Dear Chief Editor,

Thanks very much for your comments concerning our manuscript entitled “SPOC domain-containing protein 1 regulates the proliferation and apoptosis of human spermatogonial stem cells through adenylate kinase 4” (Manuscript NO.: 79114, Basic Study). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our research. We have studied the comments carefully and made corrections, which we hope meet with approval. Revised portions are marked in red on the paper. The major corrections in the paper and the responses to the reviewer’s comments are as follows:

Responds to the editor’s comments:

1. Response to comment: Abstract: “Overexpression of AK4 in SPOCD1 knockdown cells partially reversed the phenotypic changes, indicating that AK4 is a functional target gene of SPOCD1.” Any positive controls or naturally occurring systems (native cells)?

Response: Thank you very much for this suggestion. We tested the transfection efficiency by transfecting the internal reference (GAPDH) plasmid during the pre-experiment. We also overexpressed AK4 in the seminoma cell line Tcam-2 to test the efficiency of the plasmid. However, data from these pre-experiments were not provided in the manuscript. In our experimental results, the effect of AK4 overexpression was assayed by using an empty plasmid of pCMV as a negative control.

We hope this answer the editor’s questions.

2. Response to comment: “Knockdown of SPOCD1 in SSC caused a significant decrease in proliferation and self-renewal, and the induction of apoptosis.” “RNA-seq results showed that SPOCD1 deficiency significantly downregulated genes such as adenylate kinase 4 (AK4).” How did they quantitively differentiate “Knockdown of SPOCD1” from “SPOCD1 deficiency”? How did these quantitively relate to “overexpression of AK4 in SPOCD1-deficient cells?” How did they define the boundary of these concepts? Figure 4 panels clearly show that a downregulation was

observed, not a deficiency. Any mouse knock-out models?

Response: Thank you very much for this constructive comment. We think that "deficiency" in the manuscript is indeed inappropriate. The use of “Knockdown”, "inhibition," or "downregulation" would more accurately convey the meaning of the manuscript. We have replaced the relevant words in the manuscript and marked them with red lines.

3. Response to comment: Section Results should be integrated with a logical transition between subtitled paragraphs to explain why they moved to subsequent experiments.

Response: Thanks to the editor's suggestion, we have revised the subtitle and content of the results section to make the manuscript more logical. All revisions are marked with red lines.

Line 290：The subtitle was modified as " Validation of SPOCD1 distribution pattern in human testis "

Line 308： The subtitle was modified as "The role of SPOCD1 in the proliferation of human SSC line.”

Line 325：The subtitle was modified as "The influence of SPOCD1 in the apoptosis of human SSC line”

Line 338: The statements were corrected as “To explore the mechanisms of SPOCD1 in the proliferation and apoptosis of the SSC line”

Line 360： The subtitle was modified as " AK4 is responsible for the reduced proliferation of SSC line by SPOCD1 knockdown”

4. Response to comment: The limitation of the results should be pointed out based on immortalized human SSC lines. “By transfecting Large T antigen into G protein-coupled receptor 125 (GPR125)-positive human undifferentiated spermatogonia, immortalized human SSC lines were established[18]. Immortalized human SSCs maintained many properties of their primary cells and expressed many markers of primary SSCs including GFR , RET, and promyelocytic

leukemia zinc finger (PLZF)" (Section subtitled: Culture of immortalized human SSCs). However, if they had done genome evolution with culture, could they have found genome-scale changes (doi: 10.1186/s12935-014-0115-7)? It has been demonstrated genome-scale changes in the human cell line MCF-7 (doi: 10.7868/s0026898415020159), stem cell lines (doi: 10.1038/s41422-021-00592-9), and human pluripotent stem cells (hPSCs)(doi: 10.1016/j.stem.2019.04.001).

Response: As stated by the editors, we should point out the limitations of our study. Considering that the SSC line originates from primary human spermatogonia transfected with the Large T gene. It overcomes the difficulty of human spermatogonia proliferation in vitro, but inevitably, it may also produce some genome-scale changes. Our results were obtained from in vitro cultured SSC lines, which may differ from the actual situation in the testis.

Line 422-426: The statements "Additionally, considering that the SSC line originates from primary human spermatogonia transfected with the Large T gene. It overcomes the difficulty of human spermatogonia proliferation in vitro, but it may inevitably produce some genome-scale changes. Our results were obtained from in vitro cultured SSC lines, which may differ from the actual situation in the testis." Were added.

5. Response to comment: "Figure 6 Identification of the target genes of SPOC domain-containing protein 1" should be accompanied by an Excel spreadsheet listing all the genes and the expression levels.

Response: As suggested by the editor, we will provide the expression matrix as a Supplemental table.

6. Response to comment: How did they reconcile cancer-related SPOC domain-containing protein 1 (SPOCD1) from their proposed novel targets for treating male infertility? The SPOC domain containing 1 gene (SPOCD1; encoding p.Arg71Trp), at 1p35.2, was reproducibly associated with a reduced risk of gastric cancer (doi: 10.1053/j.gastro.2017.02.017). So was endometrial cancer progression (DOI: 10.1080/21655979.2022.2049026).

Response: As suggested by the editors, we do have a distance to go in treating male infertility by SPOCD1. Combined with the results already reported for SPOCD1 knockout mice, we think these results could provide a new theory for the etiology of

male infertility.

Line 35-36: the statements were revised as “Our study broadens the understanding of human SSC fate determination and may offer new theories on the etiology of male infertility.”

Line 53-54: the statements were revised as “These results broaden our understanding of human SSC fate determination and provide new theories on the etiology of male infertility.”

Line 473: the statements were revised as “Thus, our study provides new insights into regulating human SSCs and new theories on the etiology of male infertility.”

Special thanks to you for your good comments!

We tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of the paper. And here, we did not list the changes but marked them in red in the revised paper.

We appreciate the Editors’ warm work earnestly and hope that the correction will be approved.

Once again, thank you very much for your comments and suggestions.

Dear Editors and Reviewers:

Thank you for your letter and for the reviewers' comments concerning our manuscript entitled "SPOCD1 regulates the proliferation and apoptosis of human spermatogonial stem cells through AK4" (Manuscript NO.: 79114, Basic Study). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our research. We have studied comments carefully and made corrections, which we hope meet with approval. Revised portions are marked in red on the paper. The major corrections in the paper and the responses to the reviewer's comments are as follows:

Responds to the reviewer's comments:

Reviewer #1:

1. Response to comment: In their results, the authors found significant downregulation of SPOCD1 expression in some NOA patients. Plz explain in detail what is the reason behind it.

Response: Thank you for your suggestion. In our study, we explored the role of SPOCD1 in regulating SSC through in vitro cellular experiments. However, it is still difficult for us to study its role in vivo. By examining the expression pattern of SPOCD1 in normal and NOA samples, we hope to explore its relevance to disease. Of course, these results obviously remain to be confirmed, and we thought the descriptions in the manuscript were overstated. We have made changes to emphasize the possible correlation between them. Similarly, according to the reviewer's suggestion, we can subsequently explore the correlation between SPOCD1 and NOA in a large sample using computerized deep learning and screening for genetic mutations.

Line 367：the statement was corrected as "The abnormal expression of SPOCD1 may be associated with NOA."

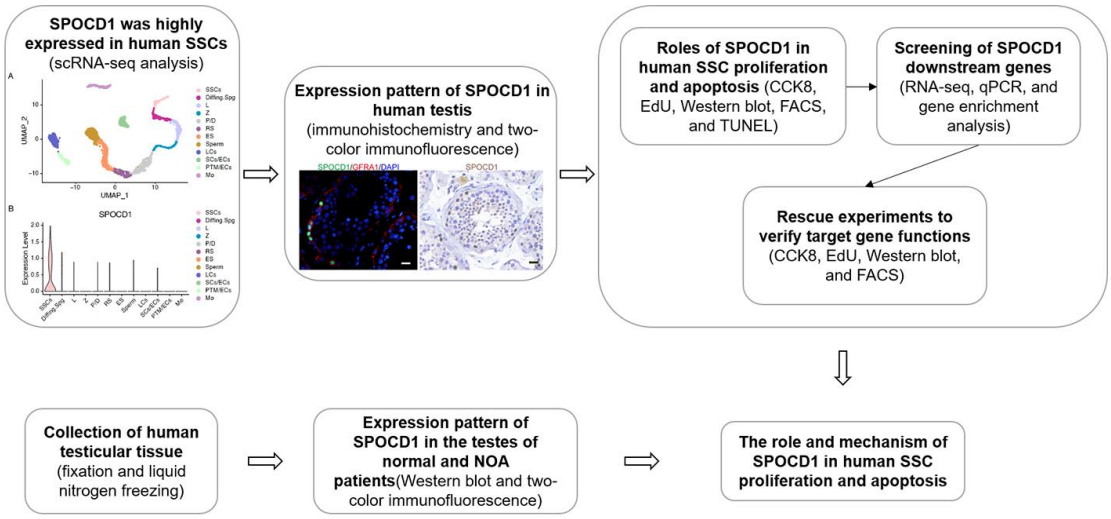
Line 381: the statement was corrected as "Our results implied that SPOCD1 downregulation may be associated with spermatogenesis dysregulation in humans"

2. Response to comment: You have collected data from 18 patients. Do you think it is enough to proof the efficacy of your proposal ?

Response: As suggested by the reviewers, 18 samples are indeed insufficient to demonstrate the association of SPOCD1 with NOA, and these results can only give us potential possibilities. However, human testis samples are very scarce, and we will expand the sample size in subsequent studies and use computerized deep learning methods to detect the association between SPOCD1 and disease. In addition, we believed that validating the expression pattern of SPOCD1 in 18 samples is relatively sufficient.

3. Response to comment: 3) You should draw some clear graphical representations (work flow Figures) in the material sections to demonstrate how your proposal works.

Response: Thanks to the constructive comments of the reviewers, we have added flowcharts to the Materials and Methods section to make our study easier for readers to understand. The study flow chart is as follows.



4. Response to comment: The authors need to study more quality journal works of recent time in this area. Read and cite : miRNA-122-5p stimulates the proliferation and DNA synthesis and inhibits the early apoptosis of human spermatogonial stem cells by targeting CBL and competing with lncRNA CASC7 (2020). RNF144B stimulates the proliferation and inhibits the apoptosis of human spermatogonial stem cells via the FCER2/NOTCH2/HES1 pathway and its abnormality is associated with

azoospermia. (2022) A classification of MRI brain tumor based on two-stage feature level ensemble of deep CNN models (2022) MiR-663a Stimulates Proliferation and Suppresses Early Apoptosis of Human Spermatogonial Stem Cells by Targeting NFIX and Regulating Cell Cycle (2018) A Deep Learning Approach using Effective Preprocessing Techniques to Detect COVID-19 from Chest CT-scan and X-ray Images (2021)

Response: Thanks to the reviewers' suggestions, we have made appropriate changes in the Introduction and Discussion sections and have cited these literatures.

Additional revisions to the manuscript include:

With the consent of all authors, we added two grant foundations.

We polished the language with the editing service to improve the readability of the manuscript.

References, abbreviations, and figure captions have also been revised.

Special thanks to you for your good comments!

We tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of the paper. And here, we did not list the changes but marked them in red in the revised paper.

We appreciate for Editors/Reviewers' warm work earnestly and hope that the correction will meet with approval.

Once again, thank you very much for your comments and suggestions.