nature portfolio

Peer Review Information

Journal: Nature Genetics
Manuscript Title: Adult human kidney organoids originate from CD24+ cells and present an advanced model for adult polycystic kidney disease
Corresponding author name(s): Professor Rafael Kramann

Editorial Notes:

Transferred manuscripts This manuscript has been previously reviewed at another journal. This document only contains reviewer comments, rebuttal and decision letters for versions considered at Nature Genetics.

Reviewer Comments & Decisions:

Decision Letter, initial version:

17th Dec 2021

Dear Rafael,

Your Article, "Adult human kidney organoids originate from CD24+ cells and present an advanced model for adult polycystic kidney disease", has now been seen by the 3 original referees. You will see from their comments below that while they find your work improved, some important points are raised. We are interested in the possibility of publishing your study in Nature Genetics, but would like to consider your response to these concerns in the form of a revised manuscript before we make a final decision on publication.

Reviewer #1 acknowledges that there is a lot of work here and doesn't raise technical issues (only one about mechanistic insight), but they don't support publication in Nature Genetics since in their view the novelty (methodological and biological) is limited.
Reviewer #2, on the other hand, who was previously negative, now supports potential publication after a relatively minor revision. They raise several points, which warrant some additional analyses but likely no major experiments.
Reviewer #3 was already positive beforehand and reiterates their support for publication, saying that this is an important contribution.

We invite you to revise your manuscript taking into account all reviewer comments. Please highlight all changes in the manuscript text file. At this stage we will need you to upload a copy of the manuscript in MS Word .docx or similar editable format.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact me if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

When revising your manuscript:

*1) Include a "Response to referees" document detailing, point-by-point, how you addressed each referee comment. If no action was taken to address a point, you must provide a compelling argument. This response may be sent back to the referees along with the revised manuscript.

*2) If you have not done so already please begin to revise your manuscript so that it conforms to our Article format instructions, available
here.
Refer also to any guidelines provided in this letter.

*3) Include a revised version of any required Reporting Summary:
https://www.nature.com/documents/nr-reporting-summary.pdf
It will be available to referees (and, potentially, statisticians) to aid in their evaluation if the manuscript goes back for peer review.
A revised checklist is essential for re-review of the paper.

Please be aware of our guidelines on digital image standards.

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Note: This URL links to your confidential home page and associated information about manuscripts you may have submitted, or that you are reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage.

Please do not hesitate to contact me if you have any questions or would like to discuss these revisions further.

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information please visit please visit www.springernature.com/orcid.

We look forward to seeing the revised manuscript and thank you for the opportunity to review your work.

Sincerely,

Tiago

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Senior Editor
Nature Genetics
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Reviewers' Comments:

Reviewer #1:
Remarks to the Author:
The authors have been very responsive to the comments from the first round of review. Technically, the work appears sound and provides another method beyond that already published to generate tubuloids from kidney. Cysts can be generated by knocking out the PKD genes, reproducing work published using iPSC kidney organoids and suggesting that these tubuloids may also be useful for modeling polycystic kidney disease. The novel aspect of the cyst modeling in this work is that the drug tolvaptan has an effect on cyst size, which it does not in iPSC-derived PKD cysts. This is surprising given that the authors' characterization of the tubuloids shows that they are primarily proximal tubule and loop of Henle. The target for tolvaptan is in the collecting duct and distal tubule. The authors show that despite the proximal tubule/loop of Henle characteristics of their tubuloids, they do in fact express the tolvaptan target AVPR2. This is not explained, and one interpretation would be that the cultured tubuloid epithelia do not correlate to any particular epithelial cell type in vivo but represent a mixed phenotype. This phenotypic mixing was identified as a drawback of monolayer culture many years ago, limiting its use in physiology and drug screening.

The manuscript provides a detailed analysis of the input cells and a tubuloid assay that appears to improve on the one already published. The relevance of the culture method for disease modeling remains unclear to me.

Reviewer #2:

Remarks to the Author:
The authors present an important finding: CD24+ cells (a possible renal progenitor population responsible for tubular repair) have a distinct transcriptional profile and appear to be the main origin of tubuloids, reinforcing their progenitor role. These experiments, together with their extensive single cell characterization of tubuloids, also provide more insight into the nature of adult kidney tissue-derived tubuloids.

Regarding their other findings, the differences in tubuloid composition between the 4-phase protocol and the published 1-phase protocol are limited. The gene-editing protocol is useful to the field. The ADPKD model is a good proof of principle, but no novel pathophysiologic or therapeutic insights are presented. The extensive ADPKD data sets can probably be of more value if analyzed in more depth.

The authors have strengthened their manuscript with their revisions and have addressed my previous points. I support publication in Nature Genetics upon addressing some final points:
• Fig. 2b,e. When were tubuli counted in the 4-phase protocol? During the long-term maintenance (phase 4), brightfield morphology looks similar between the 4-phase and 1-phase protocols (single tubule structures). If morphology changes from day 10-21 (phase 3) to long-term maintenance (phase 4), this should be made clear. In general, given some morphological and transcriptomic differences, where applicable (and not yet mentioned) state whether phase 3 or 4 tubuloids were used for an experiment.
• Fig. 2i. A substantial part of the 4-phase protocol cells (especially at day 21) lowly express the epithelial cell marker EPCAM. Are these indeed epithelial cells?
• Fig. 2i. Tubuloids seem to barely express markers of mature proximal tubule cells (e.g. LRP2, SLC34A1, SLC22A6) that are involved in proximal tubule functon(e.g. absorption, secretion). This should be mentioned.
• Fig. 2i: The 1-phase protocol tubuloids contain quite some GATA3+ AQP3+ cells, which could indicate the presence of collecting duct cells.
• Throughout the manuscript, "increased tubuloid complexity" to indicate multiple tubuli in the 4-phase protocol seems not the appropriate wording, as e.g. the cell type composition in the 4-phase tubuloids is quite similar to the 1-phase protocol, indicating equal or decreased (no collecting duct cells) complexity in that sense. State as is: the 4-phase protocol shows multiple tubuli within a tubuloid (in the early phase only?), whereas the 1-phase tubuloids consist of single tubule structures.
• How usable are the early (phase 3) tubuloids? I assume that 10-21 days after establishment, there are sufficient tubuloids for only a few experiments. It would require frequent sorting from fresh human kidney tissue for series of experiments, which is not easily doable for many research groups. The late (phase 4) tubuloids seem more useful as these can be cultured and passaged on the long term. If this holds true, then this should be stated and the characterization of the late phase tubuloids should be emphasized in the text.
• The authors have put effort in establishing valuable ADPKD single cell sequencing data sets (in vivo and PKD-WT vs. PKD-KO tubuloids), yet the analysis is quite shallow (showing a few selected genes and focused at cluster identity). Why not include: 1. Unbiased differential gene expression between PKD-WT and PKD-KO tubuloids. 2. Unbiased differential gene expression between healthy and ADPKD tissue (ideally within the different clusters to see which changes occur in the specific nephron cell types). 3. Comparison of unbiased differentially expressed genes in ADPKD tissue with changes between PKD-WT and PKD-KO organoids.

Minor points:
• Fig. 2i and supp. Fig. 6b. Given absent expression of CRB2 and high expression of AQP1, the 'PEC-like' identity of this cluster seems questionable. Also, the thin ascending limb LOH markers used (CRYAB, TACSTD2, CLDN3) do not seem very specific in fig. 1h.
• In the methods section, elaborate how tubuloid formation rate (fig. 1k, 2d) was determined.
• The vasopressin receptor is expressed in only a small % of tubuloids (fig. 6j). Were there only a few responsive cysts upon treatment or did the majority of cystic tubuloids respond to AVP treatment?

Reviewer #3:
Remarks to the Author:
In the revised version of their manuscript, the authors have addressed all my concerns and also added substantial new data that further improved the manuscript. In general, the data are of very high quality.
I think this is a very important manuscript for the following reasons:
1. At difference with previous studies, it reports a protocol for organoids development that uses a specific tubular subpopulation of the kidney, making it standardized and more reproducible.
2. It provides a detailed single cell RNA characterization demonstrating that these organoids fully resemble human disease and this is very important for a disease that cannot be adequately reproduced using animal models and a crucial step forward in the organoids use for modeling of ADPKD.
3. It provides proof of concept that these organoids represent a suitable system for compound screening by using tolvaptan, the only currently approved drug for ADPKD, and showing a significant effect on cyst size in tubuloids but no effect in gene-edited iPSC organoids, further confirming that this model resembles human disease pathophysiology and can be used for research and drug screening for this important genetic disease.

Author Rebuttal to Initial comments

We would like to thank all the reviewers for their careful assessment of our manuscript, which we feel has helped us significantly to improve the impact of our work. We have taken all the comments into account and present an improved manuscript, providing new data and analyses in response to the reviewer's comments.

Reviewers' Comments:

Reviewer #1:
Remarks to the Author:
The authors have been very responsive to the comments from the first round of review. Technically, the work appears sound and provides another method beyond that already published to generate tubuloids from kidney.

Thank you!

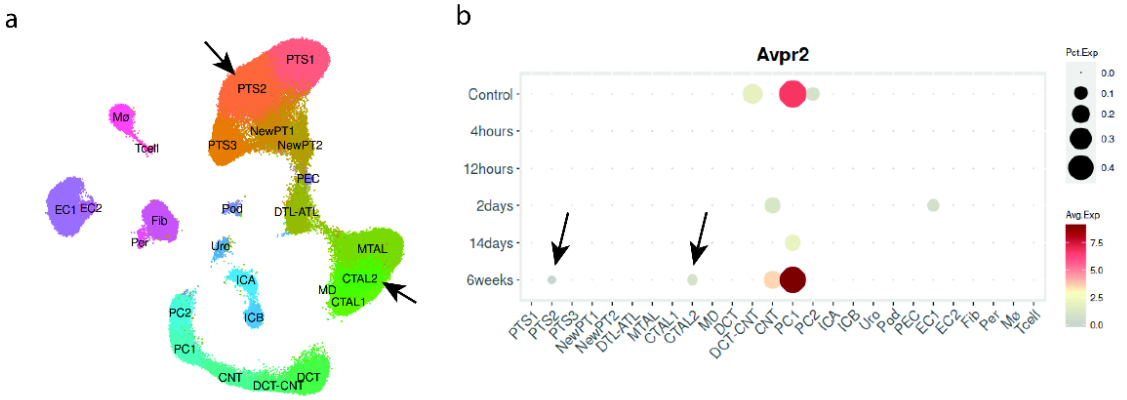
Cysts can be generated by knocking out the PKD genes, reproducing work published using iPSC kidney organoids and suggesting that these tubuloids may also be useful for modeling polycystic kidney disease. The novel aspect of the cyst modeling in this work is that the drug tolvaptan has an effect on cyst size, which it does not in iPSC-derived PKD cysts. This is surprising given that the authors' characterization of the tubuloids shows that they are primarily proximal tubule and loop of Henle. The target for tolvaptan is in the collecting duct and distal tubule. The authors show that despite the proximal tubule/loop of Henle characteristics of their tubuloids, they do in fact express the tolvaptan target AVPR2. This is not explained, and one interpretation would be that the cultured tubuloid epithelia do not correlate to any particular epithelial cell type in vivo but represent a mixed phenotype. This phenotypic mixing was identified as a drawback of monolayer culture many years ago, limiting its use in physiology and drug screening. The manuscript provides a detailed analysis of the input cells and a tubuloid assay that appears to improve on the one already published. The relevance of the culture method for disease modeling remains unclear to me.

The finding that tolvaptan does not have an effect on cyst size in gene-edited iPSCs has been made by us (Fig. 6i) and also other groups (Shimizu T et al. Biochem Biophys Res Commun 2020, PMID 32819584). We agree that it is an important novel finding that tolvaptan has an effect on cyst size in gene-edited tubuloids, suggesting that these tubuloids are a superior model of ADPKD. We have now used Symphony (Kang et al. Nat Com 2021, PMID: 34620862), a novel computational tool that allows mapping of single cell data to a reference atlas. This allowed us to map the tubuloid scRNA-seq data to the human kidney scRNA-seq data we have generated and confirmed our initial annotation since the vast majority of tubuloid derived cells mapped to proximal tubule and loop of Henle (New Extended Data Fig. 19a-e). These data argue strongly against the mixed phenotype the reviewer suggests and clearly confirm our annotation of tubuloids primarily consisting of proximal nephron parts (PT and LOH).

Several studies have shown that cysts in ADPKD can derive from all parts of the nephron including the PT and the LOH (Baert et al. Kidney Int 1978 PMID: 713285; Wilson et al. NEJM 2004, PMID: 14711914; Devuyst et al.. Am J Physiol 1996,PMID: 8760258; Schäfer et

al. Kidney Int. 1994, PMID: 7933831; Starremans et al. Kidney Int 2008, PMID: 18385665; Hopp et al. J Clin Invest 2012, PMID: 23064367; Gresh et al. EMBO J. 2004, 1657-68, PMID: 15029248). We have now performed staining for the LOH marker SLC12a1 and the PT marker LTA in human ADPKD tissue, showing that various cyst lining cells in human ADPKD express these loop of Henle or PT-specific markers (New Extended Data Figs. 19f-h). In healthy kidneys, AVPR2 is indeed expressed primarily in endothelial cells and cells of the distal nephron (collecting duct and distal convoluted tubule). However, there is some controversy about whether the receptor is also expressed in the loop of Henle. Mutig et al. reported the presence of AVPR2 in the loop of Henle particularly within the thick ascending limb in rat, human, and mouse kidneys (Mutig et al. Am J Physiol Renal Physiol. 2007; PMID: 17626156). The antidiuretic effect of arginine vasopressin (AVP) is mediated by AVPR2 linked to adenylate cyclase. Previous studies revealed that AVP caused an increase of adenylate cyclase activity stimulating active Cl^- transport in the loop of Henle (thick and thin ascending limb) of the Hamster (Imai et al. Am J Physiol.1982, PMID: 6287850 and Takahashi et al. J Clin Invest. 1995, PMID:7706469). Recent data from mouse microdissected renal tubules also indicate the presence of AVPR2 in renal TAL and ATL-LOH (Poll et al. Landscape of GPCR expression along the mouse nephron. Am J Physiol Renal Physiol. 2021; 321: F50–F68; Chen et al. Systems Biology of the Vasopressin V2 Receptor: New Tools for Discovery of Molecular Actions of a GPCR. Annu Rev Pharmacol Toxicol. 2022; 62:595-616). Due to sparsity scRNA-seq studies can not detect lowly expressed mRNA and thus do not help to answer this controversy. We propose that after injury AVPR2 can be upregulated. In mouse acute kidney injury data from Benjamin Humphreys' Lab (Kirita et al. PNAS 2020, PMID: 32571916) both PT and CTAL cells show upregulation of AVPR2 after ischemic injury (see Rebuttal figure 1 below). While we did not pick up AVPR2 expression in our human ADPKD kidney single-cell sequencing data due to the sparsity of the scRNA-seq approach we have now performed single-molecule FISH as well as immunostaining that indicates AVPR2 expression across most cyst lining cells in human ADPKD kidney tissue (New Extended Data Fig. 20h-j).

Furthermore, to clarify whether AVPR2 is expressed in $PKD1^{-/-}$ or $PKD2^{-/-}$ tubuloids, we quantified AVPR2 expression via real-time RT-qPCR. Our results show an increased expression level of AVPR2 in $PKD1^{-/-}$ or $PKD2^{-/-}$ tubuloids as compared to control tubuloids (New Extended Data Fig. 20g). This is in line with pioneering studies by Torres et al. (Torres et al. Nat Med. 2004 PMID: 14991049) and work from other groups (Gattone et al. Developmental Genetics 1999; PMID: 10322639), showing upregulation of AVPR2/VPV2R in ADPKD.



Rebuttal Fig. 1 **a)** UMAP representation of integrated single-cell RNA sequencing data from a mouse ischemia reperf from Kirita et al. PNAS 2020. **b)** Average expression of murine AVPR2 across all cell type clusters over the time course of the ischemia experiment. Arrows indicate AVPR2 expression in the subclusters PTS2 and CTAL2 at the 6 week time point. Data from http://humphreyslab.com/SingleCell/

Reviewer #2:
Remarks to the Author:
The authors present an important finding: CD24+ cells (a possible renal progenitor population responsible for tubular repair) have a distinct transcriptional profile and appear to be the main origin of tubuloids, reinforcing their progenitor role. These experiments, together with their extensive single cell characterization of tubuloids, also provide more insight into the nature of adult kidney tissue-derived tubuloids.

Regarding their other findings, the differences in tubuloid composition between the 4-phase protocol and the published 1-phase protocol are limited. The gene-editing protocol is useful to the field. The ADPKD model is a good proof of principle, but no novel pathophysiologic or therapeutic insights are presented. The extensive ADPKD data sets can probably be of more value if analyzed in more depth.

We appreciate these suggestions and have performed additional analyses of the human ADPKD dataset and a better comparison to the tubuloids as suggested. To compare the human healthy and ADPKD tissue to the control and PKD gene-edited tubuloids in an unbiased fashion, we have mapped the tubuloid populations to our reference data from human kidneys using the Symphony method and identified that most clusters from tubuloids actually map to PT_4 and TAL_2 clusters in human data, validating our tubuloid annotation (New Extended Data Fig. 19a-e). Furthermore, we now provide a better characterization of the human healthy versus ADPKD tissue based on differential expression analyses, pathway over-representation and gene-set enrichment analyses, sub-cluster and ligand-receptor analyses (New Extended Data Figs. 13-17). To compare our tubuloid and human data in greater detail, we also performed unbiased differential expression analyses between tubuloid and human-tissue data and compared the dysregulated genes at both single-gene level (e.g. direct comparison of common statistically significant up-regulated genes) and pathway level (New Fig. 5d-g and Extended Data Fig 20a-c). We also performed unbiased gene-set

enrichment analyses using fgsea tool, and over-representation analyses using reactomePA tool on the common up-regulated genes. The complete ranked-list of DE genes and identified relevant pathways that are over-represented in common up-regulated genes in human and tubuloid data are reported (New Fig. 5e-g). Overall this analysis suggested similarities between human disease and the disease modeling in tubuloids for several pathways and genes that have been also described in the literature to play an important role in ADPKD, including retinol-metabolism, RHO-GTPase, and MAPK-signaling among others as described in more detail below. We have revised the manuscript accordingly and now discuss these findings in the text.

The authors have strengthened their manuscript with their revisions and have addressed my previous points. I support publication in Nature Genetics upon addressing some final points:

Thank you!

• Fig. 2b,e. When were tubuli counted in the 4-phase protocol? During the long-term maintenance (phase 4), brightfield morphology looks similar between the 4-phase and 1-phase protocols (single tubule structures). If morphology changes from day 10-21 (phase 3) to long-term maintenance (phase 4), this should be made clear. In general, given some morphological and transcriptomic differences, where applicable (and not yet mentioned) state whether phase 3 or 4 tubuloids were used for an experiment.

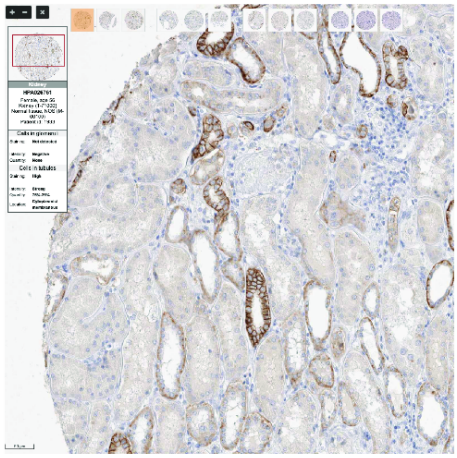
We agree and we have now included the information about whether we show data of 1-phase or 4-phase tubuloids in the figure legends and in the figures. The morphological difference between both protocols was that we detected more lumina in tubuloids generated with the 4-phase protocol as compared to the 1-phase protocol. In Fig. 2b we show the brightfield images of the 4-phase protocol and we now provide similar brightfield images from a time series of the 1-phase protocol in the revised Extended Data Fig 4g. We have further added a day 21 brightfield image of a 4-phase tubuloid in New Extended Data Fig. 4h for comparison. In these images it becomes more clear that the 4-phase tubuloids present several tubuli within one tubuloid as compared to single lumen tubuloids generated with the 1-phase protocol. This is particularly evident in early organoids between day 21 and day 30 while late organoids appear morphologically more similar. All quantifications for Fig. 2e were done at day 25. We made sure that this is described in the Fig 2e legend and the methods.

• Fig. 2i. A substantial part of the 4-phase protocol cells (especially at day 21) lowly express the epithelial cell marker EPCAM. Are these indeed epithelial cells?

We have now utilized a new computational method that allows us to map the organoid data to the human kidney data generated in an unbiased fashion. The method is called Symphony (Kang et al. Nat Com 2021, PMID: 34620862) and allows to keep the low-dimensional representation and features from one dataset (e.g. human kidney) and then map a second dataset into the same presentation / space. Using this method we mapped the tubuloid data to the human kidney data (New Extended Data Fig. 19a-e), confirming our original annotation since the vast majority of organoid cells clustered with proximal tubule and LOH cells of the human kidney (New Extended Data Fig. 19a-e). Importantly, almost all cells mapped to these two epithelial cells with some cells also mapping to DCT epithelial cells and almost no cell mapped to any other cell-type, confirming that the tubuloid cells all present epithelial cells

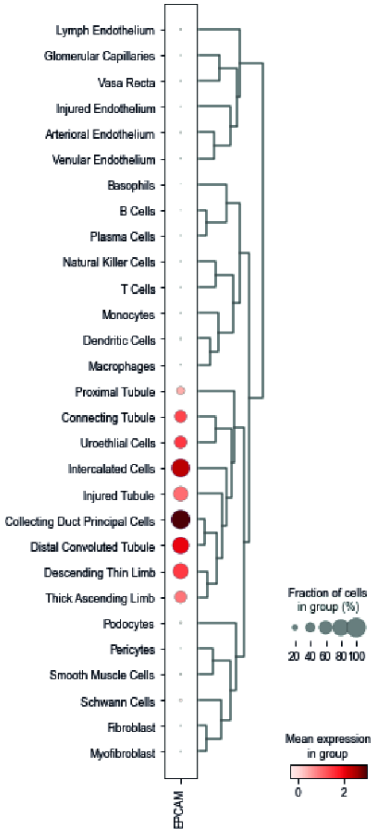
mostly from the proximal tubule and loop of Henle. We agree that EPCAM is only lowly expressed in many cells of the tubuloids. EPCAM is consistently higher expressed in distinct parts of the healthy nephron (e.g. in the distal part, as shown in a representative image from the human protein atlas, see Rebuttal Fig. 2 panel a below). To demonstrate a heterogeneous expression of EPCAM among epithelial cells of the nephron, we have also plotted EPCAM expression in our recently published human kidney scRNA-seq data (Kuppe et al. Nature 2021; PMID: 33176333) in the figure below (Rebuttal Fig. 2 panel b) showing the strongest EPCAM expression in the collecting duct and considerably less in the proximal tubule. Thus, we think different parts of the nephron show different levels of EPCAM expression already in vivo while in vitro culture of cells might further affect EPCAM expression. Nevertheless, the Symphony approach clearly verifies our annotation that the vast majority of cells in the tubuloids are epithelial cells from the PT and LOH. Furthermore, the scRNA-seq data analysis does not show expression of pan-endothelial or pan-immune cell marker genes such as PECAM1 or PTPRC (Fig2i).

a



EPCAM staining, HPA026761 human kidey

b



EPCAM average expression in human single cell data from Kuppe et al. Nature 2021

Rebuttal Fig. 2 a) EPCAM protein staining on human kidney tissue showing clear epithelial expression (arrows). **b)** Average expression of EPCAM in single cell RNA sequencing data from Kuppe et al., Nature 2021, PMID: 33176333.

• Fig. 2i. Tubuloids seem to barely express markers of mature proximal tubule cells (e.g. LRP2, SLC34A1, SLC22A6) that are involved in proximal tubule functon(e.g. absorption, secretion). This should be mentioned.

We agree and appreciate this suggestion. The marker genes of mature proximal tubule are downregulated during cell culture in vitro.

We have now added the following sentence to the manuscript:

"Expression of various marker genes of the proximal tubule such as LRP2, SLC34A1, SLC22A6 that are involved in proximal tubule function were not detected in the tubuloids using scRNA-seq. This suggests that the genes are likely downregulated in tubuloids in vitro. "

• Fig. 2i: The 1-phase protocol tubuloids contain quite some GATA3+ AQP3+ cells, which could indicate the presence of collecting duct cells.

We agree that the 1-phase tubuloids show expression of GATA3 and AQP3. The mapping of tubuloid cells from this 1-phase tubuloid to the human kidney scRNA-seq space using Symphony indicates that these cells are not collecting duct derived, but confirms our annotation with cells being similar to proximal tubule and TAL-LOH while we also observed some DCT cells (New Extended Data Fig. 19c). The DCT population might be similar to collecting duct (at least for some of the marker genes) and we propose a transitional cell state of cells in the nephron where neighboring cells show similar marker profiles that shift gradually throughout the nephron as compared to being completely distinct cell-types (Schreibing and Kramann Nat. Rev. Nephrol 2022; PMID: PMID: 35301441). The cell-population we annotated as DCT also showed the highest expression of GATA3 and AQP3 within the 1-phase tubuloid.

• Throughout the manuscript, "increased tubuloid complexity" to indicate multiple tubuli in the 4-phase protocol seems not the appropriate wording, as e.g. the cell type composition in the 4-phase tubuloids is quite similar to the 1-phase protocol, indicating equal or decreased (no collecting duct cells) complexity in that sense. State as is: the 4-phase protocol shows multiple tubuli within a tubuloid (in the early phase only?), whereas the 1-phase tubuloids consist of single tubule structures.

We appreciate this comment; we have rephrased this throughout the manuscript as suggested.

• How usable are the early (phase 3) tubuloids? I assume that 10-21 days after establishment, there are sufficient tubuloids for only a few experiments. It would require frequent sorting from fresh human kidney tissue for series of experiments, which is not easily doable for many research groups. The late (phase 4) tubuloids seem more useful as these can be cultured and passaged on the long term. If this holds true, then this should be stated and the characterization of the late phase tubuloids should be emphasized in the text.

Our disease modeling in this paper was entirely done by CRISPR/Cas9 editing of these early tubuloids at day 22 with subsequent experiments. In our hands, the early tubuloids generated from one patient were suitable for several experiments including gene editing and drug

studies. On average we were able to isolate 0.8-1x10^5 $CD24^+$ cells from a small cortical wedge biopsy from one patient. From these cells we were able on average to establish 8-10 wells with 3D tubuloid culture and this resulted on average in >100-150 tubuloids in each well at day 21 (about 1-1.5% organoid formation rate, Methods and Fig. 2e). These tubuloids can be frozen and shipped and used for disease modeling after thawing and expansion. We agree that likely not every research group can isolate these cells regularly from patients. Here generating many tubuloids at one point and then freezing these for use at later timepoints with thawing and subsequent expansion would however be suitable for many experiments. We agree that the long-term organoids might be a good model as well. Unfortunately, we have not done drug perturbation or gene-editing studies in the late-stage organoids.

• The authors have put effort in establishing valuable ADPKD single cell sequencing data sets (in vivo and PKD-WT vs. PKD-KO tubuloids), yet the analysis is quite shallow (showing a few selected genes and focused at cluster identity). Why not include: 1. Unbiased differential gene expression between PKD-WT and PKD-KO tubuloids. 2. Unbiased differential gene expression between healthy and ADPKD tissue (ideally within the different clusters to see which changes occur in the specific nephron cell types). 3. Comparison of unbiased differentially expressed genes in ADPKD tissue with changes between PKD-WT and PKD-KO organoids.

We appreciate this suggestion. We have now performed a more thorough and unbiased analyses of the human ADPKD data and also a comparison of human healthy kidney tissue versus human ADPKD kidney tissue as compared to control versus PKD-KO tubuloids.

We now report the quality assessment of the human kidney tissue nuclear RNA-sequencing data (New Extended Data Fig. 13c). We performed unbiased differential expression analyses for all cell-types in our human kidney tissue data and report the top up and downregulated genes (New Fig. 5b, New Extended Data Fig 13f, New Supplementary Table S1). We further performed gene set enrichment analyses with KEGG, PID, Biocarta and GO Molecular Function pathway genesets using fgsea tool (New Extended Data Fig. 14) and overrepresentation analysis using up-regulated differentially expressed obtained from ADPKD vs control comparison in human biopsies (New Extended Data Fig. 15) as well as receptor ligand analyses of the human tissue data (New Extended Data Fig. 16). We also performed subclustering of the main cell types, particularly for the epithelial populations and did unbiased differential gene expression analyses as well as overrepresentation analyses in the most expanded subpopulations (New Extended Data Fig 17).

We have added the following new paragraph to the manuscript describing the human kidney tissue snRNA-seq data:

"Using differential gene expression analysis we observed increased expression of genes with a proposed role in processes associated with ADPKD including upregulation of AKAP12 in proximal tubule and LOH (PT_1 and TAL), MET, LRRK2 in the DCT (DCT_1) and VCL in DCT and collecting duct (DCT_1, IC-A and PC-CD/CNT) (Fig. 5b, Supplementary Table S1 and Extended Data Fig. 13f). Ectopic AKAP12 expression in renal epithelia has been shown to result in abnormally long primary cilia and to affect epithelial morphology and

function.[35] MET has been reported as a key player in cystogenesis.[36] LRRK2 is involved in ciliogenesis and its depletion has been reported to cause rapid ciliary loss.[37] The VCL gene encodes for vinculin, which is a structural protein of the focal adhesion complex which is proposed to be dysregulated in polycystic kidney disease.[38]

[....]

We further performed gene set enrichment analysis with subsequent pathway (KEGG, PID and BIOCARTA) and GO-Term analyses (Extended Data Fig. 14). These analyses pointed towards enrichment of pro-inflammatory pathways in various immune cell populations including macrophages, B-cells and mast cells (Extended Data Fig. 14 and Supplementary Table 5 - 8). Furthermore we observed enrichment of various terms associated with cytoskeleton, focal adhesion and adherens junction, tight junctions as well as MAPK signaling in various epithelial cells (Extended Data Fig. 14 and Supplementary Table 5 - 8), in line with a reported role of these processes and pathways in ADPKD.

Overrepresentation analysis of the human ADPKD kidney tissue snRNA-seq data as compared to the healthy kidney data pointed towards PI3-Akt and MET signaling as well as extracellular matrix processes including integrin signaling in PT (Extended Data Fig. 15 and Supplementary Table 9). Moreover, this analysis showed overrepresentation of PI3-Akt, BRAF, MAPK and RHO-GTPase signaling in DCT and MET, MAPK signaling among various other pathways in TAL and collecting duct epithelium (Extended Data Fig. 15 and Supplementary Table 9). Many of these pathways have been reported as key players in ADPKD.[36,40,46,47]

To understand receptor-ligand interaction in this dataset we used CrossTalkeR[48]. The analyses pointed towards increased signaling between epithelial cell types and immune cells as well as fibroblasts (Extended Data Fig. 16a-c). Major pathways associated with cellular cross-talk involved focal adhesion, ECM-receptor signaling, PI3K-Akt, Notch, MAPK and ErbB and MET signaling (Extended Data Fig. 16d-f).

We next performed subclustering analysis of the epithelial populations with a focus on principal cells and intercalated cells of the collecting duct, distal convoluted tubule, the proximal tubule and the thick ascending limb of the loop of Henle (Extended Data Fig. 17). We observed expansion of cell-states from the collecting duct principal cells (PC-CD_CNT) that showed increased expression of SYNE2 and VCL suggesting that these might be a cyst-lining cell-state (Extended Data Fig 17a). Among the DCT and the intercalated cells of the collecting duct (IC) we identified a cell-state in ADPKD with increased expression of PKHD1 (Extended Data Fig 17b-c). Among PT we identified expansion of various cell-states that showed expression of TGFBR2, PKHD1, PDEA1 (Extended Data Fig. 17d) and among TAL we observed an ERBB4 expressing cell-state (Extended Data Fig. 17e). We then performed overrepresentation analysis (ORA) with reactome pathways using a subset (log-fold change > 1, adjusted p-value < 0.01) of up-regulated differentially expressed genes in subclusters with >30 cells per condition. Our ORA analyses pointed towards enrichment of various pathways that have been reported to play a major role in ADPKD including MAPK, BRAF, RHO signaling in IC cell-state 0, BRA, MAPK and extracellular matrix interactions in PC-DC_CNT cell-state 0, RHO-GTPAse, and extracellular matrix interaction in PT cell-state 1, MAPK and BRAF signaling and Gap junction degradation in DCT cell-tate 1 and extracellular matrix organization, cell junction organization and Type 1 hemidesmosome assembly in TAL cell-state 2 (Extended Data Fig. 17f and Supplementary Table 10 and 11). Many of these genes have been reported to be involved in ADPKD pathogenesis.

Overall this data provides an unbiased snRNA-seq atlas of human ADPKD as compared to healthy human kidney."

To specifically **compare the human tissue to the tubuloids in an unbiased fashion** we performed differential gene expression analysis in the tubuloids and the human data comparing controls to ADPKD or gene-editing as suggested. Again, in an unbiased manner, we mapped tubuloid clusters to cell-types observed in human data using Symphony and identified that most tubuloid clusters map to PT_4 and TAL_2 (New Extended Data Fig. 19a-e). We then compared all differentially expressed genes between PT_4 and TAL_2 in the human tissue to the cells that mapped to PT_4 and TAL_2 from the tubuloids, which represented the vast majority of tubuloid cells (57% and 37% of 7963 total cells) mapped to TAL_2 and PT_4, respectively. Out of 329 genes found to be statistically significantly differentially expressed (adjusted p-value <0.05) in PT_4 population between human ADPKD tissue and donor biopsies, 124 genes were up-regulated, while 32 were down-regulated in both organoids and human, respectively (New Extended Data Fig. 20a). For TAL_2, out of a total of 689 common DE genes, 133 were up-regulated and 159 were down-regulated in both organoids and human datasets, respectively (New Extended Data Fig. 20a). Via a gene set enrichment analysis of all differentially expressed genes in human ADPKD vs healthy kidney tissue and gene edited versus control tubuloids focussing on PT-4 and TAL-2 in human tissue and the cells that mapped to PT-4 and TAL-2 from tubuloids we identified common pathways (KEGG) (New Fig. 5d). This analysis indicated enriched pathways in human disease and gene-edited tubuloids as compared to controls including KEGG_MAPK_SIGNALING_PATHWAY, KEGG_RETINOL_METABOLISM among several others (New Fig. 5d and New Extended Data Fig 20b). MAPK signaling has been reported to be active in cyst lining cells and it has been suggested that cAMP could contribute directly to ERK activation via PKA, Rap-1 and B-Raf to promote cyst growth (Nagao et al. Kidney Int 2003, PMID: 12631108; Yamaguchi et al. Kidney Int 2003; PMID: 12753285). Retinoic acid has been demonstrated to induce transcription of PKD1 (Islam et al. AM J Physiol Renal Physiol 2008; PMID: 18922886) and transgenic mice overexpressing a functional human PKD1 gene develop renal cysts (Pritchard et al. Hum Mol Genet 2000; PMID: 11063721 and Puri et al Biochem Biophys Res Commun 2006; PMID: 16510125).

We next focused on the top common significantly expressed genes in PT_4 and TAL_2 between human tissue (disease vs control) and the tubuloid derived cells that mapped to human tissue PT_4 and TAL_2 in Symphony (gene-edited vs controls). Among the common significantly upregulated genes in human disease and gene edited tubuloids as compared to controls we identified SYNE2, PLEKHA1, BIRC3, RHOU, EGR1 (New Fig. 5e-f). SYNE2 also known as nesprin-2 has been reported to be involved in ciliogenesis via remodeling of the actin cytoskeleton (Dawe et al. J Cell Sci 2009; PMID: 19596800). Interestingly, PLEKHA1 has been reported to be associated with kidney function in genome-wide association studies (Chasman et al. Human Molecular Genetics 2012; PMID: 22962313). BIRC3 is a transcriptional target of YAP, a key member of the Hippo pathway which has been reported to be altered in human ADPKD (Happe et al. J Pathol 2011; PMID: 21381034, Müller et al Pediatric Nephrology 2020; PMID: 31297585). Of note, increased BIRC3 expression has also been observed in a previous transcriptomic study of ADPKD (Almeida et al. Human Genomics

2016; PMID: 27871310). ERG1 is a transcription factor that binds to the NDRG1 promoter and NDRG1 has been reported as a regulator of cystogenesis downstream of N-myc (Kim et al. Proteomics 2012; PMID: 23212942).

We then performed a gene set enrichment analysis to compare the common reactome-terms enriched in PT_4 and TAL_2. This analysis indicated enrichment of terms such as tight junction interaction, cell-junction organization in PT-4 and RHO-GTPase cycle in TAL-2 (New Fig. 5g). Tight junction composition has been reported to be altered in ADPKD (Yu et al. J Pathol 2008; PMID: 18666097) and an impaired formation of desmosomal junctions has also been reported in ADPKD (Russo et al. Histochem Cell Biol 2005; PMID: 16187067). Furthermore, work from several groups has demonstrated an important role of the Rho family of GTPases in cystogenesis (Rogers et al. Kidney Int. 2003; PMID: 12675838, Cai et al. Genes Development 2018; PMID: 29891559). Of note, we also observed common increased expression of the Rho family member RHOU in TAL of human tissue and tubuloids (Fig. 5f).

Overall, this unbiased comparison between human and tubuloid data indicates similarities between human disease and disease modeling. We have revised the paragraph in the main manuscript describing all these findings (new text is marked in red in the revised manuscript).

Minor points:
• Fig. 2i and supp. Fig. 6b. Given absent expression of CRB2 and high expression of AQP1, the 'PEC-like' identity of this cluster seems questionable. Also, the thin ascending limb LOH markers used (CRYAB, TACSTD2, CLDN3) do not seem very specific in fig. 1h.

Annotation of these rare cell-types in the kidney is challenging. It is almost impossible to differentiate between de-differentiated proximal tubule epithelial cells and PECs and both cells express CD24 (Figure 1 g and Smeets et al., J Pathol 2013, PMID: 23124355), which we have used as a marker to isolate the cells for tubuloid generation. Therefore, we tried an unbiased annotation approach based on the proposed guideline markers of the kidney precision medicine project (KPMP) (El-Achkar et al. Physiol Genomics 2021 PMID: 33197229). This allowed us to annotate all identified cell-cluster based on the KPMP reference in an unbiased fashion. The annotation of tubuloid cells was clearly difficult since various standard markers were downregulated in the tubuloids. The KPMP markers suggested that this population might be PECs and the population was also separate from other PT populations in the UMAP. Using Symphony we now also mapped the tubuloid populations to our human kidney data as a reference (New Extended Data Fig. 19a-e). This data indicates that the cells we had annotated as PEC-like in the tubuloids map to PT and/or PT_3/PEC in the human data. Therefore, and since it remains unclear, we have now adjusted the annotation in the revised manuscript and call the cells PT/PEC. To be very transparent we only used the guideline markers of the KPMP for the annotation which were CRYAB, TACSTD2 and CLDN3 for the ATL. We agree that these do not seem very specific in Figure 1h.

We have now added the following sentence to the revised manuscript:

"Of note, it is difficult to conclusively differentiate between PECs and de-differentiated proximal tubule cells, and we therefore decided to annotate these two small populations PT/PEC."

• In the methods section, elaborate how tubuloid formation rate (fig. 1k, 2d) was determined.

We apologize for not having this information included. We have now added the following paragraph to the methods:

"To measure organoid formation rate, we seeded 40µl of mixture contained 10,000 sorted CD24^{+} cells and 70% matrigel into a well of a 24-well plate and counted the tubuloid number using a counting grid (Stemgrid-6, 2700, StemCell) at day 22. Organoid formation rate was calculated using the formula: organoid formation rate (%)=(tubuloid number per well/ 10000) x100%."

• The vasopressin receptor is expressed in only a small % of tubuloids (fig. 6j). Were there only a few responsive cysts upon treatment or did the majority of cystic tubuloids respond to AVP treatment?

We observed that the majority of cysts from PKD1$^{-/-}$ and PKD2$^{-/-}$ tubuloids responded to 15-20uM tolvaptan treatment. Our results also demonstrated that tolvaptan significantly inhibited cAMP expression level (Figure 6e), suggesting its acting via AVPR2. Furthermore, to clarify whether AVPR2 is expressed in PKD1$^{-/-}$ or PKD2$^{-/-}$ tubuloids, we quantified AVPR2 expression via real-time RT-qPCR. Our results exhibited an increased expression level of AVPR2 in PKD1$^{-/-}$ or PKD2$^{-/-}$ tubuloids as compared to control tubuloids (New Extended Data Fig. 20g). This is in line with pioneering studies by Torres et al. (Torres et al. Nat Med. 2004 PMID: 14991049) and work from other groups (Gattone et al. Developmental Genetics 1999; PMID: 10322639), showing upregulation of AVPR2/VPV2R in ADPKD.

Reviewer #3:
Remarks to the Author:
In the revised version of their manuscript, the authors have addressed all my concerns and also added substantial new data that further improved the manuscript. In general, the data are of very high quality.
I think this is a very important manuscript for the following reasons:
1. At difference with previous studies, it reports a protocol for organoids development that uses a specific tubular subpopulation of the kidney, making it standardized and more reproducible.
2. It provides a detailed single cell RNA characterization demonstrating that these organoids fully resemble human disease and this is very important for a disease that cannot be adequately reproduced using animal models and a crucial step forward in the organoids use for modeling of ADPKD.
3. It provides proof of concept that these organoids represent a suitable system for compound screening by using tolvaptan, the only currently approved drug for ADPKD, and showing a significant effect on cyst size in tubuloids but no effect in gene-edited iPSC organoids, further confirming that this model resembles human disease pathophysiology and can be used for research and drug screening for this important genetic disease.

Thank you!

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Decision Letter, first revision:

Our ref: NG-A58858R

5th May 2022

Dear Rafael,

Thank you for submitting your revised manuscript "Adult human kidney organoids originate from CD24+ cells and present an advanced model for adult polycystic kidney disease" (NG-A58858R). It has now been seen by reviewer #2 and their comments are below. The reviewer finds that the paper has improved in revision, and therefore we'll be happy in principle to publish it in Nature Genetics, pending minor revisions to comply with our editorial and formatting guidelines.

We are now performing detailed checks on your paper and will send you a checklist detailing our editorial and formatting requirements soon. Please do not upload the final materials and make any revisions until you receive this additional information from us.

Thank you again for your interest in Nature Genetics. Please do not hesitate to contact me if you have any questions.

Congratulations!

Sincerely,

Tiago

Tiago Faial, PhD
Senior Editor
Nature Genetics
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Reviewer #2 (Remarks to the Author):

The authors have adequately addressed my remaining questions and further strengthened their manuscript. This work provides significant advances to the field (as described in my previous review report) and I support publication in Nature Genetics.

nature portfolio

Final Decision Letter:

In reply please quote: NG-A58858R1 Kramann

9th Sep 2022

Dear Rafael,

I am delighted to say that your manuscript entitled "Adult human kidney organoids originate from CD24+ cells and present an advanced model for adult polycystic kidney disease" has been accepted for publication in an upcoming issue of Nature Genetics.

Over the next few weeks, your paper will be copyedited to ensure that it conforms to Nature Genetics style. Once your paper is typeset, you will receive an email with a link to choose the appropriate publishing options for your paper and our Author Services team will be in touch regarding any additional information that may be required.

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Sincerely,

Tiago

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