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Unveiling Spatial and Pathway-Level Metabolic Programs in Cancer Through Transcriptome-Guided Flux Analysis

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Unveiling Spatial and Pathway-Level Metabolic Programs in Cancer Through Transcriptome-Guided Flux Analysis

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COMPUTATIONAL FLUXOMICS

To completely characterize the metabolism of an organism/cell/tissue we need **fluxomics**

$$S + E \rightleftarrows C \rightarrow P + E$$

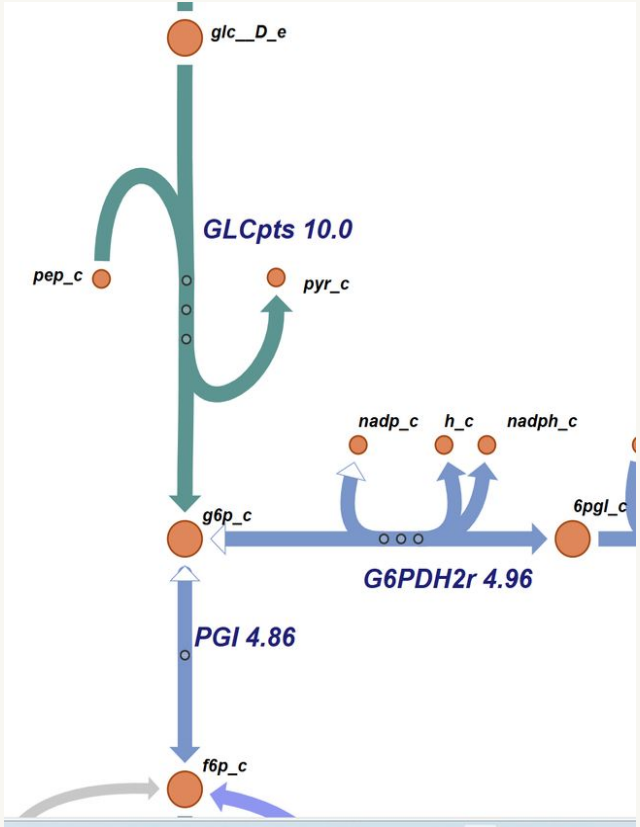
For each reaction: direction, rate (and compartment)

The bottleneck

- Experimental determination of metabolic fluxes (^{13}C isotope tracing) is **complex, costly, not scalable** to hundreds of samples, and **not available** for single-cell/spatial data.
- A single omics (transcriptomics, proteomics, metabolomics) does not tell us **which metabolic reactions are active or at what rate**.

Our approach

Derive metabolic flux distributions from **transcriptomics** (bulk, single-cell, spatial), integrating gene expression into **constraint-based metabolic models (CBM)**.



METABOLIC NETWORKS

A metabolic network is characterized by a **stoichiometric matrix S** encoding all biochemical reactions— each row a metabolite, each column a reaction, each entry the stoichiometric coefficient.

R1: $2A + B \rightarrow C + 3D$

R2: $A + 3B \rightarrow C + E$

R3: $A \rightarrow 2B + D + E$

R4: $4B \rightarrow D + A$

	R1	R2	R3	R4	R5	R6
A	-2	-1	-1	1	0	0
B	-1	-3	2	-4	-2	3
C	1	1	0	0	1	-1
D	3	0	1	1	-1	1
E	0	1	1	0	2	-4

Why a core network?

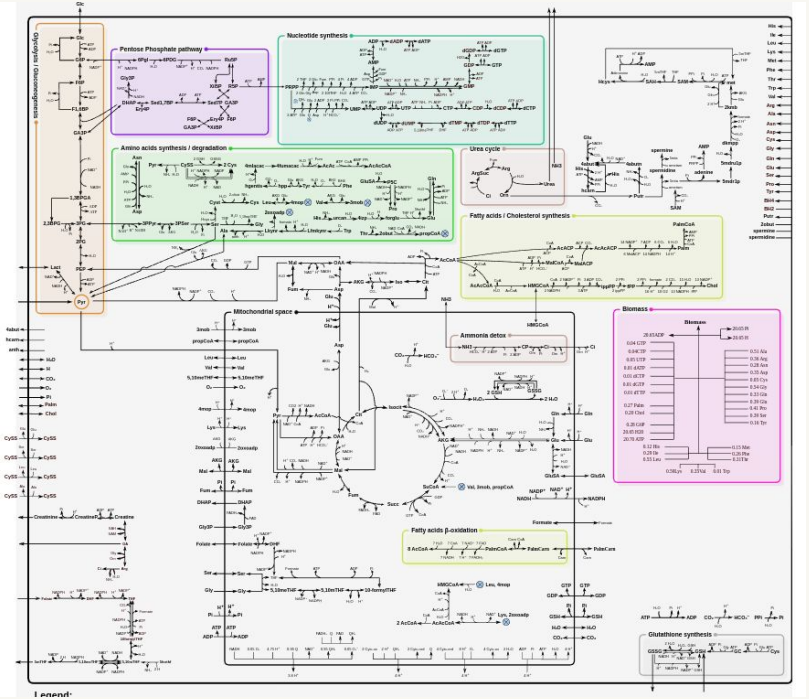
- Genome-scale networks are computationally demanding and hard to interpret.
- A core network like **ENGRO2** focuses on **central carbon metabolism**, energy production and biosynthesis — the reactions most relevant to cancer phenotypes.

RECON3D/Human 2

~10,000 reactions
~2000 genes
all known human metabolism

ENGRO2

~500 reactions
~500 genes
core carbon & energy metabolism



METHODOLOGY

FROM TRANSCRIPTOMICS TO REACTIONS: RAS

GPR rules (Gene–Protein–Reaction)
Each reaction is annotated with a logical rule specifying which genes encode its enzyme.

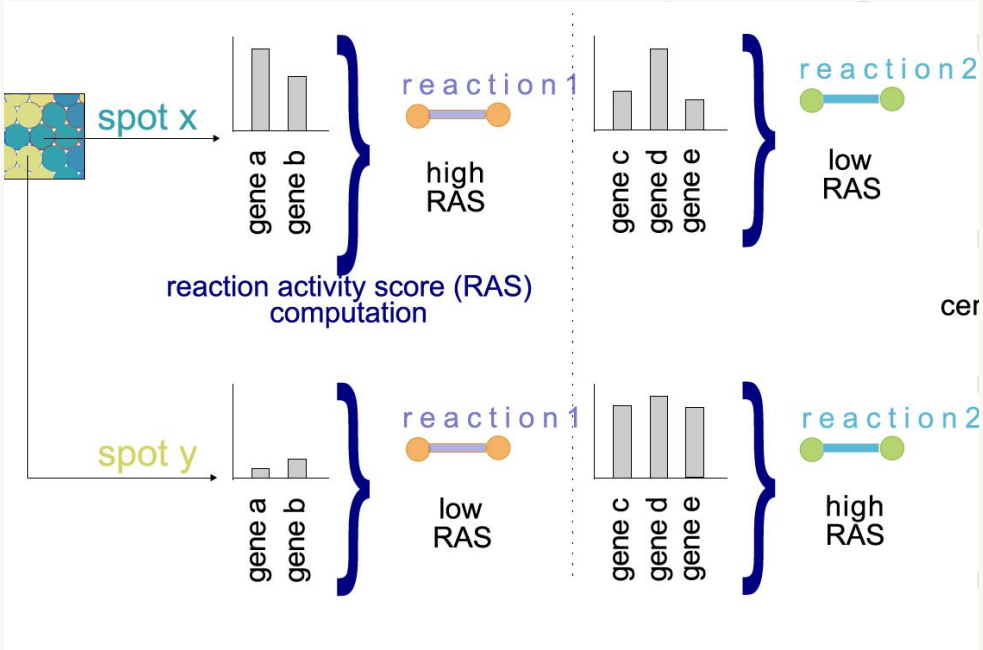
OR logic — isozymes: any isoform can catalyze

LDHA OR LDHB ⟶ RAS = expr(LDHA) + expr(LDHB)

AND logic —complex: all subunits required

NDUFV1 AND NDUFV2 AND NDUFS1 ⟶ RAS = min(expr(…))

Result
Each reaction has a **Reaction Activity Score (RAS)** per sample: a reaction-centric omic layer derived from gene expression.



METHODOLOGY

RELATIVE CONSTRAINTS AND FLUX SAMPLING

The CBM model

The metabolic network is represented as stoichiometric matrix **S**. Assuming steady-state:

Sv = 0

with capacity constraints: v_min ≤ v ≤ v_max

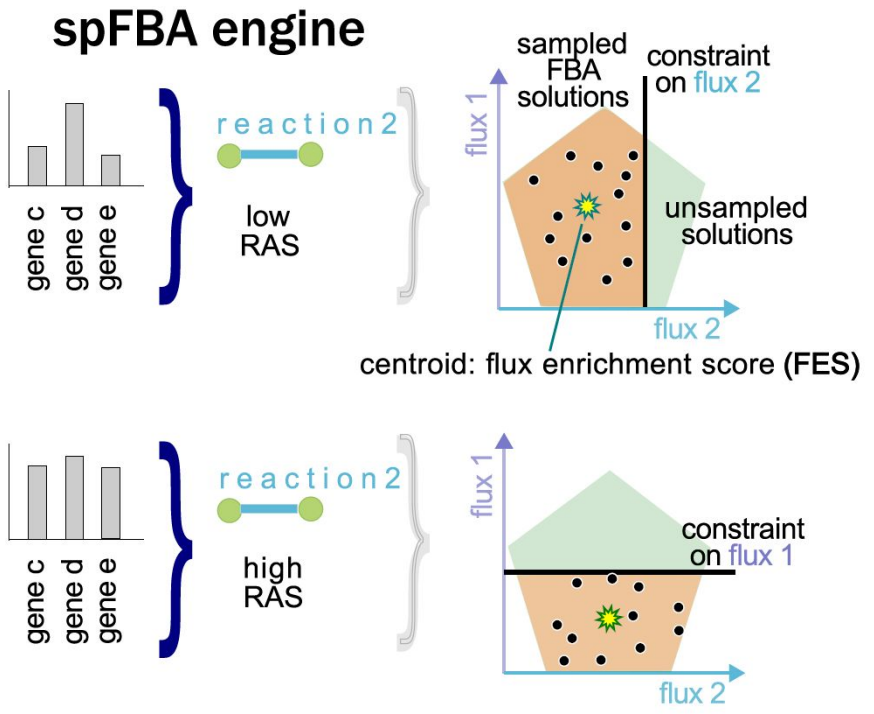
RAS as relative constraints

RAS values proportionally scale the capacity bounds of each reaction —**sample-specific relative bounds** derived from gene expression.

Flux sampling

Rather than optimizing a single objective (the classical FBA), we **sample the entire feasible flux space**.

Output: **flux state distribution** per reaction per sample.



METHODOLOGY

NON-CANONICAL CYCLE IN CANCER

Biological question

Which cancer cell lines rewire the TCA cycle toward aerobic glycolysis — and can this be detected from transcriptomics?

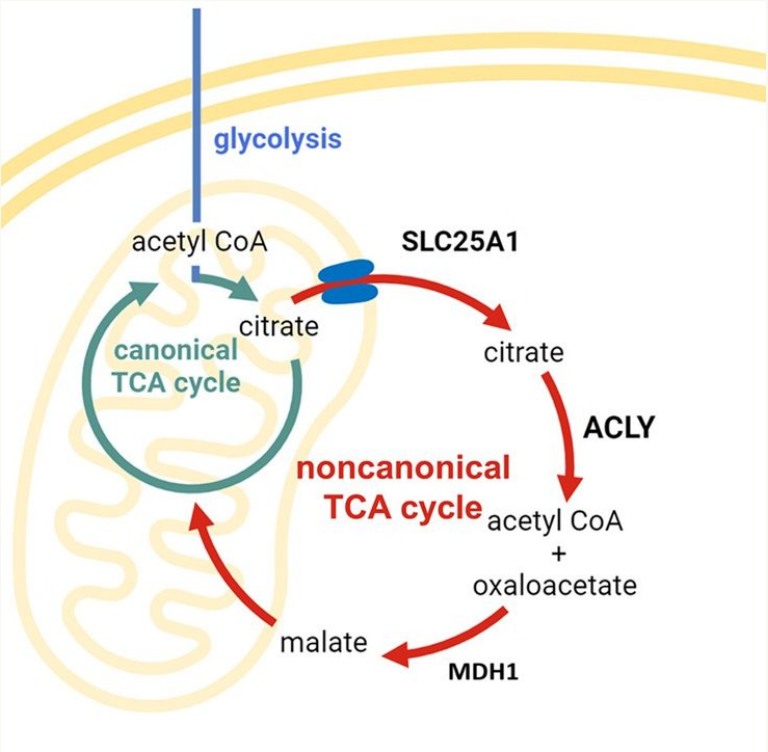
The Citrate–Malate cycle

Citrate exported from mitochondria via **SLC25A1** → cleaved by **ACLY** into OAA + AcCoA → OAA reduced to **malate** by MDH1 → malate re-imported.

This non-canonical loop redirects citrate toward biosynthesis of fatty acids and cholesterol.

Data used

Transcriptomics from **513 cancer cell lines** (24 tumor types)

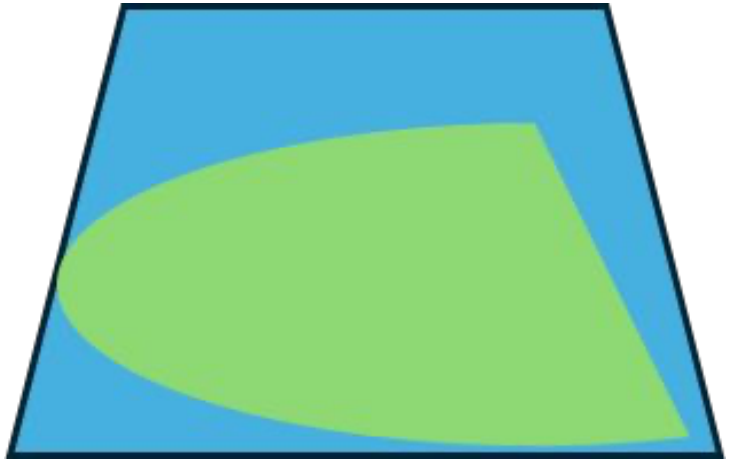


PAPER 1

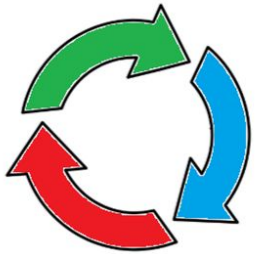
NON-CANONICAL TCA CYCLE QUANTIFICATION

To quantify the activity of the non-canonical TCA cycle, we defined two complementary metrics:

- **Cycle Propensity (CP)** was defined as the fraction of total flux samples having **active fluxes** of non TCA cycle.
- **Cycle Intensity (CI)** measures the average intensity of non TCA cycle conditional on activation.



Active flux



Non active flux

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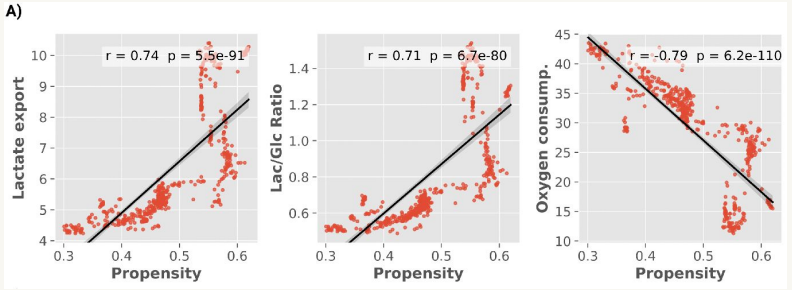
CP: METABOLIC EFFECT AND TRANSCRIPTIONAL LANDSCAPE

Glycolysis correlations

CP co-varies with O_2, lactate and Lac/Glc ratio across all 513 CCLE lines.



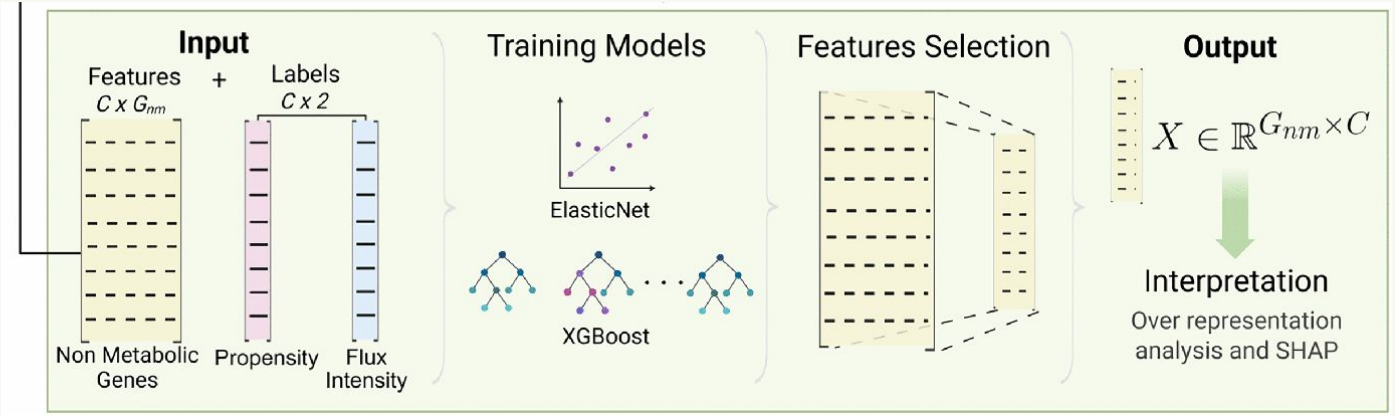
Transcriptional landscape

A regression model (ElasticNet + XGBoost) on(**non metabolic) genes** predicts CP with $\mathbf{R^2 \approx 0.81}$.

ORA reveals enrichment in:

- **EMT**
- **Tumor stemness**
- **Angiogenesis**

…



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THE spFBA WORKFLOW

The spFBA framework takes as input

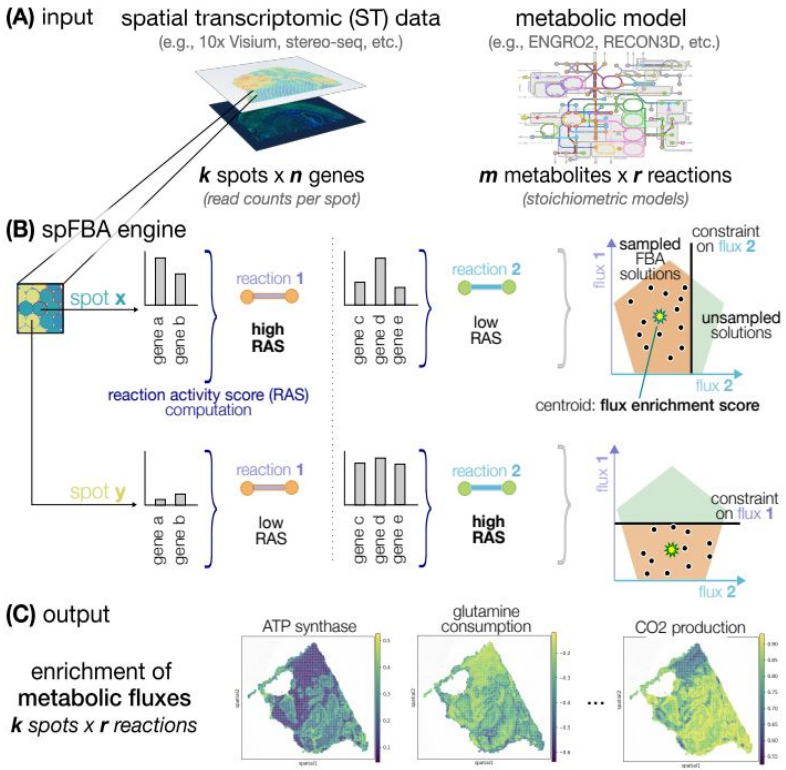
- any **metabolic network** model
- a **spatial transcriptomic** (ST) dataset containing k spatial spots.

The fluxomics for each spot is obtained by **integrating** ST into the network to obtain k metabolic networks.

spFBA outputs k × r **Flux Enrichment Scores**, providing a score for each reaction in each spatial spot.

We tasted the framework on

- a public Clear Cell Renal Cell Carcinoma (ccRCC) dataset
- in-house Colorectal cancer (CRC) dataset



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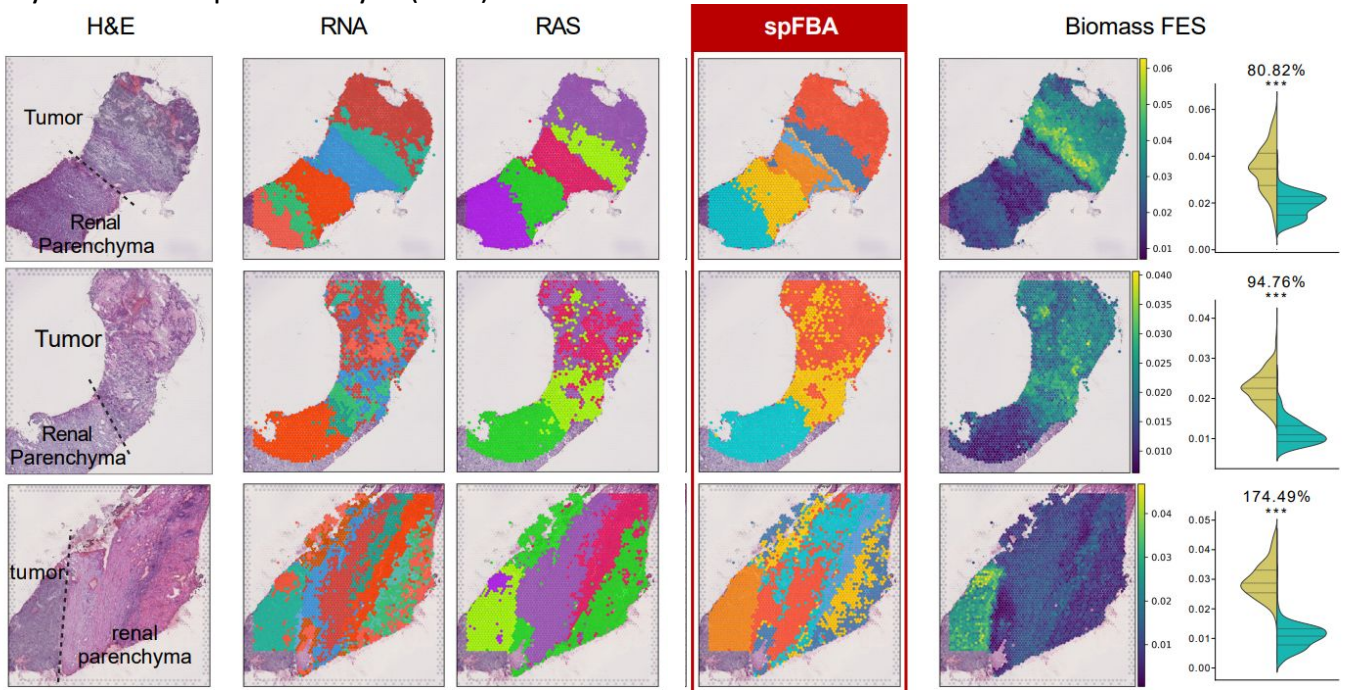
SPFBA WELL RECAPITULATES THE TISSUE ARCHITECTURE

- Tumour regions (Clear Cell Renal Cell Carcinoma) show increased Biomass Flux Enrichment Score (FES)
- Clusters **derived** from spFBA exhibit a coherent spatial distribution that closely aligns with the histological architecture (H&E) revealed also by the transcriptomics layer (RNA)



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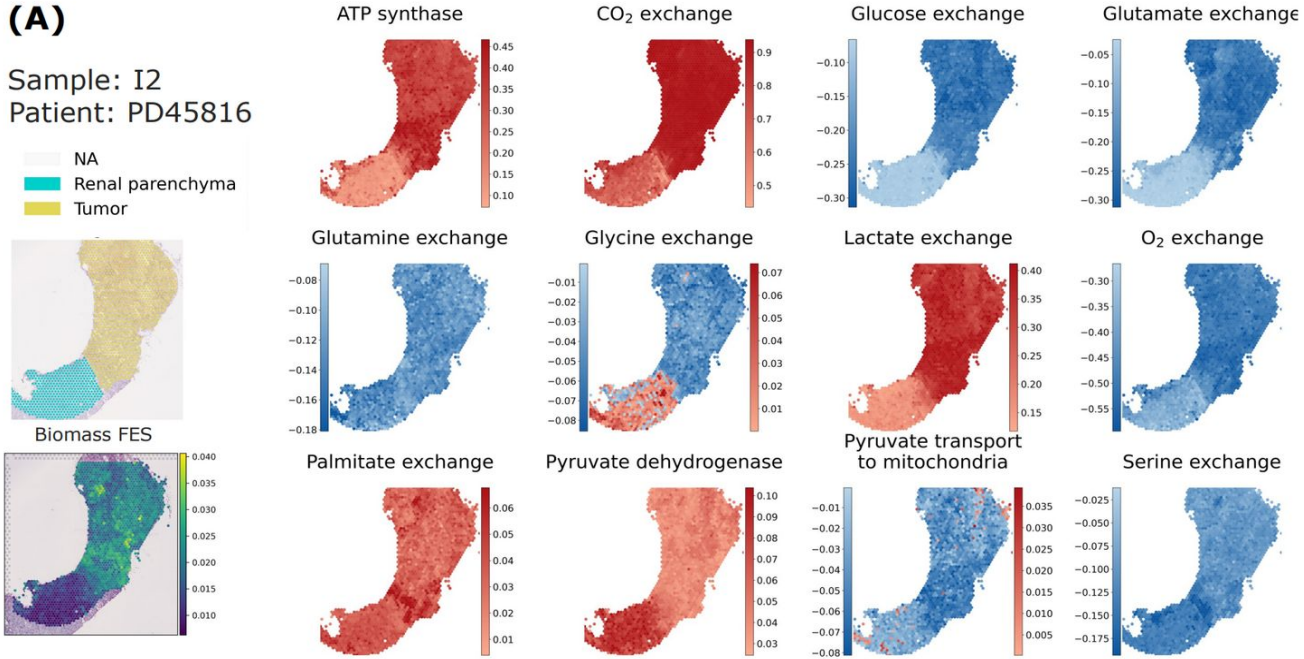
A MAJOR CANCER METABOLIC HALLMARK IS DETECTED

- The proliferation can be associated with the presence of **Warburg effect**.
- Higher glucose uptake and lactate production even in presence of oxygen



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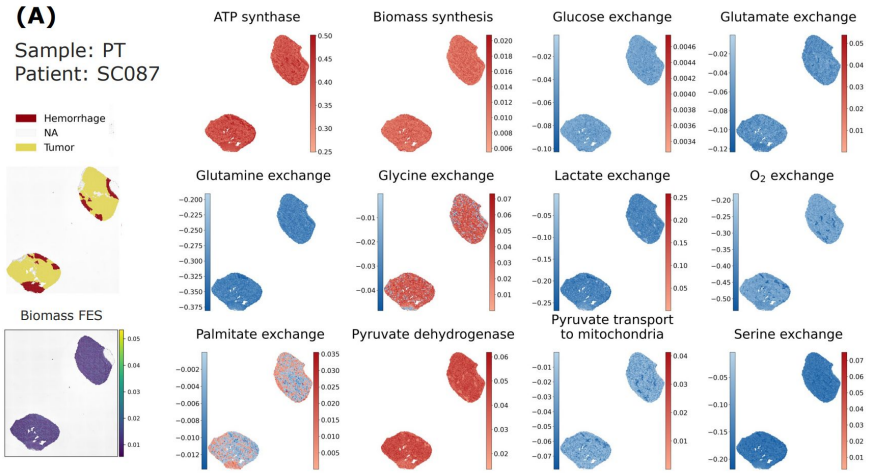
CRC AND METASTASIS METABOLISM

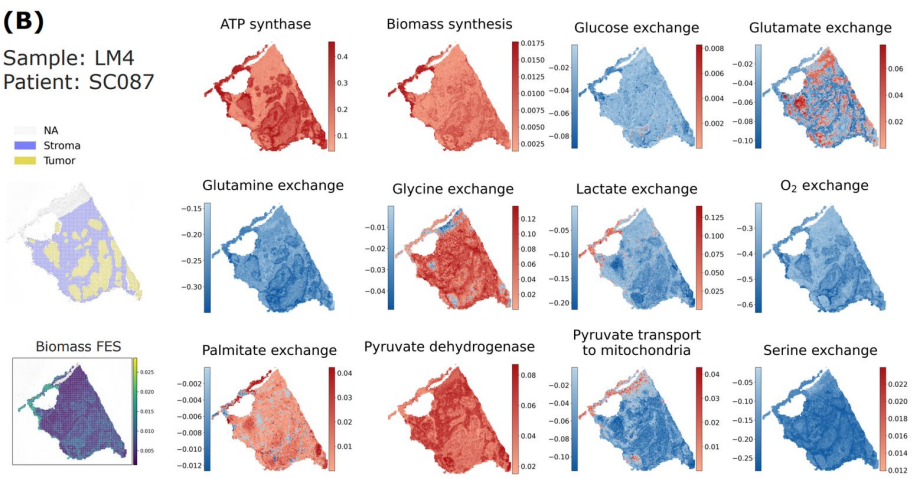
CSB Meeting 2026

- The CRC samples and the liver metastasis show a **lactate consumption**.
- Exogenous lactate could be available from various bacteria able to produce lactate in the colon
- Liver metastasis mimics tissue-of-origin metabolic traits, that can be supported by the pre-existent context of lactate accumulation, where glycolysis of stroma cells supports adjacent cancer cells .

Colon tissue



Liver metastasis



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CONCLUSIONS

- **RAS + flux sampling:** scalable pipeline to derive fluxomics from transcriptomics
- **Cit-Mal cycle:** CP and CFI stratify the 513 CCLE lines; a ML model links non-canonical TCA cycle activity to non metabolic genes
- **spFBA:** extends the RAS + flux sampling framework to spatial transcriptomics, revealing tumor-type-specific metabolic niches and recapitulate tissue architecture from gene expression alone

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