

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
<input type="checkbox"/>	<input checked="" type="checkbox"/> The exact sample size (<i>n</i>) for each experimental group/condition, given as a discrete number and unit of measurement
<input type="checkbox"/>	<input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
<input type="checkbox"/>	<input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
<input checked="" type="checkbox"/>	<input type="checkbox"/> A description of all covariates tested
<input type="checkbox"/>	<input checked="" type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
<input type="checkbox"/>	<input checked="" type="checkbox"/> A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
<input type="checkbox"/>	<input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
<input checked="" type="checkbox"/>	<input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
<input checked="" type="checkbox"/>	<input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
<input type="checkbox"/>	<input checked="" type="checkbox"/> Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	All sequencing data was obtained using Nextseq 550 or Nextseq 2000, with CASAVA 1.8 used for base calling and demultiplexing.
Data analysis	Sequencing data were processed using Cutadapt, FASTX-Trimmer, and Bowtie followed by additional processing using custom Python scripts. All analysis and visualization was done with R. All custom codes are available upon request.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our [policy](#)

The sequencing data generated for this study are deposited in NCBI's Gene Expression Omnibus (GEO) under accession number s GSE263661 (Ribosome Profiling) and GSE263659 (RNA-seq).

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

Use the terms *sex* (biological attribute) and *gender* (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.

Reporting on race, ethnicity, or other socially relevant groupings

Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status). Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.) Please provide details about how you controlled for confounding variables in your analyses.

Population characteristics

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."

Recruitment

Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

Ethics oversight

Identify the organization(s) that approved the study protocol.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

No statistical methods were used to predetermine sample size.

Data exclusions

No data were excluded outside of the predetermined standards for next-generation sequencing experiments, such as gene coverage, as described explicitly in the text.

Replication

Results were validated through analysis of sequencing data from two biological replicates.

Randomization

Randomization was not relevant to this study. Significance was assessed by using the whole population of genes/proteins or codons/residue positions within the genome/proteome, as described in the text.

Blinding

None of the experiments involved blinding.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern
<input checked="" type="checkbox"/>	<input type="checkbox"/> Plants

Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

Antibodies

Antibodies used	Hsp-60 (DSHB) Pas-7 (DSHB, CePAS7) Actin (DSHB, JLA20) Tubulin (DSHB, AA4.3) Sec61 α (Santa Cruz, sc-393182) uL16(Biomol (#AP17603)) NAC $\alpha\beta$ was raised in rabbits in-house.
Validation	Hsp-60 (DSHB) - Monoclonal antibody verified for immunoblot for C.elegans. Used in over 16 publications. Pas-7 (DSHB) - Monoclonal antibody verified for immunoblot for C.elegans. Used in multiple publications including 10.1126/science.aaa5335. Actin (DSHB) - Monoclonal antibody confirmed for immunoblot in multiple species including C.elegans. Used in hundreds of publications. Tubulin (DSHB) - Monoclonal antibody confirmed for immunoblot in multiple species including C. elegans. Used in hundreds of publications. Sec61 α (Santa Cruz, sc-393182) - validated by manufacturer for immunoblot in multiple species. Used in multiple publications for C.elegans including 0.1126/science.aaa5335 and 10.1016/j.molcel.2019.06.030. uL16(Biomol (#AP17603)) - validated by manufacturer for immunoblot in multiple species including C. elegans. NAC $\alpha\beta$ - raised in-house and validated for detection of C. elegans NAC through immunoblot. Used in 5 publications.

Animals and other research organisms

Policy information about [studies involving animals; ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals	The Bristol N2 strain of C. elegans was used in this study.
Wild animals	This study did not involve wild animals.
Reporting on sex	Sex information was not collected in this study.
Field-collected samples	This study did not involve samples collected from the field.
Ethics oversight	No ethical approval was required for the use of C. elegans.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Plants

Seed stocks	<i>Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.</i>
Novel plant genotypes	<i>Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.</i>
Authentication	<i>Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.</i>

Supplementary information

NAC controls nascent chain fate through tunnel sensing and chaperone action

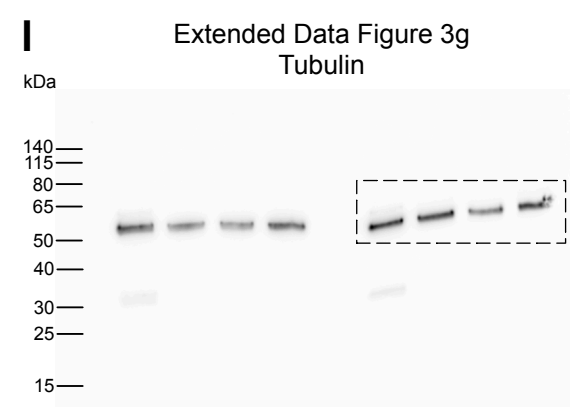
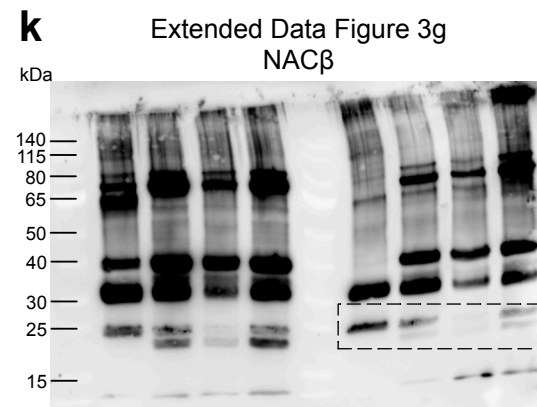
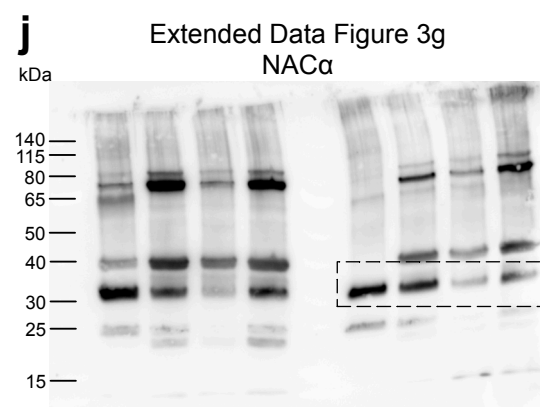
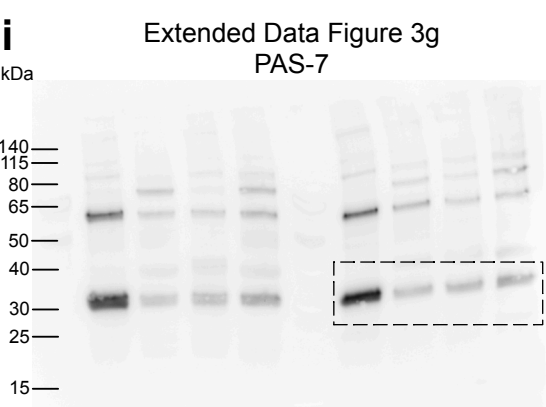
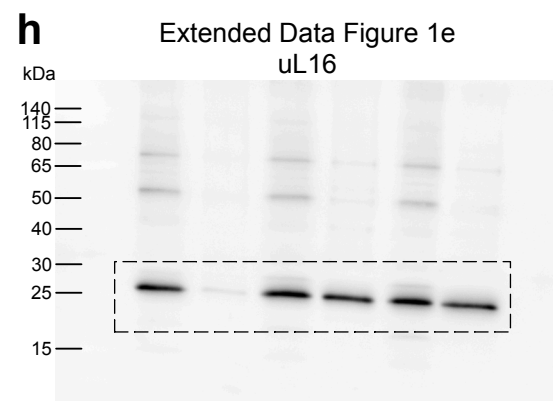
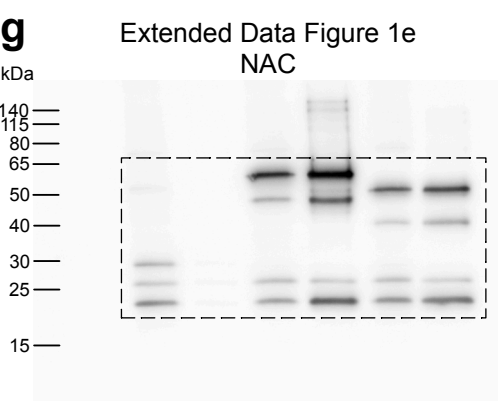
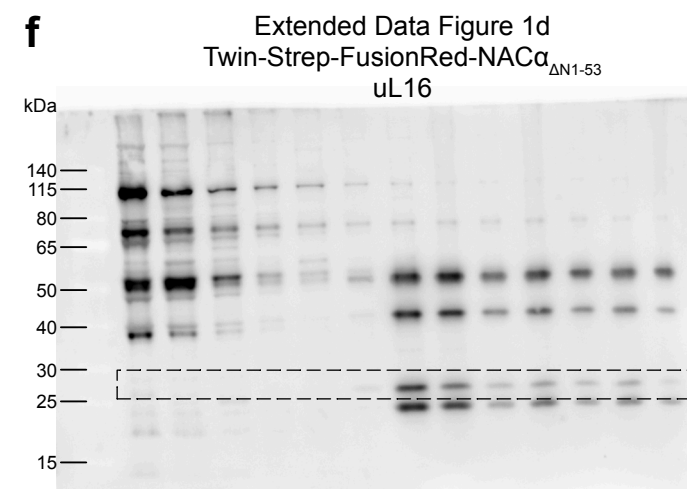
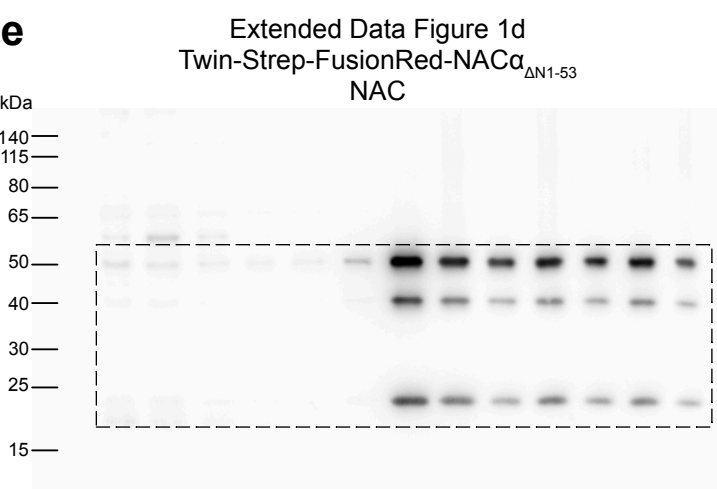
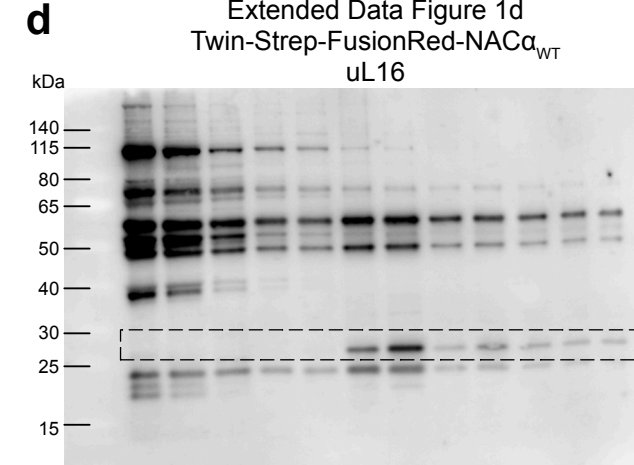
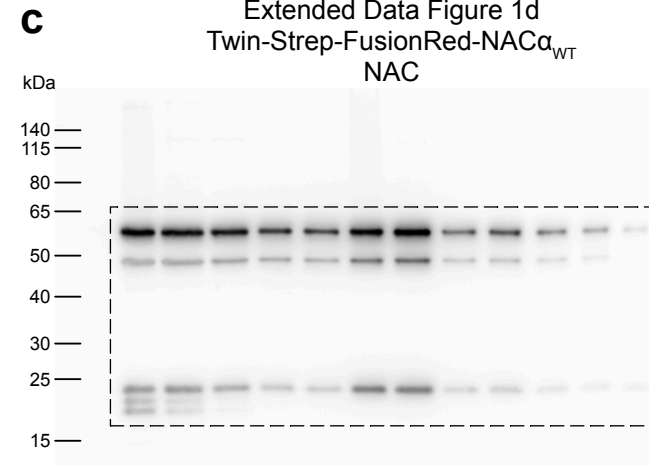
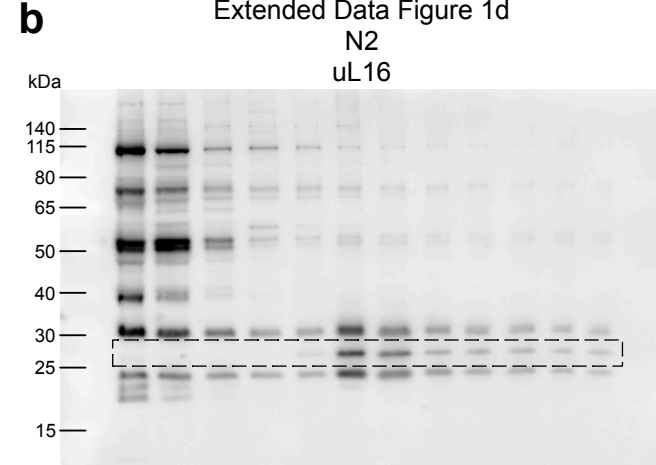
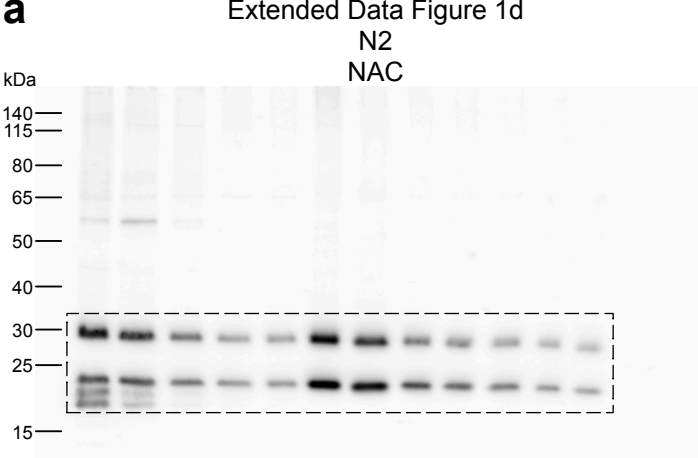
In the format provided by the
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Table of contents:

SI Figure 1. Uncropped immunoblots from Extended Data Figures. a-f, Immunoblots for Extended Data Fig. 1d showing migration of wildtype NAC (**a**), Twin-Strep-NAC (**c**), Twin-Strep-NAC Δ N1-53 (**e**), and ribosomal proteins (**b**, **d**, and **f**) on sucrose gradient. **g-h**, Immunoblots for Extended Data Fig. 1e showing the effective IP against NAC (**g**) to isolate NAC-bound ribosomes (**h**). **i-l**, Immunoblots of Extended Data Fig. 3g to verify the knockdown efficiency of PAS-7 (**i**), NAC α (**j**), NAC β (**k**), and loading control Tubulin (**l**).

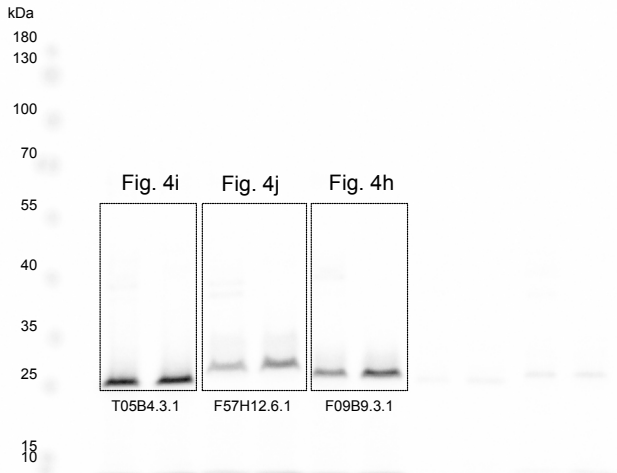
SI Figure 2. Uncropped fluorescence scan of gel from Extended Data Figures. a, Fluorescence scan of gels shown in Extended Data Fig. 7e to detect BODIPY labeled loading control as well as translation intermediates and products. **b**, Fluorescence scan of gels shown in Extended Data Fig. 7h to detect BODIPY labeled loading control as well as translation intermediates and products.

SI Figure 3. Uncropped autoradiography of gels from figures. a, Autoradiography to visualize crosslinking between nascent chain and NAC shown in Fig. 4h-j. **b**, Autoradiography to visualize crosslinking between nascent chain and NAC as shown in Extended Data Fig. 6.

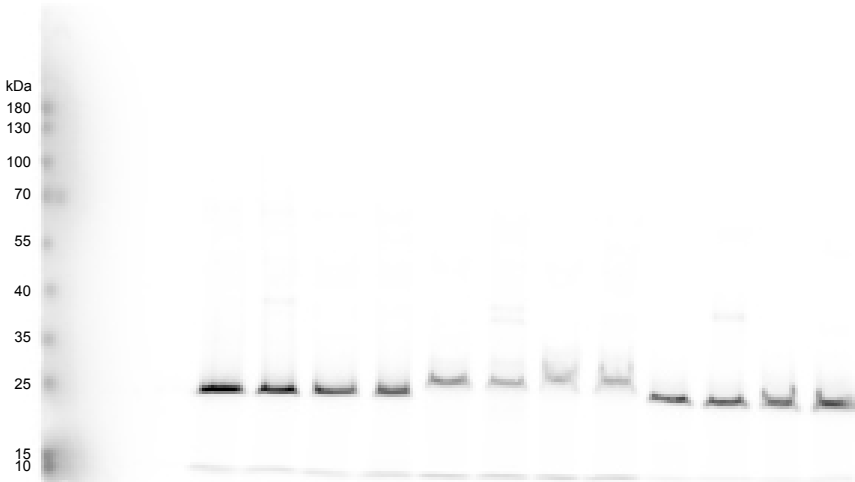


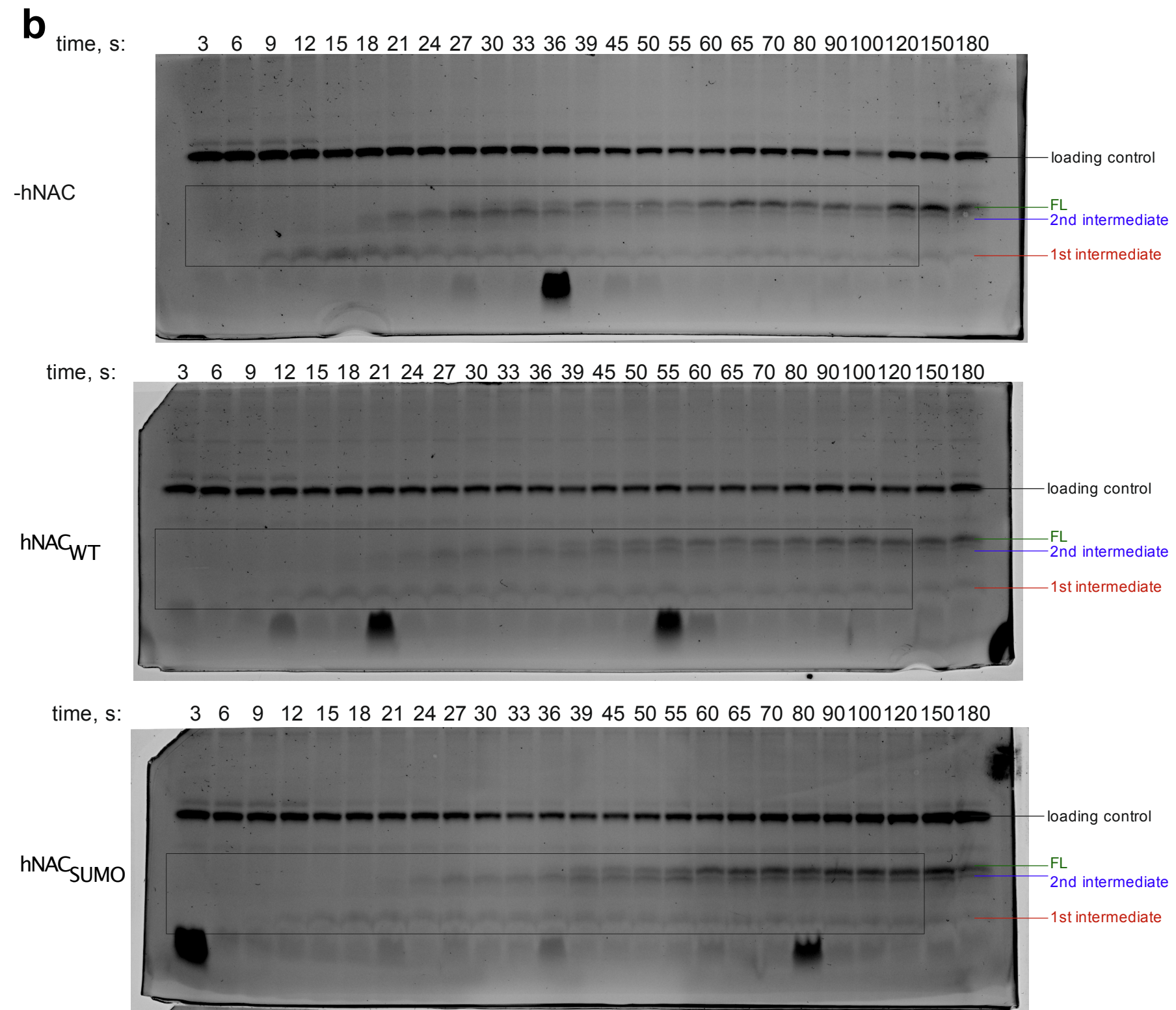
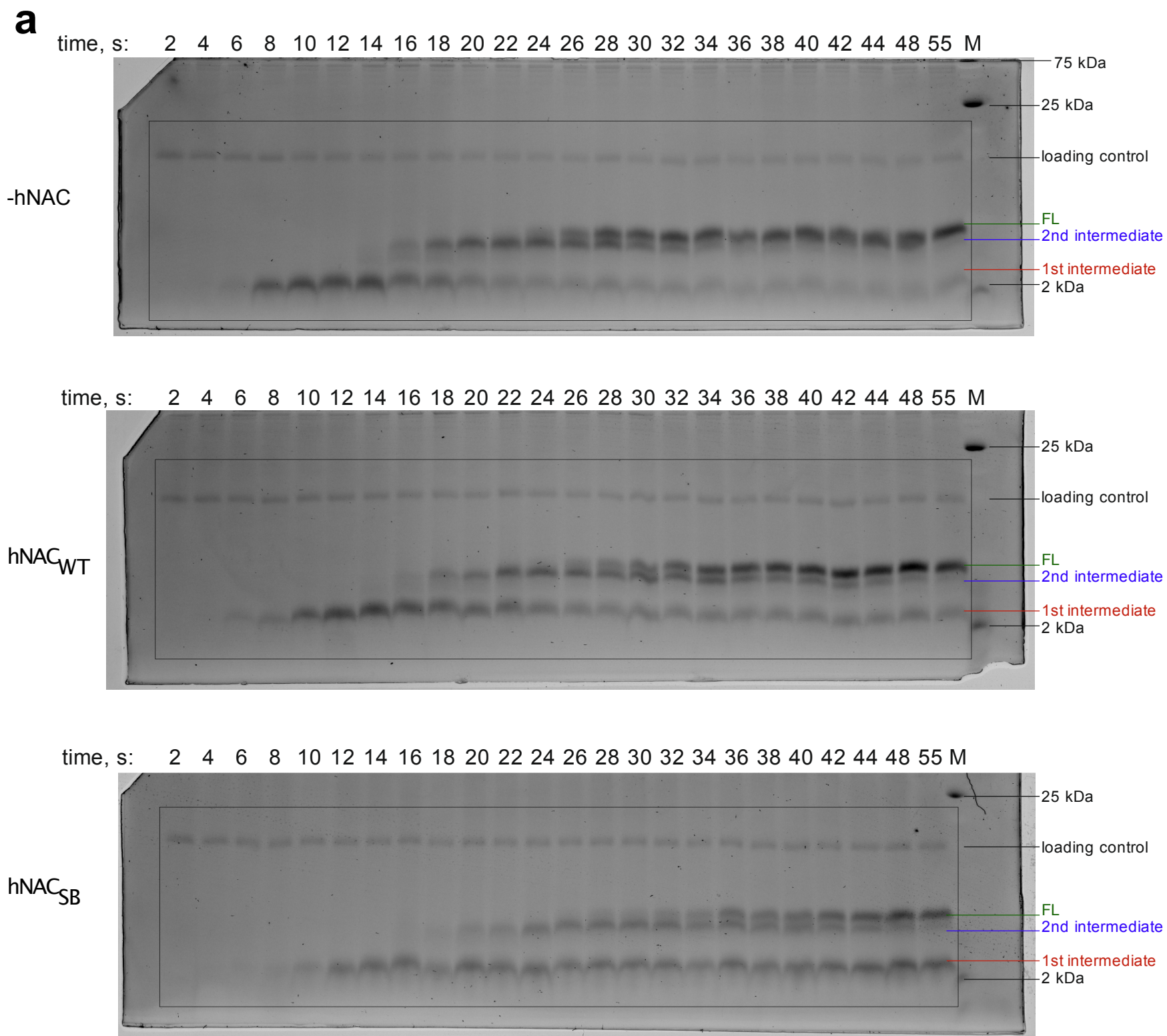
a

Fig. 4h,i,j -NAC interacts with short NCs

**b**

Ext. Fig. 6: Bpa-crosslinking of NAC and ribosome nascent chain complexes





The intensity of each *in vitro* translated peptide (1st, 2nd intermediate and full-length product (FL)) was analyzed via Image Studio software and normalized against the loading control loaded on the same gel as marked. The areas marked in the box appear in the Extended Data Figure 7.