Supplementary data

Supplementary Table 1. Primers used to generate DNA templates for *in vitro* transcription

Name	Sequence	Description
CKOligo-226	TAATACGACTCACTATAGGGGACCTGCGGCGGGTCGC	FW primer for hTR (+206~+225)
CKOligo-72	GCATGTGTGAGCCGAGTCCTGG	RV primer for hTR (+430~+451)
CKOligo-85	AAAGCGAACTGCATGTGTGAGCCGA	RV primer for hTR (+437~+461)
CKOligo-86	TTTTTAAAGCGAACTGCATGTGTGAGCCGA	RV primer for hTR (+437~+461) +5a
CKOligo-79	AACTGCATGTGTGAGCCGAGTCCT	RV primer for hTR (+432~+455)
CKOligo-80	TTTTTAACTGCATGTGTGAGCCGAGTCCT	RV primer for hTR (+432~+455) +5a
CKOligo-96	TTTTTAGAGCGAACTGCATGTGTGAGCCGA	RV primer for hTR (+437~+461) +5a (U460C)
CKOligo-64	TACGATTTAGGTGACACTATAGGGTTGCGGAGGGTGGGCCTGG	FW primer for hTR (+1)
CKOligo-72	GCATGTGTGAGCCGAGTCCTGG	RV primer for hTR (+430~+451)
CKOligo-85	AAAGCGAACTGCATGTGTGAGCCGA	RV primer for hTR (+437~+461)
CKOligo-86	TTTTTAAAGCGAACTGCATGTGTGAGCCGA	RV primer for hTR (+437~+461) +5a
CKOligo-79	AACTGCATGTGTGAGCCGAGTCCT	RV primer for hTR (+432~+455)
CKOligo-80	TTTTTAACTGCATGTGTGAGCCGAGTCCT	RV primer for hTR (+432~+455) +5a
CKOligo-96	TTTTTAGAGCGAACTGCATGTGTGAGCCGA	RV primer for hTR (+437~+461) +5a (U460C)

Supplementary Table 2. Plasmids used for transfection

Plasmid name	Description
pMG80	WT hTR in pACGFP1-1
pCMV3-LARP3-HA	LARP3 overexpression

Supplementary Table 3. Antibodies used for Western blotting in this study

Target	Source	Cat#/reference	Dilution
DHX36	Bethyl Laboratories	A300-525A	1:5000
DKC1	Bethyl Laboratories	A302-591A	1:5000
NHP2	Proteintech Group	15128-1-AP	1:1000
NOP10	ABclonal	A18250	1:2000
NAF1	Bethyl Laboratories	A303-911A	1:1000
GAR1	Proteintech Group	11711-1-AP	1:1000
TCAB1 (WRAP53)	Proteintech Group	14761-1-AP	1:1000
LARP3	Bethyl Laboratories	A303-902A	1:15000
LARP7	Bethyl Laboratories	A303-723A	1:10000
MePCE	Bethyl Laboratories	A304-184A	1:5000
PARN	Bethyl Laboratories	A303-561A	1:10000
TUBULIN	ABclonal	AC012	1:5000
Goat anti-rabbit IgG (H+L)-HRP	CROYEZ	C04003	1:5000
Anti-mouse IgG-heavy and light chain cross-adsorbed antibody	Bethyl Laboratories	A90-516D6	1:5000

Supplementary Table 4. shRNAs used in cell culture and the interfering RNA (RNAi) analyses in this study

Target	Target Sequence (5′ to 3′)
Luc (Control)	GCGGTTGCCAAGAGGTTCCAT
LARP3	GCTGAAATGAAATCTCTAGAA
LARP7	CTTGAGCTGTTCTTGGGAGAT
MePCE	GAACTACTACCGAATCCAATT
PARN	CCCAGACTCTTGGATACTAAA

Supplementary Table 5. Primers used for qRT–PCR

Name	Sequence	Target
CKOligo-14	FW: GCGAAGAGTTGGGCTCTGTCA	Total hTR
CKOligo-15	RV: TTCCTCTTCCTGCGGCCTGAAA	
CKOligo-16	FW: CTTTCAGGCCGCAGGAAGAGGAA	Extended hTR
CKOligo-17	RV: GGTGACGGATGCGCACGAT	
CKOligo-18	FW: TGCTGAGGATTTGGAAAGGG	HPRT
CKOligo-19	RV: ACAGAGGGCTACAATGTGATG	
CKOligo-20	FW: GATCCTCTAGACTCCACCTCTC	ATP5β
CKOligo-21	RV: AGAAAGTTCATCCATACCCAGG	
CKOligo-22	FW: ACATCGCTCAGACACCATG	GAPDH
CKOligo-23	RV: TGTAGTTGAGGTCAATGAAGGG	

Supplementary Table 6. Primers used for *in situ* hybridization

Name	Sequence	Target
CKFISH-1	Cy3-GCCCTTCTCAGTTAGGGTTA	hTR
CKFISH-2	Cy3-AAGTCAGCGAGAAAAACAGC	
CKFISH-3	Cy3-TCTAGAATGAACGGTGGAAG	
CKFISH-4	Cy3-CCAGCAGCTGACATTTTTTG	
CKFISH-5	Cy3-GCTGACAGAGCCCAACTCTT	
CKFISH-6	Cy3-GTCCCACAGCTCAGGGAATC	
CKFISH-7	Cy3-CATGTGTGAGCCGAGTCCTG	

Supplementary figure legends

Supplementary Fig. 1 (**a**) Schematic showing human telomerase biogenesis and purification. (**b**) The *in vitro* 3′-end processing assay with ^{32}P-labelled hTR fragments (nucleotides from 206 to 461 with an oligo A tail) was carried out in 293T cell extracts at 37°C for the indicated times. RNA was purified and resolved by a 6% polyacrylamide gel containing 8 M urea. Actin was the loading control.

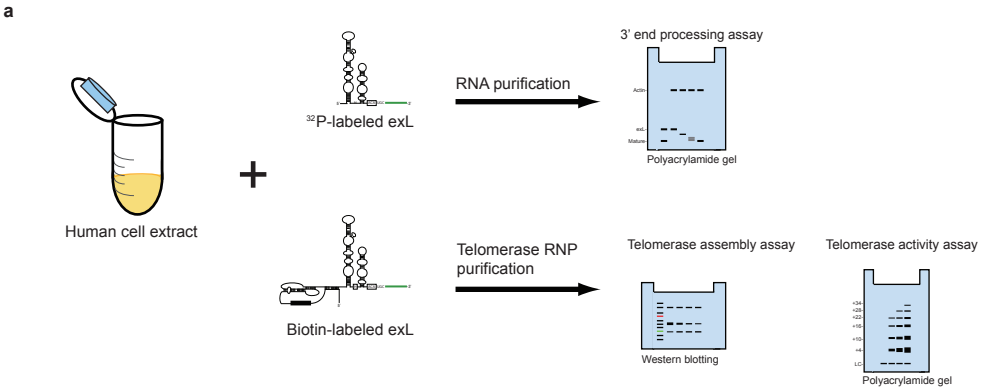
Supplementary Fig. 2 (**a**) The *in vitro* 3′-end processing assay with ^{32}P-labelled wild-type or U460C mutant hTR fragments (from nucleotides 206 to 461 with an oligo A tail) was carried out in 293T cell extracts at 37°C for the indicated times. RNA was purified and resolved on a 6% polyacrylamide gel containing 8 M urea. Actin was the loading control.

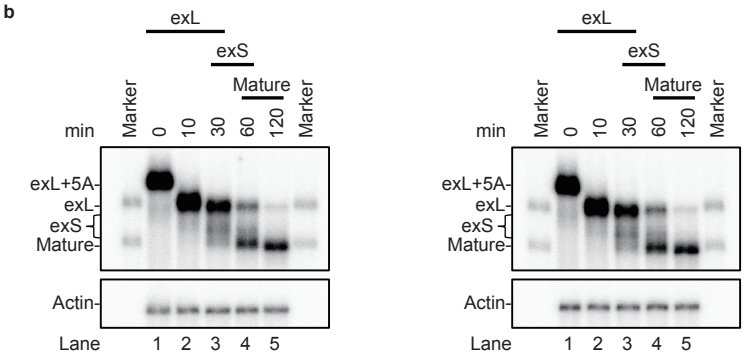
Supplementary Fig. 3 (**a** and **b**) The *in vitro* 3′-end processing assay with ^{32}P-labelled hTR fragments (from nucleotides 206 to 461 with an oligo A tail) was carried out with cell extracts prepared from 293T cells treated with either shRNA targeting LARP3 (**a**) or transfected with a LARP3 plasmid (**b**) at 37°C for the indicated times. RNA was purified and resolved on a 6% polyacrylamide gel containing 8 M urea. Actin was the loading control.

Supplementary Fig. 4 (**a**) Western blot analysis of extracts prepared from K562 cells treated with shRNA targeting luciferase or LARP3. (**b**) The *in vitro* 3′-end processing assay with ^{32}P-labelled hTR fragments (from nucleotides 206 to 461 with an oligo A tail) was carried out with the indicated cell extracts. RNA was purified and resolved on a 6% polyacrylamide gel containing 8 M urea. Actin was the loading control. (**c**) Endogenous DKC1 was immunoprecipitated and subjected to telomerase activity assay.

Supplementary Fig. 5 (**a**) Western blot analysis of extracts prepared from 293T cells treated with shRNA targeting luciferase, LARP7, or MePCE. (**b**) The *in vitro* 3′-end processing assay with ^{32}P-labelled hTR fragments (nucleotides from 206 to 461 with an oligo A tail) was carried out with the indicated cell extracts. RNA was purified and resolved on a 6% polyacrylamide gel containing 8 M urea. Actin was the loading control. (**c**) Telomere lengths were determined by TRF analysis of gDNA prepared from 293T cells treated with shRNA targeting luciferase, LARP7, or MePCE.

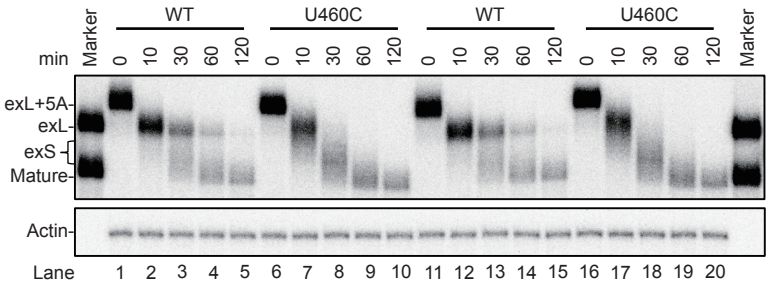
Supplementary Figure 1.





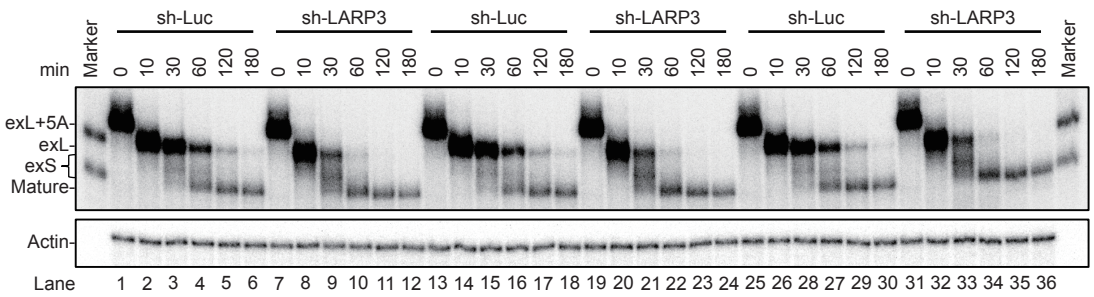
Supplementary Figure 2.

a



Supplementary Figure 3.

a



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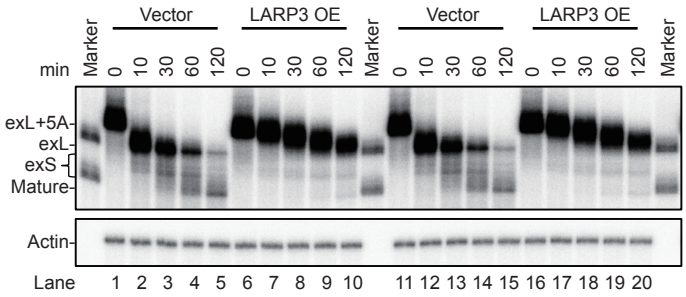
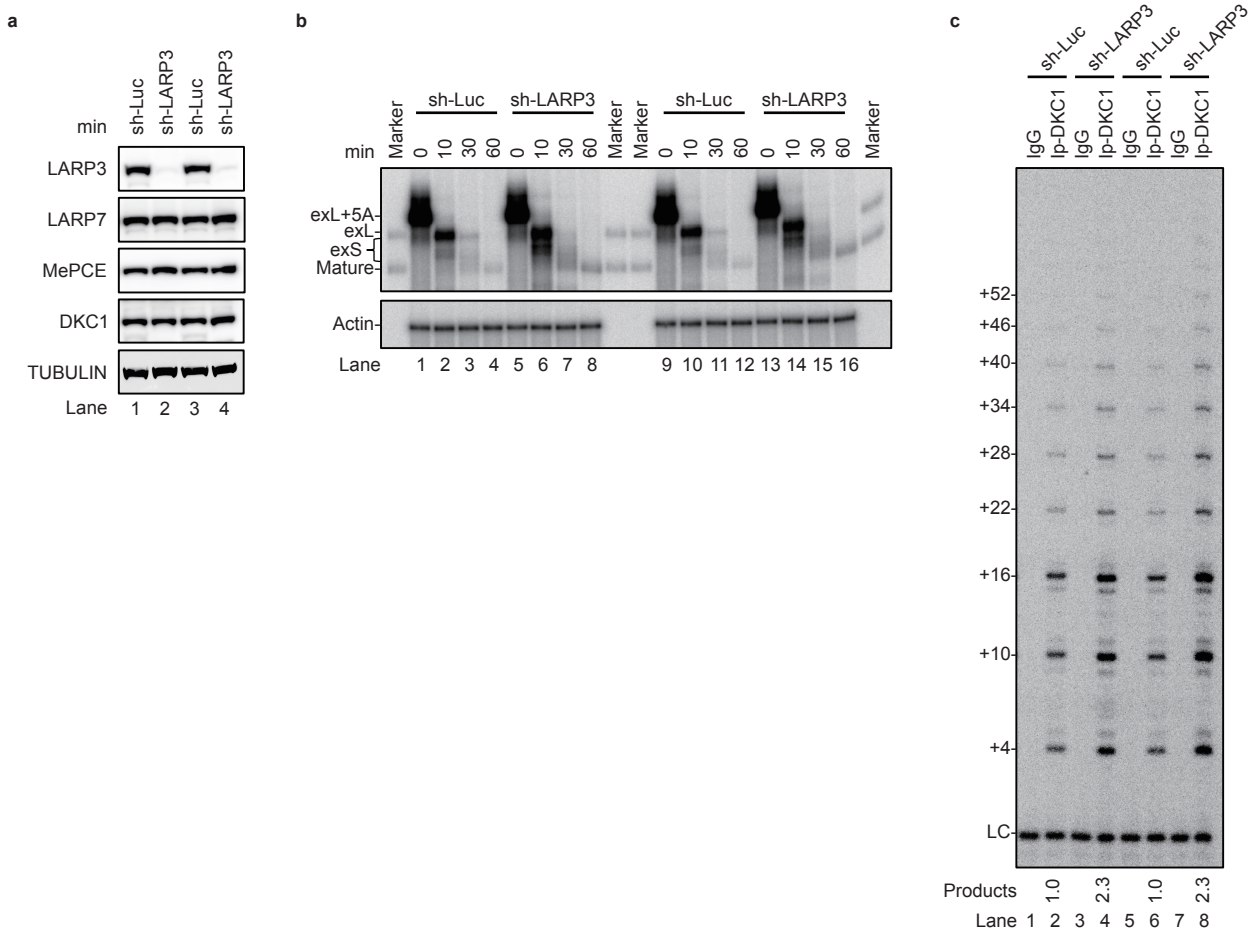


Supplementary Figure 4.



Supplementary Figure 5.