1 **Induction of transposable element expression is central to innate sensing**

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24 **SUMMARY**

25 Evidence indicates that transposable elements (TEs) stimulate innate sensing pathways in

26 various pathologies but it is not clear whether they are sensed during normal physiological

27 responses. Here we show that, during activation with an exogenous pathogen associated

28 molecular pattern (PAMP), dendritic cells (DCs) epigenetically remodel heterochromatin at TEs

29 by depleting the methyltransferase *Suv39h1* and reducing histone-3 lysine-9 trimethylation

30 (H3K9me3). TLR4 signaling activates TE expression to enhance innate responses through the

31 DNA sensor cGAS. Cytosolic cGAS-bound DNA comprised LINE1 TEs as the predominant

32 endogenous ligands. Concordantly, LINE1 inhibition attenuated the type-I IFN response to LPS

33 and rescued influenza virus infection. We propose that in healthy cells, exogenous PAMPs

34 epigenetically activate self-derived PAMPs (LINE1) that engage cGAS to enhance responses.

35 These data explain why pathogens employ redundant and broad innate immune

36 countermeasures, to suppress activation of host PAMPs and illustrate a hitherto unappreciated

37 role for host genome-derived PAMPs in response to pathogens.

38

39 **INTRODUCTION**

40 The ability to distinguish self from non-self is a central principle of immunity. Invading pathogens

41 must be recognized as non-self to trigger an adequate response while self-antigens must be

42   tolerated to avoid autoimmunity. Innate detection of pathogens depends on the recognition of

43   pathogen associated molecular patterns (PAMP) by pattern recognition receptors (PRR). PRRs

44   include the Toll-like receptors (TLR) that sample extracellular and endosomal spaces for

45   PAMPs (*1*) and various sensors that detect RNA and DNA (*2*). Upon PAMP detection, PRRs

46   trigger signaling cascades that induce transcription factors to activate defensive gene

47   expression. A key set of early defense genes include the type 1 interferons (IFN-I) that bind the

48   ubiquitously expressed interferon receptor (IFNAR1/2) to activate JAK/STAT signaling and

49   downstream transcription factors. The subsequent expression of up to 2000 interferon

50   stimulated genes (ISGs) induces a defensive state in infected and neighboring uninfected cells

51   through complex and incompletely understood mechanisms (*3*).

52

53   Recognition of foreign nucleic acid is a key step in sensing of pathogens, particularly viruses.

54   Host nucleic acid sensors recognise nucleic acid in a non-sequence-specific way. Chemical

55   differences in RNA structure such as phosphorylated 5' ends, rather than the 5' cap found in

56   mammalian mRNA, or long double-stranded (ds) RNAs, distinguish pathogen RNAs that are

57   detected by the PRRs RIG-I or MDA5, respectively (*4, 5*). Upon ligand binding, RIGI and MDA5

58   converge on the adaptor MAVS that activates TBK1 to phosphorylate IRF3 (Hornung et al.,

59   2006; Li et al., 2009). In contrast, DNA sensing depends on DNA localization, rather than

60   chemistry. DNA is not expected in the cytoplasm, and cytoplasmic DNA is sensed by the DNA

61   sensor cyclic GMP-AMP synthase (cGAS) to activate IFN production (*6*). Upon binding to DNA,

62   cGAS produces the cyclic dinucleotide 2'3'cGAMP (di-cyclic GMP-AMP monophosphate) that

63   binds the adaptor protein STING and induces conformational changes leading to IRF3

64   activation(*7, 8*). Therefore, both RNA and DNA sensing culminate in IRF3-driven transcription of

65   type-I IFN.

66

67   The fact that nucleic acid sensing is not sequence specific blurs the fundamental distinction

68   between self and non-self. Indeed, an expanding and complex literature describes dysregulation

69   of various types of host-derived retrotransposons leading to activation of either RNA or DNA

70   sensors, production of type-I IFN and/or inflammation (*9-13*). Retrotransposons are

71   transposable elements (TEs) that are broadly characterized by the presence (in endogenous

72   retroviruses, ERVs) or absence (in LINE and SINE) of long terminal repeats (LTRs). TE

73   expression can lead to the generation of dsRNA (*14*) and TEs encoding intact reverse

74   transcriptase can produce dsDNA copies from their RNA genome for *de novo* integration into

75   host genomes (*14-16*). Alternatively, RT-defective TEs, such as SINE and ETn, coopt the

76 reverse transcriptase of other TEs to generate dsDNA(*17, 18*). Therefore, expression of TEs

77 can generate nucleic acids that act as endogenous PAMPs and possibly drive deleterious

78 immune responses. Given this potential to cause disease, it is striking that nucleic acid sensors

79 have not evolved to avoid undesirable triggering by TE. Rather, it is assumed that in normal

80 tissues TEs should be transcriptionally silenced and epigenetic histone modifications, including

81 H3K9me3, are known to repress TE expression (*19*).

82

83 However, comparison of TE silencing between closely related apes revealed highly evolved

84 control mechanisms suggesting that regulation of TE expression rather than complete silencing

85 may be important in normal physiology (*20*). In this paper, we hypothesize that regulated

86 expression of TEs is, in fact, part of a normal innate immune response. We tested this

87 hypothesis in the context of the prototypic innate immune response to Gram negative bacterial

88 lipopolysaccharide (LPS) and the response to infection with the single-stranded negative sense

89 RNA virus influenza A (IAV). We show that stimulation of the anti-bacterial (*1*) and anti-viral

90 receptor (*21*) TLR4 by LPS rapidly depletes H3K9me3 at LINE1 and SINEB1 loci by reducing

91 expression of the histone methyltransferase *Suv39h1* and concomitantly inducing TE

92 transcription through MYD88 and TRIF signaling. We find that the consequent LINE1

93 expression boosts the type-I IFN response to LPS in dendritic cells (DCs) by providing nucleic

94 acid ligands for the cGAS/STING DNA sensing pathway.

95

96 Interrogation of cGAS-bound DNA in LPS stimulated cells, by immunoprecipitation and high-

97 throughput sequencing (HTS), identified LINE1 as a principal cytosolic cGAS ligand.

98 Furthermore, targeted genome-wide silencing of LINE1 elements using a CRISPR-dCas9-KRAB

99 system confirmed that LINE1 act as endogenous PAMPs to activate cGAS and contribute to the

100 consequences of LPS sensing. Crucially, single cell RNA sequencing (scRNA-seq) revealed

101 that several distinct TLR pathways can activate the same set of TEs, suggesting a common

102 mechanism among diverse innate stimuli. Indeed, inhibition of LINE1 expression, SUV39H1

103 overexpression, and treatment with reverse transcriptase inhibitors all rescued IAV infection of

104 DCs, demonstrating the impact of derepressed TE expression on the host response to a

105 pathogen encounter.

106

107 We propose that stimulation of cGAS via expression of reverse transcribing TEs contributes to

108 the normal physiological response to pathogen-derived PAMPs, exemplified here by LPS and

109 IAV infection. Our work establishes a new model in which TE expression is a central

110   component of inflammatory innate immune responses. This is important because it suggests

111   that pathogens must suppress recognition not just of their own PAMPs, but also that of TE

112   nucleic acids derived from the host, perhaps explaining why so many pathogens encode

113   apparently redundant innate immune countermeasures. Further, it defines a novel approach for

114   investigation of diverse inflammatory and autoimmune pathologies and provides a new impetus

115   for therapeutic innovation.

116

117   **RESULTS**

118   **LPS induces *Suv39h1* regulated TE expression**

119   In order to understand the role of TE expression during normal innate immune responses, we

120   treated WT bone marrow-derived dendritic cells (BMDC) with the prototypical TLR4 ligand LPS

121   and performed high throughput mRNA sequencing (mRNAseq). LPS triggered robust TE

122   expression that manifested as waves of transcription—for example, cluster one at two to four

123   hours, and cluster two at six to 18hrs (Figure 1A). TEs from the three retrotransposon

124   superfamilies were all differentially expressed (DE) at the individual element level upon LPS

125   treatment (Figure 1J). We hypothesized that distinct TE families might be uniquely targeted for

126   expression by LPS stimulation. Thus, we compared the proportion of elements in different

127   families before and after treatment using the hypergeometric test that revealed specific

128   enrichment of LINE1 and different ERV families whereas SINE families did not significantly

129   enrich (Figure S1A). Therefore, LPS selectively induces transcription of distinct TE families.

130

131   Transcriptional control of TEs remains incompletely understood, particularly during normal

132   physiology. TLR4 activates the canonical transcription factors *Nfkb* (*Rela* and *Relb*) and *Irf3* via

133   the MYD88 and TRIF signaling pathways, respectively (*22-24*). The simplest hypothesis for

134   LPS-induction of TE expression is that *Nfkb* and *Irf3* directly induce TE transcription. To test

135   this, we performed differential expression analysis of LPS-induced TE transcripts in cells

136   deficient for either *Myd88* or *Trif* (Figure 1B) and distinguished four groups of LPS-induced TE

137   as follows—*Myd88* (*Myd88*-dependent)*, Trif* (*Trif*-dependent)*, Myd88*- and *Trif*-dependent

138   (*Myd88/Trif-dependent*)*, Myd88*- and *Trif*-independent (independent) (Figure 1C, D, S1B). The

139   *Myd88-, Myd88/Trif*-, and *Trif*-dependent groups contained the majority of LPS-induced, DE

140   total LINE1 whereas the *Myd88-,* and *Myd88/Trif*-dependent groups selectively induced a

141   majority of DE intact LINE1 (Figure 1D, see Materials and Methods for definition of intactness).

142   Similar numbers of DE LTR elements were detected in the *Myd88, Myd88/Trif*, and *Trif* groups,

143   and the *Trif* pathway selectively induced relatively more SINE elements (Figure S1B),

3

144 distinguishing different modes of transcriptional regulation across the three retrotransposon

145 classes. These observations reveal a previously unappreciated role for *Myd88/Nfkb* and *Trif/Irf3*

146 in driving TE expression in addition to gene induction.

147

148 Our results indicate that autonomous TE expression is part of the normal transcriptional

149 landscape downstream of classical LPS/TLR4 signaling. Concordantly, using LINE1 as an

150 exemplar of TE, we found that RELA, RELB, and IRF3 binding motifs were enriched in the 5'

151 promoter region of intact elements (Figure 1E). As a positive control for our observations, YY1,

152 a known LINE1 transcription factor in human (*25*) and mouse (*26*) also mapped to the LINE1

153 promoter region (Figure 1E). Altogether, our observations suggest that LPS directly regulates

154 TE expression via canonical TLR4-induced transcription factors.

155

156 Although the *Myd88*-dependent group contained the most LINE1 elements (Figure 1C, D), it

157 contributed a relativey intermediate amount to the total DE LINE1 mRNA, assessed by

158 distribution of total LINE1 expression from mRNA-seq data (21-31%, Figure 1F). Meanwhile, the

159 *Myd88/Trif*-dependent group contributed the largest proportion of total DE LINE1 mRNA (55-

160 65%), whereas elements dependent on *Trif* alone contributed the least (12-20%, Figure 1F).

161 Therefore, co-regulation by *Myd88* and *Trif* induced greater TE RNA expression, compared to

162 either pathway alone. We conclude that both pathways contribute to induction of TE expression

163 downstream of LPS detection by TLR4.

164

165 TEs are transcriptionally silenced by epigenetic modifications (*19*) such as H3K9me3 which is

166 catalyzed by histone-lysine N-methyltransferases SUV39H1/2 and SETDB1, the latter

167 cooperating with TRIM28. Previous work showed that SUV39H1 selectively trimethylated H3K9

168 at intact LTR and LINE1 loci in a mouse embryonic fibroblast cell line (*27*). Two studies have

169 subsequently shown that, in cell lines, the HUSH complex also targets evolutionarily young TEs

170 in collaboration with SETDB1 and TRIM28 (*28, 29*). Intriguingly, we found that LPS stimulation

171 of BMDC rapidly and selectively depleted *Suv39h1* and *Setdb1* mRNA (Figure 1G, S1C).

172 *Trim28*, and HUSH complex members (*Morc2a, Mphosph8*) were also transiently decreased at

173 the mRNA level. *Fam208a* (HUSH) and *Suv39h2* transcript levels were relatively stable in the

174 early phase of the response but increased at later time points (Figure S1C). LPS treatment also

175 induced several histone lysine demethylases belonging to the KDM family that target H3K9me3

176 (Figure S1C). Strikingly, *Suv39h1* was also lost at the protein level on LPS stimulation of BMDC

177 whereas *Setdb1* and *Mpp8* were not (Figure 1H), indicating that additional mechanisms such as

178   proteosomal targeting may impact *Suv39h1* expression. These results suggest that LPS

179   stimulation rapidly remodels H3K9me3 heterochromatin with a key role for *Suv39h1*.

180

181   We therefore directly interrogated the effect of *Suv39h1* depletion on TE expression. In fact,

182   mRNAseq revealed that knockout of *Suv39h1* in BMDCs (Figure 1G) significantly induced TE

183   expression, even in the absence of LPS, as compared to WT cells (Figure 1I). Consistent with

184   the notion that *Suv39h1* is a negative regulator of TE expression, LPS treatment of *Suv39h1*KO

185   cells further enhanced expression of the same TEs induced by LPS in WT cells, with similar

186   kinetics (Figure1A, J). For example, LPS induced 2603 differentially expressed LINE1 elements

187   in *Suv39h1*KO compared to 1435 elements in WT (Figure 1J). We conclude that *Suv39h1* is a

188   critical regulator of the magnitude of TE expression downstream of LPS stimulation.

189

190   Because evolutionarily younger (intact) elements have been implicated as PAMPs in diverse

191   settings (*13, 30, 31*), we focused on the direct impact of *Suv39h1* expression on these elements

192   specifically. Analyzing only intact elements reiterated that *Suv39h1* regulated expression of

193   LINE1, LTR and SINE (Figure 1K). Specifically, *Suv39h1* KO had the greatest effect on DE of

194   individual TE copies belonging to young LINE1 families (L1Md_F, L1Md_T, L1Md), to SINEB1,

195   and to ERV1 and ERVK (Figure S1D-F), consistent with a previous study that used a mouse

196   embryonic cell line (*27*). *Suv39h1* knock-out increased the number of DE intact LINE1 by 3 fold,

197   upon LPS stimulation, compared with WT cells, whereas expression of truncated LINE1

198   elements was less impacted, approximately 2-fold (Figure 1K). Importantly, the expression of

199   intact elements supports the model that the transcripts we are measuring represent autonomous

200   TE transcription rather than transcriptional read-through of, for instance, intronic TEs or

201   lncRNAs (*32, 33*).

202

203   Critically, the three groups of *Myd88/Trif*-regulated TE were represented in DE LINE1 elements

204   in *Suv39h1*KO when compared to WT cells (Figure 1F), consistent with *Suv39h1* being a

205   negative regulator of these elements. We hypothesize that LPS drives specific TE expression

206   through simultaneous activation of *Myd88/Trif* to provide transcriptional activation via NFkb and

207   IRF3 while concomitant loss of the negative regulator *Suv39h1* enhances TE transcriptional

208   permissiveness. Thus, transcription factor specificity, and loss of H3K9me3 at TE loci due to

209   SUV39H1 depletion, drive TE induction and determine the magnitude of expression.

210

211   We next used publicly available RNAseq data from ENCODE to ask whether LPS induces TE

212   expression in human cells. Data from primary human monocyte-derived DCs demonstrated that,

213   as for mouse BMDCs, untreated human cells expressed low levels of LINE, SINE, and LTR

214   elements (Figure S1G, H) that were strongly increased by LPS treatment (Figure S1H). In

215   particular, the largest effects were observed on LINE and SINE that peaked at two hours (Figure

216   S1H). We saw that low levels of the relatively young, full length LINE1 L1PA elements and SINE

217   transcripts were expressed at steady state but that LPS greatly boosted their transcription along

218   with one copy of ERVK (LTR) (Figure S1I-K). Therefore, our observations of TE expression

219   during inflammatory responses in mouse cells translate directly to humans with the difference

220   that LTR expression may be relatively more restricted in humans.

221

222   Based on our analysis of transcriptional control of TEs by LPS, we hypothesized that additional

223   innate sensors would induce TEs. Using differential expression of TEs in our bulk mRNA-seq

224   datasets, we established a TE signature score, and used it to follow TE induction in a publicly

225   available single-cell RNA-seq data set from BMDCs (*34*). This analysis revealed induction of the

226   same TEs at single cell resolution as in bulk mRNAseq data following LPS treatment (Figure

227   1L). Stimulation of BMDCs with either a TLR2 (PAM_3CSK_4) or a TLR3 ligand (non-transfected

228   dsRNA mimetic poly(I:C), PIC) that stimulate the *Myd88-* and *Trif-* pathways, respectively,

229   induced robust TE expression (Figure 2J). Strikingly, these disparate PAMPs mimicking diverse

230   pathogen stimuli, activated expression of the same TEs as defined by our LPS-based signature

231   score, but with different magnitudes and kinetics of activation. Therefore, these data expand our

232   observations with different protocols, and support our hypothesis that induction of TE

233   expression is central to innate immune sensing. Altogether, these data suggest that

234   physiological depletion of *Suv39h1* following LPS detection, selectively permits expression of

235   TEs.

236

237   **Innate activation regulates TE heterochromatin dynamics**

238   Given that *Suv39h1* was uniquely depleted at both the mRNA and protein levels we sought to

239   establish the overlap between enhanced TE expression and *Suv39h1*-dependent, LPS-

240   sensitive, H3K9me3 peaks. H3K9me3 chromatin immunoprecipitation followed by high

241   throughput sequencing (ChIP-seq) of WT and *Suv39h1*KO BMDCs allowed us to establish

242   *Suv39h1*-dependent H3K9me3 patterns. Our analysis identified H3K9me3-bound regions, with

243   approximately 80% overlap of H3K9me3 peaks among biological replicates of the same time

244   point and background (Figure S2A). Consistent with the demonstrated role for *Suv39h1* in

245 establishing H3K9me3 marks on TEs (*27*), we observed 126,000 H3K9me3 peaks at TE loci in

246 *Suv39h1*WT reduced to 92,000 in *Suv39h1*KO BMDCs (Figure S2B). We reasoned that by

247 filtering the WT BMDC H3K9me3 peaks for corresponding peaks in *Suv39h1*KO BMDC, then

248 the remaining set of H3K9me3 peaks should represent those that are specifically regulated by

249 *Suv39h1*. This analysis identified 75,000 peaks that we termed *Suv39h1*-dependent peaks

250 (Figure S2B). Importantly, more peaks were identified as *Suv39h1*-dependent than were

251 apparently lost in *Suv39h1*KO cells (Figure S2B, see methods) because our analysis included

252 some peaks which were found in proximal but distinct positions in *Suv39h1*KO cells. We

253 attribute these peaks to compensatory methylation by *Setdb1,* which normally targets a set of

254 TEs that is broader but overlaps with *Suv39h1* targets (*19*). Altogether, our analyses suggested

255 that *Suv39h1* maintained 58% of the H3K9me3 peaks in untreated WT cells (Figure 2A, S2B).

256 Strikingly, when compared to *Suv39h1*-independent peaks, *Suv39h1* H3K9me3 peaks were

257 significantly enriched in LINE1 (60%), whereas LTR (26%), and SINE (7%) were relatively

258 depleted (Figure 2A). Of note, and consistent with the impact of *Suv39h1* knockout on TE

259 expression (Figure S1D-F), *Suv39h1*-dependent peaks at LINE1 were primarily enriched on

260 evolutionarily younger elements (L1Md_F, L1Md_T, L1Md_A) (Figure S2C). Peak inspection

261 revealed robust and selective enrichment of H3K9me3 at the 5' end and to a much lesser extent

262 at the 3'end of intact LINE1 elements (Figure 2B). Deletion of *Suv39h1* abolished H3K9me3 at

263 the flanking regions as well as within the bodies of LINE1 elements (Figure 2B, S2C). Although

264 *Suv39h1*-dependent H3K9me3 was not especially enriched on SINE elements relative to

265 *Suv39h1*-independent peaks (Figure 2A), a subset of peaks on SINEB1 were *Suv39h1*-

266 dependent (Figure S2D). In contrast, the impact of *Suv39h1* deletion was minimal at other TEs

267 such as SINEB2, ERV1, ERVL, and ERVK (Figure S2F), consistent with reduced enrichment of

268 *Suv39h1*-dependent H3K9me3 peaks at these elements (Figure 2A). Again, we hypothesize

269 that the reduced effect of *Suv39h1*KO on H3K9me3 at these elements may reflect

270 compensatory methylation by *Setdb1* (*19, 35*)*.*

271

272 Next, we treated WT BMDCs with LPS to compare *Suv39h1*-dependent and LPS-sensitive

273 H3K9me3 peaks by ChIP-seq. Consistent with LPS-driven loss of *Suv39h1* expression, LPS

274 stimulation of WT BMDCs readily extinguished H3K9me3 at specific loci, totaling 35% of

275 *Suv39h1*-dependent H3K9me3 peaks (Figure 2D). Concordantly, these peaks were lost at

276 LINE1 and SINEB1 (Figure 2E, S2F). Among LINE1 elements that lost *Suv39h1*-dependent

277 H3K9me3 during LPS challenge (Figure 2E), the L1Md_F2 subfamily was disproportionately

278 enriched (47%) compared to other LINE1 subfamilies (Figure 2F), indicating direct impact of

279   *Suv39h1* loss on these elements during LPS treatment. In contrast, although LPS stimulated

280   LTR element expression (Figure 1), it did not extinguish H3K9me3 peaks at ERV1 and ERVK

281   elements as readily as at LINE1 and SINEB1 (Figure S2G), consistent with the reduced effect of

282   *Suv39h1* knock-out on steady-state H3K9me3 at these loci (Figure S2E). We propose *Suv39h1-*

283   independent regulation of these elements downstream of LPS. Our data support a model in

284   which LPS-induced depletion of SUV39H1 (Figure 1G, H) facilitates heterochromatin loss to

285   directly regulate de-repression of specific LINE1 and SINEB1 elements. This model is

286   consistent with observations that disruption of epigenetic pathways that silence LINE1 causes

287   their expression and activation of innate immunity (*13, 30, 31*). We hypothesize that specific

288   TEs are regulated as part of normal innate immune physiology and might naturally contribute

289   PAMPs to enhance, amplify, or broaden a physiological innate immune response.

290

291   **TE expression and inflammation**

292   We next sought evidence for TE contributing endogenous PAMP that might be detected and

293   therefore contribute to LPS-driven inflammatory responses. We first examined the effect of

294   overexpressing *Suv39h1* on LPS treatment of RAW264.7 macrophages (Figure 3A). *Suv39h1-*

295   overexpression (SUV39H1oe) significantly dampened the LPS-mediated induction of a

296   luciferase construct controlled by the ISG54 promoter construct measured as a surrogate for

297   IFN-I production and ISG induction (ISG54-luciferase). LPS induction of the endogenous ISG

298   VIPERIN was also repressed by SUV39H1oe (Figure 3B). This effect was confirmed at the level

299   of signal transduction with reduced levels of phosphor-TBK1 and -IRF3 but unaffected levels of

300   total TBK1/IRF3 protein (Figure 3D), consistent with Suv39h1oe reducing PAMP levels and thus

301   downstream PRR signaling. Importantly, SUV39H1oe did not impact ISG54-luciferase

302   production after stimulation with a STING ligand, 2'3'cGAMP (Figure 3C), demonstrating that

303   cells overexpressing *Suv39h1* were capable of producing WT levels of ISG activation

304   downstream of DNA sensing activation.

305

306   Conversely, complete ablation of *Suv39h1* had opposite effects. LPS-stimulated *Suv39h1*KO

307   cells produced significantly higher levels of IFN-I (IFN) mRNA and protein than their WT

308   counterparts (Figure S3A, B), exhibiting enhanced phosphor-TBK1, -p65, and IRF3 signal-

309   transduction (Figure S3C). Concordantly, *Suv39h1*KO cells exhibited a more vigorous and

310   sustained anti-viral response to LPS than WT cells, evidenced by the upregulation of ISGs

311   (Figure 3E, S3D). We conclude that *Suv39h1* levels, up or down, determine the magnitude of

312   the IFN-I/ISG response following LPS stimulation.

313

314   In parallel experiments, we found that *SUV39H1* depletion with shRNA in primary human

315   monocyte-derived DCs also significantly enhanced LPS induced ISG SIGLEC-1 expression

316   whereas it had less pronounced effects on expression of the NFkB-regulated activation marker

317   CD86 (Figure S3E,F). Our data are therefore consistent with SUV39H1 regulation of LPS

318   responses being conserved in human cells.

319

320   We hypothesized that enhanced steady-state expression of TEs in *Suv39h1*KO cells should

321   also amplify baseline ISG expression. In fact, gene set enrichment analysis (GSEA) of RNAseq

322   data revealed that *Suv39h1*KO BMDCs exhibited a significantly augmented type-I IFN/anti-viral

323   gene signature at baseline compared to WT cells ($P < 10^{-10}$) (Figure 3F, blue). Further, ablation

324   of the type-I IFN receptor (*Ifnar*) in WT and *Suv39h1*KO BMDCs, abolished baseline differences

325   in ISG expression (*P > 0.05*) (Figure 3F, gold), confirming that these ISG steady-state

326   differences resulted from enhanced type-I IFN signaling. In a second cell type, in RAW264.7

327   macrophages, depletion of *Suv39h1* with shRNA (Figure S3G, H) also enhanced the baseline

328   ISG expression as measured by ISG54-luciferase production (Figure S3I). These data suggest

329   that *Suv39h1*KO is not directly impacting ISG expression because increased ISG expression

330   levels in *Suv39h1*KO cells is IFN dependent.

331

332   To further probe whether ISG and cytokine loci are directly regulated by *Suv39h1* and

333   H3K9me3, we analyzed H3K9me3 peaks in ISG loci and compared them directly with

334   mRNAseq data from the same samples. H3K9me3 assessment by ChIPseq revealed that

335   ISGs, and *Ifnb* itself, are not associated with Suv39h1-sensitive H3K9me3 marks (Figure S3J).

336   Furthermore, both WT and KO cells broadly maintained H3K9me3 peaks in the same set of

337   ISGs, and in fact, *Suv39h1*KO cells maintained slightly more peaks (Figure S3J). In addition, the

338   presence or absence of H3K9me3 peaks did not correlate with ISG mRNA expression at

339   baseline (Figure S3J). Therefore, direct regulation of ISGs by *Suv39h1* and H3K9me3 does not

340   explain the enhanced ISG response in *Suv39h1*KO cells. Rather, our data suggest that

341   *Suv39h1* loss, either downstream of LPS signaling, or due to *Suv39h1*KO, drives TE expression

342   which activates innate immune sensing and IFN production.

343

344   **cGAS is required for *Suv39h1*-mediated effects on IFN-I**

345   We hypothesized that LPS-induced TE expression is detected by innate sensors thereby

346   contributing endogenous PAMP to LPS responses. Nucleic acid sensors are thought to detect

9

347   TE-derived PAMPs when TE are expressed in various experimental systems: knock out of

348   epigenetic regulators (*13*), models of senescence/aging (*30, 31*), cancer (*9, 36*). Furthermore, in

349   the autoinflammatory condition, Aicardes Goutierres syndrome (AGS), individuals defective for

350   the nuclease TREX1, which ordinarily degrades cytoplasmic DNA, experience cGAS-dependent

351   (*37*) IFN-I-driven, inflammation resulting from either chromatin leakage into the cytosol or from

352   TE-derived DNA (*38, 39*) with the contribution of each still debated. It is unclear whether TEs

353   contribute PAMP to natural, physiological innate immune sensing.

354

355   We therefore explored the contribution of *Suv39h1*-regulated TE expression to LPS responses.

356   We first assessed activation of the DNA-sensing cGAS pathway by measuring STING

357   phosphorylation (Konno et al., 2013) in LPS activated WT BMDCs by immunoblot. In this

358   experiment, we increased the number of cells loaded into gel wells (8.5×10^5) and transfected a

359   low dose of 2'3'cGAMP to allow comparison of phosphor-STING as a control. Strikingly, LPS

360   induced STING phosphorylation and this was lost in $Cgas^{-/-}$ cells, consistent with endogenous

361   PAMP detection by cGAS (Figure 4A). LPS-activation of IRF3 detected by WB was only

362   modestly reduced by cGAS knock out, consistent with cGAS-independent LPS-induced

363   canonical TRIF and MYD88 activation of IRF3 (Figure 4A). IRF3 activation by phosphorylation is

364   a complex process, and one possibility is that cGAS impacts IRF3 phosphorylation at sites that

365   are important for activation but that are not detected here (Robitaille et al., 2016). As expected,

366   transfection of BMDCs with 2'3'cGAMP (250-500ng/ml) induced STING and IRF3

367   phosphorylation independently of cGAS (Figure 4A). Concordantly, LPS stimulation of $Cgas^{-/-}$

368   BMDCs induced significantly less IFNB (type I IFN) protein as compared to WT cells (Figure

369   S4A). As a control, *Cgas* ablation blocked HT-DNA-induced IFNB whereas 2'3'cGAMP

370   transfection induced similar IFNB levels in both WT and $Cgas^{-/-}$ cells as expected since

371   2'3'cGAMP activates STING directly (Figure S4B). Similar results were obtained using freshly

372   isolated splenic DCs where LPS treatment of $Cgas^{-/-}$ cells also triggered reduced expression of

373   the ISGs VIPERIN and CCL5 relative to WT cells (Figure S4B). Again, transfected 2'3'cGAMP

374   induced normal ISG responses in both WT and $Cgas^{-/-}$ cells as expected. Importantly, surface

375   expression of CD86, a NFkB-regulated gene, was not impacted by *Cgas*KO (Figure S4B).These

376   data indicate a contribution of cGAS/STING DNA sensing in LPS-induced activation of the IFN-

377   I/anti-viral pathway.

378

379   Interpretation of these experiments with $Cgas^{-/-}$ cells is somewhat complicated by previous

380   observations of reduced baseline expression of ISGs in $Cgas^{-/-}$ cells (*40*). We reasoned that

10

381 cGAS may constantly detect host/TE DNA, leading to low levels of IFN production, which prime

382 innate immune responses in general, and that this effect is lost in *Cgas* knockouts. If this is the

383 case, then ablation of tonic IFN-I signaling by *Ifnar* KO should eliminate the cGAS contribution

384 to LPS responses. To investigate this possibility, we generated *Ifnar*$^{-/-}$ and *Cgas*$^{-/-}$*Ifnar*$^{-/-}$

385 RAW264.7 macrophages with CRISPR-Cas9. Critically, LPS induction of IFNB was significantly

386 compromised in *Cgas*$^{-/-}$*Ifnar*$^{-/-}$ cells, as compared to *Ifnar*$^{-/-}$ cells (Figure S4G),

387 demonstrating cGAS dependence in *Ifnar*$^{-/-}$ cells. We therefore conclude that the reduction of

388 LPS-induced IFN-I production by *Cgas*$^{-/-}$ cells did not depend on the steady-state differences

389 in baseline IFN signaling in *Cgas*$^{-/-}$cells because we identified dependence on cGAS in *Ifnar*$^{-}$

390 $^{/-}$ cells. As controls, both WT and *Cgas*$^{-/-}$ RAW264.7 macrophages responded similarly to

391 stimulation with IFNB by producing comparable levels of ISG54-luciferase (Figure S4H),

392 demonstrating intact *Ifnar/Stat1* signaling that was effectively abrogated upon *Ifnar* deletion

393 (Figure S4H). Further, LPS stimulation of *Ifnar*$^{-/-}$ RAW264.7 macrophages induced IFNB

394 production (Figure S4G) but did not induce the ISG, VIPERIN (Figure S4I), as expected. We

395 conclude that cGAS dependence of LPS-induced type-I IFN production is not due to reduced

396 tonic IFN signaling in *Cgas*$^{-/-}$ cells.

397

398 To further probe the role of the cGAS pathway during LPS responses, we used a small

399 molecule STING inhibitor (STINGi, H151) (*41*). Acute inhibition of STING permits assessment

400 of the role of the cGAS/STING pathway in the absence of baseline effects. Strikingly, STING

401 blockade significantly attenuated LPS-induced expression of specific ISGs (Figure 4B). As

402 controls, *Myd88*KO in BMDCs dampened LPS-induced ISG responses while *Trif* ablation

403 completely abolished them (Figure 4B), and H151 prevented activation of ISG expression after

404 transfection with the cGAS ligand HT-DNA (Figure 4B), as expected. Similarly, LPS induction of

405 the ISG, MDA5, measured by western blot was strongly reduced in *Cgas*$^{-/-}$ cells (Figure 4A).

406 Unexpectedly, *Mavs* deletion had no effect on ISG induction by LPS (Figure 4B) whereas the

407 responses to transfected PIC, or influenza A virus/Puerto Rico 8/1934/H1N1 (IAV/PR8)

408 infection, were attenuated (Figure S4C,D). These results suggest that *Mavs*-dependent sensing

409 of TE-derived RNA does not contribute to LPS responses.

410

411 We next sought to confirm these observations in RAW264.7 macrophages using CRISPR-Cas9

412 genetic ablation of *Mavs,* in WT or *Cgas*$^{-/-}$ cells(Figure S4E). LPS induction of ISG54-

413 luciferase expression was dependent on *Myd88*, *Trif,* and *Cgas* because individual knock outs

414 showed significant impairment of IFN-I activation, recapitulating our results in BMDCs (Figure

415   4C,D). Once again, inhibition of RNA sensing by deletion of *Mavs* in a WT or *Cgas*$^{-/-}$

416   background*,* did not affect the LPS-induced ISG response (Figure 4D). As a control, ablation of

417   *Cgas* and *Mavs* effectively blocked induction of IFN-I following transfection with HT-DNA or PIC

418   (polyI:C), respectively (Figure S4F). Collectively, these data verify that cGAS-dependent

419   sensing contributes to LPS responses, but that MAVS-dependent RNA sensing does not.

420

421   Our data suggest a mechanism in which *Trif* and *Myd88*-dependent genes, LPS induces *Trif*

422   and/or *Myd88*-dependent TE expression (Figure 1B-D, S1B, D-F) that can be detected by

423   cGAS. Importantly, while deletion of *Trif* or *Myd88* significantly blocked TE induction, inhibition

424   of STING with H151 left TE induction intact (Figure 4E). Thus TE induction is downstream of

425   LPS, and cGAS/STING activation is downstream of TE expression. Together, these data are

426   consistent with a model in which the defect in LPS-triggered ISG responses in *Cgas*$^{-/-}$ cells

427   stems from an inability to sense TE-derived DNA. Based on this model, we hypothesized that

428   the enhancement of IFN production by *Suv39h1* depletion required *Cgas.* Indeed, *Cgas* deletion

429   in RAW264.7 macrophages blocked enhancement of LPS-induced IFN-I production by *Suv39h1*

430   shRNA (Figure 4G). Notably, enhancement of the LPS-induced IFN-I response in *Suv39h1*-

431   depleted cells was independent of *Ifnar*, confirming our observations in BMDCs, and dependent

432   on *Cgas* (Figure S4I). Therefore, depletion of *Suv39h1* augments LPS-induced IFN in a *Cgas*-

433   dependent manner and is independent of tonic IFN signaling.

434

435   Consistent with *Suv39h1*-knockdown experiments, SUV39H1oe did not suppress LPS-induced

436   IFN-I when *Cgas* was ablated (Figure 4F, compare to Figure 3B, note the difference in scale).

437   Importantly, SUV39H1oe did not impact cGAS protein levels (Figure 3D) ruling out a direct

438   effect on cGAS expression. Furthermore, SUV39H1oe did not restrict induction of IFN when

439   cGAS was bypassed by direct activation of STING with its ligand, 2'3'cGAMP (Figure 3C),

440   consistent with a role for *Suv39h1* in suppressing expression of endogenous cGAS ligands.

441   Thus, *Cgas* is required for *Suv39h1* regulation of the type-I IFN response to LPS*.* These data

442   are consistent with a model in which cGAS detects *Myd88/Trif*-driven TE expression following

443   the physiological depletion of *Suv39h1* by LPS detection.

444

445   **cGAS binds LINE1 elements in LPS stimulated cells**

446   The impact of *Cgas*/*Sting* blockade on the LPS-dependent IFN-I response suggested that LPS

447   induces expression of TE that are reverse transcribed into DNA and detected by cGAS. We

448   therefore sought evidence for LPS-induced cytosolic DNA. We isolated cytosolic fractions by

449   digitonin extraction (*42*) and retained nuclei for standardized quantification. Real time-qPCR

450   analysis of RNAse-treated cytosolic fractions from BMDCs revealed that LPS significantly

451   boosted cytoplasmic levels of LINE1 and ERVK DNA (Figure 5A). Furthermore, pretreatment

452   with a reverse transcriptase inhibitor cocktail (emtricitabine, tenofovir, and nevirapine) effectively

453   ablated the increased cytosolic DNA levels (Figure 5A) and restricted expression of the ISG

454   VIPERIN (Figure S5A). Therefore, reverse-transcribed TE DNA is induced by LPS, and reverse

455   transcriptase inhibition reduces LPS-induced ISG expression.

456

457   We next sought to understand the nature of the DNA activating cGAS during an LPS response

458   using cross-linked immunoprecipitation of a tagged endogenous cGAS allele (eGFP-cGAS)

459   from BMDCs (Gentili et al., 2019), followed by high through-put sequencing (HTS). We first

460   biochemically fractionated BMDCs and confirmed efficient separation of the cytosol and nucleus

461   by western blot and qPCR (Figure S5B,C). Western blots revealed cytosolic as well and nuclear

462   cGAS protein (Figure S5B), as previously observed (*43-45*). Nuclear cGAS is not expected to

463   sense chromatin (*45-50*), but it may sense de novo nuclear TE DNA, for instance. We therefore

464   isolated endogenous cGAS-bound DNA from the cytosolic and nuclear compartments

465   separately, after LPS stimulation, and subjected them to HTS. Because cGAS produces

466   2'3'cGAMP when bound to dsDNA, single-stranded DNA and RNA-DNA hybrids (*51-54*) we

467   used an approach that would preserve all DNA species in the sequencing libraries (see

468   materials and methods) to capture a global view of cGAS-bound nucleic acid. To avoid aberrant

469   post-lysis binding between DNA and cGAS (*55*), we cross-linked the cells before lysis, ensuring

470   exclusive detection of cytosolic DNA species bound to cGAS *in vivo*. Furthermore, after pull-

471   down, the immunoprecipitates were stringently washed with detergent and high salt buffer,

472   precluding a significant contribution for residual post-lysis binding events; cGAS-DNA

473   interactions are expected to occur with weak affinity (Kd=10µM) (*56, 57*).

474

475   Inspection of all possible DNA species in the cGAS-bound fraction revealed striking differences

476   relative to the cytosolic input (Figure 5B). Most notably, LINE1 DNA was profoundly enriched

477   on cytosolic cGAS compared to input (5.1-fold, 40% versus 8%) (Figure 5B, C) whereas LTR

478   families accumulated at much lower densities (Figure 5B, C). Conversely, cytosolic cGAS was

479   depleted of satellite, SINE, and simple repeat DNA relative to input (Figure 5B,C). A similar

480   trend was observed for the nuclear pool of cGAS although it did not approach the same

481   magnitude of fold enrichment or statistical significance (Figure 5B,C). For example, LINE1

482   enriched weakly (1.68-fold, 30% versus 18%) on nuclear cGAS (Figure 5B), agreeing with a

13

483   recent report (Gentili et al., 2019). Importantly, Figure 5C illustrates that significant enrichment

484   of LINE1 on cytosolic cGAS cannot be explained by genomic frequency of LINE1 copies

485   because the enrichment of LINE1 in the cytosolic input fraction (8%) is significantly below the

486   expected genomic frequency (18%), and this is further supported by the statistical test used to

487   compare cGAS-IP versus input (hypergeometric test) that takes into account genomic

488   frequency.

489

490   To broaden our observations to a different cell type, we transduced RAW264.7 macrophages

491   with lentivirus carrying eGFP-tagged cGAS and performed cross-linked IP from the cytosol

492   followed by qPCR after LPS treatment (Figure S5D). Again, IP of cGAS from these cells

493   revealed clear enrichment of LINE1 compared with non-TE DNA (Figure S5D) after LPS

494   treatment. Although mitochondrial DNA has previously been shown to stimulate the

495   cGAS/STING pathway (*58*), we did not detect appreciably enriched mitochondrial DNA on cGAS

496   in either LPS treated BMDCs or RAW264.7 macrophages (Figure S5D,E), suggesting that

497   mitochondria are not an important source of DNA PAMP downstream of LPS detection

498   (mitochondria are not expected to be damaged by LPS exposure). We conclude that LINE1 is

499   the primary endogenous ligand of cytosolic cGAS downstream of LPS stimulation. This is

500   consistent with LPS-induced epigenetic derepression of LINE1 following depletion of the LPS-

501   sensitive H3K9 methyltransferase, *Suv39h1*.

502

503   To better understand the contribution of the different cGAS fractions to the LPS response, we

504   compared fold changes in binding densities of TE subfamilies for LPS-treated versus untreated

505   cells in each fraction. The largest changes corresponded to strong increases in LINE1 and LTR

506   binding densities on cGAS whereas we detected only modest changes associated with nuclear

507   cGAS binding (Figure S5F), consistent with overall enrichment (Figure 5B, C). Enumeration of

508   unique TE copies contributing to the largest fold-changes in the different fractions identified the

509   LINE1 subfamilies L1Md_F2 and L1_Mus3 as the most highly enriched (Figure 5D), reflecting

510   the specific regulation of these elements by *Suv39h1* (Figure 2F, S2E). Of note, these elements

511   were among the youngest (Figure 5D, least diverged) and most highly expressed LINE1

512   elements in terms of mRNA (Figure S1C, Figure 5D), linking their availability for cGAS binding

513   to transcriptional activation by LPS.

514

515   To confirm whether cGAS-dependent LPS-induced IFN-I requires direct cGAS-DNA binding and

516   enzymatic activity, we reconstituted *Cgas*$^{-/-}$ RAW macrophages with WT cGAS (cGAS-FL), a

517   cGAS DNA-binding mutant that lacks the DNA-binding N-terminal 160aa and harbors mutations

518   in its zinc-thumb domain that prevent bound DNA from activating cGAS enzymatically (cGAS-

519   DBM) (*59*), or a catalytically-dead cGAS mutant (cGAS-CD, E225A/D227A) that cannot produce

520   2'3'cGAMP (Kranzusch et al., 2013). Reconstitution levels of the WT and cGAS-mutants are

521   shown in Figure S5G. Because cGAS-FL elevated IFN-I in untreated cells (Figure S5H), we

522   subtracted baseline values from stimulation induced IFN-I following treatment with HT-DNA,

523   2'3'cGAMP, or LPS (Figure S5I). Critically, LPS-induced ISG54-luciferase expression was

524   selectively restored by cGAS-FL, but not by either cGAS mutant (Figure S5I). Accordingly, HT-

525   DNA transfection selectively activated cGAS signaling when cGAS-FL but not when cGAS-DBM

526   or cGAS-CD were used (Figure S5I). In contrast, 2'3'cGAMP stimulated ISG54-luciferase in all

527   settings, confirming that downstream signaling functioned normally (Figure S5I). These data

528   reveal: 1) a specific requirement for cGAS during LPS stimulation and 2) that DNA-induced

529   cGAS enzymatic activity is necessary for cGAS enhancement of LPS-triggered ISG responses.

530

531   Because *Suv39h1* regulates the magnitude and kinetics of LINE1 expression during LPS

532   challenge, we predicted that *Suv39h1* deletion would enhance LINE1 DNA binding by cGAS.

533   Indeed, LINE1 was detectable on cGAS at low levels in untreated WT cells, but LPS treatment

534   significantly increased LINE1 density (Figure 5E). Furthermore, in *Suv39h1*KO BMDCs, steady-

535   state LINE1 densities reached magnitudes similar to that found on cGAS in LPS-treated WT

536   cells. As expected these were further enriched upon LPS stimulation (Figure 5E). In contrast, as

537   a control, SINE DNA revealed disparate trends with no statistically supported differences

538   (Figure 5F). As a control for our HTS data, cGAS-IP, followed by qPCR, detected LPS-induced

539   enrichment of LINE1 and LTR (ERVK) DNA on cytosolic cGAS whereas genic DNA such as

540   *Gapdh* was not detected (Figure 5G). Comparison of the densities of DNA elements bound to

541   cGAS exhibited significant and selective enrichment of LINE1 in *Suv39h1*KO relative to WT

542   cells (Figure S5J,K). Examination of LINE1 elements precipitating with cGAS revealed that

543   *Suv39h1* KO boosted the same LINE1 subfamilies that bound cGAS with high frequency in WT

544   cells after LPS stimulation (Figure 5F, S5L). Thus, *Suv39h1*-regulated LINE1 elements bind

545   cGAS following LPS detection.

546

547   **LINE1 elements act as endogenous PAMPs**

548   To provide further validation for our model, we sought evidence for the role of LINE1s as

549   endogenous PAMPs that induce IFN-I by targeting them for epigenetic repression using a

550   CRISPR dCas9-KRAB fusion construct. In this system, the mutant Cas9 (dCas9) binds DNA but

551   is incapable of generating dsDNA breaks that would likely occur in excess when targeting

552   numerous LINE1 elements and cause cell death (*60, 61*). The fused KRAB domain recruits

553   epigenetic factors such as SETDB1, a H3K9me3 methyltransferase, driving

554   heterochromatization and subsequent transcriptional silencing of the target region (*61*). As a

555   positive control, we targeted the 5' untranslated region of *Itgax*, the gene encoding the highly

556   expressed integrin and myeloid marker, CD11b. Using two different guide RNAs (gRNAs), we

557   succeeded in selectively repressing CD11b while maintaining MHC-II expression (Figure S5M).

558

559   We next designed gRNAs to target 20,000-110,000 LINE1 elements belonging to the L1Md

560   family, and specifically enriched either at the L1Md_F2 sub-family (gRNA_1 and gRNA_2) that

561   bound at high levels to cGAS after LPS treatment or, as a negative control, at L1Md_A elements

562   (gRNA_3) which bound at low levels to cGAS (Figure S5N). Consistent with the notion that

563   elements that bind cGAS at higher levels should contribute the largest impact to cGAS-

564   dependent IFN-I production, gRNA_1 and gRNA_2 significantly impeded IFN-I production;

565   gRNA_3, however, had no effect (Figure 5H). Importantly, differential analysis of mRNA-seq

566   data (Ctrl versus gRNA-expressing RAW cells) confirmed specific repression of L1Md

567   expression by gRNA_1 and gRNA_2 (Figure 5I, J). Of note, the efficiency of LINE1 suppression

568   by gRNA_1 was similar in both LPS-treated and untreated cells (Figure 5J, dark green bars)

569   whereas gRNA_2 suppressed LINE1 in untreated cells but lost efficiency upon LPS exposure

570   (Figure 5J, light green bars). The effects on LINE1 suppression were mirrored in the IFN-I anti-

571   viral response as revealed by GSEA of anti-viral gene expression (Figure 5K); whereas gRNA_1

572   effectively attenuated anti-viral gene expression in both untreated and LPS treated cells,

573   gRNA_2 selectively suppressed steady state expression, suggesting that its repressive effect

574   was limited to baseline TE expression and overcome by a strong LPS-induced transcriptional

575   stimulus. These effects cannot be explained by direct suppression of anti-viral genes because

576   neither gRNA_1 nor gRNA_2 target sequences were appreciably enriched in the proximity of

577   ISG loci (Figure S5O). Therefore, LINE1 expression is required for optimal induction of IFN-I by

578   LPS.

579

580   Critically, in *Cgas*$^{-/-}$ macrophages, the suppressive effect of gRNA_1 and gRNA_2 on IFN-I

581   production was lost (Figure S5P), similar to *Suv39h1oe* in *Cgas*$^{-/-}$ cells (Figure 4F). We

582   conclude that LPS-induced LINE1 expression acts through cGAS to augment the IFN-I/anti-viral

583   response. This observation is consistent with L1Md elements acting as LPS-induced

584   endogenous cGAS ligands that amplify the type-I IFN response.

16

585

Transposable element expression restricts RNA virus infection

587 Our data support a model where innate immune responses proceed through two phases: 1)

588 initial detection of pathogen-derived PAMP drives expression of cytokines, IFN-I, and TE

589 through classical innate signaling pathways and 2) once TE expression reaches a critical

590 threshold, cell autonomous detection of TE expression by cGAS/STING, and possibly other

591 sensors, amplifies the anti-microbial response (Figure 6A). Whereas bacteria encode both RNA

592 and DNA ligands, viral genomes typically encode one or the other, providing a useful tool to

593 physiologically test our model. For example, various RNA viruses, including influenza, activate

594 TLR4 (*21*), and although they do not contain DNA, they can, unexpectedly, activate and/or

595 express inhibitors for the cGAS/STING pathway (*62, 63*). IAV infection has previously been

596 shown to induce LINE1 expression (*64, 65*). We therefore tested the effect of manipulating the

597 mechanism of innate immune amplification, that we have described, on IAV infection.

598

599 Srikingly, infection of WT BMDCs with IAV/PR8 selectively triggered depletion of SUV39H1

600 protein and induced expression of LINE1 ORF1p but did not appreciably affect SETDB1 or

601 MPP8 levels at the time point queried (7 hrs post infection) (Figure6B). IAV infection can be

602 assessed by cell survival and measurement of viral nucleoprotein (NP) (Figure S6). We

603 therefore tested the physiological outcome of IAV/PR8-induced SUV39H1 depletion by

604 measuring the impact of SUV39H1 overexpression on IAV/PR8 infection. Indeed, SUV39H1

605 overexpression rescued IAV infection, leading to reduced cell survival and higher levels of NP

606 expression (Figure 6C), and consistent with the effect of SUV39H1 overexpression on cGAS-

607 dependent LPS-induced IFN-I (Figure 3B, 4G). Next, we tested whether LINE1 suppression

608 would rescue viral infection, hypothesizing that the depletion of SUV39H1 upon IAV/PR8

609 infection would promote innate detection of LINE1 PAMP, similar to LPS stimulation. Strikingly,

610 suppression of LINE1 expression with dCas9-KRAB (Figure 6D) also rescued IAV/PR8

611 infection. We conclude that blockade of host LINE1 expression during viral infection reduced the

612 murine innate immune response against IAV, leading to enhanced infection. Consistent with our

613 model, we detected cGAS-dependent induction of phosphor-STING and the ISG MDA5 by IAV

614 infection in DCs (Figure 6E). Furthermore, small molecule blockade of STING significantly

615 increased IAV NP expression, consistent with STING signaling inhibiting RNA virus infection in

616 these cells (Figure 6F). Concordantly, addition of a reverse transcriptase inhibitor cocktail,

617 known to be active against TE including LINE1 (Banuelos-Sanchez et al., 2019), rescued IAV

618 replication and enhanced virus-induced cell death (Figure 6G). Thus, consistent with our model,

17

619   overexpression of SUV39H1, suppression of LINE1 expression, inhibition of STING, and

620   inhibition of reverse transcription, all rescued IAV infection in DC through suppression of innate

621   responses. These observations support our model that innate activation induces TE expression

622   and reverse transcription-catalyzed TE DNA that is detected by cGAS to broaden and

623   strengthen host defenses.

624

625   **DISCUSSION**

626   In this study, we introduce a new paradigm for the mechanism of innate immune sensing in

627   which endogenous PAMPs are produced within cells exposed to exogenous pathogen-derived

628   PAMP. We propose that these endogenous PAMPs are detected by classical innate sensing

629   pathways, amplifying and broadening responses to pathogens. Specifically, we discovered that

630   LPS induces LINE1 expression, which is detected by cGAS to contribute to LPS-elicited IFN-I

631   responses. We find that LPS activates LINE1 expression by driving SUV39H1 depletion that

632   leads to loss of H3K9me3 at TE loci, combined with MYD88/TRIF-driven transcriptional

633   activation. This discovery is important because it reveals an entirely new level of regulation of

634   innate immune responses and ensuing inflammation that may explain inconsistencies in the

635   field.

636

637   **Epigenetic regulation of TE in innate immune responses**

638   The notion that TE should be permanently switched off derives from the observation that TE

639   expression is typically associated with pathology, particularly autoinflammation and oncogenesis

640   (*66-68*). We propose that our observation of regulated TE expression, and sensing by cGAS

641   after LPS exposure, evidences an essential role for TE expression in normal innate immune

642   responses. This is supported by our observation that disparate PAMPs also activate TE

643   expression (Figure 1L).

644   Indeed, we hypothesize that diverse innate detection pathways converge on distinct epigenetic

645   regulators to induce TE PAMPs that enhance innate immunity such as type-I IFN anti-viral

646   responses.

647

648   Previous studies of TE regulation have suggested that expression of the youngest LINE1 is

649   epigenetically regulated by an evolutionary Red Queen style arms race akin to that between

650   host and pathogen (*20*). We hypothesize that such studies, characterizing the evolution of

651   LINE1 regulation, demonstrate how host genomes have evolved to specifically regulate their

652   expression when required. Strikingly, our data highlight the importance of LINE1 regulation in

18

653   enhancing innate immunity and provide a direct example of the evolutionary benefits of retaining

654   the capacity to regulate TE expression, rather than simply suppress it. Epigenetic marks such as

655   H3K9me3 and DNA methylation switch gene expression on and off, and we propose that TE are

656   simply another example of sequences that are epigenetically regulated in normal physiology,

657   rather than comprising a distinct class of elements that must be permanently silenced (*19, 69*).

658

DNA sensing of TE in innate immune responses

660   Our results highlight a central role for the DNA sensor cGAS in the LPS driven innate immune

661   response. Interrogation of cGAS-bound DNA in LPS stimulated cells identified LINE1 DNA as a

662   principal cytosolic cGAS ligand and our data demonstrating that reverse transcriptase inhibitors

663   blocked LPS-induced cytosolic DNA, and that TE mRNA correlated with cGAS-bound DNA

664   (Figure 5D), strongly implicate reverse transcription as a key step in PAMP generation. This was

665   previously suggested in the AGS model, *Trex1*^{−/−} mice (*70*). Indeed, RTI have been used

666   successfully to treat AGS patients (*71*), reducing IFNa protein levels and ISG expression. In

667   addition, in another study RTI treatment depleted LINE1 cDNA and senescence-associated

668   inflammation (*30, 31*). Importantly, baseline expression of LINE1 RT, which promiscuously

669   reverse transcribes host RNA, may generally prime innate immune sensing and IFN-I responses

670   (*72-76*).

671

672   Our analyses indicated that the major changes in cGAS-bound DNA species during LPS

673   stimulation occurred in the cytosol. The relative contribution of the nuclear and cytosolic cGAS

674   pools to IFN-I production is not clear although it is clear that the nucleus potently restricts cGAS

675   activation due to high affinity interactions with histones H2A and H2B (*45-50, 77*). Although

676   nuclear cGAS is not expected to sense chromatin, it could conceivably sense nuclear de novo

677   LINE1 DNA, per se. Indeed, LPS induced modest changes in binding between nuclear cGAS

678   and TEs, but the majority of cGAS-TE interaction occurred in the cytoplasm. Moreover,

679   condensation of cytosolic cGAS on DNA creates lipid droplets that can co-sediment with nuclei

680   (Barnett et al., 2019; Du and Chen, 2018), suggesting that our observations of changes in the

681   binding of nuclear cGAS with TEs are overestimated by cytoplasmic contamination.

682

683   Previous studies have demonstrated the activation of cGAS by mitochondrial DNA (mtDNA)

684   (*58*). We ruled out a role for mtDNA, as it did not appreciably enrich on cGAS in either of the two

685   cell systems interrogated here, BMDCs and RAW264.7 macrophages (Figure S5D, E). Thus,

19

686   mtDNA binding to cGAS may be restricted by context such as certain types of viral infection (*78*)

687   and pathogenic mutations in humans that permit leakage of mtDNA into the cytosol (*58*).

688

689   We were surprised to find in our experiments that RNA sensing of TE did not play a significant

690   role in LPS responses because endogenous RNA PAMPs are expected to be produced more

691   readily than DNA PAMPs which require reverse transcription from an RNA template.

692   Furthermore, RNA encoded by a ribosomal pseudogene has been implicated as an endogenous

693   PAMP during infection, leading to a more potent anti-viral response against a variety of viruses

694   including DNA viruses HSV-1 and EBV, and RNA virus IAV (*79*). It will be interesting to further

695   investigate the impact of LPS on RNA sensing.

696

697   **Infection**

698   We hypothesize that induction of endogenous PAMP after detection of exogenous PAMP is a

699   powerful mechanism to broaden and amplify defensive responses. Indeed, a recent study

700   showed that in a human lung epithelial cell line, IAV infection triggered sumoylation-dependent

701   degradation of TRIM28, TE induction, and ultimately led to MAVS-dependent, STING-

702   *independent,* sensing of, presumably, de-repressed ERV RNA (*64*). Furthermore, another

703   publication suggested that a host commensal skin bacteria could induce ERV elements via

704   activation of TLR2, and this triggered innate immune responses via cGAS/STING in

705   keratinocytes that promoted adaptive T cell responses (*80*). Importantly, these responses were

706   inhibited by reverse transcriptase inhibitors, indicating that reverse transcribed ERV cDNA was

707   detected by cGAS and concurring with our data describing cGAS-mediated detection of reverse-

708   transcribed LINE1 elements downstream of TLR4 signaling. These observations support our

709   model that TE expression is an integral component of innate sensing that amplifies the primary

710   innate response through subsequent cell-autonomous sensing events (Figure 6E).

711

712   Our model is entirely consistent with observations of viruses evolving antagonists for sensing

713   pathways that they are not expected to activate. For instance, RNA viruses block DNA sensing

714   pathways (*81*). Furthermore, RNA viruses such as SARS-CoV2 (*62*), measles, and Nipah virus

715   (*82*) also trigger cGAS/STING sensing, and SARS-CoV2 at least has been demonstrated to

716   induce LINE1 expression (*65, 83*). Concordantly, RNA viruses influenza (*84*), SARS-CoV (*85,*

717   *86*), SARS-CoV2 (*87*), Porcine epidemic diarrhea virus (*88*), Dengue virus (*89*), Yellow fever

718   virus (*90*), Zika virus (*91*), and Hepatitis C virus (*92*) all inhibit the cGAS/STING DNA sensing

719   pathway. Our model provides an explanation if detection of virus infection induces endogenous

20

720   PAMP production. Consistent with this, we show that STING inhibition, reverse transcriptase

721   inhibitors, LINE1 silencing, or overexpression of SUV39H1, all rescue IAV infection of mouse

722   DC.

723

724   **Cancer**

725   We propose that our model is applicable beyond infection. Natural TE expression correlates

726   with better anti-tumor T cell responses (*93*) and the notion that enhanced TE expression drives

727   an inflammatory response that is correlated with better clinical outcome, particularly after

728   immunotherapy, is supported by studies that enhanced TE expression and the ensuing

729   inflammatory responses either by small molecule inhibitors of DNA methylation or genetic

730   ablation of epigenetic regulators(*9, 94-96*). Likewise, disruption of histone methylation by

731   ablation of *SETDB1* led to TE dsRNA sensing and IFN-I responses in different cell lines

732   including acute myeloid leukemia (*10*) and non-small-cell lung cancer(*11*). In addition, *Setdb1*

733   deletion in a mouse tumor model led to enhanced T cell responses through presentation of TE-

734   derived peptides rather than innate immune sensing (*97*). We speculate that a lack of innate

735   recognition of TEs in this study may be linked to defective innate sensing pathways frequently

736   encountered in cancer cells (Konno et al., 2018; Sutter et al., 2021). Based on our model, we

737   hypothesize that high levels of TE expression and ensuing inflammation in tumors represents a

738   natural innate immune defense against transformation. Concordantly, tumor cell intrinsic

739   suppression of DNA sensing, for example via STING mutation, or suppression of TE induction

740   by *Setdb1*, is common (*97-99*).

741

742   **Inflammation and Aging**

743   The capacity of TEs to elicit innate immunity has also been linked with diseases other than

744   cancer, particularly inflammatory disease. Patients with Aicardies Goutiere Syndrome (AGS)

745   suffer an interferonopathy from early development which has been associated with TE

746   expression, reverse transcription, and activation of cGAS (*37, 39, 71*). LINE1 DNA has also

747   been revealed as a source of STING-dependent neuroinflammation in a *TREX1*-mutant model

748   of AGS (*12*). To date, no associations have been made between the H3K9me3 pathway and

749   AGS, although a recent paper linked diminished SUV39H1 expression with inflammation in the

750   lungs of patients with chronic obstructive pulmonary disease (COPD) (*100*). Our data are

751   consistent with these observations and may provide mechanistic explanation. TE expression

752   has even been suggested as a driver of aging-associated inflammation (*30, 31*). Collectively,

753   this body of literature provides numerous examples of TE-derived nucleic acids acting as

21

754 endogenous PAMPs to activate innate sensors. However, the interpretation in each case has

755 been that disease is enhanced or caused by the failure to keep TE expression switched off.

756

757 We propose, based on our observations, that the expression of TE to drive innate sensing is a

758 natural physiological response to detection of pathogen and/or danger signals. Thus, disease

759 stems from inappropriate regulation rather than simply failure to keep TE expression

760 suppressed. For example, in cancer, we hypothesize that TE expression and activation of innate

761 immunity is a response to transformation, evolved to activate adaptive immunity against

762 transformed cells. We expect that the response of a cell, or cancer, to PAMP will vary

763 depending on the active sensing pathways in each case. For example, many cell lines and

764 cancers are defective for sensing (*98, 99*), and therefore, whether an RNA or DNA detection

765 response dominates will depend on individual circumstances.

766

767 Boosting of an incipient innate immune response by autologous sensing of TE-derived PAMP is

768 pivotal to our new model. To emphasize this and distinguish their role from that of conventional

769 exogenous PAMP, we propose calling TE that are sensed during innate responses to infection

770 TRAnsposable element Molecular Patterns (TRAMPs). In the two-phase mechanism (Figure

771 6H) that our data suggest, the initiating response, arising from direct recognition of exogenous

772 PAMPs expressed by the infecting organism, activates expression of cytokines, ISGs, and,

773 importantly, TRAMPs. In the amplifying phase, cell autonomous recognition of upregulated

774 TRAMPs, by additional sensors including cGAS, amplifies the innate response. This model

775 might explain how extremely low levels of exogenous viral PAMP such as in the case of natural

776 HIV infection, for instance, can lead to a measurable inflammatory response. We imagine a

777 threshold at which TRAMPs become dominant. This threshold can be shifted in disease states.

778 For example, in AGS, the threshold is shifted to the left by, for example, defects that lead to

779 increases in TRAMP levels (*TREX1*, *SAMHD1*). By contrast, in certain cancers, defects in

780 nucleic acid sensing (cGAS, STING) shift the threshold to the right. Defects of epigenetic

781 regulators could shift the threshold in either direction.

782

783 TE are not genetic junk left over from genome evolution. Rather, they are essential for the

784 regulation of normal physiology. We anticipate that investigation of mutations and mechanisms

785 regulating TRAMP expression will provide mechanistic insight into diverse drivers of

786 inflammation in a wide range of conditions, including aging, cancer, and infection. This work

22

787 could provide diagnostic tools and suggest novel therapeutic targets to transform our

788 management of human disease.

789

790 **FIGURE LEGENDS**

791 **Figure 1** S*uv39h1* expression determines the magnitude of TE transcription during LPS

792 challenge, see also Figure S1. (A) Heatmap showing Pearson Correlation-based hierarchical

793 clustering of the most highly expressed TEs by normalized expression (FPKM) in WT (n=3) and

794 *Suv39h1*KO (n=4) BMDCs. Relative values are represented by z-score. (B) Volcano plot

795 exhibiting differentially expressed (DE) LINE1 elements in 2hr LPS-treated *Myd88$^{-/-}$* (left) or

796 *Trif$^{-/-}$* (right) BMDCs compared with WT control cells. n=3 per genotype (*Myd88$^{+/+}$*, *Trif$^{+/+}$*,

797 *Myd88$^{-/-}$*, *Trif$^{-/-}$*) per time point. Red dots signify significantly expressed elements, adjusted *P*

798 value < 0.05. (C, D) Bar graphs representing the number of total (C) or intact (D) LINE1

799 elements dependent on the indicated signaling pathway for significant expression as determined

800 by differential analysis of mRNAseq data such as that presented in panel B. (E) Density plots of

801 IRF3, RELA, RELB, and YY1 transcription factor motif sequence (JASPAR database)

802 enrichment along the sequence of full-length LINE1 elements. (F) Stacked bar plots showing the

803 percentage of total DE LINE1 mRNA FPKM in *Suv39h1*WT and KO BMDCs at steady state and

804 during LPS treatment that derives from each transcriptionally-defined group in panels C and D.

805 (G) mRNA sequencing data showing that histone methyltransferase *Suv39h1* transcript levels

806 are quickly depleted during LPS challenge in BMDCs. WT (n=3), KO (n=4). (H) Western blot of

807 SETDB1, MPP8, SUV39H1, and Histone H3 in whole cell extracts from WT c57BL/6 BMDCs left

808 untreated or stimulated with LPS for the indicated times. (I) Volcano plots displaying DE LINE1,

809 LTR, and SINE element mRNA from sequencing data, comparing *Suv39h1*WT and *Suv39h1*KO

810 BMDCs at steady- state. WT, n=3; KO, n=4. (J) Volcano plots displaying DE LINE1, LTR, and

811 SINE element mRNA from sequencing data, comparing untreated (UT) to 2hr LPS-treated

812 within WT or *Suv39h1*KO BMDCs. WT, n=3; KO, n=4. (K) Quantification of differentially

813 expressed *intact* (top) or degenerate (bottom) TE classes in 2hr LPS-treated versus untreated

814 within WT or *Suv39h1*KO BMDCs. (L) A TE signature score based on DE LINE1, LTR, and

815 SINE in bulk mRNAseq data of WT BMDCs was projected onto a previously published

816 scRNAseq dataset (Shalek et al., 2015) to compare TE expression induction by LPS,

817 Pam$_3$CSK$_4$ (PAM), or poly(I:C) (PIC) treatment at the indicated time intervals. Significance cutoff

818 for differential analyses in B-D and I-L was defined as an adjusted *P* value less < 0.05 and a

819 log$_2$ fold change ≥ 2. In C and D, the number of elements per group represents the sum of all

820 significantly expressed LINE1 elements at 0, 1, 2, and 4hrs of LPS treatment when comparing

821 WT to KO cells (*Myd88*^{+/+}vs *Myd88*^{−/−}, *Trif*^{+/+}vs *Trif*^{−/−}). In G, statistical significance was

822 determined by one-way ANOVA and Bonferonni ad-hoc analysis comparing untreated to LPS-

823 treated WT BMDCs. *$P<0.05$, ** $P <0.001$, ***$P<0.001$, ****$P<0.0001$.

824

825 **Figure 2** LPS induces rapid depletion of H3K9me3 at transposable elements in dendritic cells,

826 see also Figure S2. (A) Left, stacked bar chart summarizing proportion of *Suv39h1*-dependent

827 (red) and –independent (white) H3K9me3 peaks in unstimulated WT BMDCs from H3K9me3

828 ChIP-seq data. Right, pie chart exhibiting percent enrichment (% indicated by emboldened

829 numbers) of specific DNA species in *Suv39h1*-dependent (middle) and –independent (right)

830 H3K9me3 peaks. Below, heatmap summarizing statistically significant enrichment of DNA

831 elements in *Suv39h1*-dependent versus *Suv39h1*-independent H3K9me3 peaks. (B) H3K9me3

832 ChIP-seq reads aligned by the 5' end of intact *Suv39h1*-dependent LINE1 elements in WT and

833 *Suv39h1KO* cells at steady state. Summary data are depicted as histograms at the top of the

834 read alignment. (C) Pie chart showing percent enrichment of LINE1 subfamilies marked by

835 *Suv39h1*-dependent H3K9me3 peaks displayed in B. Percent enrichment is indicated by

836 emboldened numbers as in panel A. (D) Bar chart showing the percentage of total *Suv39h1*-

837 dependent H3K9me3 peaks lost during LPS challenge. (E) Aligned *Suv39h1*-dependent LINE1

838 H3K9me3 peaks in untreated, two, and four hours LPS treated WT BMDCs. (F) Pie chart

839 showing percent enrichment of LINE1 subfamilies in the LPS-sensitive H3K9me3 peaks on

840 LINE1 in panel E. Percent enrichment is indicated by emboldened numbers as in panel A. (A-F)

841 WT n=3, KO n=3 mice per experimental condition. In panels B and E, the x-axis coordinates

842 refer to kilobases. Statistical significance in panel A was determined by a two-proportions z-test

843 for comparison of proportions in two independent samples. Standard statistical analysis were

844 used for peak calling of ChIPseq data (see materials and methods for detailed protocol.

845

846 **Figure 3** LPS-elicited physiological depletion of SUV39H1 promotes a type-I IFN response in

847 myeloid cells independently of direct regulation of ISG loci, see also Figure S3. (A) Western blot

848 of SUV39H1 in RAW264.7 macrophages transduced with empty lentivector or with a SUV39H1

849 overexpression (OE) lentiviral vector. (B) Left, RAW264.7 macrophages transduced with

850 lentivirus control (n=5, closed circles) or SUV39H1 overexpressing lentivirus (n=5, open circles)

851 were stimulated for 8 hours with the indicated concentrations of LPS and the IFN-I response

852 measured was measured by ISG54-driven luciferase assay. Right, flow cytometric analysis of

853 intracellular expression of the ISG, VIPERIN, by APC-control and SUV39H1 overexpressing

854 RAW264.7 marophages stimulated as in left panel. (C) Left, RAW264.7 macrophages

855   transduced with lentivirus as in B and stimulated with the STING ligand 2'3'cGAMP for 8hrs and

856   the IFN-I response measured by ISG54-driven luciferase assay as in B. Right, flow cytometric

857   analysis of ISG, VIPERIN, expression in untreated and 2'3'cGAMP transfected cells after 8hrs.

858   (D) Western blot analysis of innate signal transduction pathways elicited in control and

859   SUV39H1 overexpressing RAW264.7 macrophages by LPS stimulation for the indicated times.

860   (E) Volcano plot depicting differential gene expression in WT versus *Suv39h1*KO BMDCs at

861   two hours of LPS stimulation. anti-viral genes with an adjusted *P* value <0.05 and FC ≥ 2 are

862   indicated in red. (F) Gene set enrichment analysis (GSEA) comparing expression of anti-viral

863   genes from mRNAseq data between the following groups: untreated *Suv39h1*KO x *Ifnar*$^{+/+}$ (n=4)

864   versus *Suv39h1*WT x *Ifnar*$^{+/+}$ (n=3) BMDCs (Blue); untreated *Ifnar*$^{-/-}$*Suv39h1*KO (n=3) versus

865   *Ifnar*$^{-/-}$ x *Suv39h1*WT BMDCs (n=3) (gold). (D) Western blots of the indicated total proteins

866   and phosphor-proteins during LPS treatment of BMDCs with the following genotypes from left to

867   right: *Ifnar*$^{+/+}$x*Suv39h1*WT*, Ifnar*$^{+/+}$x*Suv39h1*KO*, Ifnar*$^{-/-}$x*Suv39h1*WT and *Ifnar*$^{-/-}$x

868   *Suv39h1*KO. Western blots represent one of three experiments where similar results were

869   obtained. In B and C, statistical significance was determined by two-way ANOVA and

870   Bonferonni ad-hoc analysis: *P<0.05, ** P <0.001, ***P<0.001, ****P<0.0001. In (E), significance

871   cutoff for differential gene expression analysis was defined as an adjusted *P*-value < 0.05 and a

872   fold change ≥ 2. WT, n=3 mice per group; KO, n=4. In (F), statistical significance was GSEA;

873   *Suv39h1*WT x *Ifnar*$^{+/+}$ (n=3)*, Suv39h1*KO x *Ifnar*$^{+/+}$ (n=4)*, Suv39h1*WT x *Ifnar*$^{-/-}$ (n=3), and

874   *Suv39h1*KO x *Ifnar*$^{-/-}$ (n=3).

875

876   **Figure 4** LPS elicits cGAS-dependent but not MAVS-dependent ISG expression, see also

877   Figure S4. (A) Western blots of the indicated proteins and phosphor-proteins in WT and *Cgas*$^{-}$

878    $^{/-}$BMDCs treated as follows: LPS treatment was for 4 hours; cGAMP (250-500ng/ml), 2 hours.

879   (B) The indicated ISGs indicated in bold italics on the left were measured by mRNAseq of

880   steady-state and LPS-stimulated (4hrs, 100ng/ml) WT, *Myd88*$^{-/-}$, *Trif*$^{-/-}$ BMDCs or by RT-

881   qPCR of WT and *Mavs*$^{-/-}$ BMDCs treated with STING inhibitor (STINGi, H-151) or vehicle

882   control (DMSO) at steady state or stimulated with the indicated LPS dose (33 or 100ng/ml). As a

883   positive control for inhibition of the cGAS/STING pathway, WT BMDCs were treated with

884   STINGi or DMSO and then transfected with 1μg/ml HT-DNA for 5 hours. ISG expression was

885   calculated relative b-Actin by the delta Ct method. (C) Scatter plot of ISG54-Luciferase assay of

886   conditioned supernatants from WT, *Myd88*$^{-/-}$, or *Trif*$^{-/-}$ RAW264.7 macrophges stimulated

887   with the indicated concentration of LPS. (D) Scatter plot of ISG54-Luciferase assay of

25

888 conditioned supernatants from WT, *Cgas*⁻/⁻, *Mavs*⁻/⁻, and *Cgas*⁻/⁻ x *Mavs*⁻/⁻ RAW264.7

889 macrophages that were stimulated with the indicated concentration of LPS. (E) The indicated

890 LINE1families indicated in bold on the left were measured by mRNAseq of steady-state and

891 LPS-stimulated (4hrs, 100ng/ml) WT, *Myd88*⁻/⁻, *Trif*⁻/⁻ BMDCs or by RT-qPCR of WT and

892 *Mavs*⁻/⁻ BMDCs treated with STING inhibitor (STINGi, H-151) or vehicle control (DMSO) at

893 steady state or stimulated with the indicated LPS (100ng/ml) for 4hrs. As a positive control for

894 inhibition of the cGAS/STING pathway. (F) ISG54-driven luciferase assay as in C and D. WT

895 and *Cgas*⁻/⁻ RAW264.7 macrophages in which *Suv39h1* was left intact (shCtrl) or depleted

896 (*shSuv39h1*) with lentiviral-derived shRNA were treated with the indicated concentrations of

897 LPS for 8. WT (*shCtrl*, n=8; *shSuv39h1*, n=8), *Cgas*⁻/⁻(*shCtrl*, n=8; *shSuv39h1*, n=8). (G)

898 ISG54-driven luciferase assay as in C,D, and F. *Cgas*⁺/⁺ or *Cgas*⁻/⁻ RAW cells that were

899 transduced with APC control or SUV39H1 overexpression lentivectors and stimulated with the

900 indicated concentrations of LPS for 8hrs (n=10 per group). B-G, Statistical significance was

901 determined by two-way ANOVA with Bonferonni's ad hoc analysis for individual

902 comparisons:*$P<0.05$, ** $P <0.001$, ***$P<0.001$, ****$P<0.0001$.

903

904

905 **Figure 5** LINE1 is an endogenous cGAS PAMP, see also Figure S5. (A) qPCR of digitonin-

906 extracted, RNAse-treated cytosolic extracts from untreated or LPS stimulated (100ng/ml, 4hrs)

907 WT BMDCs that were pretreated with an RTI cocktail or DMSO vehicle control. (B) High-

908 throughput sequencing of DNA extracted from the following fractions from LPS-treated BMDCs

909 expressing *Cgas*$^{gfp/gfp}$: cytosolic input fraction, cytosolic cGAS, nuclear input fraction, and

910 nuclear cGAS. The pie charts display the enrichment in each fraction visualized as the

911 distribution of the sum of the read densities (FPKM≥1) per unique element belonging to the

912 indicated DNA species. N=2 for each sample. (C) Hypergeometric statistical comparison of read

913 density proportions in panel B between input and the respective cGAS-IP within each cellular

914 fraction (indicated in bold on right). Red color denotes —$\log_{10}$ (*P* value); grey, no statistical

915 difference. (D) Correlation between the number of individual LINE1 elements within each

916 subfamily expressed as mRNA and detected in the top 10% of fold changes in cytosolic cGAS-

917 bound DNA. Dot size indicates the DNA density (FPKM) of cGAS-bound LINE1 families and the

918 color scale indicates the average sequence divergence from consensus of the elements

919 detected on cGAS. Less divergence indicates evolutionarily younger elements. (E) Scattered

920   dot plots exhibiting FPKM of cGAS-bound unique LINE1 element DNA (FPKM ≥ 1 and FC ≥ 2

921   over input) in untreated and treated *Suv39h1*WT*xCgas*$^{egfp/egfp}$ and *Suv39h1*KO*xCgas*$^{egfp/egfp}$

922   BMDCs. Red bars represent median values; box ends represent quartiles. Statistical analysis

923   was performed with one-way ANOVA followed by Bonferroni's ad hoc analysis for individual

924   comparison between samples. (F) Scattered dot plots as in E except FPKM values represent

925   density of unique SINE element DNA bound to cGAS. Statistical analysis was performed as in

926   E. (G) qPCR of LINE1, ERVK, and genomic *Gapdh* DNA bound to cytosolic cGAS

927   immunoprecipitated with anti-GFP magnetic beads from BMDC cytosolic fractions as in B and E

928   (filled green bars). Controls include control-bead IP from *Cgas*$^{egfp/egfp}$(hashed green bars), GFP-

929   bead (filled grey bars) and control-bead IP (hashed grey bars) from *Cgas*$^{+/+}$ BMDCs.

930   (H) ISG54-Luciferase assay of resting and LPS-stimulated (8hrs) WT RAW264.7 cells

931   expressing mutant d(dead)Cas9 KRAB fusion protein and control or LINE1-targeted gRNAs.

932   (I) Representative volcano plot comparing differentially expressed (DE) LINE1 elements in

933   gRNA_1 versus Ctrl cells stimulated with LPS. (I) Bar chart representing the log$_2$ fold-change

934   between DE LINE1 elements (Adjusted *P* value <0.05) in Ctrl ("Down in gRNA") and gRNA-

935   expressing cells ("Up in gRNA") at steady state or after LPS treatment. (K) Above, GSEA of the

936   effect of the specified gRNA versus control on anti-viral gene expression in resting and LPS-

937   stimulated cells. Line color indicates the specific comparison. Below, gene rank plots. Each bar

938   represents an anti-viral gene and its position along the x-axis indicates relative enrichment in

939   the indicated group: left indicates enrichment in control cells; right, LINE1-targeted gRNA

940   expressing cells. bar color corresponds to the same color used to specify the specific

941   comparison above. *P* value to the right indicates statistical significance of enrichment of anti-

942   viral gene expression in the control group versus LINE1-targeted gRNA-expressing cells. NES,

943   normalized enrichment score. A, E, F, and H, statistical significance was determined by 2-way

944   ANOVA, and Bonferonni's ad hoc analysis was used for individual comparisons. A, E, F, H:

945   *P<0.05, ** P <0.001, ***P<0.001, ****P<0.0001.

946

947   **Figure 6** Induction of TE expression enhances host fitness during RNA virus infection. (A)

948   Schematic illustrating the induction of endogenous PAMPs upon detection of microbial PAMPs.

949   Microbial infection triggers IFN-I, cytokines and TEs. Subsequent detection of TE PAMP

950   amplifies the innate immune response. (B) Western blots of the indicated proteins in in mock-

951   treated or IAV/PR8-infected (7hrs) WT BMDCs. (C) Western blots of the indicated proteins and

952   phosphor-proteins in mock-treated or IAV/PR8-infected (7hrs) WT and *Cgas*$^{-/-}$BMDCs. (D)

953   Quantification of IAV/PR8 NP expression in DCs 5 dpi in the presence of DMSO vehicle or

27

954 STING inhibitor (STINGi, H-151). (E) DCs treated with DMSO vehicle or a RTI cocktail were

955 infected with the indicated dilution of IAV/PR8 and NP expression(left) and percentage survival

956 (right) were quantified by flow cytometry at 5dpi. (F) DC expressing lentivector control (APC) or

957 overexpressing SUV39H1(oe) were infected with IAV/PR8 as in E and %survival and NP

958 expression quantified 5dpi as in E. (G) DC expressing mutant d(dead)Cas9 KRAB fusion protein

959 and control or LINE1-targeted gRNA_1 as described in figure 5 were infected with IAV/PR8 at

960 the indicated dilution and NP expression(left) and percentage survival (right) quantified by flow

961 cytometry. (H) Schematic displaying a revised mechanism for innate sensing of pathogen or

962 that proceeds through two phases, in which TE expression is a central component. Initial

963 detection of a pathogen occurs through classical sensing pathways that stimulate expression of

964 defensive immune genes as well as TEs. Over time, TRAMP expression is amplified and may

965 constitute the majority of detected PAMP in the latter phase. A critical threshold for TE detection

966 may be surpassed once TRAMP concentration overcomes restriction factors such as the

967 cytosolic DNase *Trex1,* for example. Grey arrow heads indicate increased or decreased

968 expression; black arrows, impact on critical threshold; question marks indicate that the given

969 condition is likely to impact the critical threshold but has not been directly tested. D-G, statistical

970 significance was determined by 2-way ANOVA and Bonferonni's ad hoc analysis for individual

971 comparisons: $*P<0.05$, ** $P <0.001$, $***P<0.001$, $****P<0.0001$.

972

973 **Figure S1** Ablation of *Suv39h1* significantly enhances LPS-induced intact TE transcription,

974 reflecting loss of H3K9me3 during LPS challenge, related to Figure 2. (A) Bubble plot exhibiting

975 percentage enrichment and statistical comparison of the proportion of differentially expressed

976 TE in LPS treated versus untreated BMDCs in WT or *Suv39h1*KO cells. Statistical significance

977 $(P < 0.05)$ was determined by a two-proportions z-test. $-\log_{10}$ (P value) is indicated by color

978 scale, bubble size indicates percentage of TE enrichment within each comparison. (B) mRNA

979 sequencing data showing expression levels (FPKM) of H3K9me3 methyltransferases or

980 pathway members (top, red lines) during LPS treatment: HUSH complex (*Fam208a,*

981 *Mphosph8, Morc2a*) and other H3K9me3 remodelers/heterochromatin factors *Setdb1, Trim28,*

982 *Suv39h2, and Hp1a (Cbx5)*. (Bottom, blue lines) mRNA sequencing data showing modulation of

983 of histone lysine demethylases expression (FPKM): *Kdm1a/b, Kdm2a/b, Kdm4a/b*, and *Kdm5c*.

984 (C-E) Summarized data of total numbers of intact LINE1 (C), SINE (D) and LTR (E) that were

985 differentially induced in either *Suv39h1*WT or KO BMDCs when LPS treated samples were

986 compared with steady state. (F) Mean average plot of human LINE, SINE, and LTR elements

987 comparing 4hr LPS treated to untreated monocyte-derived DCs from human PBMCs. Blue dots,

988   TEs with a log$_2$FC 2, and average FPKM1; Red dots, full length LINE1 transcripts with a log$_2$FC

989   2, and average FPKM1. (G) Enumeration of total TE numbers filtered by our criteria in panel e

990   from each indicated class at the different time points of LPS stimulation compared to untreated

991   cells. (H, I) Applying the same filters as in panel F, only the full length TE copies from each

992   indicated class are displayed for LINE1 and LTR (H) and SINE (I). I, Pie charts exhibiting the

993   relative proportions of expressed full length LINE1 copies enriched in untreated (UT, top) or

994   LPS-treated (4hrs, bottom) cells.

995

996

997   **Figure S2** *Suv39h1*-dependent H3K9me3 peaks are primarily localized at transposable

998   elements, related to Figure 1. (A) Percentage among three biological replicates of H3K9me3

999   peaks with significant overlap. (B) The number of H3K9me3 peaks in UT *Suv39h1WT* and

1000   *Suv39h1KO* BMDCs and the total number of *Suv39h1*-dependent peaks that were determined

1001   by subtraction of *Suv39h1*KO H3K9me3 peaks from WT H3K9me3 peaks. (C) Correlation

1002   between divergence of LINE1 versus the number of elements copies containing a *Suv39h1*-

1003   dependent H3K9me3 peak. (D) H3K9me3 peaks at SINEB1 elements in *Suv39h1*WT and

1004   *Suv39h1*KO BMDCs aligned at 5' end with summary data at top as in Figure 2B, E. (E)

1005   Summary of H3K9me3 reads in the indicated elements that were aligned at their 5' ends as in

1006   Figure 2B, E (top). (F) Aligned SINEB1 peaks as in D, in WT BMDCs stimulated with LPS for

1007   the indicated duration. (G) Summary data of aligned ERV1 (top) and ERVK (bottom) H3K9me3

1008   peaks in WT cells stimulated with LPS for the indicated times. D-G, the x-axis coordinates refer

1009   to kilobases. Statistical peak calling was performed as detailed in the methods section.

1010

1011   **Figure S3** Loss of *Suv39h1* amplifies the LPS-induced anti-viral response independently of

1012   direct regulation of gene loci, related to Figure 3. (A) *Ifnb1* expression from mRNAseq data of

1013   *Suv39h1*WT and KO BMDCs that were treated with LPS for the indicated times. Data are

1014   displayed as FPKM. (B) ELISA quantification of Type-I IFN (IFNb) protein levels in conditioned

1015   supernatants from *Suv39h1WT* (n=4) or *Suv39h1KO* (n=5) BMDCs treated with LPS for the

1016   indicated times. (C) Western blots of the indicated proteins and phosphor-proteins in

1017   *Suv39h1*WT and KO BMDCs that were treated with LPS (100ng/ml) for the specified durations.

1018   (D) Heatmap displaying semi-supervised clustering (Pearson correlation) of anti-viral (ISG) and

1019   inflammatory gene expression in WT and *Suv39h1KO* BMDCs stimulated with LPS for the

1020   indicated times. Data are from the same mRNA-seq data set used for TE analysis in Fig. 1. (E)

1021   Flow cytometric data quantifying SIGLEC1 (ISG) and CD86 (NFkB-regulated) surface

1022   expression on mDCs transduced with nontargeting or one of three different SUV39H1-targeting

1023   lentiviral shRNA constructs (n=4 patient samples per condition). (F) Western blot of SUV39H1,

1024   demonstrating targeted depletion by three different lentiviral shRNAs in freshly cultivated human

1025   monocyte-derived DCs (mDCs). (G) qPCR data exhibiting the time course of *Suv39h1* mRNA

1026   expression during LPS treatment in RAW macrophages transduced with the designated

1027   lentiviral shRNAs (n=2). (H) Western blot of SUV39H1, SETDB1, and gp96 (loading control) to

1028   assess shRNA-mediated knockdown in untreated RAW264.7 macrophages transduced with the

1029   indicated lentiviral shRNA particle. (I) ISG54-Luciferase assay data comparing luciferase

1030   concentration in conditioned supernatants of untreated RAW264.7 in which *Suv39h1* was

1031   depleted by lentiviral encoded shRNA (*shSuv39h1*, n=4) or not (shGFP, n=4). (J) Left, heatmap

1032   showing z-scores of ISG mRNA expression (FPKM) in untreated *Suv39h1*WT (n=3) or

1033   *Suv39h1*KO (n=4) BMDCs, and right, the corresponding H3K9me3 peak status from H3K9me3

1034   ChIPseq data (WT, n=3; KO, n=3): red, H3K9me3 peak positive, grey, no H3K9me3 peak

1035   detected. Several genes along with *Ifnb1* are designated in bold to the right as exemplars of

1036   ISGs with a grey or red box indicating H3K9me3 peak status. Statistical differences in panels A,

1037   B, and E were determined by two-way ANOVA, followed by Bonferroni's ad hoc analysis for

1038   individual comparison between groups:*$P<0.05$, ** $P <0.001$, ***$P<0.001$, ****$P<0.0001$. In I, a

1039   two-tailed student's t-test was performed to determine statistical significance ($P < 0.05$).

1040

1041

1042   **Figure S4** The cGAS pathway of DNA sensing, but not the RNA-sensing MAVS pathway is

1043   required for an optimal IFN-I/anti-viral response following stimulation with LPS, related to Figure

1044   4. (A) ELISA quantification of IFNb protein concentration in conditioned supernatants from

1045   *Cgas*$^{+/+}$(n=3) and *Cgas*$^{-/-}$ (n=3) BMDCs stimulated with LPS or with transfected HT-DNA,

1046   2'3'cGAMP. (B) Compiled flow cytometric data showing the percentage of Viperin$^+$ or CCL5$^+$

1047   CD11c$^+$CD11b$^+$MHC-II$^+$ splenic *Cgas*$^{+/+}$ (n=3) and *Cgas*$^{-/-}$ (n=3) DCs stimulated *ex vivo* with

1048   10-fold dilutions of LPS (top, 100ng/ml highest concentration) or bottom, with 2'3'cGAMP. (C)

1049   Western blot of the indicated total and phosphor-proteins following stimulation of WT and

1050   *Mavs*$^{-/-}$BMDCs with transfected poly(I:C) (5hrs) or infection with IAV/PR8 (7hrs). (D) RT-qPCR

1051   data of the ISGs, 5hrs after transfection of poly(I:C) in WT and *Mavs*$^{-/-}$BMDCs. ISG expression

1052   was calculated relative to b-Actin by the delta Ct method. (E) Western blot displaying protein

1053   expression of MAVS, cGAS, and gp96 after CRISPR-Cas9-targeted deletion of *Mavs* in WT and

1054   *Cgas*$^{-/-}$ RAW264.7 macrophages. (F) Functional validation of CRISPR-Cas9-targeted *Mavs*

1055   deletion in RAW264.7 macrophages displayed in E; top, transfection of poly(I:C), 2µg/ml;

1056   bottom, transfection of HT-DNA, 1µg/ml. (G) ELISA quantification of IFNB concentration in

1057   conditioned supernatants of untreated or LPS (100ng/ml) treated RAW264.7 macrophages that

1058   were deleted for *Ifnar1/2* by CRISPR-Cas9 and the transduced with control lentiviral particles

1059   (shGFP) or shRNA lentiviral particles targeting *Suv39h1 (shSuv39h1).* (H, I) Functional

1060   validation of CRISPR-Cas9 targeted deletion of *Ifnar* in RAW264.7 macrophges. (C) ISG54-

1061   driven luciferase assay measuring responsiveness of *Ifnar$^{+/+}$Cgas$^{+/+}$, Ifnar$^{+/+}$Cgas$^{-/-}$, Ifnar$^{-/-}$*

1062   *Cgas$^{+/+}$,* and *Ifnar$^{-/-}$Cgas$^{-/-}$* cells to overnight stimulation with titrated recombinant IFNB

1063   protein.Error bars represent standard deviation and bar graphs represent the mean. Statistical

1064   significance was determined with two-way ANOVA and Bonferonni's ad hoc analysis for

1065   individual comparisons. A, B, D, and G, statistical significance was determined with two-way

1066   ANOVA and Bonferonni's ad hoc analysis for individual comparisons.

1067

1068   **Figure S5** Immunoprecipitation of endogenous cGAS from nuclear and cytosolic extracts

1069   followed by NGS reveals significant changes in cytosolic cGAS-DNA binding activity, related to

1070   Figure 5. (A) Flow cytometric quantification of expression of the ISG VIPERIN in untreated or

1071   8hr LPS-stimulated WT BMDCS that were pretreated with DMSO vehicle or an RTI cocktail. (B)

1072   Western blot revealing WT cGAS and the eGFP knock-in cGAS (KI) allele from whole cell lysate

1073   (WCE), cytosolic (C) and nuclear (N) fractions, as well as immunoprecipitation of cross-linked

1074   cytosolic and nuclear cGAS species. RAB6 and H3 serve as cytosolic and nuclear loading

1075   controls, respectively. Arrowheads indicate lanes displaying immunprecipitated eGFP-cGAS.

1076   (C) qPCR of the indicated amplicons in digitonin-extracted RNAse-treated cytosolic DNA and

1077   pellet fractions from untreated BMDCs. (D) qPCR of indicated DNA species that bound to an

1078   overexpressed eGFP-tagged cGAS contstruct in WT (shControl, n=4) and *Suv39h1* (n=4)

1079   knockdown RAW cells following LPS stimulation. (E) Pie chart depicting the relative proportions

1080   of raw reads in the indicated DNA species from NGS of cytosolic cGAS-IP. (F) Left, comparison

1081   of the fold changes in LPS versus untreated BMDCs of TE binding density on endogenous

1082   cGAS in cytosolic and nuclear fractions. Right, variance describing the fold changes in the

1083   binding density of the indicated TE class enriched on nuclear and cytosolic cGAS following LPS

1084   treatment (n=2 per condition). An F-test was used for statistical comparison of variances. (G)

1085   Flow cytometric plots displaying lentiviral-based expression levels of the indicated GFP-cGAS or

1086   SFFV-BFP control constructs used for experiments in Figure S5H, I. (H) ISG54-driven luciferase

1087   assay quantification of baseline IFN-I production by *Cgas$^{-/-}$* RAW cells following lentiviral

1088   reconstitution with different cGAS constructs—full-length WT (cGAS-FL, n=12), catalytically-

1089   dead (cGAS CD, n=7), DNA binding mutant (cGAS-DBM, n=7), SFFV (empty vector control). (I)

31

1090   ISG54-driven luciferase assay quantification of IFN-I production by *Cgas*$^{-/-}$ RAW cells following

1091   lentiviral reconstitution with different cGAS constructs—full-length WT (cGAS-FL, n=12),

1092   catalytically-dead (cGAS-CD, n=7), DNA binding mutant (cGAS-DBM, n=7), SFFV (empty

1093   vector control)—and titrated stimulation with HT-DNA, 2'3'cGAMP, or LPS. Baseline values

1094   were subtracted from treated values to compare the response between groups reconstituted

1095   with distinct cGAS constructs. (J) Distribution of the cytosolic cGAS-IP read density sums

1096   (FPKM≥1) among all classes of DNA species in *Suv39h1KO* BMDCs. (K) Hypergeometric

1097   statistical comparison of the *Suv39h1*KO BMDC cytosolic cGAS-IP read density sums in panel

1098   E with WT cytosolic cGAS IP (Figure 5B). (L) Violin plot exhibiting the difference in the number

1099   of cytosolic cGAS-bound LINE1 elements by family between *Suv39h1*KO and *Suv39h1*WT

1100   BMDCs. (M) Inhibition of cell surface myeloid marker CD11b on RAW264.7 using the dCas9-

1101   KRAB transcriptional inhibitor system with two different gRNAs directed to the promoter region

1102   of the *Itgax* locus. Top, flow cytometric dot plots of CD11b (y-axis) and MHC Class II (MHC-II, x-

1103   axis); bottom, quantification of flow cytometric data for percentage of CD11b and MHC-II

1104   expressing cells and the MFI of each marker (n=3 per condition). (N) *In silico* prediction of the

1105   number of LINE1 gRNA targets (y-axis) according to the number of allowable mismatches

1106   between gRNA and target sequences (color intensity). Above, pie charts exhibiting enrichment

1107   of gRNA target sequences in LINE1 families. The most highly enriched family is denoted at the

1108   top of each pie chart and the corresponding gRNA number is listed below. (O) Scatter dot plot

1109   displaying the number of gRNA target sequences upstream of ISGs (blue circles) versus

1110   targeted LINE1 elements (red circles) . (P) ISG54-Luciferase assay of IFN-I and ISG induction

1111   in resting and LPS-stimulated (8hrs) *Cgas*$^{-/-}$ RAW264.7 cells expressing mutant dCas9 KRAB

1112   fusion protein and the indicated gRNA (Ctrl, gRNA_1, or gRNA_2; n=4 per group). Statistical

1113   significance was determined by 2-way ANOVA and Bonferonni's ad hoc analysis for individual

1114   comparisons:*$P<0.05$, ** $P <0.001$, ***$P<0.001$, ****$P<0.0001$.

1115

1116   **Figure S6** IAV/PR8 infection can be assessed by flow cytometric measurement of cell survival

1117   and viral nucleoprotein expression, related to figure 6. Top, flow cytometric dot plots of survival

1118   5 days post infection (dpi) of DCs with IAV/PR8. Bottom, flow cytometric dot plots of IAV/PR8

1119   NP protein expression 5 dpi with IAV/PR8 in the same sample as the corresponding plots

1120   above.

1121

1122   **ACKNOWLEDGMENTS**

1152   AUTHOR CONTRIBUTIONS

1153   D.C.R. conceptualized, established, and performed histone ChIP-seq, cGAS-IP-seq protocols,
1154   cGAS-IP-seq library preparation, cell stimulations, cytosolic DNA extraction, CRISPR-dCas9-
1155   targeted suppression of LINE1 elements, lentiviral knockdown of *Suv39h1*, cGAS reconstitution,
1156   and SUV39H1 overexpression, IFNB and cytokine measurements, RNA-seq, and FACS
1157   analysis. M.G. made eGFP-cGAS lentiviral constructs and performed shRNA-targeted
1158   knockdown of SUV39H1 and stimulations in primary human MDDCs. N.B. and T.H. assisted
1159   D.C.R. with various experiments. P.B. performed analysis of RNAseq data from MDDCs,
1160   BMDCs and CRISPR-Cas9-KRAB suppression of LINE1. C.G. and M.Y. performed statistical
1161   analyses and mapping of H3K9me3-ChIP, cGAS-IP sequencing, and mRNA-seq of Suv39h1WT
1162   and KO BMDCs. N.M. supervised M.G. and contributed mice with an eGFP-tagged endogenous
1163   cGAS allele. D.C.R, P.B., M.Y., and C.G. analyzed data. D.C.R., G.J.T., and R.M. wrote the
1164   manuscript.
1165

1166   DECLARATION OF INTERESTS

1167   The authors declare no competing interests.

1168

1169 **MATERIALS AND METHODS**

1170 **Mice**

1171 *Suv39h1*KO(Peters et al., 2001) mice were from Thomas Jenuwein. *Mb21d1* (*Cgas*)$^{-/-}$ (*B6(C)-*

1172 *Mb21d1$^{tm1d(EUCOMM)Hmgu}$/J*) and *Mavs*$^{-/-}$ (B6;129-*Mavs^{tm1Zjc}*/J) mice were from Jackson

1173 laboratory. cGAS-eGFP-Knock-in mice, a gift from Nicolas Manel (*Mb21d1$^{(tm1Ciphe)}$;* CIPHE) were

1174 generated by targeted in-frame insertion of an enhanced GFP sequence after the ATG start

1175 codon of the endogenous *Mb21d1* locus. *Trif*$^{-/-}$(C57BL/6J-*Ticam1$^{Lps2/J}$*) and *Myd88*$^{-/-}$

1176 (B6.129P2(SJL)-*Myd88$^{tm1.1Defr}$/J*) were from Catherine Werts (Pasteur Institute) and *Ifnar*$^{-/-}$

1177 (B6.129S2-*Ifnar1^{tm1Agt}/Mmjax*) mice were originally from JAX. C57BL/6N mice were originally

1178 from Charles Rivers Laboratories. All mice were housed in SPF animal facilities, and live animal

1179 experiments were performed in accordance with the European Veterinary Department (Project

1180 Authorization N:02465.02).

1181

1182 **Cell Culture**

1183 Bone marrow-derived dendritic cells were cultivated in 20ng/ml GMCSF (Miltenyi) in IMDM

1184 (VWRI3390) supplemented with 10% fetal bovine serum (Eurobio), Penicillin/Streptomycin,

1185 50μM -mercaptoethanol, minimal non-essential amino acids, and 2mM Glutamax (all from Life

1186 Technologies) and referred to as I-10 medium. Briefly, fresh bone marrow was collected from

1187 two of each—ilium, femur, and tibia—by centrifugation. Five million bone marrow cells were

1188 seeded on untreated 10cm plates (VWR) in 10mls of I-10 medium. On day 3, an additional

1189 10mls of I-10 medium was added, followed by collection and replenishment of 10 mls on day six

1190 and on day eight if needed for use at day ten. BMDC clusters were harvested on day 8-10 in 5

1191 mls of comlete I-10 media at room temperature and then stimulated at 1-2x10^6 cells per well of

1192 an untreated 6-well plate (Sigma M9062-100EA) in 2 mls of I-10 medium without GMCSF.

1193

1194 **Cell stimulations**

1195 Cell stimulations were performed for the indicated times with mammalian cell culture-derived

1196 mouse IFNB (PBL, 12405), herring testes (HT)-DNA (1-4μg/ml as indicated; Sigma D6898-

1197 250MG), LPS (100ng/ml, Invivogen), high molecular weight poly (I:C) (1-2μg/ml, Invivogen), or

1198 2'-3'cGAMP (4μg/ml, Invivogen) for the specified times. For cytosolic delivery of HT-DNA,

1199 poly(I:C), or 2'3'cGAMP, 12μg of PAMP and 12μl of Lipofectamine^{TM} (Life Technologies,

1200 11668019) were separately diluted in OptiMEM (ThermoFisher 3195062), incubated for 5

1201 minutes at room temperature, then combined, mixed by pipetting, and rested for an additional

1202 15 minutes at RT. Liposome-containing PAMPs were then diluted in complete medium to make

1203 a concentrated working stock for cell stimulation. STING inhibitor (H-151, MedChem Express)

1204 stock solution was stored in DMSO at -80°C. Cells were pretreated with H-151 or DMSO vehicle

1205 for 2hrs before subsequent stimulation with PAMP or infection with IAV/PR8.

1206

1207 RAW264.7-ISG-Lucia cell lines (all from Invivogen) deleted for *Tmem173*(rawl-kostg*),*

1208 *Mb21d1*(rawl-kocgas*), Mda5* (rawl-komda5*), and Rig-I*(rawl-korigi*)* carried a Lucia luciferase

1209 reporter construct under control of an IFN-inducible ISG54 promoter (Invivogen) and were

1210 cultured in DMEM containing 10% Serum (Fischer Scientific), 1% Penicilin/Streptomycin

1211 (Fischer Scientific) and Zeocin (1μg/ml; Invivogen). Cell stimulations were performed for the

1212   indicated times with HT-DNA, LPS, poly(I:C), or 2'-3'cGAMP, at the indicated concentrations in
1213   6-well or 96-well tissue culture treated plates (Costar) following the same protocol as for
1214   BMDCs.
1215

1216   **Dendritic cell infection wit IAV**
1217   IAV/PR8/34 H1N1 was purchased from Charles River Laboratories and was stored at -80°C.
1218   Both BMDCs (Figure 6C) or the previously described JAWS II (ATCC)(*101*) mouse bone
1219   marrow-derived dendritic cell line (Figure 6B, D-G) was used for infection experiments. Briefly,
1220   BMDCs or JAWS II cells were plated in serum-free IMDM media supplemented with non-
1221   essential amino acids and infected with IAV/PR8 for 1hour at 37°C, before addition of complete
1222   media with a final concentration of 10%FBS. Infections were allowed to proceed for the times
1223   specified in figure legends. For RTI, treatment, emtricitabine and disoproxil fumarate were
1224   administered two hours prior to innoculation and maintained at 10µM for the duration infection
1225   (120hrs). DMSO vehicle control was administered alongside RTI treatment as a control.
1226

1227   **Generation of *Ifnar*$^{-/-}$ and *Mavs*$^{-/-}$ RAW264.7 macrophage cell lines with CRISPR**
1228   WT RAW264.7 macrophages were deleted using recombinant Cas9 protein (IDT, Alt-R S.p. HiFi
1229   Cas9 Nuclease V3) complexed with Tracr RNA and gRNAs targeting either *Ifnar1* (IDT pre-
1230   design gRNA Mm.Cas9.IFNAR1.1AA, Mm.Cas9.IFNAR1.1AB), *Ifnar2* (IDT pre-design gRNA,
1231   Mm.Cas9.IFNAR2.1AA, Mm.Cas9.IFNAR2.1AB), or *Mavs* (IDT pre-design gRNA,
1232   Mm.Cas9.Mavs.1AA, Mm.Cas9.Mavs.1AL). Briefly, gRNAs and Tracr RNA duplexes were
1233   generated by melting at 95°C for 5 minutes followed by downramping to 20°C at 5°C/minute.
1234   Duplexed RNA was then combined with recombinant Cas9 in duplex buffer for 15 minutes at
1235   room temperature to form Cas9 ribonucleoproteins (RNPs). WT RAW264.7 cells were subjected
1236   to two rounds of nucleofection with Cas9-RNPs using the SF Cell Line 4D-Nucleofector X Kit S
1237   (Lonza) and the 4D Nucleofector apparatus precisely as specified by the manufacturer. *Ifnar1*
1238   and *Ifnar2* gRNAs were delivered in the first and second rounds of nucleofection, respectively;
1239   both *Mavs* gRNAs were delivered in first and second rounds. *Ifnar* deletion was confirmed by
1240   luciferase assay as described under "Luciferase assay" in response to 8 hrs stimulation with
1241   titrated recombinant mammalian IFNB (PBL, 12405); *Mavs* deletion was confirmed by western
1242   blot.
1243

1244   **Suppression of LINE1 using CRISPR-dCas9-KRAB**
1245   In brief, two lentiviral vectros were used to target endogenous LINE1 elements in RAW264.7
1246   macrophages. gRNAs were cloned into the puro-resistant pKLV2-U6gRNA5(BbsI)-
1247   PGKpuro2ABFP-W(Tzelepis et al., 2016) vector. A second vector expressing dCas9-KRAB
1248   under control of the SFFV promoter was co-transduced into cells(Gilbert et al., 2014) for
1249   epigenetic suppression of gRNA targets. gRNAs against LINE1 5' regions were generated using
1250   CRISPOR and selected based on their predicted *in silico* enrichment in full length LINE1
1251   elements and paucity of genic targets.
1252

1253   **Generation of human monocyte derived DCs (MDDCs)**
1254   Buffy coats were prepared from peripheral adult human blood and CD14^{+} monocytes were
1255   isolated by magnetic separation (Miltenyi 130-050-201). CD14^{+} monocytes were differentiated

1256   into DCs (MDDCs) in RPMI containing Glutamax, supplemented with 10%FBS (GIBCO),
1257   50µg/ml Gentamicin (GIBCO), 0.01M HEPES (GIBCO), 10ng/ml GM-CSF (Miltenyi premium
1258   grade), and 50ng/ml IL-4 (Miltenyi premium grade) at a density of 10^6 cells/ml. Cells were
1259   harvested on day 4 for further analyses.
1260
1261   **Lentiviral transduction of human cells**
1262   Lentiviral particles were produced as previously described(Gentili et al., 2019). Briefly, 293FT in
1263   one well of a 6-well were transfected with 1µg psPAX2, 0.4µg pCMV-VSV-G and 1.6µg of a
1264   lentiviral vector (for human shSUV39H1, Sigma, TRCN0000157251, TRCN0000275323,
1265   TRCN0000275322) using TransIT 293 (Mirus, Biomedex) in Optimem (GIBCO). 2.6µg of
1266   pSIV3 and 0.4µg of pCMV-VSV-G were transfected into 293FT cells to generate SIV-VLPs.
1267   Twelve to fourteen hours later, the medium was changed to 3 mls of MDDC culture medium
1268   without cytokines. 30-32 hours later, supernatants were harvested, passaged over a 0.45µm
1269   filter, and used immediately for transduction of freshly isolated CD14$^+$ monocytes at 1.5×10^6
1270   cells/well of a 6-well plate in 1ml of MDDC culture medium. 1 ml of each lentiviral particles and
1271   SIV-VLPS were added to each well for 3mls final volume in the presence of protamine (8µg/ml).
1272   48hrs after transduction, puromycin (2µg/ml, Invivogen) was added for seletion of shRNA-
1273   expressing cells. Cells were harvested on day 4 of culture, plated at 50,000 cells per well of a
1274   96-well round bottom plate, and stimulated overnight with the indicated PAMPs.
1275
1276   **Luciferase assay**
1277   Production of type-I IFN production in RAW264.7 macrophages was measured by production of
1278   secreted luciferase under the control of the interferon-inducible ISG54 promoter. Briefly,
1279   conditioned supernatants were collected after stimulation, and 10µl was quantified for secreted
1280   luciferase (Renilla) activity in the presence of luciferase substrate (Quanti-Luc; rep-qlc2,
1281   Invivogen). Luminescence was recorded on a FLUOstar OPTIMA microplate reader (BMG
1282   Labtech).
1283
1284   **IFN ELISA**
1285   IFNb in conditioned supernatants was measured with the VeriKine Mouse Interferon Beta ELISA
1286   Kit (PBL, 42400-1) precisely as specified in the manufacturer's protocol.
1287
1288   **RNA purification, Sequencing, and qPCR**
1289   Following stimulation, BMDCs were harvested on ice, collected by centrifugation and
1290   immediately dissolved in Trizol (Life Technologies, 15596018) and stored at -80°C until further
1291   processing. RNA was separated from DNA and protein by chloroform extraction. Briefly, 100µl
1292   chloroform was added to 1 ml of Trizol, mixed by shaking for 1 min and incubated for 5 minutes
1293   at RT. After centrifugation (20,000 x *g* for 25 minutes at 4°C), 400µl of the RNA-containing
1294   aqueous phase was removed and combined with 1µg of RNAse-free glycogen (ThermoFischer)
1295   and 500µg ice-cold isopropanol. The mixture was frozen overnight at -80°C and thawed on ice
1296   before centrifugation (20,000 x *g* for 25 minutes at 4°C). The RNA pellet was air-dried for 30-
1297   60minutes in a laminar hood, resuspended in 10µl RNAse/DNAse-free water, and the DNA
1298   removed by in-solution DNAse digestion (Qiagen's RNase-Free DNase Set, #Cat 79254) for 30
1299   minutes at room-temperature. The resulting DNA-free RNA prep was further purified over

1300   Quiagen columns using the Qiagen RNeasy mini kit and eluted in RNase/DNase-free water.

1301   RNA preparations performed with this protocol routinely yielded an A260/280 ratio above 2 and

1302   averaging 2.08. HTS libraries were constructed using the TruSeq Stranded mRNA kit from

1303   Illumina and sequenced to a depth of at least 50×10^6 reads per sample using 100bp paired-end

1304   sequencing.

1305

1306   For qPCR, cDNA was generated from 500ng of RNA using random hexamers (Promega,

1307   C1181) and the SuperScript III First-Strand kit (Themro Fisher, 18080051) in a 20µl reaction.

1308   2µl of RNaseH was added and the reaction carried out at 37°C for 20 minutes. Following cDNA

1309   synthesis, 160µl of RNAse/DNase-free H_2O was added, and 4µl of this was used per qPCR

1310   reaction containing 1µl of 5µM primers and 5µl of SybrGreen reagent (LifeTechnologies,

1311   4367659). 10µl reactions were performed in a 384 well plate (Thermo Fisher, 4483319) on a

1312   Viia7 thermal cycling system (Applied Biosystem).

1313

1314   **PolyA$^+$ RNA-Seq Analysis**

1315   **Sequencing Data Processing**

1316   FASTQC [*http://www.bioinformatics.babraham.ac.uk/projects/fastqc/*.] was run on all samples to

1317   assess the quality of sequencing. Raw sequences were mapped to the mouse mm10 reference

1318   genome (Refseq) with STAR aligner (Spliced Transcripts Alignment to a Reference, version

1319   2.5.0a) (Dobin et al., 2013). We used the option *--bamRemoveDuplicatesType UniqueIdentical*

1320   to mark all multimappers, and duplicate unique mappers, *--outFilterMultimapNmax 100* that

1321   correspond to the number of read alignments which will be output only if the read maps fewer

1322   than this value.

1323

1324   **TE Quantification and Intactness**

1325   We used the Homer Software as previously described. However, we considered only TEs for

1326   the analysis if the row sum across all samples was FPKM 5. LINE1 and LTR elements greater

1327   than or equal to 4kb in length were considered intact. SINE elements were considered intact

1328   when they diverged less than 10% from their consensus sequences.

1329

1330   **Differential Analysis**

1331   Using the raw reads matrix, we filtered out the non-expressed genes and TE separately by

1332   requiring more than 5 reads in at least 2 samples for each features and normalized using

1333   DESeq2 R-package v1.18.1 (Love et al., 2014). Then, the differential expression analysis was

1334   performed using DESeq2, only the Benjamini Hochberg (BH) adjusted p-value below 0.05 were

1335   considered as significant.

1336

1337   **PCA**

1338   The PCA was performed using the Ade4 package(S Dray, 2007) of the R software (version

1339   3.3.2). The barycenters were computed from the set of observations in each condition and

1340   projected into the PCA plot.

1341

1342   **Chromatin Immunoprecipitation (ChIP)-sequencing**

1343 Histone ChIP experiments were performed as previously specified (Blecher-Gonen, et al 2013)

1344 with several changes. Briefly, harvested BMDCs were collected by centrifugation at 350 x g for

1345 5 minutes and resuspended in 1ml of I-10 medium. Cells were cross-linked with 1%

1346 formaldehyde (Euromedex) for 8 minutes at room temperature and the reaction quenched in

1347 150mM glycine for 10 minutes. Cross-linked cells were collected by centrifugation (350 x g for 5

1348 minutes at room temperature) and washed twice with cold PBS. Pelleted cells were

1349 subsequently lysed to isolate cross-linked chromatin. For cytosolic extraction, cells were first

1350 suspended at 1x10^6 cells/100μl of lysis buffer 1 (LB1) (50mM Hepes (pH7.5), 140mM NaCl,

1351 1mM EDTA (pH 8.0) , 10% glycerol, 0.75% NP-40, 0.25% Triton X-100) with protease inhibitors

1352 (Roche 11873580001) and Na$_3$VO$_4$ (180μg/ml) for 10 minutes on ice, followed by centrifugation

1353 at 500 x g for 5 minutes at 4°C to separate cytosol and nuclei. Nuclear pellets were then

1354 dissolved in lysis buffer X (LBX) (50mM Tris pH 8.0, 5mM EDTA pH 8.0, 0.25%SDS)

1355 supplemented inhibitors at 10^{6}cells/100μl and incubated on ice for an additional 10 minutes, and

1356 then sonicated with a Bioruptor Pico (Diagenode) for 11 cycles (30 seconds on/off) at 4°C.

1357 Lysates were cleared by centrifugation at 20,000 x g for 20 minutes at 4°C, snap frozen on

1358 liquid N$_2$, and stored at -80°C until immunoprecipitation. Samples were thawed on ice and

1359 diluted 1:1 with dilution buffer X (DBX) (50mM Tris pH8.0, 0.9%NP40, 350mM NaCl), followed

1360 by the addition of either anti-H3K9me3 (Diagenode) or control rabbit IgG (Diagenode) and

1361 rotated overnight at 4°C. The following day, 20μl of DBX-washed Magna ChIP Protein W+G

1362 magnetic beads (Millipore) were added to each sample and rotated for 1hr at 4°C, followed by

1363 washes and de-crosslinking exactly as described previously(Blecher-Gonen et al., 2013). DNA

1364 fragments were purified by two-sided selection with SPRI beads (Beckman Coulter B23318)

1365 according to the manufacturer's guidelines and Illumina sequencing libraries prepared with the

1366 TruSeq ChIP Library Preparation Kit. 150bp paired-end sequencing of pooled libraries was

1367 performed on a HiSeq 2500 (Illumina) platform in rapid run mode to yield 50x10^6 reads per

1368 sample.

1369

1370

1371 **Cross-linked cytosolic and nuclear cGAS immunoprecipitation and next**

1372 **generation sequencing (NGS)**

1373 Cytosolic cGAS immunoprecipitation was carried out similar to histone ChIP

1374 experiments with minor adjustments. Anti-GFP (ChromoTek) or control beads

1375 (ChromoTek) were added to the appropriate cell number as indicated by the

1376 manufacturer and incubated overnight with rotation at 4°C. The following day, samples

1377 were collected with a magnet, transferred to a 96-well qPCR plate and washed, de-

1378 crosslinked and purified as for histone ChIP. Purified cGAS-bound DNA was sheared to

1379 a mean size of 200bp in 100μl of direct elution buffer (10 mM Tris-HCl (pH 8.0), 5 mM

1380 EDTA (pH 8.0), 300 mM NaCl and 0.5% SDS (vol/vol) with a Covaris S220. Sheared

1381 DNA was purified with Agencourt AMPure XP beads (Beckman Coulter). Illumina-based

1382 libraries were prepared to preserve ssDNA, RNA:DNA hybrids, and dsDNA. Briefly,

1383 DNA fragments were tailed and ligated with an adaptor, followed by primer-based PCR

1384 extension of complementary DNA. A second adaptor was ligated to the 5' end of the

1385 template strand. dsDNA species were amplified and indexed using barcoded primers for

1386 Illumina-based high throughput sequencing, and 150bp paired-end sequencing of

1387   pooled libraries was performed on a HiSeq 2500 (Illumina) platform in rapid run mode to

1388   yield 50×10^6 reads per sample.

1389

1390   **Mapping**

1391   ChIP-seq reads were mapped to mouse genome build mm10 with Bowtie2 (v2.1.0)

1392   (Langmead and Salzberg, 2012)using the global alignment mode allowing 1 mismatch in

1393   seed alignment. We processed only mapped reads.

1394

1395   **Transposable elements and genes quantification.**

1396   For computing quantification of TE and gene levels, genome-mapped reads were used

1397   with Homer Software (using analyzeRepeats.pl script)(Heinz et al., 2010). We used

1398   different options : (i) –*noCondensing* to report the number of reads for each repeat

1399   element separately or (ii) –*condenseL1* to condense reads from each repeat name on

1400   one entry. Finally, the number of reads mapped to each TE was normalized to its length

1401   and total number of genome-aligned reads (FPKM value, Fragments Per Kilobase of

1402   exon model per Million mapped reads using (-rpkm). TE and genes with RPKM0.1 were

1403   excluded. Using these parameters, 85,795 unique TEs and genes were kept for

1404   analysis.

1405

1406   **Human MDDC RNAseq dataset**

1407   Publicly available RNA seq datasets from untreated and LPS stimulated human MDDCs

1408   were downloaded from ENCODE (GSE94180) and aligned to the human genome using

1409   the GRCh38/hg38 assembly.

1410

1411   **ChIP-Seq Analyses**

1412   **Mapping**

1413   H3K9me3 ChIP-seq reads were mapped to mouse genome build mm10 with Bowtie2 (v2.1.0)

1414   using the global alignment mode allowing 1 mismatch in seed alignment (Langmead and

1415   Salzberg, 2012). We processed only mapped *reads* with high mapping quality (*MAPQ>=20*)

1416   using samtools (v0.1.8).

1417

1418   **Transposable elements and genes quantification**

1419   For computing quantification of TEs and genes levels, we used genome-mapped reads were

1420   used with Homer Software (v4.7) (using analyzeRepeats.pl script) (Heinz et al., 2010). We used

1421   different options : (i) –noCondensing to report the number of reads for each repeat element

1422   separately or (ii) -condenseL1 to condense reads from each repeat name on one entry. Finally,

1423   the number of reads mapped to each TE was normalized to its length and total number of

1424   genome-aligned reads (FPKM value, Fragments Per Kilobase of exon model per Million

1425   mapped reads using (-rpkm).

1426

1427   **Peak Calling and Annotation**

1428   Peak calling was performed with SICER v1.1 using parameters : '-W 200 -G 600', peaks at 1 %

1429   FDR threshold(Xu et al., 2014). Annotation of Peaks was performed using Homer Software (with

1430   AnnotatedPeaks.pl script).

1431
Bigwig files and coverage visualization
All Bigwig files were generated with merged biological replicates to improve the sensitivity by increasing the depth of read coverage and normalized over own input ChIP-seq files using --BamCompare (with options: --effectiveGenomeSize 2719482043 for mm10 and --binSize 10 and –normalizeUsing BPM) from deepTools (v3.1.1) (Ramirez et al., 2014). For heatmap visualization were generated with --PlotHeatmap after to compute the matrix of scores per genome regions generated by the tool --ComputeMatrix from deepTools Software. Steady state H3K9me3 Suv39h1-dependent elements in WT vs KO (Figure 1C) were selected using *bedtools intersect* (default parameters) with WT peak and *subtract* (-A) with KO peak. H3K9me*3 Suv39h1*-dependent elements in untreated vs LPS-treated (2hr and 4hr) were selected using *bedtools intersect* (default parameters) with UT peak and *subtract* (-A) with WT 2hr peak and WT 4hr peak.

PAGE and Western Blot
For some proteins including SUV39H1, cells were lysed with LBX supplemented with EDTA-free protease inhibitors (Roche), phosphatase inhibitors (Halt phosphatase, ThermoFischer), and benzonase. They were incubated on ice for 30 minutes, vortexed at high speed for 15 seconds and then centrifuged for 10 minutes at 13,000x*g,* aliquoted, snap frozen on liquid nitrogen and stored at -80°C until further processing. Samples were thawed on ice and denatured in Laemmli sample buffer supplemented with fresh beta-mercaptoethanol for 15 minutes at 95°C. For phosphorylated and corresponding total protein levels including IRF3 and STING, cells were lysed in ice cold RIPA buffer (50mM Tris HCL, 150mM NaCl, 0.1% SDS, 0.5% DOC, 1%NP-40, EDTA-free protease inhibitor (Roche), phosphatase inhibitors (Halt phosphatase, ThermoFischer), and benzonase (ThermoFisher)). Lysates were incubated for 30 minutes on ice, vortexed for 15 seconds at high speed, and centrifuged for 8 minutes at 8,000 x *g*. Clarified lystates were aliquoted, snap frozen on liquid nitrogen and stored at -80°C. Samples were thawed on ice and denatured in Laemmli sample buffer supplemented with fresh beta-mercaptoethanol for 15 minutes at 95°C. Lysate from 10^5 cells were separated on 4–15% Mini Protean TGX Stain-Free gels (BioRad) and dry transferred to PVDF membranes (Bio-Rad) with the Trans-Blot Turbo Transfer System (Bio-Rad). In experiments where phosphor-STING was measured, lysate from $8.5x10^5$ cells was loaded per lane. Blots were blocked with TBS 0.05% Tween20 (TBST) and 5% non-fat milk (Carnation, total protein) or 10% Roche blocking reagent (phosphor-proteins) for 1hr at RT, rinsed in TBST, and then rocked overnight at 4°C in TBST 5% BSA (Fraction V, 04-100-812-C, Euromedex) with primary antibodies: cGAS (1:1000; 31659, CST), gp96 (1:1000; adi-spa-850-D, Enzo), Phosphor-IRF3 (Ser396)(1:1000; 29047, CST), Phosphor-STING (1:1000; CST), Phosphor-NF-KappaB p65 (Ser536)(1:1000; 3033, CST), Phosphor-TBK1/NAK (Ser172) (1:1000; 5483, CST), SUV39H1(1:500; 8729, CST), TBK1 (1:1000; 3504, CST), anti-RAB6 (1:1000, sc-310; Santa Cruz Biotechnology), Histone H3 (1:1000, CST), MDA5 (1:1000, CST). The following morning, blots were washed three times for ten minutes each in TBST at RT and then rocked for 1hr at RT in blocking buffer containing secondary antibodies (1:10,000; Jackson ImmunoResearch): anti-rat-horseradish-peroxidase (HRP) (112-035-143) or anti-rabbit-HRP (111-036-046). Following 4 more washes, membranes

40

1475 were coated with an HRP substrate (Enhanced Chemiluminescence, 32106; Thermo Fisher

1476 Scientific) and the images captured using a BioRad Chemidoc imager.

1477

Flow Cytometry

1479 Surface staining, flow cytometry, and washes were performed in FACS buffer (2%FBS, 0.5mM

1480 EDTA, PBS). BMDCs were surface-labeled with fluorochrome-coupled antibodies to the

1481 following antigens (all 1:400): MHCII-v450 (Invivogen, 48-5321-82), CD11b-PerCP-Cy5.5

1482 (Invivogen, 45-0112-82), CD11c-PE-Cy7(Invivogen, 25-0114-82), CD86-APC(BD Biosciences,

1483 561964). Dead cells were identified by APC-Cy7 live-dead (eBioscience, L34975) staining. For

1484 intracellular staining, BMDCs were fixed and permeabilized using the eBioscience FoxP3

1485 staining kit according to the manufacturer's recommendations, followed by incubation with anti-

1486 Viperin-PE (BD Biosciences,565196) with 1%normal mouse/rat serum (Sigma), and FC block

1487 (1:200; BD Biosciences, 553141) in permeabilization buffer for 45 minutes at RT. Cells were

1488 washed twice in permeabilizatoin buffer and a second time in FACS buffer before flow

1489 cytometric analysis. Flow cytometric data were collected on either a MacsQuant (Miltenyi) or a

1490 FACSVerse(BDbiosciences).

1491

Cytosolic DNA isolation and RTI

1493 Cytoslic DNA from 2×10^6 BMDCs was isolated as previously described (*42*). After LPS

1494 stimulation, BMDCs were harvested from 6-well dishes, washed twice in PBS, and resuspended

1495 in 200µl of digitonin buffer (150mM NaC, 50mM HEPES pH7.4, 25µg ml^{-1} digitonin (Sigma)).

1496 Samples were rotated end-over-end for 20 minutes at 4°C, cleared by centrifugation at 18,000 x

1497 *g* for 10 minutes to collect nuclei and cellular debris which were then dissolved in LBX and

1498 saved as the pellet fraction for normalization. Soluble material was treated with DNase-Free

1499 RNaseA (20µg) (Thermo Fisher, ENO531) at 37°C for 1hr, followed by proteinase K (20µg)

1500 (Thermo Fisher AM2548) for 1hr at 56°C. DNA was further purified with MinElute columns

1501 (Quiagen, 28004) or with AmpureXP beads (Beckman Coulter) according the manufacturer's

1502 guidelines and eluted in 250µl of DNase/RNase-free H_2O. 4µl of DNA extract from cytosol or the

1503 pellet fraction were amplified as described for qPCR of cDNA. For reverse transcriptase inhibitor

1504 treatment, a combination of emtricitabine, tenofovir disoproxil fumarate, and nevirapine were

1505 maintained at 10µM each in BMDC cultures from day 1 until the end of treatment. For control,

1506 DMSO vehicle was maintained alongside RTI-treated cultures.

1507

Lentivirus and shRNA

1509 Lentiviral shRNA (pLKO.1) plasmids for mouse Suv39h1 (7µg) (Sigma TRCN0000097439,

1510 TRCN0000097440, TRCN0000097441, TRCN0000097443) or control shRNAs for GFP (Sigma)

1511 and luciferase (Sigma) were combined with 7µg of psPAX2 packaging and 0.7µg of CMV-VSVg

1512 envelope vectors in 1ml of OptiMEM medium. 45µl of room temperature Trans-IT 293

1513 Transfection Reagent (Euromedex, Mirus 2700) was then added and the mixture incubated at

1514 RT for 30 minutes. All contents were then delivered to HEK293FT cells that were plated in

1515 10mls of DMEM (Gibco), 10%FBS (VWR, 11543387), 1%Penicillin, Streptomycin (Gibco) at 2-

1516 3×10^6 cell/10cm plate the previous night. The following morning, BSA (Sigma A7979) was

1517 added to a final concentration of 1% and the viral supernatants harvested 24hrs later by

1518 passaging through a 0.45µm filter. 2.5 mls of supernatant were then added to 0.5×10^6 cells in

1519   0.5mls in a 6-well tissue culture-treated dish () and left overnight. Cells were washed the next
1520   day and subjected to puromycin selection (2μg/ml, Invivogen ant-pr-5) 48hrs later. Knockdown
1521   efficiency was assessed by western blotting or qPCR as specified.
1522
1523   **SUV39H1 cloning and overexpression**
1524   For mouse SUV39H1 overexpression studies, a previously described lentiviral expression
1525   plasmid carrying RFP reporter and puromycin resistance genes (pL-
1526   SFFV.Reporter.RFP657.PAC, Addgene#61395) was used with the following modifications. After
1527   AgeI digestion, PacI and AbsI sites were introduced and one of the AgeI sites preserved
1528   downstream of the SFFV promoter by polylinker ligation using the following oligos:
1529   ACCGGTTTGGGATTAATTAAAATCACCTCGAGGCAGTCCGGT and
1530   TGGCCAAACCCTAATTAATTTTAGTGGAGCTCCGTCAGGCCA. In brief, oligos were
1531   phosphorylated with T4 PNK (NEB) for 30 minutes at 37°C, denatured at 95°C for 5 minutes
1532   and then ramped down to 25°C at 5°C per minute. Ligation proceeded overnight at 16°C using a
1533   thermocycler, and bacterial transformation of NEB 10-beta competent E. coli (NEB)
1534   accomplished by heat shock at 42°C. Minipreps were confirmed by Sanger sequencing.
1535   Sequential digestion of modified pL-SFFV-RFP with AgeI and BspEI were carried out at 37°C
1536   with column purification between reactions (QIAquick, Quiagen).   A mouse SUV39H1 gBlock
1537   was synthesized (IDT) and cloned into AgeI/BSpEI-digested pL-SFFV-RFP using Gibson
1538   assembly (NEB) for 1hour at 50°C. NEB10 (NEB) bacteria were transformed and selected on
1539   ampicillin plates after 14hrs at 37°C. Minipreps were grown at 30°C over night with shaking at
1540   250rpm. Plasmids constructs were confirmed by Sanger sequencing. The final SUV39H1
1541   plasmid contained a P2A signal followed by the RFP reporter gene, and an internal ribosome
1542   entry site (IRES), followed by the puromycin resistance gene.
1543
1544   **cGAS reconstitution**
1545   Human cGAS WT open reading frame was amplified by PCR from cDNA prepared from
1546   monocyte-derived dendritic cells and was previously described (Gentili et al., Science 2015).
1547   Murine cGAS WT open reading frame was amplified by PCR from cDNA prepared from C57BL6
1548   murine bone-marrow derived dendritic cells and was previously described (Gentili et al., Science
1549   2015). Human cGAS 161-522 C395A/C396A, and E225A/D227A were obtained by overlapping
1550   PCR. pTRIP-SFFV was generated by substitution of the CMV promoter with the SFFV promoter
1551   from GAE-SFFV-GFPWPRE and was previously described (Gentili et al., Science 2015, Raab
1552   et al., Science, 2016). Murine cGAS cDNA , Human cGAS 161-522 E225A/D227A, and Human
1553   cGAS 161-522 C395A/C396A were cloned in frame to EGFP in pTRIP-SFFV-EGFP to obtain
1554   pTRIP-SFFV-EGFP-ms cGAS, pTRIP-SFFV-EGFP-cGAS 161-522 C395A/C396A, and pTRIP-
1555   SFFV-EGFP-FLAG-cGAS 161-522 E225A/D227A. Leniviral particles were generated as
1556   described and used to transduce WT RAW264.7 macrophages.
1557
1558   **Software**
1559   Sequencing data was analyzed with R packages and R-Studio; heatmaps were generated using
1560   Morpheus software from the Broad Institute (https://software.broadinstitute.org/morpheus/).
1561   Flow cytometry data was analyzed with FloJo (Tree Star) v9.9.5. Graphs and statistical analysis

42

1562   were generated with Prism/Graphad (Version 7). Figure layouts were constructed with
1563   Prism/Graphpad, Adobe Illustrator, and Adobe Photoshop.
1564
1565

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Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure S1

Figure S2

Figure S3

Figure S4

Figure S5

Mock   IAV/PR8

Live/Dead (APC-Cy7)

82.5   66.9

Survival

v450 (Empty) ⟶

v450 (Empty)

0.148   38.5

Replication

anti-PR8/NP (AF488) ⟶