Dear Editor and Reviewers

We sincerely thank the editor and all the reviewers for their valuable feedback that has improved the quality of our manuscript. We have carefully checked every sentence in the revised draft to eliminate/reduce any possible grammar errors, and the revision has been proofread by a native English biologist from Editage, a professional publishing service company, for better reader comprehension.The reviewer comments are laid out below in *italicized font* and specific concerns have been numbered.

Our responses are given in normal font and changes/additions to the manuscript are given in the yellow text, as follows：

Reviewer #1:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: The authors have developed a well-constructed manuscript in the context of Human Pluripotent Stem Cell (hPSC)-derived kidney organoids. The manuscript's development is acceptable, with a good level of detail and a proper structure and coherence. I commend the authors for an excellent review of the paradigm. However, in order to enhance the scientific quality of the manuscript, I suggest the following corrections: I believe that the structure of the introduction could be improved, and there could be more emphasis on whether the potential uses of organoids focus on End-Stage Renal Disease (ESRD) patients (with the possibility of autotransplantation) or if they could also improve Chronic Kidney Disease (CKD) treatment, as it is not entirely clear. Furthermore, it could be supported with data illustrating the need for the treatment of patients with renal disease, including incidence, prevalence, and its evolution over time. I consider that the last section (future direction) could be enhanced. Firstly, consider creating a specific subsection on strengths and weaknesses of current experimental models. Secondly, delve deeper into the molecular biology aspects that are deemed relevant for future research, as well as the priorities in overcoming the problems described in the weaknesses subsection. Kind regards.

1.Comment: To emphasise whether the potential use of organoids focuses on patients with end-stage renal disease (with the possibility of autotransplantation) or whether they may also improve the treatment of chronic kidney disease.

1. Reply: First of all, we would like to thank the reviewers for their professional questions, we have learnt from the relevant literature that the ultimate goal current research in organoid technology is to produce transplantable organs. However, there are still a lot of challenges to be overcome by existing renal organoid technology in this pursuit. The first challenge is related to size. The largest kidney organoid currently is only a few millimeters in diameter, which is like a grain of sand in the palm of your hand compared to the kidney, which is about 12*6*3cm in size. The second challenge is associated with vascularisation. Kidney-like organs are unable to produce a complete network of blood vessels, and therefore, cannot perform functions such as producing urine by filtration from blood as normal human kidneys do. There is still a long way to

go before a functional kidney organ can be transplanted. In a recent new study, researchers have used human stem cell-derived kidney organoids to identify genes that are essential for maintaining healthy kidney repair. Researchers have exposed kidney organoids to the chemotherapy drug cisplatin, and, through a drug screen, identified a repair compound that could rescue the tissue and prevent cisplatin-induced damage to kidney organoids in a cisplatin-induced chronic kidney disease (CKD) model of CKD progression, promising a new therapeutic modality.

2. *Comment:Use data to demonstrate the need for treatment of patients with kidney disease, including incidence, prevalence and its evolution over time.*

2. Reply: After reviewing the data, we have added data on the epidemiology of CKD. According to the Global Burden of Disease Alliance, CKD will be one of the top five diseases causing loss of life expectancy by 2040. CKD is a serious global health problem with increasing incidence and prevalence, affecting approximately 10%of the global population and leading to end-stage renal failure. Currently the main treatments for kidney disease include drugs, dialysis and kidney transplantation. By 2020, the number of people receiving renal replacement therapy exceeded 2.5 million, and was expected to double to 5.4 million by 2030, making it a serious and growing public health concern for which the search for effective treatments is urgent.

3. *Comment:It is suggested to create a specific subsection in the last section on the strengths and weaknesses of the current experimental model. Secondly, delve into aspects of molecular biology that are considered relevant to future research.*

3. Reply: We have added in the last part that the organoid model is a model used to study and simulate the function of human organs, which has the advantages of being able to replace animal experiments, being controllable, highly reproducible, and accelerating the development of drugs, etc. By using organoid models, researchers can gain an in-depth understanding of the structure and function of the organs, and explore the internal substrates and interaction modes, which can provide more in-depth knowledge and understanding. However, challenges remain in using such systems as experimental models and transplants; current methods of inducing renal organoids do not generate all cell types in the kidney, especially diverse stromal cells, and higher-order renal structures with vascular systems have not yet been established. To address these issues, more in-depth investigations of renal developmental processes are required, while the development of renal organoids in combination with novel technologies such as gene editing and species chimerism is expected to advance the transplantation of renal organoids The maturation of organoids is a major challenge that requires more detailed analyses of developing kidneys at the level of individual cells. Ultimately, organoid renal structures will need to be equipped with renal elements, collecting ducts, ureters, interstitia, and vascular flow, in order to generate transplantable kidneys.

We have revised the text to address your concerns and hope that is the paper in now clearer. Please see page 2 of the revised manuscript, lines 19–28, 33-40 and page 12, lines 16–26,32-36. We did our best to improve the manuscript and have made appropriate changes in the manuscript in line with your comments and suggestions. The changes made would not influence the overall content and framework of the paper.

We appreciate for Editors/Reviewers' work earnestly, and we hope that the corrections will be met with approval.

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

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Dear Editor,

We sincerely appreciate your valuable feedback, which has improved the quality of our manuscript. We have revised the manuscript accordingly, and please see our point-by-point responses below. All amendments are indicated by red font in the revised manuscript.

Specific Comments to Authors: The authors have examined Human Pluripotent stem cell-derived kidney Organoids: Current progress and challenges, including an extensive list of 75 references, one table, and three figures. The topics covered range from the process, types of ESCs and iPSCs, isolation, methods, and characterization to their clinical applications. While the EIC appreciated the authors' efforts, the EIC believed the manuscript requires significant revisions for clarity to attract much more readership.

The manuscript should be more concise on progress and challenges, with a balanced distribution of content, focusing on its core theme as suggested by the title: Current progress and challenges. This emphasis should be reflected in the Abstract, which lacks the necessary information. For instance, Section 2, which discusses the process, need not be exhaustive; a brief mention spanning a few sentences should suffice. Additionally, instances of redundancy need to be addressed, as detailed in point #2 below. The challenges were not fully discussed in organoid heterogeneity and in vivo functionality, which is not highly reproducible beyond biomarkers (Table 1).

Response:

We understood your comment, Just as you are concerned, We have made the greatest effort to improve the manuscript based on your opinions and suggestions, deleting redundant parts in the second section, and adding some discussion on the current challenges in the second, third, and fourth sections respectively. In the second part, we added a discussion on the fact that currently generated kidney organoids are incomplete,

lacking complete vascular network structure and distal tubules with filtration function, and although researchers and others have now developed a new protocol for inducing ureteric bud-like organs from PSCs, which have the properties of collector host cells, the generated cells are incomplete, and intercalated cells generation remains a challenge. In the third part, it is discussed that under physiological conditions in vivo, the kidney's intrarenal immune system consists mainly of macrophages (CD68, MHCII) and dendritic cells (CD11C, BDCA-1, DC-SIGN). Immune cells can be detected in almost all tissues of the human body, but almost all current in vitro organoid models lack immune cells, although protocols have been developed for co-culture of organoids with immune cells, however, due to the relative novelty of kidney organoids, co-culturing with immune cells has not yet been established, and in the future, this may be a novel way for kidney organoids to produce immune cells. In the fourth part, it is discussed that the induction protocols currently studied can only produce immature kidney organoids. Thus, only diseases that exhibit abnormalities early in embryonic development can be established by kidney organoids, hence the need to develop more mature kidney organoids in the future in order to be able to model and study delayed-onset diseases. This revision is located on page 5, lines 5-6, page 7, lines 1-7, page 9, lines 24-29, page 10, lines 1-5, and page 14, lines 17-20 in the revised manuscript.

Specific comments:

#1Not enough referencing:

1.These illustrations lack reference to workflow characteristics: How could iPSCs differentiate functionally into ectoderm, mesoderm, and endoderm tissues (not just biomarker profiling)?

Response: iPSCs are reprogrammed to obtain PSCs similar to ESCs, and can be differentiated to ectoderm through Wnt, BMP4 signaling pathway, the addition of Activin A, BMP4 induces the differentiation of iPSCs to mesoderm, and Activin A can induce the differentiation of iPSCs to endoderm(Clevers H. Cell. 2016 Jun 16;165(7):1586-1597. PMID: 27315476.), in the text has been cited in the literature, and at present, in

the figure, we have merged the ESCs and iPSCs together for hPSCs.

2.ESCs could differentiate functionally and structurally into all three types of tissues, which is not well-established for iPSCs. The figure 1 marked fibroblasts to derive iPSCs; however, the authors cited Ref #16, which was in vivo origin.

Response: Thank you for your guidance. In Figure 1A, it is not just fibroblasts that can obtain iPSCs; iPSCs can be generated from somatic cells (such as fibroblasts, peripheral blood ,hair follicles, or urine cells) through gene editing technology to reprogram them into embryonic stem cell-like cells(Doss MX, Cells. 2019 Apr 30;8(5):403. PMID: 31052294). In Figure 1A, only fibroblasts were labeled, which was our mistake. Regarding the error in the citation of reference 16, the manuscript has been deleted. At the same time, we have modified the image content, combining iPSCs and ESCs into Hpsc. We hope to receive your approval.

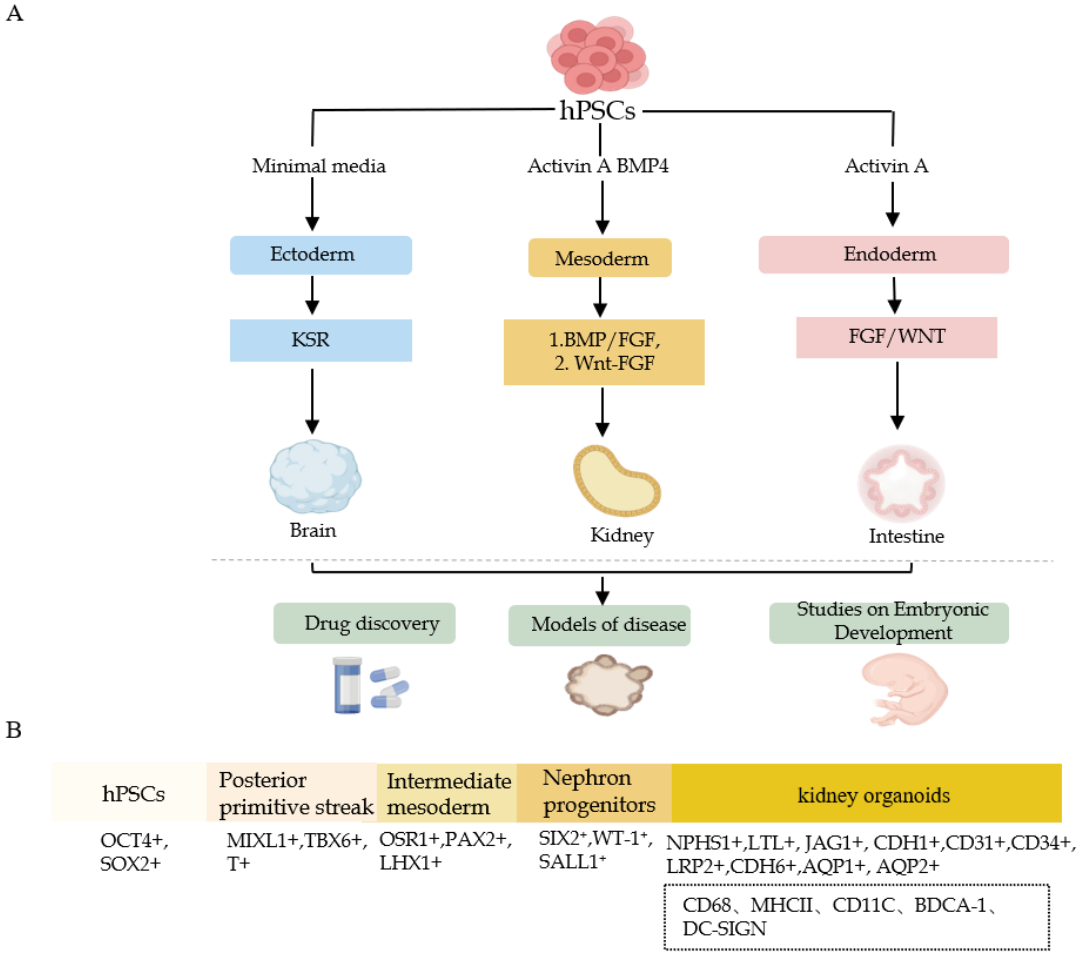

Figure 1 Overview of human pluripotent stem cell-derived organoids.A: Schematic signaling of endodermal, mesodermal, and ectodermal triodermal organoids derived from hPSCs by addition of different inducing factors; B: The process of generating hPSCs-derived kidney organoids involves the following consecutive stages: Generation of the posterior primitive streak, intermediate mesoderm, nephron progenitor cells, and kidney organoids. The dotted box shows immune cells not currently induced in kidney organoids in vitro.hPSCs: Human pluripotent stem cells; Knockout serum replacement (KSR) is a growth-promoting substitute that does not require serum.

3.Could the authors clarify PSCs cited came from ESCs, not from iPSCs, which differ from ESCs?

Response: Thank you for pointing this out. Here we have not cited enough literature, this part of the article has added three additional papers to support that iPSCs can be induced to differentiate into endoderm, mesoderm and ectoderm organoids through the addition of different germ layer inducing factors. PSCs include iPSCs and ESCs,

iPSCs and ESCs have similar regenerative ability, and both can be differentiated into endoderm, mesoderm and ectoderm through the addition of different inducing factors, compared with ESCs, iPSCs are less controversial in ethical issues, but the operation is complicated and the reprogramming is inefficient, and the mutation may be induced.

#2 – Not appropriate context manifested in the structure

Response: Thank you for your guidance. We have revised the concluding part of the manuscript.

#3: page 6: "Several procedures for differentiating pluripotent cells from kidney cells have recently been developed (Table 1)." The sentence is illogical.

Response: We thank the editors for pointing out our error, due to our negligence, the sentence logic was written incorrectly, and it has been corrected in the article as “In recent years, numerous protocols have been published on the differentiation of PSCs into kidney organoids”.

#4: page 10, “Additionally, the use of CRISPR/Cas9 to edit hPSCs, introduce specific mutations, and compare them with samples from homogeneous genetic backgrounds is another approach for studying inherited kidney diseases[48,49].” What was the author's elaboration on this approach?

Response: Thank you for your question. CRISPR/Cas9 technology is a powerful gene editing tool that can pinpoint to a specific gene and edit it to alter the expression of that gene. CRISPR/Cas9 technology is widely used in disease modeling studies of the kidney organoids, so no specific description was made in the manuscript.