Supplementary Figures

Supplementary Figure S1

Depletion of TIP5 upregulates 45S pre-rRNA transcription levels. (**A**) Western blot of nuclei extracts from shRNA-TIP-1 and -2 and shRNA control cells using anti-TIP5 and anti-UBF antibodies. (**B**) qRT-PCR of TIP5 mRNA from shRNA-TIP5 and control cells. Values were normalized to GAPDH mRNA levels. Depletion of TIP5 enhances rDNA transcription. qRT-PCR. 45S pre-rRNA levels in stable shRNA-TIP5 cells (**C**) and in NIH3T3 10 days after infection with a retrovirus expressing miRNA sequences directed against TIP5 (**D**). Values were normalized to GAPDH mRNA levels and to control cells. Error bars are defined as the s.d. of three independent experiments.

Supplementary Figure S2

(**A**) Western blot showing similar levels of CENP-A protein in both shRNA-control and shRNA-TIP5 cells. To normalize protein loading, the levels of tubulin were monitored using an anti-tubulin antibody. (**B**) Cellular localization of centromers in NIH3T3 cells. Indirect immunofluorescence analysis of NIH3T3 cells with anti-UBF and anti-CENP-A antibodies. The merge panel shows co-localization of some centromers with the perinucleolar periphery.

Supplementary Figure S3

Profile of S-phase progression of shRNA-TIP5 and control cells. FACS analysis. Cells were maintained at confluence in DMEM/10% FCS for 2 days before reseeding ($2 \cdot 10^6$ cells in a 10 cm diameter dish) and culturing for 18 h in medium containing 1 μg/ml aphidicolin (Sigma) to arrest cells at G1/S phase boundary. After release from the aphidicolin block, cells were collected at the indicated times for FACS analysis.

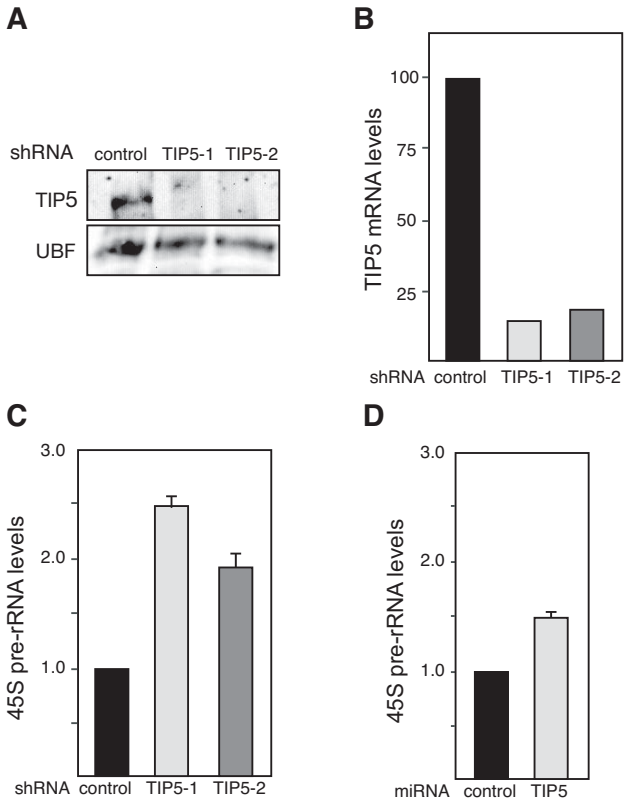
Supplementary Figure S4

rDNA polymorphism at +42/+43 marks rDNA variants. (**A**) Schema representing rDNA polymorfisms at +42/+43 (A, T and G sequences). Arrows indicate PCR primers used to amplify rDNA sequences from -165 to +83 (total rDNA) and from -165 to +64 (A, T and

G-sequences). (**B**) rDNA variant sequences. rDNA sequences were amplified from -1 to +155 from NIH3T3 genomic DNA. PCR product was directly sequenced and the region from +32 to +50 is shown. Sequences of plasmids containing rDNA-A and -T are shown. (**C**) Establishment of a polymorphic-specific qPCR. Specificity of the primers was assayed by amplification of v-rDNA plasmids. Values were normalized to the amounts amplified with total rDNA primer. (**D**) CpG methylation profile of v-rDNA variants from mouse liver (L), brain (B) and heart (H). (**E**) Depletion of TIP5 upregulates rDNA transcription at all v-rDNA genes. qRT-PCR from rRNA synthesized by v-rDNA in NIH3T3 cells 10 days after infection with a retrovirus expressing miRNA-TIP5. Data were normalized to GAPDH mRNA levels and to control cells.

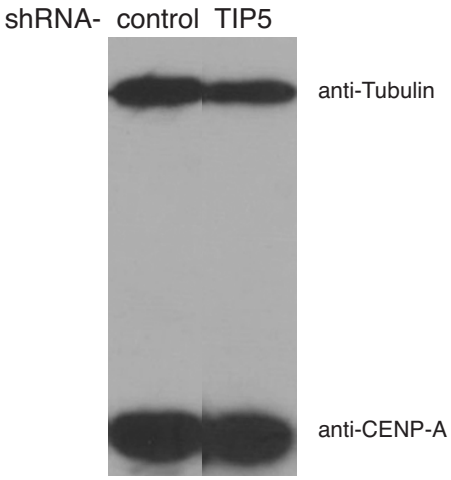
Supplementary Figure S5

(**A**) BrdU incorporation assay. Cells were incubated with 10 μM BrdU for 30 min, stained with antibodies against BrdU, and percentage of cells in S phase of two independent experiments was estimated. (**B**) Western blot of cellular lysates from shRNA-control and shRNA-TIP5 cells using antibodies against Cyclin A. Anti-actin antibodies were used to ensure that equal amounts of proteins were analyzed.



Supplementary Figure S1

A



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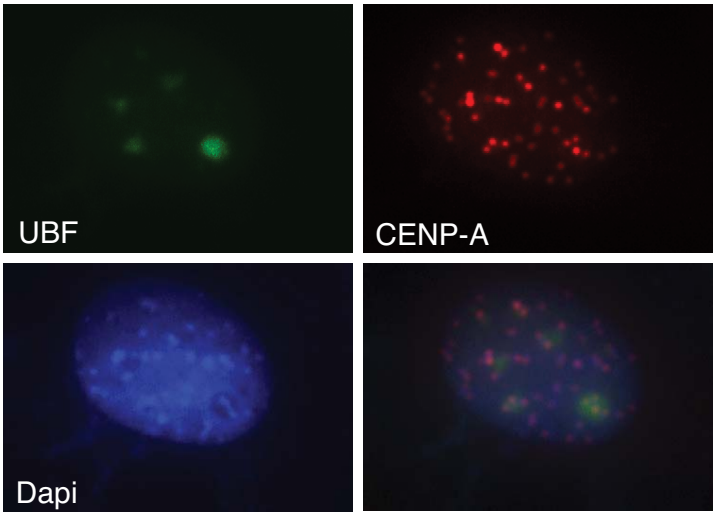
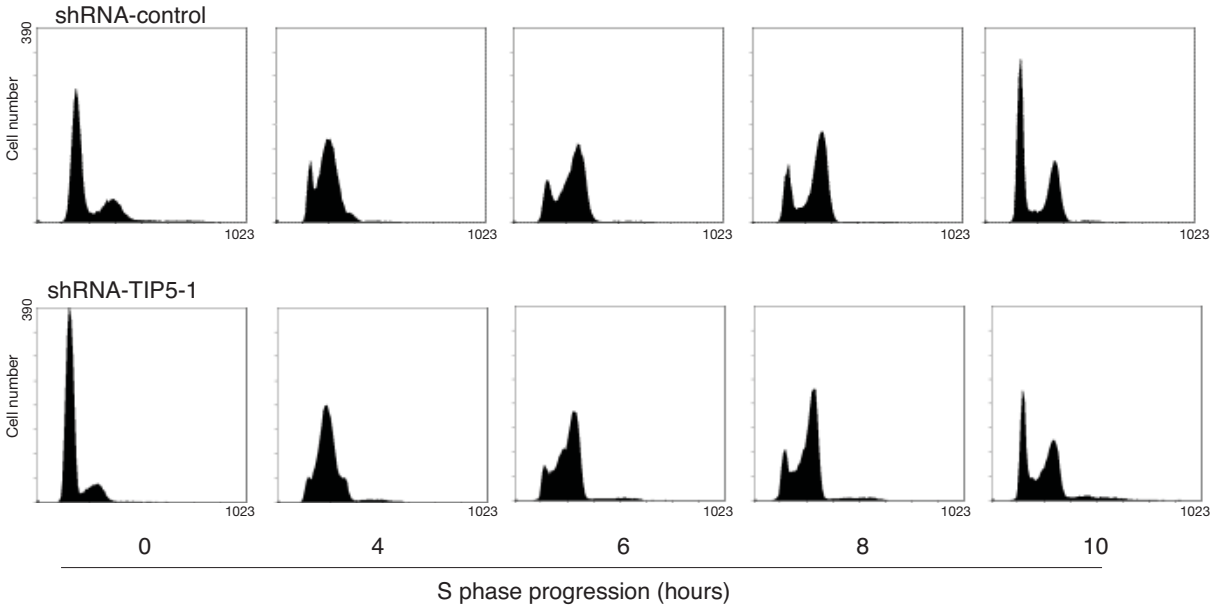
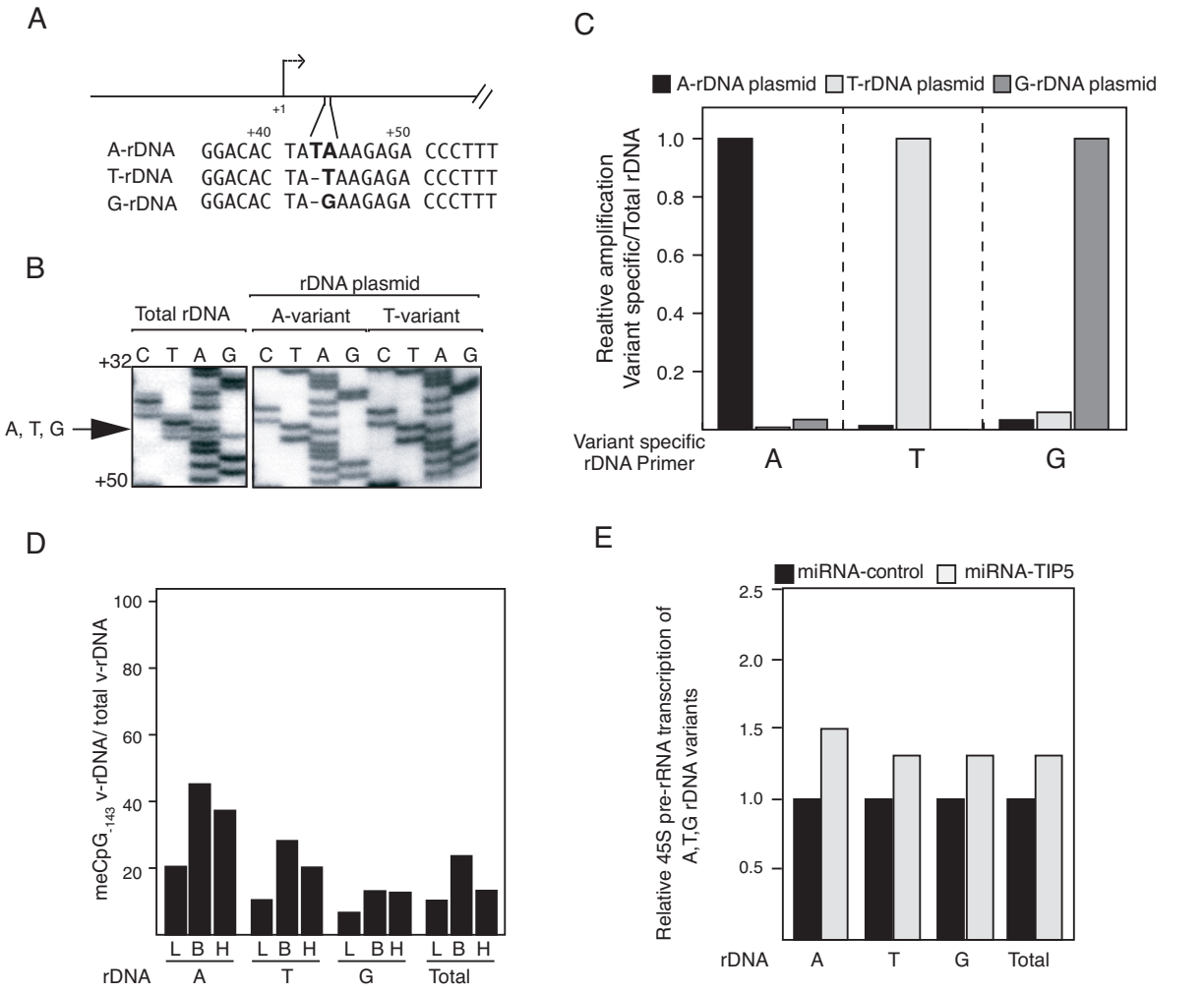


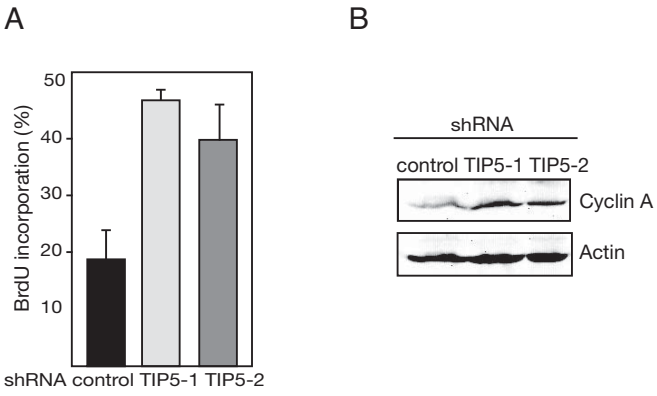
Supplementary Figure S2



Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure S5