Title

The common *TMEM173 HAQ, AQ* alleles rescue CD4 T cellpenia, restore T-regs, and prevent *SAVI (N153S)* inflammatory disease in mice.

Short Title

TMEM173 HAQ, AQ alleles prevent SAVI disease.

Authors

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Abstract

The significance of STING (encoded by the *TMEM173* gene) in tissue inflammation and cancer immunotherapy has been increasingly recognized. Intriguingly, common human *TMEM173* alleles R71H-G230A-R293Q (*HAQ)* and G230A-R293Q (*AQ*) are carried by ~60% of East Asians and ~40% of Africans, respectively. Here, we examine the modulatory effects of *HAQ, AQ* alleles on STING-associated vasculopathy with onset in infancy (SAVI), an autosomal dominant, fatal inflammatory disease caused by gain-of-function human *STING* mutations. CD4 T cellpenia is evident in SAVI patients and mouse models. Using STING knock-in mice expressing common human *TMEM173* alleles *HAQ*, *AQ*, and *Q293*, we found that *HAQ, AQ*, and *Q293* splenocytes resist STING-mediated cell death *ex vivo,* establishing a critical role of STING residue 293 in cell death. The *HAQ/SAVI(N153S)* and *AQ/SAVI(N153S)* mice did not have CD4 T cellpenia. The *HAQ/SAVI(N153S), AQ/SAVI(N153S)* mice have more (~10-fold, ~20-fold, respectively) T-regs than *WT/SAVI(N153S)* mice. Remarkably, while they have comparable TBK1, IRF3, and NFκB activation as the *WT/SAVI*, the *AQ/SAVI* mice have no tissue inflammation, regular body weight, and normal lifespan. We propose that STING activation promotes tissue inflammation by depleting T-regs cells *in vivo*. Billions of modern humans have the dominant *HAQ, AQ* alleles. STING research and STING-targeting immunotherapy should consider *TMEM173* heterogeneity in humans.

Teaser

Common human *HAQ, AQ TMEM173* alleles dominate the gain-of-function *SAVI(N154S) TMEM173* mutant in mice.

MAIN TEXT

Introduction

STING drives cytosolic DNA-induced type I IFNs production [1]. Recent research revealed that STING promotes inflammation in a variety of inflammatory diseases, including nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, kidney injury, neurodegenerative diseases, cardiovascular diseases, obesity, diabetes, and aging [2-9]. The type I IFNs-independent function of STING has also emerged [9-12]. For example, initially described as a type I interferonopathy [13], recent studies in STING-associated vasculopathy with onset in infancy (SAVI) mouse models showed that SAVI is largely independent of type I IFNs [14-17]. In a *STING N153S* mouse model of SAVI, crossing *N153S* mice to IRF3/IRF7, and IFNAR1 knockout mice, N153S mice still developed spontaneous lung diseases [14]. JAK inhibitors were used to block type I IFNs signaling for SAVI patients with mixed success [17-20]. For example, in a review of JAK inhibition in 18 SAVI patients, incomplete response to treatment happened in 7/18 (38%) of patients [21]. Furthermore, 2 patients died of respiratory failure despite this treatment [21]. Both radioresistant parenchymal and/or stromal cells and hematopoietic cells influence SAVI pathology in mice [22,23]. The observation is important because it predicts that allogeneic stem cell transplantation may not work in human SAVI patients. Indeed, lung transplantations did not show improvement in SAVI patients [18,24]. Patients died at 3- and 9 months post-lung transplant [18,24]. How STING drives inflammation *in vivo,* independent of type I IFNs, remains unknown. Consequently, there is no curative care for SAVI.

Characterized as an innate immune sensor, STING expression is, paradoxically, high in CD4 T cells [13,25]. Furthermore, STING activation kills mouse and human CD4 T cells *ex vivo* [26-28]. SAVI patients and mouse models had CD4 T cellpenia [13,28]. STING was first discovered as MPYS for its cell growth inhibition and cell death function in mouse B lymphoma cells [29]. STING-mediated cell death is cell type dependent. For example, while STING activation kills human endothelial cells, primary and cancerous T cells, it does not kill mouse MEFs, BMDCs, or BMDMs [30-32]. Second, STING-mediated cell death is type I IFNs-independent [28,30,32]. Multiple cell death pathways, *i.e.,* apoptosis, necroptosis, pyroptosis, ferroptosis, and PANoptosis, are proposed [32-34]. Last, the *in vivo* biological significance of STING-mediated CD4 T cell death is not clear [28,32]. In humans, SAVI patients with constitutively activated STING have low CD4 T cell numbers [13], and type I IFNs are dispensable for STING-mediated human CD4 T cell death [28]. Different from SAVI mice, SAVI patients (*N154S* or *V155M*) had normal counts of CD8 T and B cells [13].

The human *TMEM173* gene is highly heterogeneous [35,36]. ~50% of people in the U.S. carry at least one copy of non-*WT TMEM173* allele [35]. Among them, the R71H-G230A-R293Q (*HAQ*) is the 2nd most common *TMEM173* allele carried by ~23% of people in the U.S. [35]. However, in East Asians, *WT/HAQ* (34.3%), not *WT/WT* (22.0%), is the most common *TMEM173* genotype [36]. Critically, the *HAQ* allele was positively selected in modern humans outside Africa [37]. Anatomically modern humans outside Africa are descendants of a single Out-of-Africa Migration 50,000~70,000 years ago. ~1.4% of Africans have the *HAQ* allele, while ~63.9% of East Asians are *HAQ* carriers [37]. Haplotype analysis revealed that *HAQ* was derived from G230A-R293Q (*AQ*) allele [37]. Importantly, the *AQ* allele was negatively selected outside Africa [37]. ~40.1% of Africans are *AQ* carriers, while ~0.4% of East Asians have the *AQ* allele [37]. *TMEM173* alleles often have a dominant

negative effect likely because the protein STING exists as a homodimer [29,38]. SAVI is an autosomal dominant inflammatory disease [13]. *WT/HAQ* individuals had reduced Pneumovax23®-induced antibody responses compared to *WT/WT* individuals (NCT02471014) [39]. Notably, *AQ* responds to CDNs and produces type I IFNs *in vivo* and *in vitro* [37,40,41], but the *AQ* allele was negatively selected in non-Africans [37]. In contrast, the *HAQ* allele, defective in CDNs-type I IFNs responses [35,36,39,42,43], was positively selected in non-Africans [37], indicating that the CDNs-type I IFNs independent function of STING was essential for the survival of early modern humans outside of Africa.

In this study, we discovered, surprisingly, that the *HAQ*, *AQ* splenocytes are resistant to STING-mediated cell death. We generated *HAQ/SAVI(N153S)* and *AQ/SAVI(N153S)* mice and found that the *HAQ, AQ* alleles prevent CD4 T cellpenia, increasing/restoring T-regs and alleviating/stopping tissue inflammation in SAVI mice, thus providing evidence for the *in vivo* significance of type I IFNs-independent, STING-mediated cell death and potential *AQ*-based curative therapy for SAVI patients.

Results

STING activation kills mouse spleen CD4, CD8 T, and CD19 B cells *ex vivo*.

We first used the synthetic non-CDNs STING agonist diABZI [44] to induce lymphocyte death because diABZI induces cell death without the need for lipid transfection or detergent for cell permeabilization [31,45] and diABZI is in clinical trials (NCT05514717). Splenocytes from C57BL/6N mice were treated with diABZI in culture, and cell death was determined by Annexin V and Propidium Iodide stain. Splenocyte cell death could be detected as early as 5 hours post diABZI treatment (Figure S1A). Dosage responses showed that ~25ng/ml diABZI could kill 70% of splenocytes (Figure S1B). Similarly, STING agonists DMXAA and synthetic CDNs RpRpss-Cyclic di-AMP killed mouse spleen CD4, CD8 T cells, and CD19 B cells (Figure 1A). Thus, STING activation readily induces mouse lymphocyte death *ex vivo*.

TBK1 activation is required for STING-mediated mouse spleen cell death *ex vivo*.

STING activation can lead to apoptosis, pyroptosis, necroptosis, or ferroptosis [28-34,45,46]. We then treated mouse splenocytes with apoptosis, pyroptosis, necroptosis inhibitors, STING inhibitors H-151, C-176, and palmitoylation inhibitor 2-bromopalmitate (2-BP), followed by diABZI stimulation. Inhibitors for NLRP3 (MCC950), RIPK1 (Necrostatin-1), RIPK3 (GSK872), Caspase-1 (VX-795), Caspase-3 (Z-DEVD-FMK), Caspase 1,3,8,9 (Q-VD-Oph), ferroptosis (liproxstatin-1) did not affect diABZI-induced splenocyte cell death *ex vivo* (Figure S1B, S1C). The STING inhibitors H-151, C-176, and 2-BP also could not prevent diABZI-induced cell death (Figure S1C) though they inhibited diABZI-induced IFNβ production (Figure S1D). Instead, the TBK1 inhibitor BX-795 abolished diABZI-induced splenocyte death (Figure S1C).

BX-795 is a multi-kinase inhibitor, including 3-phosphoinositide-dependent protein kinase 1 (PDK1) and TBK1 (IC_{50s} = 6 and 11 nM, respectively). However, the treatment of PDK1 inhibitor GSK2334470 (IC50 = 10nM) did not prevent diABZI-induced splenocyte death (Figure 1B). In contrast, GSK8612, a highly potent and selective inhibitor for TBK1, prevented diABZI-induced splenocyte death (Figure 1B). Thus, TBK1 activation is likely critical for STING-mediated splenocyte cell death *ex vivo*.

HAQ, AQ, Q293 STING knock-in mouse splenocytes are resistant to STING-mediated cell death *ex vivo*.

HAQ and *AQ* are common human *TMEM173* alleles [35-37]. Previously, we reported that *HAQ* knock-in mice are defective in CDNs-induced immune responses, while CDNs responses in *AQ* knock-in mice are similar to WT mice [37]. We treated splenocytes from *HAQ* and *AQ* mice with diABZI *ex vivo* and found, surprisingly, that both *HAQ* and *AQ* splenocytes were resistant to diABZI-induced cell death (Figure 1C, 1D). In comparison, IFNAR1$^{-/-}$ splenocytes were killed by diABZI, confirming that STING-mediated lymphocytes death are type I IFNs-independent (Figure 1C, 1D) [28,30,32].

HAQ and *AQ* share the common A230 and Q293 residues changes. We thus generated a *Q293 TMEM173* knock-in mouse. Notably, the Q293 splenocytes were resistant to STING agonists 2'3'-cGAMP, RpRpss-Cyclic di-AMP, and diABZI-induced cell death (Figure 1E, 1F). Thus, the residue 293 of STING is critical for its cell death function.

149 ***WT/HAQ*, *WT/AQ* mouse splenocytes are partially resistant to STING-mediated cell**
150 **death *ex vivo*.**
151 *WT/HAQ* (34.3%) is the most common human *TMEM173* genotype in East Asians, while
152 *WT/AQ* (28.2%) is the 2nd most common *TMEM173* genotype in Africans [36]. We generated
153 *WT/HAQ*, *WT/AQ* mice and treated their splenocytes with mouse STING agonist DMXAA.
154 *WT/HAQ* and *WT/AQ* splenocytes were protected from 25µg/ml DMXAA-induced cell
155 death (Figure 1G). 100µg/ml DMXAA could kill *WT/HAQ* and *WT/AQ* splenocytes, albeit
156 less than *WT/WT* cells (Figure 1G). Thus, the *HAQ* and *AQ* alleles are dominant and likely
157 impact STING activation even in heterozygosity.
158
159 **STING activation kills primary human CD4 T cells but not CD8 T or CD19 B cells.**
160 STING agonists-based clinical trials in humans have been disappointing (NCT02675439,
161 NCT03010176, NCT05514717) [47,48]. We showed that the human *TMEM173* gene might
162 undergo natural selection during the out-of-Africa migration [37] sensitive to evolutionary
163 pressure. Thus, we investigated STING-mediated death in primary human lymphocytes.
164 Human explant lung cells from the *WT(R232)/WT(R232)* donors were treated with
165 STING agonists 2'3-c GAMP, RpRpss-Cyclic di-AMP, diABZI for 24 hours in culture.
166 Lymphocyte cell death was determined by Propidium Iodide staining. Different from
167 mouse lymphocytes, diABZI and RpRpss-Cyclic di-AMP killed human CD4 T but not
168 CD8 T or CD19 B cells (Figure 2A, 2B). Human CD8 T and CD19 B cells are resistant to
169 500ng/ml diABZI-induced cell death (Figure S2A).
170
171 ***WT/HAQ* human CD4 T cells are resistant to low doses of diABZI-induced cell death.**
172 *WT/HAQ* mouse splenocytes are resistant to low-dose diABZI-induced cell death (Figure
173 1G). To extend our observation into primary human T cells, we obtained lung explants
174 from *WT/WT* and *WT/HAQ* individuals (Figure S2B) and treated them with diABZI in
175 culture. 25ng/ml diABZI killed *WT/WT*, but not *WT/HAQ*, human lung CD4 T cells (Figure
176 2C).
177
178 **diABZI induces cell death in *STING-KO* human THP-1 cells reconstituted with *WT***
179 **human *STING (R232)* but not *HAQ, AQ or Q293* human *STING* allele.**
180 To further determine cell death influenced by human *TMEM173* alleles *HAQ, AQ,* and
181 *Q293*, we used the *STING-KO* THP-1 cell line because STING agonist induces type I IFNs
182 and cell death in *STING-KO* THP-1 cells expressing *WT* human STING [34] (Figure S2C,
183 S2D). We, thus, generated stable THP-1 *STING-KO* lines expressing *HAQ, AQ, WT,* or
184 *Q293 TMEM173* allele. Cell death was determined by Annexin-V staining. diABZI killed
185 THP-1 *STING-KO* lines expressing *WT* but not *HAQ, AQ,* or *Q293 TMEM173* allele
186 (Figure 2D, 2E). No cell death was induced in the *Q293* THP-1 cells stimulated by 20 to
187 200 ng/ml of diABZI (Figure 2F). diABZI also did not induce STING activation in *Q293*
188 THP-1 cells (Figure 2G). Notably, 50ng/ml diABZI induced p-IRF3 activation and type I
189 IFNs in *AQ* THP-1 cells but not *HAQ* THP-1 cells (Figure 2H, 2I), indicating that the
190 STING-cell death and STING-IRF3-Type I IFNs pathways can be uncoupled.
191
192 ***HAQ* and *AQ* alleles rescue the lymphopenia and suppress myeloid cell expansion in**
193 ***SAVI(N153S)* mice.**

The *in vivo* significance of the STING/MPYS-cell death is unclear. Furthermore, multiple cell death pathways, i.e., apoptosis, necroptosis, pyroptosis, ferroptosis, and PANoptosis, are proposed [32-34]. The uncertainty likely results from studies using different cell types (primary cells vs cancer cell lines); species (human vs mouse); STING agonists (cGAMP, which requires cell permeabilization by detergents or lipid transfection, vs diABZi, DMXAA that can directly cross the membrane) [26,27,30,31,49]. Critically, which mechanism is relevant *in vivo*, causing T cellpenia is not known. To clarify the *in vivo* significance and mechanisms of STING-mediated cell death, we turned to SAVI mice.

SAVI is an autosomal dominant, inflammatory disease caused by one copy of a gain-of-function *TMEM173* mutant (*WT/SAVI*) [13]. CD4 T cellpenia was found in SAVI patients and SAVI mouse models [13,14]. STING activation in SAVI mice is independent of ligands and happens *in vivo*. We thus generated *HAQ/SAVI(N153S)* and *AQ/SAVI(N153S)* mice aiming to establish the *in vivo* significance and mechanism of STING-cell death.

First, *HAQ/SAVI(N153S)* and *AQ/SAVI(N153S)* mice had reduced splenomegaly compared to *WT/SAVI(N153S)* mice though their spleens were still larger than the littermates *WT/HAQ* and *WT/AQ* (Figure 3A, 3B). Next, *HAQ/SAVI(N153S)* and *AQ/SAVI(N153S)* mice had similar spleen B cells and CD4 T cell numbers as the *WT/HAQ*, *WT/AQ* littermates (Figure 2B, 2C). Their $CD8^+$ T cells were lower than their *WT* littermates but much higher than the *WT/SAVI(N153S)* mice (Figure 3D). Third, spleen myeloid cell numbers, i.e., neutrophils, $Ly6C^{hi}$ monocytes and F4/80 macrophages, were all reduced by half compared to *WT/SAVI(N153S)* mice (Figure 3E~3H). Last, splenocytes from *HAQ/SAVI*, *AQ/SAVI* mice were partially resistant to diABZI, DMXAA-induced cell death *ex vivo* (Figure S3). Notably, the *HAQ/SAVI(N153S)* and *AQ/SAVI(N153S)* mice also had restored bone marrow monocytes (Figure S4). Thus, *HAQ* and *AQ* alleles prevent lymphopenia and suppress myeloid cell expansion in *SAVI(N153S)* mice.

The *HAQ* allele alleviates and the *AQ* allele prevents *SAVI(N153S)* disease in mice. *SAVI(N153S)* disease is characterized by early onset, failure to thrive (low body weight), persistent lung inflammation, decreased lung function, and young death in humans and mouse models [13,17,49,50]. The *HAQ/SAVI(N153S)* mice weighed more and had an improved lifespan than the *WT/SAVI(N153S)* mice (Figure 4A, 4B). The lifespan, airway resistance, and tissue inflammation (lung, liver) were also improved in *HAQ/SAVI(N153S)* mice compared to the *WT/SAVI(N153S)* mice (Figure 4C, 4D, 4J, 4K). However, the pulmonary artery pressure was still elevated in *HAQ/SAVI(N153S)* mice (Figure 4E). Remarkably, the *AQ/SAVI(N153S)* mice had similar body weight and lifespan as the *WT/AQ* mice (Figure 4F, 4G). The airway resistance, pulmonary artery pressure, and tissue inflammation in *AQ/SAVI(N153S)* were similar to the *WT/AQ* littermates (Figure 4H, 4I, 4J, 4K). Thus, the *HAQ* allele alleviates and the *AQ* allele prevents inflammatory SAVI disease in mice. Interestingly, lungs from *HAQ/SAVI, AQ/SAVI* had similarly reduced infiltration of $Ly6G^+CD11B^+$ neutrophils and $Ly6G^-Ly6C^+CD11B^+$ inflammatory monocytes compared to *WT/SAVI* mice (Figure S5).

diABZI induces similar STING, TBK1, IRF3, NFκB activation in the *AQ/SAVI(N153S)* and *WT/SAVI(N153S)* bone-marrow-derived-macrophage (BMDM).

239 SAVI was characterized as type I interferonopathy [17]. However, several studies showed
240 that type I IFN signaling and IRF3 activation were dispensable for SAVI disease [14,15,23,50].
241 *AQ* allele prevents SAVI disease (Figure 4). However, diABZI-treated *AQ/SAVI(N153S)*
242 and *WT/SAVI(N153S)* BMDM had similar TBK1-IRF3 activation and IFNβ production
243 (Figure 5A, 5B). diABZI treatment caused IκBα degradation, and similar TNF production
244 in *WT/SAVI* and *AQ/SAVI* BMDM (Figure 5C, 5D). Furthermore, diABZI activation led to
245 STING protein degradation in *WT/SAVI* and *AQ/SAVI* BMDM (Figure 5E). Last, using
246 cleavable crosslinker dithiobis (succinimidyl propionate (DSP), we showed that STING in
247 *WT/SAVI, AQ/SAVI* BMDM forms a similar dimer *in situ* (Figure 5F). Thus, the *AQ/SAVI*
248 BMDM had similar STING degradation, TBK1, IRF3, NFκB activation, and dimerization
249 as the *WT/SAVI* BMDM.
250
251 ***The HAQ* allele increased, and the *AQ* allele restored T-regs in *SAVI(N153S)* mice.**
252 IFNγ was proposed to drive SAVI disease [15,23,38]. We confirmed that *WT/SAVI* CD4 T cells
253 were enriched with IFNγ^+ cells (Figure 6A). However, *WT/SAVI* mice have CD4 T
254 cellpenia. Thus, the total numbers of spleen IFNγ^+ CD4 T cells were comparable in
255 *WT/SAVI* and *AQ/SAVI* mice (Figure 6A). In contrast, the *HAQ/SAVI mice* had decreased
256 IFNγ^+ CD4 T cells (Figure 6A).
257 The induction of Foxp3 expression in T-reg cells during ongoing autoimmune
258 inflammation resolved inflammation and pathology in mice [51]. CD4 T cellpenia depletes
259 CD4 T-regs. Indeed, *WT/SAVI* mice had ~20-fold reduction of spleen FoxP3$^+$ T-regs
260 compared to *AQ/SAVI* or *WT/WT* littermates (Figure 6B). The *HAQ/SAVI* mice also had
261 ~10-fold more T-regs than the *WT/SAVI* littermate (Figure 6B).
262

263

Discussion

This study, using the *HAQ, AQ, SAVI(N153S) TMEM173* knock-in mice, reveals the *in vivo* significance and mechanism of STING-mediated CD4 T cell death. *HAQ, AQ* alleles prevent CD4 T cellpenia, and increase/restore CD4 T-regs in SAVI mice. The results are consistent with previous finding that the impaired CD4 T cell proliferation by the *SAVI(V155M)* mutant could be rescued by the addition of the *HAQ* allele *in vitro* [27]. STING has been increasingly implicated in inflammatory diseases such as nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, cardiomyopathy, obesity, diabetes, neurodegenerative diseases, aging, and kidney injury, many of which are independent of type I IFNs [2,3,9]. It is tempting to suggest that STING activation in CD4 T cells leads to CD4 T-regs depletion that break tissue tolerance and exacerbates tissue inflammation.

Human immunodeficiency virus (HIV) primarily infects CD4 T cells and might activate the STING pathway in CD4 T cells [52-57]. The loss of CD4 T cells is the hallmark of untreated HIV infection [58,59], and the measurement of CD4 T cell count is a central part of HIV care. We found that *HAQ* and *AQ* CD4 T cells are resistant to STING-mediated cell death. Mogensen and colleagues reported that *HAQ/HAQ* was enriched in HIV-infected long-term nonprogressors [42]. These *HAQ/HAQ* individuals had reduced inhibition of CD4 T cell proliferation and a reduced immune response to DNA and HIV [42]. It is likely that HIV infection activates STING-cell death pathway in CD4 T cells. In *HAQ/SAVI* and *AQ/SAVI* mice, one copy of *HAQ*, *AQ* allele suppressed CD4 T cell death. *HAQ*, *AQ* carriers might have fewer HIV-induced CD4 T cell death, thus being long-term nonprogressors in HIV infection-induced Acquired immunodeficiency syndrome (AIDS) [42]. Targeting STING to prevent CD4 T cell death might be a valid therapy for AIDS.

Activating the STING pathway is a promising strategy for cancer immunotherapy [60-66]. Multiple STING agonists are in clinical trials [47,48]. Recently, the safety issue emerged in some STING agonist trials [47,48]. For example, in the STING agonist, ADU-S100, clinical trial, Grade 3/4 treatment-related adverse events were reported in 12.2% of 41 pretreated patients (NCT02675439)[47,48]. The National Institutes of Health defines grade 3 as "incapacitating; unable to perform usual activities; requires absenteeism or bed rest." In a clinical trial using STING antibody-drug-conjugate (ADC) that conjugates diABZI to anti-HER2 Ab, a Grade 5 (fatal) serious adverse event was recorded and deemed related to the STING-ADC (NCT05514717). SAVI disease, driven by overreacting STING, is often fatal [13]. *AQ*, to a less degree, *HAQ*, suppress mortality in SAVI mice. Future STING clinical trials should be based on human *TMEM173* genotype to achieve safe and effective responses.

Mechanistically, apoptosis, pyroptosis, ferroptosis, necroptosis, and PANoptosis have all been reported in STING-mediated cell death [28-34,45,46]. Different cell types and STING agonists used likely contributed to the inconsistency and complexity. Here, we focused on lymphopenia in the SAVI mice that avoids ligand-dependent, non-physiological dosage in STING-mediated cell death. *HAQ* and *AQ* alleles could prevent CD4 T cellpenia in the SAVI mice strongly indicating that residue A230 or Q293 prevent STING-mediated CD4 T cell death *in vivo*. Splenocyte from *Q293* mice were resistant to STING agonists-induced cell death *ex vivo*. Thus, it is likely that the Q293 residue is critical for STING-mediate lymphopenia. Notably, Q293 is outside the C-terminal tail (CTT) (residues 341–379 of human STING) critical for TBK1 recruitment and IRF3 phosphorylation [67] or miniCTT domain (aa343–354) [27], or the UPR motif (aa322–343) [49] important for T cells death *in*

310 *vitro*. Further studies are needed to understand how the aa293 of STING mediates cell
311 death *in vivo*. Noteworthy, *AQ/SAVI* cells had similar TBK1-IRF3, NFκB activation and
312 STING degradation as the *WT/SAVI* cells. Yet, *AQ/SAVI* mice did not have CD4 T
313 cellpenia as *WT/SAVI* mice suggesting that the canonical STING-TBK1-IRF3/NFκB
314 pathway, likely STING oligomerization, is not sufficient for the induction of cell death at
315 the physiological condition.
316 We used the *WT/N153S* knock-in SAVI mouse model that spontaneously develop lung
317 inflammation, T cell cytopenia, and early mortality, mimicking pathological findings in
318 human SAVI patients[16]. Using the *WT/N153S* SAVI mouse model and human Jurkat T cell
319 line, it was proposed that STING activation causes chronic ER stress and unfolded protein
320 response, leading to T cell death by apoptosis [49]. Furthermore, the study showed that
321 crossing *WT/N153S* mice to the OT-I mice reduced ER stress and restored $CD8^+$, but not
322 $CD4^+$, T cells [49]. The restoration of $CD8^+$T cells reduces inflammation and lung disease [49].
323 However, human *WT/N154S* SAVI patients have normal $CD8^+$ T cells numbers [13], and
324 primary human $CD8^+$ T cells are largely resistant to STING-agonists-induced cell death *ex*
325 *vivo* (Figure 2A) [28]. Thus, it is puzzling how restoring $CD8^+$ T cells can rescue SAVI
326 phenotypes since the SAVI patients already have normal $CD8^+$ T cells numbers.
327 Finally, it is unexpected that both *HAQ* and *AQ* alleles are resistant to cell death. Our
328 previous studies showed that the *HAQ* and *AQ* alleles have opposite functions [37]. AQ-
329 STING, not HAQ-STING, responds to CDNs [35-37,39-43,68]. *AQ* mice are lean while *HAQ*
330 mice are fat [37]. Most importantly, *HAQ* was positively selected, while *AQ* was negatively
331 selected, in modern humans outside Africans [37]. Thus, the death pathway of STING is also
332 distinct from the STING function that was naturally selected. It is worth noting that the *AQ*
333 allele does better than the *HAQ* allele in suppressing SAVI disease. Thus, besides
334 preventing cell death, additional mechanism by the *AQ* allele, e.g. fatty acid metabolism
335 [37,69], is involved in curing SAVI.
336 **The limitations of the study**. The poor transferability of mouse to humans is a major issue
337 in STING research [47,48]. The present study used *AQ/SAVI* and *HAQ/SAVI* mice.
338 Confirmation is needed in humans with the identification and evaluation of people who are
339 *AQ/SAVI*, *HAQ/SAVI*.
340

Materials and Methods

Experimental Design. The study was designed to reveal (i) the *in vivo* significance of the type I IFNs-independent, STING-dependent cell death function; (ii) the interplay between common *STING* alleles *HAQ, AQ* and the rare, gain-of-function SAVI STING mutation; (iii) the driver for the inflammatory SAVI disease. Mouse splenocytes, primary human lung cells, human THP-1 cells and *HAQ, AQ, SAVI* knock-in mice were used to establish the *in vivo* significance and human relevance. All the repeats were biological replications that involve the same experimental procedures on different mice. Where possible, treatments were assigned blindly to the experimenter by another individual in the lab. When comparing samples from different groups, samples from each group were analyzed in concert, thereby preventing any biases that might arise from analyzing individual treatments on different days. All experiments were repeated at least twice.

Mice. *WT/SAVI(N153S)* mice were purchased from The Jackson Laboratory. *HAQ*, *AQ* mice were previously generated in the lab [36,37]. The *Q293* mice were generated by Cyagen Biosciences. Briefly, the linearized targeting vector was transfected into JM8A3.N1 C57BL/6N embryonic stem cells. A positive embryonic stem clone was subjected to the generation of chimera mice by injection using C57BL/6J blastocysts as the host. Successful germline transmission was confirmed by PCR sequencing. The heterozygous mice were bred to Actin-flpase mice [The Jackson Laboratory, B6.Cg-Tg (ACTFLPe)9205Dym/J] to remove the neo gene and make the *Q293* knock-in mouse. Age- and gender-matched mice (2 – 6 month old, both male and female) were used for indicated experiments. *WT/SAVI* (male) x *WT/HAQ* (female), *WT/SAVI* (male) x *WT/AQ* (female) breeders were set up to generate *HAQ/SAVI*, *AQ/SAVI* mice. Mice were housed at 22°C under a 12-h light-dark cycle with ad libitum access to water and a chow diet (3.1 kcal/g, Teklad 2018, Envigo, Sommerset, NJ) and bred under pathogen-free conditions in the Animal Research Facility at the University of Florida. Littermates of the same sex were randomly assigned to experimental groups. All mouse experiments were performed by the regulations and approval of the Institutional Animal Care and Use Committee at the University of Florida, IACUC202200000058.

Reagents. Recombinant human IFNβ (R&D, cat no. 8499-IF-010/CF), diABZI (Invivogen, cat no. 2138299-34-8), 2'3'-cGAMP (Invivogen, cat no. tlrl-nacga23-02), DMXAA (Invivogen, cat no. tlrl-dmx), H151 (Invivogen, cat no. inh-h151), RpRpSS-Cyclic di-AMP (Biolog, cat no. C118), THP1-Dual™ KO-STING Cells (Invivogen, cat no. thpd-kostg). All other chemical inhibitors are from Selleckchem. Mouse TNF alpha ELISA Ready Set Go. (eBioscience, cat no. 88-7324). Mouse IFN-Beta ELISA Kit (PBI, cat no. 42400).

Generation of THP-1 KO-STING cells stably expressing human *TMEM173* alleles. THP1-Dual™ KO-STING Cells in 6-well plate were transfected with 1µg *TMEM173* plasmid (in pcDNA 3.1 vector) with Lipofectamine LTX and Plus Reagent (Invitrogen, cat no: A12621) according to the manufacturer's instructions. Transfecting Plasmid DNA into THP-1 Cells Using Lipofectamine LTX Reagent | Thermo Fisher Scientific - US. 48hrs after the transfection, the cell medium was changed. G418 (1mg/ml) was added to the culture to select STING expressing THP-1 cells. The G418-resistant cells were established

387 and expanded.
388
389 **Histology.** Lungs and livers were fixed in 10% formalin, paraffin-embedded, and cut into
390 4-µm sections. Lung, liver sections were then stained for hematoxylin-eosin. All staining
391 procedures were performed by the histology core at the University of Florida. Briefly,
392 tissue sectins were immersed Harris Hematoxylin for 10 seconds, then washed with tap
393 water. Cleard sections were re-immersed in EOSIN stain for ~30 seconds. The sections
394 were washed with tap water until clear, then dehydrate in ascending alcohol solutions
395 (50%,70%,80%,95% x 2, 100% x 2). Afterwards, the sections werer cleared with xylene
396 (3 - 4 x). The sections were mounted on glass slide with permount organic mounting
397 medium for visulization.
398
399 **Lung Function.** Pulmonary function was evaluated using an isolated, buffer-perfused
400 mouse lung apparatus (Hugo Sachs Elektronik, March-Huggstetten, Germany), as
401 previously described [70]. Briefly, mice were anesthetized with ketamine and xylazine and a
402 tracheostomy was performed, and animals were ventilated with room air at 100 breaths/min
403 at a tidal volume of 7 µl/g body weight with a positive end-expiratory pressure of 2 cm
404 H2O using a pressure-controlled ventilator (Hugo Sachs Elektronik, March-Huggstetten,
405 Germany).
406
407 **Isolation of lung cells.** Cells were isolated from the lung as previously described [71]. The
408 lungs were perfused with ice-cold PBS and removed. Lungs were digested in DMEM
409 containing 200µg/ml DNase I (Roche, 10104159001), 25µg/ml Liberase TM (Roche,
410 05401119001) at 37°C for 2 hours. Red blood cells were then lysed and a single cell
411 suspension was prepared by filtering through a 70-µm cell strainer.
412
413 **BMDM activation**
414 BMDMs were induced from mouse bone marrow cells cultured in RPMI 1640 (cat#11965;
415 Invitrogen) with 10% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate, 10 mM HEPES
416 buffer, 1% nonessential amino acids, 50 mM 2-ME, 1% Pen/Strep, with 20ng/ml M-CSF
417 (Kingfisher, RP0407M) for 10 days [36]. STING agonists were added into the culture (no
418 transfection or membrane permeabilization).
419
420 **Flow cytometry.** Single cell suspensions were stained with fluorescent-dye-conjugated
421 antibodies in PBS containing 2% FBS and 1mM EDTA. Surface stains were performed at
422 4°C for 20 min. For intracellular cytokine or transcription factor staining of murine and
423 human cells, cells were fixed and permeabilized with the Foxp3 staining buffer set
424 (eBioscience, cat no 00-5523-00). Cells were washed and stained with surface markers.
425 Cells were then fixed and permeabilized (eBioscience, cat no. 00-5523-00) for intracellular
426 cytokine stain. Data were acquired on a BD LSRFortessa and analyzed using the FlowJo
427 software package (FlowJo, LLC). Cell sorting was performed on the BD FACSAriaIII
428 Flow Cytometer and Cell Sorter. More information on the antibodies used can be found in
429 supplementary table S1.
430
431 **Human lung explants**
432 Human lung explants were procured at the Lung Transplant Center, Division of Pulmonary,

Critical Care and Sleep Medicine, Department of Medicine, University of Florida. Donor and patients consent was obtained for a research protocol (UF IRB201902955-Treatment with IFNβ Induces Tolerogenic Lung Dendritic Cells in Human advanced lung disease). Healthy donor lungs were surgically removed postmortem, perfused, small pieces were cut from the right middle and lower lobes for research purpose, and stored in cold Perfadex ® at 4°C for no more than 12 hrs before processing. Ex planted lungs from emphysema lung transplant patients were stored in cold Perfadex ® at 4°C for no more than 12 hrs before the process. No lung explants were procured from prisoners.

Statistical Analysis. To gain statistical power, we employ three~four mice/groups to characterize lung immunity. Ten mice/group to monitor animal health. The statistical justification for group size was calculated using the SAS program to calculate the animal numbers. The analysis was carried out using a standard error of 0.5 for immunological assays, and a power of 0.9. All data are expressed as means ± SEM. Statistical significance was evaluated using Prism 9.0 software. Comparisons between two groups were analyzed by performing an unpaired Student's *t* test. Comparisons between more than two groups were analyzed by performing a one-way analysis of variance (ANOVA) with Tukey's multiple comparisons test.

453 **References**

454 1 Barber, G. N. STING: infection, inflammation and cancer. *Nat Rev Immunol* **15**, 760-770
455 (2015).
456 2 Skopelja-Gardner, S., An, J. & Elkon, K. B. Role of the cGAS-STING pathway in
457 systemic and organ-specific diseases. *Nat Rev Nephrol* **18**, 558-572 (2022).
458 3 Bai, J. & Liu, F. cGASSTING signaling and function in metabolism and kidney diseases.
459 *J Mol Cell Biol* **13**, 728-738 (2021).
460 4 Li, H., Guo, X., Aquino, E. & Wu, C. Mini review: STING activation during non-
461 alcoholic fatty liver disease. *Front Nutr* **10**, 1139339 (2023).
462 5 Gulen, M. F., Samson, N., Keller, A., Schwabenland, M., Liu, C., Gluck, S., Thacker, V.
463 V., Favre, L., Mangeat, B., Kroese, L. J., Krimpenfort, P., Prinz, M. & Ablasser, A.
464 cGAS-STING drives ageing-related inflammation and neurodegeneration. *Nature* **620**,
465 374-380 (2023).
466 6 Szego, E. M., Malz, L., Bernhardt, N., Rosen-Wolff, A., Falkenburger, B. H. & Luksch,
467 H. Constitutively active STING causes neuroinflammation and degeneration of
468 dopaminergic neurons in mice. *Elife* **11** (2022).
469 7 Gao, L., Zhang, J., Yang, T., Jiang, L., Liu, X., Wang, S., Wang, X., Huang, Y., Wang,
470 H., Zhang, M., Gong, T., Ma, L., Li, C., He, C., Meng, X. M. & Wu, Y. STING/ACSL4
471 axis-dependent ferroptosis and inflammation promote hypertension-associated chronic
472 kidney disease. *Mol Ther* (2023).
473 8 Yan, M., Li, Y., Luo, Q., Zeng, W., Shao, X., Li, L., Wang, Q., Wang, D., Zhang, Y.,
474 Diao, H., Rong, X., Bai, Y. & Guo, J. Mitochondrial damage and activation of the
475 cytosolic DNA sensor cGAS-STING pathway lead to cardiac pyroptosis and hypertrophy
476 in diabetic cardiomyopathy mice. *Cell Death Discov* **8**, 258 (2022).
477 9 Gao, K. M., Marshak-Rothstein, A. & Fitzgerald, K. A. Type-1 interferon-dependent and
478 -independent mechanisms in cyclic GMP-AMP synthase-stimulator of interferon genes-
479 driven auto-inflammation. *Curr Opin Immunol* **80**, 102280 (2023).
480 10 Yamashiro, L. H., Wilson, S. C., Morrison, H. M., Karalis, V., Chung, J. J., Chen, K. J.,
481 Bateup, H. S., Szpara, M. L., Lee, A. Y., Cox, J. S. & Vance, R. E. Interferon-
482 independent STING signaling promotes resistance to HSV-1 in vivo. *Nat Commun* **11**,
483 3382 (2020).
484 11 Wu, J., Dobbs, N., Yang, K. & Yan, N. Interferon-Independent Activities of Mammalian
485 STING Mediate Antiviral Response and Tumor Immune Evasion. *Immunity* **53**, 115-126
486 e115 (2020).
487 12 Pham, A. T., Oliveira, A. C., Albanna, M., Alvarez-Castanon, J., Dupee, Z., Patel, D., Fu,
488 C., Mukhsinova, L., Nguyen, A., Jin, L. & Bryant, A. J. Non-Interferon-Dependent Role
489 of STING Signaling in Pulmonary Hypertension. *Arterioscler Thromb Vasc Biol* **44**, 124-
490 142 (2024).
491 13 Liu, Y., Jesus, A. A., Marrero, B., Yang, D., Ramsey, S. E., Sanchez, G. A. M.,
492 Tenbrock, K., Wittkowski, H., Jones, O. Y., Kuehn, H. S., Lee, C. R., DiMattia, M. A.,
493 Cowen, E. W., Gonzalez, B., Palmer, I., DiGiovanna, J. J., Biancotto, A., Kim, H., Tsai,
494 W. L., Trier, A. M., Huang, Y., Stone, D. L., Hill, S., Kim, H. J., St Hilaire, C.,
495 Gurprasad, S., Plass, N., Chapelle, D., Horkayne-Szakaly, I., Foell, D., Barysenka, A.,
496 Candotti, F., Holland, S. M., Hughes, J. D., Mehmet, H., Issekutz, A. C., Raffeld, M.,
497 McElwee, J., Fontana, J. R., Minniti, C. P., Moir, S., Kastner, D. L., Gadina, M., Steven,
498 A. C., Wingfield, P. T., Brooks, S. R., Rosenzweig, S. D., Fleisher, T. A., Deng, Z.,

Boehm, M., Paller, A. S. & Goldbach-Mansky, R. Activated STING in a vascular and pulmonary syndrome. *N Engl J Med* **371**, 507-518 (2014).

14 Luksch, H., Stinson, W. A., Platt, D. J., Qian, W., Kalugotla, G., Miner, C. A., Bennion, B. G., Gerbaulet, A., Rosen-Wolff, A. & Miner, J. J. STING-associated lung disease in mice relies on T cells but not type I interferon. *J Allergy Clin Immunol* **144**, 254-266 e258 (2019).

15 Stinson, W. A., Miner, C. A., Zhao, F. R., Lundgren, A. J., Poddar, S. & Miner, J. J. The IFN-gamma receptor promotes immune dysregulation and disease in STING gain-of-function mice. *JCI Insight* **7** (2022).

16 Warner, J. D., Irizarry-Caro, R. A., Bennion, B. G., Ai, T. L., Smith, A. M., Miner, C. A., Sakai, T., Gonugunta, V. K., Wu, J., Platt, D. J., Yan, N. & Miner, J. J. STING-associated vasculopathy develops independently of IRF3 in mice. *J Exp Med* **214**, 3279-3292 (2017).

17 Fremond, M. L., Hadchouel, A., Berteloot, L., Melki, I., Bresson, V., Barnabei, L., Jeremiah, N., Belot, A., Bondet, V., Brocq, O., Chan, D., Dagher, R., Dubus, J. C., Duffy, D., Feuillet-Soummer, S., Fusaro, M., Gattorno, M., Insalaco, A., Jeziorski, E., Kitabayashi, N., Lopez-Corbeto, M., Mazingue, F., Morren, M. A., Rice, G. I., Riviere, J. G., Seabra, L., Sirvente, J., Soler-Palacin, P., Stremler-Le Bel, N., Thouvenin, G., Thumerelle, C., Van Aerde, E., Volpi, S., Willcocks, S., Wouters, C., Breton, S., Molina, T., Bader-Meunier, B., Moshous, D., Fischer, A., Blanche, S., Rieux-Laucat, F., Crow, Y. J. & Neven, B. Overview of STING-Associated Vasculopathy with Onset in Infancy (SAVI) Among 21 Patients. *J Allergy Clin Immunol Pract* **9**, 803-818 e811 (2021).

18 Kim, D., Song, K. B., Choi, E. J. & Yu, J. A 17-Year-Old Girl Diagnosed With STING-Associated Vasculopathy With Onset in Infancy (SAVI) After Lung Transplantation. *Chest* **162**, e249-e252 (2022).

19 Volpi, S., Insalaco, A., Caorsi, R., Santori, E., Messia, V., Sacco, O., Terheggen-Lagro, S., Cardinale, F., Scarselli, A., Pastorino, C., Moneta, G., Cangemi, G., Passarelli, C., Ricci, M., Girosi, D., Derchi, M., Bocca, P., Diociaiuti, A., El Hachem, M., Cancrini, C., Toma, P., Granata, C., Ravelli, A., Candotti, F., Picco, P., DeBenedetti, F. & Gattorno, M. Efficacy and Adverse Events During Janus Kinase Inhibitor Treatment of SAVI Syndrome. *J Clin Immunol* **39**, 476-485 (2019).

20 Tang, X., Xu, H., Zhou, C., Peng, Y., Liu, H., Liu, J., Li, H., Yang, H. & Zhao, S. STING-Associated Vasculopathy with Onset in Infancy in Three Children with New Clinical Aspect and Unsatisfactory Therapeutic Responses to Tofacitinib. *J Clin Immunol* **40**, 114-122 (2020).

21 Dai, Y., Liu, X., Zhao, Z., He, J. & Yin, Q. Stimulator of Interferon Genes-Associated Vasculopathy With Onset in Infancy: A Systematic Review of Case Reports. *Front Pediatr* **8**, 577918 (2020).

22 Gao, K. M., Chiang, K., Jiang, Z., Korkmaz, F. T., Janardhan, H. P., Trivedi, C. M., Quinton, L. J., Gingras, S., Fitzgerald, K. A. & Marshak-Rothstein, A. Endothelial cell expression of a STING gain-of-function mutation initiates pulmonary lymphocytic infiltration. *Cell Rep* **43**, 114114 (2024).

23 Gao, K. M., Motwani, M., Tedder, T., Marshak-Rothstein, A. & Fitzgerald, K. A. Radioresistant cells initiate lymphocyte-dependent lung inflammation and IFNgamma-dependent mortality in STING gain-of-function mice. *Proc Natl Acad Sci U S A* **119**, e2202327119 (2022).

24 Picard, C., Thouvenin, G., Kannengiesser, C., Dubus, J. C., Jeremiah, N., Rieux-Laucat, F., Crestani, B., Belot, A., Thivolet-Bejui, F., Secq, V., Menard, C., Reynaud-Gaubert, M. & Reix, P. Severe Pulmonary Fibrosis as the First Manifestation of Interferonopathy (TMEM173 Mutation). *Chest* **150**, e65-71 (2016).

25 Jin, L., Getahun, A., Knowles, H. M., Mogan, J., Akerlund, L. J., Packard, T. A., Perraud, A. L. & Cambier, J. C. STING/MPYS mediates host defense against Listeria monocytogenes infection by regulating Ly6C(hi) monocyte migration. *J Immunol* **190**, 2835-2843 (2013).

26 Larkin, B., Ilyukha, V., Sorokin, M., Buzdin, A., Vannier, E. & Poltorak, A. Cutting Edge: Activation of STING in T Cells Induces Type I IFN Responses and Cell Death. *J Immunol* **199**, 397-402 (2017).

27 Cerboni, S., Jeremiah, N., Gentili, M., Gehrmann, U., Conrad, C., Stolzenberg, M. C., Picard, C., Neven, B., Fischer, A., Amigorena, S., Rieux-Laucat, F. & Manel, N. Intrinsic antiproliferative activity of the innate sensor STING in T lymphocytes. *J Exp Med* **214**, 1769-1785 (2017).

28 Kuhl, N., Linder, A., Philipp, N., Nixdorf, D., Fischer, H., Veth, S., Kuut, G., Xu, T. T., Theurich, S., Carell, T., Subklewe, M. & Hornung, V. STING agonism turns human T cells into interferon-producing cells but impedes their functionality. *EMBO Rep* **24**, e55536 (2023).

29 Jin, L., Waterman, P. M., Jonscher, K. R., Short, C. M., Reisdorph, N. A. & Cambier, J. C. MPYS, a novel membrane tetraspanner, is associated with major histocompatibility complex class II and mediates transduction of apoptotic signals. *Mol Cell Biol* **28**, 5014-5026 (2008).

30 Gulen, M. F., Koch, U., Haag, S. M., Schuler, F., Apetoh, L., Villunger, A., Radtke, F. & Ablasser, A. Signalling strength determines proapoptotic functions of STING. *Nat Commun* **8**, 427 (2017).

31 Kabelitz, D., Zarobkiewicz, M., Heib, M., Serrano, R., Kunz, M., Chitadze, G., Adam, D. & Peters, C. Signal strength of STING activation determines cytokine plasticity and cell death in human monocytes. *Sci Rep* **12**, 17827 (2022).

32 Murthy, A. M. V., Robinson, N. & Kumar, S. Crosstalk between cGAS-STING signaling and cell death. *Cell Death Differ* **27**, 2989-3003 (2020).

33 Li, C., Liu, J., Hou, W., Kang, R. & Tang, D. STING1 Promotes Ferroptosis Through MFN1/2-Dependent Mitochondrial Fusion. *Front Cell Dev Biol* **9**, 698679 (2021).

34 Song, C., Liu, D., Liu, S., Li, D., Horecny, I., Zhang, X., Li, P., Chen, L., Miller, M., Chowdhury, R., Issa, M., Shen, R., Yan, Y., Zhang, F., Zhang, L., Zhang, L., Bai, C., Feng, J., Zhuang, L., Zhang, R., Li, J., Wilkinson, H., Liu, J. & Tao, W. SHR1032, a novel STING agonist, stimulates anti-tumor immunity and directly induces AML apoptosis. *Sci Rep* **12**, 8579 (2022).

35 Jin, L., Xu, L. G., Yang, I. V., Davidson, E. J., Schwartz, D. A., Wurfel, M. M. & Cambier, J. C. Identification and characterization of a loss-of-function human MPYS variant. *Genes Immun* **12**, 263-269 (2011).

36 Patel, S., Blaauboer, S. M., Tucker, H. R., Mansouri, S., Ruiz-Moreno, J. S., Hamann, L., Schumann, R. R., Opitz, B. & Jin, L. The Common R71H-G230A-R293Q Human TMEM173 Is a Null Allele. *J Immunol* **198**, 776-787 (2017).

589 37 Mansouri, S., Gogoi, H., Patel, S., Katikaneni, D. S., Singh, A., Aybar-Torres, A., de
590 Lartigue, G. & Jin, L. MPYS Modulates Fatty Acid Metabolism and Immune Tolerance
591 at Homeostasis Independent of Type I IFNs. *J Immunol* **209**, 2114-2132 (2022).
592 38 Patel, S. & Jin, L. TMEM173 variants and potential importance to human biology and
593 disease. *Genes Immun* **20**, 82-89 (2019).
594 39 Sebastian, M., Hsiao, C. J., Futch, H. S., Eisinger, R. S., Dumeny, L., Patel, S., Gobena,
595 M., Katikaneni, D. S., Cohen, J., Carpenter, A. M., Spiryda, L., Heldermon, C. D., Jin, L.
596 & Brantly, M. L. Obesity and STING1 genotype associate with 23-valent pneumococcal
597 vaccination efficacy. *JCI Insight* **5** (2020).
598 40 Yi, G., Brendel, V. P., Shu, C., Li, P., Palanathan, S. & Cheng Kao, C. Single nucleotide
599 polymorphisms of human STING can affect innate immune response to cyclic
600 dinucleotides. *PLoS One* **8**, e77846 (2013).
601 41 Patel, S., Blaauboer, S. M., Tucker, H. R., Mansouri, S., Ruiz-Moreno, J. S., Hamann, L.,
602 Schumann, R. R., Opitz, B. & Jin, L. Response to Comment on "The Common R71H-
603 G230A-R293Q Human TMEM173 Is a Null Allele". *J Immunol* **198**, 4185-4188 (2017).
604 42 Nissen, S. K., Pedersen, J. G., Helleberg, M., Kjaer, K., Thavachelvam, K., Obel, N.,
605 Tolstrup, M., Jakobsen, M. R. & Mogensen, T. H. Multiple Homozygous Variants in the
606 STING-Encoding TMEM173 Gene in HIV Long-Term Nonprogressors. *J Immunol* **200**,
607 3372-3382 (2018).
608 43 Ruiz-Moreno, J. S., Hamann, L., Shah, J. A., Verbon, A., Mockenhaupt, F. P.,
609 Puzianowska-Kuznicka, M., Naujoks, J., Sander, L. E., Witzenrath, M., Cambier, J. C.,
610 Suttorp, N., Schumann, R. R., Jin, L., Hawn, T. R., Opitz, B. & Group, C. S. The
611 common HAQ STING variant impairs cGAS-dependent antibacterial responses and is
612 associated with susceptibility to Legionnaires' disease in humans. *PLoS Pathog* **14**,
613 e1006829 (2018).
614 44 Ramanjulu, J. M., Pesiridis, G. S., Yang, J., Concha, N., Singhaus, R., Zhang, S. Y.,
615 Tran, J. L., Moore, P., Lehmann, S., Eberl, H. C., Muelbaier, M., Schneck, J. L.,
616 Clemens, J., Adam, M., Mehlmann, J., Romano, J., Morales, A., Kang, J., Leister, L.,
617 Graybill, T. L., Charnley, A. K., Ye, G., Nevins, N., Behnia, K., Wolf, A. I., Kasparcova,
618 V., Nurse, K., Wang, L., Puhl, A. C., Li, Y., Klein, M., Hopson, C. B., Guss, J.,
619 Bantscheff, M., Bergamini, G., Reilly, M. A., Lian, Y., Duffy, K. J., Adams, J., Foley, K.
620 P., Gough, P. J., Marquis, R. W., Smothers, J., Hoos, A. & Bertin, J. Design of
621 amidobenzimidazole STING receptor agonists with systemic activity. *Nature* **564**, 439-
622 443 (2018).
623 45 Messaoud-Nacer, Y., Culerier, E., Rose, S., Maillet, I., Rouxel, N., Briault, S., Ryffel, B.,
624 Quesniaux, V. F. J. & Togbe, D. STING agonist diABZI induces PANoptosis and DNA
625 mediated acute respiratory distress syndrome (ARDS). *Cell Death Dis* **13**, 269 (2022).
626 46 Tang, C. H., Zundell, J. A., Ranatunga, S., Lin, C., Nefedova, Y., Del Valle, J. R. & Hu,
627 C. C. Agonist-Mediated Activation of STING Induces Apoptosis in Malignant B Cells.
628 *Cancer Res* **76**, 2137-2152 (2016).
629 47 Meric-Bernstam, F., Sweis, R. F., Kasper, S., Hamid, O., Bhatia, S., Dummer, R.,
630 Stradella, A., Long, G. V., Spreafico, A., Shimizu, T., Steeghs, N., Luke, J. J.,
631 McWhirter, S. M., Muller, T., Nair, N., Lewis, N., Chen, X., Bean, A., Kattenhorn, L.,
632 Pelletier, M. & Sandhu, S. Combination of the STING Agonist MIW815 (ADU-S100)
633 and PD-1 Inhibitor Spartalizumab in Advanced/Metastatic Solid Tumors or Lymphomas:
634 An Open-Label, Multicenter, Phase Ib Study. *Clin Cancer Res* **29**, 110-121 (2023).

635 48 Meric-Bernstam, F., Sweis, R. F., Hodi, F. S., Messersmith, W. A., Andtbacka, R. H. I.,
636 Ingham, M., Lewis, N., Chen, X., Pelletier, M., Chen, X., Wu, J., McWhirter, S. M.,
637 Muller, T., Nair, N. & Luke, J. J. Phase I Dose-Escalation Trial of MIW815 (ADU-
638 S100), an Intratumoral STING Agonist, in Patients with Advanced/Metastatic Solid
639 Tumors or Lymphomas. *Clin Cancer Res* **28**, 677-688 (2022).
640 49 Wu, J., Chen, Y. J., Dobbs, N., Sakai, T., Liou, J., Miner, J. J. & Yan, N. STING-
641 mediated disruption of calcium homeostasis chronically activates ER stress and primes T
642 cell death. *J Exp Med* **216**, 867-883 (2019).
643 50 Motwani, M., Pawaria, S., Bernier, J., Moses, S., Henry, K., Fang, T., Burkly, L.,
644 Marshak-Rothstein, A. & Fitzgerald, K. A. Hierarchy of clinical manifestations in SAVI
645 N153S and V154M mouse models. *Proc Natl Acad Sci U S A* **116**, 7941-7950 (2019).
646 51 Hu, W., Wang, Z. M., Feng, Y., Schizas, M., Hoyos, B. E., van der Veeken, J., Verter, J.
647 G., Bou-Puerto, R. & Rudensky, A. Y. Regulatory T cells function in established
648 systemic inflammation and reverse fatal autoimmunity. *Nat Immunol* **22**, 1163-1174
649 (2021).
650 52 Monroe, K. M., Yang, Z., Johnson, J. R., Geng, X., Doitsh, G., Krogan, N. J. & Greene,
651 W. C. IFI16 DNA sensor is required for death of lymphoid CD4 T cells abortively
652 infected with HIV. *Science* **343**, 428-432 (2014).
653 53 Doitsh, G., Galloway, N. L., Geng, X., Yang, Z., Monroe, K. M., Zepeda, O., Hunt, P.
654 W., Hatano, H., Sowinski, S., Munoz-Arias, I. & Greene, W. C. Cell death by pyroptosis
655 drives CD4 T-cell depletion in HIV-1 infection. *Nature* **505**, 509-514 (2014).
656 54 Jakobsen, M. R., Olagnier, D. & Hiscott, J. Innate immune sensing of HIV-1 infection.
657 *Curr Opin HIV AIDS* **10**, 96-102 (2015).
658 55 Silvin, A. & Manel, N. Innate immune sensing of HIV infection. *Curr Opin Immunol* **32**,
659 54-60 (2015).
660 56 Altfeld, M. & Gale, M., Jr. Innate immunity against HIV-1 infection. *Nat Immunol* **16**,
661 554-562 (2015).
662 57 Krapp, C., Jonsson, K. & Jakobsen, M. R. STING dependent sensing - Does HIV actually
663 care? *Cytokine Growth Factor Rev* **40**, 68-76 (2018).
664 58 Gubser, C., Pitman, M. C. & Lewin, S. R. CD4(+) T cell signatures in HIV infection. *Nat*
665 *Immunol* **20**, 948-950 (2019).
666 59 Morou, A., Brunet-Ratnasingham, E., Dube, M., Charlebois, R., Mercier, E., Darko, S.,
667 Brassard, N., Nganou-Makamdop, K., Arumugam, S., Gendron-Lepage, G., Yang, L.,
668 Niessl, J., Baxter, A. E., Billingsley, J. M., Rajakumar, P. A., Lefebvre, F., Johnson, R.
669 P., Tremblay, C., Routy, J. P., Wyatt, R. T., Finzi, A., Douek, D. C. & Kaufmann, D. E.
670 Altered differentiation is central to HIV-specific CD4(+) T cell dysfunction in
671 progressive disease. *Nat Immunol* **20**, 1059-1070 (2019).
672 60 Hines, J. B., Kacew, A. J. & Sweis, R. F. The Development of STING Agonists and
673 Emerging Results as a Cancer Immunotherapy. *Curr Oncol Rep* **25**, 189-199 (2023).
674 61 Samson, N. & Ablasser, A. The cGAS-STING pathway and cancer. *Nat Cancer* **3**, 1452-
675 1463 (2022).
676 62 Liu, Y., Lu, X., Qin, N., Qiao, Y., Xing, S., Liu, W., Feng, F., Liu, Z. & Sun, H. STING,
677 a promising target for small molecular immune modulator: A review. *Eur J Med Chem*
678 **211**, 113113 (2021).

63 Zheng, J., Mo, J., Zhu, T., Zhuo, W., Yi, Y., Hu, S., Yin, J., Zhang, W., Zhou, H. & Liu, Z. Comprehensive elaboration of the cGAS-STING signaling axis in cancer development and immunotherapy. *Mol Cancer* **19**, 133 (2020).

64 Corrales, L., Glickman, L. H., McWhirter, S. M., Kanne, D. B., Sivick, K. E., Katibah, G. E., Woo, S. R., Lemmens, E., Banda, T., Leong, J. J., Metchette, K., Dubensky, T. W., Jr. & Gajewski, T. F. Direct Activation of STING in the Tumor Microenvironment Leads to Potent and Systemic Tumor Regression and Immunity. *Cell Rep* **11**, 1018-1030 (2015).

65 Fu, J., Kanne, D. B., Leong, M., Glickman, L. H., McWhirter, S. M., Lemmens, E., Mechette, K., Leong, J. J., Lauer, P., Liu, W., Sivick, K. E., Zeng, Q., Soares, K. C., Zheng, L., Portnoy, D. A., Woodward, J. J., Pardoll, D. M., Dubensky, T. W., Jr. & Kim, Y. STING agonist formulated cancer vaccines can cure established tumors resistant to PD-1 blockade. *Sci Transl Med* **7**, 283ra252 (2015).

66 Barber, G. N. Innate immune DNA sensing pathways: STING, AIMII and the regulation of interferon production and inflammatory responses. *Curr Opin Immunol* **23**, 10-20 (2011).

67 Liu, S., Cai, X., Wu, J., Cong, Q., Chen, X., Li, T., Du, F., Ren, J., Wu, Y. T., Grishin, N. V. & Chen, Z. J. Phosphorylation of innate immune adaptor proteins MAVS, STING, and TRIF induces IRF3 activation. *Science* **347**, aaa2630 (2015).

68 Movert, E., Bolarin, J. S., Valfridsson, C., Velarde, J., Skrede, S., Nekludov, M., Hyldegaard, O., Arnell, P., Svensson, M., Norrby-Teglund, A., Cho, K. H., Elhaik, E., Wessels, M. R., Raberg, L. & Carlsson, F. Interplay between human STING genotype and bacterial NADase activity regulates inter-individual disease variability. *Nat Commun* **14**, 4008 (2023).

69 Vila, I. K., Chamma, H., Steer, A., Saccas, M., Taffoni, C., Turtoi, E., Reinert, L. S., Hussain, S., Marines, J., Jin, L., Bonnefont, X., Hubert, M., Schwartz, O., Paludan, S. R., Van Simaeys, G., Doumont, G., Sobhian, B., Vlachakis, D., Turtoi, A. & Laguette, N. STING orchestrates the crosstalk between polyunsaturated fatty acid metabolism and inflammatory responses. *Cell Metab* **34**, 125-139 e128 (2022).

70 Cai, J., Gehrau, R., Tu, Z., Leroy, V., Su, G., Shang, J., Mas, V. R., Emtiazjoo, A., Pelaez, A., Atkinson, C., Machuca, T., Upchurch, G. R., Jr. & Sharma, A. K. MicroRNA-206 antagomiRenriched extracellular vesicles attenuate lung ischemiareperfusion injury through CXCL1 regulation in alveolar epithelial cells. *J Heart Lung Transplant* **39**, 1476-1490 (2020).

71 Mansouri, S., Katikaneni, D. S., Gogoi, H., Pipkin, M., Machuca, T. N., Emtiazjoo, A. M. & Jin, L. Lung IFNAR1(hi) TNFR2(+) cDC2 promotes lung regulatory T cells induction and maintains lung mucosal tolerance at steady state. *Mucosal Immunol* **13**, 595-608 (2020).

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Conceptualization: LJ
Methodology: AAT, LAS, ATP, AME
Investigation: AAT, LAS, ATP, AKS, AJB, LJ
Supervision: LJ
Writing—original draft: LJ
Writing—review & editing: LJ, AJB, AKS

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Figure and Captions

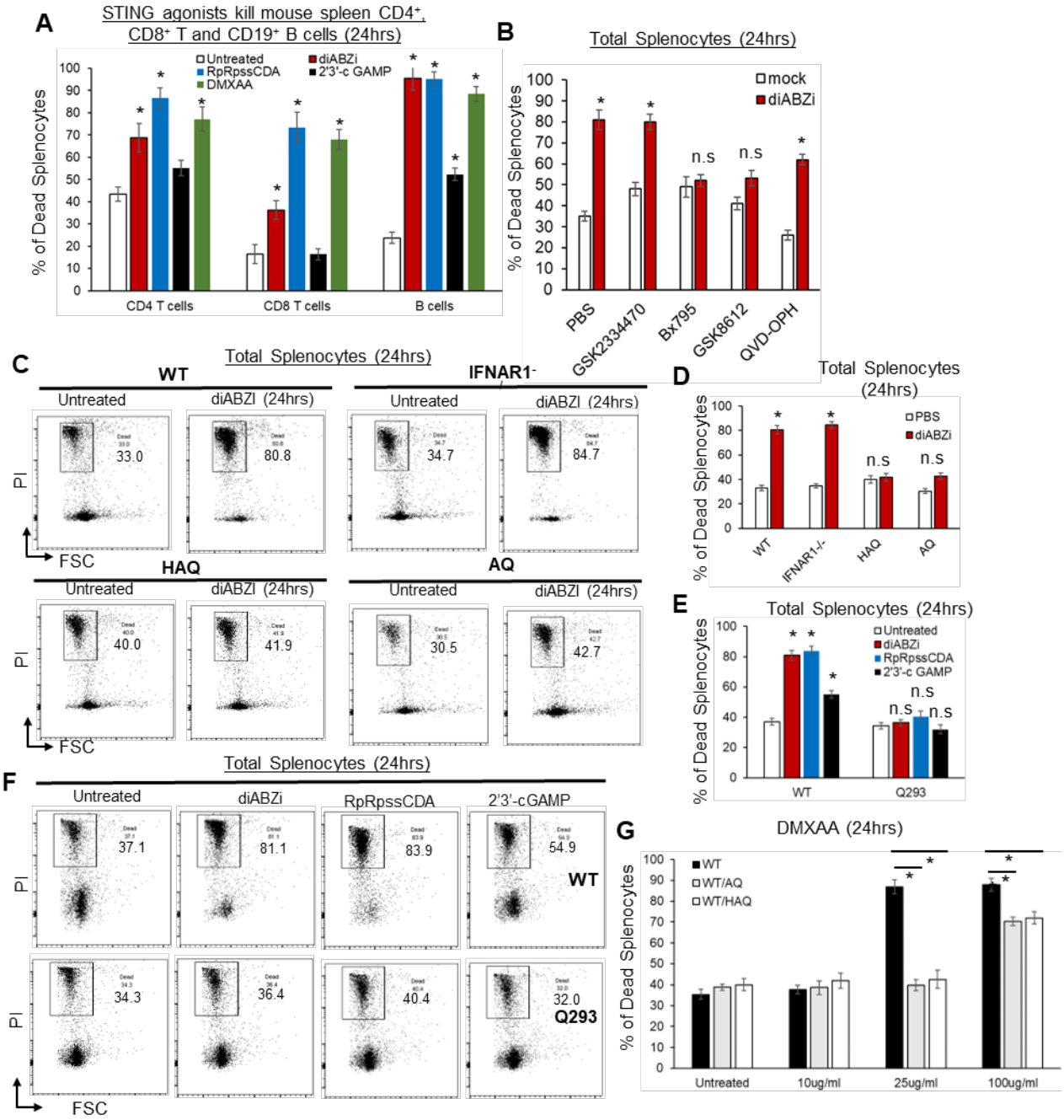

Fig. 1. Splenocytes from *HAQ, AQ*, and *Q293* mice are resistant to STING-mediated cell death *ex vivo*. (A). C57BL/6N splenocytes were treated directly (no transfection) with diABZI (100ng/ml), RpRpss-Cyclic di-AMP (5 µg/ml) or 2′3′-cGAMP (10µg/ml), DMXAA (25µg/ml) for 24hrs in culture. CD4, CD8 T cells and CD19 B cells death were determined by PI staining. (**B**). Splenocytes from C57BL/6N mice were pre-treated with indicated small molecules, GSK2334470 (1.25µM), GSK8612 (2.5µM), Bx-795 (0.5µM), QVD-OPH (25µM) for 2hrs.

747 diABZI (100ng/ml) was added in culture for another 24hrs. Dead cells were
748 determined by PI staining. (**C-D).** Flowcytometry of *HAQ*, *AQ*, IFNAR1$^{-/-}$ or
749 C57BL/6N splenocytes treated with diABZI (100ng/ml) for 24hrs. Cell death was
750 determined by PI staining. (**E-F)**. *Q293* or the *WT* littermates splenocytes were
751 treated with diABZI (100ng/ml), RpRpss-Cyclic di-AMP (5 µg/ml) or 2′3′-cGAMP
752 10µg/ml) for 24h. Cell death was determined by PI staining. (**G).** *WT/HAQ*, *WT/AQ*,
753 or *WT/WT* littermates splenocytes were treated with DMXAA (10, 25 or 100µg/ml)
754 for 24h. Cell death was determined by PI staining. Data are representative of three
755 independent experiments. Graphs represent the mean with error bars indication
756 s.e.m. *p* values are determined by one-way ANOVA Tukey's multiple comparison
757 test (**A, E, G**) or unpaired student T-test (**B, D**). * $p<0.05$. n.s: not significant.
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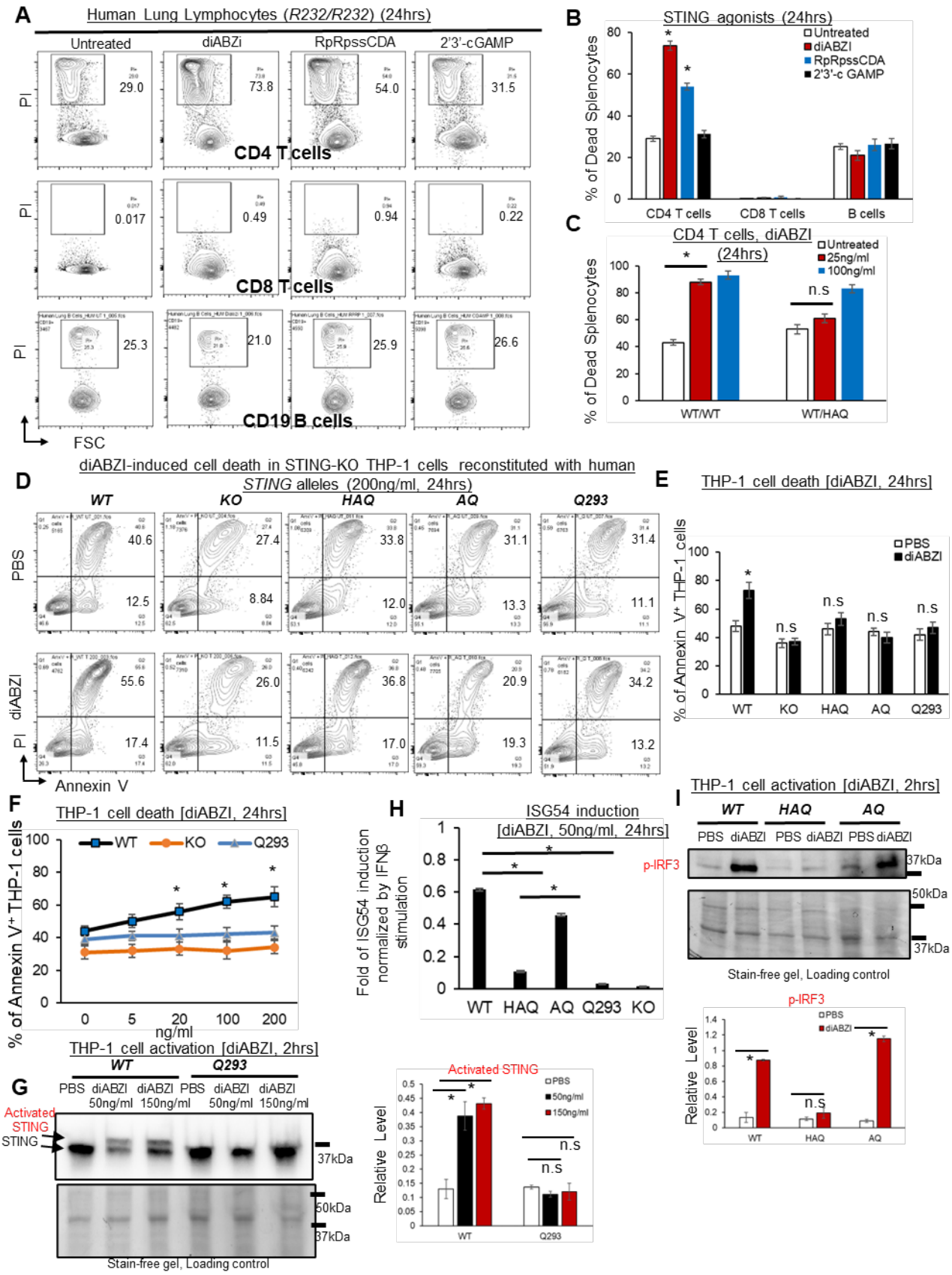
A
Human Lung Lymphocytes (R232/R232) (24hrs)
Untreated
diABZi
RpRpssCDA
2'3'-cGAMP
PI
29.0
73.8
54.0
31.5
CD4 T cells
0.017
0.49
0.94
0.22
CD8 T cells
25.3
21.0
25.9
26.6
CD19 B cells
FSC
B
STING agonists (24hrs)
Untreated
diABZI
RpRpssCDA
2'3'-c GAMP
% of Dead Splenocytes
CD4 T cells
CD8 T cells
B cells
C
CD4 T cells, diABZI (24hrs)
Untreated
25ng/ml
100ng/ml
n.s
WT/WT
WT/HAQ
D
diABZI-induced cell death in STING-KO THP-1 cells reconstituted with human STING alleles (200ng/ml, 24hrs)
WT
KO
HAQ
AQ
Q293
PBS
diABZI
40.6
27.4
33.8
31.1
31.4
12.5
8.84
12.0
13.3
11.1
55.6
26.0
36.8
20.9
34.2
17.4
11.5
17.0
19.3
13.2
Annexin V
E
THP-1 cell death [diABZI, 24hrs]
PBS
diABZI
% of Annexin V+ THP-1 cells
n.s
WT
KO
HAQ
AQ
Q293
F
THP-1 cell death [diABZI, 24hrs]
WT
KO
Q293
% of Annexin V+ THP-1 cells
ng/ml
H
ISG54 induction [diABZI, 50ng/ml, 24hrs]
Fold of ISG54 induction normalized by IFNβ stimulation
WT
HAQ
AQ
Q293
KO
I
THP-1 cell activation [diABZI, 2hrs]
WT
HAQ
AQ
PBS
diABZI
p-IRF3
37kDa
50kDa
Stain-free gel, Loading control
Relative Level
G
THP-1 cell activation [diABZI, 2hrs]
WT
Q293
PBS
diABZI 50ng/ml
diABZI 150ng/ml
Activated STING
STING
37kDa
50kDa
Stain-free gel, Loading control
Activated STING
50ng/ml
150ng/ml
Relative Level

760

Fig. 2. *HAQ* , *AQ*, and *Q293* human cells are resistant to STING agonists-induced death. (A-B). Total human Lung cells from *WT/WT* individuals were treated with diABZi (100ng/ml) for 24hrs. Cell death in CD4, CD8 T cells and CD19 B cells were determined by PI staining. (**C**). Total lung cells from a *WT/HAQ* (2 individuals) and a *WT/WT* (3 individuals) were treated with diABZi (25, 100ng/ml) for 24h. Cell death in CD4 T cells was determined by PI staining. (**D-E**). STING-KO THP-1 cells (Invivogen,, cat no. thpd-kostg) were stably reconstituted with human *WT (R232), HAQ*, *AQ*, *Q293*. Cells were treated with diABZI (200ng/ml) in culture for 24 hrs. Dead cells were determined by Annexin V staining. (**F).** STING-KO THP-1 cells stably reconstituted with human *WT (R232), Q293* were treated with indicated dose of diABZI for 24hs in culture. Dead cells were determined by Annexin V staining. (**G**). STING-KO THP-1 cells stably reconstituted with human *WT (R232), Q293* were treated with indicated dose of diABZI for 2hs in culture. STING activation was detected by anti-STING antibody (Proteintech, #19851-1-AP). (**H**). STING-KO THP-1 cells stably reconstituted with human *WT (R232), HAQ*, *AQ*, *Q293* were treated with 50ng/ml diABZI in culture for 24hrs. ISG-54 reporter luciferase activity was determined in cell supernatant and normalized to 10ng/ml IFNβ-stimulated ISG-54 luciferase activity. (**I).** STING-KO THP-1 cells stably reconstituted with human *WT (R232), HAQ*, *AQ* were treated with 50ng/ml diABZI in culture for 2hrs. STING and IRF3 activation were determined by anti-STING antibody and anti-p IRF3 antibody (CST, Ser396, clone 4D4G). Densitometry was determined by ImageLab 5. Data are representative of three independent experiments. Graphs represent the mean with error bars indication s.e.m. *p* values determined by one-way ANOVA Tukey's multiple comparison test (**B, C, F, H, G**) or unpaired student T-test (**E, I**). * $p<0.05$, n.s: not significant.

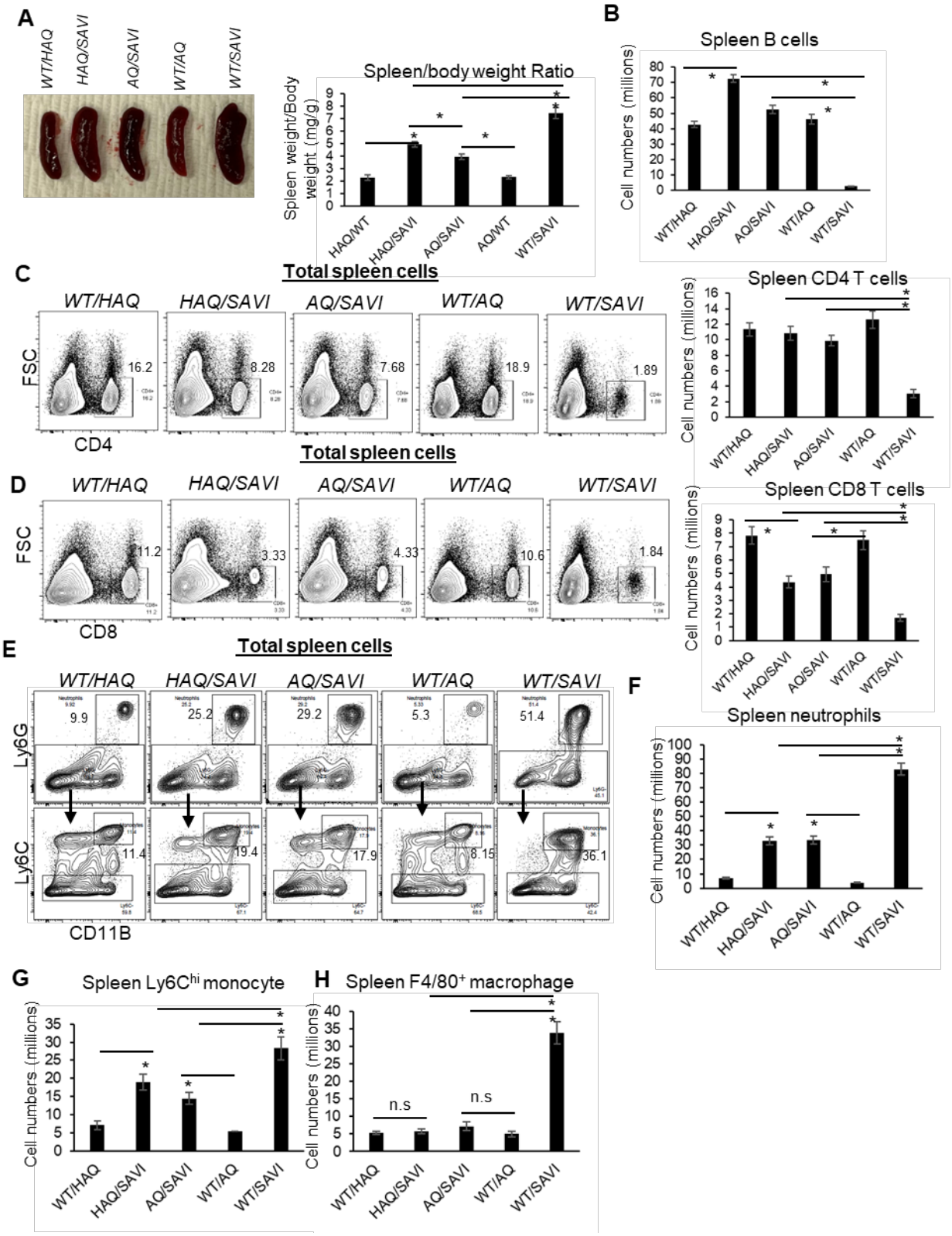

Fig. 3. *HAQ* and *AQ* rescue the lymphopenia and suppress myeloid cell expansion in *SAVI (N153S)* mice. (A). The size and weight of spleens from *WT/HAQ,*

HAQ/SAVI, AQ/SAVI, WT/AQ, WT/SAVI. **(B-D).** Spleen $CD19^+$ B cells, CD4, CD8 T cells were determined in the indicated mice by Flow. (**E-H).** Spleen $Ly6G^+$ neutrophils, $Ly6C^{hi}$ monocytes and $F4/80^+$ macrophage was determined in the indicated mice by Flow. Data are representative of three independent experiments. n=3~5 mice/group. Graphs represent the mean with error bars indication s.e.m. *P* values are determined by one-way ANOVA Tukey's multiple comparison test. * $p<0.05$, n.s: not significant.

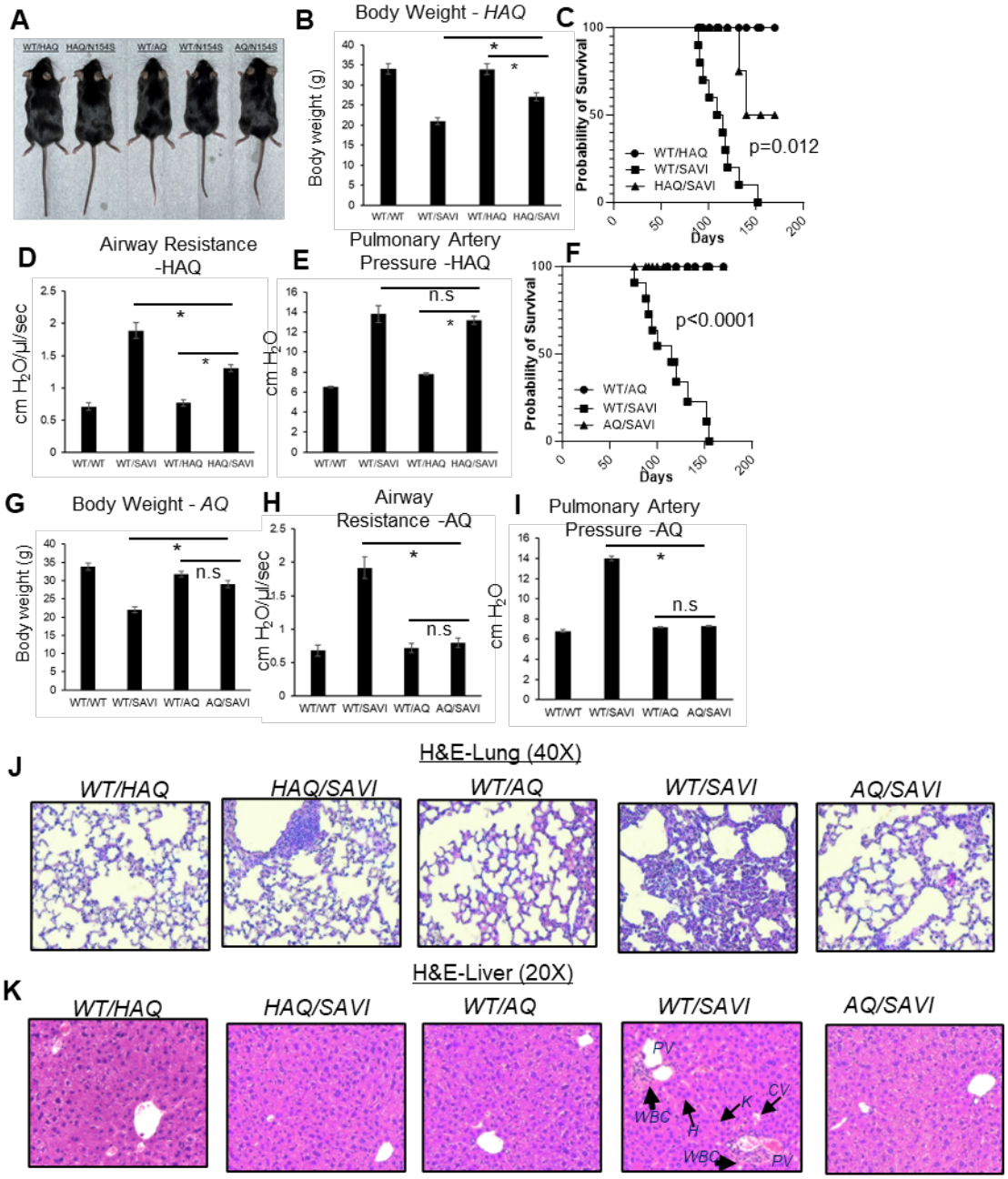

Fig. 4. *HAQ* and *AQ* alleles prevent *SAVI(N153S)* disease in mice. (A, B, G). The size and body weight of *HAQ/SAVI*, *AQ/SAVI*, *WT/SAVI* and their littermates *WT/HAQ*, *WT/AQ* mice. (**D, E, H, I**). Airway resistance, and pulmonary artery pressure were determined as described in **Materials and Methods**. **(C, F).** *HAQ/SAVI, AQ/SAVI, WT/SAVI* (10 mice/group), were monitored for survival by Kaplan-Meier. (**J, K**). Representative hematoxylin and eosin (H&E) staining of lung, liver sections from indicated mice. n=3~5 mice/group. Data are representative of 3 independent experiments. Graphs represent the mean with error bars indication s.e.m. *p* values are determined by one-way ANOVA Tukey's multiple comparison test. * $p<0.05$,

813 **$p<0.01$. n.s.: not significant. **WBC**: white blood cells; **H**: hepatocytes; **K**: Kupper
814 cells; **PV**: portal vein; **CV**: central vein.
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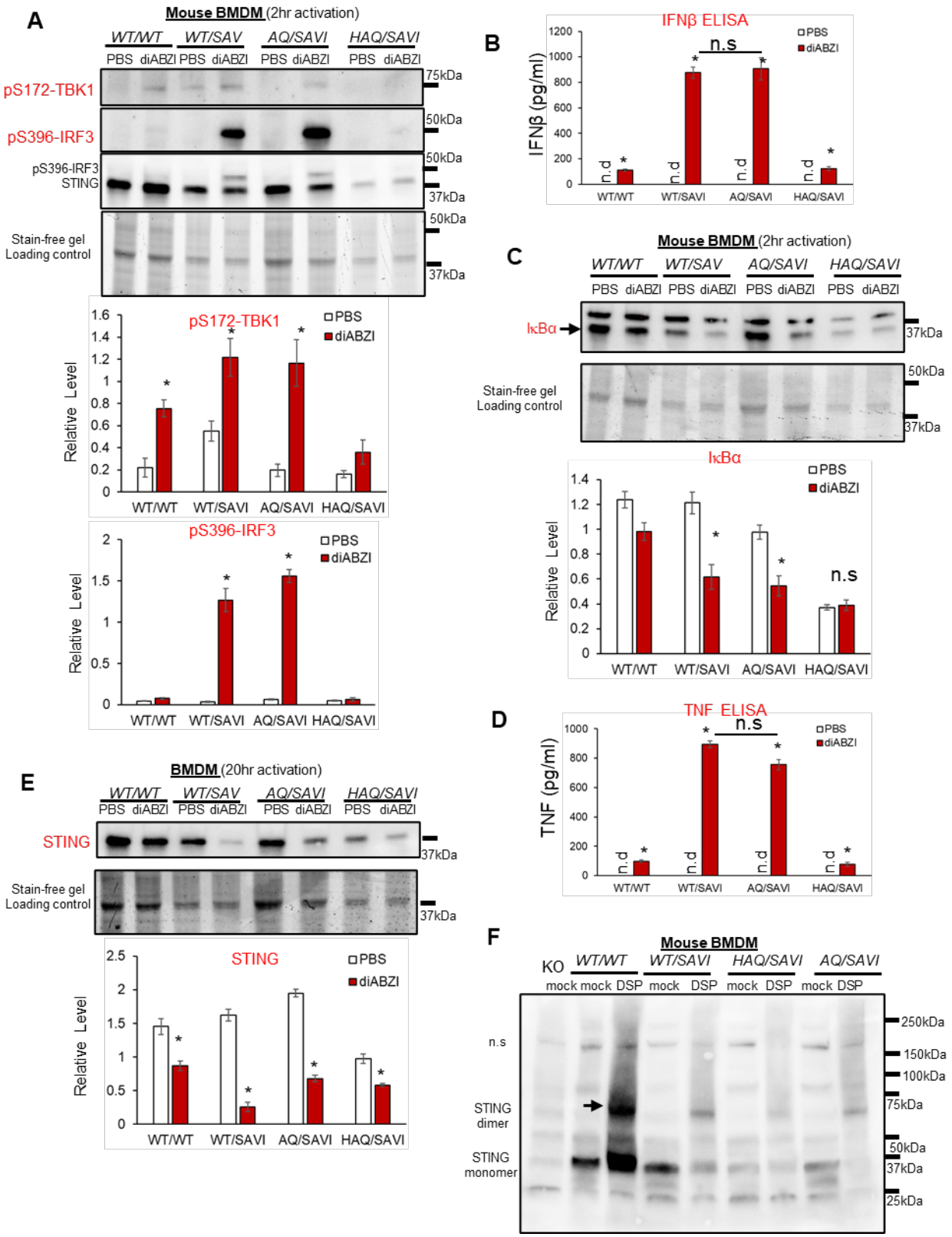

Fig. 5. *AQ/SAVI(N153S)* cells had similar TBK1-IRF3, NFκB activation and STING degradation as the *WT/SAVI(N153S)* cells. (A, C). BMDM from *WT/WT,*

822 *WT/SAVI, HAQ/SAVI* and *AQ/SAVI* were treated with 100ng/ml diABZi in culture
823 for 2 hrs. Cells were lysed and run on a 4~20% Mini-PROTEAN TGX Stain-Free
824 Precast Gel. The blot was probed for phospho-Thr172-TBK1 antibody (CST, clone
825 D52C2), phosphor-Ser 396-IRF3 (CST, clone 4D4G), STING (Proteintech,
826 #19851-1-AP) and IκBα (CST, clone 44D4) antibody. **(E).** BMDM from *WT/WT,*
827 *WT/SAVI, HAQ/SAVI* and *AQ/SAVI* were treated with 100ng/ml diABZi in culture
828 for 24 hrs. Cells were lysed and run on a 4~20% Mini-PROTEAN TGX Stain-Free
829 Precast Gel. The blot was probed for STING antibody (Proteintech, 19851-1-AP).
830 (**B, D**). IFNβ and TNF were determined by ELISA in the cell supernatant from **E**.
831 (**F).** BMDM from *WT/WT, WT/SAVI, HAQ/SAVI* and *AQ/SAVI* were treated with
832 400μM cleavable chemical crosslinker DSP (Pierce™, cat no: PG82081) in PBS
833 for 1 hr at 4°C. Cells were washed with PBS and lysed in RIPA buffer. Whole cell
834 lysate was mixed with 4x Laemmli Sample Buffer (BioRad, cat no 1610747)
835 containing 5% 2-mercaptoethanol, heated at 95°C for 10 min and, run on a 4~20%
836 Mini-PROTEAN TGX Stain-Free Precast Gel. The blot was probed for STING
837 antibody (Proteintech, 19851-1-AP). Densitometry was determined by ImageLab
838 5. Data are representative of three independent experiments. Graphs represent the
839 mean with error bars indication s.e.m. *p* values are determined by unpaired student
840 T-test (**A-E**) or one-way ANOVA Tukey's multiple comparison test (**D, B**). *
841 $p<0.05$, ** $p<0.01$, *** $p<0.001$. n.s.: not significant; n.d: not detected.
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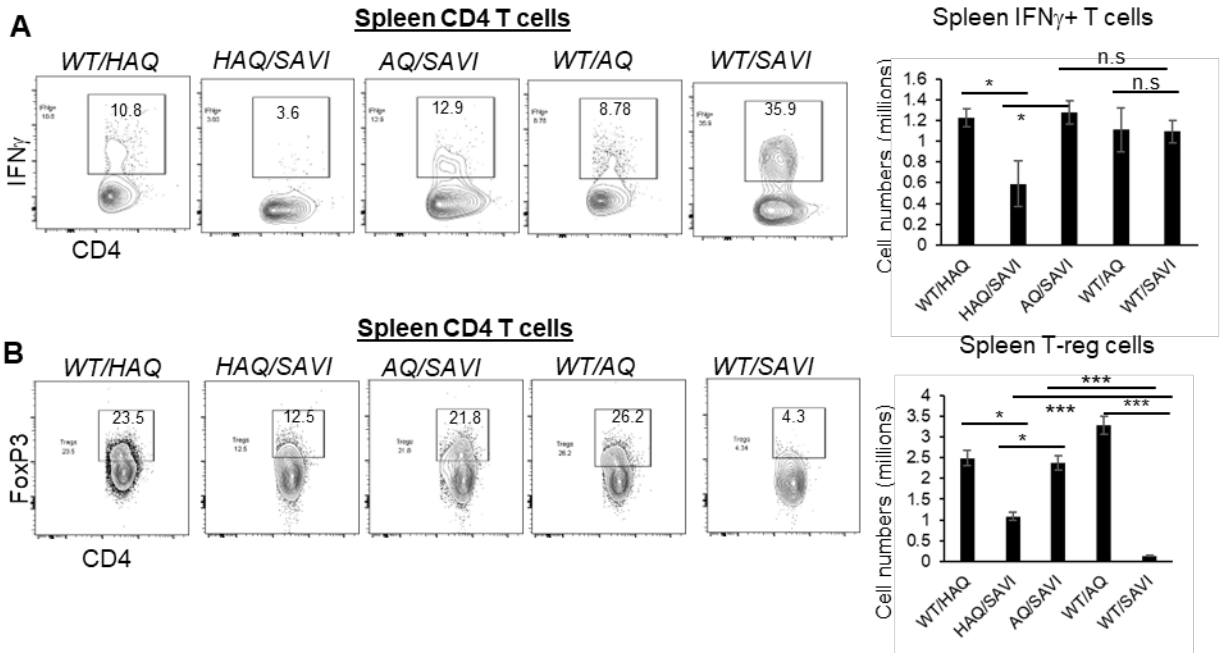

Fig. 6. *HAQ/SAVI(N153S)* and *AQ/SAVI(N153S)* cells had 10-fold and 20-fold increased spleen T-regs compared to *WT/SAVI* mice. (A). Flow cytometry analysis of IFNγ producing spleen $CD4^+$ T cells from *WT/HAQ, WT/AQ, WT/SAVI, HAQ/SAVI* and *AQ/SAVI* mice. (**B**). Flow cytometry analysis of $CD4^+$ $FoxP3^+$ spleen T-regs. n=3~5 mice/group. Data are representative of 3 independent experiments. Graphs represent the mean with error bars indication s.e.m. *p* values are determined by one-way ANOVA Tukey's multiple comparison test. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. n.s.: not significant.

Supplementary Materials for

The common *TMEM173 HAQ, AQ* alleles rescue CD4 T cellpenia, restore T-regs, and prevent SAVI (N153S) inflammatory disease in mice.

Alexandra Aybar-Torres *et al.*

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This PDF file includes:

Figs. S1 to S5

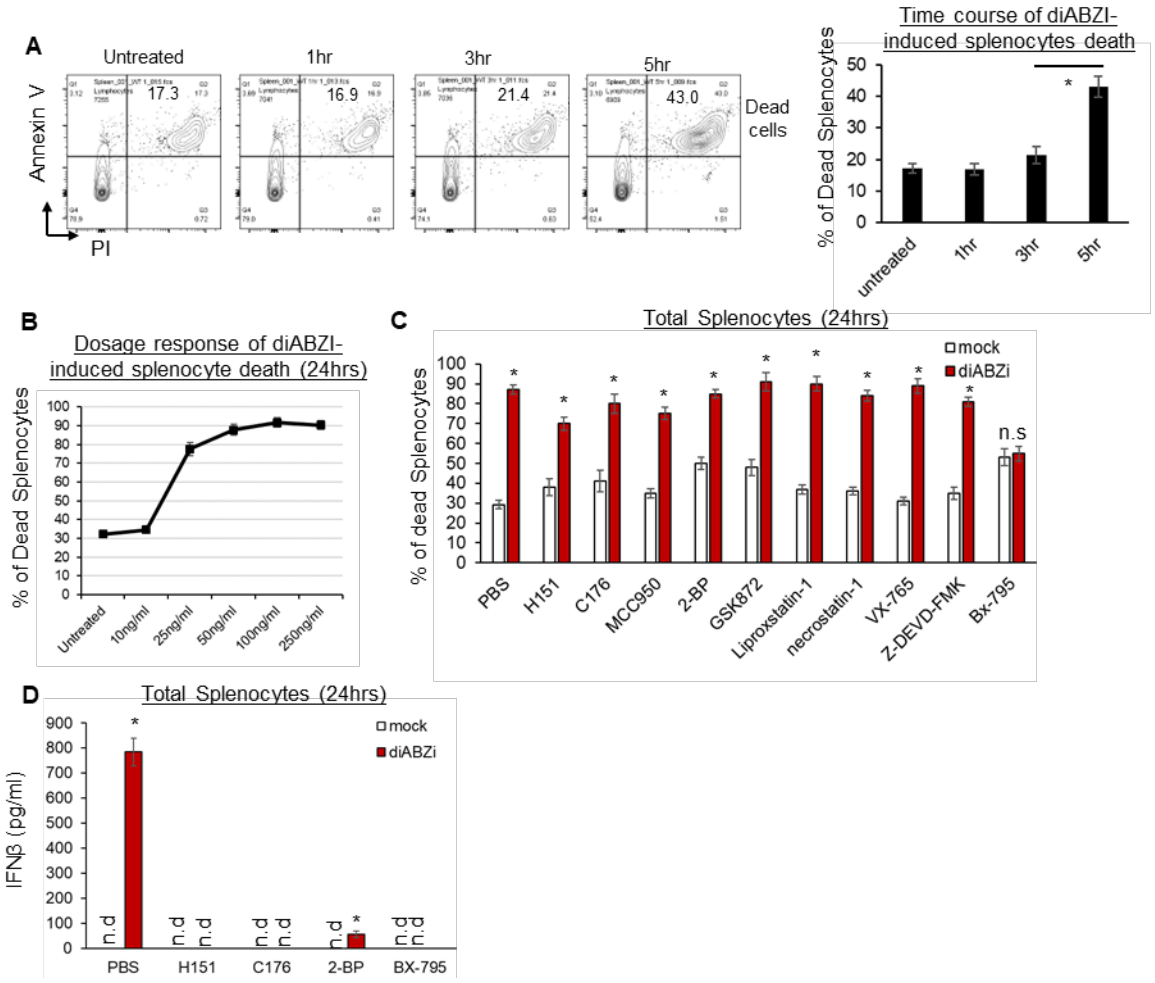

Fig. S1.

Bx-795 inhibits diABZI-induced mouse splenocyte death. (A). Splenocytes from C57BL/6N mice were treated with 100ng/ml diABZI in culture for the indicated time. Dead cells were determined by PI and Annexin V staining. (**B).** Splenocytes from C57BL/6N mice were treated with indicated dose of diABZi in culture for 24hrs. Dead cells were determined by PI staining. **(C)**. Splenocytes from C57BL/6N mice were pre-treated with indicated small molecules, H151 (10µg/ml), C176 (1µM), MCC950 (1.25µM), 2-BP (20µM), GSK872 (312.5nM), Liproxstatin-1 (1µM), necrostatin-1 (10µM), VX-765 (1µM), Z-DEVD-FMK (25µM), Bx-795 (0.5µM) for 2hrs. diABZi (100ng/ml) was added in culture for another 24hrs. Dead cells were determined by PI staining. (**D**). IFNβ was determined in the cell supernatant from **C** by ELISA. Data are representative of three independent experiments. Graphs represent the mean with error bars indication s.e.m. n=3~5 mice/group. *p* values are determined by one-way ANOVA Tukey's multiple comparison test (**A, D**) or unpaired student T-test (**C**). * $p<0.05$. n.s: not significant. n.d: not detected.

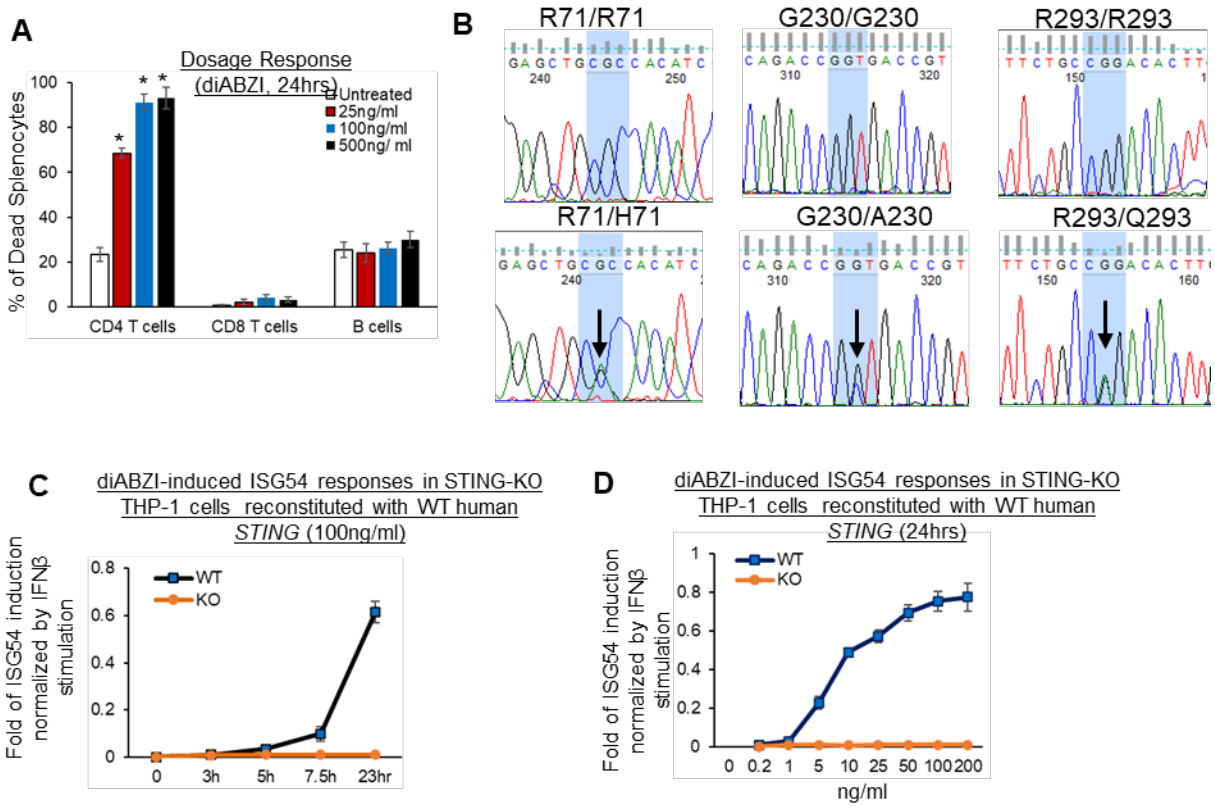

Fig. S2.

STING activation in primary human cells and THP-1 cells reconstituted with *WT* human *STING*. (A). Total human Lung cells from *WT/WT* individuals were treated with indicated dose of diABZi for 24hrs. Cell death in CD4, CD8 T cells and CD19 B cells were determined by PI staining. **(B).** Sequencing results of human *TMEM173* gene in *WT/WT* and *WT/HAQ* individuals. **(C-D)**. STING-KO THP-1 cells stably reconstituted with human *WT (R232)* were treated with indicated dose of diABZI for indicated time in culture. ISG-54 reporter luciferase activity was determined in cell supernatant and normalized to 10ng/ml IFNβ-stimulated ISG-54 luciferase activity. Data are representative of at least two independent experiments. Graphs represent the mean with error bars indication s.e.m. *p* values determined by one-way ANOVA Tukey's multiple comparison test. * $p<0.05$, n.s: not significant**.**

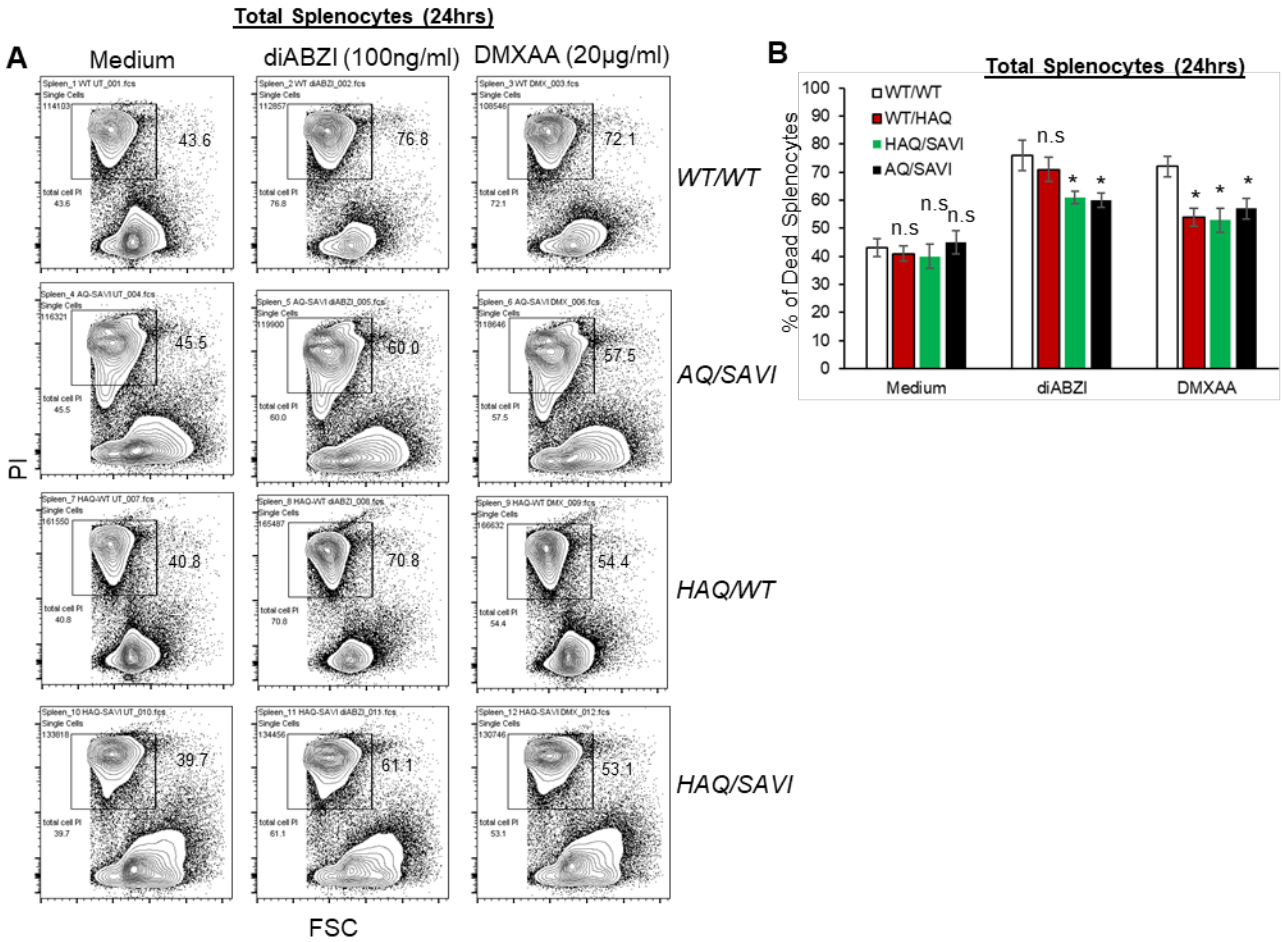

Fig. S3.

Splenocytes from HAQ/SAVI, AQ/SAVI partially resist to STING-activation-induced cell death ex vivo. **(A-B).** Flow cytometry of *HAQ/SAVI*, *AQ/SAVI*, *WT/WT* or *WT/HAQ* splenocytes treated with diABZI (100ng/ml) or DMXAA (20µg/ml) for 24hrs. Cell death was determined by PI staining. Data are representative of three independent experiments. Graphs represent the mean with error bars indication s.e.m. *p* values are determined by one-way ANOVA Tukey's multiple comparison test. * $p<0.05$. n.s: not significant.

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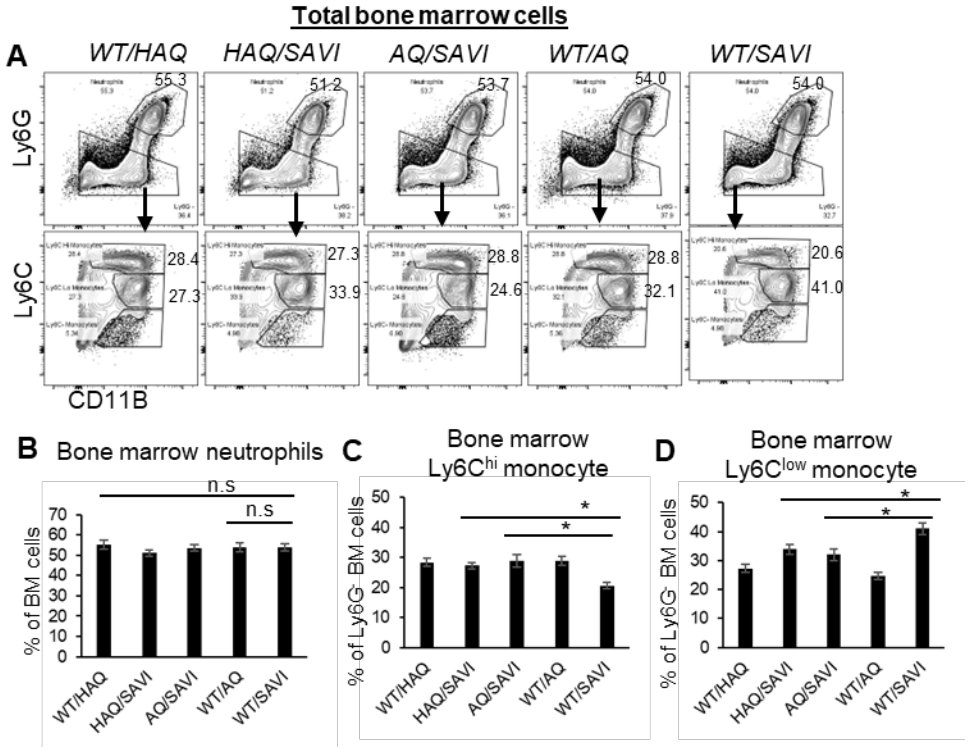

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910 **Fig. S4.**

911 ***HAQ* and *AQ* restore bone marrow monocytes in *SAVI (N153S)* mice.** (**A-D).** Flow analysis of
912 $Ly6G^{+}CD11B^{+}$ neutrophils and $Ly6G^{-}Ly6C^{+}CD11B^{+}$ bone marrow monocytes from HAQ/SAVI,
913 AQ/SAVI, WT/SAVI and their littermates. n=3~5 mice/group. Data are representative of three
914 independent experiments. Graphs represent the mean with error bars indication s.e.m. *P* values are
915 determined by one-way ANOVA Tukey's multiple comparison test. * $p<0.05$, n.s: not significant.

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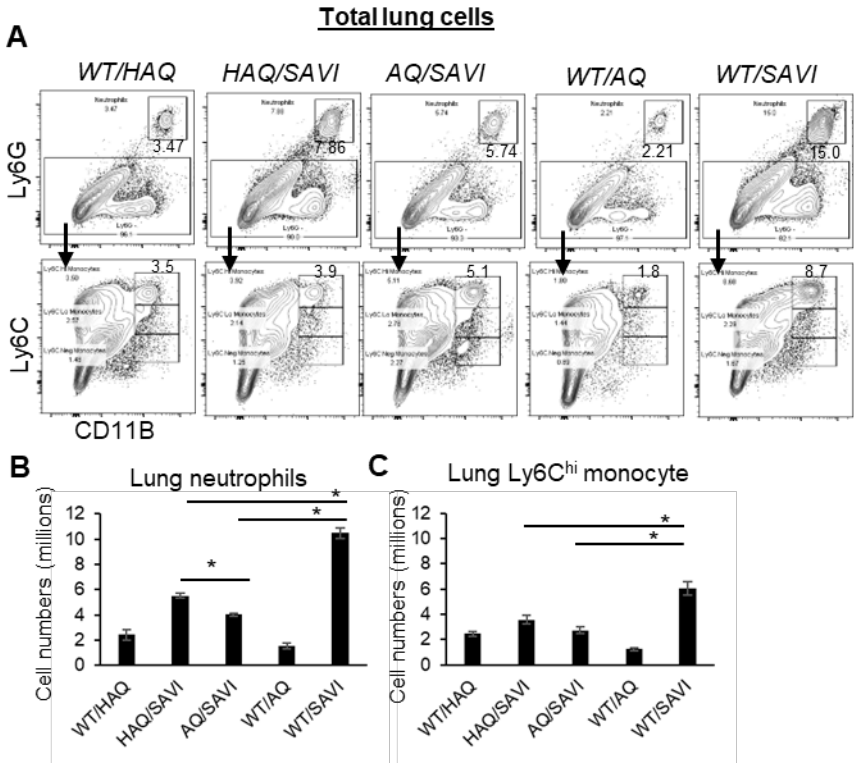

Fig. S5.

***HAQ, AQ* suppress lung myeloid cells infiltration in *SAVI(N153S)* mice**. **(A-C).** Flow analysis of lung $Ly6G^{+}CD11B^{+}$ neutrophils and $Ly6G^{-}Ly6C^{+}CD11B^{+}$ monocytes from *HAQ/SAVI, AQ/SAVI, WT/SAVI and their littermates WT/HAQ, WT/AQ* mice. n=3~5 mice/group. Data are representative of three independent experiments. Graphs represent the mean with error bars indication s.e.m. *P* values are determined by one-way ANOVA Tukey's multiple comparison test. * $p<0.05$.