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 (2·10^6 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 polymorphisms 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
(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.
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Supplementary Figure S2
Supplementary Figure S3
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