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Uncovering the spatial landscape of molecular interactions within the tumor microenvironment through latent spaces

Atul Deshpande, Melanie Loth, Dimitrios N. Sidiropoulos, Shuming Zhang, Long Yuan, Alexander T. F. Bell, Qingfeng Zhu, Won Jin Ho, Cesar Santa-Maria, Daniele Gilkes, Stephen R. Williams, Cedric R. Uytingco, Jennifer Chew, Andrej Hartnett, Zachary W. Bent, Alexander V. Favorov, Aleksander S. Popel, Mark Yarchoan, Ashley Kiemen, Pei-Hsun Wu, Kohei Fujikura, Denis Wirtz, Laura D. Wood, Lei Zheng, Elizabeth M. Jaffee, Robert A. Anders, Ludmila Danilova, Genevieve Stein-O'Brien, Luciane T. Kagohara, Elana J. Fertig

Summary

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First round of review: Number of reviewers: 2
2 confidential, 0 signed
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Second round of review: Number of reviewers: 2
2 original, 0 new
2 confidential, 0 signed
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Editorial decision letter with reviewers' comments, first round of review

Dear Elana,

I hope this email finds you well. The reviews of your manuscript are back and I've appended them below. On balance, the reviewers appreciate the goals of the work presented here; they've provided constructive comments that are intended to strengthen an already strong manuscript and that are aligned with our hopes for the paper. Accordingly, we're happy to invite a revision.

In addition to the Reviewers' comments, to help guide this revision, here are a few points of guidance:

While I agree that the deconvolution methods you reference in the penultimate paragraph of the Introduction are the natural conceptual context of the current work, I am concerned, in part because of some of Reviewer 2's comments (particularly point 2), that some readers may assume that the purpose of SpaceMarkers is to infer which cell type interacts with which (i.e. similar to e.g. CellPhoneDB and CellChat). While you do reference CellPhoneDB in the Discussion, I would advise you to briefly discuss these methods for inferring cell-cell interactions and how the aims of SpaceMarkers differ from them. Indeed it seems to me that SpaceMarkers is complementary to those approaches given that they focus on identifying cell-cell interactions rather than the molecular changes resulting from the interactions. Additionally, I'd advise you to be explicit about that transfer learning in matched scRNA-seq data does not allow identification of pairs of interacting cell types, only the cell types in which the resulting molecular changes occur (if I understand correctly).

Additionally, I'd also like to be explicit about an almost philosophical stance that we take at Cell Systems.

We believe that understanding how approaches fail is fundamentally interesting: it provides critical insight into understanding how they work. We also believe that all approaches do fail and that it's unreasonable, even misleading, to expect otherwise. Accordingly, when papers are transparent and forthright about the limitations and crucial contingencies of their approaches, we consider that to be a great strength, not a weakness. While the manuscript already embodies this attitude through the example applications aimed at demonstrating the range of applicability of SpaceMarkers, many of the reviewers' comments suggest that more explicit description of the boundaries of applicability is necessary.

I hope you find this feedback helpful. If you have any questions or concerns, I'm always happy to talk, either over email or by Zoom. More technical information and advice about resubmission can be found below my signature. Please read it carefully, as it can save substantial time and effort later.

I look forward to seeing your revised manuscript.

All the best,
Bernadett

Bernadett Gaal, DPhil
Editor-in-Chief, Cell System

Reviewers' comments:

Reviewer #1: This paper by Deshpande et al. presented SpaceMarkers, a new bioinformatics algorithm to estimate molecular changes from spatially overlapping cellular processes using spatial transcriptomics (ST) data. They applied this approach to model molecular changes from tumor-immune cell interactions in Visium spatial transcriptomics data. This approach is quite new, an extension of their previous method CoGAPS (a Bayesian non-negative matrix factorization approach) to identify genes with significantly higher expression in the latent features overlap region. The manuscript is described well and is easy to read. I have some concerns about the manuscript as below:

- In the method part (4.3), the authors described the linear model (Dij = APij + eij) as unable to capture molecular changes but the residual from the same model is used for calculating nonlinear effects using CoGAPS. Please clear the confusion regarding this. Moreover, CoGAPS residual introduces the non-linearity (higher or lower) in this method, any other way non-linearity can be introduced, will be good to discuss in the discussion part.

- In the same method part (4.3) the authors introduced the non-linear effect using the non-linear term f(A,P) with adding linear model which is good approach but not used for further. Not sure why this equation is added in the method part. Secondly, authors claim that "as non-linear term f is unknown and may change each pair of patterns, therefore this approach is infeasible", could you please describe with reference?

- I could not find the table of parameters kernel width Wp and outlier threshold taup that optimized the spatial autocorrelation of the residual (equation r(si)). Could you please provide the table in your test data sets "PDAC metastatic lymph node", "Invasive Breast Ductal Carcinoma", and "HCC"? Good to see how their value changes over the datasets or is fixed.

- Analysis of SpaceMakers in matched scRNAseq data: ST data is normalized by log2 but how scRNAseq data is normalized? Do you confirm that the normalization method is the same in both types of data? Please describe in details.

- Figure 5, S3, and S4: CoGAPS factorization revealed total 9 patterns in each spot however only 3 patterns (1, 2, 8) are dominant patterns in tissue. Do you think that other patterns have no role in tumor and immune cell interaction?

- 4.4 last line of the first paragraph: "On each of the input datasets, the algorithm was tested for a range of nPatterns (latent feature)" could you describe this in detail for the term "range".

Reviewer #2: In this submitted work entitled "Uncovering the spatial landscape of molecular interactions within the tumor microenvironment through latent spaces", Dr. Deshpande and colleagues proposed a novel algorithm, named SpaceMarkers, to infer molecular changes from tumor-immune interactions from latent space analysis of Visium spatial transcriptome (ST) data. The authors applied this approach to ST data generated on a lymph node metastasis from pancreatic cancer, a breast ductal carcinoma, and a hepatocellular carcinoma. Overall, this manuscript is well written, the data are interesting, and SpaceMarkers could be a useful tool to infer tumor-immune interactions using ST data. I would suggest the following improvement to the work:

Major comments:
1. Are the authors able to reproduce their results using a different latent space estimation method and clarify whether SpaceMarkers is dependent on the Bayesian matrix factorization method CoGAPS?

2. To state that the differentially expressed genes and molecular pathways are indeed resulting from inter-cellular interactions between immune and tumor compartments in spatially overlapping regions, instead of the confounding factors such as variation in cellular compositions between two spatial regions, Can the authors validate, either experimentally or computationally, one of their discovered cellular interactions?

3. It is unclear to me whether CoGAPS and SpaceMarkers are limited to samples with large tumor areas and clear tumor boundaries, are their methods also work for samples with diffused tumor cells?

4. The analyses were mainly focused on profiling tumor-immune interactions, can their methods be used to profile interaction between different types of immune cells or between immune and stromal cells?

5. It is well known that tumor-infiltrating immune cells are phenotypically highly heterogeneous, can SpaceMarkers further predict the precise cell types or states involved in which those molecular changes occur? It is unclear from the Figure 5C.

6. It is unclear to me whether the spatial interaction region simply marks the tumor edge.

7. Interactions between Tumor and immune cells exist not only at tumor edge, but also can occur in the tumor body with infiltrating immune cells. the latter was not explored in the manuscript.

8. SpaceMarkers relies on spot-based colocalization, however, interacting immune and tumor cells can in adjacent or nearby but not the same spots.

9. The pie graphs in figure 3B, there are many spots contain both DCIS and invasive cancer, are those spots confirmed by pathologists?

Minor comments:
1. What are "others" in figure 3B?

2. Figure 3C and 4E, what are the minimum values?

3. How each pattern's region of influence is determined?

4. How does the resolution of the CoGAPS influence the interaction? In Figure 4B, only 9 out of 16 patterns are shown for highRes, the authors are suggested to show the relationship for all the patterns they identified.

5. Some of the .tiff files are low resolution and the figures are blurry such as 5C.

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Authors' response to the reviewers' first round comments

Attached.

Editorial decision letter with reviewers' comments, second round of review

Dear Elana,

I'm very pleased to let you know that the reviews of your revised manuscript are back, the peer-review process is complete, and only a few minor, editorially-guided changes are needed to move forward towards publication.

In addition to the final comments from the reviewers, I've made some suggestions about your manuscript within the "Editorial Notes" section, below. Please consider my editorial suggestions carefully, ask any questions of me that you need, make all warranted changes, and then upload your final files into Editorial Manager.

I'm looking forward to going through these last steps with you. Although we ask that our editorially-guided changes be your primary focus for the moment, you may wish to consult our formatting checklist to make the final steps to publication go more smoothly. More technical information can be found below my signature, and please let me know if you have any questions.

All the best,

Bernadett

Bernadett Gaal, DPhil
Editor-in-Chief, Cell Systems

Editorial Notes

Transparent Peer Review: Thank you for electing to make your manuscript's peer review process transparent. As part of our approach to Transparent Peer Review, we ask that you add the following sentence to the end of your abstract: "A record of this paper's Transparent Peer Review process is included in the Supplemental Information." Note that this ***doesn't*** count towards your 150 word total!

Also, if you've deposited your work on a preprint server, that's great! Please drop me a quick email with your preprint's DOI and I'll make sure it's properly credited within your Transparent Peer Review record.

Manuscript Text:

- We don't allow "priority claims" (e.g. new, novel, etc.). For a discussion of why, read: http://crosstalk.cell.com/blog/getting-priorities-right-with-novelty-claims, http://crosstalk.cell.com/blog/novel-insights-into-priority-claims.

STAR Methods: Note that Cell Press has recently changed the way it approaches "availability" statements for the sake of ease and clarity. Please revise the first section of your STAR Methods as follows, noting that the particular examples used might not pertain to your study. Please consult the STAR Methods guidelines for additional information.

RESOURCE AVAILABILITY

Lead Contact: Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Jane Doe (janedoe@qwerty.com).

Materials Availability: This study did not generate new materials. *-OR-* Plasmids generated in this study have been deposited at [Addgene, name and catalog number]. *-OR-* etc.

Data and Code Availability:

- **Source data statement** (described below)
- **Code statement** (described below)
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

Data and Code Availability statements **have three parts and each part must be present. Each part should be listed as a bullet point, as indicated above.**

Instructions for section 1: Data. The statements below may be used in any number or combination, but at least one must be present. They can be edited to suit your circumstance. Please ensure that all datatypes reported in your paper are represented in section 1. For more information, please consult this list of standardized datatypes and repositories recommended by Cell Press.

• [Standardized datatype] data have been deposited at [datatype-specific repository] and are publicly available as of the date of publication. Accession numbers are listed in the key resources table.

• [Adjective] data have been deposited at [general-purpose repository] and are publicly available as of the date of publication. DOIs are listed in the key resources table.

• [De-identified human/patient standardized datatype] data have been deposited at [datatype-specific repository]. They are publicly available as of the date of publication until [date or delete "until"]. Accession numbers are listed in the key resources table.

• [De-identified human/patient standardized datatype] data have been deposited at [datatype-specific repository], and accession numbers are listed in the key resources table. They are available upon request until [date or delete "until"] if access is granted. To request access, contact [insert name of governing body and instructions for requesting access]. [Insert the following when applicable] In addition, [summary statistics describing these data/processed datasets derived from these data] have been deposited at [datatype-specific repository] and are publicly available as of the date of publication. These accession numbers are also listed in the key resources table.

• Raw [standardized datatype] data derived from human samples have been deposited at [datatype-specific repository], and accession numbers are listed in the key resources table. Local law prohibits depositing raw [standardized datatype] datasets derived from human samples outside of the country of origin. Prior to publication, the authors officially requested that the raw [adjective] datasets reported in this paper be made publicly accessible. To request access, contact [insert name of governing body and instructions for requesting access]. [Insert the following when applicable] In addition, [summary statistics describing these data/processed datasets derived from these data] have been deposited at [datatype-specific repository] and are publicly available as of the date of publication. These accession numbers are also listed in the key resources table.

• The [adjective] data reported in this study cannot be deposited in a public repository because [reason]. To request access, contact [insert name of governing body and instructions for requesting access]. [Insert the following when applicable] In addition, [summary statistics describing these data/processed datasets derived from these data] have been deposited at [datatype-specific or general-purpose repository] and are publicly available as of the date of publication. [Accession numbers or DOIs] are listed in the key resources table.

• This paper analyzes existing, publicly available data. These accession numbers for the datasets are listed in the key resources table.

• [Adjective or all] data reported in this paper will be shared by the lead contact upon request.

Instructions for section 2: Code. The statements below may be used in any number or combination, but at least one must be present. They can be edited to suit your circumstance. ***If you are using GitHub, please follow the instructions here to archive a "version of record" of your GitHub repo at Zenodo, then report the resulting DOI. Additionally, please note that the Cell Systems strongly recommends that you also include an explicit reference to any scripts you may have used throughout your analysis or to generate your figures within section 2.***

• All original code has been deposited at [repository] and is publicly available as of the date of publication. DOIs are listed in the key resources table.

• All original code is available in this paper's supplemental information.

• This paper does not report original code.

Instructions for section 3. Section 3 consists of the following statement: Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

In addition,

STAR Methods follows a standardized structure. Please reorganize your experimental procedures to include these specific headings in the following order: LEAD CONTACT AND MATERIALS AVAILABILITY (including the three statements detailed above); EXPERIMENTAL MODEL AND SUBJECT DETAILS (when appropriate); METHOD DETAILS (required); QUANTIFICATION AND STATISTICAL ANALYSIS (when appropriate); ADDITIONAL RESOURCES (when appropriate). We're happy to be flexible about how each section is organized and encourage useful subheadings, but the required sections need to be there, with their headings. They should also be in the order listed. Please see the STAR Methods guide for more information or contact me for help.

Please ensure that the standardized datasets generated in this paper has been archived in at least one datatype-specific repository recommended by Cell Press (e.g. GEO, PRIDE, etc.). If your data are not standardized, we recommend that you deposit them in a general purpose repository recommended by Cell Press. Please provide your datasets' accession numbers/DOIs in Deposited Data section of the Key Resources Table. Thank you!

Please ensure that original code has been archived in a general purpose repository recommended by Cell Press and that its DOI is provided in the Software and Algorithms section of the Key Resources Table. If you've chosen to use GitHub, please follow the instructions here to archive a "version of record" of your GitHub repo at Zenodo, complete with a DOI. Thank you!

Currently, you don't have a **Key Resources Table** (KRT). Note that the key resources table is required for manuscripts with an experimental component, and if a purely computational manuscript links to any external datasets (previously published or new), code-containing websites (e.g. a GitHub repo, noting that DOIs are strongly preferred), or uses non-standard software, it needs to include a key resources table that details these aspects of the paper. Purely computational or theoretical papers that don't contain any external links and use standard software don't require a key resources table, although you're welcome to include one if you like. For details, please refer to the Table Template or feel free to ask me for help.

Thank you!

Reviewer comments:

Reviewer #1: The authors have done a substantial improvement of the manuscript and have addressed my concerns.

Reviewer #2: The authors have done a great job in addressing the comments and have improved the manuscript. My last suggestion is to improve the illustration (some text in the figures, keys are a bit

small and the images in some panels are blurry) and if possible, make their Visium data together with the high-resolution H&E images publicly accessible.

Reviewer #1

In the method part (4.3), the authors described the linear model (Dij = APij + eij) as unable to capture molecular changes but the residual from the same model is used for calculating nonlinear effects using CoGAPS. Please clear the confusion regarding this. Moreover, CoGAPS residual introduces the non-linearity (higher or lower) in this method, any other way non-linearity can be introduced, will be good to discuss in the discussion part.

We thank the reviewer for bringing this confusion to our attention. We have rephrased the text in (4.3) to clarify that a change in the model assumption enables use of the residuals to estimate the molecular changes due to inter-pattern interactions. To further introduce alternative applications of SpaceMarkers, we have also added an extension of the method to the output of STdeconvolve (Miller et al., 2021) in Figure S4 of the revised manuscript. Finally, as suggested we revised paragraph 4 in the discussion to suggest other ways to introduce non-linearity, which can even supplement the existing DE and residual modes of SpaceMarkers.

In the same method part (4.3) the authors introduced the non-linear effect using the non-linear term f(A,P) with adding linear model which is good approach but not used for further. Not sure why this equation is added in the method part.

As suggested, we corrected the notation in (4.3) following the introduction of f(A,P) to introduce terms representing the estimate of f(A,P).

Secondly, authors claim that "as non-linear term f is unknown and may change each pair of patterns, therefore this approach is infeasible", could you please describe with reference?

We agree with the reviewer that the language here is vague, and we also understand that it comes across as a strong statement. We have moderated our statement in (4.3), and clarify the scope of the paper which is limited to detecting the excess effects from inter-pattern interactions.

I could not find the table of parameters kernel width Wp and outlier threshold taup that optimized the spatial autocorrelation of the residual (equation r(si)). Could you please provide the table in your test data sets "PDAC metastatic lymph node", "Invasive Breast Ductal Carcinoma", and "HCC"? Good to see how their value changes over the datasets or is fixed.

We thank the reviewer for bringing this to our notice. We now include the optimized parameters in supplementary Table S1.

Analysis of SpaceMakers in matched scRNAseq data: ST data is normalized by log2 but how scRNAseq data is normalized? Do you confirm that the normalization method is the same in both types of data? Please describe in details.

We have included the scRNAseq preprocessing and normalization approach in the revised Methods section under the heading “ProjectR analysis with matched single-cell RNAseq data”. Although the normalization methods are different, ProjectR has been shown to be able to perform transfer learning successfully across the different data types and modalities as shown in (Stein-O’Brien et al., 2019).

- Figure 5, S3, and S4: CoGAPS factorization revealed total 9 patterns in each spot however only 3 patterns (1, 2, 8) are dominant patterns in tissue. Do you think that other patterns have no role in tumor and immune cell interaction?

As the three dominant patterns (1,2,8) explain most of the gene expression in their respective regions, we hypothesize that their role in the tumor-immune interaction is similarly dominant. To illustrate the application of SpaceMarkers, we focus on these dominant patterns. However, the less dominant patterns could represent rarer cell types or minor processes and their interaction analysis could reveal key findings in the understanding of the tumor biology. Accordingly, we note the same at the end of the revised Results section 2.6[1].

4.4 last line of the first paragraph: "On each of the input datasets, the algorithm was tested for a range of nPatterns (latent feature)" could you describe this in detail for the term "range"

We revised the manuscript to provide the details for nPatterns in Table 1 in the Methods section, with the settings used for SpaceMarkers analysis typed in boldface.

Reviewer #2

Are the authors able to reproduce their results using a different latent space estimation method and clarify whether SpaceMarkers is dependent on the Bayesian matrix factorization method CoGAPS?

SpaceMarkers is compatible with other latent space estimation methods, as illustrated by the example shown in Figure S4. Here we have performed SpaceMarkers analysis on the output of STdeconvolve (Miller et al., 2021). The STdeconvolve output and SpaceMarkers results are made available as supplementary data in S4B of the revised manuscript.

To state that the differentially expressed genes and molecular pathways are indeed resulting from inter-cellular interactions between immune and tumor compartments in spatially overlapping regions, instead of the confounding factors such as variation in cellular compositions between two spatial regions, Can the authors validate, either experimentally or computationally, one of their discovered cellular interactions?

We welcome the reviewer’s comments, and focus our revision on further computational validation. We have included an additional SpaceMarkers analysis of a clinical sample from a pancreatic cancer premalignant lesion where we identified confounding factors using gene set

[1] Previously 2.5, changed to 2.6 to account for addition of a result in 2.3

overrepresentation and confirmed it using an independent tissue classification algorithm (see revised Figure 3, Figure S2, and Result Section 2.3)[2]. This example illustrates identification of confounding effects as well as their mitigation when using SpaceMarkers in the residual mode. We include suggestions in the second paragraph of the revised discussion section for experimental validation in future work.

It is unclear to me whether CoGAPS and SpaceMarkers are limited to samples with large tumor areas and clear tumor boundaries, are their methods also work for samples with diffused tumor cells?

In its current version, SpaceMarkers is more successful in resolving the interactions between large tumor areas. However, this could also be a limitation of the granularity of the Visium technology which prevents us from having rich spatial data on a smaller scale which could be beneficial for diffused tumor cells. We point this out in the fourth paragraph of the revised Discussion section and also note the importance of continued development of SpaceMarkers for different tumor types as well as other spatial technologies in paragraph 3 of the revised Discussion section.

The analyses were mainly focused on profiling tumor-immune interactions, can their methods be used to profile interaction between different types of immune cells or between immune and stromal cells?

Yes, SpaceMarkers can be used to profile interactions between different types of immune cells and between immune and stromal cells, provided we have latent features associated with the different cell types. In fact, we have provided the SpaceMarkers output from interaction between Pattern 1 (immune) and Pattern 2 (stroma) for the high resolution breast cancer analysis in the supplementary zip file. These were not featured in our results because we chose to focus on the interaction of immune cells with the different tumor types. In our analysis, we added an additional sample from a pancreatic premalignant lesion and further demonstrated the applicability of SpaceMarkers to the interactions between additional cell types in this sample as Figure 3 and a new subsection of the results in our revised manuscript. We have also clarified in paragraph 5 of Discussion in the revised manuscript to note that SpaceMarkers is generally applicable to interaction between any pair of cell types, including stromal cells and immune cells.

It is well known that tumor-infiltrating immune cells are phenotypically highly heterogeneous, can SpaceMarkers further predict the precise cell types or states involved in which those molecular changes occur? It is unclear from the Figure 5C.

The ability of SpaceMarkers to predict the precise cell types is limited to the detail presented by the Visium technology and the latent features. The spot-based Visium technology limits the ability to resolve individual immune cells, leading to a broad pattern representing the overall

[2] Sections previously numbered 2.3-2.5 have now been renumbered to 2.4-2.6. Similarly, Figures 3-5 have been renumbered to 4-6.

immune signature. However, when any two patterns representing two cell types are spatially interacting, SpaceMarkers can help identify which cells are involved in those molecular changes such as shown in now revised Figure 6C. We address these points in paragraphs 5 and 6 of the Discussion section.

It is unclear to me whether the spatial interaction region simply marks the tumor edge. Interactions between Tumor and immune cells exist not only at tumor edge, but also can occur in the tumor body with infiltrating immune cells. The latter was not explored in the manuscript.

The spatial interaction region is obtained as a result of identifying overlapping hotspots of each pattern's influence. This involves using a spatial kernel-smoothing approach to model the influence of the cells extending to the neighboring spots, and subsequent thresholding. Although we see some immune activity in the tumor interior, it is not significant to be counted as a hotspot of immune influence. Consequently the interaction region is limited to the area near the tumor boundary. We have clarified the same in the revised Result section 2.4.

SpaceMarkers relies on spot-based colocalization, however, interacting immune and tumor cells can in adjacent or nearby but not the same spots.

SpaceMarkers accounts for adjacent and nearby spots, which is modeled by performing a Kernel-based smoothing of the patterns. We have modified the text in the Introduction, Methods, and Discussion to clarify this point.

The pie graphs in figure 3B, there are many spots contain both DCIS and invasive cancer, are those spots confirmed by pathologists?

Our opinion is that CoGAPS is going beyond what the human pathologist can detect given the quality of the image. When a pathologist annotates an image, they usually will label a spot as either DCIS or invasive carcinoma, but not both at the same time. This is something that may be possible only by looking at the transcriptional profile of the spots. To this end, this observation is consistent with the results of the clustering performed by 10x's SpaceRanger software, where a few spots in the DCIS lesion are labeled the same way as a majority of the invasive cancer spots as seen in https://cf.10xgenomics.com/samples/spatial-exp/1.3.0/Visium_Human_Breast_Cancer/Visium_Human_Breast_Cancer_web_summary.html.

Minor Comments

What are "others" in figure 3B?

"Others" represents the sum total of the gene expression in each spot contributed by the other patterns identified by CoGAPS not explicitly shown in the figure. We have updated the Figure captions to clarify these.

Figure 3C and 4E, what are the minimum values?

We have revised the figure captions to note that FDR<0.05 for the pathways shown in the figures.

How each pattern's region of influence is determined?

SpaceMarkers identifies hotspots of each pattern's influence by using a spatial kernel-smoothing approach to model the influence of the cells extending to the neighboring spots, and subsequent thresholding. We have modified the text in the Methods section to clarify this.

How does the resolution of the CoGAPS influence the interaction? In Figure 4B, only 9 out of 16 patterns are shown for highRes, the authors are suggested to show the relationship for all the patterns they identified.

As the CoGAPS resolution increases, the heterogeneity of the patterns, and consequently the corresponding SpaceMarker genes increases to reflect different biological processes associated with the different interactions (see Section 2.5). Since many of the patterns had very minimal footprint in the sample, we do not focus on them in the main manuscript figure or its associated text. However, for the sake of completeness, we have included the equivalent figure using all 16 patterns in the revised Figure S3.

Some of the .tiff files are low resolution and the figures are blurry such as 5C.

We will upload higher resolution files for the final version of the manuscript.

Miller, B. F., Atta, L., Sahoo, A., Huang, F., & Fan, J. (2021). Reference-free cell-type deconvolution of pixel-resolution spatially resolved transcriptomics data. In *bioRxiv* (p. 2021.06.15.448381). https://doi.org/10.1101/2021.06.15.448381

Stein-O'Brien, G. L., Clark, B. S., Sherman, T., Zibetti, C., Hu, Q., Sealfon, R., Liu, S., Qian, J., Colantuoni, C., Blackshaw, S., Goff, L. A., & Fertig, E. J. (2019). Decomposing Cell Identity for Transfer Learning across Cellular Measurements, Platforms, Tissues, and Species. *Cell Systems*, *8*(5), 395–411.e8.