Electronic Supplementary Material (ESI) for RSC Chemical Biology.

Supplemental Information

RT-qPCR as a screening platform for mutational and small molecule impacts on structural stability of RNA tertiary structures

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***Table S1**. Nucleic acid sequences for synthesis of the RNA constructs. SHAPE cassette added to the 3'-end of MALAT1 is shown in red.*

Name	Sequence
MALAT1-WT DNA template	GAAGGTTTTTCTTTTCCTGAGAAAACAACACGTATTGTTTTCTCAGGTTTTG CTTTTTGGCCTTTTTTCTAGCTTAAAAAAAAAAAAAGCAAAATCGATCCGGTT CGCCGGATCCAAATCGGGCTTCGGTCCGGTTC
MALAT1-WT Forward Primer	GAAATTAATACGACTCACTATAGGAAGGTTTTTCTTTTCCTGAGAAAACAAC ACGTATT
MALAT1 SHAPE cassette Reverse Primer	mG[mA]ACCGGACCGAAGCCC
MALAT1-U13C DNA template	GGAAGGTTTTTCTCTTCCTGAGAAAACAACACGTATTGTTTTCTCAGGTTT TGCTTTTTGGCCTTTTTCTAGCTTAAAAAAAAAAAAAGCAAA ATCGATCCGGTTCGCCGGATCCAAATCGGGCTTCGGTCCGGTTC
MALAT1-U13C Forward Primer	GAAATTAATACGACTCACTATAGGAAGGTTTTTCTCTTCCTGAGAAAACAAC ACGTATT
SARS-CoV-2 pseudoknot template	GCAGCTTAATACGACTCACTATAGTTTGCGGTGTAAGTGCAGCCCGTCTTA CACCGTGCGGCACAGGCACTAGTACTGATGTCGTATACAGGGCTTTTGAC ATC
SARS-CoV-2 pseudoknot forward perimer	GCAGCTTAATACGACTCACTATAGTTTGCGGTGTAAGTGC
SARS-CoV-2 pseudoknot reverse primer	mGmATGTCAAAAGCCCTGTATACGACATCAGTACTAGTGCC

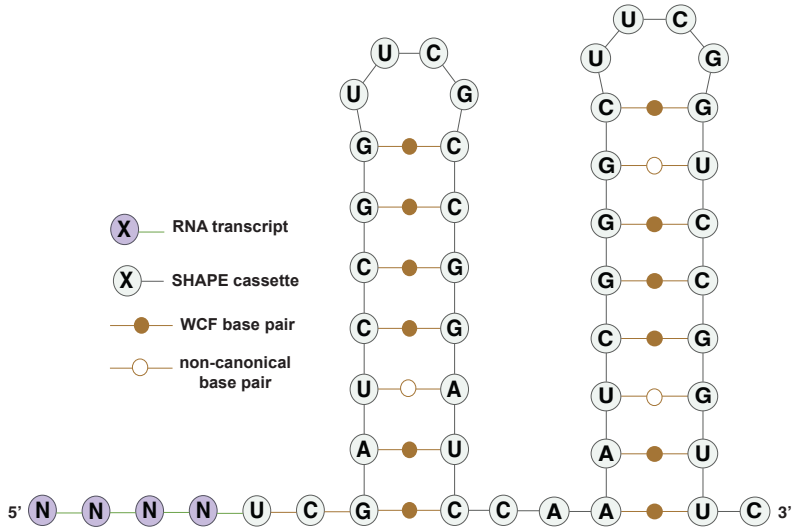

Figure S1. Representation of structure and sequence of the SHAPE cassette (orange) as reported by Wilkinson and co-workers.[1]

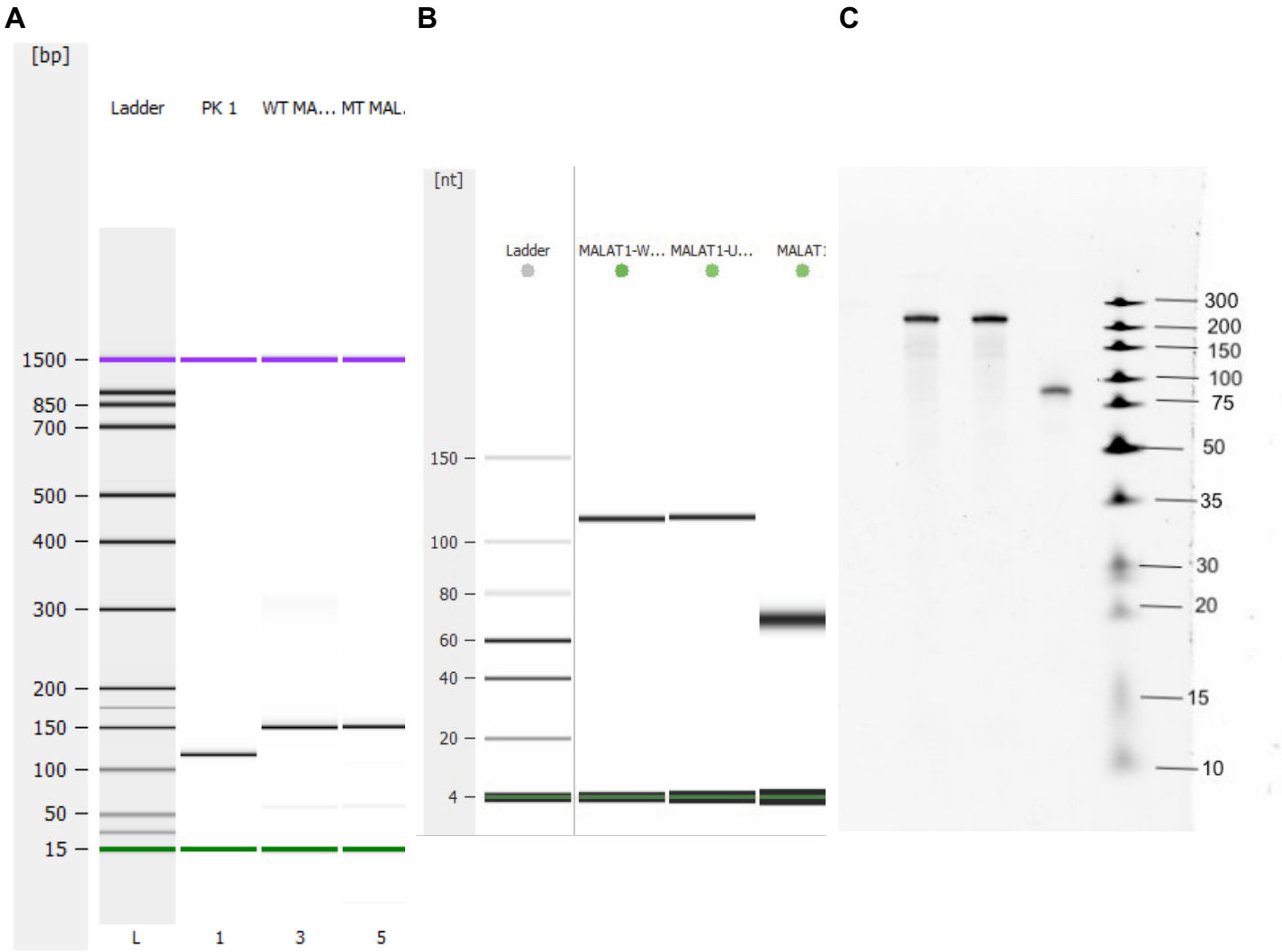

Figure S2. Purity analysis of constructs used in the study. (A) Agilent DNA high-resolution Chip of the PCR products indicate purifty of all constructs. The samples are arranged as SARS-CoV-2 pseudoknot, MALAT1 wild-type, and MALAT1 mutant, respectively. (B) Small RNA Agilent chip of the RNA constructs used in the RT-qPCR assay. Samples are arranged as MALAT1 wild type, MALAT1 mutant, and MALAT1 triple helix without the SHAPE cassette for reference, respectively. (C) 10% TBE denaturing gel with the RNA constructs used in the RT-qPCR assay. Samples are arranged as MALAT1 wild-type MALAT1 mutant, SARS-CoV-2 pseudoknot, and ladder, respectively.

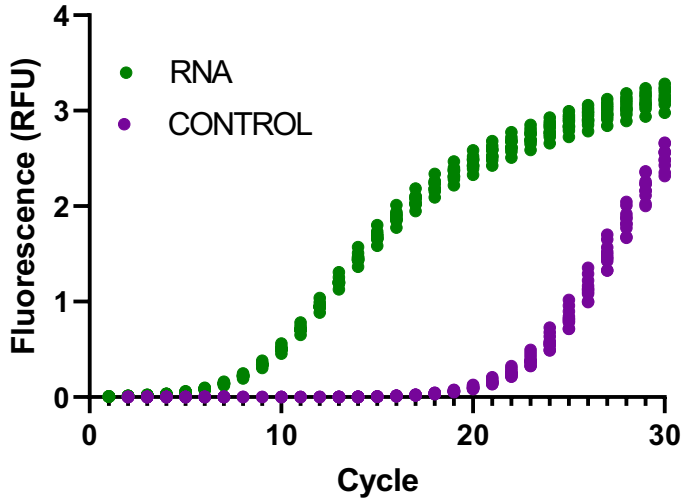

Figure S3. Raw curve of z'-factor experiment with amplification of MALAT1_WT or control. The experiment was run in two independent replicates with 10 wells of RNA and 10 wells of controls (no RNA) each. Z-factor was calculated as previously reported.[2]

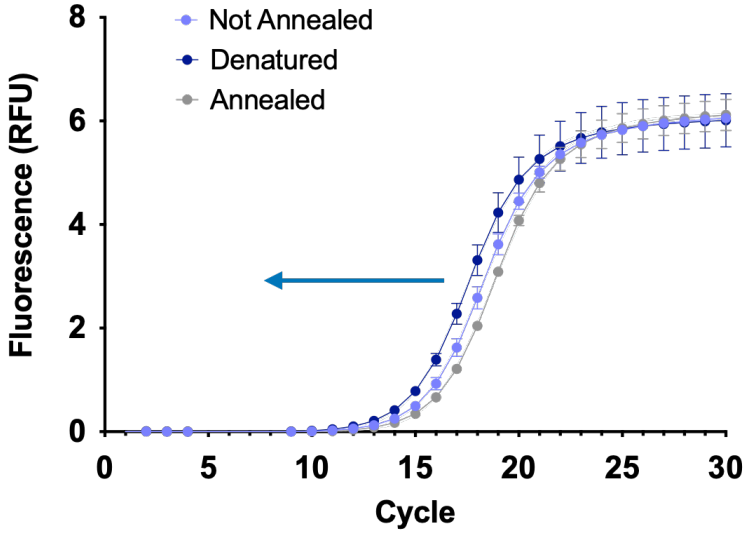

Figure S4. Denatured control of the MALAT1 wild type ensures the RT-qPCR assay reports on construct structuredness. The MALAT1 WT sample was either not annealed (light blue), denatured in 100μM DTT and 10μM RNA prior to dilution to 100nM and addition to the plate, or annealed as performed with small molecules treatment. Decrease in C_t value of the not annealed and DTT-denatured conditions confirm that the assay reports on and is influenced by structuredness of the construct.

References

1. Wilkinson, K. A.; Merino, E. J.; Weeks, K. M., Selective 2′-hydroxyl acylation analyzed by primer extension (SHAPE): quantitative RNA structure analysis at single nucleotide resolution. *Nature Protocols* **2006,** *1* (3), 1610-1616.
2. Patwardhan, N. N.; Cai, Z.; Newson, C. N.; Hargrove, A. E., Fluorescent peptide displacement as a general assay for screening small molecule libraries against RNA. *Org Biomol Chem* **2019,** *17* (7), 1778-1786.