Supporting Information

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Methods

TRAP Assay. *T*elomeric *R*epeat *A*mplification *P*rotocol assays were performed using either the *TeloTAGGG* Telomerase PCR ELISA (Roche) or the Trapeze Detection Kit (Chemicon) to analyze telomerase activity in the testis or intestine, respectively. Briefly, cells were isolated from testis as described in *Methods* and from intestine using a chelation protocol (1) and sorted into GFP^+ and GFP^- populations using either a FACSAria or a FACS VantageSE flow cytometer. Telomerase activity was assessed using cell extracts from 1,000–2,000 GFP^+ or GFP^- cells, as indicated.

RT-PCR. Total RNA was extracted from FACS purified GFP^+ and GFP^- male germ cells or intestinal cells from adult mTert-GFP mice using TRIzol reagent (Sigma–Aldrich) per the manufacturer's protocol. RNA was then concentrated using RNeasy MinElute columns (Qiagen) and first strand cDNA synthesis was performed using the iScript Select cDNA Synthesis Kit (Bio-Rad). One tenth the volume (2 μl) of cDNA was used to amplify either mTert or β-actin transcripts yielding 107-bp and 390-bp products, respectively, using the following primer sets: mTert forward, 5′-TGCAGGAACTGATGTGGAAG-3′; mTert-reverse, 5′-GATCCTCTCCCTCAGACGGT-3′; β-actin-forward, 5′-GCCCTAGACTTCGAGCAAGA-3′, β-actin-reverse, 5′-CAGTGAGGCCAGGATAGAGC-3′. All PCRs were performed using an annealing temperature of 60°C and 40 cycles of amplification.

Ki67 FACS Analysis. Total bone marrow cells were isolated from *mTert*-GFP or wild-type mice and stained for LT-HSCs ($Flk2^-$KSL) as described in *Methods*. Cells were then fixed with 4% PFA-PBS, permeabilized in 0.1% Triton-PBS, refixed, and stained with a Ki67 rabbit monoclonal antibody (diluted 1:1; NeoMarker catalog no. # RM-9106-R7). Pacific Blue goat anti-rabbit Ig (1:100; Invitrogen) was used as a secondary antibody. Data were collected using a FACSAria flow cytometer and subsequently analyzed using FlowJo.

1. Mariadason JM, *et al*. (2005) Gene expression profiling of intestinal epithelial cell maturation along the crypt-villus axis. *Gastroenterology* 128:1081–1088.

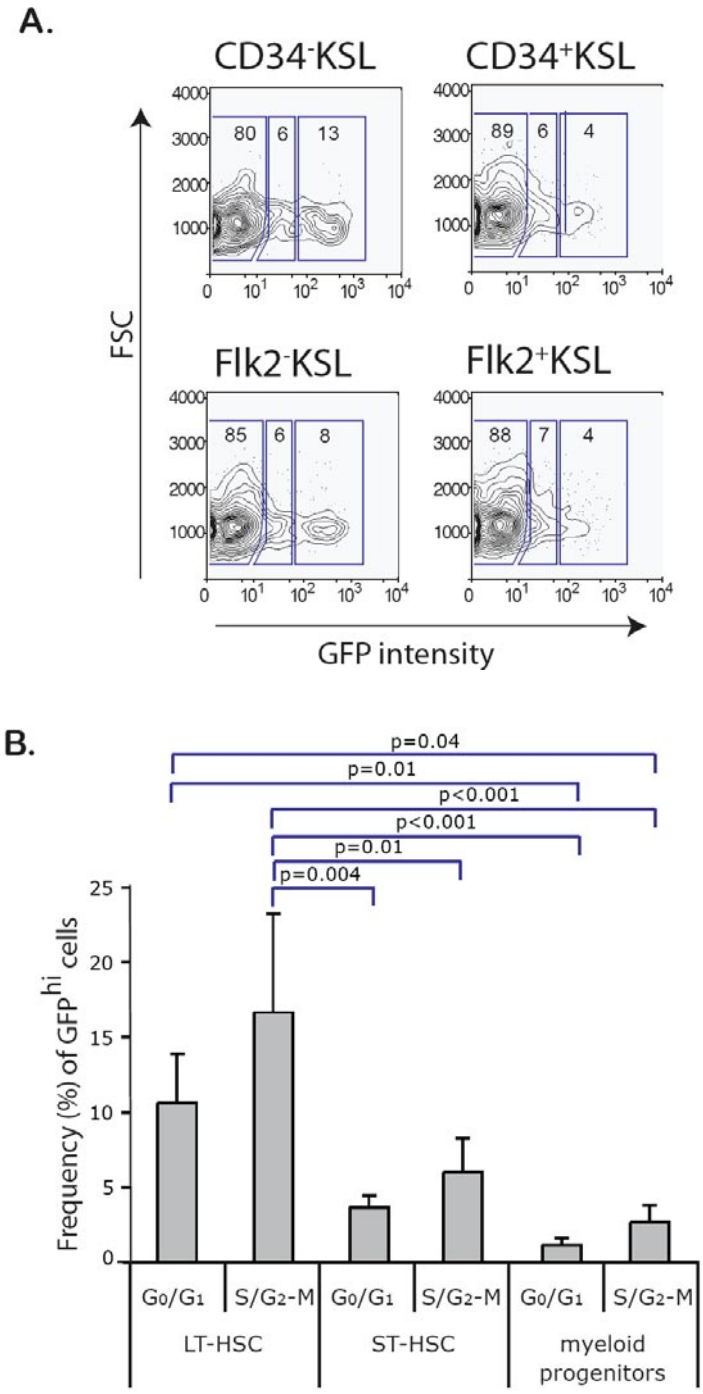
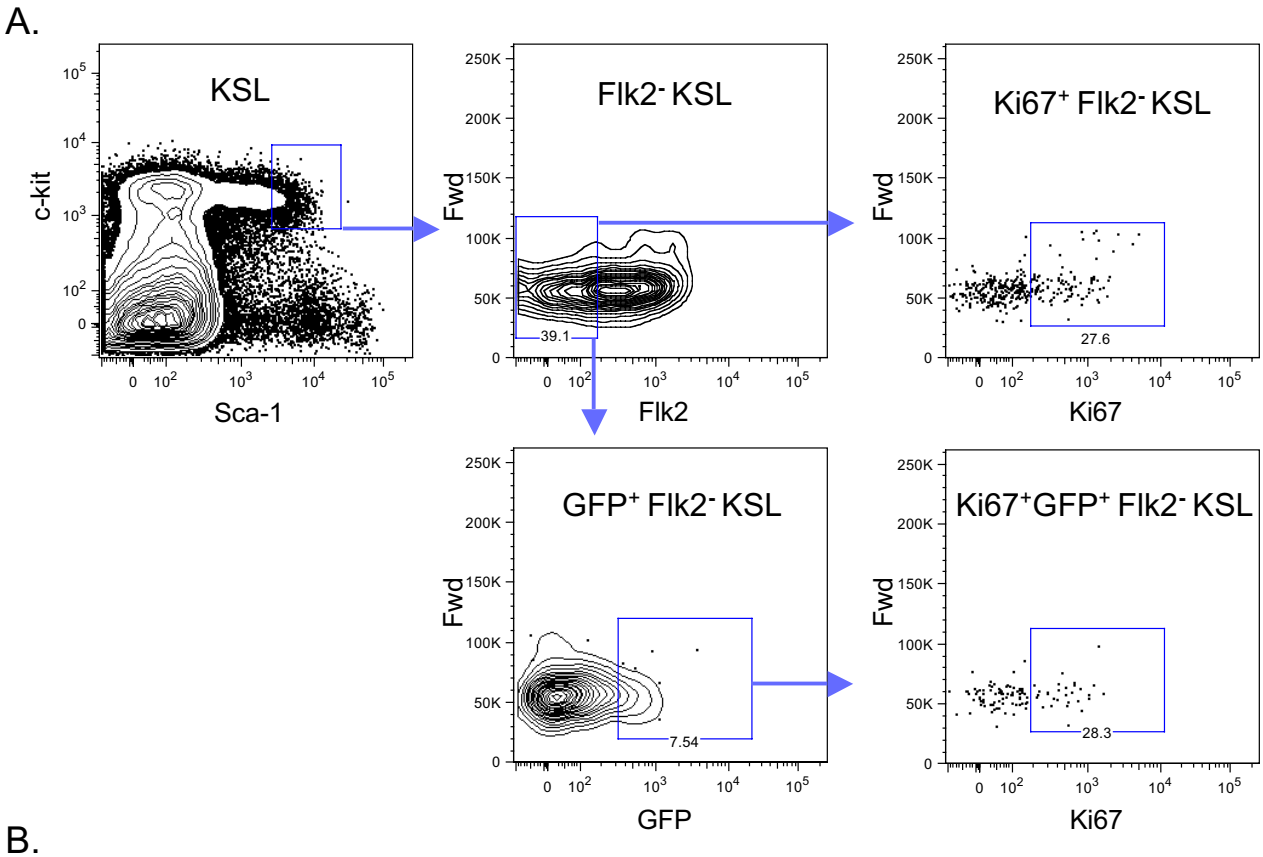


Fig. S1. Phenotypic analysis of GFP expression and cell cycle regulation in mTert-GFP mice. (*A*) Representative FACS plots for LT HSC [c-Kit$^+$Sca-1$^+$Lin$^-$ (KSL) and CD34$^-$ or Flk2$^-$] and ST HSC (KSL and CD34$^+$ or Flk2$^+$) illustrate the gates corresponding to the three GFP populations: GFP$^-$, GFP^{lo}, GFP^{hi}. (*B*) Cell cycle analysis was performed by using Hoechst dye labeling followed by multicolor FACS analysis. Pooled results (mean ± SEM) are from two independent experiments (n = 4 for each group; ANOVA, P = 0.003); results from post hoc Fisher's (PLSD) analysis indicated.



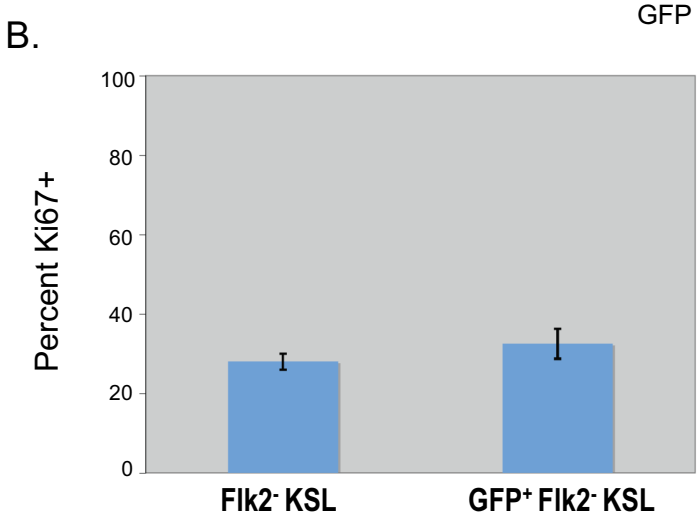


Fig. S2. No difference in proliferation status (Ki67$^+$) between mTert-GFP$^+$ and control LT-HSCs (Flk2-KSL). (*A*) Flow-cytometry analysis of lineage-negative bone marrow cells from mTert-GFP or WT mice. Long-term reconstituting hematopoietic stem cells, defined as c-Kit$^+$Sca-1$^+$Lin$^-$ (KSL) and Flk2$^-$, were analyzed for either Ki67 (*Upper Right*) or for GFP^{Hi} expression followed by Ki67 (*Lower Right*). (*B*) Comparison of percent Ki67$^+$ LT HSCs in control (Flk2$^-$KSL) vs. GFP (GFP$^+$Flk2$^-$KSL) populations. Data presented are from two experiments performed in duplicate or triplicate; mean ± SEM, $P = 0.3$, Student's *t* test.

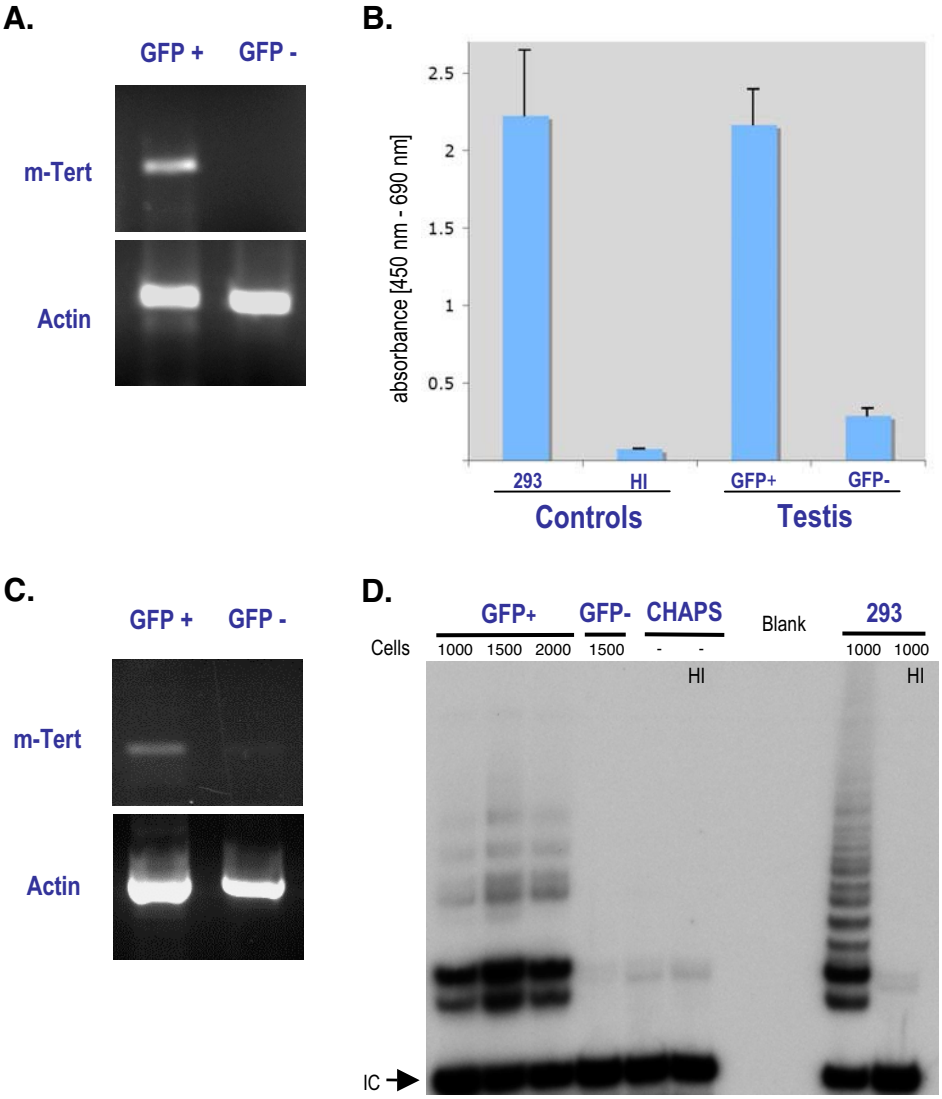


Fig. S3. Telomerase expression and activity colocalize with GFP$^+$ testis and intestinal cells. (*A*) Analysis of mTert and actin mRNA levels in FACS-purified GFP$^+$ or GFP$^-$ testis cells from adult mTert-GFP mice by RT-PCR. (*B*) Analysis of telomerase activity in 1000 FACS-purified GFP$^+$ or GFP$^-$ testis cells performed by using the *Telo*TAGGG Telomerase PCR ELISA (Roche) according to the manufacturer's instructions. Telomerase-expressing 293 cell extract served as a positive control and heat inactivated (HI) 293 cell extract served as a negative control. The data shown are from 2–4 experiments, each performed in duplicate. Comparison between absorbance values (mean ± SEM) from GFP$^+$ and GFP$^-$ populations was performed by using Student's *t* test, $P < 0.001$. (*C*) Analysis of mTert and actin mRNA levels in FACS-purified GFP$^+$ or GFP$^-$ intestinal cells from adult mTert-GFP mice by RT-PCR. (*D*) Analysis of telomerase activity in FACS-purified GFP$^+$ or GFP$^-$ intestinal cells performed by using the Trapeze Detection Kit (Chemicon) according to the manufacturer's instructions. Telomerase-expressing 293 cell extract served as a positive control, and HI 293 cell extract along with CHAPS buffer ± HI served as a negative control. The number of cells used in each lane is indicated. IC corresponds to an internal PCR control band. Heat inactivation controls for each GFP$^+$ and GFP$^-$ sample were negative for telomerase activity (data not shown).