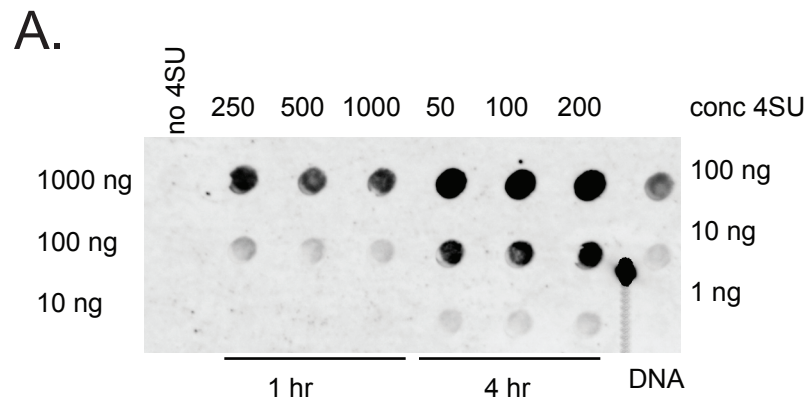
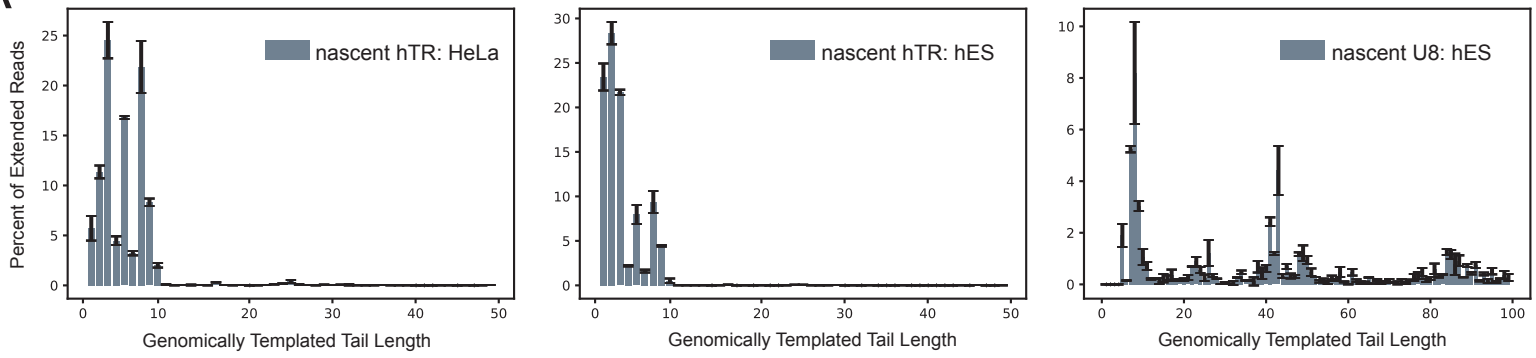


S1 related to Fig. 1

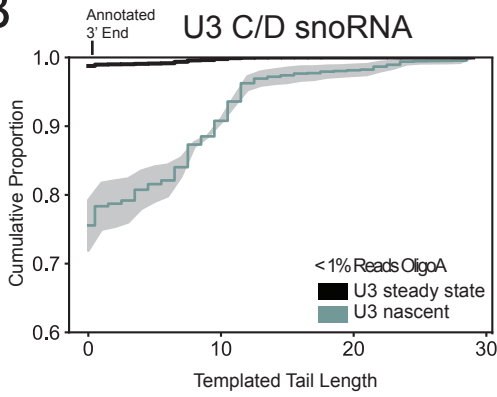


S2 related to Fig. 2

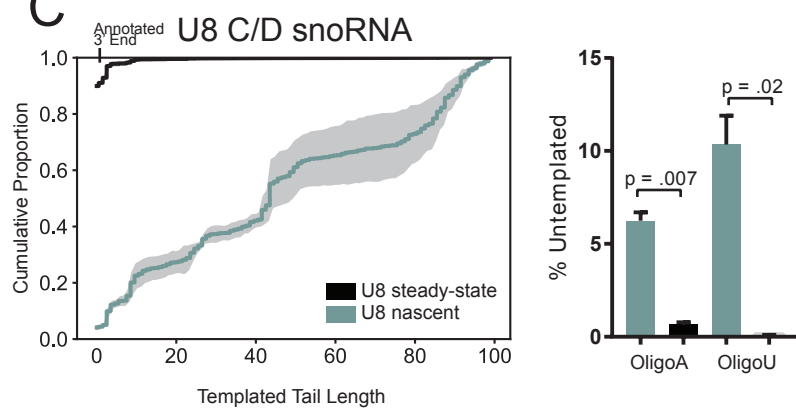
A



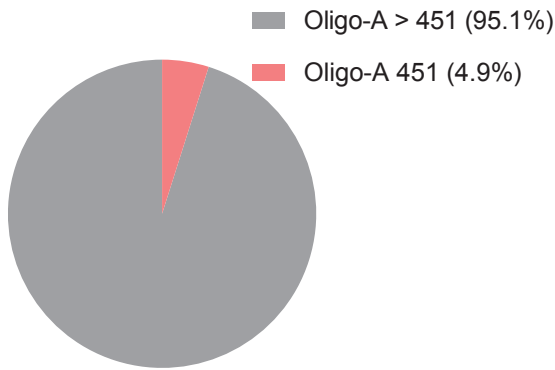
B



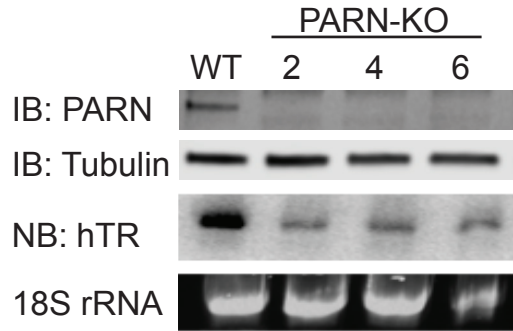
C



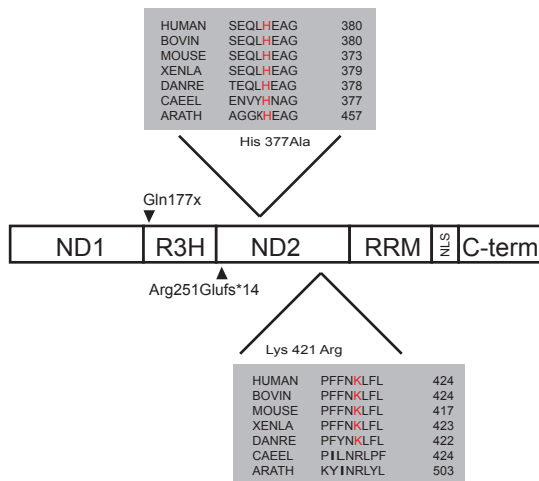
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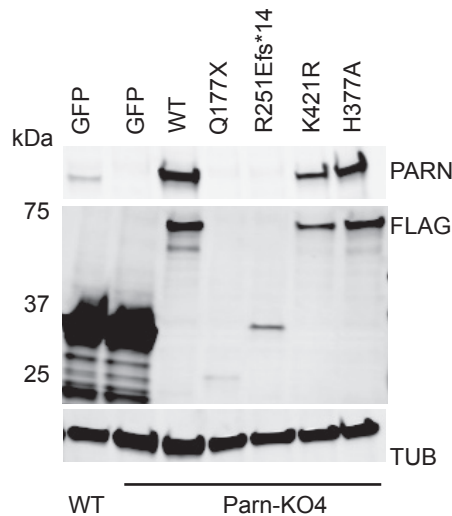
E



F

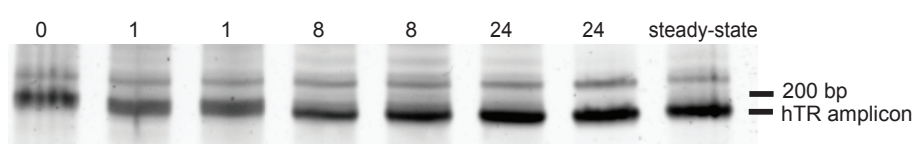


G

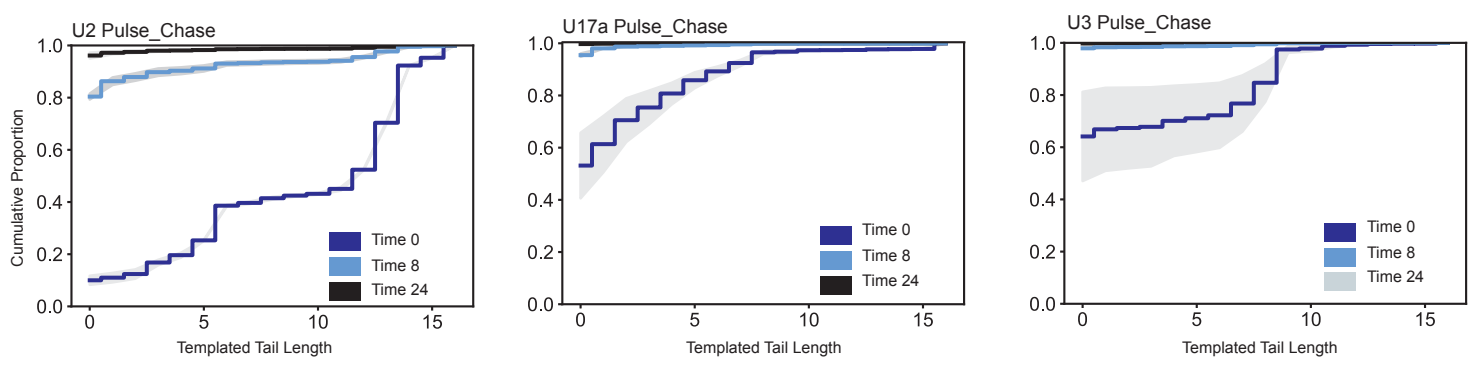


S3 related to Fig. 3

A



B



C

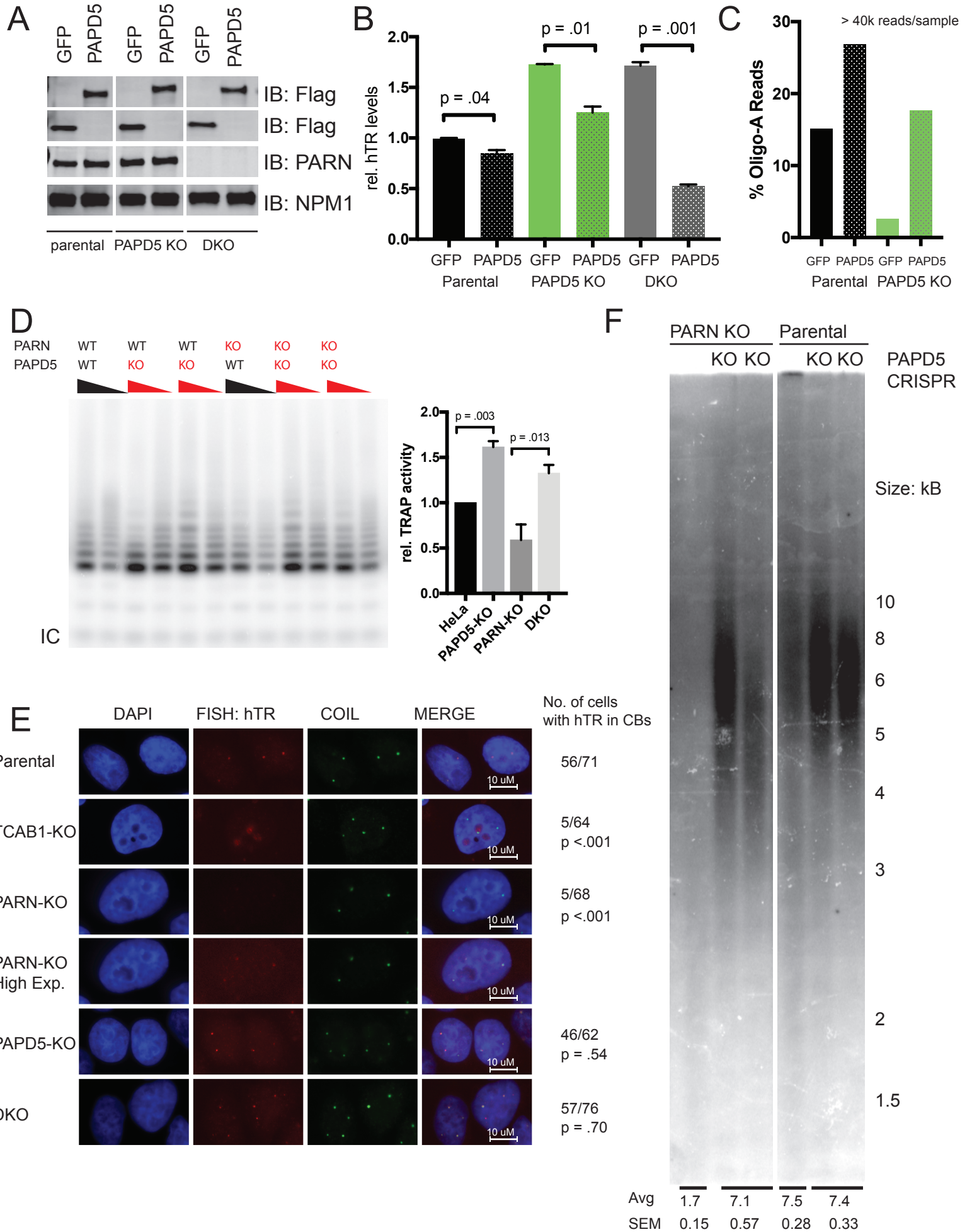
Reference	Cell Type	Protocol	hTR $t_{1/2}$	k_{decay}	R	k_{mat}	$t_{1/2 mat}$
Moon 2015	HEK293	ActD	14.5	.048	.2	.19	3.63
Shukla 2016	HeLa	ActD	25.2	.028	.24	.09	7.96
Roake This study	HeLa	Nascent RNAend-Seq				.14	4.94

$$\frac{dM}{dt} = k_{mat}P - k_{decay}M = 0$$

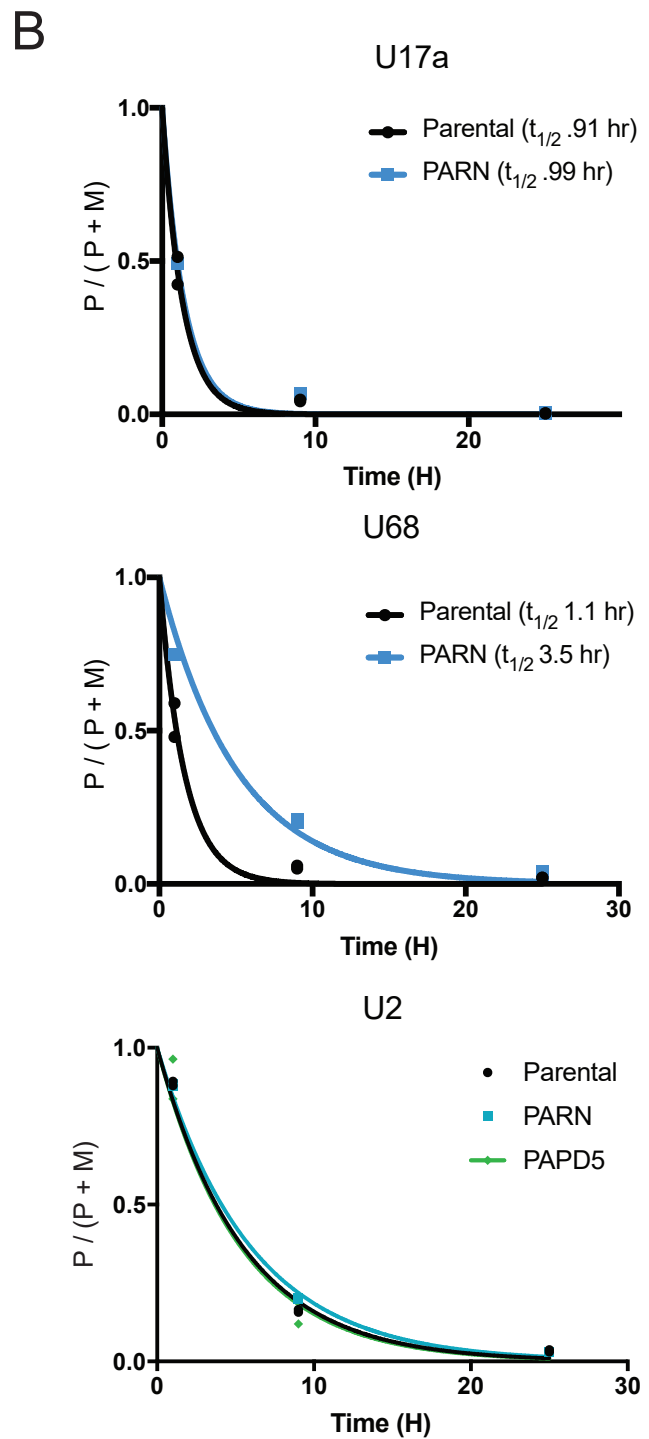
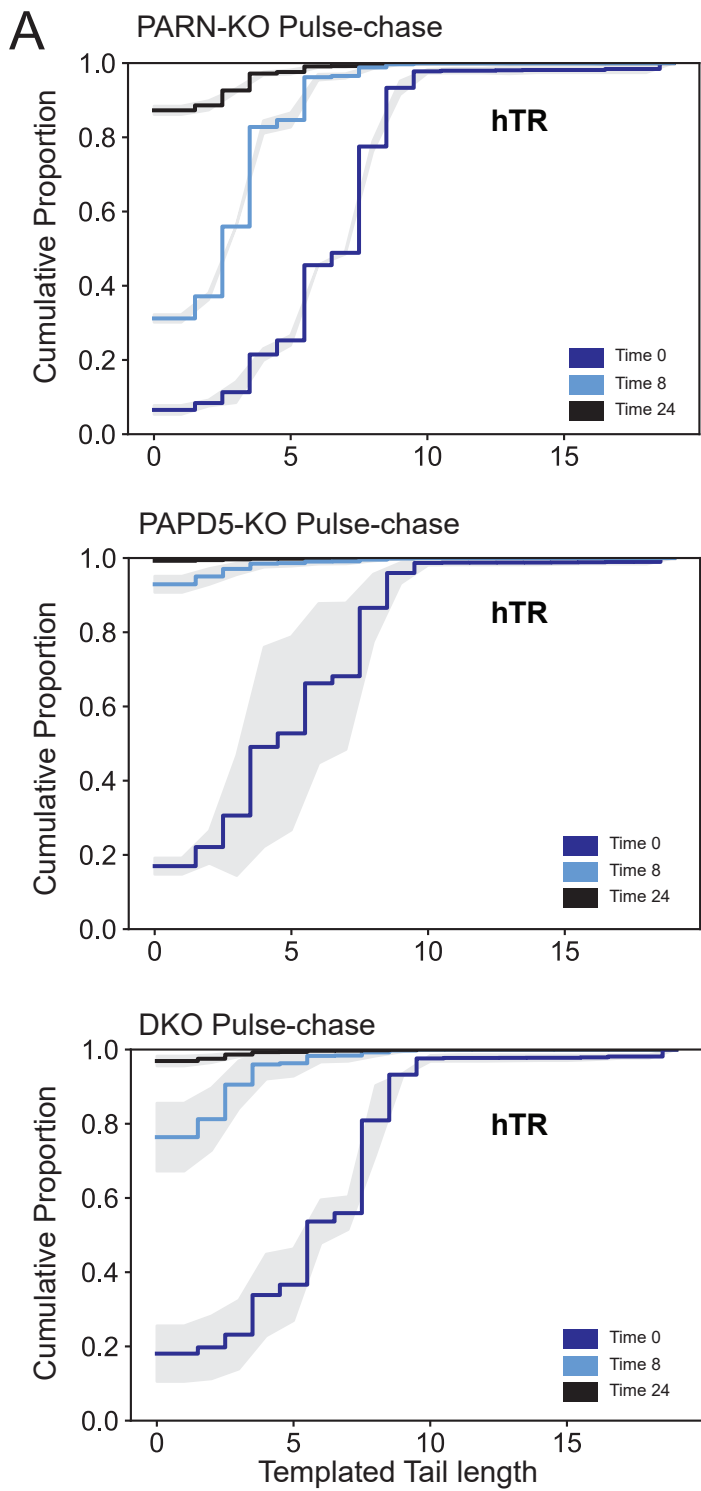
$$R = \frac{P}{P + M}$$

$$k_{mat} = k_{decay} \left(\frac{1}{R} - 1 \right)$$

S4 related to Fig. 4

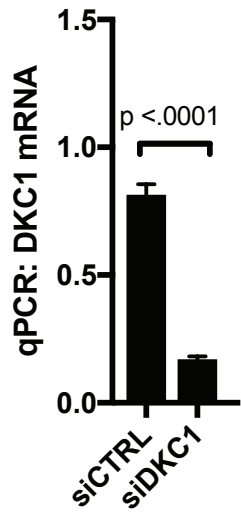


S5 related to Fig. 4

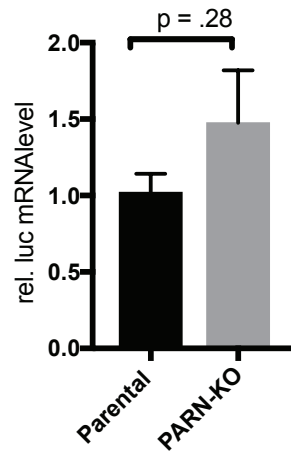


S6 related to Figure 5

A.



B.



Supplementary Figures

Figure S1 related to Figure 1:

A. Streptavidin (SA) dot blot of biotinylated RNA to estimate levels of 4SU incorporation in the indicated conditions. Cells were labeled with 50 – 1000 μ M 4SU for 1 hr or 4hrs and 10 – 1000 ng of RNA were blotted. A biotin labeled DNA oligo was loaded as a positive control at 1- 100 ng. Biotinylated RNA from 4SU naïve cells was used as a negative control.

Figure S2 related to Figure 2

A. The relative abundance of different length hTR and U8 precursors in HeLa and hES nascent RNA. Error bars represent S.E.M from 2 biologic replicates.

B. Nascent RNAend-Seq of U3, a box C/D snoRNAs transcribed from an independent polIII locus. Gray shading represents 95% CI from 2 biologic replicates. U3 is 25 kb from the nearest coding transcript. U3 is oligoadenylated at a very low rate, with ~ 2% of nascent molecules having a short (mean = 1.4 nt) untemplated tail.

C. Nascent RNAend-Seq of U8, a box C/D snoRNA transcribed from an independent polIII locus. U8 is embedded 216 nts upstream of the end of the 3' UTR of the gene TMEM107. For U8, it is possible that some of the long transcripts represent the transcripts of the host gene, TMEM107, although no transcripts were found with significant polyA tails (max A tail length = 16 nts). U8 nascent transcripts are found with either oligoU (10% of reads) or oligoA tails (6% of reads) but rarely both (<1% of reads).

D. Distribution of oligo-A marks on nascent hTR molecules. 95% of oligo-A reads are on extended (> 451 nt) hTR molecules. 5% are on mature hTR-451 or shorter forms of hTR.

E. Northern blot (N.B.) probed for hTR in parental (WT) and PARN-KO cells

F. PARN gene diagram showing location of three patient mutations (Gln177X, R251Efs*14, K421R) and catalytic dead PARN (H377A). Shaded gray boxes show conservation of mutated amino acids. ND = nuclease domain. RRM = RNA recognition motif.

G. W.B. of HeLa or PARN-KO clone 4 HeLa cells transiently transfected with FL-GFP or FL-PARN cDNA. FL-PARN mutants are labeled by amino acid change.

Figure S3 related to Figure 3

A. 3'RACE amplicon for hTR in pulse-chase experiment shown a time 0, time 1, time 8, and time 24. The 200 bp size marker is shown. The mature hTR amplicon is 189 bp.

B. Nascent RNAend-Seq of U2, U17a, U3, in pulse-chase experiment. Each curve represents an average of two biologic replicates and grey shading represents a 95% CI. Curves were drawn at time 0, time 8, and time 24.

C. Following a kinetic model, we can represent the change in the amount of mature hTR by the following equation: $\frac{dM}{dt} = k_{mat}P - k_{decay}M$ where P is the amount of precursor, M is the amount of mature hTR, k_{mat} is the maturation rate constant, and k_{decay} is the decay rate constant. We can also measure the ratio of precursor in steady state conditions as $R = \frac{P}{P+M}$. Using these two equations and assuming steady-state conditions where $\frac{dM}{dt} = 0$ we acquire the equation $k_{mat} = k_{decay} \left(\frac{1}{R} - 1 \right)$. Using decay rates from label-free experiments in the literature (using the RNA polIII inhibitor Actinomycin D), we estimate k_{mat} and compare it to the values we obtain using our 4SU approach.

Figure S4 related to Figure 4

A. WB for expression of FL-PAPD5 or FL-GFP in parental HeLa, PAPD5-KO HeLa, or PARN/PAPD5 DKO HeLa.

B. qRT-PCR for hTR relative to parental HeLa+GFP and normalized to GAPDH. Error bars represent S.E.M.

C. Percentage of hTR reads with untemplated A tails in the parental or PAPD5-KO cells expressing either GFP or FL-PAPD5. Each bar represents > 40k reads.

D. Telomeric Repeat Amplification Protocol (TRAP) assay to measure telomerase activity in the indicated cells. For each sample, two lanes are shown, and the second lane is a 3x dilution of the lysate in the first. IC: internal control. TRAP activity quantified at right represents two independent experiments.

E. RNA-FISH for hTR and IF for coilin. Cells with visible hTR foci in Cajal bodies (CBs) were counted and significance was analyzed with Fisher's Exact Test.

F. Telomere length of the indicated cells by telomere restriction fragment southern blot. DNA ladder is shown in kB. Mean telomere length calculated from 3-5 clones per genotype and variance represented as S.E.M.

Figure S5 related to Figure 4

A. Cumulative proportion of molecules with the indicated templated tail lengths is plotted in PARN-KO, PAPD5-KO, and DKO cells at time 0 (dark blue) time 8 (light blue) and time 24 (black).

B. Proportion of U17a precursors is plotted for pulse chase experiment as in Figure 4a. From fitted exponential decay curves, the $t_{1/2}$ of conversion is obtained ($t_{1/2} = .91$ for parental cells and $t_{1/2} = .99$ for PARN KO cells). Proportion of U68 precursors is plotted for pulse chase experiment. From fitted exponential decay curves, the $t_{1/2}$ of conversion is obtained ($t_{1/2} = 1.1$ for parental cells and $t_{1/2} = 3.2$ for PARN KO cells). Plot of precursor fraction versus time for U2 snRNA in cells of the indicated genotypes.

Figure S6 related to Figure 5

A. qRT-PCR for DKC1 mRNA 48 hrs post siCTRL or siDKC1. Signal is normalized to GAPDH. Results show approximately 4-fold knockdown in siDKC1. Error bars represent S.E.M.

B. qRT-PCR for luciferase mRNA in cells transfected with U1-H/ACA in parental HeLa and PARN-KO HeLa cells. Luciferase cDNA was mixed with U1-H/ACA constructs during transfection to control for transfection efficiency.

Table S1 related to STAR Methods: Primers and oligos

Oligo Name	Sequence	Description
sgPARN01	GCCGACTTCTTCGCCATCGA	CRISPR guide RNA exon2
sgPARN02	CCGACTTCTTCGCCATCGAT	CRISPR guide RNA exon2
sgPARN03	ACCTGAAAACCTCCCCATCGA	CRISPR guide RNA exon2
sgPAPD503	CAGGATCGAGAGTGTAATTA	CRISPR guide RNA Exon3
Univ RT	CTACGTAACGATTGATGGTGCCTACAG	3' RACE primer
TERC R1	CACCGCGAAGAGTTGGGCTCTG	3' RACE primer
U2 R1	TTCTCGGCCTTTTGGCTAAG	3' RACE primer
U3 R1	CGTGTAAGAGCACCGAAAACC	3' RACE primer
E1 R1	CGTGGATACACCCGGGAGGTCACTCTC	3' RACE primer
U68 R1	TTGCACCTAAACCCAAGAATC	3' RACE primer
U8 R1	TTACCTGTTCTCCTCCGGA	3' RACE primer
RAT R1	GGAGTTTATATGGAAACCTTAGACG	3' RACE primer
RAT probe	ACGTCTAAGGGTTTCCATATAAACTCCTT	NB probe
U4 Probe	CGGATAAACCTCATTGGCTACGATACTGCC	NB probe
U6 Probe	GGCCATGCTAAATGTTGTTCT TCTCTGTATCGTTCCAATT	NB probe
TERC11F	CGCTGTTTTTCTCGCTGACT	qPCR primer
TERC11R	GCTCTAGAATGAACGGTGGAA	qPCR primer
TERCext1R	CCACCAACAGGAAAGCGAAC	qPCR primer
TERCext2R	CCAACAGGAAAGCGAACTGC	qPCR primer
GAPDH60F	AGCCACATCGCTCAGACAC	qPCR primer
GAPDH60R	GCCCAATACGACCAAATCC	qPCR primer
B2M F	ACACCAAGTTAGCCCCAAG	qPCR primer
B2M R	ACCCAGACACATAGCAATTCAG	qPCR primer
U1 F	TGGCAGGGGAGATACCATGA	qPCR primer
U1 R	TGCAGTCGAGTTTCCCACAT	qPCR primer
Luc F	AGGTCTTCCCGACGATGA	qPCR primer
Luc R	GTCTTTCCGTGCTCCAAAAC	qPCR primer
5'ETS F	GAACGGTGGTGTGTCTGTTT	qPCR primer (Nguyen le et al., 2015)
5'ETS R	GCGTCTCGTCTCGTCTCACT	qPCR primer (Nguyen le et al., 2015)
U3 F	ACCGAAAACCACGAGGAAG	qPCR primer
U3 R	CCAAGCAACGCCAGAAAG	qPCR primer
TS* primer	AATCCGTCGAGCAGAGTT	Labeled TRAP primer
TRF primer	CCCTAACCTAACCCTAA	Labeled TRF primer
hTR FISH	GCCTTCTCAGTTAGGGTTA; AAGTCAGCGAGAAAAACAGC; TCTAGAATGAACGGTGGAAAG; CCAGCAGCTGACATTTTTT; GCTGACAGAGCCCAACTCTT; GTCCACAGCTCAGGGAATC; CATGTGTGAGCCGAGTCCTG	RNA FISH

Table S2 related to Figure 2 and Figure 4: Genotypes of Cells Used In This Study

Name	Sequence
WT PARN Exon 2	GCC GAC TTC TTC GCC ATC GAT GGG GAG TTT TCA A D F F A I D G E F S
PARN-KO L4 D28E_fs D28R_fs	GCC GAC TTC TTC GCC ATC GA- -GG GAG TTT TCA GCC GAC TTC TTC GCC ATT CGA TGG GGA GTT TTC
PARN-KO A2 I27S_fs I27M_fs	GCC GAC TTC TTC GC - ATC GAT GGG GAG TTT TCA GCC GAC TTC TTC GC - - - - GAT GGG GAG TTT TCA
WT PAPD5 Exon 3	GTG AAC AGG ATC GAG AGT GTA ATT AAG GAG CTC V N R I E S V I K E L
PARN/PAPD5 DKO (2/3het) T8 E149A_fs I152L_fs E149K	GTG AAC AGG ATC G - - - - - - - - - - - - - - - - - - CTC GTG AAC AGG ATC GAG AGT GTA -TT AAG GAG CTC GTG AAC AGG AT - - - - - - - - - - - - - - - - - -T AAG GAG CTC
PARN/PAPD5 DKO T10 K153* I152N_fs S150N_fs	GTG AAC AGG ATC GAG AGT GTA ATT TAA GGA GCT C GTG AAC AGG ATC GAG AGT GTA AAT TAA GGA GCT C GTG AAC AGG ATC GAG A - - - - - - - - - - - - - - - - - -
PAPD5 T4 K153* (7) V151G_fs (11)	GTG AAC AGG ATC GAG AGT GTA ATT TAA GGA GCT C GTG AAC AGG ATC GAG AGT G - - - - - - - - - - - - - - - - - -
PAPD5 T6 I152L_fs (3) I152N_fs (1) K153* (1)	GTG AAC AGG ATC GAG AGT GTA - TT AAG GAG CTC GTG AAC AGG ATC GAG AGT GTA AAT TAA GGA GCT C GTG AAC AGG ATC GAG AGT GTA ATT TAA GGA GCT C

Table S3 related to Figure 2, Figure 3, Figure 4: hTR 3'RACE-Seq Numbers

File	TotalReads	<= 451 (%)	> 451 no A (%)	> 451 + A (%)	> 451 (%)	AtailMean	EtailMean	ShortMean
DKO_08_T0	22262	0.153	0.547	0.300	0.847	1.461	7.424	4.359
DKO_08_T08	62764	0.729	0.103	0.168	0.271	2.399	3.922	1.744
DKO_08_T24	96005	0.964	0.010	0.026	0.036	2.988	4.051	1.790
DKO_10_T0	19843	0.207	0.535	0.258	0.793	1.567	7.440	4.852
DKO_10_T08	80857	0.795	0.066	0.140	0.205	2.760	3.539	1.648
DKO_10_T24	77849	0.974	0.009	0.017	0.026	2.794	3.910	1.717
HeLa_01_T0	24941	0.236	0.405	0.359	0.764	1.813	7.745	4.335
HeLa_01_T05	32801	0.444	0.165	0.391	0.556	2.521	3.862	1.692
HeLa_01_T08	37372	0.670	0.060	0.270	0.330	2.639	3.524	1.631
HeLa_01_T12	51597	0.874	0.016	0.110	0.126	3.281	3.139	1.673
HeLa_01_T24	33399	0.964	0.005	0.030	0.036	3.358	3.824	1.591
HeLa_02_T0	15839	0.264	0.381	0.355	0.736	1.837	7.687	4.918
HeLa_02_T05	54756	0.478	0.154	0.368	0.522	2.436	3.783	1.613
HeLa_02_T08	48471	0.670	0.050	0.279	0.330	2.697	3.414	1.535
HeLa_02_T12	54809	0.875	0.016	0.110	0.125	3.003	3.526	1.539
HeLa_02_T24	37353	0.954	0.008	0.038	0.046	3.275	4.092	1.473
HeLa_nascent1_1hr	29304	0.242	0.394	0.364	0.758	2.198	6.123	2.450
HeLa_nascent1_4hr	19253	0.511	0.144	0.345	0.489	3.027	4.550	1.584
HeLa_nascent2_1hr	25104	0.246	0.383	0.371	0.754	2.304	5.911	2.056
HeLa_nascent2_4hr	19846	0.595	0.098	0.308	0.405	3.202	3.750	1.366
HeLa_unbound1_1hr	28739	0.808	0.036	0.156	0.192	2.949	3.752	1.960
HeLa_unbound2_1hr	28904	0.807	0.038	0.155	0.193	2.999	3.549	2.152
hES_nascent1	20480	0.269	0.329	0.402	0.731	2.142	4.243	1.791
hES_nascent2	12360	0.280	0.362	0.358	0.720	1.986	4.152	1.870
hES_unbound1	18197	0.823	0.035	0.142	0.177	2.441	3.594	3.354
hES_unbound2	13188	0.886	0.020	0.094	0.114	1.998	3.117	3.399
PAPD5_04_T0	27945	0.161	0.577	0.261	0.839	1.380	5.619	2.938
PAPD5_04_T08	47156	0.920	0.046	0.034	0.080	1.708	4.045	1.674
PAPD5_04_T24	145143	0.993	0.004	0.003	0.007	1.557	4.535	1.906
PAPD5_06_T0	21886	0.177	0.586	0.237	0.823	1.316	6.914	5.038
PAPD5_06_T08	102572	0.935	0.036	0.028	0.065	1.875	3.742	1.686
PAPD5_06_T24	88848	0.992	0.004	0.004	0.008	1.720	3.942	1.754
PARN_A2_T0	13761	0.060	0.497	0.442	0.940	2.055	7.342	6.141
PARN_A2_T08	43465	0.290	0.112	0.597	0.710	3.817	4.135	1.591
PARN_A2_T24	19898	0.855	0.022	0.123	0.145	4.827	4.081	1.430
PARN_GFP	164858	0.578	0.066	0.356	0.422	4.503	4.675	1.709
PARN_L4_T0	18946	0.070	0.481	0.449	0.930	1.986	7.442	5.417
PARN_L4_T08	23855	0.298	0.122	0.580	0.702	3.748	4.208	1.453
PARN_L4_T24	40708	0.861	0.017	0.121	0.139	4.754	4.070	1.613
PARN_rescue	183225	0.870	0.019	0.111	0.130	4.030	4.049	2.128
Total_DKO_08	223251	0.789	0.065	0.146	0.211	2.874	4.299	1.731
Total_DKO_10	270446	0.872	0.039	0.089	0.128	2.977	3.993	1.895
Total_HeLa	200036	0.817	0.031	0.152	0.183	3.205	4.167	1.871
Total_PAPD5_04	318255	0.952	0.020	0.028	0.048	2.237	3.930	1.712
Total_PAPD5_06	210867	0.946	0.026	0.027	0.054	2.035	4.050	1.757