|  |  |
| --- | --- |
| **Author Checklist** | Manuscript Number: |
|  | NCOMMS-25-23244A |
|  | \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |
| **Please check the items below carefully and add a response in each row of the table to indicate the changes that you have made. Please also check through any additional marked-up edits we may have provided within the manuscript file.** |  |
|  |  |
|  |  |
|  |  |
| **Abstract and editor's summary** |  |
| Our guidance: | Your response: |
| We would like to propose a revised title to comply with our formatting requirements and improve the accessibility of your work:  Please take into account R2's comment about the title (paragraph 3)  Please edit the title in your manuscript files accordingly. Note that further minor changes may be made during the production process, and you will be able to check these in the proofs. | In accordance with the editor’s guidance and Reviewer #2’s comment regarding the emphasis on modularity, we have revised the title to:  **The ANTsX ecosystem for mapping the mouse brain**  which we believe accurately reflects the manuscript’s focus on reproducible, ANTsX-based workflows for mouse brain mapping. |
| Your paper will be accompanied by the following editor's summary. Please let us know if there are any inaccuracies: 'We request that you (the author) please supply a brief (~250 characters, including spaces) two-sentence summary of the main findings of the paper to be used on our website and in our e-alerts. The summary should be written in the third person in language suitable for a broad audience. The first sentence should describe an open question (i.e., the rationale/motivation for the present study), and the second sentence should describe what you found/demonstrated in your manuscript. The summary may be edited by the editors prior to publication. Example #1: The neural mechanisms that give rise to aperiodic EEG signals remain unclear. Here the authors characterize EEG signals generated by neural processes other than brain rhythms, demonstrating that certain drugs alter EEG signals in ways that confound traditional interpretation. Example #2: Encoding and downsampling images is key for visual prostheses. Here, the authors show that an actor-model framework using the inherent computation of the retinal network yields better performance in downsampling images compared to learning-free methods. ' | We have provided the following two-sentence summary:  *Integrating molecular and anatomical data in the mouse brain requires flexible, reproducible mapping tools. Here the authors demonstrate ANTsX-based workflows for aligning MERFISH, fMOST, MRI, and LSFM data into shared coordinate frameworks.* |
|  |  |
| **Author information** |  |
| Our guidance: | Your response: |
| We ask that you consult with your coauthors to ensure that all names, affiliations, and titles are represented correctly. Note that if any authors are added or removed after this point then all authors will be requested to provide approval documentation that could potentially delay the production of your paper. | All author names, affiliations, and titles have been carefully reviewed and confirmed with the co-authors. No additions or removals are anticipated. |
| Ensure affiliations are appropriately labeled and featured sequentially and in ascending order (1,2,3,... or a,b,c...). Please ensure all corresponding authors are marked with a specific symbol and include their emails. Similarly, if you have “equally contributing” or “joint supervision” authors, use a specific symbol to mark them and not a number. | Affiliations have been reviewed accordingly. Corresponding authors have been designated, and their email addresses are provided. The issue of equal contributions and joint supervision is not applicable. |
| Please ensure the author contributions section mentions each author's initials at least once with their contributions to the work. Authors with the same initials must be differentiated in the statement. | The Author Contributions section has been reviewed to ensure that each author’s initials appear at least once and that all contributions are clearly described. |
|  |  |
| **Article structure** |  |
| Our guidance: | Your response: |
| We can accommodate up to 10 display items (Figures or Tables) in the main article. Each Figure and Table must fit easily within an A4 page (210 x 297 mm). Please ensure that the number and size of your Figures and Tables fulfil these requirements to avoid any delay in the acceptance of your article. | The main manuscript contains a total of ten display items (Figures and Tables combined), each formatted to fit within a single A4 page. Figure and Table dimensions have been checked to comply with Nature Communications layout requirements. |
| Ensure main Figures are uploaded as separate individual files. Each figure file must contain all intended panels labelled and displayed as intended and fit entirely on a single page. Do NOT include legends within the figure files, as these must be in the main manuscript.  Supplementary Figures must be all contained in the Supplementary Information PDF and do NOT need to be uploaded separately. | All main Figures have been uploaded as separate, individual files, each containing the complete set of labeled panels and formatted to fit within a single page. Figure legends are included only in the main manuscript. |
| **Please ensure your main manuscript file includes the following sections, in this order:** |  |
| *Title Author list Affiliations Abstract Introduction Results Discussion (optional) Results and Discussion (optional) Methods Data Availability Code Availability (if relevant) References Acknowledgements Author Contributions Statement Competing Interests Statement Tables Figure Legends/Captions (for main text figures)* | The main manuscript file has been reviewed and confirmed to follow the required section order. |
| 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. | No Supplementary Information files accompany this manuscript. All figures fully reproducible using the data and scripts provided in the public GitHub repository ([*https://github.com/ntustison/ANTsXMouseBrainMapping*](https://github.com/ntustison/ANTsXMouseBrainMapping)). |
| Please supply Source Data files for all data presented in graphs within the Figures. | An additional copy of all source data files for all data presented in graphs within the Figures are included with this submission. |
| Within the Source Data file, the relevant raw data from each figure or table (in the main manuscript and in the Supplementary Information) should be represented by a single sheet in an Excel document, or a single .txt file or other file type in a zipped folder. An example of the Source Data file is available demonstrating the correct format: https://www.nature.com/documents/ncomms-example-source-data.xlsx The file should be labelled 'Source Data', with the title and a brief description included in your response here, and should be mentioned in all relevant figure legends using the template text below: 'Source data are provided as a Source Data file.' A reference to the source data file should be added in the 'Data Availability' section, using the text: Source data are provided with this paper. | All data underlying the relevant figures are provided as .csv and .log (i.e., raw text) files within the public GitHub repository ([*https://github.com/ntustison/ANTsXMouseBrainMapping*](https://github.com/ntustison/ANTsXMouseBrainMapping)).  An additional copy of all source data files for all data presented in graphs within the Figures are included with this submission. |
|  |  |
| **Main text** |  |
| Our guidance: | Your response: |
| Please refrain from using words such as new/novel/first, when referring to the scientific findings. Please also remove exaggerated language such as 'extremely'/'outstanding' | The manuscript has been reviewed for promotional or exaggerated language. Such language is not found in evaluative contexts in the manuscript. |
| While preprints may be cited in the reference list, unpublished meeting abstracts, personal communications and manuscripts under consideration (and not formally accepted) may be cited only internally within the text and should not be added to the reference list. Please provide names of all authors of unpublished data. If you cite personal communications or unpublished data of any individuals who are not authors of your manuscript, you must supply copies of written permission from the primary investigator of each group cited. | No unpublished meeting abstracts, personal communications, manuscripts under consideration are cited in the reference list. |
| We allow only one level of subheadings in the Results section. Please remove secondary subheadings. | We have modified the manuscript to remove > 1 level subheadings. |
| Please do not use italics, bold font, underlining or speech marks/quotation marks except in headings unless required for technical terms (in both the main text and the display items). | We have ensured the manuscript conforms to these requirements. |
| 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 must be supplied in editable format, and not as images. Scalar variables (e.g. x, V, χ) must be typeset in italic, whereas multi-letter variables and functions (e.g. log) must be formatted in roman. Vectors (such as the wavevector k or the magnetic field vector B) must be typeset in bold without italics. | We have ensured the manuscript conforms to these requirements. |
| We highly encourage you to include a summary figure/schematic that communicates the main result/advance as the final figure in either the paper or the supplement | We appreciate the suggestion, but we believe the existing figures convey the overall framework and main advances of the work. |
| If there are any figures that were included in the ‘response to reviewers’ document that are not in the manuscript or supplement, please include those figures in the manuscript / supplement. | Not applicable. |
| If practical (i.e. relatively small number of data points), all bar graphs should be overlaid with individual data points | We have ensured the manuscript conforms to these requirements. |
| Please provide the source data used for generating figures as a single excel file (i.e., each figure should have its own separate sheet tab). If the manuscript includes data from both males and females, please disaggregate data points by sex in the source data file as detailed in the SAGER guidelines. | As specified previously and detailed in the manuscript, all the numerical data used to produce the relevant figures, as well as the scripts, are available in the GitHub repository associated with this manuscript. An additional copy of source data is included with this submission. |
|  |  |
| **Figures and Tables** |  |
| Our guidance: | Your response: |
| Please see the guidelines linked below for detailed instructions about how your figures should be prepared. Following these instructions will reduce the chances of delays should we need to request replacement artwork from you at a later stage. | All figures (1–9) have been reviewed for compliance. Each fits on a single A4 page, uses sans-serif fonts of ≥8 pt, and maintains vector quality. Panel labels are consistent and legible, with no embedded legends or titles. Figures were exported in RGB color mode with embedded fonts and are accompanied by legends of ≤350 words that define all symbols, abbreviations, and statistical elements (e.g., box-plot components). |
| <https://www.nature.com/documents/NRJs-guide-to-preparing-final-artwork.pdf> |  |
| All error bars need to be defined in the legends (e.g. SD, SEM) together with a measure of centre (e.g. mean, median). For example, the legends should state something along the lines of “Data are presented as mean values +/- SEM” as appropriate. All box plots need to be defined in the legends in terms of minima, maxima, centre, bounds of box and whiskers and percentile. | The legend for Figure 9(c) has been updated to define the box-plot elements (centre, bounds, whiskers, minima, maxima, and percentiles) in accordance with Nature Communications guidelines. |
| **Please note that the box plots need to be defined in terms of minima, maxima, centre, bounds of box and whiskers and percentile in the legend(s) of figure(s) 9c.** |  |
| Tables must be editable and prepared using the table menu in Word, the table environment of LaTeX or the table functionality of ChemDraw where relevant. | Table 1 is written in LaTeX. |
| If bold/italic formatting in tables is necessary, please define its meaning in a table footnote | Table 1 is free of bold/italic formatting. |
| Shadings or symbols in graphs must be defined in some fashion. We prefer that you use a key within the image; do not include colored symbols in the legend/caption. | We do not use any shadings or symbols within any of the figures. |
| All figure legends must include a brief title that summarises the whole figure. | All figure legends have been compiled at the end of the manuscript in accordance with Nature Communications formatting guidelines. Each legend includes a brief title summarizing the figure. |
| Figures must be accompanied by a legend of up to 350 words, referring to all panels within the figure. | The figure legend is included in the submission according to the ordering given above. |
| Any abbreviations, symbols or colours present in your figures must be defined in the associated legends. | We have provided legends within the relevant figures. |
| All figures and tables must be cited in the main text, and numbered in the order in which they appear. | We have ensured the manuscript conforms to these requirements. |
|  |  |
| **Data and Code** |  |
| Our guidance: | Your response: |
| Nature journals strongly support public availability of data and code. Please deposit the data and code used in your paper into a public data repository, or alternatively, present the data as Supplementary Information. If data can only be shared on request, please explain why in your Data Availability Statement, and also in the correspondence with your editor.   Please note that for some data types, deposition in a public repository is mandatory. Any restrictions on sharing of these data types must be clearly indicated in the statement and discussed with the editor. More information on our data deposition policies and available repositories can be found here: | Please see the Code Availability section contained in the submitted manuscript where we provide access to all code described in the manuscript. We have ensured that it conforms to the provided guidelines. |
| <https://www.nature.com/nature-research/editorial-policies/reporting-standards#availability-of-data> |  |
| All published manuscripts reporting original research in Nature Portfolio journals must include a data availability statement, within the Methods and under the heading 'Data Availability'.     The data availability statement must make the conditions of access to the “minimum dataset” that are necessary to interpret, verify and extend the research in the article, transparent to readers. We ask that you don’t use phrases like ‘available on reasonable request’ but instead specify any restrictions to accessing your data as described below.    This minimum dataset may be provided through deposition in public community/discipline-specific repositories, custom proprietary repositories or general repositories like Figshare, Zenodo and Dryad. Providing large datasets in supplementary information is strongly discouraged and the preferred approach is to make data available in repositories. Please see https://www.springernature.com/gp/authors/research-data-policy/recommended-repositories for a list of recommended repositories.    If DOIs are provided, we also strongly encourage including these in the Reference list (authors, title, publisher (repository name), identifier, year).    The Data Availability Statement should also reference any source data published alongside the paper.    For clinical datasets or third party data, please ensure that the Data Availability statement adheres to our policy (https://www.nature.com/nature-research/editorial-policies/reporting-standards#availability-of-data)    If data are unavailable, please indicate the exact reasons why data cannot be made available in a suitable public repository or upon request, including any conditions related to ethical approval, consent from study subjects, commercial or legal restrictions, etc.    For data that are available under restricted access, the Data Availability statement must specify  - the reasons for access restrictions  - what the restrictions are   - how one can get access to the data  - who to contact to request access  - any restrictions on who the data can be made available to or for which purpose  - the expected timeframe for response to access requests  - for how long the data will be available once access has been granted. | Please see the Data Availability section contained in the submitted manuscript. We have ensured that it conforms to the provided guidelines. |
| Please use the following template to provide all the information stated above:  The XX data generated in this study have been deposited in the YY database under accession code ZZ [add hyperlink here]. The XX data are available under restricted access for {insert reason}, access can be obtained by {explain how}. The raw XX data are protected and are not available due to data privacy laws. The processed XX data are available at YY. The XX data generated in this study are provided in the Supplementary Information/Source Data file. The XX data used in this study are available in the YY database under accession code ZZ [Add hyperlink here]. | Please see the Code and Data Availability sections contained in the submitted manuscript. We have ensured that these sections conform to the provided guidelines. |
| Specific advice on your Data Availability Statement: |  |
| Please include a “Code Availability” section after the “Data Availability” section and please provide active links to both the data and the code repository (e.g., a GitHub link). Where applicable, the data availability statement should also link to any publicly available datasets that are used in your manuscript. | We have ensured the manuscript conforms to these requirements. |
| Please be sure to include the final version of your reporting summary in your submission as a supplementary information file. The reporting summary will be published alongside the paper. 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 |  |
| We notice that you have deposited your code in a Github repository, which we fully support. We strongly encourage you in addition to make your code citable by obtaining a DOI for the Github repository in order to provide a permanent reference to the version of the code used in this study and improve reproducibility. This can be done by linking the repository to Zenodo, following the instructions here: https://guides.github.com/activities/citable-code/ Please cite the Github repository in your manuscript text or Code Availability statement and in your reference list: authors, title (this paper), repository name, DOI identifier, year. Alternatively, you can deposit the code in Gigantum or Code Ocean for the same purpose. | We have ensured the manuscript description of the code conforms to the specified requirements including linking to Zenodo. |
|  |  |
| **Methods** |  |
| Our guidance: | Your response: |
| Sufficient details of the experiments must be provided in the Methods section such that they could be reproduced without reference to published papers. Use of the term "as described previously" is not encouraged. | We have ensured the manuscript conforms to these requirements while adhering to the space limitations dictated by the journal. |
|  |  |
| **References** |  |
| Our guidance: | Your response: |
| All references must be cited in numerical order. The reference list will be formatted according to the Nature style by our journal production team, however please ensure that references contain all of the information required, eg:  Kurumada, S., Takamori, S. & Yamashita, M. An alkyl-substituted aluminium anion with strong basicity and nucleophilicity. <i>Nat. Chem.</i> <b>12</b>, 36–39 (2020). | We use bibtex in combination with the nature citation style language file to ensure that citations are properly given. |
|  |  |
| **End matter** |  |
| Our guidance: | Your response: |
| Please thoroughly review our policy on Competing Interests (http://www.nature.com/authors/policies/competing.html) and include a detailed statement in your final manuscript file, and in our manuscript tracking system. Please ensure the statements are identical in both. Be specific about how each point stated relates to the research, list applicable author initials, and/or patent numbers. If there are no competing interests, a negative statement (“The authors declare no competing interests”) must be included. | A negative Competing Interests statement has been provided. |
| Nature Portfolio defines Competing Interest (CI) as financial and non-financial interests (including but not limited to funding, employment, stocks, shares, patents, personal or professional relationships with individuals or institutions, and unpaid membership advocacy) that could be perceived to directly undermine the objectivity, integrity, and value of a publication, or could be seen as having an influence on the judgments and actions of authors with regard to objective data presentation, analysis, and interpretation.  Please thoroughly review our policy on Competing Interests and include a detailed statement both in your final manuscript file and in our manuscript tracking system. Please ensure the statements are identical in both. Be specific about how each point stated relates to the research and list applicable author initials, and/or patent numbers.  If there are no competing interests, a negative statement must be included. | A negative Competing Interests statement has been provided. |
| <https://www.nature.com/nature-research/editorial-policies/competing-interests> |  |
| Please confirm that all relevant funding awarded to each author is described in the Acknowledgements section. List each grant number, followed by the initials of the author who received it. | We have ensured the manuscript conforms to these requirements. |
|  |  |
|  |  |
| **Preparing your manuscript files** |  |
| Our guidance: | Your response: |
| Unless otherwise stated please limit individual file sizes to approximately 30MB. We strongly encourage the use of repositories for large datasets or source data due to size considerations. |  |
| Please supply a brief (maximum 250 characters, including spaces) summary of the main findings of the paper to be used on our website and in our e-alerts. The summary should be written in the third person in language suitable for a broad audience. The summary may be edited by the editors prior to publication. Please provide this summary in your cover letter. | We propose the following two-sentence summary for use on the journal website and e-alerts: Mapping diverse mouse brain data into shared reference spaces is key for integrating structural, molecular, and developmental studies. The authors demonstrate how the ANTsX ecosystem enables reproducible alignment across these modalities.  (Need to provide the summary in the cover letter). |
| Please supply the main manuscript file in either Microsoft Word or LaTeX format | The main manuscript file is provided as a latex file. |
| Please provide figures as individual vector files with editable text. Acceptable file types for figures are .ai, .eps, .pdf, .ppt or Chem Draw for fully editable vector-based art. For detailed guidance on figure preparation, see https://www.nature.com/documents/aj-artworkguidelines.pdf | All figures are provided as .pdf files. Furthermore, we have ensured that all the scripts which produce figures produce .pdf files as output. |
| The use or adaptation of previously published images is strongly discouraged. 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. 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.  If any elements of your submitted work have been created with BioRender you will need to ensure you have obtained a publication license from BioRender, adhering to the user requirements as outlined within the license. The reference for BioRender created graphics should be present in the accompanying legend of the display material it is present in.  A copy of the publication license should be uploaded to our system as a related manuscript file upon resubmission.  For more information please see the BioRender knowledge article here: https://help.biorender.com/hc/en-gb/articles/21283116932765-CC-BY-publishing-and-reader-permissions  For more information on what constitutes ownership by a third party, please contact our Editorial Assistant at naturecommunications@nature.com | We have revised Figure 7 to remove suspected third party content. |
| Please check in particular: |  |
| **Please note that suspected third party content is present in figure 7.** |  |
|  |  |
| **Forms to complete** |  |
| Our guidance: | Your response: |
| **Reporting Summary** |  |
| Please revise the Reporting Summary according to the requests below. After making the requested changes, please be sure to include the final version of your Reporting Summary in your submission as a supplementary information file. 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. | We have revised the Reporting Summary accordingly. |
| Please update your current checklist or download from: |  |
| https://www.nature.com/documents/nr-reporting-summary.pdf |  |
|  |  |
| **Reporting Summary** |  |
| Our guidance: | Your response: |
| **General Instructions** |  |
| Please list the name(s) of the corresponding author(s) in the 'Corresponding author(s): ' field. | We added the corresponding authors. |
| Please mention the last updated date in 'Last updated by author(s):' section. | We provided the requested date. |
| **Statistics** |  |
| Checkbox 6: Please mark this field as "confirmed", as data corresponding to measures of central tendency like mean and measures of variation like standard error of mean, standard deviation, etc. have been referenced in the manuscript. | We have marked the field as confirmed. |
| **Field Specific Reporting** |  |
| **Life Sciences Study Design** |  |
| Please describe how the number of datasets involved in the study was determined and explain why these are sufficient for the analysis. | We have revised our original response to more fully address the issues of sample size. |
|  |  |
|  |  |
|  |  |
|  |  |
| **You will need to upload:** |  |
| Completed Third Party Rights Table (if relevant) | N/A |
| A point-by-point response to the reviewers' comments | X |
| A completed copy of this checklist | X |
| The main manuscript file in either Microsoft Word or LaTeX format | X |
| Separate Figure files | X |
| Separate Source Data files | X |
| Inventory of Supporting Information | N/A |
| Reporting Summary | X |
| ChemDraw files | N/A |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |
|  |  |