

LETTERS

MUC1 deficiency induces the nasal epithelial barrier dysfunction *via* RBFOX3 shortage augment ubiquitin-proteasomal degradation in allergic rhinitis pathogenesis

To the Editor,

Allergic rhinitis (AR) is a global health problem with the self-reported prevalence arising significantly.¹ Additionally, the damaged epithelial barrier has been hypothesized to be linked to the AR development.² Our previous research revealed that Mucin 1 (MUC1) deficiency promotes the nasal epithelial barrier dysfunction by inhibiting the expression of tight junction protein CLDN1, while the regulatory mechanism remains unknown.³ RBFOX3, which has been confirmed as a splicing regulator,⁴ is involved in the CLDN1 ubiquitin degradation in lung.⁵ Thus, we aimed to characterize the MUC1-RBFOX3-CLDN1 degradation system in AR pathogenesis.

As in normal human lung tissue, CLDN1 has been observed to be highly expressed in RBFOX3-positive cells⁵; the similar phenotype was evaluated in the nasal mucosa tissue of AR patients, and nasal epithelial cells labeling ubiquitin with RBFOX3 deficiency were observed in AR patients (Figure 1A). Furthermore, CLDN1 expression and RBFOX3 expression were significantly downregulated in the nasal mucosa of AR patients (Figure 1B–D and Figure S5A). Besides, MUC1 deficiency has been demonstrated to reduce CLDN1 expression in AR pathogenesis.³ Taken together, these results suggest that CLDN1 ubiquitination regulated by RBFOX3 may be involved in MUC1-mediated AR pathogenesis.

The ubiquitin-proteasome degradation pathway is the main destination of ubiquitin-modified substrate proteins, which are responsible for the degradation of 80%–85% proteins in eukaryotes.⁶ After establishing the AR mice model with the ubiquitin-proteasome inhibitor MG132 nasal administration (Figure S1), CLDN1 and RBFOX3 were observed to be depressed in the nasal mucosal tissue of AR mice, which is deteriorated in MUC1 deficiency (Figure 2A–D and Figure S5B). Meanwhile, the expression levels of CLDN1 and RBFOX3 were significantly increased in AR mice treated with MG132 (Figure 2A–D and Figure S5B). These findings suggest that MUC1-mediated ubiquitin-dependent CLDN1 degradation could be involved in AR pathogenesis. Moreover, electron microscopy illustrated the protective effect of MG132 treatment on the damages of the nasal epithelial tight junction (Figure 2E). Additionally, the nasal epithelial permeability was receded in AR mice with MG132 nasal

administration (Figure 2F). Together, these results highlight MG132 nasal administration to diminish the dysfunction of nasal epithelial barrier caused by AR. At the same time, compared with AR mice, the number of nasal scratching and the level of serum IgE were significantly decreased in MG132-treated mice (Figure S1F,G). In rhinitis mice with MG132, the density of whiskers decreased, and the symptoms of nasal redness and swelling were significantly relived (Figure S1E). Besides, the differences in nasal mucosal inflammation were not observed in rhinitis mice with or without MG132 treatment (Figure S1C,D). Thus, MG132 nasal administration was indicated to partially relieve allergic rhinitis.

In vitro, ubiquitin-dependent CLDN1 degradation was examined to be generated by Th2 marker IL-4 stress (Figure S2). We knocked out MUC1 and RBFOX3 expression in bronchial epithelium transformed with Ad12-SV40 2B (BEAS-2B) cells (Figure S3A). Then, immunoblots showed that RBFOX3 expression level was depressed in MUC1 cells and CLDN1 expression level was significantly decreased in MUC1 cells and RBFOX3 cells (Figure S3B,C). Additionally, MG132 treatment complemented CLDN1 deficiency caused by IL-4 stimulation (Figure S3C). Furthermore, RBFOX3 overexpression in wild-type (WT) and MUC1 cells upregulated CLDN1 expression and binding capacity (Figure S3D,E). Therefore, we hypothesized that IL-4 stress generated upon CLDN1 depression could be the result of MUC1-mediated defect in the expression of RBFOX3. The purified CLDN1 protein was used as bait for binding assays with stimulated epithelial cell extracts (Figure S4A). Nano LC-MS/MS analysis revealed two E3 ubiquitin ligase interacted with CLDN1 directly: Smurf2 and MARCH5 (Figure S4B). Contrary to expectations, this study only investigated elevated levels of MARCH5 in simulated epithelial cells (Figure S4C), while the expression differences of Smurf2 and MARCH5 in AR mice were not monitored (Figure S4D).

In conclusion, our study demonstrates MUC1 deficiency inhibits CLDN1 expression *via* RBFOX3 shortage augment ubiquitin-proteasomal degradation in AR. Additionally, MG132 nasal administration relieves AR by diminishing nasal epithelial barrier dysfunction.

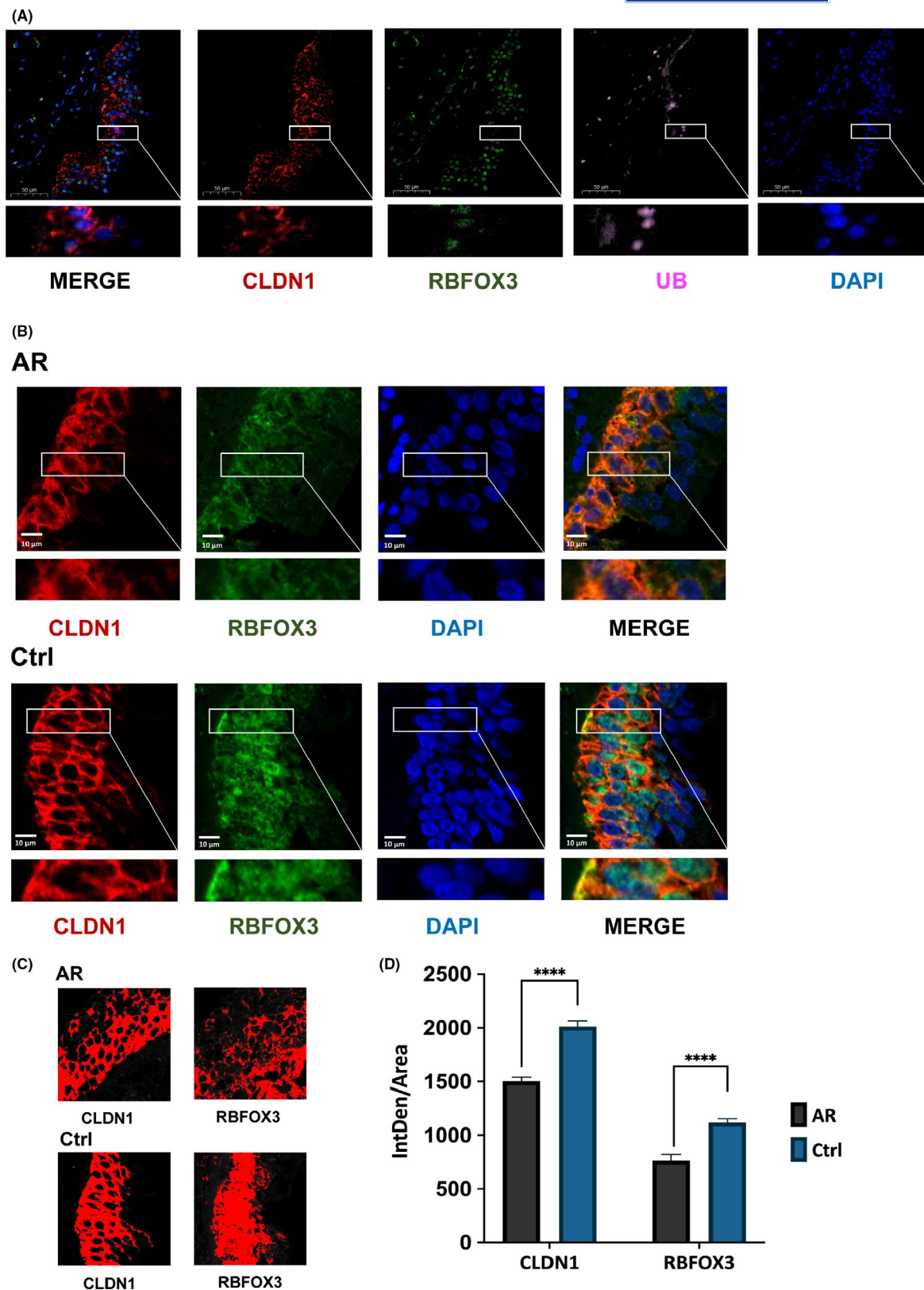


FIGURE 1 Characterization of CLDN1 expression and RBFOX3 expression in the nasal mucosa of AR patients. Panels A and B, human nasal mucosal tissues from AR patients and control subjects were immunostained with anti-CLDN1 (red), anti-RBFOX3 (green), and anti-Ubiquitin (pink) antibodies. DAPI (blue) was used to stain nuclear. Inside the white square is the view of the enlarged picture below. Panel C, Image J was used to calculate the integrated density per area (IntDen/Area). The red area exhibited the selected area after threshold (Default). Panel D, the IntDen/Area of CLDN1 and RBFOX3 in nasal mucosal tissues of AR patients ($n = 10$) and control subjects ($n = 10$) were calculated by GraphPad. Values are means $\pm$ S.E. ****: $p < 0.0001$

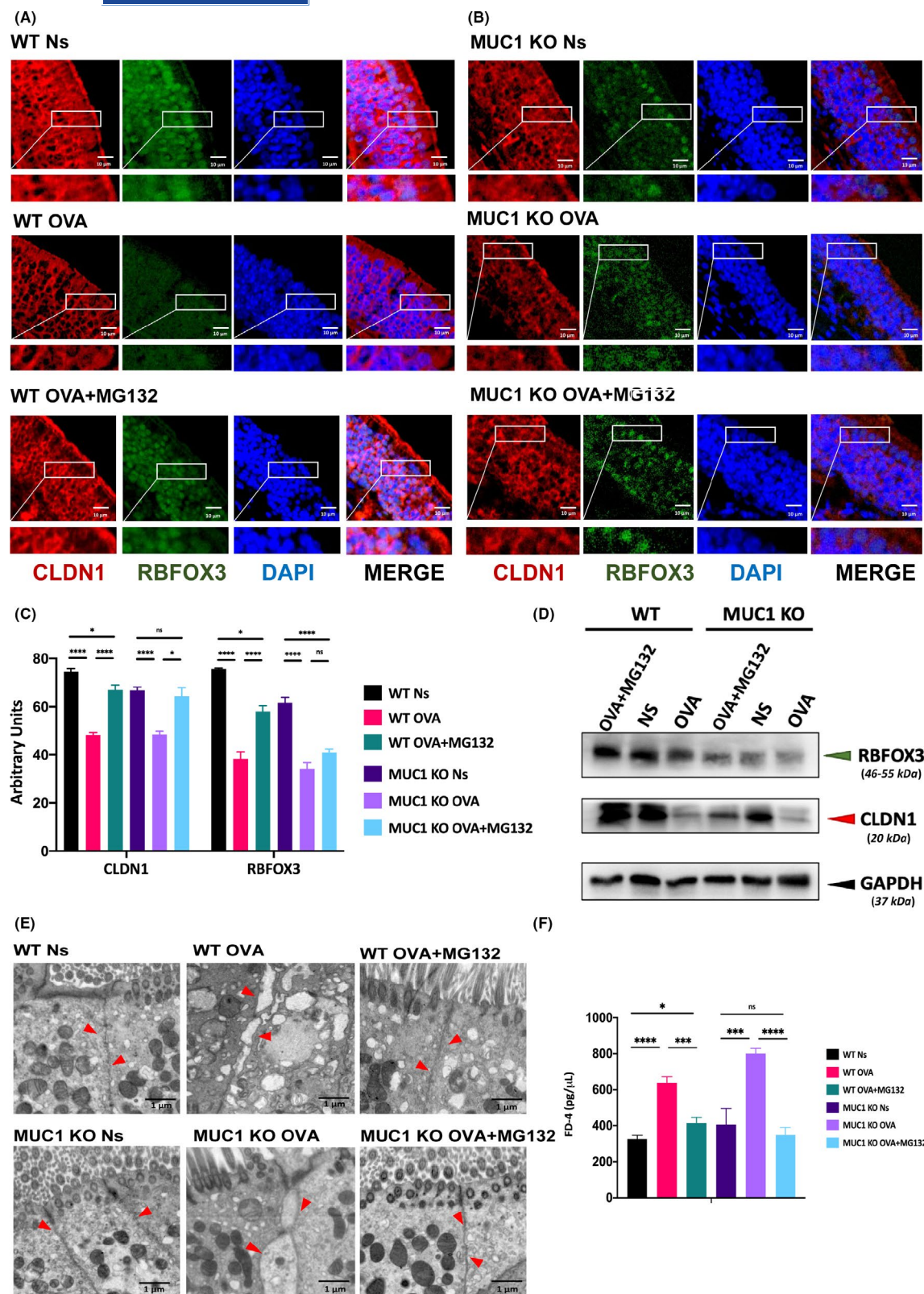


FIGURE 2 Functions of MG132 nasal administration in the nasal mucosa of AR mice. Panels A and B, nasal mucosa tissues were immunostained with anti-CLDN1 (red) and anti-RBFOX3 (green) antibodies. DAPI (blue) was used to stain nuclear. Inside the white square is the view of the enlarged picture below. Panel C, the arbitrary units were measured by Image J and calculated by GraphPad. Panel D, immunoblot analysis evaluated CLDN1 and RBFOX3 expression levels. Panel E, the tight junctions (indicated by arrows) of nasal epithelial cells. Panel F, the serum FITC-dextran levels. Values ($n = 6$) exhibit as means $\pm$ S.E. ns: $p > 0.05$, *: $p < 0.05$, **: $p < 0.001$, ****: $p < 0.0001$. NS: normal saline, OVA: intranasal OVA challenge, OVA+MG132: intranasal OVA challenge with MG132 nasal administration

KEYWORDS

allergy treatment, ENT (rhinitis, sinusitis, nasal polyps...), epithelium, rhinitis

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CONFLICT OF INTEREST

All authors declare that there is no competing interest in relation to this work.

AUTHOR CONTRIBUTIONS

Chunyi Zhang involved in conceptualization, methodology, investigation, formal analysis, validation, visualization, and writing—original draft. Yiyan Wang involved in methodology, investigation, validation, resources, and data curation. Wenjing Liao contributed to resources and investigation. Tianhao Liang, Weixing Liu, Junyang Xie, and Xiaofen Wang contributed to resources. Wenju Lu and Pingchang Yang involved in writing—review and editing, and visualization. Xiaowen Zhang involved in conceptualization, funding acquisition, resources, supervision, and writing—review and editing.

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