1

Clonal inactivation of telomerase

promotes accelerated stem cell differentiation

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20
21

1 Summary

Telomerase is intimately associated with stem cells and upregulated in cancer, where it serves essential

roles through its catalytic action in elongating telomeres, nucleoprotein caps that protect chromosome

ends[1]. Overexpression of the telomerase reverse transcriptase (TERT) enhances cell proliferation

through telomere-independent means, yet definitive evidence for such a direct role in stem cell function

has yet to be revealed through loss-of-function studies. Here, we show that conditional deletion of TERT

in spermatogonial stem cells (SSCs) markedly impairs competitive clone formation. Using

lineage-tracing from the *Tert* locus, we find that TERT-expressing SSCs yield long-lived clones, but that

selective TERT-inactivation in SSCs causes accelerated stem cell differentiation thereby disrupting

clone formation. This requirement for TERT in clone formation is bypassed by expression of a

catalytically inactive TERT transgene and occurs independently of the canonical telomerase complex.

TERT inactivation induces a genome-wide reduction in open chromatin evident in purified SSCs, but not

in committed progenitor cells. Loss of TERT causes reduced activity of the MYC oncogene and

transgenic expression of MYC in TERT-deleted SSCs efficiently rescues clone formation. These data

reveal a required catalytic activity-independent role for TERT in preventing stem cell differentiation, forge

a genetic link between TERT and MYC and suggest new means by which TERT may promote

tumorigenesis.

Main Text

Telomerase is enriched in tissue stem cells and activated by somatic promoter mutations in many

cancers [2-4]. The core of the telomerase enzyme is comprised of the catalytic subunit TERT and the

telomerase RNA component (*Terc*), a small non-coding RNA scaffold that encodes the template for

telomere addition[1]. The critical requirement for telomerase in long-term cell viability is conserved from

single cell eukaryotes to humans. Cell proliferation in the absence of telomerase results in a lag phase

that is initially well tolerated while telomere reserves are ample. But, proliferation for extended periods in

the absence of telomerase culminates in senescence or cell death as telomeres progressively shorten

and eventually become dysfunctional. This is particularly evident in laboratory mice, which have very

long telomeres (40-80 kb vs 5-15 kb in humans). Telomerase knockout mice are initially viable, but

subsequent intergenerational breeding results in severe tissue defects in the advanced generations[5-7].

These findings established the paradigm that TERT is required only for its role in synthesizing telomeres.

In contrast with these loss-of-function studies, overexpression studies have sugested that TERT

promotes cell proliferation independent of its enzyme function. Conditional transgenic TERT expression

caused proliferation of hair follicle stem cells[8], skin basal layer keratinocytes[9] and kidney podocytes[10].

These effects of TERT were separable from telomere synthesis because they also occurred with a

catalytically inactive TERT allele, or in mice lacking *Terc*[8-10]. In this context, TERT has been shown to

activate MYC, WNT and NFkB pathways[8-14]. However, the role of non-canonical functions of TERT in

tissue stem cells remains unclear due to the lack of definitive and immediate phenotype in TERT

knockout mice.

Tissue homeostasis and carcinogenesis are shaped by cell competition, a mechanism to optimize cell

composition in tissues by promoting replacement of damaged or unfit cells with more robust neighboring

cells[15]. In renewing mammalian tissues including intestine, skin, and testis, competitive repopulation is a

fundamental property of adult stem cells[16]. Stem cell competition results in a dominant expansion of

more fit "winner clones" and an elimination of less fit "loser clones" at the niche[17]. This competitive behavior is also characteristic of carcinogenesis during which oncogenic mutations drive cells to clonally expand their territory through a process of super-competition[18]. Among tissues where competitive repopulation has been ovserved, testis shows high telomerase activity and exhibits unusual telomere dynamics in that telomere lengths are preserved with aging, in contrast to the progressive shortening seen in other human tissues. We previously found that spermatogonial stem cells (SSCs) express high levels of TERT and TERT is downregulated with lineage commitment [7]. To understand the telomere-independent role of TERT in stem cells, we developed a system to mark single SSCs expressing TERT coupled with the ability to conditionally inactivate TERT using a lineage tracing approach. Combining with transgenic rescue experiments and genomic assays, these studies establish TERT as a mediator of stem cell competition in the testis, where it supports stem cell function through a non-canonical mechanism independent of telomere synthesis.

Results

High TERT expression marks long-term SSCs that undergo stem cell competition

Spermatogenesis is a dynamic process to produce sperm, composed of mitosis, meiosis and

post-meiotic maturation (Extended Data Fig. 1a,b). In the testis, SSCs reside within a functionally and

morphologically heterogeneous population undifferentiated spermatogonia (US). Singly isolated A_{single}

(A_s) US undergo incomplete cytokinesis, subsequently producing progressively elongating chains of

interconnected cells (A_{pr} - A_{16}) (Extended Data Fig. 1b)[19]. Maturation of US yields differentiating

spermatogonia (DS), which is accompanied by loss of stem cell potential. To measure *Tert* expression in

distinct spermatogonia subpopulations, we purified MCAM^{high} KIT$^-$ US (US-h), MCAM^{med} KIT$^-$ US (US-m)

and MCAM^{med} KIT$^+$ DS (Extended Data Fig. 1a-c). Among these subpopulations, *Tert* mRNA expression

was high in both US-h and US-m cells, and sharply decreased in DS cells (Extended Data Fig. 1d).

Tert-Tdtomato reporter mice also showed high Tdtomato expression both in US-h and US-m (Extended

Data Fig. 1e). These data indicate that the entire US population exhibits high *Tert* expression.

To functionally study TERT-expressing spermatogonia, we developed a lineage tracing assay using

Tert$^{CreER/+}$; *Rosa26$^{lsl-Tdtomato/+}$* (*Tert$^{CreER/+}$*) mice, in which TERT-expressing cells are permanently labeled

upon tamoxifen-dependent activation of CreER to express Tdtomato (Fig. 1a,b) [20]. Marking SSCs results

in long-lived Tdtomato$^+$ clones, also known as patches, comprised of many daughter cells produced by

the labeled SSC [21]. If committed progenitor cells are labeled, only a small transient clone is generated

and these cells are lost through differentiation (Fig. 1a,b). Sparse labeling allows rare SSCs to be

marked, and in this context each patch derives from a single SSC. Thus, measuring the number of

Tdtomato$^+$ patches allows a quantitative assessment of stem cell self-renewal activity.

At two days after tamoxifen injection, TdTomato was detected similarly throughout the population of US

but not in KIT$^+$ cells (Extended Data Fig. 2a-c). At three months, marking TERT$^+$ cells yielded labeled

patches (Fig. 1c and Extended Data Fig. 2e). As we varied the dose of administered tamoxifen from 0.25

mg to 4 mg, patch number increased in a dose-dependent manner (Fig. 1d, and Extended Data Fig. 2d).

Patch length remained constant below 1mg but increased above 2mg, reflecting the fusion of two

independently labeled clones with high dose (Fig. 1e). In mice treated with 1mg tamoxifen and traced for

one year, patch length increased, clone number decreased, and the total aggregated Tdtomato$^+$ patch

length (mean patch number times mean patch length) remained constant compared with those traced

for three months, consistent with previously described stochastic competition with unlabeled clones (Fig.

1d-f, and Extended Data Fig. 2d) [21]. At both three months and one year, patches were comprised of cells

throughout the spermatogenic lineage (Extended Data Fig. 2f). Altogether, these data show that

TERT-expressing SSCs generate long-lived clones and exhibit competition within the stem cell pool.

TERT deletion impairs SSC-mediated clone formation

To investigate a direct and immediate role for TERT in stem cells, we developed a competitive clone

formation assay using *Tert$^{CreER/flox}$:Rosa$^{lsl-Tdtomato/+}$* (*Tert$^{CreER/flox}$*) mice (Fig. 1g, and Extended Data Fig.

3a-c). In this strain, activation of CreER induces Tdtomato labeling and concomitantly inactivates *Tert* in

the same TERT$^+$ cell, enabling one to trace the fate of cell clones deriving from SSCs in which *Tert* has

been somatically deleted in an environment where most neighboring cells retain TERT expression. At

two days after 1mg tamoxifen treatment, GFRA1$^+$ A$_s$ and A$_{pr}$ clones were marked indistinguishably in

both *Tert$^{CreER/+}$* and *Tert$^{CreER/flox}$* mice (Fig. 1h,i). Pulse labeling efficiently eliminated *Tert* mRNA in

Tdtomato$^+$ US (Fig. 1j,k). At three months and six months, deletion of TERT caused a marked reduction

in the number of Tdtomato$^+$ clones and diminished mean patch length (Fig. 1l-n). Correspondingly, the

total aggregated Tdtomato$^+$ patch length was sharply reduced (Fig. 1o). Together, these findings

indicate that TERT-deletion compromises long-term clone formation ability of SSCs under theses

competitive conditions.

TERT promotes SSC competition independent of its catalytic activity and the telomerase

complex

To determine whether the effect of TERT in enhancing SSC competition depends on its catalytic activity,

we developed a system to rescue the defect in TERT-deleted cells using tetracycline-regulated TERT

transgenes: either one that is wild-type (TetO-TERT) or one that is catalytically inactive (TetO-TERTci)

due to a single amino acid substitution in the catalytic site [9]. We produced compound mouse strains in

which activation of CreER simultaneously deletes the floxed *Tert* gene while inducing expression of

transgenic *Tert* by triggering expression of the tetracycline transactivator (tTA) that binds and activates

the *TetO* promoters (Fig. 2a). We analyzed how TERT loss affected clone formation in

Tert$^{CreER/flox}$*:Rosa*$^{lsl\text{-}Tdtomato/lsl\text{-}tTA}$ (*Tert*$^{CreER/flox}$) vs. *Tert*$^{CreER/+}$*:Rosa*$^{lsl\text{-}Tdtomato/lsl\text{-}tTA}$ (*Tert*$^{CreER/+}$) controls and we

compared these results with restoration of Tert expression in *Tert*$^{CreER/flox}$*:Rosa*$^{lsl\text{-}Tdtomato/lsl\text{-}tTA}$*:TetO-Tert*

(*Tert*$^{CreER/flox}$ +*Tert*) mice or *Tert*$^{CreER/flox}$*:Rosa*$^{lsl\text{-}Tdtomato/lsl\text{-}tTA}$*:TetO-Tertci* (*Tert*$^{CreER/flox}$ +*Tertci*) mice (Fig. 2a).

Transgenic *Tert* and *Tertci* expression was induced at a physiological range by treating mice with low

dose doxycycline, which suppresses binding of tTA to the TetO promoter (Extended Data Fig. 4a).

Transgenic expression of wild-type *Tert*, but not *Tertci*, restored telomerase activity (Extended Data Fig.

4b). Intriguingly, expression of either *Tert* or *Tertci* transgenes fully rescued patch number, average

patch length, and total patch length (Fig. 2b-e). Furthermore, expression of TERTci significantly

increased total patch length compared to control *Tert*$^{CreER/+}$ mice (Fig. 2e). These results indicate that

TERT promotes enhanced stem cell competition independent of its catalytic activity.

Terc encodes the RNA template for telomere addition and serves as the central scaffold for assembly of

the telomerase complex. Therefore in the absence of *Terc*, TERT and the other components of

telomerase fail to associate [1,22]. To determine whether formation of the telomerase complex is required

for the TERT-dependent SSC clone formation, we produced first generation

Terc$^{-/-}$*:Tert*$^{CreER/flox}$*:Rosa*$^{lsl\text{-}Tdtomato/+}$ (G1 *Terc*$^{-/-}$*:Tert*$^{CreER/flox}$) mice (Extended Data Fig. 4c. In this strain,

telomerase was inactive in testes (Extended Data Fig. 4d). Three months after tamoxifen treatment,

conditional deletion of *Tert* in *Terc*-deficient mice impaired patch number, patch length, and total patch

length (Extended Data Fig. 4e -h). These findings uncouple the requirement for TERT in stem cell

competition from engagement in the classical telomerase complex.

Somatic deletion of *Tert* in mice with very long telomeres is unlikely to cause telomere dysfunction [7,23,24].

When telomeres become short, however, a DNA damage response activates the p53 tumor suppressor

protein, triggering either cell death or senescence [25]. To understand whether the impaired clone

formation in TERT-deleted SSCs is mediated by p53, we generated

Trp53^{flox/flox}:Tert^{CreER/flox}:Rosa^{lsl-Tdtomato/+} mice and examined competitive clone formation (Extended Data

Fig. 5a). At day five, the deletion efficiency of the *Trp53-flox* alleles in Tdtomato$^+$ US was 44.4%

(Extended Data Fig. 5b,c). We found that deletion of p53 failed to rescue the impaired clone formation

associated with *Tert* inactivation, as measured by patch number, patch length, and total patch length

(Extended Data Fig. 5d-g). Consistent with these findings, we found no accumulation of γH2AX, a

marker of DNA damage, in *Tert^{CreER/flox}* mice, whereas γH2AX was elevated in G6 *Tert^{Tdtomato/Tdtomato}* mice

with dysfunctional telomeres (Extended Data Fig. 5h,i). Taken together, the effects of TERT in promoting

stem cell-derived clone formation is independent of catalytic activity, the DNA damage response and the

canonical telomerase complex.

Accelerated differentiation and impaired proliferation of SSCs lacking TERT

The preferential elimination of conditionally TERT-deleted SSCs could be caused by accelerated

differentiation, impaired proliferation, or increased apoptosis. To distinguish among these possibilities,

we investigated how clonal deletion of TERT influences SSC fate across at time points. At 14 days after

tamoxifen administration, Tdtomato$^+$ A$_s$ and A$_{pr}$ US were significantly decreased in *Tert^{CreER/flox}* mice, and

the reduction of those cells was rescued by transgenic expression of either TERT or TERTci (Fig. 2f and

Extended Data Fig. 6a). At six weeks when a comparable frequency of labeled patches were found in

Tert^{CreER/+} and *Tert^{CreER/flox}* mice, labeled clones harbored significantly fewer US (Fig. 2g,h Extended Data

Fig. 6b,c). These results indicate that TERT deletion promotes differentiation of US. To understand how

TERT loss affects cell proliferation, we measured BrdU incorporation seven days after tamoxifen

administration. The percentage of US incorporating BrdU was significantly reduced in $Tert^{CreER/flox}$

animals and proliferation was rescued with transgenic expression of *Tert* or *Tertci* (Fig. 2i and Extended

Data Fig. 6d). There was no increase in apoptosis by cleaved-PARP staining in $Tert^{CreER/flox}$ mice, but

apoptosis was elevated in testes from G6 $Tert^{Tdtomato/Tdtomato}$ mice with critically short telomeres (Extended

Data Fig. 6e,f). Taken together, impaired clone formation of TERT deleted SSCs is caused by

accelerated differentiation and decreased proliferation.

TERT deletion compromises chromatin accessibility in SSCs but not in committed progenitors

To understand how TERT deletion affects global chromatin structure, we performed ATAC-seq, which

allows an assessment of chromatin accessibility genome-wide [26]. To first define the patterns of

chromatin changes during normal spermatogenesis, we purified US-h, US-m, and DS from $Tert^{CreER/+}$

mice that had been injected with tamoxifen seven days prior to isolation, while spermatocytes (SP) and

round spermatids (RS) were purified based on differential *Tert* promoter activity in $Tert^{Tdtomato/+}$ mice [7].

Principal component analysis (PCA) revealed that US-h and US-m clustered together in the lower left

quadrant, consistent with their similar gene expression patterns (Extended Data Fig. 7a) [27]. DS cells

localized in the upper left quadrant, while SP and RS populations were clustered together in the right

lower quadrant (Extended Data Fig. 7a), indicating that the PC1 axis captures the changes in global

chromatin state associated with differentiation. Similarly, Pearson correlation hierarchical clustering

showed a high correlation in open chromatin patterns among spermatogonia subpopulations, but abrupt

changes of chromatin accessibility globally upon entry to meiosis (Extended Data Fig. 7b). The number

of unique ATAC-seq peaks and promoter chromatin accessibility around transcription start sites were

highest in US-h and US-m and decreased significantly during differentiation into DS and SP (Fig. 3a,b,

and Extended Data Fig. 7c,d). The promoter region of *Tert* was accessible in US-h, US-m and DS, but

inaccessible in SP and RS, consistent with the expression pattern of TERT during spermatogenesis

(Extended Data Fig. 8a) [7]. Altogether, these data reveal that SSCs show a marked increase in chromatin

accessibility, indicating that a global reduction in chromatin accessibility occurs during spermatogenesis.

To understand how conditional TERT-deletion influences chromatin accessibility in SSC populations, we

performed ATAC-seq on US-h, US-m and DS isolated from $Tert^{CreER/flox}$ mice. Pearson correlation

hierarchical clustering showed that the overall pattern of chromatin accessibility in TERT-deleted cells

remained similar to that of $Tert^{CreER/+}$ controls (Extended Data Fig. 7b). However, PCA revealed that

TERT deletion caused a shift in the US-h and US-m along the differentiation axis, whereas TERT loss

had no discernible effect on committed DS (Fig. 3c). Deletion of TERT in the US populations caused a

marked reduction in the number of open chromatin peaks and diminished chromatin accessibility

surrounding TSSs to a level resembling the control DS (Fig. 3a,b,d,e and Extended Data Fig. 7e). In

contrast, peak number was unaffected by TERT deletion in DS (Fig. 3 a,b,d,e and Extended Data Fig.

7e). This reduction in open chromatin peaks was evident in genes associated with stemness in SSCs,

including *Ret*, *Gfra1*, *Cdh1*, and *Zbtb16*, whereas those associated with differentiation including *Kit*,

Prm2, and *Prm3* remained unchanged (Extended Data Fig. 8b-d). Pathway analysis revealed that genes

in the MAPK signaling pathway, which promotes self-renewal of SSCs [28], were particularly enriched

among those showing loss of open chromatin peaks in the TERT-deleted undifferentiated

spermatogonia (Extended Data Fig. 7f). Taken together, conditional inactivation of TERT caused a loss

of open chromatin selectively in the stem cell containing population but not in committed progenitors,

consistent with accelerated stem cell differentiation caused by TERT deletion.

TERT promotes competitive clone formation of SSCs through MYC

To understand how TERT promotes competitive clone formation in SSCs, we examined gene

expression in TERT-deleted US-h cells by RNA-seq seven days after tamoxifen administration. PCA

showed that TERT-deleted US-h clustered separately from TERT[+] controls (Fig. 4a). Using strict cutoffs

for significance, there were 23 genes downregulated and 116 genes upregulated in TERT-deleted US-h

(Extended Data Fig. 9a). Gene set enrichment analysis revealed that spermatogenesis-related genes

were upregulated in TERT-deleted US-h, consistent with their accelerated differentiation (Extended Data

Fig. 9b). Several gene sets were downregulated in TERT-deleted US-h cells, including E2F targets and

G2M checkpoints, reflecting the quantitative reduction in proliferation in these cells (Extended Data Fig.

9c). The most significantly downregulated gene set was 'MYC targets v1' and a second gene set 'MYC

targets v2' was also represented (Fig. 3b, and Extended Data Fig. 9c). Consistent with this, MYC protein

was significantly decreased in TERT-deleted US and MYC levels were restored by transgenic

expression of TERT or TERTci (Fig.4c, and Extended Data Fig.9d,e). *Myc* mRNA levels remained

unchanged, suggesting that TERT promotes MYC expression at the post-transcriptional level (Extended

Data Fig. 9f).

MYC is a transcription factor that promotes cell competitiveness by regulating cell proliferation, growth,

and metabolism [29-32]. MYC has been shown to promote SSC self-renewal and also an important

oncogene [33-35]. Given the genetic links between TERT and MYC, we hypothesized that MYC

overexpression might rescue the failure of clone formation in TERT-deleted SSCs. To test this idea, we

intercrossed *Tert*[CreER/flox]*: Rosa*[lsl-Tdtomato/lsl-tTA] mice with *TetO-human MYC* transgenic mice (*Tert*[CreER/flox]

+MYC) (Fig. 4d). This system allows simultaneous deletion of the residual TERT allele and activation of

transgenic MYC selectively in a lineage of TERT-expressing stem cells. To limit expression levels of

transgenic MYC, mice were treated with doxycycline, which reduced transgenic *MYC* mRNA levels by

5.6 fold (Extended Data Fig. 9g). Three months after tamoxifen treatment, the defects in clone formation

associated with TERT loss were dramatically rescued by MYC expression, as measured by patch

number, patch length and total patch length (Fig. 4e-h). MYC expression also restored the number of

GFRA1^{+} cells, proliferation and expression of MYC target genes (Fig. 4i,j and Extended Data Fig. 9h).

Taken together, these results indicate that the marked defect in stem cell competition associated with

TERT loss is due to impaired MYC function and establish an epistatic relationship between TERT and

MYC.

Discussion

TERT is expressed in many stem cell compartments governed by competitive repopulations and TERT upregulation is selected for during human carcinogenesis. By tracing the fate of individual SSCs *in vivo*, we found that loss of TERT severely compromises competitive clone formation and accelerates stem cell differentiation. In this context, TERT-deleted stem cells represent "loser" clones outcompeted by TERT-proficient "winner" clones. This requirement for TERT in competitive clone formation is independent of telomeres and TERT catalytic function; instead, TERT maintains chromatin accesiblity across many genes expressed in stem cells. These findings establish TERT as a key determinant regulating competition between tissue stem cells by favoring self-renewal and disfavoring differentiation (Extended Data Fig. 10). This result is surprising in that germline inactivation of TERT in mice is well tolerated initially, although SSC function is ultimately compromised by telomere dysfunction after many generations of telomerase-deficiency. Competitive behavior of SSCs may expain why germline mutations in *Tert* do not cause an obvious phenotype in laboratory mice. Selection of winner clones and elimination of loser clones in the testis may be determined in part based on competition for limiting niche signals [36]. This niche-dependent competition between TERT-proficient and TERT-deficient cells may amplify cell-autonomous defects caused by acute TERT deletion, while promoting elimination of TERT-deleted SSCs. Homogeneous deletion of TERT throughout the organism does not create a TERT-dependent competition, and in this context TERT-deficient cells are capable of self-renewal and differentiation to produce sperm. Thus, germline gene inactivation identifies only one layer of activity, whereas clonal, conditional gene deletion can reveal deeper aspects of gene function.

While TERT levels are important in maintaining stem cell function, they may play a similar role during carcinogenesis. TERT is upregulated in many human tumors through non-coding mutations in the proximal TERT promoter. These TERT promoter mutations show strong positive selection during tumor progression, as the prevalence of these mutations increases substantially from pre-invasive to invasive

stages. Our data establishing an epistatic relationship between TERT and MYC provide support for this

model. MYC activity is central in determining outcomes in cell competition; cells expressing higher MYC

outcompete those with lower MYC and MYC represents a key node in many human cancers [29-32]. TERT

upregulation in cancer likely increases telomerase activity and stabilizes telomeres, thereby preventing

the negative effects of dysfunctional telomeres on cell cycle and cell survival. At the same time, TERT

upregulation may enhance cell competition through MYC and its non-canonical effects in promoting

self-renewal and preventing differentiation. MYC can also control TERT levels by directly activating

transcription of *Tert* through promoter binding [37-39]. Therefore TERT and MYC may comprise a positive

feedback loop that promotes cell competition in various tissues during development, homeostasis and

carcinogenesis. Our findings using clonal deletion in SSCs add important new complexity to our

understanding of TERT function. Taken together, this study establish a role for TERT in promoting clonal

competition in stem cells *in vivo* with important implications for understanding tissue homeostasis, cancer

development and telomere maintenance.

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Methods

Animals. All animal protocols were approved by the Institutional Animal Care and Use Committee at

Stanford University. All experiments are in compliance with the ethical regulations of Stanford University.

Tert-CreER, TetO-Tert, TetO-Tertci, Tert-Tdtomato mice were previously reported [7,10,20].

Rosa-lslTdtomato [40], *Rosa-lsl-tTA* [41], *TetO-hMYC* [42], *Terc-KO* [43], *Trp53-flox* [44] mice were purchased from

The Jackson laboratory. Tamoxifen (Cayman) was dissolved in corn oil (Sigma-Aldrich) at 5 - 20mg/ml

by incubating at 50℃ for 30min with mixing every 5min. Two- to three-month-old mice were

administrated with 0.25 - 4mg per 25g body weight tamoxifen by oral gavage or intra-peritoneal injection.

Doxycycline (Sigma-Aldrich) was dissolved in drinking water in light-protected bottles at 1 or 3 μg/ml and

changed every 4 days. BrdU (Sigma-Aldrich) was dissolved in PBS at 10mg/ml and intra-peritoneally

injected at 1.25mg per 25g body weight at 2 hours before sacrifice.

Generation of TERT-flox mice

9kb fragment of TERT locus was subcloned and Lox-Puro-lox cassette from pBS.DAT-LoxStop plasmid

(kindly gifted from David Tuveson) was inserted at the BsiWI site in the second intron. Another loxP

sequence and NdeI site were inserted at the KasI site in the sixth intron. The targeting vector was

linearized and electroporated into J1 mouse ES cells. After positive selection with puromycin, correctly

targeted ES clones were selected by long-range PCR and Southern blotting, and then injected into

C57BL6 blastocysts to generate the knock-in line. To remove floxed Puro cassette, the knock-in line was

crossed with CMV-cre mice [45] and puro-negative TERT-floxed mice were selected by PCR and Southern

blotting using genomic DNA from tail tips. *TERT$^{flox/+}$* mice were born at normal Mendelian frequency.

Lineage tracing assay.

After tamoxifen injection, testes were detunicated, dissociated using fine forceps in PBS containing

1mg/ml collagenase IV (Worthington) for 10min to remove interstitial cells, and placed in cold PBS.

Images were captured with a fluorescent dissection microscope and the patch number and length were

measured with ImageJ. Total patch length was calculated by multiplying the patch number by the average patch length.

Whole-mount Immunofluorescence of seminiferous Tubules

Seminiferous tubules were dissociated using fine forceps in PBS containing 1mg/ml collagenase IV for 10min, fixed with 4%PFA at 4℃ for 2h, cleared with 0.1% Igepal CA-630 (Sigma-Aldrich) in PBST, and dehydrated and rehydrated by immersing in a gradient of methanol diluted with PBST (25%, 50%, 75%, 100%, 75%, 50%, 25%) at 4℃ for 5min each. After washing in PBST, tubules were incubated in blocking buffer (0.5% BSA PBST) (Sigma-Aldrich), followed by incubation with antibodies in Immuno shot immunostaining Mild (Cosmo Bio) at 4℃ for two days. After extensive wash with PBST, tubules were incubated with secondary antibodies in blocking buffer at room temperature for 90min, washed with PBST, and then mounted in Vectashield with DAPI (Vector laboratories). Images were captured on a Leica SP5 confocal microscope and processed in Photoshop.

Section immunostaining

Testes were detunicated, fixed with 4% PFA at 4℃ overnight, incubated in a gradient ethanol, xylen, embedded in paraffin, and cut into 5-µm sections. After rehydration, antigen retrieval using Antigen retrieval solution citric acid or tris-based (Vector) for 10min in a pressure cooker. Sections were blocked, incubated with primary antibody at 4℃ overnight. After washing with PBS, sections were incubated with secondary antibodies at room temperature for 1h and mounted in Vectashield with DAPI. For co-staining using rabbit anti-RFP antibodies and rabbit anti-PLZF antibodies, sections were antigen retrieved, incubated with anti-RFP antibody, then with HRP-conjugated anti-rabbit secondary antibody as described above, and signals were detected with TSA plus Cyanine 3 system (Perkin Elmer). Those antibodies were stripped off by antigen retrieval, and sections were further stained with BrdU,

cleaved-PARP, or PLZF antibodies. For BrdU detection, those slides were treated with 2N HCl for 20min,

incubated with rabbit ant-PLZF and rat anti-BrdU antibodies at 4℃ overnight, and signals were detected

by Alexa488-conjugated anti-rat IgG and Cy5-conjugated anti-rabbit IgG antibodies. For cleaved-PARP

detection, slides were incubated with rabbit ant-PLZF and mouse anti-cleaved PARP antibodies at 4℃

overnight, and signals were detected by Alexa488-conjugated anti-mouse IgG and Cy5-conjugated

anti-rabbit IgG antibodies. For triple staining using rabbit anti-RFP, rabbit anti-PLZF, and rabbit c-MYC

antibodies, sections were stained with anti-Tdtomato antibody using TSA plus Cyanine 3 system, and

antibodies were stripped off. Then those sections were stained with anti-MYC antibody with TSA plus

fluorescein system (Perkin Elmer) followed by antigen retrieval to remove antibodies. Finally, the

sections were further stained with anti-PLZF antibody and Cy5 conjugated anti-rabbit IgG. Slides were

mounted in Vectashield with DAPI. Images were captured on a fluorescent microscope and processed in

Photoshop. Signal intensity of c-MYC signal was quantified with Image J.

TRAP assays (Telomeric Repeat Amplification Protocol).

A two-step TRAP procedure was performed as previously reported[46]. Extract fractions from whole testis

at 3 weeks or FACS-sorted undifferentiated spermatogonia were incubated with telomeric primers for a

30 min initial extension step at 30°C in a PCR machine, followed by 5 min inactivation at 72°C. Without

purification, 1μl of the extended reaction was PCR amplified (cycles of 30 seconds at 94°C, followed by

30 seconds at 59°C) in presence of ^{32}P end-labeled telomeric primers that has been purified using a

micro-spin G-25 column (GE healthcare). PCR reactions were resolved by 9% polyacrylamide gel

electrophoresis at room temperature, and the gel was exposed to a phosphor-imager and scanned by a

Typhoon scanner. The scanned image was quantitated using the TotalLab Quant software.

Representative gel images were presented among at least 2 repeats.

FACS analysis

Testes were detunicated, lightly dissociated in PBS, and incubated in PBS containing DNase I

(Worthington) and 1mg/ml collagenase I (Worthington) at 32℃ for 10 min. Cells were centrifuged at

250g for 5min and supernatant was removed. After repeating collagenase I treatment, testicular cells

were further digested with TrypLE Express (GIBCO) at 32℃ for 15 min. During enzymatic digestions,

seminiferous tubules were mechanically fragmented with vigorous pipetting every 5min. Cells were

sequentially filtered with 70 μm and 40 μm strainers, resuspended in cold FACS buffer (2% FBS, 1mM

EDTA in PBS), and incubated with antibodies on ice for 30min. After PBS wash, cells were resuspended

in cold FACS buffer containing DAPI, and analyzed and sorted with a BD Aria II (BD Biosciences). Data

was analyzed with FlowJo software. The list of antibodies is available in supplemental table.

RNA in situ hybridization

5 days post labeling, testes were collected and Tdtomato+ undifferentiated spermatogonia were

FACS-sorted, and cytospun at 300 r.p.m. for 5 min onto slides. Slides were fixed in 4% (v/v) PFA for 30

min at room temperature and processed for single-molecule RNA FISH using RNAscope 2.5 HD

Reagent KIT-RED (Advanced Cell Diagnostics) and probes against mouse *Tert* or *Trp53* (Advanced Cell

Diagnostics) according to the manufacturer's instructions.

Quantitative RT-PCR

For qRT-PCR, cells were directly sorted into Trizol LS (Thermo Fisher Scientific) by FACS and mixed

with 100ng yeast tRNA as carrier. RNA was purified Direct-zol RNA Microprep (Zymo Research) and

cDNA was synthesized using oligo-dT and SuperScript IV First-Strand Synthesis system (Thermo Fisher

Scientific). For qRT-PCR of *Tert* and mouse *Myc*, Universal Probe Library Probe #066 and #72 (Roche)

were used, respectively. For these reactions, TaqMan Fast Advanced Master Mix (Thermo Fisher

Scientific) were used. For other qRT-PCR, PowerUp SYB Green Master Mix (Thermo Fisher Scientific)

was used according to the product manual. PCR analysis was done with a 7900HT Fast Real-Time PCR

System machine (ABI). List of primers is available in supplemental table.

RNA-sequencing

US-h cells were directly sorted into Trizol LS by FACS and RNA was purified using Direct-zol RNA

Microprep. Genomic DNA was digested with on-column DNase treatment. RNA quality was checked by

Bioanalyzer 2100 (Agilent). RNA-seq libraries were constructed using SMARTer Stranded Total

RNA-seq Kit v2 – Pico Input Mammalian (Clontech), starting from 5ng total RNA. cDNA was synthesized

and amplified according to the manual. After the rRNA removal step, cDNA was amplified with 13 cycles

of PCR reactions. Quality of purified cDNA libraries was confirmed by Bioanalyzer 2100 and

concentration of cDNA. Libraries were sequenced on the Illumina NextSeq platform, generating about

16-24 million 75-bp paired-end reads per library. Four biological replicates per sample were analyzed.

Raw reads were trimmed by TrimGalore 0.4.0 (Babraham Bioinformatics), mapped to mm10 by tophat

2.0.13 [47], analyzed by the DEseq2 packages [48].

ATAC-sequencing

ATAC-seq libraries were made as described previously [49] using the Omni-ATAC protocol. Adjustments

to the protocol were made to reflect two primary features of the cell types profiled in this work. First, the

amount of Tn5 transposase added to each reaction was modulated to maintain proportionality with the

number of cells assayed. For example, a normal reaction uses 50,000 cells and 2.5 ul of Tn5

transposase in a 50 ul reaction. In the case of rarer spermatogonial stem cells, only 5,000 cells could be

obtained so only 0.25 ul of Tn5 transposase was used in a 50 ul reaction. The difference in volume was

adjusted using water. Second, the ploidy of each cell type was taken into account and the amount of Tn5

was adjusted based on ploidy as well. For example, round spermatid cells are haploid, so transposition

of 50,000 cells would require 1.25 ul of Tn5 transposase in a 50 ul reaction. Similarly, spermatocytes are

4N meiotic cells so the amount of Tn5 transposase was increased proportionately and the amount of

water in the reaction was reduced. In all cases, regardless of cell number or ploidy, the reaction volume

of the transposition reaction was kept constant at 50 ul. All ATAC-seq reactions were performed using

homemade Tn5 transposase and Tagment DNA (TD) buffer [50]. Downstream amplification and

purification of libraries was performed as described previously [26,49,51].

5   ATAC-seq data pre-processing was completed using the PEPATAC pipeline

(http://code.databio.org/PEPATAC/). The mm10 genome build (https://github.com/databio/refgenie) was

used for alignment. Briefly, all fastq files were first trimmed to remove Illumina Nextera adapter

sequence using Skewer [52] with "-f sanger –t 20 –m pe –x" options. FastQC

(http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc) was used to validate proper trimming and check

overall sequence data quality. Bowtie2 [53] was then used for pre-alignments to remove reads that would

map to chrM (revised Cambridge Reference Sequence), alpha satellite repeats, Alu repeats, ribosomal

DNA repeats, and other repeat regions with "-k 1 -D 20 -R 3 -N 1 -L 20 -i S,1,0.50 -X 2000 –rg-id" options.

Bowtie2 was then used to align to the mm10 reference genome using "--very-sensitive -X 2000 --rg-id"

options. Samtools [54] was used to sort and isolate uniquely mapped reads using "-f 2 -q 10 -b -@ 20"

options. Picard (http://broadinstitute.github.io/picard/) was used to remove duplicates. Then the bam

files were merged by conditions, and MAC2 [55] was used to call peaks with parameter "-q 0.05 --nomodel

--shift 0". The narrow peaks were then filtered by the ENCODE 7 hg19 blacklist, as well as peaks that

extend beyond the ends of chromosomes. Bedtools [56] was used to retrieve the reads of the called peaks

for each sample with multicov module. All the samples have similar sequencing depth, mitochondrial rate,

and duplication rate. The spermatocyte and the round spermatid samples have similar sequencing depth

compared to all other samples, but slightly higher mitochondrial rate and lower duplication rate, so have

more final reads after initial processing and filtering. To make all the samples comparable for the

statistical analysis, we used final reads as the normalization factor. R package "DESeq2" [48] was used for

statistical analysis to identify significant peaks between different conditions. The differential peaks were

called between US-h CreER/+ and US-h CreER/flox samples. Peaks with FDR < 0.01 and fold change

larger than 2 or smaller than -2 were considered as significant. R packages "ChIPseeker" [57] was used for

peak annotation. Package "ngsplot" [58] was used for visualization of cumulated peak signal.

Statistics: No statistical methods were used to predetermine sample sizes. When comparing two

groups, p values were determined by two-sided unpaired t-test. When comparing more than two groups,

p values were determined by one-way ANOVA with Tukey's test. Values are presented as mean ± SEM.

The animals were randomly assigned to each experimental or control group. Graphs were generated by

the Prism 8.

Data availability: The source data for the RNA-seq study are available in the NCBI Gene Expression

Omnibus (GEO) repository under accession number GSE14659. All other data that support the finding

of this study are available from the corresponding author upon reasonable request.

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Figure legends

Fig. 1. TERT deletion impairs SSC-mediated clone formation. (a) $Tert^{CreER/+}Rosa^{lsl-Tdtomato/+}$ mice. **(b)**

Lineage tracing using *Tert-CreER*. **(c)** Epifluorescence of Tdtomato and bright field image of untangled

seminiferous tubules in a single whole testis at 3 months after 1mg tamoxifen injection. Scale bars, 5mm.

(d-f) Mean patch number (d), mean patch length (e), and total patch length (f) after pulse labeling with

indicated tamoxifen dose and span (n=6, 6, 6, 4, 6, 5 mice from left to right). **(g)**

$Tert^{CreER/flox}:Rosa^{lsl-Tdtomato/+}$ mice. **(h)** Whole mount immunofluorescence of GFRA1 and Tdtomato. Scale

bars, 100μm. **(i)** Quantification of Tdtomato$^+$ cells within GFRA1$^+$ A_s and A_{pr} cells (n=4 mice per group).

(j) In situ hybridization against *Tert* mRNA in purified Tdtomato$^+$ US at 5 days post-tamoxifen treatment.

Undifferentiated spermatogonia from $Tert^{CreER/CreER}$ mice was used as negative control. Scale bars,

40μm. **(k)** Quantification of foci of *Tert* mRNA (n=232, 243, 341 cells from left to right). **(l)**

Epifluorescence of Tdtomato in untangled seminiferous tubules at 3 months and 6 months

post-tamoxifen injection. Scale bars, 5mm. **(m-o)** Quantification of mean patch number (m), mean patch

length (n), and total patch length (o) (n=7, 7, 9, 8 mice from left to right). Data are represented as Mean ±

SEM.

Fig. 2. TERT promotes SSC competition independent of its catalytic activity and the telomerase

complex. (a) $Tert^{CreER/flox}:Rosa^{lsl-Tdtomato/lsl-tTA:}$ *TetO-Tert or -Tertci* mice. **(b)** Epifluorescence for Tdtomato

in untangled seminiferous tubules at 3 months after tamoxifen injection. Scale bars, 5mm. **(c-e)**

Quantification of mean patch number (c), mean patch length (d), and total patch length (e) (n=6 mice per

group). **(f)** Quantification of TdTomato$^+$, GFRA1$^+$ A_s and A_{pr} cells at 7 or 14 days post labeling (n=5, 5, 5,

5, 6, 6, 5, 5 mice from left to right). **(g)** Mean patch number at 6 weeks after tamoxifen injection (n=5

mice per group). **(h)** Quantification of percent Tdtomato$^+$ clones containing PLZF$^+$ cells (n=5,4 mice from

left to right). **(i)** Quantification of BrdU$^+$ cells in Tdtomato$^+$, PLZF$^+$ undifferentiated spermatogonia (n=6, 5,

5, 6 mice from left to right)**.** Data are represented as Mean ± SEM.

Fig. 3. TERT deletion compromises chromatin accessibility in SSCs. (a) Peak calls from ATAC-seq

data. Peak calls from each cell type are shown individually. Color indicates the type of genomic region

overlapped by the peak. **(b)** Average tag density of ATAC-seq reads around transcription start sites. **(c)**

PCA of ATAC-seq data from US-h, US-m and DS cells purified from $Tert^{CreER/+}$ versus $Tert^{CreER/flox}$ mice.

(d) Heatmap representation of 11656 peaks that are significantly different between $Tert^{CreER/+}$ and

$Tert^{CreER/flox}$ in US-h, US-m, and DS or significantly more open either in SP or RS. Each row represents

one ATAC-seq peak. Color represents the relative ATAC-seq accessibility. **(e)** Venn diagrams of the

peaks in US-h, US-m and DS cells from $Tert^{CreER/+}$ and $Tert^{CreER/flox}$ mice.

Fig. 4. MYC rescues competitive clone formation of TERT-deleted SSCs. (a) PCA of RNA-seq data

from Tdtomato$^+$ US-h. **(b)** Significant down-regulation of MYC target genes in $Tert^{CreER/flox}$ US-h cells.

RNA-seq data was analyzed using gene set enrichment analysis. **(c)** Quantification of mean signal

intensity for MYC staining in PLZF$^+$ undifferentiated spermatogonia using testicular cross sections at 7

days after labeling. **(d)** $Tert^{CreER/flox}$:$Rosa^{lsl-Tdtomato/lsl-tTA}$:$TetO$-$hMYC$ mice. **(e)** Epifluorescence for

Tdtomato in untangled seminiferous tubules. Scale bars, 5mm. **(f to h)** Quantification of mean patch

number (f), mean patch length (g), and total patch length (h) (n=6, 8, 6 mice from left to right). **(i)**

Quantification of Tdtomato$^+$ GFRA1$^+$ A_s and A_{pr} clones at 14 days post labeling detected by whole mount

immunofluorescence (n=5 mice per group). **(j)** Quantitative analysis of Tdtomato$^+$ BrdU$^+$ PLZF$^+$

undifferentiated spermatogonia using testicular cross sections at 7 days post labeling (n=5 mice per

gropu)**.** Data are represented as Mean ± SEM.

Extended Data Figure 1. TERT expression in spermatogonia subpopulations. (a)

Spermatogenesis: seminiferous tubules are composed of four layers of male germ cells.

Undifferentiated and differentiating spermatogonia located in the basement layer undergo mitosis. They

translocate to the second layer when entering into meiosis and differentiate into spermatocytes. Haploid

spermatids produced by meiosis move toward luminal side during post-meiotic maturation. **(b)** A

functional, morphological, and gene expression heterogeneity in spermatogonia subpopulations. **(c)**

Flowcytometry analysis of wild-type testicular cells stained with α6-Integrin, MCAM, and KIT.

α6-Integrin-high cells were further separated into US-h, US-m, and DS based on KIT and MCAM

expression. **(d)** qRT-PCR of *Tert* and spermatogonia markers in FACS-sorted US-h, US-m, and DS.

Expression was normalized with *Actb* (n=3 mice per group). Data are represented as Mean ± SEM. **(e)**

Flowcytometry analysis of testicular cells from *Tert*[Tdtomato/+] mouse. α6-Integrin-high cells were gated and

further separated according to KIT and MCAM expression. *Tert-Tdtomato* expression was compared in

US-h, US-m, and DS.

Extended Data Figure 2. Marking and linage tracing of TERT^{+} cells. **(a)** Immunofluorescence of

Tdtomato and E-cadherin, a membrane marker of undifferentiated spermatogonia, in whole-mount

seminiferous tubules at day two. Scale bars, 100μm. **(b)** Quantification of Tdtomato^{+} cells in E-cadherin[+]

undifferentiated spermatogonia (n=4 mice per group). Data are represented as Mean ± SEM. **(c)**

Whole-mount immunofluorescence of KIT and Tdtomato in a *Tert*[CreER/+]*:Rosa*[lsl-Tdtomato/+] testis at two days

after high dose tamoxifen injection. Scale bars, 100μm. **(d)** Epifluorescence of Tdtomato in untangled

seminiferous tubules at 3-month (3m) or 1-year (1yr) after pulse labeling; injected tamoxifen dose shown

(0.25 – 4mg). Scale bars, 5mm. **(e)** Singly isolated Tdtomato[+] patch. Scale bar, 1mm. **(f)** Cross sections

of testes immunostained with antibodies to Tdtomato at 3-month or 1-year after labeling. Scale bar,

50μm.

Extended Data Figure 3. Conditional deletion of TERT in SSCs using *Tert-flox* mice. (a) *Tert-flox*

targeting strategy and Southern blot strategy. **(b)** Southern blot analysis of genomic DNA from mouse

tails. Locations of 5' and 3' probes are shown in A. **(c)** TRAP assay using mouse embryonic fibroblasts

from *Tert-flox* mice. Cells were transfected with adenovirus to express LacZ or Cre.

Extended Data Figure 4. (a) qRT-PCR analysis of *Tert* and *Tertci* in purified Tdtomato$^+$ undifferentiated

spermatogonia at day 7. Effects of doxycycline addition to drinking water on *Tert* mRNA level.

Expression was normalized with *Actb* (n=3 mice per group). **(b)** Telomere repeat amplification protocol

(TRAP) assay using purified Tdtomato$^+$ undifferentiated spermatogonia from indicated genotypes. **(c)**

Tert$^{CreER/flox}$:Rosa$^{lsl-Tdtomato/+}$:Terc$^{-/-}$ mice. **(d)** TRAP assay using whole testes from *Tert$^{CreER/+}$:Terc$^{+/+}$* and

Tert$^{CreER/+}$:Terc$^{-/-}$ mouse. **(e)** Epifluorescence of Tdtomato in untangled seminiferous tubules. Scale bars,

5mm. **(f-h)** Quantification of mean patch number (d), mean patch length (e), and total patch length (f)

(n=7, 6, 8, 7). Data are represented as Mean ± SEM**.**

Extended Data Figure 5. Persistent clone loss phenotype of TERT-deleted spermatogonial stem

cells with conditional P53 deletion. (a) *Tert$^{CreER/flox}$:Rosa$^{lsl-Ttomato/+}$:Trp53$^{flox/flox}$* mice. **(b)** *In situ*

hybridization against *Trp53* mRNA. Tdtomato$^+$ undifferentiated spermatogonia were sorted out at 5 days

post labeling and stained. **(c)** Quantification of foci number of *Trp53* mRNA (n=174 and 135 cells from

left to right). **(d)** Epifluorescence of Tdtomato in untangled seminiferous tubules. Scale bars, 5mm. **(e-g)**

Quantitative data of patch number (e), average patch length (f), and total patch length (g) (n=6, 6, 7,7

mice from left to right). **(h)** Immunofluorescence of γH2AX, PLZF, and Tdtomato in testicular cross

sections. *Tert$^{Tdtomato/Tdtomato}$* (G6) mice were used as positive control for γH2AX staining. Scale bars, 30μm.

(i) Quantification of γH2AX-positive unidiferentiated spermatogonia (n=4 mice per group). Data are

represented as Mean ± SEM**.**

Extended Data Figure 6. Apoptosis in TERT-deleted undifferentiated spermatogonia. (a)

Immunofluorescence for GFRA1 and TdTomato at 14 days post labeling with tamoxifen. Scale bars,

100μm. **(b)** Epifluorescence for TdTomato in untangled seminiferous tubules at 6 weeks post labeling.

Scale bars, 5mm. **(c)** Immunofluorescence for Tdtomato and PLZF, a marker of undifferentiated

spermatogonia, using testis cross-sections at 6 weeks after tamoxifen injection. Scale bars, 50μm. **(d)**

Immunofluorescence for Tdtomato, PLZF, and BrdU using testis cross sections. Scale bars, 50μm. **(e)**

Immunofluorescence of PLZF, Tdtomato, and apoptosis marker, cleaved-PARP (cPARP), on testicular

cross-sections at 7 days after labeling. $Tert^{Tdtomato/Tdtomato}$ (G6) mice were used as positive control for

cleaved-PARP staining. Scale bars, 50μm. **(f)** Quantification of Tdtomato⁺ cleaved-PARP⁺

undifferentiated spermatogonia (n=4 mice per group). Data are represented as Mean ± SEM**.**

Extended Data Figure 7. Global changes of open chromatin structure in undifferentiated

spermatogonia after acute deletion of TERT. (a) Principal component analysis (PCA) of ATAC-seq

data. Colors represent indicated cell types from *Tert*-heterozygous mice. Arrow indicates direction of

differentiation. **(b)** Pearson correlation heatmaps of ATAC-seq samples. **(c)** Heatmap representation of

7597 peaks showing significant differences across US-h, US-m, DS, SP, and RS. Each row represents

one ATAC-seq peak. Color represents relative ATAC-seq accessibility. **(d and e)** Venn diagrams

showing the number of peaks found in spermatogonia subpopulations. **(f)** KEGG pathway analysis of

differential peaks between $Tert^{CreER/+}$ and $Tert^{CreER/flox}$ US-h.

Extended Data Figure 8. Changes in open chromatin structure at specific loci after acute deletion

of TERT. (a-d) Read distribution of ATAC-seq data across different cell types. **(a)** *Tert* locus. **(b)** *Ret* and

Gfra1 loci. These genes are dominantly expressed in US-h. **(c)** *Cdh1* and *Zbtb16* loci. These genes are

dominantly expressed in both US-h and US-m. **(d)** *Kit* and *Prm1/Prm2* loci. *Kit* is dominantly expressed

in DS and *Prm1/Prm2* are dominantly expressed in RS.

Extended Data Figure 9. Down-regulation of multiple signaling pathways in TERT-deleted US-h.

(a) Volcano plot of RNA-seq data of purified Tdtomato⁺ US-h from $Tert^{CreER/+}$ and $Tert^{CreER/flox}$ mice.

Genes showing the significant changes (more than 2-fold change and P<0.0001) were colored with red.

(b and c) gene set enrichment analysis using RNA-seq data of US-h purified from $Tert^{CreER/+}$ and

$Tert^{CreER/flox}$ mice. nES: normalized enrichment score. **(d)** Immunofluorescence for MYC, PLZF, and

Tdtomato at day 7 after tamoxifen labeling. Scale bars, 50μm. **(e)** Quantification of mean signal

intensities for PLZF staining in undifferentiated spermatogonia (n=68, 71, 66, 78 cells from left to right).

(f) qRT-PCR analysis of *Myc* mRNA in purified Tdtomato$^+$ US at day 7. Doxycycline was added to

drinking water. Expression was normalized with *Actb* (n=3 mice per group). **(g)** qRT-PCR analysis of

transgenic human *MYC* mRNA in purified Tdtomato$^+$ US at day 7 after tamoxifen labeling. Doxycycline

was added to drinking water. Expression was normalized with *Actb* (n=3 mice per group). n.d.; not

detected. **(h)** qRT-PCR analysis of MYC pathway genes that were significantly down-regulated in gene

set enrichment analysis in figure 4b. Tdtomato$^+$ US-h cells were purified at day 7 post labeling by FACS

and cDNA was synthesized. Expression level was normalized with *Actb* (n=6,6,5,5,5 mice from left to

right).

Extended Data Figure 10. Model for SSC competition driven by non-canonical functions of TERT.

Summary schematic of catalytic activity-independent functions of TERT in stem cell competition.

TERT-deleted SSCs are progressively eliminated from SSC pool through cell competition, reducing the

contribution of TERT-deleted SSCs to spermatogenesis over time (left). In wild-type SSCs, TERT

promotes competitive clone formation by up-regulating MYC protein. In SSCs lacking TERT,

downregulation of MYC protein promotes rapid differentiation, impaired proliferation, and global loss of

chromatin accessibility. MYC expression bypasses the requirement for non-canonical TERT, restoring

SSC-mediated clone formation.

Hasegawa et. al. Figure 2

Hasegawa et. al. Figure 3

Hasegawa et. al. Figure 4

Hasegawa et al. Extended Data Fig.2

Hasegawa et al. Extended Data Fig.7

Hasegawa et. al. Extended Data Fig.10