

Suppression of Hippo Pathway by Food Allergen Exacerbates Intestinal Epithelia Instability and Facilitates Hypersensitivity

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Scope: Hippo signaling is a crucial pathway in innate immune responses, but the relationship between food allergy and Hippo pathway is unknown. The aim of this work is to investigate the regulation of food allergy by Hippo pathway and reveal the molecular mechanisms.

Methods and results: Two food allergens tropomyosin and ovalbumin are used to challenge a mouse model and CMT93 intestinal epithelia cell model. The allergic responses and the activation of Hippo pathway are tested in these models. In the mouse model, both allergens triggered significant allergic responses, and Hippo pathway is suppressed after allergen challenge. In CMT93, both allergens upregulate the expression of allergic cytokines thymic stromal lymphopoietin, interleukin (IL)-25, and IL-33. In TAZ KD CMT93, the Hippo pathway is blocked, and the expression of allergenic cytokines are also suppressed.

Conclusions: Both in vivo and in vitro data demonstrate that the two food allergens suppressed Hippo pathway by downregulating TAZ expression, resulting in intestinal epithelia instability, and finally leading to hypersensitivity reactions. These findings provide potential therapeutic targets and molecular markers for food allergy, and provide dietary guidelines for allergenic individuals.

80% of shrimp allergic subjects. Moreover, shellfish tropomyosin has multiple IgE-binding sites that cross-react with IgE from cockroaches and termites allergic patients.^[6,7] Similarly, ovalbumin (OVA) comprises 54% of the protein in egg white, and is the main allergen in egg, frequently inducing allergic reactions especially in children.^[8,9] The food allergy is a quite complicated physiological process involving various cell types such as the intestinal epithelial cell, dendritic cell, T cell, B cell, and mast cell, and is distinguished from other adverse reactions to food.^[10] Due to its complexity, the detailed mechanisms of food allergy is not fully understood, and the therapeutic strategies are limited currently.^[11]

Hippo pathway controls organ size by regulating cell growth, proliferation, and apoptosis, and participates in development, oncogenesis and immune responses.^[12–14] The Hippo pathway comprises a core kinase cassette that consists of a pair of related serine/threonine kinases termed MST1/2 and LATS1/2,

together with the adaptor protein SAV1. After upstream signaling stimulation, these kinases are activated and phosphorylate the transcriptional coactivator YAP1 and TAZ, making them remaining in the cytoplasm and undergoing rapid degradation in proteasome, so as to suppress the expression of downstream target proliferation-promoting genes such as CTGF and Cyr61.^[15,16]

Recent studies have demonstrated critical functions of Hippo signaling in innate immune responses against pathogens and autoimmune diseases.^[17] The expression of FOXO1, IL-7 receptor, and BCL2 was impaired in MST1-deficient T cells, resulting in immunodeficiency phenotype include a progressive loss of naïve T cells, followed by recurrent bacterial and viral infections and autoimmune manifestations.^[18] On the other hand, TAZ can enhance the differentiation of inflammatory TH17 cells and attenuated the development of immunosuppressive Treg cells, providing a potential therapeutic target of TH17-mediated inflammatory diseases.^[19] Nevertheless, the understanding of how Hippo signaling regulate immune responses is limited, and the relationship between Hippo pathway and food allergy is currently unknown.

1. Introduction

Food allergies can induce severe anaphylactic reactions. In recent years, the prevalence of food allergies has increased rapidly, and $\approx 8\%$ children and 4% adults have allergic symptoms to one or more foods. Therefore, food allergy has become a global health issue and has emerged as an unanticipated “second wave” of the allergy epidemic.^[1–4] Food allergens are usually proteins that can induce hypersensitive reactions. The major allergy responses ascribe to only limited kinds of proteins, which are considered as the predominant allergens of foods.^[5] For example, tropomyosin (TM) is the major allergen of shellfish, interacting with more than

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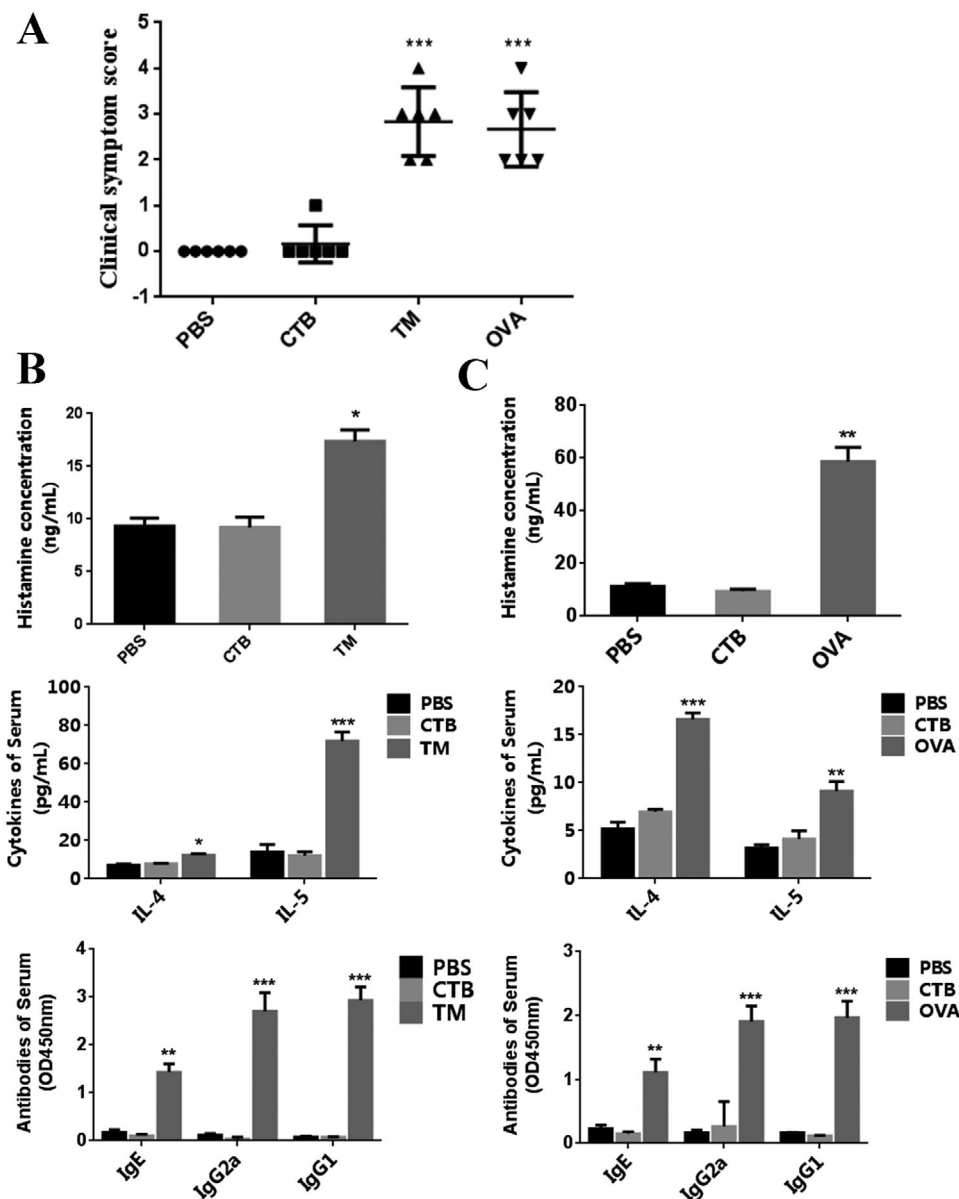


Figure 1. Food allergens induced hypersensitivity reactions in the mouse model. A) Clinical symptom score of the model mice. B,C) The concentrations of serum histamine, allergenic cytokines (IL-4/5), and antibodies (IgE, IgG2a, and IgG1) were measured in TM-induced food allergy mouse model (B) or OVA-induced food allergy mouse model (C). Data in graphs are expressed as means $\pm$ SEM, significant differences are indicated by * p < 0.05, ** p < 0.01, and *** p < 0.001.

Overall, this study was to investigate the potential links between food allergy and Hippo signaling pathway, and reveal the underlying mechanisms. To this aim, an in vivo mouse model and an in vitro intestinal epithelial cell model were established using shrimp TM and hen egg OVA to simulate food allergy in human bodies. Based on these approaches, we found that food allergens inhibited Hippo signaling pathway to disturb the immune system and therefore exacerbate allergic responses. These results identified the relationship between Hippo pathway and food allergy, provided insights for intensive investigations on food allergy, and revealed potential targets for controlling food allergy.

2. Results

2.1. Food Allergy Symptoms Induced by Allergen

To investigate the molecular mechanisms of food allergy, a food allergy mouse model was established by intragastric gavage (IG) of purified egg allergen OVA or shrimp allergen TM accompanied with cholera toxin subunit B (CTB) as the mucosal adjuvant. As shown in **Figure 1**, while the application of CTB alone only slightly triggered allergenic responses, the presence of either TM or OVA resulted in severe clinical symptoms. In addition, allergen administration significantly triggered histamine,

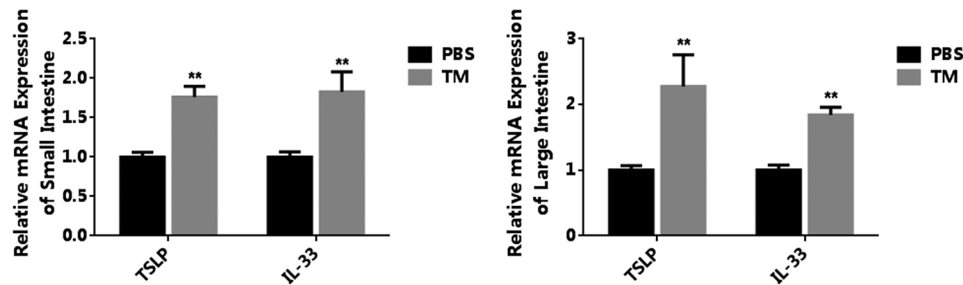


Figure 2. Allergens changed the production of allergenic cytokines in the intestine. The production of allergenic cytokines TSLP and IL-33 in small intestine (left) and large intestine (right) was determined in the TM-induced food allergy mouse model. Data in graphs are expressed as means $\pm$ SEM, significant differences are indicated by * p < 0.05, ** p < 0.01, and *** p < 0.001.

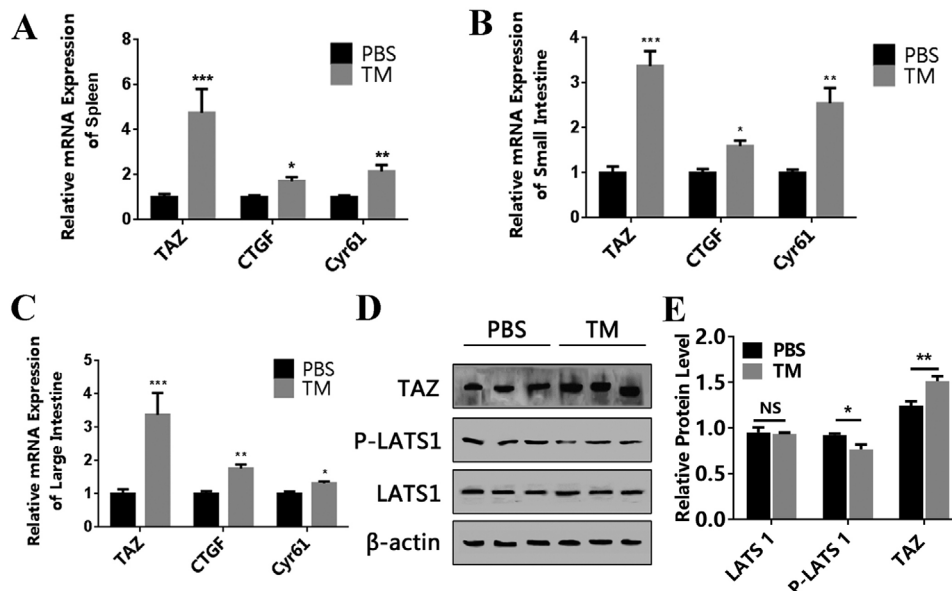


Figure 3. TM inhibited Hippo signaling in vivo. The mRNA expression of TAZ, CTGF, and Cyr61 in A) spleen, B) small intestine, and C) large intestine was determined in the TM-induced food allergy mouse model. D) The protein level of TAZ, LATS1, and phosphorylated LATS1 were measured by Western blot, the expression of β -actin was used as the loading control. E) Quantified Western blot result based on (D). Data in graphs are expressed as means $\pm$ SEM, significant differences are indicated by * p < 0.05, ** p < 0.01, and *** p < 0.001.

Th2-type allergenic cytokines (interleukin [IL]-4 and IL-5), as well as allergen-specific antibodies (IgE, IgG2a, and IgG1) production. These results indicated that TM and OVA successfully induced food allergy responses in the constructed mouse model, which can be used in subsequent experimental research.

It has been reported that food allergens can stimulate the intestinal epithelial cells, impaired the epithelial barrier function, and induced the production of allergenic cytokines such as thymic stromal lymphopoietin (TSLP), IL-25, and IL-33.^[20,21] Consistently, in the TM-allergy mouse model, the expression of TSLP and IL-33 were significantly upregulated in both small and large intestines (Figure 2). However, the mechanisms of how food allergen regulated epithelial function required further investigation.

2.2. Food Allergens Inhibited Hippo Signaling in Mouse

It has been reported that Hippo pathway participates in epithelial function and immune responses.^[22,23] In order to elucidate

whether Hippo pathway is involved in the allergic responses of intestinal epithelia, the activity of Hippo pathway under allergen stimulation was assessed. Based on the food allergy mouse model, the expressions of Hippo pathway related genes were measured by quantitative PCR (qPCR) in spleen, large intestine, and small intestine. As shown in Figure 3, TM increased the expression of TAZ, a core transcriptional coactivator and regulator of the Hippo pathway. In addition, the expression of CTGF and Cyr61, the Hippo pathway downstream target genes involved in the stability of intestinal epithelia, were also upregulated. Furthermore, Western blot revealed that TM treatment upregulated TAZ protein level and suppressed the phosphorylation of the core kinase LATS1 in spleen (Figure 3D,E). Similarly, under OVA treatment, the expression of TAZ, CTGF, and Cyr61 were also upregulated in spleen, large intestine, and small intestine (Figure S2, Supporting Information). These data all indicated the inhibition of Hippo pathway during TM-induced food allergy process, implying the relationship between food allergy, Hippo pathway, and epithelial function.

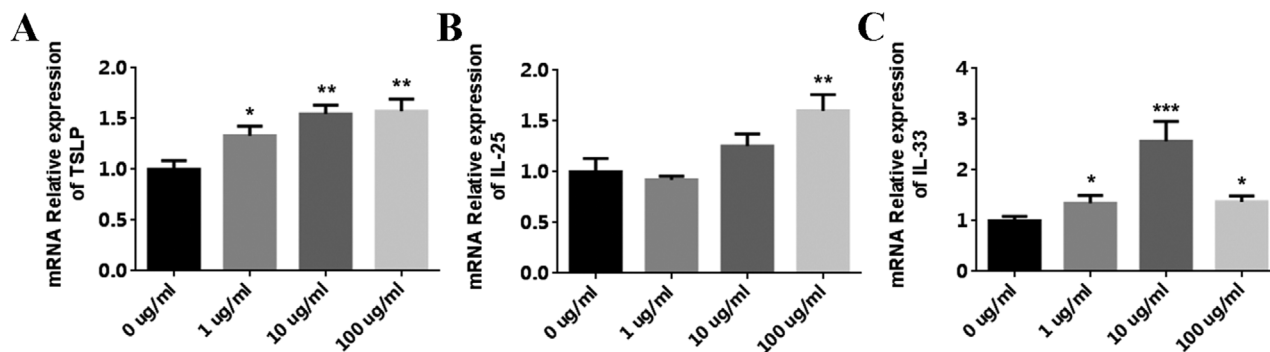


Figure 4. TM changed the expression of allergenic cytokines in CMT93. CMT93 cells were treated with indicated concentration of TM, and the production of allergenic cytokines, including A) TSLP, B) IL-25, and C) IL-33 was determined by qPCR. Data in graphs are expressed as means $\pm$ SEM, significant differences are indicated by * p < 0.05, ** p < 0.01, and *** p < 0.001.

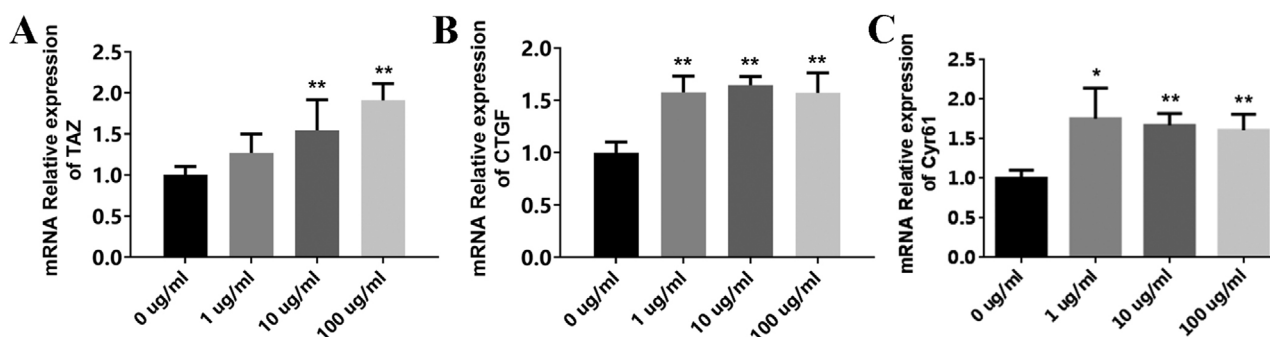


Figure 5. OVA inhibited Hippo signaling in CMT93. CMT93 cells were treated with indicated concentration of OVA, and the production of Hippo-related genes, including A) TAZ, B) CTGF, and C) Cyr61 was determined by qPCR. Data in graphs are expressed as means $\pm$ SEM, significant differences are indicated by * p < 0.05, ** p < 0.01, and *** p < 0.001.

2.3. Food Allergens Induced Allergic Cytokines Production by Intestinal Epithelia

To intensively investigate how food allergens stimulate intestinal epithelial barrier, an intestinal epithelial model based on mouse colon cell line CMT93 was established. The cells were treated with representative food allergens including TM (Figure 4) and OVA (Figure S3, Supporting Information) to mimic food allergen process in vivo, and the expression of epithelial allergenic cytokines including TSLP, IL-25, and IL-33 were detected by qPCR.

As shown in Figure 4 and Figure S3, Supporting Information, the expression of TSLP, IL-25, and IL-33 were all upregulated after TM or OVA stimulation. Most of these effects showed a dose-dependent manner on allergens, indicating that TM and OVA significantly induced the allergic responses of epithelial cells. Nevertheless, high concentration ($100 \mu\text{g mL}^{-1}$) of TM and OVA cannot maximize the expression of IL-25 and IL-33, respectively, which may due to the non-specific side effects.

2.4. Food Allergens Inhibited Hippo Signaling Pathway in Intestinal Epithelia

Based on the mouse model, Hippo pathway was considered as a major signaling mediating the allergic responses of epithelia. To further validated the effect of Hippo pathway, the expression of pathway-related genes was quantified by qPCR in the

cell model. OVA dose-dependently increased the expression of TAZ (Figure 5A). In addition, the expression of CTGF and Cyr61 were also upregulated by OVA (Figure 5B,C). Similar results were also observed in TM-stimulated cells (Figure S4, Supporting Information). TM dose-dependently upregulated the expression of TAZ, meanwhile the expression of CTGF and Cyr61 were also increased.

2.5. Hippo Pathway Mediates the Allergic Responses of Epithelia

To further reveal the direct role of Hippo pathway in food allergy activity, a TAZ knock-down CMT93 cell line (TAZ KD) was established by short hairpin RNA (shRNA), in which Hippo pathway was consistently activated.^[24] qPCR results confirmed that TAZ transcription was reduced to less than 50% in TAZ KD (Figure 6A), and the expression of CTGF and Cyr61 was also suppressed (Figure 6B,C), confirming the consistent activation of Hippo pathway. Based on this, these cells were further treated by OVA, and the inhibition of Hippo pathway in both wild type (WT) and TAZ KD cells were assessed by downstream genes expression. As shown in Figure 6, compared with WT cells, the up-regulation of TAZ, CTGF, and Cyr61 expression induced by OVA treatment were totally blocked. Identical results were observed under TM treatment (Figure 6A–C). These results verified the consistent activation of Hippo pathway in the TAZ KD cell line.

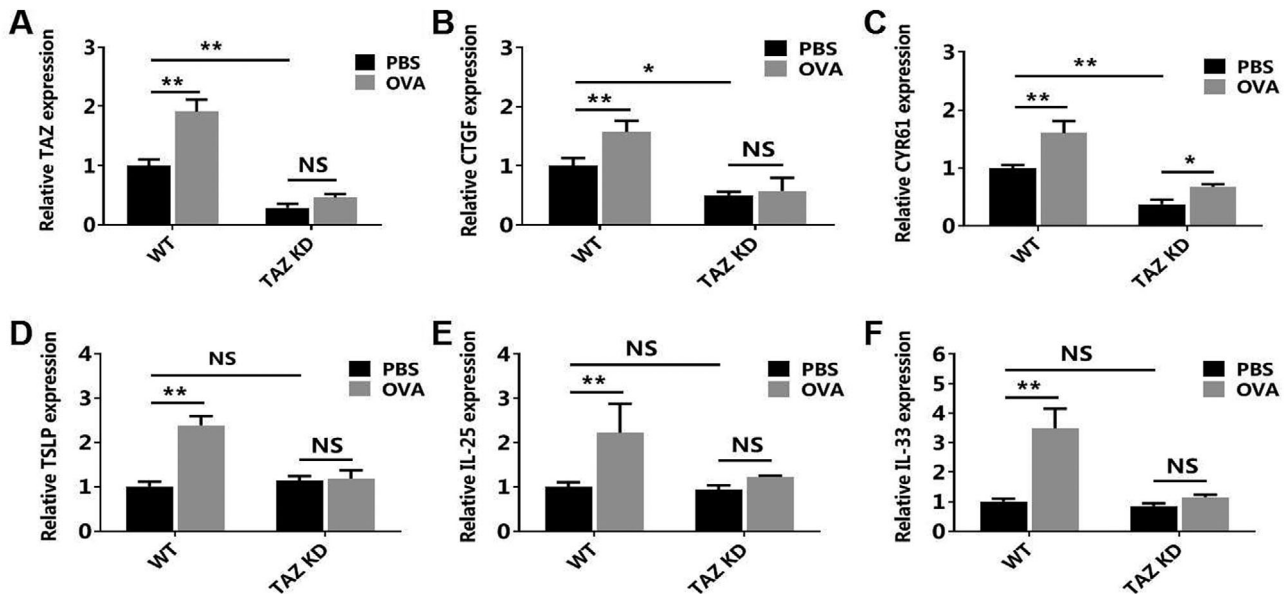


Figure 6. TAZ was required for OVA-induced allergenic responses. Both WT and TAZ KD CMT93 cells were treated with OVA or PBS (negative control), the expression of A) TAZ, B) CTGF, C) Cyr61, D) TSLP, E) IL-25, and F) IL-33 was determined by qPCR. Data in graphs are expressed as means $\pm$ SEM, significant differences are indicated by * p < 0.05, ** p < 0.01, and *** p < 0.001.

In addition to the activation of Hippo pathway, the expression of allergenic cytokines with allergen stimulation were also analyzed in TAZ KD cell line. As shown in Figure 6, while OVA significantly increased TSLP, IL-25, and IL-33 expressions in WT CMT93, this effect was not significant in TAZ KD. Similarly, for cells stimulated by TM, the increment of TSLP, and IL-33 expression were blocked in the absence of TAZ, and the upregulation of IL-25 was also impaired (Figure S5, Supporting Information).

3. Discussion

Hippo signaling pathway plays crucial role in regulating the human immune system, but the detailed mechanisms are not fully understood and the participation in food allergic diseases related to human autoimmunity is not clear.^[17] In this study, we evaluated the relationship between Hippo signaling pathway and food allergy using both in vivo and in vitro approaches, and found that food allergens suppressed TAZ-dependent Hippo pathway to exacerbate intestinal epithelia instability and facilitates hypersensitive reactions.

We first found that the expression of Hippo-related genes, including TAZ, CTGF, and Cyr61, in various organs of food allergic mice showed a significant upward trend, indicating the inhibition of Hippo signaling pathway. Notably, the gene CTGF has been proved to play a key role in allergic asthma.^[25] In agreement, a previous study demonstrated that TAZ can enhance TH17 differentiation and attenuates Treg differentiation, leading to the occurrence of autoimmune diseases.^[19] Although food allergy was not involved in that study, it has been revealed that allergenic function of TH17 as well as the anti-allergenic function of Treg.^[26,27] These findings strongly implied the potential regulatory role of Hippo pathway in food allergy process.

Hippo pathway is crucial in regulating solid organ size, which can be regulated by mechanotransduction, cell adhesion, and

contact inhibition.^[28] Consistently, our previous results showed the low expression level of Hippo pathway-related genes in suspension cells such as most of the immune cells (data not shown). Therefore, we first tested intestinal epithelial cells as the potential target cells of allergen-regulated Hippo pathway, which highly expresses Hippo-related genes and participate in food allergy at the same time. As expected, food allergens significantly promoted the expression of genes downstream of Hippo pathway in the mouse intestinal epithelial cell line CMT93. As no other cells were present in this system, we concluded that allergens directly inhibited Hippo pathway in intestinal epithelial cells, rather than mediated by other cell types, implying intestinal epithelial cells to be the direct receptor of food allergens and may transduce the signaling to downstream cells. Similarly, various previous studies as well as our data have demonstrated that food allergy will impair the intestinal epithelia stability, resulting in the producing of specific cytokines such as TSLP, IL-25, and IL-33, thus triggering downstream immune responses.^[29] Consistently, in the CMT93 cell model, we also revealed the upregulation of these cytokines after allergen stimulation, implying the allergen-triggered Hippo pathway suppression in intestinal epithelial cells could be a pilot event in food allergy processing. The direct suppression of Hippo pathway made it to be a reliable molecular marker for food allergy diagnosis, our results of two different allergens also supported the robustness of the marker. Additionally, as a conserved pathway in mammals including mouse and human,^[30] Hippo could also be a promising food allergy marker in clinical applications.

When the Hippo pathway is suppressed, LATS1/2 failed to phosphorylate YAP1 and TAZ, thus these cotranscriptional factors can enter the nuclear to facilitate target genes transcription, resulting in subsequent physiological responses.^[31] Noticeably, after allergens stimulation, the upregulation of TAZ was observed both in vitro and in vivo. Based on this, we reasoned that TAZ is a crucial mediator in the Hippo pathway in response to

allergen stimulation. To validate the critical role of TAZ, as well as Hippo pathway, in food allergen-induced intestinal epithelial instability, the TAZ KD CMT93 cell line was established. As expected, all the allergen-induced reactions, including the expression of Hippo-downstream genes, as well as the production of allergenic cytokines, were blocked in TAZ KD cells. These results strongly demonstrated that food allergens suppressed Hippo pathway to exacerbate intestinal epithelia instability, which subsequently resulted in the production of allergenic cytokines and facilitated hypersensitivity. But how food allergens downregulated TAZ expression and what is the direct receptor molecular of allergens require far more investigations. On the other hand, since the consistent activation of Hippo pathway by suppression TAZ could block most of the allergic responses, TAZ, as well as other crucial regulators in Hippo pathway, were considered to be potential therapeutic targets for food allergy.

It has long been investigated what makes a protein allergenic.^[32] Previous studies have revealed various general characters of different allergens, but the question has not been fully understood currently. In this study, we found that food allergens suppressed the Hippo pathway in intestinal epithelial cells. Interestingly, TM and OVA showed quite similar responses on Hippo pathway. Therefore, the suppression of Hippo pathway would be a general character of at least a part of food allergens, and the investigation of food allergen-suppressed Hippo signaling might reveal the mechanisms of how allergens induce allergenic responses. Particularly, because similar allergens may show comparable activity in suppressing Hippo pathway, whether this event can facilitate the cross-reactivity of different allergens is an interesting question to be solved.

Based on our finding, Hippo pathway would be a potential target for managing food allergies in a universal way. On the other hand, the suppression of Hippo pathway is potentially a general molecular marker for diagnosing and even predicting food allergies. On the other hand, Hippo pathway would be a promising target for preventing and treating food allergy. However, before practical applications, far more investigations are required to reveal the detailed underlying mechanisms, and clinical researches are also required for validating the exact physiological relevant between Hippo pathway and food allergy.

Overall, based on the above results, we conclude that food allergens suppressed Hippo pathway by downregulating TAZ expression, resulting in intestinal epithelia instability, and finally leading to hypersensitivity reactions. These findings partially revealed the molecular mechanisms of food allergy, threw light on further investigations on food allergy, and provided potential therapeutic targets and molecular markers for food allergy.

4. Experimental Section

Ethics Approval and Consent to Participate: This study was carried out in strict accordance with the recommendations of the National Guidelines for the Care and Use of Laboratory Animals of China. The Zhejiang Gongshang University Laboratory Animal Welfare Ethics Review Committee approve all the animal procedures.

Materials: TM was extracted and purified as previously described from acetone powder prepared with whole bodies of peeled frozen *Litopenaeus vannamei*.^[33] The protein purity and concentration of the fractions were determined using the SDS-PAGE and BCA Protein Assay Kit (KeyGEN BioTECH, China), respectively. OVA were purchased from Sigma (MO,

USA). LAT1 and Phospho-LAT1 (Thr1079) antibodies were bought from Cell Signaling Technology (MA, USA), TAZ antibody was from Santa Cruz Biotech (CA, USA), and β -actin antibody was from Boster Biological Technology (Wuhan, China). IL-4 Mouse Uncoated Enzyme-linked immunosorbent assay (ELISA) Kit with Plates and IL-5 Mouse ELISA Kit was from Thermo Fischer Scientific (CA, USA). Histamine (HIS) ELISA Kit was from MULTISCIENCES LIANKE) BIOTECH (Hangzhou, China).

Animals and Immunization Protocol: Thirty female BALB/c mice (aged 6–8 weeks, weighted 18–22 g) were purchased from and housed in the Laboratory Animal Center of Hangzhou Normal University (Hangzhou, China) and fed a basic diet (XIETONG ORGANISM, China) (Table S1, Supporting Information). Groups of ten mice were treated according to the sensitization protocol, with the challenges performed according to Huang and Liu et al.,^[34,35] and the protocol is illustrated in Figure S1, Supporting Information. In brief, each mouse was immunized by IG administration of purified TM (3 mg for sensitization and 9 mg for challenging each time) or OVA (10 mg for sensitization and 30 mg for challenging each time), together with recombinant CTB (250 μ g each time) as an adjuvant, for a total of 200 μ L of PBS, as indicated. The negative control group (PBS group) received an equal volume of PBS only, the adjuvant control group (CTB group) received an equal dose of CTB without TM or OVA.

Clinical Symptoms Evaluation: The appearance of clinical symptoms combined with anaphylaxis and diarrhea was observed during 30–60 min after the last challenge. Symptoms were evaluated according to a scoring system combining the systems reported by Tan et al.^[36] and Zhang et al.^[37] as follows: 0, no symptoms, normal stools; 1) repetitive mouth/ear scratching and ear canal digging with hind legs, a few wet and unformed stools; 2) decreased activity, self-isolation, and puffiness around eyes and/or mouth, wet and unformed stools with some yellow mucus; 3) periods of motionlessness for more than 1 min, lying prone on stomach, and decreased activity, more wet and unformed stools with a lot of yellow mucus; 4) no response to whisker stimuli and reduced or no response to prodding, a larger number of wet and unformed stools with moderate perianal staining of the coat; 5) tremor, convulsion, and death, severe watery stool with severe perianal staining of the coat.

ELISA: OVA-specific and TM-specific IgE, IgG2a, IgG1 were measured by ELISA as previously described.^[38,39] Briefly, plates (Costar, Corning Life Sciences, USA) were coated with purified TM (10 μ g mL⁻¹) or OVA (100 μ g mL⁻¹) overnight at 4 °C. After washing, wells were blocked with blocking solution (Sangon Biotech, China) for 1 h at 37 °C. After washing again, serum samples were added (diluted 1:6 for IgE measurement, 1:200 for IgG1 and IgG2a) and incubated for 2 h at 37 °C. Then, bound antibodies were detected using horseradish peroxidase-labeled goat monoclonal anti-mouse IgE, IgG1, and IgG2a (all were diluted 1:6000) (Southern Biotechnologies Associates, USA) and the substrate 3,3',5,5'-tetramethylbenzidine (eBioscience, USA). The absorbance was determined with a VersaMax microplate reader (Molecular Devices, USA) at 450 nm. The levels of IL-4, IL-5, and histamine from the serum were measured using appropriate ELISA kits according to the manufacturer's guidance (MULTI SCIENCES, China).

Western Blot: Spleens isolated from mouse were grinded in liquid nitrogen, and the total protein was extracted by Tissue Total Protein Lysis Buffer (Sangon Biotech, China), and the Western blot analysis was conducted with indicated antibodies as previously reported.^[40] 40 μ g of total protein extract was loaded for each sample.

Cell Culture: The CMT93 cells were originally obtained from American Type Culture Collection (Virginia, USA). The cells were maintained in DMEM (KeyGENBioTECH, Nanjing, China) containing 10% fetal bovine serum and 1% Penicillin-Streptomycin Solution (Sangon Biotech, Shanghai, China), and cultured at 37 °C with 5% CO₂.

Knock-Down of TAZ Gene in CMT93: The sequence of the shRNA targeting TAZ mRNA was obtained from MISSION shRNA database (Sigma-Aldrich, USA) and cloned into the lentiviral knock-down vector pLKO.1-Puro (Addgene, USA). To prepare lentiviral particles, the vector was co-transfected with packaging plasmids (pLP1, pLP2, and pLP/VSVG) into HEK293T cells. Viral supernatants were harvested 48 h after transfection and applied to CMT93 with 10 μ g mL⁻¹ polybrene (Sigma-Aldrich, USA). 24 h later, the cells were incubated with 8 μ g mL⁻¹ puromycin to eliminate

uninfected cells. After 1 week of selection, the knock-down efficiency was verified by qPCR, and the cells were ready for subsequential experiments. The primers for shRNA cloning are:

- 1) Forward-5'-CCGGCCAGGATTCAAGCACAGCTAACTCGAGTTAGCTG-TGCTTGAATCCTGGTTTTC-3'
- 2) Reverse-5'-AATTCAAAAACAGGATTCAAGCACAGCTAACTCGAGTT-AGCTGTGCTTGAATCCTGG-3'.

Gene Expression Quantification by Real-Time qPCR: Cells were planted in 24-well culture plates and treated with OVA (1, 10, or 100 $\mu\text{g mL}^{-1}$), TM (1, 10, or 100 $\mu\text{g mL}^{-1}$), or PBS as indicated for 48 h.^[41]

Total RNA of cell samples was extracted using the E.Z.N.A. Total RNA Kit II (Omega Bio-Tek, USA). The reverse transcription of RNA to cDNA was performed using the HiScript II qRT Super kit (Vazyme Biotech, USA). The real-time quantitative PCR analysis was performed using the SYBR Green I kit (Vazyme Biotech, USA) with the LightCycler 480 II system (Roche, USA). The expression of GAPDH was served as the internal standards for calibration. The sequences of qPCR primers are listed in Table S2, Supporting Information.

Statistical Analysis: All the statistical results were analyzed with three replicates according to a completely randomized design. All data were performed by analysis of variance or Student's *t*-test. Data were analyzed statistically by repeated measures using GraphPad Prism software, and *p* < 0.05 was considered as statistically significant.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

animal models, basic mechanisms, biomarkers, food allergy, signal transduction

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1 **Supporting Information**

2 **Table S1.** The nutrition facts of mouse feed.

Feed composition	Weight (g per 1000 g feed)
Essential amino acid	97
Non-essential amino acid	79
Carbohydrates (Dextrin, Sucrose)	619
Cellulose	50
Corn oil	100
Minerals	43
Vitamins (Including choline)	12
Total	1000

3

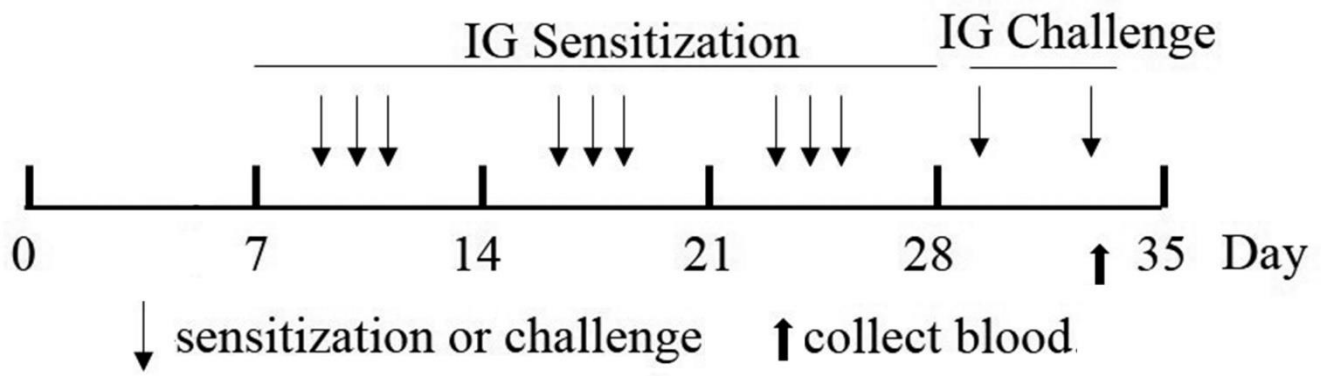
4 **Table S2.** Primers used for qPCR analysis.

Genes	Forward (5' – 3')	Reverse (5' – 3')
TSLP	CAGCTTGTCTCCTGAAAATCG	AAATGTTTTGTCGGGGAGTG
Il-33	GACACATTGAGCATCCAAGG	AACAGATTGGTCATTGTATGTAC
Il-25	CTACAGACAGGCTCCCACATGGACC	CCTGCTGCTTCAGGTAGGGCTTTC

GAPDH	CACACCGACCTTCACCATTTT	GAGACAGCCGCATCTTCTTGT
TAZ	GAGCAACATGGACGAGATGG	AGGTTAGAAAGGGCTCGCTT
CTGF	GGGCCTCTTCTGCGATTTC	ATCCAGGCAAGTGCATTGGTA
Cyr61	AGCGGGCAGTGCTGTGA	GTCGTCCAGGGAGTCCTTAATG

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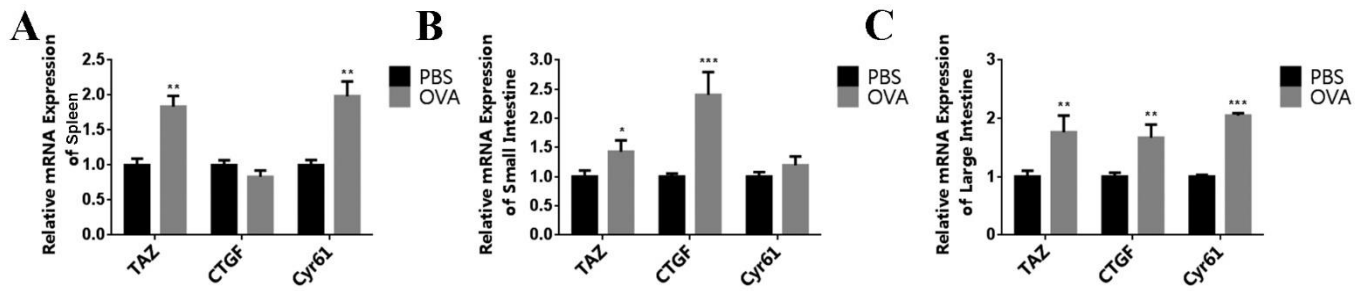


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8 **FIGURE S1** Experimental protocol of the food allergy mouse model.

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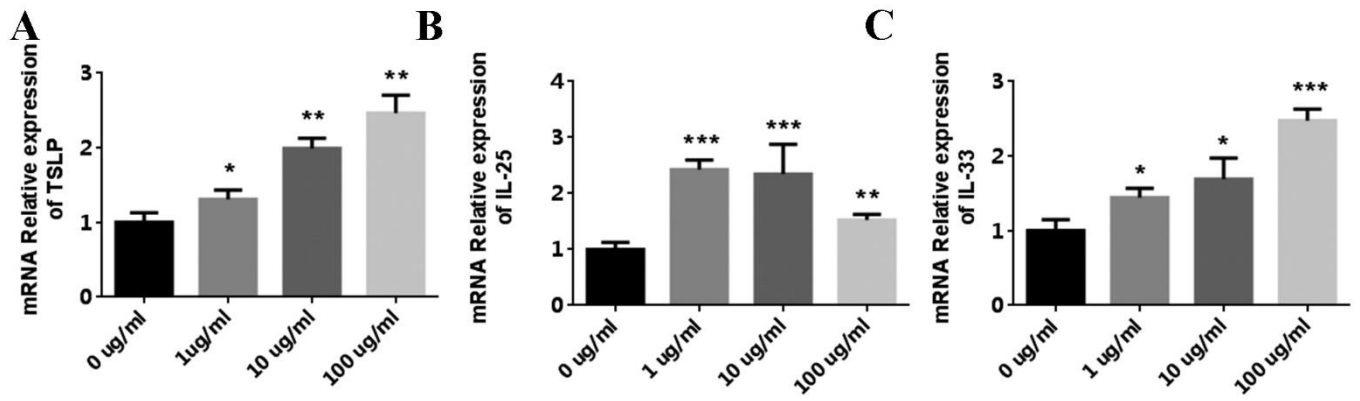
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FIGURE S2 OVA inhibited Hippo signaling in vivo. The mRNA expression of TAZ, CTGF and Cyr61 in spleen (A), small intestine (B) and large intestine (C) was determined in the OVA-induced food allergy mouse model. Data in graphs are expressed as means $\pm$ SEM, significant differences are indicated by * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.



17

18 **FIGURE S3** OVA changed the expression of allergenic cytokines in CMT93. CMT93 cells

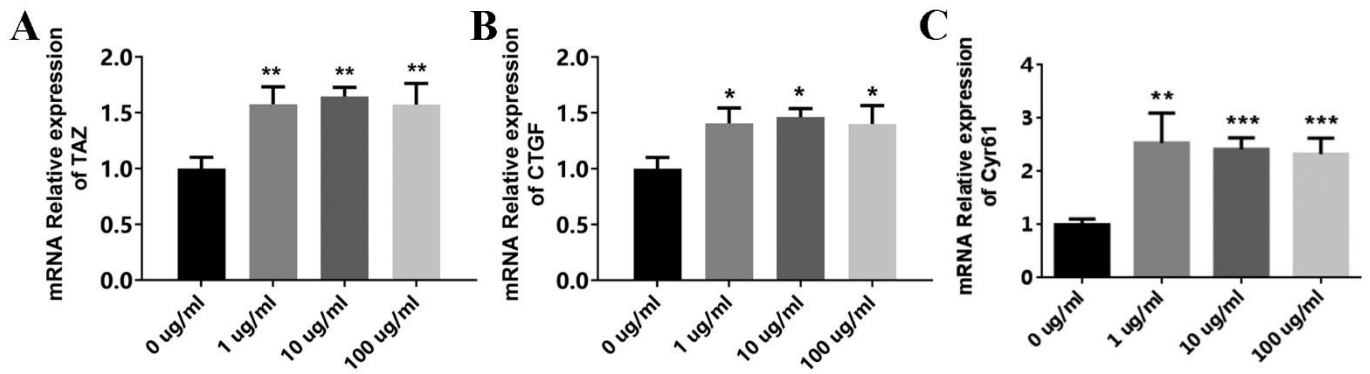
19 were treated with indicated concentration of OVA, and the production of allergenic

20 cytokines, including TSLP (A), IL-25 (B), and IL-33 (C) was determined by qPCR. Data in

21 graphs are expressed as means $\pm$ SEM, significant differences are indicated by * $p < 0.05$,

22 ** $p < 0.01$ and *** $p < 0.001$.

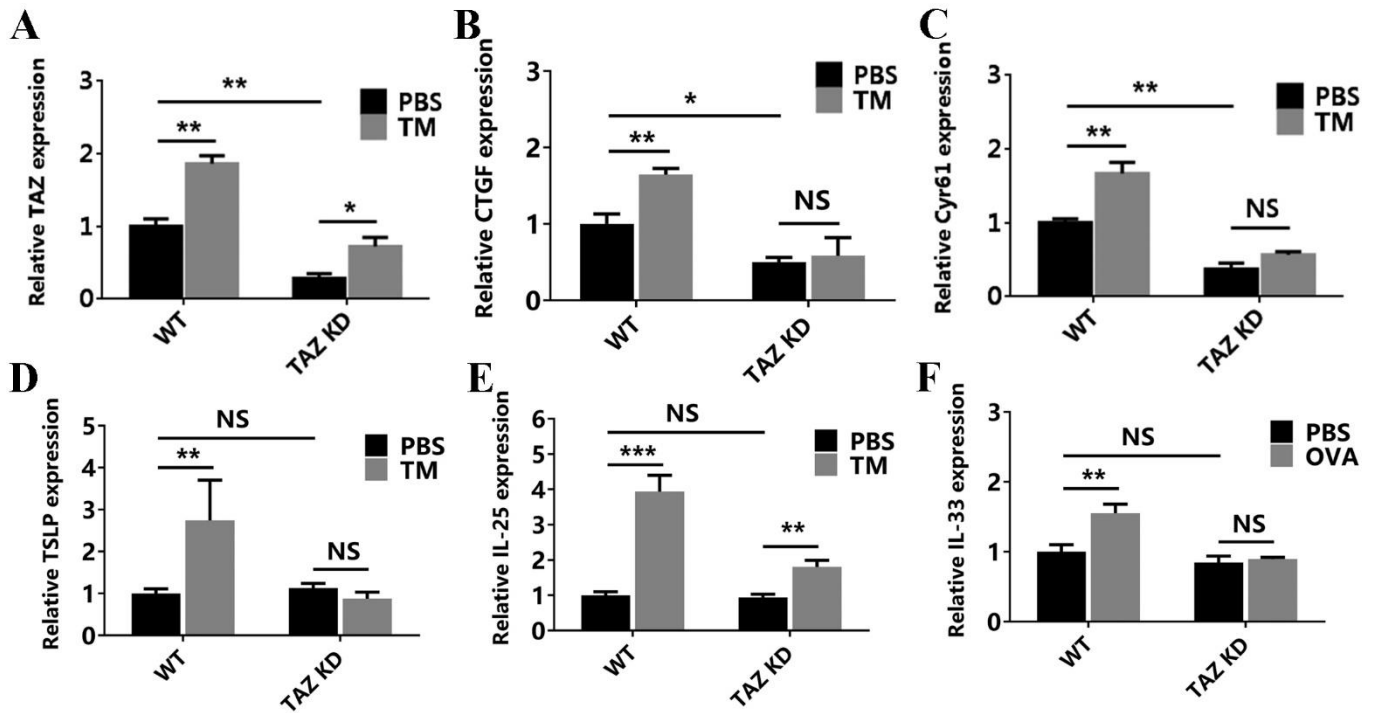
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25 **FIGURE S4** TM inhibited Hippo signaling in CMT93. CMT93 cells were treated with
 26 indicated concentration of TM, and the production of Hippo-related genes, including TAZ
 27 (A), CTGF (B), and Cyr61 (C) was determined by qPCR. Data in graphs are expressed as
 28 means $\pm$ SEM, significant differences are indicated by * $p < 0.05$, ** $p < 0.01$ and *** $p <$
 29 0.001.

30



31

32 **FIGURE S5** TAZ was required for TM-induced allergenic responses. Both WT and TAZ

33 KD CMT93 cells were treated with TM or PBS (negative control), the expression of TAZ

34 (A), CTGF (B), Cyr61 (C), TSLP (D), IL-25 (E), and IL-33 (F) was determined by qPCR.

35 Data in graphs are expressed as means $\pm$ SEM, significant differences are indicated by * $p <$

36 0.05, ** $p < 0.01$ and *** $p < 0.001$.