**Lee et al., Supplemental figure legends**

**Lee et al., Figure S1 Mitosis-specific TRF1 mobility shift.**
**(a)** Interphase and mitotic HeLa cells were immunoblotted with antibodies raised against the N-terminal 319 residue of TRF1 (top panel) or anti-Abl antibodies, with the mitosis-specific mobility shift of Abl as control. **(b)** 35S-TRF1 synthesized by TNT was incubated with Xenopus cell cycle extracts, showing a obvious mitosis-specific mobility shift.

**Lee et al., Figure S2 Identification of domains and residue responsible for the Pin1 and TRF1 interaction.**
(**a**) Pin1 WW domain, but not PPIase domain interacts with TRF1. Cells expressing TRF1 were incubated with glutathione-agarose beads containing GST, GST-Pin1 or GST-Pin1 truncated mutants. After washing, binding proteins were subjected to immunoblotting analysis with anti-HA 12CA5 mAb. (**b**) Pin1 binds to the TRF homology domain of TRF1. Cells expressing Xpress tagged TRF1 and its different truncated mutants were subjected to GST-Pin1 pulldown, followed by immunoblotting using anti-Xpress antibodies. (**c**) Pin1 binding to TRF1, but not TRF1T149A. Cells were transfected with GFP-TRF1, TRF1S11A, TRF1T149A, TRF1T324A or TRF1T351A vector and then subjected by GST-Pin1 pulldown, followed by immunoblotting with anti-TRF1 antibodies. Full scans for **c** are presented in Fig. S12.

**Lee et al., Figure S3 Cdk inhibitor abolishes TRF1 binding to Pin1.**
TRF1-transfected HT1080 cells were treated with various kinase inhibitors or control buffer, followed by GST-Pin1 pulldown assay. Selective kinase specificity of the inhibitors are Roscovitine for Cdks, Chelerythine for PKCs, U0126 for MEKs, JNK inhibitor II for JNKs, SB203580 for p38 MAP kinases, GSK-3 inhibitor X for GSK-3s and H89 for PKA.

**Lee et al., Figure S4 Pin1 knockdown increases protein stability of TRF1 in HT1080 cells.**
**(a)** Generation of stable Pin1 knockdown cells. HT1080 cells were infected with Pin1-shRNA or control constructs, followed by selection for stable with puromycin. Cells were then lysed and subjected to immunoblot with anti-Pin1 or anti-b-actin antibodies. **(b, c)** Inhibition of Pin1 increases endogenous TRF1 protein stability. Pin1-shRNA stably expressing HT1080 cells were treated with cycloheximide (100 g/ml) for indicated time points, followed by immunoblotting analysis with anti-TRF1, anti-b-actin or anti-Pin1 antibodies (**b)** and semi-quantification using b-actin as a loading control and relative TRF1 levels at time 0 as 100% (**c**).

**Lee et al., Figure S5. Pin1 knockdown in various human cell lines.**
(**a**) Pin1 knockdown in normal telomerase-negative cells. WI38 Cells were stably infected with Pin1-shRNA lentiviruses and vector viruses and selected for stable pools, followed by immunoblotting analysis with anti-Pin1 or anti-actin antibodies.
**(b)** Pin1 knockdown in on cells overexpressing TERT and TER. HT1080 cells were infected with TERT retroviruses and TER lentiviruses, and then with Pin1-shRNA lentiviruses, or control vectors and were selected triple stable cell pools, followed by immunoblots or quantitative RT-PCR for TER and GAPDH.
**(c)** Pin1 knockdown in TRF1 silenced cells. HT1080 cells were infected with TRF1-shRNA retroviruses and then with Pin1-shRNA lentiviruses, or control vectors and selected for double infected stable cell pools, followed by immunoblots with varios antibodies.

**Lee et al., Figure S6 Pin1 knockdown causes gradual and progressive telomere shortening in HT1080 cells.**
TRF lengths in two additional independent Pin1-shRNA and one vector control HT1080 stable clones were measured at different PDs using genomic Southern blotting.

**Lee et al., Figure S7 Pin1 knockdown does not induce telomere shortening in telomerase-negative GM847 cells.**
TRF lengths in two independent Pin1-shRNA and one vector control GM847 stable clones were measured at different PDs using genomic Southern blotting.

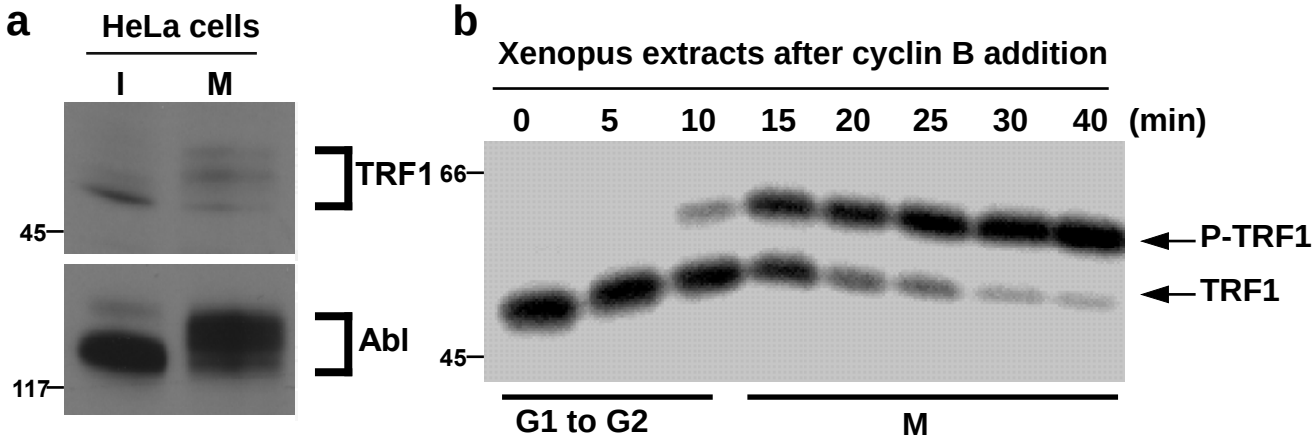
**Lee et al., Figure S8 The concomitant ablation of TRF1 in Pin1 silenced cells corrects the observed telomere shortening.**
TRF lengths in two additional independent TRF1-shRNA and Pin1-shRNA cells and one vector control HT1080 stable pools were measured at different PDs using genomic Southern blotting.

**Lee et al., Figure S9 TRF1 knockdown causes telomere elongation in MEFs.**
TRF1-shRNA or vector control interphase MEFs were fixed and and hybridiized with a Cy3-labeled PNA $(CCCTAA)_3$ probe, followed by quantifying their telomere fluorescence intensity at over 1,000 telomeres.

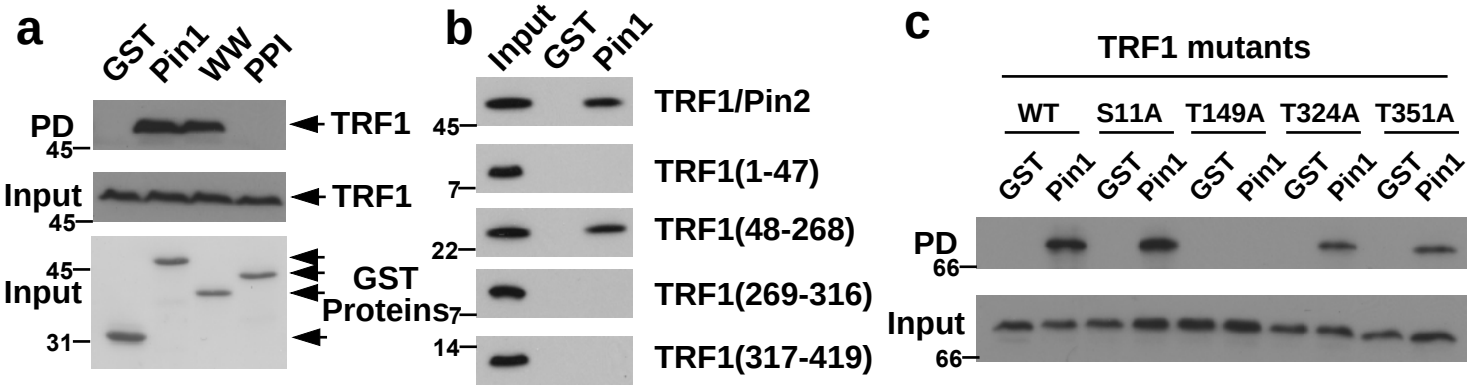
**Lee et al., Figure S10 Pin1 knockout causes telomere loss in mice.**
Total splenocytes were isolated from Pin1 WT and Pin1 KO littermates, and then hybridized with a FITC-labeled telomere PNA probe, followed by DNA counterstaining with LDS751. The stained cells were directly subjected to the FACSan flow cytometry **(a)** or after sorting using B cell marker CD45R/B220 **(b)**, with cells containing the G0/G1 DNA content being analyzed.

**Lee et al., Figure S11 Pin1 knockout causes accelerated telomere loss in mice.**
Metaphase spreads were prepared from Pin1+/+ and Pin1-/- splenocytes and hybridiized with a FITC-labeled PNA $(CCCTAA)_3$ probe, followed by quantifying their telomere fluorescence intensity at over 1,000 telomeres.

**Lee et al., Figure S12 Full scans of key Western blots presented in the manuscript.**




Lee et al., Figure S1

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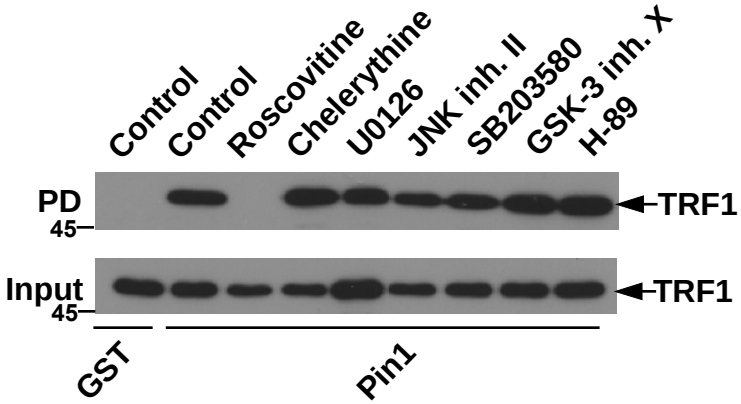
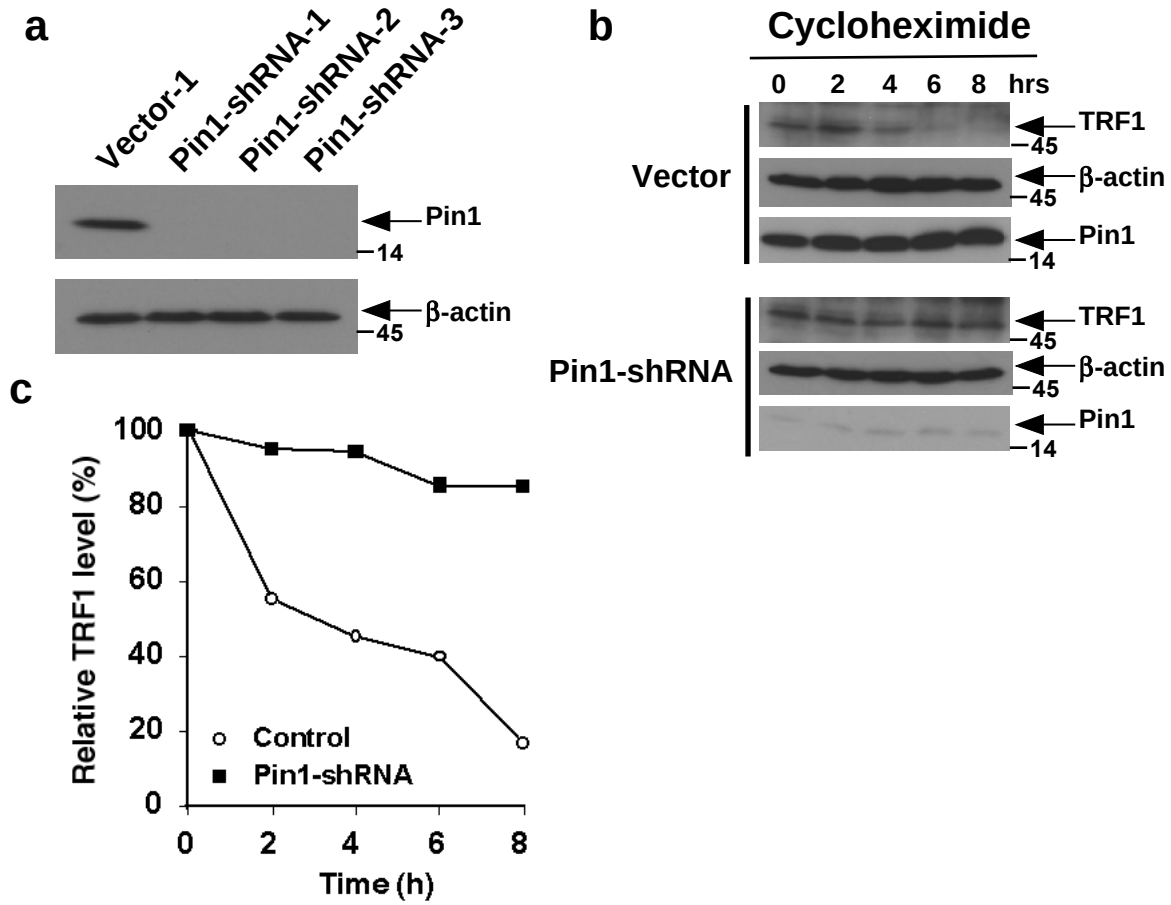



Lee et al., Figure S3

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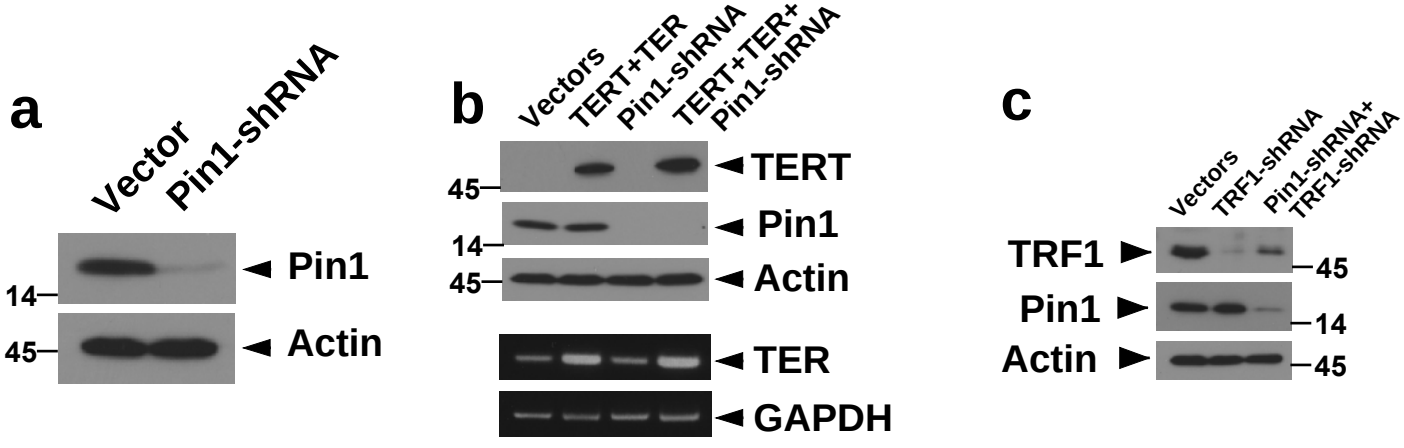


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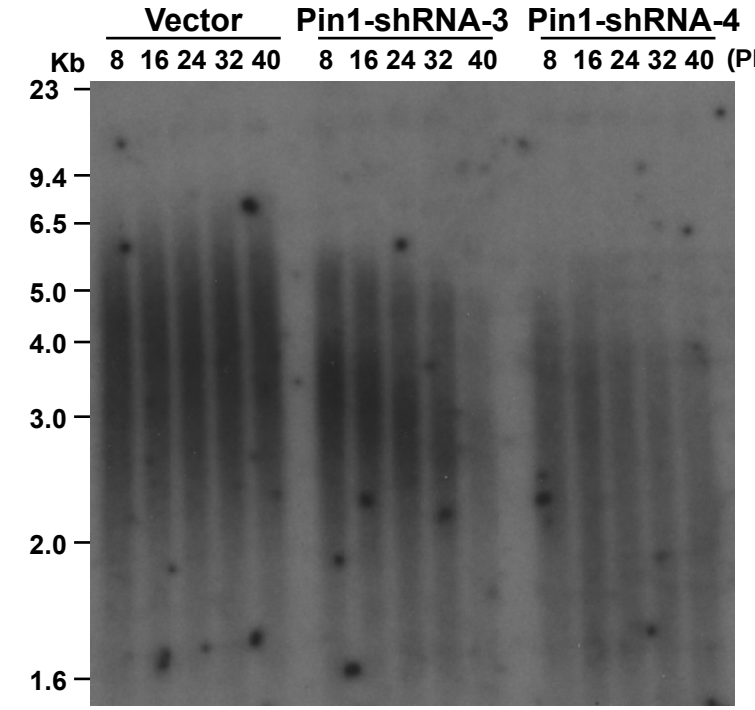


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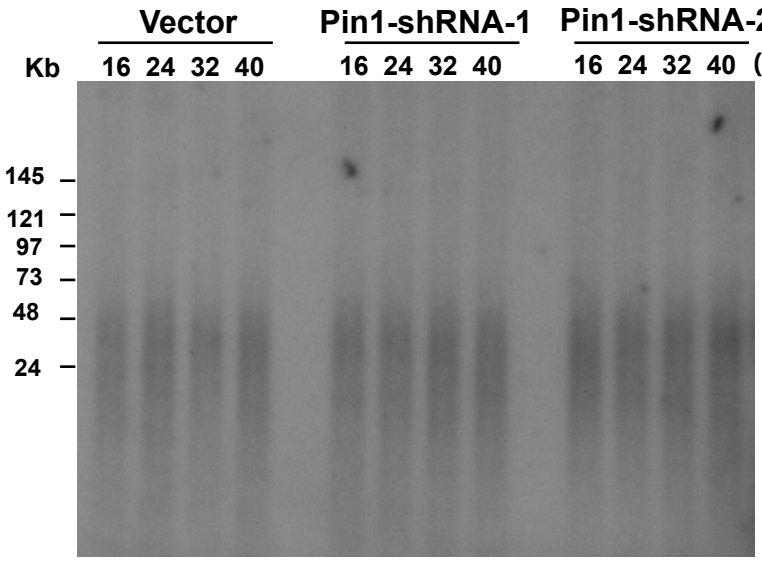


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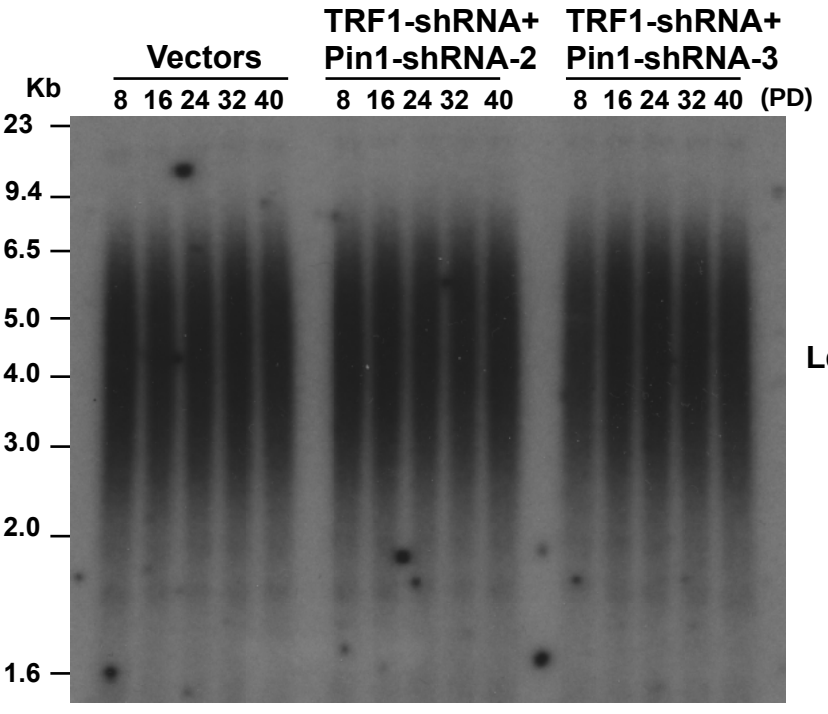


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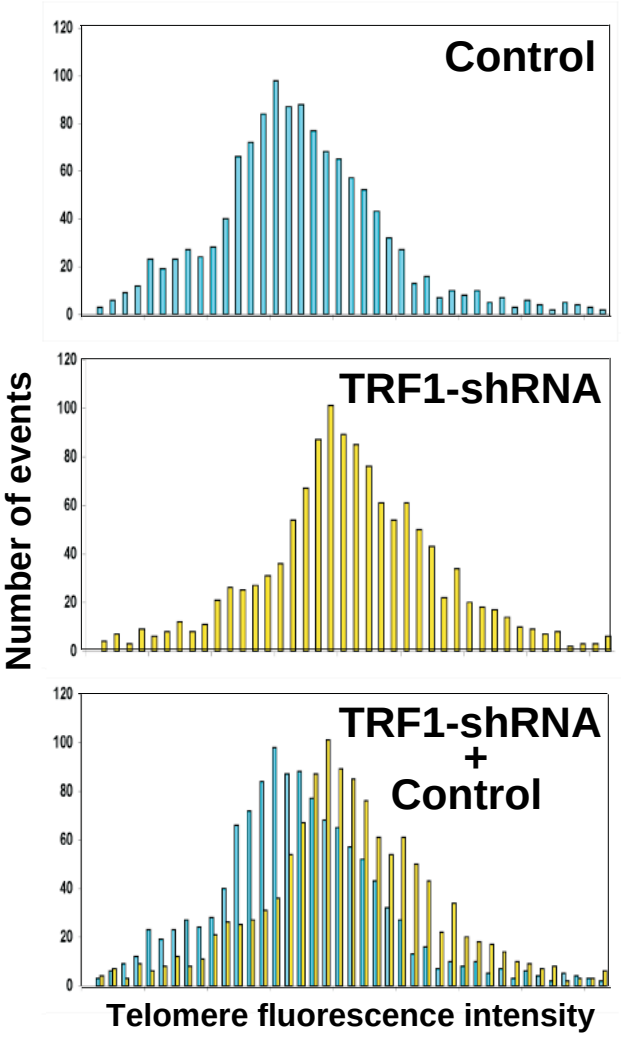


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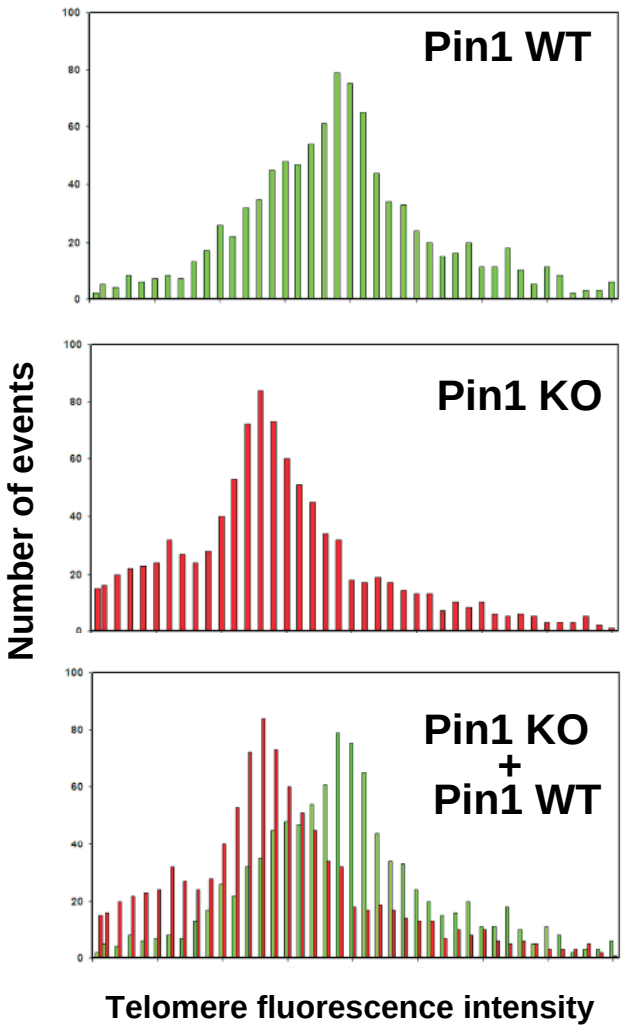


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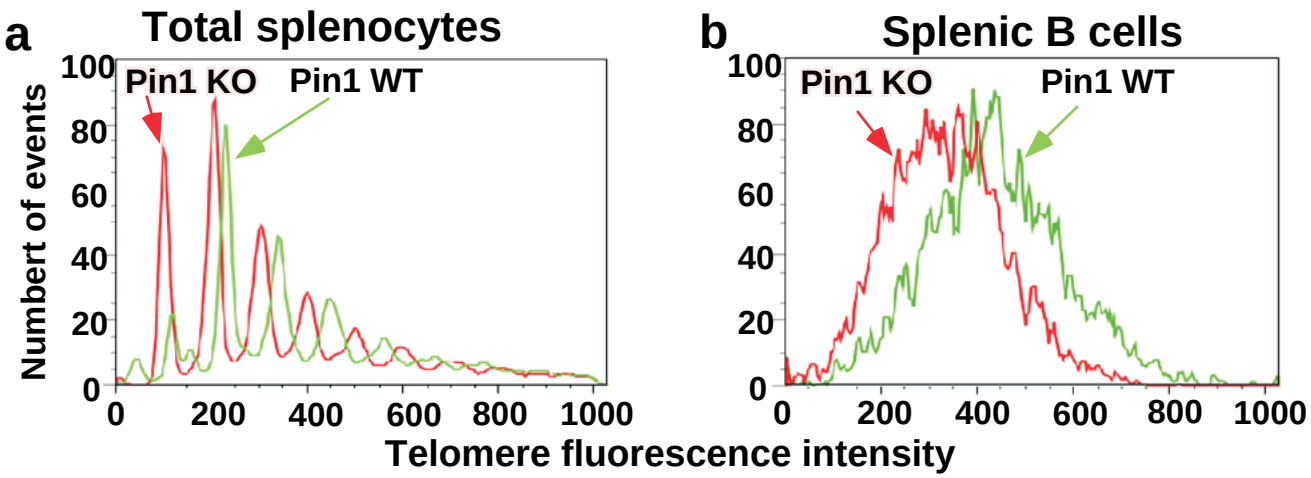


Lee et al., Figure S10

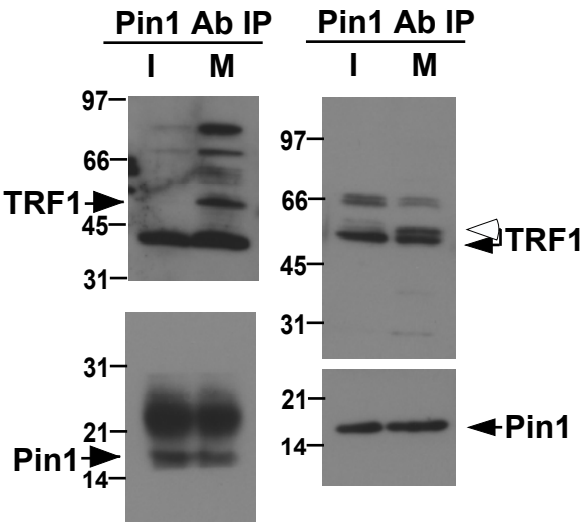


Fig. 1f

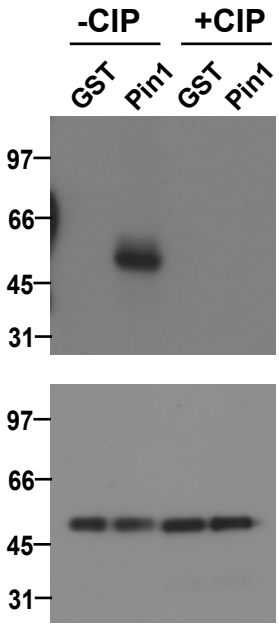


Fig. 1i

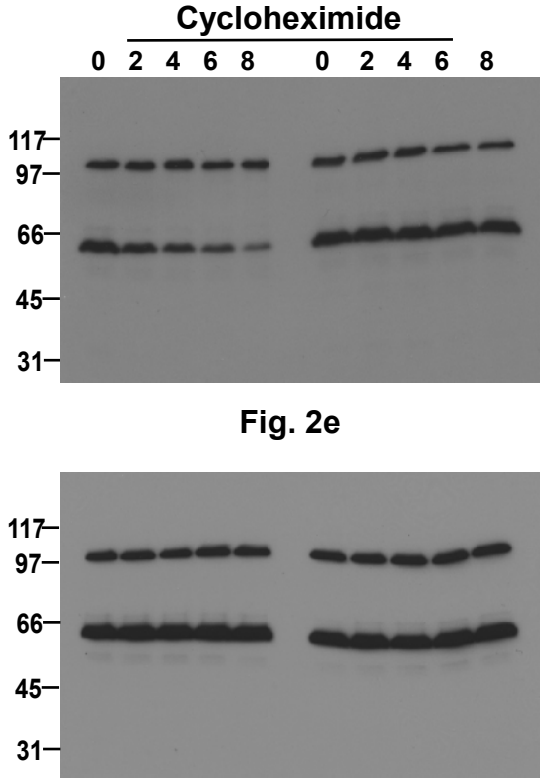


Fig. 2e

Fig. 2i

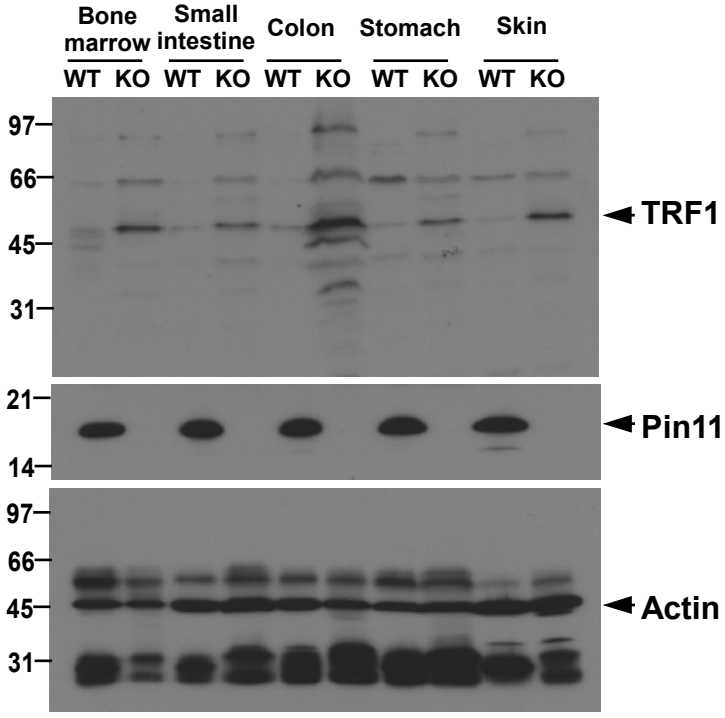


Fig. 5c

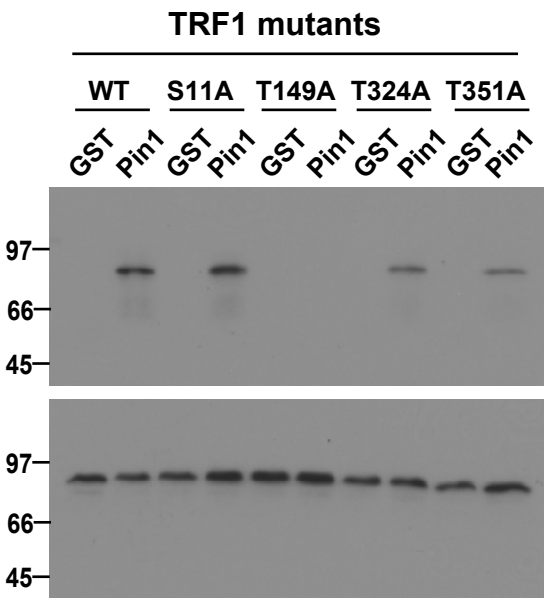


Fig. S2c