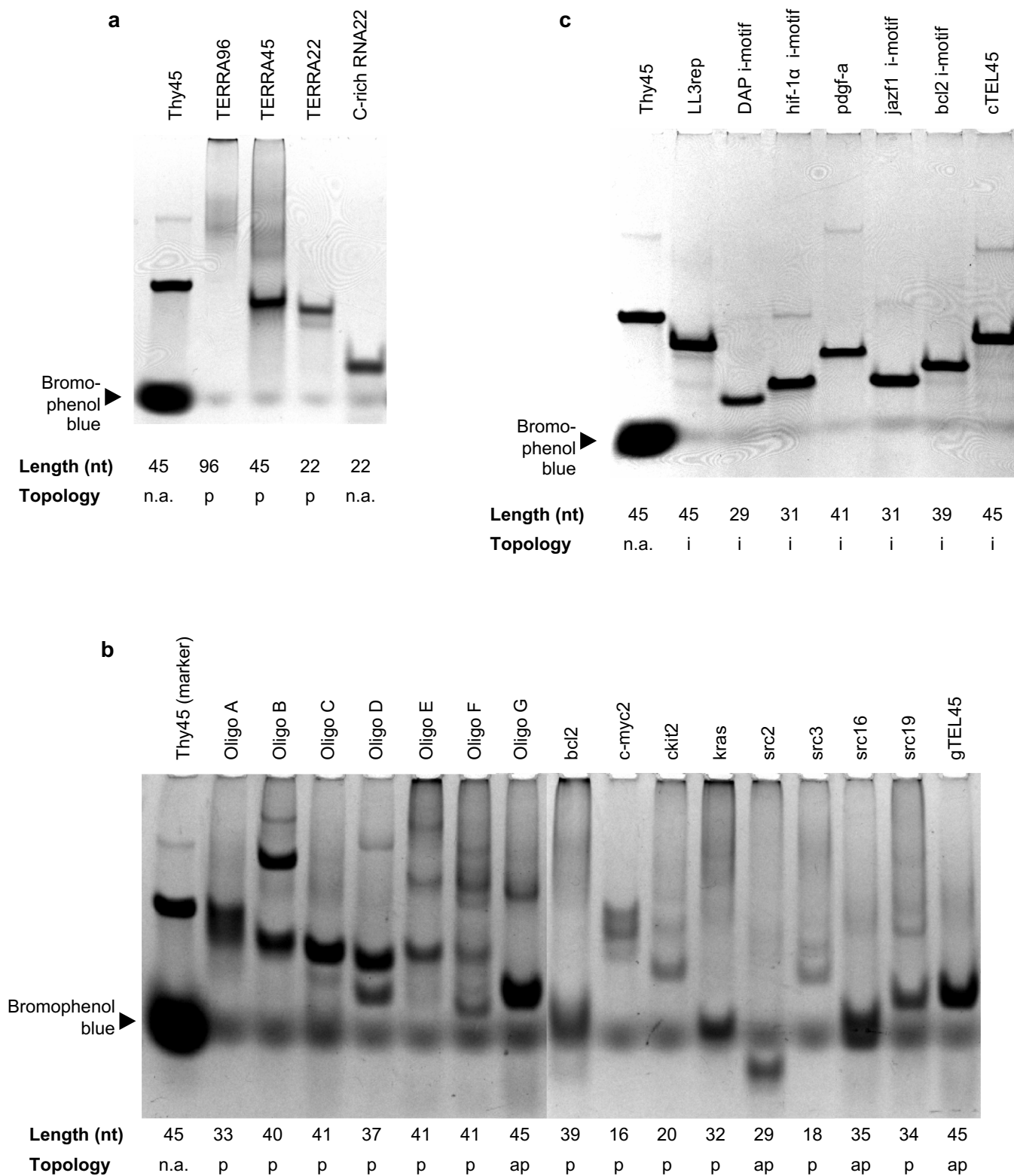
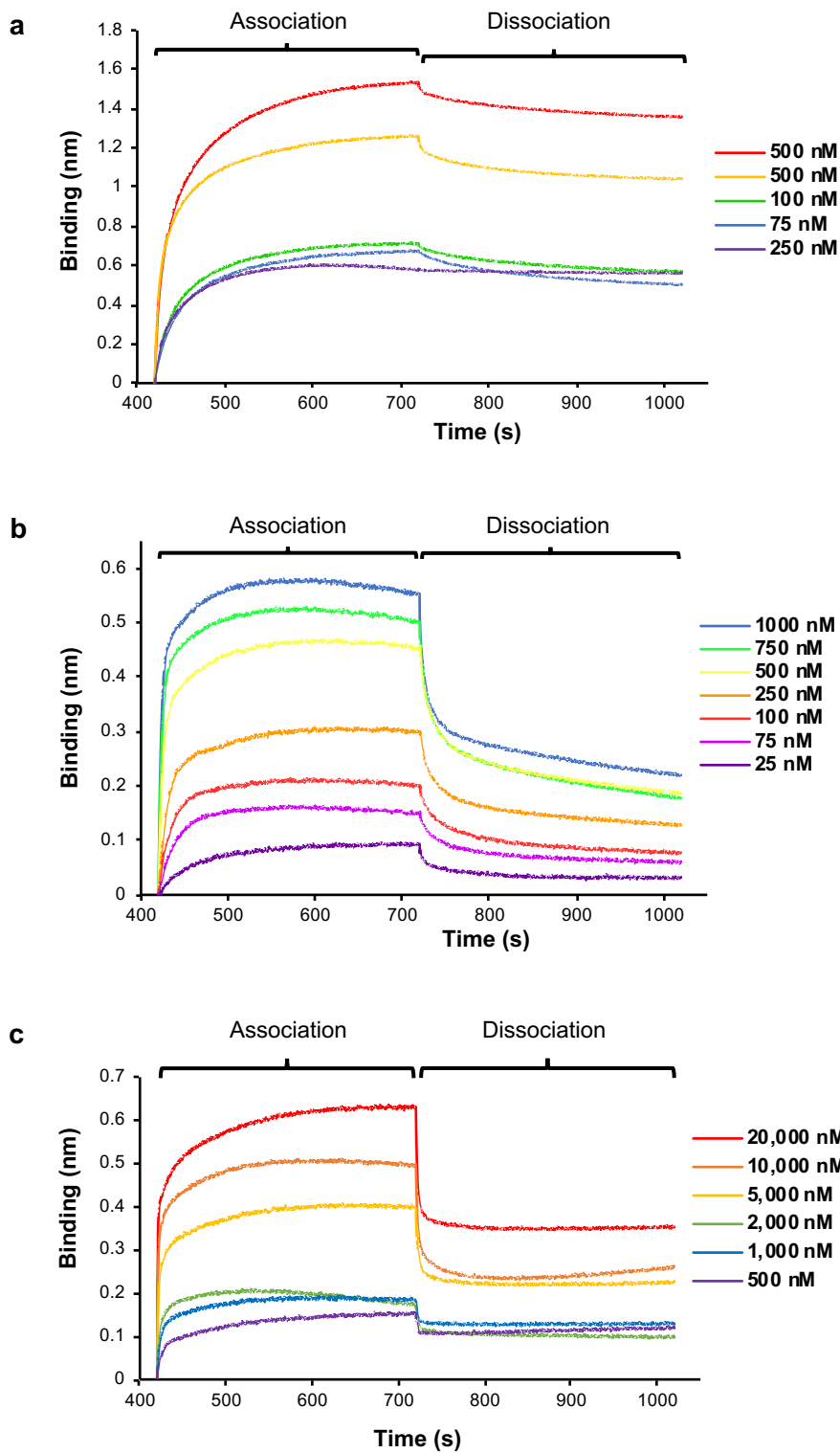


Name	Sequence	Length	Topology
TERRA96	rGrGrG rUrUrA rGrGrG rUrUrA rGrGrG rUrUrA rGrGrG rUrUrA rGrGrG rUrUrA rGrGrG rUrUrA rGrGrG rUrUrA rGrGrG rUrUrA rGrGrG rUrUrA rGrGrG rUrUrA rGrGrG rUrUrA rGrGrG rUrUrA rGrGrG rUrUrA rGrGrG rUrUrA rGrGrG rUrUrA rGrGrG rUrUrA	96	p
TERRA45	rGrGrG rUrUrA rGrGrG rUrUrA rGrGrG rUrUrA rGrGrG rUrUrA rGrGrG rUrUrA rGrGrG rUrUrA rGrGrG rUrUrA rGrGrG	45	p
TAM-TERRA45	rGrGrG rUrUrA rGrGrG rUrUrA rGrGrG rUrUrA rGrGrG rUrUrA rGrGrG rUrUrA rGrGrG rUrUrA rGrGrG rUrUrA rGrGrG rU/TAM/	46	p
mutTERRA45	rGrGrG rUrUrA rGrUrG rUrUrA rGrUrG rUrUrA rGrGrG rUrUrA rGrUrG rUrUrA rGrGrG rUrUrA rGrUrG rUrUrA rGrGrG	45	pf
TERRA22	rArGrG rGrUrU rArGrG rGrUrU rArGrG rGrUrU rArGrG rG	22	p
rC-rich22	rArCrC rCrArA rUrCrC rCrArA rUrCrC rCrArA rUrCrC rC	22	i
gTEL45	GGG TTA GGG TTA GGG TTA GGG TTA GGG TTA GGG TTA GGG TTA GGG	45	ap
Thy45	TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT	45	n/a
Oligo A	TGG GTG GGT GGG TGG GTG GGT GGG TGG GTG GGT	33	p
Oligo B	TGG GTT GGG TTG GGT TGG GTT GGG TTG GGT TGG GTT GGG T	40	p
Oligo C	TGG GTT AGG GTG GGT TAG GGT GGG TTA GGG TGG GTT AGG GT	41	p
Oligo D	TGG GTG GGT TAG GGT GGG TGG GTG GGT TAG GGT GGG T	37	p
Oligo E	TGG GTG GGT TAG GGT TAG GGT GGG TGG GTT AGG GTT AGG GT	41	p
Oligo F	TGG GTT AGG GTT AGG GTG GGT GGG TTA GGG TTA GGG TGG GT	41	p
Oligo G	TGG GTT AGG GTT AGG GTT AGG GTG GGT TAG GGT TAG GGT TAG GGT	45	ap
bcl2	AGG GGC GGG CGC GGG AGG AAG GGG GCG GGA GCG GGG CTG	39	p
c-myc2	GGG TGG GTA GGG TGG G	16	p
ckit2	GGG CGG GCG CCA GGG AGG GG	20	p
kras	AGG GCG GTG TGG GAA GAG GGA AGA GGG GGA GG	32	p
src2	GGG GCA GCT GGG TCG CTC GGG GAA CGG GG	29	ap
src3	GGG AGG GAG GGC TGG GGG	18	p
src16	GGG CTG CTG GGA CTG GTT GGG AGG GCA CTC TGG GG	35	ap
src19	GGG TCC CCT GGG CCT GGG CGG GAG CGG GGA GGG G	34	p
LL3rep	TCG TTC CGT TTT TCG TTC CGT TTT TCG TTC CGT TTT TCG TTC CGT	45	i
DAP i-motif	CCC CCG CCC CCG CCC CCG CCC CCG CCC CC	29	i
hif-1 α i-motif	CGC GCT CCC GCC CCC TCT CCC CTC CCC GCG C	31	i
pdgf-a	CCG CGC CCC TCC CCC GCC CCC GCC CCC GCC CCC CCC CCC CC	41	i
jazf1 i-motif	CCC CCC CCG CCC CCG CCC CCG CCC TCC CCC C	31	i
bcl2 i-motif	CAG CCC CGC TCC CGC CCC CTT CCT CCC GCG CCC GCC CCT	39	i
cTEL45	CCC TAA CCC TAA CCC TAA CCC TAA CCC TAA CCC TAA CCC TAA CCC	45	i

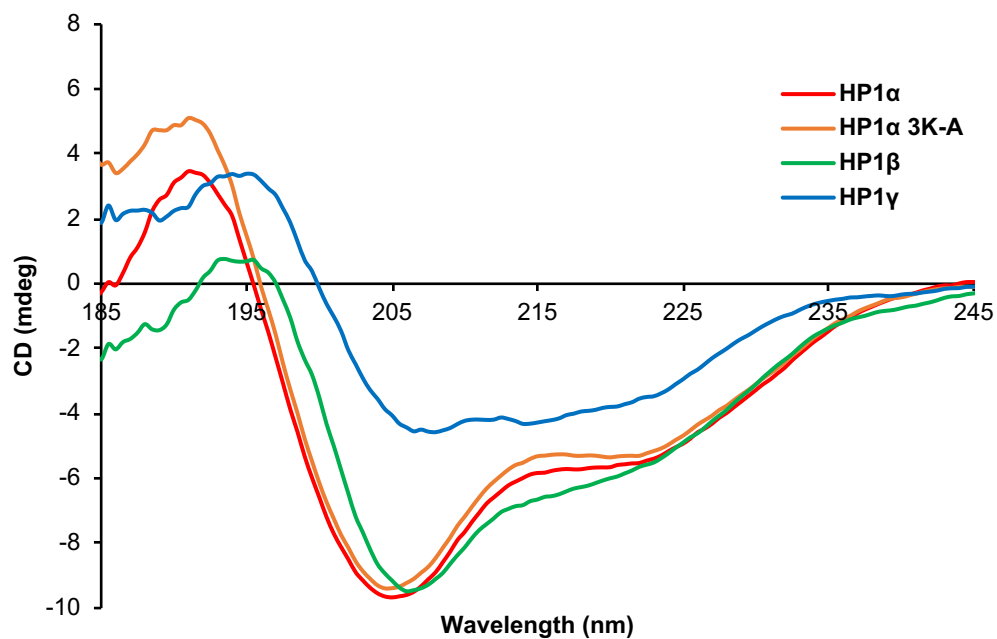
Supplementary Table 1. Sequences used in Biolayer Interferometry (BLI) and Circular Dichroism (CD) spectroscopy. G4 topology (as determined by CD spectroscopy) of each oligonucleotide listed where (a) denotes anti-parallel, (p) denotes parallel topology, (pf) denotes partially folded and (i) denotes i-motif.



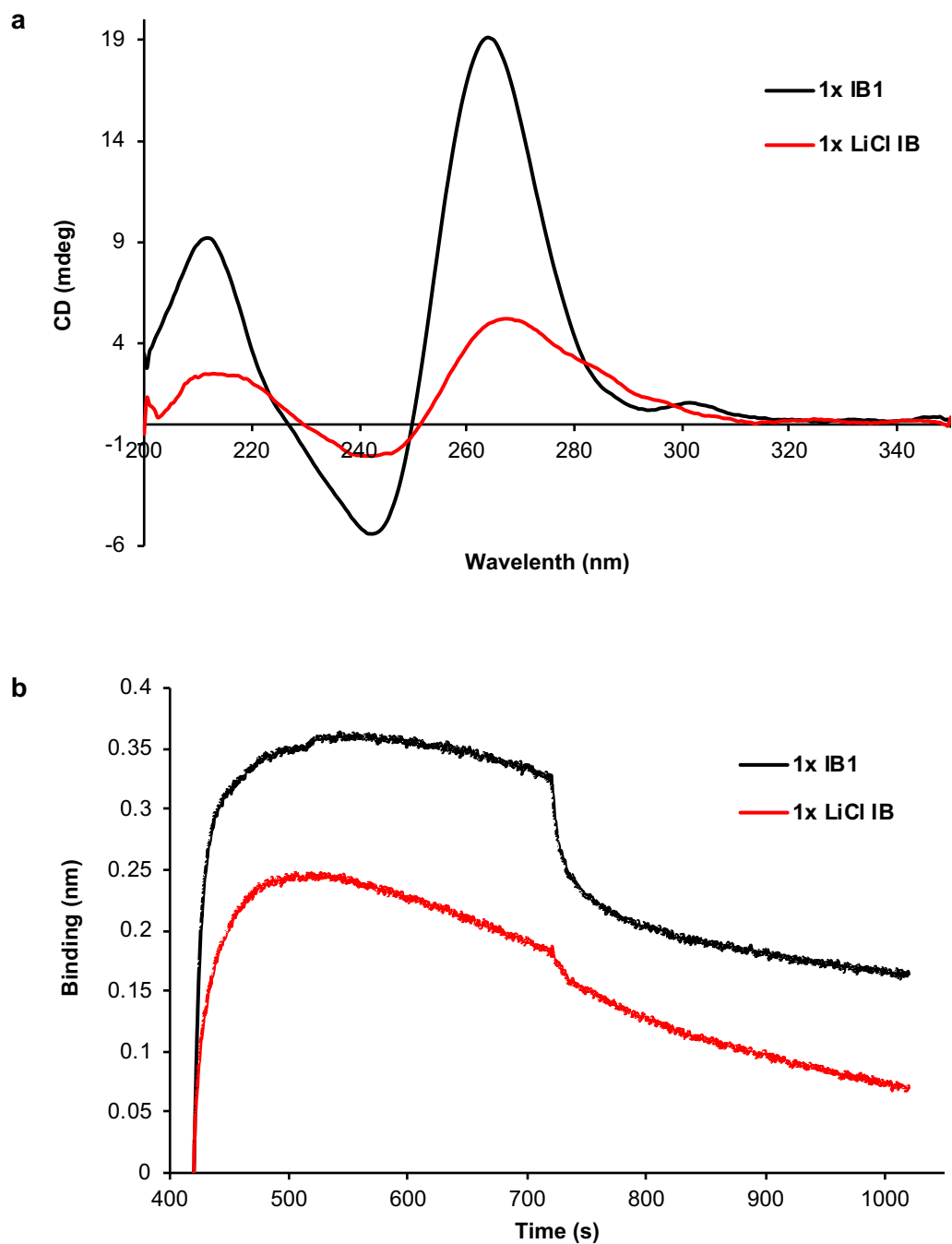
Supplementary Figure 1. Oligonucleotides separated by native 20% PAGE. A) Folded RNA oligonucleotides at pH 8. B) Folded DNA oligonucleotides at pH 8. C) i-Motif forming oligonucleotides at pH 7. Below the gel image is the length and DNA/RNA topology (as determined by CD spectroscopy) of each oligonucleotide listed where (a) denotes anti-parallel G4, (p) denotes parallel G4 topology and (i) denotes i-motif.



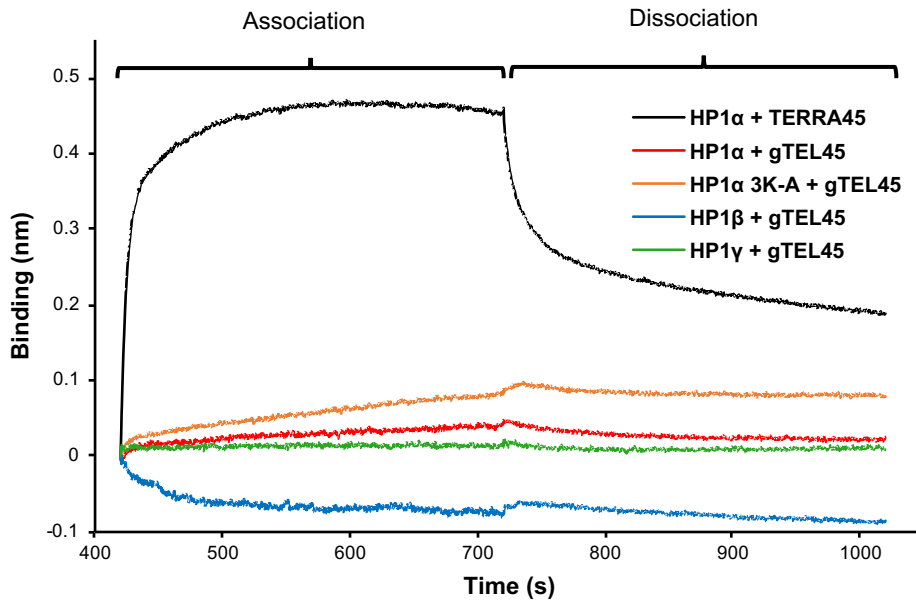
Supplementary Figure 2. The affinity of HP1 α for TERRA determined by BLI. A) Binding and dissociation curves for HP1 α with TERRA96 titrated from 75 nM to 500 nM. Association rate constant, $k_{\text{on}} = 1.59 \times 10^5 (\pm 0.09 \times 10^5) \text{ s}^{-1} \text{ M}^{-1}$; dissociation rate constant, $k_{\text{off}} = 4.0 \times 10^{-4} (\pm 0.4 \times 10^{-4}) \text{ s}^{-1}$; equilibrium constant, $K_{\text{D}} = 2.5 (\pm 0.3) \text{ nM}$. B) Binding and dissociation curves for HP1 α with TERRA45 titrated from 25 nM to 1,000 nM. Association rate constant, $k_{\text{on}} = 2.19 \times 10^5 (\pm 0.08 \times 10^5) \text{ s}^{-1} \text{ M}^{-1}$; dissociation rate constant, $k_{\text{off}} = 1.60 \times 10^{-2} (\pm 0.04 \times 10^{-2}) \text{ s}^{-1}$; equilibrium constant, $K_{\text{D}} = 74.4 (\pm 0.4) \text{ nM}$. C) Binding and dissociation curves for HP1 α with TERRA22 titrated from 500 nM to 20,000 nM. Association rate constant, $k_{\text{on}} = 4.10 \times 10^5 (\pm 0.08 \times 10^5) \text{ s}^{-1} \text{ M}^{-1}$; dissociation rate constant, $k_{\text{off}} = 3.9 \times 10^{-2} (\pm 0.2 \times 10^{-2}) \text{ s}^{-1}$; equilibrium constant, $K_{\text{D}} = 940 (\pm 60) \text{ nM}$.



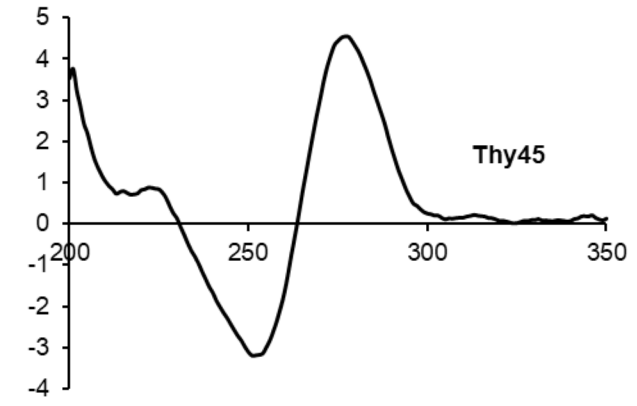
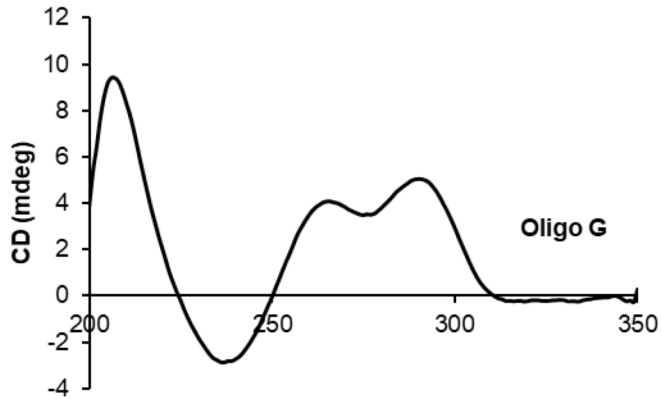
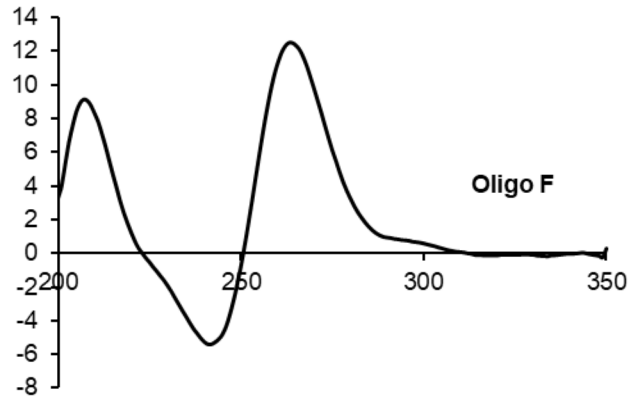
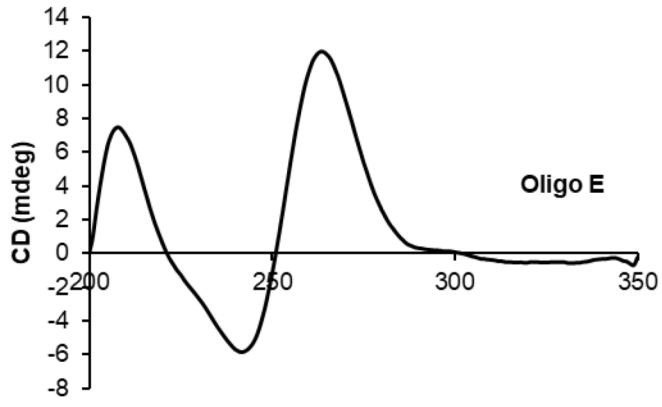
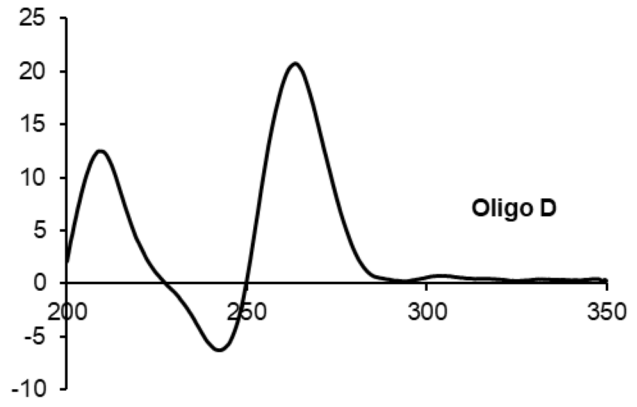
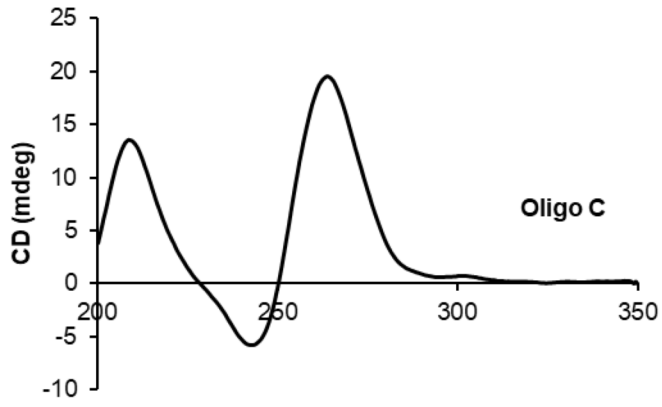
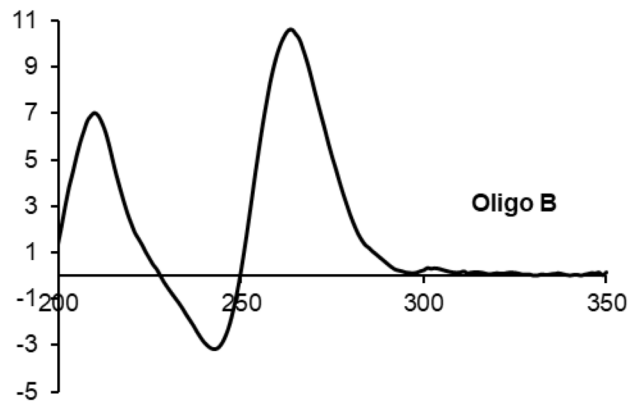
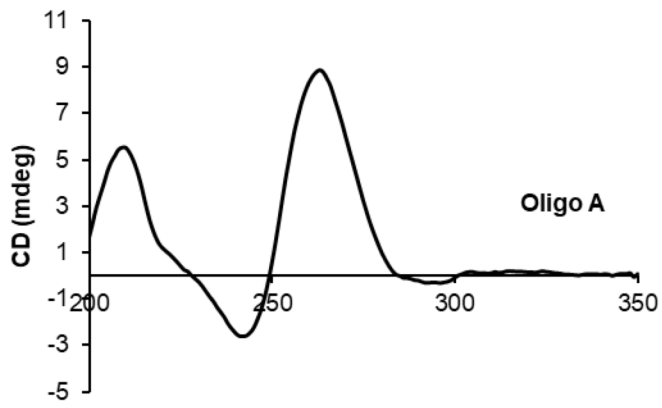
Supplementary Figure 3. CD spectra of HP1 α , HP1 β , HP1 γ , and HP1 α 3K-A at 1.4 mg/mL in 1x IB1 recorded in a 0.1 mm path length cuvette.



Supplementary Figure 4. TERRA45 in buffer containing lithium chloride reduces the interaction with HP1 α . A) CD spectra of TERRA45 in 1x Interaction Buffer 1 (1x IB1: 100 mM KCl, 50 mM NaCl, 20 mM NaH₂PO₄/Na₂HPO₄, pH 8) and TERRA45 in 1x LiCl Interaction Buffer (1x LiCl IB: 100 mM LiCl, 70 mM Tris-HCl, pH 8) at 10 μ M recorded in a 0.1 mm path length cuvette. B) BLI of TERRA45 binding to HP1 α in 1x IB1 or in 1x LiCl IB.



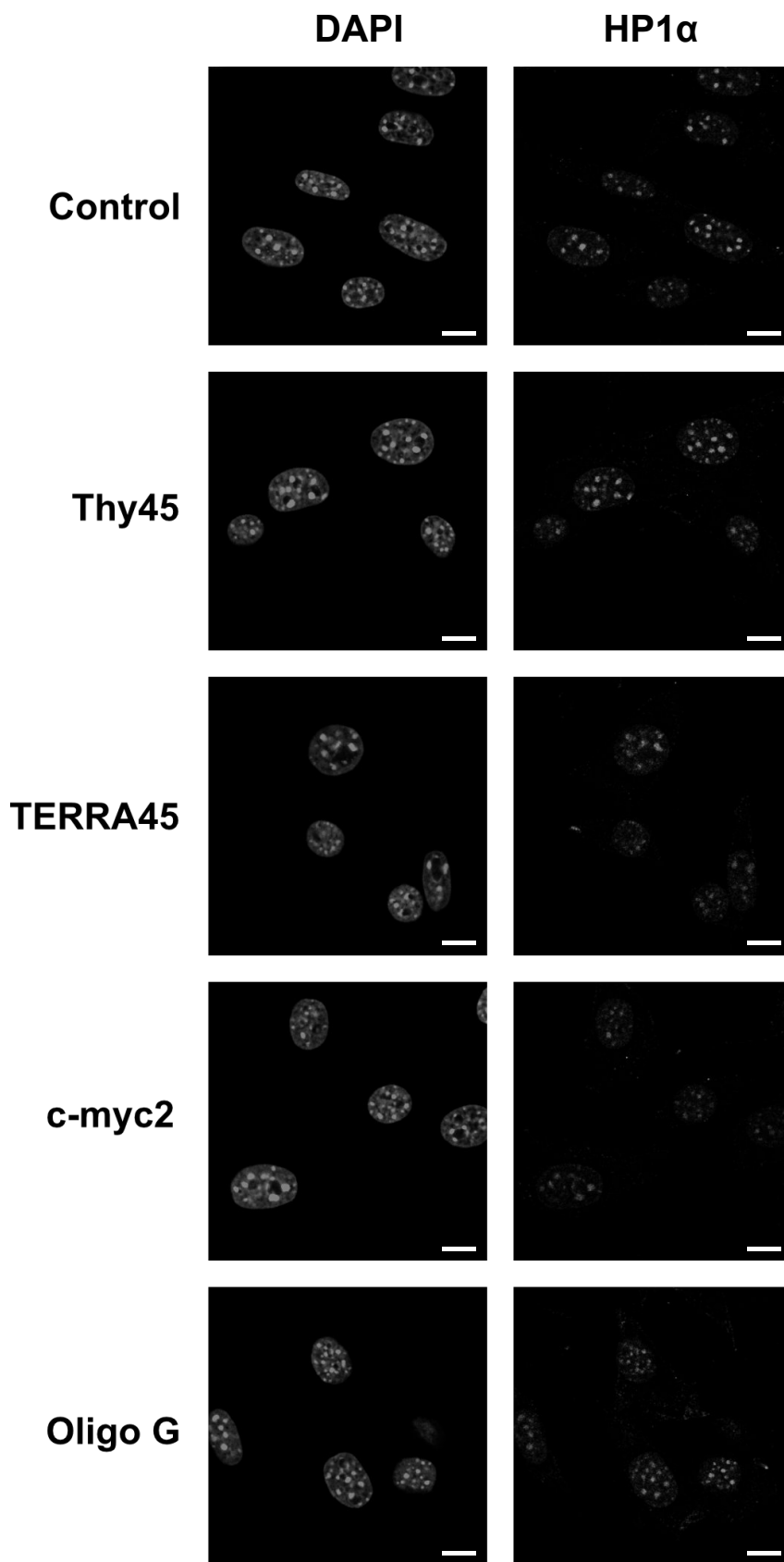
Supplementary Figure 5. BLI analysis to determine an interaction between either HP1 β , HP1 γ , or HP1 α 3K-A and gTEL45, the telomeric DNA oligonucleotide that forms an anti-parallel G4. HP1 α binding to TERRA45 is shown for reference.



Wavelength (nm)

Wavelength (nm)

Supplementary Figure 6. CD spectra of DNA oligonucleotides, Oligo A-G, which have various telomeric DNA repeat sequences, and Thy45 DNA.



Supplementary Figure 7. Immunofluorescence analysis of asynchronous mouse NIH3T3 cells that were permeabilised before incubation with either 2.5 μ M of Thy45, TERRA45, c-myc2, Oligo G or without oligonucleotide (control). After fixation, nuclear DNA was stained with DAPI and HP1 α visualised by antibody detection. Representative confocal sections are shown. Scale bars, 10 μ m.