bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

CellDART: Cell type inference by domain adaptation of single-cell and spatial transcriptomic data

Sungwoo Bae[1,2,†], Kwon Joong Na[3,4,†], Jaemoon Koh[5], Dong Soo Lee[1,2,6], Hongyoon Choi[2,6,*], Young Tae Kim[3,4,*]

[1]Department of Molecular Medicine and Biopharmaceutical Sciences, Graduate School of Convergence Science and Technology, Seoul National University, Seoul, Republic of Korea.; [2]Department of Nuclear Medicine, Seoul National University Hospital, Seoul, Republic of Korea.; [3]Department of Thoracic and Cardiovascular Surgery, Seoul National University Hospital, Seoul, Republic of Korea; [4]Seoul National University Cancer Research Institute, Seoul National University College of Medicine, Seoul, Republic of Korea; [5]Department of Pathology, Seoul National University Hospital, Seoul, Republic of Korea; [6]Department of Nuclear Medicine, Seoul National University College of Medicine, Republic of Korea

[†]These authors contributed equally to this work

Correspondence

Hongyoon Choi, MD., Ph.D.

Department of Nuclear Medicine, Seoul National University Hospital

101, Daehak-ro, Jongno-gu, Seoul 03080, Republic of Korea

Tel: +82-2-2072-2802, Fax: +82-2-745-0345, E-mail: chy1000@snu.ac.kr

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Young Tae Kim, MD., Ph.D.

Department of Thoracic and Cardiovascular Surgery, Seoul National University Hospital

101 Daehak-ro, Jongno-gu, Seoul, Republic of Korea, 03080

Tel: +82-2-2072-3161, Fax: +82-2-745-0345, E-mail: ytkim@snu.ac.kr

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Abstract

Deciphering the cellular composition in genome-wide spatially resolved transcriptomic data is a critical task to clarify the spatial context of cells in a tissue. In this study, we developed a method, CellDART, which estimates the spatial distribution of cells defined by single-cell level data using domain adaptation of neural networks and applied it to the spatial mapping of human lung tissue. The neural network that predicts the cell proportion in a pseudospot, a virtual mixture of cells from single-cell data, is translated to decompose the cell types in each spatial barcoded region. First, CellDART was applied to mouse brain and human dorsolateral prefrontal cortex tissue to identify cell types with a layer-specific spatial distribution. Overall, the suggested approach was superior to the other computational methods in predicting the spatial localization of excitatory neurons. Furthermore, CellDART elucidated the cell type predominance defined by the human lung cell atlas across the lung tissue compartments and it corresponded to the known prevalent cell types. CellDART is expected to help to elucidate the spatial heterogeneity of cells and their close interactions in various tissues.

Keywords: spatially resolved transcriptomics; single-cell genomics; domain adaptation; deep learning; cell label transfer

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Main

Rapid progress in spatially resolved transcriptomics helped to comprehensively characterize the spatial interaction of cells in a tissue[1, 2]. Breakthrough technologies enabled capturing genome-wide spatial gene expression at a resolution of several cells[3] to the single-cell[4-6] and even subcellular levels[7]. These methods have been used in various disease models to decipher spatial maps of genes of interest and culprit cells[8-12]. Furthermore, emerging computational approaches facilitated the spatiotemporal tracking of specific cells and elucidated cell-to-cell interactions by preserving the spatial context[12-14]. However, there is an inherent limitation in the spatial transcriptomic analysis that each spot or bead covers more than one cell in most cases. Even with a high-resolution technique, a small portion of several cells can be contained in the same spatial barcoded region. In addition, a tissue with a high level of heterogeneity, such as cancer, consists of a variety of cells in each small domain of the tissue[15]. Thus, the identification of different cell types in each spot is a crucial task to understand the spatial context of pathophysiology using a spatially resolved transcriptome.

In this regard, recent computational tools have focused on integrating different types of transcriptomic data, particularly spatially resolved transcriptomic and single-cell RNA-sequencing (scRNA-seq) data[14, 16-23]. These tools have utilized the cell type signatures defined by scRNA-seq and transferred the cell labels into spatial transcriptomic data. The majority of the approaches applied a statistical model or a matrix decomposition to infer the cell fraction in each spot [18, 20-22]. Meanwhile, calculating the proportion of cell types defined by scRNA-seq data from spots of spatially resolved transcriptomic data can be considered a domain adaptation task[24, 25]. A model that predicts cell fractions from the gene expression profile of a group of cells can be transferred to predict the spatial cell-type distribution.

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

In this paper, we suggest a method, CellDART, that implements adversarial discriminative domain adaptation (ADDA)[26] to infer the cell fraction in spatial transcriptomic data. The randomly selected cells from scRNA-seq data constitute a pseudospot in which the fraction of cells is known. The neural network model that extracts the cell fraction from the gene expression of a pseudospot is adapted to a different domain where spatial transcriptomic data are present. Consequently, the joint analysis of spatial and single-cell transcriptomic data elucidates the spatial cell composition and unveils the spatial heterogeneity of the cells. We utilized the proposed method to provide a resource for spatial mapping of the human lung cell atlas using the spatially resolved transcriptome of human lung tissue.

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Results

Decomposition of spatial cell distribution with CellDART in human and mouse brain data

The performance of CellDART was assessed in publicly available single-nucleus (also considered single-cell data) and spatial transcriptomic autopsy samples of the human dorsolateral prefrontal cortex (DLPFC), each of which was obtained from two different subject groups with no neurological disorders. Additionally, single-cell and spatial datasets acquired from the mouse brain were utilized. First, both single-cell datasets were preprocessed, and the cells were named after the annotation data provided by the original papers[27, 28]. The 33 and 29 annotated cell clusters from the human and mouse brains were visualized by t-distributed stochastic neighbor embedding (t-SNE) plots (**Supplementary Fig. 1a, b**), and marker genes for each cluster were extracted (**Supplementary Fig. 1c, d and Supplementary Tables 1, 2**). The cell clusters showed distinct gene expression patterns represented by cell type-specific marker genes.

A specific number of cells (k = 8) were randomly sampled from the single-cell data with random weights to generate pseudospots (number of pseudospots = 20000). Then, composite gene expression values were computed based on marker genes (**Fig. 1a**). A neural network was trained to accurately decompose the pseudospots, and another network, the domain classifier, was trained to discriminate spots of real spatially resolved transcriptomes from pseudospots. During the training process, the weights of neural networks were updated to predict cell fractions and fool the domain classifier to avoid discriminating spots and pseudospots (**Fig. 1a**). As a result, the neural network, source classifier, was trained to estimate cell fractions in both the pseudospots and the real spatial spots as an adversarial domain adaptation process.

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

The layer-specific excitatory neuron fraction in each spot was predicted by CellDART and spatially mapped to the tissue. In the case of mouse brain tissue, 7 excitatory neurons showed spatially restricted patterns in a specific cortical layer (**Fig. 1b**). In addition, the 10 excitatory neuron clusters in the human brain presented layer-specific distribution patterns across the six cortical layers (layers 1 to 6) (**Fig. 2a and Supplementary Fig. 2a**). Additionally, the spatial density of human non-neuronal cells was estimated with a neural network (**Supplementary Fig. 2b**). Astrocytes were mainly located in layer 1 and layer 6, while oligodendrocyte cluster 3 (*Oligos_3*), which showed an approximately 10 to 60 times higher cell fraction than the other two clusters (*Oligos_1* and *Oligos_2*), was predominantly localized in white matter. Endothelial cells, microglia, and macrophages were spatially distributed across the six cortical layers with low cell proportions.

Comparison of CellDART with other integration tools in human brain tissue

The capability of CellDART to accurately assign cell types in spatial spots was compared with that of three computational tools: Scanorama, Cell2location, and RCTD. The three methods were employed to decipher the spatial distribution of excitatory neurons and non-neuronal cells in the DLPFC dataset.

Scanorama showed a few excitatory neurons of cortical layer-specific distribution patterns, whereas *Ex_2_L5*, *Ex_4_L6*, *Ex_9_L5_6*, and *Ex_10_L2_4* excitatory neurons were distributed differently from the known cortical distribution (**Supplementary Fig. 3a, b**). Astrocytes and oligodendrocytes did not show consistent cell distribution patterns across the cell subtypes. Endothelial cells, microglia, and macrophages were predominantly localized in layer 1, layer 6, and the white matter according to the Scanorama analysis (**Supplementary Fig. 3c**).

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

In the case of Cell2location, neither excitatory neurons nor non-neuronal cells showed layer-specific localization patterns except for a few cell types (*Ex_4_L6*, *Oligos_1*, and *Micro_Macro*) (**Supplementary Fig. 4**).

Finally, for RCTD, a few excitatory neurons (*Ex_2_L5* and *Ex_10_L2_4*) exhibited a high cell fraction in the corresponding cortical layer of a known layer specificity; however, other excitatory neurons presented heterogeneous patterns of distribution (**Supplementary Fig. 5a, b**). Additionally, in the non-neuronal cells, the spatial distribution was relatively uneven and not layer-specific except for three oligodendrocyte cell clusters (**Supplementary Fig. 5c**).

Receiver operating characteristic (ROC) curve analysis was implemented to compare the performance of the four different tools in predicting the layer-specific distribution of excitatory neurons (**Fig. 2b**). The spatial spots in the DFPLC data were classified into a specific layer, layer 1 to layer 6, white matter, or unknown, with manual annotation data[29] based on the tissue morphology and marker genes[30]. The ROC curves for 10 excitatory neurons revealed that CellDART has overall good prediction accuracy, with an area under the curve (AUC) ranging from 0.629 in *Ex_7_L4-6* to 0.759 in *Ex_4_L6*. On the other hand, Scanorama and RCTD exhibited relatively low discriminative accuracy in several cell types with AUCs below 0.5 and comparable AUCs with CellDART for a few cell types. In addition, Cell2location showed several cell types with AUCs below 0.5 and a maximum AUC of 0.591. The confidence interval of the AUC was generated by bootstrapping, and the results were compared among the four methods (**Supplementary Fig. 6**). In general, CellDART showed superior performance in predicting layer-specific localization patterns across all excitatory neurons.

Discovery of spatial heterogeneity of human lung tissue with CellDART

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

CellDART was further applied to normal lung spatial transcriptomic data. Human lung tissue was obtained from one patient who underwent lobectomy for surgical resection of lung cancer. The two normal lung tissues were dissected far from the tumor and pathologically confirmed to have no tumor cells (**Supplementary Fig. 7**). The demographic features of the patient are summarized in **Supplementary Table 3**. The publicly available human lung cell atlas data were used for spatial mapping of lung cell types using CellDART. They consisted of scRNA-seq from three human normal lung tissues[31]. The single-cell data were embedded in low-dimensional space by a t-SNE plot, and 57 cell clusters showed discrete gene expression patterns (**Supplementary Fig. 8a**). The marker genes selected in each cell cluster were pooled and utilized in the downstream analysis (**Supplementary Fig. 8b and Supplementary Table 4**). After the generation of pseudospots, CellDART was trained to assign the proportion of cells in the spatial spots. The tissue slides from two spatial datasets (lung 1 and lung 2) were manually segmented, and each spot was classified into 7 categories: alveolar space, bronchial epithelium, fibrous stroma, immune cluster, terminal bronchiole, vessels, and unknown region (**Fig. 3a, b**). In addition, the cell types were classified into five categories based on a previous study[31]. An average scaled cell proportion of spots in the same tissue domain was calculated, and the values were expressed with heatmaps.

In both the lung 1 and lung 2 datasets, each cell type showed different distribution patterns across the segmented tissue domains (**Fig. 3c, d**). Among the 'airway epithelial' cells (blue color on the left side of the heatmaps), proximal ciliated, ciliated, mucous, and club were mainly localized in bronchial epithelium or terminal bronchiole. 'Alveoli epithelial' cells (orange color) were localized in the alveolar space of lung 2 data. 'Muscle stromal' cells (red color) were mainly distributed in unknown stroma in the lung 1 data and terminal bronchioles or vessels in the lung 2 data. 'Immune' cells (brown color), particularly B-cells, monocytes, and dendritic cells, were predominantly located in the immune cluster tissue domain. Finally,

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

'endothelial' and 'other stromal' cells did not present spatially localized patterns of distribution.

For the next step, cell types that showed highly different cell fractions across the tissue domains were selected (**Supplementary Table 5**). The top 7 cell types were ranked by the ratio of the scaled cell fraction in the specific tissue domain compared to the other domains and were mapped to the tissue (**Fig. 4a, b**). The cell types with an average scaled cell fraction in the domain below 0.2 were excluded. For lung 1 tissue, 'proximal basal' and 'proximal ciliated' cells, which were previously described as 'airway epithelial' cells (blue color), were predominantly distributed in the bronchial epithelium or terminal bronchiole tissue domain (**Fig. 4a**). Most 'immune' cell types (brown color) were localized in the immune cluster domain except for 'classical monocytes', which are commonly found in bronchial epithelium. In lung 2 tissue, 'ciliated', 'proximal ciliated', 'club', and 'mucous' cells, which are included in 'airway epithelial' (blue color) cells, were mainly located in the terminal bronchiole domain (**Fig. 4b**). 'Capillary intermediate 2' included in the 'endothelial' cell type (green color) was localized in the alveolar space domain, while another endothelial cell type, 'artery', was mainly located in the fibrous stroma and vessels. Additionally, 'alveolar epithelial type 2' in the 'alveoli epithelial' (orange color) was predominantly distributed in the alveolar space domain. In summary, CellDART could precisely localize the spatial distribution of heterogeneous cell types in normal lung tissue.

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Discussion

CellDART, which adapts the domain of single-cell and spatial transcriptomic data, could be flexibly applied to brain and lung tissues to decompose the spatial distribution of various cell types. The suggested approach was capable of accurately predicting the layer-specific localization of excitatory neurons and non-neuronal cells in the brain. Additionally, CellDART was superior to the other three computational tools, Scanorama, Cell2 location, and RCTD, in spatially localizing multiple excitatory neuron subtypes. Moreover, our domain adaptation method deciphered the spatial distribution patterns of various lung cells across the different tissue compartments.

CellDART can be adopted for spatial transcriptomic data to portray the cellular landscape of the tissue by preserving the spatial context. In the brain, six cortical layers and the white matter contain different cell types, and the heterogeneous cells in each layer shape distinct functional characteristics[32]. Therefore, it is crucial to precisely decompose brain cell types in spatial transcriptomic data to comprehensively analyze the spatial crosstalk among cells. In the mouse brain tissue, layer-specific excitatory neurons revealed localized patterns of distribution across the cortical layers (**Fig. 1b**). Additionally, in the validation study with human DLPFC tissue, not only layer-specific excitatory neurons but also glial cells such as astrocytes and oligodendrocytes showed spatially restricted patterns (**Fig. 2 and Supplementary Fig. 2b**). Three astrocyte subtypes, Astros_1, Astros_2, and Astros_3, were predominantly located in L1 and L6. It has been reported that astrocytes form cortical layer-specific morphological and gene expression features[32-34]; however, the abundance of excitatory neurons in the mid cortical layers may have masked the presence of diverse astrocyte populations. Meanwhile, one of the oligodendrocyte subtypes, Oligos_3, which presented a higher absolute cell fraction than the other subtypes, was localized in the white

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

matter. This finding is in line with a previous study showing that oligodendrocytes are highly restricted in white matter compared to gray matter[35]. On the other hand, the spatial distribution of glial cells did not match the known localization patterns and was not layer-specific in Scanorama and Cell2location, respectively (**Supplementary Fig. 3, 4**). In the case of RCTD, oligodendrocytes showed strong localization patterns in white matter; however, other glial cells did not present a layer-specific distribution (**Supplementary Fig. 5**).

Our suggested method, CellDART, was further applied to human lung tissues where a mixture of various cells was present across the tissue compartments[31]. The cell types that exhibited a high proportion in a specific tissue domain corresponded with a previous paper presenting the cell type predominance in the lung compartments (**Figs. 3, 4**). In addition, when the top 7 highly localized cell types in each tissue compartment were listed, the selected cell types were shared between the lung 1 and lung 2 tissues (**Supplementary Table 5**). More specifically, for the alveolar space tissue domain, three alveolar epithelial cells (‘alveolar epithelial type 1, 2,’ and ‘signaling alveolar epithelial type 2’) and two capillary cells (‘capillary intermediate 2 and ‘capillary aerocyte’) overlapped in both tissues. The cell types were also shared in the fibrous stroma (‘fibromyocyte’, ‘mesothelial’, and ‘myofibroblast’), immune cluster (‘B’, ‘OLR1+ classical monocyte’, ‘EREG+ dendritic’, and ‘plasmacytoid dendritic’), and terminal bronchiole (‘proximal basal’, ’proximal ciliated’, ‘differentiating basal’, and ‘ciliated’) tissue domains. In short, CellDART can accurately assign prevalent cell types in the tissue compartments and is reproducible across replicates of the tissue. Considering the heterogeneous cell types in the lung, our resource of spatially resolved cell types derived from human lung tissue data provides the spatial distribution of cell types and may be used as controls to analyze pathologic patterns of various lung diseases.

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

There are two important issues to consider before applying CellDART to transfer cell labels. First, the density of cells may vary in the different regions of the tissue. In our method, the sampled number of cells in a pseudospot is fixed during the training; however, the domain adaptation process aligns the pseudospot to the spatial data, and the impact of spatial cell density variance on the result may be attenuated. In addition, the small population of cells in the spatial data may be neglected during the prediction of the cell proportion. The proportion of those cell types can be masked due to other predominant cell types in the spatial spots. In that case, CellDART can be implemented for the corresponding subpopulation of cells by extracting the marker genes for the subclusters.

In conclusion, CellDART is capable of estimating the spatial cell compositions in complex tissues with high levels of heterogeneity by aligning the domain of single-cell and spatial transcriptomics data. The suggested method may help elucidate the spatial interaction of various cells in close proximity and track the cell-level transcriptomic changes while preserving the spatial context.

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Methods

Human brain cortex data

A publicly available Visium spatial transcriptomics dataset obtained from the DLPFC of postmortem neurotypical humans was downloaded from the data repository provided by the paper[29]. Among the 12 slides, 'tissue 151673' with 3639 spots and 33538 genes was selected. The count matrix for the tissue and brain layer information for each spot (cortical layers 1-6 and white matter) was added, and spots with no layer information were excluded from further analysis. Single-nucleus transcriptomic data acquired from the DLPFC of a healthy human control group (n=17) were utilized for the joint analysis[28]. The count matrix for 35212 cells and 30062 genes and the cell type annotation were included in the analysis.

Mouse brain data

Visium spatial transcriptomics dataset for the mouse brain was downloaded from the 10X Genomics Data Repository. The ‘Mouse Brain Serial Section (Sagittal-Anterior)’ slide, which contains 2695 spots and 32285 genes, was utilized for the CellDART analysis. For the joint analysis, scRNA-seq data obtained from the mouse primary visual cortex and anterior lateral motor cortex were selected. The count matrix was comprised of 23178 cells and 45768 genes. The layer-specific excitatory neuron types [L2/3 IT (intratelencephalic), L4, L5 IT, L5 PT (pyramidal tract), L6b, L6 CT (corticothalamic) and L6 IT] were determined based on the markers discovered in a previous study[27].

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Normal human lung data

Two normal lung samples were acquired from lung specimens from one patient who underwent surgical resection for lung cancer. We acquired samples and embedded them in optimal cutting temperature (OCT) compound in the operating room and stored them at -80°C until cryosectioning. For cryosectioning, samples were equilibrated to -20°C with a cryotome (Thermo Scientific, USA). Sections were imaged and processed for spatially resolved gene expression using the Visium Spatial Transcriptomic kit (10X Genomics, USA). The protocol of this study was reviewed and approved by the institutional review board of Seoul National University (Application number: H-2009-081-1158). 'Lung 1' consists of 1591 spots and 36601 genes, and 'Lung 2' consists of 2683 spots and 36601 genes. Single-cell data from the normal lung tissue of three subjects were downloaded and utilized for the integrated analysis[31]. The count matrix for 65662 cells and 26485 genes and the cell labels were included in the downstream analysis.

Preprocessing spatial and single-cell datasets

All of the preprocessing steps were performed with Python (version 3.7) with the Scanpy toolkit (version 1.5.1)[36]. The count matrices for both spatial and single-cell datasets were normalized with the 'scanpy.pp.normalize_total' function such that gene expression was comparable between spots or cells. For the single-cell data, the counts were log-transformed (scanpy.pp.log1p) followed by scaling (scanpy.pp.scale) and dimensionality reduction by principal component analysis (scanpy.tl.pca). Finally, the cells were represented with a t-distributed stochastic neighborhood embedding (t-SNE) plot (scanpy.tl.tsne and

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

scanpy.pl.tsne) and were named based on the annotation data from publicly available datasets.

Meanwhile, the top 20 and 10 highly expressed marker genes for each cell cluster from brain and lung samples were extracted with the Wilcoxon rank-sum test ('scanpy.tl.rank_genes_groups') based on the log-normalized count. Multiple comparison correction with the Benjamini-Hochberg method was applied, and genes were ranked by the corrected p-values. All of the cell type markers were pooled to form cell signature genes (**Supplementary Fig. 9a**). The intersection between the cell signature genes and all provided genes from the spatial data was obtained. The downstream analysis was performed only with these intersecting genes.

For the next step, k cells were randomly selected from the mouse or human brain (k = 8) and lung single-cell datasets (k = 10). Random weights were given to each cell to mimic the cases in which only the portion of the cells are contained in a spatial spot (**Supplementary Fig. 9a**). The virtual mixture of the cells was defined as a 'pseudospot'. A total of 20000 pseudospots were generated, and the composite gene expression values were calculated for each pseudospot. Then, the log-normalized count matrices for single-cell, pseudospot, and spatial spot data were scaled such that the value lies between 0 and 1 in each cell or spot.

CellDART: Cell type inference with domain adaptation

The modified ADDA algorithm[26] was applied to develop a model to predict cell type proportions for each spot (**Fig. 1**). The training of neural networks was implemented based on Keras (version 2.3.1), TensorFlow (version 1.14.0) and scikit-learn (version 0.24.1) packages.

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

First, a feature embedder that computes 64-dimensional embedding features from the gene expression data of either spatial spots or pseudospots was defined. The feature embedder was comprised of two fully connected layers, each of which underwent batch normalization and activation by the ELU function. The outputs of the first layer and second layer have 1024 and 64 dimensions, respectively. Source and domain classifiers were defined such that they could predict the cell fraction in each spot and discriminate pseudospots from spots, respectively. The domain classifier consisted of two fully connected layers. The first layer with 32-dimensional output was connected to the embedded features. After batch normalization, ELU activation, and dropout, another layer to discriminate real spots from pseudospots was applied. The source classifier is directly connected to the embedded features of the feature extractor as a one-layer model connected to the feature embedder. Therefore, the feature extractor attached to either of the classifiers was named a source or domain classification model. The source and domain classification model shared the feature extractor.

The loss function of the source classifier that predicts cell type proportions was defined by Kullback-Leibler divergence (KLD). KLD is decreased when the distribution of predicted and real cell type proportions is similar. For the initialization of weights of feature embedder and source classifier, initial training to predict cell type proportions for pseudospots was performed. The optimization process can be summarized by these formulae:

$$L_s = KLD\ (Y_s || S(f(X_s))) = -\sum_k Y_{k,s} \log(\frac{S(f(X_s), k)}{Y_{k,s}})$$

$$L_{adv} = -logD\big(f(X_s)\big) - \log\ (1 - D(f(X_r))$$

$$L_D = -logD\big(f(X_r)\big)$$

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

$X_s: Pseudospots, \quad X_r: Real\ spots\ with\ unknown\ distribution, \quad Y_s: Distribution\ of\ pseudospots$

f: feature embedder, S: source classification model, D: domain classification model

First, the model was trained to minimize L_s as a pre-training process. As an adversarial loss, it is typical to train the model with the standard loss function with inverted labels as in the above formula. Thus, two optimization processes were applied (**Supplementary Fig. 9b**). The networks were optimized to minimize L_s and L_{adv} with fixed weights of the domain classifier. Then, the domain classifier was trained to minimize L_D with fixed weights of the feature embedder, *f*. These two processes were repeated with a training parameter of the number of iterations. The iteration number was set to 3000, the minibatch size was 512, and the learning rate for the training domain classifier was 0.005. The loss weights between the source and domain classifiers were 1:0.6 in brain tissues and 1:1 in lung tissues.

Finally, the trained model, CellDART, predicted the cell fraction in each spot from the spatial data, and the results for each cell type were spatially mapped to the tissue by the 'scanpy.pl.spatial' function. Additionally, the distribution of cell type compositions across the brain layer was represented with the 'scanpy.pl.stacked_violin' function.

Comparison to other tools

For the DLPFC datasets, the performance of CellDART was compared with three other computational tools: Scanorama[16], Cell2location[20], and RCTD[21]. Briefly, Scanorama aligns the single-cell and spatial datasets based on the mutual nearest neighbors between the two. Meanwhile, Cell2location assumes that the count matrix from spatial data follows a negative binomial distribution and can be decomposed into a linear combination of cell type

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

signatures. RCTD postulates that counts in spatial spots follow a Poisson distribution and applies the maximum likelihood method to estimate cell proportions. These three toolkits were applied, and cell density from Cell2location and cell fraction from Scanorama and RCTD were spatially mapped to the tissue. For Cell2location analysis, we set the total number of cells, cell types, and groups of cell types (for example, excitatory neurons group) in each spatial spot as 8, 9, and 5, respectively. For RCTD, we selected 'full mode', which does not restrict the number of cells in each spot.

The DLPFC spatial data contain the brain layer information (layer 1 to layer 6, white matter, and unknown), and the single-cell data have ten layer-specific excitatory neuron clusters (*Ex_1_L5_6*, *Ex_2_L5*, *Ex_3_L4_5*, *Ex_4_L6*, *Ex_5_L5*, *Ex_6_L4_6*, *Ex_7_L4_6*, *Ex_8_L5_6*, *Ex_9_L5_6*, and *Ex_10_L2_4*). The layer specificity of excitatory neurons was determined by the cell types identified from the single-nucleus RNA-seq data[28] with the layer markers suggested by several studies[30, 37, 38]. Receiver operating characteristic (ROC) analysis was performed to determine whether the spatial cell fraction of excitatory neuron clusters could differentiate the specific cortical layer. The cell fraction and layer information of each spot were bootstrapped, and 1000 null distribution samples for the area under the curve (AUC) value were calculated. The ROC curves and AUC values were compared between CellDART, Scanorama, Cell2location, and RCTD. The statistical analysis and visualization were implemented with scikit-learn and matplotlib (version 3.3.4).

Spatial mapping of lung cells to normal lung tissue data: investigation of the spatial heterogeneity of the cells

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

The boundary of the tissue structures in two lung samples (lung 1 and lung 2) was delineated by a pathologist on H&E staining images, and the spots were classified into 6 domains: alveolar space, bronchial epithelium, fibrous stroma, immune cluster, terminal bronchiole, and vessels. The uncertain region of the tissue was named 'unknown' and more specifically 'unknown stroma' if the corresponding region was stromal tissue. After transferring the single-cell cluster labels to the spatial data, the minimum and maximum cell fraction values across all spots were scaled to 0 and 1, respectively. The cell types were divided into six categories based on where the cells were commonly found[31] ('airway epithelium', 'alveoli epithelium', 'endothelial', 'muscle stromal', and 'other stromal'). The average scaled cell fraction in each tissue domain according to cell types was visualized with a seaborn clustermap function (version 0.11.1), and the cell type categories were color-coded and presented on top. For the next step, the cell types showing highly different cell fractions across the histological domains were selected, and their spatial composition was mapped to the tissue. Cell type selection was performed with the Wilcoxon rank-sum test, and Benjamini-Hochberg corrected p-values were computed. The cell types in each tissue domain were ranked based on a ratio of the average scaled cell fraction in a specific tissue domain to the rest of the domains. The cell types with an average scaled fraction below 0.2 were excluded from further analysis. An adjusted p-value below 0.05 was considered significant.

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Code availability

Python source code for CellDART is uploaded on https://github.com/mexchy1000/CellDART.

Acknowledgements

This research was supported by the National Research Foundation of Korea (NRF-2020M3A9B6037195 and NRF-2020M3A9B6038086).

Author contributions

H.C. and Y.T.K. conceived the study. S.B., K.J.N., and H.C. designed the experiments. K.J.N. and Y.T.K. mainly contributed to acquire spatially resolved transcriptomic data. H.C. initially developed a new algorithm and S.B. modified and optimized the analytic method. H.C., D.S.L. and Y.T.K. devised the data analysis. S.B. mainly conducted the data analysis. J.K. conducted histologic image analysis. All authors contributed to the interpretation of the data and wrote the paper.

Competing interests

The authors declare no competing interests.

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Figure legends

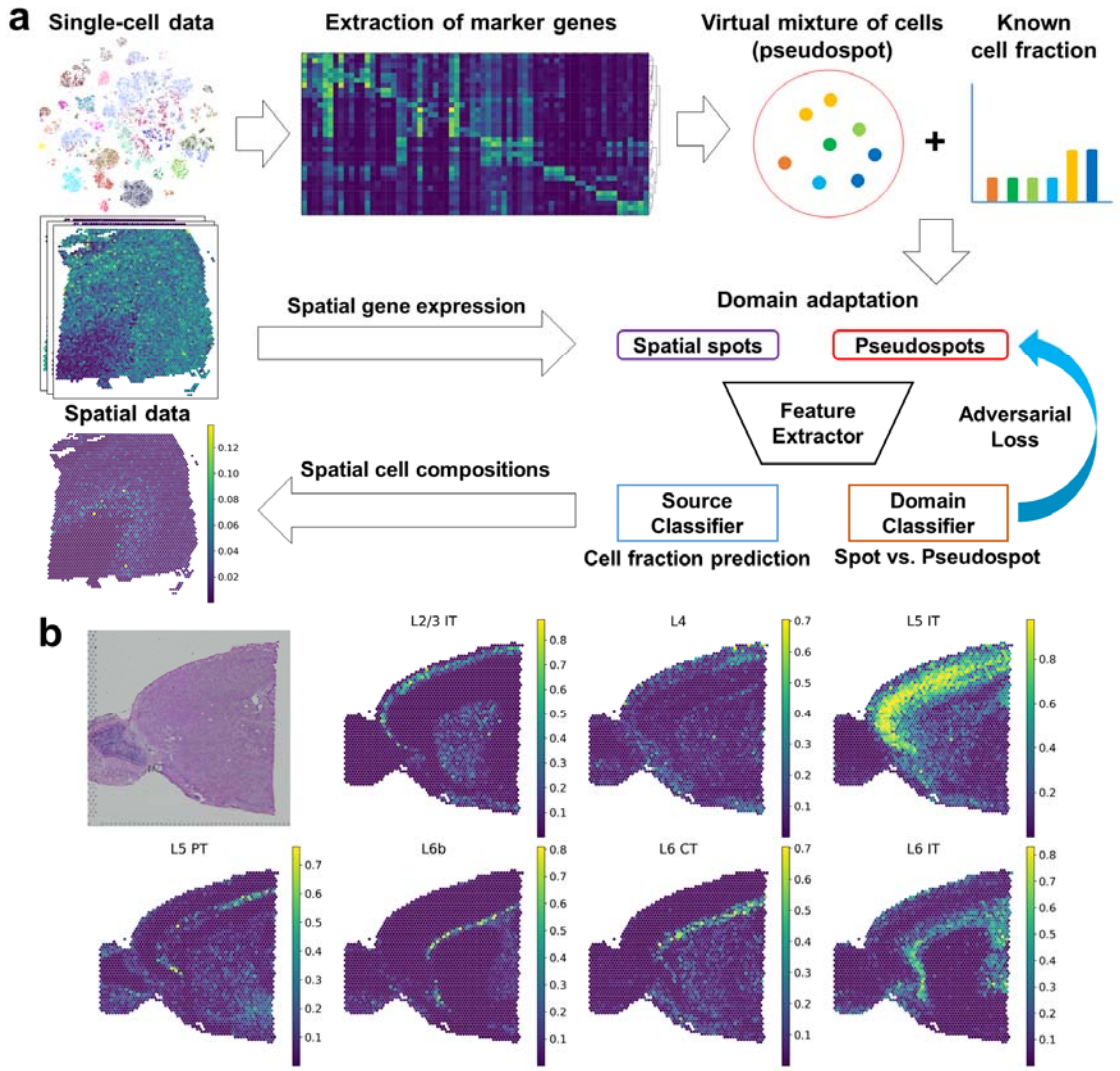


Fig. 1. CellDART analysis in human and mouse brain tissues

a, Schematic diagram for CellDART analysis. The human dorsolateral prefrontal cortex (DLPFC) dataset was preprocessed, and the marker genes for each cell cluster were extracted. The shared genes between the pooled cluster markers and the spatial transcriptomic data were selected for the downstream analysis. Then, 20000 pseudospots were generated by randomly selecting 8 cells from the single-cell data and giving them random weights. A feature extractor with a source and domain classifier was trained to estimate the cell fraction from the

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

pseudospot and distinguish the pseudospots from the spatial spots. First, weights of the neural network were updated except for the domain classifier. Next, the data label for the spot and pseudospot was inverted, and only the domain classifier was updated. Finally, the trained CellDART model was applied to spatial transcriptomics data to estimate the cell proportion in each spot.

b, Spatial mapping of 7 layer-specific mouse excitatory neurons predicted by CellDART. The figure in the top left corner shows the mouse brain tissue slide. Colormaps present the maximum and minimum values for the corresponding cell fraction.

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

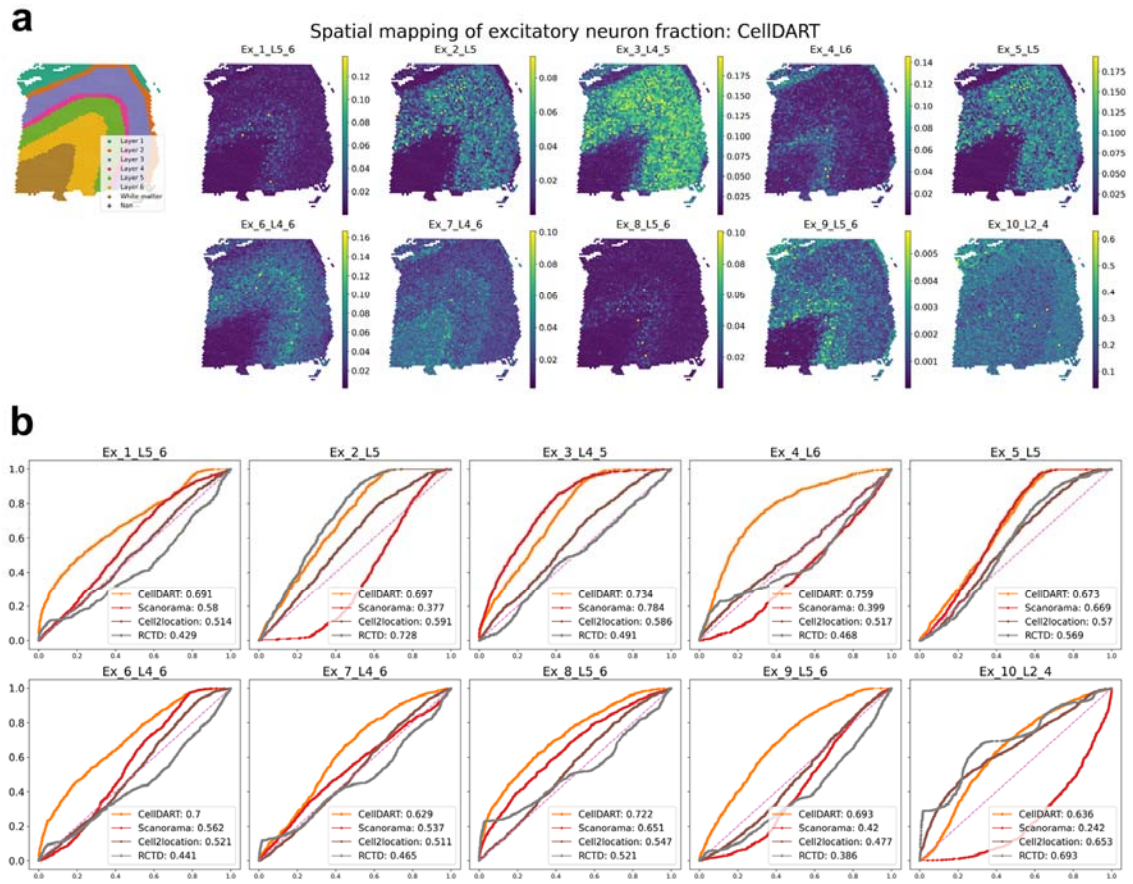


Fig. 2. Implementation of CellDART in a human dorsolateral prefrontal cortex dataset.

a, Spatial mapping of 10 layer-specific excitatory neurons predicted by CellDART. The figure in the top left corner shows the layer annotation for each spatial spot. The layer consists of cortical layers 1 to 6 and white matter. 'Nan' represents the spot without the layer information. Colormaps present the maximum and minimum values for the corresponding cell fraction.

b, Receiver operating characteristic (ROC) analysis for predicting the layer-specific distribution of excitatory neurons. The computational tools CellDART, Scanorama, Cell2location, and RCTD, which estimate cell types in the spatial spots, were compared by means of the area under the curve (AUC). The ROC curves for CellDART, Scanorama, Cell2location, and RCTD are color-coded, and AUC values are presented in the lower right

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

corner of each plot.

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

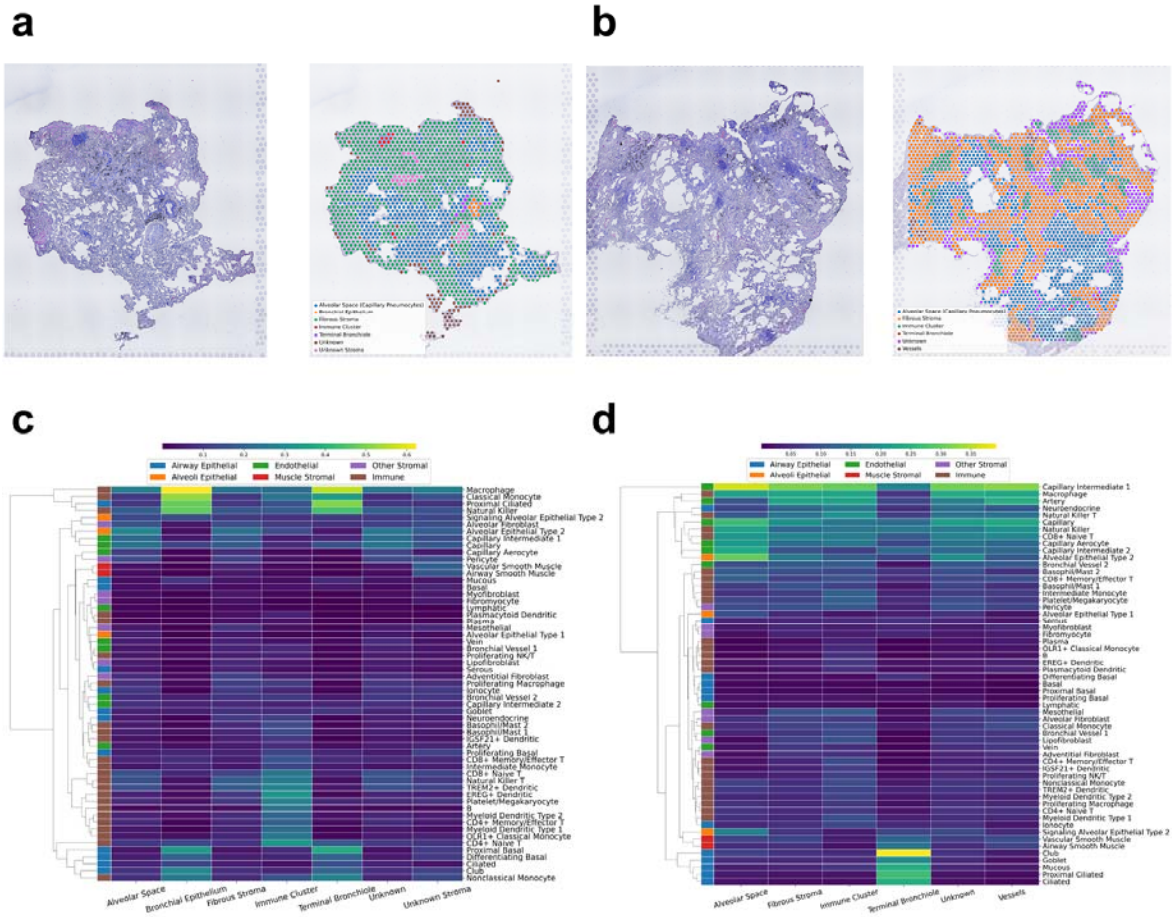


Fig. 3. Application of CellDART in human lung data to decipher the tissue microenvironment

a-b, Segmentation of the histological structures in normal lung tissue (a) 1 and (b) 2. The tissue was divided into six domains: alveolar space (capillary pneumocyte), bronchial epithelium, fibrous stroma, immune cluster, terminal bronchiole, and vessels. Uncertain stromal tissue was defined as 'unknown stroma', and the other unspecified areas were defined as 'unknown'.

c-d, Heatmaps for the average scaled cell fraction in each histological domain of (c) lung 1 and (d) 2 tissues. The cell types were classified into 6 categories ('alveolar epithelial', 'alveoli epithelial', 'endothelial', 'muscle stromal', 'other stromal', and 'immune') based on

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

the original paper of the human lung cell atlas[31] and color-coded on top of the heatmaps. Additionally, hierarchical clustering was performed based on cell fraction profiles across the tissue compartment to visualize the similarity between the cell types.

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

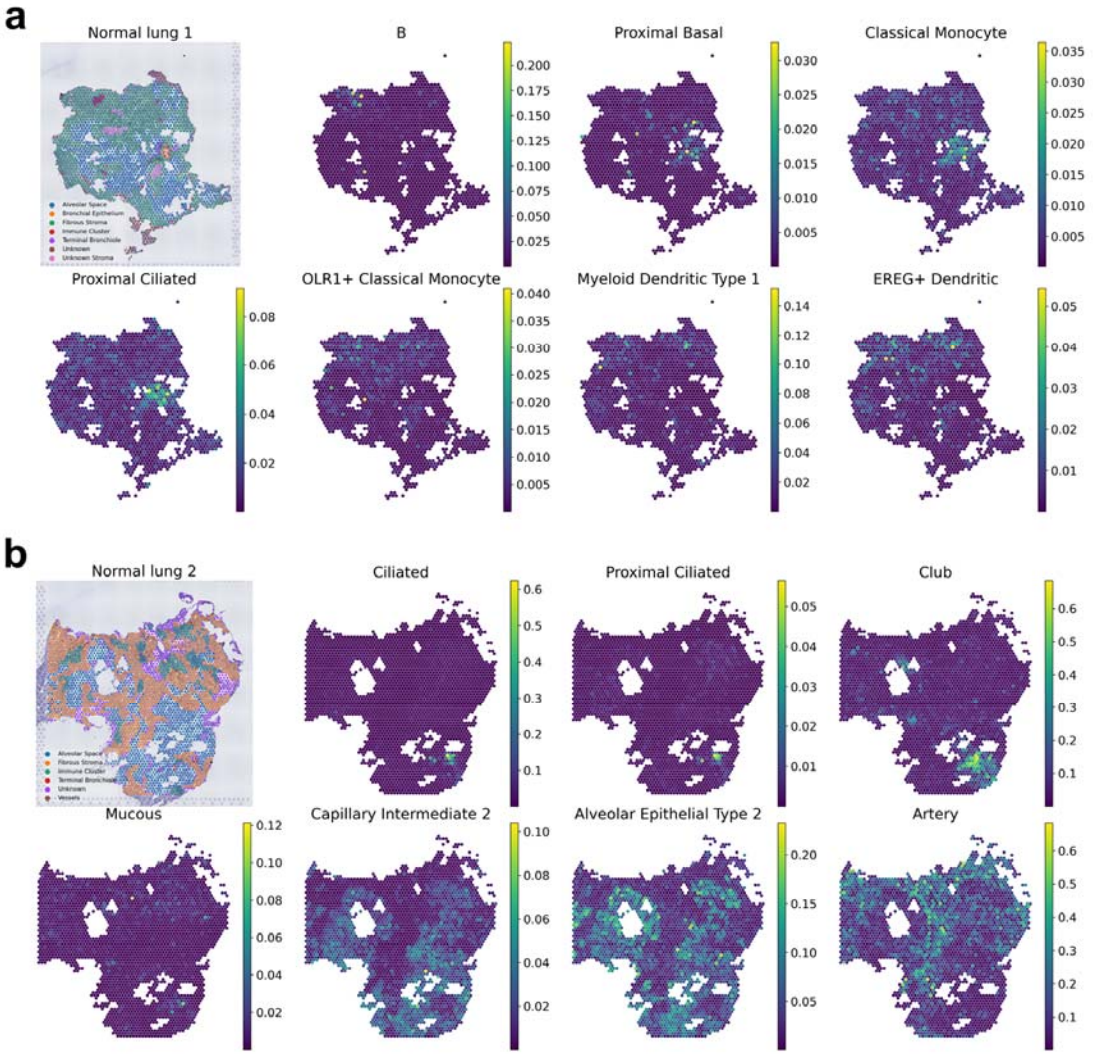


Fig. 4. Spatial compositions of tissue compartment-specific cell types in the human lung

a-b, Spatial mapping of the lung cell fraction for the top 7 cell types predominant in a specific tissue compartment of the (a) lung 1 and (b) 2 datasets. The cell types were ranked based on the average scaled cell fraction in a specific tissue domain compared to the other domains. The figure in the top left corner shows the classification of the tissue domain for each spatial spot. Colormaps present the maximum and minimum values for the corresponding cell fraction.

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

References

1. Waylen, L.N., Nim, H.T., Martelotto, L.G. & Ramialison, M. From whole-mount to single-cell spatial assessment of gene expression in 3D. *Commun Biol* **3**, 602 (2020).
2. Larsson, L., Frisen, J. & Lundeberg, J. Spatially resolved transcriptomics adds a new dimension to genomics. *Nat Methods* **18**, 15-18 (2021).
3. Stahl, P.L. et al. Visualization and analysis of gene expression in tissue sections by spatial transcriptomics. *Science* **353**, 78-82 (2016).
4. Rodriques, S.G. et al. Slide-seq: A scalable technology for measuring genome-wide expression at high spatial resolution. *Science* **363**, 1463-1467 (2019).
5. Vickovic, S. et al. High-definition spatial transcriptomics for in situ tissue profiling. *Nat Methods* **16**, 987-990 (2019).
6. Stickels, R.R. et al. Highly sensitive spatial transcriptomics at near-cellular resolution with Slide-seqV2. *Nat Biotechnol* **39**, 313-319 (2021).
7. Cho, C.S. et al. Seq-Scope: Submicrometer-resolution spatial transcriptomics for single cell and subcellular studies. Preprint at https://www.biorxiv.org/content/10.1101/2021.01.25.427807v1 (2021).
8. Berglund, E. et al. Spatial maps of prostate cancer transcriptomes reveal an unexplored landscape of heterogeneity. *Nat Commun* **9**, 2419 (2018).
9. Thrane, K., Eriksson, H., Maaskola, J., Hansson, J. & Lundeberg, J. Spatially Resolved Transcriptomics Enables Dissection of Genetic Heterogeneity in Stage III Cutaneous Malignant Melanoma. *Cancer Res* **78**, 5970-5979 (2018).
10. Carlberg, K. et al. Exploring inflammatory signatures in arthritic joint biopsies with Spatial Transcriptomics. *Sci Rep* **9**, 1-10 (2019).
11. Chen, W.T. et al. Spatial Transcriptomics and In Situ Sequencing to Study Alzheimer's Disease. *Cell* **182**, 976-991 (2020).

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

12. Ji, A.L. et al. Multimodal Analysis of Composition and Spatial Architecture in Human Squamous Cell Carcinoma. *Cell* **182**, 497-514 (2020).

13. Pham, D.T. et al. stLearn: integrating spatial location, tissue morphology and gene expression to find cell types, cell-cell interactions and spatial trajectories within undissociated tissues. Preprint at https://www.biorxiv.org/content/10.1101/2020.05.31.125658v1 (2020).

14. Dries, R. et al. Giotto: a toolbox for integrative analysis and visualization of spatial expression data. *Genome Biology* **22**, 1-31 (2021).

15. Dagogo-Jack, I. & Shaw, A.T. Tumour heterogeneity and resistance to cancer therapies. *Nat Rev Clin Oncol* **15**, 81-94 (2018).

16. Hie, B., Bryson, B. & Berger, B. Efficient integration of heterogeneous single-cell transcriptomes using Scanorama. *Nat Biotechnol* **37**, 685-691 (2019).

17. Stuart, T. et al. Comprehensive Integration of Single-Cell Data. *Cell* **177**, 1888-1902 (2019).

18. Welch, J.D. et al. Single-Cell Multi-omic Integration Compares and Contrasts Features of Brain Cell Identity. *Cell* **177**, 1873-1887 (2019).

19. Andersson, A. et al. Single-cell and spatial transcriptomics enables probabilistic inference of cell type topography. *Commun Biol* **3**, 565 (2020).

20. Kleshchevnikov, V. et al. Comprehensive mapping of tissue cell architecture via integrated single cell and spatial transcriptomics. Preprint at https://www.biorxiv.org/content/10.1101/2020.11.15.378125v1 (2020).

21. Cable, D.M. et al. Robust decomposition of cell type mixtures in spatial transcriptomics. *Nat Biotechnol* (2021).

22. Elosua-Bayes, M., Nieto, P., Mereu, E., Gut, I. & Heyn, H. SPOTlight: seeded NMF regression to deconvolute spatial transcriptomics spots with single-cell transcriptomes.

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

Nucleic Acids Res (2021).

23. Teng, H., Yuan, Y. & Bar-Joseph, Z. Cell Type Assignments for Spatial Transcriptomics Data. Preprint at https://www.biorxiv.org/content/10.1101/2021.02.25.432887v1 (2021).

24. Dai Yang, K. et al. Multi-domain translation between single-cell imaging and sequencing data using autoencoders. *Nat Commun* **12**, 1-10 (2021).

25. Zhou, X. et al. scAdapt: Virtual adversarial domain adaptation network for single cell RNA-seq data classification across platforms and species. Preprint at https://www.biorxiv.org/content/10.1101/2021.01.18.427083v1 (2021).

26. Tzeng, E., Hoffman, J., Saenko, K. & Darrell, T. Adversarial Discriminative Domain Adaptation. In Proceedings of the IEEE conference on computer vision and pattern recognition, 7167-7176 (2017).

27. Tasic, B. et al. Shared and distinct transcriptomic cell types across neocortical areas. *Nature* **563**, 72-78 (2018).

28. Nagy, C. et al. Single-nucleus transcriptomics of the prefrontal cortex in major depressive disorder implicates oligodendrocyte precursor cells and excitatory neurons. *Nat Neurosci* **23**, 771-781 (2020).

29. Maynard, K.R. et al. Transcriptome-scale spatial gene expression in the human dorsolateral prefrontal cortex. *Nat Neurosci* **24**, 425-436 (2021).

30. Hawrylycz, M.J. et al. An anatomically comprehensive atlas of the adult human brain transcriptome. *Nature* **489**, 391-399 (2012).

31. Travaglini, K.J. et al. A molecular cell atlas of the human lung from single-cell RNA sequencing. *Nature* **587**, 619-625 (2020).

32. Bayraktar, O.A. et al. Astrocyte layers in the mammalian cerebral cortex revealed by a single-cell in situ transcriptomic map. *Nat Neurosci* **23**, 500-509 (2020).

bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

33. Lanjakornsiripan, D. et al. Layer-specific morphological and molecular differences in neocortical astrocytes and their dependence on neuronal layers. *Nat Commun* **9**, 1623 (2018).

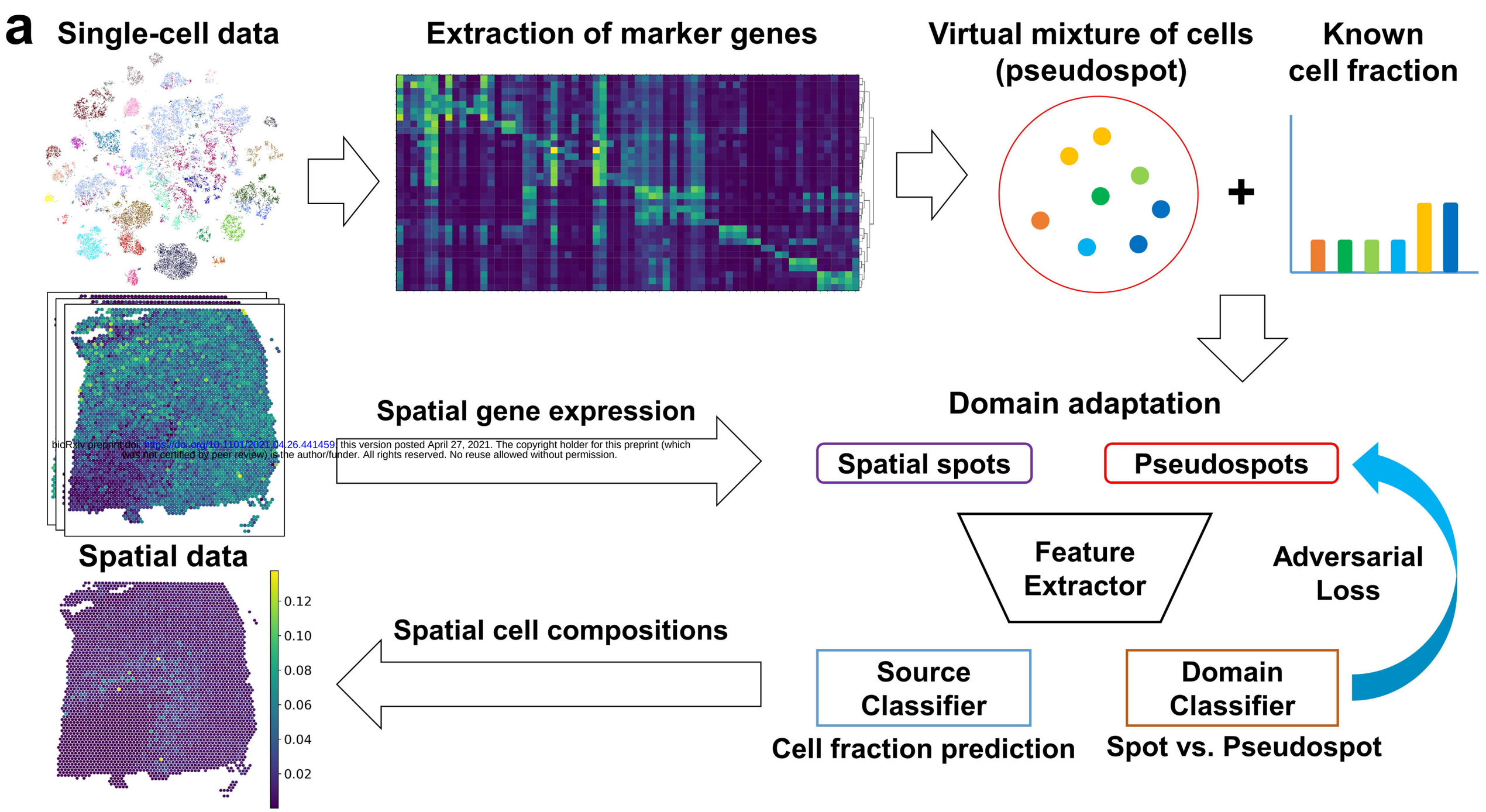
34. Batiuk, M.Y. et al. Identification of region-specific astrocyte subtypes at single cell resolution. *Nat Commun* **11**, 1220 (2020).

35. Valerio-Gomes, B., Guimaraes, D.M., Szczupak, D. & Lent, R. The Absolute Number of Oligodendrocytes in the Adult Mouse Brain. *Front Neuroanat* **12**, 90 (2018).

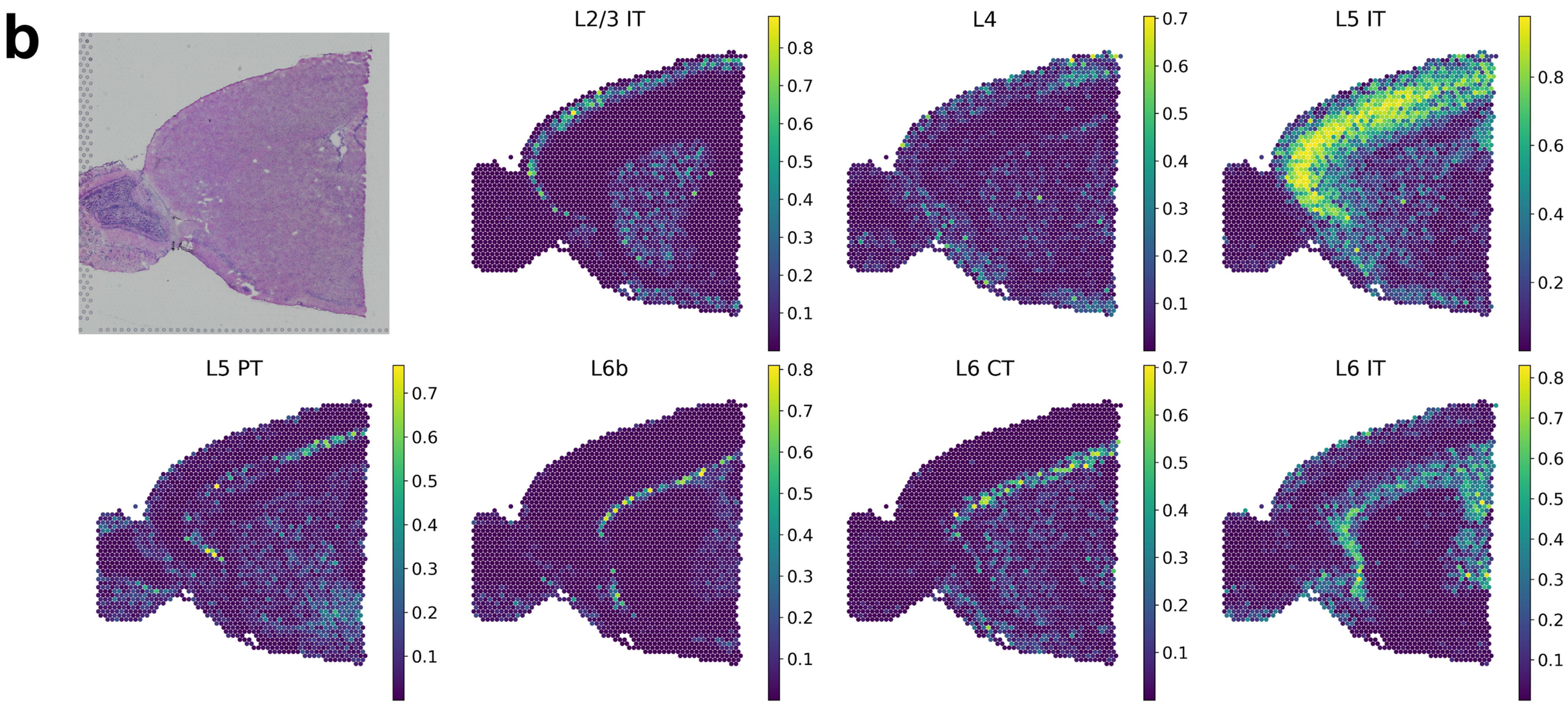
36. Wolf, F.A., Angerer, P. & Theis, F.J. SCANPY: large-scale single-cell gene expression data analysis. *Genome Biol* **19**, 15 (2018).

37. Lake, B.B. et al. Neuronal subtypes and diversity revealed by single-nucleus RNA sequencing of the human brain. *Science* **352**, 1586-1590 (2016).

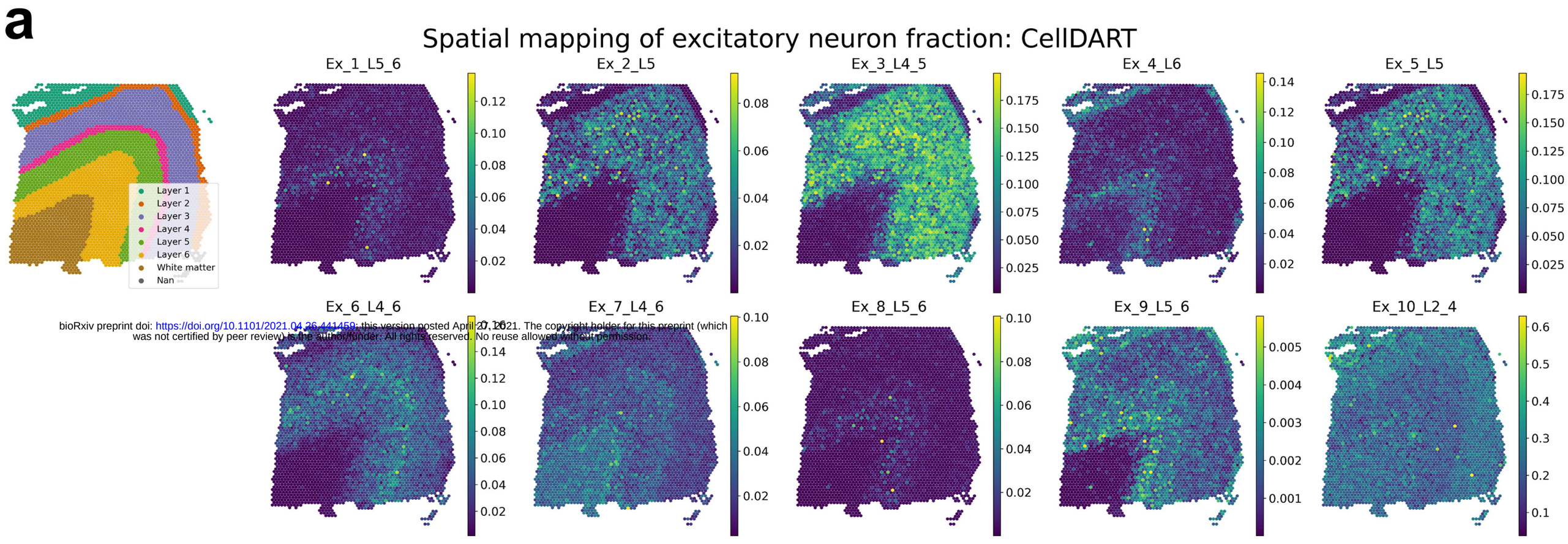
38. Habib, N. et al. Massively parallel single-nucleus RNA-seq with DroNc-seq. *Nat Methods* **14**, 955-958 (2017).

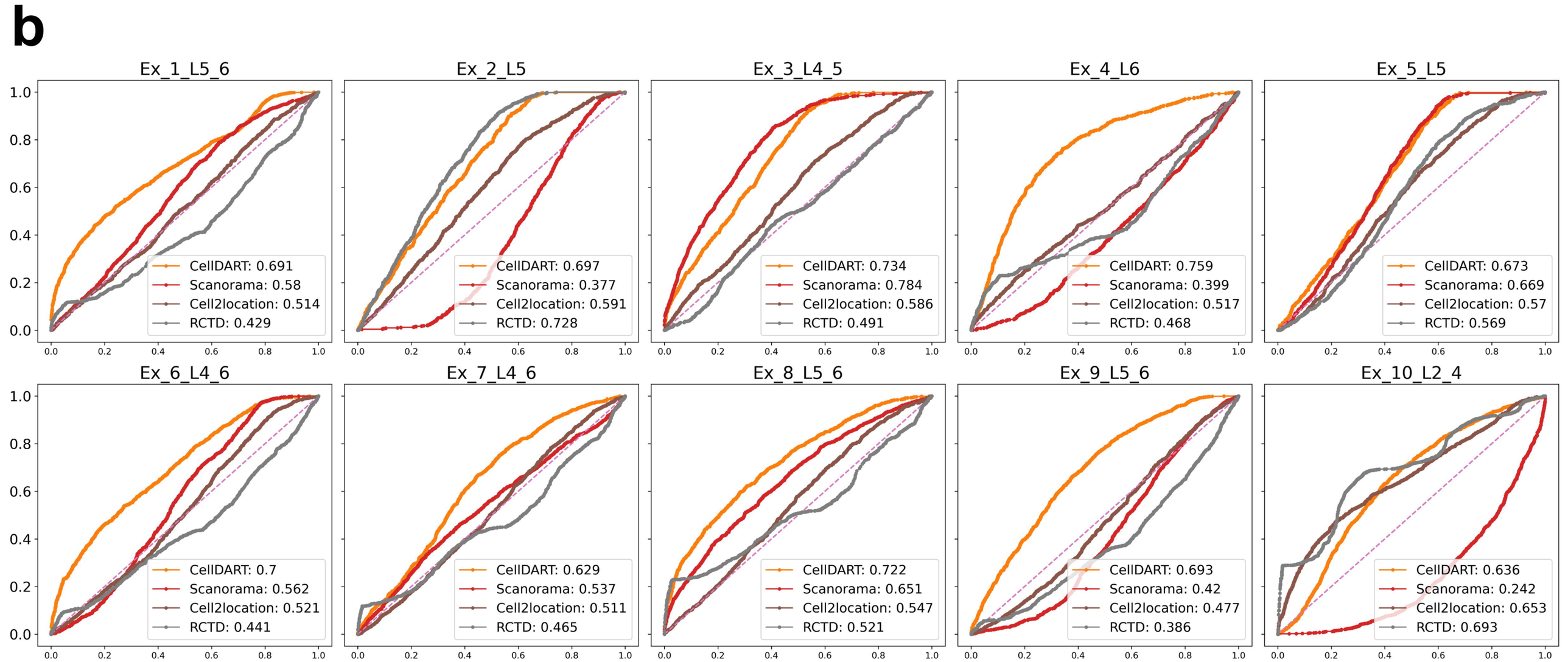


bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

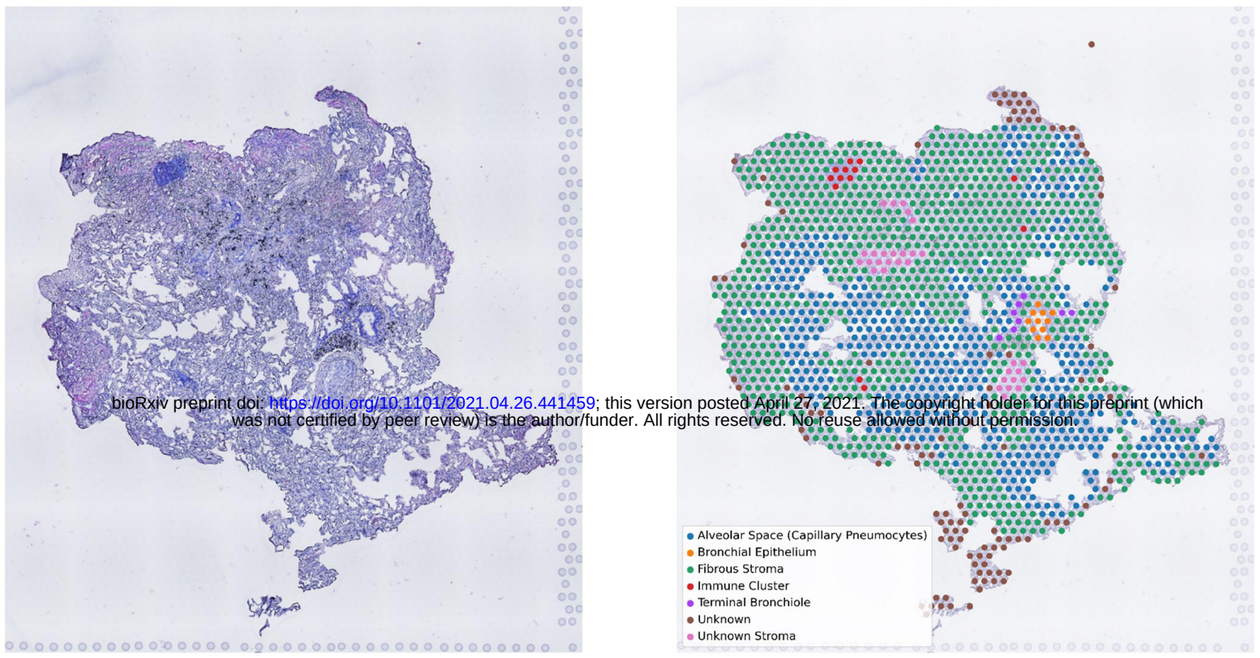


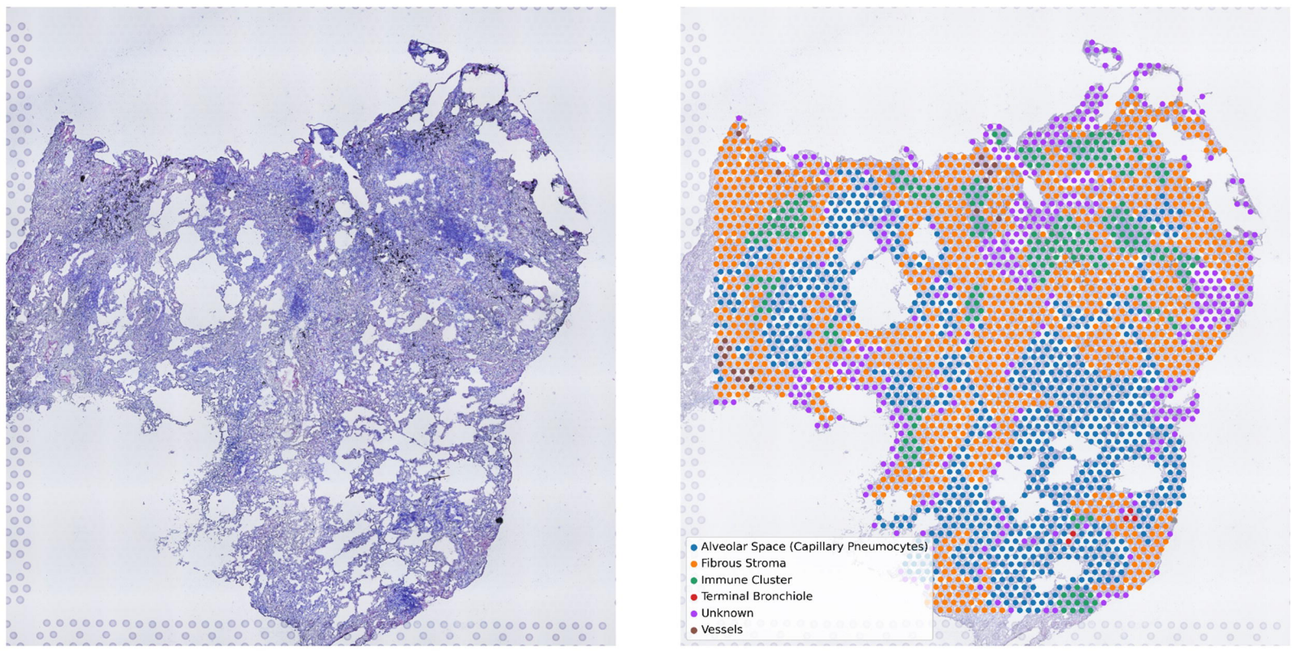
bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.

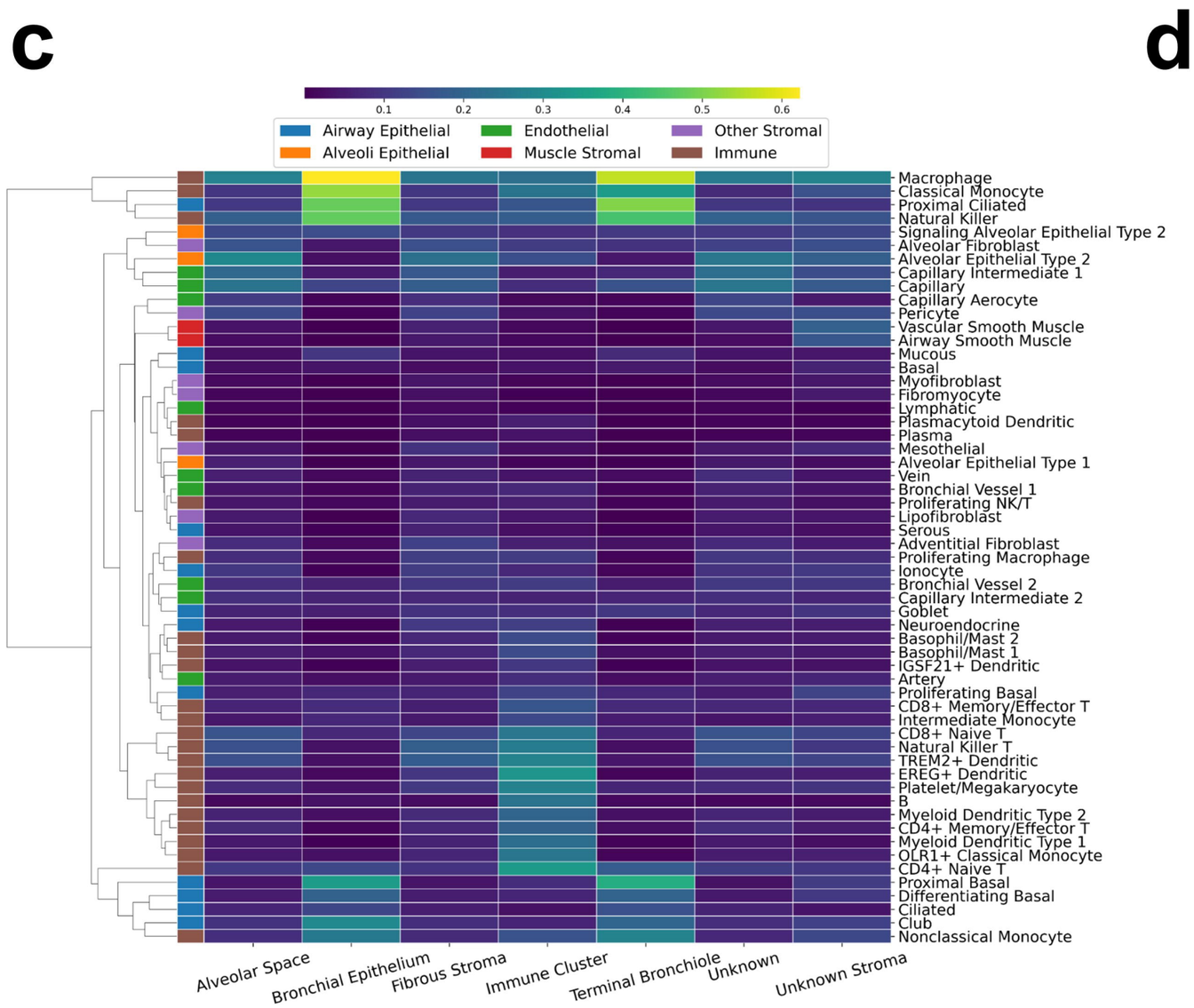


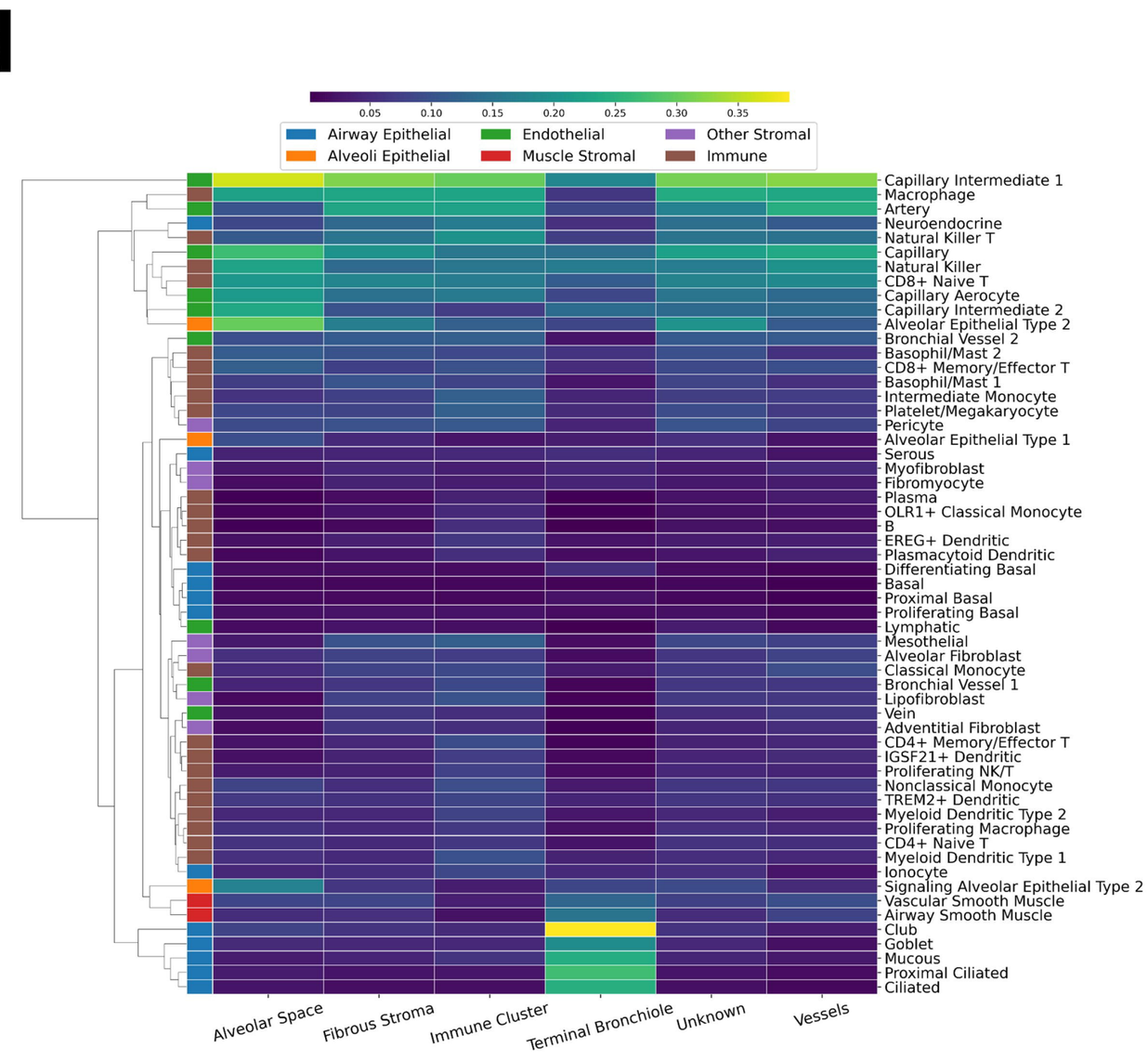


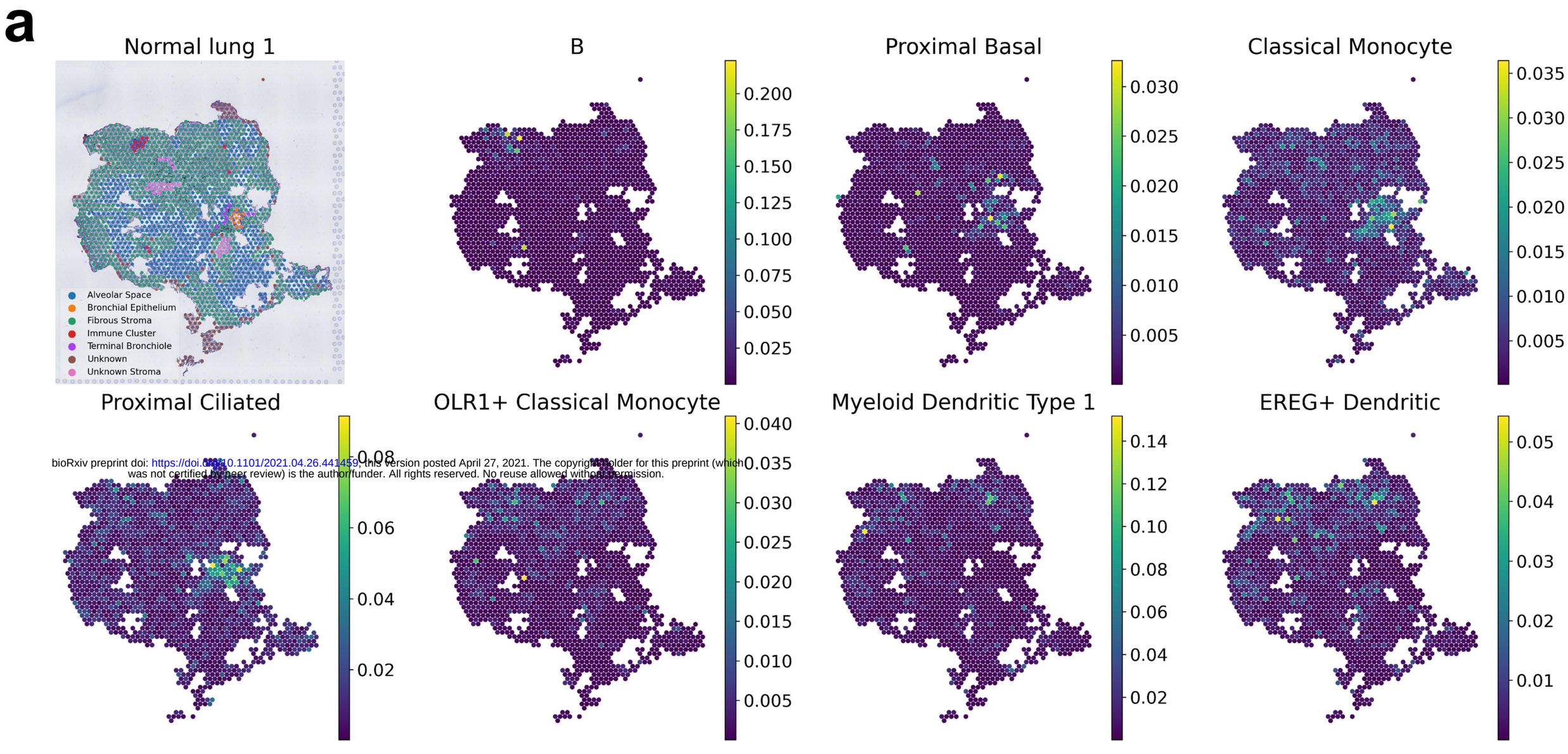
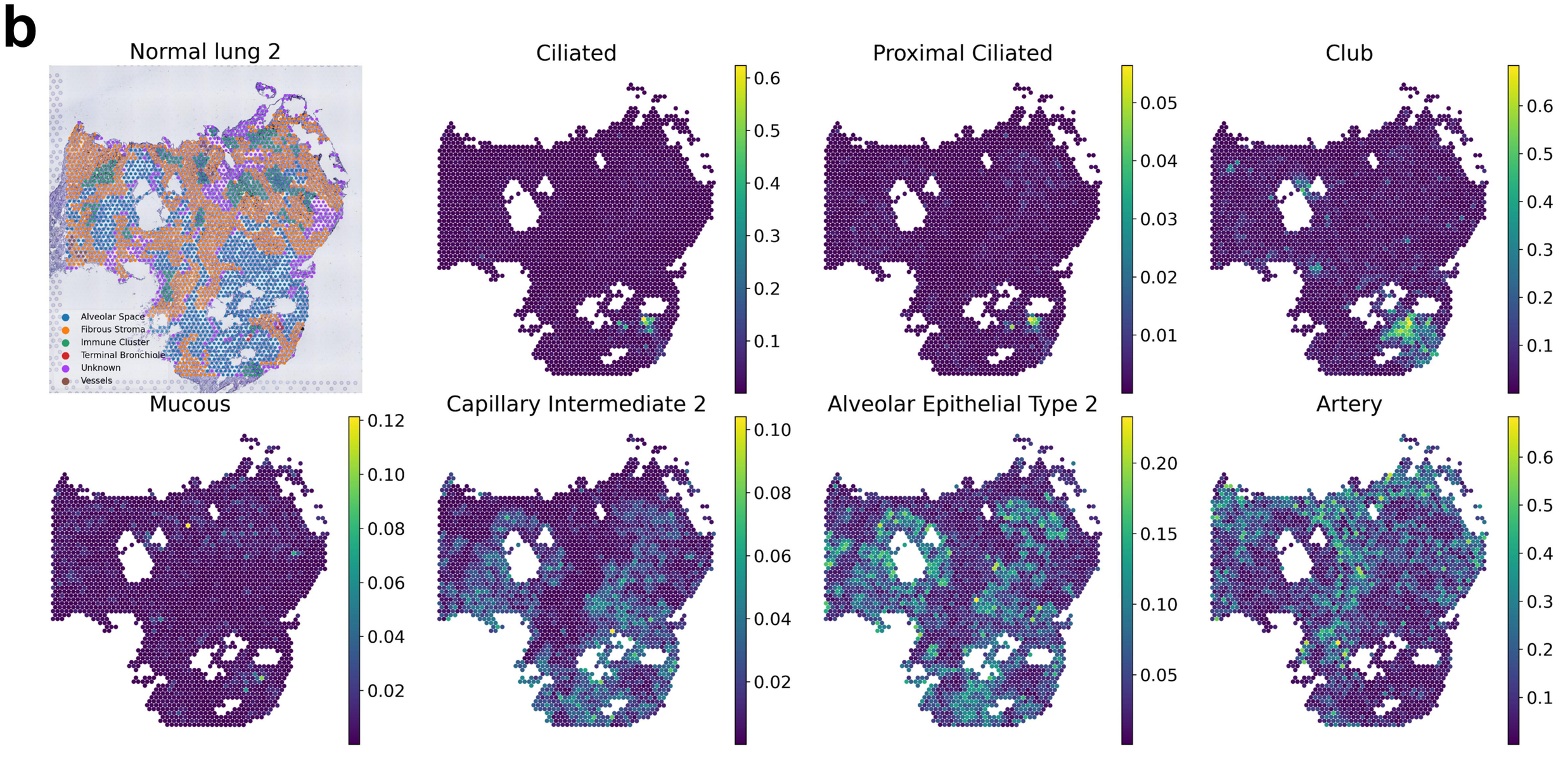
bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459; this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.











bioRxiv preprint doi: https://doi.org/10.1101/2021.04.26.441459, this version posted April 27, 2021. The copyright holder for this preprint (which was not certified by peer review) is the author/funder. All rights reserved. No reuse allowed without permission.