

## Supporting Information

### Human *NEIL3* gene expression is regulated by epigenetic-like oxidative DNA modification

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## **Additional Methods**

### Plasmid Construction

The plasmids were constructed from the psiCHECK2 plasmid (Promega) that has coding sequences for the Renilla luciferase (Rluc) and firefly luciferase (luc) genes. The luc gene is regulated by the HSV-TK promoter and was not modified allowing expression from this luciferase to be used as the internal standard. The data were collected via a dual-glo luciferase assay (Promega). The Rluc gene was originally regulated by the SV40 early enhancer/promoter, which we modified by removing the TATA-box and replacing it with the *NEIL3* potential G-quadruplex sequences (PQS) of interest. Additionally, the *NEIL3* PQS was flanked by recognition sequences for the Nt.BspQ1 nicking endonuclease. Insertion of the PQS and nicking endonuclease recognition sequences was achieved using restriction free cloning. The cloning experiment was conducted in a 25- $\mu$ L reaction with 1x Phusion reaction buffer (NEB), 200  $\mu$ M dNTPs, 1  $\mu$ M each of the forward and reverse primers, 1,000 ng of psiCHECK2 plasmid, 3% DMSO, and 0.5 units of Phusion DNA polymerase. The PCR was initiated with a 98 °C denaturation step for 30 sec followed by 30 cycles of PCR consisting of a 98 °C denaturation step for 20 sec, a 55 °C annealing step for 30 sec, a 72 °C extension step for 5 min, and a post step consisting of 98 °C for 8 sec followed by 72 °C for 5 min. After the 30 cycles, a final extension step of 72 °C for 5 min was conducted. Following PCR, the samples were digested with Dpn1 by adding 2.3  $\mu$ L of Dpn1 reaction buffer and 5 units of Dpn1 to the PCR reaction vessel. The Dpn1 reaction was conducted for 2 hr at 37 °C followed by heat quenching at 80 °C for 20 min.

Following the PCR and Dpn1 reactions, the sample was then transformed in to NEB 5-alpha competent *E. coli* cells following the manufacturer's protocol. After transformation, 50-100  $\mu$ L from the transformation was spread on a pre-warmed agar plate containing ampicillin (100  $\mu$ g/mL). The plates were incubated overnight at 37 °C. Next, individual colonies were picked and each grown overnight at 37 °C in 5 mL of lysogeny broth media containing ampicillin (100  $\mu$ g/mL). The plasmid DNA from each grown sample was then purified using a miniprep kit (Qiagen) following the manufacturer's protocols. Last, ~200 ng of plasmid and 1  $\mu$ M sequencing forward primer in 15  $\mu$ L of 10 mM Tris (pH 8.0) was submitted for Sanger sequencing at the DNA Sequencing Core facility at the University of Utah.

Insertion of site-specific modifications into the plasmids was achieved following literature protocols.<sup>1,2</sup> Specifically, 5  $\mu$ g of plasmid with Nt.BspQ1 recognition sequences flanking the site in which the DNA base modification will be inserted was placed in 50  $\mu$ L of 1x Nt.BspQ1 reaction buffer with 5 U of Nt.BspQ1. The reaction was placed at 50 °C for 60 min followed by heat quenching at 80 °C for 20 min. Next, 1 nmole (~1000x) of 5'-phosphorylated synthetic oligomer with the site-specific modification was added to the quenched reaction. The modified oligomers were synthesized by solid-phase synthesis in the DNA/Peptide Core Facility at the University of Utah. The oligos were cleaved, deprotected, and HPLC purified following protocols previously outlined.<sup>3</sup> The plasmid oligomer mixture was thermal cycled by heating at 80 °C for 2 min followed by cooling on ice for 2 min, which was repeated four times. The nicks were then sealed using T4 DNA ligase, by adding 6  $\mu$ L of 10x ligase buffer and 800 units of ligase. The ligation reaction was left at 20 °C for 4 h. The modified plasmids were purified from the protein and excess insertion oligomer using an Ultra Clean PCR cleanup kit (Mo Bio) following the manufacturer's protocol. Plasmid concentrations were determined by nanodrop UV-vis measurements.

To confirm the DNA modifications were introduced into the plasmid, we applied a protocol established in our laboratory, in which the modification was removed by a DNA glycosylase to yield a ligatable gap for sequence that we reported on.<sup>4</sup> By ligating the gap, Sanger sequencing provided a characteristic nucleotide loss at the modification site to confirm the presence of the modification.

#### Primer Sequences

##### *NEIL3* PQS Insertion Primer 1

5'- CAG TTC CGC CCA TTC TCC GCC CCA TGG CTG ACG CTC TTC TAG GGT GCT GTT  
TGG GCG GGG CCT GGG CGG GGC CGC TCT TCT GCA GAG GCC GAG GCC GCC TCG  
GCC TCT GAG CT

##### *NEIL3* PQS Insertion Primer 2

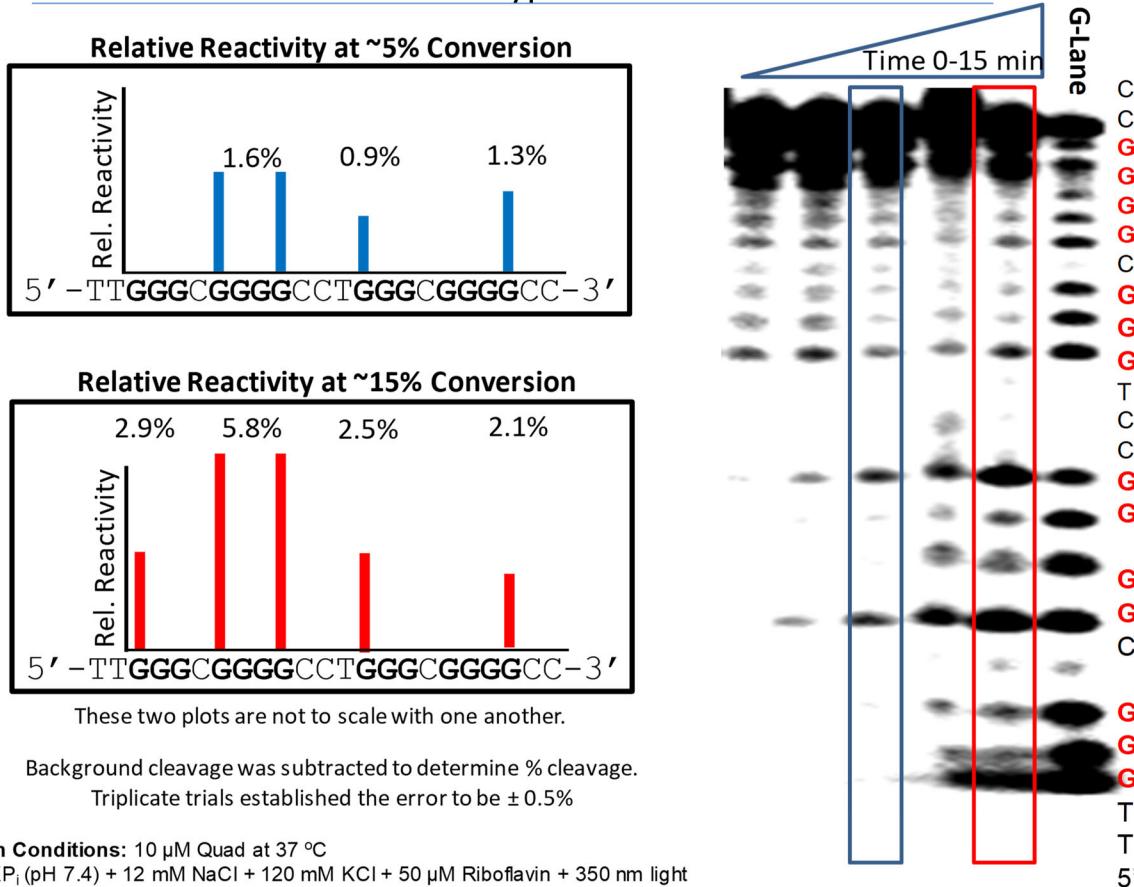
5'- AGC TCA GAG GCC GAG GCG GCC TCG GCC TCT GCA GAA GAG CGG CCC CGC CCA  
GGC CCC GCC CAA ACA GCA CCC TAG AAG AGC GTC AGC CAT GGG GCG GAG AAT  
GGG CGG AAC TG

##### Sequencing Primer

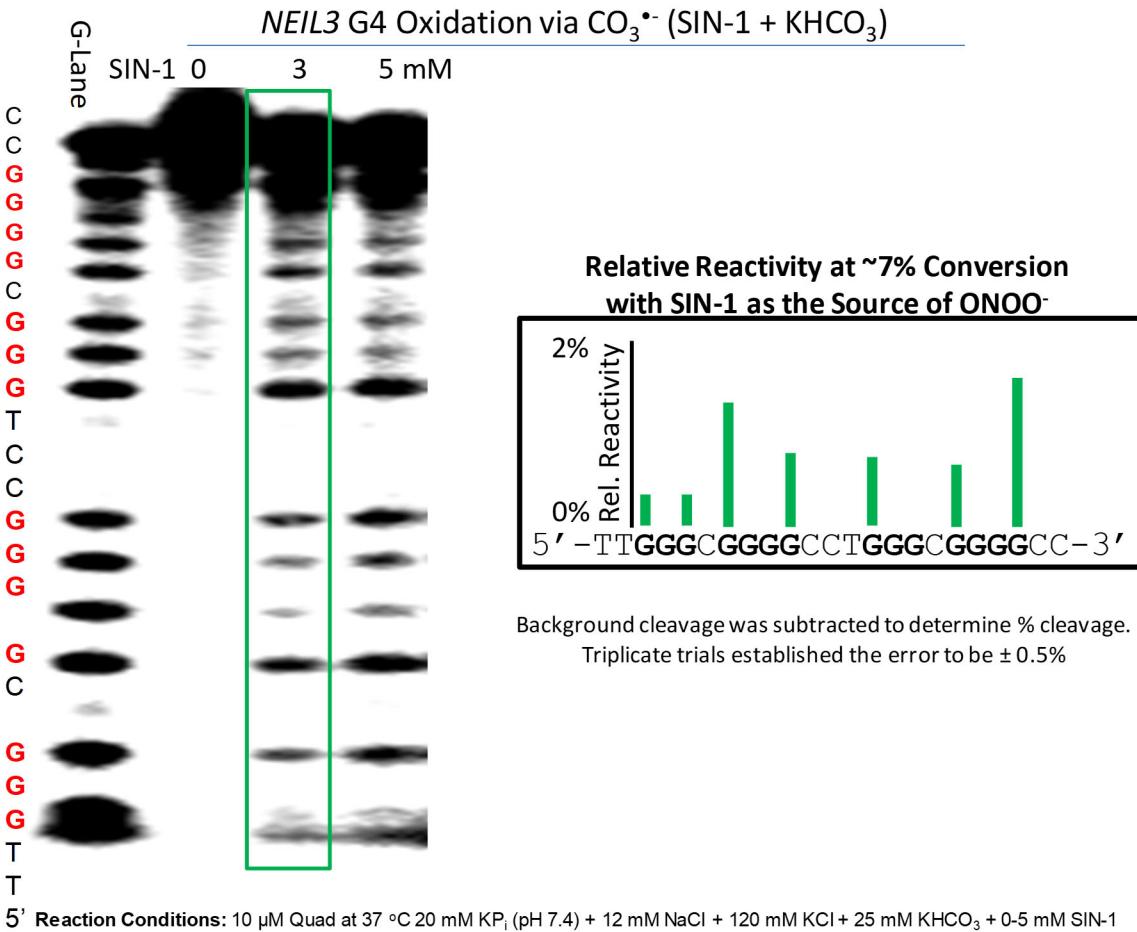
5`- TTT TTC ATG CAT  
CTC AAT TAG TCA GCA ACC ATA GT

**Figure S1.** Determination of oxidation prone sites in the *NEIL3* PQS by PAGE

*NEIL3* G4 Oxidation via the Type I Photooxidant Riboflavin



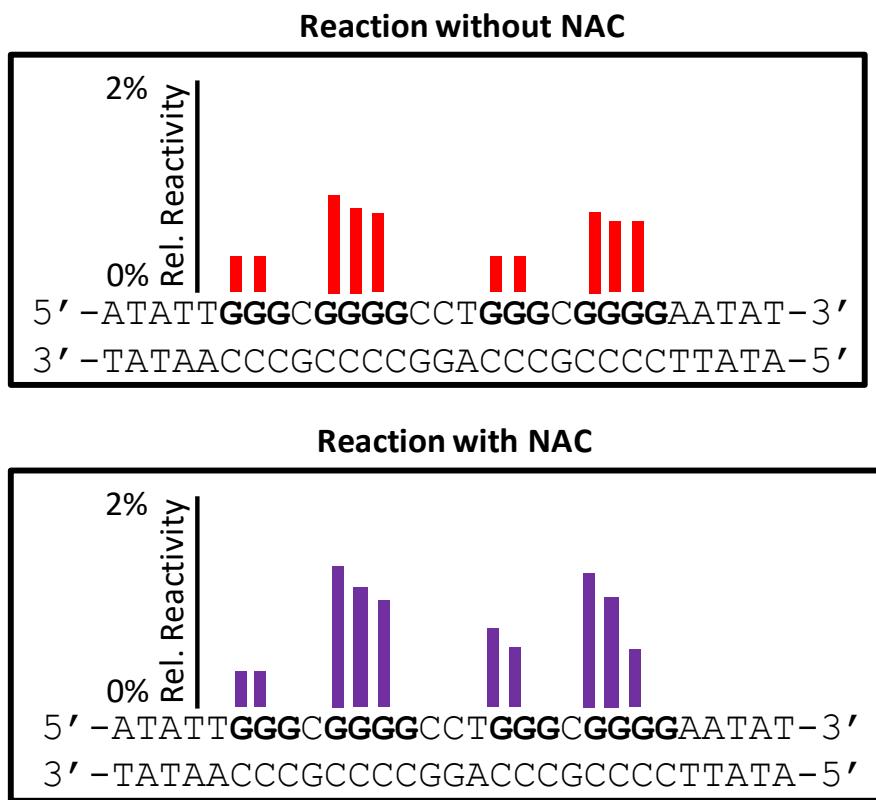
The Maxam-Gilbert G-lanes were conducted in 20  $\mu$ L of ddH<sub>2</sub>O by allowing 500 pmoles of *NEIL3* G4 react with 1% DMS for 30 sec followed by quenching with 1 M  $\beta$ -mercaptoethanol. The reaction was dialyzed against ddH<sub>2</sub>O overnight and then treated with the piperidine and sequencing protocol outlined for the oxidation reactions in the text.



See previous gel caption for the Maxam-Gilbert G-lane reaction conditions.

**Figure S2.** The presence of NAC does not impact oxidation sites in the *NEIL3* PQS

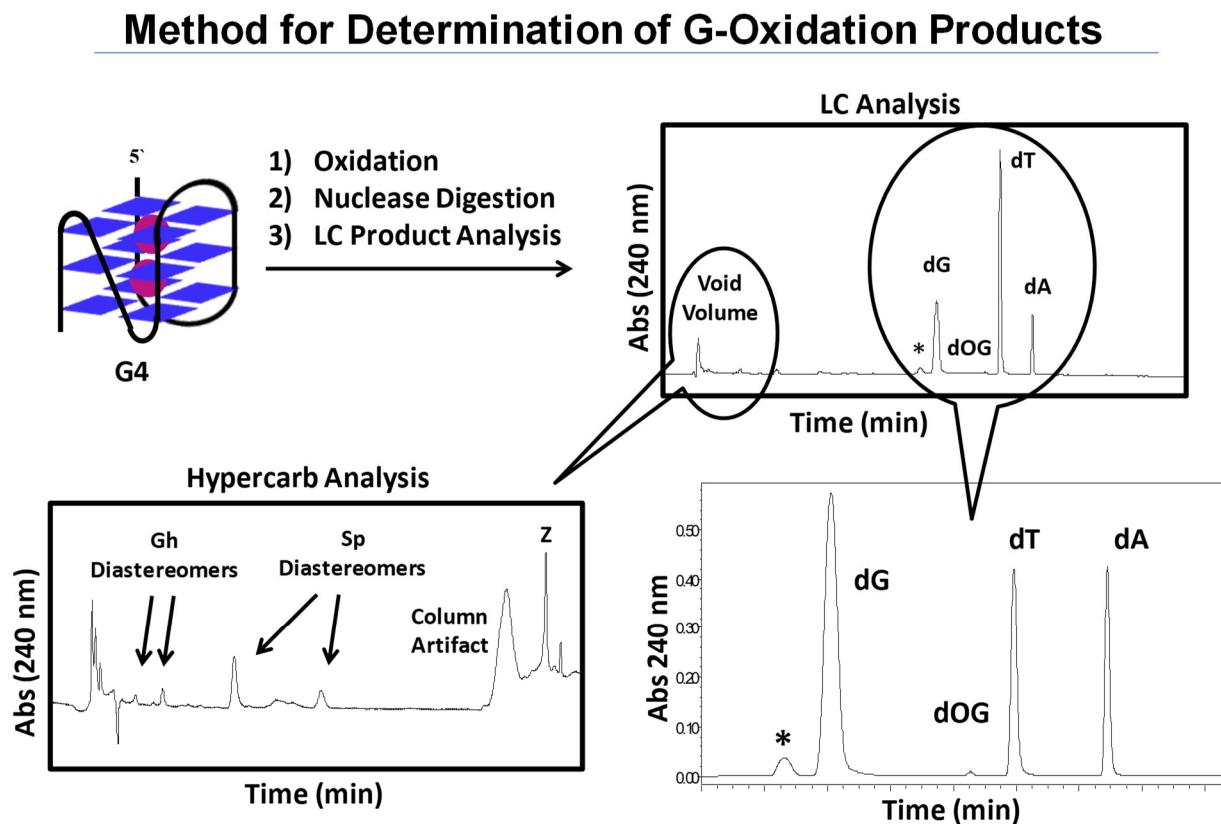
Plots to compare riboflavin reactions with and without NAC present



Background cleavage was subtracted to determine % cleavage.

TriPLICATE trials established the error to be  $\pm 0.5\%$

**Figure S3.** Outline of method to quantify G oxidation products from the *NEIL3* PQS



Nuclease Digestion: 10 nmol of oxidized DNA at <10% conversion to product followed by [1] DNase 3 h (2 U); [2] Nuclease P1 18 h (1 U q 9 h); [3] SVPD 18 h (1 U q 9 h); [4] CIP (10 U); protein removal, and then HPLC analysis RP-HPLCA = 20 mM NH<sub>4</sub>OAc pH 7 B = MeCN; Hypercarb A = 0.1% acetic acid in ddH<sub>2</sub>O. \*Inosine from deamination of A

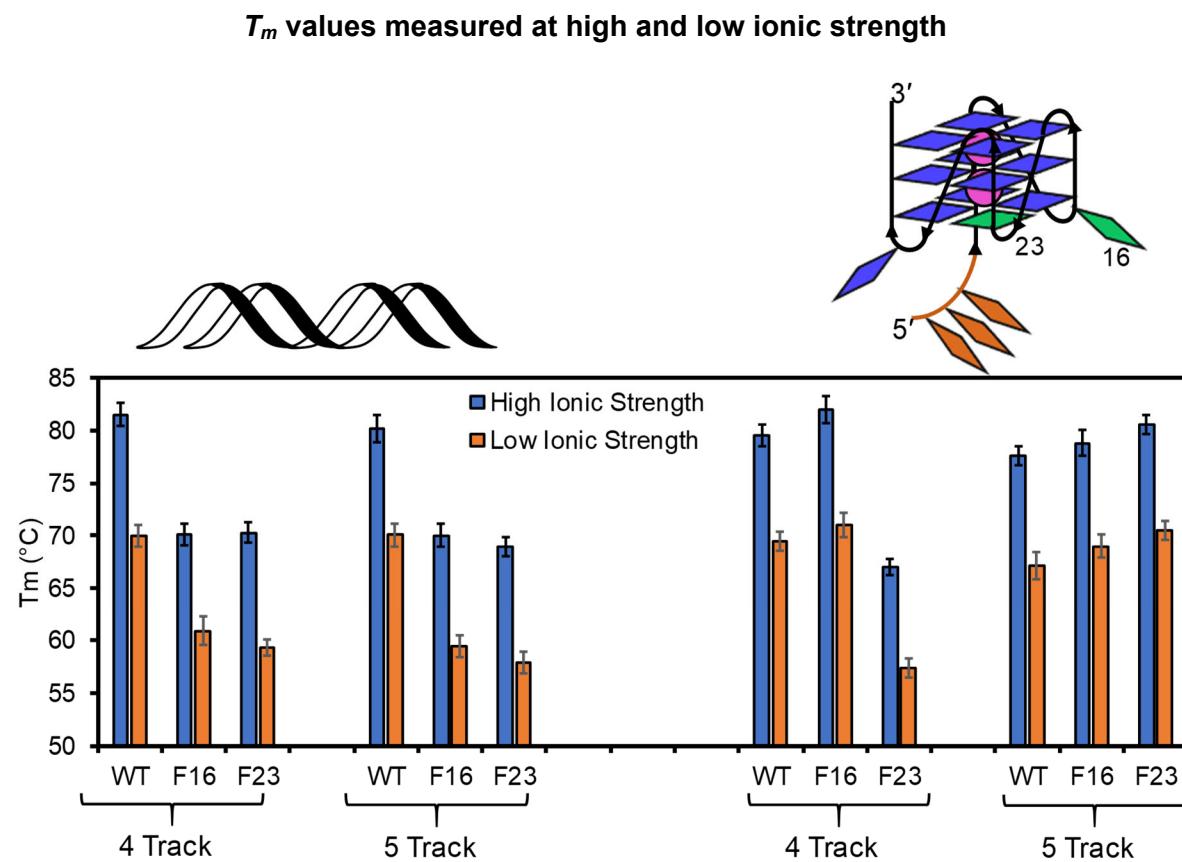
The method to quantify G oxidation products from the *NEIL3* PQS was established on the human telomere G4 sequence and previously reported.<sup>5,6</sup>

#### Table of Average Relative Amounts of Each G Oxidation Product Detected

Average Relative Amounts (%)											
		NEIL3 G4		NEIL3 Duplex				NEIL3 G4		NEIL3 Duplex	
Riboflavin		Riboflavin				CO <sub>3</sub> <sup>2-</sup>				CO <sub>3</sub> <sup>2-</sup>	
	NAC-	NAC+		NAC-	NAC+		NAC-	NAC+		NAC-	NAC+
OG	1.4	8.8		2.1	10.8		1.3	n.d.		1.5	n.d.
Sp	43.2	67.7		21.6	51.5		77.6	n.d.		24.8	n.d.
Gh	2.4	5.5		44.3	64.8		19.6	n.d.		66.8	n.d.
Z	53.0	18.0		2.1	1.8		1.5	n.d.		7.4	n.d.

\*The average error for each measurement on the basis of triplicate measurements is ~10% of the value.

**Figure S4.** The CD and  $T_m$  analysis to verify folding under APE1 reaction conditions



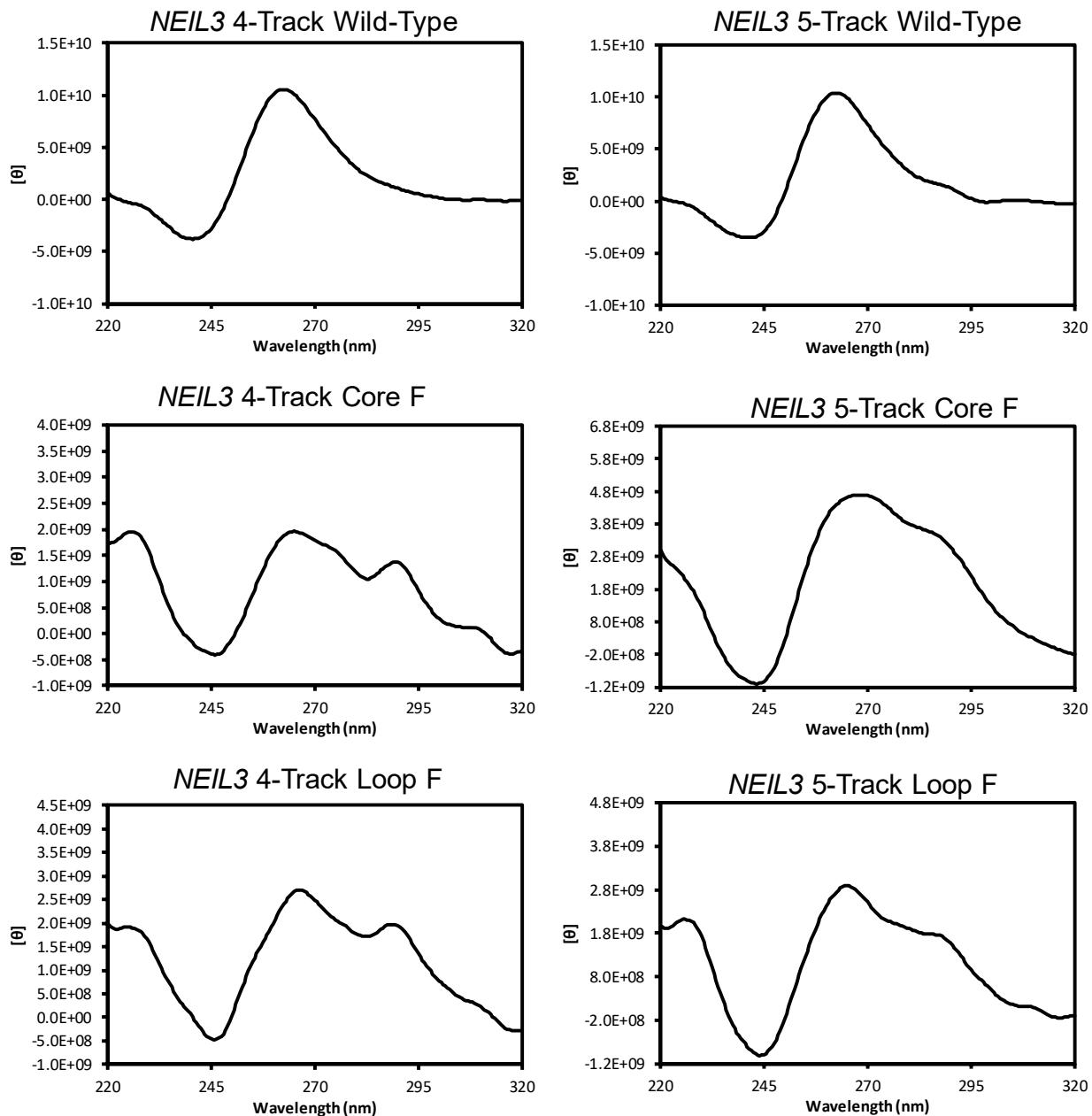
High ionic strength

= 20 mM KP<sub>i</sub> pH 7.4, 120 mM KCl, 12 mM NaCl

Low ionic strength (i.e., APE1 buffer)

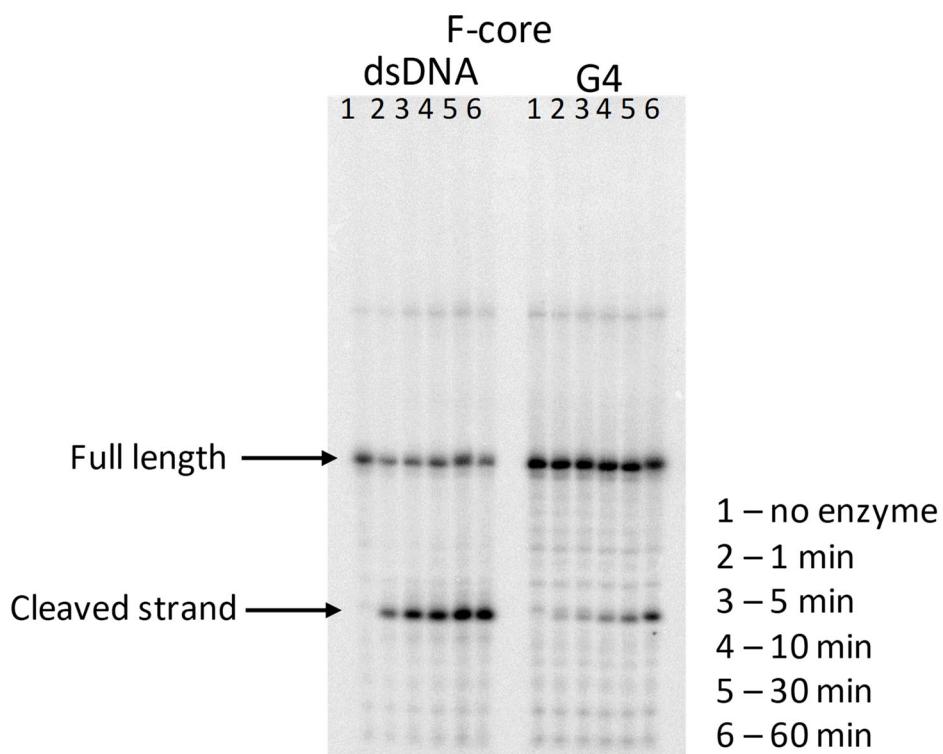
= 20 mM Tris pH 7.9 and 50 mM KOAc

### CD Spectra for the NEIL3 G4 Folds in the APE1 Buffer



The CD spectra for the wild-type sequences suggest parallel-stranded G4s in the APE1 buffer and the spectra are nearly identical to those reported for the same sequence in high ionic strength buffer as previously reported.<sup>7,8</sup> The presence of an F at any position and strand length supports a mixture of folds that is similar to our previous report for the same sequence with the modification OG.<sup>7</sup>

**Figure S5.** Sequencing PAGE analysis to monitor the APE1 reaction on the *NEIL3* PQS

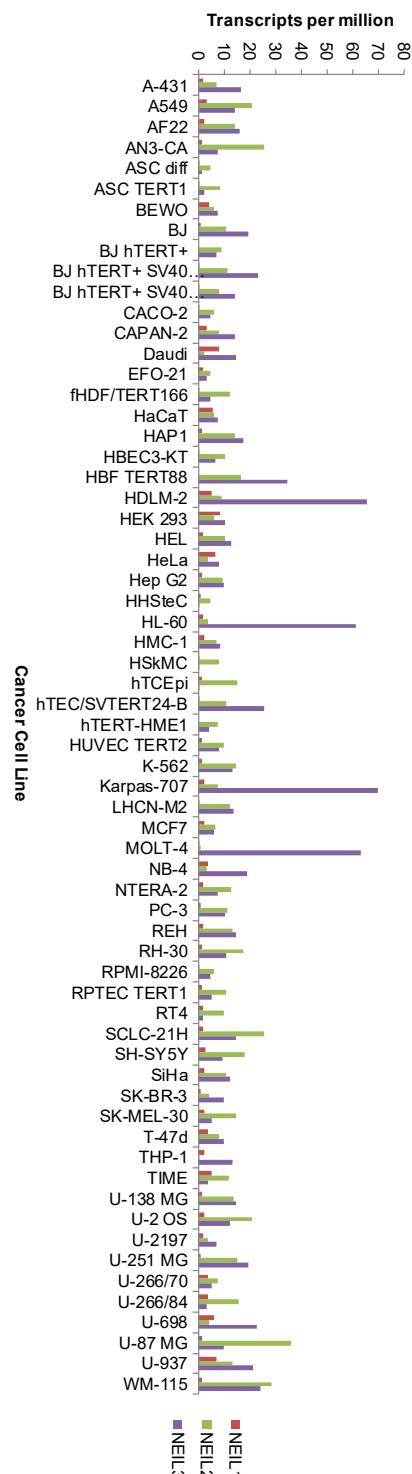


The example gel provided is for the four-track *NEIL3* PQS with an F at a core position (i.e., G23) in the dsDNA or G4 contexts.

**Table of the averages and standard deviations from the PAGE analysis of the APE1 cleavage reactions.**

<b>NEIL3 Four-track core F</b>					
time point	Avg. % Yield dsDNA	Std. Dev. dsDNA		Avg. % Yield G4	Std. Dev. G4
0	0.0	0.0		0.0	0.0
1	56.4	0.8		4.4	1.1
5	67.8	1.6		5.0	2.2
10	68.6	0.4		8.5	2.7
30	71.3	0.0		19.3	2.6
60	78.5	0.4		25.8	2.4
<b>NEIL3 Four-track loop F</b>					
time point	Avg. % Yield dsDNA	Std. Dev. dsDNA		Avg. % Yield G4	Std. Dev. G4
0	0.0	0.0		0.0	0.0
1	5.2	0.6		1.5	0.2
5	40.3	1.9		0.8	0.4
10	65.1	1.0		1.1	0.1
30	70.7	1.5		2.1	1.1
60	80.2	0.6		2.6	0.6
<b>NEIL3 Five-track core F</b>					
time point	Avg. % Yield dsDNA	Std. Dev. dsDNA		Avg. % Yield G4	Std. Dev. G4
0	0.0	0.0		0.0	0.0
1	13.7	5.5		0.1	0.0
5	35.3	6.7		0.2	0.2
10	44.7	5.4		0.4	0.4
30	50.4	0.7		0.8	0.7
60	65.9	5.2		3.9	0.7
<b>NEIL3 Five-track loop F</b>					
time point	Avg. % Yield dsDNA	Std. Dev. dsDNA		Avg. % Yield G4	Std. Dev. G4
0	0.0	0.0		0.0	0.0
1	20.9	5.6		0.5	0.6
5	59.6	7.9		1.3	0.2
10	71.8	6.4		0.6	0.4
30	80.9	0.7		1.6	0.4
60	82.1	0.5		3.6	1.2

**Figure S6.** NEIL expression levels in cancer cells and genes with OGG1 ChIP-Seq peaks containing a PQS.



## Genes with OGG1 ChIP-Seq peaks containing a PQS

Chromosome	Loci	Start	Loci	Gene
chr13	20957200	20957418	ENST00000453800	PPIAP27
chr13	25474753	25474969	ENST00000255283	ATP8A2
chr13	43957622	43957672	ENST00000629019	LINC00284
chr13	52718553	52718661	ENST00000448904	LECT1
chr13	97964672	97965240	ENST00000357602	IPO5
chr13	98391183	98391233	ENST00000600190	FARP1
chr13	100551103	100551239	ENST00000376250	GGACT
chr13	102165127	102165752	ENST00000376131	FGF14
chr13	102753262	102753396	ENST00000322527	CCDC168
chr13	107311950	107312239	ENST00000375915	FAM155A
chr13	108592661	108592796	ENST00000356711	MYO16
chr13	112784585	112784635	ENST00000459908	ATP11A
chr13	112784585	112784635	ENST00000459908	ATP11A
chr13	113056800	113056951	ENST00000423251	MCF2L
chr13	113057340	113057532	ENST00000423251	MCF2L
chr13	113057340	113057532	ENST00000423251	MCF2L
chr12	2446207	2446557	ENST00000615400	CACNA1C
chr12	4621987	4622717	ENST00000545990	AKAP3
chr12	5636607	5636752	ENST00000546188	ANO2
chr12	5636607	5636752	ENST00000546188	ANO2
chr12	6516037	6516282	ENST00000545732	NCAPD2
chr12	9605264	9605326	ENST00000229402	KLRB1
chr12	9841161	9841360	ENST00000537723	KLRF1
chr12	13593463	13594187	ENST00000628166	GRIN2B
chr12	15345521	15345683	ENST00000545023	PTPRO
chr12	15742297	15742424	ENST00000535752	EPS8
chr12	17120969	17121158	ENST00000481703	RPL7P40
chr12	21014815	21014951	ENST00000421593	SLCO1B7
chr12	21812559	21812728	ENST00000261200	ABCC9
chr12	22384224	22384594	ENST00000536558	ST8SIA1
chr12	22677263	22677375	ENST00000544191	ETNK1
chr12	24438084	24438336	ENST00000429944	RP11-444D3.1
chr12	25182926	25183101	ENST00000354189	CASC1
chr12	27446252	27446357	ENST00000500498	ARNTL2-AS1
chr12	27545225	27545357	ENST00000535575	PPFIBP1
chr12	42140162	42140215	ENST00000398675	GXYLT1

chr12	42225597	42225647	ENST00000327791	YAF2
chr12	44250357	44250864	ENST00000550623	TMEM117
chr12	46836848	46836906	ENST00000546940	SLC38A4
chr12	49915657	49915745	ENST00000549734	RP11-70F11.8
chr12	51638593	51638660	ENST00000545061	SCN8A
chr12	53035926	53036479	ENST00000552905	RP11-983P16.4
chr12	55006283	55006369	ENST00000547559	RP11-616L12.1
chr12	55298382	55298879	ENST00000358433	OR6C6
chr12	55705233	55705480	ENST00000553804	ITGA7
chr12	58903558	58904077	ENST00000552267	LRIG3
chr12	62220946	62221226	ENST00000549456	FAM19A2
chr12	62220946	62221226	ENST00000549456	FAM19A2
chr12	63005210	63005261	ENST00000550738	LDHAL6CP
chr12	65040162	65040349	ENST00000360528	RP11-168J19.2
chr12	65133849	65134335	ENST00000536787	APOOP3
chr12	67979303	67979495	ENST00000355509	GS1-410F4.5
chr12	68289169	68289430	ENST00000357874	MDM1
chr12	69798157	69798340	ENST00000550847	RAB3IP
chr12	70061332	70062058	ENST00000549419	LINC01481
chr12	77771625	77771846	ENST00000549993	RP11-781A6.1
chr12	81068459	81068542	ENST00000549175	ACSS3
chr12	83148742	83148856	ENST00000464696	RPL6P25
chr12	86815733	86815893	ENST00000621808	MGAT4C
chr12	95654742	95655041	ENST00000552554	PGAM1P5
chr12	95654742	95655041	ENST00000552554	PGAM1P5
chr12	95654742	95655041	ENST00000552554	PGAM1P5
chr12	95654742	95655041	ENST00000552554	PGAM1P5
chr12	95654742	95655041	ENST00000552554	PGAM1P5
chr12	95654742	95655041	ENST00000552554	PGAM1P5
chr12	95654742	95655041	ENST00000552554	PGAM1P5
chr12	95655302	95655468	ENST00000552554	PGAM1P5
chr12	95655302	95655468	ENST00000552554	PGAM1P5
chr12	95655302	95655468	ENST00000552554	PGAM1P5
chr12	95655302	95655468	ENST00000552554	PGAM1P5
chr12	95733693	95733775	ENST00000343702	NTN4
chr12	95837178	95837498	ENST00000553194	RP11-536G4.2
chr12	95837178	95837498	ENST00000553194	RP11-536G4.2
chr12	95879983	95880158	ENST00000344280	CCDC38
chr12	97440867	97440988	ENST00000538559	RMST
chr12	98988506	98988919	ENST00000549558	ANKS1B
chr12	98988506	98988919	ENST00000549558	ANKS1B
chr12	102131529	102131953	ENST00000417507	PARPBP

chr12	103287469	103287703	ENST00000550650	C12orf42
chr12	104006152	104006317	ENST00000360814	GLT8D2
chr12	105371991	105372092	ENST00000548336	C12orf75
chr12	108287233	108287489	ENST00000412676	CMKLR1
chr12	108287233	108287489	ENST00000412676	CMKLR1
chr12	109809429	109809586	ENST00000418703	TRPV4
chr12	110947089	110947258	ENST00000547607	LINC01405
chr12	111113672	111113735	ENST00000397643	CUX2
chr12	113876559	113876712	ENST00000545145	RBM19
chr12	115876176	115876331	ENST00000546414	RP11-110L15.2
chr12	117222409	117222501	ENST00000317775	NOS1
chr12	117635841	117635996	ENST00000425217	KSR2
chr12	117681970	117682415	ENST00000425217	KSR2
chr12	118169914	118170092	ENST00000419821	TAOK3
chr12	119073610	119073727	ENST00000537730	RP11-364C11.2
chr12	119243523	119243611	ENST00000536009	RP11-64B16.5
chr12	119397804	119397873	ENST00000535685	CCDC60
chr12	119489905	119489955	ENST00000537366	RP11-768F21.1
chr12	121145611	121145689	ENST00000535250	P2RX7
chr12	121458400	121459252	ENST00000377069	KDM2B
chr12	121506733	121507010	ENST00000377069	KDM2B
chr12	121747199	121747297	ENST00000541467	TMEM120B
chr12	121747549	121747607	ENST00000541467	TMEM120B
chr12	121748101	121748182	ENST00000541467	TMEM120B
chr12	121787821	121787929	ENST00000267205	RHOF
chr12	123654445	123654655	ENST00000623811	RP11-338K17.6
chr12	123807858	123808103	ENST00000447853	DNAH10
chr12	123807858	123808103	ENST00000447853	DNAH10
chr12	124476960	124477194	ENST00000542927	NCOR2
chr12	124476960	124477194	ENST00000542927	NCOR2
chr12	125089766	125089983	ENST00000418937	AACS
chr12	125529002	125529196	ENST00000534945	TMEM132B
chr12	125529002	125529196	ENST00000534945	TMEM132B
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chr12	126459367	126459658	ENST00000536421	RP5-944M2.4
chr12	130439268	130439845	ENST00000535703	RIMBP2
chr12	130439268	130439845	ENST00000535703	RIMBP2
chr12	130439268	130439845	ENST00000535703	RIMBP2
chr12	130439268	130439845	ENST00000535703	RIMBP2
chr12	130439268	130439845	ENST00000535703	RIMBP2
chr12	131016957	131017174	ENST00000376682	ADGRD1
chr12	131918777	131918827	ENST00000540647	ULK1
chr12	132267772	132268271	ENST00000424720	GALNT9

chr12	132267772	132268271	ENST00000424720	GALNT9
chr12	132267772	132268271	ENST00000424720	GALNT9
chr12	132267772	132268271	ENST00000424720	GALNT9
chr12	132267772	132268271	ENST00000424720	GALNT9
chr11	1170703	1170942	ENST00000621226	MUC5AC
chr11	1170703	1170942	ENST00000621226	MUC5AC
chr11	1175637	1175689	ENST00000621226	MUC5AC
chr11	1861666	1862101	ENST00000421485	LSP1
chr11	1893131	1893370	ENST00000391480	PRR33
chr11	7066049	7066189	ENST00000299481	NLRP14
chr11	7234021	7234160	ENST00000408776	MIR302E
chr11	8425013	8425352	ENST00000473980	STK33
chr11	8853907	8854308	ENST00000526155	ST5
chr11	14307068	14307219	ENST00000529237	RRAS2
chr11	16375879	16376105	ENST00000528252	SOX6
chr11	22113044	22113266	ENST00000530837	CTD-2019O4.1
chr11	29724650	29724748	ENST00000498657	RPL7AP58
chr11	31063410	31063578	ENST00000597505	DCDC1
chr11	32631607	32631691	ENST00000335185	CCDC73
chr11	33788423	33788527	ENST00000529152	RP11-646J21.7
chr11	40729995	40730188	ENST00000534577	LRRC4C
chr11	50337178	50337238	ENST00000533230	RP11-574M7.1
chr11	59312448	59312781	ENST00000534535	OR5AN2P
chr11	59724095	59724341	ENST00000300150	STX3
chr11	59769852	59769919	ENST00000530498	STX3
chr11	61391234	61391292	ENST00000515837	TMEM216
chr11	63214920	63215512	ENST00000525620	SLC22A10
chr11	63221312	63221508	ENST00000525620	SLC22A10
chr11	68817792	68817853	ENST00000376618	CPT1A
chr11	69813564	69813888	ENST00000334134	FGF3
chr11	70122462	70122666	ENST00000531349	ANO1
chr11	70125236	70125356	ENST00000531349	ANO1
chr11	70787037	70787519	ENST00000470759	SHANK2
chr11	72708911	72708961	ENST00000464109	RPS12P20
chr11	78183572	78183644	ENST00000529350	KCTD21
chr11	84726628	84727341	ENST00000398309	DLG2
chr11	86995799	86995982	ENST00000531827	RP11-736K20.6
chr11	87044945	87045100	ENST00000305494	TMEM135
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chr16	29197362	29197957	ENST00000563477	RP11-426C22.5
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chr16	81689240	81690266	ENST00000565680	CMIP
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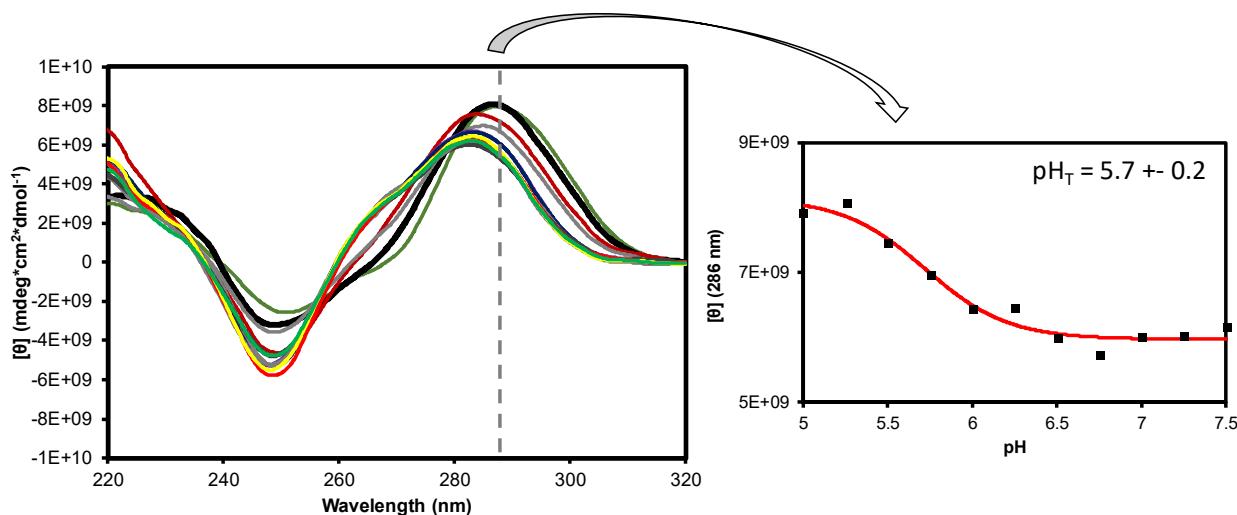
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chr1	237922047	237922530	ENST00000450208	RP11-193H5.2
chr1	239510121	239510171	ENST00000481779	CHRM3
chr1	240022911	240023168	ENST00000447095	FMN2
chr1	240895747	240896087	ENST00000446183	RGS7
chr1	241684755	241684918	ENST00000437684	WDR64
chr1	241878454	241878606	ENST00000521202	EXO1
chr1	242300520	242300595	ENST00000536534	PLD5
chr1	243938630	243938776	ENST00000440494	RP11-278H7.1
chr1	244601757	244602042	ENST00000415043	CYCSP5
chr1	244640838	244640937	ENST00000487449	C1orf101
chr1	244691192	244691264	ENST00000418162	DESI2
chr1	244968370	244968759	ENST00000607453	RP11-156E8.1
chr1	245078339	245078712	ENST00000427529	EFCAB2
chr9	2927177	2927428	ENST00000426329	CARM1P1
chr9	3340112	3340649	ENST00000382004	RFX3
chr9	3341208	3341283	ENST00000382004	RFX3
chr9	3861693	3861820	ENST00000324333	GLIS3
chr9	5179300	5179863	ENST00000381641	INSL6
chr9	5685755	5685837	ENST00000545641	RIC1
chr9	19418605	19418718	ENST00000340967	ACER2
chr9	22743595	22743900	ENST00000453177	CLIC4P1
chr9	33736566	33736816	ENST00000422764	LINC01251
chr9	36058139	36058556	ENST00000479053	RECK
chr9	41229400	41230423	ENST00000612572	MIR4477A
chr9	41316706	41316848	ENST00000618030	RP11-187C18.5
chr9	41945108	41945737	ENST00000618777	CNTNAP3B
chr9	62799226	62799877	ENST00000427509	LINC01410
chr9	62799226	62799877	ENST00000427509	LINC01410
chr9	63818790	63818845	ENST00000455245	DUX4L50
chr9	67723662	67724532	ENST00000617851	RP11-12A20.6
chr9	87743961	87744682	ENST00000410476	AL160279.1
chr9	92866866	92867054	ENST00000395505	ZNF484
chr9	94791324	94791434	ENST00000428313	C9orf3
chr9	95958750	95958975	ENST00000470362	ERCC6L2
chr9	98798035	98798216	ENST00000471846	ANKS6
chr9	110982396	110982517	ENST00000374431	LPAR1
chr9	112127237	112127287	ENST00000355396	SUSD1
chr9	121895479	121896014	ENST00000373778	TTLL11
chr9	124585467	124585590	ENST00000487099	NR6A1
chr9	129478665	129478723	ENST00000578012	AL353803.1

chr9	130325224	130325288	ENST00000611173	HMCN2
chr9	130841823	130842070	ENST00000318560	ABL1
chr9	131726547	131726721	ENST00000484033	RN7SL328P
chr9	132010769	132010922	ENST00000357028	MED27
chr9	132577322	132577430	ENST00000263610	BARHL1
chr9	132799760	132799810	ENST00000476719	AK8
chr9	133392570	133392697	ENST00000462310	STKLD1
chr9	133393531	133393738	ENST00000462310	STKLD1
chr9	133393531	133393738	ENST00000462310	STKLD1
chr9	133791953	133792566	ENST00000406606	VAV2
chr9	133791953	133792566	ENST00000406606	VAV2
chr9	133851677	133852006	ENST00000406606	VAV2
chr9	134141220	134141351	ENST00000358625	WDR5
chr9	134550017	134550299	ENST00000446184	RP11-473E2.3
chr9	134550017	134550299	ENST00000446184	RP11-473E2.3
chr9	134550017	134550299	ENST00000446184	RP11-473E2.3
chr9	134550017	134550299	ENST00000446184	RP11-473E2.3
chr9	134550017	134550299	ENST00000446184	RP11-473E2.3
chr9	134684930	134685982	ENST00000464187	COL5A1
chr9	134726380	134726971	ENST00000469093	COL5A1
chr9	135091542	135091960	ENST00000371793	OLFM1
chr9	136010668	136010902	ENST00000371753	NACC2
chr9	136427638	136428349	ENST00000371712	INPP5E
chr9	136802898	136803109	ENST00000471502	RP11-216L13.18
chr9	136802898	136803109	ENST00000471502	RP11-216L13.18
chr9	136802898	136803109	ENST00000471502	RP11-216L13.18
chr9	136810613	136810725	ENST00000357466	RABL6
chr9	136810613	136810725	ENST00000357466	RABL6
chr9	136810613	136810725	ENST00000357466	RABL6
chr9	137466521	137466894	ENST00000492278	PNPLA7
chr9	138056856	138056906	ENST00000371355	CACNA1B
chr8	13256459	13256804	ENST00000509922	DLC1
chr8	22963915	22964156	ENST00000522278	PEBP4
chr8	26778777	26778917	ENST00000380586	ADRA1A
chr8	30920636	30920832	ENST00000523019	RP5-1009N12.1
chr8	32188788	32188940	ENST00000517967	NRG1
chr8	39482120	39482329	ENST00000460383	ADAM3A
chr8	49944544	49944602	ENST00000521316	SNTG1
chr8	49944544	49944602	ENST00000521316	SNTG1
chr8	54331803	54331875	ENST00000362342	RNU105C
chr8	54331803	54331875	ENST00000362342	RNU105C
chr8	63935893	63935943	ENST00000523191	RP11-32K4.1
chr8	67139120	67139885	ENST00000508335	RP11-453N18.1

chr8	68001847	68002013	ENST00000517617	PREX2
chr8	69127654	69127841	ENST00000520239	GS1-44D20.1
chr8	73218849	73219129	ENST00000518337	RP11-956J14.2
chr8	85643266	85643610	ENST00000604378	REXO1L8P
chr8	92031740	92031918	ENST00000613886	RUNX1T1
chr8	99336999	99337374	ENST00000522802	VPS13B
chr8	99808339	99808551	ENST00000523025	RP11-402L5.2
chr8	120363323	120363464	ENST00000440844	COL14A1
chr8	121093480	121093740	ENST00000517739	RP11-369K17.1
chr8	124874253	124874363	ENST00000528090	LINC00964
chr8	133194708	133195127	ENST00000220856	WISP1
chr8	139757038	139757130	ENST00000389328	TRAPPC9
chr8	144234495	144234558	ENST00000544576	MROH1

**Figure S7.** *NEIL3* i-motif pH-dependent stability determined by CD analysis



The four- or five-track *NEIL3* i-motif stabilities were determined. The stabilities for these sequences were determined by folding the sequences in Britton-Robinson buffer (lithium salts of 20 mM phosphate, 20 mM acetate, and 20 mM borate doped with 140 mM KCl) preadjusted to pH values 5.0 to 8.0 in increments of 0.25. The i-motifs were folded by direct addition of the sequences to each buffer studied allowing folding to occur for 30 min followed by CD analysis (see figure on this page). The CD intensities at 286 nm were plotted as a function of pH to obtain the titration curve that was fit to the Henderson-Hasselbalch equation to determine the transition pH ( $\text{pH}_T$ ), which is a value that reports on the stability of the fold.<sup>9</sup> The five-track *NEIL3* i-motif had a  $\text{pH}_T = 5.7 \pm 0.2$  and the four-track *NEIL3* i-motif had a  $\text{pH}_T = 5.3 \pm 0.3$ . These values are consistent with a previous report from our laboratory.<sup>10</sup> Previously our lab studied model i-motifs and found those with purine-rich loops were the least stable,<sup>11</sup> and inspection of the *NEIL3* i-motif sequences identify loops rich in purines supporting the low stabilities measured for these sequences.

Four-track *NEIL3* i-motif = 5'-GG CCCC G CCC AGG CCCC G CCC AA

Five-track *NEIL3* i-motif = 5'-GG CCCC G CCC AGG CCCC G CCC AACAGCA CCC TA

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