Supporting Information

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SI Materials and Methods

Generation of *Dkc1*$^{\Delta 15}$ ES Cells and Mice. The *Dkc1*^{Floxed15} targeting construct was made by standard subcloning methods as described (1) (see Fig. S1). A stop codon was introduced into exon 14 by using the QuikChange kit (Stratagene). ES cells (RW-4, Siteman Cancer Center ES cell core at Washington University School of Medicine) were transfected with the *Dkc1*^{Floxed15} construct and selected with Geneticin (Sigma). To generate *Dkc1*$^{\Delta 15}$ ES cells, *Dkc1*^{Floxed15} cells were transiently transfected with a plasmid expressing Cre, and the resulting clones were selected for loss of neomycin resistance. ES cells were cultured on mitomycin C-treated STO cell (CRL-2225; American Type Culture Collection) feeder layers in ES culture medium (DMEM, 17% ES qualified FCS) supplemented with 200 mM L-glutamine, 10 mM Hepes, 10 mM nonessential amino acids, 1,000 units/ml leukemia inhibitory factor, and 0.14 mM β-mercaptoethanol. *Dkc1*$^{\Delta 15}$ mice were derived from *Dkc1*^{Floxed15} ES cells by breeding with mice carrying the *EIIA-Cre* transgene.

RNA Isolation. Total RNA was extracted from ES cells and mouse tissues by using TRIzol reagent (Invitrogen). A rotor-stator homogenizer was used to thoroughly disrupt and homogenize mouse tissues. RNA was aliquoted and stored at −80°C for further use.

Southern and Northern Blot Analysis. Southern and Northern blotting was performed by using the classical high-salt buffer method. Hybond-N membrane was used according to the manufacturer's instructions. For analysis of snoRNAs, 5 μg total RNA was electrophoresed through 6% polyacrylamide/7 M urea gels and transferred onto Hybond-N membranes by using a Transblot SD semidry blotting system (Bio-Rad). Oligonucleotide probes for the detection of snoRNAs were as described in ref. 1.

Real-time RT/PCR. Real-time RT-PCR was carried out by using Power SYBR-Green PCR master mix (Applied Biosystems) and SuperScript II Moloney murine leukemia virus transcriptase and RNase inhibitors (Invitrogen) and a 7900HT real-time PCR system equipped with SDS software (Applied Biosystems).

Measurement of Telomere Length. ES cells or spleen cells were embedded in agarose plugs by using a CHEF agarose plug kit according to the manufacturer's instructions (Bio-Rad). DNA embedded in the plug was extracted, digested with MboI, and electrophoresed through a 1% agarose gel for 20 h at 6 V/cm, 1- to 6-s switch time by using CHEF DR-III pulse-field system (Bio-Rad). A [γ-^{32}P]ATP-labeled $(CCCTAA)_4$ probe was used in the in-gel hybridization procedure (2).

Telomerase Repeat Amplification Protocol (TRAP). ES cells were lysed by using CHAPS lysis buffer. Telomerase activity was measured by TRAP assay with the TRAP-EZE telomerase detection kit (INTERGEN), according to the manufacturer's protocol.

Pulse–Chase Analysis of rRNA Processing. ES cells were preincubated for 45 min in methionine-free medium and then incubated for 30 min in medium containing L-[*methyl*-^{3}H]methionine (50 μCi/ml). The cells were then chased in nonradioactive fresh medium for various times. Total RNA was separated on 1.25% agarose–formaldehyde gel and transferred to nylon membrane. The membranes were sprayed with EN^{3}HANCE spray (PerkinElmer) and exposed to x-ray films at −80°C.

Western Blot analysis. Total protein from cells and mouse tissues was prepared by using RIPA lysis buffer (1×TBS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS, 0.004% sodium azide, and 1× protease inhibitor mixture). Nuclear extracts were prepared by using NE-PER nuclear and cytoplasmic extraction reagents (Pierce). Protein concentration was measured by using the Bio-Rad protein assay.

Immunofluorescence and Immuno-FISH. Immunofluorescence was performed with a standard paraformaldehyde technique (fixed in PBS-buffered 4% paraformaldehyde for 10 min, permeabilized with 0.5% Triton-PBS for 15 min, blocked with 30% normal goat serum for 1 h). Primary antibody was used at 1/500 in 1.5% normal goat serum for 2 h. After washing with PBS, cells were incubated with a secondary goat anti-rabbit IgG conjugated with FITC and/or Alexa Fluor 568 at 1/1,000 in 1.5% normal goat serum for 45 min. All blocking and incubation steps were carried out at room temperature. After immunostaining, telomeric FISH was performed. Briefly, slides were fixed for 2 min in 4% paraformaldehyde, dehydrated in a 70%, 80%, and 100% ethanol, and air dried. DNA was denatured for 4.5 min at 80°C in hybridization buffer containing 0.3 μg/ml $(C_3TA_2)_3$-FITC-labeled telomeric peptide nucleic (PNA) probe, 70% formamide, 12 mM Tris·HCl (pH 7.2), 5 mM KCl, 1 mM $MgCl_2$, 0.001% Triton X-100, and 2.5 mg/ml acetylated BSA. After denaturation, hybridization was continued for 2 h at room temperature in a humidified chamber. Slides were washed twice for 15 min each with 70% formamide and 2× SSC then washed three times for 5 min each with 50 mM Tris·HCl (pH 7.2), 150 mM NaCl, and 0.05% Tween 20. Slides were then dehydrated with an ethanol series and air dried. Finally, slides were counterstained with DAPI and covered by mounting medium. The cells were examined at a magnification of ×1,000 by using a fluorescence microscope (Nikon). FITC, Texas red, Alexa Fluor 568, and DAPI images were overlapped by using ISIS FISH imaging software (Metasystems).

Antibodies. The sources of antibodies were as follows: anti-γ-H2AX-S139 (ab2893; Abcam), anti-p53 (ab26; Abcam), anti-p21 (ab7960; Abcam), and anti-ATM-phospho-S1981 (ab2888; Abcam). Anti-dyskerin was as described in ref. 1. Anti-β-actin was used as total protein loading control (ab20272; Abcam), and anti- TATA box-binding protein was used as nuclear loading control (ab818; Abcam).

Mice. *Terc*$^{-/-}$ (3), *Tert*$^{-/-}$ (4), *p53*$^{-/-}$ (5), and *EIIA-Cre* mice (6) were obtained from R. DePinho, L. Harrington, The Jackson Laboratory, and H. Westphal, respectively, and maintained in a C57BL6 background.

Establishment of Primary Mouse Embryonic Fibroblasts (MEFs). *Dkc1*$^{\Delta 15}$ and control wild-type (WT) male MEF cells were prepared from a cross of *Dkc1*$^{\Delta 15/+}$ females with WT male mice. Primary MEFs were isolated from 13.5-day mouse embryos and harvested for analysis after two to three passages. Cells were cultured in DMEM supplemented with 10% FBS, 100 units/ml penicillin, and 100 μg/ml streptomycin and maintained at 37°C in a humidified atmosphere of 3% O_2 and 10% CO_2.

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Fig. S1. Deletion of exon 15 of mouse Dkc1 gene. (*A*) Diagram depicting the *Dkc1*^{floxed15} construct, showing exon 15 flanked by *lox*P sites. The IRES-Neo element had been cloned into a unique StuI site in the 3′-untranslated region. The positions of the probes are shown. The Mpp1 probe was a 450-bp cDNA from *Mpp1* gene, the Neo probe was a 601-bp fragment from the Neo element. The asterisk represents the induced stop codon at the end of exon 14 of the *Dkc1* gene. Deletion of exon 15 (*Dkc1*$^{\Delta 15}$) in the early zygote was obtained by breeding with EIIa-Cre mice. Mice were then backcrossed with C57BL6 for at least four generations. All experiments were performed in C57BL6 background $n \geq 4$ unless indicated differently, in the absence of the EIIa-Cre transgene. (*B*) Southern blot analysis of G1 mice. DNA was extracted from liver and spleen of G1 mice. Fifteen micrograms of DNA was digested by BstEII and hybridized with *MPP1* probe. Correct WT and *Dkc1*$^{\Delta 1}$ cells are expected to yield 3.8-kb and 3.3-kb bands, respectively.