



# Single-microbe RNA sequencing uncovers unexplored specialized metabolic functions of keystone species in the human gut

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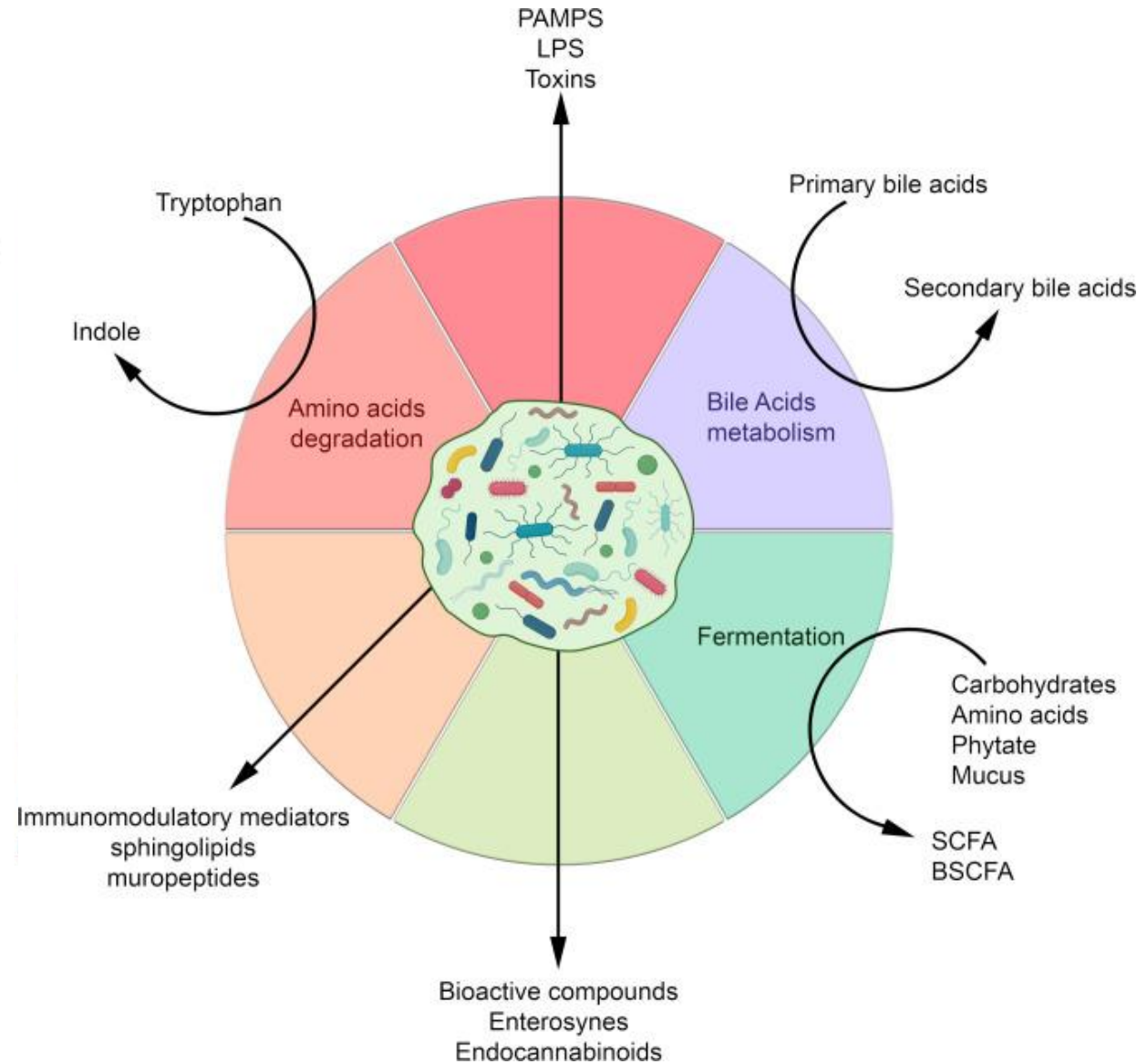
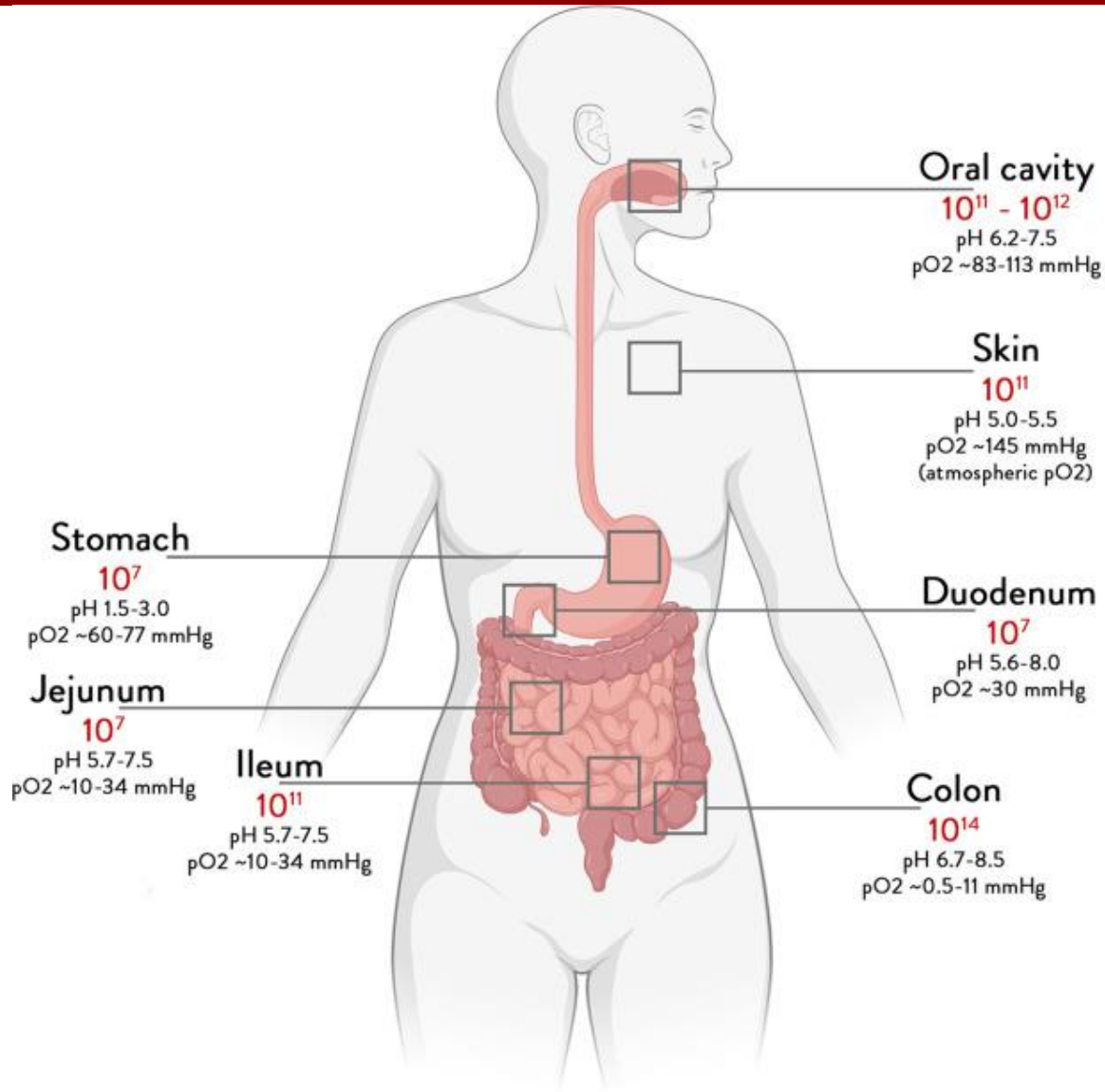


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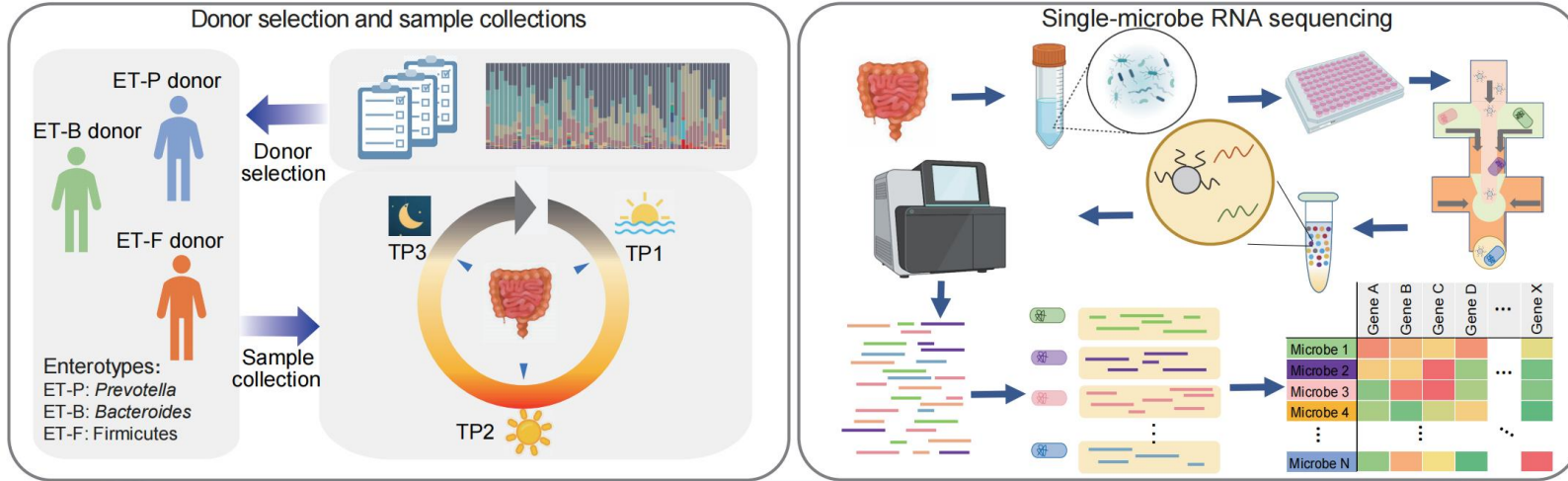


# Introduction

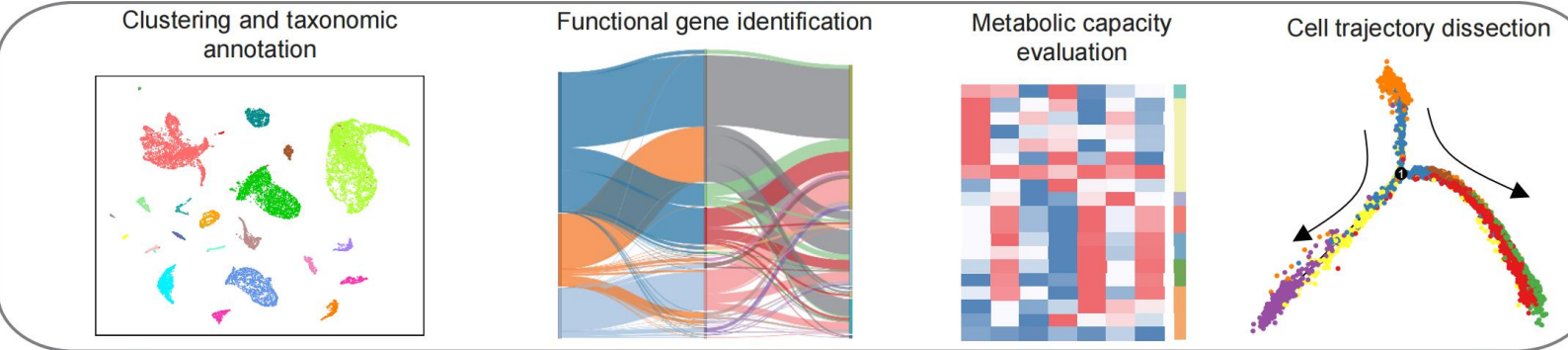




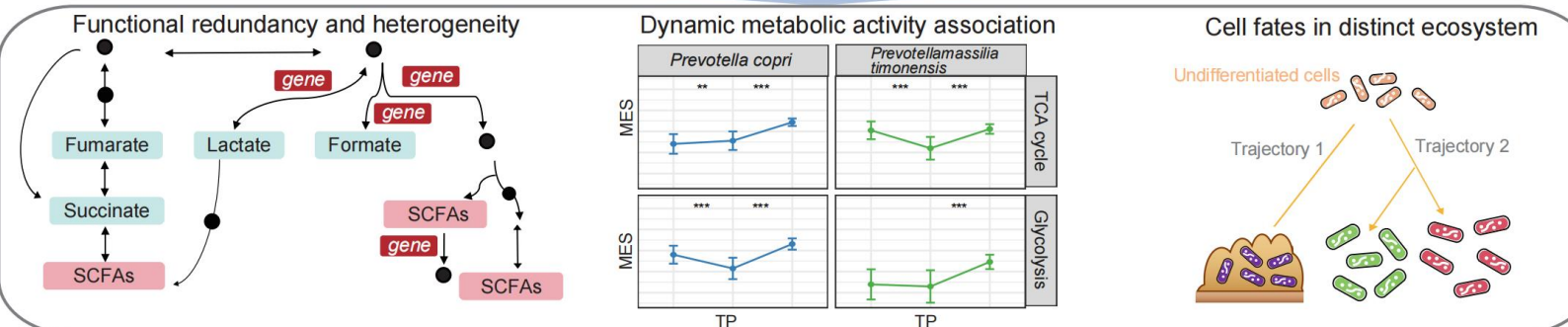
# Highlight



## Integrated analysis



## Species-function characterization



- Apply a novel gut-specific single-microbe RNA sequencing and analytical framework on three healthy donors with distinct enterotypes.
- Identified diurnal dynamic activities and metabolic heterogeneity involved in central carbon metabolism in the gut microbiome.
- Demonstrated *Megamonas funiformis*, a keystone species in Asian populations, can effectively improve mineral absorption through exogenous phytic acid degradation.

# Experimental design and single-microbe RNA sequencing of the human gut microbiome

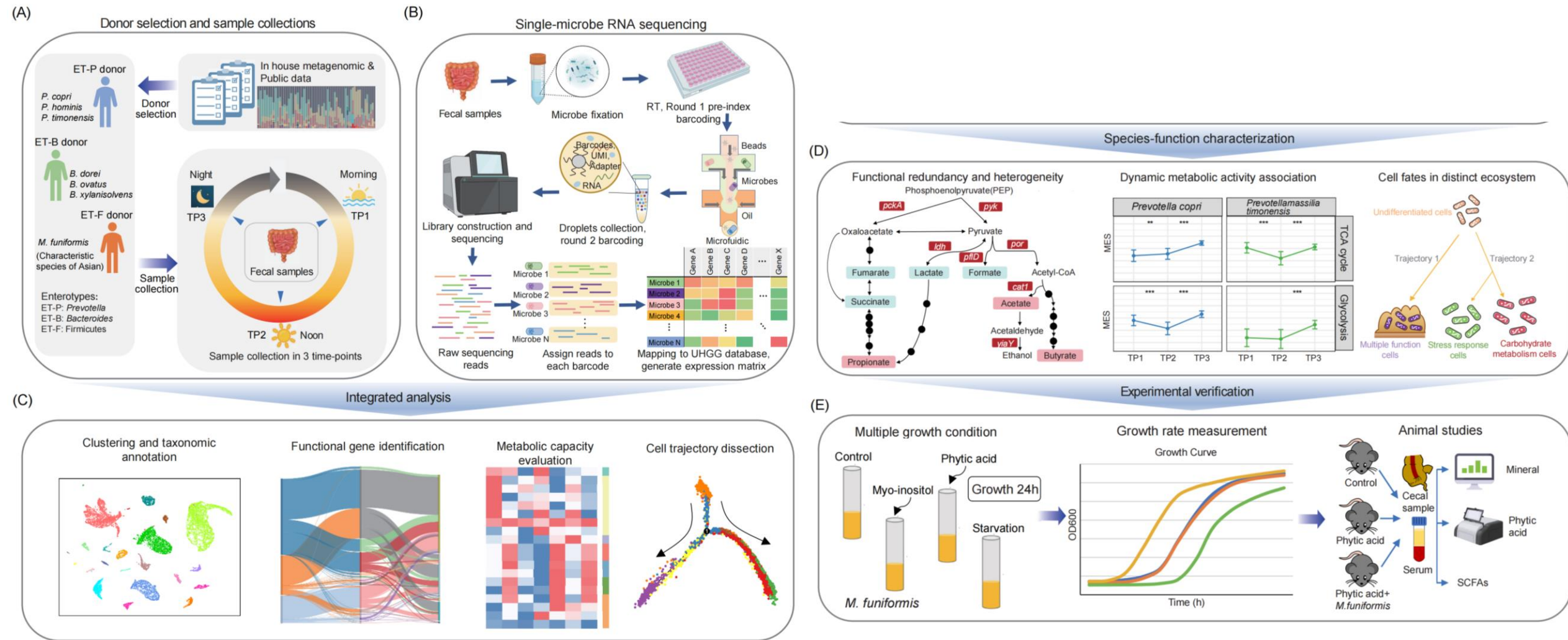


Figure 1 Overview of the experiment design and single-microbe ribonucleic acid (RNA) sequencing of the human gut microbiome.

# Single-microbe transcriptional landscape reveals species-specific functional characterizations in the human gut

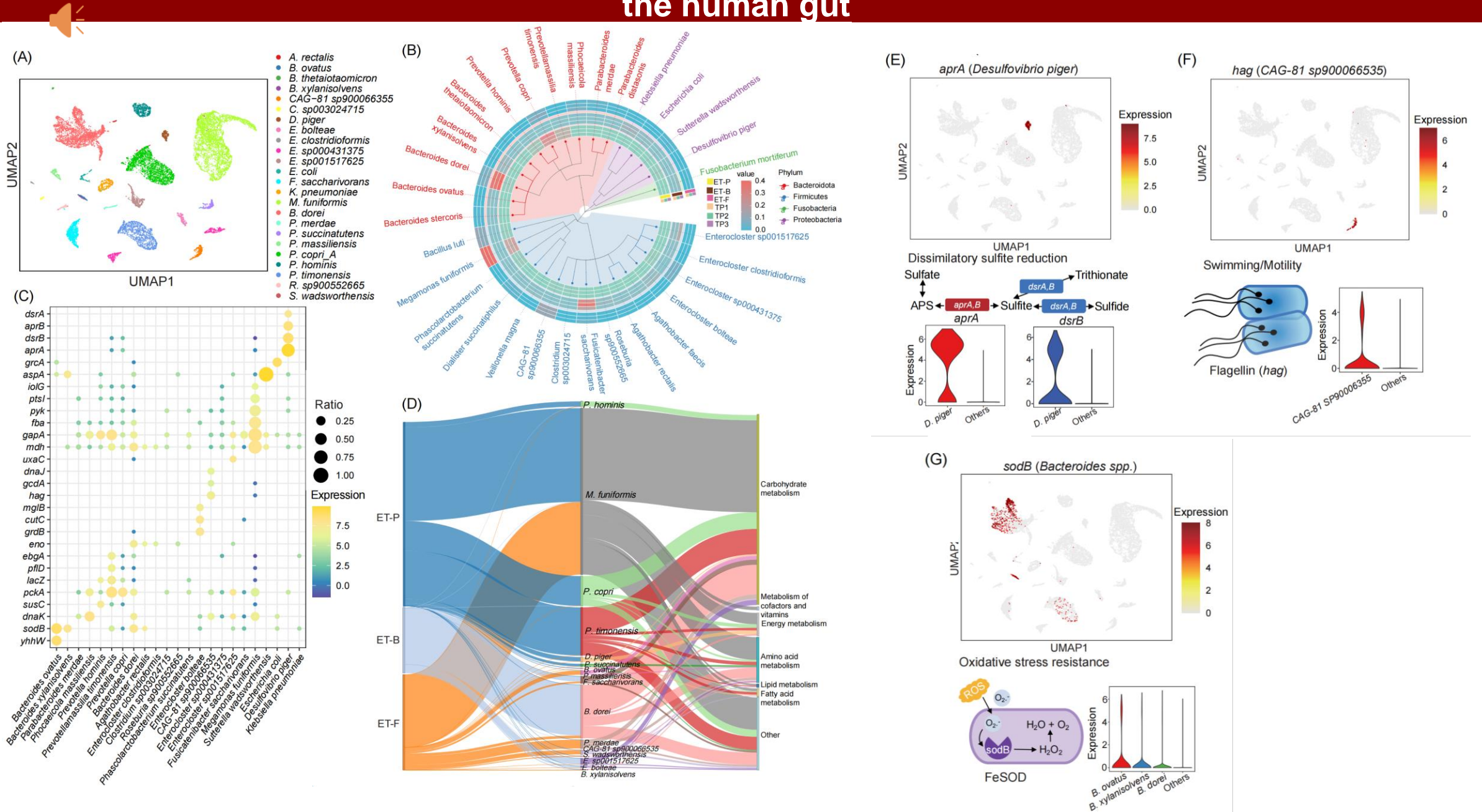


Figure 2 Single-microbe transcriptional landscape of the human gut and the species-specific functional characterizations.

# Functional redundancy and complementarity of SCFAs related central carbon metabolism

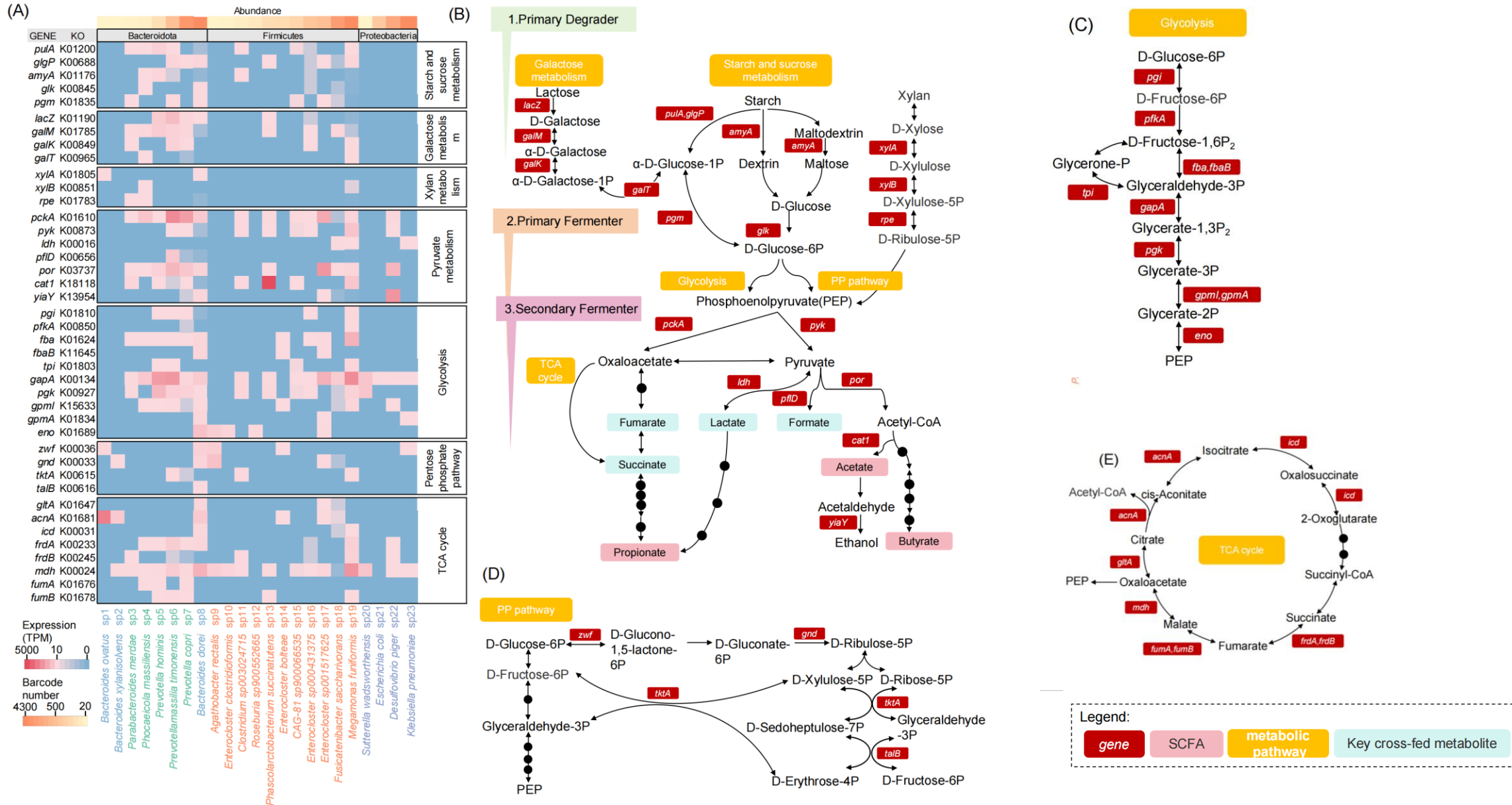
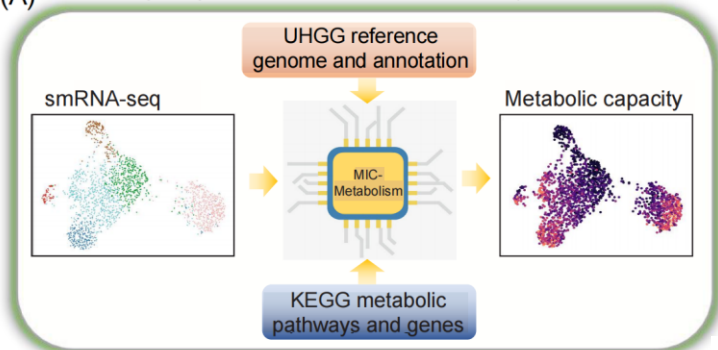


Figure 3 Functional redundancy and complementarity of short-chain fatty acids (SCFAs) related central carbon metabolism.

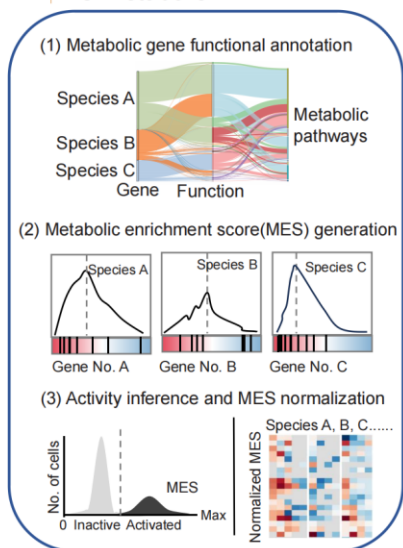


# Heterogeneity and covariations of single-microbe metabolic functions in the human gut

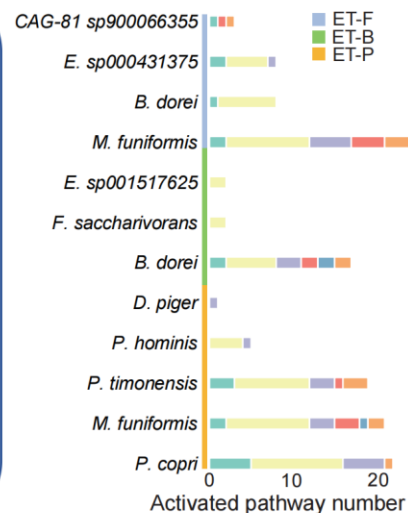
## (A) Inferring single-microbe metabolic capacity



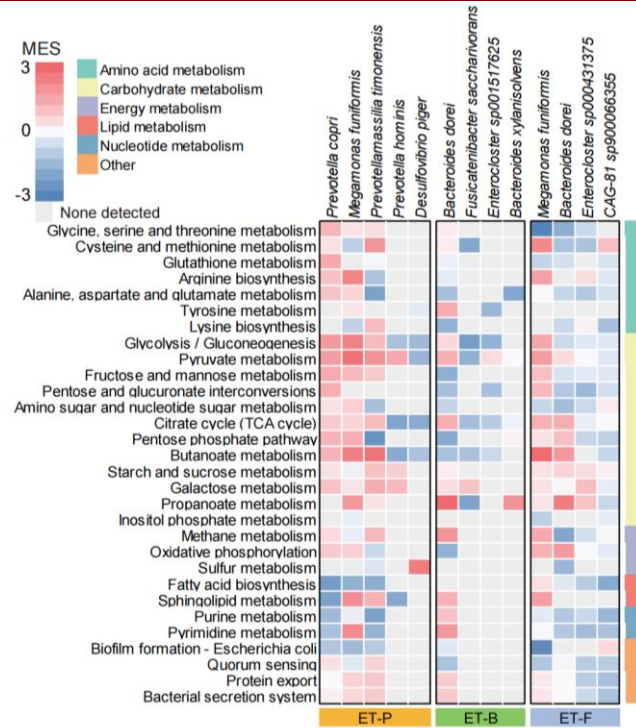
## (B) MIC-Metabolism



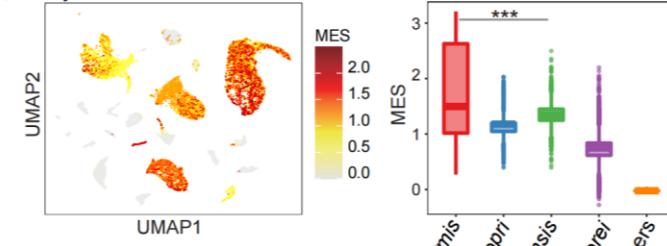
## (C)



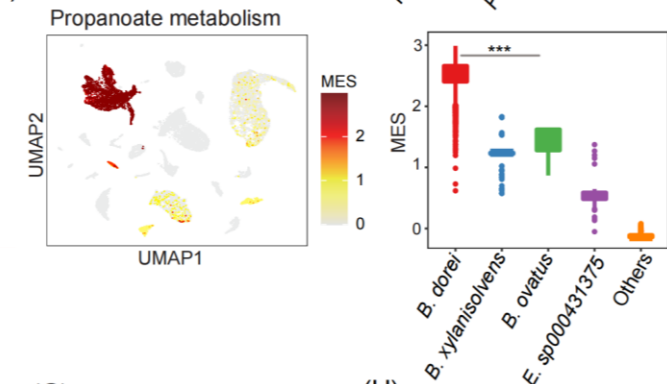
## (D)



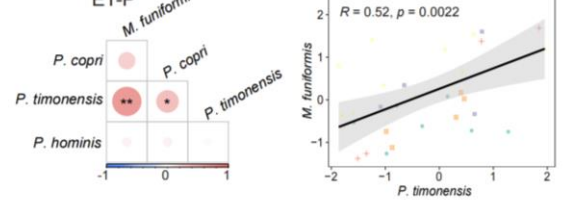
## (E) Pyruvate metabolism



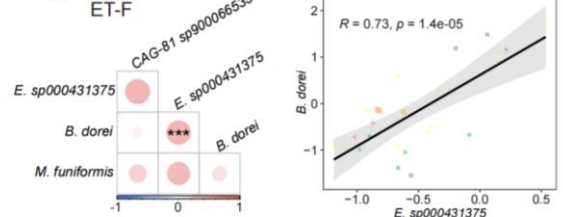
## (F) Propanoate metabolism



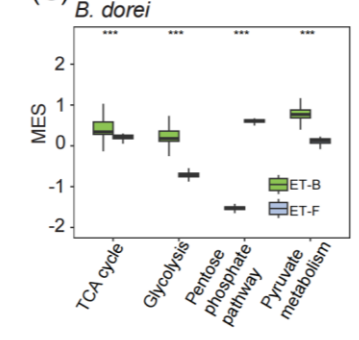
## (I) ET-P



## (J) ET-F



## (G) B. dorei



## (H) M. funiformis

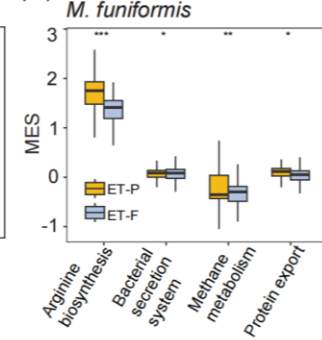


Figure 4 Heterogeneity and covariations of single-microbe metabolic functions in the human gut.



# Diurnal dynamic activities associated with sub-population heterogeneous

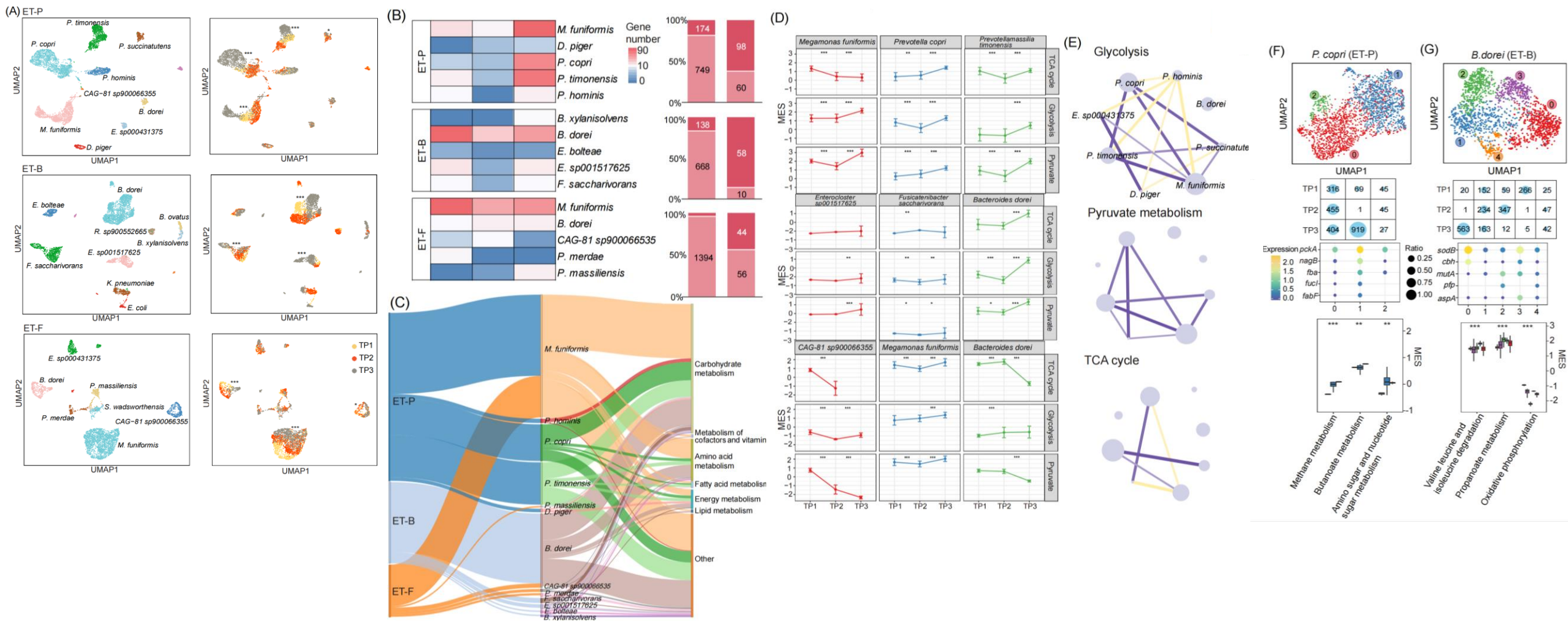


Figure 5 Single-microbe RNA sequencing captures the diurnal dynamic activities of the human gut microbiome.

# Cell state transitions of *Megamonas funiformis* in distinct colon ecosystems

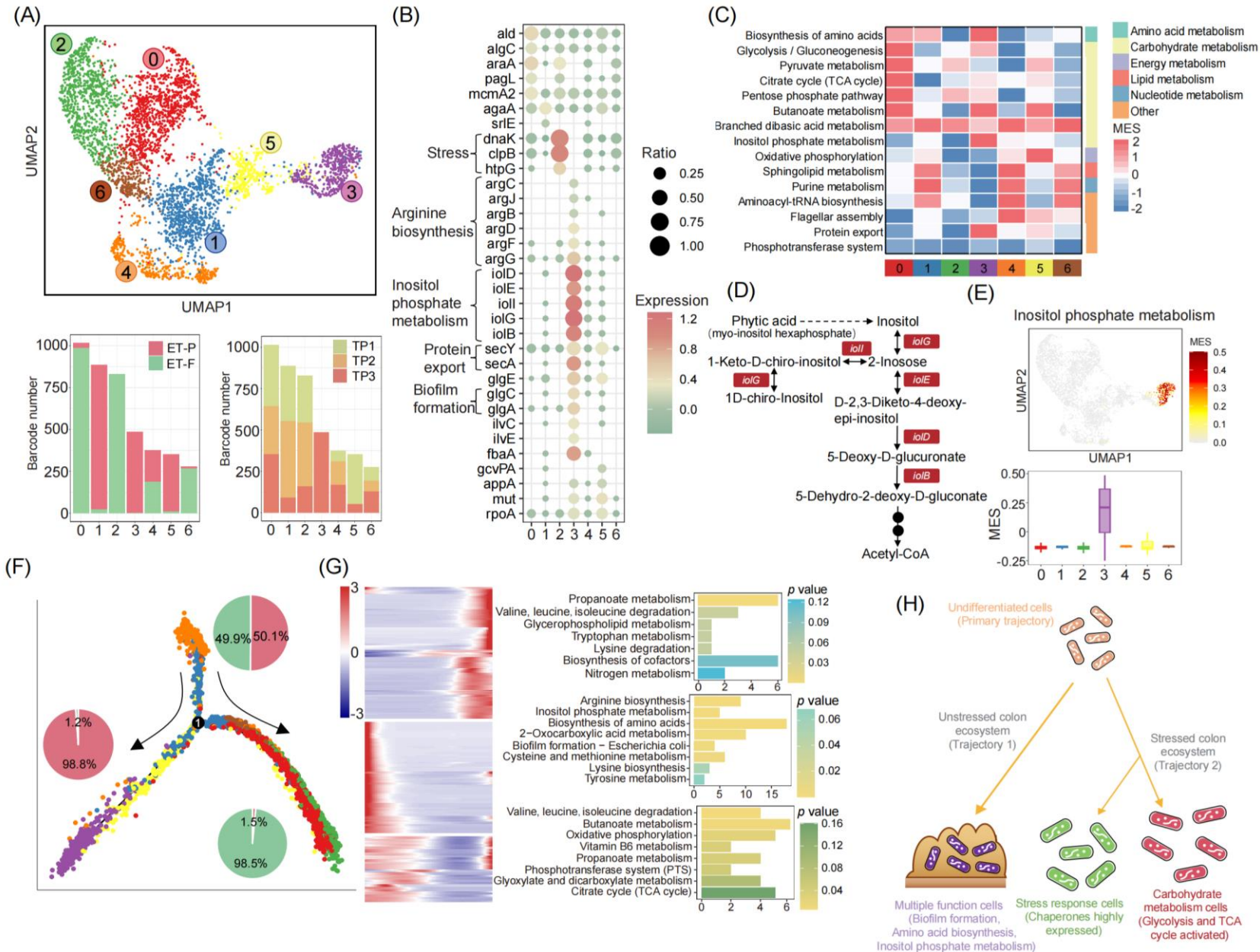


Figure 6 Functional trajectory analysis uncovering *M. funiformis*'s state transitions in distinct colon ecosystems.



# *M. funiformis* improve mineral absorption through exogenous phytic acid degradation

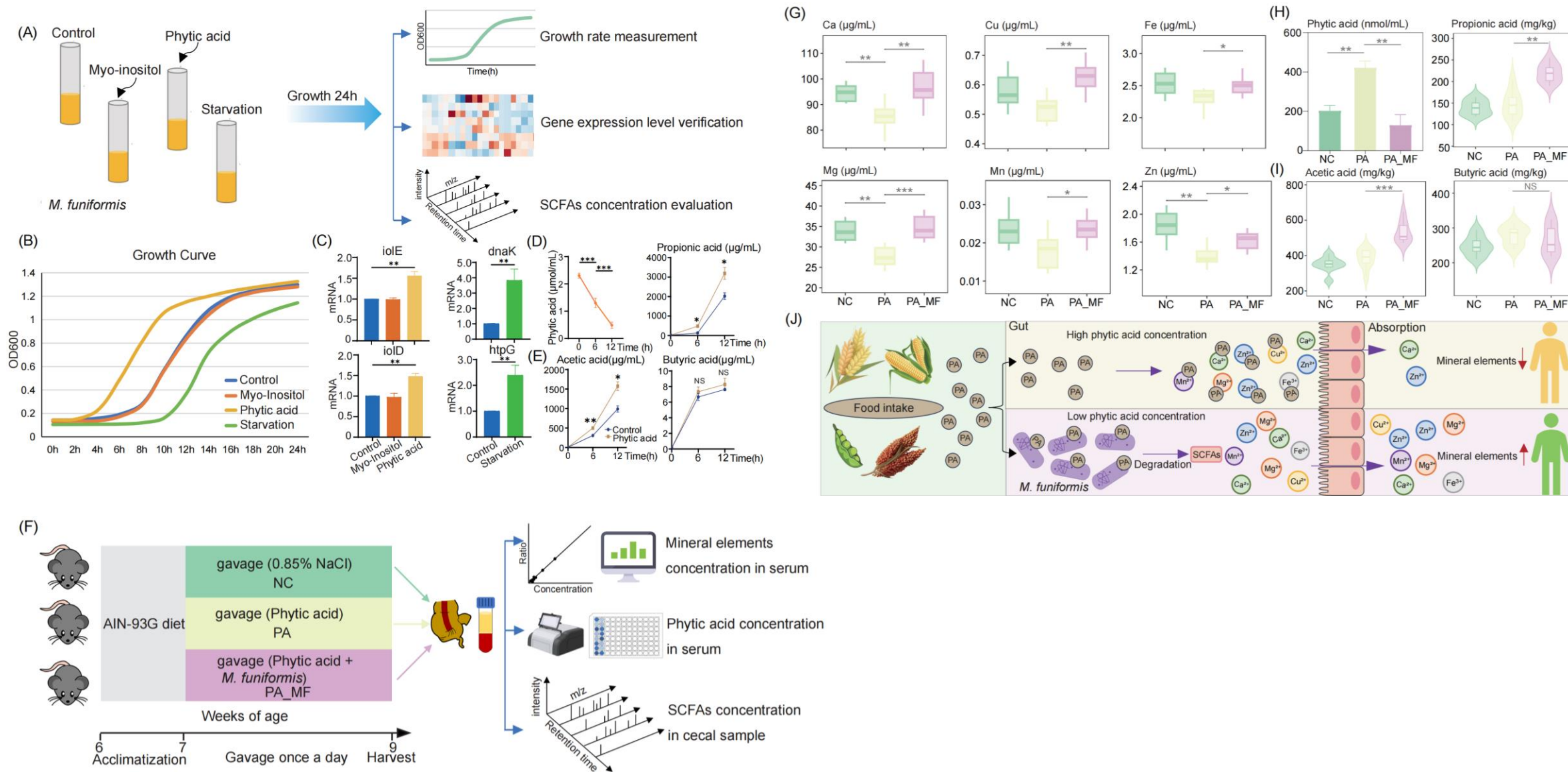


Figure 7 *M. funiformis* improve mineral absorption through exogenous phytic acid degradation.



# Summary

- ❑ Single-microbe RNA sequencing enables precise resolution of microbial dynamic transitions across distinct ecological niches, significantly advancing our understanding of inter-individual 'species-function' heterogeneity in the gut microbiome.
- ❑ The developed single-microbe RNA sequencing and analytical framework provides an efficient strategy for identifying keystone functional species with both biological significance and clinical relevance in gut microbial ecosystems
- ❑ We identified a novel function of *M. funiformis* in enhancing mineral absorption through exogenous phytic acid degradation




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