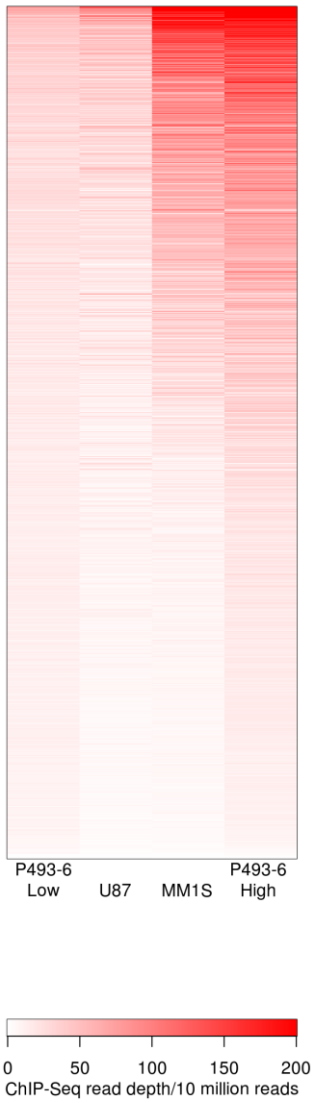
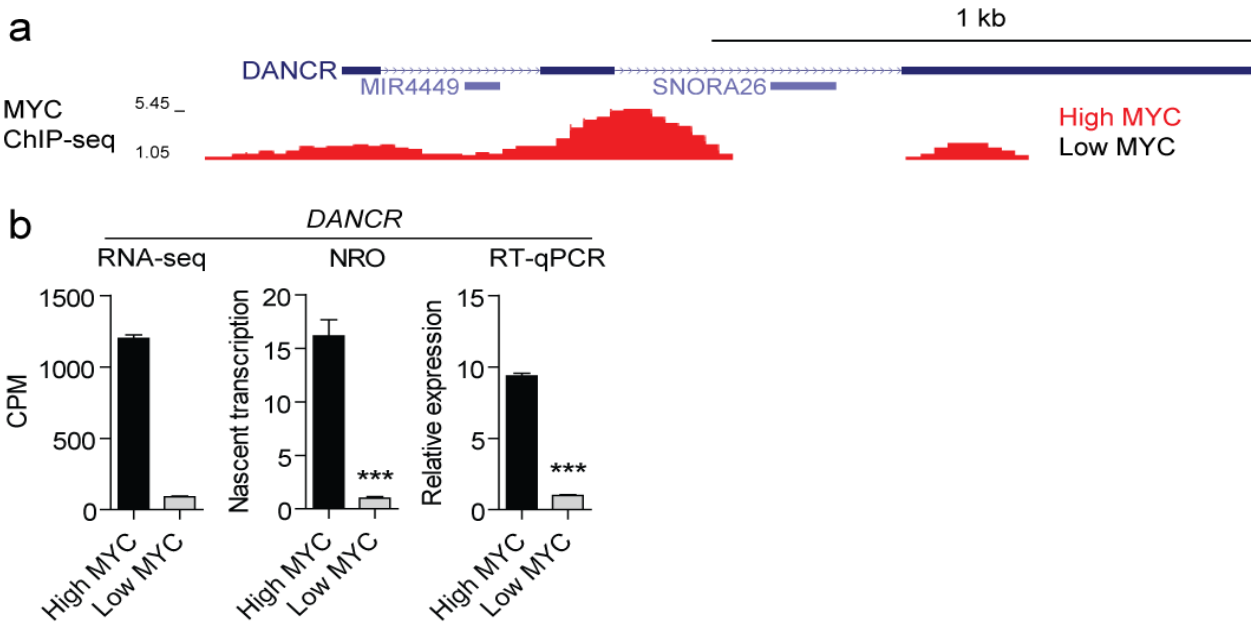
MYC regulates the non-coding transcriptome

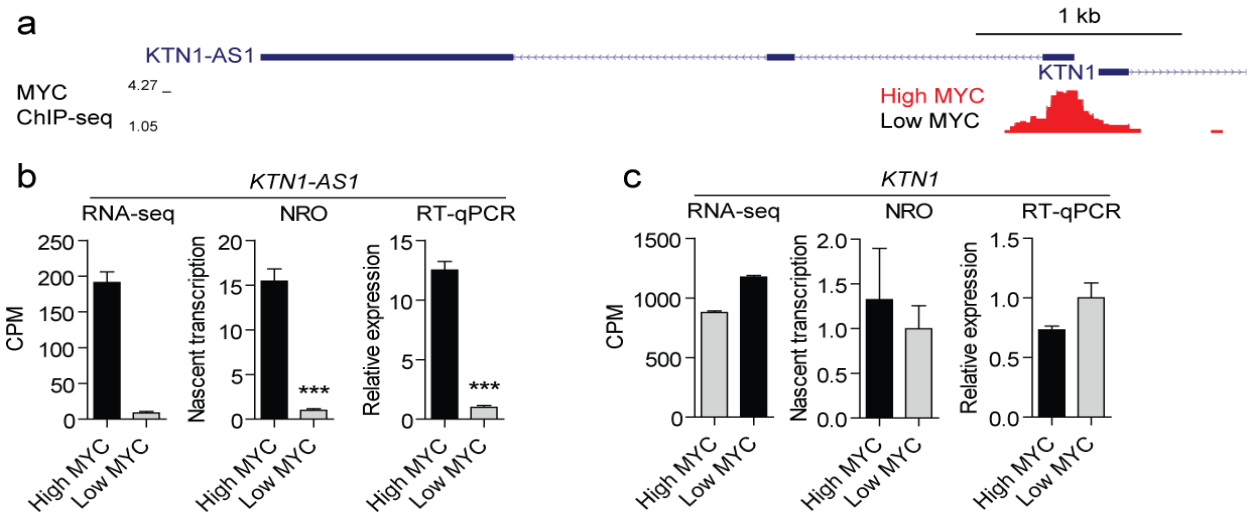
Supplementary Material



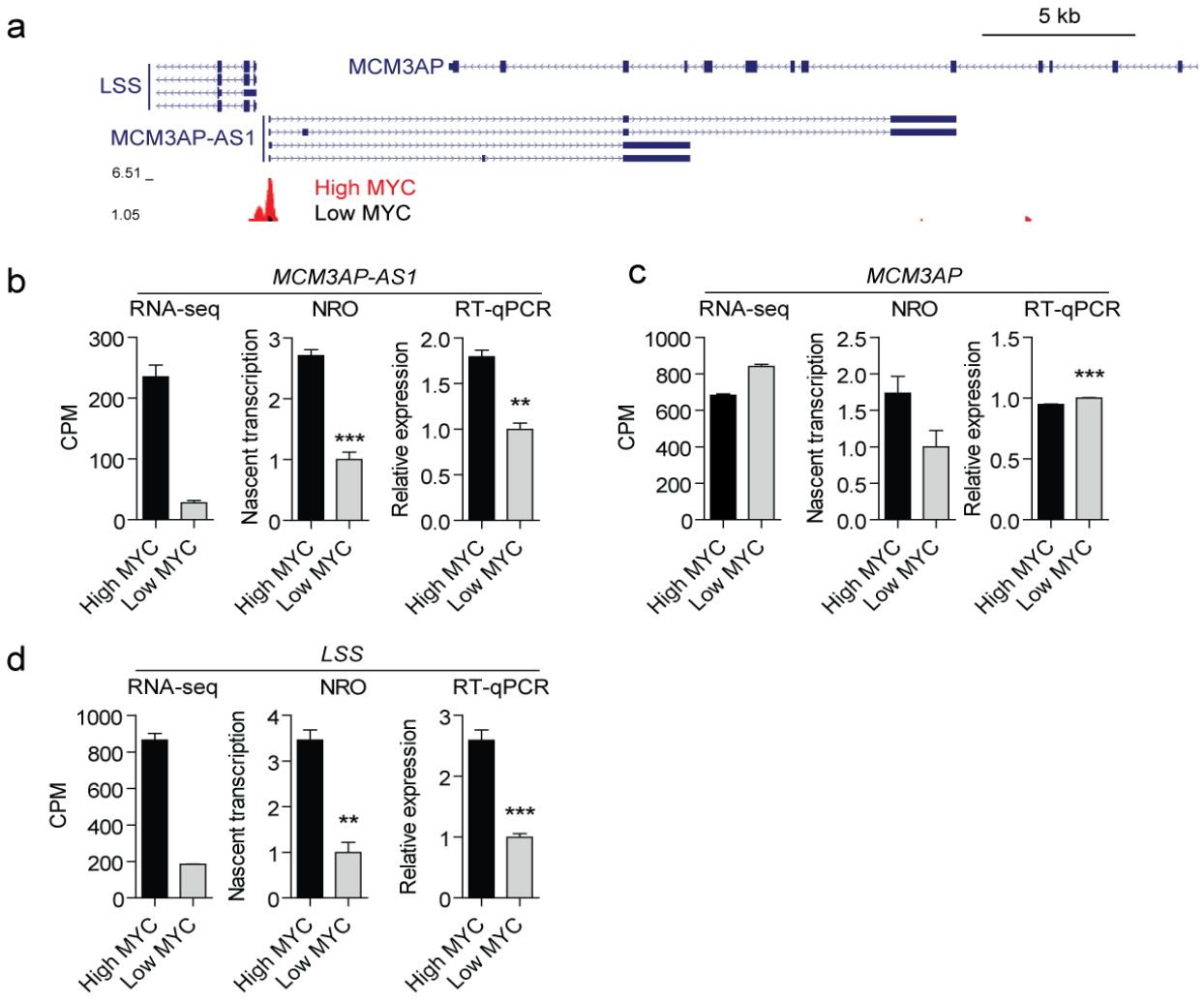
Supplemental Figure 1: MYC binds to non-coding RNA promoters in P493-6, MM.1S and U87 cells. The number of ChIP-seq reads (normalized by the total number of reads) within 3 kb of the promoters was determined for lncRNAs expressed in any of the three cell lines. Columns are arranged by increasing amounts of MYC, and rows are ordered by the average ChIP-seq intensity in the four datasets.



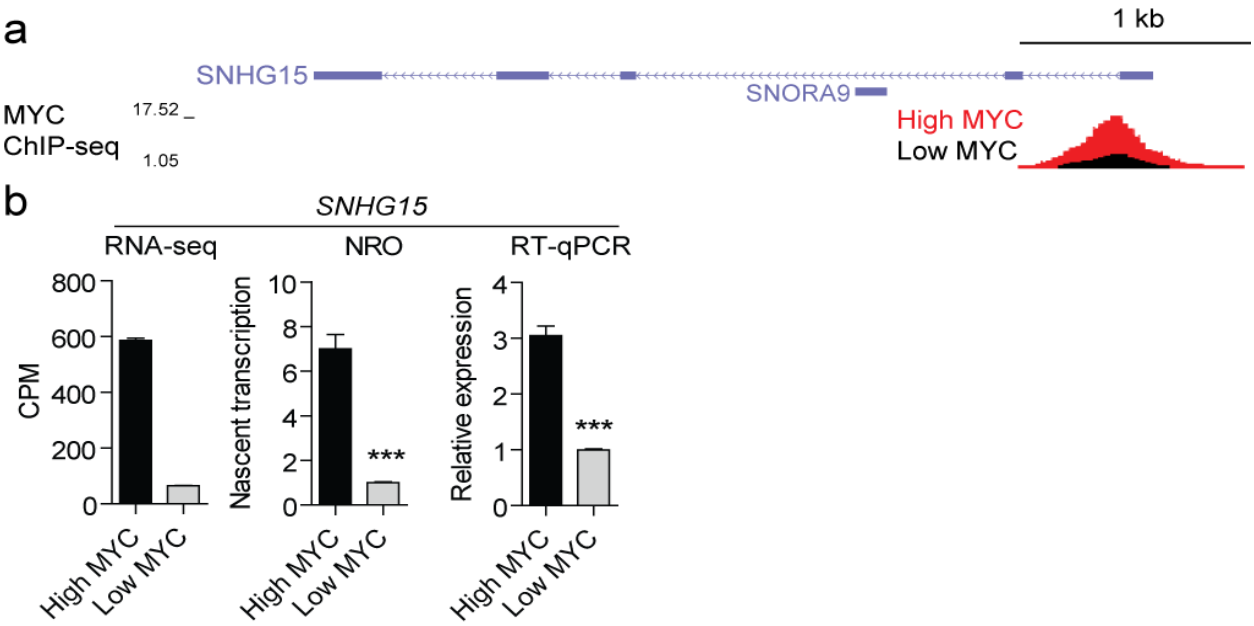
Supplemental Figure 2: Validation data for the MYC-regulated lncRNA *DANCR*. (a) MYC ChIP-seq data were visualized in the UCSC genome browser for the *DANCR* locus. The locations of miRNA precursor/snoRNAs are indicated. (b) Expression of *DANCR* in high MYC and low MYC conditions was determined by RNA-seq, NRO and steady-state RT-qPCR. Values are mean + SEM, n=3, ***P<0.001.



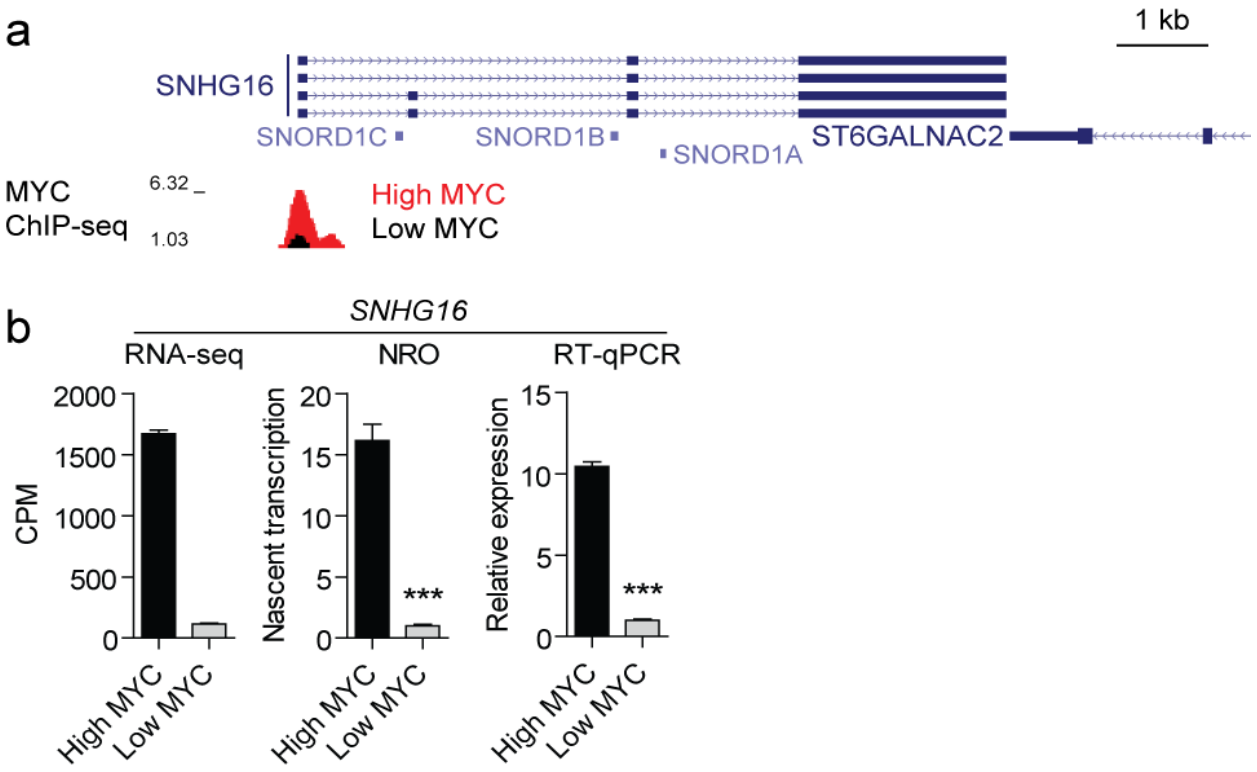
Supplemental Figure 3: Validation data for the MYC-regulated lncRNA *KTN1-AS1*. (a) MYC ChIP-seq data were visualized in the UCSC genome browser for the *KTN1-AS1* locus. *KTN1-AS1* shares a bi-directional promoter with *KTN1*. Expression of (b) *KTN1-AS1* and (c) *KTN1* in high MYC and low MYC conditions was determined by RNA-seq, NRO and steady-state RT-qPCR. Values are mean + SEM, n=3, ***P<0.001.



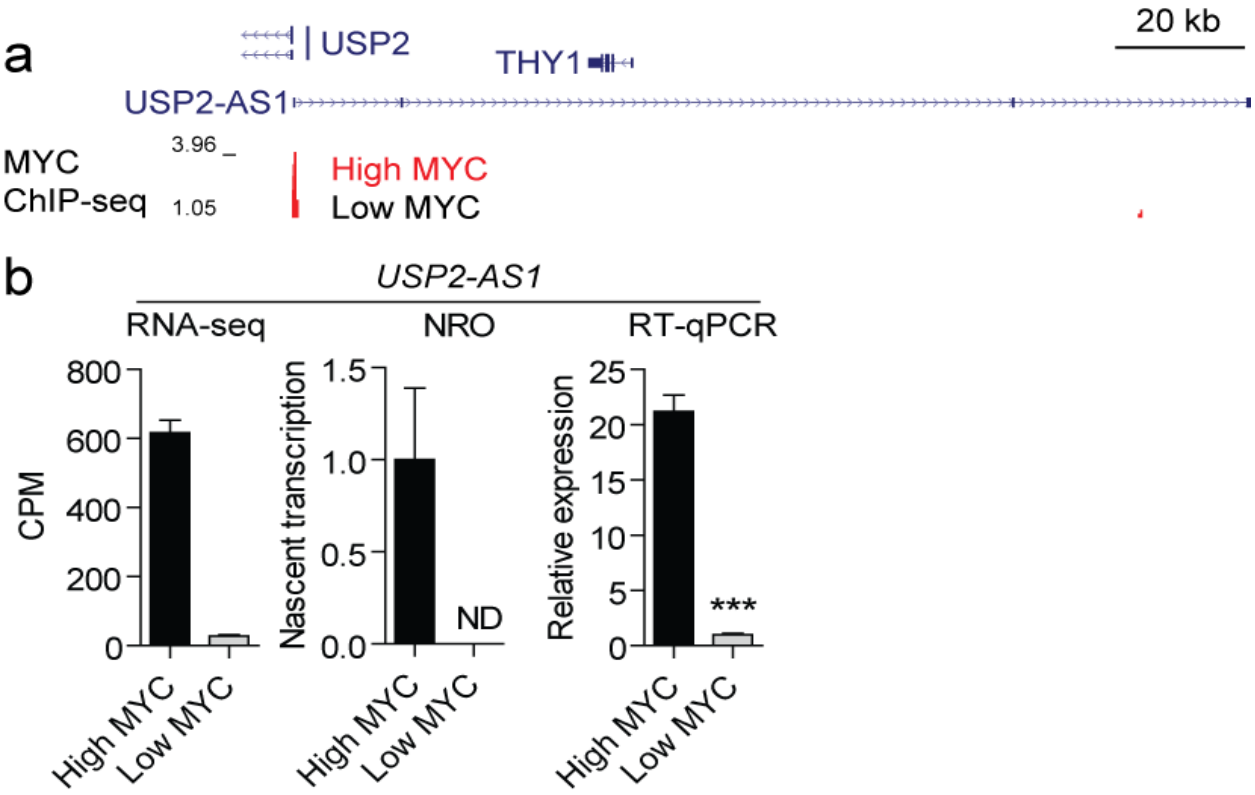
Supplemental Figure 4: Validation data for the MYC-regulated lncRNA *MCM3AP-AS1*. (a) MYC ChIP-seq data were visualized in the UCSC genome browser for the *MCM3AP-AS1* locus. The *MCM3AP-AS1* gene is an antisense lncRNA that overlaps with the 3′ terminus of the protein-coding gene *MCM3AP*. Additionally, *MCM3AP-AS1* is derived from a bidirectional promoter shared with a second protein-coding gene *LSS*. Expression of (b) *MCM3AP-AS1*, (c) *MCM3AP*, and (d) *LSS* in high MYC and low MYC conditions was determined by RNA-seq, NRO and steady-state RT-qPCR. *MCM3AP* was unaffected by MYC expression levels suggesting that there is no regulatory relationship between it and *MCM3AP-AS1*. Conversely, LSS was concordantly up-regulated with *MCM3AP-AS1* suggesting that MYC transcriptionally activates transcription of this promoter in both directions. Values are mean + SEM, n=3, **P<0.01, ***P<0.001.



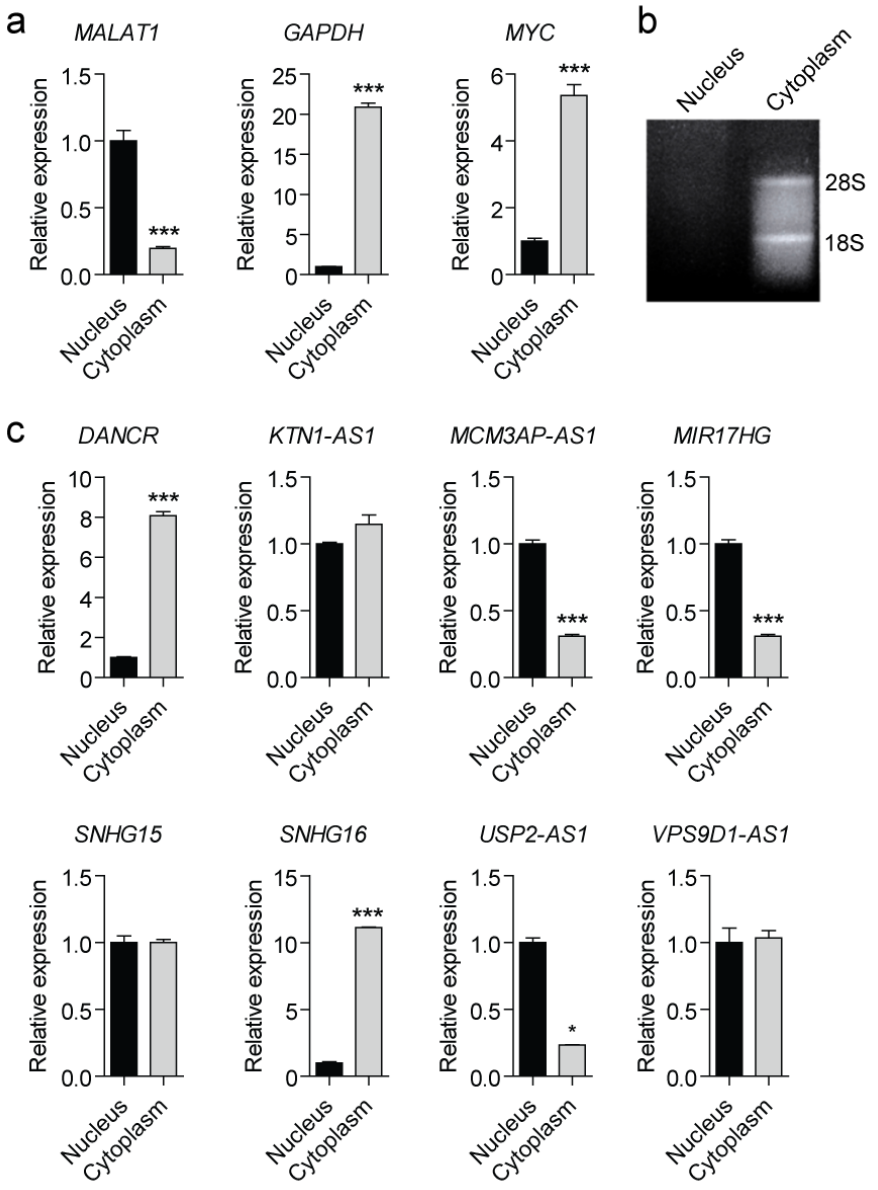
Supplemental Figure 5: Validation data for the MYC-regulated small nucleolar (sno) RNA host gene *SNHG15*. (a) MYC ChIP-seq data were visualized in the UCSC genome browser for the *SNHG15* locus. Locations of snoRNAs are indicated. (b) Expression of *SNHG15* in high MYC and low MYC conditions was determined by RNA-seq, NRO and steady-state RT-qPCR. Values are mean + SEM, n=3, ***P<0.001.



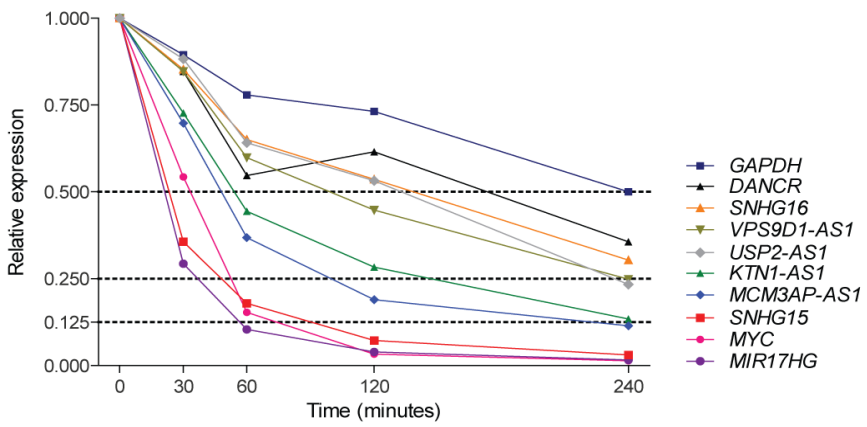
Supplemental Figure 6: Validation data for the MYC-regulated snoRNA host gene *SNHG16*. (a) MYC ChIP-seq data were visualized in the UCSC genome browser for the *SNHG16* locus. Locations of snoRNAs are indicated. (b) Expression of *SNHG16* in high MYC and low MYC conditions was determined by RNA-seq, NRO and steady-state RT-qPCR. Values are mean + SEM, n=3, ***P<0.001.



Supplemental Figure 7: Validation data for the MYC-regulated lncRNA *USP2-AS1*. (a) MYC ChIP-seq data were visualized in the UCSC genome browser for the *MCM3AP-AS1* locus. (b) Expression of *USP2-AS1* in high MYC and low MYC conditions was determined by RNA-seq, NRO and steady-state RT-qPCR. Values are mean + SEM, n=3, ***P<0.001, ND not detected.



Supplemental Figure 8: Nucleocytoplasmic distribution of lncRNAs. P493-6 cultures were separated into nuclear and cytoplasmic fractions. (a) Cellular fractionation was confirmed by measuring the transcript abundance of *MALAT1* (nuclear) or *GAPDH* and *MYC* (cytoplasmic) in either fraction. (b) Total RNA from both fractions was separated by agarose gel electrophoresis. 18S and 28S ribosomal RNAs were observed in the cytoplasmic fraction only. (c) The relative expression of the validated lncRNAs was determined in the nucleus and cytoplasm. All values are mean + SEM, n=3, *P<0.05, ***P<0.001.



Supplemental Figure 9: Transcript stability of lncRNAs. P493-6 cultures were treated with Actinomycin D and cultures harvested at a series of time points later. RNA was extracted, RT-qPCR performed and transcript half-times ($t_{1/2}$) determined as described in Materials and Methods. Transcript decay curves are shown for the representative lncRNAs with *GAPDH* and *MYC* included for comparison.

Supplemental Table 1: A census of lncRNAs expressed in P493-6 cells and presented as counts per million with high MYC and low MYC as regulated by 0.1 ug/mL doxycycline at 24 hours. Average $\log_2$(fold change) as well as significance values are included.

Supplemental Table 2: List of RT-qPCR primers used in this study. Note that primer sets were designed to be suitable for both steady-state gene expression analysis and nuclear run-on.

	FWD	REV
RLuc	GTAACGCTGCCTCCAGCTAC	CCAAGCGGTGAGGTACTTGT
MYC	GCAAAAGCTCATTTCTGAAGAGG	TTCATAGGTGATTGCTCAGGAC
GAPDH	CGCTGAGTACGTCGTGGAGTC	GCAGGAGGCATTGCTGATGA
MALAT1	GCGTAATGGAAAGTAAAGCCC	CAAACACCTCACAAAACCCC
RPLP0	GAAACTCTGCATTCTCGCTTC	GGTGTAATCCGTCTCCACAG
RPL10	CCTCTTTCCCTTCGGTGTG	AATCTTGGCATCAGGGACAC
DANCR	GCTGACCCTTACCCTGAATAC	CTTCGGTGTAGCAAGTCTGG
KTN1-AS1	CGTGAAAGGTGGGATCTGTAG	GAACTGCCTGTCTCGTATAGC
MCM3AP-AS1	CACCTCTTGCTCACTCTGATG	TCTACTTCCCAGCTCACTCTAG
MIR17HG	GACCTGTCTAACTACAAGCCAG	TGTTCTCCAGGAAGTTGCAG
SNHG15	CCTTGGCACCTTAATTGAGC	ATTCTGGAAGCAGAGAACCTG
SNHG16	CTGCTATCATAGAGACCAAGGAG	TTACTGGCACGAGGACAAAG
USP2-AS1	ACCAAATCCCCAGACCAATG	AGCGAGATGAAGAGTTTACACC
VPS9D1-AS1	ACTCCCATTTTCTCATCCCG	GGAGAGCAAAGTCCCAGAAG
KTN1	TCTGAGAGTGTACCTCGAGAC	GCACAGGCTTTTGTTTCTTTTC
LSS	AGGTAGGTCCACGTCTGC	GCCCCTACAAGACCGAG
MCM3AP	GTCTCCTTTGCAGTCTGGAAG	TTGCTGTGAGGATGTCTGTG
VPS9D1	CTCAGACGTCCCCAGGAACTG	GCCAGACAAGGACAGCTCGTTC