

# Characterizing Cellular Metabolism via Flux Enrichment Scores: A Versatile Framework for Bulk, Single-Cell, and Spatial Transcriptomics

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## Introduction

Cellular metabolism is **highly heterogeneous**, shaped by tissue context, developmental stage, and cell-cell interactions, and plays a central role in physiology, disease, and embryonic development.

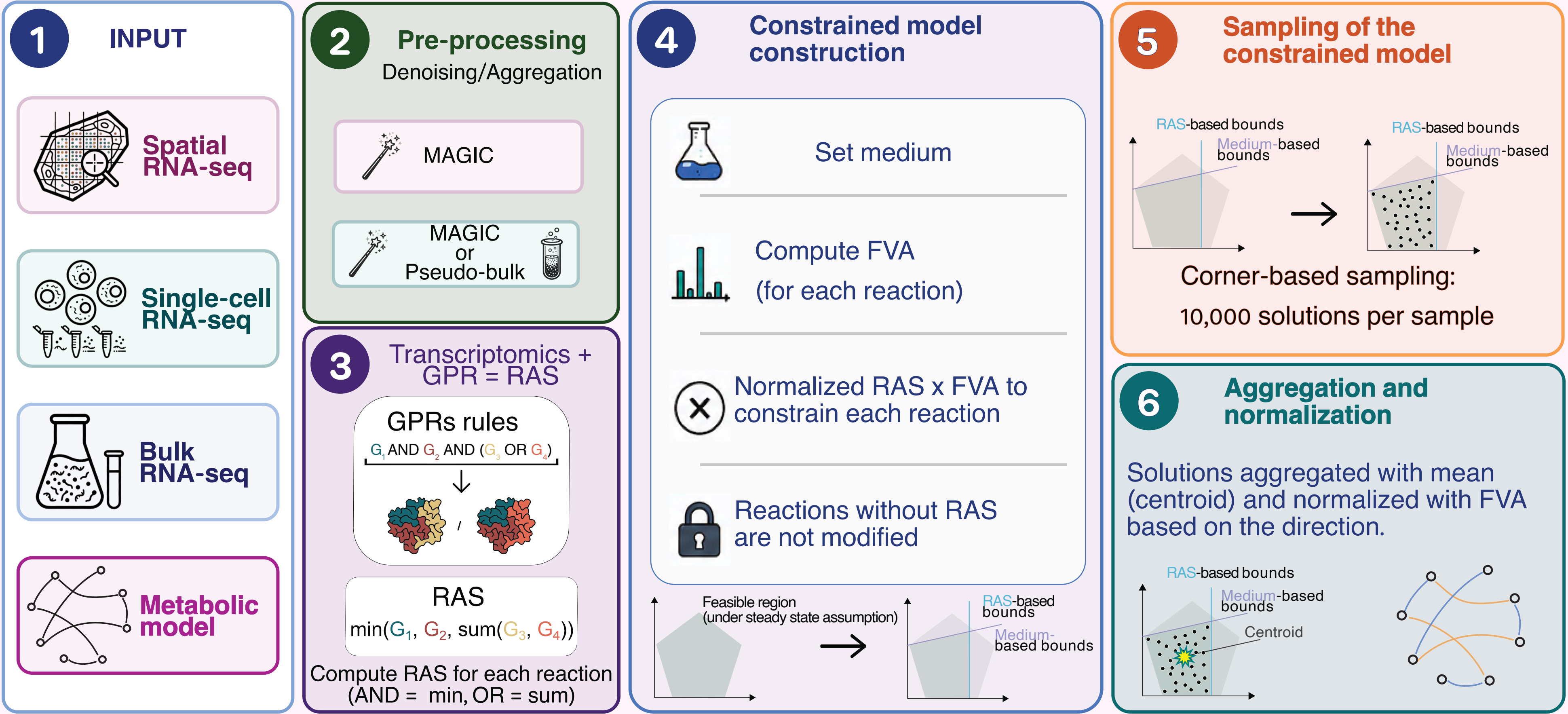
Current methods for inferring metabolic fluxes (e.g. traditional MFA) **fail to capture this heterogeneity** and are typically tied to a **single data modality**.

Recent advances in **transcriptomics** (bulk, single-cell, and spatial) provide an unprecedented view of cellular states, but a unified **computational framework** to translate them into **metabolic fluxes** is still missing.

To address this gap, we developed a **versatile framework** that leverages transcriptomics from any modality to enrich metabolic Flux Enrichment Score (FES) at the appropriate resolution (sample, cell, or spot).

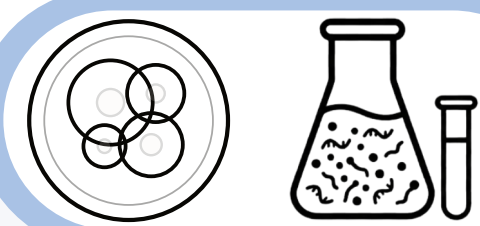
We applied it to three case studies: **human embryo development** (bulk + single-cell), **clear cell Renal Cell Carcinoma** (Visium), and **Colorectal Cancer with matched liver metastases** (Stereo-seq + Visium HD).

## Framework



Framework available on Galaxy with **COBRAXy**: Lapi, et al., Bioinformatics, Volume 42, Issue 2 (2026)

MAGIC: van Dijk D, et al. Cell, 2018; 174, 716-729.e27



### Bulk: human embryo

#### Metabolic remodeling in human embryo peri-to-post implantation

Bulk RNA-seq + paired **exo-metabolomics** from 3D embryo-like structures (hPSC), peri (D4) vs post (D6).

Three layers converge on a **coordinated shift**

→ **Reduced glycolytic output** in Post

**Less glucose uptake** and **less lactate release** (metabolomics), downregulated glycolytic RAS, lower glycolytic fluxes, consistent across non-limiting and oxygen-limiting simulations

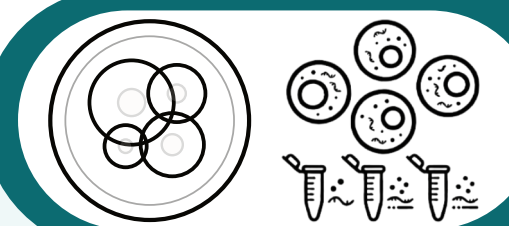
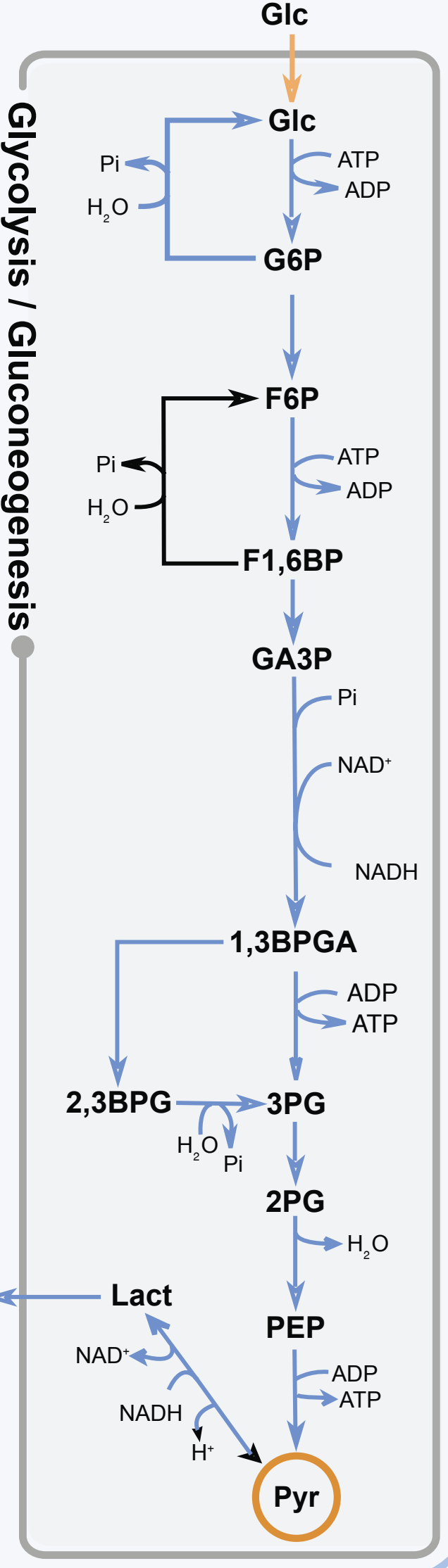
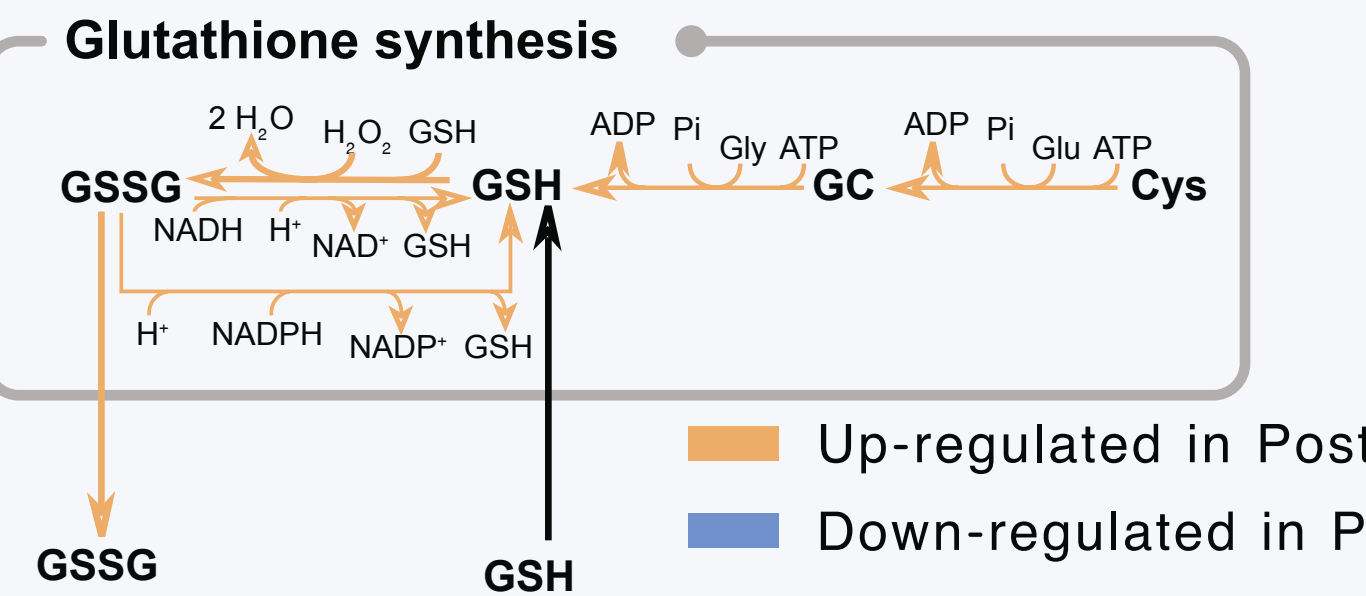
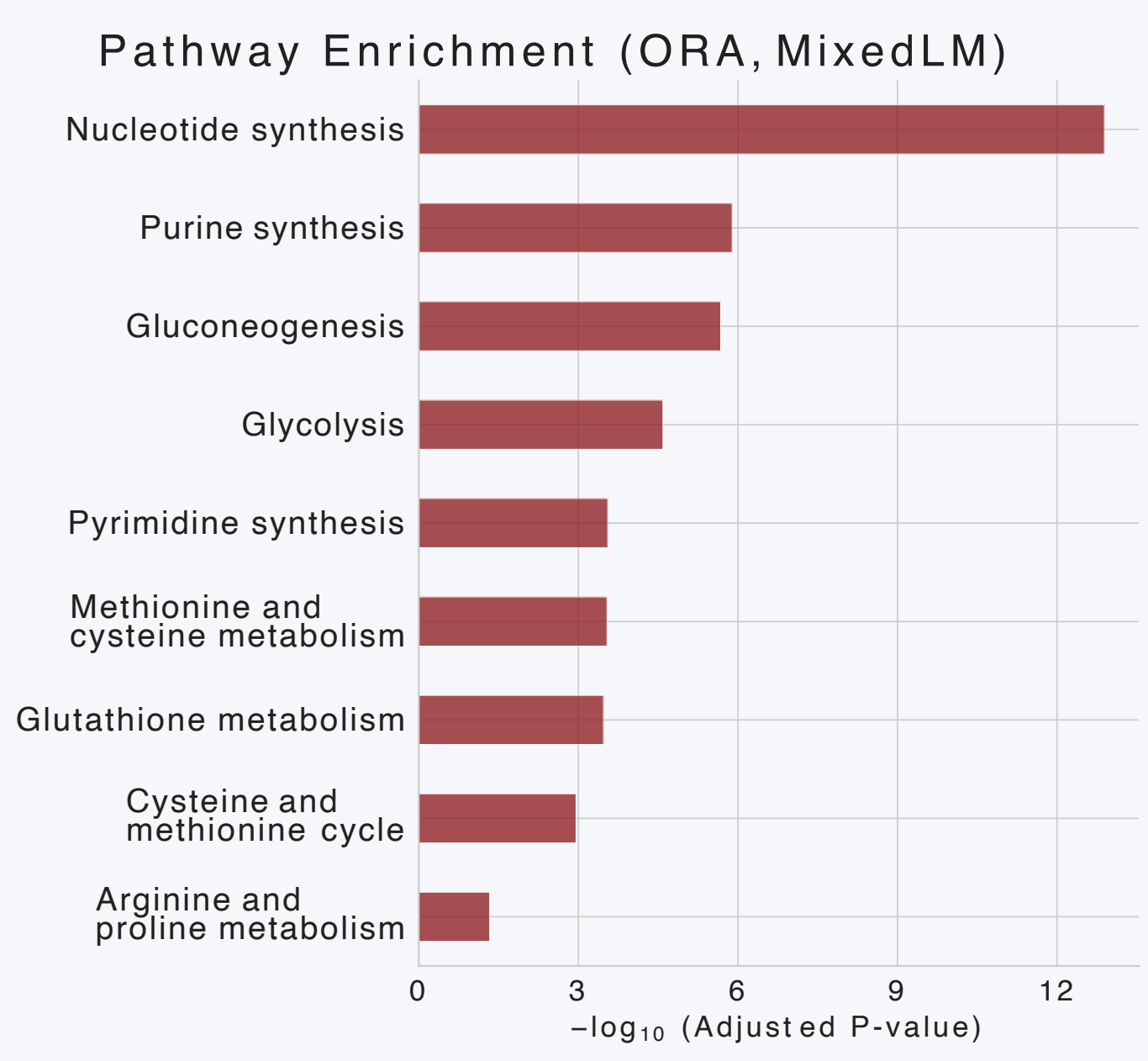
→ **Reinforced redox/antioxidant capacity** in Post

Coordinated upregulation of **glutathione synthesis/recycling** at the RAS and flux level, supported by cysteine/cystine-related genes

→ **Stable mitochondrial backbone**

TCA cycle largely unchanged; oxygen availability modulates magnitude but not direction of the remodeling

→ **Downregulated fatty acid metabolism** and re-patterned amino acid usage (serine, glutamine, glutamate transport)

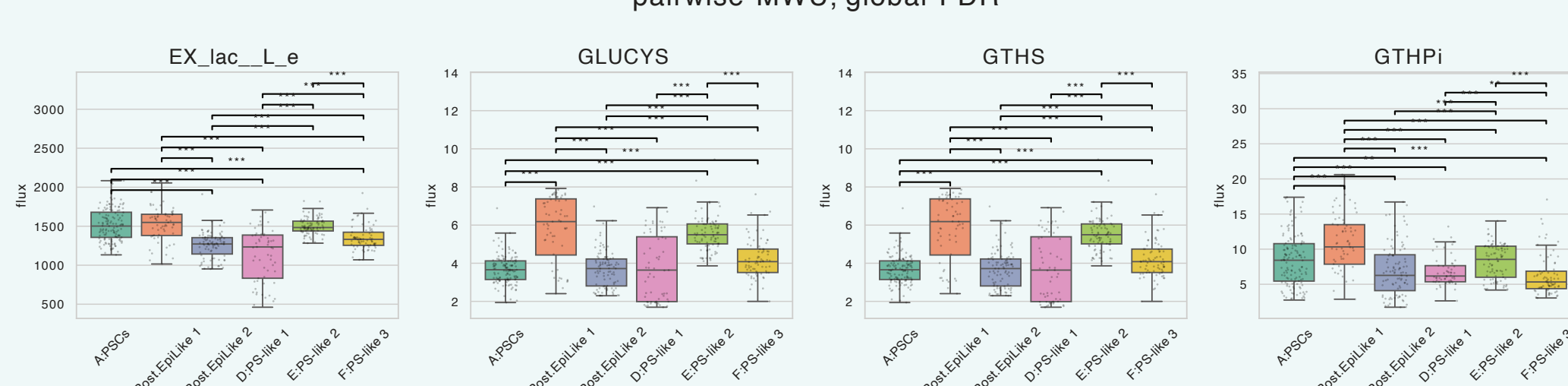


### Single-cell: human embryo

#### Single-cell validation in human embryo peri-to-post implantation

We validated the framework on a published **single-cell RNA-seq dataset of HES3-MIXL1-GFP reporter human gastruloids**, spanning a developmental trajectory from **PSCs** through **Post Epi-like** to **Primitive Streak-like** stages.

After MAGIC, we applied spFBA with the same setup used on the embryo bulk data.



Bulk findings are **recovered at single-cell resolution**

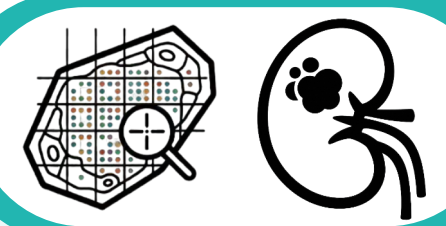
→ **Lactate secretion declines along the trajectory**: recovers the reduced glycolytic output seen in bulk

→ **Glutathione synthesis (GTHS) peaks** at the Post Epi-like stage, matching the D6 post-implantation peak in bulk

→ **Redox cycling (GTHPI, GDR)** coordinately upregulated → reinforced redox capacity

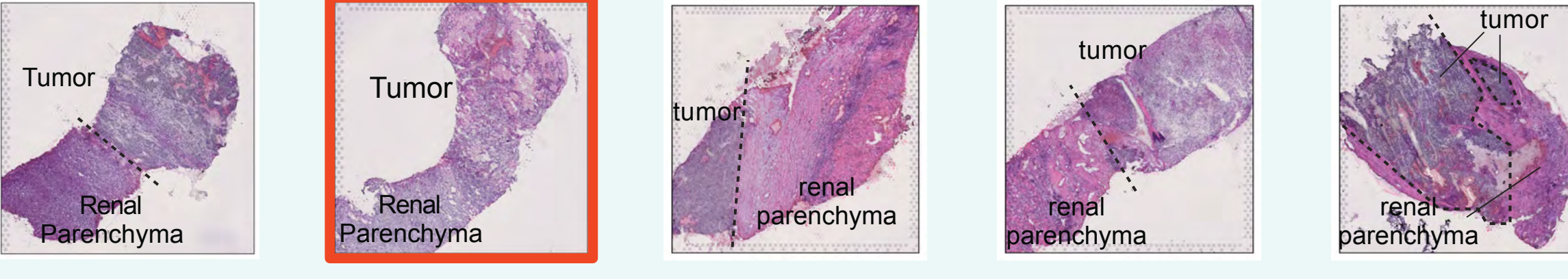
**Same metabolic program across data modalities and models**

Single-cell data: Zorzan, I., et al. Nat Cell Biol 28, 49–65 (2026)

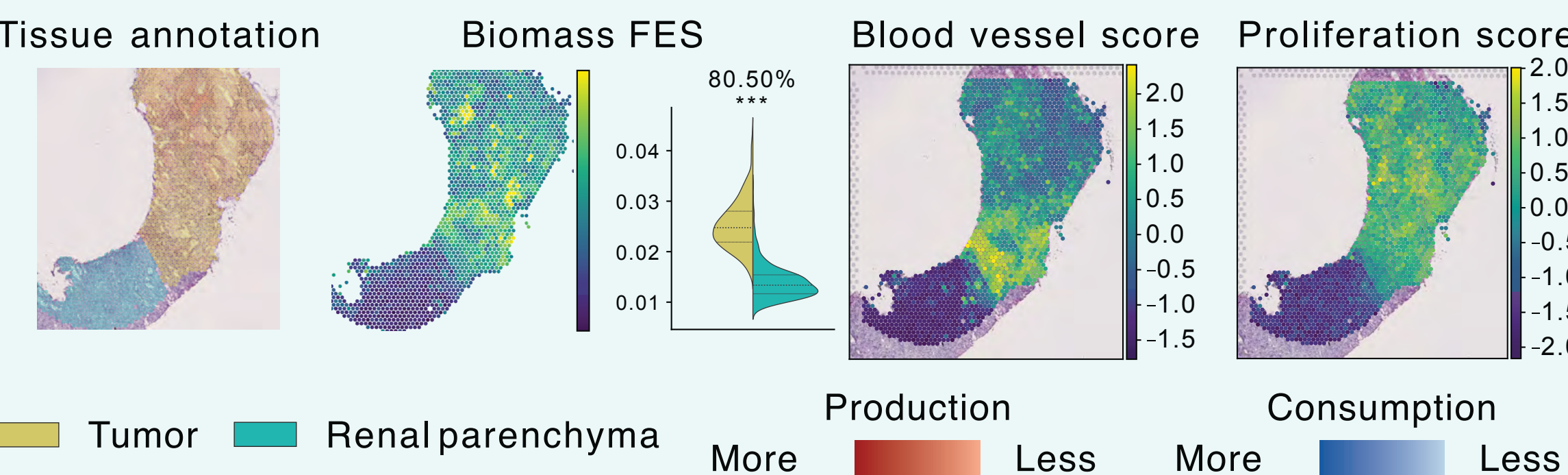


### Clear cell Renal Cell Carcinoma

To evaluate the method with spatial transcriptomics, we analyzed a public ccRCC dataset containing five tumor-normal boundary samples.



Three metabolic hallmarks emerge consistently across all five samples, and predicted fluxes align with independent tissue markers:

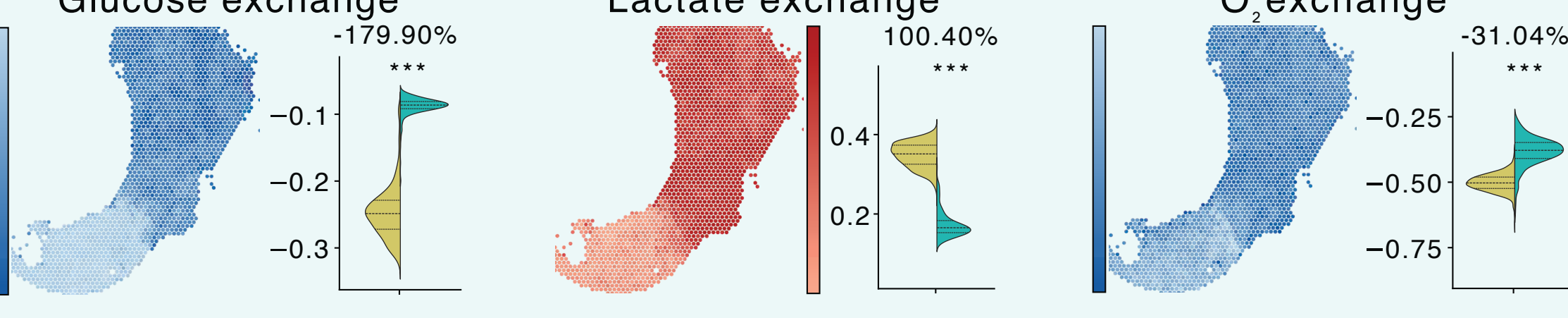


**Sustained proliferation:**

→ **Increased biomass FES in tumor regions** → Co-localizes with the proliferation score → validates FES as a growth-rate readout

**Vascular-oxidative coupling:**

→ **O<sub>2</sub> exchange mirrors the blood vessel score** → oxidative fluxes track tissue vascularization



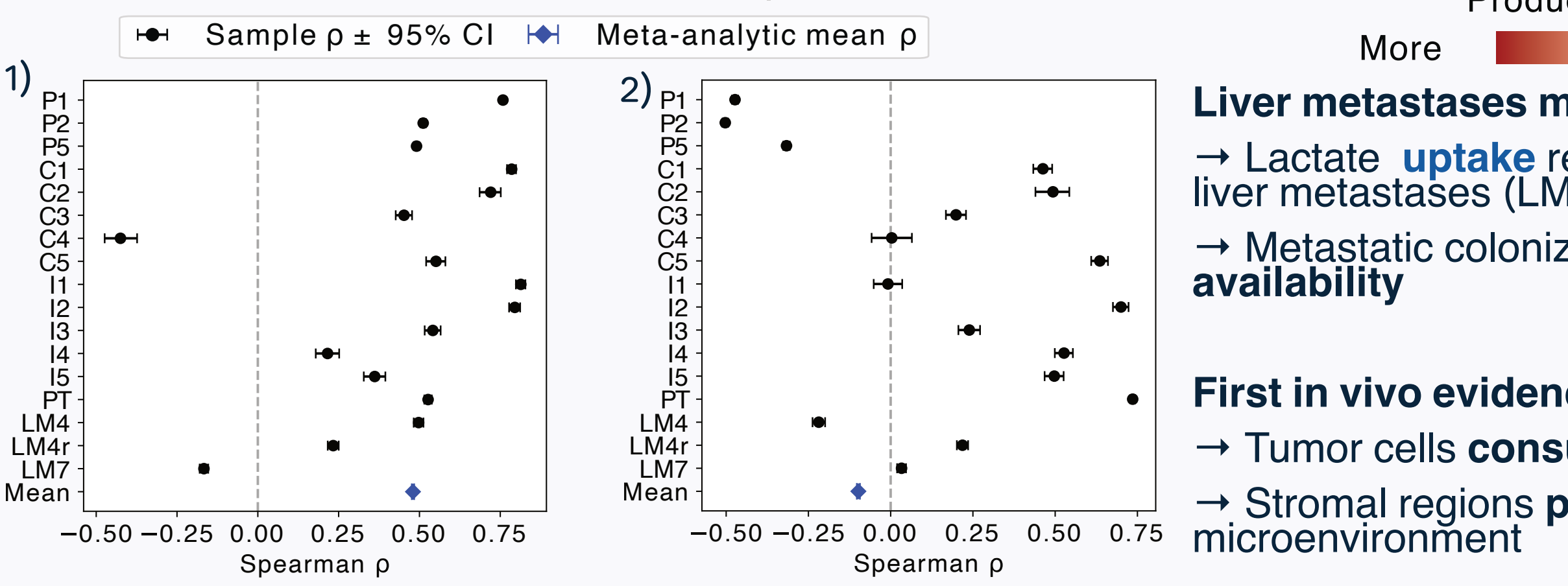
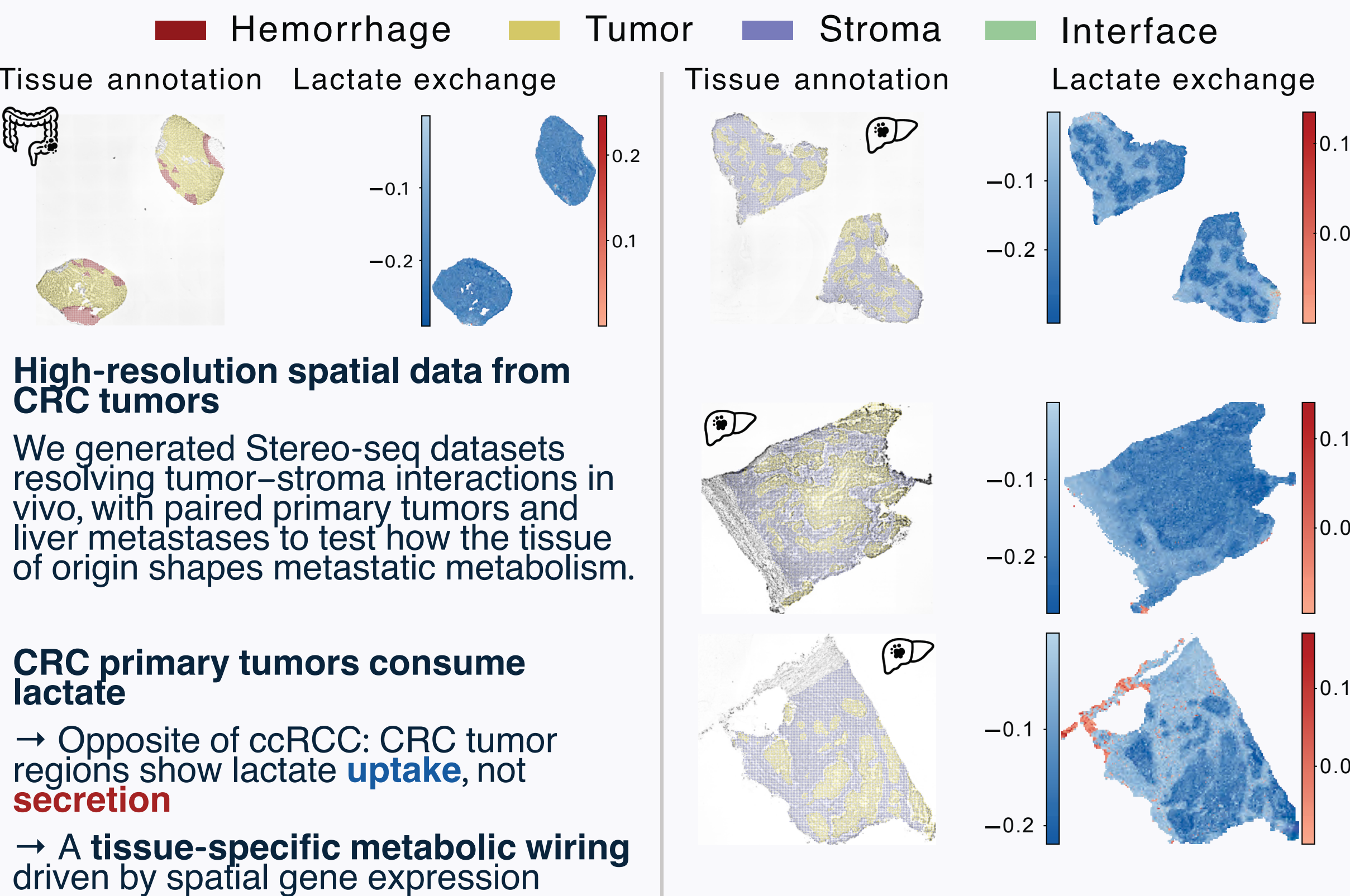
**Warburg effect:**

→ **Higher glucose uptake in tumor** → **Increased lactate secretion**

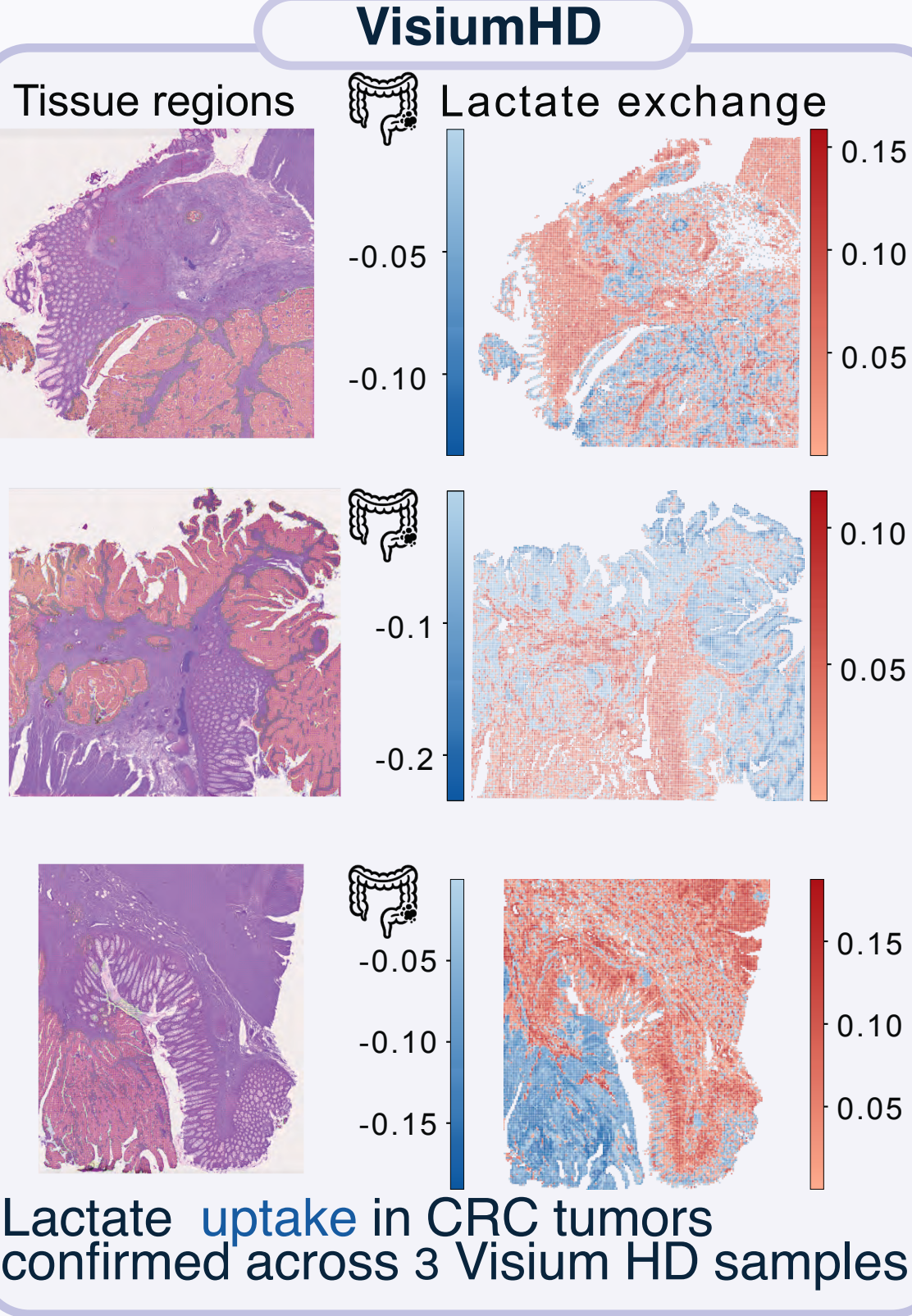
spFBA: Maspero, D., Marteletto, G., Lapi, F. et al. npj Syst Biol Appl 12, 32 (2026)



### Colorectal cancer and liver metastasis



1) Biomass FES correlates with the Proliferation score across samples (Spearman ρ)  
2) O<sub>2</sub> exchange correlates with the Blood vessel score across samples (Spearman ρ)



**Lactate uptake in CRC tumors confirmed across 3 Visium HD samples**

**Liver metastases mimic tissue-of-origin traits**

→ Lactate **uptake** retained in both primary tumors (PT) and liver metastases (LM)

→ Metastatic colonization may depend on **niche metabolite availability**

**First in vivo evidence of the reverse Warburg effect**

→ Tumor cells **consume** lactate

→ Stromal regions **produce** it, sustaining a lactate-rich microenvironment