Whole-body clonal mapping identifies giant dominant clones in zebrafish skin epidermis

Hsiao-Yuh Roan, Tzu-Lun Tseng and Chen-Hui Chen
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Original submission

First decision letter

MS ID#: DEVELOP/2021/199669

MS TITLE: Whole-body clonal mapping identifies giant dominant clones in zebrafish skin epidermis

AUTHORS: Hsiao-Yuh Roan, Tzu-Lun Tseng, and Chen-Hui Chen

I have now received two 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.

As you will see, the referees express interest in your work, but have some significant criticisms that need to be addressed before we can consider publication. Reviewer 1 considers that the methodological novelty of your work partially compensates for limited mechanistic insights whereas reviewer 2 considers that much more in depth analysis is needed to understand the phenomena you describe; my opinion is that you do indeed need to address the concerns raised by reviewer 2, and to a lesser extent, reviewer 1. If you are able to revise the manuscript along the lines suggested, I will be happy receive a revised version of the manuscript. Please also note that Development will normally permit only one round of major revision.

We are aware that you may be experiencing disruption to the normal running of your lab that make experimental revisions challenging. If it would be helpful, we encourage you to contact us to discuss your revision in greater detail. Please send us a point-by-point response indicating where you are able to address concerns raised (either experimentally or by changes to the text) and where you will not be able to do so within the normal timeframe of a revision. We will then provide further guidance. Please also note that we are happy to extend revision timeframes as necessary.

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.

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Reviewer 1

Advance summary and potential significance to field

This manuscript describes a transgenic system for stochastically labeling and tracing clones in the basal skin layer of zebrafish. The tool and technique are powerful, and the authors use them to make the intriguing observation that there are occasional “giant dominant” cell clones in the skin, which are more frequent in the anterior region of the animal. They also find that these dominant clones are less frequent in a laminin mutant, providing proof-of-principle that this approach can be used to detect genetic regulators of epithelial cell behaviors.

Comments for the author

The manuscript is well written, the figures are appealing, the experimental design is sound, and the data were analyzed rigorously. Each experiment is sound (and interesting), but I have three major comments, only one of which (#3) requires additional experiments.

1. Since the significance of this manuscript lies in the introduction of a new tool, descriptive observations about the nature of skin cell clones, and a simple proof-of-principle genetic experiment, this manuscript would be a better fit for the “Techniques and Resources” section of the journal than the “Research Articles” section. The manuscript falls somewhat short of providing mechanistic clarity into a developmental process, which I would expect for a research article. However, the technique is promising and opens up exciting possibilities, so, as a “Technique”, it would broadly interest epithelial developmental biologists.

2. This transgenic line is called “basebow”, reflecting its derivation from “brainbow” technology. However, Brainbow (and other -bows) are fittingly named because they feature the recombination of multiple fluorescent protein gene copies, thus combinatorially creating dozens of colors that allow researchers to simultaneously track many clones. If I understand this line correctly, it is a single copy integration that only allows recombination into two alternative colors — blue and green. Moreover, the distinct colors are not as important to the technique as the physical separation between clones, which is the feature allowing researchers to identify them as arising from clonal events. For this reason, I suggest that the authors come up with a different name that better reflects the functional features of the technique.

3. One potentially powerful aspect of this approach that the manuscript does not fully exploit is the ability to track specific clones at multiple intervals over long periods of development. This feature should allow researchers to understand the dynamics that shape clones. For example, do dominant clones expand all at once at a particular developmental period, or do they grow at a steadily faster rate than other clones? How often do clones shrink through cell death? Are clones always contiguous, or do they ever split apart and become separated through migration, creating what appear to be separate clones that actually arose from the same progenitor? This sort of tracking—-following clones every few days over a long time period (perhaps a month?)—-should be technically possible and could provide mechanistic insight into key observations in the manuscript. Since giant dominant clones make up almost 5% of clones in the anterior region, if the authors are lucky, they may be able to catch one by tracking only ~20 clones in this way.

Reviewer 2

Advance summary and potential significance to field

This study by Chen et al. details how basal keratinocytes contribute to regional differences in the skin. The zebrafish is an ideal model for imaging skin development and the basebow line can provide new insight into clonal expansion from skin stem cells. The manuscript identified regional differences in the anterior and posterior half of the body whereby the anterior half produces giant clonal lineages from a single precursor.

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Comments for the author

While this study is interesting, there substantial questions that need to be addressed prior to consideration for publication in Development. The manuscript lacks details and hence, it is difficult to understand the rationale for certain experiments. Moreover, the main idea of the paper is that there are giant clones that populate large body surfaces. However, the analysis of such giant clones is insufficient at this point, and needs more detailed characterizations, such as determining the relationship between the clonal size and cell size within these clones, as well as the proliferation rate, which could contribute to the clonal size differences.

1. Can the authors discuss the krt19 transgene, which does not express in the fins of early embryonic and larval zebrafish and but is expressed in the fin of adult fish. How is the skin of the fins maintained and which stem cells contribute to these compartments early on?

2. The statement that the anterior half of the fish is 1.8-fold larger than the posterior half seems rather arbitrary since the division line between anterior and posterior was drawn arbitrarily. Is it possible that the proportional clone size within each body half is simply coincidence? Therefore, this statement would benefit from a better justification by drawing parallels from other systems or better experimental evidence. It rather seems that the anterior clones are larger in size due to the stretching of the skin in this area whereas the body is slimmer in the tail and thus the skin may not stretch. Are individual cells within each clone also different in size, which would support this theory?

3. Fig. 3C was listed to show extensive clonal growth but the images do not have a scalebar. It is therefore difficult to assess whether the clonal growth is substantial.

4. The authors cite a DeLeon manuscript that is still in preparation, which needs to be removed. This also raises the question how sure the authors are that this specific line cited only expresses in basal epithelial cells given that the basebow line shows expression in both SEC and BECs? Because of this, it is unclear how the authors calculated the number of basal cells since this line could contain both cell types.

5. Especially with regards to Fig. 4 the manuscript text would benefit from a more thorough explanation how these numbers of clone:body surface area ratio were calculated, given that this is one of the main points of the paper. For example what does the following statement mean: “Thus, when scaling proportionally with the expansion of the fish body surface area,….”. How was the body surface measured over time?

6. What does the (cutoff ratio: 0.2%) refer to in text for Fig. 4? I assume total surface area? If so, how were these rather arbitrary clone sizes determined as Giant, dominant, near-dominant and ordinary? Is this based on precedence in other systems? This seems rather random and no rationale is provided.

7. What does the word “scale” refer to in this sentence: “Remarkably, we found that during post-embryonic skin growth, a few dominant clones readily emerge to occupy up to 0.6% of the animal body surface, which is about ~20-fold larger than expected for clones that scale proportionally (Fig. 4C).” I am unsure about the meaning of this sentence.

8. Rather than dominance of one clonal lineage over another, could the larger clones simply be a result of a more rapid division whereas smaller clones did not divide as many times within the same time, hence the differences in clonal size? I would suggest to quantify proliferation rate in time-lapse movies to determine if there might be such differences.

9. I am unsure about this sentence: “Because the spatiotemporal correlation between lamb1a expression and the clonal expansion behavior in the BEC population, ….” What is the relationship between the spatiotemporal expression of lamb1a (which seems like an overstatement based on qPCR data) and clonal expansion? I would suggest to better explain lamb1 at this point such that an inexperienced reader does not have to search the literature for its possible roles in clonal expansion.

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10. It is unclear to me how the lamb1asde1 allele is sequestering Laminin protein in skin BECs (Fig. 5B,C)? Is this an overexpression phenotype or lack of degradation, is it rather a lack of secretion out of BECs? This allele is not described and hence the observed phenotype is difficult to understand. What does “mislocalized” precisely describe?

11. What is the relationship between lamb1 and sde1? Do the authors mean the lamb1sde1 mutant background or is sde1 another mutation? There should be consistent labeling of lines and mutants to make it easier for the reader to follow.

12. In Fig. 5, what is the difference between sde1/+ and sde1?

13. I don’t see a difference in clonal size looking at the data shown in Fig.
5F. Since sde1 has less clones analyzed than sde1/+, is this data sufficient to draw conclusions about the number of clones with a given size especially if the clone sizes are arbitrarily assigned?

14. What exactly is quantified in graphs 4D, E and 5H? The clone size as percentage of total size set to 100%, or the percentage of clones with a given size? I assume the latter, but if so, the terminology should be changed to Clones (%).

15. In the discussion the authors state that “… Lamb1a, is transiently induced in the skin BEC population during post-embryonic growth”. However, Fig. S5 shows that there is a higher expression during adult stages while the difference between AS and PS in juvenile fish is not significant. It is unclear what precisely does the “relative expression” compare? Perhaps this leads to the confusion. Overall this manuscript would benefit from better explanations especially the last paragraph describing Figure 5 is difficult to follow due to the omission of many important details.

16. Fig. 2J, 3, etc. showing clonal analyses, how many animals were analyzed?

Other comments:
Fig. S1: I would remove graph 1D showing only BECs, or separate the graph in 1E to only show SECs to make it consistent and less redundant.
Fig. 1E does not show 3D rendering but rather an orthogonal view
There are a number of missing and incorrectly spelled words throughout the manuscript, including the materials and methods. Hence the manuscript would benefit from thorough editing using an editing service.

First revision

Author response to reviewers' comments

Reviewer 1 Advance Summary and Potential Significance to Field:

This manuscript describes a transgenic system for stochastically labeling and tracing clones in the basal skin layer of zebrafish. The tool and technique are powerful, and the authors use them to make the intriguing observation that there are occasional “giant dominant” cell clones in the skin, which are more frequent in the anterior region of the animal. They also find that these dominant clones are less frequent in a laminin mutant, providing proof-of-principle that this approach can be used to detect genetic regulators of epithelial cell behaviors.

Reviewer 1 Comments for the Author:

The manuscript is well written, the figures are appealing, the experimental design is sound, and the data were analyzed rigorously. Each experiment is sound (and interesting), but I have three major comments, only one of which (#3) requires additional experiments.

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1. Since the significance of this manuscript lies in the introduction of a new tool, descriptive observations about the nature of skin cell clones, and a simple proof-of-principle genetic experiment, this manuscript would be a better fit for the "Techniques and Resources" section of the journal than the "Research Articles" section. The manuscript falls somewhat short of providing mechanistic clarity into a developmental process, which I would expect for a research article. However, the technique is promising and opens up exciting possibilities, so, as a "Technique", it would broadly interest epithelial developmental biologists.

Response (1.1): We thank the reviewer for the encouraging comments on the study. As suggested, we will submit the revised version as a Techniques and Resources article.

2. This transgenic line is called "basebow", reflecting its derivation from "brainbow" technology. However, Brainbow (and other -bows) are fittingly named because they feature the recombination of multiple fluorescent protein gene copies, thus combinatorially creating dozens of colors that allow researchers to simultaneously track many clones. If I understand this line correctly, it is a single copy integration that only allows recombination into two alternative colors —blue and green. Moreover, the distinct colors are not as important to the technique as the physical separation between clones, which is the feature allowing researchers to identify them as arising from clonal events. For this reason, I suggest that the authors come up with a different name that better reflects the functional features of the technique.

Response (1.2): We thank the reviewer for the excellent point and would like to explain the imaging system we used in this study. To capture clonal behavior on a centimeter scale, we coupled an epifluorescence microscope with a highly sensitive sCMOS camera. Although the macroscopic platform enables whole-body monitoring of fluorescent protein-tagged clones in juvenile zebrafish, the system falls short of allowing us to differentiate fluorescence signals at varying levels, which is key for imaging additional colors generated with Brainbow technology. Thus, the imaging system is most sensitive when capturing primary colors (red, green, and blue) and not the secondary or tertiary colors that occur from differentially titrated mixes of primary colors. Of note, we determined that the transgenic line can generate additional colors, which can be visualized by imaging with a confocal microscope. Therefore, the line carries multiple integrated copies of the transgene; however, our imaging method restricted the number of colors we could successfully track in our experiments. Thus, we respectfully suggest that "basebow" may be a proper name for this transgenic line. For clarification, we have revised the text to comment on this point on page 8, paragraph 2:

> "Of note, although the platform enables fluorescence imaging on a centimeter scale, the system falls short of being able to differentiate fluorescence signals at varying levels, which is a requirement for tracking secondary and/or tertiary colors generated with Brainbow technology."

3. One potentially powerful aspect of this approach that the manuscript does not fully exploit is the ability to track specific clones at multiple intervals over long periods of development. This feature should allow researchers to understand the dynamics that shape clones. For example, do dominant clones expand all at once at a particular developmental period, or do they grow at a steadily faster rate than other clones? How often do clones shrink through cell death? Are clones always contiguous, or do they ever split apart and become separated through migration, creating what appear to be separate clones that actually arose from the same progenitor? This sort of tracking—-following clones every few days over a long time period (perhaps a month?)—-should be technically possible and could provide mechanistic insight into key observations in the manuscript. Since giant dominant clones make up almost 5% of clones in the anterior region, if the authors are lucky, they may be able to catch one by tracking only ~20 clones in this way.

Response (1.3): We thank the reviewer for the insightful comment. As suggested, we performed same-animal same-clone tracking at 21, 25, and 28 dpf. By plotting the growth trajectories of 17 clones from 6 animals, we concluded that individual BEC clones grow at distinct rates. We found that dominant clones expand at a faster pace on average than non-dominant clones. Also, we were unable to detect any clearly split or shrunken clones in the examined cases. We have now included the tracking scheme and result in Fig. S6, and described the finding on page 12, paragraph 1:

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> "To determine the expansion dynamics that may lead to the appearance of a dominant clone, we performed same-animal same-clone tracking at 21, 25, and 28 dpf (Fig. S6A,B). Intriguingly, although individual BEC clones appeared to grow at distinct rates, we determined that dominant clones expand at a faster pace on average than non-dominant clones (Fig. S6C,D). Of note, we failed to detect any clearly split or shrunken clones in the examined cases (n = 17)."

Reviewer 2 Advance Summary and Potential Significance to Field:

This study by Chen et al. details how basal keratinocytes contribute to regional differences in the skin. The zebrafish is an ideal model for imaging skin development and the basebow line can provide new insight into clonal expansion from skin stem cells. The manuscript identified regional differences in the anterior and posterior half of the body whereby the anterior half produces giant clonal lineages from a single precursor.

Reviewer 2 Comments for the Author:

While this study is interesting, there substantial questions that need to be addressed prior to consideration for publication in Development. The manuscript lacks details and hence, it is difficult to understand the rationale for certain experiments. Moreover, the main idea of the paper is that there are giant clones that populate large body surfaces. However, the analysis of such giant clones is insufficient at this point, and needs more detailed characterizations, such as determining the relationship between the clonal size and cell size within these clones, as well as the proliferation rate, which could contribute to the clonal size differences.

1. Can the authors discuss the krt19 transgene, which does not express in the fins of early embryonic and larval zebrafish and but is expressed in the fin of adult fish. How is the skin of the fins maintained and which stem cells contribute to these compartments early on?

Response (2.1): We thank the reviewer for the comment. In response, we would like to explain the "fin fold" structures of early embryonic and larval zebrafish. These primitive fin structures last only for a short time period during early development. As a simple, flattened epidermal extension, the structure contains no basal stem cell layer (i.e., no P63-positive cells), a feature which differs from juvenile and adult fin tissues that emerge at later stages. Thus, the Tg(krt19:H2A-mCherry) line, which labels only the BEC stem cell population, has no expression in the fin fold region. We have revised the legend of Figure 4A for clarification.

2. The statement that the anterior half of the fish is 1.8-fold larger than the posterior half seems rather arbitrary since the division line between anterior and posterior was drawn arbitrarily. Is it possible that the proportional clone size within each body half is simply coincidence? Therefore, this statement would benefit from a better justification by drawing parallels from other systems or better experimental evidence. It rather seems that the anterior clones are larger in size due to the stretching of the skin in this area whereas the body is slimmer in the tail and thus the skin may not stretch. Are individual cells within each clone also different in size, which would support this theory?

Response (2.2): We thank the reviewer for the comment and would like to highlight that our macroscopic imaging platform may represent the first used to study clonal growth behavior on a centimeter scale. To the best of our knowledge, similar, parallel studies from other systems or models have not been performed, presumably due to technical challenges.

Response (2.3): To determine whether the large anterior clones may be caused by the stretching of the skin in the region, we determined average basal cell numbers on both the anterior region and the posterior region of the fish body surface. Using the Tg(krt19:H2A-mCherry) line, which labels the whole BEC population, we determined that the skin basal cells are evenly spaced on different body regions. We identified no marked difference between the anterior region and the posterior region at 21 and 28 dpf. Thus, we conclude that skin tissues are not more stretched on the anterior region. Individual clone sizes are positively correlated with the basal cell numbers within each clone. We have now included these new results as Fig. 4C and 4D, and the finding is

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described on page 11, paragraph 1.

3. Fig. 3C was listed to show extensive clonal growth but the images do not have a scalebar. It is therefore difficult to assess whether the clonal growth is substantial.

Response (2.4): We have now added a scale bar to Fig. 3C.

4. The authors cite a DeLeon manuscript that is still in preparation, which needs to be removed. This also raises the question how sure the authors are that this specific line cited only expresses in basal epithelial cells given that the basebow line shows expression in both SEC and BECs? Because of this, it is unclear how the authors calculated the number of basal cells since this line could contain both cell types.

Response (2.5): As suggested, we have removed the reference and characterized further the expression profile of *Tg(krt19:H2A-mCherry)* at different developmental stages. By staining with DAPI and anti-P63 (the basal stem cell marker) at 8 and 32 dpf, we determined that the mCherry expression in this transgenic line is indeed restricted to the basal cell population. We detected no ectopic expression in the SEC layer. Thus, we conclude that this line can provide a fair estimate of the basal cell number in live animals. We have now included these new histological results as Fig. S4, revised the text on page 10, paragraph 2, and updated the materials and methods section on page 17, paragraph 2.

5. Especially with regards to Fig. 4 the manuscript text would benefit from a more thorough explanation how these numbers of clone:body surface area ratio were calculated, given that this is one of the main points of the paper. For example, what does the following statement mean: "Thus, when scaling proportionally with the expansion of the fish body surface area,….". How was the body surface measured over time?

Response (2.6): We thank the reviewer for the comment. In Fig.4, we used the *Tg(krt19:H2A-mCherry)* line to determine the total number of basal cells in an animal. Because fish body surfaces are relatively flat and the basal cells are evenly spaced across different body regions (Fig. 4A-4D), we can estimate the percent body surface area occupied by each 8 dpf basal cell in a 2-dimensional space (i.e., 1/3109 = 0.03%). If all 8 dpf basal cells can grow and make the same contributions, the ratio is expected to remain constant regardless of fish body surface area expansion. To clarify our rationale for the comparison, we have now revised the text on page 11, paragraph 1:

> "Thus, when a clone grows proportionally with the fish body surface area expansion, an ordinary BEC clone is expected to occupy 0.03% of the body surface area in a 2D space (i.e., 1/3109 = 0.03%; assuming all 8 dpf basal cells can grow and make the same contributions)."

6. What does the (cutoff ratio: 0.2%) refer to in text for Fig. 4? I assume total surface area? If so, how were these rather arbitrary clone sizes determined as Giant, dominant, near-dominant and ordinary? Is this based on precedence in other systems? This seems rather random and no rationale is provided.

Response (2.7): Yes, the ratio "0.2%" refers to the total body surface area. Because an ordinary BEC clone is expected to occupy "0.03%" of the body surface area, we set a specific cutoff at "0.1%" for dominant clones, and a series of other cutoffs that each cover a two-fold change in size. While the clone size classification may seem arbitrary, we respectfully suggest that the presentation is more informative for understanding trend changes in overall clone sizes. For clarification, we have now revised the text on page 11, paragraph 1:

> "Of note, we set a specific size cutoff at 0.1% for dominant clones, which is about 3-fold higher than the theoretical ratio of an ordinary clone (0.03%)."

7. What does the word "scale" refer to in this sentence: "Remarkably, we found that during post- embryonic skin growth, a few dominant clones readily emerge to occupy up to 0.6% of the animal body surface, which is about ~20-fold larger than expected for clones that scale proportionally (Fig. 4C)." I am unsure about the meaning of this sentence.

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Response (2.8): We have revised the text to clarify the expected value and replace the word "scale" on page 11, paragraph 1:

> "Remarkably, we found that during post-embryonic skin growth, a few dominant clones readily emerge. Some dominant clones occupy up to 0.6% of the animal body surface, which is about 20-fold larger than expected (0.03%) for ordinary clones that grow proportionally (Fig. 4E)."

8. Rather than dominance of one clonal lineage over another, could the larger clones simply be a result of a more rapid division whereas smaller clones did not divide as many times within the same time, hence the differences in clonal size? I would suggest to quantify proliferation rate in time-lapse movies to determine if there might be such differences.

Response (2.9): Yes. Because clone sizes positively correlate with the basal cell number within each clone (Response 2.3), we expect that there are more cell divisions in larger clones. As suggested, we performed a time-lapse imaging at 21, 25, and 28 dpf to chart the growth trajectories of 17 individual clones from 6 animals. We determined that dominant clones indeed expand at a faster pace than non-dominant clones. We have now included these new results as Fig. S6, and the finding is described on page 12, paragraph 1:

> "To determine the expansion dynamics that may lead to the appearance of a dominant clone, we performed same-animal same-clone tracking at 21, 25, and 28 dpf (Fig. S6A,B). Intriguingly, although individual BEC clones appeared to grow at distinct rates, we determined that dominant clones expand at a faster pace on average than non-dominant clones (Fig. S6C,D). Of note, we failed to detect any clearly split or shrunken clones in the examined cases (n = 17)."

9. I am unsure about this sentence: "Because the spatiotemporal correlation between lamb1a expression and the clonal expansion behavior in the BEC population, …." What is the relationship between the spatiotemporal expression of lamb1a (which seems like an overstatement based on qPCR data) and clonal expansion? I would suggest to better explain lamb1 at this point such that an inexperienced reader does not have to search the literature for its possible roles in clonal expansion.

Response (2.10): We thank the reviewer for the comment and apologize for not making the rationale clear. In our previous study, we determined that lamb1a is transiently induced during tailfin regeneration, and its expression is restricted to the skin basal cell population. Here, we determined that lamb1a has a similar expression profile during post-embryonic skin growth. Thus, we hypothesized that lamb1a may have a specific role in supporting the basal cell-mediated clonal growth behavior. For clarification, we have now revised the text on page 13, paragraph 1.

10. It is unclear to me how the lamb1asde1 allele is sequestering Laminin protein in skin BECs (Fig. 5B,C)? Is this an overexpression phenotype or lack of degradation, is it rather a lack of secretion out of BECs? This allele is not described and hence the observed phenotype is difficult to understand. What does "mislocalized" precisely describe?

Response (2.11): The lamb1a^sde1 allele is a temperature-sensitive allele. When the allele-carrying animals are kept at the restrictive temperature, the laminin protein with lost function becomes stuck in the BEC cytosol, presumably due to a failure in secretion. Because deposition of the laminin protein at the basement membrane is key for its functions, the allele provides a direct method for manipulating laminin activities in intact and live animals. For clarification, we have now revised the text as the following on page 13, paragraph 1:

> "Upon shifting the animals from 28°C to 34°C, homozygous lamb1a^sde1 allele carriers had Laminin protein sequestered in skin BECs (*sde1*, Fig. 5B,C), preventing its proper deposition at the basement membrane (Chen et al., 2015)."

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11. What is the relationship between lamb1 and sde1? Do the authors mean the lamb1sde1 mutant background or is sde1 another mutation? There should be consistent labeling of lines and mutants to make it easier for the reader to follow.

Response (2.12): We have revised the text on page 13, paragraph 1 to label the lamb1a mutant allele as “lamb1a^sde1” and the lamb1a homozygous mutant as “sde1” for consistency.

12. In Fig. 5, what is the difference between sde1/+ and sde1?

Response (2.13): We referred the heterozygous lamb1a^sde1 mutant as sde1/+ and the homozygous mutant as sde1. For clarification, we have now clearly stated this in the text on page 13, paragraph 1.

13. I don’t see a difference in clonal size looking at the data shown in Fig. 5F. Since sde1 has less clones analyzed than sde1/+, is this data sufficient to draw conclusions about the number of clones with a given size especially if the clone sizes are arbitrarily assigned?

Response (2.14): Because of variation in sample sizes, we used the D'Agostino-Pearson normality test to determine whether the data have a parametric distribution in all figures. If the data set had a normal distribution, we determined the p-value by a two-tailed Student’s t-test. Otherwise, if the data set failed to pass the test, we determined the significance by a two-tailed Mann-Whitney test. With these standard statistical methods, we concluded that the 33% difference seen in Fig. 5F is significant. Of note, the clone sizes shown in Fig. 5F are not arbitrarily assigned, but measurements of actual size. We have now checked and ensured that the details of the statistical analyses are reported for each figure.

14. What exactly is quantified in graphs 4D, E and 5H? The clone size as percentage of total size set to 100%, or the percentage of clones with a given size? I assume the latter, but if so, the terminology should be changed to Clones (%).

Response (2.15): Yes, we meant “the percentage of clones with a given size”. We thank the reviewer for the careful reading and have changed the labeling to “Clones (%)” in Fig. 4F, 4G, and 5H.

15. In the discussion the authors state that “… Lamb1a, is transiently induced in the skin BEC population during post-embryonic growth”. However, Fig. S5 shows that there is a higher expression during adult stages while the difference between AS and PS in juvenile fish is not significant. It is unclear what precisely does the “relative expression” compare? Perhaps this leads to the confusion. Overall this manuscript would benefit from better explanations, especially the last paragraph describing Figure 5 is difficult to follow due to the omission of many important details.

Response (2.16): We have revised the text to clearly state which groups are compared in the RT-qPCR assays on page 13, paragraph 1.

16. Fig. 2J, 3, etc. showing clonal analyses, how many animals were analyzed?

Response (2.17): We have now added the animal numbers to the figure legends.

Other comments:

Fig. S1: I would remove graph 1D showing only BECs, or separate the graph in 1E to only show SECs to make it consistent and less redundant.

Response (2.18): As suggested, we have removed Fig. S1D for consistency.

Fig. 1E does not show 3D rendering but rather an orthogonal view

Response (2.19): Yes, we have revised the text on page 7, paragraph 2.

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There are a number of missing and incorrectly spelled words throughout the manuscript, including the materials and methods. Hence the manuscript would benefit from thorough editing using an editing service.

Response (2.20): We have carefully checked the entire manuscript as advised.

Second decision letter

MS ID#: DEVELOP/2021/199669

MS TITLE: Whole-body clonal mapping identifies giant dominant clones in zebrafish skin epidermis

AUTHORS: Hsiao-Yuh Roan, Tzu-Lun Tseng, and Chen-Hui Chen

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. You will be pleased to see that the referees are happy with your revisions and there is just one issue for you to consider prior to publication regarding inclusion of a supplemental figure in the main text.

Reviewer 1

Advance summary and potential significance to field

This manuscript presents a useful new tool and method for monitoring basal cell clones in the zebrafish skin.

Comments for the author

This manuscript presents a useful new tool and method for monitoring basal cell clones in the zebrafish skin. Revisions have improved the manuscript, and the authors' responses clarified some misunderstandings—for example, why the images do not appear to show clones with multiple hues despite being based on the -bow system. Text revisions in response to Reviewer 2's excellent and thoughtful comments clarify the experimental approach and rationale for several experiments. I particularly appreciate the new experiment tracking clones over several days which is now reported in Figure S6. This experiment highlights a strength of the method, and provides some insight into how "giant clones" arise. If possible, it would be appropriate to include this figure as a main figure, rather than a supplemental figure.

Reviewer 2

Advance summary and potential significance to field

This study contributes to our knowledge of basal keratinocyte development in zebrafish, which is an understudied field.

Comments for the author

No more comments.

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Second revision

Author response to reviewers' comments

As advised, we have now included Figure S6 as a main figure (new Figure 5).

Third decision letter

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MS TITLE: Whole-body clonal mapping identifies giant dominant clones in zebrafish skin epidermis

AUTHORS: Hsiao-Yuh Roan, Tzu-Lun Tseng, and Chen-Hui Chen
ARTICLE TYPE: Techniques and Resources Article

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

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