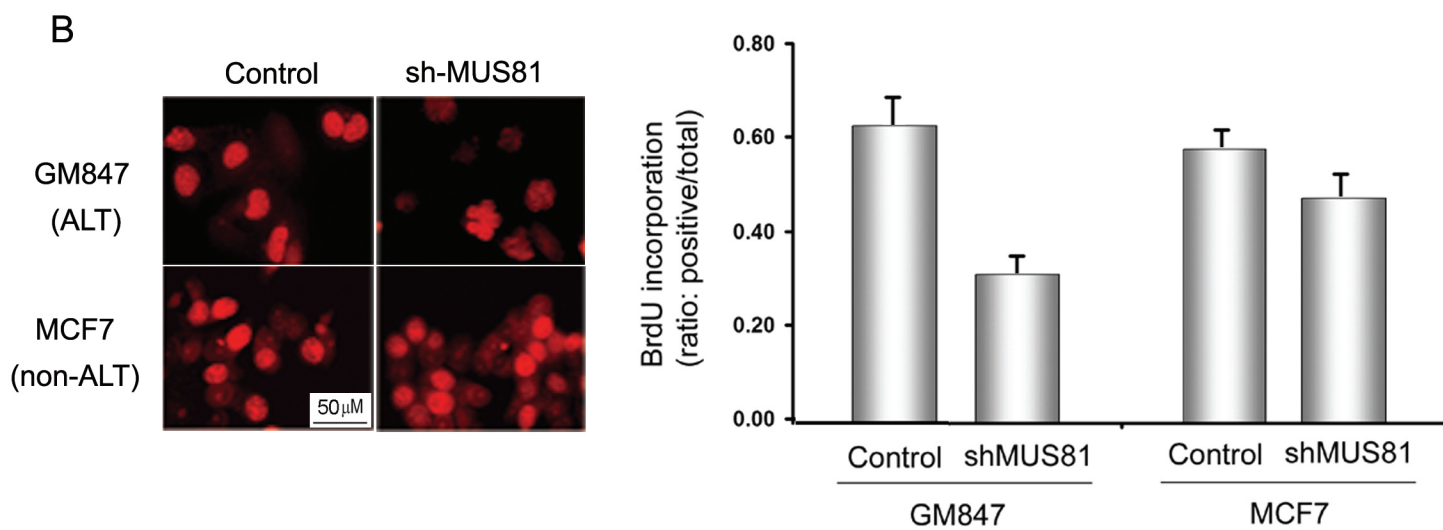
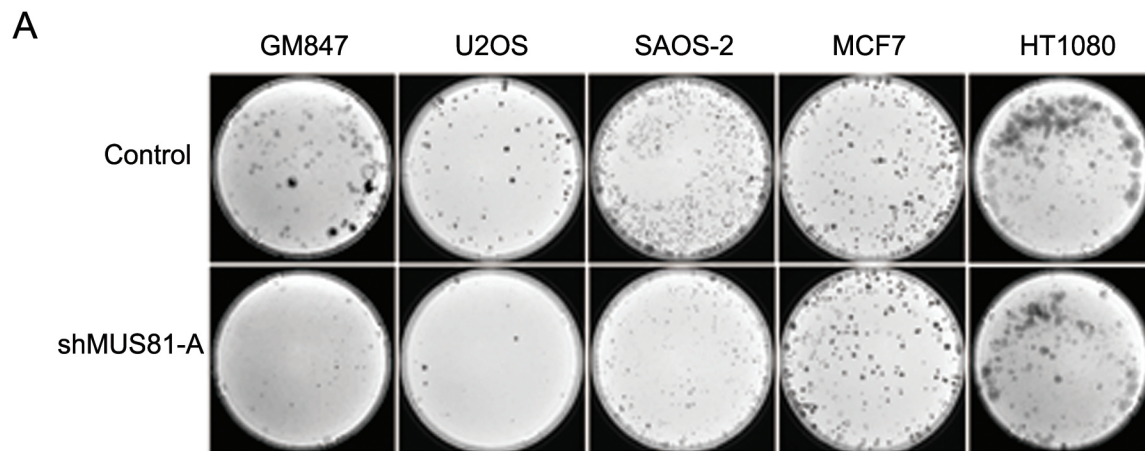
	0	5h	9h	13h	13h*	-Met	HU
<b>G1</b>	85	55	8	9	5	88	22
<b>S</b>	11	40	87	46	7	8	72
<b>G2/M</b>	4	5	5	45	88	4	6

D

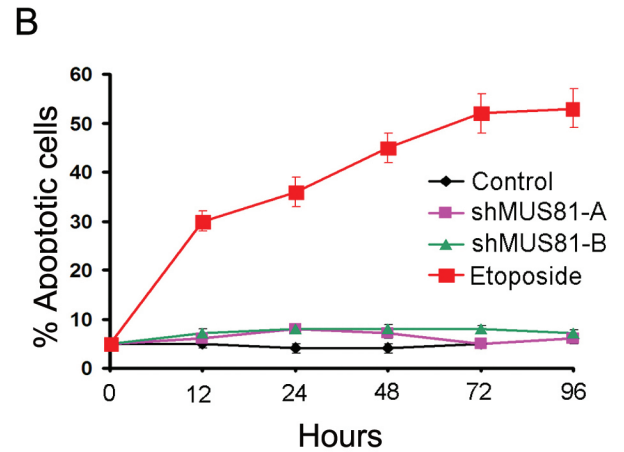
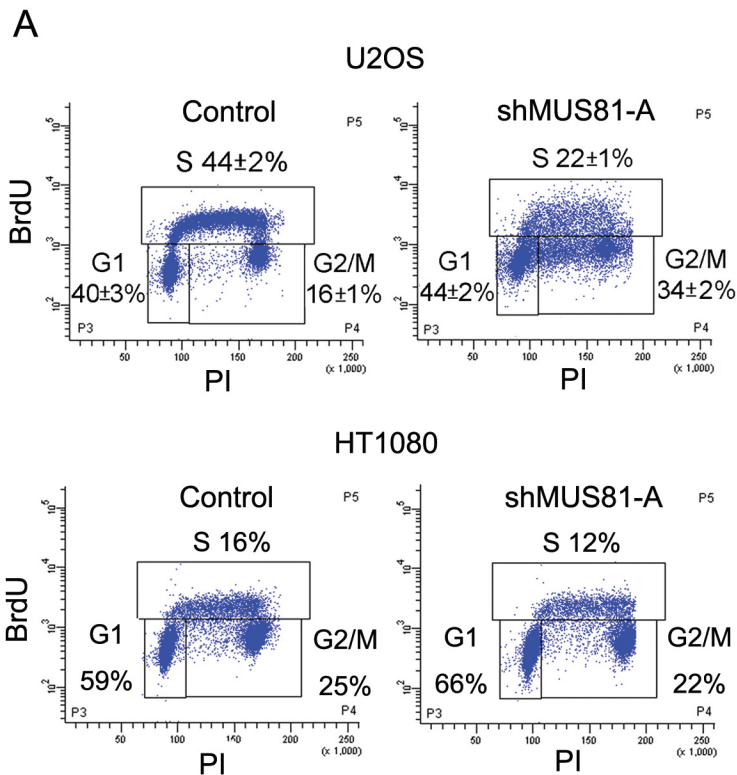


E





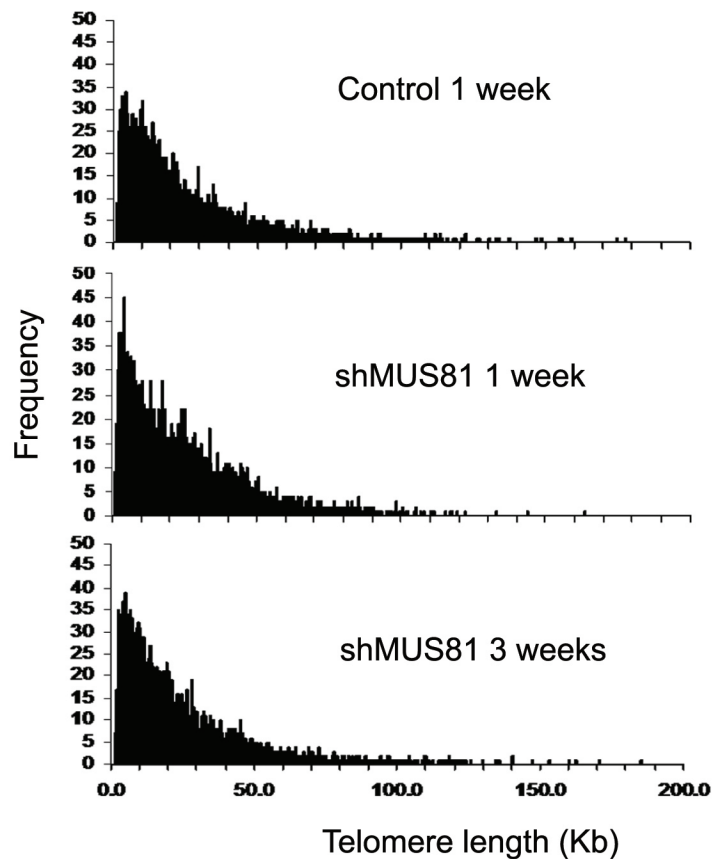
Yang Fig. S3



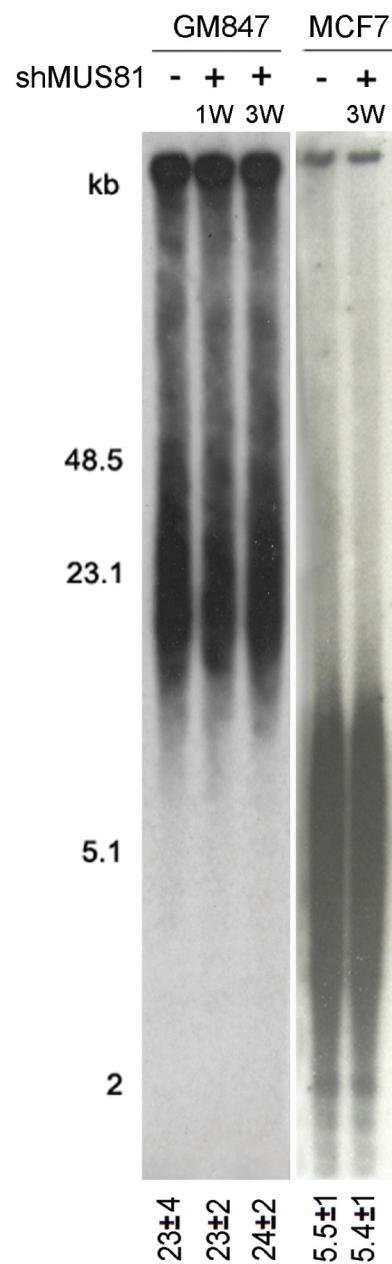
Yang Fig. S4

	<b>T-SCE</b> (per chromosome)	<b>Telomere loss</b> (per chromosome)
<b>MCF7</b>		
Control	0.008±0.03	0.008±0.01
shMUS81A	0.01±0.01	0.01±0.01
shMUS81B	0.005±0.005	0.005±0.001
<b>HT1080</b>		
Control	0.001±0.01	0.001±0.01
shMUS81A	0.001±0.001	0.001±0.002
shMUS81B	0.0008±0.005	0.0008±0.002
<b>MEF</b>		
MUS81+/+	0.03±0.01	0.01±0.001
MUS81-/-	0.03±0.002	0.016±0.001

A

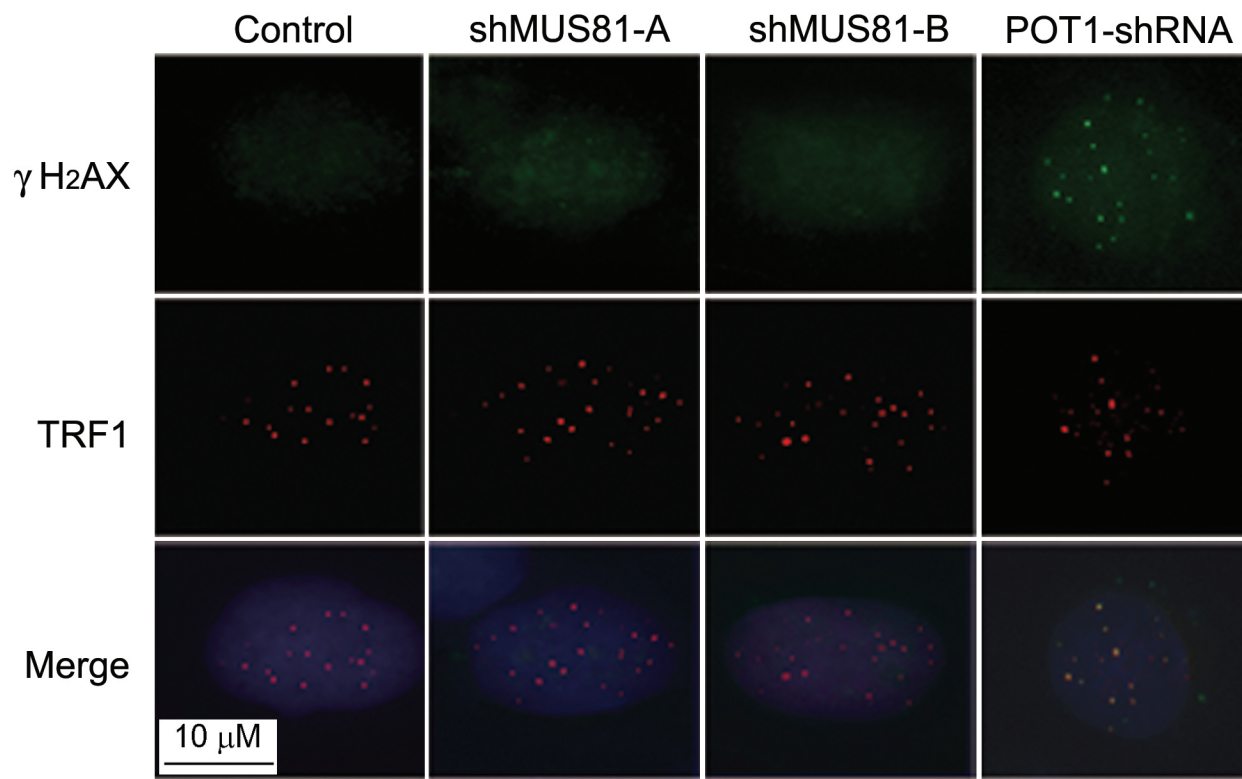


B

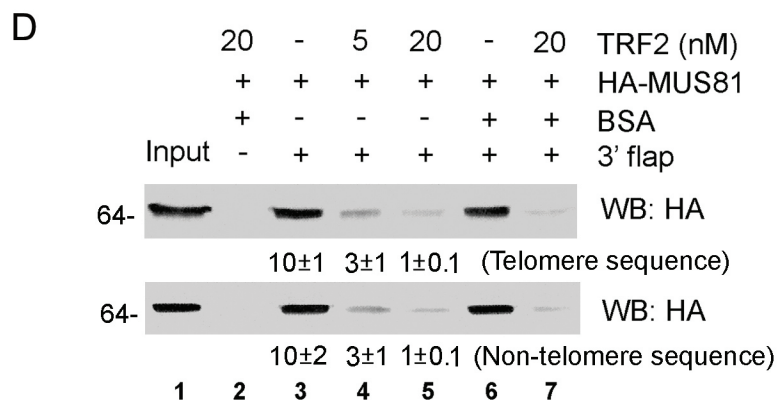
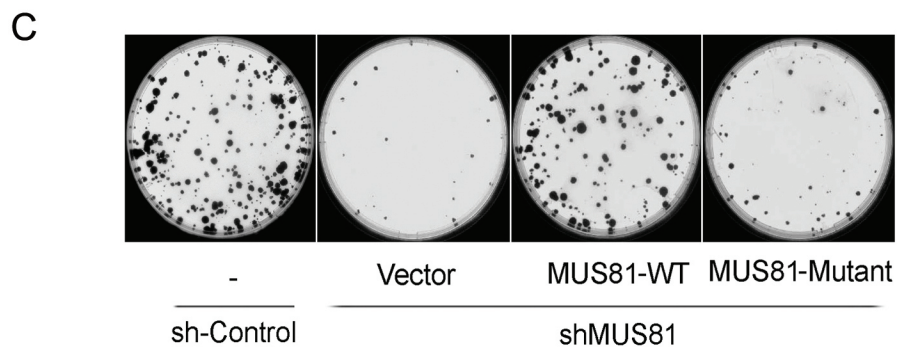
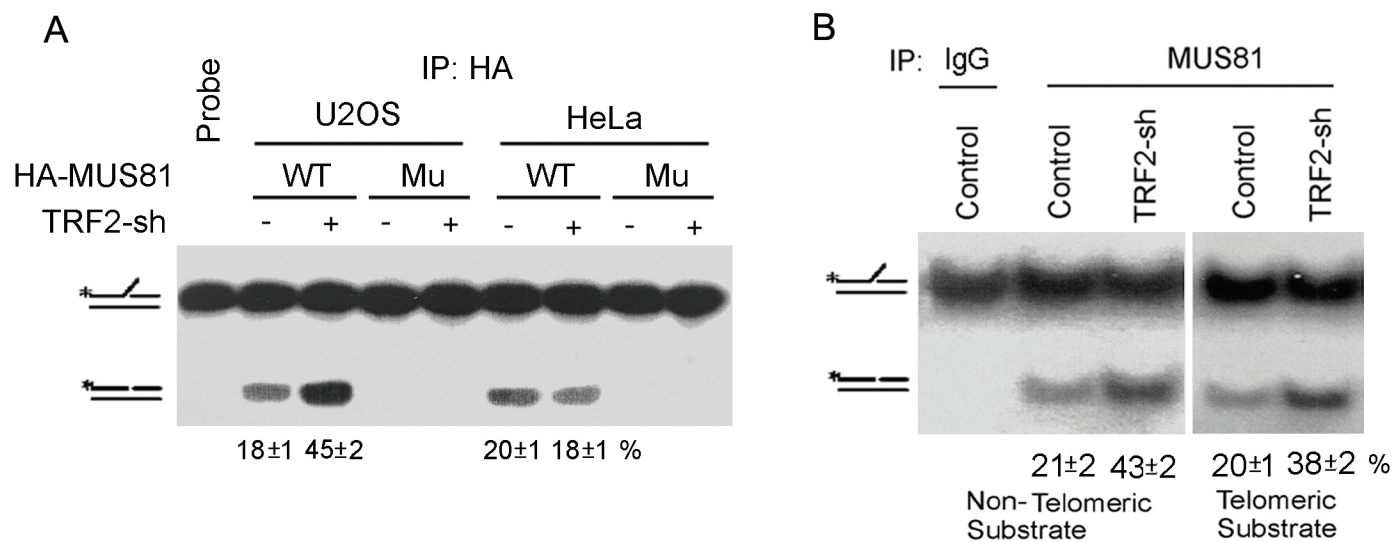


	GM847-Control	shMUS81-1W	shMUS81-3W
Number of telomeres	5857	9102	6390
Mean length (Kb)	23.2±20.8 (±3.8)*	22.3±20.8 (±3.1)*	21.4±20.3 (±2.9)*
% Telomeres < 5Kb	15.5±0.02	16.3±0.02	15.4±0.02
% Telomeres > 50 Kb	6.9±0.01	5.1±0.02	5.1±0.02

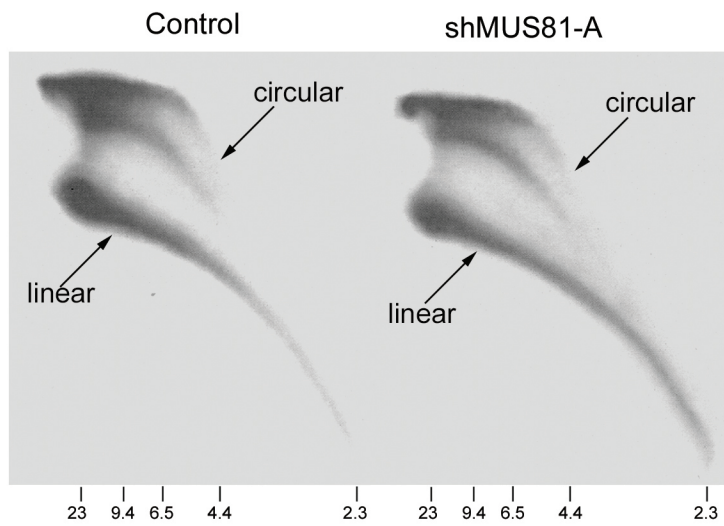
Yang Fig. S6







Yang Fig. S8



# Yang Fig. S9

Fig 1C

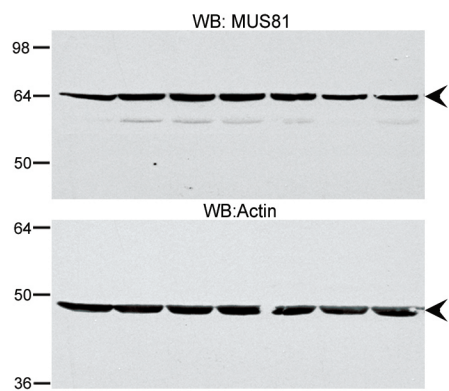


Fig 2A

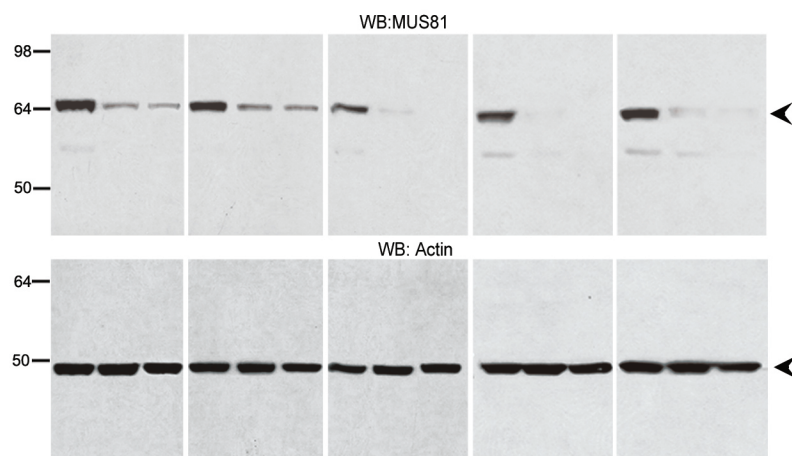


Fig 3B

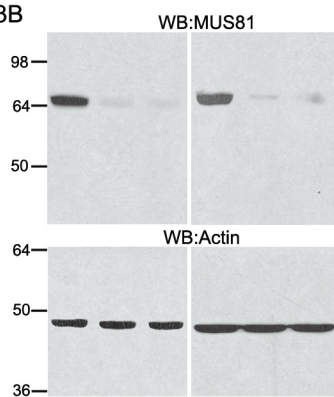


Fig 4A

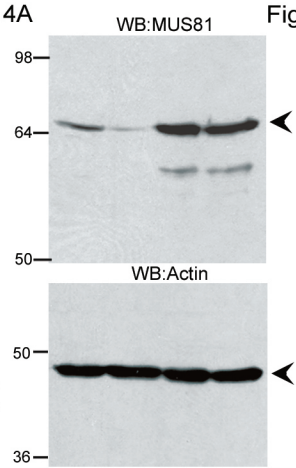


Fig 4B

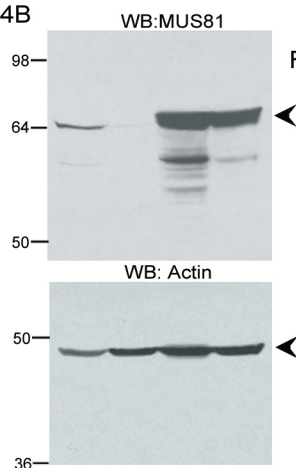


Fig 5A

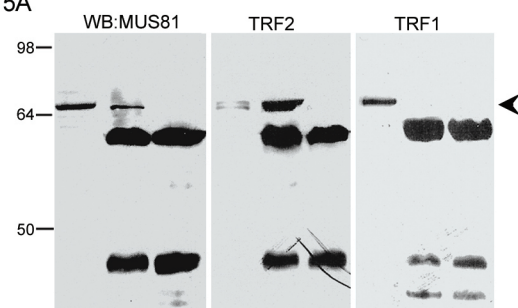


Fig 5B

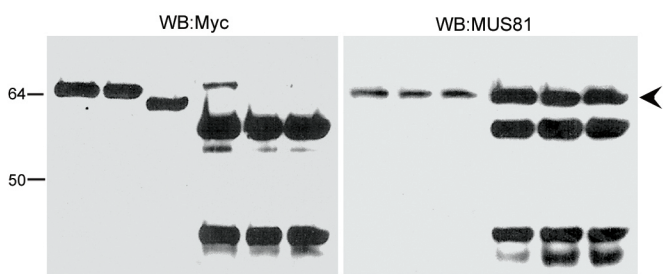


Fig 5C

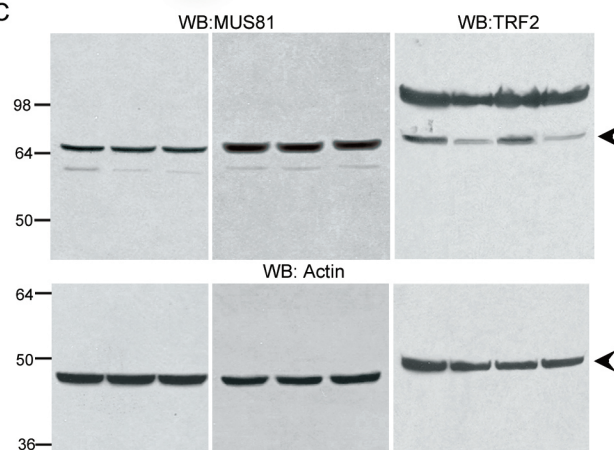


Fig 5D

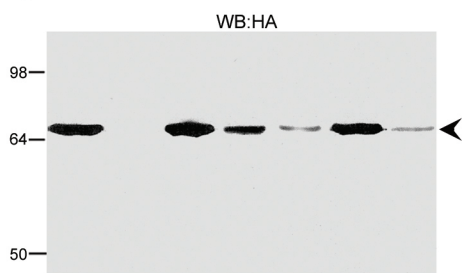


Fig 5F

