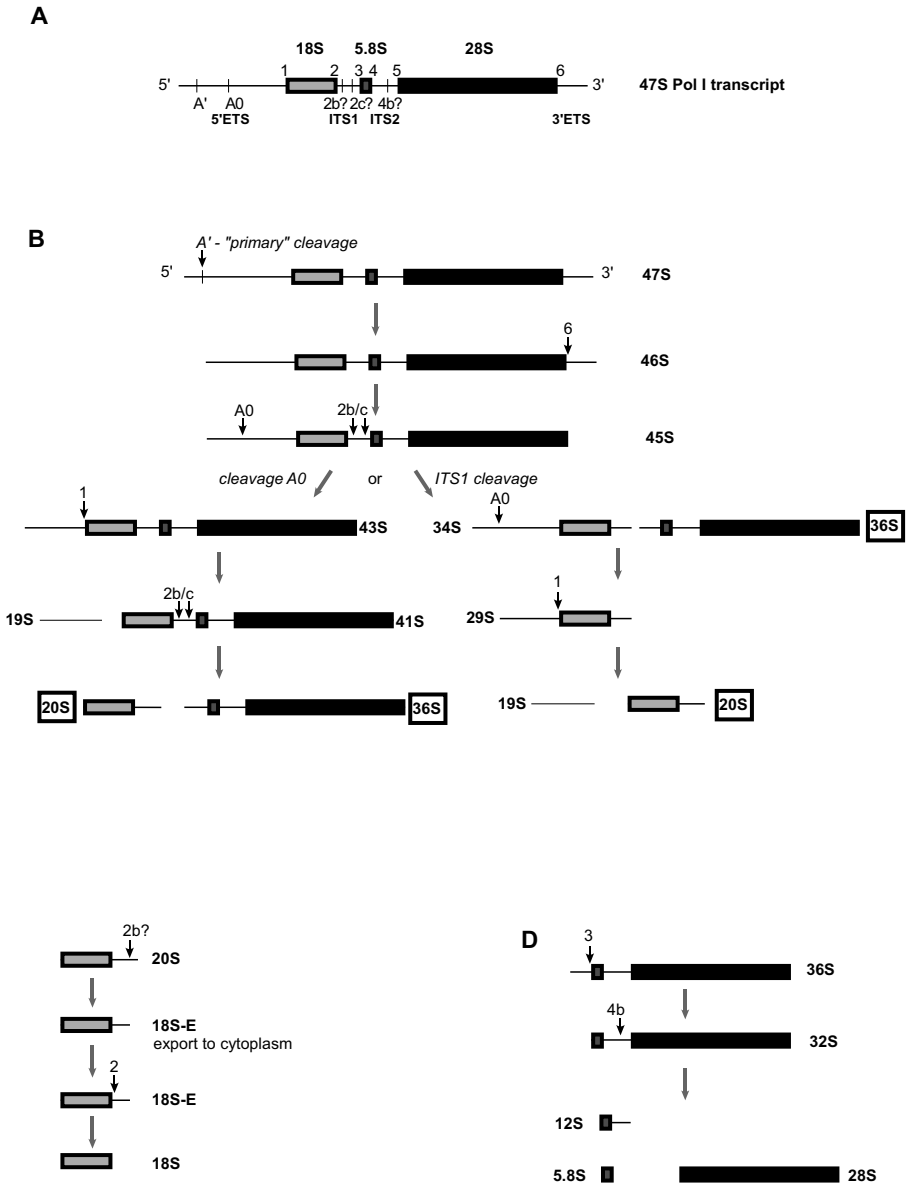
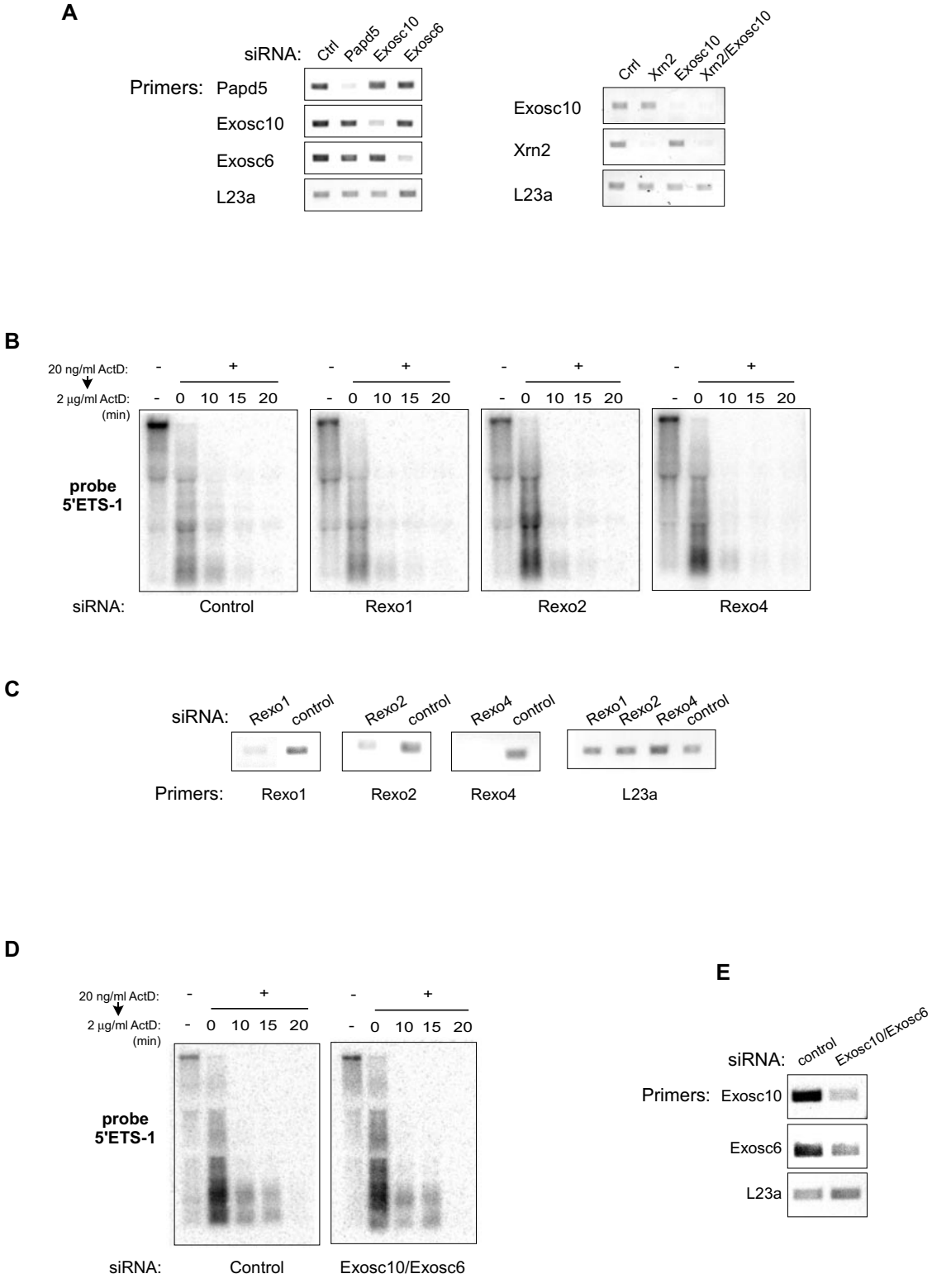
Supplementary Figure 1

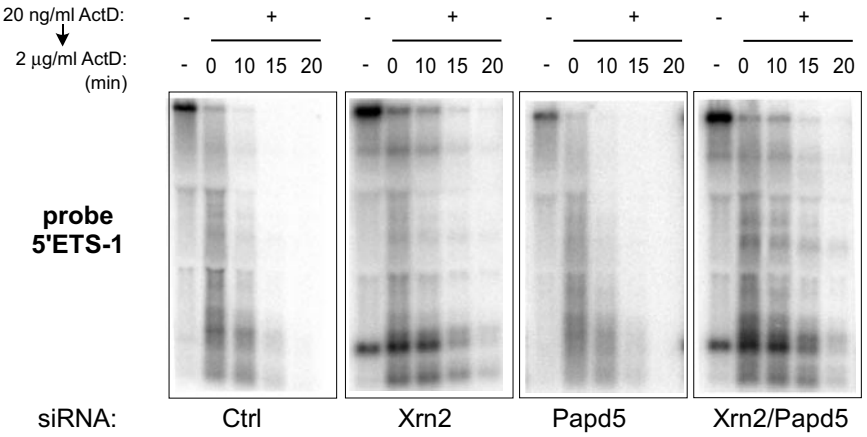


Supplementary Figure 2



Supplementary Figure 3

A



B

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. Outline of pre-rRNA processing in mouse cells.

(A) Structure of the mouse RNA polymerase I transcript (47S pre-rRNA) showing major processing sites. The cleavage sites that were proposed to exist but whose location was not previously mapped are labeled with a question mark. (B) Early events in pre-rRNA processing. 47S pre-rRNA is first cleaved at the primary cleavage site A' to yield 46S, from which the 3'ETS is removed by cleavage at site 6. Both cleavages are normally rapid, resulting in the abundant 45S pre-rRNA. Cleavages in the 5'ETS and ITS1 occur next in a varying order, yielding the 20S and 36S pre-rRNAs. There are at least two cleavage sites in the ITS1, 2b and 2c, but these sites were not precisely mapped or described as separate in many previous studies. (C) Generation of mature 18S rRNA in the pre-40S ribosomal subunit by processing of the 20S pre-rRNA. (D) Generation of 5.8S and 28S rRNAs in the pre-60S subunit by processing of 36S and 32S pre-rRNAs. The 32S is a highly abundant pre-rRNA that is slowly cleaved in the ITS2 to yield the mature 5.8S and 28S rRNAs.

Figure S2. Decay of ActD-induced short Pol I transcripts is unaffected by knockdowns of 3' exonucleases or a simultaneous knockdown of Exosc6 and Exosc10.

(A) Verification of the siRNA-mediated knockdown efficiency by RT-PCR. Ribosomal protein L23a mRNA was used as a control. (B) Northern hybridization with a 5'ETS probe to evaluate decay rates of pre-rRNA transcripts in cells transfected with siRNAs against several putative mouse 3'-5' exonucleases: Rexo1 (locus id, NM_025852), Rexo2 (NM_024233), Rexo4 (NM_207234). The assay was performed as described for Fig. 1C and D, and equal amounts of total RNA were loaded in each lane. (C) Verification of the siRNA knockdown efficiency by RT-PCR. (D, E) The same assays

performed with a double knockdown of Exosc10 (mouse homolog of the yeast exonuclease Rrp6) and Exosc6 (mouse homolog of the core exosome subunit Mtr3).

Figure S3. Synergistic effect of the Xrn2 and Papd5 knockdowns on the decay of ActD-induced short Pol I transcripts.

(A) The hybridization assay as described for Fig. 1 C and D was performed with a double knockdown of Xrn2 and the mouse Trf4 homolog Papd5; equal amounts of total RNA were loaded in each lane. (B) Verification of siRNA knockdown efficiency by RT-PCR. Ribosomal protein L23a mRNA was used as a control.

SUPPLEMENTARY TABLE 1

Oligonucleotides used in pre-rRNA analysis

Name	Sequence (5' to 3')	Position*
5'ETS-1	AGAGAAAAGAGCGGAGGTTCGGGACTCCAA	346-375
5'ETS-3	AGCTCCCCACGGGAAAGCAATGAGTCTCTC	1294-1323
5'ETS-4	ACAATGACCACTGCTAGCCTCTTTCCCTT	2809-2837
5’ETS-5	ATCGGGAGAAACAAGCGAGATAGGAATGTCTTA	602-634
ITS1-1a	ACGCCGCCGCTCCTCCACAGTCTCCCGTT	5877-5905
ITS1-1c	TTCTCTCACCTCACTCCAGACACCTCGCTCCACA	5975-6008
ITS1-4	GTATCGGTATTTCGGGTGTGAGCGAACTCA	6848-6877
ITS2-3	GGTCAGAAAGGGGGGACACGCGCCCAGCCG	8094-8123
ITS2-4	TCGCATCGGCGCACGGACGC	7821-7840
ITS2-5	TCCCTCCCCGAACTCGCAACA	7853-7873
ITS2-pEx1	GGGGTGGAGGATCTTACTCA	7956-7975
pEx-5.8S-19	GATCCACCGCTAAGAGTCGT	6876-6895
3'ETS-1	AGAGCGACGGAAGGGGAAAGAGAAACGAAC	12993-13022

* Site of oligonucleotide complementarity to the 47S pre-rRNA sequence (Genbank entry X82564).