Supplementary Material to:

Research Paper

Human RioK3 is a novel component of cytoplasmic pre-40S pre-ribosomal particles

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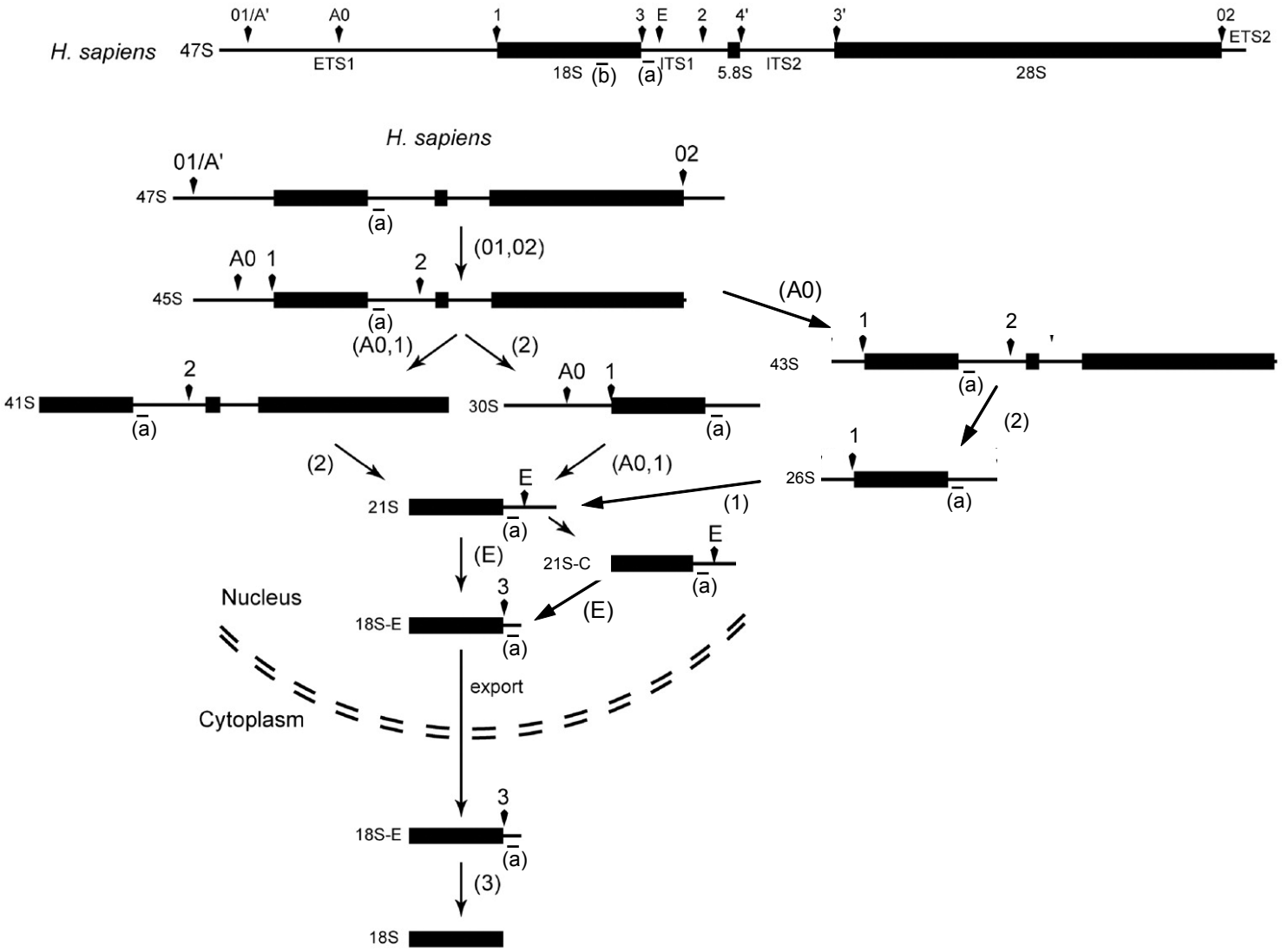
FIGURE S1. Scheme of the pre-rRNA processing pathway in HeLa cells. The position of the oligonucleotide probes (probe (a) hybridizing with the ITS1 and probe (b) hybridizing with 18S rRNA) used to detect the pre-rRNAs and rRNAs analysed in this study is shown on the precursors. Their sequences are given in Table S1.

FIGURE S2. Immunoprecipitation of pre-40S particles under conditions where RioK3 level is increased. The IP experiment was performed with aliquots of cytoplasmic extracts prepared from transiently transfected cells (by scramble or RpL11 siRNAs) corresponding to about 500 µg protein, instead of 40S fractions as described in the Materials and Methods section. (*A*) Western-blot analysis of the proteins precipitated with anti-hLtv1 serum (IP-hLtv1) or non-immune serum (IP N-immun) from cytoplasmic extracts prepared with cells treated with Rpl11 siRNAs (siRpl11) or scramble siRNAs (si scramble). Total proteins were extracted from the beads pellets (IP) or from $1/10^{th}$ of the initial input extract used for immunoprecipitation (Input) and analyzed by Western blot with antibodies against hLtv1, RioK3, RioK2 and actin. (*B*) Quantification of the IP yield. Protein amounts in both IP samples (from scramble siRNA and siRpl11-treated cell extracts) and in inputs of the siRpl11-treated cell extract are expressed as % of the protein amounts measured in the scramble siRNA-treated cell inputs. (*C*) Northern blot analysis of the co-precipitated RNAs from the siRpl11-treated cell extract. RNAs loaded were extracted from the bead pellet (IP) obtained following immunoprecipitation performed with 40 µl of extract or directly from the same amount of input extract (Input). 18S and

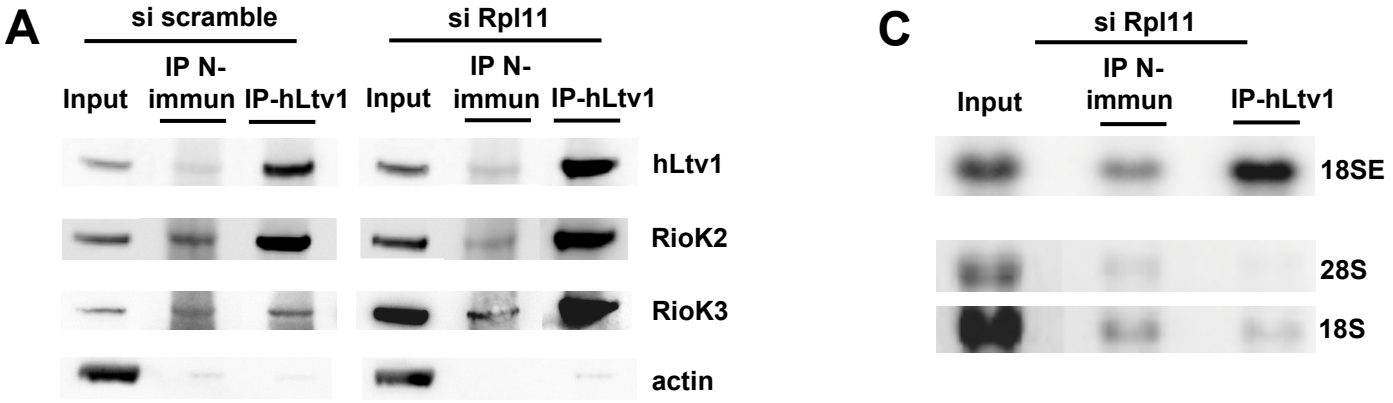
28S rRNAs were detected by ethidium bromide staining and 18S-E pre-rRNA by the Northern procedure using the 5'-ITS1 probe.

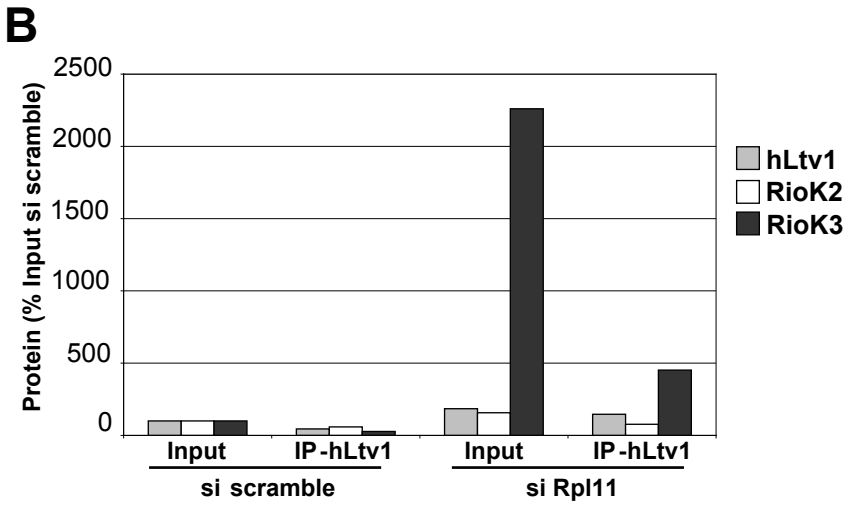
FIGURE S3. RioK3 mRNA levels are not increased relative to RioK2 and hLtv1 mRNA levels when LSU biogenesis is impaired. The levels of RioK3, RioK2, hLtv1 and Gapdh mRNAs were investigated by semi-quantitative RT-PCR using different dilutions of total RNAs extracted from HeLa cells 48 h after transfection with scramble (scramble siRNA) or siRpl11 siRNAs (Rpl11 siRNA). Ethidium bromide staining of PCR fragments produced was quantified and values obtained for RioK3, RioK2, hLtv1 fragments were normalized to those obtained for Gapdh. The enrichment of RioK3, RioK2, hLtv1 mRNAs in siRpl11-treated cells relative to scramble siRNA-treated cells was determined (indicated below lane "dilution 1, Rpl11 siRNA").

Supplemental Fig. 1



Supplemental Fig. 2





Supplemental Fig. 3