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1 **STING-dependent induction of neutrophilic asthma exacerbation in** 2 **response to house dust mite.**

3 Yasmine Messaoud-Nacer, PhD¹, Elodie Culerier, MSc¹, Stéphanie Rose, MSc¹, Isabelle
4 Maillat, MSc¹, Bernard Malissen, PhD², Urszula Radzikowska, PhD^{3,4}, Milena Sokolowska,
5 MD^{3,4}, Gabriel VL da Silva, PhD⁵, Michael R Edwards, PhD⁶, David J Jackson, PhD⁶, Sebastian
6 L Johnston, MD, PhD⁶, Bernhard Ryffel, MD, PhD¹, Valerie F. Quesniaux, PhD¹, and
7 Dieudonnée Togbe, PhD^{1*}

8
9 **ORCID ID** : DT (0000-0001-6658-414X); VFQ (0000-0003-2907-7995), BR (0000-0003-
10 2897-8230), SLJ (0000-0003-3009-9200); MS (0000-0001-9710-6685), UR (0000-0002-7341-
11 9764), BM (0000-0003-1340-9342).

12
13 ¹*Experimental and Molecular Immunology and Neurogenetics, INEM UMR7355 University of*
14 *Orleans and CNRS, 3B Rue de la Ferrollerie 45100 Orleans-Cedex 2, France.*

15 ²*Centre d'Immunophénomique (CIPHE), Aix Marseille Université, INSERM, CNRS, 13288,*
16 *Marseille, France.*

17 ³*Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Herman-*
18 *Burchard-Strasse 9, 7265 Davos Wolfgang, Switzerland*

19 ⁴*Christine Kühne – Center for Allergy Research and Education (CK-CARE), Herman-*
20 *Burchard-Strasse 1, 7265 Davos Wolfgang,*

21 ⁵*Ribeirão Preto Medical School, University of Sao Paulo. Avenida Bandeirantes, 3900 - CEP*
22 *14049-900. Ribeirao Preto, Sao Paulo, Brazil.*

23 ⁶*National Heart and Lung Institute, Imperial College Londont, London W2 1PG, United*
24 *Kingdom; Asthma UK Centre in Allergic Mechanism of Asthma, London, United Kingdom;*
25 *Imperial College Healthcare NHS Trust, The Bays, S Wharf Rd, London W2 1NY, United*
26 *Kingdom.*

27 **Corresponding author: Dieudonnée Togbe (dtogbe@cnrs-orleans.fr)*

28
29 **Running head:** STING activation induces neutrophilic asthma exacerbation

30 31 **Acknowledgements**

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38

39

40 **Abstract** (248 words)

41 **Background:** Severe refractory, neutrophilic asthma remains an unsolved clinical problem.
42 STING agonists induce a neutrophilic response in the airways, suggesting that STING
43 activation may contribute to the triggering of neutrophilic exacerbations. We aim to determine
44 whether STING-induced neutrophilic lung inflammation mimics severe asthma.

45 **Methods:** We developed new models of neutrophilic lung inflammation induced by house dust
46 mite (HDM) plus STING agonists diamidobenzimidazole (diABZI) or cGAMP in wild-type,
47 and conditional-STING-deficient mice. We measured DNA damage, cell death, NETs,
48 cGAS/STING pathway activation by immunoblots, N1/N2 balance by flow cytometry, lung
49 function by plethysmography, and Th1/Th2 cytokines by multiplex. We evaluated diABZI
50 effects on human airway epithelial cells from healthy or patients with asthma, and validated the
51 results by transcriptomic analyses of rhinovirus infected healthy controls *vs* patients with
52 asthma.

53 **Results:**

54 DiABZI administration during HDM challenge increased airway hyperresponsiveness,
55 neutrophil recruitment with prominent NOS2⁺ARG1⁻ type 1 neutrophils, protein extravasation,
56 cell death by PANoptosis, NETs formation, extracellular dsDNA release, DNA sensors
57 activation, IFN γ , IL-6 and CXCL10 release. Functionally, STING agonists exacerbated airway
58 hyperresponsiveness. DiABZI caused DNA and epithelial barrier damage, STING pathway
59 activation in human airway epithelial cells exposed to HDM, in line with DNA-sensing and
60 PANoptosis pathways upregulation and tight-junction downregulation induced by rhinovirus
61 challenge in patients with asthma.

62 **Conclusions:** Our study identifies that triggering STING in the context of asthma induces cell
63 death by PANoptosis, fueling the flame of inflammation through a mixed Th1/Th2 immune

64 response recapitulating the features of severe asthma with a prognostic signature of type 1
65 neutrophils.

66 **Keys words:** DNA sensing, cGAMP, diABZI, cell death, asthma exacerbation.

67

68

69 **Abbreviations**

70 AHR: airway hyperresponsiveness, AIM2: absent in melanoma 2, BAL, BronchoAlveolar
71 Lavage; BALF, BronchoAlveolar Lavage Fluid; cGAMP: cyclic Guanosine monophosphate-
72 Adenosine MonoPhosphate, cGAS: cyclic GMP-AMP synthase, DDX41: DEAD-box helicase
73 41, diABZI: symmetry-related amidobenzimidazole-based compounds, GSDMD: Gasdermin
74 D, GEO: Gene Expression Omnibus, hAEC, human airway epithelial cells, HBEC: human
75 bronchial epithelial cells, HDM: house dust mite, HE: hematoxylin and eosin, IFI204: IFN- γ -
76 Inducible protein 204, IRF3: IFN regulatory factor 3, ISG: Interferon Stimulated Gene, LDH:
77 Lactate DeHydrogenase, MLKL: Mixed Lineage Kinase domain Likepseudokinase, MPO:
78 myeloperoxidase, NETs: Neutrophil Extracellular Traps, NLRP3: NOD-, LRR- and Pyrin
79 Domain-Containing Protein 3, PAS: Periodic acid Schiff, RNA-Seq : RNA sequencing,
80 STING: STimulator of INterferon Genes, TBK1: TANK-binding kinase 1, WT: wild type,
81 ZBP1: Z-DNA-Binding Protein 1.

82

83 **Introduction** (455 words)

84 The most common reaction to several allergens such as house-dust mite (HDM) in allergic
85 asthma endotype is IgE production depending on high Th2 responses with IL-4, IL-5 and IL-
86 13 release, mucus overproduction, eosinophil recruitment, epithelial metaplasia and airway
87 remodeling ¹. The ability of an allergen to initiate the first steps of type 2 immune response is
88 based on direct epithelial cell damage and damage of associated cells forming the respiratory
89 barrier. Allergenic toxins or proteases may cause necrotic cell death leading to a strong Th2
90 response ². Severe asthma is often associated with Th2 low and mixed endotype, increased
91 neutrophils and extracellular DNA (eDNA) in patient sputum, with an increase in type 1
92 cytokines IL-1 β and IL-6 at the late onset of the disease, and unresponsiveness to
93 corticosteroids or specific biologicals ^{1,3-6}. However, the molecular mechanism underlying
94 severe neutrophilic asthma is more complex and remains unsolved.

95 Extracellular host-derived DNA has been associated with allergic type 2 immune responses.
96 Indeed, host DNA released from dying cells acts as a damage associated molecular pattern
97 (DAMP) that mediates e.g. the adjuvant activity of alum ⁷. The detection of extracellular host-
98 derived dsDNA is ensured by cyclic GMP–AMP synthase (cGAS) which converts ATP and
99 GTP into cyclic dinucleotide 2'3'cGAMP. The latter, in turn binds and activates the ER-
100 resident adaptor protein Stimulator of Interferon Genes (STING) which activates the
101 transcription factors NF-kB and IRF3, and the production of pro-inflammatory cytokines
102 including tumor necrosis factor (TNF α), IL-6 and type I IFNs (IFN α/β) ⁸.

103 Cytosolic dsDNA accumulated in airway epithelia of ovalbumin (OVA)- or HDM-challenged
104 mice ⁹. Deletion of cGAS in airway epithelial cells attenuated OVA or HDM induced allergic
105 airway inflammation, while cGAS promoted Th2 immunity likely by regulating airway
106 epithelial GM-CSF ⁹. Further, 2'3'cGAMP or c-di-GMP produced after activation of cGAS by
107 extracellular DNA, was shown to function as an adjuvant promoting type 2 allergic lung

108 inflammation when administered during sensitization with HDM, with increased of serum IgE
109 and eosinophils in the airways, an effect mediated by IL-33 and STING signaling pathway ^{10,11}.
110 Recently, we showed that endotracheal administration of cGAMP or the synthetic STING
111 agonist diamidobenzimidazole (diABZI) led to lung inflammation with neutrophilic response
112 in the broncho-alveolar space, cell death via PANoptosis, loss of epithelial barrier function, and
113 release of self-dsDNA and NETs ¹².
114 Here, we asked whether activating STING pathway during challenges after immunization with
115 the widely distributed house-dust mite allergen might affect the allergic response and trigger a
116 neutrophilic exacerbation. We show that cGAMP and furthermore diABZI administration
117 during challenge in mice exacerbated HDM-induced lung resistance, cell death by PANoptosis,
118 neutrophil recruitment with a prominent N2 to N1 switch, protein extravasation and
119 extracellular dsDNA release in the airways, with an overexpression of Th1 cytokine IFN γ . We
120 report differential expression of DNA-sensing and PANoptosis pathways after rhinovirus
121 challenge in patients with asthma.

122

123 **Methods** (565 words)

124 ***Mice***

125 Female C57BL/6Rj mice were purchased from Janvier Laboratories (Le Genest St Isle, France).
126 Wild type mice and mice deficient for STING (STING^{-/-})¹³, cGAS (cGAS^{-/-})¹⁴ were bred and
127 housed under specific pathogen free conditions at CNRS animal facility (TAAM UAR44,
128 Orleans, France). STING-OST^{fl} were crossed to LysM^{Cre} mice to generate STING-OST^{fl}
129 LysM^{cre} mice. They were maintained in a 12-h light-dark cycle with food and water ad libitum,
130 following European and local legislation. Age-matched, 8- to 12- week-old mice were used for
131 experiments. All animal experiments complied with the French Government animal experiment
132 regulations and ARRIVE guidelines. The protocols were submitted to the “Ethics Committee
133 for Animal Experimentation of CNRS Campus Orleans” under the number CLE CCO 2019-
134 2017 and 2020-2006, and approved by the French Minister under APAFIS #25876 and #26195.

135 ***Induction of allergic airway inflammation and bronchoalveolar lavage (BAL)***

136 Mice were anesthetized with 2% Isoflurane (ISO-VET, Netherlands) were sensitized with HDM
137 on day 0 and 7 (25 µg/mouse, i.n.) and challenged with HDM (Stallergenes Greer) intranasally
138 on 3 consecutive days (10 µg/mouse, i.n. on day 14-16), in the absence or together with cGAMP
139 (at 1, 3 or 10 µg/mouse, i.t.), diABZI compound 3 (at 0.1 or 1 µg/mouse, i.t) or Poly(I:C) (at 60
140 or 200 µg/mouse, i.t). Mice were analyzed on day 17, 24 hours after the last HDM instillation
141 (Fig. 1A).

142 Bronchoalveolar lavage (BAL) was performed 24h after the last challenge by flushing lung
143 tissue four times with 0.5 mL of cold NaCl 0.9% via tracheal intubation with a cannula. BALF
144 was collected, cells counted and cytopspins performed. The supernatant of the first lavage was
145 collected after centrifugation and stored at -80°C for dsDNA and mediator quantifications. The
146 left lung lobe was harvested for histology, the post caval lung for RNA extraction and qPCR
147 analysis and the right lobes for Western blot analysis and cytokine measurement. Protein

148 extravasation in the BALF was measured by Pierce™ BCA Protein Assay (ThermoFisher®,
149 Massachusetts).

150

151 *Cell Culture and stimulation*

152 Human airway epithelial cells (hAEC; Epithelix, Switzerland) isolated from bronchial biopsies
153 (passage 1) were maintained in 75 cm² flasks in the hAEC serum-free culture medium
154 (Epithelix). hAEC (passage 8) were seeded in 24-wells plate at 5 x 10⁵ cells/well, when they
155 reached 80% confluence. Cells were stimulated with HDM at 100 µg/mL alone or in
156 combination with diABZI (10 µM), cGAMP (14 µM) or Poly(I:C) (100 µg/mL) for 2 or 24h at
157 37°C and 5% CO₂. Human airway bronchial epithelium (MUCILAIR™; Epithelix,
158 Switzerland), from a single healthy or donor with asthma were maintained in MUCILAIR™
159 serum-free medium (Epithelix) and stimulated on the apical face, with HDM at 100 µg/mL
160 alone or in combination with diABZI, cGAMP or Poly(I:C) as above for 6h. Supernatant from
161 hAEC and MUCILAIR™ cultures were collected for mediator measurements and cells
162 harvested for mRNA and proteins analysis.

163

164 *Statistical Analysis*

165 Statistical analysis was performed with GraphPad Prism 9.0 software (San Diego). To
166 determine whether the data come from Gaussian distribution, column statistic using Shapiro-
167 Wilk normality test is performed before running the statistical analysis. Statistical significance
168 was determined by non-parametric Kruskal-Wallis multiple-comparisons tests followed by
169 Dunn post-test or two-way ANOVA followed by Dunn post-test as indicated in figure legends.
170 All data are shown as mean ± SEM. Saline control data are compared with HDM group. HDM
171 +/- agonist groups are compared. P value <0.05 was considered significant. *p <0.05, **p
172 <0.01, ***p <0.001 and ****p <0.0001.

173

174

175 **Results** (2614 words)

176 **Synthetic STING agonist diABZI triggers a neutrophilic asthma exacerbation in HDM**
177 **sensitized mice.**

178 To evaluate how STING activation by agonists modulate type 2 immune response, we tested
179 their effects during the challenge phase of HDM-induced allergic lung inflammation in mice.
180 We compared the effect of the natural STING agonist cGAMP, and of the potent, non-
181 nucleotidyl STING agonist diABZI¹⁵, to the TLR3 agonist dsRNA Poly(I:C), all known to
182 induce IRF3-type 1 IFN response. Wild type BL6 (WT) mice sensitized with HDM on day 0
183 and 7 (25 µg/mouse, i.n.) and challenged on day 14-16 (10 µg/ mouse, i.n.) received either
184 diABZI (1 µg/mouse, i.t.), cGAMP (10 µg/mouse, i.t.), or Poly(I:C) (200 µg/mouse, i.t.), doses
185 determined from previous titrations (**Suppl Fig E1-3**), and were analyzed on day 17, 24-hours
186 after the last challenge (**Fig 1A**). First, we evaluated airway hyperresponsiveness (AHR), one
187 of the hallmarks of allergic asthma known to correlate with disease severity^{16,17}. There was a
188 significant increase of AHR in HDM-challenged WT mice at a dose of 200 mg/mL of
189 methacholine (MCh), as compared to saline control (**Fig 1B**). Administration of diABZI during
190 HDM challenge further increased AHR significantly, while cGAMP increased AHR only at
191 200 mg/mL of MCh, and Poly(I:C) had no effect, compared to untreated HDM-challenged mice
192 (**Fig 1B**). DiABZI and cGAMP administration had little effect on eosinophil recruitment in the
193 airways (**Fig 1C**), while they strongly increased neutrophil recruitment (**Fig 1D**) in HDM-
194 challenged mice. Interestingly, diABZI and cGAMP promoted a subsets of neutrophils
195 expressing inducible nitric oxide synthase (iNOS/NOS2) corresponding to type 1 neutrophils
196 (N1, NOS2⁺ARG1⁻) while decreased the frequency of arginase 1 (ARG1) expressing type 2
197 neutrophils (N2, NOS2⁻ARG1⁺) (**Fig 1E, Suppl Fig E4A-D**). This increase in N1 neutrophils

198 was associated with higher myeloperoxidase (MPO) in BALF and lung (**Fig 1F-G**), total protein
199 extravasation (**Suppl Fig E4E**) and dsDNA release in BALF (**Fig 1H**). There was also an
200 increase in macrophages, but not in lymphocytes influx in BAL (**Suppl Fig E4A-C**) or Th2
201 cytokines IL-4, IL-5 and IL-13 (**Suppl Fig E4F-K**). The exacerbation effect was observed
202 especially with diABZI, inducing a strong IFN γ response (**Fig 1I**) with CCL11, IL-6 and TNF
203 release in the airways (**Suppl Fig E4L-P**). Further, IFN α/β , the end-products of STING
204 pathway activation were increased in BALF upon diABZI administration (**Fig 1J, K**).

205

206 STING agonists induced a narrowing of the airways with severe infiltration of inflammatory
207 cells in the peribronchial area, epithelial injury, bronchiolitis, alveolitis and decreased goblet
208 cells hyperplasia and production of mucus together with a decrease in *Muc5ac* transcripts in the
209 lung compared to HDM-challenged mice (**Fig 1L-P**), while TLR3 agonist caused no major
210 changes (**Fig 1L-O**). Muc5AC protein concentration doubled in BALF after administration of
211 STING or TLR3 agonists while it was essentially unaffected in the lung (**Fig 1Q-R**). STAT6,
212 the transcription factor involved in *Muc5ac* gene induction and mucus hypersecretion¹⁸ was
213 phosphorylated in the lung of HDM challenged mice and this was increased by STING or TLR3
214 agonists (**Fig 1S, Suppl Fig E4Q**).

215 Thus, administration of the potent STING agonist diABZI during HDM challenge augmented
216 HDM-induced lung resistance, together with a strong neutrophilic response characterized by
217 neutrophil recruitment, MPO expression, protein extravasation, overexpression of Th1 cytokine
218 IFN γ and extracellular dsDNA release in the airways.

219

220 **NETosis and PANoptosis as main sources of airway dsDNA fueling STING-triggered**
221 **exacerbation of HDM inflammation.**

222 Extracellular dsDNA release is an active process in several chronic inflammatory diseases.
223 Therefore, we first assessed the ability of neutrophils to form NETs^{19,20}. Indeed, MPO staining
224 demonstrated that unlike HDM-treated mice that showed localized cellular staining, the lung of
225 cGAMP- and more importantly diABZI-challenged mice exhibited the typical morphological
226 shape of NETs (**Fig 2A**), with citrullinated histone H3 (Cit-H3) revealed by immunoblot (**Fig**
227 **2B, suppl Fig E5A**). In contrast, Poly(I:C) did not result in the formation of NET, with
228 intracellular MPO staining and absence of Cit-H3 (**Fig 2A-B**). DNA damage was documented
229 by increased γ H2AX after both diABZI and cGAMP challenges, but less after Poly(I:C)
230 administration (**Fig 2B, suppl Fig E5A**).

231 NETs comprise host DNA fibers coated with cytoplasmic proteases, making host DNA
232 available to activate DNA sensors. Indeed, cGAS, IFI204 and/or DDX41 expression increased
233 in the lung of HDM and STING agonist challenged mice (**Fig 2C, suppl Fig E5B**). Further,
234 there was evidence of inflammasome activation, likely involving AIM2 and/or NLRP3, with
235 non-canonical caspase-11 upregulation, resulting in cleavage of IL-1 β and IL-18 release after
236 STING trigger (**Fig 2D, suppl Fig E5C**).

237 NETs associated molecules such as MPO, neutrophil elastase and defensins may promote lung
238 injury, and directly induce epithelial and endothelial cell death^{19,21}. HDM-immunized and
239 challenged mice co-administered with STING or TLR3 agonists induced caspase 3 cleavage
240 indicative of apoptosis in the lung tissue, but also cell death markers of necroptosis and
241 pyroptosis with upregulation of Mixed Lineage Kinase domain Likepseudokinase (MLKL) and
242 Gasdermin D (GSDMD), and GSDMD cleavage indicative of PANoptosis (**Fig 2E, suppl Fig**
243 **E5D**). We further assessed specific components of the macromolecular complexes regulating
244 PANoptosis by immunoblot. Indeed, Z-DNA binding protein 1 (ZBP1), caspase-8 and RIPK3
245 were upregulated after challenge with STING or TLR3 agonists in HDM-treated mice (**Fig 2E,**
246 **suppl Fig E5D**). We also demonstrated a co-localization of PANoptosis components Caspase-

247 8, ASC and RIPK3 proteins in macrophages from BAL cells (**Fig 2F**) which is absent in
248 neutrophils (**Suppl Fig E5E**).

249 Therefore, neutrophils induced during HDM challenge with STING agonists undergo NETs
250 formation, expose host DNA to activate innate immune DNA sensors, inducing lung tissue
251 damage and death by PANoptosis, amplifying DNA release as a positive feedback loop.

252

253 **STING dependence of the neutrophilic exacerbation of HDM-induced response.**

254 To evaluate the STING specificity of diABZI induced asthma exacerbation, response to the
255 extracellularly released dsDNA, mice deficient for STING ($STING^{-/-}$) or cGAS ($cGAS^{-/-}$) were
256 sensitized and challenged with HDM with or without diABZI as above (**Fig 3A**). DiABZI-
257 induced increase of neutrophils was absent in the airways of HDM-challenged $STING^{-/-}$ mice,
258 and reduced in $cGAS^{-/-}$ mice (**Fig 3B**), as were total inflammatory cells, macrophages and
259 lymphocytes, or the decreased recruitment of eosinophils (**Suppl Fig E6A-D**). In addition,
260 diABZI-induced increase of MPO and extracellular dsDNA (**Fig 3C-E**), or protein
261 extravasation, $IFN\gamma$, TNF, IL-6, and CXCL10 (**Fig 3F-K**) release were abolished in the airways
262 of HDM-challenged $STING^{-/-}$ mice, while they were barely affected in $cGAS^{-/-}$ mice. Because
263 allergic airway inflammation triggers Th2 immune response, we evaluated Th2 cytokines and
264 found increased IL-4 and IL-5 transcripts and proteins in HDM-challenged mice (**Suppl Fig**
265 **E6E, F and H, I**). DiABZI further increased Th2 cytokine IL-13 and chemokines CCL11 and
266 CCL24 in the airways, and this was absent in $STING^{-/-}$ and reduced in $cGAS^{-/-}$ mice (**Suppl Fig**
267 **E6 G, J-L**). Pulmonary CXCR2⁺ and CXCR4⁺ expressing neutrophils subsets increased after
268 diABZI challenge were reduced in $STING^{-/-}$ and $cGAS^{-/-}$ mice (**Suppl Fig E6 O-R**).

269 The increased leukocyte infiltration, epithelial injury, with decreased goblet cells, mucus
270 staining (**Fig 3L-O**) and *Muc5ac* transcript expression (**Fig 3P**) after diABZI administration in
271 HDM-challenged WT mice were abolished in $STING^{-/-}$ mice, while they were barely affected

272 in cGAS^{-/-} mice. DiABZI and HDM co-administration upregulated *Tmem173* (STING)
273 transcript expression in WT and cGAS^{-/-} mice (**Fig 3Q**), while the increase in *Mb21d1* (cGAS)
274 transcripts was abolished in STING^{-/-} mice (**Fig 3R**).

275 IL-13 mRNA expression, a Th2 cytokine necessary for mucus metaplasia, increased after
276 diABZI and HDM co-administration in WT mice, while it was barely affected in STING^{-/-} or
277 cGAS^{-/-} mice. IL-13 binding triggers a STAT6-dependent transcriptional program involving the
278 activation of *Serpin1* and *Clca1* (protein calcium-activated chloride channel 1) whose
279 transcripts were overexpressed after diABZI administration in HDM-challenged WT mice but
280 not in STING^{-/-} mice (**Fig 3S-V**). On the other hand, the transcripts for *Socs1*, a negative
281 regulator of *Muc5ac* expression²², was overexpressed after diABZI co-administration in WT
282 mice but not in STING^{-/-} mice (**Fig 3W**), in line with the fact that STING activation was shown
283 to enhance SOCS1 expression²³.

284 Thus, diABZI-induced asthma exacerbation is mediated by STING pathway activation in terms
285 of neutrophil recruitment, release of extracellular dsDNA and pro-inflammatory cytokines in
286 the airways, increased epithelial injury with decreased goblet cells and mucus production, while
287 the involvement of cGAS is more limited.

288

289 **STING-deficient macrophages and granulocytes mitigate asthma exacerbation in vivo.**

290 Neutrophilic inflammation is observed during asthma exacerbation but also in the airways of
291 patients with severe asthma²⁴. In addition to neutrophils, macrophages are one of the main lung
292 cell types responsible for the uptake of cGAMP *in vivo*²⁵. We investigated the contribution of
293 both cell types in mice by generating conditional STING deficient mice. We crossed mice
294 carrying STING-OST floxed alleles (STING-OST^{fl}) with LysM^{cre} transgenic mice expressing
295 Cre under the control of the LysM promotor to obtain STING-OST^{fl}LysM^{cre/+} mice deficient
296 for STING gene in myeloid cells such as granulocytes and macrophages, as well as STING-

297 OST^{fl}LysM^{+/+} control mice (**Suppl Fig E7 A-D**). Administration of cGAMP during HDM
298 challenges resulted in a reduction of eosinophils and an increase in total cells, neutrophils, MPO
299 and self dsDNA in BALF of STING-OST^{fl}LysM^{+/+} control mice (**Fig4 A-E** and **Suppl Fig**
300 **E7E, G**). These parameters were partially reduced in STING-OST^{fl}LysM^{cre/+} suggesting that
301 myeloid cells, but also other cell types contribute to shape the neutrophilic response after
302 STING trigger (**Fig4 A-E**). cGAMP induced an increase in type I IFN α/β , as well as pro-
303 inflammatory cytokines TNF α and IL-6 production in HDM-treated STING-OST^{fl}LysM^{+/+}
304 control mice that were partially reduced in STING-OST^{fl}LysM^{cre/+} (**Fig 4 F-I**). The increase in
305 peribronchial leukocytes infiltration and epithelial injury following cGAMP administration in
306 STING-OST^{fl}LysM^{+/+} mice was abrogated in STING-OST^{fl}LysM^{cre/+} (**Fig 4J, K**). Moreover,
307 protein extravasation in BALF was reduced in STING-OST^{fl}LysM^{cre/+} mice in comparison with
308 control mice (**Suppl Fig E7F**), which was suggestive of a less severe barrier disruption.
309 Neutrophils infiltrating the lung of mice lacking STING in granulocytes/macrophages, were
310 tested for their ability to form NETs. BAL cells from STING-OST^{fl}LysM^{+/+} control mice
311 challenged with HDM and cGAMP showed the formation of a weblike structure positive for
312 MPO and Cit-H3 staining (**Fig 4L**), suggestive of NET formation. In contrast, this process was
313 reduced in STING-OST^{fl}LysM^{cre/+} group (**Fig 4L**), in line with the reduced histopathological
314 damage observed.

315 Thus, these data indicate that granulocytes/macrophages, but also other cell types, contribute to
316 the development of STING-dependent neutrophilic asthma exacerbation *in vivo*.

317
318 **Epithelial cells respond to diABZI and may contribute to STING agonist-induced**
319 **neutrophilic asthma exacerbation.**

320 Allergens cause airway epithelium damage and structural changes including downregulation of
321 group I tight junction and extracellular matrix (ECM) expression which are often associated
322 with asthma exacerbations and severity²⁶⁻²⁸. The transmembrane proteins Zona-occludens-1

323 (ZO-1) are scaffold proteins important for cell-cell adhesion in healthy tissue and main
324 regulators of epithelial permeability in allergic lung inflammation²⁷. After confirming the role
325 of diABZI on immune cells infiltration, we investigated its effect on human airway epithelium.
326 HDM led to ZO-1 downregulation in lung tissue compared to saline control (**Fig 5A**), as
327 expected²⁷. STING agonists DiABZI and cGAMP, and Poly(I:C) further downregulated ZO-1
328 expression as evidenced by immunofluorescence staining (**Fig 5A**). To evaluate the ability of
329 diABZI to activate STING pathway in epithelial cells, human airway epithelial cells (hAEC)
330 from bronchial biopsies were stimulated for 2h or 24h with HDM alone or in combination with
331 diABZI, cGAMP or Poly(I:C). There was evidence of STING pathway activation with the
332 detection of phosphorylated STING, TBK1 and IRF3 when hAEC were co-stimulated with
333 diABZI detectable at an early stage, 2h post stimulation (**Fig 5B, suppl Fig E8A**). cGAMP and
334 Poly(I:C) co-stimulation also induced TBK1 phosphorylation at 2h, that persisted after 24h with
335 diABZI and cGAMP. Moreover, there was evidence of epithelial DNA damage in hAEC,
336 revealed by phosphorylation of γ H2AX at 2h and 24h post stimulation (**Fig 5C, suppl Fig**
337 **E8A**), and some activation of necroptosis with the phosphorylation of MLKL at 2h (**Fig 5C,**
338 **suppl Fig E8A**). Activating STING with diABZI in cells sensitized with HDM yielded in the
339 secretion of inflammatory cytokines starting from 2h post stimulation that were sustained after
340 24h, such as CXCL8, IFN β , CXCL10, IL-6 and TNF- α (**Fig 5 D-M**).

341 To further characterize the contribution of epithelial barrier, air liquid interface (ALI) culture
342 of bronchial epithelial cells isolated from the lung of healthy donor and patient with asthma
343 were performed and stimulated for 16h with HDM alone or in combination with diABZI,
344 cGAMP or Poly(I:C) (**Fig 5 N**). DiABZI induced an increased release of lactate dehydrogenase
345 (LDH), IFN α , IFN β , IFN λ , CXCL10, IL-6 and TNF- α protein and overexpression of *MUC5AC*
346 transcript in healthy epithelial cells sensitized with HDM (**Fig 5 O-V**), and less in epithelial
347 cells from patient with asthma (**Fig 5 O-V**). Further, diABZI induced epithelial DNA damage

348 evidenced by the phosphorylation of γ H2AX in epithelial cells from patient with asthma,
349 compared to healthy cells (**Fig 5 W**).

350 Thus, diABZI affected airway epithelium cells by activating STING pathway, impairing tight
351 junctions, and inducing epithelial DNA damage, necroptosis and secretion of inflammatory
352 cytokines, that may further fuel the lung inflammation.

353

354 **Viral infection of human bronchial epithelial cells from patients with asthma aggravates**
355 **inflammation and weakens epithelial barrier both *in vitro* and *in vivo*.**

356 To ascertain the clinical relevance of these findings, we first analyzed the different expression
357 of STING and PANoptosis pathways in PBMC from healthy controls or patients with severe
358 asthma (**Suppl FigE9**). Indeed, host double-stranded DNA released by NETosis promote
359 rhinovirus-induced asthma exacerbations ²⁹. Therefore, we analyzed DNA-sensing,
360 PANoptosis, tight junction, and neutrophils gene signatures from two independent studies
361 addressing Rhinovirus (RV) infection in patients with asthma, either *in vitro* in differentiated
362 primary bronchial epithelial cells (HBECs) from healthy and individuals with asthma ³⁰, or *in*
363 *vivo* in bronchial brushing from intranasally RV infected healthy controls and patients with
364 asthma ^{31,32} (**Supl FigE10**).

365 Human epithelial cells from patients with asthma stimulated *in vitro* with RV-A16 (**Fig. 6A**)
366 showed an upregulation of genes involved in DNA-sensing and STING pathway (**Fig.6B**) such
367 as the DNA sensors ZBP1, IFI16 and the cytosolic TRIM21. *IRF3* responsible of type I/III IFNs
368 induction and anti-viral defense was also upregulated in the cells of these patients, concomitant
369 with *IFN- λ* expression, while STING itself was not significantly downregulated. Analysis of
370 tight-junction gene sets (**Fig.6C**) revealed the reduced expression of a whole cluster of genes
371 such as *OCN*, *CLD3*, *CLDN4*, *NRAS*, *HCLS1*, *MYH10* in virus-infected cells from patients
372 with asthma in comparison to the controls. Moreover, analysis of PANoptosis related genes set

373 revealed the upregulation and a tendency to upregulation of several components of the
374 PANoptosome such as the pivotal *ZBP1*, *PYCARD*, *CASPASE-8*, *RIPK1*, *CASPASE-1*, and
375 *FADD* after viral infection of the cells from patients with asthma compared to the controls
376 (**Fig.6D**), which was not visible before RV infection (**Suppl Fig E10A-D**). Mucus genes such
377 as *MUC5AC* and *MUC5B* were also upregulated (**Suppl Fig E10E-F**).

378 Bronchial brushings from patients with asthma experimentally infected *in vivo* with RV-A16³¹
379 (**Fig. 6E**) showed a decrease in a group of tight junction-related gene such as *CLDN15*, *TJAP1*,
380 *AKT2*, *SRC*, *CGN*, *EXOC3*, *LLG2*, *CLDN22*, compared to similarly infected healthy controls,
381 while *CLDN12* and *MYL12B* genes were increased (**Fig. 6F**). PANoptosis components showed
382 a tendency toward an increase 4 days after *in vivo* infection in patients with asthma compared
383 to controls (**Fig.6G**), in contrast to the non-infected controls and patients with asthma, where
384 the PANoptosis components were downregulated (**Suppl Fig E9G-I**). Moreover, the gene
385 expression of IL-18 (*IL18*) was higher after infection in patients with asthma compared to
386 healthy individuals (**Fig.6G**), in line with inflammasome activation³². Interestingly, the
387 *NOS2/ARG1* gene expression balance was disrupted in bronchial brushings. *NOS2* was
388 significantly increased after infection in patients with asthma compared to the controls, while
389 *ARG1* was downregulated in patients with asthma (**Fig.6H-I**). Overall, these analyses show an
390 involvement of DNA sensing pathway, weakening of the epithelial barrier and upregulation of
391 pro-inflammatory markers such as *NOS2* after RV infection in patients with asthma.

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399 **Discussion** (791 words)

400 Here we demonstrate the existence of a tight link between STING activation during type 2
401 allergic response challenge and asthma exacerbation, with reproduction of hallmark features of
402 severe asthma and epithelial damage. Indeed, administration of synthetic STING agonist
403 diABZI during HDM challenge induces asthma exacerbation by increasing epithelial damage,
404 dsDNA release, DNA damage, cell death by NETosis and PANoptosis, and neutrophils influx
405 in the lung. We highlight the partial requirement for STING expression in
406 granulocytes/macrophages to induce asthma exacerbation. We show that co-stimulation of
407 human epithelial cells with diABZI and HDM induces IL-6, TNF- α , type I IFN α/β and type III
408 IFN λ release *in vitro*, with DNA damage and cell death. These results have important
409 implications revealing STING agonists as inducers of asthma exacerbation and offer a
410 mechanism through which dsDNA release after cell death triggers Th1 response and promotes
411 neutrophilic asthma exacerbation.

412 Host DNA released from dying cells was shown to mediate alum adjuvant activity ⁷, and
413 cGAMP may function as a type 2 adjuvant promoting allergic asthma when administered during
414 sensitization with HDM ¹⁰. Our previous findings showing neutrophilic response induced by
415 STING agonists in the airways ¹² prompted us to ask whether triggering STING during HDM-
416 challenge might affect the allergic response and promote a neutrophilic exacerbation.

417 The potent STING agonist diABZI administered during HDM-challenge strongly exacerbated
418 neutrophil recruitment and the release of IFN γ , CXCL1/KC and CXCL10/IP10 in the airways.
419 It induced some cell stress with extracellular dsDNA release, mobilized DNA sensors, and
420 promoted an IFN α/β response. Importantly, administration of diABZI during HDM-challenge
421 functionally exacerbated HDM-induced lung resistance, while Poly(I:C) did not affect lung
422 function. We verified that diABZI-induced neutrophilic response in HDM-challenged mice is
423 fully dependent on STING pathway, as it is absent in STING-deficient mice, and showed a

424 limited involvement of cGAS. The increased neutrophil recruitment, together with the presence
425 of extracellular DNA, MPO and Cit-H3 was indicative of NETs formation, that were indeed
426 visible in the airways of HDM challenged mice after cGAMP or diABZI administration. Thus,
427 STING agonist induction of NETosis may contribute to the neutrophilic exacerbation of HDM-
428 induced responses.

429 STING agonists led to a reduction of goblet cell numbers and of mucus transcript in the lung
430 of HDM-challenged mice, together with a strong release of mucus protein in the airways. A
431 possible explanation is that diABZI induced epithelial barrier damage, documented by
432 epithelium cell tight junction ZO-1 protein expression, leading to Goblet cells shedding and
433 release of their mucus content, that may clog the airspace and contribute to the exacerbation of
434 HDM-induced lung resistance.

435 Several soluble mediators regulate the recruitment of neutrophils in the airways ²⁴. Indeed,
436 diABZI induced IFN γ , CXCL1/KC and CXCL10/IP10 release *in vivo* in the BAL of HDM-
437 challenged mice, and CXCL8 *in vitro* in the supernatant of human epithelial cells. These
438 mediators are potent neutrophil chemoattractants in the lung, CXCL10 facilitating the
439 recruitment of Th1 cells producing IFN γ which, in turn, upregulates chemotaxis receptors of
440 human neutrophils ³³. Functional type I IFN signaling in neutrophils induces neutrophil
441 maturation, and limits their lifespan by acting on both extrinsic and intrinsic apoptosis pathways
442 but also by influencing the NETosis process ³⁴. Indeed, we show increased mature neutrophils
443 expressing high CXCR2 or CXCR4 in the airways after diABZI during HDM-challenge.

444 Type I IFNs are essential regulators of neutrophil polarization, as shown in cancer, driving
445 neutrophils from anti-inflammatory, pro-tumoral N2 phenotype into pro-inflammatory, anti-
446 tumoral N1 capable of cytotoxicity ^{34,35}. Here, we demonstrate that STING agonists strongly
447 induced N1 (NOS2⁺ARG1⁻) subset. This phenotypic shift from N2 (ARG1⁺NOS2⁻) observed at
448 HDM baseline response toward N1 pro-inflammatory after STING agonist challenge, may

449 shape neutrophils function and enroll them in NETs formation process, in part responsible of
450 lung tissue damage. Conversely, the N2 (ARG1⁺ NOS2⁻) profile of Poly(I:C)-induced
451 neutrophils may explain the lack of NET in the lung of Poly(I:C) treated HDM challenged mice.
452 NOS2 and ARG1 are important enzymes regulating both macrophage and neutrophil functions
453 and markers of alternative activation^{36,37}. NOS2 has multiple roles in the airways, its expression
454 being highest in severe asthma, while ARG1 decreases³⁸⁻⁴¹. We report increased NOS2
455 expression in RV-A16 infected patients with asthma, as compared to healthy controls. Further,
456 transcriptomic analyses of RV-A16 infected cells from patients with asthma also indicated an
457 involvement of DNA sensing, PANoptosis, and weakening of the epithelial barrier. Our study
458 is the first to demonstrate the induction of this particular subset of N1, NOS2⁺ARG1⁻ neutrophils
459 following STING activation.

460 Triggering STING in an allergic context may shape the inflammatory response toward a mixed
461 Th1/Th2 immune response and recapitulate features of severe neutrophilic asthma with
462 increased lung resistance, NETosis and PANoptosis, increased epithelial injury, release of
463 extracellular dsDNA and pro-inflammatory cytokines in the airways. Thus, targeting the
464 STING pathway might be considered in severe asthma including seasonal respiratory viral
465 exacerbation.

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470 **Author Contributions**

471 Y.M.-N. performed most experiments with the assistance of E.C. and S.R. B.M. generated the
472 STING-OST^{fl} mouse model. Y.M.-N., D.T., B.R. and V.Q. conceived the project and designed
473 the experiments, Y.M.-N., D.T., B.R., V.Q., G.dS., M.E., D.J., S.J., M.S. and U.R. analyzed
474 and interpreted the data. Y.M.-N, D.T., B.R. and V.Q. wrote the manuscript. All authors had
475 the opportunity to discuss the results and comment on the manuscript.

476

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493

494 **Conflict of interest statement**

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514

515 **Ethics Statement**

516 All animal experiments complied with the French Government animal experiment regulations
517 and ARRIVE guidelines. The protocols were submitted to the "Ethics Committee for Animal

518 Experimentation of CNRS Campus Orleans” under the number CLE CCO 2019-2017 and 2020-
519 2006, and approved by the French Government animal experiment regulatory authorities under
520 APAFIS #25876 and #26195. In vivo rhinovirus infection in controls and patients with asthma was
521 approved by the St. Mary`s Hospital Research Ethics Committee (United Kingdom), permission number
522 09/H0712/59 (observational trial registration number NCT01159782) ^{31,32}.

523

524 **Data Availability Statement**

525 All datasets generated and analyzed during this study are included in this published article and
526 its Supplementary Information files. Publicly available Transcriptome data from bronchial
527 brushings from control individuals and patients with asthma infected in vivo with RV are available under
528 accession number: GSE185658 ³². Publicly available RNAseq data GSE61141 were downloaded from
529 the NCBI gene expression omnibus ^{30,32}. Additional data are available from corresponding author
530 on reasonable request. The PBMCs scRNAseq datasets from patients with severe asthma and
531 control individuals analyzed in the present study, have been deposited in the Sequence Read
532 Archive (SRA) repository under the accession number GSE172495 ⁴².

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637

638 **Figures legends**

639 **Figure 1: Endogenous STING agonists promote lung neutrophilia and exacerbate allergic**
640 **airway inflammation.**

641 **(A)** Mice sensitized with HDM on day 0 and 7 (25 µg/mouse, i.n.) and challenged with HDM
642 on day 14-16 (10 µg/mouse, i.n.) received either diABZI (1 µg/mouse, i.t.), cGAMP (10
643 µg/mouse, i.t.), or Poly(I:C) (200 µg/mouse, i.t.) on day 14-16, and were analyzed on day 17.

644 **(B)** Airway resistance to increased doses of methacholine (50-200 mg/mL Mch) was measured
645 24h after the last HDM/agonist challenges. **(C)** Eosinophil and **(D)** neutrophil counts in BAL.

646 **(E)** Flow cytometry analysis of NOS2/ARG1 expressing neutrophils pre-gated on singlet cells,
647 and CD45⁺CD11b⁺Ly6G⁺F4/80⁻SiglecF⁻ cells. **(F, G)** Myeloperoxidase (MPO) concentrations

648 in BALF and lung determined by ELISA. **(H)** dsDNA measured in the acellular fraction of the

649 BAL. **(I)** IFN γ , **(J)** IFN- α and **(K)** IFN- β concentrations in BALF measured by multiplex
650 immunoassay. **(L)** Histology of lung sections stained with PAS, with semi quantitative
651 pathology scoring of **(M)** goblet cells, **(N)** peribronchial infiltrates and **(O)** epithelial injury.

652 Bars, left panel: 2.5 mm, right panel: 250 µm. **(P)** *Muc5ac* transcripts measured by real-time
653 PCR **(Q, R)** *Muc5ac* protein in BALF and lung measured by ELISA. **(S)** Immunoblots of
654 phospho-STAT6 and STAT6 with Actin β as a reference. Data were presented as mean \pm SEM

655 with n = 8 mice per group. Each point represents an individual mouse. *p < 0.05, **p < 0.01,

656 ***p < 0.001, ****p < 0.0001 (Nonparametric Kruskal–Wallis with Dunn’s post-test).

657

658 **Figure 2: NETosis, apoptosis, pyroptosis and necroptosis (PANoptosis) as main sources**
659 **of airway dsDNA exacerbating HDM-induced lung inflammation.**

660 Mice sensitized and challenged with HDM received either diABZI, cGAMP, or Poly(I:C) as in Figure
661 1, and were analyzed on day 17. **(A)** Visualization of NETs formation in lung tissue with the
662 staining of DNA dye DAPI (Blue) and MPO (Yellow). Bars (20 µm). Tissue fluorescence

663 intensity (TFI) of MPO staining in lung. **(B)** Immunoblot highlighting DNA damage with
664 expression of Cit-H3 phospho- γ H2AX and γ H2AX with Actin β as a reference. **(C)** Immunoblot
665 of STING axis including phospho-STING, STING dimer, STING, phospho-TBK1, TBK1 and
666 DNA sensors including cGAS, DDX41 and IFI204 with Actin β as a reference. **(D)** Immunoblot
667 of inflammasome activation including AIM2, NLRP3, Caspase-11, cleaved caspase-11, IL-1 β ,
668 cleaved IL-1 β and IL-18 with Actin β as a reference. **(E)** Immunoblot of cell death axis showing
669 cleaved caspase-3, caspase-3, MLKL, cleaved GSDMD, GSDMD, ZBP1, caspase-8, and
670 RIPK3 with Actin β as a reference. **(F)** Confocal microscopy showing Caspase-8 (green), ASC
671 (red), RIPK3 (far-red/turquoise blue) and DNA dye DAPI (cyan) in BAL cells showing the
672 colocalization of PANoptosome components. Bars, 2 μ m.

673

674 **Figure 3: STING dependence of the neutrophilic airway inflammation exacerbation.**

675 **(A)** Wild-type (WT), STING^{-/-} or cGAS^{-/-} mice were sensitized with HDM on day 0 and 7 (25
676 μ g/mouse, i.n.), challenged with HDM on day 14-16 (10 μ g/mouse, i.n.) without or with diABZI
677 (1 μ g/mouse, i.t.), and analyzed on day 17. **(B)** Neutrophils count in BAL. **(C, D)** MPO
678 concentration in BALF and lung determined by ELISA. **(E)** dsDNA concentration in the BAL
679 acellular fraction. **(F)** Protein concentration in BALF. **(G)** IFN- γ in BALF determined by
680 multiplex immunoassay. **(H, I)** TNF- α in BALF and lung determined by ELISA. **(J)** IL-16 and
681 **(K)** CXC10/IP-10 concentration in BALF measured by ELISA. **(L)** Histology of lung tissues
682 stained with PAS of WT, STING^{-/-} and cGAS^{-/-} mice, with semi quantitative pathology scoring
683 of **(M)** peribronchial infiltrates, **(N)** goblet cells and **(O)** epithelial injury. Bars, upper panel: 2.5
684 mm, lower panel: 250 μ m. **(P-W)** *Muc5ac*, *Tmem173*, *Mb21d1*, *Il-13* *Serpin1*, *Clc1*, *Spdef* and
685 *Socs1* transcripts measured by real-time qPCR. Data were presented as mean \pm SEM with n =
686 6 ~ 8 mice per group. Each point represents an individual mouse. *p < 0.05, **p < 0.01, ***p
687 < 0.001, ****p < 0.0001 (Nonparametric Kruskal–Wallis with Dunn’s post-test).

688

689

690 **Figure 4: STING-deficient macrophages and granulocytes mitigates asthma**

691 **exacerbation *in vivo* in the lung.**

692 **(A)** STING-OST^{fl}LysM^{cre/+} or STING-OST^{fl}LysM^{+/+} mice were sensitized with HDM on day 0
693 and 7 (25 µg/mouse, i.n.), challenged with HDM on day 14-16 (10 µg/mouse, i.n.) without or
694 with cGAMP (10 µg/mouse, i.t.), and analyzed on day 17. **(B)** Eosinophils and **(C)** Neutrophils
695 counts in BAL. **(D)** MPO concentration in BALF measured by ELISA. **(E)** dsDNA measured
696 in the acellular fraction of the BAL. **(F)** Concentration of IFN-α and **(G)** IFN-β in BALF
697 measured by luminex immunoassay. **(H)** IL-6 concentration **(I)** and TNF-α concentration in
698 BALF measured by ELISA. **(J)** Lung tissue histology with PAS staining of STING-
699 OST^{fl}LysM^{cre+} or STING-OST^{fl}LysM^{+/+} mice with semi quantitative pathology scoring of **(K)**
700 peribronchial infiltrates, epithelial injury and goblet cells. **(L)** Visualization of NET in BAL
701 with the staining of DNA dye DAPI (blue), MPO (Green), CitH3 (Red). Bars, 20 µm. Data were
702 presented as mean ± SEM with n = 8 ~ 13 mice per group. Each point represents an individual
703 mouse. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 (Nonparametric Kruskal–Wallis
704 with Dunn’s post-test).

705

706 **Figure 5. Epithelial cells contribute to the process of STING agonists-induced neutrophilic**
707 **asthma exacerbation.**

708 **(A)** Immunofluorescence staining of Zonulin-1 (ZO-1) (RED) in lung tissue sections from mice
709 treated by HDM and challenged or not with diABZI, cGAMP, or Poly(I:C) as in Figure 1,
710 counterstained with DAPI (Blue). Upper panel: 20µm, lower panel: 5µm. Immunoblots of
711 human airway epithelial cell (hAEC) stimulated with HDM at 100 µg/mL alone or in
712 combination with diABZI (10 µM), cGAMP (14 µM) or Poly(I:C) (100 µg/mL) for 2 or 24h

713 showing **(B)** STING pathway activation including phospho-STING, STING, phospho-TBK1,
714 TBK1, phospho-IRF3, IRF3, phospho-STAT6 and STAT6, with Actin β as a reference. **(C)**
715 DNA damage and cell death axis including phospho- γ H2AX, γ H2AX, phospho-MLKL, MLKL
716 and Actin β as a reference. **(D-M)** Cytokines concentration of **(D, I)** IL-8/CXCL8, **(E, J)** IFN-
717 β , **(F, K)** IP-10/CXCL10, **(G, L)** IL-6 and **(H, M)** TNF- α in cell culture supernatant were
718 measured by multiplex immunoassay at 2h **(D-H)** and 24h **(I-M)**. Data were presented as mean
719 \pm SEM with n = 3 independent wells from the same donor. **(N)** Human airway epithelium
720 (MUCILAIR™) from a single healthy or donor with asthma were restimulated on the apical
721 face, with HDM at 100 μ g/mL alone or in combination with diABZI at 10 μ M, cGAMP at 14
722 μ M or Poly(I:C) at 100 μ g/mL. At 6h post-stimulation, **(O)** Lactate dehydrogenase (LDH)
723 concentration was determined in the supernatant. **(P-U)** Multiplex immunoassay of IFN- α **(P)**,
724 IFN- β **(Q)**, IFN- λ **(R)**, IP-10/CXCL-10 **(S)**, IL-6 **(T)** and TNF- α **(U)** concentrations in the
725 supernatant. **(V)** *MUC5AC* transcripts measured by real-time PCR. **(W)** Immunoblot from
726 epithelial cell homogenates from healthy and patients with asthma showing the expression of
727 phospho- γ H2AX with Actin β as a reference. Data were presented as mean \pm SEM with n = 3
728 independent wells from the same donor. *p < 0.05. **(D-M)** Nonparametric Kruskal–Wallis with
729 Dunn’s post-test.

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732

733 **Figure 6. Upregulation of DNA sensing and PANoptosis pathways upon rhinovirus**
734 **infection in patients with asthma**

735 (A) Human bronchial epithelial cells (HBECs) from patients with asthma (n = 6) and healthy
736 controls (n=6), were infected with RV-A16 for 24h, and subjected to transcriptome analysis.

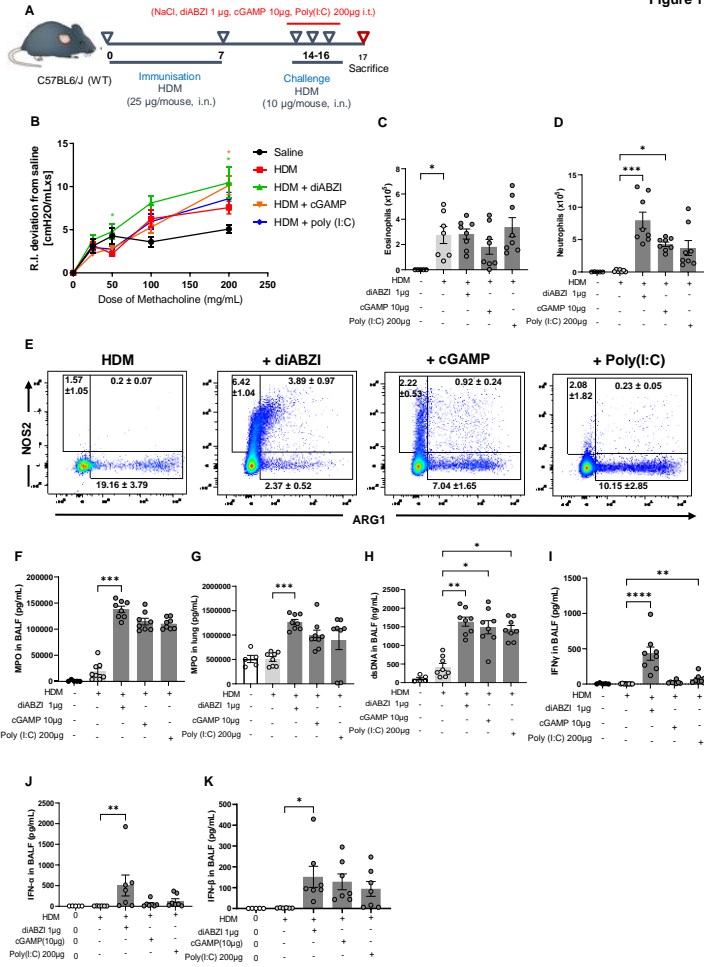
737 (B-E) Heatmaps presented together with the corresponding log₂ fold change (FC) expression
738 changes (black bars) of (B) STING pathway-related genes, (C) Tight junction genes set, (D)
739 PANoptosis-related genes, after in vitro RV-A16 infection in controls compared to HBEC from
740 patients with asthma. Transcriptomic data were processed with the workflow available on
741 <https://github.com/uzh/ezRun>.

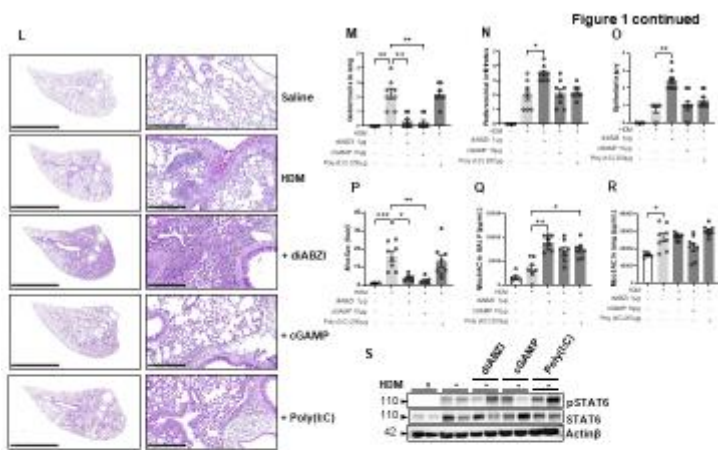
742 (E) Experimental in vivo RV RV-A16 infection in patients with asthma (n=17) and healthy
743 individuals (n=7): Transcriptomic analysis of bronchial brushings 14 days before and 4 days
744 after in vivo infection. (F-G) Heatmaps presented together with the corresponding log₂ fold
745 change (FC) expression changes (black bars) of (F) Tight junction genes set, (G) PANoptosis-
746 related genes, after in vivo RV-A16 infection in controls compared to patients with asthma. (H)
747 Heatmaps of neutrophilic signature before (right panel) and after (left panel) infection in
748 controls compared to patients with asthma. (I) Violin plots representing gene expressing of
749 *NOS2* and *ARG1* in healthy individual and patients with asthma, before (lower set) and after
750 (Upper set) infection. Data was analyzed with Bioconductor microarray analysis workflow
751 [[https://www.bioconductor.org/packages/release/workflows/vignettes/arrays/inst/doc/arrays.ht](https://www.bioconductor.org/packages/release/workflows/vignettes/arrays/inst/doc/arrays.html)
752 [ml](https://www.bioconductor.org/packages/release/workflows/vignettes/arrays/inst/doc/arrays.html)].

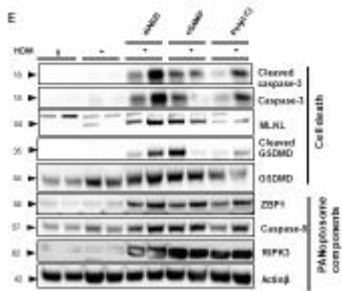
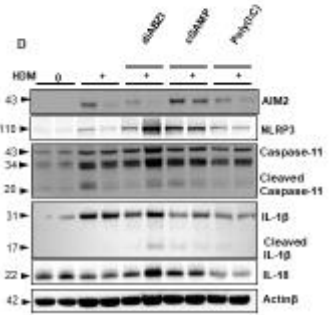
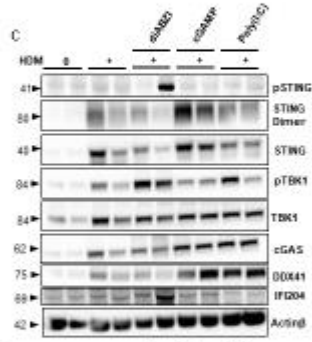
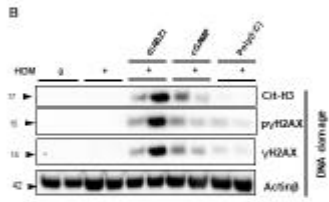
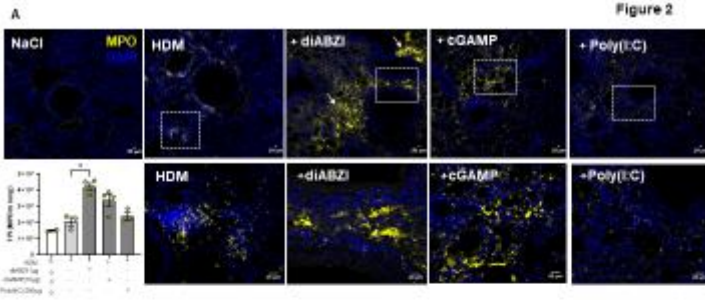
753 All Heatmaps display normalized gene expression across the groups (row normalization).
754 Asterisks demonstrate significantly changed genes with threshold $p < 0.05$. p-value: * < 0.05 ;
755 ** < 0.005 ; *** < 0.0005 , **** < 0.00005 . Publicly available data under accession number:
756 GSE185658 and GSE61141. Source data are provided as Source Data files.

757

Figure 1



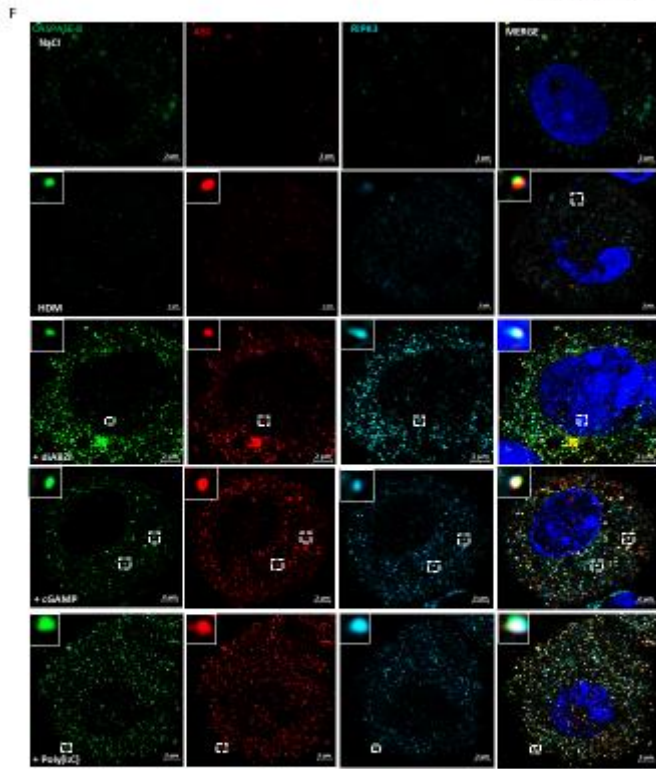




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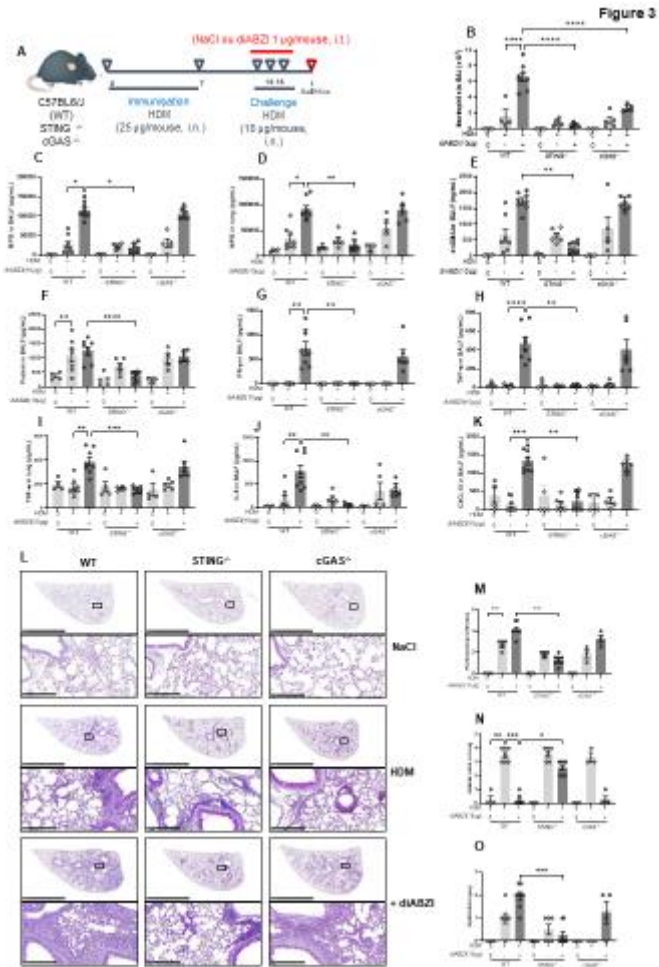
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Figure 2 continued



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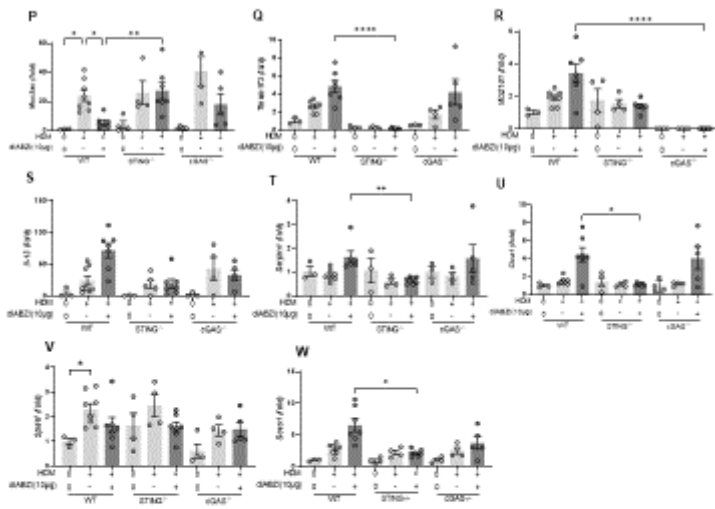
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Figure 3 continued



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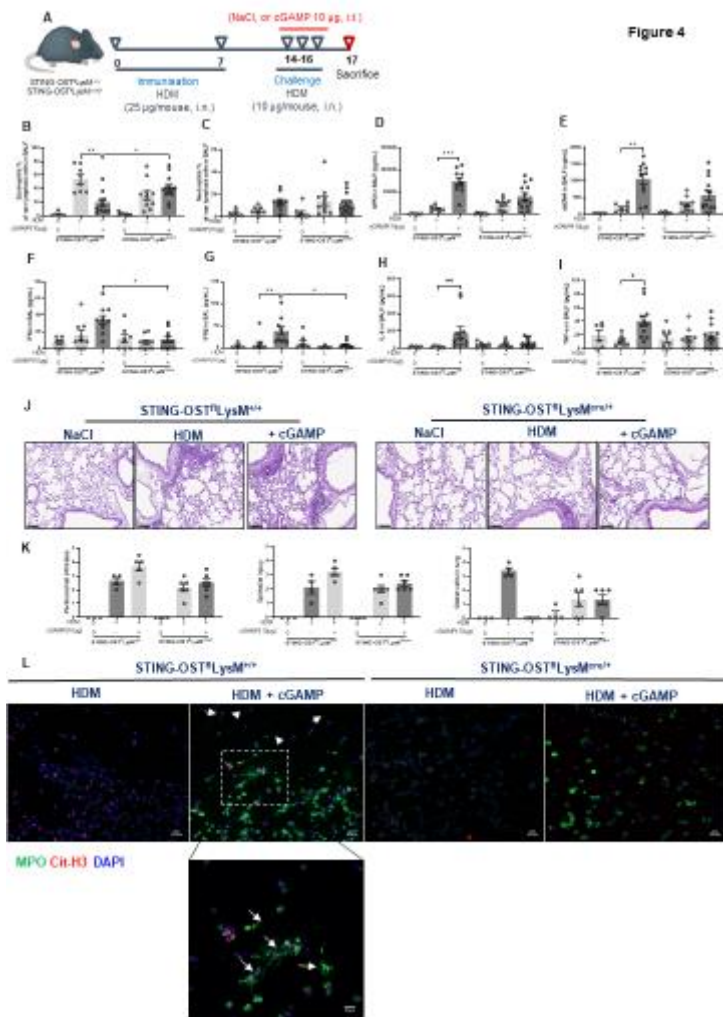
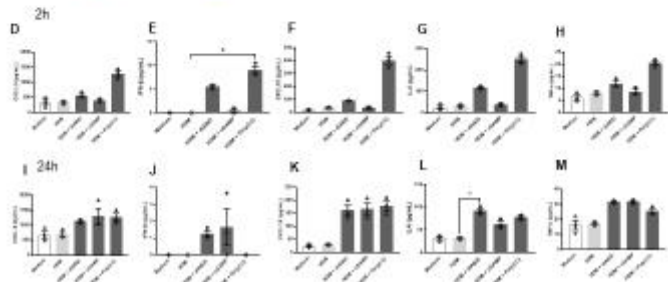
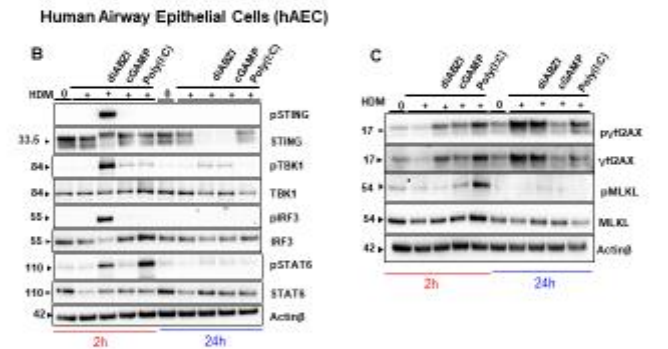
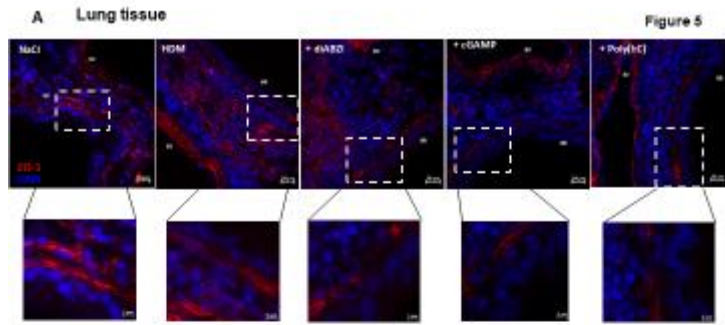


Figure 4

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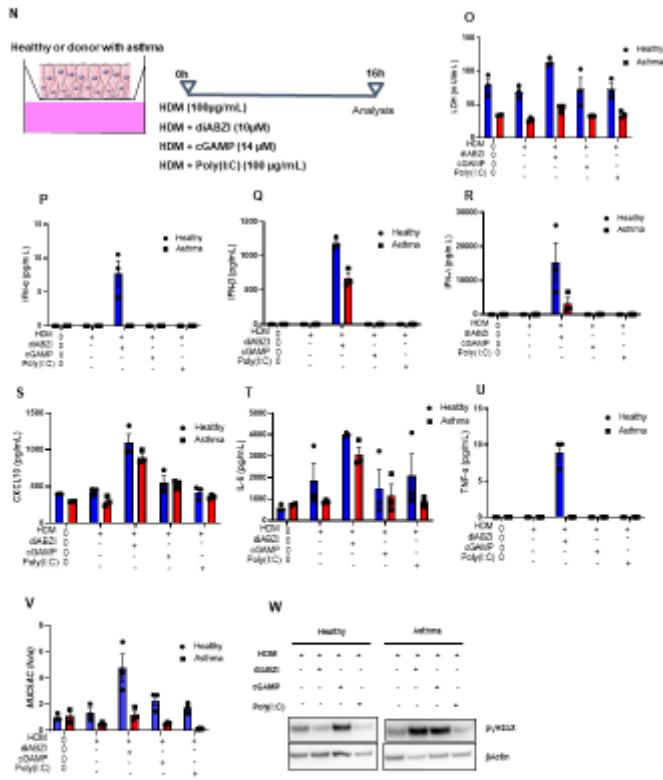
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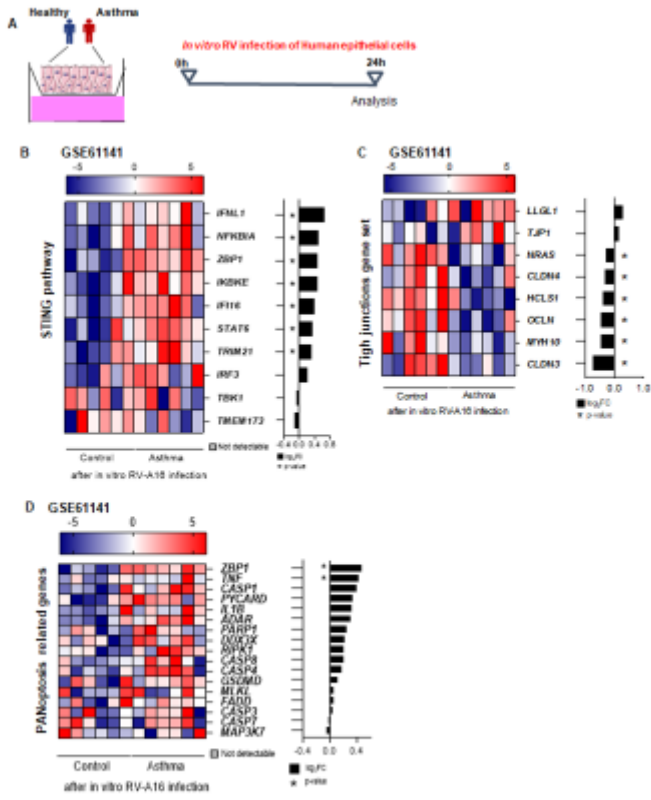
Figure 5 continued



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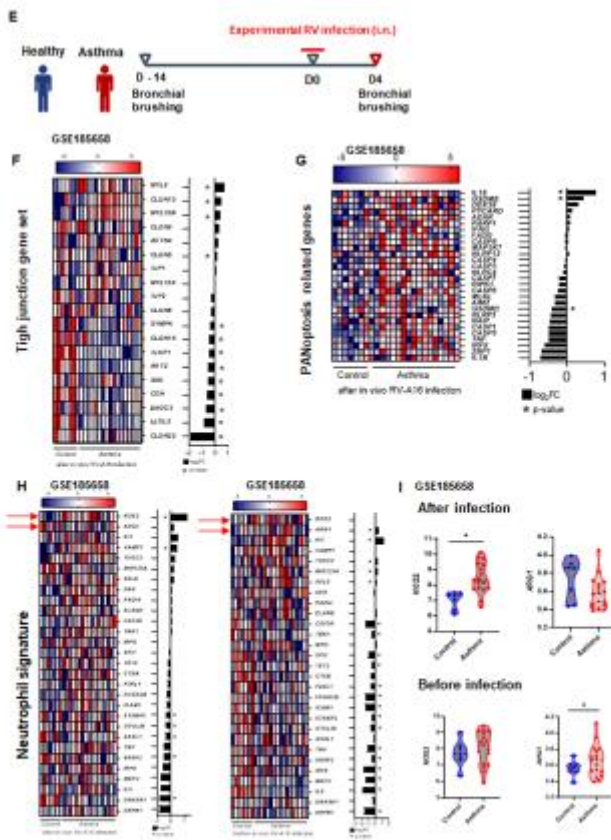
Figure 6



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Figure 6 continued



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