Ischemic Str🧠ke Vis(ium)alized

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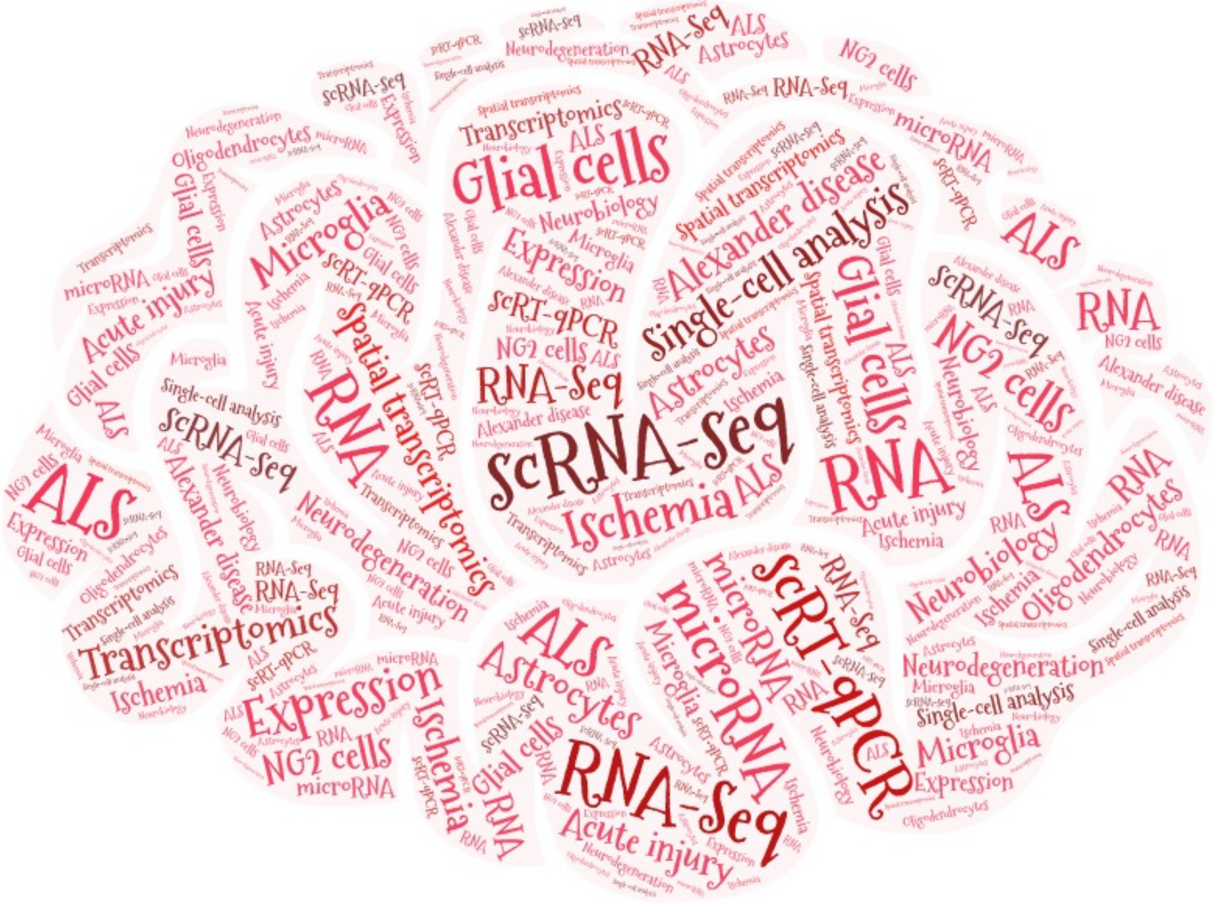
Laboratory of Gene Expression

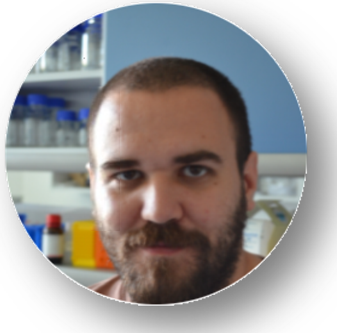
- Gene expression analysis
  - Bulk RNA-seq
  - Single-cell RNA-seq
  - Spatial transcriptomics
- Method development and QC
- Glial cell biology
  - Acute injury
  - Neurodegeneration



Projects with 10x Genomics technology

- Single-cell/nucleus RNA-seq
  - Neurobiology - stroke, amyotrophic lateral sclerosis, Alexander disease (mouse models; human iPSCs and organoids)
  - Developmental biology - craniofacial bones formation (mouse embryos)
  - Healing/regeneration (Xenopus tadpoles)
  - Gynaecology and Obstetrics - Asherman's syndrome (patient‘s sample)
  - Plant biology - embryonic cells in Arabidopsis and Sorghum

- Spatial transcriptomics
  - Neurobiology - stroke (mouse model)
  - Developmental biology - craniofacial bones formation (mouse embryos)



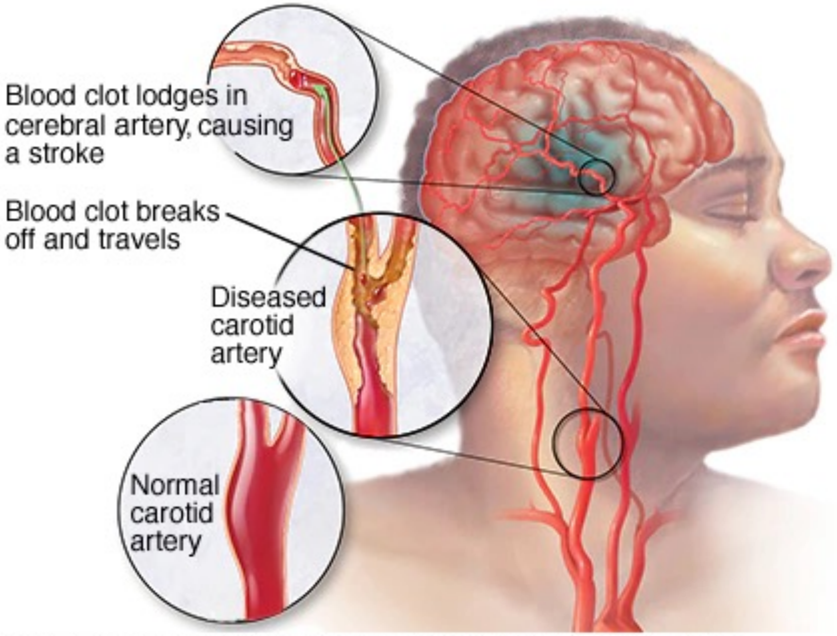
Pavel Abaffy

Daniel Zucha

Ischemic brain injury (stroke)

- Critical reduction in blood flow caused by either sudden or gradual occlusion of cerebral arteries
- Blockage of blood circulation causes neurologic deficits

- Main pathologic changes includes
  - Energy depletion, calcium overload, reactive oxygen species generation, inflammatory response, and ion imbalance
- Changes detrimental to basic cell functions leading to cell death



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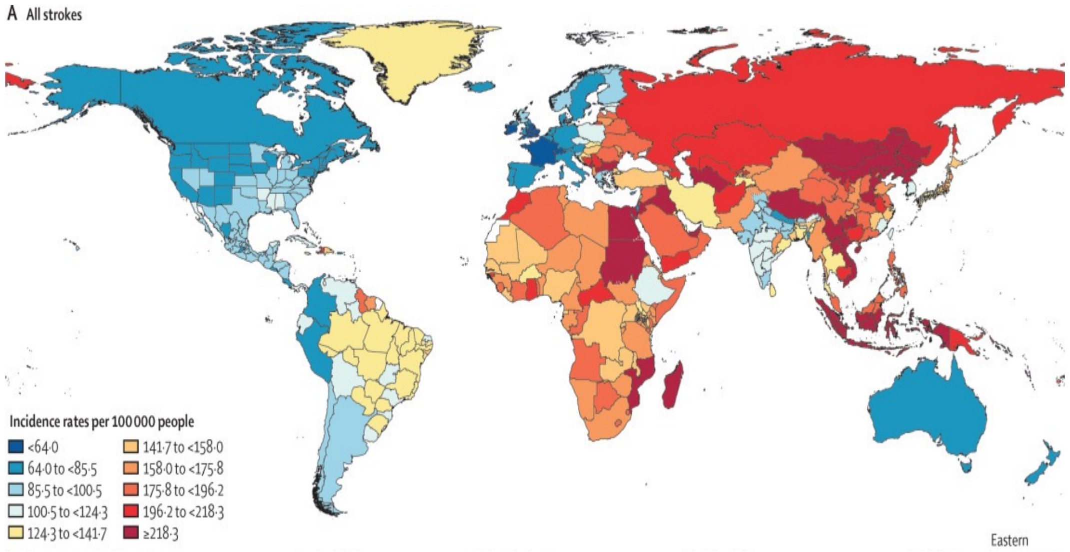
Ischemic brain injury (stroke)

- Affects over 12 millions people per year world-wide
  - Second leading cause of death (6.5 millions)
  - Third leading cause of death and disability combined

-> Major health care and economic burden

- Demand on development of new neuroprotective strategies
  - >1000 drugs investigated
  - >100 tested in clinical trials
- Early clot lysis remains the sole approved therapy



GBD 2019 Stroke Collaborators, 2021

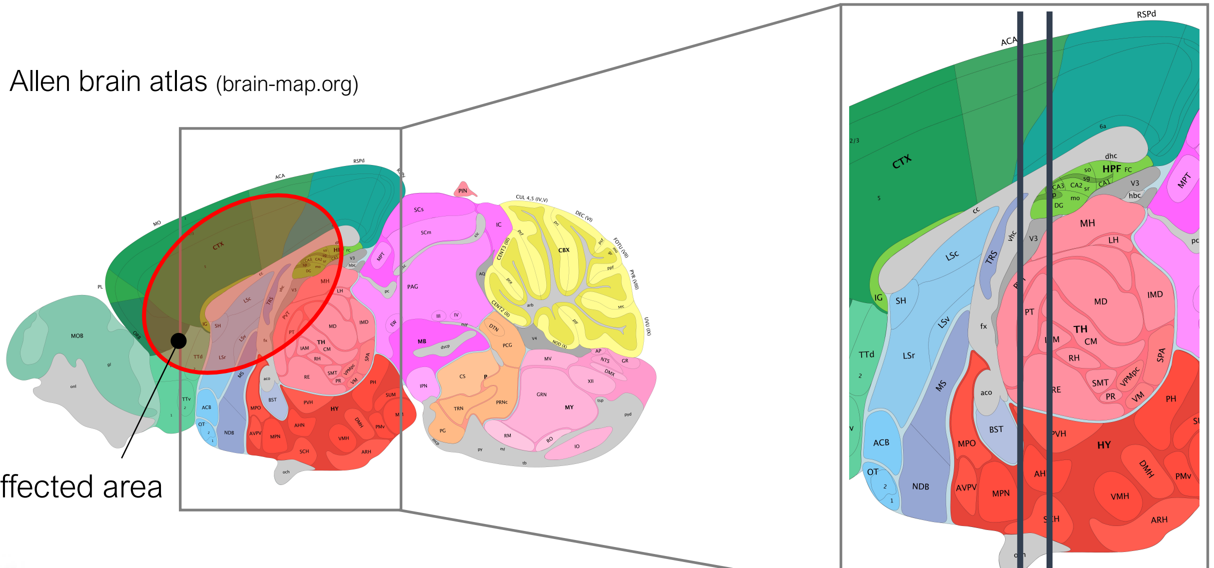
Ischemic brain injury (stroke)

- The major reasons causing the lack of new therapeutics
  a) Complexity of the disease involving interactions of many cell types
  b) Existence of heterogeneity exaggerating the complexness of the disease
  c) Temporal and spatial factors defining the role of cell types in the disease

- Unique opportunity to apply recent technologies for transcriptomic analysis
  - Single-cell RNA-sequencing
  - Spatial transcriptomics
  - Integrative analysis

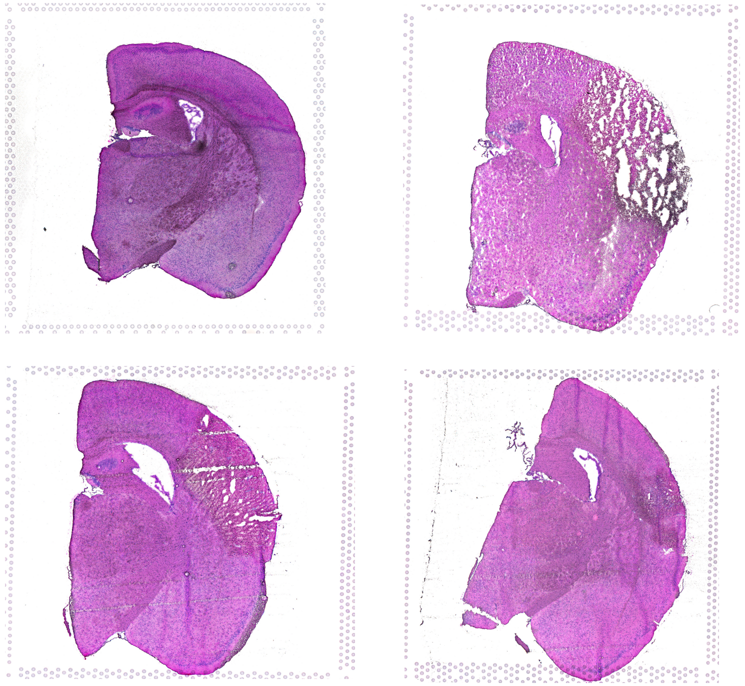
Experimental model

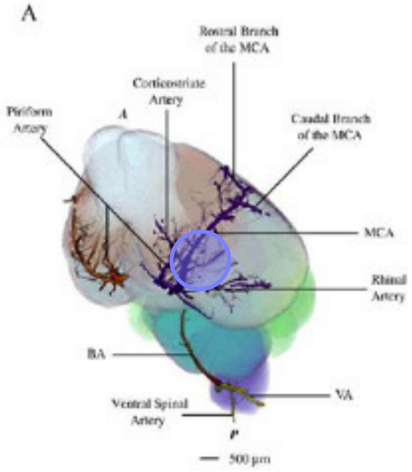
Allen brain atlas (brain-map.org)



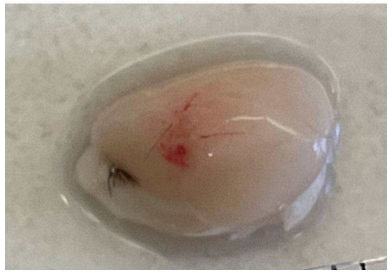
Affected area

Pre-defined area of interest



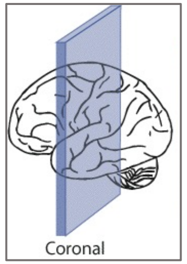


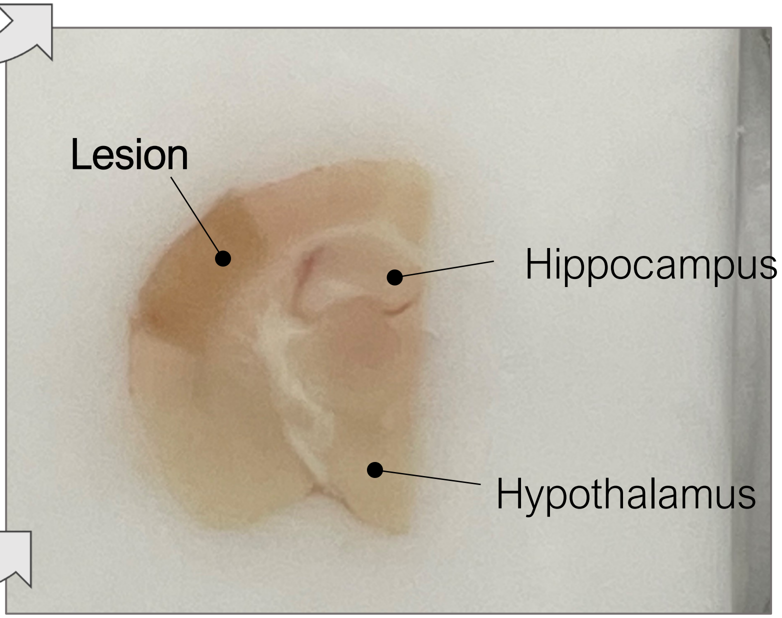
Middle cerebral artery occlusion (MCAO)



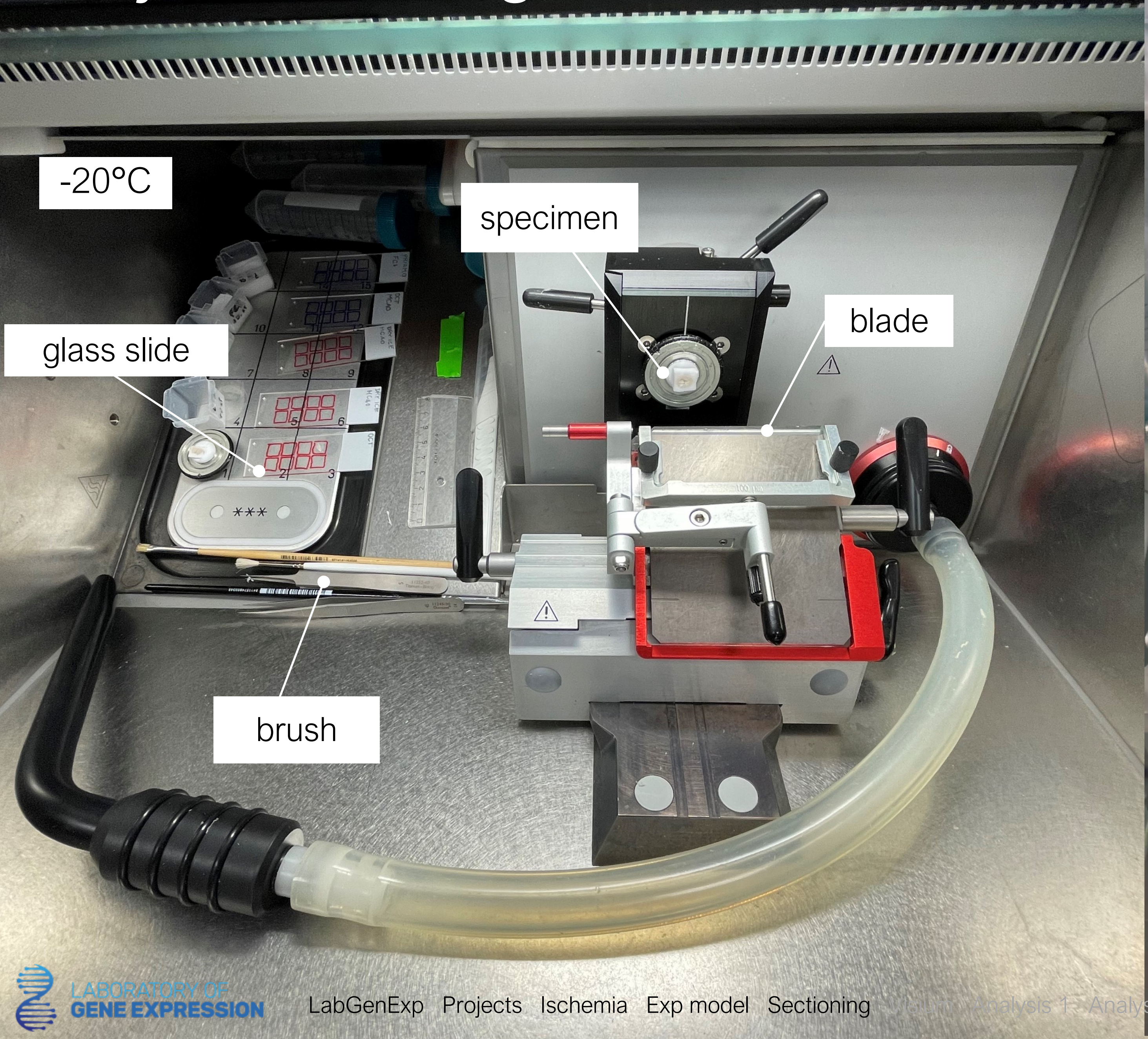
m. musculus, ipsilesional hemisphere

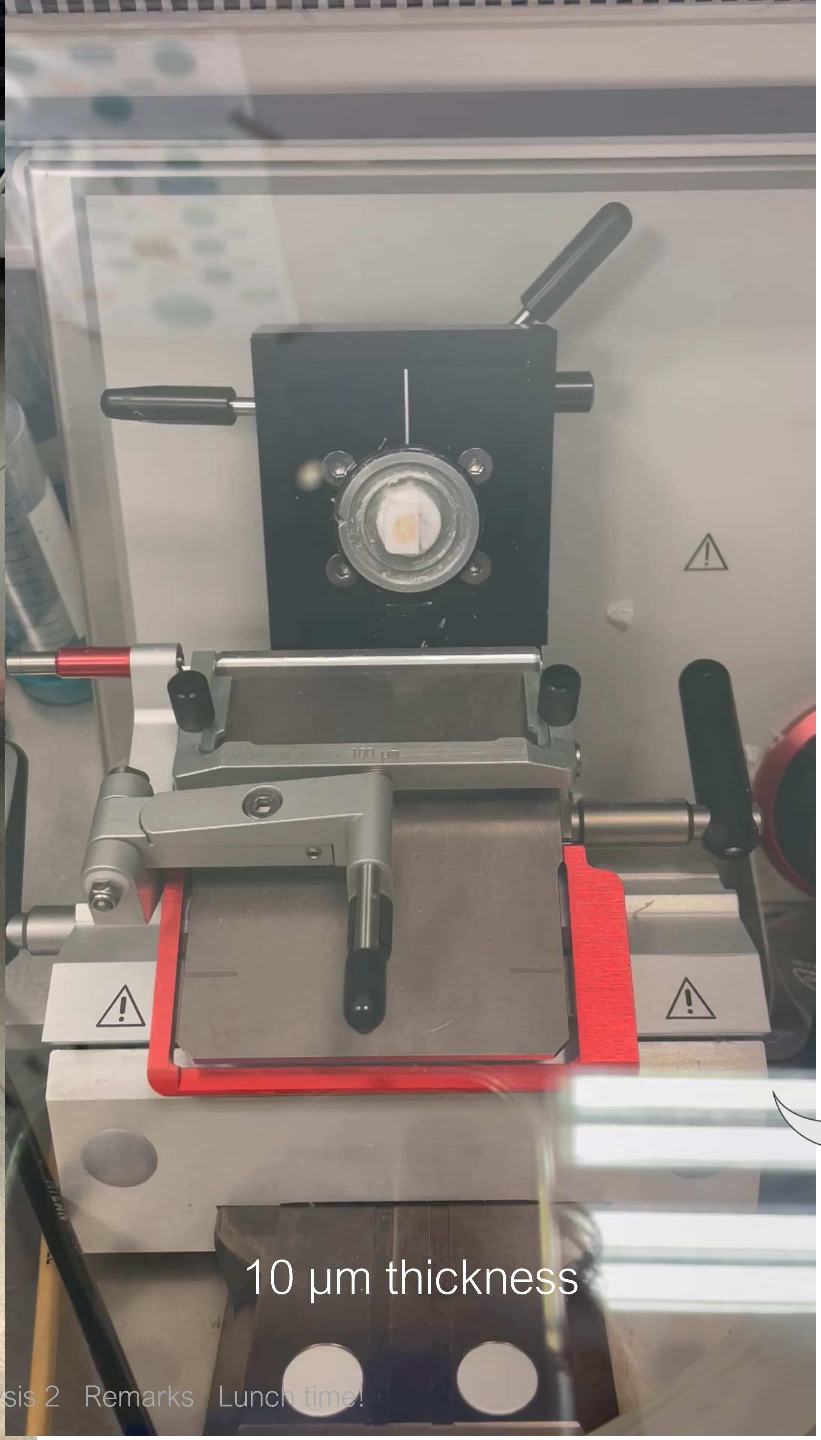
Freeze on dry ice





Cryosectioning





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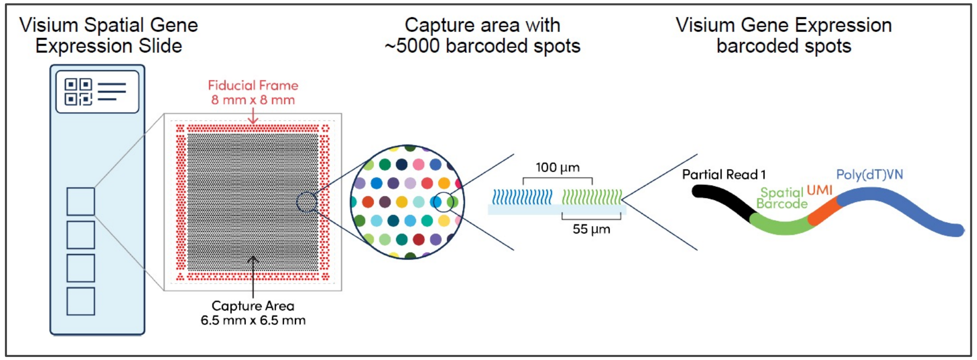
Critical step

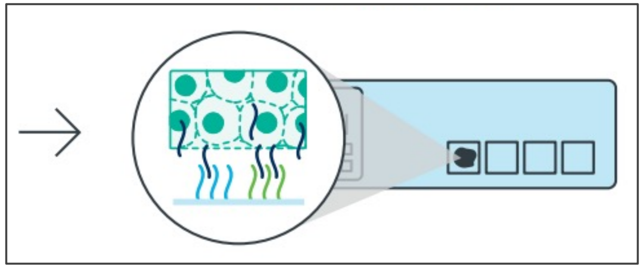
Patience

Practice

Precision

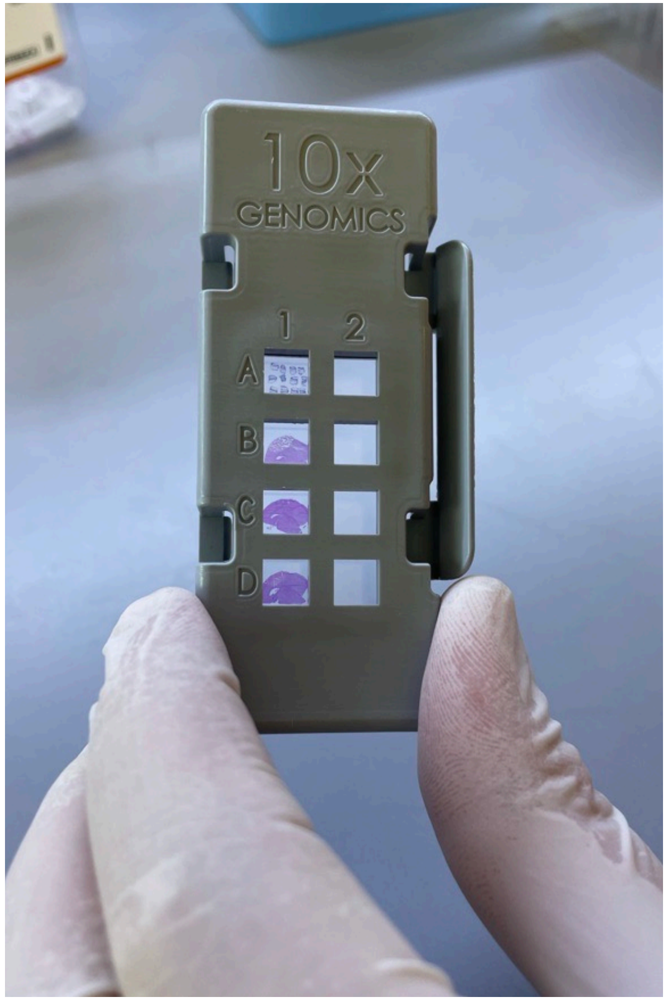
Visium





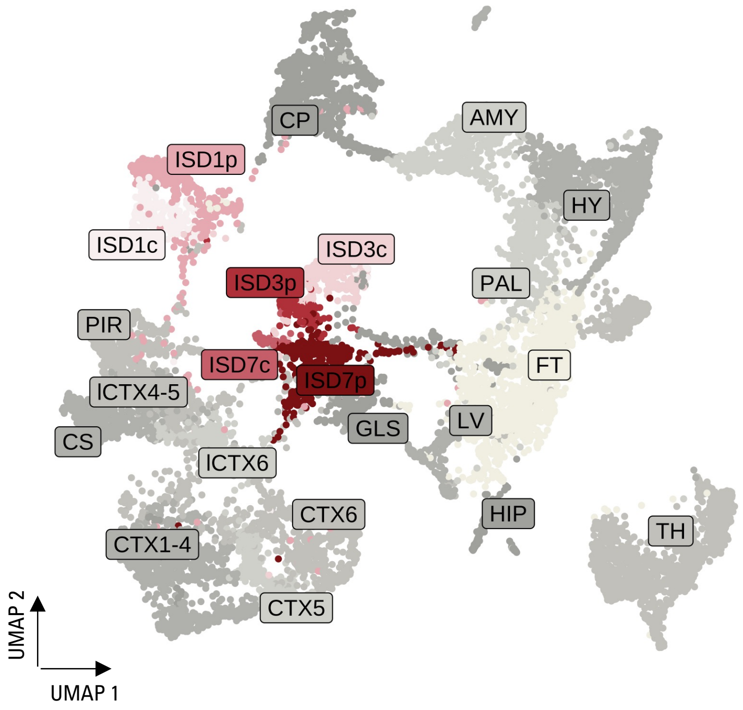
Transcriptome-wide with spatial localization

Not single-cell resolution



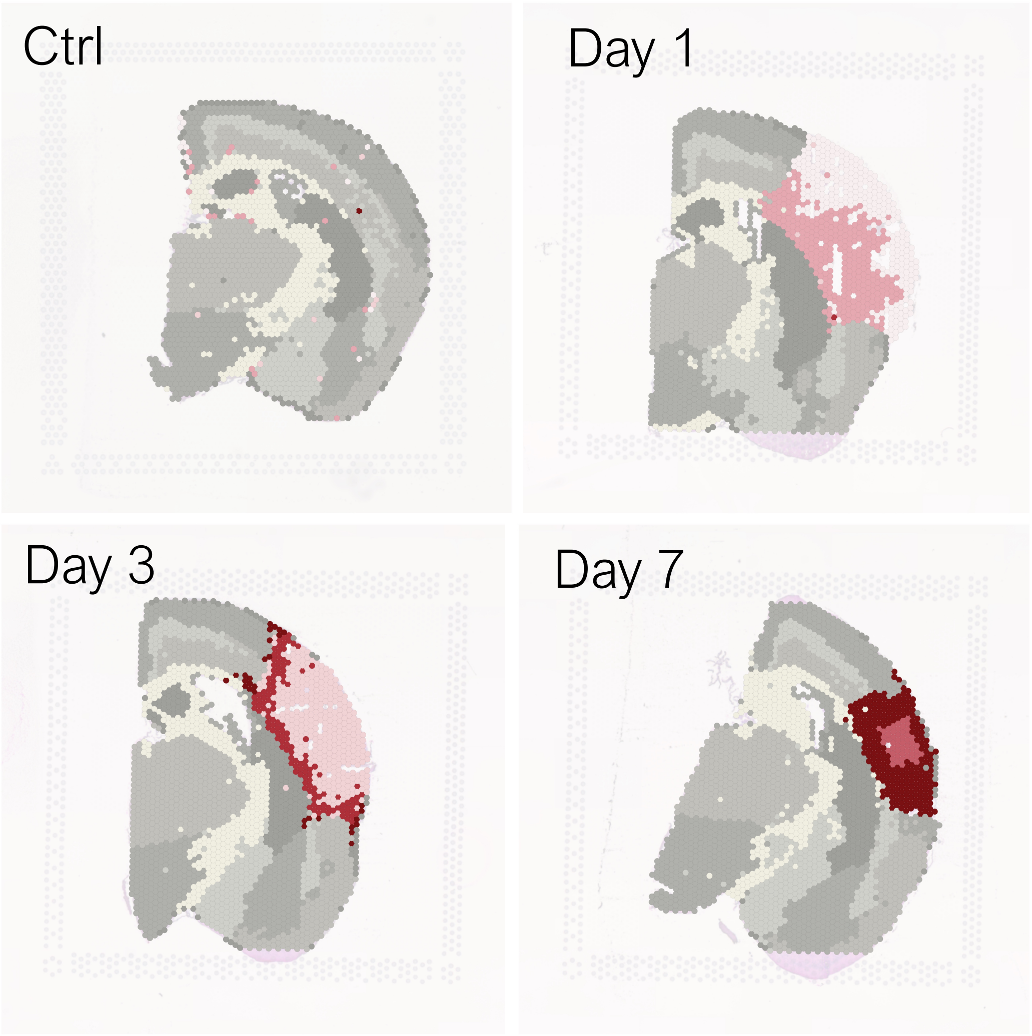
100 % concentrated and enjoying!:-)

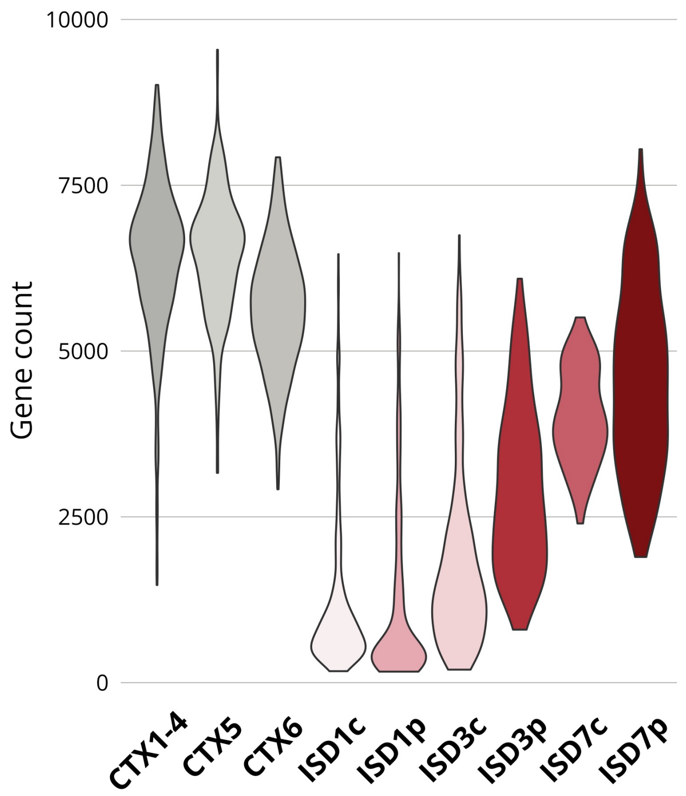
UMAP clustering



Basic Analysis – 10X Loupe Browser

Advanced – R (Seurat), Python (scanpy)

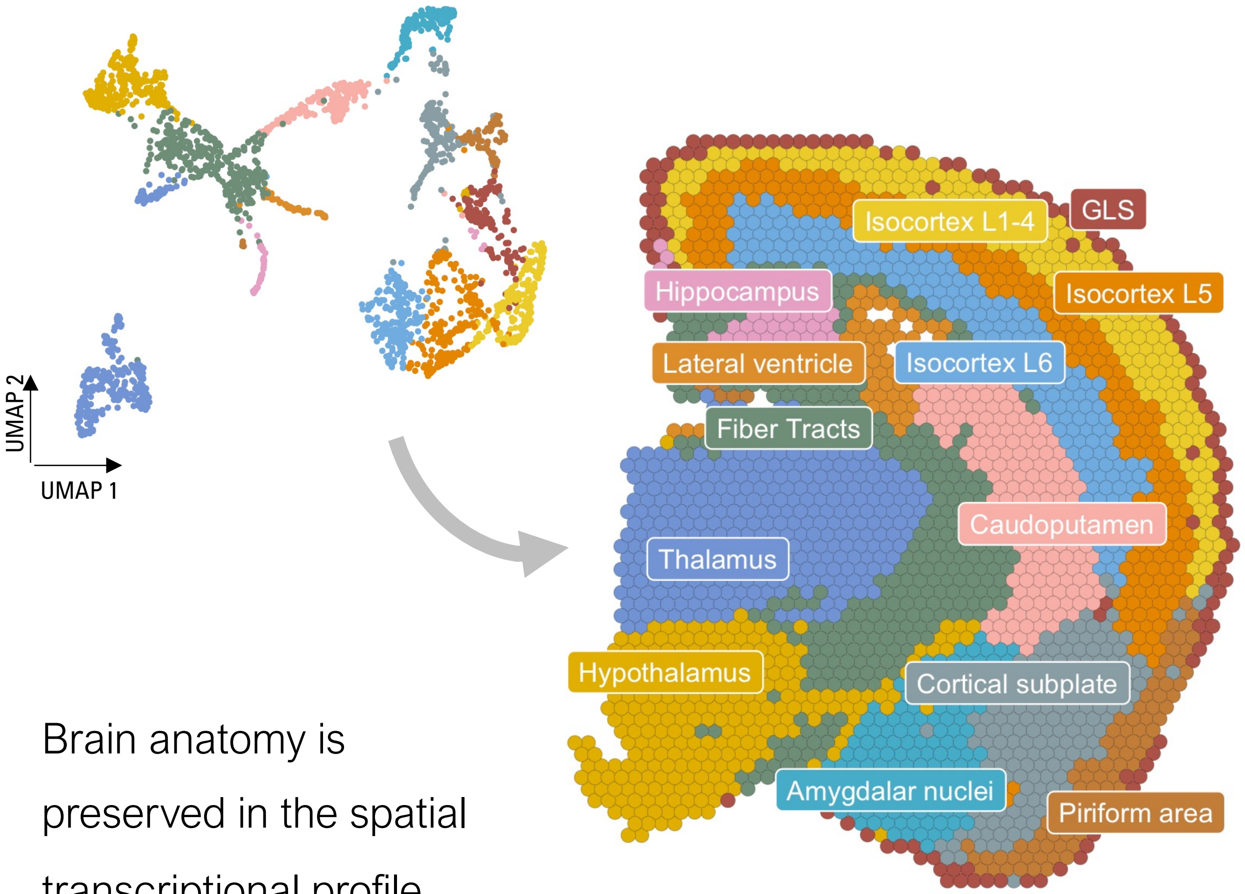




Gene abundance across the cortical landscape

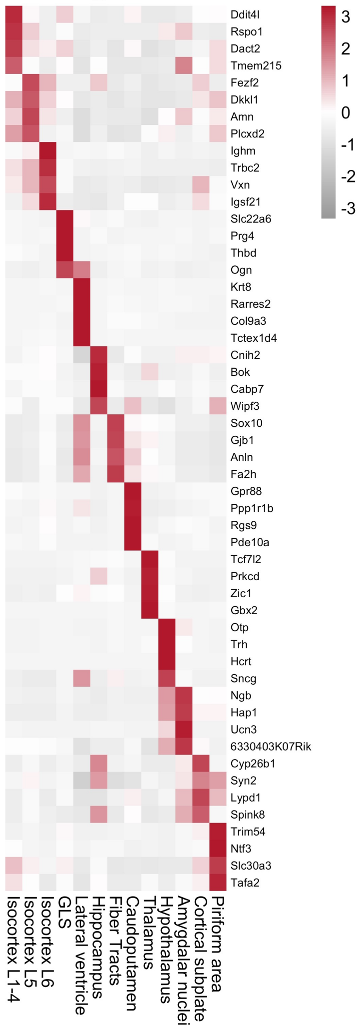
(IS = ischemic area; c = center, p = periphery)

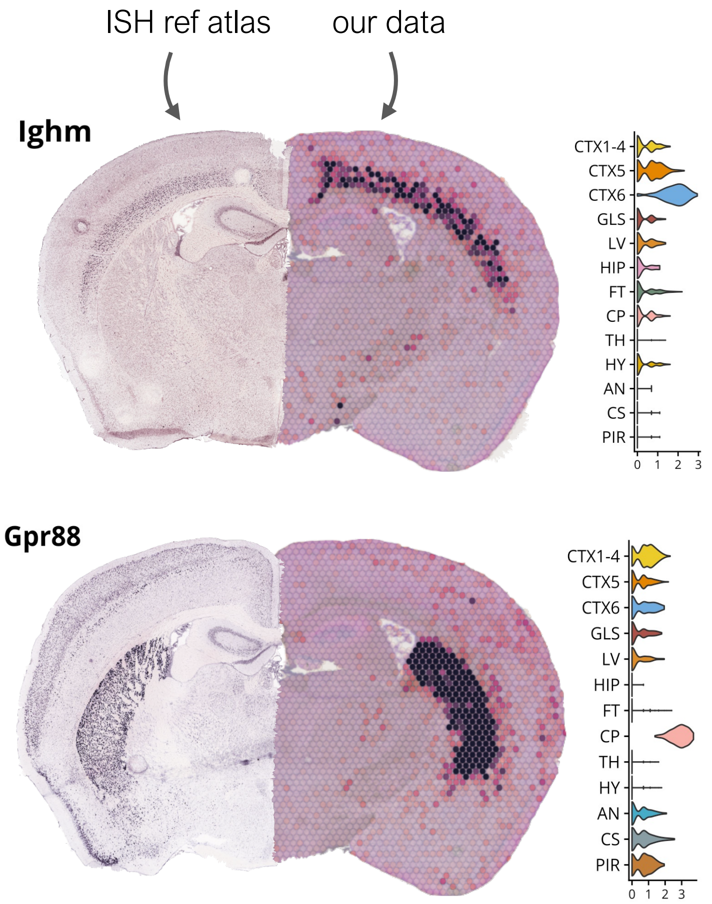
Data robustness



Brain anatomy is preserved in the spatial transcriptional profile

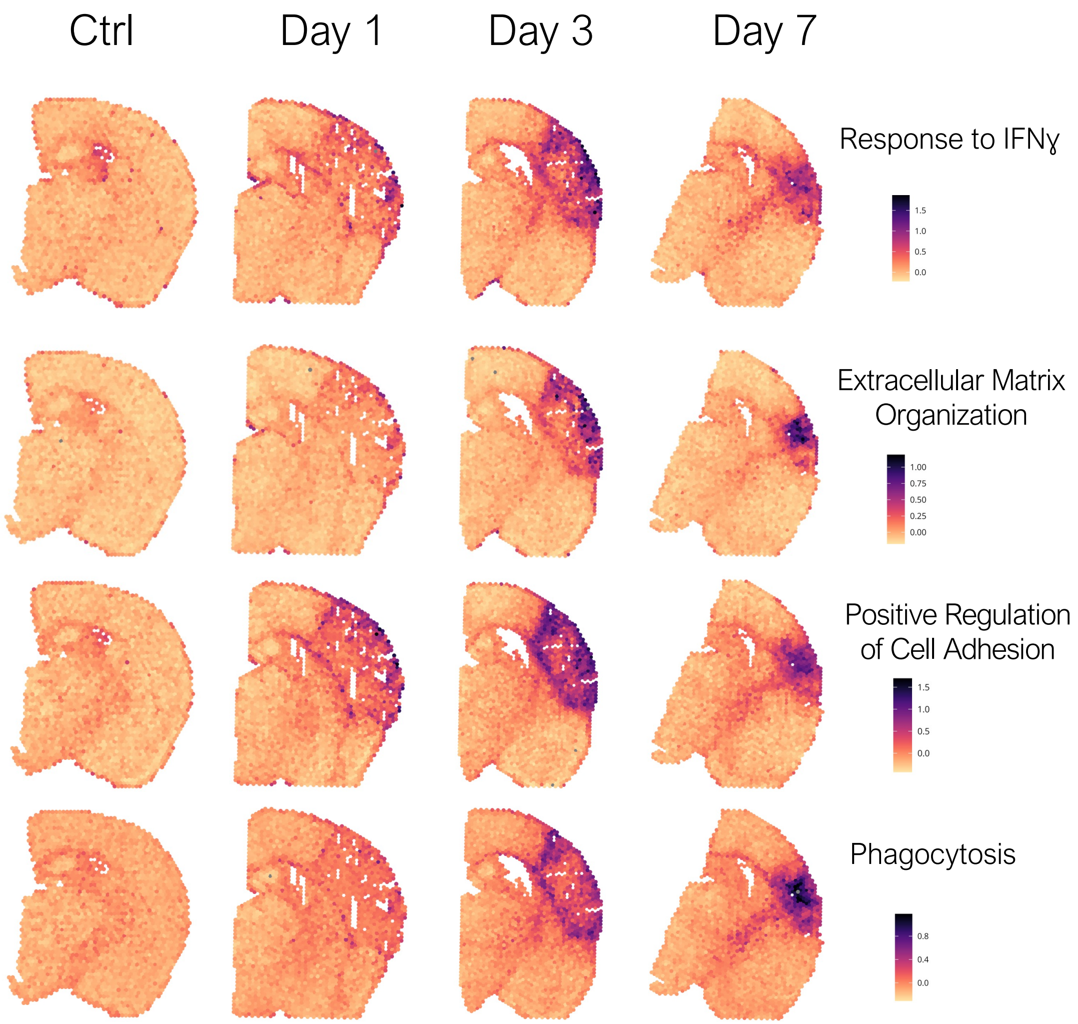
Brain Region Markers

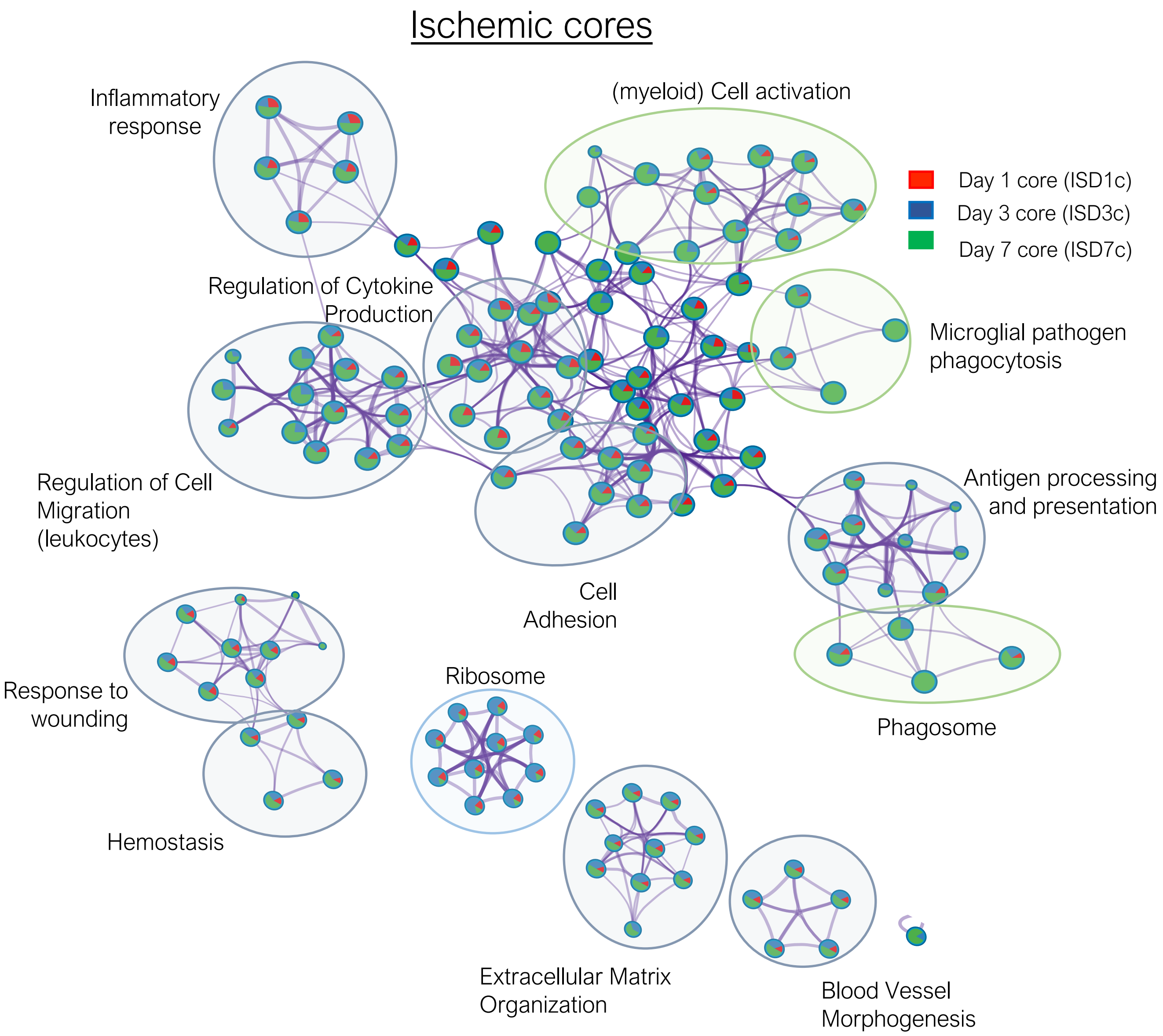




Marker validation with Allen Brain Atlas *in-situ hybridization* atlas

Functional Annotation




Ischemic cores

Clustering of the **Gene Ontology** terms

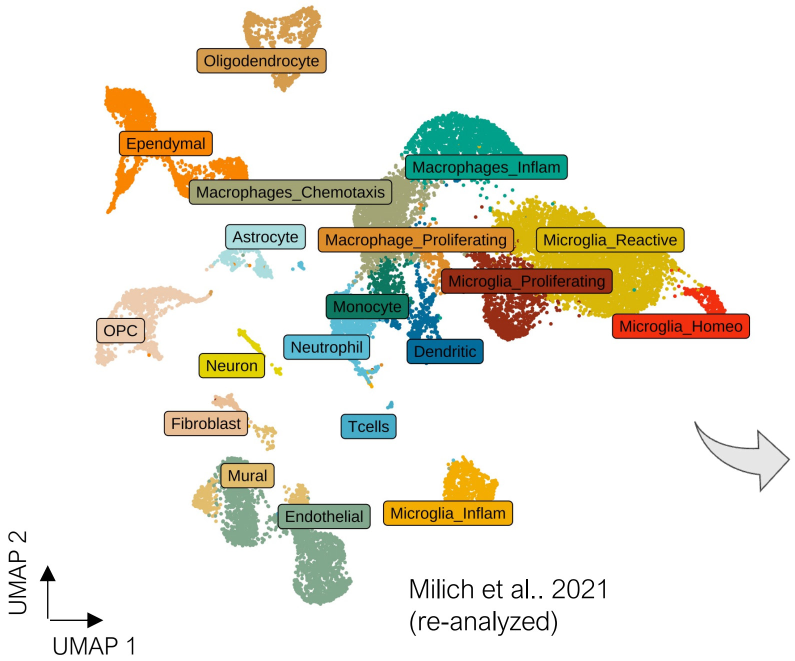
I demand… single-cells! (deconvolution)

JEM Journal of Experimental Medicine

TECHNICAL ADVANCES AND RESOURCES

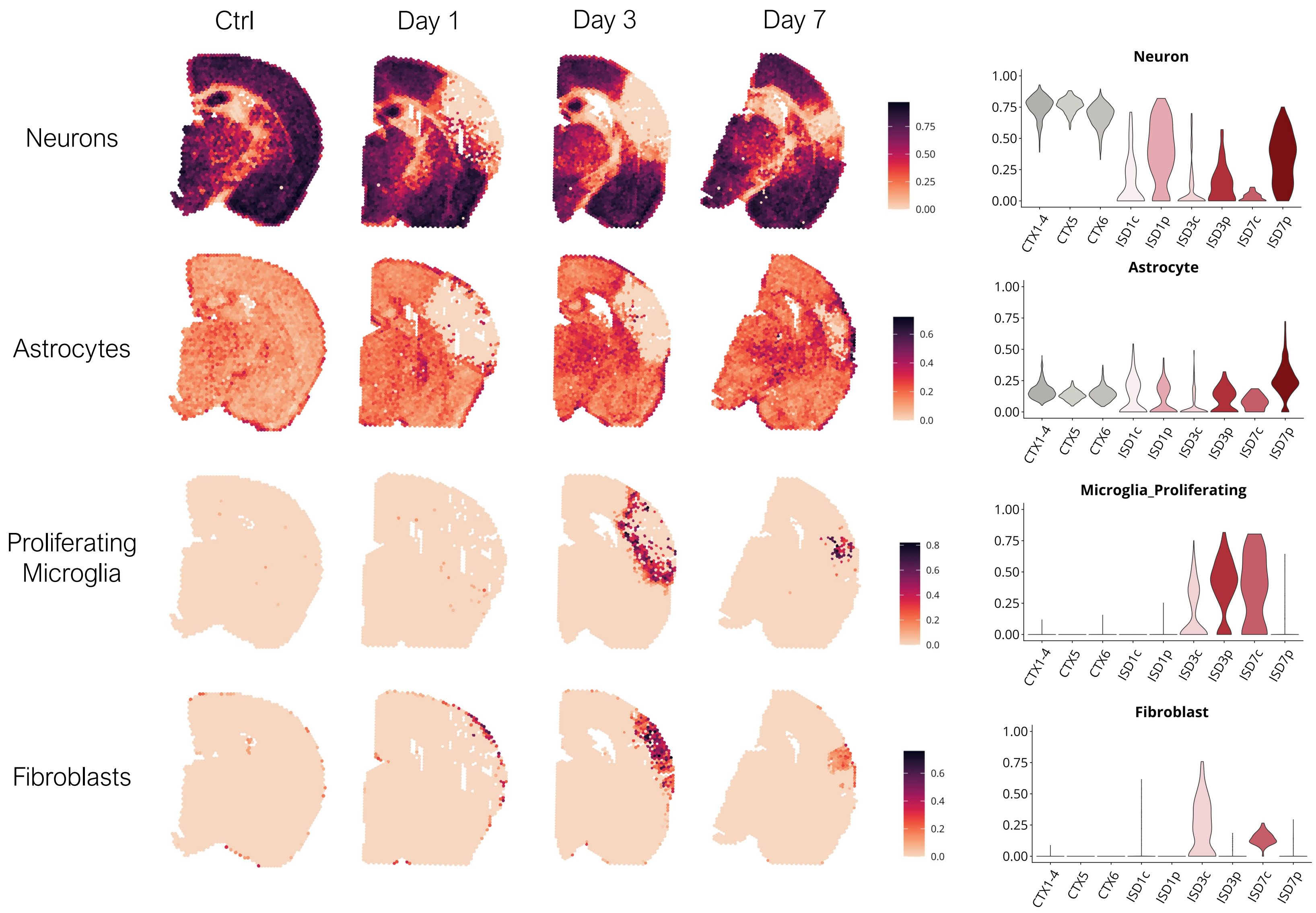
Single-cell analysis of the cellular heterogeneity and interactions in the injured mouse spinal cord

Lindsay M. Milich[1,2]*, James S. Choi[1]*, Christine Ryan[1,2], Susana R. Cerqueira[1], Sofia Benavides[1], Stephanie L. Yahn[1,2], Pantelis Tsoulfas[1], and Jae K. Lee[1]



Milich et al.. 2021
(re-analyzed)

- Ctrl – Day 1 – Day 3 – Day 7
- “full” cell type diversity coverage
- Good quality data



“None ever wished it longer than it was.”

~ Samuel Johnson on Milton’s ‘Paradise Lost’ (1779)

Concluding remarks

- Insightful and context-preserving view – allows for exploration
- Efficient results sharing is key – interactive web tool
- Hidden treasures – looking beyond the ischemic lesion

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Thank y🧠u for your attention!