Supplemental Methods

Primary cells and cell lines

Fibroblast cultures and iPSCs were generated from NHSF2 and *PARN*-mutant skin fibroblasts as described (17, 30). *PARN*-mutant patient fibroblasts were immortalized by transduction with pBABE-hTERT-puro retroviral vectors. iPSCs were maintained in E8 medium (Life Technologies) on hES-qualified Matrigel matrix (BD Biosciences). HEK 293 were sub-cultured and expanded using trypsin 0.05% and DMEM 10% FCS.

RNA interference and cDNA expression

short-hairpin RNA (shRNA) constructs. Duplex oligonucleotides encoding shRNA targeting human *PAPD5* (ENA accession: FR872509) or luciferase control (Supp. Table S4) were cloned into the pLKO.1-puro vector and pLKO.1-blast vectors (Addgene #10878 and #26655, gifts of D. Root). shRNA constructs targeting human *PARN* (NM_002582) were as described (17).

cDNA expression constructs. Codon-optimized cDNA encoding the *PAPD5* open reading frame (ENA accession: FR872509) with an N-terminal FLAG tag was synthesized (Integrated DNA Technologies), and cloned into pLX304 (blasticidin^{R}; Addgene #25890, gift of D. Root). Lentiviral vectors encoding *EGFP* and *PARN* were cloned into pLX301 (puromycin^{R}; Addgene #25895; gift of Dr. D. Root) and pLX304 (17). Retroviral and lentiviral vectors pBABE-hTERT-puro

(Addgene #1171; gift of R. Weinberg), p-MIG-*DKC1* and pHIV7/SF-U3-TER-500 and controls were previously described (30).

Viral vector production and transduction. Retroviral particles were produced by co-transfection of HEK 293T cells with retroviral vectors, VSV-G and Gag-Pol packaging plasmids as described (30). Lentiviral particles were produced by co-transfection of HEK 293T cells with lentiviral vectors, pCMV_dR8.91 and pCMV_VSV-G as described (17). For knockdown and overexpression experiments, HEK 293 cells, fibroblasts and iPSCs were transduced with viral vectors in the presence of protamine sulfate (10 µg/ml) for 8-12 hours. For *TERC* and *DKC1* experiments, transduced HEK 293 cells and fibroblasts were sorted for mCherry or EGFP expression and cultured without further selection. For antibiotic selection in shRNA and other overexpression experiments, HEK 293 cells and fibroblasts were cultured in puromycin (1-2 µg/ml) and/or blasticidin (5-10 µg/ml), and iPSCs were cultured in puromycin (0.2 µg/ml) and/or blasticidin (3-5 µg/ml).

RNA isolation, cDNA synthesis and quantitative RT-PCR

RNA was isolated using TRIzol (Ambion). After DNase treatment (Turbo DNA-free, Ambion), cDNA was synthesized using SuperScript III Reverse Transcriptase (Invitrogen). qPCR was performed using SsoAdvanced Supermix (Bio-Rad) and primers *PAPD5*_L/R and *POLR2A*_L/R (Supp. Table S4) in a CFX96 Real-Time PCR detection system (Bio-Rad). Quantification of *PAPD5*

was normalized to *POLR2A.* Graphing and statistical analysis was performed using GraphPad Prism.

Western blot analysis

Cell lysates were subjected to SDS–PAGE and transferred to PVDF membranes using standard procedures. Proteins were detected using antibodies in Supp. Table S5 and Clarity Western ECL reagent (Biorad). Quantification was performed using the ChemiDoc Touch imaging system (BioRad).

Northern blots

Formaldehyde/agarose gel electrophoresis. Total RNA was separated on 1.5% agarose/formaldehyde gels, transferred to Hybond N+ membranes (Amersham), and hybridized with α-^{32}P-dCTP-labeled *TERC* probe (TERC_L2/TERC_R (Supp. Table S4)) in ULTRAhyb (Life Technologies). Signals were normalized to *18S* rRNA by ethidium bromide staining. Quantification was performed using ImageJ.

***TERC* RNA decay**

10^6 iPSCs were treated with 5 µg/ml of actinomycin D (Life Technologies) and harvested in TRIzol. Purified RNA was subjected to Northern blot. *TERC* signals were normalized to *18S* rRNA. Decay slopes were determined by simple linear regression and transcript half-life was calculated as the x-intercept at y=0.5, using GraphPad Prism.

3′ rapid amplification of cDNA ends (RACE)

3′ RACE was performed as described (17). Total RNA was ligated to 5′-adenylated, 3′-blocked adapter (New England Biolabs) with T4 RNA ligase KQ (New England Biolabs). cDNA was synthesized using universal RT primer and SuperScript III. PCR amplification was carried out using TERC_L/universal RT primers (Supp. Table S4) with SsoAdvanced Supermix (Bio-Rad). PCR products were run on 3% agarose gels.

RNA profiling and transcriptome analysis by next generation sequencing

3′ end sequencing of TERC RNA

TERC RNA 3′ ends were profiled as previously described (17). Briefly, RACE products were ligated and amplified using barcoded Illumina adapters, and sequenced on an Illumina MiSeq platform. Reads mapping to the TERC gene were analyzed using custom developed Perl scripts.

RNA-seq

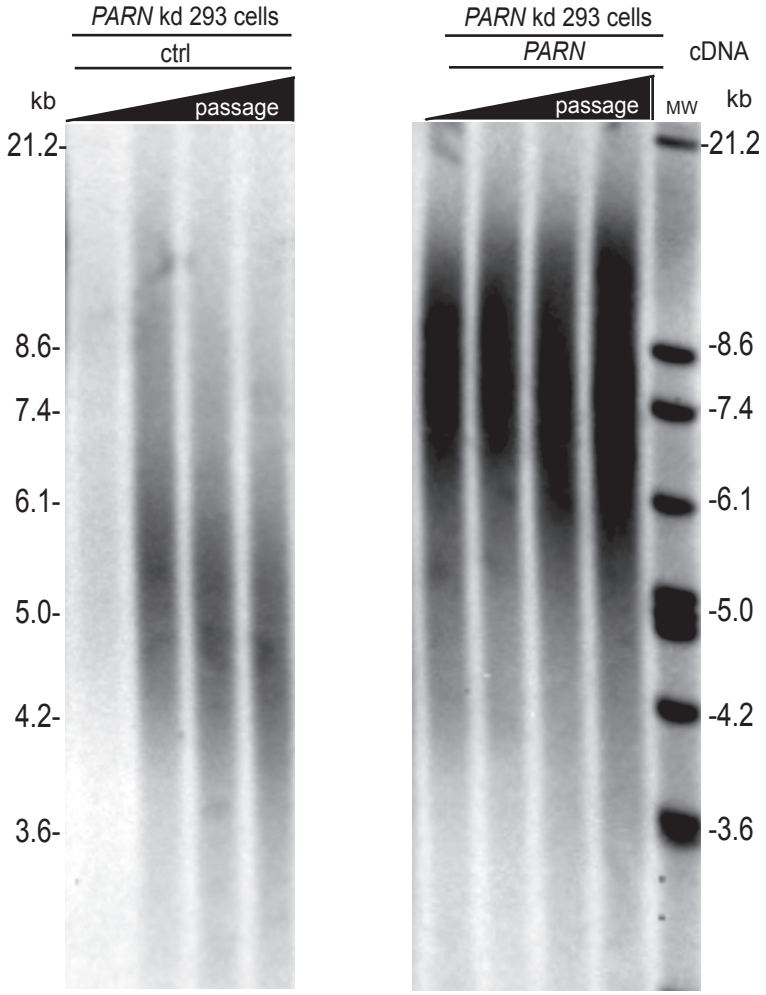
Transcriptome analysis of the alterations in coding and non-coding RNAs from pairs of *PAPD5* versus luciferase control knockdown cell lines (HEK 293, WT iPSC, Patient 1 clone 1 and clone 2 iPSCs) was performed using methods previously described (17). Briefly, total RNA was processed using the Total RNA with RiboZero Gold kit, and barcoded libraries were pooled and sequenced on two lanes of HiSeq 2500 High Output single read 50 bases format. Mapping and

analysis to find genes that were commonly differentially expressed after PAPD5 knockdown was as previously described (17). We used the fold-change in TERC in HEK 293 and Patient 1 clone 1 and clone 2 iPSCs as a threshold to define genes as differentially expressed in a paired comparison manner. The *TERC*-defined thresholds (ln(1+TPM)), were: HEK 293 (0.25); Patient 1 clone 1 iPSC (0.67); Patient 1 clone 2 iPSC (1.24). Individual transcripts altered by PAPD5 knockdown to a degree exceeding this fold change were compared to define those that were commonly altered in all 3 cell types or in 2 of 3 comparisons (Supp. Table S2-S3). We then asked whether the number of commonly altered genes in each category of transcript was different than what would be expected by chance using the Chi-squared test (Supp. Table S1).

Telomerase activity and telomere length

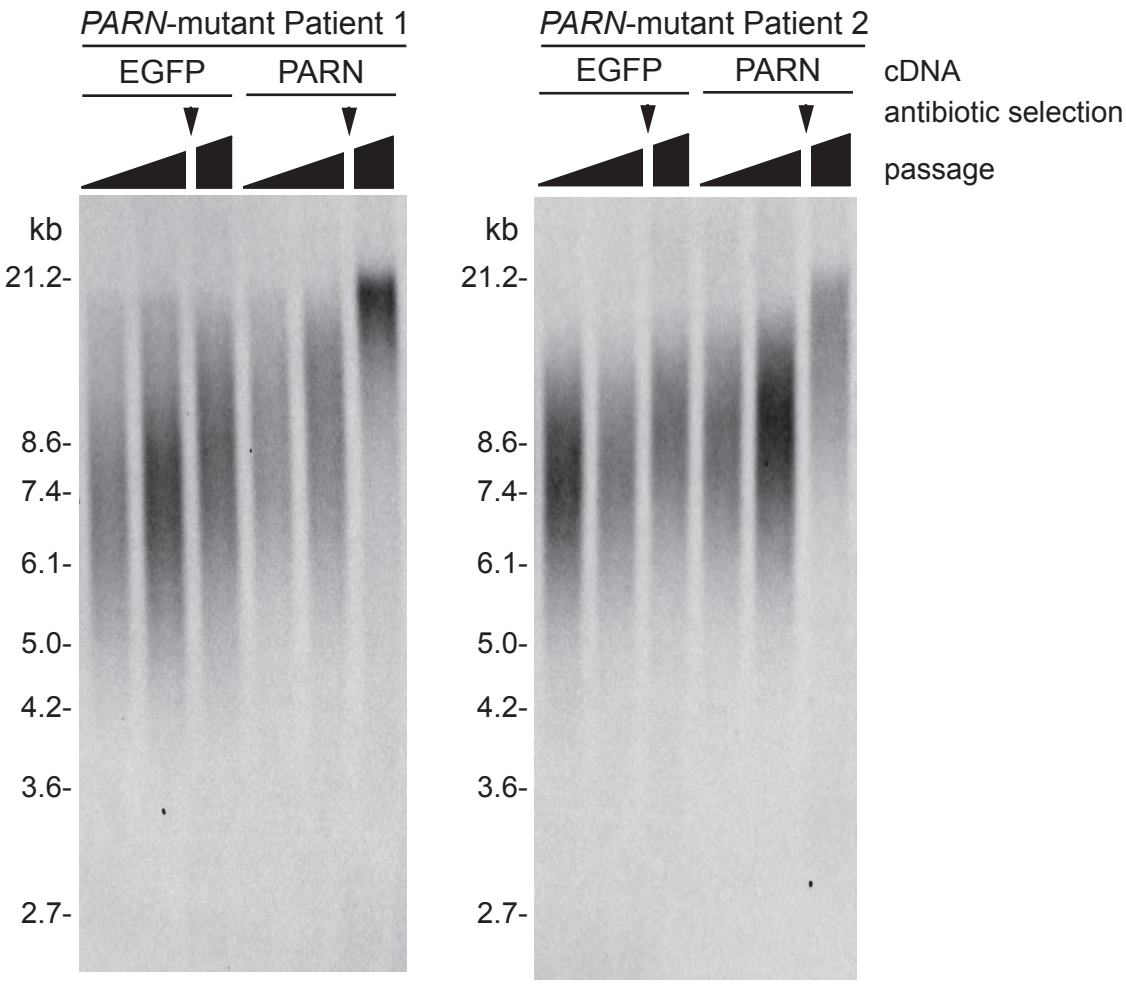
Telomere repeat amplification protocol (TRAP) and terminal restriction fragment (TRF) length analysis were performed as described (17).

Supplemental Figure 1



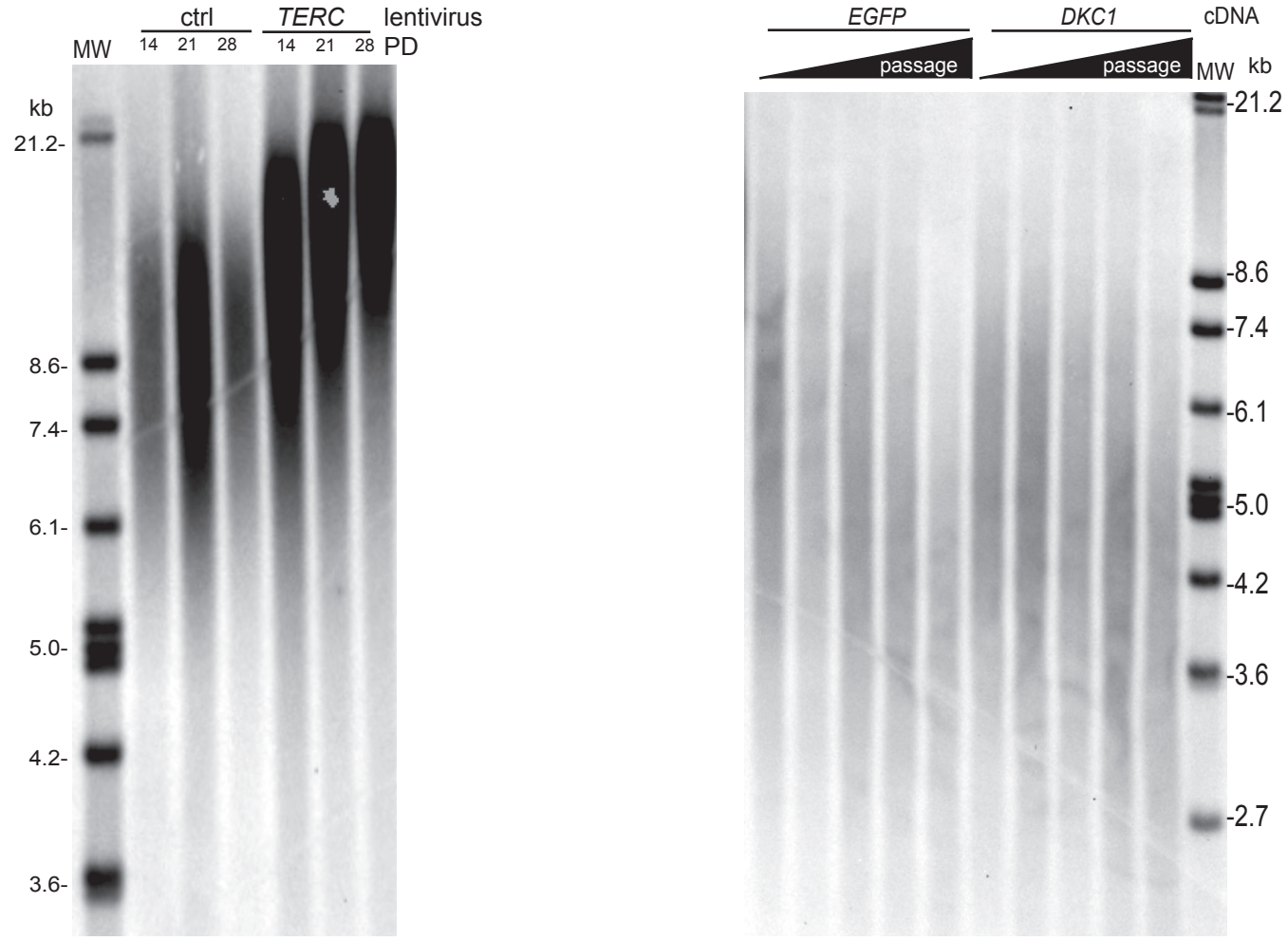
Telomere length in *PARN* kd 293 cells after *PARN* overexpression
Southern blot of telomere length by telomere restriction fragment length analysis in *PARN* kd 293 cells. Cell were transduced with lentivirus containing vector alone (ctrl) or encoding *PARN.* Telomere length was followed for 12 passages. (~6 weeks)

Supplemental Figure 2



Telomere elongation in immortalized *PARN*-mutant fibroblasts after PARN rescue
Southern blot of telomere length by telomere restriction fragment length analysis in *TERT*-immortalized, *PARN*-mutant patient fibroblasts. Cells were transduced with lentivirus encoding either EGFP or PARN and also carrying a blasticidin resistance cassette. Blasticidin selection was performed at the time point indicated by the arrowhead.

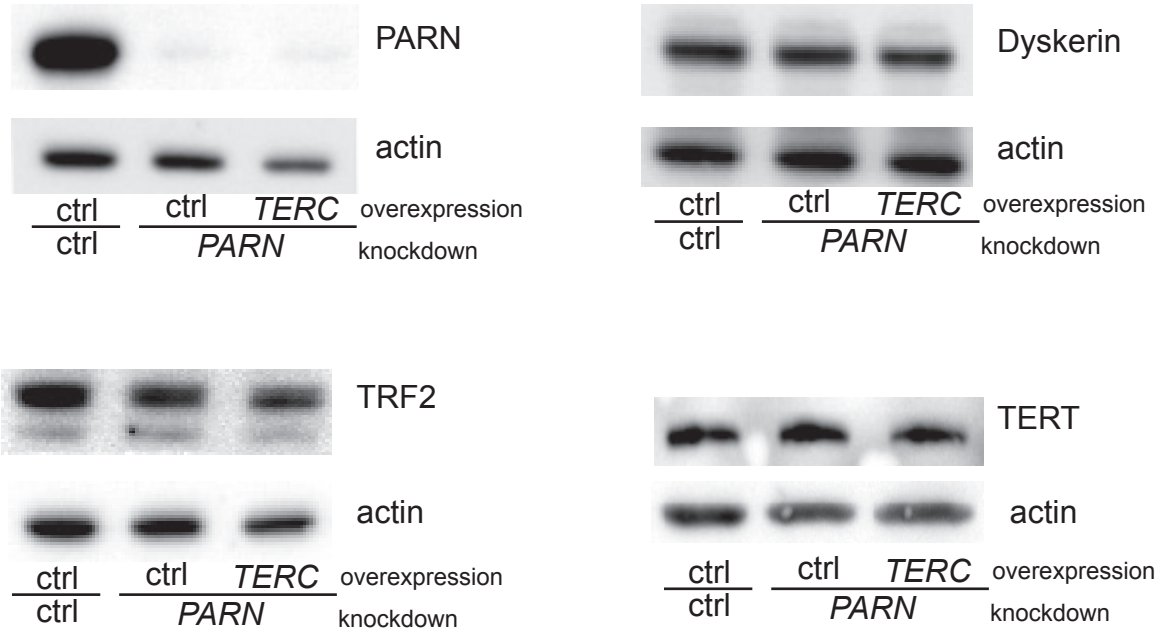
Supplemental Figure 3



Telomere length in 293 cells

(a) Southern blot of telomere length by telomere restriction fragment length analysis in 293 cells. Cell were transduced with lentivirus encoding *TERC* vs control (ctrl) **(b)** Southern blot of telomere length by telomere restriction fragment length analysis in *PARN* kd 293 cells. Cell were transduced with lentivirus encoding either *EGFP* or *DKC1.* Telomere length was followed for 12 passages (~12 weeks) after sorting for GFP+ transduced cells.

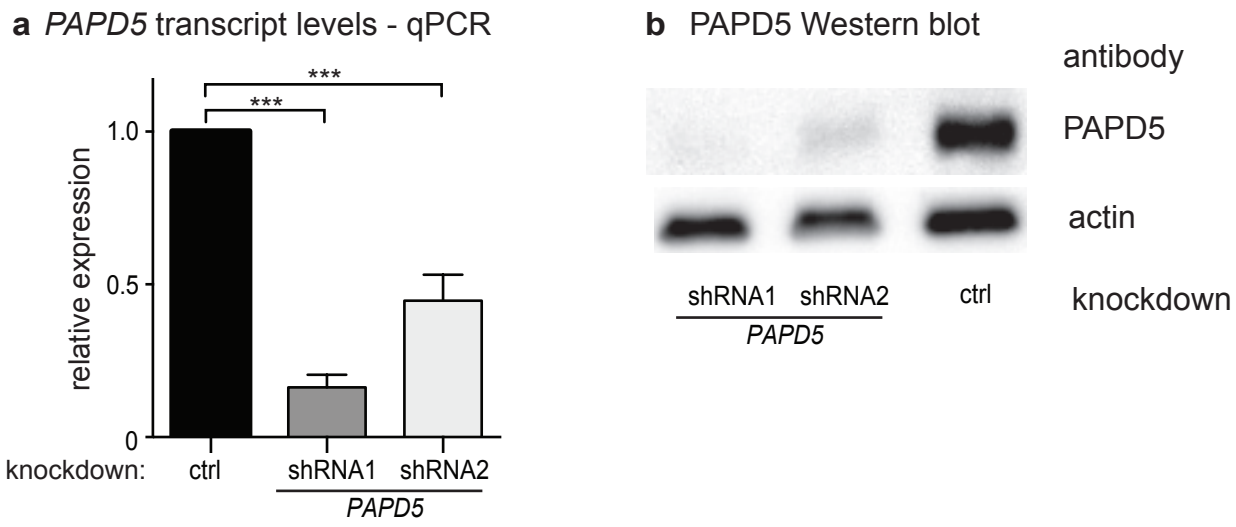
Supplemental Figure 4



Expression of telomere related proteins in *PARN*-kd cells with and without *TERC* overexpression

Western blots for PARN, Dyskerin, TRF2 and TERT of cell lysates from HEK293 cells transduced with either control or *PARN* shRNA and *PARN*-kd cells with *TERC* overexpression. Actin is shown as a loading control. Cells are those used in Fig. 1b.

Supplemental Figure 5

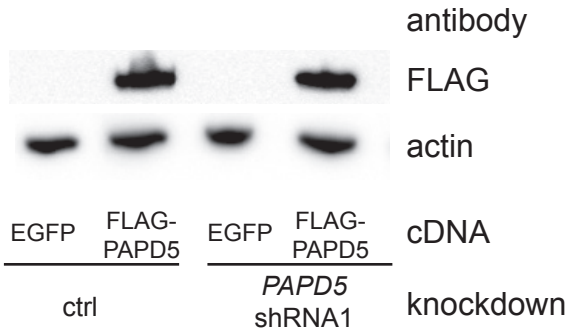


***PAPD5* knockdown in HEK293 cells**

a. Quantitative PCR (qPCR) of *PAPD5* transcripts from HEK 293 cells transduced with lentiviruses expressing shRNAs against luciferase (ctrl) versus *PAPD5*. n =3 biological replicates. Error bar indicates standard deviation, and significance is indicated by P value ≤ 0.001 (***)

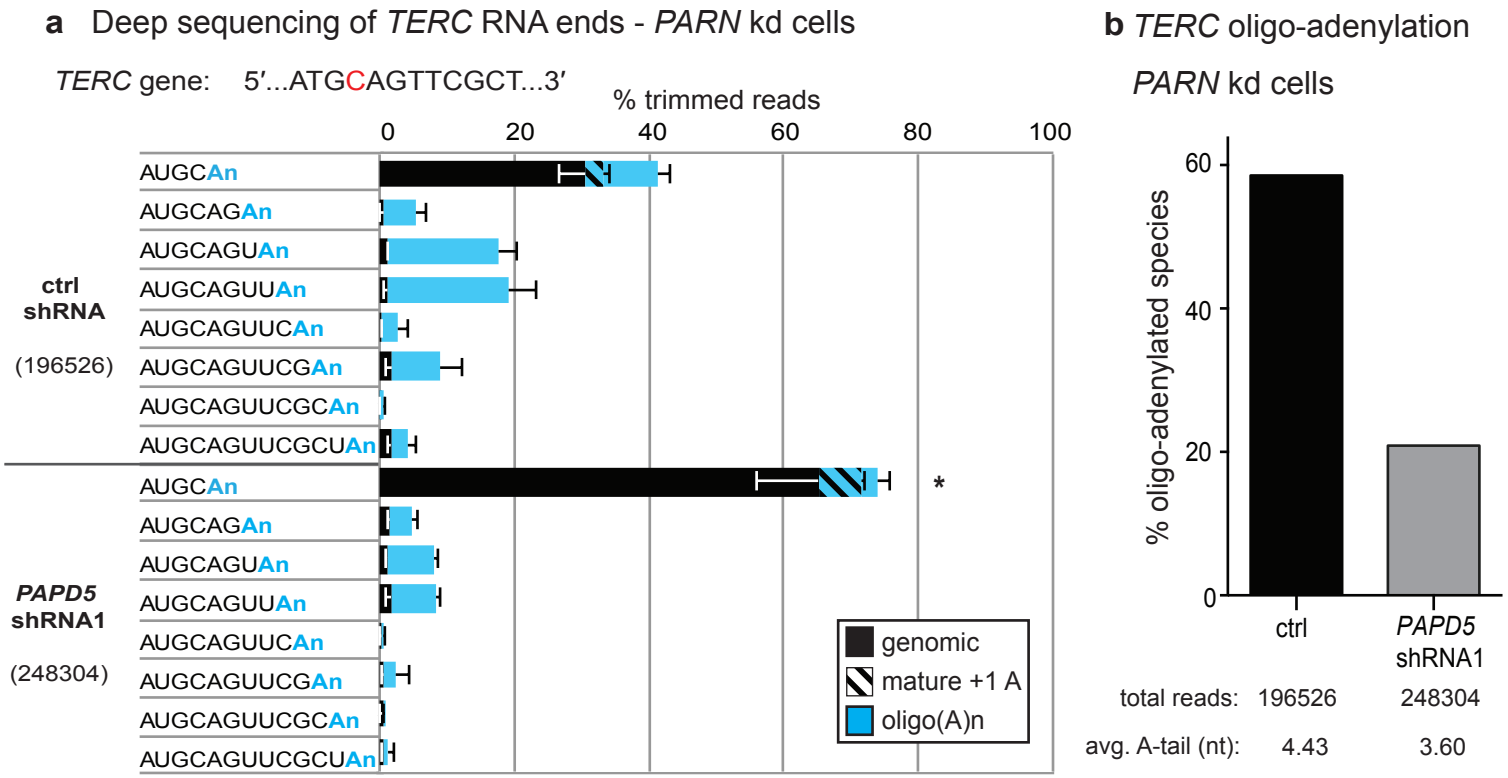
b. Immunoblot of PAPD5 and actin protein levels in HEK293 cells transduced with lentivirus encoding shRNA against luciferase (ctrl) or *PAPD5*.

Supplemental Figure 6



Overexpression of *PAPD5* in control and *PAPD5* knockdown cells
Western blots of cell lysates from control knockdown versus *PAPD5* knockdown HEK 293 cells, with lentiviral overexpression of either a cDNA encoding FLAG-tagged, codon-optimized PAPD5 or EGFP. Actin is shown as a loading control. Cells are those used in Fig. 2e.

Supplemental Figure 7

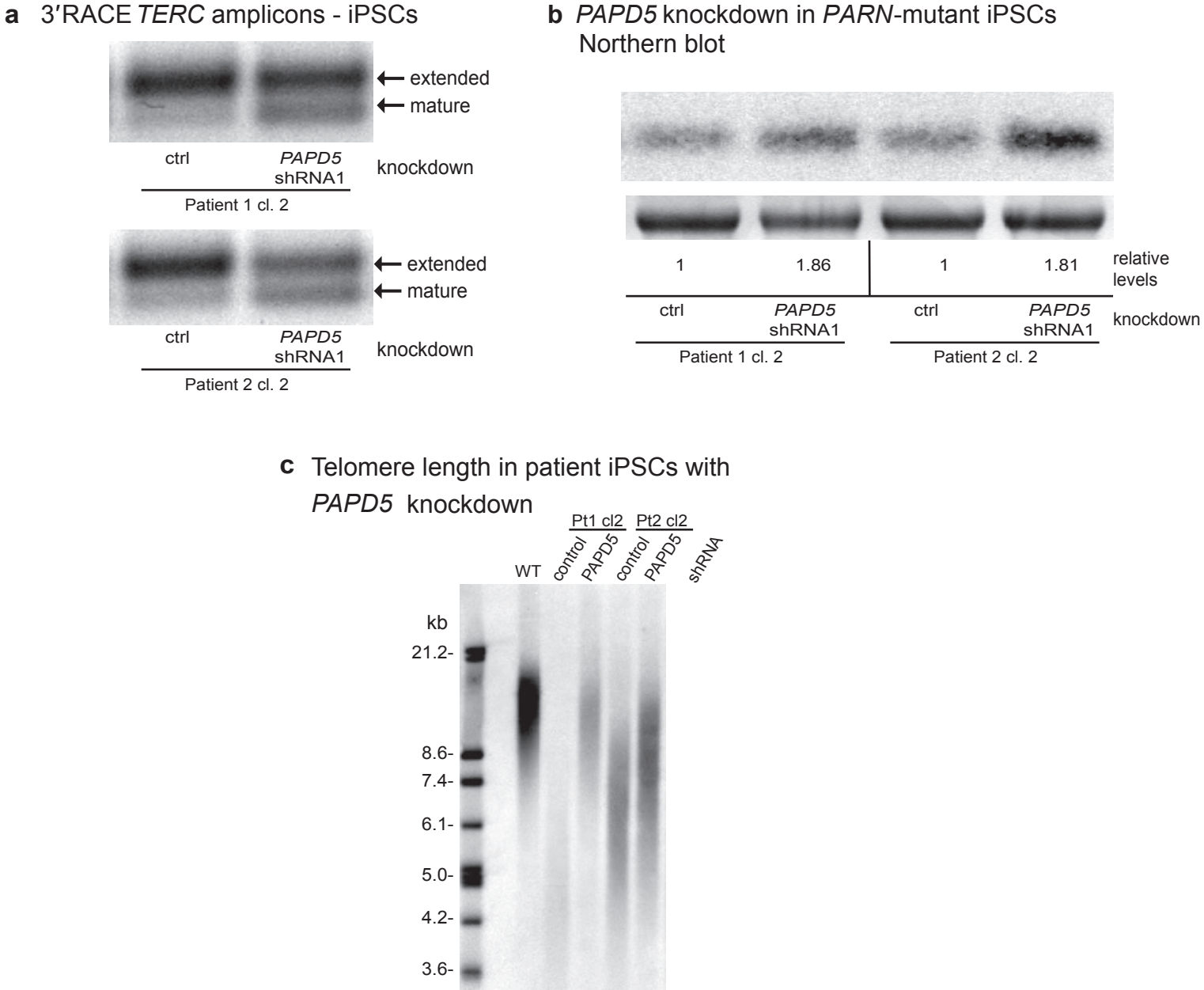


Deep sequencing of *TERC* 3'ends after *PAPD5* knockdown in *PARN*-deficient HEK293 cells

a. 3' RACE PCR products from *PARN* knockdown HEK 293 cells transduced with lentivirus encoding shRNA against *PAPD5* versus control were subjected to deep sequencing, as in Fig. 2c. Proportions are averaged from 2 biological replicates for each group. Error bars represent standard deviations. For statistical evaluations, mature *TERC* forms and oligo(An) ends for all genomically-extended *TERC* species were compared between control and *PAPD5* knockdown cells in a two-tailed t-test. Significance for difference in proportion of mature *TERC* is indicated by P value ≤ 0.05 (*).

b. Oligo(An) species as a proportion of total reads are indicated in control versus *PAPD5* knockdown, *PARN*-deficient HEK293 cells. The A-tail length in nucleotides (nt) was averaged (avg) over the entirepopulation of oligo(An) species for each condition and is indicated.

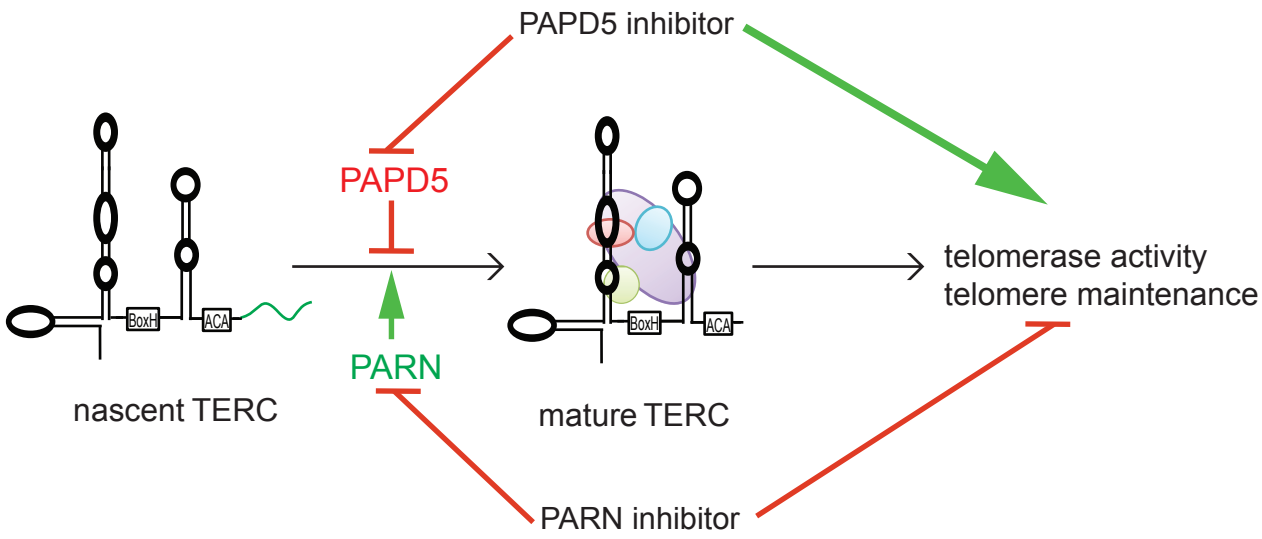
Supplemental Figure 8



***PAPD5* knockdown in *PARN*-mutant patient iPSCs (additional clones)**

(a) 3' RACE TERC amplicons from normal (WT) versus PARN-mutant iPSCs stably transduced with lentivirus encoding shRNA directed against *PAPD5* versus luciferase as a control (ctrl). **(b)** Northern blot of TERC RNA from normal versus PARN-mutant iPSCs stably transduced with lentivirus encoding shRNA directed against *PAPD5* versus luciferase. **(c)** TRF length analysis of *PARN*-mutant patient iPSCs, 12 passages (~6 weeks) after transduction.

Supplemental Figure 9



Model - reciprocal regulation of *TERC* maturation by PARN and PAPD5
Model demonstrating the reciprocal regulation of *TERC* levels by PAPD5 and PARN, and the potential for therapeutic manipulation of telomerase in degenerative or malignant disorders.

Supplemental Table S1

RNA-Seq transcriptome analysis of the effects of *PAPD5* knockdown: transcripts altered in excess of the fold increase in TERC RNA

		Altered in any 2 of 3 pair-wise comparisons: HEK 293 and *PARN*-mutant Patient 1 iPSC clones 1 and 2			**Altered in both pair-wise *PARN*-mutant comparisons: Patient 1 iPSC clones 1 and 2**		
	Total genes analyzed	Obs	Exp	P-value	Obs	Exp	P-value
Decreased							
mRNA	19698	30	33.7	0.34	4	3.97	1
lincRNA	7670	7	13.1	0.07	1	1.55	0.97
snoRNA	454	**7**	**0.78**	**5.9E-11**	0	0.09	1
snRNA	1848	7	3.2	0.05	1	0.37	0.83
Increased							
mRNA	19698	47	53.6	0.15	3	3.31	1
lincRNA	7670	*12*	*20.9*	*0.03*	0	1.29	0.42
snoRNA	454	**11**	**1.2**	**4.0E-17**	**2**	**0.08**	**2.0E-7**
snRNA	1848	**11**	**5.0**	**0.01**	0	0.31	1

mRNA: protein-coding mRNA; lincRNA: long intergenic non-coding RNA; snoRNA: small nucleolar RNA (includes box H/ACA, C/D, scaRNA); snRNA: small nuclear RNA
Obs: observed; Exp: expected by chance
Bold: significantly more than expected by chance
Italic: significant fewer than expected by chance

Supplemental Table S2

Genes whose transcript levels are altered in excess of the fold-change in *TERC* levels, in pair-wise comparisons of PAPD5 knockdown versus control cells

	Altered in all 3 of 3 pair-wise comparisons: **HEK 293 and *PARN*-mutant Patient 1 iPSC clones 1 and 2**	**Altered in both pair-wise *PARN*-mutant comparisons:** **Patient 1 iPSC clones 1 and 2**
Decreased	*none*	CHURC1-FNTB
		DEC1
		GARS
		RNU6-39P
		RP4-533D7.6
		RP4-794H19.1
Increased	SNORD100	AC018867.1
		GPAA1
		SNORA73
		SNORD100
		USP7

Supplemental Table S3

Genes whose transcript levels are altered in excess of the fold-change in *TERC* levels, in any 2 of 3 pair-wise comparisons of PAPD5 knockdown versus control: HEK 293 cells and *PARN*-mutant Patient 1 clones 1 and 2

Decreased	Increased
AARSD1	AC004076.7
AC069499.1	AC018867.1
AL592183.1	AC112719.2
ANP32B	ANKMY1
AP001628.6	AP001059.7
BASP1	BPI
CD52	BRPF3
CHURC1-FNTB	C12orf79
CTC-273B12.7	C16orf59
DDX56	C16orf93
DEC1	C18orf8
DXO	C1orf127
FKSG63	C5orf22
G0S2	CGRRF1
GARS	CH507-513H4.5
HLA-DPA1	COMMD3-BMI1
MILR1	CTC-435M10.3
MTRR	CTD-2302E22.2
MVP	CTD-2313N18.7
NDUFS7	CYBA
NPIPA1	DNASE1L1
OFCC1	DPP7
POLR3GL	EIF4ENIF1
PRR4	FXYD1
PSMC3IP	GABARAP
RNU6-24P	GEMIN4
RNU6-307P	GPAA1
RNU6-314P	LINC01360
RNU6-341P	NDUFA7
RNU6-39P	NOMO3
RNU6-48P	PACRG
RNU6-606P	PIAS3
RP11-101E3.5	PMM2
RP11-2B6.3	PRKAB1
RP11-397H6.1	PTCD3
RP11-686D22.5	PVR
RP11-70F11.11	RFXANK
RP11-895M11.3	RMRP
RP4-533D7.6	RNU4-86P
RP4-794H19.1	RNU6-1099P
SCARNA1	RNU6-1275P
SNORA21	RNU6-373P
SNORA31	RNU6-45P
SNORA55	RNU6-714P
SNORA68	RNU7-140P
SNORD11	RNU7-154P
snoU13	RNU7-181P
SPRR2G	RNU7-77P
TARS	RNVU1-15
TMEM141	RP11-160E2.6
U2AF1L4	RP11-171I2.3
	RP11-245A18.1
	RP11-338N10.3
	RP11-38C17.1
	RP11-430H10.1
	RP11-577B7.1
	RP11-644F5.10
	RP3-526F5.2
	RP4-539M6.19
	RP6-24A23.6
	RPAP1
	SCARNA12
	SIGIRR
	SLC25A16
	SLC6A6
	SLX1B
	SMTNL2
	SNORA13
	SNORA5C
	SNORA73
	SNORD100
	SNORD116-15
	SNORD33
	SNORD38A
	SNORD46
	SNORD89
	SNORD92
	SULT1A3
	TRMT1
	UBE2F
	USP7

Supplemental Table S4

DNA oligos and primers used in this study

Oligo/Primer name	DNA Sequence
PAPD5_L	AGGGAGTCGTGGGTCTGCATGAA
PAPD5_R	ATATCTGGACGTCAGCGCTGGG
POLR2A_L	GCTTGATGCGGGTGCTGAGTGA
POLR2A_R	GTCCTGGCGGTTGACTCCGTGT
TERC_L	CTCTGTCAGCCGCGGGTCTCTC
Universal RT primer	CTACGTAACGATTGATGGTGCCTACAG
TERC_L2	GGGTTGCGGAGGGTGGGCCT
TERC_R	GCATGTGTGAGCCGAGTCCTGG
Luciferase shRNA	CCGG**CGCTGAGTACTTCGAAATGTC**CTCGAGGACA TTTCGAAGTACTCAGCGTTTTTG
PAPD5 shRNA 1	CCGG**CGATGTTGGAAGGAGTTCATA**CTCGAGTATG AACTCCTTCCAACATCGTTTTTG
PAPD5 shRNA2	CCGG**GCCACATATAGAGATTGGATA**CTCGAGTATC CAATCTCTATATGTGGCTTTTTG

Supplemental Table S5

Antibodies used for Western blot in this study

Antibody	Source	Catolog#	Dilution
Anti-FLAG HRP	Sigma	A8592	1:1000
Anti-PAPD5	Atlas	HPA042968	1:1000
Anti-TERT	Rockland	600-401-252S	1:500
Anti-Dyskerin	Santa Cruz	Sc-48794	1:5000
Anti-TRF2	Santa Cruz	Sc-8528	1:1000
Anti-PARN	Abcam	Ab188333	1:5000
Anti-Actin HRP	Santa Cruz	Sc-1615	1:5000
Goat anti-rabbit	Bio-rad	170-5046	1:15000
Anti-goat	Jackson ImmunoResearch	705-030-147	1:10000