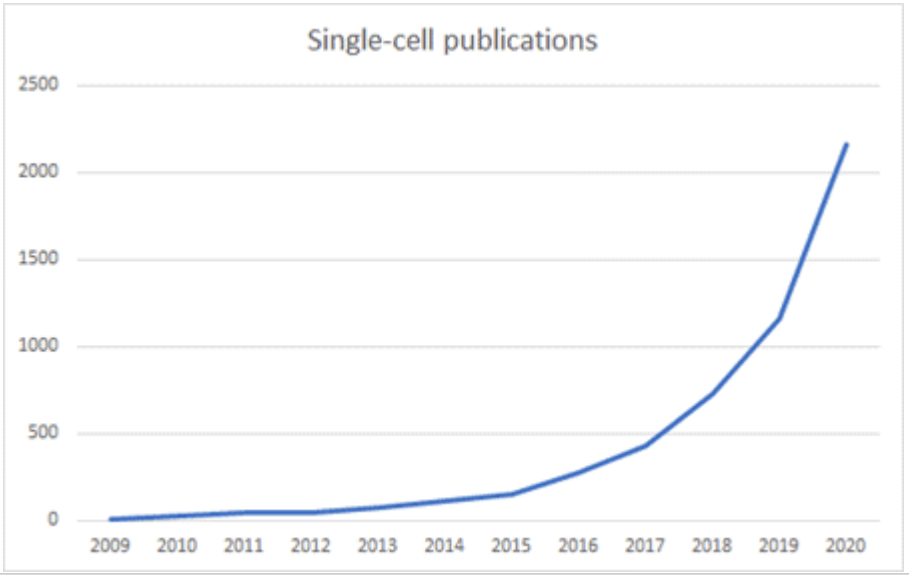
Spatiotemporal transcriptomic characterization of wound healing and regeneration in amphibians and of ischemic brain injury in mice

Mikael Kubista

Radek, Lukas, Daniel

Single cell profiling - 2005



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Gene expression profiling in single cells from the pancreatic islets of Langerhans reveals lognormal distribution of mRNA levels

Martin Bengtsson[1,2,4], Anders Ståhlberg[2], Patrik Rorsman[1,3], and Mikael Kubista[2]

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doi: 10.1101/gr.3820805
Genome Res. 2005. 15: 1388–1392
Cold Spring Harbor Laboratory Press

- » Abstract *Free*
- Full Text *Free*
- Full Text (PDF) *Free*

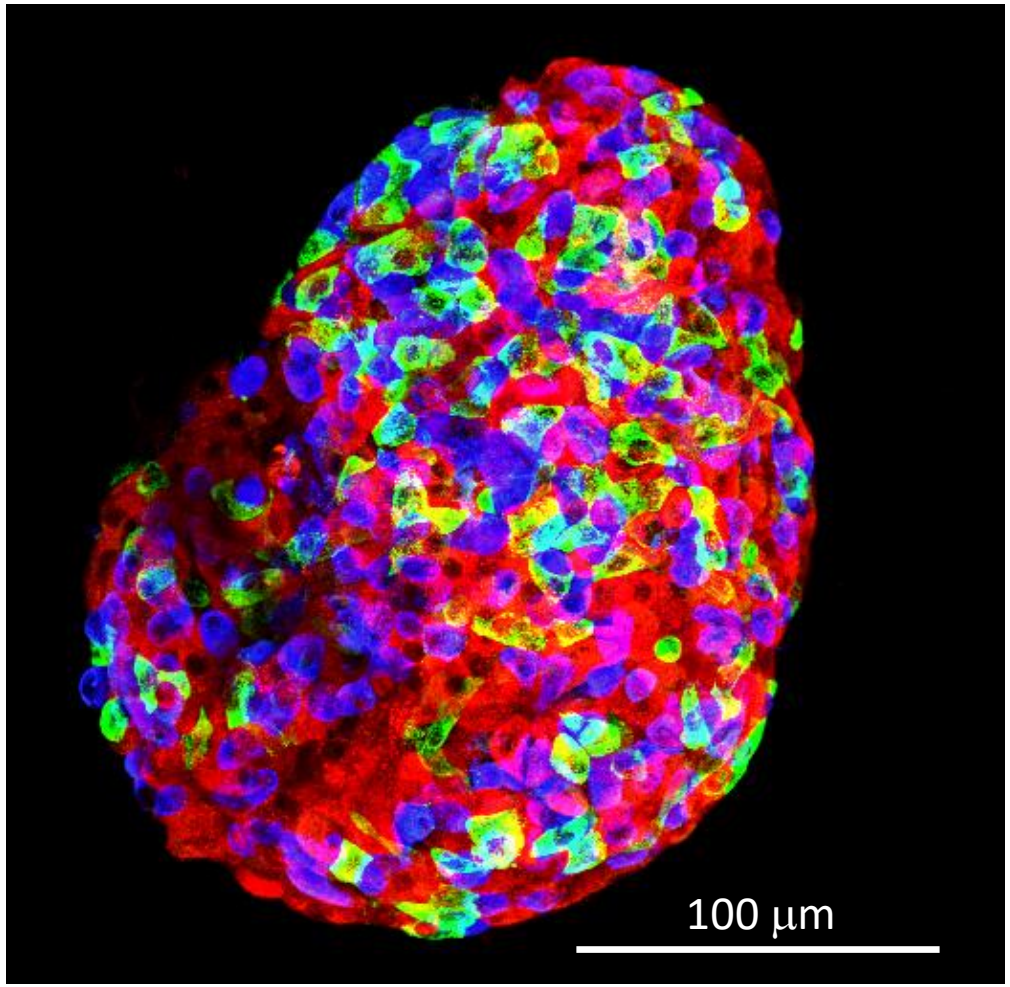
Tissue is heterogeneous

δ-cells (<5%)

α-cells (20%)

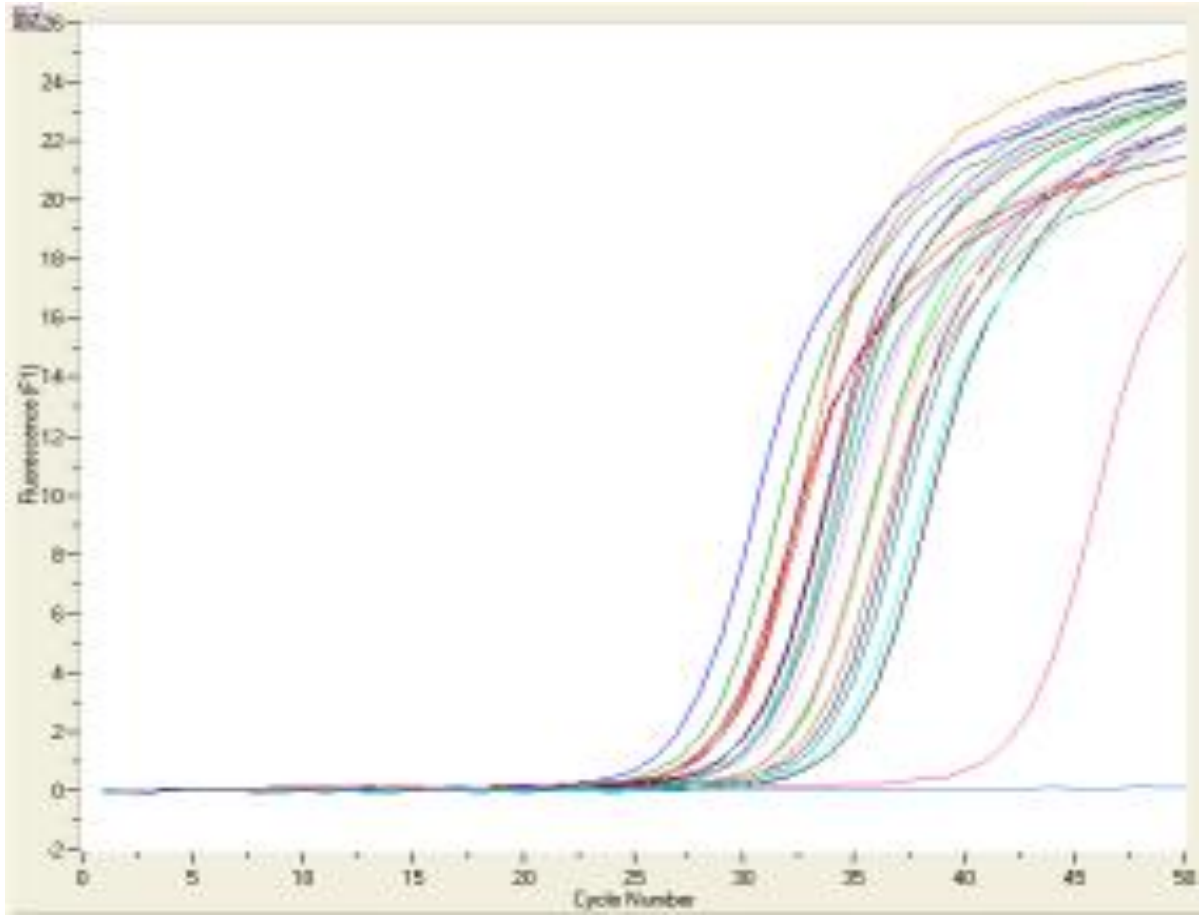
β-cells (75%)

Islet of Langerhan

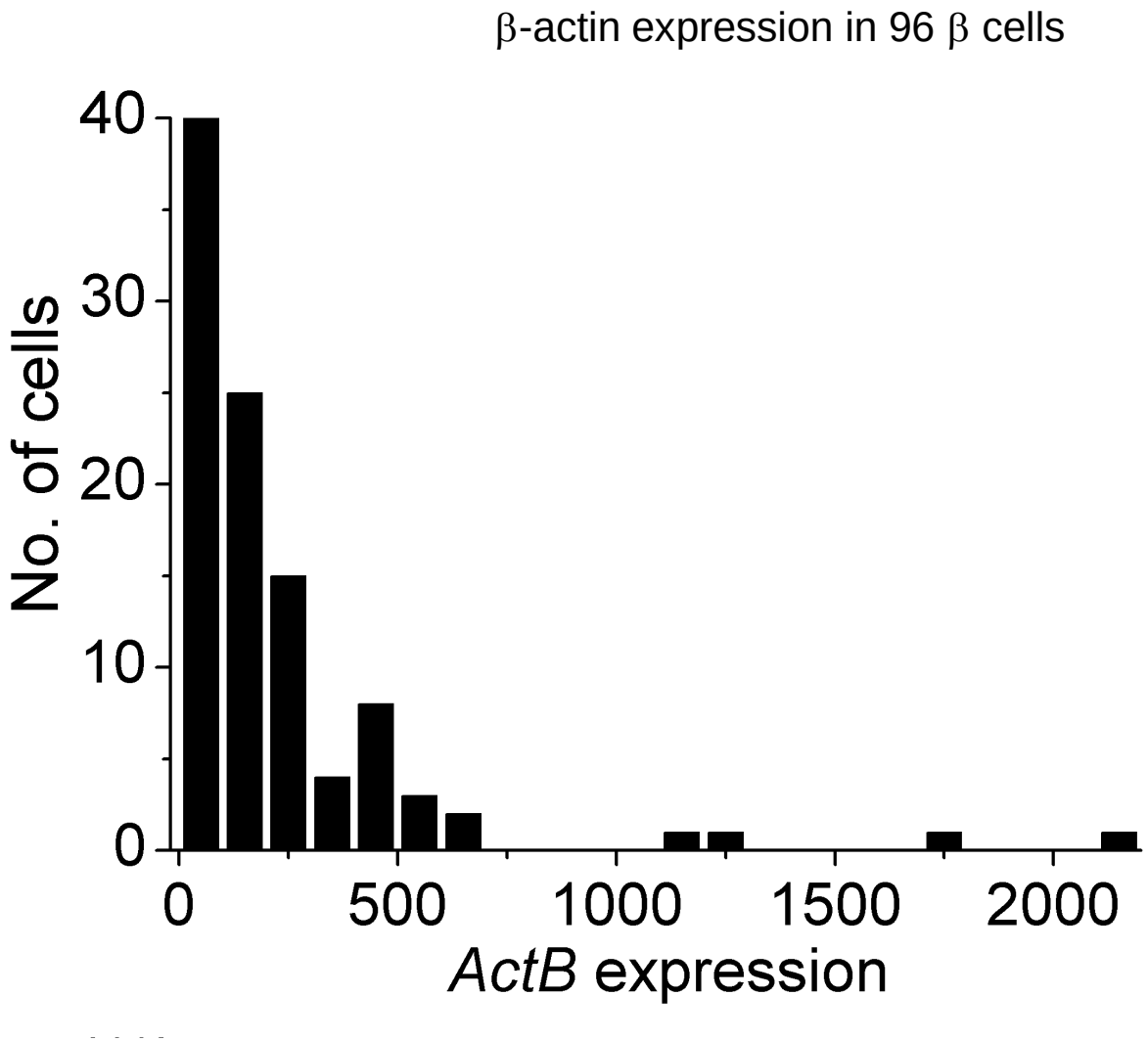


Large cell-to-cell variation

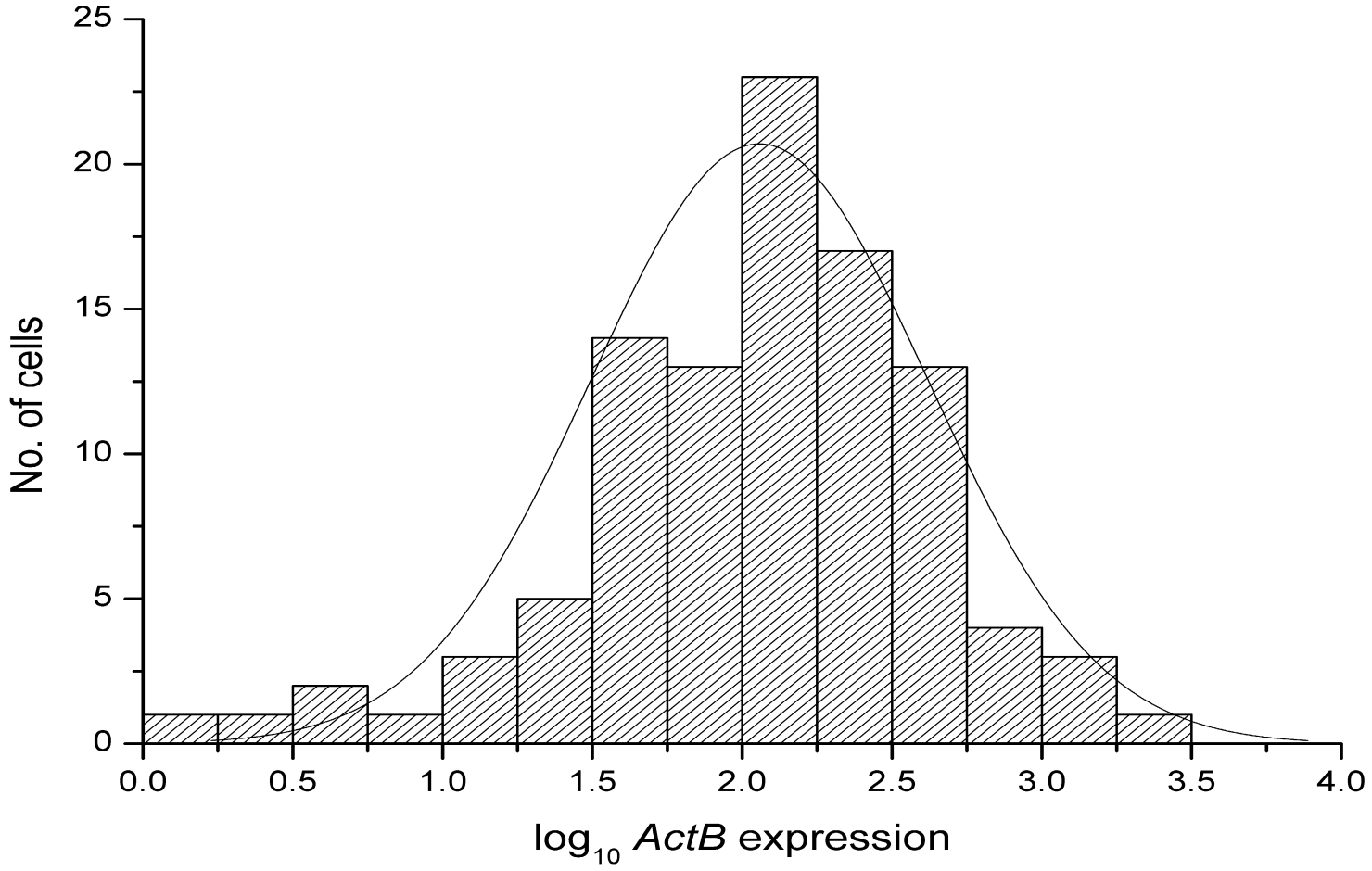
Expression of Ins1 in MIN6 cells



Skewed distribution in linear scale

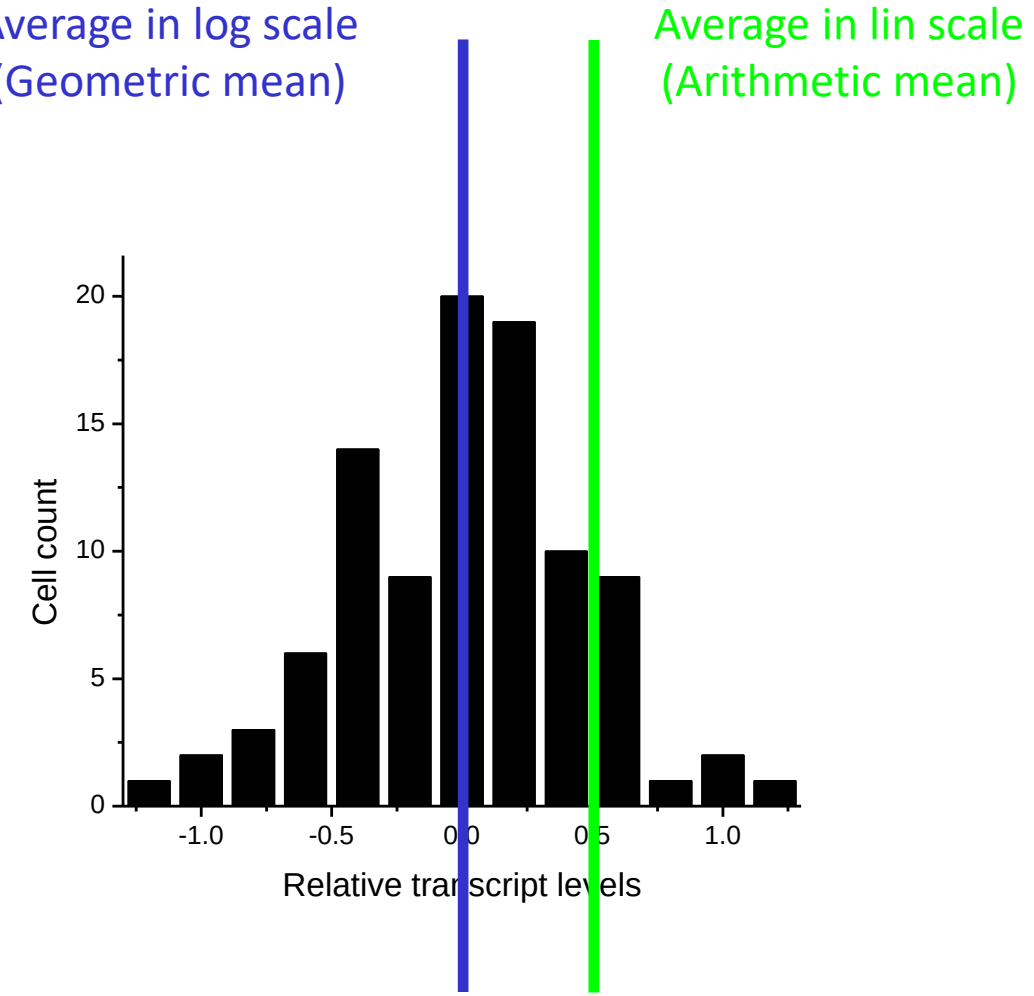


normal in logarithmic scale....



M. Bengtsson, A. Stålberg, P. Rorsman, M. Kubista, Genome Research (2005) 1388-1392

The typical (median) cell



GENE EXPRESSION

Which mean do you mean?

There is considerable variation in gene-expression levels between individual cells. Bengtsson *et al.* show that these levels are distributed log-normally rather than normally, which implies that the arithmetic mean does not represent the situation in a typical cell. They also show that the levels of expression of different genes in the same cell do not generally correlate, and suggest that mechanistic conclusions can be drawn when they do.

Using reverse transcriptase quantitative real-time PCR, they measured the transcript levels of 5 genes in 169 mouse pancreatic cells. For each gene the results were distributed log-normally across the sample cells, making the geometric mean a more appropriate representation of the data than the more commonly quoted arithmetic mean. For the insulin genes, *Ins1* and *Ins2*, up to 9-fold differences were found between the arithmetic and geometric means.

Of the five genes studied, only *Ins1* and *Ins2* expression levels correlated at the level of the individual cell. Levels of *ActB*, the β-actin gene, correlated with these two only at the overall population level, whereas levels of the final two genes did not correlate with any of the others. This indicates that expression-level differences in individual genes are not due to cells having different levels of overall transcription. The authors suggest that genes that correlate at the individual cell level are coordinately regulated, whereas those that correlate at the population level merely respond to the same environmental stimuli.

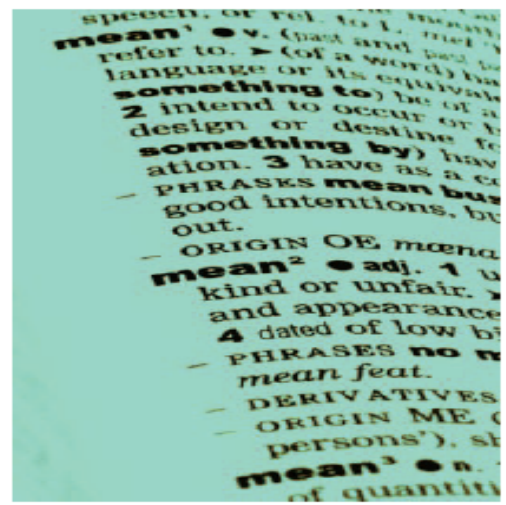
The importance of these findings is demonstrated by the fact that we might have underestimated the effect of glucose on insulin expression by almost 4-fold, which could be important in the administration of therapeutic insulin.

Patrick Goymer

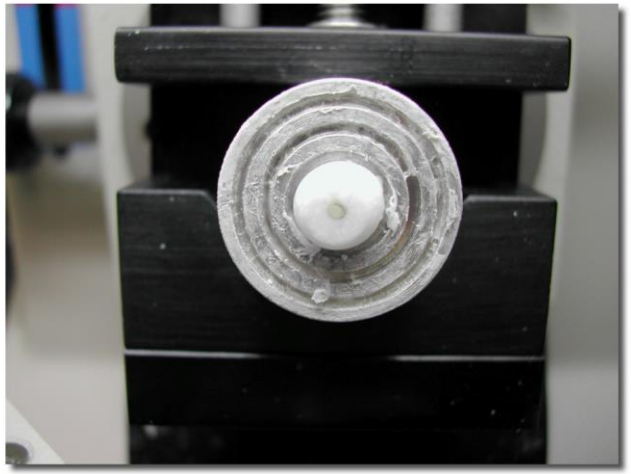
References and links

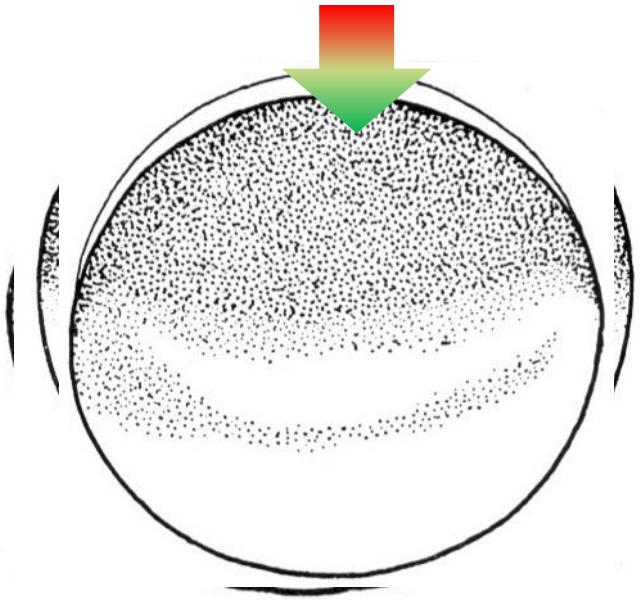
ORIGINAL RESEARCH PAPER

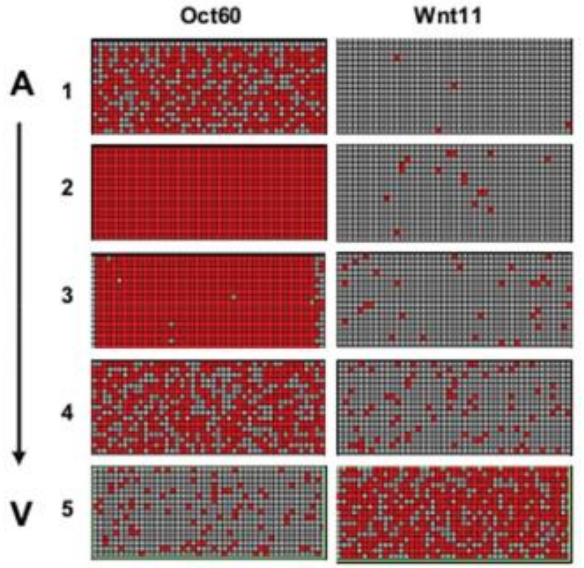
Bengtsson, M. *et al.* Gene-expression profiling in single cells from the pancreatic islets of Langerhans reveals lognormal distribution of mRNA levels. *Genome Res.* **15**, 1388–1392 (2005)



Intracellular profiling - 2007







Intracellular expression profiles measured by real-time PCR tomography in the Xenopus laevis oocyte. R. Sindelka, J. Jonak, R. Hands, S.A. Bustin, M. Kubista, Nucleic Acids Research, 2007, 1–6,

Laboratory aims

(A) Introduce and develop new methods for expression profiling and utilize them in our research.

(B) Cell fate determinants during early development.

(C) Glial cells in acute and neurodegenerative disorders.

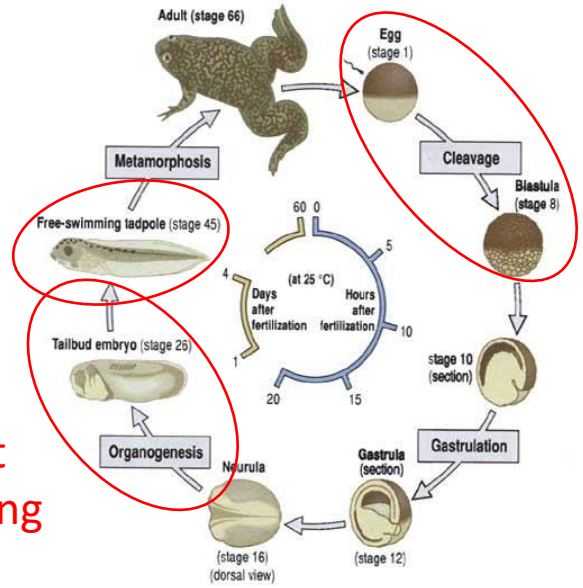
(D) Expression regulation of wound healing and regeneration.

GeneCore

GeneCore offers service in the field of single-cell and spatial transcriptomics.

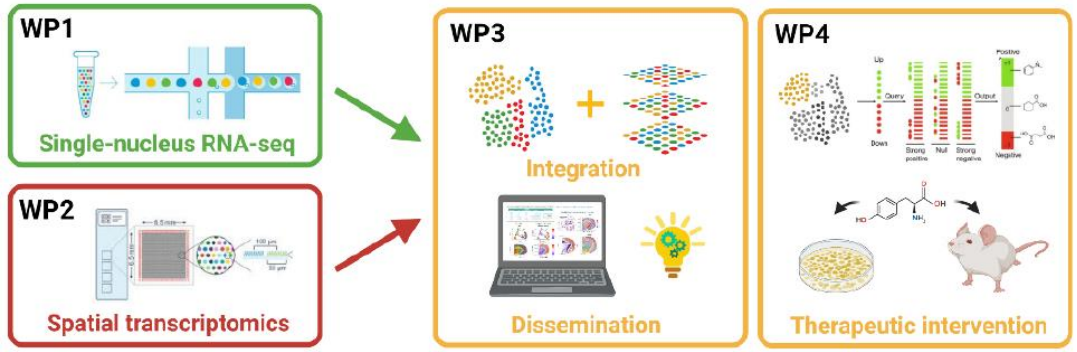
(E) Make the methods and bioinformatic solutions available through BIOCEV Gene Core

Regeneration



Asymmetrical localization of biomolecules

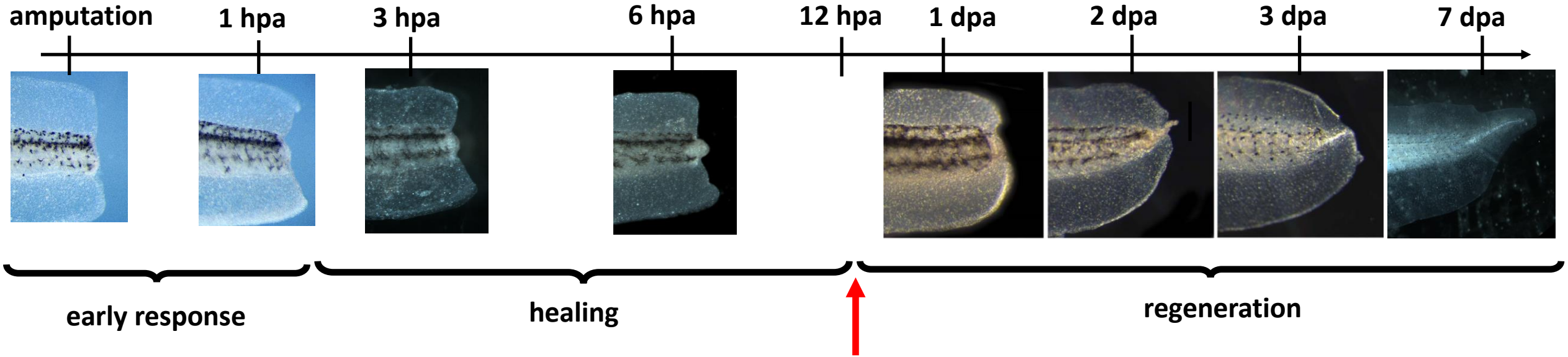
Epidermis development
Wound healing



https://www.ibt.cas.cz/en/core-facilities/gene-core-quantitative-and-digital-pcr

ibt

Xenopus laevis tail regeneration

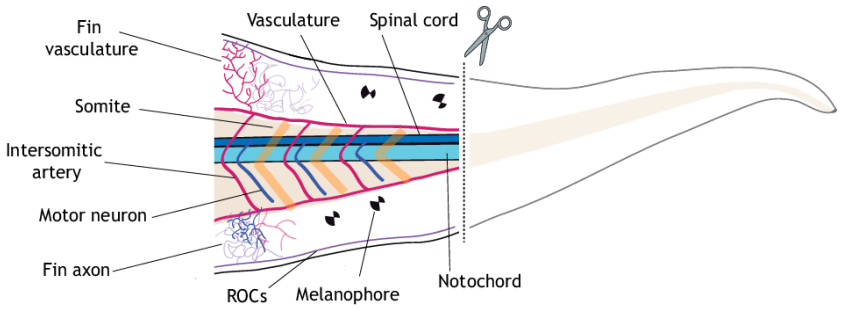


Haemostasis

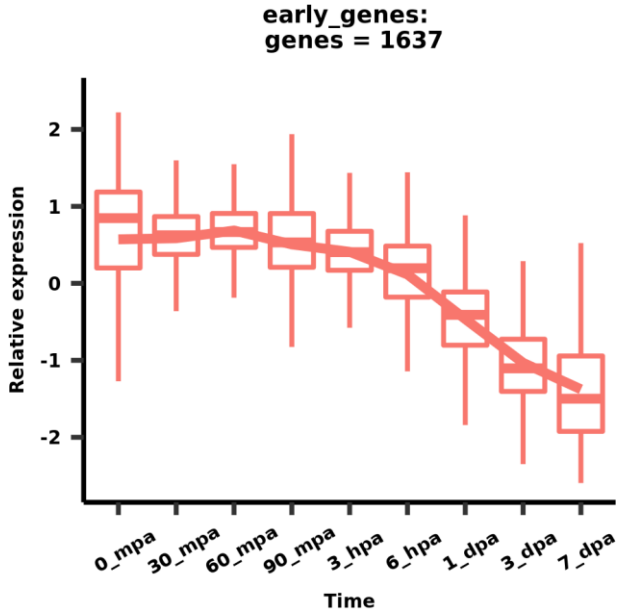
Inflammation

Proliferation

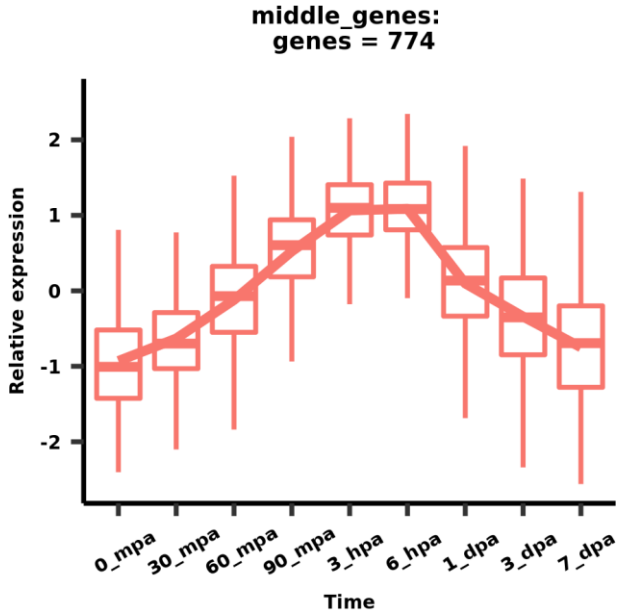
Remodeling



Bulk RNA-Seq analysis



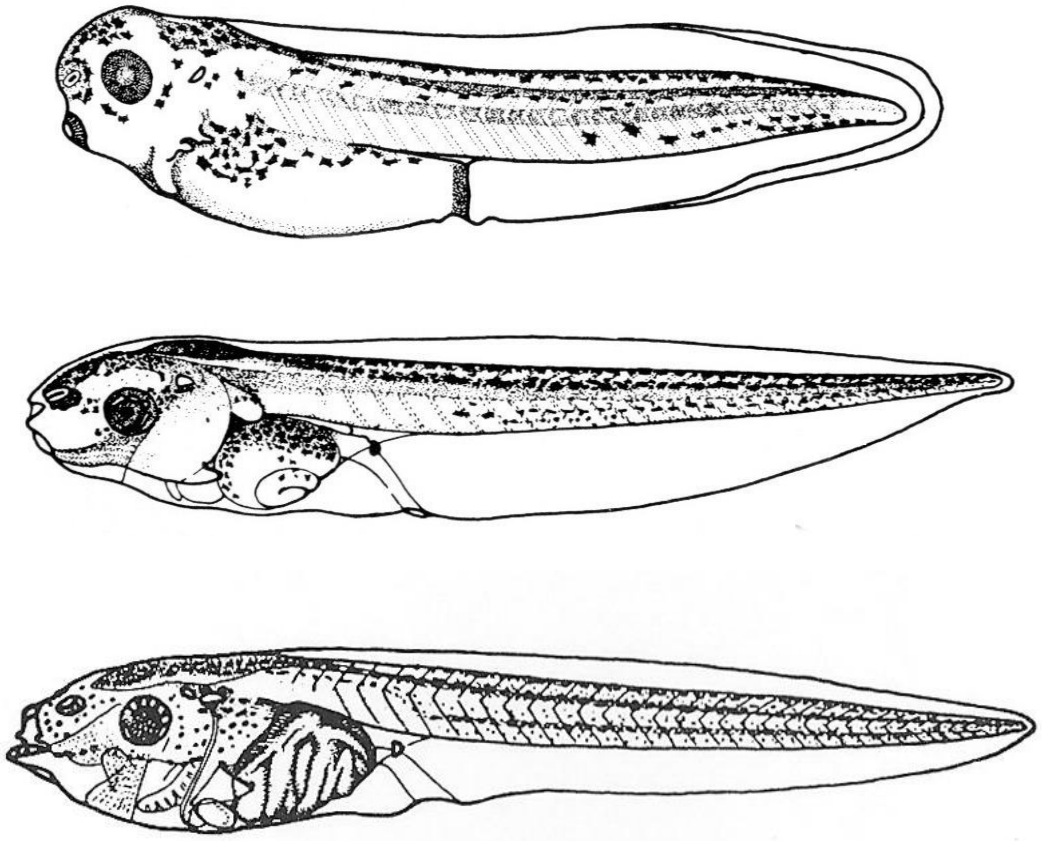
- actin-mediated muscle cell contraction
- ATP/NADH metabolic process
- calcium ion transmembrane transport
- carbohydrate biosynthetic process
- nucleotide phosphorylation

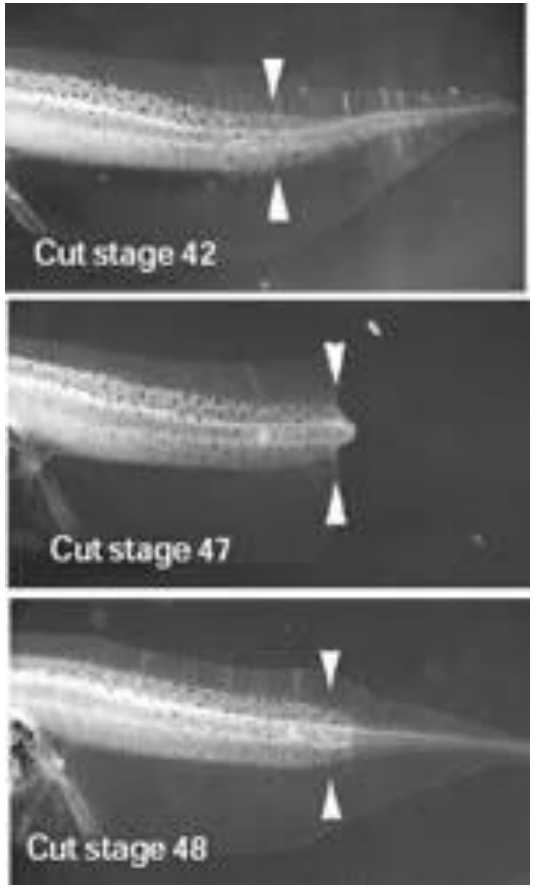


- cell-cell adhesion
- collagen catabolic process
- cytokine-mediated signaling pathway
- epidermis development
- external encapsulating structure organization
- leukocyte migration
- multi-multicellular organism process

ibt

Refractory phase of *X. laevis* development

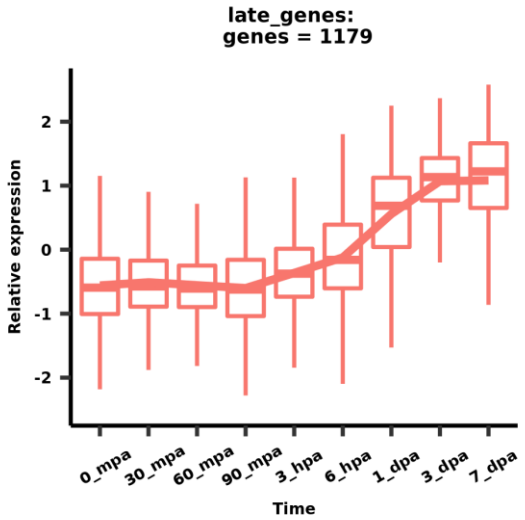




- start of feeding
- immune system maturation
- signaling pathway differences

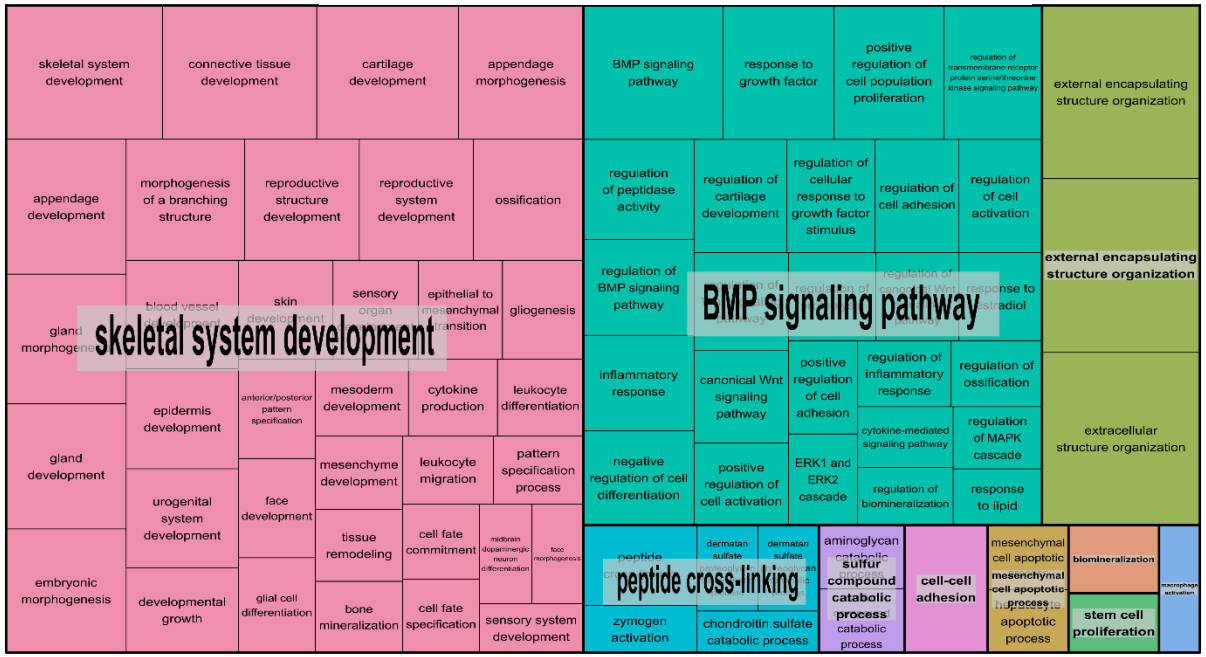
Similar early and middle phases of regeneration!

Healing is a key phase for regeneration

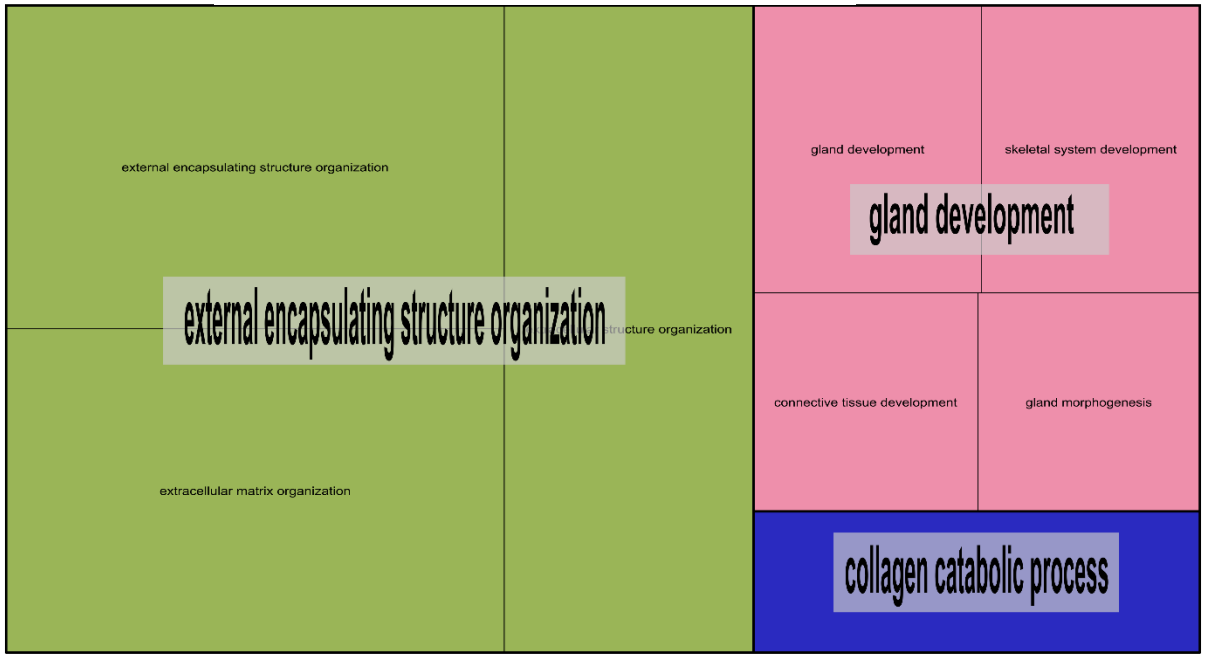


- organism development
- signaling pathways
- extracellular modifications

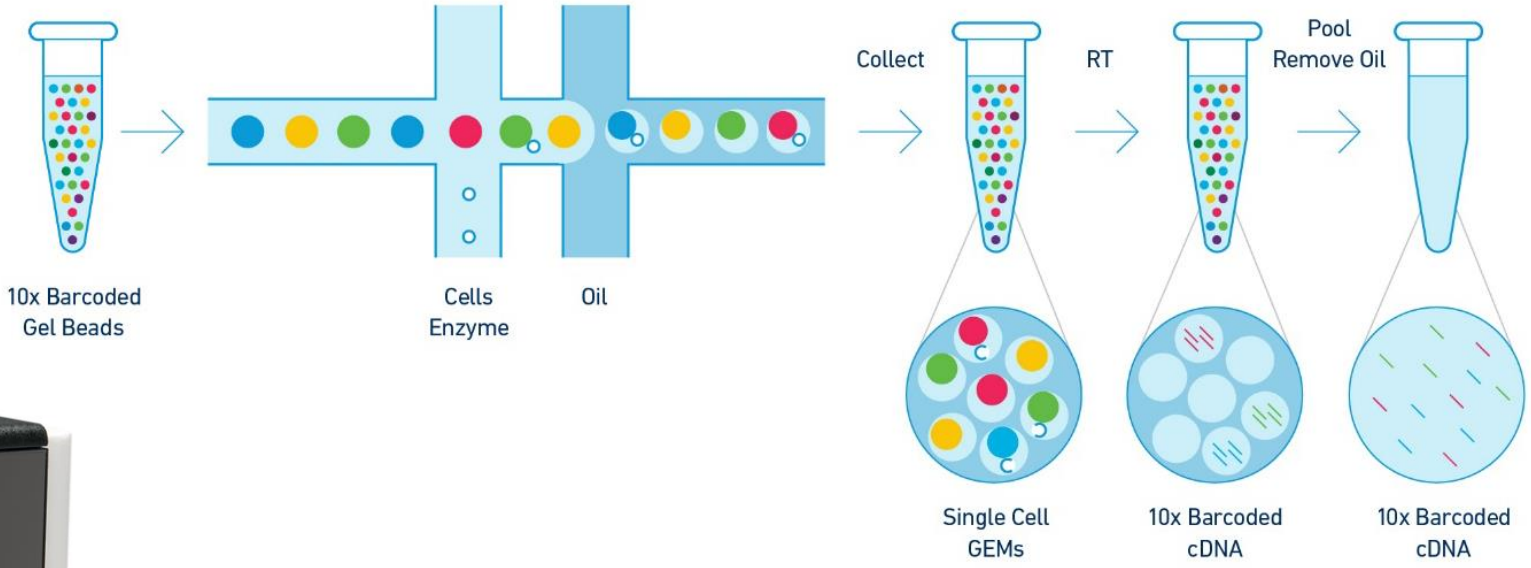
Regenerative – late phase



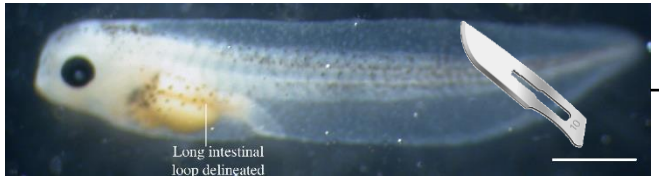
Refractory – late phase

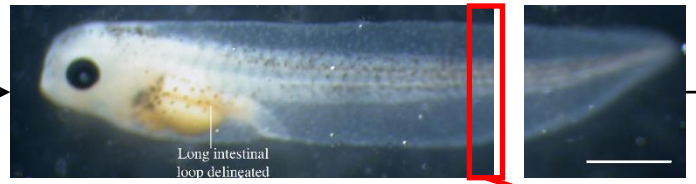


Chromium System (10x Genomics)

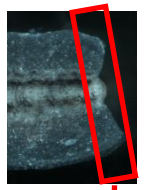


scRNA-Seq: Experimental design





0 hpa



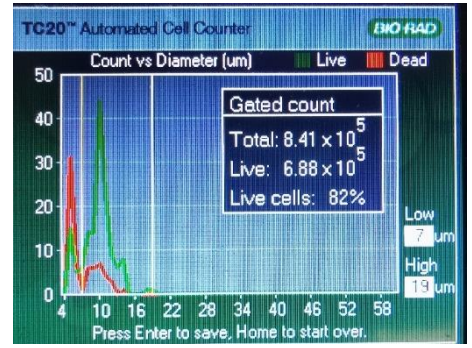
3 hpa

6 hpa

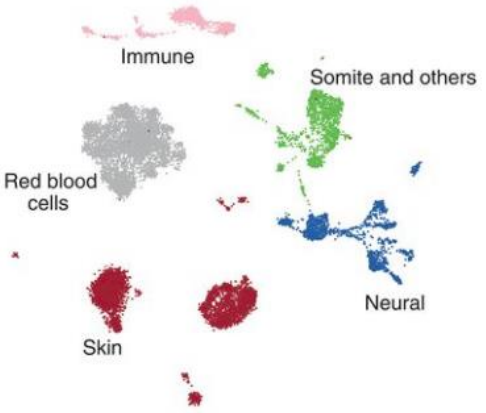
24 hpa

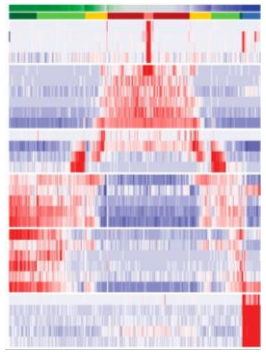
tissue dissociation

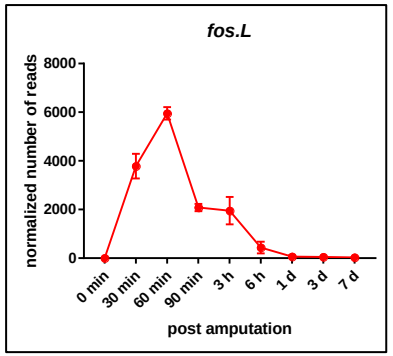
quality control and cell suspension validation

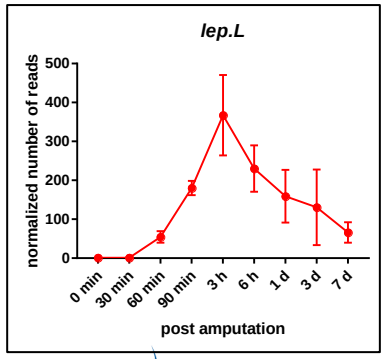


data analysis

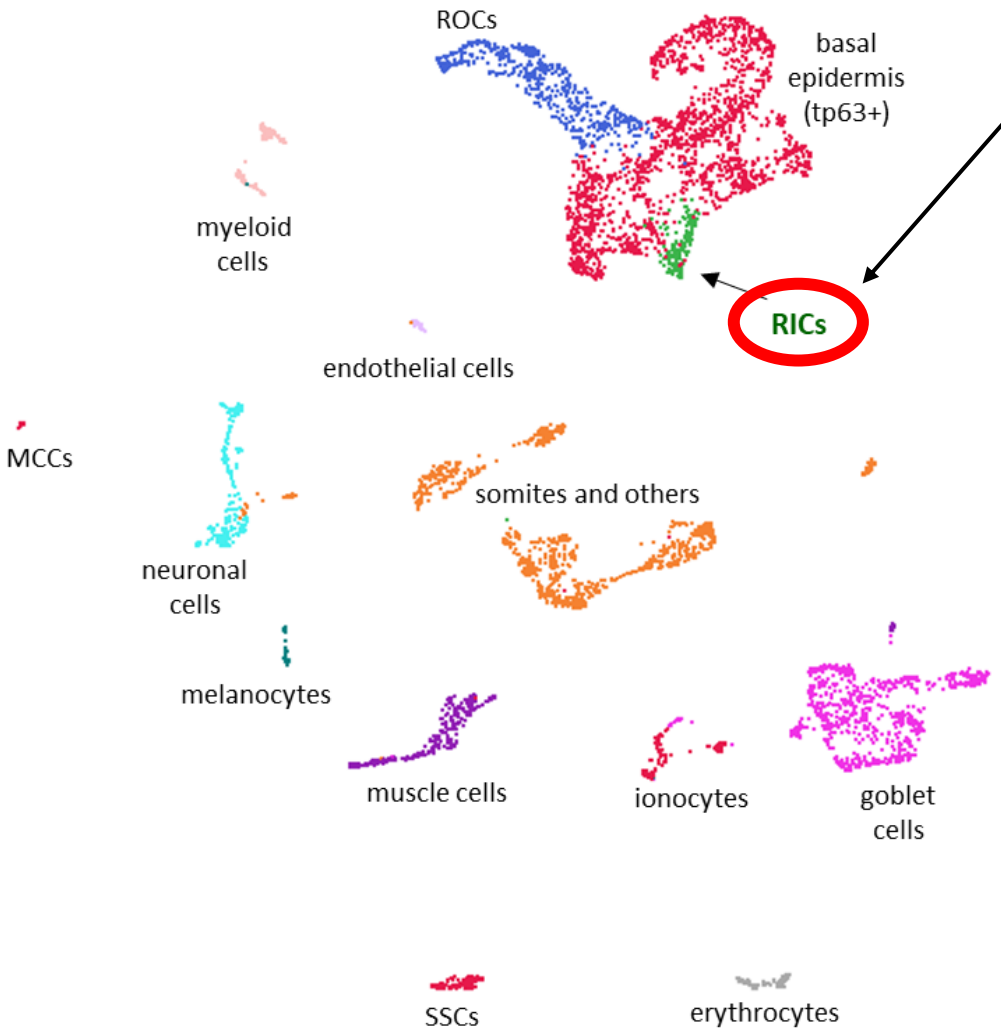




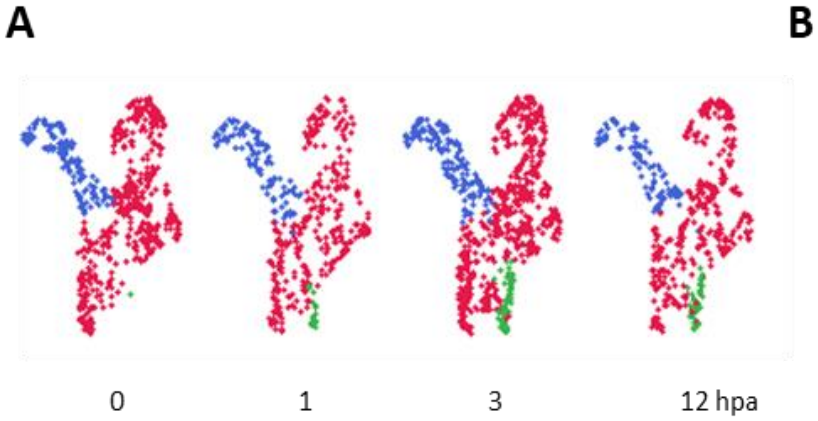


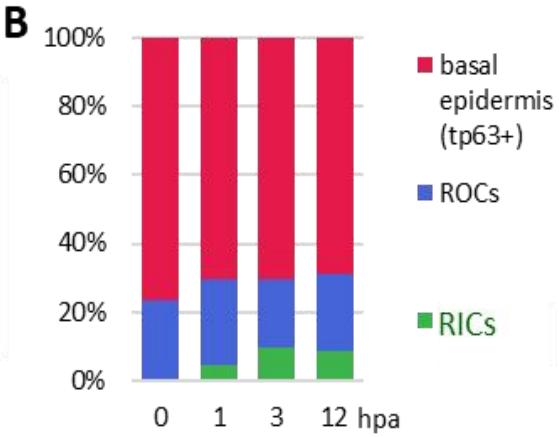


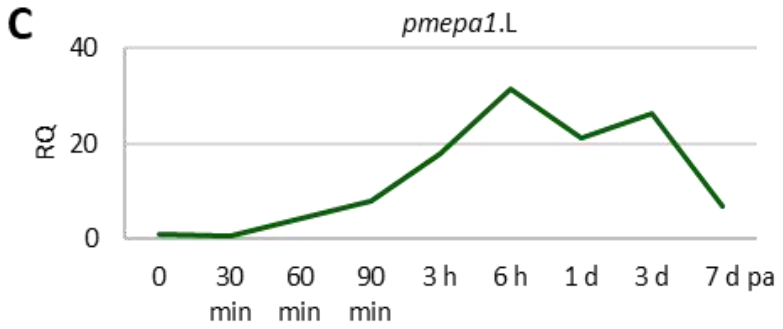
scRNA-Seq during healing phase

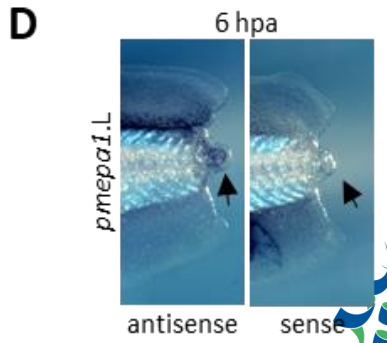


- Remodelling enzymes
- TGF-beta inhibitors
- Genes involved in DNA repair and stress response



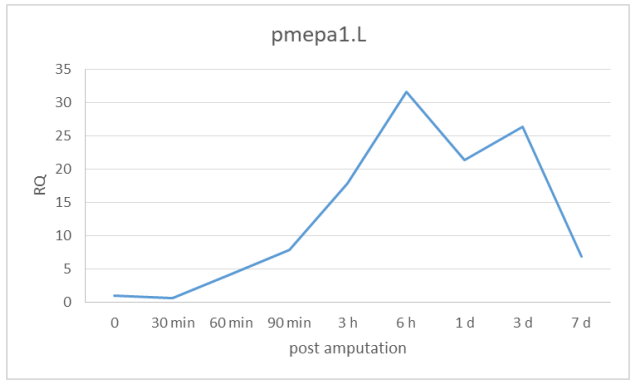




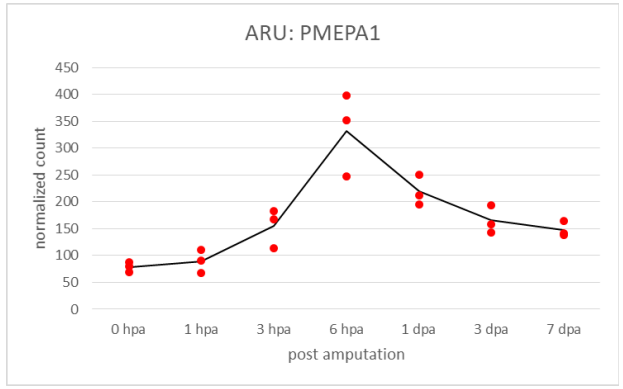


Evolutionary conservation

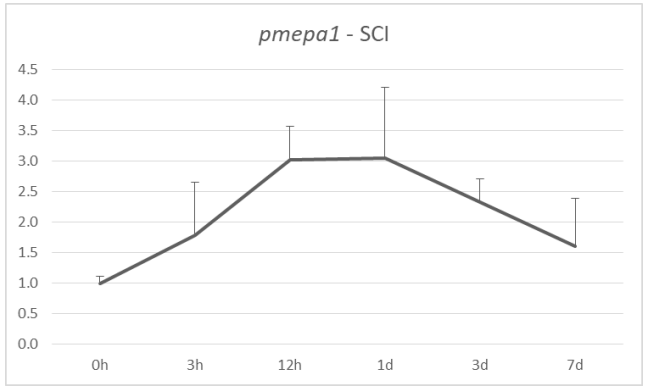
X. laevis tail



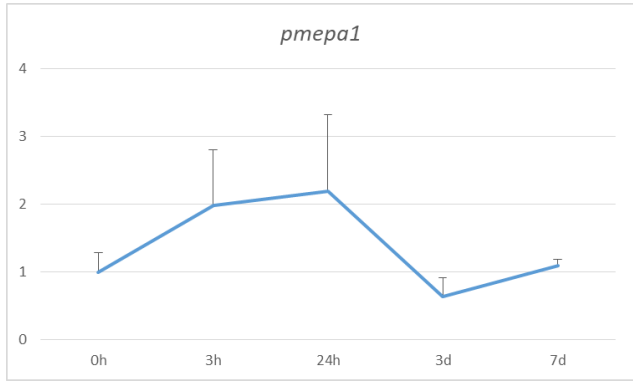
sturgeon fin



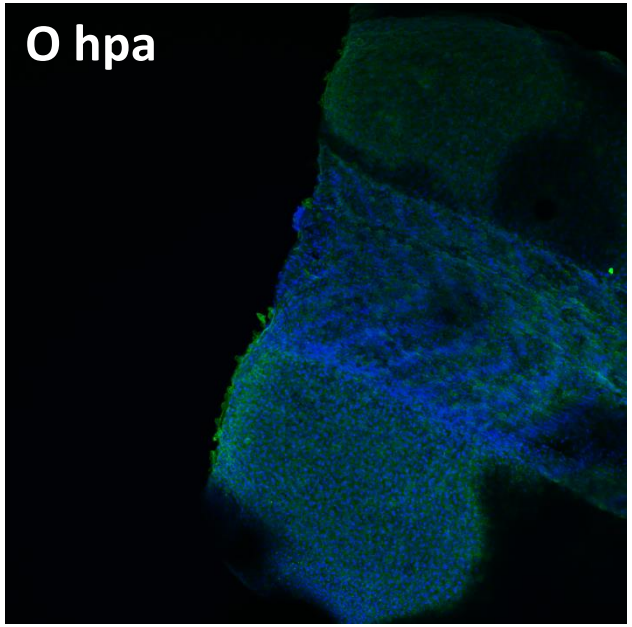
rat spinal cord

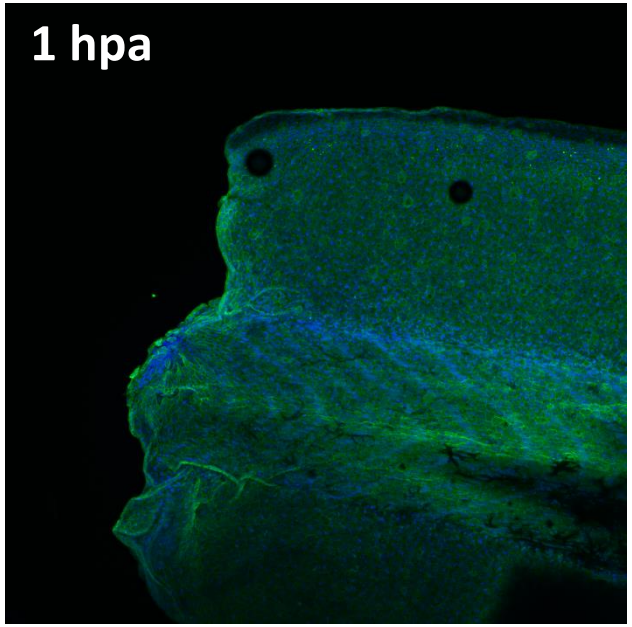


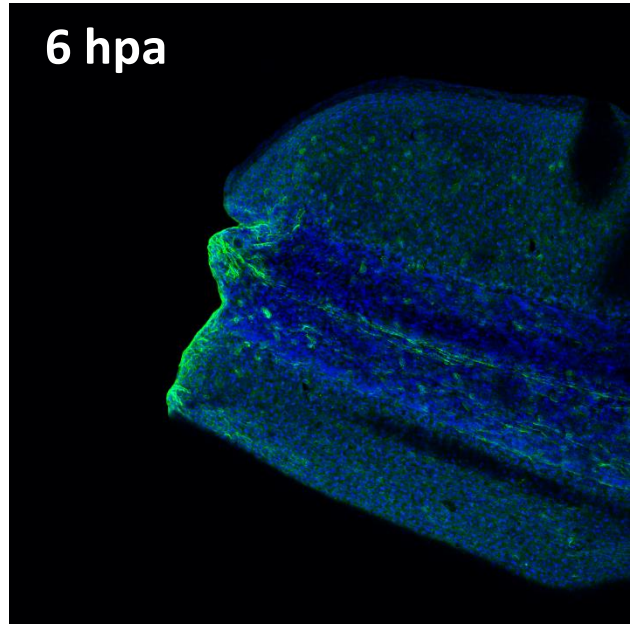
mouse liver



IHC (*Xenopus*)

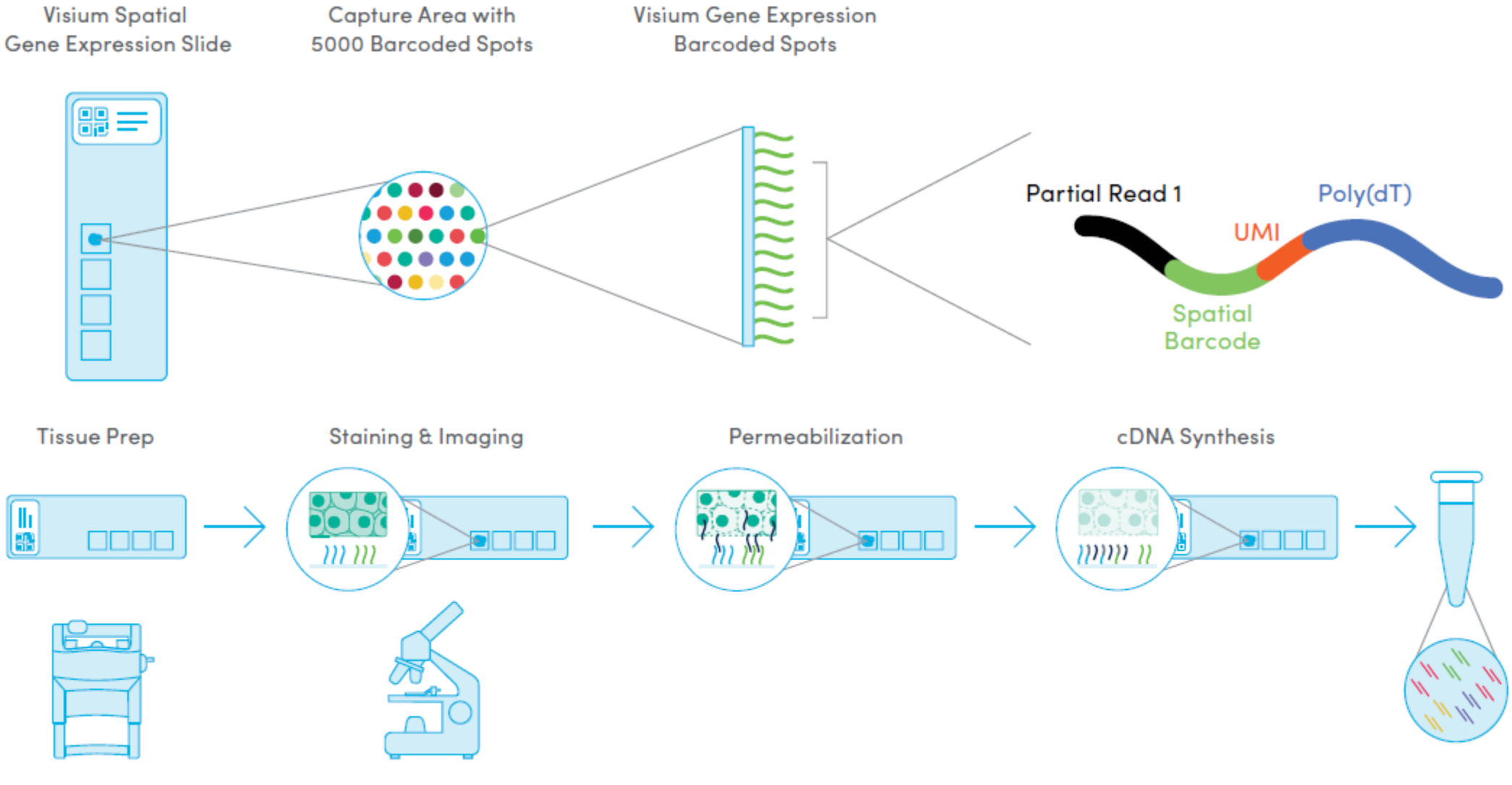




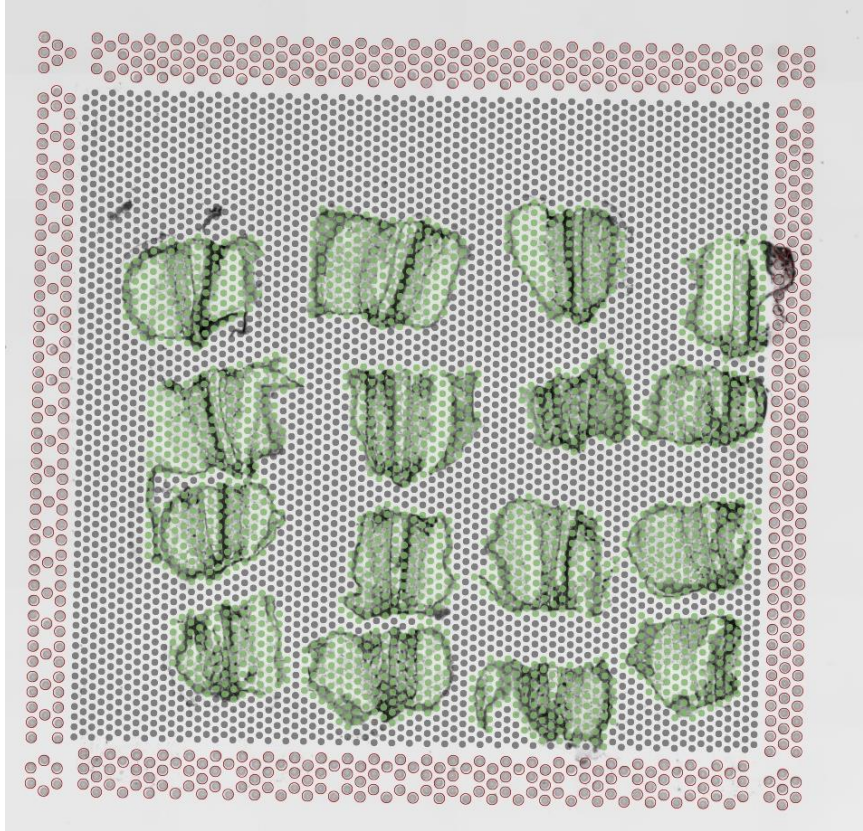


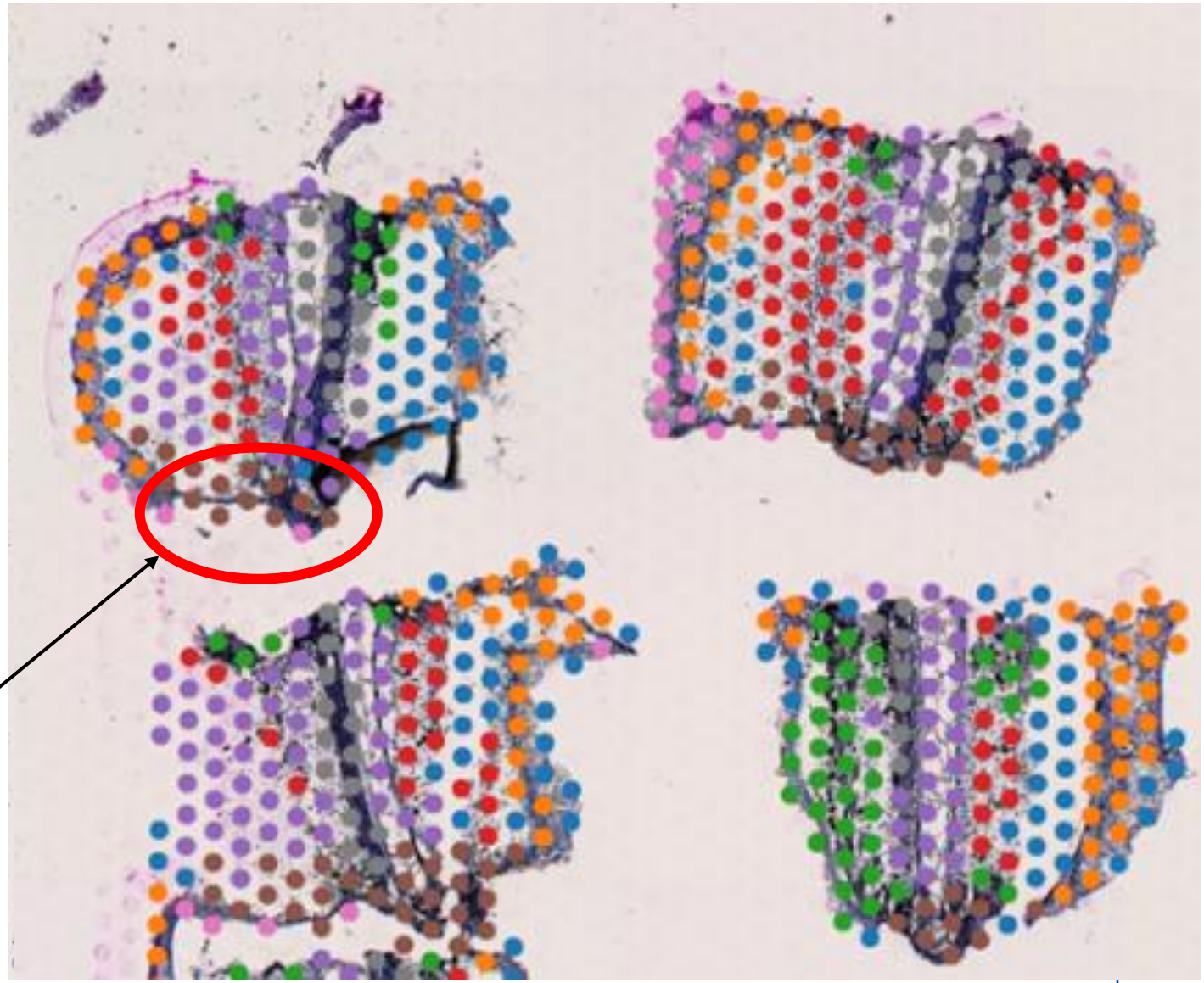
Visium system

- 4 capture areas on a 6.5mm2 area (average 1–10 cells captured per spot dependent on tissue type)
- spot contains hundreds of millions of oligonucleotides to capture mRNA
- 1 day tissue and library preparation workflow



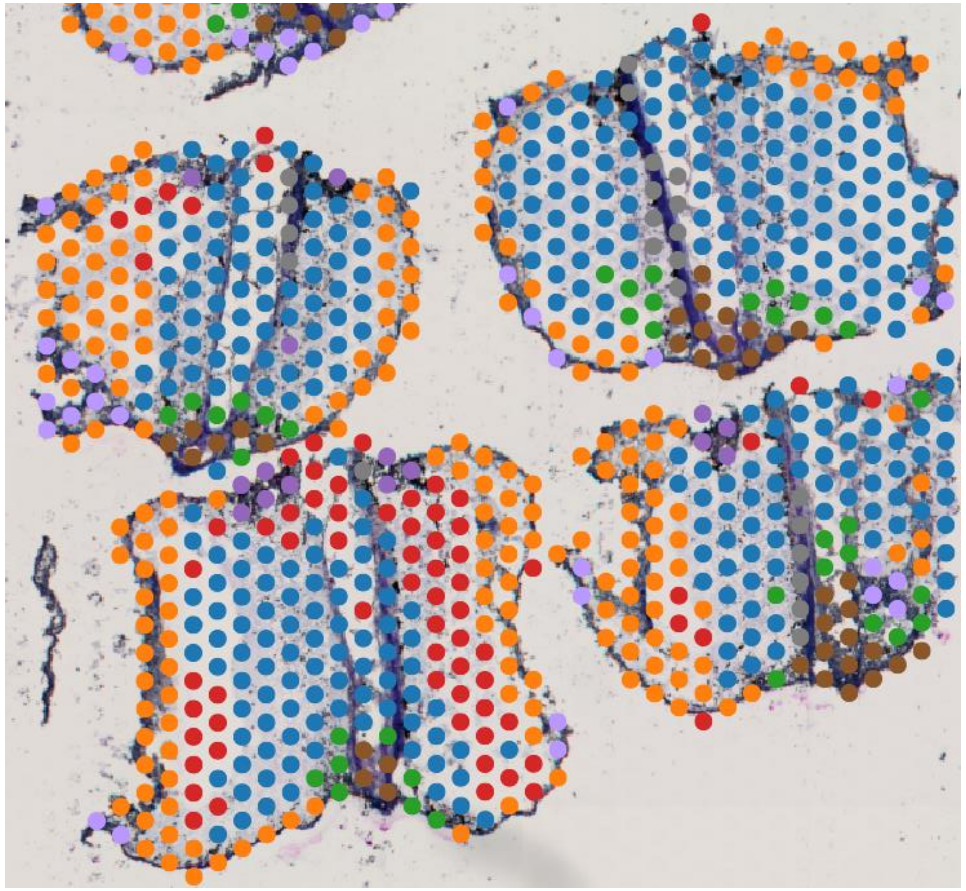
Spatial transcriptomics



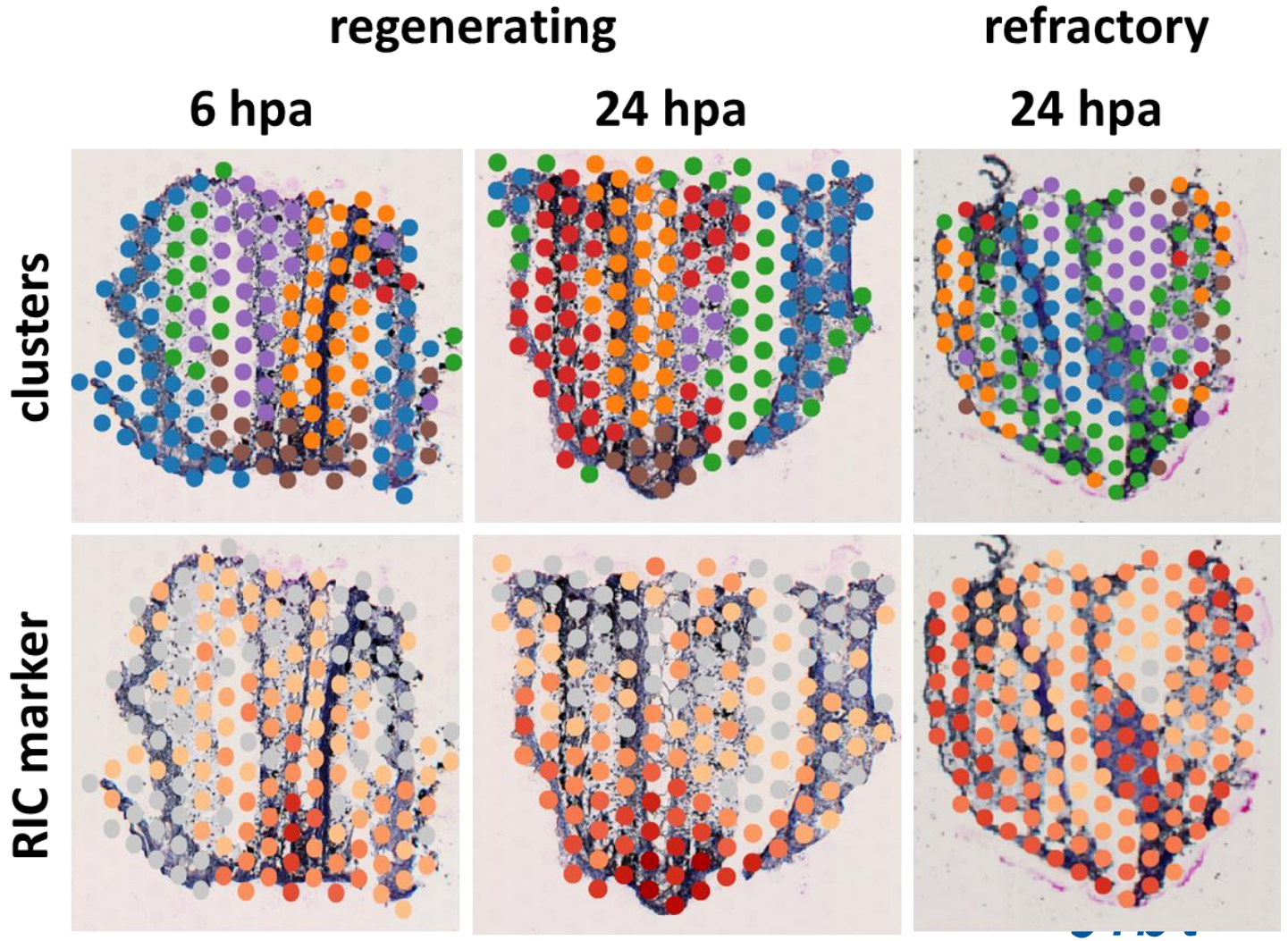


- remodelling enzymes
- TGF-beta inhibitors
- ECM components

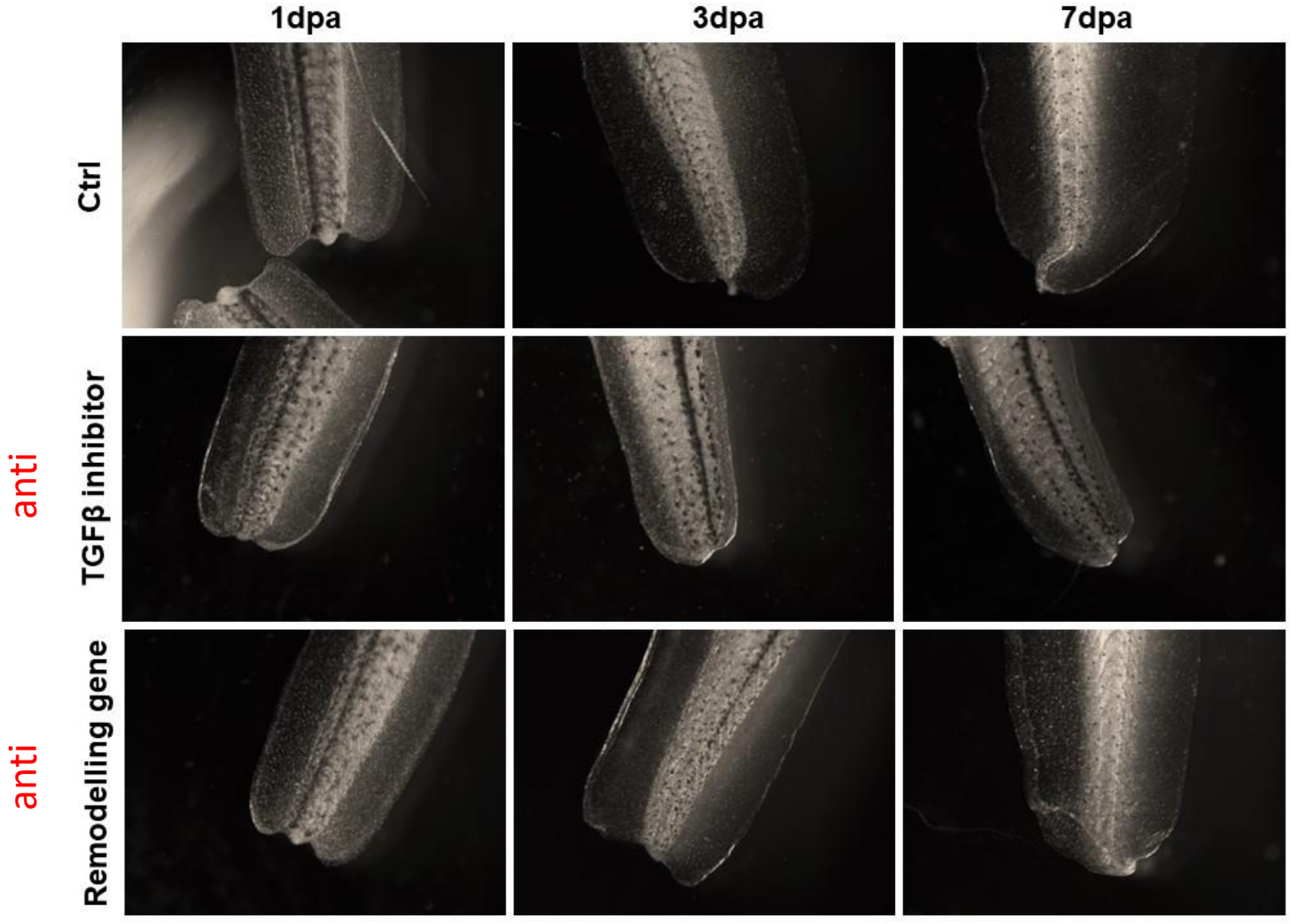
Refractory spatial transcriptomics

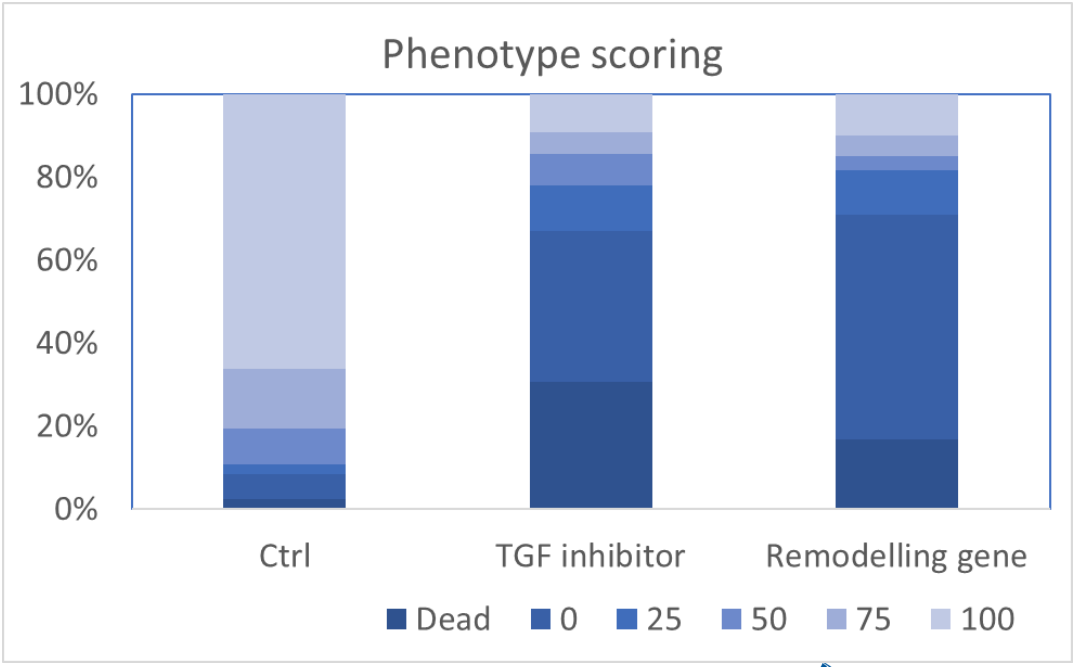


- remodelling enzymes
- TGF-beta inhibitors

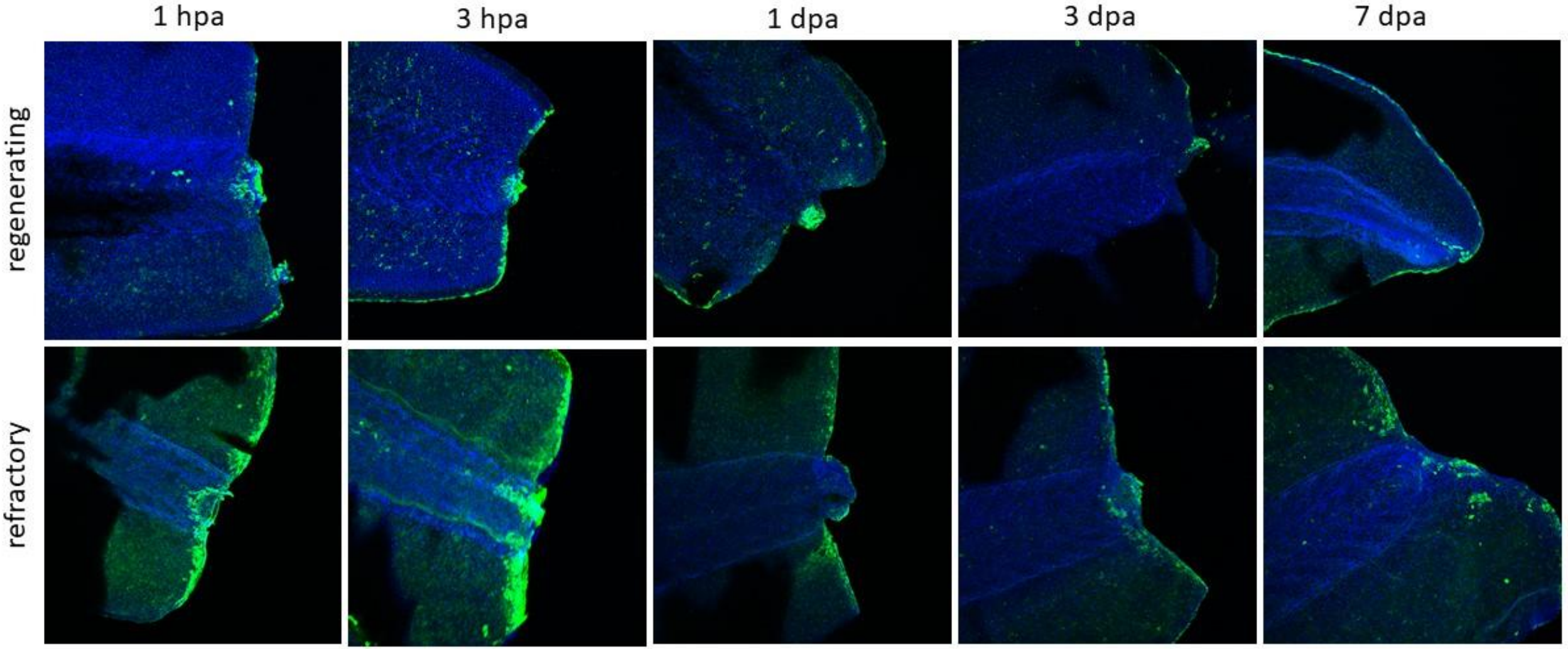


Loss of function when RIC markers are inhibited

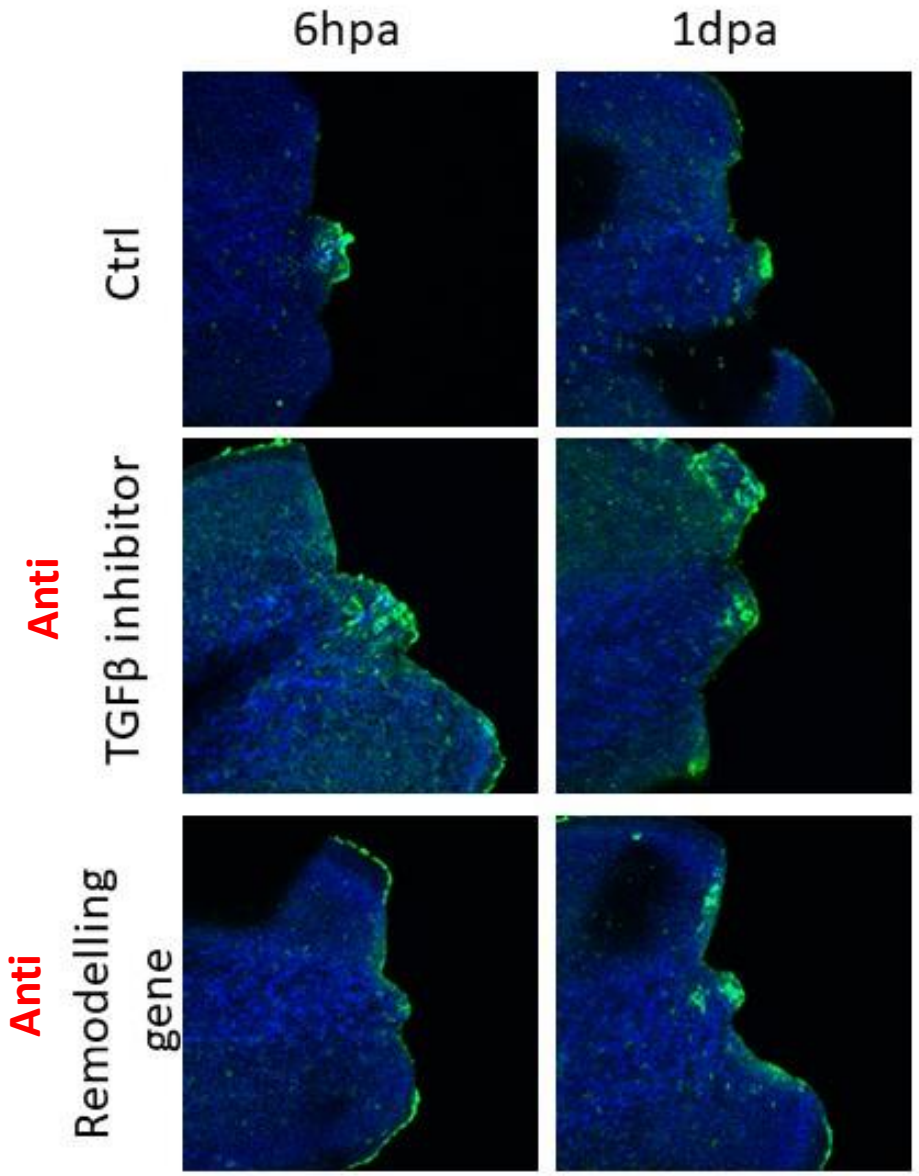




Scarring in refractory stage inhibits regeneration



Scarring appears in RIC loss of function models



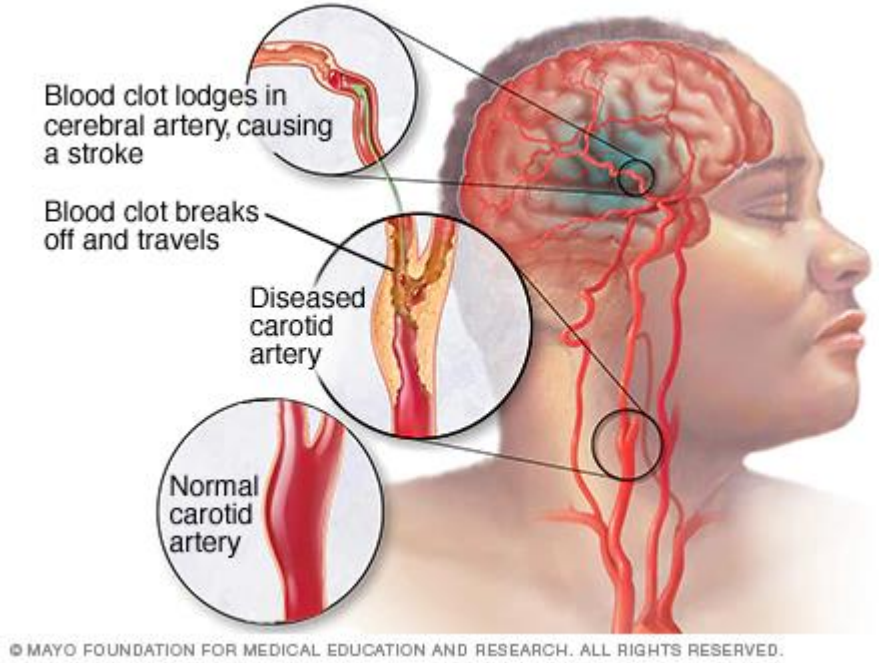
Summary

- The end of the healing phase is the initiation step for effective regeneration.
- *X. laevis* regeneration requires Regeneration Initiation Cells (RICs) that express remodeling enzymes and TGFβ inhibitors.
- Extracellular matrix modifications are important for regeneration initiation.

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
  - Neuronal death
  - Inflammatory response
  - Structural changes

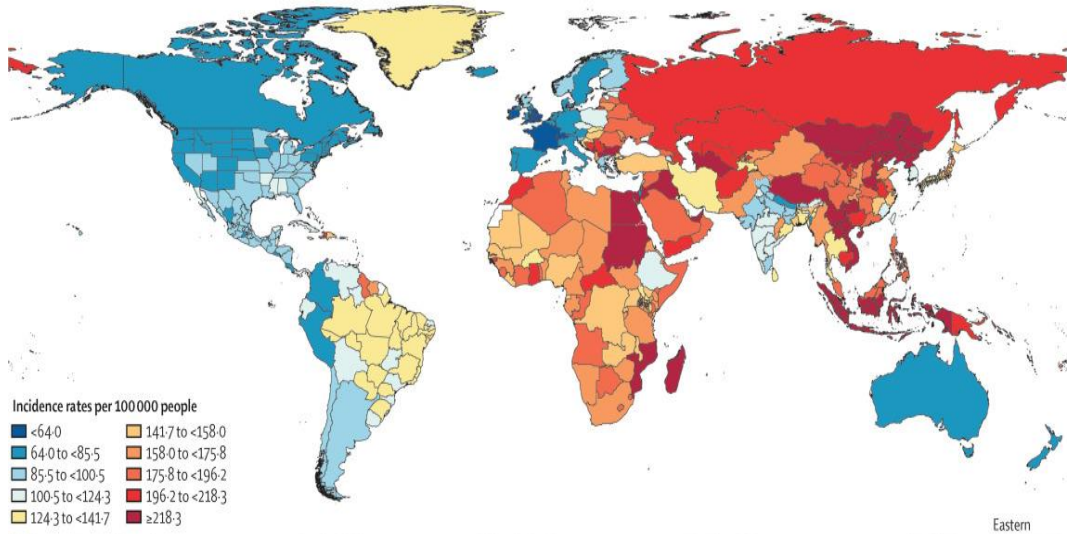


Ischemic brain injury (stroke)

- 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 treatment strategies
  - >1000 drugs investigated
  - >100 tested in clinical trials
- Early mechanical thrombectomy and thrombolysis remain the sole therapies



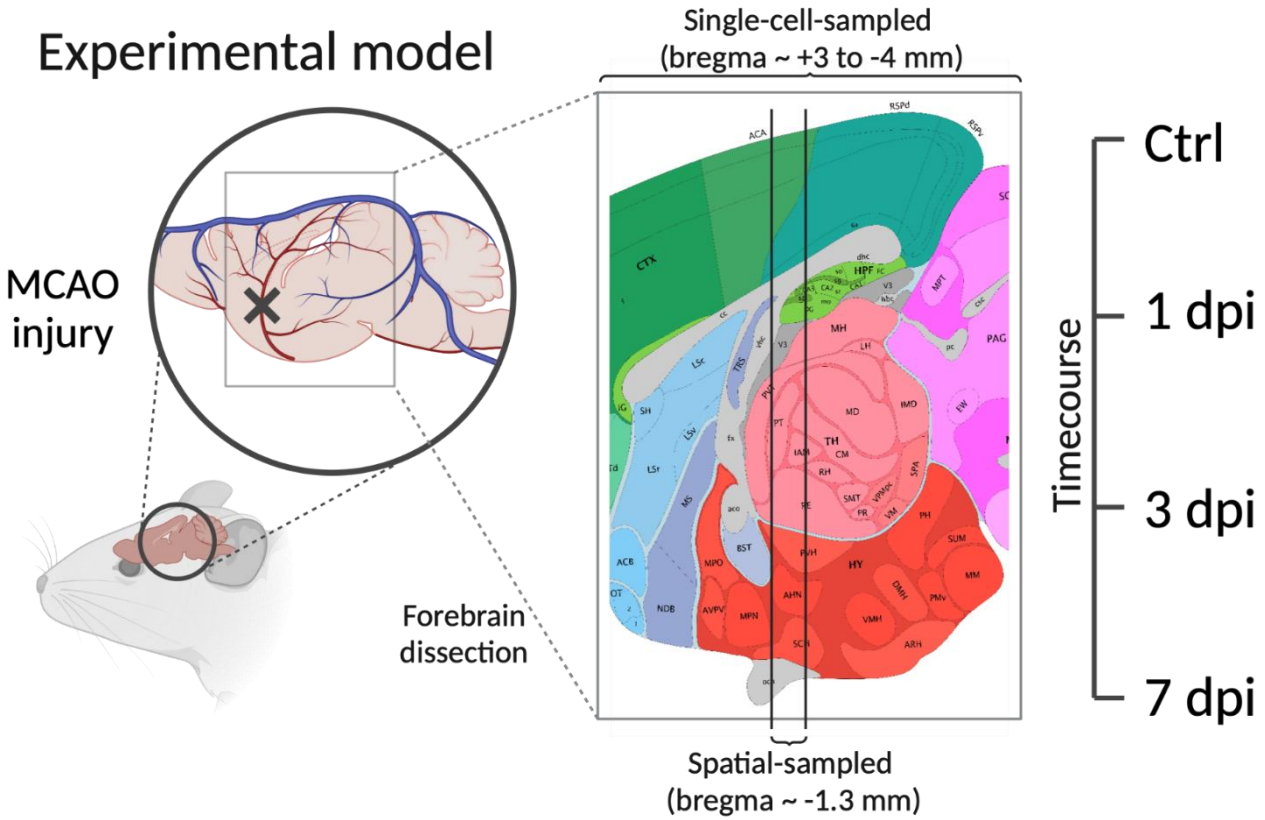
GBD 2019 Stroke Collaborators, 2021

Ischemic brain injury (stroke)

- New therapeutics are challenging to develop because of
  a) Disease complexity
  b) Cell heterogeneity
  c) Temporal and spatial factors

- Unique opportunity for the latest omics and advanced computional methods
  - Spatial transcriptomics (ST)
  - Single-cell/nucleus RNA-sequencing (sc/snRNA-seq)
  - Integrative analysis

Experimental design

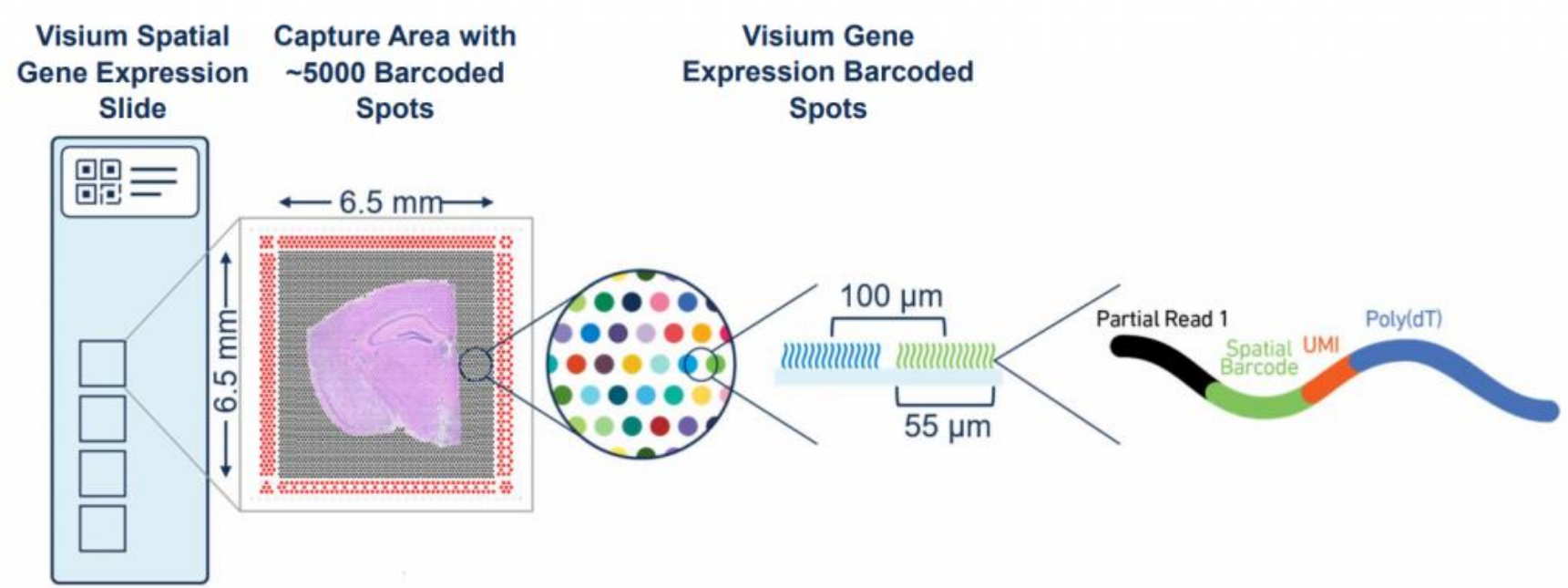


- MRI
- Spatial transcriptomics
- Sc/snRNA-seq
- Bulk RNA-seq
- IHC

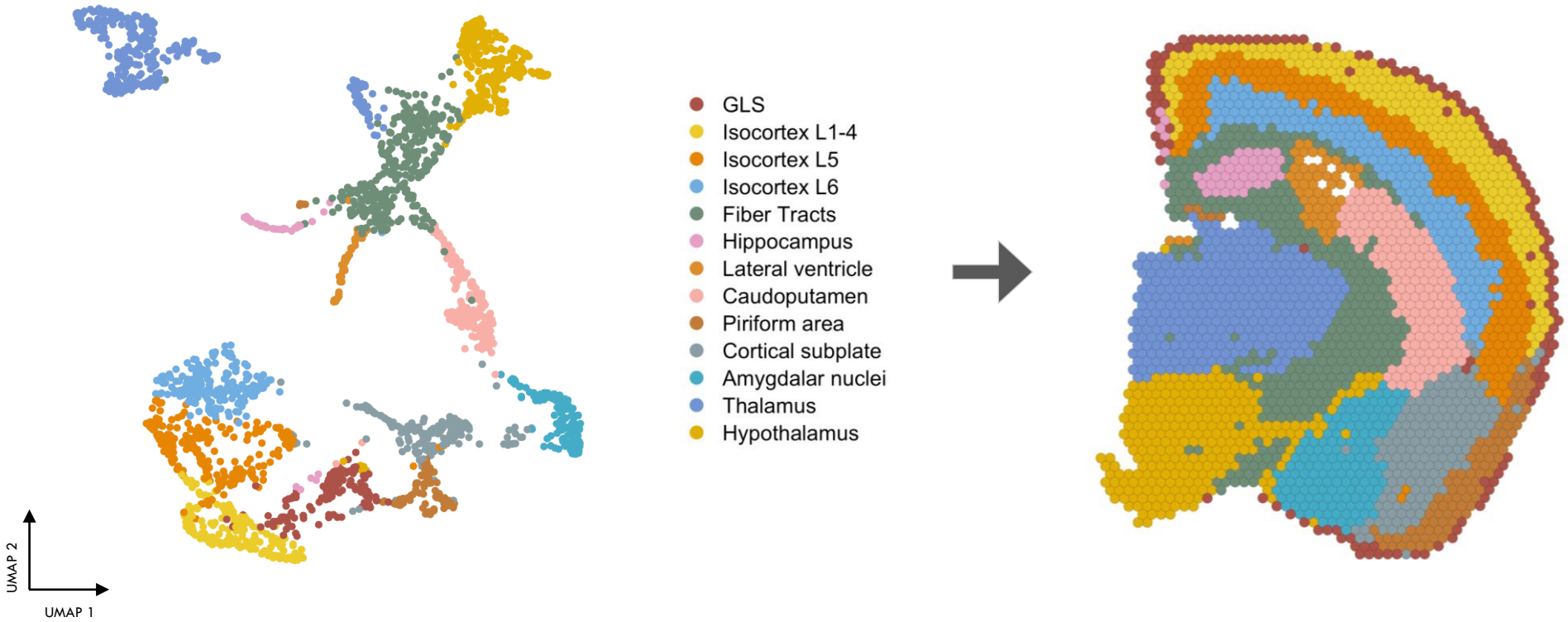
- Data analysis

Visium Spatial Gene Expression, 10x Genomics

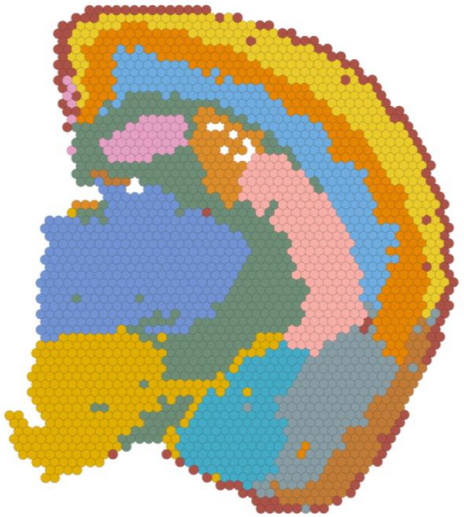
- Genome-wide analysis
- 55 µm resolution
- 6.5 x 6.5 capture area

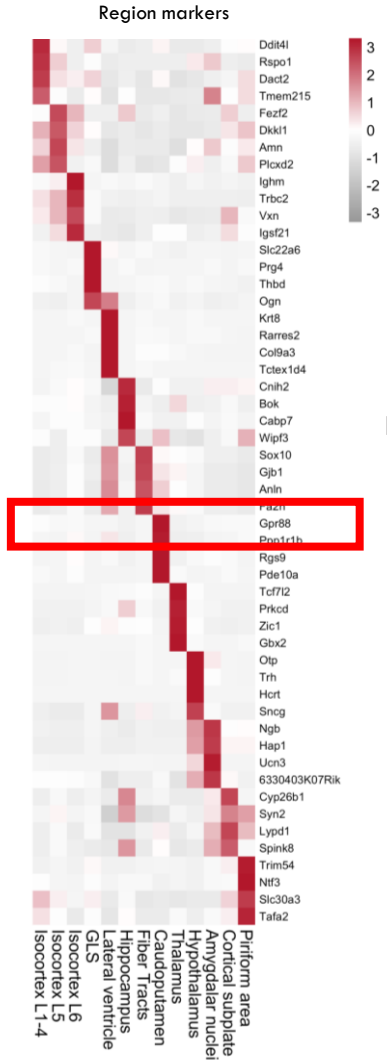


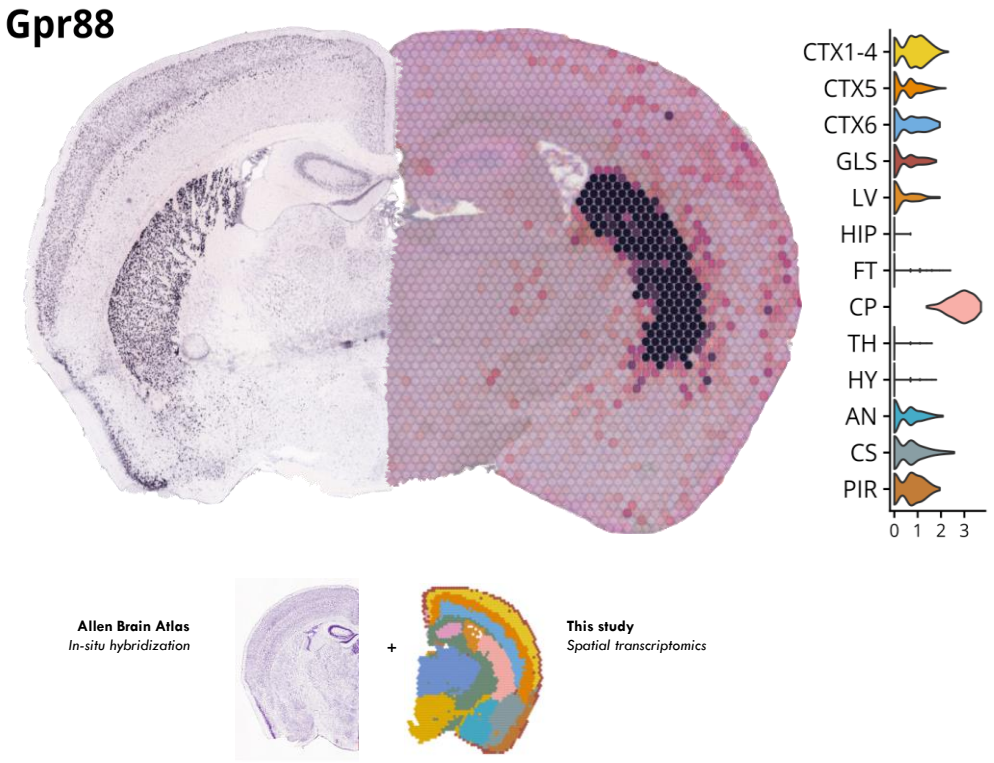
ST robustly captures mouse brain anatomy



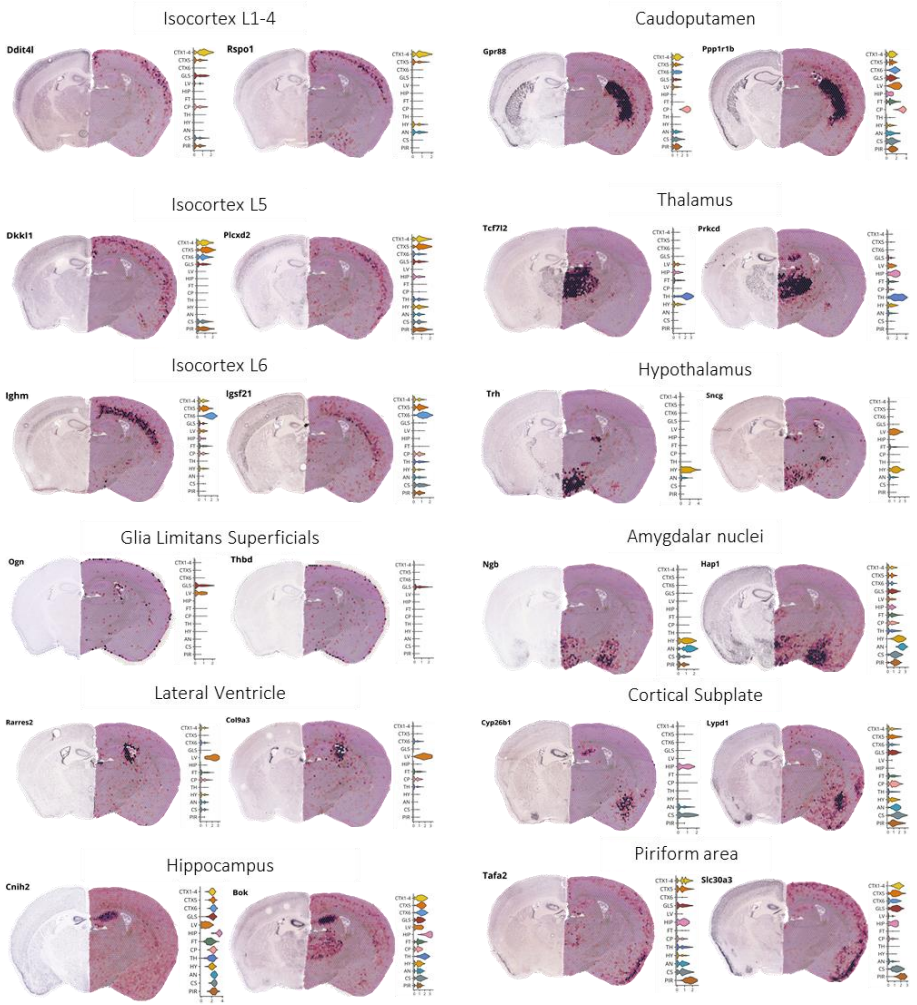
ST identifies brain region-specific genes

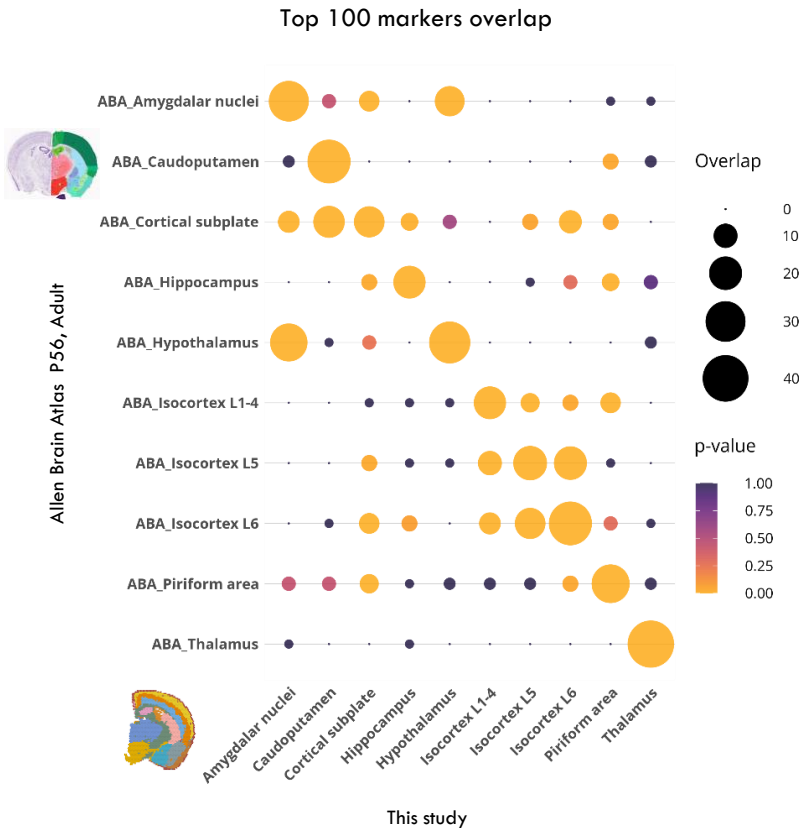




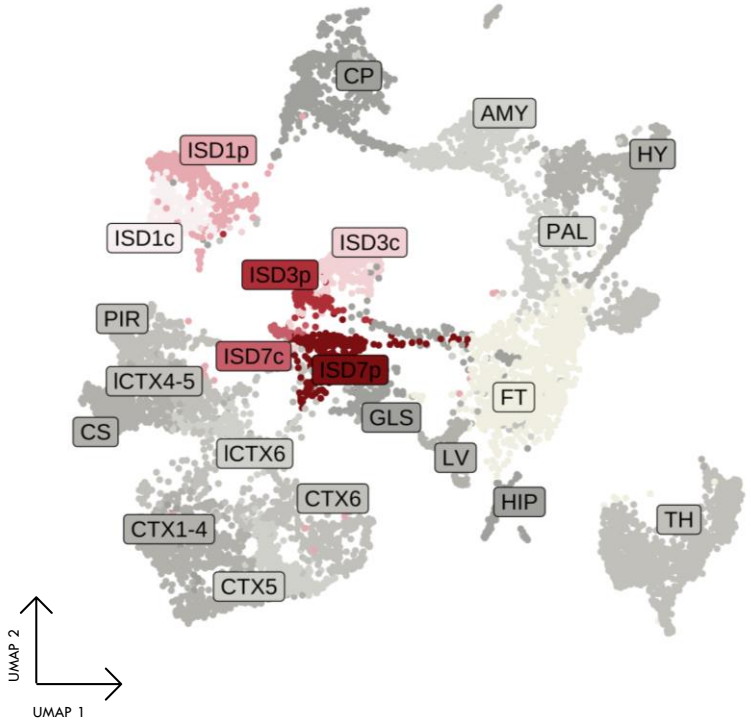


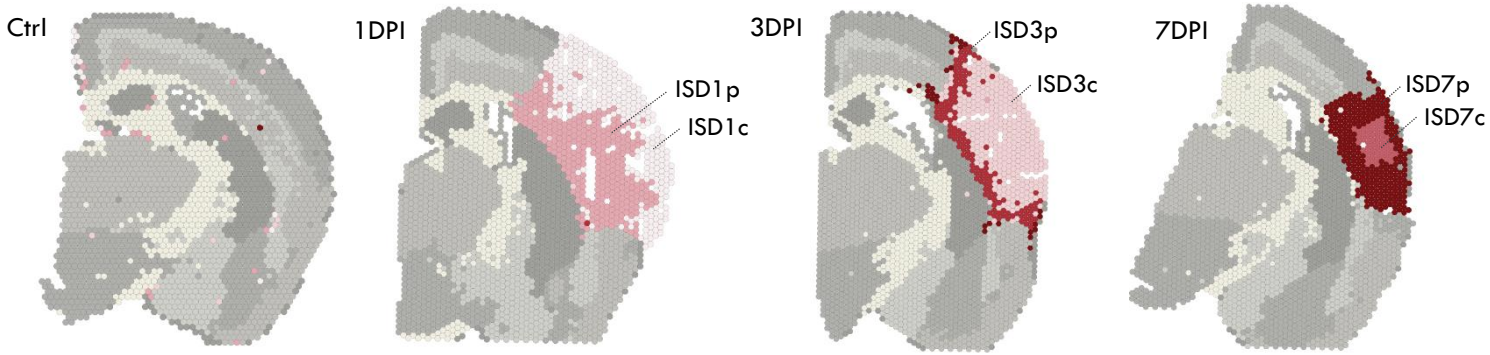
ST data correlates with reference ISH atlas

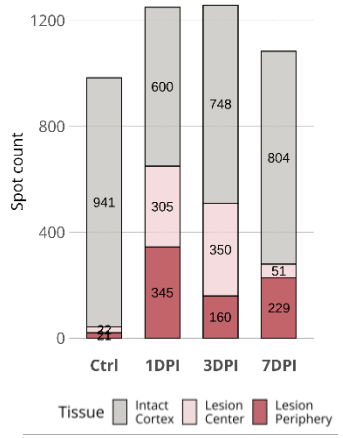


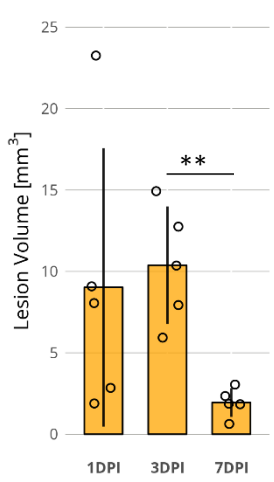


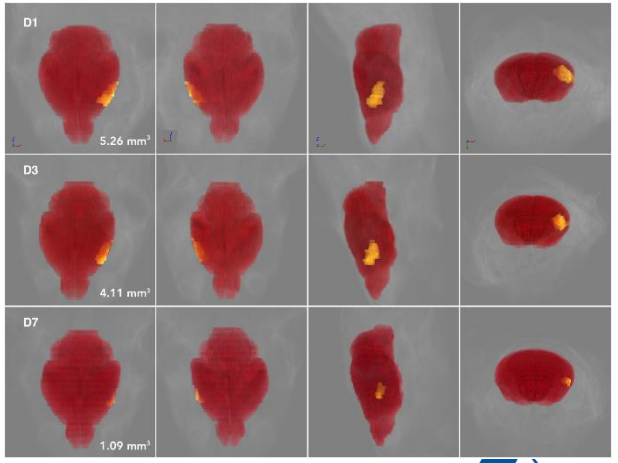
ST revealed injury-induced disruption of cortical gene expression landscape











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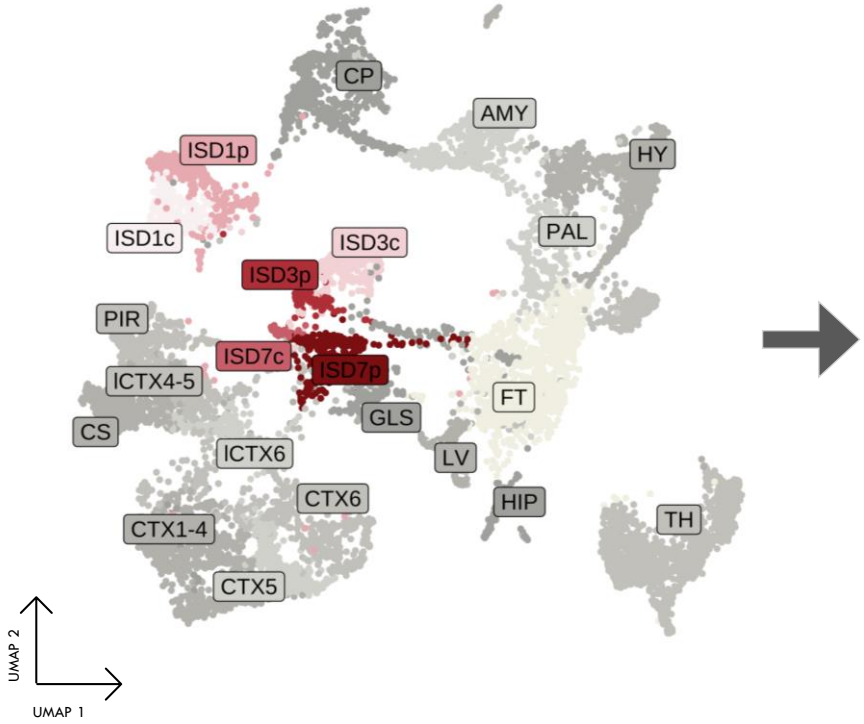
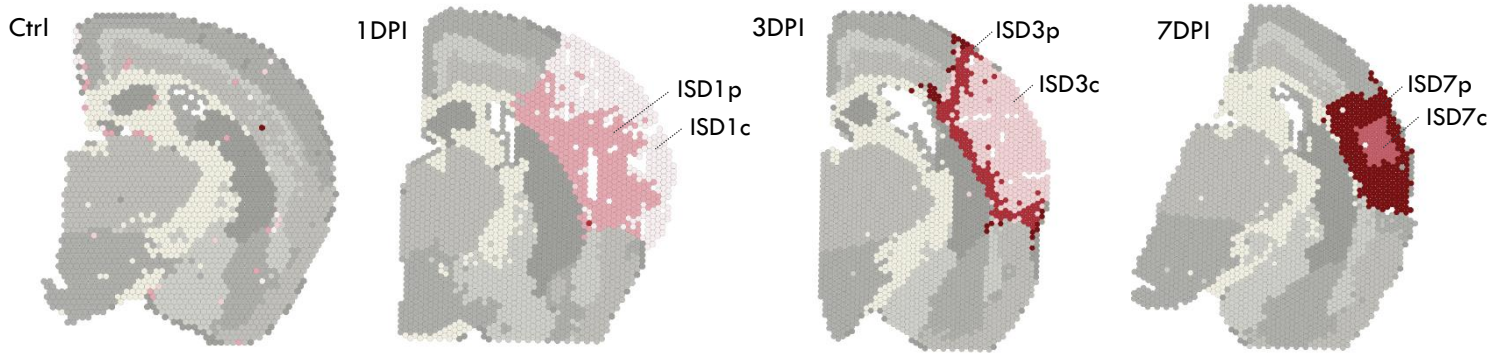
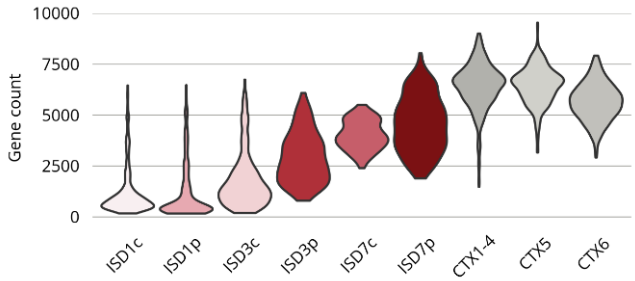


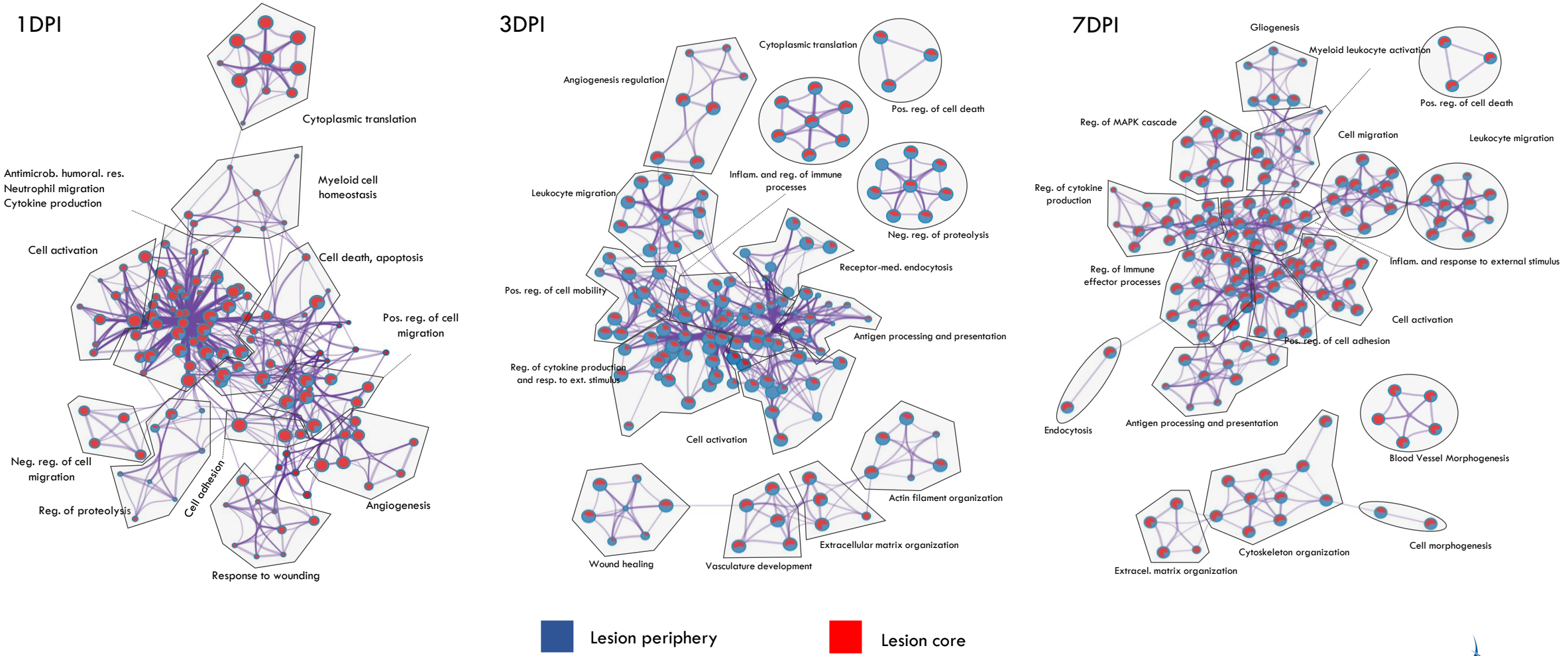




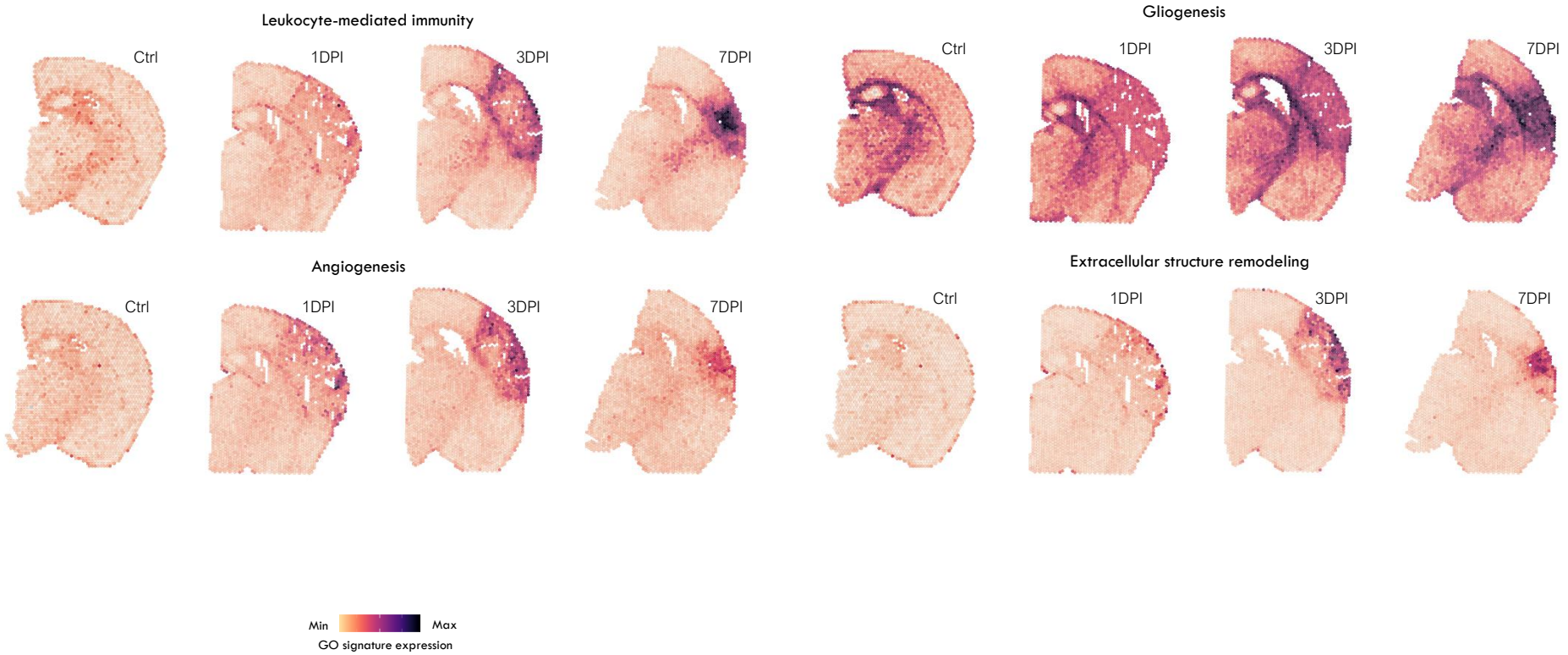
# of DEG

	ISD1c	ISD1p	ISD3c	ISD3p	ISD7c	ISD7p
Up	164	41	333	397	775	294
Down	86	36	192	332	1130	107

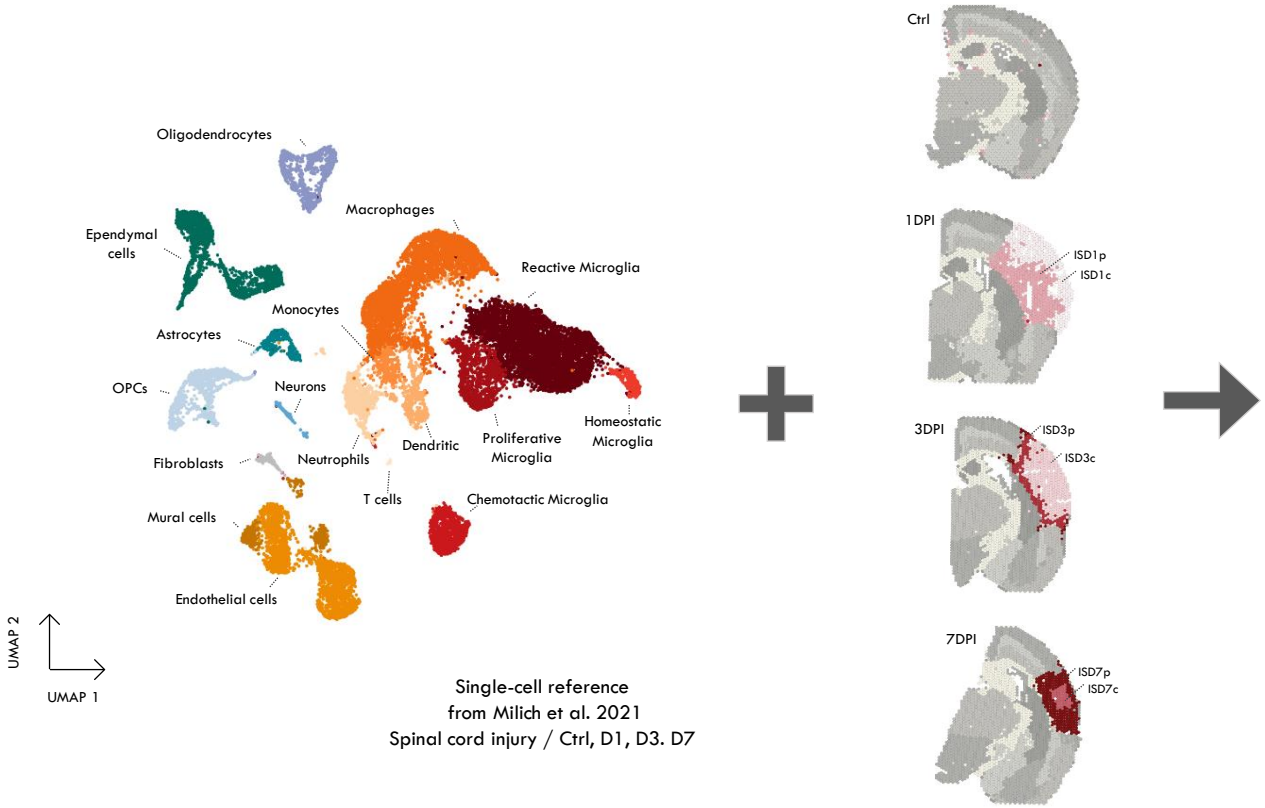
Cell activation, inflammation and tissue remodeling - hallmarks of coordinated response to ischemic damage



ST allowed mapping of spatially localized processes



Deconvolution showed dynamic changes in cell type compositon in the ischemic lesion



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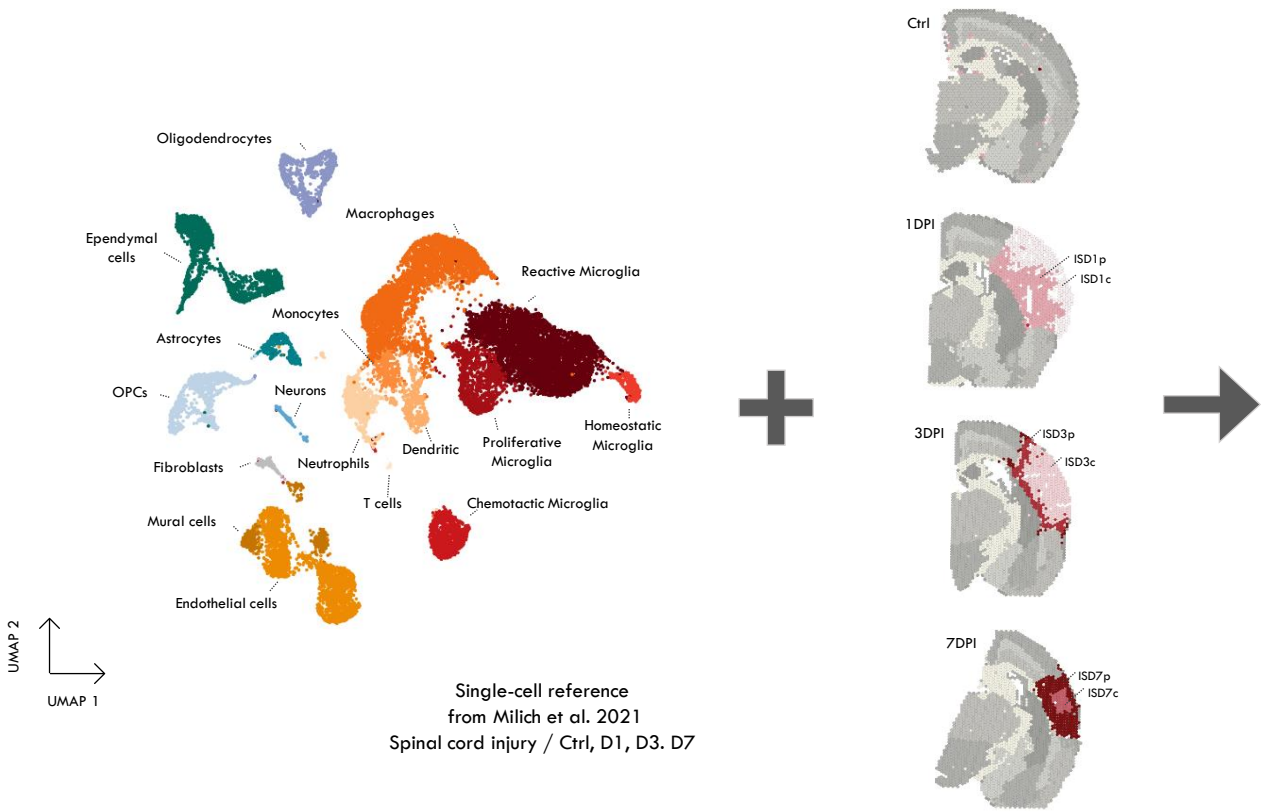

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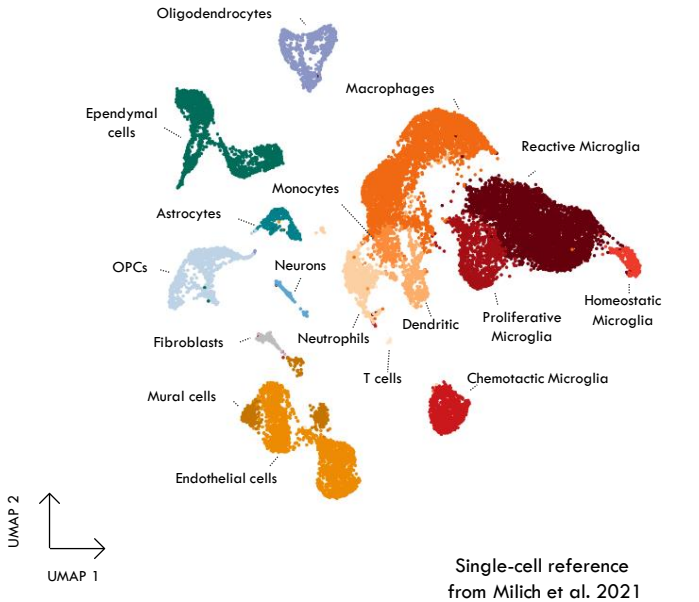
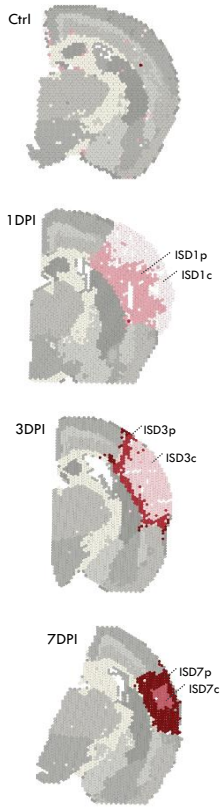
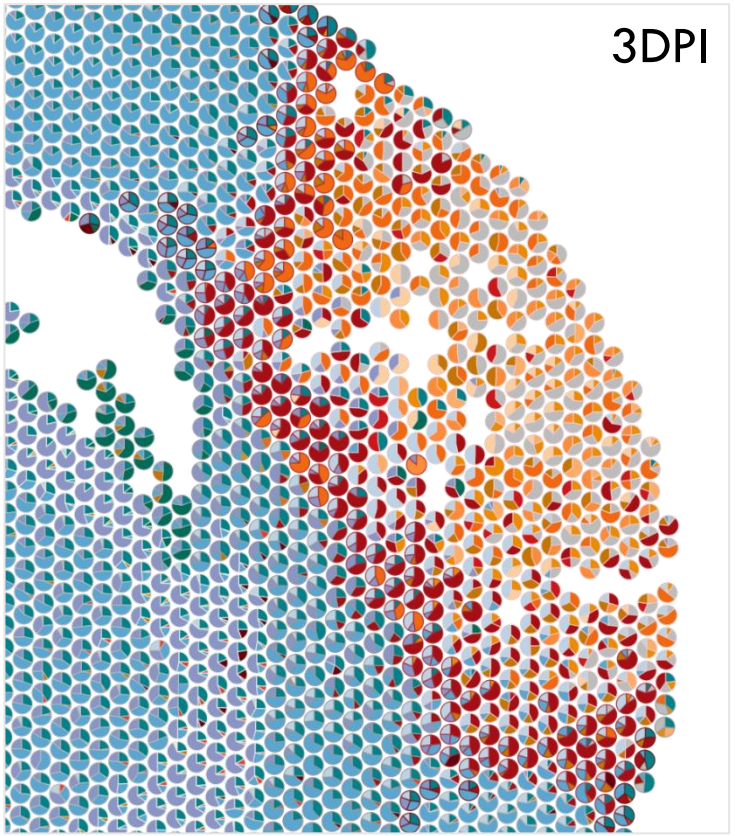


Single-cell reference
from Milich et al. 2021
Spinal cord injury / Ctrl, D1, D3. D7





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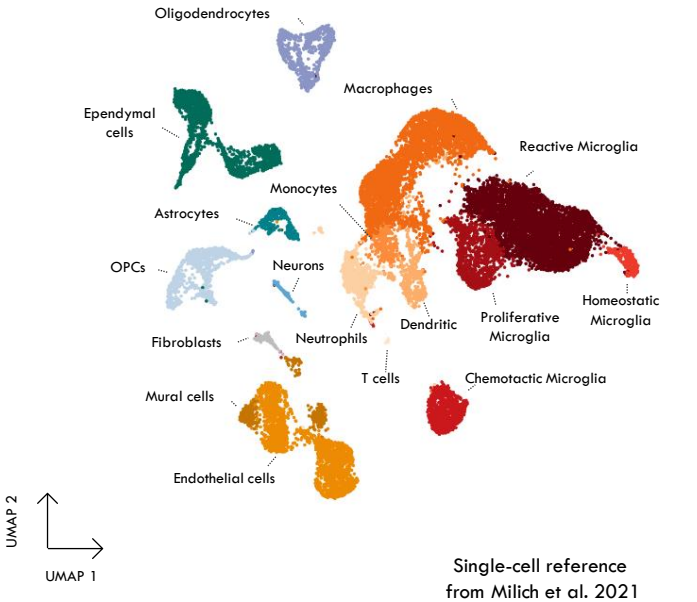

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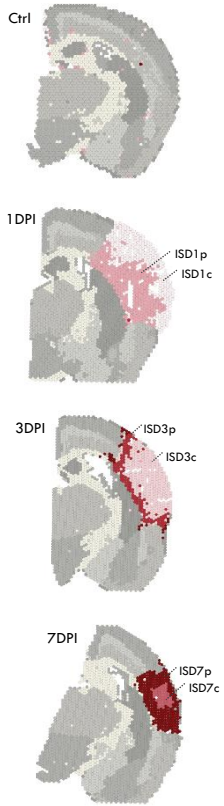

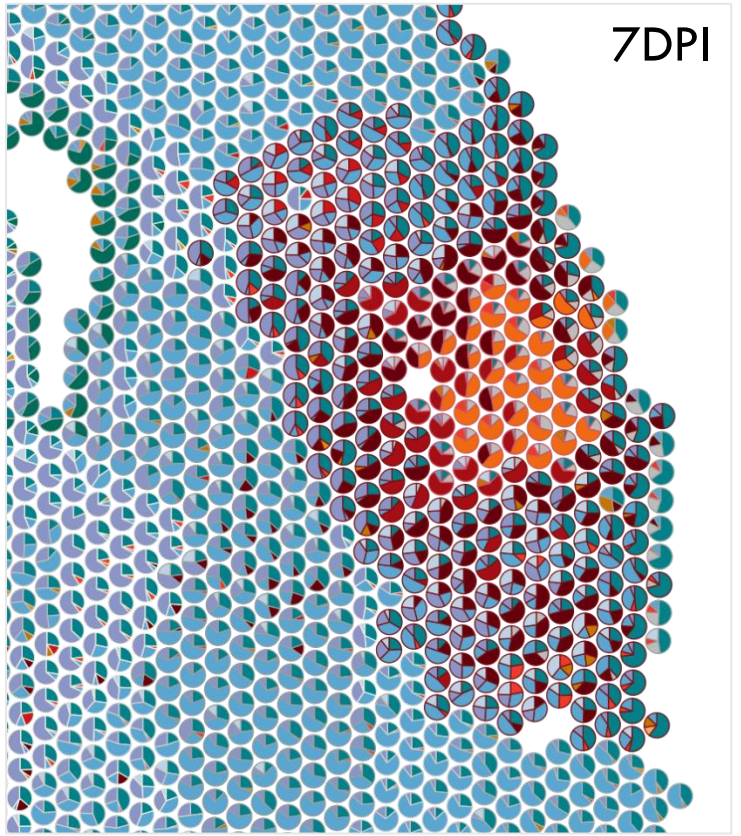

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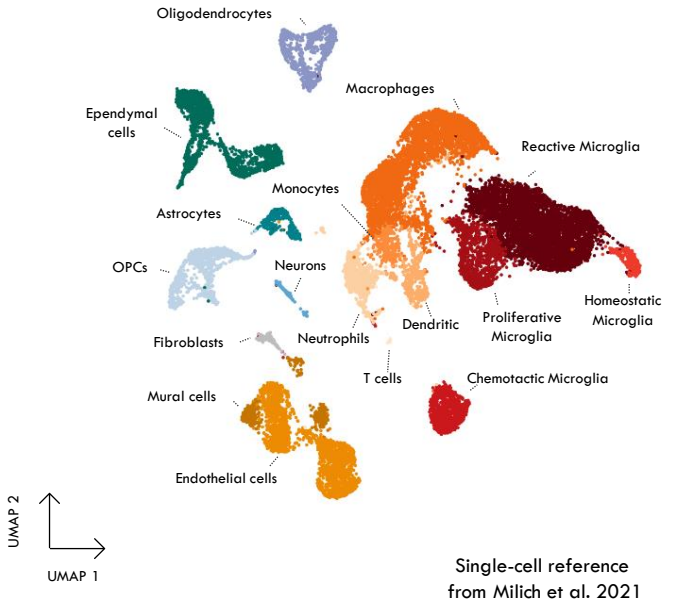

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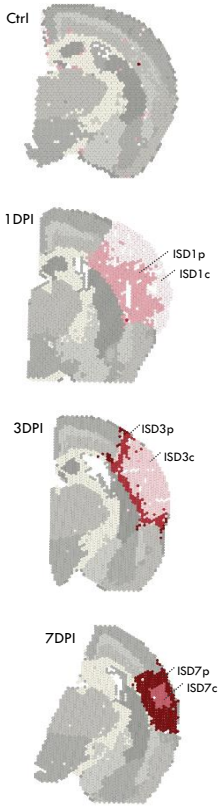

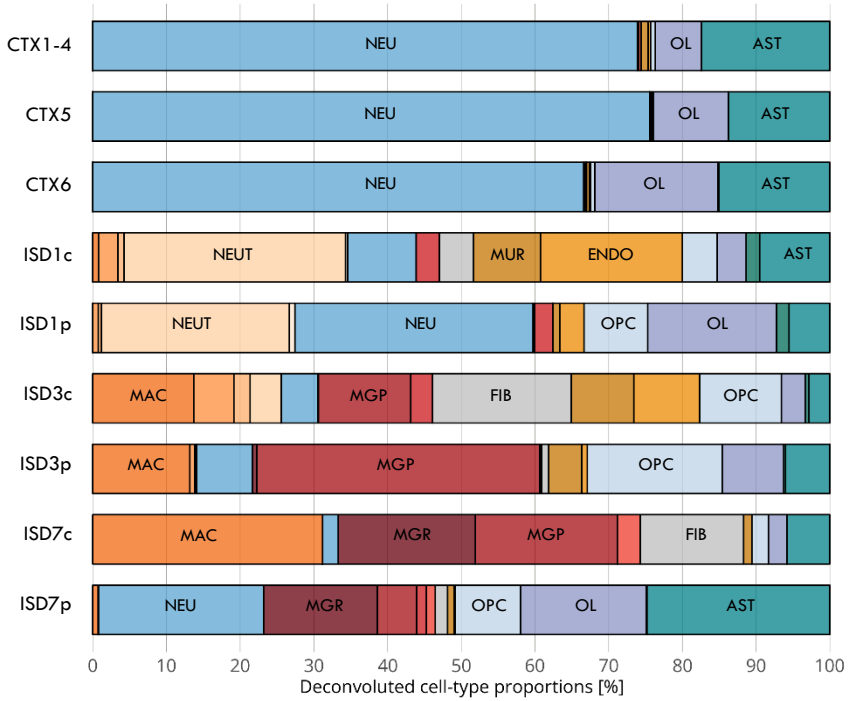

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- Ravindra Nara
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- Katerina Simk
- Paulina Kikinc
- Pavel Abaffy
- Viktoria Iegor
- Daniel Zucha

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