

## Oncofetal reprogramming drives phenotypic plasticity in WNT-dependent colorectal cancer

Corresponding Author: Dr Ernesto Guccione

This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Nature Genetics.

Version 0:

Decision Letter:

30th Aug 2024

Dear Ernesto,

Your Article, "Oncofetal reprogramming fuels phenotypic plasticity in WNT-driven colorectal cancer" has now been seen by 2 referees. Reviewer #2 is fully satisfied with the revised manuscript. However, Reviewer #1 has a remaining concern regarding the simultaneous need of the OnF and SC states for complete drug response. We are interested in the possibility of publishing your study in Nature Genetics, but would like to consider your response to this concern in the form of a revised manuscript before we make a final decision on publication. Therefore, we suggest you to make Reviewer #2's required textual changes. Please do not hesitate to get in touch if you would like to discuss these issues further.

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

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

When revising your manuscript:

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

\*2) If you have not done so already please begin to revise your manuscript so that it conforms to our Article format instructions, available

[here](http://www.nature.com/ng/authors/article_types/index.html).

Refer also to any guidelines provided in this letter.

\*3) Include a revised version of any required Reporting Summary: <https://www.nature.com/documents/nr-reporting-summary.pdf>

It will be available to referees (and, potentially, statisticians) to aid in their evaluation if the manuscript goes back for peer review.

A revised checklist is essential for re-review of the paper.

Please be aware of our [guidelines](https://www.nature.com/nature-research/editorial-policies/image-integrity) on digital image standards.

Please use the link below to submit your revised manuscript and related files:

Link Redacted

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

We hope to receive your revised manuscript within four to eight weeks. If you cannot send it within this time, please let us know.

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

Nature Genetics is committed to improving transparency in authorship. As part of our efforts in this direction, we are now requesting that all authors identified as 'corresponding author' on published papers create and link their Open Researcher and Contributor Identifier (ORCID) with their account on the Manuscript Tracking System (MTS), prior to acceptance. ORCID helps the scientific community achieve unambiguous attribution of all scholarly contributions. You can create and link your ORCID from the home page of the MTS by clicking on 'Modify my Springer Nature account'. For more information please visit [www.springernature.com/orcid](http://www.springernature.com/orcid).

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

Sincerely,  
Chiara

Chiara Anania, PhD  
Associate Editor  
Nature Genetics  
<https://orcid.org/0000-0003-1549-4157>

Referee expertise:

Referee #1:

Referee #2:

Referee #3:

Reviewers' Comments:

Reviewer #1:

Remarks to the Author:

My concerns raised in the previous review have been satisfactorily addressed.

Regarding the terminology adjustment, I agree with the authors that there is a transition of LGR5+ stem cell state to a hybrid state, but from the data demonstrated in Fig.4c-d, it shows clearly that both OnF and stem cell state alone were downregulated after FOLFIRI treatment (OnF: 7.04% to 5.67%; SC: 24.5% to 12%), while the hybrid state was remarkably activated from 9.66% to 44.4%. This indicates that it is the hybrid state, but not the pure OnF state, that is induced to drive drug tolerance. This is a very important finding as their subsequent in vivo data confirms that depletion of both states simultaneously (but not alone) is required to yield complete response to FOLFIRI. I'd therefore argue that the author needs to improve the clarity of this key finding in the abstract and the text. For instance, in the revised abstract, it states "...targeting the OnF program in combination with the current standard of care is pivotal for achieving effective and durable CRC treatment.". This is inaccurate since targeting either OnF or SC state alone yield the same partial drug response. Only simultaneous targeting of both OnF and SC state shows complete drug response (Fig.4I). Therefore, the accurate statement would be "simultaneous targeting of both the OnF and the canonical stem cell program in combination with...." to reflect their key findings.

Reviewer #2:

Remarks to the Author:

This revised version adequately addresses our previous comments, we therefore support its publication and congratulate the authors for their work.

Michael Tyler and Itay Tirosh

Version 1:

Decision Letter:

Our ref: NG-A66151R

23rd Sep 2024

Dear Ernesto,

thank you for submitting your revised manuscript "Oncofetal reprogramming fuels phenotypic plasticity in WNT-driven colorectal cancer" (NG-A66151R). We find that the paper has improved in revision, and therefore we'll be happy in principle to publish it in Nature Genetics, pending minor revisions to satisfy to comply with our editorial and formatting guidelines.

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

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

Congratulations!

Sincerely,  
Chiara

Chiara Anania, PhD  
Associate Editor  
Nature Genetics  
<https://orcid.org/0000-0003-1549-4157>

Version 2:

Decision Letter:

In reply please quote: NG-A66151R1 Guccione

11th Dec 2024

Dear Dr. Guccione,

I am delighted to say that your manuscript "Oncofetal reprogramming drives phenotypic plasticity in WNT-dependent colorectal cancer" has been accepted for publication in an upcoming issue of Nature Genetics.

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

After the grant of rights is completed, you will receive a link to your electronic proof via email with a request to make any corrections within 48 hours. If, when you receive your proof, you cannot meet this deadline, please inform us at [rjsproduction@springernature.com](mailto:rjsproduction@springernature.com) immediately.

You will not receive your proofs until the publishing agreement has been received through our system.

Due to the importance of these deadlines, we ask that you please let us know now whether you will be difficult to contact over the next month. If this is the case, we ask you provide us with the contact information (email, phone and fax) of someone who will be able to check the proofs on your behalf, and who will be available to address any last-minute problems.

Your paper will be published online after we receive your corrections and will appear in print in the next available issue. You can find out your date of online publication by contacting the Nature Press Office ([press@nature.com](mailto:press@nature.com)) after sending your e-proof corrections.

You may wish to make your media relations office aware of your accepted publication, in case they consider it appropriate to organize some internal or external publicity. Once your paper has been scheduled you will receive an email confirming the publication details. This is normally 3-4 working days in advance of publication. If you need additional notice of the date and time of publication, please let the production team know when you receive the proof of your article to ensure there is sufficient time to coordinate. Further information on our embargo policies can be found here: <https://www.nature.com/authors/policies/embargo.html>

Before your paper is published online, we shall be distributing a press release to news organizations worldwide, which may very well include details of your work. We are happy for your institution or funding agency to prepare its own press release,

but it must mention the embargo date and Nature Genetics. Our Press Office may contact you closer to the time of publication, but if you or your Press Office have any enquiries in the meantime, please contact [press@nature.com](mailto:press@nature.com).

Acceptance is conditional on the data in the manuscript not being published elsewhere, or announced in the print or electronic media, until the embargo/publication date. These restrictions are not intended to deter you from presenting your data at academic meetings and conferences, but any enquiries from the media about papers not yet scheduled for publication should be referred to us.

Please note that *Nature Genetics* is a Transformative Journal (TJ). Authors may publish their research with us through the traditional subscription access route or make their paper immediately open access through payment of an article-processing charge (APC). Authors will not be required to make a final decision about access to their article until it has been accepted. [Find out more about Transformative Journals](https://www.springernature.com/gp/open-research/transformative-journals)

**Authors may need to take specific actions to achieve [compliance](https://www.springernature.com/gp/open-research/funding/policy-compliance-faqs) with funder and institutional open access mandates.** If your research is supported by a funder that requires immediate open access (e.g. according to [Plan S principles](https://www.springernature.com/gp/open-research/plan-s-compliance)) then you should select the gold OA route, and we will direct you to the compliant route where possible. For authors selecting the subscription publication route, the journal's standard licensing terms will need to be accepted, including [those licensing terms](https://www.nature.com/nature-portfolio/editorial-policies/self-archiving-and-license-to-publish) will supersede any other terms that the author or any third party may assert apply to any version of the manuscript.

If you have any questions about our publishing options, costs, Open Access requirements, or our legal forms, please contact [ASJournals@springernature.com](mailto:ASJournals@springernature.com)

If you have posted a preprint on any preprint server, please ensure that the preprint details are updated with a publication reference, including the DOI and a URL to the published version of the article on the journal website.

To assist our authors in disseminating their research to the broader community, our SharedIt initiative provides you with a unique shareable link that will allow anyone (with or without a subscription) to read the published article. Recipients of the link with a subscription will also be able to download and print the PDF.

As soon as your article is published, you will receive an automated email with your shareable link.

You can now use a single sign-on for all your accounts, view the status of all your manuscript submissions and reviews, access usage statistics for your published articles and download a record of your refereeing activity for the Nature journals.

An online order form for reprints of your paper is available at <https://www.nature.com/reprints/author-reprints.html>. Please let your coauthors and your institutions' public affairs office know that they are also welcome to order reprints by this method.

If you have not already done so, we strongly recommend that you upload the step-by-step protocols used in this manuscript to protocols.io. protocols.io is an open online resource that allows researchers to share their detailed experimental know-how. All uploaded protocols are made freely available and are assigned DOIs for ease of citation. Protocols can be linked to any publications in which they are used and will be linked to from your article. You can also establish a dedicated workspace to collect all your lab Protocols. By uploading your Protocols to protocols.io, you are enabling researchers to more readily reproduce or adapt the methodology you use, as well as increasing the visibility of your protocols and papers. Upload your Protocols at <https://protocols.io>. Further information can be found at <https://www.protocols.io/help/publish-articles>.

Sincerely,  
Chiara

Chiara Anania, PhD  
Associate Editor  
Nature Genetics  
<https://orcid.org/0000-0003-1549-4157>

Click here if you would like to recommend Nature Genetics to your librarian  
<http://www.nature.com/subscriptions/recommend.html#forms>

\*\* Visit the Springer Nature Editorial and Publishing website at [http://editorial-jobs.springernature.com?utm\\_source=eJP\\_NGen\\_email&utm\\_medium=eJP\\_NGen\\_email&utm\\_campaign=eJP\\_NGen](http://editorial-jobs.springernature.com?utm_source=eJP_NGen_email&utm_medium=eJP_NGen_email&utm_campaign=eJP_NGen) for more information about our career opportunities. If you have any questions please click [here](mailto:editorial.publishing.jobs@springernature.com).

**Open Access** This Peer Review File is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

In cases where reviewers are anonymous, credit should be given to 'Anonymous Referee' and the source.

The images or other third party material in this Peer Review File are included in the article's Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.

To view a copy of this license, visit <https://creativecommons.org/licenses/by/4.0/>

Reviewer #1:

Remarks to the Author:

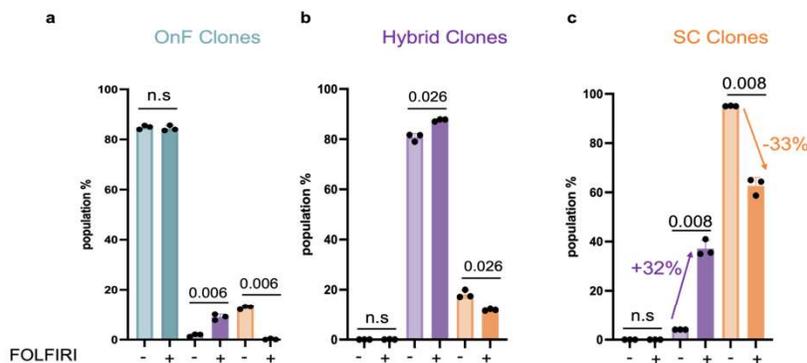
My concerns raised in the previous review have been satisfactorily addressed.

Regarding the terminology adjustment, I agree with the authors that there is a transition of LGR5+ stem cell state to a hybrid state, but from the data demonstrated in Fig.4c-d, it shows clearly that both OnF and stem cell state alone were downregulated after FOLFIRI treatment (OnF: 7.04% to 5.67%; SC: 24.5% to 12%), while the hybrid state was remarkably activated from 9.66% to 44.4%. This indicates that it is the hybrid state, but not the pure OnF state, that is induced to drive drug tolerance. This is a very important finding as their subsequent in vivo data confirms that depletion of both states simultaneously (but not alone) is required to yield complete response to FOLFIRI. I'd therefore argue that the author needs to improve the clarity of this key finding in the abstract and the text. For instance, in the revised abstract, it states "...targeting the OnF program in combination with the current standard of care is pivotal for achieving effective and durable CRC treatment.". This is inaccurate since targeting either OnF or SC state alone yield the same partial drug response. Only simultaneous targeting of both OnF and SC state shows complete drug response (Fig.4I). Therefore, the accurate statement would be "simultaneous targeting of both the OnF and the canonical stem cell program in combination with...." to reflect their key findings.

We are pleased that all reviewers were satisfied with the revised manuscript.

In response to Reviewer #1, we have adjusted the terminology in the text to clarify the points raised. We would like to bring to this reviewer's attention that the marginal fluctuation in the pure OnF state OnF: 7.04% to 5.67% is not statistically significant ( $p = 0.096$ ; Fig. 4d).

We acknowledge that it is perhaps challenging to draw definitive conclusions from the concurrent analyses of all 3 cells states (**Fig. 4c-d**). To address this, we conducted a more detailed assessment of individually sorted clones, which are predominantly in either an OnF, Stem, or Hybrid state, to evaluate their response to FOLFIRI. Below, we present a more detailed breakdown of the changes observed in each state across the various clones in response to FOLFIRI (**panels a-c**). These data provide compelling evidence that:



1. The proportion of cells in the pure OnF state remains largely unchanged (**panel a** below - break downs of original **Ext. Data Fig. 4j**). Notably, there's a significant increase in hybrid cells, which is better understood in light of data presented in **panel c**, discussed point 2 below.

The **persistence of cells with an active OnF program**—encompassing both pure OnF and Hybrid cells—demonstrates their **inherent resistance to FOLFIRI**. As requested by this reviewer, we have revised the terminology in the text to clarify that cells "with an active OnF program" includes both the pure OnF and hybrid states.

2. Conversely, we observed a specific and **substantial reduction of cells in the pure LGR5+ SC state** (-33%), which coincided with a corresponding increase in the hybrid state (+32%) (most

evident in **Panel c**). This demonstrates that cells in the pure **LGR5<sup>+</sup> SC** state are sensitive to FOLFIRI, and it suggests an activation of the of the OnF program in the surviving SCs.

In line with this reviewer's request, we have modified the abstract to clarify 1) that drug-tolerant states (pure OnF and Hybrid) are characterized by an active OnF program, and 2) that the pure/canonical LGR5<sup>+</sup> SC state is sensitive to FOLFIRI.

This provides a rationale for **combining FOLFIRI, which targets the pure LGR5<sup>+</sup> SC state**, with modalities/**therapies that inhibit the OnF program** (such as DT or inhibitors of YAP and AP-1) **to achieve a more effective and durable treatment for CRC, as demonstrated in our Fig. 4I-q.**

In brief, the experiments in **Fig. 4I-q** provide compelling evidence that targeting cells with an active OnF program -pure OnF and Hybrid- in combination with FOLFIRI is an effective strategy. Further targeting the pure LGR5<sup>+</sup> SC state program, as suggested, is redundant with the use FOLFIRI, which is sufficient to deplete this population (**panel c**). Mechanistically, FOLFIRI depletes canonical LGR5<sup>+</sup> SCs, but not entirely; those who survive this treatment have activated the OnF program, entering a hybrid state. Therefore, successful targeting of this program or its drivers (YAP/AP-1) enhances the effectiveness of FOLFIRI by eliminating these hybrid cells as well as the pure OnF cells.

Please provide a point-by-point response to the following requests:

-As previously requested, please include an **Ethics** section as a first paragraph of the Methods and a **Code Availability Statement**.

-As previously mentioned, even if your study doesn't include the use of custom codes, you should please provide a Code Availability Statement clearly stating that you did not use custom codes. **The reader should be directed to details of software packages in the appropriate vignette within the Methods**. However, we strongly recommend that ***all*** code used should be deposited in a DOI-minting repository such as Zenodo, Gigantum or Code Ocean and the link cited in the Methods-only reference list. Authors are encouraged to manage subsequent code versions and to use a license approved by the open source initiative. Additional details can be found here: <https://www.nature.com/nature-portfolio/editorial-policies/reporting-standards#availability-of-computer-code> and <https://www.nature.com/articles/s41588-023-01411-0>.

- Please check and confirm that full version numbers are provided for all software packages.

A paragraph providing the ethics statement has been added at the beginning of the Methods section. A Code Availability Statement has also been included, and the details of the software packages and relevant citations have been verified.

-Main Fig. 1 d-l: scatter plots need color scale?

These panels do not require a color scale, as the colors represent different samples or conditions. Labels and a legend have been provided for clarity.

-Main Fig. 2a: color scale needs label?

Color scale is now labeled.