Supplemental information for:

Dinuclear Nickel(II) Supramolecular Helicates Down-regulate Gene Expression in Human Cells by Stabilizing DNA G-quadruplexes Formed in the Promoter Regions

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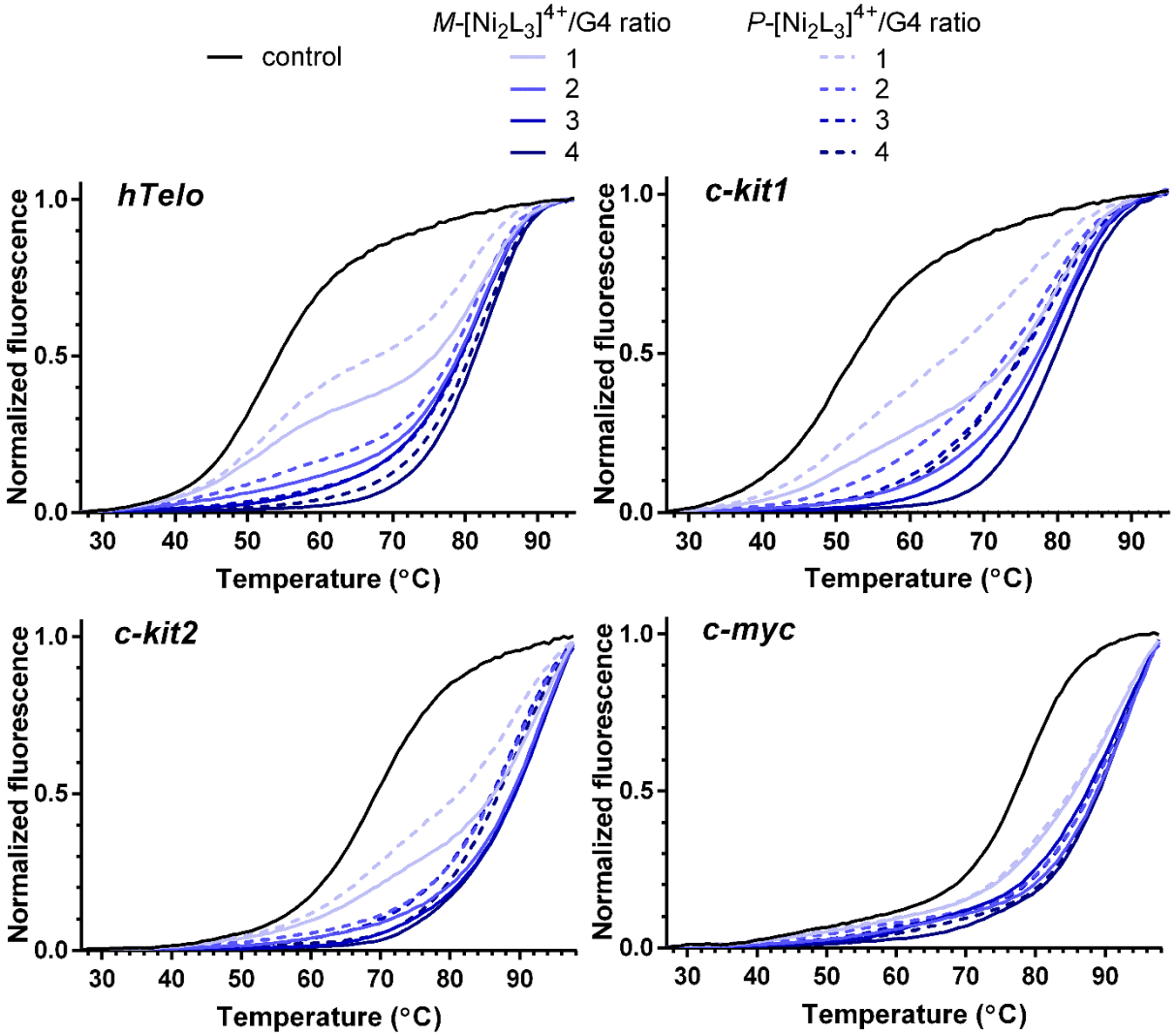

Figure S1. FRET melting curves for *hTelo*, *c-myc*, *c-kit1*, and *c-kit2* DNA G4s (0.4 μM) mixed with *M*- and *P*-$[Ni_2L_3]^{4+}$ at different cylinder/G4 ratios (indicated in the figure). The buffer conditions were 10 mM potassium phosphate (pH 7).

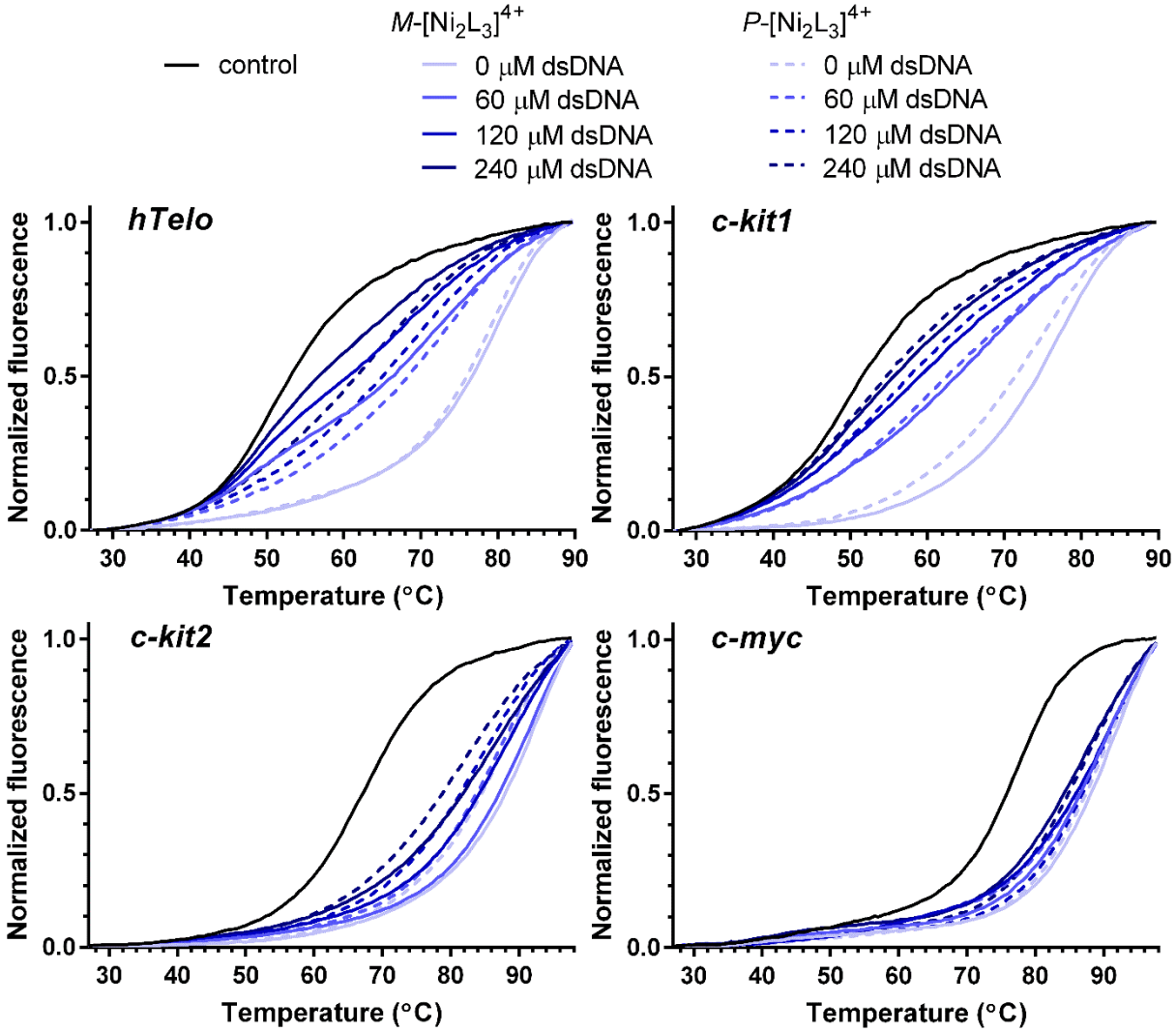

Figure S2. FRET melting curves for *hTelo*, *c-myc*, *c-kit1*, and *c-kit2* DNA G4s (0.4 μM) in the absence and in the presence of 0.8 μM *M*- and *P*-$[Ni_2L_3]^{4+}$ and increasing concentrations (indicated in the figure) of dsDNA. The buffer conditions were 10 mM potassium phosphate (pH 7).

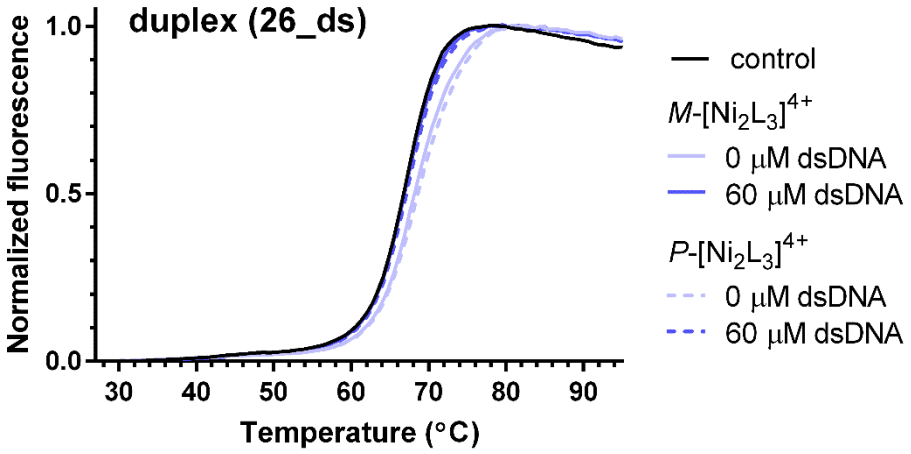

Figure S3. FRET melting curves for the DNA duplex (0.4 µM) in the absence and in the presence of 0.8 µM *M*- and *P*-$[Ni_2L_3]^{4+}$ and 60 µM dsDNA. The buffer conditions were 10 mM potassium phosphate (pH 7).

Stabilizing ability of the cylinders towards G4s in the presence of ds DNA

This quantification can only be done very approximately based on the available data. If we take into account the results obtained using the FRET assay for the selectivity of the cylinders towards *c-kit1* (0.4 µM) in the presence of double-stranded B-DNA (dsDNA) (240 µM) (in this case, the smallest selectivity of the cylinders towards G4 was observed), then the stabilizing effect of the cylinders decreased to about 10% - T_m dropped from 26.4 °C to 3.4 °C for the M-enantiomer and from 24.2 °C to 2.3 °C for the P-enantiomer (Table S1). If the concentration of *c-kit1* and dsDNA according to the number of bases are compared, then the concentration of *c-kit1* is 8.4 µM (0.4 x 21, since it is a 21-mer) and the concentration of dsDNA is 240 µM, which means that dsDNA is in ~29-fold surplus. If even with such an excess of dsDNA cylinders stabilize G4, it seems reasonable to conclude that they show a certain, albeit weak, selectivity towards G4. If the same comparison is made in terms of binding sites, it is possible to assume that on dsDNA, due to its length, the cylinder binds to 5 base pairs and, at the same time, also one binding site to G4. Then, a comparison of the concentration of binding sites results for 24 µM dsDNA (240 µM / 10 bases) versus 0.4 µM for G4, which corresponds to a ratio of 60:1, i.e., an even greater excess of binding sites on dsDNA compared to G4. In this case, the selectivity of the cylinders appears even higher.

The loss of the stabilizing ability of the cylinders towards G4s (0.4 µM) in the presence of 240 µM dsDNA is shown in Table S1. The data in this table indicate that the selectivity of the cylinders towards G4 over ds DNA is highest in the case of *c-myc* and *c-kit2* and lowest in the case of *c-kit1*.

Table S1. Loss of the stabilizing ability of the cylinders towards G4s (0.4 μM) in the presence of 240 μM ds DNA.

Cylinder	ΔT_m (°C) in the absence of dsDNA	ΔT_m (°C) in the presence of 240 μM dsDNA	% of stabilization loss
c-myc			
M-$[Ni_2L_3]^{4+}$	13.2	10.0	24.2
P-$[Ni_2L_3]^{4+}$	13.4	10.3	23.1
hTelo			
M-$[Ni_2L_3]^{4+}$	28.3	13.3	53.0
P-$[Ni_2L_3]^{4+}$	27.6	13.2	52.2
c-kit1			
M-$[Ni_2L_3]^{4+}$	26.4	3.4	87.1
P-$[Ni_2L_3]^{4+}$	24.2	2.3	90.5
c-kit2			
M-$[Ni_2L_3]^{4+}$	23.2	18.0	22.4
P-$[Ni_2L_3]^{4+}$	20.2	14.1	30.2

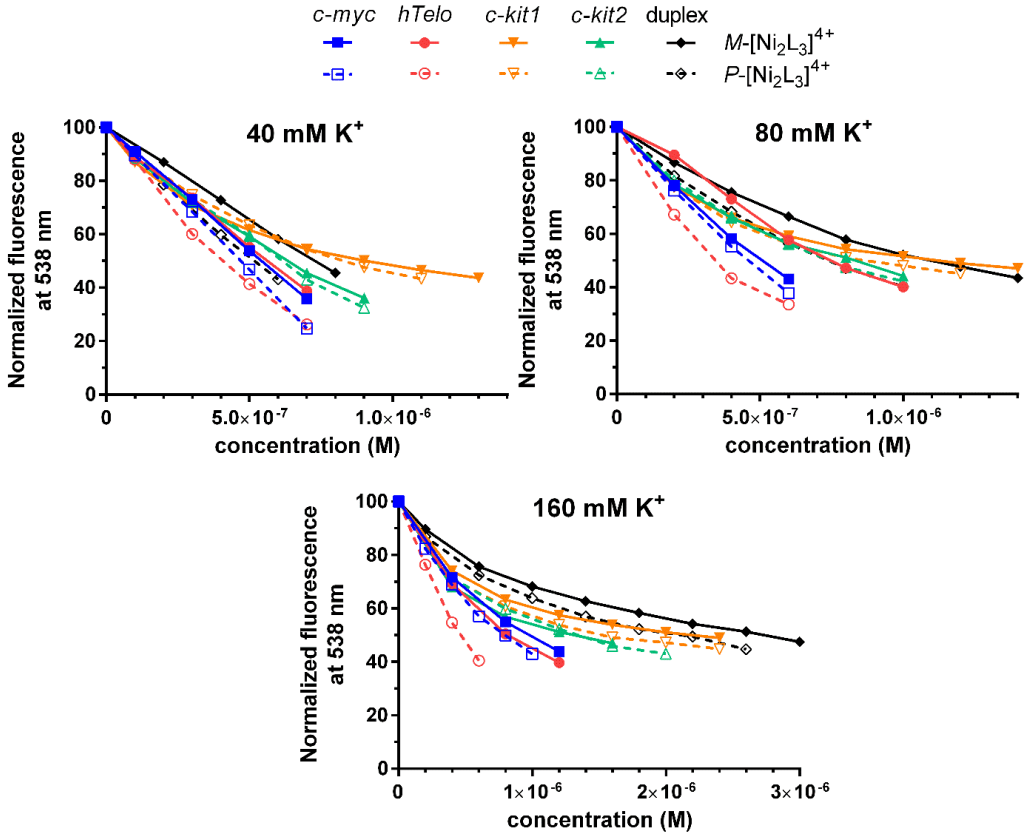

Figure S4. Displacement of thiazole orange from *hTelo*, *c-myc*, *c-kit1*, and *c-kit2* DNA G4s and from DNA duplex (26_ds) by *M*- and *P*-$[Ni_2L_3]^{4+}$ in 10 mM potassium phosphate (pH 7) and various concentrations of KCl.

Table S2. DC_{50} values (μM) for DNA G4s (*hTelo*, *c-myc*, *c-kit1*, *c-kit2*) and DNA duplex (26_ds) in 10 mM potassium phosphate buffer (pH 7) and in the presence of 30, 70, and 150 mM KCl determined by FID upon addition of cylinders.

Cylinder	TO displacement (DC_{50})				
	hTelo	***c-myc***	***c-kit1***	***c-kit2***	**duplex**
40 mM K^+					
***M*-$[Ni_2L_3]^{4+}$**	0.55 ± 0.01	0.54 ± 0.01	0.89 ± 0.03	0.62 ± 0.02	0.71 ± 0.04
***P*-$[Ni_2L_3]^{4+}$**	0.40 ± 0.01	0.42 ± 0.03	0.82 ± 0.02	0.61 ± 0.01	0.50 ± 0.03
80 mM K^+					
***M*-$[Ni_2L_3]^{4+}$**	0.71 ± 0.03	0.51 ± 0.01	1.08 ± 0.05	0.79 ± 0.04	1.09 ± 0.04
***P*-$[Ni_2L_3]^{4+}$**	0.34 ± 0.01	0.46 ± 0.01	0.85 ± 0.04	0.75 ± 0.02	0.74 ± 0.02
160 mM K^+					
***M*-$[Ni_2L_3]^{4+}$**	0.85 ± 0.06	1.06 ± 0.08	1.89 ± 0.09	1.35 ± 0.05	2.9 ± 0.1
***P*-$[Ni_2L_3]^{4+}$**	0.43 ± 0.03	0.70 ± 0.07	1.76 ± 0.08	1.27 ± 0.06	2.5 ± 0.2

Table S3. Binding selectivity indexes of cylinders towards *hTelo*, *c-myc*, *c-kit1*, and *c-kit2* DNA G4s in 10 mM potassium phosphate buffer (pH 7) and in the presence of 30, 70, and 150 mM KCl.

Cylinder	Selectivity (DC_{50})			
	duplex/ *hTelo*	**duplex/ *c-myc***	**duplex/ *c-kit1***	**duplex/ *c-kit2***
40 mM K^+				
***M*-$[Ni_2L_3]^{4+}$**	1.29 ± 0.08	1.31 ± 0.08	0.80 ± 0.05	1.15 ± 0.07
***P*-$[Ni_2L_3]^{4+}$**	1.25 ± 0.08	1.2 ± 0.1	0.61 ± 0.04	0.82 ± 0.05
80 mM K^+				
***M*-$[Ni_2L_3]^{4+}$**	1.54 ± 0.09	2.14 ± 0.09	1.01 ± 0.06	1.38 ± 0.09
***P*-$[Ni_2L_3]^{4+}$**	2.18 ± 0.09	1.61 ± 0.06	0.87 ± 0.04	0.99 ± 0.04
160 mM K^+				
***M*-$[Ni_2L_3]^{4+}$**	3.4 ± 0.3	2.7 ± 0.2	1.53 ± 0.09	2.1 ± 0.1
***P*-$[Ni_2L_3]^{4+}$**	5.8 ± 0.6	3.6 ± 0.5	1.4 ± 0.1	2.0 ± 0.2

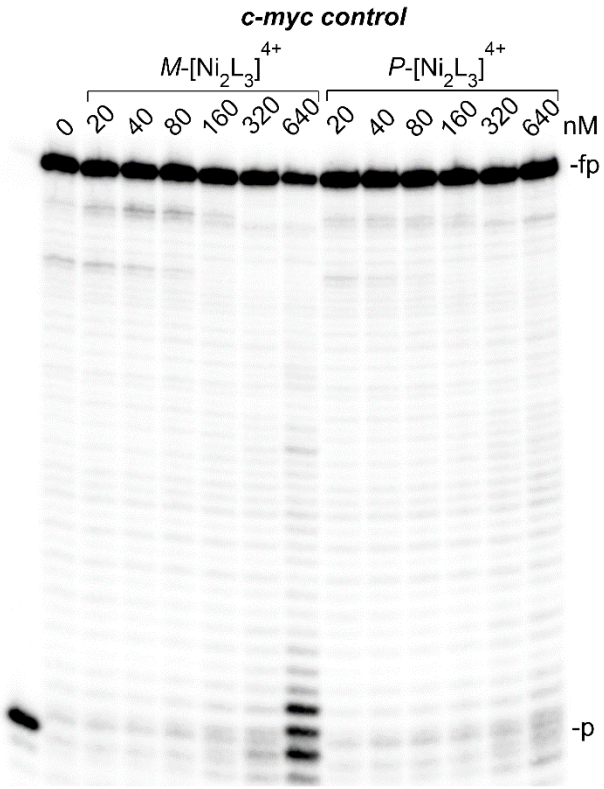

Figure S5. Autoradiogram of a 12% PAA sequencing gel with products of *Taq* polymerase DNA synthesis across *c-myc* control template (30 nM) at 55 ºC in the presence of increasing concentrations of *M*- and *P*-$[Ni_2L_3]^{4+}$. *fp* and *p* correspond to full-length product and primer, respectively.