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Supplementary Materials for

PES1 is a critical component of telomerase assembly and regulates cellular senescence

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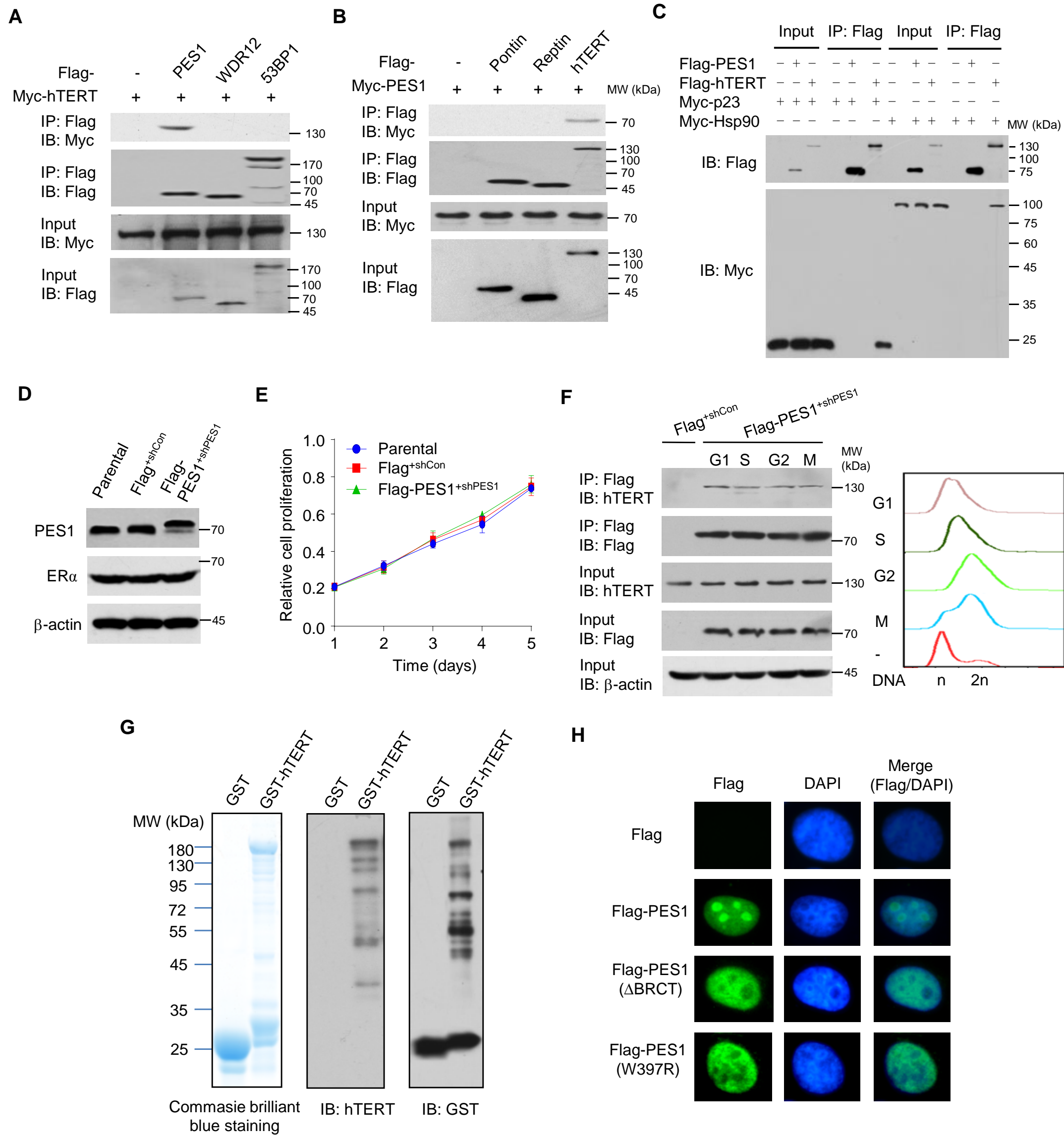


Fig. S1. PES1 specifically interacts with hTERT. (A) Co-IP analysis of interaction between hTERT and PES1, WDR12 or 53BP1 in HEK293T cells transfected with Myc-hTERT and Flag-tagged PES1, WDR12 or 53BP1. (B) Co-IP analysis of interaction between PES1 and Pontin, Reptin or hTERT in HEK293T cells transfected with Myc-PES1 and Flag-tagged Pontin, Reptin or hTERT. (C) Co-IP analysis of interaction between PES1 or hTERT and p23 or Hsp90 in HEK293T cells transfected with Flag-PES1 or Flag-hTERT and Myc-p23 or Myc-Hsp90. (D) Immunoblot analysis with the indicated antibodies in MCF-7, MCF7-Flag-PES1+shCon and MCF7-Flag-PES1+shPES1 cells. (E) Cell proliferation assays in MCF-7, MCF7-Flag-PES1+shCon and MCF7-Flag-PES1+shPES1 cells. (F) Interaction between PES1 and hTERT in different cell cycles. MCF7-Flag-PES1+shPES1 cells were treated with or without Aphidicolin or Nocodazole to obtain cells in different cell cycles. Cell lysates were subject to co-IP with anti-Flag, followed by immunoblot with the indicated antibodies. Flow cytometry analysis of DNA content in cells was shown on the right. β-actin was used as a loading control. (G) Purified GST-hTERT protein was subject to SDS-PAGE and commasie brilliant blue staining (left) or immunoblot with anti-hTERT (middle) or anti-GST (right). (H) Immunofluorescence with anti-Flag to detect localization of WT and mutant Flag-PES1 in MCF7 cells transfected with the corresponding vectors.

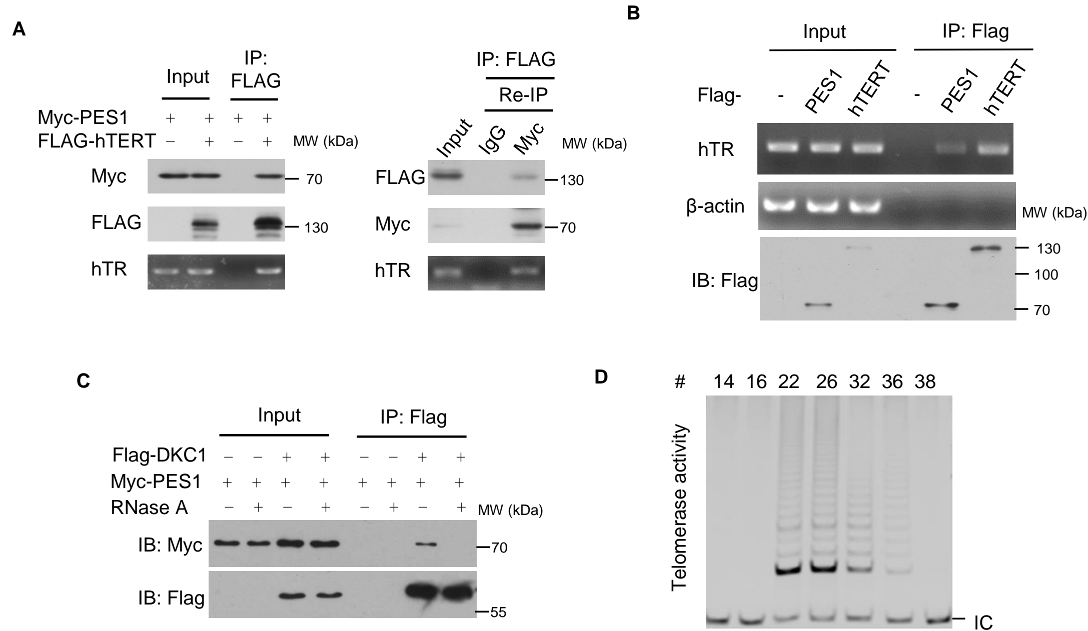


Fig. S2. PES1 forms a complex with hTERT and hTR. (**A**) HepG2 cells transfected with Myc-PES1 or Myc-PES1 plus Flag-hTERT were immunoprecipitated with anti-Flag. The immune complexes were eluted with Flag peptide and re-IP using anti-Myc or normal IgG. The resulting precipitates were used for assessment of Flag-hTERT and Myc-PES1 expression by immunoblot. hTR levels were analyzed by RT-PCR. (**B**) HepG2 cells were transfected with Flag-tagged PES1 or hTERT or empty vector, and immunoprecipitated with anti-Flag agarose. The precipitates were used for detection of Flag-PES1 or Flag-hTERT expression by immunoblot. hTR levels were examined by RT-PCR. β-actin was used as a negative control for hTR determination. (**C**) MCF7 cells transfected with the indicated plasmids were treated with or without RNase A (0.1 mg/ml), and immunoprecipitated with anti-Flag. The immune complexes were analyzed by immunoblot with the indicated antibodies.(**D**) FPLC chromatographic elution from Fig. 2B were subjected to TRAP assay.

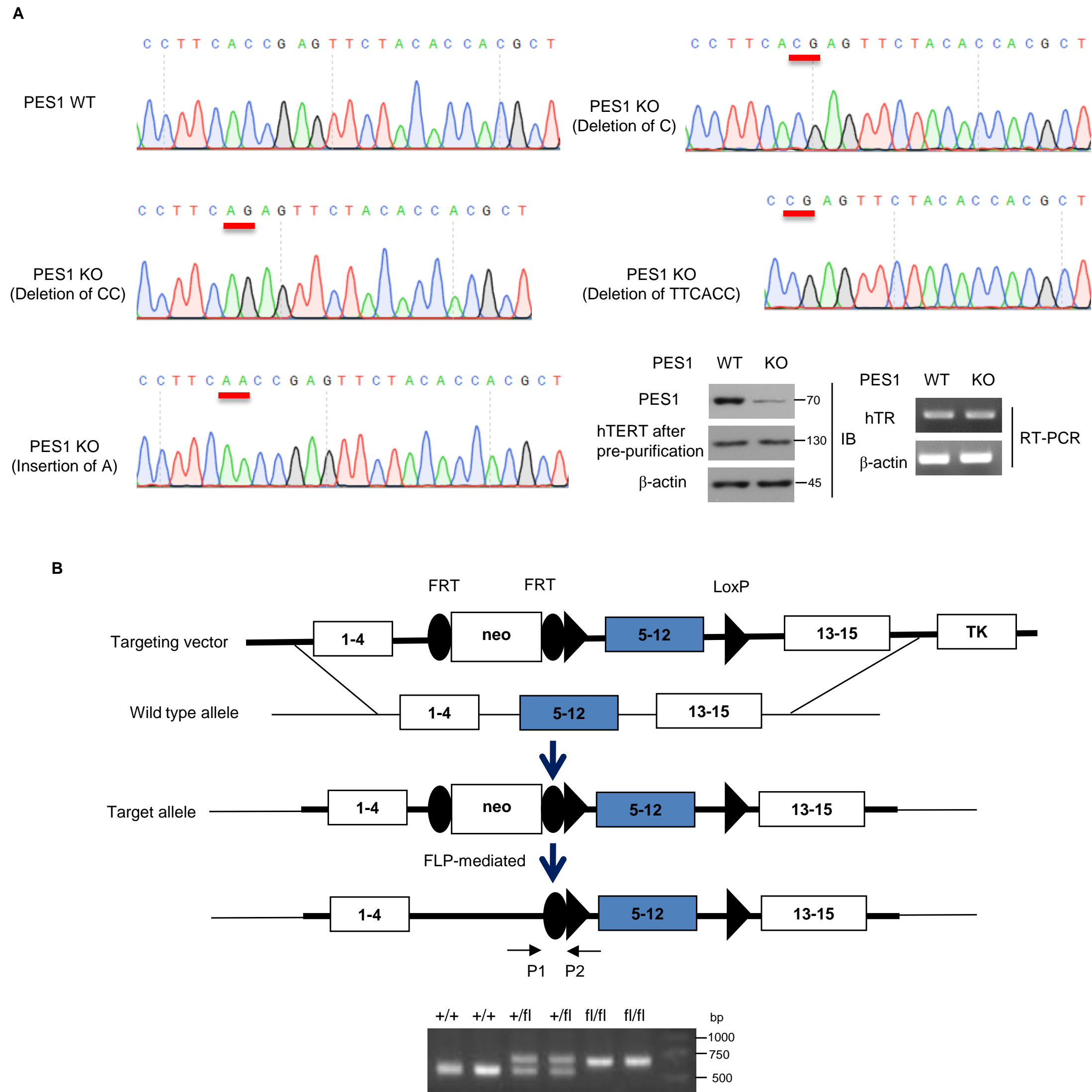


Fig. S3. Identification of PES1 KO MCF7 cell line and PES1 KO mice. (**A**) Mixed PES1 KO MCF7 clones were identified by DNA sequencing. Expression of PES1, hTERT and β-actin were determined by immunoblot, and hTR and β-actin mRNA levels were examined by RT-PCR. To increase the specificity of hTERT detection, cells were immunoprecipitated with anti-hTERT from Abbexa, followed by immunoblot with anti-hTERT from Abcam (hTERT after pre-purification). (**B**) Generation of PES1-floxed mice. Upper panel shows schematic representation of the targeting vector, PES1 genomic locus and PES1 mutated locus. The targeting vector was designed to replace exon 5 (E5) to E12. Lower panel shows genotyping analysis of wild-type, PES1$^{+/fl}$ and PES1$^{fl/fl}$ mice using primers P1 and P2.

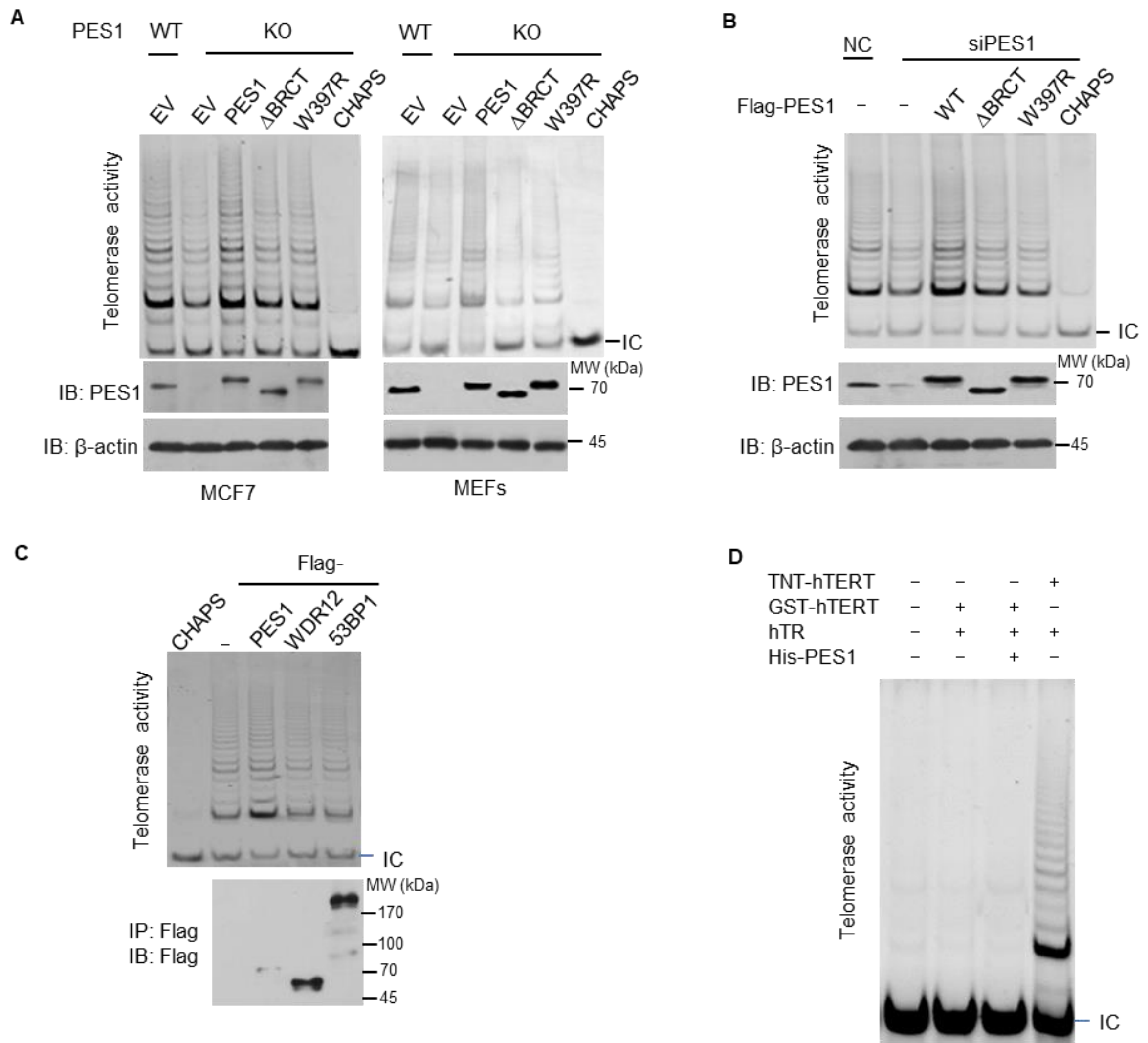


Fig. S4. PES1 modulates telomerase activity and telomere length. (A) PES1 WT or KO MCF7 cells (left panel) and MEFs (right panel) were transfected with the indicated plasmids and cell extracts were analyzed by TRAP and immunoblot. EV, empty vector. CHAPS buffer was used as a negative control for TRAP assay. IC, internal control. (**B**) siRNA-mediated PES1 knockdown HepG2 cells were transfected with Flag-tagged PES1, PES1 (△BRCT), PES1 (W397R) or empty vector, and subjected to TRAP assay. Immunoblot shows the expression of Flag-tagged proteins. NC, control siRNA. siPES1, PES1 siRNA. (**C**) TRAP assay in MCF7 cells transfected with Flag-tagged PES1, WDR12 or 53BP1. (**D**) *In vitro* translated hTERT or purified GST-hTERT was incubated with *in vitro* transcribed hTR. Telomerase activity was determined by TRAP assay with or without purified His-PES1.

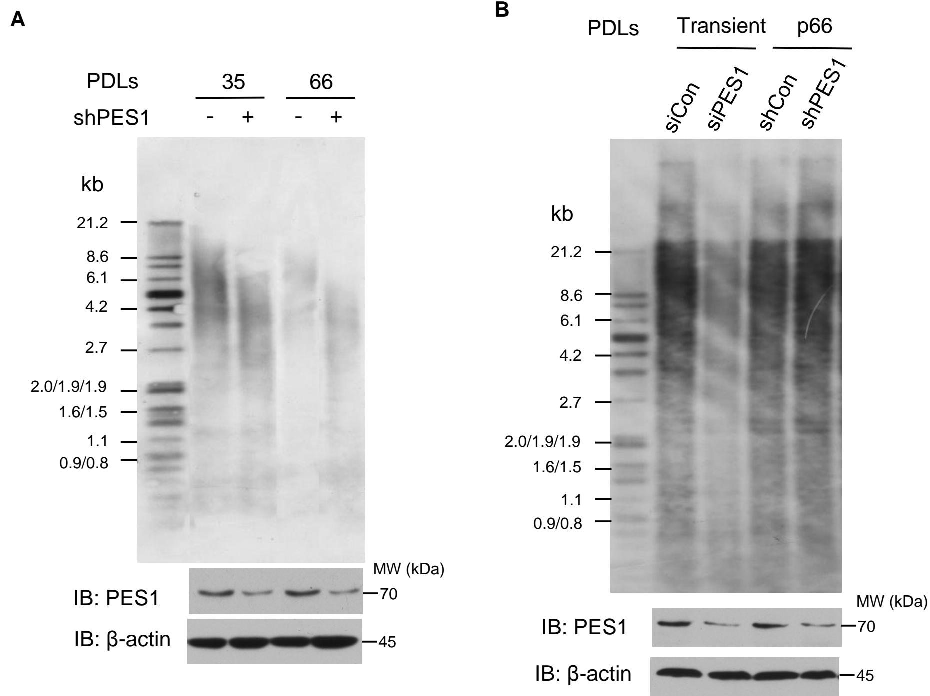
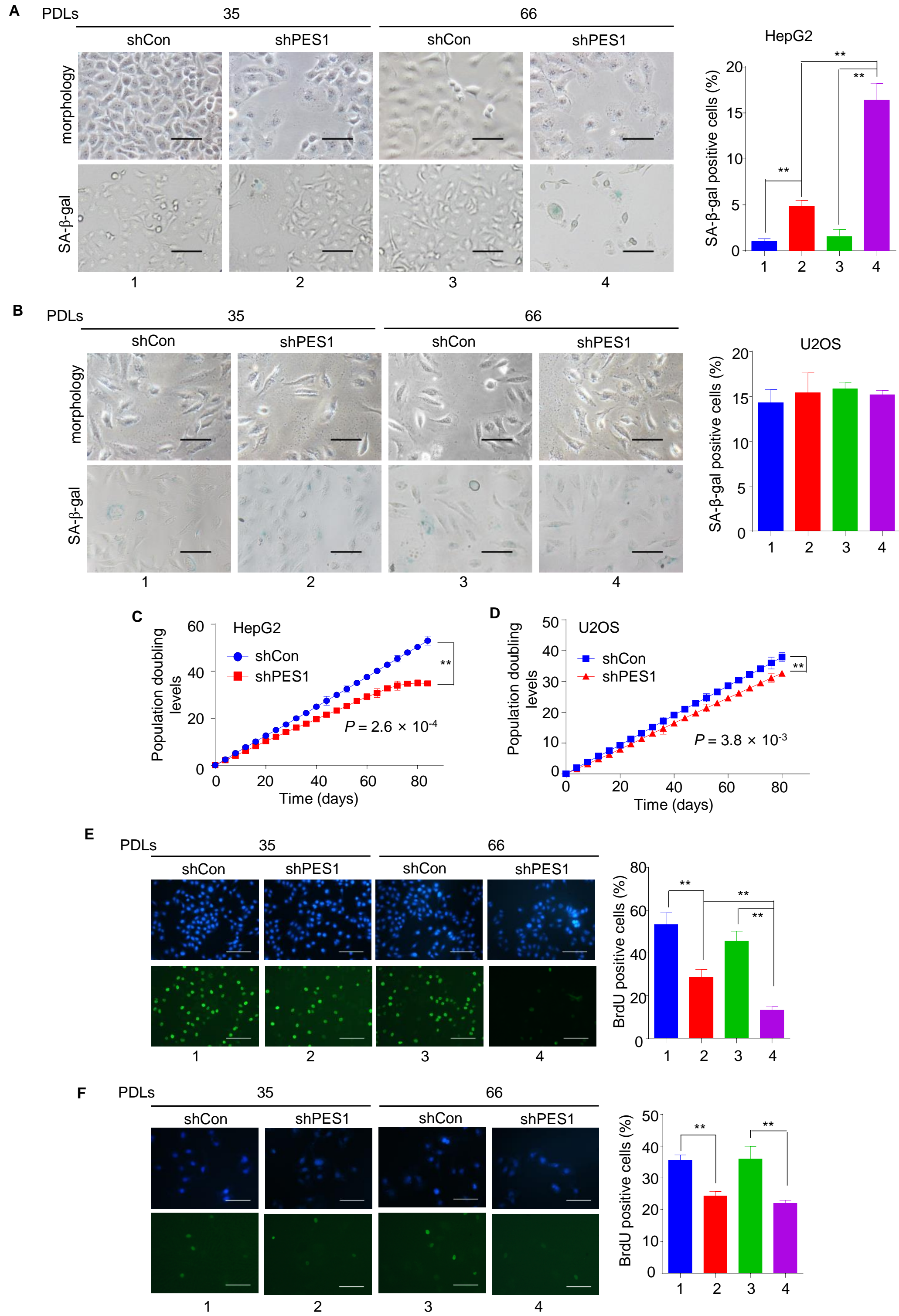
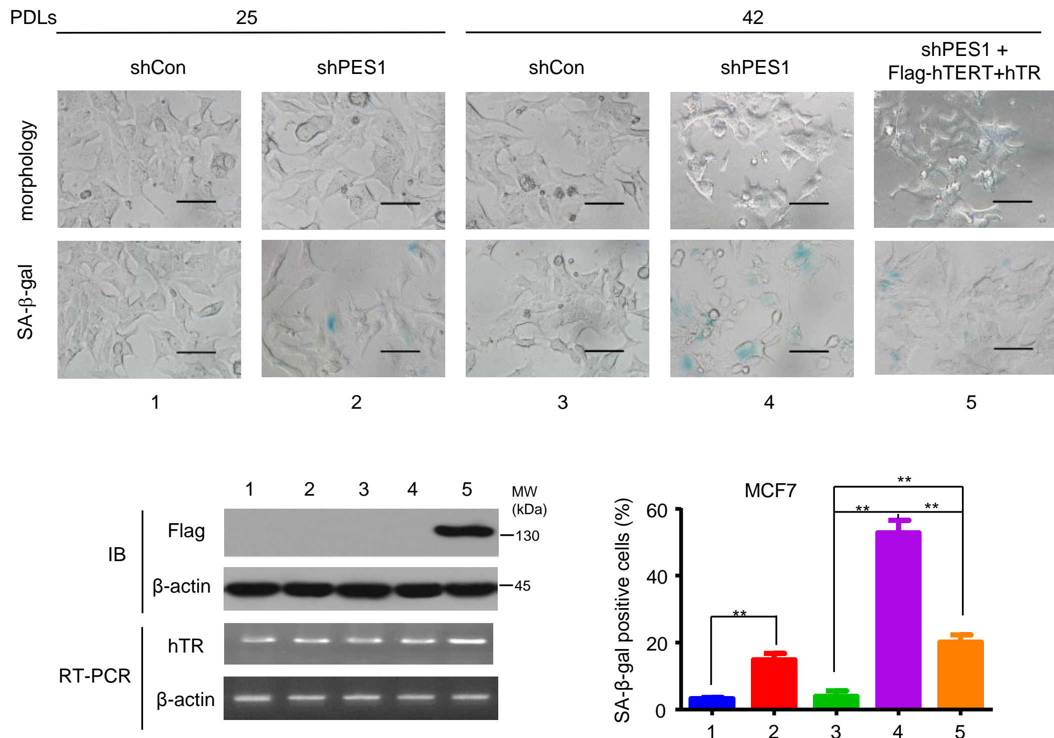


Fig. S5. PES1 modulates telomere length. (**A**) HepG2 monoclones stably expressing PES1 shRNA or control shRNA were harvested at the indicated PDs and subjected to TRF analysis. Representative immunoblot indicates PES1 expression. (B) Telomerase-negative U2OS cells transiently transfected with PES1 siRNA or control siRNA, or stably expressing PES1 shRNA or control shRNA at the indicated PDLs were harvested and analyzed by TRF experiments.


A
PDLs
35
66
shCon
shPES1
shCon
shPES1
morphology
SA-β-gal
1
2
3
4
HepG2
SA-β-gal positive cells (%)
**
B
PDLs
35
66
shCon
shPES1
shCon
shPES1
morphology
SA-β-gal
U2OS
SA-β-gal positive cells (%)
C
HepG2
shCon
shPES1
Population doubling levels
P = 2.6 × 10-4
Time (days)
D
U2OS
shCon
shPES1
Population doubling levels
P = 3.8 × 10-3
Time (days)
E
PDLs
35
66
shCon
shPES1
shCon
shPES1
BrdU positive cells (%)
F
PDLs
35
66
shCon
shPES1
shCon
shPES1
BrdU positive cells (%)

G



H

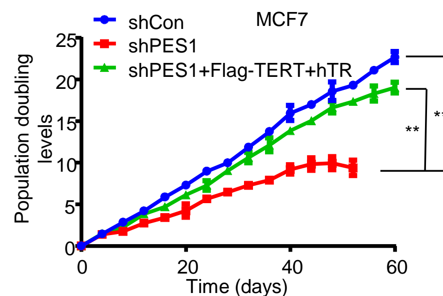
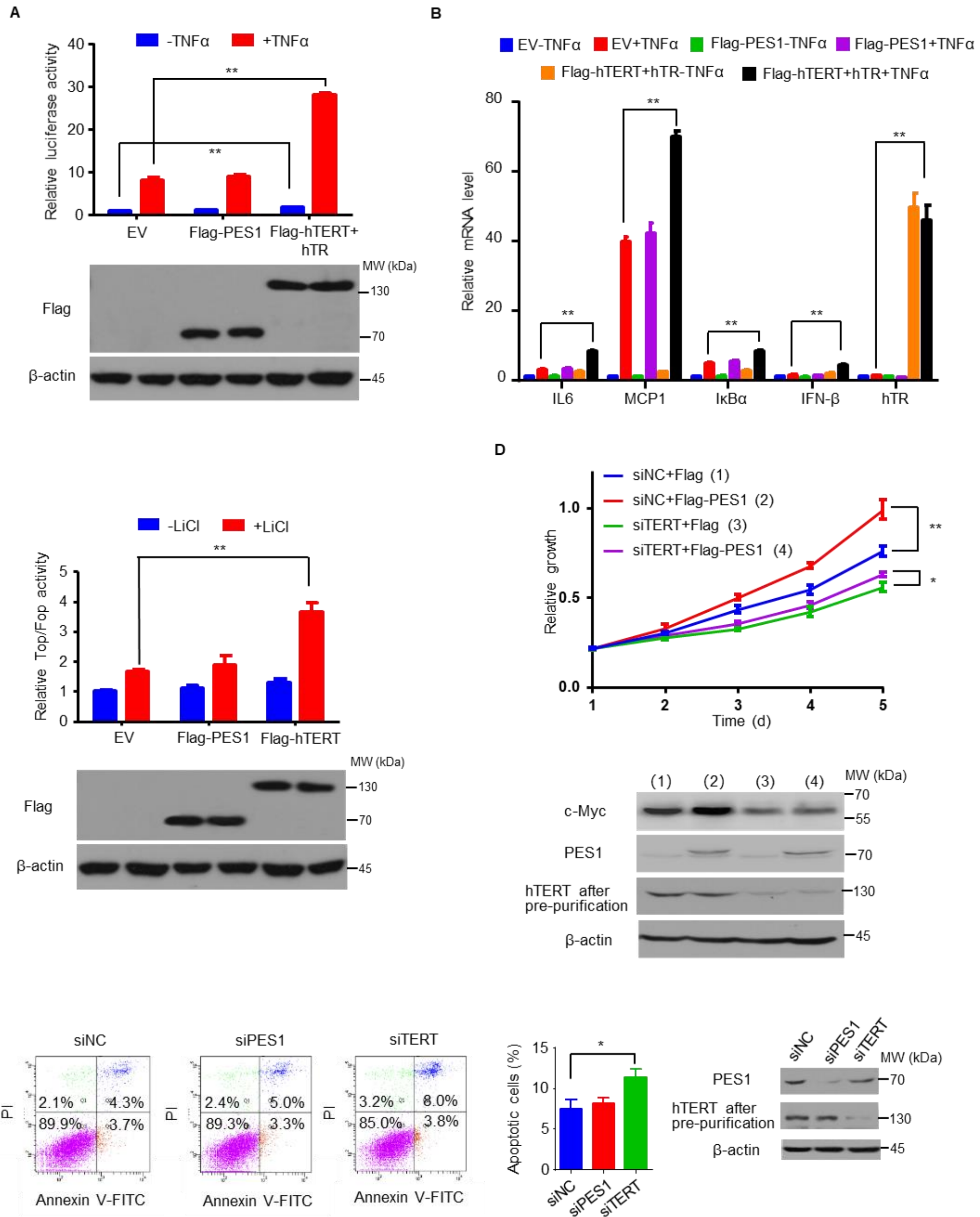


Fig. S6. PES1 KD induces cellular senescence. (**A** and **B**) Cells from fig. S4D (A) and fig. S4F (B) were captured by microscopy (upper panel) and subjected to SA-β-gal staining (lower panel). The percentage of SA-β-gal–positive cells on right was calculated from 5 randomly chosen fields. At least 150 cells were analyzed per experiment. Scale bar, 50 μm. Data shown are mean ± SD of 3 independent experiments. $^{**}p < 0.01$. (**C** and **D**) Growth curves of HepG2 (C) or U2OS (D) cells stably expressing control shRNA or PES1 shRNA and beginning at P34 PDLs. Data shown are mean ± SD of 3 independent experiments. $^{**}p < 0.01$. (**E** and **F**) Cells from fig. S5A (E) and fig. S5B (F) were labeled with BrdU and stained with anti-BrdU. Scale bar, 100 μm. Data shown are mean ± SD of 3 independent experiments. $^{**}p < 0.01$. (**G**) Representative MCF7 monoclones stably expressing PES1 shRNA or control shRNA and Flag-hTERT plus hTR were harvested at the indicated PDLs, captured by microscopy (upper panel) and subjected to SA-β-gal staining (lower panel). Scale bar, 50 μm. Data shown are mean ± SD of 3 independent experiments.$^{**}p < 0.01$. (**H**) Growth curves of representative MCF7 monoclones stably expressing PES1 shRNA or control shRNA and Flag-TERT plus hTR from 34 PDLs. $^{**}p < 0.01$.


A
-TNFα
+TNFα
Relative luciferase activity
40
30
20
10
0
**
**
EV
Flag-PES1
Flag-hTERT+
hTR
MW (kDa)
Flag
130
70
β-actin
45
B
EV-TNFα
EV+TNFα
Flag-PES1-TNFα
Flag-PES1+TNFα
Flag-hTERT+hTR-TNFα
Flag-hTERT+hTR+TNFα
Relative mRNA level
80
60
40
20
0
**
IL6
MCP1
IκBα
IFN-β
hTR
C
-LiCl
+LiCl
Relative Top/Fop activity
5
4
3
2
1
0
**
EV
Flag-PES1
Flag-hTERT
MW (kDa)
Flag
130
70
β-actin
45
D
siNC+Flag (1)
siNC+Flag-PES1 (2)
siTERT+Flag (3)
siTERT+Flag-PES1 (4)
Relative growth
1.0
0.5
0.0
**
*
1
2
3
4
5
Time (d)
(1)
(2)
(3)
(4)
MW (kDa)
c-Myc
70
55
PES1
70
hTERT after
pre-purification
130
β-actin
45
E
siNC
siPES1
siTERT
PI
2.1%
4.3%
89.9%
3.7%
2.4%
5.0%
89.3%
3.3%
3.2%
8.0%
85.0%
3.8%
Annexin V-FITC
Apoptotic cells (%)
15
10
5
0
*
siNC
siPES1
siTERT
MW (kDa)
PES1
70
hTERT after
pre-purification
130
β-actin
45

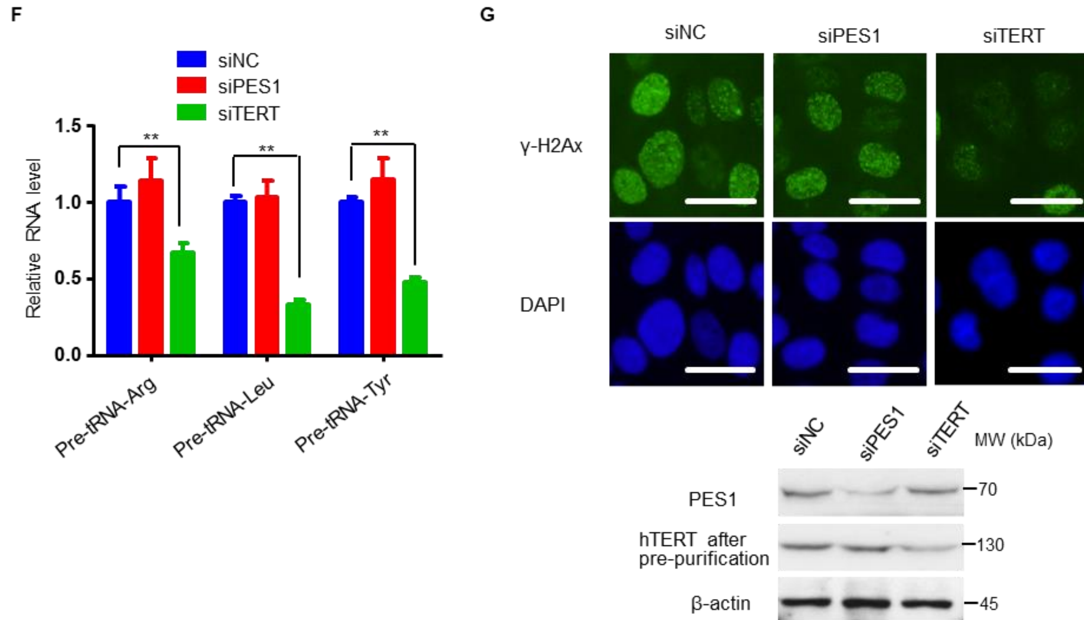


Fig. S7. Effects of PES1 on modulation of noncanonical functions of TERT. (**A**) Luciferase assay in MCF7 cells transfected with the NF-κB-Luc reporter and Flag-PES1 or Flag-hTERT plus hTR. Cells were treated with or without 10 ng/ml TNFα for 16 h. (**B**) qRT-PCR analysis in MCF7 cells transfected with the indicated vectors and treated with or without 10 ng/ml TNFα for 2 h. (**C**) Luciferase assay in MCF7 cells transfected with the Top-Flash/Fop-Flash reporters and Flag-PES1 or Flag-hTERT. Cells were treated with 30 mM LiCl for 6 h. Data shown are mean ± SD of triplicate measurements that have been repeated 3 times with similar results (A-C). $^{**}p < 0.01$. (**D**) Cell proliferation assay in MCF7 cells transfected with TERT siRNA (siTERT) or control siRNA (siNC) and Flag-PES1 or empty vector (Flag). Cell viability was assessed at the indicated times. $^{**}p < 0.01$ versus empty vector at day 5. $^{*}p < 0.05$ versus siTERT at day 5. Immunoblot analysis with c-Myc, PES1 and TERT is shown. (**E** and **F**) MCF7 cells were transfected with siPES1 or siTERT. Apoptosis was determined by flow cytometric analysis (E) and expression of tRNA pre-transcripts was assessed by qRT-PCR analysis (F). Immunoblot analysis of PES1 and TERT is shown (E). Data are shown as the mean ± SD of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$. (**G**) HMEC-TERT cells transfected with siPES1 or siTERT were treated with cisplatin (17 μM) for 12 h and immunostained with anti-γ-H2AX and DAPI. Scale bar, 20 μm. Immunoblot analysis of PES1 and TERT is shown.

Table S1. siRNAs and shRNAs for PES1 and hTERT.

Target*	Sequences
siPES1-1	ACCCAAACACAAGAAGAAGGUUAAC
siPES1-2	GGCAGGCCAGAGGACCUAAGUGUGA
siPES1-3	UGUCAACAAGUUCCGUGAAUACAAG
sihTERT-1	AGGCACUGUUCAGCGUGCUCAACUA
sihTERT-2	CCUCUGUGCUGGGCCUGGACGAUAU
sihTERT-3	GCCUGUUUCUGGAUUUGCAGGUGAA
shPES1-1	ACACAAGAAGAAGGTTAAC
shPES1-2	CCAGAGGACCTAAGTGTGA
shPES1-3	CAAGTTCCGTGAATACAAG
simPES1-1	GGTGTTCTGGCTGCCTTGAATTTCT
simPES1-2	GCGCAAGGTCTTCCTGTCCATTAAA
simPES1-3	GCTCTACTCTGAACCTCCTTCCTCA

*siRNAs against PES1 and hTERT were purchased from Invitrogen (Stealth RNAi). A mixture of the three siRNAs or shRNAs against PES1 and hTERT was used for transfection. siPES1 and simPES1 are used for knockdown of human and mouse PES1, respectively.

Table S2. Optimized coding sequence of hTERT.

ATGCCGCGTGCGCCGCGTTGTCGTGCAGTCCGTAGCCTGCTGCGTTCGCACTATCGTGAAGTTCTGCC
GCTGGCGACCTTTGTACGTCGTCTTGGTCCGCAGGGCTGGCGTCTGGTTCAGCGTGGTGACCCGGCAG
CATTCCGTGCGCTGGTAGCTCAGTGCCTGGTGTGCGTTCCGTGGGATGCGCGCCCGCCGCCGGCTGCC
CCGAGCTTCCGTCAGGTAAGCTGCCTGAAAGAGCTGGTTGCGCGTGTTCTGCAGCGCCTGTGTGAAC
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AGCGCTATTGGCAAATGCGTCCGCTGTTTCTGGAACTGCTGGGCAACCATGCACAGTGCCCGTACGGC
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