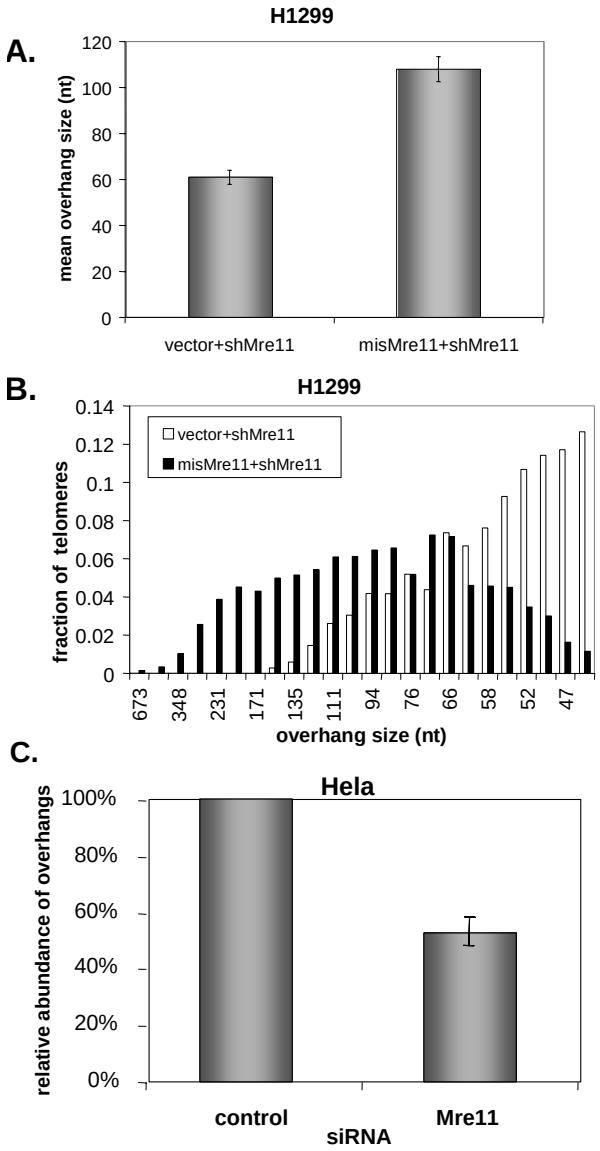
Supplemental Materials and Methods:

Cloning of Mre11 cDNA and site-directed mutagenesis. Human Mre11 cDNA was amplified from Hela cells by RT-PCR. SnaBI and SalI sites were introduced in the PCR primers and amplified cDNA was cloned into the pWZL-blast retroviral vector. Sequence was confirmed by sequencing to ensure that no mutation was introduced. The RNAi-untargeted Mre11 (1226 A→U) was made by site-mutagenesis using QuikChange Site-directed Mutagenesis Kit (Stratagene) following protocol provided by manufacturer. Primers used were: 5'-CCAAAGTTGATCTCTTCACCTGTTTTT TCCTTTTGT-3' and 5'-ACAAAAGGAAAA AACAGGTGAAGAGATCAACTTTGGG-3'. H1299 cells were infected with retrovirus and selected for stable blasticidin resistance.

***Terminal restriction fragment analysis (TRF)*:** Telomere length was determined by TRF analysis as described (Herbert et al., 2003).

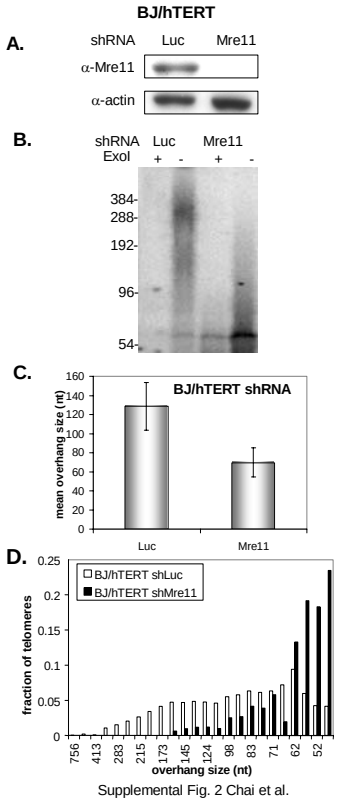
Supplemental Figures:

Supplemental Fig. 1. Overhang shortening induced by Mre11 RNAi is specific. H1299 cells expressing vector pWZL-blast only or an untargeted Mre11 (mis Mre11) were infected with retrovirus carrying Mre11 shRNA and selected for puromycin resistance. Seven days after selection, proteins were extracted for immunoblotting and DNA was isolated at the same time for telomere overhang protection assay. (A) The weighted mean overhang sizes of G-overhangs. Co-expression of Mre11 shRNA and the untargeted Mre11 (mis Mre11) rescued the overhang reduction caused by Mre11 knockdown. (B) Overhang size distribution in H1299 cells co-expressing Mre11 shRNA and the untargeted Mre11 (mis Mre11). The signal from each size region was divided by its size in nucleotides in order to compensate for increased probe hybridization with size. The resulting value was then expressed as a fraction of the total from all the regions spanning the entire lane above 45 nucleotides. (C) Relative abundance of G-overhangs from Mre11-RNAi Hela cells based on non-denaturing in-gel hybridization assays. Results are representative of two independent experiments with duplicate samples in each. Error bars represent one standard deviation. The results using the in-gel hybridization assay confirm those shown in the text Figure 1 using the overhang protection assay.



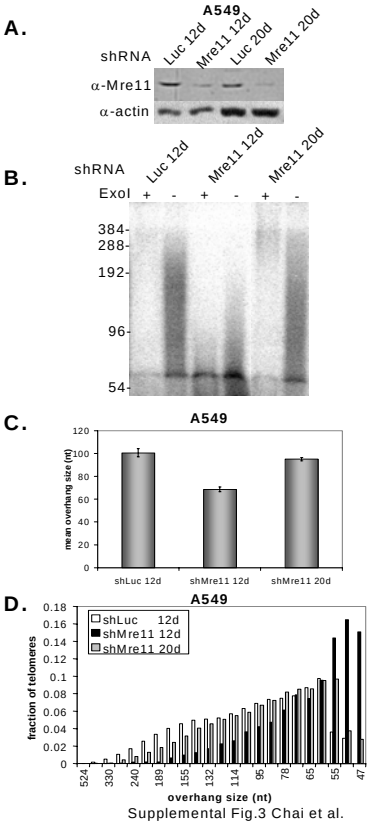
Supplemental Fig. 1 Chai et al.

Supplemental Fig. 2. Reducing Mre11 expression induces overhang shortening in BJ normal human fibroblasts expressing hTERT. (A) Western blot showing reduced Mre11 expression in BJ/hTERT cells after stably expressing shRNA targeting Mre11. Cells were collected 16 days after selection. Proteins were extracted for immunoblotting and DNA was isolated at the same time for the telomere overhang protection assay. (B) Telomere overhang protection assay using BJ/hTERT cells with reduced Mre11 expression. ExoI digests 3' overhangs and the ExoI plus (+) lanes show the background. (C) The weighted mean overhang sizes of G-overhangs from Mre11-RNAi BJ/hTERT cells. Results are representative of three independent experiments. Error bars represent one standard deviation. (D) Overhang size distribution in BJ/hTERT expressing Mre11 shRNA. The fraction of short overhangs was significantly increased in Mre11-RNAi BJ/hTERT cells.



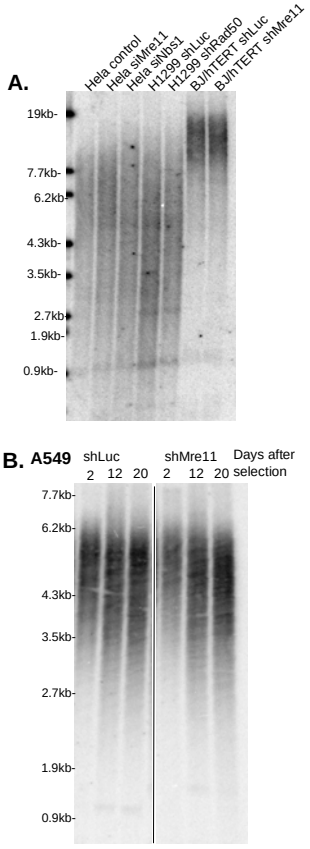
Supplemental Fig. 2 Chai et al.

Supplemental Fig. 3. Overhang shortening caused by MRN reduction is transient. A549 cells stably expressing luciferase (Luc) and Mre11 shRNA were collected 12 days and 20 days after selection. (A) Western blot showing reduction of Mre11 expression in A549 cells was stably maintained for at least 20 days after selection. Proteins were extracted for immunoblotting and DNA was isolated at the same time for telomere overhang protection assay in (B). (C) The weighted mean overhang sizes of G-overhangs from Mre11 diminished A549 cells. Results are representative of two independent experiments. Error bars represent one standard deviation. (D) Overhang size distribution of A549 cells with reduced Mre11 expression. Gels from the overhang protection assay were analyzed by Imagequant as described (Chai et al., 2005). Both the mean overhang size and overhang distribution were restored to normal after 20 days of selection.



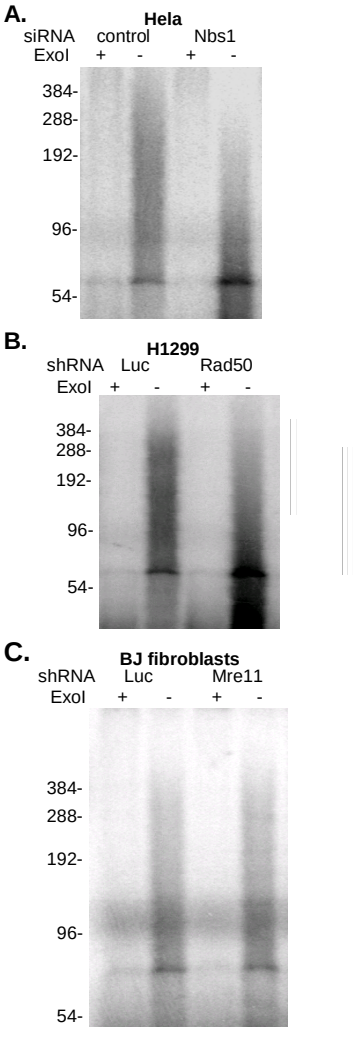
Supplemental Fig.3 Chai et al.

Supplemental Fig. 4. MRN reduction does not alter telomere length. (A) DNA from Hela cells transfected with control siRNA, Mre11 siRNA, and Nbs1 siRNA; H1299 cells stably expressing luciferase shRNA (Luc) and Rad50 shRNA; BJ/hTERT cells stably expressing luciferase shRNA and Mre11 shRNA was used for telomere restriction fraction analysis. The same DNA was used for overhang size measurement. No alteration of telomere length or loss of bulk telomere DNA was observed. (B) A549 cells stably expressing luciferase and Mre11 shRNA were collected 2 days, 12 days and 20 days after selection (the doubling time of A549 is approximately 30 hrs). DNA was isolated and used for telomere restriction fraction analysis. The same DNA was used for overhang size measurement in Supplemental Fig. 3. No alteration of telomere length was observed.



Supplemental Fig. 4 Chai et al.

Supplemental Fig. 5. Overhang Protection assays
(A) Telomere overhang protection assay using Hela cells with reduced Nbs1 expression. (B) Telomere overhang protection assay using H1299 cells with reduced Rad50 expression. (C) Telomere overhang protection assay using BJ cells with reduced Mre11 expression (seven days after selection). ExoI digests 3' overhangs and the ExoI plus (+) lanes show the background.



Supplemental Fig. 5 Chai et al.