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mTORC1 Sensing Glutamate Signal to Promote Porcine Intestinal Development by Accelerating Intestinal Stem Cell Expansion

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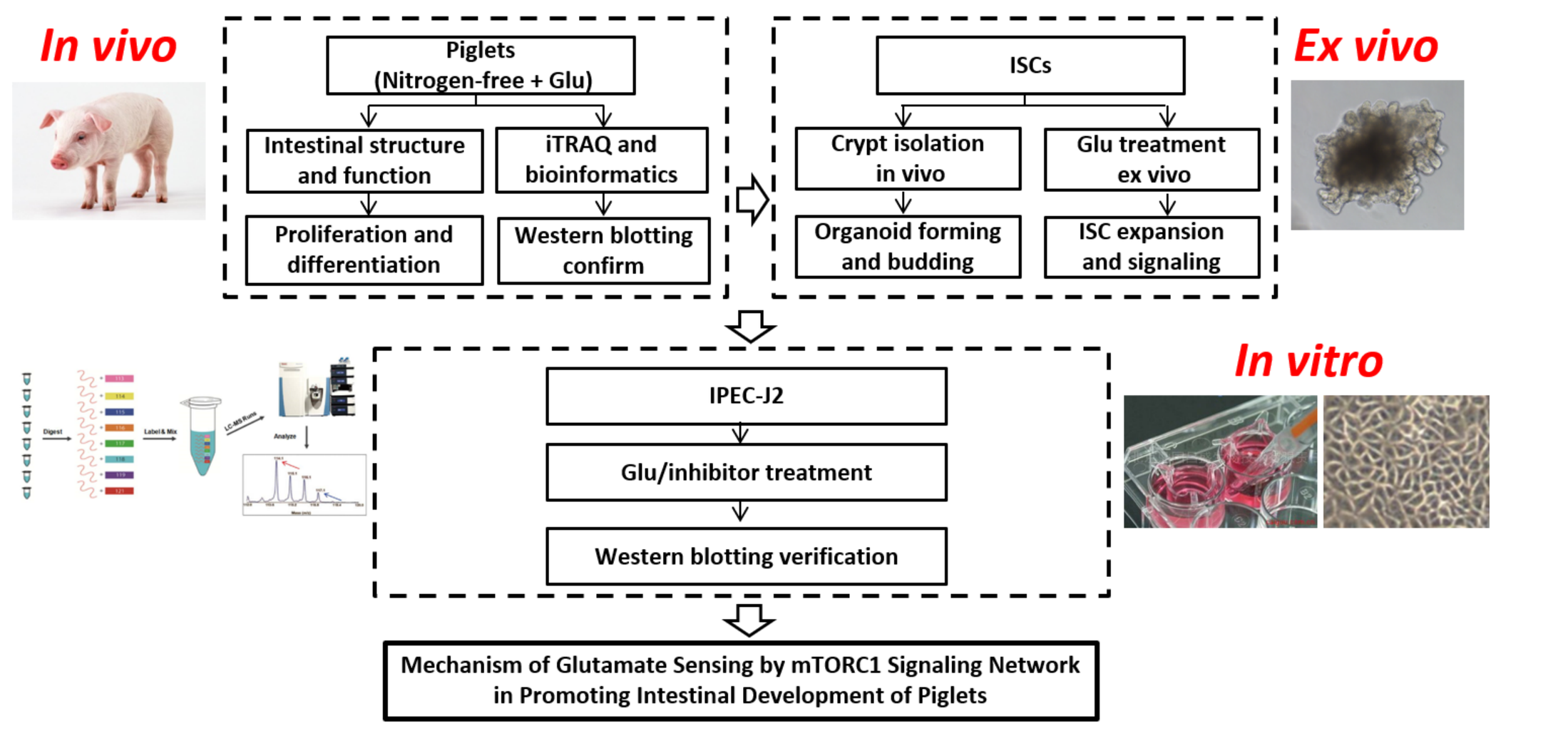
Introduction

Mechanistic target of rapamycin complex 1 (mTORC1) coordinates cell growth and metabolism with environmental cues, such as amino acids, growth factors, and energy. Glutamate (Glu) is a primary metabolic fuel for the intestinal epithelium and extensively involves numerous physiological processes. Crypt intestinal stem cells (ISCs) driven intestinal epithelial renewal that needs a continuous energy supplement. However, the effects of Glu on the expansion of porcine ISCs and intestinal epithelial development remain unclear. Therefore, the objective of this study was to investigate the underlying mechanism that Glu promotes intestinal development.

Experimental design

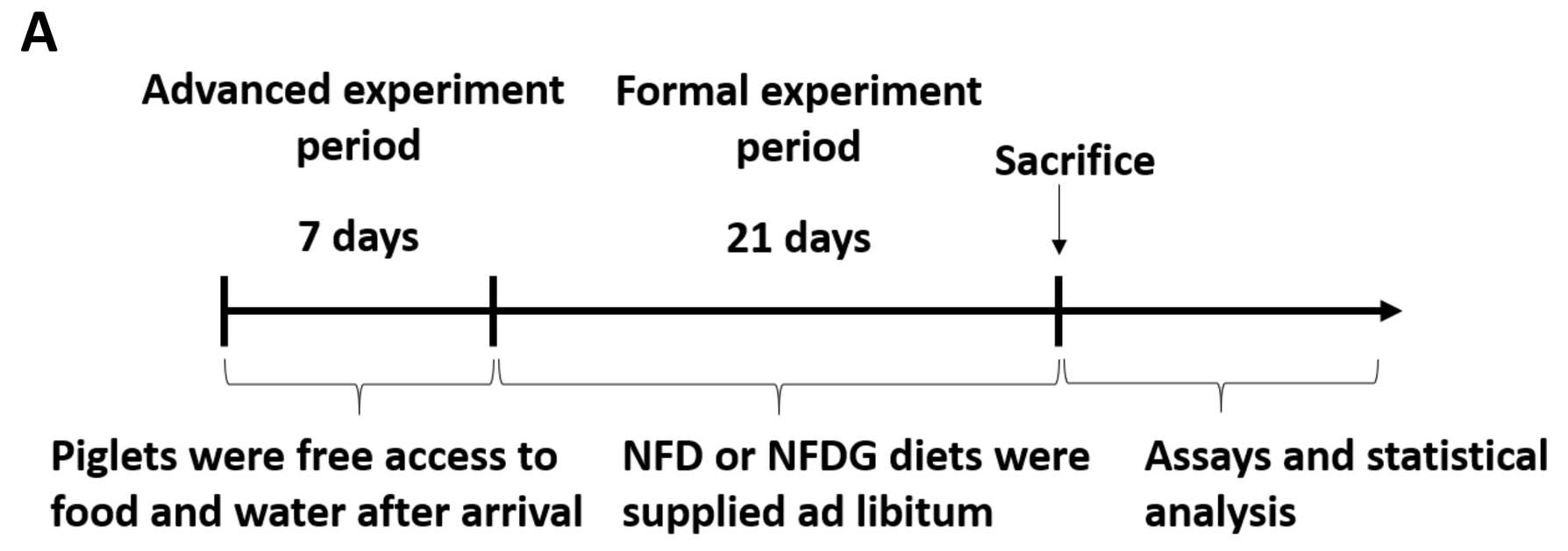


Materials and methods

- Crossbred weaned piglets (Duroc×Landrace×Large White) & IPEC-J2 & Porcine ISCs
- NFD = Nitrogen-free diet; NFDG = NFD + 1.00% glutamate
- iTRAQ & Hematoxylin-Eosin staining & Scanning Electron Microscope & Immunohistochemistry & Immunofluorescence & 3-D culture & Docking & Western blotting & WES & MTT & EdU

Results

1. Dietary supplementation with 1.00% Glu can effectively improve the intestinal epithelial development.



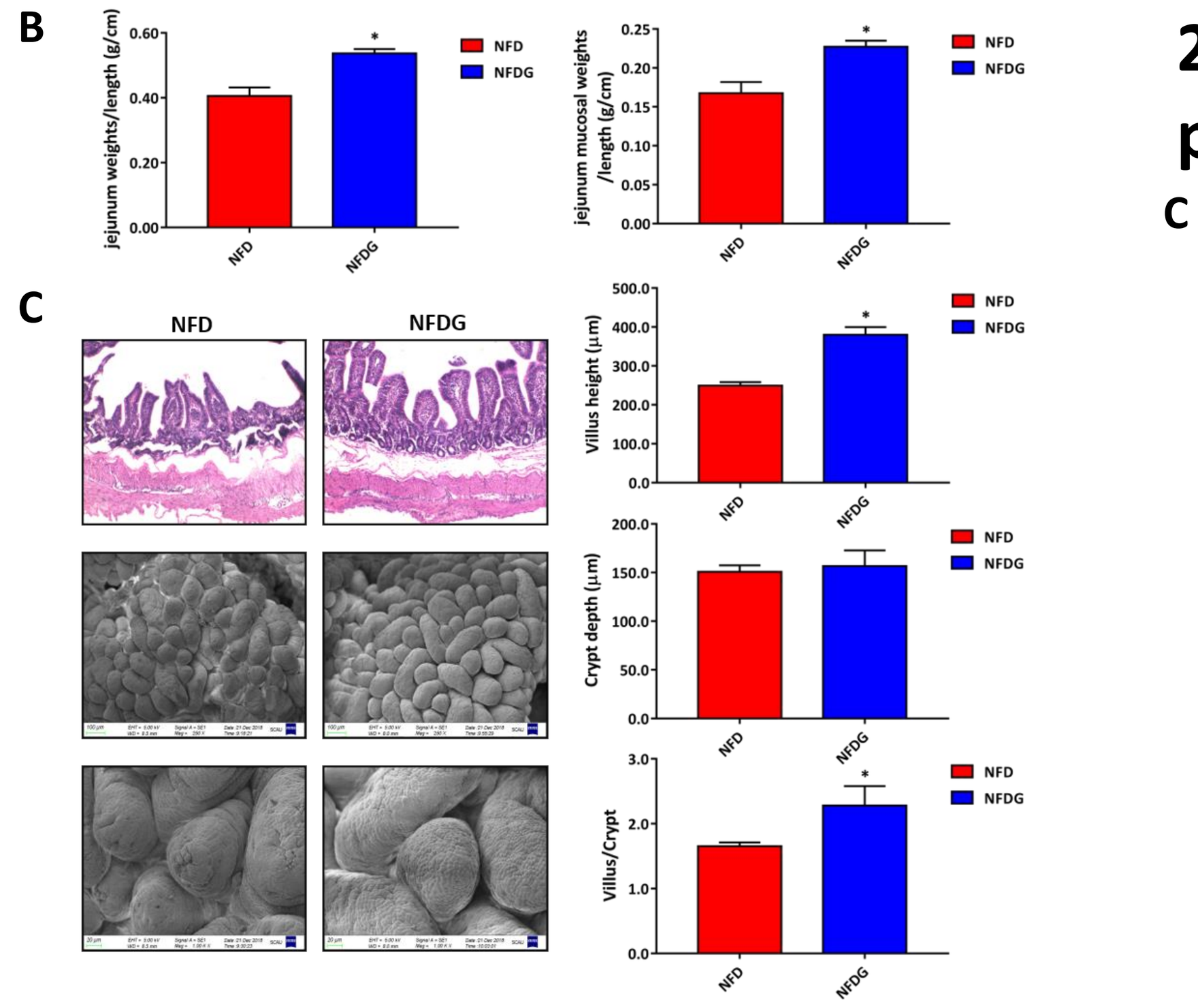


Figure 1. Dietary supplementation with 1.00% Glu increased the jejunum weight and mucosal weight of weaned piglets, and improved the morphological structure of jejunum.

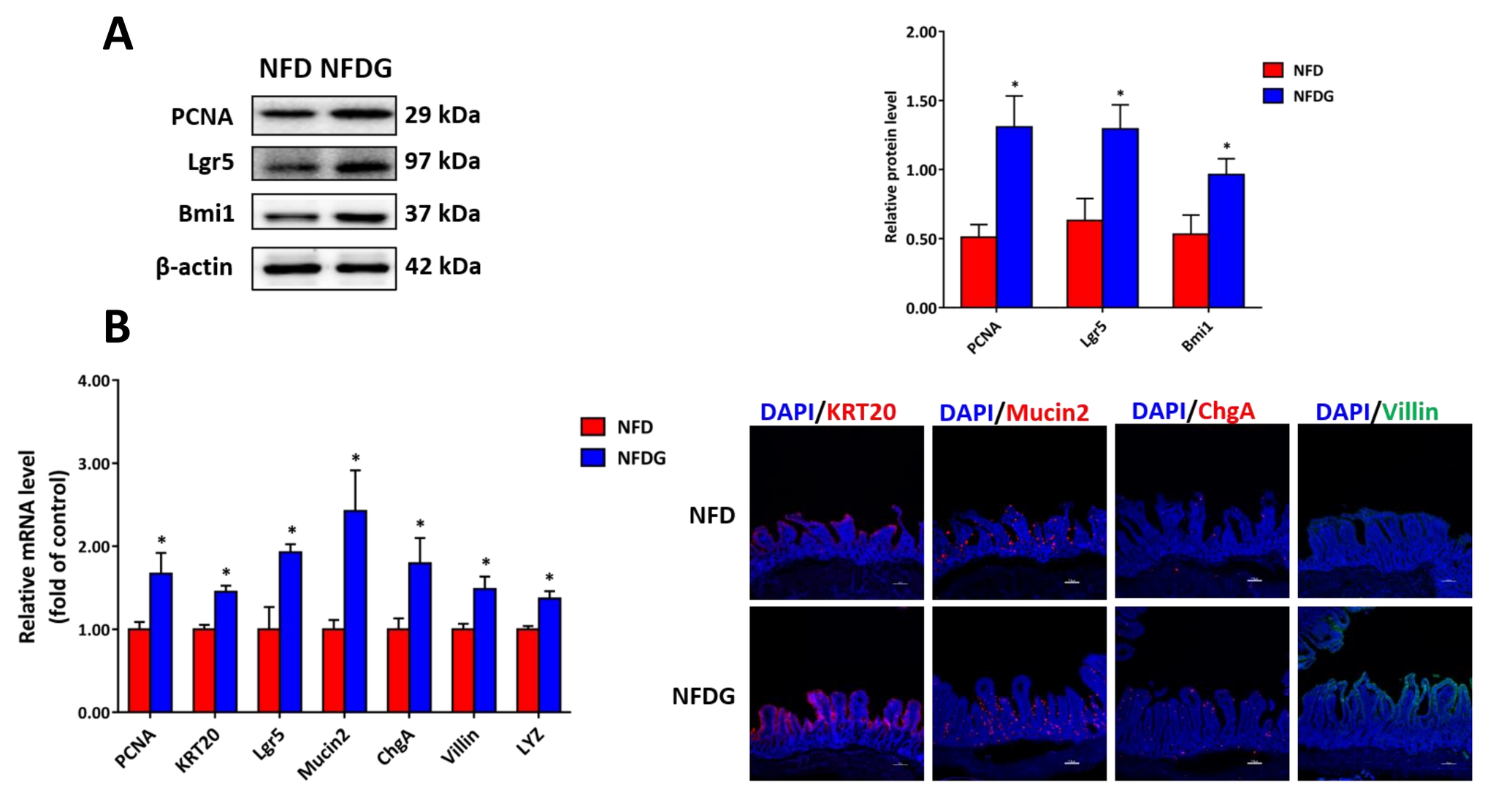
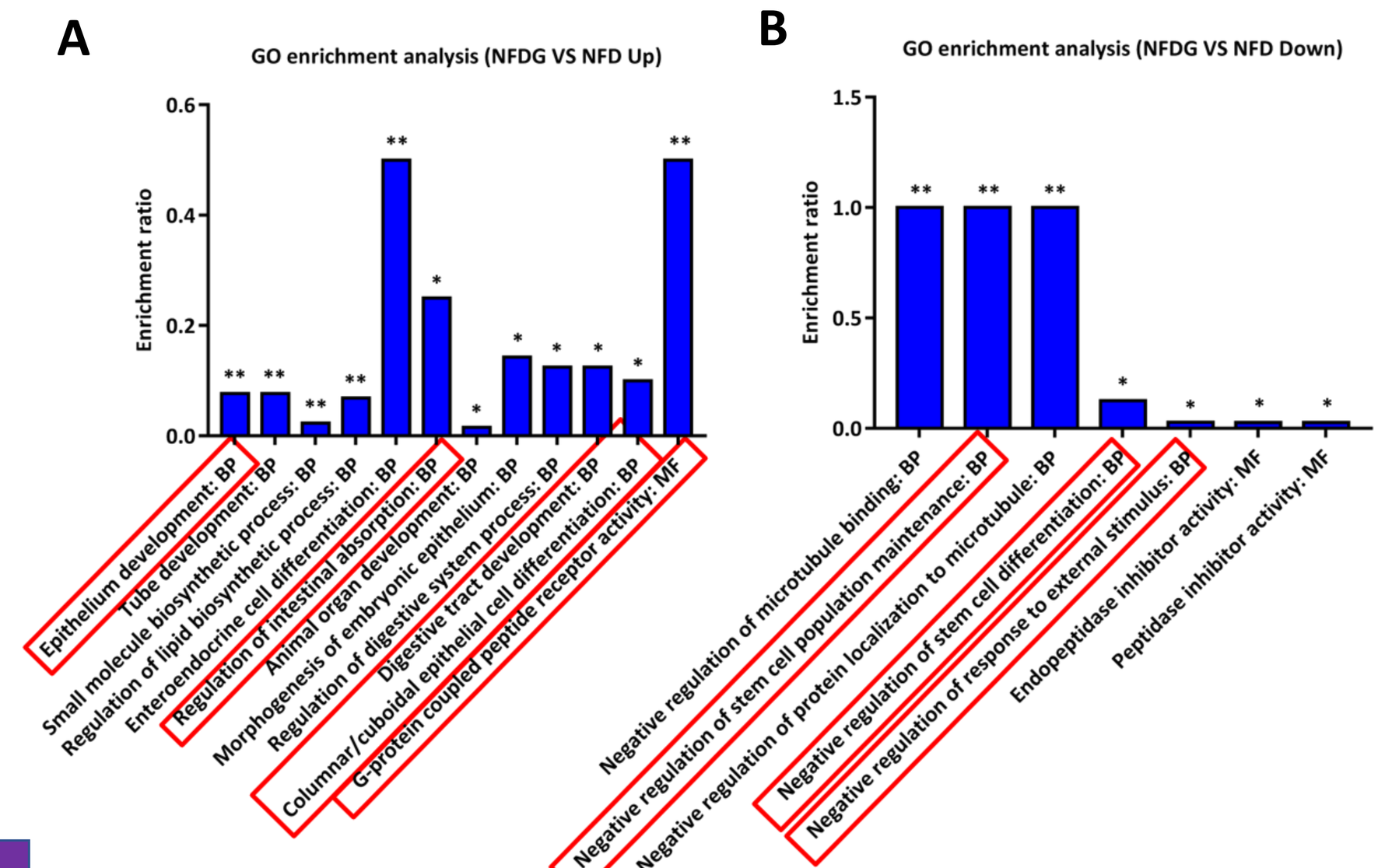


Figure 2. Dietary supplementation with 1.00% Glu promoted the proliferation and differentiation of jejunal epithelial cells.



2. mTORC1 signaling involves in Glu promotes porcine intestinal development.

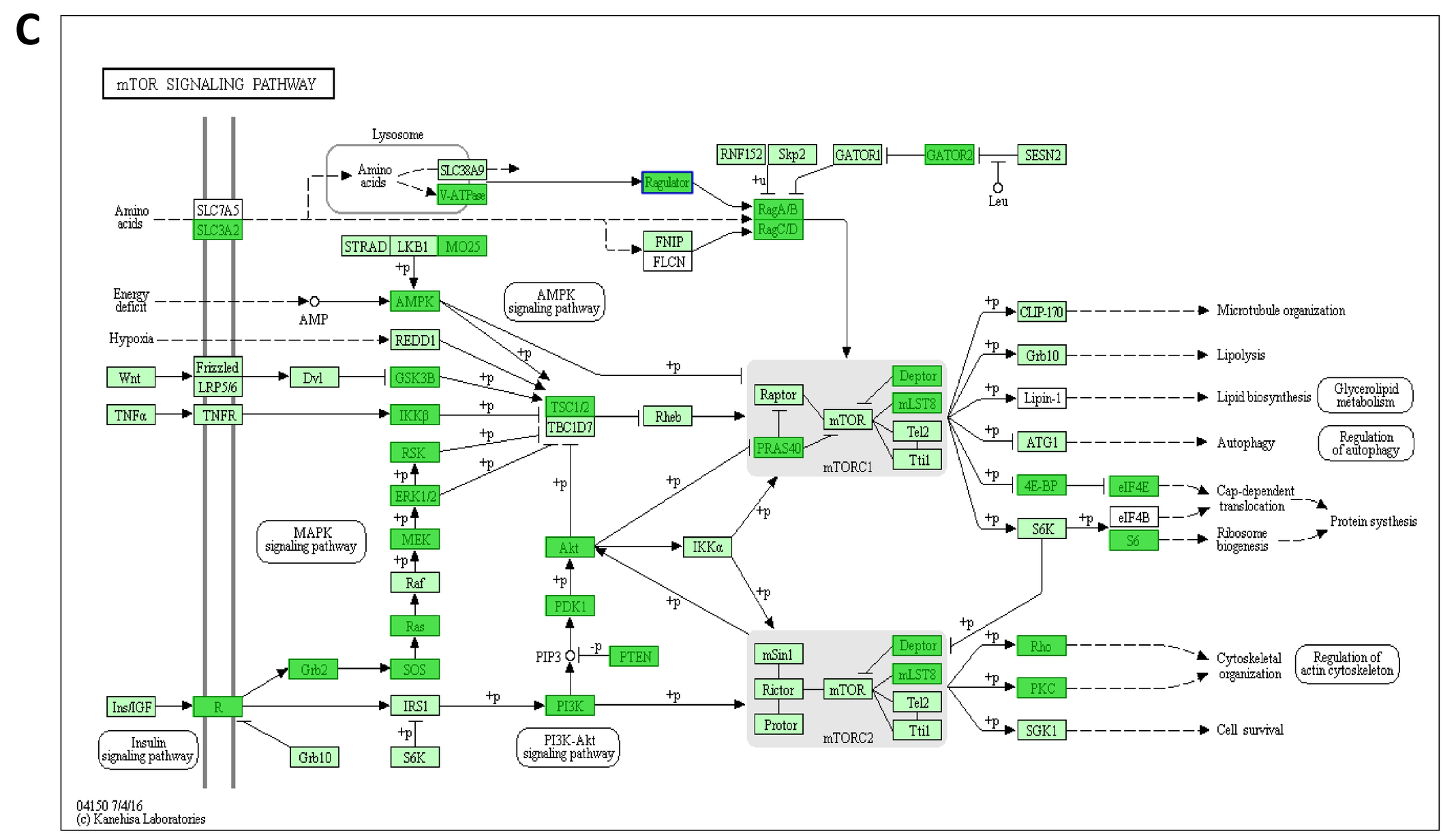


Figure 3. iTRAQ analysis showed that mTORC1 and its upstream Insulin/PI3K/Akt pathway, MEK/ERK pathway involved in intestinal epithelial development.

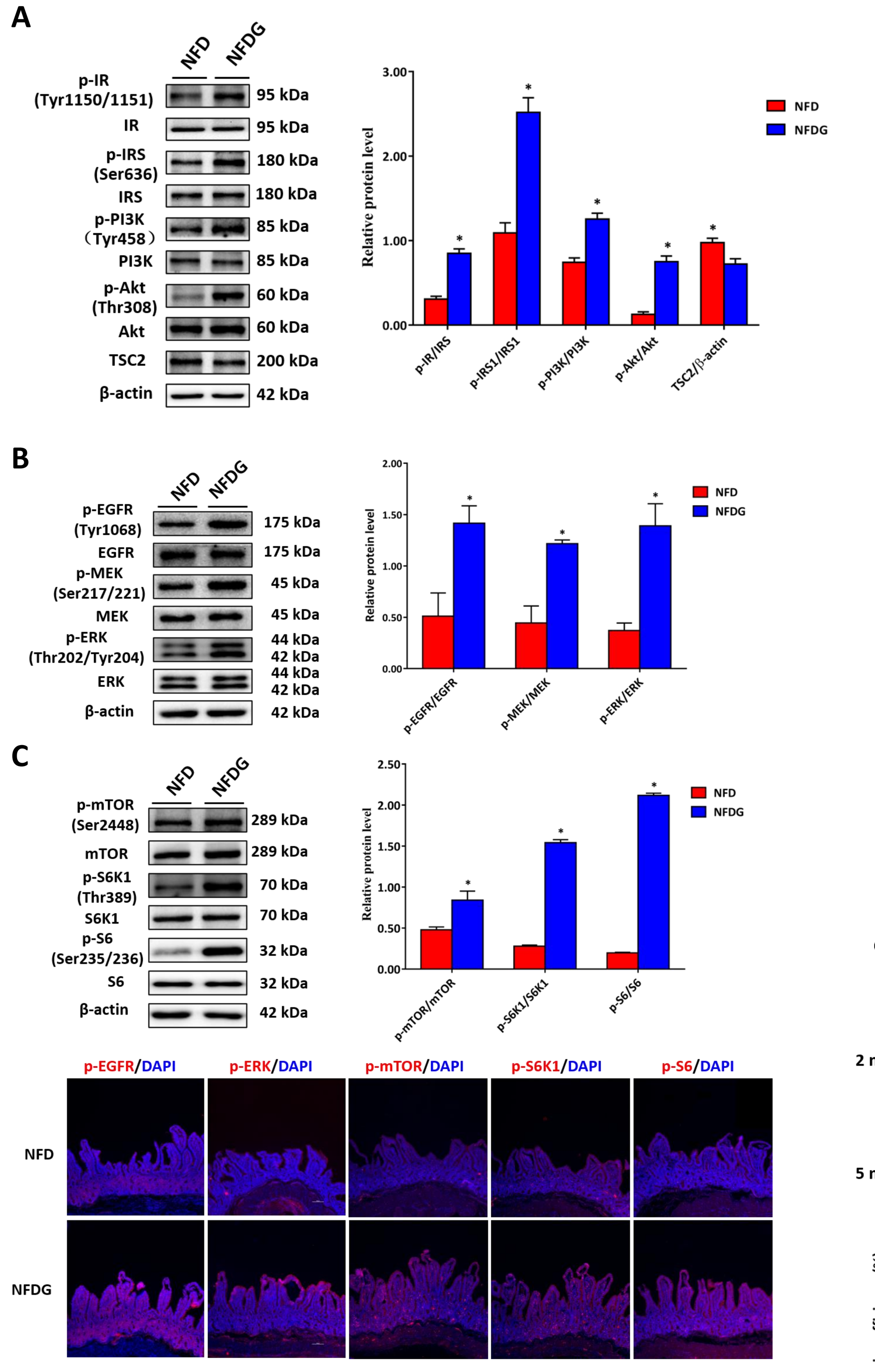


Figure 4. Dietary glutamate activated the IR/PI3K/Akt pathway, EGFR/MEK/ERK pathway, and mTORC1 signaling.

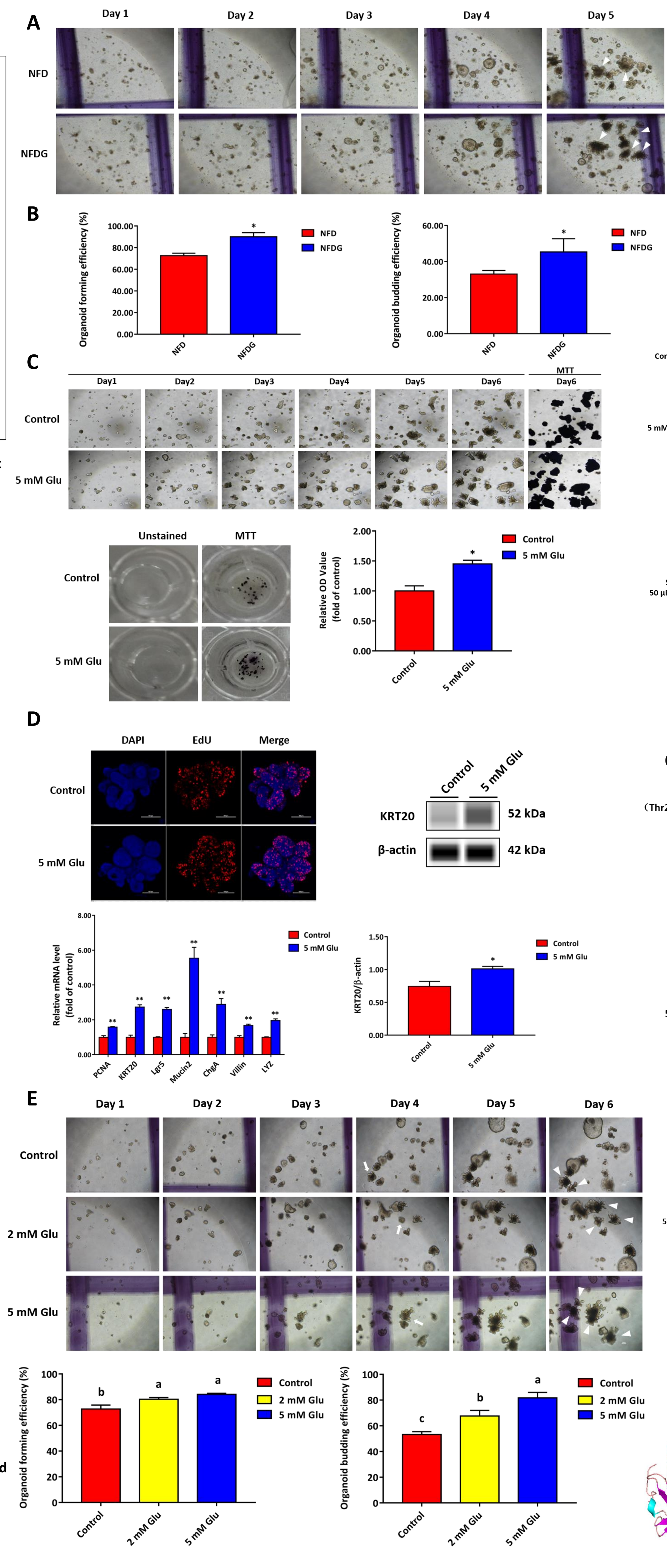
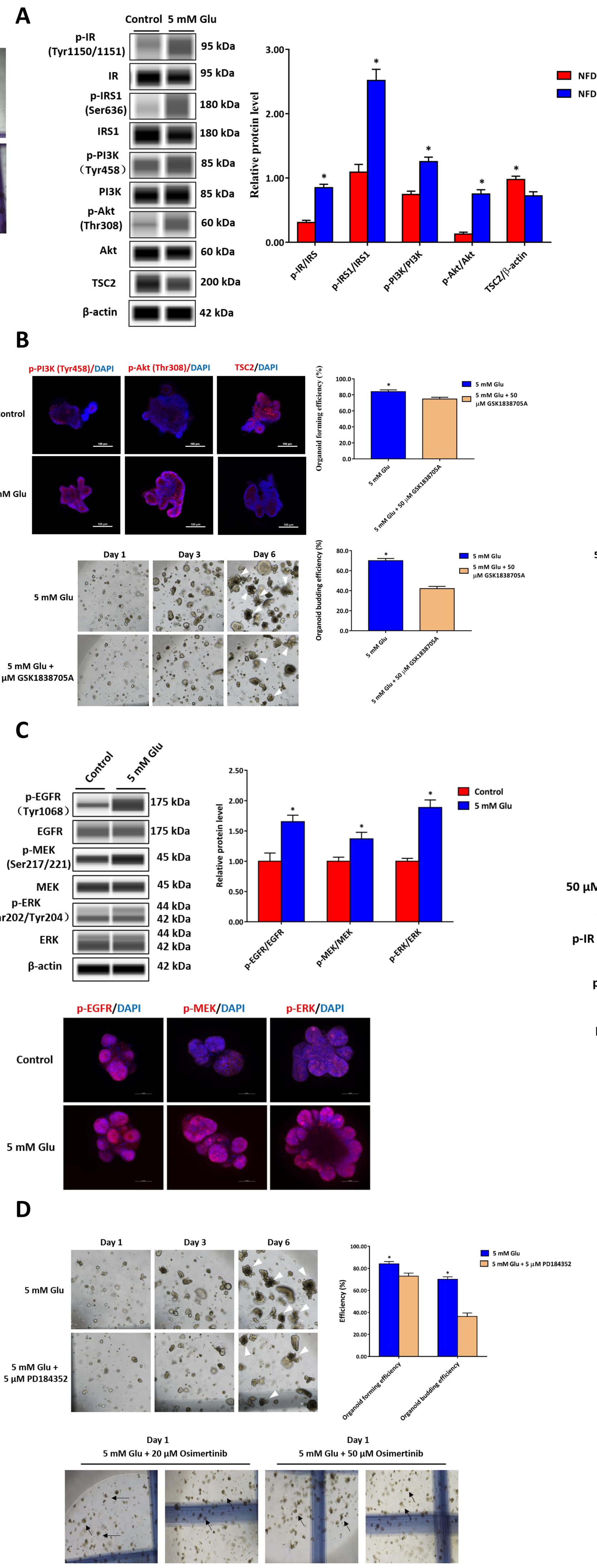


Figure 5. Glutamate significantly improved the growth of intestinal organoid.



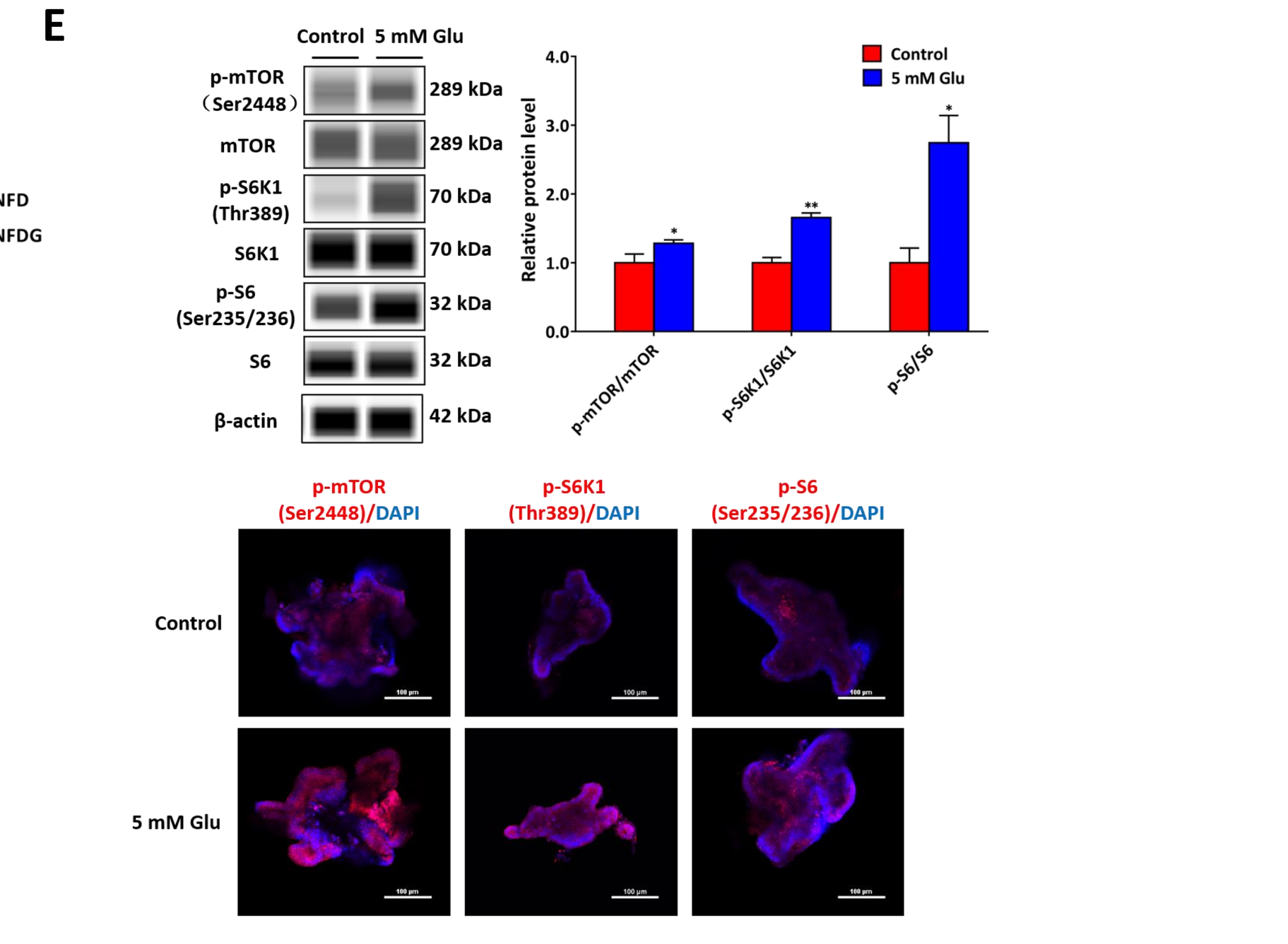


Figure 6. Glutamate promoted the expansion of intestinal stem cells, which may be related to its upregulation of IR/PI3K/Akt pathway and EGFR/MEK/ERK pathway, and activation of mTORC1 signaling pathway.

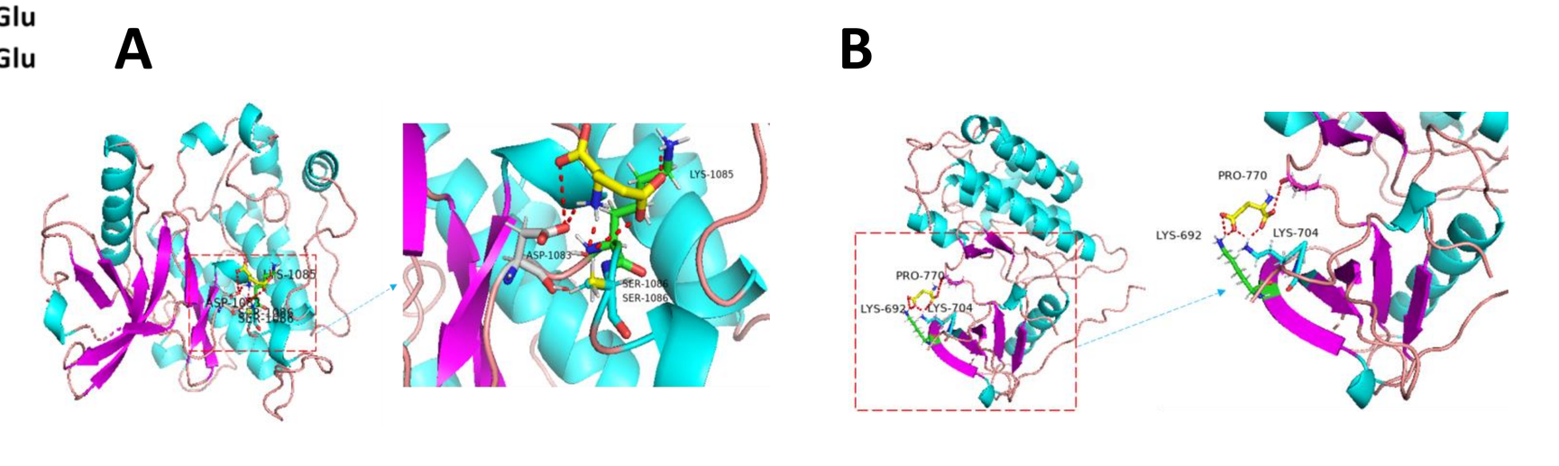


Figure 7. Molecular docking between glutamate and receptor (IR/EGFR).

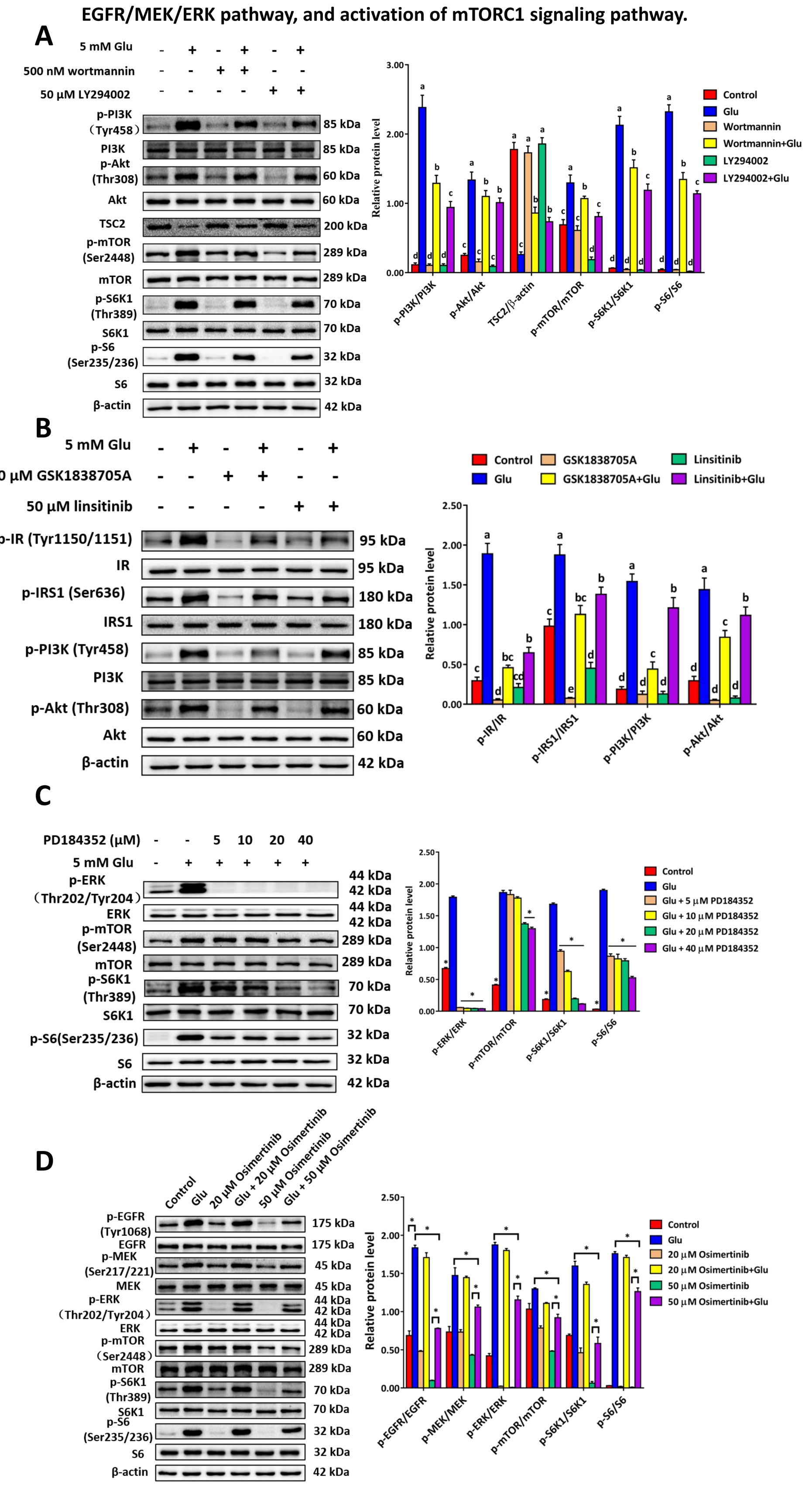
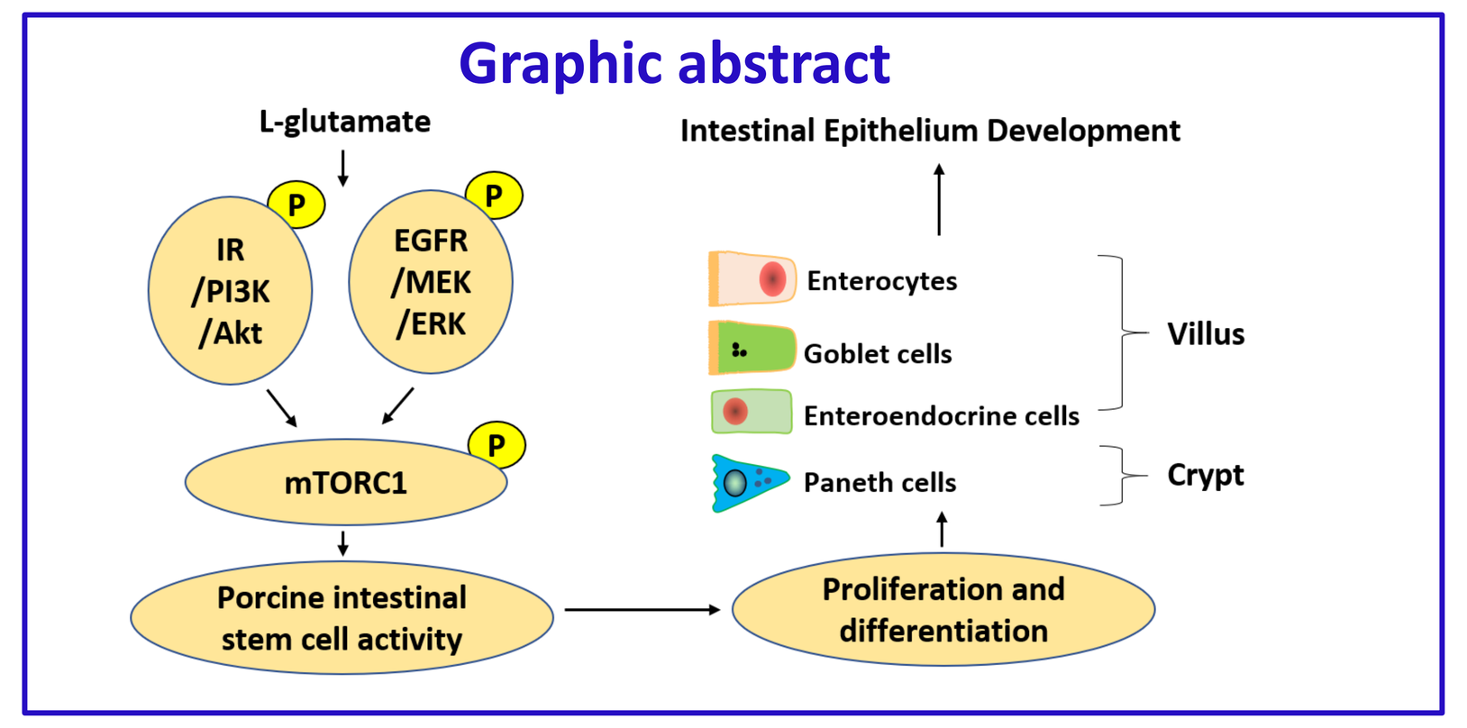


Figure 8. Glu-induced mTORC1 signaling activation via IR/PI3K/Akt pathway and EGFR/MEK/ERK pathway in IPEC-J2 cell line.

Conclusion

In summary, mTORC1 integrated Glu signal via IR/PI3K/Akt and EGFR/MEK/ERK pathway stimulated ISC expansion and ultimately promoted intestinal epithelial development.



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