Supplementary Data

-Supplementary Figures 1-5
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Supplementary Figure 1
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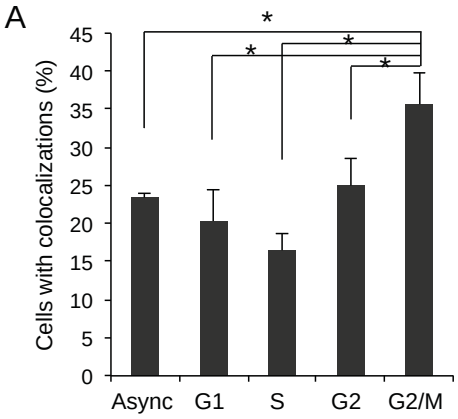

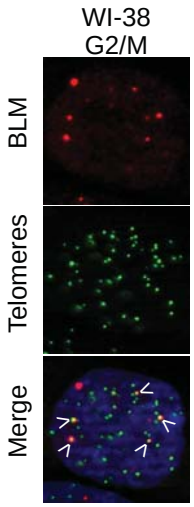

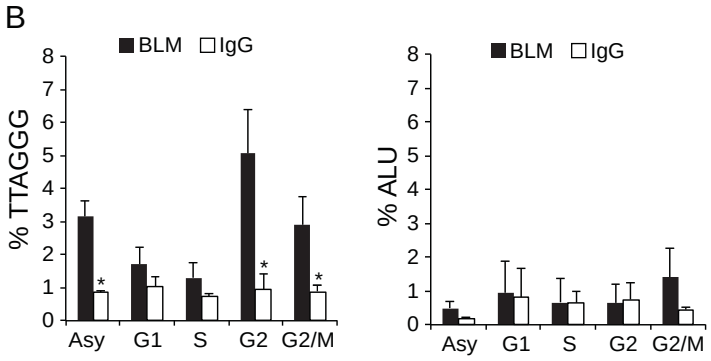

Supplementary Figure 2
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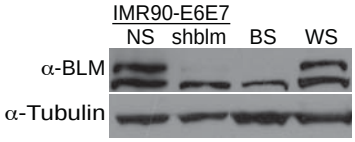

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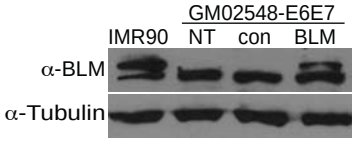

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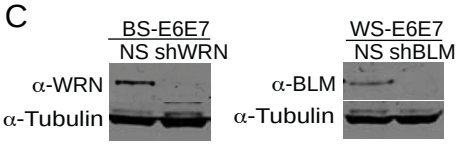

Supplementary Figure 3
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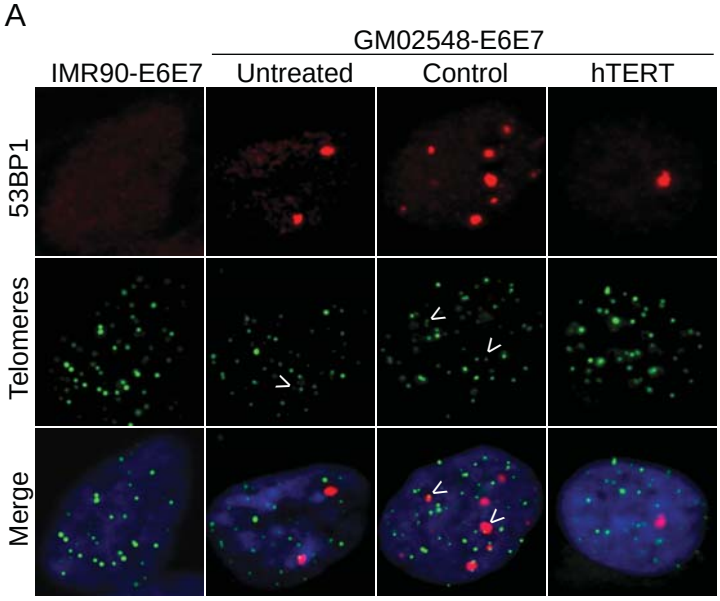

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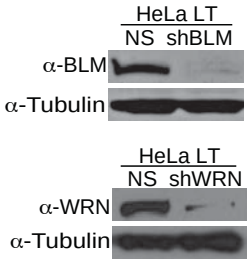

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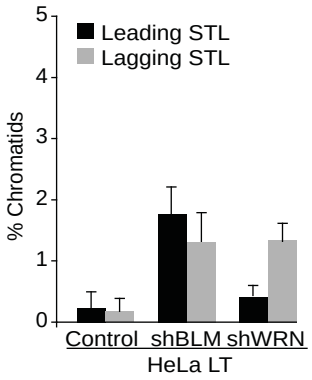

Supplementary Figure 4
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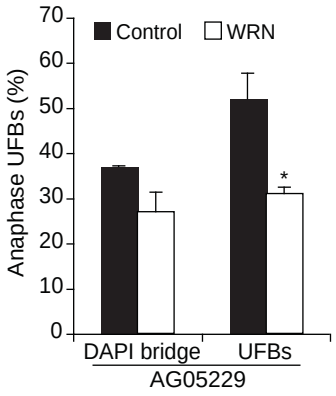

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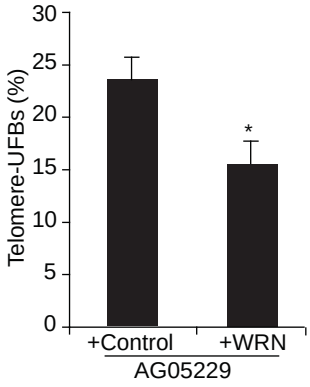

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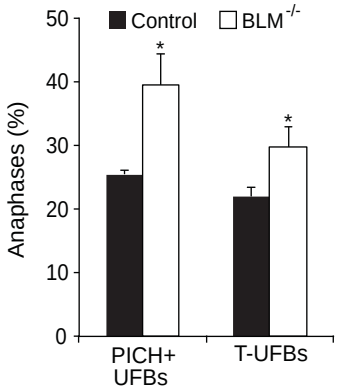

Supplementary Figure 5
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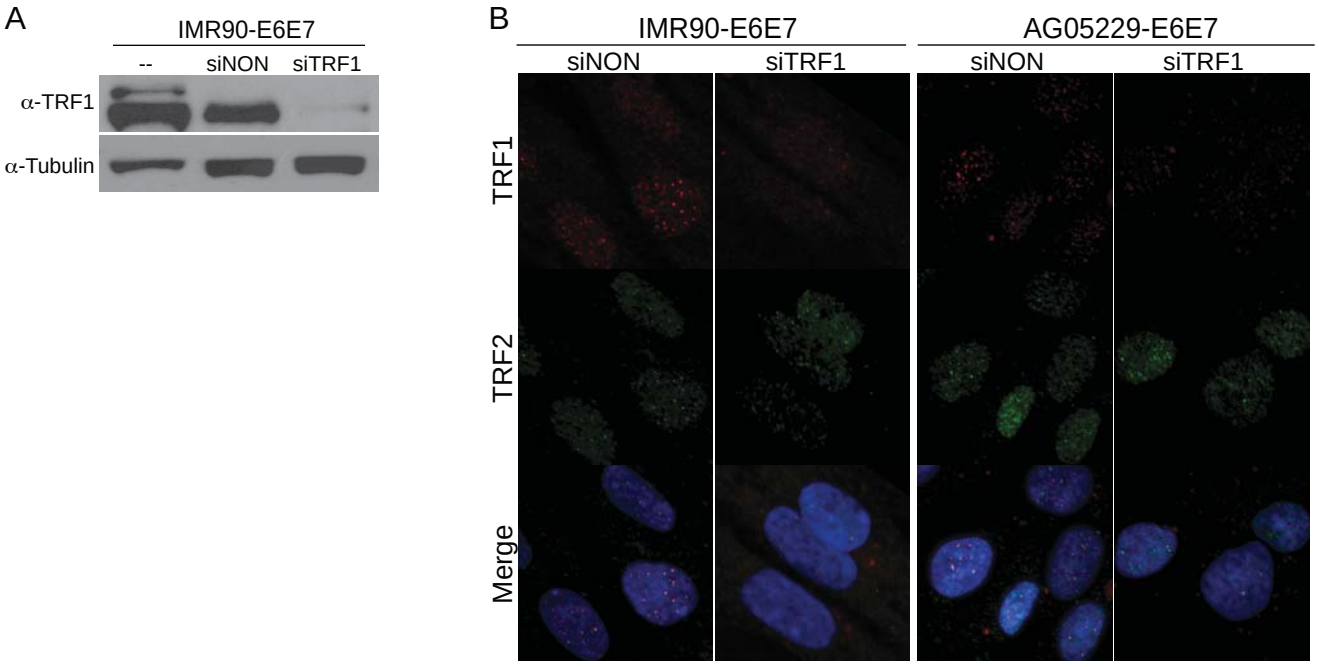

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Supplementary Table 1

Frequency of Telomere Defects after TRF1 suppression

	Telomere defects (% chromatids)		
	siNON	siBLM	siTRF1
IMR90-E6E7	2.8	4.1	5
AG05229-E6E7	5.1	7.2	12.2

Supplementary Legends

Supplementary Figure 1. **(A)** Quantification of IF-FISH data, showing the percentage of WI38 cells with over two colocalization events. At least 100 cells were counted per time-point and values were averaged from four independent experiments. Error bars represent the standard deviation and *p* values were calculated with a Student's t-test (*p<0.05). **(B)** Quantification of ChIP data of synchronized IMR90 cells. The values shown here are the percentage of telomeric (TTAGGG) or control (ALU) DNA bound by the BLM antibody at the indicated cell cycle stage; the fraction of DNA bound by an IgG antibody serves as negative control. Error bars represent the standard deviation of three independent experiments.

Supplementary Figure 2. BLM knockdown in normal fibroblasts or BLM overexpression in BS cells. **(A)** Western blot showing BLM expression in IMR90-E6E7 fibroblasts transduced with a retrovirus expressing non-specific shRNA (NS) or shRNA targeting BLM, in GM02548-E6E7 Bloom syndrome cells (BS) and in AG05229-E6E7 Werner syndrome cells (WS). **(B)** Western blot showing BLM expression in Bloom's syndrome cells after full length BLM was retrovirally delivered in GM02548-E6E7 BS fibroblasts. **(C)** Western blot of WRN or BLM expression upon shRNA mediated targeting, using Tubulin as loading control. In all three experiments, IMR90 and BS fibroblasts were treated with puromycin to generate stable cell lines.

Supplementary Figure 3. (A) DNA damage foci and telomere damage-induced foci (TIFs) are elevated in BS fibroblasts. Production of IMR90-E6E7 and GM02548-E6E7 fibroblast cell lines expressing hTERT was described previously. Cells grown on glass coverslips were fixed for IF-FISH with 53BP1 (red) and a telomeric probe (green). 53BP1 foci indicated a DNA damage event; and these foci were counted as a TIF when localized with telomeric signal (A) Examples of IF-FISH in indicated fibroblast cell lines, representing DNA damage events and/or 53BP1-positive TIFs, which are indicated with white arrows. **(B)** Western Blot of control HeLa LT cells or cells expressing shBLM or shWRN RNAs. α-tubulin serves as loading control. **(C)** Quantification of STL of leading and lagging strand telomeres of control HeLa LT cells or cells expressing shBLM or shWRN RNAs. The error bars represent the standard deviation of three independent experiments.

Supplementary Figure 4. Reconstituted WRN in AG05229 cells reduces the frequency of BLM-positive UFB formation and suppresses UFB formation at telomeres. AG05229 cells were transduced with a vector control or the full length WRN cDNA. Cells were selected with puromycin to generate stable cell lines and fixed for IF-FISH with BLM (red) and a telomeric probe (green). **(A)** Quantification of the percentage of anaphases with UFBs and **(B)** the percentage of UFBs that extend from telomeres (T-UFBs). Values were averaged from at least 30 anaphases per cell line, from two independent experiments. In both panels, error

bars represent standard deviation and *p* values were calculated by a Student's t-test (*$p<0.05$ and **$p<0.005$). **(C)** Control **I**MR90-E6E7 cells or **I**MR90-E6E7 cells expressing shBLM RNAs were stained with anti-PICH antibody and a FITC labeled telomeric probe. Cells were counted, imaged and analyzed for PICH-positive UFBs and UFBs extending from telomeric foci. Average values were calculated from two independent experiments where at least 100 anaphases were counted for PICH-positive staining and at least 25 anaphases were analyzed for TUFBs. Error bars represent standard deviation and *p* values were calculated by a Student's t-test (*$p<0.05$).

Supplementary Figure 5. **(A)** Western analysis and **(B)** IF analysis of shRNA based TRF1 suppression in IMR90-E6E7 and AG05229-E6E7 cells, using antibodies against TRF1 (red) and TRF2 (green).

Supplementary Table 1. Frequency of chromatids with telomere defects in fibroblast cells following TRF1 knockdown. IMR90-E6E7 and AG05229-E6E7 WS cells were treated with siRNA against TRF1 or a non-specific siRNA (Dharmacon). After 72 hours, cells were prepared for FISH of metaphase chromosomes. Telomere defects (TDs) were determined by analysis of metaphase spreads; at least 1500 chromatids were counted per cell line.