

# Direct cleavage and activation of gasdermin B by asthma trigger allergens

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To the Editor:

Recent fine-mapping studies have pointed to gasdermin B (*GSDMB*) as a potential asthma susceptibility gene in 17q21 locus, the strongest and most highly replicated signal in genome-wide association studies<sup>1</sup>. The *GSDMB* protein is a member of the gasdermin family that, when cleaved, triggers an inflammatory cell death known as pyroptosis<sup>2</sup>. Caspase-1 and granzyme A have been shown to cut *GSDMB* at specific sites to release the N-terminal fragment of the protein (*GSDMB*-NT) that has the ability to induce pyroptosis in cells, including airway epithelial cells<sup>3,4</sup>. These findings suggest that the role of *GSDMB* in asthma lies in its ability to be activated through cleavage to induce pyroptosis; however, it remains unclear whether *GSDMB* cleavage and activation occur in the context of asthma.

Common asthma trigger allergens often possess protease activities that cause airway epithelial injury and inflammation<sup>5,6</sup>. We thus tested whether the allergens directly cleave *GSDMB*. Incubation of extracts from house dust mite (HDM), a common asthma trigger, with lysates from human bronchial epithelial cells, which express endogenous *GSDMB*<sup>3</sup>, resulted in *GSDMB* cleavage as evidenced by the appearance of a smaller protein around 17kD (Figure 1A). Since the *GSDMB* antibody used in the Western blotting targets the C-terminus of the protein, the 17kD protein band likely represents the C-terminal *GSDMB* fragment. Such *GSDMB* cleavage was also observed when lysates from cells expressing C-terminal-FLAG-tagged *GSDMB* were mixed with HDM extract (Figure 1B). Furthermore, mold or cockroach extract also cleaved tagged *GSDMB* (Figure 1C). The cleavage of *GSDMB* protein by all allergen extracts resulted in a single product of similar size (about 17 kD), suggesting a specific cutting site.

To identify the cleavage site, we incubated recombinant full-length *GSDMB* with HDM extract and resolved the cleaved protein products on SDS-PAGE (Figure 1D). We excised the putative 17 kD C-terminal fragment (*GSDMB*-CT, Figure 1D) and determined the N-terminal amino acid sequence of the fragment via Edman sequencing (Supplemental Figure S1, Figure 1E). Despite some ambiguities, the first ten amino acid residues of the 17 kD *GSDMB*-CT largely map to position 245 to 254 (SLGSEDSRNM) of the full length *GSDMB* protein (Figure 1E). This result indicates that *GSDMB* was cleaved immediately after the lysine residue at position 244 (K244). Interestingly, granzyme A also cuts *GSDMB* at the same K244 site<sup>4</sup>. To confirm K244 as the site of cleavage, we mutated lysine 244 to alanine (K244A) in *GSDMB* and tested whether the mutant protein can be cleaved by HDM. As shown by Western blotting, HDM was able to cleave wild type (WT) *GSDMB* but failed to cleave K244A *GSDMB* as evidenced by the absence of the 17 kD fragment (Figure 1F).

The cleavage of *GSDMB* by HDM is expected to release an N-terminal fragment of 244 amino acids (*GSDMB*-NT-K244) (Figure 2A). We next tested whether *GSDMB*-NT-K244 triggers pyroptosis. Transfection of *GSDMB*-NT-K244 induced cell morphological changes characteristic of pyroptosis, including rounding up

and detachment (Figure 2B). LDH release assay confirmed increased toxicity in these cells (~3.4 fold) as compared to cells transfected with the full-length GSDMB (Figure 2C). Consistent with our previous finding on GSDMB-NT shortened by a functional asthma-associated splice variant<sup>3</sup>, transfection of a truncated GSDMB-NT from the variant (NT-K231var) did not induce pyroptosis (Figure 2B,C).

While future studies are needed to identify the specific proteases within the allergen extracts that cleave GSDMB, our current study demonstrates that asthma triggers such as HDM can directly cleave and activate GSDMB, thus providing biochemical evidence linking GSDMB-mediated pyroptosis to asthma.

### Figure Legends:

**Figure 1 .** Asthma triggers cleave GSDMB. (A) Western blot showing cleaved GSDMB from differentiated airway epithelial cell lysates mixed with HDM extracts or 293T cell lysates (transfected with GSDMB) mixed with increasing concentrations of (B) HDM extracts and (C) mold or cockroach extracts. (D) Cleavage of recombinant GSDMB by HDM allergen. Box marks the fragment subjected to sequencing. (E) Upper panel: Results of the Edman Sequencing of the first 10 amino acids. Residues that were identified with low degree of certainty are in parentheses. Lower panel: Diagram depicting the position of the identified amino acids in the GSDMB protein. (F) *In vitro* cleavage assay in 293T lysates transfected with WT-GSDMB or K244A and incubated with HDM extracts. Anti-GSDMB (A) or anti-FLAG antibody (B,C,F) was used to detect full-length and cleaved GSDMB.

**Figure 2.** GSDMB-NT generated by HDM cleavage induces pyroptosis. (A) Schematic diagrams showing the WT GSDMB and the resulting GSDMB-NT fragments (NT-K244 and NT-K231var) after HDM cleavage. (B) Representative images of cells transfected with indicated plasmids. Blue arrowheads mark pyroptotic cells. (C) LDH release assay from 293T cells transfected with the indicated plasmid constructs. Results indicate mean +/- SEM of n=6 from a representative experiment. \**p* <0.05.

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Q.L. a co-founder, scientific advisor and shareholder of Vesigen Therapeutics. All other co-authors declare no competing interests.

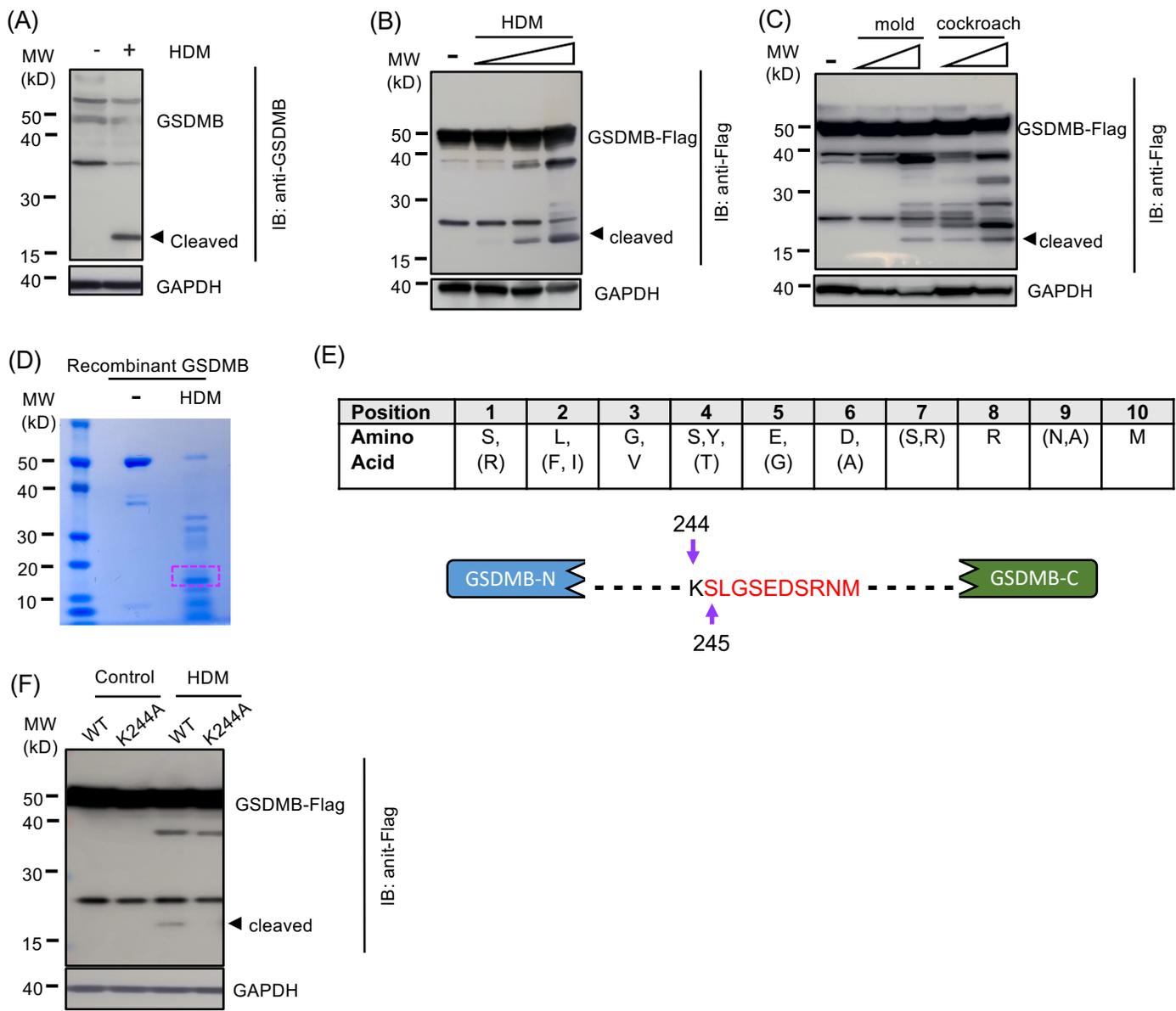


Figure 1\_Panganiban et al

