Supplemental material

Gasdermin B, an Asthma-susceptibility Gene, Promotes MAVS-TBK1 Signaling and Airway Inflammation

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I. Supplementary methods

Mice

To generate a human GSDMB transgenic mouse line with conditional expression in airway epithelial cells, we subcloned the open reading frame (ORF) of human GSDMB into pTRE2 vector immediately downstream of pTet-responsive PhCMV-1 promoter, which was co-injected with CC10-rtTA (kindly provided by Dr. Chun Lee at Brown University) into the perinucleus of fertilized FVB/NJ ova at the Brigham and Women's Hospital Transgenic Core facility. Mice were then housed in the Brigham and Women's Hospital animal facility with a 12-h-light/12-h-dark cycle with free access to food and water. Mice used in each batch of experiments were sex- and age-matched. All animal experiments were approved by the Animal Care and Use Committee (IACUC) at the Brigham and Women's Hospital.

Reagents and Antibodies

The details of reagents and antibodies were listed in **Table E3**. The Human rhinovirus 16-A was gifted from Dr. James Gern at University of Wisconsin School of Medicine and Public Health Madison.

Quantification of lung inflammation

Mice were harvested and lungs were dissected as previously described [1], fixed in 10% phosphate-buffered formalin. Fixed tissues were sent for

paraffin-embedding, sectioning and hematoxylin-and-eosin (H&E) staining (InvivoEx, Boston, Massachusetts, USA). The degree of inflammation surrounding airways was evaluated based on HE staining from four lung lobes of each mouse. Airways around 300–600 μm in diameter were chosen for quantification of the inflammation using scores including 0 (normal, immune cells infiltration per airway $\leqslant$10), 1 (minimal, 10 <immune cells infiltration per airway $\leqslant$ 50), 2 (mild, 50<immune cells infiltration per airway $\leqslant$ 100), 3 (moderate, 100<immune cells infiltration per airway $\leqslant$ 200), 4 (severe, 300<immune cells infiltration per airway). The average lung inflammation score for each experimental group is based on scores from five mice with more than 80 airways per group. The inflammation score was conducted by two independent researchers in a double-blinded way.

Immunofluorescence staining

For immunofluorescence staining, lung slides were rehydrated and subjected to antigen retrieval with Tris-HCl buffer (100 mM, pH 9.5). After one hour of blocking with 15% BSA in 0.2% Triton-X/PBS at room temperature, slides were incubated with various primary antibodies at 4°C overnight including Anti-GSDMB antibody, CCL5/RANTES Antibody, CC10 Antibody. Following incubation with Alexa Fluor conjugated secondary antibodies, lung slides were counter-stained with DAPI in ProLong gold anti-fade mounting reagent. Images were captured with ZEISS Axio Imager 2.

CRISPR/Cas9 *GSDMB* gene knockout in NHBE and BEAS-2B cells

CRISPR/Cas9-mediated genome engineering was used to generate the *GSDMB* knockout (KO) in human normal bronchial epithelial cells following the Zhang laboratory protocol [2]. sgRNAs were cloned into Bbs1 site of pSpCas9(BB)-2A-GFP plasmid (Addgene; 48138) (guide-RNA target sequence was AGAGAACTTTCTTTGGATGC). Following single-cell sorting on GFP-positive cells, Western blotting was used to screen colonies carrying *GSDMB* gRNA.

To generate *GSDMB* knockout lines, BEAS-2B cells were infected with Lentivirus containing *GSDMB* sgRNA CRISPR All-in-One (Human) (abm, Cat.K0911016, now 227241110603) for 48 hours followed by puromycin(2 μg/ml) selection for 7 days. Next, cells were serially diluted and plated in 96-well plates to generate individual colony as we have done previously followed by expansion and sequencing verification [3, 4].

Stable shRNA-mediated *RIG-I* and *MDA5* knockdown

GFP or GSDMB-overexpressing BEAS-2B cells were infected with the lentiviral vector carrying a scrambled shRNA (sc-61480-SH, Santa Cruz Biotechnology, Dallas, Texas, USA) or the MDA5 shRNA (sc-61010-SH, Santa Cruz Biotechnology) or the RIG-I shRNA (sc-61480-SH, Santa Cruz Biotechnology) followed by selection with puromycin (2 ug/ml) in the medium

for 7 days.

GSDMB stable overexpression

GSDMB (isoform 3) was stably overexpressed in NHBEs or BEAS-2B cells by infecting cells with lentivirus containing Gasdermin like (GSDMB) Human Tagged ORF (Cat. RC219044L1V, Origene, Rockville, Maryland, USA) for 48 hours followed by puromycin selection using protocol as previously described [5, 6]. Overexpression of *GSDMB* was determined by Western blot analysis.

Transfection of Poly (I:C) in cells

BEAS-2B cells seeded in 6-well plate at 90% confluency were transfected with 2 μg/mL HMW ploy (I:C), LMW ploy (I:C) or 3p-hpRNA by Lipofectamine 3000. Six hours after transfection, cell pellet was collected for qPCR or Western Blotting.

Cytokine release assay

The supernatant was collected for ELISA measurements with Human RANTES ELISA Kits or IFN beta Human ELISA Kit following the protocol.

Cell viability and microscopy-based cytotoxicity assays

Relevant cells were transfected with poly(I:C) for 6 or 24 hours. Cell death was quantified by the lactate dehydrogenase (LDH) assay with the CytoTox 96

Non-Radioactive Cytotoxicity Assay kit as previously described [4, 6].

For microscopy-based cytotoxicity analysis, cells were seeded in 24-well plates in culture media with 1 μg/ml propidium iodide followed by imaging using a fluorescence microscope (BZ-X810, Keyence, Itasca, Illinois, USA).

Quantitative RT-PCR

Total RNA was extracted from cells using the Direct-zol™ RNA MiniPrep with TRI-Reagent® according to the manufacturer's instructions. For RT-PCR analysis, cDNA was generated with High-Capacity cDNA Reverse Transcription Kit with RNase and was analyzed by TaqMan™ Fast Advanced Master Mix or Applied Biosystems Power SYBR Green PCR Master Mix. All data were normalized to the expression of reference gene. *GSDMB* (sense: 5'-CCCAACAAGGAGACGATGAATA-3'; anti-sense: 5'-GACTTTCCTAAACAGGATGAAGC-3'; probe: 5'-ACAAAATCCTTTCCAGAAGAGAAGGATGGT-3') and the counterpart human *GAPDH* (Hs.PT.39a.22214836), human XBP1 (Hs.PT.58.1903847), human ACTB (Hs.PT.39a.22214847) or mouse *Gapdh* (Mm.PT.39a.1) probes are purchased from Integrated DNA Technologies IDT. Other primer sequences were listed in **Table E4.**

Immunoprecipitation and Immunoblot Analysis

For immunoblot, the cells or tissues were lysed in low-salt lysis buffer (50 mM HEPES, 150 mM NaCl, 1 mM EDTA, 1.5 mM MgCl2, 10% glycerol, 1% Triton X-100) containing protease inhibitor and phosphatase inhibitor. The soluble fraction was obtained by centrifuging the mixture at 12,000 × g for 5 minutes at 4°C. The concentration of total protein in the lysate was determined using a Bio-Rad Protein Assay, and the samples were loaded with 4x Laemmli Sample Buffer, and separated by pre-cast 5-12% SDS-PAGE gel. The proteins were then transferred to polyvinylidene fluoride (PVDF) membranes and further probed with indicated antibodies, including Anti-GSDMB antibody, Phospho-TBK1/NAK (Ser172) (D52C2) XP® Rabbit mAb, Anti-NAK/TBK1 antibody [EP611Y], Phospho-IRF-3 (Ser396) (4D4G) Rabbit mAb, Anti-IRF3 antibody, Anti-GFP antibody, DYKDDDDK Tag (D6W5B) Rabbit mAb, Anti-HA antibody produced in rabbit as well as either Goat anti Mouse IgG (H+L) Secondary Antibody or Donkey anti-Rabbit IgG (H+L) Secondary Antibody. In addition, β-actin was directly probed by using Anti-β-Actin antibody [AC-15] (HRP). At last, proteins were detected by SuperSignal West Femto Chemiluminescent Substrateor or Pierce ECL Western Blotting Substrate. Images of blotting were captured by using the G:Box system (Syngene, Federick, MD, US).

For immunoprecipitation, protein lysates were prepared from cells after transfection with plasmids and/or poly (I:C) in low-salt lysis buffer and incubated overnight with anti-FLAG beads. The beads were washed five times

with low-salt lysis buffer, and the immunoprecipitates were processed for immunoblotting as described above.

RNA-protein binding assay in vitro

Plasmids for HA-, or FLAG-tagged GSDMB and various mutants, including HA-GSDMB (AA. 83-417), HA-GSDMB (AA. 275-417) and HA-GSDMB (AA. 83-274) were cloned into the pLenti CMV Blast empty vector and expressed in BEAS-2B cells and immunoprecipitated with Flag or HA beads overnight at 4°C, respectively. Next, beads were washed with low-salt lysis buffer and immunoprecipitates were eluted with 3X Flag-peptides (5 μg/ml) or influenza Hemagglutinin (HA) Peptide (5 μg/ml) to obtain Flag-GSDMB or HA-GSDMB protein complex. Purified protein complex or purchased GSDMB or GSDMD protein from OriGene Technologies was pre-incubated with biotin-labeled poly(I:C) or poly (dA:dT) at indicated concentration for 1h. Then, Streptavidin beads or Neutravidin beads were added into the protein-RNA mixture for 30 minutes to pull down biotin-labeled poly(I:C) and beads were washed three to five times with PBS. Immunoprecipitated protein samples were boiled with 4x Laemmli Sample Buffer and resolved by SDS-PAGE for western blotting.

Gene expression correlation analysis in airway epithelial cells

RNA-sequencing data was generated from bronchoscopy brushings samples, primarily bronchial epithelial cells from 45 subjects participating in the Asthma

BioRepository for Integrative Genomic Exploration (Asthma BRIDGE) study, as previously described [7]. In addition, correlation between these genes and *GSDMB* gene expression was performed using a linear regression between expression levels of two genes. Detailed information on the subjects in the Asthma Bridge cohort is shown in **Table E1**.

Additional RNA-sequencing data in nasal epithelium was obtained from the Genes-environments & Admixture in Latino Americans (GALA) II study (N=695, GSE152004) samples [8]. This dataset contains normalized expression data for 441 asthmatics and 254 healthy controls. We extracted the expression data for five genes including *RANTES, OASL, ISG15, ISG20 and CXCL10.* Then, we computed the log2 transformed values of gene expression for correlation analysis. Based on these values, we first analyzed marginal associations of these genes with asthma affection status using a logistic regression with log2 transformed expression of the corresponding gene as the predictor.

GSDMB protein structural simulation and prediction

GSDMB protein 3D folding simulation was performed with OpenStructure (OST) engine [9], primarily based on templates homology of available structure models in Protein Data Bank [10]. Protein sequence of GSDMB lacking matchable homology templates in the Protein Data Bank was simulated by Monte Carlo techniques [11]. Using QMEAN Z-score analysis [12], we ranked all models and used the model with the highest score for virtual ligand

screening with EADock [13]. The top 5 scored ligand candidates were listed in **Figure E8**. Distribution of protein electrical charge shown in **Figure 5e** was calculated based on the type of each amino acid in the protein sequence, with a window sliding method. The score of charge for a.a. was defined as below: "D"(Aspartic acid) and "E" (Glutamic acid) for -1, "K" (lysine) and "R" (Argine) for +1, and "H" (Histidine) for +0.5. The resolution is defined as Bin Size / Total Sequence Length. The score of charge was calculated by R (4.0.0), and the distribution map was plotted with ggplot2 package (3.3.3).

Correlation between asthma GWAS variants and gene expression in human asthmatic airway epithelial cells

Gene expression data in human airway epithelial cells from normal and asthmatics (GSE172368) were obtained from a previously published paper [14]. Gene expression levels were calculated as normalized counts, following the methodology described in the aforementioned paper. To assess the ISG scores for individual samples, we utilized the 'ssgsea' function from the GSVA R package(v1.38.2), p-value is calculated using t-test. The signature genes for the interferon response were obtained from the GSEA MSigDB database. For visualization purposes, a heatmap was generated using the heatmap R package(v1.0.12), employing the normalized counts with gene-wise scaling.

Supplementary Table E1. Demographic characteristics of AsthmaBridge bronchial epithelium subcohort

N	45
Female (%)	68.9
Mean Age (years)	40.4
African American	75.6
White	17.8
Other	6.7
Has Asthma (%)	62.2

Supplementary Table E2. Demographic characteristics of the patients, from whom airway epithelial cells (AECs) were collected and treated with RV

Sample size (N)	95
Gender (% Female)	41
Mean Age (Range)	45 (18-73)
Asthma (Ever, N)	42

* The sample composition is derived from the study conducted by Marcus M. Soliai and colleagues [14].

Supplementary Table E3. Antibodies and reagents

REAGENT or RESOURCE	SOURCE
Rabbit monoclonal anti-TBK1 antibody	Abcam (ab40676)

Rabbit monoclonal anti-Phospho-TBK1 antibody	Cell Signaling Technology (5483S, Danvers, Massachusetts, USA)
Rabbit monoclonal Anti-IRF3 antibody	Abcam (ab68481)
Rabbit monoclonal Anti-Phospho-IRF3 antibody	Cell Signaling Technology (4947S)
Chicken polyclonal Anti-GFP antibody	Abcam (ab13970)
Mouse monoclonal HRP Anti-beta Actin antibody	Abcam (ab49900)
Rabbit monoclonal antibody Anti-DYKDDDDK Tag	Cell Signaling Technology (14793S)
Anti-FLAG® Affinity Isolated Mouse Monoclonal Antibody [M2]	Sigma-Aldrich (F1804-200UG)
Anti-HA antibody produced in rabbit	Sigma-Aldrich (H6908-100UL)
Goat polyclonal anti-Mouse IgG(H+L) Secondary Antibody	Novus Biologicals (NBP1-75957, Centennial, Colorado, USA)
Donkey polyclonal anti-Rabbit IgG (H+L) Highly	Thermo Fisher Scientific (31458, Pittsburgh, Pennsylvania, USA)
Agarose Gel Anti-FLAG® Mouse Monoclonal Antibody [M2]	Sigma-Aldrich (A2220-5ML)
Anti-GSDMB antibody [EPR20841]	Abcam (ab215729)
Donkey anti-rabbit IgG (Alexa Fluor 568)	Invitrogen (A10042, Waltham, Massachusetts, USA)
Donkey anti-mouse IgG H&L (Alexa Fluor® 488)	Invitrogen (A-21202)
CCL5/RANTES Antibody	Novus Biologicals (NB120-10394)
CC10 Antibody (T-18): sc-9772	Santa Cruz (sc-9772)
Poly(I:C) (HMW) /LyoVec	Invitrogen (tlrl-piclv)
Biotin labeled TLR3 agonist	Invivogen (tlrl-picb)
ProLong™ Gold Antifade Mountant with	LIFE TECHNOLOGIES

DAPI	CORPORATION (P36935, Carlsbad, California, USA)
Pierce™ NeutrAvidin™ Agarose	Thermo Fisher Scientific (29200)
Streptavidin C1 Dynabeads	Thermo Fisher Scientific (65-001)
Applied Biosystems Power SYBR Green PCR Master Mix	Thermo Fisher Scientific (43-687-02)
IFN beta Human ELISA Kit	Fisher Scientific (414101)
Human RANTES ELISA Kits	Thermo Fisher Scientific (EHRNTS2)
Richard-Allan Scientific™ Periodic Acid-Schiff (PAS) kit	Thermo Fisher Scientific (87007)
Opti-MEM® Reduced Serum Medium	Life Technologies (P2318223)
Lipofectamine™ 3000 Transfection Reagent	Life Technologies (L3000015)
High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor 1000 reactions	Life Technologies (4368813)
TaqMan™ Fast Advanced Master Mix	LIFE TECHNOLOGIES CORPORATION (4444964)
Mouse CCL5/RANTES DuoSet ELISA	R&D Systems (DY478-05, Minnneapolis, Minnesota, USA)
Anti-HA antibody produced in rabbit	Sigma-Aldrich (H6908-100UL)
ANTI-FLAG® M2 Affinity Gel,purified immunoglobulin, buffered aqueous glycerol solution	Sigma-Aldrich (A2220-5ML)
3x FLAG® Peptide,lyophilized powder	Sigma-Aldrich (F4799-4MG)
Doxycycline hyclate, ≥98% (HPLC)	Sigma-Aldrich (D9891-100G)
Sucrose	Sigma-Aldrich (573113-5KG)
GSDMB (NM_001165958) purified	OriGene Technologies (TP328330)

human protein	
Complete™, Mini, EDTA-free Protease Inhibitor Cocktail,Tablets provided in EASYpacks,	Sigma-Aldrich(4693159001)
Phosstop™- phosphatase inhibitor tablets	Sigma-Aldrich(4906845001)
4x Laemmli Sample Buffer	BioRad (#1610747)
Protein Assay Kit	BioRad (#5000001)
pre-cast 5-12% SDS-PAGE gel	Bio-Rad (#4561093 and #4561096)
Polyvinylidene fluoride (PVDF) membranes	Bio-Rad (#1620264)
SuperSignal West Femto Chemiluminescent Substrate	Thermo Fisher Scientific (A38556)
Pierce ECL Western Blotting Substrate	Thermo Fisher Scientific (32209)
CytoTox 96 Non-Radioactive Cytotoxicity Assay kit	Promega (G1780, Madison, Wisconsin, USA)
Propidium iodide	BD Bioscience (556463, Lake Franklin, New Jersey, USA)
Direct-zol RNA Miniprep Plus	Zymo Research(R2071, Irvine, California, USA)
TaqMan™ Fast Advanced Master Mix	LIFE TECHNOLOGIES CORPORATION(4444965, Carlsbad, California, USA)

Supplementary Table E4. Primers used in RT-PCR assays.

Gene name	Primers
human IFNβ	sense: 5'- ATGACCAACAAGTGTCTCCTCC-3'

	anti-sense:5’-GGAATCCAAGCAAGTTGTAGCTC-3’
human IFNλ1	sense: 5’- CACATTGGCAGGTTCAAATCTCT-3’
	anti-sense:5’-CCAGCGGACTCCTTTTTGG-3’
human IFNλ2/3	sense: 5’- CTGCCACATAGCCCAGTTCA-3’
	anti-sense:5’-AGAAGCGACTCTTCTAAGGCATCTT-3’
human RANTES	sense: 5’- CTCTGGGTTGGCACACACTT-3’
	anti-sense:5’- AACAGAGAGGATTTCGTTTCCG-3’
human OASL	sense: 5’- CCATTGTGCCTGCCTACAGAG-3’
	anti-sense:5’- CTTCAGCTTAGTTGGCCGATG-3’
Human GAPDH	sense: 5’-GTCGCTGTTGAAGTCAGAGG -3’
	anti-sense:5’-GAAACTGTGGCGTGATGG -3’
Human CHOP	sense: 5'- GCACCTCCCAGAGCCCTCACTCTCC -3’;
	anti-sense:5’-GTCTACTCCAAGCCTTCCCCCTGCG
Human BIP	sense: 5'-CGAGGAGGAGGACAAGAAGG-3';
	anti-sense:5’- CACCTTGAACGGCAAGAACT-3';
Human XBP1s	sense: 5'-TGCTGAGTCCGCAGCAGGTG-3';
	anti-sense:5’-GCTGGCAGGCTCTGGGGAAG-3';
m*Cxcl10*	sense: 5’-CCAAGTGCTGCCGTCATTTTC-3’
	anti-sense:5’- GGCTCGCAGGGATGATTTCAA-3’
m*Viperin*	sense: 5’-ATAGTGAGCAATGGCAGCCT-3’
	anti-sense:5’- AACCTGCTCATCGAAGCTGT-3’
m*Ifit1*	sense: 5’-CAAGGCAGGTTTCTGAGGAG-3’,

	anti-sense:5’- GACCTGGTCACCATCAGCAT-3’
m*Oasl2*	sense: 5’-TTGTGCGGAGGATCAGGTACT-3’
	anti-sense:5’- TGATGGTGTCGCAGTCTTTGA-3’
m*Mx1*	sense: 5’-GACCATAGGGGTCTTGACCAA-3’
	anti-sense:5’- AGACTTGCTCTTTCTGAAAAGCC-3’
m*Isg15*	sense: 5’-GGTGTCCGTGACTAACTCCAT-3’
	anti-sense:5’- TGGAAAGGGTAAGACCGTCCT-3’
m*Gapdh*	sense: 5’-GAAGGGCTCATGACCACAGT-3’
	anti-sense:5’-GGATGCAGGGATGATGTTCT-3’
m*Ifi44*	sense: 5’- GACAAGAGGCATTGCTGTGTT-3’
	anti-sense:5’- CGTGTTTGCTGAACCAGGTCT-3’
m*Chop*	sense: 5’-CCAACAGAGGTCACACGCAC-3’
	anti-sense:5’- TGACTGGAATCTGGAGAGCGA-3’
m*Bip*	sense: 5’-ACCCTTACTCGGGCCAAATT-3’
	anti-sense:5’- AGAGCGGAACAGGTCCATGT-3’
m*Xbp1s*	sense: 5’-GAGTCCGCAGCAGGTG-3’
	sense: 5’-GAGTCCGCAGCAGGTG-3’
m*Xbp1t*	sense: 5’-CACCTTCTTGCCTGCTGGAC-3’
	anti-sense:5’- GGGAGCCCTCATATCCACAGT-3’

II. Supplementary Figure

Figure E1. *ORMDL3* has no correlation with *ISGs* in asthmatic epithelial cells

(**a**) Expression of *RANTES, OASL, ISG15*, *ISG20* and *CXCL10* were analyzed in Asthma BRIDGE samples. Raw expression levels were log2-transformed and quantile-normalized. Then, the linear correlation between the expression levels of each pair of genes was tested using the t-test for Pearson's correlation coefficient. (**b**) Expression of *GSDMB* in BEAS-2B cells transfected with high molecular weight (HMW) poly (I:C) for 6 hours (hr). (**c-d**) Expression of *GSDMB* in BEAS-2B cells infected with RSV line 19 (MOI=1) for 24 hours (c) or RV-A16 (MOI=1) for 72 hours (d). (**e**) Enhanced induction of *GSDMB* by RV-A16 (MOI=1) infection for 72 hours in BEAS-2B cells is inhibited by the TBK1 inhibitor BX795 (1μM). $P < 0.05$; ** $P < 0.01$ (Student's t-test). GAPDH was used as the reference gene for all RT-PCR measurements.

Figure E2 GSDMB promotes interferons expression

(a) mRNA level of *IFNβ, IFNλ1* and *IFNλ2/3* in BEAS-2B cells with overexpression of *GSDMB* and transfected with (HMW) poly (I:C) for 6 hours. **(b)** Extracellular *IFNβ* protein level was measured in BEAS-2B cells with overexpression of *GSDMB* and transfected with HMW poly (I:C) for 6 hours. **(c)** Expression of *IFNβ, IFNλ1 and OASL* in normal human bronchial epithelial cells (nHBEs) with overexpression of *GSDMB* after transfection of HMW poly

(I:C) for 6 hours. **(d-e)** RNA level of *IFNβ, IFNλ1* and *IFNλ2/3* in BEAS-2B cells with overexpression of GSDMB and transfected with low molecular weight (LMW) poly (I:C) (d) or 3p-hpRNA for 6 hours (e), respectively. *, $p<0.05$; ** $p < 0.01$ (two-way ANOVA).

Figure E3 GSDMB promotes MAVS-TBK1 signaling upstream of TBK1

Enhanced induction of *RANTES* and *OASL* by HMW poly (I:C) transfection in GSDMB-overexpressing cells is inhibited by the TBK1 inhibitor BX795 (low, 1μM; high, 10 μM) in BEAS-2B cells. *, $p<0.05$; ** $p < 0.01$ (two-way ANOVA).

Figure E4 The regulation of GSDMB on the MAVS-TBK1 signaling is independent of pyroptosis

(**a**) Propidium iodide (PI) staining and cell morphology images of BEAS-2B cells with overexpression of GSDMB post transfection with high molecular weight (HMW) poly(I:C) (2 μ g/mL) for 6 hours or 24 hours. (**b**) Lactate dehydrogenase (LDH) levels in supernatants from BEAS-2B cells with overexpression of GSDMB and post transfection with HMW poly(I:C) (2 μg /mL) for 6 hours or 24 hours. (**c**) BEAS-2B cells with or without GSDMB overexpression were transfected with HMW poly(I:C) (2 μg /mL) for 6 hours. THP-1 cells were pre-incubated in culture medium with 100 ng/ml phorbol 12-myristate 13-acetate (PMA) overnight followed by transfection with HMW poly(I:C) (2 μg /mL) for 6 hours as a positive control for IL-1β induction as

measured by ELISA. (**d**) BEAS-2B cells with or without GSDMB were pretreated with YVAD (50 uM) for 1 hour and then transfected with high HMW poly (I:C) for 6 hours. Then, the mRNA level of RANTES was measured. (e) Expression of *IFNλ2/3* in BEAS-2B cells transfected with HMW poly (I:C) for 6 hours. Mean ± SEMs shown from at least three independent biological replicates. Scale bar, 100 μm; *, p<0.05 (two-way ANOVA).

Figure E5 GSDMB promotes MAVS-TBK1 signaling independent of ER stress. The mRNA levels of *BIP*, *CHOP*, *XBP1* and splicing *XBP1* (*XBP1s*) were detected in GFP or GSDMB-overexpressing BEAS-2B cells transfected with or without HMW poly (I:C) (2 μg/mL) for 6 hours. Mean ± SEMs shown from at least three independent biological replicates. *, p<0.05 (two-way ANOVA).

Figure E6 Knockout of *GSDMB* decreases IFN response

(**a**) Knockout efficiency of *GSDMB* in BEAS-2B cells by *GSDMB* sgRNA was evaluated by qPCR (relative to GAPDH). **(b-c)** mRNA levels of *IFNβ, IFNλ1* and *IFNλ2/3* (b) or extracellular *IFNβ* (c) in BEAS-2B cells with knockout (KO) of *GSDMB* and transfected with HMW poly (I:C) for 6 hours. (**d**) *GSDMB* sgRNAs were delivered into nHBEs by lenti-viral system. Single-colonies were selected and the extracts were analyzed by immunoblot analysis. (**e**) Expression of *IFNλ1* in nHBEs with knockout (KO) of *GSDMB* and transfected

with HMW poly (I:C) for 6 hours. Mean ± SEMs shown from at least three independent biological replicates. *, $p<0.05$; ** $p < 0.01$ (two-way ANOVA).

Figure E7 The functions of GSDMB on MAVS-TBK1 signaling are independent of MDA5 and RIG-I

(**a**) Expression of *RANTES* in *GFP*- or *GSDMB*-overexpressing cells with *MDA5* depletion, followed by transfection of HMW poly (I:C) for 6 hours. (**b**) Expression of *RANTES* and *OASL* in *GFP*- or *GSDMB*-overexpressing cells with RIG-I depletion, followed by transfection of LMW poly (I:C) for 6 hours. (**c**) BEAS-2B cells were transfected or added with HMW poly (I:C) or LMW poly (I:C) at 2 μg/ml for 6 hours. Expression levels of *RANTES* and *OASL* were analyzed by real-time PCR. Mean ± SEMs shown from three independent biological replicates. For A and B, two-way ANOVA. For C, *, $p<0.05$ (Student's t-test).

Figure E8 Virtual ligand screening of GSDMB indicating potential binding of GSDMB with nucleic acid.

Figure E9 Confirmation of *Cc10-hGSDMB* mice

(a) Inducible expression of Flag-tagged human *GSDMB* in mouse airway epithelial cells shown by qPCR (relative to *Gapdh*) or immunofluorescence staining (green: anti-GSDMB; red:anti-CC10; blue:DAPI) (**b**) from either *Cc10*-h*GSDMB* mice treated with Doxycycline (DOX, 2 μg/ml) or Sucrose

(Suc). (**c**) Sequence alignment analysis of *Cc10*-h*GSDMB* mice. (**d**) Expression levels of GSDMB as shown by delta Ct values in normal bronchial epithelial cells (NHBEs) cultured at submerge or air-liquid interface (ALI) condition, BEAS-2B cells expressing either *GFP* or *GSDMB*, human lung tissue and mouse lung tissue from DOX-treated *Cc10*-h*GSDMB* mice. Deta CT values of *GSDMB* in various samples are relative to *GAPDH*). (**e**) The mRNA levels of ER stress-related genes were measured by qPCR from either control or GSDMB+ mice treated with Doxycycline (DOX).

Figure E10 GSDMB enhances RSV-induced IFN response and inflammation

(a) Illustration of the mouse model with RSV infections in the neonatal stage. Wild type (WT), *Cc10*-h*GSDMB* neonatal mice were repetitively nasally infected with $2x10^4$ pfu RSV line 19 (n = 5) or PBS (n= 5) at the age of Day 7, Day 14, Day 21, Day 56. Four days after the last infection, mice were harvested. (**b**) Hematoxylin-eosin (H&E) staining images of lung tissues and large airways. Scale bar, 100 um (black) or 20 um(white). (**c**) The mRNA levels of *Cxcl10*, *Viperin*, *Ifi44*, *Ifit1*, *Oasl2*, *Mx1* and *Isg15* in murine lungs as measured by qPCR. *, $p<0.05$ two-way ANOVA.

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