SUPPLEMENTARY METHODS

RNA interference.

10,000 MEFs per well (24-well plate) were reverse-transfected with 10 nM siRNA-lipofectamine 2000 mix (4.5 µl lipofectamine 2000/ µl siRNA at 40 µM) for 24 h, rinsed, and stimulated or not with 500 nM OHT. All siRNAs were synthesized as pre-annealed Dicer-substrates by Integrated DNA Technologies (m = 2'OMe modification; r = RNA base and Uppercase = DNA base). Sequences used were: mouse siSTING#1: m-siSTING1-S rCrCrArArCrArGrCrGrUrCrUrArCrGrArGrArUrUrCrUrGGA m-siSTING1-AS rUrCrCrArGrArArUrCrUrCrGrUrArGrArCrGrCrUrGrUrUrGrGrUrU; mouse siSTING#1Mut: m-siSTING1-Mut-S rCrCrArArCrArGrCrGrUrCrUrArCrGrArGrArUrUrCrUrGGA m-siSTING1-Mut-AS rUrCrCrArGrArArUrCrUrCrGrUrArArArArGrCrUrGrUrUrGrGrUrU; mouse siSTING#2: MMC.RNAI.N028261.12.1_2nmS rGrCrUrGrUrArUrArCrArArUrCrArCrArGrUrGrGrGrCrUGG; MMC.RNAI.N028261.12.1_2nmA rCrUrUrArGrGrGrArGrGrUrArUrArCrArUrGrArUrCrUrArUrUrArU; mouse sicGAS#1: m-cGAS1-S rGrGrArUrUrGrArGrCrUrArCrArArGrArArUrArUrUrArUGA; m-cGAS1-AS rUrCrArUrArArUrArUrUrCrUrUrGrUrArGrCrUrCrArArUrCrCrUrG; mouse sicGAS#2: MMC.RNAI.N173386.12.1_2nmS rArGrArUrUrGrArArArCrGrCrArArArGrArUrArUrCrUrCGG; MMC.RNAI.N173386.12.1_2nmA rCrCrGrArGrArUrArUrCrUrUrUrGrCrGrUrUrUrCrArArUrCrUrCrA; non-targeting control siNC5: siNC5S3 mCmArUmArUmUrGrCrGrCrGmUrAmUrAmGrUmCrGrCrGrUrUmAG; siNC5AS12 rCrUmArArCrGrCrGmArCmUrAmUrArCrGrCrGrCrArArUmArUmGmGmU.

Reverse transcription quantitative real-time PCR (RT-qPCR).

Total RNA was purified from cells using the innuPREP Micro RNA Kit (Analytik Jena), the mirVana miRNA isolation kit (Life Technologies), the ISOLATE II RNA Mini Kit (Bioline) or the AllPrep DNA/RNA kit (Qiagen). For mRNA quantification, cDNA was synthesized from isolated RNA using the High-Capacity cDNA archive kit (Life Technologies) according to the manufacturer's instructions. RT-qPCR was carried out with the SensiFAST™ SYBR® Hi-ROX Kit (Bioline) on the HT7900 RT-PCR system (Life Technologies). Each PCR was carried out in technical duplicate. Mouse and human 18S were used as reference genes. Each amplicon was sequence-verified and used to generate a standard curve for the quantification of gene expression (used in each run). Melting curves were used in each run to confirm specificity of amplification. Values were considered as not detected (nd) when the amplification cycle was greater than 36 or that of the non-template water control. The primers used were the following: Mouse Rn18S: mRn18s-FWD GTAACCCGTTGAACCCCATT; mRn18s-REV CCATCCAATCGGTAGTAGCG; Mouse Ifit1: mIfit1-RT-FWD GAGAGTCAAGGCAGGTTTCT; mIfit1-RT-REV TCTCACTTCCAAATCAGGTATGT; Mouse Rsad2: mRsad2-FWD CTGTGCGCTGGAAGGTTT; mRsad2-REV ATTCAGGCACCAAACAGGAC; Mouse Ifnb1: mIfnb1-FWD CCCTATGGAGATGACGGAGA; mIfnb1-REV CCCAGTGCTGGAGAAATTGT; Mouse Irf1: mIrf1-FWD CAGGACTTGGATATGGAAAGGG; mIrf1-REV

GTCTTCGGCTATCTTCCCTTC; Mouse Ifih1: mIfih1-FWD TCTTGGACACTTGCTTCGAG; mIfih1-REV TCCTTCTGCACAATCCTTCTC; Mouse Oas3: mOas3-FWD GTACCACCAGGTGCAGACAC; mOas3-REV GCCATAGTTTTCCGTCCAGA; Mouse Oasl1: mOasl1-FWD CTCTCTATGCCCTGGAGCTG; mOasl1-REV TTTGTCCCCTGAGCTGTCTC; Mouse Isg15: mIsg15-FWD CAATGGCCTGGGACCTAAAG; mIsg15-REV TAAGACCGTCCTGGAGCACT; Mouse Tnfaip3: mTnfaip3-RT-FWD AGCTGAGATCAAGCCAGCAT; mTnfaip3-RT-REV GTGGGCCACAGAACCTACTC; Mouse Sting: mSting-RT-FWD TGGCCTTCTGGTCCTCTATAA; mSting-RT-REV CTGGTAAGATCAACCGCAAGTA; Mouse Cxcl10: mCxcl10-RT-FWD GCTGCCGTCATTTTCTGC; mCxcl10-RT-REV CACTGGGTAAAGGGGAGTGA; Mouse cGas: mcGas-RT-FWD GGCCGAGACGGTGAATAAA; mcGas-RT-REV TCACAAGATAGAAAGCACCTGT; Human RSAD2: hRSAD2-RT-FWD TGGTGAGGTTCTGCAAAGTAG; hRSAD2-RT-REV GTCACAGGAGATAGCGAGAATG; Human IFIT1: hIFIT1-FWD TCACCAGATAGGGCTTTGCT; hIFIT1-REV CACCTCAAATGTGGGCTTTT; Human IFIT2: hIFIT2-RT-FWD TTATTGGTGGCAGAAGAGGAAG; hIFIT2-RT-REV CCTCCATCAAGTTCCAGGTG; Human 18S: h18S-FWD CGGCTACCACATCCAAGGAA; h18S-REV GCTGGAATTACCGCGGCT. Cre: Cre-FWD CGT ACTGACGGTGGGAGAAT; Cre-REV CCCGGCAAAACAGGTAGTTA.

Immunofluorescence.

Cells were plated on coverslips and fixed at various time points following OHT stimulation or camptothecin (5 µM for 2 h) in 10% formalin. Cells were permeabilized using 0.2% Triton X-100 and blocked with 5% BSA. Cells were then incubated for 1 h with a 1/200 dilution of Alexa Fluor® 647 conjugated rabbit monoclonal anti-PhosphoHistone-H2A.X (Ser139) (#9720 Cell Signaling). The nucleus was stained using Hoechst 33342 10 µg/ml (#H3570 Life Technologies). For the dsDNA staining, cells were incubated with a 1/50 dilution of anti-DNA (#AC-30-10 Novus Biological) for 1 h followed by 3 washes. A dilution of 1/1000 of goat anti-mouse AlexaFluor 568 IgM antibody was used as a secondary antibody. All washes/dilutions were carried out in PBS supplemented with 1 mM $CaCl_2$ and 1 mM $MgCl_2$. Confocal imaging was performed using API DeltaVision Widefield and a 40X objective. Percentages of phospho-H2A.X-positive cells were determined by counting cells with bright foci per field (using a 20X objective), relative to the total amount of cells per field (determined with Hoechst staining), and averaged from five fields for each coverslip (with two coverslips for each independent experiment). Percentages of cytoplasmic anti-dsDNA-positive cells were determined by counting bright cytoplasmic foci per field (using a 20X objective), relative to the total amount of cells per field and averaged from 10 fields for each coverslip (with two coverslips for each independent experiment). Overall, at least 100 cells have been counted per condition per independent experiment. Analyses were performed using ImageJ v1.49.

Genome-wide microarray analyses.

Total RNA was purified from *Dicer1*$^{f/f}$ x *Cre/ESR1*$^{+}$ and *Dicer1*$^{f/wt}$ x *Cre/ESR1*$^{+}$ MEFs (96 h after +/- OHT treatment) and *Dicer1*$^{wt/wt}$ x *Cre/ESR1*$^{+}$ and *Dicer1*$^{wt/wt}$ x *Cre/ESR1*$^{-}$ (48 h after +/- OHT treatment), using the ISOLATE II RNA Mini Kit (Bioline). Cy3-labeled cRNA was prepared from 0.1 µg total RNA using the One-Color Low input Quick Amp labeling Kit (Agilent, v6.6 September 2012), followed by RNeasy column purification (Qiagen). 600 ng of Cy3-labeled cRNA was fragmented at 60°C for 30 min in 1x Agilent fragmentation buffer and 2x Agilent blocking agent. On completion, 25 µl of 2x Agilent gene expression hybridization buffer was added and 43 µl of sample hybridized for 17 h at 67°C in a rotating Agilent hybridization oven with one Agilent SurePrint G3 Mouse Gene Expression 8 x 60K (028005) microarray. After hybridization, microarrays were sequentially washed 1 min at room temperature with GE wash buffer 1 (Agilent) and 1 min with 37°C GE wash buffer 2 (Agilent). Slides were scanned on the Agilent C DNA microarray scanner using one-color scan settings for 8x60k array slides. The scanned images were analyzed with Feature Extraction Software 11.0.1.1 (Agilent). Feature-extracted files are available in GEO (GSE72024), and were analyzed with GeneSpring 13.0 (Agilent) using Percentile shift (75th percentile), and a Baseline median of all parameters to normalize the data. Of 55,821 probes, 99 were significantly correlated with Rsad2 expression (A_52_P670026) (Pearson $0.95 \leq r \leq 1.0$), among which were 67 coding genes, including 52 annotated as type-I IFN induced (≥ 2 fold) in the *Interferome* v2.01 database (8). Normalized data for selected genes were processed with the GENE-E matrix visualization and analysis platform (Broad Institute) for the creation of expression heat maps.

Cre-overexpression studies.

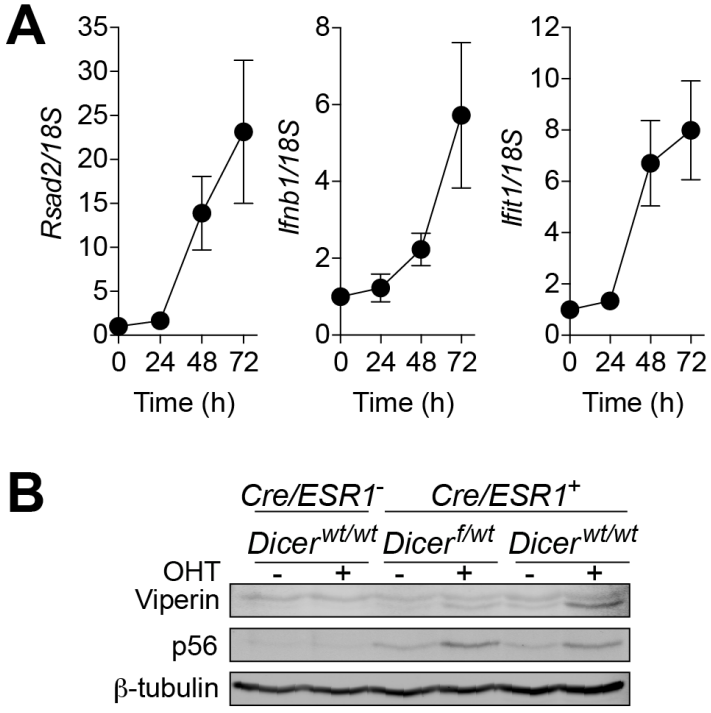
Stable A549 cells and *Sting*$^{+/+}$ or *Sting*$^{-/-}$ primary MEFs expressing Cre or Citrine (Control) from pRP-Cre or pRP-Citrine retroviral constructs were generated as follows. Briefly, 60% confluent LentiX^{TM} 293T cells were transfected with 10 µg of pRP-Cre/Citrine, 1 µg of VSG-g and 10 µg of gag-pol constructs, with the BES-adapted method of calcium phosphate. Medium was replaced 24 h after transfection. Two days after transfection, viral-containing supernatants were filtered at 0.45 µm and supplemented with 8 µg/ml of polybrene (#107689, Sigma), and added to 50% confluent A549 cells or MEFs. Cells were rinsed on day 3 and further expanded in 2 µg/ml puromycin for another nine days for A549 cells (12 days from infection), and 3 weeks for the MEFs. A synthetic Cre-R173K mutant gene (synthesised and fully confirmed by IDTDNA) was used to replace the native Cre sequence within pRP-Cre.

Co-culture studies.

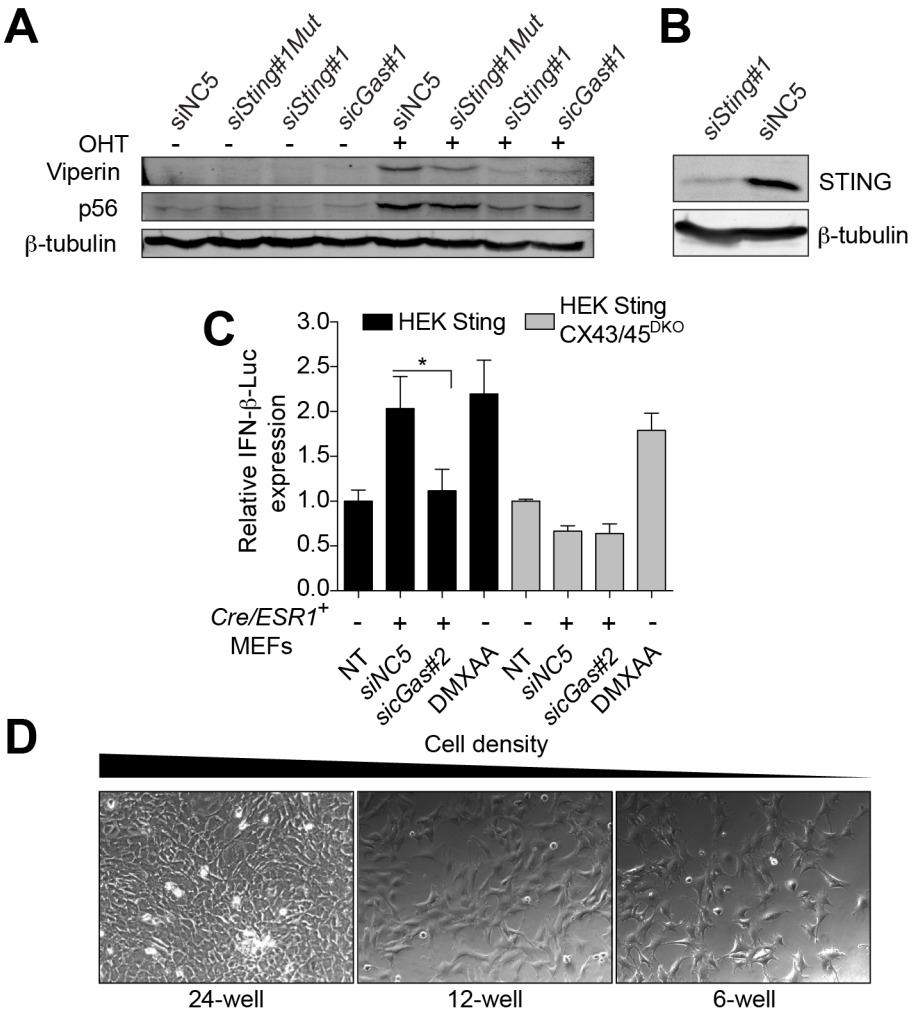
~30,000 *Dicer1*$^{f/f}$ x *Cre/ESR1*$^{+}$ and *Dicer1*$^{wt/wt}$ x *Cre/ESR1*$^{+}$ MEFs were treated for 24 hrs with 10 nM of sicGAS#2 or the control siNC5. The cells were then co-cultured in a 24-well plate for 48 hrs with ~60,000 HEK cells transiently expressing an IFN-β-Luciferase reporter (pLuc-IFN-β, kind gift from K. Fitzgerald). Following cell lysis with 100 µl Glo lysis buffer (Promega) per

well, 40 µl of Luciferase Assay Reagent (Promega) were added to 15 µl of lysate prior to luminescence quantification with a Fluostar OPTIMA (BMG LABTECH) luminometer.

SUPPLEMENTARY DATA



Supplementary Figure S1. Activation of an antiviral response in *Dicer1*$^{f/f}$ x *Cre/ESR1*$^{+}$ and *Dicer1*$^{f/wt}$ x *Cre/ESR1*$^{+}$ cells but not in control *Dicer1*$^{wt/wt}$ x *Cre/ESR1*$^{-}$ cells. (**A**) RT-qPCR analysis of ISGs in *Dicer1*$^{f/wt}$ x *Cre/ESR1*$^{+}$ MEFs after 24 h OHT treatment. Data are shown relative to 24 h non-OHT-treated cells (data are presented as mean of three independent experiments in biological duplicate ± s.e.m.). (**B**) Viperin and p56 levels in BMDMs treated with OHT, 72 h after OHT stimulation. Data shown are representative of a minimum of three independent mice for each genotype.



Supplementary Figure S2. STING controls ISG induction by Cre-ER^{T} in *Dicer1*$^{f/f}$ x *Cre/ESR1*$^{+}$ MEFs. **(A)** *Dicer1*$^{f/f}$ x *Cre/ESR1*$^{+}$ MEFs were transfected with 10 nM of the indicated siRNAs overnight, prior to 24 h OHT stimulation. Viperin and p56 levels were analyzed 48 h after stimulation (*siNC5* is a non-targeting siRNA and *siSting#1Mut* is a control *siSting#1* sequence mutated to prevent Ago2-mediated mRNA cleavage). Blots are representative of three independent experiments. **(B)** Validation of STING down-regulation in *Cre/ESR1*$^{+}$ MEFs transfected with 10 nM siRNA for 48 h. Blots are representative of two independent experiments. **(C)** HEK WT, Sting and Sting CX43/45^{DKO} cells expressing an IFN-β-Luciferase reporter were cultured alone (NT and DMXAA conditions), or co-cultured 48 hrs with *Dicer1*$^{f/f}$ x *Cre/ESR1*$^{+}$ MEFs pre-treated for 24 hrs with 10 nM siNC5 or sicGAS#2. IFN-β-Luciferase expression was reported relative to the NT condition for each cell line (data presented are averaged from two independent experiments in biological triplicate ± s.e.m. with unpaired Mann-Whitney U test to the sicGAS condition shown). **(D)** 12, 000 *Dicer1*$^{f/f}$ x *Cre/ESR1*$^{+}$ MEFs were seeded in 24, 12 or 6-well plates, prior to 24 h treatment with OHT and expansion for another 48 h (in Figure 5E). Pictures shown are representative of the cell density at this time point (i.e. 72 h after stimulation) for each type of well.