EXPERIMENTAL PROCEDURES OF SUPPLEMENTAL DATA

Transient transfection in HEK293T cells and immunoprecipitation — 2μg of each plasmid DNA was transiently transfected into HEK293T cells using TransIT-LT-1 Transfection Reagents (Milus). After 24-48 hrs incubation, transfected cells were rinsed with PBS, harvested, and lysed in the ice-cold lysis buffer (50mM Tris-HCl [pH7.5], 20% glycerol, 1mM EDTA, 150mM NaCl, 0.5% Triton X-100, 0.02% SDS, 1mM DTT, and a protease inhibitor cocktail (Roche)). After incubation on ice for 5 min, 5M NaCl was added until reaching a final concentration of 400mM NaCl, and the incubation was continued for 5 min. Then, an equal volume of cold-water was added and mixed. The treated samples were centrifuged and supernatants were collected as total cell extracts. Extracts were immunoprecipitated with anti-GFP polyclonal antibody (MBL) or anti-MYC monoclonal antibody (9E10) overnight at 4°C. The immunoprecipitated samples were analyzed by SDS-PAGE and immunoblotted with mouse anti-FLAG (M2, Sigma), anti-MYC or anti-GFP antibodies

Indirect Immunofluorescence Analysis—Harvested cells were suspended in hypotonic buffer (10mM Tris-HCl [pH7.5], 20mM NaCl, 5mM KCl, 1mM CaCl₂, 0.5mM MgCl₂, and 50% Glycerol), fixed with 4% paraformaldehyde and with 70% ethanol. After spotting onto glass slides by centrifugation, the fixed cells were permeabilized with 0.1% TritonX-100 and incubated with anti-FLAG or anti-GFP antibodies. Alexa-488 or Alexa-568 conjugated goat IgG were used as secondary antibodies. Labeled cells were then examined by fluorescence microscopy (Axioplan II, ZEISS).

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Western Blotting—Cell extractes lysed and sonicated into sample buffer were analyzed by SDS-PAGE and immunoblotted with anti-TRF1 (Iwano et al. 2004), anti-FLAG or anti-MYC antibodies.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Telomeric localization of GFP-mTPP1, GFP-mPOT1a, and EGFP-mPOT1b. (**A**) *mTRF1* △ ES cells that expressed FLAG-mTRF1 were transfected with either the GFP-mTPP1, -mPOT1a, or -mPOT1b expression construct. FLAG-mTRF1 and GFP fusion molecules were detected by anti-FLAG (red) and anti-EGFP (green) antibodies. The two immunofluorescence images (red and green) for each cell type were superimposed (Merge) to evaluate co-localization (yellow). (**B-F**) Telomeric foci formation of GFP-mTPP1, -mPOT1a and -mPOT1b in different *mTRF1 cond-KO* ES cell lines with and without OHT treatment. Used cell lines were following: (**B**) *mTRF1 cond-KO* cells, (**C**) *mTRF1 cond-KO* cells that

expressed FLAG-mTRF1, (**D**) *mTRF1 cond-KO* cells that expressed FLAG-cmTRF1 (**E**) *mTRF1 cond-KO* cells that over-expressed FLAG-mTRF2, (**F**) *mTRF1 cond-KO* cells that expressed FLAG-TIN2-cmTRF1.

Supplemental Figure 2. Interaction of mTIN2 with mTRF1 or cTRF1. HEK293T cells were co-transfected with the MYC-mTIN2 expression construct and either the FLAG-mTRF1 or the FLAG-cTRF1 expression construct (Input). Cell lysates were immunoprecipitated with anti-MYC antibody and subjected to Western blot analysis (WB). * The asterisk in the upper panel indicates a signal from the anti-mouse IgG heavy chain.

Supplemental Figure 3. Alignment for mouse and chicken TRF1. Alignment for mouse and chicken TRF1 was analyzed in BLAST site of NCBI homepage http://www.ncbi.nlm.nih.gov/blast/Blast.cgi. Dark and light grey box indicates TRFH and Myb domain of both TRF1. Red line shows replaced site of mouse and chicken TRF1 to make cmTRF1.

Supplemental Figure. 4 Interaction of cmTRF1 with telomeric components. FLAG-cmTRF1 and GFP-fused telomeric proteins (FLAG-mTRF1 and GFP-mTIN2 as a positive control) were co-transfected in HEK293T cells and these lysates were immunoprecipitated with anti-GFP polyclonal antibody. Western blot analysis was performed by using anti-FLAG or anti-GFP antibody. * indicates non-specific band.

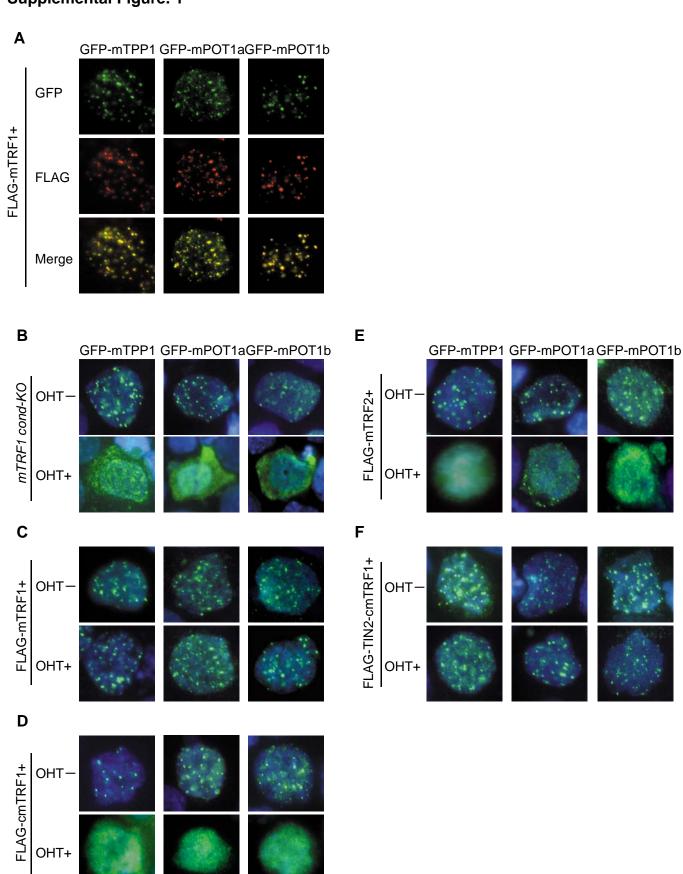
Supplemental Figure 5. Protein expression and <u>telomere</u> dysfunction-<u>i</u>nduced <u>f</u>oci (TIFs) induction at telomere.

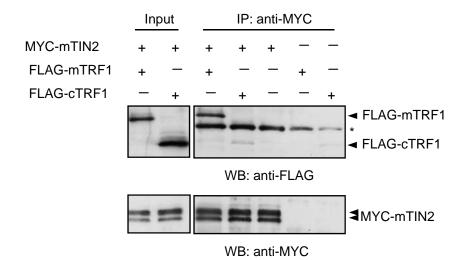
(A) and (C) Expression of *loxP*-mTRF1 and transfected proteins in each *mTRF1 cond-KO* ES cell line with or without OHT treatment were examined by Western blotting using anti-FLAG or anti-mTRF1 antibodies. (B) and (D) TIF formation in different *mTRF1 cond-KO* ES cell lines with or without OHT treatment was examined by immuno-FISH analysis using an anti-phospho-Histone H2A.X (Serine 139) antibody (γH2AX, green) and the Cy3-PNA probe (red). Used cell lines were following: (A) and (B) *mTRF1 cond-KO* ES cells and them expressing FLAG-mTRF1 (FLAG-mTRF1+) or expressing FLAG-cmTRF1 (FLAG-cmTRF1+), (C) and (D) *mTRF1 cond-KO* ES cells that expressed FLAG-mTRF2 (FLAG-TRF2+) or FLAG-TIN2-cmTRF1 (FLAG-TIN2-cmTRF1+).

Supplemental Figure 6. ChIP analysis for mTRF2-associated telomeric DNA. Chromatin was prepared from the indicated genotypes of mTRF1 cond-KO ES cells treated with or without

(OHT- or OHT+), before (input) and after immunoprecipitation with antibody against mTRF2. (A) and (B) are the autoradiograms of TRF2-associated telomeric DNA (ChIP DNA) that correspond to graphs in Fig. 2(B) and 3(C), respectively. The black triangles below each panel indicate the levels of serial dilutions of the ChIP DNA. The Mock lane contains the ChIP DNA without any antibody added. The 100% signals were taken as the ratio of lane 4/lane 1 in the top row of each blot in (A) and the ratio of the bottom/top rows of lane 1 in left panel in (B).

Supplemental Figure 7. Protein expression in mTRF1-present or absent cell lines used for TRF analysis. Protein expression level in mTRF1-present or absent ES cell lines expressing indicated proteins was checked by Western blotting. Used cell lines were following: (**A**) *mTRF1 cond-KO* ES cells expressing FLAG-mTRF1, FLAG-mTRF2 or FLAG-TIN2-cmTRF1. (**B**) *mTRF1 cond-KO*, *mTRF2*+ ES cells that expressed MYC-cmTRF1 or MYC-TIN2-cmTRF1. * indicates no-specific band.





Mouse	64	${\tt WMLDFLCLSLCRAFRDGRSEDFRRTRDSAEAIIHGLHRLTAYQLKTVYICQFLTRVASGK}$	123
		$ \begin{tabular}{lllllllllllllllllllllllllllllllllll$	
Chicken	28	WVLEFSCCCLCRYFVEECEAEFRRWRDVAQAVSNGFSKVTTHQKKMVYLCQLLIRIAEGK	87
Mouse	124	ALDAQFEVDERITPLESALMIWNSIEKEHDKLHDEIKNLIKIQAVAVCMEIGSFKEA	180
		L+ FE D I+PLESAL W +E+E KL H+EI+ LI+IQ VAV ME G +KEA	
Chicken	88	RLECHFENDTTISPLESALSFWTLLEREESKLNTLHEEIRRLIQIQVVAVYMEKGYYKEA	147
Mouse	181		240
Chicken	148	AEVLERLFTDSESHKPLRMKLAAIVKSKDPYVPLLQSFSYSLLLSKVKSYVKLFLKENRT	207
u	0.41	TEL WAARTING THE KARTOA OKORORO ATNITOHOTE VOL NIKEKOVNIGO OGTETERI VIDTVO	000
Mouse	241		300
Chiakan	200		251
GITCKEII	200	NFLLWAA INGVESE	201
Mouse	301	STRSHKNALSOLKHRRAPSDESRNEARTGTLOCETTMERNRRTSGRNRLCVSENOPDT-D	359
Modoc	001		000
Chicken	252		295
on one			200
Mouse	360	DKSGRRKRQTWLWEEDRILKCGVKKYGEGNWAKILSHYKFNNRTSVMLKDRWRTMKRLK	418
		D R+RQ W +EED+ LK GV+++G GNW KIL H FNNRTSVMLKDRWRT+ ++K	
Chicken	296	DLPCSRRRQPWTYEEDKKLKSGVREFGVGNWTKILIHGDFNNRTSVMLKDRWRTLCKIK	354
	Chicken Mouse Chicken Mouse Chicken Mouse Chicken Mouse Chicken	Chicken 28 Mouse 124 Chicken 88 Mouse 181 Chicken 148 Mouse 241 Chicken 208 Mouse 301 Chicken 252	W+L+F C LCR F + +FRR RD A+A+ +G ++T +Q K VY+CQ L R+A GK Chicken 28 WVLEFSCCCLCRYFVEECEAEFRRWRDVAQAVSNGFSKVTTHOKKMVYLCQLLIRIAEGK Mouse 124 ALDAGFEVDERITPLESALMIWNSIEKEHDKLHDEIKNLIKIQAVAVCMEIGSFKEA L+ FE D I+PLESAL W +E+E KL H+EI+ LI+IQ VAV ME G +KEA Chicken 88 RLECHFENDTTISPLESALSFWTLLEREESKLNTLHEEIRRLIQIQVVAVYMEKGYYKEA Mouse 181 EEVFERIFGDPEFYTPLERKLLKIISOKDVFHSLFQHFSYSCMMEKIQSYVGDVLSEKSS EV ER+F D E + PL KL I+ KD + L Q FSYS ++ K++SYV L E + Chicken 148 AEVLERLFTDSESHKPLRMKLAAIVKSKDPYVPLLQSFSYSLLLSKVKSYVKLFLKENRT Mouse 241 TFLMKAATKVVENEKARTQASKDRPDATNTGMDTEVGLNKEKSVNGQQSTETEPLVDTVS FL++AATK VE+E G + V NK +V ++ E Chicken 208 NFLLQAATKQVESE

