

Substrate-induced assembly and functional mechanism of the membrane protein insertase SecYEG-YidC

Max Busch, Cristian Rosales-Hernandez, Michael Kamel, Yulia Schaumkessel, Eli van der Sluis, Otto Berninghausen, Thomas Becker, Roland Beckmann, and Alexej Kedrov

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Dear Prof. Kedrov,

Thank you for submitting your manuscript for consideration by the EMBO Journal. It has now been seen by two referees whose comments are enclosed. As you will see, both referees express interest in your manuscript and are broadly in favor of publication, pending satisfactory minor revision.

Given the referees' positive recommendations, I would like to invite you to submit a revised version of the manuscript, addressing the comments of all three reviewers. I should add that it is EMBO Journal policy to allow only a single round of revision, and acceptance of your manuscript will therefore depend on the completeness of your responses in this revised version.

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We generally allow three months as standard revision time. As a matter of policy, competing manuscripts published during this period will not negatively impact on our assessment of the conceptual advance presented by your study. However, we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed.

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

Yours sincerely,

Cornelius Schneider, PhD
Editor
The EMBO Journal
c.schneider@embojournal.org

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Please also check that the title and abstract of the manuscript are brief, yet explicit, even to non-specialists.

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- a point-by-point response to the referees' comments, with a detailed description of the changes made (as a word file).
- a word file of the manuscript text.
- individual production quality figure files (one file per figure)
- a complete author checklist
- Expanded View files (replacing Supplementary Information)
- a Reagents and Tools Table as part of the Methods section

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We realize that it is difficult to revise to a specific deadline. In the interest of protecting the conceptual advance provided by the work, we recommend a revision within 3 months (10th Jun 2026). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions. Use the link below to submit your revision:

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Referee #1:

This manuscript provides a clear structural basis for understanding how SecYEG and YidC work together to insert multi-pass membrane proteins into the membrane, which is a fundamental and important biological process. Although membrane protein insertion has been studied for many years, structural information that captures dynamic intermediate states at near-atomic resolution has been limited. In this respect, the present study directly addresses this important question and represents a meaningful and well-designed effort.

The authors used fusion constructs of NuoK with SecM of different lengths to generate stalled, cotranslational intermediate states. These intermediates were analyzed by cryo-electron microscopy. This approach allowed the authors to capture defined stages of membrane insertion and to provide structural information about the relative positions and interactions of SecYEG and YidC. The reconstitution of these complexes and their high-resolution structural analysis are technically demanding. The overall quality of the structural data appears to be high.

Importantly, the study shows that YidC can be positioned at the backside of SecY during membrane insertion. This observation adds a new perspective to the current understanding of bacterial membrane protein insertion. Therefore, the study makes a valuable contribution to the field.

An earlier version of this work was posted on bioRxiv. The present manuscript appears to have been carefully revised since then, likely after peer review. The overall quality of the manuscript is high. The data presentation and discussion are clear and well organized. At this stage, I have no major comments.

Minor comments:

1. It would be useful to include a table summarizing the cryo-EM data collection and refinement statistics in the Supplementary Information.
2. When comparing the current structures with previously reported structures, the corresponding PDB IDs should be clearly indicated in the figure legends. Please add the missing PDB IDs where necessary.

Referee #2:

In this manuscript, the authors combined biochemistry, cryo-EM and AlphaFold predictions to characterize a bacterial co-translational SecYEG-YidC complex when engaged by the nascent membrane protein NuoK. In the bacterial translocation field, YidC has long been thought to operate at the lateral gate of SecYEG, helping to insert and chaperone transmembrane helices (TMHs) as they exit the Sec translocation channel. Recent work in mammalian and yeast systems have found that a substantial proportion of membrane insertion occurs independently of the Sec lateral gate, instead occurring "behind" Sec via other factors, some of which are evolutionarily related to YidC. The authors set out to determine whether in bacteria, a similar "back-of-Sec" mechanism of insertion might also operate. Their findings show that YidC is recruited to SecYEG in response to the substrate NuoK, and that nascent insertion intermediates of NuoK are observed behind a closed SecYEG adjacent to YidC. The structures are consistent with AlphaFold predictions of the SecYEG-YidC complexes, and reveal interesting insights into how a substrate can engage these two complexes. This suggests that the basic mechanisms of membrane insertion are broadly conserved in bacteria and eukaryotes. The work is of broad interest for EMBO Journal's readership, and will surely stimulate more work in this area. I therefore recommend publication pending some revisions to the writing.

Major points:

- 1) It seems that nascent NuoK is inserted in the opposite orientation in nanodiscs to what is found in native membranes. Although this is discussed at the end of the paper, with some plausible explanations provided, it is not mentioned in the results section where the structure is described. This leads a reader to wonder what is happening and whether the findings are biologically relevant. I recommend a more direct approach to this problem by changing the following: (i) when the structure is first presented, directly and unambiguously state that the topology is backwards; (ii) mention that although this might represent an artefact with respect to NuoK, the fact that an insertion intermediate is being observed nonetheless makes the structure interesting and interpretable from the general standpoint of the mechanism of membrane insertion; (iii) tell the reader that this issue will be discussed in more detail later.
- 2) Similar to point 1, it is useful to be a bit clearer in the results about the fact that TMH2/3 are probably inserted shortly after termination, but that the strategy of analysing a stalled ribosome-bound intermediate helps one to visualise the likely insertion process structurally. This way, a reader is not left until the end of the paper to realise what has been done. The authors may want to mention whether they think that insertion occurs before or after the ribosome dissociates from SecYEG under normal conditions. I feel it probably occurs before the ribosome dissociates, meaning that the structures that are seen are valid proxies of the physiologic pathway, but it remains possible that insertion occurs via YidC after the ribosome and substrate have

dissociated from SecYEG. Both possibilities should probably be mentioned.

3) The interpretation that the conserved Arg is not involved in insertion is questionable. The position of TMH2 in this intermediate likely represents an endpoint location, and it is not possible to infer the path TMH2 took to reach this point. It is formally possible that Arg366 is on that path, especially considering that previous work tested its importance by growth assay (PMID: 25466392), simulation (PMID: 34873152), and other methods (such as PMID: 35148995).

4) NuoK-86 should have 36 amino acids between the site of stalling and TMH3, which is enough to span the entire ribosome exit tunnel and more. Yet, half of TMH3 is proposed to be inside the exit tunnel. I am not entirely sure why this is the case. Is it possible that the TMH assigned to TMH2 of NuoK could be TMH3 in the correct topology, and that TMH2 is what is barely visible in the density, and TMH1 is not visible? This would reconcile the opposite topology problem. In other words, how certain are the authors that their model for the substrate is correct given the rather modest resolution? Some explanation and discussion of this point might be useful.

Minor points:

Pg. 2, Line 9 - "leaves the translocon" is not quite right since it implies that the nascent chain passes from inside SecYEG through the back wall, which is of course not what seems to happen. Rephrase. Perhaps "leave the cytosolic vestibule of SecYEG" or something like that.

Pg. 2, Line 14 - The insertase machinery is not elusive; YidC and SecY have been known for decades. May want to delete "elusive"

Pg. 2, Line 14 - "prove" is too strong; perhaps "suggest an evolutionary conservation..." is better.

Pg. 3 Line 2 - IMPs do not constitute 30% of the genome content; they constitute 30% of the protein-coding genes. Genomes have lots of other content. Also, better not to equate protein with nucleic acids, so could instead say 30% of protein coding genes encode IMPs...

Pg. 14, line 32 - Sup. Fig. 15 should be 16.

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The authors used fusion constructs of NuoK with SecM of different lengths to generate stalled, cotranslational intermediate states. These intermediates were analyzed by cryo-electron microscopy. This approach allowed the authors to capture defined stages of membrane insertion and to provide structural information about the relative positions and interactions of SecYEG and YidC. The reconstitution of these complexes and their high-resolution structural analysis are technically demanding. The overall quality of the structural data appears to be high.

Importantly, the study shows that YidC can be positioned at the backside of SecY during membrane insertion. This observation adds a new perspective to the current understanding of bacterial membrane protein insertion. Therefore, the study makes a valuable contribution to the field.

An earlier version of this work was posted on bioRxiv. The present manuscript appears to have been carefully revised since then, likely after peer review. The overall quality of the manuscript is high. The data presentation and discussion are clear and well organized. At this stage, I have no major comments.

Authors: We thank the Referee for the interest in our work and for the positive evaluation of the manuscript in its current form!

Minor comments:

Referee 1, Comment 1: It would be useful to include a table summarizing the cryo-EM data collection and refinement statistics in the Supplementary Information.

Authors: We apologize for leaving out the table from the original submission! The information is included in the revised version (Appendix Table S2).

Referee 1, Comment 2: When comparing the current structures with previously reported structures, the corresponding PDB IDs should be clearly indicated in the figure legends. Please add the missing PDB IDs where necessary.

Authors: Thank you for the point! The missing ID's have been added to the legends of the Figure 3E and Figure EV1B (former Suppl. Figure 2B).

Referee #2:

In this manuscript, the authors combined biochemistry, cryo-EM and AlphaFold predictions to characterize a bacterial co-translational SecYEG-YidC complex when engaged by the nascent membrane protein NuoK. In the bacterial translocation field, YidC has long been thought to operate at the lateral gate of SecYEG, helping to insert and chaperone transmembrane helices (TMHs) as they exit the Sec translocation channel. Recent work in mammalian and yeast systems have found that a substantial proportion of membrane insertion occurs independently of the Sec lateral gate, instead occurring "behind" Sec via other factors, some of which are evolutionarily related to YidC. The authors set out to determine whether in bacteria, a similar "back-of-Sec" mechanism of insertion might also operate. Their findings show that YidC is recruited to SecYEG in response to the substrate NuoK, and that nascent insertion intermediates of NuoK are observed behind a closed SecYEG adjacent to YidC. The structures are consistent with AlphaFold predictions of the SecYEG-YidC complexes, and reveal interesting insights into how a substrate can engage these two complexes. This suggests that the basic mechanisms of membrane insertion are broadly conserved in bacteria and eukaryotes. The work is of broad interest for EMBO Journal's readership, and will surely stimulate more work in this area. I therefore recommend publication pending some revisions to the writing.

Authors: We thank the Referee for recognizing the impact of our study, and the constructive suggestions for the revision!

Major points:

Referee 2, Comment 1: It seems that nascent NuoK is inserted in the opposite orientation in nanodiscs to what is found in native membranes. Although this is discussed at the end of the paper, with some plausible explanations provided, it is not mentioned in the results section where the structure is described. This leads a reader to wonder what is happening and whether the findings are biologically relevant. I recommend a more direct approach to this problem by changing the following: (i) when the structure is first presented, directly and unambiguously state that the topology is backwards; (ii) mention that although this might represent an artefact with respect to NuoK, the fact that an insertion intermediate is being observed nonetheless makes the structure interesting and interpretable from the general standpoint of the mechanism of membrane insertion; (iii) tell the reader that this issue will be discussed in more detail later.

Authors: Thank you for the suggestion. We totally agree with the Referee that despite the unexpected orientation of the specific nascent protein, the identified insertion route via SecYEG-YidC may be taken by many other clients, in agreement with the recent work of Keenan and co-workers (PMID 41116047), so the visualization of the nascent chain path represents the major impact of the study. For the revised manuscript, we have followed the suggestion of the Reviewer and indicated the inverted topology of NuoK in the early part of the Results chapter (page 7), where we also suggest the general importance of the insertion route available for various clients.

Referee 2, Comment 2a: Similar to point 1, it is useful to be a bit clearer in the results about the fact that TMH2/3 are probably inserted shortly after termination, but that the strategy of analysing a stalled ribosome-bound intermediate helps one to visualise the likely insertion

process structurally. This way, a reader is not left until the end of the paper to realise what has been done.

Authors: Thank you for considering this aspect. Recognizing the differences between the naturally occurring insertion and the stalled intermediate employed for the structural analysis is indeed highly important, so we explain those early in the Results section, when introducing the in vitro system (page 6). We revised the text (lines 19-23) to make this point clear.

Referee 2, Comment 2b: The authors may want to mention whether they think that insertion occurs before or after the ribosome dissociates from SecYEG under normal conditions. I feel it probably occurs before the ribosome dissociates, meaning that the structures that are seen are valid proxies of the physiologic pathway, but it remains possible that insertion occurs via YidC after the ribosome and substrate have dissociated from SecYEG. Both possibilities should probably be mentioned.

Authors: We thank the Referee for the suggestion. Insertion of the NuoK TMH 2 should occur in a co-translational manner, and the event is illustrated by the NuoK-70 construct in our work (pages 10-11, Figure 6A-C). In respect to NuoK TMH 3, we would like to avoid the speculation/over-interpretation of the structural data. In the Discussion chapter (page 15, lines 23-32), we refer to the recent studies which show that YidC-type insertases are involved in post-translational insertion of C-terminal helices, which is indeed likely scenario for NuoK (also included in Figure 8). It is indeed possible that the ribosome remains bound to SecYEG at that stage, but it is unlikely to be involved in routing of the released polypeptide chain.

Referee 2, Comment 3: The interpretation that the conserved Arg is not involved in insertion is questionable. The position of TMH2 in this intermediate likely represents an endpoint location, and it is not possible to infer the path TMH2 took to reach this point. It is formally possible that Arg366 is on that path, especially considering that previous work tested its importance by growth assay (PMID: 25466392), simulation (PMID: 34873152), and other methods (such as PMID: 35148995).

Authors: We agree with the Referee and modify the text accordingly to indicate possible interactions of Arg-366 with NuoK at different insertion stage (page 10, lines 19-21). Furthermore, the legend of Figure 5C has been re-phrased to avoid mis-/over-interpretation.

Referee 2, Comment 4: NuoK-86 should have 36 amino acids between the site of stalling and TMH3, which is enough to span the entire ribosome exit tunnel and more. Yet, half of TMH3 is proposed to be inside the exit tunnel. I am not entirely sure why this is the case. Is it possible that the TMH assigned to TMH2 of NuoK could be TMH3 in the correct topology, and that TMH2 is what is barely visible in the density, and TMH1 is not visible? This would reconcile the opposite topology problem. In other words, how certain are the authors that their model for the substrate is correct given the rather modest resolution? Some explanation and discussion of this point might be useful.

Authors: The Referee is absolutely correct in respect to the length of the nascent chain residing within the ribosomal exit tunnel: The helices of NuoK are fully exposed from the ribosome, as explained in the experiment design (page 5, lines 13-16) and confirmed by the cryo-EM data (Figure 4A). The resolution of the nascent chain within the tunnel is below 3 Å and it allows visualization and counting of individual residues to the mouth of the tunnel, where the flexible glycine-rich linker is positioned (summarized on page 9, lines 8-19; Figure 4 A and B). The chain formed a continuous density with the folded alpha-helix starting from the tunnel mouth. As we could not identify additional densities under the tunnel, which could indicate

unaccounted parts of the nascent chain, the helix was assigned to NuoK TMH 3 with high confidence.

Minor points:

Pg. 2, Line 9 - "leaves the translocon" is not quite right since it implies that the nascent chain passes from inside SecYEG through the back wall, which is of course not what seems to happen. Rephrase. Perhaps "leave the cytosolic vestibule of SecYEG" or something like that.

Authors: Thank you for the correction. We have modified the sentence, hoping to resolve this ambiguity, while remaining within the size limit of the abstract, i.e. 175 words,

Pg. 2, Line 14 - The insertase machinery is not elusive; YidC and SecY have been known for decades. May want to delete "elusive"

Authors: Done!

Pg. 2, Line 14 - "prove" is too strong; perhaps "suggest an evolutionary conservation..." is better.

Authors: Done!

Pg. 3 Line 2 - IMPs do not constitute 30% of the genome content; they constitute 30% of the protein-coding genes. Genomes have lots of other content. Also, better not to equate protein with nucleic acids, so could instead say 30% of protein coding genes encode IMPs...

Authors: The text has been modified accordingly.

Pg. 14, line 32 - Sup. Fig. 15 should be 16.

Authors: Thank you for the correction! In the revised version, the figure appears as Figure EV5.

Dear Prof. Kedrov,

Thank you for submitting a revised version of your manuscript which we find has addressed the concerns raised by both referees. There remain only a few mainly editorial points that have to be addressed before I can extend formal acceptance of the manuscript:

- As we are switching from a free-text author contribution statement towards a more formal statement based on Contributor Role Taxonomy (CRediT) terms, please remove the present Author Contribution section and instead specify each author's contribution(s) directly in the Author Information page of our submission system during upload of the final manuscript. See <https://casrai.org/credit/> for more information.
- FIGURE CALLOUTS: all callouts should be listed sequentially; missing callouts for Fig. 7A, 7C and Dataset EV2
- APPENDIX 1 FILE WITH ToC: Appendix file needs to be in PDF format; title page should contain "Appendix for + ms title" and ToC with the page numbers for the listed items; nomenclature correct; Appendix tables should be included in Appendix PDF with the nomenclature Appendix Table S1-S2 and listed in ToC
- Please provide suggestions for a short 'blurb' text prefacing and summing up the conceptual aspect of the study in two sentences (max. 250 characters), followed by 3-5 one-sentence 'bullet points' with brief factual statements of key results of the paper; they will form the basis of an editor-written 'Synopsis' accompanying the online version of the article. Please also provide an altered synopsis image, making sure that the aspect ratio conforms to our website's format - it should be exactly 550 pixels wide and 300 pixels high.
- Figure reuse between Figure 5B and Appendix Figure S8B.
Not stated in the figure legend. If purposeful re-use of the image has occurred, please state this clearly in the figure legend.
- Sections need to be named and the order should be corrected: Title page - Abstract - Introduction - Results - Discussion - Methods - Data Availability - Acknowledgements - Disclosure and Competing Interests Statement - References - Figure Legends - Table(s) - Expanded View Figure Legends.

Please let me know if you have any questions regarding any of these points. You can use the link below to upload the revised files.

Thank you again for giving us the chance to consider your manuscript for The EMBO Journal. I look forward to receiving the final version.

With best regards,

Cornelius Schneider

Cornelius Schneider, PhD
Editor | The EMBO Journal
c.schneider@embojournal.org

Please refer to our figure preparation guideline in order to ensure proper formatting and readability in print as well as on screen:

<https://link.springer.com/journal/44318/submission-guidelines#cms-Figure-and-data-presentation>

Use the link below to submit your revision:

<https://emboj.msubmit.net/cgi-bin/main.plex>

All editorial and formatting issues were resolved by the authors.

Dear Prof. Kedrov,

I am pleased to inform you that your manuscript has been accepted for publication in the EMBO Journal.

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If you have any questions, please do not hesitate to contact the Editorial Office. Thank you for your contribution to The EMBO Journal.

Yours sincerely,

Cornelius Schneider, PhD
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This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: [10.31222/osf.io/9sm4x](https://doi.org/10.31222/osf.io/9sm4x)). Please follow the journal's guidelines in preparing your

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The data shown in figures should satisfy the following conditions:

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- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical
- if $n < 5$, the individual data points from each experiment should be plotted. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data

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- a specification of the experimental system investigated (eg cell line, species name).
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- an explicit mention of the biological and chemical entity(ies) that are being measured.
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- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
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- definitions of statistical methods and measures:
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Newly Created Materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
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Antibodies	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
For antibodies provide the following information: - Commercial antibodies: RRID (if possible) or supplier name, catalogue number and or/clone number - Non-commercial: RRID or citation	Yes	Reagents and tools table and in Methods section
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Microbes: provide species and strain, unique accession number if available, and source.	Yes	Reagents and Tools table and in Methods section
Human research participants	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
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Design

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Experimental study design and statistics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Include a statement about sample size estimate even if no statistical methods were used.	Not Applicable	
Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, have they been described?	Not Applicable	
Include a statement about blinding even if no blinding was done.	Not Applicable	
Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	Not Applicable	
If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification .		
For every figure, are statistical tests justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?	Not Applicable	

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In the figure legends: state number of times the experiment was replicated in laboratory.	Not Applicable	
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Ethics	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Studies involving human participants : State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval.	Not Applicable	
Studies involving human participants : Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	Not Applicable	
Studies involving human participants : For publication of patient photos , include a statement confirming that consent to publish was obtained.	Not Applicable	
Studies involving experimental animals : State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval. Include a statement of compliance with ethical regulations.	Not Applicable	
Studies involving specimen and field samples : State if relevant permits obtained, provide details of authority approving study; if none were required, explain why.	Not Applicable	

Dual Use Research of Concern (DURC)	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Could your study fall under dual use research restrictions? Please check biosecurity documents and list of select agents and toxins (CDC): https://www.selectagents.gov/sat/list.htm .	Not Applicable	
If you used a select agent, is the security level of the lab appropriate and reported in the manuscript?	Not Applicable	
If a study is subject to dual use research of concern regulations, is the name of the authority granting approval and reference number for the regulatory approval provided in the manuscript?	Not Applicable	

Reporting

The MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives. Journals have their own policy about requiring specific guidelines and recommendations to complement MDAR.

Adherence to community standards	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
State if relevant guidelines or checklists (e.g., ICMJE, MIBBI, ARRIVE, PRISMA) have been followed or provided.	Not Applicable	
For tumor marker prognostic studies , we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	Not Applicable	
For phase II and III randomized controlled trials , please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	Not Applicable	

Data Availability

Data availability	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Have primary datasets been deposited according to the journal's guidelines (see 'Data Deposition' section) and the respective accession numbers provided in the Data Availability Section?	Yes	The cryo-EM structural data have been deposited in the Electron Microscopy Data Base (EMDB) and the Protein Data Bank (PDB) repositories, additional data is included in Appendix dataset S1
Were human clinical and genomic datasets deposited in a public access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement?	Not Applicable	
Are computational models that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided?	Not Applicable	
If publicly available data were reused, provide the respective data citations in the reference list .	Not Applicable	