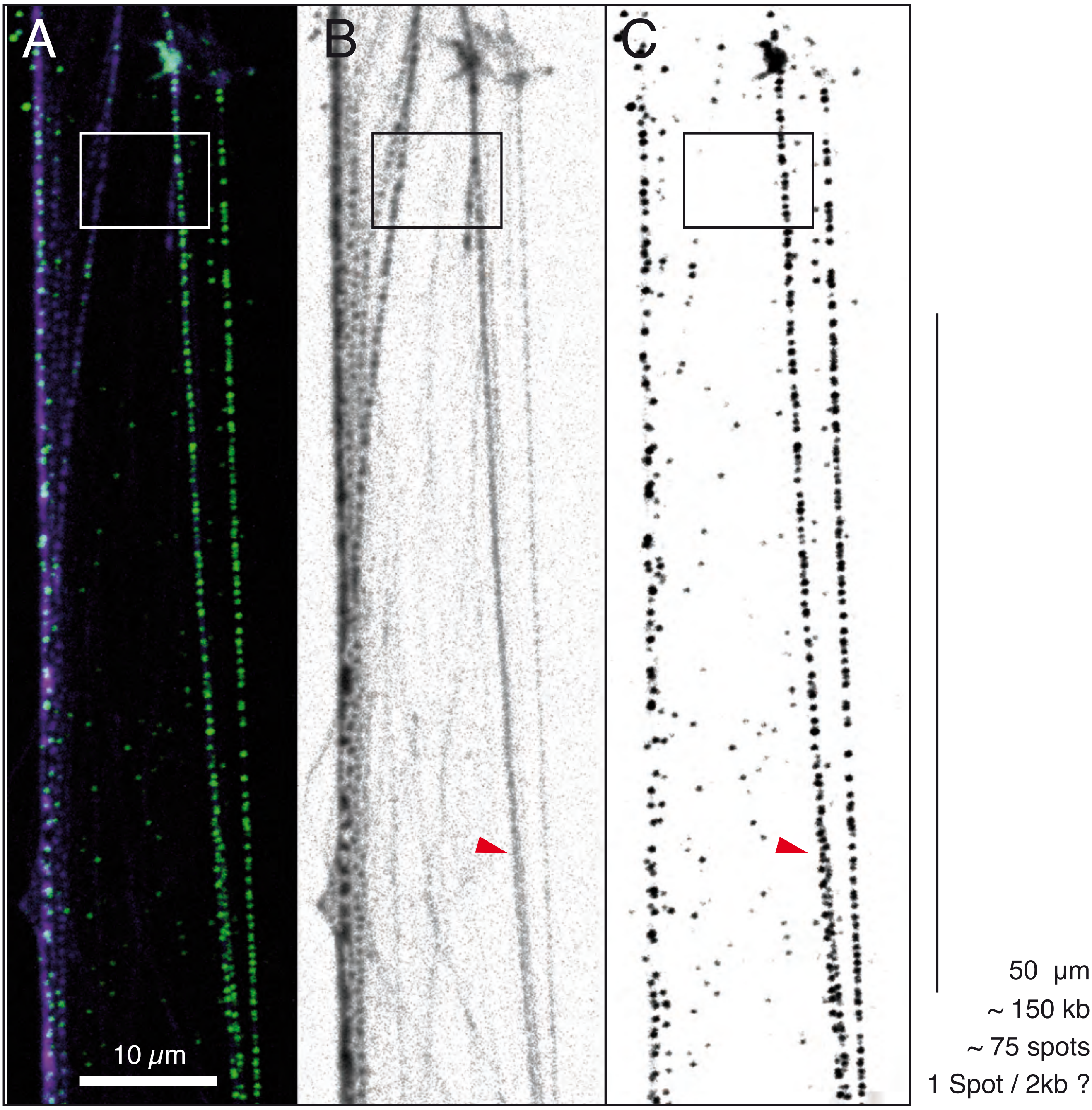

Supplementary Figure 1
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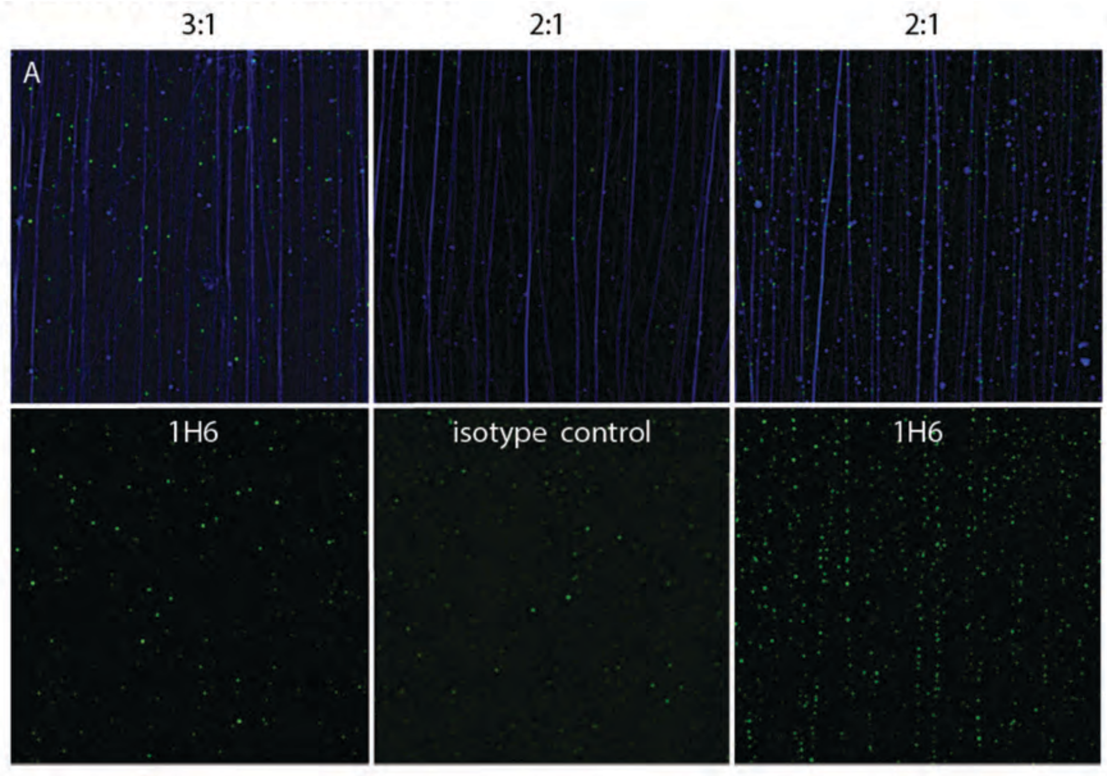

Supplementary Figure 2
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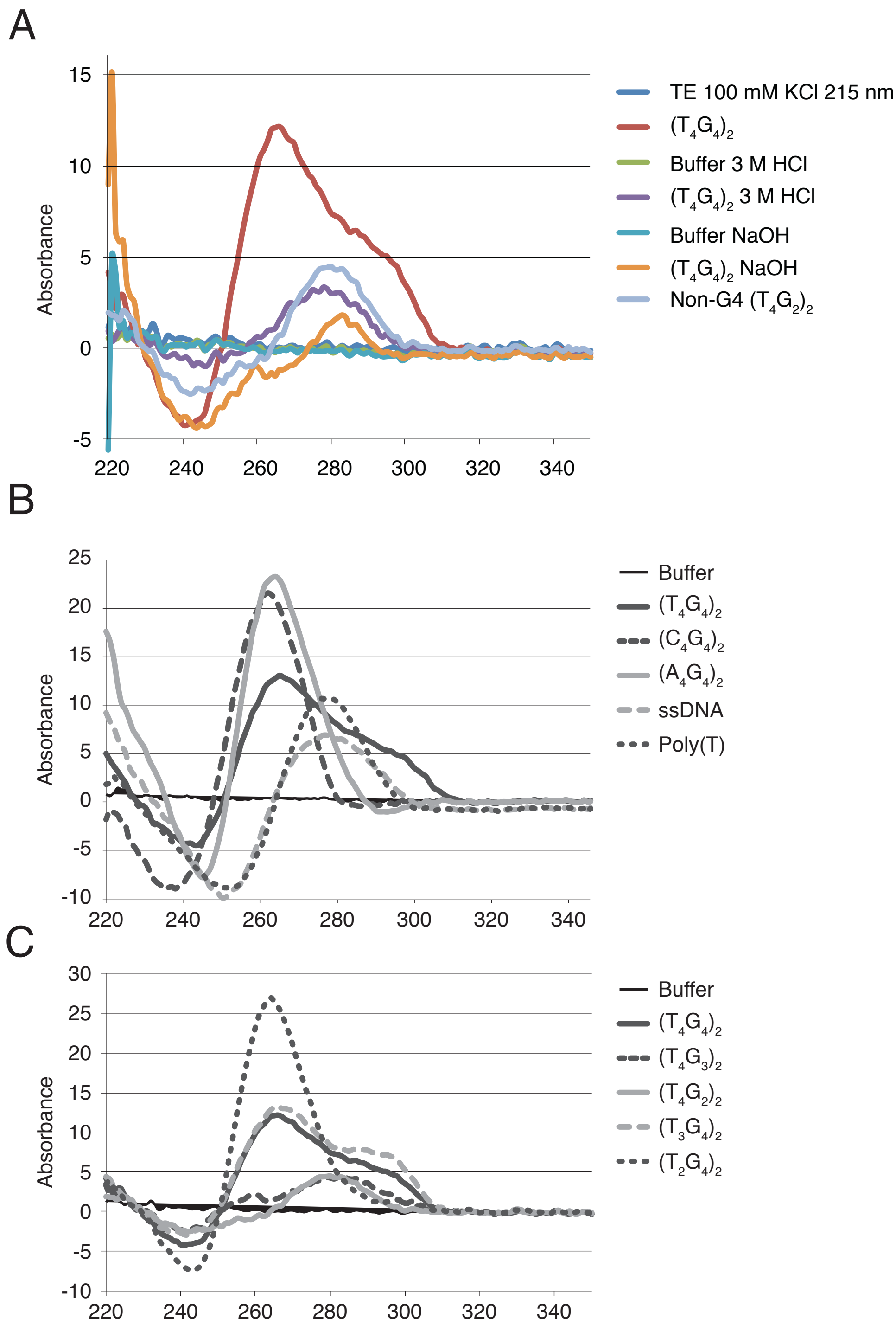

Supplementary Figure 3
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Supplementary Information

Supplementary Figure 1.

1H6 appears to react with subsets of DNA fibers.

(A-C) DNA fibers from mouse embryonic stem cells were stained with 1H6 antibody (green) and a DNA dye (purple). Note that DNA fibers show variable staining with 1H6 (boxed area) and that antibody staining is more apparent after separation of the fibers (red arrows). (B) Inverted image of DNA dye; (C) Inverted image of 1H6 antibody staining. Assuming DNA is fully stretched (300 nm for 1000 base pairs) and dots represents individual binding sites of 1H6 the spacing of individual antibodies is estimated to be in the order of 1 per every 2 kb. Scale bar 8 μm.

Supplementary Figure 2.

Binding of 1H6 to DNA fibers depends on the acidity of the fixative.

DNA fibers from HEK cells were fixed by immersion in different fixatives containing methanol and acetic acid followed by immediate washes with PBS (no air drying), 1H6 staining was only observed when the fixative contained acetic acid at a 2:1 ratio relative to methanol and not when the fixative contained methanol and acetic acid at a 3:1 ratio. This observation provides an explanation for the finding shown in Supplementary Figure 1. When fibers were allowed to dry to air after fixation the DNA could be exposed to variable acidity with variable DNA denaturation of DNA fibers as a result.

Supplemental Figure 3: G4 structures are denatured under various conditions. **(A)** G4 structures were folded as described in the methods. After formation, structures were incubated with 3M HCl (purple line), or 3M NaOH (light brown line) and CD analysis was performed to reveal formation state of the oligonucleotides. In both cases CD measurements reveal that DNA is no longer in G4 formation. **(B, C)** CD analysis of G4 structures. Circular Dichroism spectroscopy shows characteristic maximum at 260 nm and minimum at 240 nm that confirmed G4 formation. **(B)** CD analysis of (T4G4)2, (A4G4)2, (C4G4)2. All tested regions formed G4 structures. **(C)** CD analysis of (T4G4)2, (T3G4)2 (T2G4)2, (T4G3)2 (T4G2)2.

Supplementary Table S1

Name	5'	sequence	n	3'	Binding	G4	Assay	Reference
Oxy2 = (T4G4)2	bio	TTTTGGGGTTTTGGGG	16		y	þ	ELISA, CD, MST	(1)
Tet4	bio	TTGGGGTTGGGGTTGGGGTTGGGG	24		y		ELISA	(2)
Ver3	bio	TGGGGGTTAGGGTTAGGGT	19		y		ELISA	(3)
Ver4	bio	AGGGTTAGGGTTAGGGTTAGGG	22		n		ELISA	(4)
GQ	bio	TAGAAACTACGGCGGCGGCGGAATCGTAGA	30		n		ELISA	Data not shown
Mutant	bio	GGGTTGCGGAGGGTGGGCCTGGGAGGGGTGGTGGCCAT	38		n		ELISA	Data not shown
hTR GQ	bio	TAGACCCTGCAACGTCAGCGTAGTCGTAGC	30		n		ELISA	Data not shown
Oxy2 duplex	bio	TTTTGGGGTTTTGGGG	16		n		ELISA	Data not shown
Oxy2 complement	bio	AAAACCCCAAAACCCC	16		n		ELISA	Data not shown
gp054a	bio	GGGGTTGGGGCTGGGGTTGGGGTTTTT	27		y		ELISA	(5)
un2	bio	GGGGGCGAGGGGCGGGAGGGGGCGAGGGGTTTTT	34		y		ELISA	(5)
un3	bio	GGGAGGAGCGGGGGGAGGAGCGGGTTTTT	29		y		ELISA	(5)
hTel	bio	TCGAGGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAGGGTTAC	54		y		ELISA	(5)
ssDNA	bio	AAAAACTACTGCACGCTCGCTACGACGACACTGTCGCGCATACAAGCTGCAAAAA	55		n	×	ELISA, CD	(5)
Motif_1_RepB		GGGCCTGGGGCTAGGACTGGGCTGGGG	27	bio	n		ELISA	Data not shown
Motif_2_RepB		GGGCTGGACTGGATTGG	17	bio	n		ELISA	Data not shown
Motif_3_RepB		GGGGCAGGGGCAGGGGCAGGGG	22	bio	n		ELISA	Data not shown
Motif_4_RepB		GGGGCTGGGGCTGGGGCTGGGG	22	bio	y		ELISA	Data not shown
Motif_5_RepB		GGGGCAGGGGCTGGGGCTGGGGCAGGGG	28	bio	y		ELISA	Data not shown
Motif_6_RepB		GGGGCTGGGGCTGGGGCAGGGG	22	bio	n		ELISA	Data not shown
Motif_7_RepB		GGGGCTGGGCCTGGGGCTGGG	21	bio	y		ELISA	Data not shown
4G	bio	TTTTTTTTTTTGGGG	15		y		ELISA	Data not shown
3G	bio	TTTTTTTTTTTTGGG	15		y		ELISA	Data not shown
2G	bio	TTTTTTTTTTTTTGG	15		y		ELISA	Data not shown
1G	bio	TTTTTTTTTTTTTTG	15		y		ELISA	Data not shown
noG = poly(T)	bio	TTTTTTTTTTTTTTT	15		y	×	ELISA, CD, MST	this study
3G_TT	bio	TTTTTTTTTTGGGTT	15		y		ELISA	Data not shown
2G_TT	bio	TTTTTTTTTTTTGGTT	16		y		ELISA	Data not shown
1G_TT	bio	TTTTTTTTTTTTTTGTT	17		y		ELISA	Data not shown
Poly(T)13AG	bio	TTTTTTTTTTTTTAG	15		y		ELISA	Data not shown
Poly(C)14G	bio	CCCCCCCCCCCCCCG	15		n		ELISA	Data not shown
Poly(A)14G	bio	AAAAAAAAAAAAAAG	15		n		ELISA	Data not shown
(T4C4)2	bio	TTTTCCCCTTTTCCCC	16		n		ELISA	This study
(T4A4)2	bio	TTTTAAAATTTTAAAA	16		n		ELISA	This study
(A4G4)2	bio	AAAAGGGGAAAAGGGG	16		n	þ	ELISA, CD, MST	This study
(C4G4)2	bio	CCCCGGGGCCCCGGGG	16		n	þ	ELISA, CD, MST	This study
(T3G4)2	bio	TTTGGGGTTTGGGG	14		y	þ	ELISA, CD, MST	This study
(T2G4)2	bio	TTGGGGTTGGGG	12		n	þ	ELISA, CD	This study
(T4G3)2	bio	TTTTGGGTTTTGGG	14		n	×	ELISA, CD, MST	This study
(T4G2)2	bio	TTTTGGTTTTGG	12		n	×	ELISA, CD	This study
Poly(T)8	bio	TTTTTTTT	8		n		ELISA	This study
Poly(T)6	bio	TTTTTT	6		n		ELISA	This study
Poly(T)4	bio	TTTT	4		n		ELISA	This study
Poly(T)2	bio	TT	2		n		ELISA	This study
poly(A)	bio	AAAAAAAAAAAAAAA	15		n		ELISA	This study
poly(C)	bio	CCCCCCCCCCCCCCC	15		n		ELISA	This study
Bio-poly(T)4-Bio	bio	TTTT	4	bio	y		ELISA	This study
Hairpin	bio	GCGCTTTTGCGCTTTTGCGCTTTTGCGC	28		n		ELISA	This study
RNA Oxy2	bio	TTTTGGGGTTTTGGGG	16		n		ELISA	Data not shown

Supplementary Table 1
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REFERENCES

1. Sen, D. and Gilbert, W. (1990) A sodium-potassium switch in the formation of four-stranded G4-DNA. *Nature*, **344**, 410-414.
2. Williamson, J.R. (1994) G-quartet structures in telomeric DNA. *Annu Rev Biophys Biomol Struct*, **23**, 703-730.
3. Williamson, J.R., Raghuraman, M.K. and Cech, T.R. (1989) Monovalent cation-induced structure of telomeric DNA: The G-Quartet model. *Cell*, **59**, 871-880.
4. Wang, Y. and Patel, D.J. (1993) Solution structure of a parallel-stranded G-quadruplex DNA. *J Mol Biol*, **234**, 1171-1183.
5. Artusi, S., Perrone, R., Lago, S., Raffa, P., Di Iorio, E., Palu, G. and Richter, S.N. (2016) Visualization of DNA G-quadruplexes in herpes simplex virus 1-infected cells. *Nucleic acids research*, **44**, 10343-10353.