The C-terminal tail of the NEIL1 DNA glycosylase interacts with the human mitochondrial single-stranded DNA binding protein

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Supplemental Information

1. Supplemental Methods
2. Supplemental Figures

1. Supplemental Methods

1.1. DNA glycosylase activity assay

Glycosylase/lyase assays were performed with γ-^{32}P labeled Sp1-containing substrate (20 nM) and increasing concentrations of full-length NEIL1 in 20 mM HEPES, pH 7.5, 150 mM NaCl, 2 mM EDTA (pH 8.0) with 200 µg/ml BSA at 25 °C for 30 minutes [1]. The assay was performed in absence or presence of mtSSB at 0.5 or 10-fold molar excess. The reactions were stopped with the addition of an equal volume of formamide loading buffer (98% formamide, 5 mM EDTA, 0.1% xylene cyanol and 0.1% bromophenol blue) to assay for both glycosylase and lyase activities. The reaction products were separated from the uncleaved substrates with a 12% (w/v) denaturing polyacrylamide gel and quantified with an isotope imaging system (Molecular Imaging System, Bio-Rad).

1.2. Protein painting and mass spectrometry

Protein painting experiments were performed as described previously [2]. Briefly, NEIL1-FL, mtSSB, and NEIL1-mtSSB complex each 200 pmol (total 50 µl) were mixed with 100 molar excess of disodium;1-amino-9,10-dioxo-4-[3-(2-sulphonatooxyethyl sulphonyl)anilino]anthracene-2-sulphonate (RBB) and incubated for 5 min. The His-tag has not been cleaved from full-length NEIL1 while mtSSB was untagged. In total 5 molecular paints were tested initially and RBB was determined to be the best paint molecule to use for our NEIL1-mtSSB complexes. The NEIL1-mtSSB complex was prepared by mixing the two proteins on ice followed by incubation for 1 hr. The protein-RBB mixture was then passed through a size exclusion column (Sephadex G25, Roche) to separate excess RBB by centrifugation at 1000 x g for 1 min. The flow through containing the protein/complex was then denatured by the addition of 2 M urea, reduced using 10 mM dithiothretol for 15 min at room temperature, and alkylated with 50 mM

iodoacetamide. Thereafter, trypsin (Promega) digestion was performed with 1:100 w/w trypsin:protein ratio for 1 hr at room temperature. The protease reaction was stopped using glacial acetic acid and tryptic peptides were purified with Zip-Tips (Millipore) followed by mass spectrometry (MS) analysis. The HPLC mobile phases consisted of 3% acetonitrile and 0.2% formic acid in water (solvent A), and 3% water and 0.2% formic acid in acetonitrile (solvent B). Briefly, the samples were loaded onto a C18 pre-column (Agilent Technologies) using 5% solvent B. The eluted sample from pre-column was then loaded onto a separating Hypersil Gold C18 column (ThermoFisher Scientific). Peptide elution was performed using a gradual linear gradient (0 - 40%) of solvent B. During the elution, the peptides were ionized by electrospray ionization and subjected to MS analysis using the LTQ-Orbitrap XL hybrid mass spectrometer. The RAW files were generated using XCalibur software (ThermoFisher Scientific) and protein identification was performed with the Mascot server. Each sample was searched against a custom database containing the sequences for NEIL1 and mtSSB. Search parameters were set to 10 ppm peptide mass tolerance, 0.6 Da fragment mass tolerance, and semi-tryptic enzyme specification. Two missed cleavages were allowed. Analyses for identification of interface residues was performed as previously described [2]. Briefly, tryptic peptides obtained from individual painted proteins were subtracted from those obtained from the painted complex. The resulting peptides contained residues from the protein-protein interaction interface.

1.3. Preparation of mitochondrial extracts and western-blot analysis

Mitochondria from HCT-116 cells were isolated as described previously [3]. The mitochondrial pellets were treated with 0.5 mg/ml Proteinase K and heated for 5 min at 95 ºC followed by centrifugation at 10,000 x g for 5 min. The pellet was resuspended in Laemmli buffer and proteins were separated by SDS-PAGE. The proteins were

transferred to a PVDF membrane and blocked with 3% bovine serum albumin at room temperature for 1 hr. The membrane was incubated overnight with anti-NEIL1 ab (1:200) (Santa Cruz sc-271164) at 4 ºC followed by incubation with an anti-mouse horseradish peroxidase conjugated secondary antibody for 1 hr at room temperature. The proteins were detected by chemiluminescence (Super Signal West Femto; ThermoScientific) on a ChemiDoc imager (Bio-Rad). Images were visualized using Image Lab (Bio-Rad).

2. Supplemental Figures

Figure Legends:

Supplementary Figure 1: NEIL1 is present in purified human mitochondrial extracts. Western-blot analysis was performed by loading purified mitochondrial extracts from HCT-116 cells treated with 0.5 mg/ml Proteinase K. Purified NEIL1-FL was also loaded as a positive control. The membrane was probed with an anti-NEIL1 antibody (Santa Cruz), an anti-VDAC1 antibody as a mitochondrial loading control, and an anti-lamin A/C antibody for nuclear control.

Supplementary Figure 2: (A) General scheme of the protein painting procedure adapted from Luchini A. et. al. [2]. (i) Proteins are combined with 100 molar excess of molecular paints for 5 minutes. (ii) Unbound paint molecules are washed away using a mini size-exclusion chromatography spin column. (iii) The protein complex is dissociated and denatured using 2 M urea. (iv) Proteins are linearized by reduction with dithiothreitol (DTT) and further alkylated with iodoacetamide. (v) Linearized proteins are treated with trypsin. (vi) Tryptic fragments are analyzed by mass-spectrometry. The molecular paints block solvent accessible trypsin cleavage sites. Therefore, tryptic fragments are generated only from unpainted interaction regions of the protein complex. (B) Peptides

identified by MS in NEIL1 and mtSSB when 5 paints MV, CR, AO50, RBB and ANSA were used individually to coat the protein surface. (C) NEIL1 tryptic peptides generated before *(top panel)* and after *(bottom panel)* treatment with paint molecule RBB shown in red mapped on NEIL1 sequence. (D) mtSSB tryptic peptides generated before *(top panel)* and after *(middle panel)* treatment with paint molecule RBB shown in red mapped on the mtSSB sequence. *Bottom panel* indicates a cartoon representation of the mtSSB homotetramer with the peptide (107-132) obtained after RBB treatment highlighted in red. The peptide is present at the intramolecular interface. (E) NEIL1 and mtSSB tryptic peptides generated before and after treatment of the NEIL1-mtSSB complex with paint molecule RBB. The peptides generated exclusively for the coated complex (Set B - Set A) are colored in green. (F) Structural model of full-length NEIL1 generated using Phyre2 [4] showing the location of peptides obtained after complex formation.

Supplementary Figure 3: Far-western analysis to determine the minimal construct of NEIL1 required for an interaction between NEIL1 and mtSSB. 50 pmol of all NEIL1 constructs, bovine serum albumin (negative control), glutathione S-transferase (negative control), and 10 pmol of mtSSB were loaded onto the gel. Far-western analysis was performed and proteins were transferred to a PVDF membrane, denatured, slowly renatured on the membrane, and incubated with 1 mg/ml HEK-293T whole cell extract and probed with an anti-mtSSB antibody to detect an interaction.

Supplementary Figure 4: SEC analysis of NEIL1-Δ100-mtSSB, mtSSB-DNA, and NEIL1-DNA-mtSSB complexes. (A) NEIL1-Δ100 does not form complex with mtSSB. *Top panel*, elution profiles from Superdex 200 column chromatography. Black line, NEIL1-Δ100-mtSSB tetramer (1:1 molar ratio) mixture; red line, mtSSB alone; and grey line, NEIL1-Δ100 alone. The two proteins elute as separate peaks. *Bottom panel*, SDS-

PAGE analysis of fractions collected after SEC. Lane 1, marker; lane 2, NEIL1-Δ100; lane 3, mtSSB; lane 4, NEIL1-Δ100-mtSSB mixture input prior to gel filtration; lane 5-10, fractions 29-34 obtained after gel filtration corresponding to elution volume 14.5-17 ml. 0.5 ml fractions were collected for all samples. (B) SEC elution profile of mtSSB (red), NEIL1 (yellow), mtSSB-DNA (blue), NEIL1-DNA (magenta), NEIL1-DNA-mtSSB (green) complexes. The NEIL1-DNA-mtSSB complex elutes (elution volume, 12.2 ml) before the mtSSB-DNA complex (elution volume, 13.1 ml) and NEIL1-DNA complex (elution volume, 13.44 ml).

Supplementary Figure 5: Scattering curves of (A) NEIL1, (B) mtSSB, (C) mtSSB-DNA, (D) NEIL1-mtSSB, and (E) NEIL1-DNA-mtSSB complex. The Guinier region showing linearity for each protein/complex is shown in the inset.

Supplementary Figure 6: mtSSB inhibits NEIL1 glycosylase activity on a single-stranded DNA substrate containing Sp1. Decrease in DNA glycosylase activity (decrease in product formation) was observed when the substrate was incubated with 0.5 and 10 molar excess of mtSSB. As expected, mtSSB alone does not cleave the DNA substrate.

References:

[1] A. Prakash, B.L. Carroll, J.B. Sweasy, S.S. Wallace, S. Doublié, Genome and cancer single nucleotide polymorphisms of the human NEIL1 DNA glycosylase: activity, structure, and the effect of editing, DNA repair, 14 (2014) 17-26.

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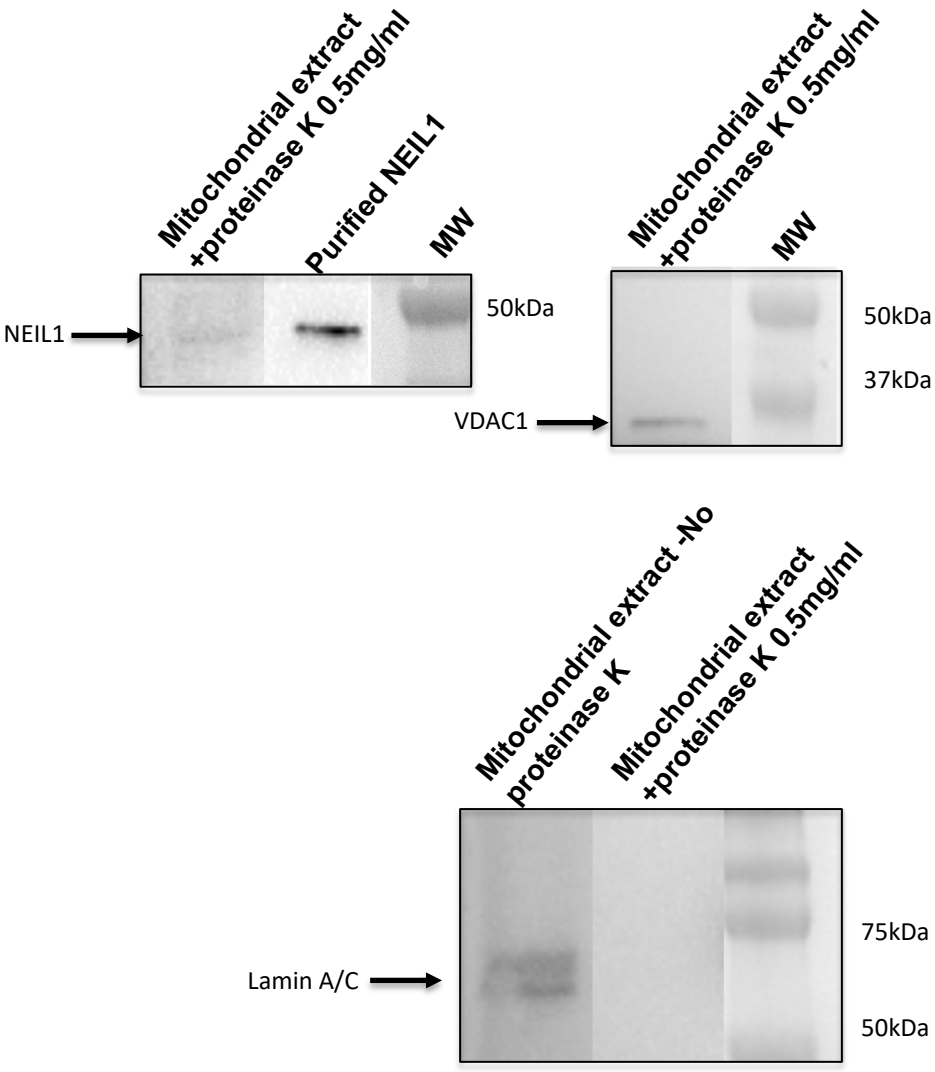
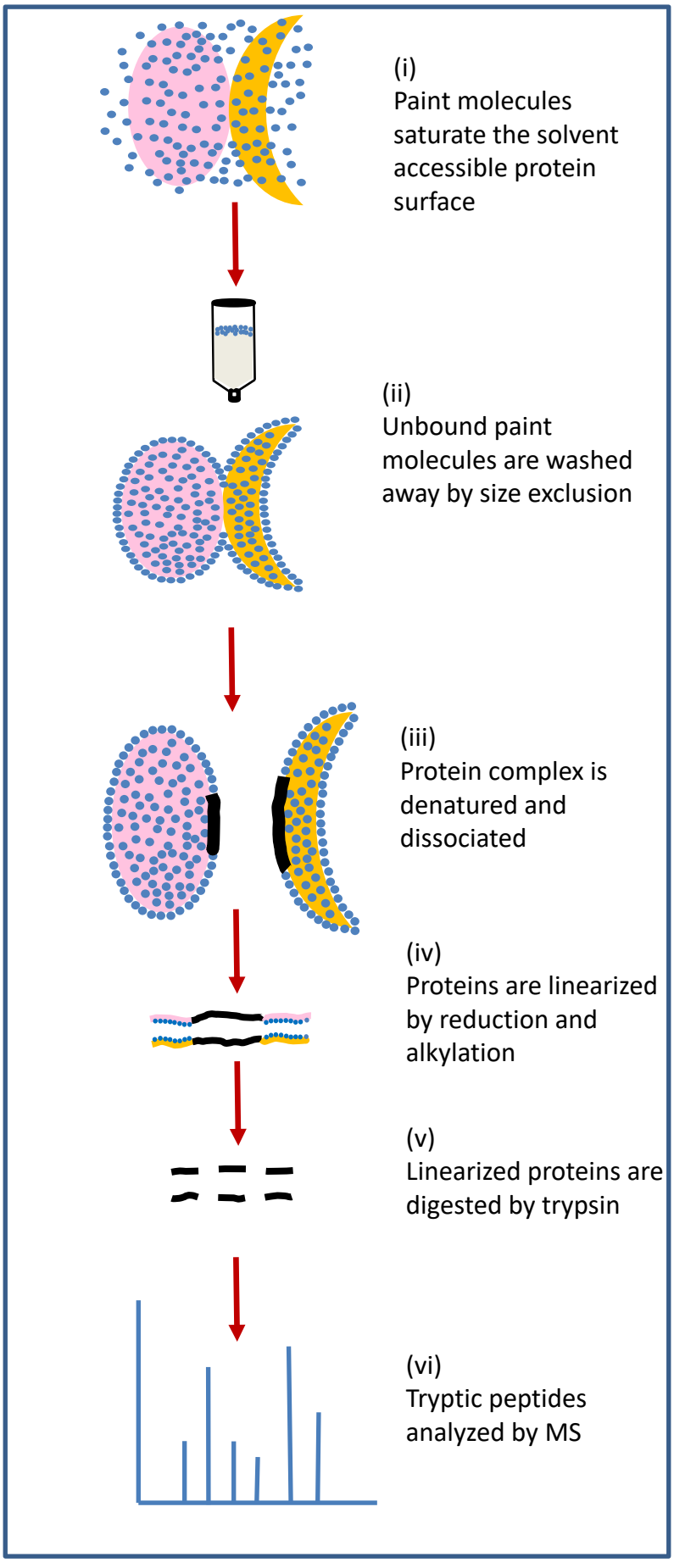
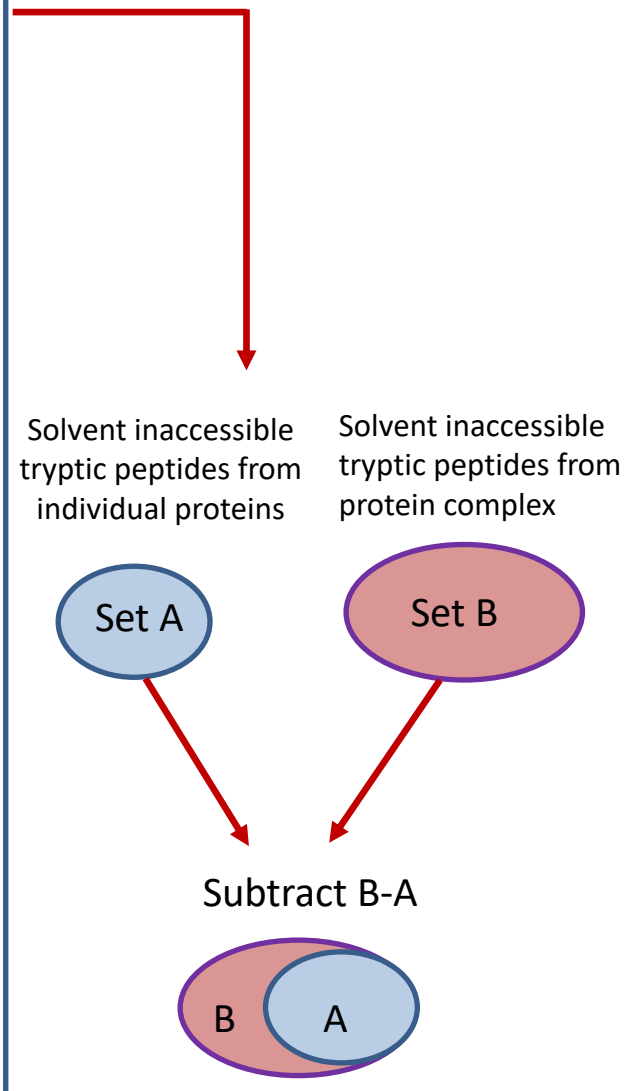
[4] L.A. Kelley, S. Mezulis, C.M. Yates, M.N. Wass, M.J. Sternberg, The Phyre2 web portal for protein modeling, prediction and analysis, Nature protocols, 10 (2015) 845-858.

Supplemental Figure 1



Supplemental Figure 2A





Differentially cleaved trypsin sites representing protein-protein interaction points.

Supplemental Figure 2B

MV- N-(4-{bis[4-(dimethylamino)phenyl]methylene}-2,5-cyclohexadien-1-yli- dene)methanaminium chloride

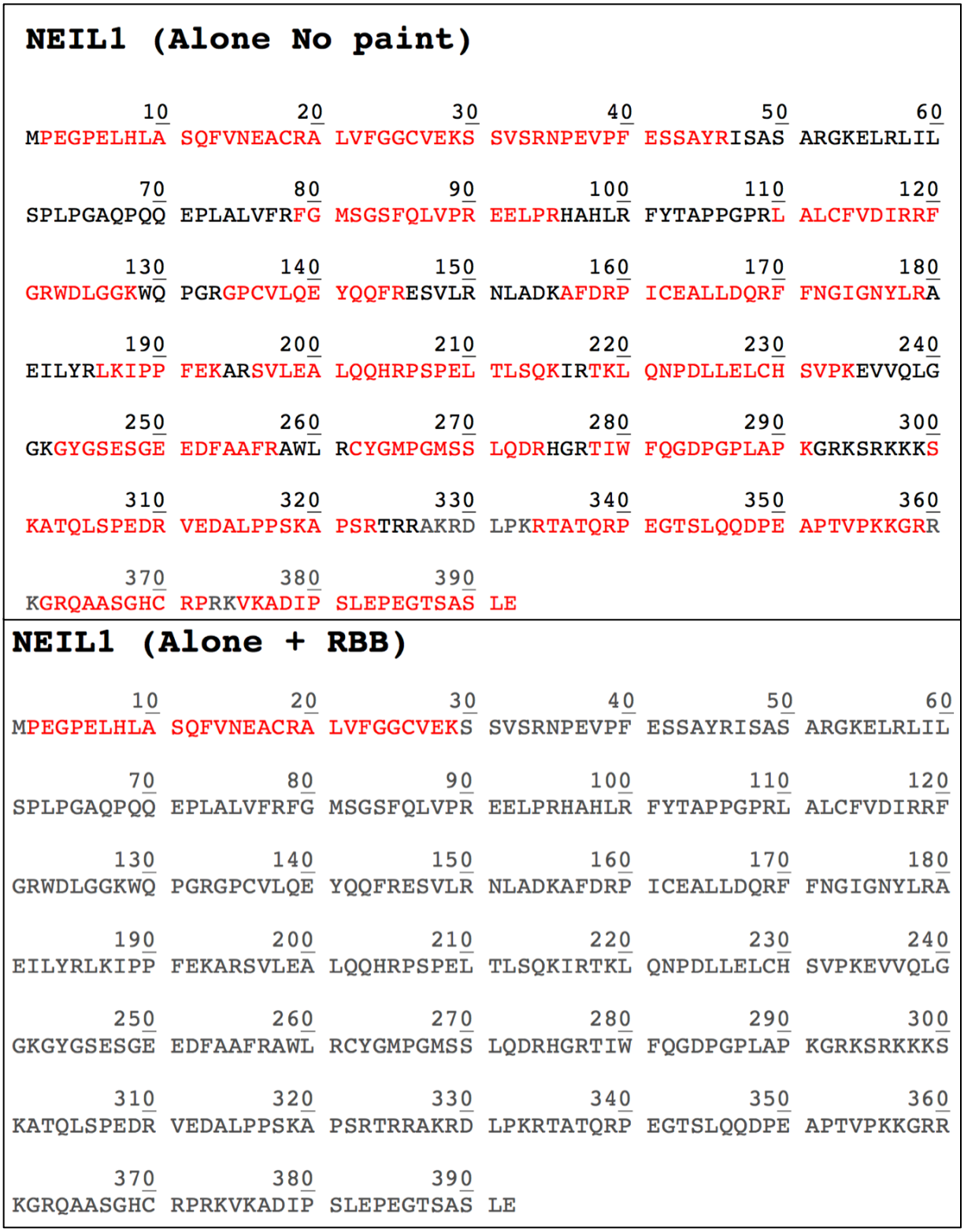
AO50- sodium 4-(4-(benzyl-et-amino)-ph-azo)-2,5-di-cl-benze- nesulphonate (AO50)

RBB- disodium; 1-amino-9,10- dioxo-4-[3-(2-sulphonatooxyethylsulphonyl) anilino] anthracene-2-sulphonate

CR- disodium; 4-amino- 3-[[4-[4-[(1-amino-4-sulphonatonaphthalen-2-yl)diazenyl] phenyl] phenyl] diazenyl] naphthalene-1-sulphonate

ANSA- 3,30 -Diethylthiacarbocyanine iodide (DECI; Sigma), 8-Anilino-1-naphthalenesulphonic acid

Supplemental Figure 2C



Supplemental Figure 2D

mtSSB (Alone No Paint)

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         10          20          30          40          50          60
ESETTTSLVL ERSLNRVHLL GRVGQDPVLR QVEGKNPVTI FSLATNEMWR SGDSEVYQLG

         70          80          90         100         110         120
DVSQKTTWHR ISVFRPGLRD VAYQYVKKGS RIYLEGKIDY GEYMDKNNVR RQATTIIADN

        130
IIFLSDQTKE KE
```

mtSSB (Alone + RBB)

```
         10          20          30          40          50          60
ESETTTSLVL ERSLNRVHLL GRVGQDPVLR QVEGKNPVTI FSLATNEMWR SGDSEVYQLG

         70          80          90         100         110         120
DVSQKTTWHR ISVFRPGLRD VAYQYVKKGS RIYLEGKIDY GEYMDKNNVR RQATTIIADN

        130
IIFLSDQTKE KE
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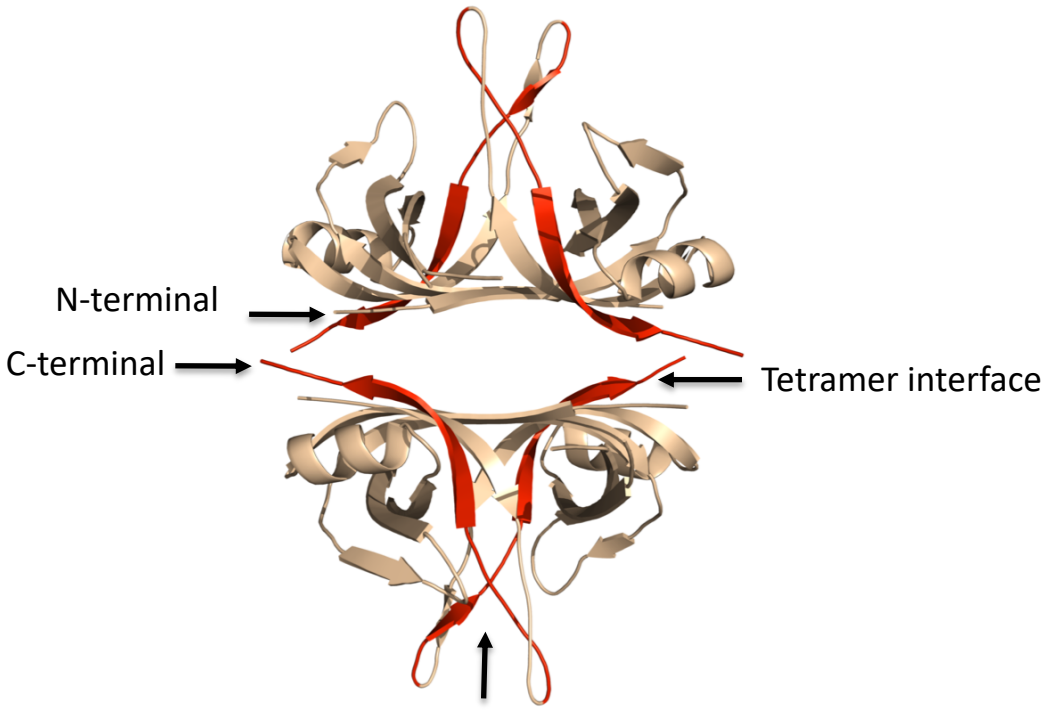
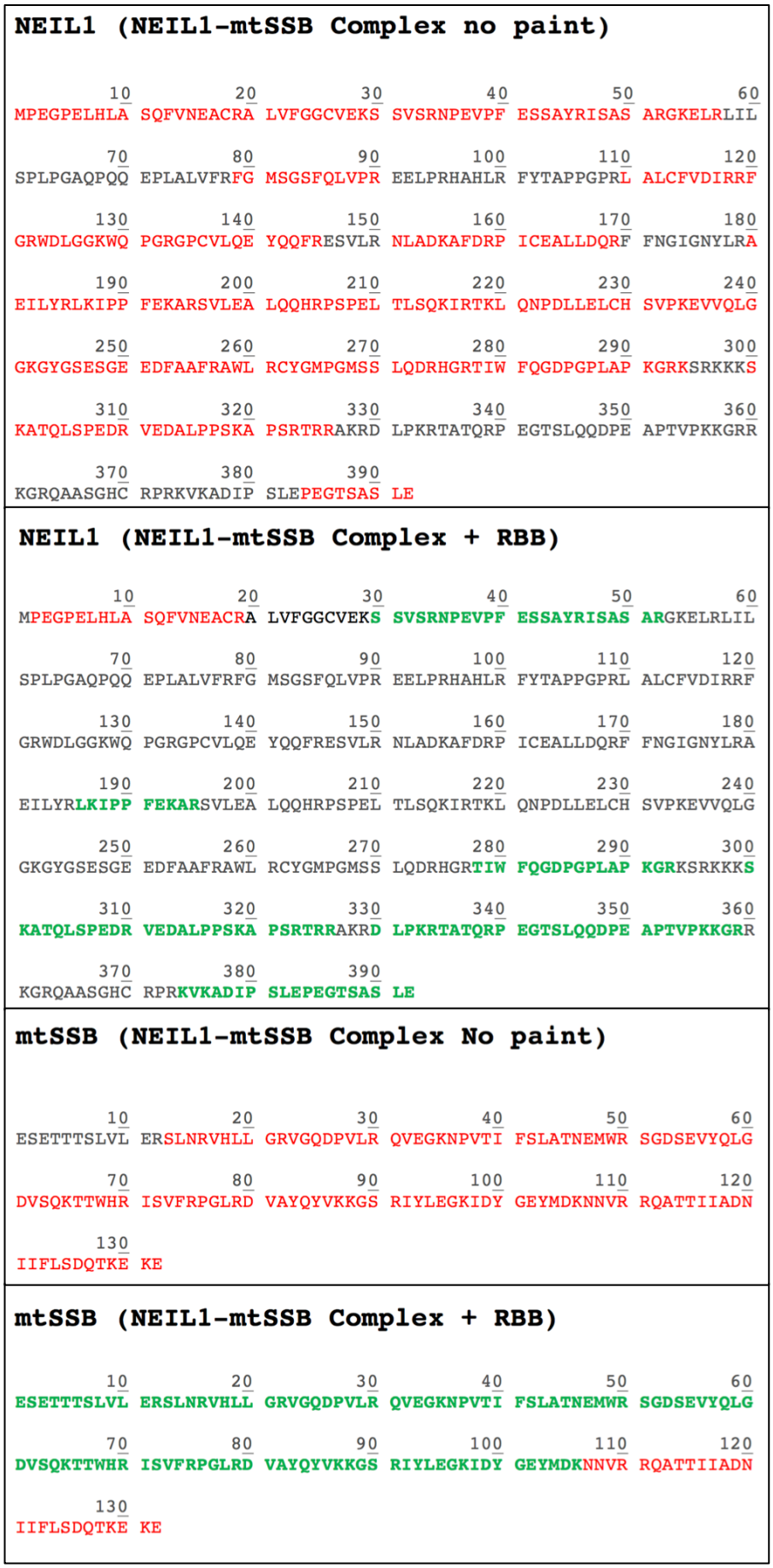


mtSSB tetramer (PDB ID: 3ULL)

Supplemental Figure 2E



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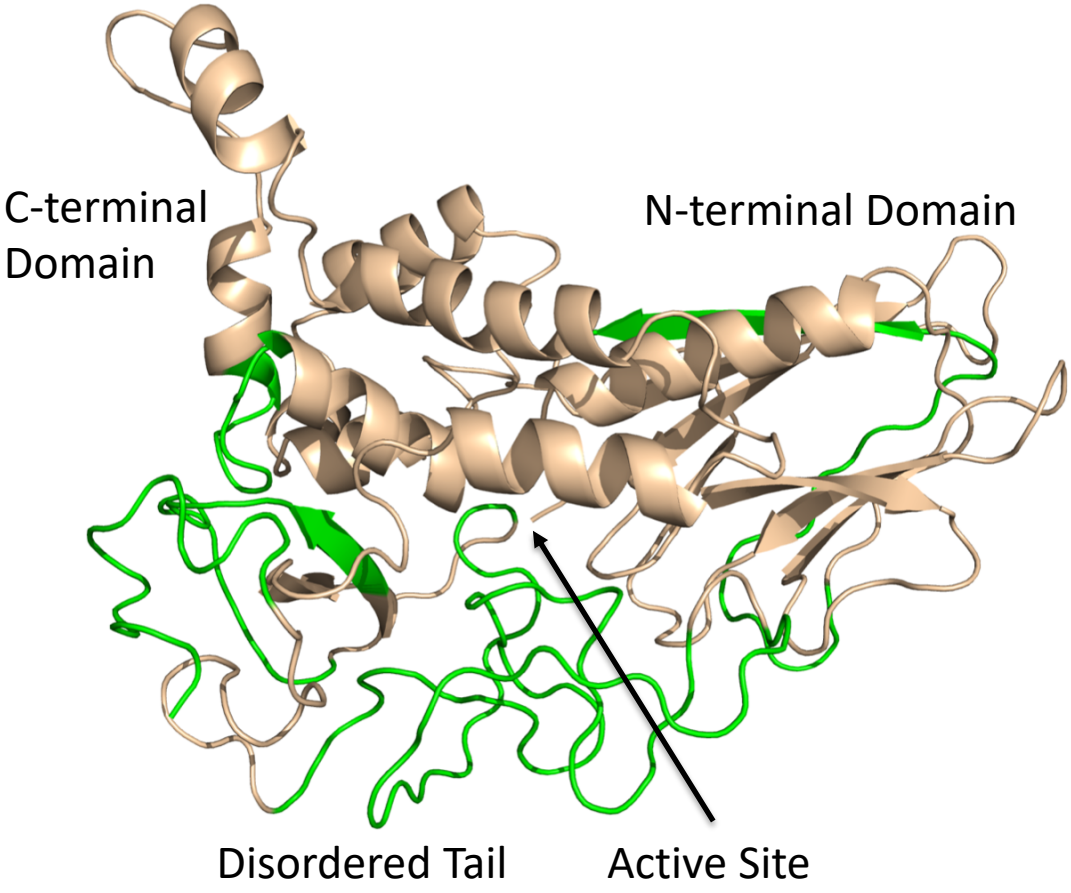

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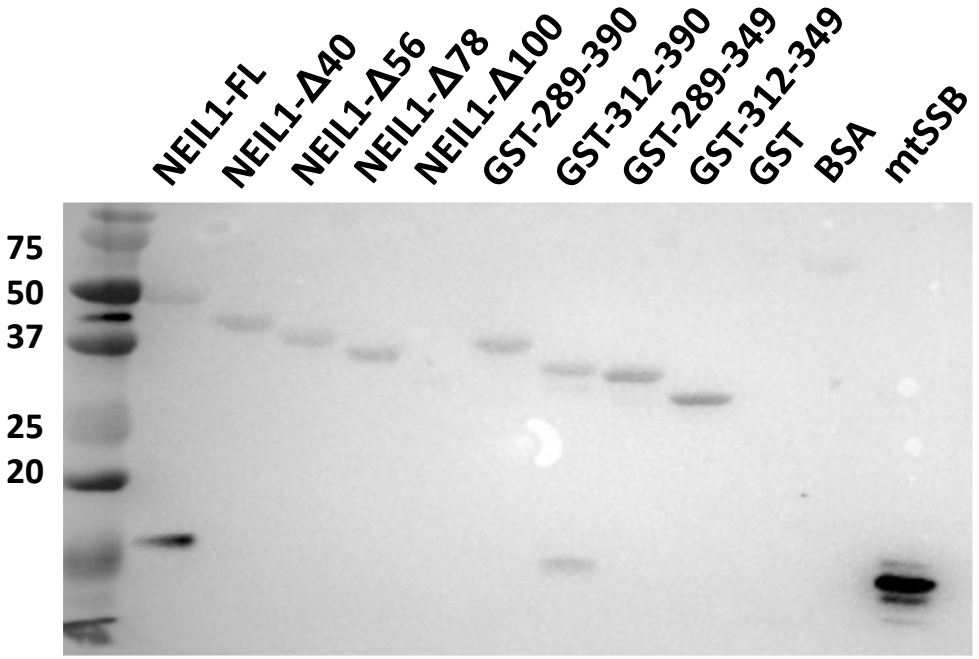
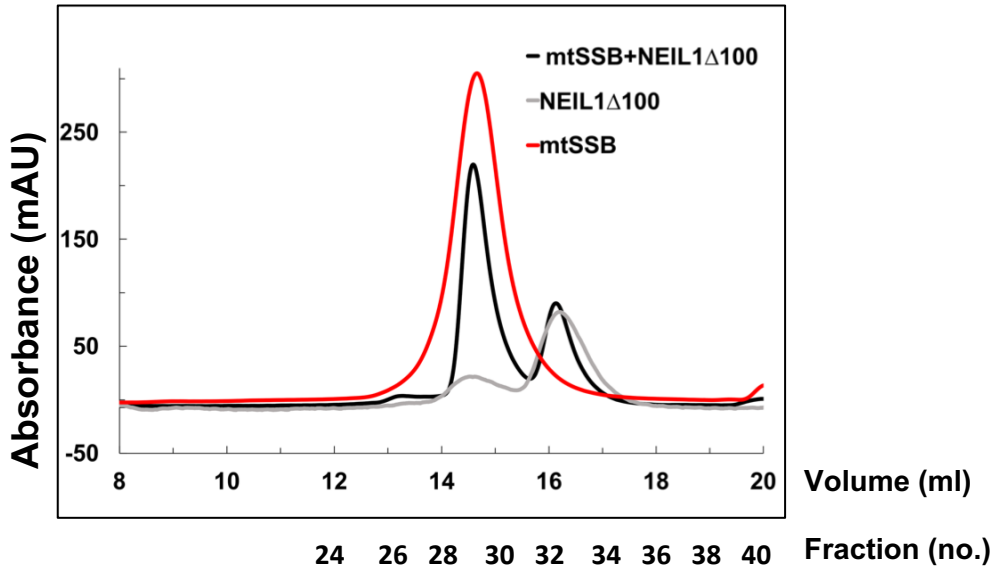
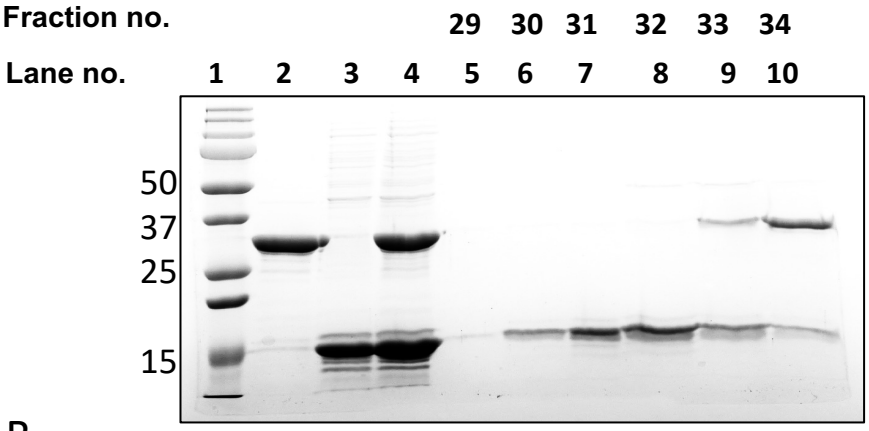


Far western
HEK-293T Whole cell extract

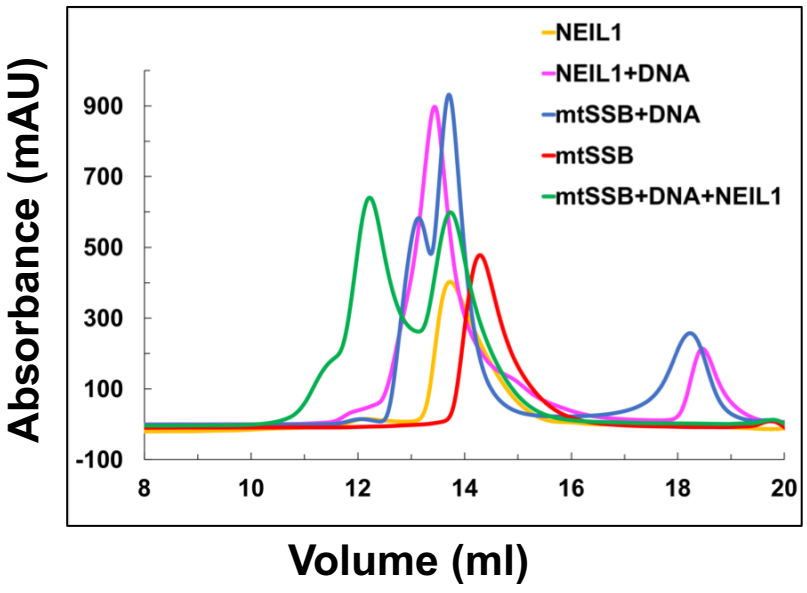
Supplemental Figure 4

A

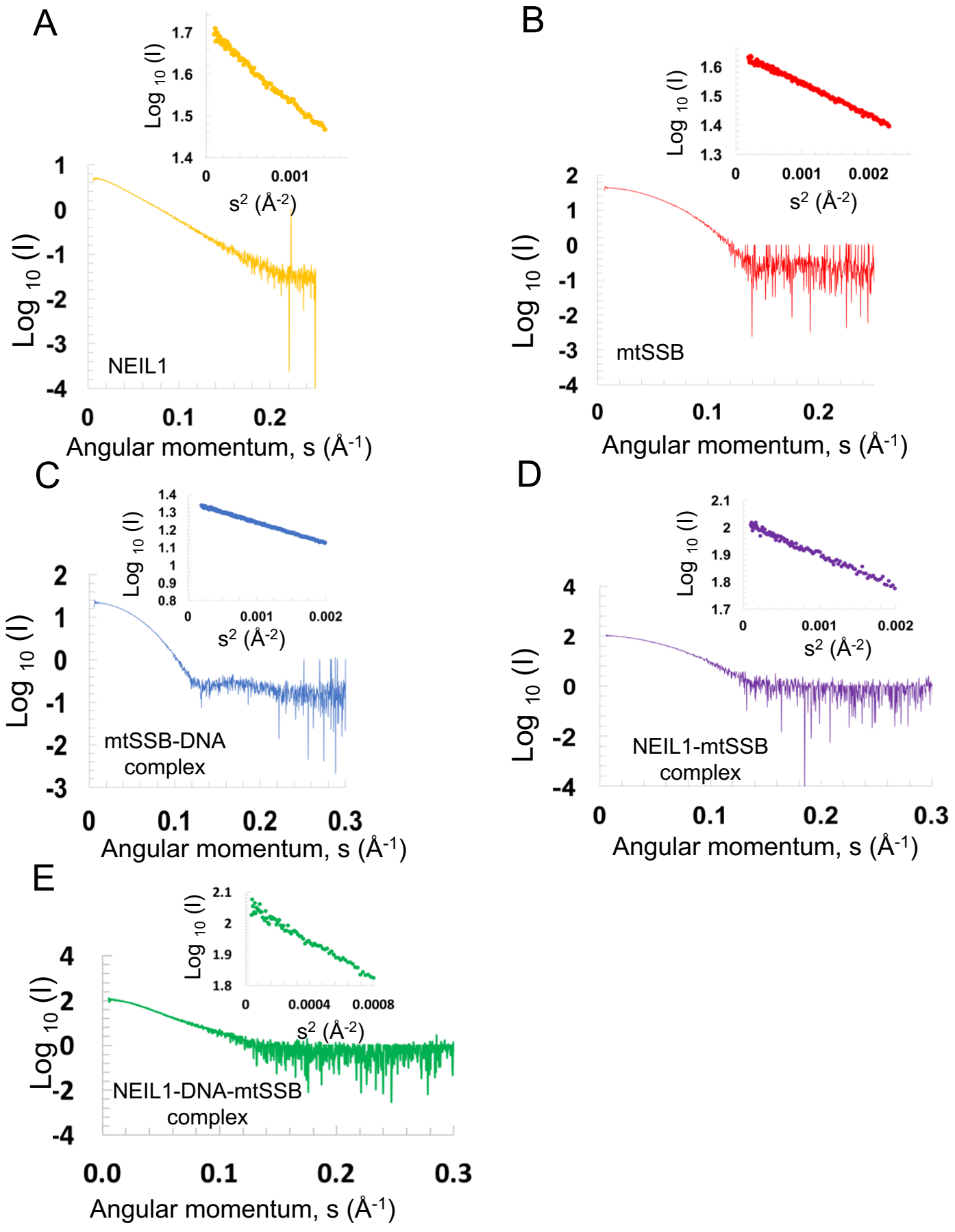




B



Supplemental Figure 5



Supplemental Figure 6