nature portfolio

Peer Review File

Chemerin attenuates acute kidney injury by inhibiting ferroptosis via the AMPK/NRF2/SLC7A11 axis

Corresponding Author: Professor Xinzhong Huang

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

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
In the study “Chemerin attenuates acute kidney injury by inhibiting ferroptosis via the AMPK/NRF2/SLC7a11 axis”, Ma and colleagues identified the regulatory role of chemerin in AKI. They found the expression of chemerin was decreased in AKI, while overexpression of chemerin could alleviate AKI both in vivo and in vitro. They investigated the mechanism that chemerin could activate AMPK to induce the phosphorylation of NRF2, stimulating the expression of SLC7a11 and increase the biosynthesis of GSH to prevent the renal injury from ferroptosis. Although they have comprehensively explored the effect of chemerin on AKI, the molecular function of chemerin on ferroptosis regulation lacks novelty and data in this manuscript are of low quality and integrity.
There are major concerns:
1. The relationship of chemerin and ferroptosis should be inferred. Why do they choose SLC7a11 as the main target? This part seems to lack logic and continuity.
2. In the part of mechanism exploration, the authors directly proposed the target genes and pathways, and did not use experimental data or references to make inferences, nor designed exclusive experiments, so the conclusions have a certain orientation.
3. AKI can be caused by a variety of causes, so it is recommended to use more than 2 animal models in experimental investigations.
4. To investigated the knock down effect of chemerin, 2 or more siRNAs should be designed to avoid the off-target effects of siRNAs.
5. E-cadherin is not commonly used as a tubular marker, while its expression is influenced under tubular injury. To investigate the expression of chemerin and CMKLR1, I would suggest to use classic markers of renal tubular cell. For instance, LTL could be used as a marker of proximal tubular cell under normal condition.
6. The quality of the fluorescence image should be improved, and the key parts should be enlarged and emphasized.
7. The time line in Fig.2A is incorrect.
8. The writing of this article should be improved, and some grammar mistakes need to be corrected.

Reviewer #2

(Remarks to the Author)
The authors found that chemerin plays a beneficial role in AKI by repressing ferroptosis in renal tubular cells, which is likely due to activation in the axis of AMPK/NRF2/SLC7a11. Overall, the study is well designed and performed. Some minor concerns should be addressed.

(1) The authors only used one acute kidney injury model to study the renal protective effect of chemerin. How about other acute kidney injury models? For example, models such as LPS, cisplatin, and folic acid.
(2) The method detecting ferroptosis should be improved, the author should use a technique to assay cell death.
(3) The authors should supplement the protein expression levels of p-AMPK/AMPK and p-NRF2/NRF2 in kidney tissues of AKI mice treated with chemerin.
(4) How is the correlation between the level of chemerin and renal biochemical indicators in AKI patients?
(5) It is recommended to compare the fluorescence intensity of CMKLR1 and chemerin with the corresponding fluorescence intensity of renal tubular epithelial cell markers.

Reviewer #3

(Remarks to the Author)
Chemerin, a multifunctional adipokine, is biosynthesized as a 163-amino-acid prepro-chemerin (1-163) and is secreted as 143-amino-acid prochemerin (21-163) upon cleavage of an N-terminal signal peptide. This pro-chemerin requires extracellular C-terminal processing to produces active chemerin species, ChemS157 and ChemF156. Chemerin-9, a peptide consisting of the ultimate nine amino acids, retains most of the activity of the full-size protein to activate CMKLR1. In this study, the authors found that the serum chemerin levels in AKI patients were reduced, application of chemerin-9 improved renal function in I/R mice model. They further identified that chemerin activated AMPK/NRF2/SLC7a11 axis to inhibit ferroptosis.
However, the authors did not demonstrate the clear mechanisms in this present study. We donnot think this paper is suitable for publication in this journal.
There are some major concerns:
1. In the I/R mice model, chemerin-9 was applied, whereas chemerin was used to treat TCMK-1 cells. Why? I wonder whether chemerin-9 can work in TCMK-1 cells? Furthermore, stimulating mouse cells with human-derived chemerin appears to be inappropriate.
2. The tubular injury should be determined by PAS staining not by HE.
3. In figure 2, the ferroptotic marker is time dependent increased after IRI?
4. Why you use H_2O_2 to mimic IRI in vitro?
5. A mistake should be addressed, Page 12, line 1, “PeproTech, AF-100-21C” is refer to as Animal-Free Recombinant Human TGF-β1 (CHO derived).
6. For fig.3, n=3 is not acceptable; at least 6 mice/group are required.
7. Regarding ferroptosis, the cell death assay such as PI staining, cell viability assay (ATP) are required.
8. Lipid-ROS is a key indicator of ferroptosis, and this data is missing.
9. To detect IL-6 and TNF-α, the ELISA assay is more reliable. What is the relationship between IL-6 and TNF-α and ferroptosis.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
In the revised manuscript “Chemerin attenuates acute kidney injury by inhibiting ferroptosis via the AMPK/NRF2/SLC7A11 axis”, the authors have addressed my questions.

Reviewer #2

(Remarks to the Author)
The authors addressed all my concerns, I have no further comment, thank you.

Reviewer #3

(Remarks to the Author)

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

In the study “Chemerin attenuates acute kidney injury by inhibiting ferroptosis via the AMPK/NRF2/SLC7a11 axis”, Ma and colleagues identified the regulatory role of chemerin in AKI. They found the expression of chemerin was decreased in AKI, while overexpression of chemerin could alleviate AKI both in vivo and in vitro. They investigated the mechanism that chemerin could activate AMPK to induce the phosphorylation of NRF2, stimulating the expression of SLC7a11 and increase the biosynthesis of GSH to prevent the renal injury from ferroptosis. Although they have comprehensively explored the effect of chemerin on AKI, the molecular function of chemerin on ferroptosis regulation lacks novelty and data in this manuscript are of low quality and integrity.

There are major concerns:

1. The relationship of chemerin and ferroptosis should be inferred. Why do they choose SLC7a11 as the main target? This part seems to lack logic and continuity.

Thank the referee for these constructive comments. During the revision, we conducted RNA-Seq analysis using kidney samples from C9 treated I/R model mice. Unfortunately, due to limited availability of samples from Sham group, we analyzed only the I/R model mice and C9-treated I/R model mice. Our RNA-Seq results revealed 3103 differentially expressed genes in the kidneys of C9-treated mice. KEGG and GO analysis indicated that these genes are involved in various biological processes, including glutathione metabolic process, unsaturated fatty acid biosynthetic process, response to iron ion, and ferroptosis. Notably, *Slc7a11* was enriched in both the glutathione metabolic process (GO:006749) and ferroptosis (mmu04216) clusters. These findings strongly suggest that ferroptosis plays a role in the protective effects of chemerin in AKI, with *Slc7a11* likely being a key molecule. These results have been included in the revised manuscript as Figure 2D-G.

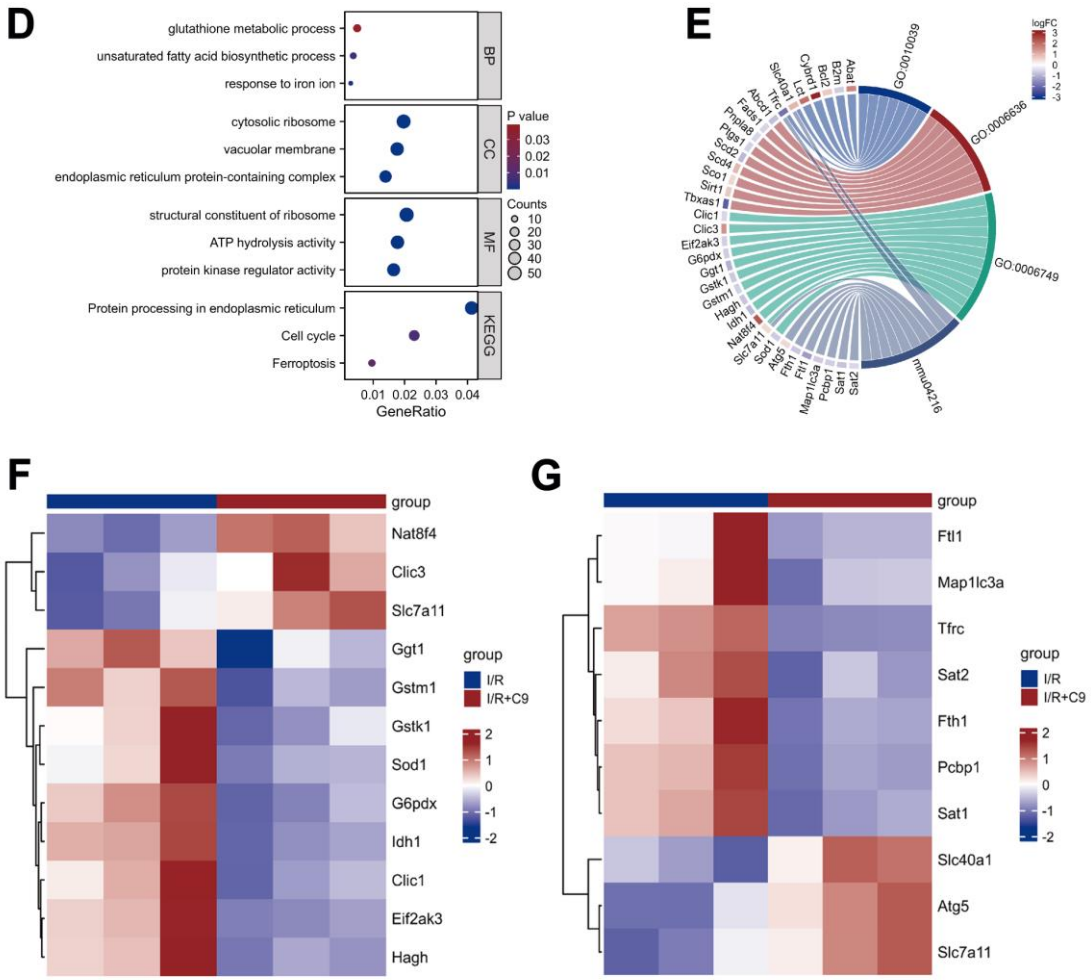


Figure 2D-G. RNA-Seq analysis showing that ferroptosis is involved in chemerin-mediated renal protective effects in I/R mice, and that *Slc7a11* is likely a key molecule.

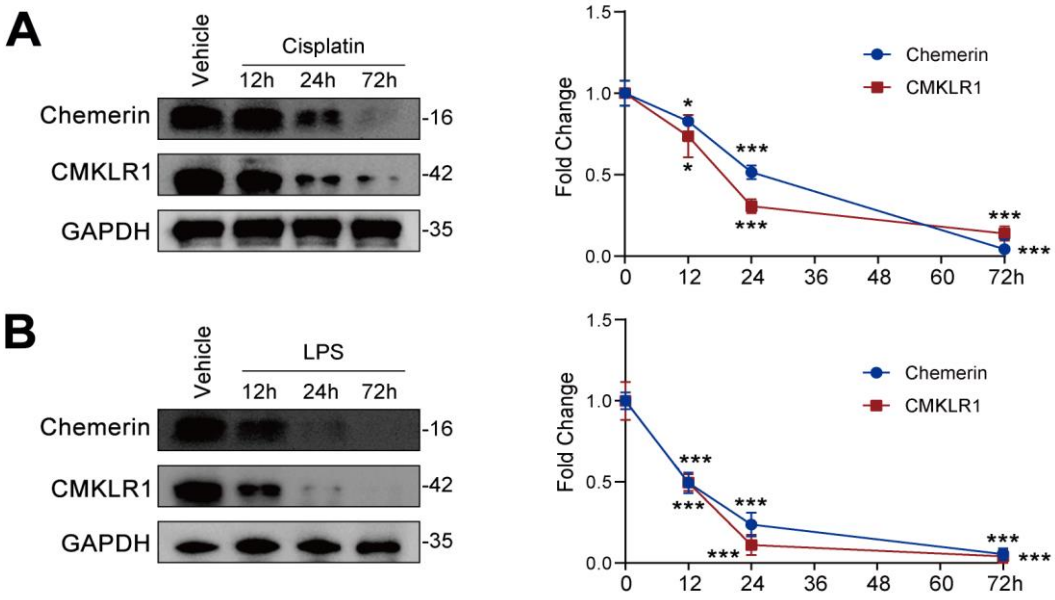
2. In the part of mechanism exploration, the authors directly proposed the target genes and pathways, and did not use experimental data or references to make inferences, nor designed exclusive experiments, so the conclusions have a certain orientation.

Thank the referee for this critical comment. As mentioned above, we performed RNA-Seq to explore the underlying mechanism for the chemerin-induced protective effects in AKI. Bioinformatics analysis showed that the differentially expressed genes are enriched in ferroptosis related pathways, including glutathione metabolic process, unsaturated fatty acid biosynthetic process, response to iron ion, and ferroptosis. Notably, *Slc7a11* was up-regulated in both glutathione metabolic process and ferroptosis clusters. These newly developed data provided supportive evidence for our proposed mechanisms in the present study, and these data were presented as Figure 2D-G in the revised manuscript.

3. AKI can be caused by a variety of causes, so it is recommended to use more than 2 animal models in experimental investigations.

Thank the referee for this constructive comment. During the revision, we utilized two additional AKI animal models induced by cisplatin or LPS in mice. Kidney samples were

collected at 12, 24, and 72 h post-injection with cisplatin or LPS and subsequently subjected to western blot analysis. Our data showed that the expression levels of chemerin and CMKLR1 were decreased following treatment with cisplatin or LPS, which is consistent with the findings observed in the I/R model mice. These data further support the notion that chemerin is involved in the proper function of the kidney. In the revised manuscript, these results were presented as supplementary Figure 1A, B.



Supplementary Figure 1A-B. Expression of chemerin and CMKLR1 in the kidneys of AKI model mice induced by cisplatin or LPS.

4. To investigated the knock down effect of chemerin, 2 or more siRNAs should be designed to avoid the off-target effects of siRNAs.

Thank the referee for this constructive comment. For chemerin knockdown, we initially selected one siRNA (si-*Chem*-1) based on its knockdown efficiency for subsequent functional studies. As suggested, we now present the knockdown efficiencies of all three siRNAs tested and have included another siRNA (si-*Chem*-3) for functional studies, in addition to si-*Chem*-1. Our data showed that ATP production was further reduced by si-Chem-3 in erastin-treated cells, and this reduction could be partially reversed by the application of recombinant chemerin. In the lipid peroxidation assay, oxidized lipids were exacerbated by si-*Chem*-3, but chemerin application partially reversed this effect. These changes in ATP production and lipid peroxidation are consistent with the results observed for si-*Chem*-1. The newly developed data were presented in Figure 4A, B, and Supplementary Figure 4A-C in the revised manuscript.

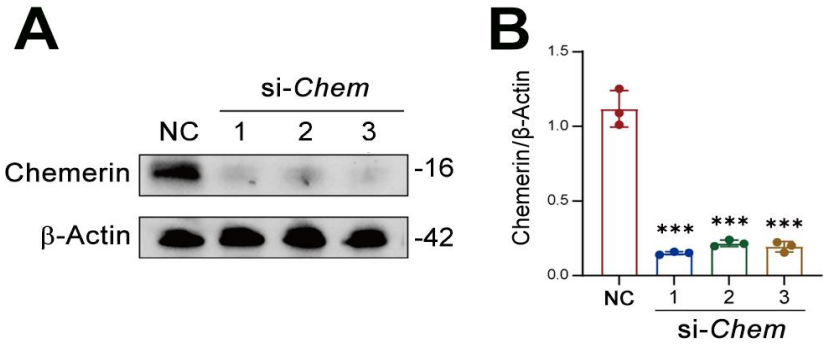
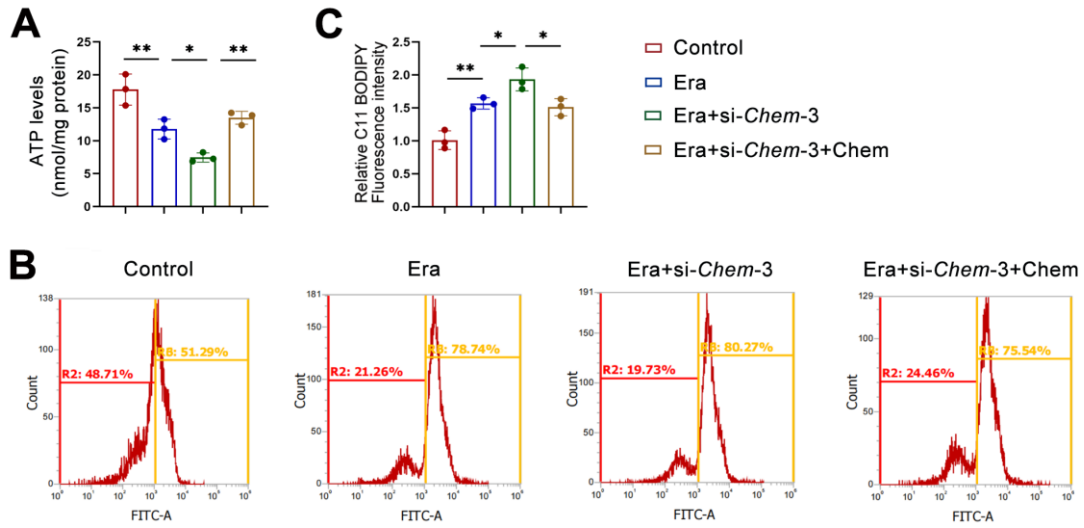


Figure 4A, B. Chemerin knockdown.



Supplementary Figure 4A-C. Knockdown of chemerin by si-*Chem*-3 aggravates ATP reduction and lipid peroxidation.

Additionally, in the *Slc7a11* knockdown experiments, we used another siRNA targeting *Slc7a11* (si-*SLC*-2) for functional studies. Our data showed that, similar to si-*SLC*-1, knockdown of SLC7a11 with si-*SLC*-2 abolished the chemerin-induced increase in ATP production. Moreover, the reduced lipid peroxidation observed in chemerin-treated cells was also reversed by si-*SLC*-2. These findings were presented in Figure 6A, B, and Supplementary Figure 7A-C in the revised manuscript.

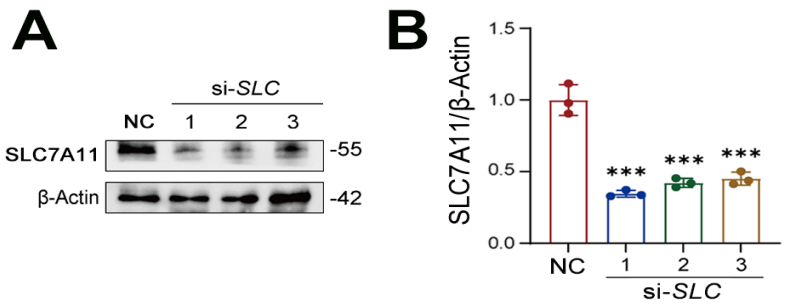
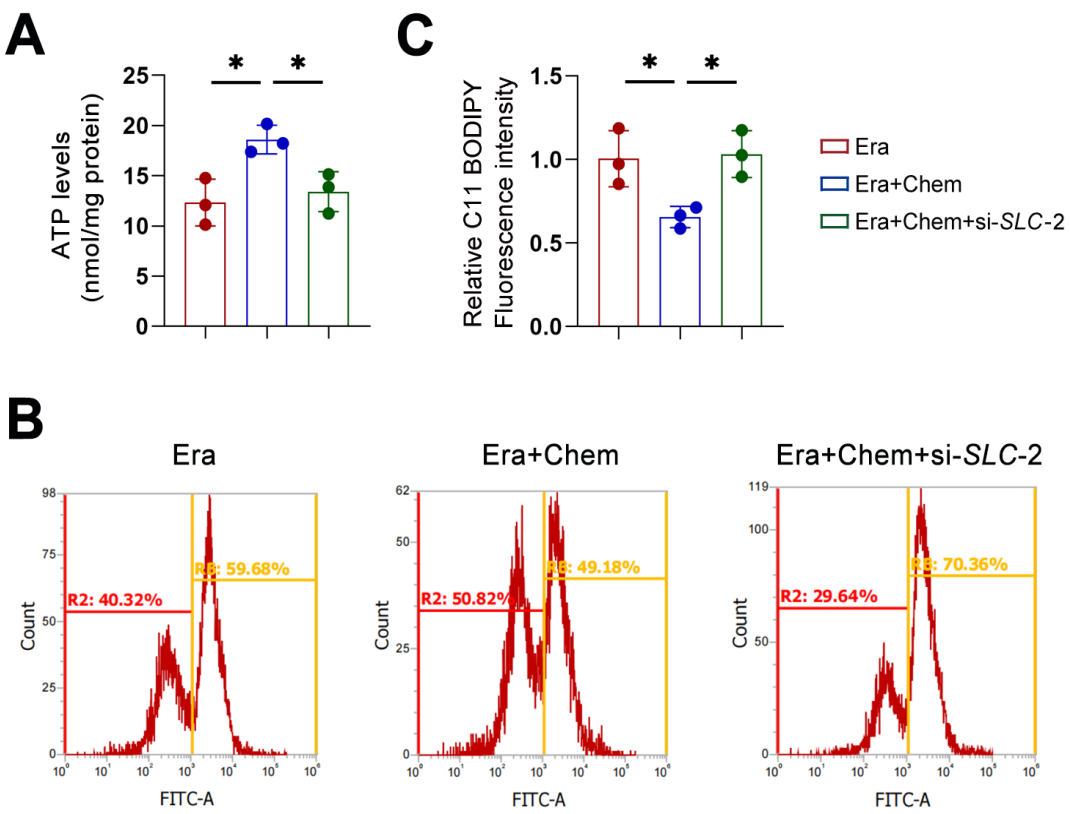


Figure 6A-B. SLC7A11 knockdown.



Supplementary Figure 7A-C. Knockdown of SLC7A11 abolished chemerin-induced changes in ATP production and lipid peroxidation.

5. E-cadherin is not commonly used as a tubular marker, while its expression is influenced under tubular injury. To investigate the expression of chemerin and CMKLR1, I would suggest to use classic markers of renal tubular cell. For instance, LTL could be used as a marker of proximal tubular cell under normal condition.

We agree with the referee's observation that E-cadherin is not commonly used as a tubular marker. LTL is a classic marker for renal tubular cells, as suggested by the reviewer. However, LTL is not available through our local distributors. As an alternative, we used AQP1 and CK19 as markers for renal tubular cells [Ref. 1; Ref. 2] and re-performed the immunofluorescence experiments. Our results showed that chemerin co-localized with AQP1 and CK19 in the kidney, and its expression was reduced in the I/R model mice. These newly developed data were presented in Figure 1D-F of the revised manuscript.

References:

1. Khundmiri SJ, Chen L, Lederer ED, Yang CR, Knepper MA. Transcriptomes of Major Proximal Tubule Cell Culture Models. J Am Soc Nephrol. 2021;32(1):86-97.

2. Nightingale J, Patel S, Suzuki N, et al. Oncostatin M, a cytokine released by activated mononuclear cells, induces epithelial cell-myofibroblast transdifferentiation via Jak/Stat pathway activation. J Am Soc Nephrol. 2004;15(1):21-32.

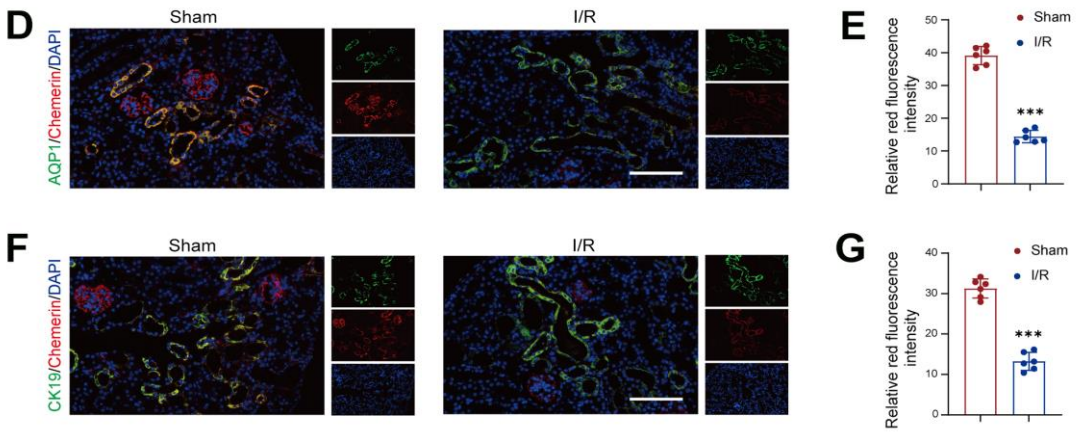


Figure 1D-F. Expression of chemerin, AQP1, and CK19 in the kidneys of sham and I/R mice.

6. The quality of the fluorescence image should be improved, and the key parts should be enlarged and emphasized.

Thank the referee for this critical comment. During the revision, we re-arranged the fluorescence images with enlarged images, which were presented in supplementary Figure 3A, C, E, and G, supplementary Figure 5A, C, E, and G, supplementary Figure 6A, C.

7. The time line in Fig.2A is incorrect.

Thank the referee for this correction. In the revised manuscript, we corrected the second 24 h into 0 h in Figure 2A.

8. The writing of this article should be improved, and some grammar mistakes need to be corrected.

During the revision, we polished the language using the Wiley Editing Services.

Letter from the editor

Wiley Editing Services

Message from your editor, editor's first name

Dear Author,

It was a pleasure working on your document. Do go through my changes and comments in the edited file, as well as the notes in this document.

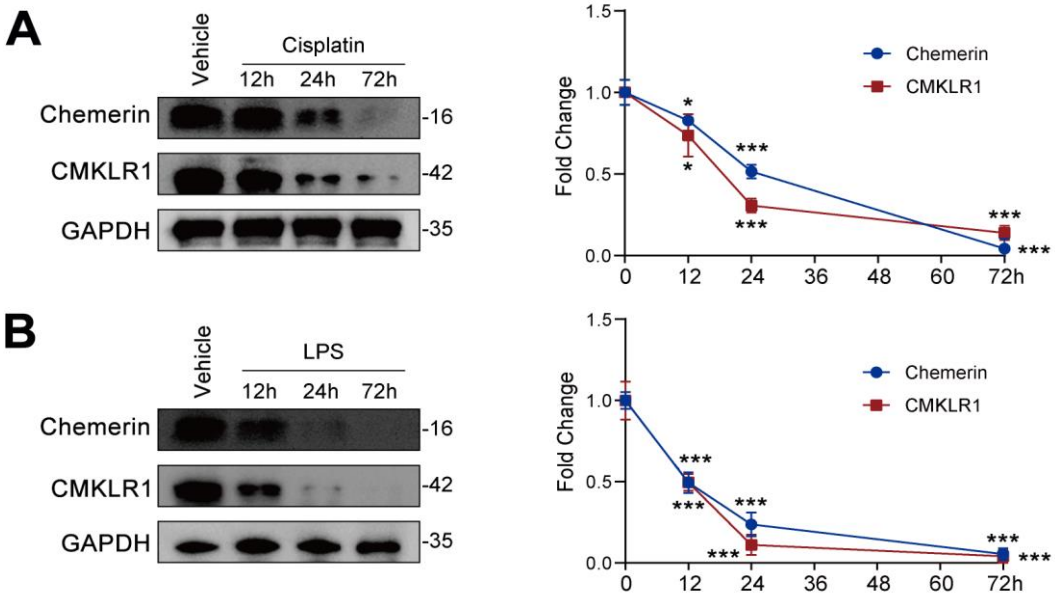
Please send me your feedback or any questions through your account (cn.wileyeditingservices.com).

Reviewer #2 (Remarks to the Author):

The authors found that chemerin plays a beneficial role in AKI by repressing ferroptosis in renal tubular cells, which is likely due to activation in the axis of AMPK/NRF2/SLC7a11. Overall, the study is well designed and performed. Some minor concerns should be addressed.

(1) The authors only used one acute kidney injury model to study the renal protective effect of chemerin. How about other acute kidney injury models? For example, models such as LPS, cisplatin, and folic acid.

Thank the referee for this constructive comment. During the revision, we used cisplatin and LPS to generate AKI mouse models. Like I/R model mice, cisplatin- or LPS-induced AKI mice displayed reduced expression of chemerin and CMKLR1 in the kidney. These data further confirmed our notion that chemerin is involved in the AKI process, which were presented in supplementary Figure 1A, B.



Supplementary Figure 1A-B. Expression of chemerin and CMKLR1 in the kidneys of AKI model mice induced by cisplatin or LPS.

(2) The method detecting ferroptosis should be improved, the author should use a technique to assay cell death.

Thank the referee for this constructive comment. According to the suggestion, we performed a series of experiments for detecting ferroptosis, including ATP production, lipid peroxidation, and calcein/PI staining. These newly developed data were presented in Figure 4G-K, Figure 6G-K, supplementary Figure 4A-C, supplementary Figure 7A-C.

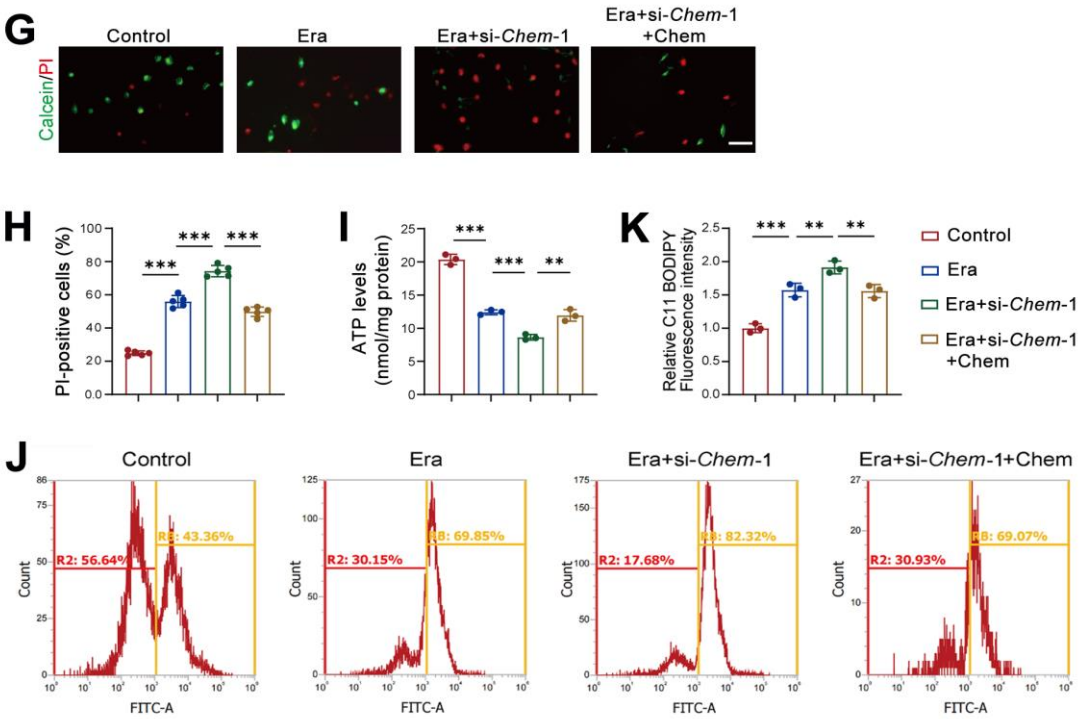


Figure 4G-K. Assays of cell death, APT production, and lipid peroxidation.

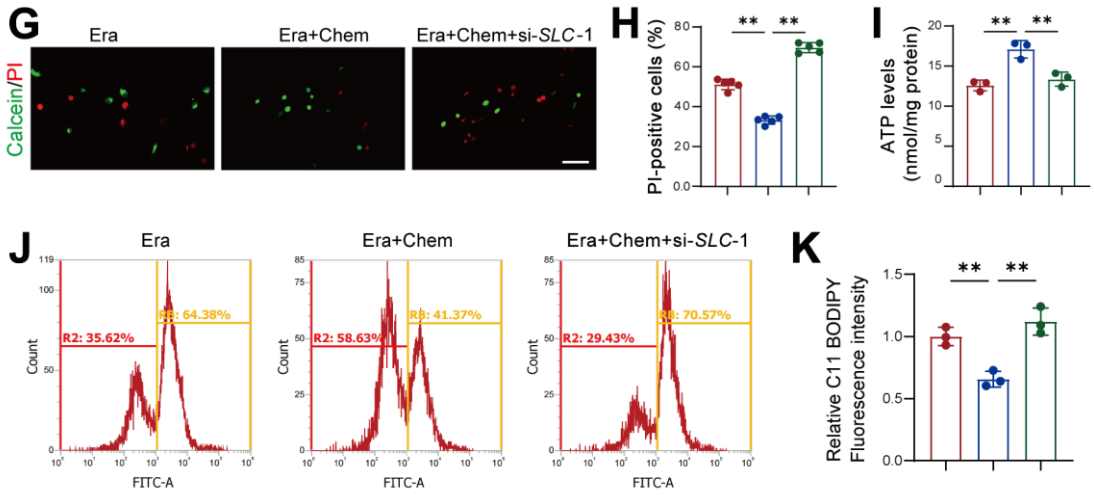
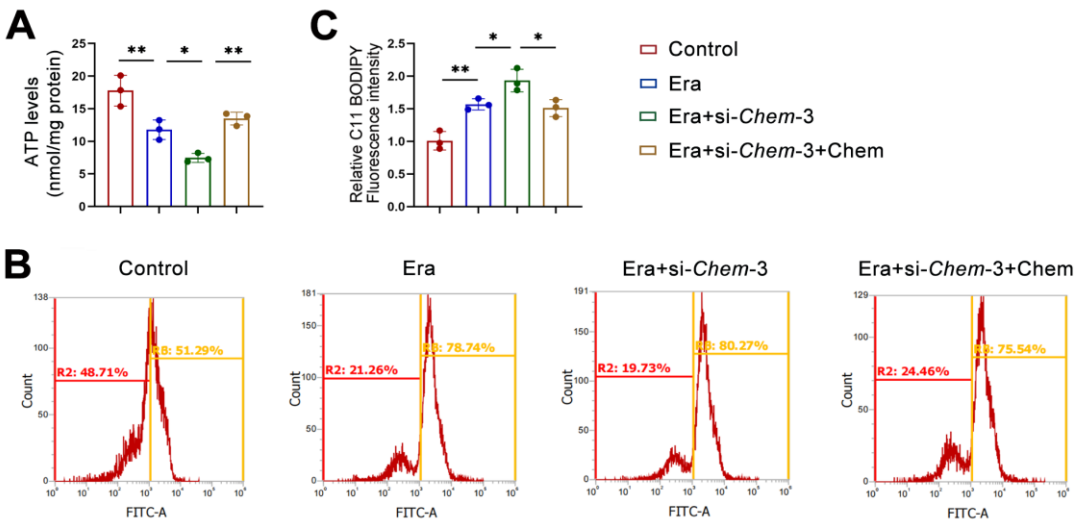
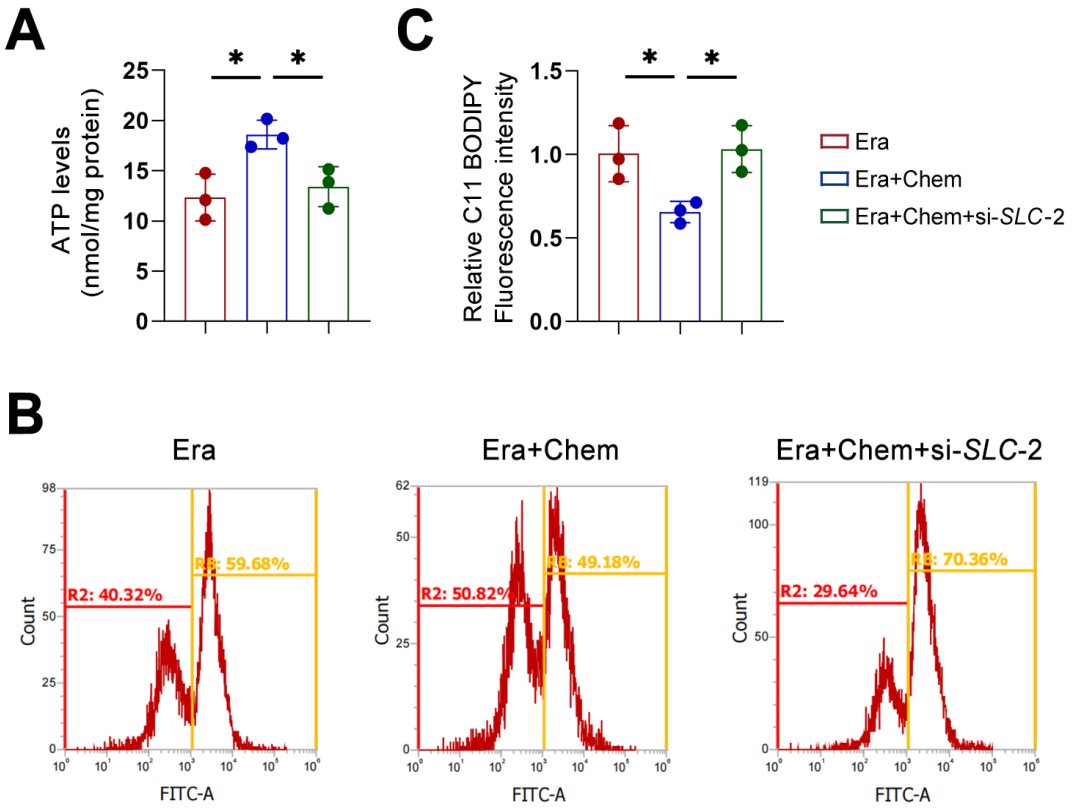


Figure 6G-K. Assays of cell death, APT production, and lipid peroxidation.



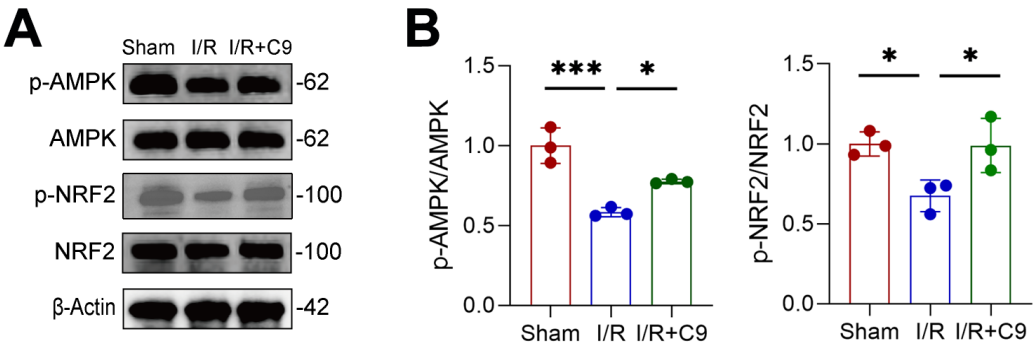
Supplementary Figure 4A-C. Assays of ATP production and lipid peroxidation.



Supplementary Figure 7A-C. Assays of ATP production and lipid peroxidation.

(3) The authors should supplement the protein expression levels of p-AMPK/AMPK and p-NRF2/NRF2 in kidney tissues of AKI mice treated with chemerin.

Thank the referee for this critical comment. According to the suggestion, we detected these proteins in the kidney. The results showed that p-AMPK and p-NRF2 decreased in I/R model mice, whereas C9 treatment reversed these declines. These data further indicate that chemerin targets the AMPK/NRF2 axis to play its nephroprotective role in AKI, which have been included in the revised manuscript as supplementary Figure 8.



Supplementary Figure 8. C9 treatment activates the AMPK/NRF2 axis in the kidneys of I/R mice.

(4) How is the correlation between the level of chemerin and renal biochemical indicators in AKI patients?

Thank the referee for this constructive and insightful comment. During the revision, we collected serum BUN and creatinine values, together with serum chemerin levels, to make two correlation analysis between chemerin and BUN or creatinine. Our data showed that serum chemerin levels are reversely correlated with serum BUN or creatinine levels. These data were presented in Figure 1I-L.

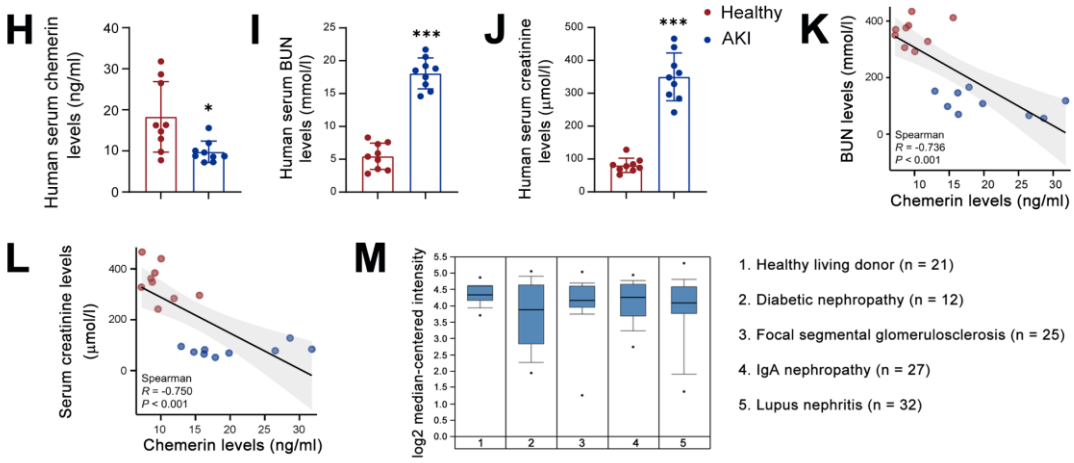
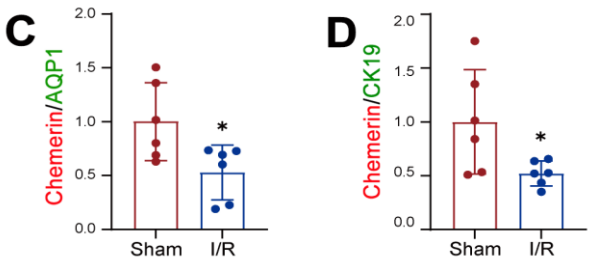


Figure 1I-L. BUN and serum creatinine levels reversely correlate with serum chemerin in humans.

(5) It is recommended to compare the fluorescence intensity of CMKLR1 and chemerin with the corresponding fluorescence intensity of renal tubular epithelial cell markers.

We thank the reviewer for this constructive comment. During the revision, we re-analyzed chemerin expression in the kidneys of I/R mice using additional renal tubular cell markers, including AQP1 and CK19. The immunofluorescence results demonstrated that chemerin expression was reduced in the kidneys of I/R mice. Notably, chemerin was co-localized with AQP1 and CK19, indicating that chemerin is primarily expressed in renal tubular cells. Following the reviewer's suggestion, we further normalized chemerin levels to AQP1 and CK19 and confirmed a decrease in chemerin expression in the kidneys of I/R mice. These data were presented in Supplementary Figure 1C, D.



Supplementary Figure 1C-D. Chemerin expression levels with normalization to AQP1 or CK19.

Reviewer #3 (Remarks to the Author):

Chemerin, a multifunctional adipokine, is biosynthesized as a 163-amino-acid prepro-chemerin (1-163) and is secreted as 143-amino-acid prochemerin (21-163) upon cleavage of an N-terminal signal peptide. This pro-chemerin requires extracellular C-terminal processing to produces active chemerin species, ChemS157 and ChemF156. Chemerin-9, a peptide consisting of the ultimate nine amino acids, retains most of the activity of the full-size protein to activate CMKLR1.

In this study, the authors found that the serum chemerin levels in AKI patients were reduced, application of chemerin-9 improved renal function in I/R mice model. They further identified that chemerin activated AMPK/NRF2/SLC7a11 axis to inhibit ferroptosis.

However, the authors did not demonstrate the clear mechanisms in this present study. We donnot think this paper is suitable for publication in this journal.

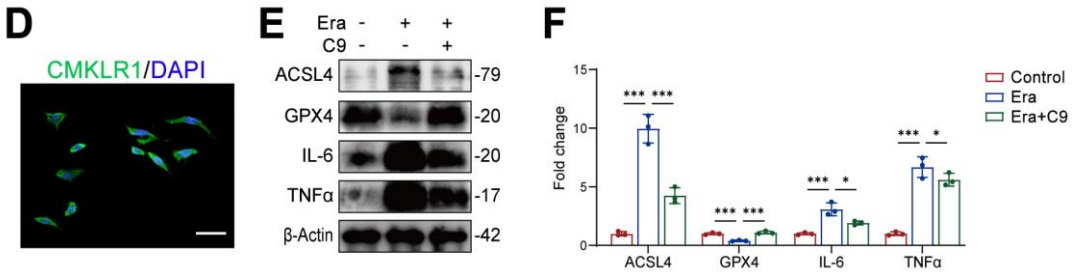
There are some major concerns:

1. In the I/R mice model, chemerin-9 was applied, whereas chemerin was used to treat TCMK-1 cells. Why? I wonder whether chemerin-9 can work in TCMK-1 cells? Furthermore, stimulating mouse cells with human-derived chemerin appears to be inappropriate.

We thank the reviewer for these critical comments. Compared to full-length chemerin, chemerin-9 (C9) is more cost-effective, especially for animal studies where larger quantities are required. As a result, we used C9 for the animal experiments, while full-length chemerin was used for the cell experiments.

Before conducting the formal studies, we confirmed that TCMK-1 cells express CMKLR1, a key receptor for chemerin. Our results indicated that TCMK-1 cells do express CMKLR1, which justified the use of recombinant chemerin in our experiments. To assess whether chemerin-9 is effective in TCMK-1 cells, we treated the cells with chemerin-9 and H_2O_2. Our data showed that both IL-6 and TNF-α levels were elevated by erastin, but chemerin-9 treatment significantly mitigated these increases. Similarly, the upregulation of ACSL4 in erastin-treated cells was abolished by chemerin-9, and the erastin-induced reduction in GPX4 was reversed by chemerin-9 treatment. Collectively, these findings clearly demonstrate that chemerin-9, like full-length chemerin, exhibits anti-ferroptotic activity in TCMK-1 cells. These results were included as Supplementary Figure 4D-F in the revised

manuscript.

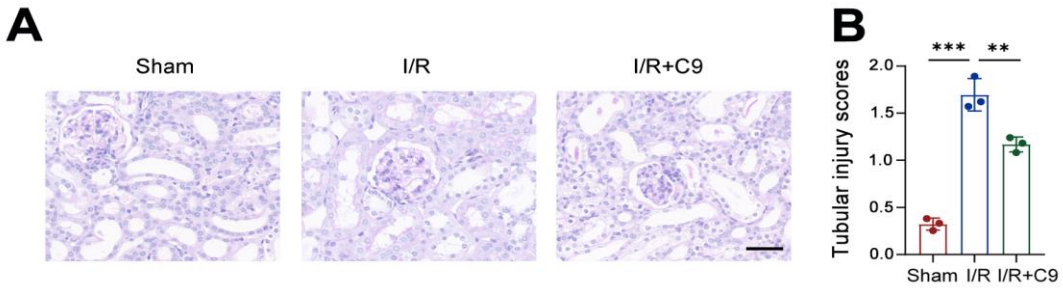


Supplementary Figure 4D-F. CMKLR1 expression in TCMK-1 cells and chemerin-9 treatment reverses the changes in ACSL4, GPX4, IL-6, and TNFα induced by erastin.

In the present study, we used the C-terminal peptide of full-length mouse chemerin (chemerin-9) both in vivo and in vitro. In our original manuscript, we incorrectly listed the catalog number of chemerin as #7116, which corresponds to the C-terminal peptide of full-length human chemerin (chemerin-9). During the revision, we realized this mistake and corrected the catalog number to #7117, which refers to the C-terminal peptide of full-length mouse chemerin. We thank the reviewer for their careful reading and attention to detail.

2. The tubular injury should be determined by PAS staining not by HE.

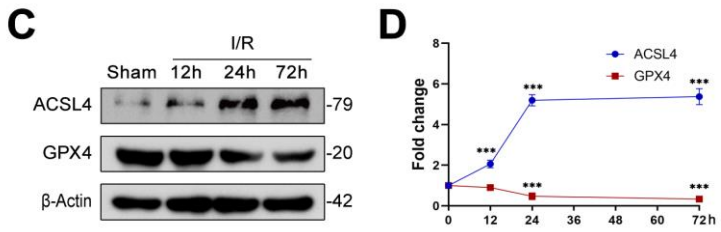
We thank the referee for this constructive comment. In response, we performed PAS staining, and the results showed that C9 treatment reduced tubular injury in I/R model mice. These data were presented in supplementary Figure 2A, B in the revised manuscript.



Supplementary Figure 2A-B. PAS staining showing that tubular injury in I/R mice was improved by C9.

3. In figure 2, the ferroptotic marker is time dependent increased after IRI?

We thank the referee for this critical comment. To address it, we analyzed ACSL4 and GPX4 expression in the kidney at 12, 24, 72 hours post-I/R. Our data showed that ACSL4 expression gradually increased over time following I/R surgery, while GPX4 expression displayed an opposite trend, decreasing after I/R surgery. These data were presented in supplementary Figure 2C, D in the revised manuscript.



Supplementary Figure 2C-D. Expression of ACSL4 and GPX4 in the kidneys of I/R mice.

4. Why you use H2O2 to mimic IRI in vitro?

I/R mouse model is considered as an AKI animal model, and oxidative stress in the kidney is a hallmark of AKI. Hydrogen peroxide (H_2O_2) is a potent inducer of oxidative stress, which was widely used in AKI cell models (Ref. 1-3). Hence, we used H_2O_2-treated cells as an *in vitro* cell model of AKI.

References:

1. Zhao ZB, Marschner JA, Iwakura T, et al. Tubular Epithelial Cell HMGB1 Promotes AKI-CKD Transition by Sensitizing Cycling Tubular Cells to Oxidative Stress: A Rationale for Targeting HMGB1 during AKI Recovery. J Am Soc Nephrol. 2023, 34(3):394-411.

2. Kormann R, Kavvadas P, Placier S, et al. Periostin Promotes Cell Proliferation and Macrophage Polarization to Drive Repair after AKI. J Am Soc Nephrol. 2020, 31(1):85-100.

3. Chen J, Matzuk MM, Zhou XJ, Lu CY. Endothelial pentraxin 3 contributes to murine ischemic acute kidney injury. Kidney Int. 2012, 82(11): 1195-1207.

5. A mistake should be addressed, Page 12, line 1, “PeproTech, AF-100-21C” is refer to as Animal-Free Recombinant Human TGF-β1 (CHO derived).

We thank the referee for this correction. TGF-β1 was used to induce fibrotic gene expression in our other studies. In the present study, mouse recombinant chemerin (HY-P75554, MedChemExpress) was used to evaluate the potential role of chemerin in ferroptosis in TCMK-1 cells. We have corrected this description in the revised manuscript.

6. For fig.3, n=3 is not acceptable; at least 6 mice/group are required.

We thank the referee for this critical comment. In most cases, an *n* of 3 is considered the minimal requirement for biomedical research.

7. Regarding ferroptosis, the cell death assay such as PI staining, cell viability assay (ATP) are required.

We thank the referee for these constructive comments. Following the suggestion, we performed PI staining and an ATP production assay during the revision. PI staining revealed that chemerin knockdown exacerbates cell death induced by erastin, while SLC7A11 knockdown abolishes the protective effect of chemerin in reducing cell death in erastin-treated cells. These results were presented in Figure 4G, H, and Figure 6G, H.

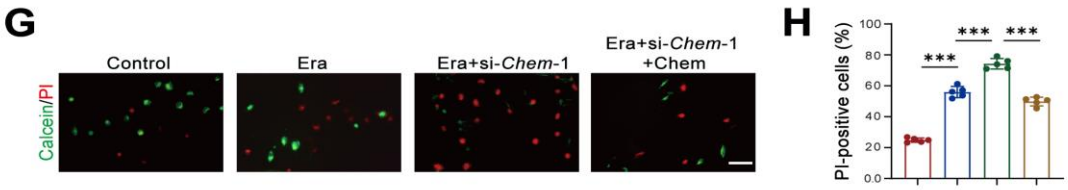


Figure 4G-H. Cell death assay.

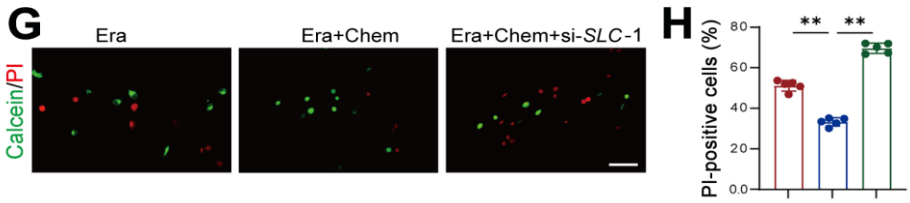


Figure 6G-H. Cell death assay.

The ATP production assay showed that chemerin knockdown further decreases ATP levels in erastin-treated cells, and SLC7A11 knockdown reduces the increased ATP levels seen in chemerin-treated cells. These data were included in Figure 4I, supplementary Figure 4A, Figure 6I, and supplementary Figure 7A in the revised manuscript.

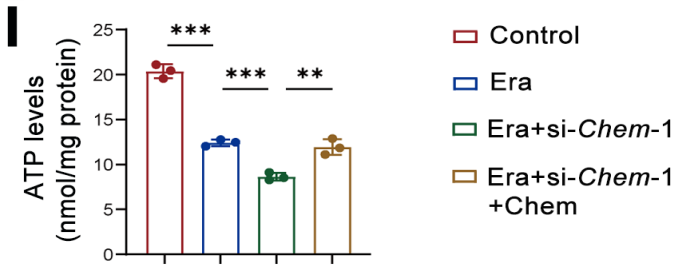
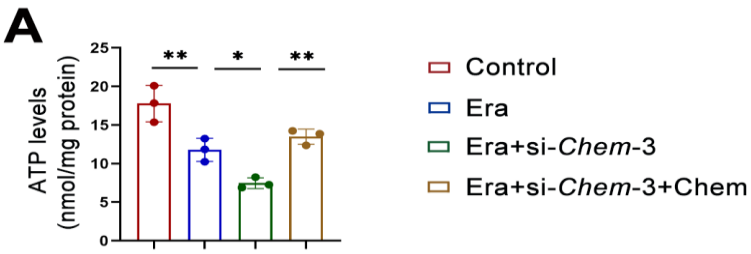


Figure 4I. ATP assay.



Supplementary Figure 4A. ATP assay.

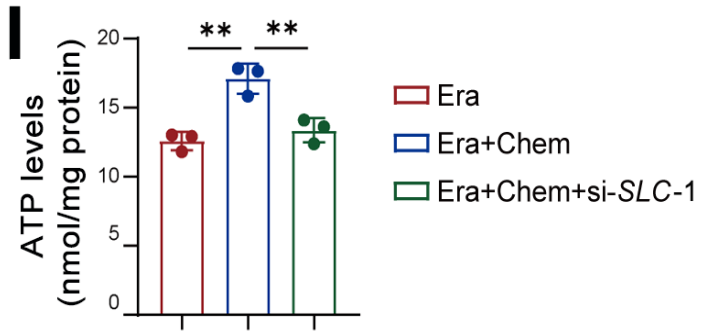
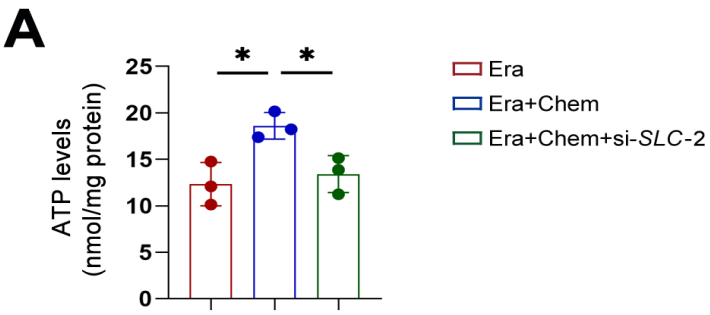


Figure 6I. ATP assay.



Supplementary Figure 7A. ATP assay.

8. Lipid-ROS is a key indicator of ferroptosis, and this data is missing.

We agree with the referee's notion that lipid-ROS is a key indicator of ferroptosis. During the revision, we conducted flow cytometry to assess lipid peroxidation. Our data showed that oxidized lipids were exacerbated by chemerin knockdown in erastin-treated cells. Additionally, SLC7A11 knockdown restored the repressed lipid peroxidation in chemerin-treated cells. These results are presented in Figure 4J, K, Supplementary Figure 4B, C, Figure 6J, K, and Supplementary Figure 7B, C in the revised manuscript.

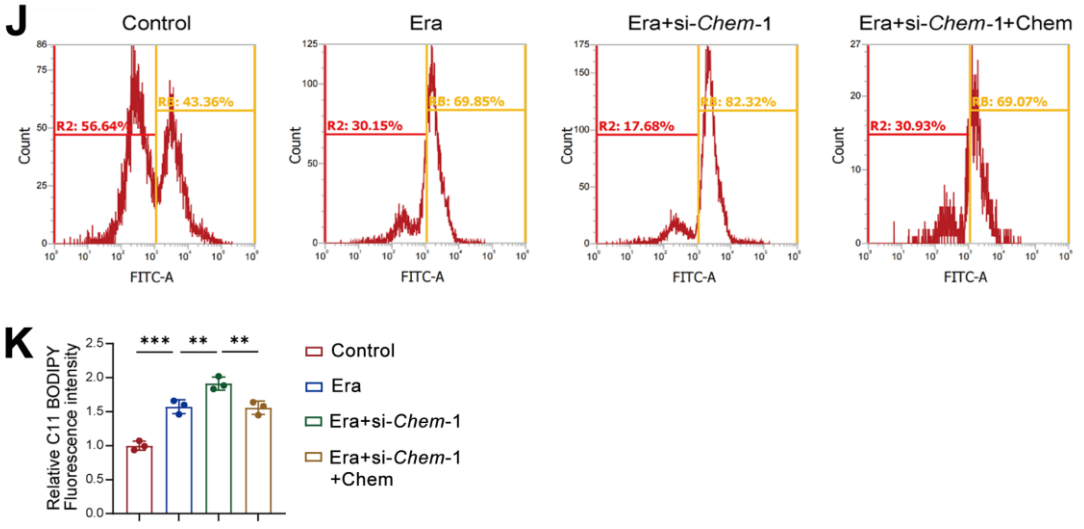
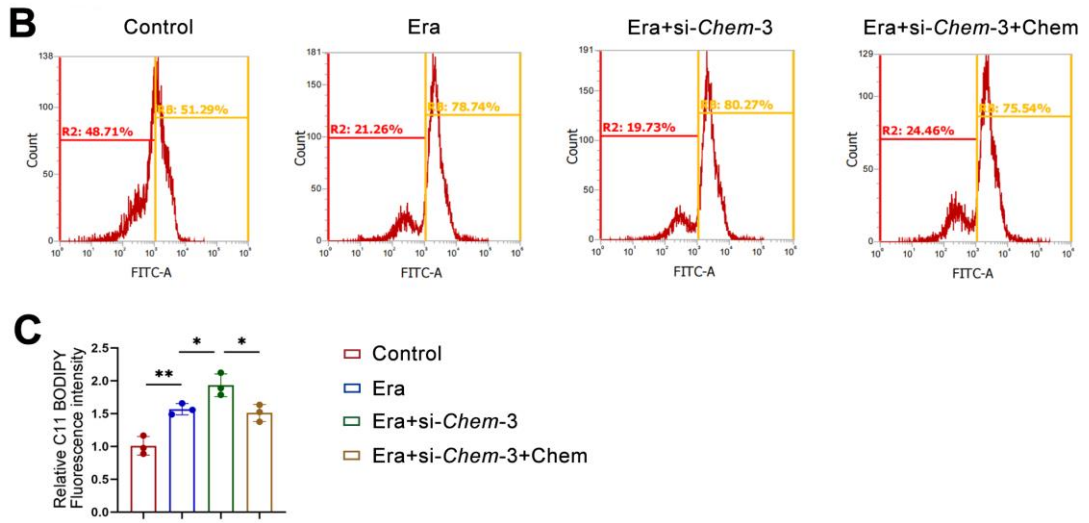


Figure 4J-K. Lipid peroxidation assay.



Supplementary Figure 4B-C. Lipid peroxidation assay.

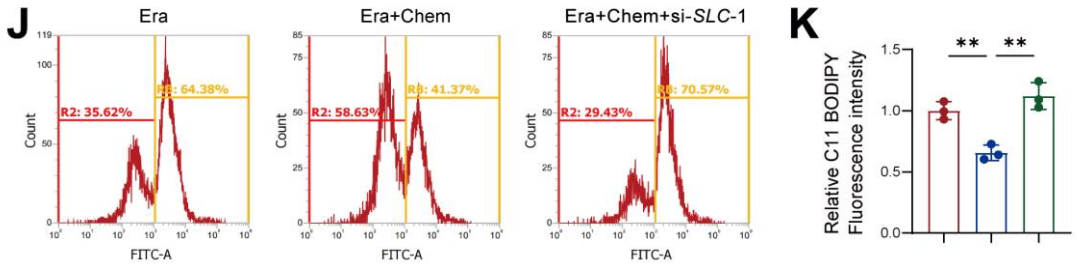
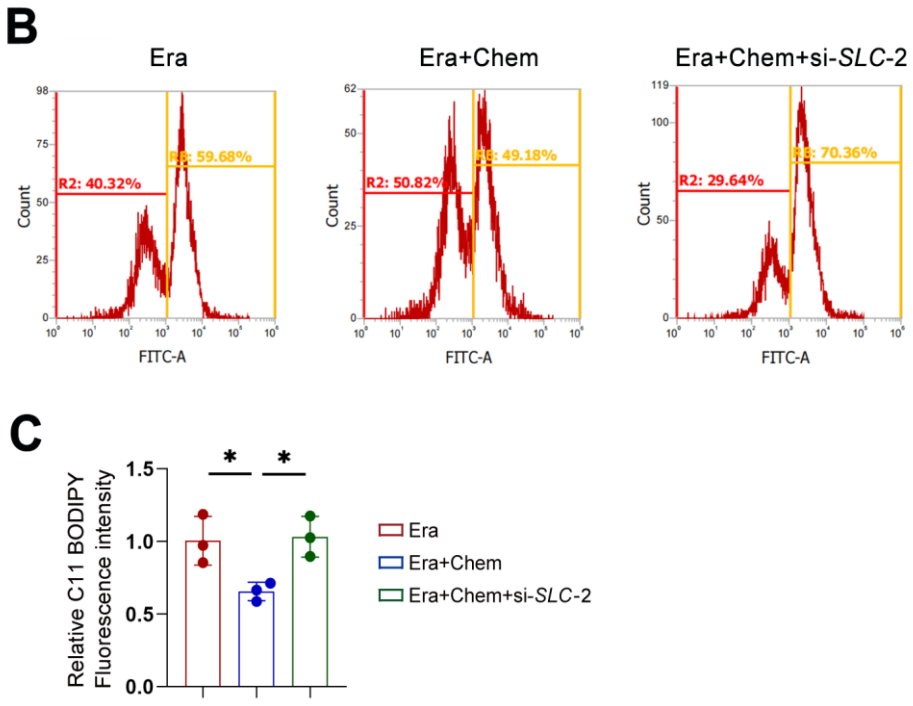


Figure 6J-K. Lipid peroxidation assay.



Supplementary Figure 7B-C. Lipid peroxidation assay.

9. To detect IL-6 and TNF-α, the ELISA assay is more reliable. What is the relationship between IL-6 and TNF-α and ferroptosis.

According to the suggestion, we measured IL-6 and TNFa using the ELISA assay. Our results showed that serum IL-6 and TNF-α increased in I/R mice, whereas C9 treatment prevented these increases. These data were presented in Figure 3C, D. In TCMK-1 cells, chemerin knockdown further increased IL-6 and TNF-α expression, and supplementation of chemerin could prevent these trends. These data were presented in Figure 5C, D.

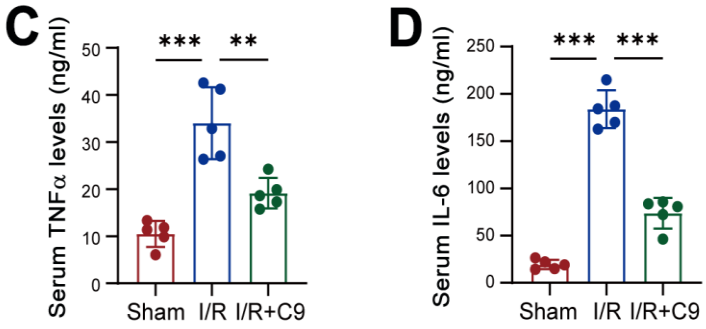


Figure 3C-D. TNFα and IL-6 assays using ELISA.

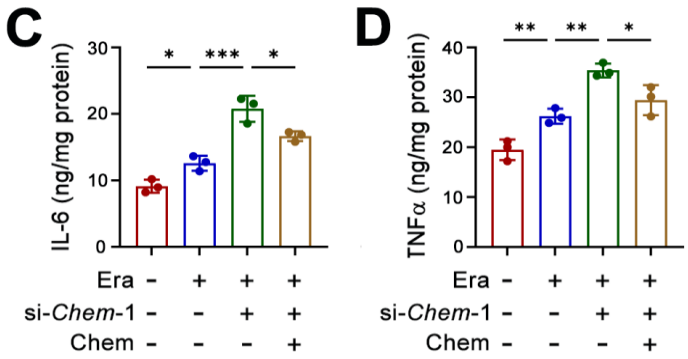


Figure 5C-D. TNFα and IL-6 assays using ELISA.

There is a growing body of evidence indicating that ferroptosis plays a significant role in inflammation, and ferroptosis inhibitors have been shown to exert anti-inflammatory effects in experimental models of various diseases. For instance, polyunsaturated fatty acids (PUFAs) and their metabolic enzymes have emerged as key regulators of crucial cellular processes in the context of inflammation [Ref. 1]. Arachidonic acid (AA), an ω-6 PUFA, is primarily found in phospholipids within cell membranes. Upon exposure to stimuli, AA is released from phospholipids by phospholipase A2 and phospholipase C as free AA, serving as a precursor for bioactive proinflammatory mediators that promote inflammatory cascades [Ref. 2; Ref. 3]. Furthermore, reactive oxygen species (ROS) generated during ferroptosis can drive the initiation and progression of inflammatory responses [Ref. 4; Ref. 5]. In light of these factors, we measured IL-6 and TNF-α in the present study.

References:

1. Colakoglu M, Tuncer S, Banerjee S. Emerging cellular functions of the lipid metabolizing

enzyme 15-Lipoxygenase-1. Cell Prolife. 2018, 51(5): e12472.

2. Sperling RI, Benincaso AI, Knoell CT, Larkin JK, Austen KF, Robinson DR. Dietary omega-3 polyunsaturated fatty acids inhibit phosphoinositide formation and chemotaxis in neutrophils. J Clin Invest 1993, 91(2): 651-660.

3. Uauy R, Mena P, Rojas C. Essential fatty acid metabolism in the micropremie. Clin Perinatol. 2000, 27(1): 71-93.

4. Lin Q, Li S, Jiang N, et al. PINK1-parkin pathway of mitophagy protects against contrast-induced acute kidney injury via decreasing mitochondrial ROS and NLRP3 inflammasome activation. Redox Biol. 2019, 26: 101254.

5. Han Y, Xu X, Tang C, et al. Reactive oxygen species promote tubular injury in diabetic nephropathy: The role of the mitochondrial ros-txnip-nlrp3 biological axis. Redox Biol. 2018, 16: 32-46.