

SUPPLEMENTARY MATERIAL

Locked Nucleic Acid Oligonucleotides Facilitate RNA•LNA-RNA Triple-Helix Formation and Reduce *MALAT1* Levels

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Table S1. Oligonucleotides and RNAs used in this study.

Synthesized oligonucleotides	
R15	5'-AAAAAAAAGCAAA-3'
L15	5'-[+A] [+A] [+A] [+A] [+A] [+A] [+A] [+A] [+G] [+C] [+A] [+A] [+A]-3'
PS-L15	5'-[+A]*[+A]*[+A]*[+A]*[+A]*[+A]*[+A]*[+A]*[+A]*[+A]*[+G]*[+C]*[+A]*[+A]*[+A]*[+A]-3'
A ₂₈	5'-AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA-3'
<i>In vitro</i> transcribed RNAs	
<i>MALAT1</i> SL	5'-GGAAGGUUUUUCUUUCCUGAGAAAACAACACGUAUU GUUUUCUCAGGUUUUGCUCUUUUGGCCUUUU-3'
<i>MALAT1</i> triple helix	5'-GGAAGGUUUUUCUUUCCUGAGAAAACAACACGUAUU GUUUUCUCAGGUUUUGCUCUUUUGGCCUUUUUCUAGCUUAA AAAAAGCAAA-3'
<i>MENβ</i> SL	5'-AGGUGUUUCUUUACUGAGUGCAGCCCAGGCCAUGGCCGCACU CAGGUUUUGCUUUACCUUCC-3'
<i>MENβ</i> triple helix	5'-AGGUGUUUCUUUACUGAGUGCAGCCCAGGCCAUGGCCGCACU CAGGUUUUGCUUUACCUUCCCAUCUGUGAAAGAGUGAGCAGGAAA AAGCAAA-3'
KHSV PAN ENE	5'-GGCUGGGUUUUCCUUGUUCGCACCGGACACCUCAGU GACCAGACGGCAAGGUUUUAUCCAGUUCGUAU-3'
<i>TWIFB1</i> dENE	5'-GGCUGUACUCUUUUCUUUGUCAUGGUUUUCUCAAUA UGAGUUUUUACAUGACAAAGUUUUUACGAGGCAGCA-3'

The “+” sign inside a square bracket [+] indicates locked nucleic acid modification, whereas the asterisk (*) denotes phosphorothioate backbone.

Table S2. Primers used for RT-qPCR experiments.

<i>MALAT1</i>	Forward: 5'-GATCTAGCACAGACCCTTCAC-3' Reverse: 5'-CGACACCATCGTTACCTTGA-3'
<i>MENβ</i>	Forward: 5'-GTGTCCACAGGTCTTAGATTCC-3' Reverse: 5'-TCTGTGTAGTAGGGTGGGATAG-3'
<i>HOTAIR</i>	Forward: 5'-GGTAGAAAAAGCAACCACGAAGC-3' Reverse: 5'-ACATAAACCTCTGTCTGTGAGTGCC-3'
<i>U6 snRNA</i>	Forward: 5'-CTCGCTTCGGCAGCACA-3' Reverse: 5'-AACGCTTCACGAATTGCGT-3'

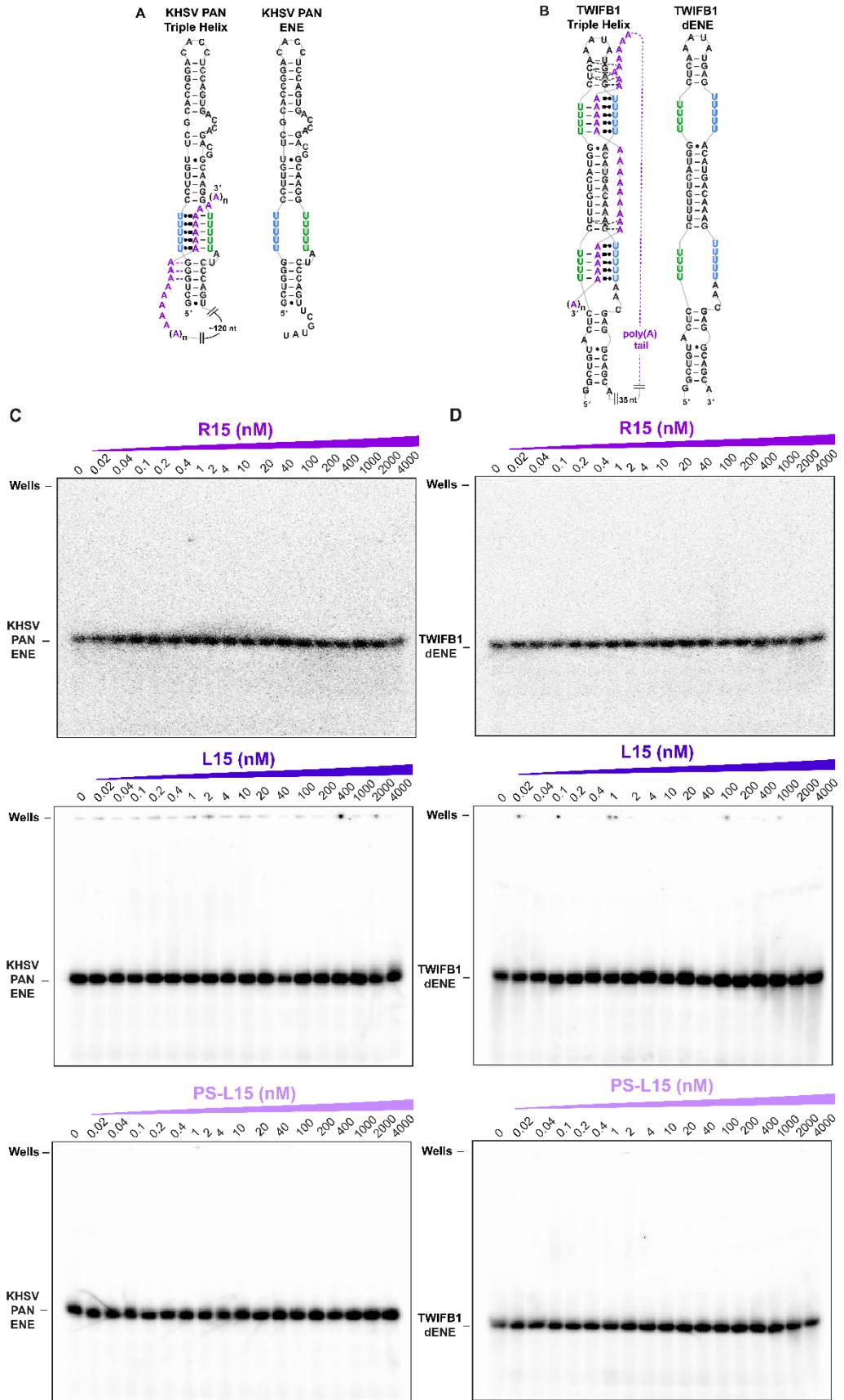


Figure S1. EMSA for R15, L15 and PS-L15 not binding to the Kaposi's sarcoma-associated herpesvirus (KHSV) *PAN* ENE and mRNA element from *Oryza sativa* hAT-type DNA transposon *TWIFBIG1* dENE (*TWIFB1* dENE) RNAs [1,2]. Schematic diagrams of (A) KHSV *PAN* triple helix and its ENE, (B) rice transposase *TWIFB1* triple helix and its dENE. The Watson-Crick and Hoogsteen interactions are represented by a solid line (|) and Leontis-Westhof notation (●—■) [3], respectively. Representative gel images for R15, L15 and PS-L15 titrated against (C) KHSV *PAN* ENE and (D) *TWIFB1* dENE.

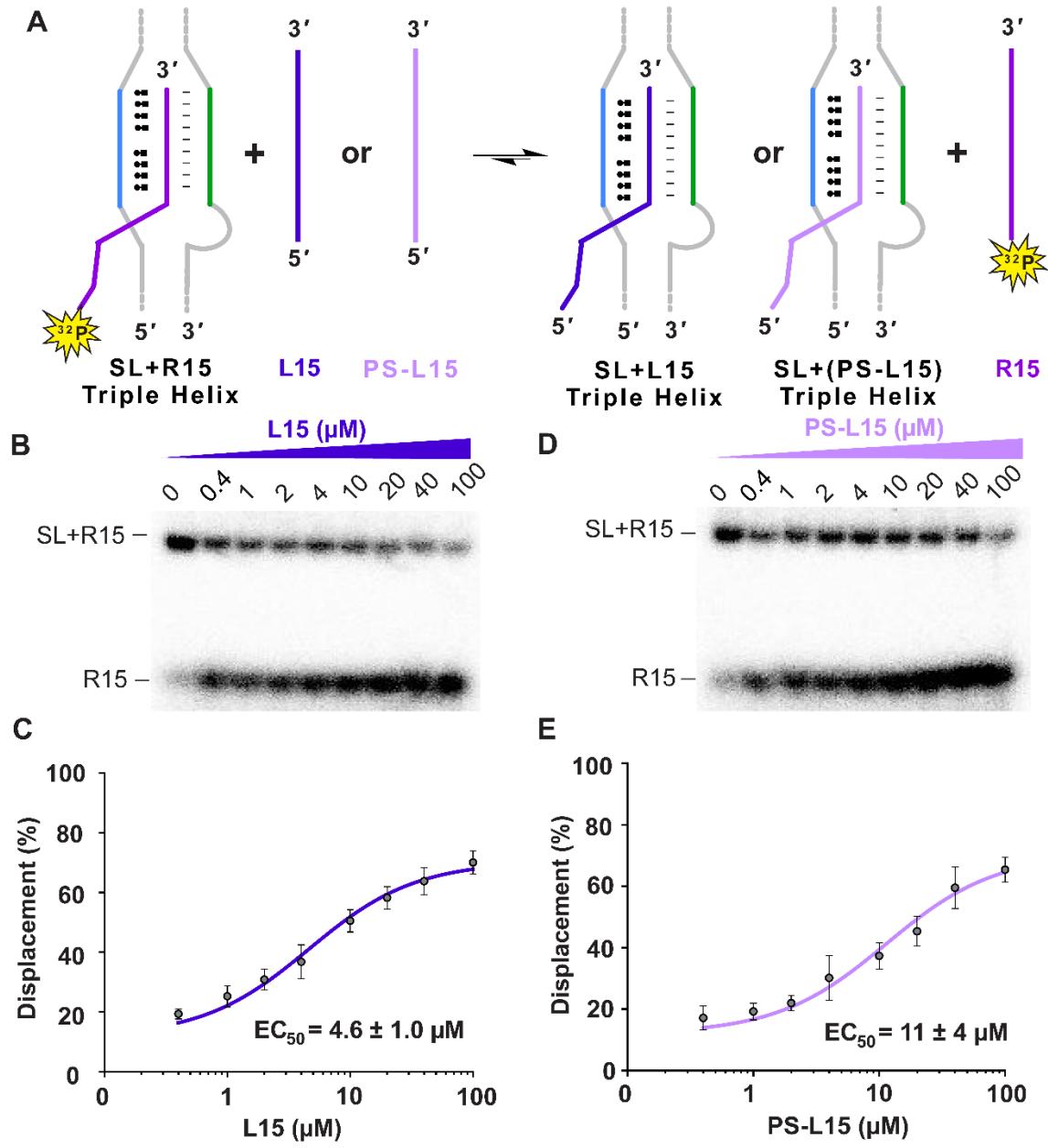


Figure S2. Competitive EMSA for the LNAs (L15 and PS-L15) displacing RNA (R15) from *MENβ* SL+R15 complex. (A) Cartoon schematic showing the displacement of $5' - [^{32}\text{P}]$ -radiolabeled R15 (purple) from SL+R15 triple helix by L15 (dark purple) or PS+L1 (light purple) to form SL+L15 or SL+(PS-L15) complexes. (B, D) Representative gel images and (C, E) binding curves for the displacement of R15 by L15 and PS-L15 from *MENβ* SL+R15 complex. The gel images show dissociation of $5' - [^{32}\text{P}]$ -radiolabeled R15 from SL+R15 complex as increasing amounts of L15 or PS+L15 are added.

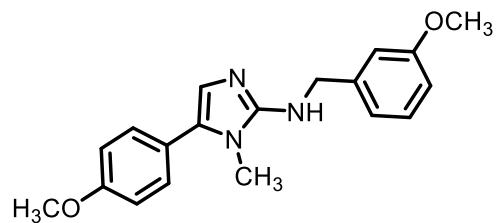


Figure S3. The chemical structure of compound **5**, a small molecule previously established to bind to the *MALAT1* triple helix [4].

REFERENCES

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