Supporting Information

Deng et al. 10.1073/pnas.1300600110

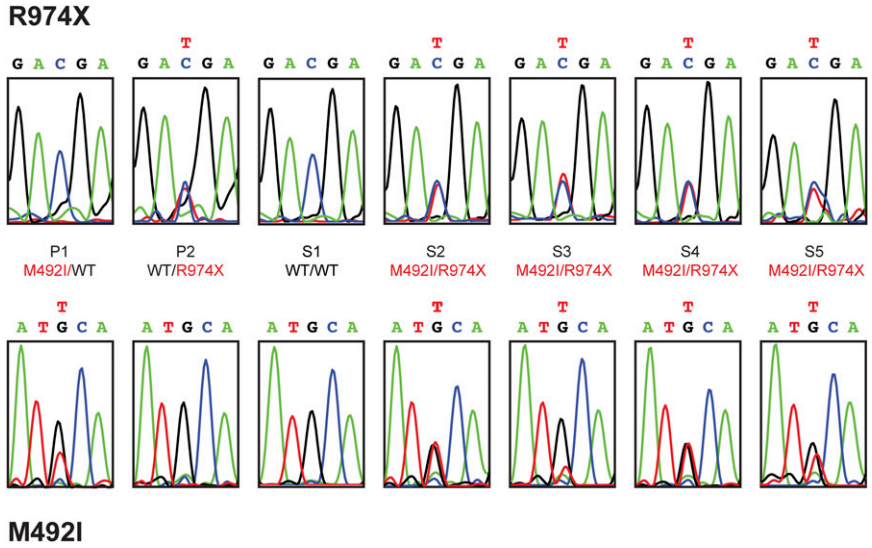

Fig. S1. Compound heterozygous regulator of telomere elongation helicase 1 (*RTEL1*) mutations were associated with Hoyeraal–Hreidarsson syndrome (HHS). PCR amplification and sequencing of exon 30 from genomic DNA validated the presence of the heterozygous R974X mutation in the affected siblings (S2, S3, S4, S5) and parent P2, and its absence in the healthy sibling S1 and in P1. PCR amplification and sequencing of exon 17 validated the presence of the heterozygous M492I mutation in the affected siblings (S2, S3, S4, S5) and parent P1, and its absence in the healthy sibling S1 and in P2. Shown are the sequencing chromatograms with the sequence above.

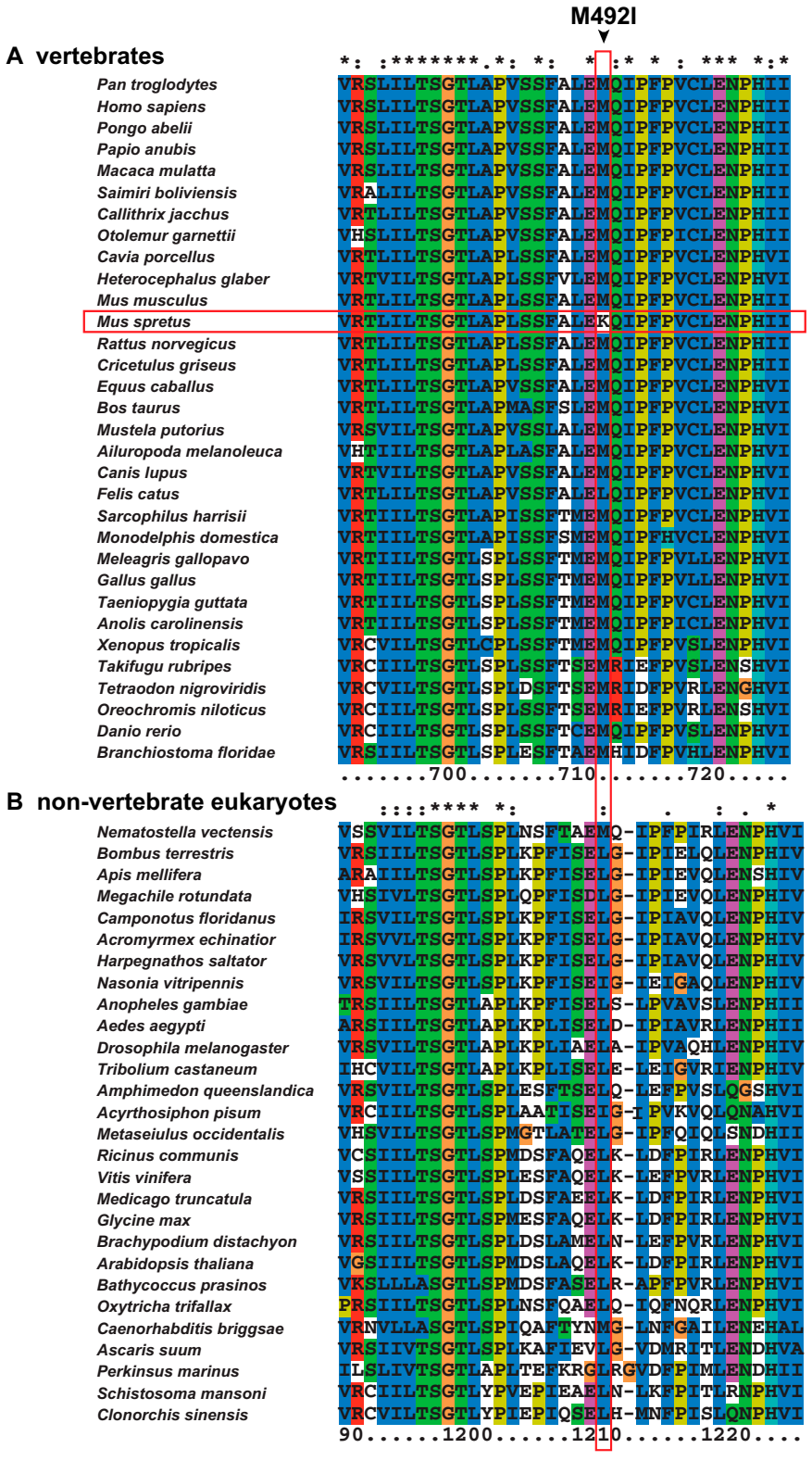

Fig. S2. Conservation of methionine 492 in RTEL1. RTEL1 ortholgs available in the GenBank were aligned using ClustalX (1). Shown is a fragment of the alignment containing methionine 492 (position in human RTEL1) for vertebrate (*A*) and nonvertebrate (*B*) species. Indicated are the M492 position and the *Mus spretus* sequence.

1. Chenna R, et al. (2003) Multiple sequence alignment with the Clustal series of programs. *Nucleic Acids Res* 31(13):3497–3500.

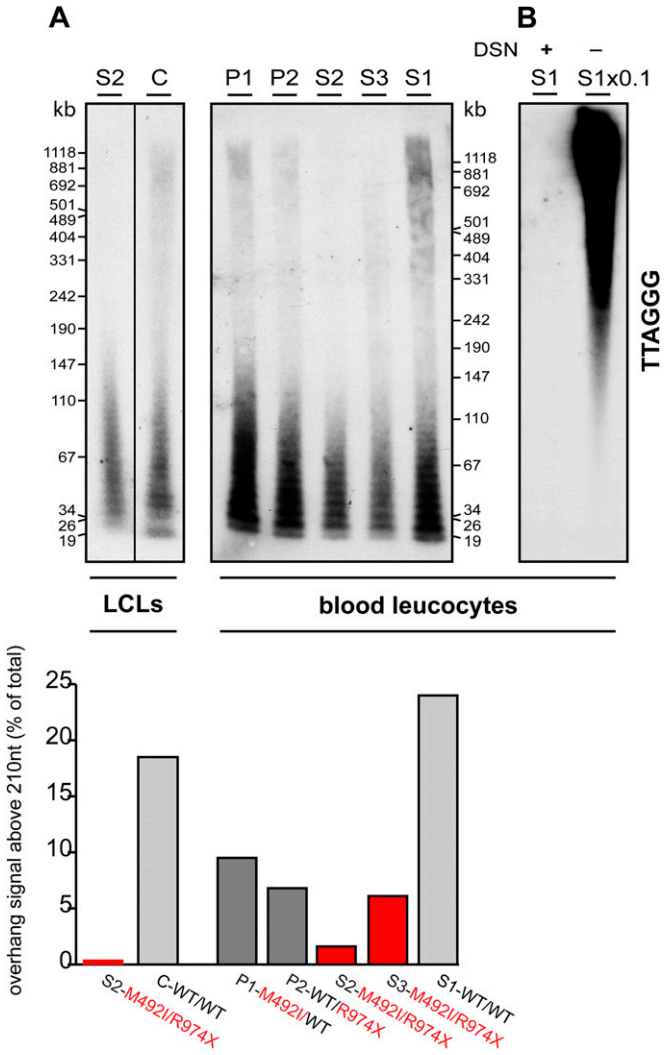

Fig. S3. Diminished telomeric overhang in the RTEL1-deficient cells. (*A*) Five micrograms of genomic DNA prepared from lymphoblastoid cell lines (LCLs) and leukocytes derived from a healthy donor (C) and from the family members, as indicated, were digested with 1 unit of duplex-specific nuclease (DSN), electrophoresed on a 4–8% (wt/vol) gradient polyacrylamide gel, transferred to a membrane, and hybridized to a C-rich probe, as described previously (1) except for the incubation of the DSN reaction at 65 °C. (*B*) Five micrograms of DSN-digested and 0.5 μg of undigested genomic DNA from S1 leukocytes were electrophoresed, transferred, and hybridized to a G-rich telomeric probe under the same conditions. The signal at the undigested DNA represents double stranded telomeric sequence, which disappeared upon DSN digestion, indicating the apparent absence of C-rich single-stranded telomeric DNA. The histograms below the lanes represent the percentage of overhang signal above 210 nt, of the total overhang signal.

1. Lamm N, et al. (2009) Diminished telomeric 3′ overhangs are associated with telomere dysfunction in Hoyeraal-Hreidarsson syndrome. *PLoS ONE* 4(5):e5666.

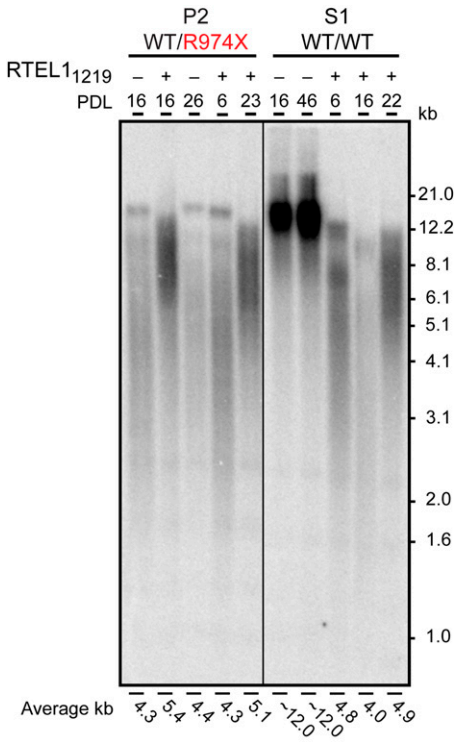

Fig. S4. RTEL1$_{1219}$ promoted telomere elongation in P2 cells and shortening in S1. P2 (RTEL1$^{M492I/WT}$) and S1 (RTEL1$^{WT/WT}$) LCLs were transduced at a late population doubling level (PDL) (~40) with lentiviruses expressing RTEL1$_{1219}$ (+) or an empty vector control (–). Genomic DNA samples were prepared from the LCLs at the indicated PDLs, digested with AluI+MboI and analyzed by Southern using a telomeric probe. The average telomere length for each sample was calculated using MATELO (1) and indicated below the lane.

1. Yehezkel S, Segev Y, Viegas-Péquignot E, Skorecki K, Selig S (2008) Hypomethylation of subtelomeric regions in ICF syndrome is associated with abnormally short telomeres and enhanced transcription from telomeric regions. *Hum Mol Genet* 17(18):2776–2789.

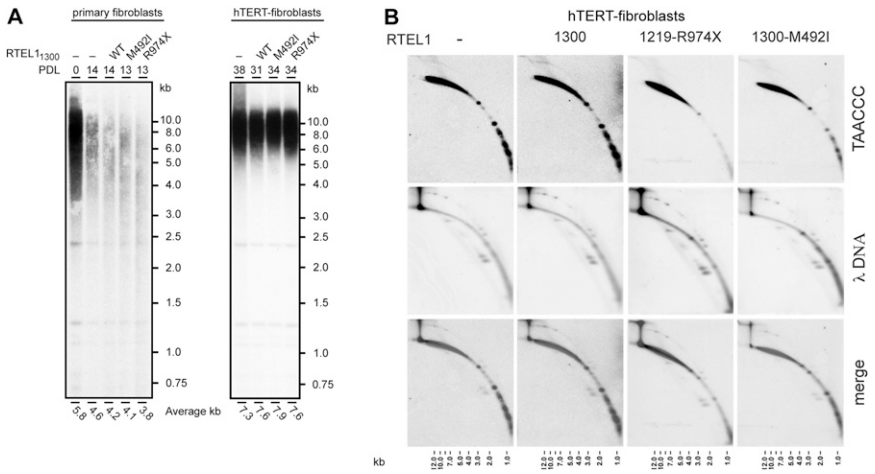

Fig. S5. Telomere length and 2D gel electrophoresis analyses of fibroblasts expressing WT and mutant RTEL1 proteins. (*A*) Genomic DNA samples were prepared from hTERT (the telomerase reverse transcriptase subunit)-negative and hTERT-positive fibroblasts expressing WT or mutant RTEL1 variants, or vector alone (–), at the indicated PDLs, and analyzed by Southern using a telomeric probe. The average telomere length for each sample was calculated using MATELO (1) and indicated below the lane. (*B*) Genomic DNA samples from the indicated hTERT-fibroblasts cell lines at PDL ~30 were digested with AluI+HinfI and analyzed together with circularized λ DNA HindIII fragments by neutral-neutral 2D gel electrophoresis, separating first on the basis of size and then on the basis of conformation. Shown are blots hybridized with C-rich telomeric probes (*Top*), or λ DNA specific probes (*Middle*). The merged images (*Bottom*) indicate the comigration of circular λ DNA HindIII fragments with the open T-circles. Note the increased T-circles in cells expressing WT RTEL1 but not R974X or M492I mutants.

1. Yehezkel S, Segev Y, Viegas-Péquignot E, Skorecki K, Selig S (2008) Hypomethylation of subtelomeric regions in ICF syndrome is associated with abnormally short telomeres and enhanced transcription from telomeric regions. *Hum Mol Genet* 17(18):2776–2789.

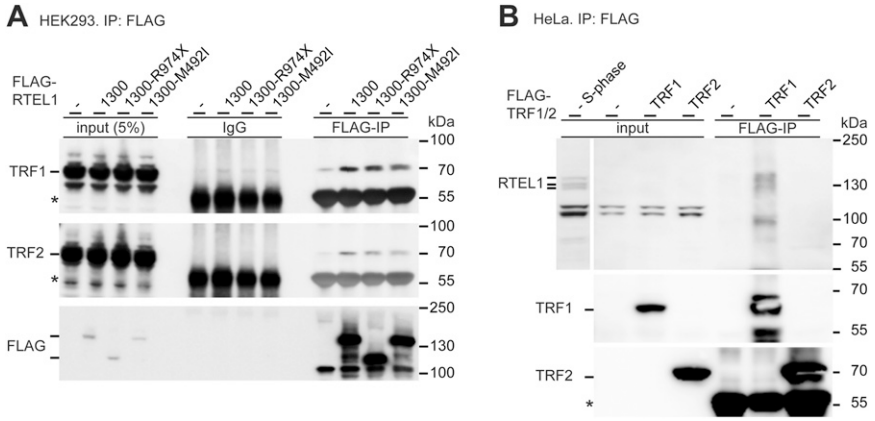

Fig. S6. RTEL1 interacted with telomeric repeat binding factor 1 (TRF1). (*A*) 293 HEK cells were transfected with an empty vector (–), or vectors expressing FLAG-RTEL1 1300, R974X, or M492I. Seventy-two hours posttransfection, cells were assayed by FLAG IP or IgG control and Western blot with the indicated antibodies. An asterisk indicates a nonspecific IgG band. (*B*) HeLa cells were transfected with an empty vector (–), or vectors expressing FLAG-TRF1 or -TRF2. Seventy-two hours posttransfection, cells were assayed by FLAG or IgG (control) IP, and Western blot with the indicated antibodies. An asterisk indicates a nonspecific IgG band.