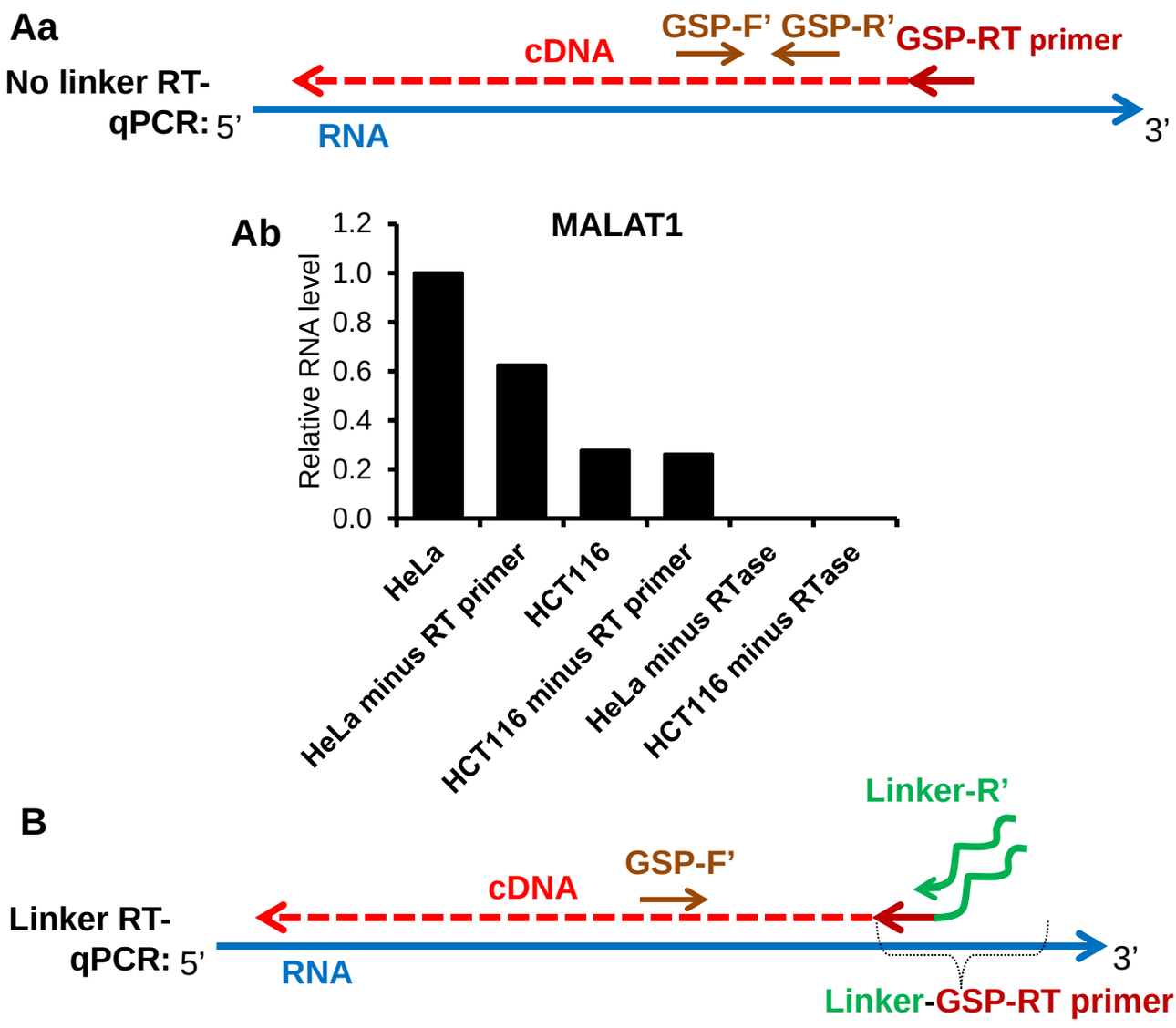
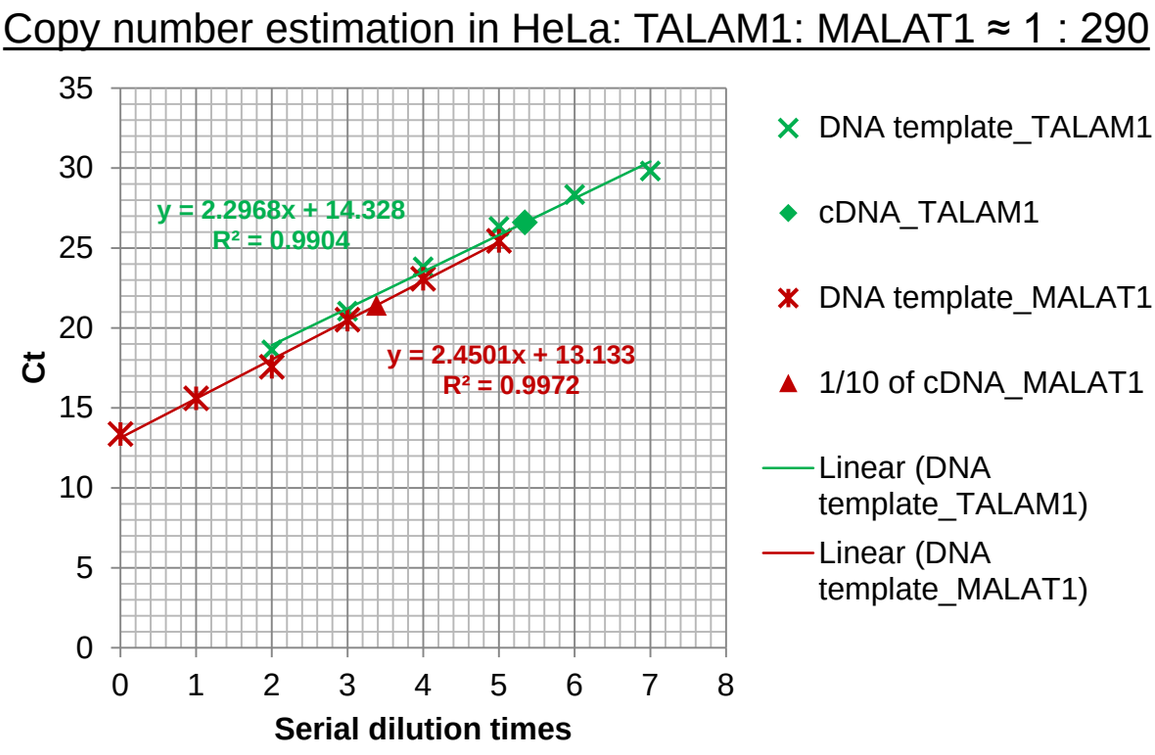
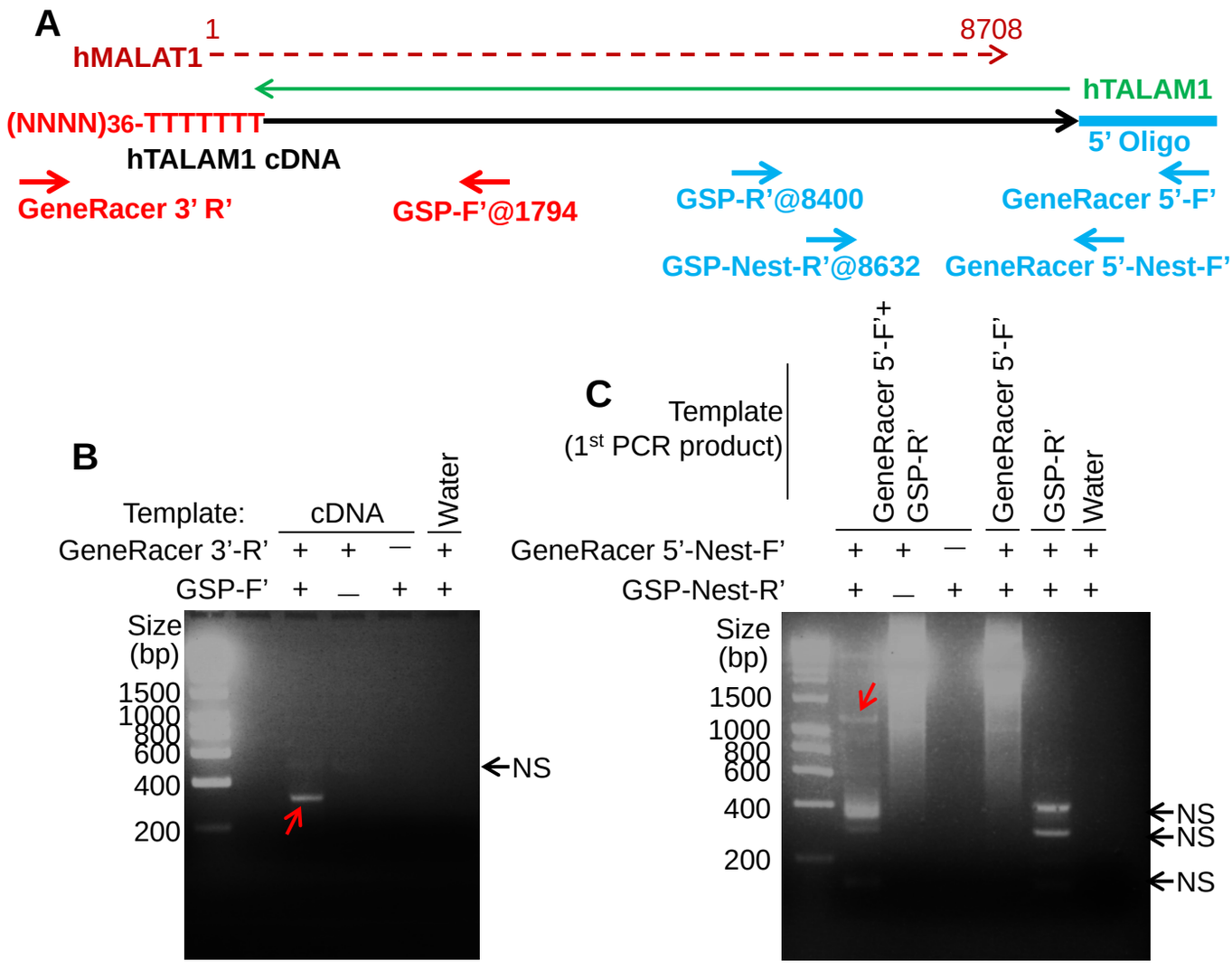
Supplementary figure S1



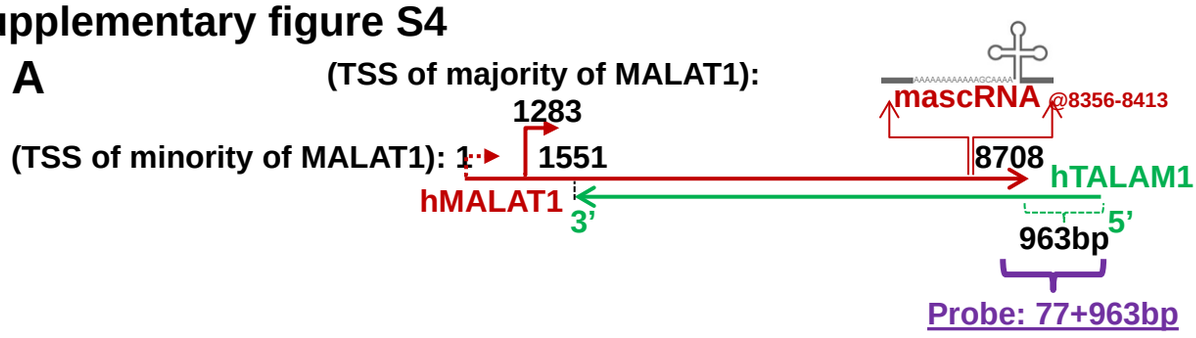
Supplementary figure S2

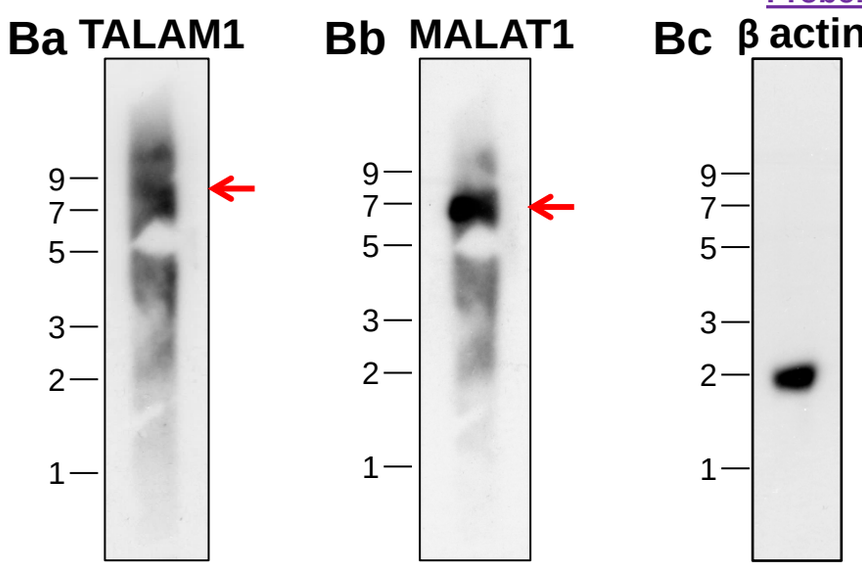


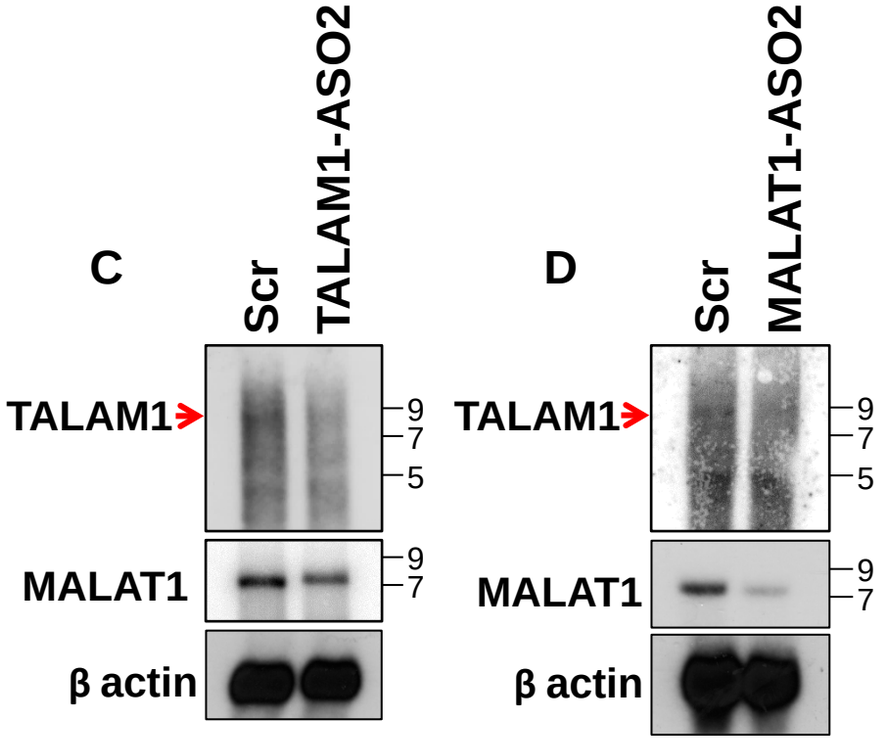
Supplementary figure S3



Supplementary figure S4

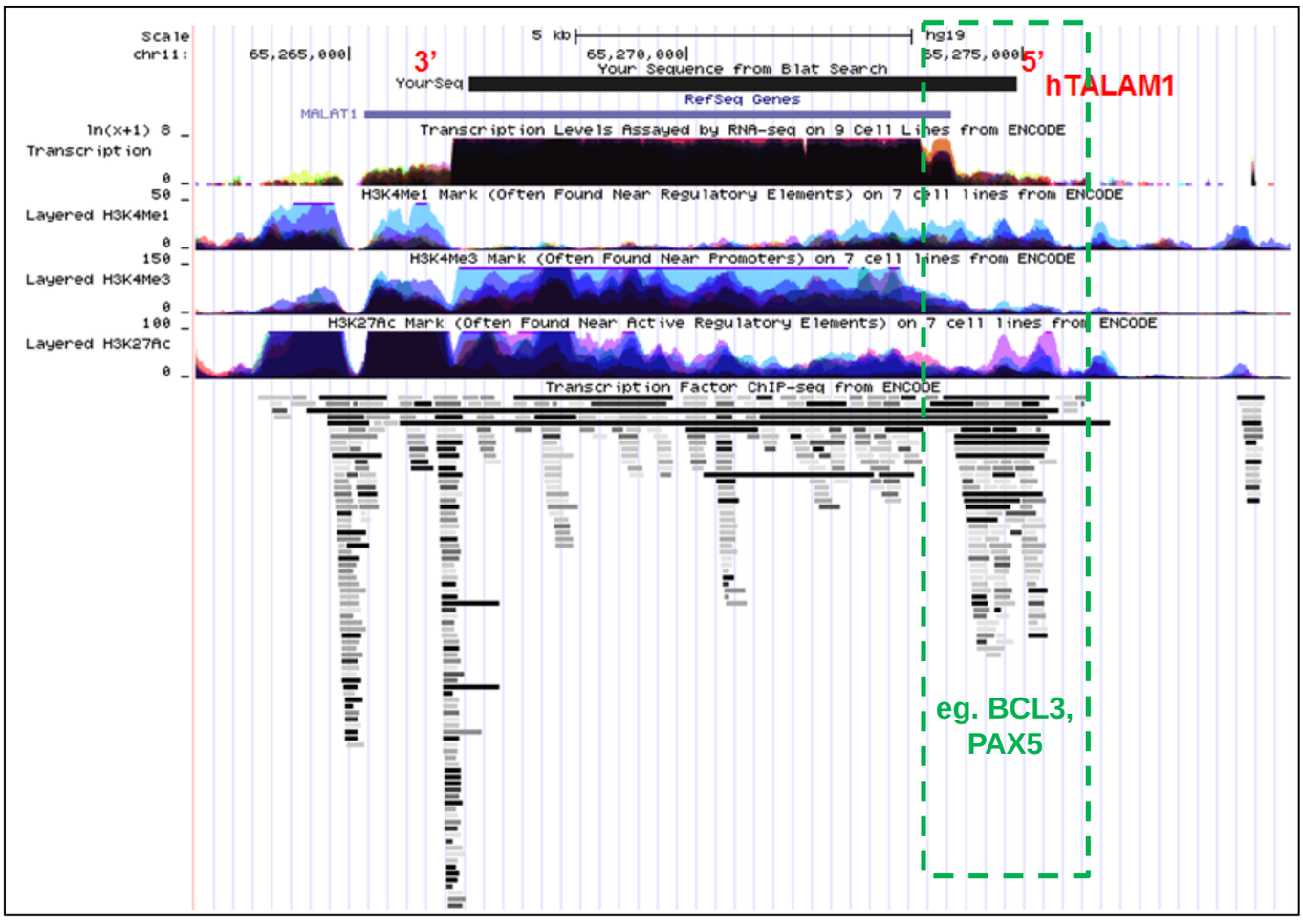






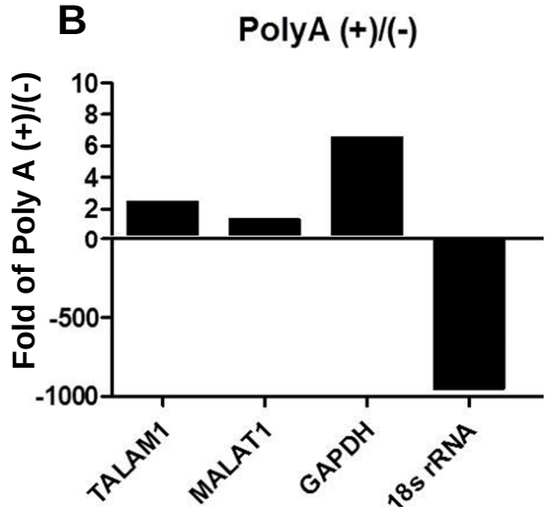
Supplementary figure S5

A

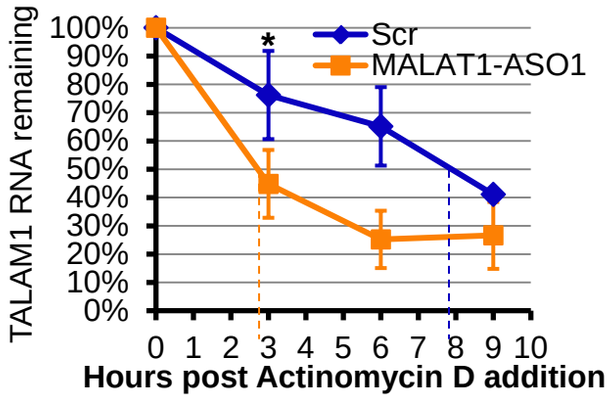


http://genome.ucsc.edu

B



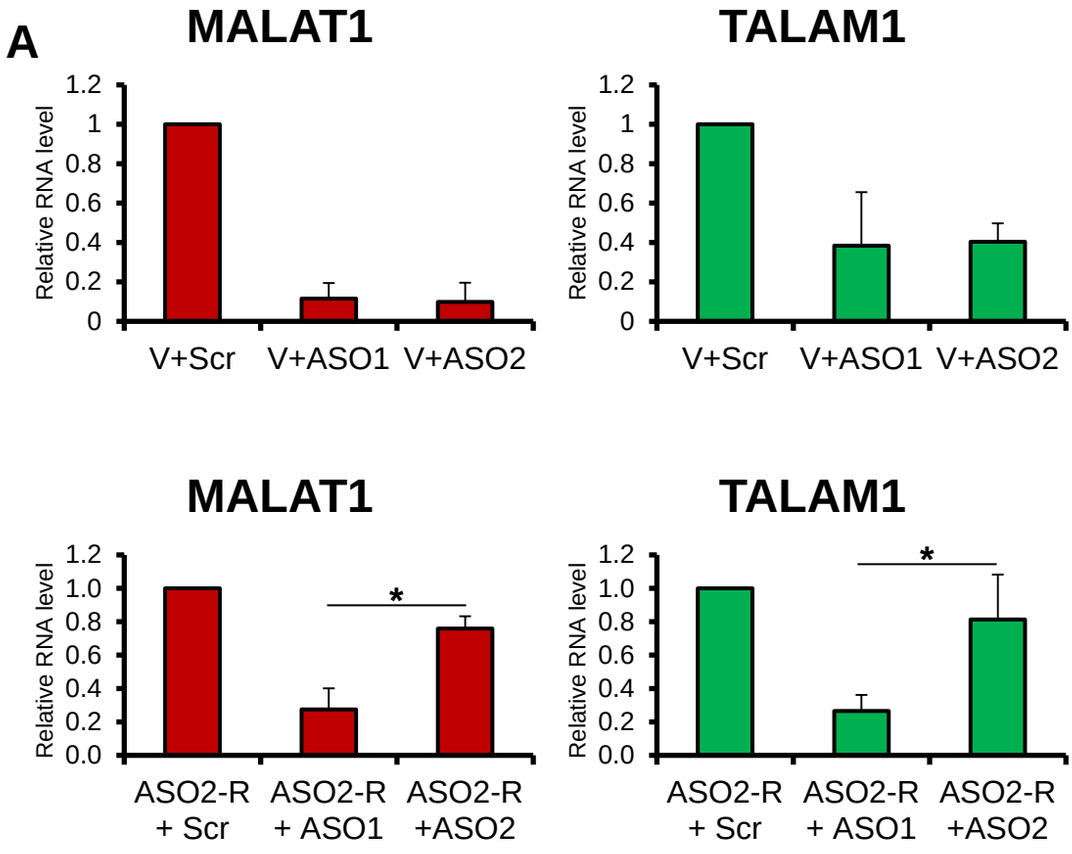
Supplementary figure S6

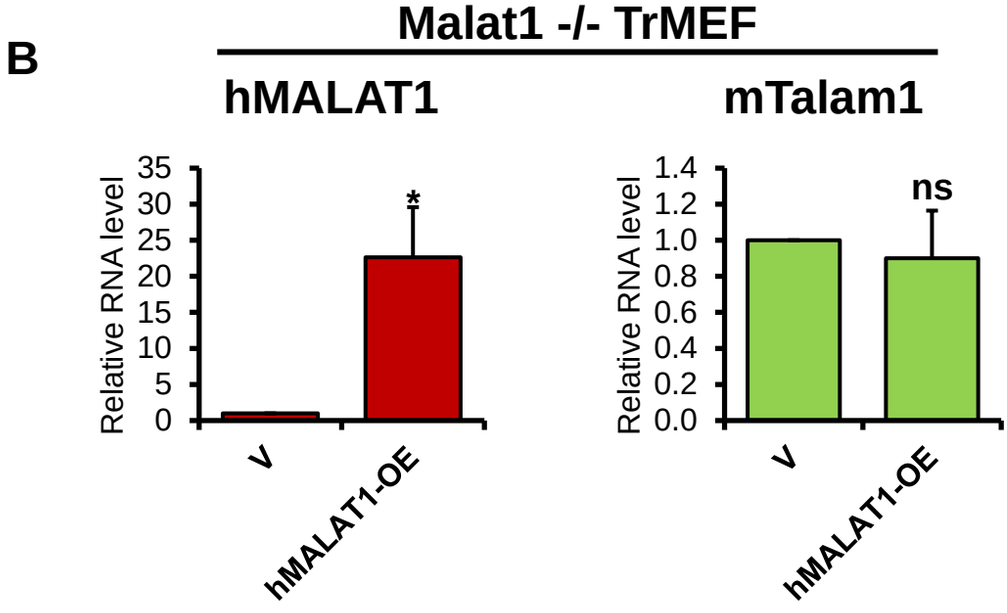


$t_{1/2}$ in Scr: 7.8hr

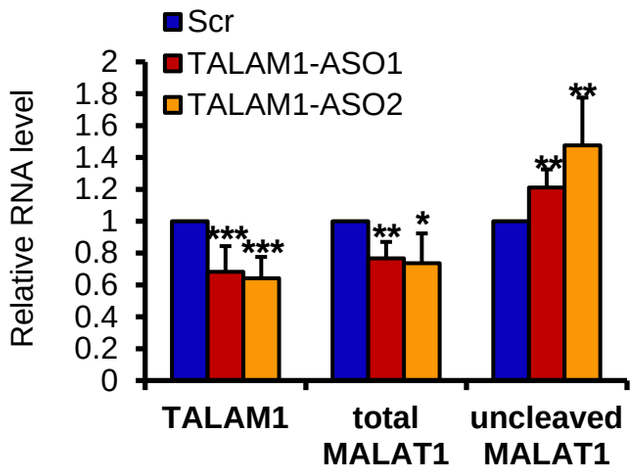
$t_{1/2}$ in MALAT1-ASO1:2.8hr

Supplementary figure S7

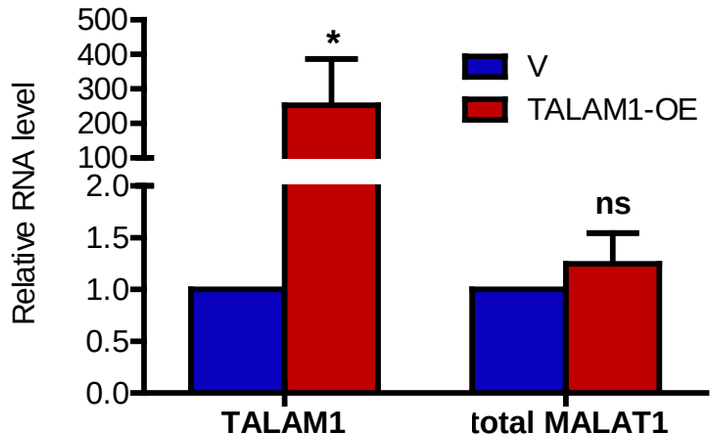




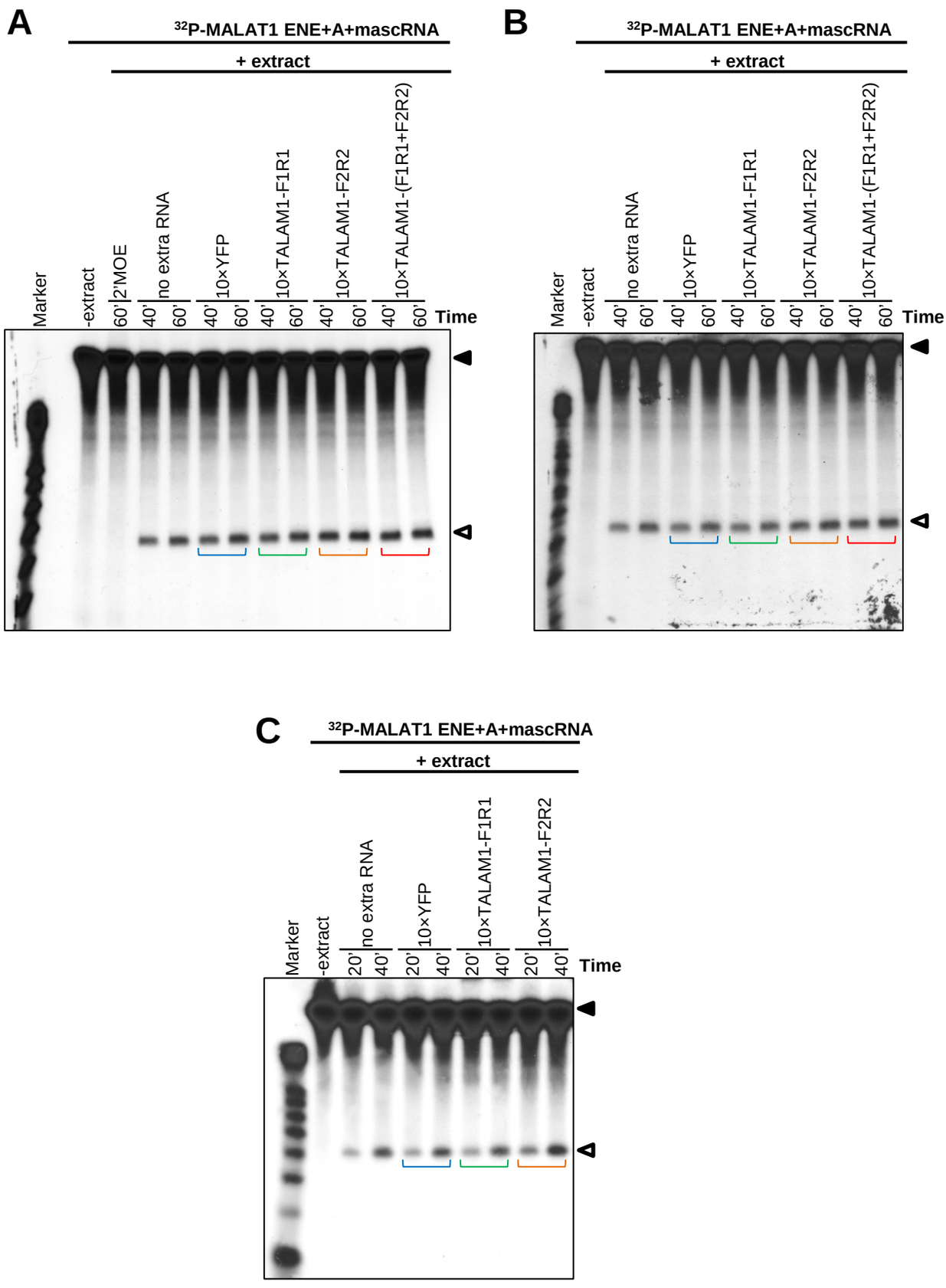
Supplementary figure S8



Supplementary figure S9



Supplementary figure S10



SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Strand-specific RT-qPCR strategy. (*A*) RT-qPCR strategy without the application of the linker sequence, which gives nonspecific amplification that is independent of the RT primer and thus is not strand-specific. This is due to the inherent property of RTase enzyme to reverse transcribe self-primed RNA template independent of RT primer(1). (*Aa)* Schematic representation of the RT-qPCR strategy without the application of the linker sequence, in which a gene-specific reverse transcription primer without tagging (GSP-RT) was used to perform reverse transcription, and then a pair of gene-specific forward and reverse primers (GSP-F' and GSP-R') was used in the qPCR reaction. (*Ab*) Non-strand-specific amplification that is independent of RT primer, using MALAT1 in HeLa and HCT116 cells as an example, shown by the comparable qPCR signals from cDNAs reverse transcribed in presence (HeLa, HCT116) and absence of RT primer (HeLa minus RT primer, HCT116 minus RT primer). Absence of genomic DNA contamination was confirmed by the minus reverse transcriptase controls (minus RTase). *(B)* Schematic representation of the strand-specific RT-qPCR (ssRT-qPCR) strategy, in which a gene-specific reverse transcription primer tagged with a linker sequence (linker-GSP-RT) was used to perform reverse transcription (2). A gene-specific forward primer (GSP-F') and the linker (linker-R') as reverse primer were used in the qPCR reaction, and strand-specific detection of RNA was shown in Figure 1A.

Supplementary Figure 2. Copy number analysis of TALAM1, relative to MALAT1. TALAM1 full length plasmid DNA at predetermined concentration (10^7 molecules/µl) was serial diluted (dilution factor:5), used as template and assayed by qPCR using TALAM1 primer set (targeting the 5' unique region of TALAM1, represented as DNA template_TALAM1) and MALAT1 primer set (targeting an overlapping region of MALAT1 and TALAM1, represented as DNA template_MALAT1). Standard amplification curves were generated by linear regression of the Ct values (Y axis) against the serial dilution times (X axis), represented by the trendlines and equations for TALAM1 and MALAT1, respectively. The RT-qPCR value from ~580 HeLa cells was determined and fit into the regression equation to calculate the copy number of TALAM1 relative to MALAT1 (For MALAT1, 1/10 of the cDNA was used, to get the Ct value in a range comparable to that of TALAM1 in order to minimize the biases from differences in qPCR amplification efficiencies). The ratio of TALAM1: MALAT1 was calculated by $10\times5^{-(X \text{ of cDNA\_TALAM1} - X \text{ of 1/10 of cDNA\_MALAT1})}$.

Supplementary Figure 3. RACE analyses of TALAM1 RNA. (*A*) Schematic representation of the oligonucleotides and PCR primers used in the RACE experiment. The red arrows in (*B*) and *(C)* point to the 3' RACE and 5' RACE (nested) PCR products, respectively. (See also Materials and Methods). NS=nonspecific.

Supplementary Figure 4. Northern blot analysis of TALAM1. (*A*) Schematic illustration of the probe used for TALAM1 Northern blot analysis. A PCR fragment containing the 963 bp 5' unique region of TALAM1 and 77 bp overlapping sequence (illustrated in purple), was used as template to generate TALAM1 Northern probe via random-priming. (*B*) Northern blot of TALAM1(Ba), MALAT1 (Bb) and β actin (Bc) in HeLa. Prominent bands are pinpointed by red arrows. Same blot in Figure Ba was stripped and reprobed for MALAT1 using strand-specific probe generated via end labeling of MALAT1-specific antisense oligos. Note: The MALAT1 band is at the expected 7kb size, distinct from the TALAM1 bands, which is above 7kb. (*C*) Northern blot of TALAM1, MALAT1 and β actin in Scr and TALAM1-ASO2 treated HeLa samples. (*D*) Northern blot of TALAM1, MALAT1 and β actin in Scr and MALAT1-ASO2 treated HeLa samples.

Supplementary Figure 5. Further characterization of TALAM1 RNA. (*A*) The genomic landscape of human *TALAM1* in UCSC genome browser. (*B*) TALAM1 RNA is enriched in the polyadenylated fraction. Poly(A) plus verse minus RNA fractionation in HeLa cells followed by RT-qPCR showed the enrichment of TALAM1 in poly(A) RNA fraction. GAPDH and 18srRNA were used as controls to show the purity of fractionation. Note that the abundant mature form of MALAT1 also manifests a moderate enrichment at the poly(A) plus fraction due to its 3' end genome-encoded A-rich tract. Positive values are the folds of enrichment in poly(A) plus over poly(A) minus, while negative values are the folds of enrichment in poly(A) minus over poly(A) plus.

Supplementary Figure 6. TALAM1 stability in HeLa cells treated with Scr and MALAT1-ASO1. Dotted lines represent the half-life of TALAM1. Data plotted are mean values with SEM, N=3 (*p<0.05, two-tailed paired Student t-test).

Supplementary Figure 7. Species-specific rescue of TALAM1 by exogenously overexpressed MALAT1. (*A*) Rescue of human TALAM1 levels by human MALAT1 in HeLa. Empty vector (V) or construct expressing human MALAT1 with point-mutations conferring resistance to MALAT1-ASO2 knockdown (ASO2-R) was first transfected into HeLa cells. 12 hours post transfection, cells were treated with Scr or MALAT1-ASO1 or ASO2 for 24 hours. Then MALAT1 and TALAM1 levels were determined via ssRT-qPCR. (*B*) Inability of human MALAT1 to rescue mouse Talam1 in transformed MEF with homozygous knockout of Malat1 (Malat1-/- Tr MEF). Empty vector (V) or construct expressing human MALAT1 (hMALAT1-OE) was transfected into Malat1-/- Tr MEF, and 24 hours post transfection, human MALAT1 and mouse Talam1 levels were determined via ssRT-qPCR. Data plotted are mean values with SD, N≥3 (biological replicates, *p<0.05, two-tailed paired Student t-test).

Supplementary Figure 8. Lower concentrations of TALAM1-ASOs show similar effects on MALAT1 as higher concentration treatment. RT-qPCR analyses of RNA extracted from HeLa cells treated with lower concentration of Scr, TALAM1-ASO1 and ASO2 at 50nM, for two rounds of knockdown. Data plotted are mean values with SD, N≥3 (biological replicates, *p<0.05, **p<0.01and ***p<0.001, two-tailed paired Student t-test).

Supplementary Figure 9. TALAM1 overexpression is not sufficient to alter total levels of MALAT1 RNA. ssRT-qPCR analyses of TALAM1 and total MALAT1 levels in HeLa cells transfected with empty vector (V) or with TALAM1-overexpression construct (TALAM1-OE). Data plotted are mean values with SD, N=4(biological replicates, *p<0.05, two-tailed paired Student t-test).

Supplementary Figure 10. Replicates of the *in vitro* processing assay of MALAT1 3' end sequence.

SUPPLEMENTARY MATERIALS AND METHODS

Cell Culture

HeLa, U2OS, MEFs were grown in DMEM containing high glucose, supplemented with 10% fetal bovine serum (FBS) (Hyclone, Logan, UT) and penicillin-streptomycin (PS). WI-38 cells were grown in DMEM containing high glucose+10% FBS +1% non-essential amino acid+PS. HCT116 cells were grown in McCoy's 5A medium+10%FBS+PS.

Antisense oligonucleotide, 2'MOE, and DNA Transfection

The ASOs were transfected to cells, at a final concentration of 100 nM for MALAT1-ASO for one time, and 200nM for TALAM1-ASO for twice (48hr) with a gap of 24 hr, using Lipofectamine RNAimax reagent (Invitrogen)(3). Lower concentration of TALAM1-ASOs at 50nM was used in Supplementary Figure S8 to minimize potential off-target effects. 2' MOE ASOs were administered at a final concentration of 100nM to cells with Lipofectamine 2000 (Invitrogen), and the cells were collected after 24hrs. Plasmid DNAs were transfected into cells using Lipofectamine 2000, and the cells were collected after 24hrs. The sequence of 2'MOE ASO to block human mascRNA cleavage is GTAGTCAAAGCAAAGACGCCGC.

Rapid Amplification of cDNA Ends (RACE)

5' and 3' RACE were performed using the GeneRacer Kit (Invitrogen), according to the manufacturer recommendations. The primers for both 5' and 3' RACE are listed below. 5-hTALAM1-R: TCCCTGCGGCGTCTTTGCTTTGA, 5-hTALAM1-Nest-R: CCTGCAG CTGGTGTTTTGAGAAGCCCTA, 3-hTALAM1-F: CGCAATTCTCCCTGCGTCATGGATTTCA.

RT-qPCR and RT-PCR

Total cellular RNA was extracted from the cells using Trizol (Invitrogen) as per manufacturer's instructions and reverse transcribed into cDNA using Multiscribe Reverse transcriptase and either Random hexamers (Applied Biosystems) or gene specific primers. qPCRs were performed using StepOne Plus system (Applied Biosystems). Transcript levels were quantitated against a standard curve by Real-time RT-PCR using the SYBR Green I fluorogenic dye and data analyzed using the StepOne plus system software. Primer sets showing comparable high efficiencies were used for the analyses. The primers to detect human and mouse MALAT1 and TALAM1 were listed below: linker-R: CGACTGGAGCACGAGGACACTGA, hMALAT1-RT: CGACTGGAGC-ACGAGGACACTGATTATTTTAATCACCTACAAC, hMALAT1-F:ATACCAAT-AGAAGGGCAATG, hTALAM1-RT:CGACTGGAGCACGAGGACACTGAGGAGTTCTTAAAT-ATCAACCA, hTALAM1-F: GCCCACAGGAACAAGTCCTA, mMALAT1-RT: CGACTGGA-GCACGAGGACACTGATTGTGGTAGGTCATCTGTTC, mMALAT1-F: AGCTTTTGAGGGCT-GACTGC, mTALAM1-RT: CGACTGGAGCACGAGGACACTGAGCCTTTAGTCTCTTCCAGATT, mTALAM1-F: GCACTAAAGACCACGGAACT.

RNA Cutoff Aassay Primer Sequences

Adaptor sequence: GCTACGTAACGGCATGACA. For uncleaved MALAT1, the forward qPCR primer is: GAGAAGCCCTACTGCTGAAA; For cleaved MALAT1, the forward qPCR primer is: GGAAGCTGATCTCCAATGCT; For internal control GAPDH, the forward qPCR primer is: CTCCTCACAGTTGCCATGTA.

Poly(A) RNA Purification

The fractionation of poly(A) plus versus minus RNAs were performed using NucleoTrap mRNA kit (Clontech). After the purification, the yield of both fractions were quantified, and equal amount of RNA were taken for RT and qPCR analysis, and the fold of enrichment in either poly(A) plus or minus fractions were calculated by normalization the qPCR values of each fractions to the yield of the corresponding fraction.

Chromatin Immunoprecipitation (ChIP)

1×10^7 control and MALAT1-KD HeLa cells were fixed using 1% formaldehyde at room temperature for 10mins, then quenched with 125mM Glycine for 5 mins, and rinsed twice with ice cold PBS. Cells were then harvested and lysed in buffer containing 1% SDS, 10mM EDTA, 50mM Tris, pH8.0. Chromatin was sonicated using Bioruptor Power-up (Diagenode) for 40~45 cycles of 30" on +30" off at high level, to obtain genomic DNA fragments tightly within 150-300 bp size range. Debris was pelleted by centrifuge at 10000g at 4°C for 10 mins. Sheared

chromatin was precleared using protein G Dynabeads (Life Technologies) for 2 h before incubation with Anti-RNA polymerase II antibody (Millipore, Cat# 05-623) or IgG overnight at 4 °C. Antibody-bound chromatin was pulled down using preblocked Dynabeads for 1.5 h. Beads were washed once each with low salt buffer (0.1% SDS; 1% Triton; 2 mM EDTA; 20 mM Tris, pH 8.0, 150 mM NaCl), once with high salt buffer (0.1% SDS; 1% Triton; 2 mM EDTA; 20 mM Tris, pH 8.0, 500 mM NaCl), once with LiCl wash buffer (10 mM Tris, pH 8.0; 1% Na-deoxycholate; 1% NP-40, 250 mM LiCl; 1 mM EDTA), and twice with TE (10 mM Tris, pH 8.0, 1 mM EDTA). Chromatin was eluted in elution buffer (1% SDS, 0.1 M sodium bicarbonate) at 65 °C twice, 10 min each. Cross-link was reversed with 0.2 M NaCl overnight at 65 °C. The eluted material was RNase A-treated followed by Proteinase K treatment at 42 °C for 2 h. DNA was purified using QIAquick PCR purification kit (Qiagen). ChIP-qPCR results were represented as percentage of IP/input signal (% input). qPCR primers used for ChIP-qPCR as depicted in Figure 2A are: TALAM1 promoter region aF: TGTTTTTCCAAAGCAGAAGGTTCT, aR: GCCCCCAATTCCTACTCGTACT, upstream 4kb of TALAM1 TSS bF: CTCACGCCGGTAA-TCCAAGT, bR: GGCCATGTTGGTCTCAAACTC, GAPDH-F: TAGAGCGGCCGCCATGT, GAPDH-R: GCAGGAGCGCAGGGTTAGT, ACTB-F: CAGGGCTTCTTGTCCTTTC, ACTB-R: GGTACTTCAGGGTGAGGAT.

RNA Stability Analysis

RNA stability analysis was carried out by incubating 5μg/ml of Actinomycin D with control and MALAT1 knockdown cells. Samples were then collected at indicated time points, and the RNA levels at specific time points were normalized to 0h time point, that is, before the addition of Actinomycin D.

In vivo RNAse Protection Assay

$4x10^6$ HeLa cells were washed in PBS, pelleted, and resuspended in 1.5 ml of lysis buffer containing 10 mM Tris pH 7.4, 3 mM $CaCl_2$, 2 mM $MgCl_2$, and 0.7% NP-40. Lysates were passed through a 27.5 gauge needle five times, then placed on ice for 10 minutes. The solution was adjusted to 125 mM NaCl, and 12.5 units/ml of DNAse I(Sigma) was added. The 1.5ml lysate was then split into two equal aliquots. RNase A (Qiagen) to a final concentration of 200 ng/ml, was added to one aliquot; the other aliquot with 250 units/ml of SUPERRnaseIn (Ambion) served as an RNAse-negative control. Samples were incubated at 37^oC for 40 minutes. TrizolLS (Invitrogen) was added to stop the reaction and extract RNA; residual DNA was removed with the DNAse I(Sigma). The protected RNAs were then subjected to Random hexamers-mediated RT-qPCR and RT-PCR. The primers to detect the overlapping region between BACE1 and BACE1-AS are BACE1-S-AS-F: TGATGGATTTGACTACAGCTTCAA, and BACE1-S-AS-R: ATGACAAGAGCATTGTGGAC-AGT. Primers for MALAT1 and TALAM1 as depicted in Figure 2A are: 1F: GCTTGAGGAAACCGCAGATA, 1R: CGTTAAAAACTTAACGCTAAGCAA, 2F: CTTCCCTAGGGGATTTCAGG, 2R: GCCCACAGGAACAAGTCCTA, 3F: AACTGGCAAGTGG-AAATGTTT, 3R: AAGACCAAGGGAGGGGAGA, 4F: CCTGGGTCACTCAGGATCACA, 4R: CACGTTCAAAGGGACAAAAGG.

Northern Blots

For mascRNA Northern, 2μg small RNAs were separated by 12% polyacrylamide/8M urea denaturing gel electrophoresis and electroblotted to Hybond N+ membrane (GE Healthcare). For TALAM1 Northern, 2~10μg poly(A) plus RNAs were separated by 1% agarose/formaldehyde gel prepared using NorthernMax denaturing gel buffer (Ambion), and transferred to Hybond N+ membrane via upward capillary transfer with 10×SSC. For northern blots using oligonucleotide probes, ULTRAhyb-Oligo Hybridization Buffer and ULTRAhyb Hybridization Buffer (Ambion) were used for oligonucleotide probes and for random-labeled probes, respectively, as per the manufacturer's instructions. Oligo probes were labeled with [γ-

^{32}P] ATP with T4 poly-nucleotide kinase (NEB). Labeling of random-labeled probes was performed with the Prime-It RmT Random Primer Labeling Kit (Stratagene). Decade Markers were used as a ladder on small RNA northern blots (Ambion). ssRNA ladder was used for normal Northern blots (NEB). Blots were visualized and quantified with X-ray film and Image J. The oligonucleotide probe sequence for human mascRNA in small RNA Northern is AGACGCCGCAGGGATTTGAACCCCGTCCTGGAAACCAGGAGTGCCAA-CCACCAGCATC; for U6 is GCTAATCTTCTCTGTATCGTTCCAATTTTAGTATATGTGCTGCCG; for MALAT1 in normal Northern is ACGAATTCAGGGTGAGGAAGTAAAAACAGGTCATCTATTCACAAAA-CTGA. The PCR fragment used as template to make random-labeled probe for TALAM1 is the 5' RACE-Nest PCR product of TALAM1, which is described in Supplementary Figure S3A,C.

In vivo Decay Assay

U2OS 2-6-3 Tet-off cells were maintained in DMEM supplemented with 10% tetracycline-approved FBS and 2 mM L-glutamine at 37 °C and 5% CO_2(4). *In vivo* decay assays were modified from the procedure described previously (5). pCMV-EYFP (EYFP-N1, Clontech) or pCMV-hTALAM1 full length was transfected into U2OS 2-6-3 Tet-Off cells with Lipofectamine 2000, 5 hours later fresh medium was added. 24 hours after 1st transfection, the pTRP-βΔ1,2-MALAT1 ENE+A+mascRNA construct was transfected(5). After 5 hours, the medium was changed into fresh medium with 50ng/ml doxycycline, and the transcriptional pulse was started ~24 hours after transfection by washing the cells twice with PBS and adding medium lacking doxycycline. At the end of a ~5 hours pulse period, the medium was removed, and fresh medium with 50 ng/ml doxycycline was added. Cells were harvested at various time points, and RNA was isolated with Trizol and analyzed by RT-qPCR. Since βΔ1,2-MALAT1 ENE+A+mascRNA is known to undergo single decay(5), the percentage of RNA remaining versus time were fit to a single-exponential decay equation: RNA remaining=A (exp(-*k* t)), as represented by the trendlines. The half-lives of reporter RNA were calculated from $t_{1/2}$=ln 2/*k*, as described previously(5). qPCR primers for βΔ1,2 are b-globin-F: CCACTCCTGATGCTGTTATG and b-globin-R: CTCACTCAGTGTGGCAAAG.

In vitro Cleavage Assay

9×10^5 HeLa cells were lysed in 300µl lysis buffer containing 10mM Tris-HCl, pH 7.5, 2.5mM $MgCl_2$, 100mM KCl, 2mM DTT, 0.2mM PMSF, 5% Glycerol, 200U/ml RNase Inhibitor and additional protease inhibitors. The cell lysate was rotated at 4^{o}C for 10mins and then subject to sonication using Bioruptor Power-up (Diagenode), 5 cycles of 30" on +30" off at high level. Debris was pelleted by centrifuge at 10000g at 4^{o}C for 5 mins. The supernatant was taken and supplemented with 0.5mM ATP, 100mM NH_4Cl, 10mM $MgCl_2$, 1mM 2-mercaptoethanol, and 0.01% Triton X-100 as final concentration. *In vitro* transcribed RNA was folded in presence of 6.4mM $MgCl_2$ and diluted RNase Inhibitor, by incubation at 65^{o}C for 2 mins, then slow cool down to 30^{o}C. Substrate ^{32}P-MALAT1, YFP and TALAM1 RNA fragments were folded separately. Substrate ^{32}P-MALAT1 was used at ~10,000CPM, ~4fmol per 15 µl reaction system. The mole ratio of YFP or TALAM1 RNA fragments to substrate ^{32}P-MALAT1 RNA was 10:1. Folded YFP or TALAM1 RNA fragments were first added to the cell lysate, followed immediately by the addition of folded substrate ^{32}P-MALAT1 to start the reaction. The reaction was carried out at 30^{o}C for indicated time. The reaction was stopped by addition of RNA loading dye and analyzed by a 12% Polyacrylamide/8M urea gel and exposure to X-ray film.

REFERENCES

1. Tuiskunen, A., Leparc-Goffart, I., Boubis, L., Monteil, V., Klingstrom, J., Tolou, H.J., Lundkvist, A. and Plumet, S. (2010) Self-priming of reverse transcriptase impairs strand-specific detection of dengue virus RNA. *The Journal of general virology*, **91**, 1019-1027.

2. Vashist, S., Urena, L. and Goodfellow, I. (2012) Development of a strand specific real-time RT-qPCR assay for the detection and quantitation of murine norovirus RNA. *Journal of virological methods*, **184**, 69-76.
3. Zong, X., Huang, L., Tripathi, V., Peralta, R., Freier, S.M., Guo, S. and Prasanth, K.V. (2015) Knockdown of nuclear-retained long noncoding RNAs using modified DNA antisense oligonucleotides. *Methods Mol Biol*, **1262**, 321-331.
4. Rafalska-Metcalf, I.U., Powers, S.L., Joo, L.M., LeRoy, G. and Janicki, S.M. (2010) Single cell analysis of transcriptional activation dynamics. *PloS one*, **5**, e10272.
5. Brown, J.A., Bulkley, D., Wang, J., Valenstein, M.L., Yario, T.A., Steitz, T.A. and Steitz, J.A. (2014) Structural insights into the stabilization of MALAT1 noncoding RNA by a bipartite triple helix. *Nature structural & molecular biology*, **21**, 633-640.