

Supplementary Material

Novel interaction interfaces mediate the interaction between the NEIL1 DNA Glycosylase and Mitochondrial Transcription Factor A

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Key words: Base Excision Repair, NEIL1 DNA glycosylase, mitochondrial transcription factor A, size exclusion chromatography, small angle X-ray scattering, HDX-MS

Running title: Characterization of the NEIL1-TFAM complex

CONTENT:

I. Supplementary Tables

II. Supplementary Legends and Figures

I. Supplementary Tables

Supplementary Table 1: Molecular Weight (MW) values determined by Size Exclusion Chromatography (SEC) using a Superdex 200 3.2/300 column

	<i>NEIL1</i>	<i>TFAM</i>	<i>NEIL1-TFAM</i>		<i>NEIL1-SD*</i>		<i>NEIL1-NSD*</i>		<i>TFAM-SD*</i>		<i>TFAM-NSD*</i>		<i>NEIL1-TFAM-SD*</i>			<i>NEIL1-TFAM-NSD*</i>		
			<i>Peak 1</i>	<i>Peak 2</i>	<i>Peak 1</i>	<i>Peak 2</i>	<i>Peak 1</i>	<i>Peak 2</i>	<i>Peak 1</i>	<i>Peak 2</i>	<i>Peak 1</i>	<i>Peak 2</i>	<i>Peak 1</i>	<i>Peak 2</i>	<i>Peak 3</i>	<i>Peak 1</i>	<i>Peak 2</i>	<i>Peak 3</i>
Calculated MW	49.1	39.9	51.0	41.8	52.8	34.4	52.8	34.4	45.4	36.3	43.6	34.4	51.0	41.8	34.4	51.0	41.8	34.4

* double-stranded DNA substrates used in these studies is described in Supplementary Figure 1 where the X represents either thymine in the non-specific DNA substrate (NSD) or tetrahydrofuran, an abasic site analog, in the specific substrate (SD)

Supplementary Table 2: SAXS and MALS data collection parameters and calculated molecular weight values

(A) Sample details																					
	NEIL1		TFAM	NEIL1-TFAM		NEIL1-SD*		NEIL1-NSD*		TFAM-SD*		TFAM-NSD*		NEIL1-TFAM-SD*		NEIL1-TFAM-NSD*					
Organism	Homo sapiens		Homo sapiens	Homo sapiens		Homo sapiens		Homo sapiens		Homo sapiens		Homo sapiens		Homo sapiens		Homo sapiens					
Source	E. coli		E. coli	E. coli		E. coli		E. coli		E. coli		E. coli		E. coli		E. coli					
UniProt ID	#Residues		Q96F14	Q00059																	
	in constructs		(1-390)	(43-246)																	
Extinction coeff. [A280, 0.1%(w/v)]	0.725		1.38																		
SEC-SAXS-MALS column, Superdex 200 Increase 10/300																					
Loading concentration (uM)	190		190	190		190		190		190		190		190		190		190			
Injection volume (µl)	300		300	300		300		300		300		300		300		300		300			
Flow rate (ml min ⁻¹)	0.7		0.7	0.7		0.7		0.7		0.7		0.7		0.7		0.7		0.7			
Buffer	25 mM HEPES pH 7.4, 5% glycerol, 300 mM NaCl, and 1 mM DTT																				
(B) Data collection parameters																					
Beamline	APS		APS	APS		APS		APS		APS		APS		APS		APS		APS			
Wavelength (Å)	1.03		1.03	1.03		1.03		1.03		1.03		1.03		1.03		1.03		1.03			
Q Range (Å ⁻¹)	0.0043-0.35		0.0043-0.35	0.0043-0.35		0.0043-0.35		0.0043-0.35		0.0043-0.35		0.0043-0.35		0.0043-0.35		0.0043-0.35		0.0043-0.35			
Temperature (°C)	25		25	25		25		25		25		25		25		25		25			
(C) Software used for data reduction, analysis and interpretation																					
SAXS data reduction											BioXTAS Raw version 2.1.0										
SAXS data analysis											BioXTAS Raw (EFA), ATSAS										
Extinction coeff. estimate											ProtParam (Expasy)										
(D) Structural parameters																					
	NEIL1		TFAM	NEIL1-TFAM		NEIL1-SD*		NEIL1-NSD*		TFAM-SD*		TFAM-NSD*		TFAM-NEIL1-SD*		TFAM-NEIL1-NSD*					
	Comp 1	Comp 2	Comp 1	Comp 1	Comp 2	Comp 1	Comp 2	Comp 1	Comp 2	Comp 3	Comp 1	Comp 2	Comp 1	Comp 2	Comp 1	Comp 2	Comp 3	Comp 1	Comp 2	Comp 3	
I(0) from P(r)	0.07 +/- 1.87e-3	0.18 +/- 8.49e-4	2.88e-4 +/- 3.83e-6	0.07 +/- 5.75e-4	0.03 +/- 3.69e-4	0.44 +/- 5.52e-4	0.02 +/- 2.48e-4	0.05 +/- 3.93e-4	0.27 +/- 4.09e-4	0.01 +/- 2.09e-4	0.15 +/- 2.27e-4	0.01 +/- 1.58e-4	0.18 +/- 2.64e-4	0.02 +/- 2.66e-4	0.02 +/- 7.16e-4	0.36 +/- 1.17e-3	0.12 +/- 3.32e-4	0.33 +/- 6.51e-4	0.19 +/- 5.21e-4	nd	
R _g (Å) from P(r)	53.85 +/- 2.23	36.25 +/- 0.36	33.83 +/- 0.93	40.26 +/- 0.78	30.69 +/- 0.69	31.87 +/- 0.09	24.39 +/- 0.27	41.92 +/- 0.38	31.53 +/- 0.11	27.83 +/- 0.57	26.77 +/- 0.06	24.07 +/- 0.36	28.33 +/- 0.06	25.1 +/- 0.65	61.51 +/- 1.38	38.5 +/- 0.24	26.32 +/- 0.12	40.78 +/- 0.15	29.61 +/- 0.1	nd	
I(0) from Guinier	0.07 +/- 9.08e-4	0.17 +/- 4.87e-4	2.87e-4 +/- 2.81e-6	0.07 +/- 4.27e-4	0.03 +/- 3.61e-4	0.44 +/- 4.85e-4	0.02 +/- 3.1e-4	0.05 +/- 4.62e-4	0.26 +/- 3.87e-4	0.01 +/- 2.26e-4	0.15 +/- 1.99e-4	0.01 +/- 1.71e-4	0.18 +/- 2.69e-4	0.02 +/- 2.78e-4	0.02 +/- 1.04e-3	0.35 +/- 9.99e-4	0.12 +/- 3.69e-4	0.32 +/- 6.37e-4	0.19 +/- 5.12e-4	nd	
R _g from Guinier	44.5 +/- 0.84	32.35 +/- 0.14	31.87 +/- 0.55	35.22 +/- 0.38	27.33 +/- 0.68	31.03 +/- 0.07	23.74 +/- 0.55	41.7 +/- 0.64	30.76 +/- 0.08	25.49 +/- 0.91	26.55 +/- 0.06	23.11 +/- 0.54	28.1 +/- 0.08	25.42 +/- 0.76	42.57 +/- 3.99	37.08 +/- 0.18	25.75 +/- 0.15	39.16 +/- 0.13	29.25 +/- 0.14	nd	
Dmax (Å)	220	152	140	171	112	126	68	127	115	90	89	73	95	96	203	148	87	154	94	nd	
(E) Molecular weight determination (kDa)																					
Expected Theoretical (Expasy)	44.7		25.6	70.3		58.3		58.3		39.2		39.2		83.9		83.9					
MW(V _p)	84.1	56.3	40.2	63.3	17.6	nd	nd	158.9	66.4	28.2	40.3	10.3	50.9	nd	231.9	107	45.8	119.9	63.2	nd	
MW(V _c)	55.9	44.6	34.2	47.7	17.3	nd	nd	136.1	57.2	30.4	38.6	19	47.2	nd	452.7	89.2	43.3	98.7	57.7	nd	
MW(S&S)	-	53.3	45	55.9	28.5	nd	nd	152.5	69.6	34	47.4	22.5	53.1	nd	nd	102.9	47.8	111.8	58.9	nd	
MW (Bayes)	62.4	49.8	36.9	53.1	16.8	nd	nd	138.2	58.1	28.9	37.7	14.2	47.7	nd	104.9	94.2	43.8	101	58.1	nd	
MW (MALS)	nd	nd	26.1	57.5	29.1	56.3	nd	78.1	57.2	nd	34.3	nd	41.2	nd	91.2**	68.7	51.6	nd	79	52.7	

* double-stranded DNA substrates used in these studies is described in Supplementary Figure 1 where the X represents either thymine in the non-specific DNA substrate (NSD) or tetrahydrofuran, an abasic site analog, in the specific substrate (SD).

“Comp” refers to different components obtained during EFA. While all the data for each component is reported in the table above, the highlighted data (light orange) corresponding to the most well-behaved species as determined by scattering curves and Kratky analysis are discussed in the main manuscript.

**The peaks used to calculate the MW values using MALS and SAXS do not necessarily correspond to the same species. The values that match best with our theoretical understanding of the complex are highlighted in light purple and further discussed.

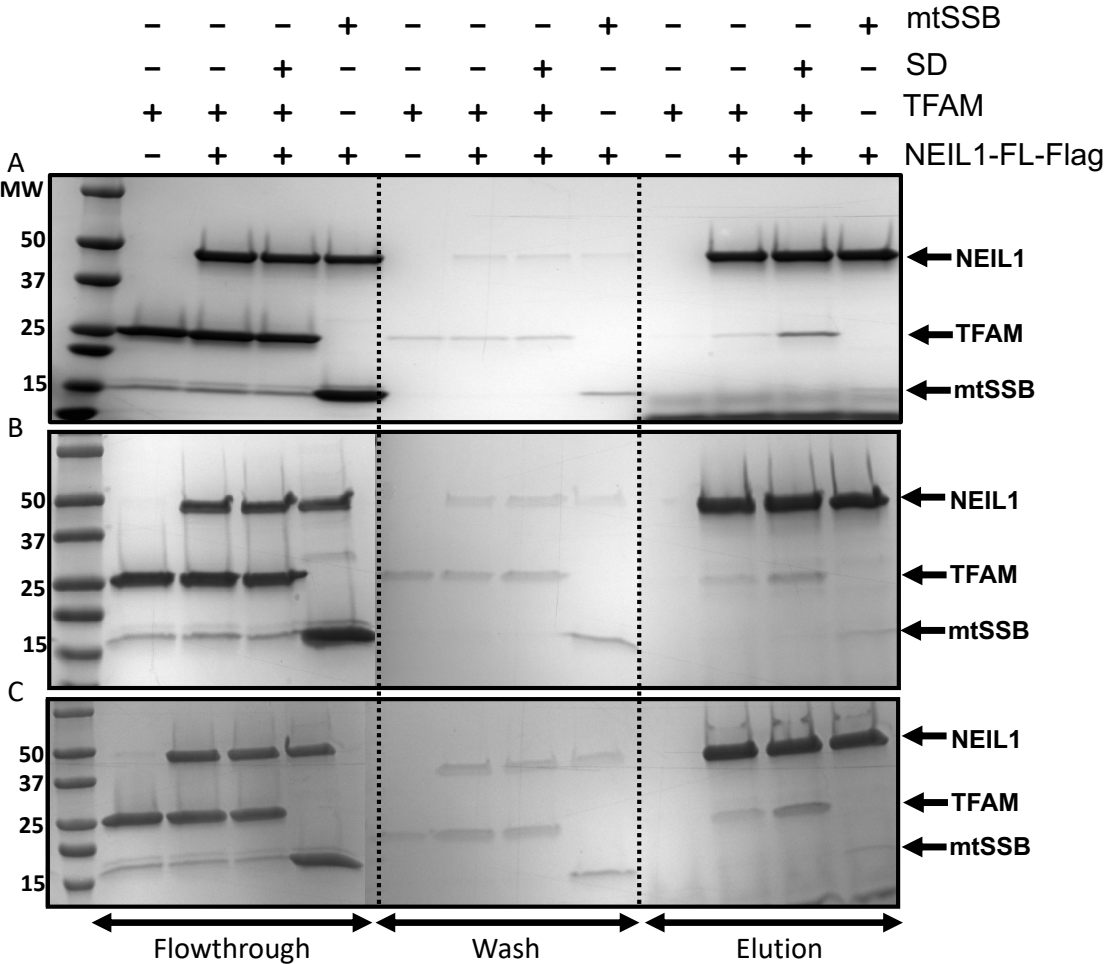
II. Supplementary Figures

Supplementary Figure 1: Sequences of the duplex DNA and RNA substrates used in our studies. In the non-specific DNA substrate (NSD), the **X** refers to a thymine and in the specific DNA (SD) sequence the **X** represents an abasic site analog, tetrahydrofuran (THF).

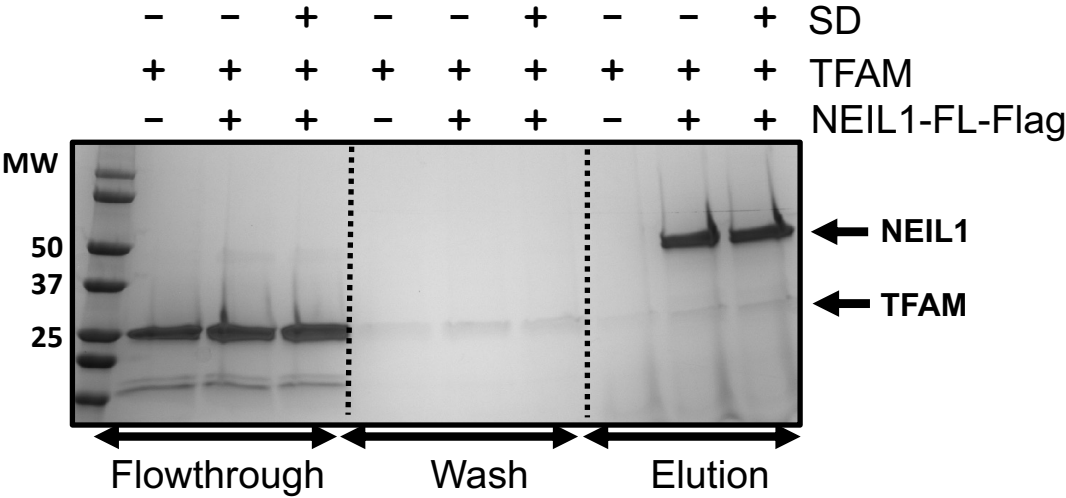
5' – ATTCAACCAAXAGCCCTGGCCG– 3' Non-Specific DNA (NSD) X=T 3' – TAAGTTGGTTATCGGGACCGGC– 5' Specific DNA (SD) X=THF

5' – rArUrUrCrArArCrCrArAXrArGrCrCrCrUrGrGrCrCrG– 3' X=THF
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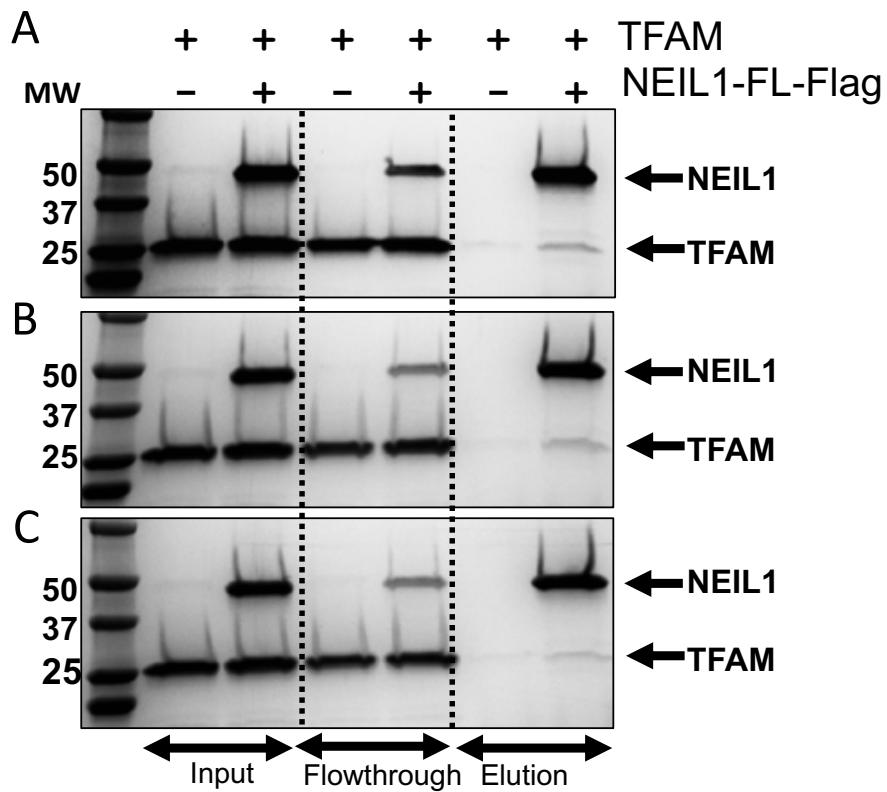
Supplementary Figure 2: Affinity pull-down experiments reveal an interaction between NEIL1 and TFAM under conditions of low salt between 50-100 mM NaCl. **(A)** Recombinantly expressed Flag-tagged NEIL1 was used to pull down TFAM in the presence and absence of a specific DNA (SD) sequence containing an abasic site under conditions of 50 mM NaCl. **(B-C)** Replicate gel analyses of the affinity pull-down experiments displayed in Figure 1 of the main manuscript using Flag-tagged NEIL1 at 100 mM NaCl.



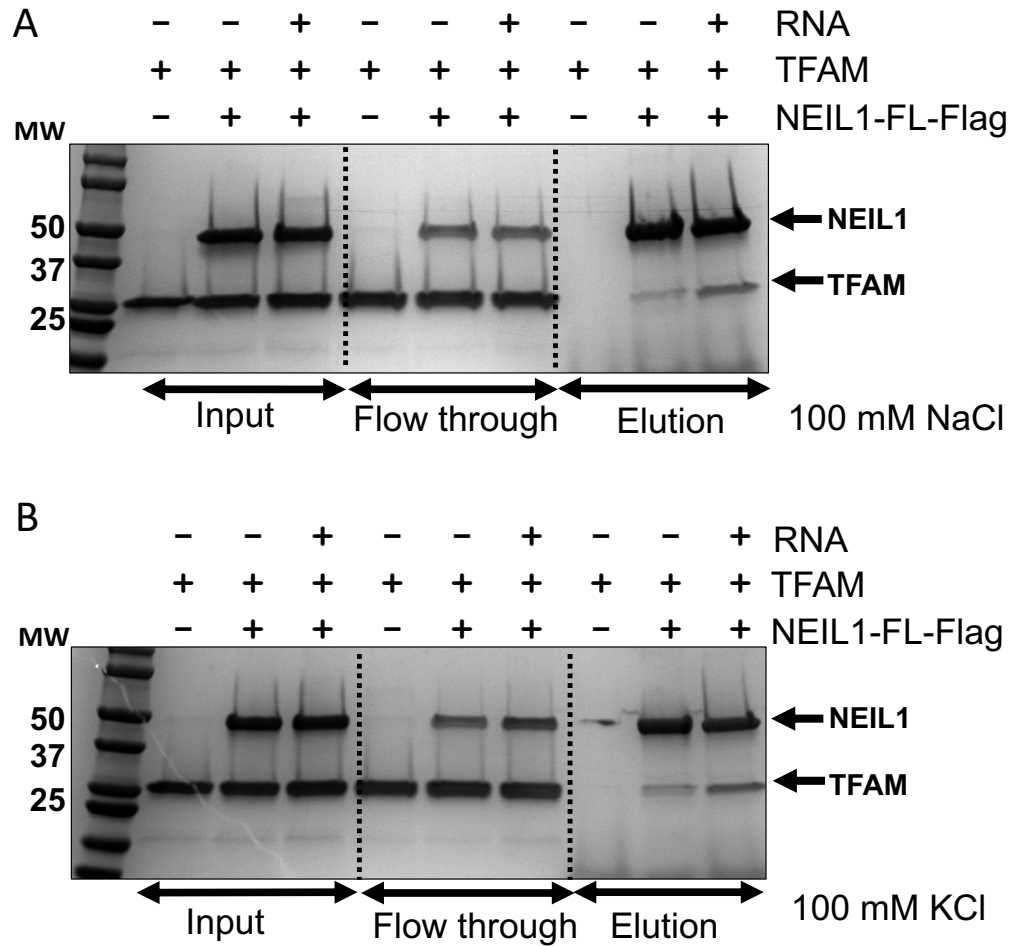
Supplementary Figure 3: Affinity pull-down experiments at 300 mM NaCl reveal a slight interaction between Flag-tagged NEIL1 and TFAM in the presence and absence of SD.



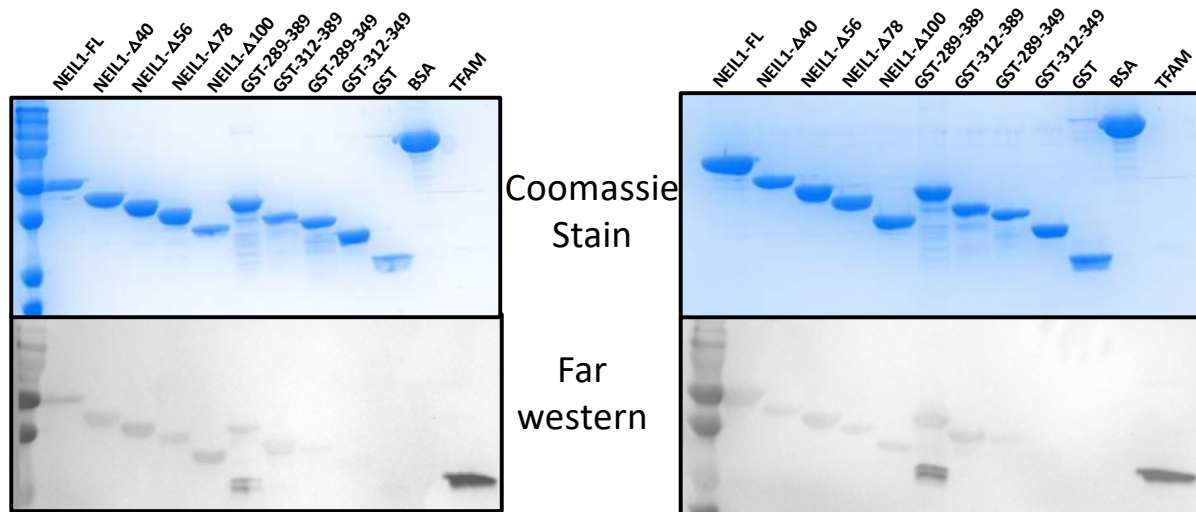
Supplementary Figure 4: Affinity flag pull-down experiments for flag tagged NEIL1 and TFAM at (A) 50 mM KCl, (B) 100 mM KCl, and (C) 150 mM KCl in the absence of nucleic acids.



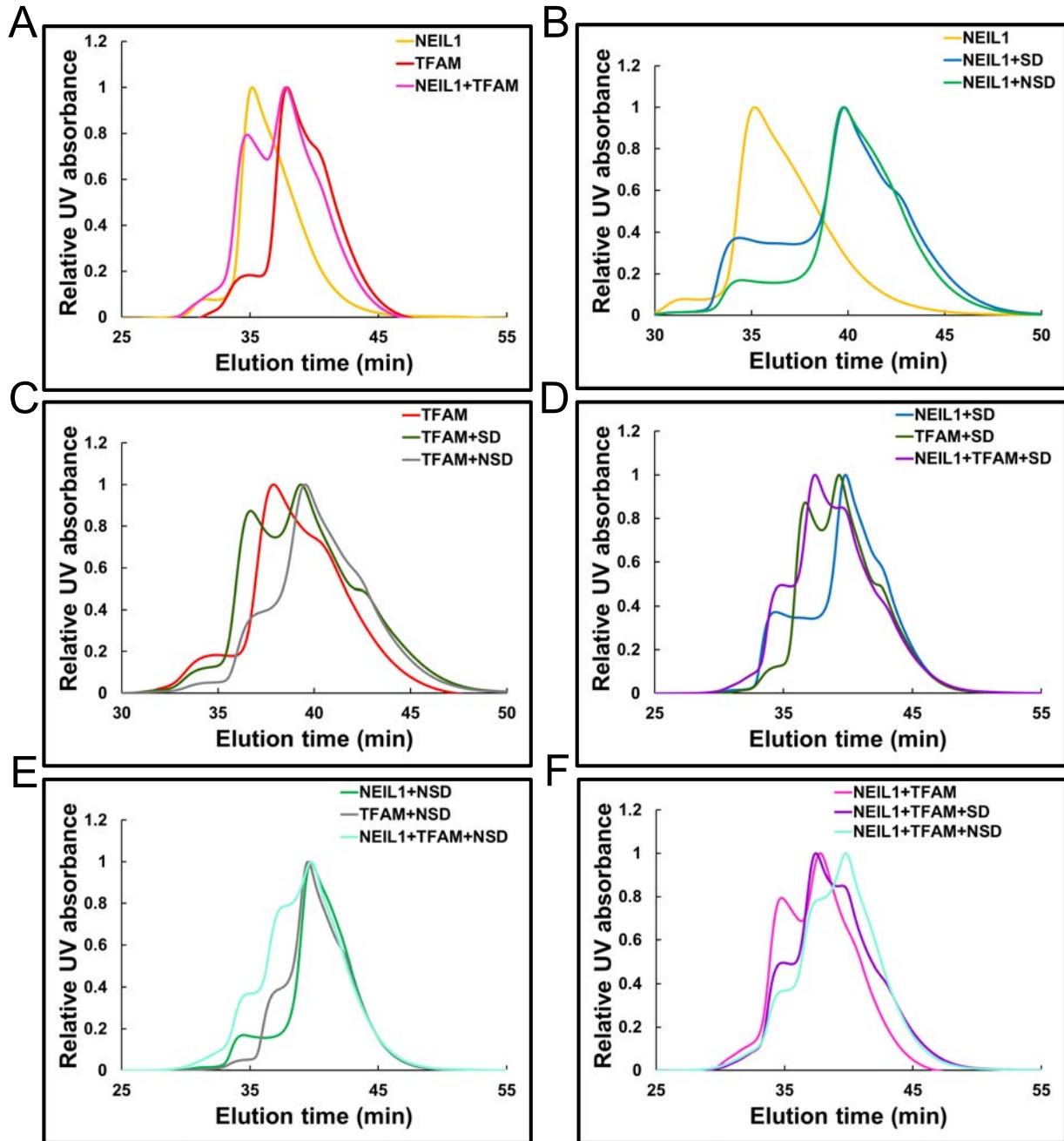
Supplementary Figure 5: Replicate gel analyses of the affinity flag pull-down experiments in the presence of RNA at (A) 100 mM NaCl and (B) 100 mM KCl.



Supplementary Figure 6: Replicate gels of the far-western analysis displayed in Figure 3 of the main manuscript reveals that the interaction between NEIL1 and TFAM is mediated by residues present within the N and C-terminal domains of NEIL1.

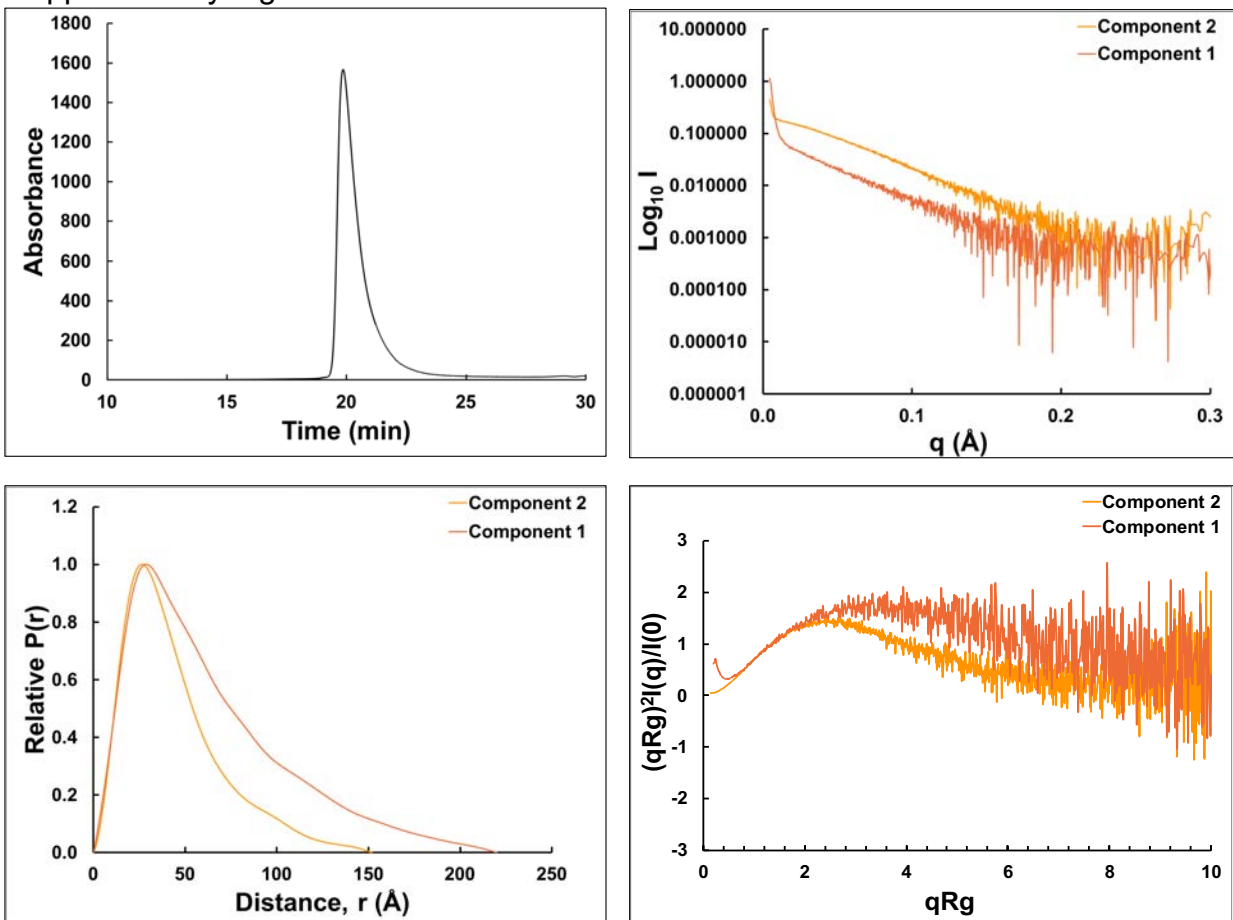


Supplementary Figure 7: Size-exclusion chromatography (SEC) to isolate complexes of NEIL1 and TFAM in the presence of DNA. **(A – F)** SEC elution profiles from a pre-calibrated Superdex 200 column for samples containing the NEIL1 and TFAM proteins alone, binary complexes of the proteins in the presence or absence of SD or NSD, or ternary complexes containing NEIL1-TFAM-SD or -NSD.

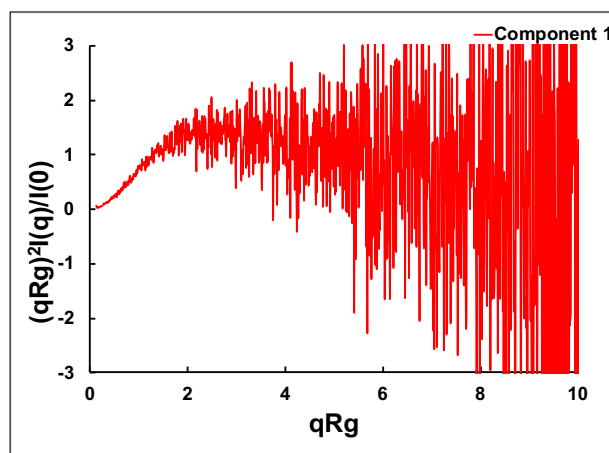
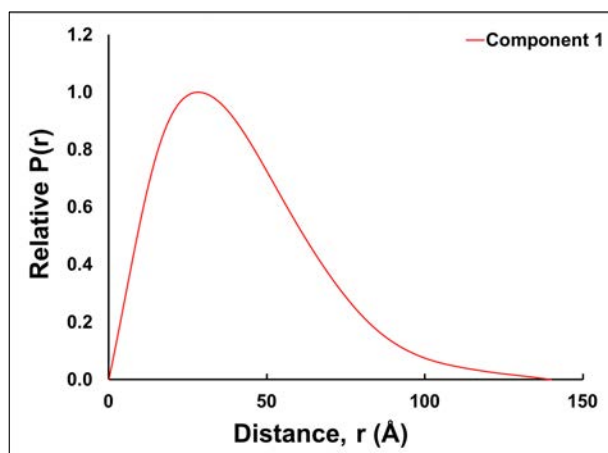
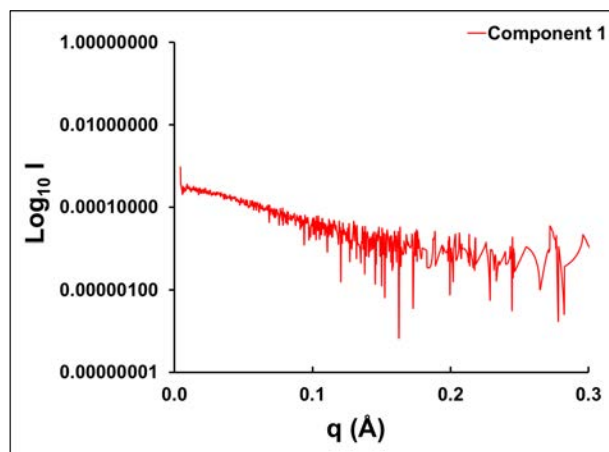
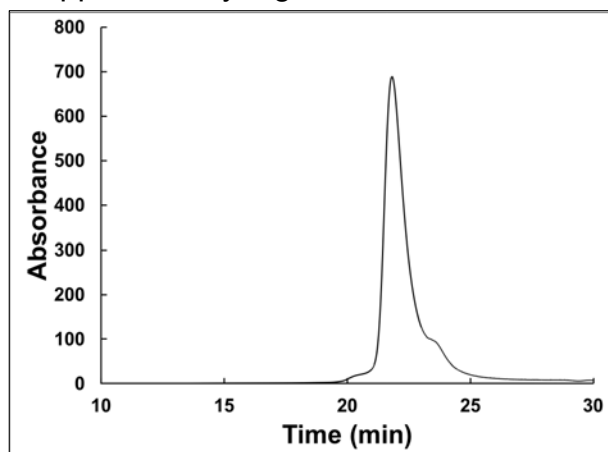


Supplementary Figure 8: SEC-SAXS analysis for NEIL1 **(A)**, TFAM **(B)**, NEIL1+TFAM **(C)**, NEIL1+SD **(D)**, NEIL1+NSD **(E)**, TFAM+SD **(F)**, TFAM+NSD **(G)**, TFAM+NEIL+SD **(H)**, TFAM+NEIL+NSD **(I)**. Evolving factor analysis was performed during SAXS data processing to extract multiple components. For each protein/sample mixture, the SEC profile from the Superdex 200 column is displayed in the top left panel, the SAXS scattering curve is at the top right, pairwise distance distribution function is displayed on the bottom left, and the dimensionless (R_g) Kratky plot is on the bottom right. I - scattering intensity, q - momentum transfer, R_g - radius of gyration, and $P(r)$ - pairwise distance distribution function.

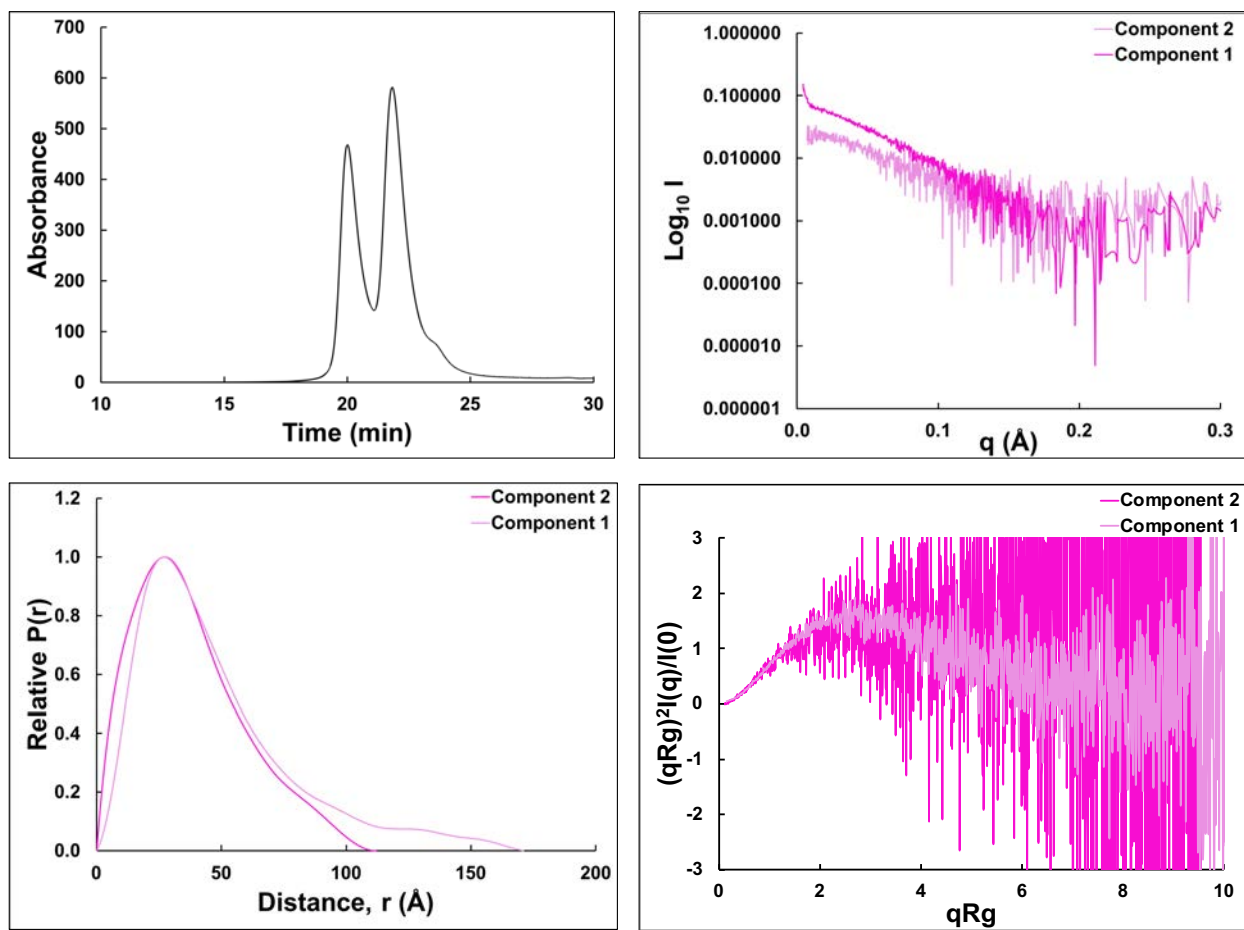
Supplementary Figure 8A: NEIL1



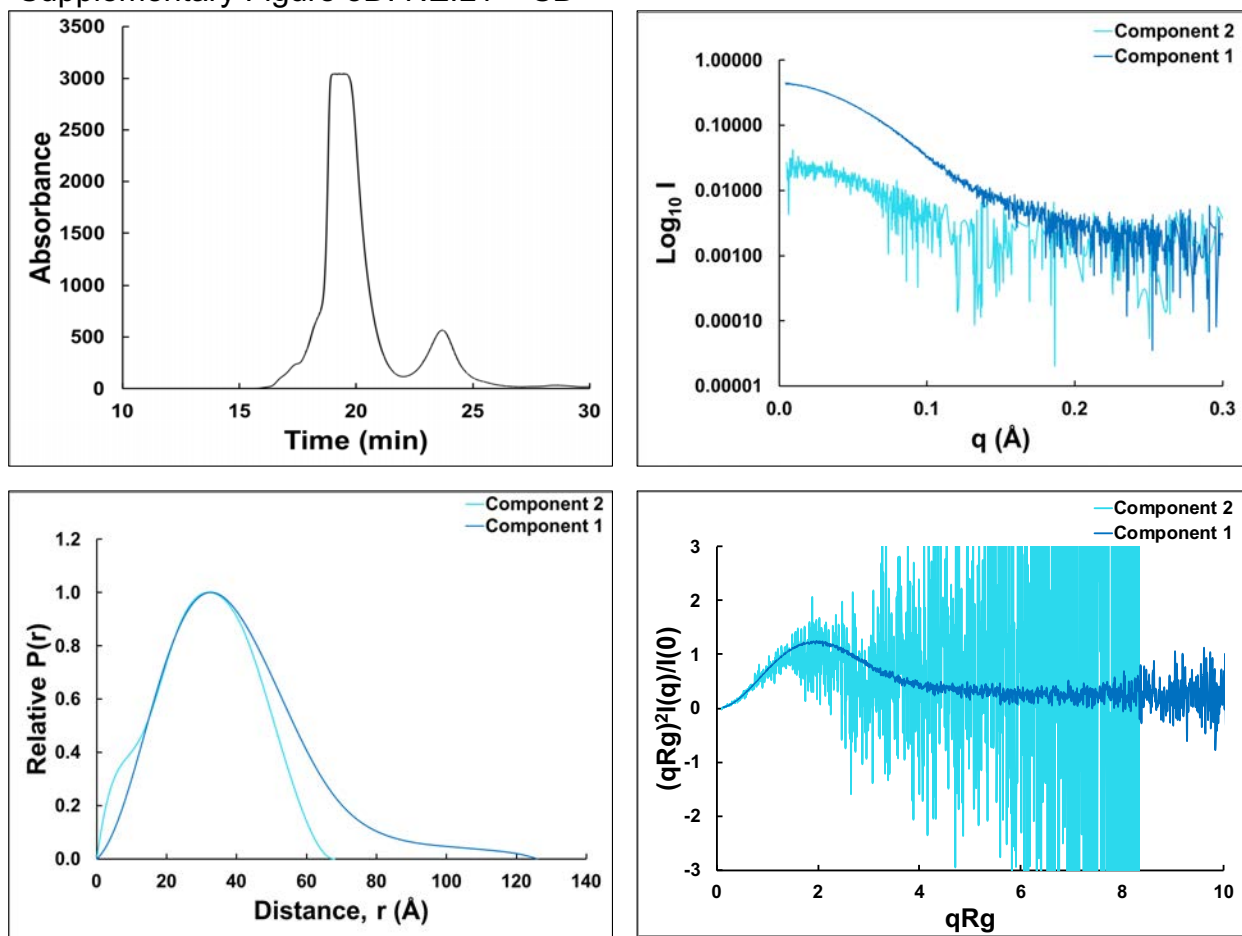
Supplementary Figure 8B: TFAM



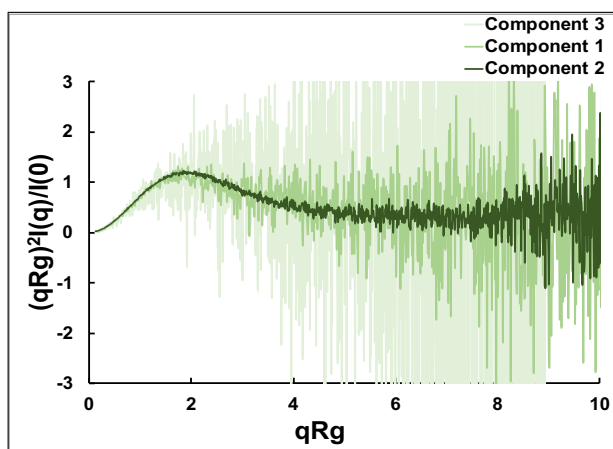
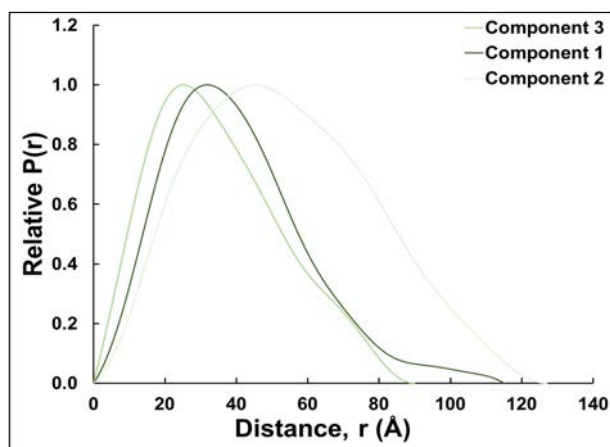
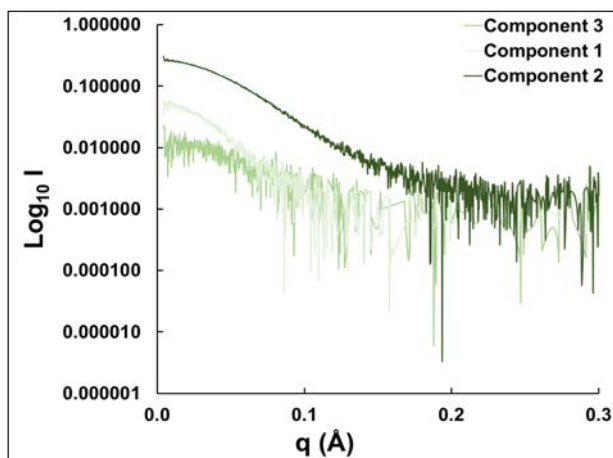
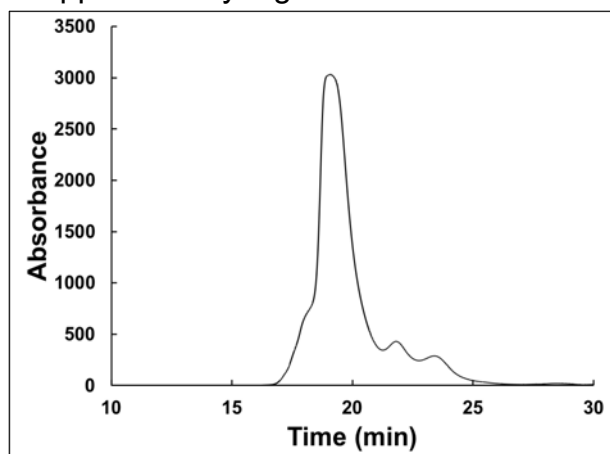
Supplementary Figure 8C: NEIL1 + TFAM



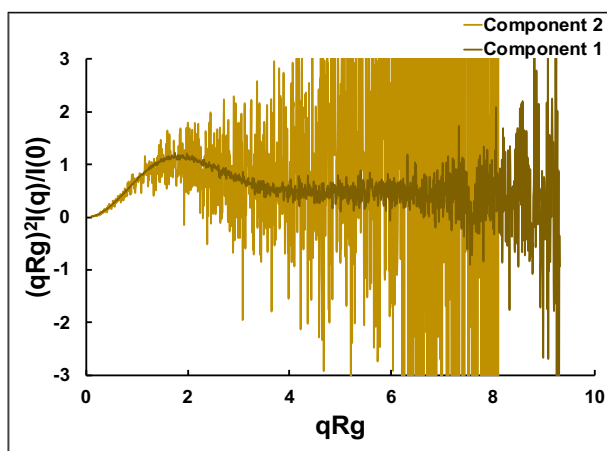
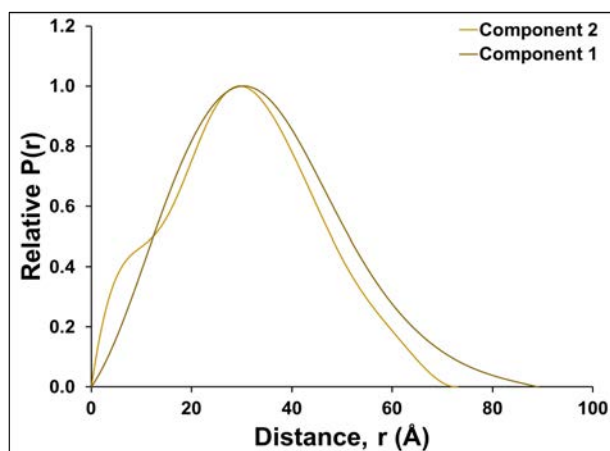
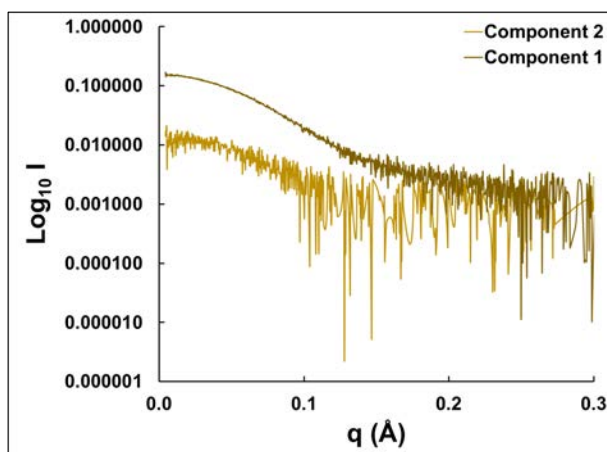
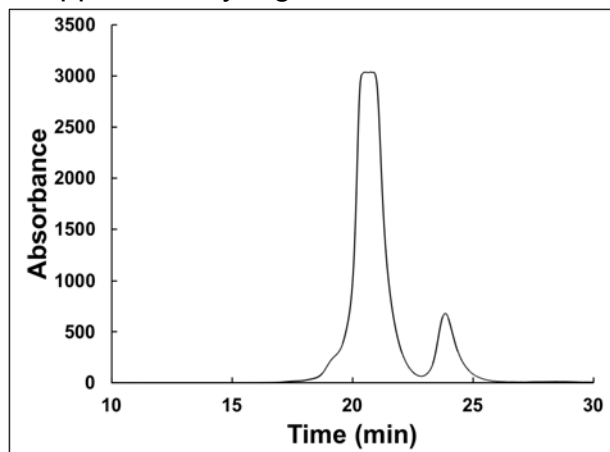
Supplementary Figure 8D: NEIL1 + SD



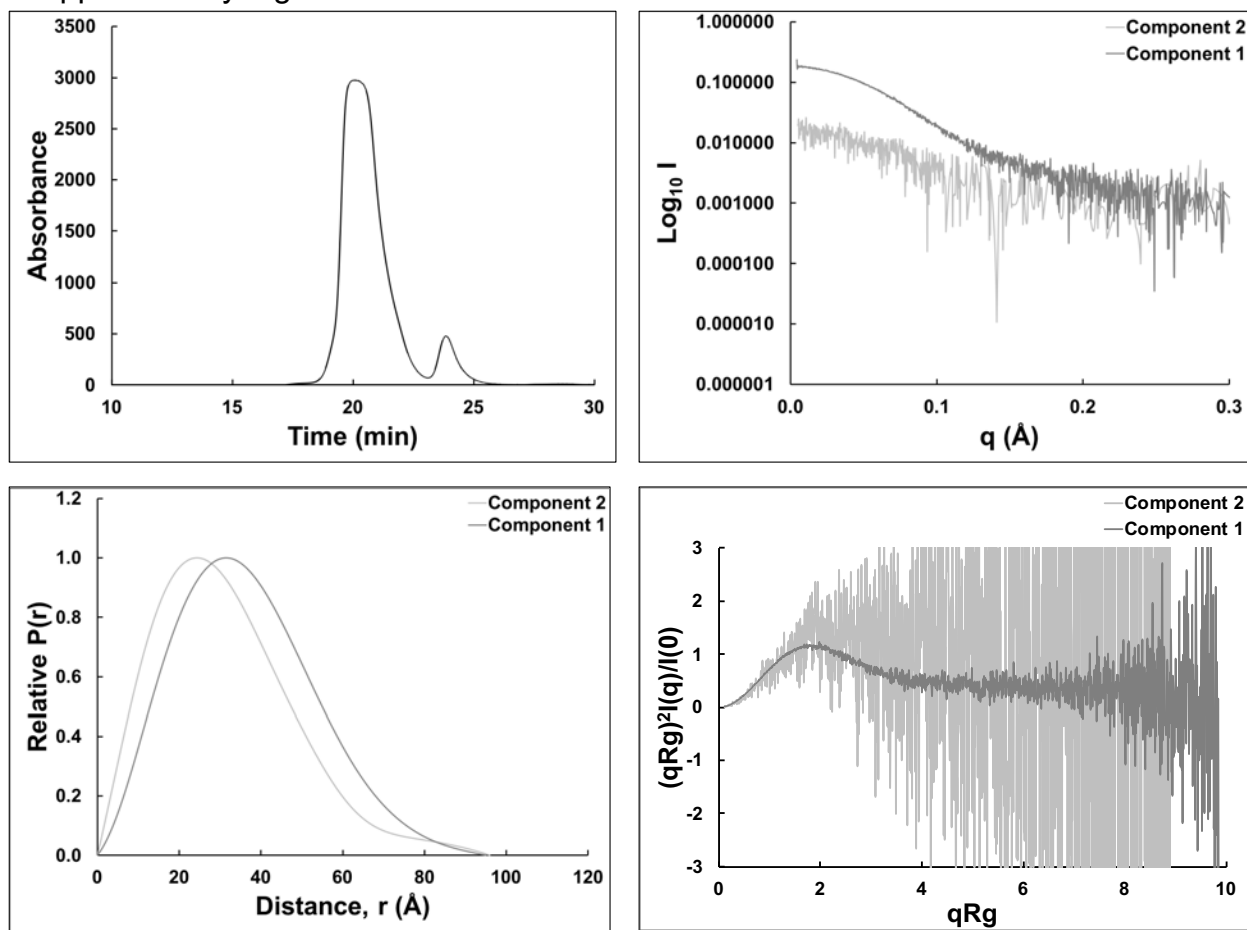
Supplementary Figure 8E: NEIL1 + NSD



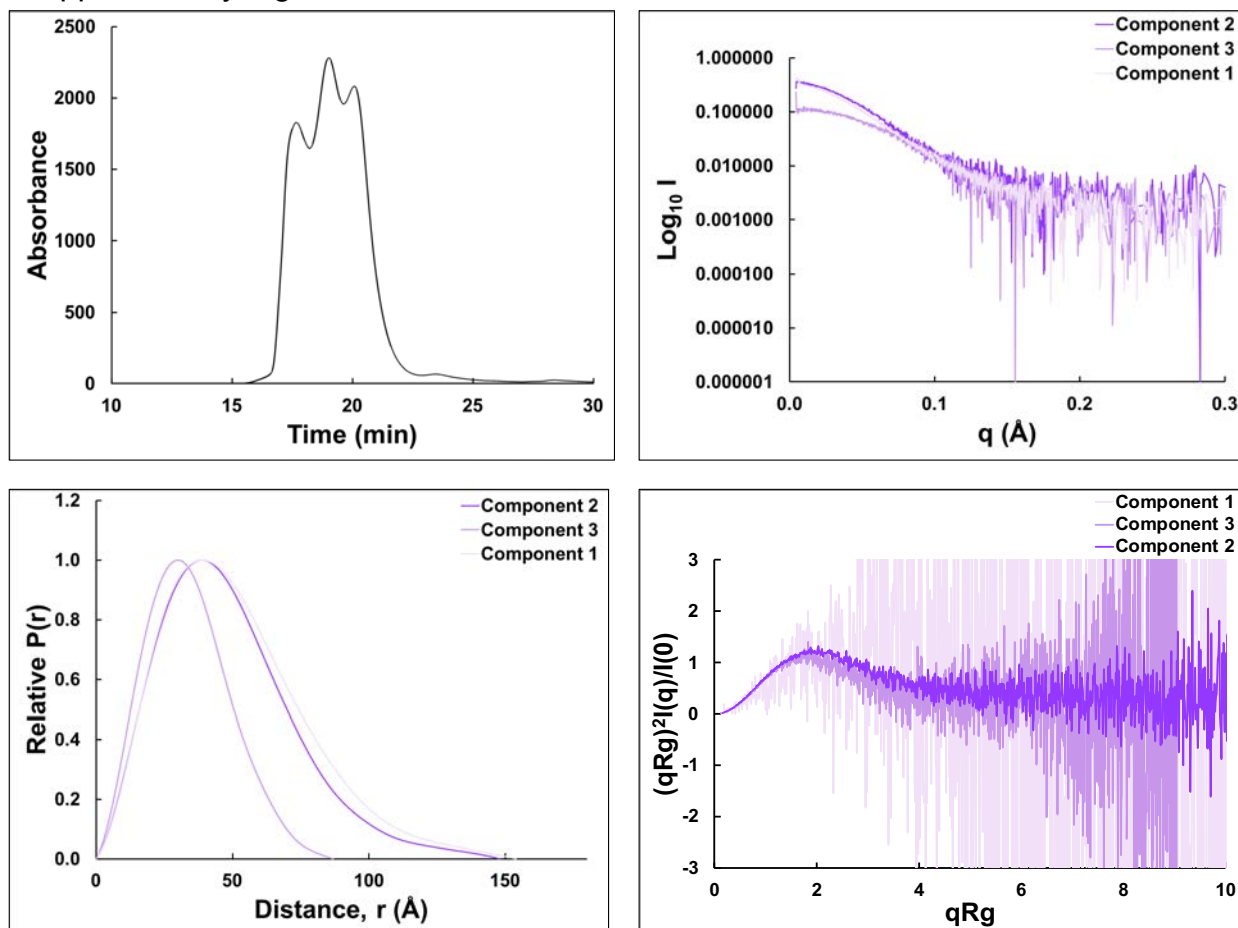
Supplementary Figure 8F: TFAM + SD



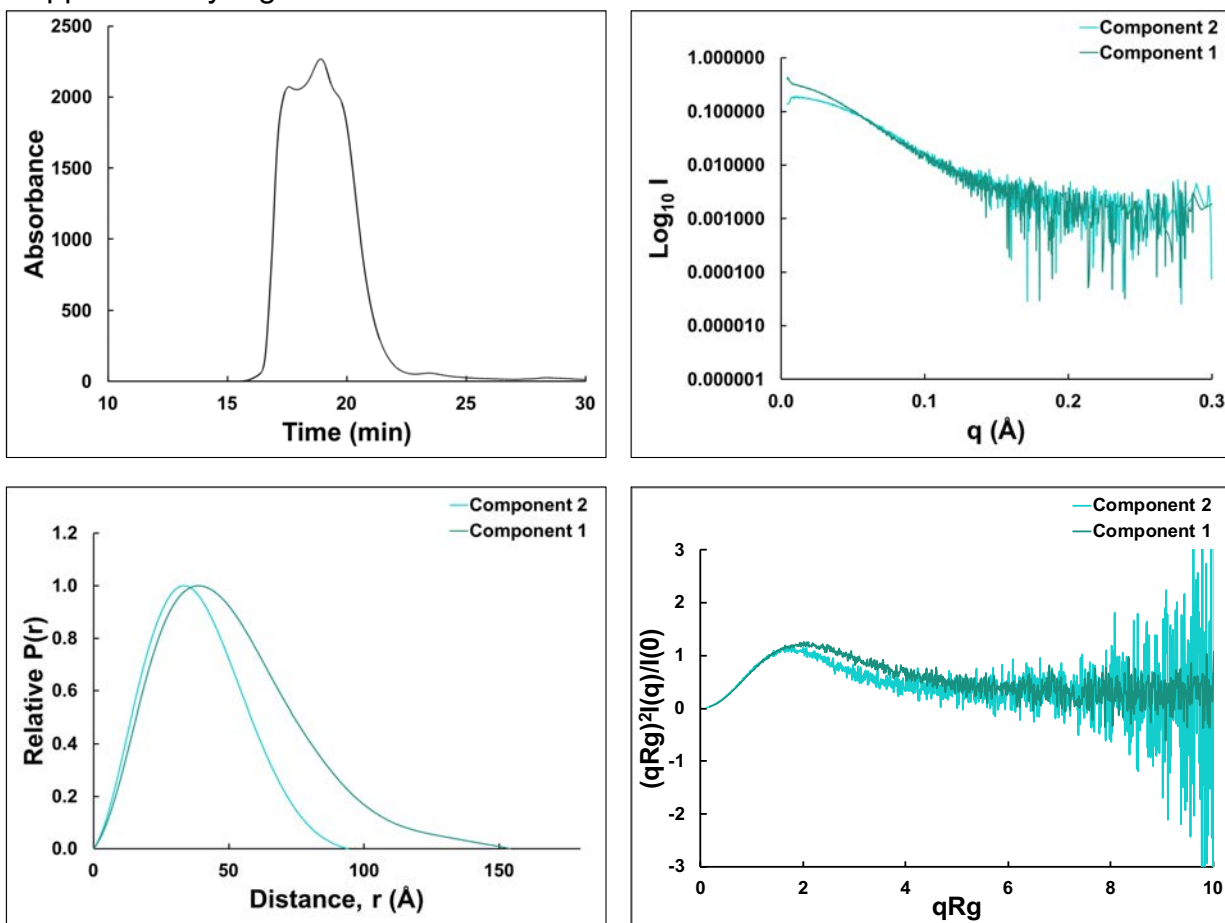
Supplementary Figure 8G: TFAM + NSD



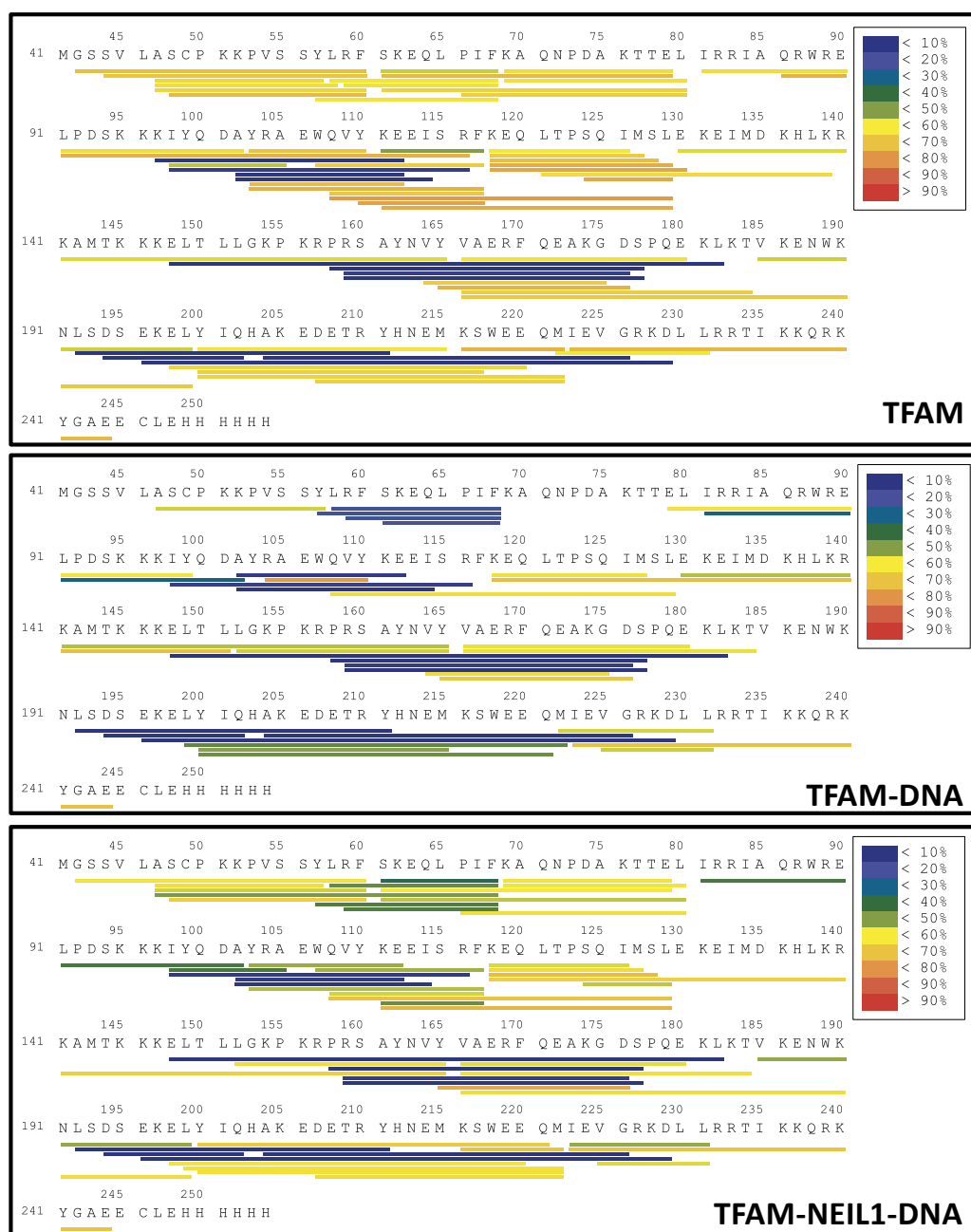
Supplementary Figure 8H: TFAM + NEIL1 + SD



Supplementary Figure 8I: TFAM + NEIL1 + NSD

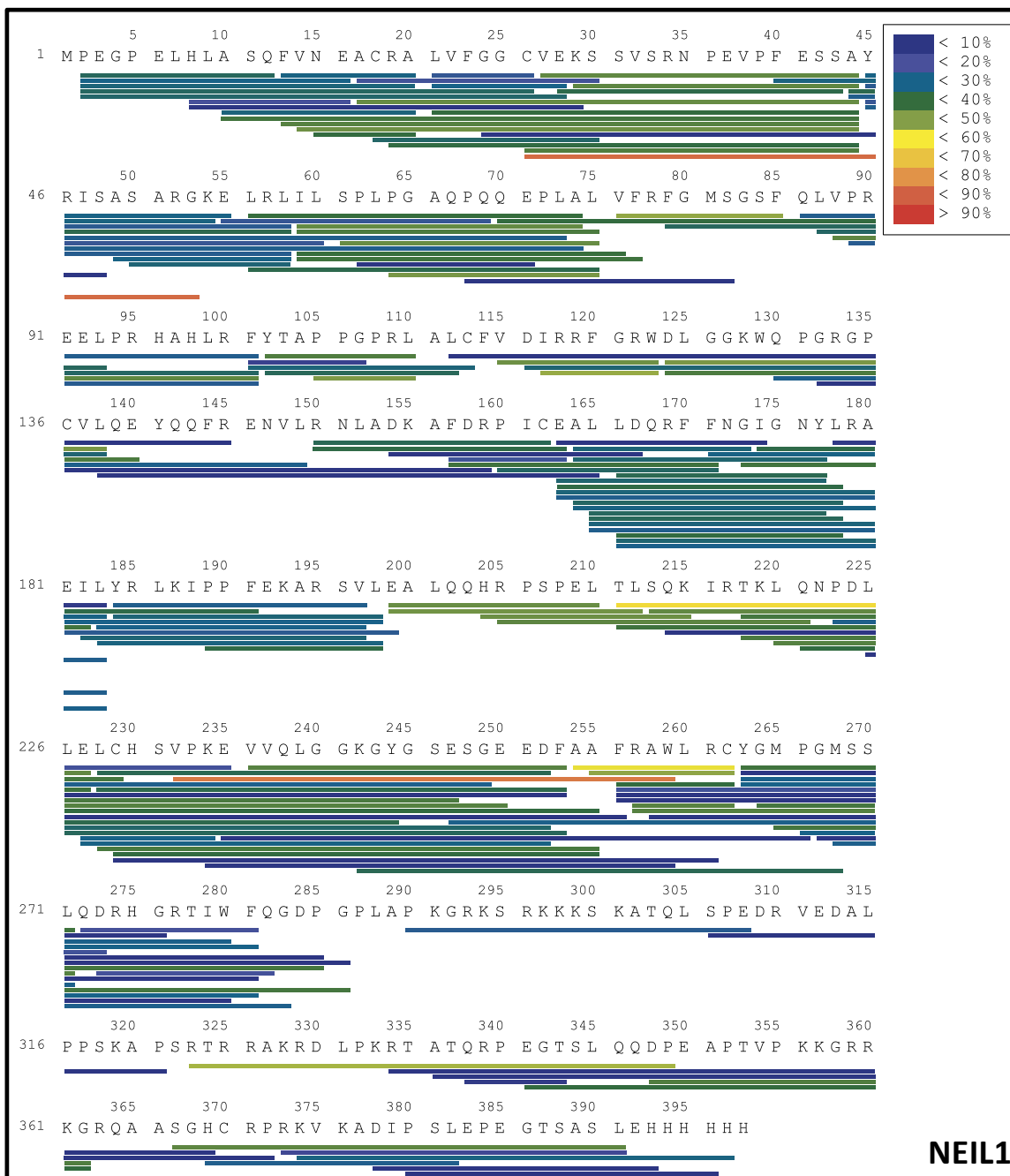


Supplementary Figure 9: Coverage maps for pepsin digested TFAM peptides in the TFAM, TFAM-DNA, and TFAM-NEIL1-DNA samples after HDX. Each colored bar represents a digested peptide monitored during the experiment. The colors indicate average deuterium uptake differences where red represents peptides with the highest uptake and blue corresponds to the lowest uptake. Peptide coverage of the protein sequence for each sample indicated 94.86% coverage for TFAM, 84.58% for TFAM-DNA, and 94.86% for the TFAM-NEIL1-DNA complex.

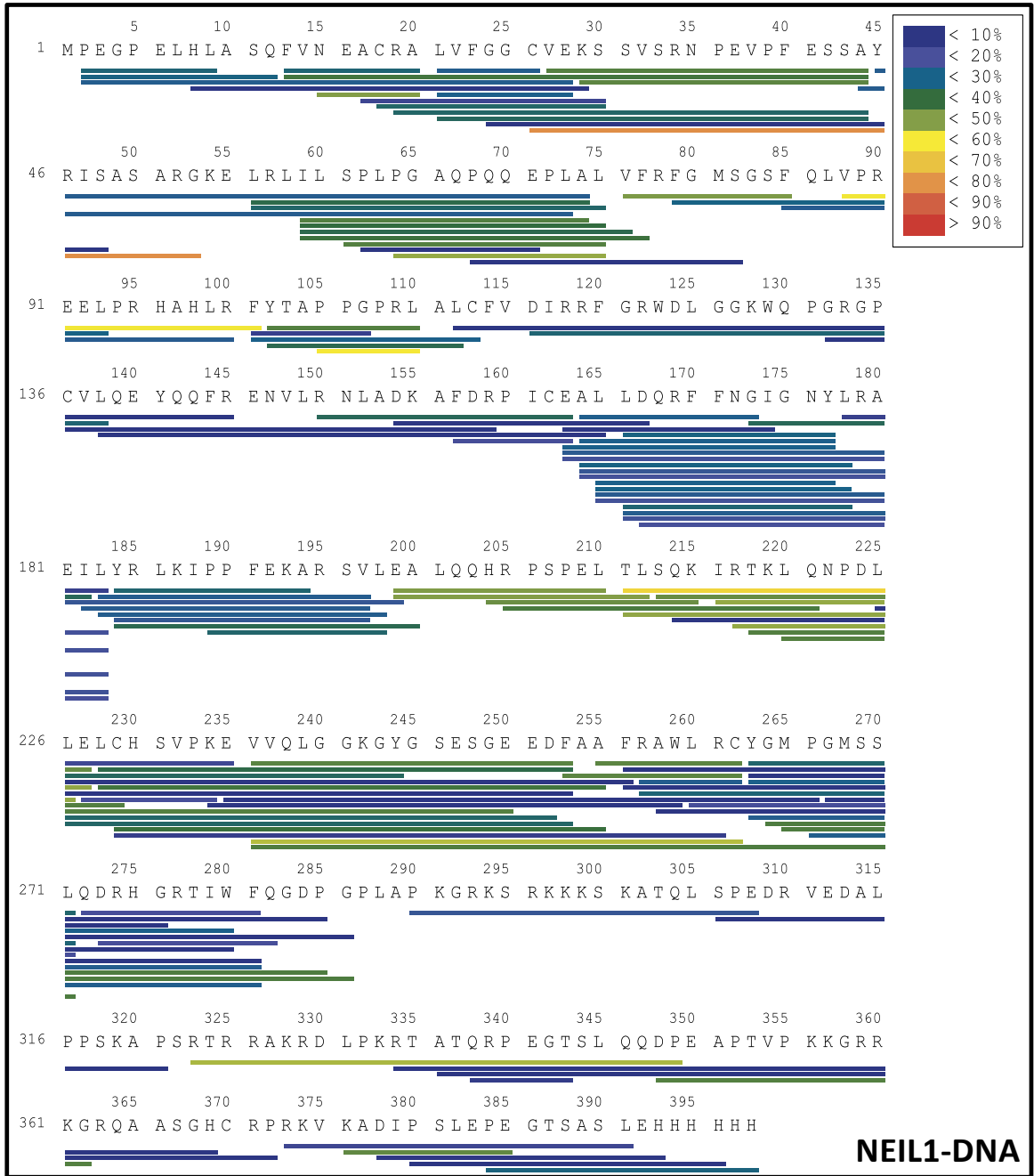


Supplementary Figure 10: Coverage maps for pepsin digested NEIL1 **(A)** and NEIL1-DNA **(B)** after HDX. Peptide coverage of the protein sequence for each sample indicated 98.74% coverage for both NEIL1 and NEIL1-DNA.

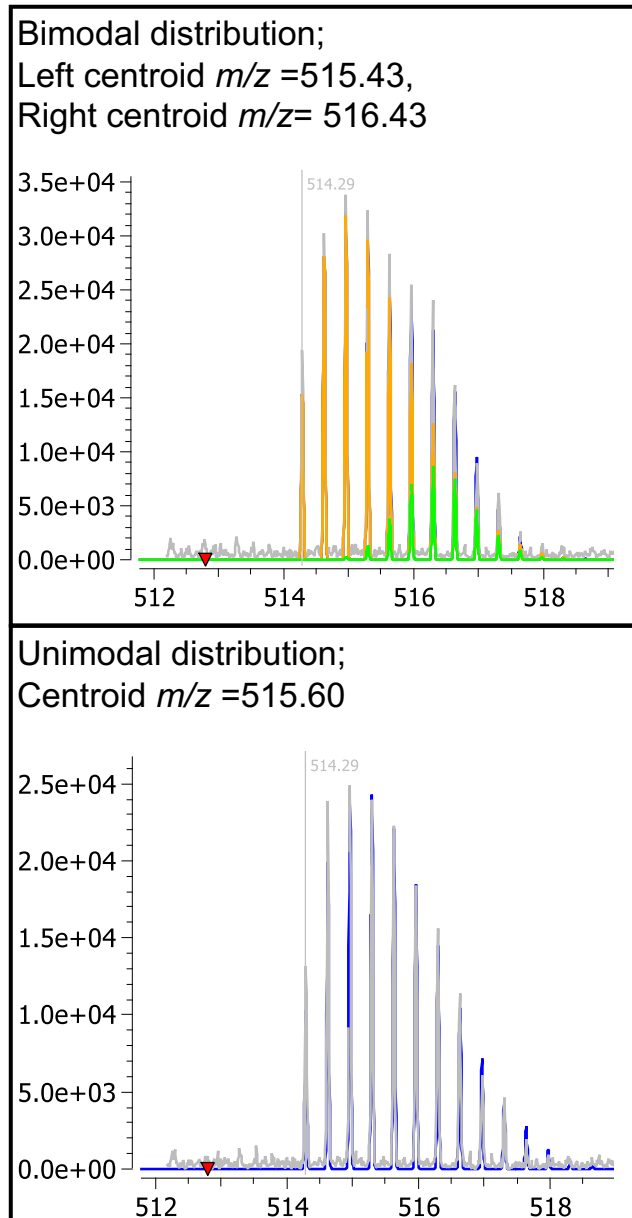
A



B



Supplementary Figure 11: Isotopic mass distribution spectra for the TFAM peptide comprising residues 57-68 at the 30s timepoint. The grey and blue colors represent experimental and theoretical clusters. The orange and green colors represent two differentially exchanged peptides when the data is fitted using bimodal distribution analysis.



Supplementary Figure 12: NEIL1 expression and cell survival. **(A)** Western blot analysis for NEIL1 expression in WT and KO Hap1 cells indicates no NEIL1 expression in the KO cell line. PCNA expression was used as the loading control. **(B)** Dose-response curves to assess Hap1 cell survival plotted as the relative fluorescent units (RFU) generated using a resazurin-based fluorescent assay versus the final concentration of MMS. Lines represent non-linear regression analysis ([Inhibitor] vs. response) performed using GraphPad Prism 8.

