

Supplementary Information

Dietary supplementation with a novel selenium-enriched *Pichia kudriavzevii* regulates gut microbiota and host metabolism in mice

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Experimental

Selection of selenium doses in animal experiments

The doses of selenium in *Pichia kudriavzevi* were selected to be < 0.01, 0.15, and 0.40 mg/kg, mirroring variations in human dietary intakes as earlier reported^{1, 2}. In mice, a dietary selenium level of 0.15 mg/kg is the threshold required for maximal expression of GPX1, which corresponds to a daily selenium intake of 55 µg in humans. By analogy, a 0.40 mg/kg selenium diet in mice parallels supplementing the human diet with 200 µg/day, which is the common amount of selenium used in clinical trials¹.

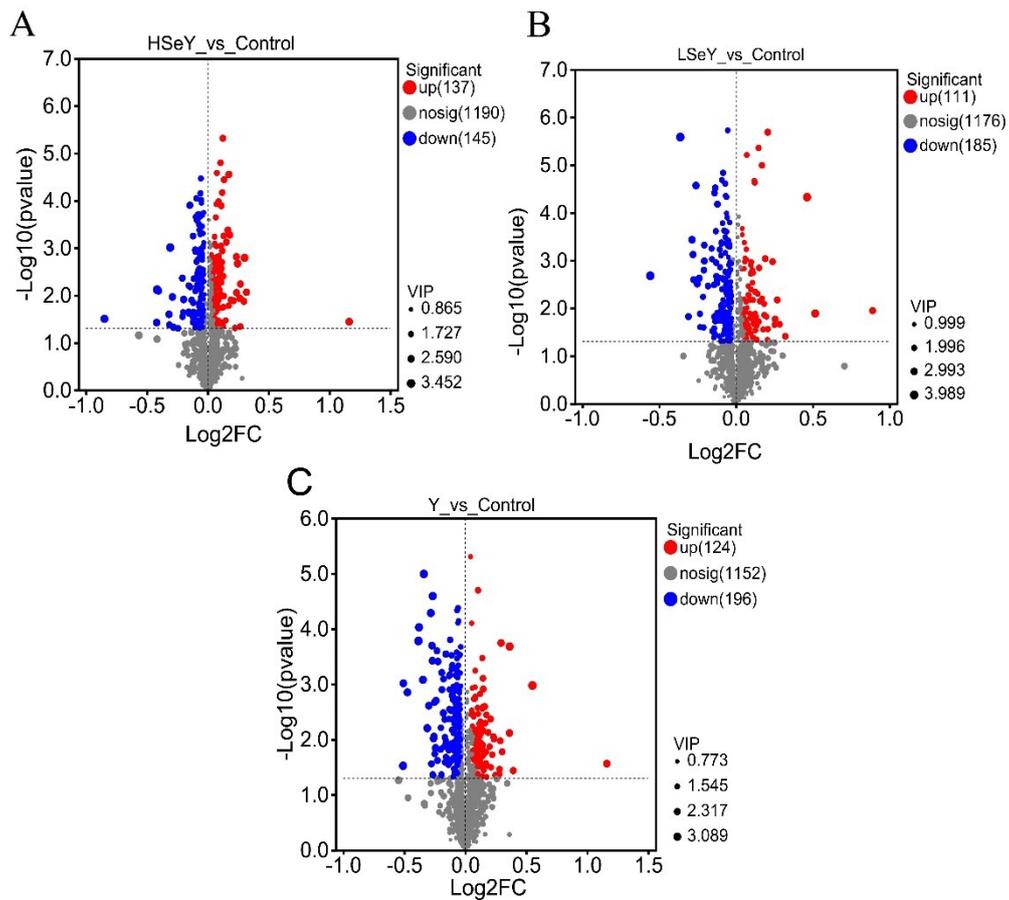


Fig. S1. Y and SeY treatment markedly impacted the gut metabolites of healthy mice.

(A) Volcano map of differential metabolites in HSeY group vs control group; (B) Volcano map of differential metabolites in LSeY group vs control group; (C) Volcano map of differential metabolites in Y group vs control group.

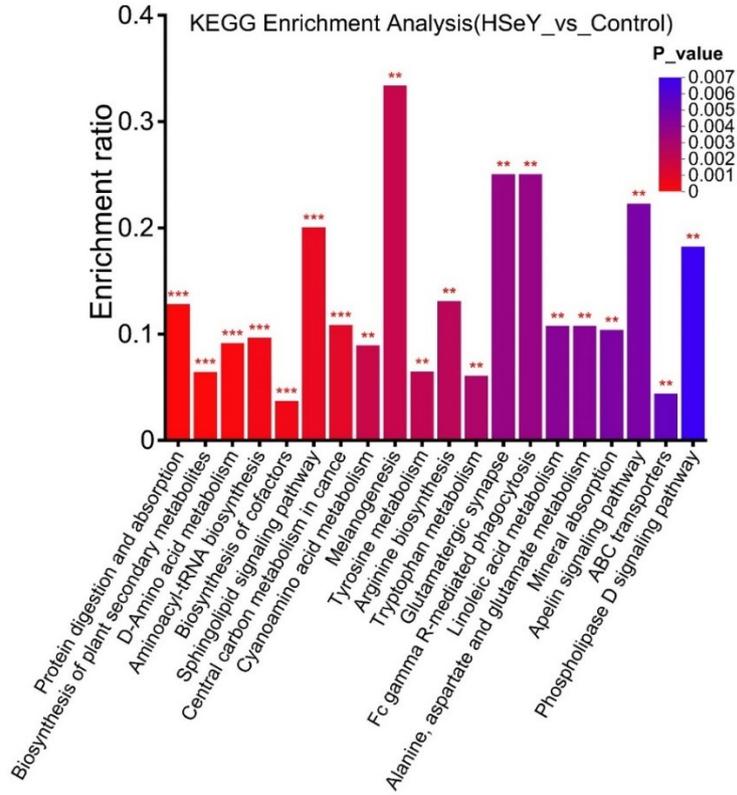


Fig. S2. Pathway enrichment analysis of differential metabolites (HSeY group vs control group).

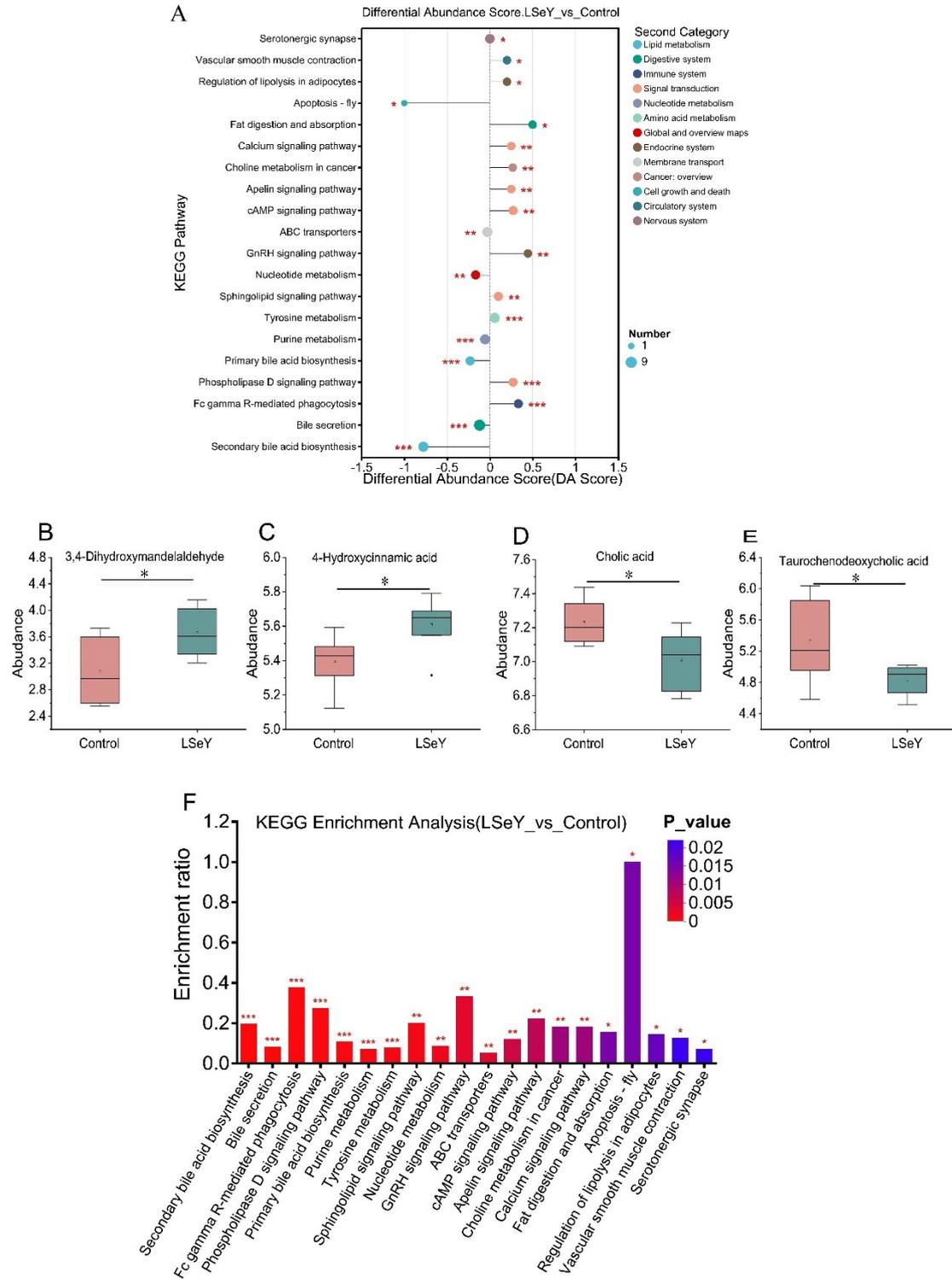


Fig. S3. Differential metabolites analysis between the control and LSeY treatment groups. (A) Map of the changed KEGG metabolic pathways after LSeY treatment; (B-E) Abundance of 3, 4-dihydroxymandelaldehyde, 4-Hydroxycinnamic acid, cholic acid, and taurochenodeoxycholic acid; (F) Pathway enrichment analysis of differential metabolites (LSeY group vs control group). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

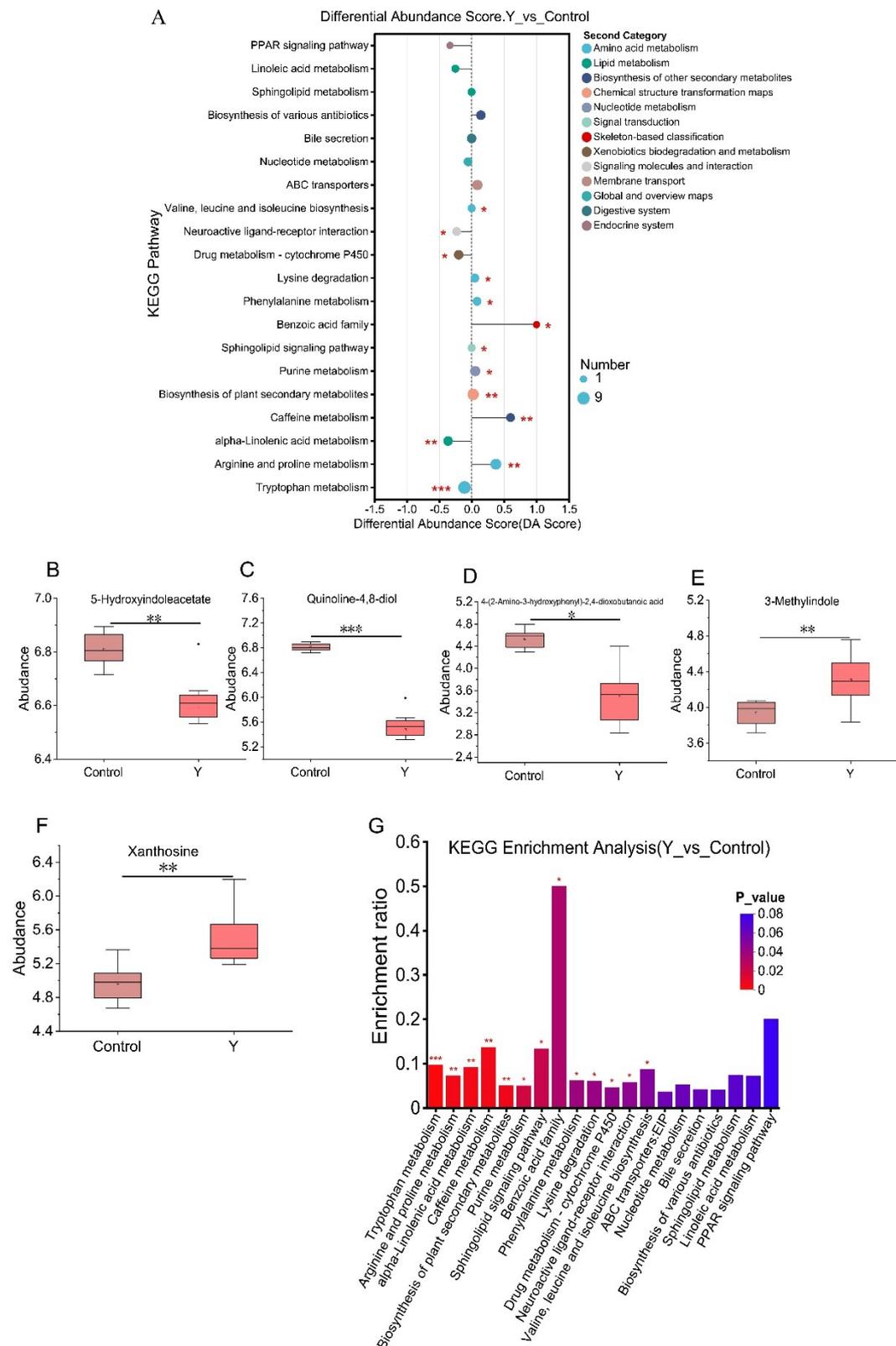


Fig. S4. Differential metabolites analysis between the control and Y treatment groups. (A) Map of the changed KEGG metabolic pathways after Y treatment; (B-F) Abundance of 5-Hydroxyindoleacetate, quinoline-4,8-diol, 4-(2-Amino 3-

hydroxyphenyl)-2,4-dihydroxybutanoic acid, 3-Methylindole, and xanthosine; (H) Pathway enrichment analysis of differential metabolites (Y group vs control group). * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Table S1. Operating conditions of GC

| Conditions | Parameters |
|--|------------|
| Injection volume (μL) | 1.0 |
| Column temperature ($^{\circ}\text{C}$) | 50 |
| Equilibration time (min) | 3 |
| Detector temperature ($^{\circ}\text{C}$) | 300 |
| Flow rate of chromatographic column (mL min^{-1}) | 4 |

Table S2. Abbreviation comparison table

| Full name | Abbreviation |
|---|---------------|
| <i>Pichia kudriavzevii</i> | Y |
| Se-enriched <i>Pichia kudriavzevii</i> | SeY |
| 0.15 mg/kg Se-enriched <i>Pichia kudriavzevii</i> | LSeY |
| 0.40 mg/kg Se-enriched <i>Pichia kudriavzevii</i> | HSeY |
| Alanine transaminase | ALT |
| Aspartate transaminase | AST |
| Lactate dehydrogenase | LDH |
| Catalase | CAT |
| Superoxide dismutase | SOD |
| Glutathione peroxidase | GPX |
| Myeloperoxidase | MPO |
| Malondialdehyde | MDA |
| Tumor necrosis factor- α | TNF- α |
| Interleukin- β | IL- β |
| Interleukin-6 | IL-6 |
| Interleukin-10 | IL-10 |
| Hematoxylin and eosin staining | H&E |
| Yeast extract peptone dextrose | YPD |
| Short-chain fatty acids | SCFAs |
| Gas chromatography | GC |
| Phosphate-buffered saline | PBS |

References

1. Q. Zhai, S. Cen, P. Li, F. Tian, J. Zhao, H. Zhang and W. Chen, Effects of dietary selenium supplementation on intestinal barrier and immune responses associated with Its modulation of gut microbiota, *Environ Sci Tech Let*, 2018, **5**, 724-730.
2. H. Chen, G. Du, X. Yan, H. Ye, Q. Guo, Z. Wang, Y. Yuan and T. Yue, Selenium-enriched *Pediococcus acidilactici* MRS-7 alleviates patulin-induced jejunum injuries in mice and its possible mechanisms, *J Agric Food Chem*, 2022, **70**, 4755-4764.