Title: Epidermal growth factor promotes intestinal secretory cell differentiation in weaning piglets via Wnt/β-catenin signaling

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**Journal:** animal

**Sup****plementary Mat****erial** **S1****:** **Analysis of** **w****estern blotting**

Protein abundances of β-actin, lysozyme, non-p-β-catenin and β-catenin were based on the western blotting analysis. The frozen samples were powdered under liquid nitrogen, and lysed in 1ml ice-cold radioimmunoprecipitation assay buffer [150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), and 50 mM Tris-HCl (pH 7.4)], supplemented with protease inhibitor phenylmethanesulfonyl fluoride (10µL/1ml). The lysates were centrifuged at 12 000 × *g* for 10 min at 4°C. Total protein content of the supernatant fluids was determined using a bicinchoninic acid protein assay kit. All samples were adjusted to an equal protein concentration and then diluted with 5 × loading buffer [0.63 ml of 0.5 M Tris-HCl (pH6.8), 0.42 ml 75% glycerol, 0.125 g SDS, 0.2ml β-mercaptoethanol, 0.2 ml 0.05% solution of bromphenol blue, and 1 ml water] and heated in boiling water for 10min to denature. The denatured proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on a 10% gradient gel. Separated proteins were transferred onto polyvinylidene fluoride (PVDF) membranes in transfer buffer. Make notches in the corner of membranes for orientation.

Immunodetection: Membranes were blocked at room temperature for 2 h with 5% non-fat dried milk in tris-buffered saline with tween (TBST), then incubated with primary antibody [β-actin (Santa, SC-47778; 1:3000 dilution); lysozyme (abcam, ab2408; 1:1000 dilution); non-p-β-catenin ([Cell Signaling Technology](http://www.sogou.com/link?url=DSOYnZeCC_qY5nWg7_9-cidQRoztLvNXreaVHN88tHY.), D2U8Y; 1:1000 dilution); β-catenin ([Cell Signaling Technology](http://www.sogou.com/link?url=DSOYnZeCC_qY5nWg7_9-cidQRoztLvNXreaVHN88tHY.), D10A8; 1:1000 dilution)] overnight at 4°C. After washing the membranes thrice in TBST, they were incubated with the corresponding horseradish peroxidase-conjugated secondary antibody for 2 h at room temperature. The developing was used to visualize the immunoreactive bands by adding chemiluminescence solution (Applygen) and using an analytical imaging system. The developing time of β-actin, lysozyme, *non-p-β-catenin*, *β-catenin* is 1s, 1min, 35s, 10s, respectively. Measurements of protein bands were made using Gel-pro Analyzer software (Media Cybernetics).

**Su****pple****m****entary** **Figure S1:**

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**Supplementary Figure S1** Effects of dietary supplement with epidermal growth factor (EGF) on lysozyme protein abundance of piglets on day 7 (A) and day 14 (B) post-weaning. Protein abundance of lysozyme was determined by western blotting. The β-actin was used as loading control. MW = molecular weight. **Sup****plementary Figure S2:**

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**Supplementary Figure S2** Effects of dietary supplement with epidermal growth factor (EGF) on the activity of Wnt/β-catenin signaling in weaning piglets. Protein abundance of non-phospho (p)-β-catenin and β-catenin was determined by western blotting. A. The protein abundance of non-p-β-catenin and β-catenin in the villus on day 7 post-weaning; B. The protein abundance of non-p-β-catenin and β-catenin in the villus on day 14 post-weaning; C. The protein abundance of non-p-β-catenin and β-catenin in the crypt on day 7 post-weaning; D. The protein abundance of non-p-β-catenin and β-catenin in the crypt on day 14 post-weaning. The β-actin was used as loading control. MW = molecular weight.