Supplementary Information

Mouse rDNA primers	Sequence
-2175/-2155 For	5'-TAGTGGTGACAAGTTTCGGGA
-1994/-1975 For	5'-GCAGACCGAGTTGCTGTAC
-1953/-1933 Rev	5'-CTAGTAACGTTGCCACCAAC
-554/-535 For	5'-GAAGCCCTCTTGTCCCCGTC
-466/447 Rev	5'-GATCCAAAGCTCCAGCTGAC
-232/-214 For	5'-GAAAGCTATGGGCCGCGGT
-160/-140 Rev	5'-CCGGACCTCAAAGGAACAAC
-20/-39 Rev	5'-AAGAACAGATAGAAAAGATCA
-21/-1 Rev	5'-ACCTATCTCCAGGTCCAATAG
+550/+570 For	5'-CTCTTGTTCTGTGTCTGCC
+745/+765 Rev	5'-GCCCGCTGGCAGAACGAGAAG
+2251/+2270 For	5'-GCATCGGTGTGTCGGCATCG
+2346/+2365 Rev	5'-CTGAGCAGTCCCACCACACC
mExoSC3(242-262) For	5′-GTCTGCTGGTCACCAAGTGTG
mExoSC3 (347-356) Rev	5′-GTGGTCCCCTTTCACAGGTAC

Table S1. Sequences of primers used in this study.

Supplementary Figure Legends

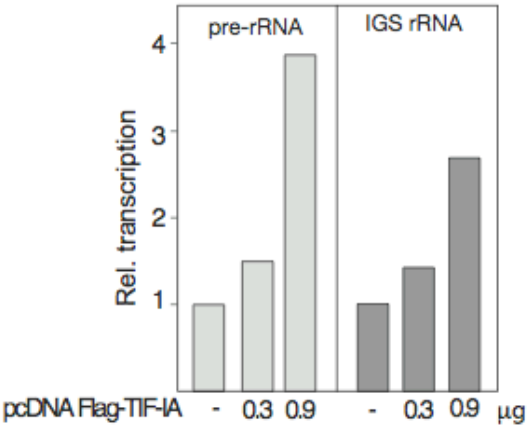
Fig. S1 Ectopic TIF-IA activates transcription from the pre-rRNA and the spacer promoter. NIH3T3 cells were transfected with pMr-1922/+130-BH (left panel, pre-rRNA) and pMr-2172/1933-BH (right panel, IGS rRNA) in the absence or presence of 0.3 and 0.9 μg of an expression vector encoding TIF-IA. Values were normalized to the levels of a co-transfected luciferase reporter gene and to GAPDH mRNA.

Fig. S2 The steady-state level of IGS rRNA does not change during the cell cycle. Levels of IGS rRNA and pre-rRNA in serum-starved NIH3T3 cells (G0), in cells arrested at at G1/S by treatment with aphidicolin (1 μg/ml, 18 h), and at G2/M by treatment with nocodazole (40 μg/ml, 16 h). Values were normalized to GAPDH and to RNA levels in asynchronous cells (As.).

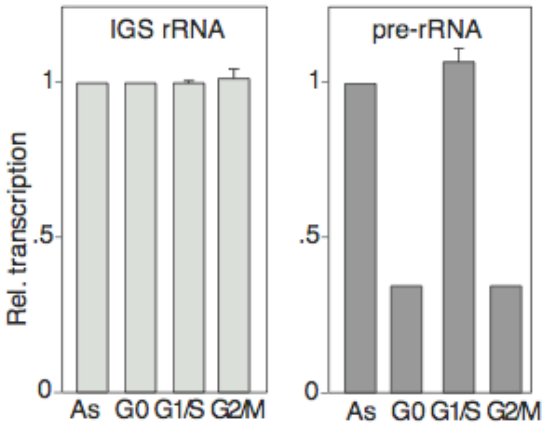
Fig. S3 Biphasic replication of rDNA. NIH3T3 cells synchronized at G1/S were released into S-phase and pulse-labeled with BrdU in 1h intervals. Nascent DNA was immunoprecipitated with anti-BrdU antibody. To control S-phase progression, a known early-replicating gene (α-

globin) and a late-replicating locus (X141) were amplified. Genomic DNA (g) from asynchronous NIH3T3 cells was used as control.

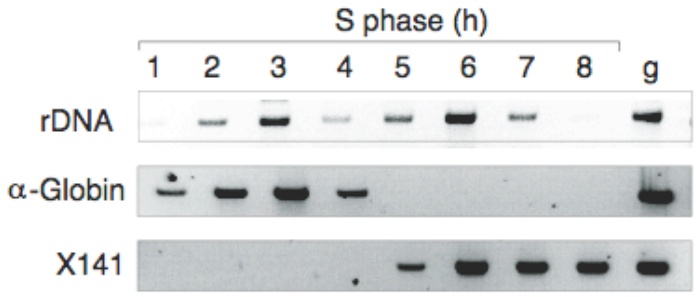
Fig. S4 IGS rRNA is degraded by the exosome. The levels of IGS rRNA, pRNA and pre-rRNA were monitored by RT-qPCR after siRNA mediated depletion of ExoSC3. The knockdown of ExoSC3 was monitored by RT-qPCR of ExoSC3 mRNA (ExoSC3). The bar diagram shows transcript levels normalized to GAPDH mRNA. Error bars indicate +/- SD.



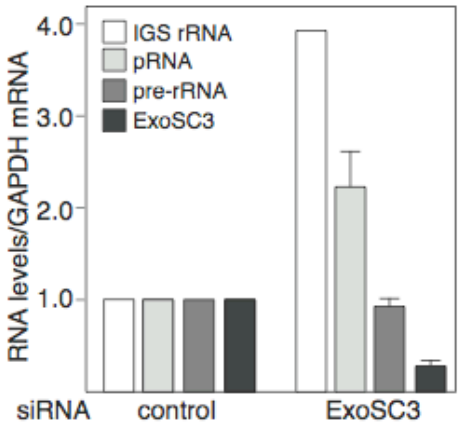
Santoro et al. Figure S1



Santoro et al. Figure S2



Santoro et al. Figure S3



Santoro et al. Figure S4