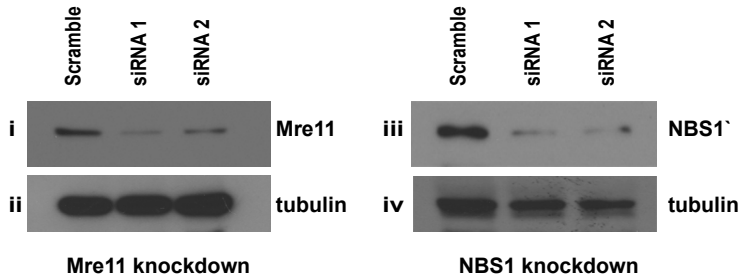

Figure. S1
ES129.1 cells were subjected to RNAi-mediated knockdown of Mre11 and NBS1. Western-blot analysis with antisera against Mre11, NBS1 and beta-tubulin, showing a reduction in the expression levels of Mre11 and NBS1, respectively.

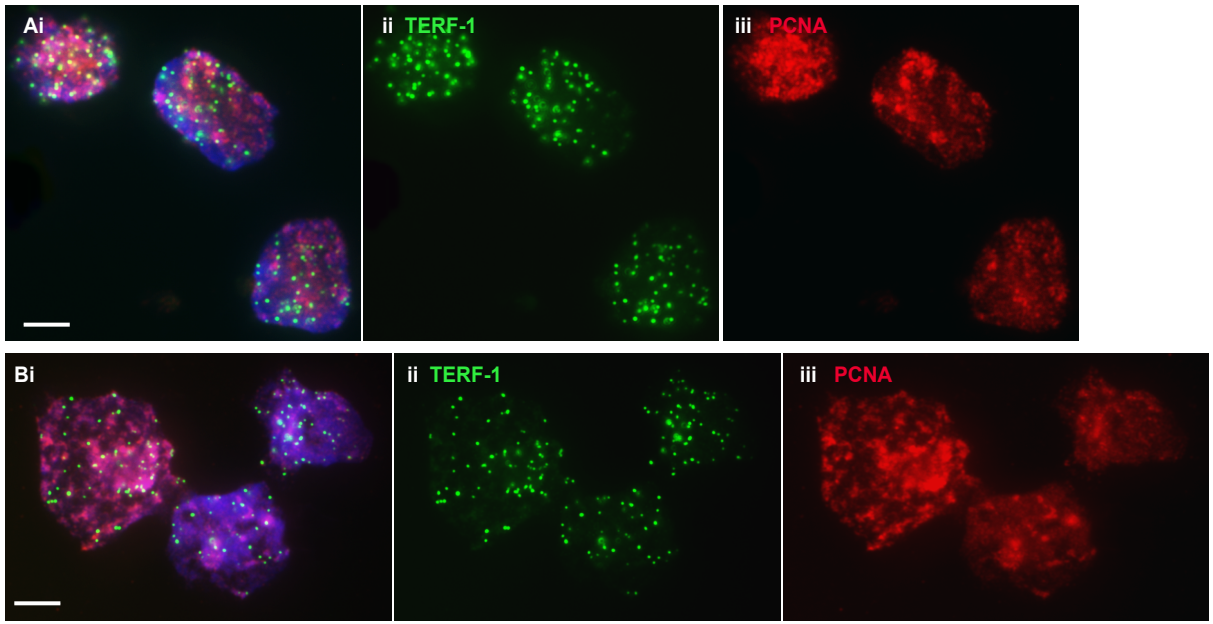

Figure S2. PCNA staining in mouse ES cells depleted of PML bodies.
RNAi-mediated knockdown of PML was performed on ES129.1 cells with specific siRNA oligonucleotides for 48hr, followed by immunofluorescence analysis. Compared to cells transfected with scramble siRNA (A), depletion of PML bodies (B) did not affect the progression of cell cycle or progression of cells through S phase as indicated by the presence of cells with positive PCNA staining (refer to Supplementary material, Table S2c quantitative analysis).

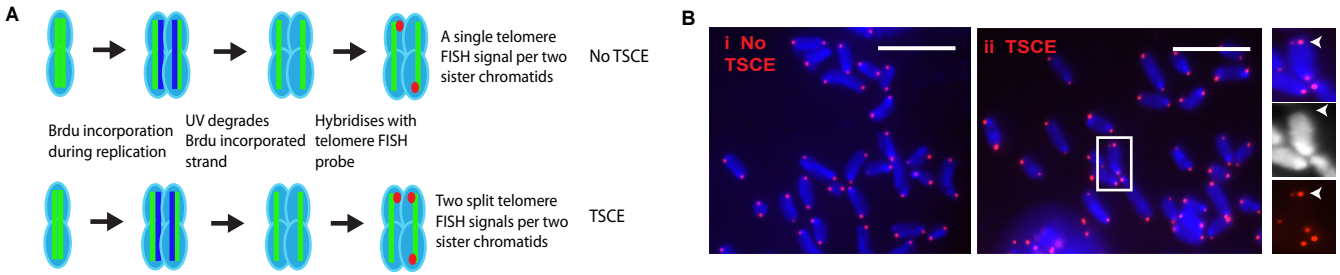

Figure S3. (A) Principle of CO-FISH. Newly replicating DNA strands incorporate BrdU during replication.
Treatments with Hoechst and UV specifically degrade these strands and leave each sister-chromatid single-stranded.
Consequently a strand-specific telomeric probe will hybridise with only one of the two sister-chromatids at the telomere and show a single signal.
When TSCE occurs after replication, splitting of the signal between both chromatids occurs and a double-signals will be detected.
The intensities of the two signals may not be symmetric, depending on the site of exchange within the telomere.
(B) CO-FISH analysis was performed with telomere probe on ES129.1 cells depleted of PML expression.
Examples of metaphase cells showing either absence (left cell) or presence (right cell) of TSCE are shown.
Data collected for CO-FISH analysis was presented in Supplementary Table S3b.

Supplementary data

Table S1a. RNAi-mediated knockdown of NBS1 and Mre11. RNAi-mediated knockdown was performed on ES129.1 cells with specific siRNA oligonucleotides against NBS1 and Mre11, and a set of scramble control siRNA oligonucleotides for 48hr. Cells were harvested for reverse-transcription and real-time PCR analysis. Expression levels of NBS1 and Mre11 were measured as Ct values and normalized against Ct values of actin to give ΔCt values (=NBS1 or Mre11 Ct value- Actin Ct value). Average ΔCt values were calculated from 3 sets of ΔCt values. ΔΔCt values were calculated as differences in ΔCt values (=NBS1 or Mre11 RNAi ΔCt - control RNAi ΔCt) of samples subjected to knockdown with specific siRNA oligonucleotides and those with control scramble siRNA oligonucleotides. Expression ratios and percentages of knockdown were calculated as 2-ΔΔCt and [1 - Expression Ratio] x100%, respectively. The data showed a reduction of 74.9% to 78.8% in NBS1 level and 77.46% to 84.69% in Mre11 level, respectively.

NBS1 knockdown N=1

Samples	Primer set	Average Ct	ΔCt	ΔΔCt	Fold of expression	% knockdown
Scramble control	NBS1 primer set 1	25.26377133	7.256732667			
NBS1 RNAi set 1		27.67566567	8.804118	1.547385333	0.342129561	**65.78704392**
NBS1 RNAi set 2		27.01070667	9.622971667	2.366239	0.193950581	**80.60494194**
Scramble control	NBS1 primer set 2	25.42374733	7.416708667			
NBS1 RNAi set 1		28.00570467	9.134157	1.717448333	0.304086077	**69.59139231**
NBS1 RNAi set 2		26.91123933	9.523504333	2.106795667	0.232162093	**76.78379074**
Scramble control	NBS1 primer set 3	25.41877167	7.411733			
NBS1 RNAi set 1		28.07520367	9.203656	1.791923	0.288786859	**71.1213141**
NBS1 RNAi set 2		27.12061967	9.732884667	2.321151667	0.200107665	**79.98923352**
Scramble control	Actin primer	18.00703867				
NBS1 RNAi set 1		18.87154767				
NBS1 RNAi set 2		17.387735				

NBS1 knockdown N=2

Samples	Primer set	Average Ct	ΔCt	ΔΔCt	Fold of expression	% knockdown
Scramble control	NBS1 primer set 1	26.837439	7.163034333			
NBS1 RNAi set 1		29.098551	9.670301667	2.507267333	0.175888452	**82.41115485**
NBS1 RNAi set 2		28.84305767	9.583289667	2.420255333	0.186823089	**81.31769115**
Scramble control	NBS1 primer set 2	27.27044767	7.596043			
NBS1 RNAi set 1		29.25820733	9.829958	2.233915	0.212581064	**78.74189356**
NBS1 RNAi set 2		28.4302395	9.1704715	1.5744285	0.335776112	**66.42238884**
Scramble control	NBS1 primer set 3	27.23280633	7.558401667			
NBS1 RNAi set 1		29.67916867	10.25091933	2.692517667	0.154693269	**84.53067305**
NBS1 RNAi set 2		28.71013367	9.450365667	1.891964	0.26944001	**73.05599904**
Scramble control	Actin primer	19.67440467				
NBS1 RNAi set 1		19.42824933				
NBS1 RNAi set 2		19.259768				

NBS1 knockdown N=3

Samples	Primer set	Average Ct	ΔCt	ΔΔCt	Fold of expression	% knockdown
Scramble control	NBS1 primer set 1	26.09737533	6.532164			
NBS1 RNAi set 1		28.05081367	9.129084	2.59692	0.165290991	**83.47090089**
NBS1 RNAi set 2		27.54149033	8.097568333	1.565404333	0.337882999	**66.2117001**
Scramble control	NBS1 primer set 2	26.304097	6.738885667			
NBS1 RNAi set 1		28.430359	9.508629333	2.769743667	0.146630419	**85.33695808**
NBS1 RNAi set 2		28.24687867	8.802956667	2.064071	0.23914027	**76.08597297**
Scramble control	NBS1 primer set 3	26.224467	6.659255667			
NBS1 RNAi set 1		28.69802267	9.776293	3.117037333	0.115259907	**88.47400929**
NBS1 RNAi set 2		28.043052	8.59913	1.939874333	0.260639142	**73.93608577**
Scramble control	Actin primer	19.56521133				
NBS1 RNAi set 1		18.92172967				
NBS1 RNAi set 2		19.443922				

NBS1 RNAi sets	Average % knockdown of NBS 1 from 3 experiments
NBS1 RNAi set 1	**78.82948223**
NBS1 RNAi set 2	**74.93420045**

Mre11 knockdown N=1

Primer set	Samples	Average Ct	ΔCt	ΔΔCt	Fold of expression	% knockdown
Mre11 primer set 1	Scramble control	24.65532633	6.132106333			
	Mre11 RNAi set 1	27.971309	9.047531333	2.915425	0.132546915	**86.74530848**
	Mre11 RNAi set 2	26.9752635	8.341522167	2.209415833	0.216221841	**78.37781588**
Mre11 primer set 2	Scramble control	24.92914567	6.405925667			
	Mre11 RNAi set 1	28.87453067	9.950753	3.544827333	0.08568418	**91.43158203**
	Mre11 RNAi set 2	27.30910167	8.675360333	2.269434667	0.207411146	**79.25888535**
Actin Primer	Scramble control	18.52322				
	Mre11 RNAi set 1	18.92377767				
	Mre11 RNAi set 2	18.63374133				

Mre11 knockdown N=2

Primer set	Samples	Average Ct	ΔCt	ΔΔCt	Fold of expression	% knockdown
Mre11 primer set 1	Scramble control	24.78264233	6.064400333			
	Mre11 RNAi set 1	26.679978	8.898928333	2.834528	0.140191618	**85.98083819**
	Mre11 RNAi set 2	25.84722033	7.890400667	1.826000333	0.282045469	**71.79545308**
Mre11 primer set 2	Scramble control	24.82210433	6.103862333			
	Mre11 RNAi set 1	26.53305967	8.75201	2.648147667	0.159524767	**84.04752328**
	Mre11 RNAi set 2	25.675893	7.719073333	1.615211	0.326417203	**67.35827966**
Actin Primer	Scramble control	18.718242				
	Mre11 RNAi set 1	17.78104967				
	Mre11 RNAi set 2	17.95681967				

Mre11 knockdown N=3

Primer set	Samples	Average Ct	ΔCt	ΔΔCt	Fold of expression	% knockdown
Mre11 primer set 1	Scramble control	23.50240933	5.053729667			
	Mre11 RNAi set 1	26.01550333	6.979964333	1.926234667	0.263114987	**73.68850127**
	Mre11 RNAi set 2	25.770029	7.124001	2.070271333	0.238114712	**76.18852879**
Mre11 primer set 2	Scramble control	23.869041	5.420361333			
	Mre11 RNAi set 1	26.44168967	7.406150667	1.985789333	0.252474689	**74.75253112**
	Mre11 RNAi set 2	26.362578	7.71655	2.296188667	0.203600263	**79.63997365**
Actin Primer	Scramble control	18.44867967				
	Mre11 RNAi set 1	19.035539				
	Mre11 RNAi set 2	18.646028				

Mre11 knockdown N=4

Primer set	Samples	Average Ct	ΔCt	ΔΔCt	Fold of expression	% knockdown
Mre11 primer set 1	Scramble control	25.50081233	6.463228667			
	Mre11 RNAi set 1	28.00497467	9.645509667	3.182281	0.110163561	**88.98364394**
	Mre11 RNAi set 2	26.8625135	9.084040167	2.6208115	0.162576259	**83.74237411**
Mre11 primer set 2	Scramble control	25.45127733	6.413693667			
	Mre11 RNAi set 1	28.394948	10.035483	3.621789333	0.081233052	**91.87669476**
	Mre11 RNAi set 2	26.773127	8.994653667	2.58096	0.167129696	**83.28703043**
Actin Primer	Scramble control	19.03758367				
	Mre11 RNAi set 1	18.359465				
	Mre11 RNAi set 2	17.77847333				

Mre11 RNAi sets	**Average % knockdown of MRE11 from 4 experiments**
Mre11 set 1	**84.68832788**
Mre11 set 2	**77.45604262**

Table S1b. FACS analysis of NBS1 and Mre11 knockdown cells. RNAi-mediated knockdown was performed on ES129.1 cells with NBS1 and Mre11 specific siRNA oligonucleotides and a set of scramble control siRNA oligonucleotides for 48hr. Cells were harvested for FACS analysis and percentage of cell population in various stages of cell cycle was shown. RNAi-mediated depletion of NBS1 and Mre11 for 48hr, respectively, did not have a significant impact on the progression of cell cycle.

Cell cycle stages		**G1**	**S**	**G2/M**
Average values (% of cell)	Scramble control RNAi	17.17	68.67	14.34
	NBS1 RNAi set#1	17.99	69.50	12.51
	NBS1 RNAi set#2	16.71	70.87	12.44

Cell cycle stages		**G1**	**S**	**G2/M**
Average values (% of cell)	Scramble control RNAi	18.35	70.18	11.64
	Mre11 RNAi set#1	17.31	73.14	9.74
	Mre11 RNAi set#2	17.94	68.95	13.44

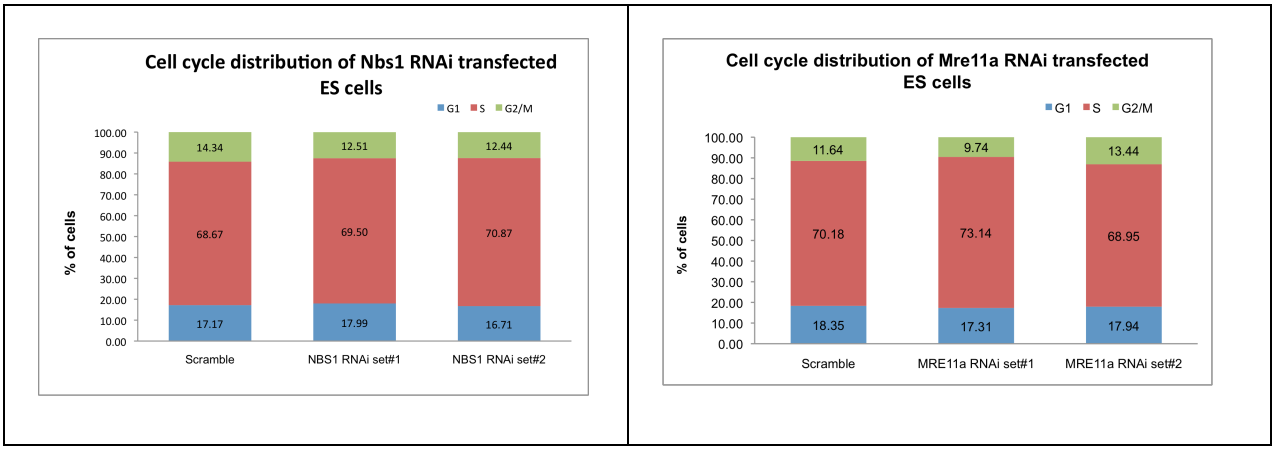

Table S1c. Immunofluorescence analysis of NBS1 and Mre11 knockdown cells. (c) RNAi-mediated knockdown was performed on ES129.1 cells with NBS1 and Mre11 specific siRNA oligonucleotides and a set of scramble control siRNA oligonucleotides for 48hr. Cells were harvested for immunofluorescence analysis and quantification of telomeric signals at PML bodies as indicated by co-staining of telomere FISH and anti-PML antibody staining signals. Percentage of cell population showing positive foci of telomeres and PML bodies remained unchanged between the control cells and those depleted of either NBS1 or Mre11. These data indicate that loss of NBS1 and Mre11 did not have a significant effect on the assembly of telomere-associated PML bodies in mouse ES cells.

		Control RNAi	**NBS1 RNAi set#1**	**NBS1 RNAi set#2**
Number of co-localised foci (% of cell population)	**<4**	38.31	48.21	46.31
	5 to 9	39.22	36.48	29.28
	10 to 14	16.25	10.32	13.97
	>14	6.22	6.84	11.85
% of cell with ≥5 positive foci		**61.69**	**51.795**	**53.69**

		Control RNAi	**Mre11 RNAi set#1**	**Mre11 RNAi set#2**
Number of co-localised foci (% of cell population)	**<4**	35.29	32.67	35.29
	5 to 9	40.14	45.81	43.58
	10 to 14	18.35	14.30	13.21
	>14	6.22	7.23	7.92
% of cell with ≥5 positive foci		**64.71**	**67.33**	**64.71**

Table S2a. RNAi-mediated knockdown of PML. RNAi-mediated knockdown of PML was performed on ES129.1 cells using 4 sets of specific siRNA oligonucleotides for 48hr. Cells were harvested for reverse-transcription and real-time PCR assays. Expression level of PML was measured as Ct values and normalized against Ct values of actin to give ΔCt values (=PML Ct value- Actin Ct value). Average ΔCt values were calculated. ΔΔCt values were calculated as differences in ΔCt values (=PML RNAi ΔCt - control RNAi ΔCt) of samples subjected to knockdown with PML-specific siRNA oligonucleotides and those with control scramble siRNA oligonucleotides. Expression ratios and percentages of knockdown were calculated as 2-ΔΔCt and [1 - Expression Ratio] x100%, respectively. The data showed a reduction of 57.80% to 76.65% in PML level. siRNA oligonucleotides set#3 and #4 were used in further experiments.

PML Primer set 1	**Average Ct**	**ΔCt**	**ΔΔCt**	**Fold of expression**	**% of Knockdown**
Scramble control	21.6266455	6.2360745			
PML RNAi set#1	22.989065	7.607481	1.3714065	0.386514247	61.3
PML RNAi set#2	23.33388	7.4574755	1.221401	0.428866044	57.1
PML RNAi set#3	24.012379	8.599505	2.3634305	0.194328513	80.6
PML RNAi set#4	24.0316495	8.440722	2.2046475	0.216937671	78.3
PML Primer set2	**Average Ct**	**ΔCt**	**ΔΔCt**	**Fold of expression**	**% Knockdown**
Scramble control	21.097066	5.706495			
PML RNAi set#1	22.217684	6.8361	1.129605	0.457040843	54.3
PML RNAi set#2	22.439604	6.5631995	0.8567045	0.552212519	44.8
PML RNAi set#3	23.2923735	7.8794995	2.1730045	0.221748385	77.8
PML RNAi set#4	23.299015	7.7080875	2.0015925	0.249724193	75.0
Actin primer	**Average Ct**				
Scramble control	15.390571				
PML RNAi set#1	15.381584				
PML RNAi set#2	15.8764045				
PML RNAi set#3	15.412874				
PML RNAi set#4	15.5909275				

PML RNAI	**Average % of knockdown**
PML RNAi set#1	**57.80**
PML RNAi set#2	**50.95**
PML RNAi set#3	**79.20**
PML RNAi set#4	**76.65**

Table S2b. FACS analysis of PML knockdown cells. RNAi-mediated knockdown of PML was performed on ES129.1 cells using PML specific siRNA oligonucleotides and a set of scramble control oligonucleotides. Cells were harvested for FACS analysis. The depletion of PML expression (after 48hr) has no immediate or significant impact on the cell cycle progression of the cell population.

Cell cycle stages	G1 (%)	S (%)	G2/M (%)
Untransfected	16.84	16.84	16.84
Control RNAi	17.96	64.30	17.76
PML RNAi set#1	16.73	64.26	19.02
PML RNAi set#2	16.15	63.23	20.63

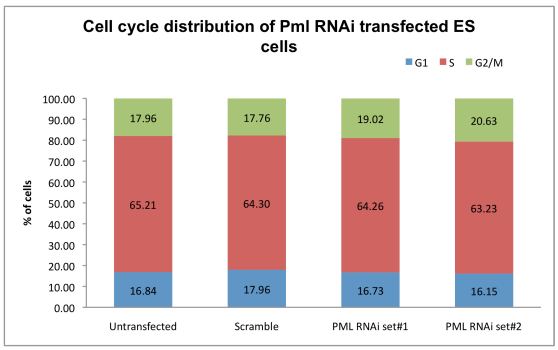

Table S2c. PCNA staining of PML knockdown cells. RNAi-mediated knockdown of PML was performed on ES129.1 cells using PML specific siRNA oligonucleotides and a set of scramble control oligonucleotides. Cells were harvested for immunofluorescence analysis using an antibody against PCNA (see Supplementary Figure S1), an antigen that is expressed in the nuclei of cells during the DNA synthesis or S phase of the cell cycle. RNAi-depletion of PML did not have any impact of percentage of cells showing PCNA staining, suggesting that the progression of cells through S phase was not affected.

		Telomere-associated PML bodies					
Time period after G1/S release (hours) Expt N=1		**0**	**2**	**4**	**6**	**8**	**10**
Scramble control	Cells with positive PCNA foci (35 cells)	4	12	26	32	12	9
	Percentage of cell population with positive PCNA foci (%)	11.00	34.29	74.26	91.43	34.26	25.71
PML RNAi	Number of cells with positive PCNA foci (35 cells)	5	10	23	29	11	10
	Percentage of cell population with positive PCNA foci (%)	**14.29**	**28.57**	**65.71**	**82.86**	**31.42**	**28.57**

		Telomere-associated PML bodies					
Time period after G1/S release (hours) Expt N=2		**0**	**2**	**4**	**6**	**8**	**10**
Scramble control	Cells with positive PCNA foci (35 cells)	6	9	23	33	10	7
	Percentage of cell population with positive PCNA foci (%)	17.14	25.71	65.71	94.28	28.57	20.00
PML RNAi	Number of cells with positive PCNA foci (35 cells)	7	13	29	32	18	12
	Percentage of cell population with positive PCNA foci (%)	**20.00**	**37.14**	**82.86**	**91.43**	**51.43**	**34.29**

Table S3a. Staining with anti-53BP1 antibody in PML knockdown cells. In PML RNAi-mediated knockdown ES129.1 cells, TIFs were determined by staining with an antibody against a DNA damage marker- 53BP1. In ES129.1 cells transfected with control scramble siRNA oligonucleotides, only 10% to 14% of cells showed ≥5 TIFs per cell. In contrast, ES129.1 cells subjected to transfection with PML-specific siRNA oligonucleotides showed an increase by 3 to 5 fold (from 10%-14% to 38%-46%) in the population of cells showing ≥5 TIFs per cell, indicating that telomere functional integrity was impaired following the loss of PML bodies. Data from the scoring and quantification was presented in Figure 4.

NO of TIFs	**Expt n=1**		**Expt n=2**		**Expt n=3**	
	Control	**PML RNAi**	**Control**	**PML RNAi**	**Control**	**PML RNAi**
<5	44	31	43	27	45	27
5 to 9	6	13	5	16	3	15
10 to 14	0	5	2	6	2	5
>14	0	1	0	1	0	3
Total	50	50	50	50	50	50
	Percentage of cell population (%)					
	Expt n=1		**Expt n=2**		**Expt n=3**	
	Control	**PML RNAi**	**Control**	**PML RNAi**	**Control**	**PML RNAi**
<5	88%	62%	86%	54%	90%	54%
5 to 9	12%	26%	10%	32%	6%	30%
10 to 14	0%	10%	4%	12%	4%	10%
>14	0%	2%	0%	2%	0%	6%
Total	100%	100%	100%	100%	100%	100%

Table S3b CO-FISH analysis in PML knockdown cells. CO-FISH assay (Supplementary Figure S2) was used to determine the rate of TSCE following the loss of PML bodies in ES129.1 cells. Number of metaphase chromosomes showing double-signals out of the total of 600 was determined, in 3 sets of experiments. No significant increase in the rate of TSCE was detected in ES129.1 cells following RNAi-mediated knockdown of PML and subsequently loss of function of PML bodies.

Expt n=1		Expt n=2		Expt n=3	
Control	PML RNAi	Control	PML RNAi	Control	PML RNAi
2/150	4/150	4/150	6/150	5/150	3/150
3/150	6/150	4/150	4/150	4/150	6/150
3/150	1/150	7/150	6/150	2/150	6/150
2/150	2/150	2/150	4/150	3/150	6/150
Average =10/600	Average =13/600	Average =17/600	Average =20/600	Average =14/600	Average =21/600
Average percentage of TSCE (%)					
Control	PML RNAi	Control	PML RNAi	Control	PML RNAi
1.7%	2.2%	2.8%	3.3%	2.3%	3.5%

Table S4. CHIP analysis in PML knockdown cells. ES129.1 cells were transfected with either scramble control or PML-specific siRNA oligonucleotides (set#2). ChIP analysis was performed using antisera against H3K9me3, H4K20me3, ATRX and TERF-1, followed by dot blot analysis by hybridization with a γ-32p ATP end-labelled telomere-specific probe. The membrane was exposed overnight on a Typhoon PhosphoImager screen. The intensities of the signals (average signals from 8 sets of experiments) were quantitated using ImageQuant software. The graph is present is Figure 6. The data showed that there were increases in the levels of H3K9me3 and H4K20me3 at telomeres by ~2.3 and 3.4 folds, respectively. In addition, there was an increase in the level of TERF-1 by 2.5 fold, whereas, ATRX level was reduced by half (0.42 fold), indicating a change in telomere chromatin properties in cells depleted of PML bodies.

		H3K9me3/H4	**H4K20me3/H4**	**ATRX/H4**	**TERF-1/H4**
Set 1	**control RNAi**	4.312372608	1.589548722	3.657718723	0.457781679
	PML RNAi	10.60924814	6.597930243	1.78199156	0.934848117
Set 2	**control RNAi**	3.497399774	1.944504368	2.547526039	0.622432459
	PML RNAi	9.099310986	6.519464544	1.686144298	0.564184344
Set 3	**control RNAi**	3.766466342	0.702050028	3.176433747	0.368899084
	PML RNAi	12.52727107	1.963834465	1.130557592	1.406845001
Set 4	**control RNAi**	3.999414621	0.186866765	2.715411761	1.079034493
	PML RNAi	9.873186165	1.734839595	0.923926565	1.430664885
Set 5	**control RNAi**	4.312372608	1.076694409	5.709135979	0.355210816
	PML RNAi	7.583572714	7.134483494	1.412933163	0.676673907
Set 6	**control RNAi**	3.056582117	1.212031806	4.835579463	0.411449347
	PML RNAi	10.89478202	6.716990087	3.411391508	1.332049875
Set 7	**control RNAi**	4.166925022	2.337286346	4.203692081	0.064487967
	PML RNAi	5.712851623	4.056942018	0.526188307	1.509585288
Set 8	**control RNAi**	5.337696703	2.398181595	2.922381585	0.924472095
	PML RNAi	10.45528454	4.557298173	1.604104277	2.951697576

N=8		**H3K9me3**	**H4K20me3**	**ATRX**	**TERF-1**
Fold of enrichment	**Control RNAi**	**4.056153724**	**1.430895505**	**3.720984922**	**0.535470993**
	PML RNAi	**9.594438407**	**4.910222827**	**1.559654659**	**1.350818624**
	Fold difference	**2.37**	**3.43**	**0.42**	**2.52**