



# Stromal $\beta$ -catenin activation impacts nephron progenitor differentiation in the developing kidney and may contribute to Wilms' tumor

Keri A. Drake, Christopher P. Chaney, Amrita Das, Priti Roy, Callie S. Kwartler, Dinesh Rakheja and Thomas J. Carroll  
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**Editor:** Liz Robertson

## Review timeline

Original submission:	17 February 2020
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Accepted:	2 June 2020

## Original submission

### First decision letter

MS ID#: DEVELOP/2020/189597

MS TITLE: Interstitial activation of beta-catenin in the developing kidney impacts nephron progenitor differentiation and may contribute to Wilms tumor

AUTHORS: Keri A. Drake, Christopher P. Chaney, Amrita Das, Priti Roy, Callie S. Kwartler, Dinesh Rakheja, and Thomas J. Carroll

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, provided that the referees' comments can be satisfactorily addressed. Please attend to all of the reviewers' comments in your revised manuscript and detail them in your point-by-point response. If you do not agree with any of their criticisms or suggestions explain clearly why this is so.

### Reviewer 1

#### *Advance summary and potential significance to field*

This is a really interesting manuscript looking at the spatial influence of beta-catenin stabilisation / activation in the formation of the developmental renal neoplasia Wilms tumor. In brief, the researchers show that it is an activation of beta-catenin within the non-medullary stroma of the developing mouse kidney that can result in a phenocopy of the blastemal expansion and nephrogenic arrest seen in Wilms' tumor. This is a significant shift in thinking around the origin of this condition and contributes also to our understanding of how patterning and morphogenesis is regulated in the kidney.

The study moves between transcriptional analyses of tumors and conditional beta-catenin activation models in which specific Cre drivers are being used to restrict the cellular location of the

activation. Contrary to what has been anticipated in the literature, activation of beta-catenin is present within both NPC and stromal regions of WT and the activation of beta-catenin in the NPCs does not result in nephrogenic rests whereas Foxd1-Cre driven activation of beta-catenin in the stroma does.

#### *Comments for the author*

The authors tried to model the likely events leading to the observation of activation in both cell type using a TCre driver. This showed no effect on development. It is possible that induction of Cre even as early as 7.5dpc still did not target the common progenitor of both populations and this may tell us something about the origin of the Foxd1 lineages. While it may have been possible to try a slightly later common progenitor driver, perhaps Eya1, I think there is no reason to ask for this as the approach then taken was to drive Cre off both the Six1 and Foxd1 locus and show that this in fact generated heterologous tissue that was described as bone-like.

A feature of Wilms' tumors are the formation of ectopic tissues, including skeletal muscle, cartilage and bone. Perhaps my only criticism here is that the staining of this tissue mass with alkaline phosphatase would suggest cartilage rather than bone necessarily. This would have been analysed by looking more closely at the histology - cartilage has clear cells within lacunae - or to stain with Alizarin red to distinguish the two. It would be good to do this. Another minor criticism is that the transcriptional comparison between human WT and the different Cre driven beta-catenin activators presented in Figure 5 is impossible to distinguish - text needs to be larger and colours need to be clearer. This also doesn't convincingly support the claim being made here, but I don't think it is terribly critical data.

#### Reviewer 2

##### *Advance summary and potential significance to field*

This paper identifies a novel mechanism of activation of beta-catenin in the renal interstitium that is seen in the developing kidney and then recapitulated in Wilms Tumors. They use a combination of transgenic knockouts coupled with interrogation of primary human Wilms tumor and expression profiling. This is an excellent example of utilization of mouse transgenic models to tease apart a clinical phenomenon.

#### *Comments for the author*

This article by Drake et al to a special edition of Development related to the developmental origins of disorders is well written and presents interesting new data. The utilization of genetic studies interrogating the role of beta catenin regulation in the developing kidney, mapping alterations in nephron progenitor differentiation to the stromal lineage and then further linking it to wilms tumor is well done and an exciting observation. In general the studies are well done and of a high quality. There are a few concerns that are listed below.

##### Major concerns

1. This paper lacks any real quantitative elements and this needs to be addressed. Even the number of animals per experiment is not well defined in many of the experiments. How many animals were looked at the various histological stages etc?
2. The assessment of the cortical stromal components seems incomplete. Marker analysis of this cell type is important and immune stains should be done for cortical stromal markers. It appears that the cortical stroma is dramatically thinned out in the stromal overexpressor. Is this driven by increased apoptosis or changes in the cell cycle?
3. The examination of the bone phenotype is superficial and needs to be expanded. Utilization of further markers and validation using real time PCR would strengthen this argument.
4. The molecular comparison of the various mutant lines and the human wilms samples is difficult to interpret as shown. It is almost impossible to read the labels and the specific genes need to be shown in a table also.

5. The caveat related to the tamoxifen experiments is whether a sufficient regime of tamoxifen was utilized and this recombination efficiency needs to be assessed to make this argument effectively.

### Reviewer 3

#### *Advance summary and potential significance to field*

This manuscript explores a very interesting aspect of Wilms Tumor (WT) biology; the influence of stromal cells on tumor pathogenesis. WT is the most common childhood kidney cancer, and pathology indicates that it derives from residual progenitor cells, nephrogenic rests, that remain after kidney development is complete.

The tumor cells display many features of nephron progenitor cells, and the field has focused on understanding how these residual nephron progenitor cells give rise to tumors. This work adds an intriguing dimension to the WT field in that it argues that the stromal cells contribute to tumor development. The authors model the influence of stroma on the maintenance of the progenitor cell phenotype in nephron progenitor cells, and show stromal expression of a WT mutation causing constitutive activation of b-catenin causes retention of the progenitor phenotype. This is an interesting and provocative finding that certainly is worthy of publication in my opinion.

#### *Comments for the author*

I have some reservations about the data and arguments being made that need to be addressed before the work can be published:

1. The data in supplemental figure 1 is foundational for the work and should be in the main figures.
2. I do not think that the authors have any support for inferring lineages in human tumors. On the one hand the stroma and the blastema do differ morphologically. On the other hand, one could argue that the strongest lineage data is the mutation analysis, and finding an identical mutation in both of these cell populations could very well suggest a common origin and lineage. Tumor cells in other systems have been shown to transdifferentiate. I think the section on the human specimens needs to be revised accordingly.
3. While the data on laser capture microdissection of the human samples is intriguing, it will not be entirely convincing until the authors can show with some markers, eg RT-QPCR that demonstrate that the blastema sample indeed is blastemal and the stromal sample is stromal. This needs to be incorporated into the figure.
4. One significant difference between the human data and the mouse data that is presented in this paper is that the human data is on a WT but the mouse data addresses the nephrogenic rests that are the precursors of WT. This needs to be clarified in the text.
5. The data in figure 5 seems quite arbitrary and poorly annotated. It needs to be presented in a more convincing way with some quantitative measure of significance for the relationships being represented.
6. Figure 6 is not particularly convincing in its current form because only a mutant is shown with no other tissue for comparison. The authors refer to supplementary figure 2E for comparison but the magnification is not comparable. The best comparison would be a section from the bCATd3;Foxd1cre at the same magnification as 6B included in figure 6.

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### **First revision**

#### Author response to reviewers' comments

#### **Response to reviewers:**

**Reviewer 1:**

**Advance Summary and Potential Significance to Field:** This is a really interesting manuscript looking at the spatial influence of beta-catenin stabilization/activation in the formation of the developmental renal neoplasia Wilms tumor. In brief, the researchers show that it is an activation of beta-catenin within the non-medullary stroma of the developing mouse kidney that can result in a phenocopy of the blastemal expansion and nephrogenic arrest seen in Wilms' tumor. This is a significant shift in thinking around the origin of this condition and contributes also to our understanding of how patterning and morphogenesis is regulated in the kidney. The study moves between transcriptional analyses of tumors and conditional beta-catenin activation models in which specific Cre drivers are being used to restrict the cellular location of the activation. Contrary to what has been anticipated in the literature, activation of beta-catenin is present within both NPC and stromal regions of WT and the activation of beta-catenin in the NPCs does not result in nephrogenic rests whereas Foxd1-Cre driven activation of beta-catenin in the stroma does.

**Reviewer 1 Comments for the Author:**

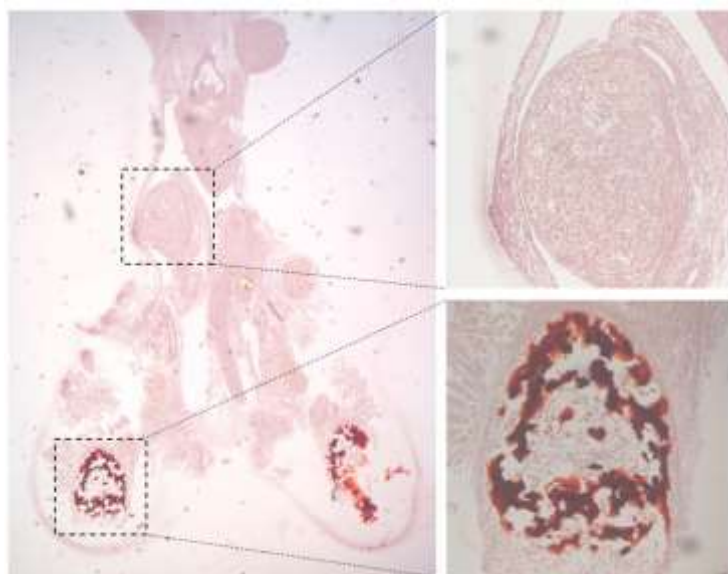
The authors tried to model the likely events leading to the observation of activation in both cell type using a TCre driver. This showed no effect on development. It is possible that induction of Cre even as early as 7.5dpc still did not target the common progenitor of both populations and this may tell us something about the origin of the Foxd1 lineages. While it may have been possible to try a slightly later common progenitor driver, perhaps Eya1, I think there is no reason to ask for this as the approach then taken was to drive Cre off both the Six1 and Foxd1 locus and show that this in fact generated heterologous tissue that was described as bone-like. A feature of Wilms' tumors are the formation of ectopic tissues, including skeletal muscle, cartilage and bone. Perhaps my only criticism here is that the staining of this tissue mass with alkaline phosphatase would suggest cartilage rather than bone necessarily. This would have been analyzed by looking more closely at the histology - cartilage has clear cells within lacunae - or to stain with Alizarin red to distinguish the two. It would be good to do this.

We agree with the reviewer that the presence of "bone-like" tissue is both very surprising and interesting. As shown in Figure 2, panel H, the histology is quite consistent with bone, in that we do not observe prominent lacunae/chondrocytes on H&E staining. However, we also do not observe osteoclasts and that the pale extracellular matrix is consistent with osteoid matrix that is not yet calcified. We have reviewed these slides with a pathologist, who agreed with the histology resembling "non-calcified" bone.

We agree with the reviewer that alkaline phosphatase staining does not differentiate between bone and cartilage (Miao and Scutt, Histochemical localization of alkaline phosphatase activity in decalcified bone and cartilage, J Histochem Cytochem. 2002), as this paper notes that alkaline phosphatase does stain some types of cartilage matrix as well as early bone formation (including osteoblasts and osteoid matrix).

We did perform Alizarin red staining on the lower half of a mutant embryo, with staining appearing in the long bone of the legs but not in the abnormally developed kidney tissue (as shown in the high magnification images below). Since Alizarin red stains calcified matrix, this may only suggest that this extracellular matrix has not yet been calcified, and given that these mutants die after birth, we are unable to perform any later analyses.

Therefore, we felt that a lack of Alizarin red staining was not very informative in further characterizing the phenotype, and it was not included in the original manuscript, since the histology helps support the distinction between bone and cartilage.



Another minor criticism is that the transcriptional comparison between human WT and the different Cre driven beta-catenin activators presented in Figure 5 is impossible to distinguish - text needs to be larger and colours need to be clearer. This also doesn't convincingly support the claim being made here, but I don't think it is terribly critical data.

We appreciate the reviewer's concerns and have revised this figure, using a different statistical representation (bar graph format instead of a UMAP) to improve the clarity of visualizing this data. Additionally, revised the results and methods of the manuscript to further explain how unsupervised classification techniques (such as neural network mapping) can be utilized to compare large, complex data sets - as we did here to support the findings that human WT show transcriptional changes that resemble stromal activation of beta-catenin in our mutant mouse model (page 11, lines 213-226):

"We next developed a more comprehensive method to compare beta-catenin targets in human WT to our mutant mouse models. Given the considerable complexity and size of the TARGET data set, we utilized a deep learning classification technique. Using a trained neural network classifier, the expression profiles for 124 Wilms tumor samples were mapped to the expression data generated from kidneys of each of the three mouse genotypes (wild type, *Six2cre;Catnb<sup>ex3/+</sup>*, and *Foxd1cre;Catnb<sup>ex3/+</sup>*), resulting in a score ranging from 0 (no match) to 1.0 (representing a perfect match). As shown in Fig. 7, nearly all the human WT samples, including 6 tumors with known activating CTNNB1 mutations, showed almost exclusive mapping to the *Foxd1cre;Catnb<sup>ex3/+</sup>* transcriptome (scores ranging from 0.55 to 0.99), with only two samples showing an appreciable degree of similarity to wild type kidneys (scores of 0.28 and 0.44), and none of the samples mapping to the *Six2cre;Catnb<sup>ex3/+</sup>* expression profile (Supplemental Figure 4). This unbiased, quantitative approach, along with our histological studies, suggest that activation of beta-catenin in the NPC lineage does not appear to transcriptionally recapitulate Wilms tumor. Interestingly, despite human WT histologically resembling normal development, this analysis suggests that it is transcriptionally more similar to the mutant kidneys with stromal activation of beta-catenin than to normal, wild type embryonic kidney."

## Reviewer 2

**Advance Summary and Potential Significance to Field:** This paper identifies a novel mechanism of activation of beta-catenin in the renal interstitium that is seen in the developing kidney and then recapitulated in Wilms Tumors. They use a combination of transgenic knockouts coupled with interrogation of primary human Wilms tumor and expression profiling. This is an excellent example of utilization of mouse transgenic models to tease apart a clinical phenomenon.

## Reviewer 2 Comments for the Author:

This article by Drake et al to a special edition of Development related to the developmental origins of disorders is well written and presents interesting new data. The utilization of genetic studies interrogating the role of beta catenin regulation in the developing kidney, mapping alterations in nephron progenitor differentiation to the stromal lineage and then further linking it to wilms tumor is well done and an exciting observation. In general the studies are well done and of a high quality. There are a few concerns that are listed below.

## Major concerns

1. This paper lacks any real quantitative elements and this needs to be addressed. Even the number of animals per experiment is not well defined in many of the experiments. How many animals were looked at the various histological stages etc?

We have clarified the methods with the following statement (page 18, line 409-411): “Data presented in figures are representative examples from one of at least three different experiments on at least three different embryos/organs. No significant variability was noted in tissues of the same genotype.” Additionally, we have included “Ns” analyzed in each figure legend.

2. The assessment of the cortical stromal components seems incomplete. Marker analysis of this cell type is important and immune stains should be done for cortical stromal markers. It appears that the cortical stroma is dramatically thinned out in the stromal overexpressor. Is this driven by increased apoptosis or changes in the cell cycle?

The reviewer is correct in the observation that the cortical stroma is lost and medullary stroma is expanded in the *Foxd1cre;Catnb<sup>ex3/+</sup>* mutant kidneys. While we did not examine cell cycle or apoptosis, our data suggests that beta-catenin drives differentiation of the medullary stroma which is ectopically expressed in mutant kidneys. We have included this data in an additional figure and describe these results in the text of the manuscript (page 9, lines 169-185):

“Previously, it has been shown that ablation of the stromal progenitor population results in abnormally maintained NPCs reminiscent of nephrogenic rests (Das et al., 2013). *Foxd1cre;Catnb<sup>ex3/+</sup>* kidneys show some hallmarks of these stroma-less kidneys, including abnormally expanded, *Six2* expressing NPCs surrounding the UB (Fig. 3, K). Given these findings, we previously hypothesized that nephrogenic stromal cells produce a signal that promotes differentiation or blocks renewal of the NPCs. We next examined the molecular phenotype of stromal cells upon activation of beta-catenin with *Foxd1Cre*. *Foxd1cre;Catnb<sup>ex3/+</sup>* kidneys show early loss of the stromal progenitor population as indicated by decreased expression of *Foxd1*, *Netrin1* and *Smoc2* (Fig. 5, G, H, and S, respectively). Instead, genes normally expressed in the medullary stroma were precociously and ectopically expressed in the cortex, including *Cpmx2*, *Sdc2*, *Dpp6*, and *Wnt5a* (Fig. 5, J, K, L, and R, respectively). Additionally, mutant kidneys showed decreased expression of the cortico-medullary stromal marker *Penk* but lacked expression of *Wnt4* in the medullary stroma (Shan et al., 2010), as shown in Fig. 5, I and T, respectively. These findings suggest that activation of beta-catenin in the stromal progenitor cells may be leading to precocious and ectopic differentiation of a more medullary stromal cell type. Given that beta-catenin has previously been shown to be necessary for the development of the medullary stroma (Yu et al., 2009, Boivin and Bridgewater, 2018, Boivin et al., 2016), our findings suggest it is also sufficient.”

We have also highlighted this in the discussion with the following additions (page 13, lines 277-290):

“Additionally, we show that the patterning of the renal interstitium is severely disrupted in *Foxd1cre;Catnb<sup>ex3/+</sup>* kidneys, with an early loss of *Foxd1* progenitor cells and a pronounced expansion of medullary interstitial markers. While distinctions between cortical and medullary interstitial cells have been previously recognized, recent work has revealed a surprising degree of heterogeneity in the embryonic renal interstitium (England, 2020) suggesting that unique sub-populations of interstitial cells regulate adjacent cell types in normal kidney development. In support of this, inactivation of beta-catenin in the stromal progenitor population (using *Foxd1Cre*) blocks development of the medullary interstitium as well as adjacent epithelial cells of the loop of

Henle (England, 2020, Yu et al., 2009). Conversely, we show here that activation of beta- catenin in the stromal progenitor population drives expression of medullary interstitial cells. We hypothesize that the abnormal interstitial patterning in the *Foxd1cre;Catnb<sup>ex3/+</sup>* kidneys disturbs the normal stromal microenvironment present in developing kidneys, leading to the altered gene expression and lack of differentiation in the NPC population. We hypothesize that a similar disruption to the stromal microenvironment contributes to Wilms tumorigenesis, as has been suggested in numerous other tumors (Clark and Vignjevic, 2015, Mao et al., 2013, Bremnes et al., 2011, Valkenburg et al., 2018, Li et al., 2007).”

3. The examination of the bone phenotype is superficial and needs to be expanded. Utilization of further markers and validation using real time PCR would strengthen this argument.

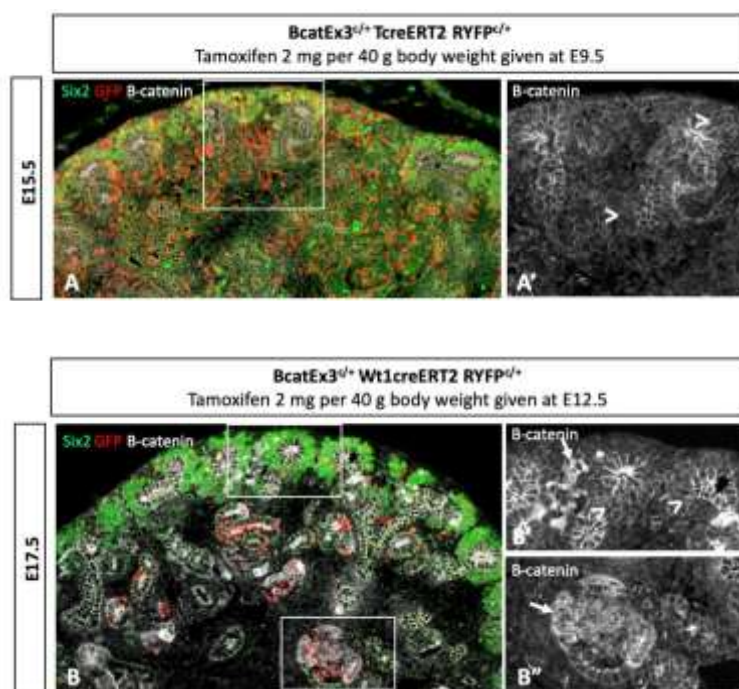
As in our response to reviewer 1, we demonstrate the presence of bone-like tissue in *Six2cre;Foxd1cre;Catnb<sup>ex3/+</sup>* mutants based on the histology and alkaline phosphatase staining. We agree that with additional experiments, it would be very interesting to characterize how “bone-like” the abnormally developing tissue is. However, we respectfully feel that pursuing these additional studies may be outside the scope of the focus of this manuscript.

4. The molecular comparison of the various mutant lines and the human wilms samples is difficult to interpret as shown. It is almost impossible to read the labels and the specific genes need to be shown in a table also.

As in our response to reviewer 1, we have revised this figure and provide additional details about the methods/gene expression profiling used to generate this data (please see response to reviewer 1 for details).

5. The caveat related to the tamoxifen experiments is whether a sufficient regime of tamoxifen was utilized and this recombination efficiency needs to be assessed to make this argument effectively.

We agree that tamoxifen inducible models introduce an additional element of variability. We administered a dose of 2 mg per 40 g body weight (and this has been added to the text of the paper in addition to the figure legend). Given our experience, as well previous publications using tamoxifen-inducible cre lines (ie: 1 mg/40g body weight used by Mugford et al, *Osr1* expression demarcates a multi-potent population..., *Dev Biol*, 2008), we expect this is a sufficient dose to induce recombination. As shown in the figure, we do see reporter expression, indicating the dose is sufficient to induce Cre-expression and recombination at the *Rosa* locus, and we expect that recombination would similarly occur at the beta-catenin exon 3 locus. To further support this, we have included additional data below showing that the same dose of tamoxifen resulted in activation of beta-catenin with *Wt1creERT2* (panel B' and B"). Though this data is not included in the paper, we have included it below to support that the dose of tamoxifen used should be sufficient to induce recombination as expected in the *TcreERT2* model.



### Reviewer 3

**Advance Summary and Potential Significance to Field:** This manuscript explores a very interesting aspect of Wilms Tumor (WT) biology; the influence of stromal cells on tumor pathogenesis. WT is the most common childhood kidney cancer, and pathology indicates that it derives from residual progenitor cells, nephrogenic rests, that remain after kidney development is complete. The tumor cells display many features of nephron progenitor cells, and the field has focused on understanding how these residual nephron progenitor cells give rise to tumors. This work adds an intriguing dimension to the WT field in that it argues that the stromal cells contribute to tumor development. The authors model the influence of stroma on the maintenance of the progenitor cell phenotype in nephron progenitor cells, and show stromal expression of a WT mutation causing constitutive activation of b-catenin causes retention of the progenitor phenotype. This is an interesting and provocative finding that certainly is worthy of publication in my opinion.

### Reviewer 3 Comments for the Author:

I have some reservations about the data and arguments being made that need to be addressed before the work can be published:

1. The data in supplemental figure 1 is foundational for the work and should be in the main figures.

[We have included this figure in the main text of the paper as Figure 1 \(page 7, lines 115\).](#)

2. I do not think that the authors have any support for inferring lineages in human tumors. On the one hand, the stroma and the blastema do differ morphologically. On the other hand, one could argue that the strongest lineage data is the mutation analysis, and finding an identical mutation in both of these cell populations could very well suggest a common origin and lineage. Tumor cells in other systems have been shown to transdifferentiate. I think the section on the human specimens needs to be revised accordingly.

[We agree with the reviewer that these observations cannot be used to infer cell lineages in human WT. We have clarified this in the manuscript text with the following revisions \(page 14, lines 292-308\):](#)

“Studies using laser microcapture techniques to analyze different components of human WT, including data from three patients in our study, demonstrate identical mutations in blastema, stroma, and epithelial components (Duhme et al., 2019, Uschkereit et al., 2007)... Although these observations are consistent with a model in which activating mutations must occur in both NPC and stromal lineages (or a common progenitor for both), we cannot rule out the possibility that the triphasic morphology of WTs is due to aberrant tumor cell differentiation (through either multi-lineage potential or an ability to transdifferentiate) or that activating mutations in epithelial structures lead to EMT. Although we feel that the molecular phenotype of tumor stroma along with the histology of mouse mutants makes these possibilities unlikely, it is clear that activation of beta-catenin alone is not sufficient to drive WT formation in mice.”

3. While the data on laser capture microdissection of the human samples is intriguing, it will not be entirely convincing until the authors can show with some markers, eg RT-QPCR that demonstrate that the blastema sample indeed is blastemal and the stromal sample is stromal. This needs to be incorporated into the figure.

Wilms tumor has been historically characterized based on its histology, and actually there is surprisingly limited data characterizing the gene expression profiles of blastema vs stroma vs epithelial components. The 3 patients examined in this report are consistent with the findings from previously published cases showing (Duhme et. al, WT1 mutant Wilms tumor..., J Pediatr Hematol Oncol, 2019 and Uschkreit et. al, Different CTNNB1 mutations..., J Med Genet, 2007). Given these supporting studies, we feel there is limited utility to confirm the tissue obtained by laser microcapture with additional techniques. Although we agree that a more comprehensive approach to profile these differing components of Wilms tumor is warranted, it is beyond the scope of this study.

4. One significant difference between the human data and the mouse data that is presented in this paper is that the human data is on a WT but the mouse data addresses the nephrogenic rests that are the precursors of WT. This needs to be clarified in the text.

We have clarified the comparison of mutant mouse kidney data to the human Wilms tumor data throughout the manuscript (page 6, line 96; page 10, line 189) as well as in the figure.

5. The data in figure 5 seems quite arbitrary and poorly annotated. It needs to be presented in a more convincing way with some quantitative measure of significance for the relationships being represented.

In agreement with reviewer 1 and 2's comments, we revised this figure and added additional explanation to the manuscript text and methods utilized, including how statistics were performed along with how the measure of relationships were defined. Please see details included in response to reviewer 1.

6. Figure 6 is not particularly convincing in its current form because only a mutant is shown with no other tissue for comparison. The authors refer to supplementary figure 2E for comparison but the magnification is not comparable. The best comparison would be a section from the bCATd3;Foxd1cre at the same magnification as 6B included in figure 6.

This figure has been revised to include a negative control (cre-negative kidney) as well as “positive” control kidneys showing nuclear expression of beta-catenin using the Six2cre and Foxd1cre lines, to further highlight that the TcreERT2; ;Catnb<sup>ex3/+</sup> model resembles wild type kidney despite cre-expression confirmed by lineage tracing.

Additionally, we have made minor revisions to the title and abstract to meet word limit requirements with changes documented in “blue” text.

We again appreciate the consideration of our revised manuscript and responses the reviewers comments.

Second decision letter

MS ID#: DEVELOP/2020/189597

MS TITLE: Interstitial activation of beta-catenin in the developing kidney impacts nephron progenitor differentiation and may contribute to Wilms tumor

AUTHORS: Keri A DRAKE, Christopher P Chaney, Amrita Das, Priti Roy, Callie S Kwartler, Dinesh Rakheja, and Thomas J. Carroll

ARTICLE TYPE: Research Article

I am happy to tell you that your manuscript has been accepted for publication in Development, pending our standard ethics checks.

Reviewer 1*Advance summary and potential significance to field*

This was provided in my initial review. The manuscript in its revised form is stronger as a results of changes to text and presentation.

*Comments for the author*

I remain disappointed that characterisation of the 'bone'like' tissue was not strengthened given the discussion about histological review and Alazarin red staining. This could at least have been added to the Supplementary data. While a minor feature of the paper, the identity of this ectopic material would be very relevant to the field. Having said that, this should not hold up publication.

Reviewer 2*Advance summary and potential significance to field*

This paper identifies a novel mechanism of activation of beta-catenin in the renal interstitium that is seen in the developing kidney and then recapitulated in Wilms Tumors. They use a combination of transgenic knockouts coupled with interrogation of primary human Wilms tumor and expression profiling. This is an excellent example of utilization of mouse transgenic models to tease apart a clinical phenomenon.

*Comments for the author*

The authors have addressed all concerns

Reviewer 3*Advance summary and potential significance to field*

See previous reviews.

*Comments for the author*

All comments have been addressed in the revision of this very interesting paper.