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 $CaCl_2$, 0.5mM $MgCl_2$, 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**) *mTRF1Δ* 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 telomere dysfunction-induced foci (TIFs) induction at telomere.

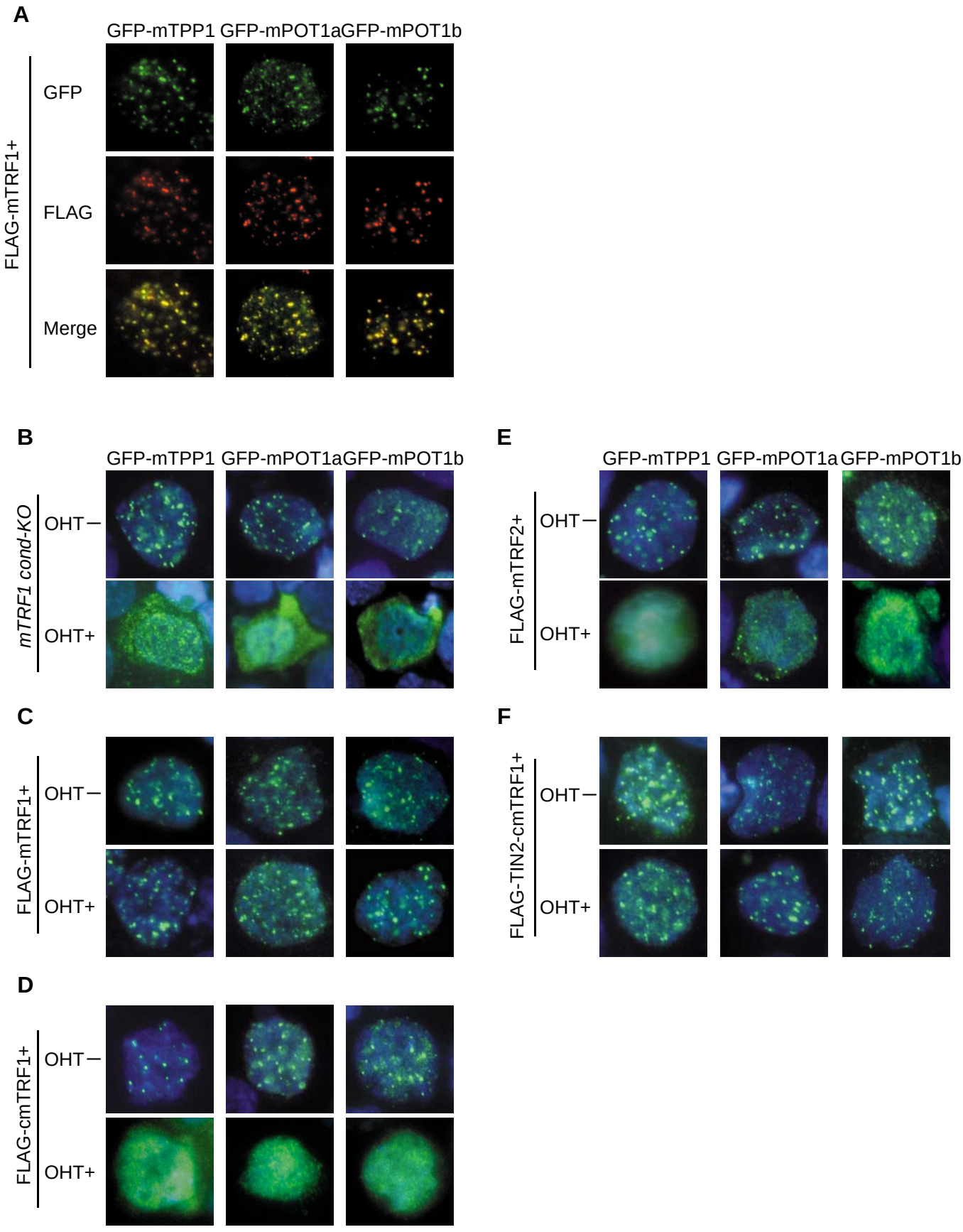
(**A**) and (**C**) Expression of *loxP*-mTRF1 and transfected proteins in each *mTRF1 cond-KO* ES cell line with or without OHT treatment were examined by Western blotting using anti-FLAG or anti-mTRF1 antibodies. (**B**) and (**D**) TIF formation in different *mTRF1 cond-KO* ES cell lines with or without 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

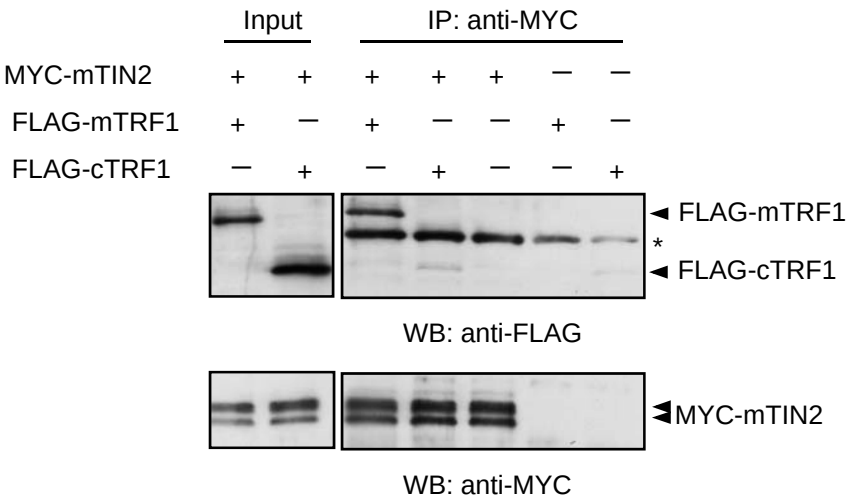
(OHT- or OHT+), before (input) and after immunoprecipitation with antibody against mTRF2. (**A**) and (**B**) are the autoradiograms of TRF2-associated telomeric DNA (ChIP DNA) that correspond to graphs in Fig. 2(**B**) and 3(**C**), respectively. The black triangles below each panel indicate the levels 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.

Supplemental Figure. 1



Supplemental Figure. 2



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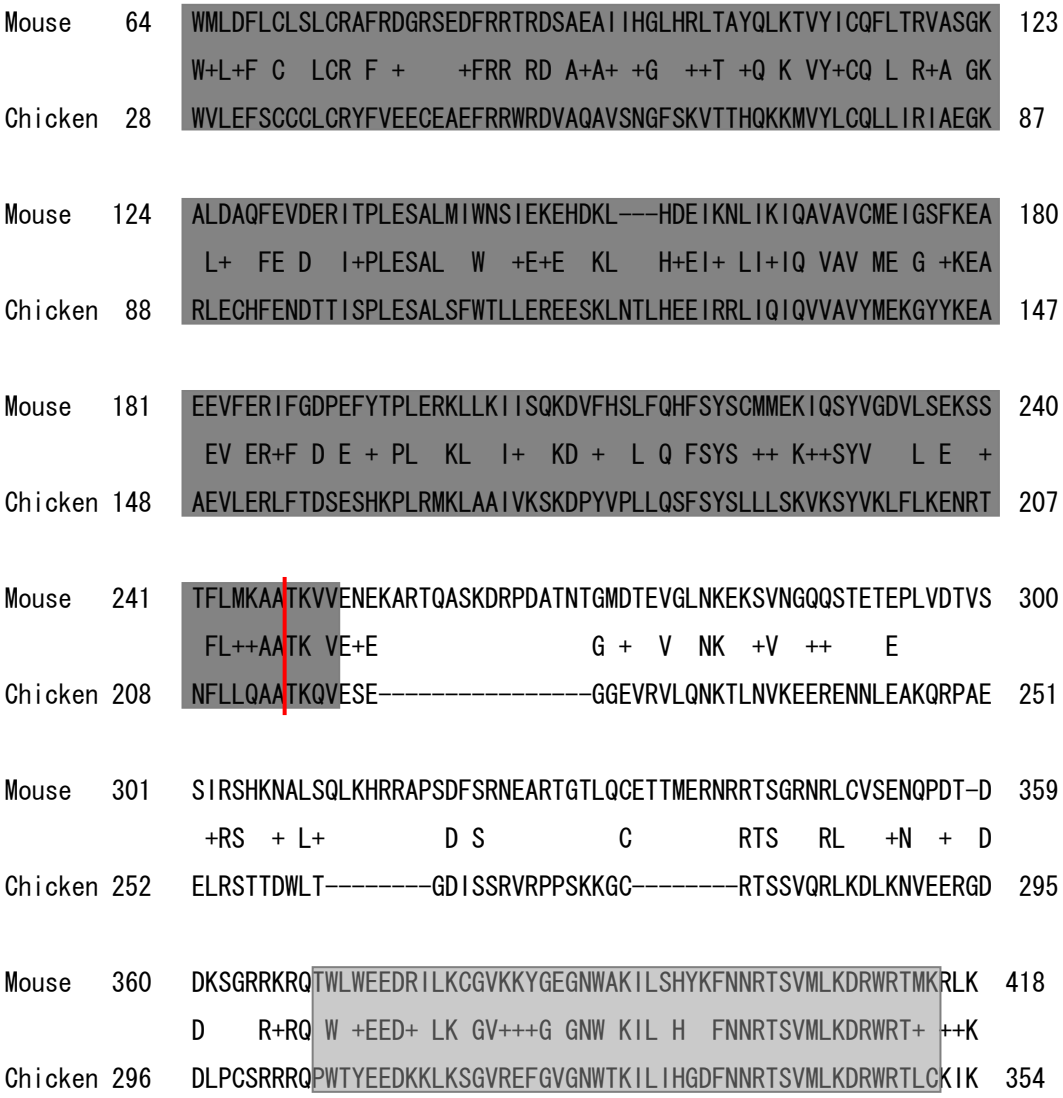

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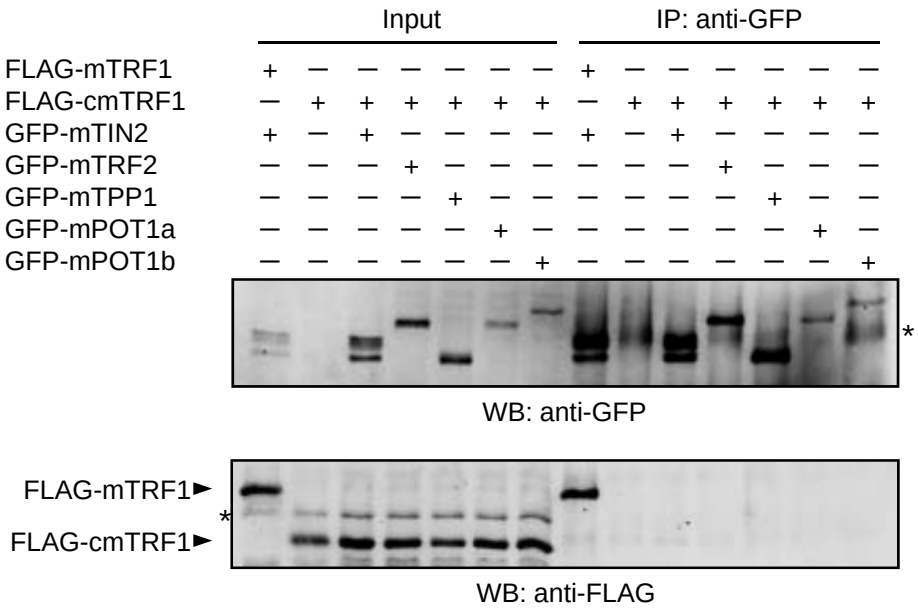

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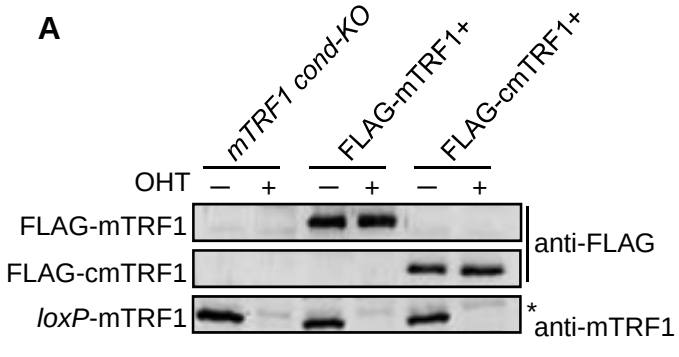
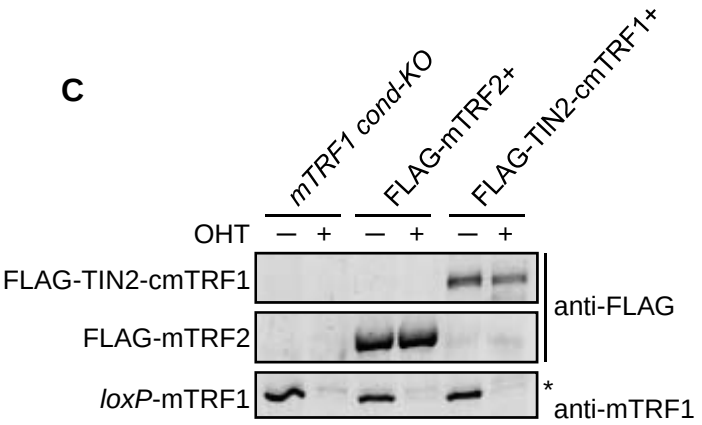
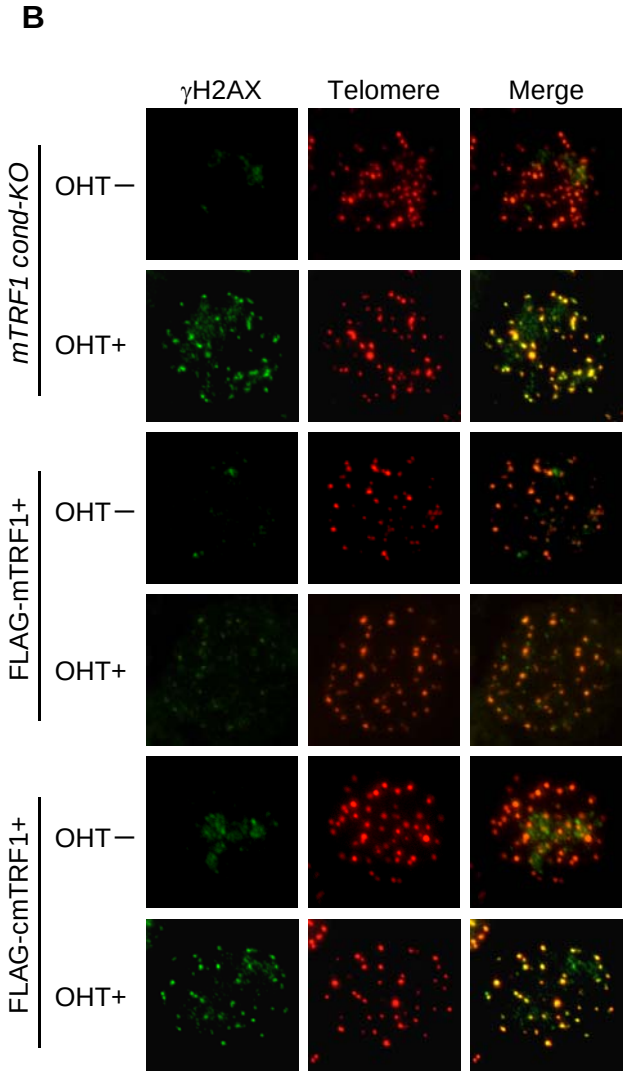
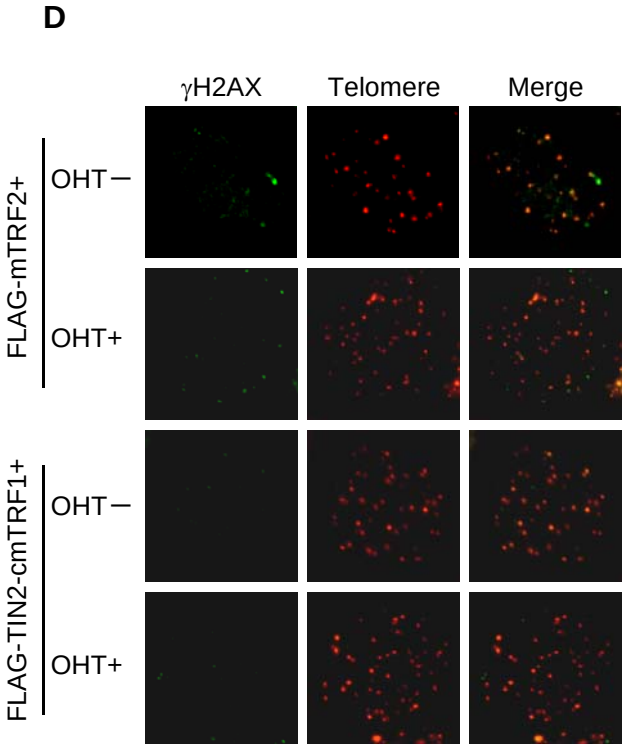
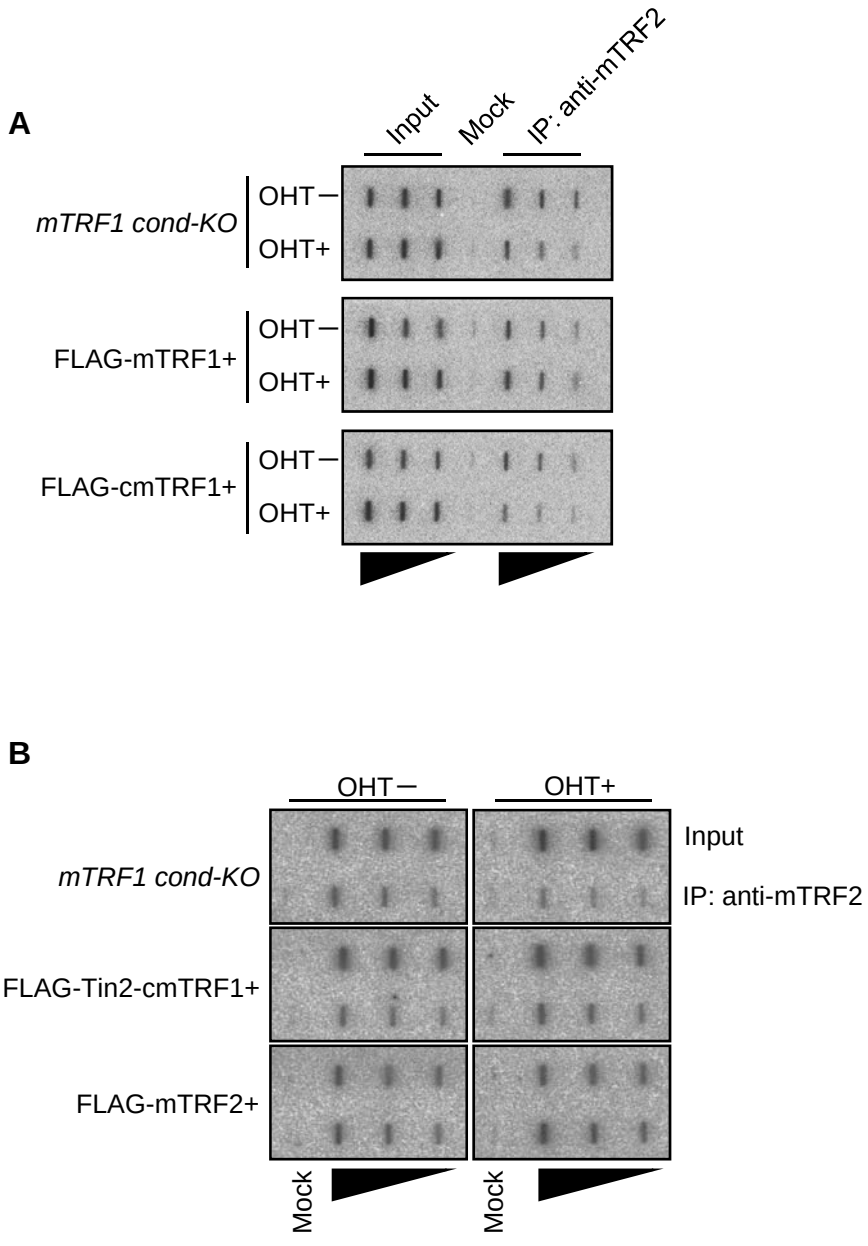








Supplemental Figure. 6



Supplemental Figure. 7