SUPPLEMENTAL MATERIAL (Poly(A)-specific ribonuclease is a nuclear ribosome biogenesis factor involved in human 18S rRNA maturation; Montellese et al. 2017)

Supplemental Table 1

List of primers used for site-directed mutagenesis, *in vitro* transcription and RNase H assays

PARN-1_siMut forward	5'-CTCCAGCATCGATTTCTTAGCAAGCC-3'
PARN-1_siMut reverse	5'-CTCTGACAAACAAATTTGACATCTGGTG-3'
PARN-H377A forward	5'-CAACTTGCCGAGGCAGGC-3'
PARN-H377A reverse	5'-TTCAGAGGCTGTGTCATAACTTGGAAAAC-3'
OHA416	5'-GGGCCGGATCCTAATACGACTCACTATAGGGTTTCCGTAGGTGAACCTGCGGAAGG-3'
OHA418	5'-GGCGGAAGCTTGCAGTGGCGGTGGGGGGGTGGGTGTGCGGAGGG-3'
ITS1-Hs-RACE	5'-CGCGAATTCGATCATTAACGGAGCCCGGAG-3'
RNaseH_1	5'-TTTACTTCCTCTAGATAGTCAAGTTCGACC-3'
RNaseH_2	5'-TGTTACGACTTTTACTTCCTCTAGATAGTC-3'

Supplemental Table 2

List of target genes and siRNA sequences used in the present study

Gene	GenBank accession #	siRNA	siRNA sequence
PARN	NM_002582.3	siPARN-1	5'-GCUCCAGCAUUGACUUUCUdTdT-3'
		siPARN-3	5'-GCAGAAACAUGCCAAAGAAdTdT-3'
		siPARN-5	5'-AAGCUAUCGGAUCCAAACCUAdTdT-3'
		siPARN-6	5'-AGGACCAGACUUGCAGCCUAAdTdT-3'
NOB1	NM_014062.2	siNOB1-1Q	5'-CGCCCUGGAGCCAAUCUUCAAdTdT-3'
		siNOB1-2Q	5'-UUGCCCAACAUCGAUCAUGAAdTdT-3'
		siNOB1-3	5'-AAGGUUAAGGUGAGCUCAUCGdTdT-3'
PAPD5	NM_001040284.2	siPAPD5-5	5'-GGACGACACUUCAAUUAUUdTdT-3'
		siPAPD5-6	5'-GGCCUUUGAUUAUGCCUACGUUGUUdTdT-3'
PAPD7	NM_006999.4	siPAPD7-3	5'-CGCCGAAAGUACUUUAGGAdTdT-3'
		siPAPD7-5	5'-CCACCACUUCCAGAACACUGAUCAUdTdT-3'
LSG1	NM_018385.2	siLSG1	5'-AGACCAAACUGGAACCAAA-3'
eIF6	NM_001267810.1	sieIF6	5'-GAGCUUCGUUCGAGAACAA-3'
CRM1	NM_003400.3	siCRM1	5'-UGUGGUGAAUUGCUUAUAC-3'
ENP1	NM_004053.3	siENP1	5'-AGCGTGCCATAGAGATGTT-3'
CK1δ	NM_001893.4,	siCK1δ	5'-CCATCGAAGTGTTGTGTAA-3'
CK1ε	NM_001894.4	siCK1ε	5'-ACATCGAGAGCAAGTTCTA-3'
RIO2	NM_018343.2	siRIO2	5'-GGAUCUUGGAUAUGUUUAA-3'
LTV1	NM_032860.4	siLTV1	5'-UGGCAGUGAUCUUCCUAAA-3'
RPS2	NM_002952	siRPS2-2	5'-CCAGGUUCAAGGCAUUUGUdTdT-3'

RPS3	NM_001005	siRPS3-2	5'-CCAGGACAGAAAUCAUUAUdTdT-3'
RPS10	NM_001014	siRPS10-1	5'-GAACCGGAUUGCCAUUUAUdTdT-3'
RPS11	NM_001015	siRPS11-1	5'-GCGCCACAAGAACAUGUCUdTdT-3'
RPS15	NM_001018	siRPS15-2	5'-UCACCUACAAGCCCGUAAAdTdT-3'
RPS17	NM_001021	siRPS17-2	5'-GCAGGUUAUGUCACGCAUCdTdT-3'
RPS21	NM_001024	siRPS21-1	5'-GGAUGGGUGAGUCAGAUGAdTdT-3'
RPS23	NM_001025	siRPS23-1	5'-GGGUCCAGCUGAUCAAGAAdTdT-3'
RPS24	NM_033022	siRPS24-1	5'-CACCGGAUGUCAUCUUUGUdTdT-3'
RPS26	NM_001029	siRPS26-1	5'-GGACAAGGCCAUUAAGAAAdTdT-3'
RPS29	NM_001032	siRPS29-1	5'-CGGUCUGAUCCGGAAAUAUdTdT-3'

Supplemental Table 3

List of probes used in Northern blot

5'ETS	5'-AGACGAGAACGCCTGACACGCACGGCAC-3'
5'ITS1	5'-CCTCGCCCTCCGGGCTCCGTTAATGATC-3'
ITS1-38	5'-GGAGGGAAGCGCGCGGCGGC-3'
ITS1-49	5'-GGTGGGTGTGCGGAGGGAAG-3'
ITS1-59	5'-GCGGTGGGGGGGTGGGTGTG-3'
ITS2-1	5'-CTGCGAGGGAACCCCCAGCCGCGCA-3'
ITS2-2	5'-GCGCGACGGCGGACGACACCGCGGCGTC-3'
18S	5'-TTTACTTCCTCTAGATAGTCAAGTTCGACC-3'
28S	5'-CCCGTTCCCTTGGCTGTGGTTTCGCTAGATA-3'

Legends of the Supplemental Figures

Supplemental Figure S1: Endo- and exonucleolytic cleavages during pre-ribosomal RNA processing in human cells

This schematic representation summarizes the endo- and exonucleolytic steps undertaken in human cells to release ribosomal RNA sequences, as reported in the literature and described in the present study. Separation between pre-40S and pre-60S precursors occurs after endonucleolytic cleavage at site 2. Cleavage at site E releases the last 18S precursor, 18S-E pre-rRNA, corresponding to the sequence of 18S rRNA flanked in 3' by a portion of ITS1, 78 or 81 nt in length. This full-length precursor is referred to as 18S-E_{FL} pre-rRNAs in the present study. In the cytoplasm, 18S-E precursors are poly-uridylated by an uncharacterized TUTase and processed into shorter pre-rRNAs[1]. These precursors are then finally cleaved by the endonuclease NOB1, which releases the 18S rRNA 3' end [1,2]. Upon knockdown of PARN, 18S-E_{FL} precursors can be exported to the cytoplasm and further processed, although less efficiently. Concerning the large ribosomal subunit pathway, two 5.8S rRNAs varying by 5 nt in their 5' extremity are produced ($5.8S_L$ and $5.8S_S$), although it is not known whether the longest form is obtained through endonucleolytic cleavage as in *Saccharomyces cerevisiae*. So far, the 3'-5' exoRNase ERI1, acting on cytoplasmic 6S precursors, has only been described in

[1] Preti, M., O'Donohue, M.F., Montel-Lehry, N., Bortolin-Cavaille, M.L., Choesmel, V. and Gleizes, P.E. (2013) Gradual processing of the ITS1 from the nucleolus to the cytoplasm during synthesis of the human 18S rRNA. *Nucleic acids research*, **41**, 4709-4723

[2] Sloan, K.E., Mattijssen, S., Lebaron, S., Tollervey, D., Pruijn, G.J. and Watkins, N.J. (2013) Both endonucleolytic and exonucleolytic cleavage mediate ITS1 removal during human ribosomal RNA processing. *The Journal of cell biology*, **200**, 577-588.

mouse cells [3]. Several exoRNases, such as XRN2 or RRP6, are also involved in the degradation of transcribed spacers released after endonucleolytic cleavages.

Supplemental Figure S2: Assessment of the functionality of HASt-tagged PARN and PARN H377A

(A) Nuclear localization of PARN assessed with an anti-PARN antibody. PARN labeling is mainly seen in nucleoli (Nol), but a weaker signal is also seen in the nucleoplasm (Np). White arrowheads indicate the putative positions of a few fibrillar centers, which correspond to unlabeled areas. Scale bar, 5 μm. (B) IF analysis of PARN after depletion of the export factor CRM1 (positive control) and 60S RBFs (LSG1, eIF6). Scale bar, 20 μm. (C) Western blot analysis shows the efficiency of the knockdowns shown in Fig. 1F. (D) IF using an anti-HA antibody revealed that the intracellular localization of HASt-tagged PARN (w.t.) is similar to that of endogenous PARN displayed in (A). Similar results were obtained when HASt-tagged PARN was silently mutated to be insensitive to the action of the siRNA PARN-1 (siMut), or further mutated to provide a catalytically-dead form of the enzyme (HASt-PARN-H377A). (E) Purification of pre-40S particles with HASt-tagged PARN (w.t.) or HASt-tagged PARN-H377A led to similar profiles after silver staining.

Supplemental Figure S3: Western blot analysis of HeLa cells expressing HASt-tagged version of **PARN and PARN H377A**

As displayed on Figure 2E, HeLa cells treated with a scramble siRNA or siRNA PARN-1 were rescued with plasmids allowing expression of either an HASt-tagged version of PARN silently mutated to render it insensitive to siRNA PARN-1 (pPARN), or a catalytically inactive PARN mutant (pPARN-H377A). The empty plasmid (pHASt) was used as a control. Total protein samples were analyzed by Western blot and revealed with antibodies directed against PARN, the HA tag, or β-actin.

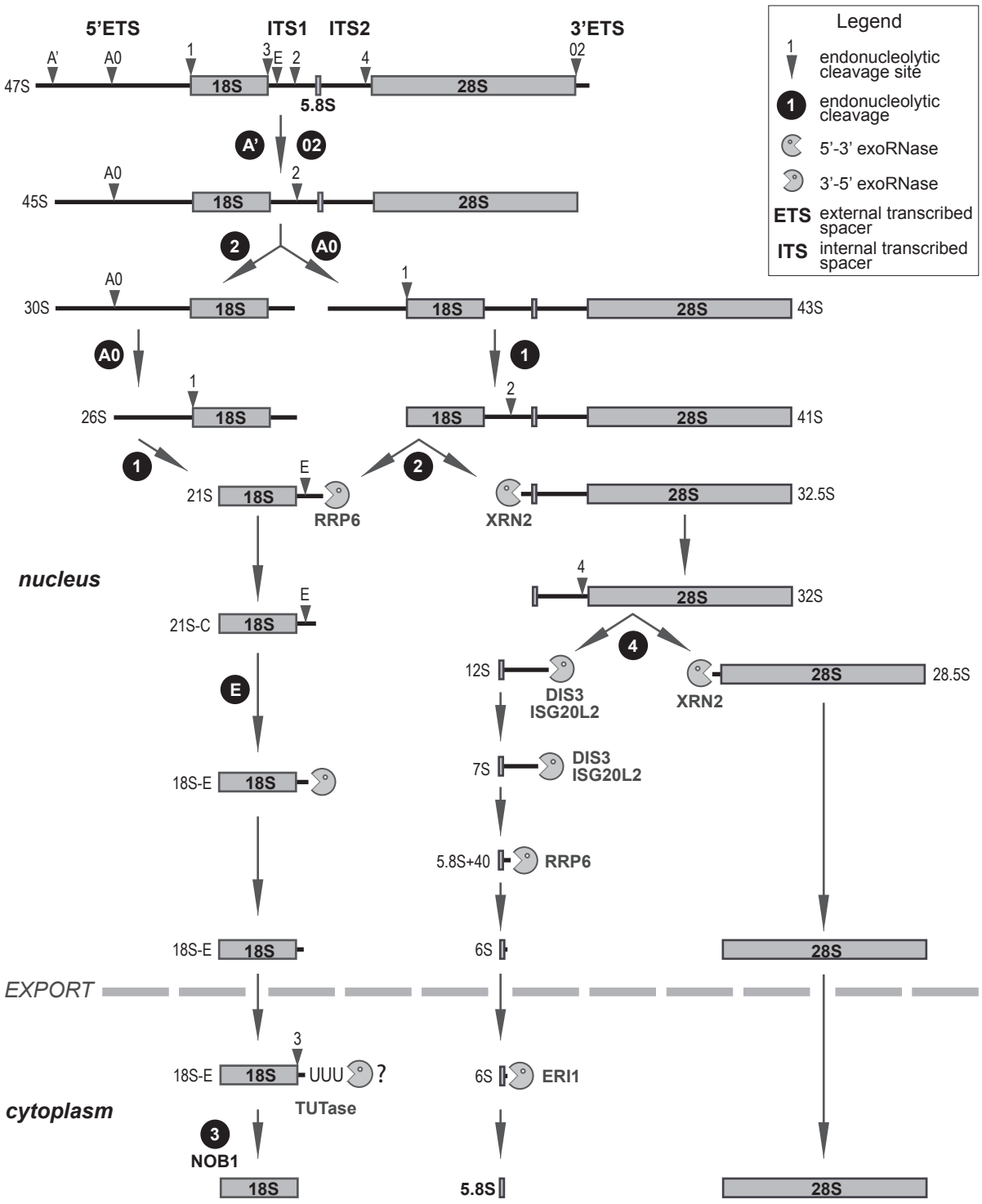
Supplemental Figure S4: Analysis of neo-synthesized RNAs in siRNA-depleted HeLa cells

In a parallel experiment to that presented on Fig. 2F, HeLa cells depleted of NOB1, or co-depleted of NOB1 and PARN, were pulse labeled with L-methyl ^{3}H methionine. Cells were harvested and total RNAs were analyzed as described in the corresponding legend. The gel on the left is identical to that displayed on Fig. 2F.

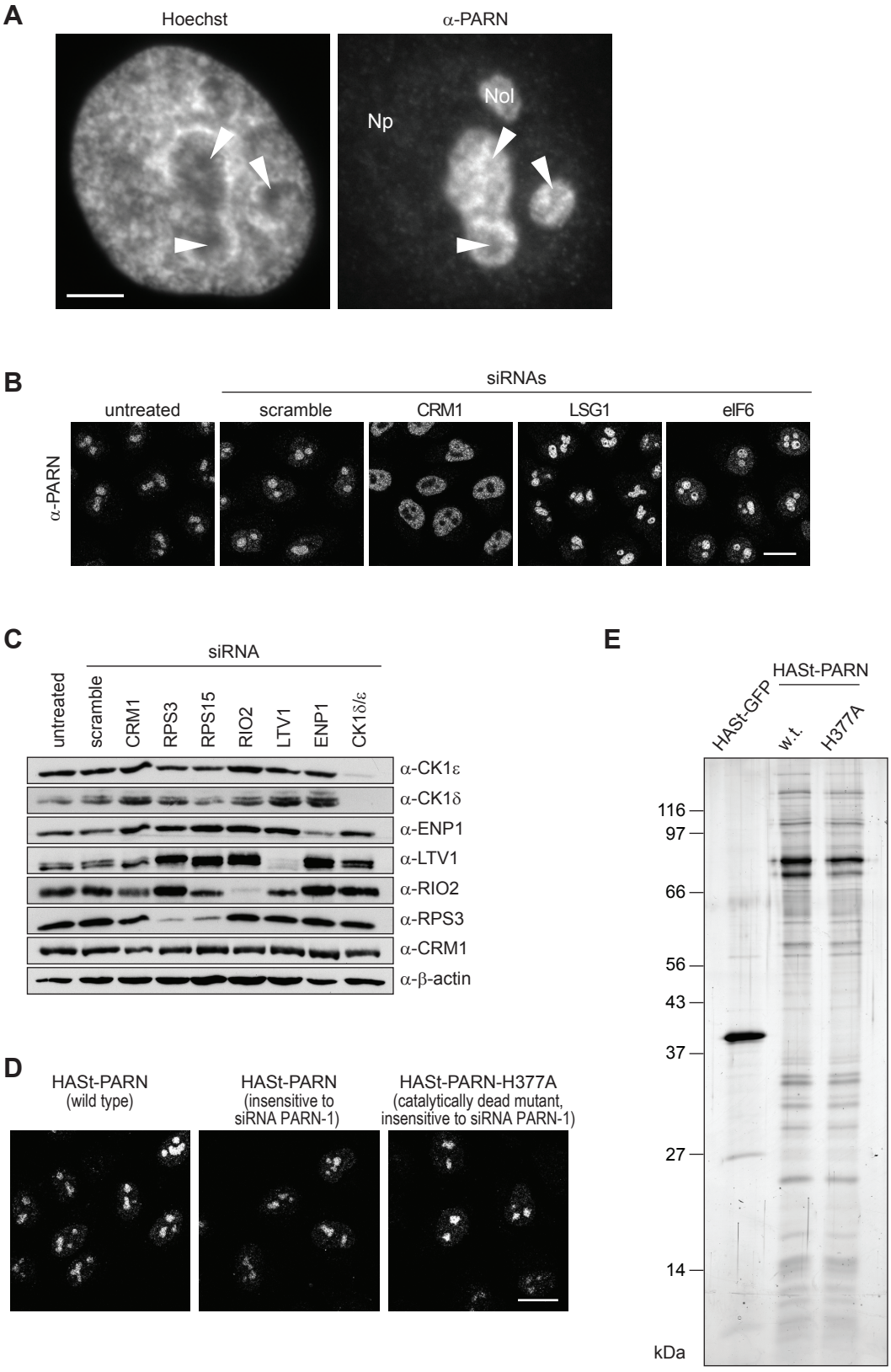
Supplemental Figure S5: Impact of PARN depletion on quality control

(A) Northern blot analyses of PARN co-depletion with RPSs that provoke an increase of 18S-E pre-rRNAs (see Fig. 4D for quantifications). (B) Northern blot analysis of RPS24, RPS23 or RPS11 depletions, which led to a strong accumulation of 30S pre-rRNAs, or of co-depletion of these RPSs with PARN. The graph presents the ratios of 30S pre-rRNA relative to 28S rRNA for each experimental condition. (C) An accumulation of abortive transcripts was induced by a low dose of actinomycin D in control cells and cells depleted of RPS26 or PARN, or co-depleted for RPS26 and PARN. Total RNAs were isolated after the indicated times and analyzed by Northern hybridization with 5'ITS1, ITS1-59 and 5'ETS probes.

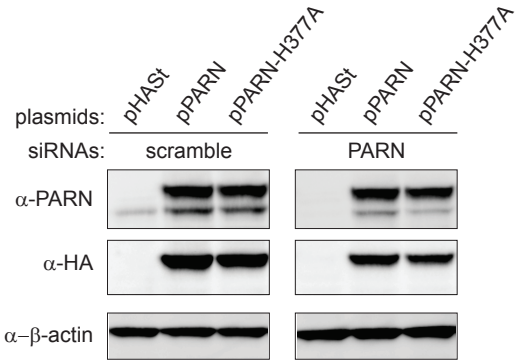
[3] Ansel, K.M., Pastor, W.A., Rath, N., Lapan, A.D., Glasmacher, E., Wolf, C., Smith, L.C., Papadopoulou, N., Lamperti, E.D., Tahiliani, M. *et al.* (2008) Mouse Eri1 interacts with the ribosome and catalyzes 5.8S rRNA processing. *Nature structural & molecular biology*, **15**, 523-530.



Supplemental Figure S1



Supplemental Figure S2



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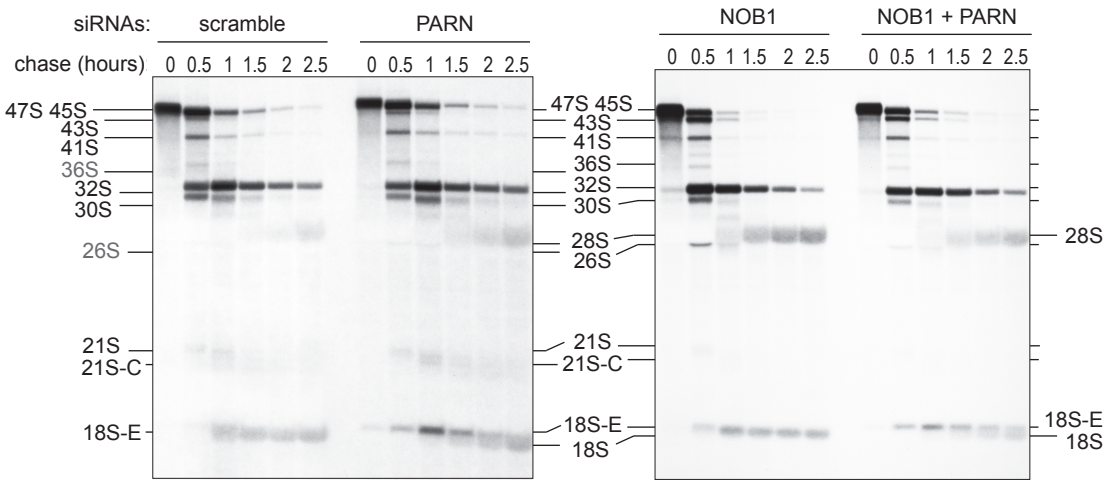
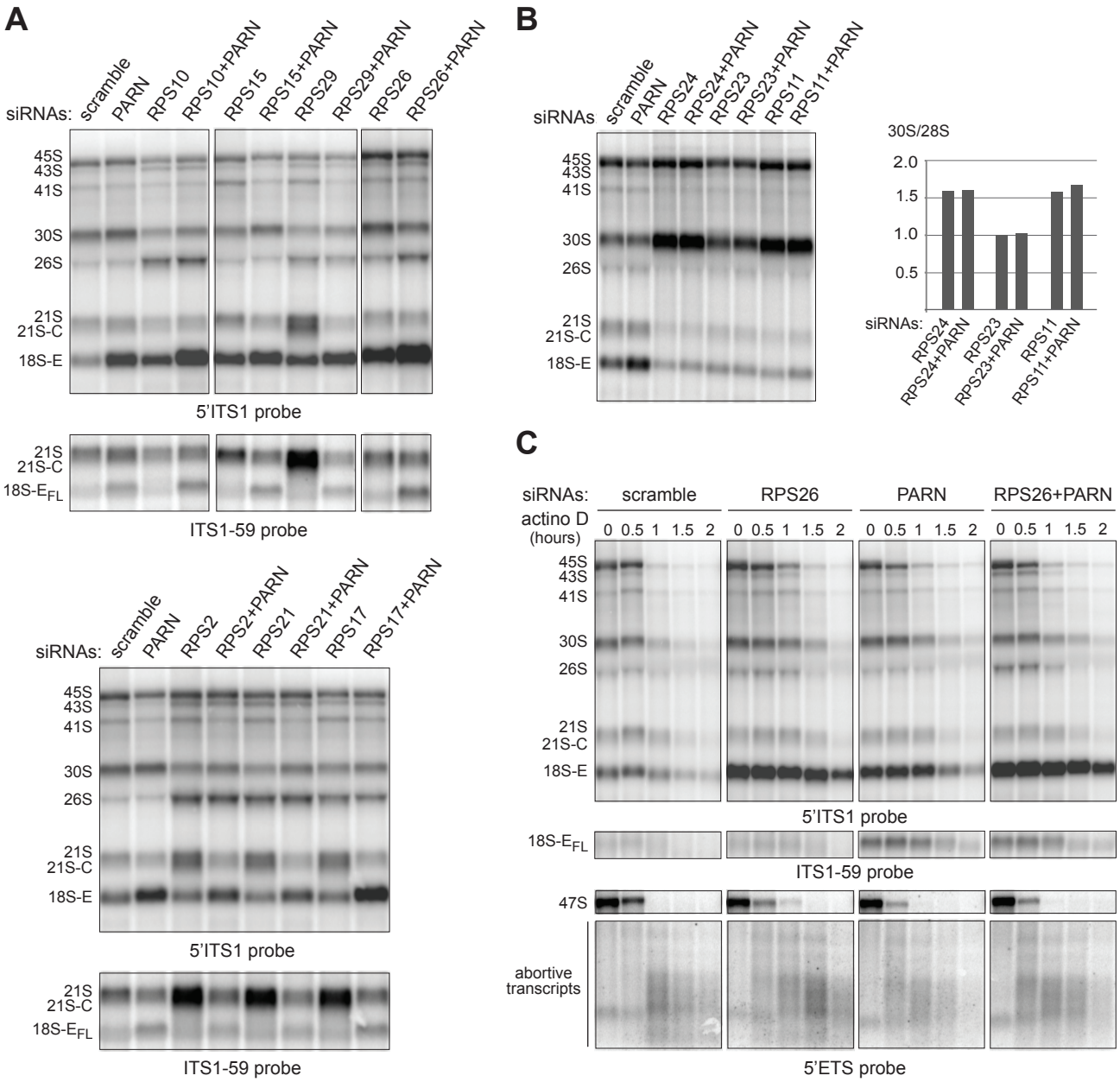


Supplemental Figure S4



Supplemental Figure S5