Antioxidants promote metabolic remodeling in cattle rumen epithelium revealed by single-cell resolution

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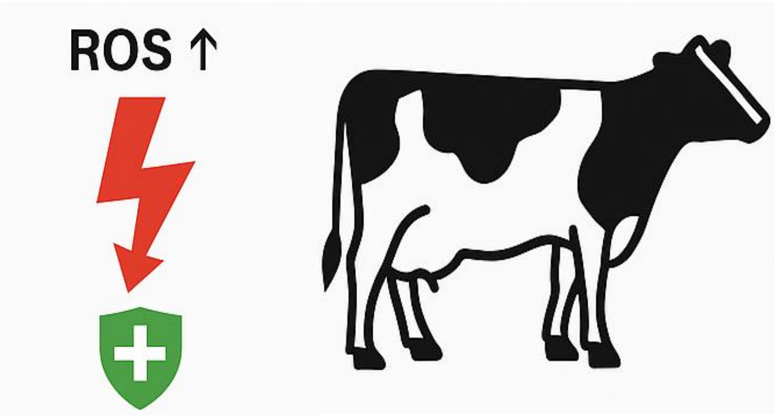
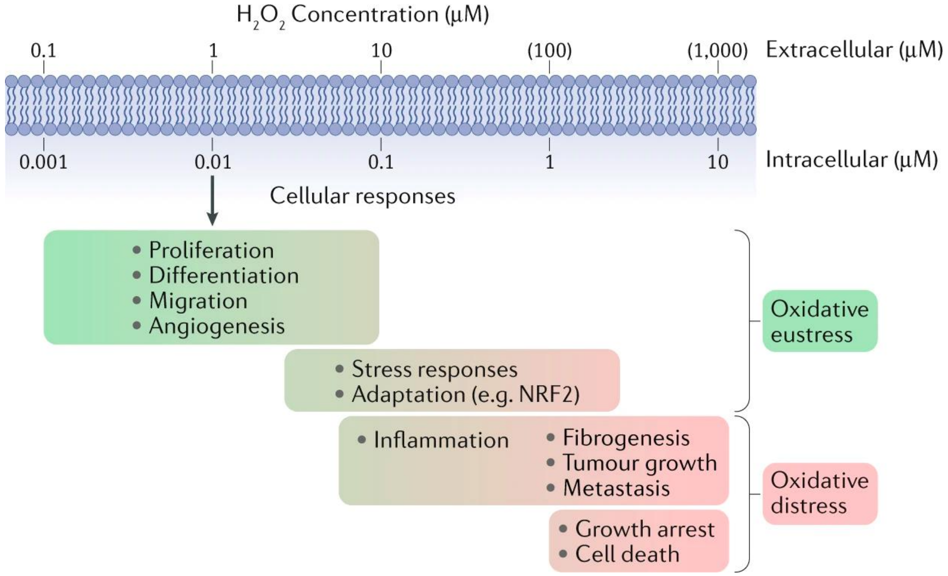
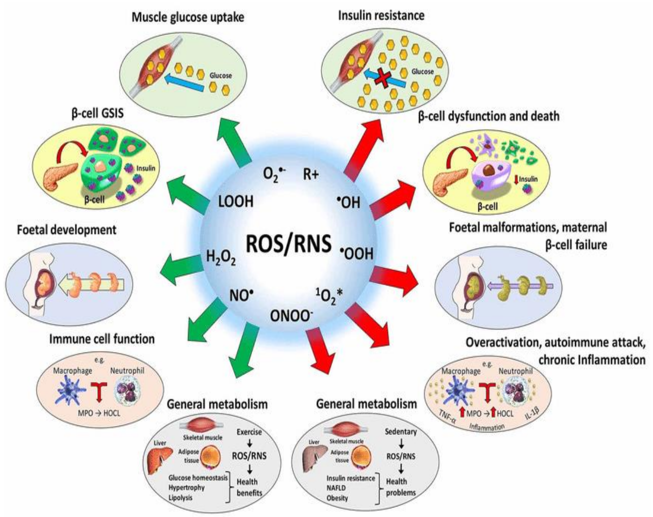
Introduction

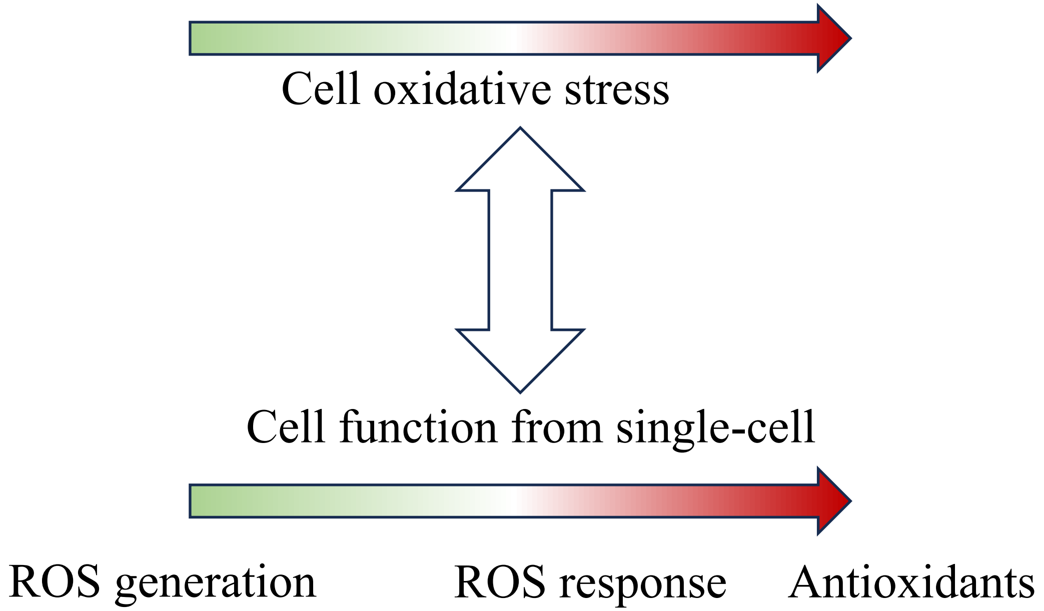




(Sies, H et al., *Nat. Rev. Mol. Cell. Biol.*, 2020)



(Newsholme et al., *Biochem. J.*, 2016)



Cell oxidative stress

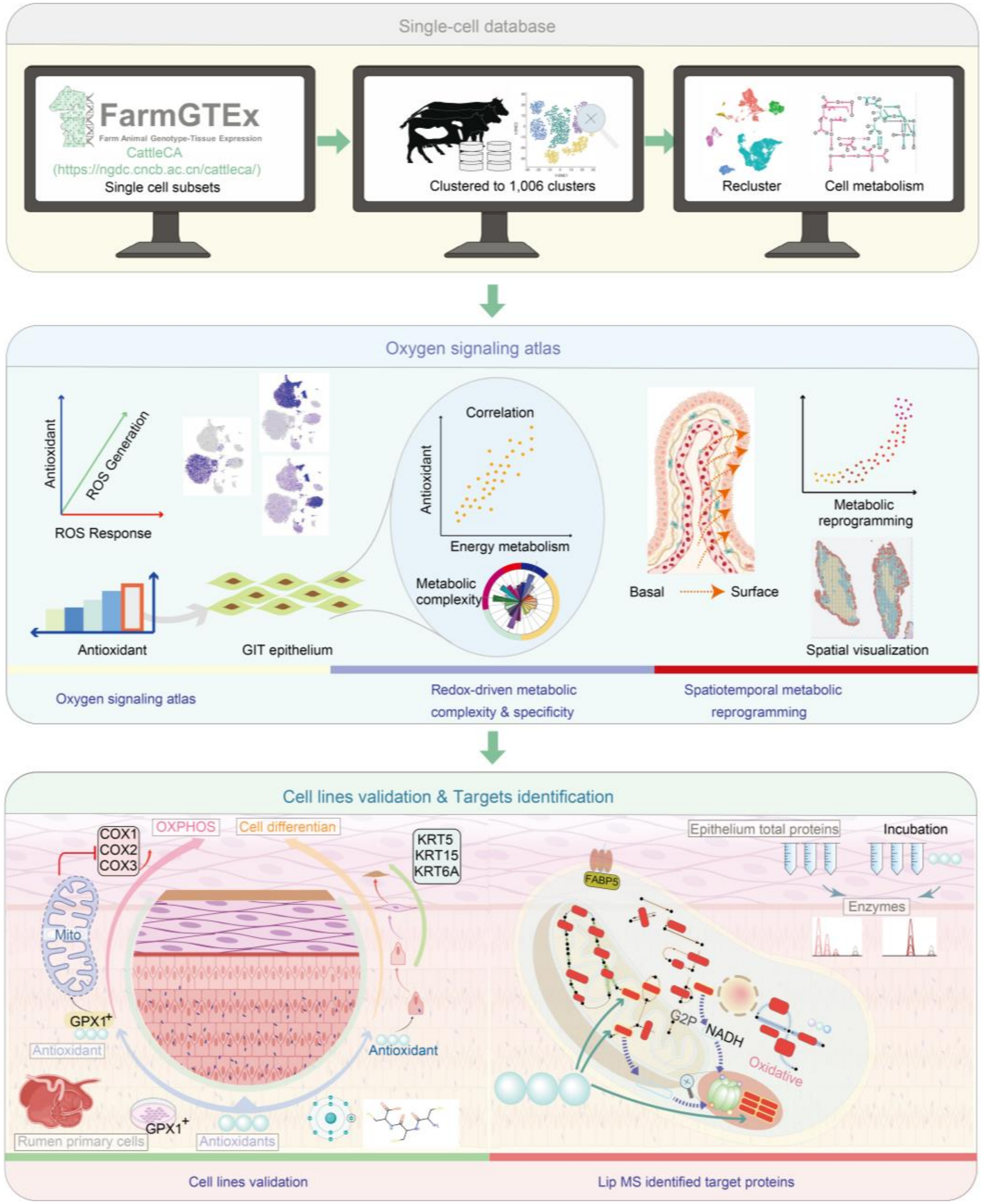
Cell function from single-cell

ROS generation

ROS response

Antioxidants

Highlights

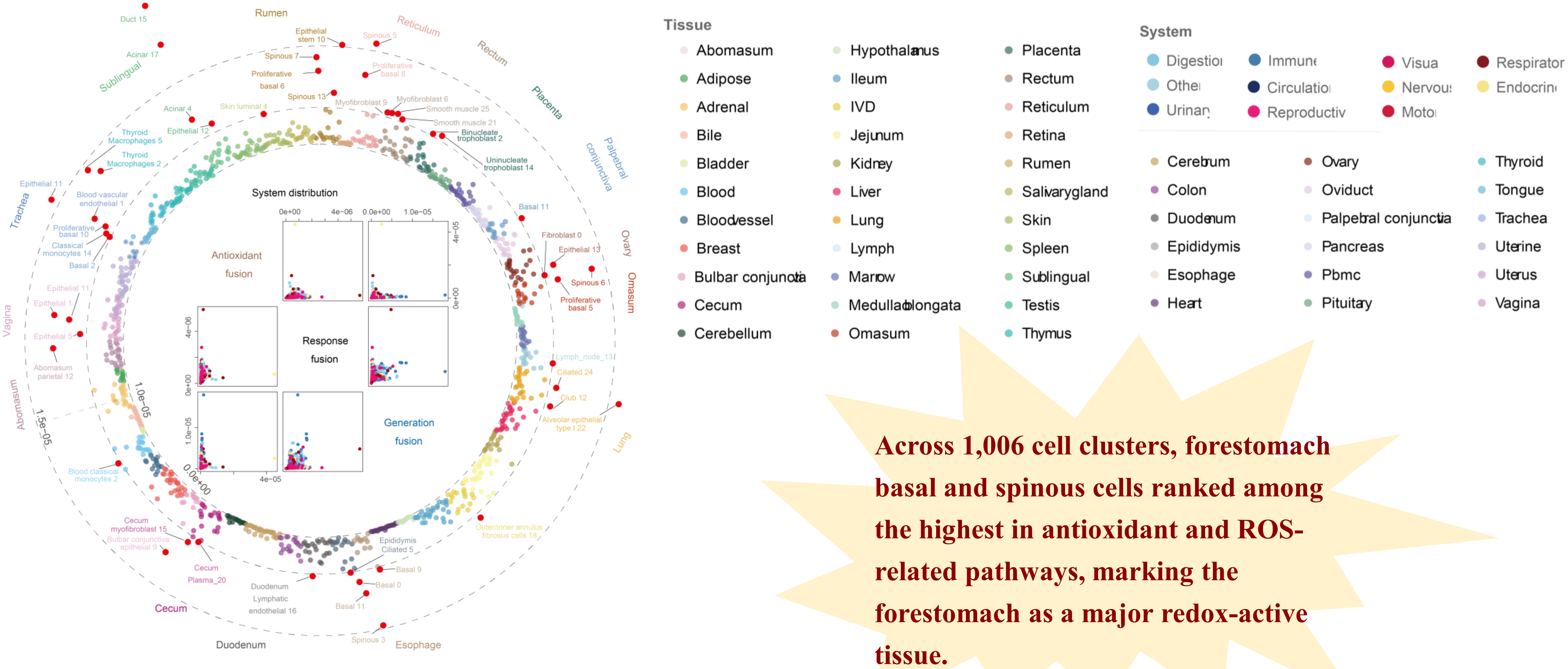


- **A single-cell transcriptomic atlas of 1.79 million bovine cells revealed the forestomach epithelium as one of the most antioxidant-active tissues.**

- **As forestomach epithelium differentiate from the basal layer towards the luminal surface, both mitochondrial respiration and antioxidant capacity progressively intensify, establishing a coupled metabolic–redox gradient.**

- **Functional assays identified GPX1 as key antioxidant target mediating OXPHOS and cell differentiation.**

A single-cell oxygen signaling atlas in dairy-cow



Across 1,006 cell clusters, forestomach basal and spinous cells ranked among the highest in antioxidant and ROS-related pathways, marking the forestomach as a major redox-active tissue.

Figure 1. Oxygen signaling mapping based on the CattleCA.

Oxygen signaling atlas in forestomach epithelium

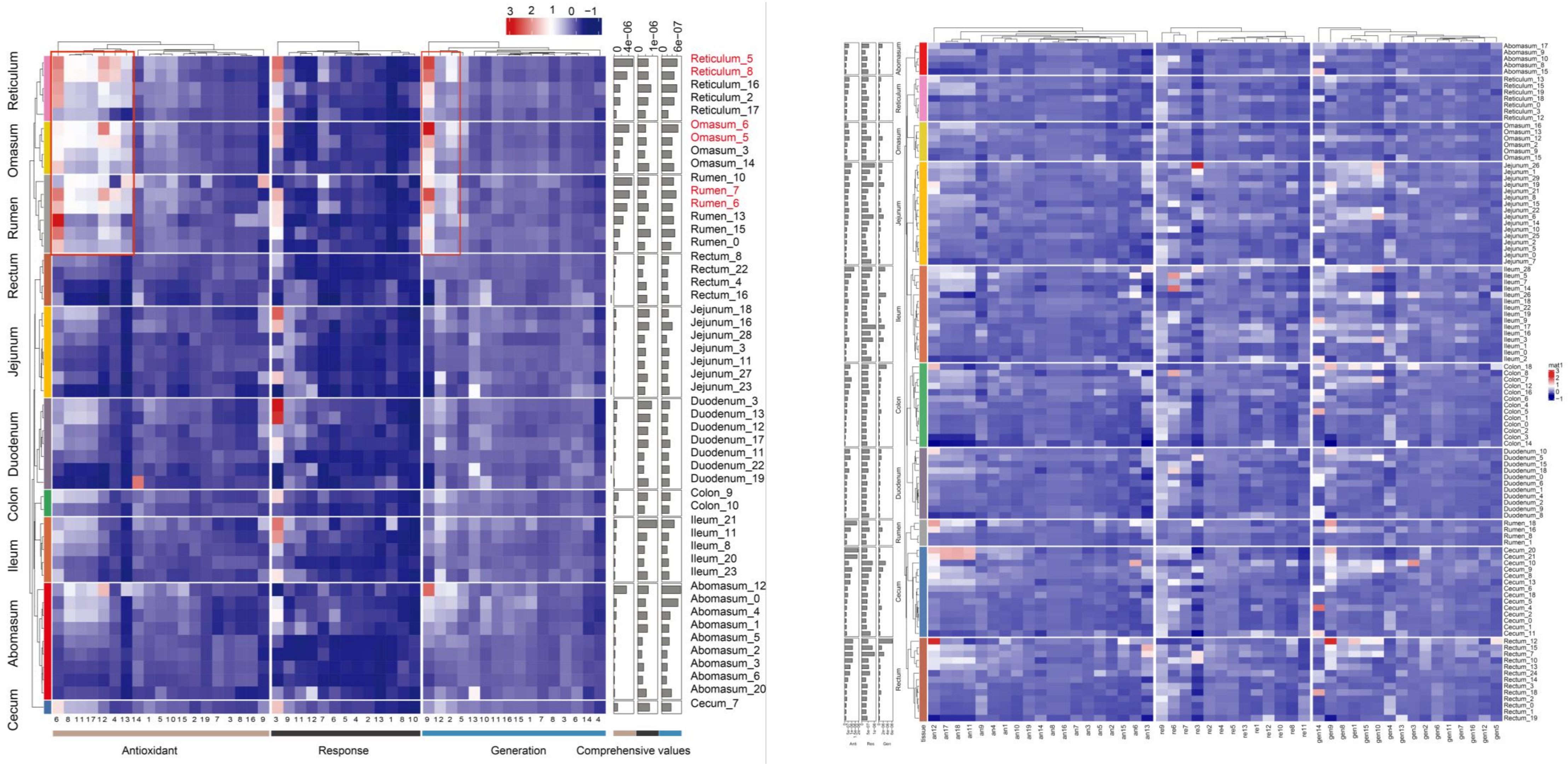


Figure 2A. Heatmap of Antioxidant, ROS Generation, and Response pathway scores across (gastrointestinal tract) GIT epithelial cell types.

Correlation between antioxidant and energy metabolism

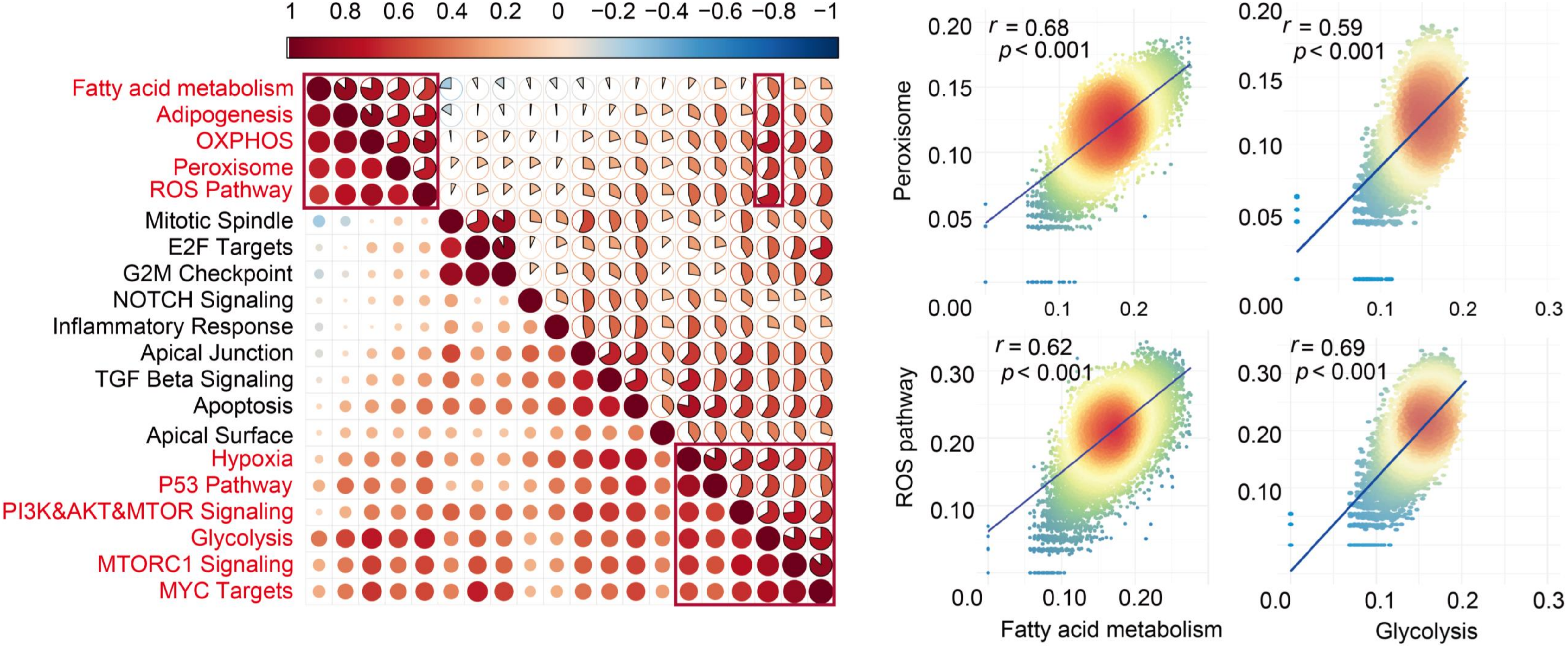
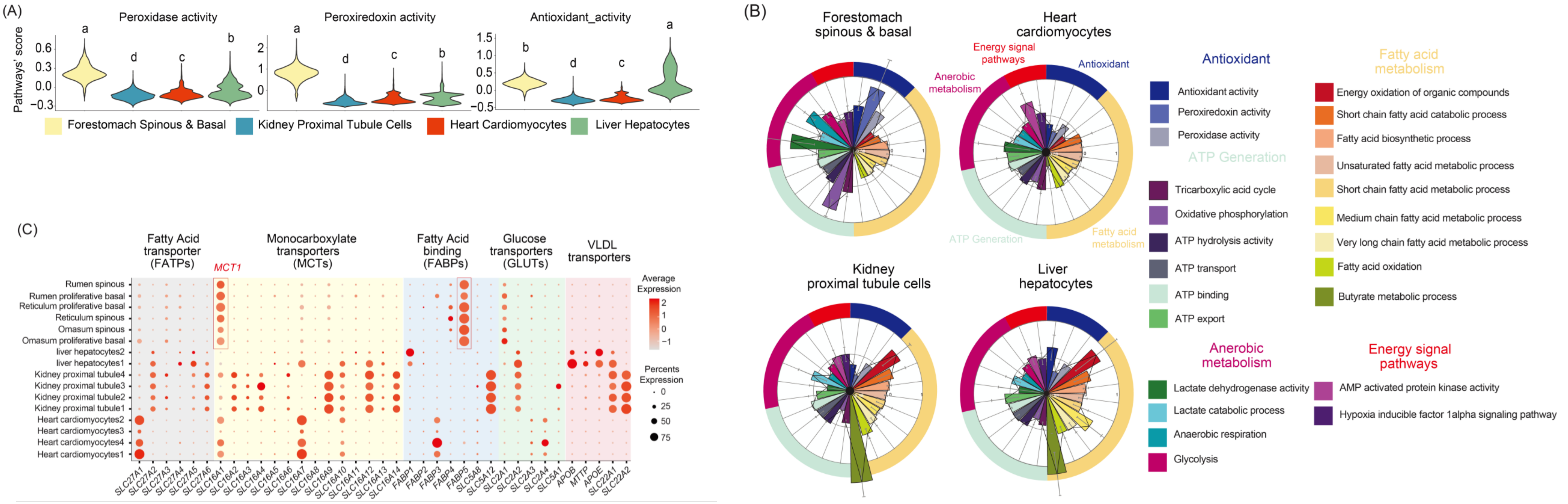


Figure 2B, C. Oxygen signaling landscape and correlation between antioxidant activity and energy metabolism in forestomach epithelium.

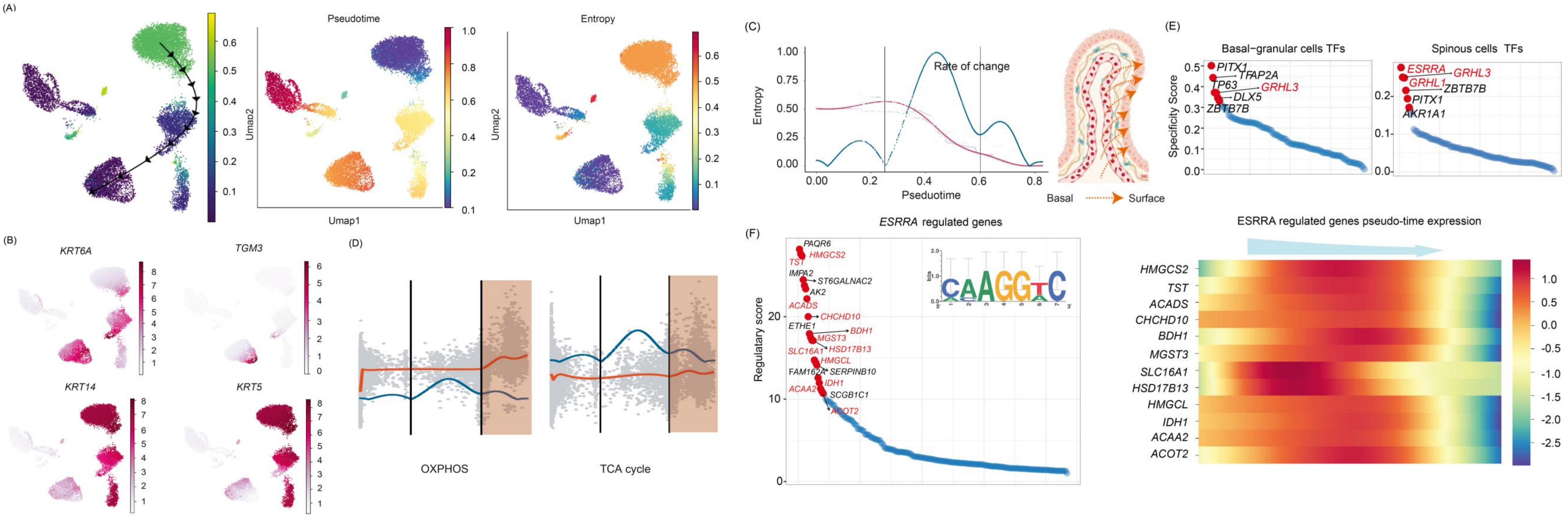
Metabolic heterogeneity between cell types



- **Forestomach epithelium displays the strongest antioxidant defenses and metabolic activity among major high-energy cell types.**

Figure 3. Metabolic landscape heterogeneity within high energy-consuming cell types.

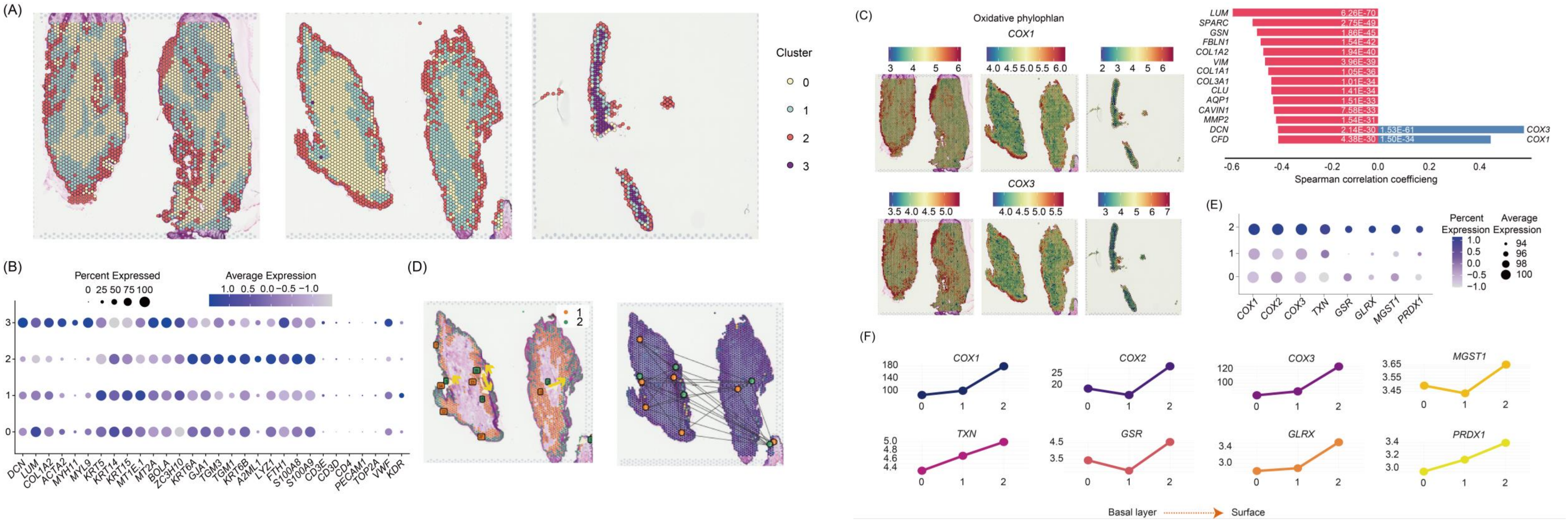
Metabolic remodeling during epithelial differentiation



- **Rumen epithelial differentiation involves a coordinated rise in mitochondrial and lipid metabolism driven by key transcription factors.**

Figure 4. Pseudotime analysis reveal metabolic remodeling in cattle forestomach epithelium.

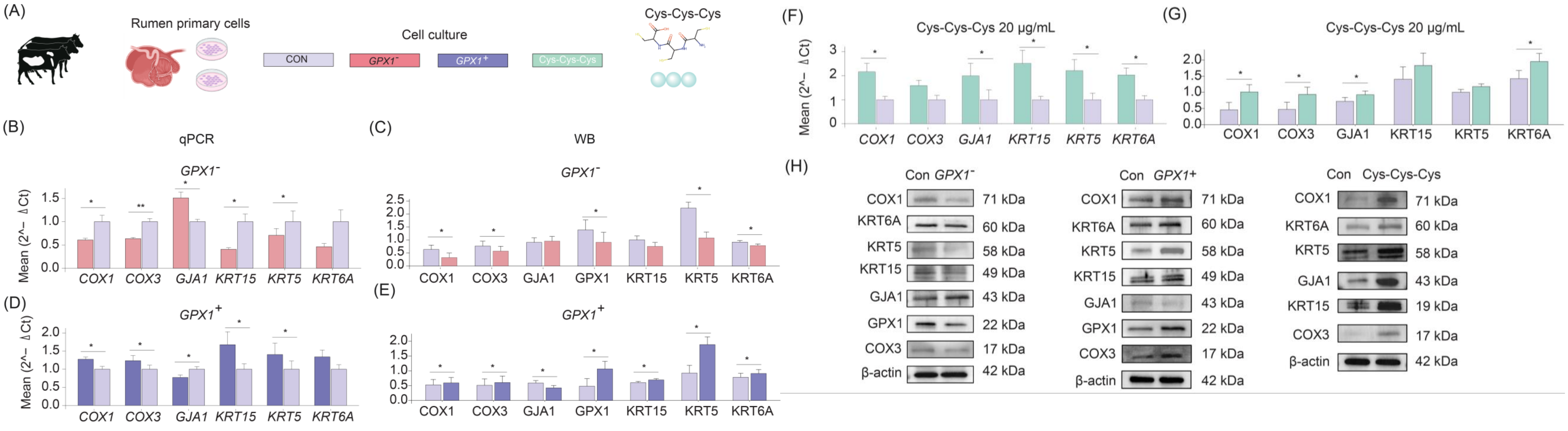
Spatial transcriptomics of rumen papillae



- **Spatial transcriptomics revealed clear basal-to-lumen metabolic gradients in the rumen epithelium, with OXPHOS and antioxidant pathways strongly upregulated in the outer differentiated layers.**

Figure 5. Metabolic remodeling and spatial heterogeneity of rumen cells uncovered through spatial transcriptomics

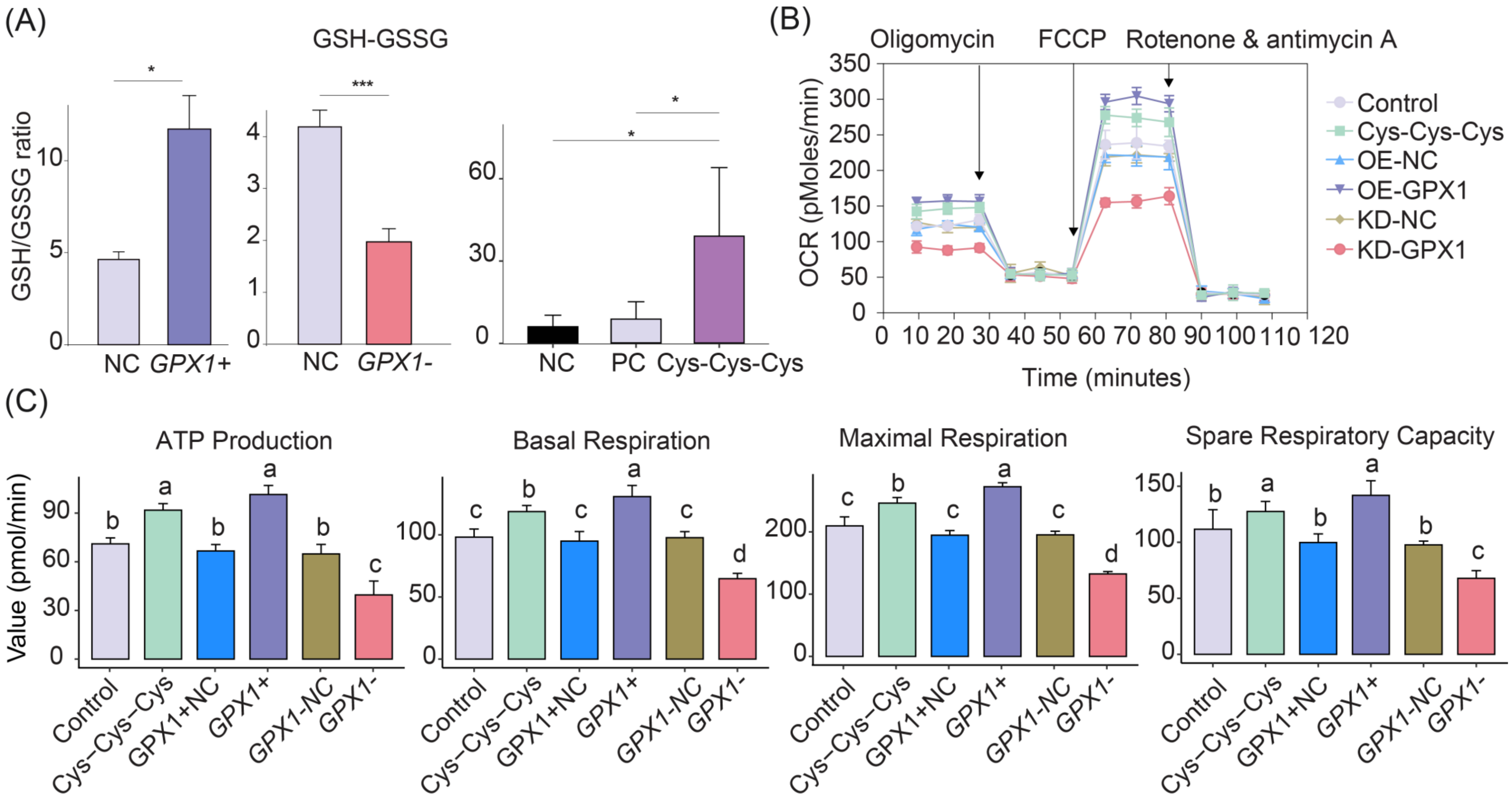
Cell assays validation



- **Antioxidant activation—through *GPX1* modulation or Cys-Cys-Cys treatment—drives coordinated increases in OXPHOS gene expression and epithelial differentiation in rumen cells.**

Figure 6. Antioxidant supports metabolic remodeling and differentiation in rumen epithelial cells.

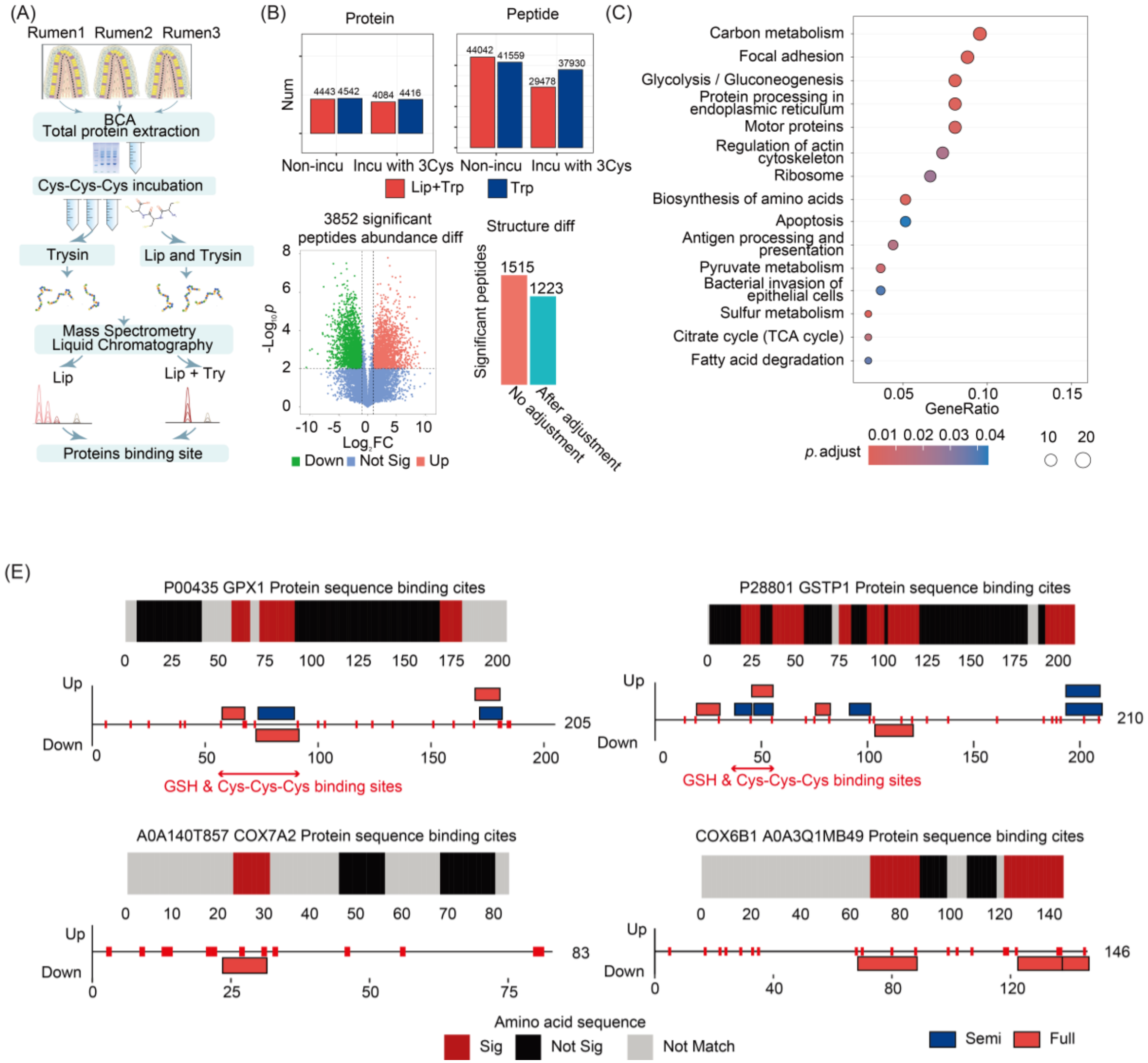
Antioxidant-driven functional phenotypes

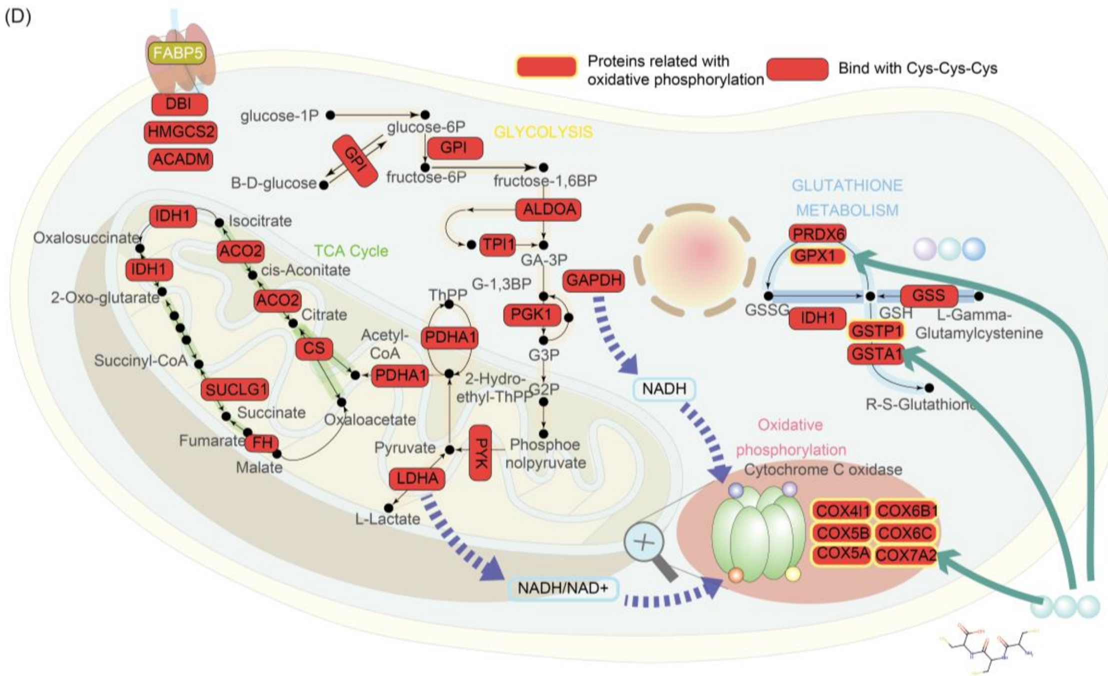


- **Antioxidant enhancement—via GPX1 overexpression or Cys-Cys-Cys—improves redox balance and mitochondrial respiration, while GPX1 knockdown impairs both, demonstrating that antioxidants directly support OXPHOS and epithelial maturation.**

Figure 7. Antioxidant modulation alters redox balance and mitochondrial respiration in rumen epithelial cells.

LiP-MS identifies Cys–Cys–Cys targets





- **LiP-MS reveals that the microbiota-derived antioxidant Cys–Cys–Cys interacts with a broad set of metabolic and redox-regulating proteins, linking antioxidant activity to OXPHOS and TCA cycle remodeling.**

Figure 8. Limited Proteolysis-Mass Spectrometry (Lip-MS) analysis reveals Cys-Cys-Cys interaction with rumen epithelial proteins.

Summary

❑ We constructed a cross-tissue redox signaling atlas encompassing approximately 1.8 million bovine cells, revealing the forestomach epithelium as a key tissue for antioxidant metabolism.

❑ Single-cell, spatial transcriptomic, and pseudotime analyses demonstrated a coordinated basal-to-luminal gradient of antioxidant activation, metabolic remodeling, and epithelial differentiation within the forestomach epithelium.

❑ Functional experiments showed that modulation of GPX1 or supplementation with the microbiota-derived tripeptide Cys–Cys–Cys enhances antioxidant capacity, oxidative phosphorylation (OXPHOS) activity, and epithelial differentiation.

❑ LiP-MS further identified GPX1 as a potential molecular target of Cys–Cys–Cys.

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