Supplemental information

Human MAF1 targets and represses active RNA polymerase III genes by preventing recruitment rather than inducing long-term transcriptional arrest

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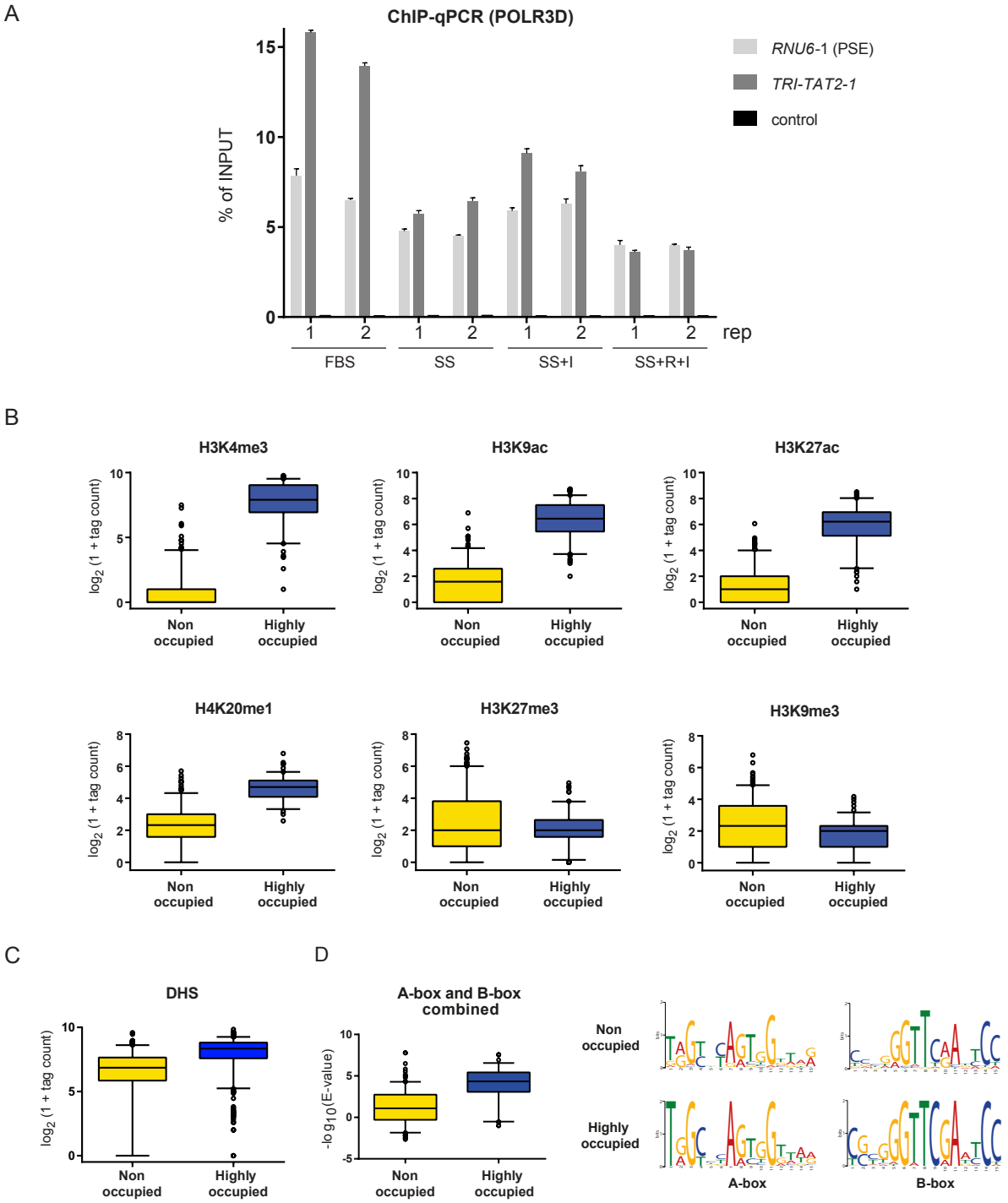
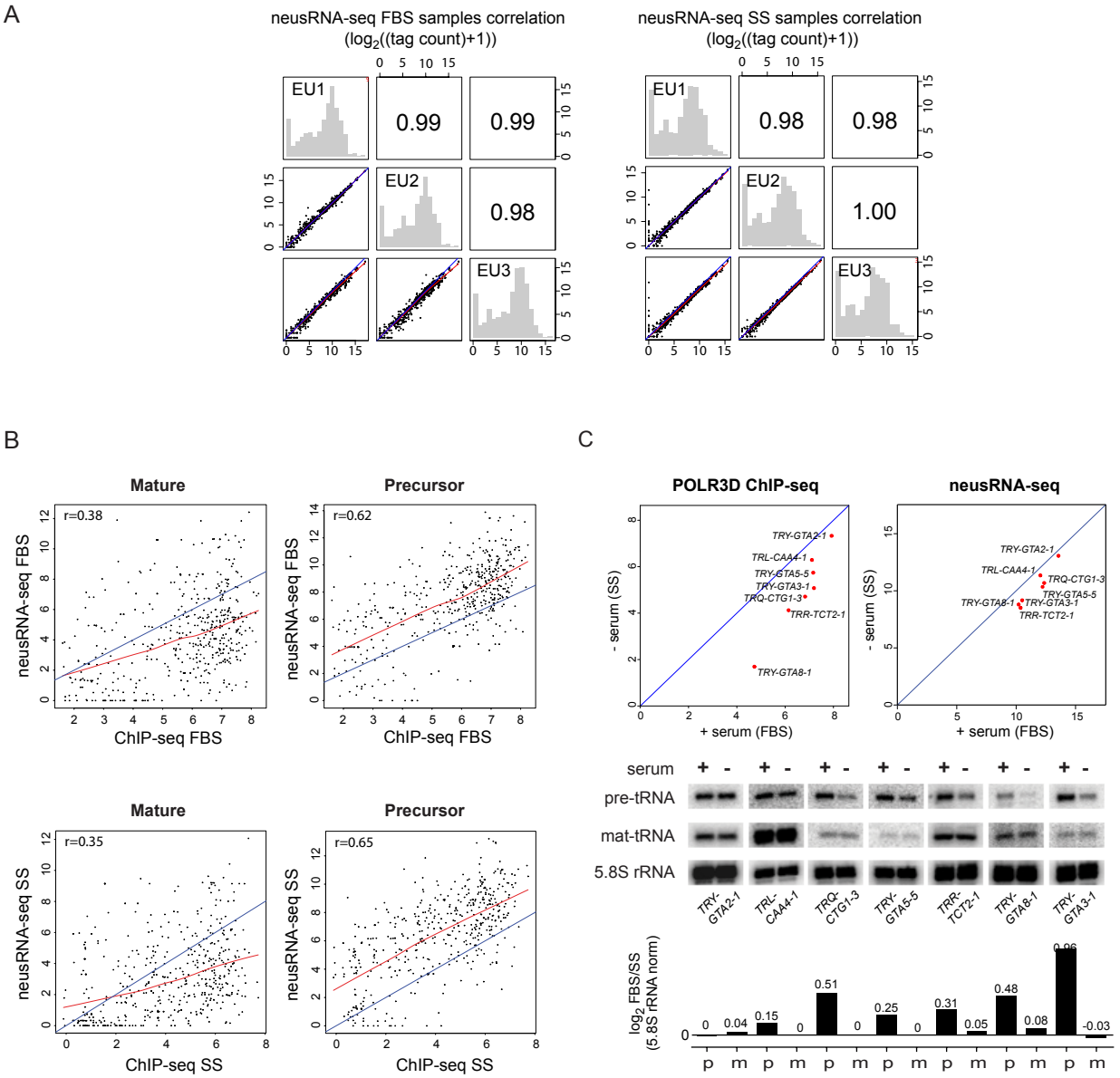
This document contains the following supplemental information:

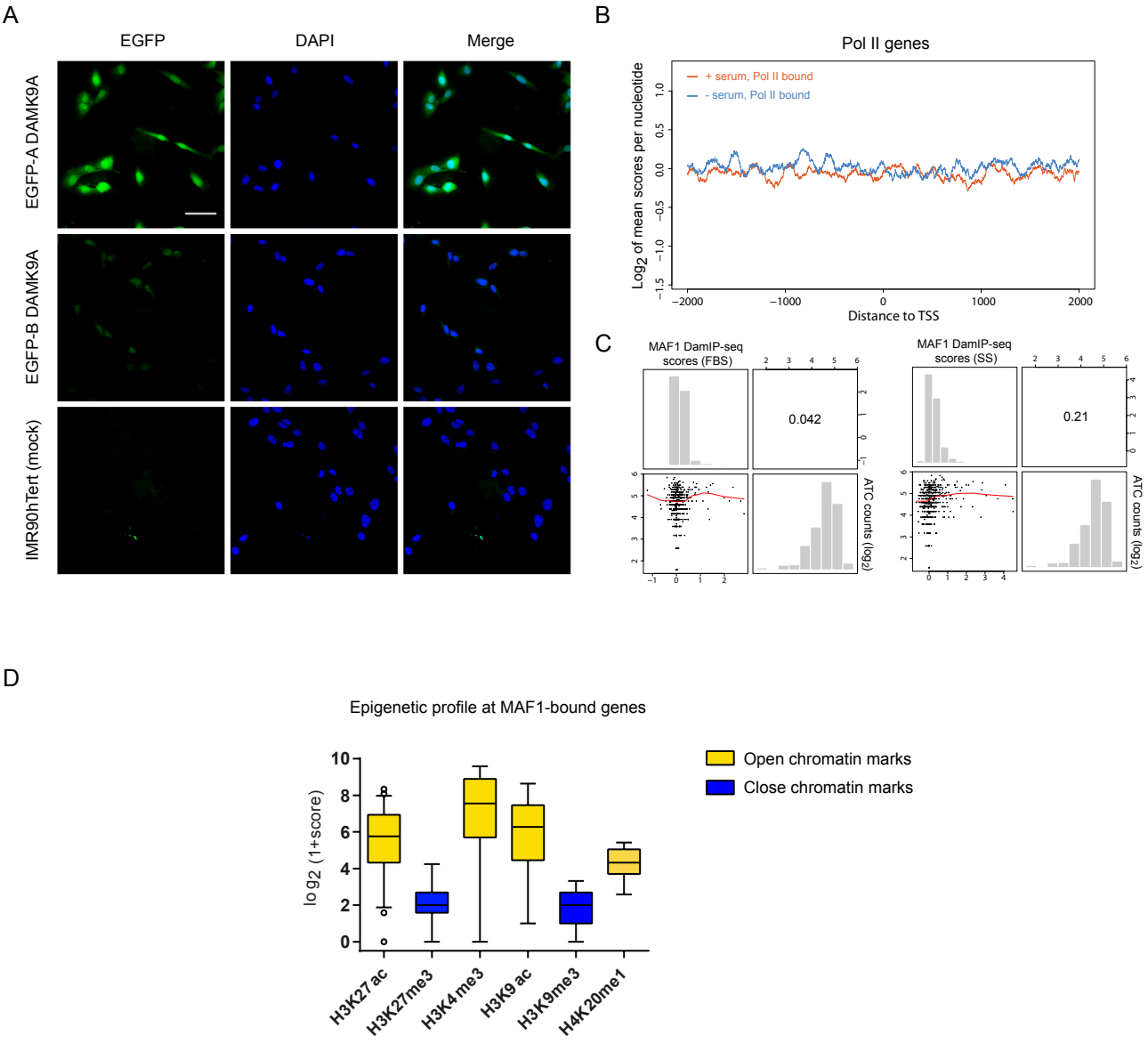
Supplemental Fig. S1



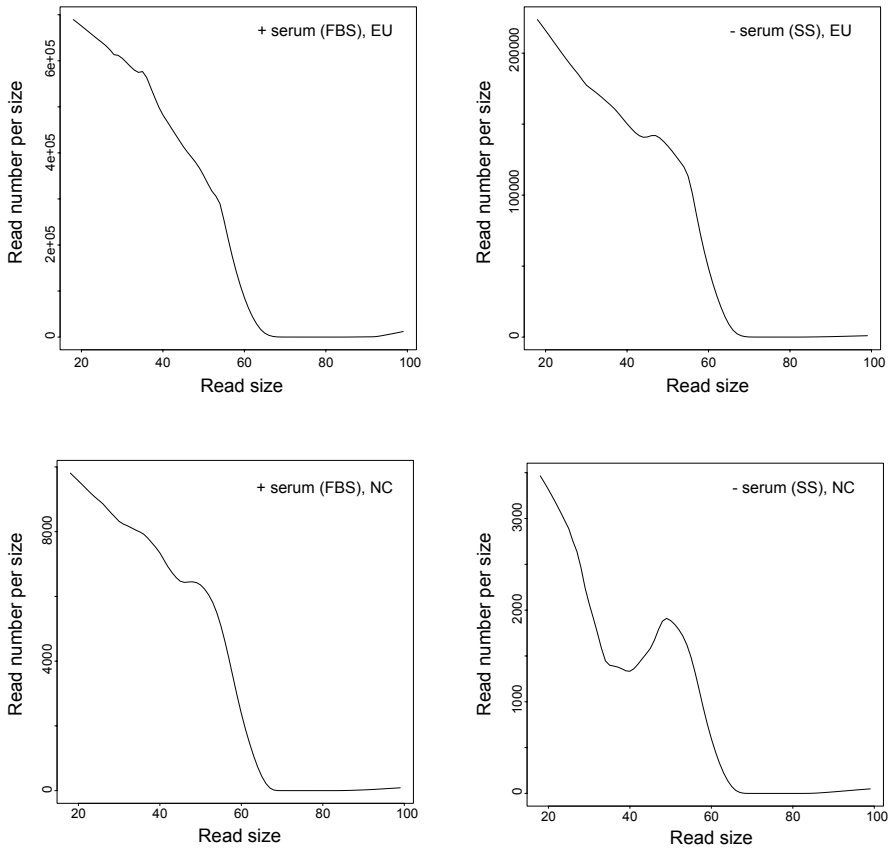
Supplemental Fig. S2



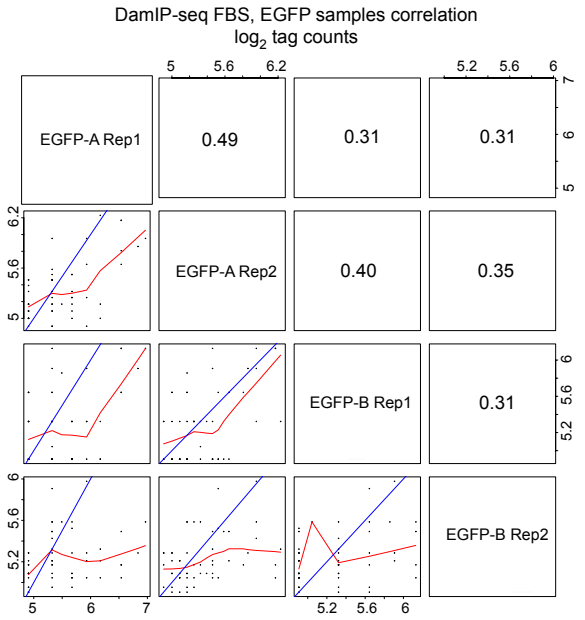
Supplemental Fig. S3

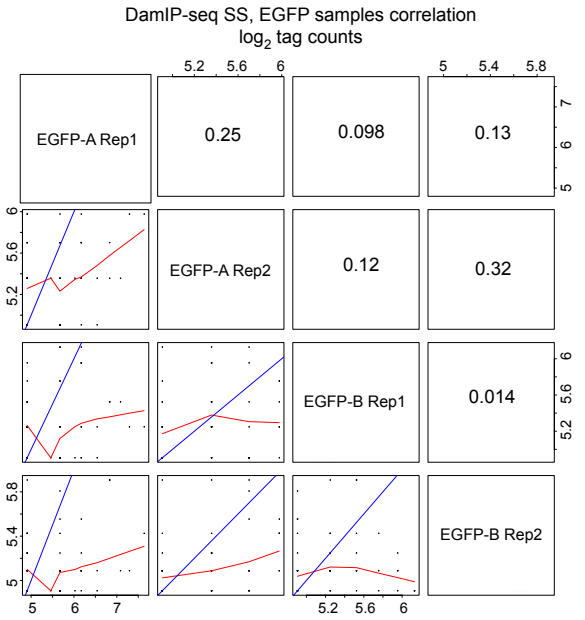


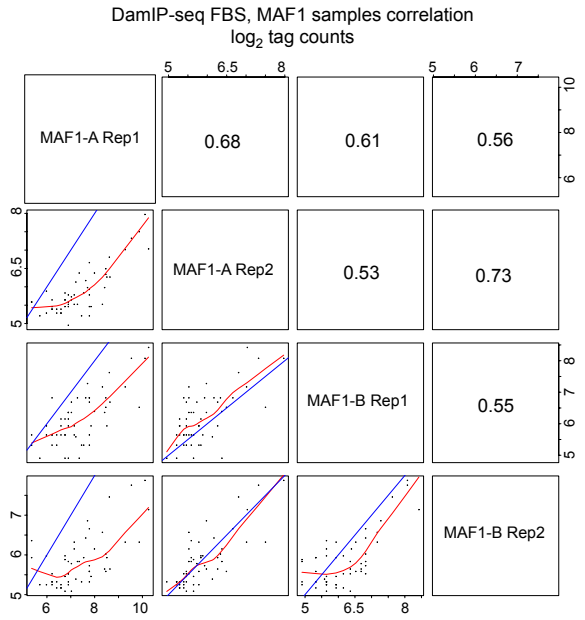
Supplemental Fig. S4

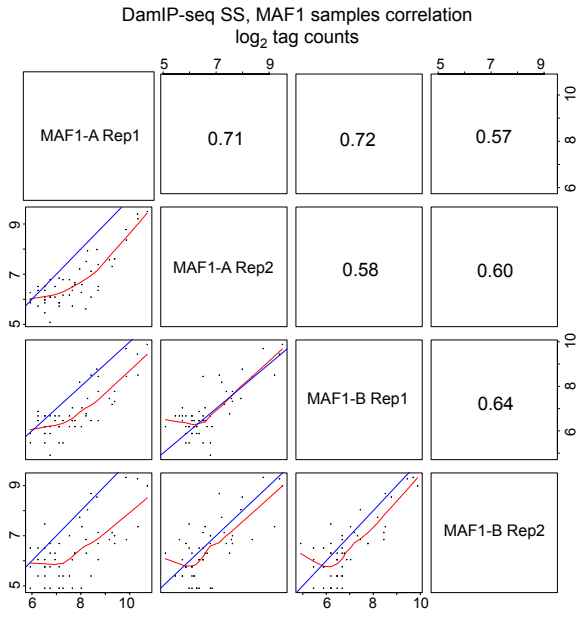


Supplemental Fig. S5









Supplemental Figure Legends (S1-S5)

Figure S1. (*A*) ChIP-qPCR validation of Pol III binding. IMR90hTert cells were grown in the presence (FBS) or absence (serum starved, SS) of fetal bovine serum for 8 hrs. 1 µM insulin was then added for 1h30' to starved cells alone (SS+I), or after 45' treatment with 2 nM Rapamycin (SS+R+I). ChIP assays from two replicates (rep 1 and 2) were performed with an antibody against the POLR3D (RPC4) subunit of Pol III and reveal binding to both type II (*TRI-TAT2-1*) and type III (*RNU6*) promoter genes. The control corresponds to an intergenic region 2 kb upstream of *RNU6* TSS. ChIP-qPCR data relative to input DNA are mean ± SD (n=3). Primers are indicated in Table S4. (*B-D*) Correlation of Pol III ChIP-seq scores with publicly available datasets and promoter conservation. Box-and-whisker plots (5-95 percentile) showing (*B*) Signal distribution for histone modification ChIP-seq experiments performed in IMR90 cells; H3K4me3 (GSM469970), H3K9ac (GSM469973), H3K27ac (GSM469966), H4K20me1 (GSM521915), H3K27me3 (GSM469968), H3K9me3 (GSM469974). (*C*) Distribution of DNaseI hypersensitive sites (DHS, GSM1008586) tag density in IMR90 cells. (*D*) Distribution of combined A-box and B-box scores (see Experimental procedures); only tRNA genes are considered. On the right panel is indicated the sequence logo of A-box and B-box promoter elements associated with non occupied or highly occupied tRNA genes. In yellow are the 226 non-occupied genes and in blue the 122 most highly occupied genes (upper quartile of Pol III-bound genes) in the Pol III ChIP-seq (FBS) experiment.

Figure S2. (*A*) Correlation between neusRNA-seq replicates in cells grown with (FBS) or without (SS) serum. Scores (Experimental procedures) for each Pol III gene ±150bp were calculated as the $\log_2$ (tag count+1) and indicated for EU labelled samples (EU1-3). The Pearson correlation coefficient (r) is given. (*B*) Scatterplot comparison of ChIP-seq and RNA-seq data. The ChIP-seq data are expressed as ChIP-seq scores (see legend of Fig. 2). neusRNA-seq scores were calculated with tags assigned uniquely to mature tRNAs or tags assigned uniquely to precursor tRNAs. Only

Pol III-occupied genes are shown. The Pearson correlation coefficient (r) is given. (*C*) RNA from IMR90hTert cells grown with or without serum for 8hrs was analyzed by Northern blotting with probes specific for pre-tRNAs or mature tRNAs. Intron-containing and 3'-extended (*TRQ-CTG1-3*) tRNAs were selected from correlation plots (FBS vs SS) of Pol III ChIP-seq (left panel) and neusRNA-seq (right panel) experiments. Representatives tRNAs of low changing (*TRY-GTA2-1* and *TRL-CAA4-1*) and high changing (*TRQ-CTG1-3*, *TRY-GTA5-5*, *TRY-GTA3-1*, *TRY-GTA8-1* and *TRR-TCT2-1*) ChIP-seq and neusRNA-seq scores were selected. p: pre-tRNA, m: mature tRNA. Expression was normalized to 5.8S rRNA.

Figure S3. (*A*) Laser scanning confocal microscopy (LSCM) maximum intensity projections of EGFP-DamK9A expressing cells and untransfected IMR90hTert cells (mock). Cells were fixed and nuclei stained with DAPI. Scale bar is 100 µM. (*B*) Metasample profiles depicting sequence coverage in a ±2 kb window surrounding the TSS of MAF1-enriched Pol II genes in +serum (FBS, orange) and -serum (SS, blue) experiments. MAF1-enriched Pol II genes are listed in Table S3. MAF1 DamIP-seq scores per nucleotide are normalized for total tag count and calculated as the $\log_2$ (MAF1/EGFP). (*C*) Limited ATC bias in DamIP-seq. ATC counts ($\log_2$) for each tRNA gene ±150 bp are plotted against MAF1 DamIP-seq scores for cells grown with (FBS, left panels) or without (SS, right panels) serum. Scores were calculated as the $\log_2$ of normalized MAF1/EGFP DamIP-seq tag counts within the gene body ±150 bp of each tRNA gene. (*D*) Scores distribution for histone modification ChIP-seq experiments performed in IMR90 cells as described in Supplemental Figure S1. Only scores at MAF1-bound genes in serum-starved cells are shown.

Figure S4. Size distribution of neusRNA-seq reads. In each plot reads from three biological replicates per condition are pooled. EU: 5-ethynyl uridine labelled cells. NC: negative control (+DMSO).

Figure S5. Correlation between DamIP-seq replicates for MAF1-occupied genes, calculated using the $\log_2$ (tag count+1) for EGFP-DamK9A and MAF1-DamK9A expressing cell lines. The Pearson correlation coefficient (r) is given.

Supplemental Experimental procedures

Plasmids. MAF1-HA-DamK9A and EGFP-HA-DamK9A sequences were purchased from *Genscript* and subcloned into pcDNA5/FRT (*Life Technologies*) under the control of the MoMuLV promoter. The cDNA encoding puromycin resistance was used to replace the hygromycin resistance coding sequence.

Antibodies. Anti-POLR3D was described previously (Canella et al., 2010) and used for ChIP as indicated or for Western blotting at 1:2000. Anti-MAF1 (cs2107) was raised in rabbits against a C-terminal peptide of human MAF1 (163-175) and used in Western blotting at 1:1000. The following commercial antibodies were used in Western blotting at the indicated dilutions: anti-RPS6-$P^{S235/236}$ (*CST, #2211*), 1:1000; anti-RPS6 (*CST, 5G10, #2217*), 1:2000; anti-AKT-P^{S473} (*CST, #92715*), 1:1000; anti-AKT (*CST, #92725*), 1:1000; anti-ERK1/2-$P^{T202/Y204}$ (*CST, D.13.14.E, #4370*), 1:2000; anti-ERK1/2 (*CST, 137F5, #4695*), 1:5000; anti-β-Actin (*Sigma, A2228*), 1:5000; anti-GFP (*Roche, #11814460001*), 1:3000. Secondary antibodies anti-rabbit Alexa Fluor 680 (*Life technologies, A21109*) and anti-mouse IRDye® 800CW (*LI-COR, 926-32210*) were used at 1:10000.

RT-PCR. 2 µg of total RNA extracted with the RNeasy kit (*Qiagen, 74104*) was reverse transcribed with Superscript III (*Life technologies, 18080093*) according to the manufacturer's instructions. The cDNA was then amplified with Phusion DNA polymerase (*NEB, M0530S*) and site-specific primers (Table S4).

Immunoblotting and Immunoprecipitation. For preparation of whole cell extracts, IMR90hTert cells were washed twice with 1X PBS and resuspended in 1X Laemmli buffer, 1% β-mercaptoethanol. For immunoprecipitation of HA-tagged MAF1-DamK9A and EGFP-DamK9A, IMR90hTert stable cell lines were washed twice with 1X PBS and collected by centrifugation (6 min, 600g, 4°C). Cell pellet was resuspended in 1X Lysis buffer 3 (10 mM Hepes pH 7.9, 0.2 mM

EDTA, 150 mM KCl, 10 mM NaCl, 1.5 mM $MgCl_2$, 1 mM DTT, 0.5 mM PMSF, 0.5% NP40, 1X protease inhibitor cocktail (*Roche, 04693159001*) and 1X PhosSTOP (*Roche, 04906837001*)) and incubated on ice for 20 min. The supernatant was cleared twice by centrifugation and proteins were quantified with the BCA assay. One mg of total protein was incubated for 2h with 1 µg of anti-HA (*Abcam, ab16918*) antibody at 4°C, followed by 2h incubation with 10 mg of protein-A sepharose beads (GE Healthcare, 17-0780-01) at 4°C under rotation. Beads were washed three times with 1X Lysis buffer 3 (w/o protease inhibitors, PhosSTOP and NP40) and resuspended in an equal volume of 2X Laemmli buffer, 2% β-mercaptoethanol. Samples were separated by SDS-PAGE on 10% acrylamide gels, transferred onto nitrocellulose, and analyzed with antibodies as described. Signals were detected by quantitative IR with the Odyssey (*LI-COR*) scanner.

ChIP-seq and ChIP-qPCR. Chromatin immunoprecipitation was carried out as described (Canella et al. 2010) with modifications. IMR90hTert cells were exposed to 1% formaldehyde for 7 minutes, after which the crosslinking reaction was stopped by addition of 0.125M glycine for 10 minutes. Chromatin extracted from 6 x 10^7 cross-linked cells was sonicated to an average size of 200-700 bp. Sheared chromatin was immunoprecipitated overnight (4°C) with 8 µl per 10^7 cells of an anti-POLR3D antibody and recovered for 1 hour at room temperature with 2 mg per 10^7 cells of protein-A beads (*GE Healthcare, 17-0780-01*) pre-blocked with 10 µg/ml of salmon sperm DNA and 10 µg/ml of BSA. After washing and crosslinking-reversal, 12 ng of purified ChIP-DNA was used for library preparation with the "ChIP-seq DNA Sample prep kit" (*Illumina, 1003473*) or analyzed by qPCR (*Applied Biosystems*, 7900HT) with the primers described in Supplemental Table S4.

Northern blotting. For Northern analysis of EU-labeled RNAs, RNA on beads was resuspended in loading buffer and resolved on a 6% acrylamide gel in parallel with 20% input RNA as described (Preti et al., 2010). Northern analysis of unlabeled RNAs was performed as described (Preti et al.,

2010) with 1 µg of a small RNA-enriched fraction as starting material. Probes are listed in Table S4.

Immunofluorescence microscopy. Mock and EGFP-DamK9A expressing IMR90hTert cells were grown on coverslips and then washed with 1X PBS. Cells were fixed for 6 min with 4% PFA and mounted on microscope slides with Vectashield mounting medium containing DAPI (*Vectashield, H1200*). LSCM maximum intensity projections were acquired with a Zeiss LSM 510 inverted confocal microscope equipped with a 40X oil-immersion lens. Images were processed with the ImageJ software.

Supplemental References

Preti, M., Ribeyre, C., Pascali, C., Bosio, M.C., Cortelazzi, B., Rougemont, J., Guarnera, E., Naef, F., Shore, D., and Dieci, G. (2010). The Telomere-Binding Protein Tbf1 Demarcates snoRNA Gene Promoters in Saccharomyces cerevisiae. Mol. Cell *38*, 614–620.