SUPPLEMENTARY DATA

Pre-folded structures govern folding pathways of human telomeric G-quadruplexes

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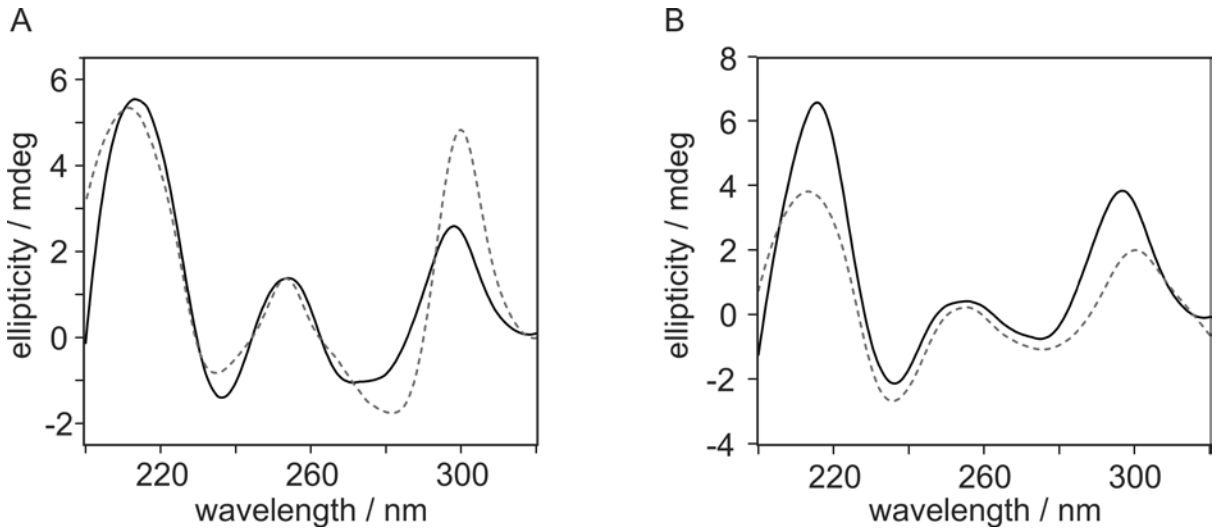

Figure S1. CD spectra. (**A**) of *htel1* at pH 7 (solid line) and pH 5 (dashed line). (**B**) of *htel2* at pH 7 (solid line) and pH 5 (dashed line). CD spectra were recorded at 5 ºC in the absence of cations that promote G-quartet formation at DNA concentration of 1.0 mM.

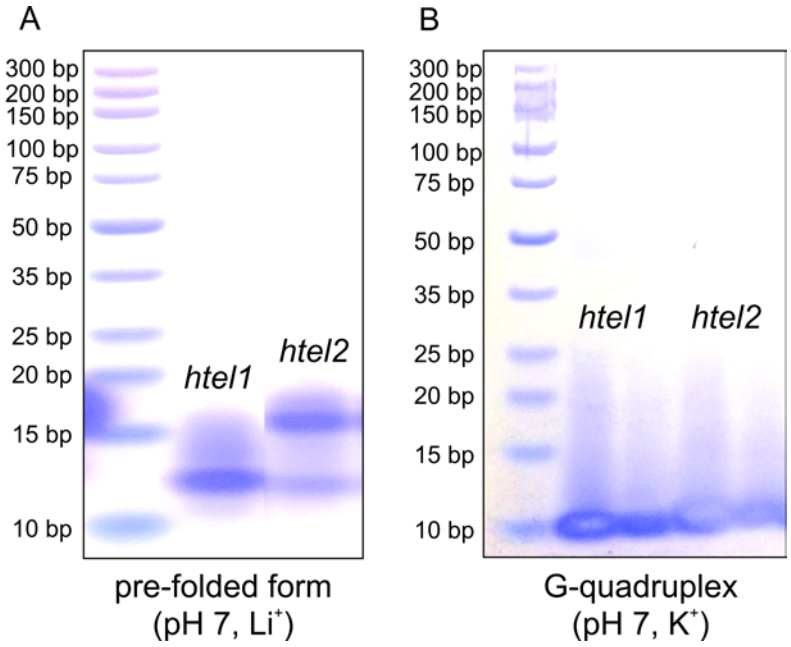

Figure S2. Native PAGE of 23-nt *htel1* d[TAGGG(TTAGGG)$_3$] and 25-nt *htel2* [TAGGG(TTAGGG)$_3$TT]. (**A**) at pH 7 in the presence of 30 mM concentration of Li^+ ions, and (**B**) at pH 7 in the presence of 70 mM concentration of K^+ ions. On the left side of each gel marker was loaded. Gels ran at 5 ºC overnight.

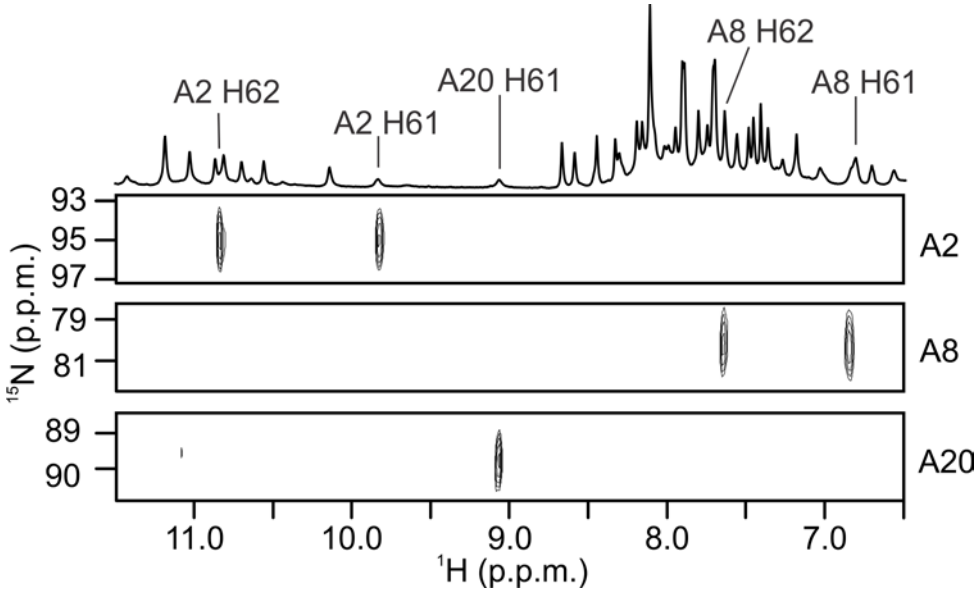

Figure S3. Assignment of the *htel1* amino resonances of adenines at pH 5. Top: 1D ^{1}H NMR spectrum of unlabelled oligonucleotide *htel1*. Bottom: 2D ^{1}H-^{15}N HSQC spectra acquired on partly (~ 8 %) residue-specific ^{15}N, ^{13}C-lablled oligonucleotides. Spectra were recorded in 10 % ^2H_2O on 800 MHz spectrometer at 5 ºC and pH 5 in the absence of cations that promote G-quartet formation at DNA concentration of 1.0 mM.

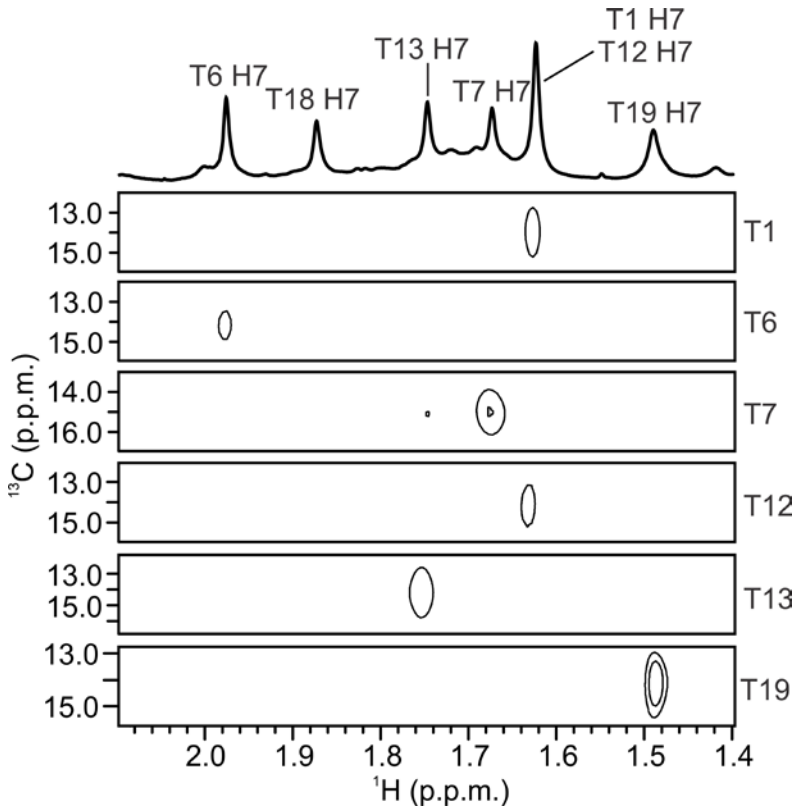

Figure S4. Assignment of the *htel1* methyl resonances of thymines at pH 5**.** Top: 1D ^{1}H NMR spectrum of unlabelled oligonucleotide *htel1*. Bottom: 2D ^{1}H-^{13}C HSQC spectra acquired on partly (~ 8 %) residue-specific ^{15}N, ^{13}C-lablled oligonucleotides. Spectra were recorded in 10 % $^{2}H_2O$ on 600 MHz spectrometer at 5 ºC in the absence of cations that promote G-quartet formation at DNA concentration of 1.0 mM.

Table S1. Imino-imino NOE connectivities determined in 2D NOESY spectrum with mixing time of 150 ms.

Stronger NOE connectivities	**Weaker NOE connectivities**
G3 H1 – T12 H3	G3 H1 – G17 H1
G3 H1 – G16 H1	G4 H1 – G11 H1
G4 H1 – ^{1}T6 H3/G9 H1	T7 H3 – T19 H3
G4 H1- G10 H1	T7 H3 – G21 H1
G5 H1 – G17 H1	
G5 H1 – T18 H3	
G5 H1 – T19 H3	
^{1}T6 H3/G9 H1 – G10 H1	
^{1}T6 H3/G9 H1 – G17 H1	
T12 H3 – G11 H1	
T18 H3 – T19 H3	
^{1}Imino protons of T6 H3 and G9 H1 have isochronous chemical shifts	

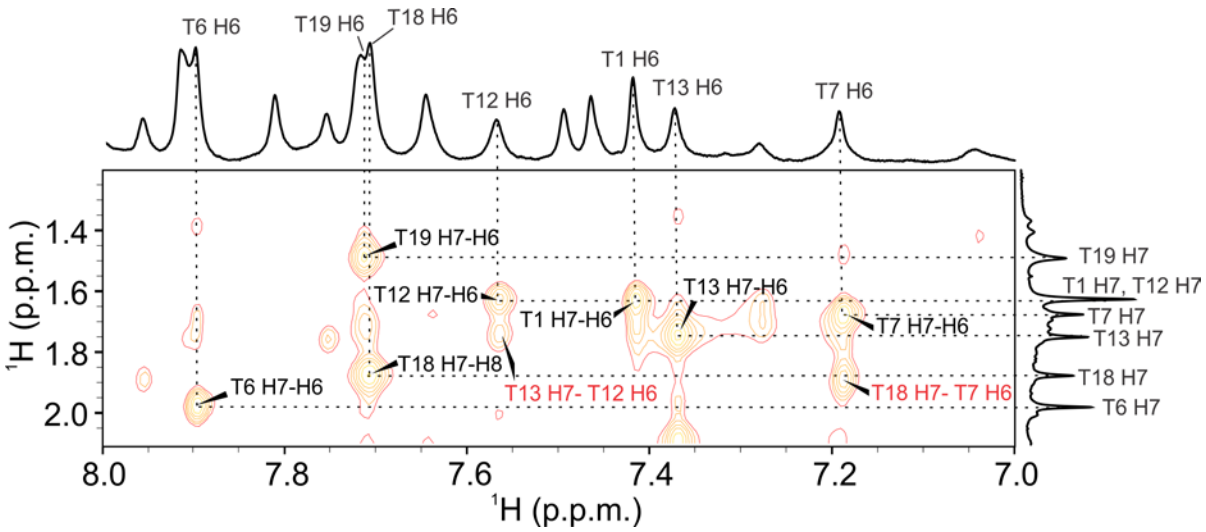

Figure S5. Aromatic-methyl region of 2D NOESY spectrum (τ_m=150 ms) of oligonucleotide *htel1*. Spectrum was recorded in 10 % ^2H_2O on 800 MHz spectrometer at 5 ºC and pH 5 in the absence of cations that promote G-quartet formation at DNA concentration of 1.0 mM.

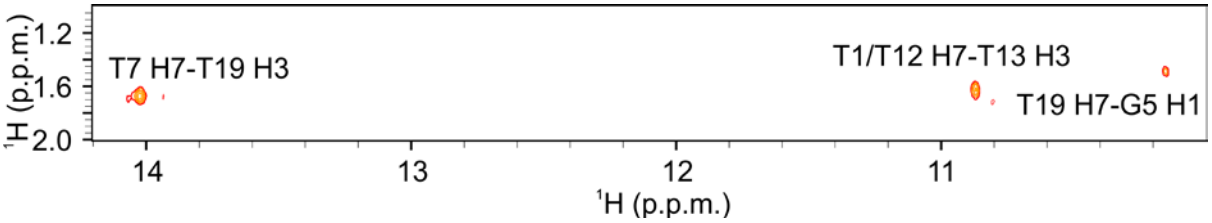

Figure S6. Imino-methyl region of 2D NOESY spectrum (τ_m=120 ms) of oligonucleotide *htel1*. Spectrum was recorded in 10 % ^2H_2O on 800 MHz spectrometer at 5 ºC and pH 5 in the absence of cations that promote G-quartet formation at DNA concentration of 1.0 mM.

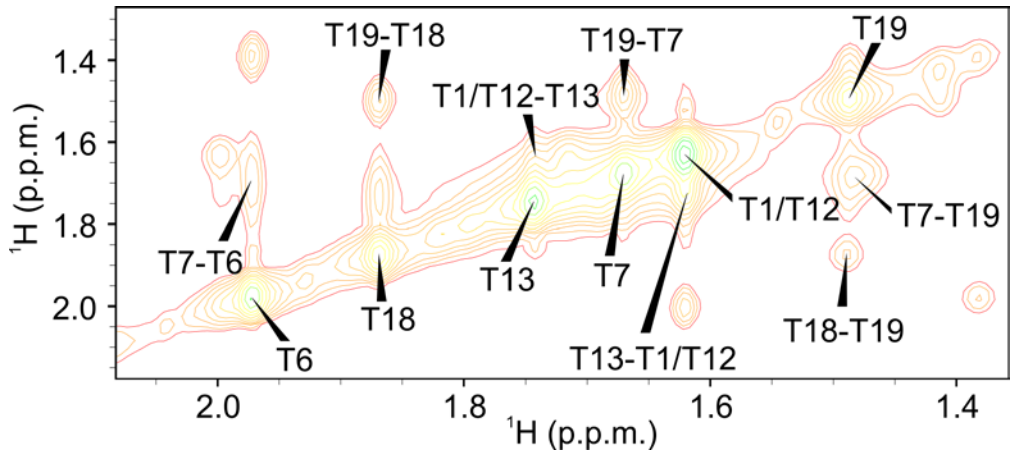

Figure S7. Methyl-methyl region of 2D NOESY spectrum (τ_m=150 ms) of oligonucleotide *htel1*. Spectrum was recorded in 10 % ^2H_2O on 800 MHz spectrometer at 5 ºC and pH 5 in the absence of cations that promote G-quartet formation at DNA concentration of 1.0 mM.

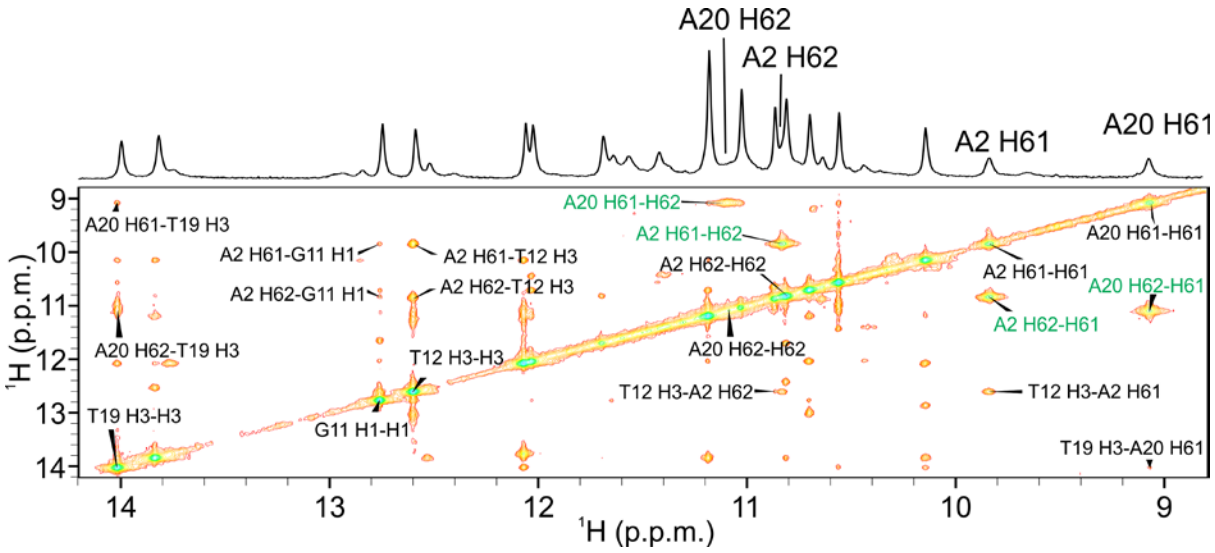

Figure S8. Amino-amino and imino-amino region of 2D NOESY spectrum (τ_m=150 ms) of oligonucleotide *htel1*. Spectrum was recorded in 10 % ^2H_2O on 800 MHz spectrometer at 5 ºC and pH 5 in the absence of cations that promote G-quartet formation at DNA concentration of 1.0 mM.

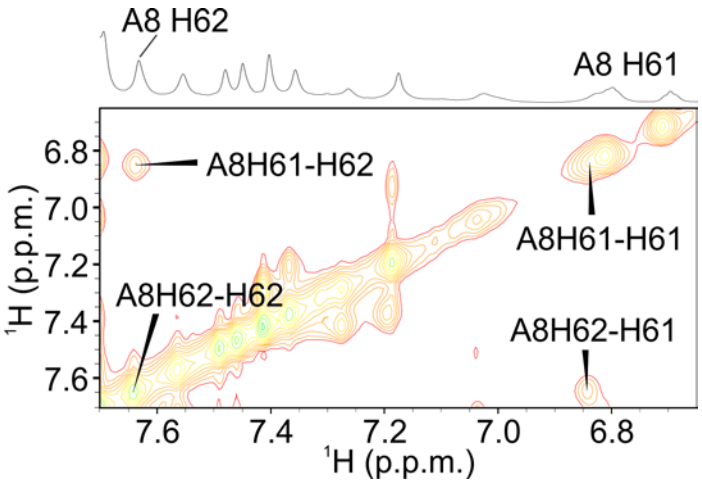

Figure S9. Amino-amino region of 2D NOESY spectrum (τ_m=150 ms) of oligonucleotide *htel1*. Spectrum was recorded in 10 % ^2H_2O on 800 MHz spectrometer at 5 ºC and pH 5 in the absence of cations that promote G-quartet formation at DNA concentration of 1.0 mM.

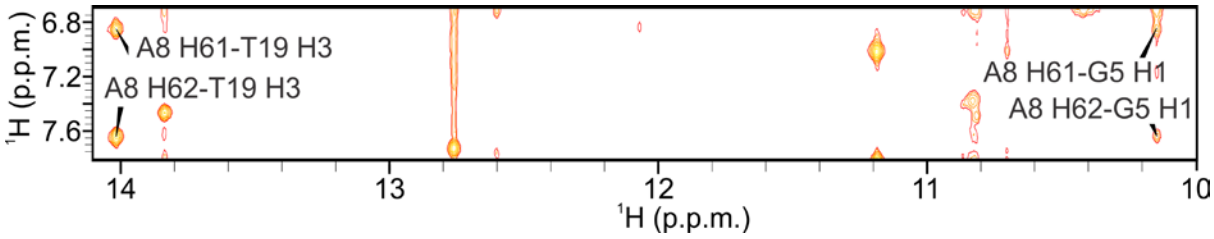

Figure S10. Imino-amino region of 2D NOESY spectrum (τ_m=150 ms) of oligonucleotide *htel1*. Spectrum was recorded in 10 % ^2H_2O on 800 MHz spectrometer at 5 ºC and pH 5 in the absence of cations that promote G-quartet formation at DNA concentration of 1.0 mM.

Table S2. NOE connectivities used to determine structure at pH 5.

H1/H3-H1/H3	H1/H3-H7	H7-H7	H7-H6 (T)	H61-H62 (A)	H61/H62-H1/H3
G3 – T12	T19 – T7	T7 – T6	T13 – T12	A2 – A2	A2 – G11
G3 – G16	G5 – T19	T19 – T18	T18 – T7	A8 – A8	A2 – T12
T6 H3/G9 – G10	T13 – T1/T12	T1/T12 – T13		A20 – A20	A8 – T19
T6 H3/G9 – G17		T19 – T7			A8 – G5
G5 – G17					A20 – T19
G5 – T18					
G5 – T19 H3					
G4 – T6/G9					
G4 – G10					
T12 – G11					
T18 – T19					
G3 – G17					
G4 – G11					
T7 – T19					
T7 – G21					

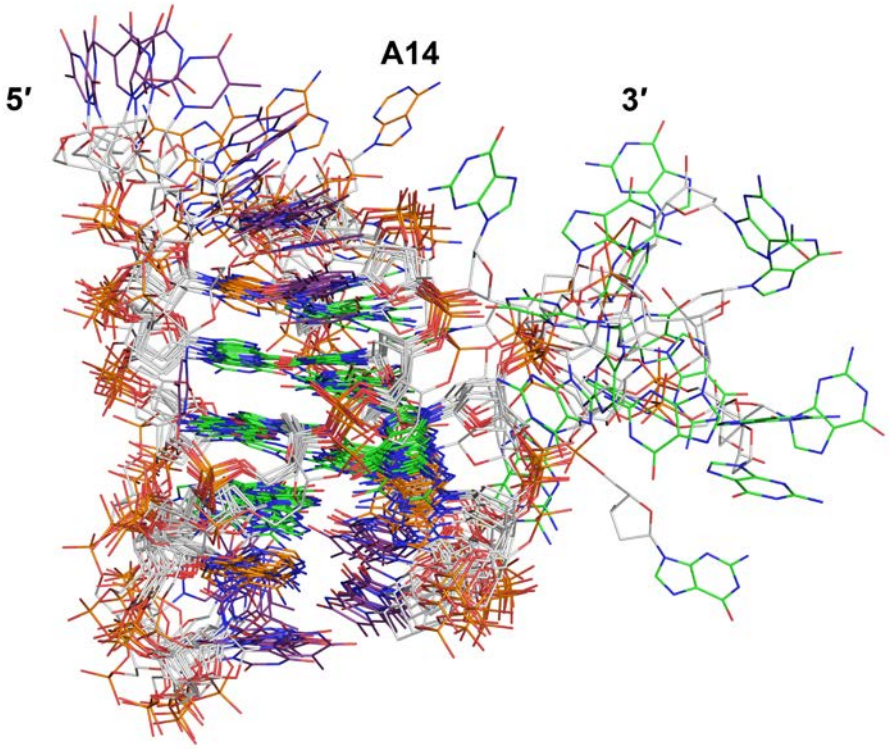

Figure S11. Structural ensemble of ten lowest-energy structures of *htel1* pre-folded form at pH 5. Guanines are coloured in green; adenines are orange; thymine residues are purple; sugar rings are grey.

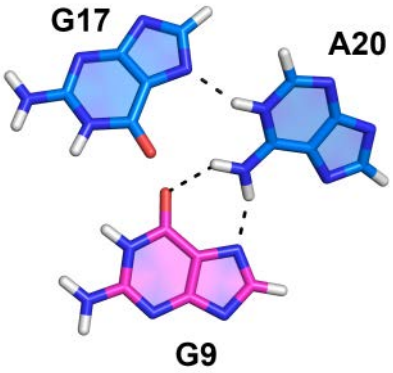

Figure S12. Hydrogen bond formation. Hydrogen bond formation between A20 and G9 as well as G17.

Table S3. Modified oligonucleotides.

	Modified oligonucleotide sequences
htel1 A2T	d(T**T**GGGTTAGGGTTAGGGTTAGGG)
htel1 A8T	d(TAGGGTT**T**GGGTTAGGGTTAGGG)
htel1 A14T	d(TAGGGTTAGGGTT**T**GGGTTAGGG)
htel1 A20T	d(TAGGGTTAGGGTTAGGGTT**T**GGG)
htel1 A2,8T	d(T**T**GGGTT**T**GGGTTAGGGTTAGGG)
htel1 A2,20T	d(T**T**GGGTTAGGGTTAGGGTT**T**GGG)
htel1 A2,8,20T	d(T**T**GGGTT**T**GGGTTAGGGTT**T**GGG)
htel1 ΔAGGG	d(TAGGGTTAGGGTTAGGGTT)
htel1 ΔGGG	d(TAGGGTTAGGGTTAGGGTTA)
htel1 ΔGG	d(TAGGGTTAGGGTTAGGGTTAG)
htel1 ΔG	d(TAGGGTTAGGGTTAGGGTTAGG)

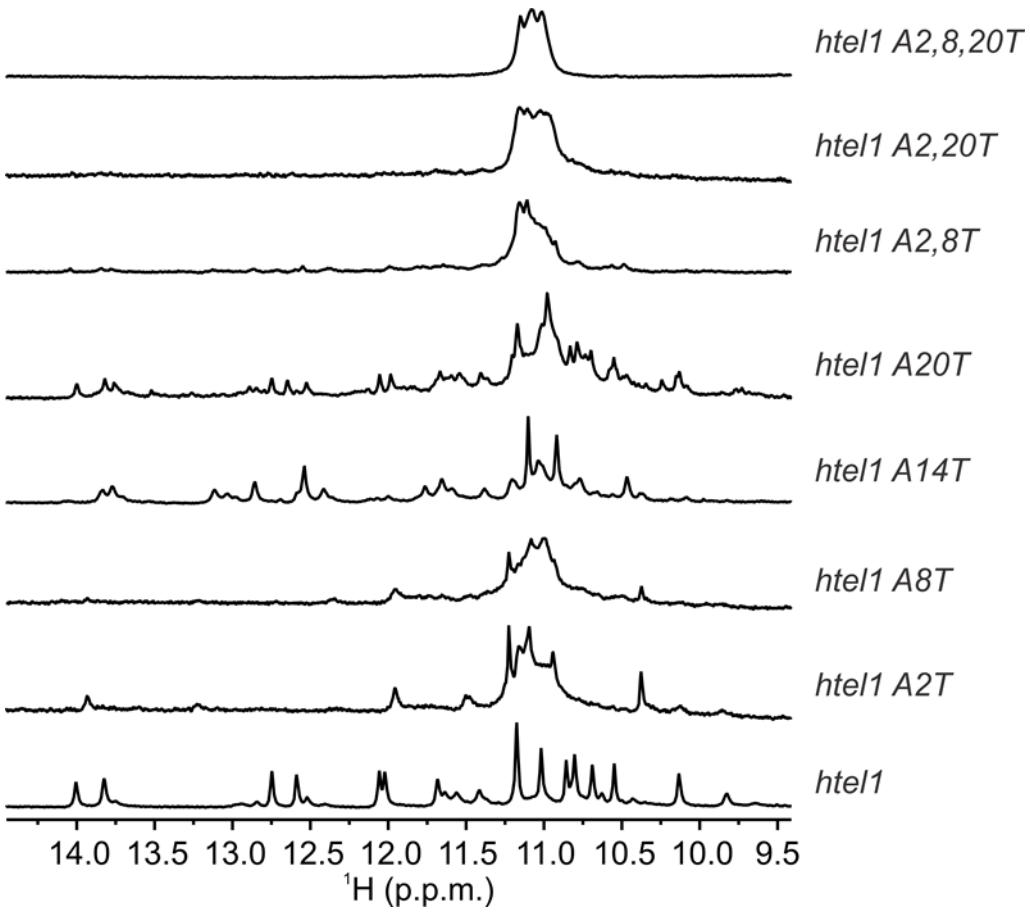

Figure S13. Comparison of imino region of 1D ^{1}H NMR spectra of oligonucleotide *htel1* (bottom) and modified sequences. Adenine residues were replaced with thymine residues in the sequence. Spectra were recorded in 10 % $^{2}H_2O$ on 800 MHZ spectrometer at 5 ºC and pH 5 in the absence of cations that promote G-quartet formation at DNA concentration of 1.0 mM.

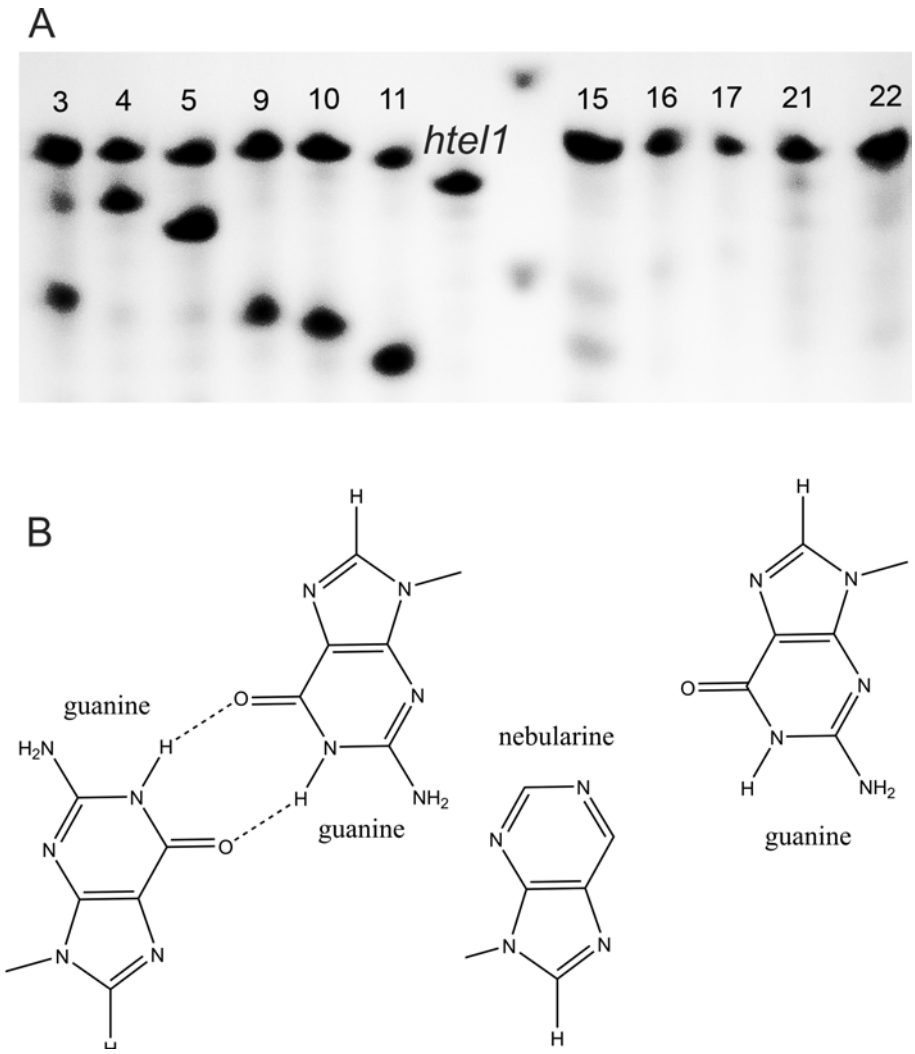

Figure S14. Site-specific replacements of guanine to nebularine residues. (**A**) Native PAGE of 23-nt *htel1* d[TAGGG(TTAGGG)$_3$] and of oligonucleotides with site-specific replacement of guanine to nebularine residues. Number above bands indicates at which position within oligonucleotide *htel1* is nebularine instead of guanine residue. (**B**) Schematic presentation of hydrogen bonds within GG N1-carbonyl base pair as well as the absence of hydrogen bonds between nebularine and guanine residues.

Table S4. Comparison of imino proton chemical shifts at pH 5 and 7. Chemical shifts were measured at 5 ºC. All shifts are referenced to DSS.

	δ at pH 5	δ at pH 7
T1	11.05	10.69
G3	10.83	11.82
G4	10.70	12.96
G5	10.16	12.88
T6	11.21	13.89
T7	10.58	10.38
G9	11.21	11.47
G10	12.09	10.56
G11	12.78	11.62
T12	12.60	~ 11.10
T13	10.87	10.51
G15	/	12.83
G16	11.72	11.57
G17	13.82	12.57
T18	12.09	13.73
T19	14.02	/
G21	11.46	11.37
G22	/	~ 11.05
G23	/	10.69
/ - Not determined because of the absence of the signal. Chemical shifts are in ppm.		