The GSDMD-mediated keratinocytes pyroptosis regulates T cell homeostasis through HMGB1/NF-κB pathway to promote atopic dermatitis

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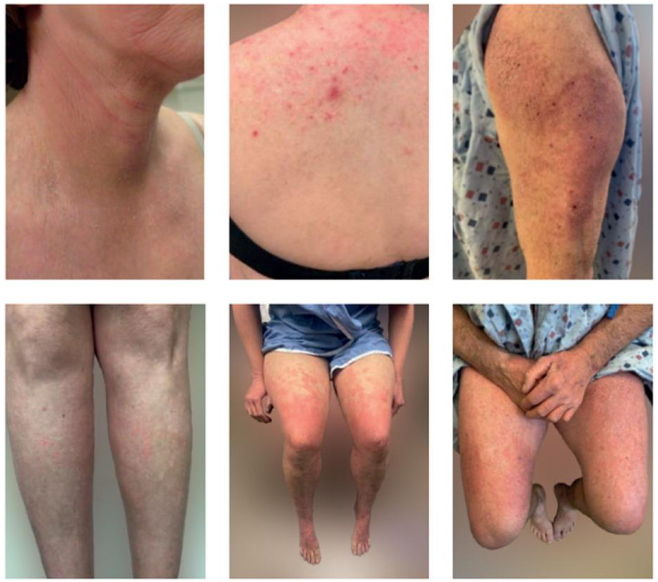
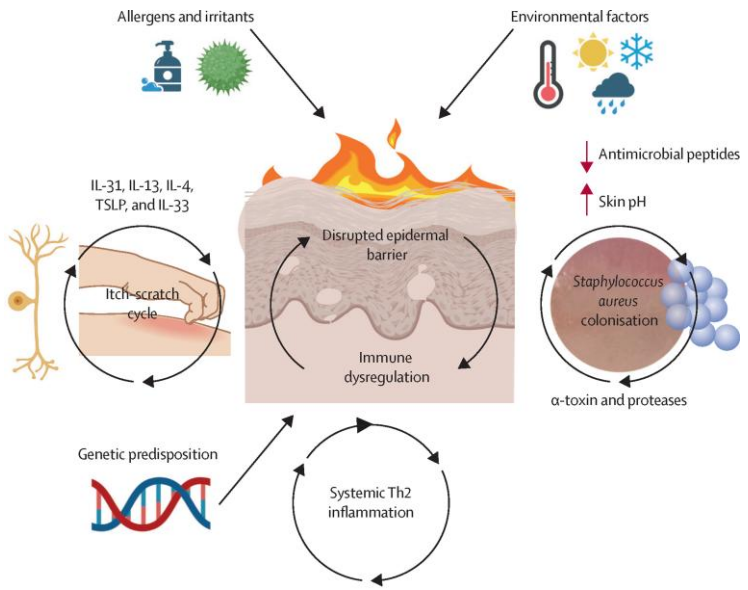
All authors declare no conflict of interest.

➢ Background

- Atopic dermatitis (AD) is a prevalent **chronic inflammatory skin disorder** characterized by a **type-II immune response**, leading to symptoms such as dry skin, intense itching, erythema, and impaired skin barrier function
- Current diagnostic approaches primarily rely on clinical features and medical history, supplemented by biomarkers like **cytokine levels, IgE, and eosinophil counts**. Treatment strategies vary from topical therapies for mild cases to systemic immunosuppressants and biologics for severe cases; however, these interventions often have limited efficacy and safety concerns





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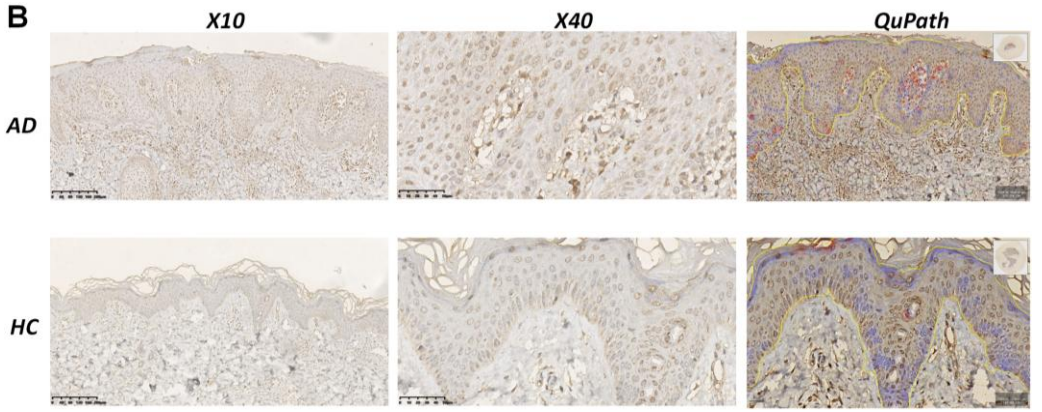
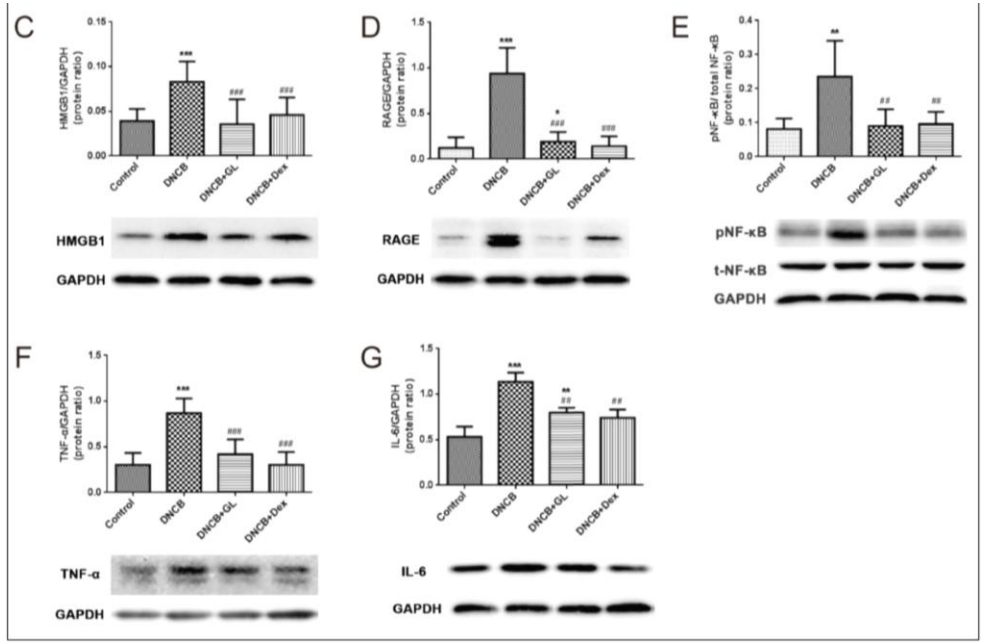
Global epidemiology of atopic dermatitis: a comprehensive systematic analysis and modelling study, Br J Dermatol. 190 (2023), 55–61.

- Recent research has highlighted **pyroptosis**, a novel form of programmed cell death marked by cell swelling, membrane rupture, and release of pro-inflammatory cytokines, as a critical player in AD pathophysiology
- **Gasdermin D (GSDMD),** a key executor of pyroptosis, is cleaved upon inflammatory stimuli to form membrane pores, facilitating cytokine release and cell death. Although it is reported to be highly expressed in AD lesions, its specific role in AD, particularly whether it regulates T cell immune homeostasis—a core aspect of AD—to promote the disease, remains unclear
- In AD mice, the **High mobility group box 1（HMGB1）**, **NF-κB**, inflammatory cytokines, IgE levels were reported to be enhanced, and the glycyrrhizin treatment restrained the HMGB1 signaling cascade and ameliorated the AD symptoms



GSDMD is upregulated in AD lesions



HMGB1, NF-κB, inflammatory cytokines enhanced AD-like symptoms in mice

Construction and verification of atopic dermatitis diagnostic model based on pyroptosis related biological markers using machine learning methods. BMC Med Genomics. 2023 Jun 17;16(1):138.
Single-cell RNA-seq reveals abnormal differentiation of keratinocytes and increased inflammatory differentiated keratinocytes in atopic dermatitis, J Eur Acad Dermatol Venereol. 37 (2023), 2336–2348.

➢ Aim of the Study

- Whether and how GSDMD regulates the pyroptosis process in keratinocytes.
- Whether GSDMD-mediated pyroptosis affects T cell activation and differentiation through the HMGB1/NF-κB pathway
- Whether targeting the GSDMD/HMGB1 axis can alleviate AD-like inflammatory phenotypes in vitro and in vivo

We propose that GSDMD may mediate keratinocyte pyroptosis by activating the HMGB1/NF-κB signaling axis, thereby disrupting T cell homeostasis and ultimately driving AD progression

➢ Methods

Vitro Models

•Pyroptosis Model: Human immortalized keratinocytes (HaCaT) establish an in vitro AD-like inflammatory environment-induced pyroptosis model

•**Genetic Intervention**: The model cells were transfected with si-GSDMD (knockdown) or oe-GSDMD (overexpression，oe) lentiviral vectors

•**Pharmacological Intervention**: The HMGB1-specific inhibitor—glycyrrhizic acid (GA, 40 μM)—was applied.

•**Assays**: Cell viability was measured using CCK-8; cell death was assessed via Annexin V/PI flow cytometry; expression of pyroptosis and inflammation-related proteins was detected by Western blot and ELISA

Vivo Models

•**AD mice Model:** An AD-like dermatitis model was successfully established in 8-week-old male C57BL/6 mice by sensitization and challenge with oxazolone (OXA), presenting symptoms such as erythema, edema, scaling, and scratching

•**Experimental Groups:**

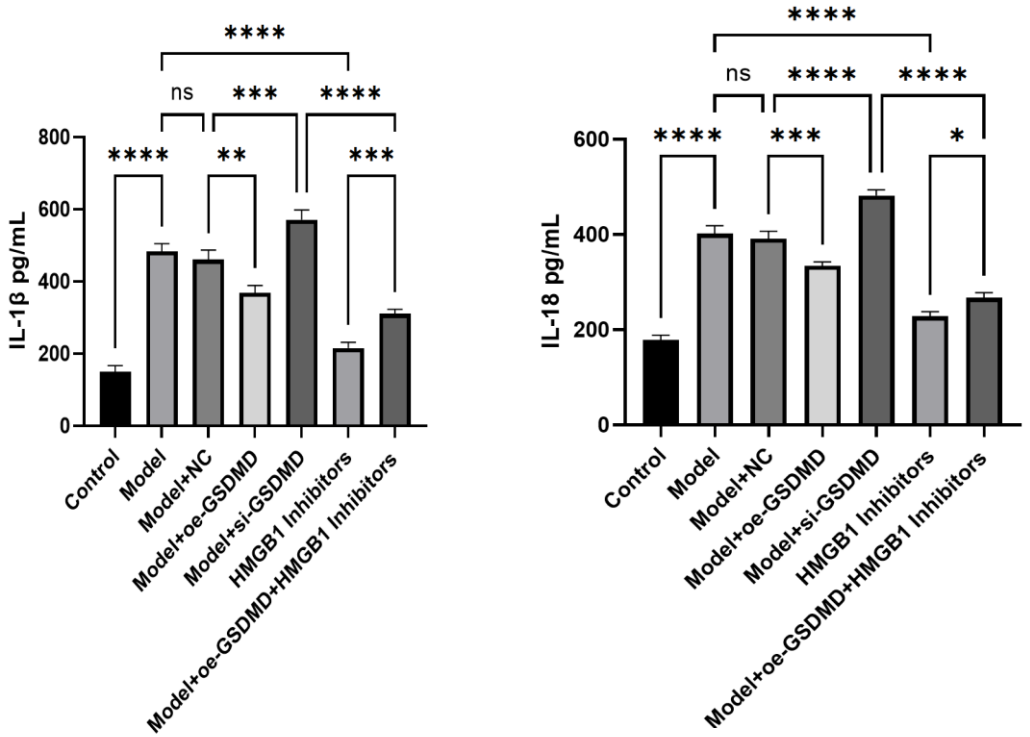
- ◦Control
- ◦Model
- ◦model + si-GSDMD
- ◦Model + oe-GSDMD
- ◦Model + HMGB1 inhibition
- ◦Model + oe-GSDMD + HMGB1 inhibition

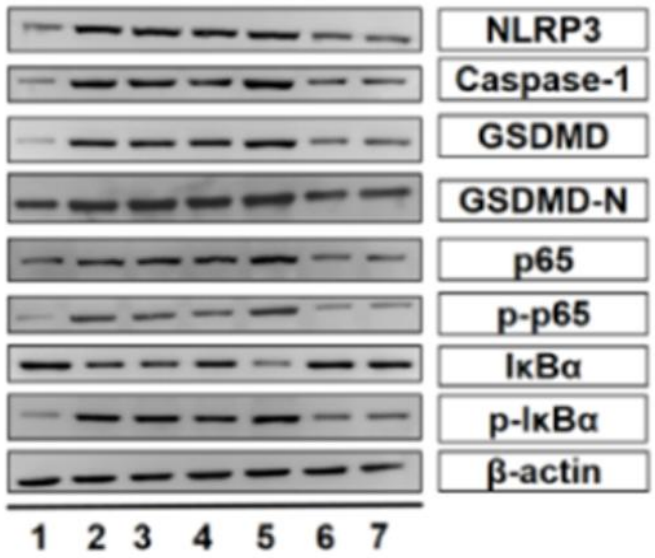
•**Sample Collection:** Skin tissues were collected for HE staining and protein analysis; serum and splenic/skin lymphocytes were collected for cytokine detection and flow cytometry

➢ Results

● The GSDMD/HMGB1 axis mediated pyroptosis in HaCaT cells

- Silencing GSDMD (si-GSDMD) reduced GSDMD expression and cell viability, while overexpression (oe-GSDMD) increased both
- Treatment with the HMGB1 inhibitor glycyrrhizic acid at 40 μM was optimal for reducing cell viability without excessive toxicity
- Both si-GSDMD and HMGB1 inhibition promoted pyroptosis in pyroptosis-like HaCaT cells and suppressed pyroptosis-related markers including NLRP3, caspase-1, GSDMD, IL-1β, and IL-18. Overexpression of GSDMD reversed these effects, but co-treatment with HMGB1 inhibitors mitigated the upregulation of these markers



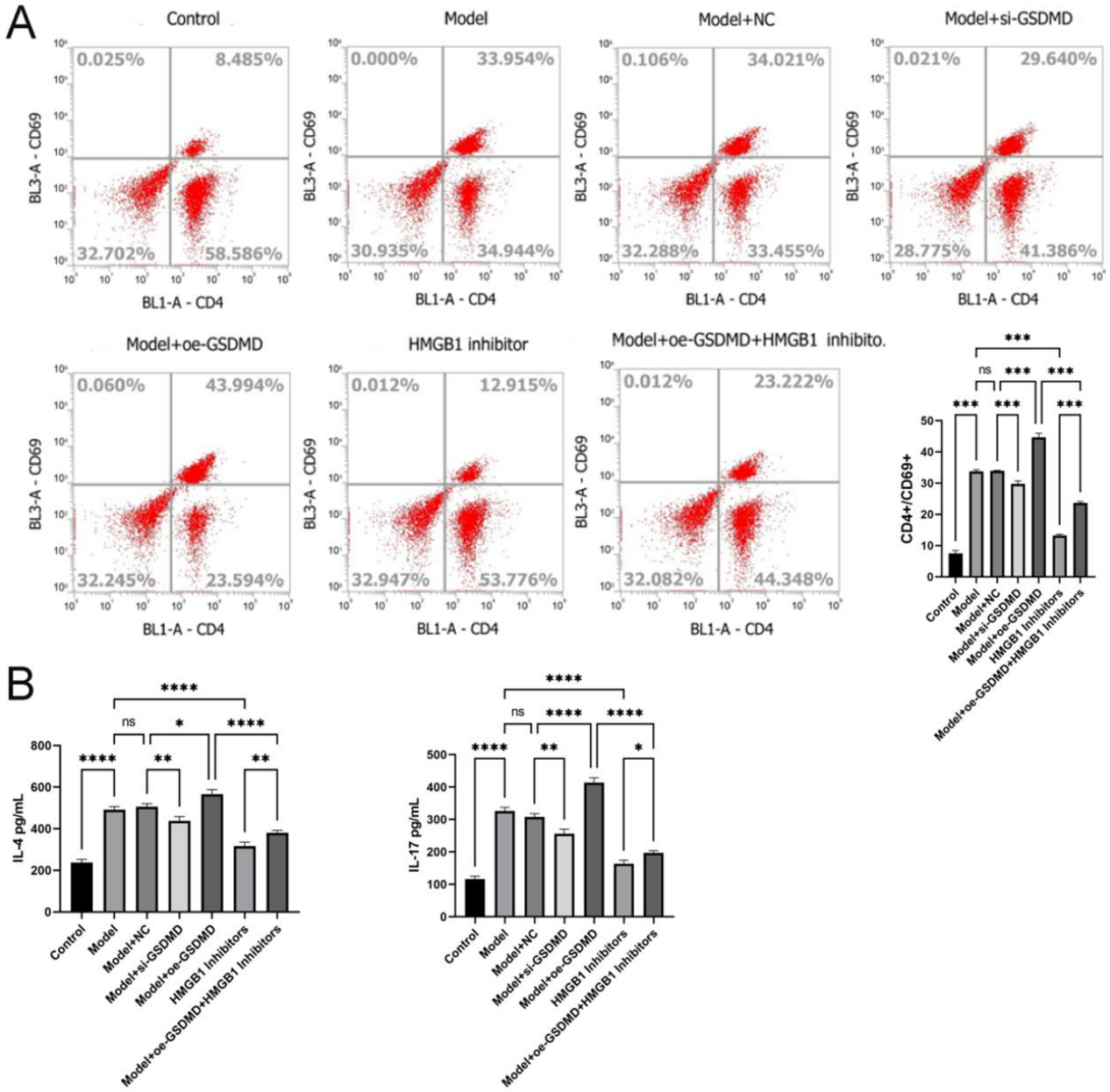


1-control
2-model
3-model+NC
4-model+si-GSDMD
5-model+oe-GSDMD
6-model+HMGB1 inhibitors
7-model+oe-GSDMD+HMGB1 inhibitors

unpublished

- **The GSDMD/HMGB1 axis contributed to the T cell activation in pyroptosis-like HaCaT cells**

  - Pyroptotic HaCaT cells strongly activated T cells (increased CD69 expression)
  - This effect was enhanced in the GSDMD overexpression group and reduced in the GSDMD knockdown or GA treatment groups
  - Changes in Th2/Th17-related cytokine levels (IL-4, IL-17) in the co-culture supernatant aligned with T cell activation trends
  - GSDMD-mediated keratinocyte pyroptosis actively regulates and amplifies surrounding T cell immune responses via HMGB1



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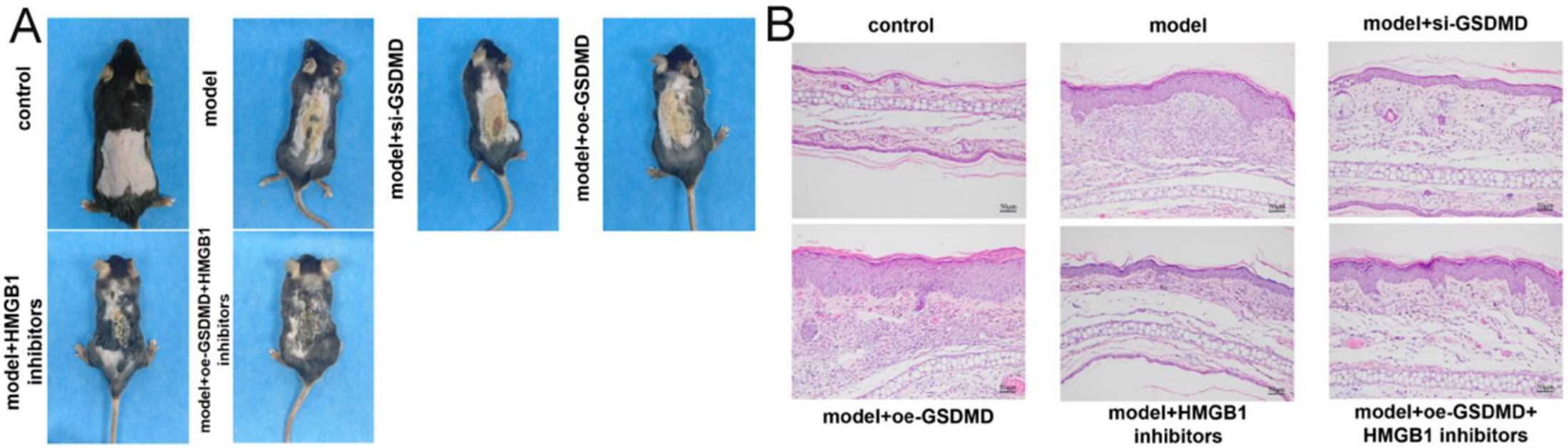
- **GSDMD activated HMGB1/NF-κB signaling to promote AD development**

•Overexpression of the GSDMD gene exacerbated skin tissue inflammation and pathological changes, while inhibition of GSDMD or HMGB1 alleviated these lesions to varying degrees

•The combined inhibition showed superior effects compared to single interventions



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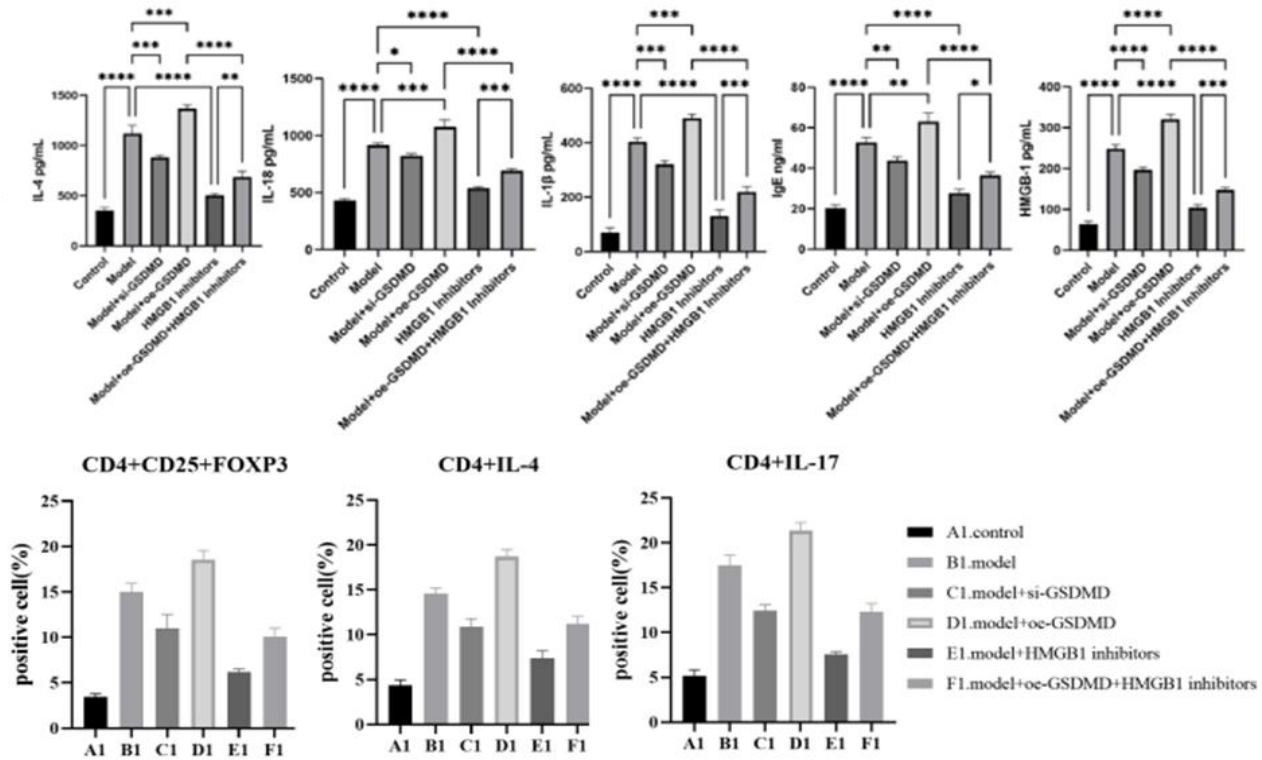
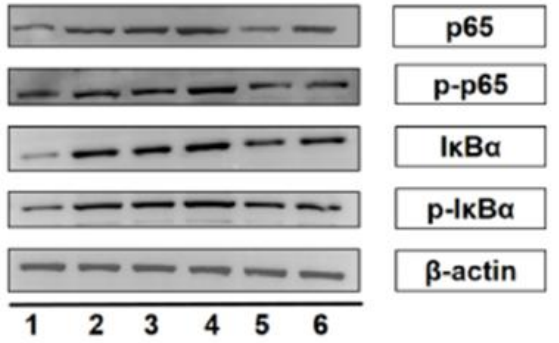
- **GSDMD activated HMGB1/NF-κB signaling to promote AD development**

•AD model mice exhibited severe dermatitis, extensive inflammatory cell infiltration, and **high expression of IL-4, IL-1β, IgE, and HMGB1**

•The GSDMD/HMGB1 axis promoted abnormal activation of **Th2, Th17, and Treg cells** in AD mice

•Western blot results showed that GSDMD activates the **NF-κB signaling pathway** (increased p-p65/p65 and p-IκBα/IκBα ratios), and this effect was blocked by the HMGB1 inhibitor





1-control
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5-model+HMGB1 inhibitors
6-model+oe-GSDMD+ HMGB1 inhibitors

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➢ Conclusion

- We elucidate a novel pro-inflammatory loop in AD: GSDMD → Pyroptosis → HMGB1 Release → NF-κB Activation → (1) Inflammatory Amplification & (2) T Cell Dyshomeostasis → AD Pathogenesis.

GSDMD-mediated keratinocyte pyroptosis promotes AD development by activating HMGB1/NF-κB signaling and disrupting T-cell homeostasis.

Targeting this axis represents a viable therapeutic strategy

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Thank you to my team and our collaborators

Thank you for your attention！