nature portfolio

Peer Review File

Cytoplasmic lattices are megadalton storage complexes in mammalian oocytes

Corresponding Author: Dr Miguel Leung

Any redactions in this file are there to maintain patient confidentiality, the confidentiality of unpublished data, or to remove third-party material.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Attachments originally included by the reviewers as part of their assessment can be found at the end of this file.

Version 0:

Reviewer comments:

Referee #1

(Remarks to the Author)
Mammalian oocytes store proteins for embryonic development on filamentous structures called cytoplasmic lattices (CPLs): however, its molecular architecture and assembly mechanisms have been poorly understood. The authors isolated CPL filaments from mouse oocytes and analyzed the structure of the core unit of CPL using cryo-EM single particle analysis. The study yielded a high-resolution map of a single CPL unit and revealed the interactions between the subunits (components), which explain many of the previous biological findings regarding CPLs. The information provided in this article is valuable. However, there are some issues that need to be clarified mainly regarding the identification of the components and cryo-EM analysis. Also, helical refinement could be employed to further elucidate the architecture of the CPL filament. Deeper discussions on some structural features are also desirable.

Major comments
1. Assignment of some subunits was dependent on a visual proteomics approach. Since the authors isolated CPL filaments from mouse oocytes, the presence of the assigned subunits could be validated using mass spectrometry. In fact, most, if not all, subunits could be confirmed by this analysis. It is highly recommended to perform mass spectrometry on the isolated CPLs.

2. Helical refinement could be employed to further elucidate the architecture of the CPL filament. The authors are encouraged to reanalyze the micrographs using helical reconstruction techniques, present the resulting filament structure, and discuss the interactions between individual CPL units within the filament. Additionally, by superimposing the reconstructed filaments onto the previously reported cryo-ET map (Jentoft et al., Cell 2023), the authors could explore potential inter-filament interactions. Such an integrative analysis would substantially enhance the quality of the manuscript.

3. The current presentation of the cryo-EM workflow in Extended Data Fig. 1 lacks details to assess the validity of the analysis. To improve transparency and reproducibility, I request a revised figure with following elements:
-Number of classes used in 2D classification
-Representative images from the 2D classification
-Number of classes used in 3D classification
-Resulting 3D volumes from the classification, along with their relative occupancies (%)
-Orientation distribution of the refined particles

4. Extended Data Fig. 2b displays only one representative subunit overlaid with the cryo-EM map. To provide a more comprehensive overview, all subunits should be shown overlaid with their corresponding EM maps.

5. The methodology of reconstruction of composite map shown in Figure 1D is unclear. The authors should provide the details of the procedure.

6. Cryo-EM analysis revealed that the PADI6 dimer forms two pentamers via head-to-head arrangement (Line 212). Is this assembly mode consistent with the crystal packing observed in previously reported PADI6 structures (Ranaivoson et al., IUCrJ 2024)?

7. It is interesting that CPL has a large characteristic cavity, which is formed by PADI6, MLRP14, UHRF1 and UBE2. However, the authors did not discuss possible biological function of this cavity. I would like the authors to estimate the electrostatic properties and size of the cavity and discuss what kind of molecules could fit into this structure.

8. Previous studies showed that UHRF1 level is increased in the nucleus of Nlrp14- and Padi6-mutant oocytes and embryos (Giaccari et al., Genes Dev.; 2024; Yan et al., Nat Genet, 2023) while it is decreased in Nlrp5-knockout oocytes (Unoki et al., Hum Mol Genet, 2024). Can the authors discuss how this happens based on their findings?

Minor comments
-Line 186: Please cite Maenohara et al., PLOS Genet. 2017. To my knowledge, this was the first paper to show that UHRF1 stored in the oocyte is essential for regulating methylation patterns in the early embryo.
-Extended data table 1 should come with additional cryo-EM validation metrics, map sharpening B-factor, CC (volume/mask), and the FSC threshold used to define model resolution.
-Extended Data Fig.1: Unit for box size should be specified (e.g., in pixels).
-Readers may be curious whether the samples were chemically crosslinked prior to vitrification.

Referee #2

(Remarks to the Author)
This study by Kilic and coworkers reveals the molecular structure and composition of cytoplasmic lattices (CPLs) in mammalian oocytes. CPLs are protein storage structures that are essential for early embryonic development and have been poorly understood until now. Using single particle cryo-EM at a resolution of 4.2 Angstroem, the authors find that CPLs from mouse oocytes are megadalton-scale complexes composed of at least 13 proteins, including PADI6, subcortical maternal complex (SCMC) components (NLRP5, TLE6, OOEP, KHDC3, ZBED3, NLRP4F), a-/b-tubulin dimers, and ubiquitination factors such as UHRF1, UBE2 and FBXW proteins. PADI6 acts as a structural scaffold by its assembly in pentamers of homodimers, while SCMC proteins organize CPL filaments. Tubulin is stored as capped, unpolymerized alpha/beta-dimer, providing a maternal reservoir for cytoskeletal reorganization post-fertilization. The authors speculate that the inclusion of ubiquitination enzymes suggests that CPLs also regulate protein stability and degradation timing during oocyte-to-embryo transition. This work solves the decades-long mystery of CPL composition and provides a structural framework for understanding infertility and developmental disorders linked to maternal factor disruption.

The manuscript is well written, providing some background information on oocyte biology and the potential impact of distinct CPL subunits in infertility. The explanation of the structural architecture could be a little more elaborate, for example, breaking down the filamentous assembly into its composing units, explaining symmetry aspects and the number of individual subunits. I have only some comments and suggestions to the manuscript.

Criticism
Where are the AI-based modelling approaches used within the study and structure reconstruction? While mentioning this in the abstract (line 16), I could not find it in the main text but only in the Methods.

Along these lines: “We find that CPLs consist of …” (line 73). How did you find this? The paragraph starting with this phrase does not explain, if and how this was identified. For example, were mass spectrometry or modelling approaches applied? The “previous functional evidence” later this paragraph (line 81) should be substantiated by citation(s).

The authors describe the five PADI6 homodimers as “ears on the sides of each globular repeat of the CPLs” (line 89) and later that “resembles a spiral staircase” (line 93). Could the authors propose a more precise or scientifically appropriate term for this ‘pot-like’ structure?

Same for the introduction of the SCMC structure that “bridge PADI6 rings at the top and bottom of every globular repeat, …” (line 110). This SCMC assembly is later described as “… giving the CPLs a chain-like appearance …” (line 121). Could the authors suggest a clearer or more suitable designation for this entity?

I very much like the explanation of the “discrete type I and type II SCMC subcomplexes”, as the authors refer to it (line 117f and Fig. 3b,c). In the type I subcomplex, the C-terminal LRR of NLRP4F binds to NLRP5 and OOEP mediated by ZBED3, whereas in the type II assembly, the N-terminal PYD and NACHT domains of NLRP4F bind to NLRP5 and OOEP via the KHDC3 subunit. Do the authors have an explanation for this asymmetry?

Could the authors comment on which element of the CPL chain assembly is proposed to be the weakest? Or in other words, is there a predetermined breaking point in the filament and is anything known about its disassembly?

Does any of the NLR proteins NLRP4f, MLRP5 or NLRP14 contain a bound ADP or ATP nucleotide? Please comment even if the site is empty.

It is not immediately becoming clear why the authors mention DARPins in the consideration of the a- and b-tubulins (line 147 and Fig. 4b). Is the comparison to a-/b-tubulin--DARPin binding providing an explanation, why only one tubulin pair is found the in the CPL structure?

Could the authors envision a similar storage capacity for (G-)actin molecules in the CPL as found here for tubulin? At least, there are actin elongation factors as FMN1 and FMN2 present in the oocyte, that are thought to be implicated in interphase microtubule binding and asymmetric spindle positioning during meiosis.

Could the entity harboring the tubulin pair have any chaperone-like activity?

Extended Data Fig. 3: It would be good, if the authors would add how many copies of each subunit are found within one chain of the CPLs. E.g., 20x PADI6 subunits, 4x NLRP5 …

The authors could provide some additional dimensions (sizes in nm) in the text or in figure displays for some of the CPL entities explained, not only those given in Fig. 1d.

Line 63: “We leveraged the reported detergent-resistance of the CPLs …” Please provide a citation.

Referee #3

(Remarks to the Author)
The mammalian oocyte contains specialized filaments called cytoplasmic lattices (CPLs), which are thought to serve as storage reserves of maternal factors required for early embryonic development. CPLs were originally visualized by electron microscopy almost 60 years ago, but a high-resolution structure has yet to be determined for this challenging sample. Previous studies have suggested that maternal factors important for fertility including the subcortical maternal complex (SCMC) and peptidylarginine deaminase 6 (PADI6) are CPL components, but it remains unclear how these factors are incorporated into the CPL filament architecture remains elusive. Further, the identities and organization of other CPL components are unknown due to a lack of a high-resolution CPL structure.

This paper from Kiliç et al. presents an impressive cryo-EM structure of CPL filaments natively isolated from murine oocytes. The high-resolution of the density maps, which in many cases feature side-chain level detail, allows the authors to use a combination of modeling tools to build a model of the CPL filament and identify CPL protein components. The structure reveals that PADI6 forms a unique pentamer of homodimers which dock on to he sides of the CPL repeating unit. It also shows how PADI6 interacts with the SCMC, which acts as a core structural component of CPLs. The authors also find that proteins important for embryonic development that were previously hypothesized to be stored in CPLs are indeed structural components. For example, / -tubulin heterodimers and ubiquitination factors like UHRF1 and UBE2 are incorporated into the filament. We believe this work represents a major advance that is appropriate in scope for publication in Nature, pending revisions to address a few concerns outlined below.

We recommend further validation of CPL component identities, as well as extending the structural analysis to probe CPL structural dynamics and higher-order architecture.

Recommendations:

1. The resolution of the CPL map allows the authors to assign the protein identity of the majority of components with high confidence. However, the authors effectively built the model by manual inspection of candidate proteins. To further support the identification of these proteins, we recommend analyzing the burst oocyte sample by mass spectrometry. This would allow the presence of all the proteins within the sample to be verified, and it may also reveal CPL component isoforms that were not discernable at the current resolution.

2. The authors focus on compositional variability within the individual CPL unit regarding the number of FBXW molecules present. Is there also conformational variability in the overall lattice architecture that is discernable by 3D variability analysis (3DVA)? Does 3DVA of multiple units of the lattice result in differences in the twist or curvature of the lattice? This may provide hints at pathways for filament assembly / disassembly.

3. In recent cryo-ET analysis of CPLs, Jentoft et al. (2023) determine a subtomogram average of five CPL filaments together that shows lower resolution density for contacts between the filaments. It would be interesting to compare the authors’ CPL model with this cryo-ET data, which may provide insights into how CPL components mediate the formation of higher-order CPL filament networks.

Minor points:

4. When uploading the final maps and models to the PDB/EMDB, the authors should address a discrepancy in the magnification used for data collection. The magnification is reported as 81,000x in Extended Data Table 1 and 130,000x in the PDB validation report.

5. In the Methods section for ‘Model Building and refinement,’ the authors note that due to resolution limitations, they were unable to identify the specific isoforms of tubulin, FBXW, and UBE2 molecules. This is an important point to note and should

be included in the main text when discussing the storage of tubulin within CPLs.

Version 1:

Reviewer comments:

Referee #1

(Remarks to the Author)
The authors have appropriately addressed almost all points that I raised: I appreciate the effort that they made to perform additional experiments and analysis and prepare additional Ext. Data figures, new Suppl. Tables, and rebuttal figures. The manuscript has been improved greatly.

- Regarding major point 1 (request for mass spectrometry analysis), I apologize my misunderstanding: while I thought that isolated CPL was used for the cryo-EM analysis, it was in fact burst oocyte sample. Because of this, and also because of the request made by Reviewer #3, they have performed mass spectrometry analysis with the burst oocyte sample. Thus, proteins identified by this analysis is not necessarily abundant in CPL. However, as the authors wrote in the response letter, it helped to confirm their abundant presence in oocytes and also to identify specific isoforms (UBE2D3, etc.). I therefore appreciate the authors' effort regarding this issue.

- Regarding major point 7 (properties of the cavity), the authors have now calculated the electrostatic properties and estimated the size of the cavity (Rebuttal Fig. 3). They then state in the response letter that they cannot make confident conclusions about specific proteins/molecules that might associate with the cavity, based solely on the information. While I agree their opinion and value their careful attitude, what they found would be worth mentioning in the manuscript. I think that the authors could briefly touch upon the findings in the manuscript and provide the data as an Ext. Data figure.

- Regarding major point 8 (question on the differential UHRF1 stability), I would respect the authors' cautious response.

Taken together, the revisions made by the authors substantially strengthen the manuscript. While I would like the authors to consider the above suggestion on major point 7, there is no other concern or suggestion.

Referee #2

(Remarks to the Author)
The authors present a thoroughly revised version of their original article. I have only two minor comments, which, however, should not affect the acceptance of the manuscript.

In Fig. 1d, the authors may also show the filament from the back side and rotate it another 90 degrees along the longitudinal axis. If I am not wrong, this view is otherwise only shown in ED Fig. 4c.

I would like to withdraw my previous comment about the author's description of a 'spiral staircase' for the assembly of the PADI6 pentamer and like to encourage them to use this illustrative description.

Referee #3

(Remarks to the Author)
The authors have revised their manuscript thoroughly, addressing the reviewers' requests and comments. The mass spectrometry results of the burst oocyte sample lend further support of protein identities in the CPL structure, and the multi-body refinement of neighboring CPL filament units characterizes the flexibility of the overall filament architecture. With their revised manuscript and figures, as well as supplemental information, the authors offer a detailed and well-presented investigation of the mammalian CPL's structure and how they built their atomic model. We support the publication of this manuscript with the present revisions.

Point-by-point response to referee comments for the manuscript
"Cytoplasmic lattices are megadalton storage complexes for tubulin and ubiquitination factors in mammalian oocytes"

We thank the editor for facilitating an efficient and helpful review of our work. We have taken the suggestions of the three expert referees on board and prepared a revised manuscript, which we believe is much improved. The main additions to this version are as follows:

1. We performed mass spectrometry on burst oocyte samples prepared in an identical manner to the samples used for cryo-EM. This confirms that all CPL subunits identified by cryo-EM are indeed present and abundant in the burst oocyte sample.
2. We now also apply state-of-the-art automated modeling tools to increase confidence in our assignment of candidate proteins. We describe these strategies in the Main Text and Methods.
3. We prepared an extensive new Data S1 document, which contains a page for each identified protein providing (i) basic details such as sequence identifier used for modeling, molecular weight, and domain organization; (ii) information about its location and stoichiometry within the CPLs; (iii) its abundance in our mass spectrometry data; (iv) model-in-map figures for each chain along with zoomed-in views showing regions with resolved side chain densities; (v) procedure for protein assignment using automated approaches, showing the model fragments used as queries and the e-values reported by the identification software; and (vi) supporting information from the literature. We believe this will serve as a useful reference for readers and will help them to make a fair assessment of data quality and confidence in protein assignment.
4. We compared our CPL structure to the reported subtomogram average of a CPL fiber/filament bundle, as suggested by referees #1 and #3. This analysis provided insight into how individual CPL filaments could interact to form higher-order assemblies and is included in a new Extended Data Figure.
5. We used Multi-Body Refinement to analyze conformational heterogeneity/flexibility on particles extracted with a large box size so as to include several neighboring subunits, as suggested by referee #3. The results are presented in a new Extended Data Figure.
6. We overhauled our Extended Data figures to accommodate the aforementioned new results as well as to include additional details of our processing strategy.

Additional revisions are described in the rest of this document, which contains our point-by-point response to the referee comments. We have also marked changes in blue font throughout the Main Text.

Referee #1 (Remarks to the Author):

Mammalian oocytes store proteins for embryonic development on filamentous structures called cytoplasmic lattices (CPLs): however, its molecular architecture and assembly mechanisms have been poorly understood. The authors isolated CPL filaments from mouse oocytes and analyzed the structure of the core unit of CPL using cryo-EM single particle analysis. The study yielded a high-resolution map of a single CPL unit and revealed the interactions between the subunits (components), which explain many of the previous biological findings regarding CPLs. The information provided in this article is valuable. However, there are some issues that need to be clarified mainly regarding the identification of the components and cryo-EM analysis. Also, helical refinement could be employed to further elucidate the architecture of the CPL filament. Deeper discussions on some structural features are also desirable.

Major comments

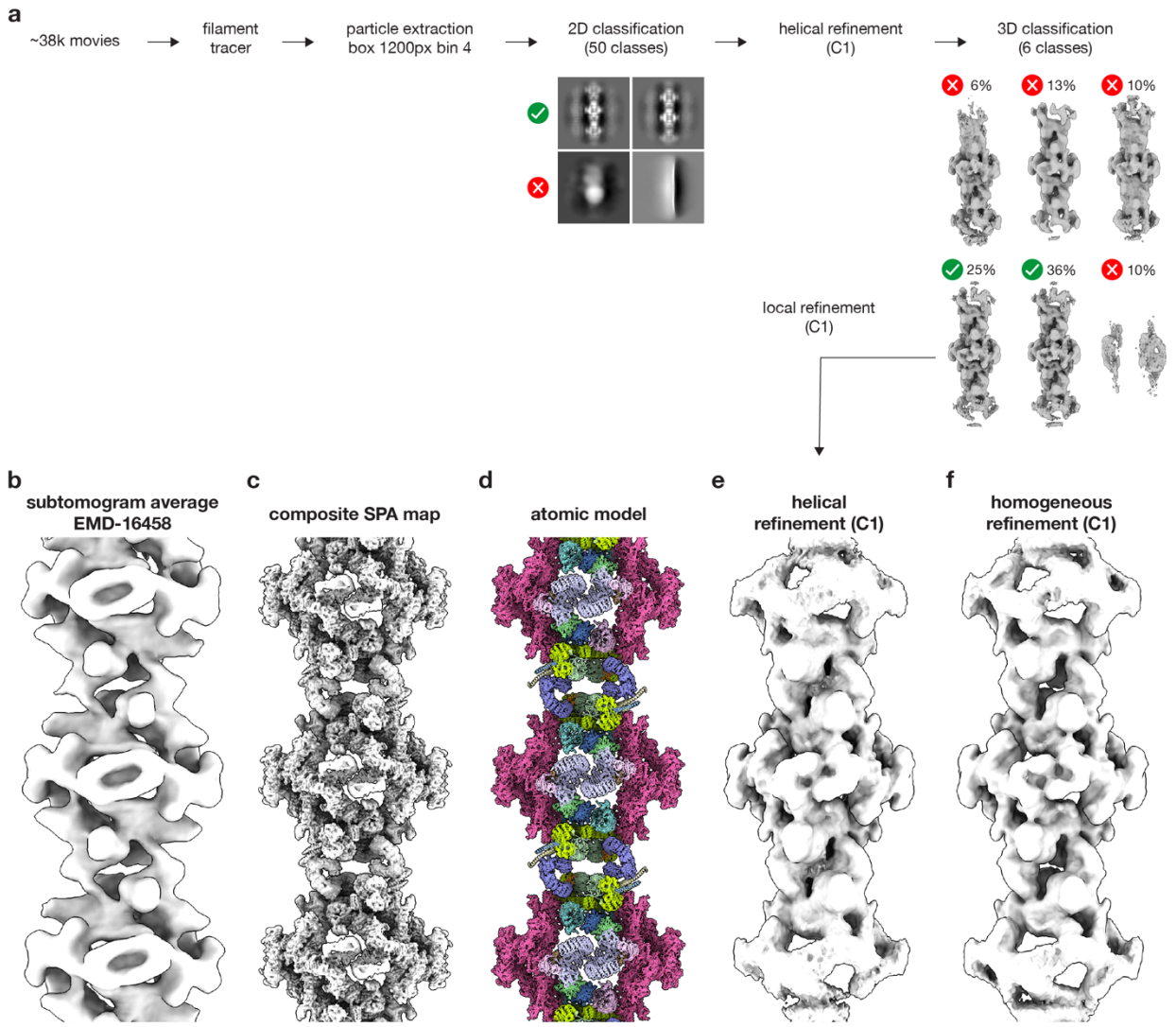
1. Assignment of some subunits was dependent on a visual proteomics approach. Since the authors isolated CPL filaments from mouse oocytes, the presence of the assigned subunits could be validated using mass spectrometry. In fact, most, if not all, subunits could be confirmed by this analysis. It is highly recommended to perform mass spectrometry on the isolated CPLs.

We would first like to clarify our workflow for imaging CPLs. We removed the ZP from mouse oocytes, washed them, then transferred them to a lysis buffer containing 0.5% Triton X-100. The burst oocyte sample was directly used to prepare cryo-EM grids *without any further purification, enrichment, or centrifugation*. During imaging, we manually searched for and targeted the CPL filaments on the grids.

To enable direct comparison, we therefore performed mass spectrometry (MS) on burst oocyte samples prepared identically to those used for cryo-EM (as also suggested by referee #3). This confirms that all assigned subunits are highly abundant in the sample (in fact all are among the top 150 proteins). We present the MS data graphically in a new Extended Data Figure 5, with a full list of identified proteins in a new Supplementary Table 1. Details of the sample preparation and MS analysis are now described in the Methods, and raw data have been deposited in PRIDE with dataset identifier PXD073451 and access token psiIqKGS2xdq. We believe this adds confidence to our assignment of CPL component proteins.

2. Helical refinement could be employed to further elucidate the architecture of the CPL filament. The authors are encouraged to reanalyze the micrographs using helical reconstruction techniques, present the resulting filament structure, and discuss the interactions between individual CPL units within the filament. Additionally, by superimposing the reconstructed filaments onto the previously reported cryo-ET map (Jentoft et al., Cell 2023), the authors could explore potential inter-filament interactions. Such an integrative analysis would substantially enhance the quality of the manuscript.

In our initial manuscript, we reconstructed a composite map (and corresponding atomic model) of the CPL filament by fitting three copies of the globular subunit map (from SPA) into the published *in situ* subtomogram average (see Fig. 1d, Extended Data Fig. 3a-c, and response to referee #1 point 5). As suggested by the reviewer, we performed helical reconstruction without symmetry in cryoSPARC (Rebuttal Figure 1a). In addition, as an alternative approach, we re-extracted our particles with a larger box size covering three CPL repeats and performed standard homogeneous refinement, also in cryoSPARC. The results from both approaches were very similar and are consistent with our model derived from fitting our original SPA data into the published subtomogram average (Rebuttal Figure 1b-f).



Rebuttal Figure 1. Structure of the CPL filament obtained by different approaches. (a) Processing workflow used to resolve the structure of a CPL filament using helical refinement in cryoSPARC. (b) *In situ* subtomogram average of a CPL filament from Jentoft et al 2023 PMID 37922900. (c) Composite map of a CPL filament constructed from fitting multiple copies of our SPA-derived maps into the subtomogram average and stitching with *volume maximum* in ChimeraX; see the new Extended Data Figure 3 for details of the stitching procedure. (d) Atomic model of a CPL filament built from the composite map. (e) Map of a CPL filament derived from a C1 helical refinement in cryoSPARC. (f) Map of a CPL filament derived from a C1 homogenous refinement in cryoSPARC.

As suggested by the reviewer, we also fit our SPA-derived models into the published subtomogram average of a CPL filament bundle from EMD-16458 (Jentoft et al 2023 PMID 37922900). The results are presented in a new Extended Data Figure 6 and described in the PADI6 section of the Main Text. This revealed that the PADI6 ring likely plays a crucial role in the assembly of individual CPL filaments into bundled fibers. We find that there are two types of contacts between filaments within a bundle, both involving the PADI6 ring: (i) a front-to-back interaction where PADI6 rings from neighboring filaments are bridged by as-yet-unidentified proteins; and (ii) a back-to-back interaction seemingly mediated by direct interaction of the PADI6 rings from neighboring filaments.

3. The current presentation of the cryo-EM workflow in Extended Data Fig. 1 lacks details to assess the validity of the analysis. To improve transparency and reproducibility, I request a revised figure with following elements:
-Number of classes used in 2D classification
-Representative images from the 2D classification
-Number of classes used in 3D classification
-Resulting 3D volumes from the classification, along with their relative occupancies (%)
-Orientation distribution of the refined particles

We have revised the presentation of our cryo-EM workflow to include the details requested and include additional information on composite map generation and protein assignment strategies. This information is distributed across Extended Data Figures 1-3.

4. Extended Data Fig. 2b displays only one representative subunit overlaid with the cryo-EM map. To provide a more comprehensive overview, all subunits should be shown overlaid with their corresponding EM maps.

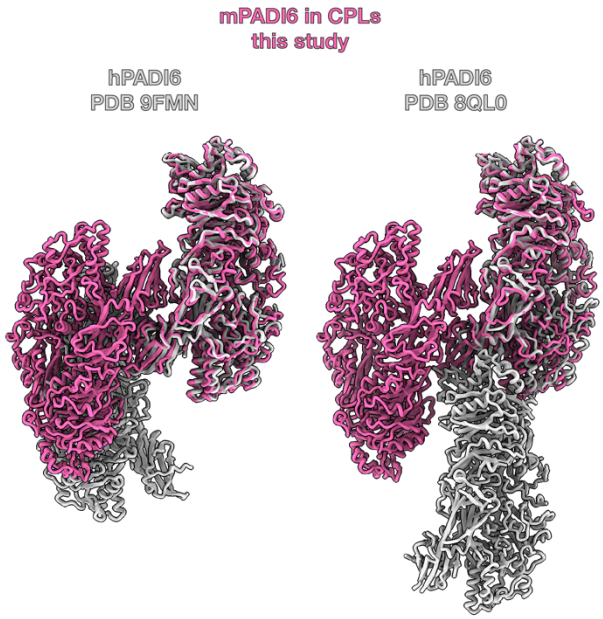
Thank you for this suggestion. We now show a model-in-map figure for every chain of every component in the Data S1 document.

5. The methodology of reconstruction of composite map shown in Figure 1D is unclear. The authors should provide the details of the procedure.

We apologize that this was unclear. We now provide a new Extended Data Figure 3 to guide the reader through the process for generating the composite maps. First, local refinements of the PADI6, SCMC, and tubulin subcomplex region were stitched together to generate a composite map of the asymmetric unit. Two copies of the asymmetric unit map were then stitched together to generate a composite map of the CPL globular repeat. Finally, three copies of the globular repeat were fit into the subtomogram average of a CPL filament (EMD-16458, Jentoft et al 2023) and stitched together to generate a composite map of the filament.

6. Cryo-EM analysis revealed that the PADI6 dimer forms two pentamers via head-to-head arrangement (Line 212). Is this assembly mode consistent with the crystal packing observed in previously reported PADI6 structures (Ranaivoson et al., IUCrJ 2024)?

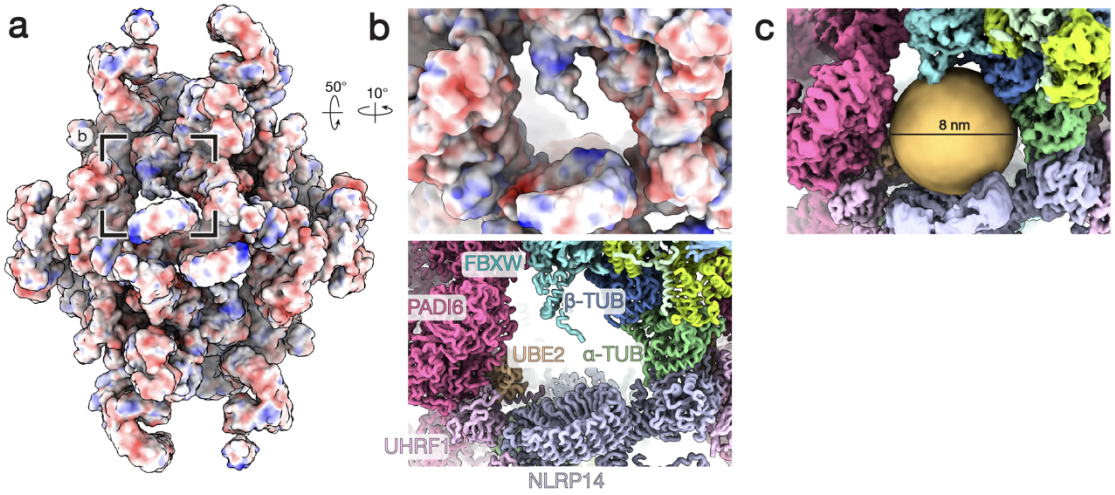
We compared PADI6 inter-dimer contacts in our structure of mouse oocyte CPLs with those in two published crystal structures of human PADI6 (PDB 9FMN from Williams et al 2024 PMID 39286527 and PDB 8QL0 from Ranaivoson et al 2024 PMID 38656308), see Rebuttal Figure 2 below. This analysis shows that the assembly mode of PADI6 in native CPLs differs from the crystal packing in previously reported structures.



Rebuttal Figure 2. Comparison of PADI6 inter-dimer contacts in native mouse cytoplasmic lattices (CPLs) and in reported crystal structures. For each structure, two adjacent PADI6 dimers are shown, with those from our CPL structure shown in pink and those from published crystal structures of human PADI6 shown in white.

7. It is interesting that CPL has a large characteristic cavity, which is formed by PADI6, MLRP14, UHRF1 and UBE2. However, the authors did not discuss possible biological function of this cavity. I would like the authors to estimate the electrostatic properties and size of the cavity and discuss what kind of molecules could fit into this structure.

We calculated the electrostatic properties of the whole CPL unit, along with the specific cavity formed among PADI6, UHRF1, UBE2, NLRP14 and α/β- tubulin dimer (Rebuttal Figure 3a). Our calculations show an enrichment of positively charged residues on the top and bottom regions of the cavity formed by FBXW and NLRP14, whereas negatively charged residues are concentrated along the sides formed by α/β- tubulin dimer and PADI6 (Rebuttal Figure 3b).



Rebuttal Figure 3. Electrostatic properties of a CPL globular repeat and the cavity formed between PADI6, UHRF1, NLRP14, and UBE2. (a) Electrostatic properties of a complete CPL globular repeat. (b) Electrostatic properties of the cavity formed by PADI6, UHRF1, NLRP14, tubulin, and UBE2. (c) A sphere with a diameter of approximately 8 nm could fit into the cavity.

We then estimated what could plausibly fit within the cavity, finding that it could accommodate a sphere of diameter ~8-nm (volume of ~268 nm^3 or $2.68x10^5$ $Å^3$) (Rebuttal Figure 3c). Assuming an average protein density of 1.43 g/cm^3 or ~0.861 $Da/Å^3$ (Quillin and Matthews 2000 PMID 10930825, Carbajal-Gonzales et al 2013 PMID 23281266), this would correspond to a maximum of ~230 kDa.

With this information alone, however, we cannot make confident conclusions about specific proteins/molecules that might associate with this cavity *in situ*. Whether it serves as a binding platform for other proteins that associate with CPLs less stably or perhaps transiently at different stages of oogenesis or early embryonic development will be an interesting topic for follow-up studies.

8. Previous studies showed that UHRF1 level is increased in the nucleus of Nlrp14- and Padi6-mutant oocytes and embryos (Giaccari et al., Genes Dev.; 2024; Yan et al., Nat Genet, 2023) while it is decreased in Nlrp5-knockout oocytes (Unoki et al., Hum Mol Genet, 2024). Can the authors discuss how this happens based on their findings?

Our structure shows that UHRF1 is a core CPL protein interacting with PADI6, UBE2, and NLRP14 through its SRA and UBL domains. Our structure therefore explains how a large pool of UHRF1 is stably retained in the oocyte cytoplasm (Maenohara et al 2017 PMID 28976982); why UHRF1 is essential for CPL stability (Uemura et al 2023 PMID 37225425); and why total UHRF1 levels are significantly reduced in $NLRP14^{-/-}$ and $PADI6^{-/-}$ oocytes (Giaccari et al 2024 PMID 38453481, Jentoft et al 2023 PMID 37922900, Williams et al 2025 bioRxiv).

However, we believe our structure alone is not sufficient to fully explain UHRF1 dynamics in the absence of other CPL proteins. Multiple factors regulate UHRF1 stability/mobility (e.g. Stella) and epigenetic reprogramming (e.g. Dnmt1, Dnmt3) in the early embryo, and it is possible that

knocking out different CPL components would have different effects on these pathways. We believe that resolving these questions requires following-up the studies mentioned with more quantitative methods, for example evaluating UHRF1 levels in both cytoplasmic and nuclear fractions of KO oocytes/embryos by quantitative mass spectrometry.

-Line 186: Please cite Maenohara et al., PLOS Genet. 2017. To my knowledge, this was the first paper to show that UHRF1 stored in the oocyte is essential for regulating methylation patterns in the early embryo.

We have now cited this publication.

-Extended data table 1 should come with additional cryo-EM validation metrics, map sharpening B-factor, CC (volume/mask), and the FSC threshold used to define model resolution.

We have added the requested values to Extended Data Table 1. Additionally, we provide per-chain CC values in Data S1.

-Extended Data Fig.1: Unit for box size should be specified (e.g., in pixels).

We have now specified the unit for box size (pixels) in Extended Data Figure 1.

-Readers may be curious whether the samples were chemically crosslinked prior to vitrification.

The samples were not chemically crosslinked prior to vitrification. As also discussed in our response to point 1 above, we directly applied the burst oocyte lysate to cryo-EM grids without any further treatment (i.e., no centrifugation, purification, or cross-linking). We have now more strongly emphasized this key feature of our approach in the Main Text.

Referee #2 (Remarks to the Author):

This study by Kilic and coworkers reveals the molecular structure and composition of cytoplasmic lattices (CPLs) in mammalian oocytes. CPLs are protein storage structures that are essential for early embryonic development and have been poorly understood until now. Using single particle cryo-EM at a resolution of 4.2 Angstroem, the authors find that CPLs from mouse oocytes are megadalton-scale complexes composed of at least 13 proteins, including PADI6, subcortical maternal complex (SCMC) components (NLRP5, TLE6, OOEP, KHDC3, ZBED3, NLRP4F), a-/b-tubulin dimers, and ubiquitination factors such as UHRF1, UBE2 and FBXW proteins. PADI6 acts as a structural scaffold by its assembly in pentamers of homodimers, while SCMC proteins organize CPL filaments. Tubulin is stored as capped, unpolymerized alpha/beta-dimer, providing a maternal reservoir for cytoskeletal reorganization post-fertilization. The authors speculate that the inclusion of ubiquitination enzymes suggests that CPLs also regulate protein stability and degradation timing during oocyte-to-embryo transition. This work solves the decades-long mystery of CPL composition and provides a structural framework for understanding infertility and developmental disorders linked to maternal factor disruption.

The manuscript is well written, providing some background information on oocyte biology and the potential impact of distinct CPL subunits in infertility. The explanation of the structural architecture could be a little more elaborate, for example, breaking down the filamentous assembly into its composing units, explaining symmetry aspects and the number of individual subunits. I have only some comments and suggestions to the manuscript.

Criticism
Where are the AI-based modelling approaches used within the study and structure reconstruction? While mentioning this in the abstract (line 16), I could not find it in the main text but only in the Methods.
AND
Along these lines: “We find that CPLs consist of …” (line 73). How did you find this? The paragraph starting with this phrase does not explain, if and how this was identified. For example, were mass spectrometry or modelling approaches applied? The “previous functional evidence” later this paragraph (line 81) should be substantiated by citation(s).

We apologize for failing to provide this information in the Main Text. We have now expanded the Main Text to include more details about the procedures for protein identification, which benefitted greatly from AlphaFold-based fitting approaches. We also summarize our strategy visually in a new Extended Data Figure 3.

In addition, we have now used more automated approaches and state-of-the-art machine learning-based modelling tools (e.g. CryoAtom, FoldSeek, findMySequence) to increase confidence in our assignment of candidate proteins. These strategies are described in the Main Text and in the Methods, with e-values/confidence scores provided in Data S1.

We also performed mass spectrometry on burst oocyte samples prepared identically to those used for cryo-EM. MS analysis confirmed that all identified proteins are highly abundant in the sample, further increasing confidence in our assignment (see also responses to referee #1 and referee #3).

We have added citations to studies providing functional evidence supporting our assignments. In addition, we summarize previous functional evidence in Supplementary Table 2, since this comprises many experiments across several studies.

The authors describe the five PADI6 homodimers as “ears on the sides of each globular repeat of the CPLs” (line 89) and later that “resembles a spiral staircase” (line 93). Could the authors propose a more precise or scientifically appropriate term for this ‘pot-like’ structure?

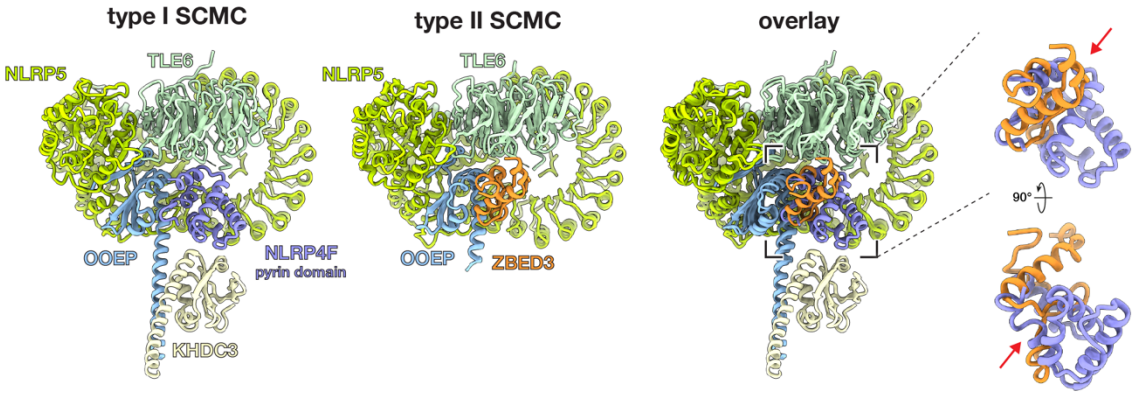
We removed the original analogy to “ears” and instead consistently refer to the PADI6 pentamer-of-homodimers as the “PADI6 ring”. We also removed the analogy to a “spiral staircase” and instead report the precise rotation and translation between neighboring PADI6 homodimers within the ring.

Same for the introduction of the SCMC structure that “bridge PADI6 rings at the top and bottom of every globular repeat, …” (line 110). This SCMC assembly is later described as “… giving the CPLs a chain-like appearance …” (line 121). Could the authors suggest a clearer or more suitable designation for this entity?

We feel that the term “bridge” is appropriate to describe how the assembly formed by type I/type II SCMC occupies the space between PADI6 rings. Likewise, we feel that the analogy to a chain is an intuitive way to describe the overall appearance of the CPL filament.

I very much like the explanation of the “discrete type I and type II SCMC subcomplexes”, as the authors refer to it (line 117f and Fig. 3b,c). In the type I subcomplex, the C-terminal LRR of NLRP4F binds to NLRP5 and OOEP mediated by ZBED3, whereas in the type II assembly, the N-terminal PYD and NACHT domains of NLRP4F bind to NLRP5 and OOEP via the KHDC3 subunit. Do the authors have an explanation for this asymmetry?

Comparing the arrangement of subunits within the type I and type II SCMC subcomplexes shows that the binding site of the NLRP4F pyrin domain clashes with the binding site of ZBED3 (see Rebuttal Figure 3 below), which could explain why ZBED3 is absent from the type I SCMC subcomplex.



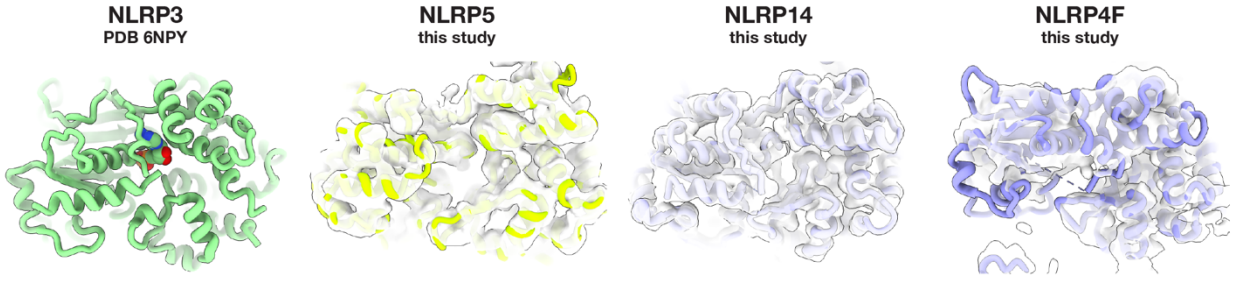
Rebuttal Figure 3. Comparison of type I and type II SCMC subcomplexes within native mouse cytoplasmic lattices. The binding site of ZBED3 would clash with the binding site of the pyrin domain of NLRP4F (red arrows in rightmost panel).

Could the authors comment on which element of the CPL chain assembly is proposed to be the weakest? Or in other words, is there a predetermined breaking point in the filament and is anything known about its disassembly?

Classical EM suggests that CPLs are present even until the 2-cell stage, although the molecular triggers or pathways of disassembly are largely unknown. Multi-body refinement performed at the request of referee #3 and presented in Extended Data Fig. 8 suggests that there is considerable flexibility at the interface between neighbouring CPL globular repeats, particularly around the NLRP4F region. However, how this flexibility ultimately relates to CPL disassembly remains to be tested in follow-up work, likewise with the mechanisms for assembly of this unique structure.

Does any of the NLR proteins NLRP4f, MLRP5 or NLRP14 contain a bound ADP or ATP nucleotide? Please comment even if the site is empty.

We inspected our maps and could not find any density indicative of bound ADP/ATP in the putative nucleotide-binding pocket of NLRP5 or NLRP14, while the density for NLRP4F is too poorly-resolved for us to make any assessments (see Rebuttal Figure 4 below). The absence of nucleotide in NLRP5 is consistent with the lack of ATP hydrolysis activity and with previous structures of reconstituted SCMC (Chi et al 2024 PMID 38177687).



Rebuttal Figure 4. Assessing possible nucleotide density for NLRP proteins in the CPL structure.

It is not immediately becoming clear why the authors mention DARPins in the consideration of the a- and b-tubulins (line 147 and Fig. 4b). Is the comparison to a-/b-tubulin--DARPin binding providing an explanation, why only one tubulin pair is found the in the CPL structure?

This panel was meant to show that the FBXW in CPLs binds to a similar site on β-tubulin as a DARPin that inhibits microtubule polymerization, suggesting that the FBXW might also have a similar effect. However, since this panel is not essential to the main message of the manuscript, we have opted to remove it in the revised version so as not to mislead the reader.

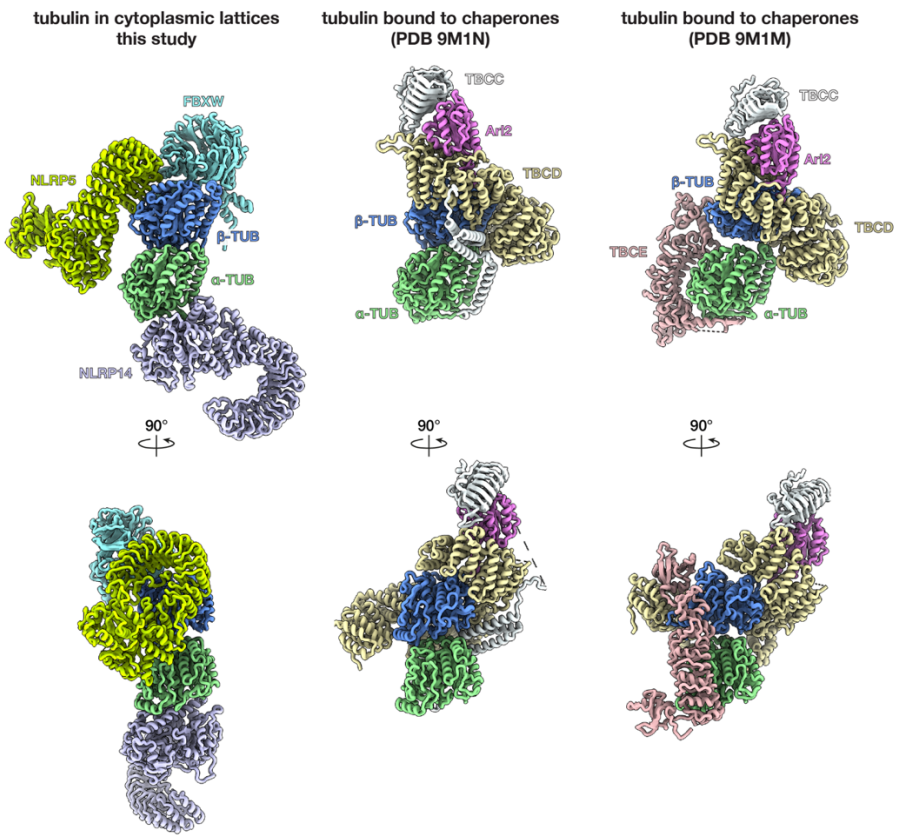
Could the authors envision a similar storage capacity for (G-)actin molecules in the CPL as found here for tubulin? At least, there are actin elongation factors as FMN1 and FMN2 present in the oocyte, that are thought to be implicated in interphase microtubule binding and asymmetric spindle positioning during meiosis.

This is an interesting question, though we did not observe any densities resembling actin or actin-binding proteins in our CPL structure. Williams et al. 2025 and Jentoft et al. 2023 performed quantitative mass spectrometry on PADI6$^{-/-}$ and TLE6$^{-/-}$ oocytes and while they report a significant decrease in some actin-binding proteins (e.g. Arp2/3 complex proteins), they did not report a significant decrease in actin or formin levels. These observations suggest a separate mechanism for actin storage in the oocytes. One possible mechanism had been proposed by Scheffler et al 2022 (PMID 35112707), where they had shown nucleus could act as a G-actin buffer in oocytes to maintain meiosis-compatible cytoplasmic F-actin organization.

Could the entity harboring the tubulin pair have any chaperone-like activity?

We compared our structure of the CPL-embedded α/β-tubulin heterodimer and its interacting partners with recently reported cryo-EM structures of tubulin bound to tubulin-specific chaperones and cofactors (PDB 9M1N and 9M1M from Seong et al 2025 PMID 41166473), see Rebuttal Figure 5 below.

This comparison shows that the binding sites of tubulin-specific chaperones differ from the binding sites of tubulin interactors within the CPLs. This suggests that if CPL components do have chaperone activity, the mechanism would differ from known chaperones. More definitive insights into this intriguing possibility will require comprehensive structural studies of CPL assembly intermediates, which we believe falls within the scope of future follow-up work.



Rebuttal Figure 5. Comparison of the α/β-tubulin heterodimer and its interacting proteins within native mouse cytoplasmic lattices with structures of tubulin bound to chaperones.

Extended Data Fig. 3: It would be good, if the authors would add how many copies of each subunit are found within one chain of the CPLs. E.g., 20x PADI6 subunits, 4x NLRP5 …

Thank you for this suggestion. We have included information about stoichiometry of each CPL protein component in the new Data S1 document, along with other basic information such as molecular weight and domain organization.

The authors could provide some additional dimensions (sizes in nm) in the text or in figure displays for some of the CPL entities explained, not only those given in Fig. 1d.

Thank you for this recommendation. We now have annotated dimensions of the CPL subcomplexes shown in Figures 2c, 3b-c, and 4a.

Line 63: “We leveraged the reported detergent-resistance of the CPLs …” Please provide a citation.

We have now added citations that report the detergent-resistance of CPLs in our revised manuscript.

Referee #3 (Remarks to the Author):

The mammalian oocyte contains specialized filaments called cytoplasmic lattices (CPLs), which are thought to serve as storage reserves of maternal factors required for early embryonic development. CPLs were originally visualized by electron microscopy almost 60 years ago, but a high-resolution structure has yet to be determined for this challenging sample. Previous studies have suggested that maternal factors important for fertility including the subcortical maternal complex (SCMC) and peptidylarginine deaminase 6 (PADI6) are CPL components, but it remains unclear how these factors are incorporated into the CPL filament architecture remains elusive. Further, the identities and organization of other CPL components are unknown due to a lack of a high-resolution CPL structure.

This paper from Kiliç et al. presents an impressive cryo-EM structure of CPL filaments natively isolated from murine oocytes. The high-resolution of the density maps, which in many cases feature side-chain level detail, allows the authors to use a combination of modeling tools to build a model of the CPL filament and identify CPL protein components. The structure reveals that PADI6 forms a unique pentamer of homodimers which dock on to he sides of the CPL repeating unit. It also shows how PADI6 interacts with the SCMC, which acts as a core structural component of CPLs. The authors also find that proteins important for embryonic development that were previously hypothesized to be stored in CPLs are indeed structural components. For example, α/β-tubulin heterodimers and ubiquitination factors like UHRF1 and UBE2 are incorporated into the filament. We believe this work represents a major advance that is appropriate in scope for publication in Nature, pending revisions to address a few concerns outlined below.

We recommend further validation of CPL component identities, as well as extending the structural analysis to probe CPL structural dynamics and higher-order architecture.

Recommendations:

1. The resolution of the CPL map allows the authors to assign the protein identity of the majority of components with high confidence. However, the authors effectively built the model by manual inspection of candidate proteins. To further support the identification of these proteins, we recommend analyzing the burst oocyte sample by mass spectrometry. This would allow the presence of all the proteins within the sample to be verified, and it may also reveal CPL component isoforms that were not discernable at the current resolution.

Thank you for this suggestion. As described in our response to reviewer #1 point 1, we analyzed the burst oocyte samples by mass spectrometry (Extended Data Fig. 5 and Supplementary Table 1). This confirmed that all subunits identified by structural analysis are indeed highly abundant in the sample. While the resolution of our maps prevents us from confidently identifying specific isoforms, we have replaced the tubulin, UBE2, and FBXW protein isoforms in the deposited model with the most abundant ones identified in our MS analysis (specifically, TUBA1A, TUBB4B, UBE2D3, and FBXW20). In addition, since we are averaging across many CPL repeats, we still

cannot exclude that different isoforms are incorporated in CPLs to different extents and thus this caveat remains in the text.

2. The authors focus on compositional variability within the individual CPL unit regarding the number of FBXW molecules present. Is there also conformational variability in the overall lattice architecture that is discernable by 3D variability analysis (3DVA)? Does 3DVA of multiple units of the lattice result in differences in the twist or curvature of the lattice? This may provide hints at pathways for filament assembly / disassembly.

We were able to resolve conformational variability between neighboring units in a CPL filament using Multi-body Refinement in RELION-5. The results presented in a new Extended Data Fig. 8. Briefly, we re-extracted and refined particles with a larger box size centered on the interface between two globular repeats (1200 pixels, binned 4x due to computational limitations). We then performed Multi-body Refinement treating each globular repeat as a separate body. Major motions involved a ~15° out-of-plane (forward/backward) bending of one globular repeat relative to its neighbour and a ~9° in-plane (side-to-side) bending between repeats.

3. In recent cryo-ET analysis of CPLs, Jentoft et al. (2023) determine a subtomogram average of five CPL filaments together that shows lower resolution density for contacts between the filaments. It would be interesting to compare the authors' CPL model with this cryo-ET data, which may provide insights into how CPL components mediate the formation of higher-order CPL filament networks.

Thank you for this suggestion. As described in our response to referee #1 point 2, fitting our models into the subtomogram average from Jentoft et al. (2023) suggests that the PADI6 ring plays a role in the higher-order assembly of CPL filaments into bundled fibers. We present the results of this analysis in a new Extended Data Fig. 6 and describe the findings in the Main Text.

Minor points:

4. When uploading the final maps and models to the PDB/EMDB, the authors should address a discrepancy in the magnification used for data collection. The magnification is reported as 81,000x in Extended Data Table 1 and 130,000x in the PDB validation report.

Thank you for pointing out this discrepancy. The correct value is 81,000x – we have fixed this in PDB 9SFP/EMD-54838 when we re-uploaded the new coordinates. We have also contacted the EMDB/PDB deposition team who have confirmed that they will amend this in associated entries EMD-54839 to EMD-54842.

5. In the Methods section for 'Model Building and refinement,' the authors note that due to resolution limitations, they were unable to identify the specific isoforms of tubulin, FBXW, and UBE2 molecules. This is an important point to note and should be included in the main text when discussing the storage of tubulin within CPLs.

We now mention in the Main Text that we cannot confidently assign specific isoforms at the current resolution, but that we have tentatively modelled tubulin, FBXW, and UBE2 models as the most abundant isoforms detected in our sample by mass spectrometry (TUBA1A, TUBB4B, FBXW20, and UBE2D3).

Point-by-point response to second round of referee comments for the manuscript:
"Cytoplasmic lattices are megadalton storage complexes in mammalian oocytes"

Referee #1 (Remarks to the Author):
The authors have appropriately addressed almost all points that I raised: I appreciate the effort that they made to perform additional experiments and analysis and prepare additional Ext. Data figures, new Suppl. Tables, and rebuttal figures. The manuscript has been improved greatly.

- Regarding major point 1 (request for mass spectrometry analysis), I apologize my misunderstanding: while I thought that isolated CPL was used for the cryo-EM analysis, it was in fact burst oocyte sample. Because of this, and also because of the request made by Reviewer #3, they have performed mass spectrometry analysis with the burst oocyte sample. Thus, proteins identified by this analysis is not necessarily abundant in CPL. However, as the authors wrote in the response letter, it helped to confirm their abundant presence in oocytes and also to identify specific isoforms (UBE2D3, etc.). I therefore appreciate the authors' effort regarding this issue.

- Regarding major point 7 (properties of the cavity), the authors have now calculated the electrostatic properties and estimated the size of the cavity (Rebuttal Fig. 3). They then state in the response letter that they cannot make confident conclusions about specific proteins/molecules that might associate with the cavity, based solely on the information. While I agree their opinion and value their careful attitude, what they found would be worth mentioning in the manuscript. I think that the authors could briefly touch upon the findings in the manuscript and provide the data as an Ext. Data figure.

Thank you for this suggestion. We included a slightly modified version of this figure as a new Extended Data Figure 9 and added a brief statement to the Discussion section about the possibility of this pocket transiently accommodating other proteins.

- Regarding major point 8 (question on the differential UHRF1 stability), I would respect the authors' cautious response.

Taken together, the revisions made by the authors substantially strengthen the manuscript. While I would like the authors to consider the above suggestion on major point 7, there is no other concern or suggestion.

Referee #2 (Remarks to the Author):
The authors present a thoroughly revised version of their original article. I have only two minor comments, which, however, should not affect the acceptance of the manuscript.

In Fig. 1d, the authors may also show the filament from the back side and rotate it another 90 degrees along the longitudinal axis. If I am not wrong, this view is otherwise only shown in ED Fig. 4c.

Thank you for this suggestion. We added the additional view to Figure 1d.

I would like to withdraw my previous comment about the author's description of a 'spiral staircase' for the assembly of the PADI6 pentamer and like to encourage them to use this illustrative description.

We have added back the references to a "spiral staircase" when describing the arrangement of the PADI6 rings.

Referee #3 (Remarks to the Author):
The authors have revised their manuscript thoroughly, addressing the reviewers' requests and comments. The mass spectrometry results of the burst oocyte sample lend further support of protein identities in the CPL structure, and the multi-body refinement of neighboring CPL filament units characterizes the flexibility of the overall filament architecture. With their revised manuscript and figures, as well as supplemental information, the authors offer a detailed and well-presented investigation of the mammalian CPL's structure and how they built their atomic model. We support the publication of this manuscript with the present revisions.