Butyrate alleviates food allergy by improving intestinal barrier integrity through suppressing oxidative stress-mediated Notch signaling

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Jialu Shi, Wenjun Mao, Yuqing Song, Yuxin Wang, Lili Zhang, Yan Xu, Huiwen Gu, Siyu Yao, Yuanhang Yao, Zhifeng Liu, Vijaya Raghavan, Jin Wang. 2025. Butyrate alleviates food allergy by improving intestinal barrier integrity through suppressing oxidative stress-mediated Notch signaling. ***iMeta*** 4: 70024. https://doi.org/10.1002/imt2.70024.

Introduction

- Food Allergy (FA) Epidemiology & Clinical challenges: Surge in prevalence → Management dilemma
- FA Pathogenesis: intestinal barrier disruption → Allergen translocation activates innate immunity
- Notch Signaling Pathway: Notch inhibition alleviates intestinal hyperpermeability (↑TJ proteins); Mechanism in FA unclear?
- Gut Dysbiosis & Protective Role of SCFAs: Gut dysbiosis may drive FA pathogenesis; SCFAs exhibit anti-allergic effects

? Unresolved: Do SCFAs regulate barrier integrity via Notch signaling?

- Study Objectives

➢ Clinical Validation: Compare fecal SCFAs (butyrate, acetate, etc.) between FA children and healthy controls (metabolomics).

➢ Model Validation: Evaluate effects of SCFAs (butyrate vs. others) in OVA-induced FA murine models.

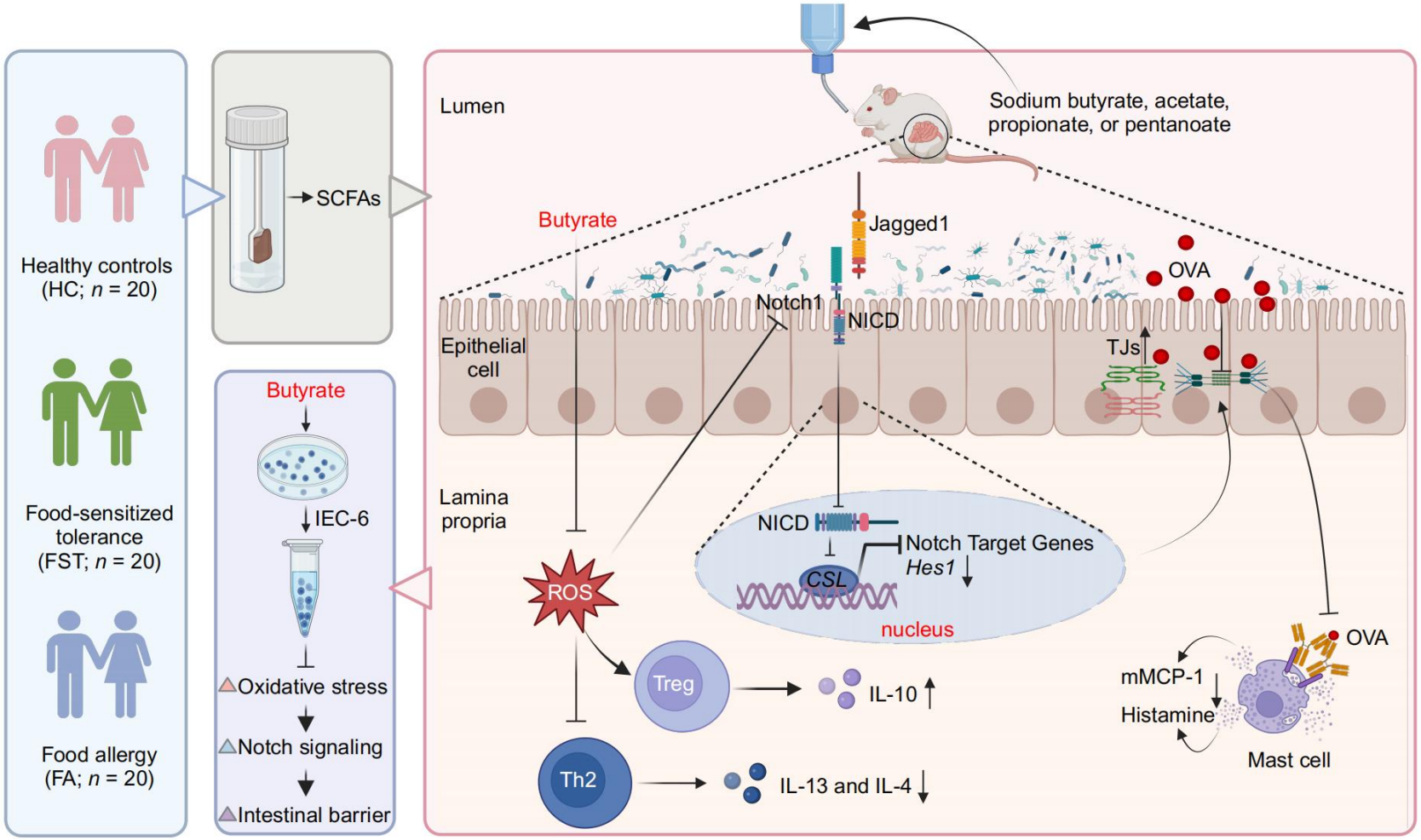
➢ Mechanism Exploration: Multi-omics integration (16S rRNA sequencing, untargeted metabolomics, transcriptomics) to decode microbiota-epithelial-immune interactions.

➢ Causal Validation: Elucidate molecular mechanisms of butyrate-Notch-barrier axis using IEC-6 cell models.

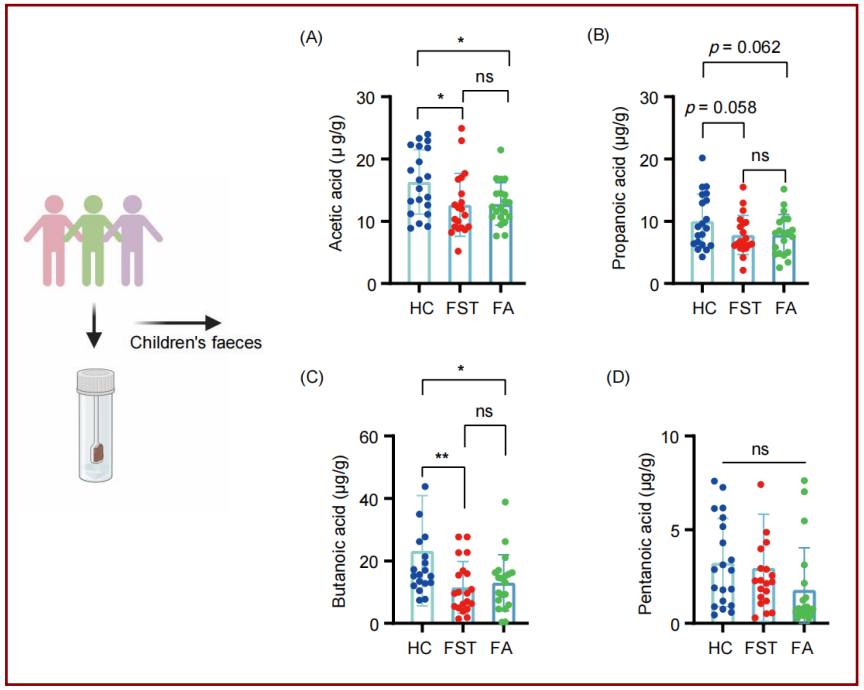
➢ Key Insight: Mechanism of butyrate-mediated barrier regulation via ROS-Notch axis clarified, offering dietary/microbiota-based targets for FA intervention.

Highlights

- Fecal SCFA levels were significantly lower in children with food-sensitized tolerance (FST) and FA compared to healthy controls (HC).
- In OVA-sensitized mouse models, butyrate exhibited the most pronounced alleviation of FA among SCFAs.
- Butyrate reduces ROS production, thereby suppressing the Notch signaling pathway in intestinal epithelial cells and enhancing intestinal barrier function.



Butyrate protected against food allergy in OVA-sensitized mice



- SCFAs may exert protective effects against FA.
- Butyrate demonstrates the most efficacy in mitigating FA symptoms among SCFAs.
- Butyrate reconfigures immune homeostasis by suppressing Th2 polarization and promoting Tregs expansion, thereby reducing allergic responses.

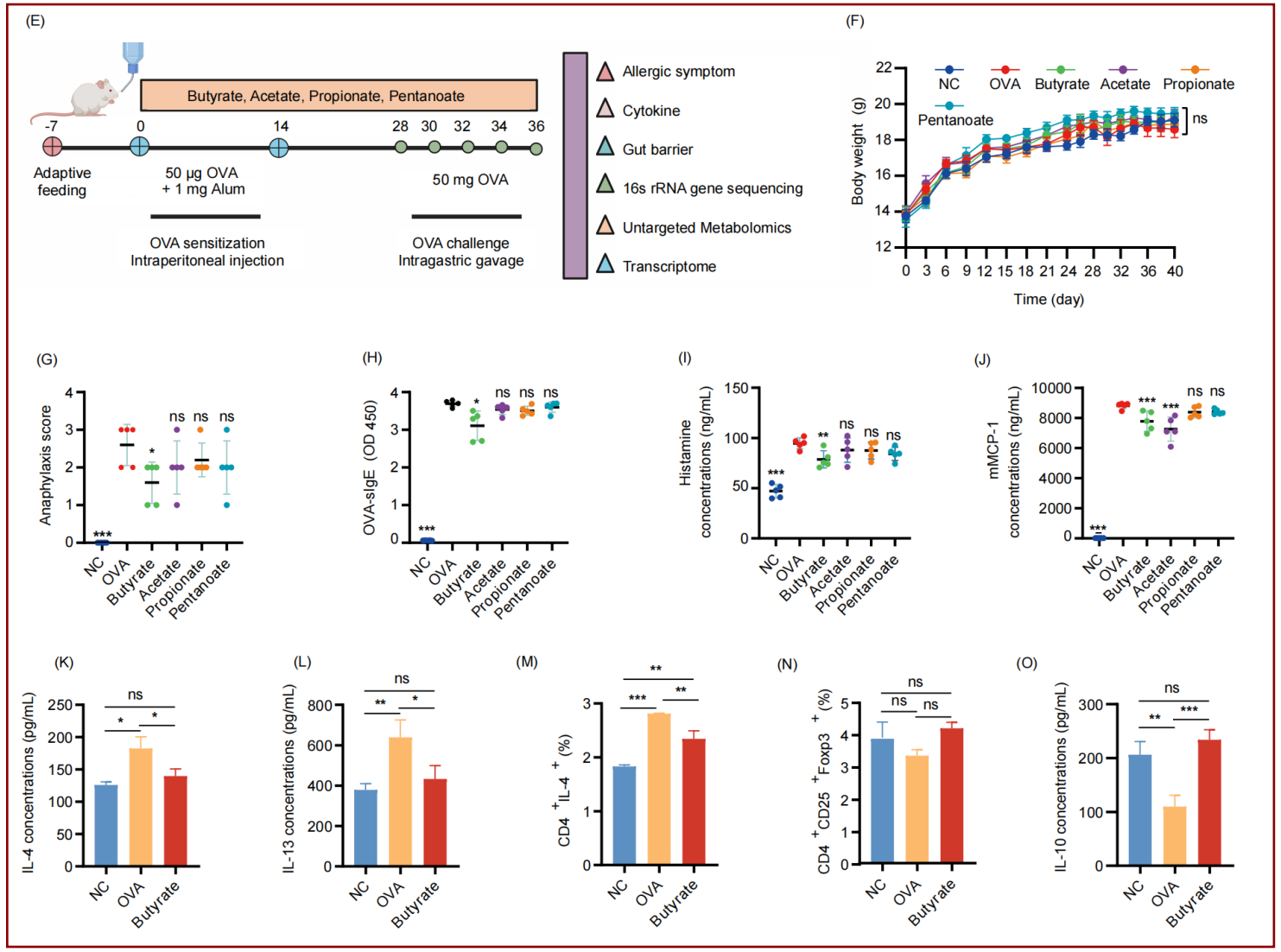


Fig1. Short-chain fatty acids (SCFAs) concentrations of children's fecal samples and the effect of SCFAs on food allergy in OVA-sensitized mice

Butyrate maintained intestinal barrier function

- Butyrate intervention improves villus height and restores goblet cell number
- Butyrate intervention upregulates tight junction proteins (Occludin/ZO-1) and Muc2 expression, enhancing intestinal barrier integrity

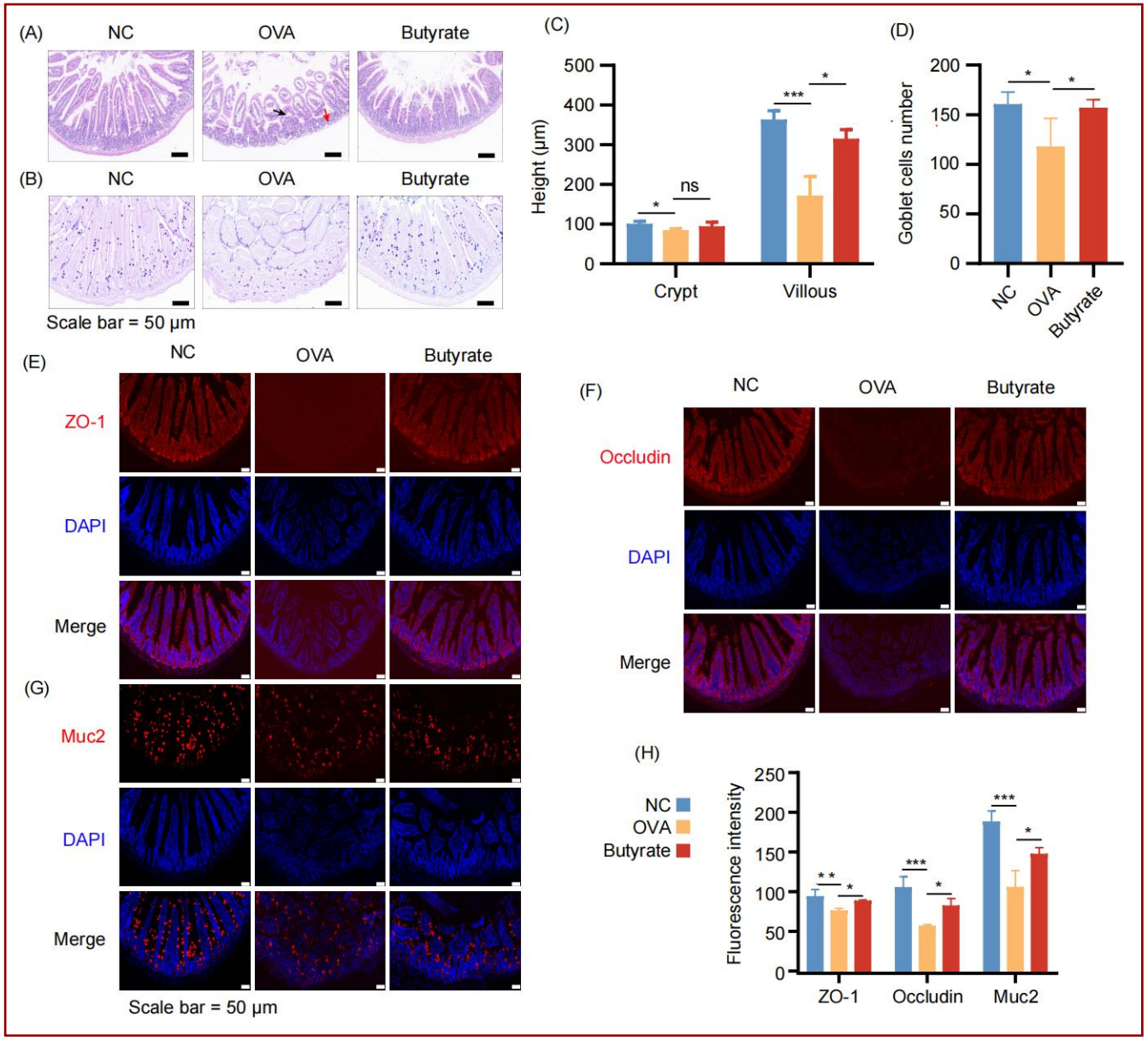
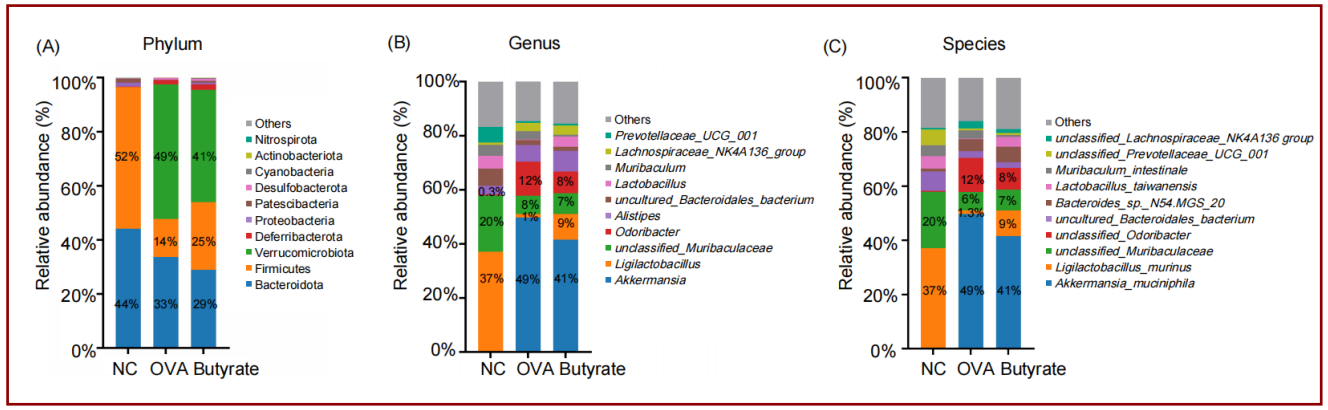


Fig2. Butyrate maintained gut tight junction integrity and mucosal barrier function

Butyrate treatment modulated gut microbiota



- PCoA analysis revealed distinct separation of gut microbiota structure among groups, with the OVA group significantly deviating from the NC group.
- OVA group: reduced Firmicutes/Bacteroidetes (F/B) ratio; decreased abundance of *L. murinus* and unclassified_Muribaculaceae; significant enrichment of Verrucomicrobiota, *A. muciniphila*, and *Odoribacter*.
- Butyrate group: enrichment of *Marvinbryantia* and *Clostridiales_bacterium_CIEAF_012*.

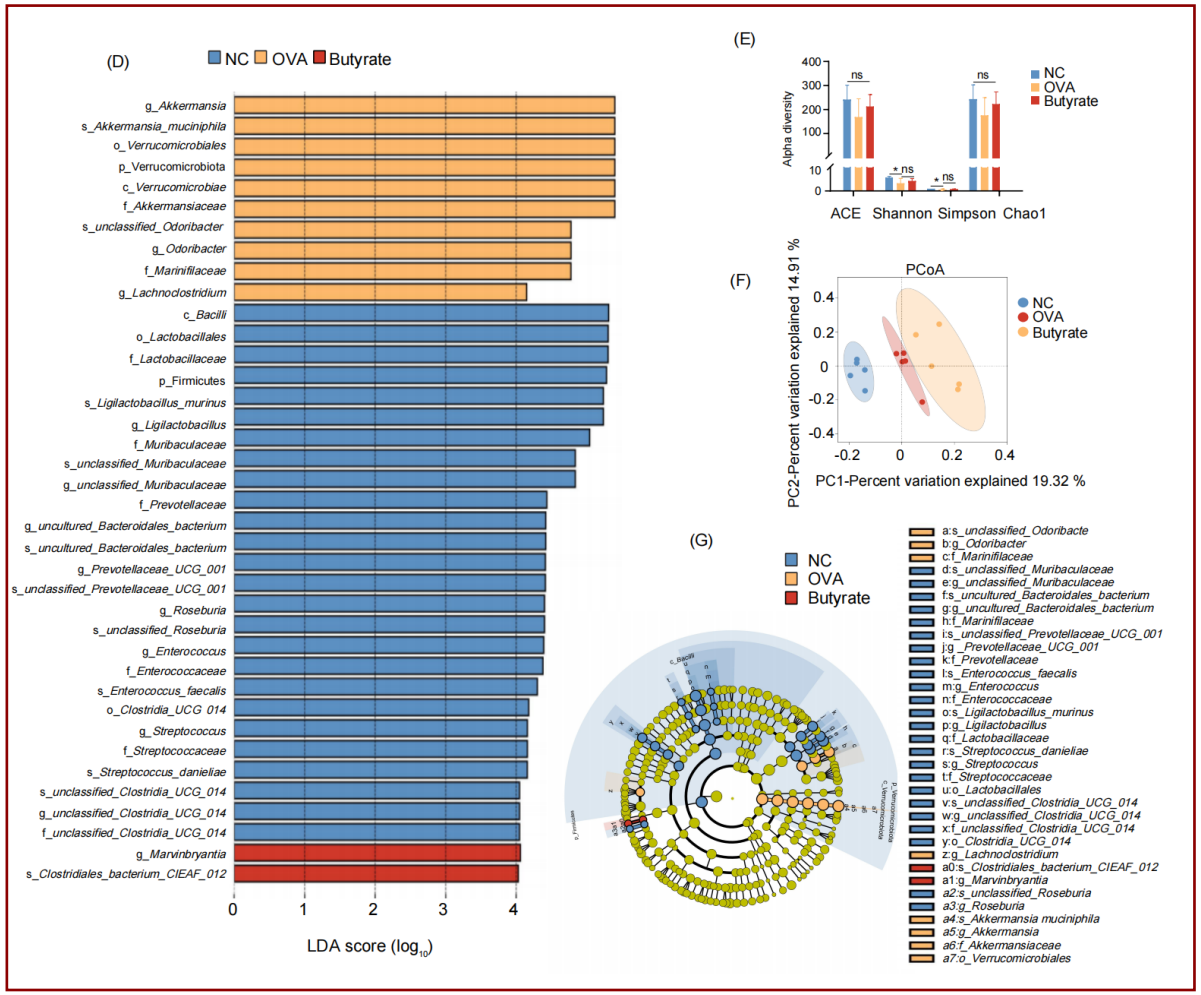


Fig.3 Butyrate treatment modulated gut microbiota in OVA-induced mice

The effect of butyrate treatment on the gut metabolites

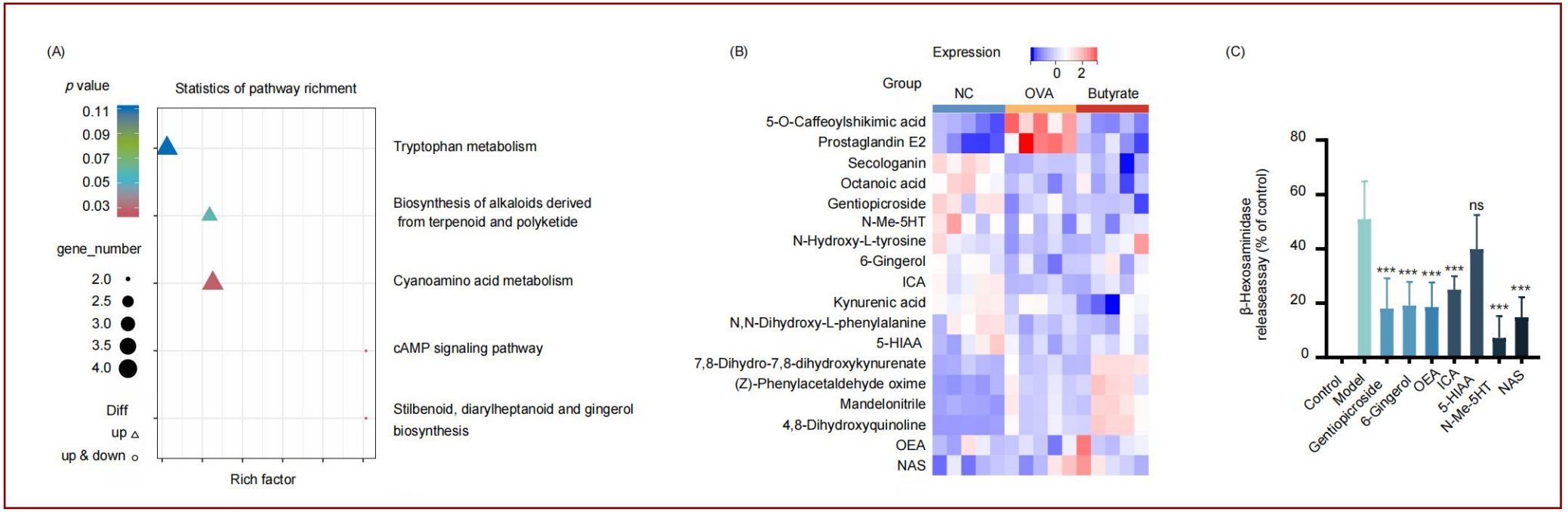


Fig4. The effect of butyrate treatment on the gut metabolites in OVA-induced mice and antiallergic properties of key gut metabolites

- Butyrate(anti-inflammatory and antioxidant metabolism↑):gentiopicroside, 6-Gingerol, oleoylethanolamide
- Butyrate(tryptophan metabolism↑):indole-3-carboxaldehyde, 5-Hydroxyindoleacetic acid, N-Methylserotonin, N-Acetylserotonin
- These differential metabolites significantly inhibit β-Hexosaminidase release

Butyrate inhibited Notch signaling pathway

- PCA analysis revealed distinct separation of IEC gene expression profiles among NC, OVA, and butyrate groups.
- Butyrate downregulated immune regulatory and inflammation-associated pathways.
- Butyrate intervention suppressed the Notch signaling pathway and reduced expression levels of key genes (*Notch1*, *Jagged1*, and *Hes1*) in the Notch pathway.

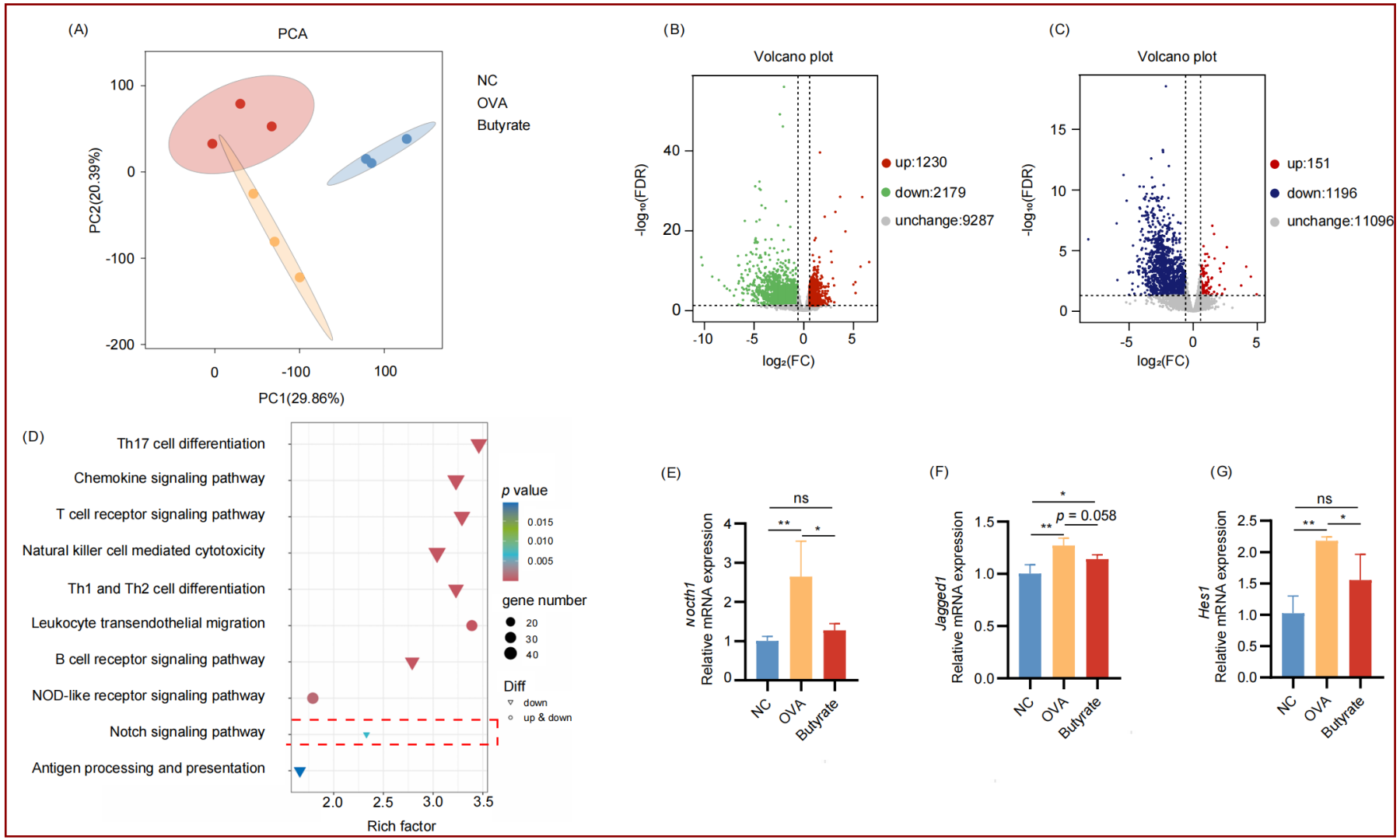


Fig5. Butyrate significantly altered intestinal epithelial cells (IECs) gene expression in OVA-induced mice

Butyrate inhibited oxidative stress and improves intestinal barrier integrity

- Butyrate suppressed the oxidative stress-Notch axis: reduced ROS accumulation, enhanced antioxidant capacity (GSH/SOD), and protected epithelial cells against oxidative damage.
- Butyrate fortified the intestinal barrier: upregulated tight junction proteins (*ZO-1*, *Claudin-1*) and *Muc2*, and reduced allergen penetration.

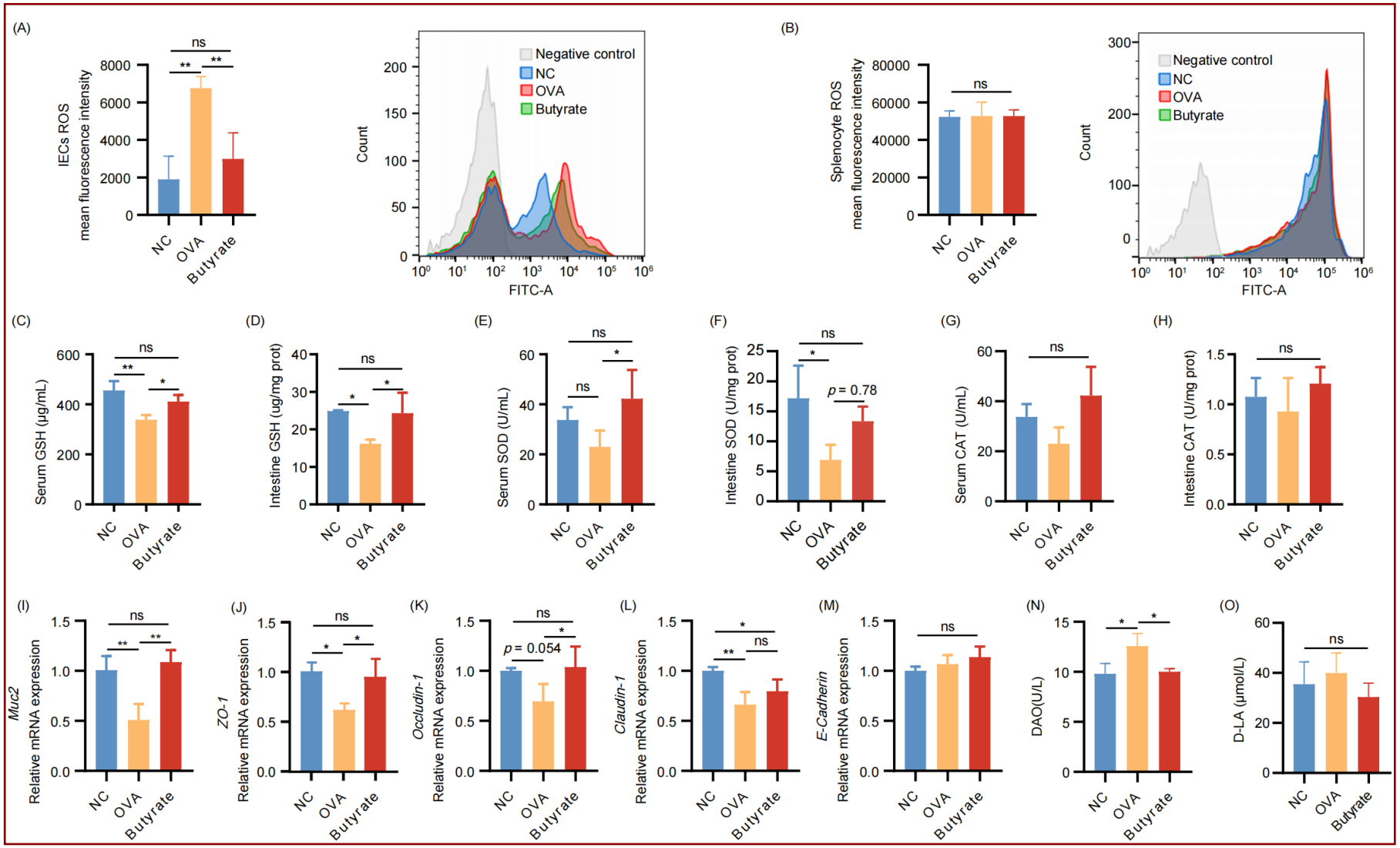


Fig6. Butyrate improved intestinal barrier integrity via inhibiting oxidative stress-mediated Notch signaling

Dose-dependent effects of butyrate on oxidative stress and Notch signaling

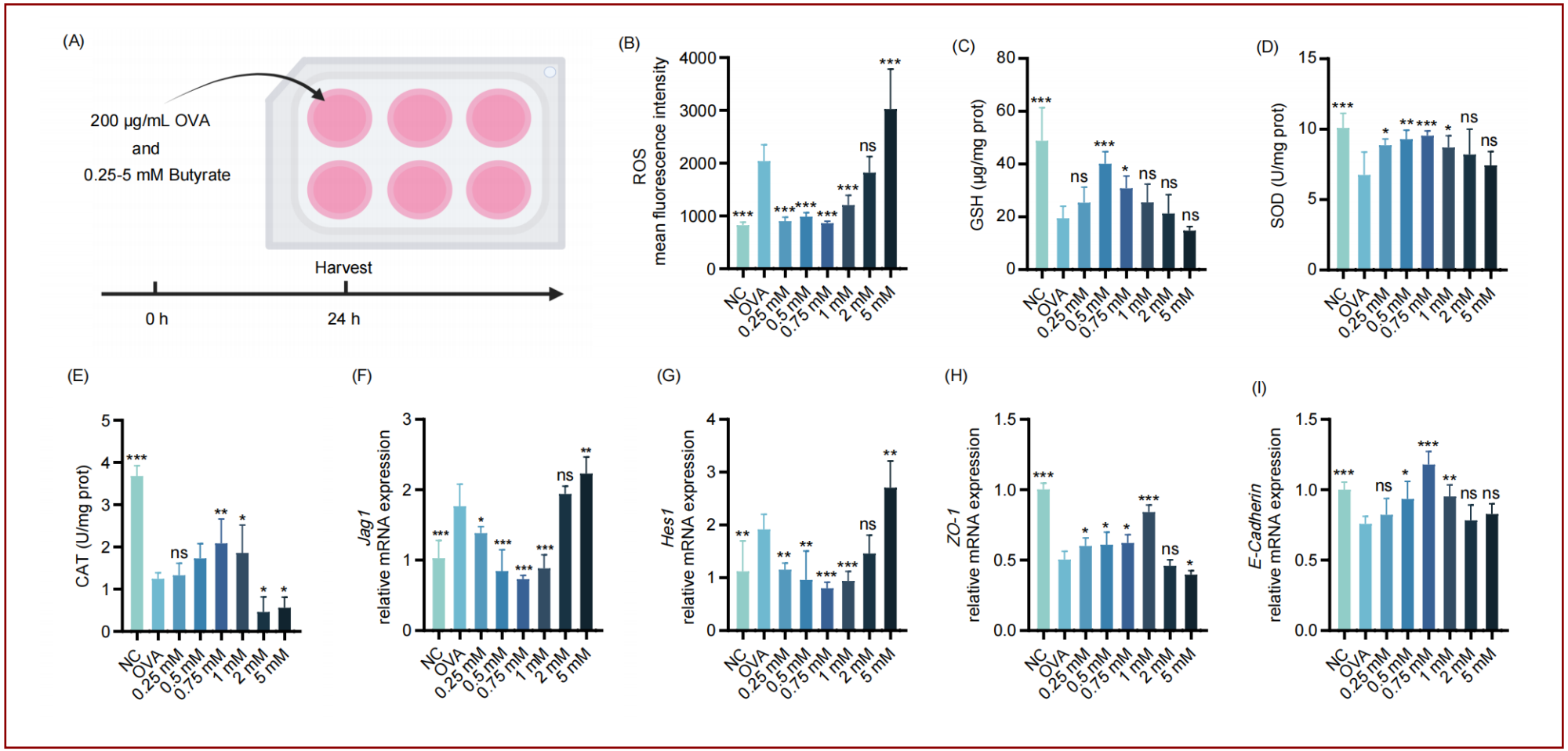


Fig7. Effect of different concentrations of butyrate on the Notch signaling in rat small intestine crypt epithelial cells (IEC-6) cells

- Butyrate (0.25-1 mM) suppressed ROS and restored antioxidant levels, while 5 mM butyrate failed to exert antioxidative effects.
- Butyrate (0.25-1 mM) inhibited *Jagged1* and *Hes1* mRNA expression, whereas 5 mM butyrate paradoxically enhanced their expression.
- Butyrate (0.5-1 mM) restored *ZO-1* and *E-Cadherin* mRNA levels, but 2-5 mM lacked intestinal barrier reparative activity.

Effect of butyrate on Notch signaling in IEC-6 cells

- Butyrate, DAPT alone, and combined treatment enhanced intestinal barrier integrity.
- Butyrate, DAPT alone, and combined treatment inhibited activation of the Notch signaling pathway.

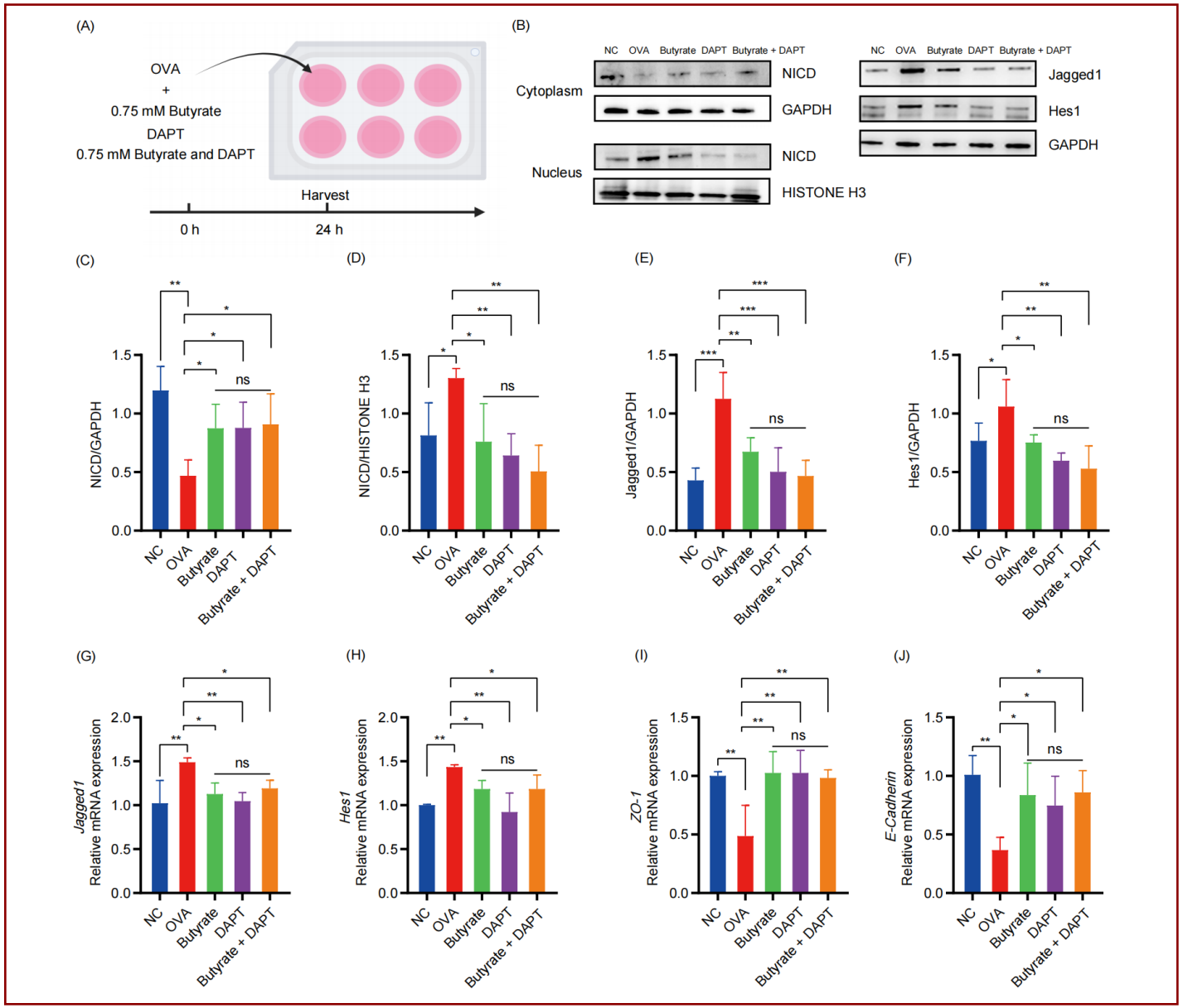


Fig8. Effect of butyrate on the Notch signaling in IEC-6 cells

Summary

- **Clinical Evidence:** Children with food allergy exhibited significantly reduced intestinal SCFA levels.
- **Animal Models:** Butyrate demonstrated superior anti-allergic effects over other SCFAs in murine models.
- **Molecular Mechanism:** Butyrate restored intestinal barrier integrity by targeting the ROS-Notch axis in intestinal epithelial cells.

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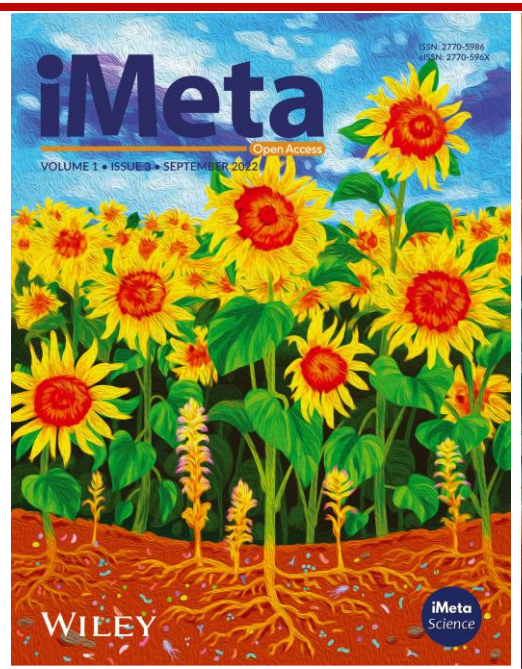

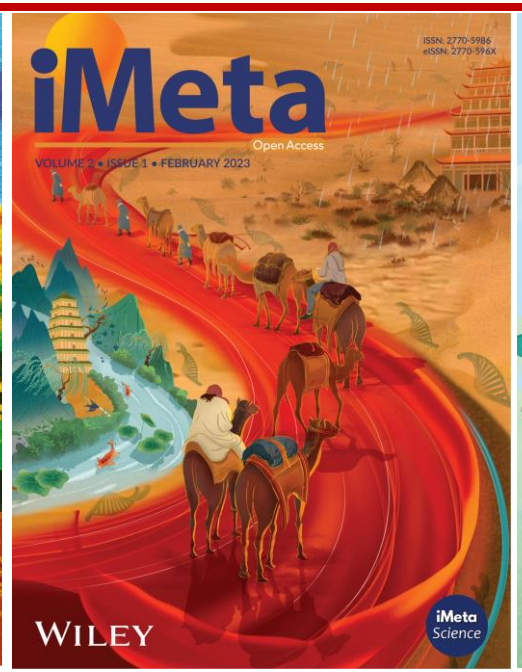

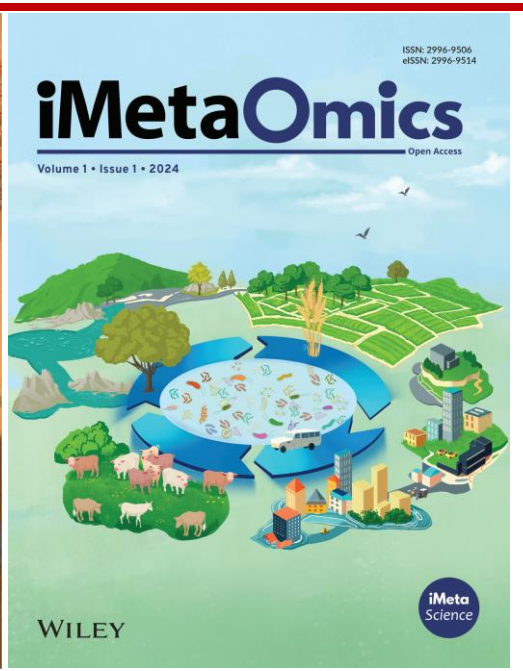

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