EMBO Molecular Medicine

# Glycine decarboxylase advances IgA nephropathy by boosting mesangial cell proliferation through the pyrimidine pathway

Yi Xiong, Fang Zeng, Kaiping Luo, Li Wang, Manna Li, Yanxia Chen, Tianlun Huang, Chengyun Xu, Gaosi Xu, and Honghong Zou

*Corresponding authors: Honghong Zou (ndefy21195@ncu.edu.cn) , Gaosi Xu (ndefy08027@ncu.edu.cn)*

## Review Timeline:

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| Submission Date: | 31st Mar 25 |
| Editorial Decision: | 21st May 25 |
| Revision Received: | 19th Aug 25 |
| Editorial Decision: | 2nd Sep 25 |
| Revision Received: | 14th Sep 25 |
| Accepted: | 16th Sep 25 |

*Editor: Zeljko Durdevic*

## Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

21st May 2025

Dear Dr. Zou,

Thank you for the submission of your manuscript to EMBO Molecular Medicine, and please accept my apologies for the delay in getting back to you, which is due to the fact that one referee needed more time to complete his/her review. We have now received feedback from the two reviewers who agreed to evaluate your manuscript.

As you will see from the reports pasted below, both referees recognize interest of the study but also raise important concerns that should be addressed in a major revision. After the cross-commenting discussion it became clear that referee #1 point 1 should be addressed by discussion and point 2 through stratified analyses or statistical adjustment for sex as a covariate. If you would like to discuss further the points raised by the referees, I am available to do so via email or video. Let me know if you are interested in this option.

Further consideration of a revision that addresses reviewers' concerns in full will entail a second round of review. EMBO Molecular Medicine encourages a single round of revision only and therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses included in the next, final version of the manuscript. For this reason, and to save you from any frustrations in the end, I would strongly advise against returning an incomplete revision. Further, when submitting the revised manuscript please be sure to add institutional email addresses for Honghong Zou in the manuscript and our submission system

We would welcome the submission of a revised version within three months for further consideration. Please let us know if you require longer to complete the revision.

I look forward to receiving your revised manuscript.

Yours sincerely,

Zeljko Durdevic

Zeljko Durdevic
Senior Editor
EMBO Molecular Medicine

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When submitting your revised manuscript, please carefully review the instructions that follow below. We perform an initial quality control of all revised manuscripts before re-review; failure to include requested items will delay the evaluation of your revision.

We require:

1) A .docx formatted version of the manuscript text (including legends for main figures, EV figures and tables). Please make sure that the changes are highlighted to be clearly visible.

2) Individual production quality figure files as .eps, .tif, .jpg (one file per figure). For guidance, download the 'Figure Guide PDF': (https://www.embopress.org/page/journal/17574684/authorguide#figureformat).

3) A .docx formatted letter INCLUDING the reviewers' reports and your detailed point-by-point responses to their comments. As part of the EMBO Press transparent editorial process, the point-by-point response is part of the Review Process File (RPF), which will be published alongside your paper.

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In case you have no data that requires deposition in a public database, please state so in this section. Note that the Data Availability Section is restricted to new primary data that are part of this study.

7) For data quantification: please specify the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments (specify technical or biological replicates) underlying each data point and the test used to calculate p-values in each figure legend. The figure legends should contain a basic description of n, P and the test applied. Graphs must include a description of the bars and the error bars (s.d., s.e.m.). See also 'Figure Legend' guidelines: https://www.embopress.org/page/journal/17574684/authorguide#figureformat

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10) We replaced Supplementary Information with Expanded View (EV) Figures and Tables that are collapsible/expandable online. A maximum of 5 EV Figures can be typeset. EV Figures should be cited as 'Figure EV1, Figure EV2" etc... in the text and their respective legends should be included in the main text after the legends of regular figures.

- For the figures that you do NOT wish to display as Expanded View figures, they should be bundled together with their legends in a single PDF file called *Appendix*, which should start with a short Table of Content. Appendix figures should be referred to in the main text as: "Appendix Figure S1, Appendix Figure S2" etc.

- Additional Tables/Datasets should be labeled and referred to as Table EV1, Dataset EV1, etc. Legends have to be provided in a separate tab in case of .xls files. Alternatively, the legend can be supplied as a separate text file (README) and zipped together with the Table/Dataset file.

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14) Every published paper now includes a 'Synopsis' to further enhance discoverability. Synopses are displayed on the journal webpage and are freely accessible to all readers. They include a short stand first (maximum of 300 characters, including space) as well as 2-5 one-sentences bullet points that summarizes the paper. Please write the bullet points to summarize the key NEW findings. They should be designed to be complementary to the abstract - i.e. not repeat the same text. We encourage inclusion of key acronyms and quantitative information (maximum of 30 words / bullet point). Please use the passive voice. Please attach these in a separate file or send them by email, we will incorporate them accordingly.

Please also suggest a striking image or visual abstract to illustrate your article as a PNG file 550 px wide x 300-600 px high.

15) Include a Reagents and Tools Table as part of the Methods section, which can be downloaded from our author guidelines (https://www.embopress.org/page/journal/17574684/authorguide#structuredmethods)

EMBO Molecular Medicine has a "scooping protection" policy, whereby similar findings that are published by others during review or revision are not a criterion for rejection. Should you decide to submit a revised version, I do ask that you get in touch after three months if you have not completed it, to update us on the status.

***** Reviewer's comments *****

Referee #1 (Remarks for Author):

In this study, the authors aimed to investigate whether glycine decarboxylase (GLDC) contributes to mesangial cell proliferation in IgA nephropathy (IgAN). The study was generally well designed and conducted; however, the following suggestions are offered for improvement:

1. GLDC is involved in pyrimidine metabolism and would be involved in proliferation in any cell. In other words, it would be involved in any disease that causes cell proliferation; it may not be specifically involved in the pathogenesis of IgAN.

2. Regarding the clinical information, there is a significant difference in the number of primary IgAN patients between males and females as well as across the different Lee stages.

3. Regarding the bioinformatics analysis, the authors used bulk RNA-seq, and the corresponding Lee stage information is necessary to enable comparison with the results shown in Figure 1A. Moreover, it remains unclear which specific cell types are affected by the GLDC-related pathways mentioned, given the limitations of bulk RNA-seq. In addition, both PDGFRβ- and GLDC-positive areas in Figure 1A appear to include extensive non-mesangial regions, whereas only a limited number of mesangial regions seem to be double-positive. If this interpretation is incorrect, appropriate negative controls should be provided to clarify the findings. Additionally, regarding Figure 1C, the IF images of GLDC should be accompanied by mesangial cell marker staining to more clearly demonstrate its localization and clarify whether GLDC is specifically expressed in mesangial cells.

4. There are some typographical errors in lines 78, 79, and 80 that should be corrected.

5 . Regarding Ref. 36, isn't it a beta II spectrin, not a beta III spectrin?

Referee #2 (Remarks for Author):

The authors validated through in vivo and in vitro experiments that GLDC promotes mesangial cell proliferation via the pyrimidine pathway, thereby accelerating the progression of IgA nephropathy. The study demonstrates a well-founded experimental rationale, comprehensive design, and clear result figures with notable scientific innovation. However, several limitations should be addressed: 1) Inaccuracies were identified in the description of certain methodological details; 2) The result figures lack sufficient explanatory annotations to ensure self-explanatory clarity; 3) The Western Blot analysis requires submission of original raw data images from at least three independent experimental replicates. These revisions are essential to strengthen methodological transparency and data reproducibility, therefore, the manuscript is recommended for acceptance pending adequate revision of the aforementioned issues.
Suggestions：
1. The abstract does not adequately introduce the research objective, methodology, key findings, or significance of the study, making it difficult to intuitively grasp the article's content. It is recommended to revise and expand the abstract to address these elements.
2. In the Methods section (2.7 Cell Culture and Treatment), the primer sequences should be compiled into a table for clarity and

ease of reference.
3. All figure panels lack descriptive legends and annotations at the bottom, compromising the self-explanatory clarity of the results. Detailed captions and labels should be supplemented to enhance interpretability.
4. A quantitative analysis of all Western Blot images is strongly advised. Additionally, raw data images from at least three independent experimental replicates must be provided to ensure reproducibility and statistical validity.
5. In the Discussion section, incorporating a mechanistic diagram to summarize the experimental validation of the study would enhance the article's visual clarity and scientific completeness.

Dear Editor:

Thank you very much for your suggestion and advice. We also would like to thank the reviewers for their valuable comments. We have thoroughly considered the comments and substantially revised the manuscript. We hope this version is much improved. A point-to-point response to the comments is given below.

Referee #1 (Remarks for Author):

1. GLDC is involved in pyrimidine metabolism and would be involved in proliferation in any cell. In other words, it would be involved in any disease that causes cell proliferation; it may not be specifically involved in the pathogenesis of IgAN.

Response: The fundamental pathology of IgAN is the proliferation of glomerular mesangial cells[1]. These proliferating mesangial cells release a series of pro-inflammatory and fibrogenic cytokines that mediate oxidative stress and complement activation, such as C3, CFH, FN1, and TGF-beta1[2-4]. This process is accompanied by matrix expansion, which leads to podocyte and proximal tubular epithelial cell injury, thereby promoting the progression of IgAN[5]. Additionally, glomerular mesangial cells can recruit macrophage infiltration through the secretion of inflammatory molecules such as MCP-1, thus affecting the formation of glomerular crescents[6, 7]. We have also found that the proliferation of glomerular mesangial cells leads to increased release of factors such as MCP-1, IL-6, and C3 (Figure S2C). Therefore, the abnormal proliferation of glomerular mesangial cells is a key pathological change occurring in the glomeruli of IgAN and is worthy of further investigation.

Both bulk RNA-seq analysis of glomeruli and immunohistochemical staining of clinical samples reveal a pathological increase in GLDC in glomeruli, particularly in glomerular mesangial cells. Moreover, clinical data analysis has uncovered a

correlation between GLDC and clinical pathology. This suggests that GLDC may be a potential therapeutic target for IgAN.

Although GLDC is generally involved in cell proliferation, it remains unknown whether GLDC participates in the abnormal proliferation of glomerular mesangial cells in IgAN. Furthermore, the mechanisms by which GLDC affects this abnormal proliferation in IgAN are also unclear. Only through experimental evidence that elucidates the regulatory mechanisms of glomerular mesangial cell proliferation can we recognize the potential importance of GLDC as a therapeutic target for IgAN. For the first time, we have discovered that under pathological conditions of IgAN, glomerular mesangial cells undergo metabolic reprogramming, with pyrimidine metabolism being one of the key altered metabolic pathways. GLDC, as a glycine metabolic enzyme, happens to be a key factor in this regulatory pathway. Under pIgA stimulation, glomerular mesangial cells enhance pyrimidine metabolism by upregulating GLDC expression, thereby affecting the proliferative capacity of glomerular mesangial cells.

As for other regulatory pathways of GLDC and the reasons for its increase under pathological conditions, we are continuing to explore these aspects. To translate our findings into clinical applications, it is essential to fully elucidate the molecular mechanisms of GLDC in disease development and its therapeutic targeting potential to ensure its potential efficacy in clinical treatment.

2. Regarding the clinical information, there is a significant difference in the number of primary IgAN patients between males and females as well as across the different Lee stages.

Response: We further collected 30 additional IgAN samples, and the final statistics are presented in revised Figure 1B. Among these samples, there were 24 male and 25 female cases. The Lee grading classification included 13 cases of Type II, 13 cases of Type III, 12 cases of Type IV, and 11 cases of Type V, in order to achieve as balanced

a distribution of sample types as possible. The result showed that the expression of GLDC was significantly correlated with Lee's grade, segmental glomerulosclerosis (S) and tubular atrophy/interstitial fibrosis (T).

3. Regarding the bioinformatics analysis, the authors used bulk RNA-seq, and the corresponding Lee stage information is necessary to enable comparison with the results shown in Figure 1A. Moreover, it remains unclear which specific cell types are affected by the GLDC-related pathways mentioned, given the limitations of bulk RNA-seq. In addition, both PDGFRβ- and GLDC-positive areas in Figure 1A appear to include extensive non-mesangial regions, whereas only a limited number of mesangial regions seem to be double-positive. If this interpretation is incorrect, appropriate negative controls should be provided to clarify the findings. Additionally, regarding Figure 1C, the IF images of GLDC should be accompanied by mesangial cell marker staining to more clearly demonstrate its localization and clarify whether GLDC is specifically expressed in mesangial cells.

Response: Previously, the PDGFRβ antibody we used lacked specificity in clinical human samples. Therefore, we replaced it with PDGFRβ and GLDC antibodies from another reagent supplier for co-staining of human clinical samples and analyzed the results (see revised Figure 1A). To clarify the co-localization of GLDC in glomeruli, we performed co-staining with CD31 (an endothelial cell marker), podocin (a podocyte marker), and claudin-1 (a parietal epithelial cell marker and a component of crescents) (see revised Figure S1C). We found that GLDC partially co-localizes with podocin, but not significantly with CD31 or claudin-1. To further compare the co-localization coefficients of GLDC with podocin and PDGFRβ, we used ImageJ for co-localization analysis. The co-localization coefficient with PDGFRβ was significantly higher than that with podocin (see revised Figure 1A), indicating that GLDC is primarily localized in glomerular mesangial cells. Additionally, we supplemented the co-localization detection of PDGFRβ and GLDC in glomerular mesangial cells of IgAN mice in revised Figure S1E. In normal mice, the expression

of GLDC in glomeruli is very weak, while in the disease mouse model, the expression is increased and co-localization sites with PDGFRβ are observed (revised Figure S1E & 6B).

4. There are some typographical errors in lines 78, 79, and 80 that should be corrected.

Response: We have corrected the errors.

5．Regarding Ref. 36, isn't it a beta II spectrin, not a beta III spectrin?

Response: It shouled be anti-βII-spectrin. We have corrected the error.

Referee #2 (Remarks for Author):

1. The abstract does not adequately introduce the research objective, methodology, key findings, or significance of the study, making it difficult to intuitively grasp the article's content. It is recommended to revise and expand the abstract to address these elements.

Response: Thank you for the comments. We have revised the Abstract section.

2. In the Methods section (2.7 Cell Culture and Treatment), the primer sequences should be compiled into a table for clarity and ease of reference.

Response: Thank you for the comments. We have compiled the sequences into tables. Please refer to the revised Table 1 and 2.

3. All figure panels lack descriptive legends and annotations at the bottom,

compromising the self-explanatory clarity of the results. Detailed captions and labels should be supplemented to enhance interpretability.

Response: Thank you for the comments. We have revised figure legends and provided more details.

4. A quantitative analysis of all Western Blot images is strongly advised. Additionally, raw data images from at least three independent experimental replicates must be provided to ensure reproducibility and statistical validity.

Response: Thank you for the comments. We have provided the quantitative analysis of all Western Blot images and raw data images.

5. In the Discussion section, incorporating a mechanistic diagram to summarize the experimental validation of the study would enhance the article's visual clarity and scientific completeness.

Response: Thank you for the comments. We have provided a mechanistic diagram in the discussion section.

References


1. Roberts IS: Pathology of IgA nephropathy. *Nat Rev Nephrol* 2014, 10(8):445-454.
2. Schmitt R, Ståhl AL, Olin AI, Kristoffersson AC, Rebetz J, Novak J, Lindahl G, Karpman D: The combined role of galactose-deficient IgA1 and streptococcal IgA-binding M Protein in inducing IL-6 and C3 secretion from human mesangial cells: implications for IgA nephropathy. *J Immunol* 2014, 193(1):317-326.
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Nyström J: Mesangial cells from patients with IgA nephropathy have increased susceptibility to galactose-deficient IgA1. *BMC Nephrol* 2016, 17:40.

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7. Lai PC, Chiu LY, Srivastava P, Trento C, Dazzi F, Petretto E, Cook HT, Behmoaras J: Unique regulatory properties of mesangial cells are genetically determined in the rat. *PLoS One* 2014, 9(10):e111452.

2nd Sep 2025

Dear Dr. Zou,

Thank you for the submission of your revised manuscript to EMBO Molecular Medicine. I am pleased to inform you that we will be able to accept your manuscript pending the following final amendments:

1) Authors: Please provide institutional email address for both corresponding authors.
2) Figures:
- The table in Figure 1B should be presented as an actual table. So, please remove it from the figure and add it to the main manuscript file as a table with a short title. All main tables should be placed in the manuscript file. Please update the callout in the main text.
- All supplementary figures should be renamed to "Figure EV1" etc. and uploaded as individual, high-resolution figure files. The legends should be added to the manuscript text, after the tables, under the heading "Expanded View Figure Legends". Please update their callouts in the main text.
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- Please upload source data checklist.
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o Figure legends:
1. Please note that the legend for figure 2 is not provided in a sequential manner. This needs to be rectified.
2. Please note that the exact p values are not provided in the legends of figures 1A, 2A-J; 3B, C; 4A-J; 5A-H; 6A, C; 7B-D.
3. Please indicate the statistical test used for data analysis in the legends of figures 2A-J; 3B, C; 4A-J; 5A-H; 6A, C.
4. Please note that the box plots need to be defined in terms of minima, maxima, centre, bounds of box and whiskers, and percentile in the legends of figures 1A, 7C, D.
5. Please note that information related to n is missing in the legends of figures 4A-H.
6. Please note that the error bars are not defined in the legends of figures 1A, 2A-J; 3B, C; 4A-J; 5A-H; 6C, 7B.
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- In Methods, provide the statement that informed consent was obtained from all human subjects and confirm 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.
- In Methods, it seems that in "Clinical information" the information about the patients added during revision is missing. Please update this paragraph.
- In Methods, provide the antibody dilutions that were used for each antibody.
- Indicate in legends exact n and exact p values, not a range, along with the statistical test used. To keep the figures "clear" some authors found providing an Appendix table Sx with all exact p-values preferable. You are welcome to do this if you want to.
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- Synopsis image: Please provide a visual abstract as a high-resolution jpeg file 550 px-wide x 300-600 pixels high to illustrate your article.
- Synopsis text: Please provide a short standfirst (maximum of 300 characters, including space) as well as 2-5 one sentence bullet points that summarise the paper as a .doc file. Please write the bullet points to summarise the key NEW findings. They should be designed to be complementary to the abstract - i.e. not repeat the same text. We encourage inclusion of key acronyms and quantitative information (maximum of 30 words / bullet point). Please use the passive voice.
- Please check your synopsis text and image before submission with your revised manuscript. Please be aware that in the proof stage minor corrections only are allowed (e.g., typos).
9) As part of the EMBO Publications transparent editorial process initiative (see our Editorial at http://embomolmed.embopress.org/content/2/9/329), EMBO Molecular Medicine will publish online a Review Process File (RPF) to accompany accepted manuscripts. This file will be published in conjunction with your paper and will include the anonymous referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript. Let us know whether you agree with the publication of the RPF and as here, if you want to remove or not any figures from it prior to publication. Please note that the Authors checklist will be published at the end of the RPF.
10) Please provide a point-by-point letter INCLUDING my comments as well as the reviewer's reports and your detailed responses (as Word file).

I look forward to reading a new revised version of your manuscript as soon as possible.

Yours sincerely,

Zeljko Durdevic

Zeljko Durdevic
Senior Editor
EMBO Molecular Medicine

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In the event of acceptance, this file will be published in conjunction with your paper and will include the anonymous referee reports, your point-by-point response and all pertinent correspondence relating to the manuscript. If you do NOT want this file to be published, please inform the editorial office at contact@embomolmed.org.

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1) a .docx formatted version of the manuscript text (including Figure legends and tables)

2) Separate figure files*

3) supplemental information as Expanded View and/or Appendix. Please carefully check the authors guidelines for formatting Expanded view and Appendix figures and tables at https://www.embopress.org/page/journal/17574684/authorguide#expandedview

4) a letter INCLUDING the reviewer's reports and your detailed responses to their comments (as Word file).

5) The paper explained: EMBO Molecular Medicine articles are accompanied by a summary of the articles to emphasize the

major findings in the paper and their medical implications for the non-specialist reader. Please provide a draft summary of your article highlighting
- the medical issue you are addressing,
- the results obtained and
- their clinical impact.
This may be edited to ensure that readers understand the significance and context of the research.
Please refer to any of our published articles for an example.

6) Author contributions: the contribution of every author must be detailed in a separate section.

7) EMBO Molecular Medicine now requires a complete author checklist (https://www.embopress.org/page/journal/17574684/authorguide) to be submitted with all revised manuscripts. Please use the checklist as guideline for the sort of information we need WITHIN the manuscript. The checklist should only be filled with page numbers were the information can be found. This is particularly important for animal reporting, antibody dilutions (missing) and exact values and n that should be indicted instead of a range.

8) Every published paper now includes a 'Synopsis' to further enhance discoverability. Synopses are displayed on the journal webpage and are freely accessible to all readers. They include a short stand first (maximum of 300 characters, including space) as well as 2-5 one sentence bullet points that summarise the paper. Please write the bullet points to summarise the key NEW findings. They should be designed to be complementary to the abstract - i.e. not repeat the same text. We encourage inclusion of key acronyms and quantitative information (maximum of 30 words / bullet point). Please use the passive voice. Please attach these in a separate file or send them by email, we will incorporate them accordingly.

You are also welcome to suggest a striking image or visual abstract to illustrate your article. If you do please provide a jpeg file 550 px-wide x 300-600px high.

9) A Conflict of Interest statement should be provided in the main text

10) Please note that we now mandate that all corresponding authors list an ORCID digital identifier. This takes <90 seconds to complete. We encourage all authors to supply an ORCID identifier, which will be linked to their name for unambiguous name identification.

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11) Include a Reagents and Tools Table as part of the Methods section, which can be downloaded from our author guidelines (https://www.embopress.org/page/journal/17574684/authorguide#structuredmethods)

*Additional important information regarding Figures

Each figure should be given in a separate file and should have the following resolution:
Graphs 800-1,200 DPI
Photos 400-800 DPI
Colour (only CMYK) 300-400 DPI"

Figures are not edited by the production team. All lettering should be the same size and style; figure panels should be indicated by capital letters (A, B, C etc). Gridlines are not allowed except for log plots. Figures should be numbered in the order of their appearance in the text with Arabic numerals. Each Figure must have a separate legend and a caption is needed for each panel.

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***** Reviewer's comments *****

Referee #2 (Remarks for Author):

I would like to commend the authors for their comprehensive and thoughtful responses to the reviewers' comments. They have addressed the concerns raised regarding the involvement of GLDC in IgA nephropathy (IgAN) by providing detailed explanations and additional data, which enhance the clarity and rigor of their findings. The inclusion of new clinical samples and the revised statistical analyses significantly strengthen their argument for GLDC as a potential therapeutic target.

Furthermore, the authors have improved the manuscript by revising the abstract for clarity, compiling primer sequences into tables, and enhancing figure legends, thereby improving the overall readability and interpretability of the figures. The incorporation of quantitative analyses and the provision of raw data further bolster the credibility of their results.

The addition of a mechanistic diagram in the discussion section effectively summarizes their findings and elucidates the proposed pathways involved in GLDC's role in mesangial cell proliferation.

Overall, the authors have successfully addressed the reviewers' critiques, and the revisions made to the manuscript render it suitable for publication. I believe this study will make a valuable contribution to the understanding of IgAN pathology and potential therapeutic interventions.

The authors addressed the remaining editorial issues.

16th Sep 2025

Dear Dr. Zou,

We are pleased to inform you that your manuscript is accepted for publication and is now being sent to our publisher to be included in the next available issue of EMBO Molecular Medicine.

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Yours sincerely,
Zeljko Durdevic

Zeljko Durdevic
Senior Editor
EMBO Molecular Medicine

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## EMBO Press Author Checklist

| | |
|---|---|
| Corresponding Author Name: Honghong Zou | |
| Journal Submitted to: EMBO Molecular Medicine | |
| Manuscript Number: EMM-2025-21727 | |

**USEFUL LINKS FOR COMPLETING THIS FORM**
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**Reporting Checklist for Life Science Articles (updated January**
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). Please follow the journal's guidelines in preparing your
**Please note that a copy of this checklist will be published alongside your article.**

**Abridged guidelines for figures**
**1. Data**
The data shown in figures should satisfy the following conditions:
- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- 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

**2. Captions**
Each figure caption should contain the following information, for each panel where they are relevant:
- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements.
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- 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.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:
  - common tests, such as t-test (please specify whether paired vs. unpaired), simple $\chi^2$ tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

**Please complete ALL of the questions below.**
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**Materials**

| 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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| For **antibodies** provide the following information:<br>- Commercial antibodies: RRID (if possible) or supplier name, catalogue number and or/clone number<br>- Non-commercial: RRID or citation | Yes | Materials and Methods |

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| Describe **inclusion/exclusion criteria** if samples or animals were excluded from the analysis. Were the criteria pre-established?<br><br>If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification. | Yes | Materials and Methods |
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| In the figure legends: state number of times the experiment was **replicated** in laboratory. | Yes | Figure legends |
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| 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**. | Yes | Materials and Method |