Methods

Mammalian cell culture

HeLa S3 cells, mTERT-/- MEFs, mTERC-/- MEFs, 293T, SW-13 and BJ cells were maintained with Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal bovine serum. MEFs were isolated from 13.5 dpc embryos. Mouse embryonic stem cells were maintained in DMEM, 20% FBS, LIF (1000 unit per ml) on feeders.

GST-pull down assay

Glutathione-S-transferase fusions of 300 amino acid fragments of Brg-1 [1] were expressed in bacteria and purified onto glutathione resin (Amersham) using standard methods. To generate GST fusions of regions flanking the Brg-1 bromodomain, amino acids 1200-1433 (E', see Fig. 1D), 1434-1565 (G), and 1566-1647 (F') were ligated in frame with a 3' stop codon into the BamH I and Sal I sites of pGEX6 vector. Immobilized GST fusions were incubated with 10 ul of ^{35}S-labeled hTERT made using the TNT T7 coupled Rabbit Reticulocyte Lysate System (Promega). After incubating for 1 hr, resins were washed with NP-40 buffer (0.5% NP-40, 1.5 mM $MgCl_2$, 25 mM HEPES, 150 mM KCl, 10 % glycerol, pH 7.5) 5 times for 10 min each at 4'C. Bound proteins were resolved in SDS-PAGE gels, fixed, treated with Amplify (Amersham) and exposed to film. Equivalent amounts of resin were Coomassie stained to determine the amount of GST fusion protein used in each pull down.

Transfection, transduction and shRNAs

HeLa, SW-13 and MEF cells were transfected with FuGENE 6 (Roche). For transduction, retrovirus was produced by triple transfection of plasmids containing Gag + Pol, VSV-G, and the expression or shRNA vector using standard calcium phosphate precipitation into 293T cells. Retroviral supernatants were filtered onto target cells twice, and selection was begun 24 hours after the final transduction (puromycin at 2 μg/ml, blasticidin at 1-2 μg/ml). Hairpin sequences targeting TERT and Brg-1 were amplified and ligated into the LMP retroviral vector as previously described [2, 3].

shBrg-1-A: 5'-TGCTG TTGAC AGTGA GCGAA GGCTC GCATC GCACA CCGAA TAGTG AAGCC ACAGA TGTAT TCGGT GTGCG ATGCG AGCCT GTGCC TACTG CCTCG GA-3'

shBrg-1-B: 5'-TGCTG TTGAC AGTGA GCGCA GCAGT CAGCG CTTAT GGTCA TAGTG AAGCC ACAGA TGTAT GACCA TAAGC GCTGA CTGCT TTGCC TACTG CCTCG GA-3'

shBrg-1-C: 5'-TGCTG TTGAC AGTGA GCGAG GCAGA AGAAA TCATC ACGGA TAGTG AAGCC ACAGA TGTAT CCGTG ATGAT TTCTT CTGCC GTGCC TACTG CCTCG GA-3'

Immunofluorescent staining

HeLa S3 and mouse embryonic stem cells were fixed with 4% paraformaldehyde and permeabilized by 0.5% Triton X-100. Primary antibodies were incubated for 1 hour and washed with PBS 3 times and further incubated with either FITC or Cy5 conjugated secondary antibodies. VectaShield (DAPI containing, Vector Labs) medium was used for mounting and the images were analyzed by Nikon Eclipse E800 microscope and SPOT Advanced software.

Reporter assays

For luciferase assays, we utilized pMegaTOPFLASH (14 wild-type TCF binding elements), pMegaFOPFLASH (8 mutant TCF binding elements), -163 Cyclin D1 (from A. Ben-Ze'ev, Weizmann Institute, Israel), TBE-1/2 (from K. Kinzler, Johns Hopkins U., Baltimore), Siamois (S01234 and S24) (from M. Brannon and D. Kimelman, U. Washington, Seattle), SBE-luc. (Vogelstein, Johns Hopkins U., Baltimore). Each luciferase reporter plasmids were co-transfected with pRL-SV40 renilla reporter plasmid (internal control). The cells were lysed with passive lysis buffer (Promega) for 10 min. and analyzed using Dual-Luciferase Reporter Assay system (Promega). Luciferase activities were determined using the Luminoscan Ascent luminometer (Labsystems) and firefly luciferase activities were normalized by renilla activities. Reporter assays using Xenopus embryos were performed as described previously [4]. 40 ng of morpholinos (StdMO, TMO) or 0.5 ng of mRNAs (β-gal, xTERT and xβ-catenin) were co-injected with

TOPFLASH plasmid into one blastomere at two cell stage for reporter assays. At stage 11-12, the embryos were collected and prepared for luciferase assay.

Constructs

Standard recombinant DNA techniques were used to construct the following plasmids: FLAG-tagged human TERT (wild type and TERT^{ci}(D712A)) fragments in pcDNA3.1, wild-type and TERT^{ci} in pLPC retroviral plasmid, mTERT in pMGIB retroviral plasmid (G. Nolan), GST-Brg-1 fragments plasmids (G. Crabtree), xTERT (F. Ishikawa)-pCS2-HA, xTERT^{ci}(D770A)-pCS2-HA (a point mutation was introduced by PCR amplification and EcoRV-EcorI digestion), xβ-catenin-pCS2 and GFP-pCS2. CMV-Flag-Smad3, CMV-Flag-Smad4 plasmids were generously provided by R. Derynck, U. California, San Francisco.

Immunohistochemistry

The small intestine was frozen sectioned and fixed with 4% paraformaldehyde for 10 min. After blocking with 1% bovine serum albumin – PBS, tissues were stained with anti-CD44 (BD Pharmingen) antibody and Cy5-conjugated secondary antibody.

Real-time PCR

Total RNA was extracted from cell lines using Trizol (Invitrogen). cDNA was generated from 1 μg of total RNA using SuperScript reverse transcriptase II (Invtirogen) and used for real-time PCR. For real-time PCR, cDNAs were amplified by QuantiTect SYBR Green PCR kit (Qiagen) and detected by iCycler iQ real-time PCR detection system (Bio-Rad). The fold differences were calculated by $2^{-\Delta\Delta Ct}$ method based on GAPDH and HPRT as internal controls. Real-time PCR primers used were as follows.

mAxin2 -F	5'-AAG CCT GGC TCC AGA AGA TCA CAA-3'
mAxin2 -R	5'-TTT GAG CCT TCA GCA TCC TCC TGT-3'
mHPRT -F	5'-AGC TAC TGT AAT GAT CAG TCA ACG-3'
mHPRT -R	5'-AGA GGT CCT TTT CAC CAG CAA-3'
mGAPDH -F	5'-GGT GAA GGT CGG TGT GAA CGG A-3'
mGAPDH -R	5'-TGT TAG TGG GGT CTC GCT CCT G-3'
mCdx1-F	5'-TCA CAG AGC GGC AGG TAA A-3'
mCdx1-R	5'-GCC AGC ATT AGT AGG GCA TAG A-3'
mSmad7-F	5'- GAC TCC AGG ACG CTG TTG GT-3'
mSmad7-R	5'- CCA TGG TTG CTG CAT GAA CT-3'
mPAI-1-F	5'- CTCCGAGAATCCCACACAG-3'
mPAI-1-R	5'- ACTTTGAATCCCATAGCATC-3'
xCdx1-F:	5'-ACCGGGATCTGGACTTTTG-3'
xCdx1-R:	5'-TTGTCTTACCGTTGGTGGTG-3'
xCdx2-F:	5'-CGGTTAGAGCTGGAGAAGGA-3'
xCdx2-R:	5'-TCATGTGGTGGGTAATGGAG-3'
xCdx4-F:	5'-CAAGACGAGGACCAAGGAAA-3'
xCdx4-R:	5'-TGG ACT GGC TGA ACT GGA A-3'
Thy1-F	5'- TTC TTG GCC TTG ATG AGG AG-3'
Thy1-R	5'- ACA GTC TCT GAA GCG TGT GG-3'
ANR5-F	5'- AAA CCC TCG GCA CAA AAT C-3'
ANR5-R	5'- GTC CCA TCA CCT CTG TCC AA-3'
NRH-F	5'- ACA TTC CCA TCC TCA AAC CC-3'
NRH-R	5'- GTT CTT GCT GTT GTC CGC T-3'
xGAPDH-F	5'- ATG CTG GTG CCG TGT ATG T-3'
xGAPDH-R	5'- CGT TGA TGA CCT TTG CGA G-3'
Siamois-F	5'- CTG CCA AGA GCA ACC ACT TT-3'
Siamois-R	5'- CAG TTT GGG TAG GGC TGT GT-3'

Primers for ChIP assays

Cyclin D1-TBE-F:	5'- CGCTC CCATT CTCTG CCGGG-3'
Cyclin D1-TBE-R:	5'- CCGCG CTCCC TCGCG CTCTT-3'

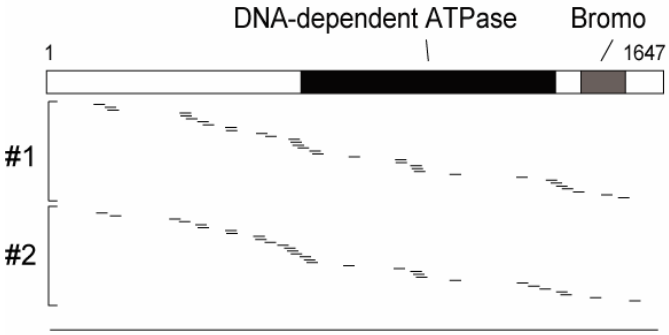
Cyclin D1-3'UTR-F:	5'- CAAGA GAAGA TTACC GCCCG AG-3'
Cyclin D1-3'UTR-R:	5'- TCCCC AGCCT TTTTG ACACC-3'
c-Myc-TBE1-F:	5'- CGTCT AGCAC CTTTG ATTTC TCCC-3'
c-Myc-TBE1-R:	5'-CTCTG CCAGT CTGTA CCCCA CCGT-3'
c-Myc-TBE2-F:	5'-CGTTT TCCTC CTTAT GCCTC TATC-3'
c-Myc-TBE2-R:	5'-GTACC AGGCT GCAGG GCGCC TCGCT-3'
c-Myc-3'UTR-F:	5'-CTAAT GTATC ACAAA GTCCT TTA-3'
c-Myc-3'UTR-R:	5'-GTGAT CTGCT CTGTT AGCTT TTGA-3'

*Primers for ChIP promoter scanning of human and mouse Axin2, c-Myc and HPRT are available upon request.

Telomerase rapid amplification protocol (TRAP) assay

Mouse tissues were homogenized in CHAPS lysis buffer and extracts used to program a standard TRAP reaction using 100 ng of protein extract (TRAPeze, Chemicon). For TRAP analysis of Xenopus embryos, 2 ug of embryos extracts in CHAP lysis buffer were used to program a TRAP reaction and purified by using nucleotide removal kit (Qiagen) for subsequent PCR amplification.

Supplementary Figures



	#1	#2
Unique peptides to Brg-1	33	32
Unique peptide to Brm	1	1
Shared peptides to Brg-1, Brm	14	15

Figure S1. Identification of Brg-1 as a TERT interacting protein by mass spectrometry.

Dual affinity purification of TERT complexes from HeLa cells identified multiple unique peptides by LC-MS/MS for Brg-1 and Brm in replicate experiments (#1 and #2). Also, Brg-1 and Brm peptides from mass-spectrometry are listed as follows.

List of peptides from mass-spectrometry

1st TERT purification

Brg-1 matched unique peptides

K.AIEEGTLEEIEEEVR.Q
K.AVATYHANTER.E
K.AVATYHANTEREQK.K
K.EVDYSDSLTEK.Q
K.EVEAQLPEKVEYVIK.C
K.GVLLTDGSEK.D
K.GVLLTDGSEKDK.K
K.GVLLTDGSEKDKK.G
K.IPDPDSDDVSEVDAR.H
K.KAENAEGQTPAIGPDGEPLDETSQM*SDLPVK.V
K.KEVEAQLPEK.V
K.KIPDPDSDDVSEVDAR.H
K.KIVDAVIK.Y
K.KKIPDPDSDDVSEVDAR.H
K.QDVDDEYGVSQALAR.G
K.QSALM*VNGVLK.Q
K.VIHVESGK.I
R.AQIM*AYK.M
R.DSDAGSSTPTTSTR.S
R.DTALETALNAK.A

R.GLDPVEILQER.E
R.GLQSYYAVAHAVTER.V
R.GPTPFNQNQLHQLR.A
R.GQPLPDHLQM*AVQGK.R
R.HEEEFDLFM*R.M
R.IQELENLPGSLAGDLR.T
R.KRDSDAGSSTPTTSTR.S
R.LDGTTKAEDR.G
R.QKIEKEDDSEGEESEEEEEGEEEGSESESR.S
R.RAFVPQLR.S
R.RDTALETALNAK.A
R.SLNDLEK.D
R.TKATIELK.A

Brg-1 and Brm shared peptides

K.ELPEYYELIR.K
K.LTQVLNTHYVAPR.R
K.VIQAGM*FDQK.S
K.VLRPFLLR.R
K.YKLNVDQK.V
K.YM*IVDEGHR.M
R.ASGKFELLDR.I
R.IGQQNEVR.V
R.LDGTTKSEDR.A
R.LM*AEDEEGYR.K
R.LM*AEDEEGYRK.L
R.QLSEVFIQLPSR.K
R.QLSEVFIQLPSRK.E
R.RLM*AEDEEGYRK.L
R.VDLNEEETILIIR.R

2nd TERT purification

Brg-1 matched unique peptides

K.AENAEGQTPAIGPDGEPLDETSQM*SDLPVK.V
K.AIEEGTLEEIEEEVR.Q
K.AVATYHANTER.E
K.AVATYHANTEREQK.K
K.EVDYSDSLTEK.Q
K.EVEAQLPEKVEYVIK.C
K.GVLLTDGSEK.D
K.GVLLTDGSEKDK.K
K.GVLLTDGSEKDKK.G
K.IPDPDSDDVSEVDAR.H
K.KAENAEGQTPAIGPDGEPLDETSQM*SDLPVK.V
K.KIPDPDSDDVSEVDAR.H
K.KKIPDPDSDDVSEVDAR.H
K.QDVDDEYGVSQALAR.G
K.VIHVESGK.I
R.AKPVVSDDDSEEEQEEDR.S
R.DSDAGSSTPTTSTR.S
R.DTALETALNAK.A

R.GLDPVEILQER.E
R.GLQSYYAVAHAVTER.V
R.GPTPFNQNQLHQLR.A
R.GQPLPDHLQM*AVQGK.R
R.HEEEFDLFM*R.M
R.HIIENAKQDVDDEYGVSQALAR.G
R.IQELENLPGSLAGDLR.T
R.KRDSDAGSSTPTTSTR.S
R.LDGTTKAEDR.G
R.RAFVPQLR.S
R.RDTALETALNAK.A
R.SDSEESGSEEEEEEEEEEQPQAAQPPTLPVEEK.K
R.SLNDLEK.D

Brg-1 and Brm shared peptides

K.ELPEYYELIR.K
K.LTQVLNTHYVAPR.R
K.TLM*NTIM*QLR.K
K.VIQAGM*FDQK.S
K.VLRPFLLR.R
K.YKLNVDQK.V
K.YM*IVDEGHR.M
R.ASGKFELLDR.I
R.IGQQNEVR.V
R.LDGTTKSEDR.A
R.LM*AEDEEGYR.K
R.LM*AEDEEGYRK.L
R.LM*EEDELPSWIIKDDAEVER.L
R.QLSEVFIQLPSR.K
R.VDLNEEETILIIR.R

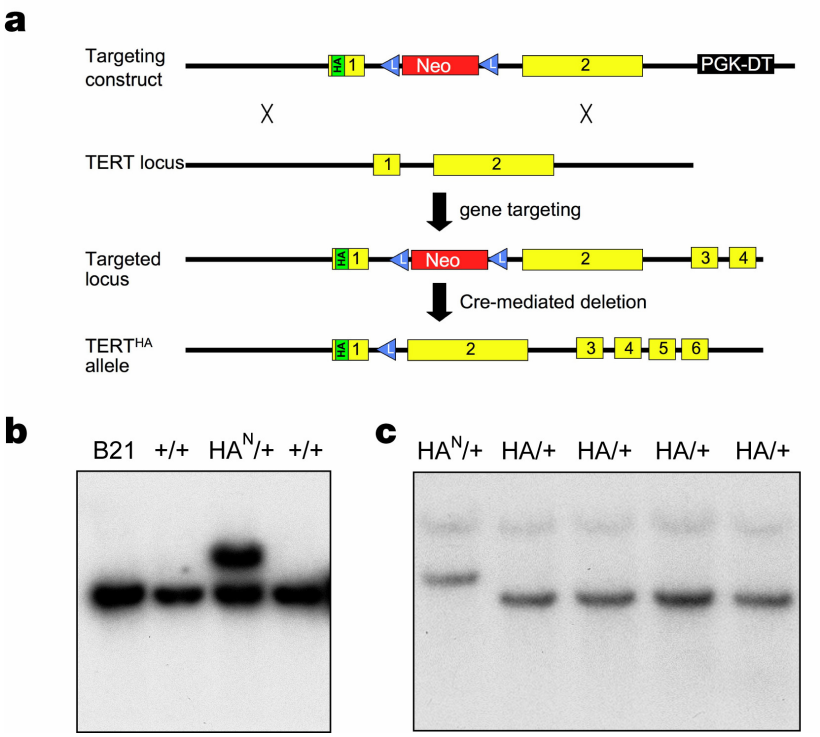


Figure S2. Establishment of TERT$^{HA/+}$ knock-in ES cells.

a, A triple HA epitope tag was cloned in frame at the initiating methionine of exon 1 in a TERT knock-in plasmid. A neo resistance gene flanked by loxP sites was used for positive selection and a PGK-DT cassette was used for negative selection.
b, G418-resistant ES clones were screened by Southern blot by digesting the DNA with BamH1 and using a 5' probe from the promoter region. HA^N indicates a correctly targeted clone that retains the floxed Neo cassette.
c, Activation of RosaCreER^{T2} using 4OHT to delete the floxed Neo cassette, was followed by isolion of independent subclones. Southern blot analysis of subclones was performed by digesting DNA with HpaI, followed by hybridization with a 3' intron 2 probe. HA/+ indicates subclones in which the floxed Neo gene was successfully deleted.

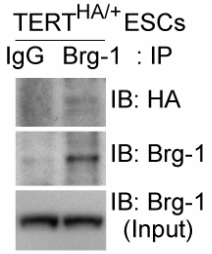


Figure S3. TERT interacts with Brg-1 in TERT$^{HA/+}$ mESCs.

Extracts from TERT$^{HA/+}$ mESCs were immunoprecipitated with IgG or Brg-1 (H-88) antibody and immunoblotted with HA (3F10) antibody to detect endogenous mTERT protein.

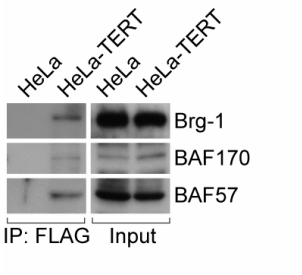


Figure S4. TERT interacts with Brg-1-associated factors BAF170 and BAF57.

HeLa and HeLa-FLAG-TERT cell extracts were immunoprecipitated with anti-FLAG antibody (M2), followed by immunoblotting with BAF170 and BAF57 antibodies. HeLa extracts served as negative controls for FLAG-TERT immunoprecipitation.

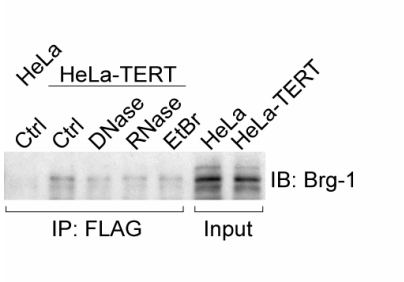


Figure S5. TERT:Brg-1 association is mediated through protein:protein contacts.

HeLa and HeLa-FLAG-TERT whole cell extracts were treated with DNase, RNase or EtBr, then immunoprecipitated with FLAG antibody and blotted with Brg-1 antibody. TERT:Brg-1 association was not affected by DNase, RNase or EtBr treatment.

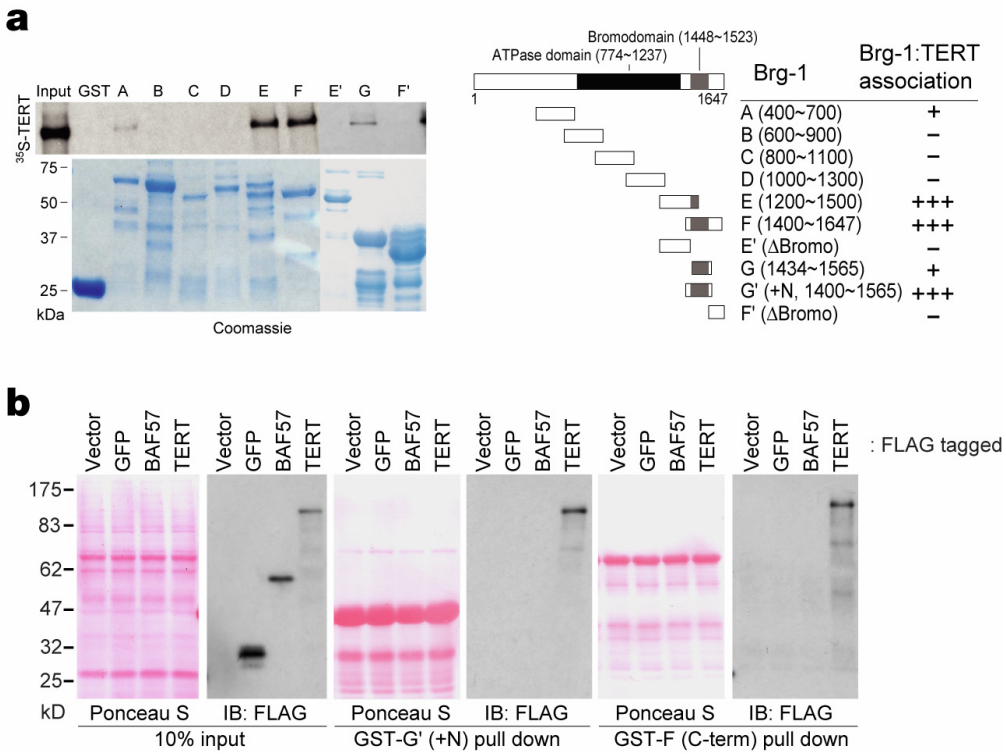


Figure S6. TERT interacts with the bromodomain of Brg-1.

a, TERT interacts with Brg-1. GST pull down experiments were performed using overlapping fragments of Brg-1 fused to GST and recombinant TERT protein expressed in rabbit reticulocyte lysate. TERT did not bind fragments comprising the DNA-dependent ATPase domain of Brg-1, previously shown to bind β-actin [5]. Instead, TERT specifically bound overlapping fragments of the bromodomain, a protein-protein interaction module that binds acetyl-lysine residues [6]. Deletion of the bromodomain from each fragment abrogated TERT binding to these GST-fusion proteins. Furthermore, TERT bound a GST protein fused to a minimal Brg-1 bromodomain. GST-Brg-1 fragments spanning the Brg-1 open reading frame were incubated with ^{35}S-labeled TERT protein in a GST-pull down analysis.
b, FLAG-tagged TERT, GFP and BAF57 were expressed in RRL, then incubated with immobilized GST-Brg-1 G' or F fragments. Bound proteins were detected by FLAG western blot. Ponceau S stained membranes show loading. Input panel (left) reflects 10% of FLAG-tagged protein input.

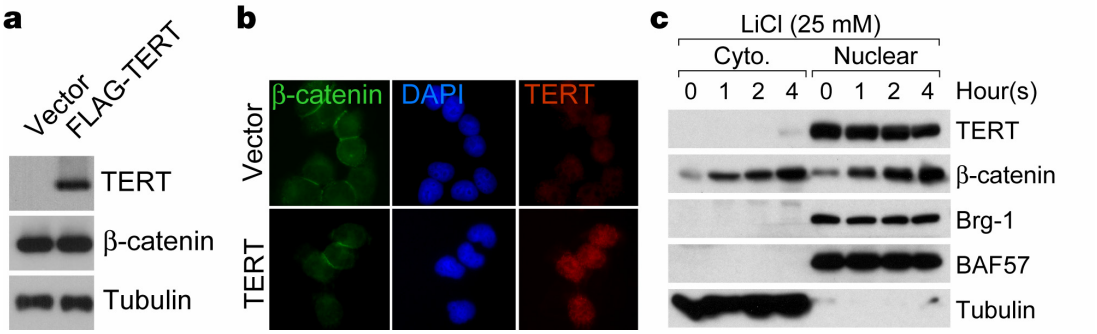


Figure S7. TERT overexpression has no effect on β-catenin protein stabilization and nuclear translocation.

a, Overexpression of TERT had no effect on steady-state levels of β-catenin. Either empty vector or FLAG-TERT plasmids were transiently transfected into HeLa cells and immunoblotted for β-catenin.
b, No nuclear translocation of β-catenin by TERT overexpression. HeLa cells grown onto chamber slides were transiently transfected with pcDNA3.1 vector only (control) or TERT-pcDNA3.1 plasmids. 24 hours after transfection, cells were stained with anti-β-catenin and TERT (rabbit polyclonal, Venteicher et al., Cell 2008) antibodies.
c, FLAG-TERT-HeLa cells were treated with LiCl at various time points and cytoplasmic and nuclear fractions were analyzed by immunoblotting. β-catenin served as a positive control, showing stabilization by LiCl. BAF57 and Tubulin served as controls for nuclear and cytoplasmic fractionation, respectively.

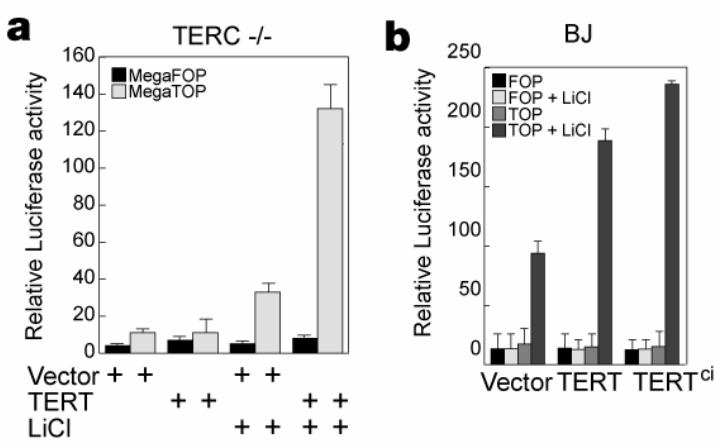


Figure S8. Telomerase reverse transcriptase activity is dispensable for stimulation of WNT/β-catenin reporters by TERT.

a, Reporter activation by TERT is independent of TERC. mTERC$^{-/-}$ MEFs were transiently cotransfected with TERT and reporter plasmids (pMegaFOP/TOP), followed by treatment with LiCl (25 mM, 24 hours). **b**, Reporter activation by TERT does not require telomerase reverse transcriptase function. Either wild-type TERT or a catalytically inactive mutant TERT^{ci} activated the TCF-dependent reporter. TERT plasmids were co-transfected with reporter plasmids into BJ cells, followed by LiCl (25 mM, 24 hours) treatment.

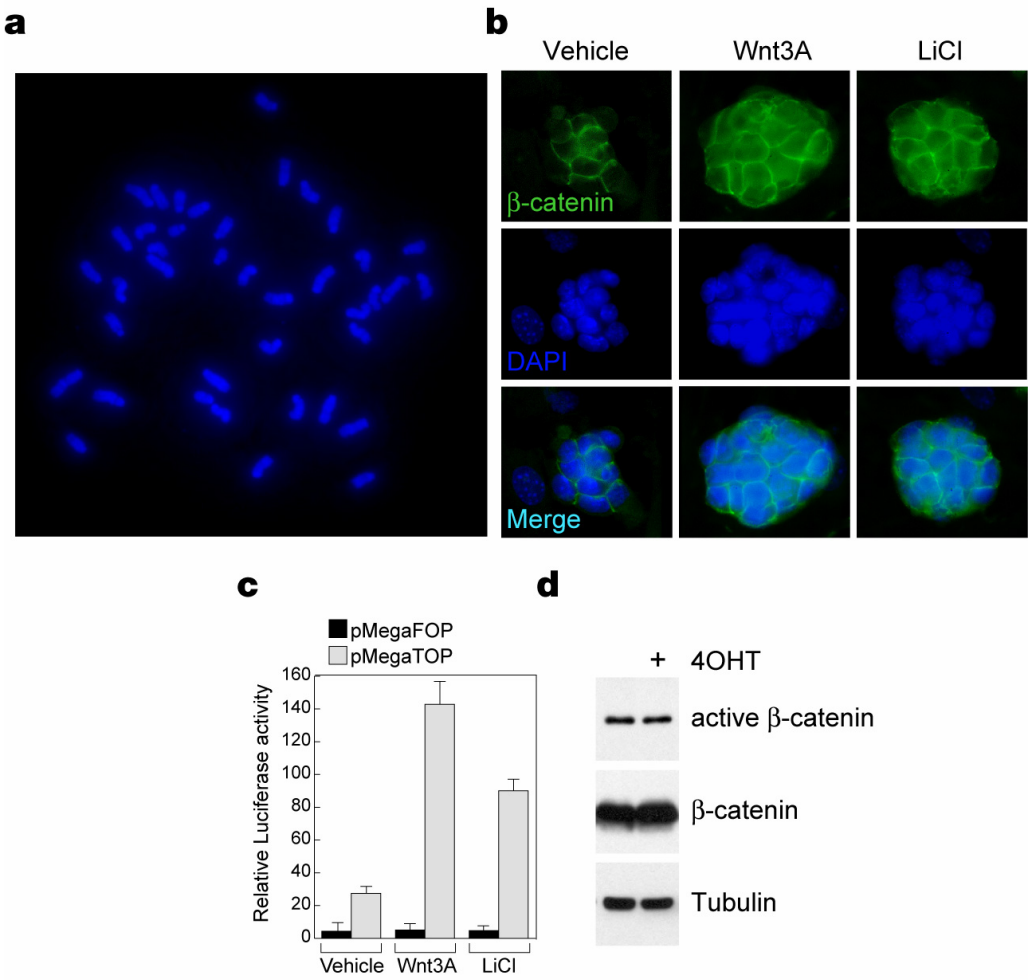


Figure S9. Characterization of TERT CKO embryonic stem cells.

a, The karyotype of TERT CKO ESCs analyzed by counting chromosomes in cells arrested with Colcemid. 98% of TERT CKO mESCs harbor 40 chromosomes (49 of 50 metaphases analyzed).
b, Intact Wnt/β-catenin signaling pathway in mESCs. TERT CKO mESCs were treated with vehicle (control), Wnt3A (200 ng/ml) or LiCl (25 mM) for 6 hours and fixed with 4% PFA for further immunostaining. After permeabilizatoin by Triton X-100 (0.5 %), the cells were incubated with anti-β-catenin antibody (1:500) and FITC-conjugated anti-mouse antibody (1:200). The stabilized β-catenin protein is detected in both cytosolic and nuclear compartments upon Wnt activation.
c, Increase of β-catenin transcriptional activity by Wnt in ESCs. ESCs were transiently transfected with reporter plasmids (pMegaTOP/FOPFLASH with pRL-SV40 as normalization control) using Lipofectamine 2000 (Invitrogen). 24 hours after transient transfection, the cells were treated with Wnt3A (200 ng) or LiCl (25 mM) for additional 24 hours to induce β-catenin transcriptional activity.
d, Absence of effects of 4OHT on β-catenin level. TERT CKO mESCs were treated with 4OHT (250 nM) for 6 hours and harvested for immunoblotting with anti-active β-catenin antibody (Upstate) and anti-β-catenin antibody. Neither active β-catenin (non-phosphorylated form) nor total β-catenin was altered in amount.

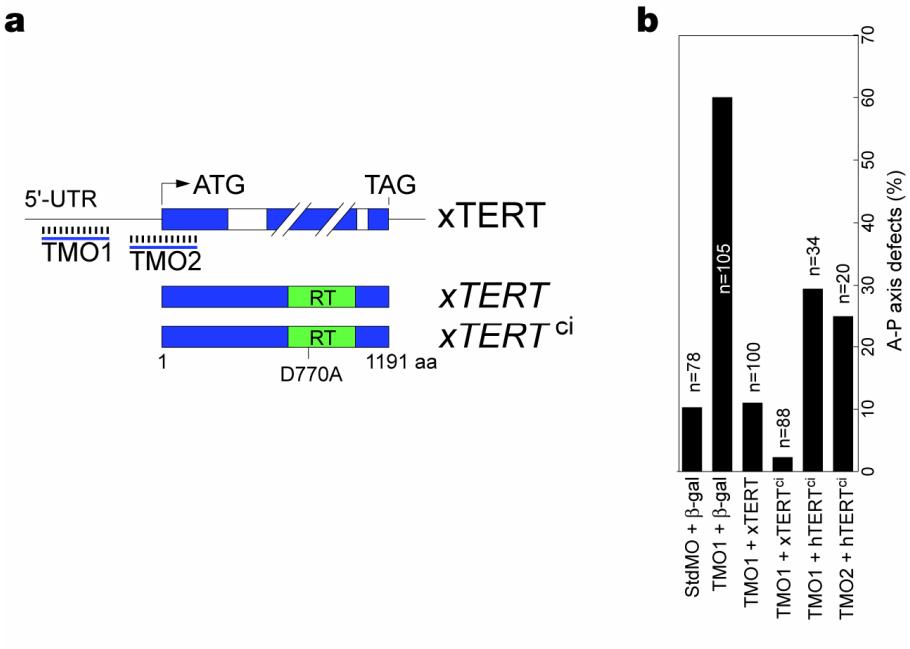


Figure S10. Rescue of A-P axis developmental defects by xTERT and hTERT.

a, Schematic diagram indicating the location of TMO1 (5'-UTR), TMO2 (translation start site) and the point mutation in xTERT^{ci}.

b, Quantitation of percent of embryos with A-P axis defects plotted from more than three-independent microinjections. The morpholinos (StdMO, TMO1 or TMO2) were co-injected with 50 pg of each mRNA into the animal pole of Xenopus embryos at one cell stage. A-P axis developmental defects were analyzed at stage 37-38. Note that TMO2 target sequence differs from the hTERT^{ci} mRNA sequence.

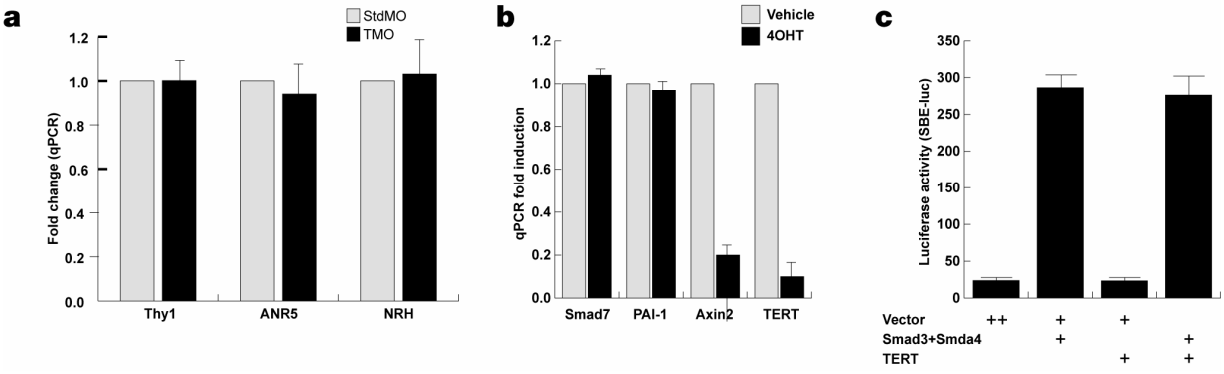


Figure S11. Absence of effects of TERT on the FGF, RA and TGF-β/Smad signaling pathways.

a, *Xenopus* embryos injected with 40 ng of morpholinos (StdMO or TMO) at the one cell stage were collected at stage 11-12 for real-time PCR of Thy1 (Retinoic acid pathway target)[7], ANR5 and NRH (FGF pathway targets)[8, 9]. xTERT knockdown does not affect RA and FGF pathway target genes (n=3), while Cdxs and Siamois were down-regulated (see Fig. 4c).
b, Smad7 and PAI-1, validated TGF-β/Smad target genes, were examined in the setting of conditional deletion of TERT in mESCs. Upon deletion of TERT by 4OHT (3 days), Smad7 and PAI-1 transcripts were unaffected while the β-catenin target gene Axin2 was down-regulated (n=3).
c, 293T cells were transiently transfected with SBE-luciferase (4 copies of Smad binding element) reporter plasmid and each expression plasmid (Smad3, Smad4 and TERT). 24 hours after transfection, cells were collected for luciferase analysis. Ectopic expression of TERT failed to enhance transcriptional activity of Smad3 and 4 (n=3). All error bars indicate standard deviation.

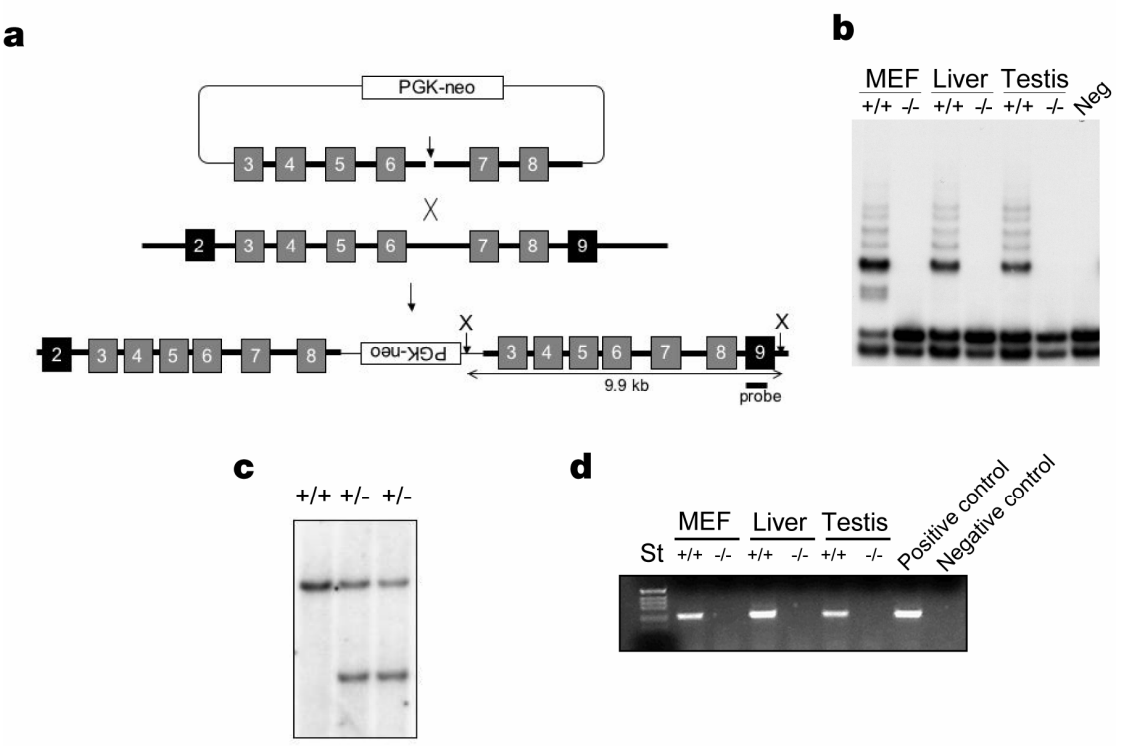


Figure S12. Generation of TERT-/- mice.

a, mTERT gene targeting strategy. Using a gene insertion method, the linearized targeting vector (exon 3-8) was integrated into the TERT locus.
b, TRAP (telomerase rapid amplification protocol) confirms the complete loss of telomerase activity in TERT-/- MEFs and tissues (liver and testis).
c, Southern blotting shows the incorporation of targeting plasmids. XbaI digested genomic DNAs were blotted with a probe from exon 9 to detect the targeted allele.
d, Semi-quantitative PCR indicates the absence of TERT transcripts in MEFs, liver and testis.

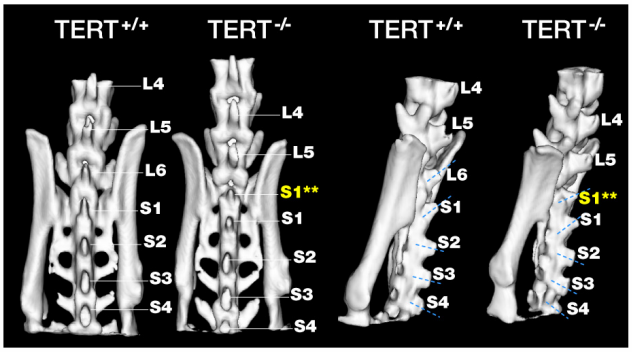


Figure S13. L6 to S1 homeotic transformation in G1 TERT-/- mice.

TERT-deficiency also causes a 6th lumbar to 1st sacral homeotic transformation. The projection of each spinous process is marked by dotted blue line in the right panel, revealing an angle characteristic of S1. Note that the lateral processes are inappropriately fused in S1**, consistent with the L6-S1 transformation. The microCT images of live mice were reconstructed and analyzed.

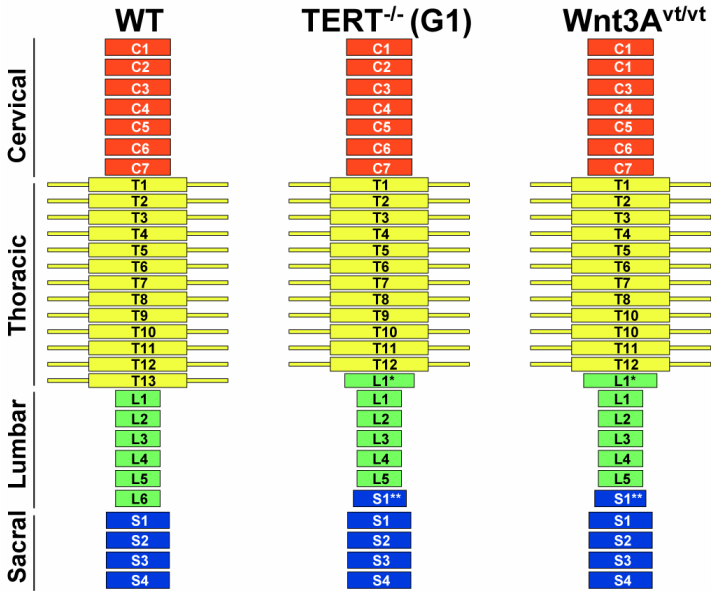


Figure S14. Schematic diagram of homeotic transformations in TERT$^{-/-}$ and Wnt3A$^{vt/vt}$ mutants.

The single (*) and double (**) asterisk indicate the T13 to L1 and L6 to S1 transformation, respectively. Also, see Supplementary Movies.

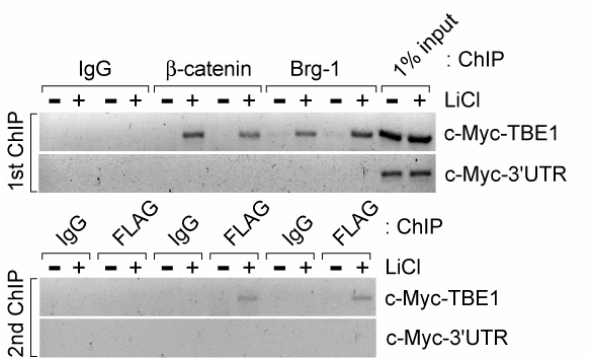


Figure S15. Sequential ChIP shows that TERT associates with Brg-1 and β-catenin at the same chromosome region.

Cross-linked chromatin fragments from FLAG-TERT HeLa cells treated with LiCl (25 mM, 6 hours) were first immunoprecipitated with IgG, β-catenin or Brg-1 antibodies (1st ChIP). Then, eluted chromatin complexes were re-immunoprecipitated with either IgG or anti-FLAG antibody to reveal that FLAG-TERT was present in both Brg-1 and β-catenin-containing complexes at the c-Myc-TBE promoter by semi-quantitative ChIP-PCR (2nd ChIP).

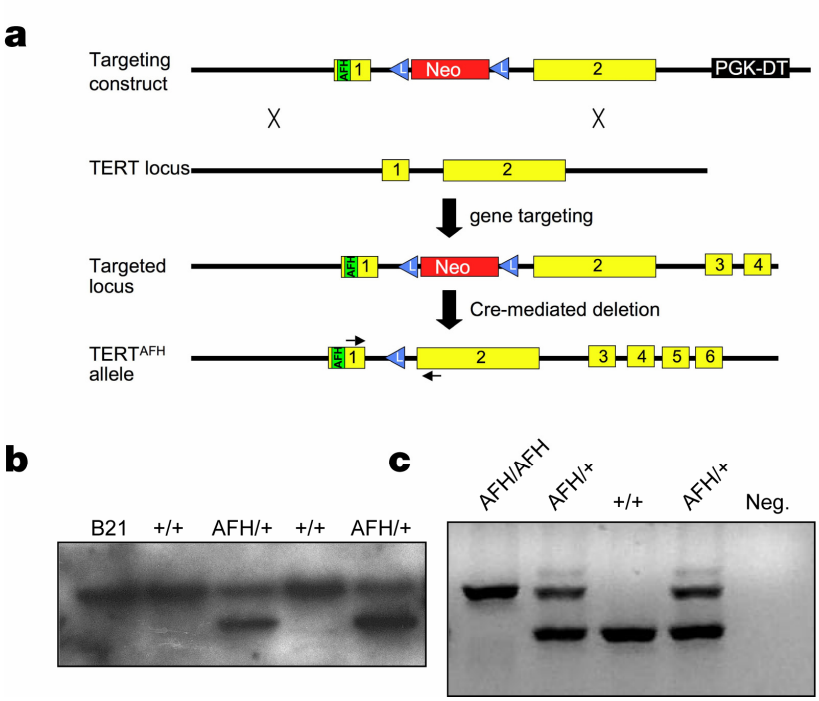


Figure S16. Generation of TERT$^{AFH//+}$ knock-in mice.

a, A composite epitope tag comprising a Staph protein A domain, Flag epitope, and triple HA epitope was cloned in frame at the initiating methionine of exon 1 in a TERT knock-in plasmid. A floxed Neo cassette was used for positive selection and PGK-DT was used for negative selection.
b, G418-resistant ES clones were analyzed by Southern blot using a HpaI digest and 3' probe from intron 2. Correctly targeted ES cells, labeled AFH/+, were used to generate knock-in mice by blastocyst injection.
c, AFH/+ mice were intercrossed with CMV-Cre mice to delete the floxed Neo cassette. The recombined allele (delta-Neo) were identified by PCR of mouse tail DNA using oligos from exons 1 and 2 (shown), which give a larger fragment than wild-type. These oligos cannot amplify the allele that retains the floxed Neo gene under these conditions.

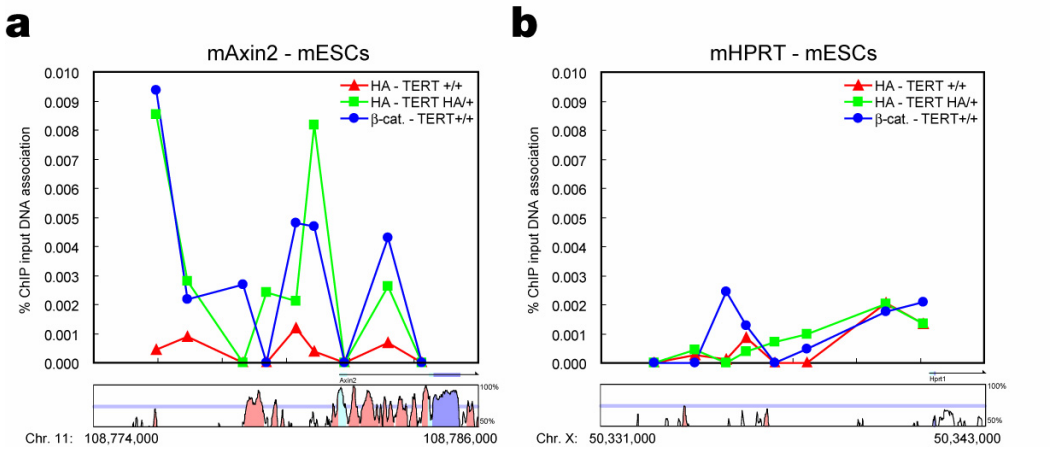


Figure S17. TERT co-occupies mAxin2 promoter with β-catenin in mTERT$^{HA/+}$ mESCs.

TERT$^{HA/+}$ and parental ESCs were used for ChIP promoter scanning analysis. Anti-HA antibody (3F10) was employed to immunoprecipitate HA-TERT protein associated with chromatin. HA ChIP on parental ESCs served as a negative control for HA-TERT IP. Endogenous β-catenin association and HA-TERT proteins bound to the Axin2 promoter in a similar pattern (a). HPRT promoter served as a negative control for ChIPs (b).

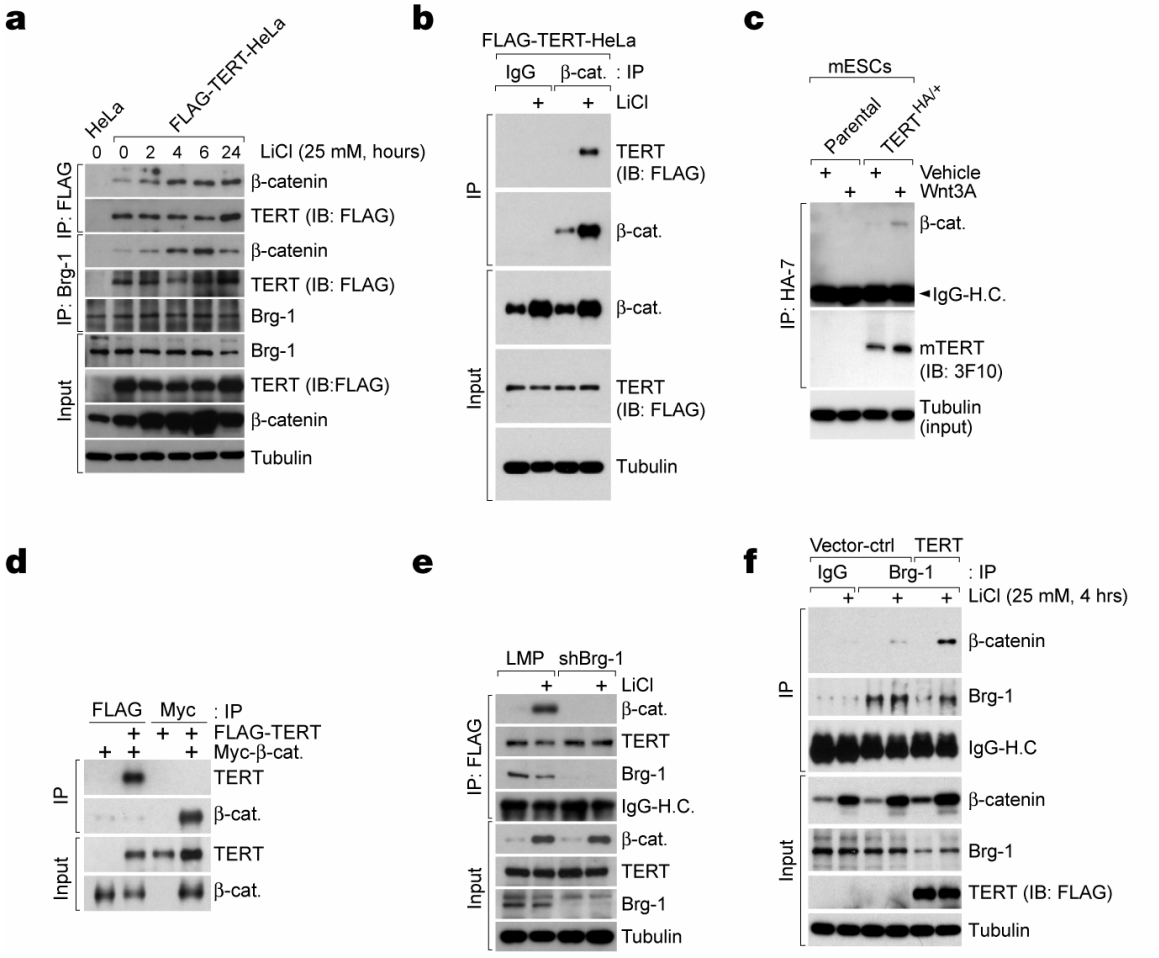


Figure S18. Association of TERT with β-catenin.

a, FLAG-TERT-HeLa cells were treated with LiCl at various time points and co-immunoprecipitated with FLAG antibody to pull down TERT-associated proteins. Similar to LiCl-induced β-catenin stabilization, TERT immediately associates with β-catenin by LiCl treatment (see top blot).
b, Reciprocal co-IP. Extracts from FLAG-TERT-HeLa cells treated with LiCl (25 mM, 4 hours) were immunoprecipitated with β-catenin antibody followed by immunoblot with FLAG antibody to detect β-catenin-associated FLAG-TERT.
c, Endogenous mouse TERT associates with β-catenin in $TERT^{HA/+}$ ESCs treated with Wnt3A ligand. $TERT^{HA/+}$ ESCs were treated with Wnt3A (200 ng/ml) for 6 hours. Extracts were immunoprecipitated with HA (HA-7) antibody, followed by immunoblotting with β-catenin and HA (3F10) antibodies.
d and **e**, TERT indirectly associates with β-catenin through Brg-1. In vitro transcribed and translated FLAG-TERT and Myc-β-catenin proteins were immunoprecipitated and western blotted reciprocally (d). HeLa-LMP (control) and HeLa-shRNA-Brg-1 cells were transiently transfected with FALG-TERT plasmid. 24 hours after transfection, cells were treated with LiCl (4 hours, 25 mM). LiCl-induced TERT:β-catenin association is abrogated by Brg-1 knockdown (compare lane 2 and 4) (e).
f, TERT increases β-catenin:Brg-1 association. HeLa-vector (control) and FLAG-TERT-HeLa cells were treated with lithium and immunoprecipitated with IgG (control) and Brg-1 (H-88) antibody. LiCl-dependent Brg-1:β-catenin association is increased by TERT overexpression (compare lane 4 and 6).

Supplementary Tables

Supplementary table 1. Penetrance of impaired A-P axis development in morpholino-treated Xenopus embryos

Xenopus embryos injected with standard control or xTERT morpholinos (40 ng at one cell stage) were analyzed for A-P axis developmental defects at stage 38-39.

Phenotype	Uninjected	StdMO	TMO1	TMO2
Normal	45	48	75	38
Defects in A-P axis development	1 (2.2 %)	4 (7.7 %)	68 (47.6 %)	124 (76.5 %)
Total number of embryos	46	52	143	162

Supplementary table 2. No T13 to L1 homeotic transformation in TERC knockout mice.

	TERC-/- *	TERC-/- **
Normal	4	13
T13 to L1 homeotic transformation	0	0

Axial skeleton of TERC germline knockout mice were analyzed for homeotic transformation. Unlike TERT-/- mice, T13 to L1 transformation was not observed in TERC-/- mice. (*: TERC knockout mice were generated by intercrossing TERC+/- with TERC+/-; **: TERC-/- mice from intercrossing TERC+/- with TERC-/- (G1).

Supplementary References

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