FGF8 induces chemokinesis and regulates condensation of mouse nephron progenitor cells

Abhishek Sharma, Marco Meer, Arvydas Dapkunas, Anneliis Ihermann-Hella, Satu Kuure, Seppo J. Vainio, Dagmar Iber and Florence Naillat
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Editor: Steve Wilson

Review timeline

Submission to Review Commons:	22 February 2022
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First revision received:	19 August 2022
Editorial decision:	12 September 2022
Second revision received:	16 September 2022
Accepted:	23 September 2022

Reviewer 1

Evidence, reproducibility and clarity

NPC condensation is essential for nephron formation, but underlying regulation mechanisms remain elusive. Previous studies have demonstrated the important role of Fgf8 for the survival of NPCs. In this manuscript, Sharma et al reveal a novel function of Fgf8. By using mouse models, quantitative imaging assays, and data-driven computational modeling, they demonstrated the crucial role of Fgf8 signaling for the coordination of NPCs behaviors to the UB, especially for NPC condensation. Generally speaking, the manuscript was well organized and written. The experiments and analysis were well done. However, I have following concerns:

1. Fig 3, I don't understand why the control lost Six2 expression (Fig 3A). Fig 3E is not consistent withFig 3d, which showed that the treatment of Fgf8 antibody significantly decreases the number of live cells. Lastly, the 3D culture matrix experiments did not provide evidence on the role of Fgf8 for NPC condensation.
2. At the end of "A model based on Fgf8-induced motility leads to robust condensation of NPC", there is not a conclusive sentence.
3. Whole kidney qPCR results are not enough to support the claim of incomplete deletion of Fgf8 in mouse models. Protein staining or mRNA detection in section is required to support the claim. In addition, clear explanation is required on how the phenotypes of Fgf8 KO mice are associated the function of Fgf8 for NPC condensation.
4. I can not understand the last sentence well "Further work is required to reveal how Fgf8 along with its receptors and inhibiting factors orchestrates NPC condensation, its "

Significance

It is novel to study the role of Fgf8 for NPC condensation as a chemokine.

Reviewer 2

Evidence, reproducibility and clarity

This is a well-written and organized manuscript that investigated the role of FGF8 in chemokinesis and condensation of nephron progenitor cells to the ureteric bud during metanephric kidney development. The results described in this present study are scientifically relevant, and the figures clearly support the content and authors' conclusions. However, there are some major and minor concerns that should be addressed by the authors.

Major concerns:

- Parametric tests are not appropriate for a small sample size; non-parametric tests should be applied to examine if data are robust. Specifically, in Figures 3F and 3G, where n=3.
- In Figure 2E it is unclear how many kidneys were analyzed since the graph shows an n=5 per genotype while the figure legend indicates an n=6. Similarly, please indicate the number of independent biological samples analyzed in panel 2G rather than only the total number of cells, and specify the statistical test used for data analysis.
- Can the authors clarify how the experiment described in Figure 3E was performed? It is unclear if NPCs were treated with FGF8 ligand (as indicated in the chart legend) or with an anti-FGF8b antibody (as described in the figure legend). Moreover, the authors stated that "the loss of Six2 expression as a result of the absence of FGF8 was not completely due to cells death as more live cells were observed", however, the number of live cells seems similar between control and FGF8-treated nephrospheres. Can the authors comment on that?
- The authors argued that "the NPCs, the Six2+ cells, accumulate around the UB tip and that NPC induction is interrupted failing the PTAs formation". Please include quantification of Lhx1-positive structures to assess the number of PTA structures in wildtype, as well as, in Pax8Cre;Fgf8n/c and Wnt4Cre;Fgf8n/c mutant kidneys.
- It is unclear if both male and female offspring were collected. If so, did the authors observe sex-related differences in outcomes?

Minor concerns:

- Abbreviations should be defined at first mention in the text (e.g. "MET" in the second paragraph of the Introduction) and in each figure/table legend (e.g. UB, CM, tNPCs, PTA in Figure 1).
- In Figure 7, please label the structures (kidney; ureters, and bladder) in the urogenital system of control and mutant mice to facilitate the reader's understanding.
- For consistency, please include an inset showing a higher magnification image for Figure 8C.
- Please revise the following sentence for clarity: "Primary antibody incubation duration and temperature was."
- The legend of Supplementary Figure 1 needs to be improved, as it does not contain all information required to fully understand the presented results.
- In Supplementary Figure 2, please include the phospho-GSK3? relative expression normalized to GSK3? expression.

Significance

This study provides provide conceptual and methodological insights relevant to the field and it will be of considerable interest to the readers.

My field of expertise: kidney development and disease (mouse models, kidney explants, cell culture). I do not have sufficient expertise to evaluate the 2D simulations of NPC condensation to the ureteric epithelium.

Author response to reviewers' comments

1. General Statements [optional]

This section is optional. Insert here any general statements you wish to make about the goal of the study or about the reviews.

We would like to thank the reviewers for their positive and constructive reviews. We have already addressed their major concerns by including additional data, especially in the new Figure 3. We also detail the planned experiments that we propose to perform to address their remaining comments. Some points are mentioned in both section 2 and 3 of the revision plan.

2. Description of the planned revisions

Insert here a point-by-point reply that explains what revisions, additional experimentations and analyses are planned to address the points raised by the referees.

Reviewer #1 (Evidence, reproducibility and clarity (Required)):

Major Comments:

1. Fig 3, I don't understand why the control lost Six2 expression (Fig 3A). Fig 3E is not consistent withFig 3d, which showed that the treatment of Fgf8 antibody significantly decreases the number of live cells. Lastly, the 3D culture matrix experiments did not provide evidence on the role of Fgf8 for NPC condensation.

The figure 3A showed a reduced expression of SIX2 expression (Red) in the NPC population in the kidney. When the NPC population is cultured without any FGF ligands, the SIX2 expression in the NPCs disappeared. Our results are similar to Dapkunas et al 2019 (Arvydas Dapkunas, Ville Rantanen, Yujuan Gui, Maciej Lalowski, Kirsi Sainio, Satu Kuure, and Hannu Sariola. Simple 3d culture of dissociated kidney mesenchyme mimics nephron progenitor niche and facilitates nephrogenesis wnt-independently. Scientific reports, 9(1):1-10, 201).

The figure 3D showed that the antibody against FGF8 prevented the SIX2 expression in the NPC population from the nephrosphere experiment. We have modified the legend of figure 3D.

We agreed with the reviewer that this result might confuse the reader. We are carrying another set of experiment for the Figure 3E where nephrospheres will be treated with and without FGF8 ligand instead of culturing a full kidney with and without FGF8 ligand for 24 hours as mentioned in figure 3E.
We would like to mention that Dapkunas et al. 2019 demonstrated that dissociated kidney mesenchyme which contain the NPC population formed spontaneously self-organized spheres with the addition of FGF2 ligand and PP2 a Src inhibitor. By staining with Pax2 antibody which is a marker for progenitors and early nephron precursors we could show similarly as in Dapkunas et al that ectopic FGF8 ligand induced PAX2 expression whereas the antibody against FGF8 did not induce PAX2 expression in the cultured nephrosphere (Figure 3E-G)

2. At the end of "A model based on Fgf8-induced motility leads to robust condensation of NPC", there is not a conclusive sentence.
We have modified the text. We have written "We conclude from these simulation results that the chemokinetic effect of FGF8 enables the niche-wide distribution of NPCs. This allows them to reach the vicinity of the UB and also to enter the sphere of influence of epithelial factors that support the immobilization of NPCs. The corresponding motility gradient that appeared in the simulations (Supplementary Fig. Sup3) is in agreement with experimental observations Combes et al. 2016 (Alexander N. Combes, James G. Lefevre, Sean Wilson, Nicholas A. Hamilton, and Melissa H. Little. Cap mesenchyme cell swarming during kidney development is influenced by attraction, repulsion, and adhesion to the ureteric tip. (2016) Developmental Biology, 418(2):297-306. The simulations also show that excess FGF8 can override the guidance of epithelial signaling and prevent mesenchymal condensation."

3. Whole kidney qPCR results are not enough to support the claim of incomplete deletion of Fgf8 in mouse models. Protein staining or mRNA detection in section is required to support the claim. In addition, clear explanation is required on how the phenotypes of Fgf8 KO mice are associated the function of Fgf8 for NPC condensation.
The qPCR results of the figure 3I and J have been carried out on the nephrospehere assays. In figure 3I, the nephrosphere assay which consist of culturing the kidney mesenchyme with ectopic FGF8 ligand for 24 hours. This showed that the NPCs markers were sustained due to FGF8 ligand. This is further confirmed with the staining of PAX2 a marker for progenitors and early nephron precursors which stained the aggregated NPC cells expressing SIX2 marker as in Dapkunas et al. 2019 (Figure 3E-G).

I can not understand the last sentence well "Further work is required to reveal how Fgf8 along with its receptors and inhibiting factors orchestrates NPC condensation, its "
We have modified the text. We have written "It is known that FGF8 ligand interacts with several FGF receptors and such interaction can also be modulated by heparan sulfate proteoglycan which will consequently regulate the gradient of FGF8 concentration (Harish et al. 2022 bioRXiv). Towards this goal a detailed ligand/receptor interactions in \textit{in vivo} is required to fully understand how FGF8 imparts its function."

Reviewer #2 (Evidence, reproducibility and clarity (Required)):

Major concerns:

- Parametric tests are not appropriate for a small sample size; non-parametric tests should be applied to examine if data are robust. Specifically, in Figures 3F and 3G, where n=3.
We would like to thank the reviewer as we did not write the correct statistical test that we have carried out. It is the 2-way Anova Sidak multiple comparison that is a non-parametric test. We have corrected the legend of the figure 3.

- In Figure 2E it is unclear how many kidneys were analyzed since the graph shows an n=5 per genotype while the figure legend indicates an n=6. Similarly, please indicate the number of independent biological samples analyzed in panel 2G rather than only the total number of cells, and specify the statistical test used for data analysis.
We would like to thank the reviewer for catching our written mistake for the figure 2E. We have analysed 5 kidneys and we have corrected the legend. We have added the number of independent biological samples analyzed in the figure 2G and the statistical test (Wilcoxon signed-rank test) used in the legend.

- Can the authors clarify how the experiment described in Figure 3E was performed? It is unclear if NPCs were treated with FGF8 ligand (as indicated in the chart legend) or with an anti-FGF8b antibody (as described in the figure legend). Moreover, the authors stated that "the loss of Six2 expression as a result of the absence of FGF8 was not completely due to cells death as more live cells were observed", however, the number of live cells seems similar between control and FGF8-treated nephrospheres. Can the authors comment on that?
The figure was mislabeled the NPC were treated with the ectopic FGF8 ligand (mistake has been corrected in manuscript).
To detect the number of cells (dead/live), the flow cytometry was utilized on a full cultured kidney for 24 hours with or without ectopic FGF8 ligand. This method requires several washing steps which can remove the dead cells during the procedure. However, we are planning to repeat the same experiment using the nephrosphere assay where the nephrosphere will be cultured with or without ectopic FGF8 ligand for 24 hours before being sorted to check the live and dead cells.

- The authors argued that "the NPCs, the Six2+ cells, accumulate around the UB tip and that NPC induction is interrupted failing the PTAs formation". Please include quantification of Lhx1-positive structures to assess the number of PTA structures in wildtype, as well as, in Pax8Cre;Fgf8n/c and Wnt4Cre;Fgf8n/c mutant kidneys.
As we never have worked with Lhx1 antibody before, we are optimizing the protocol for the staining of the Lhx1 antibody combined with Troma (epithelial marker) and Six2 (NPC population marker) antibodies to highlight the NPC population from the ureteric bud in the WT kidney slides. We have few sample slides for Pax8Cre;Fgf8n/c and Wnt4Cre;Fgf8n/c mutant kidneys and we

would like a working protocol before any staining.

- It is unclear if both male and female offspring were collected. If so, did the authors observe sex-related differences in outcomes?

We have use male and female embryos. We did not genotype for the sex of the embryos in any of the experiments. In such way the sample collection was unbiased regarding the sex of the embryos.

Minor concerns:
- Abbreviations should be defined at first mention in the text (e.g. "MET" in the second paragraph of the Introduction) and in each figure/table legend (e.g. UB, CM, tNPCs, PTA in Figure 1).
We appreciate the suggestion and include all the abbreviation in the text and in the legend of Fig1.

- In Figure 7, please label the structures (kidney; ureters, and bladder) in the urogenital system of control and mutant mice to facilitate the reader's understanding.
We appreciate the suggestion and have labelled the structures in the figure 7.

- For consistency, please include an inset showing a higher magnification image for Figure 8C.
We followed the reviewer's suggestion and have included a higher magnification image for the figure 8C.

- Please revise the following sentence for clarity: "Primary antibody incubation duration and temperature was."
We clarified the sentence and have added the temperature and the time for the staining for each staining in the table 2.

- The legend of Supplementary Figure 1 needs to be improved, as it does not contain all information required to fully understand the presented results.
We have rewritten the legend of the supplementary figure 1 as the reviewer suggested.

- In Supplementary Figure 2, please include the phospho-GSK3β relative expression normalized to GSK3β expression.
We have calculated the ratio of phospho Gsk3 to GSK3 and no statistical difference was found.

3. Description of the revisions that have already been incorporated in the transferred manuscript

Please insert a point-by-point reply describing the revisions that were already carried out and included in the transferred manuscript. If no revisions have been carried out yet, please leave this section empty.

Several minor comments regarding typos and simple errors have already been incorporated in the transferred manuscript. The changes are highlighted in blue in the revised submission.

We have addressed all the minor comments that the reviewers have kindly highlighted to us. We feel these were straightforward to do and feasible in a short time, so do not require a detailed listed plan.

As mentioned above we are planning to stain the samples WT, Pax8Cre;Fgf8n/c and Wnt4Cre;Fgf8n/c mutant kidneys with Lhx1 antibody counterstained with Troma and Six2 markers and quantify the number of observed PTA structures

Description of analyses that authors prefer not to carry out
Please include a point-by-point response explaining why some of the requested data or additional analyses might not be necessary or cannot be provided within the scope of a revision. This can be

due to time or resource limitations or in case of disagreement about the necessity of such additional data given the scope of the study. Please leave empty if not applicable.

Original submission

First decision letter

MS ID#: DEVELOP/2022/201012

MS TITLE: FGF8 induces chemokinesis and regulates condensation of mouse nephron progenitor cells

AUTHORS: Florence Naillat, Abhishek Sharma, Marco Meer, Arvydas Dapkunas, Anneliis Ihermann-Hella, Satu Kuure, Seppo J Vainio, and Dagmar Iber

Thanks for submitting your manuscript through Review commons to be considered for publication in Development. I have now looked at the manuscript, the reviews and your response to the reviews. I consider that the topic of your study is suitable for the journal and your revisions and revision plans are reasonable. Once you have completed the additional planned experiments and submitted the revised manuscript and your response to the reviews, I will contact the original reviewers to re-assess the manuscript.

Please attend to all of the reviewers' comments and ensure that you clearly highlight all changes made in the revised manuscript. Please avoid using 'Tracked changes' in Word files as these are lost in PDF conversion. I should be grateful if you would also provide a point-by-point response detailing how you have dealt with the points raised by the reviewers in the 'Response to Reviewers' box. If you do not agree with any of their criticisms or suggestions please explain clearly why this is so.

First revision

Author response to reviewers' comments

We would like to thank the reviewers for their positive and constructive comments. All our answers are written in red and the changes in the revised text are highlighted in blue in the corrected version.

Reviewer #1 (Evidence, reproducibility and clarity (Required)):

NPC condensation is essential for nephron formation, but underlying regulation mechanisms remain elusive. Previous studies have demonstrated the important role of Fgf8 for the survival of NPCs. In this manuscript, Sharma et al reveal a novel function of Fgf8. By using mouse models, quantitative imaging assays, and data-driven computational modeling, they demonstrated the crucial role of Fgf8 signaling for the coordination of NPCs behaviors to the UB, especially for NPC condensation. Generally speaking, the manuscript was well organized and written. The experiments and analysis were well done. However, I have following concerns:

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The figure 3A showed a reduced expression of SIX2 expression (Red) in the NPC population in the kidney. Nephrosphere assay has been developed and published by our collaborators (Dapkunas et al 2019). This assay is able to maintain in culture the NPC population for 24 hours using Matrigel as a base in combination with PP2 a Src inhibitor. When the NPC population is cultured without any FGF8 ligands, the SIX2 expression in the NPCs population disappeared. Our results are in line with Dapkunas et al 2019 (Arvydas Dapkunas, Ville Rantanen, Yujuan Gui, Maciej Lalowski, Kirsi

Sainio, Satu Kuure, and Hannu Sariola. Simple 3d culture of dissociated kidney mesenchyme mimics nephron progenitor niche and facilitates nephrogenesis wnt-independently. Scientific reports, 9(1):1-10, 201).

The figure 3D showed that the antibody against FGF8 prevented the SIX2 expression in the NPC population from the nephrosphere experiment. We have modified the legend of figure 3D.

We agreed with the reviewer that this result might confuse the reader. We have carried another set of experiment for the Figure 3E. Nephrosphere assays were treated with and without FGF8 ligand cultured for 24 hours instead of culturing a full kidney. Our results showed that by adding FGF8 ligand in the assay, the cells were more live than dead compared to the control. Our result is in line with the result of live/dead cell nephrosphere assays sustained by FGF2 ligand in Dapkunas et al. 2019.

We would like to mention that Dapkunas et al. 2019 demonstrated that dissociated kidney mesenchyme which contain the NPC population formed spontaneously self-organized spheres with the addition of FGF2 ligand and PP2 a Src inhibitor. By staining with Pax2 antibody which is a marker for progenitors and early nephron precursors, we showed similarly as in Dapkunas et al that ectopic FGF8 ligand induced PAX2 expression whereas the antibody against FGF8 did not induce PAX2 expression in the cultured nephrosphere assays (Figure 3F-H)

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4. I can not understand the last sentence well "Further work is required to reveal how Fgf8 along with its receptors and inhibiting factors orchestrates NPC condensation, its "
We have modified the text. We have written "It is known that the FGF8 ligand interacts with several FGF receptors and that this interaction can also be modulated by heparan sulphate proteoglycans, which consequently affect the spatio-temporal gradient of FGF8 concentration (Harish et al. 2022). Still, self-organised motility such as we observed in NPCs with FGF8, may provide flexibility to changes in the microenvironment. To fully understand how FGF8 mediates its function, a detailed elucidation of the ligand-receptor interaction in vivo is required."

Reviewer #1 (Significance (Required)):

It is novel to study the role of Fgf8 for NPC condensation as a chemokine.

Reviewer #2 (Evidence, reproducibility and clarity (Required)):

This is a well-written and organized manuscript that investigated the role of FGF8 in chemokinesis and condensation of nephron progenitor cells to the ureteric bud during metanephric kidney development. The results described in this present study are scientifically relevant, and the figures

clearly support the content and authors' conclusions. However, there are some major and minor concerns that should be addressed by the authors.

Major concerns:

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- Can the authors clarify how the experiment described in Figure 3E was performed? It is unclear if NPCs were treated with FGF8 ligand (as indicated in the chart legend) or with an anti-FGF8b antibody (as described in the figure legend). Moreover, the authors stated that "the loss of Six2 expression as a result of the absence of FGF8 was not completely due to cells death as more live cells were observed", however, the number of live cells seems similar between control and FGF8-treated nephrospheres. Can the authors comment on that?
The figure 3E was mislabeled, the NPC were treated with the ectopic FGF8 ligand (mistake has been corrected in manuscript).
To detect the number of cells (dead/live), the flow cytometry was utilized on a full cultured kidney for 24 hours with or without ectopic FGF8 ligand. This method requires several washing steps which can remove the dead cells during the procedure. However, we have carried out the same experiment using the nephrosphere assays. The nephrosphere were cultured with or without ectopic FGF8 ligand for 24 hours before being sorted to check the live and dead cells using propidium iodide. The results are summarized in the graph 3E and ectopic FGF8 ligand was able to sustain the live cells compared to the control assays.

- The authors argued that "the NPCs, the Six2+ cells, accumulate around the UB tip and that NPC induction is interrupted failing the PTAs formation". Please include quantification of Lhx1-positive structures to assess the number of PTA structures in wildtype, as well as, in Pax8Cre;Fgf8n/c and Wnt4Cre;Fgf8n/c mutant kidneys.
LHX1 is expressed in the renal vesicles, comma and S-shaped bodies (Liu et al 2018 Development). We have stained WT, Pax8Cre;Fgf8n/c and Wnt4Cre;Fgf8n/c mutant kidneys with Lhx1 antibody combined with Troma (epithelial marker) and Six2 (NPC population marker) antibodies. We wanted to highlight the NPC population from the ureteric bud in the kidney slides. We observed that Pax8Cre;Fgf8n/c kidneys contained no S-shaped bodies whereas in WT and Wnt4Cre;Fgf8n/c kidneys the 3 distinctive structures were observed. These results are summarized in the supplementary figure 6.
We have changed the sentence and wrote " LIM-class homeodomain transcription factor 1 (LHX1), is a known critical marker for nephron patterning and maturation (Kobayashi et al 2005, Liu et al. 2018).In E16.5 WT and Fgf8 n/c;W nt4Cre kidneys LHX1 stained RV, CB and SB structures whereas in Fgf8 n/c; P ax8Cre kidneys the expression was only found in RV and CB demonstrating that the maturation of the nephrons is delayed (Supplementary figure 6 A-D). These results confirm the observations from the in situ hybridisation experiments (Figure 8) suggesting that the NPCs, the SIX2+ cells, accumulate around the UB tip and that NPC induction is set back, inducing nephron development delay."

- It is unclear if both male and female offspring were collected. If so, did the authors observe sex- related differences in outcomes?
We have use male and female embryos in all experiments. We did not genotype for the sex of the embryos in any of the experiments. In such way the sample collection was unbiased regarding the

sex of the embryos. We have added in material and method sub section mouse strains and tissue collection that male and female embryos were collected.

Minor concerns:

- Abbreviations should be defined at first mention in the text (e.g. "MET" in the second paragraph of the Introduction) and in each figure/table legend (e.g. UB, CM, tNPCs, PTA in Figure 1).
We appreciate the suggestion and include all the abbreviation in the text and in the legend of figures.

- In Figure 7, please label the structures (kidney; ureters, and bladder) in the urogenital system of control and mutant mice to facilitate the reader's understanding.
We appreciate the suggestion and have labelled the structures in the figure 7.

- For consistency, please include an inset showing a higher magnification image for Figure 8C.
We followed the reviewer's suggestion and have included a higher magnification image for the figure 8C.

- Please revise the following sentence for clarity: "Primary antibody incubation duration and temperature was."
We clarified the sentence and have added the temperature and the time for the staining for each antibody staining in the table 2.

- The legend of Supplementary Figure 1 needs to be improved, as it does not contain all information required to fully understand the presented results.
We have rewritten the legend of the supplementary figure 1 as the reviewer suggested.

- In Supplementary Figure 2, please include the phospho-GSK3β relative expression normalized to GSK3β expression.
We have calculated the ratio of phospho Gsk3 to GSK3 and no statistical difference was found.

Reviewer #2 (Significance (Required)):

This study provides provide conceptual and methodological insights relevant to the field and it will be of considerable interest to the readers.

My field of expertise: kidney development and disease (mouse models, kidney explants, cell culture). I do not have sufficient expertise to evaluate the 2D simulations of NPC condensation to the ureteric epithelium.

Second decision letter

MS ID#: DEVELOP/2022/201012

MS TITLE: FGF8 induces chemokinesis and regulates condensation of mouse nephron progenitor cells

AUTHORS: Abhishek Sharma, Marco Meer, Arvydas Dapkunas, Anneliis Ihermann-Hella, Satu Kuure, Seppo J Vainio, Dagmar Iber, and Florence Naillat

I have now received all the referees reports on the above manuscript, and have reached a decision. The referees' comments are appended below, or you can access them online: please go to BenchPress and click on the 'Manuscripts with Decisions' queue in the Author Area.

The overall evaluation is positive and we would like to publish a revised manuscript in Development after you have addressed the remaining minor issues raised by the referees. Please attend to all of

the reviewers' comments in your revised manuscript and detail them in your point-by-point response.

Reviewer 1

Advance summary and potential significance to field

This manuscript investigates the role of FGF8 in chemokinesis and condensation of nephron progenitor cells to the ureteric bud during metanephric kidney development.

This study provides conceptual and methodological insights relevant to the field and should be of considerable interest to the readers of Development.

Comments for the author

The revised manuscript has been improved and most of my comments were adequately addressed. However Figure 3E is still confusing. If I comprehend the message correctly, the following sentence is contradictory
“the loss of Six2 expression as a result of the absence of FGF8 (Figure 3A, D) was not completely due to cell death, as we observed more live cells when ectopic FGF8 was added (Figure 3E)”.
Please clarify/rewrite it.

Reviewer 2

Advance summary and potential significance to field

In this manuscript, Sharma et al reveal a novel function of Fgf8. By using mouse models, quantitative imaging assays, and data-driven computational modeling, they demonstrated the crucial role of Fgf8 signaling for the coordination of NPCs behaviors to the UB, especially for NPC condensation. It is novel and significant to study the role of Fgf8 for NPC condensation as a chemokine.

Comments for the author

The reversion was improved. However I still have following minor concerns:
1. Fig3E, the labeling of control and Fgf8 treated did not match the figure.

2. Authors mentioned that "Further, in both Fgf8 n/c; Pax8Cre and Fgf8 n/c;Wnt4Cre kidneys, condensation of NPCs expressing Eya1 and Cited1 around the UB was perturbed, while the expression of Cited1 was still maintained (Figure 8K-L,N-O)."
Please provide explanation on the cause and significance of the maintained Cited expression.
"Incorrect localization pattern of NPCs" is a very obscure description.

3. The sentence "Our model predicts that this mechanism, requires a balance between autocrine chemokinesis and epithelial signaling" may need some reversion

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Second revision

Author response to reviewers' comments

We would like to thank the reviewers for their constructive comments. Please find below our answers, in red colour is our answer to the reviewer’ s comments.

Reviewer 1 Advance Summary and Potential Significance to Field:

This manuscript investigates the role of FGF8 in chemokinesis and condensation of nephron progenitor cells to the ureteric bud during metanephric kidney development. This study provides conceptual and methodological insights relevant to the field and should be of considerable interest to the readers of Development.

Reviewer 1 Comments for the Author:

The revised manuscript has been improved and most of my comments were adequately addressed. However, Figure 3E is still confusing. If I comprehend the message correctly, the following sentence is contradictory “the loss of Six2 expression as a result of the absence of FGF8 (Figure 3A, D) was not completely due to cell death, as we observed more live cells when ectopic FGF8 was added (Figure 3E)”. Please clarify/rewrite it.

We have rewritten the sentence. “As previously reported by Grieshammer et al. 2005 [26], the absence of FGF8 leads to cell death, and similar observation was found in nephrospheres lacking FGF8 as compared to ectopic FGF8 nephrosphere assays, and a similar result is observed for the live cell number (Figure 3E).”

Reviewer 2 Advance Summary and Potential Significance to Field:

In this manuscript, Sharma et al reveal a novel function of Fgf8. By using mouse models, quantitative imaging assays, and data-driven computational modeling, they demonstrated the crucial role of Fgf8 signaling for the coordination of NPCs behaviors to the UB, especially for NPC condensation. It is novel and significant to study the role of Fgf8 for NPC condensation as a chemokine.

Reviewer 2 Comments for the Author: The reversion was improved. However I still have following minor concerns:

1 Fig3E, the labelling of control and Fgf8 treated did not match the figure.

We would like to thank the reviewer for finding our mistake. We have correctly relabelled the legend of the figure 3E.

2. Authors mentioned that "Further, in both Fgf8 n/c; Pax8Cre and Fgf8 n/c;Wnt4Cre kidneys, condensation of NPCs expressing Eya1 and Cited1 around the UB was perturbed, while the expression of Cited1 was still maintained (Figure 8K-L,N-O)." Please provide explanation on the cause and significance of the maintained Cited expression. "Incorrect localization pattern of NPCs" is a very obscure description.

We have miswritten the sentence. “Eya1 and Six2 around the UB was perturbed, while the expression of Cited1 was still maintained in the Fgf8 n/c; Wnt4Cre kidneys but not in the Fgf8 n/c; Pax8Cre kidneys.” The Cited1 expression is maintained in the Fgf8 n/c; Wnt4Cre kidneys. These kidneys contained few comma-shaped body structures suggesting that the localisation of Cited1 is correctly expressed.

We have replaced “Incorrect localization pattern of NPCs“ by “Together, data shows that the anchor genes for the NPC compartment have an incorrect localization pattern, where Fgf8 is deleted via either Pax8Cre or Wnt4Cre mouse lines.”

3. The sentence "Our model predicts that this mechanism requires a balance between autocrine chemokinesis and epithelial signaling" may need some reversion.

We have rewritten the sentence.
“In our model, the specific balance between FGF8-induced NPC motility and epithelial signaling is evident.”

Third decision letter

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MS TITLE: FGF8 induces chemokinesis and regulates condensation of mouse nephron progenitor cells

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