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| MRC Integrative Epidemiology Unit  University of Bristol  Oakfield House  Bristol BS8 2BN  UK |

Dear Editor,

**Re. Exploiting horizontal pleiotropy to search for causal pathways within a Mendelian randomization framework**

We are very grateful that our manuscript has been accepted for publication by *Nature Communications*.

We have carefully checked our manuscript again to comply with the journal’s policies and format requirements. We enclosed the revised manuscript and our detailed responses to Editorial comments. Two Supplementary tables were provided as individual Supplementary Data files following the editorial comment as follows:

**Supplementary Data 1. MR of candidate traits on each outcome.** SNP, single nucleotide polymorphism; N SNPs, number of SNPs; SE, Standard error; VLDL, very low-density lipoprotein; HDLC, high density lipoprotein cholesterol; LDLC, low density lipoprotein cholesterol; CI, confidence interval. The number of SNPs used for two sample MR analysis of candidate traits on the outcome. The results were presented as IVW beta coefficient (95% CI), derived from two sample MR analyses.

**Supplementary Data 2. Proportion of unbiased estimates and AUROC value across different scenarios and methods.** The top panel of the table presents the proportion of simulations that lead to unbiased effect estimates of X on Y. The bottom panel of the table presents the ability (proportion) of the method to distinguish between simulations in which the causal effect of x on y is either null or not null. 'Raw’ = IVW random effects estimates applied to all detected instruments; ‘Removed’ = either all outliers are removed, or only outliers detected to associate with a candidate trait; ‘MVMR’ = multivariable MR using either candidate traits detected to associate with any instrument or using only candidate traits associated with outlier instruments; ‘Adjusted’ = Adjusting SNP-outcome associations for candidate traits applied either only to variants detected to be outliers, or all variants regardless of outlier status.

All authors have agreed to be listed and have approved the changes to the author list. The relevant written document with physical signatures from all authors were provided. We wish to participate in transparent peer review and to be mentioned on the journal’s twitter account with the following handles: @explodecomputer.

If there is any further information you need, please do not hesitate to get in touch with us.

Thank you very much for your time and consideration.

Yours sincerely,

Yoonsu Cho, George Davey Smith and Gibran Hemani on behalf of all authors

**EDITORIAL REQUESTS:**  
  
**TITLE PAGE**  
  
**\* To adhere to journal style, I suggest the following revision to the title. If you would like to suggest an alternative title, please ensure that it does not exceed 15 words and does not contain punctuation.  
  
"MR-TRYX is a Mendelian randomization framework that exploits horizontal pleiotropy to infer putative causal pathways"**

**Answer)** Thanks for the suggestion. We have changed the title as follows: “Exploiting horizontal pleiotropy to search for causal pathways within a Mendelian randomization framework”

**\* Changes to Author list:   
Our policy is that any changes to the author list from the initial submission must be approved by all authors. As such, please obtain written permission from all co-authors approving the addition of Eleanor Sanderson as additional co-author. You can either 1/ provide an PDF of a string of emails with electronic approval from all other authors approving the additional author, or 2/ devise a written document using the format below to obtain physical signatures from all co-authors. Please upload this material for the final submission.   
  
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**Answer)** Signed document has been provided.

**\* In the abstract, please revise/remove the sentence referring to the name of the method and William Bateson - this information is irrelevant and not understandable to a potential reader who is not familiar with this quote/concept. The abstract should focus on what was developed and what we can do with it and learn from it. You can explain your reasons for choosing this particular name in the introduction if you wish (with a bit more context than is currently given). The word limit for the abstract is 150 words.**

**Answer)** We have revised the sentence referred to William Bateson.

**\* When discussing the current work in the abstract, please use the present tense.**

**Answer)** Revised.  
  
  
**MAIN TEXT  
  
\* The final paragraph of the Introduction should summarise the major results and conclusions of this manuscript, in the present tense. Please expand on what you have found/main conclusions.**

**Answer)** Revised.

**\* Please revise the section order of the manuscript to Introduction -> Results -> Discussion -> Methods -> Data availability statement -> References -> End notes -> Figure legends -> Tables. I would suggest that you include the paragraph “Overview of MR-TRYX” (currently in Methods) as the first paragraph of the Results section. Further, some brief introductory sentences might be necessary for various parts of the results as the methods section is not before the results section anymore.**

**Answer)** The section order of the manuscript has been revised.

**\* Please shorten all subheadings in the Results section to fewer than 60 characters including spaces.**

**Answer)** Revised.   
  
  
**LANGUAGE AND STYLE**  
  
**\* Please remove language such as "new", "novel", "for the first time", "unprecedented", etc. Novelty should be clear from the context. If absolutely necessary, you can replace ‘novel’ with ‘previously unreported’ or similar.**

**Answer)** Revised.

**\* Please avoid using speech marks around words or phrases. In most cases they are unnecessary.**

**Answer)** Speech marks have been removed.

**\* Please make sure that mathematical terms throughout your manuscript and Supplementary Information (including in figures, figure axes, and legends) conform strictly to the following guidelines. Equations should be supplied in editable format, and not as images. Scalar variables (e.g. x, V, χ) should be typeset in italic, whereas multi-letter variables should be formatted in roman. Constants (e.g. ħ, G, c) should be typeset in italics (the only exceptions being e, i, π, which should be typeset in Roman) and vectors (such as r, the wavevector k, or the magnetic field vector B) should be typeset in bold without italics. In contrast, subscripts and superscripts should only be italicised if they too are variables or constants. Those that are labels (such as the 'c' in the critical temperature, T\_c, the 'F' in the Fermi energy, E\_F, or the 'crit' in the critical current, I\_crit) should be typeset in roman. To avoid doubt, unit dimensions should be expressed using negative integers (e.g. kg m^-1 s^-2, not kg/ms^2) or the word 'per'.**  
**Answer)** Revised

**\* Please label equations sequentially as (1), (2), (3), etc. Subdivisions (1a, 1b, or 1.1, 1.2) are not permitted.**

**Answer)** All equations are labelled.

**\* Wherever p-values are stated in the text and figure legends, please also state the name of the statistical test.**

**Answer)** The name of the statistical test has been provided where it is required.  
  
  
**METHODS AND DATA  
  
\* Please shorten all subheadings in the Methods section to fewer than 60 characters including spaces.**

**Answer)** Revised.

**\* We allow only one level of subheadings in the Methods section. Please remove secondary subheadings.**

**Answer)** We used one level of subheadings in the Methods section. For the simulation, we would like to keep the current format assuming this format can be transformed to the same format as the attached paper: <https://www.nature.com/articles/s41467-019-09542-x#Sec9>

**\* Please confirm that you have complied with all relevant ethical regulations for work with human participants, and that informed consent was obtained. Please state this in the Methods section, including the name of the board and institution that approved the study protocol**.

**Answer)** The present study is exempted from ethical consideration since we only used secondary data (summary statistics) which are publicly available. Confidential information from human participants will not be used at any point.

**\* All *Nature Communications* manuscripts must include a section titled "Data Availability" as a separate section after the Methods section and before the References. For more information on this policy, and a list of examples, please see** [**http://www.nature.com/authors/policies/data/data-availability-statements-data-citations.pdf**](http://www.nature.com/authors/policies/data/data-availability-statements-data-citations.pdf)

**Answer)** The data source has been provided.

**\* DATA SOURCES: Nature Research** [**policies**](https://www.nature.com/authors/policies/availability.html#data) **strongly encourage deposition of research data in public repositories and in some cases this is mandatory, and you will have been previously advised if that was the case. If you need help depositing and curating your research data (including raw and processed data, text, video, audio and images) you should consider:  
  
Contacting Springer Nature’s Research Data** [**Helpdesk**](https://www.springernature.com/gp/authors/research-data-policy/helpdesk/12327114) **for advice  
  
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Please refer to our data policies here:** [**http://www.nature.com/authors/policies/availability.html**](http://www.nature.com/authors/policies/availability.html)

**Answer)** A permanent URL for data source has been provided.

**END NOTES  
  
\* Please supply an Acknowledgements section after the References.**

**Answer)** Acknowledgements section has been added.

**\* Please update any preprints in the reference list with details of the published paper, if possible. If the paper has not been published, please cite the preprint in the style: Babichev, S. A., Ries, J. & Lvovsky, A. I. Quantum scissors: teleportation of single-mode optical states by means of a nonlocal single photon. Preprint at** [**http://arXiv.org/quant-ph/0208066**](http://arXiv.org/quant-ph/0208066) **(2002).**

**Answer)** Preprints in the reference list has been updated.  
  
  
**DISPLAY ITEMS**  
 **\* Please check whether your manuscript or Supplementary Information contain third-party images, such as figures from the literature, stock photos, clip art or commercial satellite and map data. We strongly discourage the use or adaptation of previously published images, but if this is unavoidable, please request the necessary rights documentation to re-use such material from the relevant copyright holders and return this to us when you submit your revised manuscript.**

**Answer)** We confirm that there are no third-party images in the manuscript and supplementary file.

**\* Please ensure that figure legend titles are brief - they should not occupy more than one line in the final proof.**

**Answer)** The figure legend titles have been revised.  
  
**\* Please ensure that each display item is no larger than a single A4 portrait page (260x179 mm). Please upload figures as individual high-resolution images and not embedded in the article file.**  
**Answer)** Figures in the article file have been removed. Individual images are provided with high-resolution.

**\* Data in tables must be free from bold/italic formatting unless this has been clearly defined in the footnote. Tables need to be black and white, fit onto a single A4 portrait page and can contain only one row of column titles. Finally, we are unable to merge cells or include vertical dividing lines or diagonal lines. Please format your tables accordingly. Specifically, please revise both tables so that they do not contain any merged cells.**

**Answer)** Merged cells have been removed from the tables.

**\* Please ensure that all figures are cited in the text in the order in which they appear. If a figure needs to be referred to out of order, please use the text "see below" instead of a citation.**

**Answer)** All figures are cited in the text in the order.

**\* Please define any new abbreviations, symbols or colours present in your figures in the associated legends. Please do not use symbols in your legend, instead please write out the symbols in words (blue circles, red dashed line, etc.).**

**Answer)** Figure legends have been double checked.  
  
  
**SUPPLEMENTARY INFORMATION  
  
\* We do not edit Supplementary Information files; they will be uploaded with the published article as they are submitted with the final version of your manuscript. Any tracked changes should be removed from the file and the file should be provided as a PDF file. Supplementary Figures do not need to be provided separately.**

**Answer)** We confirm that there are no tracked changes in Supplementary information files  
  
**\* Supplementary References should appear at the end of the Supplementary Information file, and should be self-contained and numbered from 1. References mentioned in both the main text and the Supplementary Information should be part of both reference lists so that the Supplementary Information does not refer to the reference list in the main paper and vice versa.**

**Answer)** Supplementary References have been double checked.

**\* Large datasets and unwieldy tables should be supplied as Supplementary Data files, whereas smaller tables should be supplied as supplementary tables. As such, please include supplementary tables 1,3 and 4 in the merged SI pdf and provide supplementary tables 2 and 5 as individual ‘Supplementary Data’ files instead (please do not merge them).**

**Answer)** Supplementary tables 2 and 5 were provided as Supplementary Data.  
  
**\* Please supply legends for each Supplementary Movie/Audio/Data file in your cover letter (not in the Supplementary Information file). Please label each files as Supplementary Movie/Audio/Data 1, etc.**

**Answer)** Legends for Supplementary data were provided in the cover letter.  
  
---------------------------------------------------------------------  
  
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**Answer)** All authors have agreed to participate in transparent peer review. The relevant statement can be found in the cover letter.

**\* An updated editorial policy checklist that verifies compliance with all required editorial policies must be completed and uploaded with the revised manuscript. All points on the policy checklist must be addressed; if needed, please revise your manuscript in response to these points. Please note that this form is a dynamic "smart pdf" and must therefore be downloaded and completed in Adobe Reader, instead of opening it in a web browser.  
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**Answer)** The completed form is provided.

**\* Your paper will be accompanied by a two-sentence Editor's summary, of between 250-300 characters including spaces, when it is published on our homepage. Could you please approve the draft summary below or provide us with a suitably edited version.**  
**“In Mendelian randomization (MR) studies, one typically selects SNPs as instrumental variables that do not directly affect the outcome to avoid violation of MR assumptions. Here, Cho et al. present a framework, MR-TRYX, that leverages knowledge of such outliers of horizontal pleiotropy to identify putative causal relationships between exposure and outcome.”**  
**Answer)** Thanks for the draft summary you provided. We would like to revise the summary as follows:

“In Mendelian randomization (MR) studies, invalid instruments that influence the outcome through other pathways then the exposure violate MR assumptions. Here, Cho et al. present a framework, MR-TRYX, that leverages knowledge of such outliers that represents horizontal pleiotropy to identify putative causal pathway for the outcome.”

**\* As part of our efforts to communicate our content to a wider audience, we endeavour to highlight papers published in *Nature Communications* on the journal’s Twitter account (**[**@NatureComms**](https://twitter.com/NatureComms)**). If you would like us to mention authors, institutions or lab groups in these tweets, please provide the relevant twitter handles in your cover letter upon resubmission.**  
**Answer)** Exploiting horizontal pleiotropy that violates MR assumption to identify putative causal risk factors for an outcome of interest.

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**Answer)** We have read and acknowledge the policy.

**\* In recognition of the time and expertise our reviewers provide to *Nature Communications*’s editorial process, as of November, 2018, we formally acknowledge their contribution to the external peer review of articles published in the journal. All peer-reviewed content will carry an anonymous statement of peer reviewer acknowledgement, and for those reviewers who give their consent, we will publish their names alongside the published article. For more information, please refer to our FAQ page at** [**https://www.nature.com/documents/ncomms-reviewer-information.pdf**](https://www.nature.com/documents/ncomms-reviewer-information.pdf)

**Answer)** We have read and acknowledge the policy.

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\* A cover letter describing your response to our editorial requests.**

**\* A separate document detailing your point-by-point response to any issues raised by our referees (please include the referees' comments in this document).**

**\* The final version of your text as a Word or TeX/LaTeX file, with any tables prepared using the Table menu in Word or the table environment in TeX/LaTeX and using the 'track changes' feature in Word.**

**\* The complete author list provided in the article file, which must match that given on our manuscript tracking system. The author list in the main article file will be used during typesetting of your article.**

**\* Production-quality versions of all figures, supplied as separate files containing all panels. To ensure the swift processing of your paper please provide the highest quality, vector format, versions of your images (.ai, .eps, .psd) where available. Please see our** [**brief guide to manuscript submission**](https://www.nature.com/documents/ncomms-submission-guide.pdf) **for further details on the figure formats we can accept. Text and labelling should be in a separate layer to enable editing during the production process. If vector files are not available then please supply the figures in whichever format they were compiled in and not saved as flat .jpeg or .TIFF files. Any chemical structures or schemes contained within figures should additionally be supplied as separate ChemDraw (.cdx) files. If your artwork contains any photographic images, please ensure these are at least 300 dpi.  
  
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**Answer)** We have carefully read the policies and double checked if our manuscript complies with the policy.

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2. In the ‘Personal profile’ tab, click on ‘ORCID Create/link an Open Researcher Contributor ID (ORCID)’. This will re-direct you to the ORCID website.   
3a. If you already have an ORCID account, enter your ORCID email and password and click on ‘Authorize’ to link your ORCID with your account on the MTS.  
3b. If you don’t yet have an ORCID account, you can easily create one by providing the required information and then clicking on ‘Authorize’. This will link your newly created ORCID with your account on the MTS.  
  
If you experience problems in linking your ORCID, please contact** [**Platform Support**](mailto:platformsupport@macmillan.com)

Answer) We have informed all co-authors.