Figure S1

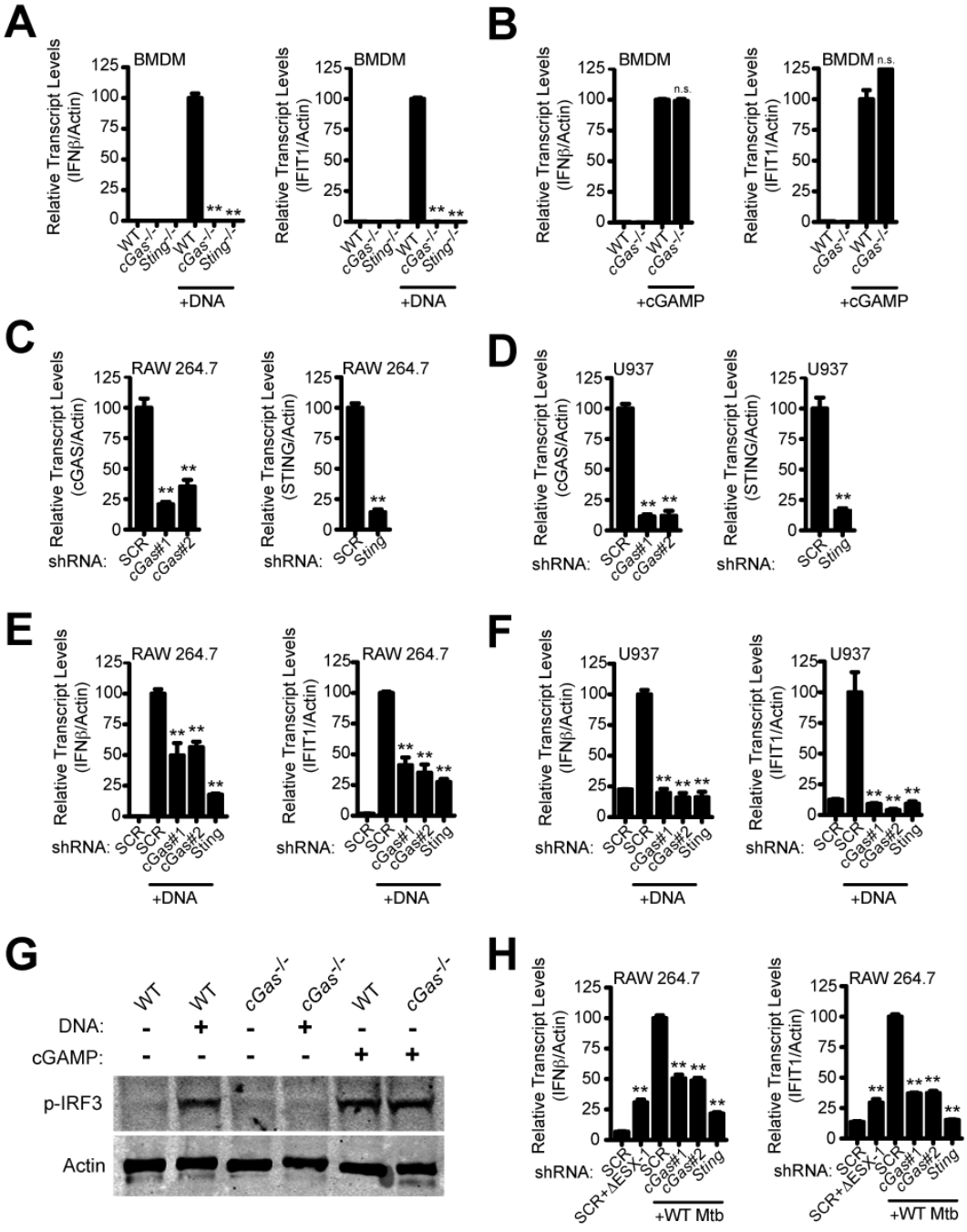
A
Relative Transcript Levels (IFNβ/Actin)
BMDM
WT
cGas-/-
Sting-/-
+DNA
Relative Transcript Levels (IFIT1/Actin)
B
n.s.
+cGAMP
C
Relative Transcript Levels (cGAS/Actin)
RAW 264.7
shRNA: SCR cGas#1 cGas#2
Relative Transcript Levels (STING/Actin)
shRNA: SCR Sting
D
U937
E
shRNA: SCR SCR cGas#1 cGas#2 Sting
F
G
DNA:
cGAMP:
p-IRF3
Actin
H
SCR+ΔESX-1
+WT Mtb

Figure S2

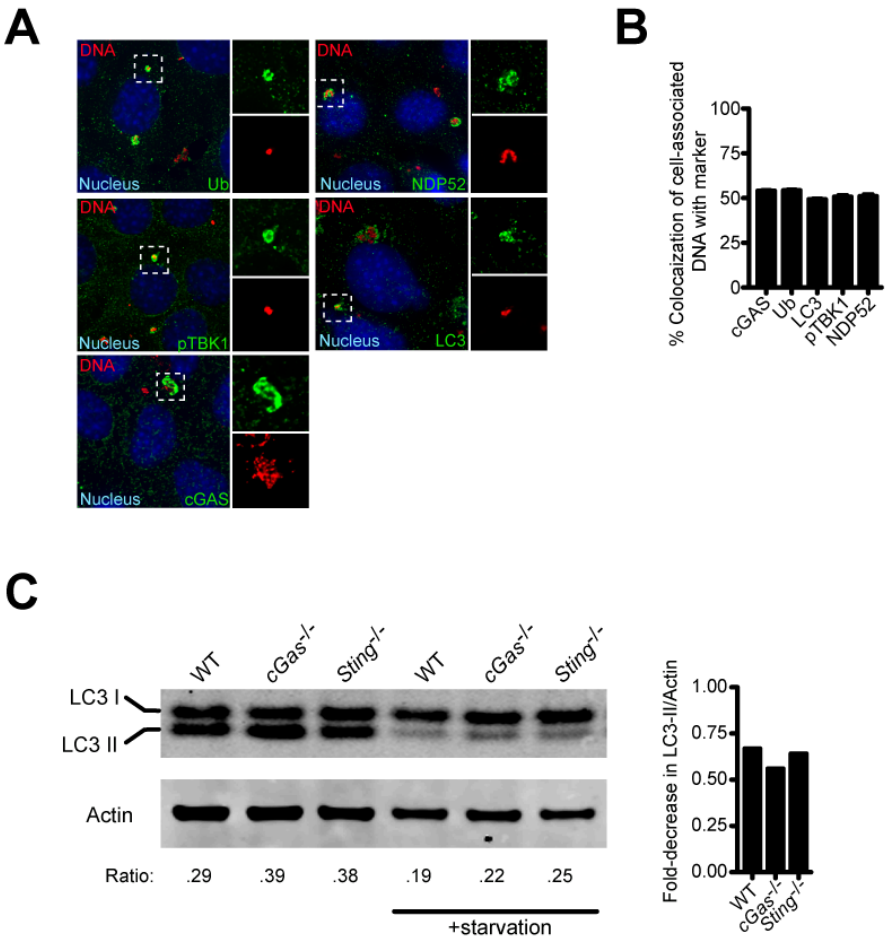
A
DNA
Nucleus
Ub
NDP52
pTBK1
LC3
cGAS
B
% Colocaization of cell-associated DNA with marker
100
75
50
25
0
cGAS
Ub
LC3
pTBK1
NDP52
C
WT
cGas-/-
Sting-/-
LC3 I
LC3 II
Actin
Ratio:
.29
.39
.38
.19
.22
.25
+starvation
Fold-decrease in LC3-II/Actin
1.00
0.75
0.50
0.25
0.00

Figure S3

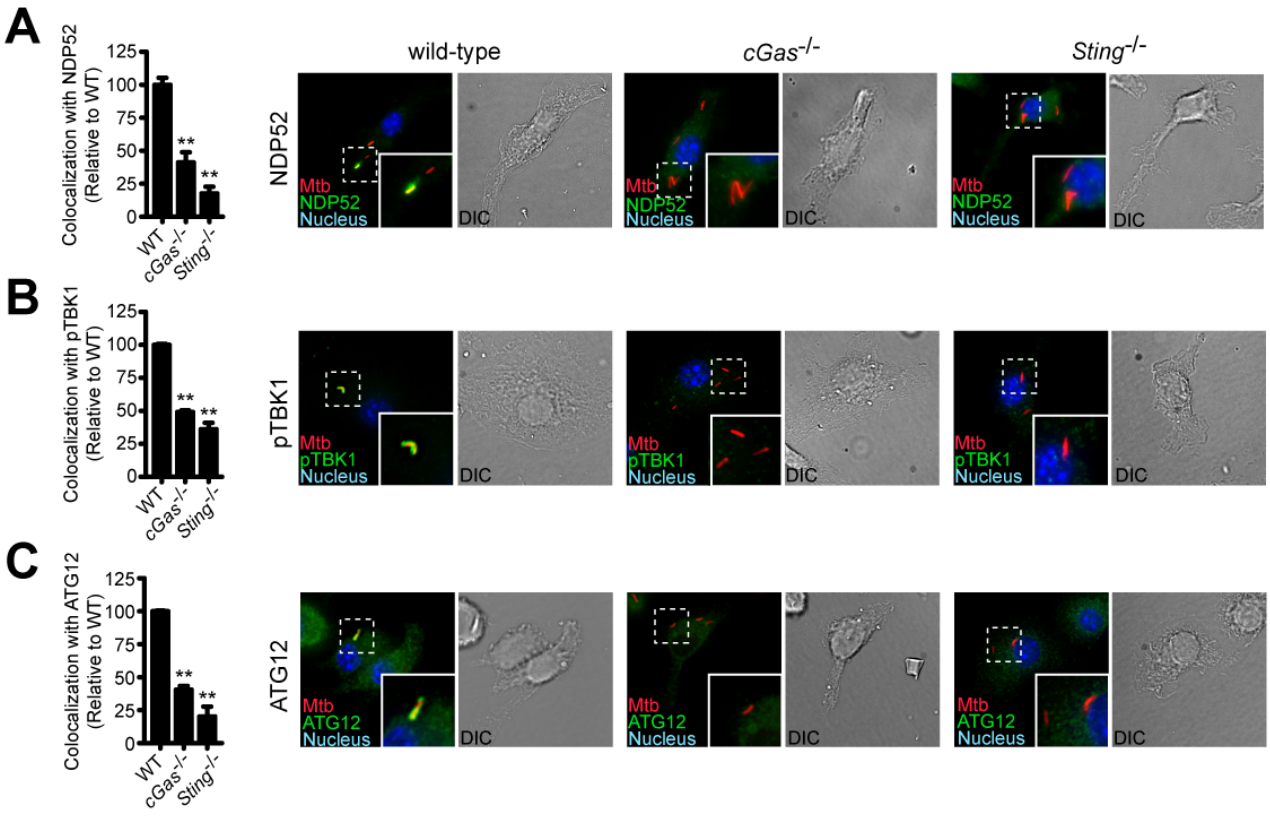
A
B
C
Colocalization with NDP52 (Relative to WT)
Colocalization with pTBK1 (Relative to WT)
Colocalization with ATG12 (Relative to WT)
125
100
75
50
25
0
**
WT
cGas-/-
Sting-/-
wild-type
cGas-/-
Sting-/-
NDP52
pTBK1
ATG12
Mtb
Nucleus
DIC

Figure S4

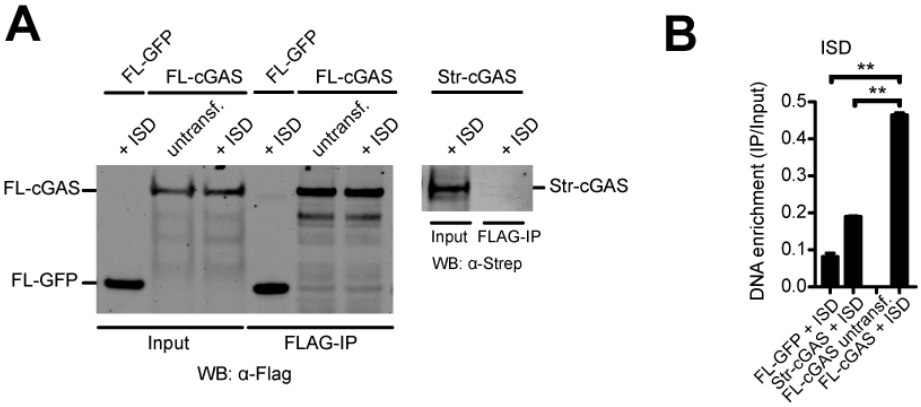
A
FL-GFP
FL-cGAS
FL-GFP
FL-cGAS
Str-cGAS
+ ISD
untransf.
+ ISD
+ ISD
untransf.
+ ISD
+ ISD
+ ISD
FL-cGAS
FL-GFP
Str-cGAS
Input
FLAG-IP
WB: α-Strep
Input
FLAG-IP
WB: α-Flag
B
ISD
**
**
DNA enrichment (IP/Input)
0.5
0.4
0.3
0.2
0.1
0.0
FL-GFP + ISD
Str-cGAS + ISD
FL-cGAS untransf.
FL-cGAS + ISD

Figure S1, related to Figure 1. cGAS is essential for induction of the cytosolic surveillence response to cytosolic DNA.
(A) WT, *cGas*$^{-/-}$, and *Sting*$^{-/-}$ BMDMs were transfected with interferon-stimulatory DNA (ISD) for 4 h, and IFN-β and IFIT1 transcript levels were measured. mRNA levels are expressed as percentages relative to transfected WT cells.
(B) WT and *cGas*$^{-/-}$ BMDMs were transfected with 2'-3'-cGAMP for 4 h, and IFN-β and IFIT1 transcript levels were measured.
(C) RAW 264.7 cells were transduced with lentivrial constructs expressing scrambled shRNA (SCR) or shRNAs targeting *cGas* or *Sting*, and mRNA levels were measured by RT-qPCR. mRNA levels are expressed as a percentage relative to SCR-expressing cells.
(D) Same as (C) but with the human monocyte cell line U937.
(E) RAW 264.7 cells stably expressing scramble-shRNA (SCR), shRNA-*cGas*, or shRNA-*Sting* constructs were transfected with ISD for 4 h, and IFN-β and IFIT1 transcript levels were measured. mRNA levels are expressed as percentages relative to transfected SCR-expressing cells.
(F) Same as (E) but with the human monocyte cell line U937.
(G) Western blot analysis of IRF3 phosphorylation in WT and *cGas*$^{-/-}$ BMDMs transfected with DNA or 2'-3'-cGAMP.
(H) RAW 264.7 cells stably expressing scramble-shRNA (SCR), shRNA-*cGas*, or shRNA-*Sting* constructs were infected with WT or ΔESX-1 *M. tuberculosis* for 4 h, and IFN-β and IFIT1 transcript levels were measured. n.s., not significant, $^{**}p < 0.005$ by two-tailed t-test comparing to treated WT or SCR.

Figure S2, related to Figure 2. cGAS is recruited to and required for targeting cytosolic DNA to the ubiquitin-mediated selective autophagy pathway.
(A) MEFs expressing FLAG-cGAS were transfected with Cy3-labelled plasmid DNA for 4 h and immunostained for 3xFLAG or indicated selective autophagy markers.
(B) Quantification of cGAS+ or selective autophagy marker-positive Cy3-DNA from (A). Differences are not statistically significant.
(C) WT, *cGas*$^{-/-}$ and *Sting*$^{-/-}$ BMDMs were starved for 30 min and LC3-II conversion was analyzed by quantitative Western blot and expressed as a ratio of LC3-II/Actin (left) and as a fold-increase of this ratio (right). Results shown are representative of at least three independent experiments.

Figure S3, related to Figure 3. cGAS is required to target *M. tuberculosis* to the ubiquitin-mediated selective autophagy pathway.
(A-C) WT, *cGas*$^{-/-}$, and *Sting*$^{-/-}$ BMDMs were infected with mCherry *M. tuberculosis* and immunostained for pTBK1 (A), NDP52 (B), and ATG12 (C) 4 h post-infection, and selective autophagy marker-positive bacteria were quantified. $^{*}p < 0.05$, $^{**}p < 0.005$ by two-tailed t-test comparing to wild-type unless otherwise indicated.

Figure S4, related to Figure 4. cGAS binds to cytosolic dsDNA after tranfection.
(A) Western blot of inputs and FLAG immunoprecipitates from untransfected or interferon-stimulatory DNA (ISD)-transfected RAW 264.7 cells stably expressing FLAG-GFP or FLAG-cGAS (left), or Strep-cGAS as a negative control (right). Whole cell lysates (Input) or

immunoprecipitates (Flag-IP) using anti-FLAG antibodies were separated by SDS-PAGE and blotted using anti-FLAG (left panel) or anti-Strep (right panel) antibodies.
(B) qPCR analysis of the abundance of ISD from DNA isolated from immunoprecipitated samples in (A) using primers specific to ISD. Amounts were normalized to abundance of ISD in inputs. Results are representative of at least three independent biological experiments and are reported as the mean ± SD (n = 3 per group). $**p < 0.005$ by two-tailed t-test.

Table S1, related to Experimental Procedures. shRNA target sequences.

Gene	Species	Target Sequence (5' – 3')
SCR	Mouse	CCTAAGGTTAAGTCGCCCT
cGas #1	Mouse	GGTGAATAAAGTTGTGGAA
cGas #2	Mouse	GAATTTGATGTTATGTTTA
Sting	Mouse	GAATGTTCAATCAGCTACA
SCR	Human	GGTTAAGTCGCCCTCGCTC
cGas #1	Human	GCTTCTAAGATGCTGTCAA
cGas #2	Human	GAATTTGATGTCATGTTTA
Sting	Human	GGGTTTACAGCAACAGCAT

Table S2, related to Experimental Procedures. RT-qPCR oligonucleotides.

Gene	Species	Forward (5' – 3')	Reverse (5' – 3')
Ifn-β	Mouse	TCCGAGCAGAGATCTTCAGGAA	TGCAACCACCACTCATTCTGAG
Ifit1	Mouse	CGTAGCCTATCGCCAAGATTTA	AGCTTTAGGGCAAGGAGAAC
Tnf-α	Mouse	ATGGCCTCCCTCTCATCAGT	GTTTGCTACGACGTGGGCTA
Actin	Mouse	GGTGTGATGGTGGGAATGG	GCCCTCGTCACCCACATAGGA
cGas	Mouse	CCACTGAGCTCACCAAAGAT	CAGGCGTTCCACAACTTTATTC
Sting	Mouse	TGGCCTTCTGGTCCTCTATAA	CTCGTAGACGCTGTTGGAATAA
Ifn-β	Human	CTTCTCCACTACAGCTCTTTCC	GCCAGGAGGTTCTCAACAATA
Ifit1	Human	CCAGAAATAGACTGTGAGGAAGG	CCCTATCTGGTGATGCAGTAAG
Actin	Human	GACCACCTTCAACTCCATCAT	CCTGCTTGCTAATCCACATCT
cGas	Human	GCCCTGCTGTAACACTTCTTAT	GGATAGCCGCCATGTTTCTT
Sting	Human	GCTGCTGTCCATCTATTTCTACT	GCCGCAGATATCCGATGTAATA

Table S3, related to Experimental Procedures. Oligonucleotides for DNA analysis of cGAS pulldowns.

Gene	Species	Forward (5' – 3')	Reverse (5' – 3')
16s	Mtb	CCGGAATTACTGGGCGTAAA	AGTACTCTAGTCTGCCCGTATC
dnaA	Mtb	CGACAACGACGAGATTGATGA	CGGTAGCGGAATCGGTATTG
IS6110	Mtb	CCCGTCTACTTGGTGTTGG	CTTCAGCTCAGCGGATTCTT
CRISPR	Mtb	GTCGTCAGACCCAAAAC	GTTTCCGTCCCCTCTC
ISD	n/a	TACAGATCTACTAGTGATCTATGAC	TGTAGATCATGTACAGATCAGT
Actin	Mouse	CTGAGTCTCCCTTGGATCTTTG	CCACAGCACTGTAGGGTTTA

Supplemental Experimental Procedures

Cell lines and cell culture. RAW 264.7 cells (ATCC TIB-71) and mouse embryonic fibroblasts (MEFs, generated from wild-type C57BL/6 mice by F. Mar) were cultured in DMEM with high glucose + 10% FBS + 20 mM HEPES + 2 mM L-glutamine. For selection, RAW 264.7 cells were grown with 5 μg/ml puromycin (Invivogen) or 150 μg/ml hygromycin (Life Technologies). U937 (ATCC CRL-1593.2) cells were cultured in suspension in RPMI + 10% FBS + 20 mM HEPES. For selection, 250 μg/ml hygromycin was added to the media. Before infection, U937 cells were plated on dishes coated with 0.1% gelatin (Sigma) and cultured with 10 ng/ml phorbol 12-myristate 13-acetate (PMA) (Sigma) for 48 h to induce differentiation. Bone marrow derived macrophages (BMDMs) were generated from wild-type C57BL/6 (The Jackson Laboratory), *cGas*$^{-/-}$ (Schoggins et al., 2014) (or (Li et al., 2013) for experiments with *L. pneumophila*), and *Sting*$^{gt/gt}$ (referred to as *Sting*$^{-/-}$)(Sauer et al., 2011) mice that were 8-12 weeks old. *cGas*$^{-/-}$ mice have a clean deletion of exon 2, which includes the catalytic domain of cGAS, and the knockout targeting cassette was removed, and the mice were fully backcrossed to the C57BL/6 background. All mice were bred and maintained in barrier facilities at Washington University in St. Louis or at the University of California, San Francisco and were handled in accordance with institutional and federal guidelines. Isolated BMDMs were cultured in DMEM + 20% FBS + 2 mM glutamine + 0.11 mg/ml sodium pyruvate + 10% M-CSF (bone marrow media).

Bacterial strains. The wild-type *Mycobacterium tuberculosis* strain used in these studies was the Erdman strain except when CDC1551 was used as described in the text. The ΔESX-1 mutant has been described previously (Stanley et al., 2003). Wild-type and ΔESX-1 mCherry-expressing strains are also previously described (Watson et al., 2012). All *M. tuberculosis* strains were cultured at 37°C in Middlebrook 7H9 broth or on 7H10 agar plates (BD Biosciences) supplemented with 10% OADC, 0.5% glycerol, and 0.1% Tween-80.

The 10403S strain of *Listeria monocytogenes* was the wild type strain used in these studies. Cultures were grown at 37°C in brain heart infusion broth or agar plates. *Salmonella typhimurium* serovar Typhimurium experiments used the SL1344 strain as the wild type strain. These cultures were grown at 37°C in LB broth or agar plates. The LPO2 *ΔflaA* strain of *L. pneumonphila* is previously described (Monroe et al., 2009) and was used as the wild type strain in order to prevent activation of the host inflammasome during infection. The *ΔsdhA* mutant is also described previously (Monroe et al., 2009). *L. pneumophila* cultures were grown at 37°C on BYCE plates or BYE media with thymidine, cysteine, and ferric nitrate.

Overexpression and knockdown cell lines. Tagged cGAS constructs were cloned using cGAS-specific primers (forward 5'- GAA GAT CCG CGT AGA AGG ACG -3'; reverse 5'- T GGG TTT CCA ATT TTT GAC AAG CTT TGA-3') and cDNA from mouse BMDMs. PCR products were cloned into Gateway pENTR1a vectors (Life Technologies) containing the indicated N-terminal tags. Constructs were Gateway cloned using LR clonase (Life Technologies) into a pLenti vector with a CMV promoter and puromycin selection marker (Campeau et al., 2009). shRNA sequences were designed using DSIR (Designer of Small Interfering RNA)(Vert et al., 2006), and are listed in Supplemental Table S1. Constructs were made by phosphorylating and annealing the primers, and ligating them into the pSicoR-GFP vector (Ventura et al., 2004) with a hygromycin selection marker and BFP.

For stably expressing cell lines, lentivirus was produced by transfecting LentiX cells (Clonetech) with expression or knockdown plasmids and the packaging plasmids, pspAX2 and pMD2.G. Cells were transduced with lentivirus and 8 μg/ml Polybrene (Millipore) overnight, allowed to recover for 24 h, and then grown under antibiotic selection for at least seven days. Expression of tagged constructs was tested by Western blot analysis, and knockdown efficiency was measured by qPCR analysis using primer sets listed in Supplemental Table S2. For transient expression of tagged cGAS in MEFs, cells were plated on cover slips in 24-well dishes and transfected the following day with 150 ng of cGAS expression vector. Cells were cultured an additional 16-24 h before use in subsequent experiments.

Bacterial infections. For RNA and cytokine analysis during *M. tuberculosis* infection, cells were plated in 6-well dishes: BMDMs and U937 cells were plated at $1x10^6$ cells/well, and RAW 264.7 cells were plated at $2x10^6$ cells/well. *M. tuberculosis* infections were performed as previously described(Ohol et al., 2010). Briefly, *M. tuberculosis* cultures were grown to log phase, and clumps were removed by low-speed centrifugation (500 *g*). Cultures were washed twice with PBS and then sonicated briefly to further remove clumps. Cells were infected at a multiplicity of infection (MOI) of 10 in DMEM + 10% horse serum with 10 min spinfection (1000 *g*). Infected cells were then washed twice with PBS and cultured in bone marrow media. Cells were harvested 4 h post-infection for RNA analysis, and culture supernatants were collected 24 h post-infection for cytokine analysis. For CFU assays, BMDMs were plated in 12-well dishes at $3x10^5$ cells/well and infected with *M. tuberculosis* as above at an MOI of 1. At the indicated time points, cells were washed twice with PBS and lysed in 0.5 ml 0.5% Triton X-100 (Sigma). Serial dilutions were made in PBS + 0.1% Tween-80 and plated on 7H10 agar plates. Colonies were enumerated 2-3 weeks post-infection to determine CFUs.

For *L. monocytogenes* infections, BMDMs were plated in 6-well dishes at $1x10^6$ cells/well. *L. monocytogenes* cultures were grown overnight at 37°C without shaking to prevent expression of flagellin to avoid activating the host inflammasome during infection. Cultures were diluted 1:10 and grown for 3 h until cultures reached log phase. To infect, bacteria and cells were washed twice with HBSS. Cells were infected in HBSS with 10 min spinfection at an MOI of 10. Infected cells were then washed twice with PBS + 50 μg/ml gentamycin, and cultured in BMDM media + 5 μg/ml gentamycin. Cells were harvested 6 h post-infection. For *S.* Typhimurium infections, BMDMs were plated at $1x10^6$ cells/well in 6-well dishes. *S.* Typhimurium cultures were grown overnight at 37°C and then diluted 1:150 in LB + 0.3M NaCl to induce expression of the type III secretion system. Cultures were grown to log phase and washed twice in warm HBSS. Cells were washed twice in warm HBSS and infected as described for *L. monocytogenes* infections, but at an MOI of 5. Additionally, cells were pretreated for 1 hr and then subsequently cultured with 100 μM Caspase-1 Inhibitor II (EMD Chemicals) to prevent activation of the host inflammasome during infection. For *L. pneumophila* infections, BMDMs were plated at $1x10^6$ cells/well in 6-well dishes. Bacteria were patched on BYCE with thymidine, cysteine and ferric nitrate and then resuspended and grown overnight at 37°C with shaking. Cells and bacteria were washed with PBS and infected as described for *L. monocytogenes* at an MOI of 1. Cells were harvested 4 h post-infection.

DNA transfections. For RNA analysis, cells were plated as described above in 6-well dishes. Cells were transfected with 2 μg of annealed ISD oligos (5'- TAC AGA TCT ACT AGT GAT CTA TGA CTG ATC TGT ACA TGA TCT ACA -3') using either Lipofectamine 2000

(Invitrogen) or PolyJet (SignaGen) as per manufacturer's instructions. ISD oligos were annealed in 5 mM Tris + 25 mM NaCl at 50 uM of each oligo by heating to 98°C for 5 min and then cooling to 25°C over 1 h. For cGAMP transfections, 2'-3' cyclic dinucleotide was purified as previously described (Diner et al., 2013) and transfected into cells using Lipofectamine 2000 (Invitrogen). Cells were harvested at 4 h post-transfection.

RNA isolation and qPCR analysis. For RNA analysis, cells were harvested in 1 ml Trizol. RNA was isolated using the PureLink RNA Mini Kit (Invitrogen), and samples were DNase treated on-column with PureLink DNase (Invitrogen). cDNA was synthesized using 0.5-1 ug total RNA and the iScript cDNA Synthesis Kit (Bio-Rad). qPCR analysis was performed using Taq DNA Polymerase (NEB) and SYBR Green I (Sigma) as a label. qPCR primers are listed in Supplemental Table S2, and values reported were in the linear range and normalized to actin or rps17 mRNA levels as indicated. Both the averages and the standard deviations of the raw values were normalized to the average of the treated (infected or transfected) wild-type sample, which was set at 100%.

Mouse infections. All procedures involving animals were conducted by following the National Institutes of Health guidelines for housing and care of laboratory animals and were performed in accordance with institutional regulations after protocol review and approval by the Institutional Animal Care and Use Committee of The Washington University in St. Louis School of Medicine. The *cGas*$^{-/-}$ mice used in these experiments were originally described by Schoggins et al. (Schoggins et al., 2014). Embryos derived from parental ES cells (line JM8.N4, Mb21d1$^{tm1a(EUCOMM)Hmgu}$) were received from ECOMM in the C57BL/6N background. The gene trap cassette was removed by crossing this mouse to FLP-deleter and Cre-deleter mice (both C57BL/6J), generating a knockout of exon 2, which contains the catalytic site of cGAS (Supplemental Figure S3D) (Schoggins et al., 2014). The resulting mice were further backcrossed to C57BL/6J to remove the FLP and Cre transgenes. Using speed congenics, we verified that the *cGas*$^{-/-}$ mice were 99% C57BL/6 and a mix of B6N and B6J. Mice heterozygous for the mutant *cGas* allele were bred, and wild-type and knockout littermates were used for infection.

The *M. tuberculosis* inoculum was prepared as described for macrophage infections. Age- and sex-matched wild-type and *cGas*$^{-/-}$ mice (male and female littermates, approximately 8-10 weeks old) were infected with approximately 100 *M. tuberculosis* using an inhalation exposure system (Glas-Col). At the indicated times, homogenized lungs and spleens were plated in serial dilutions on 7H10 agar plates to enumerate CFUs in infected mice. RNA from lungs were collected in RLT buffer + β-mercaptoethanol (Qiagen RNeasy Kit), bead beaten, and frozen at -80°C. RNA was isolated using the RNeasy Kit (Qiagen), and DNA was removed with the TURBO DNA-free Kit (Ambion). Blood was collected and serum isolated after organs were dissected. For time-to-death experiments, mice were infected as above and weighed regularly to monitor disease progression. Mouse survival was assessed until day 102 by monitoring body weight of infected mice. Previous work has demonstrated that the number of mice used in this study is sufficient to give accurate and reproducible results.

Cytokine measurements. Cell culture supernatant IFN-β levels were measured using VeriKine-HS Mouse IFN Beta Serum ELISA Kit (PBL Assay Science) according to the manufacture's instructions. IFN-β levels in mouse serum and cell culture supernatants were measured using

L929 ISRE-luciferase reporter cells as previously described (Woodward et al., 2010). Briefly, reporter cells were plated in a 96-well dish and incubated with serum or supernatants for 6-8 h. Cells were washed and reporter activity was measured using the Luciferase Reporter Assay (Promega) according to the manufacturer's instructions.

Antibodies. The following primary antibodies were used for microscopy studies: mouse monocolonal antibodies against ubiquitinylated proteins (Millipore, clone FK2), Calcoco2 (NDP52, accession number NM_001271018) (Abnova, No. H00010241_B01P), and 3xFLAG (Sigma, clone M2); and rabbit polyclonal antibodies against 3xFLAG (GenScript, No. A00170), phospho-TBK1 (Cell Signaling, D52C2 #5483S), Atg12 (Cell Signaling, #2011S), and LC3B (Life Technologies, No. L10382). Secondary antibodies used were: Alexa 488- and 647-conjugated goat anti-rabbit and Alexa 488- and 647-conjugated goat anti-mouse IgG antisera (Molecular Probes). The following primary antibodies for Western blot analysis: mouse monoclonal antibodies against actin (Sigma, clone AC-40) and 3xFLAG (Sigma, clone M2); and rabbit polyclonal antibodies against phospho-IRF3 (Cell Signaling, 404G #4947S), LC3B (Abcam, No. ab48394), and strep-tag (GenScript, No. A00626). Secondary antibodies used in Western blot analysis included IRDye680 and IRDye800 goat anti-mouse and IRDye680 and IRDye goat anti-rabbit IgG antisera (Li-Cor).

Microscopy. Cells were plated on cover slips in 24-well dishes and either transfected with 100 ng Cy3-DNA (Mirus Label IT Plasmid Delivery Control Cy3) or infected with mCherry *M. tuberculosis* as described above at an MOI of 1. At the designated time points, cells were fixed in 4% paraformaldehyde for 10 min at room temperature. The fixed cells were washed three times in PBS and permeabilized by incubating them in PBS containing 5% non-fat milk and 0.05% saponin (Calbiochem) (PBS-MS). Cover slips were incubated in primary antibody diluted in PBS-MS for 1-3 h. The cover slips were then washed three times in PBS and incubated in secondary antibody for 1 h. After two washes in PBS and two washes in deionized water, the cover slips were mounted onto glass slides using Prolong Gold Antifade Reagent (Molecular Probes). Images were acquired on a Nikon Ti-E microscope fitted with a Coolsnap HQ2 CCD camera (Photometrics) controlled by NIS-Elements 4.20 (Build 982) software (Nikon Instruments) and then deconvolulted with Huygens Deconvolution software (Scientific Volume Imaging).

Colocalization of markers with *M. tuberculosis* and cytosolic DNA. To quantify the percentage of *Mycobacterium*-containing phagosomes or cytosolic DNA puncta colocalized with different cellular markers, infected cells were visualized directly by fluorescence microscopy. A series of images were captured including internalized bacteria or cell associated DNA and the cellular marker. Overlayed fluorescent images were analyzed to enumerate the number of *Mycobacterium*-containing phagosomes or cell-associated DNA that co-localized with the corresponding marker. Bacteria or cytosolic DNA were considered positive for the presence of a marker when they contained detectable amounts of the antibody/fluorescence signal. A minimum of one hundred bacteria or DNA puncta were analyzed per cover slip for each treatment and designated post-infection time. Each experiment was completed in triplicate cover slips, and data is expressed as a percentage relative to wild-type. For triple labeling experiments, one hundred marker-positive bacteria or DNA puncta were assessed for colocalization with the second marker. For example, one hundred cGAS+ bacteria were assessed for pTBK1 colocalization. In

experiments with knockout or knockdown cells, genotypes were blinded throughout experimentation, processing, and quantification.

LC3 conversion assays. BMDMs were plated at $3x10^5$ cells/well in 12-well dishes. For DNA treatment, cells were transfected with 1.5 μg ISD and harvested 2 h post-transfection. For starvation, cells were washed twice with HBSS, incubated in HBSS to induce starvation, and harvested 30 min post-starvation. For *M. tuberculosis* infections, cells were infected at an MOI of 20 and harvested 2 h post infection. To harvest, cells were resuspended and directly lysed in 2x NuPage LDS Sample Buffer (Invitrogen) and then sonicated. Samples were run on 4-12% Bis-Tris gels in MES running buffer (Invitrogen). The same Westerns blots were split and probed with rabbit anti-LC3 antibody (Abcam) and mouse anti-actin (Sigma) as an internal loading control. Blots were imaged using IRDye secondary antibodies (Licor) and the Odyssey infrared imaging system. The ratio of LC3-II/Actin was calculated using the fluorescent intensity of each band (LC3-II and Actin) for every sample. The fold-change of this ratio was calculated by comparing the treated and untreated samples.

Biotin-DNA pulldowns. 5'-biotinylated ISD oligos (Elim Biopharm, Hayward, CA) were annealed as described above. $2x10^8$ RAW 264.7 cells expressing FL-cGAS were transfected with 40 μg of biotinylated or non-biotinylated ISD. 2 h post-transfection, cells were harvested and lysed by sonication in 50 mM Tris pH 7.4 + 150 mM NaCl + 0.075% NP-40. Cleared cell lysates were incubated with streptavidin agarose (Invitrogen) for 2 h, and beads were washed five times with lysis buffer. Proteins were eluted by resuspending the agarose in 2x NuPage LDS Sample Buffer (Invitrogen) and boiling, and precipitated proteins were visualized by Western blot analysis with anti-3xFlag antibody (Abcam).

cGAS immunoprecipitation and DNA analysis. RAW 267.4 cells were plated at $5x10^7$ cells/15cm plate. Cells were infected at an MOI of 20 or transfected with 25 μg of annealed ISD oligo using PolyJet (SignaGen). At 45 min post-infection or post-transfection, cells were washed twice with cold PBS and harvested. Cells were fixed for 5 min in 4% PFA and quenched with 1 M Tris pH 7.4 for 5 min. Cells were washed with PBS and frozen at -80°C until processing. Fixed cells were lysed in 50 mM Tris pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.1% NP-40, and 1.25% Triton X-100 with 30 min total sonication in BioRuptor water bath sonicator. Tagged constructs were immunoprecipated using M2 FLAG Magnetic Beads (Sigma) at 4°C overnight. Immunoprecipitates were washed twice with 50 mM Tris, 150 mM NaCl, 1 mM EDTA, and 0.05% NP-40 (Wash Buffer); once with Wash Buffer + 500 mM NaCl; and twice more with Wash Buffer. Immunoprecipitated proteins were eluted with FLAG peptide (synthesized by Bioneer, Inc., Alameda, CA). IP efficiency was confirmed by boiling samples to reverse crosslinks and performing SDS-PAGE and Western blot analysis with anti-3xFLAG (Sigma) and anti-2xStrep (GenScript) antibodies. Samples for DNA analysis were diluted in 10 mM Tris + 1 mM EDTA + 0.65% SDS and incubated for 16 h at 65°C to reverse crosslinks. Samples were treated with 0.2 mg/ml proteinase K (Sigma) for 2 h at 37°C and nucleic acids were extracted with phenol:chloroform:isoamyl alcohol and precipitated with ethanol. DNA pellets were resuspended in 10 mM Tris, and an equal percentage of the immunoprecipitated material was used in qPCR analysis to measure the abundance of specific DNA sequences. Primers designed to amplify ~100 bp fragments from the *M. tuberculosis* and mouse genome (listed in Supplementary Table S3) were used in qPCR analysis.

Statistics. Statistical analysis of data was performed using GraphPad Prism software (Graphpad, San Diego, CA). Two-tailed unpaired Student's t-tests were used for analysis of gene expression, microscopy images, and *in vitro* CFU assays. Unless otherwise noted, all results are representative of at least two independent biological experiments and are reported as the mean ± SD (n = 3 per group). The sample sizes used in this study were sufficient to detect differences as small as 10-20% using the statistical methods described.

Supplemental References

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