Supplemental materials

Quadruplex-forming motif inserted into 3'UTR of Ty1 retrotransposon inhibits retrotransposition in yeast

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Name	sequence 5´-3´	Use
URA3_F	GCGGCAGAAGAAGTAACAAAGG	qRT-PCR
URA3_R	ATCTTTGTCGCTCTTCGCAATG	
His3_F	CCTTCGTTTATCTTGCCTGCTC	
His3_R	TTTTCCACCTAGCGGATGACTC	
WT	CCCCGGGGCGGGGCGGGGCGGGGCGGGG	CD spectroscopy
M1	CCCCGGGGCGGGGCGAAGCGGGGCGGGG	
M2	CCCCGAAGCGAAGCGAAGCGAAGCGGGG	
Ty1_probe_F	TGGTGGAGGGAACATCGTTG	southern blot probe
Ty1_probe_R	ATTCCGGCTGGTCGCTAATC	
WT_top	CGGGGCGGGGCGGGGCGGGGC	plasmid construction
WT_bottom	GCCCCGCCCCGCCCCGCCCCG	
M1_top	CGGGGCGGGGCGAAGCGGGGC	
M1_bottom	GCCCCGCTTCGCCCCGCCCCG	
M2_top	CGAAGCGAAGCGAAGCGAAGC	
M2_bottom	GCTTCGCTTCGCTTCGCTTCG	

Table S1. Overview of oligonucleotides used in this study

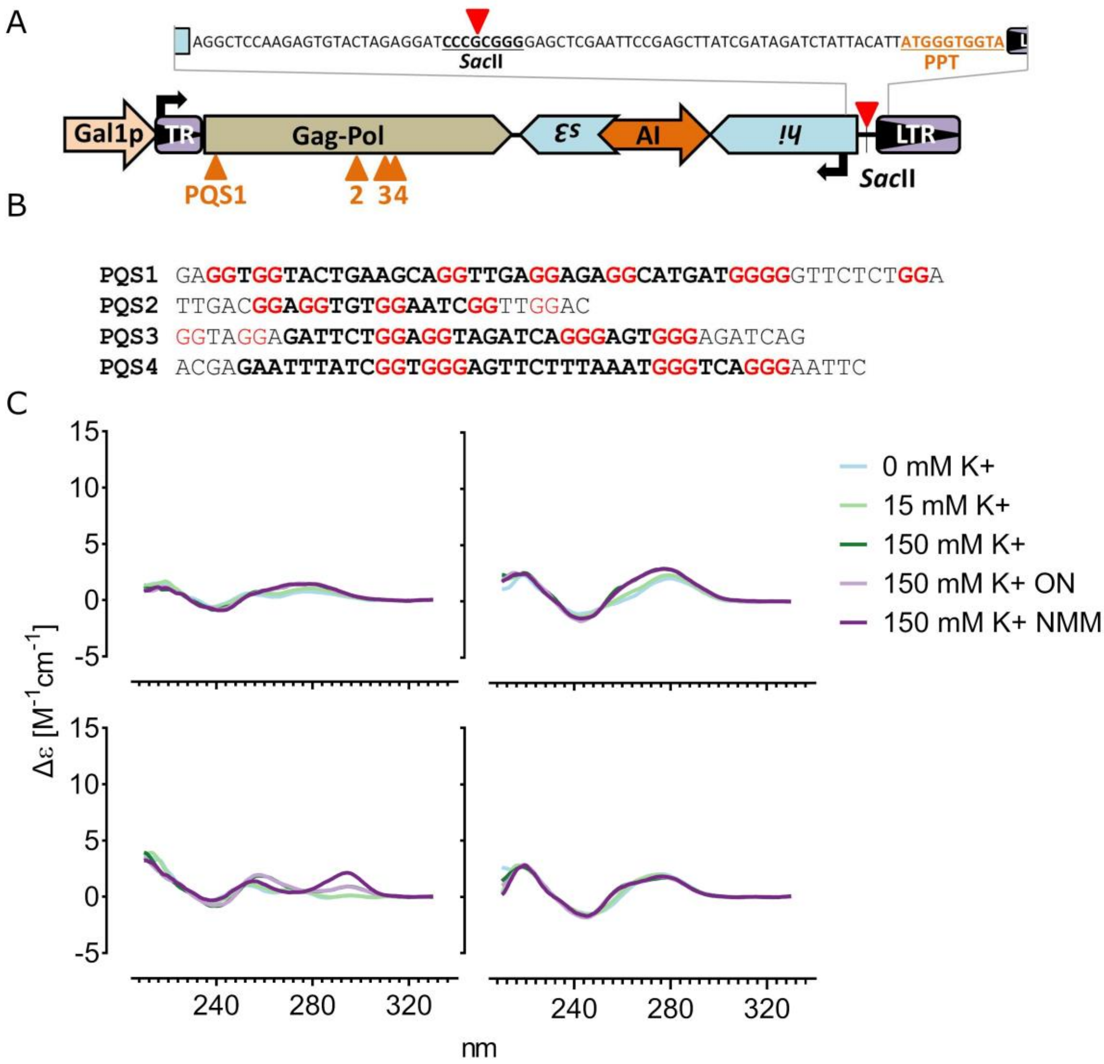

Figure S1. Analysis of endogenous PQS in *Ty1his3-AI* retrotransposon.
(A) A schematic localisation of predicted potential quadruplex forming sequences (PQS; orange triangles) within *Ty1his3-AI* retrotransposon. G-rich strand of all predicted sequences is template strand for transcription. Red triangles show the cloning *SacII* site where sequences of interest were inserted. The polypurine tract (PPT) is also highlighted. (**B**) Oligonucleotides used for CD spectroscopy measurement containing both predicted sequences (bold) and surrounding sequences. Guanines possibly forming G4 are red. (**C**) CD spectra obtained in increasing potassium concentration at 22 °C. After overnight incubation (ON) in 150mM K^+ samples were also measured with the addition of 7,5 µM NMM to force G4 formation.

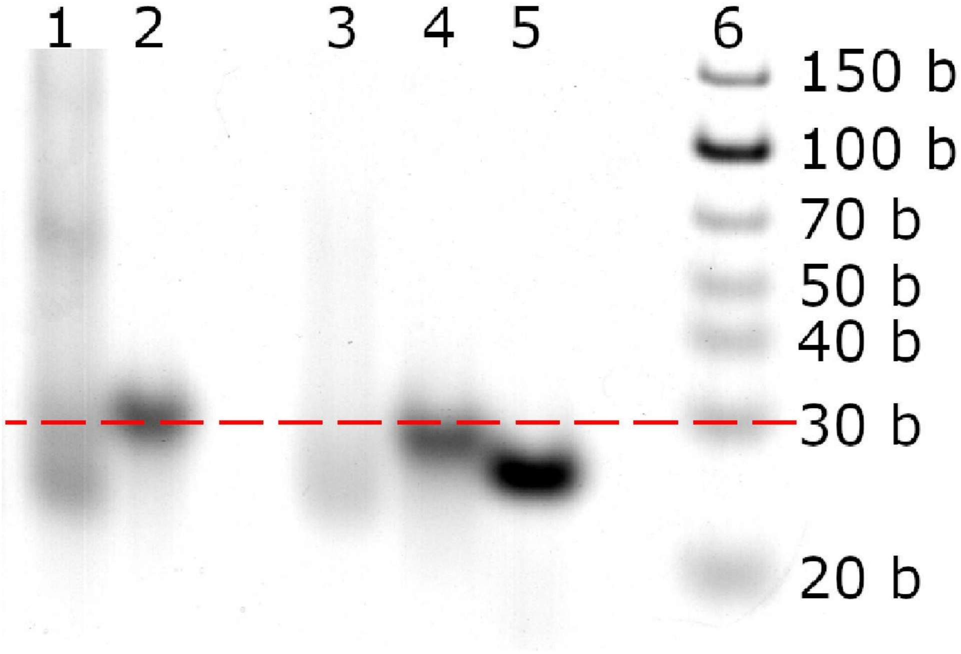

Figure S2. Native PAGE

Annealed oligonucleotides WT and M1 (lines 1 and 2), oligonucleotides WT, M1 and M2 without annealing (lines 3-5), size marker (line 6). All tested oligonucleotides adopted monomolecular organization but WT also formed a bimolecular fraction after annealing in 150 mM potassium.

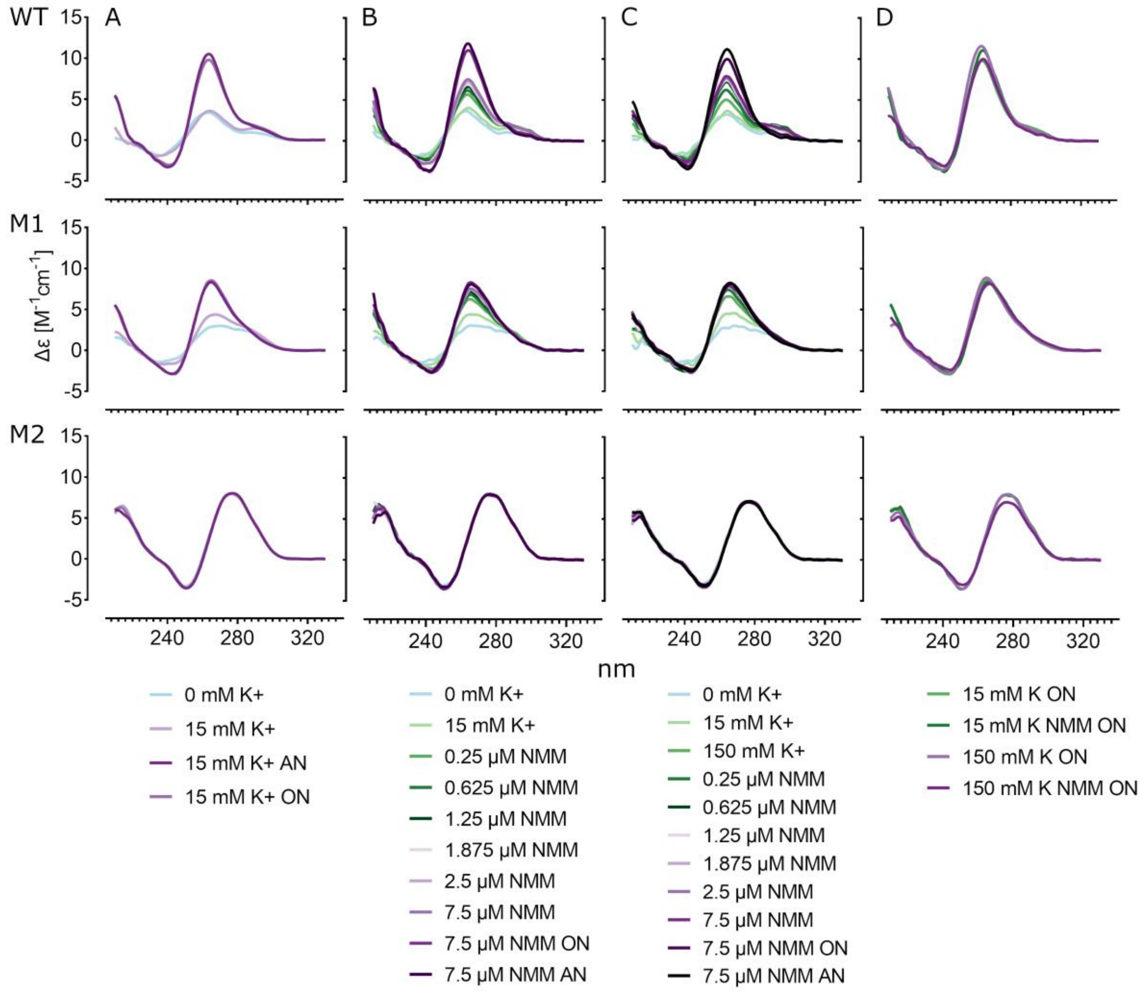

Figure S3. Effect of potassium and NMM concentration on G4 formation and kinetics measured by circular dichroism (CD). (A) CD obtained in final 15mM potassium. **(B)** Increasing NMM concentration in presence of 15mM potassium ions shows acceleration of G4 formation. **(C)** Effect of an increasing NMM concentration on the ellipticity in presence of 150mM potassium ions showing similar results as in 15mM potassium. **(D)** Comparison of overnight incubated samples in both 15mM and 150mM potassium as well as with and without NMM shows negligible differences which suggest that 15mM potassium is fully sufficient to induce G4 formation and NMM have no effect on G4 topology and orderliness. Note that M2 spectra remain unaffected by both NMM and potassium concentration. ON - overnight, AN - annealed.

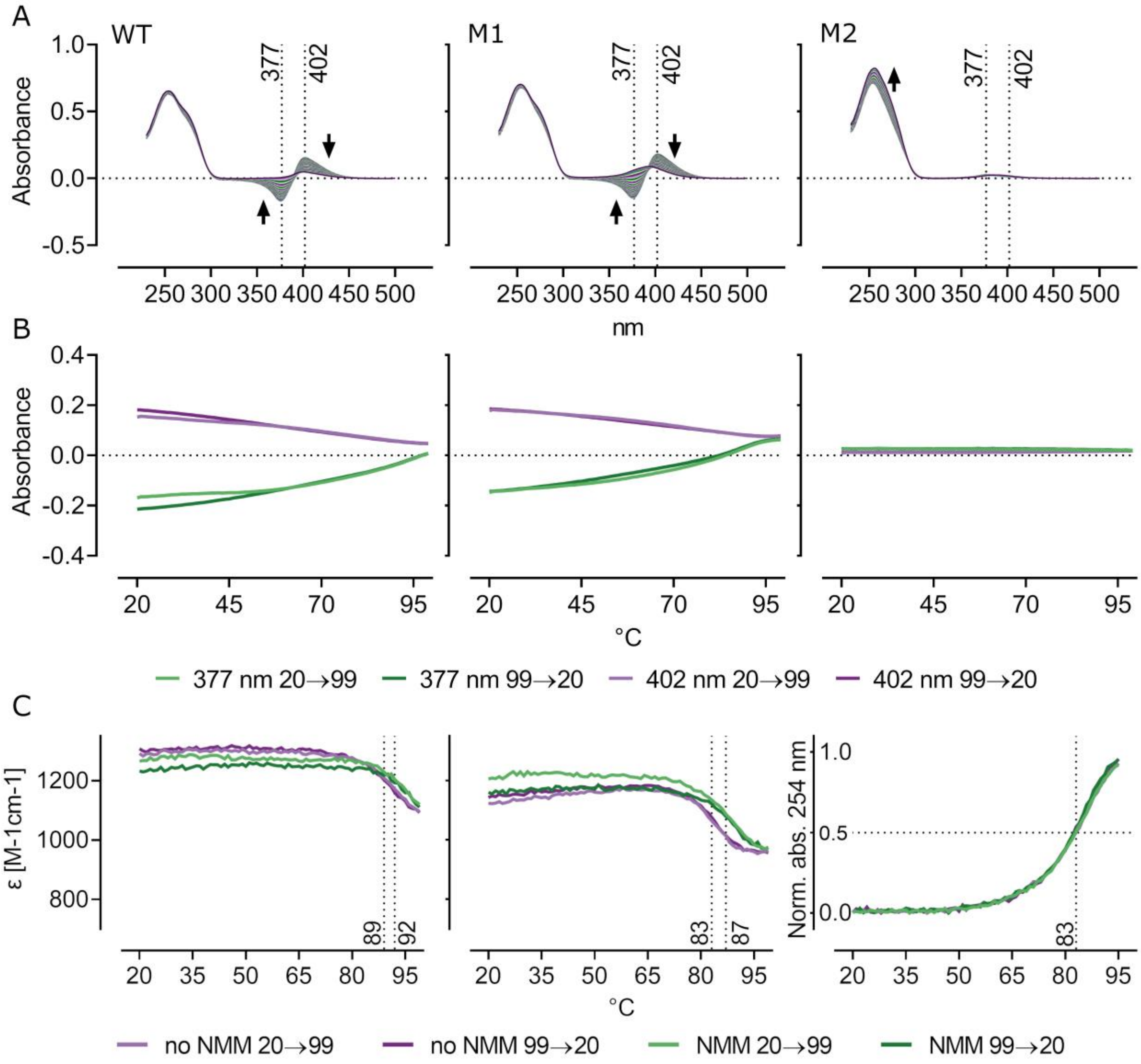

Figure S4. DNA melting assay in 150 mM potassium

(A) Full absorption spectra obtained during heating samples from 20 to 99°C in 150 mM potassium with 7.5 µM NMM present typical peaks at 377 and 402 nm of free and G4-bound NMM respectively. Black arrows indicate peak development with increasing temperature. **(B)** Absorbance at 377 and 402 nm plotted against temperature revealing a disruption of NMM-G4 interaction by increasing temperature and reassociation when cooling down. **(C)** Comparison of melting curves at 297 nm in 150 mM potassium shows a shift towards a higher temperature due to NMM presence. Indicated Tm are rough estimates calculated from normalised data. Melting curves for M2 are calculated as normalised absorbance at 254 nm.

Supplemental file 1. ***Ty1his3-AI*** **sequence**

>Ty1his3-AI
ACGGATTAGAAGCCGCCGAGCGGGCGACAGCCCTCCGACGGAAGACTCTCCTCCGTGCGTCCTCGTCT
TCACCGGTCGCGTTCCTGAAACGCAGATGTGCCTCGCGCCGCACTGCTCCGAACAATAAAGATTCTAC
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