

1 **Type I interferon potentiates metabolic dysfunction, inflammation, and accelerated aging in**
2 **mtDNA mutator mice**

3
4
5 Yuanjiu Lei¹, Camila Guerra Martinez¹, Sylvia Torres-Odio¹, Samantha L. Bell¹, Christine E.
6 Birdwell¹, Joshua D. Bryant¹, Carl W. Tong², Robert O. Watson¹, Laura Ciaccia West¹, and A.
7 Phillip West^{1*}

8
9
10 ¹Department of Microbial Pathogenesis and Immunology, College of Medicine, Texas A&M
11 University Health Science Center, Bryan, Texas, USA

12 ²Department of Medical Physiology, College of Medicine, Texas A&M University Health
13 Science Center, Bryan, Texas, USA

14
15 * Correspondence to: awest@tamu.edu

16
17 **Abstract**

18 Mitochondrial dysfunction is a key driver of inflammatory responses in human disease. However,
19 it remains unclear whether alterations in mitochondria-innate immune crosstalk contribute to the
20 pathobiology of mitochondrial disorders and aging. Using the polymerase gamma (POLG)
21 mutator model of mitochondrial DNA (mtDNA) instability, we report that aberrant activation of
22 the type I interferon (IFN-I) innate immune axis potentiates immunometabolic dysfunction,
23 reduces healthspan, and accelerates aging in mutator mice. Mechanistically, elevated IFN-I
24 signaling suppresses activation of nuclear factor erythroid 2-related factor 2 (Nrf2), which
25 increases oxidative stress, enhances pro-inflammatory cytokine responses, and accelerates
26 metabolic dysfunction. Ablation of IFN-I signaling attenuates hyper-inflammatory phenotypes by

27 restoring Nrf2 activity and reducing aerobic glycolysis, which combine to lessen cardiovascular
28 and myeloid dysfunction in aged mutator mice. These findings further advance our knowledge of
29 how mitochondrial dysfunction shapes innate immune responses and provide a framework for
30 understanding mitochondria-driven immunopathology in POLG-related diseases and aging.

31

32 **Introduction**

33 An expanding body of literature indicates that mitochondria are key regulators of the mammalian
34 innate immune response, with both beneficial and deleterious consequences for the host (1).
35 Mitochondria serve as antiviral signaling hubs and facilitate antibacterial immunity by generating
36 reactive oxygen species (ROS), but they can also promote inflammation following cellular
37 damage and stress (2–5). Recent work has demonstrated that mtDNA is a potent agonist of
38 nucleic acid sensors of the innate immune system, including Toll-like receptor 9 (TLR9), NLR
39 family, Pyrin domain containing 3 (NLRP3), and cGAS (6, 7). The cGAS-STING axis is now
40 recognized as a major driver of IFN-I and inflammatory responses to nuclear and mitochondrial
41 genome instability, and the aberrant release of mtDNA from damaged cells and tissues is
42 increasingly linked to a growing list of human diseases (6, 8–10).

43 Mitochondrial diseases (MDs) are a group of clinically heterogeneous disorders caused
44 by inherited mutations in genes that function in oxidative phosphorylation (OXPHOS) and
45 mitochondrial metabolism (11, 12). In addition to exhibiting metabolic and energetic deficits,
46 patients with MDs are more susceptible to opportunistic pathogens and also suffer elevated
47 complications arising from these infections (13–15). Although B and T cell immunodeficiencies
48 can contribute to recurrent infections in MDs (16), comparatively little is known about innate
49 immune dysregulation in patients and/or murine models. Hyperactivation of the innate immune
50 system is a key feature of sepsis, systemic inflammatory response syndrome (SIRS), and acute
51 respiratory distress syndrome, all of which occur more frequently and lead to significant mortality

52 in patients with MD (14, 15). Given the established and emerging links between mitochondria and
53 innate immunity, persistent mitochondrial dysfunction in MD could basally activate or rewire the
54 innate immune system. This could occur as loss of mitochondrial integrity and/or quality control
55 liberates mitochondrial damage-associated molecular patterns (DAMPs), such as mtDNA, which
56 engage the innate immune signaling and promote inflammatory responses that synergize with
57 metabolic impairments to drive pathology. Accordingly, elevated inflammatory cytokines have
58 been observed in patients with Alpers-Huttenlocher syndrome and mouse models of primary
59 mitochondrial disorders, suggestive of heightened innate immune activation (17, 18).

60 The mitochondrial polymerase gamma (POLG) enzyme possesses DNA polymerase and
61 3'→5' DNA exonuclease activities, and nearly 250 pathogenic mutations in *POLG* have been
62 linked to diseases including primary MDs, parkinsonism, and cancer (19–22). Mutations in *POLG*
63 represent the most prevalent single-gene cause of MD, and are implicated in a range of disorders
64 including Alpers-Huttenlocher syndrome, ataxia neuropathy spectrum, and progressive external
65 ophthalmoplegia (PEO), all of which are characterized by multiple organ pathology with varying
66 degrees of nervous, muscular, digestive, and endocrine system involvement. In recent years,
67 several mouse models of *POLG*-related disease have been reported, the most well studied of
68 which is the *POLG* mutator mouse (23, 24). These knock-in mice contain D257A substitutions in
69 the exonuclease domain, and animals homozygous for the mutant alleles exhibit disrupted
70 exonuclease function and elevated mtDNA instability. *POLG* mutator mice present pathology that
71 mirrors various aspects of human MDs, including cardiomyopathy, progressive anemia, and
72 sensorineural hearing loss. These animals also display premature aging between 6 and 9 months,
73 characterized by alopecia, osteoporosis, kyphosis, and decreased body weight, and consequently
74 die between 13-15 months of age (23–25). Mitochondrial dysfunction and inflammation are key
75 features of aging (26), yet whether the innate immune system contributes to *POLG*-related disease
76 phenotypes and premature aging of mutator mice is unknown.

77 Here, we report that mutator mice exhibit a hyper-inflammatory innate immune status that
78 is driven by chronic engagement of the cGAS-STING-IFN-I axis. Persistent IFN-I signaling
79 represses Nrf2 activity, which increases oxidative stress and aerobic glycolysis that potentiate
80 inflammation and age-related pathology in these animals. Our findings indicate that IFN-I
81 signaling is a key driver of innate immune rewiring and multi-organ pathology in mutator mice
82 and provide a strong rationale for more broadly examining IFN-I dysregulation in mitochondrial
83 diseases and aging.

84

85 **Results**

86 **Innate immune hyper-responsiveness of POLG mutator mice is regulated by monocyte and** 87 **neutrophil expansion**

88 To begin to characterize innate immune alterations in the POLG mutator model of mtDNA
89 disease, we employed a lipopolysaccharide (LPS)-induced endotoxemia model and monitored
90 circulating cytokines and survival post challenge. Interestingly, both 6- and 12-month old mutator
91 cohorts succumbed faster to intraperitoneal (i.p.) LPS challenge (Figure 1A and S1C). In-line
92 with a more rapid mortality rate, we detected elevated levels of pro-inflammatory cytokines,
93 chemokines, and type I interferons in the plasma of LPS-challenged mutator mice at all ages
94 compared to WT littermates (Figure 1B, S1A and S1B), consistent with a prior report (27).

95 As circulating leukocyte populations are key mediators of the cytokine storm that
96 contributes to endotoxin shock, we next examined the blood cell composition of 12-month old
97 cohorts. Although aged mutator mice exhibited B and T cell lymphopenia (Figure S1D and S1E)
98 (28, 29), we noted a significant expansion of innate immune cell types, namely monocyte
99 (CD11b⁺, Ly6C^{hi}) and neutrophil (CD11b⁺, Ly6G⁺) populations, by flow cytometry (Figure 1C-
100 1F). We also observed significant increases in steady-state tumor necrosis factor alpha (TNF α)
101 and monocyte chemoattractant protein 1 (MCP-1/CCL2) in the plasma of aged mutators (Figure

102 S1F). To next identify the sources of TNF α at baseline and after challenge, we employed
103 intracellular staining and multiparameter flow cytometry. B and T cell populations in mutator
104 mice did not express higher levels of intracellular TNF α before or after ex vivo LPS stimulation
105 (Figure S1G). However, CD11b⁺ cells expressed significantly more TNF α and IL-6 after
106 challenge (Figure 1G, 1J and S1H). Quantitation across multiple experiments revealed that
107 CD11b⁺Ly6C^{hi} inflammatory monocytes in mutator blood possessed the greatest intracellular
108 TNF α intensity, both at rest and after LPS stimulation (Figure 1G-1I). Although CD11b⁺Ly6G⁺
109 neutrophils were also more numerous in the mutator blood, their TNF α intensity was roughly
110 100-fold lower (Figure 1J-1L), indicating that inflammatory monocyte expansion is most likely
111 responsible for the elevated levels plasma cytokines at rest and after Toll-like receptor 4 (TLR4)
112 engagement. Similar expansion and elevated TNF α positivity was observed in monocyte and
113 neutrophil lineage cells in the bone marrow of mutator mice (Figure S1I and S1J). Finally, oral
114 infection of 9-10-month old cohorts with *Listeria monocytogenes* resulted in notably higher pro-
115 inflammatory cytokine levels in mutator plasma at day 3 (Figure S1K), but less bacterial
116 dissemination to the spleen and liver at day 5 post infection (Figure S1L). These results are
117 consistent with prior findings that Ly6C^{hi} inflammatory monocytes predominantly control oral
118 *Listeria* infection (30), and taken together, suggest that CD11b⁺ myeloid cell expansion and
119 innate immune reprogramming drive systemic hyper-inflammatory responses in mutator mice.

120 **STING regulates enhanced IFN-I and pro-inflammatory responses in POLG mutator** 121 **macrophages**

122 To define the underlying signaling pathways that shape innate immune hyper-responsiveness in
123 mutator mice, we performed RNA sequencing analysis of primary bone marrow-derived
124 macrophages (BMDMs) and peritoneal macrophages (PerMacs) at rest and after LPS challenge.
125 Pathway analysis of RNAseq datasets revealed significant elevations in interferon/IRF,
126 JAK/STAT, and NF- κ B signaling, as well as increased glycolytic metabolism and reactive

127 oxygen and nitrogen species production in mutator macrophages (Figure S2A). Gene expression
128 profiling of mutator macrophages revealed an enrichment of interferon-stimulated genes (ISG)
129 (Figure 2A and 2B), which was confirmed by qRT-PCR analyses (Figure 2C, 2D, S2B and S2C).
130 Mutator macrophages also displayed augmented pro-inflammatory cytokine RNA (Figure 2E and
131 2F) and protein levels (Figure 2G and 2H), agreeing with the intracellular cytometry data of
132 Figure 1. Moreover, mutator macrophages produced higher levels of nitrite after LPS and IFN γ
133 co-stimulation, confirming pathway analysis of RNAseq data (Figure 2I). Finally, interferon
134 stimulatory DNA (ISD) transfection to directly engage cGAS-STING revealed elevated ISG and
135 pro-inflammatory cytokine expression in mutator BMDMs (Figure 2J), demonstrating that POLG
136 mutator macrophages are broadly hyper-responsive to multiple innate immune stimuli.

137 STING is a key mediator of IFN-I and pro-inflammatory responses induced by cytosolic
138 and extracellular mtDNA (6, 7), and ablation of STING can limit mtDNA-driven inflammation in
139 mouse models of acute kidney injury and Parkinson's disease (31–33). We observed that aged
140 POLG mutator mice have significantly more circulating, cell-free mtDNA in the plasma, and we
141 also noted that LPS-challenged mutator BMDMs liberate more mtDNA into the culture media
142 (Figure S2D and S2E). We therefore reasoned that mtDNA instability and release in POLG
143 mutator macrophages might drive constitutive IFN-I and elevated pro-inflammatory responses
144 after LPS via the cGAS-STING pathway. Notably, we observed that macrophages from POLG
145 mutators crossed onto a STING-deficient background had lower ISG expression (Figure S2F and
146 S2G), as well as lower TNF α secretion (Figure S2H), when compared to STING-sufficient
147 mutators. Taken together, our data suggest that mtDNA instability and release in POLG mutators
148 engages STING, which potentiates macrophage activation to subsequent innate immune
149 challenge.

150 **The cGAS-STING-IFN-I signaling axis regulates monocyte and neutrophil expansion and**
151 **hyper-inflammatory responses in mutator mice**

152 The early, inflammatory phase of septic shock is characterized by leukocyte activation and
153 secretion of pro-inflammatory cytokines such as TNF α and IL-1 β (34). IFN-I can exacerbate
154 inflammation in response to TNF α (35) and contributes to mortality in LPS-induced sepsis (36).
155 Because we noted increased IFN-I secretion after LPS challenge (Figure 1B) and more mtDNA
156 was present in the plasma of aged POLG mutators (Figure S2D), we explored whether cGAS-
157 STING signaling contributes to increased mortality after i.p. LPS challenge. Interestingly, we
158 observed delayed LPS-induced mortality (Figure 3A) and markedly lower plasma cytokine levels
159 in mutator mice lacking cGAS or STING (Figure 3B). In line with a role for IFN-I signaling in
160 driving hyper-inflammatory responses to LPS, aged mutator mice lacking type I interferon
161 receptor subunit 1 (*Ifnar*^{-/-}) also had lower circulating levels of many cytokines compared to
162 mutator mice alone (Figure 3B, rightmost columns).

163 IFN-I signaling is known to drive peripheral myeloid expansion in murine models of lupus
164 and can modulate Ly6C^{hi} inflammatory monocyte recruitment in both infectious and sterile
165 diseases (37–41). To next assess whether cGAS-STING-IFN-I activation governs increased
166 peripheral myeloid expansion in mutator mice, we examined CD11b⁺Ly6C^{hi} monocyte and
167 CD11b⁺Ly6G⁺ neutrophil populations in double mutant cohorts. Notably, CD11b⁺Ly6C^{hi}
168 inflammatory monocyte numbers were progressively lower in cGAS-, STING-, and IFNAR-
169 deficient mutators (Figure 3C and 3D), while blood neutrophil abundance was also decreased
170 (Figure S3A and S3B). Moreover, both CD11b⁺Ly6C^{hi} monocytes (Figure 3E and 3F) and
171 CD11b⁺Ly6G⁺ neutrophils (Figure S3C and S3D) from IFNAR-deficient mutators produced less
172 TNF α after ex vivo LPS challenge. Additionally, TNF α ⁺IL-1 β ⁺ double positive leukocytes were
173 decreased in IFNAR-deficient mutator blood after LPS challenge (Figure S3E), and the elevated
174 percentage of CD11b⁺Ly6C^{hi}TNF α ⁺ cells in LPS-stimulated mutator bone marrow was ablated by
175 IFNAR knockout (Figure S3F). Collectively, these results suggest that sustained cGAS-STING-
176 IFN-I signaling in POLG mutator mice promotes peripheral myeloid expansion and innate

177 immune rewiring that potentiates systemic inflammatory responses and increases mortality to LPS
178 challenge.

179 **Antioxidant and anti-inflammatory Nrf2 signaling is suppressed in POLG mutator**
180 **macrophages**

181 To next define the mechanisms underlying elevated ISG and pro-inflammatory cytokine
182 expression in mutator monocytes and macrophages, we assayed key steps in NF- κ B, IRF, and
183 STAT1 activation during a 24-hour LPS time course. Although pathway analysis suggested
184 potentiated signaling in mutator macrophages (Figure S2A), the kinetics of NF- κ B, IRF, and
185 STAT1 activation were nearly identical between WT and mutator BMDMs following LPS
186 treatment (Figure S4A). However, in agreement with RNAseq data implicating elevated
187 glycolytic metabolism, we observed a marked increase in the extracellular acidification rate
188 (ECAR) and decreased basal and maximal oxygen consumption rates (OCR) in mutator BMDMs
189 (Figure S4B and S4C). This suggested metabolic reprogramming away from mitochondrial
190 respiration to glycolysis for enhanced inflammatory M1 macrophage activation. A recent study
191 reported that the nuclear factor erythroid 2-related factor 2 (Nrf2) transcription factor interferes
192 with LPS-induced cytokine gene expression and is therefore a major transcriptional repressor of
193 M1 macrophage polarization and inflammation (42). Our RNA profiling indicated that a
194 significant number of Nrf2-target genes were transcriptionally repressed in mutator macrophages
195 (Figure S2A and 4A). In agreement, analysis of endogenous Nrf2 by quantitative fluorescent
196 microscopy revealed markedly lower nuclear translocation in LPS-stimulated POLG mutator
197 macrophages (Figure 4B-4D).

198 To next explore whether Nrf2 suppression contributes to hyper-inflammatory phenotypes
199 in mutator macrophages, we employed two pharmacological approaches to boost Nrf2 activity.
200 Itaconate functions as an anti-inflammatory metabolite by alkylating Kelch-like ECH-associated
201 protein 1 (KEAP1) (43), which normally associates with and promotes the degradation of Nrf2.

202 Itaconate-mediated alkylation of key KEAP1 cysteine residues allows newly synthesized Nrf2 to
203 accumulate, translocate to the nucleus, and activate a transcriptional antioxidant and anti-
204 inflammatory program (43, 44). Supplementing POLG mutator PerMacs with 4-octyl-itaconate
205 (4OI) (a cell-permeable itaconate derivative) restored the expression of Nrf2 target genes (Figure
206 S4D). In addition, 4OI markedly reduced the elevated secretion of pro-inflammatory cytokines in
207 LPS-challenged mutator macrophages (Figure S4E). Treatment of macrophages with a second
208 Nrf2 stabilizer, dimethyl fumarate (DMF), also restored Nrf2 target gene expression (Figure 4E
209 and 4G), suppressed inflammatory cytokine production (Figure 4F), and reduced ISGs in mutator
210 macrophages (Figure 4G). Interestingly, we noted that KEAP1 protein was markedly elevated in
211 mutator PerMacs after LPS, while being nearly undetectable in WT cells (Figure 4G). DMF also
212 limits aerobic glycolysis and lowers cytokine secretion from activated macrophages by
213 inactivating GAPDH (45). Accordingly, DMF reduced the elevated GAPDH protein expression in
214 PerMacs from mutator mice (Figure 4G), while also reducing aerobic glycolysis in both WT and
215 mutator macrophages (Figure 4H and 4I). Together, these data indicate that Nrf2 suppression and
216 augmented aerobic glycolysis shift POLG mutator macrophages toward a more pro-inflammatory
217 state.

218 **IFN-I signaling represses Nrf2 activity and drives pro-inflammatory metabolic phenotypes** 219 **in POLG mutator macrophages**

220 Nrf2 has been recently reported to inhibit STING-dependent IFN-I responses (46–48), and IFN-I
221 signaling can limit microbial clearance by dysregulating Nrf2 target gene expression (49, 50). We
222 therefore utilized IFNAR-deficient cohorts to examine whether hyperactive IFN-I signaling
223 contributes to Nrf2 suppression in mutator macrophages. We first confirmed that IFNAR
224 depletion ablated elevations in LPS-induced ISG expression in mutator macrophages (Figure 5A).
225 Next, we utilized quantitative fluorescent microscopy and observed that *Ifnar*^{-/-} mutator
226 macrophages displayed marked restoration of Nrf2 nuclear translocation after LPS challenge

227 (Figure 5B-5D and S5A-5C). RNA and protein profiling of Nrf2 targets further confirmed that
228 ablation of IFNAR was sufficient to restore, or even hyperactivate, Nrf2 signaling in *Ifnar*^{-/-}
229 mutator macrophages (Figure 5E, 5F and S5D). In addition, the secretion of TNF α from *Ifnar*^{-/-}
230 mutator PerMacs was lowered (Figure S5E), further confirming our intracellular cytokine staining
231 results (Figure 3 and S3).

232 In line with a role for Nrf2 antioxidant programs in regulating both mitochondrial and
233 cytosolic ROS (51), we detected significantly higher levels of mitochondrial superoxide and total
234 cellular ROS in mutator BMDMs after LPS induction (Figure 5G, 5H, S5F and S5G). IFNAR
235 depletion lowered ROS in POLG WT macrophages and dramatically reduced ROS in POLG
236 mutator BMDMs. As we previously observed that POLG mutator macrophages exhibit elevated
237 KEAP1 protein after LPS stimulation (Figure 4G), we next explored whether IFN-I directly
238 affects KEAP1 abundance. Interestingly, we observed that treatment of BMDMs with
239 recombinant mIFN β augmented KEAP1 levels, leading to a concomitant reduction in Nrf2
240 abundance (Figure S5H). Overall, these results highlight that IFN-I-mediated repression of Nrf2,
241 via increased KEAP1 expression, lowers antioxidant capacity and elevates pro-inflammatory ROS
242 in mutator macrophages.

243 IFN-I signaling can shift innate immune cell metabolism from OXPHOS to aerobic
244 glycolysis due to induced breaks in the TCA cycle (52, 53). Accordingly, we found that both
245 acute and chronic IFN β treatment slightly elevated ECAR in WT BMDMs, but dramatically
246 increased ECAR in mutator macrophages (Figure S5I). In contrast, knockout of IFNAR markedly
247 reduced ECAR in resting and LPS-stimulated in mutator macrophages, while having little to no
248 effect on WT macrophages (Figure 5I). Consistent with the changes in ECAR, mutator
249 macrophages had higher LDH expression level and activity, and thus produced more L-lactate
250 than WT cells. Ablation of IFN-I signaling in POLG BMDMs largely reversed elevated LDH
251 expression (Figure 5F) and activity (Figure 5J) after LPS challenge, while also blunting L-lactate

252 levels (Figure 5K). Taken together, our results suggest that blocking IFN-I signaling restores
253 Nrf2, lowers oxidative stress and aerobic glycolysis, and blunts the hyper-inflammatory profile in
254 POLG mutator macrophages.

255 **Blocking IFN-I signaling restores Nrf2 activity, limits oxidative stress, and lowers aerobic**
256 **glycolysis in aged POLG mutator mice**

257 Several studies have linked oxidative stress to tissue dysfunction and premature aging phenotypes
258 in POLG mutator mice (27, 54–57). Therefore, we next explored dysregulation in IFN-I-Nrf2
259 crosstalk in the heart, liver, and kidney of 12-month old animals. Transcript and protein profiling
260 revealed a significant increase in ISGs, similar to that observed in POLG macrophages, which
261 was dependent on intact IFNAR signaling (Figure S6A and S6B). In addition, the expression of
262 Nrf2 and Nrf2-regulated antioxidant genes (*Nqo1*, *Gclc*, *Gclm*, *Sqstm1/p62*) was markedly lower
263 in mutator tissues compared to age-matched WT littermates, but was rescued in *Ifnar*^{-/-} mutator
264 tissues (Figure S6C and S6D). Aconitase is widely recognized as a sensitive and specific target of
265 ROS, and aconitase inactivation is a surrogate marker of oxidative stress. Therefore, to determine
266 whether IFN-I-mediated Nrf2 suppression increases oxidative stress in mutator mice, we analyzed
267 the enzymatic activity of aconitase in various tissues of WT, mutator, *Ifnar*^{-/-}, and *Ifnar*^{-/-} mutator
268 littermate cohorts. Heart, liver, and kidney extracts from mutator mice showed significantly lower
269 aconitase activity, indicative of oxidative stress; however, IFNAR deficiency largely restored
270 aconitase activity in mutator tissues, likely via boosting Nrf2-regulated antioxidant activity
271 (Figure S6E).

272 Our prior observations in macrophages and other reports indicated imbalances in
273 glycolytic metabolism and oxidative phosphorylation in mutator tissues (58), thus we next
274 examined expression of enzymes in glycolysis, the TCA cycle, and OXPHOS (Figure S6F).
275 GAPDH and LDHA were significantly elevated in aged POLG mutator tissues (Figure S6F, top
276 panel); however, *Ifnar*^{-/-} mutator cohorts exhibited decreased glycolytic enzyme expression and

277 decreased L-lactate accumulation in the plasma (Figure S6F and S6G). In addition, several TCA
278 cycle enzymes were upregulated in mutator heart and liver homogenates, which may reflect a
279 compensatory response to elevated aerobic glycolysis and OXPHOS deficiency. Consistent with a
280 role for IFN-I-mediated metabolic rewiring, TCA enzymes were largely restored to WT levels in
281 *Ifnar*^{-/-} mutator tissues (Figure S6F, middle panel). Finally, *Ifnar*^{-/-} mutator cohorts exhibited
282 modestly higher levels of some OXPHOS subunits, namely mt-CO1 and NDUFB8 in the liver,
283 compared to IFNAR-sufficient mutators (Figure S6F, bottom panel). Collectively, our tissue
284 analyses mirror the key findings from macrophage studies, and thus define chronic IFN-I
285 responses as critical regulators of Nrf2 suppression and metabolic rewiring in POLG mutator
286 mice.

287 **Ablation of IFN-I signaling improves healthspan and extends lifespan of POLG mutator** 288 **mice**

289 Age-related multi-tissue pathology, including alopecia, kyphosis, anemia, and dilated
290 cardiomyopathy, has been noted in numerous reports on POLG mutator mice (23, 24, 28, 55, 59).
291 As both elevated IFN-I signaling and Nrf2 inhibition are implicated in cardiomyopathy and
292 anemia in animal models and human patients (60–63), we reasoned that sustained imbalances in
293 IFN-I and Nrf2 signaling may contribute to the aging-related phenotypes of mutator mice. Using
294 transthoracic echocardiography, we confirmed that 9-10-month old mutator mice exhibited dilated
295 cardiomyopathy by demonstrating left ventricle dilation (wider left ventricular inner diameter at
296 diastole LVID;d) and markedly reduced systolic function (decreased left ventricular ejection
297 fraction, LVEF) (Figure 6A and 6B). Other dilated cardiomyopathy phenotypes included
298 decreased interventricular septum thickness at end-systole (IVS;s), increased left ventricular
299 internal dimension at end-systole (LVID;s) and elevated left ventricular volume at end-systole
300 (Vol;s) (Figure S7A and S7B). Remarkably, cardiac dilation and LVEF were improved in
301 IFNAR-deficient mutator cohorts (Figure 6A, 6B, S7A and S7B). In addition, histological

302 analyses revealed increased mean myofiber width and more infiltrating immune cells in mutator
303 hearts, which were both significantly reduced in IFNAR-deficient mutator sections (Figure 6C
304 and 6D). Finally, we noted a shift in the cardiac myosin heavy chain isoform from *Myh6* toward
305 *Myh7* in mutator heart homogenates, which is a well-appreciated marker of cardiac hypertrophy,
306 injury, and stress. Consistent with our echocardiographic and histological measurements, IFNAR-
307 deficient mutator hearts exhibited increased *Myh6* and reduced *Myh7* expression, indicative of
308 lower cardiomyopathy (Figure S7C).

309 POLG mice develop progressive, ultimately fatal megaloblastic anemia due to
310 hematopoietic stem cell deficits, impaired erythrocyte maturation, and increased erythrocyte
311 destruction by splenic macrophages (28, 64, 65). Remarkably, we found that *Ifnar^{-/-}* mutator
312 blood contains increased cellularity (Figure S7D), as well as higher red blood cell (RBC) counts,
313 hemoglobin (HBG) concentration, and hematocrit (HCT) levels compared to IFNAR-sufficient
314 mutators (Figure 6E). Moreover, ablation of IFNAR-signaling significantly restored splenic
315 architecture in POLG mutator mice and increased white pulp to red pulp abundance (Figure 6F).
316 We also observed widespread extramedullary hematopoiesis in the livers of mutator mice, which
317 was largely ablated in age-matched *Ifnar^{-/-}* mutator cohorts (Figure 6G), indicating that persistent
318 IFNAR signaling in POLG proofreading-deficient mice contributes to the progressive and lethal
319 anemia observed. Finally, we noted that loss of cGAS-STING-IFN-I signaling generally
320 improved body condition in 12-month old mutator mice, as indicated by less kyphosis of the spine
321 and reduced hair loss (Figure S7E). Remarkably, *Ifnar^{-/-}* mutator cohorts exhibited a 10%
322 extension in mean lifespan over IFNAR-sufficient mutator mice (Figure 6H and 6I). Altogether,
323 our data suggest that innate immune reprogramming and chronic IFN-I signaling contribute to
324 age-related anemia and cardiomyopathy, negatively impacting both healthspan and lifespan of
325 POLG mutator mice (Figure S8).

326

327 **Discussion**

328 Recent clinical case reports suggest that patients with MDs experience recurrent infections and
329 develop sepsis and/or SIRS at significantly elevated rates relative to the general population (14,
330 15, 66). In fact, one study reported that sepsis is the most frequent cause of early death in
331 pediatric MD patients (13). The innate immune system is the first line of defense against invading
332 microbes and is an important driver of hyper-inflammatory responses in sepsis and SIRS.
333 However, there is a paucity of information about innate immune system function and/or cellular
334 composition in patients with MDs or murine models. Our work begins to address this critical
335 knowledge gap in a mouse model of POLG-related MD. We have uncovered that POLG mutator
336 mice are highly susceptible to lethal endotoxin shock owing to a dramatic increase in circulating,
337 myeloid-derived innate immune cell populations and elevated levels of pro-inflammatory
338 cytokines and IFN-I in the plasma after i.p. LPS challenge. Our data suggest that CD11b⁺ myeloid
339 cell expansion, especially the CD11b⁺Ly6C^{hi} inflammatory monocyte population, is a key driver
340 of augmented cytokine secretion in mutator mice, as intracellular TNF α levels in CD11b⁺Ly6C^{hi}
341 mutator monocytes are elevated 4-fold compared to WTs. Although mutator mice do not directly
342 model any particular human MD, CD11b⁺ myeloid cell expansion in the bone marrow and spleen
343 has also been observed in the *Ndufs4*^{-/-} model of Leigh Syndrome (67, 68). Therefore, future
344 studies in other mouse models and patients are warranted to determine the degree to which
345 monocyte and neutrophil expansion/polarization occurs in MDs. Monitoring circulating MCP-
346 1/CCL2, which is basally elevated in mutator mice and is a predictive biomarker for sepsis, may
347 also aid in identifying MD patients at risk for developing life-threatening systemic inflammation
348 after infection (69, 70).

349 Mounting evidence suggests that the IFN-I-driven expansion of inflammatory monocytes
350 is deleterious in autoimmune and inflammatory disorders such as lupus and pneumonia (37, 38,
351 40, 71). Elevated IFN-I signaling during chronic pattern recognition receptor (PRR) stimulation

352 drives hematopoietic stem cell (HSC) skewing toward granulocyte/macrophage progenitors
353 (GMP), leading to emergency myelopoiesis in the bone marrow and peripheral myeloid expansion
354 (37, 72, 73). Myeloid lineage skewing in the bone marrow of mutator mice has been previously
355 reported, although the mechanisms underlying this phenotype have remained unclear (74).
356 Remarkably, the expansion of Ly6C^{hi} inflammatory monocytes and Ly6G⁺ neutrophils, systemic
357 cytokine levels, and time to death after lethal LPS challenge is significantly reduced in mutator
358 mice lacking cGAS, STING, or IFNAR. We therefore propose that mtDNA instability and release
359 in POLG mutators triggers cGAS-STING-dependent IFN-I priming, which not only drives HSC
360 skewing and peripheral myeloid expansion, but also potentiates responsiveness of circulating
361 monocytes and neutrophils to innate immune stimuli. In addition to well-characterized mtDNA
362 instability, a recent study documented dNTP pool imbalance and nuclear genome instability in
363 mutator mice (75). Future work should therefore clarify the cellular sources of mtDNA and
364 mechanisms of release, while also examining the contribution of nuclear DNA damage to
365 elevated innate immune and IFN-I signaling in POLG mice.

366 We have found that mutator macrophages are broadly hyper-responsive to pattern
367 recognition receptor agonists, although unexpectedly, the signaling kinetics of NF- κ B, IRF, and
368 STAT1 are nearly identical between WT and mutator BMDMs following LPS treatment. Instead,
369 our data indicate that the hyper-inflammatory phenotype of mutator macrophages results from
370 enhanced M1 polarization due to IFN-I dependent Nrf2 suppression and metabolic rewiring. Nrf2
371 can directly inhibit LPS-induced cytokine gene expression, and loss of Nrf2 activity is linked to
372 mitochondrial dysfunction and increased glycolysis (42, 76). Compared to cells from age-matched
373 mutator cohorts, IFNAR-deficient mutator macrophages display higher Nrf2 levels and target
374 gene expression, while also generating less ROS and pro-inflammatory cytokines after LPS
375 challenge. This dramatic restoration in Nrf2 activity in *Ifnar*^{-/-} mutators is likely due to a lessening
376 of IFN-I- and IL-10-mediated TCA cycle breaks (i.e. reduced aconitase and isocitrate

377 dehydrogenase activity), which lower carbon flux toward itaconate (53). However, consistent
378 with findings in fibroblasts and induced pluripotent stem cells (56, 58), POLG mutator BMDMs
379 also exhibit elevated ECAR and decreased OCR at rest. Thus, the restoration of Nrf2 activity in
380 *Ifnar*^{-/-} mutator macrophages may also be due to reduced aerobic glycolysis, which directs
381 pyruvate away from TCA metabolism toward lactate generation. Consistent with this notion, loss
382 of IFN-I signaling markedly blunts ECAR and lactate levels in *Ifnar*^{-/-} mutator macrophages, and
383 also lowers plasma lactate concentrations in aged mice. In sum, our results support a model
384 whereby mtDNA mutagenesis in POLG mutator macrophages potentiates IFN-I signaling, which
385 enhances Keap1 to destabilize Nrf2, while also elevating LDHA levels and activity to potentiate
386 aerobic glycolysis.

387 Our research has also uncovered that elevated IFN-I responses and reduced Nrf2 activity
388 extend to multiple organs of aged mutator mice. Genetic ablation of IFNAR is sufficient to blunt
389 potentiated ISG expression and markedly increase Nrf2 target gene abundance in the heart, liver,
390 and kidney of aged cohorts. ROS and oxidative stress have been linked to organ dysfunction in
391 aged POLG mice, with antioxidant therapy providing some benefits to overall healthspan (27, 54,
392 57, 64). We found that oxidative stress is significantly lower in *Ifnar*^{-/-} mutator organs compared
393 to IFNAR-sufficient cohorts, suggesting that IFN-I signaling triggered by mtDNA mutagenesis
394 and instability inhibits Nrf2-mediated antioxidant responses in vivo. Interestingly, Nrf2-null mice
395 display a spectrum of pathology that overlaps with POLG mutator mice, namely anemia,
396 splenomegaly, cardiomyopathy, and increased susceptibility to lethal septic shock (63, 77, 78).
397 Moreover, reduced Nrf2 activity is linked to numerous aging-related diseases (79–81), and Nrf2
398 repression was recently uncovered as a novel driver of oxidative stress and premature aging in
399 Hutchinson-Gilford progeria syndrome (HGPS) (82). Elevated IFN-I responses have also been
400 observed in HGPS and other progeroid syndromes, which share some overlapping phenotypes
401 with mtDNA mutator mice (83–85). Strikingly, we found that *Ifnar*^{-/-} mutator cohorts exhibit an

402 extension in mean survival time of roughly forty days and an overall improvement in body
403 condition compared to IFNAR-sufficient mutators, documenting that perturbed IFN-I-Nrf2
404 crosstalk is an unappreciated molecular mechanism contributing to premature aging in mutator
405 mice.

406 Cardiac and hematologic analyses indicate that blockade of IFNAR signaling during aging
407 yields striking improvements in cardiomyopathy and anemia-related phenotypes in mutator mice.
408 Mitochondrial ROS contributes to cardiomyopathy in mutator mice (55), and our genetic and
409 echocardiographic data suggest that shifting the balance from IFN-I toward Nrf2 activity lowers
410 cardiac oxidative stress and improves cardiac function in aged mutator animals. However,
411 additional IFN-I-dependent inflammatory and metabolic processes are likely dysregulated in aged
412 mutator hearts. Future research on the mutator strains described here should yield new insight into
413 roles for IFN-I dysregulation in both aging- and MD-related cardiomyopathy. Elevated IFN-I
414 signaling has also been linked to anemia in aging and in various human diseases and animal
415 models (61, 73, 86). A recent report characterized a unique subset of splenic hemophagocytes that
416 differentiate from IFN-I expanded Ly6C^{hi} monocytes, which are responsible for anemia in a
417 model of macrophage activation syndrome and lupus (37, 73). As we observed the IFN-I-
418 dependent elevation of Ly6C^{hi} monocytes in mutator mice, it is likely that the restoration of
419 peripheral erythrocyte numbers in *Ifnar*^{-/-} mutator blood is results from less GMP skewing in the
420 bone marrow and reduced destruction of erythrocytes by inflammatory hemophagocytes. Anemia
421 is the most frequent hematological abnormality observed in patients with MD, and the presence of
422 anemia negatively influences survival in patients with POLG-related disease (87, 88). Our work
423 provides a strong rationale for translational research to explore whether IFN-I-Nrf2 signaling
424 imbalances potentiate anemia in elderly/frail populations and patients with POLG-related MD.

425 In conclusion, we report that mtDNA mutator mice exhibit a hyper-inflammatory innate
426 immune status that is driven by chronic IFN-I priming and Nrf2 repression. Our work constitutes

427 the first detailed characterization of innate immune rewiring in a model of POLG-related disease
428 and may provide a mechanistic framework for understanding why some MD patients are more
429 prone to developing sepsis and SIRS following infection. Moreover, we have made the novel
430 observation that innate immune dysregulation and IFN-I-mediated inflammaging contribute to
431 several progeroid phenotypes in mutator mice. Therapeutic approaches aimed at rebalancing IFN-
432 I-Nrf2 signaling may therefore represent a promising target for limiting runaway inflammation,
433 combatting anemia, and improving overall healthspan in progeroid syndromes, mitochondrial
434 diseases, and aging.

435

436 **Materials and Methods**

437 **Mouse strains**

438 C57BL/6J, *POLG*^{D257A/D257A} mutator, *cGAS*^{-/-}, *Sting*^{-/-} (*Tmem173^{gt}*) and *Ifnar1*^{-/-} mice were
439 purchased from The Jackson Laboratory and bred and maintained in multiple vivaria at Texas
440 A&M University. *POLG*^{D257A/+} breeder pairs used to generate *POLG*^{+/+} and *POLG*^{D257A/D257A}
441 experimental mice (and *cGAS*^{-/-}, *Sting*^{-/-} and *Ifnar*^{-/-} null intercrosses) were obtained from male
442 *POLG*^{D257A/+} to female C57BL/6J (or *cGAS*^{-/-}, *Sting*^{-/-} and *Ifnar*^{-/-} null on a pure C57BL/6J
443 background) crosses. All animal experiments were approved by the Institutional Animal Care and
444 Use Committee (IACUC) at Texas A&M University.

445 **Antibodies and reagents**

446 Anti-IFIT3 was a gift from G. Sen at Cleveland Clinic and anti-VIPERIN was a gift from P.
447 Cresswell at Yale School of Medicine. Commercially obtained antibodies and reagents include:
448 antibodies for immunoblotting: rabbit anti-TBK1 (3504), -p-TBK1 (5483), -STAT1 (9172), -p-
449 STAT1 (7649), -RIG-I (4200), -p-IκBα (2859), -IκBξ (93726), -IRF1 (8478), -IRG1 (17805), -
450 NRF2 (12721), -KEAP1 (8047) (Cell Signaling Technology); rabbit anti-LDHA (19987-1-AP), -
451 p62/SQSTM1 (18420-1-AP), -IκBα (10268-1-AP), mouse anti-GAPDH (600004-1), -β-Actin

452 (66009-1) (Proteintech); mouse anti-OXPPOS (ab110413), rabbit anti-GCLC (ab207777), -PDH
453 (ab110333) (abcam); mouse anti-ACO2 (MA1-029), rabbit anti-GCLM (MA5-32783)
454 (Invitrogen); goat anti-HSP60 (N-20) (Santa Cruz Biotechnology); rabbit-OGDH (HPA020347)
455 (Sigma); antibodies and reagents used for flow cytometry included: PE/Cy7 anti-mouse TNF- α
456 Antibody (506324), PerCP/Cy5.5 anti-mouse Ly-6C Antibody (128012), FITC anti-mouse Ly-6C
457 Antibody (128006), PerCP/Cy5.5 anti-mouse IL-12/IL-23 p40 (505211) (BioLegend); Purified
458 Anti-Mouse CD16 / CD32 (2.4G2) (70-0161), violetFluor™ 450 Anti-Mouse Ly-6G (1A8) (75-
459 1276), APC Anti-Human/Mouse CD11b (M1/70) (20-0112) (Tonbo); IL6 (11-7061-81)
460 (Invitrogen); IL1 β (31202) (Cell Signaling Technology); Brefeldin A Solution (420601),
461 Monensin Solution (420701) (BioLegend); mouse TNF α (430904) and IL-6 (431304) enzyme-
462 linked immunosorbent assay (ELISA) kits were purchased from BioLegend; Griess Reagent
463 System (G2930) was purchased from Promega.

464 **Cell culture**

465 L929 cells were obtained from ATCC and maintained in DMEM (D5796) (Sigma) supplemented
466 with 10% FBS (97068-085) (VWR). BMDMs were generated from bone marrow and cultured on
467 Petri plates in DMEM containing 10% FBS plus 30% L929 culture media for 7 days. PerMacs
468 were collected from peritoneal gavage 4 days after intraperitoneal injection of 3% brewer
469 thioglycolate medium (B2551) (Sigma). Transfection of interferon-stimulatory DNA (ISD)
470 (InvivoGen) into the cytosol of BMDMs was performed using Polyethyleneimine (PEI) (43896)
471 (Alfa Aesar). Unless stated, 6×10^5 BMDMs and 1.2×10^6 PerMacs per milliliter were used in in
472 vitro experiments. The LPS-B5 Ultrapure (InvivoGen) concentration used was 200ng/ml for
473 BMDMs and 20 ng/ml for PerMacs unless otherwise indicated. The 4-octyl Itaconate (Cayman)
474 concentration was 125 μ M and the Dimethyl fumarate (Sigma) concentration was 50 μ M, and in
475 experiments cells were exposed to both treatments 6 hrs before LPS stimulation.

476 **LPS in vivo challenge and multi-analyte cytokine analysis**

477 Mice were intraperitoneally (i.p.) injected with LPS from *Escherichia coli* O55:B5 (L4005;
478 Sigma). Blood was collected using EDTA-coated tubes at indicated time, then centrifuged at
479 1,000xg for 15 min at 4C°. The upper phase of plasma was saved and subjected to LEGENDplex
480 Anti-Virus Response Panel (740446) (BioLegend) or LEGENDplex Mouse Inflammation Panel
481 (740446) (BioLegend) for cytokine analysis.

482 **Blood and bone marrow staining for flow cytometry**

483 Whole mouse blood was collected in sodium heparin tubes, and bone marrow was harvested from
484 femurs and tibia. RBCs were lysed twice with ACK lysis buffer, and leukocytes were subjected to
485 1µg/ml LPS stimulation in the presence of brefeldin A and monensin for 4 hrs. Fc receptors were
486 blocked with anti-Mouse CD16 / CD32 (2.4G2) antibody, cells stained with antibodies against
487 surface proteins, permeabilized with Foxp3/Transcription Factor Staining Buffer Kit (TNB-0607-
488 KIT) (Tonbo), and stained with antibodies against intracellular proteins. Cells were analyzed with
489 a BD Fortessa X-20.

490 ***Listeria monocytogenes* infections**

491 Age- and sex matched mice of indicated genotypes were used for infections, which were
492 performed under BSL2 containment according to protocols approved by the Texas A&M IACUC.
493 Two days prior to infection, cages' plain water bottles were replaced with bottles containing 5
494 mg/ml streptomycin in water. The night before infection, chow was removed from the mouse
495 cages. Mice were infected with log phase (O.D.600 = 0.5-1.0) *Listeria monocytogenes* (strain
496 10403S, gift from D. Portnoy) grown in BHI at 37°C. Bacteria were washed twice with warm,
497 sterile PBS, and for each mouse, an inoculum of 1x10⁸ bacteria was placed on a 3 mm piece of
498 bread with 3 ml of butter. Each mouse was individually fed one piece of *Listeria*-soaked bread
499 and butter and then returned to their cage with fresh, antibiotic-free water and chow. Colonization
500 was confirmed by assessing bacterial shedding in feces; at indicated time points, stools were
501 collected from each mouse, dissolved in PBS, and spot plated as serial dilutions on LB plates.

502 Mice were weighed prior to infection and regularly throughout to monitor their health status.
503 Bacterial burdens in spleens were determined by homogenizing spleens in 0.1% IGEPAL and
504 plating serial dilutions on LB plates. Plasma was collected using EDTA-coated tubes at indicated
505 time points for cytokine analyses.

506 **Quantitative PCR**

507 To measure relative gene expression by qRT-PCR in cells and tissues, total cellular RNA was
508 isolated using Quick-RNA microprep kit (Zymo Research). Approximately 0.5-1 ug RNA was
509 isolated and cDNA was generated using the qScript cDNA Synthesis Kit (Quanta). cDNA was
510 then subjected to qPCR using PerfeCTa SYBR Green FastMix (Quanta). Three technical
511 replicates were performed for each biological sample, and expression values of each replicate
512 were normalized against Gapdh or Rpl37 cDNA using the $2^{-\Delta\Delta CT}$ method. For mtDNA abundance
513 assessment, 2ng/ μ l of template DNA was used for qRT-PCR, and expression values of each
514 replicate were normalized against nuclear-encoded Actb. All primer sequences used for qRT-PCR
515 can be found in Extended Data Table 1.

516 **Immunoblotting**

517 Cells and tissues were lysed in 1% NP-40 lysis buffer or 1% SDS lysis buffer supplemented with
518 protease inhibitor and then centrifuged at 4°C to obtain cellular lysate. After BCA protein assay
519 (Thermo Fisher Scientific), equal amounts of protein (10–40 μ g) were loaded into 10-20% SDS-
520 PAGE gradient gels and transferred onto 0.22uM PVDF membranes. After air drying to return to
521 a hydrophobic state, membranes were incubated in primary antibodies at 4°C overnight in 1X
522 PBS containing 1% casein, HRP-conjugated secondary antibody at room temperature for 1 hour,
523 and then developed with Luminata Crescendo Western HRP Substrate (Millipore).

524 **ELISA**

525 Detached cells and debris were removed in cell culture supernatant or plasma prior to the assay by
526 centrifugation. After incubating in capture antibodies at 4°C overnight, blocking with PBS

527 containing 10% FBS at room temperature for 1 hr, standards and cell culture supernatant were
528 added into the ELISA plates, followed by incubation of detection antibodies, Avidin-HPR and
529 TMB for faster color development. Plates were washed with PBS containing 0.05% Tween-20
530 between each step.

531 **Immunofluorescence microscopy**

532 Cells were grown on coverslips and treated as described. After washing in PBS, cells were fixed
533 with 4% paraformaldehyde for 20 min, permeabilized with 0.1% Triton X-100 in PBS for 5 min,
534 blocked with PBS containing 5% FBS for 30 min, stained with primary antibodies for 1h, and
535 stained with secondary antibodies for 1h. Cells were washed with PBS containing 5% FBS
536 between each step. Coverslips were mounted with ProLong Diamond Antifade Mountant with
537 DAPI (Molecular Probes). Cells were imaged on a LSM 780 confocal microscope (Zeiss) with a
538 40 or 63x oil-immersed objective with Airyscan or a Cytation 5 (BioTek) with a 20x objective. 3-
539 5 images per sample were acquired. At least 100 cells per genotype were used to obtain statistical
540 significance for nuclear Nrf2 intensity analysis. Gen5 software (BioTek) was utilized to define
541 nuclear region, by DAPI staining, and calculate the Nrf2 fluorescent intensity in the nuclear
542 region of each cell.

543 **Metabolism assays**

544 The Seahorse XFe96 Analyzer was used to measure mitochondrial respiration and glycolysis.
545 Briefly, BMDMs and PerMacs were plated at a density of 5×10^4 cells/well or 2×10^5 cells/ well in
546 80 μ L of culture medium in Agilent Seahorse XF96 Cell Culture Microplate. DMF (50 μ M) and/or
547 LPS (10 ng/ml) were added 3 hrs after the cells were plated. Following a 16 hr incubation, cells
548 were washed and replaced with XF assay medium (Base Medium Minimal DMEM supplemented
549 with 2 mM Ala-Gln, pH 7.4) prior to analysis. Oxygen consumption rate (OCR) and extracellular
550 acidification rate (ECAR) were measured after sequential addition of glucose 25 mM, oligomycin
551 (1.5 μ M), FCCP (1.5 μ M)+ sodium pyruvate (1mM) and antimycin A (833 nM) and rotenone

552 (833 nM). L-Lactate (700510), Aconitase (705502) (Cayman); LDH (MK401) (TaKaRa) assay
553 kits were commercially purchased. Standard protocols provided with the kits were followed when
554 performing all assays.

555 **Histology**

556 Mice were euthanized and tissues were washed in PBS and incubated for 24 h in 10% formalin
557 and then transferred to 70% ethanol. Tissue embedding, sectioning and staining were performed
558 at AML Laboratories in Jacksonville, FL. Images of the H&E staining slides were acquired on
559 Lionheart FX (BioTek) with a 20x or 40x objective. Images for myofiber width quantification
560 were acquired on Nikon ECLIPSE Ts2 and a 40X objective. Ten cells from each sample, and
561 three mice of each genotype were imaged and calculated blindly using NIS-Elements software
562 (Nikon).

563 **Echocardiography**

564 Mice were depilated at the chest area the day before echocardiogram measurement. Anesthesia
565 was induced by placing mouse in a box with inhaled isoflurane at 2-3%. Afterwards, light
566 anesthesia was maintained using a nose cone with inhaled isoflurane at 0.5-1%. Mice were
567 immobilized on an instrumented and heated stage. Continuous electric cardiogram (ECG),
568 respiration, and temperature (via rectal probe) were monitored. Light anesthesia and core
569 temperature were maintained to ensure near physiological status with heart rate at range of 470-
570 520 beats per minute. Transthoracic echocardiography was performed using a VisualSonics Vevo
571 3100 system with a MX550D imaging transducer (center frequency of 40 MHz). Parasternal long-
572 axis (B-mode) and parasternal short-axis (M-mode) views of each animal were taken. Vevo LAB
573 cardiac analysis package was used to analyze the data.

574 **RNA sequencing and bioinformatic analyses**

575 Total cellular RNA from WT and *POLG* BMDMs and PerMacs was prepared using Quick-RNA
576 microprep kit (Zymo Research) and used for the Next-generation RNA sequencing procedure at

577 Texas A&M University Bioinformatics Core. RNA sequencing data were analyzed using
578 BaseSpace Sequence Hub (Illumina). In brief, STAR algorithm of RNAseq Alignment V2.0.0
579 software was utilized to align the results to reference genome *Mus musculus/mm10* (RefSeq),
580 then RNA-seq Differential Expression V1.0.0 software was used to obtain raw gene expression
581 files and determine statistically significant changes in gene expression in *POLG* macrophages
582 relative to WT. Ingenuity Pathway Analysis software (QIAGEN) was used to identify gene
583 families and putative upstream regulators in the datasets. Heat maps were generated using
584 GraphPad Prism. Upon acceptance of the manuscript, datasets will be deposited in GEO
585 according with NIH data sharing policies.

586 **Statistical analyses**

587 Error bars displayed throughout the manuscript represent s.e.m. and were calculated from
588 triplicate technical replicates of each biological sample unless otherwise indicated. For ex vivo
589 experiments, error bars were calculated from the average of duplicate or triplicate technical
590 replicates of at least 2 animals per point. For microscopy quantification, images were taken
591 throughout the slide of each sample using DAPI channel to avoid bias of selection, and at least
592 100 cells per genotype were randomly selected to obtain statistical significance. To reduce
593 potential experimental bias, samples for cardiac function (LVID;d, LVEF, IVS;s, LVID;s, Vol;s)
594 and myofiber width analysis in Figure 6 were blinded to researchers when performing analysis.
595 The identities were only revealed at the final data analysis stage. No randomization or blinding
596 was used for all other animal studies. No statistical method was used to predetermine sample size.
597 Data shown are representative of 2–3 independent experiments, including microscopy images,
598 western blots, flow cytometry and all metabolism assays.

599

500 **References and Notes**

501 1. A. P. West, Mitochondrial dysfunction as a trigger of innate immune responses and
502 inflammation. *Toxicology*. **391**, 54–63 (2017).

- 503 2. A. P. West, G. S. Shadel, S. Ghosh, Mitochondria in innate immune responses. *Nat Rev*
504 *Immunol.* **11**, 389–402 (2011).
- 505 3. S. E. Weinberg, L. A. Sena, N. S. Chandel, Mitochondria in the regulation of innate and
506 adaptive immunity. *Immunity.* **42**, 406–417 (2015).
- 507 4. E. L. Mills, B. Kelly, L. A. J. O’Neill, Mitochondria are the powerhouses of immunity.
508 *Nature Immunology.* **18**, 488–498 (2017).
- 509 5. R. J. Youle, Mitochondria-Striking a balance between host and endosymbiont. *Science.* **365**
510 (2019), doi:10.1126/science.aaw9855.
- 511 6. A. P. West, G. S. Shadel, Mitochondrial DNA in innate immune responses and inflammatory
512 pathology. *Nat Rev Immunol.* **17**, 363–375 (2017).
- 513 7. A. P. West, W. Khoury-Hanold, M. Staron, M. C. Tal, C. M. Pineda, S. M. Lang, M.
514 Bestwick, B. A. Duguay, N. Raimundo, D. A. MacDuff, S. M. Kaech, J. R. Smiley, R. E.
515 Means, A. Iwasaki, G. S. Shadel, Mitochondrial DNA stress primes the antiviral innate
516 immune response. *Nature.* **520**, 553–557 (2015).
- 517 8. A. Ablasser, Z. J. Chen, cGAS in action: Expanding roles in immunity and inflammation.
518 *Science.* **363** (2019), doi:10.1126/science.aat8657.
- 519 9. K. Nakahira, S. Hisata, A. M. K. Choi, The Roles of Mitochondrial Damage-Associated
520 Molecular Patterns in Diseases. *Antioxid. Redox Signal.* **23**, 1329–1350 (2015).
- 521 10. Z. Wu, S. Oeck, A. P. West, K. C. Mangalharra, A. G. Sainz, L. E. Newman, X.-O. Zhang, L.
522 Wu, Q. Yan, M. Bosenberg, Y. Liu, P. L. Sulkowski, V. Tripple, S. M. Kaech, P. M. Glazer,
523 G. S. Shadel, Mitochondrial DNA stress signalling protects the nuclear genome. *Nature*
524 *Metabolism.* **1**, 1209–1218 (2019).
- 525 11. G. S. Gorman, P. F. Chinnery, S. DiMauro, M. Hirano, Y. Koga, R. McFarland, A.
526 Suomalainen, D. R. Thorburn, M. Zeviani, D. M. Turnbull, Mitochondrial diseases. *Nature*
527 *Reviews Disease Primers.* **2**, 1–22 (2016).
- 528 12. J. D. Stumpf, R. P. Saneto, W. C. Copeland, Clinical and Molecular Features of POLG-
529 Related Mitochondrial Disease. *Cold Spring Harb Perspect Biol.* **5** (2013),
530 doi:10.1101/cshperspect.a011395.
- 531 13. S. Eom, H. N. Lee, S. Lee, H.-C. Kang, J. S. Lee, H. D. Kim, Y.-M. Lee, Cause of Death in
532 Children With Mitochondrial Diseases. *Pediatr. Neurol.* **66**, 82–88 (2017).
- 533 14. S. M. Kapnick, S. E. Pacheco, P. J. McGuire, The emerging role of immune dysfunction in
534 mitochondrial diseases as a paradigm for understanding immunometabolism. *Metab. Clin.*
535 *Exp.* **81**, 97–112 (2018).
- 536 15. M. A. Walker, N. Slate, A. Alejos, S. Volpi, R. S. Iyengar, D. Sweetser, K. B. Sims, J. E.
537 Walter, Predisposition to infection and SIRS in mitochondrial disorders: 8 years’ experience
538 in an academic center. *Journal of Allergy and Clinical Immunology. In Practice;*
539 *Amsterdam.* **2**, 465–468 (2014).

- 540 16. T. N. Tarasenko, S. E. Pacheco, M. K. Koenig, J. Gomez-Rodriguez, S. M. Kapnick, F. Diaz,
541 P. M. Zerfas, E. Barca, J. Sudderth, R. J. DeBerardinis, R. Covian, R. S. Balaban, S.
542 DiMauro, P. J. McGuire, Cytochrome c Oxidase Activity Is a Metabolic Checkpoint that
543 Regulates Cell Fate Decisions During T Cell Activation and Differentiation. *Cell Metab.* **25**,
544 1254-1268.e7 (2017).
- 545 17. O. Hasselmann, N. Blau, V. T. Ramaekers, E. V. Quadros, J. M. Sequeira, M. Weissert,
546 Cerebral folate deficiency and CNS inflammatory markers in Alpers disease. *Mol. Genet.*
547 *Metab.* **99**, 58–61 (2010).
- 548 18. H. M. Wilkins, I. W. Weidling, Y. Ji, R. H. Swerdlow, Mitochondria-Derived Damage-
549 Associated Molecular Patterns in Neurodegeneration. *Front Immunol.* **8**, 508 (2017).
- 550 19. M. J. Young, W. C. Copeland, Human mitochondrial DNA replication machinery and disease.
551 *Curr Opin Genet Dev.* **38**, 52–62 (2016).
- 552 20. S. Rahman, W. C. Copeland, POLG-related disorders and their neurological manifestations.
553 *Nat Rev Neurol.* **15**, 40–52 (2019).
- 554 21. B. Singh, K. M. Owens, P. Bajpai, M. M. Desouki, V. Srinivasasainagendra, H. K. Tiwari, K.
555 K. Singh, Mitochondrial DNA Polymerase POLG1 Disease Mutations and Germline
556 Variants Promote Tumorigenic Properties. *PLoS ONE.* **10**, e0139846 (2015).
- 557 22. G. Davidzon, P. Greene, M. Mancuso, K. J. Klos, J. E. Ahlskog, M. Hirano, S. DiMauro,
558 Early-onset familial parkinsonism due to POLG mutations. *Ann. Neurol.* **59**, 859–862
559 (2006).
- 560 23. G. C. Kujoth, A. Hiona, T. D. Pugh, S. Someya, K. Panzer, S. E. Wohlgemuth, T. Hofer, A.
561 Y. Seo, R. Sullivan, W. A. Jobling, J. D. Morrow, H. V. Remmen, J. M. Sedivy, T.
562 Yamasoba, M. Tanokura, R. Weindruch, C. Leeuwenburgh, T. A. Prolla, Mitochondrial
563 DNA Mutations, Oxidative Stress, and Apoptosis in Mammalian Aging. *Science.* **309**, 481–
564 484 (2005).
- 565 24. A. Trifunovic, A. Wredenberg, M. Falkenberg, J. N. Spelbrink, A. T. Rovio, C. E. Bruder, M.
566 Bohlooly-Y, S. Gidlöf, A. Oldfors, R. Wibom, J. Törnell, H. T. Jacobs, N.-G. Larsson,
567 Premature ageing in mice expressing defective mitochondrial DNA polymerase. *Nature.*
568 **429**, 417–423 (2004).
- 569 25. K. Szczepanowska, A. Trifunovic, Different faces of mitochondrial DNA mutators.
570 *Biochimica et Biophysica Acta (BBA) - Bioenergetics.* **1847**, 1362–1372 (2015).
- 571 26. J. Y. Jang, A. Blum, J. Liu, T. Finkel, The role of mitochondria in aging. *J. Clin. Invest.* **128**,
572 3662–3670 (2018).
- 573 27. A. Logan, I. G. Shabalina, T. A. Prime, S. Rogatti, A. V. Kalinovich, R. C. Hartley, R. C.
574 Budd, B. Cannon, M. P. Murphy, In vivo levels of mitochondrial hydrogen peroxide
575 increase with age in mtDNA mutator mice. *Aging Cell.* **13**, 765–768 (2014).
- 576 28. M. L. Chen, T. D. Logan, M. L. Hochberg, S. G. Shelat, X. Yu, G. E. Wilding, W. Tan, G. C.
577 Kujoth, T. A. Prolla, M. A. Selak, M. Kundu, M. Carroll, J. E. Thompson, Erythroid
578 dysplasia, megaloblastic anemia, and impaired lymphopoiesis arising from mitochondrial
579 dysfunction. *Blood.* **114**, 4045–4053 (2009).

- 580 29. A. Safdar, J. M. Bourgeois, D. I. Ogborn, J. P. Little, B. P. Hettinga, M. Akhtar, J. E.
581 Thompson, S. Melov, N. J. Mocellin, G. C. Kujoth, T. A. Prolla, M. A. Tarnopolsky,
582 Endurance exercise rescues progeroid aging and induces systemic mitochondrial
583 rejuvenation in mtDNA mutator mice. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 4135–4140 (2011).
- 584 30. C. Shi, T. M. Hohl, I. Leiner, M. J. Equinda, X. Fan, E. G. Pamer, Ly6G⁺ neutrophils are
585 dispensable for defense against systemic *Listeria monocytogenes* infection. *J Immunol.* **187**,
586 5293–5298 (2011).
- 587 31. H. Maekawa, T. Inoue, H. Ouchi, T.-M. Jao, R. Inoue, H. Nishi, R. Fujii, F. Ishidate, T.
588 Tanaka, Y. Tanaka, N. Hirokawa, M. Nangaku, R. Inagi, Mitochondrial Damage Causes
589 Inflammation via cGAS-STING Signaling in Acute Kidney Injury. *Cell Rep.* **29**, 1261-
590 1273.e6 (2019).
- 591 32. K. W. Chung, P. Dhillon, S. Huang, X. Sheng, R. Shrestha, C. Qiu, B. A. Kaufman, J. Park,
592 L. Pei, J. Baur, M. Palmer, K. Susztak, Mitochondrial Damage and Activation of the STING
593 Pathway Lead to Renal Inflammation and Fibrosis. *Cell Metabolism.* **30**, 784-799.e5 (2019).
- 594 33. D. A. Sliter, J. Martinez, L. Hao, X. Chen, N. Sun, T. D. Fischer, J. L. Burman, Y. Li, Z.
595 Zhang, D. P. Narendra, H. Cai, M. Borsche, C. Klein, R. J. Youle, Parkin and PINK1
596 mitigate STING-induced inflammation. *Nature.* **561**, 258–262 (2018).
- 597 34. G. Rackov, R. Shokri, M. Á. De Mon, C. Martínez-A., D. Balomenos, The Role of IFN- β
598 during the Course of Sepsis Progression and Its Therapeutic Potential. *Front Immunol.* **8**
599 (2017), doi:10.3389/fimmu.2017.00493.
- 700 35. S. H. Park, K. Kang, E. Giannopoulou, Y. Qiao, K. Kang, G. Kim, K.-H. Park-Min, L. B.
701 Ivashkiv, Type I interferons and the cytokine TNF cooperatively reprogram the macrophage
702 epigenome to promote inflammatory activation. *Nature Immunology.* **18**, 1104–1116 (2017).
- 703 36. M. Karaghiosoff, R. Steinborn, P. Kovarik, G. Kriegshäuser, M. Baccarini, B. Donabauer, U.
704 Reichart, T. Kolbe, C. Bogdan, T. Leanderson, D. Levy, T. Decker, M. Müller, Central role
705 for type I interferons and Tyk2 in lipopolysaccharide-induced endotoxin shock. *Nature*
706 *Immunology.* **4**, 471–477 (2003).
- 707 37. M. B. Buechler, T. H. Teal, K. B. Elkon, J. A. Hamerman, Cutting Edge: Type I IFN Drives
708 Emergency Myelopoiesis and Peripheral Myeloid Expansion during Chronic TLR7
709 Signaling. *The Journal of Immunology.* **190**, 886–891 (2013).
- 710 38. P. Y. Lee, Y. Li, Y. Kumagai, Y. Xu, J. S. Weinstein, E. S. Kellner, D. C. Nacionales, E. J.
711 Butfiloski, N. van Rooijen, S. Akira, E. S. Sobel, M. Satoh, W. H. Reeves, Type I interferon
712 modulates monocyte recruitment and maturation in chronic inflammation. *Am. J. Pathol.*
713 **175**, 2023–2033 (2009).
- 714 39. S.-U. Seo, H.-J. Kwon, H.-J. Ko, Y.-H. Byun, B. L. Seong, S. Uematsu, S. Akira, M.-N.
715 Kweon, Type I Interferon Signaling Regulates Ly6Chi Monocytes and Neutrophils during
716 Acute Viral Pneumonia in Mice. *PLOS Pathogens.* **7**, e1001304 (2011).
- 717 40. R. Channappanavar, A. R. Fehr, R. Vijay, M. Mack, J. Zhao, D. K. Meyerholz, S. Perlman,
718 Dysregulated Type I Interferon and Inflammatory Monocyte-Macrophage Responses Cause
719 Lethal Pneumonia in SARS-CoV-Infected Mice. *Cell Host Microbe.* **19**, 181–193 (2016).

- 720 41. O. Majer, C. Bourgeois, F. Zwolanek, C. Lassnig, D. Kerjaschki, M. Mack, M. Müller, K.
721 Kuchler, Type I Interferons Promote Fatal Immunopathology by Regulating Inflammatory
722 Monocytes and Neutrophils during Candida Infections. *PLOS Pathogens*. **8**, e1002811
723 (2012).
- 724 42. E. H. Kobayashi, T. Suzuki, R. Funayama, T. Nagashima, M. Hayashi, H. Sekine, N. Tanaka,
725 T. Moriguchi, H. Motohashi, K. Nakayama, M. Yamamoto, Nrf2 suppresses macrophage
726 inflammatory response by blocking proinflammatory cytokine transcription. *Nature*
727 *Communications*. **7**, 1–14 (2016).
- 728 43. E. L. Mills, D. G. Ryan, H. A. Prag, D. Dikovskaya, D. Menon, Z. Zaslona, M. P.
729 Jedrychowski, A. S. H. Costa, M. Higgins, E. Hams, J. Szpyt, M. C. Runtsch, M. S. King, J.
730 F. McGouran, R. Fischer, B. M. Kessler, A. F. McGettrick, M. M. Hughes, R. G. Carroll, L.
731 M. Booty, E. V. Knatko, P. J. Meakin, M. L. J. Ashford, L. K. Modis, G. Brunori, D. C.
732 Sévin, P. G. Fallon, S. T. Caldwell, E. R. S. Kunji, E. T. Chouchani, C. Frezza, A. T.
733 Dinkova-Kostova, R. C. Hartley, M. P. Murphy, L. A. O'Neill, Itaconate is an anti-
734 inflammatory metabolite that activates Nrf2 via alkylation of KEAP1. *Nature*. **556**, 113–117
735 (2018).
- 736 44. K. Taguchi, H. Motohashi, M. Yamamoto, Molecular mechanisms of the Keap1–Nrf2
737 pathway in stress response and cancer evolution. *Genes Cells*. **16**, 123–140 (2011).
- 738 45. M. D. Kornberg, P. Bhargava, P. M. Kim, V. Putluri, A. M. Snowman, N. Putluri, P. A.
739 Calabresi, S. H. Snyder, Dimethyl fumarate targets GAPDH and aerobic glycolysis to
740 modulate immunity. *Science*. **360**, 449–453 (2018).
- 741 46. D. Olganier, R. R. Lababidi, S. B. Hadj, A. Sze, Y. Liu, S. D. Naidu, M. Ferrari, Y. Jiang, C.
742 Chiang, V. Beljanski, M.-L. Goulet, E. V. Knatko, A. T. Dinkova-Kostova, J. Hiscott, R.
743 Lin, Activation of Nrf2 Signaling Augments Vesicular Stomatitis Virus Oncolysis via
744 Autophagy-Driven Suppression of Antiviral Immunity. *Mol. Ther.* **25**, 1900–1916 (2017).
- 745 47. C. Gunderstofte, M. B. Iversen, S. Peri, A. Thielke, S. Balachandran, C. K. Holm, D.
746 Olganier, Nrf2 Negatively Regulates Type I Interferon Responses and Increases
747 Susceptibility to Herpes Genital Infection in Mice. *Front Immunol.* **10**, 2101 (2019).
- 748 48. D. Olganier, A. M. Brandtoft, C. Gunderstofte, N. L. Villadsen, C. Krapp, A. L. Thielke, A.
749 Laustsen, S. Peri, A. L. Hansen, L. Bonefeld, J. Thyrted, V. Bruun, M. B. Iversen, L. Lin,
750 V. M. Artegoitia, C. Su, L. Yang, R. Lin, S. Balachandran, Y. Luo, M. Nyegaard, B.
751 Marrero, R. Goldbach-Mansky, M. Motwani, D. G. Ryan, K. A. Fitzgerald, L. A. O'Neill,
752 A. K. Hollensen, C. K. Damgaard, F. v de Paoli, H. C. Bertram, M. R. Jakobsen, T. B.
753 Poulsen, C. K. Holm, Nrf2 negatively regulates STING indicating a link between antiviral
754 sensing and metabolic reprogramming. *Nature Communications*. **9**, 1–13 (2018).
- 755 49. N. J. Hos, R. Ganesan, S. Gutiérrez, D. Hos, J. Klimek, Z. Abdullah, M. Krönke, N.
756 Robinson, Type I interferon enhances necroptosis of Salmonella Typhimurium-infected
757 macrophages by impairing antioxidative stress responses. *J. Cell Biol.* **216**, 4107–4121
758 (2017).
- 759 50. M. Riedelberger, P. Penninger, M. Tscherner, M. Seifert, S. Jenull, C. Brunnhofer, B. Scheidl,
760 I. Tsymala, C. Bourgeois, A. Petryshyn, W. Glaser, A. Limbeck, B. Strobl, G. Weiss, K.
761 Kuchler, Type I Interferon Response Dysregulates Host Iron Homeostasis and Enhances
762 Candida glabrata Infection. *Cell Host & Microbe*. **27**, 454–466.e8 (2020).

- 763 51. S. Kovac, P. R. Angelova, K. M. Holmström, Y. Zhang, A. T. Dinkova-Kostova, A. Y.
764 Abramov, Nrf2 regulates ROS production by mitochondria and NADPH oxidase. *Biochim*
765 *Biophys Acta*. **1850**, 794–801 (2015).
- 766 52. A. Pantel, A. Teixeira, E. Haddad, E. G. Wood, R. M. Steinman, M. P. Longhi, Direct type I
767 IFN but not MDA5/TLR3 activation of dendritic cells is required for maturation and
768 metabolic shift to glycolysis after poly IC stimulation. *PLoS Biol.* **12**, e1001759 (2014).
- 769 53. D. P. D. Souza, A. Achuthan, M. K. S. Lee, K. J. Binger, M.-C. Lee, S. Davidson, D. L. Tull,
770 M. J. McConville, A. D. Cook, A. J. Murphy, J. A. Hamilton, A. J. Fleetwood, Autocrine
771 IFN-I inhibits isocitrate dehydrogenase in the TCA cycle of LPS-stimulated macrophages. *J*
772 *Clin Invest.* **129**, 4239–4244 (2019).
- 773 54. I. G. Shabalina, M. Yu. Vyssokikh, N. Gibanova, R. I. Csikasz, D. Edgar, A. Hallden-
774 Waldemarson, Z. Rozhdestvenskaya, L. E. Bakeeva, V. B. Vays, A. V. Pustovidko, M. V.
775 Skulachev, B. Cannon, V. P. Skulachev, J. Nedergaard, Improved health-span and lifespan
776 in mtDNA mutator mice treated with the mitochondrially targeted antioxidant SkQ1. *Aging*
777 (*Albany NY*). **9**, 315–336 (2017).
- 778 55. D.-F. Dai, T. Chen, J. Wanagat, M. Laflamme, D. J. Marcinek, M. J. Emond, C. P. Ngo, T. A.
779 Prolla, P. S. Rabinovitch, Age-Dependent Cardiomyopathy in Mitochondrial Mutator Mice
780 is Attenuated by Overexpression of Catalase Targeted to Mitochondria. *Aging Cell.* **9**, 536–
781 544 (2010).
- 782 56. R. H. Hämmäläinen, K. J. Ahlqvist, P. Ellonen, M. Lepistö, A. Logan, T. Otonkoski, M. P.
783 Murphy, A. Suomalainen, mtDNA Mutagenesis Disrupts Pluripotent Stem Cell Function by
784 Altering Redox Signaling. *Cell Rep.* **11**, 1614–1624 (2015).
- 785 57. J. E. Kolesar, A. Safdar, A. Abadi, L. G. MacNeil, J. D. Crane, M. A. Tarnopolsky, B. A.
786 Kaufman, Defects in mitochondrial DNA replication and oxidative damage in muscle of
787 mtDNA mutator mice. *Free Radical Biology and Medicine.* **75**, 241–251 (2014).
- 788 58. A. Saleem, A. Safdar, Y. Kitaoka, X. Ma, O. S. Marquez, M. Akhtar, A. Nazli, R. Suri, J.
789 Turnbull, M. A. Tarnopolsky, Polymerase gamma mutator mice rely on increased glycolytic
790 flux for energy production. *Mitochondrion.* **21**, 19–26 (2015).
- 791 59. B. P. Woodall, A. M. Orogo, R. H. Najor, M. Q. Cortez, E. R. Moreno, H. Wang, A. S.
792 Divakaruni, A. N. Murphy, Å. B. Gustafsson, Parkin does not prevent accelerated cardiac
793 aging in mitochondrial DNA mutator mice. *JCI Insight.* **4** (2019),
794 doi:10.1172/jci.insight.127713.
- 795 60. A. Y. Khakoo, M. K. Halushka, J. E. Rame, E. R. Rodriguez, E. K. Kasper, D. P. Judge,
796 Reversible cardiomyopathy caused by administration of interferon alpha. *Nat Clin Pract*
797 *Cardiovasc Med.* **2**, 53–57 (2005).
- 798 61. S. Ioannou, G. Hatzis, I. Vlahadami, M. Voulgarelis, Aplastic anemia associated with
799 interferon alpha 2a in a patient with chronic hepatitis C virus infection: a case report. *J Med*
300 *Case Reports.* **4**, 268 (2010).
- 301 62. J. Chen, Z. Zhang, L. Cai, Diabetic cardiomyopathy and its prevention by nrf2: current status.
302 *Diabetes Metab J.* **38**, 337–345 (2014).

- 303 63. J.-M. Lee, K. Chan, Y. W. Kan, J. A. Johnson, Targeted disruption of Nrf2 causes
304 regenerative immune-mediated hemolytic anemia. *Proc Natl Acad Sci U S A.* **101**, 9751–
305 9756 (2004).
- 306 64. K. J. Ahlqvist, R. H. Hämäläinen, S. Yatsuga, M. Uutela, M. Terzioglu, A. Götz, S.
307 Forsström, P. Salven, A. Angers-Loustau, O. H. Kopra, H. Tyynismaa, N.-G. Larsson, K.
308 Wartiovaara, T. Prolla, A. Trifunovic, A. Suomalainen, Somatic Progenitor Cell
309 Vulnerability to Mitochondrial DNA Mutagenesis Underlies Progeroid Phenotypes in Polg
310 Mutator Mice. *Cell Metabolism.* **15**, 100–109 (2012).
- 311 65. K. J. Ahlqvist, S. Leoncini, A. Pecorelli, S. B. Wortmann, S. Ahola, S. Forsström, R.
312 Guerranti, C. De Felice, J. Smeitink, L. Ciccoli, R. H. Hämäläinen, A. Suomalainen,
313 MtDNA mutagenesis impairs elimination of mitochondria during erythroid maturation
314 leading to enhanced erythrocyte destruction. *Nat Commun.* **6**, 6494 (2015).
- 315 66. J. L. Edmonds, D. J. Kirse, D. Kearns, R. Deutsch, L. Spruijt, R. K. Naviaux, The
316 otolaryngological manifestations of mitochondrial disease and the risk of neurodegeneration
317 with infection. *Arch. Otolaryngol. Head Neck Surg.* **128**, 355–362 (2002).
- 318 67. A. Quintana, S. E. Kruse, R. P. Kapur, E. Sanz, R. D. Palmiter, Complex I deficiency due to
319 loss of Ndufs4 in the brain results in progressive encephalopathy resembling Leigh
320 syndrome. *PNAS.* **107**, 10996–11001 (2010).
- 321 68. Z. Jin, W. Wei, M. Yang, Y. Du, Y. Wan, Mitochondrial Complex I Activity Suppresses
322 Inflammation and Enhances Bone Resorption by Shifting Macrophage-Osteoclast
323 Polarization. *Cell Metabolism.* **20**, 483–498 (2014).
- 324 69. Y. Wang, Q. Liu, T. Liu, Q. Zheng, X. Xu, X. Liu, W. Gao, Z. Li, X. Bai, Early plasma
325 monocyte chemoattractant protein 1 predicts the development of sepsis in trauma patients: A
326 prospective observational study. *Medicine (Baltimore).* **97**, e0356 (2018).
- 327 70. H. Matsumoto, H. Ogura, K. Shimizu, M. Ikeda, T. Hirose, H. Matsuura, S. Kang, K.
328 Takahashi, T. Tanaka, T. Shimazu, The clinical importance of a cytokine network in the
329 acute phase of sepsis. *Scientific Reports.* **8**, 13995 (2018).
- 330 71. A. J. Lee, B. Chen, M. V. Chew, N. G. Barra, M. M. Shenouda, T. Nham, N. van Rooijen, M.
331 Jordana, K. L. Mossman, R. D. Schreiber, M. Mack, A. A. Ashkar, Inflammatory monocytes
332 require type I interferon receptor signaling to activate NK cells via IL-18 during a mucosal
333 viral infection. *J. Exp. Med.* **214**, 1153–1167 (2017).
- 334 72. M. B. Buechler, H. M. Akilesh, J. A. Hamerman, Cutting Edge: Direct Sensing of TLR7
335 Ligands and Type I IFN by the Common Myeloid Progenitor Promotes mTOR/PI3K-
336 Dependent Emergency Myelopoiesis. *The Journal of Immunology* (2016),
337 doi:10.4049/jimmunol.1600813.
- 338 73. H. M. Akilesh, M. B. Buechler, J. M. Duggan, W. O. Hahn, B. Matta, X. Sun, G. Gessay, E.
339 Whalen, M. Mason, S. R. Presnell, K. B. Elkon, A. Lacy-Hulbert, B. J. Barnes, M. Pepper, J.
340 A. Hamerman, Chronic TLR7 and TLR9 signaling drives anemia via differentiation of
341 specialized hemophagocytes. *Science.* **363** (2019), doi:10.1126/science.aao5213.

- 342 74. G. L. Norddahl, C. J. Pronk, M. Wahlestedt, G. Sten, A. Ugale, M. Sigvardsson, D. Bryder,
343 Accumulating mitochondrial DNA mutations drive premature hematopoietic aging
344 phenotypes distinct from physiological stem cell aging. *Cell Stem Cell*. **8**, 499–510 (2011).
- 345 75. R. H. Hämäläinen, J. C. Landoni, K. J. Ahlqvist, S. Goffart, S. Ryytty, M. O. Rahman, V.
346 Brillhante, K. Içay, S. Hautaniemi, L. Wang, M. Laiho, A. Suomalainen, Defects in mtDNA
347 replication challenge nuclear genome stability through nucleotide depletion and provide a
348 unifying mechanism for mouse progerias. *Nature Metabolism*. **1**, 958–965 (2019).
- 349 76. K. M. Holmstrom, L. Baird, Y. Zhang, I. Hargreaves, A. Chalasani, J. M. Land, L. Stanyer,
350 M. Yamamoto, A. T. Dinkova-Kostova, A. Y. Abramov, Nrf2 impacts cellular bioenergetics
351 by controlling substrate availability for mitochondrial respiration. *Biology Open*. **2**, 761–770
352 (2013).
- 353 77. R. Erkens, C. M. Kramer, W. Lückstädt, C. Panknin, L. Krause, M. Weidenbach, J. Dirzka, T.
354 Krenz, E. Mergia, T. Suvorava, M. Kelm, M. M. Cortese-Krott, Left ventricular diastolic
355 dysfunction in Nrf2 knock out mice is associated with cardiac hypertrophy, decreased
356 expression of SERCA2a, and preserved endothelial function. *Free Radical Biology and
357 Medicine*. **89**, 906–917 (2015).
- 358 78. R. K. Thimmulappa, H. Lee, T. Rangasamy, S. P. Reddy, M. Yamamoto, T. W. Kensler, S.
359 Biswal, Nrf2 is a critical regulator of the innate immune response and survival during
360 experimental sepsis. *J. Clin. Invest.* **116**, 984–995 (2006).
- 361 79. A. Cuadrado, A. I. Rojo, G. Wells, J. D. Hayes, S. P. Cousin, W. L. Rumsey, O. C. Attucks,
362 S. Franklin, A.-L. Levonen, T. W. Kensler, A. T. Dinkova-Kostova, Therapeutic targeting of
363 the NRF2 and KEAP1 partnership in chronic diseases. *Nat Rev Drug Discov*. **18**, 295–317
364 (2019).
- 365 80. J. Kwon, E. Han, C.-B. Bui, W. Shin, J. Lee, S. Lee, Y.-B. Choi, A.-H. Lee, K.-H. Lee, C.
366 Park, M. S. Obin, S. K. Park, Y. J. Seo, G. T. Oh, H.-W. Lee, J. Shin, Assurance of
367 mitochondrial integrity and mammalian longevity by the p62–Keap1–Nrf2–Nqo1 cascade.
368 *EMBO reports*. **13**, 150–156 (2012).
- 369 81. C. J. Schmidlin, M. B. Dodson, L. Madhavan, D. D. Zhang, Redox regulation by NRF2 in
370 aging and disease. *Free Radic. Biol. Med.* **134**, 702–707 (2019).
- 371 82. N. Kubben, W. Zhang, L. Wang, T. C. Voss, J. Yang, J. Qu, G.-H. Liu, T. Misteli, Repression
372 of the Antioxidant NRF2 Pathway in Premature Aging. *Cell*. **165**, 1361–1374 (2016).
- 373 83. A. Härtlova, S. F. Erttmann, F. A. Raffi, A. M. Schmalz, U. Resch, S. Anugula, S.
374 Lienenklaus, L. M. Nilsson, A. Kröger, J. A. Nilsson, T. Ek, S. Weiss, N. O. Gekara, DNA
375 damage primes the type I interferon system via the cytosolic DNA sensor STING to promote
376 anti-microbial innate immunity. *Immunity*. **42**, 332–343 (2015).
- 377 84. R. Kreienkamp, S. Graziano, N. Coll-Bonfill, G. Bedia-Diaz, E. Cybulla, A. Vindigni, D.
378 Dorsett, N. Kubben, L. F. Z. Batista, S. Gonzalo, A Cell-Intrinsic Interferon-like Response
379 Links Replication Stress to Cellular Aging Caused by Progerin. *Cell Reports*. **22**, 2006–2015
380 (2018).
- 381 85. Q. Yu, Y. V. Katlinskaya, C. J. Carbone, B. Zhao, K. V. Katlinski, H. Zheng, M. Guha, N. Li,
382 Q. Chen, T. Yang, C. J. Lengner, R. A. Greenberg, F. B. Johnson, S. Y. Fuchs, DNA-

383 Damage-Induced Type I Interferon Promotes Senescence and Inhibits Stem Cell Function.
384 *Cell Reports*. **11**, 785–797 (2015).

385 86. M. F. Manchinu, C. Brancia, C. A. Caria, E. Musu, S. Porcu, M. Simbula, I. Asunis, L.
386 Perseu, M. S. Ristaldi, Deficiency in interferon type 1 receptor improves definitive
387 erythropoiesis in Klf1 null mice. *Cell Death & Differentiation*. **25**, 589–599 (2018).

388 87. J. Finsterer, M. Frank, Haematological abnormalities in mitochondrial disorders. *Singapore*
389 *Med J*. **56**, 412–419 (2015).

390 88. O. Hikmat, C. Tzoulis, C. Klingenberg, M. Rasmussen, C. M. E. Tallaksen, E. Brodtkorb, T.
391 Fiskerstrand, R. McFarland, S. Rahman, L. A. Bindoff, The presence of anaemia negatively
392 influences survival in patients with POLG disease. *J. Inherit. Metab. Dis.* **40**, 861–866
393 (2017).

394

395 **Acknowledgments**

396 **General**

397 We thank Dr. Rola Barhoumi Mouneimne for assistance with confocal microscopy, Ms. Robbie
398 Moore for flow cytometry assistance, and Ms. Heidi Creed for assistance with the Vevo 3100
399 ultrasound.

400 **Funding**

401 This research was supported by awards W81XWH-17-1-0052 and W81XWH-20-1-0150 to
402 A.P.W. from Office of the Assistant Secretary of Defense for Health Affairs through the Peer
403 Reviewed Medical Research Programs. Additional support was provided by NIH grants
404 R01HL148153 to A.P.W., R01AI145287 and R01AI125512 to R.O.W., R01HL145534 to C.W.T,
405 and NIEHS P30 ESES029067. Opinions, interpretations, conclusions, and recommendations are
406 those of the author and are not necessarily endorsed by the Department of Defense or NIH.

407 **Author contributions**

408 A.P.W and Y.L. designed the experiments, analyzed the data, and wrote the manuscript. Y.L.
409 performed most of the experiments. C.G.M., S.T.O., C.E.B., and J.D.B. assisted with animal
410 experiments, sample processing, and/or data analyses. S.L.B. and R.O.W. contributed to the *L.*
411 *monocytogenes* infections, sample collection, and data analyses. C.W.T. and L.C.W. provided

012 expertise and advice on cardiac measurements and flow cytometric experiments, respectively.

013 A.P.W conceived the project and provided overall direction.

014 **Competing interests**

015 The authors declare no competing interests.

016 **Data and materials availability**

017 All data needed to evaluate the conclusions drawn herein are present in the paper and/or the

018 Supplementary Materials. Raw data will be provided upon request. RNA-Seq datasets will be

019 deposited to GEO upon acceptance of the manuscript.

020

021 **Figures**

Figure 1. POLG mutator mice exhibit a hyper-inflammatory phenotype to LPS challenge due to increased CD11b⁺ myeloid cells in the blood.

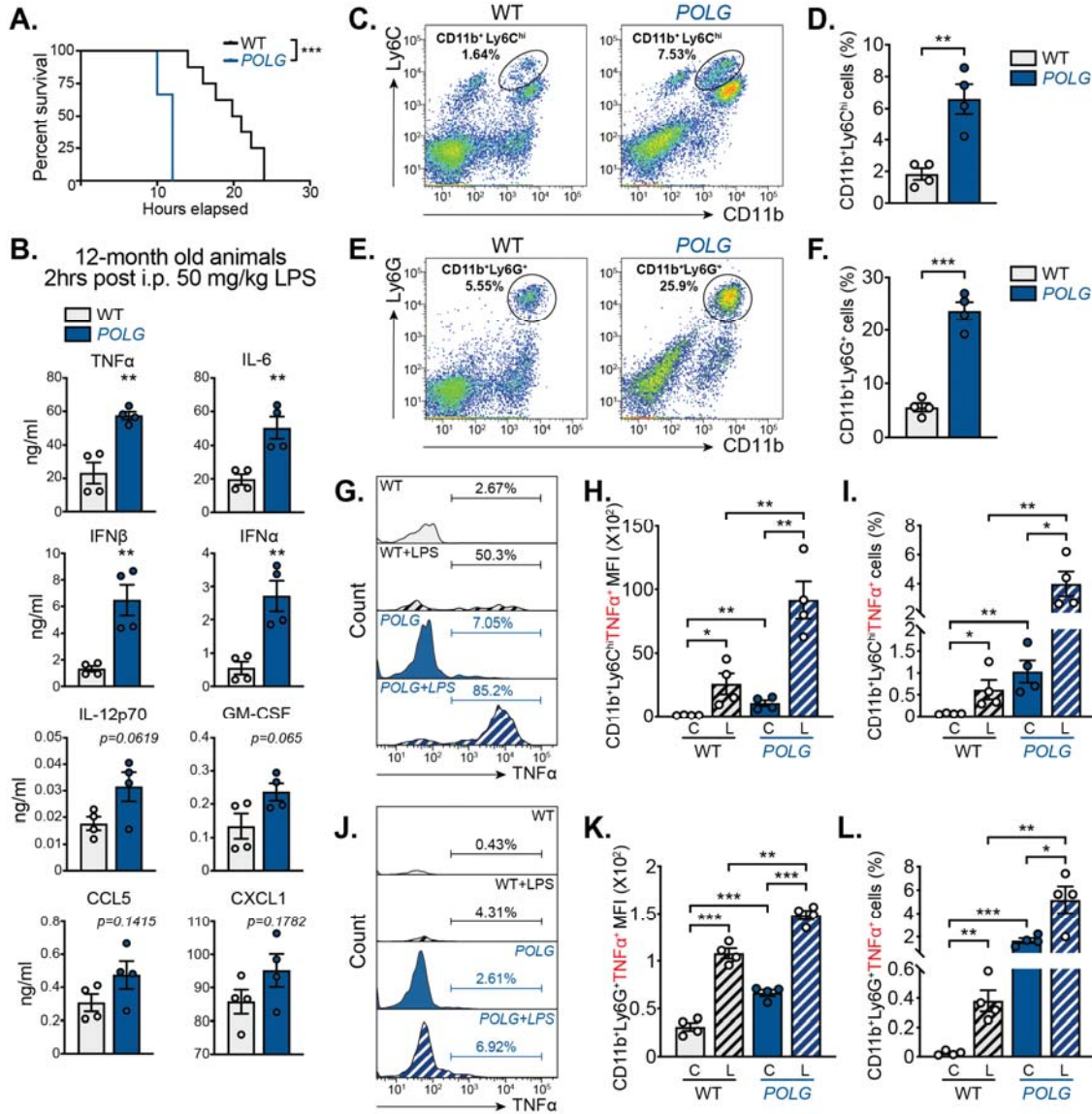


Figure 1. POLG mutator mice exhibit a hyper-inflammatory phenotype to LPS challenge due to increased CD11b⁺ myeloid cells in the blood.

(A and B) 12-month old WT (n=8) and *POLG* (n=7) mice were challenged with LPS (50 mg/kg by i.p. injection). Kaplan-Meier survival analysis was performed (A). Plasma cytokine profiles were determined by multi-analyte bead-based immunoassay on two biological duplicates and two technical duplicates per group (B). Log-rank (Mantel-Cox) test was used to compare percent survival between different groups.

30 (C and D) CD11b⁺Ly6C^{hi} inflammatory monocyte population in whole blood from 12-month old
31 WT and *POLG* mice was evaluated by flow cytometry. Pseudocolor plots are representative of 4
32 independent experiments (C) and quantification of the percentage of CD11b⁺Ly6C^{hi} cells is
33 shown in (D).

34 (E and F) CD11b⁺Ly6G⁺ blood neutrophil population in 12-month old WT and *POLG* mice was
35 determined by flow cytometry. Pseudocolor plots are representative of 4 independent experiments
36 (E) and quantification of the percentage of CD11b⁺Ly6G⁺ cells is shown in (F).

37 (G-I) CD11b⁺Ly6C^{hi}TNF α ⁺ inflammatory monocyte population in unstimulated [C] or LPS
38 challenged [L] whole blood from 12-month old WT and *POLG* mice was evaluated by flow
39 cytometry. Histograms are representative of 4 independent experiments (G). Quantification of
40 CD11b⁺Ly6C^{hi}TNF α ⁺ mean fluorescent intensity (MFI) is shown in (H), and the percentage of
41 CD11b⁺Ly6C^{hi}TNF α ⁺ cells is shown in (I).

42 (J-L) CD11b⁺Ly6G⁺TNF α ⁺ neutrophil population in unstimulated [C] or LPS challenged [L]
43 whole blood from 12-month old WT and *POLG* mice was evaluated by flow cytometry.
44 Histograms are representative of 4 independent experiments (J). Quantification of
45 CD11b⁺Ly6G⁺TNF α ⁺ MFI is shown in (K), and the percentage of CD11b⁺Ly6G⁺TNF α ⁺
46 neutrophils is shown in (L) .

47 Unless stated, statistical significance was determined using unpaired Student's t-tests. **P* < 0.05;

48 ***P* < 0.01; ****P* < 0.001. Error bars represent S.E.M.

Figure 2. POLG mutator macrophages exhibit enhanced IFN-I and pro-inflammatory responses after innate immune stimulation.

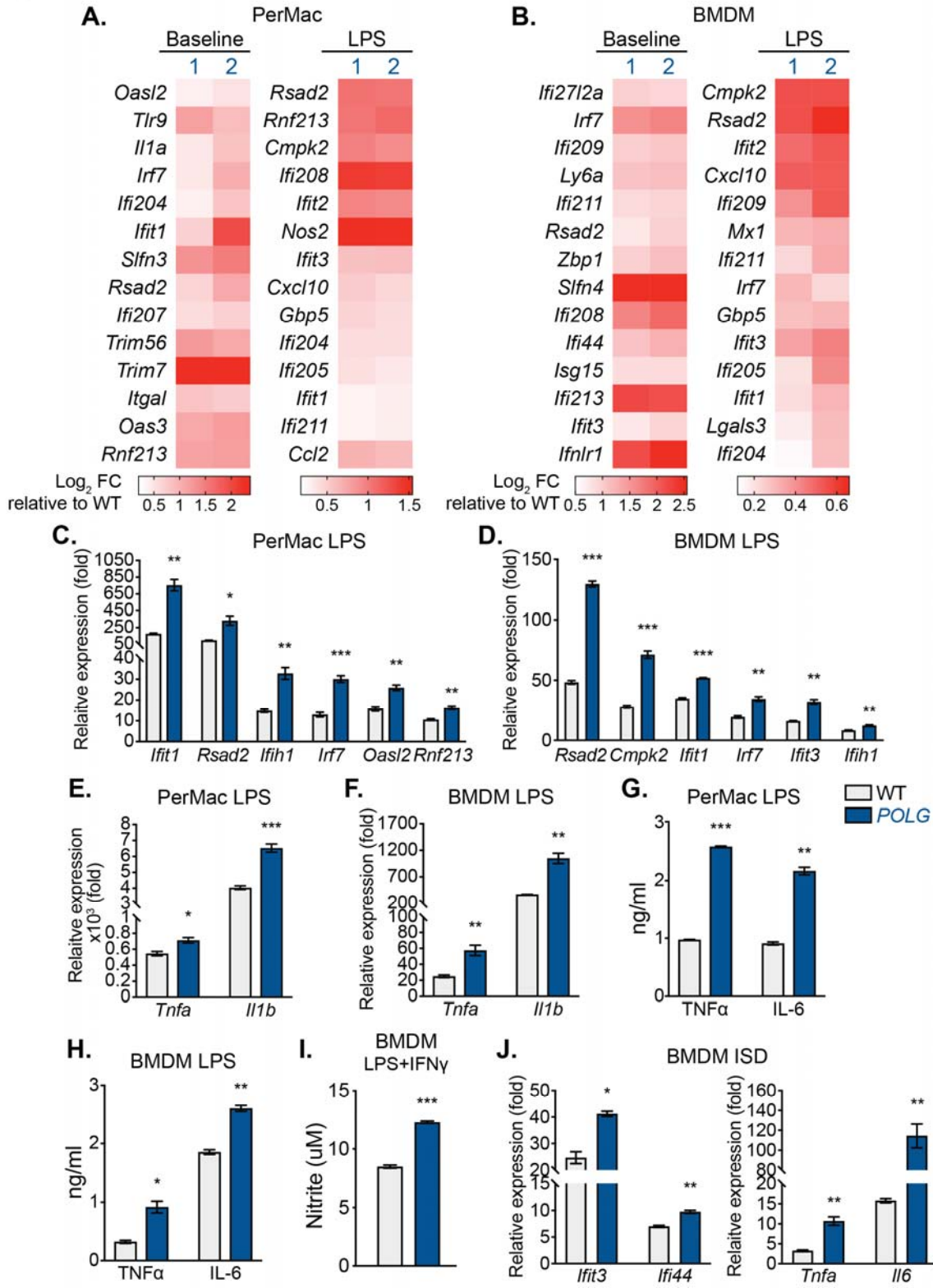


Figure 2. POLG mutator macrophages exhibit enhanced IFN-I and pro-inflammatory responses after innate immune stimulation.

052 (A and B) Heatmap of RNAseq data displaying statistically significant ($P < 0.05$ in two biological
053 replicates) differences in ISG expression between WT and *POLG* mutator peritoneal macrophages
054 (PerMacs) (A) and bone marrow-derived macrophages (BMDMs) (B) post LPS challenge
055 (200ng/ml for 6h).

056 (C-F) qRT-PCR analysis of ISG and pro-inflammatory cytokine expression in WT and *POLG*
057 PerMacs (C and E) and BMDM (D and F) after 4h or 6h of LPS stimulation.

058 (G and H) Pro-inflammatory cytokine secretion in WT and *POLG* PerMacs (G) or BMDMs (H)
059 after 4h or 6h of LPS stimulation.

060 (I) Nitrite levels in WT and *POLG* BMDM after 17h of LPS (20ng/ml) +IFN γ (50ng/ml)
061 treatment. (J) qRT-PCR analysis of ISGs and cytokines expression in WT and *POLG* BMDMs
062 after 4h of 2ug/ml ISD transfection.

063 Statistical significance was determined using unpaired Student's t-tests. $*P < 0.05$; $**P < 0.01$;
064 $***P < 0.001$. Error bars represent S.E.M.

Figure 3. The cGAS-STING-IFN-I signaling axis regulates inflammatory monocyte expansion and elevated cytokine secretion in POLG mutator mice.

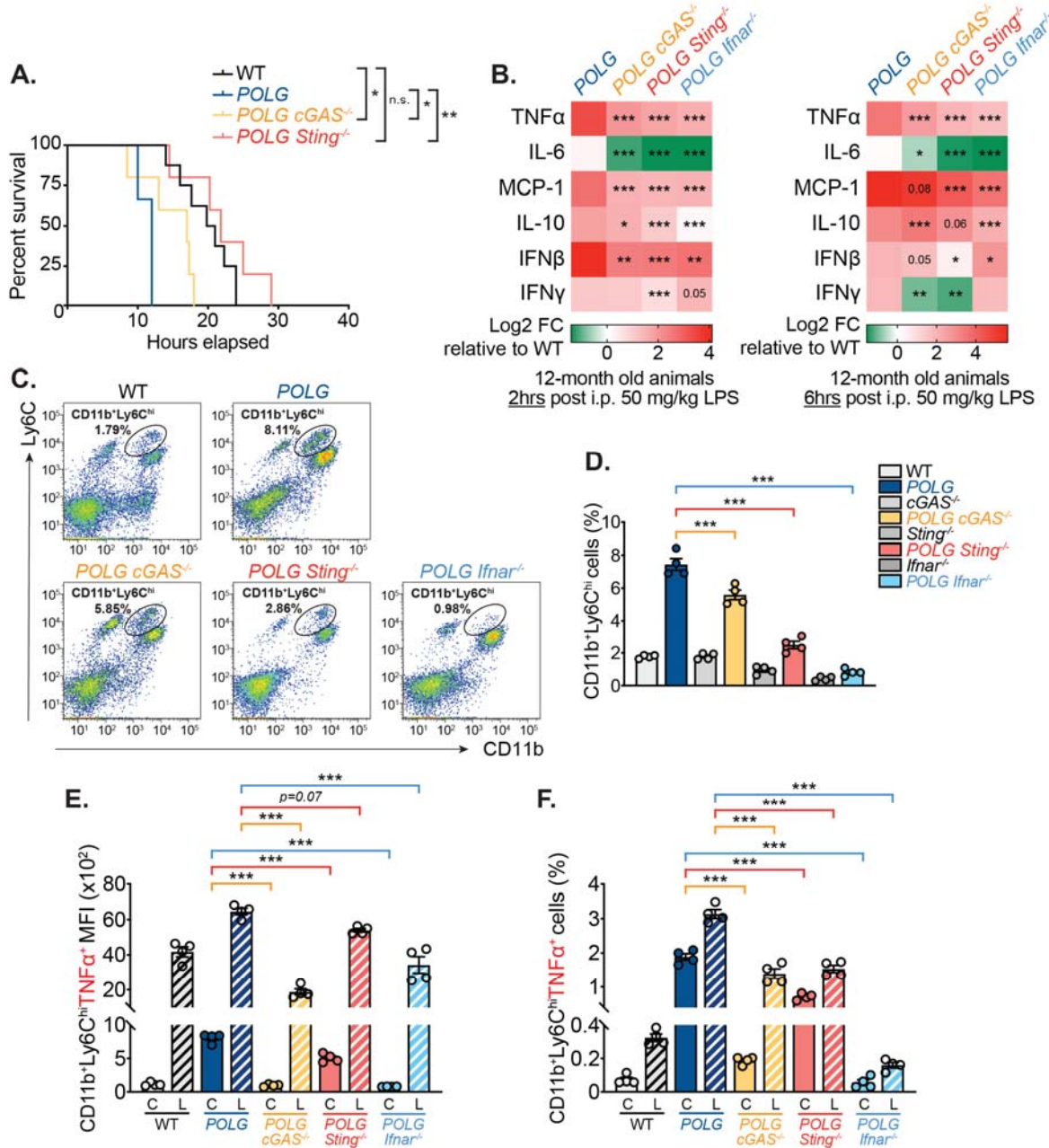
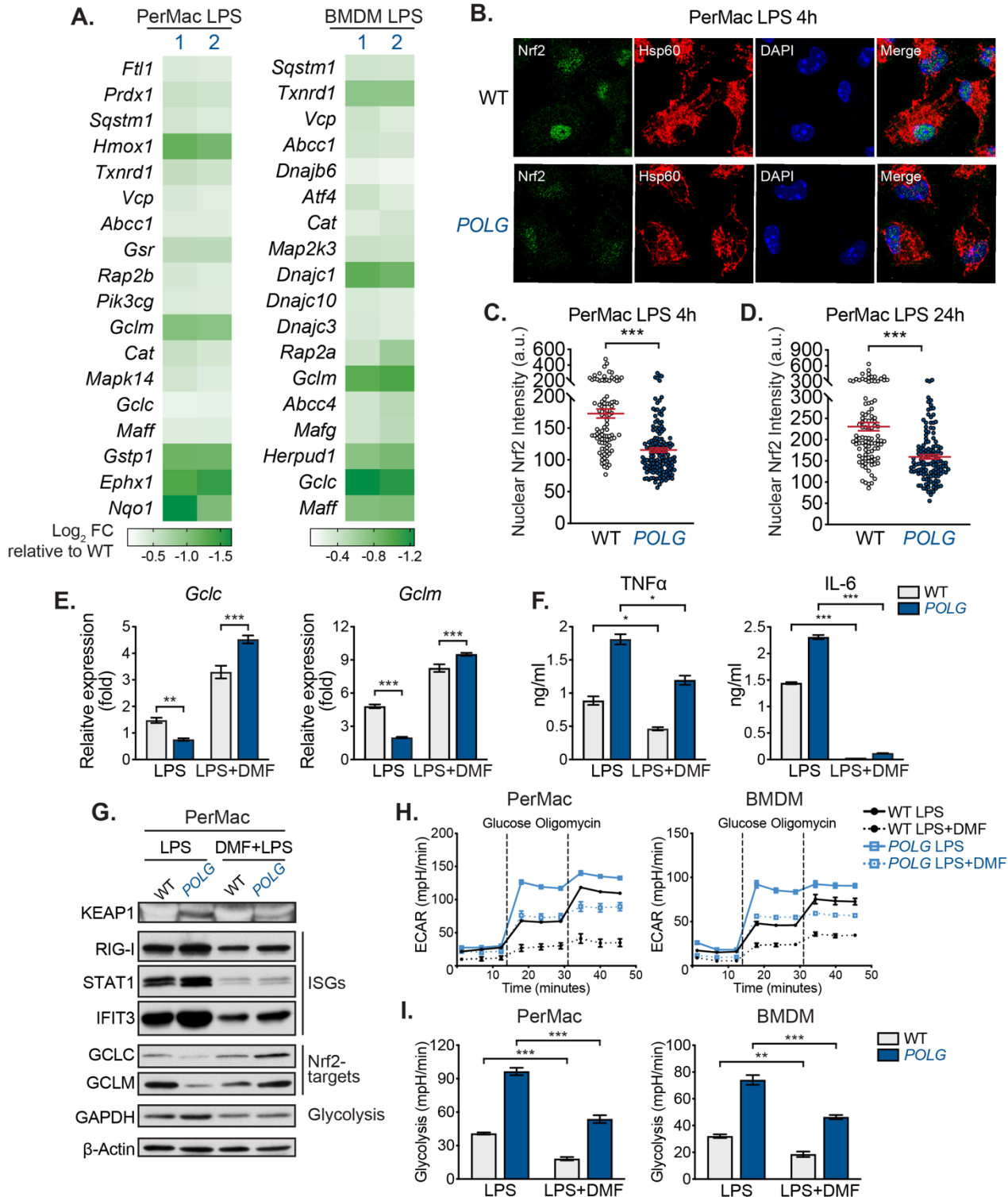


Figure 3. The cGAS-STING-IFN-I signaling axis regulates inflammatory monocyte expansion and elevated cytokine secretion in POLG mutator mice.

(A and B) 12-month old WT, *POLG*, *POLG cGAS*^{-/-}, *POLG Sting*^{-/-} and *POLG Ifnar*^{-/-} (n=5-8 per group) mice were i.p. injected with 50 mg/kg LPS. Kaplan-Meier survival analysis was performed (A). Plasma was collected at indicated time points (n=6 at 2h and n=4 at 6h) and subjected to multi-analyte cytokine analysis (B). Statistical comparisons (B) were made against

072 LPS injected *POLG* mice. Log-rank (Mantel-Cox) test was used to compare percent survival
073 between different groups. (C and D) CD11b⁺Ly6C^{hi} inflammatory monocyte population in whole
074 blood from 12-month old mice was evaluated by flow cytometry. Pseudocolor plots are
075 representative of 4 independent experiments (C) and quantification of the percentage of
076 CD11b⁺Ly6C^{hi} cells is shown in (D).
077 (E and F) CD11b⁺Ly6C^{hi}TNF α ⁺ inflammatory monocyte population in unstimulated [C] or LPS
078 challenged [L] whole blood from 12-month old cohorts was evaluated by flow cytometry.
079 Quantification of CD11b⁺Ly6C^{hi}TNF α ⁺ mean fluorescent intensity (MFI) is shown in (E), and the
080 percentage of CD11b⁺Ly6C^{hi}TNF α ⁺ cells is shown in (F).
081 Unless stated, statistical significance was determined using unpaired Student's t-tests or ANOVA.
082 * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Error bars represent S.E.M.

Figure 4. Nrf2 suppression contributes to the hyper-inflammatory phenotype of POLG mutator macrophages.



883
884 **Figure 4. Nrf2 suppression contributes to the hyper-inflammatory phenotype of POLG**
885 **mutator macrophages.**

086 (A) Heatmap of RNAseq data displaying statistically significant ($P < 0.05$ in two biological
087 replicates) differences in Nrf2 target gene expression between WT and *POLG* mutator PerMacs
088 BMDMs post LPS challenge (200ng/ml for 6h).

089 (B) Representative confocal microscopy images of LPS treated PerMacs stained with anti-Nrf2
090 and -Hsp60 antibodies and DAPI.

091 (C and D) Quantification of nuclear Nrf2 staining intensity in WT and *POLG* PerMacs 4h (C) or
092 24h (D) post LPS stimulation. a.u., arbitrary unit.

093 (E) qRT-PCR analysis of Nrf2 target gene expression in WT and *POLG* PerMacs post LPS or
094 LPS+DMF treatment.

095 (F) Pro-inflammatory cytokine secretion by WT and *POLG* PerMacs after LPS or LPS+DMF
096 treatment.

097 (G) Protein expression in WT and *POLG* PerMacs post LPS or LPS+DMF treatment.

098 (H and I) Seahorse ECAR analysis of WT and *POLG* PerMacs and BMDMs post LPS or
099 LPS+DMF exposure (H). Glycolysis rate was calculated and plotted in (I).

100 Statistical significance was determined using unpaired Student's t-tests. $*P < 0.05$; $**P < 0.01$;

101 $***P < 0.001$. Error bars represent S.E.M.

Figure 5. Elevated IFN-I signaling represses Nrf2 activity and drives pro-inflammatory metabolic phenotypes in POLG mutator macrophages.

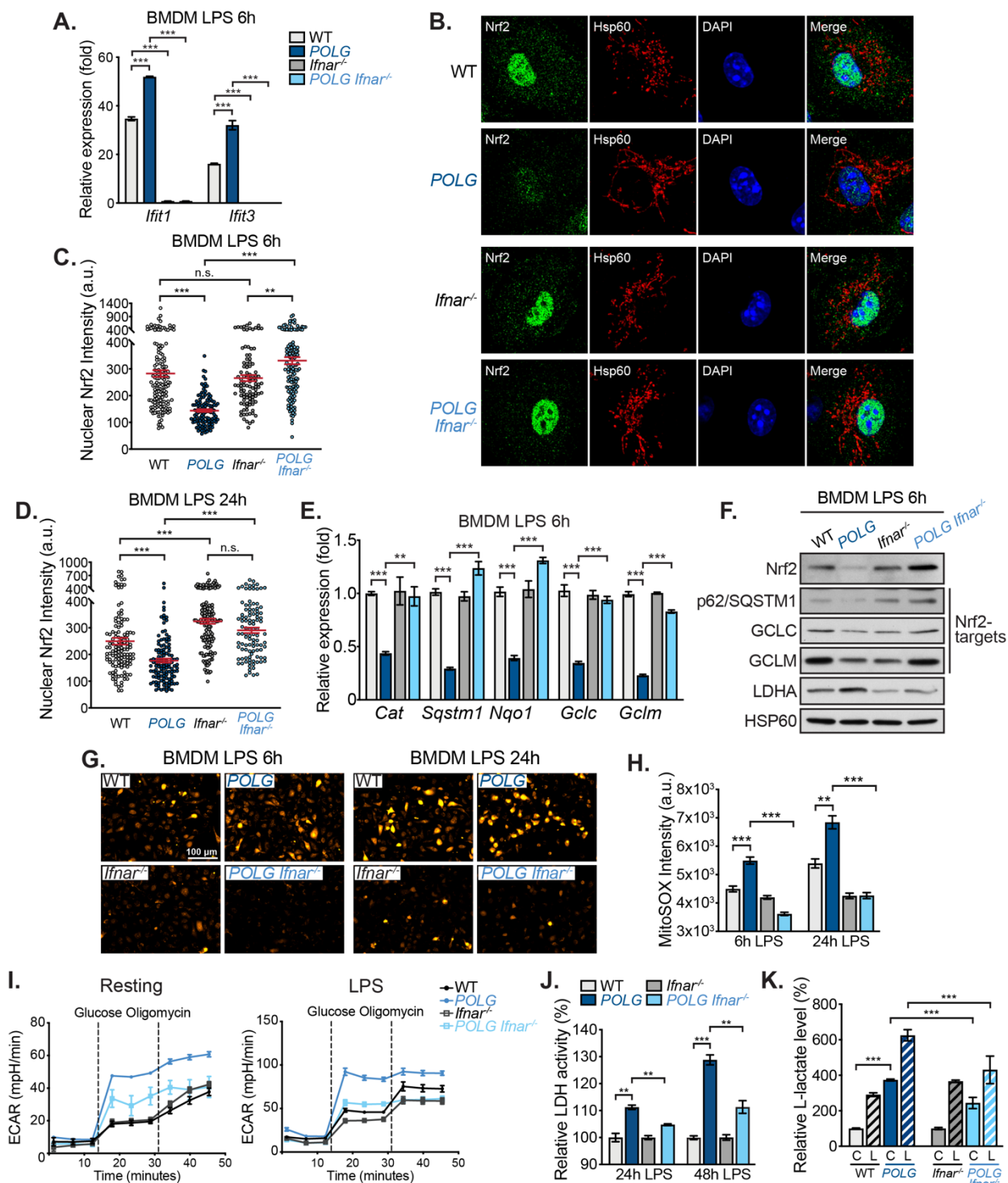


Figure 5. Elevated IFN-I signaling represses Nrf2 activity and drives pro-inflammatory metabolic phenotypes in POLG mutator macrophages.

(A) qRT-PCR analysis of ISG expression in BMDMs after LPS challenge.

006 (B) Representative confocal microscopy images of LPS treated BMDMs stained with anti-Nrf2
007 and -Hsp60 antibodies and DAPI.

008 (C and D) Quantification of nuclear Nrf2 staining intensity in BMDMs 6h (C) or 24h (D) after
009 LPS exposure. a.u., arbitrary unit.

010 (E) qRT-PCR analysis of Nrf2 target gene expression in BMDMs post LPS exposure. *POLG*
011 BMDMs were normalized to WT BMDMs, *POLG Ifnar^{-/-}* BMDMs were normalized to *Ifnar^{-/-}*
012 BMDMs.

013 (F) Protein expression of Nrf2 targets and LDHA in BMDMs after 6h LPS exposure.

014 (G) Representative microscopy images of LPS treated BMDMs stained with MitoSOX.

015 (H) Quantification of MFI of MitoSOX staining in LPS treated BMDMs.

016 (I) Seahorse ECAR analysis of BMDMs with or without overnight LPS (10ng/ml) challenge.

017 (J) Relative LDH activity in BMDMs 24h and 48h post LPS exposure (normalized to WT).

018 (K) Extracellular L-Lactate level in culture media of resting [C] or LPS exposed [L] BMDMs.
019 WT and *POLG* were normalized to WT resting BMDMs, *Ifnar^{-/-}* and *POLG Ifnar^{-/-}* were
020 normalized to *Ifnar^{-/-}* resting BMDMs.

021 Statistical significance was determined using unpaired Student's t-tests or ANOVA. * $P < 0.05$;
022 ** $P < 0.01$; *** $P < 0.001$. Error bars represent S.E.M.

Figure 6. Ablation of IFN-I signaling lessens multi-organ pathology and extends lifespan in POLG mutator mice.

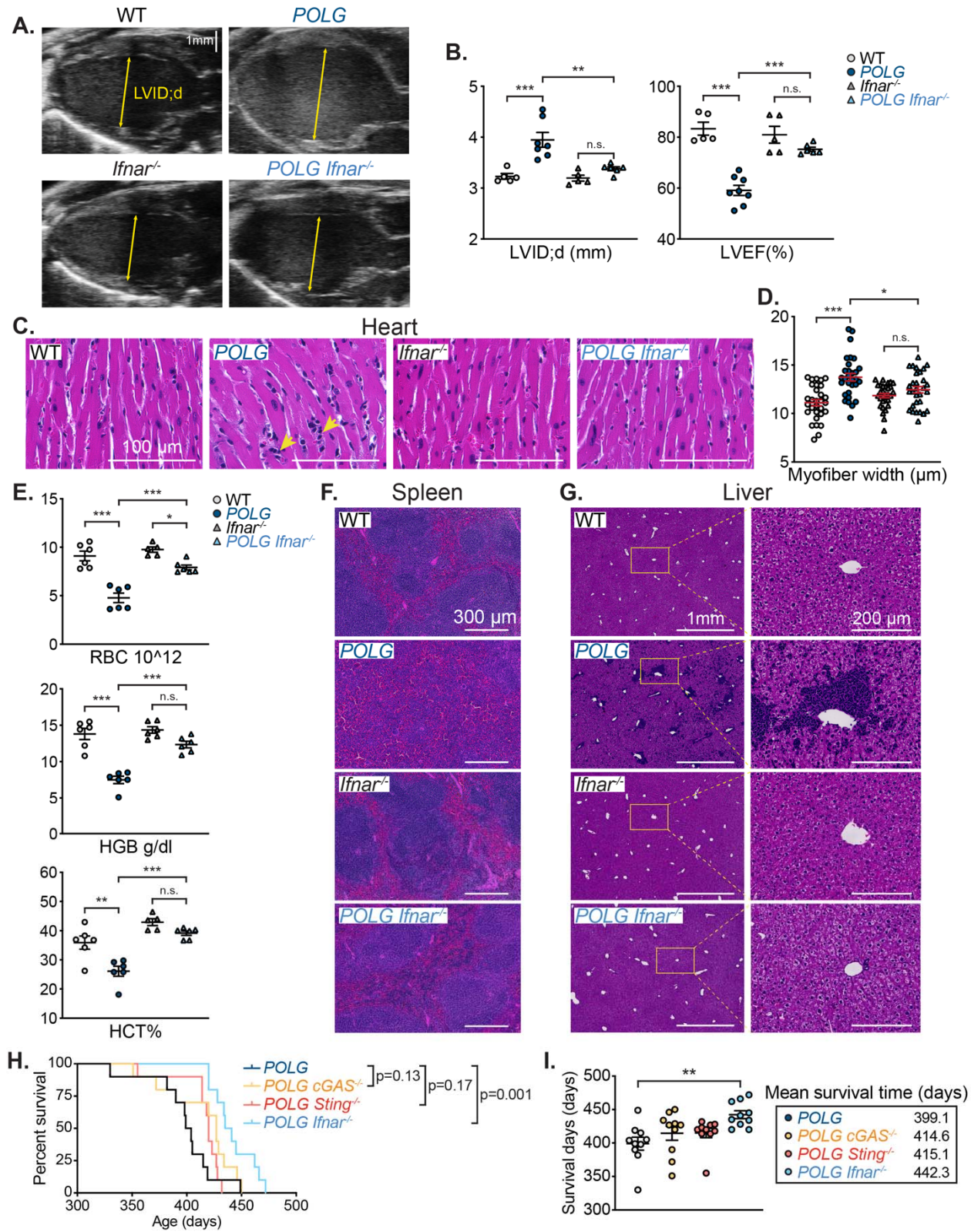


Figure 6. Ablation of IFN-I signaling lessens multi-organ pathology and extends lifespan in POLG mutator mice.

026 (A) Representative B-mode echocardiogram images of 9-10-month old WT, *POLG*, *Ifnar*^{-/-} and
027 *POLG Ifnar*^{-/-} mouse hearts.

028 (B) Left ventricular internal dimension at end-diastole (LVID;d) and left ventricular ejection
029 fraction (LVEF) calculated from M-mode or B-mode images using Vevo Lab software. n=5-8
030 animals per genotype.

031 (C and D) Representative H&E staining of heart sections from WT, *POLG*, *Ifnar*^{-/-} and *POLG*
032 *Ifnar*^{-/-} mice (C) and quantification of cardiomyocyte width (D). Yellow arrows indicate
033 infiltrating immune cells. 5 myocytes per section and 6 animals per genotype were quantified in a
034 blinded fashion (D).

035 (E) Red blood cell counts (RBC), hemoglobin concentration (HGB) and hematocrit (HCT) were
036 measured in WT, *POLG*, *Ifnar*^{-/-} and *POLG Ifnar*^{-/-} mouse whole blood using the HM5
037 Hematology Analyzer. n=6 animals per genotype.

038 (F) Representative H&E staining showing white pulp and red pulp organization in WT, *POLG*,
039 *Ifnar*^{-/-} and *POLG Ifnar*^{-/-} mouse spleens.

040 (G) Representative H&E stained liver sections from WT, *POLG*, *Ifnar*^{-/-} and *POLG Ifnar*^{-/-}
041 cohorts.

042 (H and I) Percent survival (H) and survival time (I) of *POLG*, *POLG cGAS*^{-/-}, *POLG Sting*^{-/-} and
043 *POLG Ifnar*^{-/-} mice. Log-rank (Mantel-Cox) test was used to compare percent survival between
044 different groups. n=10 animals per genotype.

045 Unless stated, statistical significance was determined using ANOVA. **P* < 0.05; ***P* < 0.01; ****P*
046 < 0.001. Error bars represent S.E.M.

047

048 **Supplementary Materials**

Figure S1. CD11b⁺ myeloid cell expansion in POLG mutator mice drives systemic hyper-inflammatory responses to innate immune challenge.

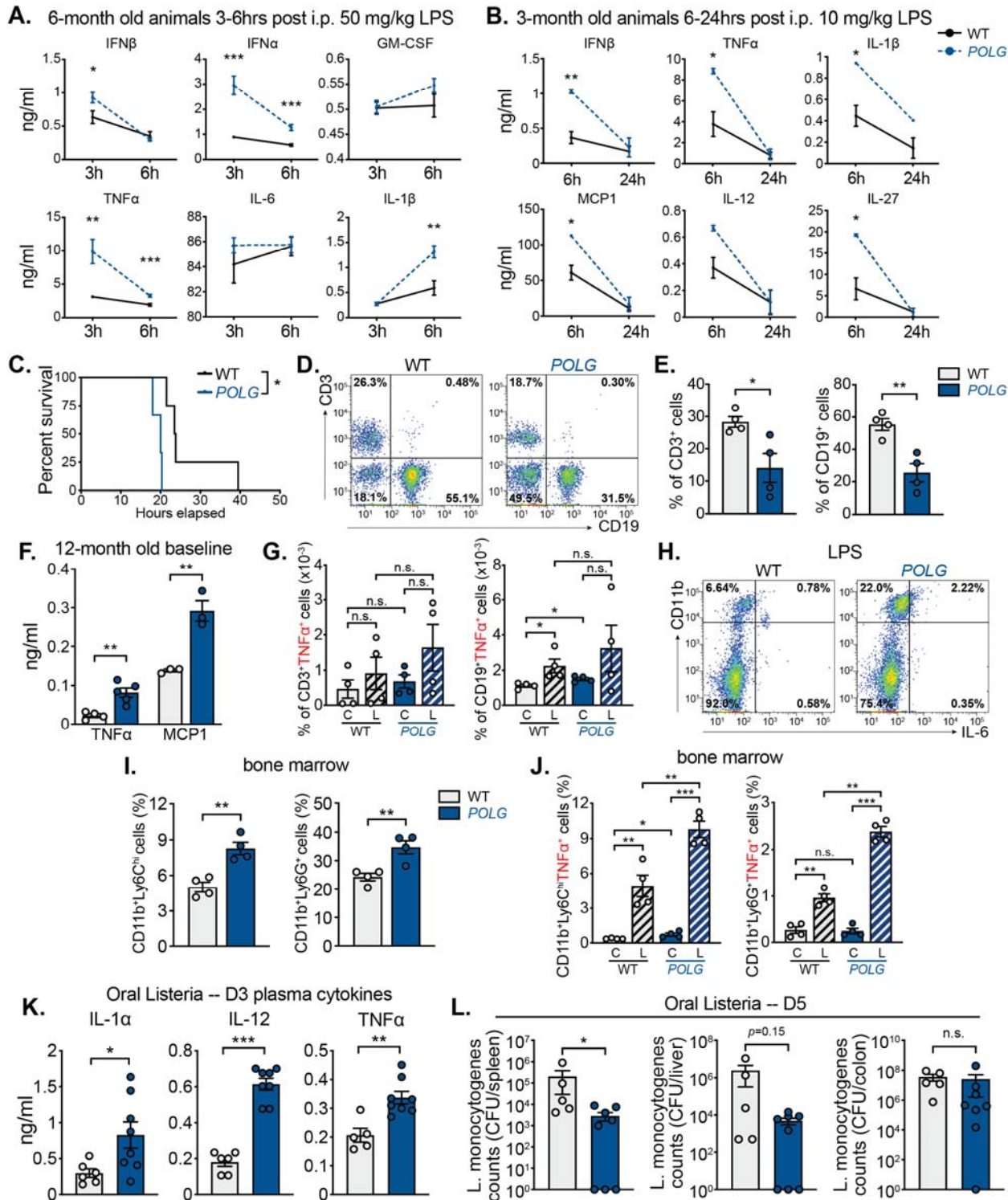


Figure S1. CD11b⁺ myeloid cell expansion in POLG mutator mice drives systemic hyper-inflammatory responses to innate immune challenge.

052 (A and C) 6-month old WT and *POLG* mice were i.p. injected with 50 mg/kg LPS. Plasma was
053 collected and subjected to multi-analyte cytokine analysis.

054 (B) 3-month old WT and *POLG* mice were i.p. injected with 10 mg/kg LPS (n=3). Plasma was
055 collected and subjected to multi-analyte cytokine analysis.

056 (C) Kaplan-Meyer survival analysis was performed on 6-month old WT and *POLG* mice (n=3-4).
057 Log-rank (Mantel-Cox) test was used to compare percent survival between different groups.

058 (D and E) T cell (CD3⁺) and B cell (CD19⁺) populations in whole blood from 12-month old WT
059 and *POLG* mice were evaluated by flow cytometry. Pseudocolor plots are representative of 4
060 independent experiments (D) and quantification is shown in (E).

061 (F) Cytokine levels in WT and *POLG* mutator plasma at baseline.

062 (G) CD3⁺TNF α ⁺ and CD19⁺TNF α ⁺ MFI in unstimulated [C] or LPS challenged [L] whole blood
063 from 12-month old WT and *POLG* mice as determined by flow cytometry.

064 (H) CD11b⁺IL-6⁺ population in LPS challenged whole blood from 12-month old WT and *POLG*
065 mice was evaluated by flow cytometry. Pseudocolor plots are representative of 4 independent
066 experiments.

067 (I) The percentage of CD11b⁺Ly6C^{hi} and CD11b⁺Ly6G⁺ cells in WT and *POLG* bone marrow as
068 determined by flow cytometry.

069 (J) The percentage of CD11b⁺Ly6C^{hi}TNF α ⁺ and CD11b⁺Ly6G⁺TNF α ⁺ populations in
070 unstimulated [C] or LPS challenged [L] bone marrow from 12-month old WT and *POLG* mice as
071 quantified by flow cytometry.

072 (K) Cytokine levels in WT and *POLG* mutator plasma 3 days post *L. monocytogenes* infection.

073 (L) *L. monocytogenes* CFU counts in mouse spleen, liver and colon D5 post infection. Mann-
074 Whitney test was used to compare CFUs in tissues between WT and *POLG* mice.

075 Unless stated, statistical significance was determined using unpaired Student's t-tests. **P* < 0.05;

076 ***P* < 0.01; ****P* < 0.001. Error bars represent S.E.M.

Figure S2. STING regulates enhanced IFN-I and pro-inflammatory responses in LPS challenged *POLG* mutator macrophages.

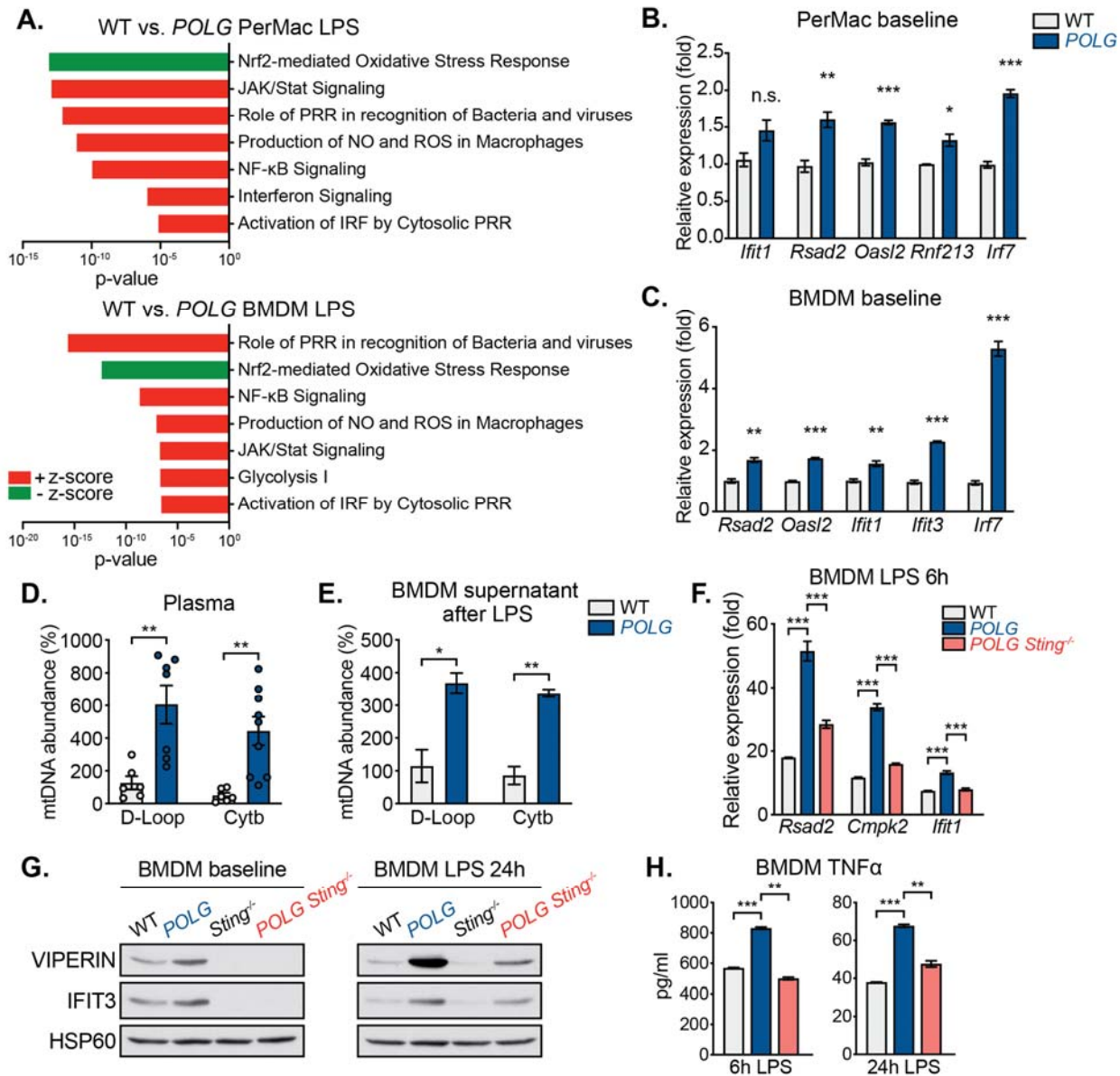


Figure S2. STING regulates enhanced IFN-I and pro-inflammatory responses in LPS challenged *POLG* mutator macrophages.

(A) Ingenuity Pathway Analysis comparing RNAseq datasets from LPS treated (200ng/ml for 6h)

WT and *POLG* PerMac and BMDMs.

(B and C) qRT-PCR analysis of ISG expression in resting WT and *POLG* PerMac (B) and

BMDMs (C).

084 (D) qPCR analysis of circulating, cell-free mtDNA abundance in 12-month old WT and *POLG*
085 mutator plasma. n=6-9 animals per genotype.

086 (E) qPCR analysis of WT and *POLG* BMDMs mtDNA release into the culture supernatant 48h
087 after 200ng/ml LPS exposure.

088 (F) qRT-PCR analysis of ISG expression in WT, *POLG* and *POLG Sting^{-/-}* BMDMs after LPS
089 challenge.

090 (G) Protein abundance of ISGs in WT, *POLG*, *Sting^{-/-}* and *POLG Sting^{-/-}* BMDMs without or with
091 LPS treatment.

092 (H) Pro-inflammatory cytokine secretion of WT, *POLG* and *POLG Sting^{-/-}* BMDMs after LPS
093 challenge.

094 Statistical significance was determined using unpaired Student's t-tests. **P* < 0.05; ***P* < 0.01;
095 ****P* < 0.001. Error bars represent S.E.M.

Figure S3. Neutrophil expansion and elevated pro-inflammatory cytokine production in POLG mutator mice is regulated by the cGAS-STING-IFN-I signaling axis.

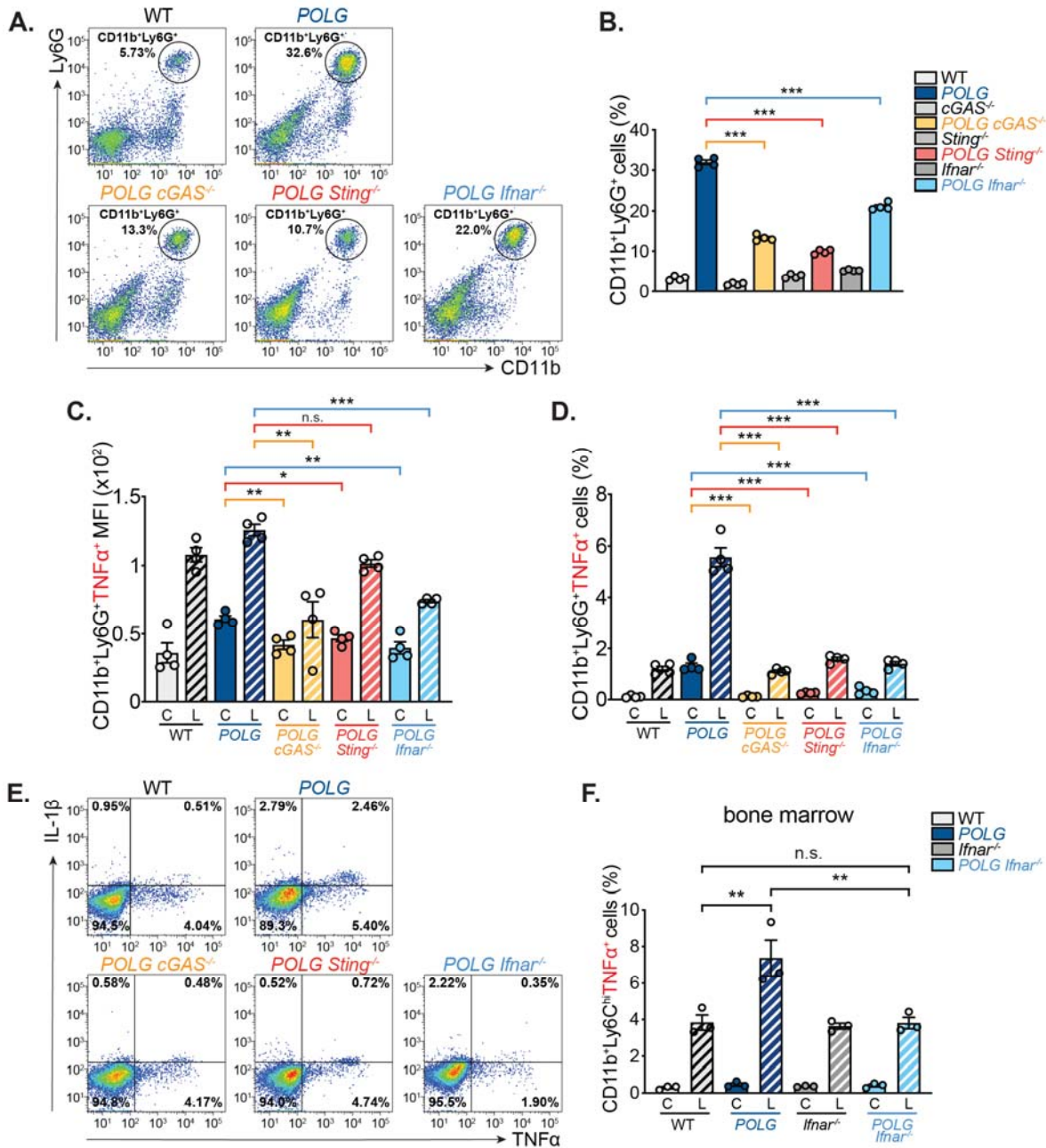


Figure S3. Neutrophil expansion and elevated pro-inflammatory cytokine production in POLG mutator mice is regulated by the cGAS-STING-IFN-I signaling axis.

(A and B) CD11b⁺Ly6G⁺ neutrophil population in the whole blood was evaluated by flow cytometry. Pseudocolor plots are representative of 4 independent experiments (A) and quantification of the percentage of CD11b⁺Ly6G⁺ cells is shown in (B).

102 (C and D) CD11b⁺Ly6G⁺TNFα⁺ neutrophil population in unstimulated [C] or LPS challenged [L]
103 whole blood from 12-month old cohorts was evaluated by flow cytometry. Quantification of
104 CD11b⁺Ly6G⁺TNFα⁺ mean fluorescent intensity (MFI) is shown in (C), and the percentage of
105 CD11b⁺Ly6G⁺TNFα⁺ cells is shown in (D).

106 (E) Intracellular TNFα and IL-1β staining was performed on LPS challenged whole blood from
107 12-month old cohorts. Pseudocolor plots are representative of 2 independent experiments.

108 (F) The percentage of CD11b⁺Ly6C^{hi}TNFα⁺ inflammatory monocyte population in unstimulated
109 [C] or LPS challenged [L] bone marrow from 12-month old cohorts as quantified by flow
110 cytometry.

111 Statistical significance was determined using unpaired Student's t-tests or ANOVA. **P* < 0.05;
112 ***P* < 0.01; ****P* < 0.001. Error bars represent S.E.M.

Figure S4. POLG mutator macrophages exhibit normal NF- κ B and IFN-I signaling kinetics in response to LPS, but displayed impaired Nrf2 activity.

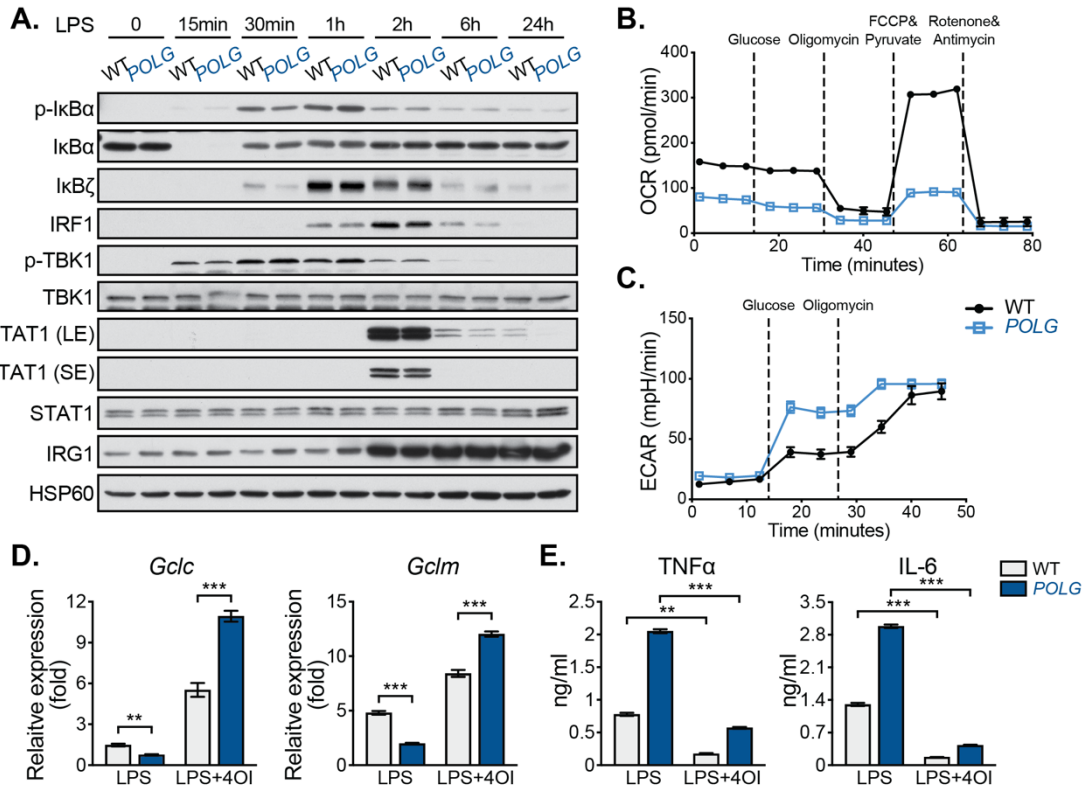


Figure S4. POLG mutator macrophages exhibit normal NF- κ B and IFN-I signaling kinetics in response to LPS, but displayed impaired Nrf2 activity.

(A) NF- κ B and IFN-I signaling pathways protein expression in WT and *POLG* BMDMs after LPS exposure over a time-course.

(B and C) Seahorse analysis of resting WT and *POLG* BMDMs represented as OCR (B) and ECAR (C).

(D) qRT-PCR analysis of Nrf2 target gene expression in WT and *POLG* PerMacs after LPS or LPS+4OI treatment.

(E) Pro-inflammatory cytokine secretion by WT and *POLG* PerMacs after LPS or LPS+4OI treatment.

Statistical significance was determined using unpaired Student's t-tests. * $P < 0.05$; ** $P < 0.01$;

*** $P < 0.001$. Error bars represent S.E.M.

Figure S5. Elevated IFN-I signaling represses Nrf2 activity and drives pro-inflammatory cytokine and metabolic phenotypes in POLG mutator macrophages.

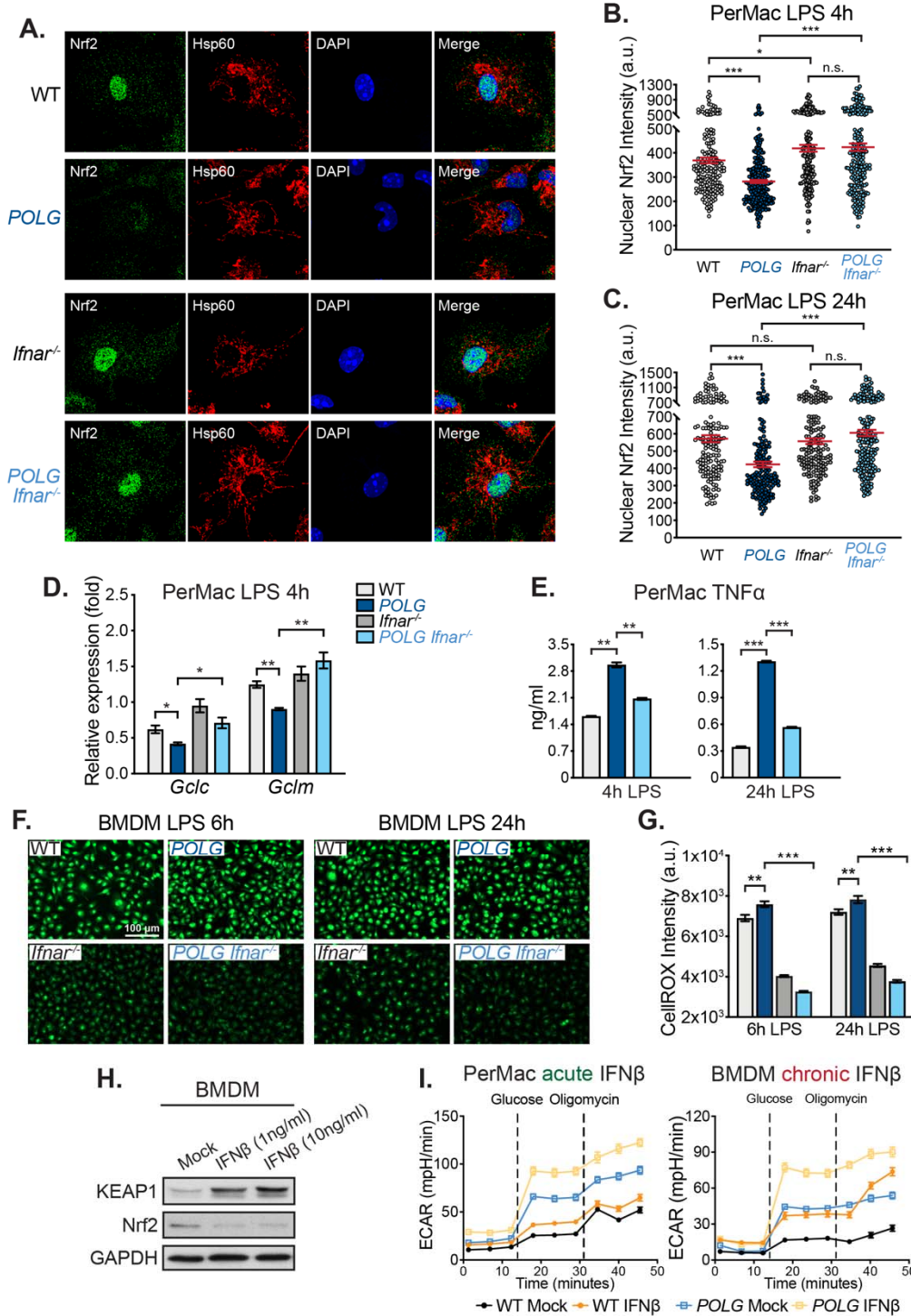
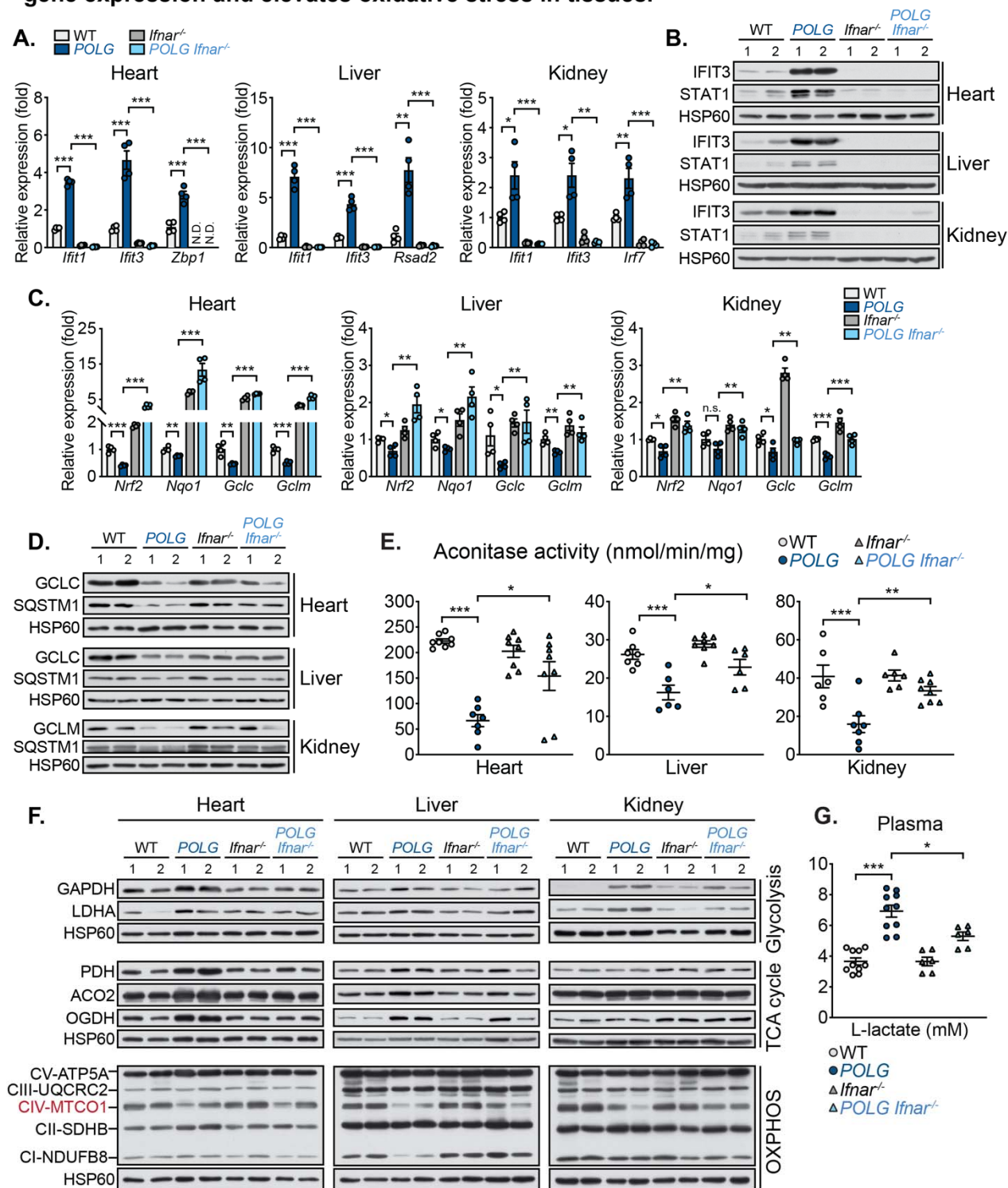


Figure S5. Elevated IFN-I signaling represses Nrf2 activity and drives pro-inflammatory cytokine and metabolic phenotypes in POLG mutator macrophages.

(A) Representative confocal microscopy images of LPS treated PerMacs stained with anti-Nrf2 and -Hsp60 antibodies and DAPI.

- 131 (B and C) Quantification of nuclear Nrf2 staining intensity in PerMacs 4h (b) or 24h (c) after LPS
132 exposure. a.u., arbitrary unit.
- 133 (D) Nrf2 target gene expression in PerMacs post LPS exposure.
- 134 (E) TNF α secretion by PerMacs 4h and 24h post LPS exposure.
- 135 (F) Representative microscopy images of LPS treated BMDMs stained with CellROX.
- 136 (G) Quantification of MFI of CellROX staining in LPS treated BMDMs.
- 137 (H) Protein expression in WT BMDM after 24h of IFN β exposure.
- 138 (I) Seahorse ECAR of WT and *POLG* PerMacs and BMDMs post-acute (24h) or -chronic (5
139 days) IFN β exposure.
- 140 Statistical significance was determined using unpaired Student's t-tests or ANOVA. * $P < 0.05$;
141 ** $P < 0.01$; *** $P < 0.001$. Error bars represent S.E.M.

Figure S6. Chronic IFN-I signaling in aged POLG mutator mice represses Nrf2 target gene expression and elevates oxidative stress in tissues.



142

143

144

Figure S6. Chronic IFN-I signaling in aged POLG mutator mice represses Nrf2 target gene expression and elevates oxidative stress in tissues.

145 (A and B) Transcript (A) and protein (B) levels of ISGs in WT, *POLG*, *Ifnar*^{-/-} and *POLG Ifnar*^{-/-}
146 mouse heart, liver and kidney. 2 animals (12-month old) per genotype are represented.

147 (C and D) Transcript (C) and protein (D) levels of and Nrf2-targets in WT, *POLG*, *Ifnar*^{-/-} and
148 *POLG Ifnar*^{-/-} mouse heart, liver and kidney. 2 animals (12-month old) per genotype are
149 represented.

150 (E) Aconitase activity in WT, *POLG*, *Ifnar*^{-/-} and *POLG Ifnar*^{-/-} heart, liver and kidney. n=4-5
151 animals per genotype and each animal was represented in duplicates.

152 (F) Protein levels of glycolytic enzymes, TCA cycle enzymes, and OXPHOS complexes in WT,
153 *POLG*, *Ifnar*^{-/-} and *POLG Ifnar*^{-/-} mouse tissues. 2 animals (12-month old) per genotype are
154 represented.

155 (G) L-lactate concentration in plasma from WT, *POLG*, *Ifnar*^{-/-} and *POLG Ifnar*^{-/-} mice. n=6-10
156 animals per genotype.

157 Statistical significance was determined using unpaired Student's t-tests. **P* < 0.05; ***P* < 0.01;
158 ****P* < 0.001. Error bars represent S.E.M.

Figure S7. Ablation of IFN-I signaling attenuates cardiomyopathy and anemia, and improves body condition of POLG mutators.

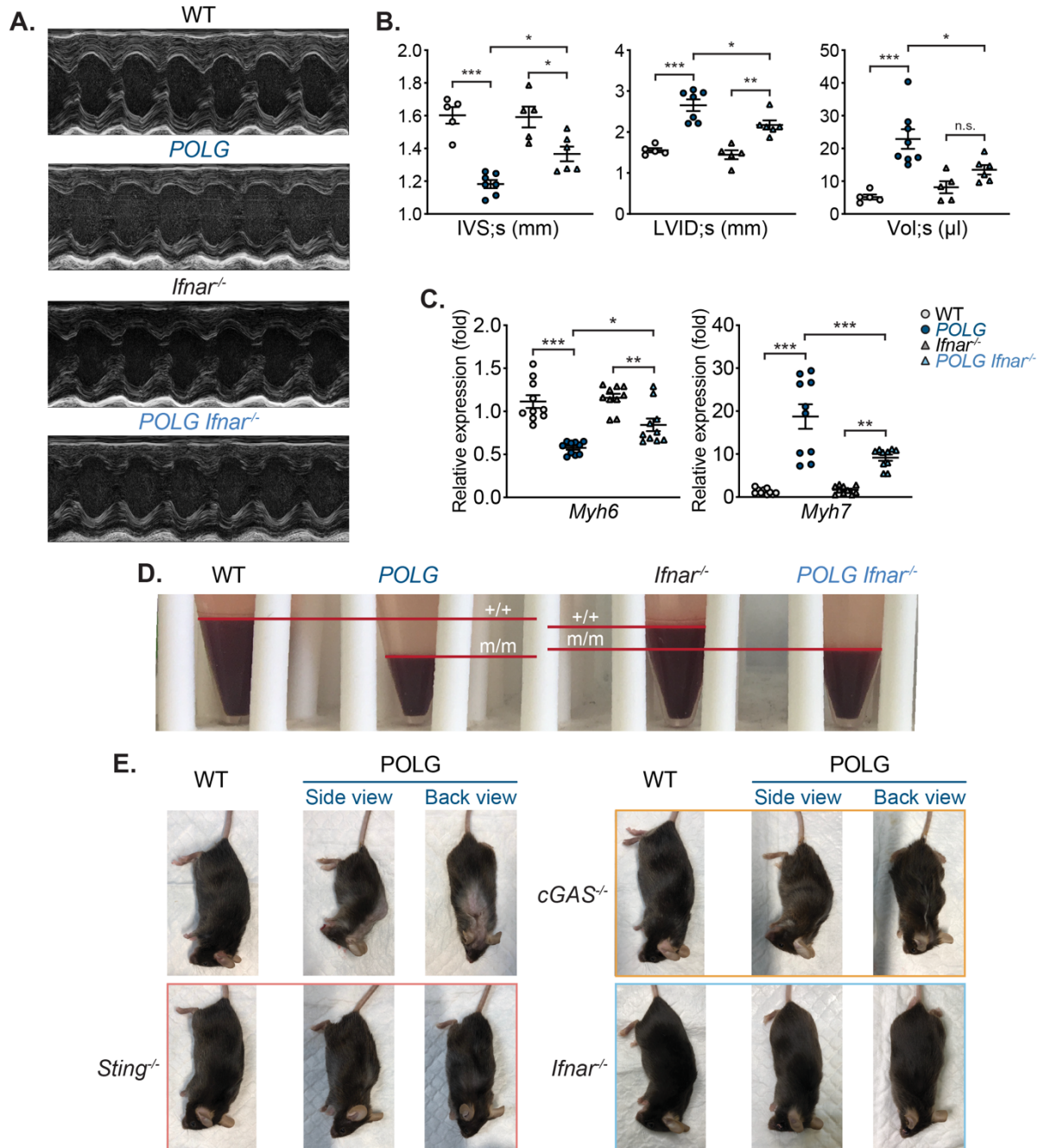


Figure S7. Ablation of IFN-I signaling attenuates cardiomyopathy and anemia, and improves body condition of POLG mutators.

(A) Representative M-mode ultrasound echocardiograms of WT, *POLG*, *Ifnar*^{-/-} and *POLG Ifnar*^{-/-} hearts.

164 (B) Interventricular septum thickness at end-systole (IVS;s), left ventricular internal dimension at
165 end-systole (LVID;s) and left ventricular volume at end-systole (Vol;s) were measured in WT,
166 *POLG*, *Ifnar*^{-/-} and *POLG Ifnar*^{-/-} mouse hearts. n=5-8 animals per genotype.

167 (C) qRT-PCR analysis of *Myh6* and *Myh7* expression from heart homogenates. n=5 animals per
168 genotype and each animal was represented in duplicates.

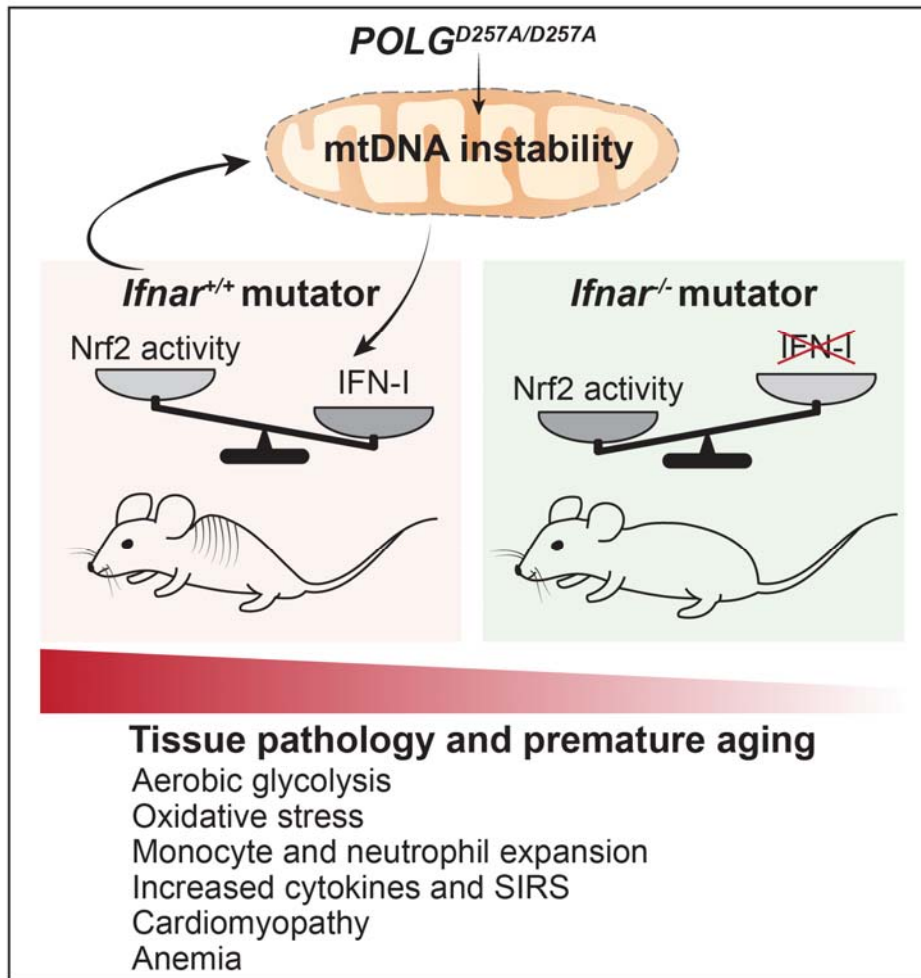
169 (D) Representative images of blood pellets from exsanguinated 12-month old mice.

170 (E) Representative images of 12-month old *POLG*, *POLG cGAS*^{-/-}, *POLG Sting*^{-/-} and *POLG*
171 *Ifnar*^{-/-} animals.

172 Statistical significance was determined using ANOVA.**P* < 0.05; ***P* < 0.01; ****P* < 0.001.

173 Error bars represent S.E.M.

Figure S8. Imbalances in IFN-I and Nrf2 signaling contribute to inflammatory and age-related phenotypes in POLG mutator mice.



174

175 **Figure S8. Imbalances in IFN-I and Nrf2 signaling contribute to inflammatory and age-**
176 **related phenotypes in POLG mutator mice.**

177 mtDNA instability and mitochondrial dysfunction in POLG mutator mice lead to elevated IFN-I
178 responses, which subsequently repress Nrf2 activity and enhance aerobic glycolysis.
179 Consequently, chronic IFN-I responses augment the expansion and inflammatory potential of
180 CD11b⁺ myeloid cells and macrophages, while also contributing to cardiomyopathy and anemia
181 in mutator mice. Genetic ablation of IFN-I signaling relieves the break on Nrf2 and significantly
182 improves healthspan by limiting myeloid reprogramming, inflammation, and tissue dysfunction in
183 mtDNA mutator mice.

