Supplemental Figures

Supp. Figure 1. G-overhang signal in cells expressing myc-Trf1

A. Autoradiogram showing native in-gel hybridisations of $(TA_2C_3)_4$ G-strand probe to MspI-, HinfI- and HaeIII-digested genomic DNA from the following cell lines: Lane 1. wild-type; 2. *Trf1*$^{-/-/-}$; 3. *Trf1*$^{-/-/-}$ myc-Trf1 clone 1 (PD 200-250); 4. *Trf1*$^{-/-/-}$ myc-Trf1 clone 2 (PD 800-900); 5. wild-type myc-Trf1 (~PD 50-80). Levels of myc-Trf1 are much higher in *Trf1*$^{-/-/-}$ myc-Trf1 clone 2 compared with clone 1.

B. Histogram showing relative G-overhang signals. The relative signal in each lane was quantified with a phosphoimager and normalised against a representative band in the TRF signal of the denatured gel. Data represent the s.e.m. of three independent experiments.

C. Immunoblot analysis of myc-Trf1 expression in the following cell lines: Lane 1. wild-type; 2. *Trf1*$^{-/-/-}$; 3. *Trf1*$^{-/-/-}$ myc-Trf1 clone 1; 4. *Trf1*$^{-/-/-}$ myc-Trf1 clone 2; 5. wild-type myc-Trf1. Immunoblot for γ-tubulin is used as a loading control.

Supp. Figure 2. Telomerase activity in *Trf1* mutant cells.

A. A representative TRAP assay performed using 1μg protein extract from the following cells: lane 2. wild-type; 4. *Trf1*$^{-/-/-}$myc-Trf1^{+}; 5. *Trf*$^{+/+/-}$; 6. *Trf*$^{+/-/-}$; 7. *Trf1*$^{-/-/-}$ PD12; 8. *Trf1*$^{-/-/-}$PD360. An intra-assay PCR internal control is indicated ('IC'). Negative control reactions involved 1. the use of water in the extension reaction instead of telomerase extract or 3. the omission of Taq polymerase.

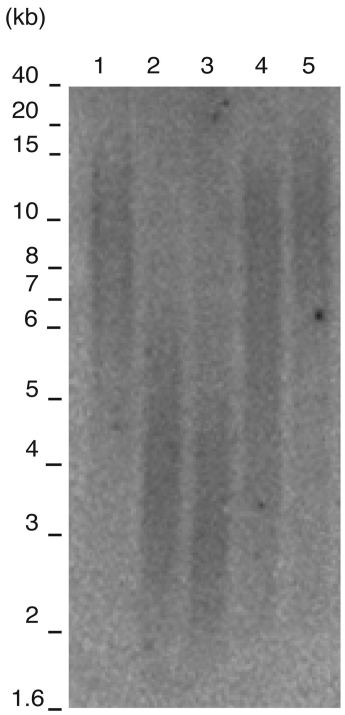
B. A representative TRAP assay showing titration of wildtype extract. Lane 1. wild-type 2μg; 2. wildtype 1μg; 3. wildtype 0.5μg; 4. no telomerase extract; 5. no Taq polymerase.

C. Histogram showing telomerase activity in the indicated cell lines. Data represent the mean values +/- standard deviation of at least three independent TRAP experiments.

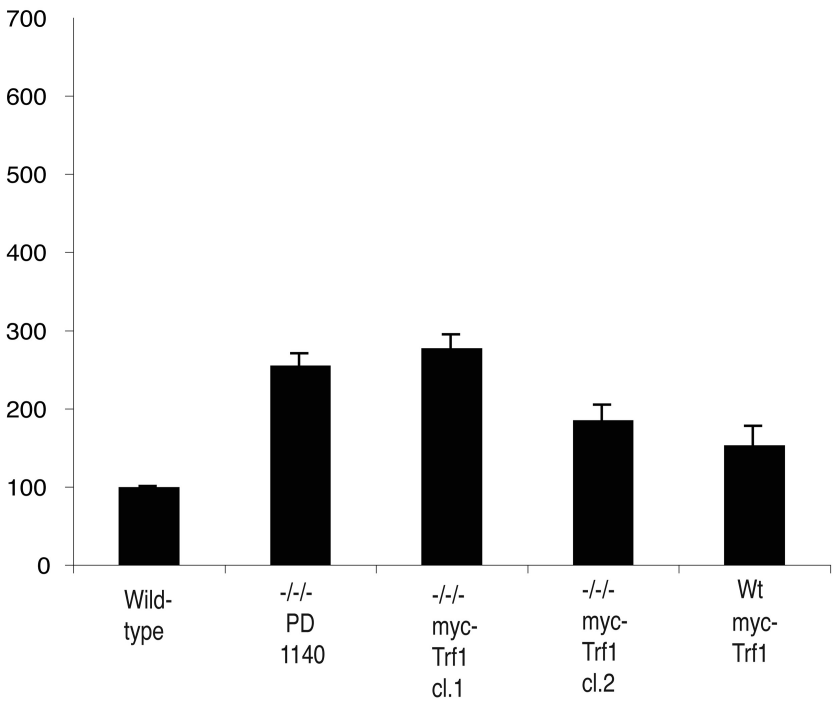
Supp. Figure 3. DNA damage response induced by electroporation of *Trf1*$^{-/-}$ cells

Wild-type or Trf1-deficient cells were either untreated or transfected with 10 μg of linearised plasmid DNA, as indicated and immunoblot analysis of H2AX phosphorylation performed 12 h post-electroporation. Plasmids transfected were pCMV-3myc (empty vector), or pCMV-3myc into which was cloned full-length chicken *Trf2*, or chicken Trf2 deleted in the regions encoding the basic domain and the myb homology domain (ΔBΔM). Size markers are shown at left in kDa and immunoblot for actin was used as a loading control.

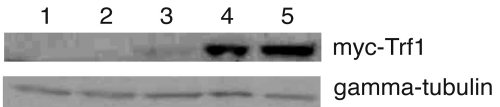
A


(kb)
1 2 3 4 5
40
20
15
10
8
7
6
5
4
3
2
1.6

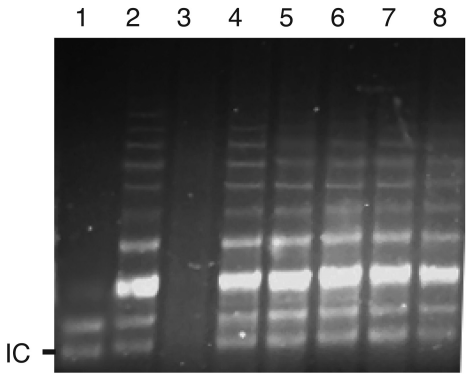
B


700
600
500
400
300
200
100
0
Wild-type
-/-/- PD 1140
-/-/- myc-Trf1 cl.1
-/-/- myc-Trf1 cl.2
Wt myc-Trf1

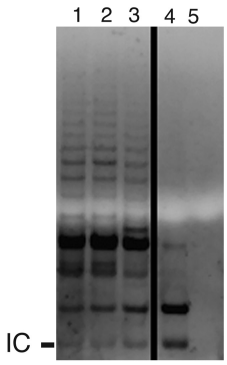
C


1 2 3 4 5
myc-Trf1
gamma-tubulin

A


1 2 3 4 5 6 7 8
IC

B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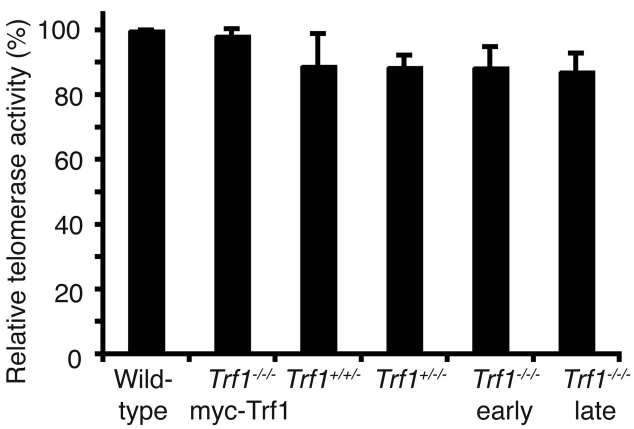
IC

C


Relative telomerase activity (%)
100
80
60
40
20
0
Wild-type
Trf1-/-/- myc-Trf1
Trf1+/+/-
Trf1+/-/-
Trf1-/-/- early
Trf1-/-/- late

Supplemental Figure 3