

## SUPPLEMENTARY INFORMATION

**Accession numbers.** Homo sapiens rDNA promoter (X01547), Mus musculus rDNA repeat unit (BK000964), Rattus norvegicus rDNA promoter (X00677), Sus scrofa rDNA promoter (L31782).

**RNase footprinting.** pRNA was transcribed with T7 RNA polymerase, labeled at the 5'-end with T4 polynucleotide kinase, and purified by electrophoresis on 8% polyacrylamide/7M urea gels. Prior to binding to TIP5, 1 fmol pRNA was renatured in binding buffer at 55°C for 20 min, slowly cooled to 20°C and incubated with 20-500 nM TIP5<sub>510-611</sub> for 10 min on ice. After addition of 0.1 µg *E. coli* tRNA and either RNase V1 (0.0003 units) or RNase T1 (0.05 units), incubation was continued for 10 min at 20°C. RNA was recovered by ethanol precipitation and analyzed by electrophoresis on 10% polyacrylamide/7 M urea gels along with partial alkaline hydrolysis or RNase T1 cleavage products as size markers.

## LEGENDS TO SUPPLEMENTARY FIGURES

**Figure S1.** RNase footprinting of TIP5/pRNA complexes.

**(A)** Electrophoretic analysis of partial RNase T1 cleavage of pRNA. 1 fmol of radiolabeled pRNA (-143/-39) was incubated with increasing amounts (20-500 nM) of purified hTIP5<sub>510-611</sub> and subjected to partial digestion with 0.05 u RNase T1 (lanes 1-5). RNA incubated with buffer only was used as control (C). A partial alkaline hydrolysis ladder (OH) and RNase T1 ladder (T1) were run alongside as size markers. The positions of guanosines are indicated.

**(B)** Same as in (A), except that the incubation mixture was digested with 0.0003 u RNase V1.

**Figure S2.** Depletion of pRNA from NIH3T3 cells.

Knockdown of pRNA by antisense LNA-DNA gapmers. NIH3T3 cells were transfected with control RNA (light bar, m) 50 nM LNA-DNA gapmers in antisense orientation (dark bar, as). The amount of pRNA levels was determined by RT-qPCR and normalized against GAPDH mRNA. Data are from two independent experiments. Error bar indicates +/-SD.



