Integrating single cell and spatial gene expression profiling of mouse organogenesis to identify and localize unknown cell types

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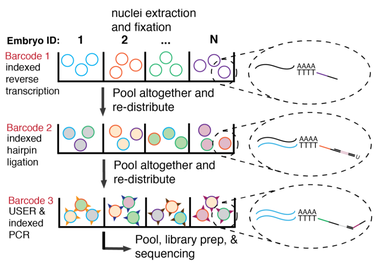
Abstract

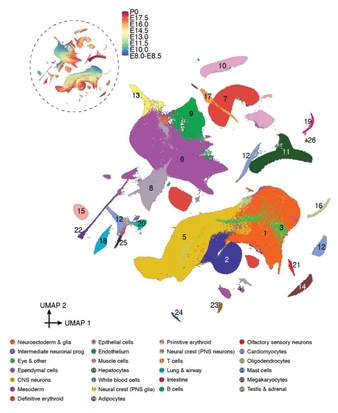
Mammalian organogenesis is a remarkable process, whereby cells rapidly proliferate and differentiate into diverse cell types. Single cell RNA-sequencing of whole embryos yields unprecedented views of development, revealing hundreds of unique cell types defined by gene expression precisely regulated in time and space. Although many methods exist to identify cell types defined by scRNAseq, annotating cells remains a challenging process. In this work, we leverage both scRNAseq and spatial gene expression profiling to identify novel cell populations during organogenesis, and discover changes in cell specification and localization in a developmental mutant. We used high-resolution single nucleus transcriptional profiling of millions of cells done by 3-level combinatorial indexing on staged mouse embryos in 2-6 hour increments from gastrulation to birth. Although an initial round of manual annotation based on marker genes and earlier atlases was fruitful, many ambiguities remained. To address these in part, we integrated matched timepoints with spatial whole transcriptome profiles of precise anatomical regions generated using the GeoMx® Digital Spatial Profiler (DSP). We used a cell type deconvolution algorithm to estimate the abundance of each cell type in each region and validated that known populations such as tissue-specific epithelial cell subtypes were localized with high accuracy. We then used this method to map trajectories derived from the lateral plate mesoderm, populations with limited research that are challenging to annotate. Next, we applied this method to understand how dysregulated cell lineage contributes to organ malformation in a developmental mutant. Absence of embryonic macrophages due to CSFR1 deficiency causes bone and brain deformities and perinatal lethality in mouse and humans. We performed massively scalable single-cell transcriptomics and GeoMx DSP on E18.5 wildtype and CSF1R-deficient mutant littermates. We find differential cell type abundance in both the scRNAseq and between matched spatial regions across many tissues, suggesting that organs beyond bone and brain are impacted by macrophage loss. In conclusion, this work provides a framework for integrating spatial data with scRNAseq in an automated pipeline to map cell populations in normal and pathological samples.

Methods: single cell RNAseq and spatial whole transcriptome profiling of mouse organogenesis

Massively scalable single cell RNAseq (sci-RNA-seq3) (1,2) enables high resolution profiling of mouse organogenesis

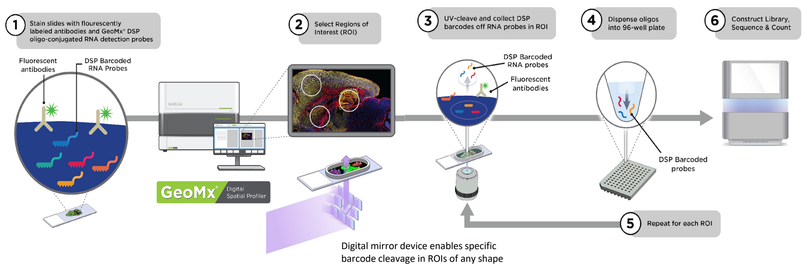
>11 million nuclei from mouse embryos were profiled at 2-6 hour increments across development by sciRNAseq using a 3-level combinatorial indexing approach (3).



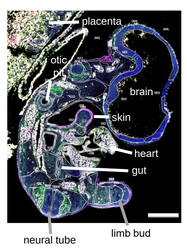


UMAP visualization of the whole dataset colored by 26 major cell type clusters.

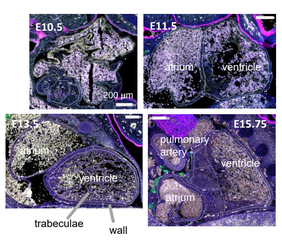
GeoMx Digital Spatial Profiling with the Mouse Whole Transcriptome Atlas (4) enables quantification of 20,175 protein-coding genes in precisely defined regions of interest



We used a publicly available spatial mouse development dataset from the NanoString Spatial Organ Atlas with four timepoints in mid-gestation development (E10.5, E11.5, E13.5, E15.75). These data include spatial whole transcriptome profiling of 15 developing mouse organs, with regions of interest precisely drawn to capture specific histological structures.

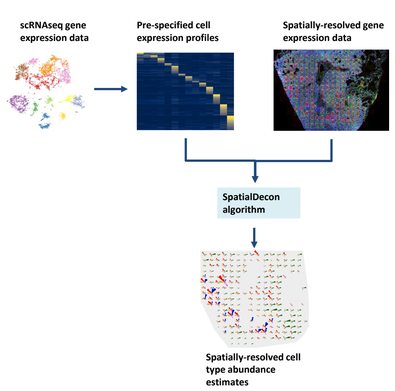


Comprehensive profiling at E10.5 with ROIs drawn to capture precise anatomical structures



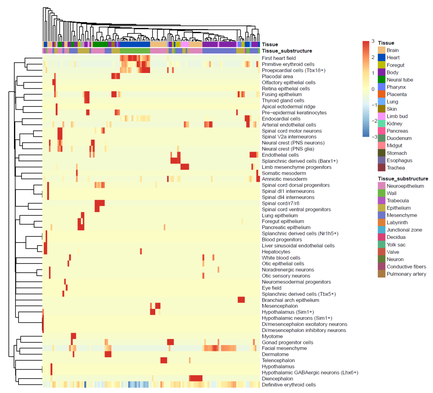
Example regions of interest in heart at all four timepoints

The SpatialDecon algorithm (5) estimates abundances of mixed cell populations in spatially resolved gene expression data using cell profiles derived from scRNAseq

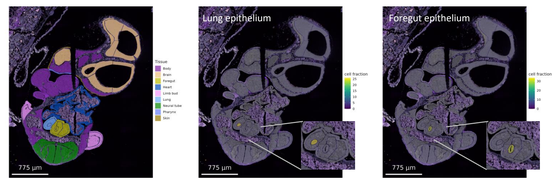


The SpatialDecon algorithm uses log-normal regression to estimate cell abundances, confidence intervals, and p-values in spatial data based on a reference gene expression profile for each cell type. We constructed cell type profiles from scRNAseq clusters and performed cell type deconvolution using the SpatialDecon R package (https://bioconductor.org/packages/release/bioc/html/SpatialDecon.html)

Major cell clusters in wild type E10.5 embryo show expected spatial patterns



Major cell clusters in the developmental scRNAseq dataset were mapped to regions of interest in age-matched E10.5 embryos. Heatmap shows enrichment of each scRNAseq cell type in each GeoMx ROI, annotated by tissue and substructure in the GeoMx data. Only cell types significantly enriched in at least one ROI are shown.



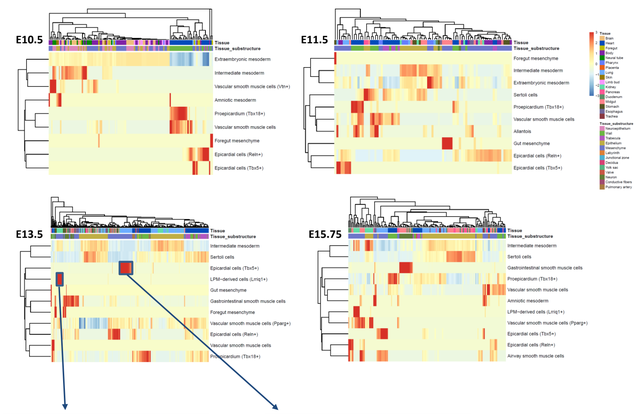
Associated with first branchial arch

Associated with skin

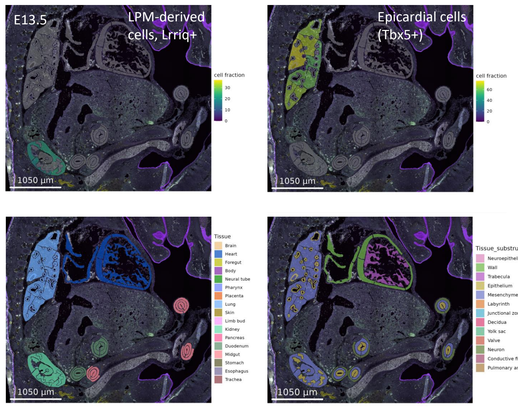
Images of an example E10.5 embryo demonstrating spatial specificity of cell types, visualized using the SpatialOmicsOverlay R package. ROIs are colored by abundance of example epithelial cell types when that cell type is significantly enriched in the ROI (FDR < 0.05). ROIs with no significant presence of the cell type are colored in grey.

Mapping lateral plate mesoderm trajectories across mid-gestation development

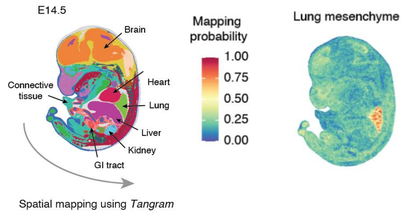
Some previously unannotated cell types can be identified and relabeled based on spatial location



Cells derived from the lateral plate mesoderm contribute to many organs in the developing embryo, but lateral plate mesoderm lineages are complex and difficult to annotate. To validate and correct annotations, we mapped sub-trajectories of lateral plate mesoderm to spatial locations at matched timepoints in mid-gestation development. Heatmaps shows enrichment of each cell type at each timepoint in each GeoMx ROI, annotated by tissue and substructure in the GeoMx data. Only cell types significantly enriched in at least one ROI are shown.

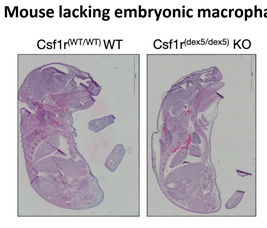


Example images from an E13.5 embryo demonstrate that spatial profiling identified that "Lrriq+ LPM derived cells" contribute to kidney cortex mesenchyme. The "Epicardial cells (Tbx5+)" label can be corrected to lung mesenchyme. These new annotations can be further validated by mapping scRNAseq data to orthogonal spatial data with Stereo-seq (6). The relabeled lung mesenchyme cell type maps to lung in this dataset as well.



Application to a developmental mutant: sciRNAseq and spatial profiling of *Csfr1* deficient embryos

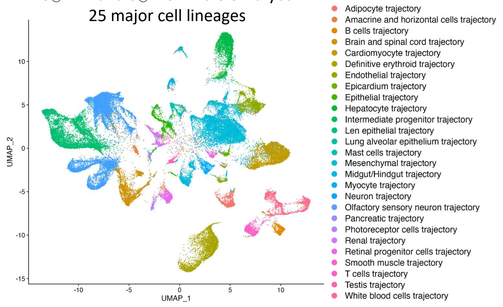
Mouse lacking embryonic macrophages exhibit profound developmental defects in multiple tissues



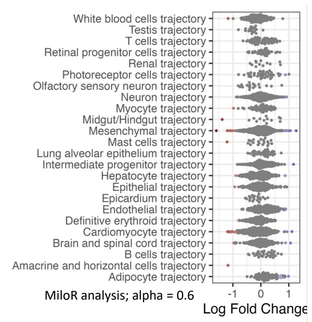
Embryonic macrophages arise from yolk-sac island progenitor cells which migrate to developing organ buds, expanding from E9.5 of mouse organogenesis to become functionally distinct long-lived tissue-resident macrophages. Loss of embryonic macrophages due to *Csfr1* deficiency causes bone and brain deformities as well as perinatal lethality in mouse and humans (7).

Littermate WT and *Csfr1* KO E18.5 embryos were profiled by sciRNAseq and cells were mapped to 25 major trajectories

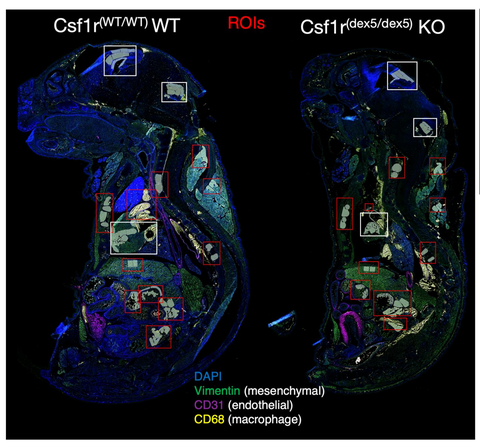
UMAP of 59,095 high-quality cells from 3♂ WT and 3♂ KO whole embryos 25 major cell lineages

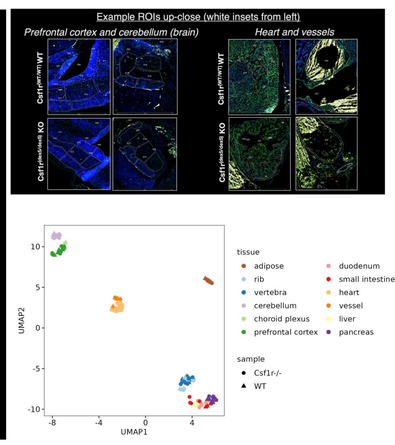


Differential abundance testing suggests minor differences in major trajectories between wild type and mutant



GeoMx Digital Spatial Profiling of 12 major tissues and substructures within those tissues in matched fixed frozen sagittal sections of littermate E18.5 WT and *Csfr1* deficient embryos

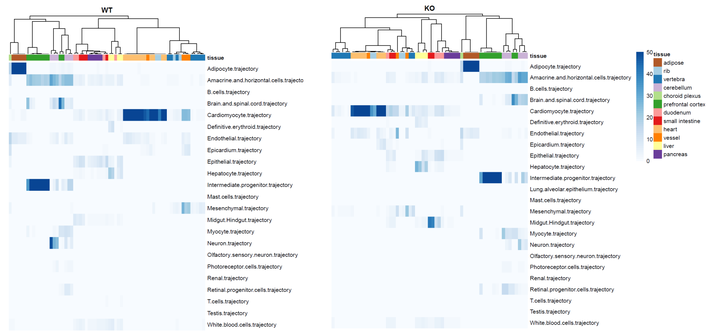




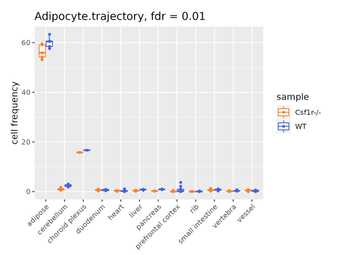
UMAP of ROIs colored by tissue and shaped by mutant status shows clear separation by tissue

Mapping major cell types spatially and identifying tissue-specific differential cell abundance in *Csfr1* mutants

Abundance and spatial patterning of cell types in each region of interest in wild type and mutant embryos using matched sciRNAseq and GeoMx DSP data



Spatial patterning of major trajectories is overall similar between wild type and mutant, with only minor differences in abundance in certain populations. For example, the adipocyte trajectory is less abundant in multiple tissues, including adipose and brain. Future work will investigate the spatial distribution and abundance of the sub-lineages of each cell type in wild type and mutant and differential gene expression in each tissue.



Conclusions

- High resolution sciRNAseq identifies novel cell types during dynamic processes such as embryonic development
- Spatial whole transcriptome profiling with GeoMx WTA can localize and annotate novel cell types identified by scRNAseq
- In developmental mutants, single cell RNAseq plus spatial gene expression data can link observed pathology to changes in cell abundance, cell localization, or cell state

References

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